CHAPTER 1
INTRODUCTION

1.1 HETEROCYCLIC COMPOUNDS

The study of heterocyclic compounds is a vast and expanding area of chemistry because of their applications in medicine, agriculture, photodiodes and other fields. Many heterocyclic compounds occur naturally and are actively involved in biology as nucleic acids (Purine and pyrimidine bases), vitamins (Thiamine B₁, Riboflavin B₂, Nicotinamide B₃, Pyridoxol B₆ and Ascorbic acid C), Heme and Chlorophyll, Penicillins, Cephalosporins, Macrolides etc [1]. Why does nature utilize heterocycles? The answer to this very valid question is due to the fact that heterocyclic compounds are able to get involved in an extraordinarily wide range of reaction types.

Though plants are the main source of heterocyclic compounds, the huge demand of these classes of compounds cannot be fulfilled only from natural plant source. Hence they are synthesized by various routes and used extensively.

Heterocyclic compounds have attracted the attention of both academic and industrial communities. Hence there is a continuous development of methodology to assemble molecules containing heterocyclic templates. A number of pesticides, antibiotics, alkaloids and cardiac glycosides are heterocyclic natural product of high significance for human and animal health. Some dyes (e.g. mauveine), luminophores, (e.g. acridine orange), pesticides (e.g. diazinon) and herbicides (e.g. paraquat) are also heterocyclic in nature.
Heterocyclic compounds are also used as additives and modifiers, and in a variety of industries including reprography, information storage, plastics, solvents, antioxidants and vulcanization accelerators.

Among the heterocycles, nitrogen, sulphur and oxygen containing ring compounds is an expanding area of research in medicinal chemistry, exhibiting a wide range of biological activities including antidepressive, analgesic, antipsychotic, antihistaminic, antimuscarinic, antitumor, antimicrobial, antiinflammatory, antiepileptic, antiviral, antineoplastic, antihypertensive, antimalarial, local anaesthetic, antianxiety, antioxidant, antitubercular, antiparkinson’s, antidiabetic, antiobesity and immunomodulatory agents.

Many natural drugs, such as papaverine, theobromine, quinine, emetine, theophylline, atropine, procaine, codeine, reserpine and morphine are heterocycles. Almost all the compounds we know as synthetic drugs, such as diazepam, chlorpromazine, isoniazid, metronidazole, azidothymidine, barbiturates, antipyrine, captopril and methotrexate are also heterocycles [2]. They have a great applicability as drugs because of two reasons such as

(I) They have specific chemical reactivity and

(II) They provide convenient building blocks to which biologically active substituents can be attached.

All these natural and synthetic heterocyclic compounds can and do participate in chemical reactions in the human body. Furthermore, all biological processes involved in our body are chemical in nature. Heterocyclic compounds participate in the chemical reaction which give fundamental manifestations of life such, as provision of energy, transmission of nerve impulses, sight metabolism and the transfer of hereditary information.
1.1.1 Quinazolines

Quinazolines (1) is a bicyclic compound earlier known as Benzo-1,3-diamine [3]. Quinazolines and their derivatives are the building blocks for approximately 150 naturally occurring alkaloids isolated from a number of families of the plant kingdom. Quinazoline was prepared by Gabriel in 1903. It is a yellow coloured compound found usually in crystalline form. The name quinazoline was proposed for its compound by Weddige as this was isomeric with the then compound Cinnoline (2) and Quinaoxaline (3). The numbering system presently followed for the quinazoline ring was suggested by Paal and Bush.

Though the chemistry of quinazoline compounds dates back to centuries old, the intensive research for the synthesis of biologically active series began only in the last few decades. The development of research on biologically active compound gained momentum when the compound, 2-Methyl-1,3-aryl-4-quinazoline derivative was synthesized. This compound has soporific and sedative action. The compound was synthesized based on the general concept that the presence of a quaternary carbon atom in the β-position is the characteristic feature for the pharmacological activity.

Up to 1968, only few derivatives of quinazoline were used as soporific, anticonvulsant and diuretic agents. By 1980, about 50 kinds of derivatives of this compound are used for other medical purposes. In the last 10-15 years, there has been a phenomenal growth in the search for biologically active quinazoline derivatives.
1.1.1.1 Methods of Synthesis of the Quinazoline Nucleus [4]

Riedel obtained a patent for quinazoline in 1905 describing the synthesis of quinazoline from \( o \)-Nitrobenzaldehyde with formamide to form \( o \)-Nitrobenzylidene-diformamide. Reduction of this with zinc and dilute acetic acid gave quinazoline, in good yield. Later Riedel's original procedure was improvised to get 65% yield. This is known as the best procedure for obtaining quinazoline.

This method has been applied to substitute quinazolines for the preparation of 6,7-Dimethoxy quinazolines through the intermediate of 6-Nitroveratraldehyde. The usual synthesis of quinazolines makes use of an \( o \)-Disubstituted benzene structure from which the quinazoline skeleton is completed by adding C-2 and N-3 in various ways. Substituents could either be in the pyrimidine ring or in the benzene ring or in both the ring.

2 and 4-Alkyquinazolines could be readily prepared from \( o \)-Acylaminobenzaldehyde or Phenyl ketones. They show typical reactions of alkyl groups placed \( ortho \) or \( para \) to a heterocyclic nitrogen atom. 2-Methyl and 2,4-Dimethylquinazoline undergo mannich reaction. Moreover, sodium hypobromide prefer to add methyl group in the fourth position in preference to first or second positions. In order to get improved yields, ammonia is passed through a fusion of the \( o \)-Ketoanilides or \( o \)-Acylaminoketone with ammonium or sodium acetate at 165-175 °C. Condensation of \( o \)-Aminoaceto or Benzophenone with oxalyl chloride gives Di-anilides which on ring closure gives 2,2-Biquinazolines.

