COMPARISON OF INHIBITORY EFFECTS OF THE PROTON PUMP-INHIBITING DRUGS OMEPRAZOLE, ESOMEPRAZOLE, LANSOPRAZOLE, PANTOPRAZOLE, AND RABEPRAZOLE ON HUMAN CYTOCHROME P450 ACTIVITIES

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ABSTRACT:
The human clearance of proton pump inhibitors (PPIs) of the substituted benzimidazole class is conducted primarily by the hepatic cytochrome P450 (P450) system. To compare the potency and specificity of the currently used PPIs (i.e., omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole) as inhibitors of four cytochrome P450 enzymes (CYP2C9, 2C19, 2D6, and 3A4), we performed in vitro studies using human liver microsomal preparations and recombinant CYP2C19. Sample analysis was done using selected reaction monitoring liquid chromatography/tandem mass spectrometry. With several systems for CYP2C19 activity (two marker reactions, S-mephenytoin 4′-hydroxylation and R-omeprazole 5-hydroxylation, tested in either human liver microsomes or recombinant CYP2C19), the five PPIs showed competitive inhibition of CYP2C19 activity with Ki of 0.4 to 1.5 μM for lansoprazole, 2 to 6 μM for omeprazole, ~8 μM for esomeprazole, 14 to 69 μM for pantoprazole, and 17 to 21 μM for rabeprazole. Pantoprazole was a competitive inhibitor of both CYP2C9-catalyzed diclofenac 4′-hydroxylation and CYP3A4-catalyzed midazolam 1′-hydroxylation (Kp of 6 and 22 μM, respectively), which were at least 2 times more potent than the other PPIs. All PPIs were poor inhibitors of CYP2D6-mediated bufuralol 1′-hydroxylation with IC50 > 200 μM. The inhibitory potency of a nonenzymatically formed product of rabeprazole, rabeprazole thioether, was also investigated and showed potent, competitive inhibition with Ki values of 6 μM for CYP2C9, 2 to 8 μM for CYP2C19, 12 μM for CYP2D6, and 15 μM for CYP3A4. The inhibitory potency of R-omeprazole on the four studied P450 enzymes was also studied and showed higher inhibitory potency than its S-isomer on CYP2C9 and 2C19 activities. Our data suggest that, although the inhibitory profiles of the five studied PPIs were similar, lansoprazole and pantoprazole are the most potent in vitro inhibitors of CYP2C19 and CYP2C9, respectively. Esomeprazole showed less inhibitory potency compared with omeprazole and its R-enantiomer. The inhibitory potency of rabeprazole was relatively lower than the other PPIs, but its thioether analog showed potent inhibition on the P450 enzymes investigated, which may be clinically significant.

Knowledge of drug interactions with the liver microsomal mixed-function oxidase system is becoming increasingly important in the safety assessment of new compounds developed for clinical use. It is of particular importance during multidrug treatment, because the pharmacodynamics of coadministered drugs may be dramatically altered by the influence of one drug on the metabolism of another (Zumbrunnen and Jann, 1998). Drug-drug interaction (DDI) is mainly due to enzyme induction or interference with drug metabolism. The latter can be assessed by measuring the influence of the drug on the cytochrome P450 (P450) system, which is responsible for the metabolism of most drugs (Papp-Jambor et al., 2002). With this understanding, it has become essential to make educated predictions for new drugs in regards to the other drugs with which they are likely, or not likely, to interact (Tucker, 1994).

Proton pump inhibitors (PPIs) have been widely used as acid inhibitory agents for the treatment of disorders related to gastric acid secretion for about 15 years. Five such agents (i.e., omeprazole, lansoprazole, pantoprazole, rabeprazole, and the more recently introduced esomeprazole) are now available (Fig. 1). Each of these available PPIs is a substituted benzimidazole, and they are all, with the exception of esomeprazole, marketed as the racemates. The rationale for developing esomeprazole (the S-enantiomer of omeprazole) was its higher metabolic stability (Åbelo et al., 2000), which leads to a higher bioavailability (Hassan-Alin et al., 2000) compared with the racemate. The resulting increase in the area under the plasma concentration-time curve (AUC) of esomeprazole provides more effective control of gastric acid secretion than that of all other PPIs given at their standard doses (Röhss et al., 2002; Lindberg et al., 2003). All of these PPIs have a common mechanism of action involving chemical rearrangement to a reactive sulfenamide that inhibits the ability of H⁺,K⁺-ATPase (the proton pump) to participate in gastric acid formation. As a result of structural and functional similarities, the PPIs share many pharmacokinetic features [e.g., similar rates of absorption, maximum plasma concentrations, and total drug absorption (Hassan-Alin et al., 2000; Andersson et al., 2001b; Vanderhoff and Tahboub, 2002)]. All PPIs are extensively metabolized in the liver via CYP2C19 and 3A4, and they all exhibit polymorphic metabolism in humans (Andersson et al., 1993; Yasuda et al., 1995; Sohn et al.,

