10 Role of Phase I Metabolism in Drug Activation and Clinical Treatment Outcomes

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10.1 SUMMARY

This chapter explores the relation between drug biotransformation and pharmacological activity. More precisely, it surveys the formation of active metabolites and exemplifies cases of metabolites as or more active than the parent drug. Because very few drug conjugates are pharmacologically active, the focus is on metabolic reactions of functionalization (i.e., phase I), namely, oxidations, reductions, or hydrolyses.

The first section sets the scene by explaining the *pharmacokinetic/pharmacodynamic (PK/PD) interplay* that underlies drug action, and the existing continuum ranging from drugs devoid of active metabolites, to drugs yielding some active metabolite(s), to prodrugs whose activity is due solely to the metabolite. *Soft drugs* are briefly exemplified since they nicely illustrate the concept of drugs designed to be rapidly metabolized to inactive, atoxic metabolites.

The second section is dedicated to drugs whose activity depends in part on the presence of one or more active metabolite(s). Thus, the drug's action can be prolonged, as exemplified with *diazepam* and some other benzodiazepines. Alternatively, a drug's

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pharmacological spectrum can be broadened by a metabolite; witness the antinociceptive activity of *codeine*, which is essentially due to its metabolite morphine. In another scenario, both the drug and its metabolite contribute comparatively to the clinical effect (e.g., *tramadol*). Finally, a drug such as *tamoxifen* is metabolized to one or more highly active metabolite(s).

The third section covers carrier-linked prodrugs, the examples presented therein having been selected to illustrate significant objectives in prodrug design. Thus, *fospropofol* was developed with the aim of overcoming a pharmaceutical hurdle, namely the low solubility of propofol. The lack of oral absorption of the anti-influenza agent Ro-64-0802 was overcome using simple physicochemical principles, namely, by masking its highly hydrophilic carboxylate group with a bioreversible ester function to yield *oseltamivir*. Another strategy to improve oral absorption is by targeting intestinal transporters, as illustrated with *valacyclovir*. The last example, *capecitabine*, exemplifies a more ambitious objective, namely, tissue targeting via organ-selective activation.

The fourth section deals with another class of prodrugs known as *bioprecursors*. Here, metabolic activation is by redox modification of a functional group, without cleavage of a promoiety. Four examples are presented, namely, *nabumetone*, a long-acting non-steroidal anti-inflammatory (NSAI) prodrug with low gastric toxicity, the two anti-aggregating agents *clopidogrel* and *prasugrel*, whose bioactivation unmasks a highly reactive sulfenic acid group, and *tirapazamine*, an antitumor bioprecursor activated by reduction to a DNA-damaging radical. Clopidogrel is treated in some detail to illustrate the influence of genetic factors on clinical outcomes.

The fifth section briefly illustrates drug–drug interactions (DDIs) at the metabolic level, taking the antiretroviral protease inhibitors (PIs) as a case in point. A concluding section then completes the chapter.

10.2 BACKGROUND

To open this chapter, we note that the continuously increasing significance of drug metabolism investigations in drug discovery and development cannot be fortuitous [1–9]. The pharmacological and toxicological consequences of drug metabolism account for this phenomenon, which simultaneously drives, and is driven by, the many methodological, factual, and conceptual advances in this discipline. In a perspective of drug discovery, a number of metabolites of established drugs were found to have comparable or improved therapeutic properties compared to their parent and have become useful drugs in their own right. In addition to the examples discussed in the text, one can single out desloratadine (from loratadine), cetirizine (from hydroxyzine), fexofenadine (from terfenadine), and oxazepam (from diazepam). Even more significant is the discovery of paracetamol, which has replaced phenacetin, its more toxic parent. As a result, the pharmacological activity of metabolites even plays a role in bioequivalence studies [10].

10.2.1 The PK–PD interplay

Basically, there are two types of interactions between bioactive compounds and biological systems [6,11]. A drug acting on a biological system elicits a pharmacological and/or a toxic response, in other words a *pharmacodynamic (PD) event*. In the present

context, we discriminate between pharmacological activities (in effect, wanted and/or favorable PD effects) and toxic effects (in fact, all adverse effects a drug or chemical can elicit). In concert with PD events, the biological system "acts" on the xenobiotic by absorbing, distributing, metabolizing, and excreting it. These are the *pharmacokinetic (PK) events*. Most of these processes are mediated by enzymes or transporters and are active in the sense that they consume energy produced by the biosystem; others in contrast are passive.

Significantly, there is a mutual interdependence between PD and PK events, since it is well known that PK events such as metabolic activation or inactivation influence PD responses. This is the main focus of this chapter. Symmetrically, the PK behavior of a xenobiotic can be markedly influenced by the overexpression of metabolizing enzymes (a typical PD response) as mediated by substrates or other xenobiotics binding to nuclear receptors [12,13]. Such a situation is exemplified in Section 10.6.

10.2.2 Drugs, Metabolites, and Bioactivities—A Continuum

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 [1], an implicit meaning we also adopt. Here, we outline the various combinations of activities to be illustrated, (i) from intrinsically active drugs having no active metabolite to (ii) intrinsically active drugs having one or more active metabolites to (iii) intrinsically inactive "drugs" (i.e., prodrugs), whose therapeutic effects necessitate metabolism to an active metabolite (i.e., bioactivation). For example, sedative-hypnotic benzodiazepines fall in two categories [14]. Some have no active metabolite, for example, the 3-hydroxylated benzodiazepines such as lorazepam, oxazepam, and temazepam, which undergo O-glucuronidation and cleavage reactions. Other intrinsically active benzodiazepines such as diazepam have one or more active metabolite(s), sometimes long-acting ones. In a quantitative perspective, some drugs have a metabolite of comparable activity (e.g., tramadol), while some others have one or more highly active ones (e.g., tamoxifen). The extreme case of prodrugs will be treated separately.

10.2.3 Bioactivation and Metabolic Conjugation

This chapter is dedicated to drug activation by phase I reactions. As a reminder, these imply the creation or modification of a functional group in a substrate molecule by reactions of oxidation or reduction (redox reactions catalyzed by oxidoreductases, EC 1) or reactions of hydrolysis catalyzed by hydrolases (EC 3) [4,5,15].

The reason for not including phase II reactions (i.e., conjugation reactions catalyzed mainly by transferases EC 2) is that only a very small number of drug conjugates are pharmacologically active (i.e., having a wanted and/or favorable PD effect). A frequently cited 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 [16].

