Bioactivation of Drugs: Risk and Drug Design

John S. Walsh[1] and Gerald T. Miwa[2]

[1]DMPK Consulting, Wake Forest, North Carolina 27587; email: jwalsh14@nc.rr.com

[2]School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599-7360; email: gerald_miwa@msn.com

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Abstract

Bioactivation through drug metabolism is frequently suspected as an initiating event in many drug toxicities. The CYP450 and peroxidase enzyme systems are generally considered the most important groups of enzymes involved in bioactivation, producing either electrophilic or radical metabolites. Drug design efforts routinely consider these factors, and a number of structural alerts for bioactivation have been identified. Among the most frequently encountered structural alerts are aromatic systems with electron-donating substituents and some five-membered heterocycles. Metabolism of these groups can lead to chemically reactive electrophiles. Strategies that have been used to minimize the associated risk involve replacing the structural-alert moiety, blocking or making metabolism less favorable, and incorporating metabolic soft spots to facilitate metabolism away from the structural-alert substituent. The metabolism of drugs to radicals usually leads to cellular oxidative stress. The formation of radical metabolites can be minimized through the use of similar approaches but remains an area less frequently considered in drug design.

INTRODUCTION

In 1841, when Alexander Ure first identified the hippuric acid in subjects who had been dosed with benzoic acid (described in Reference 1), it was unlikely that he was thinking about being the first person to describe drug metabolism or the biotransformation of a chemical catalyzed by enzymes in the body. Nor was he conducting his research to define how the drug metabolism enzymes affect the bioavailability and clearance of a drug, the exposure variability among subjects, the cause of many drug-drug interactions, or the basis for the toxicity of some drugs. Instead, he was trying to use benzoic acid as a therapy for gout because he had observed a reduction in uric acid in subjects who had taken benzoic acid. Nevertheless, this observation was the first demonstration of a biotransformation reaction in humans, and our current appreciation for the many roles drug metabolism plays in the disposition and toxicity of drugs owes its origins to Ure and others during the early to mid-nineteenth century.

Today, we recognize that drug metabolism is often an important contributor to the clearance of drugs, the determination of the dose and dosing frequency required for therapeutic utility, and the intersubject variability in drug exposure due to polymorphisms and variable expression of the drug metabolism enzymes. Drug metabolism also underlies many drug-drug interactions, the detoxification or clearance of some toxic compounds, the activation of drugs to chemically reactive electrophilic or radical products that are potentially toxic or that produce oxidative stress, and the activation or the targeting of drugs for therapy. The role of drug metabolism in pharmaceutical companies has evolved in the past several decades from simply characterizing metabolites during the drug development process to serving as a critical component of drug design during drug discovery. Reviews have been written about drug metabolism, particularly the metabolic pathways leading to drug clearance (2) or the chemical mechanisms underlying drug metabolism reactions and reactions leading to reactive intermediates (3). The strategies that scientists have employed to address the toxicities from metabolites or the drug-targeting liabilities of new chemical entities are less well reviewed. This review focuses on the enzyme mechanisms and the chemistry of the specific functional groups that lead to reactive species and on the design of strategies to eliminate these liabilities. These strategies are presented through case examples. Although these examples do not always come from a single institution and often reflect research from different groups, the chemical rationale in structure design is evident.

Nearly 170 years since Ure attempted a strategy of using drug metabolism to reduce uric acid as a treatment for gout, pharmaceutical companies are turning to drug metabolism enzymes for strategies that target drug action through tissue-specific bioactivation (4, 5). Ure would have recognized this strategy but certainly would have been surprised at the many other roles, especially in drug toxicities, now recognized for drug metabolism.

ENZYME MECHANISMS AND THE FORMATION OF CHEMICALLY REACTIVE SPECIES

Although a number of enzyme systems have been implicated in bioactivation, the ones most likely to be encountered in drug design are the CYP450 system (6), glucuronyl transferases (7), and members of the peroxidase family (8). The CYP450 system is considered the most important of these, as it is in drug metabolism in general, because of the wide range of functional groups it is capable of metabolizing. The peroxidases are structurally and mechanistically related to the CYP450 system, but they have specific differences that modify, and limit, the diversity of their functional group transformations (9). Bioactivation via the glucuronyl transferases is only of importance with regard to carboxylic acids. However, the formation of acyl glucuronides comprises

one of the most intensively studied bioactivation processes because of their proposed role in the toxicity of nonsteroidal anti-inflammatory agents (10).

The mechanism of the CYP450 catalytic cycle and the diversity of its chemistry have been extensively reviewed (11, 12). These heme-containing monooxygenases first bind substrate then undergo two sequential one-electron reductions (from CYP450 reductase and NADPH)—one before and one after binding oxygen as an axial ligand. The net effect is the reduction of oxygen and cleavage of the dioxygen bond to give high-valent ferric oxene and water. The subsequent chemistry of this species is still the subject of some debate (13, 14), but many reactions can be rationalized as an abstraction of either a single electron or a hydrogen radical from the bound substrate. Oxygen rebound may then occur to generate the closed-shell, oxidized metabolite.

This mechanism presents a number of features relevant to bioactivation. First, because electron transfer to CYP450 occurs after the binding of substrate, substrates with suitable reduction potentials can undergo one-electron reduction (instead of oxygen reduction). Thus CYP450 can act as a one-electron reductase. Second, the oxidation mechanism is not concerted, and discrete intermediates have been proposed. Metabolism occurs via one-electron processes, so with suitable substrates, there is the potential for free radical formation. These radicals are most frequently carbon centered. Third, oxygen rebound to suitable substrates can result in a range of possible electrophiles (see below), which are capable of further reactions with cellular components by two-electron processes. Whereas examples of each of these types of bioactivation processes are well documented, most drug design effort has been focused on the third example particularly because it relates to the reaction of electrophiles with proteins to form covalently bound metabolites (15). Electron-rich functional groups, including aromatic π systems, are frequently sites of oxidation.

The peroxidases differ from the CYP450s in the nature of the axial ligand to the heme and also in that the heme is covalently bound to the apoprotein (16). This difference seems to result in more limited access for substrates, and in most cases, the natural endogenous substrates for these enzymes are small molecules. The consequences of this for xenobiotic metabolism are that oxidation is thought to occur from the periphery of the porphyrin ring, resulting more frequently in free radical formation without oxygen rebound. These radicals are often heteroatom centered. Peroxidases are important enzymes in nonhepatic tissues, particularly neutrophils, and hence have been associated with a number of blood dyscrasias (17). Also of interest is the fact that during the inflammation associated with many disease states, neutrophil infiltration of tissues occurs. When this infiltration occurs in the liver, it increases the potential for drugs that normally undergo CYP450 oxidation by oxygen rebound to undergo peroxidase reactions to generate radical products instead. This raises the possibility that significant increases in free radical formation may now occur. Although still simply a hypothesis (18), the effect of neutrophil infiltration does provide a potential mechanism whereby drug metabolism might differ in healthy and disease states.

