PRINCIPLES OF DRUG METABOLISM

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1. INTRODUCTION

Xenobiotic metabolism, which includes drug metabolism, has become a major pharmacological science with particular relevance to biology, therapeutics and toxicology. Drug metabolism is also of great importance in medicinal chemistry because it influences in qualitative, quantitative, and kinetic terms the deactivation, activation, detoxification, and toxification of the vast majority of drugs. As a result, medicinal chemists engaged in drug discovery and development should be able to integrate metabolic considerations into drug design. To do so, however, requires a fair or even good knowledge of xenobiotic metabolism.

This chapter, which is written by a medicinal chemist for medicinal chemists, aims at offering knowledge and understanding rather than encyclopedic information. Readers wanting to go further in the study of xenobiotic metabolism may consult various classical or recent books [1–15], broad reviews, and book chapters [16–27].

1.1. Definitions and Concepts

Drugs are but one category among the many *xenobiotics* (Table 1) that enter the body but have no nutritional or physiological value [14]. The study of the disposition—or fate—of xenobiotics in living systems includes the consideration of their absorption into the organism, how and where they are distributed and stored, the chemical and biochemical transformations they may undergo, and how and by which route(s) they are finally excreted and returned to the environment. As for "metabolism," this word has acquired two meanings, being synonymous with disposition (i.e., the sum of the processes affecting the fate of a chemical substance in the body), and with biotransformation as understood in this chapter.

In pharmacology, one speaks of *pharmacodynamic effects* to indicate what a drug does to the body, and *pharmacokinetic effects* to indicate what the body does to a drug, two aspects of the behavior of drugs that are strongly interdependent. Pharmacokinetic effects will obviously have a decisive influence on the intensity and duration of pharmacodynamic effects, while metabolism will generate new chemical entities (metabolites) that may have distinct pharmacodynamic properties of their own. Conversely, by its own pharmacodynamic effects, a compound may affect the state of the organism (e.g., hemodynamic changes, enzyme activities, etc.) and hence the organism's capacity to handle xenobiotics. Only a systemic approach can help one appreciate the global nature of this interdependence.

1.2. Types of Metabolic Reactions Affecting Xenobiotics

A first discrimination to be made among metabolic reactions is based on the nature of the catalyst. Reactions of xenobiotic metabolism, like other biochemical reactions, are *catalyzed by enzymes*. However, while the vast majority of reactions of xenobiotic metabolism are indeed enzymatic ones, some *nonenzymatic reactions* are also well documented. This is due to the fact that a variety of xenobiotics have been found to be labile enough to react nonenzymatically under biological conditions of pH and temperature [28]. But there is more. In a normal enzymatic reaction, metabolic intermediates exist *en route* to the product (s) and do not leave the catalytic site. However, many exceptions to this rule are known, with the metabolic intermediate leaving the active site and reacting with water, with an endogenous molecule or macromolecule, or with a xenobiotic. Such reactions are also of a nonenzymatic nature but are better designated as *postenzymatic reactions*.

The metabolism of drugs and other xenobiotics is often a biphasic process in which the compound may first undergo a *functionalization reaction* (phase I reaction) of oxidation, reduction, or hydrolysis. This introduces or unveils a functional group such as a hydroxy

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Table 1. Major Categories of Xenobiotics (Modified from Ref. [14])

- Drugs
- Food constituents devoid of physiological roles
- Food additives (preservatives, coloring and flavoring agents, antioxidants, etc.)
- Chemicals of leisure, pleasure, or abuse (ethanol, coffee and tobacco constituents, hallucinogens, doping agents, etc.)
- Agrochemicals (fertilizers, insecticides, herbicides, etc.)
- Industrial and technical chemicals (solvents, dyes, monomers, polymers, etc.)
- Pollutants of natural origin (radon, sulfur dioxide, hydrocarbons, etc.)
- Pollutants produced by microbial contamination (e.g., aflatoxins)
- Pollutants produced by physical or chemical transformation of natural compounds (polycyclic aromatic hydrocarbons by burning, Maillard reaction products by heating, etc.)

or amino group suitable for coupling with an endogenous molecule or moiety in a second metabolic step known as a *conjugation reaction* (phase II reaction) [19,24]. In a number of cases, phase I metabolites may be excreted prior to conjugation, while many xenobiotics can be directly conjugated. Furthermore, reactions of functionalization may follow some reactions of conjugation, for example, some conjugates are hydrolyzed and/or oxidized prior to their excretion.

Xenobiotic biotransformation thus produces two types of metabolites, namely functionalization products and conjugates. But with the growth of knowledge, biochemists and pharmacologists have progressively come to recognize the existence of a third class of metabolites, namely xenobiotic–macromolecule adducts, also called macromolecular conjugates. Such peculiar metabolites are formed when a xenobiotic binds covalently to a biological macromolecule, usually following metabolic activation (i.e., postenzymatically). Both functionalization products and conjugates have been found to bind covalently to biological macromolecules, the reaction often being toxicologically relevant.

1.3. Specificities and Selectivities in Xenobiotic Metabolism

The words "selectivity" and "specificity" may not have identical meanings in chemistry and biochemistry. In this chapter, the specificity of an enzyme is taken to mean an ensemble of properties, the description of which makes it possible to specify the enzyme's behavior. In contrast, the term selectivity is applied to metabolic processes, indicating that a given metabolic reaction or pathway is able to select some substrates or products from a larger set. In other words, the selectivity of a metabolic reaction is the detectable expression of the specificity of an enzyme. Such definitions may not be universally accepted, but they have the merit of clarity.

What, then, are the various types of selectivities (or specificities) encountered in xenobiotic metabolism? What characterizes an enzyme from a catalytic viewpoint is first its chemospecificity, that is, its specificity in terms of the type(s) of reaction it catalyzes. When two or more substrates are metabolized at different rates by a single enzyme under identical conditions, substrate selectivity is observed. In such a definition, the nature of the product(s) and their isomeric relationship are not considered. Substrate selectivity is distinct from product selectivity, which is observed when two or more metabolites are formed at different rates by a single enzyme from a single substrate. Thus, substrate-selective reactions discriminate between different compounds, while product-selective reactions discriminate between different groups or positions in a given compound.

The substrates being metabolized at different rates may share various types of relationships. They may be chemically dissimilar or similar (e.g., analogs), in which case the term of substrate selectivity is used in a narrow sense. Alternatively, the substrates may be isomers such as positional isomers (regioisomers) or stereoisomers, resulting in substrate regioselectivity or substrate stereoselectivity. Substrate enantioselectivity is a particular case of the latter (see Section 4.2.1).

Products formed at different rates in product-selective reactions may also share various types of relationships. Thus, they may be

analogs, regioisomers, or stereoisomers, resulting in product selectivity (narrow sense), product regioselectivity, or product stereoselectivity (e.g., product enantioselectivity). Note that the product selectivity displayed by two distinct substrates in a given metabolic reaction may be different, in other words the product selectivity may be substrate-selective. The term substrate–product selectivity can be used to describe such complex cases, which are conceivable for any type of selectivity but have been reported mainly for stereoselectivity.

1.4. Pharmacodynamic Consequences of Xenobiotic Metabolism

The major function of xenobiotic metabolism can be seen as the elimination of physiologically useless compounds, some of which may be harmful, witness the tens of thousands of toxins produced by plants. The function of toxin inactivation justifies the designation of detoxification originally given to reactions of xenobiotic metabolism. However, the possible pharmacological consequences of biotransformation are not restricted to detoxification. In the simple case of a xenobiotic having a single metabolite, four possibilities exist [25], namely:

1. Both the xenobiotic and its metabolite are devoid of biological effects (at least in the concentration or dose range investigated); such a situation has no place in medicinal chemistry.
2. Only the xenobiotic elicits biological effects, a situation that in medicinal chemistry is typical of drugs yielding no bioactive metabolite, as seen for example with soft drugs.
3. Both the xenobiotic and its metabolite are biologically active, the two activities being comparable or different either qualitatively or quantitatively.
4. The observed biological activity is due exclusively to the metabolite, a situation that in medicinal chemistry is typical of prodrugs.

When a drug or another xenobiotic is transformed into a toxic metabolite, the reaction is one of *toxification*. Such a metabolite may act or react in a number of ways to elicit a variety of toxic responses at different biological levels. However, it is essential to stress that the occurrence of a reaction of toxification (i.e., toxicity at the molecular level) does not necessarily imply toxicity at the levels of organs and organisms, as discussed later in this chapter.

1.5. Setting the Scene

In drug research and development, metabolism is of pivotal importance due to the interconnectedness between pharmacokinetic and pharmacodynamic processes [14]. *In vitro* metabolic studies are now initiated early during discovery and development to assess the overall rate of oxidative metabolism, to identify the metabolites, and to obtain primary information on the enzymes involved, and to postulate metabolic intermediates. Based on these findings, the metabolites must be synthesized and tested for their own pharmacological and toxicological effects. In preclinical and early clinical studies, many pharmacokinetic data must be obtained and relevant criteria must be satisfied before a drug candidate can enter large-scale clinical trials [29,30]. As a result of these demands, the interest of medicinal chemists for drug metabolism has grown remarkably in recent years.

As will become apparent, the approach followed in this chapter is an analytical one, meaning that the focus is on metabolic reactions, the target groups they affect, and the enzymes by which they are catalyzed. This information provides the foundations of drug metabolism, but it must be complemented by a synthetic view to allow a broader understanding and meaningful predictions. Two steps are required to approach these objectives, namely (a) the elaboration of metabolic schemes where the competitive and sequential reactions undergone by a given drug are ordered and (b) an assessment of the various biological factors that influence such schemes both quantitatively and qualitatively. As an example of a metabolic scheme, Fig. 1 presents the biotransformation of propranolol (**1**) in humans [31]. There are relatively few studies as comprehensive and clinically relevant as this one, which remains as current today as it was

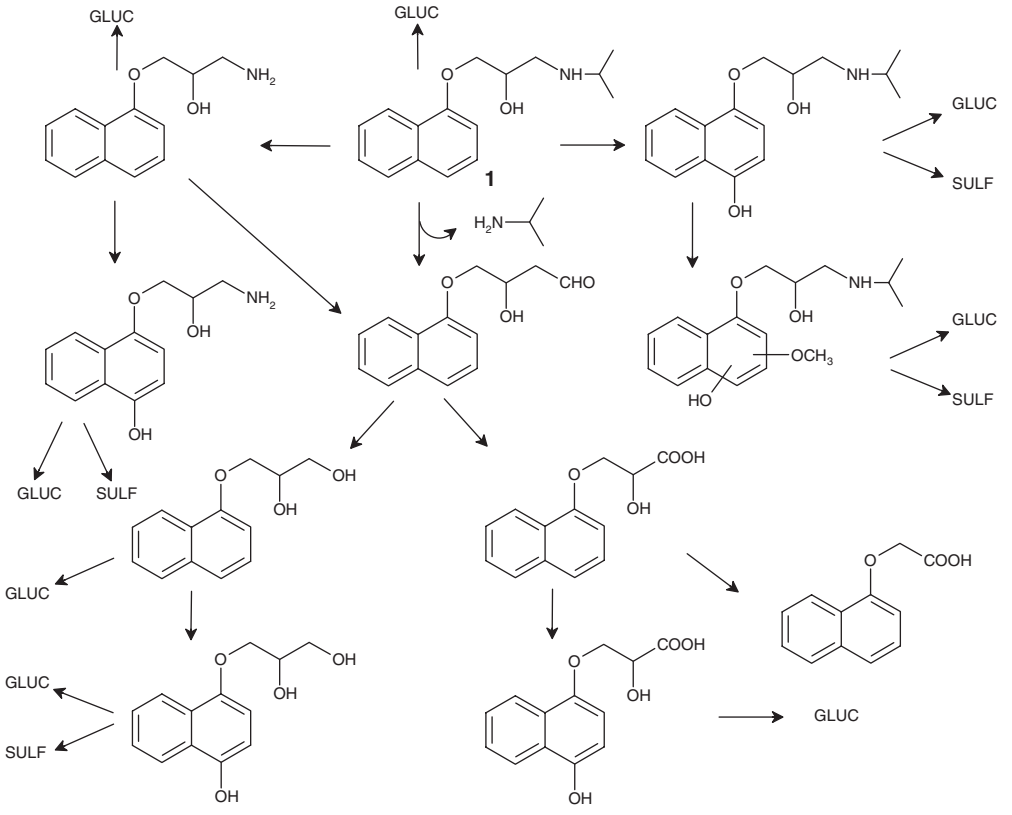

Figure 1. The metabolism of propranolol (1) in humans, accounting for more than 90% of the dose; GLUC = glucuronide(s); SULF = sulfate(s) [31].

when published in 1985. Indeed, over 90% of a dose were accounted for and consisted mainly in products of oxidation and conjugation. The missing 10% may represent other, minor and presumably quite numerous metabolites, for example, those resulting from ring hydroxylation at other positions or from the progressive breakdown of glutathione conjugates.

A large variety of enzymes and metabolic reactions are presented in Sections 2 and 3. As will become clear, some enzymes catalyze only a single type of reaction (e.g., *N*-acetylation), whereas others use a basic catalytic mechanism to attack a variety of moieties and produce different types of metabolites (e.g., cytochromes P450). As an introduction to these enzymes and reactions, we present in Table 2 an estimate of their relative importance in drug metabolism. In this table, the correspondence between the number of substrates and the overall contribution to drug metabolism does not need to be perfect, since some enzymes show a limited capacity (e.g., sulfotransferases) whereas others make a significant contribution to the biotransformation of their substrates (e.g., hydrolases).

2. FUNCTIONALIZATION REACTIONS

2.1. Introduction

Reactions of functionalization comprise oxidations (electron removal, dehydrogenation, and oxygenation), reductions (electron addition, hydrogenation, and removal of oxygen), and hydrations/dehydrations (hydrolysis and addition or removal of water). The reactions of oxidation and reduction are catalyzed by a very large variety of oxidoreductases, while various hydrolases catalyze hydrations. A

Table 2. Estimate of the Relative Contributions of Major Drug-Metabolizing Enzymes[a]

Enzymes	Overall Contribution to Drug Metabolism[b]
Cytochromes P450 (Section 2.2.1)	+ + + +
Dehydrogenases and reductases (Section 2.2.2)	+ + +
Flavin-containing monooxygenases (Section 2.2.2)	+
Hydrolases (Section 2.2.3)	+ +
Methyltransferases (Section 3.2)	+
Sulfotransferases (Section 3.3)	+
Glucuronyltransferases (Section 3.4)	+ + +
N-Acetyltransferases (Section 3.5)	+
Acyl-coenzyme A synthetases (Section 3.6)	+
Glutathione *S*-transferases (Section 3.7)	+ +
Phosphotransferases (Section 3.8)	(+)

[a] (+) very low; + low; + + intermediate; + + + high; + + + + very high.
[b] Including drug metabolites.

large majority of enzymes involved in xenobiotic functionalization are briefly reviewed in Section 2.2 [14]. Metabolic reactions and pathways of functionalization constitute the main body of this section.

2.2. Enzymes Catalyzing Functionalization Reactions

2.2.1. Cytochromes P450 Monooxygenation reactions are of major significance in drug metabolism and are mediated by various enzymes, which differ markedly in their structure and properties. Among these, the most important as far as xenobiotic metabolism is concerned are the cytochromes P450 (EC 1.14.14.1, also 1.14.13), a very large group of enzymes belonging to heme-coupled monooxygenases [4–7,12,15–18,20,22,32–36]. The cytochrome P450 enzymes (CYPs) are encoded by the *CYP* gene superfamily and are classified in families and subfamilies as summarized in Table 3. Cytochrome P450 is the major drug-metabolizing enzyme system, playing a key role in detoxification and toxification, and is of additional significance in medicinal chemistry because several CYP enzymes are drug targets, for example, thromboxane synthase (CYP5) and aromatase (CYP19). The three CYP families mostly involved in xenobiotic metabolism are CYP1 to CYP3, whose relative importance is given in Table 4.

The present section focuses on the metabolic reactions, beginning with the catalytic cycle of cytochrome P450 (Fig. 2). This cycle involves a number of steps that can be simplified as follows:

(a) The enzyme in its ferric (oxidized) form exists in equilibrium between two spin states, a hexacoordinated low-spin form that cannot be reduced, and a pentacoordinated high-spin form. Binding of the substrate to enzyme induces a shift to the reducible high-spin form (reaction a).

(b) A first electron enter the enzyme–substrate complex (reaction b).

(c) The enzyme in its ferrous form has a high affinity for diatomic gases such as CO (a strong inhibitor of cytochrome P450) and dioxygen (reaction c).

(d) Electron transfer from Fe^{2+} to O_2 within the enzyme–substrate–oxygen ternary complex reduces the dioxygen to a bound molecule of superoxide. Its possible liberation in the presence of compounds with good affinity but low reactivity (uncoupling) can be cytotoxic (reaction d).

(e) The normal cycle continues with a second electron entering via either F_{P1} or F_{P2} and reducing the ternary complex (reaction e).

(f) Electron transfer within the ternary complex generates bound peroxide anion (O_2^{2-}).

(g) The bound peroxide anion is split, liberating H_2O (reaction f).

(h) The remaining oxygen atom is an oxene species. This is the reactive form of oxygen that will attack the substrate.

(i) The binary enzyme–product complex dissociates, thereby regenerating the initial state of cytochrome P450 (reaction h).

Oxene is a rather electrophilic species, being neutral but having only six electrons in

Table 3. The Human *CYP* Gene Superfamily: A Table of the Families and Subfamilies of Gene Products [34–37]

Families	Subfamilies (Representative Gene Products)
P450 1 Family (A*ryl hydrocarbon hydroxylases; xenobiotic metabolism; inducible by polycyclic aromatic hydrocarbons*)	
	P450 1A Subfamily (CYP1A1, CYP1A2)
	P450 1B Subfamily (CYP1B1)
P450 2 Family (X*enobiotic metabolism; constitutive and xenobiotic-inducible*)	
	P450 2A Subfamily (CYP2A6, CYP2A7, CYP2A13)
	P450 2B Subfamily (CYP2B6)
	P450 2C Subfamily (CYP2C8, CYP2C9, CYP2C18, CYP2C19)
	P450 2D Subfamily (CYP2D6)
	P450 2E Subfamily (CYP2E1)
	P450 2F Subfamily (CYP2F1)
	P450 2J Subfamily (CYP2J2)
	P450 2R Subfamily (CYP2R1)
	P450 2S Subfamily (CYP2S1)
	P450 2U Subfamily (CYP2U1)
	P450 2W Subfamily (CYP2W1)
P450 3 Family (X*enobiotic and steroid metabolism; steroid-inducible*)	
	P450 3A Subfamily (CYP3A4, CYP3A5, CYP3A7, CYP3A43)
P450 4 Family (*Peroxisome proliferator-inducible*)	
	P450 4A Subfamily (CYP4A11, CYP4A20, CYP4A22)
	P450 4B Subfamily (CYP4B1)
	P450 4F Subfamily (CYP4F2, CYP4F3, CYP4F8, CYP4F11, CYP4F12, CYP4F22)
	P450 4V Subfamily (CYP4V2)
	P450 4X Subfamily (CYP4X1)
P450 5 Family	
	P450 5A Subfamily (CYP5A1)
P450 7 Family (*Steroid 7-hydroxylases*)	
	P450 7A Subfamily (CYP7A1)
	P450 7B Subfamily (CYP7B1)
P450 8 Family	
	P450 8A Subfamily (CYP8A1)
	P450 8B Subfamily (CYP8B1)
P450 11 Family (M*itochondrial steroid hydroxylases*)	
	P450 11A Subfamily (CYP11A1)
	P450 11B Subfamily (CYP11B1, CYP11B2)
P450 17 Family (S*teroid 17β-hydroxylase*)	
	P450 17A Subfamily (CYP17A1)
P450 19 Family (CYP19)	
P450 20 Family (CYP20)	
P450 21 Family (CYP21)	
P450 24 Family (CYP24)	
P450 26 Family	
	P450 26A Subfamily (CYP26A1)
	P450 26B Subfamily (CYP26B1)
	P450 26C Subfamily (CYP26C1)
P450 27 Family (M*itochondrial steroid hydroxylases*)	
	P450 27A Subfamily (CYP27A1)
	P450 27B Subfamily (CYP27B1)
	P450 27C Subfamily (CYP27C1)
P450 39 Family (CYP39)	
P450 46 Family (CYP46)	
P450 51 Family (CYP51)	

This list reports all 57 known human *CYP* gene products.

