11 Molybdenum-Containing Hydroxylases

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11.1 SUMMARY

Molybdenum-containing enzymes represent a large and growing class of enzymes, which incorporate molybdenum, a transition metal as a biologically active cofactor. Nitrate reductase, sulfite oxidase, and the dimethylsulfoxide (DMSO) reductase family are of the most prominent in this class of enzymes. Of interest to drug research are the molybdo-flavoenzymes or molybdenum-containing hydroxylases, which not only need the molybdenum cofactor (MoCo) but also require a flavin cofactor for catalytic activity. These molybdenum-containing hydroxylases consist of mainly two enzymes from this class: aldehyde oxidase (AO) EC 1.2.3.1 and xanthine oxidoreductase (XOR) EC 1.2.3.2 [1], both enzymes being present in the cytosol of the cell and found in all vertebrates. AO and XOR catalyze both oxidation and reduction reactions, but the oxidation reaction has a higher degree of prevalence *in vivo*. The substrates for oxidation

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by AO are N-heterocycles, iminium ions, and aldehydes, while XOR primarily oxidizes N-heterocycles. The reduction reactions include reductions of *N*-oxides, sulfoxides, hydroxamic acids, as well as, ring opening due to reductive cleavage. XOR is quite conserved among species and has consistent enzyme kinetics among species for a specific XOR substrate, xanthine. This may be because of its critical role in the catabolism of purines, putatively essential in the metabolism of xanthine to the terminal catabolite uric acid, its potential influence on the development of the mammary gland, and XOR's role in the longevity of lactation. AO, on the other hand, has multiple homologs, a broad substrate pool, varying kinetic parameters with the same substrate among species, and no pathophysiology has been associated with its absence. Recently, emphasis has been put on AO in drug research, due in part to two apparent reasons. The first reason is a perceived need during the early drug design phase of drug research to mitigate cytochrome P450 (CYP) metabolism in new compounds being considered for testing in clinical trials in an effort to achieve the required exposure in humans. Replacement of an aromatic substituent that can be rapidly metabolized by P450 (e.g., phenyl or naphthyl) with an azaaromatic (e.g., pyridyl, pyrimidyl, quinolinyl) can lead to a decrease in P450 catalyzed metabolism but potentially introduce oxidation catalyzed by AO [2,3]. The second reason relates to the necessary use of azaaromaric chemical scaffolds in drug design, which happen to be AO substrates, but are required to achieve specific binding interactions at the site of a therapeutic target, such as specific kinases [4–6]. A switch in emphasis from P450 to AO moves drug researchers from a drug-metabolizing enzyme family for which there is much understood and numerous experiments and strategies already established to aid in drug design, cross-species correlations, predictions of human pharmacokinetics, drug–drug interactions, and so on to an enzyme family for which there is much less understood. For AO and XOR, there is a lesser understanding of abundance in tissues, little to no understanding of polymorphisms, no species that consistently translates to humans, and sheer complexity of the assembly of the enzymes. As science helps evolve our ability to incorporate these enzymes into drug research, strategies will become easier. In this chapter, a comprehensive summary of what is known from the peer-reviewed literature about these enzymes will be provided, with emphasis on the key understandings around the molybdenum-containing hydroxylases.

11.2 STRUCTURE AND FUNCTION OF MOLYBDENUM-CONTAINING HYDROXYLASES

Molybdenum-containing hydroxylases perform both oxidation and reduction reactions, but the oxidation reaction has a higher prevalence *in vivo*. Molybdenum is an indispensable component for this enzyme family and is necessary for catalysis along with flavin adenine dinucleotide. The oxidative hydroxylation catalyzed by the molybdenum is different from monooxygenases, in that, the molybdenum hydroxylase generates reducing equivalents during the hydroxylation process (e.g. not consume them), and the final source of the oxygen added to the substrate is from water, not molecular oxygen [7]. The reaction mechanism is discussed later in detail.

The primary physiological function of XOR has been understood for quite some time. XOR has a key role in the catabolism of purines, in which it oxidizes xanthine to hypoxanthine, then into the terminal catabolite uric acid [8,9]. The high kinetic efficiency of XOR to xanthine and hypoxanthine *in vitro* and *in vivo* provides evidence

that XOR would have significant activity toward drug-containing purine moieties. In mammals, XOR exists in two interconvertible forms, xanthine dehydrogenase and xanthine oxidase, the former predominates *in vivo*, although, the likely major role of XOR is purine catabolism. Of late, evidence has been growing that XOR has a broader biological role. One possible function stems from some observations that mice that are heterozygous for a loss of function in the XOR gene are not able to maintain lactation because of membrane defects in the milk fat droplets and mammary epithelial cell disruption [10]. This loss of function indicates that XOR may have a structural role in the development of the mammary gland. Higher levels of XOR have been observed in human colostrums, the fluid that is produced by mammals during the first two to three days post partum. This observation provided a hypothesis that newborn infants have a need for maternal XOR in the first few days of life, which may affect the absorption of dietary iron from the still developing gut of the infant [11,12]. Another possible function of XOR is antimicrobial properties by inhibiting bacterial growth *in vitro* via a nitric-oxide-dependent manner [13]. This antimicrobial role has extended to *in vivo*, where again infants that receive breast milk rich in XOR have less of a chance to develop gastroenteritis than those who are fed formula [14]. XOR has also been reported to generate superoxide radicals, which may also act beneficially as an antimicrobial agent [15]. The same beneficial aspect of superoxide radical formation has also been implicated in influencing the pathophysiology of alcohol-induced liver injury, ischemia reperfusion, endothial dysfunction, hypertension, and heart failure [16,17]. However, the relevance to pathophysiology is somewhat speculative due to the large abundance of enzyme oxygen radical scavengers, such as catalase and superoxide dismutase (SOD), which should minimize the clinical effect.

The physiological function of AO is not completely understood. What is most known about AO is that it is involved in the metabolism of xenobiotics-specifically those containing aldehydes, iminium ions, and N-heterocycles are typical substrates in the oxidation reaction; and nitro-containing compounds, *N*-oxides, sulfoxides, hydroxamic acids, 1,2-benzisoxazole, and 1,2-benzisothiazole are typical substrates of AO reduction. Therefore, while it is not known, it is reasonable to assume that potential endogenous substrates may be similar in structural nature. One possible path to understand the function of AO is to recognize the tissue distribution of AO and infer mechanism. Putting this thought into practice, it has been reported that both AO and XOR are found in the skin. The function of these two enzymes in skin is still unknown, but possible functions could include elimination of biogenic waste or break down products formed by ultraviolet radiation or environmental stress and homeostatic control of vitamins [1,18]. Another possible function of AO is lipid homeostasis and lipid deposition. These links were observed through silencing the *Aox1* gene using siRNA in a 3 T3-L1 mouse cell line. An arrest of the differentiation of the adipocyte was observed and a sequential diminished lipid accumulation resulted in the mice [19]. This observation was further supported in humans with a high level of AOX1 mRNA in human adipose tissue [19]. This proadipogenic link with AOX1 has been hypothesized to be a protein–protein interaction with the ABCA-1 lipid transporter [20,21] or, perhaps affecting the enzyme activity of PPARγ and reducing the hepatic levels of AO [22].

AO has been suggested to influence the pathophysiology of numerous clinical disorders, although no direct link has been drawn. Renal toxicity is one disease that has been reported to be affected by AO oxidation due to AO generated toxic metabolites [23].

Two other clinical disorders hypothesized to be impacted by AO are amyotrophic lateral sclerosis (ALS) and alcohol-induced liver injury. It has been reported that the cause of the pathogenesis in these diseases is similar to what is believed for XOR, where the formation of oxygen radicals contribute to the manifestation of the disease [16,24,25]. Similar to the shortcomings of XORs link to disease pathophysiology, the linking of AO and oxygen radical formation to disease may also suffer from the vast abundance of oxygen radical scavengers in the liver, as well as other tissues in the body.

11.2.1 Classification and Structural Features

Mammalian AO and XOR are putatively similar in structure and considerable extrapolation of the biochemical properties of XOR has been done for AO. Overall similarity between AOX1 and XOR has been stated to be ~50% based on amino acid sequence [26]. Furthermore, the other AO forms that were discovered in mouse are even more homologous to AOX1 [27–29]. The enzymes exist as homodimers of subunits of ~150 kDa. Each monomer consists of three main domains: an 85-kDa portion containing the MoCo and substrate-binding sites on the C-terminal end, a 40-kDa FAD-containing domain in the middle, and a 20-kDa domain at the N-terminus that contains the two Fe–S clusters [1,30] (Fig. 11.1). However, it should be noted that during catalysis the passage of electrons occurs from MoCo to Fe–S to FAD, indicating that the protein must assume a tertiary structure that facilitates this order of transfer. The FAD-containing domain is where the transfer of electrons to oxygen occurs; however, for XOR when it is in the dehydrogenase form, this transfer occurs to NAD^+.

Exploration of specific amino acids within the protein that contribute remarkably to enzyme function has been done. For example, AO activity has been shown to possess considerable variability across rat strains and among individual rats. These findings stimulated investigations into the amino acid sequence differences and revealed, for example, that conversion of glycine 110 to a serine was critical for high catalytic activity [31]. The glycine residue in the catalytically active enzyme is proximal to one of the Fe–S clusters, but its replacement with serine results in an inability to form a dimer, which is essential for catalytic activity [32]. Mutagenesis studies have yielded information regarding the roles of specific amino acid residues in substrate specificity. Mutation of human XOR to convert XOR to a protein that oxidizes AO substrates has been accomplished. Mutants in which either arginine 881 was altered to a methionine or glutamic acid 803 was altered to valine, diminished oxidation of hypoxanthine and xanthine, but activity toward benzaldehyde or cinnamaldehyde, was introduced [33]. Crystallography of the mutant XOR proteins revealed that large changes in conformation were not observed, and it was postulated that the arginine and glutamic acid side chains served in the formation of hydrogen bonds with the nitrogen and carbonyl oxygen atoms of purine substrates. The hydrophobic groups in the mutants no longer offer that H-bonding potential but instead permit the binding of hydrophobic aldehydes as substrates. Studies have also been done to compare substrate specificities among AO enzymes from different species. An investigation of rabbit/monkey chimeric proteins narrowed down the region of residues from 993 to 1088 in rabbit as important in cinchonidine oxidation. Replacement of individual amino acids of this region in monkey AO, which ordinarily metabolizes cinchonidine slowly, showed that a simple conversion of valine to alanine at position 1085 imparted activity, whereas the reverse for the rabbit enzyme (conversion of alanine 1081 to valine) removed the activity [34].

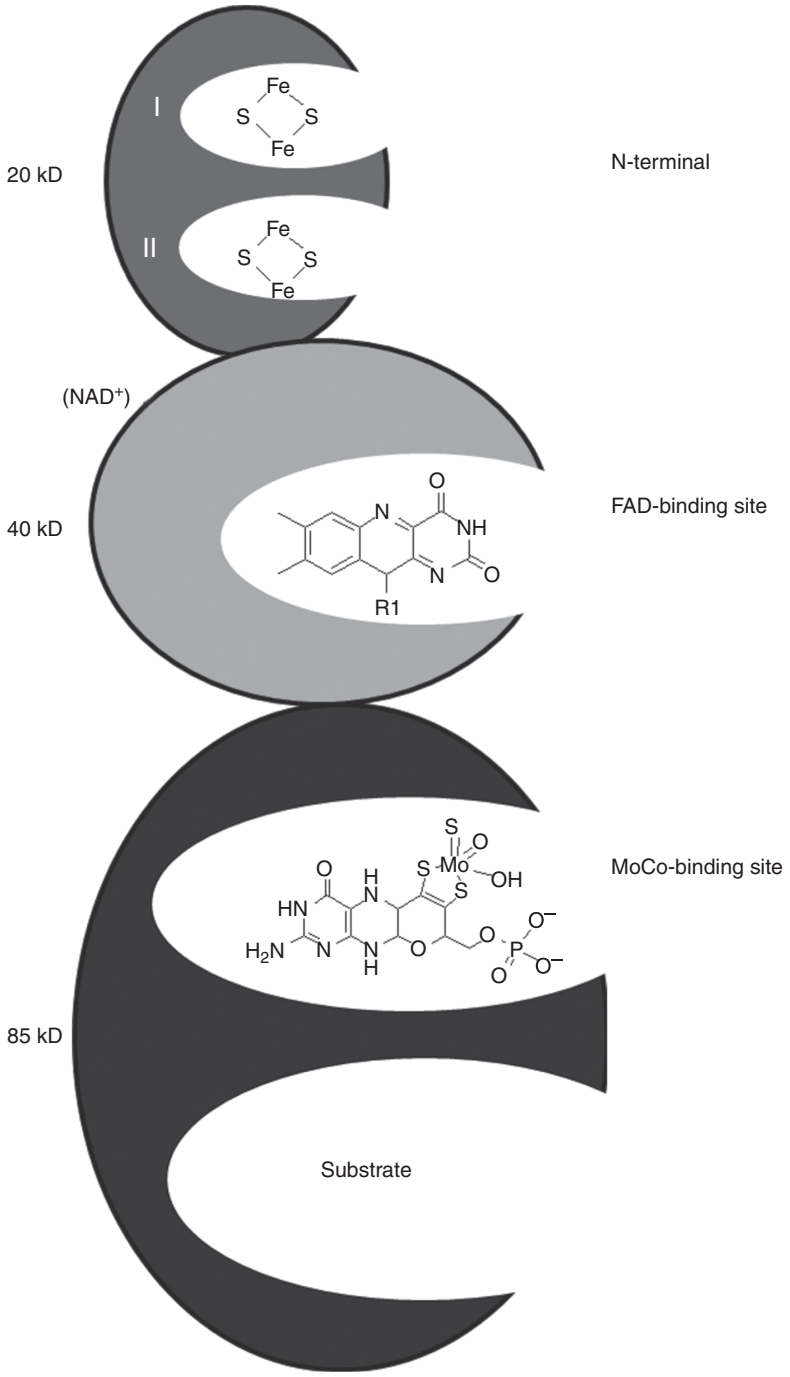


Figure 11.1 Monomeric unit of molybdenum-containing hydroxylases.

It is clear that the relatively recently acquired ability to express active AO to permit directed mutagenesis, coupled with the crystal structure of XOR (and perhaps AO in the future), will yield a much greater understanding of the roles of specific amino acids in the structure and activity of these enzymes [35–37].

While FAD and Fe–S clusters are common throughout enzymes, the MoCo is more unusual and merits a brief description. The structure of molybdopterin is shown in Fig. 11.2, and its elucidation was challenging because of its inherent instability on extraction from purified MoCo enzymes. Nevertheless, its structure elucidation represents a fascinating series of experiments and clever organic chemistry reasoning to solve this puzzle [38] (for a summary, see Leimkuehler *et al*. [39]). Much of the efforts to understand the biosynthesis of molybdopterin cofactors was obtained from bacteria as model systems, and thus must be extrapolated to mammalian systems. GTP is the common precursor. The synthesis of molybdopterin requires several enzymes catalyzing some unusual reactions, including those to add the two sulfur atoms to form the ethenedithiolate (which occurs via a thiocarboxylate on a terminal glycine of the enzyme itself), insert the Mo from molybdate, as well as sulfurase that adds the last sulfur (Fig. 11.2). The cofactor that is conjugated with cytosine monophosphate is putatively the one present in XOR and possibly AO, where as other organisms utilize other structures along this biosynthetic pathway in their respective MoCo enzymes. A detailed description of MoCo biosynthesis is described in Leimkuehler's work [39].

11.2.2 Mechanism of Catalysis

The reaction mechanism of molybdenum-containing hydroxylases has been studied extensively in XOR, and in general can be applied to AO. The reaction mediated by XOR consists of coupled reductive and oxidative half-reactions, therefore either oxidation or reduction of substrate has been observed in XOR/AO reactions. Figure 11.3 shows the redox state changes of XOR during substrate oxidation. Hydroxylation of a XOR substrate at the Mo binding site reduces Mo from VI to IV. Mo(IV) is then oxidized back to Mo(VI) via sequential electron transfer to the two Fe–S complexes. Further, electron transfer to FAD reduces FAD to $FADH_2$, which is oxidized back to FAD by O_2 to restore the enzyme to its original redox state. Kinetic studies have suggested that the different redox states are in rapid equilibrium [40–42]. The two Fe–S centers act as electron sinks to maintain Mo as VI and flavin in $FADH_2$ state for efficient reduction and oxidation.

Monooxygenases such as P450s oxidize substrate by incorporating oxygen atom from O_2 to the product. In contrast, the molybdenum hydroxylases use water as the ultimate source of the oxygen atom incorporated into the product [9,43], and generate reducing equivalents in the course of substrate hydroxylation (Fig. 11.3). Hydroxylation of XOR/AO substrates happens at the Mo center. Mo adopts a distorted square pyramidal coordination geometry, where the apical position is occupied by a Mo=O group and the four equatorial ligands are a terminal Mo=S group, two sulfurs from a pterin cofactor, and a ligand proposed to be hydroxide from water (Mo–OH) [44–48]. Previous studies have demonstrated that Mo–OH rather than the Mo=O is catalytically labile [7,49]. Oxidation of xanthine involves base-assisted nucleophilic attack on xanthine by the Mo–OH group with concomitant hydride transfer to the Mo=S group (Fig. 11.4). The concerted mechanism has been supported by both experimental evidence and computational studies [50–53]. A glutamate in the active site plays a central

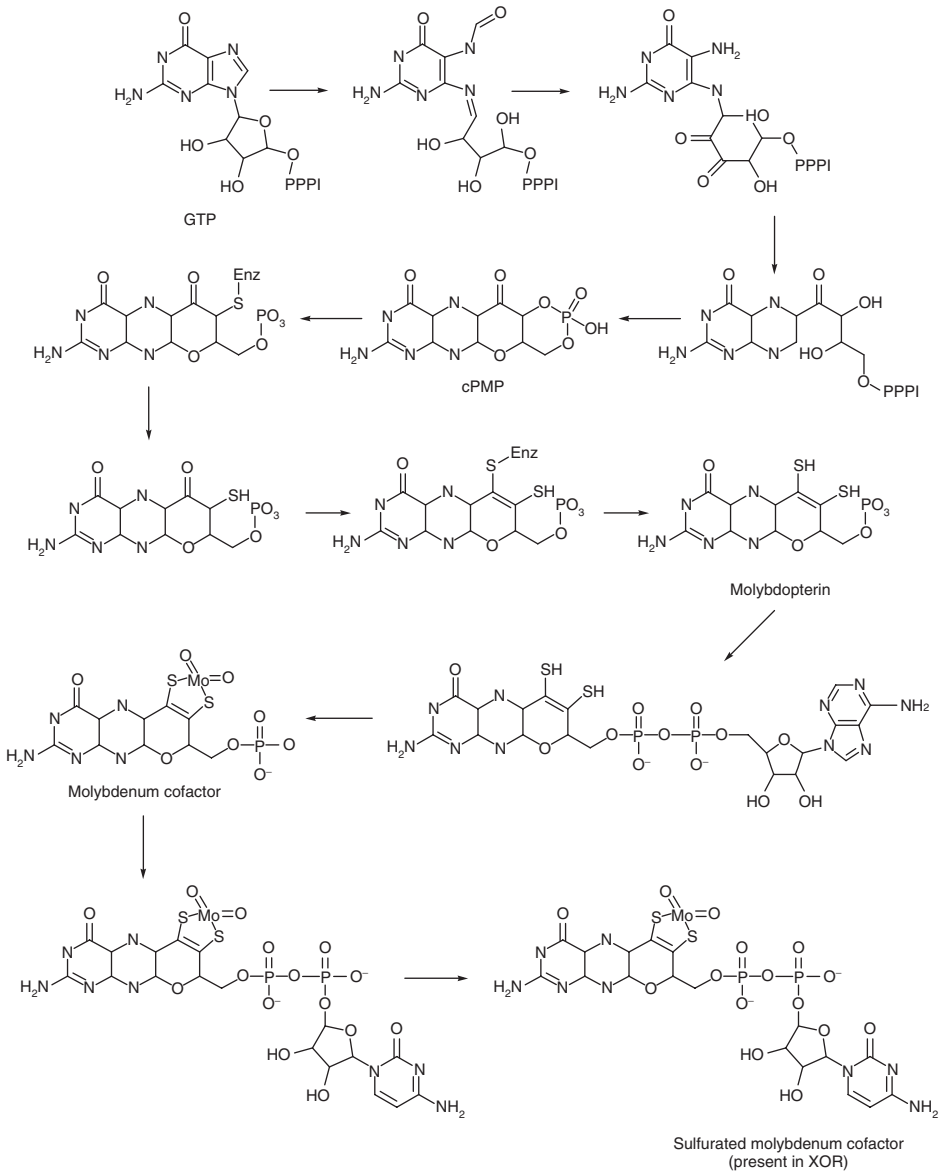


Figure 11.2 Structure of molybdenum cofactors and the pathway of their biosynthesis.

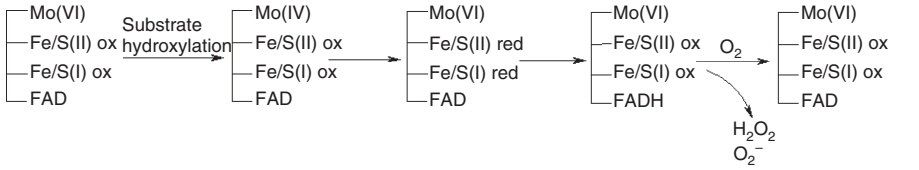


Figure 11.3 Redox state changes of XOR during substrate oxidation.

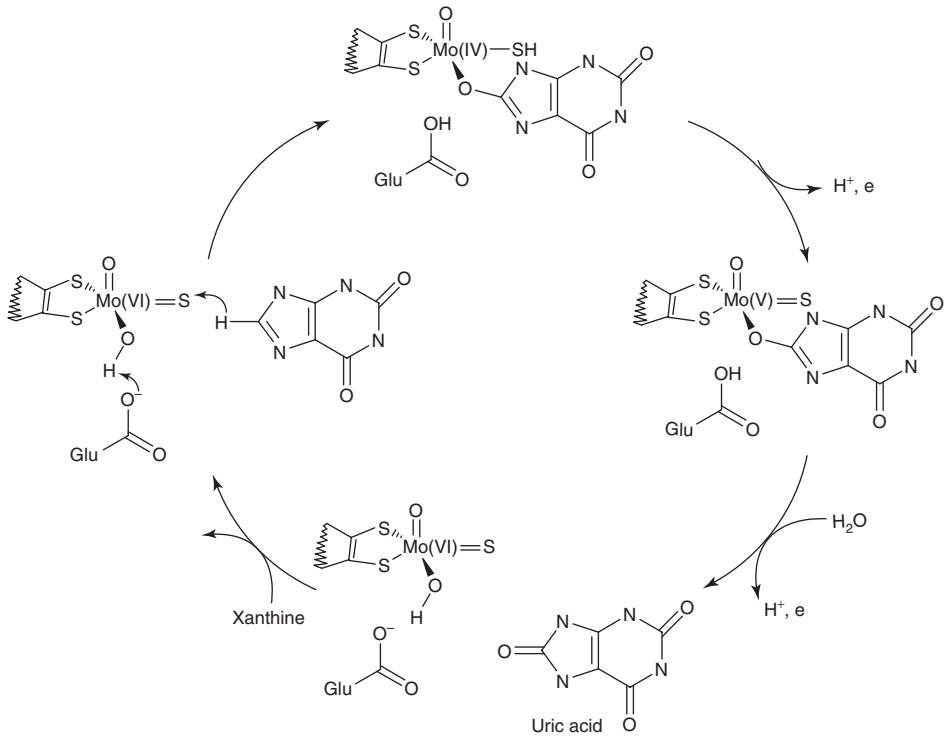


Figure 11.4 A proposed reaction mechanism for the oxidation of xanthine by XOR.

role in catalysis. Mutation of this group to alanine decreases the enzyme reduction rate by a factor of 10^7 as compared with wild-type enzyme [51]. This glutamate is also preserved in AO. Both AO and XOR oxidize a wide range of N-heterocycles and aldehydes; however, XOR shows narrower substrate specificity than AO.

11.3 GENETICS OF MOLYBDENUM-CONTAINING HYDROXYLASES

As of 2011, there had been five known molybdenum-containing hydroxylases identified in mammals. Two of the five have been previously introduced in this chapter, human XOR and AO (AOX1). The other three were identified as related mouse proteins; AO homolog 1 (AOH1), AO homolog 2 (AOH2), and AO homolog 3 (AOH3) [1,54], currently known as AOX3, AOX4, and AOX3L1, respectively [1,55,56]. The solution of multiple molybdenum-containing enzyme crystal structures [45–47,54,57] has provided an understanding of protein folding and amino acid homology, and thus made tracing the evolution of molybdenum-containing enzymes easier.