The glucuronyl transferases effect the transfer of glucuronic acid mostly to nucleophilic functional groups (19). In this case, the mechanism of formation of the glucuronide is of little interest with regard to bioactivation issues. Instead, the chemistry of the resulting glucuronide is more relevant, and, in this respect, glucuronides of carboxylic acids are by far the most important. The mechanism by which these acyl glucuronides can react with cellular nucleophiles has been reviewed extensively (20, 21). Although this mechanism has been most extensively studied in aryl propionic acids, similar reactivity might be anticipated for any aliphatic carboxylic acid. These acyl glucuronides are often stable enough to be characterized, so identifying this bioactivation pathway is usually less problematic than identifying bioactivation pathways for CYP450s or peroxidases. Consequently, in drug design, bioactivation issues involving glucuronidation are usually more easily solved.

Table 1 Structural alerts, types of reactive species produced, and the enzyme system most commonly responsible

Functional group	Reactive species	Enzyme system
Nitro aromatics	Radical	CYP450/reductase
Anilines	Electrophiles	CYP450, peroxidases
Activated aromatics	Electrophiles, radicals	CYP450, peroxidases
Propionic acids	Electrophiles	Glucuronyl transferase
Thiophenes	Electrophiles	CYP450
Furans	Electrophiles	CYP450
Formamides	Electrophiles	CYP450
3-Alkyl indoles	Electrophiles	CYP450
Thioureas	Electrophiles	CYP450
Thioamides	Electrophiles	CYP450
Thiazolidinones	Electrophiles	CYP450
Cyclopropyl amines	Radicals	CYP450
Hydrazines	Radicals	CYP450
Acetylenes	Electrophiles	CYP450
Sulfonylureas	Electrophiles	CYP450

STRUCTURAL ALERTS

Structural alerts, which are functional groups with the potential for bioactivation, comprise a risk-awareness knowledge base that is widely used in drug design. Functional-group bioactivation pathways have been extensively reviewed (22). Simple avoidance of all such alerts, however, is not practical because of the wide range of groups with bioactivation liability. **Table 1** lists some commonly encountered functional groups that are capable of undergoing bioactivation, and also shows the reactive species formed and the relevant bioactivation enzyme systems. Electrophilic species generated by CYP450 predominate. An important qualifier is that two-electron electrophilic mechanisms are significantly easier to study than radical processes and thus have been more frequently reported. In turn, this constraint has been the driving force for most drug design efforts, which have focused on eliminating electrophile formation. Radical-mediated processes, therefore, may represent an unaddressed issue in drug design.

The following sections review examples of how some of these functionalities have been implicated in adverse safety events and how an understanding of their mechanism of formation can lead to safer drugs.

STRATEGIES TO MINIMIZE BIOACTIVATION RISK

The main approaches used to minimize bioactivation risk are (*a*) eliminating the suspect functional group, (*b*) blocking the potential for metabolism, (*c*) making metabolism less favorable (most frequently by use of steric hindrance or reducing oxidation potential), and (*d*) incorporating metabolic soft spots to direct metabolism away from the suspect group. The following examples illustrate the bioactivation mechanisms of some of the groups listed in **Table 1** and the applications of these to drug design. Two of the more important groups, activated aromatics and five-membered heterocycles, are examined in greater detail.

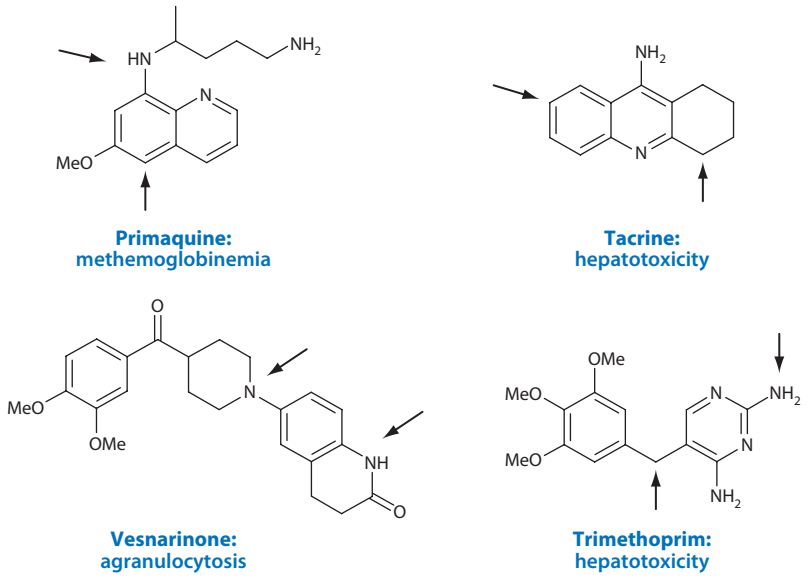


Figure 1

Examples of drugs that undergo oxidative metabolism to form quinone-type structures. The toxicity proposed to be associated with these pathways is indicated.

Blocking the Bioactivation of Activated Aromatic Systems

During drug design, activated aromatic systems (usually aniline derivatives), are perhaps the most widely encountered structural features associated with bioactivation. **Figure 1** illustrates some examples (23–25) of these functional groups, their sites of bioactivation, and the toxicity believed to be associated with them. Quinone-type metabolites are produced by oxidative metabolism across the positions indicated by arrows (quinone imines and quinone methides). Note the structural diversity that can give rise to these types of metabolite.

Nefazodone (**Figure 2**), withdrawn in 1994 because of hepatic safety concerns, also illustrates the metabolism to quinone and quinone imine electrophiles, which have been proposed as the species responsible for hepatic damage. This bioactivation pathway also provides a mechanism for aromatic N-dearylation (26).

Many nitrogen-containing aromatic systems are capable of producing quinone-like structures. These are not always easy to predict in drug design, so obtaining metabolism information, such as glutathione trapping, can be useful. As shown in **Figure 1**, activation can occur across multiple ring systems, and the presence of a free NH group often facilitates—but is not essential for—this type of metabolism. Where aromatic hydroxylation is a necessary prerequisite (e.g., in primaquine and nefazodone), blocking this site with a nonlabile substituent may be possible. Thus the bioactivation of tacrine can be blocked with a methyl substituent in the 7-position (27). Another example of this may involve the structural changes made from early- to later-stage beta blockers (**Figure 3**). The idiosyncratic peritonitis associated with practolol appears to be avoided by analogs in which the NH substituent has been replaced, as in atenolol, metoprolol, and bisoprolol.

