Highly potent and selective CYP2C19 inhibitors are not currently available. In the present study, N-3-benzyl derivatives of nirvanol and phenobarbital were synthesized, their respective (+)- and (−)-enantiomers resolved chromatographically, and inhibitor potencies determined for these compounds toward CYP2C19 and other human liver cytochromes P450 (P450s). (−)-N-3-Benzyl-phenobarbital and (−)-N-3-benzyl-nirvanol were found to be highly potent, competitive inhibitors of recombinant CYP2C19, exhibiting $K_i$ values of 79 and 250 nM, respectively, whereas their antipodes were 20- to 60-fold less potent. In human liver preparations, (−)-N-3-benzyl-phenobarbital and (−)-N-3-benzyl-nirvanol inhibited (S)-mephenytoin 4′-hydroxylase activity, a marker for native microsomal CYP2C19, with $K_i$ values ranging from 71 to 94 nM and 210 to 280 nM, respectively. At single substrate concentrations of 0.3 μM [(−)-N-3-benzyl-phenobarbital] and 1 μM [(−)-N-3-benzyl-nirvanol] that were used to examine inhibition of a panel of cDNA-expressed P450 isoforms, neither CYP1A2, 2A6, 2C8, 2C9, 2D6, 2E1, nor 3A4 activities were decreased by greater than 16%. In contrast, CYP2C19 activity was inhibited ~80% under these conditions. Therefore, (−)-N-3-benzyl-nirvanol and (−)-N-3-benzyl-phenobarbital represent new, highly potent and selective inhibitors of CYP2C19 that are likely to prove generally useful for screening purposes during early phases of drug metabolism studies with new chemical entities.

The identification of human P450 isoforms involved in a given reaction is routinely assessed by performing in vitro studies using human liver microsomes or cDNA-expressed enzymes. Such microsomal studies generally rely on the availability of potent, selective “diagnostic” inhibitors of the individual P450 isoforms, which are available for most P450s with the notable exception of CYP2C19 (Pelkonen et al., 1998). Therefore, when it is necessary to evaluate the participation of CYP2C19 in human liver microosomal metabolism, the prototypic substrate (S)-mephenytoin is generally used at high concentrations (>10 μM) because its affinity for the enzyme is low.

CYP2C19 is one of the polymorphically regulated P450 isoforms, absent in about 5% of the Caucasian population and up to 20% of the Asian population (Wrighton and Stevens, 1992). As reviewed recently by Wedlund (2000), consideration of data from in vivo studies with CYP2C19-null subjects shows that CYP2C19 is the major isozyme (>80% involvement) responsible for the oxidation of only a small number of drugs, such as the (S)-enantiomer of mephenytoin, the proton pump inhibitors omeprazole, lansoprazole, and pantoprazole, and carisoprodol. More commonly, CYP2C19 is a secondary contributor to metabolic clearance of drugs, such as phentox, diazepam, clomipramine, and citalopram (Bajpai et al., 1996; Jung et al., 1997; Wu et al., 1998; von Moltke et al., 2001). In these latter instances in particular, the availability of a potent and selective inhibitor for CYP2C19 would be expected to be a highly beneficial aid to rapidly assess the extent of the contribution of this isozyme to the metabolic clearance of an investigational agent in vitro.

Recently, we synthesized new hydantoin and barbiturate inhibitors of the human CYP2C enzymes for use in the development of comparative molecular field analysis models for these enzymes (Suzuki et al., 2000). This article describes characteristics of two of the most...
potent and selective CYP2C19 inhibitors, (+)-N-3-benzyl-nirvanol and (−)-N-3-benzyl-phenobarbital, that emerged from these efforts. The structures of N-3-benzyl-nirvanol and N-3-benzyl-phenobarbital are shown in Fig. 1.

