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## Flavin-Containing Monooxygenase S-Oxygenation of a Series of Thioureas and Thiones

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### Abstract

Mammalian flavin-containing monooxygenase (FMO) is active towards many drugs with a heteroatom having the properties of a soft nucleophile. Thiocarbamides and thiones are S-oxygenated to the sulfenic acid which can either react with glutathione and initiate a redox-cycle or be oxygenated a second time to the unstable sulfinic acid. In this study, we utilized LC-MS/MS to demonstrate that the oxygenation by hFMO of the thioureas under test terminated at the sulfenic acid. With thiones, hFMO catalyzed the second reaction and the sulfinic acid rapidly lost sulfite to form the corresponding imidazole. Thioureas are often pulmonary toxicants in mammals and, as previously reported by our laboratory, are excellent substrates for hFMO2. This isoform is expressed at high levels in the lung of most mammals, including non-human primates. Genotyping to date indicates that individuals of African (up to 49%) or Hispanic (2–7%) ancestry have at least one allele for functional hFMO2 in lung, but not Caucasians nor Asians. In this study the major metabolite formed by hFMO2 with thioureas from Allergan, Inc. was the sulfenic acid that reacted with glutathione. The majority of thiones were poor substrates for hFMO3, the major form in adult human liver. However, hFMO1, the major isoform expressed in infant and neonatal liver and adult kidney and intestine, readily S-oxygenated thiones under test, with  $K_m$ s ranging from 7–160  $\mu$ M

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and turnover numbers of 30–40 min<sup>-1</sup>. The product formed was identified by LC-MS/MS as the imidazole. The activities of the mouse and human FMO1 and FMO3 orthologs were in good agreement with the exception of some thiones for which activity was much greater with hFMO1 than mFMO1.

## Keywords

Flavin-containing monooxygenase; thioureas; thiones; drug development

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## Introduction

The flavin-containing monooxygenases (FMO) oxygenate numerous endobiotics and xenobiotics, including therapeutic drugs (Cashman, 1995; 2003; Cashman et al., 1995; Krueger and Williams, 2005; Phillips and Shephard, 2008; Ziegler, 2002). FMO, present in the endoplasmic reticulum of cells in numerous tissues, utilizes NADPH to produce a 4a-hydroperoxyflavin intermediate that oxygenates substrates in a two-electron reaction (Ziegler, 1988). Soft nucleophilic heteroatoms typically make the best substrates, but other factors, including size and charge, also determine if the compound will be a substrate (Cashman, 1995; Ziegler, 1988). In most cases, oxygenation by FMO results in a metabolite less toxic than the substrate, but there are some exceptions including two classes of chemicals, found in a variety of industrial chemicals and therapeutic drugs, thiocarbamides and thiones. The initial oxygenation of the sulfur produces the electrophilic sulfenic acid that is capable of reacting with nucleophiles, including glutathione (Decker and Doerge, 1991; Decker et al., 1992; Henderson et al., 2004; Kim and Ziegler, 2000; Poulsen et al., 1979; Russ and Waring, 1991; Vannelli et al., 2002). A number of thioureas have been demonstrated to initiate a redox cycle in which reduced glutathione and NADPH are depleted, resulting in oxidative stress and tissue injury (Krieter et al., 1984; Neal and Halpert, 1982; Smith and Crespi, 2002). If the sulfenic acid is long-lived enough and is also a good substrate for FMO, it is oxygenated to the sulfinic acid. Sulfinic acids are more reactive than the sulfenic acid metabolite and can damage the cell directly or spontaneously decompose to other products (Ji et al., 2007; Onderwater et al., 1999; 2004). Second-line anti-tuberculosis drugs such as ethionamide (thioamide) and thiacetazone (thiourea) are substrates for mammalian FMOs as well as the *Mycobacterium tuberculosis* equivalent, EtaA, (Francois et al., 2009; Henderson et al., 2008; Qian and Ortiz de Montellano, 2006). In this case, S-oxygenation represents bioactivation of a pro-drug (Dover et al., 2007; Nishida and Ortiz de Montellano, 2011; Qian and Ortiz de Montellano, 2006; Vannelli et al., 2002).

Our laboratory, and others, has demonstrated that numerous thionsulfur compounds are substrates for mammalian FMOs producing toxicity. FMOs are a super-family of enzymes. In all the mammals examined to date, there exists a single member in each family. In humans, there are five FMO genes expressed, FMOs 1–5 (Hernandez et al., 2004). Each FMO is expressed under developmental- and hormonal control, but, unlike the major monooxygenase system, the cytochrome P450s (CYPs), has not been shown to be responsive to inducers or inhibitors, other than competitive inhibition by other substrates

(Janmohamed et al., 2004; Shehin-Johnson et al., 1995; Ziegler, 1988; 2002). The majority of drug metabolism is carried out by three FMOs, FMOs 1, 2 and 3. In humans, FMO1 is the major form in fetal liver, but its expression is repressed shortly after parturition and FMO3 expression is turned on so that FMO3 is the major form in adult liver (Hines, 2006; Hines and McCarver, 2002; Koukouritaki et al., 2002). FMO1 is the major FMO in adult human kidney and intestine (Hines, 2006; Koukouritaki et al., 2002; Yeung et al., 2000). This pattern differs from most laboratory animals in that FMO1 is the major hepatic FMO; one exception is the mouse where there is a gender-specific expression of FMO3 in female liver (Janmohamed et al., 2004; Siddens et al., 2008). A number of mutations in FMO3 result in the heritable disease known as trimethylaminuria, in which patients have reduced capacity to N-oxygenate trimethylamine resulting in excretion of high levels of this noxious odorant in urine and sweat (Phillips and Shephard, 2008; Yeung et al., 2007; Zhang et al., 2003). Trimethylaminuria patients also exhibit altered metabolism of some drugs, suggesting expression of these FMO3 allelic variants could play a role in therapeutic efficacy and/or toxicity of certain drugs (Cashman et al., 2003; Shimizu et al., 2007).