Blocking the Bioactivation of Five-Membered Heterocycles

Five-membered heterocycles of most concern for bioactivation risk are furan, thiophene, and pyrrole (**Figure 4**). The biotransformations of these and other five-membered heterocycles have

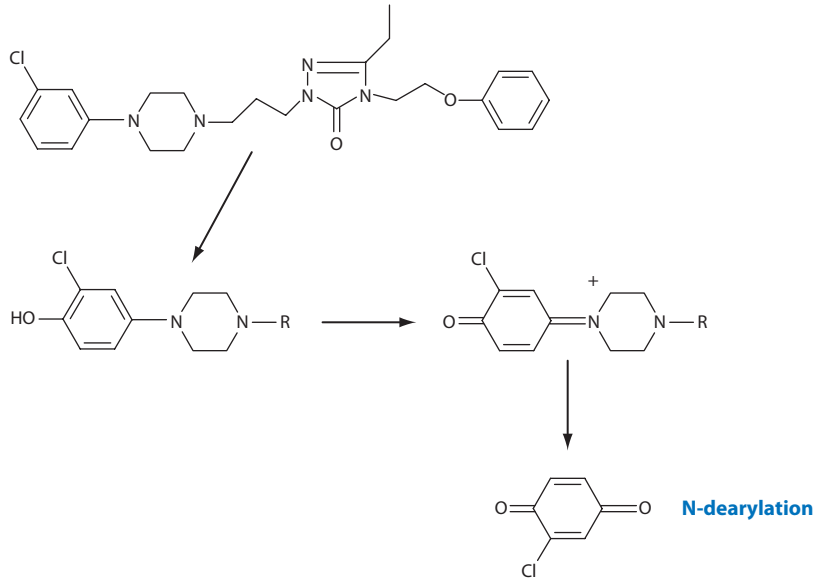
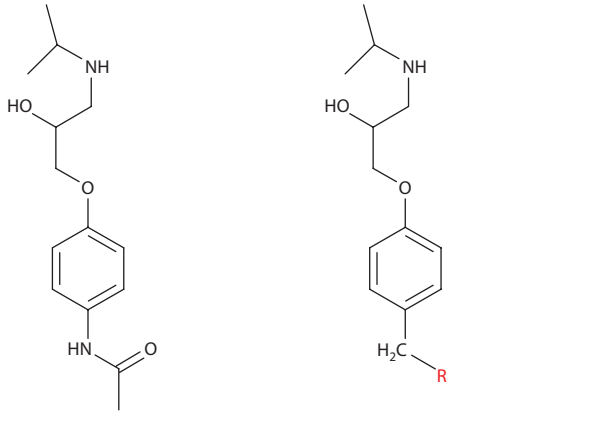


Figure 2

The bioactivation of nefazodone to quinone-type metabolites. These metabolites were inferred based on identification of their glutathione adducts. Launched by Bristol-Myers Squibb as Serzone® in 1994, the drug was withdrawn from the market in 2004.



Practolol
- **Withdrawn**
- **Idiosyncratic sclerosing peritonitis**

Atenolol R = $CONH_2$
Metoprolol R = CH_2OMe
Bisoprolol R = OCH_2CH_2OiPr

Figure 3

Replacement of suspect NH functional group required for quinone imine formation. Improved safety profiles are observed with the second-generation products listed on the right. O-dealkylation would be required for formation of quinone imines.

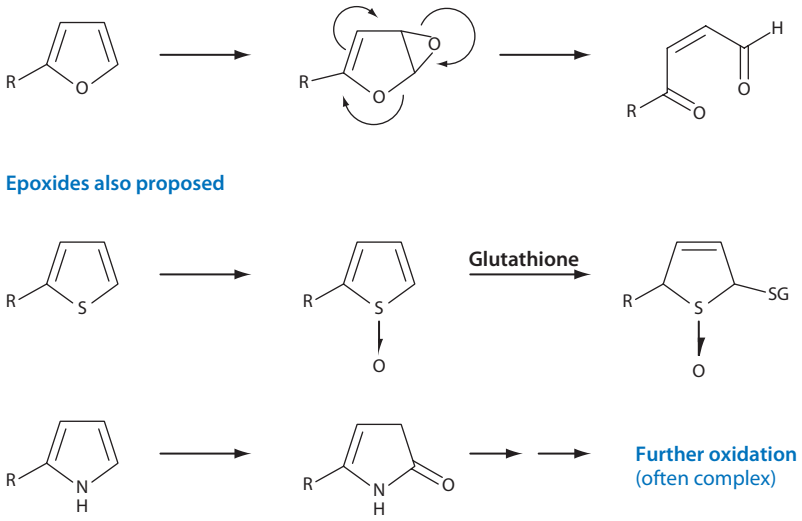


Figure 4

Oxidation of π-excessive five-membered heterocycles to electrophilic products.

been reviewed (28). Although less well-documented, their benzo-fused analogs can also be considered to have potential bioactivation liability. The delocalization of the heteroatom lone pair into the π system makes these heterocycles more reactive to electrophilic substitution as well as oxidative metabolism compared with simpler benzenoid systems. **Figure 4** illustrates how these heterocycles can undergo oxidative metabolism to electrophiles. Pyrrole metabolism can be complex. An interesting example is that of prinomide, illustrated in **Figure 5** (also see Reference 29). **Figure 6** shows a postulated mechanism for the formation of prinomide's metabolite, which involves oxidation at the site of substitution on the pyrrole ring as well as acyl migration. This illustrates that substitution may not necessarily block metabolism at a given position. In addition, even deactivated systems can undergo oxidation. For example, the 5-nitroimidazole derivative tinidazole undergoes oxidation of the imidazole ring with concomitant nitro-group migration (30). This is an unexpected pathway that may arise in part from the low molecular weight of the

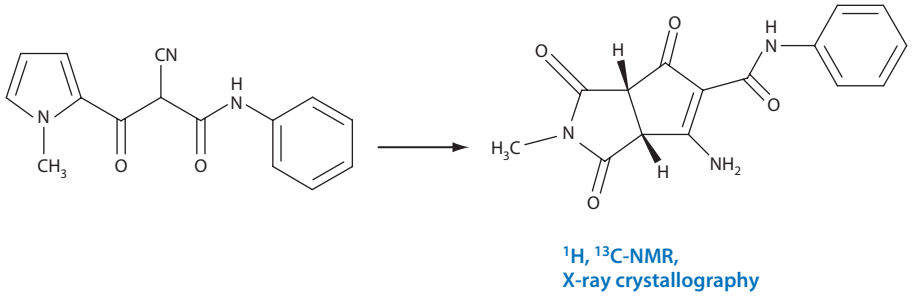


Figure 5

Metabolism of prinomide. Because of the novelty of this biotransformation, the structure was rigorously characterized, as indicated.

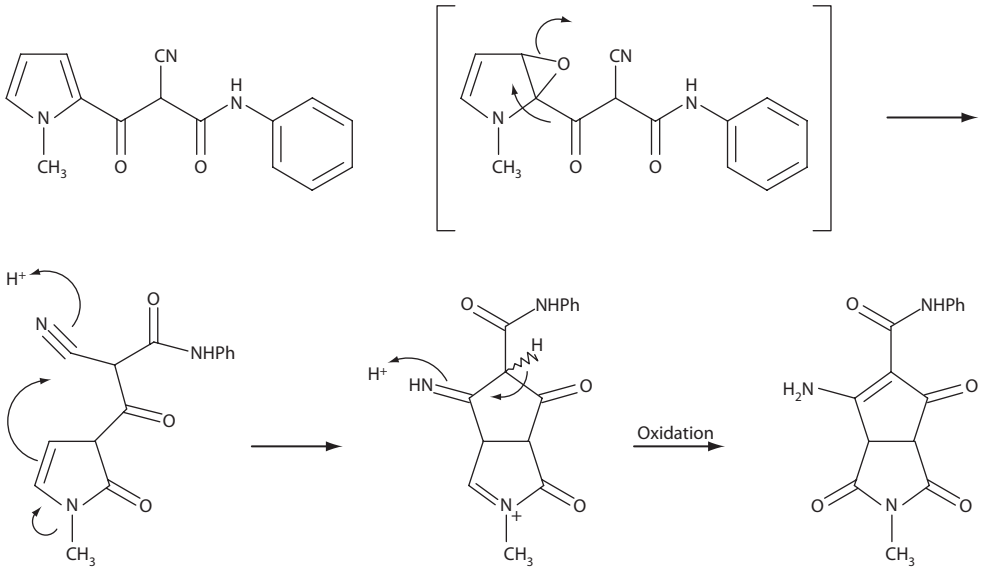


Figure 6

A possible mechanism for formation of the metabolite of prinomide, as proposed by the authors. This seems to involve oxidation of the pyrrole ring, opening of the epoxide ring with acyl migration, and enamine-type nucleophilic addition to the nitrile. A second oxidation step of the methyl iminium group gives the observed metabolite. As well as illustrating the possible complexity of pyrrole metabolism, the mechanism implies preferential oxidation of the ring at the more hindered site of substitution.