Materials and Methods

Chemicals. MFL, fluorescein, 2,7-dichlorofluorescein, coumarin, 7-hydroxy-4-methylocoumarin, and 4-hydroxymephenytoin were purchased from Sigma Chemical Co. (St. Louis, MO). CEC and 3-cyano-7-hydroxycoumarin were obtained from Molecular Probes, Inc. (Eugene, OR). 7-Hydroxycoumarin and 7-hydroxy-4-trifluoromethyl)coumarin were from Aldrich (Milwaukee, WI). DBF, MAMC, 7-hydroxy-4-(aminomethyl)coumarin, and MFC were purchased from GENERATE (Woburn, MA). Nirvanol and (S)-mephenytoin were synthesized as reported previously (Wienkers et al., 1996). Phenobarbital sodium was obtained from Spectrum Chemical Mfg. Corp. (Gardena, CA).

Racemic N-3-benzyl-nirvanol and N-3-benzyl-phenobarbital were synthesized and then resolved into their (+)- and (−)-enantiomers by HPLC according to methods that follow. (S)-Flurbiprofen, 4-hydroxyflurbiprofen, and 2-fluoro-4-biphenylacetic acid were gifts from Dr. Tim Tracy (West Virginia University, Morgantown, WV). All other chemicals and reagents were of the highest quality commercially available.

Synthesis of Racemic N-3-Benzyl-Nirvanol and N-3-Benzyl-Phenobarbital. N-3-Benzyl-nirvanol. Nirvanol (0.5 g, 2.45 mmol) was dissolved in 15 ml of N,N-dimethylformamide. Potassium carbonate (4.4 Eq) and benzyl bromide (1.1 Eq) were added, and the reaction was stirred at room temperature until TLC indicated that the starting material had been consumed. TLC plates were POLYGRAM SIL G/UV254 from Macherey-Nagel (Duern, Germany), developed with hexane/ethyl acetate (1:1, v/v). The reaction mixture was then added to 3 volumes of water and extracted with ethyl acetate. The ethyl acetate extracts were washed with 5% NaOH, water, and brine and then dried over MgSO4. The solvent was removed under reduced pressure. The product, N-3-benzyl-nirvanol, was recrystallized from hexane, and its identity confirmed by 1H NMR. Yield: 0.650 g, 90%. TLC: Rf 046. 1H NMR (DMSO-d6): δ 0.7 (3H, CH3-CH3), 2.0 (2H, CH2-CH3), 4.5 (2H, CH2-Ph), 7.3 (2H, 9H, Ph), 9.1 (s, 1H, NH).

N-3-Benzyl-phenobarbital. Phenobarbital sodium (0.5 g, 1.96 mmol) was dissolved in 15 ml of N,N-dimethylformamide. Benzyl bromide (1.1 Eq) was then added, and the solution was stirred and heated to 70°C. When TLC indicated that the starting material had all been consumed, the reaction mixture was washed with 50 ml of water, and the solution was extracted three times with ethyl acetate. The combined extracts were washed with 5% NaOH, water, and brine and then dried over MgSO4. The N-3-benzyl-phenobarbital was purified from the N,N-1,3-dibenzy derivative by silica gel chromatography with hexane/ethyl acetate (9:1, v/v), and both products were characterized by 1H NMR. The early eluting fraction was identified as N,N-1,3-dibenzy-phenobarbital. Yield: 0.235 g, 37%. TLC: Rf 0.67. 1H NMR (DMSO-d6): δ 0.9 (t, 3H, CH3-CH3), 2.5 (2H, CH2-CH3), 5.1 (4H, CH2-Ph), 7.3 (2H, 15H, Ph). The later eluting fraction was identified as N-3-benzyl-phenobarbital (0.105 g, 17%). TLC: Rf 0.58. 1H NMR (DMSO-d6): δ 0.9 (t, 3H, CH3-CH3), 2.5 (2H, CH2-CH3), 5.1 (4H, CH2-Ph), 7.3 (5H, 10H, Ph), 8.2 (2H, 1H, NH).