FMO2 is the major pulmonary isoform in most mammals, including non-human primates (Dolphin et al., 1998; Krueger et al., 2001; 2002a). An interesting genetic polymorphism exists in humans. Caucasians and Asians carry a C→T transition mutation (Dolphin et al., 1998; Furnes et al., 2003; Krueger et al., 2002b; 2004b; 2005; Whetstine et al., 2000) resulting in a premature TAG stop codon 64 amino acids from the C-terminal (g.23,238C>T; dbSNP#rs6661174; FMO2\*2). This truncated FMO2 (FMO2.2) is catalytically inactive and cannot be detected in human lung tissue by antibodies recognizing the truncated protein, presumably due to incorrect folding and rapid degradation. Approximately 27% of African-American individuals and up to 49% of individuals from Sub-Saharan Africa (Dolphin et al., 1998; Furnes et al., 2003; Krueger et al., 2002a; Veermah et al., 2008; Whetstine et al., 2000) as well as 2–7% (depending on origin) of Hispanics (Krueger et al., 2005; 2004) possess at least one *FMO2\*1* allele, coding for the full-length enzymatically active, protein. Although not yet associated with susceptibility to any lung disease, FMO2 expression may play a role in the efficacy and/or toxicity of therapeutic drugs for which lung is a target or a portal of entry.

Expressed hFMO2.1 has high activity toward a series of thiocarbamides synthesized by Allergan, Inc. We have characterized the kinetics of hFMO2.1 toward these thioureas and with a representative of the series, identified the metabolite by LC-MS/MS as the sulfinic acid. No evidence was found for further oxygenation to the sulfinic acid. Addition of glutathione to the reaction resulted in concentration-dependent disappearance of the sulfinic acid. Expressed hFMO1, hFMO3, mFMO1 and mFMO3 were tested with a series of thiones. These compounds were substrates for hFMO1 and to a lesser degree mFMO1 but were generally not oxygenated by hFMO3 or mFMO3. As seen previously by Decker et al. (1992), the major product was identified as the imidazole, indicating that hFMO1 effectively catalyzes the oxygenation of the sulfinic acid to the unstable sulfinic acid which rapidly decomposes to sulfite and the imidazole.

## Methods

### Chemicals

The compounds under study were provided by Allergan Pharmaceuticals (Irvine, CA). Other reagents and chemicals employed were obtained from Sigma/Aldrich (St. Louis, MO) and were of analytical grade.

### Protein Expression and Determination of Kinetics

Human FMOs 1, 2 and 3 and mouse Fmo1 and 3 were expressed in Sf9 insect cells utilizing baculovirus as previously described (Henderson et al. 2004; 2008). The kinetics of substrate-dependent oxidation of NADPH, in the presence of Sf9 microsomes containing 50 µg of protein, was determined by loss of absorbance at 340 nm utilizing a Cary 300 dual-beam spectrophotometer. The incubation, containing FMO in 100 mM Tricine, pH 9.5 with 1 mM EDTA and 0.1 mM NADPH, was pre-incubated at 37°C for 3 min prior to the addition of substrate (1–200 µM in ethanol (final concentration of 0.1%)). The reactions were run for 4 min and performed with two different batches of FMO-expressing Sf9 microsomes. The  $K_m$  and  $V_{max}$  values were calculated from both Lineweaver-Burk and Eadie-Hofstee plots. For the experiments involving glutathione, concentrations of 0–10 mM glutathione were utilized.

### HPLC Conditions

The reaction conditions were the same as used in the substrate-dependent NADPH oxidation with the exception that 50 µg of Sf9 microsomes expressing human or mouse FMOs was utilized, a concentration of 1 mM NADPH employed and substrate (20–500 µM) was added in ethanol or DMSO in a final incubation volume of 100 µl. The reactions were run at 37°C (with shaking), following a 3 min preincubation, for 15 min. The reactions were terminated by the addition of 100 µl methanol and transferred to ice. The incubation was centrifuged at  $10,000 \times g$  for 30 minutes at 4°C and an aliquot utilized for analysis by HPLC and/or LC/MS/MS. HPLC was performed with a Waters 2690 equipped with a Model 996 diode array detector. The column utilized was a Waters Nova-Pak C<sub>18</sub> 4 µm with dimensions of 3.9 × 150 mm. The solvent system utilized was acetonitrile as the organic phase and 10 mM ammonium formate, pH 3.2, as the aqueous phase. The solvent gradient used to elute parent compound and metabolites was 20% acetonitrile for 8 min followed by a linear gradient to 35% acetonitrile over 3 min. The acetonitrile concentration was held at 35% for 3 min and then returned to initial conditions over 3 min. The flow rate was 0.8 ml/min and the column temperature was 35°C. We allowed 5 min between injections for the column to re-equilibrate.

### LC/MS/MS

The analysis was performed with a Shimadzu (Shimadzu Scientific Instruments, Columbia, MD) Prominence CBM-20 LC with autosampler connected to a Perkin-Elmer (Applied Biosystems, Foster City, CA) Sciex API 365 mass spectrometer in positive ion mode using TurboIon spray. The runs were performed in both the MS and MS/MS modes. The column, solvents and gradient were as described above only the run was performed at room

temperature at a flow rate of 0.5 ml/min and the split ratio was 2:1. The conditions used for multiple reaction monitoring and Q1 full scan modes was as described in Henderson et al. (2008).

## Results

Thioureas have been demonstrated to be effective pulmonary toxicants and some of the Allergan compounds produced pulmonary edema in mice (data not shown). In order to assess the likelihood that FMO2 could bioactivate thioureas in humans expressing the active enzyme (FMO2.1) we determined the kinetics of S-oxygenation with expressed human FMO2.1 by substrate-dependent NADPH oxidation. The thioureas (AGN 1–7) were substrates with  $K_m$ s varying from 29–165  $\mu$ M (Table 1). As expected, urea (AGN 9) or imidazolone (AGN 10) derivatives, were not substrates for hFMO2.1 (Table 1). hFMO2.1 is also capable of S-oxygenation of thiones of similar size (AGN 8, Table 1). The size of the halogens (F, Cl, Br), *meta* and/or *ortho* to the thiourea did not appear to strikingly alter the turnover with hFMO2.1. The lowest specificity constant and highest  $K_m$  was observed with AGN 5 with chlorines in both positions. The more bulky thiourea, AGN 11 (Figure 1), was oxygenated by both human FMO1 and FMO3 (although the  $K_m$ s were both  $> 100 \mu$ M); however, the mouse orthologs, Fmo1 and Fmo3 did not demonstrate appreciable activity with this thiourea perhaps indicating a more restricted substrate access channel.