An intriguing and very rare reaction of conjugation occurs for *minoxidil*, an hypotensive agent that also stimulates hair growth. This drug is an *N*-oxide, and the actual active form responsible for the different therapeutic effects is the stable *N*-*O*-sulfate ester [17].

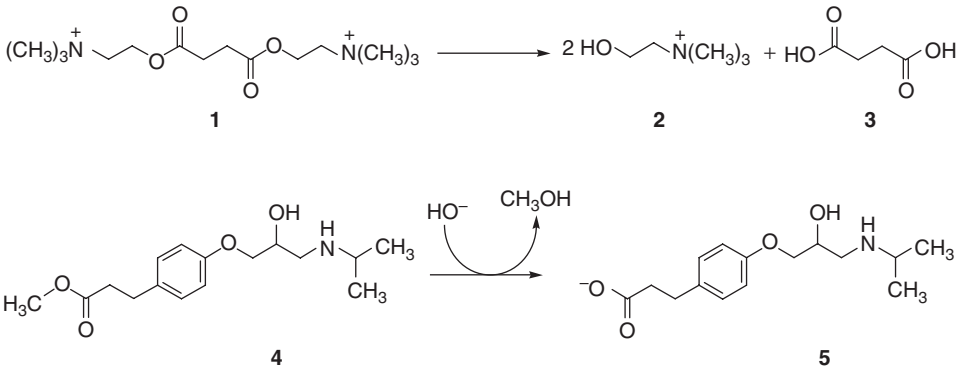

Figure 10.1 Examples of soft drugs, namely, the curarimimetic succinylcholine (**1**), which is hydrolyzed to choline (**2**) and succinic acid (**3**), and the β-blocker esmolol (**4**), whose metabolite is inactive due to the unmasking of a hydrophilic carboxylate group.

10.2.4 Drugs Having No Active Metabolite

An example of drugs designed to have neither active nor toxic metabolites is that of *soft drugs*, a concept pioneered and extensively developed by Bodor and Buchwald [18]. In practical terms, soft drugs (i) are bioisosteres of known drugs, (ii) contain a labile bridge (often an ester group), and (iii) 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 of a soft drug is *succinylcholine* (**1** in Fig. 10.1), 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 (**2**) and succinic acid (**3**) by plasma cholinesterase with a half-life of about 4 min [19,20].

Soft drugs may be found in a variety of therapeutic classes. Thus, a valuable class of soft β-blockers are aryloxypropanolamines, which possess an ester group in the para-position, as illustrated by the clinically useful *esmolol* (**4**) [21]. In this compound, as in others, the para-substituent has an intermediate polarity compatible with good receptor affinity, but hydrolysis to its metabolite **5** unmasks a carboxylate group, whose high polarity is incompatible with receptor affinity. The *in vitro* half-life of esmolol in whole blood was about 2, 13, and 23–27 min in rats, dogs, and humans, respectively. In patients, recovery from β-blockade began 2 min after discontinuation of infusion and was complete at about 18 min.

Paracetamol (**6** in Fig. 10.2; also known as *acetaminophen*) is another example of a drug having no active metabolite, but we mention it here to highlight how unexpected findings remain possible even with highly popular drugs. Recently indeed, evidence has begun to challenge the general belief by suggesting that part of the clinical effect of paracetamol might in fact be contributed by an unusual metabolite [22]. One of the pain pathways and its inhibition involve vanilloid receptors, including the capsaicin receptor (transient receptor potential cation channel, subfamily V, member 1; TRPV1). This receptor shows a marked affinity for metabolites of arachidonic acid (the so-called endovanilloids), the best known of which is anandamide. Intriguingly,

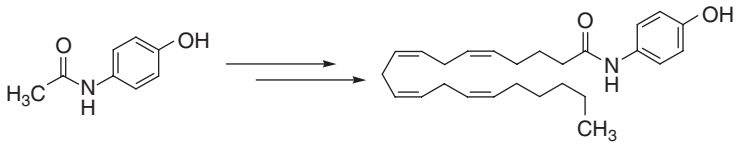

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Figure 10.2 Paracetamol (**6**) and its recently discovered metabolite *N*-arachidonoyl-4-hydroxyaniline (**7**).

N-arachidonoyl-4-hydroxyaniline (**7**), a known metabolite of paracetamol and an analog of anandamide, was shown to have a potent inhibitory effect on TRPV1. However, the clinical contribution of this metabolite in patients administered paracetamol remains to be understood.

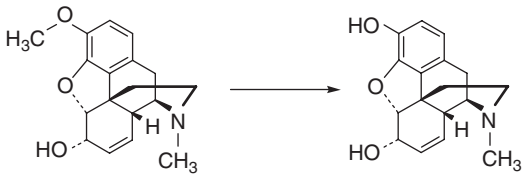
10.3 DRUGS WITH ACTIVE METABOLITES

Drugs having both intrinsic activity and active metabolite(s) may be more numerous than generally believed, but information is not always available. In this section, we examine a number of cases chosen for the different perspectives they allow on this aspect of pharmacotherapy.

10.3.1 Benzodiazepines, Drugs With or Without Active Metabolites

As mentioned above, benzodiazepine drugs can be classified into two groups, those having one or more active metabolite(s) and those having none. Criteria of half-life and duration of action allow further discrimination among these drugs [23–27]. Thus, *midazolam* and *triazolam* both have a short half-life in humans, as have their active metabolites *1-hydroxymidazolam* and *1-hydroxytriazolam*, respectively. As a result, these two drugs have a short duration of action, which makes them good short-acting hypnotics.

Benzodiazepines of longer duration of action are useful as sedatives or antiepileptics. Three examples are shown here, each of which has a different story to tell. *Clorazepate* is unusual among benzodiazepines due to the presence of its carboxylate group. Its metabolism is by decarboxylation to the long-acting *desmethyldiazepam*, followed by 3-hydroxylation to *oxazepam*. The drug does have affinity for the benzodiazepine receptor [26], but it appears incapable of crossing the blood–brain barrier and is rapidly metabolized to these two active metabolites [28]. In other words, it is a prodrug not because of lack of target affinity but for its incapacity to reach the target organ. *Diazepam* is also a sedative with a long, biphasic half-life, but one that acts both directly and via its three active metabolites, *desmethyldiazepam*, 3-hydroxydiazepam (*temazepam*), and *oxazepam. Flurazepam* is marketed mainly as a hypnotic, and indeed, its half-life is very short, the drug having disappeared from plasma 3 h after an oral dose to human volunteers [29]. An active metabolite is N^1-(2-hydroxyethyl)flurazepam, which is eliminated within a few hours. However, its N(1)-dealkylation produces *desalkylflurazepam*, a close analogue of desmethyldiazepam with an even longer half-life. As a result, flurazepam is unsuitable as a hypnotic in individuals who produce high levels of desalkylflurazepam.