ABBRVIATIONS: DDI, drug-drug interaction; P450, cytochrome P450; PPI, proton pump inhibitor; AUC, area under the plasma concentration-time curve; PM, poor metabolizer; HLM, human liver microsomes; EM, extensive metabolizer; FWHM, full-width half maximum.
The poor metabolizer (PM) phenotype of the PPIs is highly correlated with the PM phenotype of S-mephenytoin (CYP2C19), and the in vivo DDI potential of omeprazole, for example, has been investigated widely; however, the data of recently marketed PPIs, such as rabeprazole, are still not complete. Although only omeprazole has demonstrated interactions with the metabolism of other drugs in vivo, albeit regarded to be not clinically relevant, the other PPIs also show a certain potential for interacting with drugs metabolized by P450 isoforms according to previous in vitro inhibition studies (VandenBranden et al., 1996; Ko et al., 1997). In addition to P450-mediated metabolism, rabeprazole is also converted nonenzymatically to its thioether analog. Considering the relatively higher lipophilic character of this product and its relatively longer half-life in vivo compared with its parent (Yasuda et al., 1995; Shirai et al., 2001), it was also included in this study.

Based on a Medline search (1966 through August 2003), the in vitro inhibition studies of the PPIs omeprazole, lansoprazole, pantoprazole, rabeprazole, and esomeprazole on human P450 enzymes have not been described or compared thoroughly. So far, most, if not all, of the direct comparative studies referring to omeprazole have been performed with only one comparator PPI (VandenBranden et al., 1996; Ko et al., 1997; Pichard-Garcia et al., 2000). In addition, a significant variation exists between the reported inhibition studies from lab to lab, which might be caused by different marker reactions used for specific P450 enzymes, the microsome sources, and analytical methods (Ko et al., 1997). To our knowledge, there are still no published data regarding the inhibition potency of pantoprazole in vitro.

Based on the above-mentioned lack of consistent data, we investigated the potency and specificity of these PPIs as inhibitors of the four principal P450 enzymes (CYP2C9, 2C19, 3A4, and 2D6) present in human liver microsomes (HLM), which are involved in the metabolism of most drugs and also might be influenced by PPIs as reported previously to get comparable information. Other P450 enzymes were not included in this study because of the low possibility of DDIs with regard to reported data [e.g., no inhibition of omeprazole and lansoprazole on CYP1A2 activity (Ko et al., 1997)] and because other P450 enzymes (e.g., CYP2A6, 2C8, and 2E1) are not the major enzymes involved in the metabolism of the PPIs (Andersson et al., 1993; Pearce et al., 1996; VandenBranden et al., 1996). Furthermore, due to the fact that CYP2C19 is the major enzyme involved in the metabolism of all the PPIs, the inhibition potential of the PPIs on CYP2C19 activity was investigated in detail in this study with two marker reactions and using recombinant CYP2C19 as well.

**Materials and Methods**

**Chemicals and Reagents.** Bufuralol hydrochloride, 1'-hydroxybufuralol maleate, 4'-hydroxymephenytoin, 1'-hydroxymidazolam, S-mephenytoin, and sulfaphenazole were purchased from Ultrafine Chemicals (Manchester, UK). Midazolam and 4'-hydroxydiclofenac were purchased from BD Gentest (Woburn, MA). Diclofenac sodium, tinidazole (internal standard), quinidine, ketoconazole, and reduced NADPH were purchased from Sigma-Aldrich (St. Louis, MO). Ticlopidine hydrochloride was purchased from MP Biomedicals (Irvine, CA). Omeprazole sodium was obtained from AstraZeneca Bulk Production (Södertälje, Sweden). Esomeprazole sodium, R-omeprazole sodium, lansoprazole, and pantoprazole sodium sesquihydrate were obtained from AstraZeneca Process R&D (Södertälje, Sweden). 5'-Hydroxymeprazole and rabeprazole thioether were obtained from Synthece AB (Lund, Sweden), and rabeprazole was obtained from Medicinal Chemistry (AstraZeneca R&D, Malmö, Sweden). All other chemicals and reagents were of the highest commercially available quality.