Following incubation of expressed hFMO2.1 with AGN 3, a single polar metabolite was detected by HPLC (Figure 2). We performed LC-MS/MS to identify the major metabolite that was determined to be the S-oxide-sulfenic acid tautomer (Figure 3). Addition of reduced glutathione to the incubation resulted in disappearance of the sulfenic acid (Figure 4A) and in formation of a glutathione adduct, which appeared as a fragment ion  $[MH-CH_2O]^+$  at  $m/z$  538 (Figure 4B). No sulfenic acid was detected in incubations of hFMO2.1. Significant GSH-dependent reduction in sulfenic acid yield and presence of AGN 3 was observed even when the GSH was added 15 min after initiation of the incubation, suggesting that AGN 3 sulfenic acid undergoes both glutathione adduction and redox-cycling (Figure 5). These results document that expression of FMO2 in human lung is very capable of bioactivation of this series of thioureas to sulfenic acids.

In order to test the activity toward thiones of the major FMOs expressed in liver, kidney and intestine, we examined the metabolism of a series of Allergan, Inc. thiones utilizing expressed hFMO1 and hFMO3. We also thought it important to examine the expressed mouse orthologs to determine the suitability of this species as a model for FMO-dependent drug metabolism. hFMO1 S-oxygenated these thiones with  $K_m$ s ranging from 7–161  $\mu$ M (Table 2). In each example, the  $K_m$  with the hFMO1 was lower than with mFMO1. With the majority of the thiones, the  $K_m$  of the mFMO1 was so high as to be undeterminable. The thiones were poor substrates for both human FMO3 (Table 2) and mouse Fmo3 (data not shown). A  $K_m$  could not be calculated with hFMO3 or with mFmo3 for any of the thiones. At concentrations of 200  $\mu$ M, a  $K_{cat}$  an order of magnitude slower than with FMO1 was detected (data not shown).

The products formed following incubation of one of the thiones (AGN 16) were determined by LC-MS/MS. The major metabolite from HPLC gave an MS peak at minus 32 mass units from the original thione, consistent with formation of an imidazole (Figure 6). The imidazole is postulated to be formed through a two-step oxygenation to the sulfenic and sulfinic acids with subsequent decomposition, release of the sulfur as sulfite and formation of the imidazole (Figure 6). This pathway has been previously described for benzimidazoline-2-thiones by Decker et al. (1992).

## Discussion

Mammalian FMOs typically display high activity toward oxygenation of the sulfur in thioureas and thiocarbamides (Decker and Doerge, 1991; Decker et al., 1992; Henderson et al., 2004; Kim and Ziegler, 2000; Krieter et al., 1984; Neal and Halpert, 1982; Poulsen et al., 1979; Russ and Waring, 1991; Smith and Crespi, 2002; Vannelli et al., 2002). Previous work has identified the initial metabolite as the sulfenic acid. Sulfenic acids either react with cellular nucleophiles, such as glutathione, or can be oxygenated a second time to the reactive and unstable sulfinic acid. In our previous studies with expressed hFMO2 typically a single oxygenation would occur and the sulfenic acid would be eliminated in a dose-dependent manner by the addition of glutathione, even after 15 min of reaction (Henderson et al., 2004; 2008). Thiourea, phenylthiourea, ethylenethiourea and  $\alpha$ -naphthylthiourea had  $K_m$ s of 27, 29, 14 and 42  $\mu$ M, respectively and  $k_{cat}$ s of 51, 24, 48 and 33  $\text{min}^{-1}$ , respectively (Henderson et al., 2004). Rabbit FMO2 displayed  $K_m$ s for these thioureas in the same range (21–82  $\mu$ M) as the human enzyme (Henderson et al., 2004; Nagata et al., 1990). Diphenylthiourea is not a substrate for rabbit or human FMO2, apparently because the larger cross sectional area prevents the sulfur from access to the FAD hydroperoxide active site of the enzyme; FMO2 has the most restrictive active site channel for substrates (Nagata et al., 1990). The activity of hFMO2.1 toward the Allergan thioureas displays similar kinetics ( $K_m$ s of 29–165  $\mu$ M and  $k_{cat}/K_m$ s of 0.23–1.13) to that previously seen with hFMO2.1 and thiourea, ethylenethiourea, phenylthiourea and  $\alpha$ -naphthylthiourea ( $k_{cat}/K_m$  of 0.8–3.4, Henderson et al., 2004). These data reinforce the hypothesis that the major determinant of oxygenation of thioureas by FMO2 is size and the nucleophilic character of the sulfur heteroatom (Poulsen et al., 1979; Ziegler, 1988). With the AGN thioureas tested here, the size and electron-withdrawing potential of the *ortho*- and *meta*-substituted halogens did not significantly impact activity of hFMO2.1 towards S-oxygenation in any consistent way. Onderwater et al. (2006) had shown, utilizing a series of N-substituted N'-(4-imidazole-ethyl)thioureas, that the  $K_m$  of hFMO1 (but not FMO3) was reduced with the electron withdrawing capacity of the N-*p*-phenyl substituents.

The data also demonstrate that hFMO2.1 is capable of S-oxygenation of a wide range of thioureas and readily forms the sulfenic acid. The sulfenic acid is then capable of establishing a redox cycle, consuming glutathione and NADPH, resulting in oxidative stress and toxicity in the lung. Dr. Paul Ortiz de Montellano's laboratory and others have shown that 2-ethylthioisonicotinamide (ethionamide), a second line anti-tuberculosis drug, is oxygenated by human FMO (Francosis et al., 2009; Henderson et al., 2008; Nishida and Ortiz de Montellano, 2011; Qian and Ortiz de Montellano, 2006; Vannelli et al., 2002). We recently have found that, unlike the thioureas, hFMO2.1 does carry out the second S-



oxygenation to the sulfinic acid but at a slower rate (Henderson et al., 2008). This finding is important, given that ethionamide is a prodrug and is bioactivated through the sulfinic acid by a similar monooxygenase found in *Mycobacterium tuberculosis* (Dover et al., 2007; Hannouille et al., 2006). Our laboratory has recently found mice, null for Fmo2 expression, exhibit markedly lower ratios of ethionamide sulfoxide/ethionamide in plasma (Palmer et al., 2012) and pulmonary lavage fluid (Singh et al., unpublished) following dosing with this anti-tuberculosis prodrug. Taken together, these data suggest that the genetic polymorphism in expression of hFMO2 may have a number of consequences with respect to the toxicity and/or therapeutic efficacy of drugs containing a thiocarbamide or related structural moiety in the lung.

