METABOLISM OF NOMIFENSINE TO A DIHYDROISOQUINOLINIUM ION METABOLITE BY HUMAN MYELOPEROXIDASE, HEMOGLOBIN, MONOAmine OXIDASE A, AND CYTOCHROME P450 ENZYMES

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ABSTRACT:
Nomifensine is an antidepressant agent that was removed from use because of a high incidence of hemolytic anemia. It contains an N-methyl-8-aminotetrahydroisoquinoline ring which has the potential to be oxidized to quaternary dihydroisoquinolinium and isoquinolinium ions, albeit such a transformation had not been previously observed. In this report, we demonstrate the conversion of nomifensine to a dihydroisoquinolinium ion metabolite by several human enzymes. Human liver microsomes supplemented with NADPH generated the dihydroisoquinolinium ion metabolite along with other hydroxylated metabolites, whereas when supplemented with t-butyl peroxide, only the dihydroisoquinolinium ion metabolite was observed. Monoamine oxidase A, but not monoamine oxidase B, catalyzed this reaction, as well as human hemoglobin supplemented with H₂O₂. Human myeloperoxidase catalyzed this reaction in the presence of H₂O₂, and activation of the reaction was observed when incubations were conducted in the presence of acetaminophen at concentrations relevant to those measured in humans. The reaction was also observed in human whole blood. The equilibrium between the dihydroisoquinolinium ion and carbinalamine was shown to have a pK of about 11.7. The dihydroisoquinolinium ion was shown to react with cyanide and borohydride, but not glutathione. These findings suggest that the electrophilic nomifensine dihydroisoquinolinium metabolite, which can be generated by several enzymes, could be behind toxic responses to nomifensine such as hemolytic anemia and hepatotoxicity.

Nomifensine (Fig. 1) was an antidepressant drug with a unique receptor binding profile that was introduced in the 1980s (Kinney, 1985). However, it was discontinued from use because of an unacceptable incidence of drug-induced hemolytic anemia (Salama and Mueller-Eckhardt, 1985; Stonier and Edwards, 2002). Its use was also associated with reports of hepatotoxicity (Nielsen and Lund, 1981; Kummer et al., 1985). Efforts to address the mechanism of nomifensine-induced toxicity have included analysis of sera from patients which showed the presence of antibodies reactive to nomifensine and some of its reported metabolites (Salama and Mueller-Eckhardt, 1985, 1986).

Nomifensine possess an aniline group, which is a substituent in drugs frequently associated with blood and liver toxicities (Kalgutkar et al., 2005). In an examination of the metabolism of nomifensine in vivo, the observed metabolites included alterations of the phenyl ring, including conjugates suggesting the existence of a catechol intermediate metabolite (Kellner et al., 1977; Heptner et al., 1978; Hornke et al., 1980; Lindberg and Syvalahti, 1986). These represent two possibilities of structural elements in nomifensine that could underlie the toxicity of the drug. We hypothesized that the tetrahydroisoquinoline structure of nomifensine could be the chemical group responsible for toxicity, as metabolism to the dihydroisoquinolinium ion would represent a bioactivation pathway analogous to the formation of other iminium ion metabolites [e.g., 1-methyl-4-phenyl-1,2,3,6-tetrahydro-
methods (Moehrle and Biegholdt, 1988; Venkov and Vodeničar, 1990). Human hemoglobin, human myeloperoxidase, \( t \)-butyl peroxide (70%), reduced glutathione, and hydrogen peroxide (30%) were obtained from Sigma (St. Louis, MO). Monoamine oxidases were generously provided by Dr. Dale Edmondson, Emory University. Human liver microsomes, a pool from 60 individual donors, were prepared using standard procedures and maintained in an in-house bank at Pfizer.

**Incubation Procedures.** For human liver microsomes, nomifensine (0.5–500 \( \mu \)M) was incubated with human liver microsomes (0.2 mg/ml) and either NADPH (1.3 mM) or \( t \)-butyl peroxide (1.0 mM) in 0.2 ml of KH\( \text{PO}_4 \), 25 mM, pH 7.4, containing MgCl\(_2\) (3.3 mM). Reactions were commenced with the addition of cofactor and conducted at 37°C for 10 min. An initial experiment established linear conditions with respect to protein concentration and time. Reactions were terminated by the addition of 4 volumes of 0.1 M HCl and analyzed directly by HPLC-MS as described below. Incubation conditions used to generate nomifensine metabolite profiles were similar to those described above, except that the protein concentration was 1.0 mg/ml, the nomifensine concentration was 10 \( \mu \)M, and the incubation was terminated by an equal volume of CH\(_3\)CN. Analysis of these samples was done using HPLC-MS.