Table 4. Levels and Variability of Human CYP Enzymes Involved in Drug Metabolism [16]

CYP	Level of Enzyme in Liver (% of Total)	Variability Range	Percent of Drugs (or Other Xenobiotics) Interacting with Enzyme		
			As Substrates	As Inhibitors	As Inducers/Activators
1A1			3 (12)	3 (13)	6 (33)
1A2	ca. 13	ca. 40-fold	10 (15)	12 (18)	3 (25)
1B1	<1		1 (10)	1 (7)	1 (9)
2A6	ca. 4	ca. 30- to 100-fold	3 (10)	2 (6)	2 (1)
2B6	<1	ca. 50-fold	4 (9)	3 (5)	13 (no data)
2C	ca. 18	25- to 100-fold	25 (13)	27 (17)	21 (6)
2D6	up to 2.5	>1000-fold	15 (2)	22 (8)	2 (2 activators)
2E1	up to 7	ca. 20-fold	3 (16)	4 (10)	7 (7)
3A4	up to 28	ca. 20-fold	36 (13)	26 (16)	45 (17)

its outer shell. Its detailed reaction mechanisms are beyond the scope of this chapter, but some indications will be given when discussing the various reactions of oxidation catalyzed by cytochromes P450.

2.2.2. Other Oxidoreductases Besides cytochromes P450, other monooxygenases of importance are the flavin-containing monooxygenases (see Table 5), while dopamine β-monooxygenase (EC 1.14.17.1) plays only a minor role. Other oxidoreductases that can

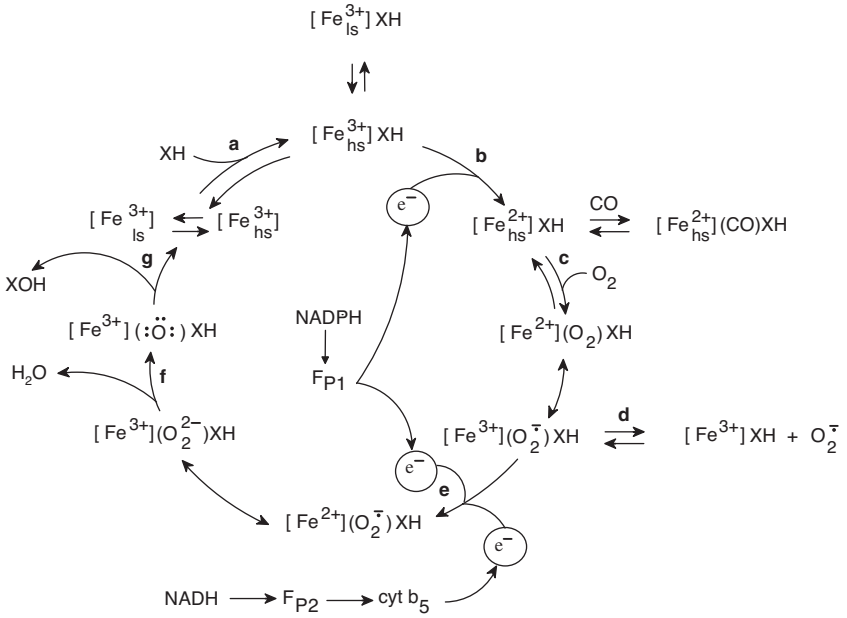

Figure 2. Catalytic cycle of cytochrome P450 associated with monooxygenase reactions. [Fe^{3+}] = ferricytochrome P450; hs = high spin; ls = low spin; [Fe^{2+}] = ferrocytochrome P450; F_{P1} = flavoprotein 1 = NADPH-cytochrome P450 reductase; F_{P2} = NADH-cytochrome b_5 reductase; cyt b5 = cytochrome b_5; XH = substrate (modified from Refs [4,14]).

[1](+) very low; + low; ++ intermediate; +++ high; ++++ very high.

Table 5. A Survey of Oxidoreductases Other than Cytochromes P450 Playing a Role in Drug Metabolism [14,22]

Enzymes	EC Numbers	*Gene Root* (or *Gene*) and Major Human Enzymes
Flavin-containing monooxygenases	EC 1.14.13.8	*FMO* (FMO1 to FMO5)
Monoamine oxidases	EC 1.4.3.4	*MAO* (MAO-A and MAO-B)
Copper-containing amine oxidases	EC 1.4.3.6	*AOC* (DAO and SSAO)
Aldehyde oxidase	EC 1.2.3.1	*AOX1* (AO)
Xanthine oxidoreductase	EC 1.17.1.4 and 1.17.3.2	*XOR* (XDH and XO)
Various peroxidases	EC 1.11.1.7 and 1.11.1.8	For example, *EPO* (EPO), *MPO* (MPO) and *TPO* (TPO)
Prostaglandin G/H synthase	EC 1.14.99.1	*PTGS* (COX-1 and COX-2)
Alcohol dehydrogenases	EC 1.1.1.1	*ADH* (ADH1A, 1B and 1C, ADH4, ADH5, ADH6, and ADH7)
Aldehyde dehydrogenases	EC 1.2.1.3 and 1.2.1.5	*ALDH* (e.g., ALDH1A1, 1A2 and 1A3, 1B1, 2, 3A1, 3A2, 3B1, 3B2, 8A1, and 9A1)
Aldo-keto reductases	In EC 1.1.1 and 1.3.1	*AKR* (e.g., ALR1, ALR2, DD1, DD2, DD3, DD4, AKR7A2, 7A3, and 7A4)
Carbonyl reductases	EC 1.1.1.184	*CBR* (CR1, CR3)
Quinone reductases	EC 1.6.5.2 and 1.10.99.2	*NQO* (NQO1 and NGO2)

play a major or less important role in drug metabolism are the two monoamine oxidases that are essentially mitochondrial enzymes, and the broad group of copper-containing amine oxidases. Cytosolic oxidoreductases are the molybdenum hydroxylases, namely aldehyde oxidase and xanthine oxidase.

Various peroxidases are progressively being recognized as important enzymes in drug metabolism. Several cytochrome P450 enzymes have been shown to have peroxidase activity. A variety of peroxidases may oxidize drugs, for example, myeloperoxidase (MPO). Prostaglandin G/H synthase (prostaglandin-endoperoxide synthase) is able to use a number of xenobiotics as cofactors in a reaction of cooxidation.

The large and important ensemble of dehydrogenases/reductases include alcohol dehydrogenases (ADH) that are zinc enzymes found in the cytosol of the mammalian liver and in various extrahepatic tissues. Mammalian liver alcohol dehydrogenases (LADHs) are dimeric enzymes. The human enzymes belong to three different classes: Class I (ADH1), comprising the various isozymes that are homodimers or heterodimers of the alpha (ADH1A, ADH2A, ADH3A), beta and gamma subunits.

Enzymes categorized as aldehyde dehydrogenases (ALDHs) include the NAD^+- and $NAD(P)^+$-dependent enzymes. They exist in multiple forms in the cytosol, mitochondria, and microsomes of various mammalian tissues. Among the aldo-keto reductases, we find aldehyde reductase, alditol dehydrogenase, a number of hydroxysteroid dehydrogenases, and dihydrodiol dehydrogenase. These enzymes are widely distributed in nature and occur in a considerable number of mammalian tissues. Their subcellular location is primarily cytosolic, and for some also mitochondrial. The carbonyl reductases belong to the short-chain dehydrogenases/reductases and are of noteworthy significance in xenobiotic metabolism. There are many similarities, including some marked overlap in substrate specificity, between monomeric, NADPH-dependent aldehyde reductase (AKR1), alditol dehydrogenase (AKR2), and carbonyl reductase (AKR3).

Other reductases that have a role to play in drug metabolism include the important quinone reductase, also known as DT-diaphorase.

2.2.3. Hydrolases Hydrolases constitute a very complex ensemble of enzymes many of which are known or suspected to be involved in xenobiotic metabolism (Table 6). Relevant en-

Table 6. A Survey of Hydrolases Playing a Role in Drug Metabolism [9,14,23]

Classes of Hydrolases	Examples of Enzymes (With Some *Gene Roots* and Human Enzymes)
EC 3.1.1: carboxylic ester hydrolases	EC 3.1.1.1: Carboxylesterase (*CES*) CES1A1, CES2, CES3
	EC 3.1.1.2: Arylesterase (*PON*, see 3.1.8.1)
	EC 3.1.1.8: Cholinesterase (*BCHE*)
EC 3.1.2: thiolester hydrolases	EC 3.1.2.20: Acyl-CoA hydrolase
EC 3.1.3: phosphoric monoester hydrolases	EC 3.1.3.1: Alkaline phosphatase (*ALP*)
	EC 3.1.3.2: Acid phosphatase (*ACP*)
EC 3.1.6: sulfuric ester hydrolases	EC 3.1.6.1: Arylsulfatase
EC 3.1.8: phosphoric triester hydrolases	EC 3.1.8.1: Paraoxonase (*PON*) PON1, PON2, PON3
	EC 3.1.8.2: Diisopropyl-fluorophosphatase
EC 3.2: glycosylases	EC 3.2.1.31: β-Glucuronidase (*GUSB*)
EC 3.3.2: ether hydrolases	EC 3.3.2.9: Microsomal epoxide hydrolase (*EPHX1*) mEH
	EC 3.3.2.10: Soluble epoxide hydrolase (*EPHX2*) sEH
EC 3.4.11: aminopeptidases	EC 3.4.11.1: Leucyl aminopeptidase (*LAP*)
EC 3.4.13 and 3.4.14: peptidases acting on di- and tripeptides	EC 3.4.14.5: Dipeptidyl-peptidase IV (*DPP4*)
EC 3.4.16 to 3.4.18: carboxypeptidases	EC 3.4.16.2: Lysosomal Pro-Xaa carboxypeptidase
	EC 3.4.17.1: Carboxypeptidase A (*CPA*)
EC 3.4.21 to 3.4.25: endopeptidases	EC 3.4.21.1: Chymotrypsin (*CTRB*)
	EC 3.4.24.15: Thimet oligopeptidase (*THOP*)
EC 3.5.1: hydrolases acting on linear amides	EC 3.5.1.4: Amidase
	EC 3.5.1.39: Alkylamidase
EC 3.5.2: hydrolases acting on cyclic amides	EC 3.5.2.1: Barbiturase
	EC 3.5.2.2: Dihydropyrimidinase (*DPYS*)
	EC 3.5.2.6: β-Lactamase

zymes among the serine hydrolases include carboxylesterases, arylesterases, cholinesterase, and a number of serine endopeptidases (EC 3.4.21). The role of arylsulfatases, paraoxonase, β-glucuronidase, and epoxide hydrolases is worth noting. Some metalloendopeptidases (EC 3.4.24) and amidases (EC 3.5.1 and 3.5.2) are also of interest [9,14,19,23].

2.3. Reactions of Carbon Oxidation and Reduction

When examining reactions of carbon oxidation (oxygenations and dehydrogenations) and carbon reduction (hydrogenations), it is convenient from a mechanistic viewpoint to distinguish between sp^3-, sp^2-, and sp-carbon atoms.

2.3.1. sp^3-Carbon Atoms Reactions of oxidation and reduction of sp^3-carbon atoms (plus some subsequent reactions at carbonyl groups) are schematized in Fig. 3 and discussed sequentially below. In the simplest cases, a nonactivated carbon atom in an alkyl group undergoes cytochrome P450-catalyzed hydroxylation. The penultimate position is a preferred site of attack (reaction 1b), but hydroxylation can also occur in the terminal position (reaction 1a) or in other positions in case of steric hindrance or with some specific cytochromes P450. Dehydrogenation by dehydrogenases can then yield a carbonyl derivative (reactions 1c and 1e) that is either an aldehyde or a ketone. Note that reactions 1c and 1e act not only on metabolites, but also on xenobiotic alcohols, and are reversible (i.e., reactions 1d and 1f) since dehydrogenases catalyze the reactions in both directions. And while a ketone is very seldom oxidized further, aldehydes are good substrates for aldehyde dehydrogenases or other enzymes, and lead irreversibly to carboxylic acid metabolites (reaction 1g). A classical example is that of ethanol, which in the body exists in redox equilibrium with acetaldehyde, this metabolite being rapidly and irreversibly oxidized to acetic acid.

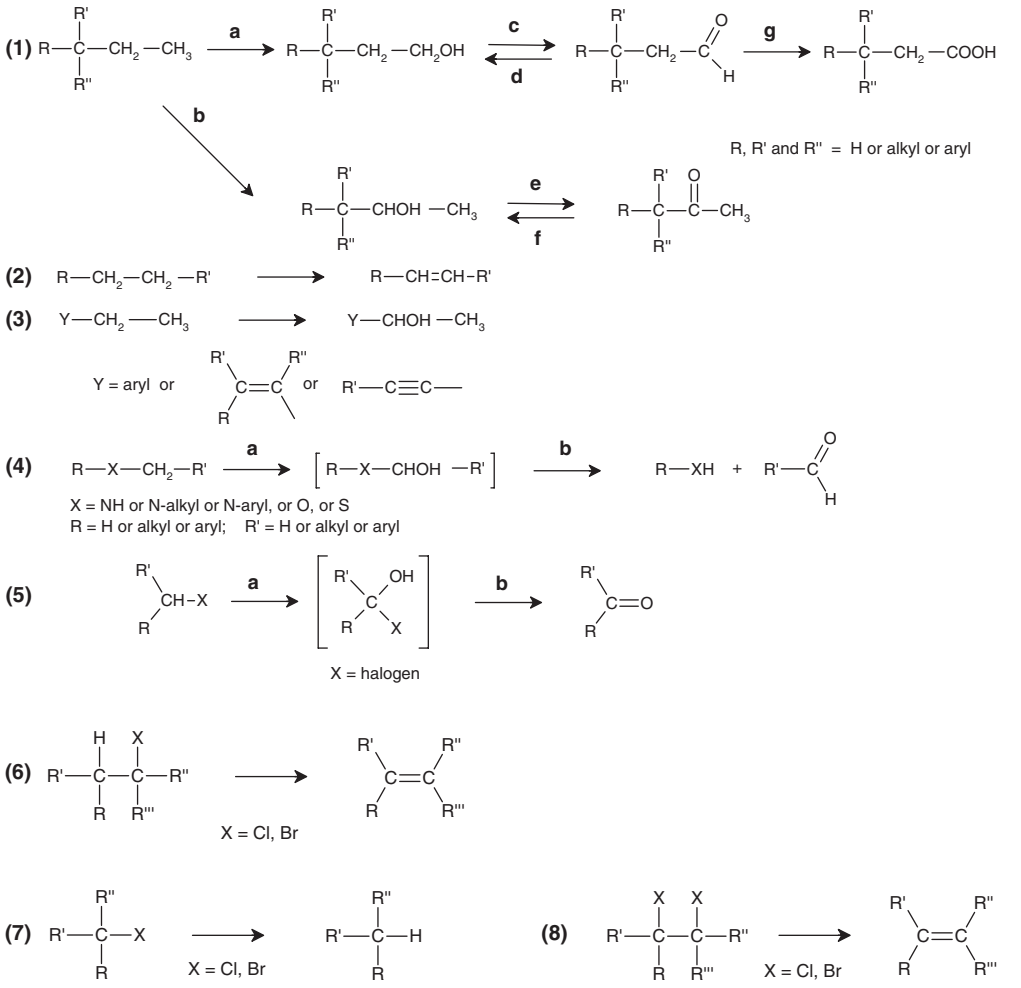

Figure 3. Major functionalization reactions involving an sp^3-carbon in substrate molecules; some reactions at carbonyl groups are also included since they often follows the former. The reactions shown here are mainly oxidations (oxygenations and dehydrogenations) and reductions (hydrogenations), plus some postenzymatic reactions of hydrolytic cleavage.

For a number of substrates, the oxidation of primary and secondary alcohol and of aldehyde groups can also be catalyzed by cytochrome P450. A typical example is the C(10)-demethylation of androgens and analogs catalyzed by aromatase (CYP19).

A special case of carbon oxidation, recognized only recently and probably of underestimated significance, is desaturation of a dimethylene unit by cytochrome P450 to produce an olefinic group (reaction 2). An interesting example is provided by testosterone, which among many cytochrome P450-catalyzed reactions undergoes allylic hydroxylation to 6β-hydroxytestosterone and desaturation to 6,7-dehydrotestosterone [38].

There is a known regioselectivity in cytochrome P450-catalyzed hydroxylations for carbon atoms adjacent (alpha) to an unsaturated system (reaction 3) or a heteroatom such as N, O, or S (reaction 4a). In the former cases, hydroxylation can easily be followed by dehydrogenation (not shown). In the latter cases, however, the hydroxylated metabolite is usually unstable and undergoes a rapid, postenzymatic elimination (reaction 4b). Depending on the substrate, this pathway produces a secondary or primary amine, an alcohol or

phenol, or a thiol, while the alkyl group is cleaved as an aldehyde or a ketone. Reactions 4 constitute a very common and frequent pathway as far as drug metabolism is concerned, since it underlies some well-known metabolic reactions of N–C cleavage discussed later. Note that the actual mechanism of such reactions is usually more complex than shown here and may involve intermediate oxidation of the heteroatom.

Aliphatic carbon atoms bearing one or more halogen atoms (mainly chlorine or bromine) can be similarly metabolized by hydroxylation and loss of HX to dehalogenated products (reactions 5a and 5b) (see later). Dehalogenation reactions can also proceed reductively or without change in the state of oxidation. The latter reactions are dehydrohalogenations (usually dehydrochlorination or dehydrobromination) occurring nonenzymatically (reaction 6). Reductive dehalogenations involve replacement of a halogen by a hydrogen (reaction 7), or *vic*-bisdehalogenation (reaction 8). Some radical species formed as intermediates may have toxicological significance.

Reactions 1a, 1b, 3, 4a, and 5a are catalyzed by cytochromes P450. Here, the iron-bound oxene (Section 2.2.1) acts by a mechanism known as "oxygen rebound" whereby a H atom is exchanged for a OH group. In simplified terms, the oxene atom attacks the substrate by cleaving a C–H bond and removing the hydrogen atom (hydrogen radical). This forms an iron-bound $HO^{\bullet}$ species and leaves the substrate as a C-centered radical. In the last step, the iron-bound $HO^{\bullet}$ species is transferred to the substrate.

Halothane (**2**) offers a telling example of the metabolic fate of halogenated compounds of medicinal interest. Indeed, this agent undergoes two major pathways, oxidative dehalogenation leading to trifluoroacetic acid (**3**) and reduction producing a reactive radical (**4**) (Fig. 4).

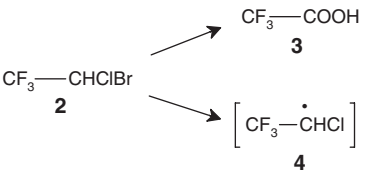

Figure 4. The structure of halothane (**2**) and two of its metabolites, namely trifluoroacetic acid (**3**) produced by oxidation, and a reactive radical (**4**) produced by reduction.

2.3.2. sp^2- and sp-Carbon Atoms Reactions at sp^2-carbons are characterized by their own pathways, catalytic mechanisms, and products (Fig. 5). Thus, the oxidation of aromatic rings generates a variety of (usually stable) metabolites. Their common precursor is often a reactive epoxide (reaction 1a) that can either be hydrolyzed by epoxide hydrolase (reaction 1b) to a dihydrodiol, or rearrange under proton catalysis to a phenol (reaction 1c). The production of a phenol is a very common metabolic reaction for drugs containing one or more aromatic rings. The *para*-position is the preferred position of hydroxylation for unsubstituted phenyl rings, but the regioselectivity of the reaction becomes more complex with substituted phenyl or with other aromatic rings.

Dihydrodiols are seldom observed, as are catechol metabolites produced by their dehydrogenation catalyzed by dihydrodiol dehydrogenase (reaction 1d). It is interesting to note that this reaction restores the aromaticity that had been lost upon epoxide formation. The further oxidation of phenols and phenolic metabolites is also possible, the rate of reaction and the nature of products depending on the ring and on the nature and position of its substituents. Catechols are thus formed by reaction 1e, while hydroquinones are sometimes also produced (reaction 1f).

In some cases, catechols and hydroquinones have been found to undergo further oxidation to quinones (reactions 1g and 1i). Such reactions occur by two single-electron steps and can be either enzymatic or nonenzymatic (i.e., resulting from autoxidation and yielding as byproduct the superoxide anion-radical $O_2^{\bullet}-$). The intermediate is this reaction is a semiquinone. Both quinones and semiquinones are reactive, in particular toward biomolecules, and have been implicated in many toxification reactions. For example, the high toxicity of benzene for bone marrow is believed to be due to the oxidation of catechol and hydroquinone catalyzed by myeloper-oxidase.