4-Methyl-2-quinazolone is prepared by heating \( o \)-Aminoacetophenone and an excess of urea at 190 °C for 20 min. Reaction of \( o \)-Aminobenzaldehyde under similar condition with urea gives 2-Quinazoline. Niementowski described the first synthesis of 4-Quinazolones in 1985. When Anthranilic acid is heated in an open container with excess formamide at 120 °C, water is expelled and a nearly quantitative conversion to 4-Quinazolone is achieved. The reaction has been carried out with a variety of substituted anthranilic acids to get the corresponding 2-Substituted 4-Quinazolones. As a rule, a higher temperature or a longer reaction
time is required for substituted anthranilic acids. It has been adapted in many aliphatic amides to get alkyl substituents in the 2nd position. At a higher temperature, yield becomes low. The yield with benzamide is found to be extremely low. But Sherril and Co-workers have obtained a 50% yield of 2-Phenyl-4-quinazolone by replacing benzamide with thiobenzamide or ethylimidobenzoate. A method of wide applicability for the preparation of 4-Quinazolones involves the direct synthesis and isolation of the desired N-Acylantranilamide. When the amide was heated above its melting point, water is removed resulting in the formation of quinazoline ring. Formylantranilamide easily yields 4-Quinazolone. 4-Quinazolone with substituents in any position can be easily obtained by heating 5-Chloro-N-acetyl anthranilide for several hours to give 6-Chloro-2-methyl-3-phenyl-4-quinazolone.

1.1.1.2 Medicinal Uses of Quinazolines

Quinazoline derivatives, which belong to the N-containing heterocyclic compounds, have caused universal concerns due to their widely and distinct biopharmaceutical activities. Researchers have already determined many therapeutic activities of quinazoline derivatives, including anticancer [5], antiinflammation [6], antibacterial [7], analgesia [8], antivirus [9], anticytotoxin [10], antispasm [11], antituberculosis [12], antioxidation [13], antimalarial [14], antihypertension [15], antiobesity [16], antipsychotic [17], antidiabetes [18], etc. They are also denovon to act as thymidyalate synthase, poly (ADP-Ribose) polymerase (PARP) [19] and proteine tyrosine kinase inhibitors.

Quinolones are known to inhibit DNA synthesis by promoting cleavage of bacterial DNA gyrase and type-IV topoisomerase, resulting in rapid bacterial death [20]. Certain drugs based on quinoline moiety, such as doxorubicin and mitoxantrone have been established as one of the most effective classes of anticancer agents in clinical use today with broad application in the treatment of several leukemia and lymphomas, as well as in combination chemotherapy of solid tumors [21]. The potent anticancer activity as well as toxic effects described for these compounds are normally ascribed, at least, to two main mechanisms viz., the one, which is associated with protein involves trapping of the protein enzyme-DNA
cleavable intermediate, whereas the other, a non-protein-associated mechanism, is related to redox cycling of the Quinoline moiety, which produces damaging free-radical species.

Similarly, various 4-Thiazolidinones have attracted considerable attention as they are also endowed with a wide range of pharmaceutical activities including anaesthetic, anticonvulsant, antibacterial and antiviral. Moreover, drug research and development have led to the discovery of new pharmacologically active agents, including imidoxy compounds, such as succinimidoxy. They also possess a strong anticonvulsant activity. 4-Thiazolidinones may be considered as phosphate bioisosteres and therefore inhibit the bacterial enzyme MurB which is involved in the biosynthesis of peptidoglycan layer of the cell wall. In addition, some thiazolidinones were recently reported as novel inhibitors of mycobacterial rhamnose synthetic enzymes. This new approach is believed to be selective, as rhamnose is not found in humans, but is essential for mycobacterial cell wall synthesis in animals [22].

1.1.1.3 Quinazoline Marketed Drugs [23]

Gefitinib (4) also known as Iressa. The chemical name is $N$-(3-Chloro-fluoro-phenyl)-7-methoxy-6-(3-morpholin-4-ylpropoxy) quinazolin-4-amine. Gefitinib is an EGFR inhibitor (epidermal growth factor receptor) which interrupts signaling through the epidermal growth factor receptor in target cells and used as anticancer drug. The trade name of Vandetaib is zactima (5). It is also known as ZD6474 and has chemical name $N$-(4-Bromo-2-fluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl) methoxy] quinazolin-4-amine. It is an antagonist of the Vascular Endothelial Growth Factor Receptor (VEGFR) and the Epidermal Growth Factor Receptor (EGFR). It is a tyrosine kinase inhibitor. The drug has a third target, which inhibits RET-tyrosine kinase activity, an important growth driver in certain types of thyroid cancer.

Evodiamine (6) inhibits the growth of certain cancer cells. It also manipulates metabolism when combined with certain drugs and influences the
secretion of catecholamines from adrenal glands [24]. Raltitrexed (7) chemically known as \( N\)-[(5-[Methyl-4-oxo-1,4-dihydroquinazolin-6-yl]methyl)amino]-2thienyl)carbonyl]-L-glutamic acid, is an antimetabolite drug used in cancer chemotherapy. It is an inhibitor of thymidylate synthase. It works by inhibiting dihydrofolate reductase, an enzyme used in the synthesis of tetrahydrofolate, thereby preventing the synthesis of thymidylate.

CB 3717 (8) is chemically known as (2S-[[4-[2-Amino-4-oxo-1H-quinazolin-6-yl]methyl-prop-2-ynylamino]benzoyl]amino]pentanedioic acid. It is used as antineoplastic agent, folic acid antagonist of enzyme Tetrahydrofolate dehydrogenase and used in cancer chemotherapy. EBE-A22 (9) [25] is used as antineoplastic agent and intercalating agent (capable to insert themselves between bases of DNA). They are used in the study of DNA.

NSC137192 (10) is chemically known as 8-Methylbenzo[f]quinazoline-1,3-diamine. The drug invention provides compositions and methods for the treatment of \( \beta \)-cell proliferative disorders that employ an A2A receptor agonist or one or more PDE inhibitors. Quinazolinone (11) [26] chemically known as Quinazolin-4(3H)-one are a class of drugs used in the treatment of cancer.

Erlotinib (12) [27] is a drug used to treat non-small cell lung cancer, pancreatic cancer and several other types of cancer. It is a tyrosine kinase inhibitor, which acts on the Epidermal Growth Factor Receptor (EGFR).
Prazosin [28] is chemically 2-[4-(2-Furoyl)piperazin-1-yl]-6,7-dimethoxyquinazolin-4-amine (13). It is a sympatholytic drug used to treat high blood pressure. It belongs to the class of alpha-adrenergic blockers, which lower blood pressure by relaxing blood vessels.
Alfuzosin (14) [29] is a $\alpha_1$ receptor antagonist used to treat Benign Prostatic Hyperplasia (BPH). It works by relaxing the muscles in the prostate and bladder neck, making it easier to urinate. Bunazosin (15) is an alpha-1 antagonist. Bunazosin was initially developed to treat benign prostatic hyperplasia (BPH). The mechanism of action is a reduction of aqueous outflow through the uveoscleral pathway resulting in lowering the intraocular pressure. It also may act to improve blood flow to the ocular nerve.

