INHIBITION OF RAT LIVER MICROSOMAL CYP1A2 AND CYP2B1 ACTIVITY BY N-(2-HEPTYL)-N-METHYL-PROPARGYLAMINE AND BY N-(2-HEPTYL)-PROPARGYLAMINE

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ABSTRACT:

(R)-N-(2-Heptyl)-N-methyl-propargylamine (R-2HMP) and (R)-N-(2- heptyl)-propargylamine (R-2HPA) are analogs of R-deprenyl, R-Deprenyl, a selective monoamine oxidase B inhibitor, is a mecha- nism-based inactivator of purified CYP2B1. The aim of the present study was to determine whether R-2HMP and R-2HPA behaved like deprenyl with respect to inhibiting cytochrome P450 (CYP450) en- zyme activity. The activities of CYP1A2 and CYP1A1 were as- sed by measuring the deethylation of 7-ethoxyresorufin by liver microsomes obtained from control and β-naphthoflavone-treated female Wistar rats, respectively. CYP2B1 activity was assessed by measuring depentylation of 7-pentoxyresorufin by liver micro- somes obtained from phenobarbital-treated rats. The activity of CYP1A1 was unaffected by 100 μM concentrations of R-deprenyl, R-2HMP, or R-2HPA. In contrast, the activities of CYP1A2 and CYP2B1 were significantly decreased. In general, the percentage of CYP1A2 activity remaining in the presence of 100 μM of one of these propargylamines ranged from 45 to 58%, whereas 10% or less of CYP2B1 activity remained. No marked differences between the various propargylamines were observed. The IC50 values for the inhibition of CYP2B1 activity by R-deprenyl, R-2HMP, and R-2HPA were found to be 2.6, 8.5, and 3.6 μM, respectively. The S-enantiomers of deprenyl, 2HMP, and 2HPA also inhibited the activity of microsomal CYP2B1. R-2HMP, R-2HPA, and S-2HPA were found to be mechanism-based inactivators of CYP2B1 activity. The inactivation constants k\text{inact} and KI were found to be as follows: R-deprenyl, 1.3 μM and 0.32 min⁻¹; R-2HMP, 0.8 μM and 0.08 min⁻¹; R-2HPA, 0.5 μM and 0.36 min⁻¹; and S-2HPA, 0.24 μM and 0.18 min⁻¹.

The cytochrome P450 (CYP450) enzymes are heme-containing proteins located in the endoplasmic reticulum and mitochondria of cells (Nedelcheva and Gut, 1994; Guengerich, 1996; Glue and Clement, 1999). Within the past 5 or 6 years, our understanding of apo- ptotic cell death has been expanded to include the CYP450 enzymes as contributors to the formation of reactive oxygen species (ROS). The findings that 1) ROS can act as one of the myriad triggers inducing apoptosis, 2) CYP450 enzymes can generate ROS, 3) CYP450 enzymes are located in the mitochondria, and 4) the mito- chondria are central to the initiation of apoptosis have put the CYP450 enzymes into the web of events leading to apoptosis (Anandatheerathavarada et al., 1997; Dalton et al., 1999; Bhagwat et al., 2000; Nebert et al., 2000).

Further support for the notion that CYP450 enzymes are involved in apoptosis comes from the demonstration that CYP450 inhibitors attenuate oxidative stress-induced apoptotic death of cultured hepatocytes (Shiba and Shimamoto, 1999). Recently, R-deprenyl was found to be an inhibitor or inactivator of CYP2B1 (Sharma et al., 1996). R-Deprenyl and the aliphatic analogs, (R)-N-2-heptyl-N-methyl-propargylamine (R-2HMP) and (R)-N-2-heptylpropargylamine (R-2HPA), reduce apoptotic death in cerebellar granule cells, PC12 cells, and in several in vivo models of apoptosis (Ansari et al., 1993; Tatton et al., 1996; Boulton et al., 1997; Paterson et al., 1998; Berry, 1999). The goal of this study was to determine whether R-2HMP and R-2HPA inhibited the activity of cytochrome CYP2B1, as has been shown for R-deprenyl. In addition, an examination of the effects of these propargylamines on the activities of CYP1A2, a normally abundant isoform, and CYP1A1, a minor but inducible isoform, was undertaken. Other propargylamines, pargyline, desmethyldeprenyl, as well as the S-enantiomers of deprenyl, 2HMP, and 2HPA were included for com- parison.

