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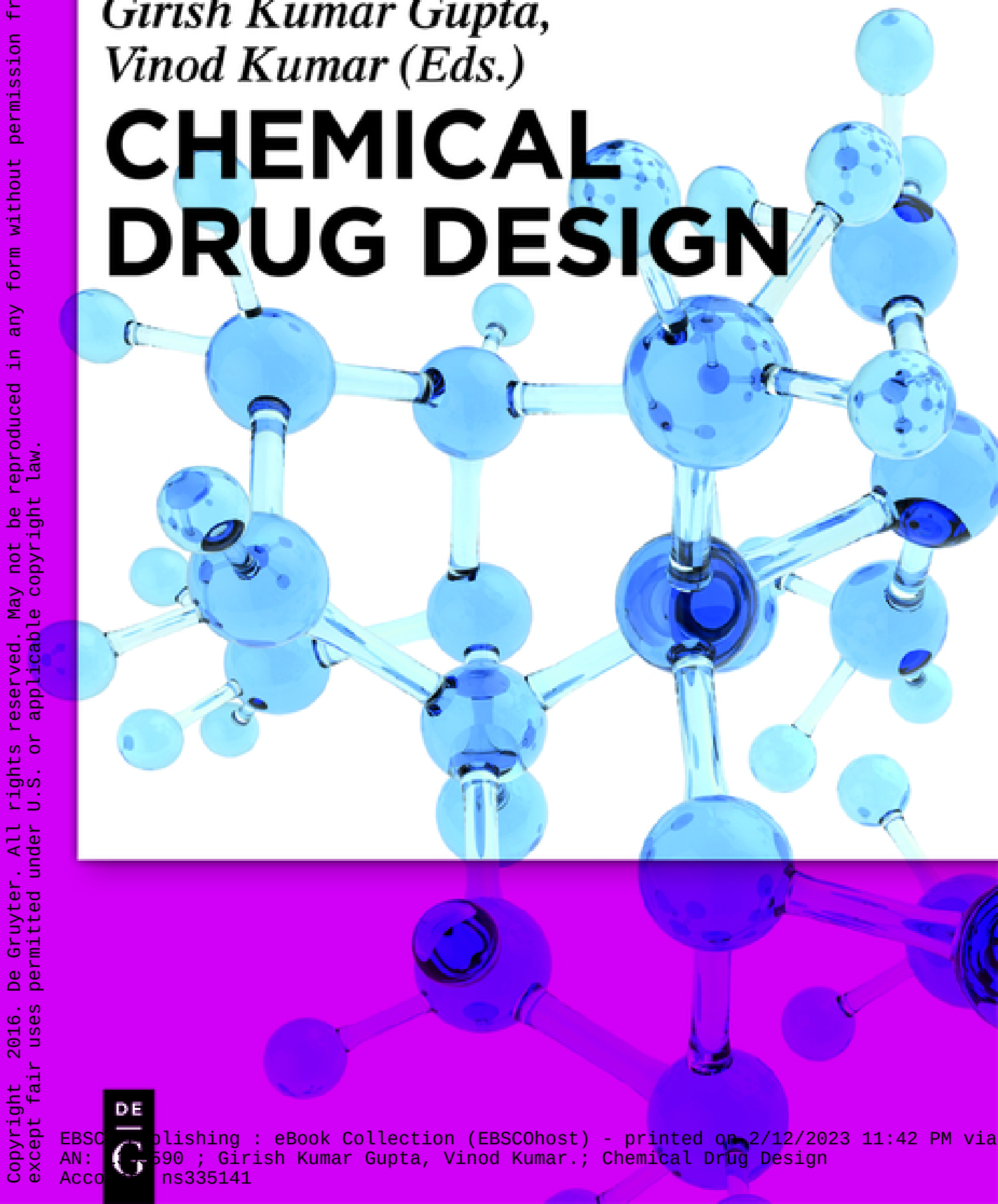

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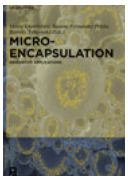

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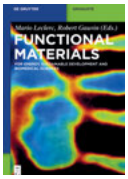

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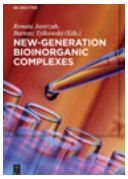

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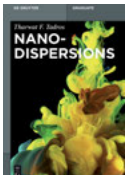

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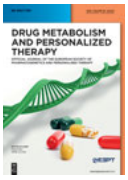

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Chemical Drug Design

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Preface

Today, drugs for practically every disease are available or in the process of developing to alleviate all diseases known to man and chemical drug design play an important role in finding new drugs or medicines based on the knowledge of a biological target. The drug molecules, generally organic substances, activate or deactivate the function of the biomolecules like proteins, lipids, carbohydrates, nucleic acids, etc. which in turn lead to the corresponding therapeutic benefits to the living being. Other classes of compounds including inorganic or organometallic compounds are also useful as drugs. Therefore, chemical drug design involves the design of small chemical compounds that can interact and bind efficiently to the biological target. In recent past, though an exhaustive study has been conducted in the field of drug design, there is still a great need of developing new drugs for future library. The present book is an attempt to present an invaluable and informative addition to field of drug design and discovery. The book contains eleven chapters contributed by international scientists and academicians focused on overview of basics in chemical drug design and the recent developments made in present scenario. The chapter 1 provides an overview of some basic techniques used in chemical drug design. The chapter 2 gives an update on recent developments in the field of drug designing paving the way for novel drug discovery and describes how knowledge of the state-of-art techniques has led researchers to find out the best method for the development of new drugs. Chapter 3 enlightens the new concept of structure and ligand based drug designing. It also covers in detail about the 2D QSAR and 3D QSAR approaches, descriptors as well as different parameters used in QSAR studies. The chapter 4 describes the drug design applied to natural products against neglected diseases. It introduces general concepts, and discusses the neglected diseases, reporting on some of the enzymatic targets studied when developing new drugs to treat these ills. The chapter 5 describes the developments made of newer hybrid drugs from natural products, especially for the treatment of infections and cancer. The advantage of this concept over a combinatorial chemistry approach is the high diversity and the inherent biological activity of the hybrids. The chapter 6 describes how does the drug metabolism play a major role in the drug design and discovery process and determine the fate of the prospective drugs. In chapter 7, the anticancer rationale of mistletoe lectins (the carbohydrate-binding proteins) is discussed. Its mechanism of discrimination between cell surface antigens of normal and cancer cells is also emphasized. The chapter 8 highlights the structural-activity relationship of different classes of typical and atypical antipsychotic drugs and provides some considerations concerning new treatment research. The chapter 9 describes the applications of high performance chromatographic techniques in the standardization of herbal drugs. The study focuses on the various chromatographic techniques, chemometric tools and interpretation of results by HCA & PCA in various guggulu samples. In chapter 10, the role of copper and its complexes with reference to toxicity level, sources, progress in the development of drugs and various important

discussions about the mode of action is discussed. Finally, chapter 11 highlights the various thiazole-containing drugs which are currently on the market or in clinical trials are discussed with a special note on their synthesis and mechanism of biological action.

The target audience of the book include students, researchers, chemists, pharmacists and professionals who are involved in different studies related to medicinal chemistry and drug design. We would like to acknowledge all the authors for their contribution and assembled the whole book. We hope that it will enhance the knowledge of scientists towards various approaches for drug design and would encourage them to dedicate their future research in understanding of relevant mechanisms and applications for the development of novel therapeutics. With this we signed off by reminding you to send feedbacks with your critiques and communiqués.

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1 Overview of chemical drug design

Abstract: Now a days identifying the novel medicines, drug design is the inventive process. Drug design that relies on the knowledge of the structure-based drug design is nothing but three-dimensional structure of the biomolecular target. The drug design approach has already proven as quite useful method in identification of many successful drugs. Computational approaches have become important tools to accelerate the development of epigenic inhibitors helping in the selection, design and lead identification of new compounds. The drug discovery research of the compound gives significant results if computational technology methods compliment in vitro experiments. These compounds may give more favorable ADME and toxicological profiles. This chapter provides an overview of some techniques used in chemical drug design to date.

1.1 Drug design

1.1.1 Introduction

Drug design, also called simply rational design or rational drug design, is the inventive process of identifying novel medicines [1]. Most commonly the drug is an organic small molecule that inhibits or activates the function of a biomolecule, which is nothing but a protein; these results are of therapeutic benefit to the patient. Drug design involves the design of molecules that are complementary in shape and charge to the biomolecular target with which they interact and therefore will bind to. Drug design frequently relies on computer modeling techniques which are often referred to as computer-aided drug design [2]. Drug design that relies on the knowledge of the three-dimensional structure of the biomolecular target is known as structure-based drug design [3].

Ligand design is nothing but design of a molecule that will bind tightly to its target [4]. The modeling techniques are successful in prediction of binding affinity of the compound. During clinical phases of drug development, there is more focus on the drug design process, especially on selection of candidate leads based on their predicted physicochemical properties and fewer complications of the drugs during development process so that it can be easily approved in the market as a drug [5]. The drug discovery research of the compound gives good results if computational technology methods compliment *in vitro* experiments. These compounds may give more favorable ADME and toxicological profiles [6].

1.1.2 Drug targets

Understanding the identity of drug targets that are encoded by the human genome is of great importance for the development of new pharmaceutical products and the allocation of resources within academic and industrial biomedical research. Many of the potential drug targets are not necessarily disease causing but must by definition be disease modifying [7]. In a specific disease modifying pathway, small molecules (receptor agonists, antagonists, inverse agonists, or modulators; enzyme activators or inhibitors; or ion channel openers or blockers) will be designed to inhibit or enhance the target function [8]. These small molecules will be designed so that they are complementary to the binding site of target [9]. The major important approach when considering the designing of small molecule is that they may not affect any other important "off-target" molecules. If the drug interacts with off-target molecules this may lead to undesirable side effects [10]. Protein networks or modules are increasingly being studied in the field of network biology using methods from graph theory, which is a growing field within computer science. Most common drugs are produced through chemical synthesis, but biopharmaceuticals (biopolymer-based drugs) produced through biological processes are becoming increasingly more common [11]. In addition, mRNA-based gene silencing technologies may have therapeutic applications [12].

1.1.3 Challenges of drug design

Any drug that is taken undergoes a number of chemical reactions in the liver as the body attempts to neutralize foreign substances. This set of reactions is well characterized, and a great deal of knowledge exists as to how drugs are modified as the body eliminates them. Scientists have worked for many years to abolish the limitations of screening by designing molecules to perform specific therapeutic tasks [13].

The general drug-target scheme suggests that three important basic tasks play a key role in structure-based rational drug design. First, the identification of an appropriate protein target for a given therapeutic need. Second, determination of the distinguishing structure of the target protein. Finally, designing a structure for a drug which interacts with the target protein. A number of technical difficulties have slowed down the work in the area of structure-based drug design [14].

1.2 Rational drug design

Rational drug design is also sometimes called drug design or rational design. The drug is most commonly an organic small molecule that activates or inhibits the function of a biomolecule such as a protein, which in turn results in a therapeutic benefit

to the patient. Biomolecules play an essential role in disease progression by either protein-nucleic acid interactions or protein-protein interactions, which lead to the alteration of metabolic processes [15–18].

Rational drug design can be broadly classified into two categories:

(a) Development of small molecules with desired properties for targets and biomolecules.
(b) Development of small molecules with predefined properties for targets, whose cellular functions and their structural information may be known or unknown. Steps related to these two approaches and evaluations of other properties in rational drug design are presented in the following figures (Figs. 1.1–1.3).

After identification of a target, then both approaches A and B for development of small molecules would require examination of several aspects (Fig. 1.3). Therefore, rational drug design would be an integral approach to drug development and drug discovery.

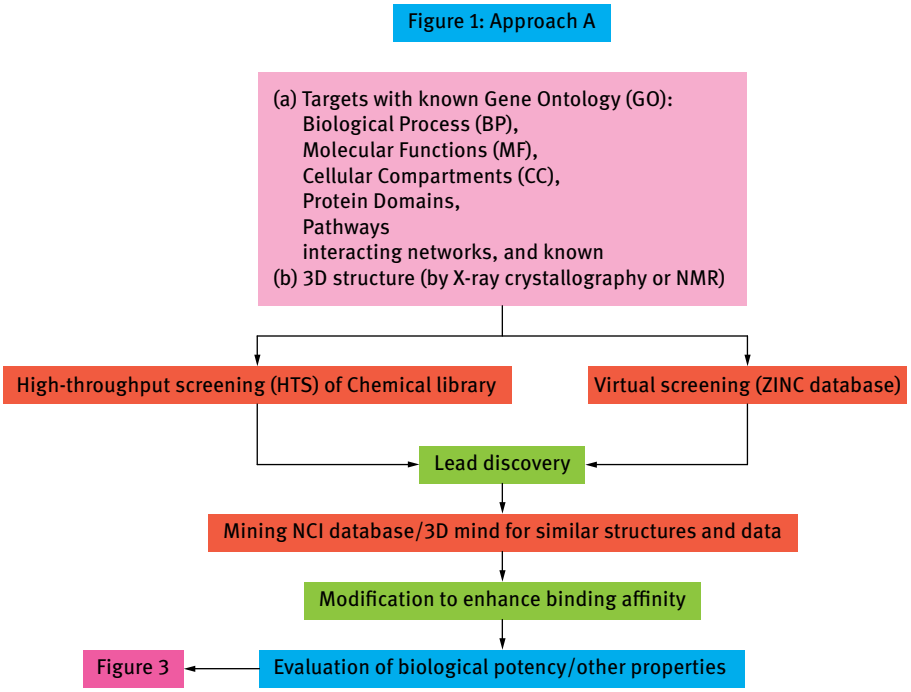

Fig. 1.1: Display of the number of possible approaches in drug design for known targets.

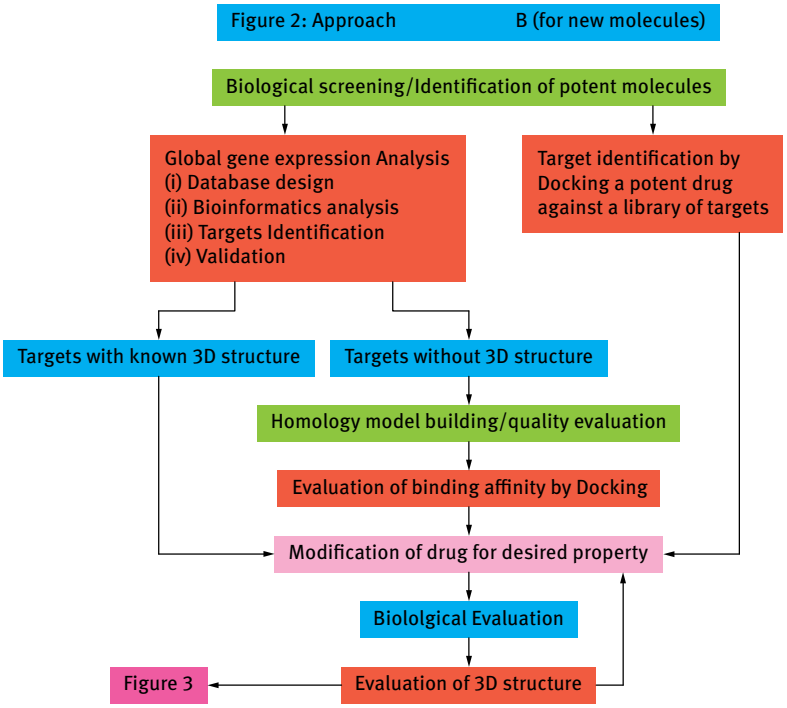

Fig. 1.2: Display of the number of possible approaches for unknown targets.

1.2.1 Structure-guided computer-aided drug design

Structure-guided methods are an integral part of drug development for known 3D structure of potential drug binding sites, which are the active sites. For a lead discovery, this is the starting point of structure-guided drug design for a known target. Once the ligand-bound 3D structure is known, a virtual screening of large collections of chemical compounds, such as ZINC [19], can be performed. Such a screening enables the identification of potential new drugs by performing docking experiments with this collection of molecules. To enhance binding and hence to improve binding affinity/specificity, a group of molecules with similar docking scores is generally used for potency determination; this is High-Throughput Screening (HTS) (Fig. 1.1).

Besides the evaluation of potency, binding specificity/affinity as well as other properties including drug-like properties (pharmacokinetics) such as log P, molar refractivity, hydrogen bond acceptor and number of hydrogen donors and molecular weight are also determined (Fig. 1.3). These parameters are important molecular properties as formulated by [20] and later developed by [21]. Toxicity predictions of the drug itself and its metabolic products can also be examined initially by computational methods; however, these properties should be verified by experimental methods.

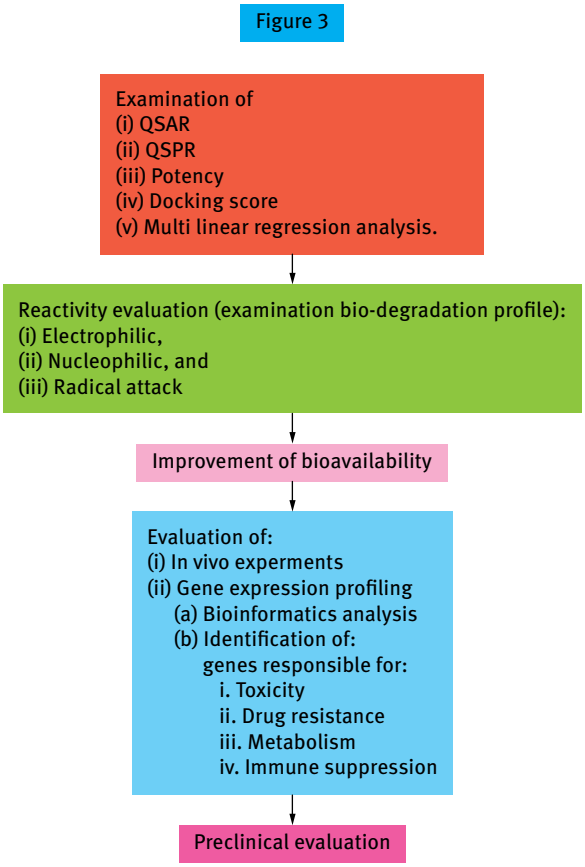

Fig. 1.3: Display of the essential properties for the improvement of drug-like properties.

The drug design approach has already been proven in that many successful drugs have been developed and some of them are already in use in the market. Among the best example we are discussing here the development of imatinib is worth mentioning, which is mainly used for the treatment of certain cancers including chronic myelogenous (or myeloid) leukemia (CML).

1.3 Ligand-based drug design

Ligand-based drug design is also called indirect drug design and deals with the information of diverse molecules that bind to the biological target of interest [24]. As an alternative, a quantitative structure-activity relationship (QSAR), which is a relationship between calculated properties of molecules and their biological activity determined through an experiment, could also be derived. These QSAR relationships

sequentially could also be used to predict the activity of latest analogs. In ligand-based drug design, you choose series of molecule that have revealed smart activity and run them in software like Sybyl 7.1, here you may get the groups such as hydrophobic, stearic and hydrophilic groups that are responsible for action [25].

1.4 Structure-based drug design

Structure-based drug design is also known as direct drug design which relies on knowledge of the three-dimensional structure of the biological target obtained through methods such as x-ray crystallography or NMR spectroscopy [26]. Using the structure of the biological target, candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics and the intuition of a medicinal chemist. Alternatively, various automated computational techniques may be used to suggest new drug candidates [27].

Structure-based drug design can be divided roughly into three main categories based on their current methods [28]. (i) The first method (virtual screening) is identification of new ligands for a given receptor. (ii) A second category is *de novo* design of new ligands [29–31]. (iii) A third method is the optimization of known ligands by evaluating proposed analogs within the binding cavity [28].

1.4.1 Finding leads

The first step in the structure-based design of new inhibitors is elucidating the three-dimensional structure of a target protein. The next step is to find a lead – the term for a compound that binds to the protein of interest; it often exhibits weak affinity or is too toxic, too unstable or has other shortcomings, yet it forms a starting point to develop molecules with improved pharmacological properties [32].

To begin to rival the complexity provided by nature, several groups have turned to screening techniques aimed at discovering tightly binding ligands from combinatorial libraries. For example, the phage display method is based on the display of a random sequence peptide on the surface of a phage. The phage library, typically including 106–108 different peptides, is mixed with the target protein, which is immobilized on the surface of a plate. Nonbinding phages are washed away while the bound ones can be used to decipher the sequence of the peptide bound to the target protein [33]. Alternatives are the affinity screening of synthetic peptide [34] and oligonucleotide [35] peptide-based libraries, chemical libraries [36, 37].

1.4.2 Optimizing leads

Screening procedures generally come up with leads that are far from perfect. These molecules then have to be optimized. At this point, the structure of the target protein in complex with the lead molecule can be extremely useful in suggesting ways to improve the affinity of the lead for the target [38].

1.4.3 Tools for structure-based drug design

Although quantitative *ab initio* prediction of binding constants remains a tremendous challenge [39, 40], a number of qualitative rules for the design of high affinity ligands can be deduced from the many crystal structures of protein-ligand complexes:

(i) Excellent steric and electronic complementarity to the target biomacromolecule is required.
(ii) A fair amount of hydrophobic surface should be buried in the complex for tight binding.
(iii) Chemical stability.
(iv) Sufficient conformational rigidity is essential to ensure that the loss of entropy upon ligand binding is acceptable.
(v) Sufficient solubility in water for inhibition tests and structural studies.
(vi) Ease of synthesis, including the avoidance of chiral centers and of 'dead-end leads'.

1.4.4 Docking algorithms

Many different strategies are currently in use for docking ligands on a target protein surface: The program GRID [41] is an example of the first strategy. Other design programs include AUTODOCK [42], LEGEND [43], and Group Build [44]. Closely related to GRID is MCSS, where thousands of copies of functional groups are simultaneously but independently positioned optimally on the protein surface by a molecular dynamics protocol [45]. A typical representative of the second strategy for docking is the program LUDI [46]. Other programs include CLIX [47], DOCK [48], GRID-like energy evaluation [49], traditional pharmacophore matching programs like ALADIN [50], FOUNDATION [51], MACCS-3D [52], ChemDBS-3D [53], CATALYST [54, 55] and BOXSEARCH [56].

1.4.5 Three-dimensional ligand databases

For many of the computer programs developed for lead discovery or inhibitor optimization, large collections of three-dimensional structures of low molecular weight compounds are required as essential input. The basic source for experimentally determined structures is the Cambridge Structural Database (CSD), containing more than 110,000 organic molecules [57, 58]). All of these contain models of the compounds obtained by structure-generation programs [59] that convert two-dimensional connection tables into three-dimensional structures. CONCORD [60] is the most popular of these programs, and has recently been used to convert 5,000,000 organic molecules of the Chemical Abstracts Service Registry file [61].

1.5 Pharmacophore-based approaches

Pharmacophore-based approaches describe the background and updated progress of pharmacophore-based drug design and provide the fundamental approach strategies on both structure-based and ligand-based pharmacophore approaches. Pharmacophore-based drug design processes include (i) pharmacophore modeling and validation; (ii) pharmacophore-based virtual screening, virtual hit profiling and lead identification [62].

1.6 Structure-based approaches

The other branch at the first decision point is used when the three-dimensional structure of the enzyme or complex is known. The process typically begins by generating a working computational model from crystallographic data, but methods to develop models of the binding site from active ligands are becoming more prevalent [63–66].

1.7 New lead generation

Generation of new lead compounds can be accomplished using *de novo* design methods to design new structures [67, 68] by searching databases [69–75] of known chemicals for particular structural features. *De novo* molecular design methods may design structures by sequentially adding or joining molecular fragments to a growing structure [76–78], by adding functionality to an appropriately sized molecular scaffold, or by evolving complete structures [79–81]. Some *de novo* design methods have concentrated on the design of diverse molecular scaffolds [82] or on the development of diverse substituents to place on a single scaffold. Methods of ligand evaluation include graphical visualization of the ligand in the binding site, substitution of

parameters from the new ligand into SAR models, and calculation of relative binding affinities [83].

1.8 Structure evaluation

Currently available evaluation methods can either provide qualitative rank ordering of a large number of molecules in a relatively short period of time [84] or generate quantitatively accurate predictions of relative binding affinities for structurally related molecules using substantial computing power [85, 86]. Methods of ligand evaluation include graphical visualization of the ligand in the binding site [87], substitution of parameters from the new ligand into SAR models, and calculation of relative binding affinities [88, 89].

1.9 Future directions

From the aforementioned introduction, it is easy to see that molecular simulation has a vital role in drug design and CADD, whether it is in protein modeling, in docking or in molecular dynamics. Rational drug design methods are continually improving, and a wider variety of drug targets are being approached by these methods. A wide variety of additional improvements can be anticipated in the future as well. Improved computer hardware will allow the use of more rigorous methods to be applied to large molecular systems. In the future, molecular simulation and computer-aided drug design can greatly influence the development of pharmaceutical industry and become a necessity before molecular experiments. In conclusion, chemical drug design is an exciting and constantly growing field of research. Its impact on quality of life and health ensure the vitality of the field.

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2 Drug designing in novel drug discovery: Trends, scope and relevance

Abstract: The severity of diseases gives rise to the need to develop new ideas for the discovery of drugs. Traditional drug development methods have been very costly and time consuming that is why computer-assisted methods have taken a center stage: they help in accelerating the whole process of drug development. Novel drugs are designed according to the specific protein target that plays an important role in a particular biological activity. Modern drug designing is based on receptor-ligand interactions according to which the drug binds inside the receptor's binding pocket and modulates its function. Drug development relies on two main approaches – structure-based and ligand-based. If the 3D structure of the drug target or receptor is available, then drug designing is done on the basis of the structural information and it is called structure-based drug designing. But if the 3D structure of the receptor is not available then drugs are developed with the help of 3D QSAR (quantitative structure-activity relationships) and pharmacophore methods, which is known as ligand-based drug designing. New drugs can also be synthesized inside the binding pockets of the receptor, called *de novo* drug design. The current chapter gives an update on recent developments in the field of drug designing paving the way for novel drug discoveries.

2.1 Introduction

Drugs have been saving man from life-threatening diseases since time immemorial. Before advancements in the medical field, doctors used to treat their patients with medicines extracted from plants. But unlike modern drugs, these traditional plant-derived extracts were not so efficient in healing wounds faster. So the need to explore new and novel drugs came to the fore. Some drugs like penicillin, warfarin etc. were discovered accidentally but saved the lives of many people. But during the 1900s the concept of drug development changed and medicines were produced to get the desired pharmaceutical effect by using multidisciplinary approaches. According to Emil Fisher's approach of lock and key, the substrate should exactly fit to the active site of the enzyme, which laid the foundation for drug-receptor interactions. The receptor will either start or block the function depending on the ligand that binds to the binding site. Furthermore, Koshland justified the fact by stating that both receptor and ligands undergo conformational changes to fit into each other. Before the genome sequencing projects, it was not so easy to work on the rational approach to drug development. However, there have been some successful projects which accomplished the

task of generating inhibitors for HIV proteinase, an important enzyme responsible for replication of the HIV virus, and this study helped in adding therapeutic value in anti-AIDS treatment [1–3].

Structure-based drug designing attained a crucial role in the development of new drugs after the completion of the human genome project and with further developments in information technology [4]. Structure determination techniques like X-ray crystallography and NMR were used to deduce the three-dimensional structure of macromolecules which formed the basis for structure-based drug designing [5]. Still, it is not very easy to design drugs for severe diseases like AIDS, cancer etc. as there is a large number of complex biomolecules whose structure determination is not so easy. The most important thing to consider while discovering drugs is that there should be no or few side effects of that drug. Bioinformatics as well as cheminformatics deal with the problems encountered in modern drug discovery approaches but they are somewhat complementary to each other. Cheminformatics tools help to look into the complex structures of small chemical lead compounds and on the other hand bioinformatics deals with the biological macromolecules [6]. Drug discovery is an interdisciplinary approach as shown in Fig. 2.1.

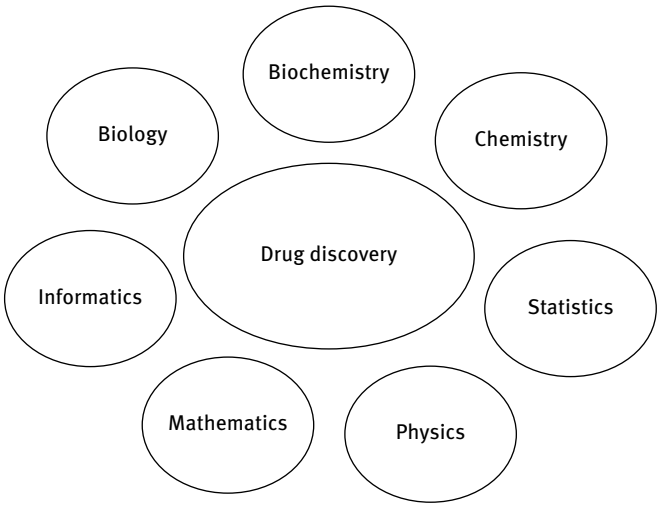

Fig. 2.1: Drug discovery: A multidisciplinary approach.

2.2 Drug designing

Traditional drug designing was quite laborious, time-consuming and less cost effective as most of the work used to be performed in wet labs. Computational tools are known to play an important role in modern drug discovery approaches since the

majority of work is performed by using them. The whole process of discovering and developing a new drug takes 10–12 years. The steps involved in the whole drug discovery process are mentioned in Fig. 2.2. Drug designing is broadly divided into two categories. One is structure-based drug designing and the other is ligand-based drug designing. Structure-based drug designing depends on the structural information of the drug target and the ligand molecules that can be easily fitted to the active sites are selected. Ligand-based drug designing does not require the 3D structure of the receptor molecules. A wide range of ligand molecules are selected from the databases which could possibly fit in the pockets of the receptor.

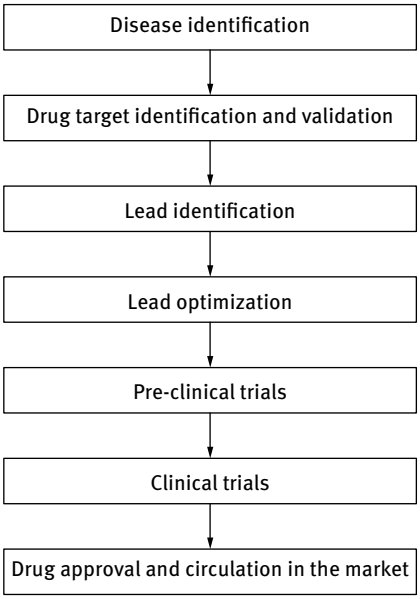

Fig. 2.2: Modern drug development process.

2.2.1 Structure-based drug designing (SBDD) or receptor-based drug designing (RBDD)

As mentioned earlier, receptor-based drug designing depends on the three-dimensional structures of the protein targets as well as the ligands. Hence structural biology plays an important part in the development of a drug. In the following the major steps in structure-based drug designing are described.

2.2.1.1 Drug target identification and validation

Drug target identification is the first step in the drug discovery process. Drug targets are those where disease-causing or virulent genes attack. These targets modulate the function of the protein depending on the molecule that binds. Some drugs act on single and specific targets, but others modulate multiple targets. The goal of these targets is to regulate the function of the protein, since for pathogenic diseases a drug binding to a target will inhibit the binding of the pathogen [4]. It should be an essential part of the cell cycle and no other pathway can inhibit its function and can easily bind to small molecules. Enzymes are the best targets, as small molecules can be easily fitted in their pocket [7].

Targets can be DNA, RNA, enzymes, GPCR's, membranes, ion channels etc. DNA is the receptor in cancer diseases so they are used in chemotherapies as drug targets. RNAs serve as the messenger between DNA and proteins so they are potential targets for drugs that bind directly to RNA or RNA-protein complexes. Enzymes such as proteases, kinases etc. are used as targets because they are involved in the catalysis of biochemical reactions. GPCR's are the signaling proteins responsible for various biological processes like cell proliferation, inflammation, neurotransmission etc. so it is obvious that they are important target proteins and 50 % of the drugs available on the market target them [8]. Membranes are used as drug targets because several antibiotics and toxins attack lipid bilayers. Sometimes species-specific genes can be used as targets because some genes are present in parasitic organisms but not in the genome of closely related free-living microorganisms so they can be the cause of pathogenicity.

Affinity chromatography is the most widely used method for target identification [9, 10]. Nonessential parts of modified small drug molecules are attached to affinity tags and protein extracts are allowed to incubate with drugs. After extensive washing, nonspecific proteins are removed and specific protein targets remain attached to the drug molecule. But this method requires extensive expertise and is time consuming. So a new method DARTS (Drug Affinity Responsive Target Stability) was proposed which does not require modification of drug molecule interfering with the drug's activity. This method relies on the fact that specific substrate bindings resist the proteases and stabilize the protein structure [11, 12]. Computational tools are now being used to distinguish targets faster. One of the important methods is reverse docking, which is the opposite to molecular docking. In reverse docking, a small molecule is allowed to bind at the predefined binding sites of the pool of targets. Li and co-workers used the computational tool "Target Fishing Dock" known as TarFisDock for this purpose [13]. Another important tool is pharmacophore modeling which saves a lot of computational time. It is a reverse screening method and measures the compatibility between the ligand molecule and the binding site structure of the target essential for the interactions. PharmMapper is a free web server which can be used for reverse screening [14]. UniDrug-Target is another computational tool used for the identification of drug targets of bacteria [15].

After the identification of the target, validation of its function becomes very important. Target validation can be done by genetic studies like gene knock-out (loss of function) and knock-in (gain of function) in animal models [16]. Other methods used for target validation are RNA interference, anti-sense RNA, antibody mediated inhibition experiments etc. Chemical validation can also be done in which various chemical compounds with well-understood functions are used. These chemical compounds show the phenotypic effect when interacted with the target and on the basis of this effect the target is validated. A benefit of using chemical validation is that druggability of the target can be analyzed [17]. It should be easy to perform experiments like recombinant expression, purification and automated assaying on a potential drug target [18].

2.2.1.2 Structure determination of drug targets

Accurate structural information about biomolecules is prerequisite for a structure-based approach. After the gene cloning of the protein target, the protein is expressed and purified. Then structure determination is done using one of the three important methods:- X-ray crystallography, NMR and homology modeling.

X-ray crystallography is the most widely used method of structure determination which provides absolute configuration of the molecules [19] and is useful for structures having weight up to 998 kDa. Crystal structures determined beyond 2.5 Å resolution are allowed in the drug discovery process [4]. Crystallographic structures can be useful in defining the complementary surfaces of leads and their targets [20]. Crystal structures of real drug targets like HIV proteinase, neuraminidase were used to synthesize drugs for AIDS and flu [21, 22]. NMR is another technique used for this purpose and useful for proteins having 50–120 kDa molecular weight. The NMR method is not as reliable as crystallography. 3D structures obtained after crystallography and NMR have been deposited in the PDB (Protein Data Bank). The three-dimensional structure of Human Serum Albumin is shown in Fig. 2.3. If 3D structures of proteins are not available in PDB, they can be modeled using modeling software. Homology modeling led to the modeling of an approximate three-dimensional structure of protein on the basis of available sequences with at least 30 % sequence identity. Swiss model is an online web modeling server [23–25]. MODELLER is a restrained-based modeling software which used to model the structure of proteins [26, 27]. Homology models are generated by using homologous structural regions of the sequences having a high degree of similarity so they could be used in drug designing [28]. But experimental methods are still more accurate and reliable than comparative modeling.

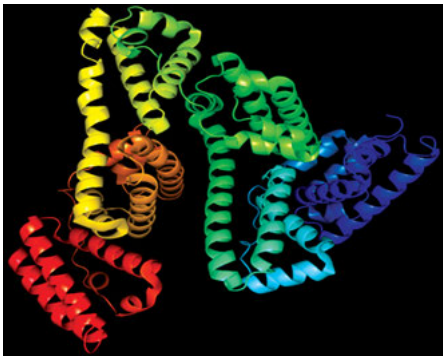

Fig. 2.3: 3D structure of human serum albumin (PDB ID – 1GNJ) [29].

2.2.1.3 Active site prediction and analysis

Identifying the binding site on a target is an important task in the structure-based drug designing procedure. The binding site can be the active site in the case of an enzyme, or an assembly site where one molecule interacts with another molecule, or a site essential for any mechanism performed by the molecule. Enzymes are the best drug targets as they have binding pockets where many ligands bind and these ligands are made to fit inside the enzyme's pocket. Ligand binding sites should have hydrogen bond donor and acceptor residues, hydrophobic characteristics and a variety of molecular surfaces. Residues involved in the interactions are conserved in nature so their substitution patterns are different from noninteracting sites.

There are different computational methods which help to predict binding sites based on different properties like volume, hydrogen bond, energy potential, hydrophobicity, residue propensity, solvent accessibility etc. [30]. The most commonly used methods are differentiated into energy-based and pure geometry-based approaches. Energy-based methods, like GRID [31], PocketFinder [32], Site Map [33] and Q-SiteFinder [34], compute the interaction energy between protein and ligand molecule. And geometry-based approaches such as POCKET [35], LIGSITE [36], Ligandfit [37] and fpocket [38] use geometric criteria to trace accessible and nonaccessible regions or energetically favorable or unfavorable regions and to identify binding sites. DoGSiteScorer is also a web server used to predict binding sites of a drug target on the basis of geometric and physicochemical properties and also estimates its druggability [39]. Par-3D is a web-based server used to predict active site residues [40]. GalaxySite is another online binding site prediction server which combines the approach of molecular docking along with the information of similar proteins with known structures [41].

2.2.1.4 Lead discovery

There are two ways to explore the lead molecule. One way is if the ligand is a known molecule, then the whole ligand molecule is allowed to bind inside the binding pockets of the target molecule by molecular docking. A three-dimensional representation of docked complex of angiotensin-converting enzyme and drug lisinopril is shown in Fig. 2.4. But if the ligand is not known, then new ligand molecules are designed according to the structure of the binding site of the target protein and this is known as *de novo* drug design.

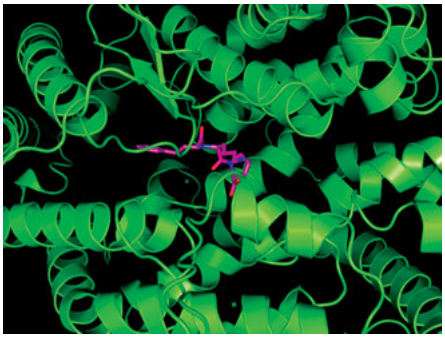

Fig. 2.4: Angiotensin-converting enzyme in complex with lisinopril (PDB ID – 1O86) [47].

High-Throughput Screening (HTS) is a testing method used to identify lead molecules and characterize their metabolic, toxicological and pharmacokinetic data. All the compounds in the library are screened against the drug target with the help of robots and detectors and a typical HTS method can test 10,000 compounds per day. It is a miniaturized cell-based assay to conduct the analysis of a large number of chemical compounds in a short time. Now a days an ultrahigh-throughput screening technique has been developed which can conduct up to 100,000 assays per day. If any compound gives a positive result in the primary screening then secondary screening is performed on it. Virtual Screening (VS) or *in silico* screening is a further computational method also used to discover novel lead molecules. Molecular docking is used to screen large compound collections in order to find leads for the targets in structure-based drug designing. Docking is a computational technique which predicts the 3D structure of the protein-ligand complex. In virtual screening, approximately 100,000 compounds can be screened per day on Linux clusters, which is equal to the speed of today's ultrahigh-throughput screening. Energy function and scoring function of ligand-protein complexes are calculated after the whole process. Energy function is the binding free energy of a ligand-receptor complex and is a heuristic approach. So scoring function is used which estimates the binding affinity of the complex [42, 43]. DOCK, FLOG, AutoDock, FlexX, ICM, LUDI, GREEN and GOLD are some of the molecu-

lar docking programs which predict binding mode of the ligand at the active site and measure its binding affinity [44]. Molecular dynamics simulation is performed after molecular docking to further improve the docking results [45].

De novo drug designing can also be used to design new ligands that are specific to the particular target. Structural features like active site composition and residues orientation in the site are completely analyzed to design a ligand molecule. Different methods are available for *de novo* drug designing like whole molecule methods, connection and site-point connection, fragment connection, sequential build-up, random connection and disconnection methods [46]. Different computational tools are present which apply different methods to analyze the active sites and construct new ligand molecules according to that.

2.2.1.5 Lead optimization

After lead identification, comes the turn of lead optimization in which the lead's favorable properties like potency and selectivity are maintained and deficiencies are removed. Lead optimization optimizes several features of the drug candidate simultaneously. The main aim of this step is to modify the lead structure to improve its absorption on the target's surface. It is not an everyday routine process and takes a lot of time to convert a lead into a drug which further undergoes preclinical trials and then clinical trials. But in case the lead candidates fail in preclinical or clinical characterization, more molecules should be in back-up to look up other molecules in series [48]. The desirable characteristics of a potential drug candidate are selectivity, potency, physicochemical properties like solubility, dissolution profile, permeability, polar surface area etc. with the drug target. The optimization process often leads to an increase in molecular weight, lipophilicity, number of rings and rotatable bonds [49]. Therefore, small and less lipophilic compounds should be chosen for optimization. Metabolic activity, ligand efficiency, off-target interactions are improved by adding functional groups which increase the molecular weight and lipophilicity. Optimization is done either by removing the parts of the drug one by one to see the importance of each part, or changing its polar functionalities like adding more hydrogen bond donors and acceptors around it. Sometimes focusing on one parameter can lead to disturbing other parameters so a single direction at a time will create problems. So multivariate optimization is done in which two or more parameters are optimized at the same time, which is more efficient. Another important thing to keep in mind is to keep an eye on the conserved part of the drug during the whole process of optimization. And these conserved parts should be replaced if they are large and lipophilic, otherwise they can create problems [50]. Out of hundreds of compounds only 10 % make the transit from lead to drug candidate as failure can occur at many steps. The preclinical stage is a cost-effective and high risk stage so transparency should be there at each step.

2.2.1.6 Preclinical trials

The preclinical phase is a very important phase in drug development as the fate of a drug is decided at this phase. Preclinical tests are performed on a drug to decide whether a drug candidate can be used to treat a particular disease without any side effects or toxicity. Toxicity, pharmacodynamic and pharmacokinetic tests are performed on rodent and mammalian models in laboratories. Toxicity profiles are performed to see the adverse effects of the drug and to set its starting doses during clinical testing. Pharmacodynamic tests tell about the relationship between effect and drug concentration. Pharmacokinetic tests are performed in the form of ADME (acronym for adsorption, distribution, metabolism and excretion) parameters. ADME deals with the determination of a drug's disposition inside the body. Pharmacodynamic studies are performed to see the effects of the drug on the body while pharmacokinetics specifies the body's action on the drug over a period of time [51]. Other key issues considered during preclinical trials are predictions about intestinal absorption, ability to cross blood-brain barrier and prediction of CYP-mediated metabolism. Different computational tools are also available which contain information about ADME/T associated properties like AdmetSAR [52], SOMEViz [53], RS-WebPredictor [54] etc. Mixing-tank models of the intestine are implemented in various commercially available programs like Intellipharm PK and Oraspotter used to predict intestinal absorption. An absorption and transit model [55] is also a basis for many computer programs such as GITA iDEA and GastroPlus [56].

2.2.1.7 Clinical trials

Clinical testing is a faster and safer way to find out whether a drug candidate is suitable for the treatment of diseases and this is performed on healthy persons as well as patients suffering from that particular disease. These trials can be done in many forms like screening trials, prevention trials, diagnostic trials, treatment trials etc. There are three main phases in clinical development, i.e., exploratory phase, confirmatory phase and life-cycle management phase, in which different types of studies are undertaken. In the first phase, the drug is tested on a small number of healthy individuals to find out about the drug's safety, safe dosage range and its side effects. In the second phase, the drug is tested on several hundred patients with the disease to check whether the drug performs its action or not. And in the third phase, patient numbers increase from hundreds to thousands and the aim is to determine its efficacy, monitor its side effects and compare it with other treatments for that disease.

2.2.1.8 Drug approval and its commercialization

After successful clinical trials, drug approval is granted by the authorities so that the drug can enter the market. In India, Central Drugs Standard Control Organization (CDSCO) and in the USA, Food and Drug Administration (FDA) allow the sale

and marketing of a new drug. These authorities primarily require companies to issue additional information about the drug regarding its safety and efficacy and then it provides the basic criteria of delivery mode of the drug and its further uses.

2.3 Applications of SBDD

The first two drugs against HIV protease, amprenavir and nelfinavir, were designed using the structure-based approach [57]. Zanamavir, tomudex and imitinab mesylate were also developed against neuraminidase, thymidylate synthase and Abl tyrosine kinase respectively [21, 58, 59]. Not only this, SBDD led to the development of specific micromolar inhibitors against HIV-1 RNA target TAR, the IL-2/IL-2Rαreceptor interaction, the VEGF/VEGF receptor and Bcl2 [4].

Structure-based drug designing led to the discovery of numerous parasitic drugs. A number of inhibitors of proteases act as drug targets in a number of diseases like malaria, leishmaniasis, schistosomiasis, African trypanosomiasis and *P. falciparum* infection [60–63] have been discovered. Another example of structure-based drug discovery is cysteine protease inhibitor or K11777 or K777 which binds to the parasite's cathepsin L-like cysteine protease, cruzainin Chagas disease [64, 65]. Moreover the development of new anti-parasitic drugs against parasitic worms also favored the success story. Amino-acetonitrile derivatives (AADS), a new class of anthelmintics proved their efficacy on a variety of nematodes and livestock helminthes [66, 67]. The 3D structure of human 5-LOX was generated on the basis of the crystal structure of rabbit 15-LOX and its binding modes were also studied by using competitive inhibitors [68].

The SBDD approach has proven to be a milestone in the fight against drug resistance and has led to the development of novel anti-cancer, anti-viral and anti-microbial therapies [69].

2.3.1 Ligand-based drug designing (LBDD)

When the 3D structure of drug receptor is not available, ligand-based drug designing helps in drug discovery and development. 3D QSAR (quantitative structure-activity relationships) and pharmacophore modeling are the most important tools used to design ligand molecules. QSAR is a computational method used to determine relationships between the structures of compounds and chemical or biological processes associated with them. The idea behind the QSAR method is that compounds having similar structure and properties produce similar activity [70]. And the pharmacophore approach is used to find common structural features of ligands.

In the 3D QSAR approach, molecular descriptors use the 3D features of the molecules to describe the QSAR model. Molecular descriptors are generated using knowl-

edge-based, quantum chemical and molecular mechanics tools. These molecular descriptors are used to develop mathematical models that describe the difference in biological activity of a molecule. A pharmacophore model is a 3D spatial arrangement of the features essential for biological activity of the compound and is generated from a group of ligands whose biological activity is known. Then the obtained 3D QSAR model is tested for stability and statistical tools are used for model development and its validation. General methodology to a build QSAR model is that first of all, ligands are identified whose biological activity is already experimentally measured. Then molecular descriptors associated with different structural features and physicochemical properties are identified and correlation between descriptors and their biological activity is established. And in the last step, statistical significance of the model's stability and its predictive power is tested. More than one output model is generated by this procedure and the best ones are selected on the basis of a scoring function and then validated. If the receptor-ligand binding complex is already understood then it can also be used to validate the model [70, 71]. Then databases are screened on the basis of the pharmacophore model for the ligand molecules. Automated methods are also available for pharmacophore and 3D QSAR studies and some of them are Catalyst, LigandScout, DISCO, GASP, PHASE, MOE and AutoGPA [72, 73].

2.4 Applications of LBDD

5-LOX (lipoxygenase) enzyme is essential in the biosynthesis of leukotrienes which are involved in various inflammatory diseases and allergic diseases. Therefore, 5-LOX is used as a drug target in the treatment of inflammatory disorders and asthma. And development of novel 5-LOX inhibitors relies on ligand-based approaches [72]. A 3D QSAR study was performed along with the CoMFA (Comparative Molecular Field Analysis) and CoMSIA (Comparative Molecular Similarity Index Analysis) approaches on 2-substituted 5-hydroxyindole-3-carboxylate derivatives to study the effect of steric, electrostatic and hydrophobic fields on the inhibitory activity of 5-LOX [74]. QSAR studies were also employed on 4-oxotiazolidines and 5-arylidine derivatives to determine the physicochemical properties essential for 5-LOX inhibition. They stated that addition of bulky groups decreases the binding affinity of 4-oxotiazolidines, presence of heteroatom and an increase in branching favors the inhibitory activity of 5-LOX [75].

Fjodorova and Novic studied the carcinogenicity of different classes of chemicals and the mechanism of their carcinogenic activity using SAR and QSAR approaches. Twelve molecular descriptors were examined that express structural and electronic features correlated with carcinogenic potency [76]. Pharmacophore and QSAR methods were used to construct the novel inhibitors for ATP synthase, a potential therapeutic target in breast cancer [71]. Novel partial agonists for peroxisome-proliferator-activated receptors (PPARs), important receptors acting as a therapeutic target in anti-diabetic and anti-metabolic syndromes, were identified using pharmacophore

modeling techniques [77, 78]. Pharmacophore model, Hypo1 was identified from the set of compounds to design new renin inhibitors for the treatment of hypertension [79].

2.5 Role of cheminformatics in drug discovery

Cheminformatics involve the use of computational tools and techniques in the process of drug discovery. Discovery of new chemical compounds using new techniques of combinatorial chemistry and high-throughput screening generate huge amounts of data. These huge amounts of data and information can only be handled by storing and retrieving them from databases [80]. And cheminformatics methods play a major role in solving many problems encountered in the whole drug development process. Many computer-based methods are used for solving problems like information storage and retrieval, chemical structure elucidation, predicting properties of lead compounds, analysis of high-throughput data, modeling of ADME-Tox properties etc. Cheminformatics tools used to draw and edit structures of chemical compounds are ISIS Draw, Chem Draw, CAS draw, ACD Chemsketch, Structure Checker, Marvin draw etc. Some cheminformatics databases are CAS, ISIS Base, ADE 3.0 and Beilstein Gmelin which store all the information regarding chemical compounds [81].

2.6 Conclusion

Structural biology, bioinformatics and cheminformatics together make the drug discovery process faster and more efficient for discovering new drugs against severe diseases. With the aid of structure determination methods, a large number of three-dimensional structures of proteins as well as ligands are available upon which structure-based drug designing fully depends. High throughput and virtual screening help in speeding up the whole process. Many life-saving drugs have been explored with the aid of structure-based approaches. Ligand-based drug development has also shown its importance in the discovery of novel inhibitors against a number of drug targets. Databases are also playing an important role as they are the rich sources of information about macromolecules and small molecules and this information can be retrieved at any time. Knowledge of the state-of-art techniques has led researchers to find out the best method for the development of new drugs. Also it is very important to know about the pitfalls of these methods so that the best approach can be selected and applied. As computer speed is increasing day by day, computer-aided drug designing becomes crucial in boosting the drug development process.

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3 Structure- and ligand-based approaches in drug designing

Abstract: This chapter enlightens the new concept of structure and ligand based drug designing. It is time saving technique and most reliable as in this method target is being modulated to obtain desired activity despite of hit and trial method which is tedious and less reliable. This chapter also comprises of different steps to be taken during the process of structure and ligand based drug designing. 2D QSAR and 3D QSAR are being discussed by including approaches, descriptors as well as different parameters used in QSAR studies.

3.1 Drug discovery

Modulation of biological targets is the most reliable rational drug design method used in drug discovery today. This method is more efficient and time saving in contrast to previously used hit-and-trial methods in which a chemical substance is first synthesized, then tested on cultured cells or animals and finally its therapeutic value is observed. Two essential points are considered for the selection of a biomolecule as a drug target: a) Evidence that the modulation of the target has therapeutic value. b) The target is druggable. Once identified the targets are than cloned and expressed to establish a screening assay [1, 2].

3.1.1 Drug discovery process and development

3.1.1.1 Target identification

The target for the drug to act upon are cellular or genetic chemicals present in the body associated with the disease. Targets have been identified and isolated by scientists. Compounds that have various interactions with drug targets are then identified [1–4].

3.1.1.2 Target validation/ prioritization

The targets used are compared with each other on the basis of their interaction for a disease as well as their efficiency in regulating biological and chemical compounds in the body that are most probably used in the development of new treatments, research analysis. Whether the drugs have the desired effect on the specific target is confirmed by several tests of the interactions of the drug with the drug target [1–4].

3.1.1.3 Lead identification

New compounds can be compared with known compounds on the basis of their resemblance to assess their potential for success. A lead compound is one that has the potential to treat disease. Lead compounds with properties needed in a drug are collected or are maintained in libraries [1–4].

3.1.1.4 Lead optimization

The properties of lead compounds are compared with the proposed compounds that have a greater potential to be developed as safe and effective medicines. During the development of compounds, basic studies are conducted in living organisms and in cells in the test tube and are compared for different properties such as metabolism and effect on the body [1–4].

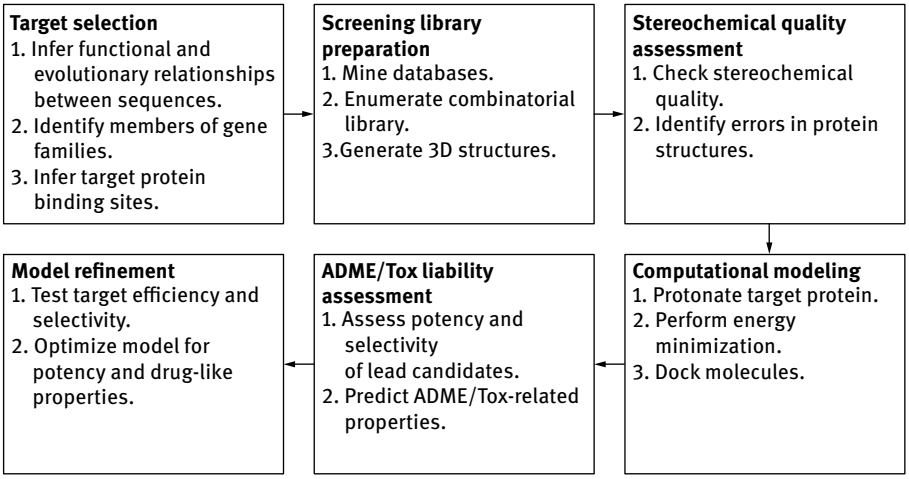

Fig. 3.1: Role of computational modeling techniques in drug design [6].

3.2 Drug design

Also termed **rational drug design**, this is an inventive process to develop a new medicine based on previous studies of the biological target [5]. Biomolecules are affected by the activating or inhibiting function of the drug. The biomolecular target to which the drug is designed to bind may be affected due to the shape and charge of the drug. Despite of drug designing it can also be known as ligand design and rely on computer modeling techniques [6]. Different roles related to these techniques are shown in Fig. 3.1

3.3 Drug designing techniques

3.3.1 Computer-Aided Drug Design (CADD)

Computational chemistry is used in CADD to enhance, discover or study drugs related to biologically active molecules. The basic goal of this method is to determine the interaction and affinity of interaction of the drug with the target. Molecular mechanics or molecular dynamics are used to predict conformational changes that occur when small molecules bind to the biological target as well as to predict the conformational change in the small molecule. Semi-empirical, ab initio quantum chemistry methods, or density functional theory are the basis for calculating optimized parameters for the molecular mechanics as well as estimating the electronic properties of the drug which influence binding affinity. Semi-quantitative prediction of the binding affinity is obtained by the molecular mechanics method. Binding affinity estimates may also be obtained by alternate knowledge. Linear regression, machine learning, neural nets or other statistical techniques are being used to derive the predictive binding affinity equations by placing the computationally derived interaction energy between small molecule and the target experimental affinities [7, 8]. Ideally, after using this technique only one compound having the highest affinity is required to be synthesized as predicted by computational methods. However, only qualitative accurate estimates of affinity are presented by the computational method. To discover an optimal molecule to be used in practice it takes several rounds of design, synthesis, and testing. The discovery of compounds has been accelerated by the use of novel small molecule structures obtained from computational methods.

Computers may be used at any of the stages of drug discovery to design a drug:

1. hit-to-lead optimization of selectivity and affinity
2. hit identification using virtual screening
3. by maintaining affinity, lead optimization of other pharmaceutical properties is achieved

3.3.2 Structure-Based Drug Design (SBDD)

Also termed **direct drug design**, this relies on the 3D structures obtained through NMR spectroscopy and X-ray crystallography of the biological target [9]. Using interactive graphics, selectivity of the drug toward the target can be designed as well as binding with high affinity to target can be predicted using the structure of biological target. New drugs can be suggested by alternatively using various automated computational procedures. Structure-based drug design methods are divided into two categories:

1. “finding” ligands for a given receptor, known as database searching and
2. “building” ligands, which is known as receptor-based drug design.

The major advantages of these methods are to predict a novel structure which is not present in any database (Fig. 3.2) [10, 11].

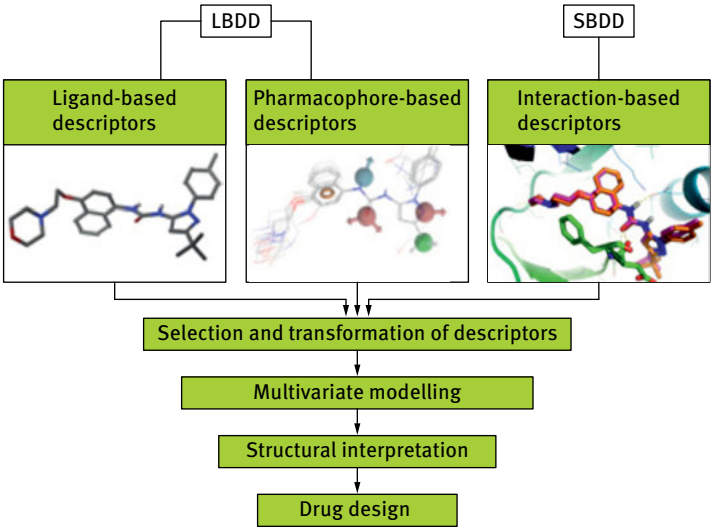

Fig. 3.2: Basic steps in drug design through SBDD and LBDD [12].

3.3.3 Ligand-based drug design

Also termed **indirect drug design** (Fig. 3.2), this depends on the study of other molecules binding to the biological target of interest. A pharmacophore model can be derived by the use of other molecules defining minimum requirements necessary for a compound to bind to the biological target [13]. Biological targets are designed according to the ligands binding to them and once it is established it will help to propose new molecular entities that can binds with the target. Quantitative structure-activity relationship (QSAR) helps to determine a correlation between the calculated properties of molecules and experimentally determined biological activities of the molecule, now this correlation is used to predict the activity of new analogs.

3.3.4 Molecular modeling

This is a method of presenting molecular structures numerically and proposing the behavior of these molecules with the equations of classical and quantum physics. It allows scientists to depict molecular data such as geometric, spectroscopic prop-

erties, energies, and bulk properties [14]. Molecular modeling refers to theoretical methods and computational techniques to mimic the behavior of the nucleus.

3.4 Quantitative Structure-Activity Relationship (QSAR)

QSAR contributes in designing and utilizing a consistent relationship between the biological activities of chemicals as well as their principle properties for a series of compounds.

A QSAR is generally represented by a linear equation

$$\text{Biological Activity} = \text{Const} + (C_1 \cdot P_1) + (C_2 \cdot P_2) + (C_3 \cdot P_3) + \ldots \quad (3.1)$$

In equation (3.1) parameters P_1 through P_n are computed for each molecule in the series and the coefficients C_1 through C_n are calculated by fitting variations in the parameters and the biological activity. Variations in the values of computed (or measured) properties to variations in biological activity for a series of molecules are represented with the help of linear models presented by the QSAR equation [15].

The common methods of displaying or presenting these data are a table in which compounds and molecular properties (or descriptors) are shown in the rows and columns respectively. QSAR general mathematical form is:

$$\text{Activity} = f(\text{physicochemical properties and/or structural properties}) \quad (3.2)$$

Different QSAR parameters, considered and studied during QSAR studies are given below:

3.5 Physicochemical parameters

(a) Electronic parameters
- ionization constant (Pka)
- sigma substituent constant (σ)
- spectroscopic chemical shift (Δ Fr)
- resonance effect (R)
- field effect (F)
- ionization potential (I)

(b) Hydrophobic parameters
- partition coefficient (log P)
- pi substituent coefficient (λ)
- Rm chromatographic factor (log Rm)
- solubility (δ)

- elution time in HPLC (log K1)
- parachor (P)

(c) Steric parameters
- Taft's steric substituent constant
- van der Waals radii
- inter atomic distances
- molar volume
- molar refractivity

3.5.1 Statistical parameters

The QSAR model is developed to predict the activity; a good correlation provides reliable prediction of biological activity. Regression analysis correlates independent X variables (e.g., physiochemical parameters, indicator variables) with the dependent Y variables (e.g., biological data) in the case of QSAR analysis. Regression analysis is determined with the help of following parameters:

- correlation coefficient (r)
- square of correlation coefficient (r^2)
- Fischer's Value (F-value)
- cross-validation
- t-test

3.5.2 History of QSAR

A century ago Crum-Brown and Fraser proposed that the physiological action of a substance can also depend on its chemical composition and constitution [16]. In 1893, Richet presented an inverse relationship between the cytotoxicities of a set of simple organic molecules and their corresponding water solubility [17]. In the 20th century, Meyer and Overton proposed that a group of compounds with their olive oil/water partition coefficients have narcotic (depressant) action [18, 19]. A thermodynamic generalization was introduced by Ferguson in 1939 which is comprised of the correlation between vehicle saturated with the drugs administered and their depressant actions [20]. The important role of ionized weak acids and bases in bacteriostatic activity was established by Albert, Bell and Roblin [21, 22]. For separating steric, polar, and resonance effects as well as the introduction of the first steric parameter, E_S was devised by Taft [23]. Hammett proposed and Taft contributed the mechanistic basis for the development of the QSAR paradigm. A partition coefficient is measured for a series using the octanol/water system, and thus leads to the formation of a new hydrophobic scale [23]. The parameter p, analogous to sigma, is defined as relative hydrophobicity of a substituent [24].

$$\Pi_x = \log P_x - \log P_H \tag{3.3}$$

P_x partition coefficients of a derivative and P_H represent the same for the parent molecule, respectively. The Hansch equation is a combination of hydrophobic constants with Hammett's electronic constants proposed by Fujita and Hansch and then extended to many forms [25].

$$\log(1/C) = a\sigma + b\pi + ck \tag{3.4}$$

Where σ is Hammett's electronic constant, π represents hydrophobicity.

Extended hydrophobicity leads to failure of linear equations and thus the Hansch parabolic equation was developed [26]:

$$\log(1/C) = a\log P - b(\log P)2 + c\sigma + k \tag{3.5}$$

Where log 1/C is biological activity, log P is hydrophobicity and σ is Hammett's electronic constant.

Other methods were also developed for structure-activity. The structure-activity study in a congeneric series using the Free–Wilson approach is described in equation (3.6) [27]:

$$BA = \sum_i a_i x_i + u \tag{3.6}$$

BA is the biological activity, a_i is the contribution of each structural feature, x_i denotes the presence $x_i = 1$ or absence $x_i = 0$ of a particular structural fragment, u is the average contribution of the parent molecule.

3.5.3 Objectives of QSAR [28]

1. **Diagnosis of mechanism:** Different parameters reveal information about the mechanism of action.
2. **Prediction of activity:** Prediction of activity by determining the relation between lipophilicity and biological activity.
3. **Optimization:** Clinical requirement helps in selecting the compound.
4. **Reduction and replacement of animals:** QSAR helps to reduce the use of animals. It is an alternative method to predict biological activity.

3.5.4 Steps Involved in a QSAR Study (Fig. 3.3) [29]

1. Selection of a series of biologically active analogues with their biological activity.
2. Calculation of various physicochemical parameters.
3. Determination of correlation matrix between various physicochemical parameters and biological activity.
4. Generation of QSAR equation.
5. Prediction of biological activity.

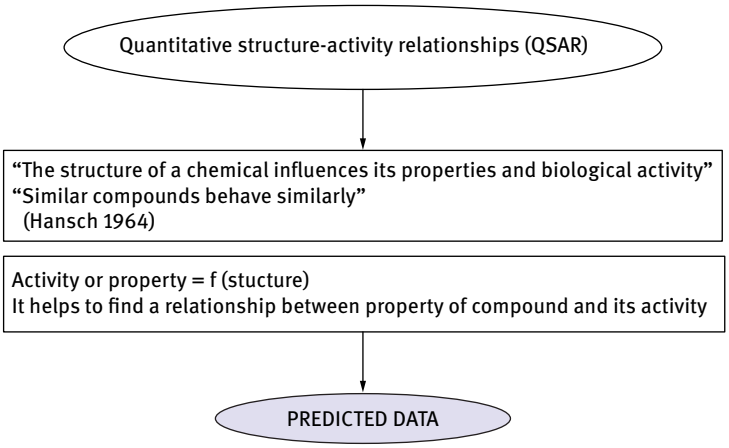

Fig. 3.3: Flow chart representing steps involved in QSAR studies [30].

3.5.5 2D QSAR

QSAR postulates requires three major factors to rationalize variations in a set of congeners which are responsible for producing a standard response [31, 32]: electronic, hydrophobic, and steric. Steric factors can be distinguished if these three factors are accounted for by the Hammett–Taft parameters and log P or π hydrophobic parameters. These could be parameterized by Taft's Es, molar refractivity or dimensional parameters such as STERIMOL. It is surprising that this simple set of principles helps a lot to explain every possible type of chemical and biological interaction. The achievement of using traditional QSAR is that it uses experimentally-based parameters, which helps in predicting the mechanistic basis of chemical and biological reactions for a series of compounds having the same or other systems [33–36]. In 2D QSAR there is no use of atomic coordinates in 3D-space as well, as the descriptors were derived from two-dimensional graph representation of a molecule. Good descriptors should characterize the molecular properties important for molecular interactions.

There are many types of software available for generating 2D-descriptors or for QSAR analysis e.g. Dragon, BuildQSAR, TSAR, Clog P, Molconn Z and MDS.

3.5.6 3D QSAR

3D QSAR requires 3D structures which are based on molecule superimposition or protein crystallography. It involves use of Lennard–Jones potential and is focused on that of the overall molecule than that of a single substituent. It helps to determine electrostatic fields and steric fields which are based on applied energy function [37]. 3D QSAR studies are performed commercially by many software programs such as MOE, GRID, COMFA, COMSIA, MOPAC etc.

3.5.7 General considerations for QSAR

1. All analogues of a series exert the same mechanism of action by acting on the same target.
2. Observed activities are of large range.
3. All analogues bind in a comparable manner.
4. Concentration in molar units (dose in g/kg is not suitable).
5. Activity data as a function of concentration (EC50, LD50).

3.5.8 Approaches to QSAR

3.5.8.1 Hansch analysis [38]

Corwin Hansch, known as the father of QSAR, contributed Hansch analysis which is the investigation of the quantitative relationship between the physicochemical substituent and biological activity of a series of compounds. Hansch analysis is used in QSAR for determination and upgradation of the pharmacological or toxicological effects. The main features of the Hansch group are two:

a) Hydrophobic substituent constant π was developed and have similarity with the Hammett equation:

$$\log P(\text{R-X}) = \log P(\text{R-H}) + \pi(\text{X}) \tag{3.7}$$

P(R-X) and P(R-H) are partition coefficients of R-X and R-H, with R-X indicating a structure derived from R-H by replacing H atom by substituent X; π (X) is the hydrophobic substituent constant, to be defined as the lipophilicity contribution of substituent X to lipophilicity when replacing H by X.

b) The multiparameter approach to QSAR:

$$\log(BA) = a \log P + b\sigma + cE_s + d \tag{3.8}$$

Equation (3.8) exemplifies a correlation with the three parameters log P, σ, Es. The general form of the Hansch equation is:

$$\log(1/C) = a\pi + b\pi + c\delta + dE_s + e \tag{3.9}$$

Where C = concentration of the drug required to analyses specific biological activity, π = hydrophobic constant that can be replaced or complemented with log P, δ = Hammett substituent constant and E_S = Taft's steric constant.

By fitting the experimental data into the regression equation the factors affecting biological activity can be identified.

3.5.8.2 Free–Wilson approach

The Free–Wilson approach depends upon the structure-activity method and is comprised of the various structural fragments which contribute to biological activity [27, 39, 40]. Equation (3.10) represents the Free–Wilson approach,

$$BA = \sum_j a_j X_j + \mu \tag{3.10}$$

The presence and absence of a particular structural feature is denoted by indicator variables. It is found that substituent effects are additive and constant toward the *de novo* approach. In equation (3.10) BA stands for biological activity; X_j stands for the j-th substituent, which carries the value of 1 if present and 0 if absent. The contribution of the j-th substituent to biological activity is represented by a_j. The summation of all activity contributions at each position must equal zero.

The Free–Wilson method has the following advantages:

- Such analysis can be conducted to obtain quantitative biological data.
- Physicochemical constants are not required.

It has the following limitations:

- Nonlinearity of the dependency of activity on substituent properties as well as the large number of molecules required that have different substituent combinations.
- Intramolecular interactions between the substituents are not handled very well.
- Extrapolation to substituents outside of those used in the study is not feasible.
- To explain a small number of compounds a very large count of variables is required, causing statistical faux pas.

This approach was improvised by Fujita and Ban in two ways [41]. The logarithm scale is used to express biological activity, so that it comes in a line with the extra thermodynamic approach, as seen in equation (3.11):

$$\log Xc = \sum_i a_i x_i + \mu \tag{3.11}$$

This allowed the other free energy related parameters to be compared with derived substituent constants.

3.5.8.3 Mixed approach

The Hansch analysis and Free–Wilson analysis have similarities and thus a further approach is based on the contribution of activities on their theoretical consistency and the numerical equivalencies. This is approach is termed the mixed approach and is represented by equation (3.12):

$$\log (1/C) = \sum_i a_i + \sum_j c_j \varphi_j + \text{const.} \tag{3.12}$$

Where, a_i = denotes the contribution for each i-th substituent, φ_j = any physicochemical property of a substituent X_j.

Molar refractivity plays a significant role as determinant of modulating ability. On the other hand molecular weight is a ubiquitous parameter in cross-resistance profiles in case of multidrug-resistance phenomena [42].