Anagrelide (16) [30] is a drug used for the treatment of Essential Thrombocytosis (ET)m or overproduction of blood platelets. It is also used in the treatment of chronic myeloid leukemia.
The trade name of Proquazone (17) is Biarison. It is chemically known as 1-Isopropyl-7-methyl-4-phenylquinazolin-2(1H)-one. It is used as a non-steroidal antiinflammatory drug.

![Figure 1.5 Antiinflammatory quinazoline drug](image)

Quinethazone (18) is marketed as Hydromox. It is chemically known as 7-Chloro-2-ethyl-4-oco-1,2,3,4-tetrahydroquinazoline-6-sulfonamide [31]. It is a diuretic drug used to treat hypertension. Fenquizone (19) is a diuretic drug used primarily in the treatment of oedema and hypertension. Ketanserin (20) is a selective serotonin receptor antagonist with weak adrenergic receptor blocking properties. This drug is used in the treatment of acute or chronic vascular hypertension.

![Figure 1.6 Antihypertension quinazoline drugs](image)
Albaconazole (UR-9825) \( (21) \) chemically known as 7-Chloro-3-[(2,4-difluorophenyl)-3-hydroxy-4-(1,2,4-triazol-1-yl)butan-2-yl]quinazolin-4-one, is a triazole antifungal. It has potential broad-spectrum antibacterial activity.

![Figure 1.7 Antibacterial quinazoline drug](image)

Febrifugine \( (22) \) [32] is a quinazolinone alkaloid first isolated from Chinese herb *Dichroa febrifuga*, but also found in the farden plant *Hydrangea*. Febrifugine has antimalarial properties and the halogenated derivative halofuginone is used in veterinary medicine as a coccidiostat.

![Figure 1.8 Coccidiostat quinazoline drug](image)

Linagliptin \( (23) \) is known by its trade name Ondero. It is a DPP-4 inhibitor. Phase III clinical trial results of linagliptin showed that the drug can effectively reduce blood sugar.
Figure 1.9 Quinazoline drug to reduce blood sugar

Quazinone (24) [33] is a cardiotonic and vasodilator drug, which was developed and marketed in the 1980s for the treatment of heart disease. It acts as a selective PDE3 inhibitor.

Figure 1.10 Quinazoline drug for heart disease

Benzouracil (25) is chemically known as 2,4(1H,3H)-Quinazolinedione. The substituted Benzouracil is used in treating or preventing an infection due to a virus from the Flaviridae family.

Figure 1.11 Antiviral quinazoline drug
NSC127213 (26) is useful as inhibitors of H1R and/or H4R for the treatment or prevention of inflammatory, autoimmune, allergic and ocular diseases. BIBN4096BS (27) is chemically known as (R(R*,S*))-N-(2-((5-Amino-1-((4-(4-pyridinyl)-1-piperazinyl) carbonyl)pentyl)amino)-1-((3,5-dibromo-4-hyroxyphenyl) methyl)-2oxoethyl)-4-(1,4-dihydro-2-oxo-3(2H)quinazolinyl)-1-piperidinecarboxamide [34]. It is a potent competitive antagonist of the relaxant effects of alpha-CGRP on human temporal artery. BIBN4096BS is a potent and selective CGRP antagonist.

Figure 1.12 CGRP antagonist quinazoline drugs

Trimetrexate (28) [35] is a nonclassical folic acid inhibitor which exhibits inhibition of the enzyme dihydrofolate reductase. It is being tested for efficacy as an antineoplastic agent against pneumocystis pneumonia in AIDS patients. Other uses include skin lymphoma. ZD 9331 (29) is a non-polyglutamatable thymidylate synthase inhibitor, used for the treatment of solid tumors and other neoplasia, including colorectal tumors.
1.1.2 Triazines

Triazines are six-membered aromatic heterocycles comprised of three carbon and three nitrogen atoms. The three isomers of triazines are 1,2,3-triazine (30), 1,2,4-triazine (31), and 1,3,5-triazine (32). The 1,3,5-triazines are the oldest and most extensively studied of the isomeric forms. Since 1,3,5-triazine is a symmetrical molecule, compounds of this type are often referred to as 3-triazines.

Triazine chemistry has a wide range of starting applications from industrial usage, to academic interests in dendritic structures which may one day come to fruition as pharmaceutically applicable molecules. Organic synthesis, using the 1,3,5-triazine derivative, 2,4,6-trichlorotriazine, cyanuric chloride, are used in organic synthesis. Due to the selective reactivity of cyanuric chloride, a plethora of targets from small molecules to large dendrimers may be synthesized.

1,3,5-Triazine was unknowingly first synthesized by Nef in 1895 by treating hydrogen cyanide with ethanol in an ether solution saturated with hydrogen.
chloride. The resulting salt was then treated with base and distilled to give 1,3,5-triazine in low yields, 10%. Nef incorrectly identified the product as a dimeric species. However, in 1954, Grundmann and Kreutzberger proved the compound to be a trimer of hydrogen cyanide, $s$-triazine.

1,3,5-Triazine is thermally stable unless heated to above 600 °C where it decomposes to form hydrogen cyanide. The triazine ring is fairly resistant to electrophilic substitution. However, it may readily undergo ring cleavage with nucleophiles and is very sensitive to hydrolysis by water and other hydroxyl compounds to a lesser degree. A variety of heterocycles can be prepared from 1,3,5-triazine by treatment with bifunctional amines or related compounds, and it may be used as an alternative for HCN in reactions [36]. The most commonly used triazine derivatives are cyanuric acid (33), melamine (34), and cyanuric chloride (35).

Triazines were first synthesized as the compound known as cyanuric acid (33). In 1776, Scheele produced cyanuric acid through the pyrolysis of uric acid. The common name of cyanuric acid was developed because, at that time, the compound was considered to be composed of cyanide groups, and it had been produced from uric acid. In 1820, Serullas repeated the work of Scheele to obtain cyanuric acid from cyanogens in water. It was discovered in 1830 that the two products were indeed the same, and the structure was elucidated by Liebig and Wohler.