Monoamine oxidase A (0.1 nmol/ml) was incubated with nomifensine (10–1000 \( \mu \)M) in 0.2 ml of 25 mM potassium phosphate, pH 7.4, at 37°C for 1 h. An initial experiment established a linear relationship between product generation and incubation time. The incubations were terminated by addition of HCl (0.8 ml of 0.1 M), and analyzed for the dihydroisoquinolinium ion by HPLC-MS.

For hemoglobin and myeloperoxidase, incubations were carried out in optical 96-well plates in a PerkinElmer plate reader (PerkinElmer, Boston, MA) with a 420-nm filter, maintained at 37°C, with readings taken every 24 s. For incubations with hemoglobin, nomifensine maleate (10–1000 \( \mu \)M) and H\(_2\)O\(_2\) (0.5 mM) were incubated with human hemoglobin (0.6 \( \mu \)M), in 0.25 ml of 25 mM KH\( \text{PO}_4 \), pH 7.4. For reactions catalyzed by myeloperoxidase, incubations consisted of nomifensine maleate (10–1000 \( \mu \)M), acetaldehyde (44 \( \mu \)M), human myeloperoxidase (1.0 U/ml) in 0.25 ml of 200 mM KH\( \text{PO}_4 \), pH 7.45. Experiments were also conducted in which the concentration of acetaldehyde was varied between 0 and 200 \( \mu \)M. Velocity calculations were done using a molar extinction coefficient for nomifensine dihydroisoquinolinium ion of 3153/cm that was determined experimentally.

Nomifensine (10 \( \mu \)M) was incubated with whole blood (0.02 ml) added to 0.98 ml of isotonic saline at 37°C for 30 min in the presence or absence of H\(_2\)O\(_2\) (0.5 \( \mu \)M). The incubations were terminated with 1 ml of CH\(_3\)CN and 0.05 ml were injected on HPLC-MS as described below.

**HPLC-MS Conditions.** For metabolite profiling, samples were injected onto a Polaris C18 column (4.6 \( \times \) 250 mm; 5-\( \mu \)m particle size; Varian, Inc., Lake Forest, CA) equilibrated in 20 mM acetic acid, adjusted to pH 4.0 with NH\(_4\)OH containing 18% CH\(_3\)CN at a flow rate of 0.8 ml/min. The CH\(_3\)CN was increased to 36% over 20 min, followed by an immediate increase to 86% CH\(_3\)CN to wash the column for 5 min. The eluent was introduced into the ionspray source of the Micromass Ultima mass spectrometer with the same tune file conditions as described above. The analyte was detected with selected reaction monitoring of the transition m/z 237 \( \rightarrow \) 146. Quantitation was accomplished using a standard curve ranging from 10.0 to 1000 ng/ml.

**Chemical Reactions and pH Titration of Nomifensine Dihydroisoquinolinium Ion.** Nomifensine iminium perchlorate (14.8 \( \mu \)M) was incubated with KCl (0–100 mM) in phosphate buffer (900 mM) at pH 7.5. Incubations with reduced glutathione (0–100 mM) were conducted at the same concentration of nomifensine dihydroisoquinolinium ion, in phosphate buffer at 500 mM, pH 7.5. Incubations (1 ml) were carried out at room temperature for 1 h, after which they were read on a Varian Cary 4000 UV/VIS spectrophotometer at 415 nm.

The pH titration was conducted similarly. To a solution of nomifensine dihydroisoquinolinium perchlorate (14.8 \( \mu \)M) was added varying amounts of NaOH (and water to keep the volume addition constant) at ambient temperature, and the pH was recorded. The absorbance at 415 nm was recorded.