Separation and Optical Rotation of Enantiomers. The enantiomers of N-3-benzyl-nirvanol were separated by HPLC using an (R,R) Whelk-O1 column (10.0 mm i.d. × 250 mm; Regis Technologies, Inc., Morton Grove, IL) with 3% isopropanol in hexane at a flow rate of 5 ml/min with UV detection at 254 nm. (+)-N-3-Benzyl-nirvanol and (−)-N-3-benzyl-nirvanol were eluted at 61.0 and 11.8 min, respectively. The enantiomers of N-3-benzyl-phenobarbital were separated using a CHIRALCEL OJ column (4.6-mm i.d. × 250 mm; Daicel Chemical Industries, Ltd., Tokyo, Japan) with 10% acetonitrile in ethanol at a flow rate of 1 ml/min. (+)-N-3-Benzyl-phenobarbital and (−)-N-3-benzyl-phenobarbital were eluted at 5.0 and 12.0 min, respectively. Individual peak fractions were collected, and the organic solvent was evaporated to dryness under reduced pressure. The purity of the resulting enantiomers was greater than 95% enantiomeric excess. Optical rotations were obtained using a Jasco P-1030 polarimeter (Jasco Co, Tokyo, Japan): (+)-N-3-benzyl-nirvanol [α]D20 +52.0° (methanol; concentration, 10 mg/ml); (−)-N-3-benzyl-nirvanol [α]D20 −45.2° (methanol; concentration, 10 mg/ml); (+)-N-3-benzyl-phenobarbital [α]D20 +43.9° (methanol; concentration, 10 mg/ml); (−)-N-3-benzyl-phenobarbital [α]D20 −43.3° (methanol; concentration, 10 mg/ml).

Enzyme Sources. CYP2C9 cDNA (Romkes et al., 1991) was obtained from Dr. J. A. Goldstein (NEIHS, Bethesda, MD) in the vector pBlueScript SK+ +/. This cDNA was inserted into the pBacPAK6 transfer vector on an XhoI/XmaI fragment behind the polyhedrin promoter to create pBPC29. Cotransfection of insect cells with Bsu36I-digested BacPAK6 viral DNA, and the pBPC29 was carried out with reagents and procedures provided by CLONTECH Laboratories, Inc. (Palo Alto, CA) to generate recombinant viral particles for expression. Suspension cultures of Trichoplusia ni insect cells were infected with recombinant viruses for CYP2C9 and CYP2C9, and the two hemoproteins were purified to near homogeneity by procedures detailed previously (Haining et al., 1996). For some experiments, Supersomes (GEN-TEST) were used. Recombinant rat NADPH-P450 oxidoreductase and human cytochrome b5 were expressed and purified from bacterial cultures in a manner described previously by Chen et al. (1998). Human liver tissue was obtained from the Human Liver Bank established in the Departments of Medicinal Chemistry and Pharmaceutics at the University of Washington. Details concerning the acquisition, storage, and preparation of the human liver microsomes used in these experiments have been described (Rettie et al., 1989).

Assay for MDM Demethylation. The activity of recombinant CYP2C9 was determined by measuring MDM demethylation activities. The incubation mixtures were in a final volume of 200 μl and contained 2.5 pmol of purified CYP2C9, 5 pmol of P450 reductase, 2.5 pmol of cytochrome b5, 5 μg of t-o-dialliloxy-sn-glycero-3-phosphocholine, 1 mM NADPH, 50 mM potassium phosphate buffer, pH 7.4, and MFL (1, 2, and 4 μM). The reaction was initiated by the addition of recombinant enzymes. Incubations were carried out at 37°C for 20 min and terminated by adding 200 μl of 40 mM 2,7-dichlorofluorescein in acetonitrile as an internal standard and 10 μl of 10% perchloric acid. After centrifugation at 8000g for 2 min, the supernatant was analyzed by HPLC using an XTerra RP18, 5-μm column (4.6-mm i.d. ×150 mm; Waters Co, Milford, MA), attached to a guard column (3.9-mm i.d. ×20 mm). The column was eluted with a linear gradient of acetone/titrate 10 mM potassium phosphate buffer, pH 8.0, that changed from a ratio of 15:85 to 35:65 over 10 min at a flow rate of 1 ml/min. Metabolites were detected fluorometrically with the excitation wavelength set at 490 nm and emission wavelength at 525 nm.