8 **9**

Figure 10.3 Codeine (**8**) and its active metabolite (**9**) produced by a CYP2D6-catalyzed reaction of O-demethylation.

10.3.2 Codeine, a Drug With a Differently Acting Metabolite

There are two main reasons why *codeine* (**8** in Fig. 10.3) is of significance in our context. First because the production of *morphine* (**9**), its active metabolite, is under strong genetic control [30]. And second, because there is a qualitative difference in the activity of drug and metabolite, in contrast with many known cases of active metabolites.

While codeine *per se* is an antitussive agent, it is often prescribed as an analgesic of medium strength. About two decades ago, reports began to appear on the lack of analgesic response in some patients administered codeine [31]. This fact was correlated with barely detectable plasma levels of morphine in some patients subsequently phenotyped as "poor metabolizers," an obsolete terminology to describe individuals lacking a given drug-metabolizing activity. The culprit enzyme, in this case, is cytochrome P450 2D6 (CYP2D6), the affected patients (ca. 5–8% of the population) having nonfunctional *CYP2D6* alleles [12].

The story does not end here, however, since there is now recent evidence that a few individuals suffered from morphine intoxication following codeine intake [32–34]. The postoperative death of a healthy two-year-old boy was also traced to morphine intoxication following normal dosage of codeine [35]. The cause of overproduction of morphine in these patients is also genetic, in this case, gene duplication or multiplication resulting in three or more functional *CYP2D6* alleles.

10.3.3 Tramadol, a Drug With a Metabolite of Comparable Activity

Tramadol (**10** in Fig. 10.4) is a frequently used centrally acting analgesic characterized by a high efficacy, low potential for dependence or abuse, and low level of side effects (no relevant respiratory depression or effects on blood pressure and heart rate) [36–39]. This drug is particularly worthy of note for its dual mechanism of action, being both a weak opioid agonist and an inhibitor of monoamine neurotransmitter reuptake. For good reasons, the drug is used as the racemic mixture of two of its four stereoisomers, both its (−)-(*1S,2S*)- and (+)-(*1R,2R*)-enantiomers contributing synergetically to its *in vivo* activity.

In experimental pharmacology, (+)-tramadol proved a more active μ-receptor agonist than its (−)-enantiomer, and it also inhibited serotonin reuptake. In contrast, the (−)-enantiomer is an inhibitor of noradrenaline reuptake in addition to its weaker opioid effects. Furthermore, both enantiomers are metabolized by CYP2D6 to the corresponding *O-desmethyltramadol* enantiomers (**11**) with little or no substrate enantioselectivity. Both enantiomers of *O*-desmethyltramadol are more active than (+)-tramadol as μ-receptor agonists, but their blood–brain barrier permeation is lower. It is noteworthy

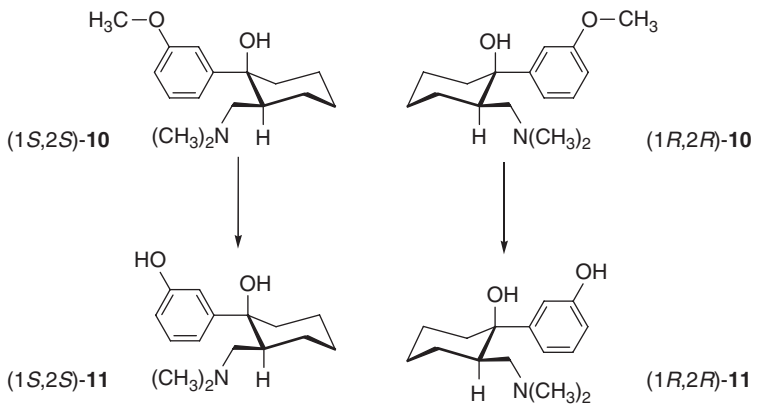


Figure 10.4 The two (active) enantiomers of *trans*-tramadol (**10**) and their even more active *O*-desmethyl metabolites.

that to the best of our knowledge, no effect on monoamine neurotransmitter reuptake has been reported for the metabolites. In clinical pharmacology, tramadol-induced analgesia is thus found to result from the combined and synergetic effects of four active entities. And while CYP2D6-deficient patients produce very little *O*-desmethyltramadol, tramadol activity is weaker but not absent in such patients [30,34].

10.3.4 Tamoxifen, a Drug Having Highly Active Metabolites

The complex and clinically highly relevant case of *tamoxifen* (**12** in Fig. 10.5) is summarized here. This estrogen receptor antagonist is extensively used for endocrine

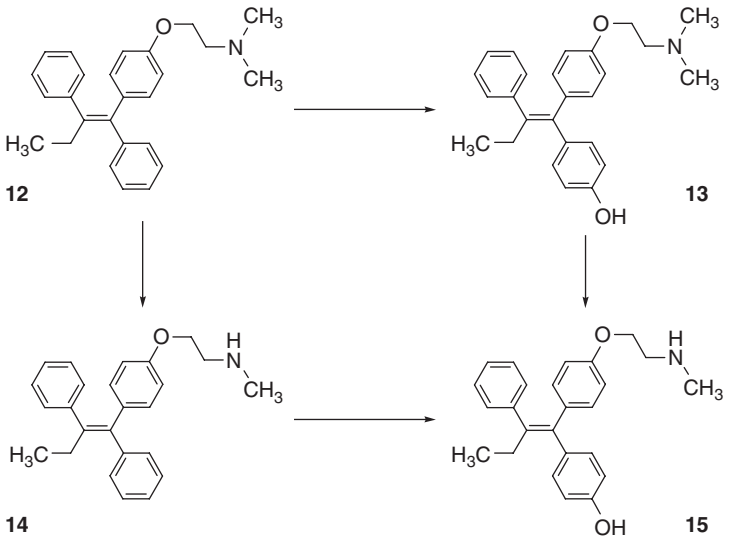


Figure 10.5 Tamoxifen (**12**) and its highly active metabolites 4-hydroxytamoxifen (**13**) and *N*-desmethyl-4-hydroxytamoxifen (**15**; endoxifen).

treatment of breast cancer [40]. Its metabolism is quite complex and leads to at least 13 oxidative metabolites [41,42], not to mention conjugation reactions. A pharmacologically significant route is aromatic oxidation to the active *4-hydroxytamoxifen* (**13**). However, the most important metabolic pathway in qualitative and quantitative terms is N-demethylation to the secondary amine *N*-desmethyltamoxifen (**14**), 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 (**15**) known as *endoxifen*.