In the reduction reaction, to an aqueous solution (0.2 ml) of nomifensine dihydroisoquinolinium perchlorate (1 mg/ml) was added 2 mg of Na\(_2\)B\(_4\)O\(_7\). The reaction was observed to change color from yellow to colorless upon addition of the reducing agent. The reaction was stirred at room temperature for 0.5 h, after which 1 ml of water was added, followed by extraction into methyl \( t \)-butyl ether (3 ml). The solvent was evaporated, and the residue was reconstituted in mobile phase and infused into an HPLC-MS instrument.

**Data Analysis.** Enzyme kinetic data were analyzed using the Enzyme Kinetics module of SigmaPlot (v8; SPSS, Chicago, IL). Data were fit to the following equations:

\[
\frac{v}{V_{\text{max}}} = \frac{[S]}{K_{\text{M}} + [S]} \quad \text{or} \quad \frac{v}{V_{\text{max}}} - \frac{[S]}{K_{\text{M}} + [S]} + \frac{[S]}{K_{\text{CLint}} + [S]}
\]

**Results**

**Metabolite Profile of Nomifensine with Peroxidases, Monoamine Oxidases, Cytochrome P450 Enzymes, and Hemoglobin.** HPLC-MS profiles of metabolic incubations of nomifensine with human liver microsomes, hemoglobin, myeloperoxidase, and monoamine oxidases are shown in Fig. 2. Hemoglobin, myeloperoxidase, MAO-A, and human liver microsomes all catalyzed turnover of nomifensine. Four metabolites were observed: nomifensine dihydroisoquinolinium ion (peak 2; retention time = 19.3 min), and three metabolites proposed to arise via monohydroxylation reactions (peaks 3, 4, and 5) because their protonated molecular ions were 16 mass units greater than those of nomifensine. The only incubation condition to yield the monohydroxy metabolites was the one containing human liver microsomes supplemented with NADPH, consistent with these metabolites arising via cytochrome P450-mediated catalysis. Human liver microsomes supplemented with NADPH also yielded the dihydroisoquinolinium ion metabolite. Interestingly, when liver microsomes were supplemented with \( t \)-butyl peroxide instead of NADPH, only the dihydroisoquinolinium ion metabolite was formed. Hemoglobin, and myeloperoxidase supplemented with H\(_2\)O\(_2\) both yielded the dihydroisoquinolinium ion, but no other metabolites. When the myeloperoxidase incubation was also supplemented with acetaldehyde, a greater rate of reaction was observed (also see below). Only a trace signal for the dihydroisoquinolinium ion was observed in incubations containing MAO-B, suggesting that this enzyme is not an active catalyst of this reaction. Finally, a trace signal was observed for the ion current at m/z 235 that eluted just after the dihydroisoquinolinium ion, indicative of an isoquinolinium ion metabolite. Interest-
ingly, an N-desmethyl metabolite was not observed, despite this type of metabolic reaction being very common for tertiary amines.

**Kinetics of Nomifensine Dihydroisoquinolinium Ion Formation.**

To gain a more quantitative understanding of the activities of these enzymes to catalyze the dehydrogenation of nomifensine to the dihydroisoquinolinium ion, the enzyme kinetics were measured. These are summarized in Table 1. For liver microsomes, the kinetic data suggested two distinguishable activities when supplemented with either NADPH or t-butyl peroxide: a low \( K_M \) activity of 26 or 11 \( \mu M \), along with a high \( K_M \) activity that did not approach saturation. This observation could be due to two or more cytochrome P450 enzymes present in liver microsomes that can catalyze this reaction. Interestingly, hemoglobin also demonstrated this biphasic enzyme kinetic behavior, despite being a pure protein. Myeloperoxidase and MAO-A demonstrated single enzyme kinetic behavior. The \( K_M \) value for MAO-A was considerably higher (\( >1 \) mM) than that for the other enzymes/proteins.

Adjusting the enzyme kinetic values to intrinsic clearance values normalized on a per nanomole protein basis allows for comparison of the nomifensine dehydrogenation activities of these enzymes to each other. The rank order of intrinsic clearances was myeloperoxidase > hemoglobin > cytochrome P450 > MAO-A.