1,3,5-Triazine represent a widely used lead structure with multitude of interesting application in numerous fields [37]. Several derivatives of $s$-triazine
show herbicidal [38], antimicrobial [39]. The replacement of a chlorine atom in cyanuric chloride by basic group is greatly facilitated by the ring nitrogen atom of the symmetrically built \(s\)-triazine nucleus. 2,4,6-trichloro-\(s\)-triazine derivatives are prepared [40] by replacement of one chlorine atom at 0-5 °C, second one at 35-45 °C and third one at 80-100 °C.

Some examples of biologically active compounds containing the 1,3,5-triazines unit [41].
For example hexamethylmelamine (HMM, 36) and 2-amino-4-morpholino-s-triazine (37) are used clinically due to their antitumor properties to treat lung, breast and ovarian cancer, respectively [42]. Hydroxymethylpentamethylmelamine (HMPMM 38) is also the hydroxylated metabolite which corresponds to the major active form of HMM. More recently, significant aromatase inhibitory activity were observed for 1,3,5-triazines of general structure (39). For the similar general structure (40) antitumor activity in human cancer and murine leukemia cell were observed [43]. The 1,3,5-triazine (41) presents potential use as siderophore (microbial iron shelter) mediated drug [44] and the general structure (42) presents potent corticotrophin-releasing factor₁ receptor antagonist activity [45]. The compounds of type (43) show potent activity against leukotriene C₄ (LTC₄) antagonist, which possess a protective effect on HCl-Ethanol induced gastric lesions [46]. More recently it was discovered that the compound (44) is a potent corticotrophin-releasing factor₁ receptor antagonist [47]. Among several other 1,3,5-triazine substituted polyamines tested, the substrate (45) presents a good in vitro activity against the protozoan parasite Trypanosoma brucei, the causative organism of Human African Trypanosomasis [48].

The diverse biological activities observed for different molecules containing the 1,3,5-triazine unit have been further explored in order to discover other new potential molecules through the synthesis of libraries by combinatorial approaches. The 1,3,5-triazine unit has also been used as a key functional group in host-guest chemistry, mainly based on the possibility to generate organized aggregates via the formation of strong three simultaneous hydrogen bonds (46).
Other applications of the 1,3,5-triazine derivatives are: as chiral stationary phases, for example, the chiral solvating agent (47) for the determination of enantiomeric excess by NMR spectroscopy and determination of absolute configuration by circular dichroism; for the preparation of luminescent, optical switches and tri-radical cation species in the case of 2,4,6-triamino-1,3,5-triazine compounds of general structure (48); as metal complexes, liquid crystals, calixarenes, polymers and optical brighteners for household washing powder (49).

They also found widespread applications in textile, plastic, and rubber industries, and are used as pesticides, dyestuffs, optical bleaches, explosives, photostabilizers and surface active agents. The chemistry of this group of compounds has been studied intensively and has been the subject of many reviews [49].

Drug delivery applications of triazine-based dendrimers were also investigated. Pegylated G3 dendrimers with molecular weights of 18 and 34 kDa with 9% and 17% iodine content by weight, respectively, were synthesized as potential macromolecular contrast agents. The development of macromolecular contrast agents is of great interest to counteract the drawbacks associated with currently used, small molecule contrast media, including toxicity, extravasation into the extracellular space, and rapid clearance from the bloodstream. Dendrimers are well suited for use as macromolecular media due to the unique properties of these molecules, including monodispersity and multivalency.

The modification of chitosan for herbicide remediation has been accomplished using triazine chemistry, as well. Treatment of chitosan iteratively
with cyanuric chloride followed by piperazine produces dendritic grafts from these flakes. Dendrons of generation one through three were synthesized on chitosan backbones of low medium, and high molecular weights. The piperazine derivatives were shown to sequester more than 99% of atrazine from an aqueous 100 ppb solution in a 24 h period.

The 1,2,4-triazine ring is a prominent structural motif found in numerous natural and synthetic biologically active compounds.

Various reviews dealing with the synthesis of condensed 1,2,4-triazines have been published [50]. The 1,2,4-triazine moieties play a vital role in many biological activities including antihypertensive [51], antiviral [52], blood-platelet aggregation inhibitory [53], analgesic, and antibacterial properties as well as some of new antiHIV [54] and anticancer agents [55].

For example well-known antiviral drug azaribine is structurally based on the 1,2,4-triazine scaffold. In addition, certain azanucleosides, for example, 6-azacytosine and 6-azauracil, bearing the 1,2,4-triazine heterocycles [56], have displayed an impressive array of biological activities, such as antimicrobial, antiviral, antiinflammatory and antimalarial activities. Furthermore, 6-azaisocytosine (3-Amino-1,2,4-triazin-5(2H)-one), an isosteric isomer of 6-azacytosine and 6-azauracil, is a great biological interest due to its resistance to deaminase [57-61].

1.1.3 Tetrazoles

1.1.3.1 Introduction

The creation of novel drugs containing a tetrazole ring as structural fragment has contributed considerably to the outstanding achievements of the pharmaceutical chemistry in the last decade.

Tetrazole is an aromatic azapyrazole. It is a five membered ring compound containing one carbon, four nitrogen and two hydrogen atoms and carries two double bonds. Tetrazole can exist in two tautomeric forms (50, 51) as shown below:
Tetrazoles are not found in nature. The simplest tetrazole is CH₄H₂. It is white to pale yellow crystalline solid with weak characteristic odour. It is soluble in water and alcohol. Due to the presence of four nitrogens, it is acidic in nature. In tetrazoles, two of the six π electrons required by the Huckel are provided by the lone pair of one nitrogen while the remaining four π electrons are provided by the other four atoms of the ring [62]. The tetrazole ring possesses a strong electron withdrawing inductive effect (-I) which surpasses the weak mesomeric effect (+M), and therefore the ring is a deactivating group [63]. It is this property that makes it possible to use tetrazoles as isosteric substituents of various functional groups in the development of biologically active substances. In the last decades, tetrazole has a renaissance especially as carboxylic acid mimicry. The biological properties of tetrazoles are due to the fact that it is metabolically more stable then the acid function [64].

1-Substituted tetrazoles have not yet been widely used for the creation of pharmaceutical products. The best known are certain derivatives of β-Lactam antibiotics and optically active tetrazole-containing antifungal preparations of the type (52), such as TAK-456 [65].
Unlike fungicidal preparations of the first- and second-generation azole type, the tetrazole-containing preparation exhibit high activity against *Candida*, *Cryptococcus* and *Aspergillus* with peroral administration. A water-soluble form TAK-457 for injections was developed on the basis of TAK-456.