Experimental Procedures

Materials. β-Nicotinamide adenine dinucleotide phosphate, reduced form, tetrasodium (NADPH), glycerol; sucrose, disodium EDTA, Trizma base, α-naphthoflavone (ANF), β-naphthoflavone (BNF), dimethyl sulfoxide (DMSO), resorufin, 7-pentoxyresorufin, proadifen, metyrapone, and pargyline were purchased from Sigma-Aldrich Canada Ltd. (Ontario, Canada). 7-Ethoxyresorufin was purchased from Molecular Probes (Eugene, OR). (R)- and (S)-Deprenyl and (R,S)-desmethyldeprenyl were obtained from Sigma/RBI (Natick, MA). R-2HMP, S-2HMP, R-2HPA, S-2HPA, (R)-N-2-heptyl-N-meth-
yramine (R-2HMA), (R)-N-2-heptylamine (R-2HA), and (R)-N-2-heptylaminopropionic acid (R-2HPCAc) were synthesized by Dr. B. Davis (Durner et al., 2000). The chemical purity of these compounds was determined by elemental analysis, NMR, and mass spectrometry to be 99%, except for R-2HPCAc, which was 95% pure. Disposable semimicro cuvettes were purchased from VWR Canada, Edmonton, AB, Canada.

Drug Treatment of Rats. Female Wistar rats (body weight, 225–250 g; Charles River, Montreal, PQ, Canada) were injected i.p. with vehicle (saline or corn oil); phenobarbital (PB), 75 mg/kg in saline; or DMSO in corn oil, 15 mg/kg i.p. once daily for four consecutive days. Phenobarbital at this dosage regimen increases the content of rat liver CYP2B1/2 20 or more times above control (Lubet et al., 1985; Masubuchi et al., 1995). NADPH at the dosage selected increases the levels of CYP1A1 (Burke et al., 1985, 1994; Oinonen et al., 1994) and is less toxic than methylicholanthrene, another CYP1A1 inducer (Zhao and Shichi, 1995). All procedures were approved by the Animal Care Committee of the University of Saskatchewan and were in accordance with the guidelines of the Canadian Council on Animal Care.

Preparation of Rat Liver Microsomes. Rat liver was rinsed with ice-cold 0.15 M KCl/0.2 M sucrose/10 mM EDTA (pH 7.2–7.2) (Nims et al., 1994), finely sliced, and homogenized (1 g to 3 ml of solution) in this ice-cold solution using a Polytron homogenizer at setting 6 with 20–30 s bursts, and centrifuged for 30 min at 9000 g at 4°C. The supernatant was centrifuged at 100,000 g for 1 h at 4°C in a Beckman L7-65 ultracentrifuge; the microsomal pellet was washed in the aforementioned solution and recentrifuged at 100,000 g for 1 h at 4°C. The final microsomal pellet was suspended in ice-cold 50 mM Tris buffer, pH 7.5, containing 20% glycerol and 1 mM EDTA, partitioned into aliquots, and stored at −70°C (Hopkins et al., 1992).

CYP1A2, CYP1A1, and CYP2B1 Assays. The O-deethylation of 7-ethoxycoumarin to 7-hydroxycoumarin by liver microsomes from control and from PB-pretreated rats can be used as a measure of CYP1A2 and CYP1A1 activity, respectively (Burke et al., 1985; Lubet et al., 1985).

The incubation mixture contained (final concentrations listed, 1-ml final volume in disposable cuvettes) the following: 50 mM Tris buffer, pH 7.5; 5 μM 7-ethoxycoumarin; 5 mM MgCl2; 50 μl of microsomal suspension (30–400 μg of protein). Inhibitors (known inhibitors or other drugs) were preincubated in this mixture for 3 min at 37°C then 1 mM NADPH was added to initiate the assay. Blank samples (no NADPH) were run routinely. Samples were incubated at 37°C and fluorescence was read at 2-min intervals for up to 10 or 20 min using an Aminco-Bowman fluorometer at an excitation wavelength of 365 nm and an emission wavelength of 460 nm. A calibration curve obtained with resorufin concentrations from 1 nM to 3 μM was run routinely.

The dealkylation of 7-pentoxyresorufin by liver microsomes from PB-pretreated rats is a measure of CYP2B1 activity (Burke et al., 1985, 1994; Lubet et al., 1985). The incubation mixture was the same as described above but using microsomes obtained from phenobarbital-pretreated rats and 5 μM 7-pentoxyresorufin as the substrate.