The thiones examined in the study were typically good substrates for hFMO1. mFMO1 appears to have a more restricted substrate channel and only slight activity was observed with mFmo1 and thiones at concentrations of 200  $\mu$ M. These thiones were poor substrates for hFMO3 and mFmo3. Thiones, capable of being S-oxygenated by human FMO, are also being used as therapeutic agents against disease such as tuberculosis (Qian and Ortiz de Montellano, 2006). It is interesting to note that with hFMO1 the orientation of the quinoline nitrogen relative to the dihydro-imidazole-3-thione results in a 10-fold or greater difference in the  $k_{cat}/K_m$  (compare AGN 16 with AGN 15 and AGN 18 in Table 2).

In summary, drugs containing the thiocarbamide or thione structure are S-oxygenated by multiple FMOs. FMO2 S-oxygenates thioureas to the sulfenic acid which may result in pulmonary oxidative stress and toxicity. Only humans (a percentage of individuals of African or Hispanic descent) expressing the full-length FMO2.1 enzyme would be expected to be susceptible. Thiones are substrates for hFMO1, expressed in fetal liver and adult intestine and kidney. Mice are unusual among mammals in that mFmo1 is expressed at appreciable levels in lung (Janmohamed et al., 2004; Siddens et al., 2008) and therefore studies of FMO-dependent toxicity of these compounds in this model must be interpreted in that light.

Thiones appear to be poor substrates for hFMO3, making it unlikely that this enzyme would be responsible for any hepatotoxicity associated with these drugs. As previously demonstrated by Decker et al. (1992), thiones are S-oxygenated by FMO first to the sulfenic acid, then the sulfinic acid which rapidly and spontaneously decomposes to the imidazole with release of  $\text{HSO}_3^-$ . In the presence of GSH, the imidazole is not formed as, with thioureas, GSH binds to the intermediates. FMO1 metabolism and bioactivation in intestine could impact oral bioavailability and in kidney result in nephrotoxicity. Our results reinforce the need to consider FMO activity in drug development for thiocarbamides and related compounds capable of S-oxygenation to reactive intermediates.

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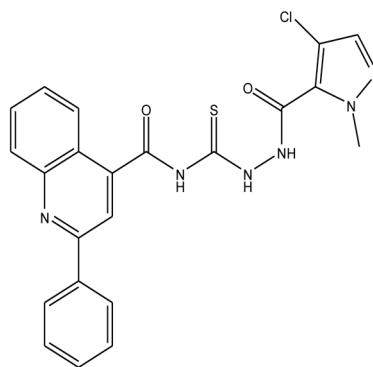
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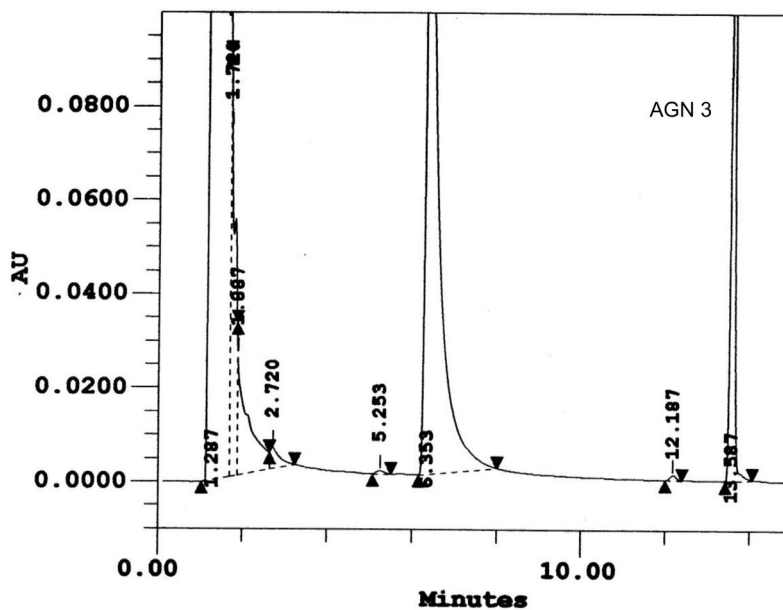
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<u>Ortholog</u>	<u>K<sub>m</sub> (μM)</u>	<u>K<sub>cat</sub> (min<sup>-1</sup>)</u>	<u>K<sub>cat</sub>/K<sub>m</sub></u>
hFMO1	130	120	0.92
mFmo1	ND	ND	
hFMO3	116	97	0.83
mFmo3	ND	ND	