The oxidation of diphenols to quinones is reversible (reactions 1h and 1j), a variety of

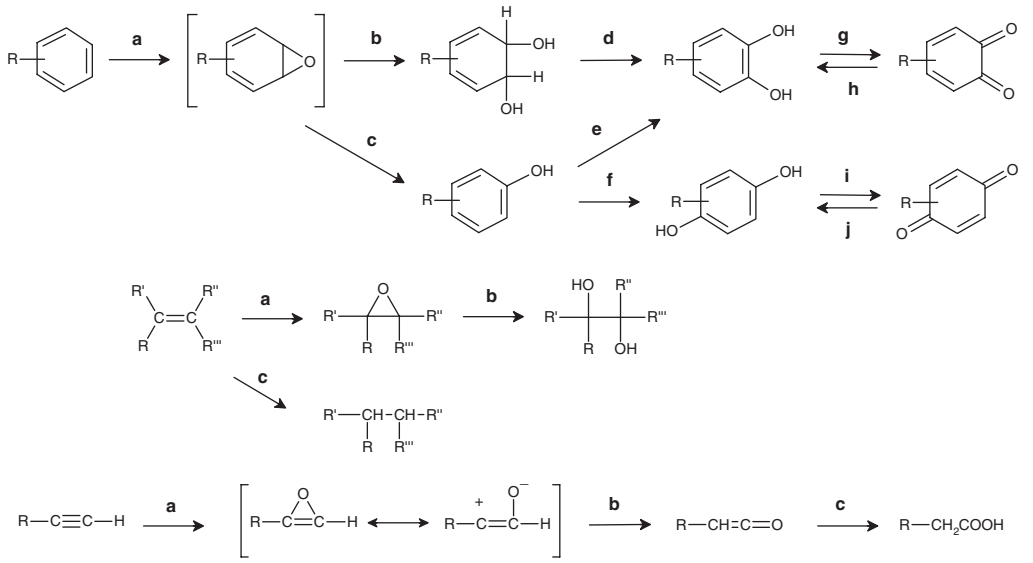

Figure 5. Major functionalization reactions involving an sp^2- or sp-carbon in substrate molecules. These reactions are oxidations (oxygenations and dehydrogenations), reductions (hydrogenations), and hydrations, plus some postenzymatic rearrangements.

cellular reductants being able to mediate the reduction of quinones either by a two-electron mechanism or by two single-electron steps. The two-electron reduction can be catalyzed by carbonyl reductase and quinone reductase, while cytochrome P450 and some flavoproteins act by single-electron transfers. The nonenzymatic reduction of quinones can occur for example in the presence of $O_2^{\bullet}-$ or some thiols such as glutathione.

Olefinic bonds in xenobiotic molecules can also be targets of cytochrome P450-catalyzed epoxidation (reaction 2a). In contrast to arene oxides, the resulting epoxides are fairly stable and can be isolated and characterized. But like arene oxides, they are substrates of epoxide hydrolase to yield dihydrodiols (reaction 2b).

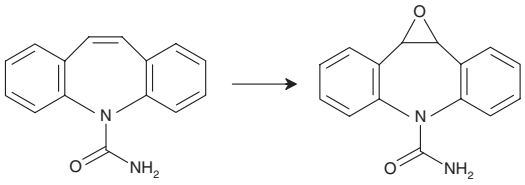

Figure 6. The structure of carbamazepine (**5**) and its 10,11-epoxide metabolite (**6**).

This is exemplified by carbamazepine (**5**), whose 10,11-epoxide (**6**) is a major and pharmacologically active metabolite in humans, and is further metabolized to the inactive dihydrodiol [39] (Fig. 6).

The reduction of olefinic groups (reaction 2c) is documented for a few drugs bearing an α,β-ketoalkene function. The reaction is thought to be catalyzed by various NAD(P)H oxidoreductases.

The few drugs that contain an acetylenic moiety are also targets for cytochrome P450-catalyzed oxidation. Oxygenation of the triple bond (reaction 3a) yields an intermediate that depending on the substrate can react in a number of ways, for example binding covalently to the enzyme, or forming a highly reactive ketene whose hydration produces a substituted acetic acid (reactions 3b and 3c).

2.4. Reactions of Nitrogen Oxidation and Reduction

The main metabolic reactions of oxidation and reduction of nitrogen atoms in organic molecules are summarized in Fig. 7. The functional groups involved are amines and amides and their oxygenated metabolites, as well as 1,4-

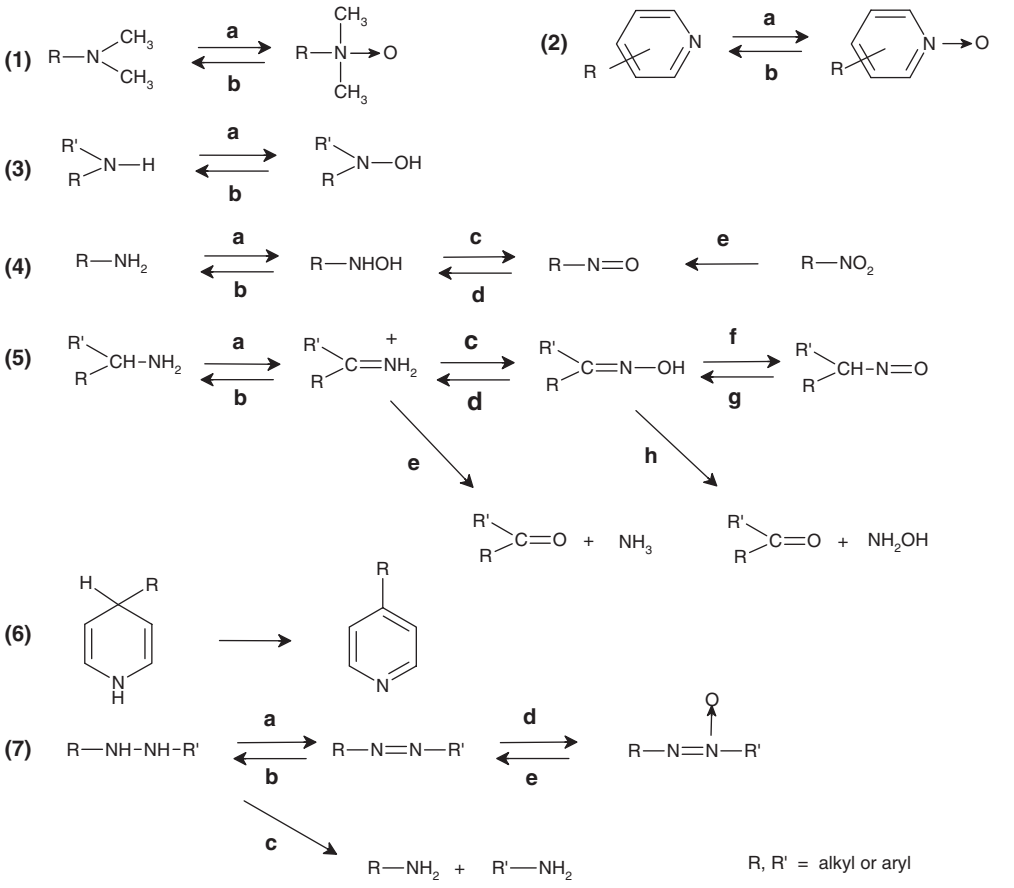

Figure 7. Major functionalization reactions involving nitrogen atoms in substrate molecules. The reactions shown here are mainly oxidations (oxygenations and dehydrogenations) and reductions (deoxygenations and hydrogenations).

dihydropyridines, hydrazines, and azo compounds. In many cases, the reactions can be catalyzed by cytochrome P450 and/or flavin-containing monooxygenases. The first oxygenation step in reactions 1–4 and 6 have frequently been observed.

Nitrogen oxygenation is an (apparently) straightforward metabolic reaction of tertiary amines (reaction 1a), be they aliphatic or aromatic. Numerous drugs undergo this reaction, the resulting *N*-oxide metabolite being more polar and hydrophilic than the parent compound. Identical considerations apply to pyridines and analogous aromatic azaheterocycles (reaction 2a). Note that these reactions are reversible, a number of reductases being able to deoxygenate *N*-oxides back to the amine (i.e., reactions 1b and 2b).

Secondary and primary amines also undergo *N*-oxygenation, the first isolable metabolites being hydroxylamines (reactions 3a and 4a, respectively). Again, reversibility is documented (reactions 3b and 4b). These compounds can be aliphatic or aromatic amines, and the same metabolic pathway occurs in secondary and primary amides (i.e., R = acyl), while tertiary amides appear resistant to *N*-oxygenation. The oxidation of secondary amines and amides usually stops at the hydroxylamine/hydroxylamide level, but formation of short-lived nitroxides (not shown) has been reported.

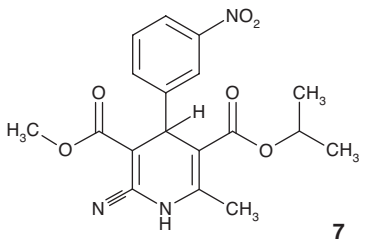

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Figure 8. The structure of nivaldipine (**7**).

As opposed to secondary amines and amides, their primary analogs can be oxidized to nitroso metabolites (reaction 4c), but further oxidation of the latter compounds to nitro compounds does not seem to occur *in vivo*. In contrast, aromatic nitro compounds can be reduced to primary amines via reactions 4e, 4d, and finally 4b. This is the case for numerous chemotherapeutic drugs such as metronidazole.

Note that primary aliphatic amines having a hydrogen on the alpha-carbon can display additional metabolic reactions shown as reactions 5 in Fig. 7. Indeed, *N*-oxidation may also yield imines (reaction 5a), whose degree of oxidation is equivalent to that of hydroxylamines [40]. Imines can be further oxidized to oximes (reaction 5c), which are in equilibrium with their nitroso tautomer (reactions 5f and 5g).

1,4-Dihydropyridines, and particularly calcium channel blockers such as nivaldipine (**7** in Fig. 8), are effectively oxidized by cytochrome P450. The reaction is one of aromatization (reaction 6 in Fig. 7), yielding the corresponding pyridine.

Dinitrogen moieties are also targets of oxidoreductases. Depending on their substituents, hydrazines are oxidized to azo compounds (reaction 7a), some of which can be oxygenated to azoxy compounds (reaction 7d). Another important pathway of hydrazines is their reductive cleavage to primary amines (reaction 7c). Reactions 7a and 7d are reversible, the corresponding reductions (reactions 7b and 7e) being mediated by cytochrome P450 and other reductases. A toxicologically significant pathway thus exists for the reduction of some aromatic azo compounds to potentially toxic primary aromatic amines (reactions 7b and 7c).

2.5. Reactions of Oxidation and Reduction of Sulfur and Other Atoms

A limited number of drugs contain a sulfur atom, usually as a thioether. The major redox reactions occurring at sulfur atoms in organic compounds are summarized in Fig. 9.

Thiol compounds can be oxidized to sulfenic acids (reaction 1a), then to sulfinic acids (reaction 1e), and finally to sulfonic acids (re-

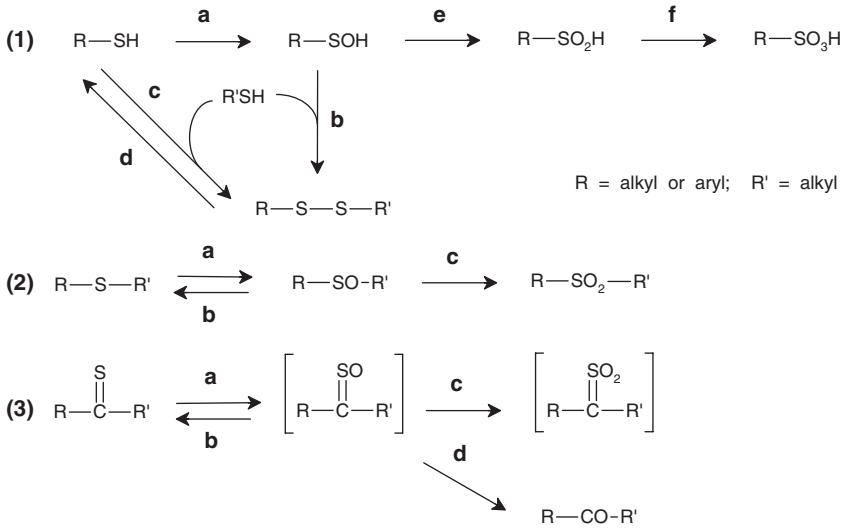


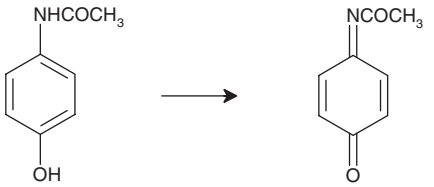
Figure 9. Major reactions of oxidation and reduction involving sulfur atoms in organic compounds.

action 1f). Depending on the substrate, the pathway is mediated by cytochrome P450 and/or flavin-containing monooxygenases. Another route of oxidation of thiols is to disulfides either directly (reaction 1c via thiyl radicals), or by dehydration between a thiol and a sulfenic acid (reaction 1b). However, our understanding of sulfur biochemistry is incomplete, and much remains to be learned. This is particularly true for reductive reactions. While reaction 1c is well-known to be reversible (i.e., reaction 1d), the reversibility of reaction 1a is unclear, while reduction of sulfinic and sulfonic acids appears unlikely.

The metabolism of sulfides (thioethers) is rather straightforward. Besides *S*-dealkylation reactions discussed earlier, these compounds can also be oxygenated by monooxygenases to sulfoxides (reaction 2a) and then to sulfones (reaction 2c). Here, it is known with confidence that reaction 2a is indeed reversible, as documented by many examples of reduction of sulfoxides (reaction 2b, while the reduction of sulfones has never been found to occur.

Thiocarbonyl compounds are also substrates of monooxygenases, forming *S*-monoxides (sulfines, reaction 3a) and then *S*-dioxides (sulfenes, reaction 3c). As a rule, these metabolites cannot be identified as such due to their reactivity. Thus, *S*-monoxides rearrange to the corresponding carbonyl by expelling a sulfur atom (reaction 3d). This reaction is known as oxidative desulfuration and occurs in thioamides and thioureas (e.g., thiopental). As for the *S*-dioxides, they react very rapidly with nucleophiles, and particularly with nucleophilic sites in biological macromolecules. This covalent binding results in the formation of adducts of toxicological significance. Such a mechanism is believed to account for the carcinogenicity of a number of thioamides.

Some other elements besides carbon, nitrogen, and sulfur can undergo metabolic redox reactions. The direct oxidation of oxygen atoms in phenols and alcohols is well documented for some substrates. Thus, the oxidation of secondary alcohols by some peroxidases can yield a hydroperoxide and ultimately a ketone. Some phenols are known to be oxidized by cytochrome P450 to a semiquinone and ultimately to a quinone. A classical example is that of the antiinflammatory drug



8 **9**

Figure 10. The structure of paracetamol (**8**) and its toxic quinoneimine metabolite (**9**).

paracetamol (**8** in Fig. 10, acetaminophen), a minor fraction of which is oxidized by CYP2E1 to the highly reactive and toxic quinoneimine **9**.

Additional elements of limited significance in medicinal chemistry able to enter redox reactions are silicon, phosphorus, arsenic, and selenium, among others (Fig. 11). Note however that the enzymology and mechanisms of these reactions are insufficiently understood. For example, a few silanes have been shown to yield silanols *in vivo* (reaction 1). The same applies to some phosphines that can be oxygenated to phosphine oxides by monooxygenases (reaction 2).

Arsenicals have received some attention due to their therapeutic significance. Both inorganic and organic arsenic compounds display an As(III)–As(V) redox equilibrium in the body. This is illustrated with the arsine-arsine oxide and arsenoxide–arsonic acid equilibria (reactions 3a and 3b and reactions 4b and 4c, respectively). Another reaction of interest is the oxidation of arseno compounds to arsenoxides (reaction 4a), a reaction of importance in the bioactivation of a number of chemotherapeutic arsenicals.

The biochemistry of organoselenium compounds is of some interest. For example, a few selenols have been seen to be oxidized to selenenic acids (reaction 5a) and then to selenic acids (reaction 5b).

2.6. Reactions of Oxidative Cleavage

A number of oxidative reactions presented in the previous sections yield metabolic intermediates that readily undergo postenzymatic cleavage of a C–X bond (X being an heteroatom). As briefly mentioned, reactions 4a and

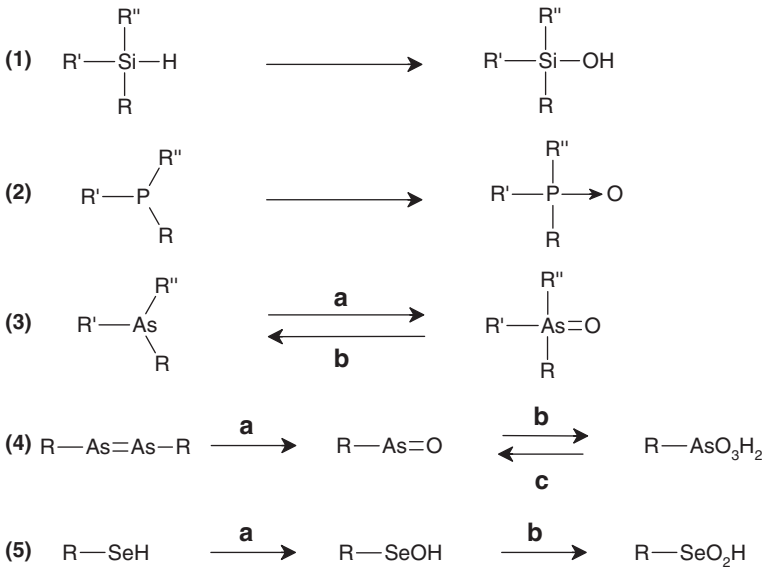


Figure 11. Some selected reactions of oxidation and reduction involving silicon, phosphorus, arsenic and selenium in xenobiotic compounds.

4b in Fig. 3 represent important metabolic pathways that affect many drugs. When X = N (by far the most frequent case), the metabolic reactions are known as *N*-demethylations, *N*-dealkylations, or deaminations, depending on the moiety being cleaved. This is aptly exemplified by the metabolic fate of fenfluramine (**10** in Fig. 12). This withdrawn drug undergoes *N*-deethylation to norfenfluramine (**11**), an active metabolite, and deamination to (*m*-trifluoromethyl)phenylacetone (**12**), an inactive metabolite that is further oxidized to *m*-trifluoromethylbenzoic acid (**13**).

When X = O or S in reactions 4 (Fig. 3), the metabolic reactions are known as *O*-dealkylations or *S*-dealkylations, respectively. *O*-Demethylations are a typical case of the former reaction. And when X = halogen in reactions

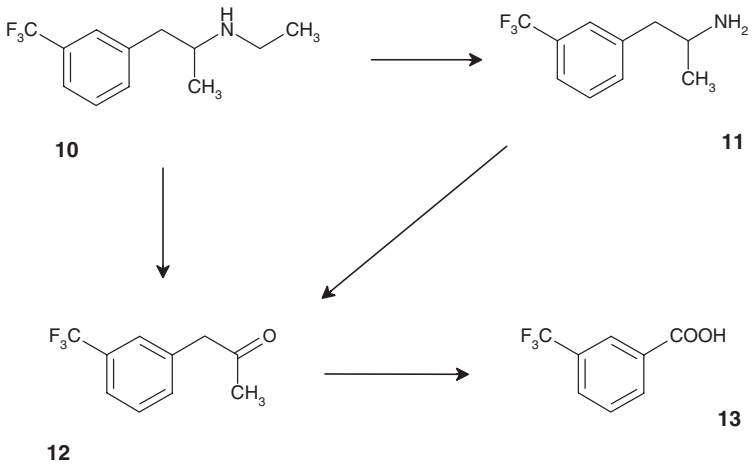


Figure 12. Fenfluramine (**10**), norfenfluramine (**11**), (*m*-trifluoromethyl)phenylacetone (**12**), and *m*-trifluoromethylbenzoic acid (**13**).

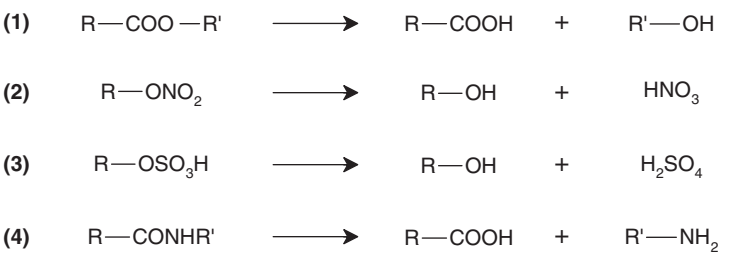


Figure 13. Major hydrolysis reactions involving esters (organic and inorganic) and amides.

5a and 5b (Fig. 3), loss of halogen can also occur and is known as oxidative dehalogenation.

