

1-Aminobenzotriazole: A Mechanism-Based Cytochrome P450 Inhibitor and Probe of Cytochrome P450 Biology

Paul R Ortiz de Montellano*

Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143, USA

Abstract

1-Aminobenzotriazole (1-ABT) is a pan-specific, mechanism-based inactivator of the xenobiotic metabolizing forms of cytochrome P450 in animals, plants, insects, and microorganisms. It has been widely used to investigate the biological roles of cytochrome P450 enzymes, their participation in the metabolism of both endobiotics and xenobiotics, and their contributions to the metabolism-dependent toxicity of drugs and chemicals. This review is a comprehensive evaluation of the chemistry, discovery, and use of 1-aminobenzotriazole in these contexts from its introduction in 1981 to the present.

Keywords: Cytochrome P450; Mechanism-based inhibition; Heme adducts; 1-Aminobenzotriazole; Benzyne; Arachidonic acid oxidation; Estradiol; Xenobiotics; Drug metabolism

Introduction

The inhibition of cytochrome P450 enzymes to study their roles in the metabolism of endogenous compounds and xenobiotics, and for potential practical purposes, began as soon as this class of enzymes was recognized in the early 1960s [1]. Initially, the most commonly used inhibitors were SKF-525A, which inhibits multiple isoforms [2], and metyrapone, which inhibits what became known as the CYP2B class of P450 enzymes, but was primarily utilized as an inhibitor of P450 enzymes in the steroid biosynthetic pathway [3]. With increasing understanding of the native complement of cytochrome P450 enzymes in humans and other species, particularly the understanding provided by genomic data, the search for cytochrome P450 inhibitors diverged into two tracks.

One direction for this search was the identification of inhibitors that are isoform specific, or as close to that as possible, as these can be used to probe the biology and/or exploit the inhibition of individual cytochrome P450 enzymes. These inhibitors could be competitive, non-competitive, quasi-irreversible, or mechanism-based [4,5]. On the other hand, agents have also been sought that are pan-specific, i.e., that broadly inhibit the complement of cytochrome P450 enzymes involved in drug metabolism, while minimally perturbing the P450 enzymes involved in biosynthetic processes. These agents include SKF-525A and ketoconazole [6,7], but this review focuses on 1-aminobenzotriazole (1-ABT), a widely employed mechanism-based inhibitor. Comparisons of 1-ABT and SKF-525A in investigations of drug metabolism by cytochrome P450 enzymes delineate the advantages of 1-ABT. For example, SKF-525A disrupts autophagy in primary rat hepatocytes, whereas 1-ABT and several other P450 inhibitors do not [8]. In another study, SKF-525A and ketoconazole were shown to inhibit CCK-induced $[[Ca^{2+}]]$ -induced oscillations in rat pancreatic acinar cells, whereas 1-ABT did not [9].

The currently available reviews on cytochrome P450 inhibition take a horizontal perspective that surveys the breadth of the field (e.g., [5,10,11]). This review pursues a vertical, comprehensive analysis of the relevant chemistry, biochemistry, and biology associated with 1-aminobenzotriazole.

Chemistry of 1-Aminobenzotriazole (1-ABT)

1-Aminobenzotriazole, abbreviated as 1-ABT, was first prepared in 1960 [12], but a more practical synthesis was subsequently described

by Campbell and Rees [13]. These authors also demonstrated that oxidation of 1-ABT by lead tetra-acetate resulted in the release of nitrogen gas and the essentially quantitative formation of benzyne, as established by its dimerization and trapping by other substrates. For example, it reacts with tetraphenylcyclopentadienone to give 1,2,3,4-tetraphenyl-naphthalene after loss of CO from the initial adduct (Figure 1) [14]. As Campbell and Rees also showed that the oxidation of 1-ABT by other chemical agents gave similar results [15].

Discovery of 1-ABT as a Cytochrome P450 Inactivating Agent

In view of the chemistry of 1-ABT, its biological oxidation by cytochrome P450 enzymes was examined to determine if this would produce benzyne within a biological environment. In effect, incubation of rat liver microsomes with 1-ABT in the presence of the cofactor NADPH led to rapid loss of the cytochrome P450 chromophore and the accumulation of a novel porphyrin [16]. Cytochrome P450 inactivation requires NADPH and is inhibited by CO, features which implicate an autocatalytic process. Glutathione does not prevent cytochrome P450 loss, which suggests that this is a mechanism-based process in which benzyne generated within the enzyme active site reacts with the heme before diffusing away from the active site cavity [16]. Exposure of isolated perfused rabbit lungs or microsomes from such lungs showed that 1-ABT caused loss of both specific P450 content and the ethoxyresorufin O-deethylation and benzphetamine N-demethylase activities [17]. These losses were detectable at concentrations of 1-ABT above 10 μ M, with maximum losses occurring at concentrations above 1 mM. At the higher concentrations of 1-ABT, the losses of P450 and benzphetamine N-demethylase activity were similar. Furthermore, covalent binding of radiolabeled 1-ABT to lung microsomes (0.3 nmol/mg protein) was similar to the spectroscopically measured loss of cytochrome P450 (0.3 nmol/mg protein) [17], suggesting a stoichiometric process. 1-ABT

***Corresponding author:** Paul R Ortiz de Montellano, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143, USA, Tel: 4154762903; E-mail: ortiz@cgl.ucsf.edu

Received March 22, 2018; Accepted March 27, 2018; Published March 31, 2018

Citation: Ortiz de Montellano PR (2018) 1-Aminobenzotriazole: A Mechanism-Based Cytochrome P450 Inhibitor and Probe of Cytochrome P450 Biology. Med Chem (Los Angeles) 8: 038-065. doi: [10.4172/2161-0444.1000495](https://doi.org/10.4172/2161-0444.1000495)

Copyright: © 2018 Ortiz de Montellano PR. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

also inhibited the metabolism of resorufin ethers in the livers and skin of mice [18] and of ethoxycoumarin by rat liver slices, isolated hepatocytes, and hepatic microsomes [19].

Based on its UV-visible spectrum, resistance to forming a zinc complex, and mass spectrometric molecular ion, the accumulated porphyrin was tentatively identified as protoporphyrin IX with a benzene group forming a bridge between two nitrogen atoms of the porphyrin (Figure 2) [20]. NMR analysis of the modified porphyrin isolated from rats treated *in vivo* with 1-ABT unambiguously confirmed this structure. In addition, a second minor fluorescent product was isolated that was identified as N-phenylprotoporphyrin IX, in which a phenyl group is attached to only one of the nitrogens of the porphyrin. It was estimated that less than 12 molecules of 1-ABT were consumed per molecule of P450 inactivated.

The oxidation of 1-ABT by P450 enzymes could be envisaged to proceed via either (path a) initial N-hydroxylation followed by dehydration and then fragmentation, or (path b) sequential hydrogen abstractions from the exocyclic nitrogen followed by fragmentation (Figure 3). Relevant experimental data is scarce, but DFT computational studies favor the two hydrogen abstraction model (path b) of Figure 3 [21]. However, as will be discussed in a later section on derivatives of 1-ABT, N-monoalkyl 1-aminobenzotriazoles also inactivate cytochrome P450 [22]. It is possible that these agents act by a different mechanism than 1-ABT itself, but if not, it would suggest that, at least in some instances, sequential removal of two hydrogens is not required for benzyne generation.

Metabolism and Pharmacokinetics of 1-ABT

The major metabolite observed in the plasma of male rats after oral administration of ^{14}C -labeled 1-ABT was the N-acetyl derivative, but the N-glucuronides of 1-ABT and of benzotriazole were also found in the urine (Figure 4) [23]. N-acetylation of 1-ABT by human N-acetyltransferases NAT1 and NAT2 has been confirmed by *in vitro* incubations in studies, which also demonstrated that 1-ABT is a time-independent inhibitor of the N-acetyltransferases in rat liver [24]. In contrast, 1-ABT was not found to be a measurable inhibitor of rat liver glucuronosyl transferases or sulfotransferases, as judged by its failure to inhibit the action of these enzymes on acetaminophen and 7-hydroxycoumarin, respectively. The absence of glucuronosyl transferase inhibition in rats was independently reported [25].

Although the benzotriazole glucuronide (Figure 4) was detected in the urine of rats, benzotriazole was not observed in the serum or urine [23]. However, benzotriazole itself was reported as the primary detectable metabolite in incubations of ^{14}C -labeled 1-ABT with guinea pig hepatic or pulmonary microsomes [26]. The authors postulated a plausible mechanism in which hydroxylation of the exocyclic nitrogen is followed by elimination of HNO to give benzotriazole (Figure 5).

Oral administration of ^{14}C -labeled 1-ABT to male rats showed that the compound is absorbed slowly, 50% of the radioactivity remaining in the stomach 6 h after dosing, with maximum plasma and tissue concentrations observed at 24 h [23]. Of the initial dose, 71% was excreted in the urine and 12% in the feces over a period of 72 h. Measurement of radioactivity in the various tissues showed that the highest tissue-to-plasma ratios were in the liver, adrenals, and kidneys, with half-lives of elimination from these tissues of ~24, 16, and 24 h, respectively. The plasma half-life was ~9 h.

Comparison of the effect of oral and intravenous 1-ABT on the bioavailability of midazolam in rats revealed that bioavailability was

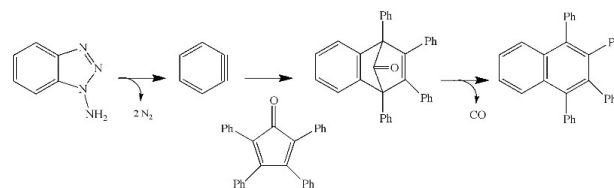


Figure 1: Oxidation of 1-ABT (compound 1) produces benzyne, which can be chemically trapped to give 1,2,3,4-tetraphenylnaphthalene.

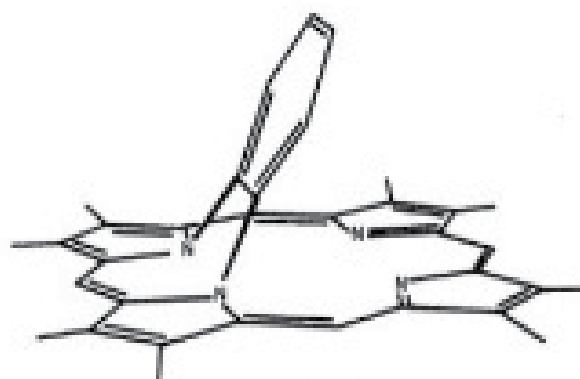


Figure 2: Heme adduct formed by reaction of the heme of cytochrome P450 with autocatalytically activated 1-ABT. The peripheral substituents on the porphyrin are only shown schematically, as isomeric structures that differ in the pattern of peripheral substitution are possible.

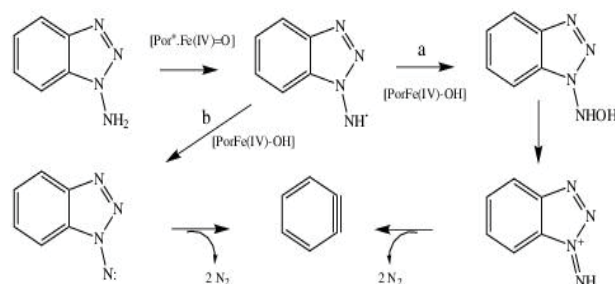


Figure 3: Two possible mechanisms for the P450-catalyzed oxidation of 1-ABT to benzyne, where $[\text{Por}^+ \text{Fe(IV)=O}]$ stands for the activated iron oxo species of cytochrome P450.

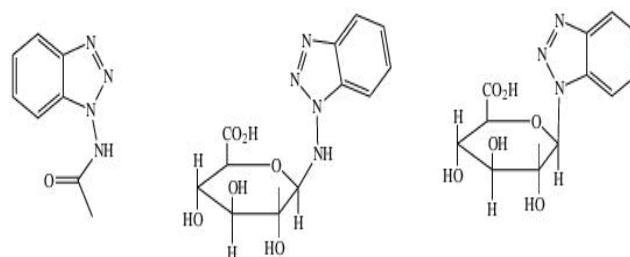


Figure 4: Metabolites of 1-ABT formed *in vivo* in rats.

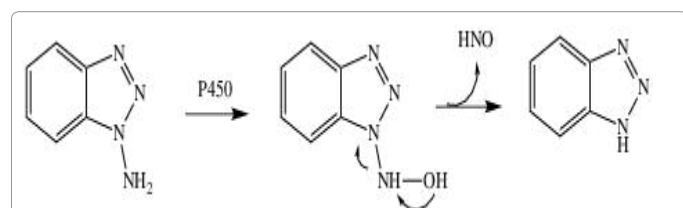


Figure 5: Possible mechanism for the P450-catalyzed conversion of 1-ABT to benzotriazole.

much lower with oral administration, which led to the conclusion that 1-ABT inactivated intestinal P450 enzymes, which are responsible for most of midazolam metabolism [27]. This led to the proposal that 1-ABT can be used to evaluate gut versus liver clearance of drugs. Using metoprolol as a probe, the AUC was found to increase 16- and 6.5-times when 1-ABT was co-administered orally and intravenously, respectively [28]. This difference was interpreted as being due to a delay in the absorption of 1-ABT. However, a different study concluded from the fact that concentration-time curves were linear and proportional to the dose that 1-ABT is rapidly absorbed into the systemic circulation in rats and has a $t_{1/2}$ of 8-13 h [29]. It has also been reported that 1-ABT delays gastric emptying in rats and can alter systemic exposure and T_{max} of coadministered agents [30]. The observation that 50 mg/kg of 1-ABT inhibited antipyrine clearance by 88% when given shortly before the antipyrine, but only by 29% when given 24 h before, suggested that the duration of 1-ABT was relatively short [31]. However, The pharmacokinetic profiles of oral doses of 1-ABT ranging from 5 to 200 mg/kg, determined in rats, dogs, and monkeys, showed that a single dose of 50 mg/kg in rats and 20 mg/kg in dogs and monkeys produced high plasma concentrations that were sustained over 24 hours [32]. This resulted in inhibition of the plasma clearance of antipyrine by 88% in rats, 96% in dogs, and 83% in monkeys. Using a serial sampling method after administration of 50, 100, and 150 mg/kg oral doses to mice and guinea pigs revealed that the plasma 1-ABT concentrations were maintained at levels of 5-100 μ M for at least 12 hours in both species [33]. *In vitro* studies indicated that the K_i values for 1-ABT as a mechanism-based inactivator were 45.6 and 193 μ M in mice and guinea pigs, respectively, with the corresponding k_{inact} values of 0.089 and 0.075 min^{-1} . Administration of 1-ABT 2 hours before intravenous antipyrine decreased plasma antipyrine clearance by approximately 95% in mice and 84-95% in guinea pigs at all the doses of 1-ABT examined [33]. Osmotic pumps can be used to maintain maximum blood concentrations of 1-ABT for at least 6 days [31]. Indeed, ALZET osmotic pumps were able to maintain the 1-ABT plasma concentration above 4.1 mg/ml over 336 hours without overt toxicity [34]. A convenient LC/MS/MS assay for the quantitation of 1-ABT in pharmacokinetic studies has been reported [35]. On the other hand, a test of the residual effects of 1-ABT and quinidine on drug disposition in rats after a 7-day washout period suggested that this period suffices to return to the original pharmacokinetics observed with naive rats [36].

Specificity of 1-ABT

Cytochrome P450

Early work showed that the cytochrome P450 enzymes induced in rat liver by phenobarbital and 3-methylcholanthrene were similarly inactivated by 1-ABT [37]. Furthermore, similar amounts of N,N'-aryl bridged porphyrin adducts were obtained from male and female rat livers after treatment with 1-ABT [38]. Other early studies supported the conclusion that 1-ABT was a relatively non-selective inactivating

agent for cytochrome P450 enzymes. It inactivated the liver, lung, and kidney P450 enzymes of uninduced, phenobarbital pretreated, and β -naphthoflavone-pretreated guinea pigs [39], the rat liver and lung enzymes before and after pretreatment with β -naphthoflavone [40], and recombinant human P450 enzymes in microsomes from baculovirus infected insect cells [7]. The use of isoform selective probe substrates indicated that pooled human liver microsomes incubated with 1 mM 1-ABT for 30 min resulted in complete inactivation of CYP2A6 and CYP3A4, but retention of a residual activity of up to 20% with CYP1A2, -2B6, -2C8, -2C9, -2C19, and -2D6 [41]. In this study, CYP2C9 was particularly resistant, with 60% of the activity remaining. A separate study indicated that 1-ABT caused a concentration dependent inhibition of CYP1A2, -2B6, -2C9, -2C19, -2D6, and -3A4 [32]. A later study showed, however, that addition of (S)-warfarin together with 1-ABT resulted in potent inactivation of CYP2C9, presumably because 1-ABT is displaced from the (S)-warfarin binding site to one more favorable for its oxidation [42]. In accord with catalysis-dependent inactivation, inhibition of baculovirus-expressed P450 enzymes is less differentiated from those of ketoconazole and SKF-525A in the absence of preincubation under turnover conditions [6].

The inactivation of CYP2E1 by 1-ABT has been extensively investigated. An early study demonstrated that 1-ABT caused loss of CYP2E1 activity but not loss of the protein, whereas CCl_4 caused loss of both the activity and the protein [43]. The modification of CYP2E1 by 1-ABT thus does not trigger rapid degradation of the P450 protein, in contrast to modification by CCl_4 . The inactivation of CYP2E1 by 1-ABT has been used to establish the role of this enzyme in the metabolism of acrylamide [44], methacrylonitrile [45], 1,3-butadiene [46], urethane [47], and chlorzoxazone [48].

CYP4A11, a human fatty acid ω -hydroxylase, is inactivated by 1-ABT, but interestingly, its L131F mutant is not [49]. In the case of CYP4A1, the homologous rat enzyme, the wild-type enzyme and its E320A mutant are not inactivated by 1-ABT, but the D323E and E320A/D323E mutants are inactivated [50]. Suppression of inactivation of CYP4A11 by the L131F mutation, and enabling of inactivation of CYP4A1 by a D323A mutation, are ascribed to alterations in steric interference to the productive binding of 1-ABT. These results are reminiscent of the finding that occupying the (S)-warfarin site of CYP2C9 enables its much more effective inactivation by 1-ABT [42].

The cytochrome P450 enzymes involved in sterol biosynthetic pathways appear to be resistant to the action of 1-ABT. In incubations with guinea pig adrenal microsomes it did not inhibit the 11-, 17 α - or 21-sterol hydroxylases [51,52]. However, 1-ABT did inhibit the activities of these enzymes when administered *in vivo* to rats or guinea pigs [53-55]. It was postulated that an extra-adrenal metabolite of 1-ABT was responsible for the *in vivo* inactivation of the adrenal enzymes despite their resistance to inactivation *in vitro*. Inactivation of CYP51 has been cited [56], but no evidence for this is available in the published literature.

It is important to note that cytochrome P450 reductase, the electron donor partner of most cytochrome P450 enzymes, is not inactivated in rats by incubation with 1-ABT in the presence of NADPH [57]. Similarly, 1-ABT does not inhibit cytochrome b_5 , which is an alternative electron donor for some P450 enzymes [25]. Comparable results were obtained in studies of P450 inactivation in a plant enzyme system [58]. The loss of P450 activities is thus due to inactivation of the hemoproteins themselves rather than their electron donor partners.

Species differences in susceptibility to inactivation of P450 metabolism have been reported. The oxidation of ketamine by human

and canine liver microsomes was markedly inhibited, but little inhibition was seen with equine microsomes [59].

1-ABT has been used to inactivate cytochrome P450 enzymes in non-mammalian species, including plants, insects, fungi, and bacteria. This aspect of 1-ABT specificity is addressed in the section that describes inactivation in these species.

Proteins other than cytochrome P450

The interactions of proteins other than the cytochrome P450 enzymes with 1-ABT has been examined to provide a context for any physiological observations that may be made when this agent is used to inactivate P450 enzymes in intact cells, tissues, or animals. Inhibition of oxidative enzymes other than the cytochromes P450, for example, would complicate specific attribution of a physiological consequence to inactivation of cytochrome P450 enzymes. The flavin monooxygenase (FMO) family of enzymes is of concern in this regard, as their oxidative activities towards heteroatomic substrates resemble those of the P450 enzymes. However, a number of studies have established that 1-ABT is neither a substrate nor an inhibitor of the FMO enzymes. Thus, 1-ABT has no effect on lung or liver FMO enzymes [17,22,60,61]. One consequence of this is that 1-ABT has been used to determine the relative roles of FMO and P450 enzymes in the oxidative metabolism of xenobiotics (Table 1).

1-ABT is reported to not be an inhibitor of horseradish peroxidase, as it does not inhibit the oxidation of o-anisidine by this enzyme [68], but measurements of the binding of benzo[a]pyrene to serum bovine albumin in the presence of horseradish peroxidase and H₂O₂ suggested that this process is inhibited by 1-ABT [69]. Based on indirect evidence, the inference has been made that 1-ABT interacts with hemoglobin [70]. However, clear and convincing evidence that 1-ABT actually inhibits horseradish peroxidase or interacts with hemoglobin is not available. In contrast, chloroperoxidase, a cytochrome P450-like enzyme with a thiolate iron ligand clearly oxidizes 1-ABT to phenol and is inactivated in the process [20].

1-ABT is a substrate for N-acetyltransferases and inhibits these enzymes [71]. 1-ABT is also reportedly a non-competitive inhibitor of mouse, rat, and human monoamine oxidases with K_i values of 7.87, 8.61, and 65.2 μM, respectively, when measured *in vitro* with S9 liver fractions [56]. It does not, however, inhibit aldehyde oxidase [72], α,β-ketone reductase [73], or 3β-hydroxysteroid dehydrogenase [53]. Although the glucuronide of 1-ABT has been observed in rat urine [23], 1-ABT has no inhibitory activity against the rat glucuronosyl transferases and sulfotransferases [24] and little if any against the human glucuronosyl transferases [74]. Indeed, in rat hepatocytes, treatment with 1-ABT increased the formation of the 1-O-acyl glucuronides of gemfibrozil, tolmetin, mefenamic acid, and diclofenac, and the S-acyl glutathione conjugates of mefenamic acid and diclofenac [75]. 1-ABT also does not significantly alter the glutathione transferase activities or glutathione levels in rat liver or kidney [25].

Studies with sandwich-cultured human hepatocytes indicate that

1-ABT does not alter the hepatic transport of rosuvastatin, atorvastatin, or midazolam [71]. Work published in a poster supported the inference that 1-ABT does not interfere with transporters, as concentrations of 1-ABT up to 1 mM did not inhibit the MDR1, Bcrp1, and MRP2 transporters [76]. In another study, the *in vivo* clearance of GNE1, an inhibitor of the p21-activated kinase PAK1, was lower than predicted [77]. The use of transporter knockout rats with and without 1-ABT administration indicated that the clearance of this agent is determined by uptake transporters rather by metabolism. These results indicate that 1-ABT does not interfere with transporters. However, 1-ABT can interact with transporter inhibitors by inhibiting the metabolism of drugs whose concentration is elevated due to transporter inhibition. As a case in point, the toxicity of nefazodone, which inhibits bile acid transport, is due to the unmetabolized drug. The toxicity of this drug is potentiated by 1-ABT, which prevents metabolism of nefazodone [78]. Similarly, inhibition of the bile acid export pump, which results in accumulation of bile acids in hepatocytes, can be potentiated by inhibition of the metabolism of pump inhibitors by 1-ABT [79].

Induction of Cytochrome P450 Enzymes

In an early study, administration of 1-ABT to rats was not found to induce the mRNA or P450 protein levels for what were then known as cytochromes P450b and P450e, nor did 1-ABT prevent the elevation of P450 mRNA levels caused by co-administration of phenobarbital or 3-methylcholanthrene [80]. However, two decades later 1-ABT was found to upregulate the expression of CYP2B6 and CYP3A4 mRNA in a concentration dependent manner in freshly isolated human hepatocytes. Indirect data suggested that 1-ABT is a CAR, but not PXR activator [81]. In another study with primary cultured rat hepatocytes, 1-ABT was found to indirectly increase CYP2B mRNA levels, possibly by inhibiting the initial step in the conversion of monocarboxylic to dicarboxylic fatty acids, thereby decreasing peroxisomal β-oxidation and increasing intracellular fatty acid levels [82].