molecule, and in part from the few alternative sites for oxidation. It is unlikely that such a pathway would occur in a larger molecule with alternative oxidation sites. This illustrates the potential limitation of attempting to block metabolism solely by making it less favorable.

Indole metabolism is generally less complex, but the pyrrole-ring system may still be subject to bioactivation through oxidation. The indole-containing drug zafirlukast has received two label warnings for hepatitis since its launch in 1994. A glutathione adduct of this compound has been identified, and a proposed mechanism for its formation is shown in **Figure 7**. It has been postulated that the electrophilic iminium species indicated in **Figure 7** may be responsible for the adverse events observed with zafirlukast (31).

Tienilic acid (**Figure 8**) is a well-studied example of a thiophene-containing drug. The thiophene ring is believed to undergo bioactivation by CYP2C9 (32, 33). This bioactivation has been proposed to be the initiating step in the autoimmune-mediated hepatitis observed for this drug, which resulted in its withdrawal from the market. The benzothiophene derivative zileuton has received U.S. Food and Drug Administration (FDA) warnings for hepatitis, and glutathione adducts of this drug have also been identified, indicating the formation of electrophiles (34). The proposed mechanism is similar to that for thiophene derivatives (**Figure 9**).

Thiazoles are heterocycles that are less π excessive than the examples discussed above but are capable, in some cases, of undergoing bioactivation by different mechanisms. In this case, oxidation and opening of the ring system may generate thioamides. An example of this is sudoxicam, whose mechanism of bioactivation is shown in **Figure 10** (also see Reference 35). The metabolism of thiourea and thioamide derivatives has been associated with a range of adverse events. Both electrophilic and radical-based oxidative stress mechanisms have been proposed (36–38).

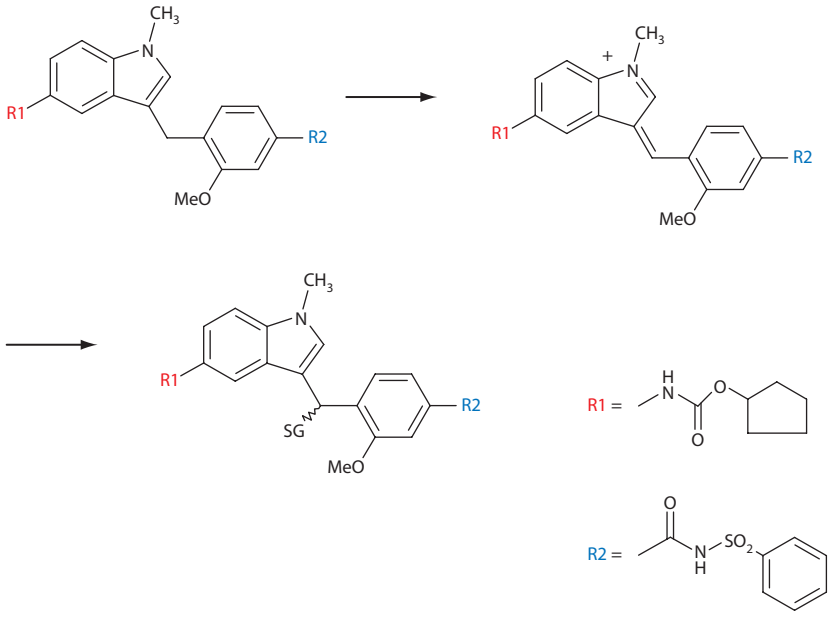


Figure 7

The metabolism of the indole ring of zafirlukast to an electrophile, yielding a metabolite with the addition of glutathione (SG). Launched by AstraZeneca as Accolate® in 1996, it received label warnings for hepatitis in 2000 and 2004.

Thiazolidinediones are also five-membered ring systems, but they are structurally distinct from the aforementioned heterocycles. This functionality has been associated with hepatotoxicity, and a number of electrophilic metabolites have been identified (39).

Five-membered heterocycles are widely encountered in drug design, and replacement in many cases is not feasible because they often constitute part of the key pharmacophore. As illustrated above, benzo-fused systems still have some liability, and metabolism may still occur at sites of substitution. However, di- or trisubstitution (often in the 2- and 5-positions) is widely employed and may provide enough steric hindrance, particularly if alternative oxidation sites are possible (i.e., metabolic switching). Thus olanzapine (**Figure 11**) is a trisubstituted thiophene that undergoes glucuronidation, N-oxide formation, and methyl oxidation (40) rather than thiophene

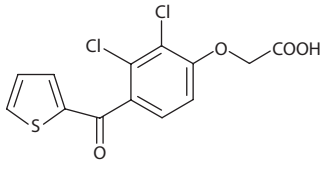


Figure 8

The structure of tienilic acid. It was removed from the market due to its observed association with hepatitis, and its metabolism has been extensively studied. Oxidation of the thiophene ring has been implicated in the toxicity.

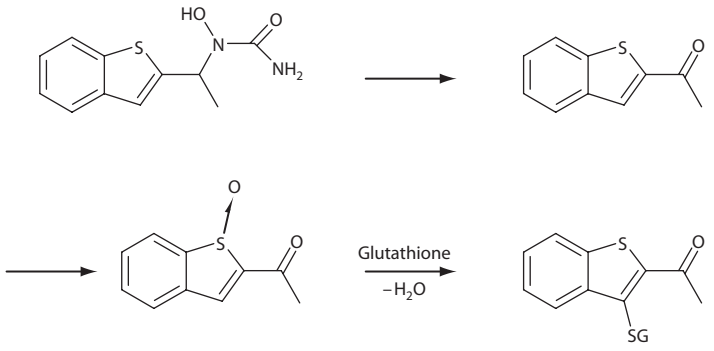


Figure 9

Zileuton (Zyflo®). Launched by Abbott Laboratories in 1997, it subsequently received a U.S. Food and Drug Administration warning for hepatotoxicity in 2004 and now requires liver-function monitoring. A benzothiophene derivative, it has been proposed to undergo bioactivation as shown.