Inhibition Studies. Microsomes were preincubated with known CYP450 inhibitors or with other drugs in the presence of substrate for 3 min prior to the addition of NADPH to initiate the enzyme assay (Roberts et al., 1995; Beebe et al., 1996). Drugs were dissolved in the Tris assay buffer, except for ANF, which was solubilized in DMSO. DMSO itself inhibits CYP450 activity (Burke et al., 1985); therefore, the ANF samples were compared with control samples with 5 μl of DMSO added. Known inhibitors were initially tested to confirm the selectivity of the assays, then other drugs at an initial concentration of 100 μM were tested for their ability to inhibit rat liver microsomal CYP1A2, 1A1, or 2B1 activities.

IC50 values were calculated for the propargyramines of interest using regression analysis (CA-Cricket Graph III software program) of the graph of the percentage of activity remaining after a 10-min incubation versus concentrations of the inhibitor (Beebe et al., 1996).

Inactivation Experiments. Microsomes were preincubated with R-depropynyl, R-2HMP, R-2HPA, S-2HPA, or R-2HA in the presence of NADPH for 0, 0.5, 1, 2, 3, 4, or 5 min. Following the various preincubation time periods, 7-pentoxyresorufin was added and fluorescence readings taken after a 10-min incubation. For comparison, proadifen, which is not a mechanism-based inactivator, was run as a negative control. The natural logarithm of the percentage of activity remaining values was plotted against the time of preincubation at each concentration of these drugs (Roberts et al., 1995; Beebe et al., 1996; Sharma et al., 1996).

The reciprocals of the slope of each drug concentration from these graphs were then calculated and plotted against the reciprocals of the respective concentrations of propargyramines. Linear regression analysis was used to calculate the kinetic constants kinit, the maximal rate constant of inactivation, and kcat, the concentration of inactivator yielding half-maximal inactivation (Roberts et al., 1995; Beebe et al., 1996; Sharma et al., 1996).

Membrane Filtration Experiments. To assess the reversibility of the inactivation of microsomal CYP2B1 by the selected propargyramines, these drugs were preincubated with liver microsomes in the presence of NADPH, and then the samples were filtered by centrifugation at 14,000 g through a Microcon YM-30 (Millipore Corporation, Bedford, MA), a filter device fitted with a YM-30 cellulose membrane with a molecular cut-off of 30,000. The retentate, typically a 40–50 μl volume, containing the recovered CYP2B1 protein (Roberts et al., 1998) was added to a 1-m1 incubation mixture containing NADPH. 7-Pentoxyresorufin was added and fluorescence readings taken following a 5-min incubation. Control samples consisted of liver microsome samples that received the same pretreatment and that were incubated at the same time as the YM-30-filtered samples but were centrifuged in a polyethylene tube (i.e., no YM-30 membrane filtration).

Protein Determination. The protein concentrations of the microsomal preparations were determined (Lowry et al., 1951). The amount of liver microsomal protein used per assay from control rats was typically 250 to 280 μg, 200 to 250 μg from PB-treated rats, and 30 to 40 μg from BNF-treated rats.

Statistical Analyses. The percentage of activities remaining in samples incubated with inhibitors was calculated using the control samples run at the same time as the inhibitor. To determine whether the reductions in the percentage of activity remaining in the presence of the tested compounds were significant, the activities of the inhibitors were compared with control samples using one-way analysis of variance and by determining significant differences with Scheffe’s test (Winer, 1962).

Results

Confirmation of CYP1A2, 1A1, and 2B1 Enzyme Activity Assays. All three P450 assays were linear up to 10-min incubation. With a 10-min incubation, the CYP1A2 assay was linear up to 400 μg of protein, the CYP1A1 assay was linear up to 50 μg of protein, and the CYP2B1 assay was linear up 380 μg of protein. The CYP2B1 activity of liver microsomes obtained from vehicle-treated rats typically was 0.3 ± 0.2 nmol/min/mg of protein. CYP1A1 activity of liver microsomes from BNF-treated rats was 4.0 ± 0.1 nmol/min/mg of protein and CYP2B1 activity of microsomes from PB-treated rats was 0.7 ± 0.03 nmol/min/mg of protein.