Assay for (S)-Mephenytoin 4'-Hydroxylation by Human Liver Microsomes. K, values for the inhibition of (S)-mephenytoin 4'-hydroxylation were assessed using three individual human liver microsomal preparations (HL134, male, 7 years, Caucasian; HL143, male, 48 years, Caucasian; and HL164, female, 50 years, Caucasian). The incubation mixtures for (S)-mephenytoin 4'-hydroxylation were in a final volume of 200 μl and contained 10 pmol of human liver microsomal P450, 1 mM NADPH, 50 mM potassium phosphate buffer, pH 7.4, and (S)-mephenytoin (20, 40, and 80 μM). The reaction was initiated by the addition of microsomes. Incubations were carried out at 37°C for 30 min and terminated by adding 50 μl of 5 μM phenobarbital in acetonitrile as an internal standard. The samples were then centrifuged at 8000g for 2 min, and 4'-hydroxymephenytoin in the supernatant was analyzed by HPLC using an XTerra RP18, 5-μm column (4.6-mm i.d. ×150 mm) attached to a guard column (3.9-mm i.d. ×20 mm). The column was eluted with a linear gradient of acetone/titrate 10 mM potassium phosphate buffer, pH 3.0, that changed from 25:75 to 30:70 over 10 min, and then isocratically with a 50:50 mix from 10 to 15 min. The flow rate was 1 ml/min with UV detection at 204 nm.
**TABLE 1**

<table>
<thead>
<tr>
<th>P450 Isoform</th>
<th>Substrate (pmol P450/incubation)</th>
<th>Enzyme (pmol P450/incubation)</th>
<th>Metabolite (μM)</th>
<th>Internal Standard (μM)</th>
<th>Mobile Phase Acetonitrile/10 mM Potassium Phosphate Buffer (pH 8.0)</th>
<th>Excitation</th>
<th>Emission</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>2.5 μM CEC</td>
<td>0.5</td>
<td>CYP1A2</td>
<td>0.5 μM HMC</td>
<td>0–10 min; 20:80–30:70 linear gradient</td>
<td>408</td>
<td>450</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>0.5 μM Coumarin</td>
<td>0.4</td>
<td>CYP2A6</td>
<td>0.1 μM HMC</td>
<td>0–5 min; 20:80–30:70 linear gradient; 5–10 min; 30:70</td>
<td>368</td>
<td>456</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>0.5 μM DBF</td>
<td>2.5</td>
<td>CYP2C8</td>
<td>40 nM DCFL</td>
<td>0–5 min; 15:85–32:68 linear gradient; 12 min 70:30</td>
<td>490</td>
<td>525</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>35 μM MFL</td>
<td>2.5</td>
<td>CYP2C9</td>
<td>40 nM DCFL</td>
<td>10–10 min; 15:85–35:65 linear gradient</td>
<td>490</td>
<td>525</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>1.5 μM MFL</td>
<td>2.5</td>
<td>CYP2C19</td>
<td>40 nM DCFL</td>
<td>10–10 min; 15:85–35:65 linear gradient</td>
<td>490</td>
<td>525</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>5.0 μM MAMC</td>
<td>2.5</td>
<td>CYP2D6</td>
<td>0.1 μM HCl</td>
<td>0–10 min; 10:90–30:70 linear gradient</td>
<td>370</td>
<td>470</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>100 μM MFC</td>
<td>5.0</td>
<td>CYP2E1</td>
<td>0.25 μM HIC</td>
<td>0–10 min; 20:80–70:30 linear gradient</td>
<td>385</td>
<td>502</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>0.5 μM DBF</td>
<td>0.5</td>
<td>CYP3A4</td>
<td>40 nM DCFL</td>
<td>0–5 min; 15:85–32:68 linear gradient; 12 min 70:30</td>
<td>490</td>
<td>525</td>
</tr>
</tbody>
</table>

CHC, 3-cyano-7-hydroxycoumarin; HC, 7-hydroxycoumarin; FL, fluorescein; HAMC, 7-hydroxy-4-(aminomethyl)coumarin; HFC, 7-hydroxy-4-(trifluoromethyl)coumarin; HMC, 7-hydroxy-4-methylcoumarin; DCFL, 2,7-dichlorofluorescein.