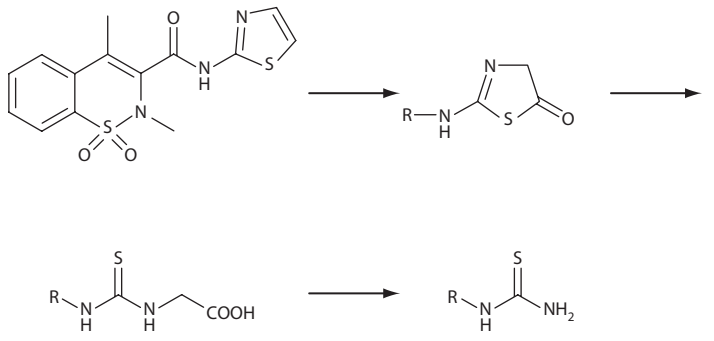


Figure 10

The metabolism of sudoxicam to a thiourea. Thioureas, in turn, may undergo oxidative metabolism to electrophiles or free radicals.

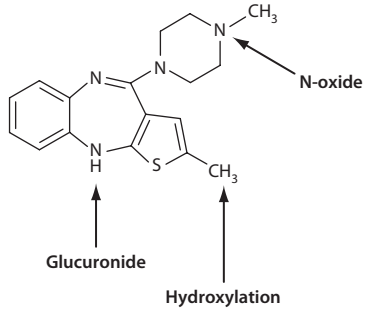


Figure 11

Olanzapine avoids oxidation of the thiophene ring because more easily metabolized sites are present, as indicated. The trisubstitution pattern of the thiophene ring may also provide some steric hindrance. It has been proposed that these alternative pathways to thiophene oxidation may contribute to olanzapine's favorable safety profile.

bioactivation. Similarly, it has been suggested that meloxicam's safer profile relative to sudoxicam results from less scission of the thiazole ring, which, in turn, arises from preferential oxidation of a methyl group (41). These examples illustrate what might be the primary strategy for reducing bioactivation risk in these ring systems: metabolic switching in association with some degree of steric hindrance on the heterocycle.

Other five-membered heterocycles such as imidazoles, oxazoles, and oxadiazoles may also undergo ring-opening reactions, but these heterocycles are usually not associated with the formation of electrophilic intermediates and toxicity. For example, triazoles and tetrazoles often undergo benign N-glucuronidation (42). These ring systems, therefore, can be used in drug design with minimal risk for bioactivation.

Metabolic Switching

There are a number of examples, in addition to five-membered heterocycles, in which the presence of metabolic soft spots can prevent metabolism of structural-alert groups. The experimental acetylenic drug DPC961 forms a glutathione adduct through the mechanism proposed in **Figure 12**. The formation of the proposed carbocation involves an interesting ring-opening reaction that involves the adjacent cyclopropyl group (43). DPC961 is a small molecule, lacking other electron-rich functionalities that would be susceptible to CYP450 oxidation. In contrast, the synthetic retinoid tazarotene (**Figure 13**), in use for psoriasis, appears to undergo sulfur oxidation and ester hydrolysis rather than acetylenic oxidation (44).

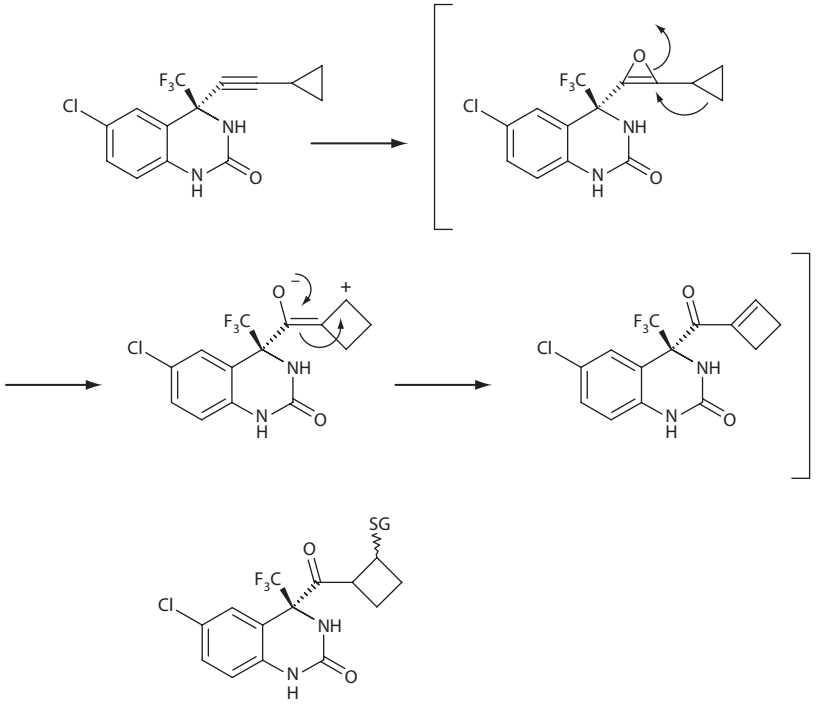


Figure 12

The metabolism of the acetylene function in DPC961 to give a glutathione adduct (glutathione is indicated as SG).

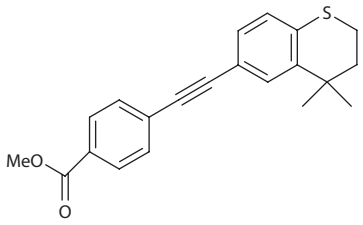


Figure 13

The presence of alternate soft spots allows tazarotene to avoid metabolism of the acetylenic function. Hydrolysis of the ester group and oxidation of sulfur are the predominate pathways of metabolism.

Ester hydrolysis is generally a favorable metabolic pathway and is likely to be part of the reason for the generally safe profile of the calcium-channel blocker nifedipine (**Figure 14**). Here, both steric hindrance of the nitro group and the propensity for ester hydrolysis (45) constitute drug design features that minimize the bioactivation risk of nitro reduction (see below).

The anxiolytic agent alpidem was withdrawn in 1995 because of its association with cases of severe hepatitis. It has been proposed that oxidation of the aromatic system to an arene oxide may be the mechanism of bioactivation (**Figure 15**). In contrast, a close analog, zolpidem, does not show this hepatotoxicity signal, and metabolism is shifted from arene-oxide formation to N-dealkylation and benzylic oxidation (**Figure 16**) (46).

Thus the addition of substituents that provide metabolic soft spots can direct metabolism away from functional groups that possess the potential for bioactivation. A list of substituents, with the metabolism for each, is shown in **Table 2**. Thus when these groups are present in a molecule, they have a high likelihood of metabolism through a benign clearance pathway composed of stable, nonelectrophilic metabolites. This is, of course, a generality, as are most aspects of rational drug design.

Making Metabolism Less Favorable: Reducing Oxidation Potential

The enzymatic oxidation of cyclopropyl amines has been extensively investigated, and some controversy remains as to the exact mechanism. It may depend on the enzyme system involved and may involve either single-electron transfer (SET) oxidation of nitrogen or hydrogen-atom transfer (HAT) (47). Although this metabolic pathway is usually associated with mechanism-based enzyme inhibition mediated by radicals or electrophiles, it is also presumed to constitute a metabolic liability for broader adverse events. If SET oxidation of nitrogen is the initiating event, then

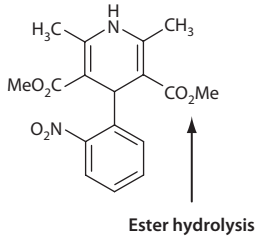


Figure 14

The structure of nifedipine. Nitro-group reduction is avoided through the presence of the ester group, a primary site of metabolism.