3.5.9 Descriptors used in QSAR

Descriptors of chemical structures help to characterize and quantify properties of the molecule or its fragments. There are two kinds of descriptors that explain the properties of a chemical:

1. Quantitative descriptors, which are based on molecular structures represented by constitutional formulas.
2. Structural descriptors, which represent the entire spectrum of the molecular or submolecular interaction leading to a particular property.

The following descriptors are described below:

3.5.9.1 Electronic descriptors [43]

They help to identify the ionization and dissociation of chemicals. The QSAR studies use only those parameters which describe the electron density distribution of molecules. The following electronic descriptors are described below:

Hammett substituent constant (σ). This is used in equations to calculate the electron-withdrawing/releasing potency of substituent on an aromatic ring. It reveals that chemical reactivity and biological activities are intimately related to their electronic attributes. Hammett employed a model reaction of the ionization of the substituted benzoic acids in water. On this basis s can be defined as the substituent constant. It represents a measure of electronic charge distribution in the benzene nucleus and is also a measure of the size of the electronic effect for a given substituent.

$$\sigma_X = \log K_x - \log K_H \tag{3.13}$$

or

$$\log (K_x/K_H) = -pK_x + pK_H \tag{3.14}$$

Thus the electron-donating substituents have negative values whereas electron-withdrawing substituents are characterized by positive values. Hammett also drew attention toward the fact that a plot of log K for ester hydrolysis versus log KA for benzoic acids for a series of molecules is linear, which suggests that substituents reflect a similar effect in dissimilar reactions. A correlation of this type suggests that proportional changes in the activation energy ΔG^{++} for such reactions are due to changes produced in structure. Hence, the universally known name for the Hammett equation is the linear free energy relationship (LFER).

Quantum chemical methods. These are used to obtain stereo-electronic descriptors such as ionization potential, polarizability, electron affinity, dipole moment, charge densities, highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO).

3.5.9.2 Steric descriptors [43]

Taft's steric substituent constant. This plays an important role in ligand-receptor interactions and transportation of it in cellular systems. Taft's E_S constant was the first steric parameter to be quantified and used in QSAR studies. It can be defined as:

$$E_s = \log (k_X/k_H) A \tag{3.15}$$

k_X represents the rates of acid hydrolysis of esters, XCH_2COOR, k_H represents the rates of acid hydrolysis of esters, CH_3COOR.

Polarizability descriptors (molar refractivity). These include a polarizability component which describes cohesion related to London dispersion forces:

$$MR = 4\pi Na/3 \tag{3.16}$$

where N is Avogadro's number and a is the polarizability of the molecule.

Equation (3.16) contains no information about shape. MR was further defined by the Lorentz–Lorenz equation:

$$MR = \frac{(n^2 - 1)!}{n^2 + 2} \cdot \frac{MW}{density} \tag{3.17}$$

MR is generally used in biological QSAR and scaled by 0.1. n represents the refractive index of the molecule.

Verloop steric parameter. Verloop's development of STERIMOL parameters occurred due to the failure of the MR descriptor; it defines the steric constraints of a given substituent along several fixed axes. Five parameters are important to define the shape: L, B1, B2, B3, and B4. L represents the length of a substituent along the bond axis between the parent molecule and the substituent; B1 to B4 stand for four different width parameters.

Verloop subsequently established three parameters for QSAR analysis: a slightly modified length L, a minimum width B1, and a maximum width B5 that is orthogonal to L [44].

3.5.9.3 Topological descriptor

Topological descriptors are 2D descriptors which reflect the connectivity between the different atoms in a molecule based on graph theory concepts [45]. The graph invariants are produced by theorems of graph theory, called topological descriptors. The topological description of a molecule provides information on the atom-atom connectivity in the molecule and encodes for the size, shape, branching, heteroatom and presence of multiple bonds [46]. These descriptors are basically used in QSPR and in QSAR studies. Descriptors help to differentiate the molecules mostly on the basis of their size, degree of branching, flexibility, and overall shape. Some examples of topological descriptors are:

Kappa indices. The kappa shape indices are derived from the atom and bonds. They attribute molecular shape, including cyclicity, spatial density, central branching and symmetry.

Wiener index (W). This is the sum of the chemical bonds in the molecule between all pairs of a heavy atom. This is equal to half the summation of D-matrix entries [45]:

$$W = 1/2 \sum_i \sum_j a_{ij}^D \tag{3.18}$$

Electrotopological states. Electrotopological states are another type of topological descriptor. This descriptor is a numerical value computed for each atom present in a molecule, it provides data related to the topological environments of the atom and also about the electronic interactions occurring due to all atoms in the molecule [47].

Molecular connectivity indices. The molecular graph is the basis of these molecular descriptors. It is calculated for a number of molecular subgraphs i.e., path-cluster, path, cluster and chain of different order [48].

3.5.9.4 Log P descriptor [43]

Partition coefficient (P) is used to measure the hydrophobicity of the solutes. Partition coefficients deal with neutral species, on the other hand distribution ratios incorporate concentrations of charged species as well. P can also be defined as the ratio of concentration of the solute in octanol to its concentration in water.

$$P = \frac{[\text{conc.}]\text{octanol}}{[\text{conc.}]\text{aqueous}} \tag{3.19}$$

Octanol was chosen as the solvent to calculate the partition coefficient as it mimic the biomembrane.

3.6 Biological parameters [43]

In QSAR analysis, to develop a meaningful model the biological data should be both accurate and precise. The accuracy of any QSAR model that is developed completely depends upon the accuracy of the data that are responsible for its development. Nonlinear characteristics of dose-response relationships cause percentage inhibition of growth at certain concentrations which are not appropriate biological endpoints. These endpoints are later converted into equieffective molar doses. Logarithmic scale is used to express biological data between log dose and response in the midregion of the log dose-response curve because of the linear relationship.

Types of biological data utilized in QSAR analysis

Source of activity	Biological parameter
Michaelis Menten constants	log 1/km
Inhibition constants	log 1/IC50

3.6.1 Statistical methods in QSAR

- **Linear regression**
- **Nonlinear regression**

3.6.1.1 Linear regression

3.6.1.1.1 Regression methods [49]

Regression analysis is an equation in which activity (or other properties) is related to descriptors. The main objective is: prediction and experimental design. A predictive model is more useful as it can be used for screening a large set of molecules or proposed molecules for a potent molecule. The applicability of a regression model will be enhanced if it also predicts previously unknown correlations between some of the properties and activities. There is no single method that has the perfect relation to predict the interpretability, and computational efficiency. Some examples are:

- Simple and multiple linear regressions are fast to interpret and very easy, but they are not applied when the number of molecules is low in comparison to the number of independent variables.
- Stepwise multiple linear regression and GFA work was done with any number of variables.
- Partial least squares creates only linear relationships, but they are capable of handling any number of independent variables.

3.6.1.1.2 Simple linear regression

For each independent variable a linear one-term equation is obtained. This helps to discover descriptors as well since it also ignores the interaction between multiple descriptors.

3.6.1.2 Multiple linear regression

Multiple regression presents a logical extension of two variable regressions in which instead of a single variable, two or more independent variables are used. A single multiple-term linear equation is produced.

3.6.1.3 Stepwise multiple linear regression

A multiple-term linear equation is produced, but not by using all independent variables. If the equation passes the test for significance then each variable is added to the equation and a new regression is obtained.

3.6.1.3.1 Principal components analysis (PCA)

The aim of PCA is to discover a reduced number of variables which help in explaining biological activity or chemical properties. Now this is attained by analysis of the correlation matrix of biological or chemical properties. It is one of the famous data-reduction techniques.

3.6.1.3.2 Principal components regression (PCR)

A principal components analysis transformation of the independent variables suggests a multiple-term linear equation. The components are selected so that the largest amount of variance of the independent variables can be obtained if some of the components are discarded. The first and second component is the direction of greatest variance that is orthogonal to all preceding independent variables. Other components are discarded sometimes so as to reduce the size of the model.

3.6.1.3.3 Partial least squares (PLS)

Fundamental relations between two matrices (X and Y), i.e., a latent approach to modeling the covariance structure, is analyzed with the help of PLS. PLS regression is used when observations have fewer variables than the matrix of predictors. PLS was first discovered by the Swedish statistician Herman Wold.

3.6.1.3.4 Nonlinear regression

A nonlinear regression prediction equation depends nonlinearly on one or more unknown parameters. It arises on the basis of the relationship between the response and the predictors. Nonlinear regression is calculated by neural networking.

3.6.1.3.5 Neural networking [50]

In neural networking regression analysis, a net is trained to predict dependent variables from a set of explanatory variables. An artificial neural network (ANN) consists of an input, hidden and output layers of neurons and nodes which are connected by bonds with each other in which each output layer node corresponds to a different dependent variable and every input layer node corresponds to a single independent variable.

3.6.1.3.6 Judging the quality of QSAR models

QSARs can be known as predictive models correlating biological activity of chemicals with descriptors derived from application of statistical tools. QSARs can be used in different disciplines and lead optimization. A good quality of biological data, the choice of descriptors and statistical methods help in producing a good quality QSAR

model. These QSAR modeling are capable of developing of robust models which are capable of making accurate and reliable predictions of biological activities of new compounds.

QSAR model validation can be done with the following strategies [51]:

1. cross-validation or internal validation
2. true external validation by applying the model on external data and
3. data randomization or Y-scrambling

The success of a QSAR model completely depends on the validation of the developed model as well as the accuracy of the large number of factors. Validation is the process used for a specific purpose by which the reliability and relevance of a QSAR model is established. Novel validation parameters for analyzing the quality of QSAR models have been developed.

3.6.1.4 Validation methods [49]

Once the regression equation is derived, its reliability and application are determined. Several procedures are available to analyze whether the size of the model is suitable for the quantity of data to be analyzed, and also to provide the efficiency of a model that can predict activity for new molecules.

1. **Internal validation** uses the dataset from which the model is derived. This method uses a reduced set of structural data to derive a new model. It helps to predict the activities of the molecules which were not included in the new model set. It is repeated again and again until all the compounds have been deleted and predicted. Cross-validation is less precise than external validation.
2. **Cross-validation [49].** Cross-validation evaluates the validity of a model on the basis of how well it predicts data. The analysis uses a "leave-one-out" scheme; each compound is left out of the model derivation and predicted in turn. In some cases more than one molecule is left out in a time. The regression is repeated many times in a cross-validation process to obtain subsets of the data.
3. **External validation** evaluates on the basis of equation generalization. The data is divided into two groups: the training set and the test set. The training set helps to derive a model, and this model is used in the prediction of the activities of the test set members.
4. **Randomization test.** An equation may have very poor predictive power even if a large number of observations and a small number of terms are available. This occurs if observations are not independent of each other. Randomization of the dependent variables is one way to test independence. A new regression can be performed by randomly reassigning the set of activity values to different molecules. This process is repeated again and again. If the random models activity prediction is comparable to the original equation, the set of observations obtained are not sufficient to assist the model.

3.7 Statistical terms in QSAR [15, 43]

Correlation coefficient (r). The correlation coefficient r helps to calculate the quality of the model. It may constitute the variance in the data. In experimental conditions due to the complexity of biological data, a correlation coefficient value above 0.90 is considered, whereas in an ideal situation the correlation coefficient is equal to or approaches 1. A value of r higher than 0.9 indicates the high statistical significance of the regression equation, while a lower value of r indicates that the substituent constant is not important enough to be considered.

Square of correlation coefficient (r^2). This is the measure of variance, mostly presented as percentage value e.g. r = 0.8 then r^2 = .8 or 80 %. The greater the value of r^2, the lower the variance which remains unaccounted for by the equation.

Standard deviation (s). The standard deviation(s) represents how far the activity values can be spread on average. This value indicates the quality of prediction by showing the amount of variability inherent in the data. The standard deviation is an absolute measure of the fitness of the quality. Ideally it should approach zero, but experimentally such a value is not attained. It should be small but the value cannot be lower than the standard deviation of the experimental data. The magnitude of s contributes to some of the experimental error in the data and it also contributes to imperfections in the biological model. A smaller number of variables and a larger data set usually lead to lower values of s.

F-statistic [49]. The F-statistic is used to compare two models differing by one or more variables, it is one of the variance-related parameters. That a more complex model is significantly better than a less complex one was established with the help of this statistic. The F-statistic is computed by equation (3.20):

$$F_{\nu_1,\nu_2,\alpha} = \frac{\sum(\hat{Y} - \bar{Y})^2/\nu_1}{\sum(Y_i - \bar{Y}_i)^2/\nu_2} \tag{3.20}$$

Where the summation in the numerator is the sum of the squares about regression with ν_1 degrees of freedom. The summation in the denominator is the residual sum of the squares with ν_2 degrees of freedom. α is the level of confidence, usually 0.05, that the result is significant.

3.7.1 Mean squares

This is obtained when the total sum of the squares is corrected for degrees of freedom. The significance of regression models is evaluated using this term. Its value is used to calculate the error of measurement in the X data.

3.7.2 Training set

This is a portion of a data set classified or used to fit a model for predicting the values that are known in the training set.

3.7.3 Test set

This is a set of data used to assess the utility of a predictive relationship and its strength. It is prepared from the whole data set to evaluate the training set.

3.7.4 Advantages of QSAR

1. Quantification of the effect of structure on activity.
2. Help to predict novel analogues for synthesis.
3. Interaction between functional groups present in a molecule having greatest activity with those of their target can be understood.
4. Prediction of biological activity.

3.7.5 Disadvantages of QSAR

1. False correlations may arise because of experimental error in biological data.
2. If the training data set is not large enough, the data collected may reflect the complete property space.
3. 3D structures of ligand binding to receptor may not be available.

3.7.6 Application of QSAR

3.7.6.1 Chemical

1. A QSAR application was used to predict boiling points [52]. In organic chemistry, a strong correlation between structure and properties may exist.
2. Hammett equation, Taft equation and pKa prediction methods were developed [53].

3.7.6.2 Biological

1. The biological activity of the molecules is measured in assays to suggest the level of inhibition or activation for a particular signal transduction or metabolic pathways.
2. QSAR has also been used in drug discovery to predict chemical structures having good inhibitory or agonistic effects on specific targets and low toxicity.
3. Partition coefficient log P is predicted to identify "druglikeness".
4. QSAR also helps in the study of interactions between the structural domains of proteins [53].

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Luciana Scotti*, Francisco J. B. Mendonça Júnior, Marcelo S. da Silva, Marcus T. Scotti

4 Drug design applied to natural products against neglected diseases

Abstract: Natural products can be defined as those compounds isolated from plants which provide a variety of lead structures used in the development of new drugs by the pharmaceutical industry. Interest in these substances has increased because of their beneficial effects on human health, such as antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor, antioxidant and antiparasitic activities. The Neglected Diseases are 17 diseases transmitted by virus, protozoa, helminthes, and bacteria, they are infections principally caused by tropical parasites, affecting mainly people who live in poor countries, and, having differing symptoms, may often lead to death. The available therapeutic drugs used to treat these diseases are either obsolete, toxic, or have questionable efficacy, possibly from encountering bacterial resistance. Discovery of new, safe, effective, and affordable active molecules is urgently needed. Natural organisms, especially plants, provide innumerable molecules with the potential to treat these diseases. This work introduces general concepts, and discusses the neglected diseases, reporting on some of the enzymatic targets studied when developing new drugs to treat these ills.

4.1 Natural products

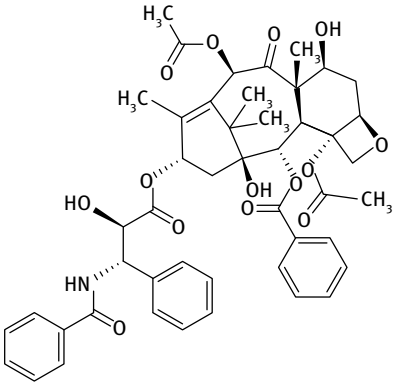
The known organic substances, in general, have all been produced by Nature, yet it is the kingdom Plantae which has contributed most significantly to the discovery of useful substances that treat human disease. The development of organic chemistry (mostly in the 19th century) occurred in parallel with the study of plants. The angiosperms are source plants of many natural products, having an undoubtedly unique development, as evidenced by the occurrence of their distinct and complex metabolites [1].

Natural products often result from an optimized evolutionary process in which chemicals have been under the selective forces of coevolution, organisms producing substances in the presence of their predators. These natural compounds have been utilized by humans since ancient times to treat and cure their diseases. Traditional Chinese medicine is the best example of natural products' efficiency, especially in the discovery of new active chemical entities. These compounds are present in fruits and vegetables and are important components of our daily diets. Of the existing plants in the world, most of which are unknown from a scientific point of view, only about 5 % of the approximately 250–500 thousand species have been biologically studied and

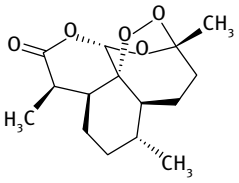
evaluated. Natural products are often phenolic compounds, flavonoids, alkaloids, or terpenes; secondary plant metabolites that may provide several benefits to our health. These benefits include cosmetic action, cardio-protective effects, anti-inflammatory activity, and usefulness in the treatment of cancer and the neglected diseases [2–4].

The use of natural products has been the single most successful strategy for discovering new medicines, and many medical breakthroughs are based on natural products. Half of the top 20 best-selling drugs are natural chemical compounds, and their total sales amount to US$ 16 billion yearly. These numbers suggest that natural chemicals may well be considered pre-optimized for bioactive potential, and therefore possess "drug-like properties" [5–7].

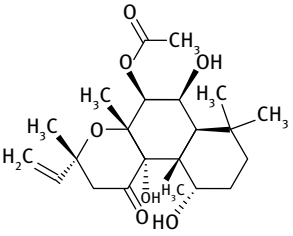
New molecules are continually being reported in the literature, many with relevant pharmacological activity, such as taxol, forskolin, and artemisinin, etc. (see the Fig. 4.1). It is important to remember that plants have contributed over the years to obtaining various widely used drugs, such as morphine, emetine, vincristine, colchicine, and rutin, etc. (Fig. 4.1). In the 1980s, consumers in the US paid more than 8 billion dollars for prescriptions with active natural products, and about 80 % of all people use natural compounds in the treatment of their diseases [8–11].



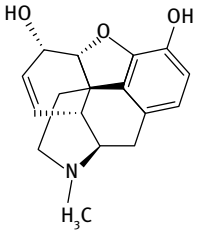
Taxol



Artemisinin



Forskolin

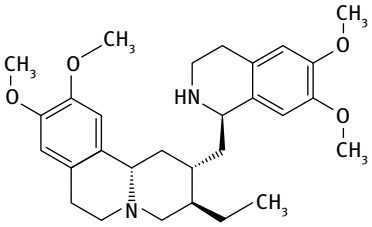


Morphine

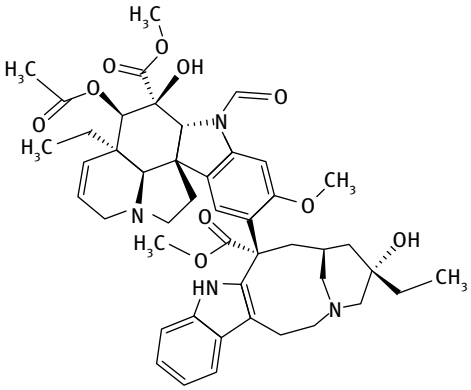
(a)

Fig. 4.1: Some natural products.

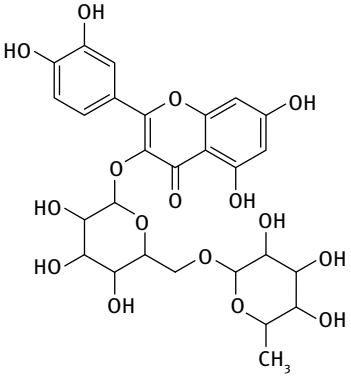
There are several therapeutically attractive metabolites from marine organisms that have proven to be effective modulators of biological targets such as phospholipases, adenosine receptors, and which are useful in tumor models. Examples of these candidates (or structural models) for drugs follow: manoalide (an irreversible inhibitor of phospholipase A_2) (Fig. 4.2), lufarolide (cytotoxic in human lymphoma cells), and Prostaglandin A_2 (Fig. 4.2) [11–15].



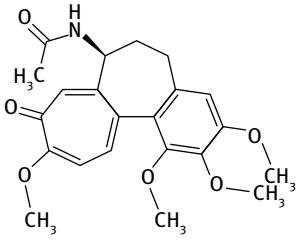
Emetine



Vincristine



Rutin



Colchicine

(b)

Fig. 4.1: (continued)

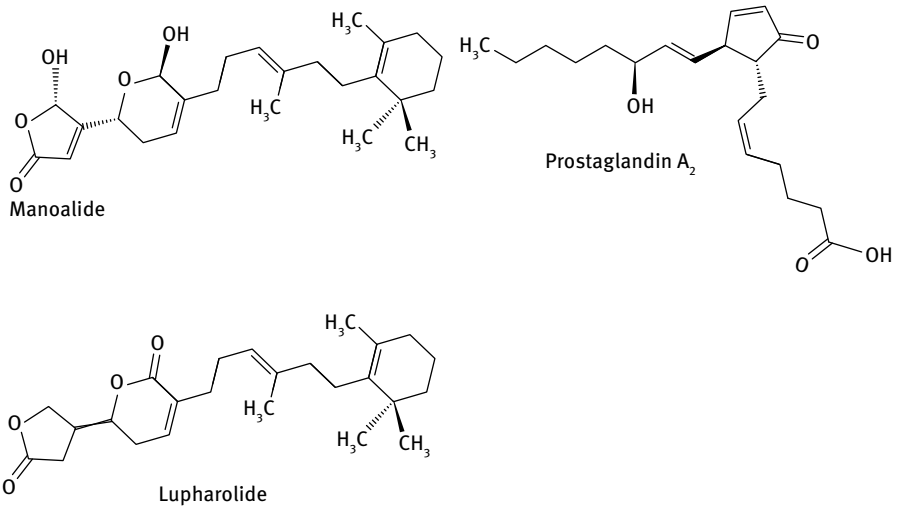


Fig. 4.2: Marine natural products.

New chemical bioactive entity (bioNCEs) studies done by industrial research laboratories have adopted techniques such as Combinatorial Chemistry to obtain more compounds. Through this technology, reactions are done in several steps, occurring in parallel or in mixtures, with few reagents. Products are reagent combinations and therefore large numbers of new compounds can be generated.

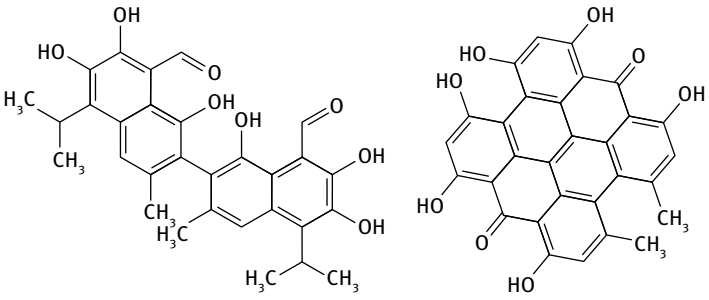
4.2 Medicinal chemistry

Medicinal chemistry, in a modern view, is dedicated to understanding the molecular mechanism, chemical relationships, and pharmacological activity involved in drug action through pharmacodynamic and pharmacokinetic factors. The introduction of new technologies has become a prime concept in medicinal chemistry, expanding its interdisciplinary character. Most drugs are small bioactive molecules that interact with specific macromolecules or receptors, resulting in their therapeutic effects. Modern computational methods can determine the different qualitative and quantitative contributions of the structural subunits of different drugs. Pharmacokinetic and toxicity factors of new drug candidates can be evaluated virtually using modern computational tools. The computer has become an inseparable ally, allowing computational studies that model the chemistry and molecular dynamics of medicinal chemistry [16–18].

Using several computational tools, researchers can create new virtual candidate ligands for receptor sites in three dimensions (3D). Pharmaceutical companies

report that spending on research and development in 2004 was about 33 billion US dollars, representing real growth year to year in investment, this does not correspond, however, to a proportional increase in discoveries of new active molecular candidates for innovative drugs in the market [19].

Natural products have had an important and decisive role in the development of modern medicinal chemistry and drug design. It has been observed (during the last 200 years) that the complexity, chemical diversity, and biological properties of natural products have all aided in the discovery of important new drugs [20]. In the last 30 years, the development of new bioassay techniques, biotech methods, bio-guided phytochemical studies, automated high-throughput screening, and high performance analytical methods have introduced both new concepts and possibilities for rational drug design and drug discovery. With the development of new spectroscopic techniques, organic chemists have been able to elucidate the complex molecular structures of natural constituents quickly. As examples: gossypol (Fig. 4.3) obtained from the cotton seed oil (*Gossypium sp*.) is widely used in China as a male contraceptive, and hypericin isolated from Saint John's wort (*Hypericum perforatum*) extract, is used as an antidepressant [21].



Gossypol Hypercin

Fig. 4.3: Gossypol and hypericin.

Another natural substance of oriental origin is artemisinin isolated from *Artemisia annua,* a plant known and used in Chinese medicine, and whose structural complexity has inspired new, useful drugs for the treatment of malaria. However, due its low solubility and inadequate pharmacokinetic properties for therapeutic use, various modified analogs have been synthesized, as shown in Fig. 4.4. SAR studies have shown that the endoperoxide function in the natural compound is the pharmacophoric subunit. Through semi-synthesis, the active compounds β-artemether, arte-ether, and sodium artesunate were obtained, and all without limitations in bioavailability (Fig. 4.4) [22].

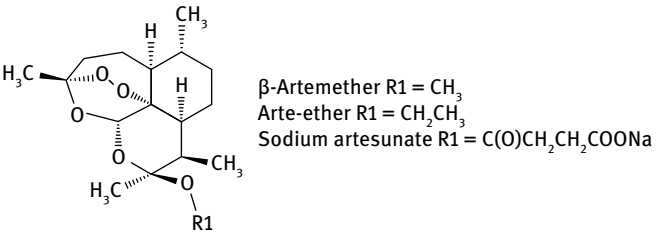


Fig. 4.4: Artemisinin derivatives.

In silico methods or CADD (Computer-aided drug design studies) are increasingly being used in both industry and in universities, they involve an understanding of the molecular interactions from both qualitative and quantitative points of view. These methods generate and manipulate three-dimensional (3D) molecular structures, calculate descriptors and the dependent molecular properties, model constructions, and employ other tools that encompass computational drug research. Analysis of the molecular structure of a given system allows relevant information to be extracted, and to predict the potential of bioactive compounds [23].

Theoretical studies using *in silico* methods have aided in the process of drug discovery. Technological advances in the areas of structural characterization, computational science, and molecular biology have contributed to faster planning of new feasible molecules [24]. Chemoinformatic studies exist which show that a large fraction of natural products are "drug-like" or at least, "lead-like" having structural and physicochemical properties that render them as potential drugs or leads. Some authors have even suggested a "natural product-likeness score" as a means to filter large chemical databases and find new entities suitable for activity testing. The use of natural products has been the subject of increasing interest in phytochemistry, biochemistry and other fields of research at the chemistry-biology-ecosystems interface.

4.3 Docking

Drug discovery is a lengthy and expensive process that can take up to 15 years, and cost upwards of billions of dollars. Most drug candidates fail in clinical trial. Of every 10,000 compounds tested, about one or two are marketed, the process wastes great resources and effort. Traditionally, new drugs have been discovered through studies of natural or synthetic compounds with biological activity. Today, theoretical methodologies have the advantages of both effectively reducing costs, and of speeding up drug discovery; which results in earlier drug marketing. As examples of this, we have: Captopril, from Bristol Myers-Squibb used for treatment of hypertension and heart failure; Dorzolamide, an anti-glaucoma agent developed by Merck; Saquinavir, which is a protease inhibitor used for HIV therapy and produced by Hoffmann-

La Roche; and Zanamivir, a neuraminidase inhibitor used for influenza treatment [25–27].

Computer-aided drug design (CADD) studies use several methodologies of computational chemistry to discover, enhance, and study drugs and their related biologically active molecules. Computational methods not only help in minimizing the number of drug candidates, but also reveal both their ADME profile and toxicological properties. Assuming (in principle) that the biological activity of a drug is generally related to its interaction with a protein or a nucleic acid, drugs are designed (*in silico*), based on their interaction (ligand with the macromolecule) observed in three dimensions, using molecular docking, in a structure-based drug design (SBDD) technique. This method is used to investigate and predict how the candidate drug (ligand) interacts at the molecular level, by binding to the target protein or nucleic acid (receptor), and to analyze the energies and interactions involved between them [28–30].

Docking involves molecular biology and computer-assisted drug design. The method's objective is to predict the predominant binding mode of a ligand to a protein, using complex ligand-protein docking of the known three-dimensional structures. Docking methods can effectively search the high-dimensional spaces which occur, and apply a scoring function that correctly ranks candidate dockings. Trough docking can be used to perform virtual screenings of large libraries of compounds, rank the results, and propose structural hypotheses on how the ligands might inhibit a target, which is invaluable when optimizing leads [31–35].

Technological advances, better performing algorithms, and increasing computing power, allow timeline molecular docking with thousands of ligands, this is of great importance in the pharmaceutical industry. In recent years, the growing number of publications based on molecular docking demonstrates its importance and effectiveness in drug discovery [36].

4.4 Neglected diseases

Just over 40 years ago in the early 1970s, the term "neglected diseases" was used for the first time on a Rockefeller Foundation program coordinated by Kenneth Warren, "The Great Neglected Diseases". In the original context, the term was used to refer to a set of diseases caused by various endemic infectious and parasitic agents (helminths, protozoa, bacteria, and viruses), in "under-developed" countries, and in low-income populations inhabiting the African, Asian, and American (South and Central America), continents. Neglected diseases do not arouse the interest of big pharmaceutical companies, treatment options are nonexistent, weak, or outdated, and do not attract research financing by governments. They are overlooked [37].

The concept itself has undergone minor modifications over the years, especially with regard to the exclusion of inappropriate or merely geographical characterizations, to some extent discriminatory, since they are a global range of diseases and

which need to be addressed within the sociopolitical and economic development of the affected countries' dimensions [38].

Currently, the World Health Organization (WHO) together with the Doctors Without Borders Organization defined the neglected diseases as: "... a set of diseases associated with poverty, poor living conditions and health inequities." Although they account for almost half the disease burden of developing countries; investments in R&D, especially by the private sector, are not traditionally prioritized for these diseases.

In order to demonstrate the degree of disinterest of the pharmaceutical industry regarding neglected diseases, Chirac and Torreele (2006) [39] surveyed a number of new chemical entities (new active ingredients) sold worldwide between 1975 and 2004, and found that in this period, of the 1,556 new chemical entities, only 21 (less than 1%) were for neglected diseases. This proportion has not changed in the last 10 years, despite significant advances in the fields of genetics, molecular and cell biology, proteomics, new biological targets discovery, computational tools for the realization of virtual screening, docking, and other techniques that are part of rational drug design.

More recently a study by the Global Funding of Innovation for Neglected Diseases (G-FINDER) published in PLoS Medicine [9] indicated that private institutions, like the pharmaceutical industry spent less than 5% of global funding on neglected diseases innovations. The George Institute for International Health supported by the Bill & Melinda Gates Foundation adds that most of the investments in this area are from philanthropic (54%), and public (41%) institutions which unfortunately, in most cases, cannot ensure long term investments.

The NGO Doctors Without Borders proposed the division of the world's diseases into: Global, Neglected, and More Neglected. WHO, in the same year, made use of an equivalent classification, which shared disease classifications in Type I (equivalent to Global Diseases), Type II (equivalent to Neglected), and Type III (equivalent to the Most Neglected) [40].

The Global diseases, or Type I, would be diseases that affect populations of all countries, like cancer, cardiovascular and nervous system diseases, inflammatory diseases, and others, which win the largest R&D investments by pharmaceutical companies.

Neglected diseases, or Type II, arouse less industry interest for being more prevalent in the populations of developing countries. These diseases, such as tuberculosis, malaria and AIDS, are known as the "big three" (responsible for 5.6 million deaths according to data from WHO [41]. If we were to consider only the level of investment by government and industry funding in the current context they could not be considered neglected.

Type III or More Neglected diseases refer to those diseases which are almost exclusive to people in poorer (developing) countries arousing very little or no interest from pharmaceutical companies, i.e. sleeping sickness, Chagas disease, leishmaniasis, dengue, filariasis, schistosomiasis, etc.

Neglected diseases refer to chronic infections which cause severe health burdens on the world's poorest people. They include diseases such as tuberculosis, leprosy, leishmaniasis, African trypanosomiasis (sleeping sickness), malaria, lymphatic filariasis, dengue, onchocerciasis, Chagas disease, and schistosomiasis. They continue to cause high rates of morbidity and mortality. Such diseases are common in Aboriginal populations; around 370 million people are classified today to belong to Aboriginal groups which include populations of Malaysia, Australia, Brazil, Venezuela, Africa, and India. These diseases kill an estimated 534,000 people worldwide every year [42].

There are also huge numbers of clinical cases for other neglected diseases, WHO registered the 2012 prevalence of leprosy to be 189,018 for 115 countries, and during the same year, 232,857 new cases were reported. It was also estimated that in 2012, malaria provoked 207 million clinical episodes, and 627,000 deaths. According to the World Health Organization, African trypanosomiasis is a serious health risk to more than 60 million people in Sub-Saharan Africa with 300,000 new cases per year, and fewer than 30,000 cases diagnosed and treated. An estimated 120 million people in tropical and subtropical areas of the world are infected with lymphatic filariasis [42].

Due to the development of multidrug resistance, the need often arises to develop new drugs for the same neglected diseases, and the market faces these setbacks; the scenario itself hinders the quest for new drugs since the process of drug development is lengthy and expensive. This quest for new drugs and drug targets is therefore ipso facto essential, and the need for new drugs will persist until pathogens are eradicated [43].

Knowledge of the genome and proteome is instrumental in the process of new drug targets development. Despite sufficient knowledge of the genome and proteome being available, little work has been done in relation to neglected diseases drug development, and though some efforts have been made in drug development, today's essential drugs to treat neglected diseases are either too expensive, unavailable in the market, highly toxic, or ineffective due to pathogen resistance. One report says that of the 336 new drugs approved for all diseases in 2000–2011, only 1 % were for malaria, and diarrheal diseases, no drugs were approved for any of the 17 WHO-listed NDs [44]. The research complexities that have led to this scenario are due to highly customized research infrastructure, narrow research focus, and lack of clinical trials. Lack of finance and expertise has also contributed greatly to this low percentage. Most newly developed therapeutic products are merely re-purposed versions of existing drugs. To review and explore the available resources has become an important process for new drug development and target identifications for neglected diseases.

The main neglected diseases, available drugs for the treatment, targets, and resistance are reported in Table 4.1. Later we will better discuss these infections, the drugs currently in use, and give some examples of studies with natural products.

Table 4.1: Current scenario of the main neglected diseases.

Disease	Causative agent	Drug Targets	Drugs	Drug resistance reported
Tuberculosis	*Mycobacterium tuberculosis*	Peroxidase/catalase T, Enoyl-[acyl-carrier-protein] reductase [NADH], DNA-directed RNA polymerase beta chain, Pyrazinamidase/ nicotinamidase, 30S ribosomal protein S12, arabinosyltransferase A, DNA gyrase	isoniazid, Rifampicin, Pyrazinamide, Streptomycin, Ethambutol Ethionamide Kanamycin Cycloserine Capreomycin Ofloxacin	Yes
Leishmaniasis	*Leishmania donovani*	Squalene synthase Farnesyl diphosphate synthase ergosterol of the cell membrane ATPase	Quinuclidine derivatives, ER-119884 and E5700 Terbinafine Ketoconazole Bisphosphonates Pentostam Amphotericin B Aminosidine Pentamidine Miltefosine	Yes
Malaria	*Plasmodium falciparum*	Cytochrome b, Dihydrofolate reductase, Dihydropteroate synthase	Artemether Artesunate Chloroquine Mefloquine Mepacrine Proguanil	Yes
Lymphatic filariasis	*Wuchereria bancrofti*	Tubulin polymerization Glutamate-gated chloride ion channels L-subtype nicotinic acetylcholine receptors	Albendazole Diethylcarbamazine Levamisole	Yes

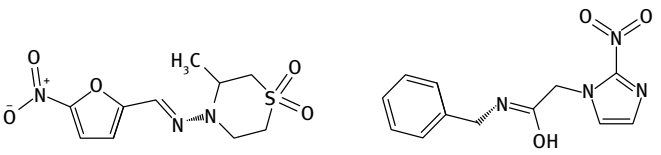
Chagas disease	*Trypanosoma cruzi*	Mode of action is chemotherapy	Benzonidazole nifurtimox	Yes
African sleeping sickness	*Trypanosoma brucei*	Ornithine decarboxylase farnesyltransferase S-adenosylmethionine decarboxylase N-myristoyltransferase	Eflornithine Pentamidine Suramin Melarsoprol Nifurtimox	Yes
Leprosy	*Mycobacterium leprae*	Dihydropteroate synthetase	Ofloxacin Minocycline Clarithromycin rifampicin Dapsone	Yes
Helminth infections	*Schistosoma mansoni* *Ascaris lumbricoides* *Trichuris trichiura* *Necator americanus* *Ancylostoma duodenale*	Bind to beta-tubulin and prevent the polymerization of the microtubules Calcium chloride ion channels	Triclabendazole Praziquantel	Yes

4.4.1 Chagas disease

Chagas disease (also called American trypanosomiasis) is a human tropical parasitic disease which occurs in the Americas, particularly in South America, caused by the hemoflagelate protozoan *Trypanosoma cruzi*. The most frequent mode of transmission is through an insect vector from the *Triatominae* subfamily commonly known as "the kissing bug". Other transmission routes include blood transfusions, and organ transplants, congenital transmission, ingestion of contaminated foods or drinks, and laboratory accidents. Chagas disease presents itself in two phases: an initial, acute phase which mostly presents mild unspecific symptoms fever, headache, muscle pain, and others (or no symptoms at all), and a chronic phase, which is asymptomatic for about 70 % of patients, yet it causes life-threatening heart, and digestive disorders in roughly 30 % [45]. The infection causes great social problems, and in addition, there are only a restricted number of effective drugs available, which carry serious side effects. Currently approved medications for treating Chagas disease are still limited to nifurtimox and benzonidazole, which were developed more than 40 years ago, but present important efficacy and safety limitations. The emergence of new drug resistant forms has pushed research for new antiprotozoal drugs.

In several structure-activity studies, flavonoids have been tested for their ability to inhibit key enzymes in the *T. cruzi* mitochondrial respiratory pathway [45]. Along with other biological effects, the inhibition of mitochondrial enzyme systems is an underlying cytotoxicity mechanism of natural products. Flavonoid compounds and analogs are naturally present in vegetables, fruits, and beverages and they are considered important components of the daily Western diet.

The use of these plant derived drug substances, and their associated derivatives, and synthetic compounds deduced from their natural product precursors, represents a major part of today's pharmaceutical market (Fig. 4.5). Natural products provide opportunities in drug discovery, leading to detailed understandings of biological pathways, and revealing the functions of the involved enzymes and receptors. They are also common constituents of medicinal plants, and the therapeutic effects of many traditional medicines have been attributed to them. These compounds exert distinct biological effects, particularly, acting as antioxidants and prophylactic agents against many diseases, among them Chagas disease.



Nifurtimox Benzonidazole

Fig. 4.5: Nifurtimox and Benzonidazole.

4.4.2 Dengue

Aedes aegypti Linn (Diptera: Culicidae), vector of dengue fever, is considered the most important disease-carrying mosquito in the world, and it has become a major international public health concern. Unlike most vectors, *Ae. aegypti* lives near human habitations, and also breeds in a variety of water containers. During its life cycle, the female mosquitoes seek blood as a source of supplemental substances, such as protein and iron to drive oogenesis. Contaminated mosquitoes transfer the dengue virus from their salivary glands to humans during the course of the bite. After infection, the virus incubates for about six days, resulting in a flu-like illness that affects infants, young children, and adults, but rarely causing death [46]. However, severe dengue is characterized by high fever which may progress to circulatory failure and death.

Because there is no treatment or vaccine available for treating or controlling dengue fever, the only methods available to control dengue fever are to eliminate its vector, either by environmental actions, such as elimination of its breeding sites or by the use of pesticides. The use of larvicides is the most successful method of controlling mosquito infestations. Three major classes of larvicides are approved for use in potable water, organophosphates (e.g. temephos), growth regulators (e.g. juvenile hormone mimics, chitin synthesis inhibitors), and bacteria toxins (e.g. *Bacillus thuringiensis*, Bti). However, resistance to pesticides has led research to look for new methods of controlling *Ae. aegypti* propagation. Natural product derivatives are a potential source for new pesticide candidates. Chemical derivatives from botanical sources may not only be selective to a target species, but also more environmentally friendly than synthetic compounds. Chemically modified monoterpenes have been evaluated for activity against adult *Ae. aegypti* mosquitoes, as well as larvae. Such studies have demonstrated the importance of double bonds and phenolic hydroxyls to larvicidal activity [47].

Thymol and carvacrol are important phenolic monoterpenoids obtained from plant essential oils. Both exhibit a large number of pharmacological effects which include; antimicrobial, antitumor, antiplatelet, analgesic, anti-inflammatory, anti-angiogenic, and insecticidal activities. Additionally, thymol and carvacrol are *generally regarded as safe* (GRAS) in food flavorings, an indication of low mammalian toxicity for starting materials.

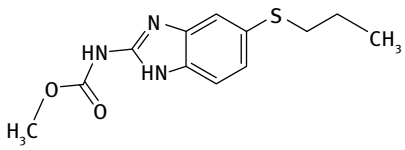
4.4.3 Lymphatic filariasis

Lymphatic filariasis (LF) is a mosquito-borne tropical disease caused by the nematode parasites *Wuchereria bancrofti*, *Brugia malayi* and *B. timori* [48]. It is the major cause of acute and chronic morbidity in 81 countries in Asia-Pacific, Africa and the Americas. Approximately 1.3 billion people living in these regions are at risk of infec-

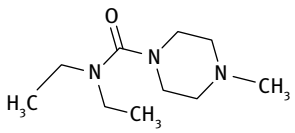
tion [49]. As of 2012, approximately 441 million persons were at risk of this disease in 33 countries. The three African countries with the largest populations at risk are Nigeria (109 million), DRC (49 million) and Ethiopia (30 million) [50].

Wuchereria bancrofti is responsible about 90 % of LF infections worldwide while B. malayi is predominant only in some parts of South and Southeast Asia, and the closely related *Brugia timori* is restricted to southeastern Indonesia [50]. The parasite is transmitted by *Culex* species mosquitoes [51]. The crippling physical effects of the disease have a huge economic and social impact, which is why lymphatic filariasis is one of the top 10 tropical diseases being targeted by the World Health Organization.

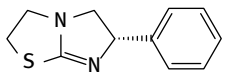
Strategies to control the disease include administration of drugs, like annual doses of diethylcarbamazine (DEC), DEC plus albendazole, diethylcarbazine, levamisole, which reduce the level of infection and prevent transmission, however these drugs have been plagued with concerns about their inability to kill the adult worms, long treatment durations, severe side effects, and the emergence of drug resistance in humans (Fig. 4.6). Therefore, emergence of drug resistance to the currently available treatment is a potential threat to the LF elimination program [49, 52, 53]. Since no effective treatment for filarial adult worms is currently available, new chemical classes of compounds with macrofilaricidal activities are now required [54].



Albendazole



Diethylcarbamazine



Levamisole

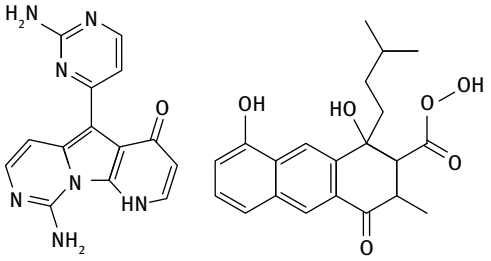
Fig. 4.6: Current drugs against LF.

The research of natural products to select and propose new structures using computer-aided drug design is very recent in the literature. Sukuru and coworkers [55] report a study that used docking methodology to screen several large databases to identify inhibitors of Brugia malayi asparaginyl-tRNA synthetase (AsnRS). Aminoacyl-tRNA synthetases (AARS) have been acknowledged as rational targets for anti-infective

drug development [56] because these enzymes are essential for viability. AARS are one of several new drug targets in human filarial parasites that have been proposed in recent years [57].

The authors used a 1.9 Å resolution closed structure of *Brugia* (AsnRS) in complex with a non-hydrolyzable analog of asparaginyl adenylate (ASNAMS) for structure-based ligand screening and design (PDB id: 2XGT) [58]. The software SLIDE was used to screen two databases of small organic molecules, The Cambridge Structural Database (CSD) [59] and the National Cancer Institute (NCI) Plated Compounds Database [60], to find potential inhibitors of *Brugia* AsnRS. SLIDE [61] is a screening and docking tool that uses distance geometry to screen and dock ligand candidates into the binding site of the target protein. Initially the Lipinski rule was applied to ensure drug-like physicochemical properties [62], selecting about 188,000 compounds, 110,000 of CSD plus 70,000 of NCI.

After the structure-based screening using docking, the authors selected several secondary metabolites. Variolin B a pyrrolopyrimidine, was originally isolated from an Antarctic sea sponge and has been shown to have antitumor and antiviral activity [63]. However the author highlights that Variolin B (Fig. 4.7) is highly cytotoxic in human Namalwa cell lines and is isosteric and shares chemistry with adenine, and it may be toxic because it binds to ATP sites. Another natural product selected by Sukuru and coworkers is Rishirilide B, isolated from *Streptomyces rishiriensis*, additionally, the authors suggest that the + enantiomer is responsible for inhibition (Fig. 4.7).

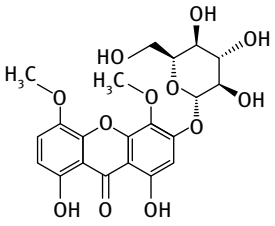


Variolin B Rishinilide B

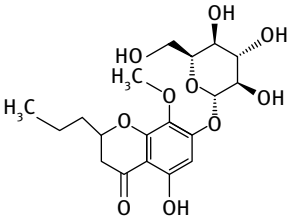
Fig. 4.7: Structures of Variolin B and Rishirilide B, potential structures with inhibition activity against *Brugia malayi* asparaginyl-tRNA synthetase.

Recently, virtual screening (VS) of plant constituents from traditional Chinese medicine (TCM) database was reported being as target asparaginyl-tRNA synthetase [64] (AsnRS; PDB ID: 2XGT). The authors selected 95 chemical constituents reported from eight plants (*Areca catechu, Omphalia lapidescens, Torreya grandia, Melia azedarach, Quisqualis indica, Cucurbita moschata, Agrimonia pilosa, Melia azedarach L*) used for the treatment of worm infection retrieved from traditional Chinese medicine (TCM) [65].

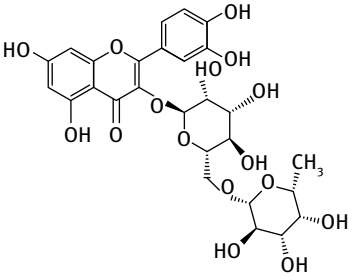
The compounds were processed using LigPrep wizard in Maestro v9.2 (Schrödinger, Inc.) [66] and prepared using the Optimized Potentials for Liquid Simulations (OPLS) 2005 force field [67]. Two VS approaches were used in this study: Docking-based virtual screening (DBVS) and E-pharmacophore (pharmacophore (energy-optimized structure-based pharmacophore)-based screening)-based virtual screening (PBVS). To perform docking the program Glide extra precision (XP) protocol [66] implemented in Maestro v9.2 was used. The NSS (5'-O-[N-(lasparaginyl)sulfamoyl] adenosine) ligand present in the active site of the target protein (AsnRS). The E-phar-



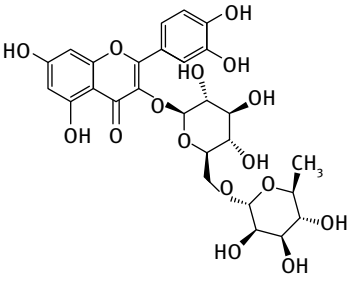
Agri 1



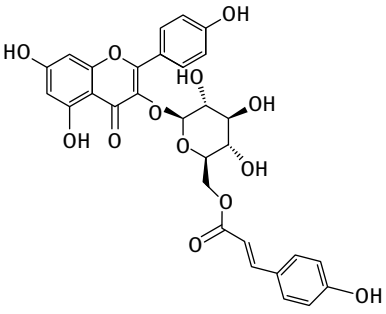
Agri 2



Agri 20



Agri 23



Agri 26

Fig. 4.8: Secondary metabolites selected by virtual screen as potential inhibitors of Brugia malayi asparaginyl-tRNA synthetase.

macophore approach uses combined characteristic of structure- and ligand-based approaches for the screening a ligand database [68], the software Glide XP was used to generate the hypothesis of pharmacophore features. Additionally for the compounds selected by virtual screening the authors performed molecular dynamics simulation using GROningen Machine for Chemical Simulations V4.6.1 (GROMACS) computational package [69] and PRODRG server [70] to generate the topology files and other force field parameter for the five selected ligands.

The compounds named as Agri 1 (1,3,8-trihydroxy-4,5-dimethoxyxanthen-9-one-3-O-beta-D-glucopyranoside), Agri 2 (7-dihydroxy-2-propylchromone 7-Obeta-D-glucopyranoside), Agri 20 (quercetin-3-Orhamnopyranosyl), Agri 23 (quercetin-3-O-rutinoside) and Agri 26 (tiliroside) (Fig. 4.8) are natural chemical constituents of the plant *Agrimonia pilosa*. From these five structures, according to the authors, Agri 1 and Agr 2 are the hits with more stable interaction with the target.

Nathan and coworkers [71] built a structural model of Glutathione-S-transferase (GST) of the lymphatic filarial parasite *Wuchereria bancrofti (Wb)* by homology modeling. Single letter code amino acid sequence of GST of W. bancrofti [72] was used as input in the BLAST software (http://www.ncbi.nlm.nih.gov/BLAST) and selected as template the porcine pclass GST 2gsr-A chain. After that, the authors used the software MODELLER6v2 to generate the 3D-structure (PDB Id: 1SFM) and verified it using the softwares PROCHECK and WHATCHECK.

The modeled 3D structure of wbGST was used for docking with GST inhibitors to find the active pocket of ligand binding sites using the Hex4.2 macromolecular docking program with default parameters of the docking module. The three structures with lowest docking energy were selected for *in vitro* study using GST enzyme isolated from *S. digitata*. Two compounds (Fig. 4.9) are able to inhibit the GST enzyme and exhibited macrofilaricidal activity *in vitro* [73].

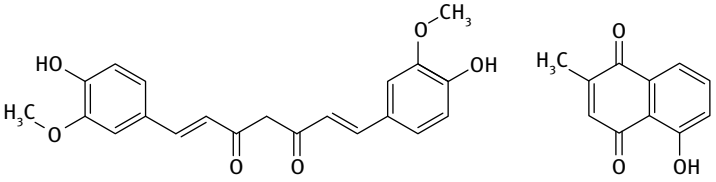


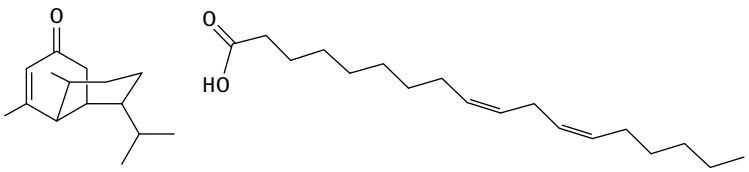
Fig. 4.9: Secondary metabolites with inhibitory activity against Glutathione-S-transferase (GST) and macrofilaricidal activity against *Setaria digitata*.

4.4.3.1 Onchocercosis

Human onchocerciasis or subcutaneous filariasis is a parasitic disease caused by the filarial worm *Onchocerca volvulus*. It is transmitted through the bites of infected *Simulium* blackflies, which carry immature larval forms of the parasite from human to human. In the human body, the larvae form nodules in the subcutaneous tissue, where they mature to adult worms. Some studies have demonstrated *in vitro* microfilarial activity of plant extracts.

Crude, hexane, chloroform, ethyl acetate, and water extracts (of *Euphorbia hirta* Linn.), (Euphorbiaceae), and *Rauvolfia vomitoria* Afzel, (Apocynaceae), were evaluated *in vitro* against *Onchocerca volvulus* microfilariae and demonstrated an ability (at different levels) to immobilize *O. volvulus* microfilariae. The extracts possess antifilarial properties. In general *E. hirta* extracts are more effective than those of *R. vomitoria*. Among the extracts, the ethyl acetate fraction was the most effective, whilst the crude extract was the least toxic to monkey kidney cell lines [74].

Metuge and colleagues demonstrated that essential oil from the roots and rhizomes of *Cyperus articulates* is active *in vitro* against *O. ochengi* microfilariae and adult worms in a dose dependent manner, and may provide a new source of antifilarial compounds [75]. Two metabolites were also isolated from *C. articulates*: AMJ1 [containing mustakone as the major component], and linoleic acid (Fig. 4.10). Both compounds were found to kill *O. ochengi* microfilariae and adult worms [76], these metabolites may provide leads for design and development of new anti-Onchocerca agents.



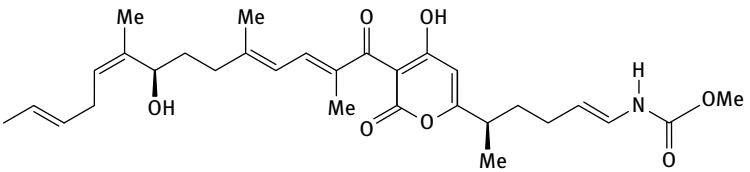


Fig. 4.10: Compounds with anti-Onchocerca activity.

Another strategy that can be used to control onchocercosis involves the depletion of the endosymbiotic bacteria *Wolbachia*, which are essential to filarial worms (lymphatic filariasis, and onchocercosis) throughout their life cycle. Elimination of *Wolba-*

chia causes a permanent block in oogenesis, embryogenesis, and development, and finally, a slow death for the adult worms.

Corallopyronin A (CorA) (Fig. 4.6) is a promising antibiotic for treatment of filarial diseases. CorA isolated from the mycobacterial strain of *Corallococcus coralloides* c127 effectively depletes *Wolbachia* from filarial nematodes, so, it is an attractive anti-Wolbachia compound for further development as an antibiotic to use in either control or elimination of filarial nematode infections [77].

4.4.4 Tuberculosis

Tuberculosis (TB) is an infectious disease caused by members of the *Mycobacterium tuberculosis* (Mtb) complex; it comprises one of the most important infectious diseases worldwide. About ten million people are affected, mainly in the poorest of countries, albeit high prevalence also occurs in developed countries, due to HIV co-infection. The reported clinical cases come in huge numbers for each of the neglected diseases. In 2013, some 9 million people around the world were reported to have contracted TB. There were roughly 1.5 million TB-related deaths worldwide (1.1 million HIV-negatives, and 400 thousand co-infected with HIV) [1]. Although anti-mycobacterial drugs have kept TB prevalence rates under control for several decades, the appearance of resistant cases of the disease brings TB to a worrying status. There has been an increase in cases of resistance to TB treatment for the current drugs: Isoniazid, Rifampicin, Pyrazinamide, Streptomycin, Ethambutol, Ethionamide, Kanamycin, Cycloserine, Capreomycin and Ofloxacin (Fig. 4.11) [78, 79].

Mycobacteria are slow-growing bacilli that possess an external cell wall composed of highly lipophilic fatty acids, the mycolic acids. The structure of this cell wall is very complex, and its highly lipophilic nature plays a role in anti-TB compound penetration. Penetration through the mycobacterial cell wall (an issue in many anti-TB molecules), must always be considered when designing new anti-TB agents, such anti-mycobacterial agents should be lipophilic. Considering the complexity of the cell wall, obtaining internally effective anti-mycobacterial agents (using the "prodrug" approach) is feasible, this while simultaneously improving both their pharmaceutical and pharmacokinetic profiles. Numerous examples of this approach are to be found in literature.

In the course of infection within the host body, tuberculosis, associated with several lipases and phospholipases, uses surface proteins to enter macrophages. During infections, *M. tuberculosis* infects macrophages; needing to acquire nutrients within the host as well as resisting the host's immune defenses. Recent studies have shown that *M. tuberculosis* exploits its lipases for hydrolyzing host cell lipids, and then uses the fatty acids released for long-term energy. Researchers have identified natural compounds as ligand inhibitors [80]. Studies in anti-tubercular drug development using natural products have observed important structural features for

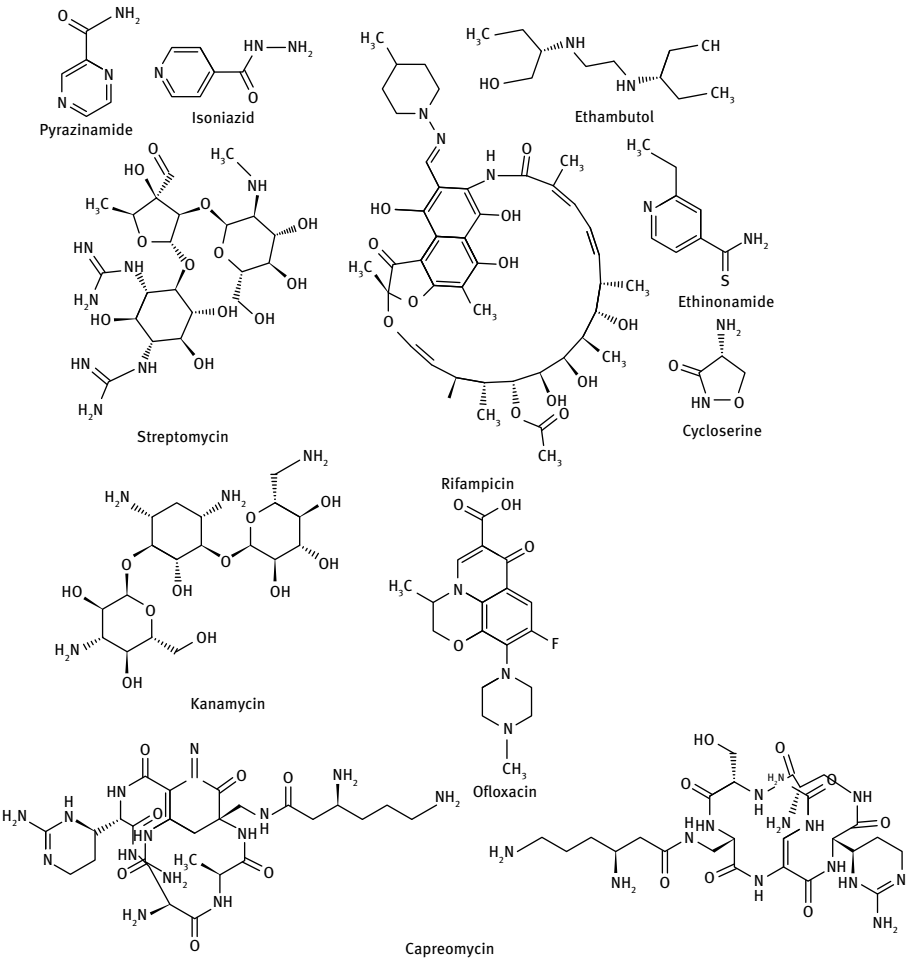


Fig. 4.11: Current drugs against tuberculosis.

ligand-binding affinity with the enzyme 3-dehydroquinate dehydratase, part of the shikimate pathway [35]. Shikimate kinase (SK) is an essential enzyme in many pathogenic bacteria, and does not have any counterparts in human cells. This makes it an attractive target for the development of new antibiotics, in particular, against the *Mycobacterium tuberculosis*.

4.4.5 Leprosy

Leprosy is a chronic infectious disease caused by the bacillus *Mycobacterium leprae*. The literature does not report a large number of plants or natural compounds with

anti-leprosy activity. Some drugs used to treat tuberculosis are also used for leprosy, such as ofloxacin and rifampicin. Other drugs currently used are minocycline, clarithromycin, and dapsone (Fig. 4.12).

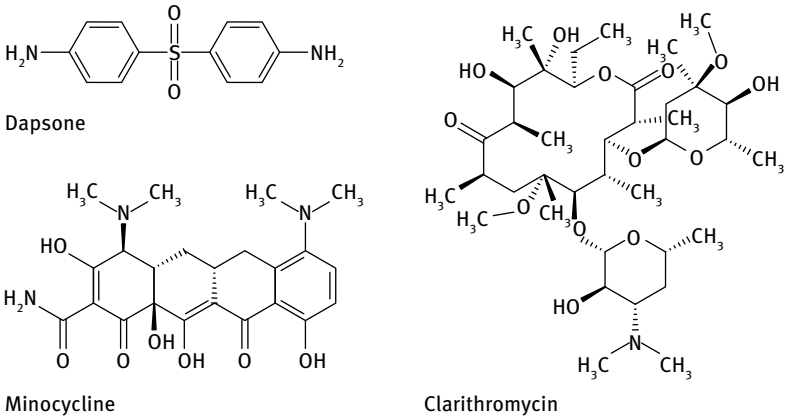


Fig. 4.12: Current drugs against leprosy.

The most recently published review by Barbosa-Filho, and colleagues in 2006 [81] listed 11 plants with their families, geographical distribution, the parts utilized, and the type of extract used in the treatment of leprosy in humans. These plants are native in India, China, Morocco, and Senegal: *Acacia catechu, Achyranthes aspera, Albizzia lebbeck, Centella asiática, Hemidesmus indicus, Lasiosiphon kraussianus, Leucaena glauca, Melia azedarach, Semecarpus anacardium, Smilax ornata, Tripterygium wilfordii*.

This review includes 17 substances isolated from higher plants and microorganisms which present inhibitory activity against *Mycobacterium leprae*. These compounds belong to the following classes: lipids (Chaulmoogric acid, and dihydro-Chaulmogric acid from *Chaulmoogra odorata*, Glucose micolate (*Nocardia rubra*), Hydnocarpic acid (*Hydnocarpus wightiana*), and Palmitic acid (*Chaulmoogra odorata*); triterpenes (Asiaticoside and Oxy-Asiaticoside from *Centella asiatica*), Boswellin acid (*Boswelia serrata*), and Fusidic acid (*Fusidium coccineum*); macrolides (Clarithromicin (*Streptomyces erythreus*), and Rifampicin (*Nocardia mediterranei*); alkaloids (Desoxyfrutoserotonin); benzenoids (Curcumin from *Curcuma longa*); flavonoids (Dalibotrin from *Dalbergia latifolia*); matansinoids (Ansamycin from *Nocardia mediterranei*); proteids (Proteoglycan-G009 from *Ganoderma lucidum*); and sulfur compounds (Allicilin from *Allium sativum*). From these substances, the compounds that were active in human trials are shown in Fig. 4.13.

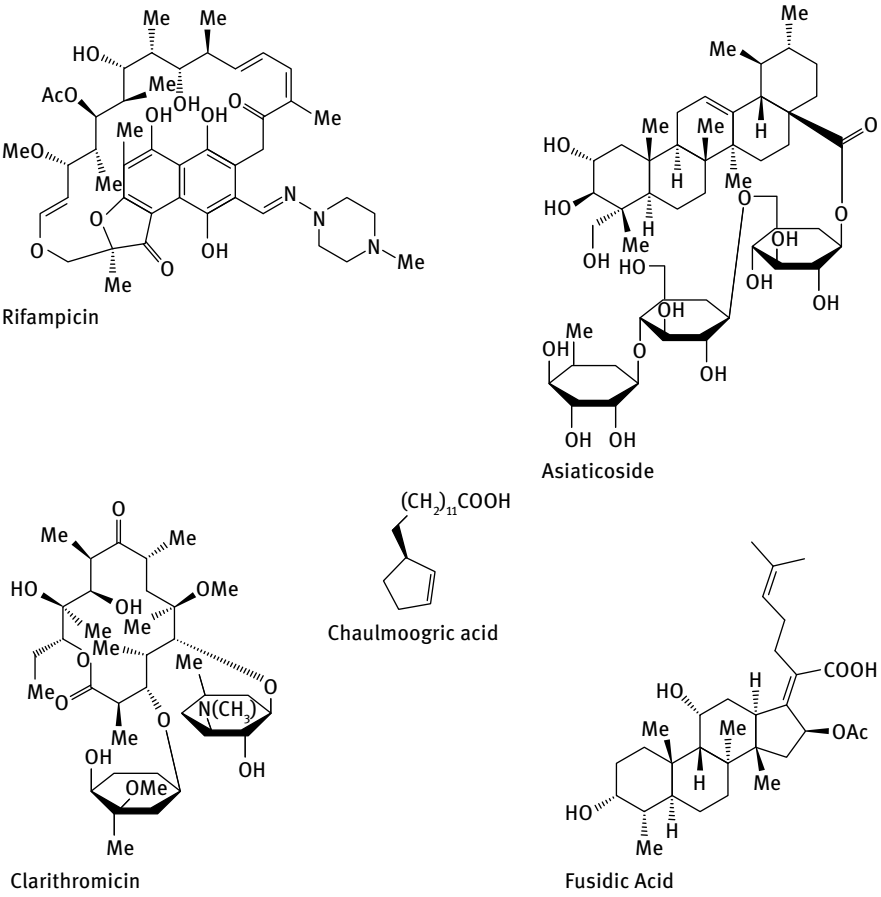


Fig. 4.13: Natural compounds showing antileprotic activity in human.

4.4.6 Helminth Infections

Diseases caused by helminths are a major burden on humanity, with from 807 to 1221 million people infected. It is estimated that up to one third of the human population are infected with parasitic helminths. The most significant agent is the roundworm *Ascaris lumbricoides*. Many individuals are infected with several helminths, or with other infectious agents such as HIV, the malarial parasites, or bacteria. In many cases, the helminth infections are not immediately life-threatening [82].

To date, there are no effective vaccines for helminth infections, and treatment is entirely chemotherapeutic. While most anthelminthic drugs (e.g. triclabendazole, praziquantel, see Fig. 4.14) are effective, affordable, and generally considered safe, not all of these diseases can be treated effectively, and resistance is emerging [83].

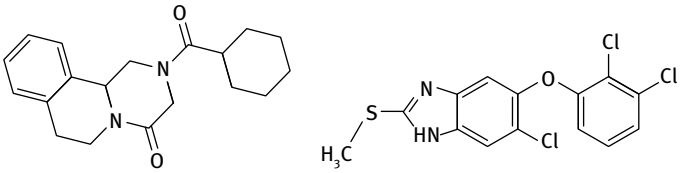


Fig. 4.14: Praziquantel and Triclabendazole.

Central carbon metabolism pathways (i.e. glycolysis, the Krebs tri-carboxylic acid cycle, the electron transport chain, and their associated pathways) are necessary for energy production in the majority of eukaryotes. Inhibition of these processes generally results in reduced ATP production and can lead to cell death. A number of common poisons target elements of carbon metabolism. The pathways of carbon metabolism are highly conserved through evolution, as are the enzymes which catalyze the individual steps. Yet, there are a few structural differences between host and parasite enzymes that can be exploited when designing species-specific inhibitors.

As an example, studies have shown that the catabolizing enzyme *Schistosoma mansoni* NAD(+) has significant structural and functional analogy to the mammalian CD38/ADP-ribosyl cyclase family, and inhibition of SmNACE by the natural product cyanidin occurs [84].

4.4.7 African sleeping sickness (*T. brucei*)

Sleeping sickness or Human African trypanosomiasis (HAT) is perhaps the most neglected disease that exists. This is because it is mainly restricted to the African continent, and affects the poorest populations, between 15 and 45 years of age. These are typically the inhabitants of rural areas who work in agriculture or fishing, yet whose lifestyles have suffered accelerated changes in demographics, populations, and the environment. When left untreated, the disease is always fatal. According to the World Health Organization, from 300 to 500 thousand people are infected, 60 million are at risk, and sleeping sickness causes from 15,000 to 30,000 deaths annually. After the bite of the vector, the parasite remains in the bloodstream, and after an asymptomatic period that can last for weeks, the acute stage (known as stage 1) begins to emerge with frequent fevers, fatigue, and vomiting. Finally the Trypanosome reaches the central nervous system (stage 2), causing great dependency on others, and even death [85, 86].

The principal drugs used to treat Human African Trypanosomiasis are pentamidine, melarsoprol, suramin, and eflornithine. Nifurtimox can also be administered in certain cases. Figure 4.15 reports the chemical structures of the substances mentioned.

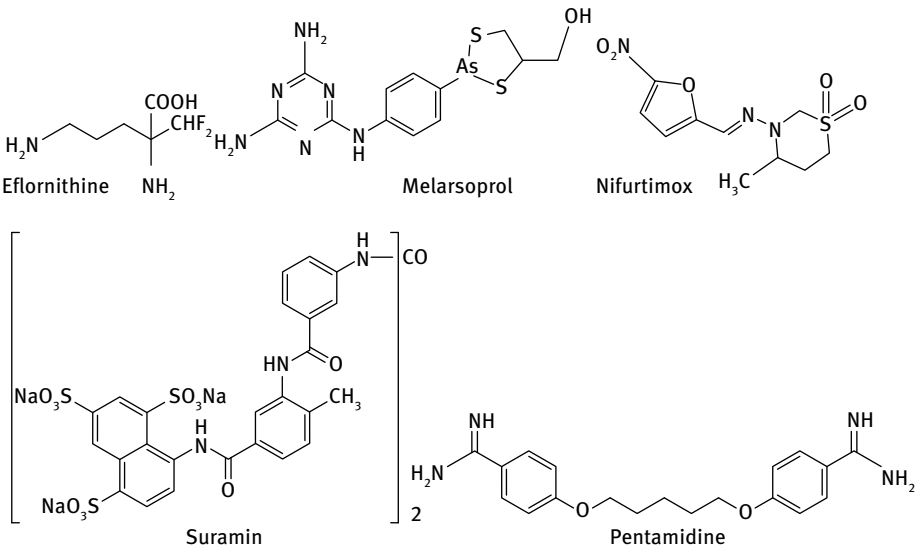


Fig. 4.15: Chemical structure of the drugs currently used in the treatment of sleeping sickness.