**TABLE 2**

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_i$ ($\mu$M)</th>
<th>Inhibitor Potency Relative to (S)-Mephenytoin</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-(−)-Mephenytoin</td>
<td>237.0</td>
<td>0.12</td>
</tr>
<tr>
<td>(S)+(−)-Mephenytoin</td>
<td>29.5</td>
<td>1.0</td>
</tr>
<tr>
<td>(−)-N-3-Benzyl-nirvanol</td>
<td>5.3</td>
<td>5.6</td>
</tr>
<tr>
<td>(+)-N-3-Benzyl-nirvanol</td>
<td>0.25</td>
<td>11.0</td>
</tr>
<tr>
<td>(+)-N-3-Benzyl-phenobarbital</td>
<td>4.7</td>
<td>6.3</td>
</tr>
<tr>
<td>(−)-N-3-Benzyl-phenobarbital</td>
<td>0.079</td>
<td>373.0</td>
</tr>
</tbody>
</table>

$K_i$ values for the inhibition of CYP2C19 by mephenytoin, N-3-benzyl-nirvanol, and N-3-benzyl-phenobarbital enantiomers

**Results**

**Inhibition of CYP2C19 by N-3-Benzyl-Nirvanol, N-3-Benzyl-Phenobarbital, and Mephenytoin Enantiomers.** All six compounds inhibited recombinant CYP2C19 in a competitive manner (data not shown). $K_i$ values for the inhibition of CYP2C19-dependent MFL activity by each of these compounds are shown in Table 2. As expected, (S)-mephenytoin was a much more potent inhibitor than (R)-mephenytoin (8-fold), although the affinity of the (S)-enantiomer for CYP2C19 was not particularly strong ($K_i = 29.5 \mu$M). Replacement of the N-3 methyl group of the mephenytoin enantiomers with a benzyl moiety resulted in a dramatic enhancement in affinity for CYP2C19 (45- to 118-fold). Expansion of the five-membered hydantoin nucleus to a six-membered barbiturate ring with retention of the N-3 benzyl moiety resulted in further increases in inhibitor potency such that the $K_i$ value for the most potent inhibitor examined, (−)-

N-3-benzyl-phenobarbital, dropped to 79 nM—an increase in affinity for CYP2C19 of 373-fold relative to (S)-mephenytoin.

**Inhibition of (S)-Mephenytoin 4’-Hydroxylation in Human Liver Microsomes by (−)-N-3-Benzyl-Nirvanol and (−)-N-3-Benzyl-Phenobarbital.** Representative Dixon plots for the inhibition of human liver microsomal (S)-mephenytoin 4’-hydroxylation by (−)-N-3-benzyl-nirvanol and (−)-N-3-benzyl-phenobarbital are shown in Fig. 2. Both (−)-N-3-benzyl-nirvanol and (−)-N-3-benzyl-phenobarbital were found to be potent competitive inhibitors of native microsomal CYP2C19 activity, exhibiting mean $K_i$ values obtained from three separate microsomal preparations of 0.24 and 0.085 μM, respectively (Table 3). These data are in good agreement with the $K_i$ values obtained from the inhibition of MFL demethylation catalyzed by recombinant CYP2C19.