In humans, only one expressed AOX gene (AOX1) is present, which differs in other mammals where multiple AOX homologs have been identified [55]. The AOX isoforms are expressed in specific tissues in different organisms, and are believed to recognize specific substrates and carry out different physiological responsibilities [54]. When AOX1 was first sequenced, it was apparent that the primary structure of the protein was quite similar to many mammalian XORs [26]. The amino acid sequences of AOX1

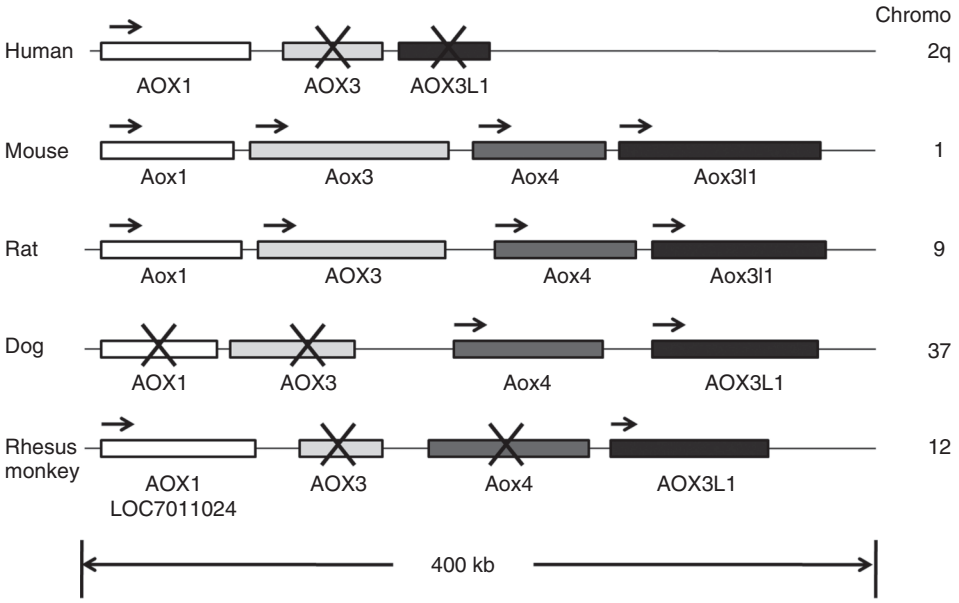


Figure 11.5 A comparison of aldehyde oxidase genes and pseudogenes present in the genomes of humans and commonly utilized animal models in pharmacokinetic and drug-metabolism studies. Othologous genes are indicated in boxes of the same shade. Pseudogenes are indicted with an "x" through the box, and the species difference in chromosomal location is indicated on the right. *Source*: Adapted from the work of Garrattini and Terao [55,59].

and XOR from many different animal species can be easily aligned along the entire length [1]. As previously mentioned in Section 11.2.1, the similarity of AOX1 and XOR reaches ~50%, which indicates a common ancestral precursor [26,28,29]. When comparing the three mouse identified homologs of AO, a 63% amino acid homology is observed [1].

Since it is widely held that the eukaryote XOR coding region is the progenitor of all AOs, Rodriguez-Trelles *et al*. [58] provided a hypothesis of how these AOs came about. They hypothesize that AOs were created from two independent duplications of the progenitor XOR and further expressed, or not, because of species-specific evolutionary suppression or deletion events. It is also hypothesized that the duplication events occurred before species divergence, due to the high similarity among vertebrate orthologous proteins, and at the gene level, with the conserved exons of the three homologs of AOX observed in many mammals (Fig. 11.5) [1,55,59].

XOR has been identified in a very wide range of mammalia, and have been purified from liver and milk in bovine, human, rat, mouse, and rabbit. Regardless of the species and the source used for isolation, there is relatively little observed variability in the kinetic parameters (K_M or V_{max},) when measured using hypoxanthine or xanthine as a substrate [1]. Furthermore, allopurinol, a very selective tight-binding XOR inhibitor, inhibits all mammalian XORs, providing further evidence of extensive homology from species to species.

The human locus for XOR maps to chromosome 2p22 [60], which in mouse maps to chromosome 17 [61]. Other animal species of interest are rat, dog, and rhesus

monkey, which map to chromosomes 6, 17, and 13, respectively. The XOR genes of both human and mouse consist of 36 short exons and are 80 and 85 kb, respectively, in length. All of the exon/intron junctions of these two orthologs are completely conserved in both position and type. In comparison with the other organism, orthologs of XOR (i.e., *Aspergillus nidulans, Caenorhabditis elegans*, and insects such as *Drosophila melanogaster, Bombyx mori*, and Calliphora vicina, the number of exons in mammalian XOR genes is much larger, but the exon/intron junctions are concordant in the various species. This suggests a preserved code from a common origin. A few functional studies to define the regulatory elements of XOR in mammals provide that the 5′-flanking regions of the two genes are lacking a canonical TATA box, which is substituted by an initiator element [62,63]. These DNA regions contain most of the elements responsible for the constitutive expression of XOR, but do not seem to contain coding for tissue and cell specificity.

The gene structures of mammalian AO are quite similar to that of reptiles and birds but tend to diverge from that of insects and amphibians [27]. The disparate number of genes of AO in different mammalian species, as mentioned previously, is thought to be due to asynchronous duplication from a precursor XOR gene, which is further influenced by subsequent suppression and deletion events [58]. Evidence of this suppression is observed in humans. The complete Human *AOX1* is mapped to chromosome 2q32.3–2q33.1 [24]. Interestingly, the XOR gene is mapped to the same chromosome in humans, although this trait is uncommon in other mammalian species, except a close relative the Chimpanzee [59]. The human AOX1 gene spans ~80 kb and contains 35 exons. Two pseudogenes, homologous to mouse AOX3 and AOX3L1, have been identified in the same genomic region of chromosome 2 (Fig. 11.5). These pseudogenes named *duplication* 1 (dupl1) and dupl2 are the vestiges of Aox3 and Aox3L1, which are transcribed to mRNAs that do not seem to code for protein products [59,64]. The observed suppression in humans is also observed in many preclinical species but to varying degrees among species (Fig. 11.5). With this variation, the prediction of human pharmacokinetics from preclinical species has proven challenging [55,59].

In humans, classical xanthinuria is a rare autosomal recessive metabolic disorder characterized by impairment of the final steps in the catabolism of purines; for example oxidation of hypoxanthine to xanthine and xanthine to uric acid by xanthine dehydrogenase. Consequently, classical xanthinuria manifests with diminished levels of uric acid and increased amounts of xanthine and hypoxanthine in the serum and urine. This displays itself clinically as a tendency to form xanthine stones in the urinary tract and xanthine precipitates in the muscles. There are two types of classical xanthinuria: type I that results from an isolated deficiency of XDH due to the autosomal recessive gene and type II that results from combined XDH and AO deficiency [65]. Xanthinuria type II has been connected with at least three individual single amino acid changes in the human molybdenum cofactor sulfurase (HMCS) gene [66–68], which when expressed catalyzes the conversion of the oxo- to the sulfido form of the MoCo required for the activity of XOR and AO. These mutations have obvious implications on XOR, because of the connection made between the HMCS expression and the lack of function. Since genetic deficiencies in the HMCS gene have not yet been associated with the pathophysiology associated with an AO related mechanism, it is hypothesized that AO is not essential for normal human function.

AO genes in other animal species' cluster are similar to what is observed in humans. In mouse, the clustering is close on chromosome 1 band c1, *Aox1* is on the most 5′ end, followed by *Aox3*, 5 kb after, and then 15 kb *Aox4*, and 9 kb after *Aox3l1* [59,64] (Fig. 11.5). In rat, the same cluster of active genes is located on chromosome 9 [69], which has similar synteny to mouse. In dogs, there are two active genes in a cluster of four on chromosome 37. The two active genes are the orthologs of mouse *Aox4* and *Aox3l1*, and the other two stretches of DNA have homology to rodent *Aox1* and *Aox3*, but these sequences do not code for active protein [1,55,56]. In a review of AO by Garattini and Terao [55,59], some unpublished work had been reported, which claims that the rhesus monkey AO cluster is located on chromosome 12. Garattini and Terao's review also claims that the two genes that code for active protein are orthologous to those of mouse *Aox1* and *Aox3l1*, and the two nonexpressing pseudogenes are similar in DNA sequence to *Aox3* and *Aox4.*

11.4 BIOTRANSFORMATIONS OF XENOBIOTICS BY XOR AND AO

Oxidation by XOR/AO involves nucleophilic attack at an electron deficient carbon, which in turn provides the insertion point of an oxygen atom to generate a carboxylic acid from aldehydes or a lactam from aromatic N-heterocycles. Both AO and XOR reduction occur at either N- or S-functional groups under anaerobic or hypoxic conditions, providing that an appropriate electron donor is present. In regard to AO, the appropriate electron donor is either benzaldehyde or 2-pyrimidinone, or other oxidized substrate; and for XOR, the electron donor is xanthine. Reduction rates are dependent on both the concentration and availability of the electron donor. Some known reduction reactions include reductions of *N*-oxides, sulfoxides, nitro-containing compounds, hydroxamic acids, as well as reductive ring opening of heterocycles. These metabolic routes attributed to XOR and AO will be elaborated on in the following sections.

11.4.1 Oxidation of Aromatic N-Heterocycles

Oxidation of N-heterocycles accounts for a majority of AO/XOR-mediated reactions. A recent survey of several current compound collections showed that 35–56% of the compounds in these collections are potential AO/XOR substrates based on the presence of an unsubstituted aromatic carbon atom adjacent to the nitrogen [2]. Figure 11.6 is a summary of heterocyclic cores that are reported to be oxidized by AO/XOR. Simple unsubstituted N-heteroaromatics such as imidazole, pyrazole, pyridine, pyrazine, and pyrimidine are not readily oxidized by XOR/AO. Substituted pyridines are more easily oxidized by AO (Fig. 11.7). Both metyrapone [70] and 1,2-bis(nicotinamideo)propane were oxidized to pyridone at the α-position [71]. A methylpyridine group in a TLR7 agonist has also been shown to be oxidized to α-pyridone by AO [72].

Substituted pyrimidines such as 2- and 4-hydroxypyrimidine were readily oxidized by AO to form uracil, the former being widely used as an electron donor for AO reduction studies *in vitro*. The conversion of hydroxypyrimidine to uracil by AO has been utilized to oxidize several 5-substituted hydroxypyrimidine (5-fluoro- and 5-ethynyl-2-pyrimidinone, Fig. 11.8) to the corresponding pyrimidine nucleoside bases to overcome low and/or inconsistent oral bioavailability of this compound class

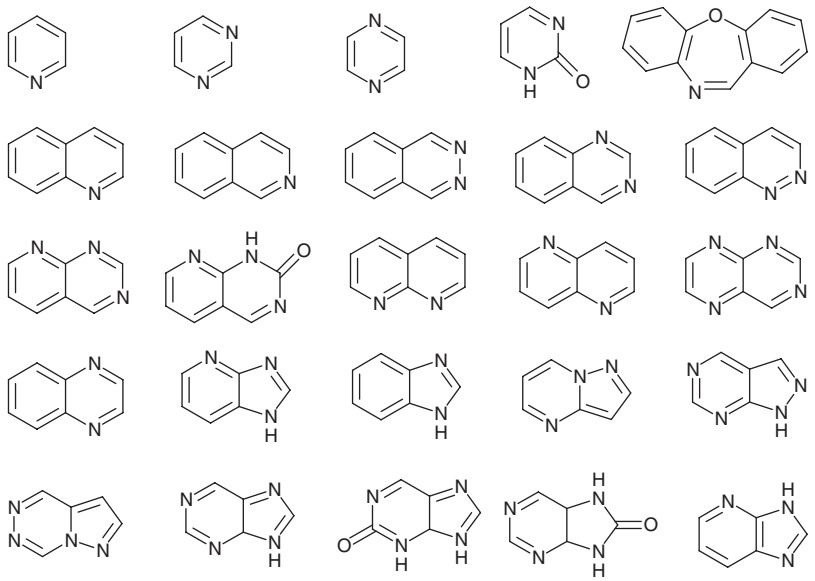


Figure 11.6 Typical aromatic heterocyclic cores in AO/XOR substrates.

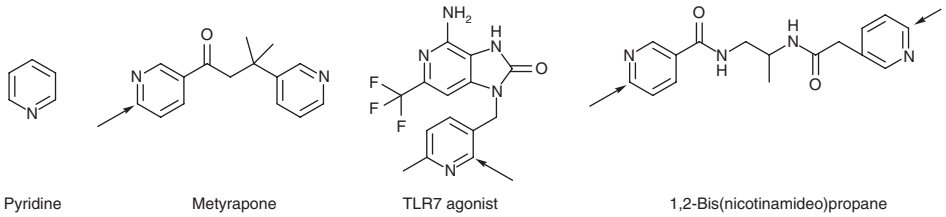


Figure 11.7 Pyridine-containing substrates of AO/XOR (arrow indicates the site(s) of oxidation).

[73,74]. This prodrug approach has also been employed in the bioactivation of 5-iodo-2-pyrimidinone-2′-deoxyribose (IPdR) to 5-iodo-2′-deoxyuridine (IUdR) [75,76] and zebularine to uridine [77]. FK3453 is an adenosine A1/2 dual inhibitor for the treatment of Parkinson's disease. Despite its satisfactory absolute bioavailability and total body clearance in animals, the plasma concentrations of FK3453 in humans were extremely low. This was attributed to AO metabolism where an oxidative metabolite of the 2-aminopyrimidine moiety was identified as a major metabolite [78]. RS-8359 is a selective and reversible MAO inhibitor containing 4-aminopyrimidine. Itoh *et al*. [79] showed that AO exhibited stereoselectivity for the S-enantiomer in rat *in vitro* (Fig. 11.9). The stereoselective AO metabolism was also observed *in vivo*. After oral administration of racemic RS-8359 to rats, mice, dogs, monkey, and humans, plasma concentrations of the R-enantiomer were substantially higher than those of the S-enantiomer [80].

The fused-ring analogs are typical targets for oxidation by AO/XOR. Fusion of benzene rings to pyridine such as quinoline, isoquinoline, and phenanthridine increases

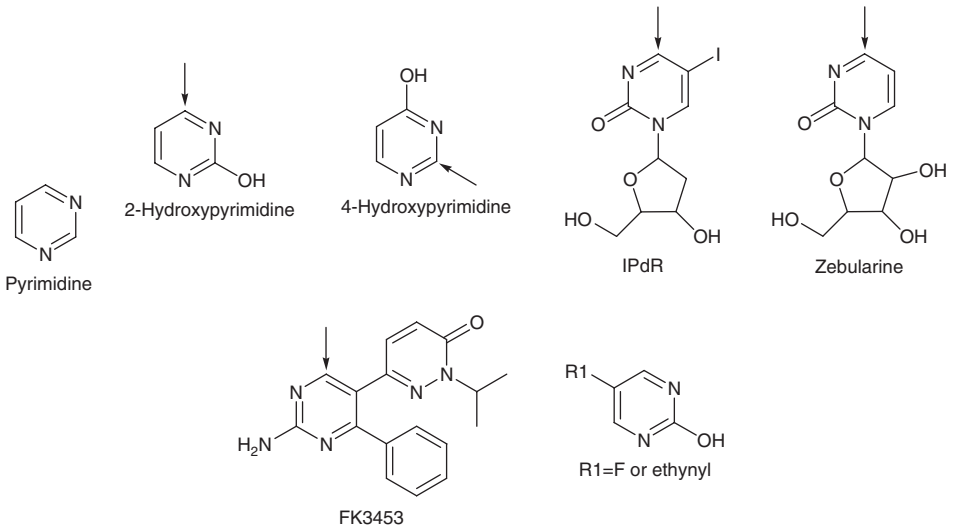


Figure 11.8 Pyrimidine substrates of AO/XOR (arrow indicates the site(s) of oxidation).

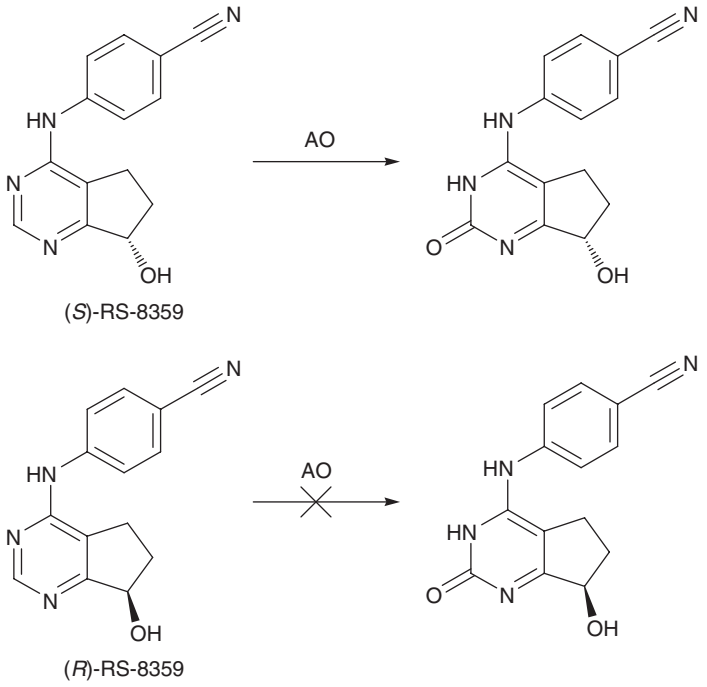


Figure 11.9 Stereoselective oxidation of RS-8359 by AO.

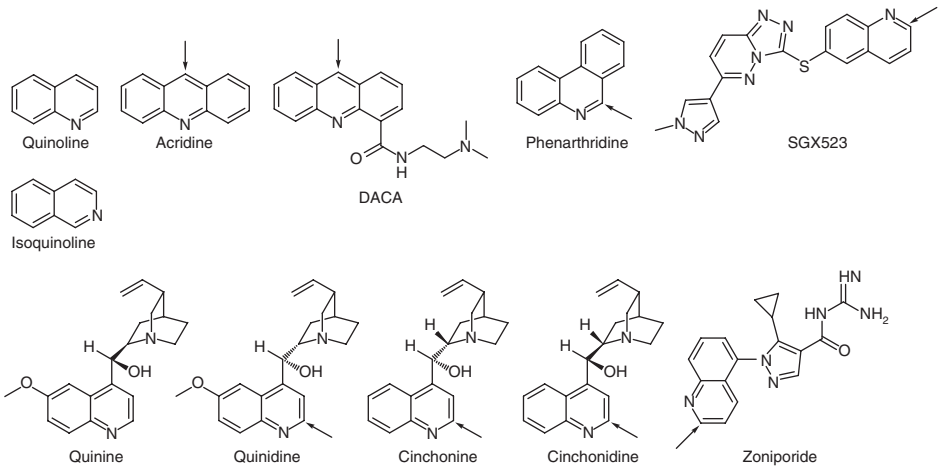


Figure 11.10 Quinoline and isoquinoline substrates of AO/XOR (arrow indicates the site(s) of oxidation).

binding to rabbit liver AO [81] (Fig. 11.10). Quinoline and isoquinoline have K_m values of 3 and 0.2 mM, respectively, while phenanthridine has higher binding affinity toward AO (K_m, 2.3 μM), which could be due to the increased lipophilicity that facilitates substrate binding to the active site. Interestingly, although phenanthridine, 3,4-benzoquinoline, is a good AO substrate, 5,6- and 7,8-benzoquinoline do not bind to the enzyme [82]. The AO oxidation of acridine is unusual in that hydroxylation takes place at carbon para position rather than ortho to the nitrogen atom [83]. The normal oxidation position, ortho to the nitrogen, is not available for enzyme attack. Similarly, the acridine anticancer agent *N*-[(2′-dimethylamino)ethyl]acridine-4-carboxamide (DACA) is rapidly converted to the acridone by human, rat, and guinea pig AO [84]. Quinine, quinidine, cinchonine and cinchonidine, four quinoline-based antimalarials are all oxidized at the quinoline ring by AO [85]. The oxidation rates of quinine and quinidine were lower than those of cinchonine and cinchonidine, probably because of the electron-donating 6′-methoxy group. Stereoselective metabolism by AO was observed for the two pairs of stereoisomers: cinchonidine and quinidine were oxidized at much higher rates than their corresponding stereoisomers, cinchonine and quinine [86]. SGX523, a quinoline-containing c-MET inhibitor in clinical development for the treatment of solid tumors, was oxidized by AO to a 2-hydroxyquinoline metabolite [23]. This metabolite has low solubility and was excreted in urine, and was suspected to form crystal deposits in renal tubules to compromise renal function in patients. The selective dopamine D3 receptor antagonist SB-277011 is another quinoline-containing compound. SB-277011 was predominantly metabolized by AO and its clearance value in human liver homogenates was 35-fold higher than that in microsomes [87]. Zoniporide, a quinoline-containing inhibitor of the sodium/hydrogen exchanger, was mainly cleared via metabolism, and the major excretory and circulating metabolite in humans was 2-oxozoniporide formed by AO [88].

Quinazoline and phthalazine, cinnoline, and quinoxaline (benzene-fused pyrimidine, pyrazine, and pyridazine) are oxidized by AO to the lactam metabolites (4-hydroxyquinazoline, 2,4-di-hydroxyquinazoline, 1-hydroxyphthalazine, 4-hydroxy-

cinnoline, 2-hydroxyquinoxaline, and 2,3-dihydroxyquinoxaline) [81] (Fig. 11.11). These compounds are weak XOR substrates. Ghafourian *et al*. [89,90] have studied the quantitative structure–activity relationship (QSAR) of phthalazine and quinazoline derivatives and the influence of substituents on AO-mediated oxidation, utilizing kinetic data reported previously. Their studies suggest that polarity of phthalazines had a negative effect on the enzyme activity, leading to the reduction of V_{max} and increase of K_m. Electron-withdrawing substituents at 2- or 4-position of quinazoline favored the AO oxidation. Carbazeran, a phthalazine-containing inotropic agent, is primarily metabolized by AO in humans to the phthalazinone. The oral bioavailability in man was too low to be measurable, although oral bioavailability in dog was ~68% because of low AO activity in dogs [91,92]. Bromidine, a quinoxaline-containing potent ocular hypotensive agent, has been shown to be metabolized by AO to quinoxaline-2,3-dione and two isomeric quinoxalinone in both ocular tissues and liver fractions [93,94]. A potential new anticancer agent XK-469 underwent extensive metabolism in human hepatocytes and in a phase 1 patient study. An oxidized product at C-3 of quinoxaline formed by AO was the predominant metabolite both *in vitro* and *in vivo* [95]. Bicyclic heteroaromatic moieties in molecules as big as macrolides can be metabolized by AO. The 1,8-naphthyridine ring system in compound **1** was shown to be vulnerable to AO in human liver cytosol. Addition of 3-hydroxyl group (compound **2**) failed to improve AO metabolic stability. However, the 1,5-naphthyridine from rearrangement of nitrogen in the ring (compound **3**) was stable in human liver cytosol [96].

Pteridines are readily oxidized by AO/XOR. Of the four carbons adjacent to the nitrogens, 2, 4, 7, but not 6 are oxidizable. Multiple oxidations can happen in pteridine (Fig. 11.11) [97,98]. However, XOR is more active in carrying out the sequential oxidation. For example, 2- and 7-hydroxypteridine were oxidized by XOR but not AO. *In vitro* studies suggest that XOR may predominate pteridine metabolism; however, methotrexate, a potent inhibitor of dihydrofolate reductase for the treatment of leukemia, was metabolized by AO [99,100]. A major metabolite of methotrexate is its 7-hydroxylated derivative.

