

Drug Metabolism

A Fascinating Link Between Chemistry and Biology

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Drug metabolism involves the enzymatic conversion of therapeutically important chemical species to a new molecule inside the human body. The process may result in pharmacologically active, inactive, or toxic metabolite. Drug metabolic process involves two phases, the occurrence of which may vary from compound to compound. In this article, we discuss the basics of drug metabolism, the process, metabolising organs and enzymes (especially CYP450) involved, chemistry behind metabolic reactions, importance, and consequences with several interesting and significant examples to epitomize the same. We also cover the factors influencing the process of drug metabolism, structure–toxicity relationship, enzyme induction and inhibition.

1. Introduction

Medicines are required for humans to cure diseases but at the same time, they are foreign objects to the body. Hence, the human body tries to excrete them at the earliest. It is highly desirable that the medicines get eliminated from the human body immediately after showing their drug action. The longer time the drug spends in the body, the greater are its side effects. The human body has a natural mechanism to eliminate these foreign objects (medicines). This is mainly facilitated by the process known as drug metabolism.

Drug metabolism may be defined as the biochemical modification of one chemical form to another, occurring usually through specialised enzymatic systems. It often involves the conversion of lipophilic chemical compounds (drugs) into highly polar derivatives that can be easily excreted from the body. (See *Figure 1*.)



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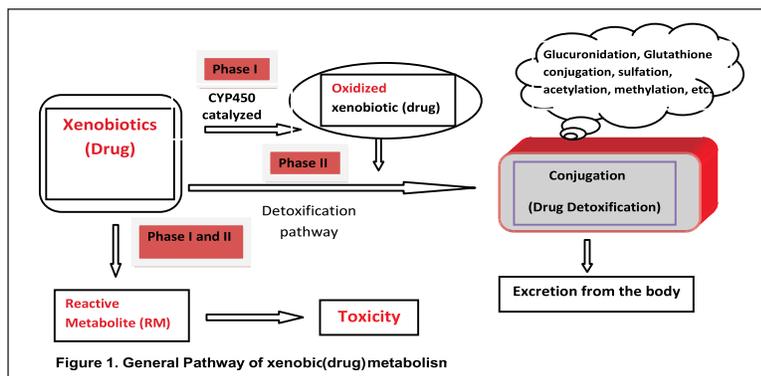
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Keywords

Drug metabolism, chemistry, CYP450, toxicity.



Figure 1. General pathway of drug metabolism.

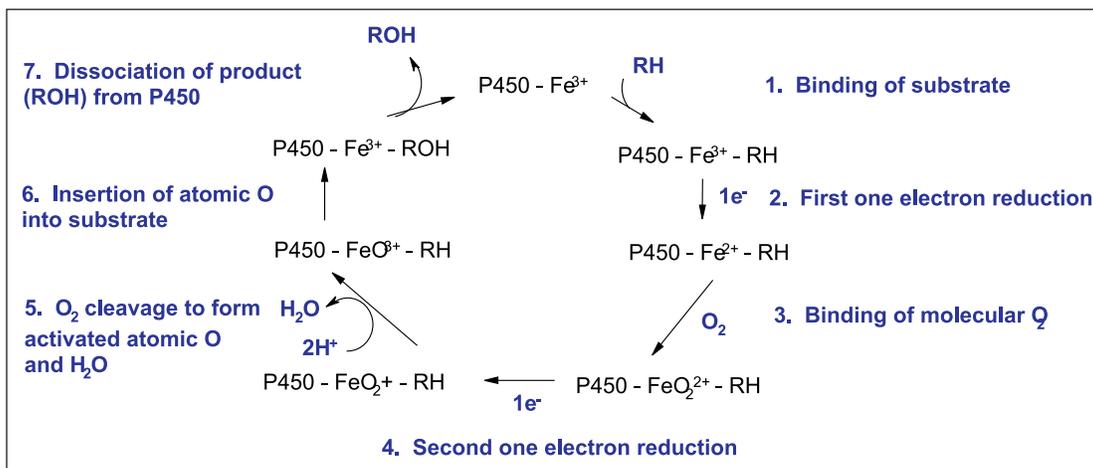


Since lipid soluble substances undergo passive reabsorption from the renal tubules into the blood stream, they accumulate in the body and lead to toxic reactions. To avoid the same, the body has been provided with an armoury in the form of a metabolic system. This system transforms lipophilic, water-insoluble and nonpolar drugs into more polar and water-soluble metabolites, easily excretable from the body. Thus, fittingly and aptly, drug metabolism is termed as a detoxification process [1,2]. Some of the metabolites are required for drug action. This phenomena is being exploited in the design of prodrugs¹. However, the same metabolic process can also lead to the generation of reactive metabolites (RM), which are toxic to the human body. This is termed as bioactivation of drugs, which depends specifically on important structural features present in these compounds [3]. Attempts are being made to bypass the formation of such reactive metabolites. To carry out all these metabolic reactions, catalytic machinery present in several organs in the human body is required, as discussed in the next section.

2. Metabolic Organs

The chemistry of drug metabolism needs an elaborate understanding – it is a fascinating and a complicated process. The primary site of drug metabolism is the smooth endoplasmic reticulum of the liver cell. This is because of the presence of large amounts of many varieties of enzymes. The drug metabolism happening in the liver is termed as hepatic metabolism. In addition to the liver, every biological tissue of the body has the ability to metabolize drugs. The drug metabolism process occurring in organs other than the

¹ See H Surya Prakash Rao, Capping Drugs: Development of Prodrugs, *Resonance*, Vol.8, No.2, 2003.



Scheme 1. The catalytic cycle of CYP450, leading to the oxidation of substrate.

The detailed mechanism of metabolism by CYPs has been described in the catalytic cycle shown in *Scheme 1* [4, 6].

There are more than 300 different CYP enzymes, which have been grouped into several families and subfamilies based on the amino-acid sequence. Out of these, 18 CYP families have been identified in mammals, comprising majorly of families CYP1, CYP2 and CYP3. The percentage contribution of various CYP isoforms in metabolism of drugs is given in *Figure 2*. A selected list of drugs which are metabolized by various families of CYP enzymes is shown in *Table 1* [5,7].

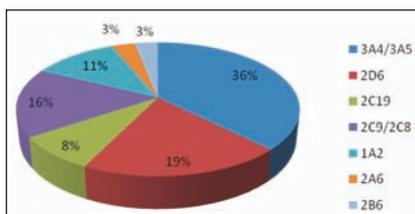


Figure 2. The percentage contribution of various CYP isoforms in drug metabolism.