The currently employed chemotherapies for sleeping sickness have serious side effects, and often cause resistance. The drugs are typically effective during only one stage of the disease, and against a single subspecies of the parasite. They are not feasible for a large part of the poorer rural populations, either because of drug cost, or by the length of necessary hospitalization. There is no vaccine to prevent infection by *T. brucei*, and if treatment is not effective, the disease is fatal. New drugs against the parasite are needed.

Docking methodology that accounts for full protein flexibility was used to identify natural product inhibitors of *T. brucei* UDP-galactose 4'-epimerase [87]. Ogungbe and Setzer in 2009 [88] investigated several natural products, submitting the compounds to docking with the enzymes rodesaine, TR, farnesyl diphosphate isomerase, and trioephosphato synthase. The authors reported the main interactions, and concluded that the compounds cissanpeloflavona, 3-geranilemodine, and ningpogenine deserve further research. In natural products studies, compounds having greater selectivity and effectiveness, yet with less toxicity, are the main objectives. As an example, glycosides were isolated from the leaves of *Pyostria* major that were active against *T. brucei* with low toxicity.

A review by Schmidt et al. (2012) [89, 90] reports that we can isolate many natural products from various plants which are active against parasites. The study discusses various classes of molecules, and their action against the parasites of neglected diseases. The plants *Aframomum sceptrum* and *Xylopia aethiopica*; and the monoterpenoids linalool, terpinolene, R-(+)-limonene, α-felandrene, S-(−)-carvone, 1.8-cineol, among others, are all active against *T. brucei*.

4.4.8 Leishmaniases

The Leishmaniases are caused by protozoan parasites from more than 20 Leishmania species, such as *Leishmania amazonensis*, *Leishmania mucocutânea*, *Leishmania tarentolae*, *Leishmania donovani*, *Leishmania cutaneous*, *Leishmania major*, *Leishmania infantum*, *Leishmania visceral*, and *Leishmania braziliensis*. The diseases can be categorized broadly into three types: visceral (VL), cutaneous, and mucocutaneous. VL is caused exclusively by species of the *Leishmania donovani* complex; it is the most severe form of leishmaniasis and may be lethal if left untreated [91].

The medicines used to treat the Leishmanias do not have the desired effectiveness, they are often toxic, they are expensive or not accessible in poorer countries (which have the highest burden of cases), and the parasites present resistance. Figure 4.16 shows the current drugs used against *Leishmania spp*.

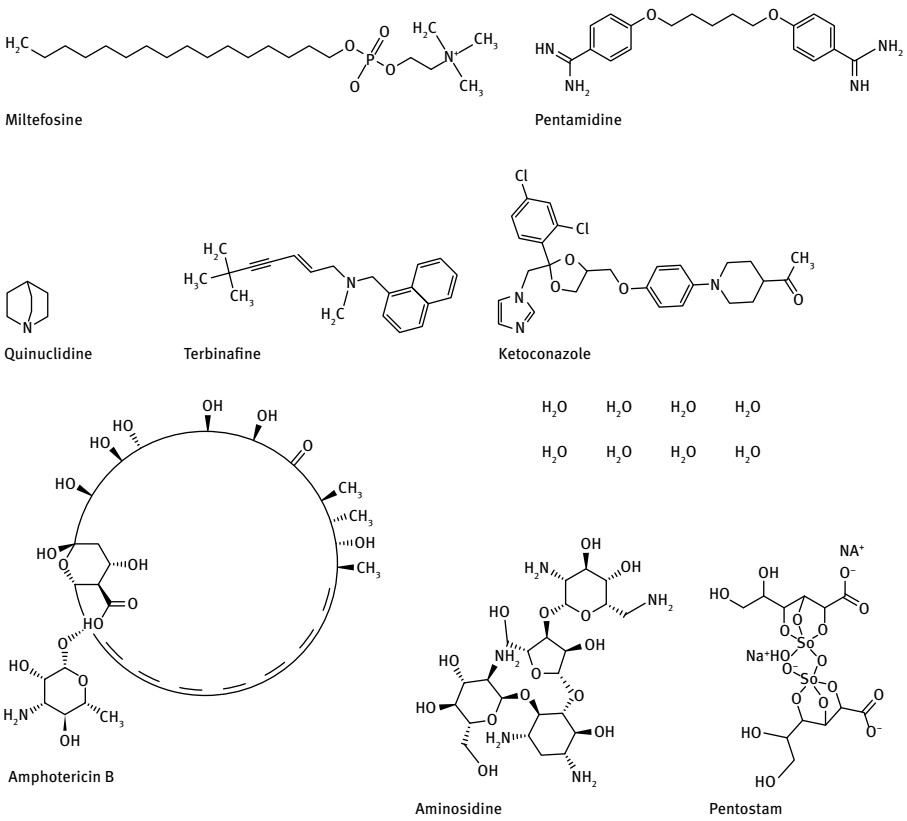


Fig. 4.16: Current drugs antileishmaniasis.

Two characteristics are important in the process of target identification for drug development: difference from the mammalian, and necessity for the survival of the pathogen. Some metabolic pathways are essential for the life of the parasite, and these reactions may involve extremely complex chemical mechanisms. Yet, a very large number of reactions are catalyzed efficiently by enzymes. Research for new antileishmania drugs involves searching for new biochemical targets: related to the mechanism of defense, the metabolism of RNA, DNA, glucose, sterols, and fatty acids, the purine pathway and nucleotides, or any parasite biochemical route that can be attacked by the drug and yet remain safe to the host.

Natural products have been a rich source of compounds with antileishmanial activity. Focusing on natural products as inhibitors, some of the main enzymatic targets studied include the following: lipid metabolism (lipid-catabolizing lipases (LdLip3 lipase)) [92]; adenosine kinase inhibition (purine pathway), phosphorylation of adenosine to AMP, and crucial for parasites [93]; *Leishmania infantum* trypanothione reductase [94] (crystal structure using in silico virtual screening of a natural product data set of 800 diverse chemical entities); marine sponge alkaloids, triterpenoids (and derivatives) of the lupane group [95].

4.4.9 Malaria

Plasmodium falciparum is the most virulent human malaria parasite, and is responsible for most of the malaria-related deaths. Although there is significant promise for a future vaccine, the only current course of action is to use some prophylaxis and/or personal protection to prevent infection from the bite of a parasite-carrying mosquito. Moreover, the emergence of drug-resistant forms of the parasite is an ever-burdening public health threat, and it curbs the likelihood of eradicating the disease. Since the discovery of the natural product quinine, many compounds with a quinoline scaffold have displayed good antimalarial activity, leading to the development of effective antimalarial agents, including chloroquine, amodiaquine, piperaquine, and mefloquine (Fig. 4.17) [96, 97].

The emergence of multi-chemo-resistant parasites could seriously undermine global malaria control; a major contributor to the problem is the emergence and spread of resistance toward most of these drugs (mentioned above) in clinical use, such as chloroquine, amodiaquine, mefloquine and pamaquine. New chemotherapeutic approaches based on innovative mechanisms of action are needed.

The parasite redox metabolism is a promising target for novel antimalarial drugs, since maintaining redox equilibrium is of fundamental importance. Novel inhibitors were selected via screening of a library of compounds from the Leibniz Institute for Natural Product Research and Infection Biology, that are active against the redox-related enzymes thioredoxin reductase, glutathione reductase, glutathione-S-transferase, glucose-6-phosphate dehydrogenase, and 6-phosphoglucono-lactonase [98].

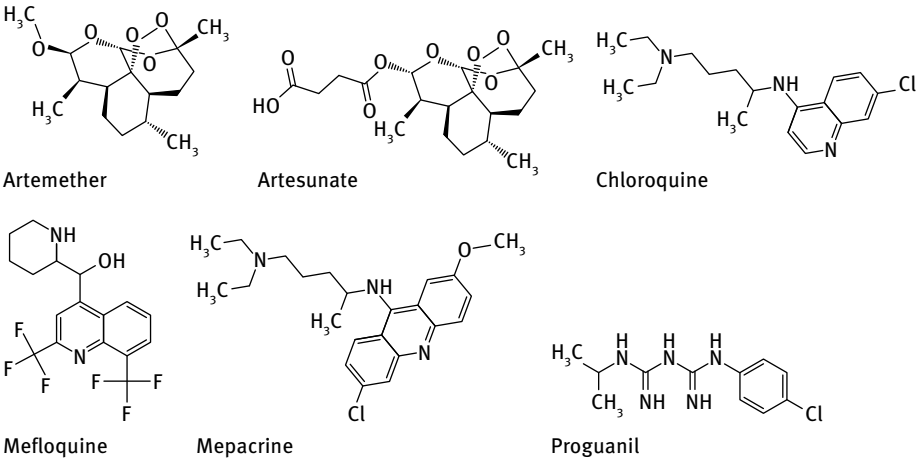


Fig. 4.17: Some drugs used against malaria.

In the search for new drugs against *Plasmodium falciparum* bi-functional dihydrofolate reductase-thymidylate synthase inhibitors, two natural compounds were found to be active: ochralifuanine and bischromone-chrobisiamine [99].

Mushrooms were identified through *in silico* methodologies, like structural modeling, protein-protein docking, and structural superimpositions, to have biologically active products that act as protease inhibitors [100].

Crude leaf extracts of *Gymnema sylvestre* (Retz) *Schult* (Asclepiadaceae), and purified gymnemagenol were studied against the early fourth-instar larvae of *Anopheles subpictus* Grassi, and *Culex quinquefasciatus Say* (Diptera: Culicidae), being malaria and filariasis vectors. The compounds were effective [101].

4.5 Final considerations

Our ancestors used syrups, macerations, teas, and other medicinal plant preparations for the treatment and cure of their diseases. With the development of technologies in medicinal chemistry, more precise studies have been performed on the bioactive compounds isolated from these natural organisms: plants, fungi, or marine organisms.

Natural products are a rich source of compounds in drug discovery. We find many scientific studies using natural products when searching for new chemotherapeutic agents.

In this context, many studies are continuing in the search for new drugs against the neglected diseases; those tropical infections that affect the poorest people on our planet, on the African, American, and Asian continents. The drugs currently used to treat these infections often have little effect, cause serious collateral problems, and

enable the emergence of resistant strains. Despite this situation, there is little real interest by pharmaceutical companies to invest in the needed research for new drugs. Many scientific studies are carried out using natural products such as enzyme inhibitors. The target (parasite) enzyme is carefully selected, and the search for natural compounds that interact as perfectly as possible commences. Molecular docking (the theoretical tool), assesses the ligand-enzyme complex. In this chapter, we have discussed the scenario involving research for new drugs using natural products against neglected diseases; the basic concepts have been introduced; the major neglected diseases described, and some theoretical examples of natural compounds were reported.

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5 Natural product hybrid compounds as drug leads

Abstract: Natural products are important source for the development of newer drugs, especially for the treatment of infections and cancer. The number of natural products is limited, combinations of parts of different natural products can be an interesting approach to develop vast library of compounds. This new approach seems to be very promising in the development of leads for medicinal applications, as the biological activity of several new hybrids exceeds that of the parent compounds. The advantage of this concept over a combinatorial chemistry approach is the high diversity and the inherent biological activity of the hybrids.

5.1 Introduction

In the last two decades, unfortunately not many newer drugs came onto the market and it is one of the most urgent needs in drug development to accelerate the process of identification of lead compounds that can be translated into drugs. Newer lead molecules are required particularly urgently in the area of antimicrobials and neglected diseases. Nature has been engineering an extensive number of substances for millions and millions of years and approximately 40 % of the marketed medicines that have been approved in the last few years are either natural products or congeners/derivatives and their analogs [1]. Natural products have played a vital and critical role especially in cancer and anti-infective therapeutics and the contribution of natural products is estimated to exceed 60 % [2].

The development of combinatorial chemistry in the mid-1990s resulted in the synthesis and development of millions of new chemical entities in the short term. The evaluation of these compounds by the use of high-throughput screening was done but unfortunately the results have not been very encouraging, which may be due to the lack of structural diversity in these compounds [3]. This led to a change in the overall strategy of drug development and scientists started looking again toward nature to get the lead molecules. In nature the compounds which have been biosynthesized with different biosynthetic pathways have shown excellent biological activities (e.g. in the structure of vitamin E, the terpenoid phytyl chain binds with the cell membrane and the phenol moiety derived from shikimic acid forms a radical trap). The diversity and stereochemistry generated in such compounds are difficult to match with synthetic compounds [4]. Keeping this in mind led to the development of a number of natural hybrid compounds by conjugating two or more natural products/synthetic compounds to form a hybrid in which the multiple functional moieties act sequentially on single or multiple receptors. These hybrids can be designed and synthesized either by traditional organic procedures or by conjugation of the corresponding biosynthetic

tools, namely by a transfer of gene clusters into a new host, which will then produce new "non-natural" natural products [5]. In recent years, a number of advancements have been made in natural hybrid compound chemistry and various strategies have been adopted to generate lead molecules out of these compounds. There are various classes of natural hybrid compounds that can be divided into following categories:

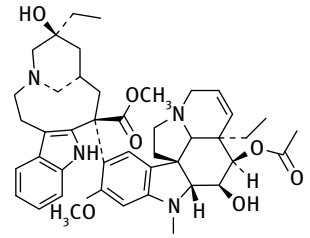
1. naturally occurring hybrids/conjugates natural products
2. synthetic hybrids/conjugates of natural products

5.2 Naturally occurring hybrids/conjugates of natural products

In nature the biosynthesis of dimeric natural products is a common character and the dimeric hybrids exhibit a vast array of biology compared to that of the monomer. Some of the best known pharmacologically important examples are the indole dimeric alkaloids vinblastine **(1)** and vincristine **(2)** from *Vinca roseus*, which are both used clinically for various types of cancers [6]. Similarly, the bisbenzylisoquinoline alkaloids like tubocurarine **(3)**, which has been extensively used for ages as a muscle relaxant, is another naturally occurring hybrid molecule, which has now been substituted by amino steroid derivatives like vecuronium bromide **(4)** and rocuronium bromide **(5)**. The two important biaryl biscarbazole alkaloids **6** and **7** were isolated from *Clausena and Murraya* genera of Rutaceae family [7]. The interesting characteristic of these alkaloids is a stereogenic axis and some molecules of this category are active against *Leischmania donovani* and also exhibit a modest fungicidal activity.

The cephalostatins are another dimeric natural product hybrid with marked pharmacological activity, but at the same time they have completely different properties to its monomer. This compound contains a pyrazine moiety, which is attached to a highly oxygenated steroid moiety on each side. Cephalostatin 1 **(8)** is the most potent compound of its class and was isolated from the marine worm *Cephalodiscus gilchristi*. The NCI 60 human cancer cell lines screening revealed that it is a highly potent compound with GI_{50} value of about 2.20 nm [8].

Another compound of this class of natural hybrids is thiomarinol **(9)** with marked antimicrobial properties. The compound **9** was isolated from the marine bacterium *Alteromonas rava* sp. nov. SANK 73390 and was observed to be a hybrid of the pseudomonic acid C analog **(10)** and holothin **(11)**. Interestingly, the antibacterial profile of **11** showed characteristics of both parent molecules and was active against Gram-positive and Gram-negative bacteria (e.g. multidrug-resistant *Staphylococcus aurea* strains), and its effects were greater than those of either parent molecules [9].



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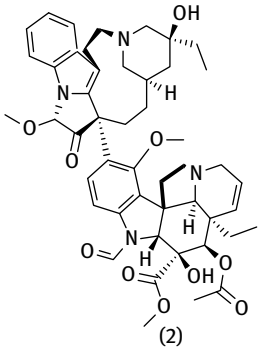

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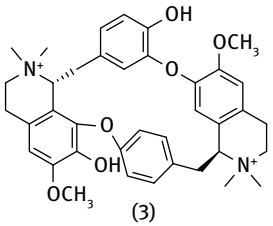

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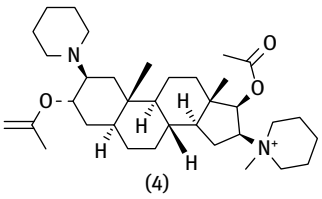

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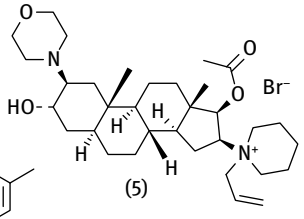

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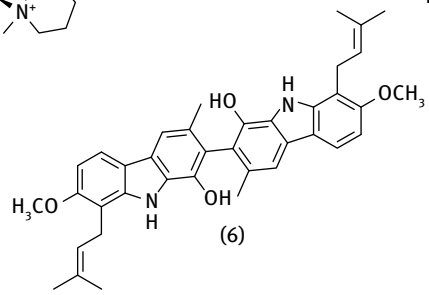

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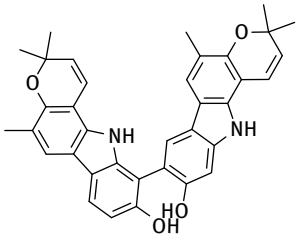

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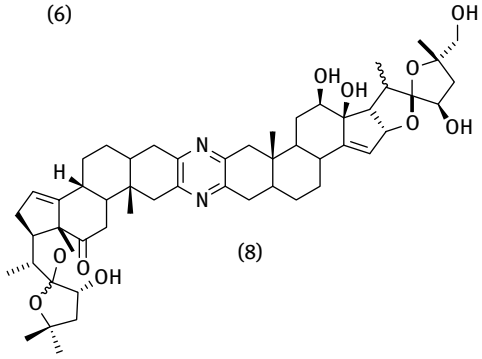

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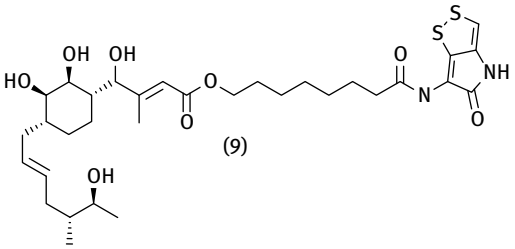

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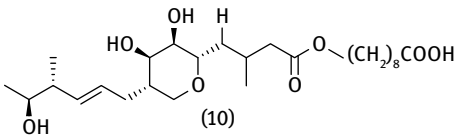

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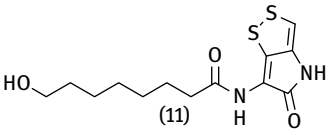

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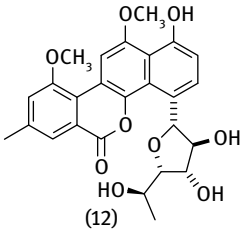

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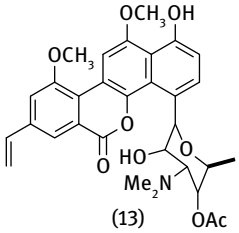

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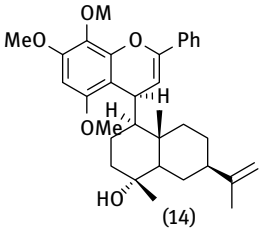

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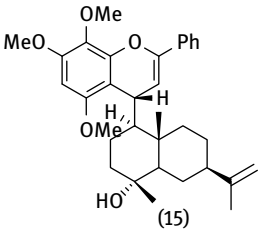

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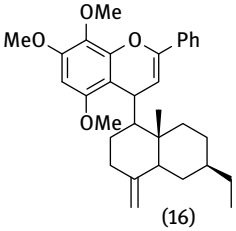

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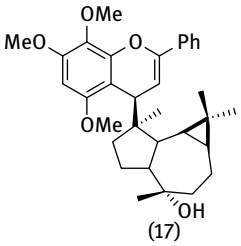

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There are a number of examples of natural hybrid compounds which originate from different biosynthetic pathways. In nature thousands of O- and N-glycosidic natural products exists, like saponines, flavones, ribonucleosides and anthracyclic glycosides, which contain a carbohydrate and another natural compound (the aglycone) and can therefore also be considered as natural product hybrids. Many C-glycosidic antineoplastic antibiotics are hybrids of carbohydrates and tetracyclines. A lot of work has been done on these classes of compounds and generally these compounds fall under the anthracycline class of natural products [10]. Gilvocarcin **(12)** and ravidomycin **(13)** represent a new class of aryl C-glycoside antitumor antibiotics that have a benzonaphthopyrone tetracycle in common and differ in the carbohydrate at C4 (a fucose unit in gilvocarcin and an amino sugar in ravidomycin) [11]. It has been observed that the amino sugar analogs are pharmacologically more efficacious. In Asia a number of plants contain secondary metabolites that are nature hybrids of fla-

vonoid and sesquiterpene moieties [12]. The fissistigmatins A–D **(14–17)** were isolated from a creeper grown in North Vietnam known as *Fissistigma bracteolatum* Chatt. (Annonaceae). These compounds are a hybrid of flavonoid and a sesquiterpene and are used in folk medicine to treat wound bleeding and as an anti-infective agent [13].

5.3 Synthetic hybrids of whole natural products

In the drug discovery process, the synthetic hybrids of natural products have been playing a vital role in the last few decades. There are a number of lead compounds that have been generated by using this strategy. The design and development of different molecular structures from natural or unnatural origin to transform or augment different moieties or to generate a compound with bi-functional feature with new properties is an interesting approach [14].

5.3.1 Coumarin-containing hybrid compounds

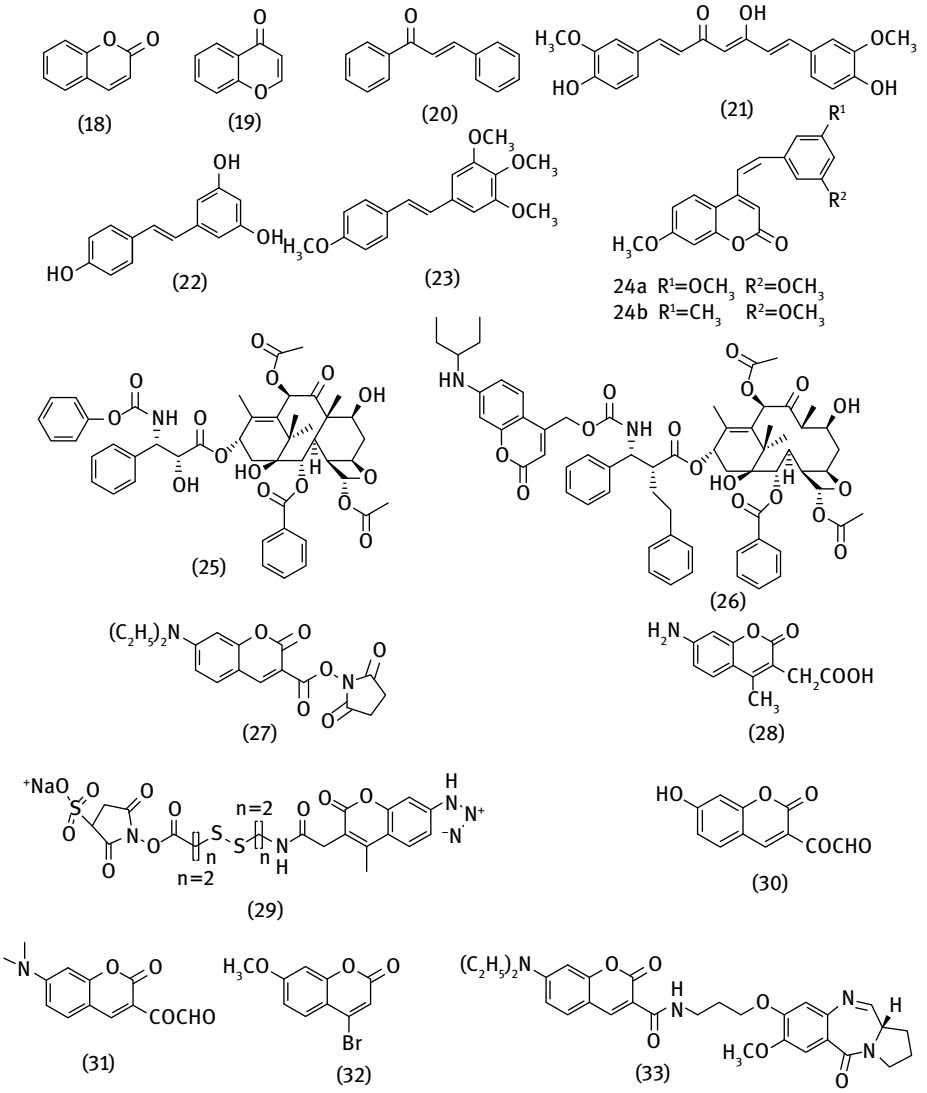
The α,β-unsaturated carbonyl moiety-containing molecules are one of the widest classes of naturally occurring compounds which includes coumarin (benz-α-pirone) **18**, flavones **19**, chalcone **20**, and curcumin **21**. These compounds showed different and diverse biological activities with low toxicity. The mechanism of action of these classes of compounds is because of their binding with thiol groups of enzymes via Michael addition at ketovinyl double bond [15]. The structure-activity relationship studies revealed that electron withdrawing moieties is favorable because it will increase the electrophilicity of the C-β and therefore facilitate the nucleophilic attack of the cellular thiols groups whereas the opposite is true for the electron donating moieties. There are a number of bioactive naturally occurring compounds with α,β-unsaturated carbonyl moiety but coumarin (1,2-benzopirone) is one of the most vital and studied class of compounds in this category. In recent times a number of natural and synthetic coumarins have been studied and were observed to show a wide array of biological activities like anti-HIV, anticoagulant, antibacterial, antioxidant, anti-inflammatory, and fluorescent labeling [16–23].

In recent years, resveratrol (3,5,4'-trihydroxy-trans-stilbene) **22** and 3,4,5,4'-tetramethoxystilbene (DMU-212, **23**) have been widely studied for various biological properties. To screen the structure-activity relationship (SAR) a number of hybrid compounds have been designed and synthesized. In one of the studies, Belluti et al. incorporated substituted *trans*-vinylbenzene moiety on coumarin backbone and evaluated for anticancer activity against lung carcinoma H460, squamous cell carcinoma A431 and melanoma JR8 [24]. The 3,5-dimethoxy- **24a** and 3,5-dimethylstilbin **24b** substitution on 7-methoxycoumarin scaffolds at C-4 position was found to exert optimum inhibitory action against H460 (0.45 ± 0.09 μM), A431 (3.44 μM) and

JR8 (3.2, 3.5 μM). In a similar study, a series of coumarin-chalcone hybrids were synthesized and screened for their cytotoxic potential against KB (oral squamous cell carcinoma), C33A (cervical carcinoma), MCF-7 (breast adenocarcinoma), A549 (lung) and one normal human NIH3T3 (mouse embryo fibroblast) [184]. The results showed that chalcone-coumarin hybrids with electron withdrawing group **21** were more efficacious in comparison to parent coumarin [25].

The aqueous solubility (log W) is one of the most important factors in the bioavailability and development of successful drug candidates [191]. Paclitaxel **(25)** is a blockbuster mitotic inhibitor and extensively used in the chemotherapy for lung, ovarian, breast, head, and neck cancers, and advanced forms of Kaposi's sarcoma [26]. Paclitaxel is highly hydrophobic, therefore it has to be administered with a non-aqueous vehicle containing detergent like Cremophor EL [27]. The use of surfactants sometimes leads to serious allergic reactions and administration is highly inconvenient to the patients. At the same time, paclitaxel also has serious side effects such as unusual bruising or bleeding, pain/redness/swelling at the injection site, fever, chills, cough, sore throat, difficulty in swallowing, dizziness, shortness of breath, severe exhaustion, skin rash, facial flushing, female infertility by ovarian damage [28]. Therefore, a number of structural modifications have been done in paclitaxel to reduce its toxicity. Recently, photoliable 7-N,N-diethylamino-4-hydroxymethyl coumarin (DECM) hybrid of paclitaxel **(26)** was synthesized to increase water solubility along with enhancement of target-specific delivery [29].

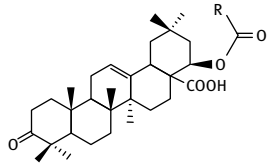
Fluorescent labeling is extensively used as target probes for detection of a specific target via fluorescence microscopy, flow cytometer or some other fluorescence reading instrument [30]. A number of fluorescent dyes like fluorescein, rhodamine, Alexa Fluors, Dylight fluors, ATTO dyes (labeling of DNA, RNA and protein), BODIPY dyes (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene), and 6-FAM phosphoramidite are used for this purpose. The structural resemblance of coumarin with fluorescent dyes imparts significant fluorescent properties to them. There are a number of examples such as 7-diethylaminocoumarin succinimidyl ester **(27)**, 7-amino-4-methyl coumarin-3-acetic acid **(28)** [31], sulfosuccinimidyl-2(7-azido-4-methycoumarin-3-acetamido)-ethyl-1,3′-dithiopropionate **(29)** [32], 7-hydroxycoumarinyl-3-glyoxal **(30)**, 7-(dimethylamino)coumarinyl-3-glyoxal **(31)** [33], and 4-bromometyl-7-methoxy-coumarin **(32)** (BrMMC) [34] which show wide applicability of coumarin and similar moieties in the biological fields. It has been found recently that 7-diethylaminocoumarin can be used as supporter in the nuclear penetration on conjugation with sequence-selective DNA-targeting agents pyrrolo [2, 1-c] [1, 4] benzodiazepine (PBD) **(33)** via varying length of spacer [35]. These hybrid compounds represent a new array of diagnostic compounds with different physical and chemical properties.



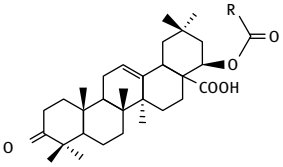
5.3.2 Triterpenoids and diterpenoid-containing hybrid compounds

Triterpenoids are members of a larger family of related structures called cyclosqualenoids. Triterpenoids are synthesized by many plants by the cyclization of squalene are extensively used in Asian traditional systems of treatment [36]. Triterpenoids are highly biological important compounds and the triterpenoid skeleton is unique among the natural products. The triterpenoids have been classified in to squalenes, lanostanes, fusidanes, dammaranes, euphanes, lupanes, oleananes,

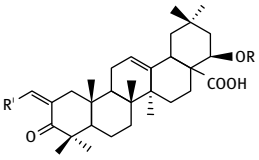
ursanes, hopanes, tetranortriterpenoids, quassinoids, and others [37]. Among each class of triterpenoids there is a remarkable array of structural diversity and a wide range of pharmacological activity. There are thousands of papers on the structure-active relationship of various classes of triterpenoids and hybrid compounds. These hybrid compounds containing triterpenoid as one the active moieties have given a number of lead molecules. Lantadenes **(34–37)** are one such triterpenoid isolated from weed *Lantana camara* L., which has been extensively studied and a number of its hybrid compounds have been developed in the last decade [38–49]. Some of these hybrid compounds have been taken up the National Cancer Institute USA and have been observed to be potent lead molecules for different types of cancers with potent inhibition of NF-κB and AP-1. Recently, functionalities such as cinnamoyl and vanillyl have shown significant NF-κB and COX inhibition activities. The conjugation



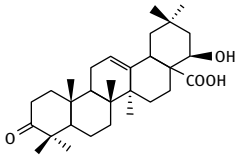
(34) R = $-(CH_2)=CH(CH_3)$
(35) R = $-(CH)=C(CH_3)_2$



(36) R = $-CH-(CH_3)-C_2H_5$
(37) R = $-C(CH_3)=CHCH_3$



(38) R' = $C_6H_5CH=CH$
R = $-COC(CH_3)=CHCH_3$
(39) R' = $C_6H_5CH=CH$
R = $COCH=C(CH_3)_2$
(40) R' = $C_6H_5CH=C(CH_3)$
R = $-COC(CH_3)=CHCH_3$
(41) R' = $C_6H_5CH=C(CH_3)$
R = $COCH=C(CH_3)_2$



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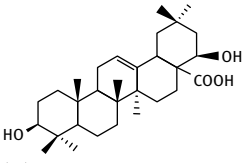

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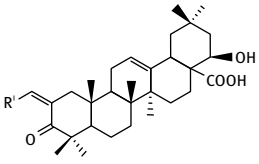
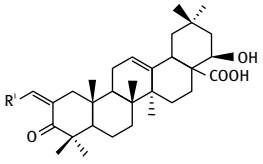
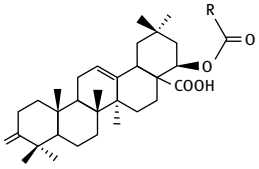


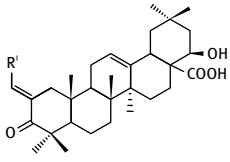
(44) R' = $C_6H_5CH=C(CH_3)$
(45) R' = $3,4-(OCH_3)_2C_6H_3$
(46) R' = $C_6H_5CH=C(CH_3)$



(47) R' = $C_6H_5CH=C(CH_3)$
(48) R' = $3,4-(OCH_3)_2C_6H_3$
(49) R' = $C_6H_5CH=CH$



(50) R = $-(CH_2)=CH(CH_3)$
(51) R = $-CH=C(CH_3)_2$

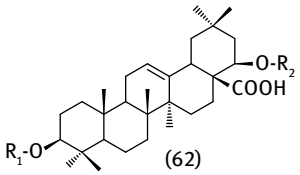


(52) R' = C_6H_5
(53) R' = $4(OCH_3)C_6H_4$
(54) R' = $4(CH_3)C_6H_4$
(55) R' = $4(Cl)C_6H_4$
(56) R' = $3(OCH_3)4(OH)C_6H_3$
(57) R' = $C_6H_5CH=CH$
(58) R' = C_5H_4N
(59) R' = $3(NO_2)C_6H_4$
(60) R' = $4N(CH_3)_2C_6H_4$
(61) R' = $4FC_6H_4$

of two different molecular entities (lantadenes and cinnamoyl/vanillyl functionality) having the same mechanism of action is an interesting approach to designing molecules that can either enhance or modulate the desired properties of parent compounds or lead to new properties. An appealing feature of this hybrid approach is that it may provide hybrids with possibilities for generating a diverse array of new compounds which may synergize each other's biological properties. In this direction, Navin et al. integrated cinnamoyl and 3-*O*-methyl-vanillyl functionalities over oleanane framework of lantadenes **(38–51)**. These hybrids of lantadenes showed marked selective toxicity against cancer cells and inhibited the activation of NF-κB and Akt signaling in A549 cells [50]. Similarly, a number of lantadene hybrid compounds have also been developed by introducing 3-arylidene moiety in the oleanane framework of lantadene **(52–61)**. The GI_{50} value of compound 3 and 12 was < 5 μM against more than 80 % cancer cell lines. The mean graph midpoint (MG_MID) value of compound 3 (MG_MID _5.69) was higher than standard cisplatin (MG_MID _5.66) while comparable in case of compound 12 (MG_MID _5.52). In the NCI's COMPARE analysis it was observed that these compounds were in a significant number of correlations with activity patterns of mechanistic set of compounds (PCC ≥ 0.60). The results indicated that these compounds can be used as template for future development to obtain more potent antitumor agents [51].

In the last few years nonsteroidal anti-inflammatory drugs (NSAIDs) have been widely studied for antineoplastic and chemopreventive activity against various different types of cancers. NSAIDs act via the depression of prostaglandin synthesis through the inhibition of COX-2, which results in the suppression of proliferation, and the possible mechanism is the increase in the apoptosis in cancer cells. To enhance the physicochemical properties or efficacy and to improve the therapeutic index, an interesting prodrug approach is used nowadays. The two molecular entities, each having its own distinct mechanism of action, can be conjugated into a single novel chemical entity [47]. In this direction, Sharad et al. designed the prodrugs of different NSAIDs **(62–72)** with the pentacyclic triterpenoid and NF-κB inhibitor 3β,22β-dihydroxy-olean-12-en-28-oic acid **(43)**. The lead prodrug **(72)** showed a marked cytotoxicity against lung adenocarcinoma cells A549 and was found to be 50-fold more active than cisplatin. The lead prodrug exerted its effect by the dual inhibition of NF-κB and COX-2 [52].

Oleanolic acid **(73)** and ursolic acid **(74)** are two very important triterpenoids, which are present in large number of plants [53]. Oleanolic acid and ursolic acid have been widely studied and showed a wide array of biological activities. These compounds also showed moderate anticancer and anti-inflammatory activity and this motivated Gordon Gribble's research group in the USA to take up structural modification of these compounds. They decided to measure their ability to block the cellular synthesis of inducible nitric oxide synthase (iNOS); an enzyme that plays a key role in the process of inflammation, as quantitative assays for evaluating the efficacy of new molecules. The synthetic plan which this group used was to modify the three "active" portions of **73** and **74**, namely, the C-3 hydroxy, the ring C double bond (C-12-C-13),



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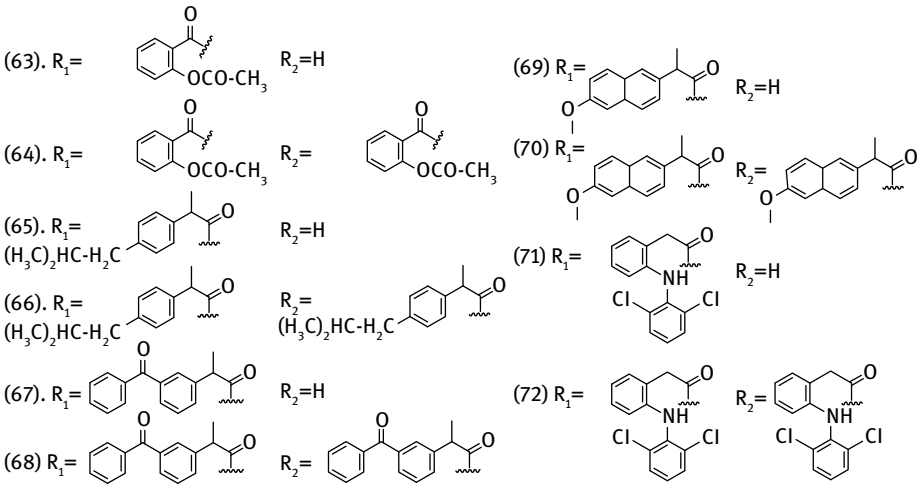
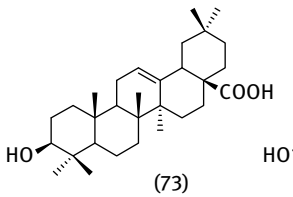


and the C-28 carboxylic acid [54]. Similarly, a number of ring-cleavage reactions were designed for the synthesis of analogs for screening.

They initially synthesized 70 oleanolic and ursolic acid congeners which were screened. The first "hit" **(75)** molecule in the iNOS assay has A-ring enone at C-3 position [55]. This synthetic triterpenoid showed significant activity in the iNOS assay with IC_{50} 6.0 μM whereas the corresponding ursolic acids showed IC_{50} 17.6 μM. The results prompted them to convert the C-ring to its corresponding enone with ring-A substitution at C-2 with an electron withdrawing group which would further activate ring A to a conjugate with addition reactions (e.g., a thio- or aza-Michael reaction). This synthetic triterpenoid design led to the lead molecule "CDDO" (2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid **(76)**, with a C-2 cyano group. The lead molecule was 400,000 times more potent than oleanolic acid in the iNOS assay. The CDDO showed marked anti-inflammatory and anticancer activity in various preclinical studies [56]. The same group further synthesized CDDO methyl ester **(77)** and CDDO imidazole **(78)** as more active analogs of CDDO. The impressive *in vivo* pharmacological profile makes it clear that clinical use in patients may be possible [57]. Although clinical investigations still are in their earliest stages, yet it has already been observed that CDDO methyl ester (bardoxolone methyl) has yielded beneficial results in patients with advanced chronic kidney disease (CKD), related to diabetic nephropathy. Remarkable results have already been reported in phase 2 trials, and a large phase 3 study is planned [58].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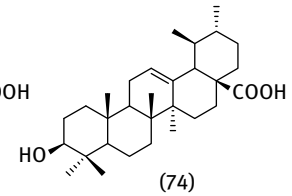
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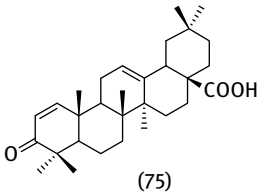
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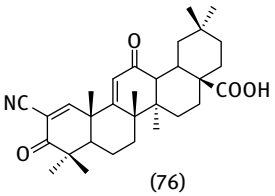
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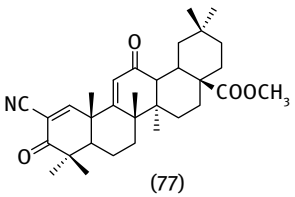
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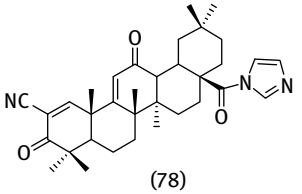
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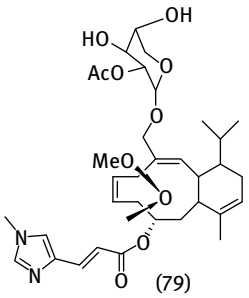
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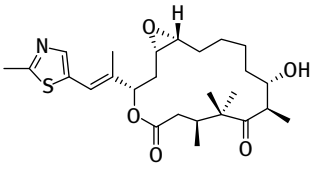
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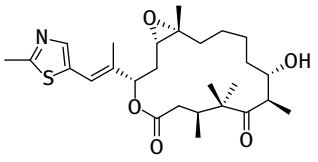
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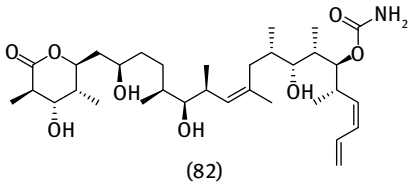
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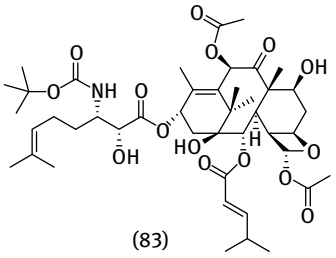
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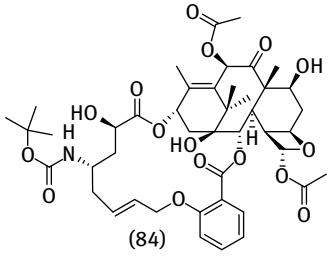
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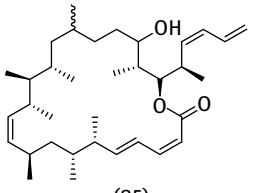
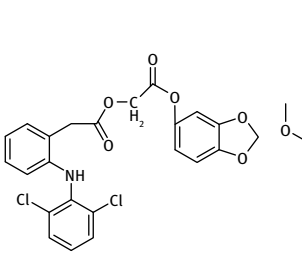

(85)

There are number of naturally occurring molecules like taxol **(25)**, eleutherobin **(79)**, epothilone A **(80)**, epothilone B **(81)**, discodermolide **(82)** and nonataxel **(83)** (a nonaromatic analog of paclitaxel), that have the ability to induce the stabilization of microtubules, which leads to mitotic arrest. Taxol is a blockbuster drug molecule which is used in the treatment of various types of cancers [59]. The structural features of taxol are quite different from other naturally occurring microtubule stabilizing molecules, but they have a common mechanism of action. These compounds have an identical pharmacophore that interacts with the receptor and on the basis of this concept a new hybrid molecule SB-TE-1120 **(84)** was developed which demonstrated cytotoxic with IC_{50} value of 0.39 μm against the human breast cancer cell line MDA-435/LCC6-WT and tubulin-binding activity [60]. On the basis of SB-TE-1120 a new generation of tubulin-directed anticancer agents was developed. In another study the conjugate-containing parts of discodermolide **(82)** and dictyostatin-1 **(85)** were synthesized as another discodermolide-like macrocycle. The hybrid, which contains the highly complicated and complex lower part of dictyostatin-1 **(85)**, was more potent (GI_{50} = 1.0–1.4 μm). The hybrid compound displaced [3H] paclitaxel stoichiometrically interacts to microtubules at about one third of the potency of discodermolide [61].

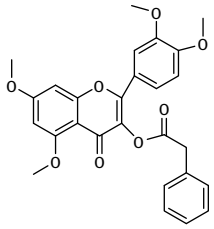
5.3.3 Natural antioxidant-containing hybrid compounds

The origin of peptic ulcer and gastrotoxicity of NSAIDs is associated with the generation of free radicals; and antioxidants are known to scavenge the free radicals and reactive oxygen species. Naturally occurring alcoholic and phenolic compounds are well known for their antioxidant, anti-inflammatory, and analgesic properties [62]. It has been observed that conjugation of these alcoholic and phenolic compounds with traditional NSAIDs not only enhances the potency of NSAIDs but also imparts gastrosparing properties. In recent years a number of natural antioxidant–NSAID hybrids have been developed. Menon et al. designed and synthesized diclofenac prodrugs with a number of antioxidants like guaiacol, eugenol, thymol, vanillin, sesamol, umbelliferone, and menthol using glycolic acid as a spacer. The results of *in vivo* anti-inflammatory and analgesic assays showed that diclofenac–sesamol ester **(86)** was the most potent among all the prodrugs and also exhibited reduced ulcerogenicity in comparison to diclofenac [63]. The potent NSAID fenbufen has active metabolite biphenylacetic acid and is believed to be more efficacious than the parent drug. Madhukar et al. conjugated two anti-inflammatory moieties, that is, 4-biphenylacetic acid and quercetin tetramethyl ether as gastrosparing NSAID, employing a hybrid synthesis approach. This approach resulted in the development of hybrid compound **(87)** that was stable against chemical hydrolysis, while it hydrolyzed rapidly in plasma to release the parent drug moieties [64]. Moreover, the lead hybrid compound exhibited significant analgesic and anti-inflammatory activity and was also less gastrotoxic than the parent drug. By using a similar approach, Sawraj et al. designed and syn-

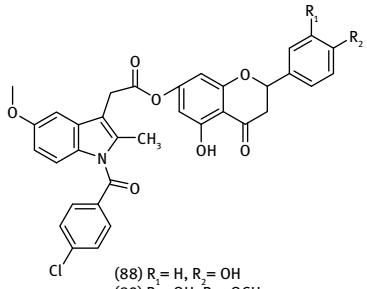
thesized hybrids of indomethacin with naringenin and hespertin. The synthesized hybrids not only retained the anti-inflammatory activity but also showed decreased ulcerogenicity than indomethacin. The synthesized indomethacin–naringenin **(88)** and indomethacin–hespertin **(89)** hybrids displayed *in vivo* anti-inflammatory and analgesic activities better than that of indomethacin [65]. Various hybrids of ibuprofen with menthol, thymol, and eugenol were designed and synthesized by Redasani et al. All the synthesized hybrids exhibited anti-inflammatory activity better than that of ibuprofen with improved ulcerogenic profile. The ibuprofen–menthol **(90)** conjugate evolved as the lead anti-inflammatory candidate, which could be considered for further development as a gastrosparing NSAID [66]. Similarly, Chandiran et al. also designed and synthesized (+)-*S*-ibuprofen conjugates with various antioxidant (thymol, guaiacol, eugenol, and menthol) hybrids with and without a spacer (CH_2COO). All the synthesized (+)-*S*-ibuprofen–antioxidant conjugates not bearing spacer between the NSAID scaffold and antioxidant moiety showed *in vivo* anti-inflammatory activity superior to that of ibuprofen, whereas their analgesic activity was also observed to be similar to that of (+)-*S*-ibuprofen. The ibuprofen–eugenol ester **(91)** demonstrated anti-inflammatory and analgesic activity higher than the other hybrids



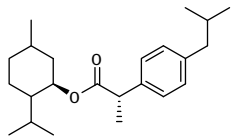
(86)



(87)



(88) R_1 = H, R_2 = OH
(89) R_1 = OH, R_2 = OCH_3



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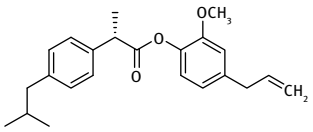

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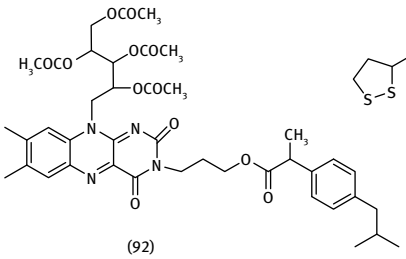

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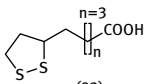

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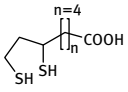

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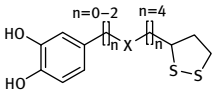


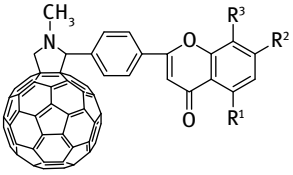
(95) X = 1,2,4-oxadiazole
(96) X = 1,3,4-oxadiazole
(97) X = 1,2,3-triazole
(98) X = Tetrazole
(99) X = Thiazole

of this series. All the conjugates also exhibited improved gastric tolerability with gastrotoxicity lower than ibuprofen [67]. Whereas the conjugates that possessed a spacer showed less activity. Riboflavin or vitamin B_2 acts as a micronutrient for normal and cancer cells. Though, rapidly dividing cancer cells need higher amounts of nutrients than normal cells. A covalent linking of micronutrients with the anticancer agents is one of the new paradigms currently being practiced to enhance the internalization of anticancer agents into the cancerous cells. Banekovich and associates synthesized dexibuprofen derivatives covalently linked to tetraacetylated riboflavin by means of alkylene spacers of changeable length [68]. Biological evaluation studies revealed that test compounds were significantly active against MCF-7 human breast adenocarcinoma and HT-29 human colon adenocarcinoma cell lines with IC_{50} values in the range of 8–15 μmol. Compound **(92)** was found to be the most cytotoxic with IC_{50}s of 7.8 and 9.3 μmol against MCF-7 and HT-29 cells, respectively.

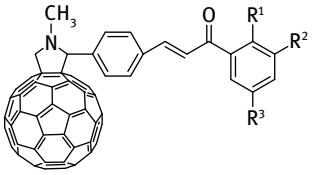
The 1, 2-dithiolane-3-pentanoic acid **(93)**, also known as α-lipoic acid (α-LA**)** is a naturally occurring antioxidant existing as R- and S-enantiomeric forms and has the ability to scavenge reactive oxygen species (ROS) and regenerate or recycle endogenous antioxidants. The α-LA is quickly taken up and reduced in cells and tissues to dihydrolipoic acid **(94)**, and it exhibits oxidative protection in both intracellular and extracellular environments. These compounds have also been involved in the regeneration of other antioxidants like vitamin C and vitamin E via redox coupling and increase intracellular glutathione levels [69, 70]. The thiol functionality of glutathione is the major contributor to oxidative defense in the brain. It has been observed that alpha-lipoate may be effective in numerous neurodegenerative disorders. Taking these studies into consideration, a number of hybrid compounds containing 1, 2-dithiolone moiety have been studied. Koufaki et al. designed hybrids with 1, 2-dithiolone scaffold by conjugating α-LA with catechol and these hybrids were evaluated on glutamate-challenged hippocampal HT22 cells [71]. In the process of designing potential neuroprotective agents, it has been observed that neuroprotective potential was increased on bioisosteric replacement of the amide group with heteroaromatic rings such as triazole, 1, 2, 4-oxadiazole, 1, 3, 4-oxadiazole, tetrazole or thiazole **(95–99)** in comparison to parent α-LA [72].

5.3.4 Fullerene-containing hybrid compounds

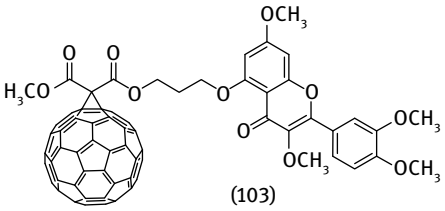
Fullerene, also well known as buckminsterfullerene, is composed of C_{60} carbon and its discovery has attracted a lot of interest because of its unusual physicochemical properties and reactivity [73]. C_{60} and its derivatives have potent ability to scavenge free radicals and this makes them suitable drug candidates for various oxidative stress induced disorders, like cardiovascular and neurodegenerative diseases [74, 75]. Many hybrid compounds were synthesized by conjugation of fullerenes with a number of antioxidants such as flavonoids and quercetin **(100–103)**. In another



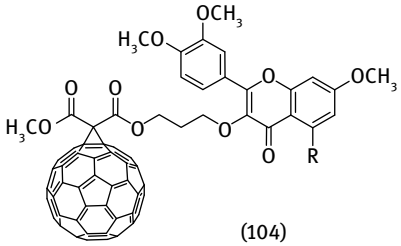
	R^1	R^2	R^3
(100a)	H	H	H
(100b)	Bn	H	H
(100c)	H	OBn	OBn



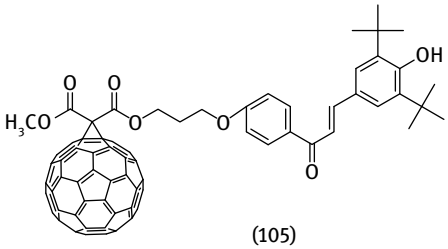
	R^1	R^2	R^3
(101a)	OH	OH	OH
(102b)	OBn	OBn	OBn



(103)



(104)



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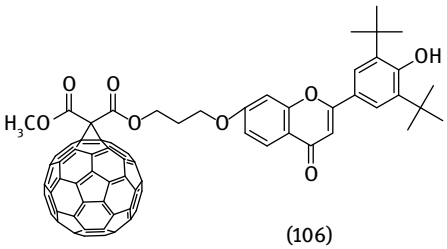

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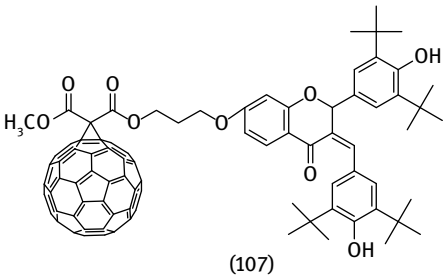
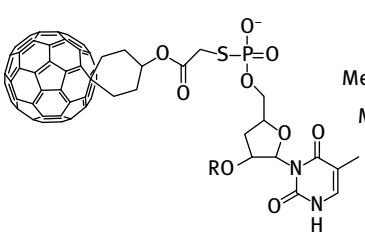
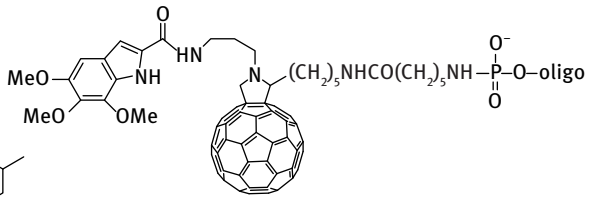


(107)



(108) R = 3'-TCTACGATGTGTTAATCCG AACATGTATAACAGCAATC



(109) oligo = 5'-TTT-TC*T-TTT-C*C*C*-C*C*C*-T-3'
C* = Methylated cytosine

report Enes et al. conjugated 3, 5-di-tert-butyl-4-hydroxyphenyl groups (BHT) with C_{60}-flavonoid conjugate **(104–107)** with synergistic free radical scavenging ability. To improve pharmacokinetic and pharmacodynamic properties, many hybrids of C60 with nucleic acids, proteins and carbohydrates were prepared. In one such study, Rubin et al. designed synthesis of C60-linked deoxynucleotide **(108)** and it interacted with light and oxygen to damage only guanosines in DNA which are closest to C60 [76]. To achieve sequence selectivity Prato et al. synthesized fullerene hybrid **(109)** containing a trimethoxyindole moiety reminiscent of the minor groove binder duocarmycin and an oligonuclotide [77].

5.4 Conclusion

The science of developing hybrid systems for generating molecular diversity through either integration or covalent conjugation of two or more diverse chemical entities has unparalleled potential. The possibility of synthesizing diverse and multifunctional entities has attracted scientists to generate new chemical entities and natural products leads emanating from them will be the bedrock of these efforts. Nature has the ability to generate extraordinarily diverse structures with highly specific stereochemistry that makes them biologically active. Modulation of structure and generation of hybrid compounds of natural products is an interesting approach to yield lead molecules for various diseases. Many reports show the efforts in creating hybrid systems that have focused on various developments of lead molecules for different arrays of diseases and with the availability of the three-dimensional structures of many receptors and access to genome sequences, development of new hybrid compounds for these new targets is likely to receive increasing attention in the next few decades.

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Komarla Kumarachari Rajasekhar*

6 Drug metabolism

Abstract: Drug metabolism or biotransformations are the chemical reactions that are responsible for the conversion of drugs into metabolites within the body before and after they have reached their sites of action. In the drug design and discovery process, drug metabolism plays a major role and determines the fate of the prospective drugs. Drug metabolism has an important role in the determination of the pharmacokinetic (PK) parameters like oral bioavailability, clearance and the half-life of the entity within the cell. Drug metabolism is very essential in the toxicity studies too. The persistence of the compounds in the systemic circulation for a long period causes toxicity and the nature of the metabolites and the reaction of the metabolites within the body must be studied thoroughly before the compounds progress to the next stage of screening in the drug discovery process; otherwise, the drugs would be rejected during the screening process. In this way, it is seen that drug metabolic studies form an integral part in drug discovery. Drug metabolism, as a discipline participating in a drug discovery team, can play an important role in identifying factors underlying the problems, facilitate the optimal selection of compounds for further development, provide information on metabolites for possible improvement in drug design, and contribute to the identification of novel, safer and better drugs. The subject of drug metabolism is dealt with in greater detail in this chapter.

6.1 Introduction

Metabolism by the host organism is one of the most important determinants of the pharmacokinetic profile of a drug. High metabolic liability usually leads to poor bioavailability and high clearance. Formation of active or toxic metabolites will have an impact on the pharmacological and toxicological outcomes. There is also potential for drug-drug interactions with coadministered drugs due to inhibition and/or induction of drug metabolism pathways. Hence, optimization of the metabolic liability and drug-drug interaction potential of the new chemical entities are some of the most important steps during the drug discovery process.

Metabolism plays an important role in drug elimination. Most of the organic compounds are lipophilic and traverse through the lipoprotein membranes of lumen walls of GIT where they undergo absorption and enter into the bloodstream from where they will go to the TARGET by passive diffusion through other membranes to exert pharmacological action. The excess or unreacted compounds are reabsorbed by renal tubules but are not excreted to a substantial extent in the urine and get deposited in the body leading to undesired actions [1].

Hence to reduce toxicity and to increase excretion, lipophilic drugs are metabolized to polar compounds. Not all metabolic routes are detoxications. Some may result in reactive toxic metabolites. Therefore, metabolism may lead to activation or inactivation of drug molecules [2].

6.1.1 General pathways of drug metabolism

Drug metabolism mainly takes place in the liver, where along with metabolism of the drugs, the excretion and thus the clearance of the drugs takes place. Drug metabolism mainly takes place in two phases – Phase I and Phase II. The phase I reactions mainly result in functionalization of the drugs whereas the phase II reactions result in the increased polarity of the drugs due to the conjugation of a polar group on the drug, thereby increasing their solubility which helps in their excretion from the body. The phase I reactions mainly involve the action of cytochrome P450 enzymes, flavin monoxygenases, etc., while the phase II reactions mainly involve the action of UDP glucuronyl transferases (UGTs, sulfotransferases, etc.) [3].

6.2 Phase I metabolism

Importance: Introduction of a polar functional group such as hydroxyl(–OH), carboxylic(–COOH), amino($–NH_2$), thiol(-SH) etc. into the DRUG/XENOBIOTIC molecule. Examples include

A. Oxidative reactions
    1. oxidation of aromatic moieties. E.g. diazepam, phenytoin, phenobarbitol etc.
    2. oxidation of Olefins. E.g. carbamazepine, secobarbitol
    3. oxidation of benzylic (tolbutamide), allylic(tetrahydrocannabinol, THC) and carbons at α or β positions to carbonyl group (benzodiazepines)
    4. oxidation of aliphatic and alicyclic carbon atoms E.g. Valproic acid, acetohexamide, phencyclidine etc.
    5. oxidations involving carbon-heteroatom systems like
        a. C-N systems(aliphatic and aromatic amines) N-dealkylation, oxidative deamination, N-oxide formation, N-hydroxylation
        b. C-O systems: O-dealkylation
        c. C-S systems: S-dealkylation, S-oxidation and desulfuration
    6. oxidation of alcohols and aldehydes
    7. miscellaneous oxidative reactions
B. Reductive reactions
    1. reduction of aldehydes and ketones
    2. reduction of nitro and azo compounds
    3. miscellaneous reduction reactions

C. Hydrolytic reactions
    1. hydrolysis of esters and amides
    2. miscellaneous hydrolytic reactions

6.2.1 Introduction of a polar functional group

This can be achieved by:
A. direct introduction of functional group
    1. aromatic hydroxylation
    2. aliphatic hydroxylation
B. modifying or unmasking existing functionalities
    1. reduction of aldehydes to alcohols
    2. oxidation of alcohols to carboxylic group
    3. reduction of azo and nitro compounds to give NH_2 moieties
    4. N-, O-, S- dealkylation to give $-NH_2$, OH and SH groups

Phase I metabolites are not sufficiently hydrophilic or inactive. Phase I reactions provide a functional group or handle to the molecule that can undergo subsequent phase II reactions.

6.2.2 Phase II reactions

Importance: These reactions form water-soluble conjugated metabolites attaching small, polar and ionizable endogenous compounds to phase I metabolite. They are as follows
- glucuronic acid conjugation
- gulfate conjugation
- conjugation with glycine, glutamine and other amino acids
- glutathione conjugation
- acetylation
- methylation

If the parent compound has polar functional groups such as carboxylic (–COOH), hydroxyl (–OH), amino ($-NH_2$), they are directly conjugated by phase II enzymes. Methylation and acetylation terminate or alter biological activity of drug molecules. Glutathione conjugation protects the body against chemically reactive compounds or metabolites. Phase I and phase II reactions complement one another in detoxifying and facilitating the elimination of drugs and xenobiotics (Fig. 6.1). E.g.: Marijuana -Δ^1-tetrahydrocannabinol (Δ^1-THC).

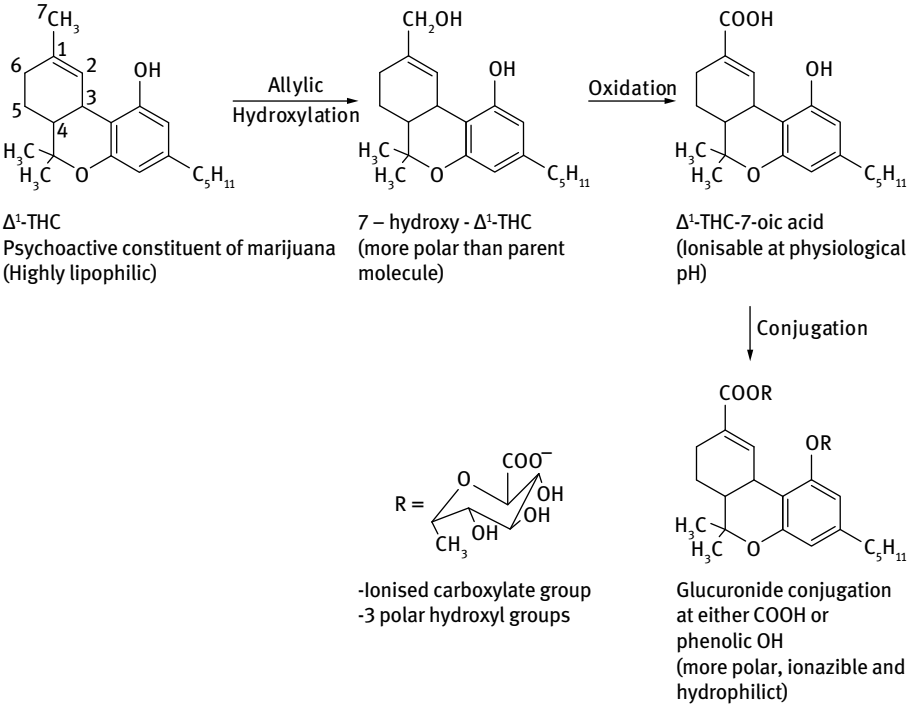


Fig. 6.1: Detoxification of Δ¹-THC.

6.2.3 Sites of drug biotransformation

The liver is the most important organ for metabolism and majority of drugs undergo metabolism in the liver [4], e.g. propoxyphene, lidocaine, propranolol, meperidine, nitroglycerine, Etazocine etc. Another important site of metabolism especially for orally administered dugs is the intestine [5]. Examples include

1. Oral isoproterenol undergoes sulfate conjugation in intestinal wall.
2. Levodopa, chlorpromazine and DES are metabolized in GIT.
3. Ester prodrugs are metabolized by esterases and lipases in the intestine.
4. Sulfadiazine, aromatic azo and nitro drugs are metabolized by bacterial flora in intestine and colon.

Other tissues include kidney, lungs, adrenal glands, placenta, brain and skin. But these sites are substrate selective, limited to particular types of reaction and their metabolic capabilities are not fully understood [6].

6.2.4 Role of cytochrome P450 monooxygenases in oxidative biotransformations [7, 8]

Mixed-function oxidases or monooxygenases catalyze conversion of molecular oxygen into activated oxygen (Fig. 6.2). Important components of this enzyme system include:

1. Cytochrome P450: responsible for transferring an oxygen atom to the substrate (R-H).
2. NADPH-dependent cytochrome P450 reductase
3. NADH-dependent cytochrome b_5

Components 2, 3 and cofactors NADPH and NADH supply reducing equivalents (electrons) needed in overall oxidative reaction.

$$\underset{\text{Xenobiotic}}{R - H} + NADPH + O_2 + H^+ \rightarrow \underset{\text{Oxidized metabolite}}{ROH} + NADP^+ + H_2O$$

NADPH-Reduced form of Nicotinamide adenosine dinucleotide phosphate (reducing agent).
$NADP^+$-Oxidized form of Nicotinamide adenosine dinucleotide phosphate (oxidizing agent).

Cytochrome P450 monooxygenases are chemically heme proteins. The heme portion is iron containing porphyrin called protoporphyrin–IX and the protein portion is apoprotein. This enzyme system is majorly present in liver and traces in lung, kidney, skin, placenta, intestine and adrenal cortex. Endoplasmic reticulum (EPR) undergoes homogenization and loses its structure to form small vesicular bodies called microsomes. Reduced form of this enzyme binds with carbon monoxide (CO) to form complex. This complex has a spectroscopic absorption maximum at 450 nm. Therefore, this enzyme system is named “Cytochrome P450”.

Its ability to metabolize an almost unlimited number of diverse substrates by a variety of oxidation transformations is a key factor in drug metabolism. This is mainly due to substrate nonspecificity and multiple forms of enzyme (polymorphism). Some of these P450 enzymes are selectively induced by various chemicals and drugs like poly aromatic hydrocarbons (induces cytochrome P448), phenobarbital and tetrachloro dibenzodioxin (induces cytochrome P450).

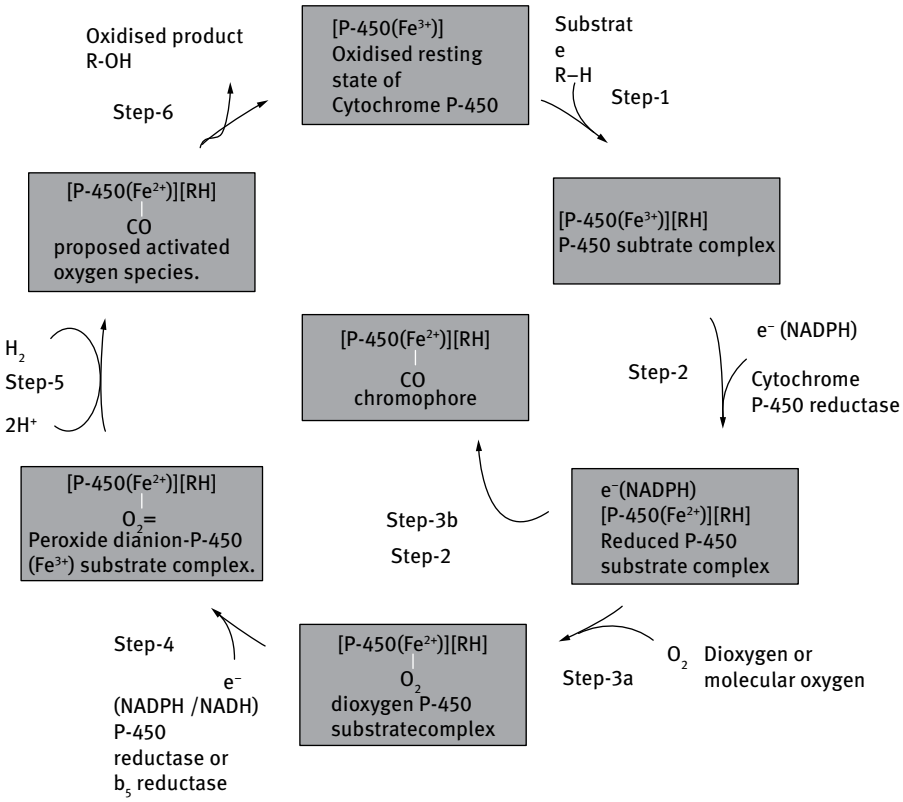


Fig. 6.2: Catalytic reaction cycle of cytochrome P450 monooxygenases.

Step 1: Substrate binding complexation:
Substrate molecule binds to enzyme which is in oxidized resting state (Fe^{3+}) and forms enzyme substrate complex.

Step 2: One electron transfer – reduction of Fe^{3+} to Fe^{2+} :
One electron from NADPH-dependent cytochrome P450 reductase is transferred to enzyme–substrate complex. This electron reduces Fe^{3+} to Fe^{2+}.

Step 3a: Dioxygen binding – complexation:
Reduced enzyme substrate complex binds to dioxygen (molecular oxygen) and forms dioxygen enzyme substrate complex.

Step 3b: Chromophore formation – CO binding:
Reduced enzyme substrate complex binds with CO and forms a complex (chromophore) which has maximum spectroscopic absorption at 450 nm.

Step 4: Reduction of 3a complex – peroxide dianion complex:
Dioxygen enzyme substrate complex undergoes one electron reduction either by

1. cytochrome P450 reductase – NADPH or by
2. cytochrome b_5 reductase – NADH

as a result, peroxide dianion enzyme substrate complex is formed.

Step 5: Formation of activated oxygen complex – activation of complex:
Water is released from the peroxide dianion enzyme substrate complex to form activated enzyme substrate complex. The activated oxygen [FeO^{3+}] in this complex is highly electron deficient and a potent oxidizing agent (Fig. 6.3).