Purine was rapidly oxidized by XOR and AO (Fig. 11.12). Purine is sequentially oxidized via hypoxanthine and xanthine to uric acid [8]. XOR is more efficient in catalyzing this sequential reaction than AO. 6-Mercaptopurine is oxidized to 6-thiouric acid via 6-thioxanthine. The first and rate limiting step in this biotransformation is catalyzed solely by XOR, whereas both XOR and AO are involved in the oxidation of 6-thioxanthine to 6-thiouric acid [101]. 6-Mercaptopurine is subject to a large drug–drug interaction when coadministered with allopurinol, a specific XOR inhibitor [102]. AO shows broader substrate specificity and oxidizes a wide variety of drugs containing purine. Both famciclovir and its deacetylated metabolite 6-deoxypenciclovir are efficient substrates of AO; however, they show little activity with XOR [103,104]. AO appears to preferentially oxidize the 8-carbon (Figure 11.13). When dosed intravenously, which bypassed intestinal XOR, both 6-mercaptopurine and 6-thioguanine were oxidized to 8-oxo metabolites, presumably catalyzed by AO [105,106]. Similarly, O^6-benzylguanine is oxidized at 8-carbon by human AO [107]. Krenitsky *et al*. [98] studied a series of substituted purines. While an electron-withdrawing ($CONH_2$, CN) or neutral (CH_3) substituent at the 6-position of purine maintained the rate of AO metabolism, an electron-donating group (NH_2, OH, SH) rendered the purine significantly less reactive (Table 11.1). Electron-donating 8-substituents also decreased AO metabolism; however, the same effect was not observed for electron-donating groups at

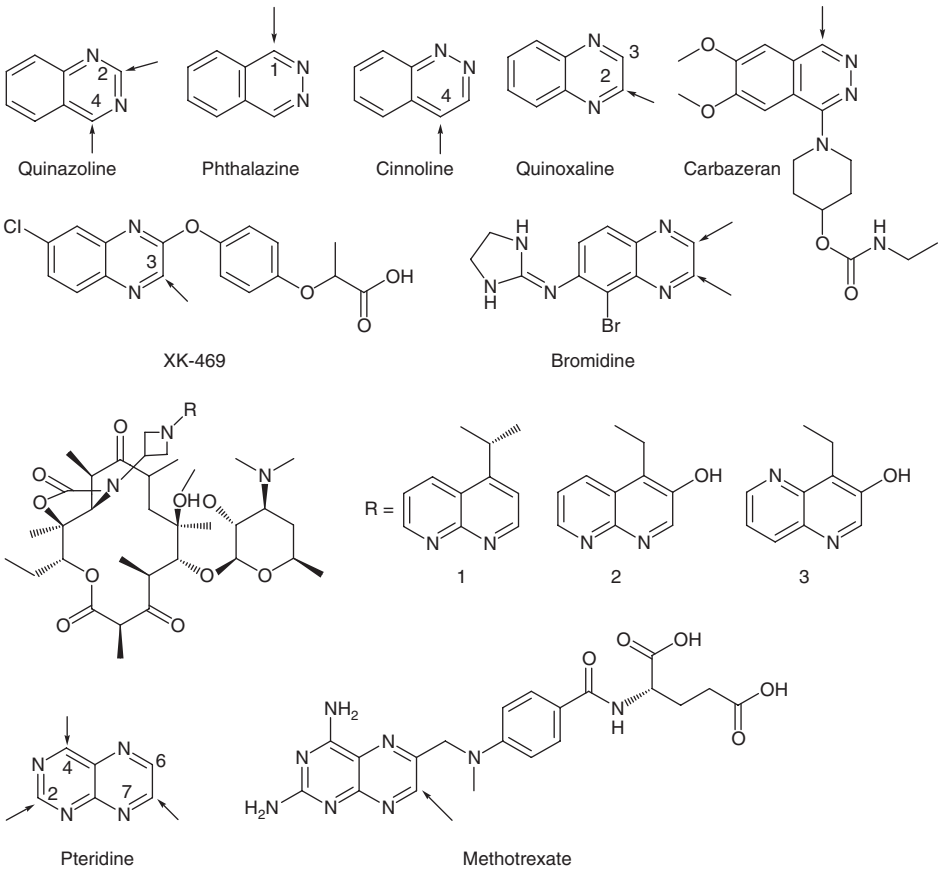


Figure 11.11 Quinazoline, phthalazine, cinnoline, quinoxaline, and pteridine substrates of AO/XOR (arrow indicates the site(s) of oxidation).

the 2-position of purine. The trend is less clear for XOR metabolism of purine analogs (Table 11.1).

In addition to the cores described above, there are other heterocyclic AO substrates reported in the literature (Fig. 11.14): pyrolopyrimidine (zaleplon) [108], dibenz[*b*, *f*]-1,4-oxazepine [109], azabenzimidazole (a gp120-CD4 inhibitors) [110], pyrazolo-triazine (a $GABA_A\alpha$ 5 inverse agonists) [111], and 8-azaquinazolinone (a CXCR3 antagonists) [112].

11.4.2 Aldehyde Oxidation

AO/XOR catalyzes the oxidation of aldehydes to carboxylic acids. Generally, drugs rarely contain an aldehyde moiety; however, aldehydes can be intermediate metabolites of alcohols. Hence AO/XOR may be involved in sequential metabolism of alcohol (the same reaction can be catalyzed by aldehyde dehydrogenase). Reactive oxygen species (ROS) such as H_2O_2 and superoxide are the byproducts of AO oxidation (Fig. 11.1). Increased generation of ROS during ethanol metabolism is proposed to

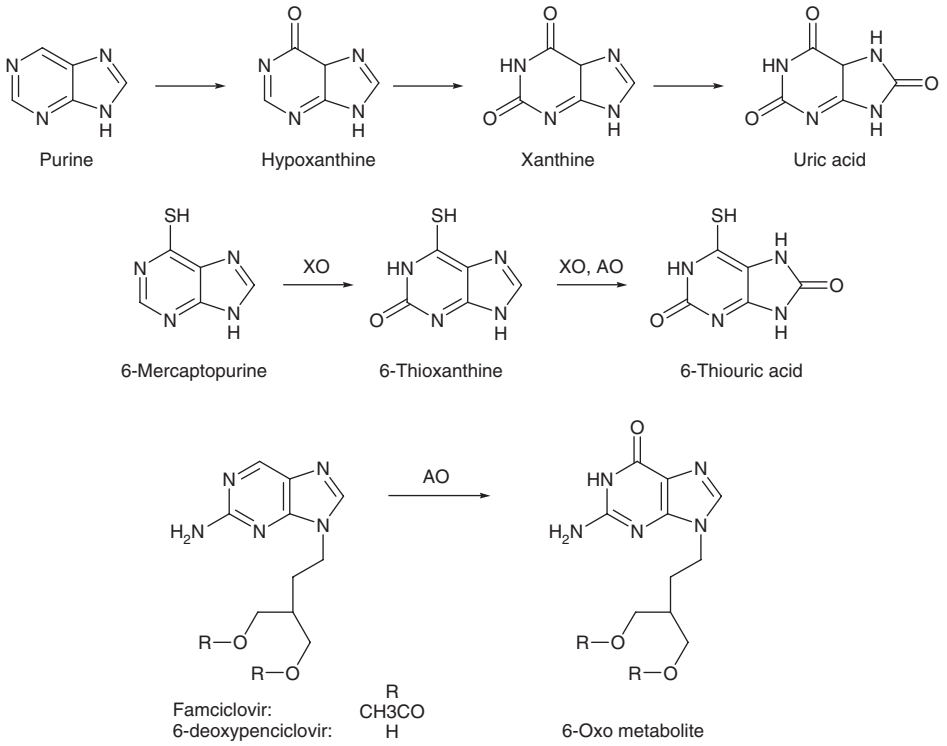


Figure 11.12 Purine substrates of AO/XOR.

contribute to alcohol-related oxidative injury to liver and pancreas [113,114]. Both aliphatic (i.e., retinal) [115] and aromatic aldehydes are oxidized by AO/XOR to the corresponding carboxylic acids. Lipophilicity appears to enhance aldehyde binding to AO, as a result, many aromatic aldehydes are excellent substrate for AO (Fig. 11.15), such as benzaldehyde, indole-3-aldehyde, vanillin, and pyridoxal [116–118]. However, these aromatic aldehydes are poor substrates of XOR.

Acid metabolites of drugs were reported to be formed by AO (Fig. 11.16). For example, tolbutamide is hydroxylated to the benzyl alcohol, by P450, further oxidized into an aldehyde by alcohol dehydrogenase and finally oxidized to the carboxytolbutamide metabolite by AO [119]. Tamoxifen is oxidized first by monoamine oxidase (MAO) to an aldehyde intermediate and subsequently oxidized to the corresponding carboxylic acid by AO [120]. Similarly, the antidepressant citalopram is oxidized by MAO to an aldehyde and further oxidized by AO to a carboxylic acid metabolite [121].

11.4.3 Oxidation of Iminium Ions

Iminium ions, with a positive charge on the nitrogen atom of C=N, facilitates nucleophilic attack to the C=N bond, therefore, they are excellent substrates for AO. For example, N^1-methylnicotinamide, which is formed by the N-methylation of nicotinamide by methyltransferase, can be oxidized by AO [122,123]. Both 2- and 4-carbons

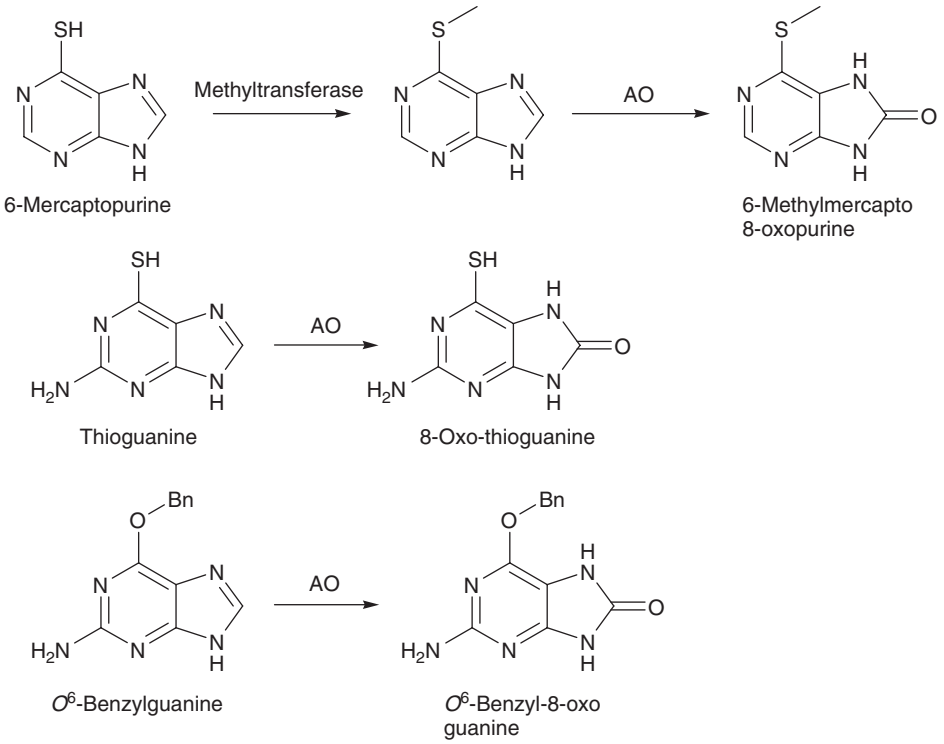


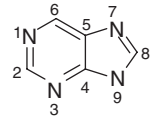
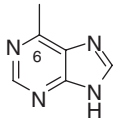
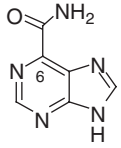
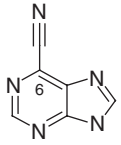
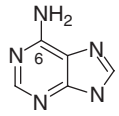
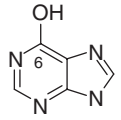
Figure 11.13 8-Oxidation of purine analogs by AO.

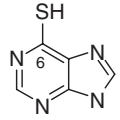
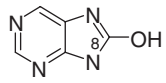
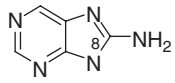
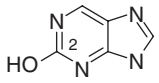
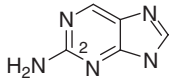
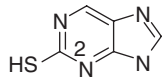
have been shown to be oxidized to form 2- and 4-pyridone, respectively, however, the ratio of pyridone metabolites varies among species [124,125]. Human AO oxidizes *N*-methylnicotinamide mainly to 2-pyridone, whereas mouse AO generates equal amounts of 2- and 4-pyridone (Fig. 11.17) [126].

Iminium ions can be generated as intermediates during the metabolism of cyclic amines such as pyrrolidines, piperidines, indoles, and dihydropyridines via CYP450 or monoamine oxidase. These iminium ions are highly reactive species and can cause toxicity [127]. AO oxidizes these iminium intermediates to lactams, which represents an important detoxification pathway for these compounds. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is an exogenous neurotoxin whose neurotoxicity is believed to be mainly because of 1-methyl-4-phenylpyridinium (MPP). MPP is generated through sequential oxidation via an intermediate 1-methyl-4-phenyl-2,3-dihydropyridinium species (MPDP). AO is one of the major enzymes, which catalyzes conversion of MPDP to MPTP lactam and therefore decreases the formation of MPP (Fig. 11.18) [128–130].

AO is involved in the formation of a number of lactam drug metabolites. For example, nicotine iminium, which is formed from P450-mediated oxidation of nicotine, is converted to cotinine by AO (Fig. 11.19) [131]. Similarly, ABT-418, prolintane, and azapetine undergo initial P450-mediated oxidation to iminium ions and further AO-mediated oxidation to lactams [132].

TABLE 11.1 Relative AO/XOR Activities of Some Purine Analogs

Purine						
AO activity	100	82	94	280	2	3
XO activity	100	<3	164	8	6	130

Purine						
AO activity	16	<1	<1	140	38	43
XO activity	17	24	<3	53	80	110

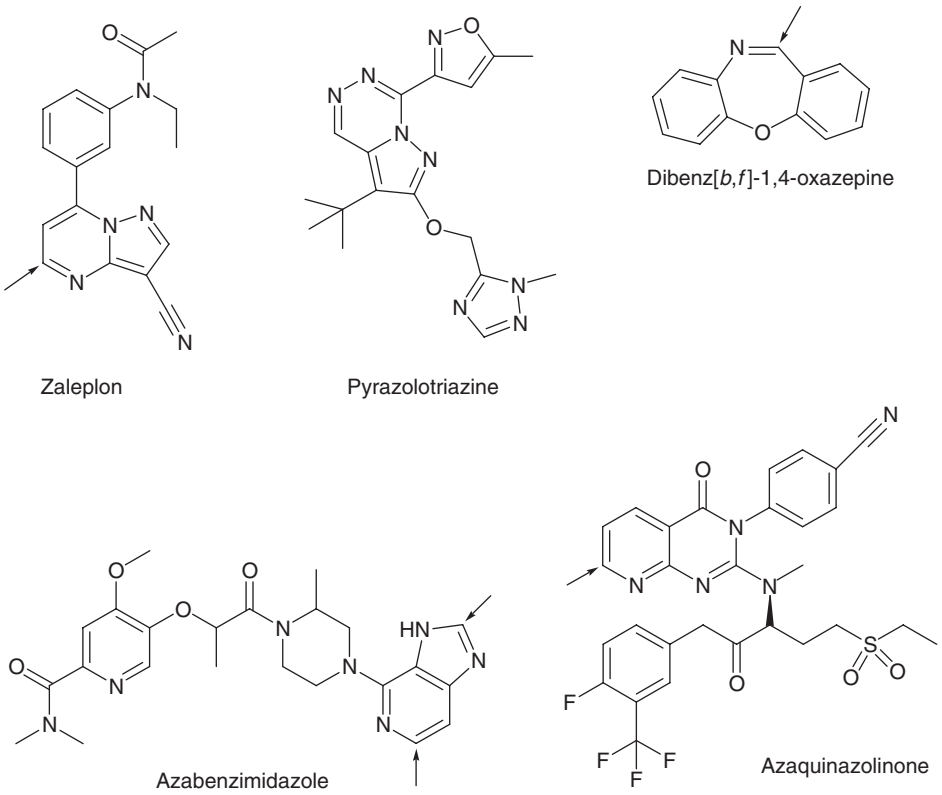


Figure 11.14 Other heterocyclic AO substrates (arrow indicates the site(s) of oxidation).

11.4.4 Reduction

AO/XOR-mediated reductions of nitro groups, *N*-oxide, nitrate, hydroxamic acids, oximes, isoxazoles, and isothiazoles have been demonstrated *in vitro*. However, the extent to which these reductions are actually carried out by AO *in vivo* is difficult to assess. This is because of several reasons: (i) *in vitro*, the incubations must be done under anaerobic conditions, as the substrate is taking the place of oxygen, (ii) electron donors such as N^1-methylnicotinamide are required *in vitro*, however, such cofactors are not identified *in vivo*, (iii) often other reducing enzymes such as P450 reductase, NADPH-benzoquinone reductase, and a recently identified molybdenum-containing mitochondrial amidoxime reducing component (mARC) can catalyze the same reduction.

11.4.5 Nitroreduction

AO and XOR are involved in nitroreduction of nitro-containing compounds (nitrated polycyclic aromatic hydrocarbons, nitrofurans, and nitroimidazoles). These nitro-containing compounds are generally mutagenic and nitroreduction is responsible for their mutagenicity. Nitroreduction is a sequential one- and/or two-electron reduction process, which generates reactive intermediates as well as ROS (Fig. 11.20). Both

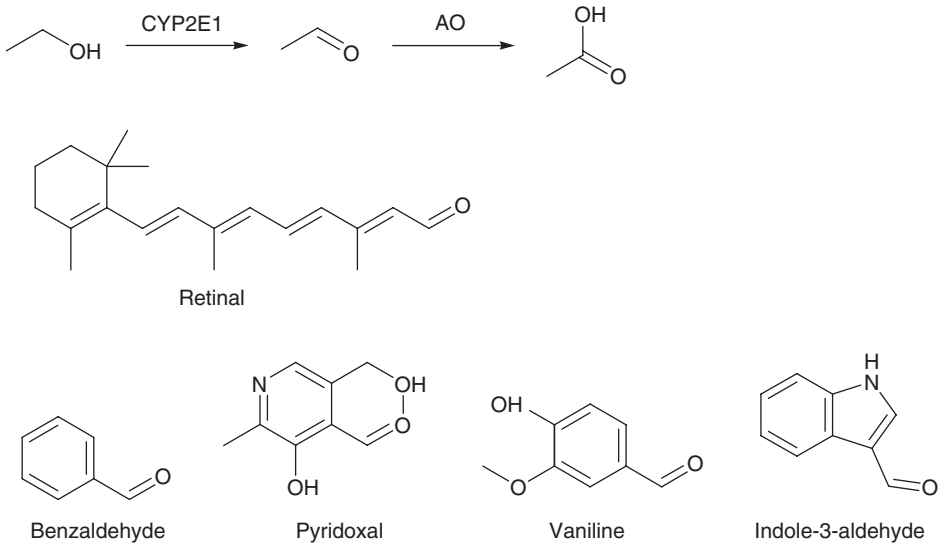


Figure 11.15 Aliphatic and aromatic aldehyde substrates of AO/XOR.

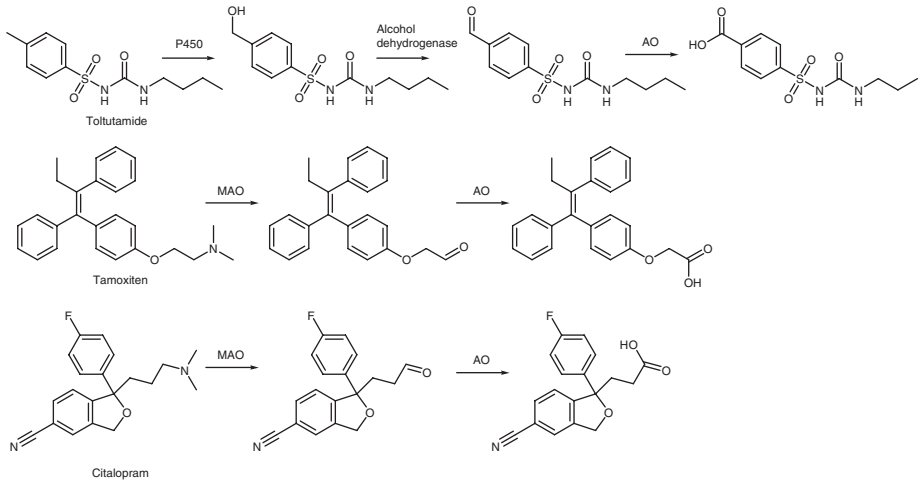


Figure 11.16 Examples of drugs that undergo sequential aldehyde oxidation by AO.

nitroso and *N*-hydroxyamino metabolites are potential electrophiles; however, only the latter derivative appears to modify DNA [133].

Nitroreduction of nitrated polycyclic aromatic hydrocarbons (2-nitrofluorene, 1-nitropyrene, and 4-nitrobisphenyl, Fig. 11.21) in animal livers has been investigated extensively, and it was reported that the reductive metabolism of these compounds is catalyzed by cytochrome P450, P450 reductase, and AO/XOR in mammalian liver [134]. Nitroheterocycles such as nitrofurazone, metronidazole, and benznidazole (Fig. 11.21) underwent XOR/AO-mediated notroreduction under anaerobic condition

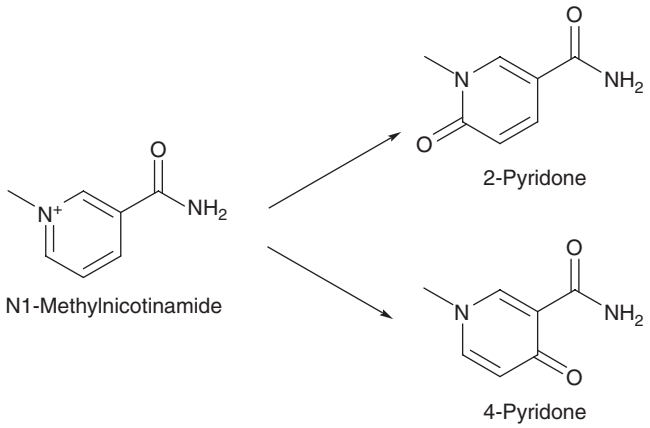


Figure 11.17 Oxidation of N^1-methylnicotinamide by AO.

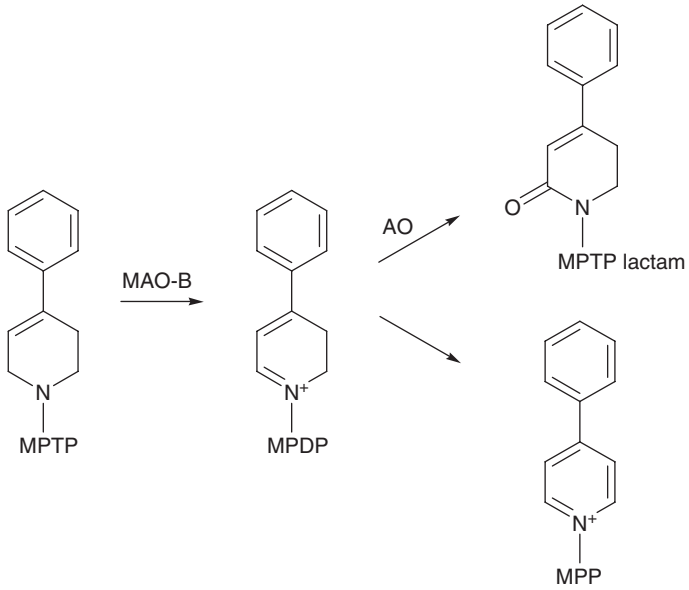


Figure 11.18 Detoxification of MPTP by AO.

[135]. P450 reductase also participated in the reaction. A number of nitroguanidine and nitromethylene insecticides have been reported to undergo nitro group reduction by rabbit liver AO. The nitro groups of clothianidin and imidacloprid was reduced by rabbit AO to nitroso and amino groups [136,137].

11.4.6 Reduction of Nitrates and Nitrites by XOR

XOR catalyzes reduction of organic and inorganic nitrates and nitrites to nitric oxide (NO). It is suggested that XOR may be an important enzyme system that produces

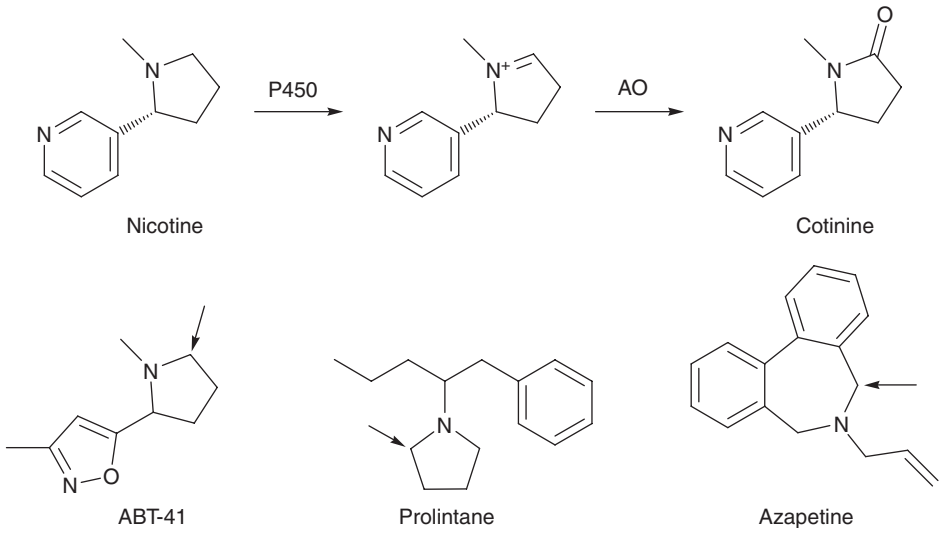


Figure 11.19 Examples of lactam metabolite formation via AO oxidation of iminium (arrow indicates site of oxidation).

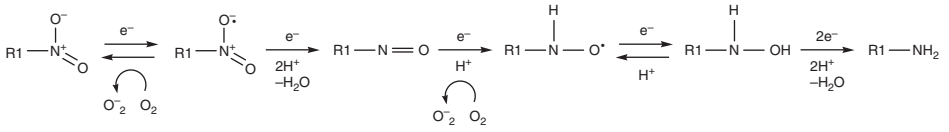


Figure 11.20 Proposed mechanism of AO/XOR reduction of nitro-containing compounds.

vasorelaxation NO via reduction of organic nitrates and nitrites such as glyceryl trinitrate, isosorbide dinitrate, isoamyl nitrite, and isobutyl nitrite (Fig. 11.22) [138–140].