**HLM and Recombinant Cytochromes P450 (rCYP).** Human liver samples (excess material removed during surgery on the liver) were obtained from the Department of Surgery I, Sahlgrenska Hospital, Göteborg, Sweden. Pooled HLM were prepared with a mixture of seven liver samples of male and female patients according to the method of Einstner et al. (1962). Recombinant human CYP2C19 was heterologously expressed in Saccharomyces cerevisiae obtained from AstraZeneca Biotech Laboratory (Södertälje, Sweden). Microsomal protein concentration was measured according to Lowry et al. (1951), using bovine serum albumin as standard. The microsomal preparations were stored at −80°C until use.

**High-Performance Liquid Chromatography Conditions.** The high-performance liquid chromatography system used included a Surveyor MS pump, a built-in degasser, a Surveyor PDA detector (Thermo Finnigan, San Jose, CA), and a CTC HTS autosampler (CTC Analytics, Zwingen, Switzerland). Chromatography was performed on a Zorbax SB C18 column (2.1 × 50 mm, 3.5 μm; Agilent Technologies, Palo Alto, CA) with an Eclipse XDB-C8 guard column (2.1 × 12.5 mm, 5 μm). The mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile, which increased linearly from 5% of solvent B to 95% B during 3.5 min at a flow rate of 0.3 ml/min. During the investigation of the effects of rabeprazole thioether on bufuralol 1'-hydroxylase (CYP2D6) and omeprazole 5'-hydroxylase (CYP2C19) activities, mobile phase of (A) 5 mM ammonium acetate (pH 7) in water and (B) acetonitrile was used to avoid the analytical interference by the inhibitor and its metabolites. In the latter case, for the analysis of 1'-hydroxybufuralol, a slower gradient profile of 5% B to 60% B in 3.5 min was used at 0.3 ml/min. The Mass spectrometric analysis was detected at 302 nm for monitoring the consumption of the PPIs, which was less than 20% of the initial concentrations after incubation with HLM.

**Mass Spectrometric Conditions.** The mass spectrometric conditions were performed using a Thermo Finnigan TSQ Quantum triple quadrupole mass spectrometer (ThermoFinnigan). The mass spectrometer was operated in the positive ionization electrospray mode. The spray voltage was set to 4.0 kV, heated capillary temperature to 325°C and its offset to 35 V, and the source collision-induced dissociation to off (except for the analysis of 4'-hydroxy diclofenac, in which a source collision-induced dissociation of 15 V was applied to assist ion desolvation, thus reducing the formation of acetonitrile adducts). Nitrogen was used as the sheath and auxiliary gas and set to 30 and 5 (arbitrary units), respectively. The argon collision gas pressure was set to 1.5 mTorr. Nitrogen was used as the sheath and auxiliary gas and set to 30 and 5 (arbitrary units), respectively. The argon collision gas pressure was set to 1.5 mTorr. The mass spectrometer was operated in the selected reaction monitoring mode, with the resolution of Q1 set at 0.7 Da FWHM. The parameters of the selected reaction monitoring transitions for the [M + H]⁺ precursor ions to selected product ions were optimized with the following typical values for the analytes and internal standard (each at their optimum collision energy): 4'-hydroxy diclofenac m/z 312.0 to 230.0; 4'-hydroxymephenytoin m/z 235.0 to 150.1; 5'-hydroxymeprazole m/z 362.1 to 214.0; 1'-hydroxybufuralol m/z 278.0 to 186.1; 1'-hydroxymidazolam m/z 342.0 to 324.1; and the internal standard tinidazole m/z 248.0 to 121.0.
Instrument control, data acquisition, and data evaluation were performed using Xcalibur software version 1.3 (Thermo Finnigan). The lower limit of quantification was 10 nM for all five hydroxylated marker metabolites in the present study.

### In Vitro Inhibition of P450 Enzyme-Specific Assays by PPIs

To measure the inhibition effects of omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole on the activities of CYP2C9, 2C19, 2D6, and 3A4, the following marker substrates with high specificity for P450 enzymes were selected: diclofenac 4'-hydroxylation for CYP2C9; S-mephenytoin 4'-hydroxylation for CYP2C19; bufuralol 1'-hydroxylation for CYP2D6; and midaflam 1'-hydroxylation for CYP3A4. R-omeprazole 5-hydroxylation was also used to reflect the activity of CYP2C19 (Abeló et al., 2000). The effects of R-omeprazole and the nonenzymatically formed degradation product of rabeprazole (i.e., rabeprazole thioether) were also investigated under the same conditions. Sulfaphenazole, ticlopidine, quinidine, and ketoconazole were chosen as model inhibitors of CYP2C9, 2C19, 2D6, and 3A4, respectively.