Step 6: Oxidation of substrate – regeneration of enzyme:
The activated oxygen [FeO^{3+}] is transferred to the substrate and the oxidized substrate (R-OH) is released from enzyme complex. Oxidized form of cytochrome P450 is regenerated. Many types of oxidative reactions carried out by cytochrome P450 are summarized schematically in Fig. 6.4.

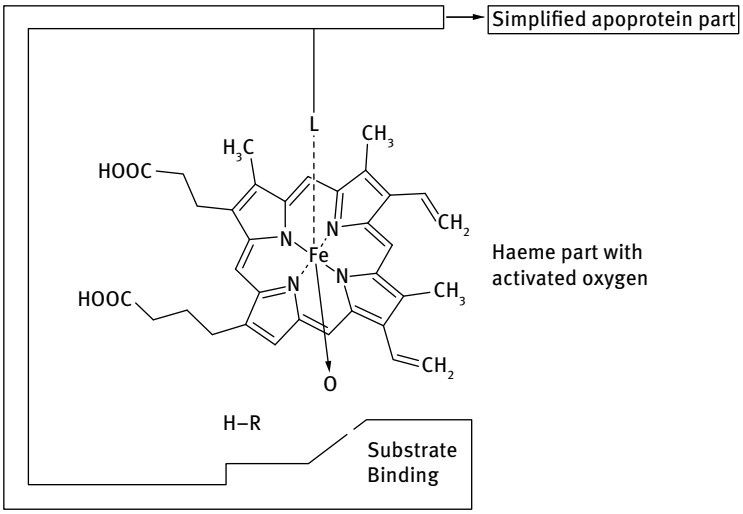


Fig. 6.3: Simplified structure of activated enzyme complex.

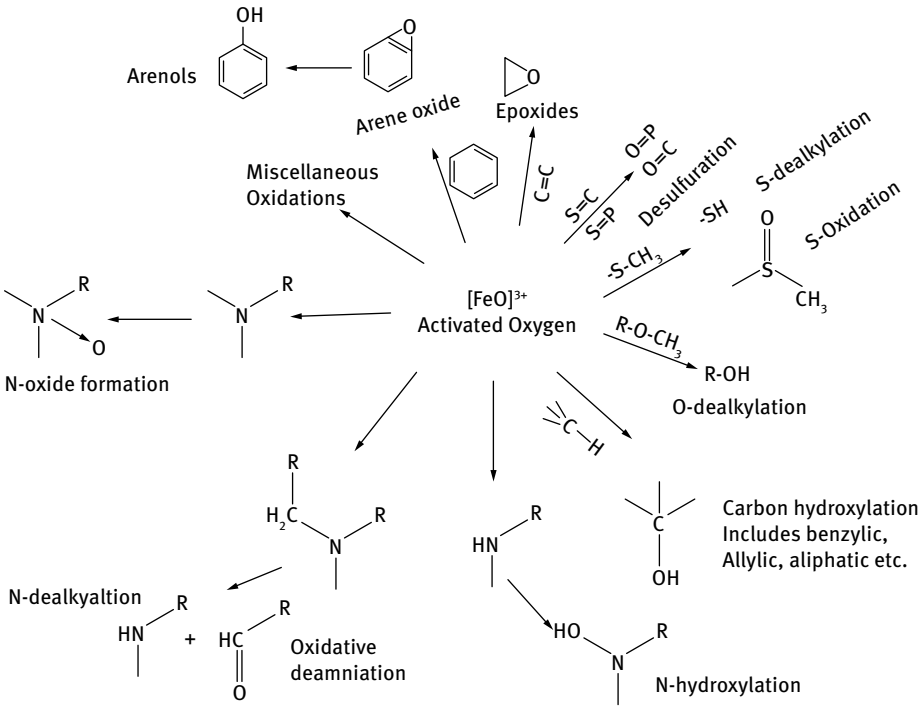


Fig. 6.4: Cytochrome P450 catalyzed reactions.

6.3 Oxidative mechanisms

6.3.1 Oxidation of aromatic moieties

Aromatic hydroxylation: This is the major route of metabolism for many drugs with phenyl rings (Fig. 6.5) in humans involving the formation of epoxide intermediate [9].

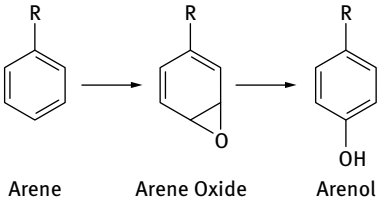


Fig. 6.5: General reaction of aromatic hydroxylation.

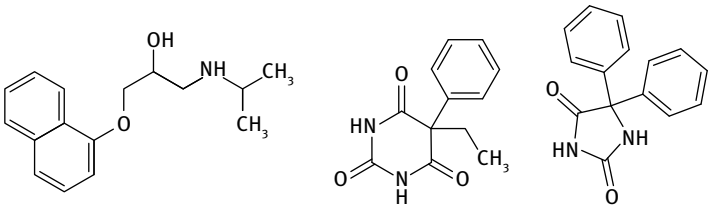


Fig. 6.6: Drugs that undergo aromatic hydroxylation as major metabolic reaction.

Drugs like propranolol (β-blocker), phenobarbital (sedative) and phenytoin (antiepileptic) undergo aromatic hydroxylation as major metabolic reaction (Fig. 6.6). Other drugs that undergo aromatic hydroxylation include phenylbutazone (anti-inflammatory), phenformin (antidiabetic), 17α-ethinyl estradiol (oral contraceptive), S(-)warfarin (anticoagulant), amphetamine (CNS stimulant) etc. In most of the cases aromatic hydroxylation occurs at para position.

The substituent present on the aromatic ring influences the ease of hydroxylation (Fig. 6.7). Aromatic hydroxylation readily occurs in drugs with electron rich or activated rings. In amphetamine (CNS stimulant) an amino alkyl side chain releases electrons into the aromatic ring and activates the ring to undergo hydroxylation preferably at para position. Drugs with electron pullers on aromatic ring or deactivated rings undergo hydroxylation very slowly and in some cases become resistant. Clonidine (antihypertensive) undergoes little aromatic hydroxylation as the ring is deactivated due to presence of two chlorine atoms. Probenecid (uricosuric agent) does not undergo aromatic hydroxylation, as the aromatic ring is deactivated [10–14].

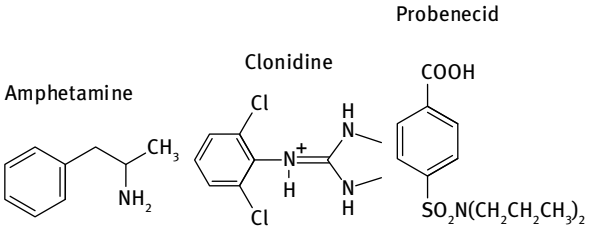


Fig. 6.7: Effect of substituents on aromatic hydroxylation.

In drugs with two or more aromatic rings, hydroxylation occurs preferentially in the more electron rich ring. Diazepam and chlorpromazine are the two drugs that preferably undergo hydroxylation in the electron rich ring (Fig. 6.8).

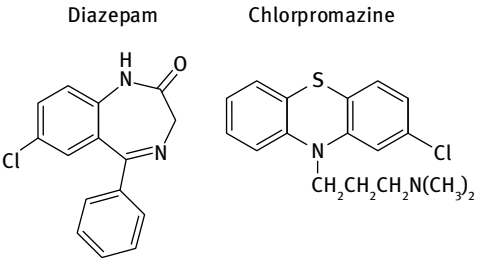


Fig. 6.8: Compounds with two aromatic rings undergoing preferential aromatic hydroxylation.

6.3.1.1 Environmental pollutants and their toxicity

Environmental pollutants like polychlorinated biphenyls (PCB) and tetrachloro dibenzo dioxin (TCDD) carry a higher number of chlorine atoms that are responsible for resistance to aromatic hydroxylation and high lipophilicity. Therefore, they remain in active form in a biological system for longer periods resulting in toxicity [15].

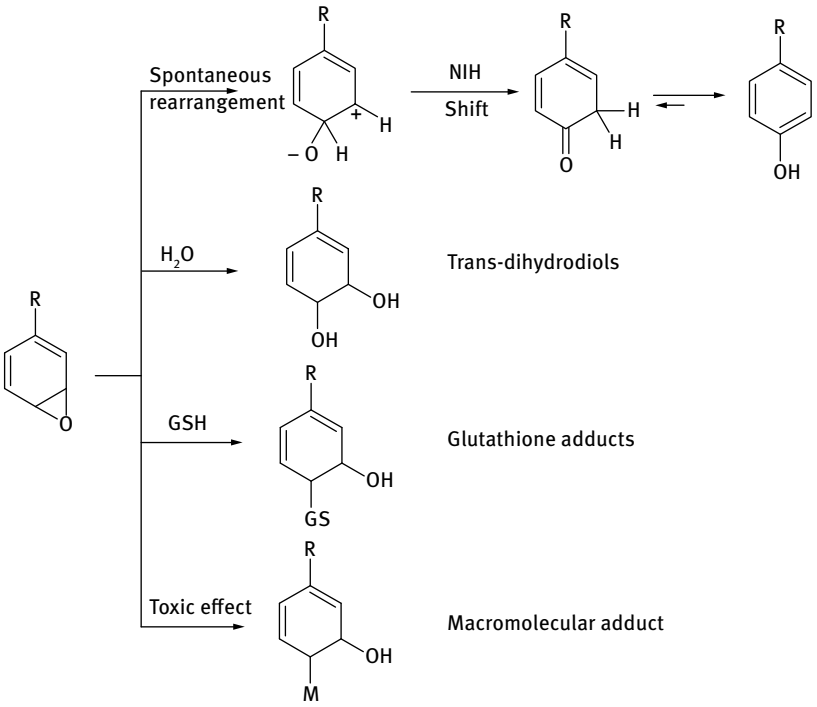


Fig. 6.9: Fate of arene oxide.

6.3.1.2 Formation and fate of arene oxide

Arene oxide is formed when a double bond in aromatic moiety is epoxidized. It binds covalently with nucleophilic groups in proteins, DNA and RNA leading to toxicity (Fig. 6.9). However, it can be detoxified in three ways i.e. spontaneous rearrangement to arenols, enzymatic hydration to trans-dihydrodiols and enzymatic conjugation with glutathione (GSH).

Arene oxide undergoes spontaneous rearrangement into arenol metabolite (Fig. 6.10). This is accompanied by a novel intramolecular hydride (deuteride) migration called "NIH shift" (NIH-National Institute of Health shift or 1, 2-deuteride shift). Arene oxide ring (epoxide ring) opens in the direction that generates most resonance stabilized carbocation (the charge on C-3 is resonance stabilized by methoxy group). In this mechanism, deuterium is retained in the molecule [16].

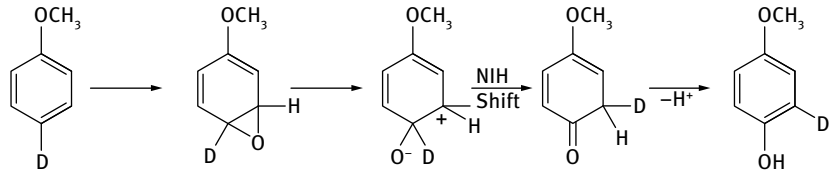


Fig. 6.10: Spontaneous rearrangement of arene oxide into arenol metabolites (NIH shift).

Arene oxide is detoxified by nucleophilic attack of water on the epoxide (arene oxide) and yields inactive trans–dihydrodiol metabolites (Fig. 6.11). Hepatic microsomal epoxide hydrase is the catalyst in this reaction and is inhibited by chemicals like cyclohexene oxide and trichloro propeneoxide (Fig. 6.12). Trans-dihydrodiols obtained from arene oxide undergo enzymatic dehydrogenation and conjugate with glucuronic acid. Naphthalene and benzo[a]pyrene are the examples for enzymatic dehydrogenation. Glutathione-S-transferases (GSH-S-transferases) catalyze the conjugation of arene oxide with glutathione [17].

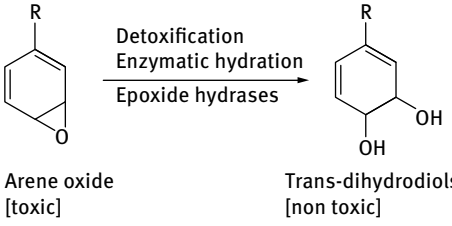
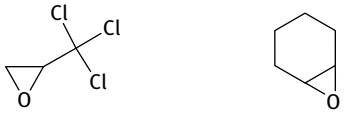


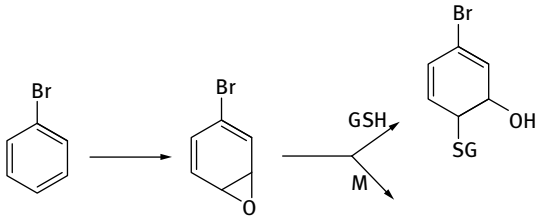
Fig. 6.11: Detoxification of arene oxide via enzymatic hydration.



1,1,1-trichloro propene-2,3-oxide Cyclohexene oxide

Fig. 6.12: Inhibitors of hepatic microsomal epoxide hydrase.

Arene oxides are highly electrophilic and reactive metabolites that react with nucleophilic groups present in biomacromolecules like DNA, RNA and other cellular components. This reaction carries undesired modifications in biomacromolecules leading to irreversible damage and cellular toxicity [18]. Bromobenzene is a classic example of cellular toxicity caused by arene oxide metabolite (Fig. 6.13).



4 - Bromobenzene Epoxide metabolite Covalent binding with macromolecules

Fig. 6.13: Cellular toxicity of bromobenzene.

6.3.2 Oxidation of olefins

The metabolic oxidation of olefinic double bonds leads to corresponding epoxide (oxirane). These epoxide metabolites are more stable than arene oxide metabolites formed from aromatic compounds. Epoxide metabolites also undergo enzymatic hydration by epoxide hydrases into trans-1,2-dihydrodiols or 1,2-diols. In addition to enzymatic hydration, epoxides undergo conjugation reaction with glutathione [19]. Carbamazepine (antiepileptic) undergoes olefinic oxidation and yields epoxide metabolite which is pharmacologically active. Other drugs (Fig. 6.14) which undergo olefinic oxidation are protryptyline (antipsychotic), cyproheptadine (H_1-antihistaminic), alcofenac (anti-inflammatory) and secobarbital (sedative).

Isolation of GSH or mercapturic acid metabolites in a biological system provides indirect evidence for the formation of epoxides (Fig. 6.15). For example, styrene in rats produces two isomeric mercapturic acid metabolites resulting from nucleophilic attack of GSH on styrene epoxide [20].

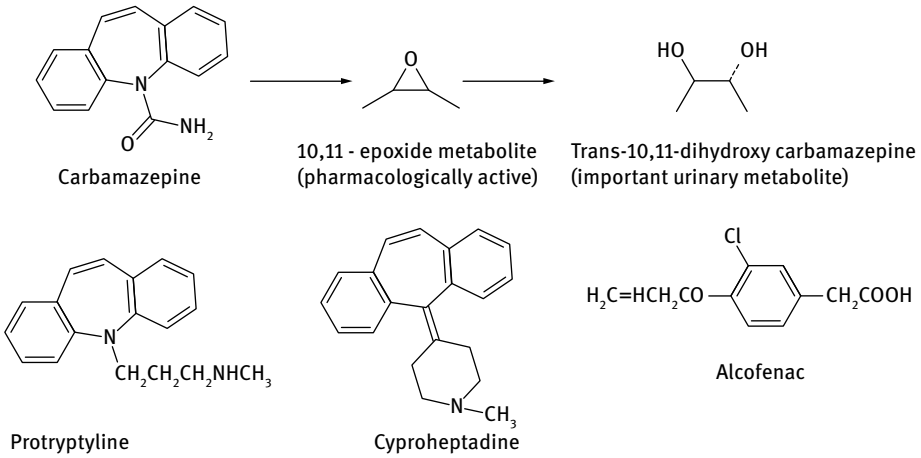


Fig. 6.14: Drugs which undergo olefinic oxidation as major metabolic reaction.

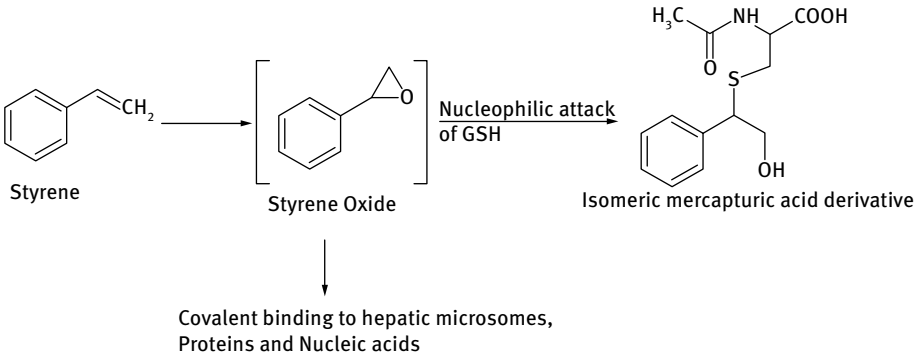


Fig. 6.15: Evidence for epoxide formation.

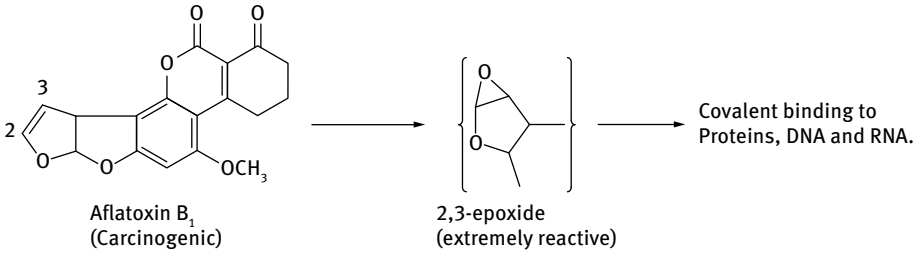
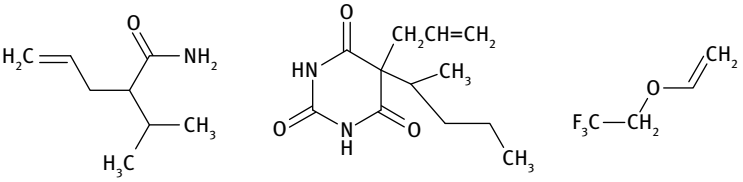


Fig. 6.16: Aflatoxin B1 exhibiting biotoxification via epoxide metabolite.

Aflatoxin B1 is a naturally occurring hepatocarcinogenic agent. It contains an olefinic double bond between C2 and C3 adjacent to cyclic ether oxygen. This olefinic double bond undergoes epoxidation to give highly reactive and toxic 2,3-epoxide metabolite (Fig. 6.16) [21].The cellular toxicity associated with some olefinic compounds can be attributed to their highly reactive epoxide metabolites [22–25].

Certain groups of olefinic compounds irreversibly damage the cytochrome P450 enzyme system (Fig. 6.17). This is due to the covalent binding of corresponding epoxides to heme portion of enzyme. For example, long term administration of allylisopropylacetamide, secobarbital and fluroxene leads to inhibition of oxidative metabolism, undesired drug interactions and prolonged pharmacological effects [26, 27].

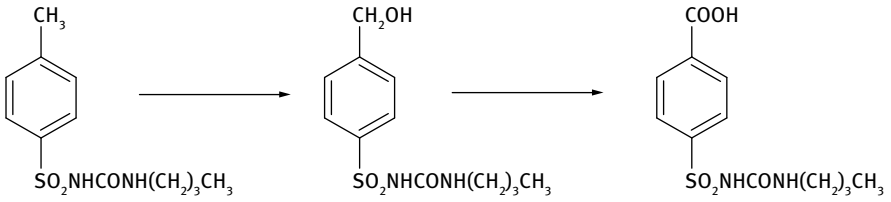


Allyl isopropyl acetamide Secobarbital Fluroxene

Fig. 6.17: Olefinic compounds involved in destruction of cytochrome P450 via epoxide formation.

6.3.3 Oxidation at benzylic carbon atom

Carbon atoms attached to an aromatic ring (benzylic position) are susceptible to oxidation thereby forming corresponding alcohol metabolites [28]. Primary alcohol metabolites undergo further oxidation to aldehydes and carboxylic acids, and secondary alcohols are converted to ketones by alcohol and aldehyde dehydrogenases. Tertiary alcohols can undergo direct conjugation with glucuronic acid [29]. Tolbutamide (oral hypoglycemic agent) has a benzylic carbon atom that upon oxidation initially yields primary alcohol metabolite and further oxidation results in the forma-



Tolbutamide Primary alcohol metabolite Carboxylic acid metabolite

Fig. 6.18: Oxidation at benzylic carbon atom in tolbutamide.

tion of carboxylic acid metabolite (Fig. 6.18). Other drugs that undergo oxidation at benzylic carbon atom are tolmetin (anti-inflammatory), imipramine, amitryptyline, debrisoquin, methaqualone, metoprolol, tetrahydrocannibol etc.

6.3.4 Oxidation at allylic carbon atoms

Oxidation at allylic carbon atoms is commonly seen in drug metabolism. Tetrahydrocannabinol, psychoactive constituent of marijuana, contains three allylic carbon atoms (C3, C6 and C7). Allylic hydroxylation occurs extensively at C7 to give major, active metabolite 7-hydroxy tetrahydrocannabinol (Fig. 6.19). 6-Hydroxy metabolite is a minor metabolite. Hydroxylation does not occur at C3 probably due to steric hindrance [30]. Other drug examples include quinidine (antiarrhythmic), hexobarbital (sedative) etc.

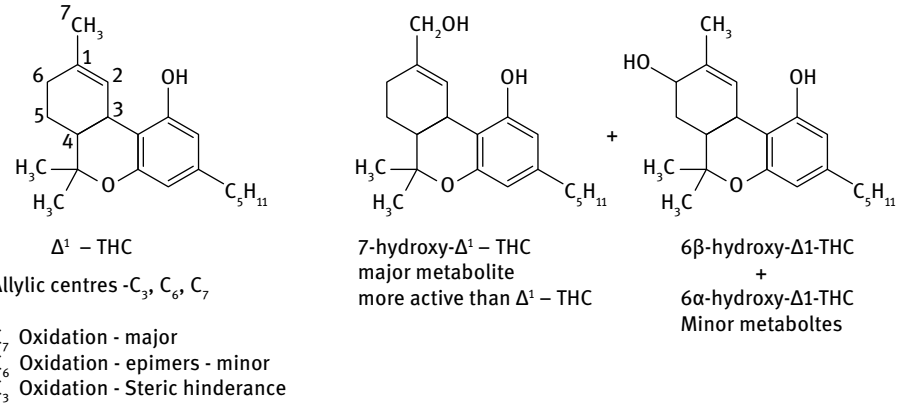
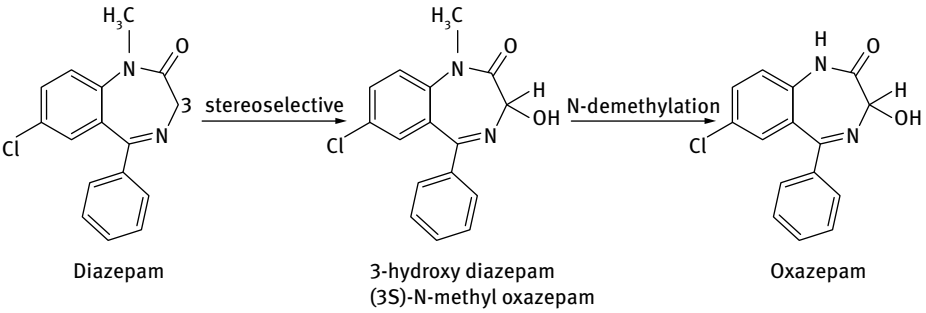


Fig. 6.19: Allylic oxidation in tetrahydrocannabinol.

6.3.5 Oxidation at carbon atoms α to carbonyls and imines

Carbon atoms α to carbonyl and imine functional groups also undergo oxidation by mixed-function oxidases. Benzodiazepines like diazepam and flurazepam undergo oxidation at third position to give 3-hydroxy metabolites. C3 in these drugs is α to both carbonyl and imine functional groups (Fig. 6.20). Hydroxylation of the carbon atom α to carbonyl functionalities generally occurs only to a limited extent in drug metabolism [31–33]. This can be seen in gluthemide (sedative and hypnotic).



Diazepam — 3-hydroxy diazepam (3S)-N-methyl oxazepam — Oxazepam

Fig. 6.20: Oxidation at carbon atoms α to carbonyls and imines in diazepam.

6.3.6 Oxidation at aliphatic and alicyclic carbon atoms

Alkyl or aliphatic carbon atoms are subject to mixed-function oxidation. Metabolic oxidation at the terminal methyl group is referred to as *ω*-oxidation, and oxidation of the penultimate carbon atom (next to the last carbon) is called *ω*–1 oxidation (Fig. 6.21). The initial alcohol metabolites formed from these enzymatic *ω* and *ω*–1 oxidations are susceptible to further oxidation to yield aldehyde, ketones, or carboxylic acids. Alternatively, the alcohol metabolites may undergo glucuronide conjugation [34].

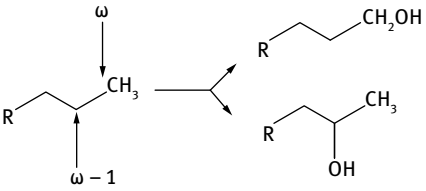


Fig. 6.21: Illustration of *ω* and *ω*–1 oxidations.

A. Aliphatic hydroxylation including both *ω* and *ω*–1 oxidations commonly takes place in drug molecules having straight or branched alkyl chains [35]. Valproic acid (antiepileptic agent) undergoes both *ω* and *ω*–1 oxidations (Fig. 6.22). Other drugs that undergo *ω* and *ω*–1 oxidations include barbiturates, meprobamate, glutethimide, ethosuximide, phenyl butazone, ibuprofen etc.

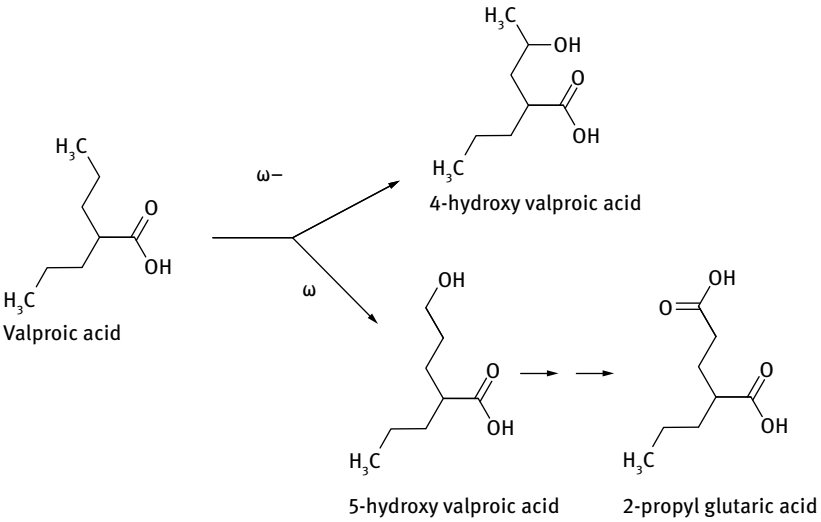


Fig. 6.22: ω and ω–1 oxidations in valproic acid.

In drugs containing a cyclohexyl ring, enzymatic alicyclic hydroxylation generally occurs at C-3 or C-4 and can lead to cis- and trans-isomers [36]. Acetohexamide, an oral hypoglycemic agent, undergoes alicyclic hydroxylation to give trans-4-hydroxy acetohexamide as major metabolite (Fig. 6.23). In phencyclidine and minoxidil, it appears that alicyclic hydroxylation of 6-membered piperidyl moiety may parallel closely the hydroxylation of cyclohexyl moiety [37].

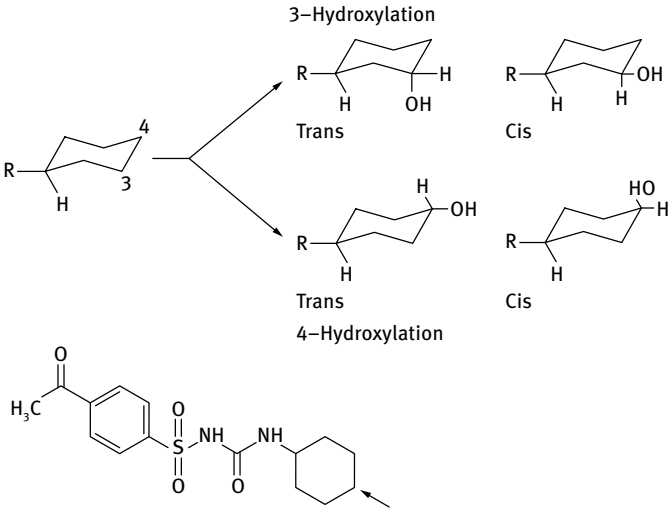


Fig. 6.23: Alicyclic hydroxylation and acetohexamide.

6.3.7 Oxidation of carbon-heteroatom bonds

Metabolic oxidation of C-N, C-O and C-S bonds (Fig. 6.24) principally involves two basic types of biotransformation process. The first one is hydroxylation of α-carbon attached to heteroatom. The intermediate is often unstable and decomposes with cleavage of C-heteroatom bond. Oxidative removal of alkyl groups from heteroatoms and oxidative deamination follow this mechanism. The second process is hydroxylation or oxidation of heteroatom (N or S only, N-hydroxylation, N-oxide formation, sulfoxide and sulfone formation). Several structural features frequently determine which pathway will predominate, especially in carbon–nitrogen systems. Metabolism of some nitrogen-containing compounds is complicated by the fact that carbon- or nitrogen-hydroxylated products may undergo secondary reactions to form other, more complex metabolic products (e.g., oxime, nitrone, nitroso, imino) [38].

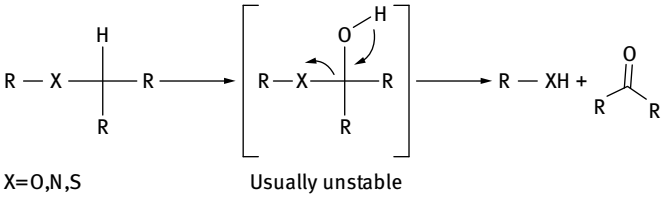


Fig. 6.24: Oxidation of carbon-heteroatom.

6.3.7.1 Oxidations involving C-N systems [39–42]

It is important to understand the metabolism of nitrogen functionalities like amine and amide, as they are found in many natural and synthetic compounds. Oxidation of C-N bonds can be divided into three basic parts:

- aliphatic (3°, 2° and 1°) and alicyclic (3° and 2°) amines
- aromatic and heterocyclic nitrogen compounds
- amides

The hepatic enzymes responsible for carrying out α-carbon hydroxylation reactions are the cytochrome P450 mixed-function oxidases. The *N*-hydroxylation or *N*-oxidation reactions, however, appear to be catalyzed not only by cytochrome P450 mixed-function oxidases but also by a second class of hepatic mixed-function oxidases called amine oxidases (sometimes called N-oxidases). These enzymes are NADPH-dependent flavoproteins and do not contain CYP. They require NADPH and molecular oxygen to carry out *N*-oxidation.

6.3.7.1.1 Aliphatic and alicyclic amines

The oxidative removal of alkyl groups from tertiary aliphatic and alicyclic amines is catalyzed by cytochrome P450 mixed-function oxidase enzymes. This reaction is commonly referred to as oxidative N-dealkylation.

6.3.7.1.1.1 3° amines

The initial step involves α-carbon hydroxylation to form a carbinolamine intermediate (Fig. 6.25), which is unstable and undergoes spontaneous heterolytic cleavage of the C–N bond to give a secondary amine and a carbonyl moiety (aldehyde or ketone).

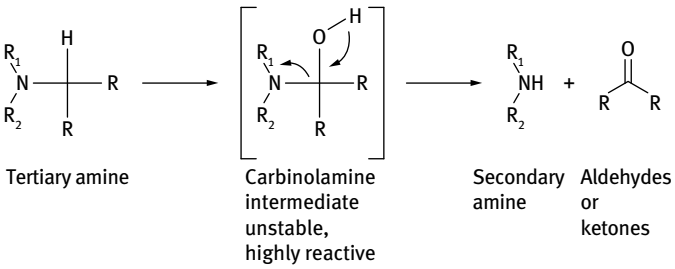


Fig. 6.25: α-carbon hydroxylation via carbinolamine pathway.

Small alkyl groups like methyl or ethyl are rapidly removed. N-dealkylation of t-butyl group by carbinolamine pathway is not possible because α-carbon hydroxylation does not occur. Removal of first alkyl group from a 3° amine occurs more rapidly than removal of second alkyl group. Bis-dealkylation of 3° aliphatic amine to 1° aliphatic amine occurs very slowly in imipramine (antidepressant) and lidocaine (local anesthetic) (Fig. 6.26). Other examples include tamoxifen, diphenhydramine, chlorpromazine and (+)α-propoxyphene. When a 3° amine contains several different removal substituents, the smaller alkyl group is removed preferentially and more rapidly. In benzamphetamine (stimulant), a methyl group is removed more rapidly than a benzyl group.

In some cases, cyclization reaction takes place as a part of N-demethylation (Fig. 6.27). Methadone upon N-demethylation yields demethylated metabolite normethadone which undergoes spontaneous cyclization reaction to form enamine metabolite (EDDP). This EDDP upon subsequent N-demethylation and isomerization of double bond gives EMDP [43].

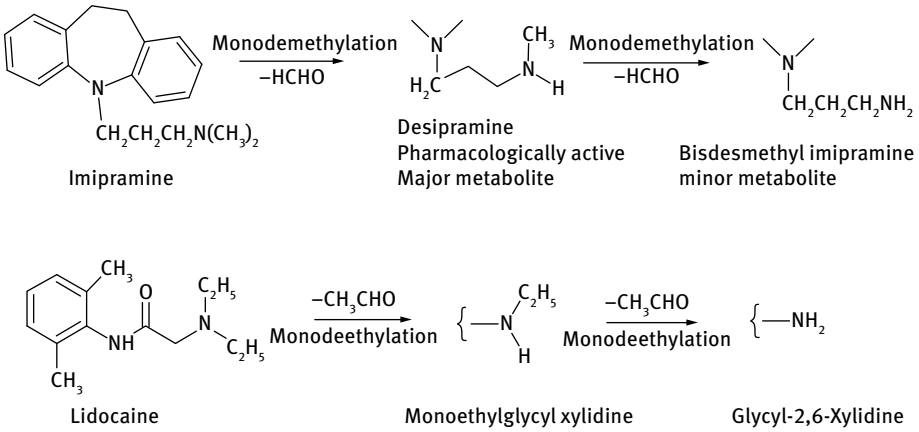


Fig. 6.26: Bis dealkylation in imipramine and lidocaine.

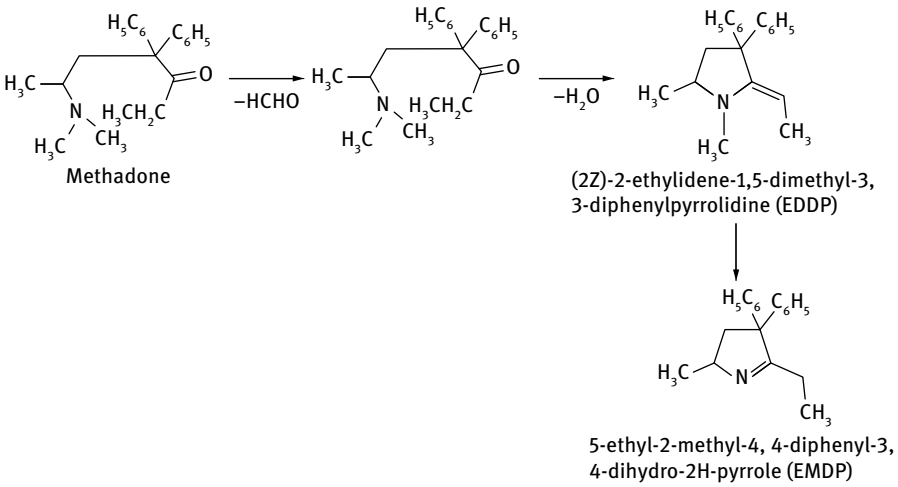


Fig. 6.27: N-demethylation involving cyclization in methadone.

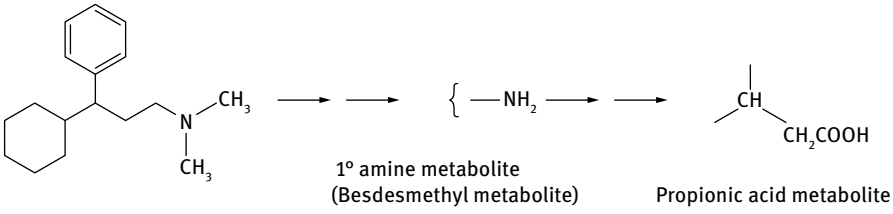


Fig. 6.28: Further oxidation of 1° amine metabolite obtained from bis-dealkylation of brompheniramine.

Sometimes 1° amine metabolite obtained from bis-dealkylation of 3° amine is susceptible to further oxidation. Brompheniramine (H_1-antihistaminic agent) exhibits this metabolic reaction (Fig. 6.28).

Alicyclic tertiary amines are susceptible to oxidative *N*-dealkylation reactions. For example, the analgesic meperidine is metabolized principally by this pathway to yield normeperidine as a major plasma metabolite in humans [44]. Other drugs like morphine and dextromethorphan also undergo N-dealkylation. N-dealkylation of t-butyl groups is not possible by α-carbon hydroxylation pathway but *in vitro* studies indicate that N-t-butyl norchlorocyclizine is metabolized to significant amounts of norchlorocyclizine where t-butyl group is removed in this reaction. The *t*-butyl group is removed by initial hydroxylation of one of the methyl groups of the *t*-butyl moiety to the carbinol or alcohol product. Further oxidation generates the corresponding carboxylic acid that, on decarboxylation, forms the *N*-isopropyl derivative. The *N*-isopropyl intermediate is dealkylated by the normal α-carbon hydroxylation (i.e., carbinolamine) pathway to give norchlorocyclizine and acetone. Indirect *N*-dealkylation of *t*-butyl groups is not observed significantly. The *N*-*t*-butyl group present in many α-adrenergic antagonists, such as terbutaline and salbutamol (Fig. 6.29), remains intact and does not appear to undergo any significant metabolism [45, 46].

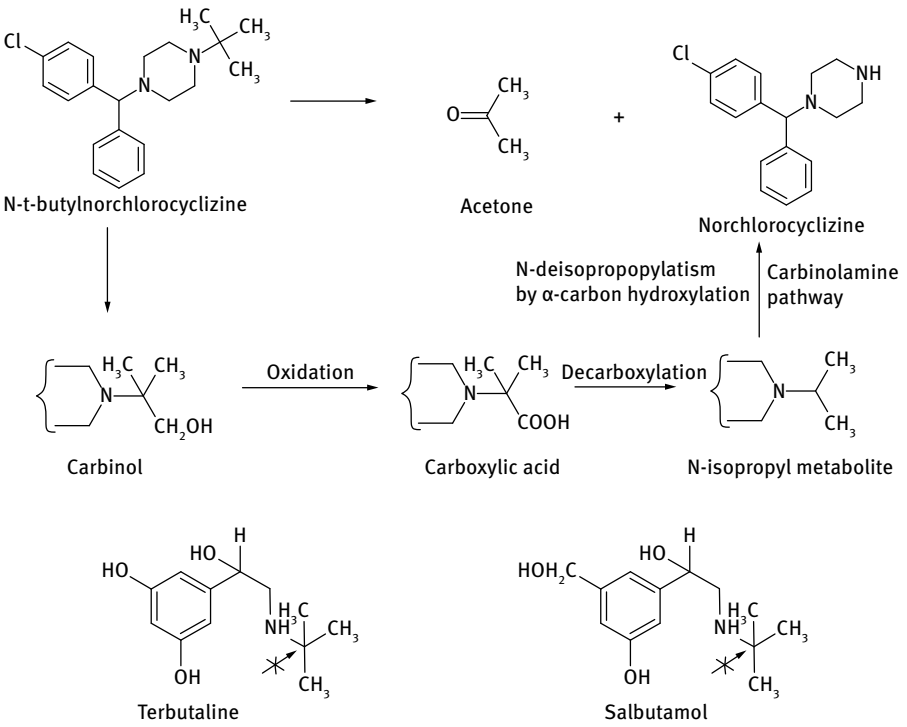


Fig. 6.29: N-dealkylation of t-butyl groups.

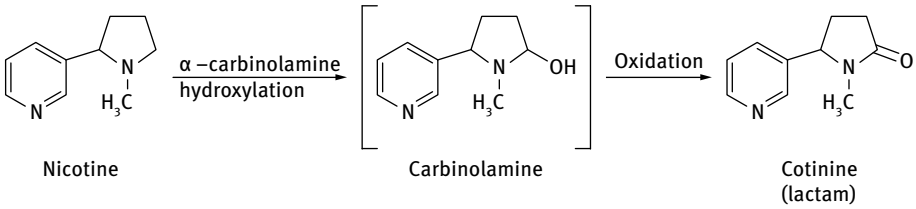


Fig. 6.30: Generation of lactam metabolite from nicotine.

Alicyclic 3° amines generate lactam metabolites by α-carbon hydroxylation reaction [47]. Nicotine (tobacco alkaloid), cyproheptadine (H_1 antihistamine) and diphenidol (antiemetic) generate lactam metabolites (Fig. 6.30).

Tertiary amines such as H_1-histamine antagonists (e.g., orphenadrine, tripelennamine), phenothiazines (e.g., chlorpromazine), tricyclic antidepressants (e.g., imipramine), and narcotic analgesics (e.g., morphine, codeine, and meperidine) reportedly form *N*-oxide metabolites. In some instances, these *N*-oxide metabolites possess pharmacological activity. A comparison of imipramine *N*-oxide with imipramine indicates that the *N*-oxide itself possesses antidepressant and cardiovascular activity similar to that of the parent drug [48].

Both 2° amines and 1° amines are susceptible to oxidative N-dealkylation, oxidative deamination or N-oxidation reactions [49]. N-dealkylation of 2° amines and 1° amines proceeds by carbinolamine pathway. The resultant metabolites from 2° amines and 1° amines are 1° amines and ammonia (in combination with carbonyls), respectively [50]. 1° amine metabolites formed from oxidative dealkylation of methamphetamine are susceptible to oxidative deamination. Oxidative deamination also involves α-carbon hydroxylation (carbinol amine pathway) [51]. If α-carbon hydroxylation does not occur then oxidative deamination is not possible as in ketamine (Fig. 6.31). Generally dealkylation of 2° amines takes place before oxidative deamination occurs.

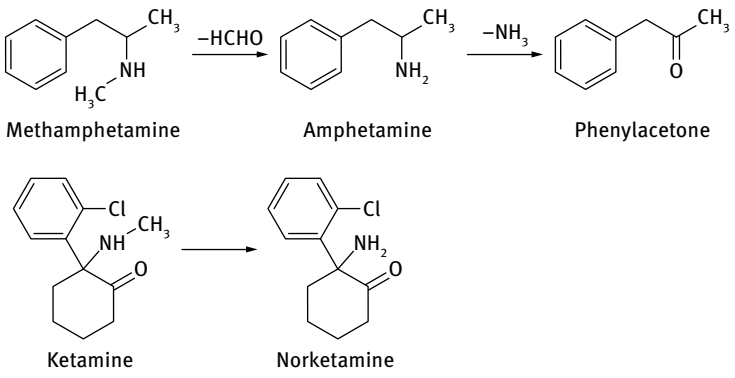


Fig. 6.31: N-dealkylation in methamphetamine and ketamine.

Direct deamination of 2° amine is an exception [52]. Some 2° alicyclic amines like phenmatrizine (anorectic agent) and methylphenidate are metabolized to their corresponding lactam derivatives [53]. Metabolic N-oxidation of 2° aliphatic and alicyclic amines leads to several N-oxygenated products. Hydroxylation of 2° amines such as N-benzylamphetamine, methyl phenindate and phenmetrazine (Fig. 6.32) generates corresponding N-hydroxylamine metabolites which are susceptible for further oxidation to form corresponding nitrone derivatives [54]. In comparison with oxida-

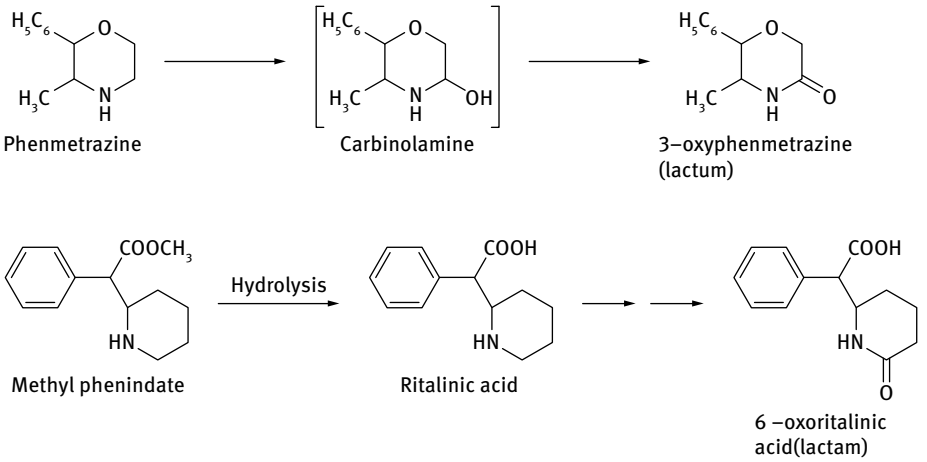


Fig. 6.32: Hydroxylation of phenmetrazine and methyl phenindate.

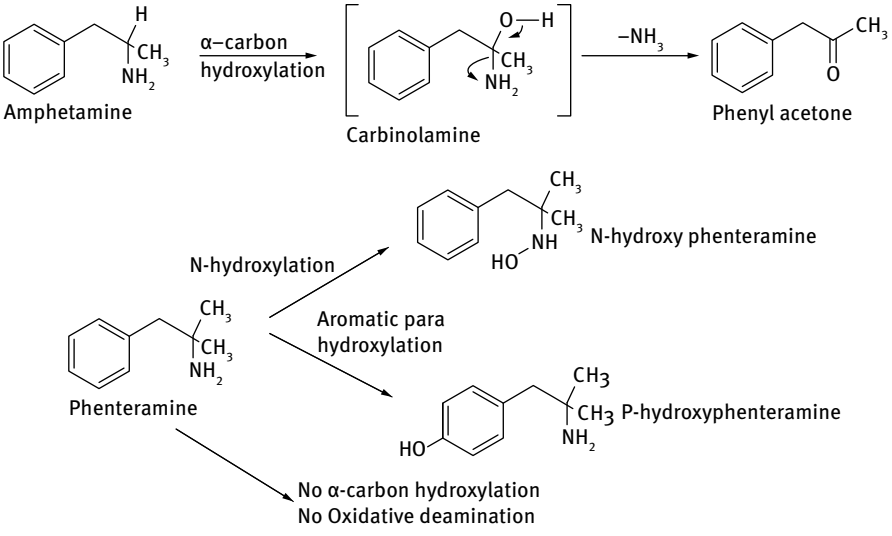


Fig. 6.33: α-substituents determining carbon or nitrogen oxidations.

tive dealkylation and deamination, N-oxidation occurs to a much lesser extent for 2° amines. Primary aliphatic amines are metabolized either by oxidative deamination (carbinolamine pathway) or by N-oxidation [55]. Examples for endogenous primary amines are neurotransmitters like dopamine, tryptamine, norepinephrine and serotonin. In amphetamine and phenteramine (Fig. 6.33), structural features, especially the α-substituents of the primary amine, often determine whether carbon or nitrogen oxidations will occur [56].

6.3.7.1.2 Aromatic amines and heterocyclic nitrogen compounds

Metabolism of aromatic amines parallels the carbon and nitrogen oxidation reactions of aliphatic amines [57]. For tertiary aromatic amines, such as *N*,*N*-dimethylaniline, oxidative N-dealkylation as well as *N*-oxide formation take place (Fig. 6.34).

Secondary aromatic amines may undergo N-dealkylation or N-hydroxylation to give the corresponding N-hydroxylamines. Further oxidation of the N-hydroxylamine leads to nitrone products, which in turn may be hydrolyzed to primary hydroxylamines (Fig. 6.35).

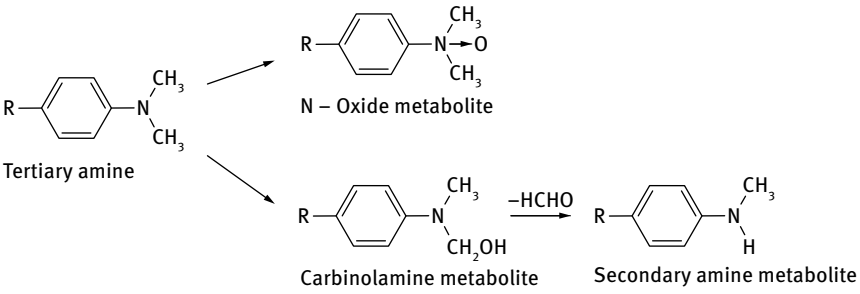


Fig. 6.34: Oxidative N-dealkylation and N-oxide formation in tertiary amines.

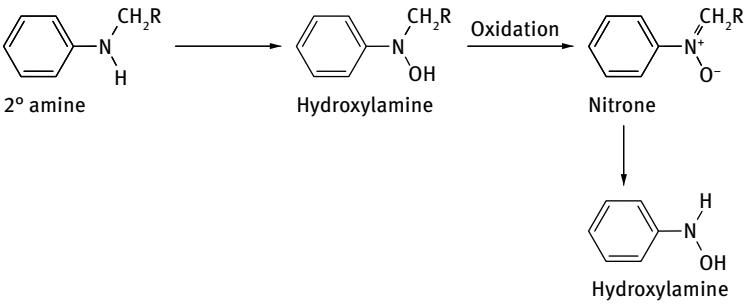


Fig. 6.35: N-dealkylation and N-hydroxylation in secondary amines.

Tertiary and secondary aromatic amines are rarely seen in medicinal agents. Primary aromatic amines are found in many drugs as well as in reduced metabolites of aromatic nitro compounds, azo compounds, and aromatic amides [58, 59]. N-Oxidation of primary aromatic amines like dapsone (Fig. 6.36) generates the N-hydroxylamine metabolites. These hydroxylamine derivatives may undergo either further oxidation to nitroso metabolites or conjugation with glucuronic acid [60].

Several aromatic amines like dapsone after bioconversion into N-hydroxy derivatives cause methemoglobinemia toxicity. N-hydroxyl amine metabolites the oxidized

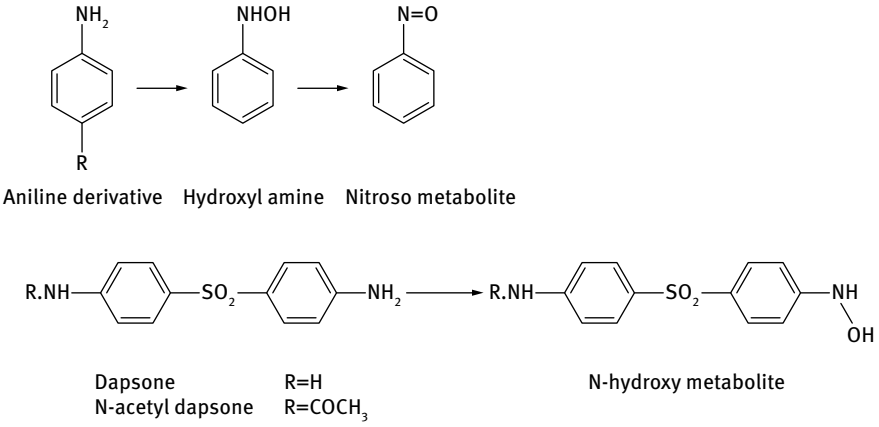


Fig. 6.36: N-Oxidation of dapsone.

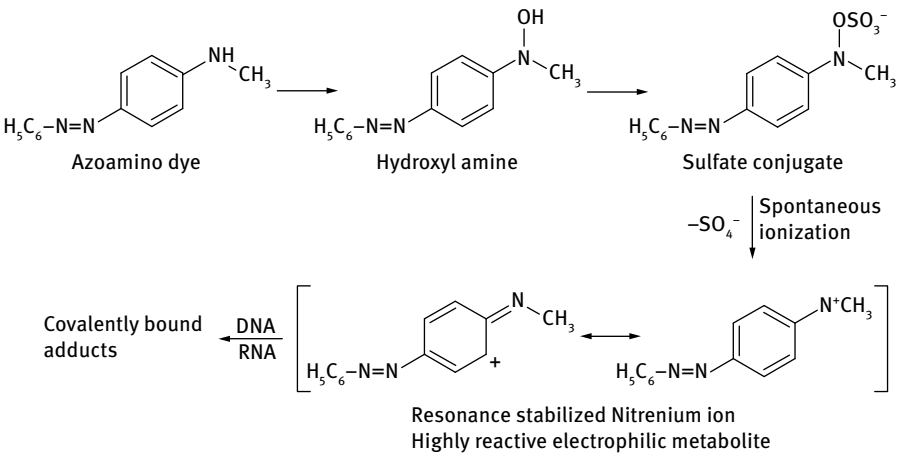


Fig. 6.37: N-oxidation of azoaminodye resulting in cellular toxicity.

ferrous form of hemoglobin to the ferric form. This oxidized form of hemoglobin is called as "methemoglobin" or "ferrrihemoglobin". Methemoglobin is unstable to transport oxygen and therefore leads to serious hypoxia and anemia [61]. N-oxidation of azoaminodyes like N-methyl-4-amino azo benzene (carcinogenic agent) (Fig. 6.37) results in the formation of potentially reactive electrophilic metabolites. These metabolites bind covalently to cellular proteins, DNA and RNA [62].

N-oxidation of the nitrogen atoms present in aromatic heterocyclic moieties of many drugs, for example trimethoprim (Fig. 6.38), occurs to a minor extent [63].

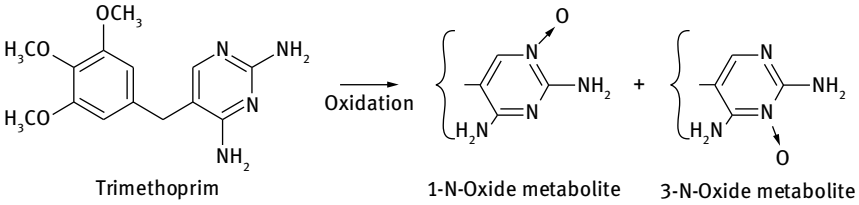


Fig. 6.38: N-Oxidation in trimethoprim.

6.3.7.1.3 Oxidation of amides

Amide functionalities are susceptible to oxidative carbon-nitrogen bond cleavage (via α-carbon hydroxylation) and *N*-hydroxylation reactions. Oxidative dealkylation proceeds via an initially formed carbinolamide, which is unstable and fragments to form the *N*-dealkylated product. For example, diazepam (Fig. 6.39) undergoes extensive *N*-demethylation to the pharmacologically active metabolite desmethyldiazepam [64].

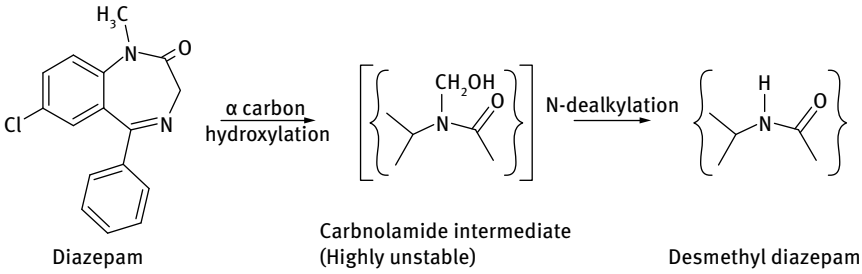


Fig. 6.39: Oxidative dealkylation in diazepam.

In the cyclic amides or lactams, hydroxylation of the alicyclic carbon α to the nitrogen atom also leads to carbinolamides. An example of this pathway is the conversion of cotinine to 5-hydroxycotinine (Fig. 6.40). This carbinolamide intermediate is in tautomeric equilibrium with the ring-opened metabolite γ-(3-pyridyl)-γ-oxo-N-methylbutyramide [65]. N-Hydroxylation of aromatic amides, for example 2-acetylaminoflurene, may lead to the formation of chemically reactive intermediates. These intermediates are carcinogenic or cytotoxic in nature [66].

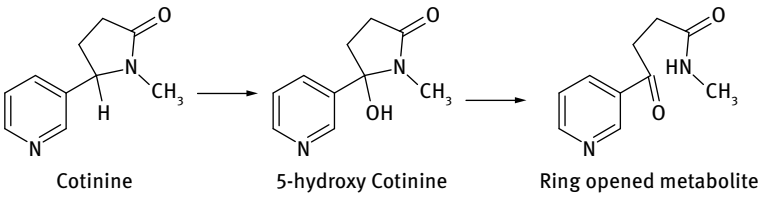


Fig. 6.40: Hydroxylation of alicyclic carbon α to the nitrogen.

6.3.7.2 Oxidation involving C-O bonds

Oxidative O-dealkylation of carbon-oxygen systems (mainly ethers) is catalyzed by microsomal mixed-function oxidases. This biotransformation involves an initial α-carbon hydroxylation to form either a hemiacetal or a hemiketal (Fig. 6.41), which undergoes carbon-oxygen bond cleavage to yield the dealkylated oxygen species (phenol or alcohol) and a carbon moiety (aldehyde or ketone). Small alkyl groups (e.g., methyl or ethyl) attached to oxygen are O-dealkylated rapidly [67]. For example, morphine is the metabolic product of O-demethylation of codeine. Other drugs that undergo O-dealkylation are indomethacin, prazosin and metoprolol.

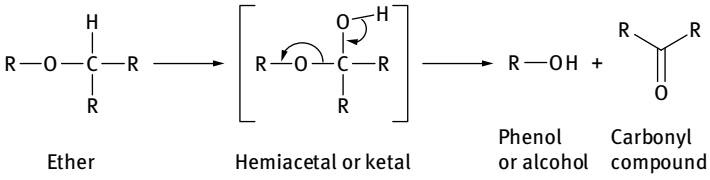


Fig. 6.41: α-carbon hydroxylation in ethers.

In drugs that have several nonequivalent methoxy groups, one particular methoxy group is O-demethylated selectively or preferentially [68]. For example, 3, 4, 5-trimethoxy phenyl moiety in both mescaline and trimethoprim undergoes 3-O-demethylation preferentially (Fig. 6.42).

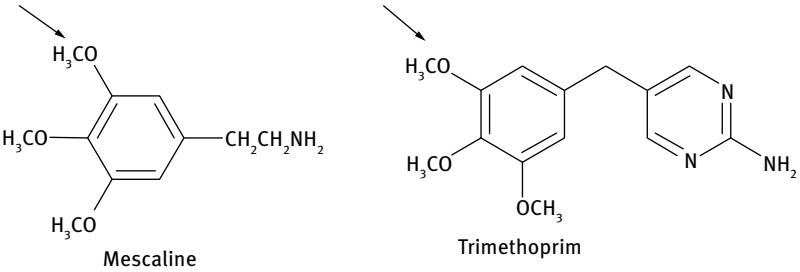


Fig. 6.42: O-demethylation in mescaline and trimethoprim.

6.3.7.3 Oxidation involving C-S bonds

Carbon-sulfur functional groups are susceptible to S-dealkylation, desulfuration and S-oxidation metabolic reactions. Both S-dealkylation and desulfuration involve oxidative cleavage of C-S bond. S-dealkylation is similar to N- and O-dealkylation and involves α-carbon hydroxylation [69]. For example, 6-(methylthio)purine is demethylated oxidatively to 6-mercaptopurine (Fig. 6.43).

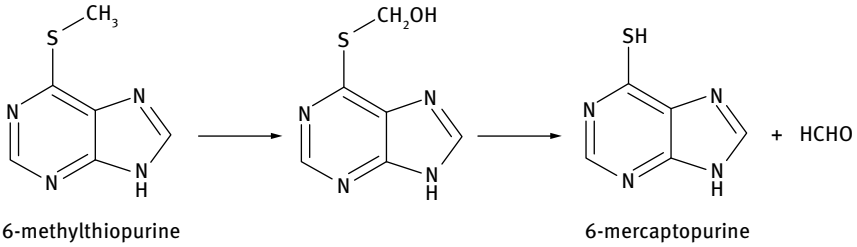


Fig. 6.43: S-demethylation in methylthiopurine.

Oxidative conversion of C=S double bonds to corresponding C=O double bond is called desulfuration [70]. A well-known drug example of desulfuration is the conversion of thiopental to its corresponding oxygen analog pentobarbital (Fig. 6.44). Desulfuration reaction also occurs with the P=S moiety (converted to P=O in the metabolite) present in several organophosphate insecticides, such as parathion [71, 72].

Organosulfur compounds commonly undergo S-oxidation to yield sulfoxide derivatives. Several phenothiazine derivatives are metabolized by this pathway. For example, both sulfur atoms present in thioridazine are susceptible to S-oxidation (Fig. 6.45). Oxidation of the 2-methylthio group yields the active sulfoxide metabolite mesoridazine. Interestingly, mesoridazine is twice as potent an antipsychotic agent as thioridazine in humans [73, 74].

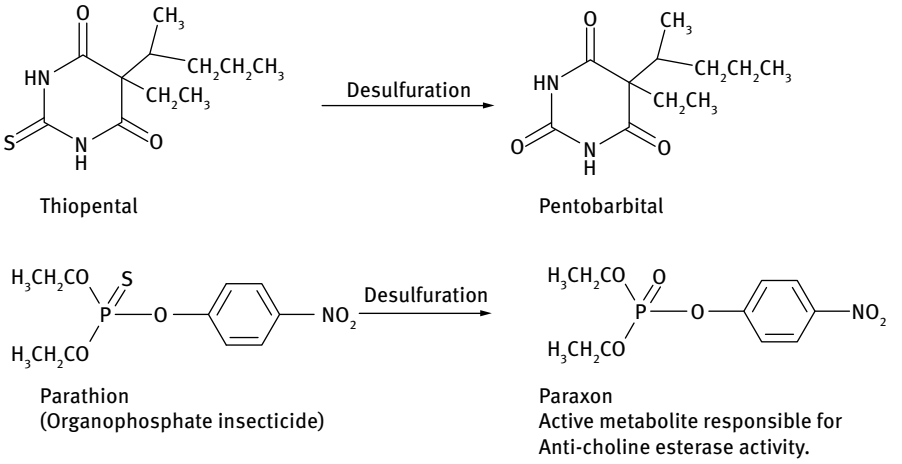


Fig. 6.44: Desulfuration of thiopental and parathion.

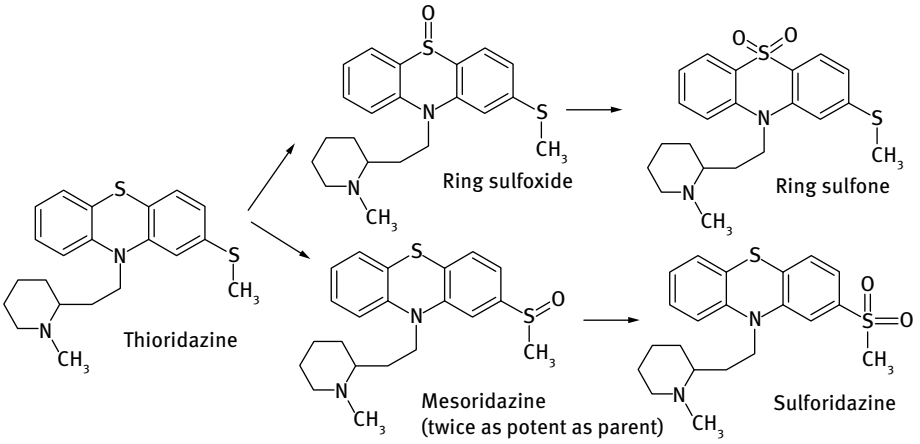


Fig. 6.45: S-oxidation in thioridazine.

S-oxidation is one of the important metabolic reactions in the H_2-histamine antagonist cimetidine and metiamide. The corresponding sulfoxide derivatives are the major human urinary metabolites [75]. Sulfoxide drugs and metabolites may be further oxidized to sulfones (-SO_2-). The sulfoxide group present in the immunosuppressive agent oxisuran is metabolized to a sulfone moiety (Fig. 6.46). Sulfoxide metabolites, such as those of thioridazine, reportedly undergo further oxidation to their sulfone derivatives [76].

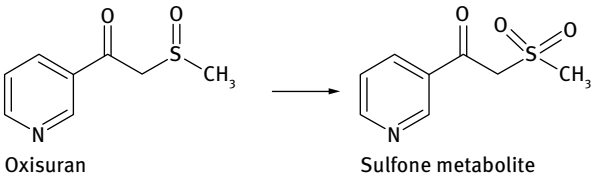


Fig. 6.46: Oxidation of sulfoxide to sulfone in oxisuran.

6.3.7.3.1 Oxidation of alcohols and aldehydes

Many oxidative processes like benzylic, allylic, alicyclic, or aliphatic hydroxylation of corresponding drugs generate alcohol or carbinol metabolites as intermediate products. If not conjugated, these alcohol metabolites are further oxidized to aldehydes or ketones. Aldehyde metabolites resulting from oxidation of primary alcohols or from oxidative deamination of primary aliphatic amines undergo simple oxidation to generate polar carboxylic acid metabolites (Fig. 6.47). Primary alcoholic and aldehyde functionalities are completely vulnerable to oxidation.

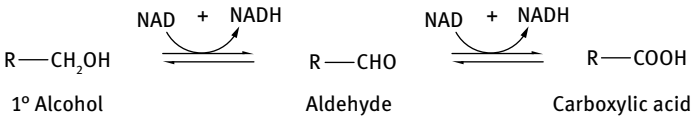


Fig. 6.47: Oxidation of alcohol and aldehyde to carboxylic acid.

Although secondary alcohols are susceptible to oxidation, this reaction is not often important because the reverse reaction, namely, reduction of the ketone back to the secondary alcohol, occurs quite readily. In addition, the secondary alcohol group, being polar and functionalized, is more likely to be conjugated than the ketone moiety (Fig. 6.48).

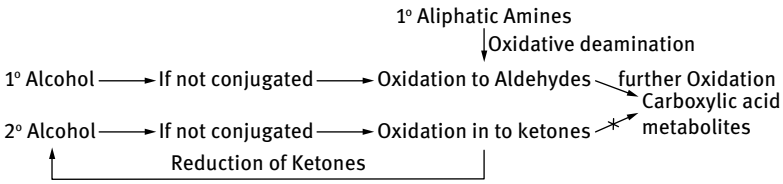


Fig. 6.48: Oxidation of primary and secondary alcohols.

The bioconversion of alcohols to aldehydes and ketones is catalyzed by soluble alcohol dehydrogenases present in the liver and other tissues. NAD^+ is required as a coenzyme, although $NADP^+$ also may serve as a coenzyme. The reaction catalyzed by alcohol dehydrogenase is reversible but often proceeds to the right because the aldehyde formed is further oxidized to the acid. Several aldehyde dehydrogenases, including aldehyde oxidase and xanthine oxidase, carry out the oxidation of aldehydes to their corresponding acids.

Soluble or microsomal dehydrogenase and oxidases are involved in oxidizing the carbinol group of the intermediate carbinolamine to a carbonyl moiety. For example, in the metabolism of medazepam to diazepam (Fig. 6.49), the intermediate carbinolamine (2-hydroxymedazepam) undergoes oxidation of its 2-hydroxy group to a carbonyl moiety [77].

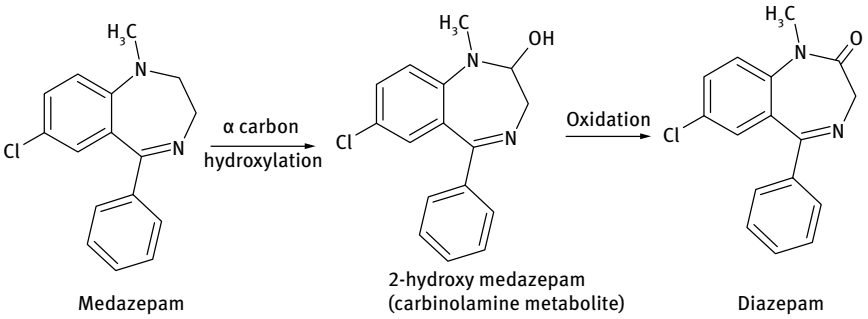


Fig. 6.49: Conversion of medazepam to diazepam.

6.3.7.3.2 Miscellaneous oxidative biotransformations

In addition to the many oxidative biotransformations discussed previously, oxidative aromatization or dehydrogenation and oxidative dehalogenation reactions also occur. Metabolic aromatization has been reported for norgestrel (Fig. 6.50). Aromatization or dehydrogenation of the ring-A present in this steroid leads to the corresponding phenolic product 17α-ethinyl-18-homoestradiol as a minor metabolite in women [78].

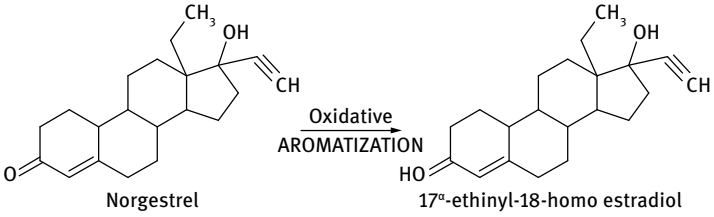


Fig. 6.50: Metabolic aromatization of norgestrel.

Many halogen-containing drugs and xenobiotics are metabolized by oxidative dehalogenation. For example, the volatile anesthetic agent halothane is metabolized to trifluoroacetic acid [79]. It has been suggested that this metabolite arises from hydroxylation of halothane to form an initial carbinol intermediate that eliminates hydrogen bromide (dehalogenation) to yield trifluoroacetylchloride (Fig. 6.51). The latter acyl chloride is chemically reactive and reacts rapidly with water to form trifluoroacetic acid. Alternatively, it can acylate tissue nucleophiles. Indeed, in vitro studies indicate that halothane is metabolized to a reactive intermediate (presumably trifluoroacetyl chloride), which covalently binds to liver microsomal proteins [80].