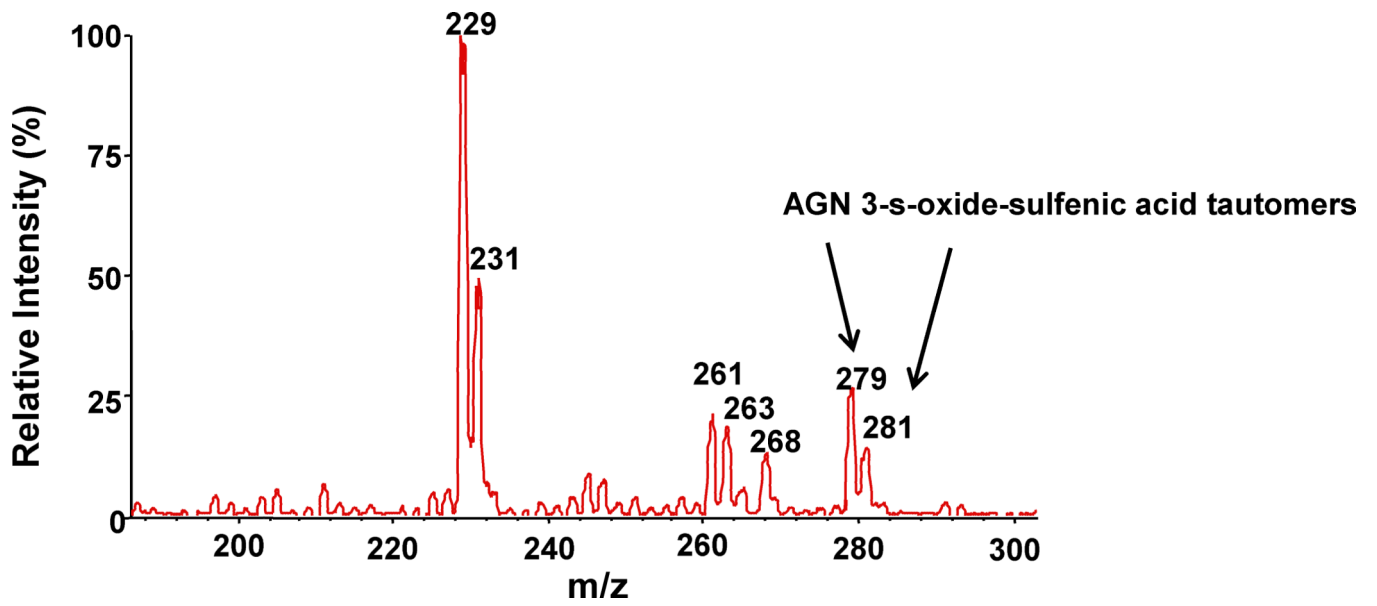
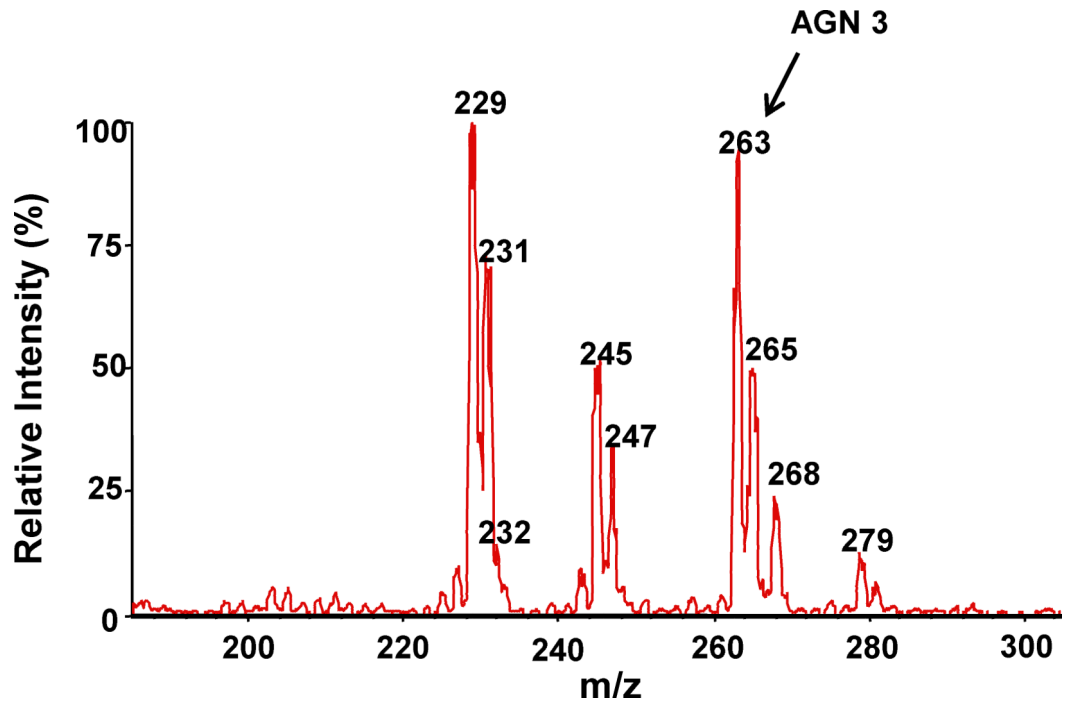
**Figure 1. S-Oxygenation of AGN 11 by hFMO1 and hFMO3 Compared to the Mouse Orthologs mFmo1 and mFmo3**

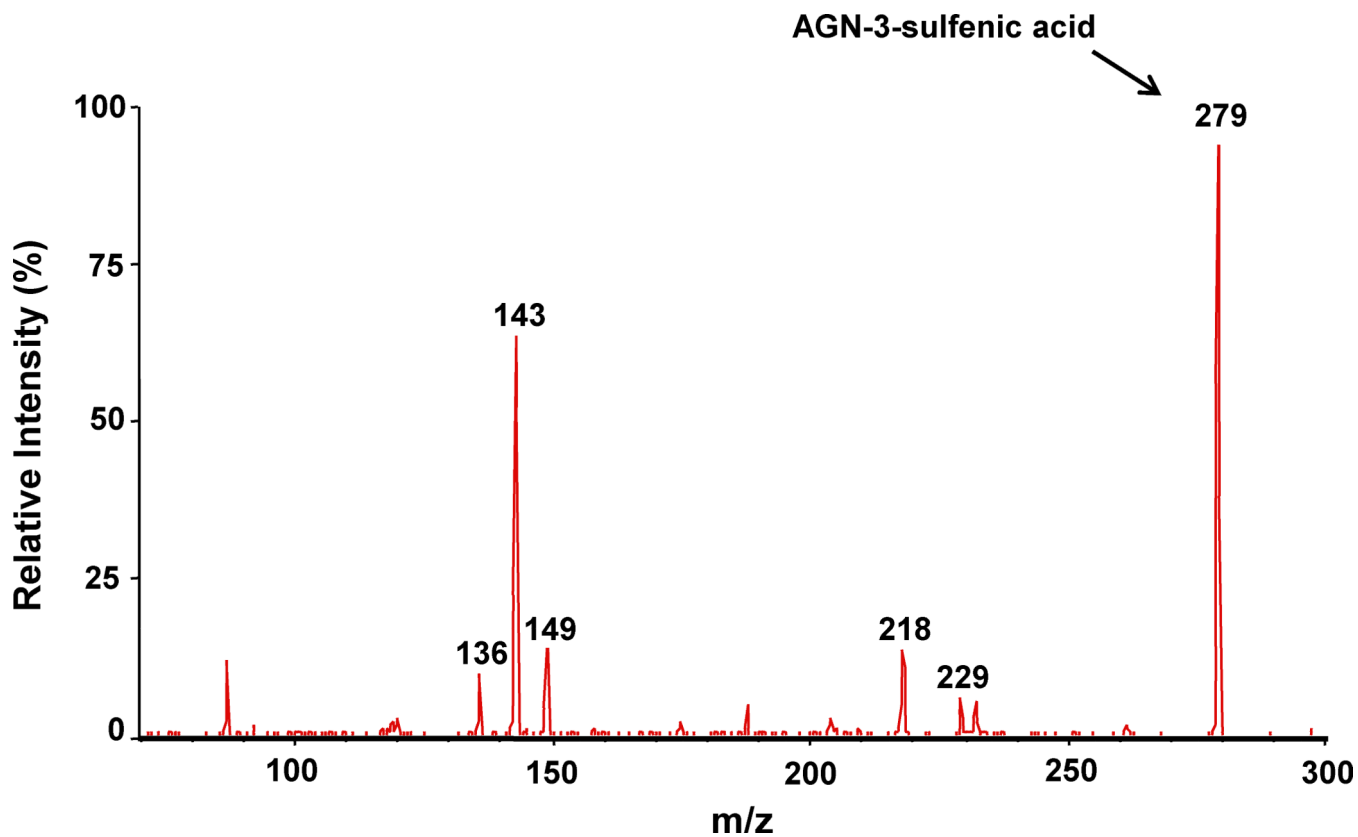
Activity of human, hFMO1 and hFMO3, as well as mouse, mFmo1 and mFmo3, toward AGN 11, a bulky thiourea. Kinetic constants were determined using Lineweaver-Burk and Eadie-Hofstee plots taking the average from two Sf9 insect cell microsomal preparations. ND indicates that the activity was too low to determine kinetic constants.