The effects of three known inhibitors on the activity of CYP1A2, 1A1, and 2B1 confirmed the selectivity of the assays. ANF (10 μM) caused the greatest reduction in CYP1A1 activity (only 1 ± 1% activity remained compared with 41 ± 1% CYP2A12 activity and 70 ± 3% CYP2B1 activity remaining), whereas 100 μM metyrapone completely abolished CYP2B1 activity with smaller effects on the other enzymes (41 ± 1% CYP1A2 and 61 ± 1% CYP1A1 activity remained). Proadifen (10 μM) effectively inhibited CYP2B1 and 1A2 activity (only 7 ± 1 and 17 ± 1% activity remained, respectively), whereas CYP1A1 was not affected.

Effects of Propargyramines and Related Compounds on CYP1A2, 1A1, and 2B1 Activity. The effects of several propargyramines and structurally similar compounds (Fig. 1) all at a concentration of 100 μM were assessed (Table 1). None of the compounds affected CYP1A1 activity except for desmethyldepenyl, in which case a slight but significant decrease was observed. The propargyramine compounds (R-depropynyl, desmethyldepenyl, pargylamine, R-2HMP, and R-2HPA) significantly reduced CYP1A2 and CYP2B1 activities compared with control. About 50 to 60% CYP1A2 activity remained in the presence of these compounds; however, only about
Inhibition of Microsomal CYP2B1 by Propargylamines. Because the propargylamines were found to have their greatest effect on CYP2B1 activity, this enzyme was selected for further study. Different concentrations of R-deprenyl, R-2HMP, and R-2HPA were incubated with microsomes for 10 min to determine their IC50 values for the inhibition of CYP2B1 activity. Graphical analyses estimated the IC50 values to be 2.6 μM for R-deprenyl, 8.5 μM for R-2HMP, and 3.6 μM for R-2HPA (Fig. 2).

Effects of Stereochemistry on Inhibition of CYP2B1 by Propargylamines. A comparison of the ability of (R)- and (S)-enantiomers of deprenyl, 2HMP, and 2HPA at concentrations of 10 μM to decrease the activity of microsomal CYP2B1 activity was undertaken. With all three propargylamines, the percentage of activity remaining in the presence of the (R)-isoform of the compounds was less than that remaining with the corresponding (S)-isoforms (Fig. 3).

Effect of NADPH Preincubation Time and Drug Concentration on CYP2B1 Inactivation. The effect of inhibitor concentration and the length of the preincubation time with NADPH on the ability of R-deprenyl, R-2HMP, R-2HPA, S-2HPA, and R-2HA to inactivate CYP2B1 activity was examined next. For comparison, proadifen was included as a negative control. With proadifen (2.5 and 5 μM), the ln percentage of CYP2B1 activity remaining values increased with increasing time of preincubation (data not shown); in contrast, with R-deprenyl, R-2HMP, R-2HPA, and S-2HPA, the ln percentage of activity remaining values decreased in a concentration-dependent manner with increasing length of preincubation time (Fig. 4). However, with 50 μM R-2HA, the ln percentage of activity remaining values did not decrease significantly as the preincubation time increased (data not shown).

Estimation of Kinetic Constants for Inactivation of Microsomal CYP2B1 Activity. The slopes (kobs) of the individual lines in Fig. 4 were obtained by linear regression analysis, and then the reciprocals of the slopes and the reciprocals of the drug concentrations were plotted to derive the kinetic constants (Fig. 5). From the equation describing the best fit for each propargylamine, the inactivation constants kmax and KI were calculated to be 1.3 μM and 0.32 min⁻¹ for R-deprenyl, 0.8 μM and 0.08 min⁻¹ for R-2HMP, 0.5 μM and 0.36 min⁻¹ for R-2HPA, and 0.24 μM and 0.18 min⁻¹ for S-2HPA.

Membrane Filtration Studies. To assess the reversibility of the inactivation of microsomal CYP2B1 activity by 3.2 μM concentrations of R-deprenyl, R-2HMP, R-2HPA, or S-2HPA, each of these drugs was preincubated with the microsomes and then filtered through a membrane that retains molecules greater than 30,000 mol. wt. For comparison, 100 μM R-2HA was included. It can be seen in Fig. 6 that filtering of the microsomal samples preincubated with each of the four propargyl compounds did not diminish significantly the inactivation of CYP2B1 activity by the propargylamines, but the effects of R-2HA were almost completely reversed by YM-30 filtration.