As discussed before, CYP450 has a heme prosthetic group, whose iron atom can occur in two oxidation states: Fe²⁺ (reduced)

Table 1. A selected list of drugs metabolized by various families of CYP enzymes.

CYP Drugs Metabolised

1A2	Amitriptyline, clozapine
2A6	Acetaminophen, amodiaquine, artesunate
2C8	Paclitaxel, torsemide
2C9	Diclofenac, ibuprofen, phenytoin
2C19	Diazepam, omeprazole, phenytoin
2D6	Amitriptyline, metoprolol, nortriptyline
2E1	Enflurane, halothane
3A4	Carbamazepine, erythromycin, zolpidem

Box 1. Compound I (Cpd I) of CYP 450: The Active Species

Cpd I is an iron(IV)-oxo heme-porphyrin radical cation species, which participates in the metabolic reaction mechanisms such as hydroxylation, epoxidation, *S*-oxidation, *N*-oxidation, etc. In the earlier studies, due to the problems in detecting and characterizing Cpd I, various suggestions for alternative oxidant species, such as Cpd 0 (ferric-hydroperoxo species) and Cpd II (one electron reduced species of Cpd I) were made. The detailed biomimetic experimental studies showed a higher reactivity of Cpd I than Cpd 0 and therefore ruled out Cpd 0 as a possible oxidant. Till date, all studies (computational as well as experimental) agree that only one oxidant, namely Cpd I is active in CYP 450 enzymes. Since the experimental studies have failed to characterize Cpd I and have yet to resolve the uncertainty associated with the status as the primary oxidant of CYP 450 enzymes, theoretical studies have been performed to establish the active species of this enzyme [8].

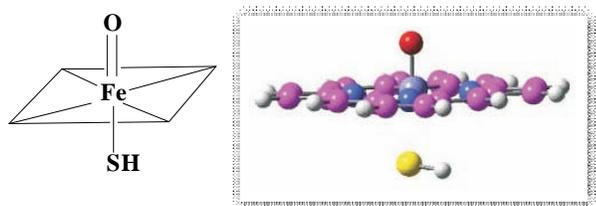
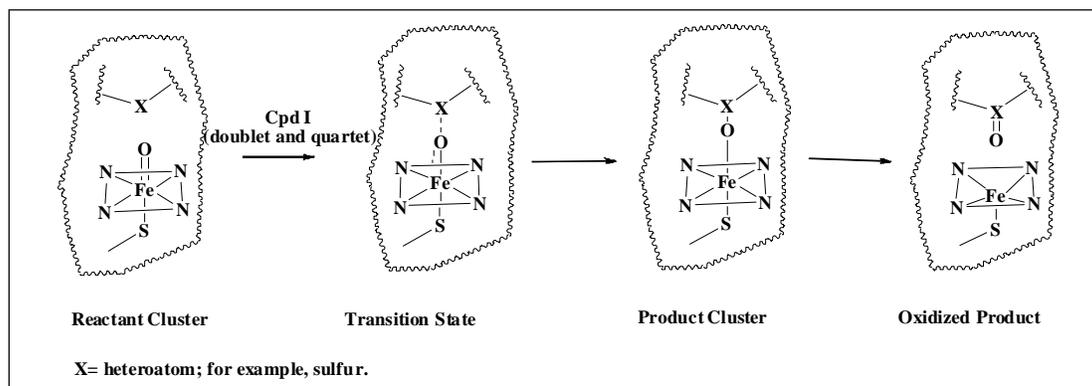


Figure A. 2D and 3D structures of the model of Cpd I (iron(IV)-oxo heme-porphyrine with SH⁻ as the axial ligand).

or Fe³⁺ (oxidized). In these enzymes, the heme prosthetic group is bound to polypeptide chain of several amino acids, through ionic interactions and one Fe-S (cysteine) covalent bond. The active species of CYP450 enzymes is Cpd I, which is responsible for the oxidation reactions by these enzymes. The model of Cpd I (iron-oxo species (Fe=O) coupled to axial cysteine ligand) is shown in Box 1 [8].

A schematic representation of heteroatom oxidation catalyzed by Cpd I is shown in Scheme 2. The first step involves the binding of

Scheme 2. The most important portion of the catalytic cycle of CYP450, leading to heteroatom oxidation of substrate.



Cpd I (iron-oxo) is the active species of CYP450.

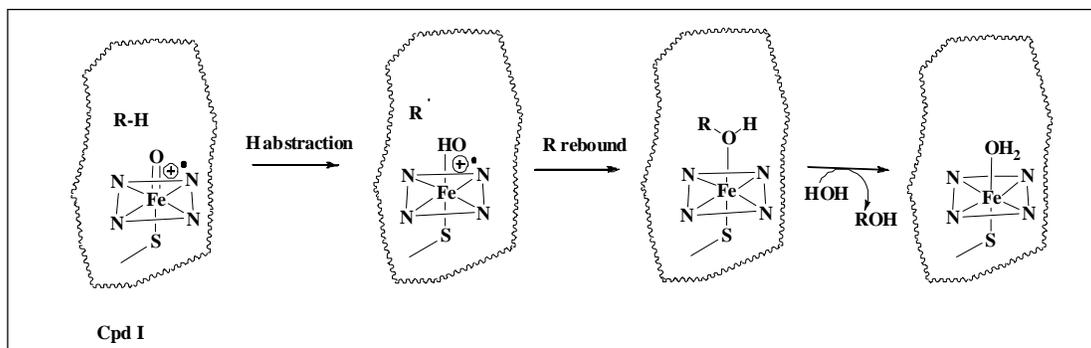
substrate in the active site of the enzyme. This leads to the formation of a reactant complex; thereafter, the oxygen transfer from Cpd I to the heteroatom of the substrate occurs [9]. This leads to the product complex where transfer of oxygen to the heteroatom is almost complete; however, the oxygen atom is still connected to heme-iron. The product containing the oxidized heteroatom leaves the active site of the enzyme in the next step.