11.4.7 *N*-Oxide and Sulfoxide Reduction

AO acts as reductase for *N*-oxide and sulfoxide under anaerobic conditions (Fig. 11.23). Nicotinamide *N*-oxide, *S*-(−)nicotine-1′-*N*-oxide, imipramine *N*-oxide, and cyclobenzaprine *N*-oxide are reduced to their corresponding parent amines when incubated in liver cytosols from rabbits, hogs, guinea pigs, hamsters, rats, and mice [141,142]. XOR also reduces *S*-(-)nicotine-1′-*N*-oxide to nicotine *in vitro* [143]. Sulfoxides (sulindac, sulfinpyrazone, phenothiazine sulfoxide, fenthion sulfoxide) are reduced by AO to their corresponding sulfides under anaerobic conditions [144–149]. Stereoselective reduction of sulfoxide has been reported for flosequinan, a racemic mixture of *R*- and *S*-sulfoxide, both rat liver and kidney cytosol reduced *R*-sulfoxide much faster than *S*-sulfoxide to the sulfide metabolite under nitrogen in the presence of electron donor 2-hydroxypyrimidine (Fig. 11.24) [150]. Both reduced amine and sulfide metabolites can be oxidized back to *N*-oxide and sulfoxide, which may prolong the residence time of these species via futile cycle of oxidation–reduction.

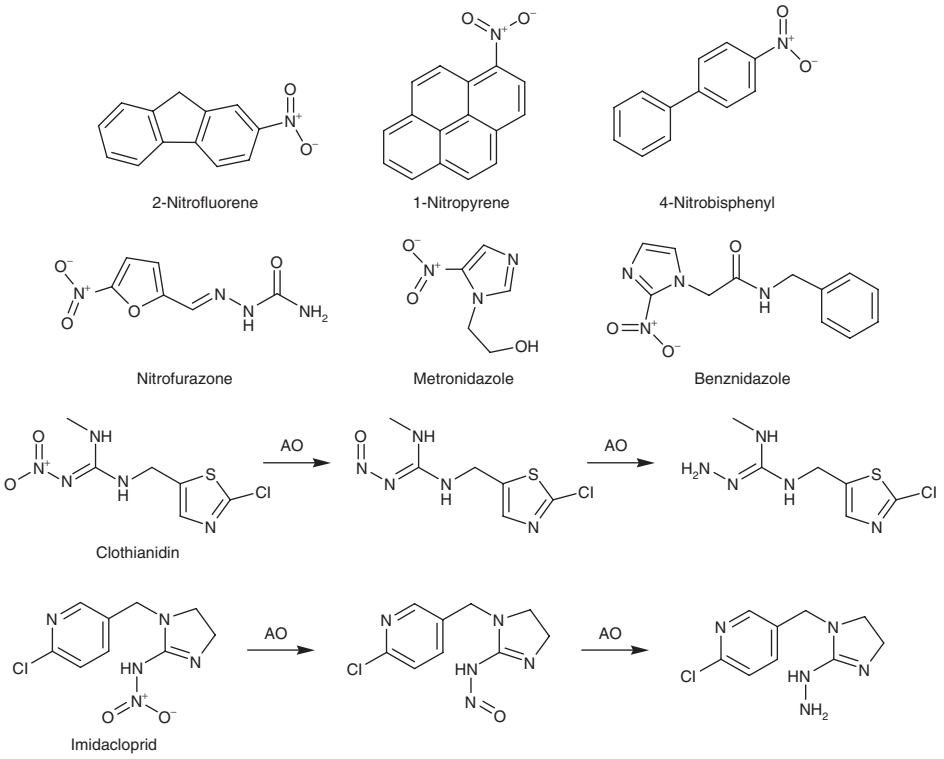


Figure 11.21 Examples of nitro-containing substrates of AO/XOR.

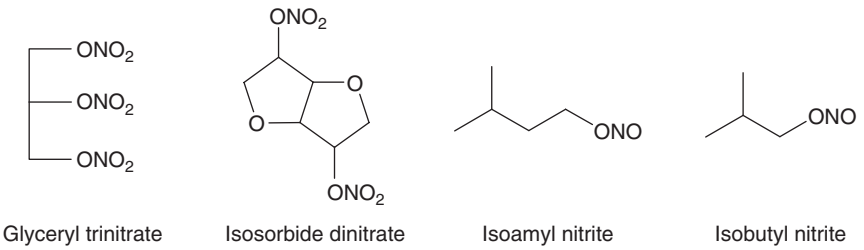


Figure 11.22 Organic nitrates and nitrites that are reduced by XOR to generate NO.

11.4.8 Reduction of *N*-Hydroxy Compounds

The reduction of *N*-hydroxy compounds such as hydroxamic acids, oximes, and *N*-hydroxyguanidines by AO/XO have been described in previous reviews [126,151]. Hydroxamic acids such as benzohydroxamic acid, nicotinohydroxamic acid, salicylhydroxamic acids, and *N*-hydroxy-2-acetylaminofluorene are reduced to their corresponding amide by AO [152,153] (Fig. 11.25). Guanoxabenz, an *N*-hydroxyguanidine metabolite of antihypertensive drug guanabenz, is reduced back to guanabenz by XO [154]. Acetophenone oxime and salicylaldoxime are reduced by AO under anaerobic

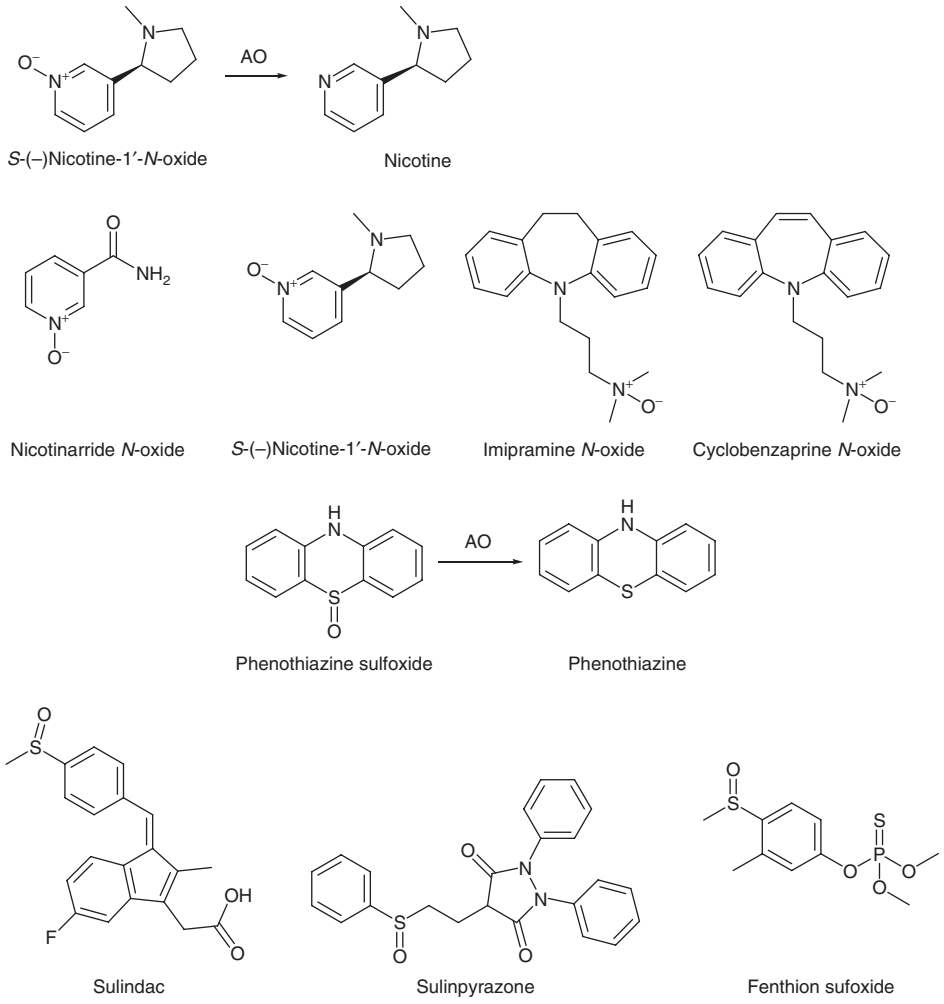


Figure 11.23 AO/XOR reduction of *N*-oxide and *S*-oxide compounds.

conditions to their corresponding ketimines, which are hydrolyzed to the oxo metabolites, whereas benzamidoxime is reduced to a ketimine [155]. Reduction of *N*-hydroxy groups is not limited to AO/XO. Recently, a new molybdenum-containing enzyme system named *mitochondrial amidoxime reducing component* (mARC) was reported to be involved in microsomal and mitochondrial reductions of hydroxylamines and amidoximes[156–158].

11.4.9 Heterocycle Reduction

AO has been suggested to be involved in the reduction of 1,2-benzisoxazole and 1,2-benzisothiazole in some drugs (Fig. 11.26). The 1,2-benzisoxazole ring of zonisamide was reductively cleaved to the ketimine intermediate, which is hydrolyzed to a keto metabolite in rat and rabbit liver cytosol [143]. It should be noted that the reductive

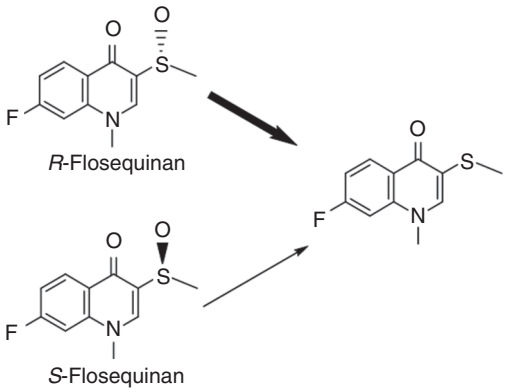


Figure 11.24 Stereoselective reduction of sulfoxide in flosequinan.

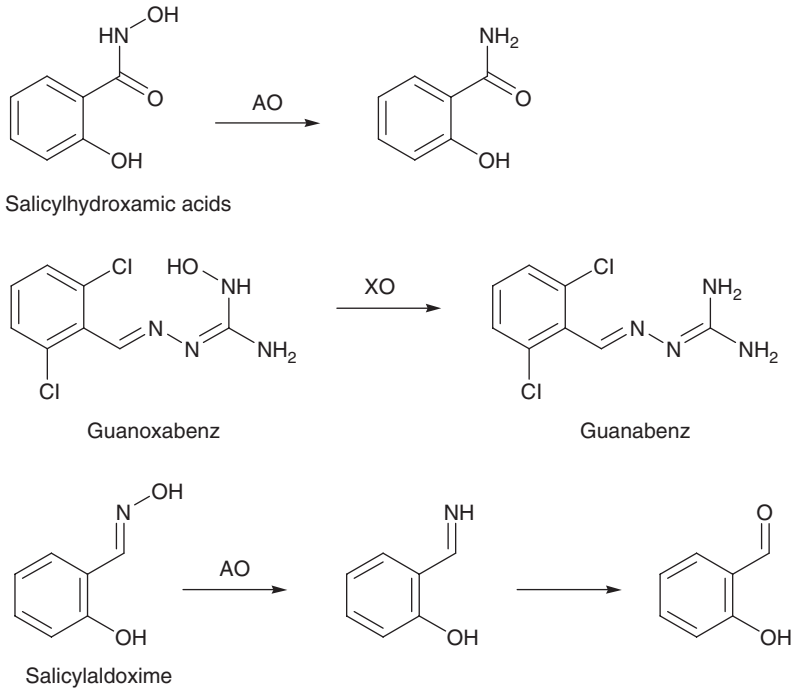


Figure 11.25 Reduction of *N*-hydroxy compounds by AO/XOR.

metabolism of zonisamide is also catalyzed by liver microsomal cytochrome P450, especially CYP3A4 [159], as well as mammalian intestinal bacteria [160]. Similarly the 1,2-benzisothiazole of ziprasidone underwent reductive cleavage of N–S bond to form a thiol intermediate. Subsequent S-methylation of the produced thiol moiety yielded a major metabolite *in vivo* [161]. *In vitro* studies suggested that AO is the major enzyme responsible for the reductive metabolism of ziprasidone [162,163].

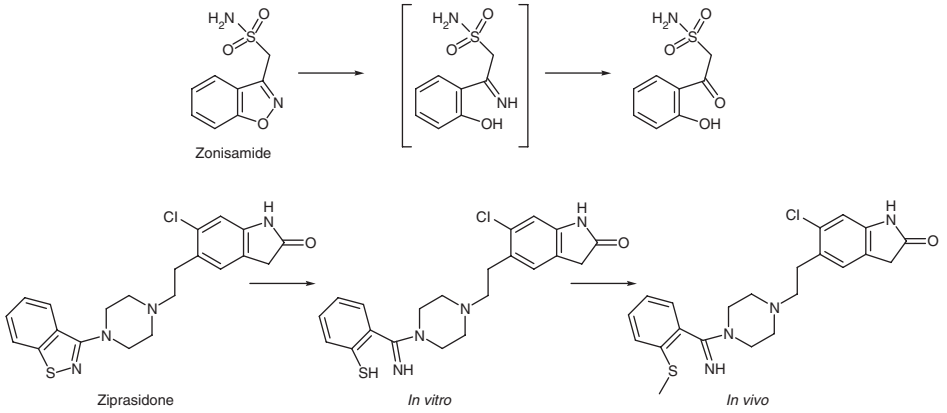


Figure 11.26 Heterocycle reduction catalyzed by aldehyde oxidase.

11.5 INHIBITORS OF MOLYBDENUM-CONTAINING HYDROXYLASES

11.5.1 Inhibitors of AO

11.5.1.1 **In Vitro.** Many inhibitors of the molybdenum-containing hydroxylases have been characterized and reported in the literature. Menadione is perhaps one of the most well-characterized inhibitors of AO, particularly *in vitro*, that does not potently inhibit XOR [164]. Thus, it is often used to distinguish between AO- and XOR-mediated metabolism in cytosolic or S-9 fractions [23,78,84,165]. Meanwhile, the use of menadione as an inhibitor of AO *in vivo* is unlikely to be effective since it is rapidly reduced by DT-diaphorase [166]. Menadione has been observed to be roughly equally potent against AO in multiple species [84,167], whereas other inhibitors of AO appear to show profound species differences for inhibitor potency. For example, estradiol was found to inhibit vanillic acid formation from vanillin in liver cytosol from human, monkey, rat, and mouse at potencies ranging from IC_{50} of 0.3 μM (human) to 12 μM (monkey) [167]. In addition, methadone and SKF-525A inhibit rat AO, with little inhibition of human AO [84,168]. These findings suggest that it is unlikely that inhibition of AO in a particular species can be directly extrapolated to human AO.

In perhaps the most comprehensive attempt to screen for inhibitors of AO, Obach *et al*. [169] tested 239 frequently used drugs for inhibition of AO utilizing phthalazine oxidation to phthalazinone as a probe reaction. One of the more interesting findings from these studies was that among the inhibitors tested, raloxifene was found to be exquisitely potent ($IC_{50} = 2.9$ nM). Other drugs from a similar therapeutic class were also identified as inhibitors, including tamoxifen, ethinyl estradiol, and estradiol, and overall, 16 drugs were found to inhibit AO with an $IC_{50} < 1\,\mu$M. Overall, there was no one particular physical property that distinguished the most potent inhibitors from the weakest ones, although most inhibitors were weakly basic in nature. From a structural perspective, a tricyclic moiety was present in many potent inhibitors, including several tricyclic antidepressants, antipsychotic agents, and phenothiazines. Of these, perphenazine was found to be the most potent ($IC_{50} = 33$ nM). Other inhibitors of AO discovered in these studies worth mentioning (Fig. 11.27) are the potent CYP3A4

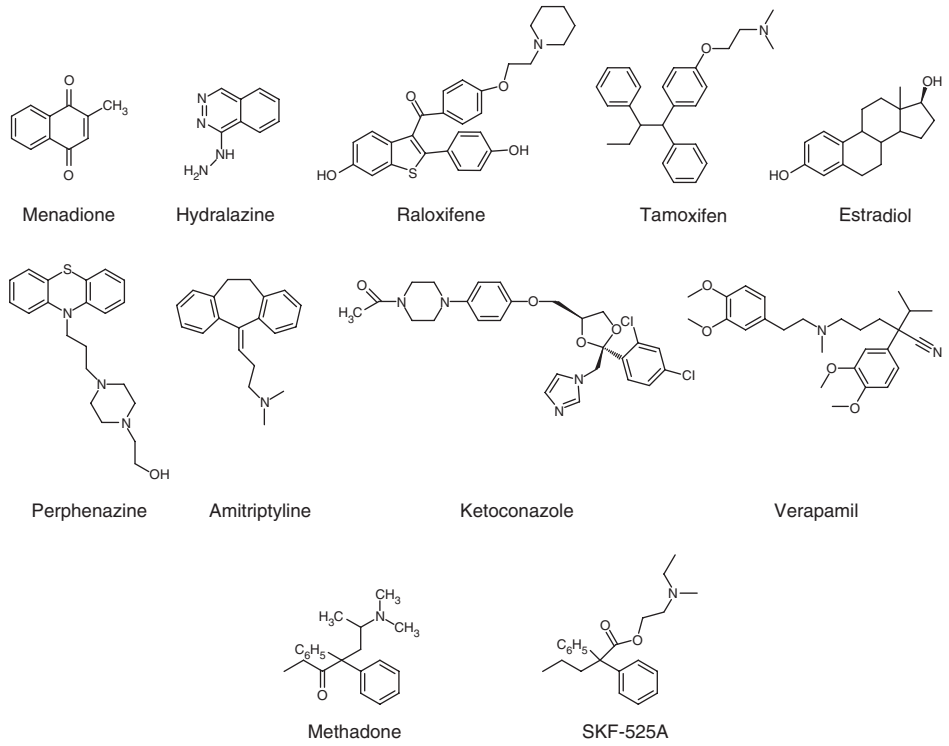


Figure 11.27 Inhibitors of aldehyde oxidase.

inhibitor ketoconazole ($IC_{50} = 3.5\,\mu M$) and the CYP3A time-dependent inhibitor erythromycin ($IC_{50} = 15\,\mu M$) and verapamil ($IC_{50} = 3.5\,\mu M$). Most recently, it has been highlighted that flavenoids, one of the largest groups of naturally occurring compounds that have been shown to modulate the activity of several drug-metabolizing enzymes, may also be effective inhibitors of AO [170,171].

As previously discussed, distinguishing AO from XOR-catalyzed reactions is relatively straightforward, as menadione selectively inhibits AO and not XOR. Raloxifene has also been found to selectively inhibit AO [172], and has been used to confirm the role of AO in the oxidation of zoniporide and zebularine [77,88]. However, when attempting to differentiate AO from cytochrome P450-mediated oxidations in more enzymatically complete systems such as hepatocytes, raloxifene likely does not possess the desired selectivity, as it is known to be potent time-dependent inhibitor of CYP3A4 [173]. In addition, menadione appears to be fairly nonselective for inhibiting AO, as it also crosses over to inhibit multiple P450 isoforms (unpublished observations, R.S. Obach). In contrast, hydralazine is reported to be a potent inhibitor of AO, both *in vitro*, and *in vivo* in rabbit [174] and guinea pig, where it was used to demonstrate the role of AO in the extensive first-pass metabolism of carbazeran [175]. Hydralazine apparently does not inhibit the cytochrome P450 enzymes (unpublished observations, R.S. Obach) [176], which makes it a potentially useful *in vitro* tool for the selective inhibition of AO in effort to understand the fraction of metabolism mediated by AO

compared to the cytochrome P450 enzymes for drugs that may have both pathways contributing to their total metabolic clearance.

11.5.1.2 **In Vivo *Drug–Drug Interactions*.** From a clinical standpoint, only one drug interaction has been attributed to inhibition of AO [177]. In a study in human volunteers, following an 800-mg oral dose of cimetidine, the oral clearance of zaleplon was significantly decreased (to 56% of control), resulting in an increase in maximum plasma concentrations (C_{max}) and area under the concentration–time curve (AUC) of zaleplon [178]. In addition, it was discovered that the 5-oxo metabolite (M2) of zaleplon was formed at a reduced rate after cimetidine administration, implicating inhibition of AO as the culprit. Follow-up *in vitro* studies by Renwick [177] demonstrated that cimetidine does in fact inhibit AO-mediated formation of M2 in a competitive fashion, albeit weakly, in both human liver cytosol ($K_i = 155\,\mu M$), and liver slices ($K_i = 506\,\mu M$). Considering this lack of potency for inhibiting AO, it is unclear whether this is the true mechanism of the observed *in vivo* interaction between cimetidine and zaleplon. However, despite the limited impact demonstrated in humans to date, it is conceivable that clinical drug–drug interactions mediated by inhibition of AO may become more prevalent. For example, there appears to be a shift in the chemical matter toward more heterocyclic-containing drug candidates in effort to reduce lipophilicity and cytochrome P450-mediated metabolism, which may result in increased metabolic clearance by non-cytochrome P450 drug-metabolizing enzymes such as AO. This observation, coupled with a number of clinically used drugs being identified as inhibitors of AO, suggests that this particular mechanism of drug–drug interaction may become more relevant in the future. The parameter that will ultimately drive the magnitude of any clinical drug–drug interaction will be fraction metabolized by AO ($f_{m,AO}$). If the $f_{m,AO}$ for any drug is >0.5, then the likelihood exists for some measurable (e.g., greater than equal to twofold) DDI in the clinic [179].

11.5.2 Inhibitors of XOR

11.5.2.1 **In Vitro.** Historically, there has been much less structural diversity among the known inhibitors of XOR, as the most effective inhibitors appear to be purine-based analogs. In addition, inhibition of XOR has been viewed as therapeutically beneficial, as it is involved in the biosynthesis of uric acid (Fig. 11.28). Allopurinol is the gold standard inhibitor of XOR, and is used therapeutically in the treatment of gout [180], a condition resulting from overproduction or underexcretion of uric acid and subsequent deposition of urate crystals in joints. Allopurinol has been characterized as a mechanism-based inhibitor of XO, whereby the product formed following hydroxylation at the 6-position (oxipurinol) complexes with the partially reduced MoCo, which prevents further electron transfer [181,182]. Xanthine oxidase enzymes may also be inactivated by cyanide, which works to remove the required sulfur atom from the molybdenum, making the enzyme catalytically inactive [183]. Arsenite also appears to be a general inhibitor of XOR and acts in a similar manner, which is an interaction with the critical sulfur atom at the molybdenum center [184]. Research into the mechanism of non-purine inhibitors has continued, with the recent report of a crystal structure of arsenite-inhibited XOR [185].

While allopurinol has been the treatment option of choice for patients suffering from gout for >30 years, a small percentage of the population suffers from adverse

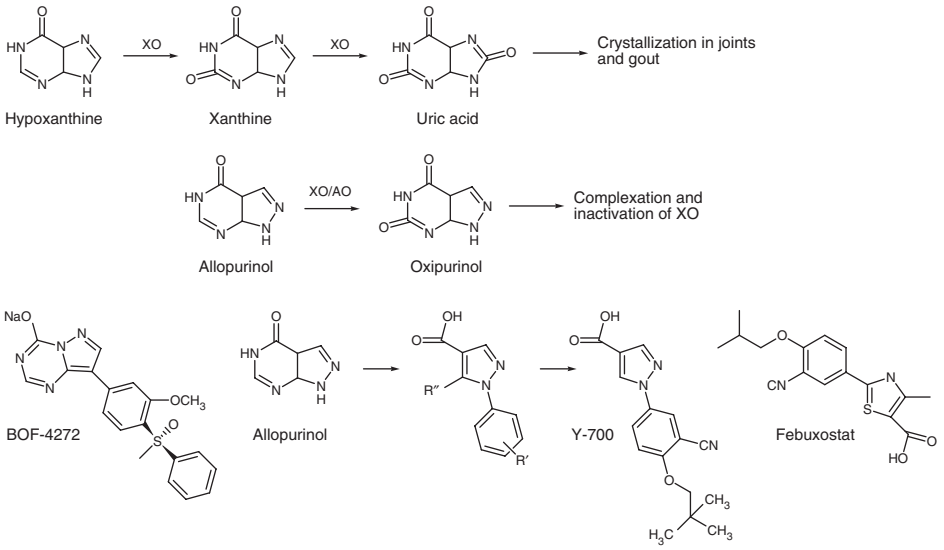


Figure 11.28 Inhibitors of xanthine oxidase.

events, including hypersensitivity reactions, toxic epidermal necrolysis, liver dysfunction, and acute nephritis [186,187]. These clinical observations have triggered numerous efforts to design potent inhibitors of XO that may be used therapeutically without the deleterious side effects. For example, BOF-4272, a pyrazolo-triazene derivative (Fig. 11.28), was found to bind tightly to both the oxidized and reduced forms of XOR, likely in the xanthine-binding site [188], and thus, was expected to inhibit the enzyme for a longer duration than allopurinol *in vivo*. This longer duration of action was in fact demonstrated *in vivo* in rats [189]. Structure–activity relationship (SAR) studies of a series of 1-phenylpyrazoles was reported in 2001 [190], with a number of compounds displaying enhanced inhibitory potency on XOR compared to allopurinol. The strategy employed here was to introduce a phenyl group at the N-1 position of allopurinol to effectively block conversion to unnatural nucleotides, as well as replacement of the 4-hydroxypyrimidine moiety with a carboxylic acid to enhance coordination with molybdenum (Fig. 11.28). From this effort, Y-700 (1-(3-cyano-4-neopentyloxyphenyl)pyrazole-4-carboxylic acid) was discovered as a potent inhibitor of XOR ($IC_{50} = 5.8$ nM) that displayed long-lasting hypouricemic effect in a rat pharmacology model, with and an ED_{20} of 0.55 mg/kg (0–6 h) and 1.4 mg/kg (0–24 h), both superior to allopurinol. Y-700 was subsequently dosed in humans where a single-oral dose (5, 20, or 80 mg) led to a dose-dependent reduction of serum uric acid levels [191]. Febuxostat, a structurally related non-purine selective inhibitor of XOR, was also found to be more potent ($K_i < 1$ nM) than allopurinol, selectively inhibiting both the oxidized and reduced form of XOR. In addition, febuxostat was more effective than allopurinol at lowering serum urate levels in patients [192], and was subsequently approved in February 2009 by the US Food and Drug Administration for the management of hyperuricemia in patients with gout (Uloric®). These few examples make it clear that a purine core is not obligatory for potent inhibition of XOR. The work to identify additional diverse inhibitors of XOR is extensive and extends beyond what we

are able to review here. For a more comprehensive summary of current XOR inhibitors, the reader is directed to a recent patent survey [193].