Discussion

Generally, it is accepted that CYP2B1 activity can be assayed with high selectivity by using 7-pentoxyresorufin as the substrate and microsomes obtained from phenobarbital-pretreated rats (Burke et al., 1985, 1994; Lubet et al., 1985). Similarly, CYP1A1 activity is assayed predominantly over CYP1A2 using liver microsomes from BNF-pretreated rats with 7-ethoxyresorufin as the substrate (Burke et al., 1985), whereas CYP1A2 activity is detected with uninduced (vehicle-treated) rat liver microsomes. The pattern of inhibition by proadifen, ANF, and metyrapone confirmed the selectivity of the assays (data not available).

TABLE 1

<table>
<thead>
<tr>
<th>Inhibitor (100 μM)</th>
<th>% CYP1A2 Activity Remaining</th>
<th>% CYP1A2 Activity Remaining</th>
<th>% CYP2B1 Activity Remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-Deprenyl</td>
<td>45 ± 2</td>
<td>100 ± 2</td>
<td>2.8 ± 0.2‡</td>
</tr>
<tr>
<td>Desmethyldeprenyl</td>
<td>65 ± 2</td>
<td>94 ± 1‡</td>
<td>11.5 ± 0.5‡</td>
</tr>
<tr>
<td>Pargyline</td>
<td>56 ± 2</td>
<td>96 ± 4</td>
<td>10.2 ± 1.1‡</td>
</tr>
<tr>
<td>R-2HMP</td>
<td>60 ± 2</td>
<td>93 ± 2</td>
<td>8.5 ± 0.4‡</td>
</tr>
<tr>
<td>R-2HPA</td>
<td>56 ± 2</td>
<td>100 ± 1</td>
<td>8.6 ± 0.4‡</td>
</tr>
<tr>
<td>R-2HMA</td>
<td>97 ± 2</td>
<td>96 ± 1</td>
<td>85.4 ± 4.9‡</td>
</tr>
<tr>
<td>R-2HA</td>
<td>107 ± 4</td>
<td>96 ± 1</td>
<td>46.9 ± 2.1‡</td>
</tr>
<tr>
<td>R-2HPCAc</td>
<td>95 ± 1</td>
<td>95 ± 1</td>
<td>106 ± 3.2</td>
</tr>
</tbody>
</table>

* Value significantly different from respective control (p < 0.05).

10% or less of CYP2B1 activity remained in the presence of these propargyl drugs.

The three compounds lacking a propargyl group, R-2HMA, R-2HA, and R-2HPCAc, are structurally related to R-2HMP and R-2HPA (Fig.
ANF and metyrapone are selective inhibitors of CYP1A2 and CYP2B, respectively (Barham et al., 1994; Halpert et al., 1994). Our results confirm a prior report that showed that R-deprenyl inhibits purified CYP2B1 but not CYP1A1 (Sharma et al., 1996). In this article, we show that several other propargylamines, (R,S)-desmethyldeprenyl, R-2HMP, R-2HPA, and pargyline also inhibited the activity of rat liver microsomal CYP2B1; furthermore, we found that these drugs inhibited microsomal CYP1A2 activity (Table 1). Structurally similar compounds lacking the propargyl function (Fig. 1) did not reduce CYP1A2 and CYP1A1 activities, but significant, although smaller, reductions in CYP2B1 activity were observed with R-2HMA and R-2HA (Table 1).

Using IC50 values as a measure of the efficacy of inhibition of microsomal CYP2B1 activity, it was found that the two alkyl compounds, R-2HMP and R-2HPA, were similar to R-deprenyl (Fig. 2). It appears that the alkyl side chain was comparable to the phenyl group of deprenyl. Further studies are in progress to determine the effect of the length and chirality of the alkyl side chain on inhibition of CYP2B1 by such compounds.

An examination of the time course of the inhibition of CYP2B1 activity by 10 μM concentrations of the (R)- and (S)-enantiomers of deprenyl, 2HMP and 2HPA, revealed that the (R)-enantiomers inhibited CYP2B1 activity to a greater extent than the respective (S)-compounds (Fig. 3). Using R- and S-2HPA, this was further explored by determining inactivation constants for these two compounds.
Kinetic constants were derived from the equations (shown in inset into the graphs) describing the best linear fit. Values shown are means, n = 6.