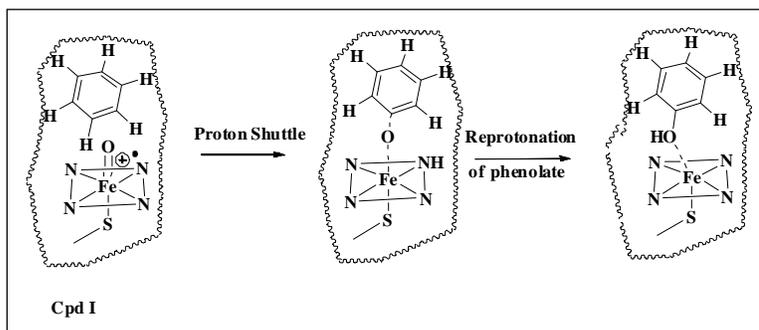
Other reaction mechanisms of drug metabolism similar to the heteroatom oxidation have been studied. Computational chemistry methods provide mechanistic details of the bioinorganic chemistry taking place inside the active site cavity of cytochromes. These methods include quantum mechanical (QM) and quantum mechanical/molecular mechanical (QM/MM) methods. The computational studies employ the use of active species, Cpd I and utilize theoretical methods in elucidating the mechanism. For example, Shaik *et al* have discussed the process of CH hydroxylation, *N*-oxidation, *S*-oxidation, epoxidation of olefins, *N*-dealkylation in various substrates [9,10].

The reaction mechanism of CH hydroxylation is shown in *Scheme 3*. This involves an initial hydrogen abstraction from the alkane (RH) by Cpd I, followed by radical rebound on the iron-hydroxo intermediate to generate the ferric-alcohol complex. Thereafter, this releases the alcohol and restores the resting state (i.e., the water complex) as shown in *Scheme 3*.

Scheme 3. The H-abstraction and rebound mechanism for CH hydroxylation.

Thiel and co-workers have further strengthened the mechanistic details through the use of QM/MM method, and established the





Scheme 4. The proton shuttle mechanism for CH hydroxylation in benzene.

basis behind several metabolic reactions, catalyzed by different CYP isoforms in the body [10]. Visser and Shaik elucidated a novel mechanism of proton shuttle for CH hydroxylation of benzene [11]. A brief description of the mechanism is shown in *Scheme 4*.

On similar lines, our group determined the mechanistic details of the process of *S*-oxidation of thiazolidinediones [12,13], and is as shown in *Figure 3*.

Thus, the mechanism varies for different reactions being catalyzed by CYPs; however, the active species (Cpd I) involved is the same. The mechanisms of these metabolic reactions vary due to the presence of different functional groups, such as *S*, *N*, alkyl, phenyl, alkene, alkyne, etc. Therefore, it becomes essential to understand the organic chemistry of metabolic reactions and the influence of steric hindrance and briefly structure–toxicity relationship.

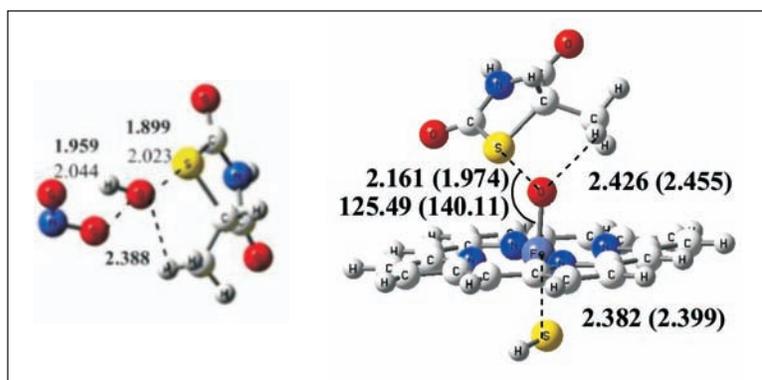


Figure 3. The 3D structures of transition state for *S*-oxidation of thiazolidinediones by HOONO and Cpd I.

Drug metabolism reactions comprise of two phases: Phase I (functionalization) reactions such as oxidation, hydrolysis; and Phase II (conjugation) reactions such as glucuronidation, sulphate conjugation.

Oxidation reactions are the most common and vital. These may occur at carbon, sulfur or nitrogen centre.

3.2 Bioorganic Chemistry of Drug Metabolism

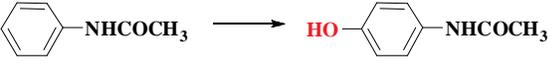
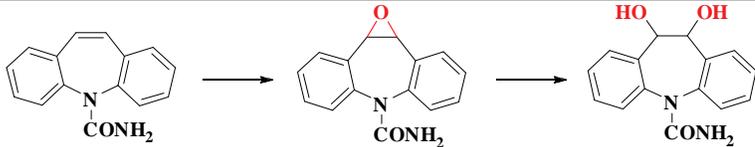
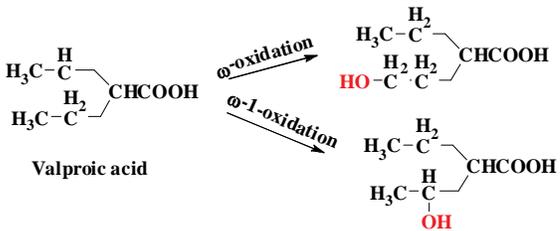
To fully understand the concept of drug metabolism, it is essential to have knowledge of the types of drug metabolism reactions. As discussed earlier, drug metabolism may be a detoxification process, or a bioactivation process, leading to varied consequences. The detoxification reactions can be divided into two broad categories: Phase I (functionalization) and Phase II (conjugation) reactions [2]. The reactions may occur (i) sequentially, (ii) independently, or (iii) simultaneously. The bioactivation process of drug metabolism will be covered later in Section 3.3. The details of both types of detoxification reactions have been described in the following section [2,7].

3.2.1 Detoxification Reactions: Phase I Reactions: These reactions are termed as the nonsynthetic reactions, and include oxidation, reduction, hydrolysis, cyclization and decyclization reactions. These reactions are carried out mostly by mixed function oxidases, usually involving CYP450 and occur in the liver. In these reactions, a polar group is either introduced or unmasked if already present. These reactions are succeeded by Phase II reactions. Most of the Phase I products are not eliminated directly; instead they undergo Phase II reactions. Various Phase I reactions are as follows, with several examples:

(i) Oxidation: This is the most commonly occurring reaction, by virtue of which hydrophilicity of the substrates is increased via the introduction of a polar functional group such as $-OH$. This reaction may occur at several centres in drugs, and thus, oxidation reactions have been classified accordingly.