There is hardly any information on the use of 2-Substituted tetrazoles in the creation of biologically active substances due to the difficulty of obtaining such compounds.

In recent years, 5-Substituted tetrazoles have been mentioned more and more frequently as nonclassical isosteres of the carboxyl group. The term “nonclassical isosterism” derives from the concept that functional groups having similar physicochemical properties can be interchangeable, while the biological activity of the initial and the new compounds will be similar. Nonclassical isosteric substituents may or may not have similar steric or electronic character, and the substituting and substituted groups may even differ in the number of atoms.

Tetrazole and 5-Substituted tetrazoles are NH acids whose acidity constants depend largely on the substituent and position. Moreover, the $pK_a$ values of 5-Alkyl- and 5-Aryltetrazoles and the corresponding carboxylic acids are quite close. Like carboxylic acids, the tetrazoles are ionized in the range of physiological pH values (~7.4) and have a planar structure. At the same time, it has been shown that ionized tetrazoles are ten times more lipophilic than the corresponding carboxylic acids [66], which in some cases enable these compounds to penetrate the cell membrane with greater ease [67].

The delocalization of the negative charge in the tetrazole ring is another important factor that must be taken into account when tetrazoles are used as isosteric substituents of the carboxyl group. It has been observed that the distribution of charge on the large surface of the molecule impede contact and reduce the capacity for bonding with the active center [68]. Therefore, it is difficult to predict in advance the pharmacological effect of substitution of a carboxyl group by tetrazole in advance.
It is worth to mention that the interest in tetrazoles as replacements for a carboxyl group has increased in recent years. The best known and most successful example of such use of tetrazole is the series of antihypertensive preparations are Losartan (53) and its analogs. Losartan belongs to the class of Angiotensin II receptor antagonists, and a large number of papers have been dedicated to it [69].

It is assumed that a preparation of the Losartan type binds to the receptor as a result of the fact that the blocker molecule enters the lipophilic “pockets” of the receptor through its lipophilic substituents at positions 2 and 4 of the imidazole. It was found that the hydrocarbon radical at position 2 must contain between three and five carbon atoms and have a normal structure. In one part of the receptor, there is a basic group bonding which requires the presence of an acidic function in the molecule, i.e., the tetrazole ring in the case of Losartan.

During the development of Losartan, a large number of compounds with various functional groups in the biphenyl part of the molecule were studied. It was established that preparations with carboxyl, amide, sulfamide and other groups were not sufficiently effective during peroral administration, and found to be extremely active during intravenous use. The effectiveness of the product in peroral use proved several times higher than in the previously investigated compounds, when the carboxyl group was replaced by tetrazole.

Compounds that may prove useful for the treatment of diabetes and contain a tetrazole ring as the acidic fragment have been studied actively in recent years. The series of perfluoroamides were studied [70].
In compound (54), the tetrazole acts as an isostere of the thiazolidinedione ring. Further study of the mechanism of the action of the compound resulted in the creation of a series of new safe preparation (55).

The development of glutamate receptor antagonists is at the present time one of the promising trends in biochemistry. These compounds are considered promising drugs against cerebral ischemia, schizophrenia and other diseases of the central nervous system. They may also prove extremely important for understanding their pharmacology and the therapeutic potential of the whole class of antagonists and agonists of glutamate receptors [71]. At present, the search is going on for selective and nonselective antagonists and potentiators of all type of glutamate receptors, and compounds of tetrazole play a significant role in these investigations. One such preparation was discovered after a study of a series of 6-Substituted decahydroisoquinoline-3-carboxylic acids [72].

Study of the biological activity showed that preparation (56) is an effective mixed antagonist of AMPA - 2-Amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionic acid and kainite receptors and at the same time has lower neurotoxicity than known antagonists of AMPA and NMDA (N-Methyl-D-aspartic acid) receptors. Another example of glutamate receptor agonists is the derivatives of isoxazoles (57).
The class of selective potentiatiors of metabotropic glutamate receptor includes compound (58).

Interestingly 5-Substituted tetrazoles are used in the synthesis of pharmaceutical preparations due to the creation of an NO synthase inhibitor.

The prodrug L-6-N-(1-Iminoethyl)lysyl-5-tetrazolylamide (59) was synthesized which is an effective inhibitor of NOS-2 synthase.

1.1.3.2 Synthesis of Tetrazoles

The 1,5-Disubstituted tetrazoles are used as isosteres of the cis-amide bond of peptides.

From the study of the amides and the corresponding tetrazoles, it was observed that the new tetrazole-containing compounds (60 and 61) can adopt almost the same steric conformations as the initial peptide. But tetrazoles are not widely
used in the synthesis of peptide preparation. Among the number of publications on
the use of 1,5-Disubstituted tetrazoles as isosteric replacements of the cis-amide
bond of peptides, it is worthy to note the synthesis of HIV-protease inhibitors.

The 1,5-Disubstituted tetrazole fragments are being studied quite actively
as the antiinflammatory agents. One such preparation is compound (62).

In addition to their antiinflammatory activity, the compounds also exhibit
weak antiulcer and analgesic activity. Another example of antiinflammatory agents
is the compounds of type (63). The principle of the action of such compounds is the
blocking of the receptors chemokines (chemotactic cytokines), which are the main
mediators of inflammatory processes in the human organism.

Habich [73] has reported the synthesis of derivatives of 3’-(5-Amino-
1,2,3,4-tetrazol-4-yl)-3’-deoxythymidines (64), which exhibited activity against the
human immuno deficiency virus.

The best known example of drugs containing a 1-Substituted
5-thiotetrazole fragment is the β-Lactam antibiotics of the cephalosporin class.
Cephalosporin and its analogs are substances related to penicillin in structure and active principle.

A typical example of antibiotics of the 1-Oxadethiacephalosporin class containing a 5-Thiotetrazole fragment is compound (65) (Latamoxef).

Antibiotics of the cephalosporin class are usually divided into generations according to their antimicrobial characteristics. Derivatives of 1-Substituted 5-thiotetrazoles are widely represented in each of the three presently existing generations of such antibiotics. Work is currently being carried out on a fourth generation of the antibiotics.