All reactions were performed in 96-well plates. Each reaction mixture consisted of the enzyme, the substrate, and 1 mM NADPH in 0.1 M Tris-hydrochloride buffer (pH 7.4) with or without test compound in a final volume of 200 μL. The initial rate of each enzyme reaction was linear with time and protein concentration. The recommended daily doses for these five PPIs are 20 mg for omeprazole, 40 mg for esomeprazole, 30 mg for lansoprazole, 40 mg for pantoprazole, and 20 mg for rabeprazole.

### Results

The inhibitory effects of the five PPIs, as well as R-omeprazole and rabeprazole thioether, on four human P450 enzymes (CYP2C9, 2C19, 2D6, and 3A4) were investigated. The apparent inhibition constants (Kᵢ) for the respective P450 enzymes were performed on the test compounds, for which the IC₅₀ values were below 200 μM. In general, the test compounds and model inhibitors were studied at four different concentrations that were chosen based on the IC₅₀ values obtained with each P450 enzyme (approximately 1/2 IC₅₀, IC₅₀, 2 × IC₅₀, and 4 × IC₅₀). The concentrations of marker substrates were chosen (approximately 1/2 Kᵢ, Kᵢ, 3 Kᵢ, and 5 Kᵢ) with regard to their Michaelis-Menten kinetics (Kᵢ and V_max) (Table 2). The vehicle controls were analyzed in parallel. The Kᵢ values were calculated by nonlinear regression analysis by fitting different models of enzyme inhibition to the kinetic data using SigmaPlot Enzyme Kinetics Module for Windows 7.0 (SPSS Inc., Chicago, IL). The inhibition mechanism was established by comparing the statistical results including the Akaike’s information criterion values of different inhibition models and selecting the one with the best fit.

### Table 1

<table>
<thead>
<tr>
<th>PPI</th>
<th>Oral Dose</th>
<th>CYP2C19 Genotype</th>
<th>Cₘₐₓ</th>
<th>AUC</th>
<th>t₁/₂GP</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omeprazole</td>
<td>20 (7th day)</td>
<td>EM</td>
<td>Mean ± S.D.</td>
<td>1.6 ± 1.0</td>
<td>2.9 ± 2.8</td>
<td>0.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>20 (7th day)</td>
<td>PM</td>
<td>Mean ± S.D.</td>
<td>3.1 ± 0.9</td>
<td>12.7 ± 7.2</td>
<td>2.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>20 (8th day)</td>
<td>PM</td>
<td>Mean ± S.D.</td>
<td>1.1 ± 0.6</td>
<td>2.2 ± 1.9</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Esomeprazole</td>
<td>40 (5th day)</td>
<td>EM</td>
<td>Mean</td>
<td>5.2</td>
<td>14.1</td>
<td>AstraZeneca, 2002, data on file</td>
</tr>
<tr>
<td>Lansoprazole</td>
<td>30 (7th day)</td>
<td>EM</td>
<td>Mean ± S.D.</td>
<td>2.2 ± 0.7</td>
<td>4.9 ± 2.1</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>30 (8th day)</td>
<td>PM</td>
<td>Mean ± S.D.</td>
<td>2.0 ± 0.3</td>
<td>4.6 ± 0.8</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Pantoprazole</td>
<td>40 (7th day)</td>
<td>EM</td>
<td>Mean ± S.D.</td>
<td>5.4 ± 1.4</td>
<td>9.5 ± 3.5</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>80 (7th day)</td>
<td>EM</td>
<td>Mean ± S.D.</td>
<td>12.8 ± 2.8</td>
<td>27.2 ± 6.5</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Rabeprazole</td>
<td>20 (7th day)</td>
<td>EM</td>
<td>Mean ± S.D.</td>
<td>1.2 ± 0.7</td>
<td>2.0 ± 0.9</td>
<td>1.0 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>20 (8th day)</td>
<td>PM</td>
<td>Mean ± S.D.</td>
<td>1.7 ± 0.9</td>
<td>3.9 ± 1.1</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>10 (8th day)</td>
<td>EM</td>
<td>Mean ± S.D.</td>
<td>0.6 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>Rabeprazole</td>
<td>20 (7th day)</td>
<td>EM</td>
<td>Mean ± S.D.</td>
<td>0.3 ± 0.2</td>
<td>2.4 ± 1.6</td>
<td>2.8 ± 0.7</td>
</tr>
<tr>
<td>Thioether</td>
<td>20 (8th day)</td>
<td>EM</td>
<td>Mean ± S.D.</td>
<td>0.6 ± 0.3</td>
<td>4.2 ± 3.2</td>
<td>3.0 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>10 (8th day)</td>
<td>PM</td>
<td>Mean ± S.D.</td>
<td>0.2 ± 0.06</td>
<td>0.8 ± 0.2</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>20 (8th day)</td>
<td>PM</td>
<td>Mean ± S.D.</td>
<td>0.26 ± 0.03</td>
<td>2.1 ± 0.3</td>
<td>6.2 ± 1.0</td>
</tr>
</tbody>
</table>