Oxidation at carbon centre: This includes oxidation at aromatic ring, olefinic centre, and aliphatic groups. Important drugs undergoing metabolism by this reaction include acetanilide (analgesic), phenylbutazone (analgesic), valproic acid (antiepileptic), carbamazepine (antiepileptic), and minoxidil (antihypertensive). (See Table 2.)



Reaction Centre	Chemical Reaction on Drug substrate	
Aromatic ring		
	Acetanilide	Paracetamol
Olefinic centre		
	Carbamazepine	Carbamazepine-10,11-epoxide
		Trans-10,11-dihydroxy carbamazepine
Aliphatic groups		
	Valproic acid	5-Hydroxy valproic acid (minor product)
		4-Hydroxy valproic acid (major product)

Oxidation at carbon-heteroatom systems: This involves reaction on C–N, C–S and C–O systems. The oxidation reactions on C–N systems comprise of *N*-dealkylation, oxidative deamination, formation of *N*-oxide, or *N*-hydroxylation. The process of *N*-dealkylation occurs in the substrates having alkyl group attached directly to nitrogen atom. Examples include: methamphetamine (antidepressant) which gets metabolized to amphetamine via *N*-dealkylation. Amphetamine further gets metabolized to phenylacetone and ammonia via oxidative deamination. (See Table 3.)

The reactions in C–S systems may involve *S*-dealkylation, desulfuration and *S*-oxidation. The mechanism of *S*-dealkylation proceeds via α -carbon hydroxylation, e.g., 6-methylmercaptapurine. The barbiturate drug, thiopental undergoes conversion to pentobarbital via desulfuration, where the cleavage of C=S bond occurs, and the product with C=O is formed. The *S*-oxidation reaction is observed in substrates with sulfide unit (-S-). Examples of drugs undergoing *S*-oxidation are methimazole (antithyroid), cimetidine (antihistaminic), ranitidine (antihistaminic), chlorpromazine

Table 2. Metabolic oxidation on carbon centre of various drug molecules.

S-oxidation reaction occurs in several classes of drugs with sulfide unit such as antidiabetics, antihistaminics and antipsychotics leading to the formation of sulfoxide metabolite.



Reaction	Chemical Reaction on Drug Substrate		
Deamination		Methamphetamine	Amphetamine + Formaldehyde
		Amphetamine	Phenylacetone + Ammonia
Cyclization		Proguanil (Prodrug)	Cycloguanil (Active Antimalarial metabolite) + Water
S-dealkylation		6-Methyl mercaptopurine	6-Mercaptopurine + Formaldehyde

Table 3. Metabolic oxidation on carbon-heteroatom systems of various drug molecules.

(antipsychotic), etc. Thiazolidinedione category of antidiabetic drugs such as rosiglitazone and pioglitazone also undergo *S*-oxidation reaction. The mechanistic details of *S*-oxidation have been elucidated using theoretical chemistry [12,13].

O-dealkylation, oxidative dehalogenation and oxidative aromatization are other important oxidation reactions at carbon centre. Drugs such as phenacetin (analgesic) and codeine (analgesic, antitussive) undergo metabolism by *O*-dealkylation. Halothane (general anaesthetic) undergoes metabolism by oxidative dehalogenation.

(ii) Reduction: The reduction reactions result in the generation of polar functional groups such as amino and hydroxyl, which may undergo further metabolic reactions. These reactions may occur on several functional groups such as carbonyl, hydroxyl, etc., as shown in *Table 4*.

Reaction Centre	Chemical Reaction on Drug Substrate
Carbonyl	$\text{Cl}_3\text{C}-\text{CHO} \cdot \text{H}_2\text{O} \longrightarrow \text{Cl}_3\text{C}-\text{CH}_2\text{OH}$ <p style="text-align: center;">Chloral Hydrate Trichloroethanol</p>
Nitrogen (N=N)	<p style="text-align: center;">Prontosil 1,2,4-Triaminobenzene Sulfanilamide</p>
Halogens	<p style="text-align: center;">Halothane</p>

The reduction reaction can take place in aliphatic and aromatic aldehydes and ketones. Drugs such as methadone (analgesic), chloral hydrate (sedative and hypnotic) and naltrexone (management of alcohol dependence) undergo this metabolic process. The *N*-containing compounds having nitro, azo or *N*-oxide undergo this metabolic reaction. For example: nitrazepam (hypnotic and anxiolytic) and prontosil (antibacterial antibiotic), which get reduced to the corresponding amines. The halogen atom present in various drugs may undergo reduction via replacement by H-atom, e.g., halothane.

(iii) Hydrolysis: These reactions generally involve a large chemical change in the substrate. The hydrolysis reactions can occur in the following functional groups, as listed in *Table 5*. Upon hydrolysis, esters lead to the formation of carboxylic acids and alcohols. Mostly, esters are administered as prodrugs, which on hydrolysis are converted to active forms, e.g., aspirin (analgesic, antipyretic). Drug molecules containing amide functionality undergo slow hydrolysis as compared to esters. These reactions are catalyzed by amidases. The reaction occurs in secondary and tertiary amides, and rarely in primary amides, e.g., procainamide (antiarrhythmic).

Table 4. Metabolic reduction reaction of various drug molecules.

Hydrolysis reactions are commonly encountered in the metabolism of ester and amide prodrugs, leading to the generation of the active metabolites.

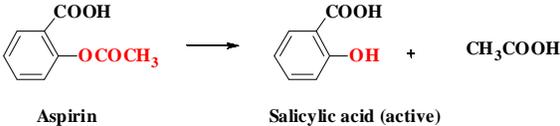
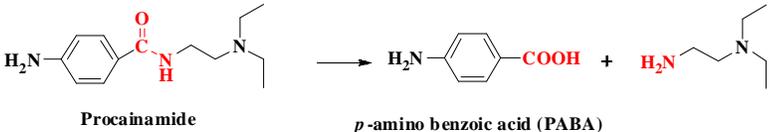
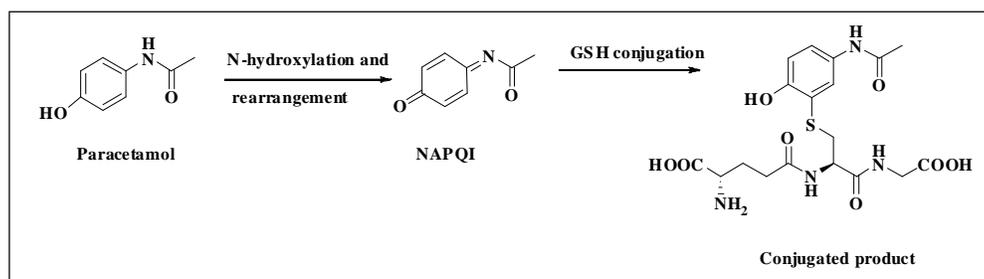
Reaction Centre	Chemical Reaction on Drug Substrate
Esters and ethers	 <p style="text-align: center;">Aspirin Salicylic acid (active)</p>
Amides	 <p style="text-align: center;">Procainamide p-amino benzoic acid (PABA)</p>

Table 5. Metabolic hydrolysis reaction of various drug molecules having different functional groups.