The antiulcer activity of derivatives of Tetrazole-5-thiols has been studied thoroughly over the last few years. Data have been published on a large number of compounds with the general structural formula (66), which are effective against ulcers caused by acetic acid derivatives e.g., indomethacin.
Tetrazole compounds were also studied for antiulcer activity. 1-Substituted 5-thiotetrazoles (67) were reported as the most effective compounds.

The antitubercular activity of 5-Thiotetrazoles was studied. The result of the investigations showed that the disulfide (68) fragment between the two electron deficient carbon atoms was necessary for the appearance of antitubercular activity.

![Image of disulfide structure](image)

The investigations opened up the way to the search for new preparations. At present antitubercular preparations based on similar structures are being developed.

Compounds (69) with various substituents at position 1 of the tetrazole ring were also studied. These products can be used as antihypertensive agents, the mechanism of action is based on the inhibition of rennin.

![Image of 2,5-disubstituted tetrazole](image)

Only very little information was available on the use of 2,5-Disubstituted tetrazoles in the synthesis of biologically active preparations. The practical uses for such compounds have not yet been found.
The compound (70) is known as glutamate receptor modulators.

A series of compounds of the type (71) was studied which exhibited antiviral activity.

1.1.3.3 Non-biological Applications of Tetrazoles

In addition to the biological activities of these classes of compounds, various tetrazole based complexes have also shown good coordination properties and are able to form stable complexes with several metal ions. Furthermore, the tetrazole ring has strong electron withdrawing property and tetrazole halides have been successfully used in organic synthesis as derivatising agents for the chemical modification of alcohols.

Some tetrazole derivatives are used for the selective recovering of palladium from the industrial wastes containing simultaneously Pd$^{2+}$, Cu$^{2+}$, Ni$^{2+}$, Fe$^{2+}$, Fe$^{3+}$, Cd$^{2+}$, Sn$^{2+}$ and Al$^{3+}$. Among the tetrazole, 1,2-Bis(1-tetrazolyl)ethane provides an effective quantitative selective precipitation of palladium from solutions.
The features of thermal decomposition and combustion of tetrazole derivatives, their capacity to self-propagate at high-temperatures decomposition and liquid phase combustion has kindled the researchers interest to choose tetrazoles for the use as the efficient components of different energetic composition. For example, Poly-1-vinyltetrazole and sodium salt of Poly-5-vinyltetrazole which have their decomposition temperature at 155 °C and 355 °C, are used as high efficient binders in the formulations for the low and high temperature generation of pure nitrogen.

Tetrazoles are also used as the components of filtering materials of the new generation for the purification of biological fluids (blood, lymph) from heavy mutations.

Since tetrazoles have the capability to form stable complexes with non-ferrous metals, they are used as modern promising anticorrosion agents for the protection of metal surfaces from the atmospheric corrosion and various aggressive environments. It has been reported that the combined application of 5-Phenyl tetrazole and benzotriazole salts in water environment resulted in synergetic effect of steel protection from corrosion.

Various types of tetrazole metal derivatives are known, where the metal atom can be linked to the heterocyclic by covalent, ionic or co-ordination bond. Hence this heterocyclic can be included in the composition of the molecule in a neutral, anionic (or) cationic form. Till recently large amount of tetrazoles containing metal derivatives are reported.

1.2 PHARMACOLOGY

Biological screening of any synthesized compounds is an important manifold in drug design. The pharmacophore or lead moiety can be selected only after the determination of functional groups which are responsible for various biological activities.

The development of modern pharmacology as a science is fairly recent and this science took shape only after the invention of screening procedures. Till that
period, the treatment was only empirical and experience alone played a dominant role. A good knowledge of the mode of action of the drug is necessary for the rational treatment of diseases. Pharmacology, being considered a branch of biology, provides the required scientific data in both animals and humans. The science of pharmacology includes some allied fields too, such as pharmacognosy, pharmacy, pharmacodynamics, pharmacokinetics, therapeutics, toxicology and chemotherapy. Pharmacognosy is a science of identification of drugs, whereas pharmacy is considered a science of identification, selection, preservation, compounding and dispensing of drugs. The quantitative biological and therapeutic effects of drugs, and drug action-chemical structure relation are studied in pharmacodynamics. However, pharmacokinetics describes the absorption, distribution, metabolism and excretion of drugs. The word “Therapeutics” refers to “To nurse” and this branch of medicine deals with the cure of diseases. The science of toxicology deals with the detection and measurement of poisons. Chemotherapy is a science, concerned with the effect of drug on microorganisms and parasites.

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.

1.2.1 Microbial Agents

Microorganisms occur nearly everywhere in nature. They are carried by air currents from the earth surface to the upper atmosphere. Even those indigenous to the ocean may find many miles away on mountain tops. Microorganisms occur most abundantly where they find food, moisture and temperature suitable for their growth and multiplication. Since the conditions that favour their survival and growth of many organisms are those under which people normally live, it is inevitable that human beings live among a multitude of microbes. They are in the air we breathe and the food we eat.
They are on the surfaces of our body in our alimentary tracts, on our months, noses and other body orifices. Microorganisms affect the well being of the people in many ways. They cause many diseases in humans and animals.

**Staphylococcus aureus**

*Staphylococcus aureus* is the causative agent of many suppurative processes ranging from localized abscesses which can occur anywhere in the body. It is a spherical bacterium. It is facultatively anaerobic, gram positive coccus, which appears as grape-like clusters when viewed through a microscope. It has large, round, golden yellow colonies, often with hemolysis, when grown on blood agar plates. Infections occur when *staphylococci* enter the body through breaks, cuts and abrasions in the skin or mucous membrane.

Although *Staphylococcus aureus* does not readily spread through tissues, the bacteria can be sometimes carried by the blood or within neutrophiles to far remove from the original abscesses. Indeed, nearly an organ or tissue may serve as it for secondary infection leading to a severe complications such as, chronic osteomyelitis (bone infection), pneumonia, meningitis and many more.