11.5.2.2 **In Vivo *Drug–Drug Interactions.*** Drug–drug interaction due to inhibition of XOR by allopurinol has been demonstrated *in vivo* with the anticancer agent 6-mercaptopurine (6-MP), which is metabolized by XOR to 8-hydroxy-6-MP, and then to thiouric acid [194]. When rhesus monkeys and humans were pretreated with allopurinol, peak plasma concentrations (C_{max}) of 6-MP following oral dose were markedly increased, in the order of 400% in monkeys and a 500% in man [102]. Allopurinol pretreatment also led to a 300% increase in plasma AUC in monkeys after oral 6-MP and a 500% increase in AUC in man. However, allopurinol pretreatment had minimal effect on the disposition of intravenously dosed 6-MP. This observed difference was concluded to be the result of inhibition of first-pass metabolism of orally dosed 6-MP by allopurinol, consistent with the known tissue distribution of XOR in the liver and intestine [195].

11.6 DISTRIBUTION OF MOLYBDENUM-CONTAINING HYDROXYLASES IN HUMANS

Subcellular distribution of molybdenum-containing hydroxylases is thought to be mainly centralized to the cytosol of the cell [116,196,197]. Therefore, these classes of enzymes are aqueous soluble proteins, and when prepared from tissue, these enzymes are primarily found in the 100,000 *g* supernatant fraction. There have been claims that both AO and XOR have been found in the mitochondrial fraction of liver homogenates from two species, rat and guinea pig [198,199], although there are claims that the activity observed could not be repeated in subsequent experiments [126]. Interestingly, Critchley *et al*. [198] provided evidence that in guinea pig liver XOR has 52% higher level of activity (μmol/min/mg) in the mitochondria than that of the cytosol. It was also found that AO in the cytosol versus the mitochondrial fraction have different substrate specificities, thus indicating at least two different forms of AO specific for a particular subcellular site. In a XOR specific study using electron microscopy by Frederiks and Vreeling-Sindelarova identified XOR in the peroxisomes of hepatocytes, and in multiple organelles of Kupffer and sinusoidal cells [196], including the rough endoplasmic reticulum, lysosomes, and endocytic vesicles. Since there is still some controversy as to the subcellular localization of both AO and XOR, more work to affirm such claims may be necessary.

The expression levels of mammalian molybdenum-containing hydroxylases have been studied with many different techniques. The techniques include immunohistochemistry, cytochemistry, Western blot, Northern blot, RT-PCR, and *in situ* hybridization of mRNA. Each one of these techniques has its strengths and weaknesses as pointed out by Garattini *et al*. [1], and Beedham [200]. The main issues with these techniques for molybdenum-containing hydroxylases are (i) the structural similarity of all the enzymes and the specificity of the antibodies used to assess the specific protein, (ii) the measurement of mRNA does not necessarily translate into active protein, and this is especially true with molybdenum-containing hydroxylases because of the complex assembly of the enzymes. However, when all the data is compiled for XOR and then AO, the results are telling. Utilizing all of these techniques, the basis of what we

have learned about the tissue distribution of XOR first came from experimental data in nonhuman species [201–203]. When we compare species, the mammary glands and the intestine seem to have higher levels of XOR than the liver. Human and cat are the exceptions to the rule, where these two species have approximately equal amounts in the intestine and liver. In most species, the proximal portion of the small intestine has the highest XOR enzymatic activity, and when quantified, the epithelial lining of the duodenum and the jejunum is rich in both mRNA and XOR protein [201,202]. A similar such abundance is supported in humans because of high observed activity in both epithelial and goblet cells throughout the villi of the intestine [204], which seems to peak midway through the villi. It has also been reported that activity, protein and mRNA indicative of XOR gene expression, is high in the human liver [201,203,205], but in human lung, expression is barely detectable [206]. Additionally, there have been observations that XOR is present in plasma [207,208]. In lactating mammary gland, acinar cells show high XOR activity compared to nonlactating mammary gland [204]. It has also been reported in humans that a large proportion (>80%) of XOR enzyme in milk is not active [209]. There are conflicting reports of XOR expression in the heart and brain [24,210,211]. Interestingly, XOR activity is increased in tumor tissue in the brain [212] and decreased or absent in aggressive breast cancer [213] hepatomas (rats) [214], and colon tumors [215]. A summary of relative amount of human XOR in tissues can be found in Table 11.2.

AO in humans is abundant in the liver [216] but has also been found in the kidney, pancreas, prostate, testis, ovary, and especially the respiratory system [25,216,217]. AO has also been observed via immunostaining in the adrenal gland, cortex, and the zona reticularis. It has been reported that AO in both the liver and adrenal glands has similar abundance and is the richest source of AO mRNA transcript [218]. AO has also been found in the glial cells in the spinal cord but not in neurons [24]. A summary of relative amount of human AO in tissues can be found in Table 11.3. An interesting comparison analysis by Garattini and Terao [55] of AO mRNA expression of human tumor and the corresponding normal tissues indicated that normal tissues produce large amounts of AO mRNA, where the corresponding neoplastic tissue produce very little. This finding is most relevant and potentially most useful for targeted drug delivery in the case of both liver and lung cancers.

11.6.1 Distribution of Molybdenum-Containing Hydroxylases in Other Species

In rodents, the distribution of AOX1 and AOX3 are overlapping. The most abundant source of these two enzymes in adult rodents is the liver. It was found that the stage of development of the mouse influences when one or the other is expressed. AOX3 is expressed in newborn mice, where AOX1 does not appear in measurable quantities until the mouse is fully grown [29]. Localization of AO in the liver of rats has been observed via histological staining. The staining of the hepatic tissue provides evidence that the distribution of the activity is not homogeneous, and seen mostly in the pericentral and not the periportal area [219]. The second richest tissue containing AOX1 and AOX3 in mouse is the lung; this observation has also been indicated in rat [220]. According to Garattini and Terao [55], mouse AOX3 mRNA is only expressed in the testis, where in mouse and rhesus monkey AOX3L1 is only expressed in the nasal mucosa. AOX1 mRNA has been detected in mouse testis, heart, and the epithelial layer of the esophagus [221], although, no translated protein can be found in either the heart

TABLE 11.2 Tissue Distribution of Xanthine Oxidoreductase (Number of "+" Denotes Relative Amount Observed, "–" Denotes None Observed)

Tissue	Human	Rat	Rabbit	Mouse	Guinea Pig	Bovine
Liver	+	++	—	++	+	+
Pericentral[a]	+	++	—	—	—	+
Periportal[a]	++	+	—	—	—	—
Kupffer cells[a]	+	—	—	—	—	+
Endothelial cells[a]	—	—	—	—	—	+
Kidney	+	+	—	+	+	—
Endothelial cells[a]	+	—	—	—	—	—
Glomerulus tubules[a]	—	+	—	—	—	—
Heart	−/+	+	+	+	—	+
Adrenals	+	+	—	—	—	—
Spleen	+	+	—	—	+	—
Skeletal muscle	+	+	+	—	—	+
Tongue	—	++	—	—	—	—
Esophagus	—	+	—	—	—	—
Stomach	—	+	—	—	—	—
Small intestine	++	+++	—	++	+	—
Large intestine	—	+	—	+	+	—
Lung	—	++	—	+	+	—
Bronchioles[a]	—	++	—	—	—	—
Alveolus[a]	—	++	—	—	—	—
Brain	−/+	—	+	—	—	—
Mammary gland	++	—	—	+	—	+
Milk	++	—	—	—	—	++

[a]Subtissue.

Source: Data adapted from the work of Christine Beedham [200].

or esophagus [221]. Interestingly, Kurosaki *et al*. [221] found that brain AO activity was much higher than what the levels of mRNA would have dictated, suggesting that the synthesis of the mouse AO is under translational and posttranslational control. Unique to only a few species, including rodent, but not human and chimpanzee, AOX4 protein is highly expressed at the Harderian gland, a specialized structure in the orbital cavity of the eye region [222]. These types of unique sources of AO may be of consequence when trying to extrapolate human pharmacokinetics from other species and using multiple AO substrates. A summary of AO species differences in tissues can be found in Table 11.3.

Moderate XOR activity has been found in mouse intestine and liver, although XOR mRNA was found at low levels in all tissues studied [203]. Only when subjected to interferon and induction of XOR was XOR mRNA found in mouse liver, intestine, kidney, heart, and lung.

Krenitsky *et al*. [223] when comparing liver and intestinal XOR activity utilizing xanthine as a substrate, found that rabbit XOR oxidase activity was lower than that of mouse, rat, or guinea pig, although, xanthine dehydrogenase activity in rabbit has been shown in heart, brain, and skeletal muscle [224]. When studying guinea pig crude extracts for AO and XOR activity, Beedham *et al*. [225] measured activity in the liver, lung, kidney, intestine, spleen, and heart. Two of the three substrates were specific for

TABLE 11.3 Tissue Distribution of Aldehyde Oxidase (Number of "+" Denotes Relative Amount Observed, "–" Denotes None Observed)

Tissue	Human	Rat	Rabbit	Mouse	Guinea Pig	Bovine
Liver	+	++	+++	+	++	++
Pericentral[a]	+	++	—	—	++	—
Periportal[a]	—	+	—	—	—	—
Kidney	+	—	++	—	+	+
Glomerulus tubules[a]	—	+	—	—	—	—
Heart	—	+	+	+	—	—
Spleen	—	—	—	+	+	+
Skeletal muscle	—	—	—	—	—	—
Tongue	—	+	—	—	—	—
Esophagus	—	++	—	+	—	+
Stomach	—	+	1/4+	—	—	—
Small intestine	—	+	—	—	+	—
Large intestine	—	+	—	+	+	—
Lung	+	++	++	+	+	+
Bronchioles[a]	—	++	—	—	—	—
Alveolus[a]	—	+	—	—	—	—
Brain	++	—	+	++	—	—

[a]Subtissue.

Source: Data adapted from the work of Christine Beedham [200].

AO (phenanthridine and phthalazine) and one was specific for XOR (xanthine). AO activity in guinea pig was observed highest in the liver, and for the kidney, the AO activity was ~40% of that observed in the liver. XOR activity was found highest in the small intestine, the jejunum, followed by the duodenum, ileum, liver, spleen, kidney, and lung. A summary of XOR species differences in tissues can be found in Table 11.2.

Unfortunately, studies on both dog and cat tissue-specific and cell-specific distributions are few, although, Terao *et al*. [56] have provided a substantial amount of AO and XOR distribution data for the dog in a single publication. It is reported that dogs are devoid of liver AO activity. Dogs, however, do express homologs of AO in very specific regions of the body. The expression of the AOX4 protein in dogs is found in the lacrimal gland, which is a functional substitute for the rodent Harderian gland. The other actively expressed AO protein, AOX3L1, is expressed similarly in the mouse and rhesus monkey, only in the nasal mucosa. On the other hand, dog XOR mRNA is ubiquitously expressed at low levels in multiple tissues [56].

11.7 XOR AND AO ACTIVITY VARIATION

Humans have a wide range of XOR activity, which spans >3.6-fold variation in enzyme activity [226]. Although the variation is substantial in humans when compared to other mammals, humans have low enzyme activity [227]. There are conflicting reports on the influence of gender on XOR activity [226,228]. Guerciolini reports that males have higher XOR activity than females, while Relling reports the opposite. These studies were conducted quite differently, and may be influenced by study specific variables. Guerciolini's study utilized cytosolic liver fractions from Caucasian patients, while

Relling's study measured caffeine metabolites in urine of both Caucasian and African-descent healthy volunteers. Unfortunately, Relling's reported results do not stratify by ethnicity and gender together to understand the impact of both at the same time. This stratification could potentially help explaining the different outcome on gender from the two studies.

Variation in active XOR has also been observed when comparing milk of different species. Abadeh *et al*. [209] found that although the expression levels were similar in both bovine and human milk, the human XOR activity for xanthine metabolism was 1–6% of that in bovine. It has been observed that a majority of this disparity can be attributed to two inactivated forms of XOR, either demolybdo-XOR or desulpho-XOR [209,229].

Early works by Krenitsky *et al*. [98,223] provided a unique insight in to the type of mammals that express high levels of AO and those which do not. In these works, it is noted that herbivores have extremely high levels of AO, where carnivores have low levels. It has been alluded that the high levels of AO in herbivores is because of certain dietary plants that might be rich in sources of AO substrates. Perhaps uniquely because of the omnivore status, humans and primates [230] possess some AOX1 activity in the liver.

Several works have displayed marked differences in AO activity within a single animal species, as well as between species [100,231], including some marked differences with famciclovir [104]. In an *in vitro* study conducted by Al-Samy *et al*., variation of hepatic AO was observed utilizing two AO substrates, DACA and benzaldehyde. Differences in both the K_M and V_{max} was observed from 13 human individual cytosolic fractions from both males and females [232]. Clearance differences between donors ranged from 16.6-fold with DACA and 2.75-fold for benzaldehyde, which is similar to other reported human AO variation for benzaldehyde [233]. Another work has reported up to 50-fold variation for benzaldehyde human AO activity [234]. Additionally, work by Yerino *et al*. [235] presented at the 2007 International Symposium of the Study of Xenobiotic (ISSX) provides a look at 23 donor cytosolic fractions with two AO substrates, which is more than any other study in the literature [232–234]. When reanalyzing the data of phthalazine or *p*-vanillin in a histogram and applying the D'Agostino and Pearson omnibus normality test, the activities of the 23 donors suggests a Gaussian distribution (Fig. 11.29).

A larger cytosolic sample set would be a more appropriate measure of population distribution, but none is presently available. The granularity of a larger study would help the hypothesis that the source of this intraspecies variability is because of multiple polymorphic enzymes distributed throughout the population [59,236]. Recently, the development of a noninvasive method for determining AOX1 activity *in vivo* [237] has been employed in the understanding of developmental AO activity in rats and Japanese children [238,239]. In Japanese children and the adults that they were compared to, a considerable interindividual variation was observed with a fourfold range in activity in individuals greater than one year of age. This method could also be employed to understand the frequency of activity in large populations and distribution modality of polymorphic AO. One of the most revealing examples of the existence of a polymorphic AO enzyme, in a species which expresses AO, was a series of works by Itoh *et al*. [31,32,240] conducted in rats. This series of experiments provided some insight as to why intra- and interstrain differences in AO activity are observed, with little or no observable change in mRNA transcription. In the earliest of the works [31], multiple

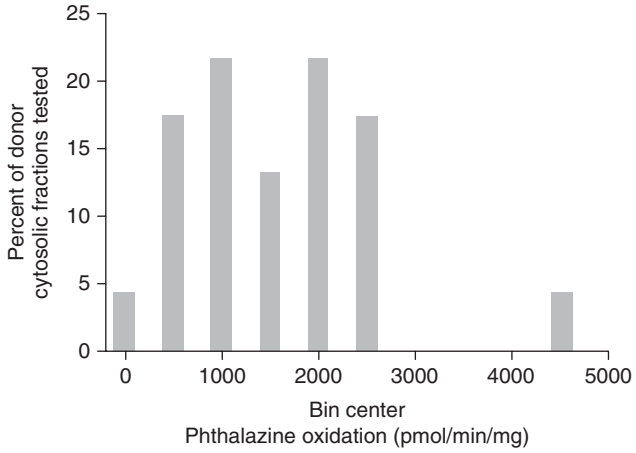


Figure 11.29 Histogram of phthalazine oxidation from 23 donors. *Source*: The data was acquired from the work of Yerino *et al*. presented at the 2007 ISSX meeting [235].

point mutations were observed, which divided the Donryu rats into ultra metabolizers (UM), extensive metabolizers (EM), and poor metabolizers (PM). As previously described in Section 11.2.1, two mutations were identified as being important, G377A and C2604T, thus translating to amino acid changes of 110Gly-Ser and 852Ala-Val. The UMs and PMs were homozygous at all four positions, where the EMs were heterozygous. Therefore, UMs translate to the amino acid at location 110 being a glycine and an alanine at amino acid 852, which differs in PMs translating to a serine and a valine, respectively. The observed specific enzyme activity in the UMs versus the EMs was greater than twofold V_{max}, and a 20- to 40-fold difference in clearance (K_M/V_{max}) of the S-enantiomer of RS-8359 was observable in the EMs versus the PMs. This effect was thought to be due to a change at amino acid position 110, which is conserved among rat species. Since this nucleic acid is near the Fe–S center, and the change shifts the isoelectric point from pH 5 to 6.2 from EMs to PMs. It was thought that this change of isoelectric point may case a conformational change of the AO. In a later study investigating interstrain difference in activity, Itoh *et al*. [240] observed that a lack of homodimerization of the enzyme was responsible for the reduced activity in-between strains of rat. Further work implicated that the same point mutations (G377A and C2604T) drive the dimerization process and effectively drives the activity [32].

Species differences are most definitely due, in part, to the expression and the differing distribution of species-specific isoforms. There are many examples of variability in AO-metabolized drugs in different species. For example, Zaleplon [108] had been reported to have marked species differences between monkey and rat. SGX523 shows acute renal failure in humans because of obstructive nephropathy from a particular poor soluble AO urinary metabolite, not seen in preclinical toxicology species. Diamond *et al*. conducted studies to understand the production of this poorly soluble metabolite in multiple species. The specific AO metabolite was formed in humans and monkeys and to a much lesser extent in rat and none in dog. Interestingly, several prodrugs of an antiviral agent, 9-(3-hydroxyproppoxy)guanine, are even activated by different molybdenum-containing hydroxylases. In rats, XOR was the responsible

enzyme, where as AO catalyzed the reaction in humans [241]. Carbazeran, a successful inotropic agent in dogs, is another interesting example of AO species differences. It has been observed that carbazeran is extensively metabolized utilizing baboon and human liver cytosol *in vitro* but stable in dogs [91,92]. The phenomenon in dog is not specific just to carbazeran. Dog AO activity has been measured with a variety of substrates and found to be very low or absent [100,223,242]. Also of interest is the data suggesting that carbazeran metabolism is species specific. Both rabbit [243] and mouse (unpublished work, Fig. 11.30) show little or no AO-specific metabolism of carbazeran. In humans, baboons, marmosets, and guinea pig, affinity in the single digit

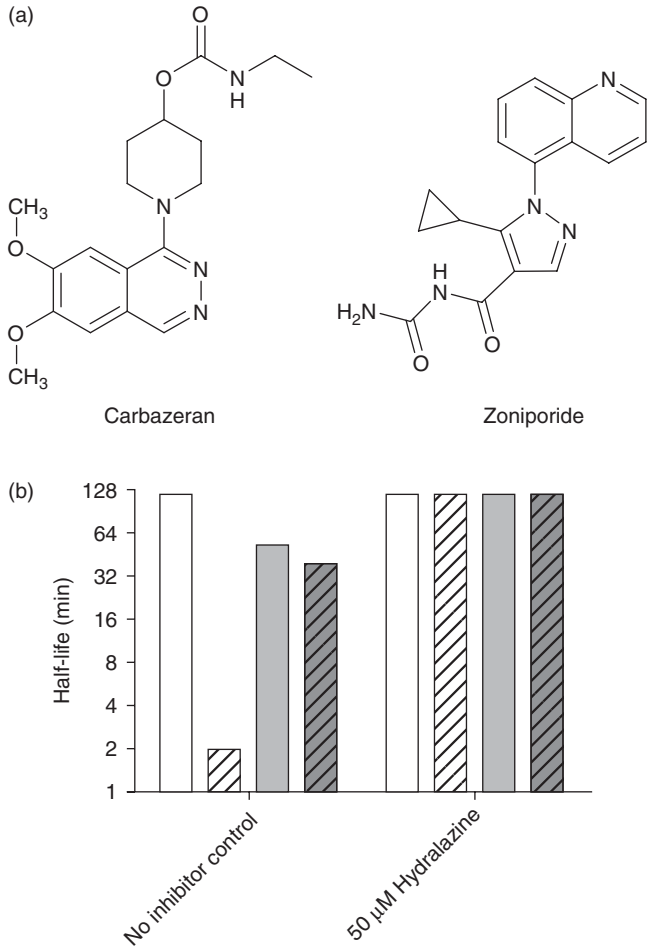


Figure 11.30 (a) Structures of carbazeran and zoniporide. (b) Unpublished results of a substrate depletion time course assessment of 1 μM carbazeran (white) and zoniporide (gray), utilizing 2 mg/mL of mouse (no pattern) and human (diagonal lines) S-9 liver fraction in the presence and absence of 50 μM hydralazine (a potent and specific AO inhibitor) [169,175] in 100 mM potassium phosphate buffer pH 7.4. Zoniporide [88] was assayed as a parent compound depletion positive control of both mouse and human AO. Maximum extrapolated half-life was 120 min. *Y*-axis displayed on the natural log scale for better visualization.

to 100 μM range with a moderate to very high V_{max} (20–150 mol/min/mg) is observed. The V_{max} for rat is much lower for carbazeran, which is similar to what is observed in the mouse (Fig. 11.30). This outcome may suggest that the rodent family may share the lack of activity with the rabbit.

11.7.1 Regulators and Inducers of Molybdenum-Containing Hydroxylases

XOR has a variety of mechanisms that govern its protein expression and level of enzymatic activity. One of the most studied is modulation by cytokines. Of the cytokines, interferon has been characterized by a number of laboratories [244–247]. The impact was shown via a mouse study using either an artificial increase in type I interferon or an interferon inducer such as bacterial lipopolysaccharide. A substantial induction of XOR was observed in multiple tissues in the mouse model [203]. In a complementary study using epithelial cells *in vitro*, a similar response was reproduced [244]. These studies suggest that this induction of XOR is a feedback mechanism to help mediate certain features of interferon activity. Studies inhibiting XOR activity do not support the involvement of XOR in influencing the therapeutic or detrimental effect of interferon [248].

In other examples of cytokines influencing XOR transcription, the proinflammatory cytokines, tumor necrosis factor α (TNF-α), interleukin-1, and interleukin-6 levels also increased XOR mRNA levels in human kidney epithelial cells [244,245]. Anti-inflammatory corticosteroids have also been found to induce XOR expression. This phenomenon is surprising, since both anti-inflammatory and proinflammatory cytokines induce XOR. Regardless, the induction triggered by both of these proinflammatory and anti-inflammatory cytokines suggests a potential XOR function in inflammation.

Other non-cytokine and exogenous stimuli are capable of inducing XOR as well. Linder [249] reports that oxygen tension has a significant effect on XOR expression, where hypoxia increases XOR, while hyperoxia tends to decrease the observed activity. It was noted that there are observations of both hypoxia and hyperoxia effecting XOR both on the transcriptional and posttranscriptional level, depending on the cell line studied and the species line was derived from. Both phorbol-12-myristate-13-acetate (PMA) and tetrachlorodibenzo-*p*-dioxin (TCDD) are inducers of XOR gene expression in many tissue types and animal species [250–256]. PMA has a questionable mechanism; it is unclear whether it directly induces or influences the production of inflammatory cytokine. Alternatively, TCDD is an environmental pollutant that induces XOR activity directly via activation of the TCDD receptor, or otherwise known as the *Ah receptor*. XOR induction derived from the TCDD receptor in the liver is suspected to be the reason for TCDD-induced liver damage. TCDD is thought to induce liver damage by the increased XOR expression and the coinciding increased production of superoxides.

Regulation of AOX has been proposed to be a consequence of multiple factors, both genetic and endogenous. Interindividual variability in AOX1 expression in humans has been suggested to be responsible for both methotrexate pharmacokinetic variations in the clinic [100]. Garattini and Steinberg [59,236] have posed that the variations seen in the methotrexate study maybe a consequence of allelic variants or polymorphic enzymes, which express a catalytically defective enzyme. Endogenous factors such as sexual steroid hormones have been shown to regulate gender-specific difference in AO levels in the liver of mice [221,257,258]. The mRNA of mouse AOX1 seems to

increase with levels of androgens, where as estrogen decreases the levels of AOX1 mRNA. This influence of androgens could be responsible for the observed difference in the non-preclinical species, the camel. A twofold increase in AO-specific activity in males opposed to female camels was observed using phenanthridine as a specific probe [259]. Another example is zebularine; when studied in cancer prone Apc(Min/+) mice, showed gender-specific expression which influenced its chemopreventive activity for intestinal polyposis [260,261]. In humans, *in vitro* and *in vivo* comparisons have taken place. The data suggests little if any gender-influenced AO activity differences. *In vitro* studies by Al-Salmy *et al*. [238] did not detect a relationship between gender and AO activity, which correlates with the aforementioned clinical study in Japanese children and adults. Additionally, with limited clinical data, penciclovir also shows little to no gender difference [262].