3.2.2 Detoxification Reactions: Phase II Reactions: The Phase II reactions follow Phase I reactions, and occur mostly in the products derived from Phase I reactions. In these reactions, a suitable moiety such as glucuronic acid, glutathione, sulphate, glycine, etc., get conjugated to the metabolites of Phase I reaction. The Phase II reactions are the real drug detoxification pathways. These are also termed as conjugation reactions, because the metabolites are conjugated with the above-mentioned moieties which are large in size and strongly polar in nature. These reactions are catalyzed by a variety of transferase enzymes, such as uridine diphosphate (UDP)-glucuronosyltransferases, sulfotransferases, glutathione transferases. These transferases also exist as a superfamily of enzymes, similar to CYPs, but they differ in the number of drugs they metabolize (lesser than CYPs). Various conjugation reactions along with the enzymes involved and examples of drugs undergoing the same are listed in *Table 6*. Glutathione conjugation is shown in *Scheme 5*.

Scheme 5. A representative scheme showing glutathione conjugation as the Phase II metabolic reaction in the body.



Conjugated Group	Endogenous Cofactor	Enzyme	Drugs
Glucuronidation	UDP glucuronic acid	UDP-glucuronosyltransferase	Chloramphenicol, morphine, paracetamol, salicylic acid, fenoprofen, desipramine, meprobamate, cyproheptadiene
Sulphation	Sulphate	Sulfotransferases	Paracetamol, salbutamol
Glycine/Glutamine conjugation	Glycine/glutamine	<i>N</i> -acyl transferases	Cholic acid, salicylic acid, nicotinic acid, phenylacetic acid
Glutathione conjugation	Glutathione	Glutathione <i>S</i> -transferases	Paracetamol, ethacrynic acid
Acetylation	Acetyl-CoA	<i>N</i> -acetyl transferases	Histamine, mescaline, procainamide, p-amino salicylic acid, isoniazid, phenelzine, hydralazine, dapsone
Methylation	<i>S</i> -adenosyl-L-methionine	Methyl transferase	Morphine, norephedrine, nicotine, histamine, isoprenaline, propylthiouracil

Thus, we have listed both Phase I and II reactions, and the functional groups on which different reactions take place in the body. We have covered the detoxification pathways of drug metabolism; however, we still have to understand the toxic effects of drug metabolism via bioactivation process.

3.3 Bioactivation Reactions: Chemistry of Reactive Metabolites and Adverse Drug Effects

The drug metabolism process can lead to the generation of Reactive Metabolites (RM) in some instances, which further lead to toxicity. The toxicity may vary from genotoxicity to immune-

Table 6. The conjugation reactions along with the enzymes involved and the examples of drugs metabolized by the same pathway.



Majority of adverse drug reactions occur owing to the formation of reactive metabolites. These are formed in the metabolism of drugs with functional groups such as furan, thiophene, epoxide, etc.

mediated Adverse Drug Reactions (ADRs). These adverse drug reactions are major obstacles of drug therapy, and to clinical drug development. The organ most affected by adverse drug reactions is the liver, which is responsible for most of the metabolic reactions [3]. These RMs are specifically termed as Chemically Reactive Metabolites (CRMs) because they possess a chemically reactive group, which leads to ADRs.

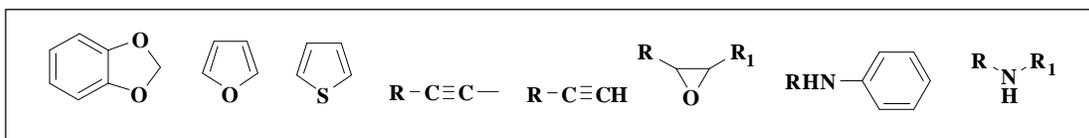
3.3.1 Structural Alerts: Several functional groups associated with the generation of CRMs constitute the structural alerts. Some well-known structural alerts are epoxides, furan, thiophene, anilines, anilides; arylacetic acids; hydrazines; thiophenes; terminal alkenes; nitroaromatics; quinones, quinone-methide, etc., as shown in *Figure 4*.

However, just the presence of these substructures that may form CRMs may not indicate the type and severity of adverse reactions. Many drugs present in the market contain one or the other structural alert, because of their higher benefit-to-risk ratio, e.g., aniline sulphonamides as anti-infective agents. In some cases, toxic effects are not seen to a large extent owing to extensive clearance. For example, raloxifene (for treatment of osteoporosis) gets cleared by glucoronidation and sulphation of phenolic OH groups, thereby minimising the amount of quinone and quinone-methide reactive metabolites formed [14].

The knowledge of structural alerts has been utilised successfully in the early phase of drug discovery, as in the case of bromofenac (ocular pain relief) that contained aniline, arylacetic acid and a bromophenyl ring as structural alerts.

Figure 4. Structural alerts associated with the generation of reactive metabolites in the body.

A structural alert which has caught everyone's attention is the carboxylic acid. Most of the Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) such as aspirin, diclofenac, etc., contain a



carboxylate function, and cause a wide range of ADRs. Therefore, it is important to understand the importance of several functional groups which may lead to toxic reactions in the body. This can be represented by structure–toxicity relationships as discussed in the next section.

3.3.2 Structure–toxicity relationship: Several methods can be utilized to qualify certain functional groups as the structural alerts. One methodology uses DEREK software. The computer program DEREK (an acronym for Deductive Estimation of Risk from Existing Knowledge) is designed to assist chemists and toxicologists in predicting likely areas of possible toxicological risk for new compounds, based on an analysis of their chemical structures. It is a knowledge-based expert system to identify various structural alerts, and the predictions are based in part on various alerts that describe structural features or toxicophores associated with toxicity. However, no *in silico* tools are available that can be utilized in predicting the occurrence of bioactivation and further toxicity in a drug candidate. Computational tools such as MetaSite [3] which can predict metabolic transformations of drug candidates, are available. It is a computational procedure which provides the structure of the metabolites formed with a ranking derived from the site of metabolism predictions. It predicts ‘hot spots’ in the molecule to help chemists focus their design of compounds to reduce CYP-mediated metabolism. It also highlights the molecular moieties that help to direct the molecule in the cytochrome cavity such that the site of metabolism is in proximity to the catalytic centre. However, it cannot predict the bioactivation pathway of a drug candidate.