Toxicity of 1-ABT

1-ABT has been reported to have no readily observable toxicity when administered to houseflies [83], wheat seedlings [84], beagle dogs [85], rats [85,86], or mice [86]. Furthermore, administration of 1-ABT to mice via an ALZET osmotic pump for 16 days caused no overt toxicity [34]. The results of a more detailed 13-week toxicological and pathologic evaluation of the effects of 1-ABT generally agree with this conclusion [87]. This study showed that in male Sprague-Dawley rats hepatic P450 levels and resorufin dealkylation activity were reduced to less than 30% of control values without obvious signs of toxicity such as changes in body weight, food consumption, or clinical appearance. Hemoglobin, hematocrit, and erythrocyte counts decreased slightly, whereas mean corpuscular hemoglobin concentration, mean corpuscular volume, red cell distribution width, and mean corpuscular hemoglobin were slightly increased. Some accumulation of lipids was suggested by an increase in liver weight, centrilobular hypertrophy, and intracytoplasmic vacuolization. Thyroid stimulating hormone was slightly elevated and triiodothyronine and thyroxine were slightly

Table 1: Some compounds for which the role of P450 versus FMO has been investigated using 1-ABT as a P450 inactivating agent.

Substrates	Enzyme source	Reference
2-Aryl-1,3-dithiolanes	Rabbit lung	[62]
Alkyl p-tolylsulfides	Rabbit lung/mini pig liver	[63]
Thiocarbamides	Rat liver	[64]
Benzimidazole-2-thiones	Rat liver	[65]
2-(Alkylthio)-1,3,4-thiadiazoles 2-(Alkylthio)-1,3-benzothiazoles	Human liver	[66]
Thiouracil derivative	Rat / dog liver	[67]

decreased. Adrenal weight was increased and hypertrophy of the zona fascicularis of the adrenal gland was observed. Some increase in kidney weight was also noted. It is likely that the changes observed are secondary to the persistent decrease in cytochrome P450 activities. The authors concluded that the effects of daily 1-ABT administration “over a 13 week period were well-tolerated under controlled laboratory conditions”.

Moorthy et al. reported that administration of 1-ABT to rats, followed by exposure for 24-, 28-, or 60-hours to >95% oxygen caused the 1-ABT-treated animals to die between 48 and 60 hours, whereas no control rats exposed to similar hyperoxic conditions died at up to 60 hours of exposure [88]. 1-ABT thus potentiated hyperoxic lung injury.

Analogues of 1-ABT

A number of analogues of 1-ABT have been examined to explore the range of structures compatible with mechanism-based inactivation of P450 enzymes. Efforts to alter the core 1-ABT structure have yielded mixed results. Compounds **3** and **4** (Figure 6) cause catalysis-dependent loss of the P450 content in microsomal incubations, but do not appear to result in the formation of a covalent heme adduct, while compound **2** causes NADPH-independent conversion of the P450 to a P420 chromophore [20].

Modifications of the aromatic hydrocarbon ring and of the exocyclic amino group, however, are tolerated. Thus, replacement of the benzene ring of 1-ABT by a naphthyl ring as in compound **7** (Table 2) yields an analogue that interacts with cytochrome P450 in much the same way as 1-ABT [20], causing both catalysis-dependent loss of the P450 chromophore and accumulation of a hepatic pigment. Although the structure of the pigment was not established, it had properties very similar to those of the N,N'-bridged porphyrin adduct obtained with 1-ABT (Figure 2).

The N-mono- and N,N-diacetyl derivatives of 1-ABT, compounds **8** and **9**, cause NADPH-dependent loss of P450 content and the formation *in vivo* of a porphyrin pigment analogous to that obtained with 1-ABT. The same is observed with the N-methyl, N-acetyl derivative **10**, N-methyl analogue **11**, and the N-butyl derivative **12** [20,22]. A porphyrin pigment was formed *in vivo* with these agents, but its identity was not determined. Compounds **13-15** represent the introduction of even larger substituents on the exocyclic nitrogen. These agents, which are more lipophilic and are generally active at lower concentrations than 1-ABT, cause catalysis-dependent loss of both P450 content and catalytic activities. In the case of the N-benzyl derivative **13**, it has been explicitly demonstrated that a hepatic pigment similar to that formed with 1-ABT is formed in phenobarbital-pretreated rats [20]. This indicates that inactivation by the N-benzyl derivative, as with 1-ABT itself, involves formation of a benzyne that adds to the P450 heme group. Although it is conceivable that the N-benzyl compound is first oxidized to 1-ABT, which then is the actual inactivating agent, it is more likely that benzyne formation is directly initiated from the N-benzyl derivative by electron abstraction from the nitrogen.

Compound **5** (Figure 6), a putative possible intermediate in the inactivation mechanism of 1-ABT (Figure 5), does not cause detectable P450 loss [20]. This indicates that this N-hydroxyl compound is not an intermediate in the action of 1-ABT. The N-nitro compound **6** could not be properly evaluated because it is a powerful electron acceptor that interferes with the P450 assay and, upon administration to rats *in vivo*, causes asphyxia [20].

Analysis of the effects of compounds **13-15** suggests that, in

addition to heme adduct formation, there are alternative, isoform-dependent inhibitory mechanisms. Thus, incubation of these agents with liver microsomes from untreated, phenobarbital-induced, or β -naphthoflavone-induced guinea pigs led to the observation of a complex with an absorption maximum at approximately 455 nm [90]. This corresponds to the formation of a metabolite-intermediate (MI) complex comparable to that formed with other amines, an inference consistent with the fact that the complex was disrupted by addition of 50 μ M potassium ferricyanide or, in the case of **14** and **15**, by sedimentation and resuspension. The formation of this complex was highest with the microsomes from phenobarbital-induced guinea pigs, and was attenuated in the presence of 1 mM glutathione. Interestingly, formation of this complex was not observed with pulmonary microsomes from guinea pigs or rabbits. Formation of the MI complex is therefore only observed with some P450 enzymes in some tissues. Further work suggested that mechanism-based inactivation was favored by CYP1A1 and CYP2B, and reversible inhibition by CYP1A2 [91]. The nature of this complex was not further characterized, but it was postulated that it might result from formation of an alkyl nitroso species according to the scheme in Figure 7. Coordination of the electron pair in the nitrogen of the nitroso group to the heme iron would then explain the MI complex. In any case, this process is ancillary and independent from that associated with the direct action of 1-ABT.

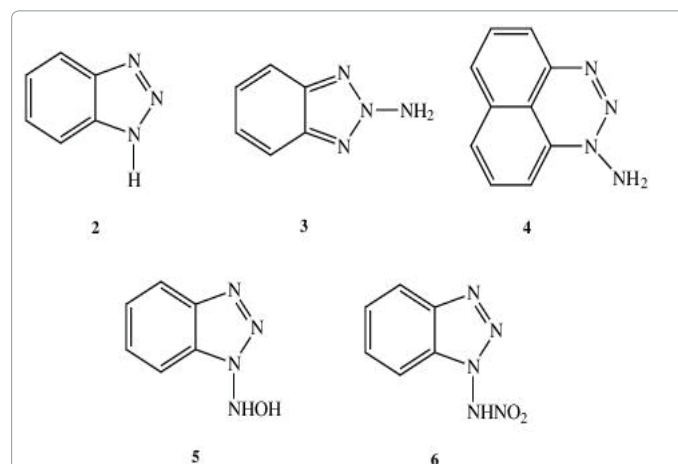


Figure 6: Analogues of 1-ABT with variants of the core 1-aminobenzotriazole structure are not effective mechanism-based inactivating agents.

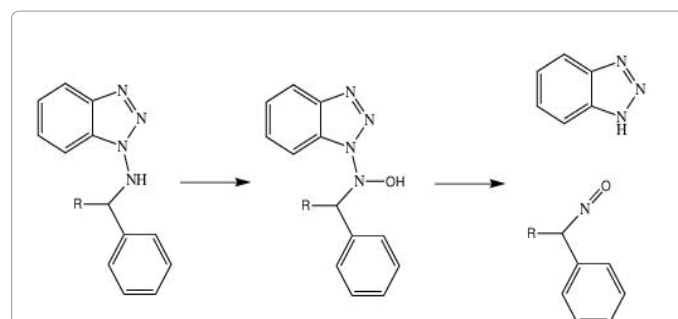


Figure 7: A possible mechanism for the formation of a nitroso compound that could coordinate to the heme iron of P450, resulting in observation of MI complexes. The reaction scheme shown is a modified version of that proposed by Sinal and Bend [90]. The R stands for H, Me, or Et, depending on whether it refers to experiments with compound **13**, **14**, or **15** (Table 2), respectively.

Rat CYP2B1 is completely inactivated *in vitro* by incubation of liver microsomes with 1 μ M N-benzyl compound **13**. Benzaldehyde, benzotriazole, 1-ABT, and an unknown metabolite of **13** tentatively assigned to a “dimer” of the inhibitor were isolated from these incubations [92]. However, the Gly478Ala mutant of CYP2B1 was not inactivated and only produced the “dimeric” metabolite. Modeling studies suggested that the Gly478Ala mutation prevented oxidation of the 1-amino group, favoring instead oxidation of the 7-benzyl carbon and preventing formation of the inactivating metabolite. Mechanistic studies of CYP2B1 inactivated by the N-benzyl derivative of 1-ABT indicated that the inactivated enzyme was reduced more slowly by NADPH-cytochrome P450 reductase [93]. The data also indicated that differences existed in the extent to which the metabolism of several compounds was inhibited. N-benzyl 1-ABT also inhibited the 7-ethoxyresorufin O-deethylase and 7-methoxyresorufin O-demethylase activities by 88 and 71%, respectively, in microsomes from β -naphthoflavone-induced guinea pig heart [94]. These activities were attributed to CYP1A1.

Both enantiomers of N-(α -methylbenzyl)-1-aminobenzotriazole [**14**] inactivated guinea pig hepatic CYP2B and CYP1A enzymes, with small differences in enantiospecificity [95].

Inactivation of guinea pig lung and liver CYP1A1, CYP2B4, and CYP4B1 by the N-benzyl and N-(α -methylbenzyl) analogues **13** and **14** has been compared by measuring changes in the oxidation of 7-ethoxyresorufin (CYP1A1), 7-pentoxoresorufin (CYP2B4) and 4-aminobiphenyl N-hydroxylation (CYP4B1) [96]. In uninduced and phenobarbital induced guinea pigs the order of inactivation was 2B1>1A1>>4B1. However, in β -naphthoflavone induced animals, the α -methyl compound inhibited lung CYP2B4, whereas even at concentrations 100-fold higher, it did not inhibit CYP4B1 and CYP1A1. The α -methyl compound was also much more active *in vivo* in inactivation of lung CYP2B4 than of the liver enzymes. In contrast, 1-ABT exhibited little isoform or tissue specificity. The authors postulated that the lung specificity of the N-substituted compounds **13** and **14** reflected selective absorption by the lipophilic aromatic amine uptake system of the lung. In these studies, inhibition by compounds **13** and **14** was not paralleled by comparable losses in P450 content, whereas with 1-ABT the activity and P450 losses were comparable. This indicates that the inhibition caused by the substituted 1-ABT analogues included mechanisms other than heme alkylation.

Inhibition of at least some cytochrome P450 enzymes by much lower concentrations of compounds **13-15** than of 1-ABT, and greater isoform and tissue selectivity, have also been reported in a number of studies [97-99]. Of particular note is the observation that N-benzyl 1-ABT inhibits CYP1A1 in guinea pig kidney in preference to the enzyme that oxidizes arachidonic acid, and in lung primarily inhibits epoxidation of arachidonic acid, whereas 1-ABT itself inhibits both epoxidation and ω -hydroxylation [100,101]. N-(α -methyl)-1-ABT has been shown to selectively inhibit dog CYP2B11 relative to CYP3A12 [102].

Incidentally, derivatives of 1-ABT obtained by laccase-catalyzed reaction of 1-ABT with 2,5-dihydroxybenzoic acid methyl ester and 2,5-dihydroxybenzoic acid ethyl ester were found to have low antimicrobial activities [103].

1-ABT in Analysis of Xenobiotic Metabolism

Bioavailability and first pass metabolism

The use of 1-ABT in a rat *in vivo* pharmacokinetic screen has

been proposed as a tool for determining the relative extent to which absorption and metabolism contribute to low oral systemic exposure of a drug [104]. The model drugs used in validating this approach were propranolol, metoprolol, and cimetidine. In another study, the low oral (19%) bioavailability of an undefined drug in Sprague Dawley rats was examined by comparing bioavailability in rats pretreated with 50 mg/kg 1-ABT 2 hours prior to both intravenous and oral administration of the drug [105]. 1-ABT caused a 71% increase in plasma clearance, a 100% increase in half-life, and a marked increase in bioavailability. Similarly, a comparison of bioavailability of oral danazol in rats with and without 1-ABT pretreatment showed that the bioavailability of danazol rose from 2% to 60% and was primarily limited by first pass metabolism [106]. Comparison of the bioavailability of a β -glucuronidase inhibitor in mice before and after pretreatment with 1-ABT established that its low bioavailability (21%) was due to first pass metabolism [107]. An investigation of intestinal permeability and first-pass metabolism of acetaminophen, verapamil, and midazolam using single-pass intestinal perfusion showed that 1-ABT increased the intestinal availability of these three drugs to 0.8-0.85 in cyanomologous monkeys, confirming that they are extensively metabolized in the intestine [108]. A significant increase in the exposure of mice to midazolam was observed when the mice were pretreated with 1-ABT [109]. The use of 1-ABT also demonstrated that intestinal metabolism, at least in part by CYP2J2, diminished the bioavailability in rats of AZ0908, a prostaglandin E synthase-1 inhibitor [110]. Using a serial blood sampling method, the administration of 1-ABT (50-150 mg/kg) 2 hours prior to a single intravenous dose of antipyrine (20 mg/kg) was shown to increase the AUC from 27.3 to upto 333 μ g h/ml and the half-life from 0.51 to up to 2.2 hours [111].

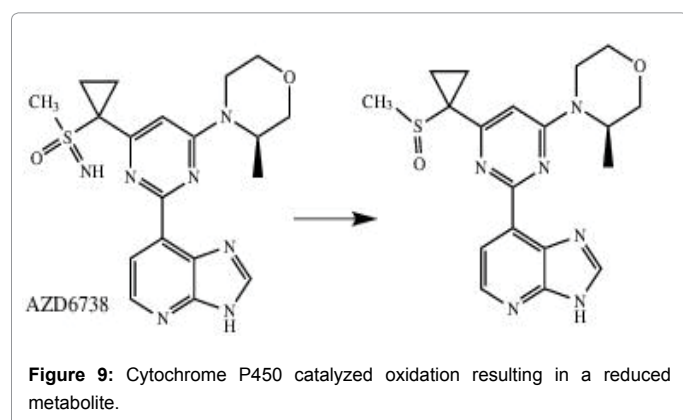
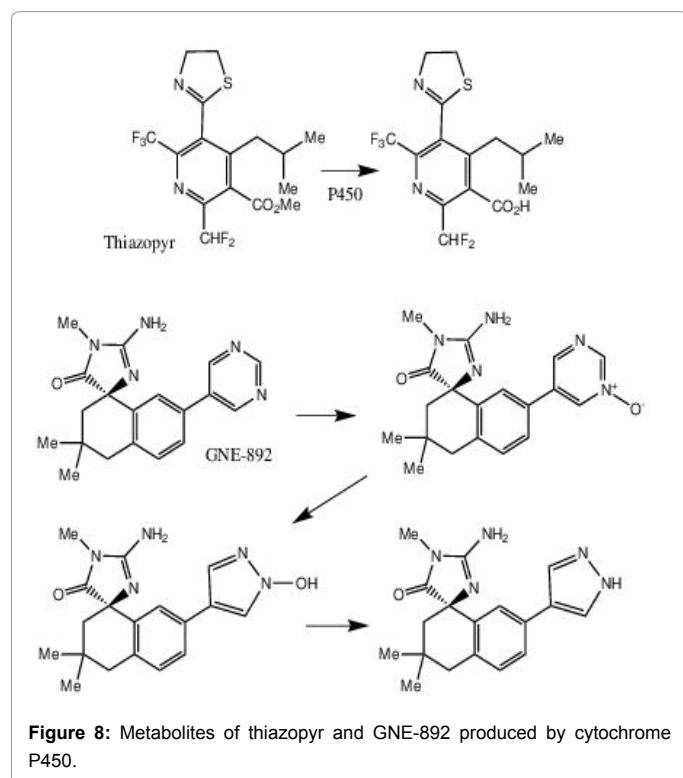
1-ABT can be used to potentiate (or “boost”) the action of a drug by decreasing its P450 metabolism. Thus, a series of 5-substituted 4-anilinoquinazolines, potent inhibitors of the erbB2 receptor tyrosine kinase, inhibited tumor growth in a mouse xenograft model when 1-ABT was employed to potentiate their action [112]. In this instance, 1-ABT increased exposure to the tumor inhibitors approximately 100-fold.

Human hepatocytes can be used to assay time-dependent inhibition of cytochrome P450 enzymes. These assays correctly distinguished the inactivation of CYP3A4 by 1-ABT and erythromycin from inhibitors that were not time-dependent: for 1-ABT, the values obtained were $K_i=22.0$ μ M and $k_{inact}=0.09$ min^{-1} [113]. 1-ABT was employed in the development of this high throughput assay system [114,115].

Clarification of oxidative mechanisms

The conversion by rat liver microsomes of the esterified pesticide thiazopyr to the free acid is inhibited by 1-ABT and is thus mediated by a cytochrome P450 enzyme rather than by an esterase (Figure 8) [116]. Using 1-ABT, cytochrome P450 catalysis in the endoplasmic reticulum was found in another instance to contribute to the instability of a TGR5 receptor agonist with a central amide bond [117]. A more exotic transformation in the metabolism of GNE-892 by rats produces two ring-contracted pyrazole compounds as minor metabolites (Figure 8). This ring contraction is inhibited by 1-ABT and is thus cytochrome P450-dependent [118].

1-ABT was recently used to establish that the deimination of the sulfoximine moiety in the ATR inhibitor AZD6738 to a sulfoxide and sulfone, a nominally reductive event, is mediated by cytochrome P450 (Figure 9) [119]. A reasonable oxidative mechanism for this transformation was postulated.



In contrast, the reductive metabolism of the dinitrobenzamide mustard anticancer prodrug PR-104, which is activated to its cytotoxic form in the liver, is not inhibited by 1-ABT, indicating that cytochrome P450 itself is not involved in this reductive transformation [120]. 1-ABT was similarly employed to establish that the metabolism of a vasoactive drug, SK&F 86466, in isolated, perfused rat lungs was not mediated by cytochrome P450 enzymes [121]. In these studies, 1-ABT administered to the rats before the lung study was shown to suppress P450 activities completely without any effect on the metabolism of the drug. A comparison of the effects of 1-ABT and inhibitors of epoxide hydrolases on the metabolism of oprozomib, an oral proteasome inhibitor, confirmed that the major metabolic pathway was epoxide hydrolysis rather than P450-catalyzed oxidation [122].

Oxidation of pharmaceutical agents

The use of 1-ABT in studies of the action of a variety of agents is summarized in Table 3.

1-ABT as a Probe of Physiological Processes

Fatty acid oxidation

Cytochrome P450 enzymes oxidize fatty acids and these oxidative reactions can be inhibited by 1-ABT. HPLC analysis of the oxidation of radiolabeled lauric acid by control and clofibrate-induced rat liver microsomes showed that 1-ABT inhibits the oxidation of lauric acid to the ω -, ω -1, and ω -2 hydroxylated derivatives [20,144,145]. Comparison with more specific inhibitors implied the presence of at least one lauric acid ω -hydroxylase and two ω -1 hydroxylases [145,146]. However, 1-ABT did not alter the ability of clofibrate to decrease serum triglyceride levels in rats, which showed that fatty acid hydroxylation was not critical for the antihyperlipidemic action of this drug [144]. Furthermore, work with primary hepatocyte cultures indicated that induction of peroxisomal β -oxidation and liver fatty acid binding protein is mediated by a 1-ABT-insensitive mechanism subsequent to P450 oxidation of long-chain fatty acids to dicarboxylic acids [147].

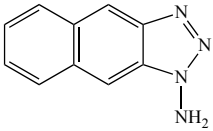
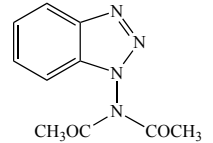
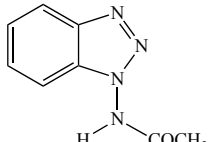
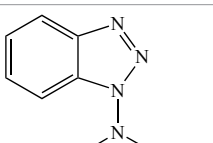
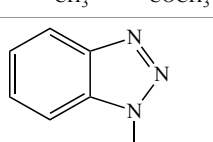
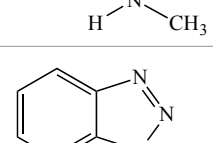
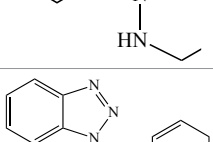
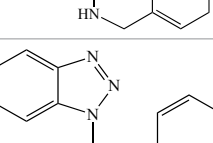
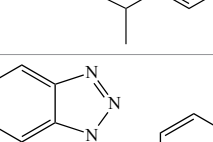
Arachidonic acid oxidation

The oxidation of arachidonic acid to both epoxyeicosatrienoic acids and 16- through 20-hydroxyeicosatetraenoic acids by pulmonary microsomes from β -naphthoflavone-induced guinea pigs was inhibited by more than 95% after preincubation with 1 mM 1-ABT [101]. Formation of the epoxide metabolites was attributed in this study largely to the guinea pig orthologue of CYP2B4. 1-ABT causes dose-dependent loss of renal cytochrome P450 content, arachidonic acid oxidation, and CYP4A protein content when administered intraperitoneally to Sprague-Dawley rats [148]. In the cortex and outer medulla, 1-ABT was a potent inhibitor of the formation of 19-hydroxyeicosatetraenoic acid (19-HETE) and 20-hydroxyeicosatetraenoic acid (20-HETE). The activity losses caused by 1-ABT returned to control levels after 72 hours. Interestingly, renal arachidonic acid epoxygenase activity was not impaired under the same conditions. A single dose of 1-ABT given to 7-week old spontaneously hypertensive rats caused an acute reduction in mean arterial pressure that persisted for at least 12 hours. The role of 20-HETE in this antihypertensive effect is supported by the finding that an analogue of 1-ABT, 1-hydroxybenzimidazole, which does not inhibit 20-HETE formation has no effect on mean arterial pressure [148]. A fluorescent 20-HETE HPLC assay, which distinguishes this metabolite from other HETEs, dihydroxyeicosatrienoic acids, and epoxyeicosatrienoic acids, was used to demonstrate that 1-ABT reduces the excretion of 20-HETE in rats and inhibits its formation by renal microsomes [149]. *In vivo*, a daily 50 mg/kg injection of 1-ABT to rats over five days greatly lowered the urinary excretion of 20-HETE and the ability of renal cortical microsomes to oxidize arachidonic acid to 20-HETE and epoxyeicosatrienoic acids [150-152]. These examples are part of an extensive body of data (Table 4) indicating that 1-ABT is a mechanism-based cytochrome P450 inactivating agent of the enzymes that oxidize arachidonic acid to its diverse metabolites.

Estradiol

As discussed in the section on 1-ABT toxicity, this inhibitor does not directly inhibit cytochrome P450 enzymes of the sterol biosynthetic pathway [51,52], although an *in vivo* study suggested that inhibition may occur via an indirect mechanism [53-56]. The further metabolism of estradiol, however, is mediated by P450 enzymes that are also involved in drug metabolism. This metabolism has a complicated effect on cell proliferation and growth, as shown in the summary of the studies of these effects carried out with the help of 1-ABT as a cytochrome P450 inhibitor (Table 5).

Table 2: Analogues of 1-ABT shown to inactivate cytochrome P450 enzymes.