The reactions of oxidative C–X cleavage discussed above result from carbon hydroxylation and are catalyzed by cytochrome P450. However, *N*-oxidation reactions followed by hydrolytic C–N cleavage can also be catalyzed by cytochrome P450 (e.g., reactions 5e and 5h in Fig. 7). The sequence of reactions 5a and 5e in Fig. 7 is of particular interest since it is the mechanism by which monoamine oxidase deaminates endogenous and exogenous amines.

2.7. Reactions of Hydration and Hydrolysis

Hydrolases catalyze the addition of a molecule of water to a variety of functional moieties [9,14,23]. Thus, epoxide hydrolase hydrates epoxides to yield *trans*-dihydrodiols (reaction 1b in Fig. 5). This reaction is documented for many arene oxides, in particular metabolites of aromatic compounds, and epoxides of olefins. Here, a molecule of water has been added to the substrate without loss of a molecular fragment, hence the use of the term "hydration" sometimes found in the literature.

Reactions of hydrolytic cleavage (hydrolysis) are shown in Fig. 13. They are frequent for organic esters (reaction 1), inorganic esters such as nitrates (reaction 2) and sulfates (reaction 3), and amides (reaction 4). These reactions are catalyzed by esterases, peptidases, or other enzymes, but nonenzymatic hydrolysis is also known to occur for sufficiently labile compounds under biological conditions of pH and temperature. Acetylsalicylic acid, glycerol trinitrate, and lidocaine are three representative examples of drugs undergoing extensive cleavage of the organic ester, inorganic ester, or amide group, respectively. The reaction is of particular significance in the activation of ester prodrugs.

3. CONJUGATION REACTIONS

3.1. Introduction

As defined in Section 1, conjugation reactions (also infelicitously known as phase II reactions) result in the covalent binding of an endogenous molecule or moiety to the substrate. Such reactions are of critical significance in the metabolism of endogenous compounds, witness the impressive battery of enzymes that have evolved to catalyze them. Conjugation is also of great importance in the biotransformation of xenobiotics, involving parents compounds or metabolites thereof [15,19,24].

Conjugation reactions are characterized by a number of criteria:

1. The substrate is coupled to an endogenous molecule sometimes designated as the endocon . . .
2. . . . which is usually polar . . .
3. . . . of medium molecular weight (ca. 100–300 Da) . . .
4. . . . and carried by a cofactor.
5. The reaction is catalyzed by an enzyme known as a transferase (Table 7).

First and above all, an endogenous molecule (called the endogenous conjugating moiety, and sometimes abbreviated as the "endocon") is coupled to the substrate. This

Table 7. A Survey of Transferases (EC 2) [15,19,24,32]

Methyltransferases (EC 2.1.1)	
S-Adenosyl-L-methionine (SAM)	EC 2.1.1.6: catechol *O*-methyltransferase (*COMT*)
	EC 2.1.1.1: nicotinamide *N*-methyltransferase (*NNMT*)
	EC 2.1.1.8: histamine *N*-methyltransferase (*HNMT*)
	EC 2.1.1.28: noradrenaline *N*-methyltransferase (*PNMT*)
	EC 2.1.1.49: arylamine *N*-methyltransferase, indolethylamine *N*-methyltransferase (*INMT*)
	EC 2.1.1.9: Thiol *S*-methyltransferase (TMT)
	EC 2.1.1.67: Thiopurine *S*-methyltransferase (*TPMT*)
Sulfotransferases (EC 2.8.2) (*SULT*)	
3′-Phosphoadenosine 5′-phosphosulfate (PAPS)	EC 2.8.2.1: aryl sulfotransferase (SULT1A1, 1A2, and 1A3)
	Thyroid hormone sulfotransferase (SULT1B1)
	SULT1C1, and SULT1C2
	EC 2.8.2.4: estrogen sulfotransferase (SULT1E1)
	EC 2.8.2.14: alcohol/hydroxysteroid sulfotransferase (SULT2A1)
	EC 2.8.2.2: hydroxysteroid sulfotransferase (SULT2B1a and 2B1b)
	EC 2.8.2.15: steroid sulfotransferase
	EC 2.8.2.18: cortisol sulfotransferase
	EC 2.8.2.3: amine sulfotransferase (SULT3A1)
UDP-Glucuronosyltransferases (2.4.1.17) (*UGT*)	
Uridine-5′-diphospho-α-D-glucuronic acid (UDPGA)	Subfamily UGT1: UGT1A1, 1A3, 1A4 to 1A10
	Subfamily UGT2A: UGT2A1 to 2A3
	Subfamily UGT2B: UGT2B4, 2B7, 2B10, 2B11, 2B15, 2B17, 2B28
	Subfamily UGT3A: UGT3A1, 3A2
	Subfamily UGT8A: UGT8
Acetyltransferases	
Acetylcoenzyme A (AcCoA)	EC 2.3.1.5: *N*-acetyltransferase (*NAT*) NAT1 and NAT2
	EC 2.3.1.56: aromatic-hydroxylamine *O*-acetyltransferase
	EC 2.3.1.118: *N*-hydroxyarylamine *O*-acetyltransferase
Acyl-CoA synthetases	
Coenzyme A (CoA)	EC 6.2.1.1: short-chain fatty acyl-CoA synthetase (*ACSS*)
	EC 6.2.1.2: medium-chain acyl-CoA synthetase
	EC 6.2.1.3: long-chain acyl-CoA synthetase (*ACSL*)
	EC 6.2.1.7: cholate-CoA ligase
	EC 6.2.1.25: benzoyl-CoA synthetase
Acyltransferases	
Xenobiotic acyl-Coenzyme A	EC 2.3.1.13: glycine *N*-acyltransferase (*GLYAT*)
	EC 2.3.1.71: glycine *N*-benzoyltransferase
	EC 2.3.1.14: glutamine *N*-phenylacetyltransferase
	EC 2.3.1.68: glutamine *N*-acyltransferase
	EC 2.3.1.65: cholyl-CoA glycine-taurine *N*-acyltransferase (*BAAT*)
	EC 2.3.1.20: diacylglycerol *O*-acyltransferase
	EC 2.3.1.22: 2-acylglycerol *O*-acyltransferase
	EC 2.3.1.26: sterol *O*-acyltransferase (*ACAT*)
Glutathione *S*-transferases (EC 2.5.1.18) (*GST*)	
(Glutathione)	Microsomal GST superfamily (homotrimers): *MGST*: MGST1 to MGST3
	Cytoplasmic GST superfamily (homodimers, and a few heterodimers):
	GSTA: Alpha class, GST A1-1, A1-2, A2-2, A3-3, A4-4, A5-5
	GSTK: Kappa class, GST K1-1
	GSTM: Mu class, GST M1a-1a, M1a-1b, M1b-1b, M2-2, M3-3, M4-4, M5-5
	GSTO: Omega class, GST O1-1, O2
	GSTP: Pi class: GST P1-1
	GSTS: Sigma class: GST S1
	GSTT: Theta class, GST T1-1, T2
	GSTZ: Zeta class, GST Z1-1

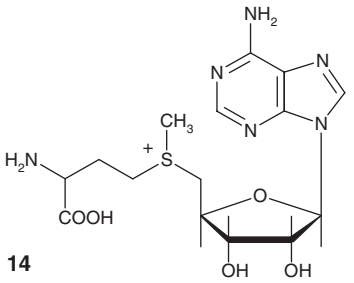


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Figure 14. *S*-Adenosyl-L-methionine (**14**).

is the absolute criterion of conjugation reactions. Second, this endogenous molecule or moiety is generally polar (hydrophilic) or even highly polar, but there are exceptions. Third, the size of the endocon is generally in the range 100–300 Da. Fourth, the endogenous conjugating moiety is usually carried by a cofactor, with the chemical bond linking the cofactor and the endocon being a high energy one such that the Gibbs energy released upon its cleavage helps drive the transfer of the endocon to the substrate. Fifth, conjugation reactions are catalyzed by enzymes known as transferases (EC 2) that bind the substrate and the cofactor in such a manner that their close proximity allows the reaction to proceed. The metaphor of transferases being a "nuptial bed" has not escaped some biochemists. It is important from a biochemical and practical viewpoint to note that criteria 2 to 5 considered separately are neither sufficient nor necessary to define conjugations reactions. They are not sufficient, since in hydrogenation reactions (i.e., typical reactions of oxidoreduction) the hydride is also transferred from a cofactor (NADPH or NADH). And they are not necessary, since they all suffer from some important exceptions.

3.2. Methylation

3.2.1. Introduction Reactions of methylation imply the transfer of a methyl group from the cofactor *S*-adenosyl-L-methionine (**14**, SAM). As shown in Fig. 14, the methyl group in SAM is bound to a sulfonium center, giving it a marked electrophilic character and explaining its reactivity. Furthermore, it becomes pharmacokinetically relevant to distinguish methylated metabolites in which the positive charge has been retained or lost as a proton.

A number of methyltransferases are able to methylate small molecules (see Table 7) [15,19,24,41]. Thus, reactions of methylation fulfill only two of the three criteria defined above, since the methyl group is small compared to the substrate. The main enzyme responsible for *O*-methylation is catechol *O*-methyltransferase, which is mainly cytosolic but also exists in membrane-bound form. Several enzymes catalyze reactions of xenobiotic *N*-methylation with different substrate specificities, for example, nicotinamide *N*-methyltransferase, histamine methyltransferase, phenylethanolamine *N*-methyltransferase (noradrenaline *N*-methyltransferase), and the nonspecific arylamine *N*-methyltransferase. Reactions of xenobiotic *S*-methylation are mediated by the membrane-bound thiol methyltransferase and the cytosolic thiopurine methyltransferase.

The above classification of enzymes makes explicit the three types of functionalities undergoing biomethylation, namely hydroxyl (phenolic), amino and thiol groups.

3.2.2. Methylation Reactions Figure 15 summarizes the main methylation reactions seen in drug metabolism [15,19,24,41]. *O*-Methylation is a common reaction of compounds containing a catechol moiety (reaction 1), with a usual regioselectivity for the *meta* position. The substrates can be xenobiotics and particularly drugs, L-DOPA being a classic example. More frequently, however, *O*-methylation occurs as a late event in the metabolism of aryl groups, after they have been oxidized to catechols (reactions 1, Fig. 5). This sequence was seen for example in the metabolism of the antiinflammatory drug diclofenac (**15** in Fig. 16), which in humans yielded 3′-hydroxy-4′-methoxy-diclofenac as a major metabolite with a very long plasma half-life.

Three basic types of *N*-methylation reactions have been recognized (reactions 2–4, Fig. 15). A number of primary and secondary amines (e.g., some phenylethanolamines and tetrahydroisoquinolines) have been shown to be substrates of *N*-methyltransferase (reaction 2). However, such reactions are seldom of significance *in vivo*, presumably due to

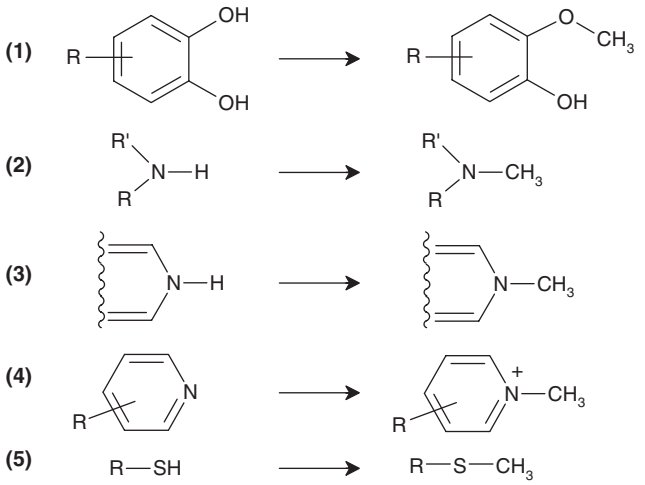


Figure 15. Major methylation reactions involving catechols, various amines, and thiols.

effective oxidative *N*-demethylation. A comparable situation involves the N–H group in an imidazole ring (reaction 3), as exemplified by histamine. A therapeutically relevant example is that of theophylline (**16**) whose *N*(9)-methylation is masked by *N*-demethylation in adult but not newborn humans.

N-Methylation of pyridine-type nitrogen atoms (reaction 4, Fig. 15) appears to be of greater *in vivo* pharmacological significance than reactions 2 and 3, and this for two reasons. First, the resulting metabolites, being quaternary amines, are more stable than tertiary or secondary amines toward *N*-demethylation. And second, these metabolites are also more polar than the parent compounds, in contrast to the products of reactions 2 and 3. Good substrates are nicotinamide (**17**), pyridine and a number of monocyclic and bicyclic derivatives.

S-Methylation of thiol groups (reaction 5) is documented for such drugs as captopril (**18**) and 6-mercaptopurine. Other substrates are metabolites (mainly thiophenols) resulting from the S–C cleavage of (aromatic) glutathione and cysteine conjugates (see below). Once formed, such methylthio metabolites can be further processed to sulfoxides and sulfones

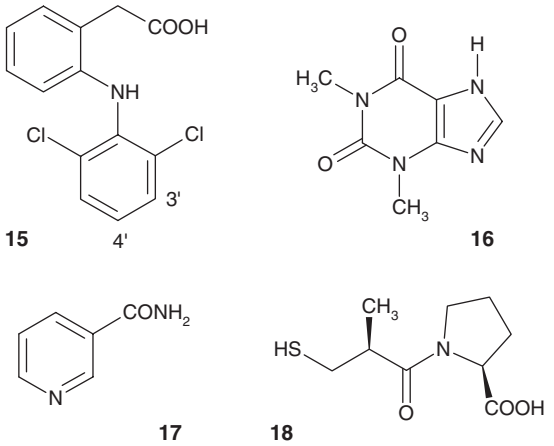


Figure 16. Diclofenac (**15**), theophylline (**16**), nicotinamide (**17**), and captopril (**18**).

before excretion (i.e., reaction 2a and 2c in Fig. 9).

From Fig. 15, it is apparent that methylation reactions can be subdivided into two classes:

(a) Those where the substrate and the product have the same electrical state, a proton in the substrate having been exchanged for a positively charged methyl group (reactions 1–3 and 5);

(b) Those where the product has acquired a positive charge, namely has become a pyridine-type quaternary ammonium (reaction 4).

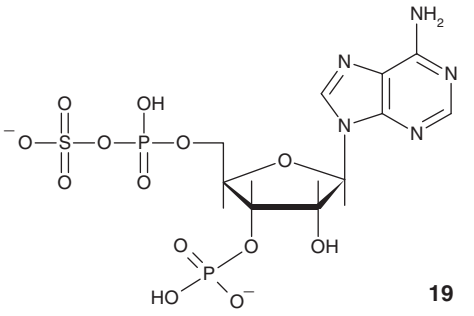


Figure 17. 3′-Phosphoadenosine 5′-phosphosulfate (**19**, PAPS).

3.3. Sulfonation

3.3.1. Introduction Sulfonation reactions consist in an SO_3 moiety being transferred from the cofactor 3′-phosphoadenosine 5′-phosphosulfate (**19**, PAPS) (Fig. 17) to the substrate under catalysis by a sulfotransferase [15,19,24,42,43]. The three criteria of conjugation are met in these reactions. Sulfotransferases, which catalyze a variety of physiological reactions, are soluble enzymes (see Table 7). The phenol sulfotransferases include aryl sulfotransferases, thyroid hormone sulfotransferase, SULT1C1 and SULT1C2, and estrogen sulfotransferase. The alcohol sulfotransferases include alcohol/hydroxysteroid sulfotransferase, hydroxysteroid sulfotransferase, steroid sulfotransferase, and cortisol sulfotransferase. There is also an amine sulfotransferase.

The sulfate moiety in PAPS is linked to a phosphate group by an anhydride bridge whose cleavage is exothermic and supplies enthalpy to the reaction. The electrophilic −OH or −NH− site in the substrate will react with the leaving SO3 moiety, forming an ester sulfate or a sulfamate (Fig. 18). Some of these conjugates are unstable under biological conditions and will form electrophilic intermediates of considerable toxicological significance.

3.3.2. Sulfonation Reactions The sulfoconjugation of alcohols (reaction 1 in Fig. 18) leads

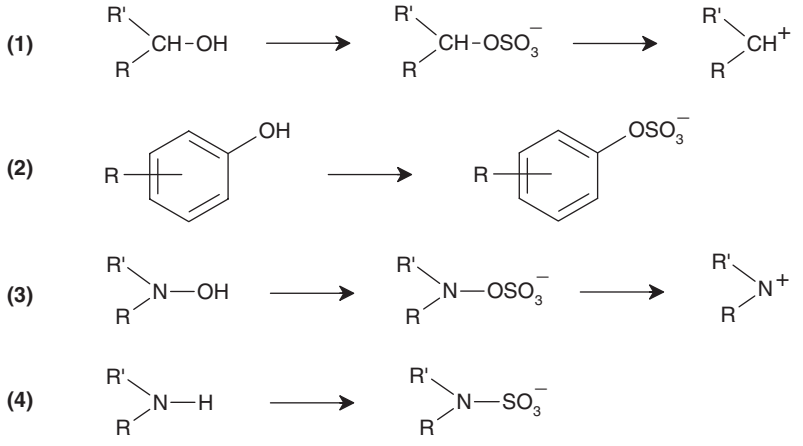


Figure 18. Major sulfonation reactions involving primary and secondary alcohols, phenols, hydroxylamines and hydroxylamides, and amines.

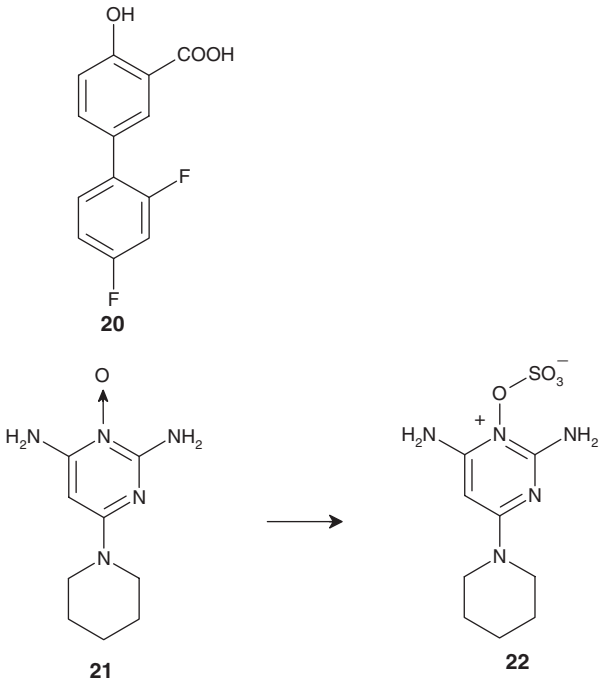


Figure 19. Diflunisal (**20**), minoxidil (**21**), and its *N*,*O*-sulfate ester (**22**).

to metabolites of different stabilities. Endogenous hydroxysteroids (i.e., cyclic secondary alcohols) form relatively stable sulfates, while some secondary alcohol metabolites of allylbenzenes (e.g., safrole and estragole) may form genotoxic carbocations. Primary alcohols, for example, methanol and ethanol, can also form sulfates whose alkylating capacity is well known [54]. Similarly, polycyclic hydroxymethylarenes yield reactive sulfates believed to account for their carcinogenicity.

In contrast to alcohols, phenols form chemically stable sulfate esters (reaction 2). The reaction is usually of high affinity (i.e., rapid), but the limited availability of PAPS restricts the amounts of conjugate being produced. Typical drugs undergoing limited sulfonation include paracetamol (**8** in Fig. 10) and diflunisal (**20** in Fig. 19).

Aromatic hydroxylamines and hydroxylamides are good substrates for some sulfotransferases and yield unstable sulfate esters (reaction 3 in Fig. 18). Indeed, heterolytic N–O cleavage produces a highly electrophilic nitrenium ion. This is a mechanism believed to account for part or all of the cytotoxicity of arylamines and arylamides (e.g., phenacetin). In contrast, significantly more stable products are obtained upon *N*-sulfoconjugation of amines (reaction 4). A few primary, secondary, and alicyclic amines are known to yield sulfamates. The significance of these reactions in humans is still poorly understood.

An intriguing and very seldom reaction of conjugation occurs for minoxidil (**21** in Fig. 19), an hypotensive agent also producing hair growth. This drug is an *N*-oxide, and the actual active form responsible for the different therapeutic effects is the *N*,*O*-sulfate ester (**22**).