Myeloperoxidase has been shown to be stimulated by the presence of various phenolic compounds (Subrahmanyam et al., 1991). The

TABLE 1

<table>
<thead>
<tr>
<th>Enzyme/System</th>
<th>Kinetic Model</th>
<th>( K_M ) (( \mu M ))</th>
<th>( V_{max} ) (nmol/min/nmol enzyme)</th>
<th>( CL_{int} ) (ml/min/nmol)</th>
<th>( CL_{int(total)} ) (ml/min/nmol)</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver microsomes + NADPH</td>
<td>Two-enzyme</td>
<td>26 ( \pm 8 )</td>
<td>1.8 ( \pm 0.2 )</td>
<td>0.0028</td>
<td>0.072</td>
<td>0.993</td>
</tr>
<tr>
<td>Liver microsomes + t-BuOOH</td>
<td>Two-enzyme</td>
<td>11 ( \pm 2 )</td>
<td>3.3 ( \pm 0.3 )</td>
<td>0.0015</td>
<td>0.30</td>
<td>0.990</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>Two-enzyme</td>
<td>21 ( \pm 2 )</td>
<td>6.7 ( \pm 0.2 )</td>
<td>0.0039</td>
<td>0.32</td>
<td>0.996</td>
</tr>
<tr>
<td>Myeloperoxidase + APAP</td>
<td>Single-enzyme</td>
<td>109 ( \pm 8 )</td>
<td>187 ( \pm 5 )</td>
<td>N.A.</td>
<td>1.7</td>
<td>0.993</td>
</tr>
<tr>
<td>Monoamine oxidase A</td>
<td>Single-enzyme</td>
<td>1130 ( \pm 360 )</td>
<td>7.7 ( \pm 2.2 )</td>
<td>N.A.</td>
<td>0.0068</td>
<td>0.998</td>
</tr>
</tbody>
</table>

\( t \)-BuOOH, \( t \)-butyl peroxide; APAP, acetaminophen; N.A., not applicable.

\( a \) For liver microsomes, a value of 0.3 nmol total P450/mg protein was used.

FIG. 2. HPLC-MS chromatograms of nomifensine incubation mixtures. Peak 1, nomifensine; peak 2, nomifensine dihydroisoquinolinium ion; peaks 3, 4, and 5, putative hydroxy metabolites possessing molecular ions 16 mass units greater than that of nomifensine.
nomifensine dehydrogenase activity of myeloperoxidase was readily observed when in the presence of acetaminophen, a phenolic drug, and the enzyme kinetics were measured in the presence of 6.7 μg/ml acetaminophen, which is similar to unbound plasma concentrations observed in vivo after typical doses of this drug. The relationship between myeloperoxidase-catalyzed nomifensine dehydrogenase activity and acetaminophen concentration, at nomifensine concentrations of 100 μM (approximately equal to $K_m$) and 1 mM (approximately 10 times $K_m$) is shown in Fig. 3. Maximal stimulation of the activity was observed at 50 μg/ml.

**Chemical Reactivity of Nomifensine Dihydroisoquinolinium Ion.** In aqueous solution, alicyclic iminium ions are in equilibrium with carbinolamines. With increasing pH, the carbinolamine form predominates. The effect of pH on the iminium ion-carbinolamine equilibrium was examined by measurement of the absorbance of nomifensine dihydroisoquinolinium perchlorate at 415 nm (an absorbance maximum peak of the conjugated iminium-aniline system). The inflection point was at a pH of about 11.7, indicating that the iminium form predominates at physiological pH (Fig. 4). Stabilization of the iminium form can be derived from the resonance stabilization imparted by the aniline ring. Other iminium ion-carbinolamine equilibria can occur at lower pH values when the iminium ion does not possess any special stabilization, such as that of the iminium ion metabolite of nicotine (Brandange and Lindblom, 1979).

To probe its potential as a reactive electrophile, nomifensine dihydroisoquinolinium ion was tested for reaction with cyanide and glutathione at physiological pH. Incubations with increasing concentrations of cyanide demonstrated a decrease in absorbance at 415 nm, with a 50:50 ratio of iminium ion and adduct (presumed to be the α-cyanoamine) occurring at about 10 mM KCN (Fig. 4).
isolate the cyanide adduct product resulted in reversal of the reaction to the dihydroisoquinolinium ion. Nomifensine dihydroisoquinolinium ion did not show any detectable reaction with GSH. Reaction between nomifensine dihydroisoquinolinium and NaBD$_4$ resulted in the generation of a deuterated nomifensine that was 1 mass unit greater than nomifensine when analyzed by mass spectrometry (m/z 240 versus 239).