Compound number	Analog	%P450 loss <i>in vitro</i>	Porphyrin pigment <i>in vivo</i>	Reference
1	1-ABT	86 ^a	yes	[20]
7		65 ^a	yes	[20]
8		32 ^a	yes	[20]
9		20 ^a	yes	[20]
10		10 ^a	yes	[20]
11		43 ^a	yes	[20,22]
12		37 ^b	nd ^d	[22]
13		25 ^c 30 ^b	nd ^d	[22,89]
14		31 ^c 37 ^b	nd ^d	[22,89]
15		2 ^c	nd ^d	[89]

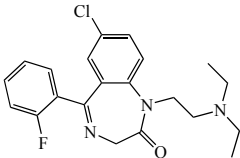
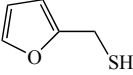
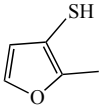
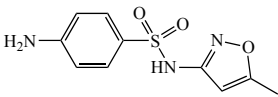
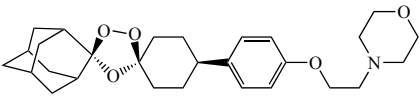
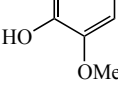
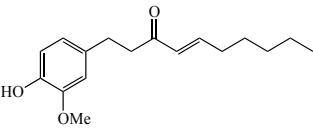
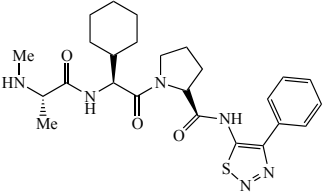
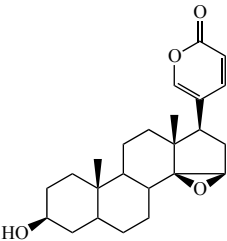
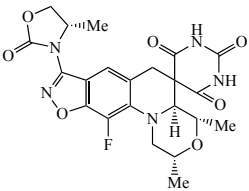
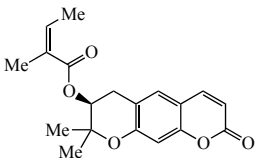
^aLoss of P450 content on incubation of phenobarbital-induced rat liver microsomes with 1 mM inhibitor for 30 min.

^bLoss of P450 content on incubation of β -naphthoflavone induced rabbit lung microsomes with 10 μ M inhibitor.

^cLoss of P450 content on incubation of untreated guinea pig pulmonary microsomes with 10 μ M inhibitor for 45 min.

^dnd=not determined

Table 3: Consequences of the inhibition of 1-ABT-mediated P450 metabolism of a range of compounds.

Compound		Effect of 1-ABT	Reference
Flurazepam		Raised flurazepam brain concentration, confirming the parent drug is the active species	[123]
Furfuryl mercaptan		Inhibited oxidation by rat to thiofuran methyl sulfoxide	[124]
2-Methyl-3-furanthiol		Inhibited oxidation by rat to thiofuran methyl sulfoxide	[124]
Sulfamethoxazole		Partially inhibited oxidation to hydroxylamine	[125]
OZ439		Partially inhibited clearance of drug in rats	[126]
CDRI 99/411		Showed two metabolites are cytochrome P450-dependent	[127]
[6]-Shogaol		Showed P450 involved in formation of desaturated metabolites	[128]
GDC-0152		Inhibited the formation of a novel metabolite	[129]
Rebisofugenin		Implicated CYP3A4 in AB-ring hydroxylation	[130]
ETX0914		Inhibited <i>in vivo</i> formation of several metabolites	[131]
Decursinol angelate		Inhibited metabolism of the drug by human liver microsomes	[132]

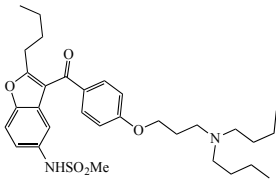
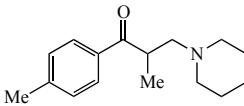
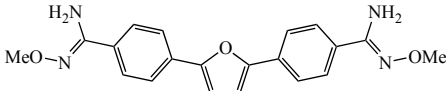
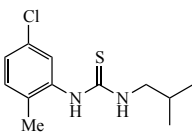
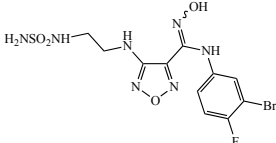
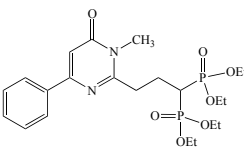
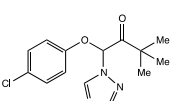
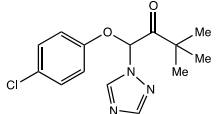
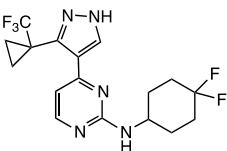
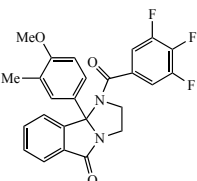
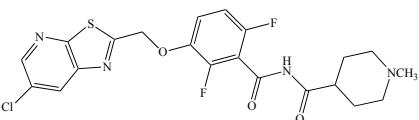
Droneradone		Almost completely inhibited metabolic clearance	[133]
Tolperisone		Inhibited metabolite formation and intrinsic clearance by human liver microsomes	[134]
DB289		Inhibited metabolite formation	[135]
N-(5-chloro-2-methylphenyl)-N'-(2-methylpropyl)-thiourea		<i>In vivo</i> , decreased GSH adduct formation by 95%	[136]
Epacadostat		Showed gut bacteria reduced the =N-OH to =NH, but liver P450s catalyzed N-dealkylation	[137]
U-91502		Showed parent drug is responsible for the cardiopulmonary toxicity	[138]
Dipeptide VLA-4 antagonist		Showed oxidative metabolism not rate limiting in excretion of this drug	[139]
Triadimefon		Established keto reduction to triadimenol is not by a P450 enzyme	[140]
Pan JNK inhibitors		Brain exposure required for therapy achieved when 1-ABT coadministered	[141]
ML375		Increased plasma exposure of the drug when administered with 1-ABT	[142]
TXY541		1-ABT increased $t_{1/2}$ and reduced the dose required for efficacy	[143]

Table 4: Effect of 1-ABT on arachidonic acid pathways.

Effector	System	Finding	Reference
Endothelin-1	Foetal lambs, guinea pigs	1-ABT inhibits contraction of ductus arteriosus by oxygen, implicating a P450-based mechanism	[153]
Acetylcholine	Isolated rat hearts	1-ABT inhibits cardiodepression by acetylcholine, implying a role for P450 and EETs	[154]
Acetylcholine leveromakalim	Rat mesenteric artery	1-ABT, unlike miconazole, did not influence endothelium-dependent hyperpolarization	[155]
Endothelium-derived hyperpolarizing factor (EDHF)	Rat mesenteric artery	As 1-ABT did not counteract the effect, EDHF released by acetylcholine is not a P450-derived arachidonic acid metabolite	[156]
High salt diet, acetylcholine	Rats	1-ABT abolished the blood pressure increase due to intrarenal acetylcholine; implicates 20-HETE but not EETs in the process	[157]
Bradykinin	Isolated rat hearts	1-ABT abolished cardiodepression by bradykinin, implying a role for P450 and 14,15-EET	[158]
Bradykinin	Perfused rat kidney	1-ABT does not inhibit bradykinin increases in perfusion of the medulla mediated by the NO system and K(Ca) channels	[159]
Bradykinin	Isolated rat heart	1-ABT prevented the decrease in left ventricular pressure caused by bradykinin	[160]
Cyclosporin A, NO	Rats	1-ABT inhibited cyclosporin A mediated increases of 20-HETE, systolic blood pressure, and renal damage	[161]
NO	Rats	1-ABT blocked the renal vasodilatory response to NO and reduced its diuretic and natriuretic effects	[162]
ATP, NO	Rats	1-ABT and NO synthase inhibitors blunted ATP mediated increases in renal arterial blood flow	[163]
Endotoxin hypotension, NO	Rats	1-ABT diminished CYP4A activity but did not prevent the NO mediated decrease in mean arterial pressure (MAP)	[164]
NO	Rats, pregnant	1-ABT prevented the increases in systolic blood pressure caused by an NO donor, suggesting a role for 20-HETE	[165]
NO, Na⁺-K⁺-2Cl⁻ cotransport	MMDD1 cells	1-ABT reversed inhibition of Na ⁺ -K ⁺ -2Cl ⁻ cotransport caused by NO, implying a role for a P450-enzyme	[166]
NO interaction	Rats, hypertensive	1-ABT attenuated elevation of blood pressure caused by L-NAME and lowered organ damage	[167]
O₂ sensing, K⁺ channel	Rat carotid body type I cells	1-ABT inhibited hypoxic depression of K ⁺ currents, suggesting a P450 enzyme is involved	[168,169]
Arachidonic acid, K⁺/Ca²⁺ channels	Rat carotid body type I cells	1-ABT did not alter the effects of arachidonic acid on K ⁺ currents, suggesting they are not mediated by a P450 enzyme	[170]
EDHF	Rat aorta smooth muscle cells	1-ABT, unlike miconazole, does not interfere with K ⁺ channel activation, negating a general P450 effect	[171]
K⁺ channels	Rabbit portal vein myocytes	1-ABT, unlike ketoconazole, had no effect on native KDR or RPV Kv1.5 current, implying P450 inhibition not critical for ketoconazole effect	[172]
K⁺ channels	Pulmonary arterial myocytes	1-ABT reversibly inhibited steady-state K ⁺ channel currents and membrane potential, but interpretation unclear	[173]
Angiotensin	Rats	1-ABT inhibited the doubling of mean arterial blood pressure mediated by angiotensin	[174]
Angiotensin	Rats	Chronic blockade of EETs and 20-HETE with 1-ABT attenuated the ANG II-induced rise in MAP by 40%	[175]
Angiotensin	Rats, hypertensive	1-ABT, angiotensin II, and endothelin individually reduced blood pressure to similar levels, and more in combination	[176]
Diabetes	Rats, diabetic	1-ABT attenuated vasoconstrictor responses to norepinephrine, endothelin-1, and angiotensin II	[177]
Postmenopause	Rats, hypertensive	1-ABT decreased blood pressure in postmenopausal, but not young, spontaneously hypertensive rats, implicating a 20-HETE contribution to postmenopausal hypertension	[178]
Pregnancy	Rats, pregnant	1-ABT decreased systolic blood pressure, urinary sodium, urinary 20-HETE, renal 20-HETE	[179]
Pregnancy	Rats, pregnant	1-ABT decreased MAP in rats with reduced uterine perfusion pressure and lowered both 20-HETE formation and CYP4A expression	[180]

Diabetes, endothelin streptozotocin	Rats, diabetic	Blockade of CaMKII, Ras-GTPase or the production of 20-HETE by 1-ABT normalized the altered vascular reactivity to ET-1 and carbachol in the carotid artery of streptozotocin-induced diabetic rats	[181]
Insulin resistance	Rats	In the presence of testosterone, the 1-ABT inhibitable Cyp4A/20-HETE system plays a key role in elevating blood pressure secondary to insulin resistance	[182]
Natriuresis, Pressure response	Rats	1-ABT decreased the pressure-natriuretic response by 50% and renal formation of 20-HETE and ETAs by 90% and 50%, respectively	[183]
Natriuresis, dopamine	Rats	1-ABT reduced the natriuretic response to dopamine by 65% and markedly reduced urine flow and sodium excretion	[184]
Deoxycorticosterone (DOCA)-salt	Rats	1-ABT reduced hypertension in a DOCA-salt model but did not prevent organ hypertrophy and proteinuria	[185]
20-HETE vs EETs	Rats	1-ABT decreased renal artery flow without altering medullary perfusion, whereas medullary NO increased	[186]
Renin Hypertension	Ren-2 transgenic rats	1-ABT in Ren-2 transgenic rats decreased blood pressure and cardiac hypertrophy, but not glomerulosclerosis, implicating P450 metabolites	[187]
Pressure response	Perfused dog renal arcuate arteries	1-ABT partially inhibited the myogenic response to elevated perfusion pressure; suggested a role for 20-HETE in the myogenic response	[188]
Balloon injury	Rat carotid arteries	1-ABT prevents the increase in 20-HETE levels caused by balloon injury and attenuated vascular smooth muscle cell remodeling,	[189]
VEGF	Rat muscle	1-ABT blocked the increase in 20-HETE and angiogenesis caused by electrical stimulation	[190]
Type II Phospholipase A2 (sPLA2-II)	Guinea pig alveolar macrophages	1-ABT prevented the inhibition of sPLA2-II expression caused by low (5 μ M) but not high (30 μ M) concentrations of arachidonic acid	[191]
GABA receptor	HEK 293 cells	1-ABT did not block the inhibitory effect of arachidonic acid on currents due to the $\alpha 1\beta 2\gamma 2$ GABA(A) receptor	[192]
Fetal bovine serum (FBS)	Murine 3T6 fibroblasts	1-ABT decreased 12(S)-HETE levels, 3T6 fibroblast growth, and DNA synthesis induced by FBS	[193]
Cholecystokinin octapeptide (CCK)	Rat pancreatic acinar cells	1-ABT, unlike ketoconazole, did not alter baseline $[Ca^{2+}]$ or CCK-evoked oscillations	[9]
Pulmonary vasodilation	Isolated perfused rat lung	1-ABT partially inhibited immediate arachidonic acid-induced pulmonary vasodilation, suggesting a role for P450 enzymes	[194]
Pulmonary vasodilation	Rat lung	1-ABT inhibited vasoconstriction caused by hypoxia and angiotensin II, but not when 1-ABT was washed out first-conclude 1-ABT does not act through a P450 mechanism	[195]
Pulmonary vasoconstriction	Isolated perfused rabbit lung	Hypoxia-induced vasoconstriction was inhibited by 1-ABT, but similar inhibition was observed under normoxia with a thromboxane analogue.	[196]
Ozone hyper-responsiveness	Mouse lung	1-ABT greatly attenuated airway sensitivity and the increases in 20-HETE caused by ozone	[197]

Table 5: Effect of 1-ABT on estradiol-dependent pathways.

Growth inhibition	Cardiac fibroblasts	Inhibition by 1-ABT suggests estradiol inhibits cardiac fibroblast growth via an estrogen receptor-independent pathway involving metabolism of estradiol to methoxyestradiols	[198]
Growth inhibition	Human glomerular mesangial cells	Estradiol inhibited, serum-induced, proliferation of glomerular mesangial cells was enhanced by P450 inducers and diminished by 1-ABT	[199]
Growth inhibition	Aortic vascular smooth muscle cells	Estradiol inhibited, serum-induced, proliferation of vascular smooth muscle cells was enhanced by P450 inducers and diminished by 1-ABT	[200]
Growth inhibition	Human coronary artery smooth muscle cells	Estradiol inhibited, serum-induced, proliferation of smooth muscle cells was enhanced by P450 inducers and diminished by 1-ABT	[201]
Growth inhibition	Human cardiac fibroblasts	Estradiol inhibited, serum-induced, proliferation of human cardiac fibroblasts was enhanced by P450 inducers and diminished by 1-ABT	[202]
Cell proliferation	Cultured rat vascular smooth muscle cells	1-ABT increased the growth promoting effect of 17 β -estradiol by inhibiting formation of 2- and 4-hydroxyestradiol and reactive oxygen species	[203]
Smooth muscle contraction	Rat aortic smooth muscle	17 β -estradiol inhibition of phenylephrine-induced contraction was not prevented by 1-ABT, suggesting a P450-independent mechanism	[204]

Table 6: Some toxicological processes in which cytochrome P450 enzymes were implicated though inhibition by 1-ABT.

Xenobiotic	End point inhibited	Reference
Acebutolol	Antinuclear antibody production	[266]
Amodiaquine	Toxicity of amodiaquine	[267]
Carbamazepine	Protein-reactive metabolite formation	[268,269]
Clopidogrel	Hepatic injury	[270]
Efavirenz	Inhibition of 8-hydroxy-efavirenz formation and hepatic cell death	[271]
Leflunomide	Cytotoxicity	[272]
Leflunomide	Hepatotoxicity	[273]
Methapyrilene	Formation of reactive metabolite and genotoxic potential	[274,275]
Ticlopidine	Protein covalent binding and hepatotoxicity	[276]
Aflatoxin B1	Cytotoxicity to 3T3 cells	[277]
	Toxicification due to CYP2C19	[278]
Capsaicin	Formation of toxic metabolite	[279]
β -Carotene / β -apo-8'-carotenal	Formation of DNA strand breaks	[280]
White snake root constituents	Cytotoxicity	[281]
Aromatic and heterocyclic amines	Genotoxicity	[282]
Benzene	Induction of micronuclei	[283]
Butylated hydroxytoluene	Quinone methide formation	[284]
Tetrahydrofuran	Hepatocellular proliferation	[285]
Dimethylbenz[a]anthracene	Pre-B lymphocyte apoptosis	[286]
Mono- and di-chlorobiphenyls	Mutagenicity assays in Chinese hamster V79 cells expressing CYP2E1 and SULT 1A1	[287]
N-(3,5-Dichlorophenyl)-succinimide	Nephrotoxic metabolite formation and renal damage	[288]
N-(3,5-Dichlorophenyl)-succinimide	Protein covalent binding and nephrotoxicity	[289]
3-(3,5-Dichlorophenyl)-2,4-thiazolidinedione	Hepatotoxicity	[290]
2-Nitropropane	Micronucleus and multinuclei formation	[291]
N-Nitrosodimethylamine	Micronucleus and multinuclei formation	[291]
NNK (4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone)	DNA strand breaks	[292]
5-Nitrobenzo[b]naphtho[2,1-d]thiophenes.	DNA adduct formation	[293]
(N-(4-((1 <i>H</i> -pyrrolo[2,3- <i>b</i>]pyridin-4-yl)oxy)-3-fluorophenyl)-1-(4-fluorophenyl) 2-oxo-1,2-dihydropyridine- 3-carboxamide)	Vacuolar degeneration and necrosis of adrenal cortex	[294]
2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin	Decreased biliary excretion of the compound in rats	[295]

Hypothermia

Studies of the effects of hypothermia on the *in vivo* pharmacokinetics of midazolam in rats showed that the K_m value for CYP3A2 remained unchanged at 37, 32, and 28°C, but the V_{max} decreased at the lower temperature [205]. 1-ABT was used in these studies to determine the contribution of this enzyme to the observed changes in pharmacokinetics.

Other endogenous pathways

SKF-93479, a histamine H2 antagonist, caused thyroid lesions in rats linked to increased plasma clearance of thyroxine and elevated TSH concentrations. Inactivation of cytochrome P450 in hepatocytes by 1-ABT did not alter the effect of SKF-93479 on the accumulation of thyroxine, indicating that the enhancement was due to the parent drug and not a metabolite [206]. Temelastine (SK&F 93944), a histamine H1 antagonist, caused thyroid lesions associated with hypertrophy and colloid depletion in rats [207]. Again, 1-ABT was used to establish that the effect of the drug was due to the parent and not an oxidatively generated metabolite. In another area, ω -hydroxyceramides are the major lipids on the corneocyte envelop in the epidermis. 1-ABT was shown to inhibit incorporation of radiolabeled acetate into this lipid in cultured human keratinocytes [208]. 1-ABT also inhibited recovery of the skin when damaged in hairless mice. These results confirmed the importance of ω -hydroxyceramides in determining the epidermal permeability of skin.

A V79-derived cell line overexpressing CYP2E1 and SULT1A1 was found to undergo much higher rates of spontaneous gene mutations and sister chromatid exchanges than control V79 cells [209]. These elevated rates were completely prevented by 1-ABT or pentachlorophenol, a SULT1A1 inhibitor, suggesting that CYP1A1 in combination with SULT1A1 activates an endogenous molecule to a mutagenic product.

Toxicity Studies

Sepsis

Sepsis is a common, sometimes fatal condition during which cytochrome P450 enzymes are significantly downregulated due to a decrease in the levels of the AhR and Arnt receptors [210-212]. 1-ABT has provided evidence that cytochrome P450 enzymes are important for the prevention of hepatic dysfunction during sepsis [212-214]. Thus, treatment with 1-ABT enhanced the levels of proinflammatory cytokines in adult male Sprague-Dawley rats after induction of sepsis [213,214]. In sepsis induced by administration of low doses of zymosan, 58% mortality was observed when 1-ABT was co-administered, in contrast to no mortality in the absence of 1-ABT [215].

Acetaminophen/Phenacetin

Intravenously administered phenacetin is oxidized in rats to acetaminophen, but this transformation is inhibited by pretreatment with 1-ABT [216], which changes the half-life of phenacetin from 34 to 230 min. In contrast, in this same system 1-ABT has little effect on the clearance, half-life, or distribution of intravenously administered acetaminophen. Both intravenous and intraperitoneal pretreatment of rat with 1-ABT greatly inhibited the systemic clearance of phenacetin, with an intraperitoneal dose of 50 mg/kg of 1-ABT resulting in 79% inhibition [85]. To determine if metabolism differences contributed to the finding that female Sprague-Dawley rats are more susceptible than male rats to acetaminophen-induced nephrotoxicity, incubations of hepatic and renal S9 fractions with radiolabeled acetaminophen in the presence of either 1-ABT or the reversible carboxyesterase

inhibitor *bis*-(*p*-nitrophenyl)phosphate were carried out [217]. No sex-dependent differences in covalent binding to protein of acetaminophen were observed and 1-ABT was found to inhibit covalent binding equally in both, whereas the carboxyesterase inhibitor had no effect on covalent binding. In a follow-up experiment, blood urea nitrogen was measured as an index of nephrotoxicity in rats after administration of radiolabeled acetaminophen. In this instance, pretreatment with either 1-ABT or tri-*o*-tolylphosphate, an irreversible carboxyesterase inhibitor, prevented elevation of blood urea nitrogen and decreased covalent binding [218]. Thus, both P450-catalyzed oxidation and deacetylation appear to contribute to nephrotoxicity. An analysis of the toxicity of acetaminophen to sinusoidal endothelial cells versus hepatocytes in C3H-HEN and Swiss Webster mice showed that it was more toxic to the sinusoidal cells, but the toxicity was suppressed by 1-ABT [219]. Studies with primary mouse hepatic parenchymal cells revealed that 1-ABT prevented covalent binding and toxicity when acetaminophen was present at concentrations lower than 5 mM, but at higher concentrations a P450-independent toxicity mechanism also intervened that appeared to be related to the presence of *p*-aminophenol [220]. This second toxicity mechanism was alleviated by a carboxyesterase inhibitor.

Cyclophosphamide

Classical P450 inhibitors such as SKF-525A did not attenuate pulmonary thymidine incorporation, a marker of tissue injury, associated with administration of cyclophosphamide, whereas pretreatment with 1-ABT lowered thymidine incorporation into lung DNA on days 3 and 10, but not on day 7. Agents, such as indomethacin and aspirin, which inhibit arachidonic acid pathways independent of cytochrome P450, similarly reduced levels of thymidine incorporation in the lung. Although differences were found in the protective effects of these agents in liver and lung, it appears that oxidation of cyclophosphamide by the cytochrome P450 system is not essential for the development of pulmonary toxicity associated with cyclophosphamide [221]. Gene therapy to increase expression of CYP2B in tumors provides a strategy for increasing tumor rather than systemic toxicity of cyclophosphamide [222]. 1-ABT, which is more inhibitory to CYP2C than CYP2B cyclophosphamide activation, increases the C_{max} and half-life of the activated anti-tumor metabolite of cyclophosphamide in rats [222].

Nevirapine

Nevirapine, a reverse transcriptase inhibitor, causes skin rash and hepatotoxicity. The 12-hydroxy metabolite causes the skin rash and deuteration of the 12-methyl in the parent drug, which inhibits 12-hydroxylation, suppresses rash formation [223]. However, blood levels of the deuterated compound were unexpectedly low due to formation of a quinone methide, which inactivates cytochrome P450. Comparable levels of nevirapine and the deuterated analogue were observed when the rats were pretreated with 1-ABT. The results led to the proposal that the quinone methide formed in the liver is responsible for the hepatotoxicity of nevirapine, whereas the skin rash is due to formation of the 12-hydroxy metabolite in the skin, probably followed by sulfation to make it a more reactive species. The role of the quinone methide in liver toxicity was substantiated by a subsequent study, again using 1-ABT and the deuterated nevirapine analog, which showed that *in vivo* the quinone methide bound covalently to liver proteins, and *in vitro* specifically to rat CYP3A1 and CYP2C11, and human CYP3A4 (Figure 10) [224].