A number of investigations have demonstrated that 4-hydroxytamoxifen and endoxifen are equipotent and many times more active as antiestrogens than the parent drug [43,44]. When taking plasma levels in consideration, it seems that the main contributor to therapeutic activity in patients is endoxifen, and to a lesser extent tamoxifen and 4-hydroxytamoxifen [45–47]. This has led to concerns that genotypic differences in CYP2D6 and the coadministration of drugs inhibiting this enzyme may have an impact on clinical outcomes in women treated with tamoxifen [48].

10.4 CARRIER-LINKED PRODRUGS

10.4.1 Introduction

What makes prodrugs different from other drugs is the fact that they are devoid of intrinsic pharmacological activity, as clearly defined by Adrien Albert [49], who coined the term. As such, prodrugs are the opposite of soft drugs (see above). *Intentional prodrugs* are by far the most frequent ones in drug research and development and are obtained by rational derivatization or modification of a known active agent. A historically important example is that of the *fortuitous prodrug prontosil*, whose *in vivo* antibacterial properties were discovered in 1932 by Gerhard Domagk, winning him the 1939 Nobel Prize in Medicine [50]. But the prodrug nature of prontosil remained unknown until 1935, when the discovery of its active metabolite sulfanilamide was the milestone that led to the creation of all antibacterial sulfonamides [51].

The prodrug concept aims at helping medicinal chemists and pharmaceutical scientists to overcome a number of development problems unsolvable by analog design [52–56]. Overcoming such hurdles translates into a number of *objectives in prodrug design*. Pharmaceutical objectives imply solving serious formulation problems caused by poor solubility, insufficient chemical stability, or poor organoleptic properties. PK objectives are currently the most important ones in prodrug research. Foremost among these is a need to improve oral bioavailability by enhancing the oral absorption of the drug and/or decreasing its presystemic metabolism; other objectives are to improve parenteral absorption, to lengthen the duration of action of the drug by slow metabolic release, and finally to achieve organ/tissue-selective delivery.

PD objectives often imply decreasing systemic toxicity, including the masking of a reactive agent, the *in situ* activation of a cytotoxic agent, and more generally improving a drug's therapeutic index. As a note of caution, it should be clear from the onset that prodrug objectives are often strongly intertwined. As demonstrated by the examples below, the prodrug concept may be a valuable strategy to disentangle PK and PD optimization in the drug discovery phases.

This section deals with prodrugs composed of the drug molecule coupled to a promoiety (also known as a *carrier*). Here, bioactivation occurs by cleavage of the

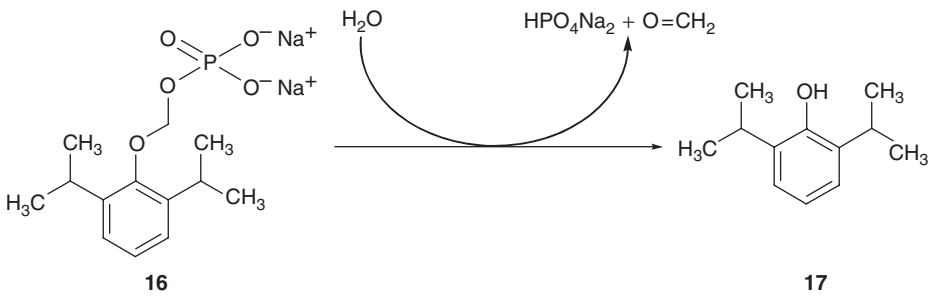


Figure 10.6 Fospropofol (**16**), a water-soluble, marketed prodrug of the sedative-hypnotic drug propofol (**17**).

link between drug and carrier, in most cases due to hydrolysis. Section 10.5 considers redox-activated prodrugs (bioprecursors).

10.4.2 Fospropofol, a Product for Improved Solubility

Propofol (**17** in Fig. 10.6) is a sedative-hypnotic, whose mechanism of action is based on an allosteric modulation of $GABA_A$ receptors, causing increased binding of γ-aminobutyric acid, its physiological ligand [57–59]. propofol provides sedation to patients undergoing uncomfortable interventions such as bronchoscopy or colonoscopy. It is also used for induction and maintenance of general anesthesia and in intensive care units for short or longer term sedation of medically ventilated adults.

Despite its advantages and extensive use, propofol suffers from a number of disadvantages such as pain at the injection site, a narrow therapeutic window, and a steep concentration–response curve bridging moderate sedation to general anesthesia. More relevant in the present context is its very low water solubility. As a result, propofol is administered intravenously as a lipid emulsion, which calls for absolute sterility. The lipid-based emulsions of propofol are fairly stable but tend to separate with the addition of other chemicals and changes in temperature or pH [59]. A most worrisome complication is propofol infusion syndrome, a rare but life-threatening event resulting from the long-term administration of high doses of the drug [60]. The syndrome is characterized by metabolic acidosis, acute renal failure, rhabdomyolysis, hyperlipidemia, and cardiac dysfunction.

The *pharmaceutical problems* associated with the poor solubility of propofol have provided a worthy justification to the development of water-soluble prodrugs. *Fospropofol* (**16**; propofol *O*-methyl phosphate disodium) has emerged as the lead candidate for a water-soluble prodrug of propofol and has recently been approved for monitored anesthesia in adults undergoing diagnostic or therapeutic procedures [61–63]. The prodrug is very stable toward chemical hydrolysis [64] and is very rapidly hydrolyzed by hepatic and endothelial alkaline phosphatases. The products of the reaction are propofol, formaldehyde (which is rapidly converted to formate), and phosphate.

Our understanding of the PK/PD relations of fospropofol underwent severe regression when it was found that the bioanalytical assay used in all PK/PD studies published up to 2009 was inadequate and yielded unreliable propofol plasma concentrations [65]. As a result, six papers were withdrawn [66], yet some unaware readers might continue to rely on conclusions found in previously published reviews.