**Inhibitory Effects of (±)-N-3-Benzyl-Nirvanol and (−)-N-3-Benzyl-Phenobarbital on the Activities of Heterologously cDNA-Expressed Human P450 Isoforms.** To probe the P450 isoform selectivity of these two potent CYP2C19 inhibitors, their inhibitory effects on the activities of the major isoforms relevant to human liver drug metabolism were determined using commercially available Supersomes. These screening experiments were performed with single inhibitor concentrations equal to 4 times the respective $K_i$ values for CYP2C19, i.e., 1 μM for (±)-N-3-benzyl-nirvanol and 0.3 μM for (−)-N-3-benzyl-phenobarbital. In preliminary experiments, we determined the $K_m$ of each metabolic reaction catalyzed by the recombinant P450 and then selected this value for the final substrate concentration to be used in each inhibition experiment (Table 1, column 2). An exception was CYP2E1, in which the substrate concentration was set at 100 μM, since determination of an accurate $K_m$ value for MFC was precluded by its limited solubility. Nonetheless, it was clear that MFC demethylation catalyzed by CYP2E1 was not saturated at this substrate concentration.
Human liver microsomes (HL164) were used as the enzyme source. Incubations were carried out at three different (S)-mephenytoin concentrations of 20 μM (closed circles), 40 μM (open circles), and 80 μM (closed squares), as described under Materials and Methods. V, velocity of 4’-hydroxymephenytoin formation.

### Table 3

<table>
<thead>
<tr>
<th>Enzyme Source</th>
<th>(+)-N-3-Benzyl-nirvanol</th>
<th>(-)-N-3-Benzyl-phenobarbital</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL134</td>
<td>0.23 ± 0.04</td>
<td>0.071 ± 0.004</td>
</tr>
<tr>
<td>HL143</td>
<td>0.28 ± 0.04</td>
<td>0.094 ± 0.004</td>
</tr>
<tr>
<td>HL164</td>
<td>0.21 ± 0.04</td>
<td>0.089 ± 0.004</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>0.24 ± 0.04</td>
<td>0.085 ± 0.012</td>
</tr>
</tbody>
</table>

Under these conditions, (+)-N-3-benzyl-nirvanol inhibited CYP2C19 by 80% with only a modest degree of inhibition (16%) toward the next most susceptible isozyme, CYP3A4 (Fig. 3A). (-)-N-3-Benzyl-phenobarbital also markedly inhibited CYP2C19 activity but without significantly affecting the metabolic activities of the other P450 isoforms tested, including CYP3A4 (Fig. 3B). In separate experiments using reconstituted preparations of CYP2C9 and an (S)-flurbiprofen 4’-hydroxylase reporter assay, the \( K_i \) values of the two most potent CYP2C19 inhibitors, (+)-N-3-benzyl-nirvanol and (-)-N-3-benzyl-phenobarbital against CYP2C9 were determined to be 83 and 16 μM, respectively (data not shown), further demonstrating the selectivity of (+)-N-3-benzyl-nirvanol and (-)-N-3-benzyl-phenobarbital for inhibition of CYP2C19.

### Discussion

Numerous studies conducted in recent years (Newton et al., 1995; Bourrie et al., 1996; Ono et al., 1996; Zhang et al., 2001) have established the use of a variety of specific, high-potency inhibitors for all of the major (and several minor) human P450 isozymes, with the notable exception of CYP2C19. Omeprazole and tranilcypramine, with \( K_i \) values of 4.1 and 8.7 μM, respectively (VandenBranden et al., 1996; Wienen et al., 1996), have occasionally been used as CYP2C19 inhibitors, but both compounds also inhibit CYP3A4 and CYP2A6 with \( K_i \) values of 79 and 0.04 μM, respectively (Lampen et al., 1995; Draper et al., 1997), and therefore do not demonstrate high selectivity. The most potent inhibitors for CYP2C19 found to date are probably norfluoxetine and ticlopidine with \( K_i \) values of 1.1 and 1.2 μM, respectively (Kobayashi et al., 1995; Ko et al., 2000). However, both drugs are also potent inhibitors of CYP2D6 (Stevens and Wrighton, 1993; Ko et al., 2000). Therefore, to our knowledge, no highly potent and isoform-specific inhibitors of CYP2C19 have been described.