3.4. Glucuronidation and Glucosidation

3.4.1. Introduction Glucuronidation is a major and very frequent reaction of conjugation. It involves the transfer to the substrate of a molecule of glucuronic acid from the cofactor uridine-5′-diphospho-α-D-glucuronic acid (**23**, UDPGA) (Fig. 20). The enzymes catalyzing this reaction are known as UDP-glucuronosyltransferases and consist in a number of proteins coded by genes of the *UGT* superfamily (see Table 7). The human UDPGT known to

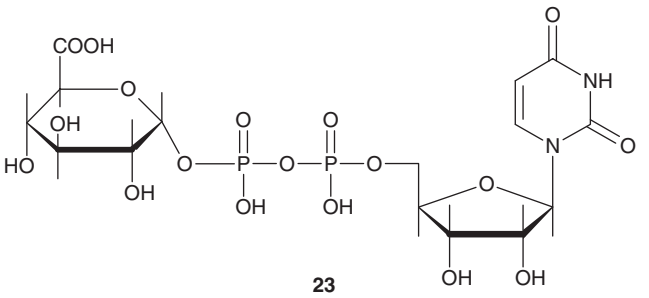


Figure 20. Uridine-5′-diphospho-α-D-glucuronic acid (**23**, UDPGA).

metabolize xenobiotics are the products of main two gene families, *UGT1* and *UGT2*. These enzymes include UGT1A1 (bilirubin UDPGTs) and several UGT1A, as well as numerous phenobarbital-inducible or constitutively expressed UGT2B [15,19,24,44,45].

In addition to glucuronidation, this section briefly mentions glucosidation, a minor metabolic pathway seen for a few drugs. These reactions are also catalyzed by UGT-glucuronosyltransferases.

3.4.2. Glucuronidation Reactions Glucuronic acid exists in UDPGA in the 1α-configuration, but the products of conjugation are β-glucuronides. This is due to the mechanism of the reaction being a nucleophilic substitution with inversion of configuration. Indeed, and as shown in Fig. 21, all functional groups able to undergo glucuronidation are nucleophiles, a common characteristic they share despite their great chemical variety. As a consequence of this diversity, the products of glucuronida-

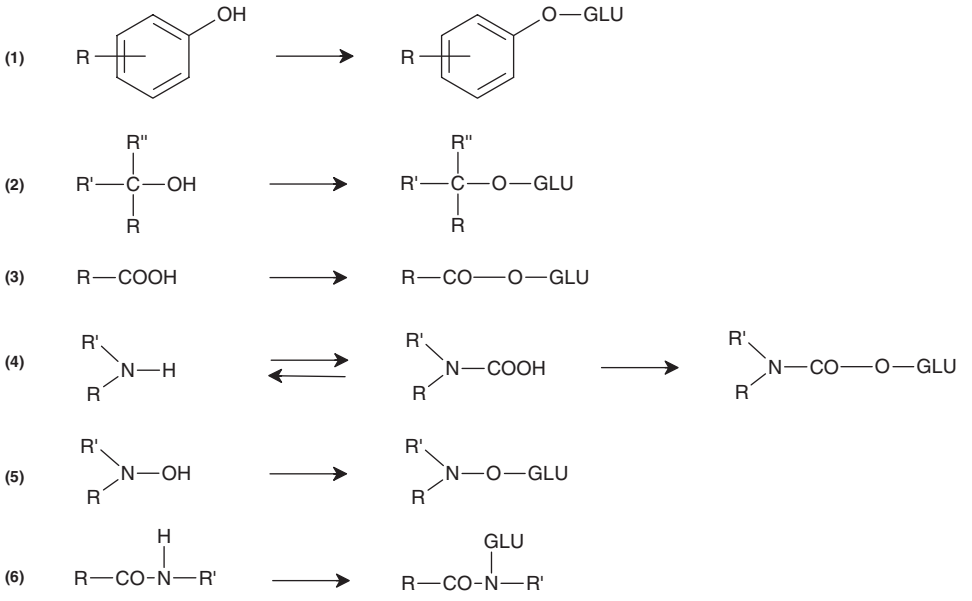


Figure 21. Major glucuronidation reactions involving phenols, alcohols, carboxylic acids, carbamic acids, hydroxylamines and hydroxylamides, carboxamides, sulfonamides, various amines, thiols, dithiocarboxylic acids, and 1,3-dicarbonyl compounds.

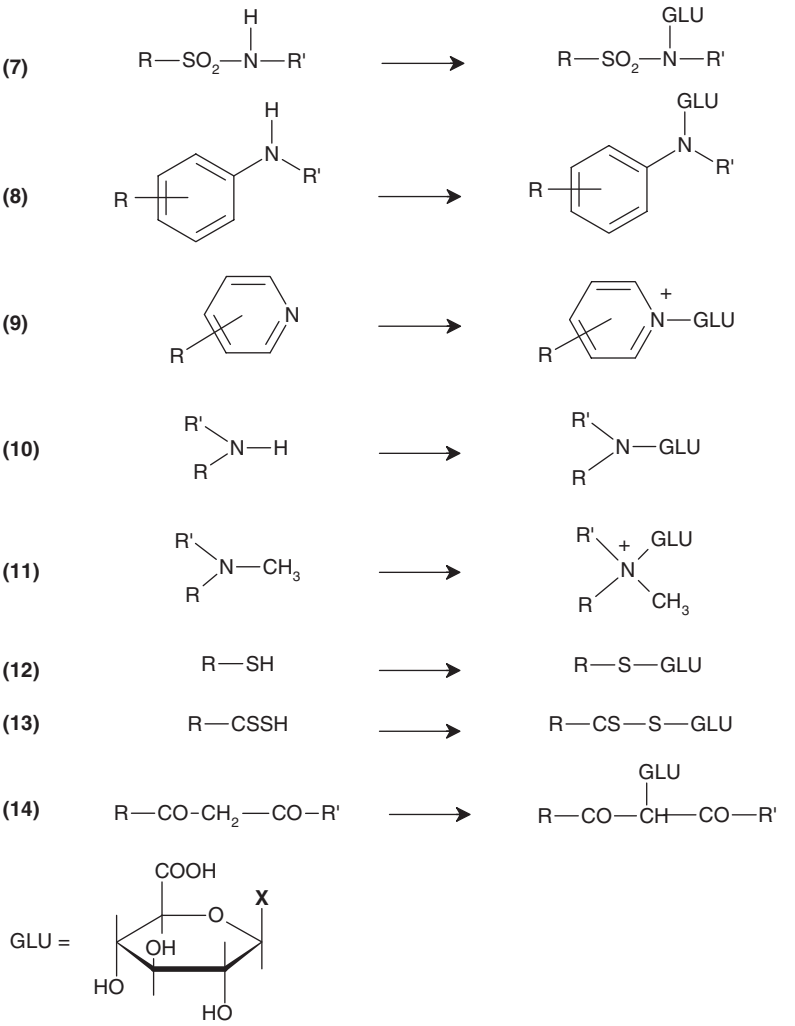
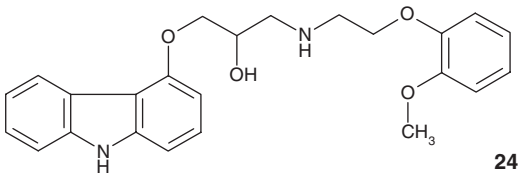


Figure 21. (*Continued*).

tion are classified as *O*-, *N*-, *S*- and *C*-glucuronides.

O-Glucuronidation is shown in reactions 1–5 (Fig. 21). A frequent metabolic reaction of phenolic xenobiotics or metabolites is their glucuronidation to yield polar metabolites excreted in urine and/or bile. *O*-Glucuronidation is often in competition with *O*-sulfonation (see above), with the latter reaction predominating at low doses and the former at high doses. In biochemical terms, glucuronidation is a reaction of low affinity and high capacity, while sulfonation displays high affinity and low capacity. A typical drug undergoing extensive glucuronidation is paracetamol (**8** in Fig. 10). Another major group of substrates are alcohols, be they primary, secondary, or tertiary (reaction 2, Fig. 21). Medicinal examples include chloramphenicol and oxazepam. Another important example is that of morphine, which is conjugated on its phenolic and secondary alcohol groups to form the 3-*O*-glucuronide (a weak opiate antagonist) and the 6-*O*-glucuronide (a strong opiate agonist), respectively.

An important pathway of *O*-glucuronidation is the formation of acyl glucuronides (reaction 3). Substrates are arylacetic acids



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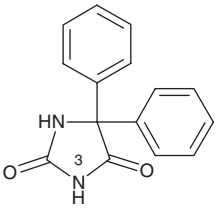

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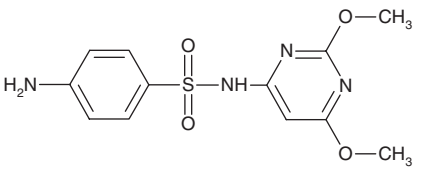

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Figure 22. Carvedilol (**24**), phenytoin (**25**), and sulfadimethoxine (**26**).

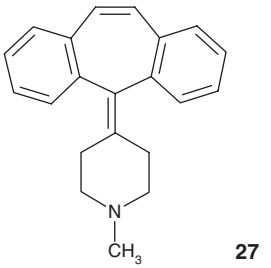
(e.g., diclofenac, **15** in Fig. 16) and aliphatic acids (e.g., valproic acid). Aromatic acids are seldom substrates, a noteworthy exception being diflunisal (**20** in Fig. 19) that yields both the acyl and the phenolic glucuronides. The significance of acyl glucuronides has long been underestimated perhaps because of analytical difficulties. Indeed, these metabolites are quite reactive, rearranging to positional isomers and binding covalently to plasma and seemingly also tissue proteins [46]. Thus, acyl glucuronide formation cannot be viewed solely as a reaction of inactivation and detoxification.

A special class of acyl glucuronides are the carbamoyl glucuronides (reaction 4 in Fig. 21). A number of primary and secondary amines have been found to yield this type of conjugate, while as expected the intermediate carbamic acids are not stable enough to be characterized. Carvedilol (**24** in Fig. 22) is one drug exemplifying the reaction, in addition to forming an *O*-glucuronide on its alcohol group and a carbazole-*N*-linked glucuronide (see below). Much remains to be understood concerning the chemical and biochemical reactivity of carbamoyl glucuronides.

Hydroxylamines and hydroxylamides may also form *O*-glucuronides (reaction 5, Fig. 21). Thus, a few drugs and a number of aromatic amines are known to be *N*-hydroxylated and then *O*-glucuronidated. The glucuronidation of N−OH groups competes with *O*-sulfation, but the reactivity of *N*-*O*-glucuronides to undergo heterolytic cleavage and form nitrenium ions does not appear to be well characterized.

Second in importance to *O*-glucuronides are the *N*-glucuronides formed by reactions 6–11 in Fig. 21, that is, amides (reactions 6–7), amines of medium basicity (reactions 8 and 9), and basic amines (reactions 10 and 11). The *N*-glucuronidation of carboxamides (reaction 6) is exemplified by carbamazepine (**5** in Fig. 6) and phenytoin (**25** in Fig. 22). In the latter case, *N*-glucuronidation was found to occur at *N*(3). The reaction has special significance for sulfonamides (reaction 7) and particularly antibacterial sulfanilamides such as sulfadimethoxine (**26** in Fig. 22) since it produces highly water-soluble metabolites that show no risk of crystallizing in the kidneys.

N-Glucuronidation of aromatic amines (reaction 8, Fig. 21) has been observed in a few cases only (e.g., conjugation of the carbazole nitrogen in carvedilol (**24**). Similarly, there are a number of observations that pyridine-type nitrogens and primary and secondary basic amines can be *N*-glucuronidated (reactions 9 and 10, respectively). As far as human drug metabolism is concerned, another reaction of significance is the *N*-glucuronidation of lipophilic, basic tertiary amines containing one or two methyl groups (reaction 11). More and more drugs of this type (e.g., antihistamines and neuroleptics), are found to undergo this reaction to a marked extent in



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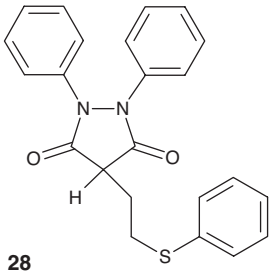

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Figure 23. Cyproheptadine (**27**) and sulfinpyrazone (**28**).

humans, for example, cyproheptadine (**27** in Fig. 23).

Third in importance are the *S*-glucuronides formed from aliphatic and aromatic thiols (reaction 12 in Fig. 21), and from dithiocarboxylic acids (reaction 13) such as diethyldithiocarbamic acid, a metabolite of disulfiram. As for *C*-glucuronidation (reaction 14), this reaction has been seen in humans for 1,3-dicarbonyl drugs such as phenylbutazone and sulfinpyrazone (**28** in Fig. 23).

3.4.3. Glucosidation Reactions A few drugs have been observed to be conjugated to glucose in mammals. This is usually a minor pathway in some cases where glucuronidation is possible. An interesting medicinal example is that of some barbiturates such as phenobarbital that yield the *N*-glucoside.

3.5. Acetylation and Acylation

All reactions discussed in this section involve the transfer of an acyl moiety to an acceptor group. In most cases, an acetyl is the acyl moiety being transferred, while the acceptor group may be an amino or hydroxyl function [15,19,24,47].

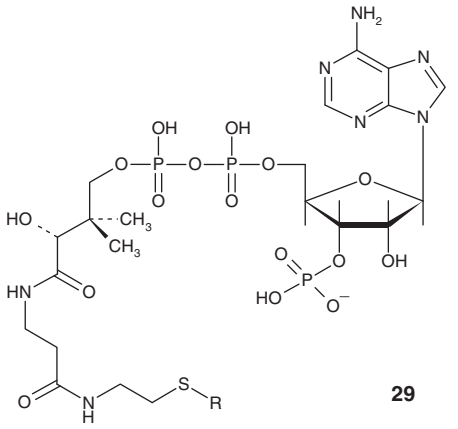

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Figure 24. Acetylcoenzyme A (**29**, when R = acetyl).

3.5.1. Acetylation Reactions The major enzyme system catalyzing acetylation reactions is arylamine *N*-acetyltransferase (see Table 7). Two enzymes have been characterized, NAT1 and NAT2, the latter as two closely related isoforms NAT2A and NAT2B whose levels are considerably reduced in the liver of slow acetylators. The cofactor of *N*-acetyltransferase is acetylcoenzyme A (CoA-S-Ac, **29** with R = acetyl) (Fig. 24) where the acetyl moiety is bound by a thioester linkage.

Two other activities, aromatic hydroxylamine *O*-acetyltransferase and *N*-hydroxyarylamine *O*-acetyltransferase, are also involved in the acetylation of aromatic amines and hydroxylamines (see below). Other acetyltransferases exist, for example, diamine *N*-acetyltransferase (putrescine acetyltransferase; EC 2.3.1.57) and aralkylamine *N*-acetyltransferase (serotonin acetyltransferase; EC 2.3.1.87), but their involvement in xenobiotic metabolism does not appear to be documented.

The substrates of acetylation, as schematized in Fig. 25, are mainly amines of medium basicity. Very few basic amines (primary or secondary) of medicinal interest have been reported to form *N*-acetylated metabolites (reaction 1), and when so the yields were low. In contrast, a large variety of primary aromatic amines are *N*-acetylated (reaction 2). Thus, several drugs such as sulfonamides and *para*-aminosalicylic acid (**30**, PAS) (Fig. 26) are

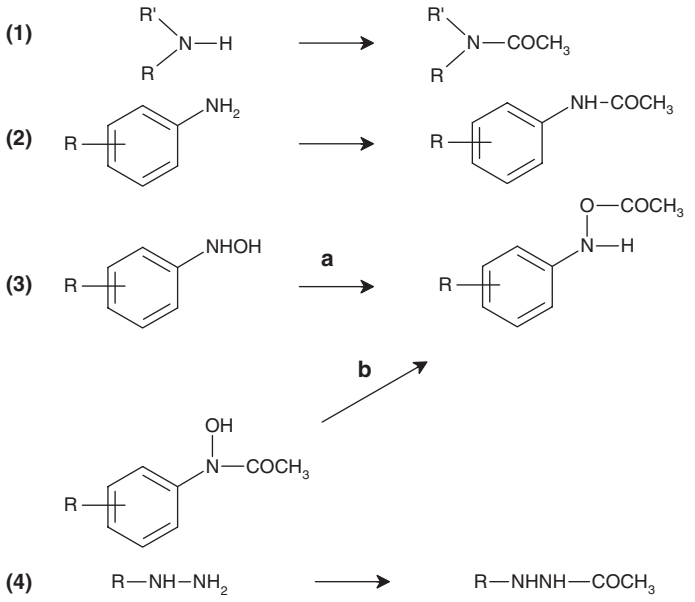


Figure 25. Major acetylation reactions involving aliphatic amines, aromatic amines, arylhydroxylamines, hydrazines and hydrazides.

acetylated to large extents, not to mention various carcinogenic amines such as benzidine.

Arylhydroxylamines can also be acetylated, but the reaction is one of *O*-acetylation (reaction 3a in Fig. 25). This is the reaction formally catalyzed by EC 2.3.1.118 with acetyl-CoA acting as the acetyl donor, the *N*-hydroxy metabolites of a number of arylamines being known substrates. The same conjugates can be formed by intramolecular *N*,*O*-acetyl transfer, when an arylhydroxamic acid (an *N*-aryl-*N*-hydroxy-acetamide) is substrate of, for example, EC 2.3.1.56 (reaction 3b). In addition, such an arylhydroxamic acid can transfer its acetyl moiety to an acetyltransferase, which can then acetylate an arylamine or an arylhydroxylamine (intermolecular *N*,*N*- or *N*,*O*-acetyl transfer).

Besides amines, other nitrogen-containing functionalities undergo *N*-acetylation, hydrazines, and hydrazides being particularly good substrates (reaction 4, Fig. 25). Medicinal examples include isoniazid (**31** in Fig. 26) and hydralazine (**32**).

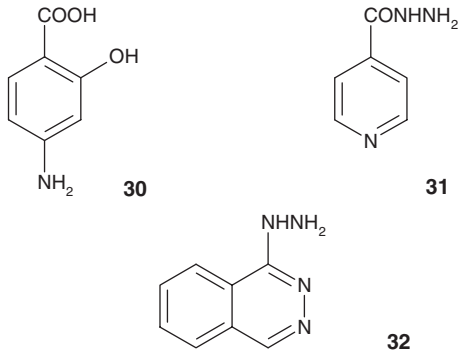


Figure 26. *para*-Aminosalicylic acid (**30**), isoniazid (**31**), and hydralazine (**32**).

3.5.2. Other Acylation Reactions A limited number of studies have shown *N*-formylation to be a genuine route of conjugation for some arylalkylamines and arylamines, and particularly polycyclic aromatic amines. There is evidence to indicate that the reaction is catalyzed by arylformamidase (EC 3.5.1.9) in the presence of *N*-formyl-L-kynurenine.

A very different type of reaction is represented by the conjugation of xenobiotic

alcohols with fatty acids, yielding highly lipophilic metabolites accumulating in tissues. Thus, ethanol and haloethanols form esters with, for example, palmitic acid, oleic acid, linoleic acid, and linolenic acid; enzymes catalyzing such reactions are cholesteryl ester synthase (EC 3.1.1.13) and fatty-acyl-ethylester synthase (EC 3.1.1.67) [48,49]. Larger xenobiotics such as tetrahydrocannabinols and codeine are also acylated with fatty acids, possibly by sterol *O*-acyltransferase (EC 2.3.1.26).

3.6. Conjugation with Coenzyme A and Subsequent Reactions

3.6.1. Conjugation with Coenzyme A The reactions described in this section all have in common the fact that they involve xenobiotic carboxylic acids forming an acyl-CoA metabolic intermediate (**29** in Fig. 24, R = xenobiotic acyl moiety). The reaction requires ATP and is catalyzed by various acyl-CoA synthetases of overlapping substrate specificity, for example, short-chain fatty acyl-CoA synthetase, medium-chain acyl-CoA synthetase, long-chain acyl-CoA synthetase, and benzoate-CoA ligase (EC 6.2.1.25) (see Table 7).

The acyl-CoA conjugates thus formed are seldom excreted, but they can be isolated and characterized relatively easily in *in vitro* studies. They may also be hydrolyzed back to the parent acid by thiolester hydrolases (EC 3.1.2). In a number of cases, such conjugates have pharmacodynamic effects and may even represent the active forms of some drugs, for example, hypolipidemic agents. In the present context, the interest of acyl-CoA conjugates is their further transformation by a considerable variety of pathways (Table 8) [15,19,24]. The most significant routes are discussed below.

3.6.2. Formation of Amino Acid Conjugates Amino acid conjugation is a major route for a number of small aromatic acids and involves the formation of an amide bond between the xenobiotic acyl-CoA and the amino acid. Glycine is the amino acid most frequently used for conjugation (reaction 1 in Fig. 27), while a few glutamine conjugates (reaction 2) and some taurine conjugates (reaction 3) have been characterized in humans. The enzymes catalyzing these transfer reactions are various *N*-acyltransferases (see Table 7), for example glycine *N*-acyltransferase, glycine *N*-benzoyltransferase, glutamine *N*-phenylacetyltransferase and glutamine *N*-acyltransferase, and cholyl-CoA glycine-taurine *N*-acyltransferase. In addition, other amino acids can be used for conjugation in various animal species, for example, alanine, as well as some dipeptides [3].