Cₘₐₓ, peak plasma drug concentration; t₁/₂GP, terminal elimination half-life.
and 20/H9262 compound). In fact, the data at low factors (e.g., substrate used, the metabolites formed from either sub-
inhibition. The enzyme kinetics in HLM could be influenced by many 
mephenytoin as substrate than in other systems measuring CYP2C19 
i value around 15/M on CYP2C19-medi-
TABLE 2

<table>
<thead>
<tr>
<th>Marker Reaction</th>
<th>P450</th>
<th>$K_i$ (μM)</th>
<th>$V_{max}$ (pmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac 4'-hydroxylation</td>
<td>2C9</td>
<td>5.3</td>
<td>1396</td>
</tr>
<tr>
<td>S-Mephenytoin 4'-hydroxylation</td>
<td>2C19</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>R-Omeprazole 5-hydroxylation</td>
<td>2C19</td>
<td>1.1*</td>
<td>29</td>
</tr>
<tr>
<td>Buturafol 1'-hydroxylation</td>
<td>2D6</td>
<td>23</td>
<td>70</td>
</tr>
<tr>
<td>Midazolam 1'-hydroxylation</td>
<td>3A4</td>
<td>4.1</td>
<td>636</td>
</tr>
</tbody>
</table>

* R-Omeprazole 5-hydroxylation was catalyzed by two enzyme systems in HLM: a high-affinity enzyme system with $K_{i,1} = 1.1$ μM and a low-affinity enzyme system with $K_{i,2} = 14$ μM, respectively.

tively. Compared with omeprazole and its R-enantiomer, the inhibitory 
effect of esomeprazole was significantly lower on diclofenac 
4'-hydroxylation, with a $K_i$ of 82 μM. The inhibitory effect of 
rameprazole thioether was about 9-fold stronger ($K_i = 6$ μM) than that 
of its parent drug (51 μM).

**CYP2C19 Activity.** The inhibitions of CYP2C19 by the PPIs 
were measured in both HLM and rCYP2C19 using S-mephenytoin 4'- 
hydroxylation as a marker reaction. Representative Dixon plots for the 
inhibitory effects of omeprazole and lansoprazole on S-mephenytoin 
4'-hydroxylase activity in HLM are shown in Fig. 2. In addition, the 
inhibition of R-omeprazole 5-hydroxylation by pantoprazole, lanso-
prazole, rabeprazole, and its thioether metabolite was also studied. 
The inhibition kinetics could best be described by applying a com-
petitive inhibition model. The inhibition constants derived from the 
various systems and marker reactions were consistent except for 
pantoprazole, which showed a higher $K_i$ value (69 μM) in HLM using 
S-mephenytoin as substrate than in the other systems, where the $K_i$ 
varied between 14 and 17 μM. Lansoprazole was the most potent 
CYP2C19 inhibitor, exhibiting a $K_i$ around 1 μM or lower depending 
on which system was used. R-omeprazole, esomeprazole, and ome-
prazole all showed $K_i$ values between 2 and 9 μM, whereas rabe-
prazole showed $K_i$ values between 17 and 21 μM. Again, the inhibitory 
effects of rabeprazole thioether ($K_i = 2–8$ μM) on CYP2C19-medi-
ated reactions were stronger than that of the parent compound.

Pantoprazole showed higher $K_i$ values in HLM using S-
mephenytoin as substrate than in other systems measuring CYP2C19 
inhibition. The enzyme kinetics in HLM could be influenced by many 
factors (e.g., substrate used, the metabolites formed from either sub-
strate or inhibitor, and the enzymes involved in the metabolism of the 
compound). In fact, the data at low S-mephenytoin concentrations (10 
and 20 μM) clearly indicated a potent inhibitory effect on S-
mephenytoin 4'-hydroxylation by pantoprazole in HLM, leading to a 
much lower $K_i$ (19 μM) than the one obtained when all tested 
concentrations were included in the calculation. Because of these 
problems in data analysis, the effect of pantoprazole on S-
mephenytoin 4'-hydroxylation in HLM should be considered as a 
gross effect, not only as an effect of a parent drug to one P450 
enzyme. Consequently, the rCYP2C19 incubation provided a much 
simpler environment for the enzyme kinetic study, and the results 
obtained might better represent the inhibitory effect of pantoprazole 
on the specific P450 enzyme activity than that in HLM. Considering 
the results of the two CYP2C19 marker reactions used in HLM and 
rCYP2C19, a $K_i$ value around 15 μM seems to be more likely for the 
inhibitory potency of pantoprazole to CYP2C19 activity.