**Pseudomonas aeruginosa**

Several species of *Pseudomonas* are pathogenic, but most are opportunistic pathogens. The most serious animal pathogens are *Pseudomonas mallei* and *Pseudomonas Pseudomallei*. *Pseudomonas mallei* is a true parasite occurring only in animal hosts. It is the causative agent of glanders, a disease to which nearly all warm blooded animals are susceptible. The species most frequently encountered in human is *Pseudomonas aeruginosa*. It is commonly found in soil and water, but can occur in many other places including hospital environment. It is the causative agent of many nosocomical infections (infections occurring in hospitals) including infections following surgery, burn infections and urinary tract infections.
Escherichia coli

*Escherichia coli* is an anaerobic gram negative bacteria. It causes acute gastroenteritis in new borns and in infants up to 2 years of age. Enteroinvasive strains invade the epithelial cells of the large intestine and cause diarrhea in children and adults. Enterotoxigenic strains produce one or both of two different toxins, a heat stable toxin (ST) and a heat label toxin (α T). Both toxins cause diarrhea in adults and infants. The other diseases caused by *Escherichia coli* are urinary tract infections, septic infections, bacterimia, pulmonary infections, abscesses, and skin and wound infections.

Aspergillus niger

*Aspergillus niger* or *A. niger* is a fungus and one of the most universal species of the genus *Aspergillus*. It causes an infection called black mold on certain fruits and vegetables, such as grapes, onions and peanuts, and is a widespread contaminant of food. It is everywhere in soil and is commonly reported from indoor environments, where its black colonies can be confused with those of *Stachybotrys* (species of which have also been called "black mould").

Candida albicans

*Candida albicans* is a diploid fungus that grows both as yeast and filamentous cells and a causal agent of opportunistic oral and genital infections in humans. Candidal onychomycosis is an infection of the nail plate by fungus caused by *Candida albicans*. RNA-binding protein Slr1 was recently discovered to play a role in instigating the hyphal formation and virulence in *Candida albicans*. Systemic fungal infections (fungemias) including those by *Candida albicans* have emerged as important causes of morbidity and mortality in immuno compromised patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation). *Candida albicans* biofilms may form on the surface of implantable medical devices. In addition, hospital-acquired infections by *Candida albicans* have become a cause of major health concerns.
**Bacillus subtilis**

*Bacillus subtilis*, known also as the hay bacillus or grass bacillus, is a Gram-positive, catalase-positive bacterium. A member of the genus *Bacillus*, *Bacillus subtilis* is rod-shaped, and has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions. It is commonly found in soil, more evidence suggests that *Bacillus subtilis* is a normal gut commensal in humans. A 2009 study compared the density of spores found in soil (~10^6 spores per gram) to that found in human feces (~10^4 spores per gram). The number of spores found in the human gut is too high to be attributed solely to consumption through food contamination. Soil simply serves as a reservoir, suggesting that *Bacillus subtilis* inhabits the gut and should be considered as a normal gut commensal.

### 1.2.2 Antimicrobial Agent

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.

### 1.2.3 Antimicrobial Action through Inhibition of Cell Metabolism

Para-amino benzoic acid (PABA) is an essential metabolite for a number of microorganisms [74]. 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.
1.2.4 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.

1.2.5 Antifungal Agents

Fungi cause a range of illnesses (mycoses) ranging from acute to chronic. 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. The fungal infection causes number of diseases like athelets foot, candidiasis, mycosis, tinea, white nose syndrome, zeaspora etc. Primary and opportunistic fungal infections continue to increase rapidly because of the increased number of immune compromised patients.

As known, not only biochemical similarity of the human cell and fungi forms a handicap for selective activity. But also the easily gained resistance the main problem encountered in developing safe and efficient antifungals.

The ideal antifungal agents should be fungicidal with broad spectrum of activity and also be suitable for oral or intravenous administration and possess good pharmacodynamic properties without development of resistance during therapy.

At present none of the clinically used drugs satisfies all these criteria. So there is a need to develop antifungal drugs.
1.2.6 Drug Administration

A drug can be applied locally or it may be administered either orally or by injection. The term application refers to the application of a material, such as a dusting powder paste, ointment, lotion or plaster on the surface of the body. Oral or enteral route is normally the most commonly followed route for drug administration, because it is safe, convenient and economical. However, this method is not suitable for administering irritant and unpalatable drugs.

1.2.7 Toxicity Studies in Animals

Toxicity studies are normally conducted in animals, including mice, rats, guinea pigs, dogs and monkeys, with a view to assess the safety of a drug. The tests, normally carried out, are classified into three types, viz., acute toxicity tests, chronic toxicity tests and special tests for teratogenicity, mutagenicity and carcinogenicity.

1.2.8 Acute Toxicity Tests

In acute toxicity tests, the chosen drug is tested by injecting graded doses into different groups of animals. The primary aim of this test is to determine the precise value of LD$\text{}_{50}$. Experiments are usually conducted with a view to studying the influence of the drug on physiological functions, including locomotion behavior and respiration. This is also supplemented by autopsy and histological examination.

The experimental animals used for this study are usually kept observed for at least fifteen days after dosing. If a positive sign persists, the experiment may be extended. Experiments are normally conducted on several species of animals. Animals of both sexes are usually employed. These studies determine the values of LD$\text{}_{50}$, ED$\text{}_{50}$ and the therapeutic index of a drug. When a combination of two or more drugs is used, additional acute toxicity tests are normally carried out.
1.2.9 **Lethal Dose, LD\(_{50}\)**

The term LD\(_{50}\) refers to a dose which is effective in producing a certain expected response in 50% of the animal group. There is another term, known as ED\(_{50}\) which is useful in understanding the potency of the drug with respect to a reference standard. The value of ED\(_{50}\) can be calculated by determining the graded response shown by the drug. However, when the response is quantal or all-or-none, ED\(_{50}\) is considered equal to LD\(_{50}\).

The safety of a drug can be understood based only on the values of both ED\(_{50}\) and LD\(_{50}\). The greater term therapeutic index refers to the ratio of LD\(_{50}\) to ED\(_{50}\). The greater the therapeutic index, the safer is the drug. It has been suggested that the therapeutic index of a drug, having a low margin of safety, has been found to be close to unity. It is of interest to determine a dose that is effective in most of the animals, namely, ED\(_{50}\) and least toxic to most of the animals of a group, viz., LD\(_{50}\).

1.2.10 **Analgesic Activity**

Pain is defined as a subjective, unpleasant, physical and psychological experience observed as a result of the stimulation of identifiable nerve fibres with defined pathways to the brain via the spinal cord. Pain often results from tissue damage that stimulates nociceptive receptors (nociceptive pain). But pain may also occur without nociception as a result of damage to neural structures (neuropathic pain or neuralgia). The former type of pain is often acute, self-limiting after healing and responds easily to analgesics, where as the latter is very difficult to treat.