Exogenous compounds, such as the previously mentioned TCDD [263], as well as methansulfonate [264], nitrosoureas [264], nitroguanidines [264], curcumin [265], and phenethyl isothiocyanate [266] have been demonstrated to induce AOX1.

11.7.2 Ontogenic Expression

No correlation has been associated with age and XOR enzyme activity, regardless of the method of analysis [226,228,267]. This also includes a study of allopurinol metabolism, which according to Turnheim *et al*. is a function of XOR activity. They observed that in elderly subjects compared to young controls, the clearance of allopurinol is unchanged. Others have reported that allopurinol is metabolized primarily by AO. If taken together, one could conclude that neither AO nor XOR vary in elderly subjects compared to younger controls. An AO-specific example is famciclovir, in which elderly subjects produced similar amount of the active metabolite penciclovir in urine as patients. This result is somewhat clouded because of the reduced renal clearance of penciclovir in the elderly [268,269]. There have been multiple studies looking at very early development stages in multiple species of both AO and XOR. In a rabbit study by Nichols *et al*., a lack of AO variation across age groups and life events was observed. Their data indicated that hepatic tissue from nonpregnant, pregnant, fetal, and newborn rabbits did not differ significantly in its ability to reduce 2,6-dichloroindophenol in the presence of acetaldehyde substrate. In another study utilizing mice at various stages of development, Holmes [270] observed that up to three weeks from birth, no XOR was present, whereas AOX-1 could be detected in a 20-day-old fetus. There has been a single study in humans conducted comparing the relative amounts of nicotinamide and its metabolites in urine of young Japanese children and adults [238]. The study population consisted of 101 children, of both sexes ranging in age from shortly after birth to 10 years of age, and 26 adults. When relative urinary product values of the children were compared to the adults, it was found that AO activity rapidly increased in a linear fashion with age to adult levels within first 365 days of life. Also of interest was that AO activity of neonates was found to be ~10% of those observed in adults. No data were available for fetal AO activity, but the expectation is that the activity would not surpass those of neonates. It was suggested that since AO activity is immature in human neonates and infants, perhaps there is a need to make dose adjustments with drugs such as methotrexate and cyclophosphamide based on AO activity to avoid increase risk of side effects including leucopenia, thrombocytopenia, liver failure, renal

failure, and nephropathy [238]. Also, when all the species data is placed together, the data indicates that there is species-specific triggering of AO expression at certain stages of early life, although this phenomenon might be homolog influenced, thus also species specific.

11.7.3 Ethnic Differences

The activity of XOR in the human population appears to follow a normal Gaussian distribution [226,271,272], although marked variation in expression and activity has been observed in some ethnic populations. As previously mentioned in Section 11.7, Relling *et al*. measured caffeine metabolites as indicators of specific drug-metabolizing enzyme activities. Utilizing ratios of the metabolite (1-methyluric acid) to substrate (1-methylxanthine), a comparison of xanthine oxidase activity was made between Caucasians, subjects from African descent, males, and females. It was found that subjects from African descent and males, in general, have lower XOR activity. Interestingly, Relling identified that the lowest xanthine oxidase activities were observed in males from African descent, and that the highest activities were observed in the females of both ethnic groups, but stated that the distributions within each gender according to ethnic group are not bimodal. However, the author later stated, in a personal communication that compared the potential impact of environmental and iatrogenic influences, such as methotrexate, exercise, interferons, smoking, and other variables on xanthine oxidase activity, the impact of gender and ethnicity is unlikely to be substantial. As far as the authors are aware, this type of ethnic analysis has not been conducted with an AO-specific substrate.

11.8 *IN VITRO–IN VIVO* CORRELATION (IVIVC)

XOR has a narrow substrate pool compared to AO, and thus as far as the authors are aware, there has not been an attempt to perform an *in vitro–in vivo* correlation. In 2010, Zientek *et al*. [273] attempted an IVIVC for human AO. Eleven test compounds were assayed using two *in vitro* systems (pooled human liver cytosol and liver S-9 fractions) to calculate scaled unbound intrinsic clearance, and were then compared to calculated *in vivo* unbound intrinsic clearance, to examine if any correlation existed. The correlation offered a means by which newly synthesized compounds that are shown to be metabolized by AO can have a prediction made for whether it will have a high, medium, or low CL'_{int} (Fig. 11.31) in the clinic.

The *in vitro* system chosen, be it pooled human liver cytosol or S-9 fraction, can be calibrated using high, medium, and low CL'_{int} selected from the 11 described in Fig. 11.31. Then a CL'_{int} can be obtained for a new compound and measured in relation to the chosen controls. The authors suggest that compounds with AO-mediated CL'_{int} less than that of zaleplon would be predicted to be low CL'_{int} *in vivo* and compounds with CL'_{int} equal to or greater than 6-deoxypenciclovir, zoniporide, or O^6-benzylguanine would be predicted to be high CL'_{int}. Thus, a relative scale has been provided that can be used for *in vitro–in vivo* correlation of AO clearance and can aid in directing drug discovery programs regarding when structural modifications are needed because of unacceptably high aldehyde-oxidase-mediated metabolism.

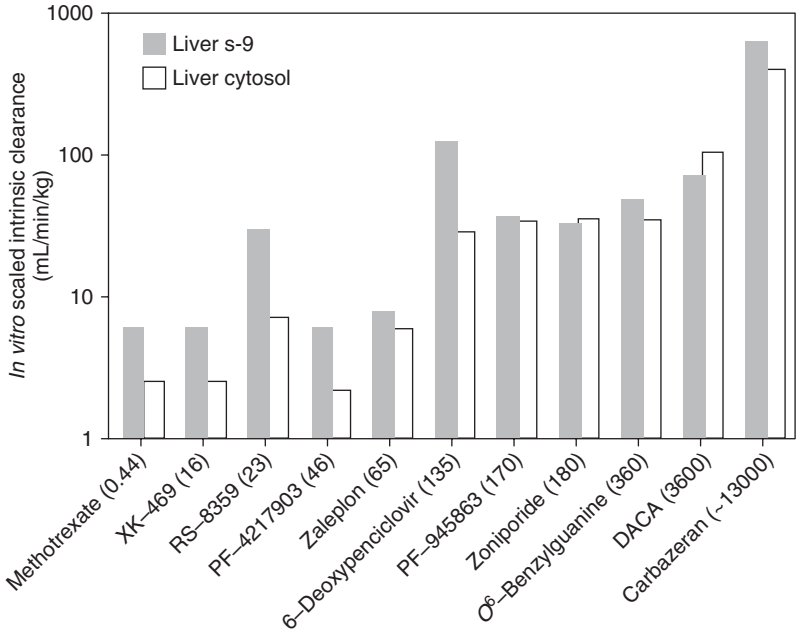


Figure 11.31 Bar graph calibrating *in vitro* scaled intrinsic clearance with *in vivo* free intrinsic clearance for 11 compounds metabolized by aldehyde oxidase in humans. Numbers in parentheses refer to estimated *in vivo* free intrinsic clearance. Bars are placed in the order of *in vivo* free intrinsic clearance (mL/min/kg). *Source*: Bar graph adapted from Zientek *et al*. [265].

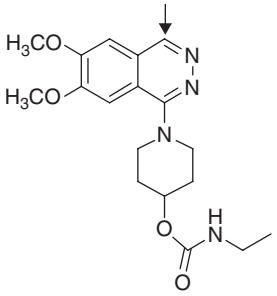
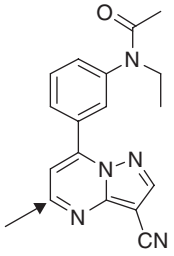
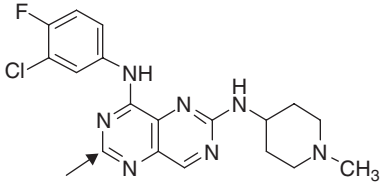
11.9 CLINICAL IMPLICATIONS

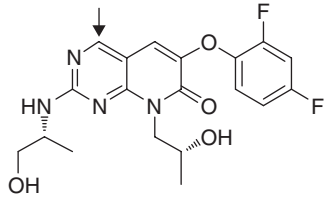
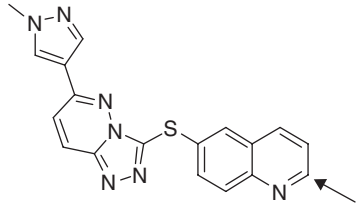
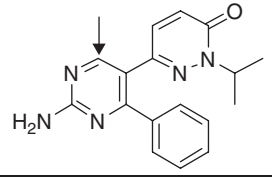
From a drug attrition standpoint, AO appears to have substantially more impact on clinical programs than XOR. Interest in AO by the pharmaceutical industry has increased dramatically of late [2,55]. Several recent literature reports have demonstrated that extensive metabolism by AO has led to the ultimate termination of clinical candidates, either due to toxicological outcomes or due to higher-than-predicted clearance in humans, yielding unacceptable pharmacokinetic properties. In each of these cases, unexpected results were encountered in the clinic, likely because metabolism of these candidates was only evaluated in liver microsomes, which are devoid of AO activity, or predictions of human clearance were extrapolated from preclinical species (e.g., rat, dog) that possessed significantly lower AO activity relative to humans. Metabolically, despite both oxidative and reductive mechanisms catalyzed by AO, oxidation of heterocyclic ring systems appears to be the most relevant pathway for drugs that have met their demise in clinical studies (Table 11.4).

11.9.1 Carbazeran

Carbazeran was an inotropic drug developed in the early 1980s that was perhaps one of the first examples of a clinical candidate that fell victim to extensive AO-mediated metabolism [92]. In early pharmacological investigations in dog, it was observed that carbazeran demonstrated good oral bioavailability (62–74%), which lead to a favorable

TABLE 11.4 Summary of Drug Candidates that have Either been Discontinued Because of Poor Oral Exposure in Early Clinical Studies, had Human Clearance Values Underpredicted, or Demonstrated Toxicity Attributed to Aldehyde Oxidase Metabolism

Drug	Structures	Preclinical Data Package Used for Human PK Predictions	Clinical Outcome	References
Carbazeran		Dog PK	<5% F-Termination	91
Zaleplon		Liver microsomes, mouse, rat, and dog PK	Clearance underpredicted (1 h half-life)	274
BIBX1382		Liver microsomes, mouse, rat, and dog PK	5% F-Termination	275

RO1		Rat, dog, and monkey PK	0.7 h half-life—termination	276
SGX523		Liver microsomes, rat and dog PK	Obstructive nephropathy—Termination	23
FK3453		Liver microsomes, rat and dog PK	M4 metabolite exposure ≫ FK3453—Termination	78

The arrow indicates the major site of metabolism by aldehyde oxidase.

pharmacodynamic profile in that species. However, following oral dosing to humans up to 350 mg, plasma levels of carbazeran were essentially undetectable, with oral bioavailability <5%. The observed profound differences in pharmacokinetic profile in dog and human has been attributed to the lack of AO activity in dog, with the predominant metabolite in both baboon and human liver cytosol being the AO-derived 4-phthalazinone metabolite [91], which illustrates the extreme importance of understanding species differences in metabolism and identifying a species with AO activity that is representative of human.

11.9.2 Zaleplon

Zaleplon is a sedative hypnotic used mainly in the treatment of insomnia. Clinically, the clearance of zaleplon was underpredicted roughly sixfold, which resulted in a short half-life in humans of 1 h [274]. It was later published that one of the predominant metabolites of zaleplon was 5-oxo zaleplon, confirmed to be AO derived by both chemical inhibition studies, and incubations conducted in the presence of $H_2^{18}O$ [165]. As it turns out, development of this drug was actually not discontinued since the short half-life actually provides advantages for a sleep aid.

11.9.3 BIBX1382

BIBX1382 was a drug candidate being evaluated clinically as an inhibitor of epidermal growth factor receptor (EGFR) for the treatment of cancer. In human liver microsomes, it was observed that BIBX1382 was mainly metabolized by CYP2D6, and in preclinical pharmacokinetic evaluations in rat and mice, oral bioavailability ranged between 50% and 100%. Following oral dosing to human patients, plasma levels were far below that expected to be efficacious (~5% oral bioavailability), and a previously uncharacterized metabolite was observed in the urine of one patient, that was also circulating in human plasma at high concentrations [275]. Retrospective experiments by Dittrich *et al*. also confirmed that BIBX1382 was metabolized by hepatic AO, and that the rhesus monkey appeared to be a suitable surrogate for human AO metabolism.

11.9.4 RO1

The pharmacokinetics of a novel p38 MAP kinase inhibitor was evaluated in six healthy male subjects following a 50-mg oral dose. Surprisingly, the observed terminal mean half-life was only 0.7 h, even though a half-life of 5.9 h had been predicted using common allometric scaling approaches based on pharmacokinetic data from rats, dogs, and monkeys [276]. The major drug metabolite of RO1 (M1) represented 220% of the AUC of parent drug, and was shown to be generated by AO.

11.9.5 SGX523

Extensive metabolism by AO may not only result in poor pharmacokinetic properties but also toxicity. SGX523 was a clinical candidate for an oncology target (c-MET, a receptor tyrosine kinase) that progressed because of favorable potency and selectivity profile for the target, as well as a comparable metabolite profile in liver microsomes from rat, dog, monkey, and humans. Thus, conventional drug safety studies were

conducted in rats and dogs to enable first-in-human (FIH) studies. However, following doses administered to human subjects of >80 mg, acute renal failure was observed [23]. Interestingly, metabolite profiling of human plasma indicated a major metabolite that was not observed *in vitro* in liver microsomes. Retrospective analysis by Diamond *et al*. demonstrated a major NADPH-independent metabolite (M11, 2-quinolinone-SGX523) when SGX523 was incubated with liver S-9 fraction, which was subsequently shown to be generated by AO. In addition, male cynomolgus monkey was shown to generate the M11 AO metabolite in substantial quantities *in vitro*, and safety studies were thus conducted in this species, where the adverse renal effects were reproduced. The ultimate cause of the renal toxicity was deemed to be the M11 metabolite that crystallized in the kidney because of poor solubility. This represents a great example of not just considering pharmacokinetic outcomes, but also toxicity outcomes, highlighting the critical importance of identifying an appropriate toxicology species with a comparable metabolite profile to humans.

11.9.6 FK3453

The most recent example of poor oral exposure in humans that was not predicted is FK3453, a novel adenosine A1/2 dual inhibitor being evaluated in Japan for the treatment of Parkinson's disease. While FK3453 underwent minimal cytochrome P450-mediated metabolism *in vitro* and demonstrated favorable preclinical pharmacokinetic properties in rat and dog, the development of this compound was discontinued because of extremely low oral exposure in humans [78]. It was again found that the poor oral exposure was the result of extensive metabolism by AO.

In summary, when one considers the first example of carbazeran failing in the clinic because of extensive AO-mediated metabolism, which is a time spanning over 25 years, it suggests that first principles in biotransformation are not being followed consistently. Understanding the complete metabolism of drug candidates, and not just the metabolism in human liver microsomes, is crucial. As medicinal chemists have successfully optimized small molecules via chemical modification to have less cytochrome P450-mediated metabolism, other drug-metabolizing enzymes such as AO may begin to play a larger role in drug biotransformation. Thus, a renewed focus by drug metabolism scientists on these types of non-P450 metabolic pathways should become standard practice. These standard practices, as suggested by Pryde *et al*., should include evaluating metabolism in cofactor-supplemented liver S-9 fraction or isolated hepatocytes [2].

11.10 FUTURE PERSPECTIVES

Since molybdenum-containing hydroxylases are a complex enzyme family, and what is known about these enzymes is in its infancy compared to that of the cytochrome P450s and perhaps uridine 5′-diphospho-glucuronosyltransferase, much work is needed to put in perspective the implications of metabolism for this class of enzyme. As the science of molybdenum-containing hydroxylases in humans and preclinical species matures by building on the foundation of the current knowledge, it will become easier to relate these enzymes in drug research. There are active efforts underway to solidify chemical structure–activity relationships, and once better understood will become a

larger part of drug design. These structural advancements will provide scientists with the understanding as to the diversity of metabolic reactions and mitigation or minimization of molybdenum-containing hydroxylase metabolism. Tools such as specific inhibitors will provide ways to elucidate the contribution of such enzymes, once universally accepted enzyme abundance values has been achieved. From the literature, there have been efforts to understand the enzyme variability in population and the reasons why. This is an area where it is believed will benefit from more intensive research. Taken together, structural understanding, contribution to metabolism, and population variability will place this enzyme family in perspective compared to, and in addition to other drug-metabolizing enzymes.

REFERENCES

1. Garattini E, Mendel R, Romao MJ, *et al*. Mammalian molybdo-flavoenzymes, an expanding family of proteins: structure, genetics, regulation, function and pathophysiology. Biochem J 2003;372(Pt 1):15–32.
2. Pryde DC, Dalvie D, Hu Q, *et al*. Aldehyde oxidase: an enzyme of emerging importance in drug discovery. J Med Chem 2010;53(24):8441–8460.
3. Rettie AE, Fisher MB. Transformation enzymes: oxidative; Non-P450. In: Woolf T, editor. Handbook of drug metabolism. New York: Marcel Dekker, Inc.; 1999. pp. 131–151.
4. Knight ZA. Small molecule inhibitors of the PI3-kinase family. Curr Top Microbiol Immunol 2010;347:263–278.
5. Knight ZA, Shokat KM. Features of selective kinase inhibitors. Chem Biol 2005; 12(6):621–637.
6. Liu Y, Gray NS. Rational design of inhibitors that bind to inactive kinase conformations. Nat Chem Biol 2006;2(7):358–364.
7. Xia M, Dempski R, Hille R. The reductive half-reaction of xanthine oxidase. Reaction with aldehyde substrates and identification of the catalytically labile oxygen. J Biol Chem 1999;274(6):3323–3330.
8. Moriwaki Y, Yamamoto T, Higashino K. Enzymes involved in purine metabolism—a review of histochemical localization and functional implications. Histol Histopathol 1999;14(4):1321–1340.
9. Hille R, Sprecher H. On the mechanism of action of xanthine oxidase. Evidence in support of an oxo transfer mechanism in the molybdenum-containing hydroxylases. J Biol Chem 1987;262(23):10914–10917.
10. Vorbach C, Scriven A, Capecchi MR. The housekeeping gene xanthine oxidoreductase is necessary for milk fat droplet enveloping and secretion: gene sharing in the lactating mammary gland. Genes Dev 2002;16(24):3223–3235.
11. Topham RW, Woodruff JH, Walker MC. Purification and characterization of the intestinal promoter of iron(3+)-transferrin formation. Biochemistry 1981;20(2):319–324.
12. Zikakis JP, Dressel MA, Silver MR. Bovine, caprine, and human milk xanthine oxidases: isolation, purification, and characterization. In: Charalambous G, Inglett G, editors. Instrumental analysis of foods. New York: Academic Press; 1983. pp. 243–303.
13. Hancock JT, Salisbury V, Ovejero-Boglione MC, *et al*. Antimicrobial properties of milk: dependence on presence of xanthine oxidase and nitrite. Antimicrob Agents Chemother 2002;46(10):3308–3310.
14. Stevens CR, Millar TM, Clinch JG, *et al*. Antibacterial properties of xanthine oxidase in human milk. Lancet 2000;356(9232):829–830.
15. Tubaro E, Lotti B, Santiangeli C, *et al*. Xanthine oxidase increase in polymorphonuclear leukocytes and macrophages in mice in three pathological situations. Biochem Pharmacol 1980;29(13):1945–1948.

16. Moriwaki Y, Yamamoto T, Higashino K. Distribution and pathophysiologic role of molybdenum-containing enzymes. Histol Histopathol 1997;12(2):513–524.
17. Berry CE, Hare JM. Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. J Physiol 2004;555(Pt 3):589–606.
18. Kishore GS, Boutwell RK. Enzymatic oxidation and reduction of retinal by mouse epidermis. Biochem Biophys Res Commun 1980;94(4):1381–1386.
19. Weigert J, Neumeier M, Bauer S, *et al*. Small-interference RNA-mediated knock-down of aldehyde oxidase 1 in 3 T3-L1 cells impairs adipogenesis and adiponectin release. FEBS Lett 2008;582(19):2965–2972.
20. Sigruener A, Buechler C, Orso E, *et al*. Human aldehyde oxidase 1 interacts with ATP-binding cassette transporter-1 and modulates its activity in hepatocytes. Horm Metab Res 2007;39(11):781–789.
21. Graessler J, Fischer S. The dual substrate specificity of aldehyde oxidase 1 for retinal and acetaldehyde and its role in ABCA1 mediated efflux. Horm Metab Res 2007; 39(11):775–776.
22. Cheung AM, Wan TS, Leung JC, *et al*. Aldehyde dehydrogenase activity in leukemic blasts defines a subgroup of acute myeloid leukemia with adverse prognosis and superior NOD/SCID engrafting potential. Leukemia 2007;21(7):1423–1430.
23. Diamond S, Boer J, Maduskuie TP Jr., *et al*. Species-specific metabolism of SGX523 by aldehyde oxidase and the toxicological implications. Drug Metab Dispos 2010; 38(8):1277–1285.
24. Berger R, Mezey E, Clancy KP, *et al*. Analysis of aldehyde oxidase and xanthine dehydrogenase/oxidase as possible candidate genes for autosomal recessive familial amyotrophic lateral sclerosis. Somat Cell Mol Genet 1995;21(2):121–131.
25. Wright RM, Vaitaitis GM, Weigel LK, *et al*. Identification of the candidate ALS2 gene at chromosome 2q33 as a human aldehyde oxidase gene. REDOX REPORT 1995;1(5): 313–321.
26. Calzi ML, Raviolo C, Ghibaudi E, *et al*. Purification, cDNA cloning, and tissue distribution of bovine liver aldehyde oxidase. J Biol Chem 1995;270(52):31037–31045.
27. Terao M, Kurosaki M, Demontis S, *et al*. Isolation and characterization of the human aldehyde oxidase gene: conservation of intron/exon boundaries with the xanthine oxidoreductase gene indicates a common origin. Biochem J 1998;332(Pt 2):383–393.
28. Terao M, Kurosaki M, Marini M, *et al*. Purification of the aldehyde oxidase homolog 1 (AOH1) protein and cloning of the AOH1 and aldehyde oxidase homolog 2 (AOH2) genes. Identification of a novel molybdo-flavoprotein gene cluster on mouse chromosome 1. J Biol Chem 2001;276(49):46347–46363.
29. Terao M, Kurosaki M, Saltini G, *et al*. Cloning of the cDNAs coding for two novel molybdo-flavoproteins showing high similarity with aldehyde oxidase and xanthine oxidoreductase. J Biol Chem 2000;275(39):30690–30700.
30. Amaya Y, Yamazaki K, Sato M, *et al*. Proteolytic conversion of xanthine dehydrogenase from the NAD-dependent type to the O2-dependent type. Amino acid sequence of rat liver xanthine dehydrogenase and identification of the cleavage sites of the enzyme protein during irreversible conversion by trypsin. J Biol Chem 1990;265(24):14170–14175.
31. Itoh K, Masubuchi A, Sasaki T, *et al*. Genetic polymorphism of aldehyde oxidase in Donryu rats. Drug Metab Dispos 2007;35(5):734–739.
32. Itoh K, Maruyama H, Adachi M, *et al*. Lack of formation of aldehyde oxidase dimer possibly due to 377G>A nucleotide substitution. Drug Metab Dispos 2007;35(10):1860–1864.
33. Yamaguchi Y, Matsumura T, Ichida K, *et al*. Human xanthine oxidase changes its substrate specificity to aldehyde oxidase type upon mutation of amino acid residues in the active site: roles of active site residues in binding and activation of purine substrate. J Biochem 2007;141(4):513–524.