Since the prediction of bioactivation pathways of possible drug candidates is a daunting task, a knowledge of structural alerts would aid in an efficient drug design, such that the structural alert existing in a toxic predecessor, would be absent in a successful successor drug candidate. This type of relationship is referred to as ‘structure–toxicity’ relationship. *Table 7* lists a set of examples of drug molecules, where the presence or absence of a functional group or an atom, results in toxic or therapeutic activity [3,14,15].

Molecular modeling methods have seen resurgence in the prediction of metabolic reactions and metabolites in drugs, which helps the chemists in the proper design of molecules.

Computational tools help in identifying structural alerts, and thus, become useful during the early stages of drug discovery.



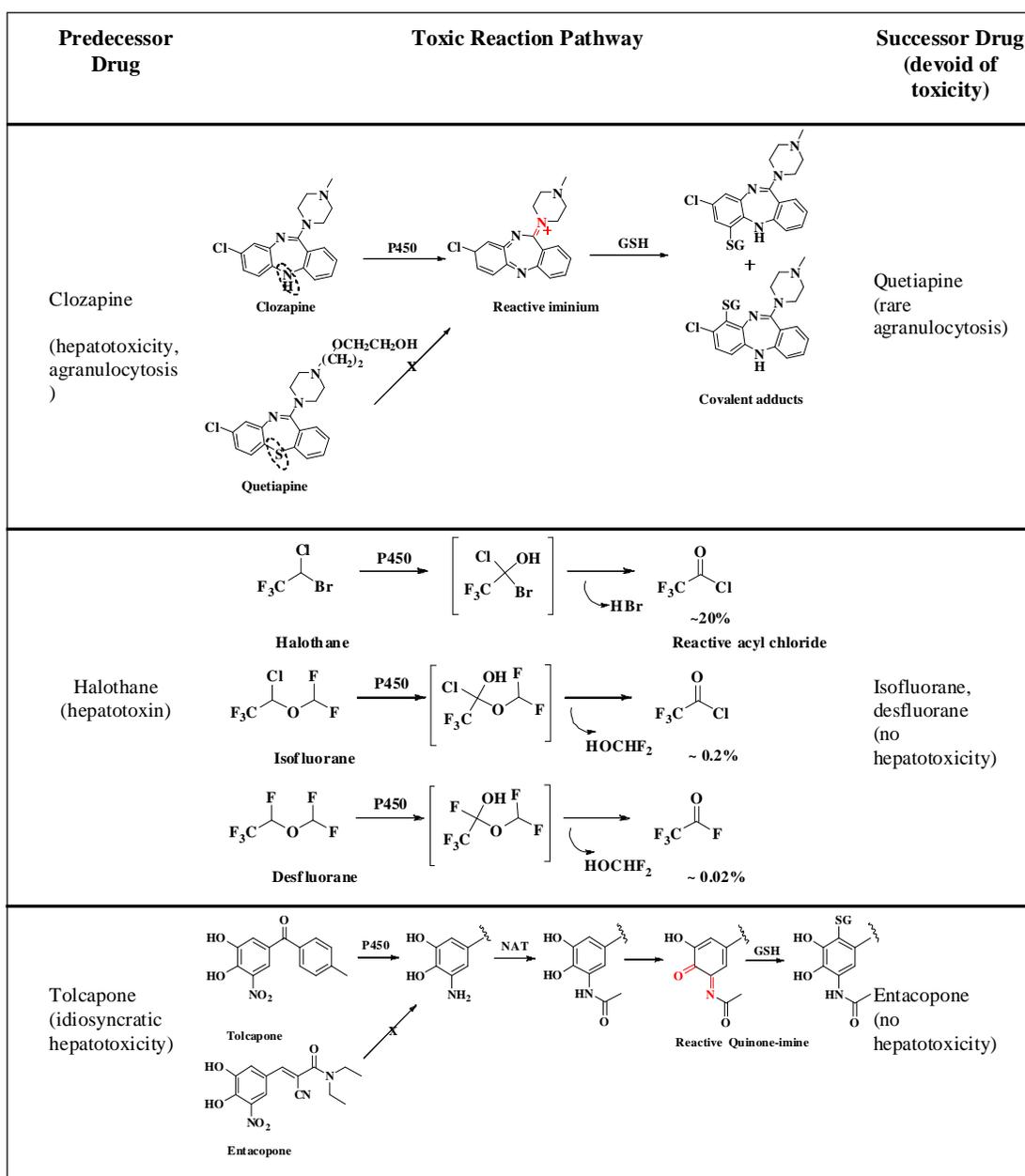


Table 7. Differential metabolism of drugs, where one is toxic and the other non-toxic, representing structure–toxicity relationship.

3.3.3 Consequences of Reactive Metabolite Formation: In most cases, the reactive metabolites (RMs) formed by CYP-mediated metabolism lead to the inactivation of enzyme, which may be reversible or irreversible in nature. The irreversible inactivation or inhibition of enzyme is termed as mechanism-based inactivation or

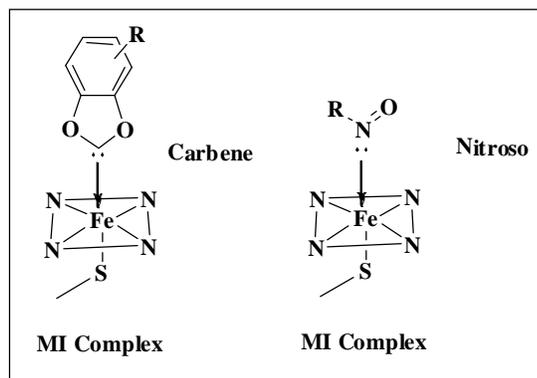


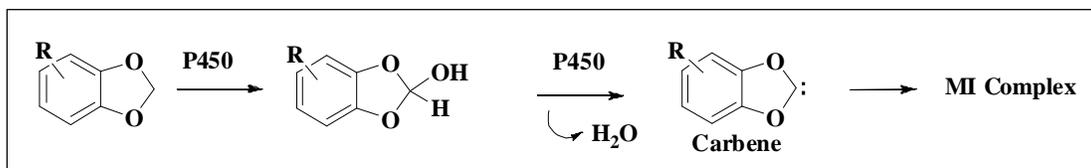
Figure 5. A representation of coordination between reactive carbene/nitroso species and heme-iron, leading to formation of metabolic-intermediate complex (MIC).