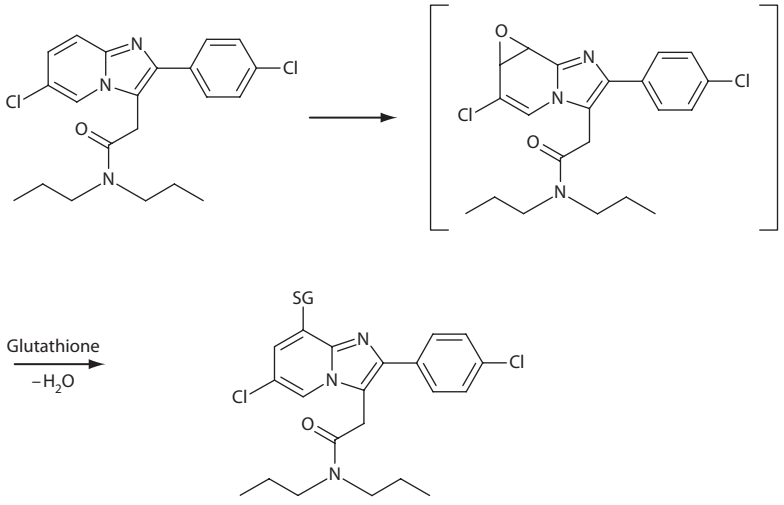


Figure 15

The metabolism of alpidem to an arene oxide and subsequent reaction with glutathione. Launched in 1991, this drug was withdrawn from the market in 1995 because of its association with cases of acute hepatitis.

reducing the SET oxidation potential at nitrogen is a rational approach to prevent this type of metabolism.

Trovafloxacin is an antibiotic that was withdrawn because of hepatotoxicity, and cyclopropylamine metabolism has recently been proposed as a possible initiating event (48). In contrast, ciprofloxacin and nevirapine do not appear to undergo this pathway of metabolism. This is likely, at least in part, because of less favorable SET oxidation resulting from aromatic substitution of the cyclopropylamine groups (**Figure 17**).

Blocking the Potential for Metabolism: Fluorination

Of the many approaches that can be used to block metabolism, one of the most widely used techniques is fluorination. An example where this has been applied is with felbamate and fluorofelbamate. Felbamate is an antiepileptic agent that has caused idiosyncratic hepatotoxicity and

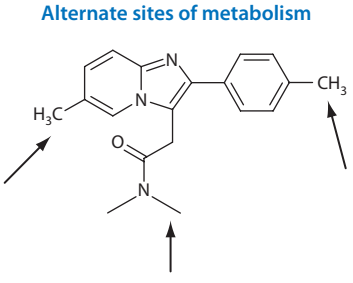


Figure 16

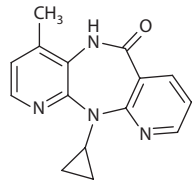
Zolpidem is a close structural analog of alpidem, but it has metabolic soft spots (benzylic methyl and N-methyl groups), which shift metabolism away from arene-oxide formation. The hepatic safety issues seen with alpidem are avoided with this drug.

Table 2 Metabolic soft spots

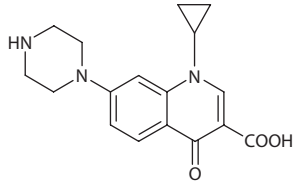
Functional group	Metabolism
Esters	Hydrolysis
Thioethers	Oxidation (to S-oxides and sulfones)
Benzylic methyl groups[a]	Oxidation (to alcohols and acids)
Cyclohexyl groups	Oxidation (to alcohols and ketones)
t-butyl groups	Oxidation (to alcohols and acids)
Phenolic and hydroxyl groups	Conjugation (to glucuronides or sulfates)
1,2,4-oxadiazoles[b]	N-O ring opening (to amides and ketones)
Tertiary aliphatic amines[c]	Oxidative N-dealkylation (to secondary amines)

[a]The possibility for formation of imino or quinone methides must be considered in para-substituted N or O benzylic systems.
[b]Although this pathway is well documented in the literature, the idea that it is frequently a prominent route of metabolism when present is a personal observation of the authors.
[c]Secondary amines frequently undergo further metabolism. This can be complex.

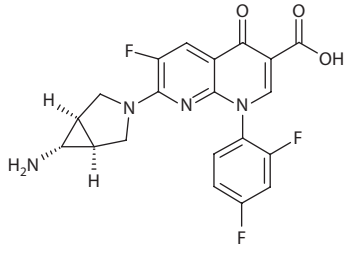
aplastic anemia, and it has been proposed that an α,β-unsaturated aldehyde (ATPAL) may be involved (49). This mechanism involves loss of the methine hydrogen with the carbamate function to produce the ATPAL (**Figure 18**). To block this process, fluorofelbamate was developed whereby the methine hydrogen, lost as a proton in formation of ATPAL, is replaced by fluorine. This blocks the formation of ATPAL, and fluorofelbamate is currently undergoing clinical trials (50).



Nevirapine
- Immune-mediated rash
- No cyclopropylamine metabolism reported



Ciprofloxacin
- Main toxicity: Gastrointestinal (well tolerated)



Trovafloxacin
- Withdrawn: hepatotoxicity

Figure 17

Some examples of cyclopropylamine-containing drugs. Nevirapine and ciprofloxacin do not undergo metabolism of the cyclopropylamine group, most likely because of less favorable oxidation of nitrogen. In contrast, it has been proposed that the adverse events seen with trovafloxacin may arise from cyclopropylamine oxidation.

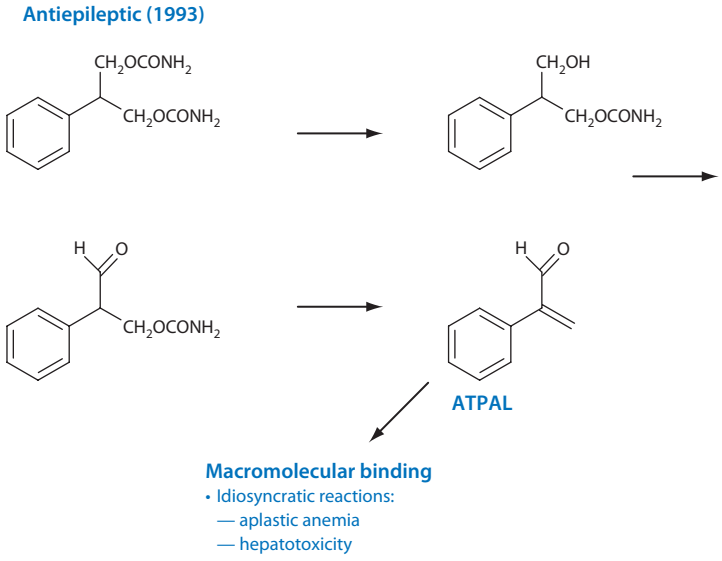


Figure 18

The metabolism of felbamate to an electrophilic Michael-type acceptor. The final step for formation involves elimination of carbamic acid, with loss of the methine hydrogen as a proton. Replacement of the methine hydrogen with fluorine blocks this pathway of metabolism. Abbreviation: ATPAL, α,β-unsaturated aldehyde.