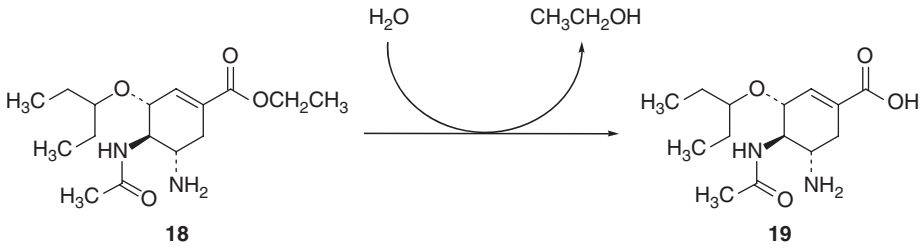


Figure 10.7 Oseltamivir (**18**), a blockbuster and an orally bioavailable prodrug of the anti-influenza agent oseltamivir carboxylate (**19**).

10.4.3 Oseltamivir, a Prodrug for Oral Absorption

Achieving improved oral absorption and generally oral bioavailability is a frequent rationale in marketed prodrugs [67,68]. Inhibitors of angiotensin-converting enzyme (ACE) such as benazepril and enalapril offer much-reviewed examples [69,70]. Here, we focus on two neuraminidase inhibitors of value against type A and B influenza in humans [71–73]. Target-oriented rational design led to highly hydrophilic agents that are not absorbed orally. One drug in clinical use is *zanamivir,* a highly hydrophilic drug administered in aerosol form.

Another active agent is Ro-64-0802 (**19** in Fig. 10.7; now known as *oseltamivir carboxylate*), which also shows high *in vitro* inhibitory efficacy but practically no oral bioavailability due to its high polarity [74,75]. In contrast, its ethyl ester prodrug *oseltamivir* (**18**) is well absorbed orally and undergoes rapid carboxylesterase 1 (CES1)-catalyzed hydrolysis *in vivo* to produce high and sustained plasma levels of the active agent [76–80]. In this example, the lack of oral absorption of the anti-influenza agent Ro-64-0802 was overcome with the help of a simple physicochemical principle, namely, the masking of a highly hydrophilic carboxylate group with a reversible ester function.

10.4.4 Valacyclovir, a Prodrug Targeting an Intestinal Transporter

Acyclovir (**21** in Fig. 10.8) is a nucleoside analogue used systemically and locally to treat herpes simplex virus (HSV-1 and -2) infections. It is also used in higher doses to treat infections from the varicella zoster virus (VZV). Acyclovir is a valuable drug, yet its oral absorption is modest (15–30%) [81]. This has lead to many potential prodrugs being prepared and examined, with the (*S*)-valyl ester being selected and now marketed as *valacyclovir* (**20**) [82,83].

This prodrug is of particular interest as its intestinal absorption is an active one mediated by the di-/tripeptide transporter PEPT1 [84], the resulting bioavailability of the active agent being ∼50–60%. This bioavailability is clearly less than expected, given the good affinity of valacyclovir for the transporter. And indeed stability studies have shown valacyclovir to undergo partial degradation in the upper lumen of the small intestine [85].

Practically, the totality of absorbed valacyclovir is activated to acyclovir by a serine hydrolase initially known as *biphenyl hydrolase-like protein* (BPHL). This enzyme, now characterized as human valacyclovirase, is specific for α-amino acid esters and

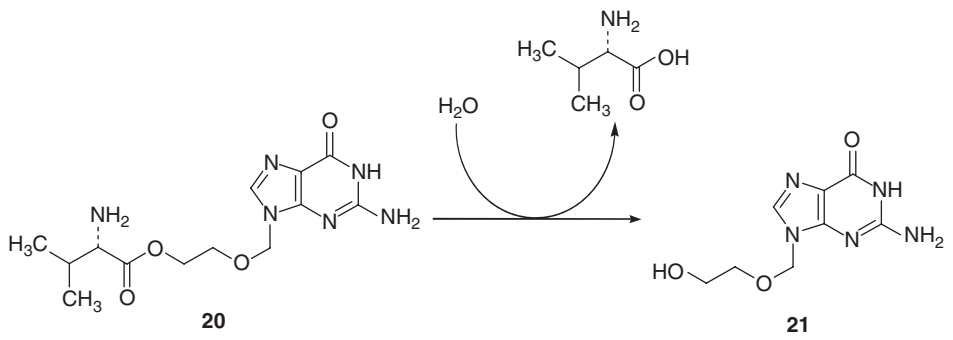


Figure 10.8 Acyclovir (**21**), a poorly absorbed antiherpes virus drug, and its (*S*)-valyl ester prodrug valacyclovir (**20**).

has been shown to hydrolyze the prodrugs of a broad range of antiviral and anticancer nucleoside analogs such as zidovudine and gemcitabine [86,87].

10.4.5 Capecitabine, a Prodrug for Targeted Delivery

The objective of organ- or tissue-selective delivery, also known as the search for the *magic bullet*, is receiving ever increasing interest in the tumor-selective delivery of anticancer agents. A clinically significant example is that of *capecitabine* (**22** in Fig. 10.9), a multistep, orally active prodrug of *5-fluorouracil* (**25**; 5-FU) [82,88]. Capecitabine is well absorbed orally and undergoes three activation steps resulting in high tumor concentrations of the active drug. It is first hydrolyzed by liver carboxylesterase (CES), the resulting metabolite being a carbamic acid that spontaneously decarboxylates to 5′-deoxy-5-fluorocytidine (**23**). The enzyme cytidine deaminase (EC 3.5.4.5), which is present in the liver and tumors, then transforms 5′-deoxy-5-fluorocytidine into 5′-deoxy-5-fluorouridine (**24**). Transformation into 5-FU (**25**) is catalyzed by thymidine phosphorylase (EC 2.4.2.4) and occurs selectively in tumor cells [89–93].

Capecitabine was first approved for the cotreatment of refractory metastatic breast cancer. Its therapeutic spectrum now includes metastatic colorectal cancer, and there are hopes that it might broaden further as positive results of new clinical trials become available. Capecitabine thus affords an impressive gain in therapeutic benefit compared to 5-FU because of its oral bioavailability and a relatively selective activation in (and thus delivery to) tumors.

10.5 BIOPRECURSORS

In contrast to the more traditional, carrier-linked prodrugs, bioprecursors do not contain a promoiety (i.e., a carrier moiety) but are activated enzymatically either by reduction or by oxidation, hence, their alternative designation as *redox-activated prodrugs* [94]. The bioactivation reaction creates either a *pharmacophoric group* (e.g., nabumetone) or a *chemically reactive group* (e.g., clopidogrel and tirapazamine).