Tienilic acid

Tienilic acid is a hepatotoxic agent that forms a glutathione adduct (Figure 11), decreases glutathione levels, and upregulates toxicity marker genes [225]. All of these effects are blocked by pretreatment with an intraperitoneal dose of 66 mg/kg of 1-ABT. The metabolism of tienilic acid, as well as several other drugs, by human hepatocytes was shown to be inhibited by 1-ABT [226]. As tienilic acid itself inactivates CYP2C9, and other studies indicate that 1-ABT is a good inactivator of most drug metabolizing forms of P450, but less so of CYP2C9 [41], a cocktail of tienilic acid and 1-ABT has been employed to approach pan-P450 inactivation [227]. A study of the cytotoxicity of bosentan in sandwich-cultured human hepatocytes showed that tienilic acid, as a CYP2C9-specific inhibitor, plus 1-ABT helped to preserve cell viability [228].

Valproic acid

To explore the mechanism by which valproic acid causes liver damage its effects on levels of 15-F_{2t}-isoprostane, a measure of oxidative stress, and the possible role of cytochrome P450 in the process, have been investigated [229]. Phenobarbital pretreatment was found to increase plasma and liver concentrations of 15-F_{2t}-isoprostane in valproic acid-treated rats by several fold. Cytochrome P450 metabolites of valproic acid were also increased. However, 1-ABT attenuated the formation of valproic acid metabolites, but did not lower the levels of 15-F_{2t}-isoprostane, suggesting that phenobarbital exacerbated liver damage by a mechanism independent of the P450 oxidation of valproic acid. An extension of this study confirmed that the (*E*)-2,4-diene metabolite of valproic acid did not contribute to the toxicity of the parent compound in hepatocytes, as shown by the failure of 1-ABT to diminish the toxicity despite its inhibitory effect on formation of the metabolite [230].

Loratadine

Desloratadine, a nonsedating antihistamine, is converted *in vivo* to 3-hydroxydesloratadine, but efforts to reproduce this transformation *in vitro* were unsuccessful. More recent work showed that cryopreserved human hepatocytes yield both 3-hydroxydesloratadine and its *O*-glucuronide [231]. 1-ABT and gemfibrozil glucuronide, a CYP2C8 inhibitor, prevented 3-hydroxydesloratadine formation. Furthermore, the formation of this metabolite required both NADPH and UDP-glucuronic acid, leading to the conclusion that 3-hydroxydesloratadine was generated by a sequence involving initial N-glucuronidation, CYP2C8 hydroxylation of the glucuronide, and glucuronidase release of the 3-desloratadine metabolite (Figure 12). The glucuronide, indicated by Glu in the figure, could conceivably be located on the other nitrogen of the molecule.

In a different vein, 1-ABT was used to demonstrate that the drug-induced phospholipidosis caused by loratadine in three-dimensional cultures of hepatocytes was due to the cytochrome P450-catalyzed formation of desloratadine [232].

Precocene

Precocene, a naturally occurring regulator of insect development, causes P450-dependent hepatic necrosis. Comparison of 24-hour cultures of rat hepatocytes with and without added calf serum showed that addition of serum decreased the toxicity of precocene and prevented glutathione loss [233]. However, although precocene was also toxic to 72-hour cell cultures, the effect of serum was attenuated and the toxicity was relatively insensitive to inhibition of P450 enzymes by 1-ABT, suggesting that a different mechanism of toxicity

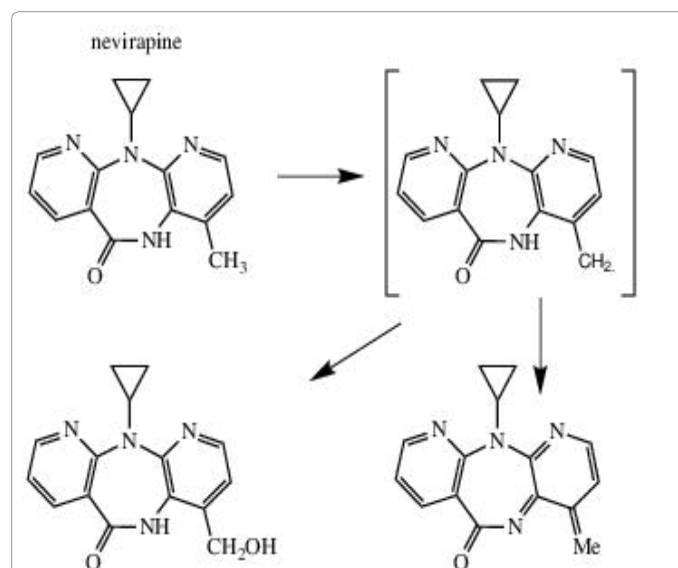


Figure 10: Metabolites of nevirapine formed by cytochrome P450 enzymes.

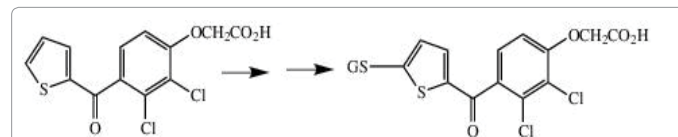


Figure 11: The cytochrome P450-catalyzed transformation of tienilic acid to a glutathione conjugate is inhibited by 1-ABT.

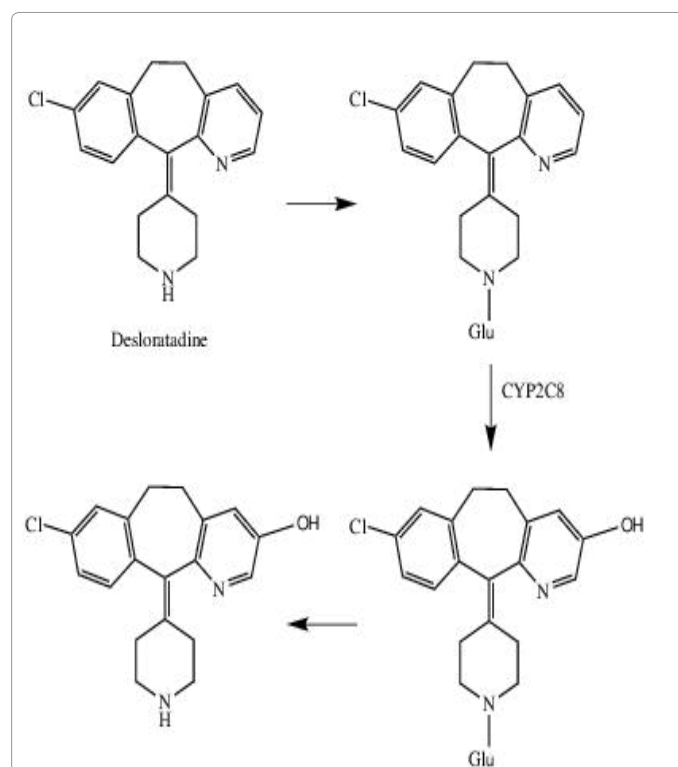


Figure 12: The hepatic metabolism of desloratadine elucidated with the help of 1-ABT.

might be operative in the older cultures [233,234]. A later study of the metabolism of precocene by rat liver slices showed that differences exist in the metabolite profiles obtained with the slices versus *in vivo* in rats, particularly in the amount of dihydrodiol products generated from the epoxide metabolite (Figure 13) by epoxide hydrolases [235]. 1-ABT decreased the extent of metabolite formation and decreased the observed toxicity of precocene, and was more effective in doing so than ketoconazole and other isoform selective P450 inhibitors.

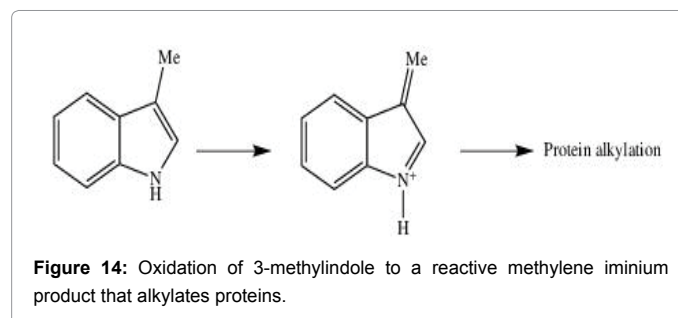
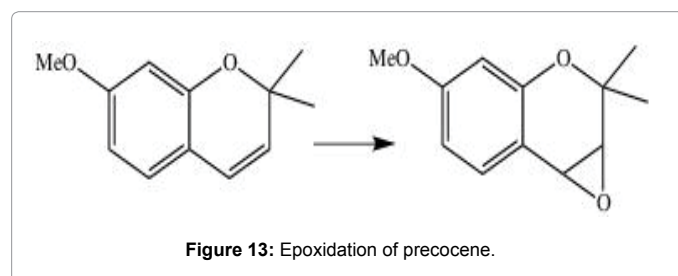
3-Methylindole

The pneumotoxicity of 3-methylindole in cattle, goats, sheep, horses, and other species appears to be mediated by a P450-generated electrophilic metabolite [236]. Thus, 1-ABT decreased 3-methylindole metabolism and its covalent binding to proteins in goat lung by 50 and 100% when present at a concentration of 10 and 100 μ M, respectively. 1-ABT also inhibited the toxicity of 3-methylindole in isolated rabbit lung cells [237,238], and abrogated the toxicity of 3-methylindole in human bronchial epithelial cells lines [239,240]. As 3-methylindole with a trideuterated methyl group is much less cytotoxic, it appears that oxidation of the methyl group produced the electrophilic metabolite involved in covalent binding and toxicity (Figure 14) [237].

10 Acrylamide/acrylonitrile

The oxidation by mice of acrylonitrile ($\text{CH}_2=\text{CHCN}$) and acrylamide ($\text{CH}_2=\text{CHCONH}_2$) to the epoxides glycidamide and cyano ethylene oxide, respectively, was suppressed by pretreatment with 1-ABT or when CYP2E1-null mice were employed. The data suggested that in mice CYP2E1 is the principal cytochrome P450 enzyme responsible for formation of these metabolites [44]. Other work showed that P450 enzymes other than CYP2E1 contribute to the oxidative metabolism of acrylonitrile and methacrylonitrile, as 1-ABT lowered the formation of cyanide derived from these compounds even in CYP2E1-null mice [241]. 1-ABT and CYP2E1-null mice were also used to show that CYP2E1 plays a major, but not exclusive, role in the oxidative metabolism of methacrylonitrile [45]. Acrylamide is a mutagen with clastogenic effects in mice. Pretreatment with 1-ABT prior to acrylamide administration greatly reduced this mutagenesis, providing strong support for the conclusion that the epoxide is the mutagenic metabolite [242]. However, 1-ABT was not found to decrease the morphological transformation of Syrian hamster embryo cells caused by acrylamide, a process that was inhibited by N-acetylcysteine. This led the authors to postulate that acrylamide itself, not its oxidative metabolite, was responsible for cell transformation [243]. In another study, the incidence of adrenal, testicular, and thyroid neoplasia in rats upon chronic exposure to acrylamide was investigated [244]. Acrylonitrile was found to increase DNA synthesis in these tissues, but not in the liver or adrenal cortex, which are not target tissues for acrylamide carcinogenesis. Pretreatment with 1-ABT reduced DNA synthesis caused by acrylamide in the adrenal medulla, but not in the testicular mesothelium or thyroid. Acrylonitrile increases oxidative stress in rat brain and cultured rat glial cells. A study of the possible causes for this effect showed that acrylonitrile decreased the activity of catalase, and increased that of xanthine oxidase, but 1-ABT-pretreatment counteracted these effects [245], suggesting that these protein alterations contribute to the increase in oxidative stress caused by acrylonitrile. Related studies in which 1-ABT was shown to decrease the extent of oxidative damage caused by acrylonitrile in rat brain concurred with the view that its P450-catalyzed oxidation was at the root of the oxidative damage [246].

Cis-2-pentenitrile triggers permanent behavioral defects in



rodents. These behavioral changes were prevented by pretreatment with 1-ABT, implicating oxidative metabolism of this substrate in its deleterious effects [247]. 1-ABT also decreased the mortality and vestibular toxicity of allylnitrile [248].

11 Halocarbons

A comparison of the toxicity of chloroform in male B6C3F₁, Sv/129 wild-type mice, and CYP2E1-null mice showed that the pathological changes caused by chloroform were only observed in wild-type mice. Furthermore, pretreatment of wild-type mice with 1-ABT completely protected against the cytotoxic effects of chloroform [249], leading to the conclusion that CYP2E1 was largely responsible for bioactivation of chloroform to a cytotoxic species. Dimethyl sulfoxide (DMSO) is known to confer some protection against chloroform-induced liver injury. To determine if this protection was due to inhibition of cytochrome P450 enzymes or some other phenomenon, the protective activities of DMSO and 1-ABT were evaluated in male Sprague-Dawley rats [250]. 1-ABT decreased covalent binding of ¹⁴C-chloroform to liver proteins by 35% and reduced the amount of radiolabel in blood 10 hours after radiolabeled chloroform administration by 50%, whereas DMSO had no effect on these parameters even though it also protected the liver. Thus, DMSO confers protection by mechanisms other than inhibition of the bioactivation of chloroform.

The metabolism of inhaled 1-bromopropane by male F344 rats and B6C3F₁ mice resulted in exhaled radiolabeled CO₂ and several glutathione-derived conjugates in the urine: *N*-acetyl-S-propylcysteine, *N*-acetyl-3-(propylsulfinyl)alanine, *N*-acetyl-S-(2-hydroxypropyl)-cysteine, 1-bromo-2-hydroxypropane-O-glucuronide, *N*-acetyl-S-(2-oxopropyl)cysteine, and *N*-acetyl-3-[(2-oxopropyl)sulfinyl]alanine (Figure 15) [251]. In rats pretreated with 1-ABT, oxidative metabolism was decreased and the single urinary metabolite was that formed by glutathione displacement of bromide from the parent compound. 1-Bromopropane also has neurotoxic effects that are difficult to study *in vivo* due to the hepatotoxicity of the compound. Administration of 1-ABT (50 mg/kg) to rats for three days inhibited 92-96% of the hepatic microsomal CYP2E1 activity, but only 62-64% of the same activity in brain microsomes. Under these conditions, it was possible to administer higher doses of 1-bromopropane and to examine their

effect on the brain [252].

1,1-Dichloro-2,2-bis(4'-chlorophenyl)ethane (DDD), 1,2-dibromoethane, and trichloroethylene are toxic to rabbit lung Clara cells, alveolar type II cells, and alveolar macrophages [253]. 1-ABT diminished the cytotoxicity of DDD and 1,2-dibromoethane to Clara cells, indicating that cytotoxic metabolite formation was mediated, at least in part, by cytochrome P450 enzymes.

The oxidative metabolism of trichloroethylene, a carcinogen present in the environment, was suggested by *in vitro* studies to be mediated by CYP2E1. To better assess the role of this enzyme, the metabolism of trichloroethylene in wild-type and CYP2E1-null mice was investigated [254]. The excretion of trichloroethylene as urinary metabolites and CO₂ was much lower, and excretion of the parent compound higher, in the CYP2E1-null than wild-type mice. Pretreatment of both types of mice with 1-ABT inhibited the metabolism of trichloroethylene. These results indicated that CYP2E1 is important in trichloroethylene metabolism, but other P450 enzymes also make a contribution.

12 Oxidative stress

Pretreatment with 1-ABT attenuated the DNA fragmentation and cell death caused by 3-amino-1,2,4-triazole plus mercaptosuccinic acid in primary cultures of rat hepatocytes. This led to the conclusion that endogenous oxidative stress was directly related to the activity of cytochrome P450 enzymes [255]. The role of cytochrome P450, particularly CYP2E1, in the cellular injury caused by ethanol has been examined in rats, Cyp2e1-knockout mice, and p47(phox)-null mice lacking nicotinamide adenine dinucleotide phosphate oxidase [256]. The extent of oxidative DNA adducts, mutagenic apurinic/apyrimidinic sites, and expression of DNA repair genes was measured. Ethanol caused an increase in these parameters in the wild-type rat and mice, and the p47(phox)-null mice, but not in the Cyp2e1-null mice. The increase in DNA repair genes was completely abolished by pretreatment with 1-ABT, implicating CYP2E1 and possibly other cytochrome P450 enzymes in ethanol-induced liver injury. The story is not simple, however, as an earlier study by the same group indicated that 1-ABT did not prevent oxidative stress-associated liver injury in rats and mice caused by alcohol, as judged by examination of liver pathology [86]. Interestingly, vitamin E and 1-ABT offered protection against oxidative stress, hepatic apoptosis, and necroinflammation associated with non-alcoholic steatohepatitis [257].

Schisandrin B (Figure 16) from the fruit of *Schisandra chinensis*, a traditional Chinese medicine, protects against CCl₄-induced hepatotoxicity and myocardial ischemia-reperfusion injury in rodents by enhancing mitochondrial glutathione status and inducing expression of the heat shock proteins Hsp25 and Hsp70 [258,259]. The finding that 1-ABT pretreatment significantly delays the *in vivo* time-course of the Schisandrin B-induced glutathione and Hsp25/70 responses in Balb/c mice suggests that cytochrome P450 enzymes facilitate the generation of reactive oxygen species in this system [259].

To determine whether cytochrome P450 enzymes contribute *in vivo* to oxidative stress through the production of reactive oxygen species, various cytochrome P450 inducers (β -naphthoflavone, phenobarbital, Aroclor 1254, isoniazid, pregnenolone 16 α -carbonitrile, and clofibrate) were examined in male Sprague-Dawley rats with and without pretreatment of the rats with 1-ABT [260]. Aroclor 1254 and phenobarbital, but not the other agents, enhanced the formation of malondialdehyde and H₂O₂ formation as well as NADPH oxidation both *in vitro* and *in vivo*. These two agents also increased the formation of F₂-isoprostanes, which are markers of oxidative stress, and these

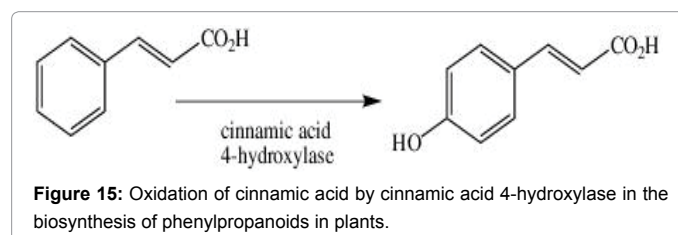


Figure 15: Oxidation of cinnamic acid by cinnamic acid 4-hydroxylase in the biosynthesis of phenylpropanoids in plants.

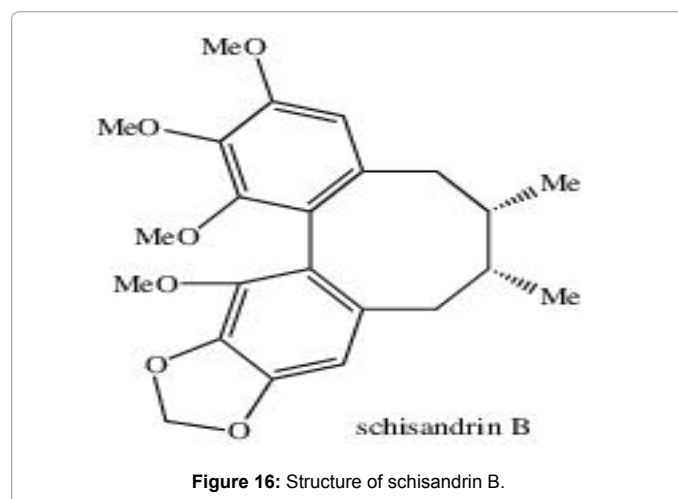


Figure 16: Structure of schisandrin B.

changes were attenuated by pretreatment with 1-ABT.

Methyl 3-(4-nitrophenyl)propionate induces tumor cell apoptosis [261]. 1-ABT inhibited the formation of reactive oxygen species and cell apoptosis, implying a role for cytochrome P450 enzymes in the process.

13 Free radical formation

1-ABT has been used to rule out a role for cytochrome P450 in the formation of certain carbon radicals. Thus, neither 1-ABT nor CO attenuated the amplitude of the ESR spectrum of the phenyl radical produced in incubations of rat hepatic microsomes or cecal microflora with 1,3-diphenyl-1-triazene [262]. Phenyl radical formation, however, was decreased by antibodies to NADPH cytochrome P450 reductase, pointing to a reductive role of this protein in radical formation. 1-ABT and CO also did not attenuate the formation of phenyl radicals from diazoaminobenzene in incubations with liver microsomes. Furthermore, the radicals were formed on incubation of diazoaminobenzene with NADPH cytochrome P450 reductase, confirming a role for this enzyme rather than cytochrome P450 in the process [263]. In contrast, 1-ABT did inhibit the formation of the CO₂⁻ radical in the metabolism of sodium formate. However, allopurinol, an inhibitor of xanthine oxidase, also inhibited this reaction, which suggested that H₂O₂ was involved in the process [264]. This inference is supported by the finding that 3-aminotriazole, a catalase inhibitor, significantly increased the level of spin trapped CO₂⁻ radical. As noted earlier, exposure for 24-, 28-, or 60-hours to greater than 95% oxygen after administration of 1-ABT to rats resulted in the death of the 1-ABT-treated animals between 48 and 60 hr, whereas no control rats exposed to similar hyperoxic conditions died at up to 60 hours of exposure [88]. 1-ABT thus markedly potentiates hyperoxic lung and brain injury. In accord with this, the inhibition by 1-ABT of oxygen radical production by CYP2E1 in the brain of mice, assayed as the delay in the onset of convulsions in a hyperbaric chamber, was not effective

and had undesirable side-effects [265].

14 Other uses of 1-ABT in analysis of xenobiotic toxicology

Many studies have been carried out that employ 1-ABT as a tool to elucidate the roles of cytochrome P450 enzymes in xenobiotic and drug toxicological processes. A summary of these is presented in Table 6. On the other hand, some toxicological processes have not been found to involve cytochrome P450, as 1-ABT did not inhibit them, and these results are summarized in Table 7.

Non-Mammalian P450 Enzymes

Plant P450 enzymes

Endogenous substrates: Cinnamic acid 4-hydroxylase is a cytochrome P450 enzyme involved in phenylalanine metabolism and the biosynthesis of phenylpropanoids in plants (Figure 15). 1-ABT efficiently inactivated this enzyme in *Helianthus tuberosus* (Jerusalem artichoke) [58], but much more slowly inactivated the enzyme responsible for the hydroxylation of lauric acid. 1-ABT also exhibited a low activity for inactivation of the lauric acid ω -hydroxylase of *Vicia sativa* L cv. *Septimane*, in contrast to the inactivation caused by 11-dodecynoic acid [301]. Microsomes from marine microalgae have P450 enzymes that oxidize cinnamic acid, fatty acids, 3-chlorobiphenyl, and isoproturon, and 1-ABT was specifically shown to abolish the oxidation of 3-chlorobiphenyl in intact cells [302].

The flavonoid 3',5'-hydroxylase of petunia hybrid flowers was inhibited by both 1-ABT and the growth regulator tetcyclasis [303]. In a more detailed study in *Solanum lycopersicum* (tomato), 1-ABT was shown to strongly inhibit the formation of flavonoids such as caffeic acid, kaempferol, and quercetin [304]. This is consistent with inhibition of cinnamic acid 4-hydroxylase. Furthermore, 1-ABT caused the accumulation of 9,12,13-trihydroxy-10(E)octadecanoic acid (Figure 17), probably by blocking further oxidative metabolism of this intermediate, whereas tetcyclasis completely suppressed its formation. These results provided clear evidence for differential roles of P450 enzymes in tomato plants.

The oxidation of tabersonine to hörhammericine in *Catharanthus roseus* hairy root cultures (Figure 18) was inhibited by 1-ABT, although it did not appear to inhibit the formation of lochnericine [305]. In contrast, clotrimazole inhibited the formation of lochnericine, indicating that different P450 enzymes catalyze these two transformations. Both agents inhibited the growth of the root cultures. Inhibition of these reactions is of potential importance in biotechnological channeling of tabersonine into the production of vindoline and the anticancer agents derived from it.

Xenobiotic metabolism: 1-ABT strongly, but differentially, inhibited the O-dealkylation of 7-ethoxycoumarin and 7-ethoxyresorufin by microsomes from manganese-induced Jerusalem artichoke, implying the action of two different P450 enzymes [306].