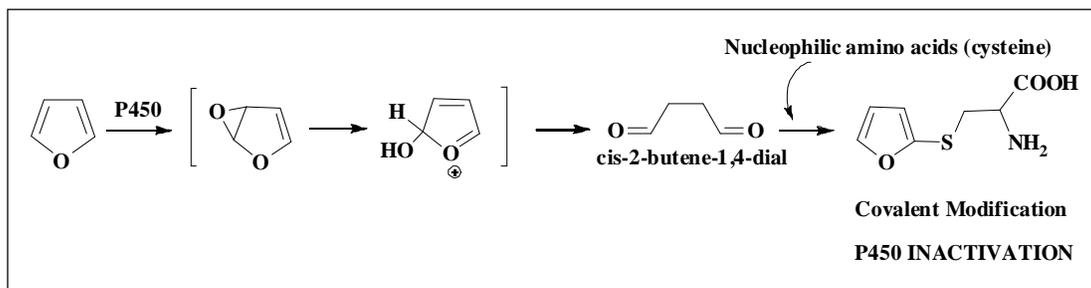
inhibition (MBI) of CYPs. The name is apt because a mechanism precludes enzyme inactivation [3,6]. This inactivation may be classified into two categories based on the mechanism:

(i) Formation of metabolic-intermediate complex (MIC) between reactive species and heme-iron, via coordination: The aliphatic and aromatic amines (nortriptyline, antidepressant; tamoxifen, anticancer) generate reactive nitroso ($R-N=O$) intermediate after various metabolic transformations, such as *N*-dealkylation, *N*-hydroxylation, etc. On the other hand, 1,3-benzdioxole moiety containing drugs lead to the generation of reactive carbene intermediate. Both the intermediates are nucleophilic in nature, and form a coordination bond with heme-iron as shown in *Figure 5*. This coordination is termed as the metabolic-intermediate complex (MIC), which leads to inhibition of CYP activity.

Scheme 6 shows the formation of reactive carbene intermediate from 1,3-benzdioxole moiety. Drugs such as stiripentol (antiepileptic), paroxetine (antidepressant), etc., possess this moiety, which acts as a perpetrator, and leads to mechanism-based inhibition of CYPs [3,6].

Scheme 6. Schematic pathway for the formation of reactive carbene species, starting from 1,3-benzdioxole moiety.





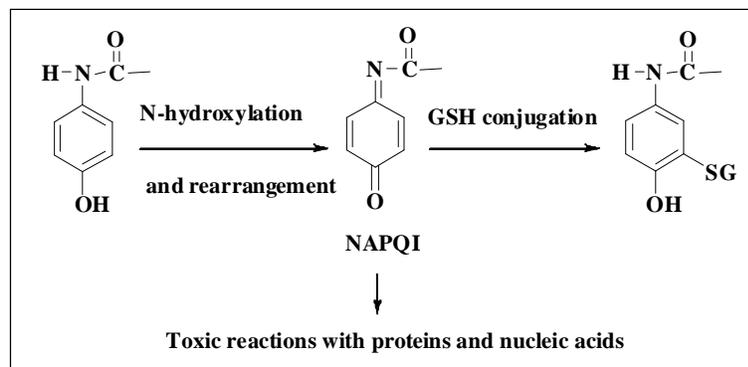
Scheme 7. Schematic pathway for the formation of reactive epoxide intermediate and nucleophilic adducts leading to MBI of CYPs, starting from furan moiety.

(ii) Covalent modification of reactive species with heme or amino-acid residues: Drugs containing the thiophene, furan, olefin, phenyl group may lead to the mechanism-based inhibition of CYPs by generating reactive epoxide intermediate. The epoxide ring opening by nucleophilic active site residues lead to formation of a covalent bond, thus inhibiting CYP activity. Other reactive species leading to MBI via covalent bond formation include quinone-imine and quinone-methide. *Scheme 7* shows the formation of reactive epoxide intermediate from furan moiety present in a few drugs such as L-754,394 (HIV protease inhibitor).

Paracetamol is the other example of a drug, whose metabolite quinone-imine (N-acetyl p-benzoquinone-imine; NAPQI) is reactive, and depletes GSH levels by binding covalently to liver macromolecules (*Scheme 8*). This results in hepatic necrosis in the body.

This CYP inactivation further translates into drug–drug interactions (DDIs), which could be deleterious to the body. For

Scheme 8. Schematic pathway for the metabolism of paracetamol to the toxic NAPQI metabolite.



example, increased cases of myopathy (muscular weakness) and rhabdomyolysis (breakdown products of damaged skeletal muscles released in blood stream) have been observed in hypertensive patients who are being administered a calcium-channel blocker and a potent CYP3A4 inactivator mibefradil together with simvastatin. Here the mechanism for DDI involves the inactivation of CYP-catalyzed metabolism process of simvastatin by mibefradil, which further results in the elevation of plasma levels of statin, leading to death. Owing to such fatal consequences, mibefradil was withdrawn from the market later [3].

Further examples include tadalafil, a drug used for treating erectile dysfunction, which is reported to be metabolized by CYP3A4 and also has inhibitory effects on CYP3A4. The inhibitory effects are proposed to be due to the presence of 1,3-benzodioxole group, which after metabolism gives rise to the corresponding carbene intermediate followed by its coordination with the heme iron as discussed before. Therefore, it has potential adverse effects on patients in its long term use. Similarly, the antidepressant drug, paroxetine acts as the potent inhibitor of CYP2D6 by generating reactive carbene intermediate, leading to a coordination bond formation with heme-iron. Since enzyme inactivation leads to potential DDIs, mechanism-based inactivation of major human CYP enzymes by new compounds is being routinely assessed in a drug-discovery paradigm.

Till now, the basic aspects of drug metabolism, bioinorganic and bioorganic chemistry of drug metabolism, detoxification and bioactivation pathways of drug metabolism have been covered. Since the intensity, therapeutic efficacy, toxicity, biological half-life and duration of pharmacological action of any drug is dependent on the rate of its metabolism, the determination of factors influencing this process becomes essential.