Conversion of Nomifensine to Nomifensine Dihydroisoquinolinium Ion by Blood. Although it is of interest that nomifensine can be oxidized to the dihydroisoquinolinium ion by hemoglobin and myeloperoxidase when supplemented with H$_2$O$_2$, potential relevance in vivo would require that this conversion can occur in intact blood cells without high concentrations of peroxide. Upon incubation of nomifensine with whole blood diluted in isotonic saline, formation of the dihydroisoquinolinium ion was observed in the presence or absence of hydrogen peroxide (Fig. 5).

Discussion

Despite its unique profile as an effective antidepressant, nomifensine was removed from use because of an unacceptable incidence rate of hemolytic anemia (Stonier and Edwards, 2002). Initiation of this adverse effect could arise via the generation of a reactive metabolite, but proof of such an intermediate for nomifensine has not been reported, despite demonstration of nomifensine antibodies in patients (Salama and Mueller-Eckhardt, 1985, 1986). Nomifensine is an aromatic amine, and compounds containing aromatic amines such as dapsone have been shown to be associated with blood and liver toxicities (Bluhm et al., 1999; Kalgutkar et al., 2005). Also, a methyl catechol metabolite of nomifensine was demonstrated in humans, suggesting the possible presence of a catechol intermediate metabolite that could be the precursor of an electrophilic ortho-quinone. In the results described in the present report, the generation of a dihydroisoquinolinium ion metabolite from nomifensine was demonstrated. Previous studies have shown that tetrahydropyridine analogs including tetrahydroisoquinolines and $\beta$-carboline derivatives are also activated to the respective N-methylisoquinolinium or N-methyl-$\beta$-carboline metabolites (Castagnoli et al., 1997) by two metabolic steps, which involve $N$-methylation by a nonspecific N-methyltransferase and subsequent oxidation by MAO (Naoi et al., 1994). The nomifensine dihydroisoquinolinium ion metabolite offers another possibility that could lead to an understanding of the toxicity of nomifensine, and possibly design of an alternate agent that possesses the desired pharmacological effects while lacking the toxic effects.

The reactivity of nomifensine dihydroisoquinolinium ion as an electrophile was probed using model nucleophiles. The pH dependence of the equilibrium between iminium ion and carbinolamine provides insight into the reactivity of the $\alpha$-carbon, when considering hydroxide ion serving as the nucleophile. The pK of this equilibrium was approximately 11.7. This value is considerably greater than that previously measured for nicotine $\Delta l'(5')$ iminium ion (pK$_N$ = 8.4; Brandange and Lindblom, 1979). It is proposed that the greater pK for nomifensine dihydroisoquinolinium ion versus nicotine iminium ion is due to the conjugation of the iminium double bond to the aromatic ring as well as the aniline nitrogen for nomifensine. Thus, the iminium ion for nomifensine can be resonance-stabilized (Fig. 6), whereas for nicotine iminium ion, this is not possible. This hypothesis is further supported by the observation that although a cyano adduct for nicotine $\Delta l'(5')$ iminium ion can be generated, it cannot be isolated, whereas 5'-cyanonicotine is a stable, isolable product from nicotine $\Delta l'(5')$ iminium ion (Peterson et al., 1987). Whether nomifensine antibodies arise from an immune response to an immunogen that is generated from a dihydroisoquinolinium ion-protein adduct is not known.

In this report, the conversion of nomifensine to nomifensine dihydroisoquinolinium ion was observed. However, in previous examinations of the metabolism of nomifensine in humans, neither the dihydroisoquinolinium ion nor any downstream products (such as the lactam) were reported (Heptner et al., 1978; Hornke et al., 1980; Lindberg and Syvalahti, 1986). But in these earlier studies, a quantitative description of the metabolite profile was not obtained; therefore, the dihydroisoquinolinium ion or any down-
Fig. 6. Nomifensine oxidation to nomifensine dihydroisoquinolinium ion along with various equilibrium phenomena. Oxidation to the iminium ion could occur via α-carbon hydroxylation to the carbinolamine, which will be in equilibrium with the iminium ion. The iminium ion can be in equilibrium with nucleophile adducts. In both of these cases, at physiological pH, the iminium ion is favored. Alternatively, it is also possible but less likely that the iminium ion could be generated by an initial N-hydroxylation of the aniline and dehydrogenation to the imine, which would be in equilibrium with the iminium ion.

stream metabolites may have been missed. Alternatively, due to its existence as a charged species, it is possible that nomifensine dihydroisoquinolinium ion could be generated and trapped within the cell or subcellular organelles. Since nomifensine dihydroisoquinolinium ion can be generated by hemoglobin and MAO-A, it could also be trapped in erythrocytes or mitochondria, respectively, and elicit toxicity. Demonstration of this conversion in whole red blood cells, even in the absence of H₂O₂, further supports this hypothesis.