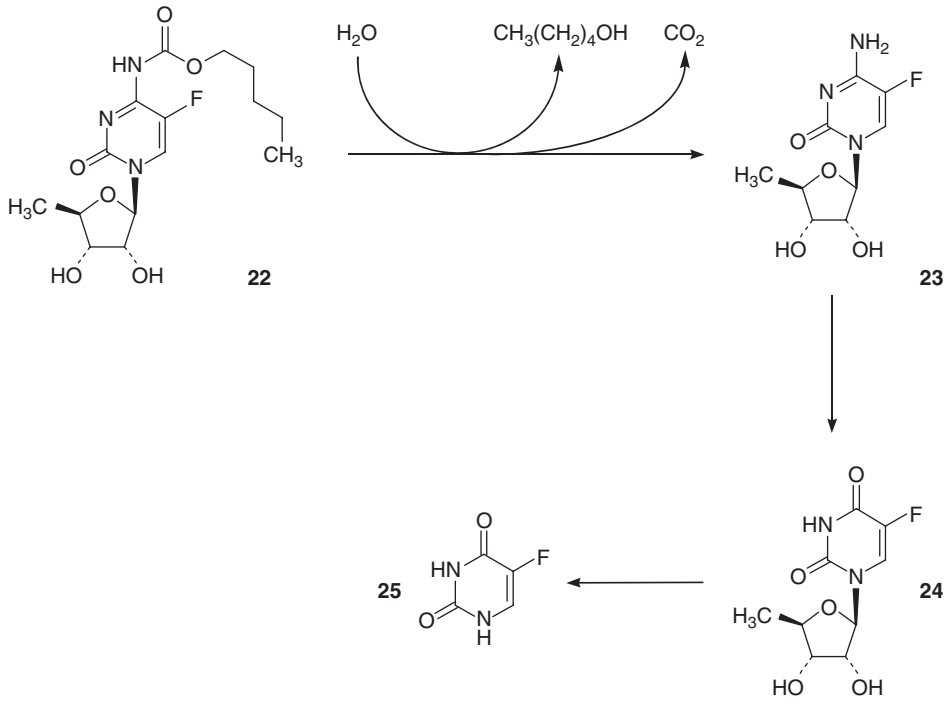


Figure 10.9 Capecitabine (**22**), a multistep, tumor-targeted prodrug of 5-fluorouracil (**25**).

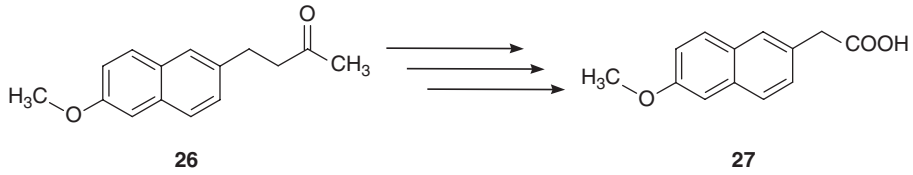


Figure 10.10 The bioprecursor nabumetone (**26**) and its metabolite 6-methoxynaphthylacetic acid (**27**), a long-acting nonsteroidal anti-inflammatory agent.

10.5.1 Nabumetone, a Long-Acting NSAI Prodrug with Low Gastric Toxicity

A rather complex and intriguing example of bioprecursors is afforded by *nabumetone* (**26** in Fig. 10.10). In humans and experimental animals, this prodrug undergoes three major initial metabolic pathways, namely, oxidative O-demethylation, carbonyl reduction, and a complex route likely involving oxidation of the terminal $-CH_3$ to $-COOH$, followed by coenzyme A conjugation and β-oxidation to *6-methoxynaphthylacetic acid* (**27**; 6MNA) [95,96]. The latter route is the major one in humans, where it accounts for at least one-third to one-half of a dose. 6MNA is the active metabolite of nabumetone. It is characterized by a very long serum half-life in humans (about 24 h, range 17–74 h), because of a slow hepatic clearance and absence of renal clearance [97,98]. The anti-inflammatory and analgesic activities of 6MNA are explained by its inhibition of cyclooxygenase, with a modest selectivity toward cyclooxygenase-2 (COX-2) [99,100].

A valuable feature of this metabolite is its affinity for synovial fluids where it reaches substantial concentrations, making it useful in chronic inflammatory arthropathies.

One of the reasons why nabumetone is discussed here is its low gastric toxicity that sets this prodrug apart from other NSAIDs [101–103]. In numerous toxicological and clinical studies, nabumetone has indeed confirmed a high therapeutic index (ratio of toxic dose overtherapeutic dose). It now appears that this low gastrointestinal toxicity is due to two factors. First, the nonacidic nabumetone has limited affinity for the gastric mucosa, where its concentrations remain low; and not being able to inhibit cyclooxygenase, it cannot cause COX-related significant damages in the gastrointestinal (GI) tract. As for its active metabolite 6MNA, its presence was undetectable in rat gastric mucosa given nabumetone orally [104]. In addition, nabumetone itself has shown an intrinsic gastroprotective effect by inhibiting neutrophil functions. Specifically, neutrophils are agents in NSAID-induced lesions in the GI tract. Indeed, NSAIDs such as indometacin facilitate their infiltration in rat gastric mucosa and induce their respiratory burst. These effects were inhibited by nabumetone.

10.5.2 Clopidogrel and Prasugrel, the Masking of a Highly Reactive Metabolite

The masking of a reactive agent to improve its therapeutic index is aptly exemplified by the successful anti-aggregating agent *clopidogrel* (**28** in Fig. 10.11) [105–107]. This compound, whose molecular mechanism of action was poorly understood for years, is now known to be a prodrug. But while its major metabolic route in humans (about 80% of a dose) is indeed one of ester hydrolysis, this reaction leads to the inactive acid. Most of the remainder of a dose of clopidogrel is activated in a three-step sequence. First, cytochromes P450s (3A and 2C19) oxidize clopidogrel to *2-oxo-clopidogrel* (**29**). This is followed by a CYP-catalyzed sulfoxidation to the *cyclic sulfoxide intermediate* **30** [106]. The latter hydrolyzes spontaneously to a highly reactive *sulfenic acid metabolite* (**31**), which forms a covalent S–S bridge with a thiol group in platelet ADP receptors, thereby causing their long-lasting inactivation. Interestingly, the same activation mechanism appears to account for the potent and irreversible inhibition of human CYP2B6 by clopidogrel [108].

The sulfenic intermediate can also be reduced by glutathione, leading to the corresponding thiol (Fig. 10.11), which until 2009 was believed to be the active metabolite [109]. This thiol has three stereogenic centers, namely, the original C(7) of (*S*)-configuration, the C(3)–C(16) double bond of (*Z*)-configuration, and C(4), whose absolute configuration appears to be (*R*).