Fluorination has also been extensively used to prevent aromatic oxidation, with some success. Two examples are shown in **Figure 19** (51, 52). However, a consideration of the CYP450 mechanism should bring to mind that oxidation of aromatic rings proceeds via oxidation of the π system and not the carbon hydrogen bond as in aliphatic systems. An example of a highly fluorinated aromatic system that is still metabolized is sitagliptin (**Figure 20**), although this is a minor pathway for overall disposition (53). Fluorination of aromatic systems may, however, fortuitously perturb metabolism to make aromatic oxidation less favorable; this is in contrast to aliphatic systems such as felbamate, in which a mechanism involving proton loss or hydrogen radical abstraction will be effectively blocked. Although the application of fluorination to block metabolism of aliphatic systems may appear favorable in a general sense, new complications may arise when fluorine

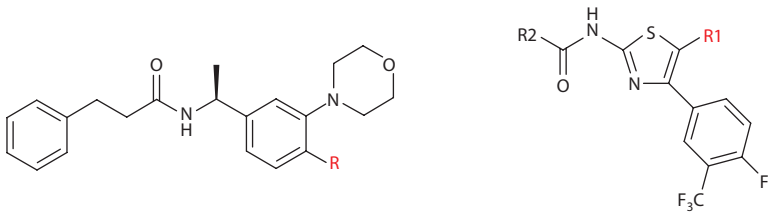


Figure 19

Examples of the successful application of aromatic fluorination to block unfavorable metabolism. R designates the site of fluorination in the left structure, and R1 designates the site of fluorination in the right structure. In both cases, replacement of H with F at these sites prevents bioactivation.

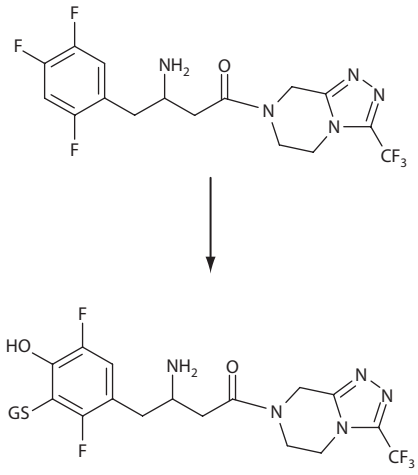


Figure 20

Sitaglipin. An example of the metabolism of a highly fluorinated aromatic ring.

acts as a leaving group. An example of this is MaxiPost™ (**Figure 21**). The covalent binding observed with this compound is proposed to be mediated by the quinone methide generated by loss of fluoride following demethylation to the phenol (54). Aromatic trifluoromethyl groups are a widely encountered function in drug design and, when electronic factors are considered important, may sometimes be considered as a replacement for nitro groups. Trifluoromethyl phenol shows indications of hepatotoxicity in vitro and forms glutathione adducts by spontaneous loss of hydrogen fluoride (HF). This loss of HF produces a quinone methide (55), which subsequently solvolyses to the carboxylic acid via an acyl fluoride (**Figure 22**). The lability of the trifluoromethyl group to hydrolysis via electrophiles has been previously noted and is not specific to phenols (56, 57), yet this remains a less well-recognized structural alert.

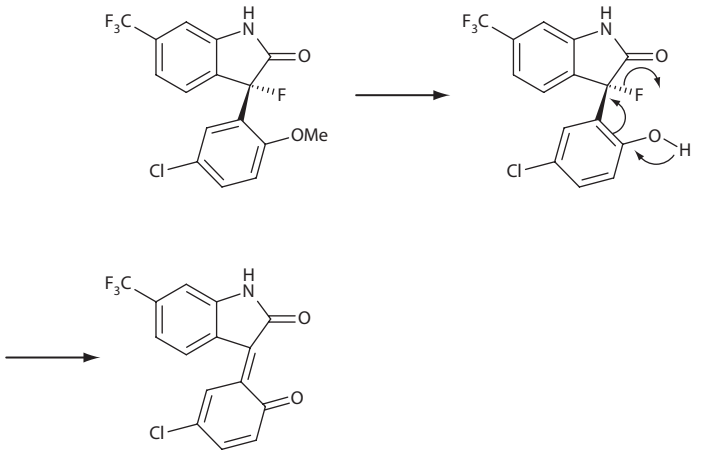


Figure 21

The metabolism of MaxiPost™ to an ortho-quinone methide, where fluorine acts as a leaving group and thus serves as a key element of the bioactivation mechanism.

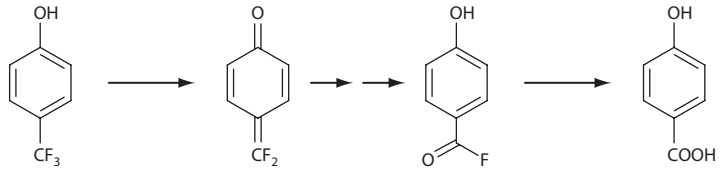


Figure 22

The hepatotoxin trifluoromethylphenol undergoes nonenzymatic solvolysis to a carboxylic acid via an electrophilic quinone methide and acyl fluoride.

In summary, the success of fluorination as a means to block metabolism is dependent on the particular chemical series. It can be a useful approach in aliphatic systems because the CYP450 mechanism involves direct hydrogen radical abstraction. It is less reliable in blocking metabolism in aromatic systems because the mechanism involves π-bond oxidation. However, it has been used extensively to perturb binding or metabolism, with favorable results. Finally, in both aliphatic and aromatic systems, fluorine may act as a leaving group during metabolism to generate electrophiles, providing an alternate bioactivation pathway. Consequently, when fluorination is intended as a strategy to prevent metabolism, each new chemical series in question should be reviewed with the above points in mind.

Metabolism to Radicals

The role of radicals in toxicity has been extensively reviewed (58–60). The underlying chemistry, however, is less well understood, and detecting radicals is much more difficult than detecting electrophiles. This difficulty has resulted in less consideration of radicals as potential mediators of toxicity during drug design.

Free radical–mediated toxicities may be associated either with the reaction of the radicals with biological macromolecules or with oxygen to induce oxidative stress. Mechanism-based inactivation of CYP450 by certain functional groups that are metabolized mostly to carbon-centered radicals has been shown to occur via covalent binding to the heme (61), and these functionalities are frequently avoided in drug design. Free radicals may also cause modifications to other macromolecules such as DNA or protein (62, 63). However, the ability to induce oxidative stress by reduction of oxygen is likely to be the most important process associated with the toxicity of radicals. Oxidative stress, an imbalance in the cell's redox state, may arise from a variety of mechanisms that usually involve the mitochondria (64). Indeed, oxidative stress is a critical element in cell death, and, consequently, it can be difficult to assess if a chemical is causing toxicity directly by oxidative stress or as a downstream consequence of a different mechanism (65). Furthermore, xenobiotic-induced oxidative stress may arise from mechanisms distinct from free radical formation (64). One well-recognized mechanism for xenobiotic-induced oxidative stress occurs via one-electron reduction of certain functional groups, usually catalyzed by CYP450 reductase (66). The resulting radical then transfers an electron to oxygen to form the superoxide anion, which may undergo further reduction to superoxide. Although these oxygen species are considered reactive, transition metal–catalyzed formation of the hydroxyl radical from these species is likely to be more important because of this radical's greater reactivity. Indeed, as oxidative stress progresses, iron is released from iron sulfur proteins; this release then enhances hydroxyl radical formation, creating an autocatalytic cycle (67, 68). The functional groups associated with this process are aryl nitro groups, quinones, and iminium species (69–72), the avoidance of which constitutes the usual