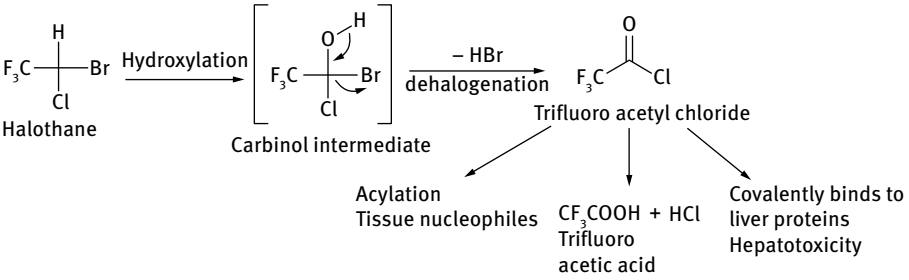


Fig. 6.51: Metabolic hydroxylation of halothane.

Chloroform is metabolized oxidatively by a similar dehalogenation pathway to yield the chemically reactive species phosgene (Fig. 6.52). Phosgene is responsible for the hepatotoxicity and nephrotoxicity associated with chloroform [81].

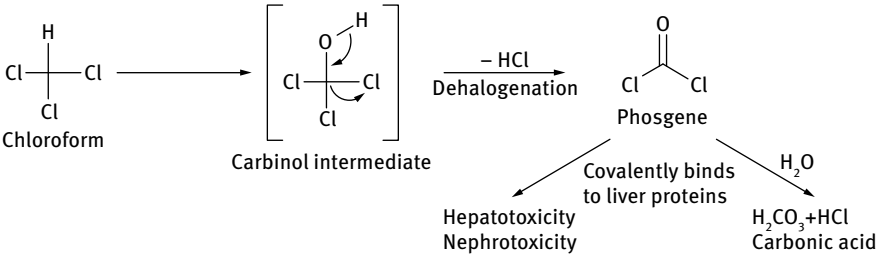


Fig. 6.52: Metabolic dehalogenation of chloroform.

The dichloroacetamide portion of the antibiotic chloramphenicol undergoes oxidative dechlorination to yield a chemically reactive oxamyl chloride intermediate that can react with water to form the corresponding oxamic acid metabolite or can acylate microsomal proteins [82]. In several instances, oxidative dehalogenation can lead to the formation of toxic and reactive acyl halide intermediates (Fig. 6.53).

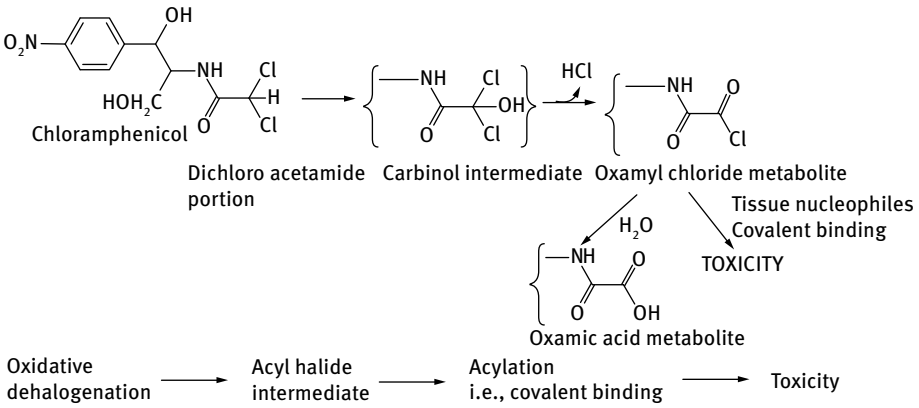


Fig. 6.53: Oxidative dehalogenation and toxicity of chloramphenicol.

6.4 Reductive reactions

Reductive reactions play an important role in the metabolism of many drugs containing carbonyl, nitro, and azo groups (Fig. 6.54). Metabolic reduction of carbonyl compounds generates alcohol metabolites, whereas nitro and azo reductions lead to amino metabolites. The hydroxyl and amino moieties of the metabolites are much more susceptible to conjugation than the functional groups of the parent compounds. Hence, reductive processes, as such, facilitate drug elimination [83].

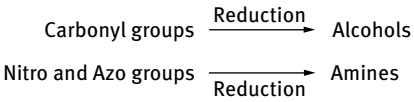


Fig. 6.54: Metabolic reduction of carbonyl, azo and nitro compounds.

Reductive reactions that are seen less frequently in drug metabolism include reduction of N-oxides to their corresponding tertiary amines and reduction of sulfoxides to sulfides. Reductive cleavage of disulfide linkages and reduction of carbon–carbon double bonds also occur, but constitute only minor pathways in drug metabolism.

6.4.1 Reduction of aldehydes and ketones

Many drugs contain carbonyl group, particularly keto group. Phase I metabolites formed by oxidative deamination of drugs also contain carbonyl group. Aldehyde

metabolites are easily oxidized to carboxylic acids and occasionally reduced to 1° alcohols (Fig. 6.55). Ketones generally resist metabolic oxidation and are reduced to secondary alcohols. Alcohol metabolites arising from reduction of carbonyl compounds generally undergo further conjugation (e.g., glucuronidation).

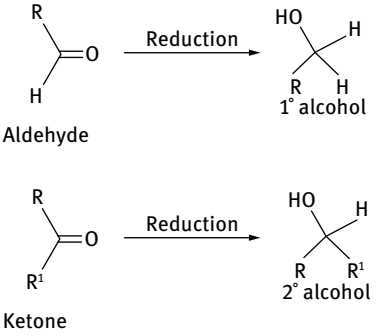


Fig. 6.55: Reduction of aldehydes and ketones.

Aldo-keto reductases are a group of diverse soluble enzymes that catalyze metabolic reduction of aldehydes and ketones. They are found in the liver and other tissues like kidney. These soluble enzymes have similar physicochemical properties and broad substrate specificities and require NADPH as a cofactor. Oxidoreductase enzymes can also reduce aldehydes and ketones. For example, the important hepatic alcohol dehydrogenase is an NAD^+ dependent oxidoreductase that oxidizes ethanol and other aliphatic alcohols to aldehydes and ketones (Fig. 6.56). In the presence of NADH or NADPH, however, the same enzyme system can reduce carbonyl derivatives to their corresponding alcohols [84].

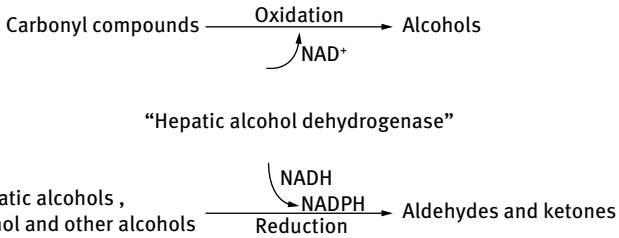


Fig. 6.56: Role of hepatic alcohol dehydrogenases in the metabolism of carbonyl compounds.

Sedative and hypnotic chloral hydrate is an example for parent aldehyde drug undergoing extensive enzymatic reduction (Fig. 6.57). Metabolic reduction of this hydrated

aldehyde yields trichloroethanol as the major pharmacologically active metabolite. Further glucuronidation of this alcohol metabolite leads to an inactive conjugated product that is readily excreted in the urine [85].

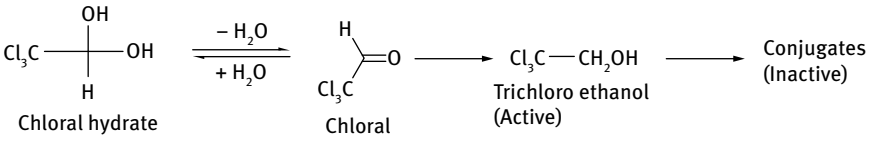


Fig. 6.57: Enzymatic reduction of chloral hydrate.

Metabolic reduction of ketones leads to the creation of an asymmetric center resulting in two possible stereoisomeric alcohols. For example, reduction of acetophenone leads to the enantiomeric alcohols (S)(–)- and (R)(+)-methylphenylcarbinol, with the (S)(–)-isomer predominating (Fig. 6.58). The preferential formation of one stereoisomer over the other is termed "product stereoselectivity" in drug metabolism. The mechanism involves a "hydride" transfer from the reduced nicotinamide moiety of the cofactor NADPH or NADH to the carbonyl carbon atom of ketone. Often, ketone reduction yields alcohol metabolites that are pharmacologically active [86].

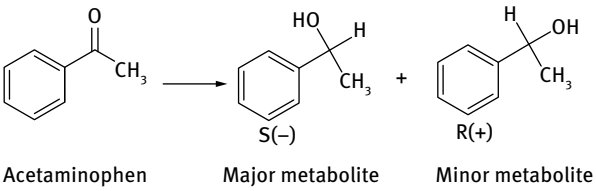


Fig. 6.58: Reduction of acetophenone leading to the enantiomeric alcohol metabolites.

Achiral drugs containing ketone groups such as oral hypoglycemic acetohexamide, usually give predominantly one enantiomer on reduction (Fig. 6.59). Acetohexamide is metabolized rapidly to give active metabolite (S)(–)-hydroxyhexamide [87].

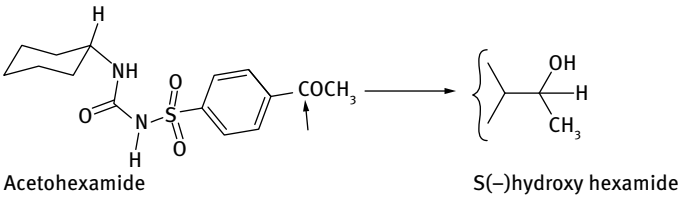


Fig. 6.59: Reduction of acetohexamide.

When chiral ketones are reduced, they yield two possible diastereomeric or epimeric alcohols. For example, the (R)(+) warfarin undergoes reduction of its side chain keto group to generate the (R, S)(+) alcohol as the major metabolite. Small amounts of the (R, R)(+) diastereomer are also formed (Fig. 6.60). In contrast, the (S)(−) warfarin undergoes little ketone reduction and is primarily hydroxylated in aromatic ring [88].

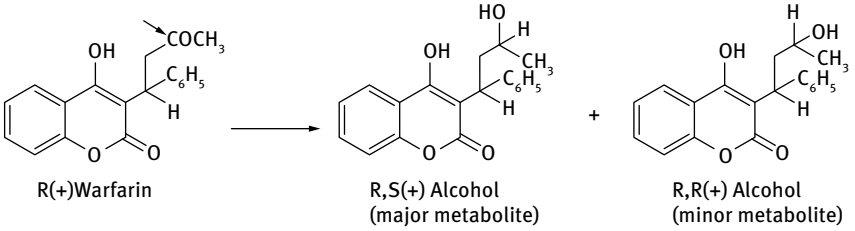


Fig. 6.60: Reduction of (R) (+) warfarin.

Oxisuran has its greatest immunosuppressive effects in those species that form alcohols as their major metabolites (humans and rats). In dog, reduction is a minor pathway and so the immunosuppressive effect is small. Oxisuran alcohols (oxisuranols) are pharmacologically active and contribute substantially to the overall immunosuppressive effect of parent drug (Fig. 6.61). The sulfoxide group in oxisuran is chiral by virtue of the lone pair of electrons on sulfur. Therefore reduction of oxisuran leads to diastereomeric alcohols [89].

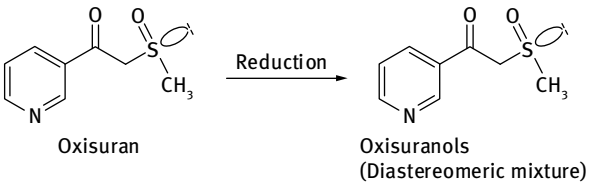


Fig. 6.61: Reduction of oxisuran into mixture of diastereomeric metabolites.

Reduction of α, β-unsaturated ketones results in reduction not only of the ketone group but of carbon-carbon double bond as well. For example norethindrone, a synthetic progestin found in many oral contraceptive drug combinations, undergoes metabolic reduction to give 3β, 5β-tetrahydro metabolite [90]. Ketones resulting from metabolic oxidative deamination processes are also susceptible to reduction (Fig. 6.62). For example, amphetamine is deaminated to phenylacetone, which is reduced subsequently to 1-phenyl-2-propanol [91].

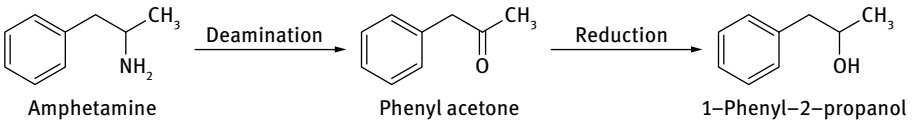


Fig. 6.62: Deamination of amphetamine to phenylacetone and subsequent reduction.

6.4.2 Reduction of nitro and azo compounds

Reduction of aromatic nitro and azo drugs leads to aromatic primary amine metabolites. Aromatic nitro compounds are reduced initially to the nitroso and then to hydroxylamine intermediates. Azo reduction proceeds via a hydrazo intermediate (-NH-NH-). This hydrazo metabolite is cleaved reductively to yield corresponding aromatic amines. Metabolic reduction of nitro compounds is catalyzed by NADPH-dependent hepatic nitro reductases present in the liver. In addition, bacterial reductases present in the intestine can reduce nitro and azo compounds, especially those that are absorbed poorly or excreted mainly in the bile [92, 93]. Various aromatic nitro drugs undergo enzymatic reduction to the corresponding aromatic amines (Fig. 6.63). For example, the 7-nitro benzodiazepine derivatives clonazepam and nitrazepam are metabolized extensively to their respective 7-amino metabolites [94].

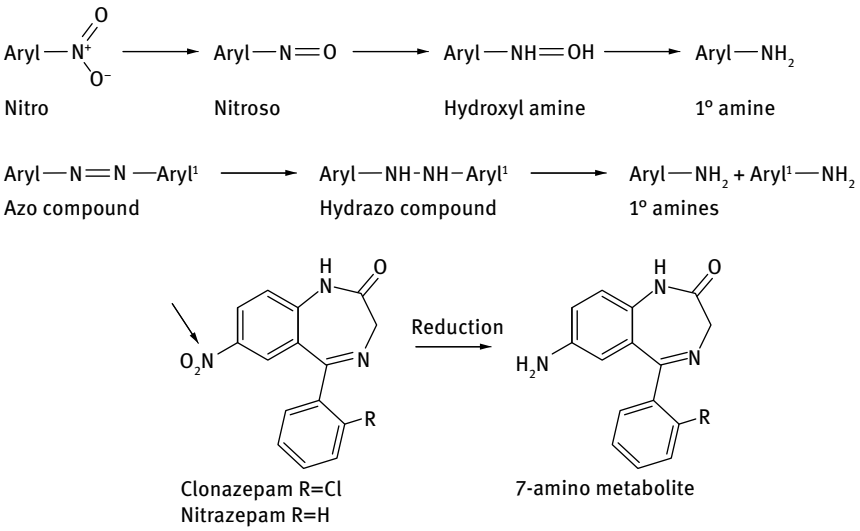


Fig. 6.63: Metabolic reduction of nitro and azo compounds.

The enzymatic reduction of azo compounds is best demonstrated by the conversion of prontosil to the active sulfanilamide metabolite in the liver (Fig. 6.64). This reaction has historical significance, for it led to the discovery of sulfanilamide as an antibacterial and eventually to the development of many of the therapeutic sulfonamide drugs. Bacterial reductases present in the intestine play a significant role in reducing azo drugs, particularly those that are absorbed poorly.

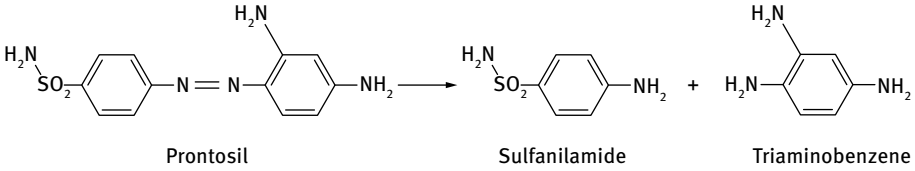


Fig. 6.64: Metabolic reduction of prontosil.

The two azo dyes tartrazine and amaranth have poor oral absorption because of the many polar and ionized sulfonic acid groups present in their structures. Therefore, these two azo compounds are metabolized primarily by bacterial reductases present in the intestine [95].

6.4.3 Miscellaneous reductions

Several minor metabolic reductive reactions also occur in xenobiotics. Reduction of *N*-oxides to the corresponding tertiary amine occurs to some extent. This reductive reaction is significant because several tertiary amines undergo oxidation to form polar and water-soluble N-oxide metabolites. If reduction of N-oxide metabolites occurs to a significant extent, drug elimination of the parent tertiary amine is hindered. N-oxide reduction is assessed by administering the pure synthetic N-oxide *in vitro* or *in vivo* and then detecting the formation of the tertiary amine. For example, imipramine N-oxide undergoes reduction [96].

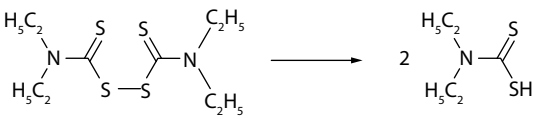


Fig. 6.65: Reductive cleavage of disulfide bond in disulfiram.

Reduction of sulfur-containing functional groups, such as the disulfide and sulfoxide moieties, also constitutes a minor reductive pathway. Reductive cleavage of the disulfide bond in disulfiram gives N,N-diethyldithiocarbamic acid as a major metabolite (Fig. 6.65).

Although sulfoxide functionalities are oxidized mainly to sulfones (-SO2-), they sometimes undergo reduction to sulfides. For example, sulindac undergoes reduction to an active sulfide metabolite that is responsible for the overall anti-inflammatory effect of the parent drug (Fig. 6.66). Sulindac or its sulfone metabolite exhibits little anti-inflammatory activity [97].

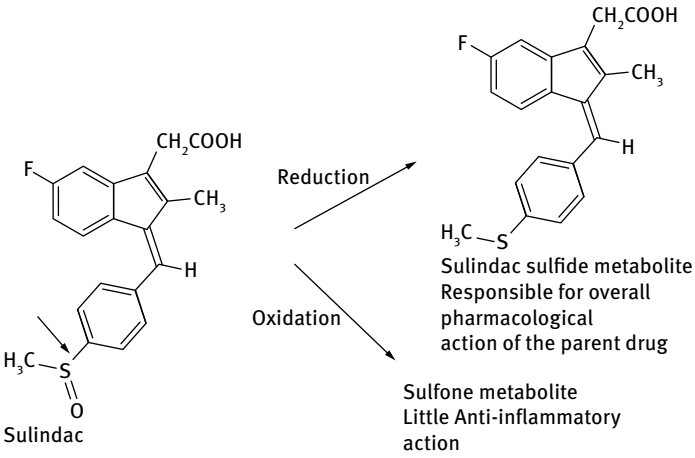


Fig. 6.66: Metabolic reduction of sulindac to pharmacologically active sulfide metabolite.

6.5 Hydrolytic reactions

6.5.1 Hydrolysis of esters and amides

The metabolic products formed (carboxylic acids, alcohols, phenols, and amines) from hydrolysis of ester and amide drugs are generally polar and functionally more susceptible to conjugation and excretion than the parent drugs. The enzymes carrying out ester hydrolysis include several nonspecific esterases found in the liver, kidney, and intestine as well as the pseudocholinesterases present in plasma. Amide hydrolysis is mediated by liver microsomal amidases, esterases, and deacylases [98].

Hydrolysis is a major metabolic pathway for drugs containing ester functionality. This is because of the relative ease of hydrolyzing the ester linkage. One of the best examples of ester hydrolysis is the metabolic conversion of aspirin to salicylic

acid. Another classic example is cocaine (Fig. 6.67). Of the two ester functional groups present in cocaine, the methyl group is hydrolyzed preferentially to yield benzoylecgonine as the major metabolite [99, 100].

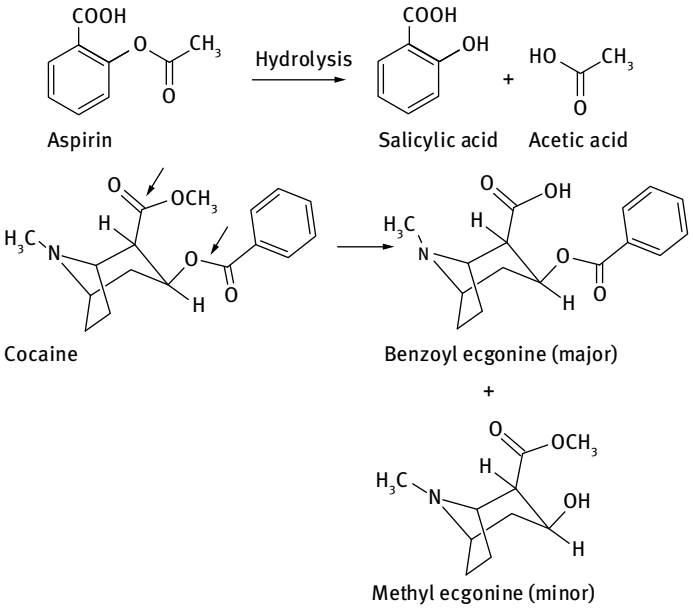


Fig. 6.67: Metabolic hydrolysis of ester group in aspirin and cocaine.

Methylphenidate is hydrolyzed rapidly to give ritalinic acid as the major metabolite. In a majority of ester drugs, ester hydrolysis leads to pharmacologically active metabolites (Fig. 6.68). For example, hydrolysis of diphenoxylate in humans leads to diphenoxylic acid (difenoxin), which is a five times more potent antidiarrheal agent than the parent ester [101, 102]. The presence of esterases in many tissues and plasma makes ester derivatives logical prodrug candidates, because hydrolysis causes the ester prodrug to revert to the parent compound. Antibiotics such as chloramphenicol and clindamycin are derivatized as their palmitate esters to minimize their bitter taste and to improve their palatability in pediatric dosage forms [103]. Amides are hydrolyzed slowly in comparison to esters. Consequently, hydrolysis of the amide bond of procainamide is relatively slow compared with hydrolysis of the ester linkage in procaine (Fig. 6.69). Drugs in which amide cleavage has been reported to occur include lidocaine, carbamazepine, indomethacin and prazosin. Amide linkages present in barbiturates (e.g., hexobarbital) as well as in hydantoins (e.g., 5-phenylhydantoin) and succinimides (phensuximide) are also susceptible to hydrolysis [104].

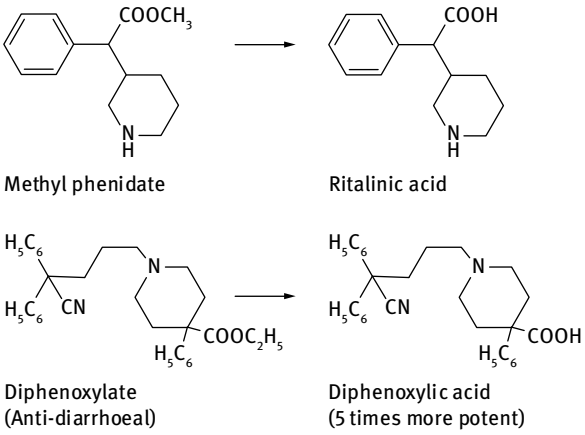


Fig. 6.68: Metabolic reduction of methylphenidate and diphenoxylate.

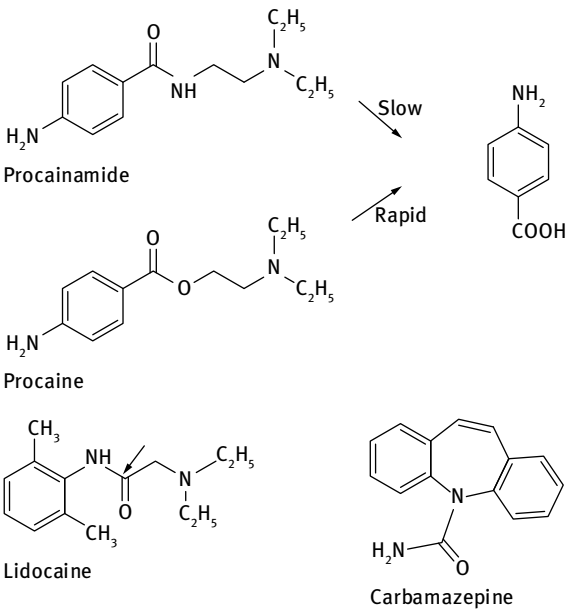


Fig. 6.69: Comparison of metabolic hydrolysis in ester and amide drugs.

6.5.2 Miscellaneous hydrolytic reactions

Recombinant human peptide drugs and hormones undergo hydrolysis at N-or C-terminal amino acids by carboxypeptidases, aminopeptidases and proteases. Examples include human insulin, growth hormone (GH), parathyroid hormone (PTH), prolac-

tin and atrial natri uretic factor (ANF) [105]. In addition to hydrolysis of amides and esters, hydrolytic cleavage of other moieties occurs to a minor extent in drug metabolism, including the hydrolysis of phosphate esters (e.g., diethylstilbestrol diphosphate), sulfonylureas, cardiac glycosides, carbamate esters, and organophosphate compounds. Glucuronide and sulfate conjugates also can undergo hydrolytic cleavage by β-glucuronidase and sulfatase enzymes.

6.6 Phase II conjugation reactions

Phase I or functionalization reactions do not always produce hydrophilic or pharmacologically inactive metabolites. Various phase II conjugation reactions can convert these phase I metabolites to more polar and water-soluble products. Many enzymes catalyze conjugation reactions by attaching small, polar, and ionizable endogenous molecules, such as glucuronic acid, sulfate, glycine, and glutamine, to the phase I metabolite or parent drug. The resulting conjugated products are relatively water-soluble and readily excretable. In addition, they generally are biologically inactive and nontoxic. Other phase II reactions, such as methylation and acetylation, do not generally increase water solubility but mainly help to terminate pharmacological activity. The role of GSH is to combine with chemically reactive compounds to prevent damage to important biomacromolecules, such as DNA, RNA, and proteins. Thus, phase II reactions are regarded as truly detoxifying pathways in drug metabolism, with a few exceptions.

A discriminating feature of most phase II reactions is that the conjugating group (glucuronic acid, sulfate, methyl, and acetyl) is activated initially in the form of a coenzyme before transfer or attachment of the group to the substrate by the suitable transferase enzyme. In other cases, such as glycine and glutamine conjugation, the substrate is activated initially. Many endogenous compounds, such as bilirubin, steroids, catecholamines, and histamine, also undergo conjugation reactions and use the same coenzymes, although they are mediated by more specific transferase enzymes [106].

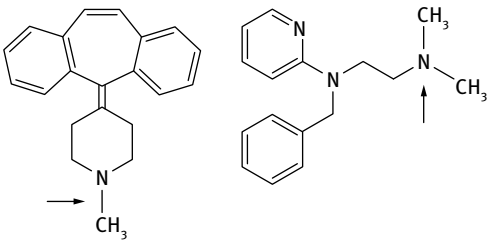
6.6.1 Glucuronic acid conjugation

Glucuronidation is the most common conjugative reaction in drug metabolism, as it greatly increases the water solubility of the conjugated product. Formation of β-glucuronides involves two steps: synthesis of an activated coenzyme, uridine-5′-diphospho-α-D-glucuronic acid (UDPGA) and subsequent transfer of the glucuronyl group from UDPGA to an appropriate substrate. The transfer step is catalyzed by microsomal enzymes called UDP-glucuronyltransferases. They are found primarily in the liver but also occur in many other tissues, including kidney, intestine, skin, lung, and brain.

The synthesis of the coenzyme UDPGA uses α-D-glucose-1-phosphate as its precursor. All glucuronide conjugates have the β-configuration or β-linkage at C-1 and hence, the term β-glucuronides. In contrast, the coenzyme UDPGA has α-linkage. In the enzymatic transfer step, nucleophilic displacement of α-linked UDP moiety from UDPGA by the substrate proceeds with complete inversion of configuration at C-1 to give the β-glucuronide. Glucuronidation of one functional group usually serves to effect excretion of the conjugated metabolite [107].

Conjugated products are classified as oxygen-, nitrogen-, sulfur-, or carbon-glucuronide, according to the heteroatom attached to the C-1 atom of the glucuronyl group. Two important functionalities, the hydroxy and carboxy, form O-glucuronides. Phenolic and alcoholic hydroxyls are the most common functional groups undergoing glucuronidation in drug metabolism. These functional groups are present in many parent drugs and arise through various phase-I metabolic reactions. Morphine and acetaminophen are a few examples of phenolic compounds that undergo considerable glucuronidation. Alcoholic hydroxyls, such as those present in chloramphenicol and propranolol are also commonly glucuronidated. Less frequent is glucuronidation of other hydroxyl groups, such as enols, N-hydroxylamines, and N-hydroxylamides [108].

The carboxy group also undergoes conjugation with glucuronic acid. For example, arylaliphatic acids, such as the anti-inflammatory agent naproxen is excreted primarily as its O-glucuronide derivatives in humans. Carboxylic acid metabolites such as those arising from chlorpheniramine and propranolol form O-glucuronide conjugates. Aryl acids like benzoic acid and salicylic acid also undergo conjugation with glucuronic acid, but conjugation with glycine is a more important pathway for these compounds [109, 110]. N-glucuronides are formed with aromatic amines, aliphatic amines, amides, and sulfonamides. Glucuronidation of aromatic and aliphatic amines is generally a minor pathway in comparison with N-acetylation or oxidative deamination. Tertiary amines, such as the antihistaminic agent cyproheptadine and tripelennamine (Fig. 6.70), form interesting quaternary ammonium glucuronide metabolites [111].



Cyproheptadine Tripelennamine

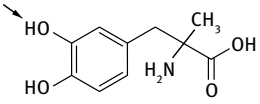
Fig. 6.70: Site of glucuronic acid conjugation in cyproheptadine and tripelennamine.

S-glucuronide conjugates have been reported for only a few drugs because the thiol group (SH) does not commonly occur in xenobiotics. For example, the thiol groups present in methimazole and propylthiouracil undergo conjugation with glucuronic acid [112]. The formation of glucuronides attached directly to a carbon atom is relatively novel in drug metabolism. Studies have shown that conjugation of phenylbutazone and sulfinpyrazone yields the corresponding C-glucuronide metabolites [113].

6.6.2 Sulfate conjugation

Conjugation of xenobiotics with sulfate occurs primarily with phenols and, occasionally, with alcohols, aromatic amines, and N-hydroxy compounds. The body uses a significant portion of the sulfate pool to conjugate numerous endogenous compounds such as steroids, heparin, chondroitin, catecholamines, and thyroxine. The sulfate conjugation process involves activation of inorganic sulfate to the coenzyme 3′-phosphoadenosine-5′-phosphosulfate (PAPS). Subsequent transfer of the sulfate group from PAPS to the substrate is catalyzed by various soluble sulfotransferases present in the liver and other tissues. Sulfate conjugation generally leads to water-soluble and inactive metabolites. However, the O-sulfate conjugates of some N-hydroxy compounds give rise to chemically reactive and toxic intermediates [114].

Phenols are the main group of substrates undergoing sulfate conjugation. Thus, drugs containing phenolic moieties are susceptible to sulfate formation. For example, the antihypertensive agent α-methyldopa is metabolized extensively to its 3-O-sulfate ester (Fig. 6.71). The β-adrenergic bronchodilators salbutamol and terbutaline also undergo sulfate conjugation as their main route of metabolism. For many phenols, however, sulfoconjugation may represent only a minor pathway. Glucuronidation of phenols is frequently a competing reaction and may predominate as the conjugative route for some phenolic drugs. The major urinary metabolite of the analgesic acetaminophen is the O-glucuronide conjugate, with the concomitant O-sulfate conjugate being formed in small amounts. In infants and young children (3–9 years) the O-sulfate conjugate is the main urinary product. The explanation for this reversal stems from the fact that neonates and young children have a decreased glucuronidating capacity because of undeveloped glucuronyltransferases or low levels of these enzymes. Sulfate conjugation, however, is well developed and becomes the main route of acetaminophen conjugation in this pediatric group [115, 116].



Methyldopa

Fig. 6.71: Sulfate conjugation in methyldopa.

Other functionalities, such as alcohols and aromatic amines can also form sulfate conjugates. These reactions, however, have only minor importance in drug metabolism. The sulfate conjugation of N-hydroxylamines and N-hydroxylamides takes place occasionally. O-Sulfate ester conjugates of N-hydroxy compounds can lead to reactive intermediates that are responsible for cellular toxicity. The carcinogenic agent N-methyl- 4-aminoazobenzene is believed to mediate its toxicity through N-hydroxylation to the corresponding N-hydroxy compound. Sulfoconjugation of the N-hydroxy metabolites yields O-sulfate esters, which are the ultimate carcinogenic species. Loss of SO_4^{2-} from the foregoing sulfate conjugates generates electrophilic nitrenium species, which may react with nucleophilic groups present in proteins, DNA, and RNA to form covalent linkages that lead to structural and functional alteration. The consequences of this are cellular toxicity (tissue necrosis) or alteration of the genetic code, eventually leading to cancer [117].

6.6.3 Conjugation with glycine, glutamine, and other amino acids

Mammalian systems use amino acids glycine and glutamine to conjugate carboxylic acids, particularly aromatic acids and arylalkyl acids. The quantity of amino acid conjugates formed from xenobiotics is minute because of the limited availability of amino acids in the body and competition with glucuronidation for carboxylic acid substrates. In contrast with glucuronic acid and sulfate, glycine and glutamine are not converted to activated coenzymes. Instead, the carboxylic acid substrate is activated with adenosine triphosphate (ATP) and coenzyme A (CoA) to form an acyl-CoA complex. This intermediate acylates glycine or glutamine under the influence of specific glycine or glutamine N-acyltransferase enzymes. The activation and acylation steps take place in the mitochondria of liver and kidney cells. Amino acid conjugates, being polar and water-soluble, are excreted mainly renally and, sometimes, in the bile.

Aromatic acids and arylalkyl acids are the major substrates for glycine conjugation. The conversion of benzoic acid to its glycine conjugate, hippuric acid is a well-known metabolic reaction. The extensive metabolism of salicylic acid to salicyluric acid is another example. Carboxylic acid metabolites resulting from oxidation or hydrolysis of many drugs are also susceptible to glycine conjugation. For example, the H_1-histamine antagonist brompheniramine (Fig. 6.72) is oxidized to a propionic acid metabolite that is conjugated with glycine [119].

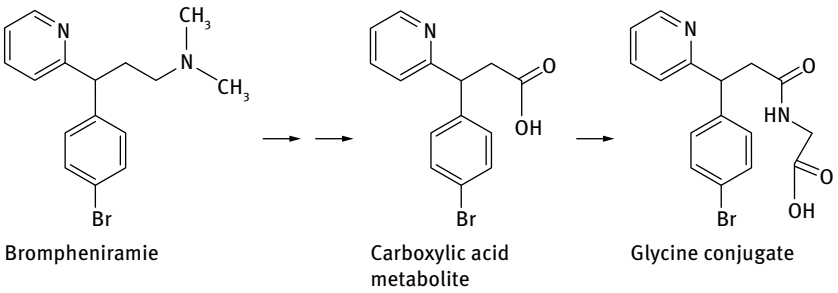


Fig. 6.72: Glycine conjugation in brompheniramine.

Glutamine conjugation occurs mainly with arylacetic acids, including endogenous phenylacetic and 3-indolylacetic acid. A few glutamine conjugates of drug metabolites have been reported. For example, the 3,4-dihydroxy-5- methoxyphenylacetic acid metabolite of mescaline (Fig. 6.73) is found as a conjugate of glutamine. Diphenylmethoxyacetic acid, a metabolite of the antihistamine diphenhydramine, is biotransformed further to the corresponding glutamine derivative [120]. Several other amino acids are involved in the conjugation of carboxylic acids, but these reactions occur only occasionally and are highly substrate and species dependent [121].

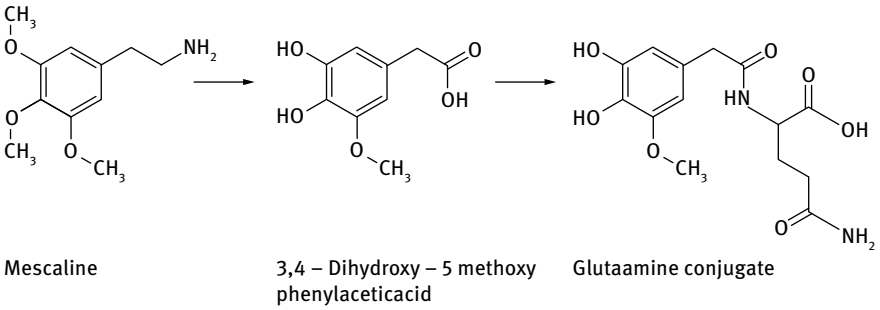


Fig. 6.73: Glutamine conjugation in mescaline.

6.6.4 GSH or mercapturic acid conjugates

GSH conjugation is an important metabolic reaction for detoxifying chemically reactive electrophilic compounds. It is accepted that reactive electrophilic species manifest their toxicity (e.g., tissue necrosis, carcinogenicity, mutagenicity, teratogenicity) by combining covalently with nucleophilic groups present in vital cellular proteins and nucleic acids. GSH protects vital cellular constituents against chemically reactive

species by virtue of its nucleophilic SH group. The SH group reacts with electrophilic compounds to form S-substituted GSH adducts [122, 123].

GSH is a tripeptide (γ-glutamyl-cysteinylglycine) found in most tissues. Drugs and phase I metabolites conjugated with GSH usually are not excreted as such, but undergo further biotransformation to give S-substituted N-acetylcysteine products called mercapturic acids. This process involves enzymatic cleavage of two amino acids (glutamic acid and glycine) from the initially formed GSH adduct and subsequent N-acetylation of the remaining S-substituted cysteine residue.

Conjugation with GSH is catalyzed by a family of cytoplasmic enzymes known as GSH S-transferases. These enzymes are found in most tissues, particularly the liver and kidney. Degradation of GSH conjugates to mercapturic acids is carried out principally by renal and hepatic microsomal enzymes. Unlike other conjugative phase II reactions, GSH conjugation does not require the initial formation of an activated coenzyme or substrate. The natural reactivity of the nucleophilic GSH toward an electrophilic substrate usually provides sufficient driving force. A major prerequisite to undergo GSH conjugation is that the substrate be sufficiently electrophilic [124].

Many aliphatic and arylalkyl halides, sulfates, sulfonates, nitrates and organophosphates possess electrophilic carbon atoms that react with GSH to form GSH conjugates. The carbon center is made electrophilic as a result of the electron-withdrawing group (e.g., halide, sulfate, and phosphate) attached to it. Nucleophilic displacement is facilitated when the carbon atom is benzylic or allylic or when there is a good leaving group (e.g., halide, sulfate). Many industrial chemicals, such as benzyl chloride, allyl chloride, and methyl iodide, are known to be toxic and carcinogenic. The reactivity of these three halides toward GSH conjugation is demonstrated by the formation of the corresponding mercapturic acid derivatives.

The metabolism of the immunosuppressive drug azathioprine to 1-methyl-4-nitro-5-(*S*-glutathionyl)imidazole and 6-mercaptopurine is an example of heteroaromatic nucleophilic substitution involving GSH. Interestingly, 6-mercaptopurine formed in this reaction appears to be responsible for azathioprine's immunosuppressive activity [125].

Arene oxides and aliphatic epoxides (or oxiranes) are a very important class of substrates that undergo conjugation and detoxification by GSH. The oxirane ring in these compounds is highly strained and, therefore, reactive toward ring cleavage by nucleophiles like GSH. Arene oxides and epoxides are intermediary products formed from CYP oxidation of aromatic compounds (arenes) and olefins, respectively. If reactive arene oxides and aliphatic epoxides are not "neutralized" or detoxified by GSH S-transferase, epoxide hydrase, or other pathways, they ultimately covalently bind to cellular macromolecules and cause serious cytotoxicity and carcinogenicity.

GSH conjugation involving substitution at heteroatoms, such as oxygen, is seen often with organic nitrates. For example, nitroglycerin and isosorbide dinitrate are metabolized by a pathway involving an initial GSH conjugation reaction. The GSH conjugate products, however, are not metabolized to mercapturic acids but instead

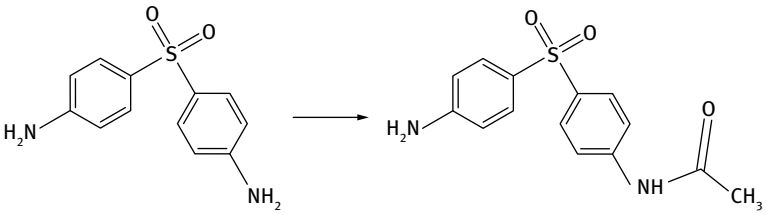
are converted enzymatically to the corresponding alcohol derivatives and glutathione disulfide (GSSG) [126].

In most instances, GSH conjugation is regarded as a detoxifying pathway that protects cellular macromolecules such as protein and DNA against harmful electrophiles. In a few cases, GSH conjugation has been implicated in causing toxicity. Often, this is because the GSH conjugates are themselves electrophilic (e.g., vicinal dihaloethanes) or give rise to metabolic intermediates (e.g., cysteine metabolites of haloalkenes) that are electrophilic. 1,2-Dichloroethane, for example, reacts with GSH to produce S-(2-chloroethyl)glutathione; the nucleophilic sulfur group in this conjugate can internally displace the chlorine group to give rise to an electrophilic three membered ring episulfonium ion. The covalent interaction of the episulfonium intermediate with the guanosine moiety of DNA may contribute to the mutagenic and carcinogenic effects observed for 1,2-dichloroethane. The metabolic conversion of GSH conjugates to reactive cysteine metabolites is responsible for the nephrotoxicity associated with some halogenated alkanes and alkenes [127].

6.6.5 Acetylation

Acetylation constitutes an important metabolic route for drugs containing primary amino groups for example, primary aromatic amines, sulfonamides, hydrazines, hydrazides, and primary aliphatic amines. The amide derivatives formed from acetylation of these amino functionalities are generally inactive and nontoxic. Because water solubility is not enhanced greatly by N-acetylation, the primary function of acetylation is to terminate pharmacological activity and detoxification [128].

The acetyl group used in N-acetylation is supplied by acetyl-CoA. Transfer of the acetyl group from this cofactor to the accepting amino substrate is carried out by soluble N-acetyltransferases present in hepatic reticuloendothelial cells. Other extrahepatic tissues, such as the lung, spleen, gastric mucosa, red blood cells, and lymphocytes, also show acetylation capability. N-Acetyltransferase enzymes display broad substrate specificity and catalyze the acetylation of several drugs and xenobiotics. Aromatic compounds with a primary amino group, such as aniline, p-aminobenzoic acid, p-aminosalicylic acid, procainamide, and dapsone (Fig. 6.74), are especially susceptible to N-acetylation. Aromatic amine metabolites resulting from the reduction of aryl nitro compounds also are N-acetylated. For example, the anticonvulsant clonazepam undergoes nitro reduction to its 7-amino metabolite, which in turn is N-acetylated. Another related benzodiazepam analog, nitrazepam, follows a similar pathway [129].



Dapsone Acetamido conjugate

Fig. 6.74: N-acetylation conjugation in dapsone.

The metabolism of several sulfonamides occurs mainly by acetylation at the N-4 position. In sulfanilamide, acetylation also takes place at the N-1 position. N-Acetylated metabolites of older sulfonamides tend to be less water-soluble than their parent compounds and have the potential of crystallizing out in renal tubules (crystalluria), thereby causing kidney damage. Newer sulfonamides are metabolized to relatively water-soluble acetylated derivatives, which are less likely to precipitate out [130].

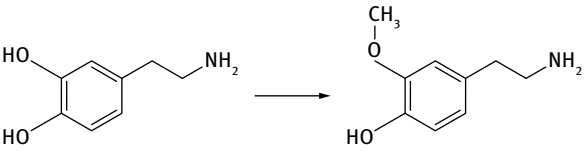
The biotransformation of hydrazine and hydrazide derivatives also proceeds by acetylation. The antihypertensive hydralazine and the MAO inhibitor phenelzine are two representative hydrazine compounds that are metabolized by this pathway. The antituberculosis drug isoniazid (INH) is metabolized extensively to N-acetylisoniazid [131]. In comparison with oxidative deamination processes, N-acetylation is only a minor pathway.

The acetylation pattern of several drugs (e.g., isoniazid, hydralazine, procainamide) in the human population displays a bimodal character in which the drug is conjugated either rapidly or slowly with acetyl-CoA. This phenomenon is termed acetylation polymorphism. Individuals are classified as having either slow or rapid acetylator phenotypes. This variation in acetylating ability is genetic and is caused mainly by differences in N-acetyltransferase activity. The proportion of rapid and slow acetylators varies widely among different ethnic groups throughout the world. Oddly, a high proportion of Eskimos and Asians are rapid acetylators, whereas Egyptians and some Western European groups are mainly slow acetylators. Other populations are intermediate between these two extremes [132].

6.6.6 Methylation

Methylation is a minor pathway for conjugating drugs and xenobiotics. Methylation generally does not lead to polar or water-soluble metabolites, except when it creates a quaternary ammonium derivative. Most methylated products tend to be pharmacologically inactive, although there are a few exceptions.

The coenzyme involved in methylation reactions is S-adenosylmethionine (SAM). The transfer of the activated methyl group from this coenzyme to the acceptor substrate is catalyzed by various cytoplasmic and microsomal methyltransferases. Methyltransferases of particular importance in the metabolism of xenobiotics include catechol-O-methyltransferase (COMT), phenol-O-methyltransferase, and nonspecific N-methyltransferases and S-methyltransferases. One of these enzymes, COMT, carries out O-methylation of important neurotransmitters such as norepinephrine and dopamine (Fig. 6.75) and thus terminates their activity. Besides being present in the central and peripheral nerves, COMT is distributed widely in other mammalian tissues, particularly the liver and kidney. The other methyltransferases mentioned are located primarily in the liver, kidney, or lungs. Transferases that specifically methylate histamine, serotonin, and epinephrine are not usually involved in the metabolism of xenobiotics [133].



Dopamine — 3 – Methoxy tyramine

Fig. 6.75: O-methylation conjugation in dopamine.

Xenobiotics that undergo methylation include catechols, phenols, amines, and N-heterocyclic and thiol compounds. Catechol and catecholamine-like drugs are metabolized by COMT to inactive monomethylated catechol products. Examples of drugs that undergo significant O-methylation by COMT include the antihypertensive (S)(-)α-methyldopa, the antiparkinsonism agent (S)(-)-dopa, isoproterenol and dobutamine. Substrates undergoing O-methylation by COMT must contain an aromatic 1,2-dihydroxy group (i.e., catechol group). Resorcinol (1,3-dihydroxybenzene) or p-hydroquinone (1,4- dihydroxybenzene) derivatives are not substrates for COMT. This explains why isoproterenol undergoes extensive O-methylation but terbutaline (which contains a resorcinol moiety) does not [134].

6.7 Factors affecting drug metabolism

Drugs are metabolized by different phase I and phase II pathways to give several metabolites and conjugates. The relative amount of any particular metabolite or conjugate is determined by the concentration and activity of the biocatalyst responsible for the biotransformation. The rate of metabolism of a drug is particularly important for its pharmacological action as well as its toxicity. For example, if the rate of

metabolism of a drug is decreased, this increases the intensity and duration of the drug action. In addition, decreased metabolic elimination may lead to accumulation of toxic levels of the drug. Conversely, an increased rate of metabolism decreases the intensity and duration of action as well as the drug's efficacy [135].

Many factors that affect drug metabolism include age, species and strain, hereditary factors, sex differences, enzyme induction and enzyme inhibition.

6.7.1 Age

In most newborn animals, metabolic enzymes are not fully developed and this results in reduction of oxidative or conjugative reactions. The ability to carry out metabolic reactions increases rapidly after birth and approaches adult levels in about 1 to 2 months. When hexobarbital (a dose of 10 mg/kg of body weight) is given to both newborn and adult mice, the newborn mouse will sleep for 6 hrs whereas adult mouse sleeps for 5 minutes [136, 137]. In humans, oxidative and conjugative capabilities of newborns are also low compared with those of adults. For example, the metabolic oxidation of tolbutamide is lower in newborns than in adults [138]. The inability of infants to conjugate chloramphenicol with glucuronic acid is responsible for the accumulation of toxic levels of this antibiotic, resulting in the gray baby syndrome [139]. In evaluating the effect of age on drug metabolism, one must differentiate between "normal" loss of enzymatic activity with aging and the effect of a diseased liver from hepatitis, cirrhosis, etc.

6.7.2 Species and strain differences

Species variation was observed in many oxidative biotransformation reactions. For example, amphetamine undergoes metabolism by two main pathways: oxidative deamination or aromatic hydroxylation. In human, rabbit, and guinea pig, oxidative deamination is the predominant pathway, whereas in the rat, aromatic hydroxylation is the more important route [140].

Phenytoin is another drug that shows marked species differences in metabolism. In the human, phenytoin undergoes aromatic oxidation to yield primarily (*S*)(−)-*p*-hydroxyphenytoin; in the dog, oxidation occurs to give mainly (*R*)(+)-*m*-hydroxyphenytoin. There is a dramatic difference not only in the position (i.e., *meta* or *para*) of aromatic hydroxylation but also in which of the two phenyl rings (at C-5 of phenytoin) undergoes aromatic oxidation [141].

Strain differences are caused by genetic variations in the amount of metabolizing enzyme present among the different strains. For example, *in vitro* studies indicate that cottontail rabbit liver microsomes metabolize hexobarbital about ten times faster than New Zealand rabbit liver microsomes [142].

6.7.3 Hereditary factors

Many hereditary factors are responsible for the large differences seen in the rate of metabolism of the drugs in humans. Hereditary factors influence the rate of oxidation of drugs such as phenytoin, phenylbutazone, isoniazid, dicumarol, and nortriptyline. The rate of oxidation of these drugs varies widely among different individuals; however, these differences do not appear to be distributed bimodally, as in acetylation. In general, individuals who tend to oxidize one drug rapidly are also likely to oxidize other drugs rapidly. Numerous studies in twins (identical and fraternal) and in families indicate that oxidation of these drugs is under genetic control [143].

Many patients state that they do not respond to codeine and codeine analogs. Now it is realized that their oxidative metabolic enzyme does not readily *O*-demethylate codeine to form morphine. This genetic polymorphism is seen in about 8 % of Caucasians, 4 % of African Americans, and less than 1% of Asians [144].

6.7.4 Sex differences

Sex differences in drug metabolism are species dependent. Rabbits and mice, for example, do not show a significant sex difference in drug metabolism. In humans, there have been a few reports of sex differences in metabolism. For instance, nicotine and aspirin seem to be metabolized differently in women and men. On the other hand, gender differences can become significant in terms of drug–drug interactions based on the drug's metabolism. For women, the focus is on drugs used for contraception. The antibiotic rifampin, a CYP3A4 inducer, can shorten the half-life of oral contraceptives [145].

6.7.5 Enzyme induction

The process by which the activity of drug-metabolizing enzyme increases is termed enzyme induction. Exposure to certain drugs, pesticides, polycyclic hydrocarbons and environmental pollutants will increase the activity of drug-metabolizing enzymes particularly mixed-function oxidases. Enzyme induction increases the rate of drug metabolism and decreases the duration of drug action [146].

Inducing agents may increase the rate of their own metabolism as well as those of other unrelated drugs, endogenous compounds and xenobiotics. Co-administration of two or more drugs may lead to serious drug interactions as a result of enzyme induction. For example, a clinically critical drug interaction occurs with phenobarbital and warfarin. Induction of microsomal enzymes by phenobarbital increases the metabolism of warfarin and markedly decreases the anticoagulant effect [147]. The ineffectiveness of oral contraceptives in women on phenobarbital or rifampin therapy

has been attributed to the enhanced metabolism of estrogens (17α-ethinylestradiol) caused by phenobarbital and rifampin induction [148, 149]. The enhanced metabolism of vitamin D3 induced by phenobarbital and phenytoin is one of the reasons for the osteomalacia seen in patients on long-term therapy with these two anticonvulsant drugs [150].

6.7.6 Enzyme inhibition

Several drugs, xenobiotics including grapefruit, and other foods can inhibit drug metabolism. With decreased metabolism, a drug accumulates, leading to prolonged drug action and serious adverse effects. Enzyme inhibition can occur by diverse mechanisms, including substrate competition, interference with protein synthesis, inactivation of drug-metabolizing enzymes, and hepatotoxicity leading to impairment of enzyme activity. Some drug interactions resulting from enzyme inhibition have been reported in humans. For example, phenylbutazone stereoselectively inhibits the metabolism of the more potent (*S*)(-)enantiomer of warfarin. This inhibition may explain the excessive hypoprothrombinemia and many instances of hemorrhaging seen in patients on both warfarin and phenylbutazone therapy. The metabolism of phenytoin is inhibited by drugs such as chloramphenicol, disulfiram, and isoniazid. Interestingly, phenytoin toxicity as a result of enzyme inhibition by isoniazid occurs primarily in slow acetylators [151].

The grapefruit–drug interaction is complex. It may be caused by the bioflavonoids or the furanocoumarins. Grapefruit's main bioflavonoid, naringin, is a weak inhibitor, but the product of the intestinal flora, naringenin is a powerful inhibitor [152].

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7 Mistletoe lectin: A promising cancer therapeutic

Abstract: Mistletoe is a group of obligate plant semi-parasites. Since ancient times, it is regarded as a medicinal plant, but current empirical studies have validated its therapeutic relevance. The biological roles have been attributed to the phytochemicals, namely, alkaloids, viscotoxins, triterpenoids, lectins and polysaccharides. Its anticancer effect has attracted most attention. Its prophylactic and curative effects against oral, breast, lung, pancreas and colon cancers have been observed. The cancer manipulative mechanisms include immune augmentation, tumor prevention, malignant tissue inhibition, moderation of chemotherapeutics side effects and DNA protection. So, overall as an adjunct therapy, it has been documented to promote patient quality of life. The mistletoe-based formulations such as Iscador, Isorel, Iscucin, Lektinol, Eurixor, Helixor, Abnoba-viscum and recombinant lectin ML-1 have been approved for commercial use. In this chapter, the anticancer rationale of mistletoe lectins (the carbohydrate-binding proteins) is discussed. Its mechanism of discrimination between cell surface antigens of normal and cancer cells is emphasized. The triumphs so far, roadblocks in pharmaceutical formation and possible fixes are outlined. The seminal findings in this field are presented here.

7.1 Introduction

Mistletoes are semi-parasitic plants in the family Santalaceae, Loranthaceae and Viscaceae of Order Santalales [1–3]. These plants vary widely in their appearance, yet most of they are characterized to have rudimentary leaves. The leaves have chlorophylls for photosynthesis, but nutritional requirement has adapted these plants to be host-dependent [4]. They have developed haustoria to siphon off nutrients from host plant xylem. These plants bear flowers and white or red berries [5] (Fig. 7.1). They grow on a variety of host trees such as sycamore, oak, eucalyptus, beech, poplar, spruce, rosewood, maple, mesquite, hawthorn, ash, sweetgum, willow, elm, linden, pine, juniper, buckeye, cottonwood, apple, almond, plum, cacao [5, 6]. Phytogeographically, mistletoes are distributed across the globe. Depending on their origin, the mistletoes have been named European (*Viscum album*), American (*Phoradendron*), Mexican (*Psittacanthus*), African (*Loranthus*), Korean, Indian (*Dendrophthoe*) etc. Some species of mistletoe found in the USA are presented in Fig. 7.1.

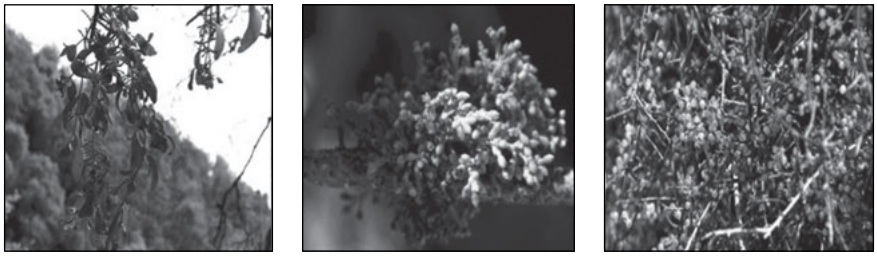

Fig. 7.1: Mistletoe plants growing in different regions of USA: **(A)** bigleaf mistletoe (*Phoradendron macrophyllum*), **(B)** dwarf mistletoe (*Arceuthobium sp.*), **(C)** desert mistletoe (*Phoradendron californicum*).

The group mistletoe encompasses hundreds of species. Some of them have been investigated for their biological significance. Viscum, Phoradendron, Arceuthobium, Amylotheca, Amyema, Peraxilla, Loranthus, Taxillus, Psittacanthus, Dendrophthoe and Scurrula are the oft-studied species. A recent review has holistically discussed their scopes in healthcare [7]. In herbal medication, mistletoes are used to treat epilepsy, hypertension, headaches, sore throat, lumbago, menopausal symptoms, infertility, diarrhea, diabetes, arthritis, rheumatism; also as aphrodisiac and narcotic [3, 8]. The immunomodulation and anticancer potential of mistletoe have been confirmed recently. Cancer is a dominant cause of mortality with tissue heterogeneity, stem cell resistance and metastasis major deterrents in conventional therapeutic regimen [9]. As the existing anticancer drugs are deficient in curing many forms of cancer and confer toxicity, benign adjunct therapies are being sought after. In this regard, mistletoes might be an untapped resource, with an interesting phytochemical repertoire. In fact, several mistletoe extracts have been approved as commercial drugs such as Iscador, Isorel, Iscucin, Eurixor, Helixor, Lektinol, Abnoba-viscum and recombinant lectin ML-1 [10, 11]. A number of reviews have discussed different facets of mistletoe in cancer mitigation [12]. More specifically, the safety and efficacy of Iscador [10], ameliorative impact on patient [13, 14], recombinant lectin aviscumine [15], lectins in modulation of apoptosis [16], meta-analysis of clinical trials [17] and macrophage activated-cytotoxicity [18] have been explored.

An array of phytochemicals such as alkaloids, viscotoxins, triterpenoids (oleanolic, betulinic acid, gallic acid, morolic acid, flavonoid (pachypodol, ombuine), saponins, β-sitosterol, stigmasterol, triacontanol, squalene, α- and β-amyrin, lupeol, lupenone, lectins and polysaccharides have been isolated from mistletoe [19–22]. This review discusses the significance of lectins, with due emphasis on its chemical aspects.

Lectins are ubiquitous carbohydrate-binding proteins, expressed in a wide range of organisms, required for recognition of carbohydrates [23]. Lectins have been detected in cells, membranes, and secretomes of all living organisms [24]. The high

specificity of plant lectins for foreign glycoconjugates (e.g. those of fungi, invertebrates and animals) mediates their pattern recognition [25]. Crucial roles of lectins in cell signaling and host-pathogen crosstalk has been well-substantiated [26]. Lectins binding to mannose, N-acetylgalactosamine, N-acetylglucosamine, N-acetylneuraminic acid and fusose have been identified so far. The Fabaceae (legume) family lectins are the most investigated [27]. Among the numerous lectins, the well-studied include concavalinA, lentil lectin, snowdrop lectin, ricin, peanut agglutinin, jacalin, hairy vetch lectin, wheat germ agglutinin, elderberry lectin etc.

7.2 Anticancer potency of bacteria/plant lectins

Diverse physiological roles of bacteria and plant lectins have been recognized. The functional variations stem from the sequence, domain, binding site and carbohydrate affinity heterogeneity [28]. In this review, focus has been laid on the relevance of lectins in cancer diagnosis and treatment.

Cyanobacteria *Microcystis viridis* has showed capacity to inhibit Hepatitis C virus by attaching to its glycosylated envelope proteins [29]. The vasorelaxant role of *Canavalia grandiflora* seed lectin has been reported [30]. The hypoglycemic and renal/hepatic ameliotrative effect by *Crataeva tapia* bark lectin has been shown [31]. Also, lectins play a pivotal role in developing assays for biomarker detection for many pathological conditions. Mushroom lectin-oligosaccharide interaction was successfully used to detect aberrant immunoglobulin G (IgG) glycosylation in Crohn's disease [32].

Plant lectins exert their anticancer effects by multifarious ways. Some of the pronounced modes are selective binding to cancer cell membranes receptors, causing cytotoxicity, shrinkage of tumor, apoptosis induction and caspase cascade activation [33]. Polyamine sequestration by lectins is considered another pathway of cancer growth inhibition. Inhibition of protein synthesis, down-regulation of telomerase activity and angiogenesis inhibition are some other mechanisms [33]. An intraperitoneal injection of *Momordica charantia* lectin at a dose 1 mg/kg/d daily could bring about 45 % remission of nasopharyngeal carcinoma xenograft tumors in nude mice [34]. *In vitro* assay showed the mechanism to be cytochrome c release and DNA damage-mediated intrinsic pathway.

The potency of *Lens culinaris* lectin in patients with breast cancer undergoing adjuvant hormone therapy has been proved [35]. Dioscorea lectin evoked apoptosis in human breast carcinoma MCF7 cells, mediated by induction of phosphatidylserine externalization and mitochondrial depolarization [36]. Chinese pinto bean lectin was purified and its anti-proliferative effect on nasopharyngeal carcinoma HONE-1 cells was demonstrated [37]. The effect of concanavalin A and *Sophora flavescens* (a legume) lectin on MCF-7 cells *in vitro* and in mice models was examined. Dose-dependent increment in the activities of caspase-3 and caspase-9 was observed. Up-regulation of Bax and Bid, and down-regulation of Bcl-2 and Bcl-X_L in the cancer cells was

monitored. In the mice study, the tumor showed shrinkage [38]. The effect of peanut agglutinin on HeLa cells as well as Dalton's lymphoma-bearing mice was evaluated [39]. Data showed that the agglutinin at a dose of 0.1–100 μg/ml prevents proliferation of HeLa cells. Excess ROS release was correlated to cell death. Autophagy has been discovered as another strategy in lectin-mediated anticancer effect. Unprecedented amount of work on this aspect has been carried out in recent times. Evidence of apoptosis and autophagy induction by lectins, via signaling pathway (Bcl-2 family, caspase family, p53, PI3K/Akt, BNIP3, Ras-Raf, ERK, and ATG families) modulation has emerged [40]. The sections below summarize the therapeutic potency and hurdles of mistletoe lectins. How given due research input, it might revolutionize cancer therapy is the focal point.

7.3 Anticancer potency of mistletoe and the underlying mechanisms

The anticancer action of mistletoe is multifaceted encompassing antimutagenic, anti-angiogenesis, antiproliferation, apoptosis, drug side-effect amelioration, post-surgery supportive care (better coping, fatigue alleviation, sleep induction, anti-depression, anxiolytic, emotional well-being) [7]. All these benefits in different models have been discussed succinctly.

The cytotoxicity of *V. album* extract on a human umbilical vein endothelial EA.hy926 cell line was determined [41]. The extract inhibited angiogenesis by manipulating vessel formation mechanism. Angiogenesis is based on activation of endothelial cells by angiogenic factors followed by basement membrane dissolution and subsequent migration of the cells toward the angiogenic signal. It leads to uncontrolled cell proliferation and formation of new blood vessels [42]. Mistletoe extract might be interfering with any of the above steps. A number of triterpenoid saponins (ursane, lupane, hopane, dammarane and germanicane) have proven their potential in apoptosis and tumor reduction [43]. In this regard, the triterpenoids saponins of *V. liquidambaricolum* were isolated and identified that exhibited cytotoxic activities against four human tumor cell lines (HeLa, SGC-7901, MCF-7, and U251) [44]. In many studies, the apoptosis has been mediated by cyclooxygenase-2/prostaglandin E2 (COX-2/PGE2), so it might have been the mechanism of above mistletoe saponin. Flavonoids, the plant polyphenols have been widely validated for their multifarious pharmacological roles. They can prevent onset, proliferation promotion and progression of cancer by multiple pathways, including signal transduction modulation [45]. The cytotoxic activities of *V. coloratum* flavonoid compounds, pachypodol and ombuine were determined against four human tumor cell lines (HeLa, SGC-7901, MCF-7, and U251) and promising results were obtained [46]. The anti-proliferative activities of the aqueous-ethanol extract of *T. sutchuenensis* on human lung adenocarcinoma A549 cells were evaluated [47]. The ethyl acetate fractions, owing to its abundant phenolic

compounds, exerted maximum antiproliferation. The effect of a commercial preparation of *V. album* extract was investigated on different leukemia cell lines (human myeloid leukemia K562, human plasmacytoma RPMI-8226 and murine lymphocytic leukemia L1210) [48]. The treatment induced apoptosis of the studied cells in culture, the mechanism being intrinsic. The protective effect of mistletoe extract on human peripheral blood mononuclear cells (PBMC) and the T-cell leukemia Jurkat cell line against alkylation was evaluated [49]. Exposure of the cells to the extract for 60–65 h followed by the alkylating agent resulted in differential activity. The PBMC cells were unaffected whereas Jurkat cells suffered loss of mitochondrial activity. The effect of *V. album* extract on B cell lymphoma cell line WSU-1 was found to be anti-proliferation, apoptosis and necrosis-mediated [50]. The triterpene extract of mistletoe, rich in oleanolic acid exerted cytotoxicity on mouse melanoma B16-F10 cells by inducing DNA cleavage, membrane disintegration and intracellular adenosine-5'-triphosphate [51]. Pretreatment with methanolic extract of *V. album* subdued the effect of chaperone proteins and increased apoptosis via caspase-3 activation in C6 glioma cells [52].

In recent times, polysaccharides from diverse sources have been isolated and characterized to have anticancer effects [53]. In this direction antitumor polysaccharides were extracted from *Scurrula parasitica*. Intraperitoneal administration in mice led to inhibition of S180 tumor growth to 54 % of the untreated tumor [54]. Induction of oxidative stress in the Ehrlich carcinoma (EAC) cells was found to be the *in vivo* cytotoxic mechanism of mistletoe leaf extracts [55]. The ethanolic extract of *P. serotinum* when injected to mice, implanted with mouse tumor TC-1 cells for 25 consecutive days, cytotoxicity was observed [56]. Oral administration of mistletoe extract at a dose 250 mg/kg daily for 10 days caused reduced chromosomal aberrations in mouse bone marrow cells [57].

Most cancer patients experience fatigue, which degrades their quality of life. So, the anti-fatigue effect of mistletoe extract was investigated [58]. A breast cancer patient with a history of decade-long recurrence was administered with this extract for two and half years. A dose-dependent reduction in fatigue was observed. Metastasis is a critical factor in therapeutic success of cancer treatments. To evaluate the efficacy of mistletoe extract against pancreatic complications, a case study was conducted [59]. After more than nine months of surgery and discontinuing chemotherapy, relapse was not detected. The remission was inferred to the mistletoe therapy. Adherence to 30 months of mistletoe therapy led to complete alleviation of anaplastic lymphoma [60]. Analysis of a questionnaire-based survey on the effect of mistletoe on cancer patients revealed better ability to cope with the psychological and physical issues [61]. Mistletoe extract at the dose 10 mg/kg inhibited the tumor growth of metastatic pancreatic cancer patients by 69 %. Elevated release of IL-2, IL-6 and IFN-γ was associated with the disease attenuation and improved survival [62].

7.4 Mistletoe lectins

In the previous sections the anticancer scopes of plant lectins as well as mistletoe extracts have been reviewed. Here, the anticancer effects of mistletoe lectin and associated mechanisms, mostly immunomodulatory aspects have been discussed. Before diving into those topics, it is important to have a fair knowledge on the structural diversity of the mistletoe lectins.

7.4.1 Phytochemistry of mistletoe lectins

Lectins belong to the family of ribosome inactivating proteins (RIPs), which encompass neural toxins like ricin and volkensin. Lectins fall under type II RIP to be precise, characterized by comparatively less toxic compounds with biological significance. Like other RIPs, they possess RNA glycosidase activity, capable of inhibiting protein biosynthesis at the ribosomal level. Due to divergent evolution, the lectins are immensely diverse. Lectin is a heterodimer of chains A and B, with catalytic and sugar binding properties, respectively [63]. Binding assays have shown that mistletoe lecithin is a sialic acid-specific lectin [64]. Analysis of its 3D structure showed its similarity to shiga toxin from the pathogen *Shigella dysenteriae* [65]. The lectins show different degrees of amino acid sequence similarities and carbohydrate specificities (galactose or N-acetyl-D-galactosamine) [66]. Evolutionarily, chain A is conserved and chain B is immensely diverse. Chain A and B are joined by a disulfide bond [67]. RIPs exert glycosylase activity on many nucleic acid substrates, which leads to the arrest of protein synthesis [68]. Chain A exhibits RNA-glycosidase activity [69] and chain B possesses lectin activity. Both components are essential for eliciting cytotoxicity and immunomodulation [70]. Three types of mistletoe lectins have been identified based on their amino acid sequence conservation, molecular mass and glycosylation patterns. The three categories are MLI, MLII, and MLIII, encoded by ml1p, ml2p, and ml3p genes, respectively. In terms of transcription, gene ml1p is most dominant, correlating well with the abundance of MLI compared to MLII and MLIII in mistletoe extract [71]. Due to sequence polymorphism, some epitopes are missing among the B chain of the three lectin types [72]. It partially explains their sugar specificity. Among the three isoforms, MLI is the most-represented mistletoe lectin, thus most characterized too. Despite being phylogenetically close to ricin it is less toxic, attributed to the amino acid polymorphism near catalytic site, galactose binding site and the bond between chain A and B [73]. Table 7.1 lists the types of lectins from *V. album* and their characteristics [74]. A symbolic diagram of the mistletoe lectin conjugate structure is illustrated in Fig. 7.2.

Table 7.1: Three isoforms of mistletoe lectins and their properties.