1-ABT has found wide use in studies of the metabolism of herbicides by plants and the role of this process in herbicide resistance. Thus, 1-ABT inhibits the metabolism of chlortoluron and isoproturon and synergizes the activities of these agents in wheat [84,307]. It also inhibits the metabolism of diclofop in wheat, but less effectively than tetcyclasis [308]. Work with suspension cultures of soybean (*Glycine max* (L.) Merr.) and rice (*Oryza sativa* L.) demonstrated that 1-ABT inhibited the metabolism of the herbicide bentazon in rice, although the data suggested that it could also reduce the uptake of bentazon by the cells [309]. The oxidation of bentazon (Figure 19) was also inhibited by 100 μ M 1-ABT in maize cell suspension cultures, although less effectively than inhibition by the alternative P450 inhibitors tetcyclasis, phenylhydrazine, and piperonyl butoxide [310]. 1-ABT also synergized the activity of the herbicides clodinafop, haloxyfop, and fenoxaprop by inhibiting their P450 metabolism in blackgrass (*Aloecurus myosuroides* Huds.) [311]. Analysis of the metabolism of cinnamic acid, lauric acid, metolalchlor, bentazon, and diazinon by microsomes from grain sorghum (*Sorghum bicolor* (L.) Moench) demonstrated differential inhibition of the metabolism of these agents by tetcyclasis, piperonyl butoxide, 1-ABT, SKF-525A, and tridiphan [312]. Resistance to the herbicidal activity of metamitron (Figure 19) has been observed in the weed *Chenopodium album* (common lambsquarters). A study of the resistant and sensitive strains of this weed taken from sugar beet fields demonstrated that the resistance was due to enhanced metabolism of metamitron in the resistant strains [313]. 1-ABT inhibited this metabolism, even though piperonyl butoxide did not. 1-ABT was used to establish that the tolerance of *Agrostis stolonifera* (creeping bentgrass) to the herbicide topramezone is due to rapid metabolism of the herbicide [314].

1-ABT was used to establish that 4-monochlorobiphenyl, an environmental pollutant, is metabolized in poplar trees by cytochrome P450 enzymes [315].

Fish P450 enzymes

The inhibition of lauric acid ω -1 hydroxylation, 7,12-dimethylbenz[a]anthracene hydroxylation, and progesterone 6 β -hydroxylation in trout was examined using several inhibitory agents [316]. These activities were used as functional markers for the trout enzymes CYP2K1, CYP1A1, and CYP3A27, respectively. 1-ABT only slightly inhibited dimethylbenz[a]anthracene hydroxylation, but potentially inhibited testosterone 6 β -hydroxylation.

Diazinon is an organophosphate pesticide that is converted to a metabolite in which the sulfur attached to the phosphorus is replaced by an oxygen atom. This metabolite is a more potent inhibitor of acetylcholinesterase than diazinon itself. In early life stages of medaka (*Oryzias latipes*), 1-ABT decreased acetylcholinesterase inhibition, presumably by inhibiting the oxidation of diazinon to the more toxic metabolite [317].

The absence of inhibition by 1-ABT provided evidence that sulfoxidation of the thiourea herbicide eptam by liver microsomes

Table 7: Some toxicological processes not inhibited by 1-ABT and therefore unlikely to involve cytochrome P450 enzymes as active participants.

Xenobiotic	End point not inhibited	Reference
Dapsone	Formation of protein adducts in normal human epidermal keratinocytes	[296]
MRL-A	Formation of protein adduct	[297]
Nimesulide	Toxicity to human and rat primary hepatocytes	[298]
p-Aminophenol	p-Aminophenol mediated changes in renal proximal tubules	[299]
Carbaryl, Quinalphos, Benomyl Carbendazim	Cytotoxicity to Fa32 and human Hep G2 cells	[300]

from striped bass was mediated by a flavoprotein monooxygenase rather than by a cytochrome P450 enzyme [318].

Insect P450 enzymes

1-ABT, which caused a loss of spectroscopically measurable cytochrome P450 and the accumulation of an N-N-bridged porphyrin in housefly microsomes [83], potentiated the activity of a carbamate insecticide in live houseflies. Based on evidence that methanol oxidation in *Drosophila melanogaster* is partially mediated by cytochrome P450 enzymes, the effect of 1-ABT and other enzyme inhibitors on methanol toxicity has been evaluated [319].

Fungal and microbial P450 enzymes

Endogenous substrates: Inhibition of the growth of *Fusarium* on cereals and their production of mycotoxins is desirable, as it results in loss of crop yield and poses a health threat to livestock and humans. 1-ABT was shown to inhibit production of the toxin 3-acetyldeoxynivalenol and the growth of the mycelia of *Fusarium graminearum* 4528 [320].

The antimicrobial sesquiterpenes enokipodins A, B, C, and D are obtained from mycelial cultures of the edible mushroom *Flammulina velutipes*. Studies of the biosynthesis of these agents using 1-ABT as a probe led to identification of three intermediates (shown in the boxes) in the biosynthetic sequence (Figure 20) [321].

A strain of *Rhodococcus erythropolis* developed by using β -myrcene as the sole carbon source showed that the major product formed by the cells from this precursor was geraniol [322]. 1-ABT decreased the production of geraniol by 73%.

Xenobiotics: 1-ABT has been used to implicate cytochrome P450 enzymes in the degradation by white-rot fungi of many compounds. In *Phlebia lindtneri*, this included the demonstration that P450 enzymes participate in the degradation of dibenzo-*p*-dioxin, dibenzofuran, diphenyl ether, chloronaphthalenes, and polycyclic aromatic hydrocarbons [323,324].

1-ABT inhibited the degradation of various chemicals by *Phanerochaete chrysosporium* and related species, including diclofenac and mefenamic acid [325], ketoprofen [326], norfloxacin and ciprofloxacin [327], diuron [328], and dibenzyl sulfoxide [329]. The growth of *P. chrysosporium* on diesel fuel was inhibited by 1-ABT, indicating that P450 enzymes are involved in the utilization of this carbon source for biomass production [330].

The degradation of xenobiotics by *Trametes versicolor*, another white-rot fungus, was also inhibited by 1-ABT, pointing to the involvement of cytochrome P450 enzymes in the degradative pathways. This included the degradation of trichloroethylene [331], 1,2,3- and 1,2,4-trichlorobenzenes [332], ibuprofen [333], naproxen [334], and diclofenac [335]. *Pleurotus ostreatus* oxidizes phenanthrene to phenanthrene *trans*-9,10-diol and this degradation is inhibited by 1-ABT [336].

1-ABT inhibited the metabolism by the filamentous fungus *Cunninghamella elegans* of phenanthrene [337], dibenzothiophene [338], and amitriptyline [339]. 1-ABT inhibited the oxidation of 4-(cyclohexylamino)benzophenone and other organic compounds by *Beauveria bassiana* [340]. A comparison of the product distribution obtained with this inhibitor versus other inhibitors indicated the involvement of more than one P450 enzyme. The oxidation of adamantane by *Streptomyces griseoplanus* was inhibited by 1-ABT [341], as was the degradation of 2,5-dimethylpyrazine by *Rhodococcus*

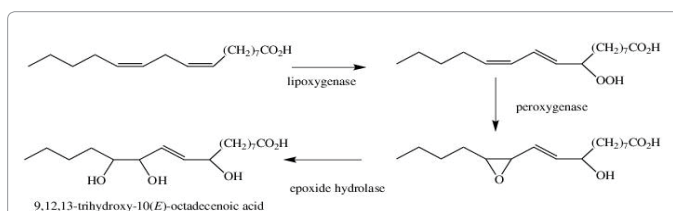


Figure 17: Formation of 9,12,13-trihydroxy-10(*E*)-octadecenoic acid in *Solanum lipoersicum* (tomato).

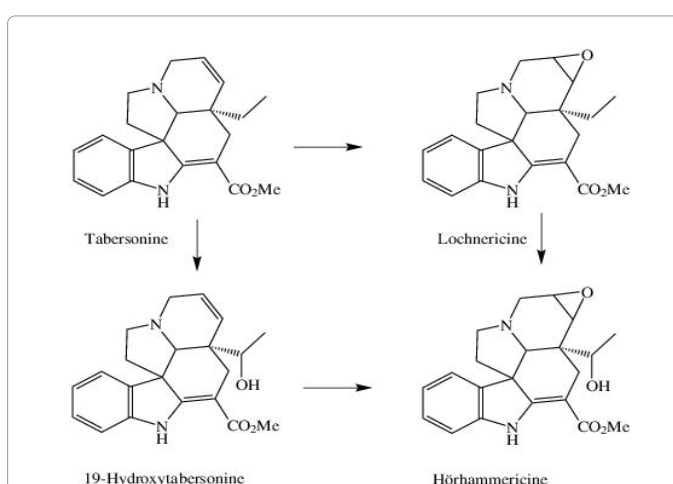


Figure 18: Pathways for the biosynthesis of hürhammericine from tabersonine in the biogenesis of vindoline.

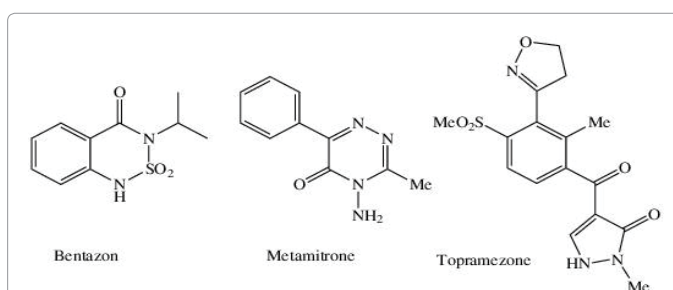


Figure 19: Structures of some of the herbicide agents whose metabolism is shown by studies with 1-ABT to involve cytochrome P450 enzymes.

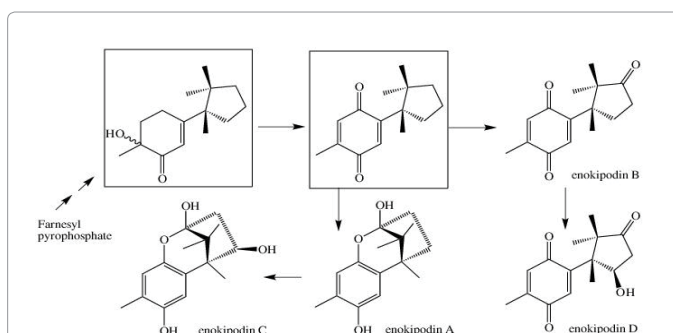


Figure 20: Two intermediates in the biosynthesis of enokipodin A, B, C, and D identified as a result of inhibition of P450 enzymes by 1-ABT. The two intermediates are shown in the boxes.

erythropolis [342], and the degradation of polychlorinated biphenyl by *Ceriporia* sp. ZLY-2010 [343].

Conclusion

1-ABT is an easily administered, mechanism-based inhibitor of most of the drug metabolizing isoforms of the cytochrome P450 family of enzymes, albeit with somewhat different sensitivities for the individual isoforms. Toxicity studies indicate that it is a relatively benign agent, even though *in vivo* it may interfere with sterol biosynthesis. It has been utilized in studies of mammals, insects, fish, and plants, using microsomal assays, intact cells, and *in vivo*. It has proven to be highly useful in situations where a pan-specific agent is required to inhibit the broad range of drug-metabolizing isoforms of cytochrome P450.

Acknowledgments

The preparation of this review was supported by National Institutes of Health grants GM25515 and AI074824.

Disclosure Statement

The author reports no conflicts of interest.

References

- Omura T (1999) Forty years of cytochrome P450. *Biochem Biophys Res Commun* 266: 690-698.
- Franklin MR, Hathaway LB (2008) 2-Diethylaminoethyl-2,2-diphenylvalerate-HCl (SKF-525A) revisited: comparative cytochrome P450 inhibition in human liver microsomes by SKF525A, its metabolites, and SKF-acid and SKF-alcohol. *Drug Metab Dispos* 36: 2539-2546.
- Rossi M (1983) Structural studies of metyrapone: A potent inhibitor of cytochrome P-450. *J Med Chem* 26: 1246-1252.
- Guengerich FP (2009) Inhibition of drug metabolizing enzymes. *Handbook of Drug Metabolism*, 2nd Ed. In: Pearson PG, Wienkers LC (eds.) pp: 203-226.
- Correia MA, Hollenberg PF (2015) Inhibition of cytochrome P450 enzymes. *Cytochrome P450: Structure, Mechanism, and Biochemistry*, 4th Ed. In: Ortiz de Montellano PR (ed.) pp: 177-259.
- Emoto C, Murase S, Sawada Y, Jones BC, Iwasaki K (2003) In vitro inhibitory effect of 1-aminobenzotriazole on drug oxidations catalyzed by human cytochrome P450 enzymes: a comparison with SKF-525A and ketoconazole. *Drug Metab Pharmacokin* 18: 287-295.
- Emoto C, Murase S, Sawada Y, Iwasaki K (2005) In vitro inhibitory effect of 1-aminobenzotriazole on drug oxidations in human liver microsomes: a comparison with SKF-525A. *Drug Metab Pharmacokin* 20: 351-357.
- Luo Y, Yang X, Shi Q (2016) The cytochrome P450 inhibitor SKF-525A disrupts autophagy in primary rat hepatocytes. *Chem Biol Interact* 255: 55-62.
- Bruce JIE, Elliott AC (2000) Pharmacological evaluation of the role of cytochrome P450 in intracellular calcium signaling in rat pancreatic acinar cells. *Br J Pharmacol* 131: 761-771.
- Kalgutkar AS, Obach RS, Maurer TS (2007) Mechanism-based inactivation of cytochrome P450 enzymes: chemical mechanisms, structure-activity relationships and relationship to clinical drug-drug interactions and idiosyncratic adverse drug reactions. *Curr Drug Metab* 8: 407-447.
- Liu J, Sridhar J, Foroozesh M (2013) Cytochrome P450 family 1 inhibitors and structure-activity relationships. *Molecules* 18: 14470-14495.
- Trave R, Bianchetti G (1960) Atti Accad Naz Lincei, Rend Classe Sci fis mat Nat 28: 652.
- Campbell CD, Rees CW (1969) Reactive intermediates. Part I. Synthesis and oxidation of 1- and 2-aminobenzotriazole. *J Chem Soc pp*: 742-747.
- Campbell CD, Rees CW (1964) A new synthesis of benzyne. *Proc Chem Soc London* 296.
- Campbell CD, Rees CW (1969) Reactive intermediates. Part III. Oxidation of 1-aminobenzotriazole with oxidants other than lead tetra-acetate. *J Chem Soc pp*: 752-756.
- Ortiz de Montellano PR, Mathews JM (1981) Autocatalytic alkylation of the cytochrome P-450 prosthetic heme by 1-aminobenzotriazole. *Biochem J* 195: 761-764.
- Mathews JM, Dostal LA, Bend JR (1985) Inactivation of rabbit pulmonary cytochrome P450 in microsomes and isolated perfused lungs by the suicide substrate 1-aminobenzotriazole. *J Pharmacol Exp Therap* 235: 186-190.
- Oldfield NF, Mortillo M, Garland WA, Mico BA (1992) Inhibition of hepatic and cutaneous biotransformation of resorufin ethers following intraperitoneal administration of 1-aminobenzotriazole. *Pharmaceut Res* 9: 1099-1102.
- Carlile DJ, Hakooz N, Houston JB (1999) Kinetics of drug metabolism in rat liver slices: IV. Comparison of ethoxycoumarin clearance by liver slices, isolated hepatocytes, and hepatic microsomes from rats pretreated with known modifiers of cytochrome P-450 activity. *Drug Metab Dispos* 27: 526-532.
- Ortiz de Montellano PR, Mathews JM, Langry KC (1984) Autocatalytic inactivation of cytochrome P-450 and chloroperoxidase by 1-aminobenzotriazole and other arylene precursors. *Tetrahedron* 40: 511-519.
- Chuanprasit P, Goh SH, Hirao H (2015) Benzyne formation in the mechanism-based inactivation of cytochrome P450 by 1-aminobenzotriazole and N-benzyl-1-aminobenzotriazole: computational insights. *ACS Catalysis* 5: 2952-2960.
- Mathews JM, Bend JR (1986) N-Alkylaminobenzotriazoles as isozyme-selective suicide inhibitors of rabbit pulmonary microsomal cytochrome P450. *Molec Pharmacol* 30: 25-32.
- Town C, Henderson L, Chang D, Mortillo M, Garland W (1993) Distribution of 1-aminobenzotriazole in male rats after administration of an oral dose. *Xenobiotica* 23: 383-390.
- Sun Q, Harper TW, Dierks EA, Zhang L, Chang S, et al. (2011) 1-Aminobenzotriazole, a known cytochrome P450 inhibitor, is a substrate and inhibitor of N-acetyltransferase. *Drug Metab Dispos* 39: 1674-1679.
- Mugford CA, Mortillo M, Mico BA, Tarloff JB (1992) 1-Aminobenzotriazole-induced destruction of hepatic and renal cytochromes P450 in male Sprague-Dawley rats. *Fund Appl Toxicol* 19: 43-49.
- Woodcroft KJ, Webb CD, Yao M, Weedon AC, Bend JR (1997) Metabolism of the cytochrome P450 mechanism-based inhibitor N-benzyl-1-aminobenzotriazole to products that covalently bind with protein in guinea pig liver and lung microsomes: comparative study with 1-aminobenzotriazole. *Chem Res Toxicol* 10: 589-599.
- Strelevitz TJ, Foti RS, Fisher MB (2006) In vivo use of the P450 inactivator 1-aminobenzotriazole in the rat: varied dosing route to elucidate gut and liver contributions to first-pass and systemic clearance. *J Pharm Sci* 95: 1334-1341.
- Boily MO, Chaurat N, Laterreur J, Leblond FA, Boudreau C, et al. (2015) In vitro and in vivo mechanistic studies toward understanding the role of 1-aminobenzotriazole in rat drug-drug interactions. *Drug Metab Dispos* 43:1960-1965.
- Parrish KE, Mao J, Chen J, Jauchico A, Ly J, et al. (2016) In vitro and in vivo characterization of CYP inhibition by 1-aminobenzotriazole in rats. *Biopharm Drug Dispos* 37: 200-211.
- Stringer RA, Weber E, Tigani B, Lavan P, Medhurst S, et al. (2014) 1-Aminobenzotriazole modulates oral drug pharmacokinetics through cytochrome P450 inhibition and delay of gastric emptying in rats. *Drug Metab Dispos* 42:1117-1124.
- Stringer RA, Ferreira S, Rose J, Ronseaux S (2016) Application of osmotic pumps for sustained release of 1-aminobenzotriazole and inhibition of cytochrome P450 enzymes in mice: model comparison with the hepatic P450 reductase null mouse. *Drug Metab Dispos* 44:1213-1216.
- Balani SK, Zhu T, Yang TJ, Liu Z, He B, et al. (2002) Effective dosing regimen of 1-aminobenzotriazole for inhibition of antipyrine clearance in rats, dogs, and monkeys. *Drug Metab Dispos* 30:1059-1062.
- Balani SK, Li P, Nguyen J, Cardoza K, Zeng H, et al. (2004) Effective dosing regimen of 1-aminobenzotriazole for inhibition of antipyrine clearance in guinea pigs and mice using serial sampling. *Drug Metab Dispos* 32:1092-1095.
- Watanabe A, Mayumi K, Nishimura K, Osaki H (2016) In vivo use of the CYP inhibitor 1-aminobenzotriazole to increase long-term exposure in mice. *Biopharm Drug Dispos* 37: 373-378.
- Soto M, Pham R, Almon V, Wagner M, Primack R, et al. (2014) Evaluation of matrix microsampling methods for therapeutic drug candidate quantification in discovery-stage rodent pharmacokinetic studies. *Bioanalysis* 6: 2135-2146.
- Deshmukh SV, Durston J, Shomer NH (2008) Validation of the use of non-