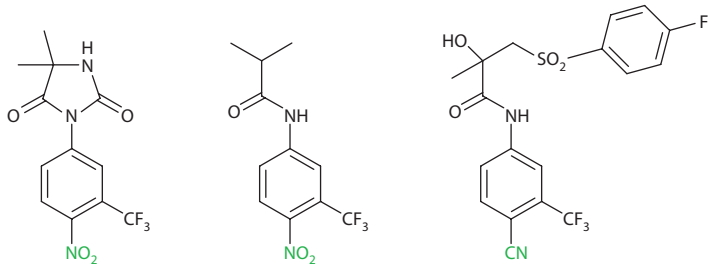


Figure 23

Nonsteroidal antiandrogens and hepatotoxicity. The redox cycling observed for nilutamide is avoided in the structural analog flutamide but does not fully eliminate the hepatic effects. Replacement of the nitro group with a cyano substituent in bicalutamide results in a nonhepatotoxic drug. Nitro reduction initially yields a radical anion, which may undergo redox cycling. Further reduction leads to electrophilic hydroxylamine and nitroso derivatives, which may cause the hepatotoxicity associated with flutamide.

drug design strategy. Clearly, the redox properties of the drug will influence the potential for toxicity by this mechanism, and it has been suggested that the reduction potential must be more positive than −0.5 V for electron transfer to occur in biological systems (73). The nonsteroidal antiandrogen nilutamide (**Figure 23**) is believed to cause hepatotoxicity by this mechanism (74). The close structural analog flutamide is still hepatotoxic but appears not to undergo redox cycling, indicating the structural sensitivity to this effect. Replacing the nitro group with cyano, as in bicalutamide, results in a nonhepatotoxic compound (**Figure 23**).

Another mechanism for xenobiotic-induced oxidative stress occurs through peroxidase (75, 76) or prostaglandin H synthase–mediated metabolism (77). This mechanism most frequently involves hydrogen radical abstraction from hydroxyl, thiol, or amine groups when they are part of an aromatic system that allows electron delocalization (78–80). These groups may, in turn, abstract a hydrogen radical from NADH to form an NAD radical that reduces oxygen to superoxide (81). These types of functional groups are difficult to avoid in drug design and may also be formed as phase I metabolites. Structural features remote from the radical site may modulate toxicity via electronic effects. Linear free-energy relationships have been established (Brown variant of Hammett σ+), suggesting that electron-releasing substituents may increase toxicity; however, lipophilicity and homolytic bond dissociation energy may also be factors (82, 83). These considerations may be too tenuous to consider in routine drug design, but in follow-on programs, where leads are sought to avoid previously observed toxicities, an awareness of this potential mechanism may be useful, particularly if any evidence of oxidative stress has been noted. In contrast to carbon-centered radicals, functions capable of reversible one-electron reduction (e.g., nitro groups) and heteroatom-centered radicals (e.g., from phenols) are functioning in a catalytic mode and thus can affect more than a stoichiometric amount of oxygen reduction.

Regarding bioactivation in drug design, most consideration is generally given to electrophiles, which can be more easily trapped and whose chemistry can be more easily rationalized (84). However, an understanding of how subsequent covalent binding can be linked to toxicities has remained elusive (85). In contrast, the downstream cellular events initiated by free radical formation, when associated with oxidative stress, are better understood. The means of identifying radicals through the use of routine methodology is the greatest limitation to screening during

discovery. However, the identification of structural features that are capable of free radical formation allows use of the same avoidance strategies employed for electrophiles, as previously illustrated for nifedipine. It is likely that further progress in avoiding free radical metabolism during drug discovery will be based on the further development of in vitro–based cellular assays (18).

CONCLUSIONS

The key issues for bioactivation and drug design remain the same as they have been for some time: how to put bioactivation data in context during drug discovery and how to assess the risk in progressing compounds with structural alerts. Currently, many companies employ a series of data-gathering stages (e.g., reactive metabolite and covalent-binding assays), which are performed and then interpreted in context with other parameters (e.g., anticipated dose, dose duration, and benefit to risk). Limitations on the predictiveness of these assays for the expression of toxicities are well recognized, and improvements on analytical methodology are unlikely to yield major advantages. Some link between bioactivation and in vivo toxicity would be of most value, and this might be achieved by assessing cellular responses to reactive metabolites. The Nrf2 system is one of the mechanisms by which cells responds to damage caused by electrophiles or oxidative stress. It is composed of a suppressed nuclear activating factor that is triggered under appropriate stress conditions, resulting in the upregulation of protective genes. Monitoring a subset of these genes through the use of TaqMan® methodology would be one way to evaluate how a cell senses risk from a compound. Furthermore, when used in conjunction with standard glutathione-trapping assays, the methodology might help guide drug discovery. However, a number of experimental issues must be addressed before this would be routinely practical.

SUMMARY POINTS

1. The major enzyme systems involved in bioactivation are the cytochrome P450 (CYP450) and peroxidase systems. Bioactivation can often be rationalized based on the enzyme mechanism. The CYP450 system generates closed-shell electrophiles primarily, and free radicals less frequently. The peroxidases primarily produce free radicals and may play a more important role in extrahepatic bioactivation and possibly also in disease states.
2. A wide range of functional groups are known to undergo bioactivation. These groups cannot always be avoided in drug design. Bioactivation risk can be avoided or minimized by (*a*) replacing the suspect functional group, (*b*) blocking metabolism, (*c*) making metabolism less favorable, and (*d*) switching to metabolic soft spots.
3. Among the most commonly encountered structural alerts in drug design are (*a*) benzenoid systems with electron-donating substituents that may be oxidized to quinone-type systems and (*b*) π electron–excessive, five-membered heterocycles. Metabolism of these and other functionalities can be avoided via the aforementioned approaches, and optimal results can be obtained through a combination of strategies.
4. Fluorination can block metabolism in aliphatic systems but may only perturb metabolism in aromatic systems. However, fluorination of aromatic systems can prove a successful approach to avoid oxidative bioactivation. Fluorine can also function as a leaving group and thus frustrate attempts to reduce bioactivation.

5. It is generally presumed that toxicity derived from electrophile formation primarily involves modification of cellular macromolecules, whereas free radical formation primarily results in oxidative stress. In general, the same approaches used to avoid electrophile formation can be applied to radicals. The CYP450 system can function as a one-electron reductase, causing redox cycling. This occurs only for a narrow range of functional groups. Peroxidases often affect hydrogen radical abstraction from heteroatoms, and some quantitative structure-activity relationships are evolving around these reactions. Free radical formation is less frequently considered in drug design and may be an underappreciated factor in the etiologies of toxicities.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Annual Review of Pharmacology and Toxicology

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Contents

Indexes

Errata

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