**CYP2D6 Activity.** No significant effects on the formation of 1'
-hydroxylated bufuralol (CYP2D6) were found, indicating that none 
of the five PPIs inhibited CYP2D6 activity in vitro ($IC_{50} > 200$ μM). 
However, rabeprazole thioether inhibited CYP2D6 activity with a $K_i$ 
of 12 μM in HLM.

**CYP3A4 Activity.** The effects of the five PPIs on CYP3A4-
mediated 1'-hydroxymidazolam formation were investigated in HLM. 
Pantoprazole showed a stronger inhibitory effect ($K_i = 22$ μM) on 
midazolam 1'-hydroxylation than omeprazole ($K_i = 42$ μM), esom-
prazole ($K_i = 47$ μM), and rabeprazole ($K_i = 51$ μM). The $IC_{50}$ 
of lansoprazole was over 200 μM. The pattern of inhibition was estab-
lished to be competitive by fitting the data to different inhibitory 
models. Omeprazole and its enantiomers showed similar inhibitory 
effects on the production of the 1'-hydroxylated metabolite of mida-
zolam. Again, the inhibitory potency of rabeprazole thioether 
to CYP3A4 activity in HLM was about 3-fold higher than that of its 
parent compound.

**Discussion**

In this study, we investigated the inhibitory effects of the five 
currently available PPIs (i.e., omeprazole, esomeprazole, lansopa-
azole, pantoprazole, and rabeprazole) on four human P450 enzymes 
(CYP2C9, 2C19, 2D6, and 3A4) using human liver microsomal prep-
arations to assess their potential of interaction with coadministered 
drugs that are metabolized by these enzymes. Several pharmacoki-
netic studies have been performed on the five PPIs in healthy S-
mephenytoin EMs and PMs (Table 1). After therapeutically repeated 
doses, the ratio of maximum plasma concentration of each PPI in 
EMs was 1 to 5 μM, with omeprazole and pantoprazole in the low and 
high ends, respectively. In PMs, the range was 2 to 10 μM, with 
rabeprazole and pantoprazole in the low and high ends, respectively. 
The ratio of the mean AUC values in PMs versus EMs was about 1-
to 2-fold for esomeprazole, 2- to 5-fold for rabeprazole, and about 
5-fold for omeprazole, lansoprazole, and pantoprazole, indicating 
considerable variation in drug exposure between EMs and PMs for 
the five compounds (Table 1). Because the $K_i$ values determined in 
our study for certain P450 enzyme and PPI combinations are in the same 
order of magnitude as the plasma levels encountered in vivo, this 
might point to the potential of DDIs for any of the five PPIs during 
multidrug treatment. PPI drug-drug interactions in vivo have been 
reviewed thoroughly (Andersson, 1996; Steinijans et al., 1997; Jung-
nickel, 2000; Andersson et al., 2001a; Gerson and Triadafilopoulos, 
2001; Fuhr and Jetter, 2002; Vanderhoff and Tahboub, 2002), and 
conclusions have been drawn that no clinically relevant DDIs exist for 
this class of compounds. However, the potential of possible clinical 
interactions is not fully explored for all PPIs (e.g., rabeprazole (Fuhr 
and Jetter, 2002)), and the population under study may also influence 
the conclusion of clinical studies (Chong and Ensom, 2003) (e.g., 
most of the rabeprazole studies were performed in Japanese men).