Several enzymes catalyze the oxidation of nomifensine to nomifensine dihydroisoquinolinium ion. The heme-containing enzymes cytochrome P450, myeloperoxidase, and hemoglobin all catalyze the reaction, probably all with a peroxidase-type mechanism. Human liver microsomes generated several metabolites, including the dihydroisoquinolinium ion, when supplemented with NADPH, but only the dihydroisoquinolinium ion was generated when supplementation was done with r-butyl peroxide. The latter cova bstrate is known to support cytochrome P450-catalyzed peroxidation reactions. MAO enzymes have a different mechanism, so that the conversion of nomifensine to nomifensine dihydroisoquinolinium ion can arise by different oxidation mechanisms. Hemoglobin-catalyzed drug oxidations have been reported previously, but these are generally considered rare (Mieyal et al., 1976; Starke et al., 1984; Giardina et al., 1995).

Myeloperoxidase-catalyzed oxidations have been implicated in the toxicity of other drugs such as clozapine (Hofstra and Uetrecht, 1993; Ip and Uetrecht, 2006). The conversion of nomifensine to the dihydroisoquinolinium ion by myeloperoxidase was stimulated by acetaminophen. A great deal of experimental data have been generated in support of acetaminophen bioactivation to reactive intermediates that cause hepatotoxicity. However, to our knowledge, investigation of acetaminophen as an agent that could activate the process of myeloperoxidase-generated reactive intermediates has not been shown. Activation occurred at acetaminophen concentrations that are relevant to unbound circulating levels attained after standard dosing of acetaminophen for analgesia (Fig. 3). This finding opens the possibility of the involvement of acetaminophen as an indirect contributor to toxicities of other drugs that involve myeloperoxidase-catalyzed processes. In a previous investigation, stimulation of myeloperoxidase by phenols had been demonstrated (Subrahmanyam et al., 1991). Furthermore, stimulation of the conversion of oxyhemoglobin to methemoglobin and peroxide by phenols has been known for many years (Wallace and Caughey, 1975). Whether this mechanism is also the one underlying the stimulation of myeloperoxidase by acetaminophen and whether acetaminophen could also stimulate nomifensine oxidation catalyzed by hemoglobin represent areas in need of further research.

Since several enzymes can catalyze this reaction, it is challenging to deconvolute the contributions that each could have to conversion in vivo. Enzyme kinetic data were gathered for this reaction for the different enzymes, in a preliminary effort to determine this. In consideration of the total quantity of hemoglobin in the body relative to the other proteins, it could be proposed that hemoglobin would be the main protein involved in this transformation in vivo. In a typical 70-kg human, estimates of the total amount of blood hemoglobin, hepatic cytochrome P450, and neutral myeloperoxidase are approximately 11,000, 19, and 2.6 μmol, respectively. Combination of these values with the intrinsic clearance values in Table 1 suggests that the contribution of hemoglobin to this reaction would far outweigh the contributions of the other enzymes. However, it must also be considered that the measurement of the enzyme kinetics for hemoglobin and myeloperoxidase in this report were made at a high concentration of hydrogen peroxide. Although the identities of several enzymes that can catalyze this reaction have been made, further work is required to delineate the contributions of each of them.

In conclusion, the conversion of the tetrahydroisoquinoline-containing drug nomifensine to a dihydroisoquinolinium ion metabolite has been demonstrated. Several enzymes and proteins can catalyze this reaction. Nomifensine dihydroisoquinolinium ion possesses electrophilic reactivity. Thus, these investigations have identified the N-alkyl tetrahydroisoquinoline ring moiety as a potential toxicophore capable of generating dihydroisoquinolinium ion metabolites. Further investigation of the metabolism and reactivity of this structure type is ongoing.

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