Mistletoe lectin types	Encoding genes	Specificity/recognition	Mol. Weight in kDa	References
MLI	ml1p	High galactose Low N-acetyl-D-galactosamine	11.5	Franz et al. 1981 [74] Franz 1991 [66]
MLII	ml2p	Comparable affinity to both galactose and N-acetyl-D-galactosamine	60	Krauspenhaar et al. 1999 [69]
MLIII	ml3p	High N-acetyl-D-galactosamine Low galactose	50	Kourmanova et al. 2004 [71]

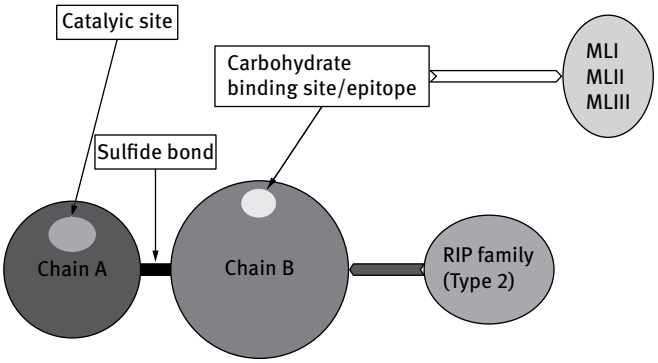

Fig. 7.2: The dimeric mistletoe lectin. Chain A is catalytic and conserved whereas chain B is carbohydrate binding and variable in its amino acid residues.

7.4.2 Anticancer mechanisms of mistletoe lectins

A very interesting review on anticancer roles of mistletoe lectins has been published. It regards anti-angiogenesis and apoptosis as the most important factors in tumor inhibition [75]. A decade later, the perception has changed and many other pathways have been implicated. The most crucial ones as validated through *in vitro*, *in vivo* and clinical trials are evaluated below.

The mechanism of human hepatocellular carcinoma SMMC-7721 cells death induced by recombinant agglutinin-I isolated from *V. album* was investigated [76]. Apoptosis occurred mediated by modulation of MAPK signaling pathways. The effect of lectin from *V. album* subsp. *coloratum* on cytokine gene expression in human colon adenocarcinoma Caco-2 cells was examined [77]. Up-regulation of the gene expression of the proinflammatory cytokines interleukin (IL)-8, tumor necrosis factor-alpha (TNF-α) and IL-6 was found to cause inhibition of the cancer cells. The anti-proliferative effect *V. album* subsp. *coloratum* lectin on HepG2A cells was analyzed. A dose

as low as 1–5 pg/ml could exert specific toxicity towards cancer cells [78]. Iscador Q attenuated the migratory and invasive potential of glioblastoma LNT-229 and LN-308 cell line [79].

A human pancreatic carcinoma PAXF 736 cell line xenografted-nude mice showed that intra-tumoral injection of mistletoe lectin extract is more efficacious than intravenous administration of the anticancer drug gemcitabine [80]. Based on the results, its inclusion in therapy against inoperable locally advanced pancreatic cancer was suggested. The effect of mistletoe lectin on human melanoma MV3 cell line xenografted-severe combined immunodeficient mice was evaluated [81]. Almost three weeks of administration was followed by euthanasia and tumor parameter analyses. Direct correlation of low dose extract and tumor shrinkage was observed. The effect of Chinese mistletoe lectin on colon cancer CT26-bearing BALB/c mice was investigated. Colon cancer progression was slowed down, which could be linked to higher proliferation of CD4+ and CD8+ T cells and increment in NK cells [82].

The effect of intravesical administration of mistletoe lectin to post-surgery bladder cancer patients was monitored [83]. Injection of the 10–5,000 ng/ml extract and bladder retention for 2 h, prevented relapse in 70 % of the total number of patients. The effect of Iscador on nonmetastatic colorectal carcinoma patients was evaluated through a cohort study and a beneficial effect was suggested [84]. A meta-analysis was conducted to evaluate the effect of Iscador, which unveiled short-time benefi-

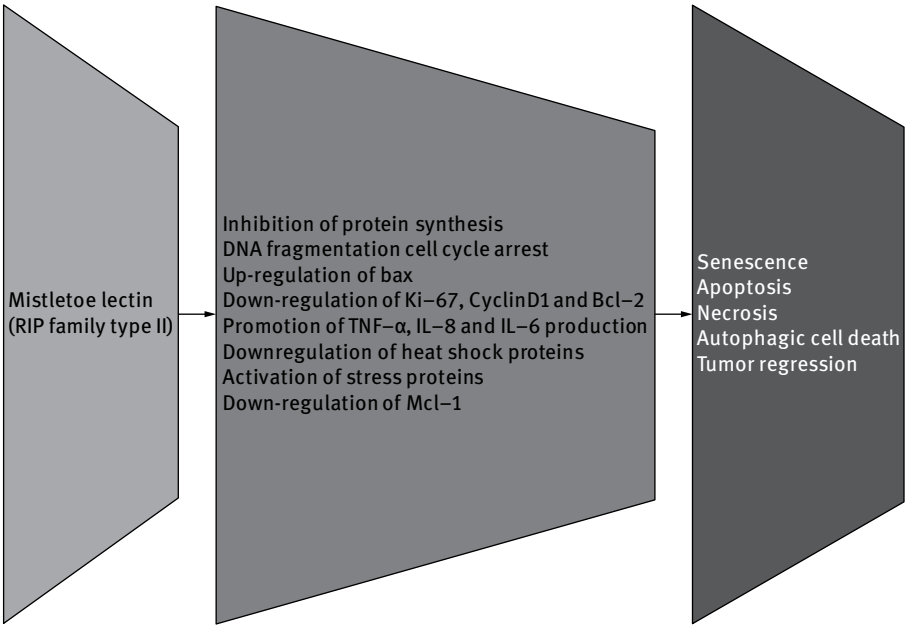

Fig. 7.3: Biochemical and immunological changes involved in the anticancer effect.

cial psychosomatic effect [85]. A questionnaire-based investigation on the usage of complementary and alternative medicine (CAM) in lung cancer patients revealed that out of the 54 % CAM users, 15 % used Iscador [86]. Mistletoe lectins when administered subcutaneously to sarcoma patients, at a dose 0.75–1.0 ng/kg, tumor remissions occurred. Binding of the lectin to CD75 ganglioside receptors of macrophages or dendritic cells and subsequent elaboration of interleukins was unveiled to be the antitumor mechanism [87].

Further, the antimutagenic potential of *V. album* L. var. coloratum agglutinin was verified in *Salmonella typhimurium* model [88]. The key anticancer mechanisms are illustrated in Fig. 7.3.

7.4.3 Immunomodulatory effect of mistletoe lectins

Evidence is emerging that many anticancer drugs exert their effect by immunomodulation. So, it seems relevant to understand the immune-stimulation properties of mistletoe lectins. Studies have shown that this lectin manipulates both innate and adaptive immune systems. In this regard, the *in vitro* and *in vivo* immunomodulatory effects of *V. album* coloratum lectin were investigated. On mitogen sensitization, it increased lymphocyte proliferation and promoted the splenic NK cell and macrophage activities. Mice study showed the increment in spleen and thymus mass [89]. Yet another study on this plant lectin confirmed immunomodulation effects. The effect of Korean mistletoe lectin on cytokine and immunoglobulin secretion was investigated. In LPS-stimulated rat small intestinal epithelial IEC-6 cell line, the lectin perturbed the IL-2, IL-5, IL-6, and tumor necrosis factor-alpha (TNF-α) secretion and skewed the Th1/Th2 balance. It led to higher IgA production from mucosal tissues and bolstered defense against pathogens [90]. Mistletoe lectin-based drugs demonstrated NK cell-mediated lysis of glioblastoma cells [79].

7.4.4 Hurdles associated with mistletoe lectins

The flurry of research undertakings on mistletoe lectins has unleashed exciting possibilities in healthcare. However, many drawbacks must be addressed before mass administration becomes a reality. Adjunct therapy using mistletoe is rampant, but, Food and Drug Administration (FDA) restricts its use in conventional cancer therapy, citing its lethal side effects. Clinical trials corroborating safety of mistletoe are scanty. The heterogeneity of extract composition has been advocated to be the reason behind the inconsistent results. Identification of several isoforms with distinct biological activities was cited as the reason for fluctuating effects [91]. This study outcome emphasized the importance of constituent and dosage standardization [92]. Possible risk of adverse reactions needs to be assessed.

7.5 Future directions

In order to fully appreciate the scope of mistletoe lectins, some major areas amenable to improvements are mentioned below. Effective purification, synergy and diagnostic study seem crucial for promoting the viability of lectin in healthcare.

Purification of lectins and subsequent structure elucidation is an important step in exploitation of their anticancer prowess. HPLC separation, Edman degradation and peptide sequence conformation by MALDI-mass spectrometry (MS) have been effective in the above objectives [93]. As lectins are highly specific and their functionality hinges on structure, technical improvement will bring significant effects. Functional differences in the three different lectin types deserve to be analyzed.

Mistletoe lectins have shown functional compatibility with other therapeutics, which might be explored to improve their contribution to oncology. Aqueous mistletoe extract in combination with doxorubicin could induce dose-dependent DNA fragmentation of Jurkat cells [94]. The combination of mistletoe lectin extract with triterpene acid extract has augmented apoptotic effect on acute lymphoblastic leukemia NALM-6 cells [95]. The synergistic anti-proliferative efficacy of mistletoe lectin type I and the peroxisome proliferator-activated receptor (PPAR-γ) ligand rosiglitazone in malignant melanoma cells was investigated [96]. Results of cell proliferation assay showed that the combined therapy is more effective than individual treatment. The combination of mistletoe drugs with other pattern recognition receptor (PRR) ligand drugs was advocated to increase its efficacy in adjuvant or even primary cancer therapy [65]. The safety and efficacy of a standardized mistletoe extract injection paired with oral administration of doxifluridine to post-surgery gastric cancer patient was evaluated [97]. Continuation of the combination therapy for 24 weeks improved immune status of the patients and lowered their diarrhea incidences. As the standard cancer drugs often cause neutropenia, benefits of using Iscador as adjuvant with gemcitabine werw investigated. As hypothesized, Iscador significantly improved the neutrophil counts of post-surgery pancreatic carcinoma patients [98]. Concurrent administration of chemotherapy and Iscador to non-small-cell lung cancer patients reduced the chemotherapy dosage and ameliorated many side effects associated with only chemotherapy use [99]. The above findings encourage the evaluation of the therapeutic efficacy and safety of pairing mistletoe lectins with other standards of care like chemo-, radio-, and hormonal therapy.

Timely diagnosis of cancers can significantly reduce the cancer mortality rate. Also, sustained drug release can more efficiently combat tumors. Mistletoe lectins can be harnessed for tumor diagnosis and drug delivery, like some other established phytolectins. Glycan profiling by the lectin microarray can be a reliable tool for tumor diagnosis. The lectin microarray analysis using grade 3, poorly differentiated tumor tissues could be determined based on the signal pattern of three lectins (*Dolichos biflorus* agglutinin, *Amaranthus caudatus* agglutinin, and *Bauhinia purpurea*) [100]. The immobilization of wheat germ agglutinin on a polymer matrix (PLGA) resulted in

strong cytostatic effect towards urothelial 5637 human BCa cancer cells [101]. A sensitive, rapid and reproducible biosensor was developed using *Sambucus nigra* agglutinin and peanut agglutinin for cancer diagnosis [102]. The antibody-lectin-based sandwich assay detected cancer antigen 15-3 in clinically important range. Wheat germ agglutinin is recognized and internalized by transformed cells. It was reported that agglutinin shows significant affinity towards four metal-based anticancer drugs, cisplatin, Pt porphyrin and two gold porphyrins. This information can be exploited for efficient drug delivery in cancer [103]. The administration routes are variable, such as oral, subcutaneous, local injection, and systemic. So, it might be a success-determining factor and ought to be optimized for maximum anticancer efficacy. Also, feasibility of tissue culture, efficiency enhancement and toxicity reduction approaches might advance cancer research. For example, *in vitro* lectin production from callus cultures of *V. album* L. var. coloratum was accomplished [104]. To nullify the undesirable effect of multiple isoforms in the lectin extract, the development of recombinant lectins might be pursued. This objective of generating lectins with fixed amino acid sequence has met with moderate success and needs to be tested further [91]. Only a few mistletoe varieties have been investigated so far for mining potential drug leads. Dwarf mistletoe and desert mistletoe have hardly been studied for functional lectin. So, bioprospecting is very important for the discovery of novel mistletoe lectins. Just recently, the anti-mitotic potential of *Phoradendron flavescens* aqueous extract was validated. As the well-known anticancer agent paclitaxel is a microtubule-binding, anti-mitotic agent, this mistletoe might have lectins of therapeutic importance [105].

7.6 Concluding remarks

As existing drugs are left inadequate by cancer heterogeneity, drug resistance, and the deleterious side effects, the urgency for development of novel candidates is perceived. In this regard, plant lectins have been pushed to the forefront of diagnostic and therapeutic research. Literature search clearly reflects the meager amount of work conducted on mistletoe lectins, despite their promising results. This lacuna motivates to take stock of the investigations done and to be done. How the toxicity can be attenuated and potency magnified remains the crux of its pharmaceutical utility. By borrowing cues from other clinically-relevant lectins and taking advantage of innovative technologies, its therapeutic implications can be broadened. This chapter is expected to be instrumental in enriching cancer research and facilitating the incorporation of this anthroposophical medicine into mainstream medication.

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Tamara Angelo* and André São Pedro

8 Antipsychotics

Abstract: Also known as neuroleptics, anti-schizophrenic and major tranquillizers, antipsychotics are drugs that selectively modify the central nervous system in order to treat psychotic syndromes, i.e. mental disorders of unknown or idiopathic origin. Among psychotic illness, schizophrenia is one of the most severe disorders due to its chronic and disabling characteristics. It is estimated to affect about 1% of the population, with a high rate of suicide: 10 to 20 times more likely than in the general population. Studies have proved that neuroleptic medication can be used to manage this psychosis due to the drug binding to brain receptors instead of, or in addition to D2 and 5HT2A, including α-1-adrenergic, α-2-adrenergic, dopamine D3, histamine H1, muscarinic, and serotonin 5-HT1A, 5-HT1D, 5-HT2C and 5-HT6 receptors. However, these bindings are not always related to therapeutic effect. Also they may lead to several adverse effects. This chapter highlights the structural-activity relationship of different classes of typical and atypical antipsychotic drugs and provides some considerations concerning new treatment research.

8.1 Introduction

Also known as *neuroleptics*, *anti-schizophrenic* and *major tranquillizers*, antipsychotics are drugs that selectively modify the central nervous system and are used in the treatment of mental disorders. Their discovery and development represented one of the greatest steps in Psychiatry. The drugs do not heal, but relieve many symptoms of mental disorders, making it possible for patients to lead meaningful lives without leaving their communities, as was the only option for most of them before [1–3].

8.2 History

The history of antipsychotics is related to the history of another group of drugs: antimalarial. For centuries the tropical plant cinchona was used to treat the symptoms of malaria. In 1820, Pelletier and Caventou isolated cinchona's alkaloidquinine, which was the only substance available against this disease for decades. Since during World War I access to medicines was difficult, synthetic alternatives were sought [2]. During this searching, the German bacteriologist Paul Ehrlich observed antimalarial effects of a phenothiazine derivate named methylene blue. Later on, it was discovered that this group of compounds had different properties like antiseptic and antihistaminic. Based on that, scientists started to synthesize several modified molecules and some of them had low antimalarial activity, but great antihistaminic effects [4, 5].

In those days, there was a belief that the histamine released during anesthesia caused sudden death. Following this premise, in 1950, the French surgeon Laborit and his colleague, the anesthetic Huguenard, studied the use of antihistamines to protect patients from adverse effects of anesthesia. Their research included promethazine and it was observed that this compound caused calming effect in the patients, unlike sedation. These findings led the physicians to test other drugs until reaching chlorpromazine, which showed a highly calming effect. The surgeons then published their research and suggested the use of this drug in Psychiatry [2, 6].

In the light of these findings, in 1952, two groups of physicians began to administer chlorpromazine to their patients – the neuropsychiatrists Hamon, Paraire and Velluz at Val de Grâce military hospital in Paris and Delay, Deniker and Harl at Saint-Anne's hospital, also in Paris. The use of crescent doses of the drug allowed the observation that chlorpromazine promoted improvement in agitated, anxious and schizophrenic patients and did not induce sleep or alter the conscience, even at high doses. Further studies showed that chlorpromazine was not limited to just a calming effect, but also had action in disorders of schizophrenic thinking. In fact, chlorpromazine was the first selective and effective treatment for schizophrenic patients [5, 6].

By that time, in the United States of America, a phytochemical substance obtained from *Rauwolfia serpentina*, so-called reserpine, had also gained prominence in psychiatric treatment, with the studies of Kline and Hollister. Thereby, chlorpromazine and reserpine were introduced in Europe and North America, simultaneously [3, 7].

The notable clinical results of these drugs inspired researchers to search for other substances with antipsychotic action. These great efforts on the study of drugs for treating mental disorders led to the beginning of the "psychopharmacological revolution" [5]. In this way, in 1958, Janssen Laboratories synthesized haloperidol, which remains one of the most used antipsychotic worldwide. Since then, researchers are seeking to understand the causes and mechanisms involved in mental disorders so they can develop more effective and safe medications [2, 7].

8.3 Etiology of schizophrenia and related psychoses

Psychosis is a term related to mental disorders of unknown or idiopathic origin. In most cases, the orientation and memory are preserved, but the emotions and thoughts are compromised. There are numerous theories regarding these syndromes. One of the most accepted hypotheses is associated with the relation between neurotransmitters and their receptors, leading to a variety of neuronal functions, with biochemical, physiological and psychological repercussions [8–11].

Among psychotic illness, schizophrenia is one of the most severe disorders due to its chronic and disabling characteristic processes. It is estimated to affect about 1 % of the population, with a high rate of suicide: 10 to 20 times more likely than in the general population. Its most common manifestations are classified into two catego-

ries: positive and negative symptoms, which may be experienced in different degrees. Positive symptoms, like hallucinations and delusions, are reflected in losing touch with reality. On the other hand, negative symptoms are related to deficits in normal functions and feelings, manifesting as disorganized speech, deficits in emotion, reduced social interaction and abnormal modes of expression, as examples [12–16]. Clinical diagnostic criteria for schizophrenia include two or more symptoms, with at least one of them being positive [17].

The etiology of schizophrenia has been studied for decades and is still not yet well defined [13, 18]. It is known, however, that it is multifactorial, with multiple small-effects and that the genetic factor is important for its development, with heritability up to 80 %. It is suggested that it is a result of various combined genetic alterations [9, 13, 19]. Moreover, recent studies point out immunogenetics and neuroinflammation as significant factors in schizophrenia processes [20–22].

Additionally, other aspects such as perinatal influence, fetal exposure to infectious or inflammatory agents, trauma in childhood or adolescence, social behavior, familial liability, migration and urbanicity have also been studied as risk factors, making a contribution to stating the considerable importance of gene-environment interaction in the manifestation of the psychosis [13, 23–25].

8.4 Potential mechanism of action of antipsychotics

Since the observation of the therapeutic effects of chlorpromazine, the "dopamine hypothesis" of schizophrenia started to be highly studied. This theory states that an excess of dopamine in the synapses may exacerbate psychotic symptoms such as delusions and hallucinations. In this way, antipsychotics with different chemical structures block dopamine D_2 receptors, reducing schizophrenic symptoms [3, 10, 11].

However, even at moderate doses, undesirable side effects are observed by the action of blocking the dopaminergic system: prolactin elevations and extrapyramidal symptoms such as tardive dyskinesia, dystonia, akathisia, and parkinsonism are some of them [1, 26].

The introduction of clozapine and the surprising observation of its fewer extrapyramidal symptoms, opened the way for the research and development of second-generation antipsychotic agents, denominated "atypical antipsychotics" or "atypical neuroleptics" [5]. Both typical and atypical antipsychotics blockade D_2 receptors, but atypical ones act specifically in the mesolimbic dopamine pathway rather than in the mesocortical and nigrostriatal pathways. This feature provides mechanism of action in the brain area hypothetically responsible for schizophrenic positive symptoms. Additionally, some atypical drugs are more potent serotonin $5HT_{2A}$ antagonist than D_2 antagonist, balancing the dopamine activity deficiency [1, 26].

According to some researchers, atypical antipsychotic may be characterized by having one or more of the following criteria: producing minimal extrapyramidal side

effects, not causing tardive dyskinesia and causing small elevation of serum prolactin levels [27]. Also, in the past decade, studies have pointed out that atypical agents may be associated with a smaller risk of death and that they may improve cognition in schizophrenic patients, which may lead to greater community adjustment [28–30]. Some investigators, however, suggest no superior efficacy of second-generation antipsychotics over the first generation, stating that individual response observation allied to judgments of efficacy, safety, and tolerability may be the guidance for therapeutic choice [13, 31, 32].

Nevertheless, it is estimated that about 49–74 % of the patients discontinue antipsychotic use due to adverse effects or treatment inefficacy [32–34]. Once some D_2 receptors are associated to the experience of reward and pleasure, dysfunction in dopaminergic pathways may lead to apathy, lack of motivation and interest in social interactions [35]. Also, studies have proved that neuroleptics can bind to brain receptors instead of, or in addition to D_2 and $5HT_{2A}$, including α-1-adrenergic, α-2-adrenergic, dopamine D_3, histamine H_1, muscarinic, and serotonin 5-HT_{1A}, 5-HT_{1D}, 5-HT_{2C} and 5-HT_6 receptors. Although these bindings are not always related to therapeutic effect, they may lead to adverse effects such as dry mouth, blurred vision, constipation, major weight gain, diabetes mellitus, hyperglycemia, dyslipidemia, cardiovascular diseases and also tardive dyskinesia, which causes involuntary muscle movements in about 4–5 % of the patients every year [14, 15, 27]. The most complicated adverse effect, however, is rare, but potentially fatal – "neuroleptic malignant syndrome", which is characterized by muscular rigidity, high fever, coma, and even death [26].

Another important feature of the relation of antipsychotics and brain receptors is that both typical and atypical drugs provide reversible binding [36]. As so, one may state that these drugs do not cure patients, but only control the psychotic symptoms and that the treatment must be continuous through life. However, several studies have questioned the maintenance of the treatment for patients with one or several psychotic episodes and the effects of antipsychotics withdrawal [14]. Therefore, further research is needed to establish risk-benefit of long-term antipsychotic treatment.

8.5 Structure-activity relationships and pharmacology of the drugs

8.5.1 Typical antipsychotic drugs

8.5.1.1 Chlorpromazine and phenothiazine drugs

Firstly synthesized in December 1951 in the French laboratories of Rhône-Poulenc, chlorpromazine appeared to be the most effective antipsychotic drug of that time. Chorpromazine is the prototypical member of the class of phenotiazinic drugs [6]. This class of drugs are amphiphilic compounds that assume positive charge at physiological pH conditions. They present an inhibitory profile on a wide range of neuron

receptors, such as α-adrenergic, serotonin, histamine and GABA-ergic receptors. However, the affinity for dopaminergic receptors is the strongest. Figure 8.1 depicts the general structure of phenothiazine.

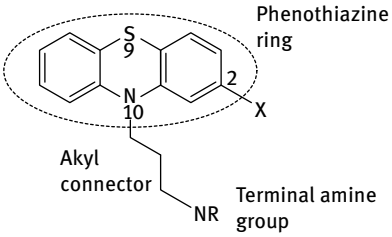


Fig. 8.1: General chemical structure of phenothiazines.

The strong antagonism of dopamine receptors by phenothiazines is related to the structure similarity of their three-dimensional configuration to the dopamine molecule [37]. As can be observed in Fig. 8.2, this similarity is clearly evidenced for chlorpromazine.

The Van der Waal's attraction between the alkyl connector and the aromatic ring substituent bound on position 2 contributes to a dopamine-like conformation favoring the affinity to dopamine receptors. The presence of the chlorine substituent in different positions would sterically hinder the approach of the alkyl connector to the ring [38]. Also, the nature of the C2 substituent is important for antipsychotic potency. Only electron withdrawing moieties can improve the affinity of the drug to the dopamine receptor. The following order of activity has been stated [39]:

$$CF_3 > Cl > H \approx COCH_3 \approx CONHNH_2$$

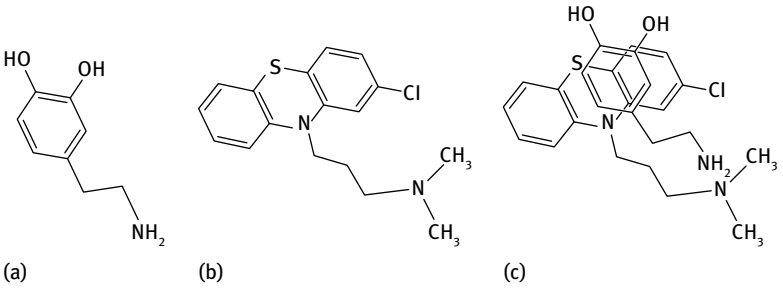
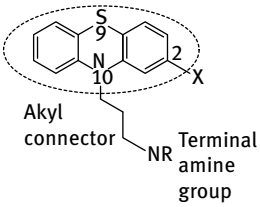
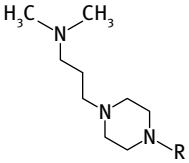
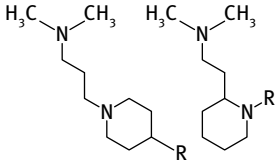
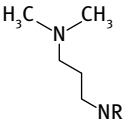


Fig. 8.2: Structural similarity between dopamine and chlorpromazine. (a) dopamine; (b) chlorpromazine; (c) superposition of chlorpromazine and dopamine structures.

Further, the size of the alkyl connector can modulate the receptor affinity of the molecule. The alkyl connector of chlorpromazine constituted of 3 carbon atoms perfectly fits to dopamine receptors. The shortening of this chain leads to an antihistaminic activity and a lack of antipsychotic activity [37, 40]. Chlorpromazine, as well as its derivatives on the group of phenothiazines, easily crosses the blood-brain barrier, which improves their effect on the central nervous system. This is possible due to the high lipophilicity of the phenothiazine ring.

Furthermore, the nature of the terminal amine group determines the antipsychotic potency, as well as the adverse effect profile of the phenothiazines. Table 8.1 lists the structures of different types of terminal amine group and also their adverse effect profiles compared with chlorpromazine [41].

Table 8.1: Phenothiazine drugs and side effect profiles.

	Phenothiazine types		
	Piperazine	Piperidine	Aliphatic
Phenothiazine ring		 Pericyazine	 Prochlorperazine	 Chlorpromazine
Examples	Pericyazine	Prochlorperazine	Chlorpromazine
Adverse Effects: Extrapyramidal	Moderate/Low	Moderate/High	Moderate
Adverse Effects: Sedative	Moderate	Moderate	High
Adverse Effects: Antimuscaric	Moderate	Moderate	Moderate
Adverse Effects: Increase in prolactin	High	High	High
Adverse Effects: Weight gain	Moderate	Moderate	Moderate
Adverse Effects: Postural hypotension	Low	Moderate	High

Regarding potency, the following general order has been established:

piperazines > piperidines > aliphatics

Taking into account the adverse effects, piperazinephenothiazines present the lowest antagonism activity to muscarinic, histamine-1 and α-1 receptors. This profile leads to a low incidence of sedation, hypotension, and other effects mediated by these receptors. On the other hand, piperidinephenothiazines show the lowest incidence of extrapyramidal side effect. Since the piperidine type has an intermediate antagonism

activity on dopamine receptor and high blocking activity on muscarinic receptors, relatively rare extrapyramidal events have been described.

8.5.1.2 Thiothixene and thioxanthene drugs

Thiothixene is a dopamine antagonist drug widely used in clinic practice due to its seldom adverse reaction upon extracorticospinal tract. It is one of the most known examples of the class of thioxanthene antipsychotics [42]. Thioxanthene drugs are alkene bioisosteres of phenothiazines. Since they are assymmetrical alkenes, they can present the Z (*cis*) or E (*trans*) conformation as can be noted in Fig. 8.3. The optimal dopamine receptor affinity is achieved only by the *cis*-isomer [43]. It has also been stated that the reduction of the double bound reduces the antipsychotic activity. The other structure-activity relationships are equivalent to those for phenothiazine drugs.

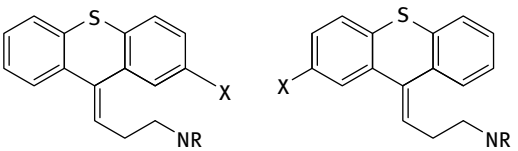


Fig. 8.3: Generic chemical structures for thioxanthene drugs. A: Z-thioxanthene; B: E-thioxanthene.

8.5.1.3 Haloperidol and butyrophenone drugs

Haloperidol is a dopamine receptor antagonist 50-fold more potent than chlorpromazine. It belongs to butyrophenone class of antipsychotic drugs and is highly effective against delusions, hallucinations and psychomotor excitement. Haloperidol is widely prescribed in emergency cases for fast-acting treatment of positive psychosis symptoms. However, the high blockage levels of dopamine receptors leads to a higher occurrence of extrapyramidal side effects when compared with other less potent typical antipsychotics. The association with promethazine is usual to alleviate the adverse effects. On the other hand, less incidence of sedation and hypotension is found [1, 44].

Figure 8.4 shows the generic structure of the butyrophenone compounds. Briefly they are tertiary amines, containing at least one aromatic ring linked by an intermediate chain to the basic amine portion.

The literature shows that the presence of fluorine as the “X” substituent on the aryl group is required for optimal activity. The butyrophenones without a substituent are two to eight times less potent than those containing fluorine on para position. The position of the “X” substituent is also pivotal. Meta- and ortho-substituted

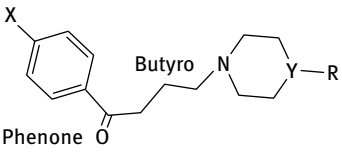


Fig. 8.4: Generic chemical structures of butyrophenone antipsychotics.

compounds are less potent than those para-substituted. Further, the isosteric replacement of the carbonyl group from the phenone portion of the molecule is related to a significant decrease in antipsychotic potency of up to ten times. The effect is also observed in case of reduction of the carbonyl moiety. Also, alterations on the three-carbon chain that connects the carbonyl group to the amine portion, such as shortening, lengthening, branching, or incorporation into a ring system, lead to a significant decrease or even complete loss of antipsychotic activity [45]. For haloperidol, the axial conformation of alcohol function depicted in Fig. 8.5 enhances the affinity to dopamine receptors [46].

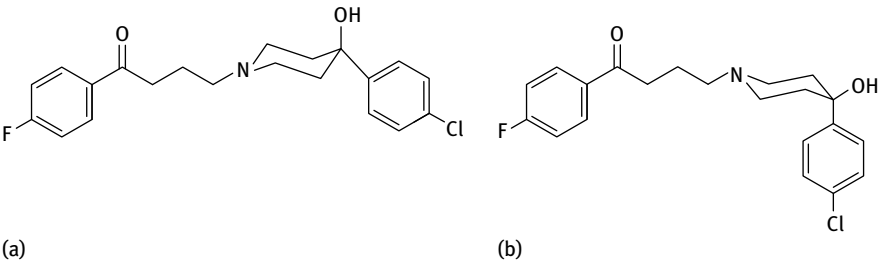


Fig. 8.5: Chemical structure of haloperidol with different conformations of alcohol moiety. A: axial and B: equatorial conformation.

Other butyrophenones present this same conformation of a hydrogen-bond donor linked to a tertiary amine as part of a 4-substituted piperidine ring, achieving optimal neuroleptic potency. As examples, droperidol, benperidol, bromperidol and trifluperidol possess this pattern of structure.

8.5.1.4 Pimozide and diphenylbutylpiperidines drugs

Pimozide is a highly specific neuroleptic drug effective against productive psychotic symptoms. As they are described in Fig. 8.6, diphenylbutylpiperidines can be considered as butyrophenone derivatives where the carbonyl group was replaced by 4-fluorophenylmethine moiety. They are commonly distinguished from butyrophenone by

their capability of breaking through autism and also to their long duration of action after oral administration. Generally they are mostly applied on maintenance therapy. The structure-activity relationship of the drugs from this group is very similar to those for butyrophenones [45, 47].

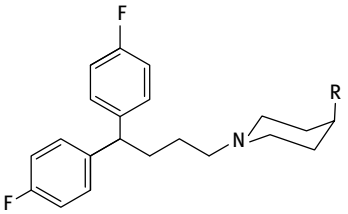


Fig. 8.6: Generic structure of diphenylbutylpiperidines.

8.5.2 Atypical antipsychotic drugs

8.5.2.1 Clozapine

Clozapine is a dibenzodiazepine drug known by its broad pleomorphic receptor pharmacology, i.e., it presents affinity for dopaminergic subtype 2 (D_2) receptor, as well as D_1, D_4, serotonergic $5HT_{2A}$ and $5HT_{2C}$, adrenergic α-1 and α-2, muscarinic M_1 and histaminergic H_1 receptors. Its relatively low affinity for D_2 receptors (38–63 %) is not enough for inducing extrapyramidal effects, which confers a significant advantage when compared to typical antipsychotics [1, 48]. In addition to this, clozapine possesses high mesolimbic selectivity, instead of those dopamine pathways preferred by typical antipsychotics – nigrostrial and mesocortical. This profile contributes to the treatment of positive symptoms of schizophrenia, as well as low incidence of extrapyramidal side effects. Additionally, the literature has shown a good efficacy of clozapine also against negative symptoms even greater than that obtained by typical neuroleptics [49, 50].

Figure 8.7 describes the chemical structure of clozapine. The seven member central ring is responsible for the affinity profile of clozapine to pleomorphic receptor. N-methylation on position 5 leads to a decrease on depressant activity. Among the 8-position substituent options, chlorine presents the strongest activity. Compared to other substituent groups, the following order potency is stated [47]:

chlorine > methyl > hydrogen > trifluoromethyl > methylthio > methoxy

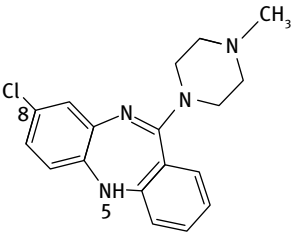


Fig. 8.7: Chemical structure of clozapine.

Several drug derivatives have been developed from the tricyclic dibenzazepineheterocycle basis, presenting similar receptor-affinity profile. As depicted in Fig. 8.8, the variation on 8-position substituent creates some important derivatives in clinic practice on antipsychotic approach. The structural variants are:

- Dibenzoxazepine (X = O)
- Dibenzodiazepine (X = NH)
- Dibenzothiazepine (X = S)

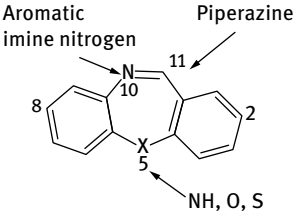


Fig. 8.8: Tricyclic dibenzazepine heterocycle basic structure.

8.5.2.2 Quetiapine

Discovered in 1984, quetiapine is a dibenzothiazepine compound with a low affinity to D_2 receptors and high affinity to serotonin-$_{2A}$ receptor ($5HT_{2A}$). Therefore, as stated for clozapine, quetiapine is less likely to produce extrapyramidal side effects than typical antipsychotics. Quetiapine also presents affinity to those receptors listed for clozapine, among them noradrenergic and histaminic receptor, which leads to an incidence of postural hypotension and sedative effects [51, 52]. The literature attributes the reduced affinity to D_2 receptors to the presence of a side aliphatic chain on the structure of quetiapine, as can be seen on Fig. 8.9 [43]. Other structure-activity relationship considerations correlate with those for clozapine due to the structural similarity.

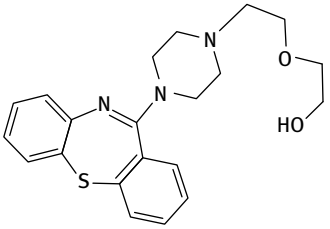


Fig. 8.9: Chemical structure of quetiapine.

8.5.2.3 Risperidone

Risperidone is a benzisoxazole derivative that presents effective activity against positive and negative symptoms comparable to haloperidol. Considering a higher D_2 receptor affinity when compared to other atypical drugs, the incidence of extrapyramidal effects is dependent on the dosage. Risperidone is also associated with an increase of prolactin release, sexual dysfunction and significant weight gain [54].

8.5.2.4 Olanzapine

Olanzapine is a thienobenzodiazepine with effective response against positive and negative symptoms of schizophrenic syndrome. It is also related to a low incidence of extrapyramidal side effect. Olanzapine also antagonizes different receptors similarly to the other atypical drugs. Figure 8.10 shows the structure of olanzapine is clearly derived from clozapine.

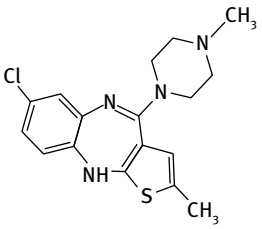


Fig. 8.10: Chemical structure of olanzapine.

8.6 Research into future treatments for schizophrenia and related psychoses

Important insights toward schizophrenia etiology, diagnosis and treatment have been provided through the last decades.

The rising number of scientific publications in the past 25 years indicates the increasingly interest in schizophrenia research. Also, the World Health Organization has focused its efforts to define the best criteria for schizophrenia diagnosis, establishing new ratings for positive symptoms, negative symptoms, mood symptoms, psychomotor symptoms and cognitive impairments, evaluated together with course specifiers [17]. A correct and early diagnosis is essential for an effective treatment.

Aiming to achieve new treatments for schizophrenia, some areas must be exploited.

Actually, there are about sixty-five antipsychotics available in the world and most of them act by blocking D_2 receptors [55]. Studies on new drugs are being developed to target glutamate receptor, PDE10A, glycine transporters and alpha-7-nicotinic acetylcholine receptor. Although some preclinical and clinical trials suggest that these action mechanisms are promising, more studies are needed to make a strong statement of their efficacy [56].

Even though medications are needed in the treatment, psychotherapies are essential to treat cognitive, emotional and behavioral deficits. They should involve not only the patient, but also the family and maybe the community, leading to social behavior improvement. This approach may be particularly helpful to prevent self-violence or violence to others and to achieve the ability to resume socializing at school and at work. Actually, several evidence-based psychotherapy modalities are already stated. It is necessary now to apply this knowledge to clinical practice [13].

A promising approach is using cranial neuroimaging data of schizophrenic patients and persons at risk of developing schizophrenia to study and obtain computerized predictions [57, 58]. Also in study are the use of repetitive transcranial magnetic stimulation (rTMS) therapy and the techniques of deep brain stimulation (DBS), aiming to treat schizophrenic symptoms based on information of brain network disturbances in the psychosis. However, effectiveness and potential side effects are not yet well established [59–61].

Regarding to the etiopathogenesis of schizophrenia, the discovery of new possible genetic alterations points out arising challenges. Studies on genetic risk markers, polymorphisms and immunity-related genes may lead to novel classification criteria and also new therapies in the future [17]. These studies will provide individual analysis and an early diagnosis, which may result in a more effective treatment and even preventive therapies.

Anyway, much needs to be done to optimize the use of already available choices. For instance, there is an estimate that in fact half of schizophrenic patients do not receive any kind of treatment. Therefore, information campaigns should fight the

stigma and discrimination and publicize the symptoms of this disorder, as well as the available treatments and mental healthcare services. Furthermore, it is necessary to improve the quality of the services by providing specialized healthcare professionals and implementing guidelines to improve clinical practice integrating the biopsychosocial model [17].

It is a long way from new research to clinical practice, but this path is already being trodden. Until then, it is important to choose the right personalized intervention for each individual patient, considering the synergistic effects of combining currently available approaches.

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Prerna Sarup*

9 Chemometric analysis: A novel tool for herbal drug analysis and designing

Abstract: High performance chromatographic techniques are the most commonly used methods in the standardization of herbal drugs. But the intricacy involved in the herbal matrices is difficult to analyze because of their complexity of chemical composition. Chemometric analysis provides a good opportunity for extracting out more useful chemical information from the original statistics. Comprehensive methods and hyphenated techniques associated with chemometrics used for extracting useful information and supplying various methods of data processing are now more and more widely used in medicinal plants, among which similarity analysis, hierarchical clustering analysis (HCA) and principal component analysis (PCA) are most commonly used techniques. This study focuses on the various chromatographic techniques, chemometric tools and interpretation of results by HCA & PCA in various guggulu samples. The results of HCA and PCA producing same inference as that of analytical techniques validated and provided proof to the results of analysis of these samples in their similarity and dissimilarity assessment. In order to evaluate the discrimination ability of the different constituents, PCA was employed using the peak areas of all peaks as input data.

Keywords: chemometrics, hierarchical clustering analysis (HCA), principal component analysis (PCA)

9.1 Introduction

This chapter aspires to present chemometrics as a significant tool in analysis and drug designing. The term chemometrics was introduced for the very first time by Svant Wold (Sweden) and Bruce Kowalski simultaneously. PCA analysis is used to decrease the size of data sets, without losing important information about the samples and identifying the major component on the basis of which the drug is designed. Chemometrics can be defined as the chemical discipline that uses mathematical and statistical methods to devise or select optimal measurement procedures and experiments and it provides maximum chemical information by analyzing chemical data. Because of the complexity of herbal matrices, it is necessary to perform a pre-processing of the data. Pre-processing is a procedure to alter the diverse factors with different units in values, giving the same change to each value that contributes to the model. The next step involves analyzing the data in terms of similarity and dissimilarity in the data. This is performed with the help of correlation coefficients, HCA (Hierarchical Cluster Analysis) and PCA (Principal Component analysis) [1].

9.1.1 Similarity analysis using correlation coefficient

Similarity analysis is the most common technique which is based on the correlation coefficient. The similarity analysis method has been successfully applied for quality evaluation of medicinal plants for further designing of herbal formulations. Cosine angle is the most established means of improving the analysis of similarities in fingerprints. The correlation coefficient and vector cosine present a better differentiation of the similarity or difference between the fingerprints from the same samples [2].

9.1.2 Principal component analysis (PCA)

The most widespread method used in the fingerprint data analysis of different plants is principal component analysis (PCA). It portrays the original measurement by discovering the dominant factors while excluding the relevant interference factors, thereby allowing a more accurate and closer estimate. PCA is a simple method for extracting relevant information from puzzling data sets. It is a way of identifying patterns in data, and highlights their similarities and differences. PCA is a special case of factor analysis that is highly useful in the analysis of many time series and the search for patterns of movement common to several series. A primary benefit of PCA arises from quantifying the importance of each dimension for describing the variability of a data set. It can also be used to compress the data without much loss of information [3].

9.1.3 Cluster analysis (CA)

Cluster analysis or clustering is the task of grouping a set of objects in such a way that objects in the same group (called cluster) are more similar to each other than to those in other groups. Cluster analysis classifies objects based on quantitative characteristics. Generally, the clustering techniques are divided into two subtypes: hierarchical and nonhierarchical. In the quality evaluation/designing of herbal plants/formulations, the most popular clustering technique is hierarchical clustering analysis (HCA). The main advantage of HCA is the flexibility to alter the similarity measurement criterion and the applied linkage method to suit different applications. Fingerprint-based PCA can directly reflect the difference between samples, whereas CA can classify objects based on their quantitative characteristics. A combination of CA and PCA has been widely used in current quality assessment of medicinal plants which plays a significant role in polyherbal drug designing [2].

9.2 Experimental

9.2.1 Material and methods

Guggulu samples (oleo gum extract obtained from *Commiphora wightii;* Family- Burseracea) were procured from different areas namely, Ajmer, Rajasthan (RG-A_1 and RG-A_2); different shops in Khari-Baoli, Delhi (RG-D_1, RG-D_2, RG-D_3, RG-D_4 and RG-D_5); Chandigarh (RG-C); Dehradun, Uttaranchal (RG-Dun), Jalandhar, Panjab (RG-J); Ludhiana, Panjab (RG-L) and Madhopur, Rajasthan (RG-M).

Also one of the Delhi samples (RG-D_5) was subjected to purification studies to evaluate the best purification method for this Ayurvedic drug [4] in terms of therapeutic efficacy and content of guggulsterones (*E*- and *Z*-), the most active bioconstituents obtained from guggulu. Purified samples were named PG-1 to PG-7 depending on the media used for purification [5, 6].

Accurately weighed, about 5 g of coarsely powdered guggulu gum (raw and purified) was extracted with ethanol (50 mL) by Soxhlet at 80 °C for 6 hours. Extracts were filtered and concentrated to dryness. The residues were reconstituted with ethanol, filtered and volume was made to 50 mL. The samples were labeled and subjected to HPLC studies.

9.2.2 HPLC system

Waters Alliance HPLC system fitted with autosampler, 2695 separation module and 2996 PDA detector were used for all HPLC studies. The data was acquired and processed through Empower software 2.

9.2.3 Column

Chromatographic separations were performed on a reverse phase C_{18} column, 250 × 4.6 mm, 5 µm. (Waters., Spherisorb®), Catalogue No. PSS831915.

9.2.4 Mobile phase

A mixture containing water, acetonitrile and methanol (35 : 55 : 10), filtered through 0.45 µm membrane filter, degassed by sonication at a flow rate of 0.8 µL/min was used in the present analysis.

9.2.5 Detector

All the samples were run for 5 min and components of the mixture were detected using PDA set at 246 nm was used.

Peak areas corresponding to each peak were noted for data analysis and evaluation of the samples.

9.3 Data analysis and quality evaluation

9.3.1 Similarity analysis (SA)

Guggulu procured from different regions varies not only in appearance but also in the content of guggulsterones as showed by our results of analysis. Owing to this variability, it was necessary that chromatographic fingerprints of different samples of guggulu should be evaluated for their similarities or differences. Figures 9.1 and 9.2 show the HPLC chromatograms of raw and purified guggulu samples respectively. Data analysis was performed using SPSS 20.0 and correlation coefficients were calculated. The guggulu samples from different areas (RG-A_1, RG-A_2, RG-D_1, RG-D_2, RG-D_3, RG-D_4, RG-D_5, RG-C, RG-Dun, RG-J, RG-L and RG-M) were set as one variable. The value of other variable was the retention times of all the peaks of all species in HPLC fingerprinting. The cross value of data under these two sets of variables was the AUC of each peak, which ranged from zero (absence of peak) to the true value of AUC at that R_t (Table 9.1).

Table 9.1: The peak intensities in HPLC chromatograms of different samples of guggulu.

R_t (min)	AUC (× 10^5)											
	RG-A_1	**RG-A_2**	**RG-D_1**	**RG-D_2**	**RG-D_3**	**RG-D_4**	**RG-D_5**	**RG-C**	**RG-Dun**	**RG-J**	**RG-L**	**RG-M**
3.07	0	0	0	0	0	0	0.23	0	0	0	0	0
7.96	0.39	0.28	0.52	0.25	0.87	0.49	0.59	0.39	0.51	0.32	0.42	0.39
8.76	0	0	0	0.37	0	0	0	0	0	0	0	0
9.90	0.17	0.12	0.52	0.36	0.51	0	0.65	0	0.21	0	0.11	0.17
11.11	0.77	0.73	0.59	1.00	0.84	0.44	1.00	0.60	0.60	0.55	0.69	0.53
12.33	0	0	0	0.39	0	0	0	0.012	0	0	0	0
13.30	1.75	1.56	2.12	1.93	1.82	1.63	2.13	1.29	3.03	1.71	2.09	2.54
14.46	0.51	0.33	0.66	0.97	0.59	0.84	0.78	0.59	0.46	0.48	0.34	0.29

RG-A_1 and **RG-A_2** (Ajmer, Rajasthan); **RG-D_1**, **RG-D_2**, **RG-D_3**, **RG-D_4** and **RG-D_5** (Delhi); **RG-C** (Chandigarh); **RG-Dun** (Dehradun); **RG-J** (Jalandhar); **RG-L** (Ludhiana) and **RG-M** (Madhopur).

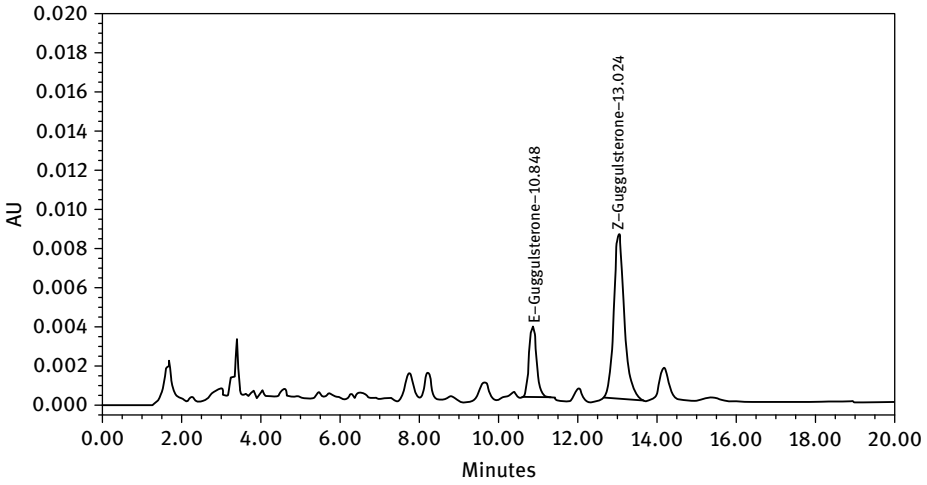

Fig. 9.1: Representative HPLC chromatogram of raw guggulu sample.

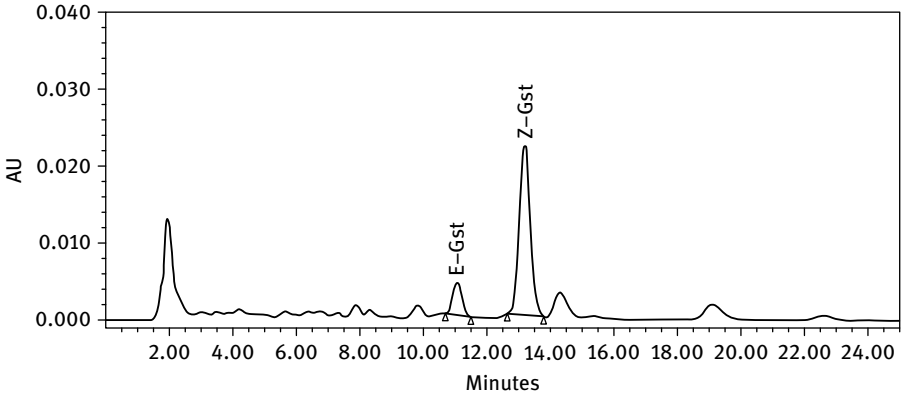

Fig. 9.2: Representative HPLC chromatogram of purified guggulu sample.

9.3.2 Principal component analysis (PCA)

The PCA was performed on various samples based on the AUCs of both guggulsterones (*E*- and *Z*-) in HPLC using SPSS 20.0. The purified samples PG-1 to PG-7 were compared with raw guggulu sample, RG-D_5 for the content of guggulsterones (*E*- and *Z*-). The principal components were extracted on the basis of eigen values. Extraction of the components was done using varimax rotation. PCA analysis of both these peaks was used for studying the similarity between species and to know the points of variation. The input data used in the analysis is given in Table 9.2.

Table 9.2: Comparison of peak intensities in HPLC chromatograms for similarity analysis among different samples of purified guggulu and their comparison with raw guggulu.

R_t (min)	AUC (× Lacs)							
	RG-D_5	PG-1	PG-2	PG-3	PG-4	PG-5	PG-6	PG-7
1.92	0	1.81	3.31	0.44	0.28	0.31	2.84	0
2.3	0	0	0	0	0.53	0.42	0	0
3.07	0.23	0	0.13	0.25	0	0	0	0.20
4.18	0	0	0	0	0.10	0.21	0	0
7.96	0.59	0	0.20	0.69	0.53	0.82	0.26	0.78
8.76	0	0.18	0	0.46	0.35	0.31	0	0.29
9.90	0.65	0	0.25	0.46	0.82	0.96	1.02	0.27
11.11	1.00	1.03	1.68	1.84	1.48	1.65	2.11	0.99
13.30	2.13	2.62	2.18	2.88	4.33	5.22	4.76	2.00
14.46	0.78	0.29	0.35	0.86	0.74	0.98	0.94	0.44
19.15	0	0	0	0	0	0	0.49	0

RG-D_5 (Delhi); **PG-1** (triphla purified guggulu); **PG-2** (cow urine purified guggulu); **PG-3** (cow milk purified guggulu); **PG-4** (vasa swaras purified guggulu); **PG-5** (vasa kasaya purified guggulu); **PG-6** (nirgundi swaras with haldi curna purified guggulu) and **PG-7** (water purified guggulu).

9.3.3 Hierarchical clustering analysis (HCA)

HCA is a multivariate analysis technique that is used to sort samples into different groups. HCA of all the samples was performed using SPSS 20.0 software. The Ward's method as the amalgamation rule and the squared Euclidean distance as metric were used to establish clusters. The input data is shown in Table 9.1.

9.4 Results and discussion

9.4.1 Similarity analysis of fingerprints of different guggulu samples

The derived correlation coefficients between the chromatograms of different samples of guggulu are shown in Table 9.3. These results indicate that the two genuine samples procured from Ajmer, RG-A_1 and RG-A_2, showed almost 100 per cent matching (0.995) with each other illustrating their close chemical similarity. All samples were similar to each other. The closest sample to genuine cultivated guggulu was RG-J with degree of correlation of 0.990. The most different sample was that of RG-L with degree of correlation of 0.854. The other samples between these two extremes were in the following increasing order of similarity: RG-D_3, RG-D_2, RG-D_1, RG-D_4, RG-C, RG-D_5, RG-Dun and RG-M (Table 9.3).

9.4.2 Hierarchical clustering analysis (HCA)

A hierarchical agglomerative clustering analysis of different samples was performed based on the peak areas of all different peaks of HPLC fingerprints. The results of HCA are shown in Fig. 9.3, which highlights the chemical characteristics. Based upon their similarity, the samples could be classified into eight different clusters. At the highest level, all samples were divided into two clusters with three samples, RG-L, RG-M and RG-Dun placed in one cluster and all other samples forming the other cluster. The cluster of three samples was further divided into two clusters, where samples procured from Ludhiana and Madhopur were closer together than the sample procured from Dehradun. The other cluster of nine samples was further classified at two levels. First splitting in two clusters of five and four samples; the second level splitting of the cluster of five samples into two clusters of RG-A_1, RG-A_2, RG-J and RG-D_4, RG-C. The other cluster of four samples was split at second level and one sample of RG-D_2 was placed singly and separately to the other three samples of RG-D_1, RG-D_3 and RG-D_5. The cluster membership is shown in Table 9.4.

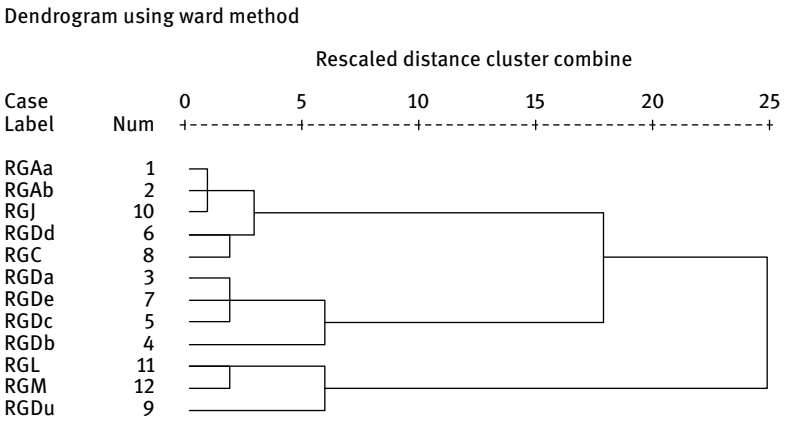

Fig. 9.3: Dendrogram showing clustering of different guggulu samples.

9.4.3 Principal component analysis (PCA)

The guggulu samples show dissimilarity and similarity among themselves to varying extents as inferred from HPLC analysis. The present study and analysis is based on the assumption that each peak in the HPLC chromatogram represent one component or group of components (unresolved under test condition) and peaks at same retention times in different chromatograms of different purified samples represent

Table 9.3: Correlation amongst different guggulu samples.

Sample code	RG-A_1	RG-A_2	RG-D_1	RG-D_2	RG-D_3	RG-D_4	RG-D_5	RG-C	RG-Dun	RG-J	RG-L	RG-M
RG-A_1	1	0.995**	0.886**	0.882**	0.879**	0.910**	0.963**	0.915**	0.965**	0.990**	0.854*	0.987**
Pearson Correlation		0.000	0.000	0.001	0.001	0.002	0.000	0.000	0.000	0.000	0.000	0.000
Sig. (2-tailed)												
RG-A_2	0.995**	1	0.869**	0.871**	0.865*	0.902**	0.971**	0.896**	0.961**	0.981**	0.848*	0.963**
Pearson Correlation	0.000		0.001	0.002	0.002	0.005	0.000	0.001	0.001	0.000	0.000	0.000
Sig. (2-tailed)												
RG-D_1	0.886**	0.869**	1	0.911**	0.958**	0.933**	0.980**	0.925**	0.977**	0.964**	0.965**	0.971**
Pearson Correlation	0.000	0.001		0.004	0.001	0.002	0.000	0.003	0.000	0.000	0.000	0.000
Sig. (2-tailed)												
RG-D_2	0.882**	0.871**	0.911**	1	0.852*	0.911**	0.918**	0.940**	0.894**	0.941**	0.913**	0.889**
Pearson Correlation	0.001	0.002	0.004		0.015	0.004	0.004	0.002	0.007	0.002	0.004	0.007
Sig. (2-tailed)												
RG-D_3	0.879**	0.865*	0.958**	0.852*	1	0.901**	0.960**	0.927**	0.924**	0.930**	0.942**	0.920**
Pearson Correlation	0.001	0.002	0.001	0.015		0.006	0.001	0.003	0.003	0.002	0.001	0.003
Sig. (2-tailed)												
RG-D_4	0.910**	0.902**	0.933**	0.911**	0.901**	1	0.910**	0.976**	0.921**	0.963**	0.923**	0.905**
Pearson Correlation	0.002	0.005	0.002	0.004	0.006		0.004	0.000	0.003	0.000	0.003	0.005
Sig. (2-tailed)												
RG-D_5	0.963**	0.971**	0.980**	0.918**	0.960**	0.910**	1	0.936**	0.950**	0.958**	0.961**	0.947**
Pearson Correlation	0.000	0.000	0.000	0.004	0.001	0.004		0.002	0.001	0.001	0.001	0.001
Sig. (2-tailed)												
RG-C	0.915**	0.896**	0.925**	0.940**	0.927**	0.976**	0.936**	1	0.918**	0.977**	0.949**	0.909**
Pearson Correlation	0.000	0.001	0.003	0.002	0.003	0.000	0.002		0.004	0.000	0.001	0.005
Sig. (2-tailed)												
RG-Dun	0.965**	0.961**	0.977**	0.894**	0.924**	0.921**	0.950**	0.918**	1	0.979**	0.989**	0.976**
Pearson Correlation	0.000	0.001	0.000	0.007	0.003	0.003	0.001	0.004		0.000	0.000	0.000
Sig. (2-tailed)												

Sample code	RG-A_1	RG-A_2	RG-D_1	RG-D_2	RG-D_3	RG-D_4	RG-D_5	RG-C	RG-Dun	RG-J	RG-L	RG-M
RG-J	0.990**	0.981**	0.964**	0.941**	0.930**	0.963**	0.958**	0.977**	0.979**	1	0.967**	0.990**
Pearson Correlation	0.000	0.000	0.000	0.002	0.002	0.000	0.001	0.000	0.000		0.000	0.000
Sig. (2-tailed)												
RG-L	0.854*	0.848*	0.965**	0.913**	0.942**	0.923**	0.961**	0.949**	0.989**	0.967**	1	0.948*
Pearson Correlation	0.000	0.000	0.000	0.004	0.001	0.003	0.001	0.001	0.000	0.000		0.000
Sig. (2-tailed)												
RG-M	0.987**	0.963**	0.971**	0.989**	0.920**	0.905**	0.947**	0.909**	0.976**	0.990**	0.948*	1
Pearson Correlation	0.000	0.000	0.000	0.007	0.003	0.005	0.001	0.005	0.000	0.000	0.000	
Sig. (2-tailed)												

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed)

Table 9.4: Cluster membership.

S. No.	Case	8 Clusters
1.	RG-A_1	1
2.	RG-A_2	1
3.	RG-D_1	2
4.	RG-D_2	3
5.	RG-D_3	4
6.	RG-D_4	5
7.	RG-D_5	6
8.	RG-C	5
9.	RG-Dun	7
10.	RG-J	1
11.	RG-L	8
12.	RG-M	8

the same component(s). PCA analysis, however, on the basis of eigen values, could extract only one principal component PC1 which was not sufficient for such an analysis. It is confirmed from the scree plot (Fig. 9.4) where the elbow of the plot lay only in the first component region.

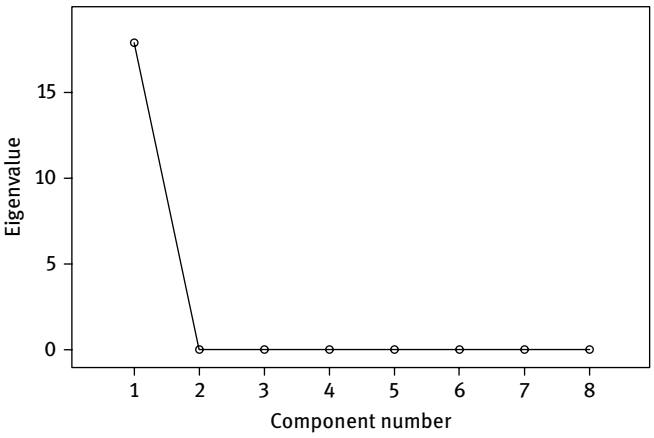

Fig. 9.4: Scree plot between eigen values and components.

9.4.4 Classification of different guggulu samples based on guggulsterone content

On the basis of content of guggulsterones, different purified samples were classified in two clusters (Table 9.5 and Fig. 9.5). The cluster 1 of RG-D_5, PG-7, PG-1, PG-2 and PG-3 was further classified into two clusters of RG-D, PG-1, PG-7 and PG-2, PG-3, whereas

there was no splitting of the other cluster even at lower level. The cluster was of PG-4, PG-5 and PG-6, which showed very similar guggulsterone content. The content of the other five samples was different from this group, where samples PG-2, PG-3 forming one cluster were different from first level splitted cluster of RG-D, PG-7 and PG-1. Clustering of different purified samples on the basis of guggulsterones are illusterated well by the dendrogram in Fig. 9.5.

Table 9.5: Cluster membership.

S.No.	Case	3 Clusters
1.	RG-D_5	1
2.	PG-1	1
3.	PG-2	2
4.	PG-3	2
5.	PG-4	3
6.	PG-5	3
7.	PG-6	3
8.	PG-7	1

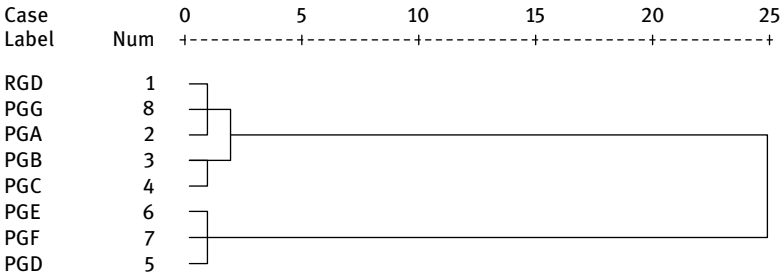

Fig. 9.5: Dendrogram showing clustering of different purified samples.

9.4.5 Principal component analysis (PCA) for similarities between differently purified samples

The purified guggulu samples through different methods show differences among themselves to varying extents as inferred from HPLC analysis. The present analysis to check similarity was based on the assumption that each peak in the HPLC chromatogram represent one component or group of components (unresolved under test condition) and peaks at same retention times in different chromatograms of different purified samples represent the same component(s). In order to evaluate the discrimination ability of the different constituents, PCA was employed using the peak areas of all peaks as input data. On the basis of eigen values, the first two principal com-

ponents PC1 and PC2 were used to provide a convenient visual aid for representing gross inhomogeneity in the data sets. It was clear from the variance matrix shown in Table 9.6 that the first two principal components contributed 97.88 % of the variance, which was further confirmed from the scree plot as the elbow of the plot lay in the first two component regions.

Table 9.6: Total variance matrix in PCA analysis of differently purified guggulu samples.

Component	Initial eigen values			Extraction sums of squared loadings		
	Total	Percent variance	Cumulative percentage	Total	Percent variance	Cumulative percentage
1.	8.234	84.194	84.194	8.234	84.194	84.194
2.	1.339	13.688	97.881	1.339	13.688	97.881
3.	0.123	1.254	99.135			
4.	0.051	0.524	99.659			
5.	0.016	0.161	99.820			
6.	0.012	0.119	99.939			
7.	0.005	0.051	99.990			
8.	0.001	0.010	100.000			

The samples were grouped in two categories based on their values in rotated component matrix scores (Table 9.7). The samples which had significant scores for the component were extracted in the respective component (Table 9.8).

These results were in agreement with the cluster analysis. The results of HCA and PCA producing the same inference validated these results and provided proof to the results of analysis of these samples in similarity assessment.

The score plot was made using the first two principal components and is shown in Fig. 9.6.

Table 9.7: Rotated component matrix in PCA analysis of differently purified guggulu samples.

Sample	Component	
	1	2
RG-D_5	0.170	0.614
PG-1	0.160	0.761
PG-2	0.461	1.113
PG-3	0.376	0.969
PG-4	1.150	0.439
PG-5	1.393	0.516
PG-6	1.416	0.559
PG-7	0.154	0.567

Table 9.8: Groupings of samples on the basis of their component scores.

Components extracted	
1	**2**
PG-4	$RG\text{-}D_5$
PG-5	PG-1
PG-6	PG-2
	PG-3
	PG-7

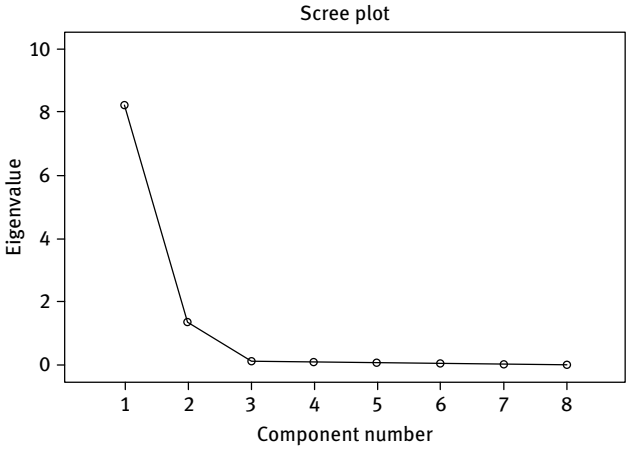

Fig. 9.6: Scree plot between eigen values and components in PCA analysis of differently purified guggulu samples.

Figure 9.7 is a graphic display of similarities in the components eluted at the respective retention times; taking into consideration the AUC of different peaks observed in HPLC analysis of differently purified guggulu samples and making comparison. Each dot in the figure represents retention times of different peaks. These dots are expected to cluster at one point, had there been no difference in chromatograms of all samples with respect to presence / absence of peak and also in the values of their AUCs. Similarly, if all peaks had been different in different samples, their dispersion would have been wide apart on different planes. The analysis of Fig. 9.7 clearly indicates that the peaks at retention times 1.92, 11.11 and 13.30 are outside the cluster showing dissimilarity, whereas most other peaks appeared in almost close cluster indicating similarity with respect to most other peaks. The three peaks appearing at a distance from the cluster indicates that these are the points of maximum variation in purified guggulu samples. It is interesting to note here that the peaks at retention time 11.11 and 13.30, which are outside the cluster showing dissimilarity, are the peaks of *E*- and *Z*- gug-

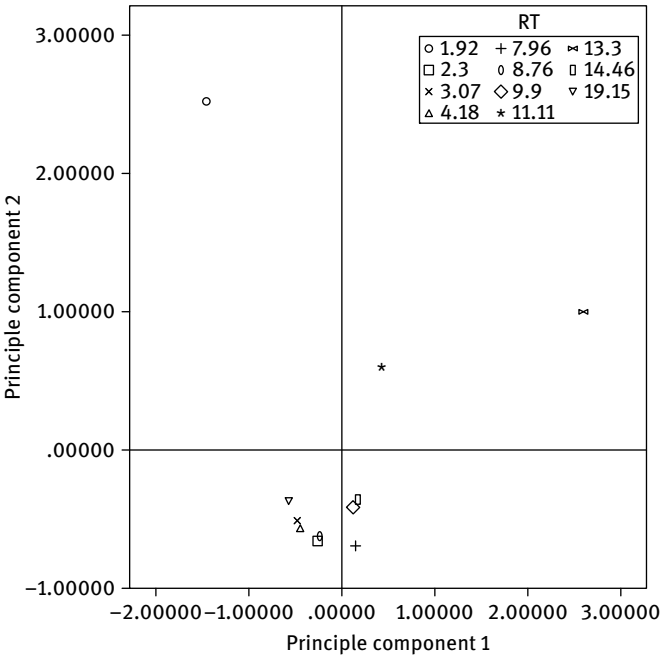

Fig. 9.7: Relation of different samples based on principal component score using retention times.

gulsterone, respectively. This clearly shows that the purification technique actually varies the content, as validated by the chemometric analysis.

9.5 Conclusion

The results of chemometric analysis clearly depict that the various guggulu samples from different geographical regions vary considerably with few showing resemblance to one another. Accordingly, we can target procurement of this particular drug of high repute from various regions. This study has led to the following conclusions:

1. The content of guggulsterones varies in different samples based on the geographical source.
2. The genuine cultivated samples from Ajmer, that is RG-A_1 and RG-A_2, closely resembled RG-J, the sample from Jalandhar which can be easily replaced with the genuine drug.
3. The content of guggulsterones alters significantly with the type of purification process employed.
4. PG-4, PG-5 and PG-6 have the maximum content of guggulsterones and thus are the best methods of purification for this drug out of the seven methods mentioned in Ayurvedic texts.