Table 8. Metabolic Consequences of the Conjugation of Xenobiotic Acids to Coenzyme A (CoA-SH) [15,24]

Depending on structure and other factors, the resulting R-CO-S-CoA intermediate may enter the following metabolic reactions:
• Hydrolysis
• Formation of amino acid conjugates
• Formation of hybrid triglycerides
• Formation of phospholipids
• Formation of cholesteryl esters
• Formation of bile acid esters
• Formation of acyl-carnitines
• Protein acylation
• Unidirectional configurational inversion of aryl-propionic acids (profens)
• Dehydrogenation and β-oxidation
• Two-carbon chain elongation

The xenobiotic acids undergoing amino acid conjugation are mainly substituted benzoic acids. In humans for example, hippuric acid and salicyluric acid (**33** in Fig. 28) are the major metabolites of benzoic acid and salicylic acid, respectively. Similarly, *m*-trifluoromethylbenzoic acid (**13** in Fig. 12), a major metabolite of fenfluramine (**10**), is excreted as the glycine conjugate. Phenylacetic acid derivatives can yield glycine and glutamine conjugates. Some drugs containing a carboxylic group do form the taurine conjugate as a minor metabolite.

3.6.3. Formation of Hybrid Lipids and Sterol Esters Incorporation of xenobiotic acids into lipids forms highly lipophilic metabolites that may burden the body as long retained residues. In the majority of cases, triacylglycerol analogs (reaction 4 in Fig. 27) or cholesterol esters (reaction 5) are formed. The enzymes catalyzing such reactions are *O*-acyltransferases (see Table 7), including diacylglycerol

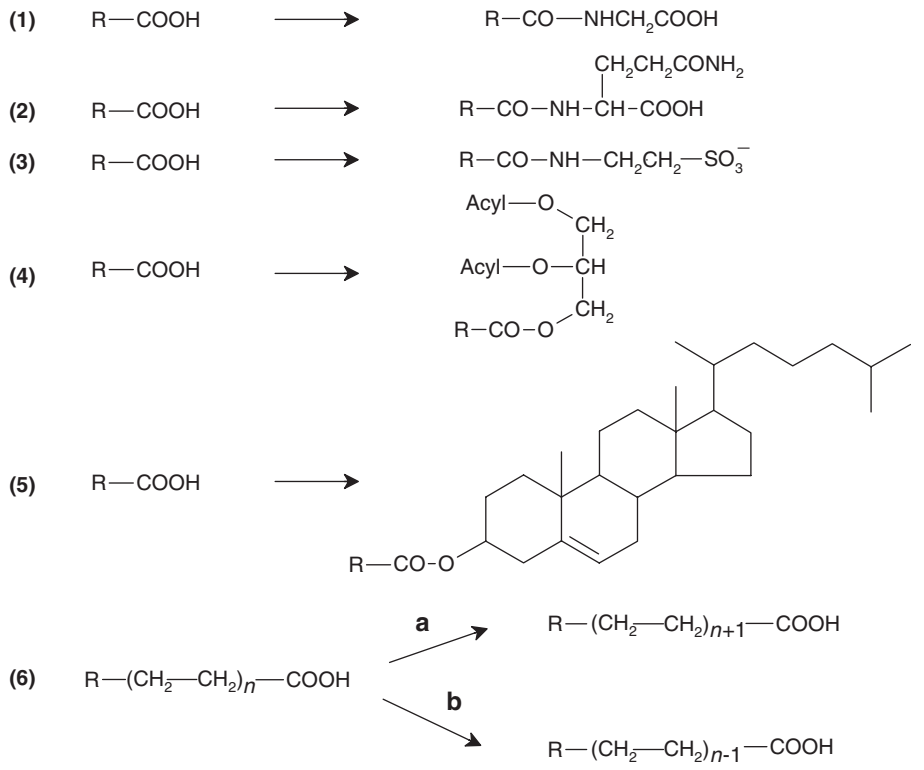


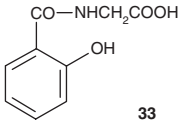
Figure 27. Metabolic reactions involving acyl-CoA intermediates of xenobiotic acids, namely conjugations and two-carbon chain lengthening or shortening. Other products of β-oxidation are shown in Fig. 30.

O-acyltransferase, 2-acylglycerol *O*-acyltransferase, and sterol *O*-acyltransferase (cholesterol acyltransferase). Some phospholipid analogs, as well as some esters to the 3-hydroxy group of biliary acids, have also been characterized [15,24,48–52].

The number of drugs and other xenobiotics that are currently known to form glyceryl or cholesteryl esters is limited, but should increase due to increased awareness of researchers. One telling example is that of ibuprofen (**34** in Fig. 28), a much used antiinflammatory drug whose (*R*)-enantiomer forms hybrid triglycerides detectable in rat liver and adipose tissue.

3.6.4. Configurational Inversion of Arylpropionic Acids Ibuprofen (**34** in Fig. 28) and other arylpropionic acids (i.e., profens) are chiral drugs existing as the (+)-(*S*) eutomer and the (−)-(*R*) distomer. These compounds undergo an intriguing metabolic reaction such that the (*R*)-enantiomer is converted to the (*S*)-enantiomer, while the reverse reaction is negligible. This unidirectional configurational inversion is thus a reaction of bioactivation, and its mechanism is now reasonably well understood (Fig. 29) [53].

The initial step in the reaction is the substrate stereoselective formation of an acyl-CoA conjugate with the (*R*)-form but not with



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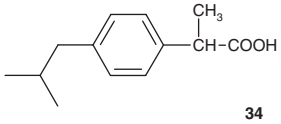

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Figure 28. Salicyluric acid (**33**) and ibuprofen (**34**).

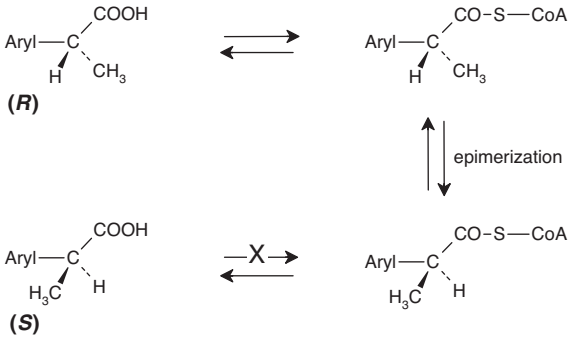


Figure 29. Mechanism of the unidirectional configurational inversion of some profens.

the (*S*)-form. This conjugate then undergoes a reaction of epimerization possibly catalyzed by methylmalonyl-CoA epimerase (EC 5.1.99.1), resulting in a mixture of the (*R*)-profenoyl- and (*S*)-profenoyl-CoA conjugates. The latter can then be hydrolyzed as shown in Fig. 29, or undergo other reactions such as hybrid triglyceride formation (see below).

3.6.5. β-Oxidation and Two-Carbon Chain Elongation In some cases, acyl-CoA conjugates formed from xenobiotic acids can also enter the physiological pathways of fatty acid catabolism or anabolism. A few examples are known of xenobiotic alkanoic and arylalkanoic acids undergoing two-carbon chain elongation, or two-, four- or even six-carbon chain shortening (reactions 6a and 6b in Fig. 27). In addition, intermediate metabolites of β-oxidation may also be seen, as illustrated by valproic acid (**35** in Fig. 30). Approximately 50 metabolites of this drug have been characterized; they are formed by β-oxidation, glucuronidation, and/or cytochrome P450-catalyzed dehydrogenation or oxygenation. Fig. 30 shows the β-oxidation of valproic acid seen in mitochondrial preparations [25,54]. The resulting metabolites have also been found in unconjugated form in the urine of humans or animals dosed with the drug.

3.7. Conjugation and Redox Reactions of Glutathione

3.7.1. Introduction Glutathione (**36** in Fig. 31, GSH) is a thiol-containing tripeptide of major significance in the detoxification and toxification of drugs and other xenobiotics [15,24,55–57]. In the body, it exists in a redox equilibrium between the reduced form (GSH) and an oxidized form (GS-SG). The metabolism of glutathione (i.e., its synthesis, redox equilibrium and degradation) is quite complex and involves a number of enzymes.

Glutathione reacts in a variety of manners. First, the nucleophilic properties of the thiol (or rather thiolate) group make it an effective conjugating agent, as emphasized in this section. Second, and depending on its redox state, glutathione can act as a reducing or oxidizing agent (e.g., reducing quinones, organic nitrates, peroxides and free radicals, or oxidizing superoxide). Another dichotomy exists in the reactions of glutathione, since these can be enzymatic (e.g., conjugations catalyzed by glutathione *S*-transferases, and peroxide reductions catalyzed by glutathione peroxidase) or nonenzymatic (e.g., some conjugations and various redox reactions).

The glutathione *S*-transferases (see Table 7) are multifunctional proteins coded by two gene superfamilies. They can act as enzymes as well as binding proteins. These enzymes are mainly localized in the cytosol as homodimers and heterodimers, but microsomal enzymes also exist [58–60]. The GST A1-2, A2-2 and P1-1 display selenium-independent glutathione peroxidase activity, a property also characterizing the selenium-containing enzyme glutathione peroxidase (EC 1.11.1.9). The GST A1-1 and A1-2 are also known as ligandin when they act as binding or carrier proteins, a property also displayed

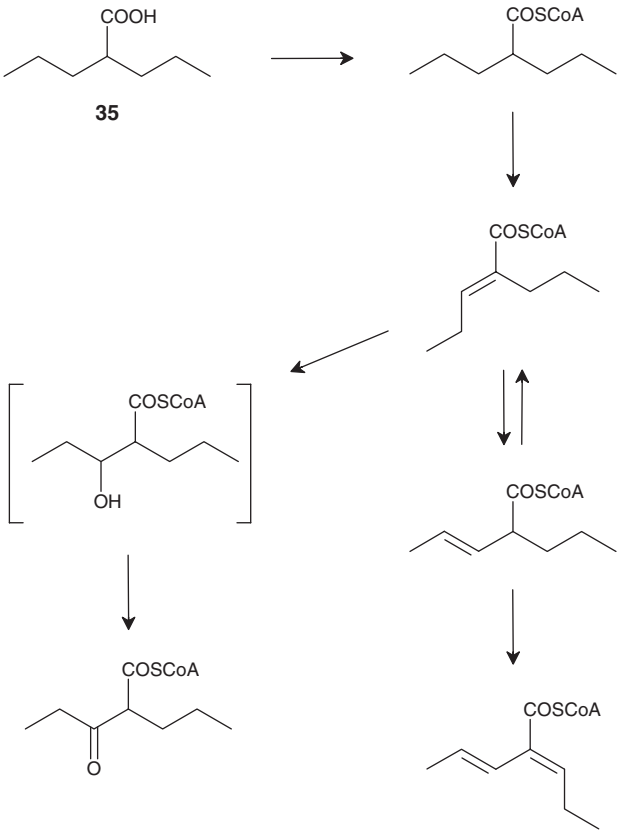
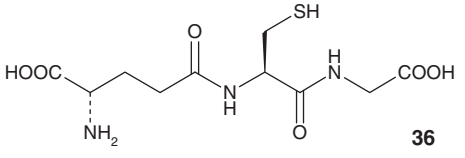


Figure 30. Mitochondrial β-oxidation of valproic acid (**35**).

by M1a-1a and M1b-1b. In the latter function, these enzymes bind and transport a number of active endogenous compounds (e.g., bilirubin, cholic acid, steroid and thyroid hormones, and hematin), as well as some exogenous dyes and carcinogens.



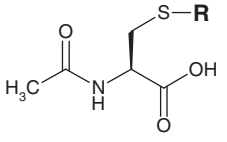


Figure 31. Glutathione (**36**) and *N*-acetylcysteine conjugates (**37**).

The nucleophilic character of glutathione is due to its thiol group (pK_a 9.0) in its neutral form and even more to the thiolate form. In fact, an essential component of the catalytic mechanism of glutathione transferase is the marked increase in acidity (pK_a 6–7) experienced by the thiol group upon binding of glutathione to the active site of the enzyme. As a result, GSTs transfer glutathione to a very large variety of electrophilic groups; depending on the nature of the substrate, the reactions can be categorized as nucleophilic additions or nucleophilic additions–eliminations. And with compounds of sufficient reactivity, these reactions can also occur nonenzymatically [61].

Once formed, glutathione conjugates (*R*-SG) are seldom excreted as such (they are best characterized *in vitro* or in the bile of laboratory animals), but usually undergo further biotransformation prior to urinary or fecal

excretion. Cleavage of the glutamyl moiety by glutamyl transpeptidase (EC 2.3.2.2), and of the cysteinyl moiety by cysteinylglycine dipeptidase (EC 3.4.13.6) or aminopeptidase M (EC 3.4.11.2), leaves a cysteine conjugate (*R*-*S*-Cys) that is further *N*-acetylated by cysteine-*S*-conjugate *N*-acetyltransferase (EC 2.3.1.80) to yield an *N*-acetylcysteine conjugate (**37** in Fig. 31, *R*-*S*-CysAc). The latter type of conjugates are known as mercapturic acids, a name that clearly indicates that they were first characterized in urine. This however does not imply that the degradation of unexcreted glutathione conjugates must stop at this stage, since cysteine conjugates can be substrates of cysteine-*S*-conjugate β-lyase (EC 4.4.1.13) to yield thiols (*R*-SH). These in turn can rearrange as discussed below, or be *S*-methylated and then *S*-oxygenated to yield thiomethyl conjugates (*R*-*S*-Me), sulfoxides (*R*-SO-Me), and sulfones (R-SO_2-Me).

3.7.2. Reactions of Glutathione The major reactions of glutathione, both conjugations and redox reactions, are summarized in Fig. 32. Reactions 1 and 2 are nucleophilic additions and additions-elimination to sp^3-carbons, respectively, while reactions 3–8 are

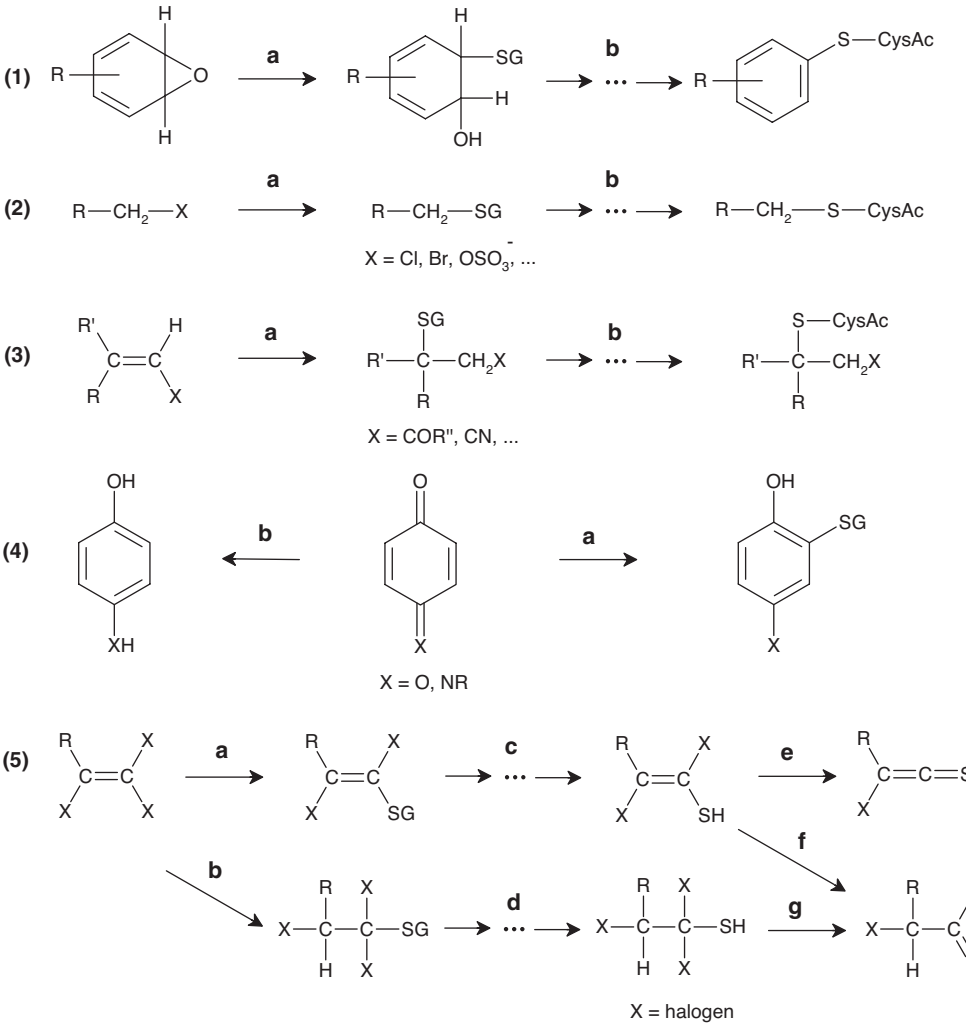


Figure 32. Major reactions of conjugation of glutathione, sometimes accompanied by a redox reaction.

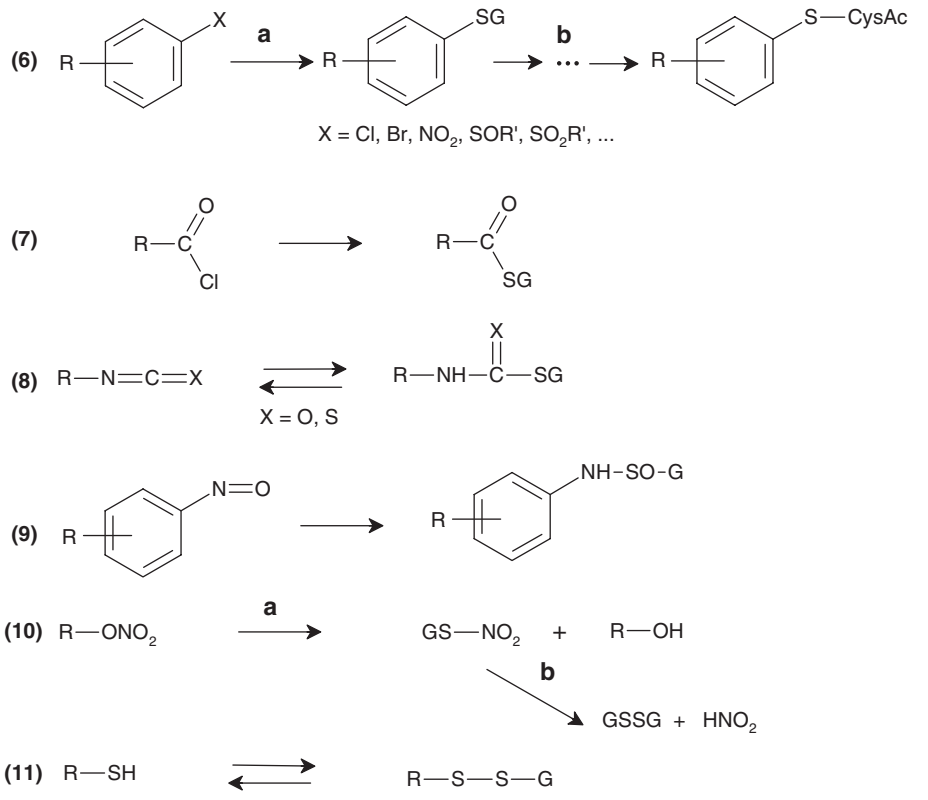


Figure 32. (*Continued*).

nucleophilic substitutions or additions at sp^2-carbons, sometimes accompanied by a redox reaction. Reactions at nitrogen or sulfur atoms are shown in reactions 9–11.

The first reaction in Fig. 32 is nucleophilic addition to epoxides (reaction 1a) to yield a nonaromatic conjugate. This is followed by several metabolic steps (reaction 1b) leading to an aromatic mercapturic acid. This is a frequent reaction of metabolically produced arene oxides (Fig. 5), as documented for naphthalene and numerous drugs and xenobiotics containing a phenyl moiety. Note that the same reaction can also occur readily for epoxides of olefins (not shown in Fig. 32).

An important pathway of addition-elimination at sp^3-carbons is represented in reaction 2a, followed by the production of mercapturic acids (reaction 2b). Various electron-withdrawing leaving groups (X in reaction 2) may be involved that are either of xenobiotic (e.g., halogens) or metabolic origin (e.g., a sulfate group). Such a reaction occurs for example at the $-CHCl_2$ group of chloramphenicol and at the NCH_2CH_2Cl group of anticancer alkylating agents.