- naive surgically catheterized rats for pharmacokinetics studies. *J Am Assoc Lab Anim Sci* 47: 41-45.
37. Ortiz de Montellano PR, Mico BA, Mathews JM, Kunze KL, Miwa GT, et al. (1981) Selective inactivation of cytochrome P-450 isozymes by suicide substrates. *Arch Biochem Biophys* 210: 717-728.
38. Wong SG, Lin EH, Marks GS (1999) Cytochrome CYP sources of N-alkylprotoporphyrin IX after administration of porphyrinogenic xenobiotics to rats. *Drug Metab Dispos* 27: 960-965.
39. Knickle LC, Bend JR (1992) Dose-dependent, mechanism-based inactivation of cytochrome P450 monooxygenases in vivo by 1-aminobenzotriazole in liver, lung, and kidney of untreated, phenobarbital-treated, and β -naphthoflavone-treated guinea pigs. *Can J Physiol Pharmacol* 70: 1610-1617.
40. Riddick DS, MacKie JE, Massey TE, Marks GS (1990) 3,5-Diethoxycarbonyl-2,6-dimethyl-4-ethyl-1,4-dihydropyridine inactivates rat liver cytochrome P-450c, but not its orthologue, rabbit lung form 6. *Can J Physiol Pharmacol* 68: 370-373.
41. Linder CD, Renaud NA, Hutzler JM (2009) Is 1-aminobenzotriazole an appropriate in vitro tool as a nonspecific cytochrome P450 inactivator? *Drug Metab Dispos* 37: 10-13.
42. Sodhi JK, Ford KA, Mukadam S, Wong S, Hop CECA, et al. (2014) 1-Aminobenzotriazole coincubated with (S)-warfarin results in potent inactivation of CYP2C9. *Drug Metab Dispos* 42: 813-817.
43. Tierney DJ, Haas AL, Koop DR (1992) Degradation of cytochrome P4502E1: selective loss after labilization of the enzyme. *Arch Biochem Biophys* 293: 9-16.
44. Sumner SCJ, Fennell TR, Moore TA, Chanas B, Gonzalez F, et al. (1999) Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. *Chem Res Toxicol* 12: 1110-1116.
45. Ghanayem BI, Sanders JM, Chanas B, Burka LT, Gonzalez FJ (1999) Role of cytochrome P450 2E1 in methacrylonitrile metabolism and disposition. *J Pharm Exp Therap* 289: 1054-1059.
46. Jackson TE, Lilly PD, Recio L, Schlosser PM, Medinsky MA (2000) Inhibition of cytochrome P450 2E1 decreases, but does not eliminate, genotoxicity mediated by 1,3-butadiene. *Toxicol Sci* 55: 266-273.
47. Hoffer U, El-Masri HA, Ghanayem BI (2003) Cytochrome P450 2E1 is the principal enzyme responsible for urethane metabolism: comparative studies using CYP2E1-null and wild-type mice. *J Pharmacol Exp Therap* 305: 557-564.
48. Muzeeb S, Pasha MK, Basha SJS, Mullangi R, Srinivas NR (2005) Effect of 1-aminobenzotriazole on the in vitro metabolism and single dose pharmacokinetics of chlorzoxazone, a selective CYP2E1 substrate in Wistar rats. *Xenobiotica* 35: 825-838.
49. Dierks EA, Zhang Z, Johnson EF, Ortiz de Montellano PR (1998) The catalytic site of cytochrome P450A11 (CYP4A11) and its L131F mutant. *J Biol Chem* 273: 23055-23061.
50. Dierks EA, Davis SC, Ortiz de Montellano PR (1998) Glu-320 and Asp-323 are determinants of the CYP4A1 hydroxylation regioselectivity and resistance to inactivation by 1-aminobenzotriazole. *Biochemistry* 37: 1839-1847.
51. Colby HD, Purcell H, Kominami S, Takemori S, Kossor DC (1994) Adrenal activation of carbon tetrachloride: role of microsomal P450 isozymes. *Toxicology* 94: 31-40.
52. Xu D, Voigt JM, Mico BA, Kominami S, Takemori S, et al. (1994) Inhibition of adrenal cytochromes P450 by 1-aminobenzotriazole in vitro. Selectivity for xenobiotic metabolism. *Biochem Pharmacol* 48: 1421-1426.
53. Xu D, Voigt J, Mico B, Colby H (1995) Inhibition of adrenal steroid metabolism by administration of 1-aminobenzotriazole to guinea pigs. *J Steroid Biochem Molec Biol* 54: 281-285.
54. Colby HD, Abbott B, Cachovic M, Debolt KM, Mico BA (1995) Inactivation of adrenal cytochromes P450 by 1-aminobenzotriazole. Divergence of in vivo and in vitro actions. *Biochem Pharmacol* 49: 1057-1062.
55. Soltis M, Colby H (1997) Inhibition of testicular steroid metabolism by administration of 1-aminobenzotriazole to rats. *Pharmacol* 56: 51-56.
56. Shaik AN, LeDuc BW, Khan AL (2017) Characterization of 1-aminobenzotriazole and ketoconazole as novel inhibitors of monoamine oxidase (MAO): an in vitro investigation. *Eur J Drug Metab Pharmacokinet* 42: 827-834.
57. di Re J, Lee C, Riddick DS (1999) Lack of mechanism-based inactivation of rat hepatic microsomal cytochromes P450 by doxorubicin. *Can J Physiol Pharmacol* 77: 589-597.
58. Reichhart D, Simon A, Durst F, Mathews JM, Ortiz de Montellano PR (1982) Autocatalytic inactivation of plant cytochrome P-450 enzymes: Selective inactivation of cinnamic acid 4-hydroxylase from *Helianthus tuberosus* by 1-aminobenzotriazole. *Arch Biochem Biophys* 216: 522-529.
59. Mössner LD, Schmitz A, Theurillat R, Thormann W, Mevissen M (2011) Inhibition of cytochrome P450 enzymes involved in ketamine metabolism by use of liver microsomes and specific cytochrome P450 enzymes from horses, dogs, and humans. *Am J Vet Res* 72: 1505-1513.
60. Cashman JR (1986) A convenient assay for the flavin-containing monooxygenase. *Analyt Biochem* 160: 294-300.
61. Furnes B, Schlenk D (2005) Extrahepatic metabolism of carbamate and organophosphate thioether compounds by the flavin-containing monooxygenase and cytochrome P450 systems. *Drug Metab Dispos* 33: 214-218.
62. Cashman JR, Williams DE (1990) Enantioselective S-oxygenation of 2-aryl-1,3-dithiolanes by rabbit lung enzyme preparations. *Molec Pharmacol* 37: 333-339.
63. Rettie AE, Bogucki BD, Lim I, Meier GP (1990) Stereoselective sulfoxidation of a series of alkyl p-tolylsulfides by microsomal and purified flavin-containing monooxygenases. *Molec Pharmacol* 37: 643-651.
64. Decker CJ, Doerge DR (1992) Covalent binding of ^{14}C - and ^{35}S -labeled thiocarbamides in rat hepatic microsomes. *Biochem Pharmacol* 43: 881-888.
65. Decker CJ, Doerge DR, Cashman JR (1992) Metabolism of benzimidazole-2-thiones by rat hepatic microsomes and hog liver flavin-containing monooxygenase. *Chem Res Toxicol* 5: 726-733.
66. Yang Y, Qiu F, Josephs JL, Humphreys WG, Xhu YZ (2012) Bioactivation of 2-(alkylthio)-1,3,4-thiadiazoles and 2-(alkylthio)-1,3-benzothiazoles. *Chem Res Toxicol* 25: 2770-2779.
67. Eng H, Sharma R, Wolfrd A, Di L, Ruggeri RB, et al. (2016) Species differences in the oxidative desulfurization of a thiouracil-based irreversible myeloperoxidase inactivator by flavin-containing monooxygenase enzymes. *Drug Metab Dispos* 44:1262-1269.
68. Martinkova M, Kubickova B, Stiborova M (2012) Effects of cytochrome P450 inhibitors on peroxidase activity. *Neuro Endocrinol Lett* 33: 33-40.
69. Kim PM, DeBoni U, Wells PG (1997) Peroxidase-dependent bioactivation and oxidation of DNA and protein in benzo[a]pyrene-initiated micronucleus formation. *Free Rad Biol Med* 23: 579-596.
70. Bedard LL, Smith GBJ, Reid KR, Petsikas D, Massey TE (2002) Investigations of the role of lipoygenase in bioactivation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in human lung. *Chem Res Toxicol* 15: 1267-1273.
71. Kimoto E, Walsky R, Zhang H, Bi Y, Whalen KM, et al. (2012) Differential modulation of cytochrome P450 activity and the effect of a-aminobenzotriazole on hepatic transport in sandwich-cultured human hepatocytes. *Drug Metab Dispos* 40: 407-411.
72. Crouch RD, Morrison RD, Byers FW, Lindsley CW, Emmitte KA, et al. (2016) Evaluating the disposition of a mixed aldehyde oxidase/cytochrome P450 substrate in rats with attenuated P450 activity. *Drug Metab Dispos* 44:1296-1303.
73. Yu L, Jiang Y, Wang L, Sheng R, Hu Y, et al. (2013) Metabolism of BYZX in human liver microsomes and cytosol: identification of the metabolites and metabolic pathways of BYZX. *PLOS One* 8: e59882.
74. Walsky RL, Bauman JN, Bourcier K, Giddens G, Lapham K, et al. (2012) Optimized assays for human UDP-glucuronosyltransferase (UGT) activities: altered alamethicin concentration and utility to screen for UGT inhibitors. *Drug Metab Dispos* 40:1051-1065.
75. Horng H, Benet LZ (2013) The effects of 1-aminobenzotriazole inhibition on the formation of acyl-glucuronide and acyl-glutathione metabolites in rat hepatocytes. *J Diagn Tech Biomed Anal* 2:1.
76. Plise EG, Halladay JS, Cheong J, Sodhi J, Salphati L (2010) Commonly used inhibitors of drug metabolizing enzymes: do they also inhibit drug transporters? *Drug Metab Rev* 42S: P190.
77. Fan PW, Chen JZ, Jauchico MA, La H, Liu N, et al. (2016) Rate-determining and rate-limiting steps in the clearance and excretion of a potent and selective p21-activated kinase inhibitor: A case study of rapid hepatic uptake and slow elimination in rat. *Drug Metab Lett* 10: 91-100.

78. Kostrubsky SE, Strom SC, Kalgutkar AS, Kulkarni S, Atherton J, et al. (2006) Inhibition of hepatobiliary transport as a predictive method for clinical hepatotoxicity of nefazodone. *Toxicol Sci* 90: 451-459.
79. Ogimura E, Sekine S, Horie T (2011) Bile salt export pump inhibitors are associated with bile acid-dependent drug-induced toxicity in sandwich-cultured hepatocytes. *Biochem Biophys Res Commun* 416: 313-317.
80. Ortiz de Montellano PR, Costa AK (1986) Dissociation of cytochrome P-450 inactivation and induction. *Arch Biochem Biophys* 251: 514-524.
81. Yang K, Koh KH, Jeong H (2010) Induction of CYP2B6 and CYP3A4 expression by 1-aminobenzotriazole (ABT) in human hepatocytes. *Drug Metab Lett* 4: 129-133.
82. Zangar RC, Novak RF (1997) Effects of fatty acids and ketone bodies on cytochromes P450 2B, 4A, and 2E1 expression in primary cultured rat hepatocytes. *Arch Biochem Biophys* 337: 217-224.
83. Feyereisen R, Langry KC, Ortiz de Montellano PR (1984) Self-catalyzed destruction of insect cytochrome P-450. *Insect Biochem* 14:19-26.
84. Gaillardon P, Cabanne F, Scalla R, Durst F (1985) Effect of mixed function oxidase inhibitors on the toxicity of chlortoluron and isoproturon to wheat. *Weed Res* 25: 397-402.
85. Mico BA, Federowicz DA, Ripple MG, Kerns W (1988) In vivo inhibition of oxidative drug metabolism by, and acute toxicity of, 1-aminobenzotriazole (ABT). A tool for biochemical toxicology. *Biochem Pharmacol* 37: 2515-2519.
86. Isayama F, Froh M, Bradford BU, McKim SE, Kadiiska MB, et al. (2003) The CYP inhibitor 1-aminobenzotriazole does not prevent oxidative stress associated with alcohol-induced liver injury in rats and mice. *Free Rad Biol Med* 35: 1568-1581.
87. Meschter CL, Mico BA, Mortillo M, Feldman D, Garland WA, et al. (1994) A 13-week toxicologic and pathologic evaluation of prolonged cytochromes P450 inhibition by 1-aminobenzotriazole in male rats. *Fundam Appl Toxicol* 22: 369-381.
88. Moorthy B, Parker KM, Smith CV, Bend JR, Welty SE (2000) Potentiation of oxygen-induced lung injury in rats by the mechanism-based cytochrome P-450 inhibitor, 1-aminobenzotriazole. *J Pharmacol Exp Therap* 292: 553-560.
89. Woodcroft KJ, Szczepan EW, Knickle LC, Bend JR (1990) Three N-aralkylated derivatives of 1-aminobenzotriazole as potent and isozyme selective, mechanism-based inhibitors of guinea pig pulmonary cytochrome P450 in vitro. *Drug Metab Disp* 18: 1031-1037.
90. Sinal CJ, Bend JR (1995) Isozyme-selective metabolic intermediate complex formation of guinea pig hepatic cytochrome P450 by N-aralkylated derivatives of 1-aminobenzotriazole. *Chem Res Toxicol* 8: 82-91.
91. Sinal C, Bend J (1996) Kinetics and selectivity of mechanism-based inhibition of guinea pig hepatic and pulmonary cytochrome P450 by N-benzyl-1-aminobenzotriazole and N-*m*-methylbenzyl-1-aminobenzotriazole. *Drug Metab Disp* 24: 996-1001.
92. Kent U, Hanna I, Szklarz G, Vaz A, Halpert J, et al. (1997) Significance of glycine 478 in the metabolism of N-benzyl-1-aminobenzotriazole to reactive intermediates by cytochrome P450 2B1. *Biochemistry* 36:11707-11716.
93. Kent UM, Pascual L, Roof RA, Ballou DP, Hollenberg PF (2004) Mechanistic studies with N-benzyl-1-aminobenzotriazole-inactivated CYP2B1: differential effects on the metabolism of 7-ethoxy-4-(trifluoromethyl)coumarin, testosterone, and benzphetamine. *Arch Biochem Biophys* 423: 277-287.
94. McCallum GP, Horton JE, Flakner KC, Bend JR (1993) Microsomal cytochrome P450 1A1 dependent monooxygenase activity in guinea pig heart: induction, inhibition, and increased activity by addition of exogenous NADPH-cytochrome P450 reductase. *Can J Physiol Pharmacol* 71: 151-156.
95. Sinal C, Hirst M, Webb C, Bend J (1998) Enantioselective, mechanism-based inactivation of guinea pig hepatic cytochrome P450 by N-(*a*-methylbenzyl)-1-aminobenzotriazole. *Drug Metab Disp* 26: 681-688.
96. Knickle LC, Philpot RM, Bend JR (1994) N-aralkylated derivatives of 1-aminobenzotriazole are potent isozyme- and lung-selective mechanism-based inhibitors of guinea pig cytochrome P450 in vivo. *J Pharmacol Exp Therap* 270: 377-385.
97. Woodcroft KJ, Bend JR (1990) N-Aralkylated derivatives of 1-aminobenzotriazole as isozyme-selective, mechanism-based inhibitors of guinea pig hepatic cytochrome P-450 dependent monooxygenase activity. *Can J Physiol Pharmacol* 68: 1278-1285.
98. Mathews JM, Bend JR (1993) N-Aralkyl derivatives of 1-aminobenzotriazole as potent isozyme and tissue selective mechanism-based inhibitors of rabbit pulmonary cytochrome P450 in vivo. *J Pharmacol Exp Therap* 265: 281-285.
99. Grimm SW, Bend JR, Halpert JR (1995) Selectivity and kinetics of inactivation of rabbit hepatic cytochromes P450 2B4 and 2B5 by N-aralkylated derivatives of 1-aminobenzotriazole. *Drug Metab Disp* 23: 577-583.
100. Knickle LC, Webb CD, House AA, Bend JR (1993) Mechanism-based inactivation of cytochrome P450 1A1 by N-aralkyl-1-aminobenzotriazoles in guinea pig kidney in vivo and in vitro: minimal effects on metabolism of arachidonic acid by renal P450-dependent monooxygenases. *J Pharmacol Exp Therap* 267: 758-764.
101. Knickle LC, Bend JR (1994) Bioactivation of arachidonic acid by the cytochrome P450 monooxygenases of guinea pig lung: the orthologue of cytochrome P450 2B4 is solely responsible for formation of epoxyeicosatrienoic acid. *Molec Pharmacol* 45:1273-1280.
102. Lu P, Singh SB, Carr BA, Fang Y, Xiang CD, et al. (2005) Selective inhibition of dog hepatic CYP2B11 and CYP3A12. *J Pharmacol Exp Therap* 313: 518-528.
103. Hahn V, Mikolasch A, Wende K, Bartrow H, Lindequist U, et al. (2010) Derivatization of the azole 1-aminobenzotriazole using laccase of *Pycnoporus cinnabarinus* and *Myceliophthora thermophila*: influence of methanol on the reaction and biological evaluation of the derivatives. *Biotechnol Appl Biochem* 56: 43-48.
104. Caldwell GW, Ritchie DM, Masucci JA, Hageman W, Cotto C, et al. (2005) The use of the suicide CYP450 inhibitor ABT for distinguishing absorption and metabolism processes in in-vivo pharmacokinetic screens. *Eur J Drug Metab Pharmacokin* 30: 75-83.
105. El-Kattan AF, Poe J, Buchholz L, Thomas HV, Brodfuehrer J, et al. (2008) The use of 1-aminobenzotriazole in differentiating the role of CYP-mediated first pass metabolism and absorption in limiting drug oral bioavailability: a case study. *Drug Metab Lett* 2: 120-124.
106. Anby MU, Nguyen TH, Yeap YY, Feeney OM, Williams HD, et al. (2014) An in vitro digestion test that reflects rat intestinal conditions to probe the importance of formulation digestion vs first pass metabolism in danazol bioavailability from lipid based formulations. *Mol Pharmaceut* 11: 4069-4083.
107. Saitta KS, Zhang C, Lee KK, Fujimoto K, Redinbo MR, et al. (2014) Bacterial β -glucuronidase inhibition protects mice against enteropathy induced by indomethacin, ketoprofen or diclofenac: mode of action and pharmacokinetics. *Xenobiotica* 44:28-35.
108. Takahashi M, Washio T, Suzuki N, Igeta K, Yamashita S (2010) Investigation of the intestinal permeability and first-pass metabolism of drugs in cynomolgus monkeys using single-pass intestinal perfusion. *Biol Pharm Bull* 33: 111-116.
109. Grimsley A, Foster A, Gallagher R, Hutchinson M, Lundqvist A, et al. (2014) A comparison of the metabolism of midazolam in C57BL/6J and hepatic reductase null (HRN) mice. *Biochem Pharmacol* 92: 701-711.
110. Bylund J, Bueters T (2013) Presystemic metabolism of AZ0908, a novel mPGES-1 inhibitor: an in vitro and in vivo cross-species comparison. *J Pharm Sci* 102: 1106-1115.
111. Watanabe A, Watari R, Ogawa K, Shimizu R, Tanaka Y, et al. (2015) Using improved serial blood sampling method of mice to study pharmacokinetics and drug-drug interactions. *J Pharmaceut Sci* 104: 955-961.
112. Ballard P, Barlaam BC, Bradbury RH, Dishington A, Hennequin LFA, et al. (2007) Neutral 5-substituted 4-anilinoquinazolines as potent, orally active inhibitors of erbB2 receptor tyrosine kinase. *Bioorg Med Chem Lett* 17: 6326-6329.
113. Li AP, Doshi U (2011) Higher throughput human hepatocyte assays for the evaluation of time-dependent inhibition of CYP3A4. *Drug Metab Lett*, 5: 183-191.
114. Li AP (2009) Evaluation of luciferin-isopropyl acetal as a CYP3A4 substrate for human hepatocytes: effects of organic solvents, cytochrome P450 (P450) inhibitors, and P450 inducers. *Drug Metab Dispos* 37: 1598-1603.
115. Doshi U, Li AP (2011) Luciferin IPA-based higher throughput human hepatocytes screening assays for CYP3A4 inhibition and induction. *J Biomol Screen* 16: 903-909.
116. Feng PCC, Solsten RT (1994) In vitro biotransformation of thiazopyr by rat liver microsomes: oxidative cleavage of a carboxylic methylester by monooxygenases. *Xenobiotica* 24: 729-734.

117. Eng H, Niosi M, McDonald TS, Wolford A, Chen Y, et al. (2010) Utility of the carboxylesterase inhibitor bis-para-nitrophenylphosphate (BNPP) in the plasma unbound fraction determination for a hydrolytically unstable amide derivative and agonist of the TGR5 receptor. *Xenobiotica* 40: 369-380.
118. Takahashi R, Ma S, Deese A, Yue Q, Kim-Kang H, et al. (2014) Elucidating the mechanism of cytochrome P450-mediated pyrimidine ring conversion to pyrazole metabolites with the BACE1 inhibitor GNE-892 in rats. *Drug Metab Dispos* 42: 890-898.
119. Jones BC, Markandu R, Gu C, Scarfe G (2017) CYP-mediated sulfoximine deamination of AZD6738. *Drug Metab Dispos* 45: 1133-1138.
120. Gu Y, Guise CP, Patel K, Abbattista MR, Li J, et al. (2011) Reductive metabolism of the dinitrobenzamide mustard anticancer prodrug PR-104 in mice. *Cancer Chemother Pharmacol* 67: 543-555.
121. Gombar CT, Burak E, Harper N, Smith BR (1988) Pulmonary clearance of vasoactive drugs: N-oxidation of SK&F 86466 in the isolated perfused rat lung. *J Pharmacol Exp Therap* 245: 402-406.
122. Wang Z, Fang Y, Teague J, Wong H, Morisseau C, et al. (2017) In vitro metabolism of oprozomib, an oral proteasome inhibitor: Role of epoxide hydrolases and cytochrome P450. *Drug Metab Dispos* 45: 712-720.
123. Capello S, Henderson L, DeGrazia F, Liberato D, Garland W, et al. (1990) The effect of the cytochrome P450 suicide inactivator, 1-aminobenzotriazole, on the in vivo metabolism and pharmacologic activity of flurazepam. *Drug Metab Dispos* 18: 190-196.
124. Lake BG, Price RJ, Walters DG, Phillips JC, Young PJ, et al. (2003) Studies on the metabolism of the thiofurans furfuryl mercaptan and 2-methyl-3-furanthiol in rat liver. *Food Chem Toxicol* 41: 1761-1770.
125. Sanderson JP, Hollis FJ, Maggs JL, Clarke SE, Naisbitt DJ, et al. (2008) Nonenzymatic formation of a novel hydroxylated sulfamethoxazole derivative in human liver microsomes: implications for bioanalysis of sulfamethoxazole metabolites. *Drug Metab Dispos* 36: 2424-2428.
126. Charman SA, Arbe-Barnes S, Bathurst IC, Brun R, Campbell M, et al. (2011) Synthetic ozonide drug candidate OZ439 offers new hope for a single-dose cure of uncomplicated malaria. *Proc Natl Acad Sci USA* 108: 4400-4405.
127. Mishra S, Manickavasagam L, Jain GK (2012) Determination of metabolic profile of anti-malarial trioxane CDRI 99/411 in rat liver microsomes using HPLC. *Biomed Chromatogr* 26: 115-122.
128. Chen H, Soroka D, Zhu Y, Sang S (2013) Metabolism of ginger component [6]-shogaol in liver microsomes from mouse, rat, dog, monkey, and human. *Mol Nutr Food Res* 57: 865-876.
129. Yue Q, Mulder T, Rudewicz PJ, Solon E, Budha N, et al. (2013) Evaluation of metabolism and disposition of GDC-0152 in rats using ¹⁴C-labeling strategy at two different positions: a novel formation of hippuric acid from 4-phenyl-5-amino-1,2,3-thiadiazole. *Drug Metab Dispos* 41: 508-517.
130. Ning J, Yu ZL, Hu LH, Wang C, Huo XK, et al. (2015) Characterization of phase I metabolism of resibufogenin and evaluation of the metabolic effects on its antitumor activity and toxicity. *Drug Metab Dispos* 43: 299-308.
131. Guo J, Joubran C, Luzietti RA, Basarab GS, Grimm SW, et al. (2017) Absorption, distribution, metabolism and elimination of ¹⁴C-ETX0914, a novel inhibitor of bacterial type-II topoisomerases in rodents. *Xenobiotica* 47: 31-49.
132. Zhang J, Li L, Tang S, Hale TW, Xing C, et al. (2015) Cytochrome P450 isoforms in the metabolism of decursin and decursinol angelate from *Koean angelic*. *Am J Chin Med* 43: 1211-1230.
133. Klieber S, Arabeyre-Fabre C, Moliner P, Marti E, Mandray M, et al. (2014) Identification of metabolic pathways and enzyme systems involved in the in vitro human hepatic metabolism of dronedarone, a potent new oral antiarrhythmic drug. *Pharmacol Res Perspect* 2: e00044.
134. Dalmadi B, Leibinger J, Szeberényi S, Borbás T, Farkas S, et al. (2003) Identification of metabolic pathways involved in the biotransformation of tolperisone by human microsomal enzymes. *Drug Metab Dispos* 31: 631-636.
135. Wang MZ, Saulter JY, Usuki E, Cheung YL, Hall M, et al. (2006) CYP4F enzymes are the major enzymes in human liver microsomes that catalyze the O-demethylation of the antiparasitic prodrug DB289 [2,5-bis(4-aminodiphenyl) furan-bis-O-methylamidoxime]. *Drug Metab Dispos* 34: 1985-1994.
136. Stevens GJ, Hitchcock K, Wang YK, Coppola GM, Versace RW, et al. (1997) In vitro metabolism of N-(5-chloro-2-methylphenyl)-N'-(2-methylpropyl) thiourea: species comparison and identification of a novel thiocarbamide-glutathione adduct. *Chem Res Toxicol* 10: 733-741.
137. Boer J, Young-Sciame R, Lee F, Bowman KJ, Yang X, et al. (2016) The role of UGT, CYP and gut microbiota in the metabolism of epacadostat (EPA) in humans. *Drug Metab Dispos* 44: 1668-1674.
138. Leong BK, Sabaitis CP, Rop DA, Jeffrey P, Parker TJ, et al. (1997) Alterations in the cardiopulmonary effects and pharmacokinetics of a bisphosphonate drug by a cytochrome P-450 inhibitor in conscious rats. *J Appl Toxicol* 17: 279-288.
139. Tang W, Stearns RA, Chen Q, Bleasby K, Teffera Y, et al. (2008) Importance of mechanistic drug metabolism studies in support of drug discovery: A case study with an N-sulfonylated dipeptide VLA-4 antagonist in rats. *Xenobiotica* 38: 223-237.
140. Kenneke JF, Mazur CS, Ritger SE, Sack TJ (2008) Mechanistic investigation of the noncytochrome P450-mediated metabolism of triadimefon to triadimenol in hepatic microsomes. *Chem Res Toxicol* 21: 1997-2004.
141. Wityak J, McGee KF, Conlon MP, Song RH, Duffy BC, et al. (2015) Lead optimization towards proof-of-concept tools for Huntington's disease within a 4-(1H-pyrazol-4-yl)pyrimidine class of pan-JNK inhibitors. *J Med Chem* 58: 2967-2987.
142. Kurata H, Gentry PR, Kokubo M, Cho HP, Bridges TM, et al. (2015) Further optimization of the M5 NAM MLPCN probe ML375: tactics and challenges. *Bioorg Med Chem Lett* 25: 690-694.
143. Kaul M, Mark L, Zhang Y, Parhi AK, Lyu YL, et al. (2015) TXA709, an FtsZ-targeting benzamide prodrug with improved pharmacokinetics and enhanced in vivo efficacy against methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 59: 4845-4855.
144. Reich NO, Ortiz de Montellano PR (1986) Dissociation of increased lauric acid omega-hydroxylase activity from the antilipidemic action of clofibrate. *Biochem Pharmacol* 35: 1227-1233.
145. Romano MC, Straub KM, Yodis LAP, Eckhardt RD, Newton JF (1988) Determination of microsomal lauric acid hydroxylase activity by HPLC with flow-through radiochemical quantitation. *Anal Biochem* 170: 83-93.
146. Ortiz de Montellano PR, Reich NO (1984) Specific inactivation of hepatic fatty acid hydroxylases by acetylenic fatty acids. *J Biol Chem* 259: 4136-4141.
147. Kaikaus RM, Chan WK, Lysenko N, Ray R, Ortiz de Montellano PR, et al. (1993) Induction of peroxisomal fatty acid β -oxidation and liver fatty acid-binding protein by peroxisome proliferators. Mediation via the cytochrome P-450IVA1 ω -hydroxylase pathway. *J Biol Chem* 268: 9593-9603.
148. Su P, Kaushal KM, Kroetz DL (1998) Inhibition of renal arachidonic acid ω -hydroxylase activity with ABT reduces blood pressure in the SHR. *Am J Physiol* 275: R426-R438.
149. Maier KG, Henderson L, Narayanan J, Alonso-Galicia M, Falck JR, et al. (2000) Fluorescent HPLC assay for 20-HETE and other P-450 metabolites of arachidonic acid. *Am J Physiol Heart Circ Physiol* 279: H863-H871.
150. Hoagland KM, Flasch AK, Roman RJ (2003) Inhibitors of 20-HETE formation promote salt-sensitive hypertension in rats. *Hypertension* 42: 669-673.
151. Dos Santos EA, Dahly-Vernon AJ, Hoagland KM, Roman RJ (2004) Inhibition of the formation of EETs and 20-HETE with 1-aminobenzotriazole attenuates pressure natriuresis. *Am J Physiol Regul Integr Comp Physiol* 287: R58-R68.
152. Sarkis A, Ito O, Mori T, Kohzaki M, Ito S, et al. (2005) Cytochrome P450-dependent metabolism of arachidonic acid in the kidney of rats with diabetes insipidus. *Am J Physiol Renal Physiol* 289: F1333-F1340.
153. Coceani F, Kelsey L, Seidlitz E, Korzekwa K (1996) Inhibition of the contraction of the ductus arteriosus to oxygen by 1-aminobenzotriazole, a mechanism-based inactivator of cytochrome P450. *Brit J Pharmacol* 117: 1586-1592.
154. Pagliaro P, Penna C, Rastaldo R, Mancardi D, Crisafulli A, et al. (2004) Endothelial cytochrome P450 contributes to the acetylcholine-induced cardiodepression in isolated rat hearts. *Acta Physiol Scand* 162: 11-20.
155. Vanheel B, Van de Voorde J (1997) Evidence against the involvement of cytochrome P450 metabolites in endothelium-dependent hyperpolarization of the rat main mesenteric artery. *J Physiol* 501: 331-341.
156. Vanheel B, Calders P, Van den Bossche I, Van de Voorde J (1999) Influence