It has been mentioned in Ayurvedic texts that administration of raw guggulu may sometimes lead to skin rashes, irregular menstruation, diarrhoea, headache, mild nausea, and with very high doses, liver toxicity [8]. In order to overcome the side effects of raw guggulu, Ayurveda describes a number of purification processes (shodhanvidhi) in different 'dravyas' i.e., fluids, which not only takes care of the adverse effects but also enhances the therapeutic activity. It is also mentioned in Ayurvedic texts that guggulu must be purified before incorporation into herbal formulations. There are a large number of commercial polyherbal anti-inflammatory formulations which are using guggulu as the chief ingredient. Thus, we can effectively design the new polyherbal guggulu formulations based on the conclusions drawn out of this study. The geographical source and the method of purification of the drug can be cautiously chosen to enhance the pharmacological activity of guggulu. The chemometric methods used in the study can be effectively employed in designing a multifaceted polyherbal formulation which will suffice to cure variety of inflammatory disorders.

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10 Copper and its complexes: A pharmaceutical perspective

Abstract: Copper came into view mainly due to its enzymatic function in the human body and directly or indirectly plays a major role in regulating human activities like maintenance of the immune system, cancer, osteoporosis, arthritis, skin disease and heart problems etc. All the disease mentioned are also governed by copper and depend upon the amount of copper consumed in daily life. Therefore, knowing about the optimum intake of copper in the diet is very crucial for human health. Copper and its complexes also possess a good recognition in the field of medical science as bioactive agents. These copper dependent bioactive agents, due to their activity, are attracting interest from scientists for their use as prospective drugs for the treatment of several diseases. In this chapter we have tried to summarize the role of copper and its complexes with reference to toxicity level, sources, progress in the development of drugs and various important discussions about the mode of action.

10.1 Introduction

Copper is an important transition metal element with atomic number 29 in the periodic table that is universally engaged in biological systems via diverse activities including embryonic development, mitochondrial respiration and regulation of hemoglobin levels etc. [1]. It pageants substantial biochemical action as a trace element or as a constituent of various exogenous compounds (copper complexes of anthranilic acid, 3,5-diisopropylsalicyclic acid, aspirin and carboxylic acid etc.) in humans. It plays a major role as a cofactor for abundant enzymes, such as cytochrome *c* oxidase, tyrosinase, ceruloplasmin, albumin, including many biomolecules and nucleic acids [2, 3]. In earlier times, copper was used as a sterilizing agent for drinking water, burns and wounds, headaches, and itching. Hippocrates, the father of modern medicine, described copper as a curing agent for leg ulcers [4, 5]. Celsus et al. described the use of copper and various copper composites for the handling of venereal disorders. The first disclosure about the role of copper and copper compounds in the immune system was confirmed when workers in copper mining had strong immune resistance to cholera during cholera epidemics that broke out in Paris [4, 5]. The French physician, Luton, reported that salt of copper (copper acetate), functions well in taking care of arthritis patients both by external and internal use of copper acetate. The pharmacological actions of copper compounds were noticed in 1895 to treat various diseases including chronic diarrhea, dysentery and cholera. A researcher from Germany also mentioned in his findings that workers in copper mining were not touched by arthritis until they ended their employment in

mining and same observation was favored by the French physician Luton's finding in 1885 [4, 5]. W. B. Saunders Company in their report recommended the dose of 0.5 g/glass of water of copper sulfate to induce vomiting [4, 5]. It was also established that copper complexes deliberately boost healing time of ulcers and wounds. In addition, it was shown that the number of radioisotopes of copper, play a very important role in radiotherapy and imaging applications [6]. Researchers have devoted more attention to exploring the chemistry of copper and its complexes, owing to its potential and attractiveness for drug development in medical sciences. However, most of the inorganic salts of copper are toxic and can form a wide range of compounds with variable oxidation states including (I), (II), and (III). Among these, (II) is the most stable state for biological activity while the others (I and III) are less stable in while forming the complexes of 4, 5, and 6 coordinated species [7]. Again, the mode of action of copper is totally different from its organometallic counterpart as a drug, because copper/copper ions alone cannot act as a drug, but when combined with some organic moiety then response is more effective. New emerging research areas like nanoscience and nanotechnology are adding new perspectives to utilizing copper and its complexes in drug delivery systems [8, 9]. In earlier times, our ancestors discovered the importance of copper through experience and keen perception. Through substantial progress in biological science, the importance of copper in relation to human health has been fairly improved to date, however, many issue are still unclear and need to be explored. Researchers are still in action to explore the function of copper in human health so as to exploit all possibilities of copper compounds as drug for human betterment. This chapter elucidates the current states of advancement of copper-based composite materials for medical applications.

10.2 Source of dietetic copper

There are several sources of copper in the human diet, but plants play a leading role toward attaining human beings' necessary copper supply. The following plants are the chief accumulators of copper from soil and water: *Aeolanthus biformifolius, Athyrium yokoscense, Azolla filiculoides, Callisneria americana, Eichhornia crassipes, Haumaniustrum robertii and Bacopa monnieri*. Therefore, the main sources of copper for the human diet are grains, sesame seeds, cashew nuts, soybeans, mushrooms, sunflower seeds, lentils, beans, nuts, potatoes, green leaves, meat, dried fruits, black pepper, yeast and oysters. Human breast milk has the maximum concentration of copper (0.25 to 6.0 mg/l) [10]. Among the manmade foods, the main sources of copper are cocoa, legumes, liver, wine (red) and organ meats [11]. However, dietary and lifestyle factors can play a very crucial role in maintaining the copper balance by preventing to acquire adequate copper either by escalated excretion or reduced absorption. People who always prefer to drink soda or bottled water instead of spring water or natural well water, are keeping themselves away from a good supply of dietetic

copper. Generally, people who reside at high altitude in mountain valleys above sea level have the benefit of copper enriched water from natural glaciers.

10.3 Recommended concentration of copper for human diet

We know that copper is a crucial element which imparts a key role in human health; to establish a recommended limit, it is mandatory to know the concentration of copper in terms of surplus and deficiency in biological activities. In the continuation of ongoing attempts to fix the copper quantity in human diet, the Food and Nutrition Board (FNB) recommended 1.5 to 3.0 mg copper for adults per day [12]. The board also suggested that the present data on copper is enough to calculate the range of requirement, but not sufficient to establish a Recommended Daily Allowances (RDA) [12, 13]. An amount of copper more than the 1.5 to 3.0 mg daily intake in diet is toxic and responsible for the malfunctioning of the human system [12, 13]. The delay in notifying the significance of copper was finally pointed out by Klevay and Medeiros in 1996 [14]. They re-evaluated the previous ten versions of the recommended per day guidelines and found that the recommended amount of copper per day in 1943 was 1–2 mg, which was significantly different from the 1958 recommendation of 2 mg per day for adults. On the other hand, in 1989 [11] recommended intake of copper was again found to be 1.2 mg per day for male adults and 0.9 mg/day for females, which was quite low compared to the recommended 2 mg per day. The intake value kept on changing from time to time by different agencies like 2 mg/day by U.S. Food and Drug Administration, 1.5–3 mg by Natural Research Council (1989), followed by the WHO suggesting 10 mg/day consumption as the reasonable higher side limit of copper [12]. Suddenly, a decrease in an intake value to 0.9 mg/day became a central discussion due to a much lower recommended value [11]. But in 2010, Chambers et al.'s [15] findings recommended an optimum consumption of copper to be 2.6 mg/day to avoid copper deficiency. The recommended low intake of copper was not an acceptable value as the estimated routine loss of copper is about 1.3 mg per day [11]. In active males, there is additional 0.34 mg/day loss of copper due to sweating, shown by Jacob in 1981 [16]. Keeping in view all the facts, Hoogenraad [17] reported that a normal person's intake of a higher quantity of copper has an unimportant or no effect on its absorption because under normal conditions, the amount of copper absorbed in the upper gastrointestinal tract is generally ~0.5 mg per day and the remaining amount of copper from dietary intake is directly eliminated without entering pathways of absorption. The Food and Nutrition Board of the National Academy of Sciences recommends daily copper amounts according to age, 0–6 months: 0.2 mg; 6–12 months: 0.22 mg; 1–3 years: 0.34 mg; 4–8 years: 0.4 mg; 9–13 years: 0.7 mg; 14–18 years: 0.89 mg; for adults: 0.9 mg; pregnant women: 1.0 mg and breast feeding women: 1.3 mg [18]. The Board also recommends around 10 mg for adult men and women per day as the higher

consumption limit [18, 19]. Study also reflects that vegetarian foods have high amount of copper compared to non-vegetarian foods.

10.4 Copper consumption and suggestions

Most nutritionists prescribe per day copper consumption in the ranges of 1 to 3 mg; however, there is no assurance to this limit. Based on the research data, daily copper intake in the range of 4 to 7 mg encouraged positive actions like reduction in harmful cellular oxidation along with lowering LDL level, and escalating HDL levels [11]. Nutritionists also proposed that the maximum limit should not be more than 10 mg copper per day. Copper grants a defensive role in all circumstances with one omission; copper should not be in daily use if one is affected by Wilson's disorder. From the reported studies [18] we know that copper and zinc compete with each other for absorption in the body. Therefore, a higher amount of copper consumption can diminish the zinc uptake and vice versa. Thus, a balance is always appreciated between these two metals ions for the betterment of human health. The recommended ratio of zinc and copper is 7:1. Moreover, one can enhance per day consumption of copper by eating copper-rich foods.

10.5 Mechanism of copper transport in humans

According to the recommendations, the minimal acceptable intake of copper for adult is about 0.9–1.3 mg per day, whereas the usual human being consumes more than that per day [20]. Intake of 2–3 mg/day of copper is safe and avoids copper deficiency. However, in a normal person, intake of a higher amount of copper has an unimportant or no effect on its absorption because under normal conditions the amount of dietary copper that first enters into the stomach and is then absorbed in the upper intestinal tract is around 0.5 mg/day which finally reaches the liver after forming complexes with proteins [21], and the remaining amount of copper is directly eliminated without entering pathways of absorption [22, 23]. Therefore, the liver is the major stockpile for intracellular copper [24]. This occurs mainly through the employment of metallothioneins that play a key role as a "mucosal block" for copper bioavailability. Copper is mainly transported across plasma membrane by CTR1 transporters and always intracellular copper remains in a complex form to prevent the oxidative damage to DNA, proteins and membrane components caused by free copper ions. Hence, copper transportation and use involves a complex interaction between transporters and binding proteins. Copper also plays a very important role as a key backbone for catalytic centers in metalloenzymes named cofactors [25]. For a smooth and well-organized transportation and distribution of cupric for biological utility, a wide range of proteins play the key roles. The family of copper-bearing proteins imparts

a major role in metal detoxification and fixing the nonionic cupric state. Such proteins are metallothioneins, prion, albumin, transcuperin, CP, phycocyanins of blue green algae and hemocyanins of blue blooded organisms. Additionally, copper forms complexes with various biomolecules (lectins and glycoproteins) and facilitates its utilization. Amino acids also exert a crucial role in uptake of copper by the intestinal membranes. Among the essential amino acids, methionine, cysteine, and glutathione are the key amino acids which have capability not only to chelate the copper ions but also have the ability to reduce copper to a monovalent state leading to its large bioavailability. Glutathione forms an intermediary complex with copper in the enterocytes before transferring the metal to other target proteins viz., superoxide dismutase or ceruloplamin, etc., thus making easy its absorption. This ability of copper to form complexes with amino acids or organic acids is broadly exploited in animal nutrition experiments [25]. As an example, copper-lysine complex has been revealed to be more effective as an enhancement in nourishment for chicks than lambs [26]. Similarly, formulations of proteins with minerals, also termed proteinates, have been found to be highly effective as nourishment for growing calves in areas with high molybdenum content in foliage, as molybdenum competitively inhibits intestinal copper uptake [27]. On the contrary, various derivatives of copper such as chlorides, acetates, sulfates and carbonates enhance its bioavailability in higher organisms.

10.6 Copper deficiency and symptoms

The first confirmation of the role of copper in the human body was pointed out in 1928 based on a diet control experiment on rats. A chemical trial on pregnant rats was performed with a marginally copper-deficient diet, and the results of the trials confirmed that the newborn progeny were different with reference to their immune and brain prominence [28]. After extended research, it came to observations that not only iron was answerable for anemia but copper also contributed equally to the development of anemia. Therefore, physicians advised copper and iron mixed supplements to treat anemia in malnourished infants [11]. In 2001 Klevay [29] discovered that generally copper deficiency is found in people who normally eat excess zinc supplements. But higher amounts of zinc affected the copper absorption process. Processed food products are usually loaded with zinc and iron. Hence, regular consumption of high amount of processed food, vitamin and mineral supplements lead to zinc and iron overload, thereby all these aspects contribute toward copper insufficiency. On other side, those who constantly choose to eat animal protein over all the other protein sources without knowing that animal protein contains a higher amount of zinc than copper [30]. The most common example of copper deficiency arising due to zinc is the “white monkey syndrome”. It is apparent that the elevated amount of the zinc trims down the level of copper which ultimately leads to loss in pigmentation level in hair and skin, that is why it is named *white monkey syndrome* [31]. Now a days, it

is quite clear that copper is absolutely an essential element for life. Though copper is part of a trace element category, however, even a minor shortage of copper may be dangerous and impart disorder. The best practice is to meet the daily requirement of essential vitamins by eating a proper diet. Today, we read about the symptoms of any metal deficiency to investigate about the disease came from animal studies. Regarding copper deficiency in humans, major symptoms are anemia, low count WBC in blood, higher tendency toward infections, weaken growth rate, cardiac impairment, bone deformity and impaired collagen and melanin synthesis [32]. All these above mentioned symptoms directly or indirectly relate to a copper shortage and can be addressed by proper intake of copper in the diet. Literature study indicates that such types of copper deficiency are not so common in human beings. But copper deficiency may increase the chance of disorder in living systems in different ways by creating trouble in the metabolism.

10.7 Copper metabolism and major diseases

No doubt copper is essential for numerous important utilities in humans and deficiency of copper ions to a certain level can lead to many diseases. For many major diseases the role of copper has been clearly established; however, the role is still unclear for many health problems. The metabolic role of copper and some the major disorders are discussed.

10.7.1 Copper homeostasis

Inorganic copper consumed via drinking water and other sources is processed directly and enters into the blood stream bypassing the liver, however, organic copper passes through the liver and is transported in a secure manner because of its toxic nature [33]. In other words, a copper homeostatic mechanism imparts a critical function in the deterrence of copper toxicity. Around 1.5 mg daily dietary copper is absorbed from the stomach and the small intestine. Absorbed copper is transported to the liver with the help of albumin and ceruloplasmin. The liver of a human being has around 10 % of the total copper present in the whole body and total content is around 80 mg in the whole body. The residual glut copper is excreted in the bile into the gut, and fecal copper production is the sum of unabsorbed eaten dietary copper and that re-excreted into the gut [22]. Copper homeostasis is harmonized by the alteration in both absorption and excretion practices. Simply, with alteration at low and high copper consumption, the output of absorption of copper goes up and down accordingly, however copper is mainly controlled in the course of excretion [23]. Copper is also situated in a number of metalloenzymes which impart a vital role in hemoglobin production, carbohydrate metabolism, the crosslinking of collagen, hair keratin and elastin along with antioxi-

dant protection mechanism [33]. In addition, a number of copper-based enzymes, like cytochrome c oxidase, superoxide dismutase, ferroxidases, monoamine oxidase, and dopamine β-monooxygenase, function to diminish reactive oxygen species (ROS) or molecular oxygen [33]. The common traits for homeostasis are anemia, leucopenia, hair loss, normocytic, depression and fatigue.

10.7.2 Oxidative-stress-related disorders

A lot of exposure to copper content can lead to a number of uninvited health impairments in vital organs of human beings. The main health problems which are directly concerned to excess exposure to copper contents are damage to kidney, liver, immunotoxicity, and toxicity expansion etc. [34]. All these health problems are indirectly concerned with oxidative damage to different membranes or proteins. The reason is that the copper ion has the ability to generate huge amount of reactive oxygen species, where a surplus of copper may perhaps result in oxidative strain related health problems, associated to its redox reactivity. It is also observed that copper can assists oxidative injury through a free radical mechanism similar to a Fenton reaction [35]. The same has been confirmed by electron spin resonance technique and further supported by copper intervening hydroxyl radical mechanism *in vivo* [36]. Apart from copper overdose, copper deficit also affects the oxidant protection arrangement resulting in increased ROS and oxidative damage to DNA, proteins and lipid that have been noticed in many clinical trials of copper deficiency.

10.7.3 Aceruloplasminemia

Ceruloplasmin is a glycoprotein surrounded by copper created in the liver and responsible for binding up to 95 % of total copper found in serum. The resulting glycoprotein accounts for the catalyzed transfer of Fe(II) to Fe(III) and is stored as transferrin. Therefore, deficiencies in serum ceruloplasmin can be answerable for accumulation of Fe(III) ions in the reticuloendothelial arrangement along with parenchymal cells. If the ceruloplasmin deficiency becomes hereditary, it can alter iron metabolism [37, 38]. There are several symptoms of aceruloplasminemia which include diabetes mellitus, retinal pigmentary degeneration and dementia etc. Histopathologic studies provided evidence for the considerable agglomeration of Fe content in the pancreas, liver, and central nervous system.

10.7.4 Wilson's disease

The main cause of Wilson's disease (WD) is a fault in copper excretion and it is a genetic disorder of copper accumulation. Simply, one can say that WD is a genetic disease concerned with copper transport and responsible for an overloaded copper development in the liver [39]. WD develops a disturbance in metabolism due to the key genetic defect in the ATP7B gene. Any defect in the ATP7B gene is responsible for abnormal copper pumping through the trans-Golgi vesicle and impairment of copper assimilation into ceruloplasmin and then finally excretion into the bile. An important input to pathophysiology of WD are copper intervening oxidative damage, commencement of cell death mechanism, and eventually outflow of copper into the plasma. The final outcomes lead to deposition of the surplus amount copper in hepatic tissues [19]. Remarkably, the hepatic copper surplus associated with WD is histopathologically distinguished by bulgy hepatocytes, inflammation, and cytoskeletal changes and ultimately directs to cirrhosis [40]. WD shows meticulous neurological indications, however after diagnosis it can be cured in different ways including the use of proper chelating agents, low consumption of copper in diet and high consumption of zinc supplements [41].

10.7.5 Menkes disease

John Menkes described Menkes disease (MD) for first time in 1962; it originates in copper deficiency. MD is a genetic impairment originated by mutation in the ATP7A gene which encodes for p-type copper carrying ATPase (ATP7A) [42]. The ATP7A is extensively articulated in humans and allows the carrying copper into the trans-Golgi arrangement for copper-dependent enzyme development including dopamine-β-hydroxylase (DBH) and arbitrates copper evacuation from cells. Copper absorption and excretion via the liver are normal in MD including copper enzyme levels; however, copper absorption in the intestinal zone severely decreases. The diminished intestinal absorption of copper results in copper being poorly distributed to cells throughout the body, as a result copper accumulates in intestine, kidneys along with a low level in brain tissues [43]. This unusual copper deposition stimulates special medical symptom including growth failure and the weakening of the nervous system [44]. It should be highlighted that the absorption by peripheral tissues is average; though excretion and intracellular copper trafficking are disordered by alteration in the ATP7A gene. As an outcome of damaged copper efflux, peripheral tissues in MD tolerant have a tendency to assemble copper in the appearance of copper metallothionein. At the clinical level, MD is distinguished by liberal neurological disorders and copper deficiency in the brain of the developing infant leading to death [45]. Death usually occurs up to the age of 10 years. Timely diagnosis and proper action with intravenous copper infusion enhance the possibility of survival for MD patients. However, therapy

can regularize signs and symptoms of the disorder only in patients with ATP7A alteration that have the mild action on the protein activity [44].

10.7.6 Alzheimer's disease

Alzheimer's disease (AD) is a chronic neurodegenerative disease which causes difficulty in remembering fresh events or simply short-term memory loss. Hebert et al. [46] reported that AD affected around 4.7 million Americans in 2010, and its occurrence is expected to be nearly triple in coming decades. Regarding the mechanism of AD, a relation between AD and copper is found where the AD patients have an augmented stage of copper in cerebrospinal fluid and plasma [47]. For example, a rabbit-based experiment concerned with AD showed that addition of an extra quantity of copper in drinking water appreciably intensified the brain AD pathology and led to failure of memorizing ability. Similarly, a society dependent study confirmed that higher consumption of copper along with high saturated fats could be allied with accelerated cognitive downturn [48]. It is assumed that copper in AD can be communicated with amyloid-based peptides in the self-aggregating plaques and neurofibrillary tangles, leading to this disease through cellular oxidative stress and copper ions may possibly oxidize the β-amyloid peptide via ROS creation. Current data supports the association among copper metabolism in AD and prion diseases, although the accurate pathway is yet to be explored. However, current research confirms that copper controls both the creation and the deposition of β-amyloid plaques. Also, it has been demonstrated that both copper excess and deficiency regulates β-amyloid peptide production and degradation [49]. It should be highlighted that copper shortage plays a key role in more ROS development and oxidative harm to proteins. Some factors which contribute to the risk of developing AD are family history, midlife hypertension, age, genetic factors, obesity, diabetes, and hypercholesterolemia [50]. However, advance research is still required to gain insight of the function of copper in Alzheimer's disease.

10.8 Copper metabolism and hypothesized health problems

Copper is a fundamental essential element with biological relevance related to connective tissue production and coding. Deficiency of copper is responsible for various diseases mentioned in Section 10.6. It is also essential in boosting immunity because of its essential role for the absorption of iron. On the other hand, overconsumption of copper leads to various side effects like cramps, diarrhea, vomiting, depression, schizophrenia, hypertension, senility and insomnia. Therefore, an optimum level is necessary to avoid the listed health problems. For the better absorption of copper, stomach should be in a state of high acid production, otherwise the absorption rate is affected. For example, antacids have an adverse effect of absorption of copper from

milk and egg proteins. Some of the common problems are elaborated below where concentration of copper directly or indirectly influences human health.

10.8.1 Skin and copper

Lysyl oxidase is one of those enzymes which absolutely depend on copper. Lysyl oxidase enzyme activity in the skin altered with low dietary consumption of copper and reduction in the action of lysyl oxidase enzyme. This reduction in the action of lysyl oxidase enzyme leads to defective collagen and elastin polymerization [51]. Collagen is a well known protein that maintains flexibility, complexion and firmness to skin. Defects in collagen lead to wrinkles, sinks, fragile bones and aneurism of major blood vessels etc. However, via stimulation and restoration of collagen synthesis it can be one possible way to remove wrinkles and tighten the skin and for that collagen building enzymes are very essential. A number of clinical trials where the supplement of required enzymes with ions of other metal ions like iron, zinc, and cobalt have been performed. However, it was observed that every time they were unsuccessful in reinstating the enzyme action, which clearly confirms that neither any minerals nor any costly cosmetics can stimulate collagen synthesis by replacing copper in lysyl enzyme. Low levels of cytochrome c oxidase, superoxide dismutage and dopamine betahydroxylase may result in neurological degeneration, generally by oxygen free radicals [52]. Some clinical trials on rats found that copper dearth in rats was responsible for cardiac amplification and anemia, which can be handled with the use of anti-glycosylation agents. A large intake of a sucrose loaded diet can also lead to copper shortage along with food stuffs that reduce peroxidation and glycation. Copper scarcity also decreases the activity of superoxide dismutage, a major antioxidant enzyme which further speeds up skin aging [53]. Cardiac nuisances are powerfully correlated with copper scarcity and reduced transmission is neither good for skin nor for whole body.

10.8.2 Immune system function and copper

For good health in everyday life, we need a robust immune system to protect us from disease by fighting irritating germs such as viruses, bacteria and parasites [11]. The immune system is composed of mainly two components, the innate and the acquired immune system. Innate immunity exists since birth and consists of mainly nonspecific protections, which take account of both structural and physiological barriers like skin and mucous membranes. Acquired immunity is adaptive and acquired after birth, and stimulated by introduction to contagious agents. The acquired immunity system involves the T-cell system of cell-intervened immunity and the B-cell system of antibody production. The cells of this system are accountable for producing antibodies,

providing memory, and killing infecting micromemory development organisms. Both systems collectively provide the integrated system of host defense. Medical history literature shows that metal plays a vital role in the immune system (as a killer of foreign microbes) and facilitates human health directly or indirectly. Montgomery et al. [54] in 1974 reported that copper may be directly concerned with the function of harmonizing the system. From the literature review it is confirmed that copper deficiency in human diet affects the immune system by reducing the activity of phagocytic cells, mainly neutrophils and macrophages. The main role of neutrophils comprises visiting the infected location infection, sticking to the endothelium and transmigration crossways the endothelium, where neutrophils are concerned in phagocytosis and assassination of foreign attacker by action of the respiratory explode [55]. The reduction in the number of travelling neutrophils due to copper shortage, results in a condition expressed as neutropenia in human and other animals [56, 57]. The reduction in cellular copper position, respiratory explodes and candidacidal action is reported in peritoneal macrophages due to copper deficiency in rats [58]. The reduced action of phagocytic cells altered the innate immune protection system and imparts a role toward the higher possibility of infections [59]. With reference to the acquired immune system, the role of copper shortage was reported while investigating the impact of splenic lymphocytes to T-cell mitogens. This reduction in response of splenic mononuclear cells (MNC) to T-cell mitogens is also reported in number of species including human being. In 2002 Bonham et al. [34] supported the role of copper in immune cells and explained that the copper deficiency is so sensitive, and immune cells are considered an accurate indicator for marginal cooper deficiency. Their study also helps in understanding how copper deficiency is liable for low interleukin 2, declined production of T-cells, and increased weakness to bacterial pathogens such as salmonella, reduction in neutrophils number and action to kill microorganisms like Candida albicans.

10.8.3 Cancer and copper

Cancer always plays key role as a killer in the developed world, therefore, a wide range of attempts are always welcome to develop the new medicine or modification in the existing medicine which can be broadly used in cancer treatment. Based on the research work involved in the treatment of cancer using copper and its complexes, it can be asserted that copper does not participate in the origin of cancer in human beings [4, 5]. Even experimental evidence showed that these complexes can stop or impede the growth of cancers cells in mice under optimum conditions where cancers are usually induced. The patients of skin cancer were treated using a mixture of copper chloride and lecithin as a medicine around 1912 [5, 11]; the use of such a mixture in the past, confirmed that the copper complexes have anticancer action. After that in 1930, a research group in France pointed out that vaccination of colloidal copper barred

tumor tissue [5, 11]. But recent investigation with mice in the USA found that [4, 5], in reality, this treatment of solid tumors with copper complexes such as copper salicylates noticeably reduced the tumor escalation and improved survival rates. The results of the work confirmed that copper compounds did not only eradicate carcinogenic cells but also caused them to revert back to standard cells. Similarly in 1984, Oberley et al. [60] reported that many copper compounds of superoxide dismutase activity impeded the impulsive growth of cancers in mice by the creation of tumor suppressor protein p53, which reduces the growth of tumors. Another study in the USA showed that colon cancer is the second most fatal type of cancer in the USA and "adenomatous polyposis coli", a gene recognized toward reduction of the development of tumors, mutates all over the development of colon cancer [5, 11]. The APC gene commands the building of the APC protein, which imparts a crucial function in numerous cellular processes. The newly formed APC protein performs as a tumor suppressor, which means that it keeps cells from mounting and dividing excessively fast in an uncontrolled manner. It keeps control of how often a cell divides, how it affixes to other cells within a tissue, and whether a cell moves within or away from a tissue. The formation of this protein also ensures that the number of chromosomes in a cell is correct following cell division. Davis and Johnson et al. [61] in 2002 said that this study's results have important connotation for the reason that more than 80 % of the people in the US does not consume a sufficient dose of copper. Any mutations in the APC gene are also accountable for Turcot syndrome, which is very much linked to familial adenomatous polyposis. Turcot syndrome is an alliance of colorectal cancer with a type of brain tumor called a medulloblastoma and about 67 % of people with Turcot syndrome have mutations in the APC gene [11]. The metal coordinated with organic moiety results in the formation of metal-based drugs which are generally used in the therapeutic anticancer applications as an anticancer agent in medical institutes. Basically we can say that ligands are not only responsible to manage the reactivity of the metal, but also impart a crucial function in determining the type of interactions required in the detection of biological target location such as DNA, protein receptors and enzymes. Any changes in the organic moieties are liable to be associated with alterations in their respective biological movement. Hence, to cover the advance developments in chemistry (metal type, oxidation state, number of coordinated sites in ligands and geometry of the coordinated ligands) involved in metal-based anticancer agents, researchers working in the area of cancer research. The therapeutic property of copper and other metal complexes in modern medicine might be initiated by the discovery of the anticancer properties of cisplatin [62, 63]. Therefore, widely, the clinical use of cisplatin to fix the particular form of cancer, this also encouraged a novel generation of efficient, selective and metal-based cancer therapeutics, thereby representing the prospective of metal complexes as anticancer agents. In view of the fact that cisplatin was initiated for chemotherapy of cancer, an exploration for metal complexes with antiproliferative action has started. A variety of copper complexes were found to be cytotoxic, this is because copper complexes have catalytic properties to reactive oxygen species and

can rupture DNA strands. However, in many cases, possibly more refined mechanisms are involved. Roy and Cini et al. [64, 65] reported that meloxicam and piroxicam form copper complexes and these complexes exhibit strong binding with DNA, disrupting its structure and stopping transcription as a result. Chen et al.'s studies and some clinical trials by the governmental sector described finding that disulfiram used as a drug in alcoholism treatment forms *in vivo* copper complexes which perform as a proteasome inhibitor, and selectively brings apoptosis in breast tumors [66, 67]. Currently "disulfiram and copper gluconate" are undergoing phase trials 1 for treatment of solid tumors with metastases in the liver.

10.8.4 Arthritis and copper

Arthritis is a type of joint disorder that involves enormous pain and infection of the joint. Osteoarthritis (OA) is a sluggish disintegration of the articular cartilage and bone propagation in joint margins that gradually gets worse over time. On the other hand, rheumatoid arthritis (RA) is a chronic inflammatory syndrome in which the synovial membranes of the joints and other tissues are sullied via an autoimmune response. RA is mainly observed by the commencement of an inflammatory and coagulation development [11]. The main oxidative trauma in OA is associated with nitric oxide but in RA it is radical oxygen intermediates [68]. Human ceruloplasmin (CP) is a multidirectional protein produced in the liver and having the capacity to bind with copper and acts as a copper-binding protein. Any change in the level of serum copper beyond the average, can lead to different inflammatory syndromes in humans [69]. The enhancement of serum copper in inflammation could be owing to the boost of ceruloplasmin, which is an acute-phase protein [70, 71]. This phase of CP acts as a sensitive stage, reactive protein to anxiety and suffering conditions. CP oxidizes ferrous to ferric and gets reduced the strength of ferrous accessible for producing risky oxidant group. CP also has capacity to act as an effective inhibitor of leukocyte myeloperoxidase (MPO) which is a key factor of oxidants *in vivo*. Therefore, CP imparts both superoxide dismutase and NO oxidase tasks and diminishes the production of reactive oxygen (ROS) and nitrogen species, with an ensuing tissue-protective outcome [68]. CP surrounds six cupredoxin-type domains, each one having 150–190 amino acids [72]. The oxygen reductase role of CP is related with the trinuclear copper center, at the interface among domains 1 and 6, while the ferroxidase action is consummated by the copper site in domain 6. Remarkably, preparations of CP sanitized from human plasma undergo spontaneous deprivation, probably due to trace quantity of as yet undisclosed plasma protease(s), producing bulky fragments and changing CP activities. [72]. Literature reported that CP antioxidant inhibits 5-lipoxygenase and other leukocyte proteins associated with inflammatory and septic processes. The unusual discharge of MPO from activated neutrophils intensifies inflammation and tissue injury and is the source of the commencement of various diseases, even in the

lack of infection. In brief, plasma MPO and thrombin concentration are surprisingly elevated in patients with rheumatoid arthritis (RA), an inflammatory autoimmune ailment most commonly affecting the synovial membrane of flexible joints [72]. The simultaneous occurrence of CP and thrombin in the blood at their higher level and in the synovial fluid of RA patients impelled us to test the assumption that thrombin might act together with CP *in vivo* and influence the several roles of this protein. It is also well accepted that the main function of ceruloplasmin is to neutralize the free oxygen radicals responsible for disturbance in metabolism results in arthritis [73, 74]. The literature study disclosed that copper can help minimize the painful inflammation. Many scientific papers reported that use of copper complexes to treat arthritis with mixing of many anti-arthritis drugs like ibuprofen and aspirin, demonstrated the better action than ibuprofen and aspirin without copper. For example, copper aspirinate is additionally effective in the cure of rheumatoid arthritis than aspirin alone. However, research related to rheumatoid arthritis showed some inconsistency regarding copper on various diseases. For example, people with rheumatoid arthritis were found to have elevated serum copper levels compared to normal. Subsequent research confirmed that serum copper is a physiological response to inflammation, rather than due to inflammation. Though, in evidently contradictory cases, copper compounds were effectively applied to treat many arthritic conditions and inflammations. It is also confirmed that people with rheumatoid arthritis are usual copper deficient due to habitual diet: typically they consume too much fat without enough fiber, zinc, magnesium and copper.

10.8.5 Osteoporosis and copper

Osteoporosis is known as a disorder characterized by low bone mass and micro-architectural weakening of bone tissue ensuing in amplified bone fragility and therefore increasing a risk of fracturing [75]. Simply, osteoporosis is a disease linked to weakening of bone strength and breaking. There are various factors that play a major role in the growth of osteoporosis such as sex, smoking, alcohol intake, exercise, age, menopause, body weight, sunlight, thyroid, low calcium intake, scarcity of other trace elements and vitamins. Copper contributes toward healthy bones and diminishes the possibility of osteoporosis. But, severe Cu deficit is known to originate skeletal abnormalities. Osteoporosis is connected with genetically determined malabsorption of copper that arises in Menkes disease [76]. The function of copper in bone metabolism can be correlated chiefly to the copper-dependent enzyme that is lysyl oxidase, for which copper performs as a cofactor. This enzyme is essential for the development of lysine-derived crosslinks in collagen and elastin [77]. Literature on animal based studies have shown that the action of this enzyme is enhanced in response to an augment in dietary copper intake [78]. Copper also imparts in the inhibition of bone resorption, during its accomplishment as a cofactor for Superoxide dismutase (SOD),

which is an antioxidant enzyme having two atoms of Zn and Cu centers. The SOD performs as a free radical scavenger, neutralizing the superoxide radicals created by osteoclasts through bone resorption. Research work based on copper deficient animals point out that a deficiency of copper is marked by an inhibition in osteoclast role, but no alteration in osteoblast action [79]. The German physician Rademacher, many years ago, established that copper supplement in patients speeds up curing of damaged bones. So, proper dietary copper can solve osteoporosis in humans as well as in animals. Copper shortage is highly associated with scoliosis; skeletal abnormalities, bone fractures and lowers bone calcium levels to below normal. A survey on elderly people found restoring the loss of bone-mineral density after providing copper supplementation of 3 mg daily for two years. In a parallel study, it is also confirmed that healthy adult males on 0.7 mg per day for six weeks display an amplified rate of bone breakage [80, 81].

10.8.6 Pregnancy and copper

Copper is a fundamental trace element for human beings. The smooth running of metabolism in humans depends upon the number of enzymes and a sufficient number of enzymatic activities depend upon the copper. For example enzymes like cytochrome c oxidase, lysyl oxidase, tyrosinase, superoxide dismutase copper, dopamine-b-hydroxylase, monoamineoxidase and ceruloplasmin provide enzymatic activities in the presence of the optimum level of copper. Any type of copper deficiency responsible for enzymatic deficiency in the human body can prompt a number of nutritional and vascular disorders [11]. It is mentioned in the literature that serum concentrations of certain crucial trace elements like copper and zinc are changed during pregnancy [82]. This alteration in concentration of trace elements leads to complications in mothers and infants. The copper level generally increases with increasing in gestational age [83]. In 1930 a sheep farm at Western Australia, it was observed that a number of newborn lambs were unable to stand and lost coordination of their action. abnormality was due structural malfunctioning in the brain areas concerned with learning, memory, and responsible for coordination and movement. However, after a long study it was suggested that the mother sheep (pregnant sheep) were nourished on a territory where the grass grown did not flourish with sufficient amounts of copper content. The main reason for the lamb deaths was due to insufficient copper that damaged their nervous system and brain [11]. A number of other studies also highlighted that copper insufficiency at the time of pregnancy can lead to many biochemical and structural deformities, owing to a decrease in connective tissue metabolism, energy production and free radical protection mechanisms [84, 85].

10.8.7 Heart disease and copper

Several scientists have drawn a correlation between copper deficiencies and heart problems. Copper may participate in the vital function of cardiovascular disorder through coagulation cascade. Any alteration in copper amount can be accountable for the growth of coagulopathy like atrial thrombosis [86]. The contribution of copper as a cofactor in enzymes is crucial for coagulation and may participate up to certain levels, but its contribution is more crucial for free radical oxidation. Copper deficiency is also reported in people who have died from ischemic heart syndrome. In addition, hemoglobin production levels reduce with copper deficit and this reduction may lead to an iron-resistant hypochromic, and microcytic anemia [87]. Copper also plays a role in the control of cholesterol, therefore, it is assumed that a metabolic imbalance between copper and zinc is a key source of coronary heart disease. There are a number of heart problems reported in medical literature closely associated with copper deficiency such as systolic and inflammation, blood clotting, diastolic hypertension, and atherosclerosis [88]. In the mentioned heart diseases, the reason for some diseases was found to be directly linked to a diminishing in key activities of specific enzymes whose action depends on the copper. Three further nonspecific mechanisms of harmful effects in cardiovascular disorder due to copper insufficiency comprise glycation, peroxidation and nitration. Of these three systems, any one can create health troubles independently and collectively they can cause more lethal disease. Coronary artery disorder in people exhibiting a number of risk features, connected to copper insufficiency in rats, such as hypertriglyceridemia and hypercholesterolemia. Additionally favor this alliance between copper and heart disorder through unusual lipid metabolism. This abnormal metabolism is again favored by an increased cholesterol level which further hinders copper absorption and retention. Dyslipidemias, such as improved LDL cholesterol and diminished HDL cholesterol levels, have also been reported in copper-deficient rats [88]. The entire mentioned metabolic problem is very common now a days in human being. Researchers have reported that copper complexes can participate in strengthening heart muscles and reducing heart attacks. In the above process, copper imparts a major function of anti-inflammatory agent and copper sources can be given as dietary supplements for avoiding and controlling coronary heart disease. In a survey based on heart attack rates, it was found that in France heart attack rates are significantly lower than other parts of Europe. This is due to the consumption of red wine, having a sufficient copper level [11]. Therefore, it is concluded that a particular level of copper is required to maintain good health. The copper level in diets can be maintained by proper food selection and food must be enriched with multivitamin, mushrooms, nuts and legumes. Other than copper intake we need awareness of some risk factors that enhance the possibilities of heart disorder such as hypertension, cigarette smoking, diabetes and hypercholesterolemia. Therefore, to avoid the possibilities of heart problems we should take care of the other

mentioned problems too. No doubt, based on research data it is expect that copper may be a vital module for future medicine.

10.9 Copper complexes as an emerging tool in pharmaceutical sciences

Out of all copper present in the human body more is concentrated in the brain and heart than in any other tissue organs and mainly stored as copper thionein. Through various metalloenzymes including tyrosinase, cytochrome c oxidase, dopamine-3-hydroxylase, pyridoxal-requiring monamine oxidases, and superoxide dismutase, copper controls the metabolic rate of the heart and brain. Copper deficiency can lead to brain disease in infants, anemia, and heart diseases. To know the considerable advances of metal ion drug combinations over parent organic drugs, many copper complexes in combination with nonsteroidal anti-inflammatory drugs (NSAIDs) have been studied. There are many such drugs which were included in the investigation, for instance aspirin, ibuprofen, tolfenamic acid, niflumic acid, oxicams, ketoprofens, diclofenac naproxen, and many others [89]. Recently Psomas et al. [90] have successfully synthesized many copper complexes with formula [Cu(X)(Y)(Z)] where X is the antimicrobial drug flumequine with additional ancillary ligands Y and Z and have observed remarkable binding interactions of these complexes with DNA and human serum albumin, which opens a new window for future use of complexes as antimicrobial, antitumor and antioxidant. The best possible example can start with cisplatin in which successful trials and treatment of various cancers were done using coordination chemistry as the basis for synthesizing metal-based drugs. The metal toxicity effect of platinum only permits dose-limiting use and endorses further research in the search of new metal ion complex with less toxicity to normal cells. Based on the findings it is clear that copper complexes have shown optimistic results due to the fact that copper is less toxic and may be a good substitute for Pt for cancer cells. Copper and its coordination chemistry is widely explored due to its redox active and strong complex forming ability. The reason is that the redox couple of Cu(II)/Cu(I) can be easily tuned by varying different donors, geometry, electronic and steric factors, and chelating ability of the ligands. A large variety of copper complexes were employed for clinical trial as anticancer drugs and in the following section we will explain some important sets of such complexes. In 2010, Leabu et al. [91] successfully synthesized a mononuclear Cu(II) complex using a bidentate ligand of S and N donor atoms (1A, Fig. 10.1). Due to presence of the thiosemicarbazone moiety in the ligand, the complex showed excellent anticancer properties after testing *in vitro* on HeLa cells. The author has synthesized different complexes where the coordination number and geometry around the metal center varies, keeping thiosemicarbazone ligand the same. All the complexes show good antiproliferation activity and in the range of 1 to 10 μM. (The antibacterial and antiviral properties of thiosemicarbazone were widely explored,

however antitumor activity was tested recently). Gulea et al. [92] in 2008 successfully synthesized a binuclear Cu(II) complex using similar a thiosemicarbazone moiety with slight modification on the Schiffbase side chain by incorporating pyridyl moiety (1B, Fig. 10.1). The complex is in distorted bipyramidal geometry where a sulfur atom from the thiosemicarbazone moiety served as a bridging entity. The authors successfully synthesized various other complexes using different copper precursors and finally ended up with resulting complexes with monomer or dimer or coordination polymer types. After biological tests it was found that all the complexes showed a strong interaction with calf thymus DNA and toxicity against V79 cells even in very low concentration of ~2.5 μM range. Natarajan et al. [93] in 2011 made a slight modification in their ligand backbone to observe the effect of ligand substitution at N terminal of thiosemicarbozole framework and subsequently its biological activities. They successfully synthesized monomeric four coordinated Cu(II) complexes using either $CuCl_2$ or $Cu(NO_3)_2$ as copper sources. After biological activity tests for all the complexes it was found that the complex [Cu(L)Cl](MeOH), (1C, Fig. 10.1) showed less toxicity toward cancer cells highly selective over similar complexes in this series, while the nitrate complex $[Cu(L)NO_3]$ strongly interacted with calf thymus DNA with a strong binding constant in the order of ~10^6 M^{-1}. Dalovic et al. [94] in the same year 2011 made slight modifications to obtain a set of Cu(II) complexes by attaching pyrazole unit to the thiosemicarbazone moiety. The complex ended up with a square-pyramidal geometry around Cu(II) ion via thiolate, N-donors and Cl-bridging (1D, Fig. 10.1). Though the authors synthesized two complexes with slight variation of substituent on the pyrazole unit, both the complexes showed a dose-dependent cytotoxicity toward REH, HL60, C6, mouse-L929, and B16 cells. By comparison of the similar experiment with uncoordinated ligands it was found that there is no cytotoxic activity. It confirms that metal has a key and prominent role. Peng et al. [95] in 2008 synthesized Cu(II) complexes of thiosemicarbazone types with additional binding site of thiol or O-donors (1E, Fig. 10.1). Here the ligands act as a four donor type and the authors synthesized different complexes by varying substituent at the N-terminals. After biological activity tests it was found that these complexes show inhibited proliferation for SK-N-DZ NB cells and are also responsible for cell cycle arrest for S-phase. The complex 1E showed stronger anticancer activity than unsubstantiated analog of 1E copper (II) complexes. In a parallel approach, several authors have also tried to explore the anticancer activity of dithiocarbamates copper complexes. Among them Dou et al. [96, 97] have successfully synthesized Cu(II) dithiocarbamates (1F, Fig. 10.1). The authors prepared various bis-complexes by varying the substituent in the middle C-atom. Bioactivity tests confirmed that the complexes have the ability to induce apoptosis in cancer cells. Another complex of the above analog was prepared (1G, Fig. 10.1) and showed good cytotoxic properties *in vitro* against A431 and 2008 cancer cells in extremely low concentration range. Various complexes of dithiolates derivatives have been reported in molecular form of [Cu(amine)(L)] where L = dithiolates derivatives. Various neutral, mixed-ligand copper(II) complexes were synthesized and tested for both *in vitro* and

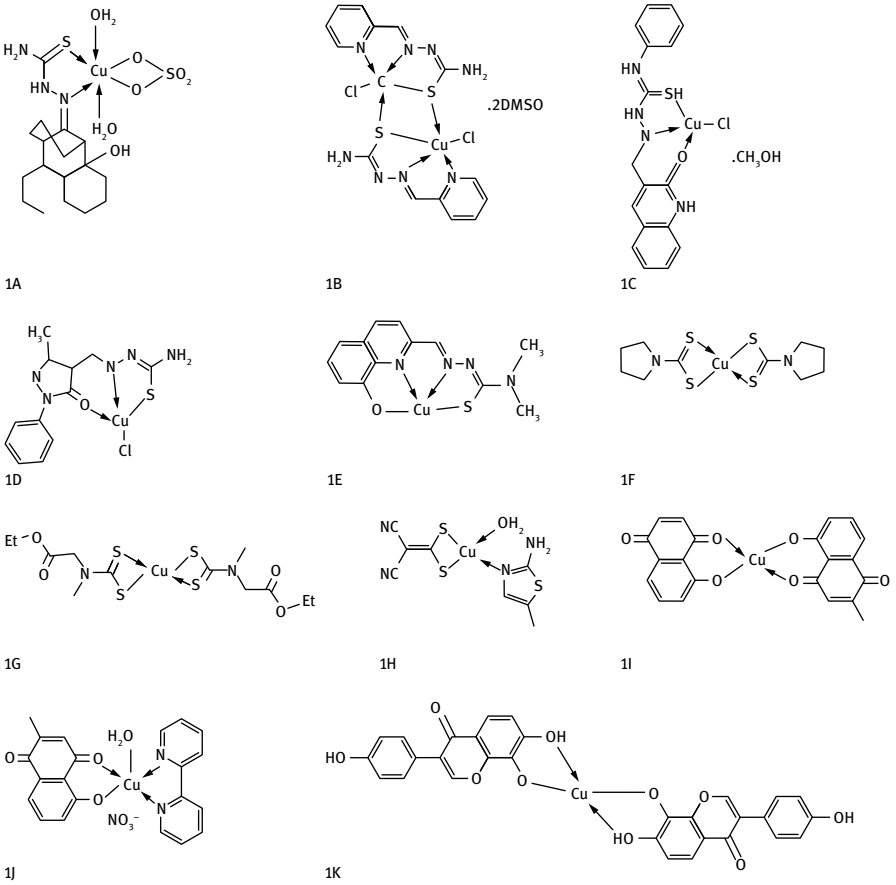

Fig. 10.1: Various copper complexes having anticancer properties.

in vivo antiproliferative activity tests. Among them the complex (1H, Fig. 10.1) showed a significant increase in lifespan of leukemia P388 in mice *in vivo* condition. The good biological activity of these complexes is due to their high polar and electrostatic interaction with biomolecules rather than their hydrophobic nature. 1,4-naphthoquinone is a good natural product used for treatment of cancer, arthritis, dysmenorrhea. Orvig et al. [98] synthesized various complexes of 1,4-naphthoquinone derivatives in pure or mixed form. Among them, the complexes (1I and 1J, Fig. 10.1) have shown anticancer activity for various cancer cells including BEF-7404, NCL-H460, CNE2, NCI-H460 etc. These complexes have strong cytotoxic ability and inhibited topo-1 character to a greater extent than free plumbagin. These complexes also have good DNA binding ability due to their strong noncovalent interaction with DNA base pairs. Having O-donor binding in the family of isoflavones has good biological activities and antitumor actions. A copper complex of such a ligand (IK, Fig. 10.1) was found to have

stronger DNA binding ability potent toward human cancer cells compared to free isoflavone [99].

Metal complexes of hesperetin and naringenin (O-donor chelating ligands) with copper(II) have been found to have more effective inhibitor properties toward SGC-7901 and HepG2 cancer cells. It is also confirmed that these complexes (2A, Fig. 10.2) [100] have better anticancer activity compared to free ligands. Recently it was found that 2-thenoyltrifluoroacetone, a well-known diacetone derived analog ligand, acts as a mitochondrial electron flux inhibitor. The copper complex of this ligand gives planar bis-complex (2B, Fig. 10.2). Anticancer activity results of the complex show an excellent result against leukemia (K562) cells [101]. The good bioactivity test of this complex is due to its bulky hydrophobic and electronegative F-atoms. A benzoic acid derivative mononuclear copper complex (2C, Fig. 10.2) [102] was found to be highly redox active and to selectively kill CEM/ADR5000 leukemia cells while leaving normal cells unharmed. Recently the flavonoid family of benzopyrazone derivatives yielded a bis-copper complex having two perchlorate ions as axial ligands.

The complex (2D, Fig. 10.2) provides excellent antitumor activity toward A375 cells which has comparable results to cisplatin. Pyrazole–pyridine ligands are good donor ligands with N, N sites for effective metal binding properties [103]. A dimer of such a ligand (2E, Fig. 10.2) [104] with ancillary chloride ions occupying both bridged and axial positions was found to have the highest activity due to flexible free thioether groups. The anticancer activities of the complexes toward HT1080 cancer cells was found to be active even at the concentration value of 3 μM0. Several other complexes of square-planar geometries were also found to be very active toward cancer cells. A bis-substituted complex (2F, Fig. 10.2) [105] showed a promising IC50 value toward HL-60 and WM-115 cancer cells in the range of 6.5–8.0 μM. Several other Cu(II) complexes of benzimidazole derivatives having tri-or tetra-dentate types also show similar anticancer activities Among them an octahedral Cu(II) complex having three coordinate sites from ligand origin and remaining sites occupied by counter anions and solvent water molecules (2G, Fig. 10.2) [106]. This complex showed a promising anticancer activity due to benzimidazole units and the cupric ion was not a requirement for biological activity. Interestingly, toward K562 cancer cells having less toxicity for normal bone marrow cells.

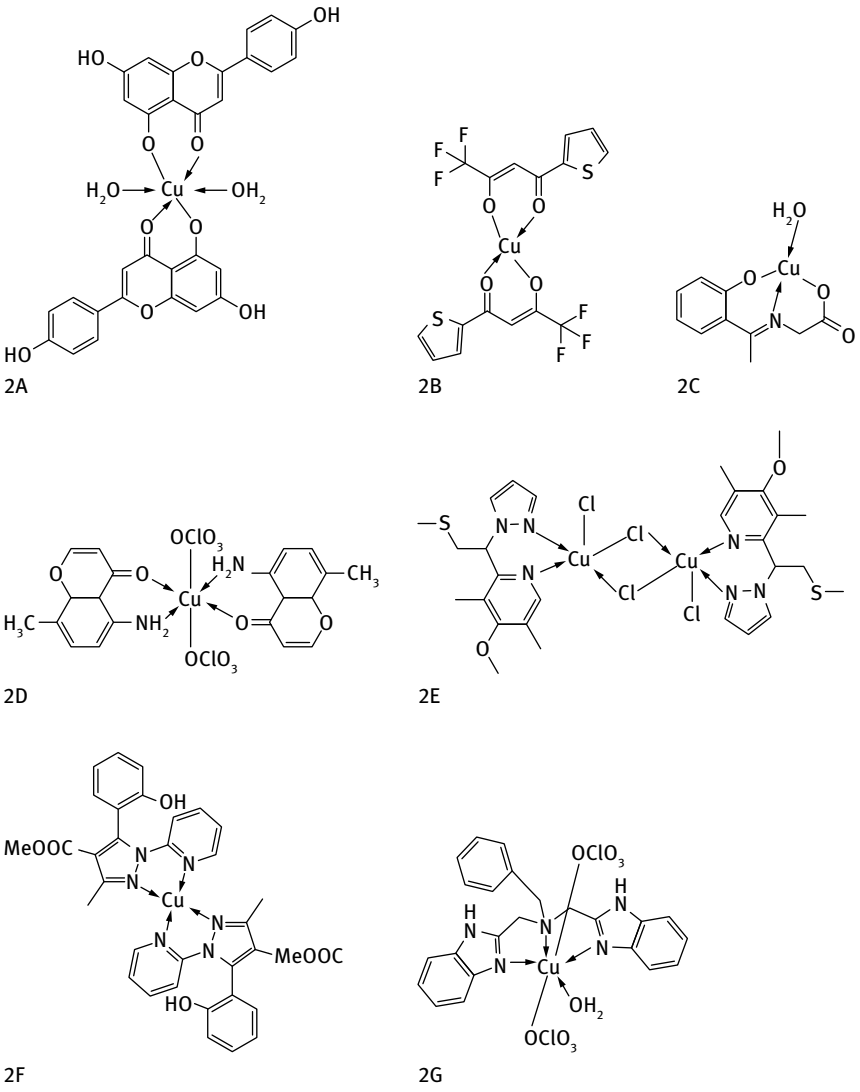

Fig. 10.2: Various copper complexes with O,N-donor ligands having anticancer properties.

In conclusion, the described copper complexes and biological activity have been found to be most promising and are good candidates for investigation in *in vivo* experiments leading to clinical trials. New design and more targeted molecular insights can provide crucial mechanistic aspects of copper species for bio-applications such as drugs against cancer and other diseases.

10.10 Concluding remarks

Based on the extensive discussion and literature data it is quite clear that copper plays an important role in human metabolism. Use of copper over the long era of human development till today is a sign of a promising future for copper and copper compounds in useful ways. Based on existing clinical drugs and further developments, copper complexes may offer greater potential over platinum-based drugs in terms of less toxicity and a different scale activity with a novel mechanism. Again it seems a long journey ahead in terms of integrating experiential screening, getting appropriate knowledge on identifying proper copper species and their metabolic action via true rational designing for future applications.

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11 Thiazole: A privileged scaffold in drug discovery

Abstract: Heterocyclic structures have enormous biomedical applications. The heterocyclic core present in various molecules is directly linked to some bioactivity known as pharmacophore whereas a few structural motifs frequently found in various bioactive molecules are termed 'privileged structures'. It is the minimum structural subunit, common in drugs or lead compounds, which provide ligand points for more than one type of bioreceptor. A focused compound collection based on 'privileged scaffolds' may provide high quality leads for further drug development. Several structural motifs are highlighted as privileged scaffolds in literature. Based on our exhaustive literature survey, a heterocyclic '1,3-Thiazole', moiety was observed in several natural products and drugs confirming thiazole as a Privileged Scaffold in drug discovery. This chapter will provide several synthetic approaches toward the construction of this interesting ring system with diverse substitutions. Detailed structures of various thiazole-containing drugs which are currently on the market or in clinical trials are discussed with a special note on their synthesis and mechanism of biological action.

11.1 Introduction

Heterocyclic structures have enormous biomedical applications. The majority of the best-selling drugs currently in use are organic small molecules comprising one or more heterocyclic rings. Most of these drugs are of natural product origin having complex polyfunctional framework. Therefore, new heterocyclic structures can be used as an important tool to explore biologically relevant chemical space [1, 2]. The heterocyclic core present in the molecules is directly linked to some bioactivity called pharmacophore whereas a few structural motifs frequently found in various bioactive molecules are termed 'privileged structures' [3]. Privileged structures are the most fascinating molecular scaffolds in pharmaceutical research [4]. Several drugs currently on the market consist of privileged structures [5]. It is the minimum structural subunit, common in drugs or lead compounds, which provides ligand points for more than one type of bioreceptor. The desired selectivity for a particular target may be modulated through judicious molecular modifications.

There is a continuous need for new molecules to be introduced as drugs into the market [6]. Between 1994 and 2001, just 22 new molecules were approved for their use as pharmaceuticals. Whereas a decade ago (in 2005), only 20 new molecular entities were introduced into the market. Only a small percentage of total number of druggable targets have been explored so far to design and develop drugs, leaving ample opportunities for new small molecule therapeutics intervention [7]. This failure of innovation in new drug discovery has multiple origins such as regulatory hurdles,

dealing with complex diseases, failure of new technologies such as combinatorial chemistry and high-throughput screening, blockbuster entities and "me-too" drugs as well as the improper exploration of new chemical space [8]. The pharmaceutical industries are transforming their business model to overcome these challenges and there is a paradigm shift to an "open" model in drug development [9]. In the year 2014, FDA approved a total of 44 drugs and it remains an excellent year for pharmaceutical innovation [10]. More precise understanding of disease processes and faster discovery of biomarkers are key to this success. Identifying biomarkers in diseases and then developing drugs that target these biomarkers may result in better efficacy and minimal side effects.

A focused compound collection based on "privileged scaffolds" may provide high quality leads for further drug development. A privileged structure in a molecule positions the functional groups in a right direction to achieve the optimal interaction with the desired biomolecule. Stockwell et al. provided the most comprehensive listing of privileged scaffolds in the literature and offered some thoughts on how new privileged scaffolds might be identified and exploited [5]. Indole, quinoline, isoquinoline, purine, quinoxaline, quinazolinone, tetrahydroisoquinoline, tetrahydroquinoline, benzoxazole, benzofuran, 3,3-benzopyran, chromone, coumarin, carbohydrates, steroids and prostanoic acid are listed as privileged scaffolds found in both drugs and natural products. On the other hand, benzodiazepines, arylpiperidines, arylpiperazines, benzylpiperidine, benzothiophene, dihydropyridines, benzimidazoles and biphenyltetrazoles were found primarily in drugs and 3-substituted-3-hydroxy-2-oxindoles, 5-7-5 lactone ring systems, 6,6-spiroacetals were found mostly in natural products. In addition, dihydropyrimidone, indolizine, biphenyl, triazaspirodecanone, *N*-acylhydrazone, pyrrolinone, hydroxyamate, *trans*-lactam/lactone, hexahydroisoindole, benzimidazolone, indoline, 2-arylbenzothiazole, imidazolequinoxaline, spiroindanylpiperidine, aminopyridazine, 1,4-pyrazolodiazepin-8-one, rhodanine, pyranopyridone and pyranoquinolone are also highlighted as privileged scaffolds.

Based on our exhaustive literature survey, a heterocyclic '1,3-Thiazole' moiety containing both sulfur and nitrogen atoms was observed in several natural products and drugs. Thiazoles having planar and aromatic structure are the members of azole heterocycles that includes imidazoles and oxazoles. The greater aromaticity in thiazoles is characterized by a larger π-electron delocalization than the corresponding oxazoles. A strong diamagnetic ring current in thiazoles is evidenced by the chemical shift (7.27–8.77 ppm) of the ring protons in ^{1}H NMR spectroscopy [11]. C-5 is the primary site for electrophilic substitution in thiazole whereas the C-2 position remains the site for nucleophilic substitution (Fig. 11.1).

Sulfur-containing compounds such as coenzyme A (**1**), coenzyme B (**2**), coenzyme M (**3**), (*S*,*S*)-adenosylmethionine (**4**), biotin (**5**), lipoic acid (**6**) and molybdopterin (**7**) are present in many living organisms (Fig. 11.2) [12]. A thiazole-containing compound such as thiamin pyrophosphate (**8**) is also a part of the living system and is involved in many cellular processes.

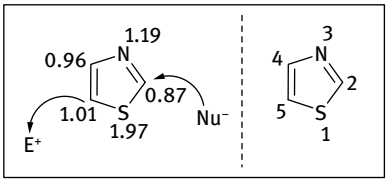


Fig. 11.1: Molecular and electronic structure of 1,3-thiazole.

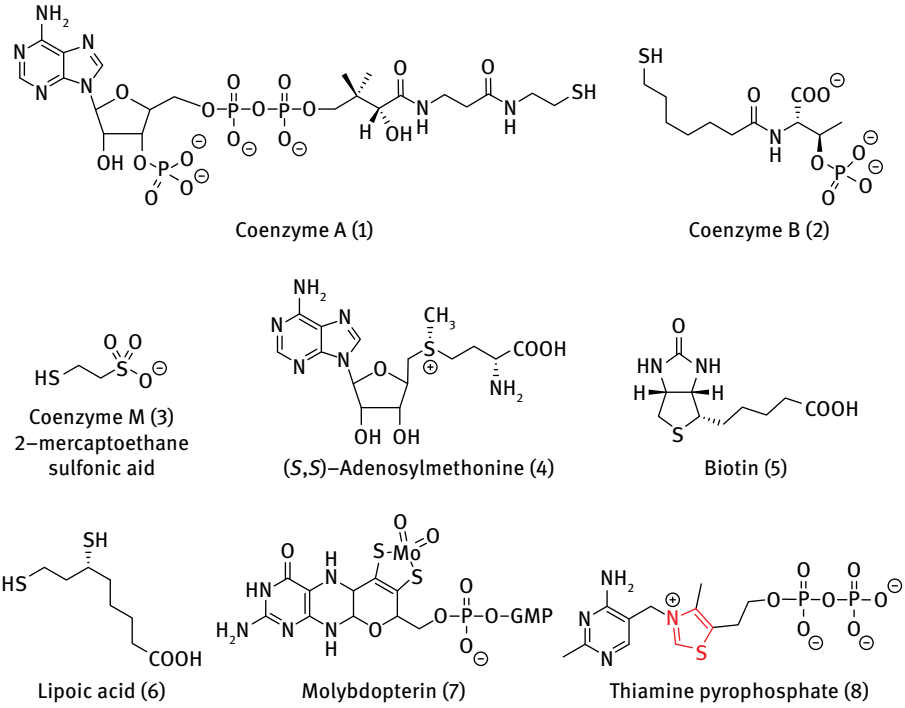


Fig. 11.2: Sulfur-containing compounds involved in many cellular processes.

Many natural and synthetic products comprise thiazole rings with varied biological properties, such as antiviral, anticancer, antibacterial, antifungal, anticonvulsant, antiparkinsonian and anti-inflammatory activities. Apart from natural products and synthetic drugs, thiazole rings were also observed in many fluorescent dyes, polymers, insecticides, antioxidants and liquid crystals [13]. The thiazole-containing natural products such as dolabellin (**9**), archazolide A (**10**), mycothiazole (**11**), WS75624 B (**12**), epothilone B (**13**), cystothiazole A (**14**), and tubulysine D (**15**) (Fig. 11.3) are derived from cysteine peptide precursors. This biosynthesis involves sequential transformations such as coupling, cyclization and oxidation to furnish a thiazole subunit [14]. Large numbers of natural macrolactam products derived from heterocyclic amino

acids are composed of thiazole rings. Bistratamides are a family of such macrolactams (e.g. bistratamide C (**16**), Fig. 11.3) isolated from *Lissoclinum bistratum*.

Overall, the thiazoles are highly fascinating molecular scaffolds in pharmaceutical research. A significant amount of natural products and the drugs currently on the market or in clinical trials comprise the thiazole ring as the important structural subunit, which is able to provide ligand points for more than one type of bioreceptor. In short, "*Thiazoles are the Privileged Scaffolds in Drug Discovery*". This chapter will provide several synthetic approaches toward the construction of this interesting ring system with diverse substitutions. Detailed structures of various thiazole-containing drugs which are currently on the market or in clinical trials will be discussed further with a special note on their synthesis and mechanism of biological action. A specialized benzene fused thiazole product known as benzothiazoles were earlier highlighted as privileged scaffolds [15] and are not discussed in this chapter.

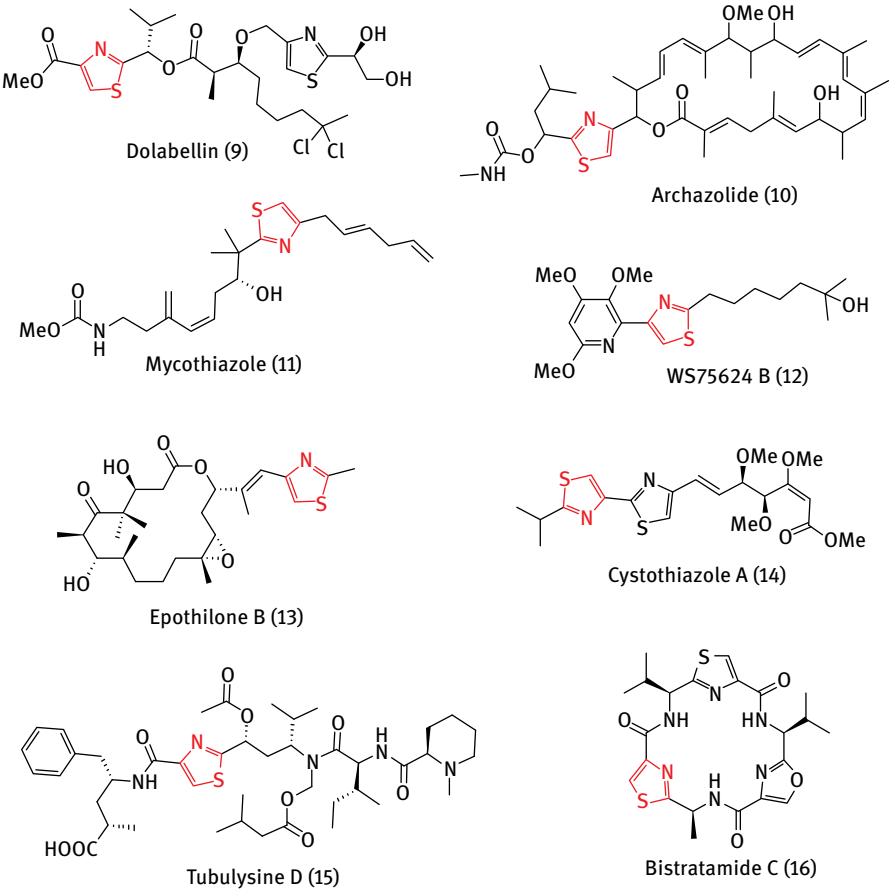


Fig. 11.3: Structures of marine natural products containing thiazole ring.

11.2 Synthetic routes for the construction of the thiazole ring

A large number of reports dealing with the synthetic approaches and biological properties of thiazole-based compounds have been published over the last century. Most recent developments in thiazole synthesis, from both synthetic and mechanistic point of view are discussed in this section. Emphasis is given to novel synthetic methods and new insights into existing methodologies for the selective construction of the thiazole ring.

Syntheses of thiazoles are classified depending on the number of components which join to form a five-membered ring system. In the case of monocyclic compounds such as thiazoles, thiazolines and thiazolidines, the most common syntheses are based on the following disconnections, which are classified as Type I to V as shown in Fig. 11.4.

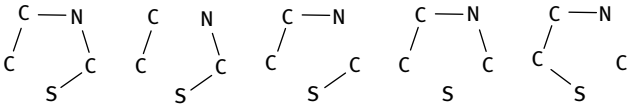
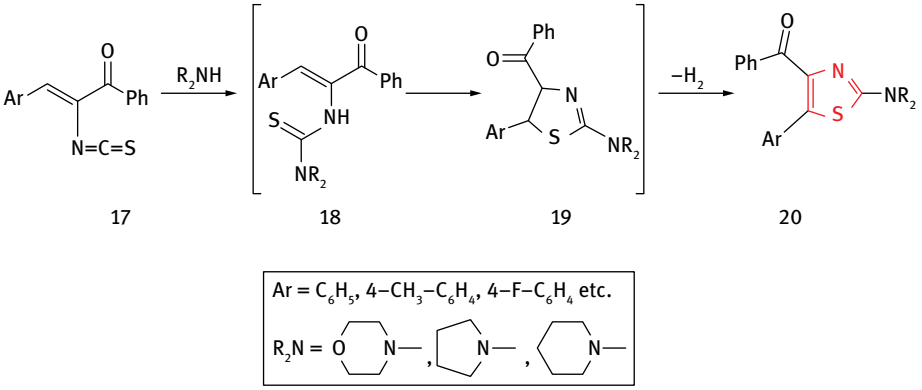


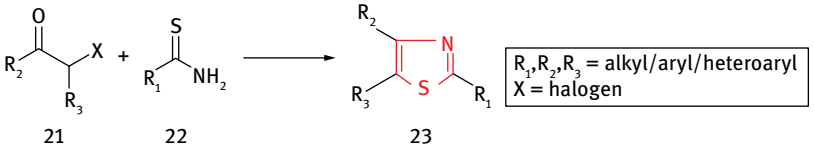
Fig. 11.4: The common disconnections for thiazole ring construction.

Thiazole synthesis from isothiocyanates is an ideal example of Type-I approach. The reaction of isothiocyanates **17** with aliphatic secondary amines provide 2,4,5-trisubstituted thiazoles (**20**) in good yields. The formation of thiazoles **20** involves an addition reaction between isothiocyanate **17** and aliphatic secondary amine to give thiourea intermediate **18**. This thiourea on further intramolecular Michael addition (S-attack) cyclizes to dihydrothiazole intermediate **19**, which on heating spontaneously undergoes dehydrogenation to give the aromatic thiazole ring **20** by aerial oxidation (Scheme 11.1) [16].

The second approach is the synthesis of thiazoles from α-functionalized carbonyl compounds (Hantzsch thiazole synthesis). This type of synthesis was first described by Hantzsch, a German chemist, in 1887. Hantzsch thiazole synthesis involves the condensation of a compound bearing two heteroatoms on the same carbon (N-C-S, **22**) and α-halogenated carbonyl compound (**21**). Most commonly thioamides, thioureas, ammonium thiocarbamate or dithiocarbamate and their derivatives react with α-haloketones (**21**) yielding a variety of thiazole derivatives (**23**) (Scheme 11.2) [17].

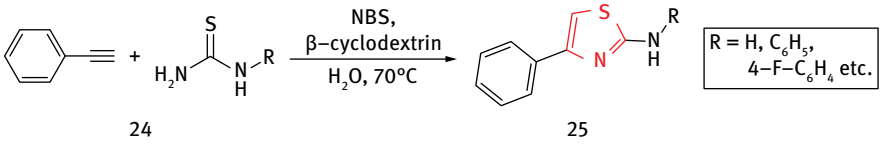


Scheme 11.1: Synthesis of trisubstituted thiazoles from isothiocyanates.