The tissue injuries produce local prostaglandins which activate peripheral pain fibres. NSAIDs prevent the synthesis of these prostaglandins and so reduce the pain. They may act directly to prevent conduction of pain impulses in the central nervous system, and thereby relieve pains. The brain and spinal cord play a major role in central pain mechanisms. The dorsal horn of the spinal cord is endowed with several neurotransmitters and receptors including somatostatin, neuropeptide Y,
inhibitory amino acid, nitric oxide, endogenous opioids and the monoamines which are the major targets for pain and inflammation.

The tail immersion test is considered to be selective to examine compounds acting through opioid receptor. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while nonsteroidal antiinflammatory drugs inhibit only peripheral pain. On the other hand, acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from the tissue phospholipid. The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathways.

1.2.11 Antiinflammatory Activity

Inflammation is the first biological response of the immune system to infection or irritation. The word ‘inflammation’ comes from the Latin “inflammo”, meaning “I set alight, I ignite”. A variety of stimuli, such as physical damage, ultraviolet irradiation, microbial invasion, and immune reactions are responsible for inflammation. Inflammation is characterized by redness, heat, swelling and pain. When healing is complete, the inflammatory process usually subsides. However, inflammation is sometimes inappropriately triggered by an innocuous agent, 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, and anti-inflammatory or immunosuppressive drugs may be required to modulate the inflammatory process. Based on timing and pathological features, there are two major categories of inflammation, such as acute and chronic. Chronic inflammatory disease is characterized by persistent inflammation. On the other hand, acute inflammation occurs over seconds, minutes, hours and days.

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rheumatoid arthritis. In such cases, the defense reactions themselves may cause progressive tissue injury, and anti-inflammatory or immunosuppressive drugs may be required to modulate the inflammatory process.

Inflammation is triggered by the release of chemical mediators from injured tissues and migrating cell. The specific chemical mediators vary with the type of inflammatory process and include amines, such as histamine and 5-hydroxtryptamine; lipids, such as the prostaglandins; small peptides, such as bradykinin; and larger peptides, such as interleukin-1.

Inflammation is implicated in several chronic-degenerative diseases like cancer, diabetes and hypertension. These health problems cause high mortality and morbidity levels around the world. Other inflammation-related diseases like infections by bacteria, virus and protozoa or autoimmune diseases like arthritis or Alzheimer’s are relevant. The main treatments used to prevent or minimize the progression of inflammation include non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids.

Most of the antiinflammatory drugs now available are potential inhibitors of cyclooxygenase (COX) pathway of arachidonic acid metabolism which produces prostaglandins.

Prostaglandins are hyperalgesia, potent vasodilators and also contribute to erythema, edema and pain. Hence, for treating inflammatory diseases, analgesic and antiinflammatory agents are required. Nonsteroidal antiinflammatory drugs (NSAIDs) are the most clinically important medicine used for the treatment of inflammation-related diseases like arthritis, asthma, and cardiovascular disease. Nonsteroidal antiinflammatory drugs (NSAIDs) are among the most widely used medications due to their efficacy for a wide range of pain and inflammatory conditions. However, the long-term administration of NSAID may induce gastrointestinal ulcers, bleeding and renal disorders due to their nonselective inhibition of both constitutive (COX-1) and inducible (COX-2) isoforms of the cyclooxygenases enzymes.
Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1-2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages.

Indomethacin is a strong antiinflammatory analgesic which is mainly used in the treatment of inflammatory joint disease. A common side-effect of this drug is headache. Like many other antiinflammatory drugs, indomethacin can cause gastric irritation, ulceration and haemorrhage. Indomethacin can rarely cause agranulocytosis.

1.2.11.1 Nonsteroidal Antiinflammatory Drugs (NSAIDs)

The nonsteroidal anti-inflammatory drugs (NSAIDs) are a group of chemically dissimilar agents that have as their primary effect the inhibition of prostaglandin synthesis. These compounds nonselectively inhibit the two isoforms of the cyclooxygenase (COX-1 and COX-2) and thus prevent the metabolism of cellular arachidonic acid (AA) and the upregulation of prostaglandin formation, which otherwise lead to an increase of vascular permeability, edema, hyperalgesia, pyrexia and inflammation.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used:

1. To treat mild pain (*analgesic* action);
2. To treat rheumatoid arthritis (*antiinflammatory* action);
3. To treat pyrexia, i.e. the high temperature of fevers (*antipyretic* action);
4. To prevent myocardial infarctions (*antiplatelet* action).
1.2.12 Cytotoxicity Study

Cancer is the second leading cause of death after cardiovascular disease. Cancer is a multifactorial, multifaceted and multi mechanistic disease that requires a multidimensional treatment process for control and prevention. There are more than hundred varieties of cancer. A tumour is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of normal tissue and continues in the same manner after cessation of the stimuli which have initiated it.

In the past two decades, there have been a remarkable revolution in the field of tumour chemotherapy. In recent years, a great progress has been made in the treatment of cancer and a substantial number of new anticancer small molecules have been synthesized. This was made possible due to the basic understanding of molecular and cellular biology, understanding the mechanism of cellular division, tumour immunology, detailed information of fundamental factors involved in both viral and chemical carcinogenesis and the improved investigative techniques. Consequently, there is great unmet medical need for new anticancer small molecule therapeutics.

Breast cancer is the most commonly occurring cancer in women, comprising almost one third of all malignancies in females. It is second to lung cancer in causing mortality. The lifetime risk of a woman developing invasive breast cancer is 12.6% i.e. 2 out of 8 normal females will develop cancer at some point in her life. The death rate for breast cancer has been slowly declining over the past decade [75].

Colorectal cancer ranked 3rd among the most incident forms of nonskin cancer. Colorectal cancer is also one of the leading causes of cancer deaths. There are well established screening techniques that are available to reduce the number of deaths caused by colorectal cancer each year. But these procedures are not utilized properly by the population at risk. Additionally, those at risk for colorectal cancer may not have access to this type of preventative care.
Cytotoxic drugs remain the mainstay of cancer chemotherapy. Anticancer drugs have been developed from a variety of sources ranging from material products to synthetic drugs, but most of it suffers from the drawback of high toxicity. It is therefore important to discover novel cytotoxic agents with wide spectrum of activity that differ from current agents.

1.2.13 Introduction to Molecular Docking

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 [76].