Two criteria that can be used to define a particular drug as a mechanism-based inactivator are that the drug-induced CYP450 inhibition is dependent on the length of the preincubation time with NADPH and on the concentration of inactivator (Roberts et al., 1995; Beebe et al., 1996; Sharma et al., 1996). R-Deprenyl has been reported previously to be a mechanism-based inactivator of purified CYP2B1 (Sharma et al., 1996). By these criteria, R-deprenyl, R-2HMP, R-2HPA, and S-2HPA were mechanism-based inactivators since their inactivation of microsomal CYP2B1 activity increased with increasing time of preincubation in a concentration-dependent manner (Fig. 4). In contrast to the propargylamines, R-2HA, which inhibited CYP2B1 activity (Table 1) but lacks the propargyl group, did not fit these criteria. Its inactivation of CYP2B1 was not affected by varying the NADPH preincubation time.

The $k_{inact}$ and $K_I$ values, derived from Fig. 5, found here (1.4 μM and 0.32 min$^{-1}$) for the inactivation by R-deprenyl are similar to those reported for purified CYP2B1 (1.1 μM and 0.23 min$^{-1}$) (Sharma et al., 1996). The $K_I$ values for R-2HMP, R-2HPA, and S-2HPA were 0.8, 0.5, and 0.24 μM, respectively. The maximal rates of inactivation, $k_{inact}$, were similar for R-deprenyl and R-2HPA (0.32 and 0.36 min$^{-1}$) and for R-2HMP and S-2HPA were somewhat lower (0.08 and 0.18 min$^{-1}$). The rate of inactivation of S-2HPA was less than that of R-2HPA. We have shown that R-2HMP is metabolized in the rat to R-2HPA (Durden et al., 2000). Perhaps this demethylation step by the liver microsomes accounts for the differences in the $k_{inact}$ constants of R-2HPA and R-2HMP. As yet, it is not known whether the demethylation is involved in the formation of a metabolite intermediate complex or whether S-2HMP is demethylated. Further studies are necessary to determine this.

To determine whether the effects of the four propargylamines of interest, R-deprenyl, R-2HMP, R-2HPA, and S-2HPA, inactivated CYP2B1 activity irreversibly, microsomes were preincubated with 3.2 μM concentrations of these drugs and then spun through a cellulose membrane with a molecular cut-off of 30,000. The propargyl compounds with molecular weights of about 200 would pass through the filter, unless they were bound to the enzyme. The percentage of inhibition of CYP2B1 activity of these samples was not significantly different from microsomes that had not been spun through a membrane (Fig. 6); thus, it appears that these drugs inactivate the enzyme irreversibly because separating the protein from the inactivator made no difference. In contrast, the percentage of inhibition of CYP2B1 activity by R-2HA was almost completely reversed by filtration through the YM-30 membrane (Fig. 6).

R-Deprenyl, R-2HMP, and R-2HPA have been reported to reduce apoptotic cell death in a variety of systems (Ansari et al., 1993; Tatton et al., 1996; Boulton et al., 1997; Paterson et al., 1998; Berry, 1999). One may speculate that these compounds might act, at least partially, by inhibiting the actions of CYP450 enzymes, such as CYP2B1, 1A2, or others, and by doing so, decrease the production of ROS, which in turn decreases ROS-initiated apoptotic cell death. Because, however, the (S)-enantiomers of these drugs are not antiapoptotic (Paterson et al., 1998; Berry, 1999), but were inhibitors of microsomal CYP2B1 activity, inactivation of CYP450 enzymes cannot be the sole mechanism whereby R-deprenyl, R-2HMP, and R-2HPA interfere with apoptotic cell death.

In summary, the results presented here demonstrated that a number of propargylamines (pargylene, R-deprenyl, desmethyldeprenyl, R-2HMP, R-2HPA, or S-2HPA) inhibited the rat liver microsomal activities of CYP1A2 and CYP2B1, but not CYP1A1. All the tested propargylamines were more effective inhibitors of liver microsomal CYP2B1 activity than of CYP1A2. The inhibition of CYP2B1 activity exhibited some preference for the (R)- over the (S)-enantiomers of deprenyl, 2HMP, and 2HPA. This is the first report concerning the inhibition by the alkylpropargylamines, 2HMP and 2HPA, of CYP450 enzyme activity. Furthermore, it appears that R-2HMP, R-2HPA, and S-2HPA, like R-deprenyl, are mechanism-based inhibitors of CYP2B1 activity.

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N-(2-HEPTYL)-PROPARGYLAMINES INHIBIT MICROSONAL CYP450

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