34. Fukiya K, Itoh K, Yamaguchi S, *et al*. A single amino acid substitution confers high cinchonidine oxidation activity comparable with that of rabbit to monkey aldehyde oxidase 1. Drug Metab Dispos 2010;38(2):302–307.
35. Adachi M, Itoh K, Masubuchi A, *et al*. Construction and expression of mutant cDNAs responsible for genetic polymorphism in aldehyde oxidase in Donryu strain rats. J Biochem Mol Biol 2007;40(6):1021–1027.
36. Alfaro JF, Joswig-Jones CA, Ouyang W, *et al*. Purification and mechanism of human aldehyde oxidase expressed in Escherichia coli. Drug Metab Dispos 2009;37(12):2393–2398.
37. Itoh K, Asakawa T, Hoshino K, *et al*. Functional analysis of aldehyde oxidase using expressed chimeric enzyme between monkey and rat. Biol Pharm Bull 2009;32(1):31–35.
38. Rajagopalan KV, Johnson JL. The pterin molybdenum cofactors. J Biol Chem 1992; 267(15):10199–10202.
39. Leimkuehler SW, Margot M, Rajagopalan KV. The history of the discovery of the molybdenum cofactor and novel aspects of its biosynthesis in bacteria. Coord Chem Rev 2011;255(9–10):1129–1144.
40. Hunt J, Massey V. Studies of the reductive half-reaction of milk xanthine dehydrogenase. J Biol Chem 1994;269(29):18904–18914.
41. Harris CM, Massey V. Kinetic isotope effects and electron transfer in the reduction of xanthine oxidoreductase with 4-hydroxypyrimidine. A comparison between oxidase and dehydrogenase forms. J Biol Chem 1997;272(36):22514–22525.
42. Harris CM, Massey V. The reaction of reduced xanthine dehydrogenase with molecular oxygen. Reaction kinetics and measurement of superoxide radical. J Biol Chem 1997;272(13):8370–8379.
43. Murray KN, Watson JG, Chaykin S. Catalysis of the direct transfer of oxygen from nicotinamide N-oxide to xanthine by xanthine oxidase. J Biol Chem 1966;241(20):4798–4801.
44. Romao MJ, Archer M, Moura I, *et al*. Crystal structure of the xanthine oxidase-related aldehyde oxido-reductase from D. gigas. Science 1995;270(5239):1170–1176.
45. Huber R, Hof P, Duarte RO, *et al*. A structure-based catalytic mechanism for the xanthine oxidase family of molybdenum enzymes. Proc Natl Acad Sci U S A 1996; 93(17):8846–8851.
46. Enroth C, Eger BT, Okamoto K, *et al*. Crystal structures of bovine milk xanthine dehydrogenase and xanthine oxidase: structure-based mechanism of conversion. Proc Natl Acad Sci U S A 2000;97(20):10723–10728.
47. Truglio JJ, Theis K, Leimkuhler S, *et al*. Crystal structures of the active and alloxanthine-inhibited forms of xanthine dehydrogenase from Rhodobacter capsulatus. Structure 2002; 10(1):115–125.
48. Okamoto K, Matsumoto K, Hille R, *et al*. The crystal structure of xanthine oxidoreductase during catalysis: implications for reaction mechanism and enzyme inhibition. Proc Natl Acad Sci U S A 2004;101(21):7931–7936.
49. Howes BD, Bray RC, Richards RL, *et al*. Evidence favoring molybdenum-carbon bond formation in xanthine oxidase action: 17Q- and 13 C-ENDOR and kinetic studies. Biochemistry 1996;35(5):1432–1443.
50. Skibo EB, Gilchrist JH, Lee CH. Electronic probes of the mechanism of substrate oxidation by buttermilk xanthine oxidase: role of the active-site nucleophile in oxidation. Biochemistry 1987;26(11):3032–3037.
51. Leimkuhler S, Stockert AL, Igarashi K, *et al*. The role of active site glutamate residues in catalysis of Rhodobacter capsulatus xanthine dehydrogenase. J Biol Chem 2004;279(39):40437–40444.
52. Amano T, Ochi N, Sato H, *et al*. Oxidation reaction by xanthine oxidase: theoretical study of reaction mechanism. J Am Chem Soc 2007;129(26):8131–8138.
53. Alfaro JF, Jones JP. Studies on the mechanism of aldehyde oxidase and xanthine oxidase. J Org Chem 2008;73(23):9469–9472.

54. Mahro M, Coelho C, Trincao J, *et al*. Characterization and crystallization of mouse Aldehyde Oxidase 3 (mAOX3): from mouse liver to E. coli heterologous protein expression. Drug Metab Dispos 2011;39(10):1939–1945.
55. Garattini E, Terao M. Increasing recognition of the importance of aldehyde oxidase in drug development and discovery. Drug Metab Rev 2011;43(3):1–13.
56. Terao M, Kurosaki M, Barzago MM, *et al*. Avian and canine aldehyde oxidases. Novel insights into the biology and evolution of molybdo-flavoenzymes. J Biol Chem 2006;281(28):19748–19761.
57. Romao MJ, Huber R. Crystal structure and mechanism of action of the xanthine oxidase-related aldehyde oxidoreductase from Desulfovibrio gigas. Biochem Soc Trans 1997;25(3):755–757.
58. Rodriguez-Trelles F, Tarrio R, Ayala FJ. Convergent neofunctionalization by positive Darwinian selection after ancient recurrent duplications of the xanthine dehydrogenase gene. Proc Natl Acad Sci U S A 2003;100(23):13413–13417.
59. Garattini E, Fratelli M, Terao M. Mammalian aldehyde oxidases: genetics, evolution and biochemistry. Cell Mol Life Sci 2008;65(7–8):1019–1048.
60. Xu P, Zhu XL, Hueckstead TP, *et al*. Assignment of human xanthine dehydrogenase gene to chromosome 2p22. Genomics 1994;23(1):289–291.
61. Cazzaniga G, Terao M, Lo Schiavo P, *et al*. Chromosomal mapping, isolation, and characterization of the mouse xanthine dehydrogenase gene. Genomics 1994;23(2):390–402.
62. Chow CW, Clark M, Rinaldo J, *et al*. Identification of the rat xanthine dehydrogenase/oxidase promoter. Nucleic Acids Res 1994;22(10):1846–1854.
63. Clark MP, Chow CW, Rinaldo JE, *et al*. Correct usage of multiple transcription initiation sites and C/EBP-dependent transcription activation of the rat XDH/XO TATA-less promoter requires downstream elements located in the coding region of the gene. Nucleic Acids Res 1998;26(7):1801–1806.
64. Garattini E, Fratelli M, Terao M. The mammalian aldehyde oxidase gene family. Hum Genomics 2009;4(2):119–130.
65. Simmonds HA. Hereditary xanthinuria. In Orphanet Encyclopedia; 2003.
66. Ichida K, Matsumura T, Sakuma R, *et al*. Mutation of human molybdenum cofactor sulfurase gene is responsible for classical xanthinuria type II. Biochem Biophys Res Commun 2001;282(5):1194–1200.
67. Yamamoto T, Moriwaki Y, Takahashi S, *et al*. Identification of a new point mutation in the human molybdenum cofactor sulferase gene that is responsible for xanthinuria type II. Metabolism 2003;52(11):1501–1504.
68. Peretz H, Naamati MS, Levartovsky D, *et al*. Identification and characterization of the first mutation (Arg776Cys) in the C-terminal domain of the Human Molybdenum Cofactor Sulfurase (HMCS) associated with type II classical xanthinuria. Mol Genet Metab 2007;91(1):23–29.
69. Kurosaki M, Terao M, Barzago MM, *et al*. The aldehyde oxidase gene cluster in mice and rats. Aldehyde oxidase homologue 3, a novel member of the molybdo-flavoenzyme family with selective expression in the olfactory mucosa. J Biol Chem 2004;279(48):50482–50498.
70. Damani LA, Crooks PA, Cowan DA. Metabolism of metyrapone. III. Formation of an alpha-pyridone metabolite by rat hepatic soluble enzymes. Drug Metab Dispos 1981;9(3):270–273.
71. Ishigai M, Ishitani Y, Orikasa Y, *et al*. Metabolism of 2(R,S)-1,2-bis(nicotinamido)propane, a new agent with anti-vasospasm activity, in rats and rabbits. Arzneimittelforschung 1998;48(5):429–435.
72. Tran TD, Pryde DC, Jones P, *et al*. Design and optimisation of orally active TLR7 agonists for the treatment of hepatitis C virus infection. Bioorg Med Chem Lett 2011;21(8):2389–2393.

73. Guo X, Lerner-Tung M, Chen HX, *et al*. 5-Fluoro-2-pyrimidinone, a liver aldehyde oxidase-activated prodrug of 5-fluorouracil. Biochem Pharmacol 1995;49(8):1111–1116.
74. Porter DJ, Harrington JA, Almond MR, *et al*. 5-ethynyl-2(1H)-pyrimidinone: aldehyde oxidase-activation to 5-ethynyluracil, a mechanism-based inactivator of dihydropyrimidine dehydrogenase. Biochem Pharmacol 1994;47(7):1165–1171.
75. Chang CN, Doong SL, Cheng YC. Conversion of 5-iodo-2-pyrimidinone-2′-deoxyribose to 5-iodo-deoxyuridine by aldehyde oxidase. Implication in hepatotropic drug design. Biochem Pharmacol 1992;43(10):2269–2273.
76. Kinsella TJ, Kunugi KA, Vielhuber KA, *et al*. Preclinical evaluation of 5-iodo-2-pyrimidinone-2′-deoxyribose as a prodrug for 5-iodo-2′-deoxyuridine-mediated radiosensitization in mouse and human tissues. Clin Cancer Res 1998;4(1):99–109.
77. Klecker RW, Cysyk RL, Collins JM. Zebularine metabolism by aldehyde oxidase in hepatic cytosol from humans, monkeys, dogs, rats, and mice: influence of sex and inhibitors. Bioorg Med Chem 2006;14(1):62–66.
78. Akabane T, Tanaka K, Irie M, *et al*. Case report of extensive metabolism by aldehyde oxidase in humans: pharmacokinetics and metabolite profile of FK3453 in rats, dogs, and humans. Xenobiotica 2011;41(5):372–384.
79. Itoh K, Yamamura M, Muramatsu S, *et al*. Stereospecific oxidation of the (S)-enantiomer of RS-8359, a selective and reversible monoamine oxidase A (MAO-A) inhibitor, by aldehyde oxidase. Xenobiotica 2005;35(6):561–573.
80. Takasaki W, Yamamura M, Nozaki A, *et al*. Stereoselective pharmacokinetics of RS-8359, a selective and reversible MAO-A inhibitor, by species-dependent drug-metabolizing enzymes. Chirality 2005;17(3):135–141.
81. Stubley C, Stell JG, Mathieson DW. The oxidation of azaheterocycles with mammalian liver aldehyde oxidase. Xenobiotica 1979;9(8):475–484.
82. Stubley C, Stell JGP, Investigation of the substrate-binding site of aldehyde oxidase. J Pharm Pharmacol 1980;2(S1):51.
83. McMurtrey KD, Knight TJ. Metabolism of acridine by rat-liver enzymes. Mutat Res 1984;140(1):7–11.
84. Schofield PC, Robertson IG, Paxton JW. Inter-species variation in the metabolism and inhibition of N-[(2′-dimethylamino)ethyl]acridine-4-carboxamide (DACA) by aldehyde oxidase. Biochem Pharmacol 2000;59(2):161–165.
85. Beedham C, Al TY, Smith JA. Role of guinea pig and rabbit hepatic aldehyde oxidase in oxidative *in vitro* metabolism of cinchona antimalarials. Drug Metab Dispos 1992;20(6):889–895.
86. Itoh K, Yamamura M, Takasaki W, *et al*. Species differences in enantioselective 2-oxidations of RS-8359, a selective and reversible MAO-A inhibitor, and cinchona alkaloids by aldehyde oxidase. Biopharm Drug Dispos 2006;27(3):133–139.
87. Austin NE, Baldwin SJ, Cutler L, *et al*. Pharmacokinetics of the novel, high-affinity and selective dopamine D3 receptor antagonist SB-277011 in rat, dog and monkey: *in vitro*/*in vivo* correlation and the role of aldehyde oxidase. Xenobiotica 2001;31(8–9):677–686.
88. Dalvie D. Cross-species comparison of the metabolism and excretion of zoniporide: contribution of aldehyde oxidase to interspecies differences. Drug Metab Dispos 2010. DOI: 10.1124/dmd.109.030783.
89. Ghafourian T, Rashidi MR. Quantitative study of the structural requirements of phthalazine/quinazoline derivatives for interaction with human liver aldehyde oxidase. Chem Pharm Bull (Tokyo) 2001;49(9):1066–1071.
90. Beedham C, Critchley DJ, Rance DJ. Substrate specificity of human liver aldehyde oxidase toward substituted quinazolines and phthalazines: a comparison with hepatic enzyme from guinea pig, rabbit, and baboon. Arch Biochem Biophys 1995;319(2):481–490.
91. Kaye B, Rance DJ, Waring L. Oxidative metabolism of carbazeran *in vitro* by liver cytosol of baboon and man. Xenobiotica 1985;15(3):237–242.

92. Kaye B, Offerman JL, Reid JL, *et al*. A species difference in the presystemic metabolism of carbazeran in dog and man. Xenobiotica 1984;14(12):935–945.
93. Acheampong AA, Shackleton M, Tang-Liu DD. Comparative ocular pharmacokinetics of brimonidine after a single dose application to the eyes of albino and pigmented rabbits. Drug Metab Dispos 1995;23(7):708–712.
94. Acheampong AA, Chien DS, Lam S, *et al*. Characterization of brimonidine metabolism with rat, rabbit, dog, monkey and human liver fractions and rabbit liver aldehyde oxidase. Xenobiotica 1996;26(10):1035–1055.
95. Anderson LW, Collins JM, Klecker RW, *et al*., Corporate author FDA, Corporate author K-C-I, and Corporate author NC-IR. Metabolic profile of XK469 (2(R)-(4-(7-chloro-2-quinoxalinyl)oxyphenoxy)- propionic acid; NSC698215) in patients and *in vitro* : low potential for active or toxic metabolites or for drug-drug interactions. Cancer Chemother Pharmacol 2005;56(4):351–357.
96. Magee TV, Ripp SL, Li B, *et al*. Discovery of azetidinyl ketolides for the treatment of susceptible and multidrug resistant community-acquired respiratory tract infections. J Med Chem 2009;52(23):7446–7457.
97. Hodnett CN, McCormack JJ, Sabean JA. Oxidation of selected pteridine derivatives by mammalian liver xanthine oxidase and aldehyde oxidase. J Pharm Sci 1976;65(8): 1150–1154.
98. Krenitsky TA, Neil SM, Elion GB, *et al*. A comparison of the specificities of xanthine oxidase and aldehyde oxidase. Arch Biochem Biophys 1972;150(2):585–599.
99. Jordan CGM, Rashidi MR, Laljee H, *et al*., Corporate author UB, and Corporate author SKB. Aldehyde oxidase-catalysed oxidation of methotrexate in the liver of guinea-pig, rabbit and man. J Pharm Pharmacol 1999;51(4):411–418.
100. Kitamura S, Sugihara K, Nakatani K, *et al*., Corporate author UH, Corporate author D, and Corporate author SRI. Variation of hepatic methotrexate 7-hydroxylase activity in animals and humans. IUBMB-Life 1999;48(6):607–611.
101. Rashidi MR, Beedham C, Smith JS, *et al*. *In vitro* study of 6-mercaptopurine oxidation catalysed by aldehyde oxidase and xanthine oxidase. Drug Metab Pharmacokinet 2007;22(4):299–306.
102. Zimm S, Collins JM, O'Neill D, *et al*. Inhibition of first-pass metabolism in cancer chemotherapy: interaction of 6-mercaptopurine and allopurinol. Clin Pharmacol Ther 1983;34(6):810–817.
103. Clarke SE, Harrell AW, Chenery RJ. Role of aldehyde oxidase in the *in vitro* conversion of famciclovir to penciclovir in human liver. Drug Metab Dispos 1995;23(2):251–254.
104. Rashidi MR, Smith JA, Clarke SE, *et al*. *In vitro* oxidation of famciclovir and 6-deoxypenciclovir by aldehyde oxidase from human, guinea pig, rabbit, and rat liver. Drug Metab Dispos 1997;25(7):805–813.
105. Keuzenkamp-Jansen CW, van Baal JM, De Abreu RA, *et al*. Detection and identification of 6-methylmercapto-8-hydoxypurine, a major metabolite of 6-mercaptopurine, in plasma during intravenous administration. Clin Chem 1996;42(3):380–386.
106. Kitchen BJ, Moser A, Lowe E, *et al*. Thioguanine administered as a continuous intravenous infusion to pediatric patients is metabolized to the novel metabolite 8-hydroxy-thioguanine. J Pharmacol Exp Ther 1999;291(2):870–874.
107. Roy SK, Gupta E, Dolan ME. Pharmacokinetics of O6-benzylguanine in rats and its metabolism by rat liver microsomes. Drug Metab Dispos 1995;23(12):1394–1399.
108. Kawashima K, Hosoi K, Naruke T, *et al*. Aldehyde oxidase-dependent marked species difference in hepatic metabolism of the sedative-hypnotic, zaleplon, between monkeys and rats. Drug Metab Dispos 1999;27(3):422–428.
109. Furnival B, Harrison JM, Newman J, *et al*. The fate of dibenz[b,f]-1,4-oxazepine (CR) in the rat. Part II. Metabolism *in vitro*. Xenobiotica 1983;13(6):361–372.

110. Tran TD, Adam FM, Calo F, *et al*. Design and optimisation of potent gp120-CD4 inhibitors. Bioorg Med Chem Lett 2009;19(17):5250–5255.
111. O'Connor D, Jones P, Chambers MS, *et al*. Aldehyde oxidase and its contribution to the metabolism of a structurally novel, functionally selective GABAA alpha5-subtype inverse agonist. Xenobiotica 2006;36(4):315–330.
112. Du X, Gustin DJ, Chen X, *et al*. Imidazo-pyrazine derivatives as potent CXCR3 antagonists. Bioorg Med Chem Lett 2009;19(17):5200–5204.
113. Shaw S, Jayatilleke E. The role of cellular oxidases and catalytic iron in the pathogenesis of ethanol-induced liver injury. Life Sci 1992;50(26):2045–2052.
114. Wright RM, McManaman JL, Repine JE. Alcohol-induced breast cancer: a proposed mechanism. Free Radic Biol Med 1999;26(3–4):348–354.
115. Ambroziak W, Izaguirre G, Pietruszko R. Metabolism of retinaldehyde and other aldehydes in soluble extracts of human liver and kidney. J Biol Chem 1999;274(47):33366–33373.
116. Beedham C. Molybdenum hydroxylases: biological distribution and substrate-inhibitor specificity. Prog Med Chem 1987;24:85–127.
117. Beedham C, Peet CF, Panoutsopoulos GI, *et al*. Role of aldehyde oxidase in biogenic amine metabolism. Prog Brain Res 1995;106:345–353.
118. Panoutsopoulos GI, Kouretas D, Beedham C. Contribution of aldehyde oxidase, xanthine oxidase, and aldehyde dehydrogenase on the oxidation of aromatic aldehydes. Chem Res Toxicol 2004;17(10):1368–1376.
119. McDaniel HG, Podgainy H, Bressler R. The metabolism of tolbutamide in rat liver. J Pharmacol Exp Ther 1969;167(1):91–97.
120. Ruenitz PC, Bai X. Acidic metabolites of tamoxifen. Aspects of formation and fate in the female rat. Drug Metab Dispos 1995;23(9):993–998.
121. Rochat B, Kosel M, Boss G, *et al*. Stereoselective biotransformation of the selective serotonin reuptake inhibitor citalopram and its demethylated metabolites by monoamine oxidases in human liver. Biochem Pharmacol 1998;56(1):15–23.
122. Shibata K. Tissue Distribution of N1-Methyl-2-pyridone-5-carboxamide- and N1-Methyl-4-pyridone-3-carboxamide-forming N1-Methylnicotinamide Oxidases in Rats. Agric Biol Chem 1989;53(12):3355–3356.
123. Egashira Y, Isagawa A, Komine T, *et al*. Tryptophan-niacin metabolism in liver cirrhosis rat caused by carbon tetrachloride. J Nutr Sci Vitaminol (Tokyo) 1999;45(4):459–469.
124. Taylor SM, Stubley-Beedham C, Stell JG. Simultaneous formation of 2- and 4-quinolones from quinolinium cations catalysed by aldehyde oxidase. Biochem J 1984;220(1):67–74.
125. Felsted RL, Chaykin S. N1-methylnicotinamide oxidation in a number of mammals. J Biol Chem 1967;242(6):1274–1279.
126. Kitamura S, Sugihara K, Ohta S. Drug-metabolizing ability of molybdenum hydroxylases. Drug Metab Pharmacokinet 2006;21(2):83–98.
127. Masic LP. Role of cyclic tertiary amine bioactivation to reactive iminium species: structure toxicity relationship. Curr Drug Metab 2011;12(1):35–50.
128. Wu E, Shinka T, Caldera-Munoz P, *et al*. Metabolic studies on the nigrostriatal toxin MPTP and its MAO B generated dihydropyridinium metabolite MPDP+. Chem Res Toxicol 1988;1(3):186–194.
129. Yoshihara S, Ohta S. Involvement of hepatic aldehyde oxidase in conversion of 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP+) to 1-methyl-4-phenyl-5,6-dihydro-2-pyridone. Arch Biochem Biophys 1998;360(1):93–98.
130. Yoshihara S, Harada K, Ohta S. Metabolism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in perfused rat liver: involvement of hepatic aldehyde oxidase as a detoxification enzyme. Drug Metab Dispos 2000;28(5):538–543.
131. Brandange S, Lindblom L. The enzyme "aldehyde oxidase" is an iminium oxidase. Reaction with nicotine delta 1′(5′) iminium ion. Biochem Biophys Res Commun 1979;91(3):991–996.

132. Rodrigues AD, Ferrero JL, Amann MT, *et al*. The *in vitro* hepatic metabolism of ABT-418, a cholinergic channel activator, in rats, dogs, cynomolgus monkeys, and humans. Drug Metab Dispos 1994;22(5):788–798.
133. Fu PP. Metabolism of nitro-polycyclic aromatic hydrocarbons. Drug Metab Rev 1990; 22(2–3):209–268.
134. Tatsumi K, Kitamura S, Narai N. Reductive metabolism of aromatic nitro compounds including carcinogens by rabbit liver preparations. Cancer Res 1986;46(3):1089–1093.
135. Bartel LC, Montalto de Mecca M, Castro JA. Nitroreductive metabolic activation of some carcinogenic nitro heterocyclic food contaminants in rat mammary tissue cellular fractions. Food Chem Toxicol 2009;47(1):140–144.
136. Dick RA, Kanne DB, Casida JE. Identification of aldehyde oxidase as the neonicotinoid nitroreductase. Chem Res Toxicol 2005;18(2):317–323.
137. Dick RA, Kanne DB, Casida JE. Substrate specificity of rabbit aldehyde oxidase for nitroguanidine and nitromethylene neonicotinoid insecticides. Chem Res Toxicol 2006; 19(1):38–43.
138. Doel JJ, Godber BL, Goult TA, *et al*. Reduction of organic nitrites to nitric oxide catalyzed by xanthine oxidase: possible role in metabolism of nitrovasodilators. Biochem Biophys Res Commun 2000;270(3):880–885.
139. Doel JJ, Godber BL, Eisenthal R, *et al*. Reduction of organic nitrates catalysed by xanthine oxidoreductase under anaerobic conditions. Biochim Biophys Acta 2001; 1527(1–2):81–87.
140. Li H, Cui H, Liu X, *et al*. Xanthine oxidase catalyzes anaerobic transformation of organic nitrates to nitric oxide and nitrosothiols: characterization of this mechanism and the link between organic nitrate and guanylyl cyclase activation. J Biol Chem 2005;280(17):16594–16600.
141. Kitamura S, Tatsumi K. Involvement of liver aldehyde oxidase in the reduction of nicotinamide N-oxide. Biochem Biophys Res Commun 1984;120(2):602–606.
142. Kitamura S, Tatsumi K. Reduction of tertiary amine N-oxides by liver preparations: function of aldehyde oxidase as a major N-oxide reductase. Biochem Biophys Res Commun 1984;121(3):749–754.
143. Sugihara K, Kitamura S, Tatsumi K. Involvement of mammalian liver cytosols and aldehyde oxidase in reductive metabolism of zonisamide. Drug Metab Dispos 1996; 24(2):199–202.
144. Tatsumi K, Kitamura S, Yamada H. Involvement of liver aldehyde oxidase in sulfoxide reduction. Chem Pharm Bull (Tokyo) 1982;30(12):4585–4588.
145. Kitamura S, Suzuki T, Kadota T, *et al*. *In vitro* metabolism of fenthion and fenthion sulfoxide by liver preparations of sea bream, goldfish, and rats. Drug Metab Dispos 2003;31(2):179–186.
146. Duggan DE. Sulindac: therapeutic implications of the prodrug/pharmacophore equilibrium. Drug Metab Rev 1981;12(2):325–337.
147. Pay GF, Wallis RB, Zelaschi D. A metabolite of sulphinpyrazone that is largely responsible for the effect of the drug on the platelet prostaglandin pathway. Biochem Soc Trans 1980;8(6):727–728.
148. Lee SC, Renwick AG. Sulphoxide reduction by rat and rabbit tissues *in vitro*. Biochem Pharmacol 1995;49(11):1557–1565.
149. Tatsumi K, Kitamura S, Yamada H. Sulfoxide reductase activity of liver aldehyde oxidase. Biochim Biophys Acta 1983;747(1–2):86–92.
150. Kashiyama E, Yokoi T, Odomi M, *et al*. Stereoselective S-oxidation and reduction of flosequinan in rat. Xenobiotica 1999;29(8):815–826.
151. Beedham C. Molybdenum hydroxylases. In: Ioannides C, editor. Enzyme systems that metabolise drugs and other xenobiotics. Wiley-VCH; 2001. p. 576.