**Figure 2. HPLC of the Major Metabolite Following Incubation of AGN 3 (see Table 1) with hFMO2**

Incubation of hFMO2 with the thiourea AGN 3 in the presence of NADPH produced a single polar metabolite (peak at 6.4 minutes). The parent compound eluted at 13.6 minutes under these conditions.





**Figure 3.** LC-MS/MS of the polar metabolite of AGN 3. **A.** MS scan of the parent AGN 3 thiourea ( $m/z$  263); there appears to be a small amount of contamination with formamidine sulfenic acid; **B.** MS scan of the major polar metabolite ( $m/z$  279 & 281) identified as the S-oxide-sulfenic acid tautomer; **C.** MS/MS scan of the AGN 3 sulfenic acid metabolite ( $m/z$  279).



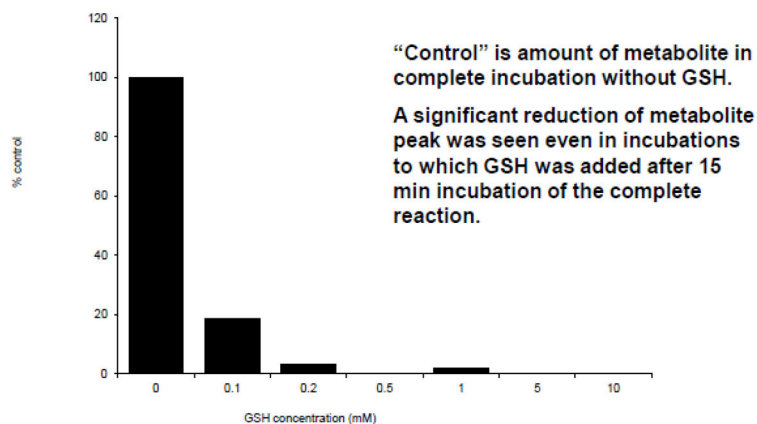


Figure 4A.

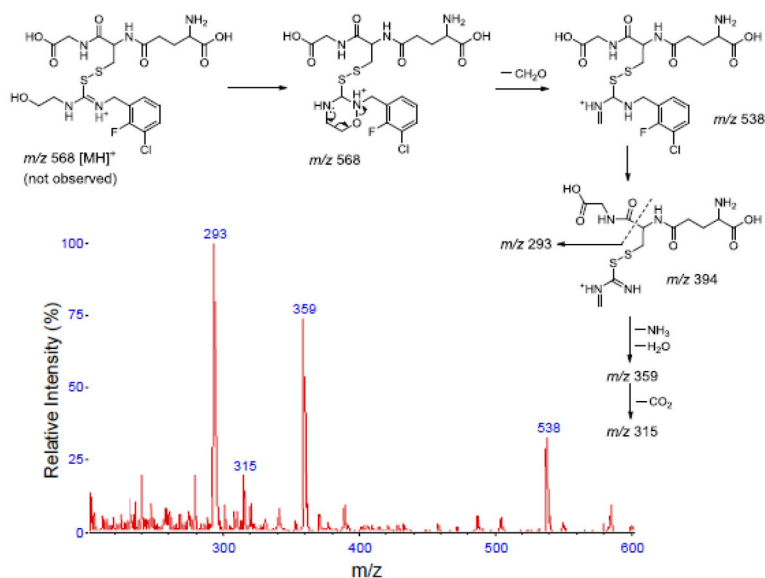
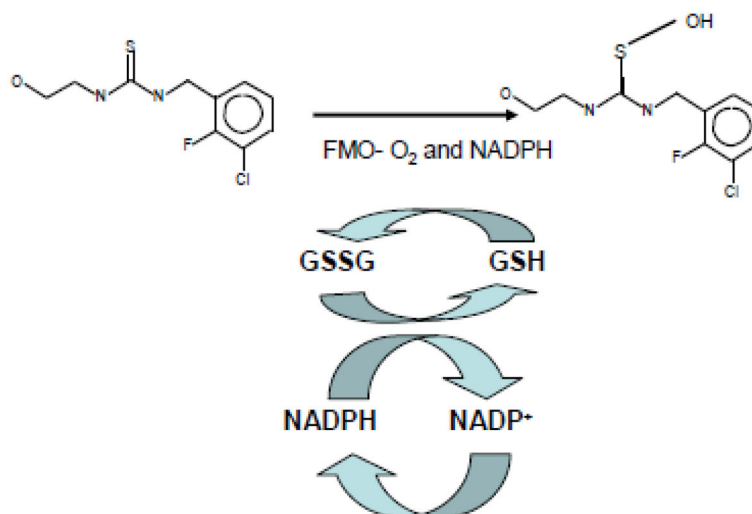