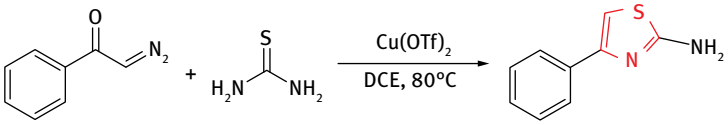
Scheme 11.2: Hantzsch thiazole synthesis.

Several thiazoles (**25**) were also synthesized from alkynes (**24**). β-Cyclodextrin was utilized as a phase transfer catalyst and the reaction in aqueous medium resulted in good yields. Initially, phenylacetylene (**24**) was reacted with one equivalent of NBS and thiourea in the presence of β-cyclodextrin, resulting in lower yields of thiazoles (**25**), however when two equivalents of NBS were used, the reaction proceeded efficiently (Scheme 11.3) [18].



Scheme 11.3: Synthesis of 2-amino-4-aryl thiazoles from alkynes.

Yadav et al. reported an efficient and selective method for the coupling of α–diazoketones (**26**) with thiourea in the presence of 10 mol% of copper (II) triflate to make the corresponding 2-aminothiazoles (**27**) in excellent yield (Scheme 11.4) [19].



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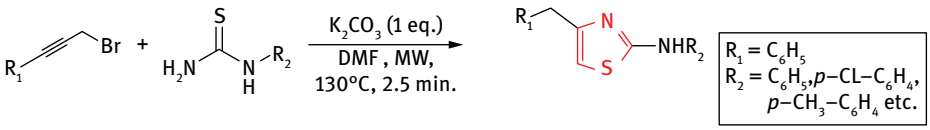
Scheme 11.4: Synthesis of 2-amino-4-phenyl thiazoles from α–diazoketones.

Castagnolo and co-workers reported a microwave mediated domino alkylation-cyclization reaction of propargyl bromides (**28**) with thioureas to synthesize 2-aminothiazoles (**29**) in high yields (Scheme 11.5) [20].



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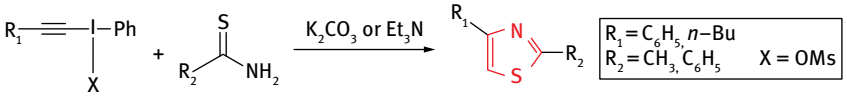
Scheme 11.5: Synthesis of 2-amino-4-benzyl thiazoles from propargyl bromides.

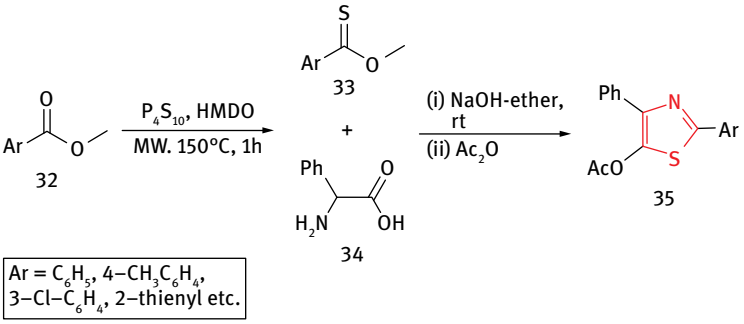
A series of disubstituted thiazoles **31** were synthesized by Miyamoto et al. via the cyclocondensation of 1-alkynyl(phenyl)-λ^3-iodanes (**30**) with thioureas or thioamides in the presence of a base, such as potassium carbonate or triethylamine (Scheme 11.6) [21].



30 31

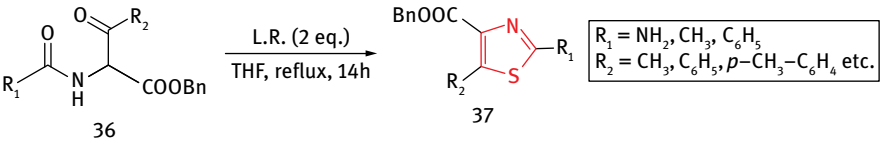
Scheme 11.6: Synthesis of 2,4-disubstituted thiazoles using hypervalent iodine reagents.

Several 2,4-disubstituted-5-acetoxythiazoles (**35**) were synthesized by reacting methyl thiobenzoate derivatives (**33**), obtained from methyl benzoate **32**, with racemic phenylglycine (**34**) in a two-phase solvent system composed of 3N NaOH and ether. The coupled product on subsequent treatment with acetic anhydride resulted in desired thiazole derivatives **35** (Scheme 11.7) [22].



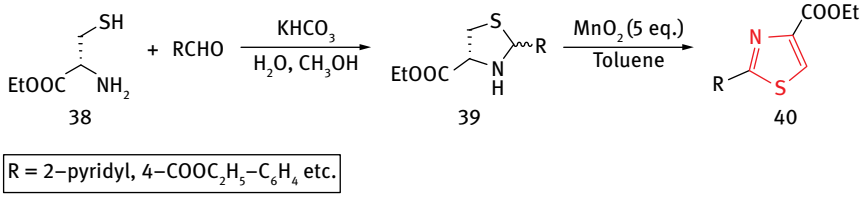
Scheme 11.7: Synthesis of trisubstituted thiazoles from phenylglycine.

A trisubstituted diverse library of thiazoles (**37**, Scheme 11.8) was synthesized by Cervera et al. which involves the double acylation of a protected glycine to α-amido-β-ketoesters (**36**). The intermediate **36** on reaction with Lawesson's reagent resulted in the formation of the desired benzyl 2,5-substitutedthiazole-4-carboxylate scaffold (**37**) [23].



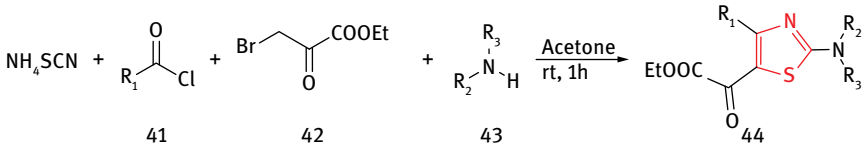
Scheme 11.8: Synthesis of trisubstituted thiazoles using Lawesson's reagent.

Several reports on the two stage synthesis of thiazoles involving cyclization of cysteine side-chains onto carbonyl groups followed by oxidative aromatization are reported in the literature. Following this approach, L-cysteine ethyl ester (**38**) was reacted with variously substituted aldehydes to afford the corresponding thiazolidine derivatives **39**, which on further oxidation with MnO_2 resulted in disubsituted thiazoles **40** as shown in Scheme 11.9 [24].



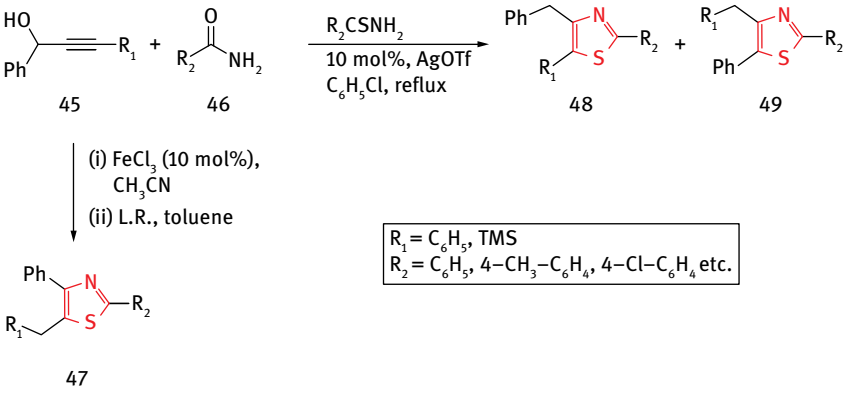
Scheme 11.9: Synthesis of 4-carbethoxy thiazoles from L-cysteine methyl ester.

An efficient synthesis of ethyl 2-dimethylaminothiazole-4-carboxylates (**44**) was described by Yavari et al. via a four component reaction between acid chlorides (**41**), α-bromoethyl pyruvate (**42**), secondary amines (**43**) and ammonium thiocyanate (Scheme 11.10) [25].



Scheme 11.10: Multicomponent synthesis of trisubstituted thiazoles.

Three differently substituted thiazoles (**47–49**) have been synthesized from commercially available propargylic alcohols (**45**) and amides (**46**) using Lawesson's reagent [26]. A diverse set of compounds were synthesized by these authors using a wide range of secondary propargylic alcohols and/or tertiary propargylic alcohols bearing both terminal as well as internal alkyne groups. Several trisubstituted thiazoles with functional groups, such as cyclopropyl, cyclohexenyl, halogens, esters and methoxy groups are synthesized under these reaction conditions (Scheme 11.11).



Scheme 11.11: Synthesis of trisubstituted thiazoles from propargylic alcohols.

11.3 Thiazole as privileged scaffold in synthetic drugs

A wide range of pharmacological activities in thiazole-containing natural and synthetic products including several drugs in clinical use (Table 11.1) illustrate well the importance of this heterocycle in today's life. Elaboration of a few of these molecules in terms of their structure, synthetic route and mechanism of biological action are discussed further.

11.4 Ritonavir

Ritonavir (**50**) is the first and the only thiazole-containing antiretroviral drug from the protease inhibitor class used to treat HIV infection and AIDS. Structurally, it is a carboxamide of L-valine and 2,5-diamino-1,6-diphenylhexan-3-ol linked to two thiazole rings. At one end, 5-thiazolylmethanol is coupled via a carbamate linker while other end forms a urea linkage with 1-(2-isopropylthiazol-4-yl)-*N*-methylmethanamine.

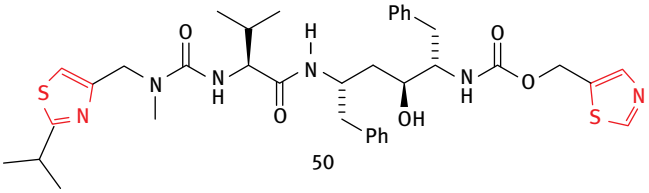


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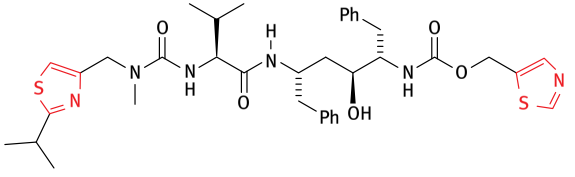
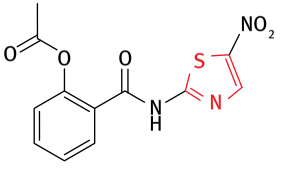
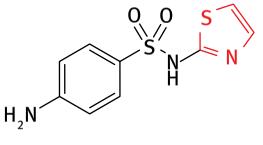
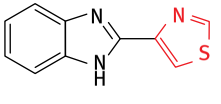
Fig. 11.5: Chemical structure of ritonavir.

Ritonavir, originally developed as HIV protease inhibitor, is frequently prescribed with a highly active antiretroviral therapy (HAART). It potently induces CYP 1A2 and inactivates cytochrome P450 3A4 (CYP 3A4), a major human drug-metabolizing enzyme. The CYP3A4 inhibition increases the plasma concentrations of other anti-HIV drugs improving the overall clinical efficacy of HAART [27].

The effect of ritonavir on the redox properties of the hemoprotein was investigated by Sevrioukova et al. [28]. The study of the CYP 3A4-ritonavir binding reaction by kinetic and equilibrium analysis and the 2.0 Å X-ray structure of this complex confirmed ritonavir as a type II ligand that perfectly fits into the CYP 3A4 active site cavity. This investigation also confirmed the irreversible binding of ritonavir to the heme iron via thiazole nitrogen, decreasing the redox potential of the protein and preventing its reduction with the redox partner, cytochrome P450 reductase. Based on their observations, the thiazole nitrogen of ritonavir is the likely iron ligand in both ferric and ferrous CYP 3A4 and therefore concluded that the thiazole and isopropyl thiazole groups of ritonavir are strictly required for the observed inhibitory activity [29].

Table 11.1: Structure, pharmacological activity and clinical use of thiazole-containing drugs.

No.	Name	Structure	Pharmacological activity	Clinical use
1	Ritonavir		HIV protease inhibitor class, induces CYP 1A2 and inactivates Cytochrome P450 3A4 enzyme and increases plasma concentration of other anti-HIV drugs.	Treatment of HIV infection and AIDS. Frequently prescribed with a highly active antiretroviral therapy (HAART).
2	Nitazoxanide		Prevent the production of acetyl CoA within anaerobic bacteria and parasites by inhibiting pyruvate/ ferredoxin reductase. Effective inhibitors of HBV and in some cases of hepatitis C virus (HCV) replication in cell cultures.	Treatment of illness caused by *Cryptosporidium parvum* or *Giardia lamblia* and other protozoa and helminthes infections. Also used for the treatment of chronic hepatitis B and C infection as well as small intestinal bacterial overgrowth.
3	Sulfathiazole		Sulfonamides are competitive inhibitors of a bacterial enzyme, dihydropteroate synthetase. This inhibition blocks the synthesis of dihydrofolic acid and decreases the amount of metabolically active tetrahydrofolic acid.	Sulfonamides are broad-spectrum, bacteriostatic anti-infectives.
4	Thiabendazole		Thiabendazole inhibits the mitochondrial helminth-specific enzyme, fumarate reductase with anthelminthic property.	Thiabendazole is used primarily as fungicide in fruits and vegetables and as an antiparasitic agent to control roundworms which attack animals and humans.

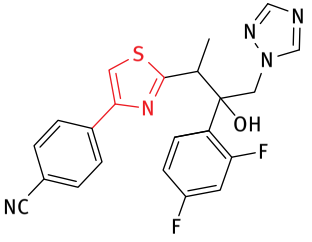
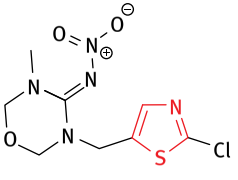
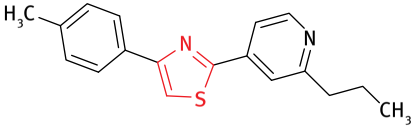
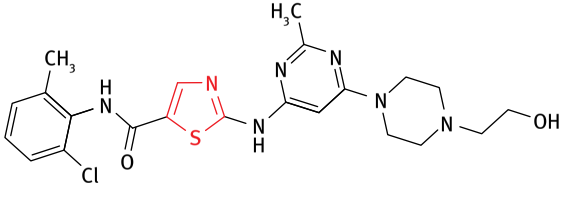
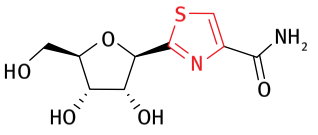
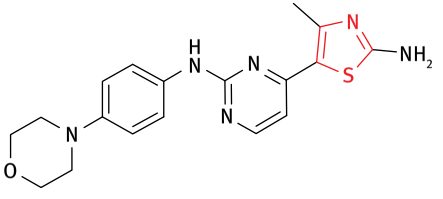
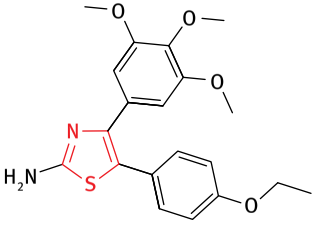
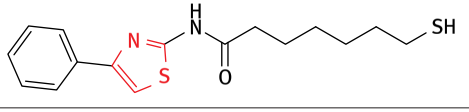
No.	Name	Structure	Pharmacological activity	Clinical use
5	Ravuconazole		Ravuconazole is a triazole antifungal agent from the class ergosterol biosynthesis inhibitor. Inhibits Cytochrome P-450 dependent C-14 demethylation of lanosterol, a key step in ergosterol biosynthesis.	Antifungal agent.
6	Thiamethoxam		Known to paralyze the muscles of insects by affecting the transfer of information between nerve cells via interfering with nicotinic acetylcholine receptors in the central nervous system.	Thiamethoxam is a systemic insecticide in the class of neonicotinoids. It has a broad spectrum of activity against many types of insects.
7	Fatostatin		Inhibits activation of sterol regulatory element-binding protein (SREBP).	Potential drug candidate to block adipogenesis. It can help combat obesity and may help diabetes. It also displays high antitumor activity in prostate cancer.
8	Dasatinib		Bcr-Abl and Src family tyrosine kinase inhibitor.	Used for the treatment of chronic myelogenous leukemia (CML), Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph + ALL) and advanced prostate cancer.

Table 11.1: (continued)

No.	Name	Structure	Pharmacological activity	Clinical use
9	Tiazofurin		It is an inosine-5'-monophosphate (IMP) dehydrogenase inhibitor.	It possesses significant activity against both human lymphoid, lung tumor cell lines and murine-implanted human ovarian cancers.
10	CYC116		Inhibitor of Aurora kinases A and B and VEGFR2.	Useful in the treatment of hematological and solid tumors.
11	TR-644		Inhibits VEGF-induced phosphorylation of VE-cadherin (VE-cadherin is crucial for controlling the state of adherence junctions, which in turn regulate endothelial cell–cell adhesion, cell motility, morphogenesis and intracellular signaling pathways).	Microtubule depolymerizing activity.
12	NCH-31		Histone deacetylase (HDAC) inhibitor.	Useful in the treatment of cancer and antiproliferative agent.

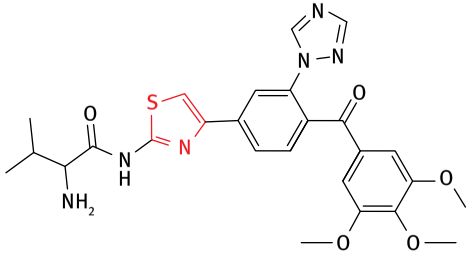
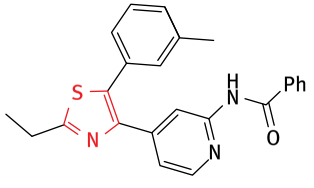
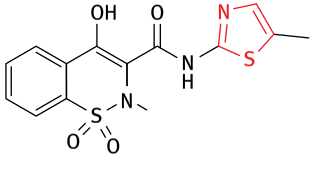
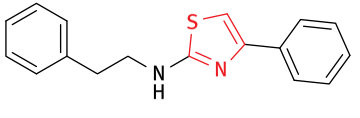
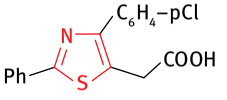
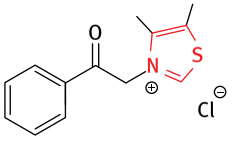
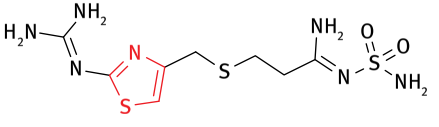
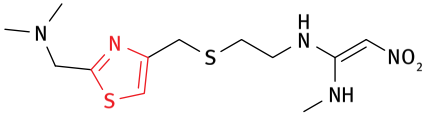
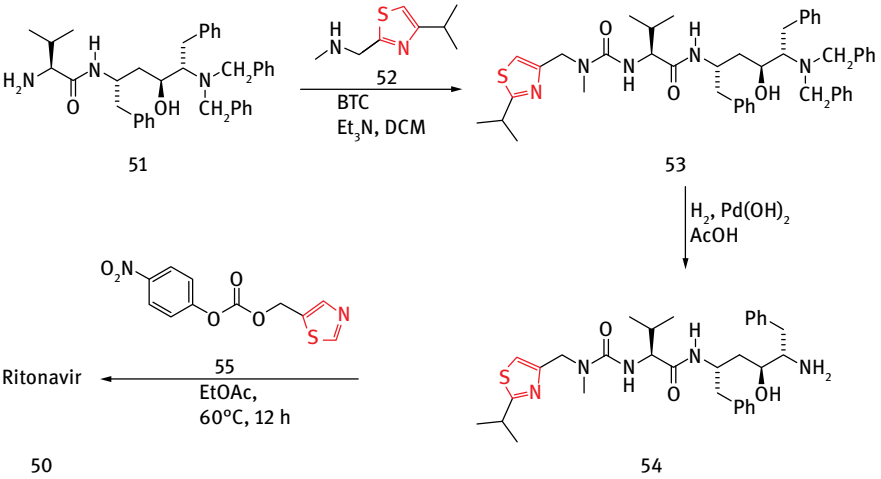
No.	Name	Structure	Pharmacological activity	Clinical use
13	CKD-516		Inhibitor of tubulin polymerization.	Used against murine and human solid tumors.
14	TAK-715		p38 MAP kinase inhibitor.	Used in the treatment of chronic inflammatory diseases such as rheumatoid arthritis (RA) and inflammatory bowel disease (IBD).
15	Meloxicam		COX-2 inhibitor.	NSAID with analgesic and fever reducer effects.
16	Fanetizole		COX-2 inhibitor.	NSAID and immunoregulating activity.

Table 11.1: (continued)

No.	Name	Structure	Pharmacological activity	Clinical use
17	Fentiazac		COX-2 inhibitor.	NSAID for joint and muscular pain and has analgesic and antipyretic activity
18	Alagebrium		Disruption of crosslinks caused by advanced glycation end products (AGEs).	Reduces systolic blood pressure.
19	Famotidine		Histamine H_2-receptor antagonist (H2RA) that inhibits stomach acid production.	Used to treat duodenal ulcers, gastric ulcers and gastroesophageal reflux disease.
20	Nizatidine		In addition to the acid-suppressing effect, the H2RA nizatidine also has a prokinetic action by suppressing acetylcholine esterase.	Nizatidine is a more effective H2RA than famotidine in the maintenance therapy of patients with reflux esophagitis.

Ritonavir has an elongated shape (Fig. 11.5), which can enter the CYP 3A4 active site with either the thiazole or isopropyl-thiazole end. Based on the observations of Koudriakova and co-workers, CYP 3A4 mediates the hydroxylation of the latter group and is unlikely to serve as a heme ligand suggesting a positional/conformational rearrangement when ritonavir docks to CYP 3A4 with the isopropylthiazole head to bring the opposite thiazole moiety in the vicinity of the heme iron. The authors also suggested a possibility of dissociation and re-entry of the drug in the active site pocket to achieve the appropriate orientation [30].

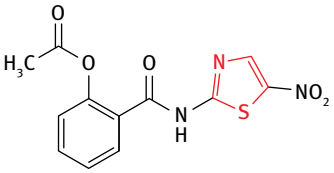
During the synthesis of ritonavir (Scheme 11.12), the urea linked disubstituted thiazole ring was introduced by the reaction of dipeptide (**51**) with (4-isopropylthiazol-2-yl)-*N*-methylmethanamine (**52**), using triphosgene [bis(tri-chloromethyl)carbonate (BTC)] and Et_3N in DCM to furnish 3-((S)-1-((2S,4S,5S)-5-(dibenzylamino)-4-hydroxy-1,6-diphenylhexan-2-ylcarbamoyl)-2-methylpropyl)-1-((2-isopropylthiazol-4-yl)methyl)-1-methylurea (**53**). Hydrogenation of **53** in the presence of Pearlman's catalyst [$Pd(OH)_2/C$] afforded intermediate **54**, this on further heating with 4-nitrophenyl (thiazol-5-yl)methyl carbonate (**55**) in ethyl acetate for 12 hours resulted in Ritonavir **50** [31].



Scheme 11.12: Synthesis of ritonavir.

11.5 Nitazoxanide

Nitazoxanide, a 2-hydroxybenzoyl-*N*-(5-nitrothiazol-2-yl)amide also known as NTZ (**56**, Fig. 11.6) is a broad spectrum anti-infective agent effective against anaerobic bacteria, viruses, and parasites. It belongs to a nitro heterocyclic class of antiviral drug named thiazolides having potential to treat chronic hepatitis C.

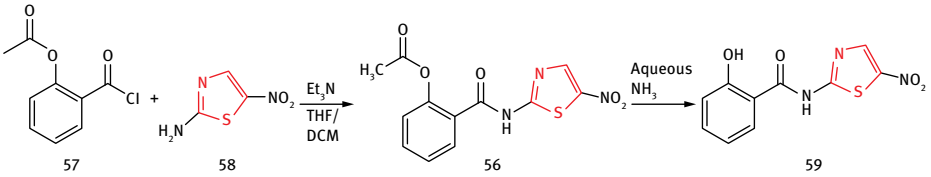


56

Fig. 11.6: Structure of nitazoxanide.

The aminothiazole derivative nitazoxanide **56**, (Fig. 11.6) is a carboxamide of acetylsalicylate and 2-amino-5-nitrothiazole. Originally, nitazoxanide was developed as an antiparasitic agent, particularly indicated against *Cryptosporidium parvum*. This broad-spectrum anti-infective agent is active against anaerobic bacteria and also used as an antiprotozoal and anthelmintic agent. The antiviral activity of this drug was noted in the late 1990s during the treatment of cryptosporidiosis in AIDS patients [32, 33].

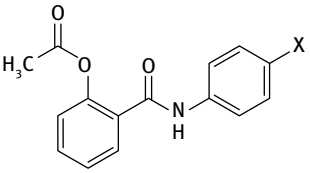
The synthesis of nitazoxanide and related analogs can be achieved from commercially available acetylsalicyloyl chloride (**57**). An anhydrous coupling condition with 2-amino-5-nitrothiazole (**58**) in the presence of Et_3N in THF was suggested because of the low nucleophilicity of amine [34]. Navarrete-Vazquez et al. reported similar synthesis which involves acylation of 2-amino-5-nitrothiazole with acetylsalicyloyl chloride in DCM [32]. Tizoxanide (**59**) was also synthesized by these authors using simple alkaline hydrolysis of nitazoxanide required for further SAR studies (Scheme 11.13).



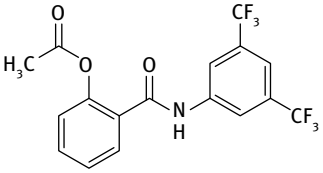
57 58 56 59

Scheme 11.13: Synthesis of nitazoxanide (**56**) and tizoxanide (**59**).

Nitazoxanide prevents the production of acetyl CoA within anaerobic bacteria and parasites by inhibiting pyruvate/ferredoxin reductase (PFOR) [34]. Nitazoxanide was found to be active against various DNA and RNA viruses. Several of its close analogs were found to be effective inhibitors of HBV and in some cases of hepatitis C virus (HCV) replication in cell cultures [35, 36]. Synthesis, structure-activity relationships and excellent correlation of activities of a wide range of thiazolides against HBV for intracellular virions was reported by these authors. Among the several substituted thiazole-containing analogs, the best activity against HBV was observed for thiazolides with electron-withdrawing groups at C(5'), especially 5'-nitro and 5'-halo derivatives. A set of salicyloyl anilides was also synthesized and evaluated wherein aminothiazole was replaced by various substituted anilines (Fig. 11.7). The therapeutic selectivity index (SI) was obtained for each analog as a ratio of the cytotoxicity (CC_{50}) to efficacy (EC_{90}, the drug concentration at which a 10-fold depression of intracellular HBV DNA was observed relative to the average levels in untreated cultures). The activity details for the nitazoxanide and few salicyloyl anilides against HBV replication are summarized in Table 11.2.



60: X = Cl; 61: X = Br; 62: X = I



63

Fig. 11.7: A set of salicyloyl anilides.

Table 11.2: Activities of NTZ and its analogs against HBV replication.

Compound	CC_{50} µM	EC_{50} (VIR) µM	EC_{90} (VIR) µM	SI (VIR)
56	>100	0.12	0.83	>121
60	>100	3.50	9.70	11
61	>100	0.76	3.00	>33
62	>100	0.20	1.20	>83
63	>100	3.80	14.00	>7.10

11.6 Sulfathiazole

Sulfathiazole (**64**) is one of the most potent sulfonamides and is a typical example of this family of bacteriostatic drugs [37]. It has one mono-substituted thiazole with amino group at position-2 which is involved in sulfonamide linkage with 4-amino-

benzenesulfonic acid. Pure sulfathiazole forms five crystalline polymorphs [38] and several (over 100) solvates of sulfathiazole were described in literature [39]. Among all the polymorphs, imide tautomer **65** is dominant, in which the thiazole ring nitrogen bears the proton (Fig. 11.8) [40].

An organosulfur compound, sulfathiazole was used as oral and topical antimicrobial before the discovery of less toxic alternative drugs like quinolones [41]. Currently, sulfathiazole is used in combination with sulfabenzamide and sulfacetamide as a topical antibacterial preparation for the treatment of vaginal infections.

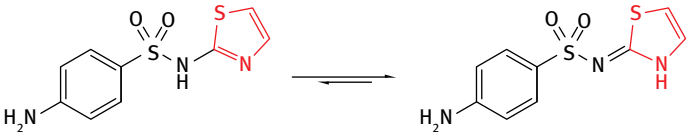
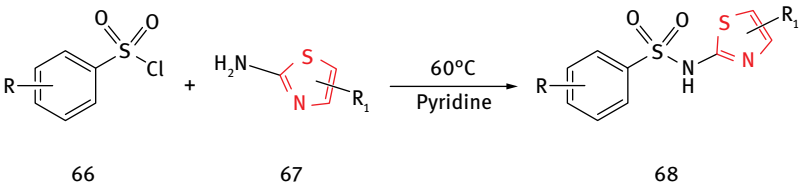


Fig. 11.8: Chemical structure of sulfathiazole and its tautomer.

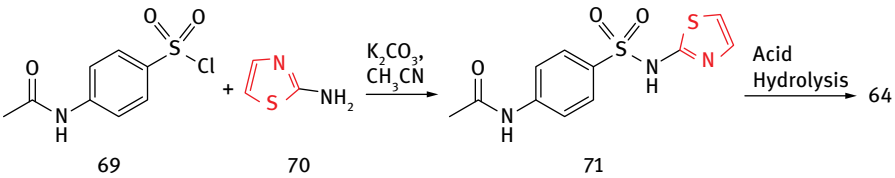
Sulfonamides are structural analogs of *p*-aminobenzoic acid (PABA) and competitively inhibit dihydropteroate synthetase, a bacterial enzyme responsible for the incorporation of PABA into dihydrofolic acid (an immediate precursor of folic acid). This inhibition decreases the amount of metabolically active tetrahydrofolic acid, a cofactor for the synthesis of purines and thymidine [42]. Sulfathiazole is also known to compete with sodium pyruvate for the active site of carboxylase [43]. Dorfman et al. proved that sulfathiazole inhibits the nicotinamide-stimulated respiration of dysentery bacilli when grown on a medium deficient in nicotinamide [44].

Argyropoulou et al. synthesized sulfathiazole derivatives **68** by heating suitable heteroarylamines **67** with the selected benzensulfonylchlorides **66** in pyridine for several hours (Scheme 11.14) [45]. During this transformation, a nucleophilic addition of the NH_2 group to the sulfonyl functionality of the benzenlsulfonylchlorides results in the formation of desired products.



Scheme 11.14: Synthesis of sulfathiazoles.

Among the several reports on the synthesis of sulfathiazoles, a safe and convenient synthesis was reported by Sarojini et al. (Scheme 11.15) where acetonitrile was used as a solvent in place of pyridine, which is a potential health hazard [46].



Scheme 11.15: Safe and convenient synthesis of sulfathiazoles.

11.7 Thiabendazole

Thiabendazole (**72**) is a thiazole coupled benzimidazole (TBZ, Fig. 11.9) well known as anthelmintic agent which expels parasitic worms from the body without any adverse effects [47].

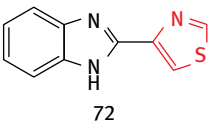


Fig. 11.9: Chemical structure of thiabendazole.

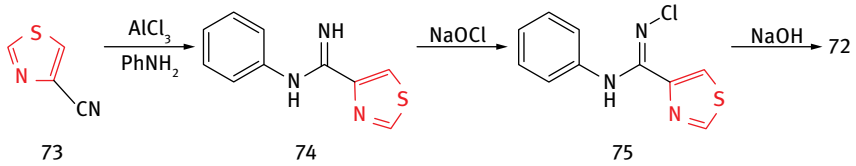
It is used primarily to control fungal diseases in plants. It is a prophylactic agent used as a preventive measure for Dutch elm disease and also used for the treatment of aspergillosis [48]. TBZ is an antiparasitic and demonstrates significant anthelmintic activity for gastrointestinal parasites in several wild animals and humans without any undesirable effects [49]. Fungicidal activities of several coordination compounds of TBZ have also been studied. It is proposed that metal complexes induce toxic effects through generation of reactive oxygen species (ROS) [50]. Recently several metal complexes of TBZ have been screened for their antitumor activities, and a platinum complex exhibits antileukemic activity against HI-60 cells [51].

Thiabendazole was prepared by Brown et al. by reacting 4-thiazolecarboxamide with *o*-phenylenediamine using a polyphosphoric acid as a catalyst [47]. Grenda et al. described the synthesis of thiabendazole from *N*-arylamidine hydrochloride **73** [52].

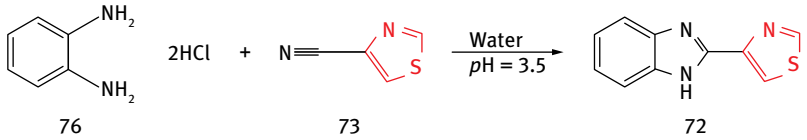
During the process, the intermediate arylamidine **74** was prepared by the $AlCl_3$ catalyzed addition of aniline to the nitrile functionality of 4-cyanothiazole **73**. Amidine

74 was then converted to its *N*-chloro analog **75** which on base treatment undergoes a nitrene insertion reaction to produce thiabendazole **72.**

Parziale et al. described an efficient process for the preparation of thiabendazole from *o*-phenylenediamine dihydrochloride **76** and 4-cyanothiazole [53].



Scheme 11.16: Synthesis of thiabendazole.



Scheme 11.17: Synthesis of thiabendazole in acidic medium.

11.8 Fatostatin

Fatostatin is a 2-pyridyl-4-tolyl thiazole derivative (Fig. 11.10) which inhibits activation of sterol regulatory element-binding protein (SREBP) by selectively blocking the activation of SREBP transcription factors and preventing the biosynthesis and accumulation of fat.

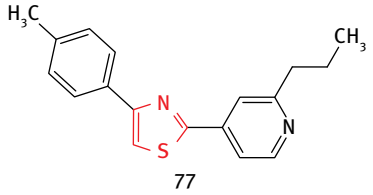
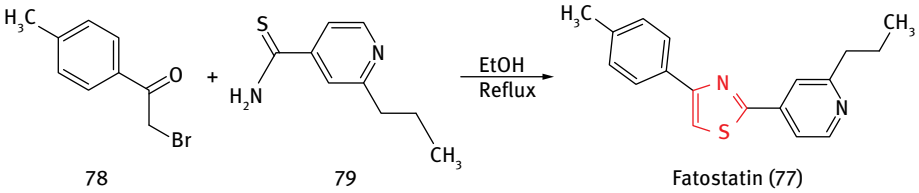


Fig. 11.10: Chemical structure of fatostatin (**77**).

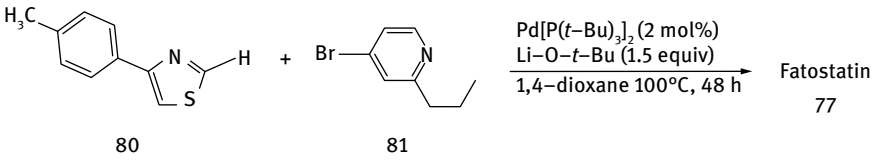
Fatostatin is a disubstituted thiazole to which an alkyl substituted pyridine and a phenyl ring are linked at C2 and C4 positions respectively. Fatostatin is a disubstituted thiazole with pyridine and a phenyl ring is linked to a central thiazole ring.

The central thiazole ring was constructed by reacting thioamide derivative (**79**) with α-bromoketone (**78**) in warm ethanol to yield 4-phenyl-2-(pyridin-4-yl)thiazole (**77**) (Scheme 11.18) [54].

Itami et al. synthesized fatostatin in 53 % yield by the reaction of 4-(*p*-tolyl)thiazole (**80**) with 4-bromo-2-propylpyridine (**81**) under the influence of $Pd[P(^tBu)_3]_2$/LiO^tBu (Scheme 11.19) [55].



Scheme 11.18: Preparation of fatostatin (**77**) using Hantzsch thiazole synthesis.



Scheme 11.19: Synthesis of fatostatin by Itami et al.

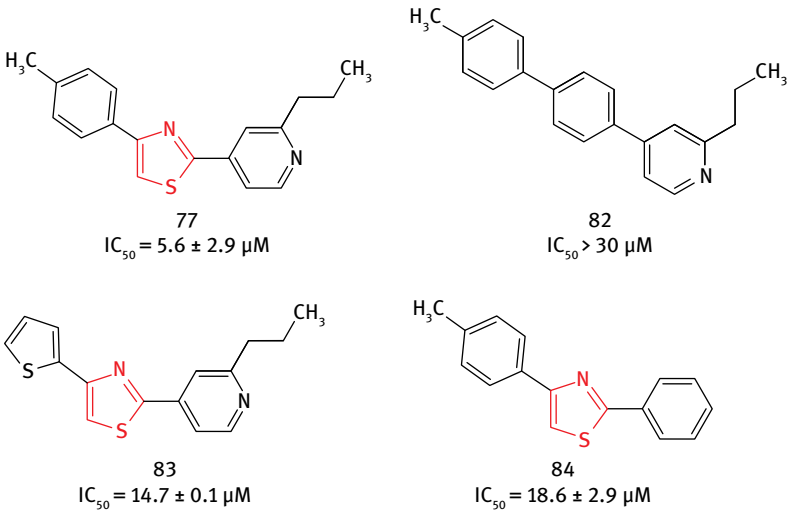


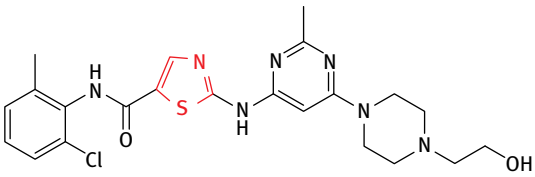
Fig. 11.11: IC_{50} values of fatostatin and its analogs.

The appropriately substituted aromatic rings in fatostatin were found to be important for its activity. Replacement of any one of the three aromatic rings drastically affected its activity (Fig. 11.11). In particular, replacement of the thiazole moiety with a phenyl group resulted in a linear compound (**82**) with 6-fold decrease in potency, suggesting the importance of the central thiazole moiety.

Further, a thiophene substitution in place of *p*-tolyl group resulted in a tri-heteroaryl system (**83**), with reduced potency. Similarly, two phenyl substituted compound **84** was found to be less active than the original compound [54].

11.9 Dasatinib

Dasatinib (**85**, Fig. 11.12), also known as BMS-354825, is orally active tyrosine kinase inhibitor which selectively inhibit Bcr/Abl, Src, c-Kit, ephrin receptors and approved for its use in patients with chronic myelogenous leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph + ALL). Dasatinib (Trade Name: Sprycel) is produced and sold by Bristol-Myers Squibb and Ostuka Pharmaceutical Limited. It is being evaluated for various types of other cancers.

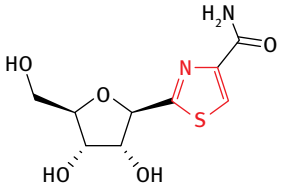


85

Fig. 11.12: Chemical structure of dasatinib (**85**).

Structurally, it is 2,5-disubstituted thiazole having amino pyrimidine group at position-2 of thiazole, whereas position-5 is substituted by aniline through an amide bond. Lombardo et al. gave the first synthesis of dasatinib in 2004 [56]. It was prepared from 2-chlorothiazole (**86**) in 61% overall yield (Scheme 11.20). The process involves the reaction of 2-Chloro-6-methylphenyl isocyanate (**87**) with lithium anion of thiazole **86** in THF which resulted in the corresponding carboxamide **88** which was further protected as the 4-methoxybenzyl derivative (**89**). Displacement of the 2-chloro substituent on the thiazole with the sodium salt of 4-amino-6-chloro-2-methylpyrimidine at reflux provided compound **90**. Removal of the 4-methoxybenzyl (PMB) group by TfOH/TFA and further reaction with 1-(2-hydroxyethyl)piperazine in dioxane resulted in the formation dasatinib **85**.

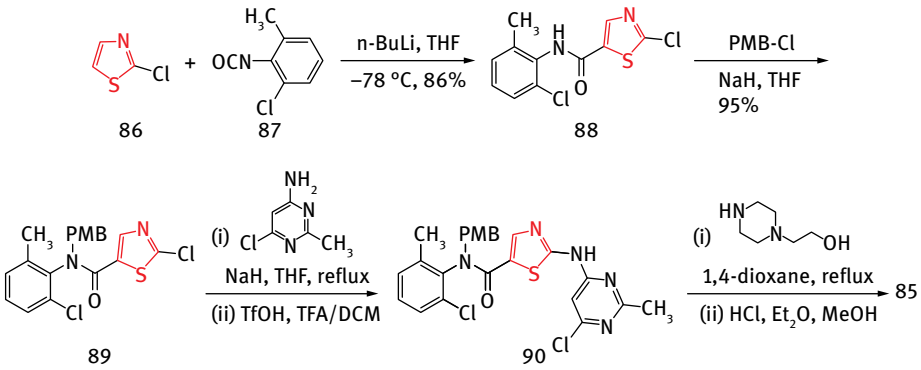
Dasatinib was found to be a highly potent, ATP competitive inhibitor of both Src and Bcr-Abl, with measured *K*i values of 16 ± 1.0 pM and 30 ± 22 pM, respectively. It



91

Fig. 11.13: Structure of tiazofurin (**91**).

also inhibited other Src-family members as shown in Table 11.3. Dasatinib demonstrated considerable activity against c-kit and PDGFRβ and over 100-fold selectivity for all other kinase targets in the panel [56].



Scheme 11.20: Synthetic outline for the synthesis of dasatinib (**85**).

Table 11.3: Kinase selectivity profile of dasatinib **85**.

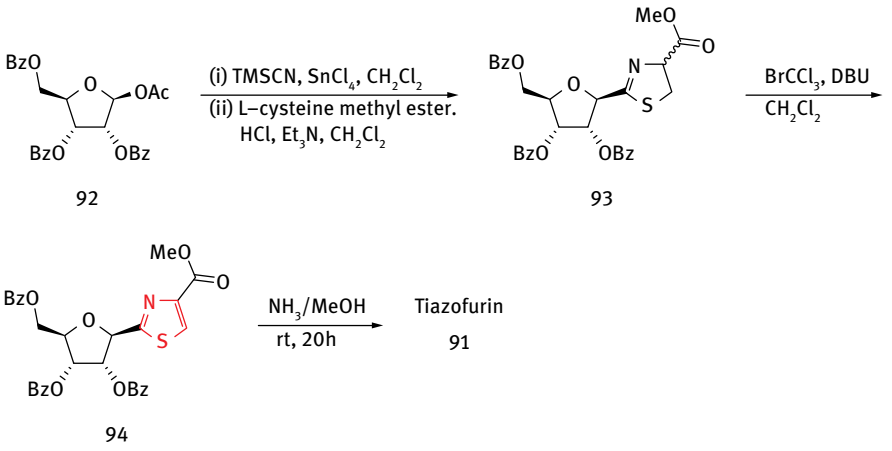
Kinase	Enzyme IC_{50} (nM)	Kinase	Enzyme IC_{50} (nM)
Bcr-Abl	< 1.0	Her2	710
Src	0.5	FGFR-1	880
Lck	0.4	MEK	1700
Yes	0.5	VEGFR-2	> 2000
c-Kit	5.0	CDK2	> 5000
PDGFRβ	28	IKK	> 10000
p38	100	AKT	> 50000
Her1	180	FAK	> 50000

11.10 Tiazofurin

Tiazofurin (**91**, Fig. 11.13) is a synthetic thiazole *C*-nucleoside antitumor agent with significant activity against both human lymphoid, lung tumor cell lines and murine-implanted human ovarian cancers [57]. It is an inosine-5'-monophosphate (IMP) dehydrogenase inhibitor.

Tiazofurin (**91**) is converted *in vivo* into the active metabolite thiazole-4-carboxamide adenine dinucleotide (TAD), an analog of NAD that prevents *de novo* guanine nucleotide synthesis via inhibition of IMP dehydrogenase (EC 1.1.1.205) [58]. The consequent decrease in cellular GTP and deoxy GTP concentrations interrupts DNA and RNA synthesis in rapidly-dividing tumor cells. Tiazofurin proved effective in reducing the leukemic cell burden in acute myelogenous leukemia patients, but was found to be toxic for general clinical application.

Tiazofurin (**91**), a tetrahydrofuran substituted thiazole, was obtained from dehydrogenation of thiazoline (**93**). Thiazoline **93** was prepared by reaction of commercially available 1'-acetoxy-2',3',5'-tri-*O*-benzoyl-β-D-ribofuranose (**92**) with L-cysteine methyl ester in the presence of triethylamine (Type V approach). Oxidation of thiazoline (**93**) to thiazole (**94**) was carried out with the help of DBU and 1-bromo-1,1,1-trichloro methane. Ester aminolysis and deprotection of benzoyl group was carried out by stirring **94** in methanolic ammonia (Scheme 11.21) [58].



Scheme 11.21: Synthesis of tiazofurin (**91**).

Franchetti et al. synthesized thiophenfurin (**95**) and furanfurin (**96**) to investigate the effect of isosteric replacement of thiazole ring of **91** by thiophene and oxazole heterocycle (Fig. 11.14). Considerable loss in the activity was observed during this investigation [59].

Thiophenfurin (**95**) was found to be less active as an antitumor agent both *in vitro* and *in vivo* whereas the furanfurin (**96**) proved to be completely inactive. The inactivity of **96** was found to be because of its poor ability to be converted to furan-3-carboxamide adenine dinucleotide (FFAD) in target cells [59, 60].

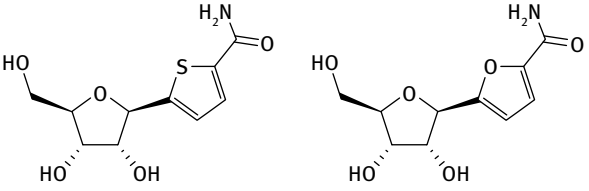


Fig. 11.14: Structure of thiophenfurin (**95**) and furanfurin (**96**).

11.11 CYC116

CYC116 (**97**, Fig. 11.15), a 2-aminothiazole derivative, is an orally-available inhibitor of Aurora kinases A and B with 50-fold more potency than other CDKs and is less potent to VEGFR2.

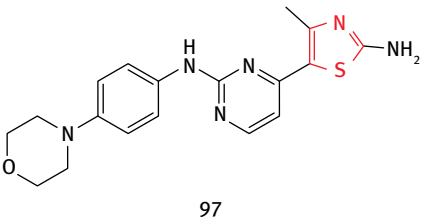
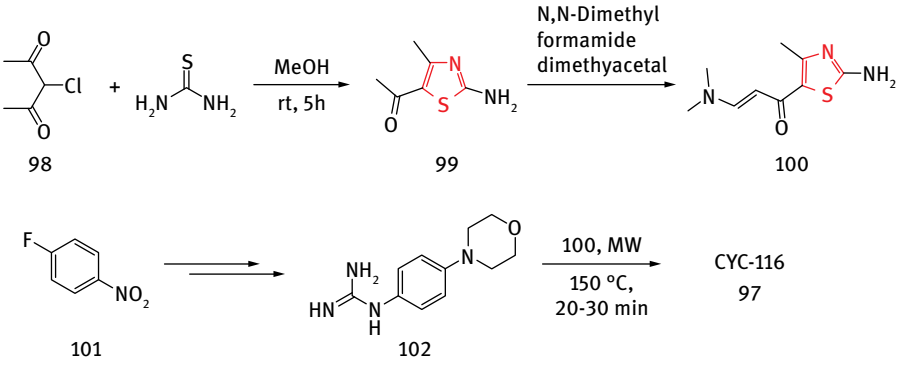


Fig. 11.15: Chemical structure of CYC116 (**97**).

CYC116 is a potent ATP-competitive inhibitor of Aurora A, B and C kinases. It inhibits Aurora A and B (IC_{50} = 44 and 19 nM respectively) in cancer cells and induces a phenotype characterized by delayed entry into mitosis, and defective tubulin polymerization, centrosome function, and spindle formation. The spindle checkpoint is inactivated and cytokinesis is inhibited by CYC116. These defects result in the generation of polyploid, multinucleated cells and cell death. CYC116 (**97**) is a broad spectrum cytotoxic agent against cultured human tumor cells from various tissues and is particularly sensitive to pancreatic, leukemia and NSCLC type of cancer. It (**97**) also has activity against the tyrosine kinase FMS-related kinase 3 (Flt-3) and vascular endothelial growth factor receptor 2 (VEGFR2) kinase which plays a key regulatory role in the

angiogenesis pathway. The anti-angiogenesis activity of this inhibitor was confirmed in chicken chorio-allantoic membrane assay [61].

Synthetic outline for this 5-heteroarylpyrimidino-2-aminothiazole class (**97**) of compound is shown in Scheme 11.22. Briefly, 2-amino-5-acetylthiazole (**99**) was prepared from thiourea and 3-chloro-2,4-pentadione (**98**) and was converted to the corresponding enaminones (**100**) by heating in *N*,*N*-dimethylformamide dimethyl acetal. The enaminones were then condensed with the appropriate phenylguanidines **102** at elevated temperature (150 °C) under microwave to form CYC116. 1-(4-Morpholinophenyl)guanidine (**102**), was synthesized from 4-fluoronitrobenzene (**101**) and morpholine in the presence of base [61].



Scheme 11.22: Synthesis of CYC116 (**97**).

11.12 NCH-31

NCH-31 (**103**) is an effective histone deacetylase (HDAC) inhibitor (Fig. 11.16). The transcriptional repression is the consequence of condensed chromatin structure which results because of the deacetylation of histone lysine residues, whereas the over acetylation in this process is associated with open chromatin configuration and activation of transcription. Inhibition of histone deacetylases causes histone hyperacetylation which leads to the disruption of the chromatin structure and the transcriptional activation of genes associated with cancer.

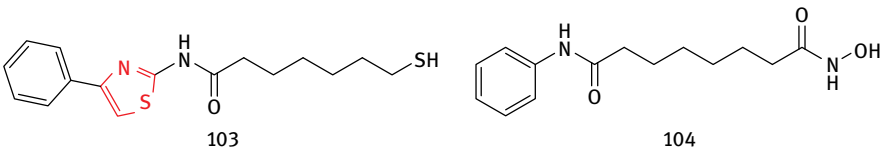
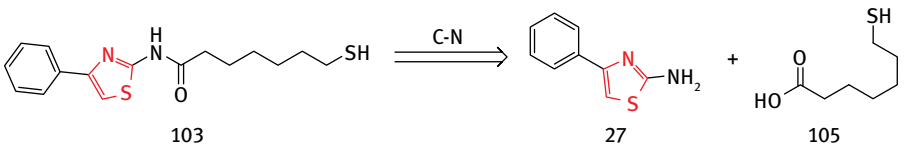


Fig. 11.16: Structure of HDAC inhibitors NCH-31 (**103**) and SAHA (**104**).

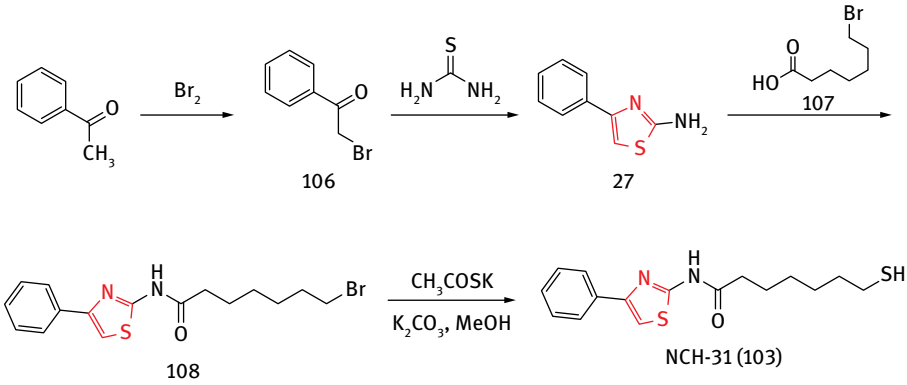
Several hydroxamic acid derivatives like Vorinostat (SAHA, **104**, Fig. 11.16), TSA, CRA 024781, SB-939 etc. have been reported in literature which act as the HDAC inhibitors [62]. The hydroxamic acid derivatives are known to be associated with poor pharmacokinetic properties and severe toxicity [63]. NCH-31 (**103**) is the first potent thiolate histone deacetylase inhibitor in which the hydroxamic acid group of SAHA is replaced by a thiol with improved activity [64]. The aminothiazole derivative (NCH-31, **103**) is a carboxamide of 7-mercaptoheptanoic acid (**105**) and 2-amino-4-phenyl thiazole (**27**) [65, 66]. The synthesis of this drug can be achieved from two starting materials such as **103** and **105** as shown in Scheme 11.23.



103 27 105

Scheme 11.23: Retrosynthetic analysis of NCH-31 (**103**).

2-Amino-4-phenyl thiazole (**27**) has been synthesized through Hantzsch thiazole synthesis using α-bromomethyl ketones (**106**) and thiourea as starting materials. The 7-mercaptoheptanoic acid unit (**105**) was introduced at position-2 of thiazole by the reaction of compound **27** with 7-bromoheptanoic acid (**107**) and further, thiolation of **108** with potassium ethanethiolate to yield NCH-31 (Scheme 11.24) [64].

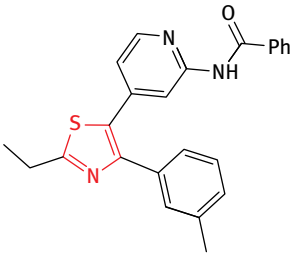


106 27 108

Scheme 11.24: Synthesis of NCH-31 (**103**).

11.13 TAK-715

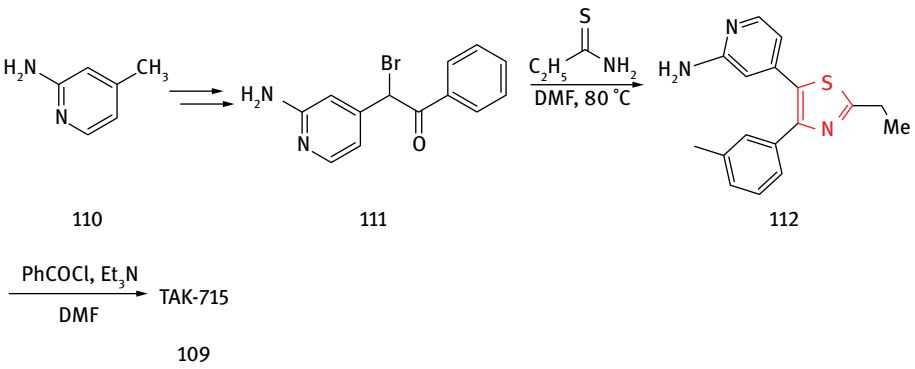
TAK-715 (**109**), a 2,4,5-substituted thiazole (Fig. 11.17), is known to exhibit potent inhibitory activity against p38 mitogen-activated protein (MAP) kinase and under clinical investigation for the treatment of rheumatoid arthritis (RA). The p38 MAP kinase inhibitors have demonstrated their application for the treatment of several chronic inflammatory diseases.



109

Fig. 11.17: Structure of TAK-715 (**109**).

N-[4-[2-Ethyl-4-(*m*-tolyl)-1,3-thiazol-5-yl]-2-pyridyl]benzamide (TAK-715, **109**) was synthesized by the acylation of 4-phenyl-5-pyridyl-1,3-thiazole (**112**) in presence of triethyl amine in *N*,*N*-dimethylformamide. 4-Methyl-2-aminopyridine (**110**) was used as starting material for the synthesis of 4-phenyl-5-pyridyl-1,3-thiazoles (**112**), where **110** was treated with LDA and 1-benzoyl-2-methylaziridines followed by bromination to furnish α-bromo ketone (**111**). Compound **111** on heating with propanethioamide in DMF resulted in the formation of the key intermediate **112** (Scheme 11.25) [67].



110 111 112

109

Scheme 11.25: Synthesis of TAK-715 (**109**).

A docking simulation between TAK-715 and p38 MAP kinase (Fig. 11.18) presented by Miwatashi et al. [67] confirmed that the phenyl ring of the benzamide moiety interacts with the hydrophobic groove between Leu108 and Gly110. Two hydrogen bonds between the amino pyridyl moiety and the kinase backbone Met109 amide along with an additional hydrogen bond interaction between Lys53 and thiazole nitrogen was observed during this study.

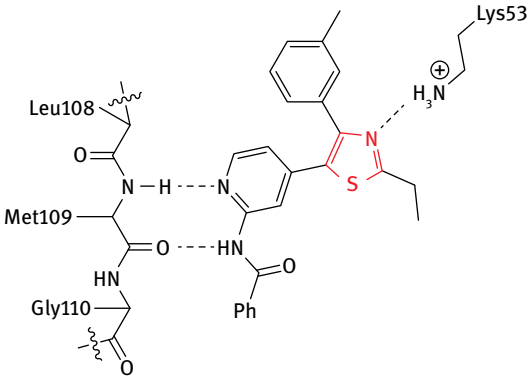
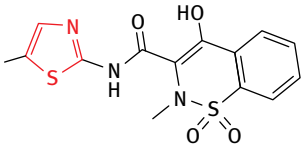


Fig. 11.18: Key interactions of TAK-715 with p38 MAP kinase (CYP 3A4).

11.14 Meloxicam

Meloxicam is an enol-carboxamide class of nonsteroidal anti-inflammatory drug (NSAID) developed by Boehringer-Ingelheim.

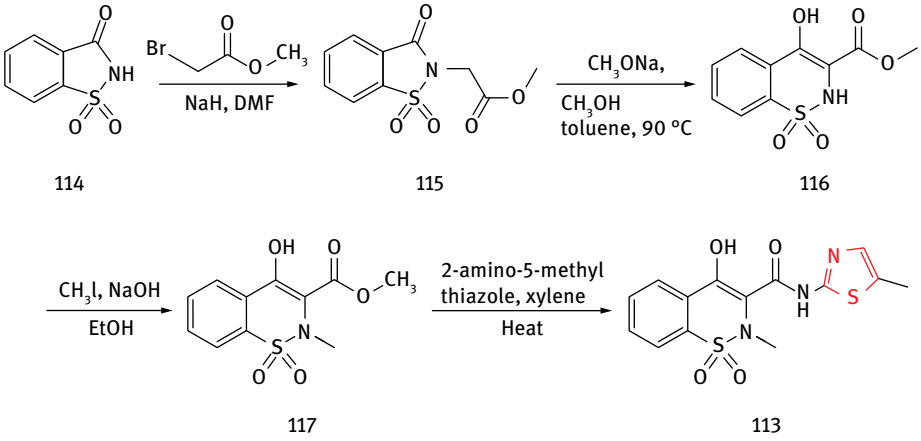


113

Fig. 11.19: Structure of meloxicam **(113).**

A process for meloxicam synthesis is shown in Scheme 11.26. The starting material benzo[d]isothiazol-3(2H)-one-1,1-dioxide (**114**) was treated with α-bromomethyl acetate in presence of sodium hydride to give **115**, which further undergoes rearrangement (in presence of sodium methoxide) and methylation to produce **117**. Reaction of **117** with 2-amino-5-methyl thiazole afforded meloxicam (**113**) [68].

Meloxicam is known to inhibit the enzyme cyclooxygenase (COX), responsible for converting arachidonic acid into prostaglandin H_2 (PGH2, the first step in the synthesis of prostaglandin). It selectively inhibits COX-2 over COX-1 at its low therapeutic doses [68].



Scheme 11.26: Synthesis of meloxicam (**113**).

To explore the SAR in this class of drug, the thiazole moiety was replaced by other heterocyclic moieties viz pyridine and oxazole (Fig. 11.20). Screening data at 10, 1, and 0.1 µg/mL were obtained for compounds **113**, **118** and **119**, and IC_{50} values (µM) were generated (Table 11.4). A comparison of **113** with piroxicam (**118**) showed different inhibitory profiles for COX-1 and COX-2. While **118** showed some selectivity for COX-2 in a microsomal assay, COX-2 inhibition plateaued at 60 %. Comparatively, **113** showed about 80 % inhibition and had 75-fold selectivity for COX-2 at the IC_{50}. Replacement of thiazole by oxazole (**119**) diminishes the activity against COX-1 and COX-2. The screening results suggest that the potency of **113** [IC_{50}: 0.49 µM and its 75-fold selectivity for COX-2] are not improved by such structural modification [68].

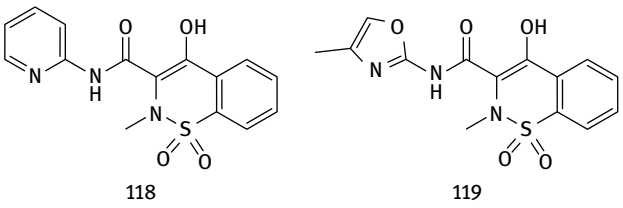
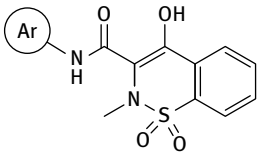
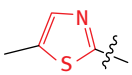
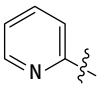
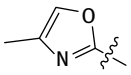


Fig. 11.20: Synthetic analogs of meloxicam.

Table 11.4: Percentage inhibition of compounds against COX-1 and COX-2.

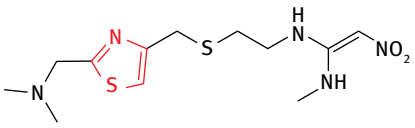


Compound (Heteroaryl group)	%Inhibition of COX-2			IC_{50}	%Inhibition COX-1			IC_{50}
	10 µg/ml	1 µg/ml	0.1 µg/ml		10 µg/ml	1 µg/ml	0.1 µg/ml	
 113	77	72	24	0.49	39	−1	−7	36.6
 118	62	58	25	59 % @ 100 µM	35	10	−7	1.3
 119	−7	4	13	–	−7	5	5	–

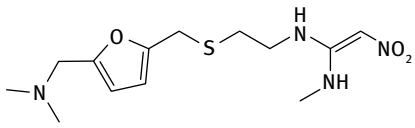
11.15 Nizatidine

Nizatidine (**120**) is a potent histamine H_2-receptor antagonist marketed in 1987 by Eli Lilly under the brand names Tazac and Axid. It inhibits stomach acid production and is used for the treatment of gastric ulcers, duodenal ulcers, peptic ulcers, stress ulcers and gastroesophageal reflux disease. Structurally, it has elongated shape consisting of 2,4-disubstituted thiazole moiety. Nizatidine differs from ranitidine (**121**) by the substitution of thiazole moiety in place of furan.

Chemically, *N*-2-[2-(dimethylamino)methyl-4-thiazolyl]methylthioethyl]-*N*'-methyl-2-nitro-1,1-ethenediamine (**120**) is an off-white crystalline solid having solubility in water. Nizatidine and ranitidine are reversible competitive inhibitors of histamine



Nizatidine (120)

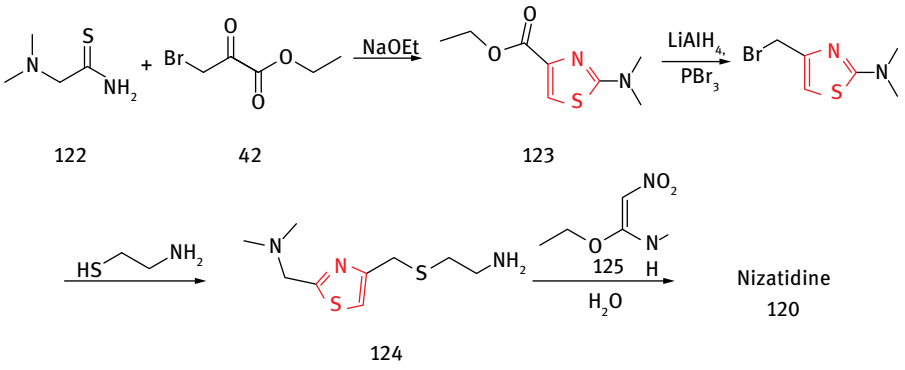


Ranitidine (121)

Fig. 11.21: Structure of nizatidine (**120**) and its structural analog ranitidine (**121**).

at the histamine H2-receptors, particularly found in the gastric parietal cells. This results in decreased gastric acid secretion, gastric volume, and reduced concentration of hydrogen ions.

During the synthesis of **120**, the key intermediate 4-carbethoxy thiazole (**123**) was constructed by the reaction of 1-(*N*,*N*-dimethyl)thioacetamide (**122**) with α-bromoethyl pyruvate (**42**). Compound **123** on reduction with lithium aluminum hydride and further reaction with phosphorus tribromide and 2-aminoethanethiol afforded 2,4-disubstituted thiazole (**124**). Reaction of **124** and 1-ethoxy-*N*-methyl-2-nitroetheneamines (**125**), obtained by the reaction of trialkyloxonium tetrafluoroborate with *N*-methyl-2-nitroacetamide in an aprotic solvent, afforded nizatidine (Scheme 11.27) [69].



Scheme 11.27: Synthetic outline for nizatidine (**120**).

11.16 Famotidine

Famotidine (**126**) is another thiazole-containing histamine H_2-receptor antagonist. Like nizatidine and ranitidine it also inhibits stomach acid production and is commonly used for the treatment of peptic ulcer disease and gastroesophageal reflux disease. Unlike cimetidine (**127**), the first H_2 antagonist, famotidine (**126**) has no effect on the cytochrome P450 enzyme system, and does not interact with other drugs (Fig. 11.22).

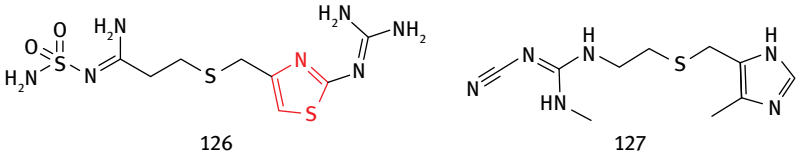
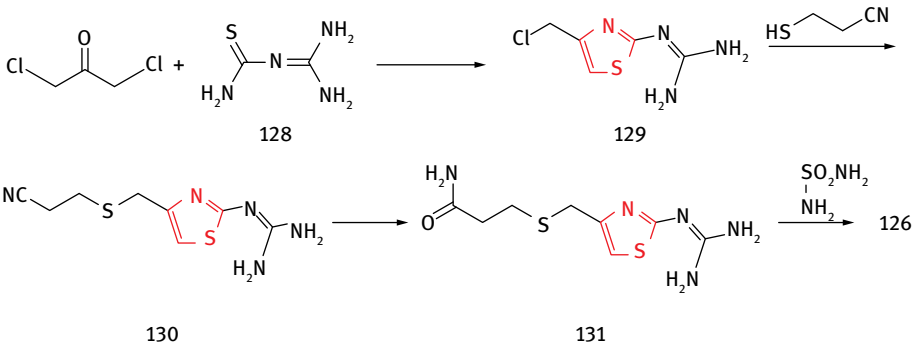
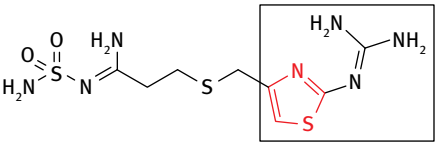


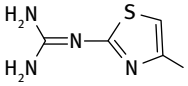
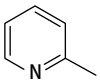
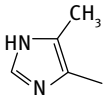
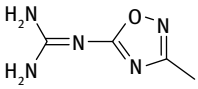
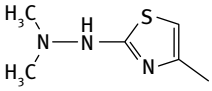
Fig. 11.22: Structure of famotidine (**126**) and cimetidine (**127**).



Scheme 11.28: Synthetic outline for famotidine (**126**).

Table 11.5: Structure and H_2-receptor antagonist activities of famotidine and its analogs.



Comp. No.	Heteroaryl Group	ED_{50}, M
126		$(2.7 \pm 0.3) \times 10^{-7}$
126a		$> 10^{-4}$
126b		$> 10^{-4}$
126c		$> 10^{-4}$
126d		$> 10^{-4}$
127	**Cimetidine**	$(2.7 \pm 0.3) \times 10^{-6}$

The synthetic route employed in the preparation of famotidine (**126**) illustrated by Yanagisawa et al. is given in Scheme 11.28 [70]. During the synthesis, the 4-chloromethyl derivative of thiazole (**129**) was obtained from the reaction of chloro acetylchloride with amidinothiourea (**128**). 2-(4-(Chloromethyl)thiazol-2-yl)guanidine (**130**) on reaction with sodium salt of 3-mercaptopropionitrile and further hydrolysis resulted in 3-((2-(aminothiazol-4-yl)methyl)thiopropanamide (**131**). The desired *N*'-sulfamoylimidamide (**126**) was synthesized by the reaction of compound **131** with sulfuric diamide.

To study the structure-activity relationship in famotidine (**126**), a number of structurally related analogs were synthesized by Yanagisawa et al. [71] in which thiazole moiety was replaced by other heteroaromatic substituents. As shown in Table 11.5, the analog **126a** was found to be inactive up to 10^{-4} M concentration, whereas the imidazole analog **126b** having similar substituent as that of cimetidine (**127**) was also found to be inactive. The 1,2,4-oxadiazole analog **126c** was virtually devoid of any activity *in vitro* and interestingly, the 2-(2,2-dimethylhydrazinyl)-4-methylthiazole substitution (**126d**) also lost the activity suggesting the importance of appropriate substituent and the overall electron density on the thiazole ring [71].

11.17 Conclusion

1,3-Thiazole moiety, a member of azole heterocycle, containing both sulfur and nitrogen atoms was observed in several natural products and drugs. Several sulfur-containing compounds are present in many living organisms and a thiazole-containing compound such as thiamin pyrophosphate is also a part of the living system and is involved in many cellular processes. Many natural and synthetic products comprise thiazole rings with varied biological properties, such as antiviral, anticancer, antibacterial, antifungal, anticonvulsant, antiparkinsonian and anti-inflammatory activities. Apart from natural products and synthetic drugs, thiazole rings were also observed in many fluorescent dyes, polymers, insecticides, antioxidants and liquid crystals. The thiazole-containing natural products are derived from cysteine peptide precursors involving sequential biotransformations such as coupling, cyclization and oxidation to furnish a thiazole subunit. Overall, thiazoles are highly fascinating molecular scaffolds in pharmaceutical research with a significant amount of natural products and drugs currently on the market or in clinical trials comprising thiazole ring as the important structural subunit, which is able to provide ligand points for more than one type of bioreceptor. In short, "*Thiazoles are the Privileged Scaffolds in Drug Discovery*".

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