152. Kitamura S, Sugihara K, Tatsumi K. Reductase activity of aldehyde oxidase toward the carcinogen N-hydroxy-2-acetylaminofluorene and the related hydroxamic acids. Biochem Mol Biol Int 1994;34(6):1197–1203.
153. Sugihara K, Tatsumi K. Participation of liver aldehyde oxidase in reductive metabolism of hydroxamic acids to amides. Arch Biochem Biophys 1986;247(2):289–293.
154. Dambrova M, Uhlen S, Welch CJ, *et al*. Identification of an N-hydroxyguanidine reducing activity of xanthine oxidase. Eur J Biochem 1998;257(1):178–184.
155. Tatsumi K, Ishigai M. Oxime-metabolizing activity of liver aldehyde oxidase. Arch Biochem Biophys 1987;253(2):413–418.
156. Kotthaus J, Wahl B, Havemeyer A, *et al*. Reduction of N(omega)-hydroxy-L-arginine by the mitochondrial amidoxime reducing component (mARC). Biochem J 2010; 433(2):383–391.
157. Havemeyer A, Grunewald S, Wahl B, *et al*. Reduction of N-hydroxy-sulfonamides, including N-hydroxy-valdecoxib, by the molybdenum-containing enzyme mARC. Drug Metab Dispos 2010;38(11):1917–1921.
158. Gruenewald S, Wahl B, Bittner F, *et al*. The fourth molybdenum containing enzyme mARC: cloning and involvement in the activation of N-hydroxylated prodrugs. J Med Chem 2008;51(24):8173–8177.
159. Nakasa H, Komiya M, Ohmori S, *et al*. Characterization of human liver microsomal cytochrome P450 involved in the reductive metabolism of zonisamide. Mol Pharmacol 1993;44(1):216–221.
160. Kitamura S, Sugihara K, Kuwasako M, *et al*. The role of mammalian intestinal bacteria in the reductive metabolism of zonisamide. J Pharm Pharmacol 1997;49(3):253–256.
161. Prakash C, Kamel A, Gummerus J, *et al*. Metabolism and excretion of a new antipsychotic drug, ziprasidone, in humans. Drug Metab Dispos 1997;25(7):863–872.
162. Miao Z, Kamel A, Prakash C. Characterization of a novel metabolite intermediate of ziprasidone in hepatic cytosolic fractions of rat, dog, and human by ESI-MS/MS, hydrogen/deuterium exchange, and chemical derivatization. Drug Metab Dispos 2005;33(7):879–883.
163. Beedham C, Flanagan LM, Obach RS, Corporate author UB, and Corporate author P. The reduction of ziprasidone to dihydroziprasidone by guinea pig liver aldehyde oxidase. Drug Metab Rev 2006;38(Suppl. 1):138.
164. Rajagopalan KV, Fridovich I, Handler P. Hepatic aldehyde oxidase. I. Purification and properties. J Biol Chem 1962;237:922–928.
165. Lake BG, Ball SE, Kao J, *et al*. Metabolism of zaleplon by human liver: evidence for involvement of aldehyde oxidase. Xenobiotica 2002;32(10):835–847.
166. Thor H, Smith MT, Hartzell P, *et al*. The metabolism of menadione (2-methyl-1,4-naphthoquinone) by isolated hepatocytes. A study of the implications of oxidative stress in intact cells. J Biol Chem 1982;257(20):12419–12425.
167. Sahi J, Khan KK, Black CB. Aldehyde oxidase activity and inhibition in hepatocytes and cytosolic fractions from mouse, rat, monkey and human. Drug Metab Lett 2008; 2(3):176–183.
168. Robertson IG, Gamage RS. Methadone: a potent inhibitor of rat liver aldehyde oxidase. Biochem Pharmacol 1994;47(3):584–587.
169. Obach RS, Huynh P, Allen MC, *et al*. Human liver aldehyde oxidase: inhibition by 239 drugs. J Clin Pharmacol 2004;44(1):7–19.
170. Pirouzpanah S, Hanaee J, Razavieh SV, *et al*. Inhibitory effects of flavonoids on aldehyde oxidase activity. J Enzyme Inhib Med Chem 2009;24(1):14–21.
171. Rashidi MR, Nazemiyeh H. Inhibitory effects of flavonoids on molybdenum hydroxylases activity. Expert Opin Drug Metab Toxicol 2010;6(2):133–152.
172. Obach RS. Potent inhibition of human liver aldehyde oxidase by raloxifene. Drug Metab Dispos 2004;32(1):89–97.

173. Baer BR, Wienkers LC, Rock DA. Time-dependent inactivation of P450 3A4 by raloxifene: identification of Cys239 as the site of apoprotein alkylation. Chem Res Toxicol 2007;20(6):954–964.
174. Johnson C, Stubley-Beedham C, Stell JG. Hydralazine: a potent inhibitor of aldehyde oxidase activity *in vitro* and *in vivo*. Biochem Pharmacol 1985;34(24):4251–4256.
175. Critchley DJ, Rance DJ, Beedham C. Biotransformation of carbazeran in guinea pig: effect of hydralazine pretreatment. Xenobiotica 1994;24(1):37–47.
176. Svensson CK, Knowlton PW, Ware JA. Effect of hydralazine on the elimination of antipyrine in the rat. Pharm Res 1987;4(6):515–518.
177. Renwick AB, Ball SE, Tredger JM, *et al*. Inhibition of zaleplon metabolism by cimetidine in the human liver: *in vitro* studies with subcellular fractions and precision-cut liver slices. Xenobiotica 2002;32(10):849–862.
178. King_Pharmaceuticals. Sonata (zaleplon) capsules: FDA Approved Labeling Text 12.10.07; 2007.
179. Obach RS. Predicting drug-drug interactions from *in vitro* drug metabolism data: challenges and recent advances. Curr Opin Drug Discov Devel 2009;12(1):81–89.
180. Rundles RW. Allopurinol in gouty nephropathy and renal dialysis. Ann Rheum Dis 1966;25(6 Suppl):694–696.
181. Massey V, Komai H, Palmer G, *et al*. On the mechanism of inactivation of xanthine oxidase by allopurinol and other pyrazolo[3,4-d]pyrimidines. J Biol Chem 1970; 245(11):2837–2844.
182. Spector T, Hall WW, Krenitsky TA. Human and bovine xanthine oxidases. Inhibition studies with oxipurinol. Biochem Pharmacol 1986;35(18):3109–3114.
183. Massey V, Edmondson D. On the mechanism of inactivation of xanthine oxidase by cyanide. J Biol Chem 1970;245(24):6595–6598.
184. Hille R, Stewart RC, Fee JA, *et al*. The interaction of arsenite with xanthine oxidase. J Biol Chem 1983;258(8):4849–4856.
185. Cao H, Hall J, Hille R. X-ray Crystal Structure of Arsenite-Inhibited Xanthine Oxidase: mu-Sulfido,mu-Oxo Double Bridge between Molybdenum and Arsenic in the Active Site. J Am Chem Soc 2011;133(32):12414–12417.
186. Emmerson BT. The management of gout. N Engl J Med 1996;334(7):445–451.
187. Hande KR, Noone RM, Stone WJ. Severe allopurinol toxicity. Description and guidelines for prevention in patients with renal insufficiency. Am J Med 1984;76(1):47–56.
188. Okamoto K, Nishino T. Mechanism of inhibition of xanthine oxidase with a new tight binding inhibitor. J Biol Chem 1995;270(14):7816–7821.
189. Sato S, Tatsumi K, Nishino T. A novel xanthine dehydrogenase inhibitor (BOF-4272). Adv Exp Med Biol 1991;309A:135–138.
190. Ishibuchi S, Morimoto H, Oe T, *et al*. Synthesis and structure-activity relationships of 1-phenylpyrazoles as xanthine oxidase inhibitors. Bioorg Med Chem Lett 2001; 11(7):879–882.
191. Yamada I, Fukunari A, Osajima T, *et al*. Pharmacokinetics/pharmacodynamics of Y-700, a novel xanthine oxidase inhibitor, in rats and man. Nucleosides Nucleotides Nucleic Acids 2004;23(8–9):1123–1125.
192. Becker MA, Schumacher HR Jr., Wortmann RL, *et al*. Febuxostat compared with allopurinol in patients with hyperuricemia and gout. N Engl J Med 2005;353(23):2450–2461.
193. Kumar R, Darpan, Sharma S, *et al*. Xanthine oxidase inhibitors: a patent survey. Expert Opin Ther Pat 2011;21(7):1071–1108.
194. Elion GB. Actions of purine analogs: enzyme specificity studies as a basis for interpretation and design. Cancer Res 1969;29(12):2448–2453.
195. Huh K, Yamamoto I, Gohda E, *et al*. Tissue distribution and characteristics of xanthine oxidase and allopurinol oxidizing enzyme. Jpn J Pharmacol 1976;26(6):719–724.

196. Frederiks WM, Vreeling-Sindelarova H. Ultrastructural localization of xanthine oxidoreductase activity in isolated rat liver cells. Acta Histochem 2002;104(1):29–37.
197. Rouquette M, Page S, Bryant R, *et al*. Xanthine oxidoreductase is asymmetrically localised on the outer surface of human endothelial and epithelial cells in culture. FEBS Lett 1998;426(3):397–401.
198. Critchley DJ, Rance DJ, Beedham C. Subcellular localisation of guinea pig hepatic molybdenum hydroxylases. Biochem Biophys Res Commun 1992;185(1):54–59.
199. Igo RP, Mackler B, Duncan H. Liver aldehyde oxidase: the nature of hematin component. Arch Biochem Biophys 1961;93:435–439.
200. Beedham C. Molybdenum hydroxylases. In: Loannides C, editor. Enzyme systems that matabolise drugs and other xenobiotics. Chichester: John Wiley and Sons, Ltd; 2002. pp. 147–187.
201. Kurosaki M, Li Calzi M, Scanziani E, *et al*. Tissue- and cell-specific expression of mouse xanthine oxidoreductase gene *in vivo* : regulation by bacterial lipopolysaccharide. Biochem J 1995;306(Pt 1):225–234.
202. Moriwaki Y, Yamamoto T, Yamakita J, *et al*. Comparative localization of aldehyde oxidase and xanthine oxidoreductase activity in rat tissues. Histochem J 1998;30(2):69–74.
203. Terao M, Cazzaniga G, Ghezzi P, *et al*. Molecular cloning of a cDNA coding for mouse liver xanthine dehydrogenase. Regulation of its transcript by interferons *in vivo*. Biochem J 1992;283(Pt 3):863–870.
204. Linder N, Rapola J, Raivio KO. Cellular expression of xanthine oxidoreductase protein in normal human tissues. Lab Invest 1999;79(8):967–974.
205. Harrison R. Structure and function of xanthine oxidoreductase: where are we now? Free Radic Biol Med 2002;33(6):774–797.
206. Saksela M, Lapatto R, Raivio KO. Xanthine oxidoreductase gene expression and enzyme activity in developing human tissues. Biol Neonate 1998;74(4):274–280.
207. Battelli MG, Abbondanza A, Musiani S, *et al*. Determination of xanthine oxidase in human serum by a competitive enzyme-linked immunosorbent assay (ELISA). Clin Chim Acta 1999;281(1–2):147–158.
208. Battelli MG, Musiani S, Valgimigli M, *et al*. Serum xanthine oxidase in human liver disease. Am J Gastroenterol 2001;96(4):1194–1199.
209. Abadeh S, Killacky J, Benboubetra M, *et al*. Purification and partial characterization of xanthine oxidase from human milk. Biochim Biophys Acta 1992;1117(1):25–32.
210. Xu P, Huecksteadt TP, Harrison R, *et al*. Molecular cloning, tissue expression of human xanthine dehydrogenase. Biochem Biophys Res Commun 1994;199(2):998–1004.
211. Hellsten-Westing Y. Immunohistochemical localization of xanthine oxidase in human cardiac and skeletal muscle. Histochemistry 1993;100(3):215–222.
212. Kokoglu E, Belce A, Ozyurt E, *et al*. Xanthine oxidase levels in human brain tumors. Cancer Lett 1990;50(3):179–181.
213. Linder N, Lundin J, Isola J, *et al*. Down-regulated xanthine oxidoreductase is a feature of aggressive breast cancer. Clin Cancer Res 2005;11(12):4372–4381.
214. Ikegami T, Natsumeda Y, Weber G. Decreased concentration of xanthine dehydrogenase (EC 1.1.1.204) in rat hepatomas. Cancer Res 1986;46(8):3838–3841.
215. Weber G, Kizaki H, Tzeng D, *et al*. Colon tumor: enzymology of the neoplastic program. Life Sci 1978;23(7):729–736.
216. Yasuhara A, Akiba-Goto M, Aisaka K. Cloning and sequencing of the aldehyde oxidase gene from Methylobacillus sKY4400. Biosci Biotechnol Biochem 2005; 69(12):2435–2438.
217. Moriwaki Y, Yamamoto T, Takahashi S, *et al*. Widespread cellular distribution of aldehyde oxidase in human tissues found by immunohistochemistry staining. Histol Histopathol 2001;16(3):745–753.

218. Shyamsundar R, Kim YH, Higgins JP, *et al*. A DNA microarray survey of gene expression in normal human tissues. Genome Biol 2005;6(3):R22.
219. Moriwaki Y, Yamamoto T, Yamakita J, *et al*. Zonal distribution of allopurinol-oxidizing enzymes in rat liver. Adv Exp Med Biol 1998;431:47–50.
220. Moriwaki Y, Yamamoto T, Yamaguchi K, *et al*. Immunohistochemical localization of aldehyde and xanthine oxidase in rat tissues using polyclonal antibodies. Histochem Cell Biol 1996;105(1):71–79.
221. Kurosaki M, Demontis S, Barzago MM, *et al*. Molecular cloning of the cDNA coding for mouse aldehyde oxidase: tissue distribution and regulation *in vivo* by testosterone. Biochem J 1999;341(Pt 1):71–80.
222. Terao M, Kurosaki M, Barzago MM, *et al*. Role of the molybdoflavoenzyme aldehyde oxidase homolog 2 in the biosynthesis of retinoic acid: generation and characterization of a knockout mouse. Mol Cell Biol 2009;29(2):357–377.
223. Krenitsky TA, Tuttle JV, Cattau EL Jr., *et al*. A comparison of the distribution and electron acceptor specificities of xanthine oxidase and aldehyde oxidase. Comp Biochem Physiol B 1974;49(4):687–703.
224. Wajner M, Harkness RA. Distribution of xanthine dehydrogenase and oxidase activities in human and rabbit tissues. Biochim Biophys Acta 1989;991(1):79–84.
225. Beedham C, Bruce SE, Rance DJ. Tissue distribution of the molybdenum hydroxylases, aldehyde oxidase and xanthine oxidase, in male and female guinea pigs. Eur J Drug Metab Pharmacokinet 1987;12(4):303–306.
226. Guerciolini R, Szumlanski C, Weinshilboum RM. Human liver xanthine oxidase: nature and extent of individual variation. Clin Pharmacol Ther 1991;50(6):663–672.
227. Parks DA, Granger DN. Xanthine oxidase: biochemistry, distribution and physiology. Acta Physiol Scand Suppl 1986;548:87–99.
228. Relling MV, Lin JS, Ayers GD, *et al*. Racial and gender differences in N-acetyltransferase, xanthine oxidase, and CYP1A2 activities. Clin Pharmacol Ther 1992;52(6):643–658.
229. Godber B, Sanders S, Harrison R, *et al*. $>$or $=$ 95% of xanthine oxidase in human milk is present as the demolybdo form, lacking molybdopterin. Biochem Soc Trans 1997;25(3):519 S.
230. Holmes RS, Vandeberg JL. Aldehyde dehydrogenases, aldehyde oxidase and xanthine oxidase from baboon tissues: phenotypic variability and subcellular distribution in liver and brain. Alcohol 1986;3(3):205–214.
231. Jordan CG, Rashidi MR, Laljee H, *et al*. Aldehyde oxidase-catalysed oxidation of methotrexate in the liver of guinea-pig, rabbit and man. J Pharm Pharmacol 1999;51(4):411–418.
232. Al-Salmy HS. Individual variation in hepatic aldehyde oxidase activity. IUBMB Life 2001;51(4):249–253.
233. Rodrigues AD. Comparison of levels of aldehyde oxidase with cytochrome P450 activities in human liver *in vitro*. Biochem Pharmacol 1994;48(1):197–200.
234. Sugihara K, Kitamura S, Tatsumi K, *et al*. Differences in aldehyde oxidase activity in cytosolic preparations of human and monkey liver. Biochem Mol Biol Int 1997;41(6):1153–1160.
235. Yerino P, Toren P, Parkinson A. Abstracts from 8th International ISSX Meeting: a comparison of p-vanillin and phthalazine as substrates for human aldehyde oxidase. Drug Metab Rev 2007;39(s1):362.
236. Steinberg KK, Relling MV, Gallagher ML, *et al*. Genetic studies of a cluster of acute lymphoblastic leukemia cases in Churchill County, Nevada. Environ Health Perspect 2007;115(1):158–164.
237. Sugihara K, Tayama Y, Shimomiya K, *et al*. Estimation of aldehyde oxidase activity *in vivo* from conversion ratio of N1-methylnicotinamide to pyridones, and intraspecies variation of the enzyme activity in rats. Drug Metab Dispos 2006;34(2):208–212.

238. Tayama Y, Miyake K, Sugihara K, *et al*. Developmental changes of aldehyde oxidase activity in young Japanese children. Clin Pharmacol Ther 2007;81(4):567–572.
239. Tayama Y, Moriyasu A, Sugihara K, *et al*. Developmental changes of aldehyde oxidase in postnatal rat liver. Drug Metab Pharmacokinet 2007;22(2):119–124.
240. Itoh K, Maruyama H, Adachi M, *et al*. Lack of dimer formation ability in rat strains with low aldehyde oxidase activity. Xenobiotica 2007;37(7):709–716.
241. Harrell AW, Wheeler SM, East P, *et al*. Use of rat and human *in vitro* systems to assess the effectiveness and enzymology of deoxy-guanine analogues as prodrugs of an antiviral agent. Drug Metab Dispos 1994;22(1):124–128.
242. Rodrigues AD, Kukulka MJ, Ferrero JL, *et al*. *In vitro* hepatic metabolism of ABT-418 in chimpanzee (Pan troglodytes). A unique pattern of microsomal flavin-containing monooxygenase-dependent stereoselective N′-oxidation. Drug Metab Dispos 1995;23(10):1143–1152.
243. Beedham C, Bruce SE, Critchley DJ, *et al*. Species variation in hepatic aldehyde oxidase activity. Eur J Drug Metab Pharmacokinet 1987;12(4):307–310.
244. Page S, Powell D, Benboubetra M, *et al*. Xanthine oxidoreductase in human mammary epithelial cells: activation in response to inflammatory cytokines. Biochim Biophys Acta 1998;1381(2):191–202.
245. Pfeffer KD, Huecksteadt TP, Hoidal JR. Xanthine dehydrogenase and xanthine oxidase activity and gene expression in renal epithelial cells. Cytokine and steroid regulation. J Immunol 1994;153(4):1789–1797.
246. Falciani F, Ghezzi P, Terao M, *et al*. Interferons induce xanthine dehydrogenase gene expression in L929 cells. Biochem J 1992;285(Pt 3):1001–1008.
247. Dupont GP, Huecksteadt TP, Marshall BC, *et al*. Regulation of xanthine dehydrogenase and xanthine oxidase activity and gene expression in cultured rat pulmonary endothelial cells. J Clin Invest 1992;89(1):197–202.
248. Terada LS, Arnold PD. Xanthine oxidase does not mediate the antiproliferative effects of interferon-gamma in human umbilical vein endothelial cells. J Interferon Res 1993;13(6):419–422.
249. Linder N. Expression and regulation of human xanthine oxidoreductase. Program for developmental and reproductive biology. Helsinki: University of Helsinki; 2005. pp. 1–47.
250. Cameron GS, Pence BC. Effects of multiple applications of tumor promoters and ultraviolet radiation on epidermal proliferation and antioxidant status. J Invest Dermatol 1992;99(2):189–192.
251. Kim HW, Murakami A, Nakamura Y, *et al*. Screening of edible Japanese plants for suppressive effects on phorbol ester-induced superoxide generation in differentiated HL-60 cells and AS52 cells. Cancer Lett 2002;176(1):7–16.
252. Pence BC, Reiners JJ Jr. Murine epidermal xanthine oxidase activity: correlation with degree of hyperplasia induced by tumor promoters. Cancer Res 1987;47(23):6388–6392.
253. Reiners JJ Jr., Pence BC, Barcus MC, *et al*. 12-O-tetradecanoylphorbol-13-acetate-dependent induction of xanthine dehydrogenase and conversion to xanthine oxidase in murine epidermis. Cancer Res 1987;47(7):1775–1779.
254. Reiners JJ Jr., Rupp T. Conversion of xanthine dehydrogenase to xanthine oxidase occurs during keratinocyte differentiation: modulation by 12-O-tetradecanoylphorbol-13-acetate. J Invest Dermatol 1989;93(1):132–135.
255. Reiners JJ Jr., Thai G, Rupp T, *et al*. Assessment of the antioxidant/prooxidant status of murine skin following topical treatment with 12-O-tetradecanoylphorbol-13-acetate and throughout the ontogeny of skin cancer. Part I: Quantitation of superoxide dismutase, catalase, glutathione peroxidase and xanthine oxidase. Carcinogenesis 1991;12(12):2337–2343.
256. Smith JK, Carden DL, Korthuis RJ. Activated neutrophils increase microvascular permeability in skeletal muscle: role of xanthine oxidase. J Appl Physiol 1991;70(5):2003–2009.

257. Ventura SM, Dachtler SL. Development and maturation of aldehyde oxidase levels in fetal hepatic tissue of C57BL/6 J mice. Enzyme 1980;25(4):213–219.
258. Ventura SM, Dachtler SL. Effects of sex hormones on hepatic aldehyde oxidase activity in C57BL/6 J mice. Horm Res 1981;14(4):250–259.
259. Al-Senni AE. Tissue distribution of molybdenum hydroxylases, aldehyde oxidase and xanthine, in male and femal camels. Global J Biotechnol Biochem 2009;4(1):43–46.
260. Yoo CB, Chuang J, Byun H, *et al*. Gender- and tissue-specific response to zebularine in Apcmin/+ mice. Conference Title: 98th Annual Meeting of the American-Association-for-Cancer-Research; 2007 April 14–18. Los Angeles (CA): Sponsor: American Association for Cancer; 2007.
261. Yoo CB, Chuang JC, Byun HM, *et al*. Long-term epigenetic therapy with oral zebularine has minimal side effects and prevents intestinal tumors in mice. Cancer Prev Res (Phila Pa) 2008;1(4):233–240.
262. Pratt SK, Pue MA, Fairless AJ, *et al*. Lack of an effect of gender on the pharmacokinetics of penciclovir following single oral dose of famciclovir. Br J Clin Pharmacol 1994; 37:493P.
263. Rivera SP, Choi HH, Chapman B, *et al*. Identification of aldehyde oxidase 1 and aldehyde oxidase homologue 1 as dioxin-inducible genes. Toxicology 2005;207(3):401–409.
264. Ohkubo M, Sakiyama S, Fujimura S. Increase of nicotinamide methyltransferase and N1-methyl-nicotinamide oxidase activities in the livers of the rats administered alkylating agents. Cancer Lett 1983;21(2):175–181.
265. Panchal HD, Vranizan K, Lee CY, *et al*. Early anti-oxidative and anti-proliferative curcumin effects on neuroglioma cells suggest therapeutic targets. Neurochem Res 2008;33(9):1701–1710.
266. Hu R, Xu C, Shen G, *et al*. Identification of Nrf2-regulated genes induced by chemopreventive isothiocyanate PEITC by oligonucleotide microarray. Life Sci 2006; 79(20):1944–1955.
267. Chung WG, Kang JH, Park CS, *et al*. Effect of age and smoking on *in vivo* CYP1A2, flavin-containing monooxygenase, and xanthine oxidase activities in Koreans: determination by caffeine metabolism. Clin Pharmacol Ther 2000;67(3):258–266.
268. Perry CM, Wagstaff AJ. Famciclovir. A review of its pharmacological properties and therapeutic efficacy in herpesvirus infections. Drugs 1995;50(2):396–415.
269. Fowles S. Pharmacokinetics of penciclovir in healthy elderly subjects following a single oral administration of 750 mg of famciclovir. Br J Clin Pharmacol 1992;34(5):450P.
270. Holmes RS. Genetics, ontogeny, and testosterone inducibility of aldehyde oxidase isozymes in the mouse: evidence for two genetic loci (Aox-I and Aox-2) closely linked on chromosome 1. Biochem Genet 1979;17(5–6):517–527.
271. Grant DM, Tang BK, Kalow W. Variability in caffeine metabolism. Clin Pharmacol Ther 1983;33(5):591–602.
272. Vistisen K, Poulsen HE, Loft S. Foreign compound metabolism capacity in man measured from metabolites of dietary caffeine. Carcinogenesis 1992;13(9):1561–1568.
273. Zientek M, Jiang Y, Youdim K, *et al*. *In vitro-in vivo* correlation for intrinsic clearance for drugs metabolized by human aldehyde oxidase. Drug Metab Dispos 2010; 38(8):1322–1327.
274. Rosen AS, Fournie P, Darwish M, *et al*. Zaleplon pharmacokinetics and absolute bioavailability. Biopharm Drug Dispos 1999;20(3):171–175.
275. Dittrich C, Greim G, Borner M, *et al*. Phase I and pharmacokinetic study of BIBX 1382 BS, an epidermal growth factor receptor (EGFR) inhibitor, given in a continuous daily oral administration. Eur J Cancer 2002;38(8):1072–1080.
276. Zhang X, Liu HH, Weller P, *et al*. In silico and *in vitro* pharmacogenetics: aldehyde oxidase rapidly metabolizes a p38 kinase inhibitor. Pharmacogenomics J 2011;11(1):15–24.