- of some phospholipase A₂ and cytochrome P450 inhibitors on rat arterial smooth muscle K⁺ currents. *Can J Physiol Pharmacol* 77: 481-489.
157. Walkowska A, Kuczeriszka M, Sadowski J, Olszynski KH, Dobrowolski L, et al. (2015) High salt intake increases blood pressure in normal rats: putative role of 20-HETE and no evidence on changes in renal vascular reactivity. *Kidney Blood Press Res* 40: 323-334.
158. Rastaldo R, Paolocci N, Chiribiri A, Penna C, Gattullo D, et al. (2001) Cytochrome P-450 metabolite of arachidonic acid mediates bradykinin-induced negative inotropic effect. *Am J Physiol Heart Circ Physiol* 280: H2823-H2832.
159. Badzyńska B, Sadowski J (2009) Differential action of bradykinin on intrarenal regional perfusion in the rat: waning effect in the cortex and major impact in the medulla. *J Physiol (Lond)* 587: 3943-3953.
160. Ceresa F, Delfino A, de Matteis A, Ferrero N, Novarino M, et al. (1999) The coronary vasculature and the myocardium. *Boll Soc Ital Biol Sper* 75: 1-8.
161. Blanton A, Nsaif R, Hercule H, Oyekan A (2006) Nitric oxide/cytochrome P450 interactions in cyclosporin A-induced effects in the rat. *J Hypertens* 24: 1865-1872.
162. López B, Moreno C, Salom MG, Roman RJ, Fenoy FJ (2001) Role of guanylyl cyclase and cytochrome P-450 on renal response to nitric oxide. *Am J Physiol Renal Physiol* 281: F420-F427.
163. Dobrowolski L, Walkowska A, Kompanowska-Jezierska E, Kuczeriszka M, Sadowski J (2007) Effects of ATP on rat renal haemodynamics and excretion: role of sodium intake, nitric oxide and cytochrome P450. *Acta Physiol (Oxf)* 189: 77-85.
164. Tuncat B, Yaghini FA, Estes A, Malik KU (2006) Inhibition by nitric oxide of cytochrome P450 4A activity contributes to endotoxin-induced hypotension in rats. *Nitric Oxide* 14: 51-57.
165. Wang MH, Wang J, Chang HH, Zand BA, Jiang M, et al. (2003) Regulation of renal CYP4A expression and 20-HETE synthesis by nitric oxide in pregnant rats. *Am J Physiol Renal Physiol* 285: F295-F302.
166. He H, Podymow T, Zimpelmann J, Burns KD (2003) NO inhibits Na⁺-K⁺-2Cl⁻ cotransport via a cytochrome P450-dependent pathway in renal epithelial cells (MMDD1). *Am J Physiol Renal Physiol* 284: F1235-F1244.
167. Benter IF, Francis I, Cojocel C, Juggi JS, Yousif MHM, et al. (2005) Contribution of cytochrome P450 metabolites of arachidonic acid to hypertension and end-organ damage in spontaneously hypertensive rats treated with L-NAME. *Auton Autacoid Pharmacol* 25: 143-154.
168. Hatton CJ, Peers C (1996) Effects of cytochrome P-450 inhibitors on ionic currents in isolated rat type I carotid body cells. *Am J Physiol* 271: C85-C92.
169. Hatton CJ, Peers C (1996) Is cytochrome P-450 involved in hypoxic inhibition of K⁺ currents in rat type I carotid body cells? *Adv Exp Med Biol* 410: 89-92.
170. Hatton CJ, Peers C (1998) Arachidonic acid inhibits both K⁺ and Ca²⁺ currents in isolated type I cells of the rat carotid body. *Brain Res* 787: 315-320.
171. Van der Voorde J, Vanheel B (1997) Influence of cytochrome P-450 inhibitors on endothelium-dependent nitro-L-arginine-resistant relaxation and cromakalim-induced relaxation in rat mesenteric arteries. *J Cardiovas Pharmacol* 29: 827-832.
172. Iftinca M, Waldron GJ, Triggie CR, Cole WC (2001) State-dependent block of rabbit vascular smooth muscle delayed rectifier and Kv1.5 channels by inhibitors of cytochrome P450-dependent enzymes. *J Pharmacol Exp Ther* 298: 718-728.
173. Yuan XJ, Tod ML, Rubin LJ, Blaustein MP (1995) Inhibition of cytochrome P-450 reduces voltage-gated K⁺ currents in pulmonary arterial myocytes. *Am J Physiol* 268: C259-C270.
174. Muthalif MM, Karzoun NA, Gaber L, Khandekar Z, Benter IF, et al. (2000) Angiotensin II-induced hypertension: contribution of Ras GTPase/mitogen-activated protein kinase and cytochrome P450 metabolites. *Hypertension* 36: 604-609.
175. Alonso-Galicia M, Maier KG, Greene AS, Cowley AW, Roman RJ (2002) Role of 20-hydroxyecosatetraenoic acid in the renal and vasoconstrictor actions of angiotensin II. *Am J Physiol Regul Integr Comp Physiol* 283: R60-R68.
176. Lima R, Yanes LL, Davis DD, Reckelhoff JF (2013) Roles played by 20-HETE, angiotensin II and endothelin in mediating the hypertension in aging female spontaneously hypertensive rats. *Am J Physiol Regul Integr Comp Physiol* 304: R248-R251.
177. Yousif MHM, Benter IF, Dunn KMJ, Dahly-Vernon AJ, Akhtar S, et al. (2009) Role of 20-hydroxyecosatetraenoic acid in altering vascular reactivity in diabetes. *Auton Autacoid Pharmacol* 29: 1-12.
178. Yanes LL, Lima R, Moulana M, Romero DG, Yuan K, et al. (2011) Postmenopausal hypertension: role of 20-HETE. *Am J Physiol Regul Integr Comp Physiol* 300: R1543-1548.
179. Wang MH, Zand BA, Nasjletti A, Laniado-Schwartzman M (2002) Renal 20-hydroxyecosatetraenoic acid synthesis during pregnancy. *Am J Physiol Regul Integr Comp Physiol* 282: R383-R389.
180. Llinás MT, Alexander BT, Capparelli MF, Carroll MA, Granger JP (2004) Cytochrome P-450 inhibition attenuates hypertension induced by reductions in uterine perfusion pressure in pregnant rats. *Hypertension* 43: 623-628.
181. Benter IF, Yousif MHM, Canatan H, Akhtar S (2005) Inhibition of Ca²⁺/calmodulin-dependent protein kinase II, RAS-GTPase and 20-hydroxyecosatetraenoic acid attenuates the development of diabetes-induced vascular dysfunction in the rat carotid artery. *Pharmacol Res* 52: 252-257.
182. Vasudevan H, Yuen VG, McNeill JH (2012) Testosterone-dependent increase in blood pressure is mediated by elevated Cyp4A expression in fructose-fed rats. *Mol Cell Biochem* 359: 409-418.
183. Williams JM, Sarkis A, Lopez B, Ryan RP, Flasch AK, et al. (2007) Elevations in renal interstitial hydrostatic pressure and 20-hydroxyecosatetraenoic acid contribute to pressure natriuresis. *Hypertension* 49: 687-694.
184. Fernandez MMF, Gonzalez D, Williams JM, Roman RJ, Nowicki S (2012) Inhibitors of 20-hydroxyecosatetraenoic acid (20-HETE) formation attenuate the natriuretic effect of dopamine. *Eur J Pharmacol* 686: 97-103.
185. Oyekan A, McAward K, Conetta J, Rosenfeld L, McGiff J (1999) Endothelin-1 and CYP450 arachidonate metabolites interact to promote tissue injury in DOCA-salt hypertension. *Am J Physiol Regul Integr Comp Physiol* 276: R766-R775.
186. Kuczeriszka M, Badzyńska B, Kompanowska-Jezierska E (2006) Cytochrome P-450 monooxygenases in control of renal haemodynamics and arterial pressure in anesthetized rats. *J Physiol Pharmacol* 57: 179-185.
187. Chábová CV, Kramer HJ, Vanecková I, Vernerová Z, Eis V, et al. (2007) Effects of chronic cytochrome P-450 inhibition on the course of hypertension and end-organ damage in Ren-2 transgenic rats. *Vascul Pharmacol* 47: 145-159.
188. Kauser K, Clark JE, Masters BS, Ortiz de Montellano PR, Ma YH, et al. (1991) Inhibitors of cytochrome P-450 attenuate the myogenic response of dog renal arcuate arteries. *Circ Res* 68: 1154-1163.
189. Orozco LD, Liu H, Perkins E, Johnson DA, Chen BB, et al. (2013) 20-Hydroxyecosatetraenoic acid inhibition attenuates balloon injury-induced neointima formation and vascular remodeling in rat carotid arteries. *J Pharmacol Exp Ther* 346: 67-74.
190. Amaral SL, Maier KG, Schippers DN, Roman RJ, Greene AS (2003) CYP4A metabolites of arachidonic acid and VEGF are mediators of skeletal muscle angiogenesis. *Am J Physiol Heart Circ Physiol* 284: H1528-H1535.
191. El Azher MA, Havet N, Singer M, Dumarey C, Touqui L (2000) Inhibition by unsaturated fatty acids of type II secretory phospholipase A2 synthesis in guinea-pig alveolar macrophages evidence for the eicosanoid-independent pathway. *Eur J Biochem* 267: 3633-3639.
192. Saxena NC (2000) Inhibition of GABA(A) receptor (GABAR) currents by arachidonic acid in HEK 293 cells stably transfected with alpha1beta2gamma2 GABAR subunits. *Pflugers Arch* 440: 380-392.
193. Nieves D, Moreno JJ (2006) Hydroxyecosatetraenoic acids released through the cytochrome P-450 pathway regulate 3T6 fibroblast growth. *J Lipid Res* 47: 2681-2689.
194. Feddersen CO, Chang S, Czartalomna J, Voelkel NF (1990) Arachidonic acid causes cyclooxygenase-dependent and -independent pulmonary vasodilation. *J Appl Physiol* 68: 1799-1808.
195. Chang SW, Dutton D, Wang HL, He LS, Stearns R, et al. (1992) Intact lung cytochrome P-450 is not required for hypoxic pulmonary vasoconstriction. *Am J Physiol* 263: L446-L453.
196. Weissmann N, Seeger W, Conzen J, Kiss L, Grimminger F (1998) Effects of arachidonic acid metabolism on hypoxic vasoconstriction in rabbit lungs. *Eur*

- J Pharmacol 356: 231-237.
197. Cooper PR, Mesaros AC, Zhang J, Christmas P, Stark CM, et al. (2010) 20-HETE mediates ozone-induced, neutrophil-independent airway hyper-responsiveness in mice. *PLoS ONE* 5: e10235.
198. Dubey RK, Gillespie DG, Zacharia LC, Rosselli M, Imthurn B, et al. (2002) Methoxyestradiols mediate the antimitogenic effects of locally applied estradiol on cardiac fibroblast growth. *Hypertension* 39: 412-417.
199. Dubey RK, Gillespie DG, Keller PJ, Imthurn B, Zacharia LC, et al. (2002) Role of methoxyestradiols in the growth inhibitory effects of estradiol on human glomerular mesangial cells. *Hypertension* 39: 418-424.
200. Barchiesi F, Jackson EK, Gillespie DG, Zacharia LC, Fingerie J, et al. (2002) Methoxyestradiols mediate estradiol-induced antimitogenesis in human aortic SMCs. *Hypertension* 39: 874-879.
201. Dubey RK, Gillespie DG, Zacharia LC, Barchiesi F, Imthurn B, et al. (2003) CYP450- and COMT-derived estradiol metabolites inhibit activity of human coronary artery SMCs. *Hypertension* 41: 807-813.
202. Dubey RK, Jackson EK, Gillespie DG, Rosselli M, Barchiesi F, et al. (2005) Cytochromes 1A1/1B1- and catechol-O-methyltransferase-derived metabolites mediate estradiol-induced antimitogenesis in human cardiac fibroblast. *J Clin Endocrinol Metab* 90: 247-255.
203. Zhang Z, Zhu C, Zhang X, Wan Y, Song J (2011) Dual effects of estrogen on vascular smooth muscle cells: receptor-mediated proliferative vs. metabolite-induced pro-senescent actions. *Steroids* 76: 309-316.
204. Gui Y, Zheng XL, Zheng J, Walsh MP (2008) Inhibition of rat aortic smooth muscle contraction by 2-methoxyestradiol. *Am J Physiol Heart Circ Physiol* 295: H1935-H1942.
205. Miyamoto H, Matsueda S, Moritsuka A, Shimokawa K, Hirata H, et al. (2015) Evaluation of hypothermia on the in vitro metabolism and binding and in vivo disposition of midazolam in rats. *Biopharm Drug Dispos* 36: 481-489.
206. Poole A, Jones RB, Pritchard D, Catto L, Leonard T (1989) In vitro accumulation of thyroid hormones by cultured rat hepatocytes and the biliary excretion of iodothyronines in rats treated with a novel histamine H2-receptor antagonist. *Toxicology* 59: 23-36.
207. Poole A, Pritchard D, Jones RB, Catto L, Leonard T (1990) In vivo biliary excretion and in vitro cellular accumulation of thyroxine by rats or cultured rat hepatocytes treated with a novel histamine H1-receptor antagonist. *Arch Toxicol* 64: 474-481.
208. Behne M, Uchida Y, Seki T, Ortiz de Montellano PR, Elias PM, et al. (2000) Omega-hydroxyceramides are required for corneocyte lipid envelope (CLE) formation and normal epidermal permeability barrier function. *J Invest Dermatol* 114: 185-192.
209. Liu Y, Glatt H (2010) Human cytochrome P450 2E1 and sulfotransferase 1A1 coexpressed in Chinese hamster V79 cells enhance spontaneous mutagenesis. *Environ Mol Mutagen* 51: 23-30.
210. Lee SH, Lee SM (2005) Suppression of hepatic cytochrome p450-mediated drug metabolism during the late stage of sepsis in rats. *Shock* 23: 144-149.
211. Zhou M, Maitra SR, Wang P (2008) The potential role of transcription factor aryl hydrocarbon receptor in downregulation of hepatic cytochrome P-450 during sepsis. *Int J Mol Med* 21: 423-428.
212. Jacob A, Zhou M, Wu R, Wang P (2009) The role of hepatic cytochrome P-450 in sepsis. *Int J Clin Exp Med* 2: 203-211.
213. Crawford JH, Yang S, Zhou M, Simms HH, Wang P (2004) Down-regulation of hepatic CYP1A2 plays an important role in inflammatory responses in sepsis. *Crit Care Med* 32: 502-508.
214. Morgan ET (2005) A role for one or more P450 enzymes in the response to sepsis. *Crit Care Med* 33: 268-269.
215. Carcillo J, Korzekwa K, Jones G, Parise R, Gillespie D, et al. (1998) The cytochrome P450 suicide inhibitor, 1-aminobenzotriazole, sensitizes rats to zymosan-induced toxicity. *Res Commun Molec Pathol Pharmacol* 102: 57-68.
216. Mico BA, Federowicz DA, Burak E, Swagdzis JR (1987) In vivo inhibition of phenacetin oxidation by suicide substrate 1-aminobenzotriazole. *Drug Metab Disp* 15: 274-276.
217. Mugford CA, Tarloff JB (1995) Contribution of oxidation and deacetylation to the bioactivation of acetaminophen in vitro in liver and kidney from male and female Sprague-Dawley rats. *Drug Metab Dispos* 23: 290-294.
218. Mugford CA, Tarloff JB (1997) The contribution of oxidation and deacetylation to acetaminophen nephrotoxicity in female Sprague-Dawley rats. *Toxicol Lett* 93: 15-22.
219. Deleve LD, Wang X, Kaplowitz N, Shulman HM, Bart JA, et al. (1997) Sinusoidal endothelial cells as a target for acetaminophen toxicity. Direct action versus requirement for hepatocyte activation in different mouse strains. *Biochem Pharmacol* 53: 1339-1345.
220. Miyakawa K, Albee R, Letzig LG, Lehner AF, Scott MA, et al. (2015) A cytochrome P450-independent mechanism of acetaminophen-induced injury in cultured mouse hepatocytes. *J Pharmacol Exp Therap* 354: 230-237.
221. Smith RD, Kehrer JP (1991) Cooxidation of cyclophosphamide as an alternative pathway for its bioactivation and lung toxicity. *Cancer Res* 51: 542-548.
222. Huang Z, Waxman DJ (2001) Modulation of cyclophosphamide-based cytochrome P450 gene therapy using liver P450 inhibitors. *Cancer Gene Ther* 8: 450-458.
223. Chen J, Mannargudi BM, Xu L, Uetrecht J (2008) Demonstration of the metabolic pathway responsible for nevirapine-induced skin rash. *Chem Res Toxicol* 21: 1862-1870.
224. Sharma AM, Li Y, Novalen M, Hayes MA, Uetrecht J (2012) Bioactivation of nevirapine to a reactive quinone methide: implications for liver injury. *Chem Res Toxicol* 25: 1708-1719.
225. Nishiya T, Kato M, Suzuki T, Maru C, Kataoka H, et al. (2008) Involvement of cytochrome P450-mediated metabolism in tienilic acid hepatotoxicity in rats. *Toxicol Lett* 183: 81-89.
226. Jinno N, Tagashira M, Tsurui K, Yamada S (2014) Contribution of cytochrome P450 and UGT-glucuronosyltransferase to the metabolism of drugs containing carboxylic acid groups: risk assessment of acylglucuronides using human hepatocytes. *Xenobiotica* 44: 677-686.
227. Yang X, Atkinson K, Di L (2016) Novel cytochrome P450 reaction phenotyping for low-clearance compounds using the hepatocyte relay method. *Drug Metab Dispos* 44: 460-465.
228. Matsunaga N, Kaneko N, Staub AY, Nakanishi T, Nunoya K, et al. (2016) Analysis of the metabolic pathway of bosentan and of the cytotoxicity of bosentan metabolites based on a quantitative modeling of metabolism and transport in sandwich-cultured human hepatocytes. *Drug Metab Dispos* 44: 16-27.
229. Tong V, Chang TKH, Chen J, Abbott FS (2003) The effect of valproic acid on hepatic and plasma levels of 15-F2t-isoprostane in rats. *Free Rad Biol Med* 34: 1435-1446.
230. Surendrass J, Chang TKH, Abbott FS (2012) Assessment of the role of in situ generated (E)-2,4-diene-valproic acid in the toxicity of valproic acid and (E)-2-ene-valproic acid in sandwich-cultured rat hepatocytes. *Toxicol Appl Pharmacol* 264: 413-422.
231. Kazmi F, Barbara JE, Yerino P, Parkinson A (2015) A long-standing mystery solved: the formation of 3-hydroxydesloratadine is catalyzed by CYP2C8 but prior glucuronidation of desloratadine by UDP-glucuronosyltransferase 2B10 is an obligatory requirement. *Drug Metab Dispos* 43: 523-533.
232. Takagi M, Sanoh S, Santho M, Ejiri Y, Kotake Y, et al. (2016) Detection of metabolic activation leading to drug-induced phospholipidosis in rat hepatocyte spheroids. *J Toxicol Sci* 41: 155-164.
233. Hammond AH, Fry JR (1994) Toxicity of precocene II in rat hepatocyte cultures: effects of serum and culture time. *Toxicol Lett* 70: 337-342.
234. Hammond AH, Garle MJ, Fry JR (1995) Mechanism of toxicity of precocene II in rat hepatocyte cultures. *J Biochem Toxicol* 10: 265-273.
235. Ly VT, Brock B (2011) Effects of CYP inhibitors on precocene I metabolism and toxicity in rat liver slices. *Chem Biol Interact* 193: 109-118.
236. Huijzer JC, Adams JD, Jaw JY, Yost GS (1989) Inhibition of 3-methylindole bioactivation by the cytochrome P450 suicide substrates 1-aminobenzotriazole and-methylbenzylaminobenzotriazole. *Drug Metab Disp* 17: 37-42.
237. Nichols WK, Larson DN, Yost GS (1990) Bioactivation of 3-methylindole by isolated rabbit lung cells. *Toxicol Appl Pharmacol* 105: 264-270.
238. Thornton-Manning JR, Nichols WK, Manning BW, Skiles GL, Yost GS (1993) Metabolism and bioactivation of 3-methylindole by Clara cells, alveolar