Several in vivo DDI studies claim a significant inhibition of 
CYP2C19 by omeprazole, [e.g., the ability of omeprazole to inhibit 
diazepam clearance in humans (Gugler and Jensen, 1985; Ishizaki et 
al., 1995)]. This seems to be due to the fact that CYP2C19 plays a 
major role in the primary metabolism of both omeprazole and diaz-
epam. In a study on genotyped subjects (Andersson et al., 1990), the 
diazepam-omeprazole drug interaction could be attributed to
CYP2C19, since no DDI was seen in PMs lacking CYP2C19 activity. This inhibitory potency has been confirmed in our present study and by others (Furuta et al., 2001). In our study, esomeprazole showed a slightly weaker inhibitory potency on CYP2C19 than omeprazole ($K_i$ value of 8 versus 2 µM in rCYP2C19). One might expect that this would be reflected in favor of esomeprazole in DDIs in vivo for, for example, typical CYP2C19 substrates, such as diazepam, phenytoin, and $R$-warfarin. However, the interaction potential for esomeprazole seems to be similar to that of omeprazole in the clinical situation. This can be explained by the higher standard dose used for esomeprazole (40 mg) and its lower clearance compared with omeprazole (20 mg) resulting in higher plasma levels and extended exposure for esomeprazole. Comparing the inhibitory potential of omeprazole and its $R$- and $S$-enantiomers, it seems that the contribution to inhibition of CYP2C19 activity by omeprazole was higher from $R$-omeprazole than from esomeprazole, which probably is due to the higher affinity of $R$-omeprazole to this enzyme (Aębë et al., 2000).

Observations that lansoprazole had no significant effect on diazepam (Leffebvre et al., 1992) or warfarin clearance in vivo (Cavanaugh et al., 1991) lead these authors to claim that lansoprazole has less significant drug interactions than omeprazole. However, later it was shown that CYP2C19 was the primary metabolizing enzyme at pharmacologically relevant concentrations of lansoprazole (1-5 µM) (Pearce et al., 1996; Table 1). The clinical correlate of these data were presented by Sohn et al. (1996), who reported in Korean subjects that PMs of $S$-mephenytoin are also PMs of lansoprazole. Our study clearly demonstrates that lansoprazole exhibits a strong competitive inhibition of CYP2C19 activity, with an average $K_i$ of about 1 µM. Thus, lansoprazole has the potential to be an equally or more potent inhibitor than omeprazole or esomeprazole. This result is also consistent with the work of Ko et al. (1997) ($K_i = 3$ µM for lansoprazole). Interestingly, this higher inhibitory potential of lansoprazole is not reflected in vivo, which suggests that the in vivo DDI studies may not have been performed under optimum conditions. Unless complementary clinical DDI studies are performed, it is difficult to conclude whether lansoprazole has clinically important DDIs with drugs such as mephenytoin and some benzodiazepine derivatives or tricyclic antidepressants, which are mainly metabolized by CYP2C19 (Zumbbrunn and Jann, 1998).

Pantoprazole showed the highest inhibition potency on CYP2C9 ($K_i, 6$ µM) of the five PPIs, although drug interactions with substrates for this enzyme have not been shown in vivo. Still, there is a potential for DDIs by pantoprazole upon coadministration with drugs mainly metabolized by CYP2C9, at least in CYP2C19 PM patients who are subjected to elevated plasma concentrations of pantoprazole. Thus, DDI studies in CYP2C19 PMs using, for example, pantoprazole and $S$-warfarin, which have a narrow therapeutic index, would be important to exclude serious effects on $S$-warfarin pharmacodynamics. A potential metabolic interaction with diazepam would be the most reasonable prediction with respect to pantoprazole because both are metabolized mainly by CYP2C19. The interaction study performed with diazepam and pantoprazole indicated that the plasma concentrations of diazepam were unchanged during pantoprazole treatment, but the plasma elimination half-life of diazepam was shorter (9%), and the apparent volume of distribution was decreased (8%) (Gugler et al., 1996). However, pantoprazole was administered intravenously, which would have avoided a potential influence on the liver enzymes by the first pass metabolism of pantoprazole.

It has been suggested that rabeprazole has significantly less drug interactions than other PPIs, and the main reason is claimed to be its nonenzyme-catalyzed degradation, which forms a thioether product.

### Table 3

**K**<sub>i</sub> (µM) values* of omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole for human P<sub>450</sub> isoforms