CYP2C19 and CYP2C9 are the most highly conserved isozymes among the human CYP2C subfamily, exhibiting 91% amino acid identity (Romkes et al., 1991) and yet having distinctive substrate, product, and inhibitor specificities. For example, sulfaphenazole is a nanomolar potency inhibitor of CYP2C9 but is not a marked inhibitor of CYP2C19 (Ono et al., 1996). CYP2C19 and CYP2C9 both metabolize phenytoin to 5-(4-hydroxyphenyl)-5-phenylhydantoin but with different prochiral stereoselectivities. Phenytoin is stereospecifically oxidized on the pro-(S)-phenyl ring by CYP2C9, whereas CYP2C19 exhibits low prochiral selectivity (Bajpai et al., 1996). However, if phenytoin is methylated at the N-3 position on the hydantoin ring, the resulting N-3-methyl-phenytoin is still metabolized by CYP2C19 but apparently no longer a substrate for CYP2C9 (Schellens et al., 1990). Furthermore, it was demonstrated recently in vitro that (R)-mephenytoin, [(+-)-N-3-methyl-phenobarbital] but not its (S)-antipode, is preferentially metabolized by CYP2C19 (Kobayashi et al., 2001). Since (S)-mephenytoin, which is also N-3-methylated on the hydantoin ring, has long been considered to be the prototypic substrate for CYP2C19, it seems clear that alkylation at the N-3 positions on hydantoin and barbiturate rings is an important structural modification that differentiates CYP2C9 and CYP2C19 substrates. Moreover, the absolute configuration of the substrate would appear also to be an essential determinant of high-affinity binding to CYP2C19.

Based on the above considerations, we prepared several enantiomerically pure N-3 substituted nirvanol and phenobarbital derivatives and tested their binding affinities for CYP2C19, as reflected in the magnitude of \( K_i \) values toward CYP2C19-dependent catalytic reactions. From these studies, (+)-N-3-benzyl-nirvanol and (-)-N-3-benzyl-phenobarbital emerged as highly potent and selective inhibitors for native and recombinant CYP2C19. Each compound exhibited a \( K_i \) value for CYP2C19 that is more than 2 orders of magnitude lower than that obtained with (S)-mephenytoin. Moreover, the antipodes of these two nanomolar inhibitors were 20- to 60-fold less potent as CYP2C19 inhibitors, and the \( K_i \) values for (+)-N-3-benzyl-phenobarbital and (+)-N-3-benzyl-nirvanol against the closely related enzyme...
CYP2C9 were 200- to 300-fold higher than for CYP2C19. Studies with a panel of recombinant P450s at inhibitor concentrations equivalent to ~4 times \( K_i \) demonstrated very high inhibitor selectivity toward CYP2C19, particularly with (−)-N-3-benzyl-phenobarbital.

As noted under Materials and Methods, synthesis of racemic N-3-benzyl-nirvanol could be achieved in high yield (90%) compared with racemic N-3-benzyl-phenobarbital (17%). This was due to the preferred formation of the dibenzyl derivative in the latter reaction, which necessitated an additional column chromatography step. Accordingly, (−)-N-3-benzyl-nirvanol, albeit a slightly less potent and less selective inhibitor of CYP2C19 than (−)-N-3-benzyl-phenobarbital, may be a more attractive synthetic target in many laboratories, particularly since it can also be prepared by direct benzylation of (S,)-(-)-nirvanol, which is easily obtained by fractional crystallization.

In conclusion, we report that (−)-N-3-benzyl-nirvanol and (−)-N-3-benzyl-phenobarbital are potent, selective inhibitors of CYP2C19. Both novel inhibitors should prove useful in the assessment of the contribution of CYP2C19 to drug metabolism in human liver microsomes. Future studies are aimed at incorporating these inhibitors and other congeners into a comparative molecular field analysis model for CYP2C19 and to use them further to delineate active-site features of the enzyme that promote high-affinity binding of N-3-alkylated hydantoins and barbiturates.

References


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SELECTIVE, POTENT CYP2C19 INHIBITORS

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