Fig. 4B

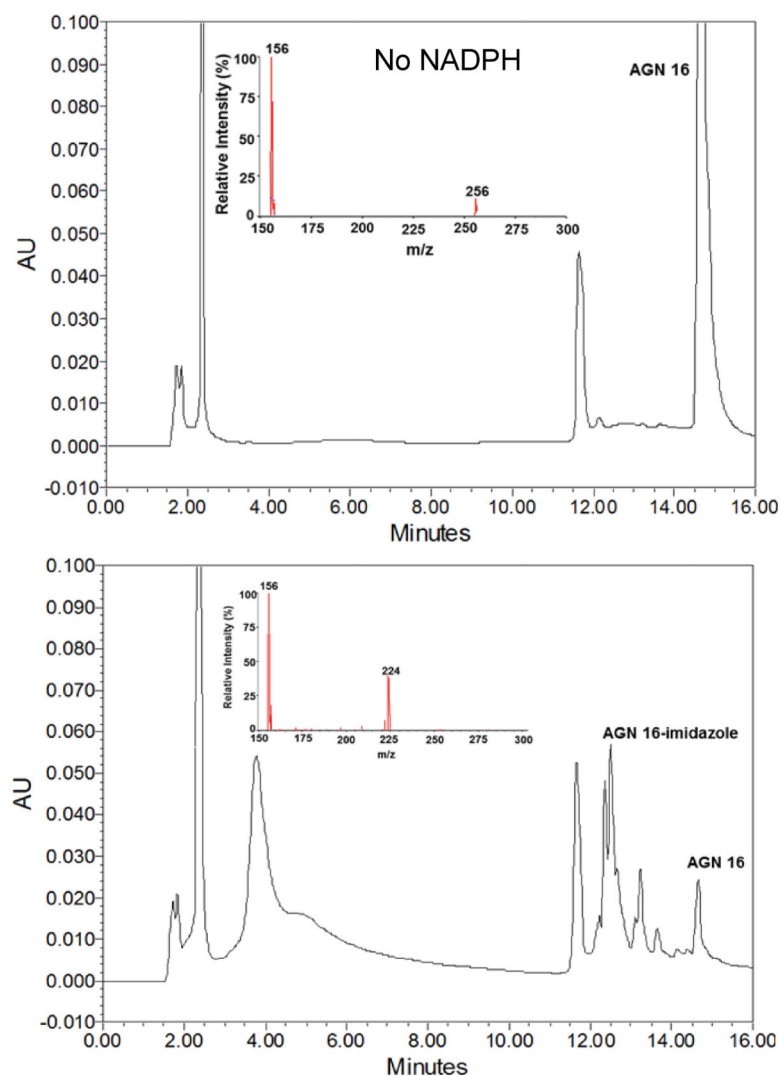
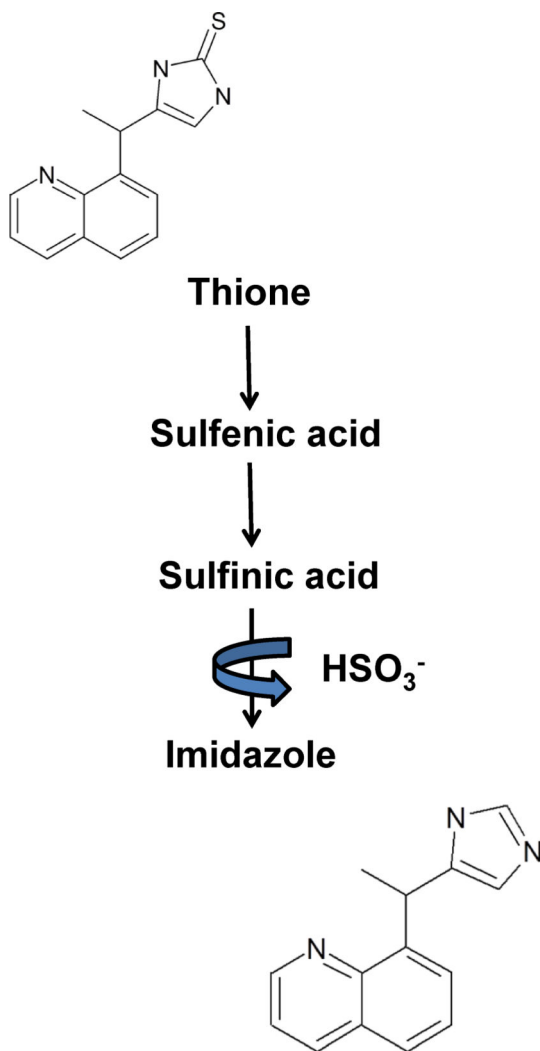
**Figure 4.**

Addition of glutathione eliminates the sulfenic acid metabolite. **A.** Concentration-dependent yield of the AGN 3 sulfenic acid in the presence of glutathione. Metabolite was reduced even when GSH was added 15 min after incubation began. The percent metabolite on the y axis is compared to the yield in the absence of glutathione. **B.** Mass spectrum and proposed mass fragmentation pathway of the glutathione adduct of AGN 3.



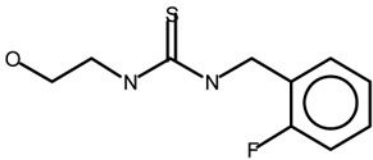
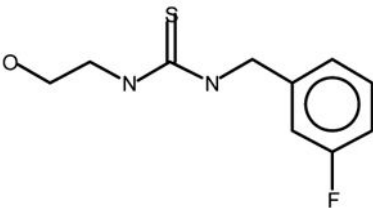
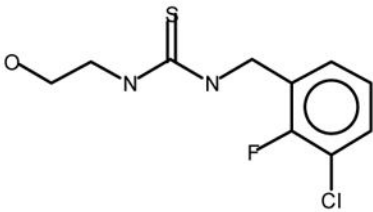
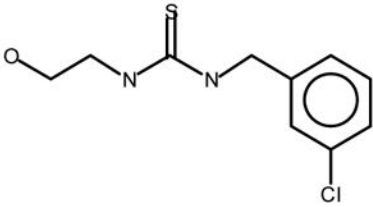
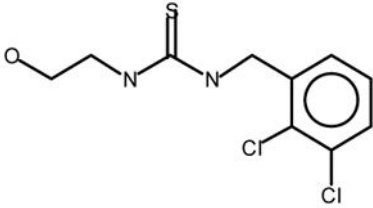
**Figure 5. Diagram of Redox-Cycling Generated by S-Oxygenation of Thiourea AGN 3 (Table 1)**

Diagram of the proposed redox cycle that occurs with AGN 3 in the presence of glutathione.



**Figure 6.** HPLC-MS Analysis of hFMO1 Conversion of the Thione AGN 16 to the Imidazole  
 LC-MS/MS analysis of hFMO1 conversion of the thione AGN 16 to the imidazole. HPLC chromatogram of AGN 16 (top right panel) with corresponding MS spectra ( $m/z$  256), following incubation with expressed hFMO1. Addition of NADPH (bottom right panel) converts most of the parent compound (14.8 min peak) to slightly more polar compounds (12.5 min peak; MS spectra  $m/z$  224), consistent with the loss of S.