The recent discovery of a sulfenic acid as the active metabolite of clopidogrel [106] raises the question of the enzymes catalyzing its formation. Indeed, sulfoxidation is a metabolic reaction catalyzed by CYPs as well as flavin-containing monooxygenases (FMOs), of which at least three enzymes exist in humans (FMO1, FMO2, and FMO3) [3,5]. This broadens the prospect of future investigations on the genetic and drug interaction factors that influence clopidogrel activation.

The metabolic pathway of activation of clopidogrel indeed offers opportunities for *genetic factors* and drug-drug interactions (DDIs) to influence its clinical outcome [110]. And indeed, patients taking clopidogrel and who were carrying loss-of-function *CYP2C19* alleles (*2, *3, *4, or *5) were recently found to have a higher rate of cardiovascular event [111]. In healthy subjects administered clopidogrel, carriers of at least one *CYP2C19* loss-of-function allele (30% of the studied population) had a relative

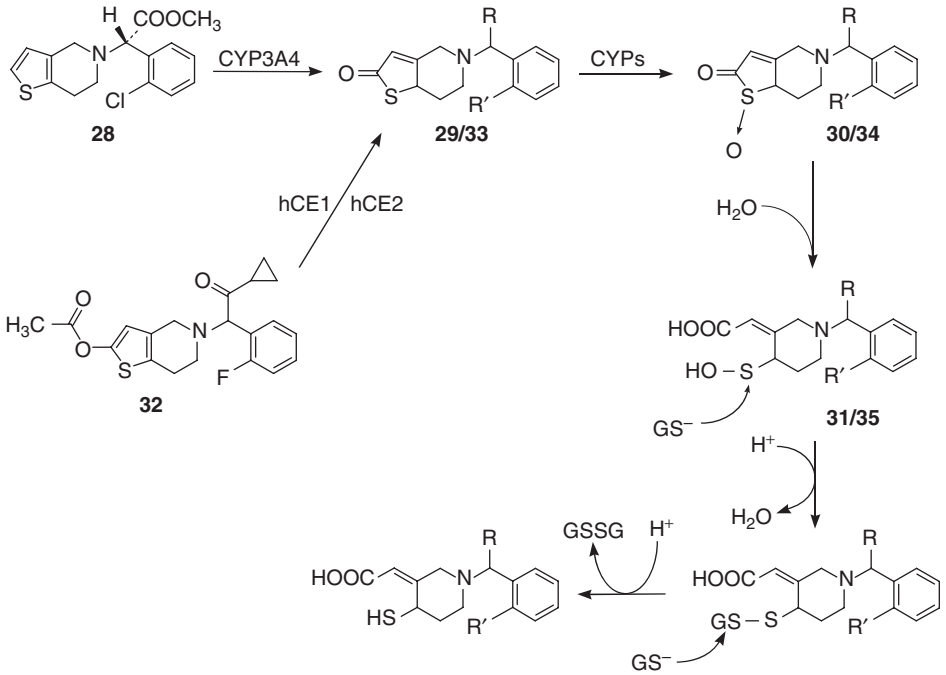


Figure 10.11 A schematic comparison of the mechanism of activation of the anti-aggregating bioprecursors clopidogrel (**28**) and prasugrel (**32**) to their (re)active sulfenic acid metabolite **31** and **35**, respectively. The lower part of the figure shows a mechanism of glutathione-mediated reduction of the sulfenic acid to the corresponding thiol, which was believed for years to be the active metabolite.

reduction of one-third in plasma exposure to the thiol metabolite, a decrease that corresponded to a reduction in maximal platelet aggregation [112]. Other studies showed that reduced-function or loss-of-function alleles of *CYP3A* genes also contributed to the phenomenon of clopidogrel resistance [113].

There are also reports of DDIs in increasing or decreasing clopidogrel activation and activity. Thus, treatment with the antituberculosis drug rifampin (a well-known CYP3A4 inducer) enhanced clopidogrel activity such that some non- and low responders became responders [113]. In contrast, proton-pump inhibitors (e.g., lansoprazole and omeprazole), which are substrates and inhibitors of CYP2C19 and 3A4, negatively affected clopidogrel response and increased platelet aggregation [114].

A recently marketed analogue of clopidogrel (**28**) is *prasugrel* (**32**) [115,116]. In contrast to the former, activation of the latter begins with a CES-catalyzed hydrolysis [117–121]. Indeed, C(2) in prasugrel carries a phenolic oxygen, whose deacetylation allows tautomerization to the thiolactone **33**, a close analogue of 2-oxoclopidogrel (**29**). Formation of the intermediate phenol is demonstrated by the isolation of its *O*-glucuronide in human plasma and urine, as well as in laboratory animals. As for the thiolactone **33**, its activation by sulfoxidation to **34** can be postulated in analogy with that of clopidogrel, followed by hydrolytic ring opening to the active *sulfenic acid metabolite* **35**, which shares the same molecular mechanism of action as the active metabolite of clopidogrel.

Prasugrel is about 10 times more potent than clopidogrel, a fact ascribable to its extensive hydrolysis to the thiolactone **33**. Cytochrome P450 has been known to be involved in prasugrel activation, a finding which at the time was difficult to reconcile with its activation by hydrolysis. The discovery of a reaction of sulfoxidation as a second and key step in the activation of thienopyridines [106] seems to resolve this difficulty, while suggesting also a complementary role for FMOs (see above). The fact that prasugrel activity appears less sensitive than clopidogrel to drug interactions and genetic factors [115] is compatible with this view.

There is one noteworthy stereochemical difference between the two prodrugs, since prasugrel is used as the racemate. As for its thiol metabolite, it was found to occur as four stereoisomers, the two most abundant ones having an (*R*)-configurated C(4) atom in addition to the original (*R*)- and (*S*)-configurations at the original center of chirality.

10.5.3 Tirapazamine, a Bioreductive Prodrug

Bioreductive prodrugs are designed to be activated by a metabolic reaction of reduction in target tissues or organisms with a low oxygen concentration. Such a state of hypoxia may favor reduction reactions because of a relative lack of oxygen, but the major factor accounting for the shift toward reduction is the hypoxia-increased expression of genes coding for reductive enzymes. A variety of chemotherapeutic agents (antibacterial, antiparasitic, or anticancer agents) are in fact prodrugs activated by a variety of reductases [122,123]. Such prodrugs include nitroarenes, quinones, amidoximes, platinum(IV) complexes, and *N*-oxides exemplified here with *tirapazamine* (**36** in Fig. 10.12) [124–128].