The reactions at sp^2-carbons are quite varied and complex. Addition to activated olefinic groups (e.g., α,β-unsaturated carbonyls) is shown in reaction 3. A typical substrate is acrolein ($CH_2{=}CH{-}CHO$). Quinones (*ortho* and *para*) and quinoneimines react with glutathione by two distinct and competitive routes, namely nucleophilic addition to form a conjugate (reaction 4a), and reduction to the hydroquinone or aminophenol (reaction 4b). A typical example is provided by the toxic quinoneimine metabolite (**9** in Fig. 10) of paracetamol (**8**). Since in most cases quinones and quinoneimines are produced by the bio-oxidation of hydroquinones and aminophenols, respectively, their reduction by GSH can be seen as a futile cycle that consumes reduced glutathione. As for the conjugates produced by

reaction 4a, they may undergo reoxidation to *S*-glutathionyl quinones or *S*-glutathionyl quinoneimines of considerable reactivity. These quinone or quinoneimine thioethers are known to undergo further GSH conjugation and reoxidation.

Haloalkenes are a special group of substrates of GS-transferases since they may react with GSH either by substitution (reaction 5a) or by addition (reaction 5b). The formation of mercapturic acids occurs as for other glutathione conjugates, but in this case S–C cleavage of the *S*-cysteinyl conjugates by the renal β-lyase (reactions 5c and 5d) yields thiols of significant toxicity since they rearrange by hydrohalide expulsion to form highly reactive thioketenes (reaction 5e) and/or thioacyl halides (reactions 5f and 5g) [62].

With good leaving groups, nucleophilic aromatic substitution reactions also occur at aromatic rings containing additional electron-withdrawing substituents and/or heteroatoms (reaction 6a). As for the detoxification of acyl halides with glutathione (reaction 7), a good example is provided by phosgene ($O{=}CCl_2$), an extremely toxic metabolite of chloroform that is inactivated to the diglutathionyl conjugate $O{=}C(SG)_2$.

The addition of glutathione to isocyanates and isothiocyanates has received some attention due in particular to its reversible character (reaction 8) [63]. Substrates of the reaction are xenobiotics such as the infamous toxin methyl isocyanate, whose glutathione conjugate behaves as a transport form able to carbamoylate various macromolecules, enzymes and membrane components. The reaction is also of interest from a medicinal viewpoint since anticancer agents such as methylformamide appear to work by undergoing activation to isocyanates and then to the glutathione conjugate.

The reaction of *N*-oxygenated drugs and metabolites with glutathione may also have toxicological and medicinal implications. Thus, the addition of GSH to nitrosoarenes, probably followed by rearrangement, forms sulfinamides (reaction 9) that have been postulated to contribute to the idiosyncratic toxicity of a few drugs such as sulfonamides [64]. As for organic nitrate esters such as nitroglycerine and isosorbide dinitrate, the mechanism of their vasodilating action is now known to result from their reduction to nitric oxide (NO). Thiols, and particularly glutathione, play an important role in this activation. In the first step, a thionitrate is formed (reaction 10a) whose *N*-reduction may occur by more than one route. For example, a GSH-dependent reduction may yield nitrite (reaction 10b) that undergoes further reduction to NO; *S*-nitrosoglutathione (GS-NO) has also been postulated as an intermediate.

The formation of mixed disulfides between GSH and a xenobiotic thiol (reaction 11) has been observed in a few cases, for example, with captopril.

Finally, glutathione and other endogenous thiols (including albumin) are able to inactivate free radicals (e.g., $R^{\bullet}$, $HO^{\bullet}$, $HOO^{\bullet}$, $ROO^{\bullet}$) and have thus a critical role to play in cellular protection [55–57]. The reactions involved are highly complex and incompletely understood; the simplest are

$$GSH + X^{\bullet} \rightarrow GS^{\bullet} + XH$$

$$GS^{\bullet} + GS^{\bullet} \rightarrow GSSG$$

$$GS^{\bullet} + O_2 \rightarrow GS{-}OO^{\bullet}$$

$$GS{-}OO^{\bullet} + GSH \rightarrow GS{-}OOH + GS^{\bullet}$$

3.8. Other Conjugation Reactions

The above Sections 3.2–3.7 present the most common and important routes of xenobiotic conjugation, but these are not the only ones. A number of other routes have been reported whose importance is at present restricted to a few exogenous substrates, or that have received only limited attention [15,24]. In the present section, two pathways of pharmacodynamic significance will be mentioned, namely phosphorylation and carbonyl conjugation (Fig. 33). In both cases, the xenobiotic substrates belong to narrowly defined chemical classes.

Phosphorylation reactions are of great significance in the processing of endogenous compounds and macromolecules. It is therefore astonishing that relatively few xenobiotics are substrates of phosphotransferases (e.g., EC

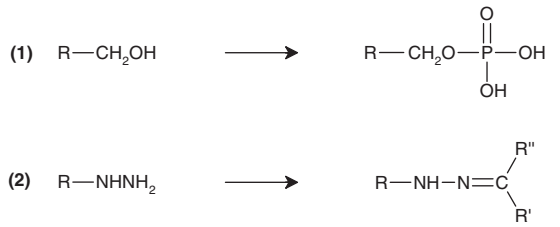


Figure 33. Additional conjugation reactions discussed in Section 3.8, namely formation of phosphate esters and of hydrazones.

2.7.1) to form phosphate esters (reaction 1 in Fig. 33). The phosphorylation of phenol is a curiosity observed by some workers. In contrast, a number of antiviral nucleoside analogs are known to yield the mono-, di- and triphosphates *in vitro* and *in vivo*, for example, zidovudine (**38** in Fig. 34, AZT) and numerous analogs. These conjugates are active forms of the drugs, being in particular incorporated in the DNA of virus-infected cells [65].

The second pathway of conjugation to be presented here is the reaction of hydrazines with endogenous carbonyls (reaction 2) [62]. This reaction occurs nonenzymatically and involves a variety of carbonyl compounds, namely aldehydes (mainly acetaldehyde) and ketones (e.g., acetone, pyruvic acid $CH_3-CO-COOH$, and α-ketoglutaric acid $HOOC-CH_2CH_2-CO-COOH$). The products thus formed are hydrazones, which may be excreted as such or undergo further transformation. Isoniazid (**31** in Fig. 26) and hydralazine (**32** in Fig. 26) are two drugs that form hydrazones in the body. For example, the reaction of hydralazine with acetyldehyde or acetone is a reversible one, meaning that the hydrazones are hydrolyzed under biological conditions of pH and temperature. In addition, the hydrazone of hydralazine with acetaldehyde or pyruvic acid undergoes an irreversible reaction of cyclization to another metabolite, methyltriazolophthalazine (**39** in Fig. 34).

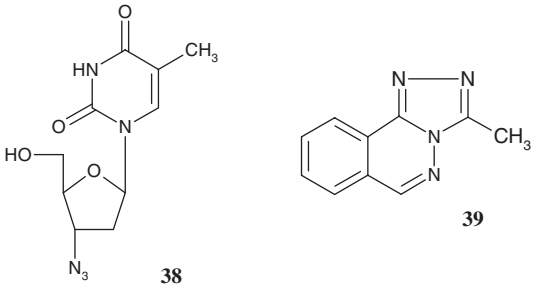


Figure 34. Zidovudine (**38**) and methyltriazolophthalazine (**39**).

4. DRUG METABOLISM AND THE MEDICINAL CHEMIST

Having presented the various reactions of drug metabolism, we now briefly review a few topics of potential interest to medicinal chemists, namely the influence of metabolism on activity and toxicity, the prediction of drug metabolism, and the biological factors that may influence biotransformation.

4.1. Metabolism and Bioactivity

The pharmacological and toxicological consequences of drug metabolism explain to a large extent the immense significance this discipline has acquired. Indeed, such consequences are of utmost relevance in fields such as drug discovery and development, clinical pharmacology and toxicology, and therapeutics.

4.1.1. Pharmacological Activity An important information in any drug's dossier is the activity (or lack thereof) of its metabolites. What should be realized, however, is that "activity" is usually understood to imply the same pharmacological target as the parent molecule [67–69]. Here, we outline various possible combinations, from a drug having no active metabolite to an intrinsically inactive "drug" (i.e., a prodrug) whose therapeutic effects necessitate metabolism to an active metabolite (i.e., bioactivation).

A number of drugs have no active metabolites, for example the 3-hydroxylated

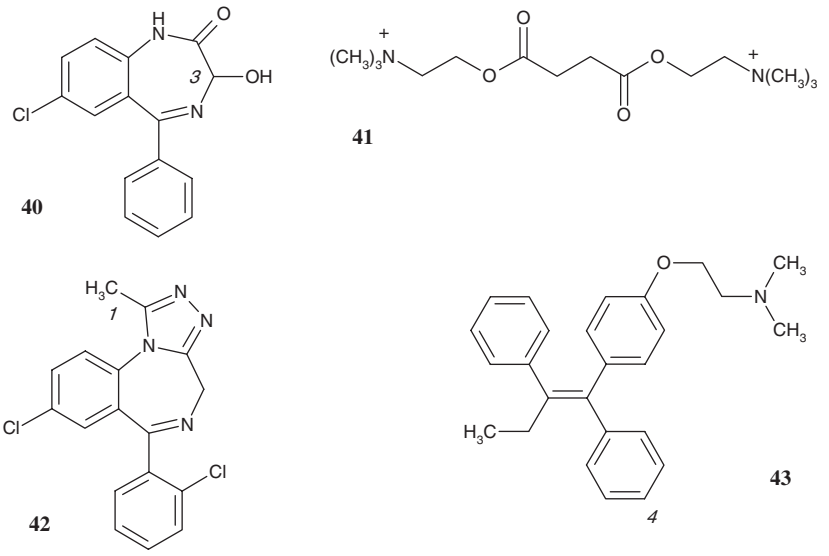


Figure 35. Oxazepam (**40**) and succinylcholine (**41**) as examples of drugs having no active metabolite. In contrast, part of the activity of triazolam (**42**) and tamoxifen (**43**) is due to one or more active metabolites.

benzodiazepines such as lorazepam, oxazepam (**40** in Fig. 35), and temazepam, which undergo *O*-glucuronidation and cleavage reactions. A case of drugs designed to have neither active nor toxic metabolites is that of soft drugs, a concept pioneered and extensively developed by Bodor and collaborators [70–72]. In practical terms, soft drugs (a) are bioisosteres of known drugs, (b) contain a labile bridge (often an ester group), and (c) are cleaved to metabolites known to lack activity and toxicity. In other words, soft drugs bear a close stereoelectronic analogy with the target drugs, but a rapid breakdown to inactive metabolites is programmed into their chemical structure. A typical example is succinylcholine (**41** in Fig. 35), although the discovery of this agent predates by decades the concept and term of soft drugs. In most individuals, this curarimimetic agent is very rapidly hydrolyzed to choline and succinic acid by plasma cholinesterase with a half-life of about four minutes [73].

Drugs having both intrinsic activity and active metabolites may be more numerous than generally believed, but information is not always available. Several benzodiazepines have one or more active metabolite(s). For example, triazolam (**42** in Fig. 35) forms the active 1-hydroxytriazolam, both agents having a short half-life compatible with the use of the drug as a short-acting short-duration hypnotic [74].

A drug whose activity owes much to metabolism is tamoxifen (**43** in Fig. 35) as summarized here. This estrogen receptor antagonist is extensively used for endocrine treatment of breast cancer. Its metabolism is quite complex and leads to at least 13 oxidative metabolites, not to mention conjugation reactions. A pharmacologically significant route is aromatic oxidation to the active 4-hydroxytamoxifen. However, the most important metabolic pathway in qualitative and quantitative terms is *N*-demethylation to the secondary amine *N*-desmethyltamoxifen, a reaction catalyzed mainly by CYP3A4. This metabolite, while reaching high plasma levels, is not as active as 4-hydroxytamoxifen and the second generation metabolite *N*-desmethyl-4-hydroxytamoxifen (known as endoxifen). Indeed, a number of investigations have demonstrated that 4-hydroxytamoxifen and endoxifen are equipotent and many times more active as antiestrogens than the parent drug [75]. When taking

plasma levels in consideration [76], it seems that the main contributor to therapeutic activity in patients is endoxifen, and to a lesser extent tamoxifen and 4-hydroxy-tamoxifen.

An extreme case of activation by metabolism is that of prodrugs, namely intrinsically inactive (or poorly active) medicinal compounds whose biotransformation produces the active agent. This case is treated in a separate chapter and will not be considered here.

4.1.2. Toxicity The toxicological consequences of the metabolism of drugs and other xenobiotics can be favorable (i.e., result in detoxification) or unwanted (i.e., result in toxification) [15,25]. The risks of toxification associated with biotransformation [77–81] have now become a major issue in drug discovery and development, where minimizing metabolic toxification is given a high priority [82–89], for example, screening for reactive intermediates and assessing toxicity [90], with metabonomics [91,92] and toxicogenomics [93,94] being increasingly useful tools.

The main types of Averse Drug Reactions (ADRs) are summarized in Table 9 [15,25,96–98]. On-target ADRs result from an exaggerated response caused by drug overdosing or too high levels of an active metabolite; they are predictable in principle and generally dose-dependent, and are labeled as Type A. Off-target ADRs result from the interaction of the drug or a metabolite with a nonintended target such as a receptor or an enzyme. They also are predictable in principle and generally dose-dependent. These two types are pharmacological in nature and fall outside the scope of the present work. ADRs caused by reactive metabolites are the ones of greatest in our context. They involve covalent binding to macromolecules, and/or oxidative stress following the formation of reactive oxygen species (ROSs). These ADRs are predictable (or rationalizable) in terms of the drug's or metabolite's structure, and they are generally dose-dependent. They are often labeled as Type C. Idiosyncratic drug reactions (IDRs) (also known as Type B ADRs) are rare to very rare, unpredictable, and apparently dose-independent. They are also poorly understood, yet appear to be usually due to reactive metabolites.

Leaving aside on-target and off-target interactions, toxic responses are mostly due to reactive metabolites acting either directly on proteins and other macromolecules, or indirectly via reactive oxygen species, reactive nitrogen species (RNSs), and reactive carbonyl species (RCSs) [99–102]. Immunological reactions (not all of which should be classified as idiosyncratic) can be against self (autoimmunity) or against normal, harmless substances (allergy) [103–105]. In some cases, the immunotoxic drug or chemical acts as a hapten (by direct reaction with a protein). In most cases, however, the drug or chemical behaves as a prohapten since it needs metabolic activation to form adducts. In both cases, a hapten-carrier conjugate is formed that may elicit an immune response.

Table 9. Types and Mechanisms of Adverse Drug Reactions (ADRs) [15,25]

On-target ADRs	Predictable in principle and generally dose-dependent. Based on the pharmacology of the drug and its metabolite(s), often an exaggerated response or a response in a nontarget tissue
Off-target ADRs	Predictable in principle and generally dose-dependent. Resulting from the interaction of the drug or a metabolite with a nonintended target
ADRs involving reactive metabolites	Predictable in principle and generally dose-dependent. A major mechanism is covalent binding to macromolecules (adduct formation) resulting in cytotoxic responses, DNA damage, or hypersensitivity and immunological reactions. A distinct (and synergetic) mechanism is the formation of reactive oxygen species and oxidative stress
Idiosyncratic drug reactions (IDRs)	Unpredictable, apparently dose-independent, and rare (<1 in 5000 cases). They might result from a combination of genetic and external factors, but their nature is poorly understood. IDRs include anaphylaxis, blood dyscrasias, hepatotoxicity, and skin reactions

Drug- or chemical-induced cytotoxicity can occur through apoptosis or necrosis, molecular mechanisms being immune injury, adduct formation, oxidative stress, and/or DNA damage. Many organs are targets, but hepatotoxicity is of particular significance due to the high metabolizing capacity of the liver [106–111]. Other organs include the kidneys (nephrotoxicity), lungs and airways (pneumotoxicity), the central nervous system (neurotoxicity), reproductive organs, bone marrow (hematotoxicity), and the skin (sensitization). As for teratogenesis, a number of embryotoxic chemicals are believed to be proteratogens activated to reactive intermediates [112].

A look at toxicophoric groups (also called toxicophores, toxophores, or toxophoric groups, see Table 10) is particularly illustrative of the unity that unlies their chemical diversity [95,113–115]. Indeed, the toxic potential of many toxicophores is explained by their metabolic toxification to electrophilic intermediates or to free radicals. In more detail, the major functionalization reactions that activate toxophoric groups include oxidation to electrophilic intermediates, reduction to free radicals, and autooxidation with oxygen reduction that leads to superoxide, other reactive oxygen species, and reactive nitrogen species. The electrophilic intermediates and the free radicals then react with bio(macro) molecules, mainly proteins and nucleic acids, producing chemical lesions. ROSs also react with unsaturated and mainly polyunsaturated fatty acids in membranes and elsewhere, leading to lipid peroxidation. Of more recent awareness is the fact that some conjugation reactions may also lead to toxic metabolites, namely reactive acyl glucuronides or conjugates with deleterious physicochemical properties [15,25].

However, it would be wrong to conclude from the above that the presence of a toxophoric group necessarily implies toxicity. Reality is far less gloomy, as only potential toxicity is indicated. Given the presence of a toxophoric group in a compound, a number of factors will operate to render the latter either toxic or nontoxic:

(a) The molecular properties of the substrate will increase or decrease its affinity and reactivity toward toxification and detoxification pathways.

(b) Metabolic reactions of toxification are always accompanied by competitive and/or sequential reactions of detoxification that compete with the formation of the toxic metabolite and/or inactivate it. A profusion of biological factors control the relative effectiveness of these competitive and sequential pathways.

(c) The reactivity and half-life of a reactive metabolite control its sites of action and determine whether it will reach sensitive sites.

(d) Dose, rate, and route of entry into the organism are all factors of known significance.

Table 10. Major Toxophoric Groups and Their Metabolic Reactions of Toxification [15,25]

Functionalization reactions
• Some aromatic systems that can be oxidized to epoxides, quinines, or quinonimines (Fig. 5, reaction 1)
• Ethynyl moieties activated by cytochrome P450 (Fig. 5, reaction 3)
• Some halogenated alkyl groups that can undergo reductive dehalogenation (Fig. 3, reaction 7)
• Nitroarenes that can be reduced to nitro anion-radicals, nitrosoarenes, nitroxides, and hydroxylamines (Fig. 7, reaction 4)
• Some aromatic amides that can be activated to nitrenium ions (reaction 3 in Fig. 7, followed by reaction 3 in Fig. 18)
• Some thiocarbonyl derivatives, particularly thioamides, which can be oxidized to *S*,*S*-dioxide (sulfene) metabolites (Fig. 9, reaction 3)
• Thiols that can form mixed disulfides (Fig. 9, reaction 1)
Conjugation reactions
• Some carboxylic acids that can form reactive acylglucuronides (Fig. 21, reaction 3)
• Some carboxylic acids that can form highly lipophilic conjugates (Fig. 27, reactions 4 and 5)

(e) Above all, there exist essential mechanisms of survival value that operate to repair molecular lesions, remove them immunologically, and/or regenerate the lesioned sites.

In conclusion, the presence of a toxophoric group is not a sufficient condition for observable toxicity, a sobering and often underemphasized fact. Nor is it a necessary condition since other mechanisms of toxicity exist, for example the acute toxicity characteristic of many solvents.

4.2. Structure–Metabolism Relationships and the Prediction of Metabolism

Two classes of factors will influence qualitatively (how ? what ?) and quantitatively (how much ? how fast ?) the metabolism a given xenobiotic in a given biological system. The second class consists in the biological factors briefly presented below. The first class of factors are the various molecular properties that will influence a metabolic reaction, most notably (a) global molecular properties such as configuration (e.g., chirality), electronic structure, and lipophilicity and (b) local properties of the target sites such as steric hindrance, electron density, and reactivity. When speaking of a metabolic reaction, however, we should be clear about what is meant. Indeed, enzyme kinetics allows a metabolic reaction to be readily decomposed into a binding and a catalytic phase. In spite of its limitations, Michaelis–Menten analysis offers an informative approach for assessing the binding and catalytic components of a metabolic reaction [116]. Here, the Michaelis constant K_m represents the affinity, with V_{max} being the maximal velocity, k_{cat} the turnover number, and K_m/V_{max} the catalytic efficiency.

4.2.1. Chirality and Drug Metabolism The influence of stereochemical factors in xenobiotic metabolism is a well-known and reviewed phenomenon [117]. Because metabolic reactions can produce more than one response (i.e., several metabolites), two basic types of stereoselectivity are seen, namely substrate stereoselectivity and product stereoselectivity (see Section 1.3). Substrate stereoselectivity occurs when stereoisomers are metabolized differently (in quantitative and/or qualitative terms) and by the "same" biological system under identical conditions. Substrate stereoselectivity is a well-known and abundantly documented phenomenon under *in vivo* and *in vitro* conditions. In fact, it is the rule for many chiral drugs, ranging from practically complete to moderate. A complete absence of substrate enantioselectivity has seldom been seen.