<table>
<thead>
<tr>
<th>Marker Reaction</th>
<th>Microsomes</th>
<th>R-Enantiomer</th>
<th>S-Enantiomer</th>
<th>Omeprazole</th>
<th>Esomeprazole</th>
<th>Lansoprazole</th>
<th>Pantoprazole</th>
<th>Rabeprazole</th>
<th>Rabeprazole</th>
<th>Population Metabolites</th>
<th>Presence of Some Metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omeprazole</td>
<td></td>
<td></td>
<td></td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-4 Hydroxylation</td>
<td>HLM</td>
<td>5.3 ± 0.4</td>
<td>164 ± 3.0</td>
<td>8.15 ± 77.9</td>
<td>20.8 ± 3.3</td>
<td>6.5 ± 1.0</td>
<td>5.0 ± 0.8</td>
<td>2.8 ± 0.0</td>
<td>2.7 ± 0.0</td>
<td>2.6 ± 0.0</td>
<td>2.5 ± 0.0</td>
</tr>
<tr>
<td>S-4 Hydroxylation</td>
<td>HLM</td>
<td>5.8 ± 0.6</td>
<td>6.2 ± 0.8</td>
<td>7.9 ± 0.5</td>
<td>4.0 ± 0.7</td>
<td>6.9 ± 0.2</td>
<td>4.5 ± 0.5</td>
<td>2.6 ± 0.0</td>
<td>2.7 ± 0.0</td>
<td>2.6 ± 0.0</td>
<td>2.5 ± 0.0</td>
</tr>
<tr>
<td>CYP2C19</td>
<td></td>
<td></td>
<td></td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td></td>
<td></td>
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<tr>
<td>S-4 Hydroxylation</td>
<td>HLM</td>
<td>17 ± 1.1</td>
<td>24 ± 0.0</td>
<td>9.3 ± 0.9</td>
<td>7.0 ± 0.3</td>
<td>1.5 ± 0.5</td>
<td>0.9 ± 0.0</td>
<td>1.1 ± 0.4</td>
<td>1.2 ± 0.3</td>
<td>1.4 ± 0.0</td>
<td>1.5 ± 0.0</td>
</tr>
<tr>
<td>CYP2C9</td>
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<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-4 Hydroxylation</td>
<td>HLM</td>
<td>NT</td>
<td>NA</td>
<td>NT</td>
<td>NA</td>
<td>0.5 ± 0.1</td>
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<td>0.3 ± 0.1</td>
<td>0.4 ± 0.1</td>
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<td>0.6 ± 0.0</td>
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<tr>
<td>CYP3A44</td>
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<td>S</td>
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<td>R</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-1 Hydroxylation</td>
<td>HLM</td>
<td>44.5 ± 5.0</td>
<td>41.9 ± 5.9</td>
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<td>46.6 ± 6.8</td>
<td>46.6 ± 6.8</td>
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</tbody>
</table>

*All the $K_i$ values were calculated with the competitive inhibition model except esomeprazole to CYP2C9-mediated diclofenac 4-hydroxylation, which showed the best fit to the mixed inhibition model.

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It has been suggested that rabeprazole has significantly less drug interactions than other PPIs, and the main reason is claimed to be its nonenzyme-catalyzed degradation, which forms a thioether product.
AUC (0–16 days) of desmethyldiazepam in the PMs compared with omeprazole or placebo (p < 0.05). The authors suggested that, since CYP3A is involved in both 3-hydroxylation of desmethyldiazepam and O-demethylation of rabeprazole, the role of CYP3A in the metabolism of desmethyldiazepam might be more dominant in PMs than in EMs; however, the reason why omeprazole did not show the same effect was not discussed. Our present results suggest that omeprazole and rabeprazole have similar affinity to CYP3A4, but the nonenzyme-catalyzed product rabeprazole thioether showed a K_i (15 μM) that indicates a higher potential for inhibition of CYP3A4 activity. Thus, inhibition by rabeprazole thioether may be the reason of the increased AUC of desmethyldiazepam after diazepam and rabeprazole coadministration in CYP3A4 PMs. Considering the relatively higher lipophilicity and lower in vivo clearance of rabeprazole thioether than its parent drug (Shirai et al., 2001), its influence on the pharmacokinetics of coadministered drugs may be worth studying further [e.g., on typical CYP2C9 and 2C19 substrates (the K_i values of rabeprazole thioether on CYP2C9 and 2C19 being 6 and 2–8 μM, respectively)].

On the other hand, omeprazole has been shown to have no inhibitory potential of CYP3A in vivo (Andersson, 1991), which is the major reason why this effect on desmethyldiazepam was not observed on omeprazole.

In conclusion, we have studied the inhibitory properties of the five currently marketed PPIs on P450 activities. Although the in vitro inhibitory properties of some of these compounds have been studied previously, this is the first complete study of all five compounds carried out and analyzed under the exact same experimental conditions. Due to the fact that these compounds are fully developed, approved by the regulatory authorities, found to have acceptable drug metabolism and pharmacokinetic properties, and are available for treatment of acid-related gastrointestinal disorders, the in vivo correlation of our in vitro data can at least partly be found in the public domain. However, the apparent lack of correlation between the in vivo DDIs reported and the predicted interaction potential from our in vitro studies may at least partly be explained by nonoptimal clinical study design or the simple fact that sufficient clinical data are not yet available. Thus, further studies are required to fully assess whether this in vitro inhibition of P450 enzymes could be a cause of concern during multidrug treatment.

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