4. Factors Influencing Drug Metabolism

Physicochemical parameters of the drug, biochemical factors like CYP induction or inhibition influence drug metabolism, and lead

The inactivation of CYPs leads to disruption of the enzymatic machinery, thus, hampering the metabolism of drugs. This leads to potential drug-drug interactions and adverse drug reactions.



The rate of drug metabolism is influenced by several factors, such as physicochemical properties of drugs, biochemical and biological factors which thereby affect the therapeutic efficacy, toxicity and duration of pharmacological action of the drug.

Enzyme induction and inhibition are opposite processes with varied effects on metabolic process and severe consequences on the drug action on the body.

to variations in the drug action in humans. Some important factors are discussed here [2,16].

4.1 Physicochemical Properties of the Drug

These include the molecular size, shape, lipophilicity, acidity/basicity, electronic characteristics, pK_a , etc. These properties influence the interaction of drug with the metabolizing enzymes and control the drug action.

4.2 Biochemical Factors

4.2.1 Metabolic Enzyme Induction: Any process that increases the rate of metabolism of a drug is termed as the metabolic enzyme induction, which results in a decrease in the duration and intensity of the drug action. Agents which carry out such effects are termed as enzyme inducers. This increase in drug metabolism arises usually due to the increased synthesis of enzyme protein. For example: barbiturates (anxiolytics) are the inducers which enhance the metabolism of coumarins, phenytoin, cortisol, testosterone, etc., because of induction. Similarly, alcohol (CNS stimulant) increases the metabolism of pentobarbital, coumarins and phenytoin because of induction. Environmental chemicals such as pesticides (DDT), and polycyclic aromatic hydrocarbons present in cigarette smoke are well known to be enzyme inducers.

4.2.2 Metabolic Enzyme Inhibition: Some chemical species block the catalytic site of cytochromes and decrease the catalytic conversion of drugs to metabolites. This process is known as enzyme inhibition, which results in an increase in duration of the drug in the body, thereby, leading to the accumulation of drug in the body and also an increase in toxicity. For example: metacholine (anti-asthmatic) inhibits the metabolism of acetyl choline by competing with it for cholinesterase. Similarly, isoniazid (antitubercular) inhibits the metabolism of phenytoin. Such influence of one drug on the metabolism of another drug leads to drug–drug interactions. Environmental chemicals such as heavy metals including nickel, mercury, arsenic are known to be potent enzyme inhibitors.



4.3 Biological Factors

The biological factors include species differences, strain differences, pharmacogenetics, ethnic variations, gender differences, age, etc. Other biological factors include diet, pregnancy, hormonal imbalance and presence of disease states in the individuals. For example, the activity of drug-metabolising enzymes decrease in people with cardiac failure. Similarly, hormonal factors during pregnancy affect the metabolic process, usually in third trimester, such as in case of anticonvulsant drugs carbamazepine and phenytoin. The circadian rhythm of an individual is also a major influencing factor on drug metabolism. Genetic polymorphism (two or more variants of an enzyme encoded by a single locus within the population) has appeared to be the common phenomenon, leading to variations in metabolic process in humans. This results in a higher or lower activity for one form of polymorphic enzyme as compared to the other form (enzyme isoforms specificity). The enzyme polymorphism is an inherited process, and thereby a major cause of inter-individual differences with respect to the rate of drug metabolism. The individuals are classified accordingly as poor metabolizers and extensive metabolizers. For example, Caucasians are poor metabolizers as compared to Asians and blacks for CYP2D6 (an isoform of CYP450) [4]. All these factors have their varied influence on the rate of metabolic process, which has a critical influence on its outcome, which may vary from a therapeutic to toxic activity as described in the next section.

Genetic polymorphism (two or more variants of an enzyme encoded by a single locus within the population) has appeared to be the common phenomenon, leading to variations in metabolic process in humans.

5. Importance and Consequences of Drug Metabolism

The drug metabolism or biotransformation process results in a variety of consequences as listed below [1,2,16]:

- Pharmacological inactivation of drugs: Here, the metabolite formed has little or absolutely no pharmacological activity. The metabolite of phenytoin (antiepileptic), *p*-hydroxy phenytoin has no pharmacological activity.
- No effect on pharmacological activity: Here, the metabolites



formed after biotransformation processes have equal and similar activity to that of the original drug. Nortriptyline possesses equal activity as that of the parent drug, amitriptyline (antidepressant).

- **Toxicological activation of drugs:** Here, the metabolites formed after biotransformation process have a high tissue reactivity, thus causing toxicity, which may include necrosis, hepatitis, etc. N-acetyl p-benzoquinoneimine which is a metabolite of paracetamol causes hepatotoxicity. Other examples such as tadalafil have been discussed earlier.
- **Pharmacological activation of drugs:** By this process, prodrugs (inactive) are metabolised to highly active drugs. Prodrug is the inactive derivative that is converted to the active component in vivo. These are generally devised when the drugs possess unattractive physicochemical and undesired pharmacokinetic properties. These are used to improve patient acceptability, improve absorption, alter metabolism, biodistribution and elimination. Ampicillin (antibiotic) is the active form of pivampicillin.
- **Changed pharmacological activity:** Here, the therapeutic activity displayed by the metabolite formed is different from that of the original drug. Isoniazid (antitubercular) is the metabolite of iproniazid (antidepressant).

6. Conclusions

Drug metabolism is a fascinating and useful link between chemistry and biology, yet a complicated one. It may result in detoxification or bioactivation of drugs. The detoxification process involves two phases of drug metabolism: Phase I converts highly lipophilic drug molecules to polar metabolites, whereas Phase II involves conjugation reactions. The metabolism includes a variety of reactions such as oxidation, reduction, hydroxylation, dealkylation, etc. Most of the metabolic reactions are carried out by a large family of heme-containing CYP enzymes. The active species of these enzymes is Cpd I (iron-oxo heme porphine with cysteine as the axial ligand), which is responsible for major oxidation and hydroxylation reactions. Besides detoxification

Drug metabolism truly links chemistry and biology in a unique and splendid manner, however, unraveling the link is a complicated task. A special emphasis on drug metabolism is a necessity in the fast moving era of adverse drug reactions and DDIs.



reactions, these enzymes can result in the formation of reactive metabolites which lead to toxicity and drug–drug interactions. Hence, extensive studies on drug metabolism to elucidate the scientific principles are in progress.

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