Michaelis–Menten analysis suggests that the molecular mechanism of substrate enantioselectivity can occur in the binding step (different affinities K_m), in the catalytic step (different reactivities, V_{max}) or in both. There are cases in which stereoselective metabolism is of toxicological relevance. This situation can be illustrated with the antidepressant drug mianserin that undergoes substrate stereoselective oxidation. In human liver microsomes, mianserin (**44** in Fig. 36) occurred by aromatic oxidation with a marked preference for its (*S*)-enantiomer, while *N*-demethylation was the major route for the (*R*)-enantiomer. At low drug concentrations, cytotoxicity toward human mononuclear leucocytes was due to (*R*)-mianserin more than to (*S*)-mianserin, and showed a significant correlation with *N*-demethylation [118]. Thus, the toxicity of mianserin seemed associated with *N*-demethylation rather than with aromatic oxidation. The chemical nature of the toxic intermediates was not established.

Product stereoselectivity occurs when stereoisomeric metabolites are generated differently (in quantitative and/or qualitative terms) and from a single substrate with a suitable prochiral center or face. Examples of metabolic pathways producing new centers of chirality in substrate molecules include ketone reduction, reduction of carbon–carbon double bonds, hydroxylation of prochiral methylenes, oxygenation of tertiary amines to *N*-oxides, and oxygenation of sulfides to sulfoxides. Product stereoselectivity may be due to the action of distinct isoenzymes, or it may result from different binding modes of a prochiral substrate to a single isoenzyme. In this case each productive binding mode will bring another of the two enantiotopic or diastereotopic target groups in the vicinity of the

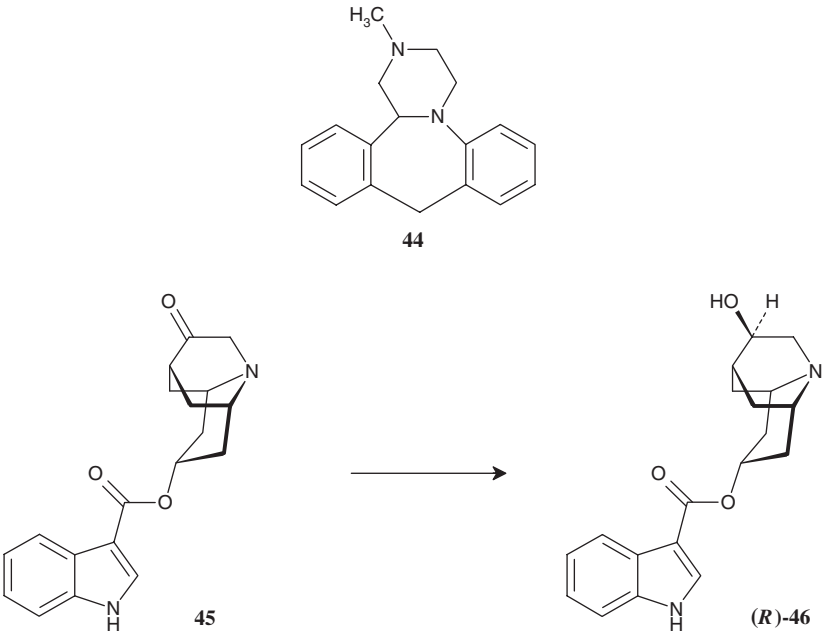


Figure 36. Mianserine (**44**), dolasetron (**45**), and its active alcohol metabolite (**46**).

catalytic site, resulting in diastereoisomeric enzyme–substrate complexes. Thus, the ratio of products depends on the relative probability of the two binding modes. In addition, the catalytic step, which involves diastereoisomeric transition states, may also influence or control product selectivity.

The potential pharmacological consequence of product-selective metabolism can be illustrated with the antiemetic 5-HT3 antagonist dolansetron (**45** in Fig. 36). This compound is prochiral by virtue of a center of prochirality at the keto group featuring two enantiotopic faces. This drug is reduced rapidly, extensively and stereoselectively in humans to its (*R*)-alcohol (**46** in Fig. 36) [119,120]. The enzymes involved are aldo-keto reductases (AKR1C1, 1C2, and 1C4) and carbonyl reductases. The metabolite proved to be manyfold more active than dolasetron, allowing the latter to be viewed as a prodrug. And significantly, the (*R*)-alcohol is markedly more active than its (*S*)-enantiomer.

It thus appears that substrate and product stereoselectivities are the rule in the metabolism of stereoisomeric and stereotopic drugs. But if the phenomenon is to be expected *per se*, it is not trivial to predict which enantiomer will be the preferred substrate of a given metabolic reaction, or which enantiomeric metabolite of a prochiral drug will predominate. This is due to the fact that the observed stereoselectivity of a given reaction will depend both on molecular properties of the substrate and on enzymatic factors (e.g., the stereoelectronic architecture of the catalytic site in the various isozymes involved). In fact, substrate and product stereoselectivities are determined by the binding mode(s) of the substrate and by the resulting topography of the enzyme–substrate complex.

4.2.2. Relations Between Metabolism and Lipophilicity Comparing the overall metabolism of numerous drugs clearly reveals a global relation with lipophilicity [116]. Indeed, there exist some highly polar xenobiotics known to be essentially resistant to any metabolic reaction, for example, saccharin, disodium cromoglycate, and zanamivir. Furthermore, many *in vivo* metabolic studies have demonstrated a dependence of biotransformation on lipophili-

city, suggesting a predominant role for transport and partitioning processes. A particularly illustrative example is offered by β-blockers, where the more lipophilic drugs are extensively if not completely metabolized (e.g., propranolol), whereas the more hydrophilic ones undergo biotransformation for only a fraction of the dose (e.g., atenolol).

This global trend is in line with the Darwinian rationale for xenobiotic metabolism, which is believed to have evolved in an animal–plant "warfare," with herbivores adaptating to the emergence of protective chemicals (e.g., alkaloids) in plants [121]. The exception to the global and direct relation between extent of metabolism and lipophilicity is offered by the vast number of human-made, highly lipophilic polyhalogenated xenobiotics, which now pollute our entire biosphere. Such compounds include polyhalogenated insecticides (e.g., DDT), polyhalogenated biphenyls, and dioxins, which have a strong propensity to accumulate in adipose tissues. In addition, these compounds are highly resistant to biotransformation in animals due in part to their very high lipophilicity, and in part to the steric shielding against enzymatic attack provided by the halo substituents.

When the results of Michaelis–Menten analyses are examined for quantitative structure–metabolism relationships (QSMRs), it is often found that lipophilicity correlates with K_m but not with V_{max} or k_{cat}. This is documented for ester hydrolysis and oxidation of various chemical series by monooxygenases and other oxidases [116]. Depending on the explored property space, the relationships between K_m and lipophilicity are linear or parabolic. Such results indicate that when relations are found between rate of metabolism and lipophilicity, the energy barrier of the reaction is largely similar for all compounds in the series, allowing lipophilicity to become the determining factor. A relevant example is provided by the CYP2D6-catalyzed oxidation of a series of fluorinated propranolol derivatives. Their K_m values spanned a 400-fold range and showed a weak but real correlation with the distribution coefficient. The same was true for the catalytic efficiency k_{cat}/K_m, but only because the k_{cat} values spanned a narrow sixfold range [122].

4.2.3. The Influence of Electronic Factors

Stereoelectronic properties may influence the binding of substrates to enzymatic active sites in the same manner as they influence the binding of ligands to receptors. For example, the K_m values of the above-mentioned propranolol derivatives were highly correlated with their basicity (pK_a), confirming that an ionic bond plays a critical role in the binding of amine substrates to CYP2D6 [122].

Electronic properties are of particular interest in structure–metabolism relationships since they control the cleavage and formation of covalent bonds characteristic of a biotransformation reaction (i.e., the catalytic step). Correlations between electronic parameters and catalytic parameters obtained from *in vitro* studies (e.g., V_{max} or k_{cat}) allow a rationalization of substrate selectivity and some insight into reaction mechanism.

An example of a quantitative SMR study correlating electronic properties and catalytic parameters is provided by the glutathione conjugation of *para*-substituted 1-chloro-2-nitrobenzene derivatives [123]. The values of $\log k_2$ (second order rate constant of the nonenzymatic reaction) and $\log k_{cat}$ (enzymatic reaction catalyzed by various glutathione transferase preparations) were correlated with the Hammett resonance σ-value of the substrates, a measure of their electrophilicity. Regression equations with positive slopes and r^2 values in the range 0.88–0.98 were obtained. These results quantitate the influence of substrate electrophilicity on nucleophilic substitutions mediated by glutathione, be they enzymatic or nonenzymatic.

Quantum mechanical calculations may also shed light on SMRs, revealing correlations between rates of metabolic oxidation and energy barrier in cleavage of the target C−H bond [124].

4.2.4. 3D-QSMRs and Molecular Modeling

When lipophilicity and electronic parameters are used as independent variables and a metabolic parameter (often assessing affinity) as the dependent variable, correlation equations are obtained for rather limited and/or related series of substrates, implying exploration of a relatively narrow structural diversity space. Also, the metabolic parameters are usually

Table 11. A Classification of *In Silico* Methods to Predict Biotransformation [14,21]

(A) "Local" methods

Methods applicable to series of compounds with "narrow" chemical diversity, and/or to biological systems of "low" complexity:

- QSAR: *linear, multilinear, multivariate* (may predict affinities, relative rates, and so on, depending on the physicochemical properties considered)
- Quantum mechanical: *MO methods* (may predict regioselectivity, mechanisms, relative rates, etc.)
- 3D-QSAR: *CoMFA*™, *Catalyst*™, *GRID/GOLPE*™, for example (may predict substrate behavior, relative rates, inhibitor behavior, etc.)
- Molecular modeling and docking (may predict substrate or inhibitor behavior, regioselectivity, etc.)
- Experts systems combining docking, 3D-QSAR and MO: *MetaSite*™, and so on

(B) "Global" methods

Methods applicable to series of compounds with "broad" chemical diversity (*and, in the future, to biological systems of "high" complexity*):

- "Meta"-systems combining (A) docking, 3D-QSAR, MO, and B) a number of enzymes and other functional proteins: *MetaDrug*™, and so on
- Databases: *Metabolite*™, *Metabolism*™, and so on (allow metabolites to be deduced by analogy)
- Rule-based expert systems: *Meta*™, *MetabolExpert*™, *Meteor*™, and so on (may predict metabolites, metabolic trees, reactive/adduct-forming metabolites, relative importance of these metabolites)

obtained from relatively simple biological systems, resulting in QSMR correlations that are typically "local" methods (Table 11) of low extrapolative capacity.

Quantum mechanical methods are also classified as local in Table 11. Here, a word of caution is necessary, since such methods are in principle applicable to any chemical system. However, they cannot handle more than one metabolic reaction or catalytic mechanism at a time, and as such can only predict metabolism in simple biological systems, in contrast to the global methods presented below.

Three-dimensional (3D) methods are also of value in SMRs, namely 3D-QSARs and the molecular modeling of xenobiotic-metabolizing enzymes (Table 11). Indeed, they represent a marked progress in predicting the metabolic behavior (be it as substrates or inhibitors) of novel compounds. An obvious restriction, however, is that each enzyme requires a specific model. 3D-QSMRs methods yield a partial view of the binding/catalytic site of a given enzyme as derived from the 3D molecular fields of a series of substrates or inhibitors (the training set). In other words, they yield a "photographic negative" of such sites, and will allow a quantitative prediction for novel compounds structurally related to the training set. Two popular methods in 3D-QSARs are CoMFA (comparative molecular field analysis) and catalyst. Numerous applications can be found in the literature.

The same is true for the molecular modeling of xenobiotic-metabolizing enzymes, which affords another approach to rationalize and predict drug–enzyme interactions. The methodology of molecular modeling is explained in detail elsewhere in this work and will not be presented here. Suffice it to say that its application to drug metabolism was made possible by the crystallization and X-ray structural determination of the first bacterial cytochromes P450 in the mid-1980s [125], followed a few years ago by human cytochromes P450 [126,127]; the crystal structure of numerous other drug-metabolizing enzymes is also available. As more and more amino acid sequences of drug-metabolizing enzymes become available, their tertiary structure can be modeled by homology superimposition with the experimentally determined template of a closely related protein (homology modeling). Known substrates and inhibitors can then be docked *in silico*. Given the assumptions made in homology modeling and in scoring functions, such models cannot give quantitative affinity predictions. However, they can afford

fairly reliable yes/no answers as to the affinity of test set compounds, and in favorable cases may also predict the regioselectivity of metabolic attack (Table 11).

The last approach among "local" systems are expert systems combining (a) 3D-models obtained by molecular modeling and (b) sophisticated QSAR approaches based on multivariate analyses of parameters obtained from molecular interaction fields (MIFs), as found in the MetaSite algorithm [128,129]. MetaSite is a specific system in the sense that it is currently restricted to the major human cytochromes P450. At the end of the procedure the atoms of the substrate are ranked according to their accessibility and reactivity. In other words, MetaSite takes the 3D stereoelectronic structure of both the enzyme and the ligand into account to prioritize the potential target sites of CYP-catalyzed oxidation in the molecule.

4.2.5. "Global" Expert Systems to Predict Biotransformation While medicinal chemists are not usually expected to possess a deep knowledge of the mechanistic and biological factors that influence drug metabolism, they will find it quite useful to have a sufficient understanding of structure–metabolism relationships to be able to predict reasonable metabolic schemes. A qualitative prediction of the biotransformation of a novel xenobiotic should allow (a) the identification of all target groups and sites of metabolic attack, (b) the listing of all possible metabolic reactions able to affect these groups and sites, and (c) the organization of the metabolites into a metabolic tree. The next desirable features would be a warning for potentially reactive/adduct-forming metabolites, and a bridge to a toxicity-predicting algorithm.

Given the available information, there exists an ever increasing interest in expert systems hopefully able to meet the above goals [130–132]. A few systems of this type are now available, for example a system known as MetaDrug combining (a) docking, 3D-QSAR, MO and (b) a number of enzymes and other functional proteins such as transporters [133,134]. Metabolic databases allow reasoning by analogy and can prove quite useful [130]. Rule-based systems are perhaps the most versatile; they include Meta [135,136], MetabolExpert [137,138], and Meteor [139–141]. Such systems will make correct qualitative or even semiquantitative predictions for a number of metabolites, but the risk of false positives must be taken seriously. This is due to the great difficulty of devising efficient filters to remove unlikely metabolites, based on the molecular properties of substrates. Taking biological factors (tissues, animal species, etc.) into account, is still far away.

4.2.6. Biological Factors Influencing Drug Metabolism A variety of physiological and pathological factors influence xenobiotic metabolism and hence the wanted and unwanted activities associated with a drug. This issue complicates significantly the task of medicinal chemists, as optimizing the pharmacokinetic property of a given lead for behavior in laboratory animals often results in clinical failure. While it is not the objective of this chapter to discuss biological factors in any depth, a brief overview will nevertheless be presented for the sake of clarity and to help medicinal chemists prioritize the challenges they face. A structured, comprehensive and amply illustrated discussion of these factors can be found in recent works [15,26,27].

It can be helpful to distinguish between interindividual and intraindividual factors that can influence the capacity of an individual to metabolize drugs (Table 12). The interindividual factors are viewed as remaining constant throughout the life span of an organism and are the expression of its genome. In contrast, the intraindividual factors may vary depending on time (age, even time of day), pathological states, or external factors (nutrition, pollutants, drug treatment).

The most significant interindividual factor to be considered in early drug development is clearly the species-related differences. The use of different species as a surrogate for investigating and predicting metabolism in humans has been and remains an indispensable step in drug development. In parallel, there has been a steady switch during early phases of drug discovery from obtaining metabolism data with animal tissues and whole animals to screening studies with human

Table 12. Biological Factors Affecting Xenobiotic Metabolism [15,26,27]

Interindividual Factors	Intraindividual Factors
Constant for a given organism	*Variable during the lifetime of a given organism*
Animal species	Physiological changes: Age
	Biological rhythms
	Pregnancy
Genetic factors (genetic polymorphism)	Pathological changes: Disease
	Stress
Sex	External influences: Nutrition
	Enzyme induction by xenobiotics
	Enzyme inhibition by xenobiotics

cDNA expressed enzymes, cell fractions (microsomes), and cells. Nevertheless, a greater understanding of the molecular, genetic, and physiological differences between humans and animal models is still required for correctly interpreting pharmacological safety studies required by regulatory agencies [142].

Genetic factors may result in polymorphic biotransformations that may cause serious problems at the stage of clinical trials and postmarketing. As much as possible, lead optimization may try to avoid affinity for cytochromes P450 known to exhibit phenotypic differences. After the specific CYP(s) involved in the metabolism of a drug are known, how does this information relate to the overall metabolism of a drug in a diverse population? The ability for an individual to metabolize a drug is dependent on the nature (genotype), location and amount of enzyme present. Studying such factors is the field of pharmacogenetics and pharmacogenomics. At the level of populations, ethnopharmacology investigates the differences in proportions of "normal" and slow metabolizers observed in different populations, a study complicated by the unavoidable influence of external factors such as nutrition and lifestyle cannot be excluded. Comparable arguments are valid for sex-related differences in metabolism, which in humans are known for a limited number of drugs only [143].

Intraindividual factors are numerous and for some difficult to investigate. A major one is age, since differences between the levels of metabolism enzymes for the fetal and neonatal (first four weeks postpartum) liver versus the adult liver have been observed in both animals and humans [144]. The problem is also significant for the growing population of elderly patients, where a marked reduction is usually observed. The origin of such differences is quite complex and often related to other physiological changes such as reduction in liver blood flow and liver size. As the number of older individuals increase in the population, especially those aged 80–90 years, adaptations in drug therapy and posology as related to metabolism become increasingly important.

There is increasing evidence that drug metabolism may undergo some variations due to diurnal, monthly and yearly rhythms. Thus, it is well established that nearly all physiological functions and parameters can vary with the time of day, for example, heart rate, blood pressure, hepatic blood flow, urinary pH, and plasma concentrations of hormones, other signal molecules, glucose, and plasma proteins [145].

A number of diseases may sometimes influence drug metabolism, although no generalization appears in sight. First and above all, diseases of the liver will have a direct effect on enzymatic activities in this organ, for example, cirrhosis, hepatitis, jaundice, and tumors. Diseases of extra-hepatic organs such as the lungs and the heart may also affect hepatic drug metabolism by influencing blood flow, oxygenation, and other vital functions. Renal diseases have a particular significance due to a decrease of the intrinsic xenobiotic-metabolizing activity of the kidneys, and a decreased rate of urinary excretion. Infectious and inflammatory diseases have been associated with differential expression of various CYP enzymes.

Interference with the metabolism of a drug by inhibition of the primary enzymes involved in its metabolism can lead to serious side-effects or therapeutic failure. Inhibitors of the CYP enzymes are considered to be

the most problematic, being the primary cause of drug–drug and drug–food interactions [16,146].

A comparable situation is created by enzyme induction, that is, when the amount and activity of a metabolizing enzyme has increased following exposure to a drug or chemical. In contrast to inhibition, induction is generally a slow process caused by an increase in the synthesis of the enzyme. A drug that causes induction may increase its own metabolism (autoinduction) or increase metabolism of another drug. Clinically, drug induction is considered less of a problem than drug inhibition, but there are some marked exceptions. Primary interest has been directed toward the CYP enzymes for which a number of classes of inducing agents have been identified.

5. CONCLUDING REMARKS

Drug discovery and development are becoming more complex by the day, with physicochemical, pharmacokinetic and pharmacodynamic properties being screened and assessed as early and as simultaneously as possible. But the real challenge lies with the resulting deluge of data, which must be stored, analyzed and interpreted. This calls for a successful synergy between humans and algorithmic machines, in other words between human and artificial intelligence. However, making sense of the data, interpreting their analyses, and rationally planning subsequent steps is an entirely different issue. The difference is a qualitative one, and it is the difference between information and knowledge.

This chapter is about both. Our first objective in writing it was obviously to supply useful information, namely structured data as exemplified by the classification of metabolic reactions (Sections 2 and 3).

Information becomes knowledge when it is connected to a context from which it receives meaning. This treatise is about medicinal chemistry, and indeed medicinal chemistry is the context of our chapter and of all others. By discussing the connection between drug metabolism and the medicinal chemistry context (Section 4), we have tried to be true to our second objective that was to present medicinal chemists with meaningful information. Had this chapter been written for a treatise of molecular biology, much of the basic information would have been the same, but the context and the chapter's meaning would have been different. This also tells us that medicinal chemistry itself needs a context to acquire meaning, the context of human welfare to which responsible medicinal chemists are proud to contribute [147].

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