**Table 1**S-Oxygenation by hFMO2 of thioureas with varying *o*- and *m*-halogen substituents.

Compound	$K_m$ ( $\mu\text{M}$ )	$K_{cat}$ ( $\text{min}^{-1}$ )	$k_{cat}/K_m$
 AGN1	55	36	0.65
 AGN2	74	42	0.57
 AGN3	29	23	0.79
 AGN4	31	35	1.13
 AGN5	165	38	0.23

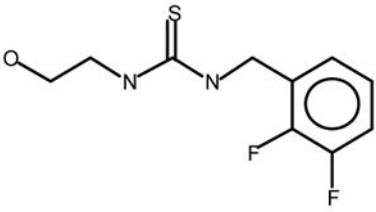
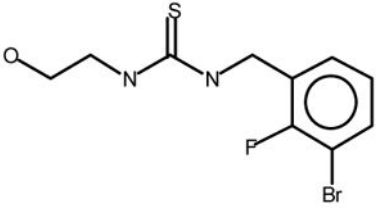
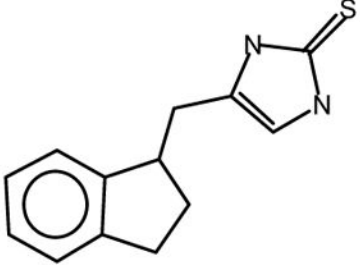
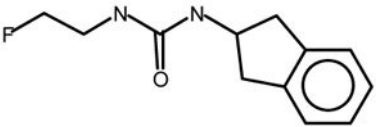
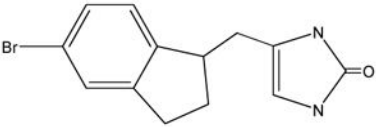
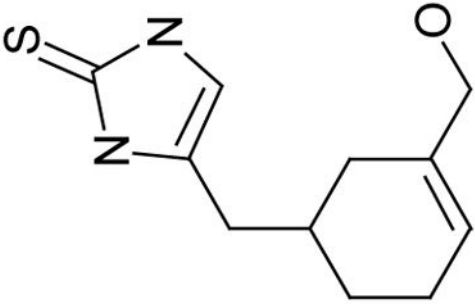
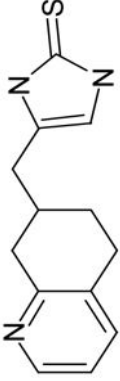
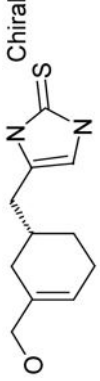

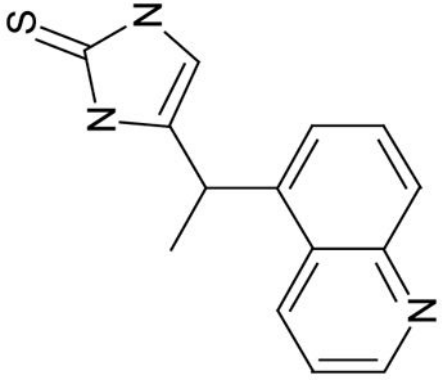
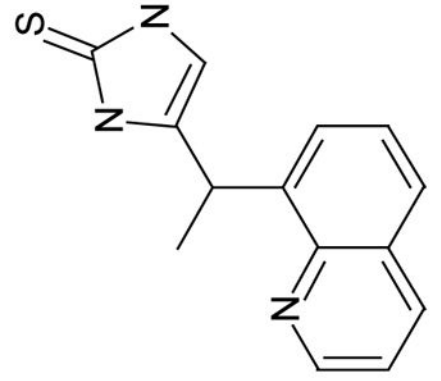
Compound	$K_m$ ( $\mu\text{M}$ )	$K_{cat}$ ( $\text{min}^{-1}$ )	$k_{cat}/K_m$
 AGN6	51	34	0.67
 AGN7	51	33	0.65
 AGN8	36	25 A thione derivative	0.69
 AGN9	No Activity – urea derivative		
 AGN10	No activity – imidazolone derivative		

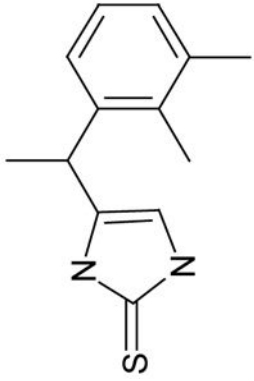
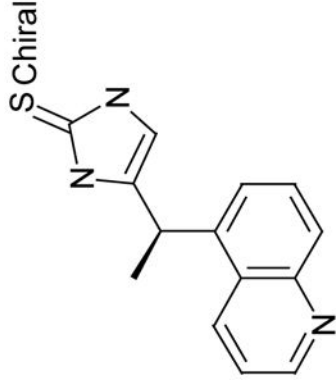
Table 2

S-oxygenation of thiones by human and mouse FMOs.

Compound	hFMO1		mFMO1		hFMO3		
	$K_m$ ( $\mu\text{M}$ )	$k_{\text{cat}}$ ( $\text{min}^{-1}$ )	$k_{\text{cat}}/K_m$	$K_m$ ( $\mu\text{M}$ )	$k_{\text{cat}}$ ( $\text{min}^{-1}$ )	$k_{\text{cat}}/K_m$	$K_m$
	139	49	0.35	159	47	0.35	ND
	40	40	1.00	40	48	1.20	ND
	161	40	0.24	ND	23 @ 200 $\mu\text{M}$	--	ND
							



Compound	hFMO1			mFMO1			hFMO3		
	$K_m$ ( $\mu\text{M}$ )	$k_{\text{cat}}$ ( $\text{min}^{-1}$ )	$k_{\text{cat}}/K_m$	$K_m$ ( $\mu\text{M}$ )	$k_{\text{cat}}$ ( $\text{min}^{-1}$ )	$k_{\text{cat}}/K_m$	$K_m$	$k_{\text{cat}}/K_m$	$K_m$
 AGN15	111	33	0.30	ND	28 @ 200 $\mu\text{M}$	--	ND	--	ND
 AGN16	7	38	5.43	ND	34 @ 200 $\mu\text{M}$	--	ND	--	ND

Compound	hFMO1			mFMO1			hFMO3		
	$K_m$ ( $\mu\text{M}$ )	$k_{\text{cat}}$ ( $\text{min}^{-1}$ )	$k_{\text{cat}}/K_m$	$K_m$ ( $\mu\text{M}$ )	$k_{\text{cat}}$ ( $\text{min}^{-1}$ )	$k_{\text{cat}}/K_m$	$K_m$	$k_{\text{cat}}/K_m$	$K_m$
	31	41	1.32	ND	40 @ 200 $\mu\text{M}$	--	ND	--	ND
AGN-17 	60	30	0.50	ND	27 @ 200 $\mu\text{M}$	--	ND	--	ND
AGN18 