This much-studied agent is inactivated by two-electron reduction steps catalyzed by quinone reductase, losing its two oxygen atoms. Its activation, in contrast, is to a radical (**37**) by a one-electron reduction catalyzed by NADPH-cytochrome P450 reductase and multiple reductases in the nucleus. Loss of water yields the *benzotriazinyl radical* **38**, whose strong reduction potential allows it to cause DNA single-strand and double-strand breaks, thereby being reduced to **39**. Back oxidation of the radical **37** by molecular oxygen is a reaction of inactivation, which can occur in aerobic cells. In other words, cellular toxicity will depend first and foremost on oxygen levels in the cells. Of interest is also the fact that simple monosubstitution of tirapazamine can alter its lipophilicity enough to markedly improve extravascular transport and activity against target cell [128].

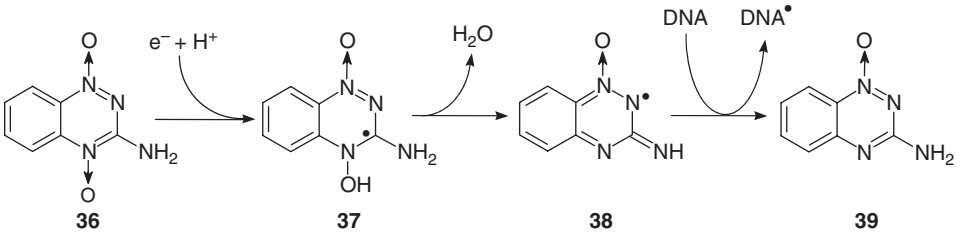


Figure 10.12 Tirapazamine (**36**), a bioreductive antitumor prodrug, and its molecular mechanism of activation to the benzotriazinyl radical **38**, which reacts with DNA.

10.6 METABOLIC DRUG–DRUG INTERACTIONS: THE CASE OF ANTI-HIV PROTEASE INHIBITORS (PIs)

DDIs at the level of metabolism result from drug A inducing or inhibiting one or more enzyme(s) acting on drug B [13]. Other types of DDIs involve interactions at the level of transporters or receptors. As such, DDIs result from a biological system being altered by the action of a xenobiotic, a process we have classified as a PD response. Significantly, this biological response is of indirect interest in our context and offers a perfect illustration of the PK/PD interplay outlined in Section 10.2.1. To illustrate the phenomenon, this section gives a short overview of DDIs produced by or seen among PIs used in antiretroviral therapy. The focus will be on the PI *ritonavir* (RTV), a classical and well-known inhibitor of the metabolism of other drugs [129–133].

RTV was first used at high doses as the sole PI in anti-HIV combinations. Its high toxicity at high doses and a less favorable profile have led to its current use being restricted to that of a pharmaco-enhancer of other PIs such as *atazanavir*, *darunavir* (DRV), *fosamprenavir, indinavir, lopinavir, saquinavir*, and *tipranavir*. Indeed, RTV is a substrate of CYP3A, the efflux transporter P-glycoprotein (P-gp), and to a lesser extent CYP2D6. More importantly, RTV is a potent inhibitor of CYP3A4, an inhibitor of CYP2D6 and P-gp, and a moderate inhibitor of CYP2C9.

The antiretroviral drugs with which RTV is being associated have their own inhibitory effects, resulting in complex effects. For example, the AUC and C_{max} of the C-C chemokine receptor type 5 (CCR5) antagonist *maraviroc* were increased four- and twofold, respectively, by a DRV-RTV combination [132]. In contrast, the DRV-RTV combination modestly increased or decreased the AUC and C_{max} of some reverse transcriptase inhibitors. As for agents in other therapeutic classes (e.g., antimicrobials, proton-pump inhibitors, and antidepressants), the effects of the DRV-RTV combination varied from drug to drug. For example, the AUC and C_{max} of ketoconazole increased two- to threefold, while those of sertaline decreased twofold; smaller effects were seen in the PK behavior of other drugs.

To complicate matters further, RTV is now known to be an enzyme inducer in addition to being an inhibitor [130]. Thus, it is an inducer of CYP3A, although its inhibitory interactions with this enzyme are far more prevalent and may be related to "inhibition-associated induction." At high doses, RTV induces CYP1A2, thereby increasing the metabolic clearance of theophylline, caffeine, and olanzapine, and CYP2C9 (acenocoumarol, phenytoin, phenobarbital, etc.). At high and low doses, it also induces CYP2B6 (bupropion, meperidine, efavirenz, sertaline, etc.), CYP2C19 (methadone, proton-pump inhibitors, etc.), and UDP-glucuronosyltransferases (zidovudine, raltegravir, valproate, oral contraceptives, oxazepam, morphine, nonsteroidal anti-inflammatory drugs, etc.).

10.7 CONCLUSION

This chapter follows a double thread. Along the main thread, as obvious for the layout, we see the intrinsic activity of the drug decreasing, while that of the metabolite(s) increases. Thus, the reader moves from (i) drugs with no active metabolite (Section 10.2.4) to (ii) drugs having active metabolite(s) (Section 10.3) to (iii) intrinsically inactive prodrugs. For the vast majority of (active) drugs having active

metabolites, bioactivation is found to occur by CYP-catalyzed oxidation, as illustrated here with benzodiazepines, codeine, tramadol, and tamoxifen. Among prodrugs, a discrimination is made between carrier-linked prodrugs, whose activation is by cleavage (generally hydrolytic) of a promoiety (Section 10.4), and bioprecursors (Section 10.5), where a redox reaction creates a pharmacophoric or reactive group.

The second thread in the chapter is that of biological levels of complexity and it may seem to be followed erratically. Indeed, metabolic bioactivation may be discussed at various biological levels, from the molecular and enzymatic to the clinical. Here, there is no logical sequence, but we have tried to achieve a reasonable balance that would appeal to most readers. Depending on the breadth and value of the available information, some examples were indeed focused mainly on clinical outcomes, while others deal in greater details with biochemical aspects. Sometimes a convincing causal link can be deduced between the biomolecular and clinical levels. In other cases, the causal link may fail to be fully convincing, pointing to a need for further studies.

While these were not the proper topic of the chapter, pharmacogenetic differences and DDIs are also briefly discussed and exemplified in later sections. These serve to strengthen the link we tried to draw between the biochemical and the clinical levels of drug metabolism.

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