- macrophages, and subcellular fractions from rabbit lungs. *Toxicol Appl Pharmacol* 122: 182-190.
239. Nichols WK, Mehta R, Skordos K, Macé K, Pfeifer AM, et al. (2003) 3-Methylindole-induced toxicity to human bronchial epithelial cell lines. *Toxicol Sci* 71: 229-236.
240. Weems JM, Yost GS (2010) 3-Methylindole metabolites induce lung CYP1A1 and CYP2F1 enzymes by AhR and non-AhR mechanisms, respectively. *Chem Res Toxicol* 23: 696-704.
241. El Hadri L, Bhanas B, Ghanayem BI (2005) Comparative metabolism of methacrylonitrile and acrylonitrile to cyanide using cytochrome P4502E1 and microsomal epoxide hydrolase-null mice. *Toxicol Appl Pharmacol* 205: 116-125.
242. Adler ID, Baumgartner A, Gonda H, Friedman MA, Skerhut M (2000) 1-Aminobenzotriazole inhibits acrylamide-induced dominant lethal effects in spermatids of male mice. *Mutagenesis* 15: 133-136.
243. Park J, Kamendulis LM, Friedman MA, Klaunig JE (2002) Acrylamide-induced cellular transformation. *Toxicol Sci* 65: 177-183.
244. Lafferty JS, Kamendulis LM, Kaster J, Jiang J, Klaunig JE (2004) Subchronic acrylamide treatment induces a tissue-specific increase in DNA synthesis in the rat. *Toxicol Lett* 154: 95-103.
245. Zhang H, Kamendulis LM, Klaunig JE (2002) Mechanisms for the induction of oxidative stress in Syrian hamster embryo cells by acrylonitrile. *Toxicol Sci* 67: 247-255.
246. Pu X, Kamendulis LM, Klaunig JE (2006) Acrylonitrile-induced oxidative DNA damage in rat astrocytes. *Environ Mol Mutagen* 47: 631-638.
247. Saldaña-Ruiz S, Hernández-Mir G, Sedó-Cabezón L, Cutillas B, Llorens J (2012) Vestibular toxicity of cis-2-pentenitrile in the rat. *Toxicol Lett* 211: 281-288.
248. Boadas-Vaello P, Jover E, Saldaña-Ruiz S, Soler-Martin C, Chabbert C, et al. (2009) Allylnitrile metabolism by CYP2E1 and other CYPs leads to distinct lethal and vestibulotoxic effects in the mouse. *Toxicol Sci* 107: 461-472.
249. Constan AA, Sprankle CS, Peters JM, Kedderis GL, Everitt JI, et al. (1999) Metabolism of chloroform by cytochrome P450 2E1 is required for induction of toxicity in the liver, kidney, and nose of male mice. *Toxicol Appl Pharmacol* 160: 120-126.
250. Lind RC, Begay CK, Gandolfi AJ (2000) Hepatoprotection by dimethyl sulfoxide. III. Role of inhibition of the bioactivation and covalent bonding of chloroform. *Toxicol Appl Pharmacol* 166: 145-150.
251. Garner CE, Sumner SCJ, Davis JG, Burgess JP, Yueh Y, et al. (2006) Metabolism and disposition of 1-bromopropane in rats and mice following inhalation or intravenous administration. *Toxicol Appl Pharmacol* 215: 23-36.
252. Zong C, Garner CE, Huang C, Zhang X, Zhang L, et al. (2016) Preliminary characterization of a murine model for 1-bromopropane neurotoxicity: Role of cytochrome P450. *Toxicol Lett* 258: 249-258.
253. Nichols WK, Covington MO, Seiders CD, Safiullah S, Yost GS (1992) Bioactivation of halogenated hydrocarbons by rabbit pulmonary cells. *Pharmacol Toxicol* 71: 335-339.
254. Kim D, Ghanayem BI (2006) Comparative metabolism and disposition of trichloroethylene in Cyp2e1-/- and wild-type mice. *Drug Metab Dispos* 34: 2020-2027.
255. Shiba D, Shimamoto N (1999) Attenuation of endogenous oxidative stress-induced cell death by cytochrome P450 inhibitors in primary cultures of rat hepatocytes. *Free Rad Biol Med* 27: 1019-1026.
256. Bradford BU, Kono H, Isayama F, Kosyk O, Wheeler MD, et al. (2005) Cytochrome P450 2E1, but not nicotinamide adenine dinucleotide phosphate oxidase, is required for ethanol-induced oxidative DNA damage in rodent liver. *Hepatology* 41: 336-344.
257. Nan Y, Wu W, Fu N, Liang B, Wang R, et al. (2009) Antioxidants vitamin E and 1-aminobenzotriazole prevent experimental non-alcoholic steatohepatitis in mice. *Scand J Gastroenterol* 44: 1121-1131.
258. Chiu PY, Mak DHF, Poon MKT, Ko KM (2005) Role of cytochrome P-450 in schisandrin B-induced antioxidant and heat shock responses in mouse liver. *Life Sci* 77: 2887-2895.
259. Chen N, Chiu PY, Leung HY, Ko KM (2012) Cytochrome P-450-catalyzed reactive oxygen species production mediates the (-)-schisandrin B-induced glutathione and heat shock responses in H9c2 cardiomyocytes. *Indian J Pharmacol* 44: 204-209.
260. Dostalek M, Brooks JD, Hardy KD, Milne GL, Moore MM, et al. (2007) In vivo oxidative damage is associated with barbiturate response but not other cytochrome P450 inducers. *Molec Pharmacol* 72: 1419-1424.
261. Sun X, Ai M, Wang Y, Shen S, Gu Y, et al. (2013) Selective induction of tumor cell apoptosis by a novel P450-mediated reactive oxygen species (ROS) inducer methyl 3-(4-nitrophenyl)propionate. *J Biol Chem* 288: 8826-8837.
262. Kadiiska MB, De Costa KS, Mason RP, Mathews JM (2000) Reduction of 1,3-diphenyl-1-triazene by rat hepatic microsomes, by cecal microflora, and in rats generates the phenyl radical metabolite: an ESR spin trapping investigation. *Chem Res Toxicol* 13: 1082-1086.
263. Ress NB (2002) NTP technical report on the metabolism, toxicity and predicted carcinogenicity of diazoaminobenzene. *Toxic Rep Ser* 73: 1-23.
264. Dikalova AE, Kadiiska MB, Mason RP (2001) An in vivo ESR spin-trapping study: Free radical generation in rats from formate intoxication - role of the Fenton reaction. *Proc Natl Acad Sci USA* 98: 13549-13553.
265. Whelan HT, Bajic DM, Karlovits SM, Houle JM, Kindwall EP (1998) Use of cytochrome-P450 monooxygenase 2E1 isozyme inhibitors for delay seizures caused by central nervous system oxygen toxicity. *Aviat Space Environ Med* 69: 480-485.
266. Muta K, Fukami T, Nakajima M (2015) A proposed mechanism for the adverse effects of acebutolol: CES2 and CYP2C19-mediated metabolism and antinuclear antibody production. *Biochem Pharmacol* 98: 659-670.
267. Shimizu S, Atsumi R, Itokawa K, Iwasaki M, Aoki T, et al. (2009) Metabolism-dependent hepatotoxicity of amodiaquine in glutathione-depleted mice. *Arch Toxicol* 83: 701-707.
268. Lillibridge JH, Amore BM, Slattery JT, Kalhorn TF, Nelson SD, et al. (1996) Protein-reactive metabolites of carbamazepine in mouse liver microsomes. *Drug Metab Dispos* 24: 509-514.
269. Wu Y, Sanderson JP, Farrell J, Drummond NS, Hanson A, et al. (2006) Activation of T cells by carbamazepine and carbamazepine metabolites. *J Allergy Clin Immunol* 118: 233-241.
270. Zhai Y, Wang L, Yang F, Feng G, Feng S, et al. (2016) The mechanism and risk factors of clopidogrel-induced liver injury. *Drug Chem Toxicol* 39: 367-374.
271. Bumpus NN (2011) Efavirenz and 8-hydroxyefavirenz induce cell death via a JNK- and BimEL-dependent mechanism in primary human hepatocytes. *Toxicol Appl Pharmacol* 257: 227-234.
272. Shi Q, Yang X, Greenhaw J, Salminen WF (2011) Hepatic cytochrome P450s attenuate the cytotoxicity induced by leflunomide and its active metabolite A77 1726 in primary cultured rat hepatocytes. *Toxicol Sci* 122: 579-586.
273. Seah QM, New LS, Chan EY, Boelsterli UA (2008) Oxidative bioactivation and toxicity of leflunomide in immortalized human hepatocytes and kinetics of the non-enzymatic conversion to its major metabolite, A77-1726. *Drug Metab Lett* 2: 153-157.
274. Graham EE, Walsh RJ, Hirst CM, Maggs JL, Martin S, et al. (2008) Identification of the thiophene ring of methapyrilene as a novel bioactivation-dependent hepatic toxicophore. *J Pharmacol Exp Therap* 326: 657-671.
275. Priestley CC, Regan S, Park BK, Williams DP (2011) The genotoxic potential of methapyrilene using the alkaline Comet assay in vitro and in vivo. *Toxicology* 290: 249-257.
276. Shimizu S, Atsumi R, Nakazawa T, Izumi T, Sudo K, et al. (2011) Ticlopidine-induced hepatotoxicity in a GSH-depleted rat model. *Arch Toxicol* 85: 347-353.
277. Li AP, Uzgaré A, Yumiko S, Laforge YS (2012) Definition of metabolism-dependent xenobiotic toxicity with co-cultures of human hepatocytes and mouse 3T3 fibroblasts in the novel integrated discrete multiple organ co-culture (IdMOC) experimental system: results with model toxicants aflatoxin B1. *Chem Biol Interact* 199: 1-8.
278. Li AF, Shen GL, Jiao SY, Li H, Wang Q (2012) Metabolic detoxification of bakuchiol is mediated by cytochrome P450 enzymes in human liver microsomes. *Beijing Da Xue Xue Bao* 44: 431-436.
279. Reilly CA, Ehlhardt WJ, Jackson IA, Kulanthaivel P, Mutlib AE, et al. (2003) Metabolism of capsaicin by cytochrome P450 produces novel

- dehydrogenated metabolites and decreases cytotoxicity to lung and liver cells. *Chem Res Toxicol* 16: 336-349.
280. Yeh SL, Wu SH (2006) Effects of quercetin on beta-apo-8'-carotenal-induced DNA damage and cytochrome P1A2 expression in A549 cells. *Chem Biol Interact* 163: 199-206.
281. Beier RC, Norman JO, Irvin TR, Witzel DA (1987) Microsomal activation of constituents of white snakeroot (*Eupatorium rugosum* Houtt) to form toxic products. *Am J Vet Res* 48: 583-585.
282. Marczylo T, Ioannides C (1999) Evidence for the presence of a microsomal NADH-dependent enzyme system that can bioactivate aromatic amines in the liver of rats and mice. *Toxicology* 134: 127-141.
283. Jiang H, Lai Y, Hu K, Wei Q, Liu Y (2014) Human CYP2E1-dependent and human sulfotransferase 1A1-modulated induction of micronuclei by benzene and its hydroxylated metabolites in Chinese hamster V79-derived cells. *Mutat Res* 770: 37-44.
284. Bolton JL, Thompson JA (1991) Oxidation of butylated hydroxytoluene to toxic metabolites. Factors influencing hydroxylation and quinone methide formation by hepatic and pulmonary microsomes. *Drug Metab Dispos* 19: 467-472.
285. van Ravenzwaay B, Gamer AO, Leibold E, Kaufmann W (2003) Effect of cytochrome P450 inhibition of tetrahydrofuran-induced hepatocellular proliferation in female mice. *Arch Toxicol* 77: 459-464.
286. Mann KK, Matulka RA, Hahn ME, Trombino AF, Lawrence BP, et al. (1999) The role of polycyclic aromatic hydrocarbon metabolism in dimethylbenz[a]anthracene-induced pre-B lymphocyte apoptosis. *Toxicol Appl Pharmacol* 161: 10-22.
287. Zhang C, Lai Y, Jin G, Glatt H, Wei Q, et al. (2016) Human CYP2E1-dependent mutagenicity of mono- and dichlorobiphenyls in Chinese hamster (V79)-derived cells. *Chemosphere* 144: 1908-1915.
288. Nyarko AK, Kellner-Weibel GL, Harvison PJ (1997) Cytochrome P450-mediated metabolism and nephrotoxicity of N-(3,5-dichlorophenyl)succinimide in Fischer 344 rats. *Fundam Appl Toxicol* 37: 117-124.
289. Henesey CM, Harvison PJ (2002) Renal damage, metabolism and covalent binding following administration of the nephrotoxicant N-(3,5-dichlorophenyl)succinimide to male Fischer 344 rats. *Toxicology* 170: 187-200.
290. Crincoli CM, Patel NN, Tchao R, Harvison PJ (2008) Role of biotransformation in 3-(3,5-dichlorophenyl)-2,4-thiazolidinedione-induced hepatotoxicity. *Toxicology* 250: 100-108.
291. Deng H, Gao H, Liu Y (2011) Biotransformation enzyme-dependent formation of micronucleus and multinuclei in cell line V79-hCYP2E1-hSULT1A1 by 2-nitropropane and N-nitrosodimethylamine. *Mutat Res* 726: 84-87.
292. Yeh SL, Wang WY, Huang CS, Hu ML (2006) Flavonoids suppress the enhancing effect of beta-carotene on DNA damage induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in A549 cells. *Chem Biol Interact* 160: 175-182.
293. King LC, Kohan MJ, Brooks L, Nelson GB, Ross JA, et al. (2001) An evaluation of the mutagenicity, metabolism, and DNA adduct formation of 5-nitrobenzo[b]naphtho[2,1-d]thiophenes. *Chem Res Toxicol* 14: 661-671.
294. Zhang D, Flint O, Wang L, Gupta A, Westhouse RA, et al. (2012) Cytochrome P450 11A1 bioactivation of a kinase inhibitor in rats: use of radioprofiling, modulation of metabolism, and adrenocortical cell lines to evaluate adrenal toxicity. *Chem Res Toxicol* 25: 556-571.
295. Jackson JA, Birnbaum LS, Diliberto JJ (1998) Effects of age, sex, and pharmacologic agents on the biliary elimination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in F344 rats. *Drug Metab Dispos* 26: 714-719.
296. Vyas PM, Roychowdhury S, Khan FD, Prinsinzano TE, Lamba J, et al. (2006) Enzyme-mediated protein haptenation of dapsone and sulfamethoxazole in human keratinocytes: I. Expression and role of cytochrome P450s. *J Pharm Exp Therap* 319: 488-496.
297. Aloysius H, Tong VW, Yabut J, Bradley SA, Shang J, et al. (2012) Metabolic activation and major protein target of a 1-benzyl-carboxyazetidine sphingosine-1-phosphate-1 receptor agonist. *Chem Res Toxicol* 25: 1412-1422.
298. Zhou L, Pang X, Xie C, Zhong D, Chen X (2015) Chemical and enzymatic transformations of nimesulide to GSH conjugates through reductive and oxidative mechanisms. *Chem Res Toxicol* 28: 2267-2277.
299. Shao R, Ring SC, Tarloff JB (1997) Coincubation of rat renal proximal tubules with hepatic subcellular fractions potentiates the effects of para-aminophenol. *Fundam Appl Toxicol* 39: 101-108.
300. Dierickx PJ (1999) CYP1/2 activation and glutathione-dependent cytotoxicity of four pesticides in Hep G2 and Fa32 cells. *Toxicol in Vitro* 13: 779-783.
301. Salaün JP, Simon A, Durst F, Reich NO, Ortiz de Montellano PR (1988) Differential inactivation of plant lauric acid omega- and in-chain hydroxylases by terminally unsaturated fatty acids. *Arch Biochem Biophys* 260: 540-545.
302. Pflugmacher S, Sandermann H Jr (1998) Cytochrome P450 monooxygenases for fatty acids and xenobiotics in marine macroalgae. *Plant Physiol* 117: 123-128.
303. Menting JGT, Scopes RK, Stevenson TW (1994) Characterization of flavonoid 3',5'-hydroxylase in microsomal membrane fraction of petunia hybrida flowers. *Plant Physiol* 106: 633-642.
304. Aghofack-Nguemezi J, Fuchs C, Yeh SY, Huang FC, Hoffmann T, et al. (2011) An oxygenase inhibitor study in *Solanum lycopersicum* combined with metabolite profiling analysis revealed a potent peroxxygenase inactivator. *J Exp Bot* 62: 1313-1323.
305. Morgan JA, Shanks JV (1999) Inhibitor studies of tabersonine metabolism in *C. roseus* hairy roots. *Phytochemistry* 51: 61-68.
306. Werck-Reichhart D, Gabriac B, Teutsch H, Durst F (1990) Two cytochrome P450 isoforms catalyzing O-de-ethylation of ethoxycoumarin and ethoxylresorufin in higher plants. *Biochem J* 270: 729-735.
307. Cabanne F, Huby D, Gaillardon P, Scalla R, Durst F (1987) Effect of the cytochrome P450 inactivator 1-aminobenzotriazole on the metabolism of chlortoluron and isoproturon in wheat. *Pest Biochem Physiol* 28: 371-380.
308. McFadden JJ, Frear DS, Mansager ER (1989) Aryl hydroxylation of diclofop by a cytochrome P450 dependent monooxygenase from wheat. *Pest Biochem Physiol* 34: 92-100.
309. Sterling TM, Balke NE (1990) Bentazon uptake and metabolism by cultured plant cells in the presence of monooxygenase inhibitors and cinnamic acid. *Pest Biochem Physiol* 38: 66-75.
310. Gronwald JW, Connelly JA (1991) Effect of monooxygenase inhibitors on bentazon uptake and metabolism in maize cell suspension cultures. *Pest Biochem Physiol* 40: 284-294.
311. Letouzé A, Gasquez J (2003) Enhanced activity of several herbicide-degrading enzymes; a suggested mechanism responsible for multiple resistance in blackgrass (*Alopecurus myosuroides* Huds.). *Agronomie* 23: 601-608.
312. Moreland DE, Corbin FT, McFarland JE (1993) Effects of safeners on the oxidation of multiple substrates by grain sorghum microsomes. *Pest Biochem Physiol* 45: 43-53.
313. Aper J, Mechant E, Rubin B, Heyerick A, Callebaut G, et al. (2012) Absorption, translocation and metabolism of metamitron in *Chenopodium album*. *Pest Manag Sci* 68: 209-216.
314. Elmore MT, Brosnan JT, Armel GR, Kopsell DA, Best MD, et al. (2015) Cytochrome P450 inhibitors reduce creeping bentgrass (*Agrostis stolonifera*) tolerance to topramezone. *Plos One* 10: e0130947.
315. Zhai G, Lehmler HJ, Schnoor JL (2013) Inhibition of cytochromes P450 and the hydroxylation of 4-monochlorobiphenyl in whole poplar. *Environ Sci Technol* 47: 29-35.
316. Miranda CL, Henderson MC, Buhler DR (1998) Evaluation of chemicals as inhibitors of trout cytochrome P450s. *Toxicol App Pharmacol* 148: 237-244.
317. Hamm JT, Wilson BW, Hinton DE (2001) Increasing uptake and bioactivation with development positively modulate diazinon toxicity in early life stage medaka (*Oryzias latipes*). *Toxicol Sci* 61: 304-313.
318. Cashman, JR, Olsen LD, Young G, Bern H (1989) S-oxygenation of eptam in hepatic microsomes from fresh- and salt-water striped bass (*Morone saxatilis*). *Chem Res Toxicol* 2: 392-399.
319. Wang SP, Hu XX, Meng QM, Muhammad SA, Chen RR, et al. (2013) The involvement of several enzymes in methanol detoxification in *Drosophila melanogaster* adults. *Comp Biochem Physiol B Biochem Mol Biol* 166: 7-14.
320. Mathies A, Walker F, Buchenauer H (1999) Interference of selected fungicides, plant growth retardants as well as piperonyl butoxide and 1-aminobenzotriazole in trichothecene production of *Fusarium graminearum*

- (strain 4528) in vitro. *J Plant Dis Protect* 106: 198-212.
321. Ishikawa NK, Tahara S, Namatame T, Farooq A, Fukushi Y (2013) Use of cytochrome P450 inhibitors in studies of enokipodin biosynthesis. *Brazil J Microbiol* 44: 1285-1290.
 322. Thompson ML, Marriott R, Dowle A, Grogan G (2010) Biotransformation of beta-myrcene to geraniol by a strain of *Rhodococcus erythropolis* isolated by selective enrichment from hop plants. *Appl Microbiol Biotechnol* 85: 721-730.
 323. Mori T, Kondo R (2002) Oxidation of dibenzo-p-dioxin, dibenzofuran, biphenyl, and diphenyl ether by the white-rot fungus *Phlebia lindtneri*. *Appl Microbiol Biotechnol* 60: 200-205.
 324. Mori T, Kitano S, Kondo R (2003) Biodegradation of chloronaphthalenes and polycyclic aromatic hydrocarbons by the white-rot fungus *Phlebia lindtneri*. *Appl Microbiol Biotechnol* 61: 380-383.
 325. Hata T, Kawai S, Okamura H, Nishida T (2010) Removal of diclofenac and mefenamic acid by the white rot fungus *Phanerochaete sordida* YK-624 and identification of their metabolites after fungal transformation. *Biodegradation* 21: 681-689.
 326. Marco-Urrea E, Pérez-Trujillo M, Cruz-Morató C, Caminal G, Vicent T (2010) White-rot fungus-mediated degradation of the analgesic ketoprofen and identification of intermediates by HPLC-DAD-MS and NMR. *Chemosphere* 78: 478-481.
 327. Prieto A, Möder M, Rodil R, Adrian L, Marco-Urrea E (2011) Degradation of the antibiotics norfloxacin and ciprofloxacin by a white-rot fungus and identification of degradation products. *Bioresour. Technol* 102: 10987-10995.
 328. da Silva Celho-Moreira J, Bracht A, da Silva de Souza AC, Oliveira RF, Babeto de Sá-Nakanishi A, et al. (2013) Degradation of diuron by *Phanerochaete chrysosporium*: role of ligninolytic enzymes and cytochrome P450. *BioMed Res Internat* 2013: 1-9.
 329. Van Damme JD, Wong ET, Dettman H, Gray MR, Pickard MA (2003) Dibenzyl sulfide metabolism by white rot fungi. *Appl Environ Microbiol* 69: 1320-1324.
 330. Kanaly RA, Hur HG (2006) Growth of *Phanerochaete chrysosporium* on diesel fuel hydrocarbons at neutral pH. *Chemosphere* 63: 202-211.
 331. Marco-Urrea E, Parella T, Gabarrell X, Caminal G, Vicent T, et al. (2008) Mechanistics of trichloroethylene mineralization by the white-rot fungus *Trametes versicolor*. *Chemosphere* 70: 404-410.
 332. Marco-Urrea E, Pérez-Trujillo M, Caminal G, Vicent T (2009) Dechlorination of 1,2,3- and 1,2,4-trichlorobenzene by the white-rot fungus *Trametes versicolor*. *J Hazard Mater* 166: 1141-1147.
 333. Marco-Urrea E, Pérez-Trujillo M, Vicent T, Caminal G (2009) Ability of white-rot fungi to remove selected pharmaceuticals and identification of degradation products of ibuprofen by *Trametes versicolor*. *Chemosphere* 74: 765-772.
 334. Marco-Urrea E, Pérez-Trujillo M, Blázquez P, Vicent T, Caminal G (2010) Biodegradation of the analgesic naproxen by *Trametes versicolor* pellets and identification of intermediates using HPLC-DAD-MS and NMR. *Bioresour Technol* 101: 2159-2166.
 335. Marco-Urrea E, Pérez-Trujillo M, Criuz-Morató C, Caminal G, Vicent T (2010) Degradation of the drug sodium diclofenac by *Trametes versicolor* pellets and identification of some intermediates by NMR. *J Hazard Mater* 176: 836-842.
 336. Bezalel L, Hadar Y, Cerniglia CE (1997) Enzymatic mechanisms involved in phenanthrene degradation by the white rot fungus *Pleurotus ostreatus*. *Appl Environ Microbiol* 63: 2495-2501.
 337. Lisowska K, Diugonski J (2003) Concurrent corticosteroid and phenanthrene transformation by filamentous fungus *Cunninghamella elegans*. *J Steroid Biochem Mol Biol* 85: 63-69.
 338. Schlenk D, Bevers RJ, Vertino AM, Cerniglia CE (1994) P450 catalyzed S-oxidation of dibenzothiophene by *Cunninghamella elegans*. *Xenobiotica* 24: 1077-1083.
 339. Zhang D, Evans FE, Freeman JP, Duhart B, Cerniglia CE (1995) Biotransformation of amitriptyline by *Cunninghamella elegans*. *Drug Metab Disp* 23: 1417-1425.
 340. Holland HL, Morris TA, Nava PJ, Zabic M (1999) A new paradigm for biohydroxylation by *Beauveria bassiana* ATCC 7159. *Tetrahedron* 55: 7441-7460.
 341. Mitsukura K, Kondo Y, Yoshida T, Nagasawa T (2006) Regioselective hydroxylation of adamantane by *Streptomyces griseoplanus* cells. *Appl Microbiol Biotechnol* 71: 502-504.
 342. Rappert S, Li R, Kokova M, Antholz M, Nagorny S, et al. (2007) Degradation of 2,5-dimethylpyrazine by *Rhodococcus erythropolis* strain DP-45 isolated from a waste gas treatment plant of a fishmeal processing company. *Biodegradation* 18: 585-596.
 343. Hong CY, Kim HY, Lee SY, Kim SH, Lee SM, et al. (2013) Involvement of extracellular and intracellular enzymes of *Ceriporia* sp. ZLY-2010 for biodegradation of polychlorinated biphenyls (PCBs). *J Environ Sci Health A Tox Hazard Subst Environ Eng* 48: 1280-1291.