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-mephentoin 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 (Kᵢ 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 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 (Röhm 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.,...
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′-hydroxybufuralol, 1′-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 Synthecell 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 Einster 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 buforalol 1′-hydroxylase (CYP2D6) and omeprazole 5-hydroxylase (CYP2C19) activities, a 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 detector was set at 302 nm for monitoring of the consumption of the PPIs, which was less than 20% of the initial concentrations after incubation with HLM.

**Mass Spectrometric Conditions.** The mass spectrometric analyses 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′-hydroxydiclofenac, 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. The mass spectrometer was operated in the selected reaction monitoring mode, with the resolution of Q1 set at 0.5 Da FWHM and that of Q3 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′-hydroxydiclofenac *m/z* 312.0 to 230.0; 4′-hydroxyphenytoin *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.

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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, C219, 2D6, and 3A4, the following marker substrates with high specificity for P450 enzymes were selected: diclofenac 4'-hydroxylation for CYP2C9; 5-methoxytetramic 4'-hydroxylation for CYP2C19; bufuralol 1'-hydroxylation for CYP2D6; and mephenytoin 4'-hydroxylation for CYP2C19. R-omeprazole 5-hydroxylation was also used to reflect the activity of CYP2C19 (Abel 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. Sulfaphenazon, 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 incubation conditions used for CYP2C9, 2D6, and 3A4 activities were 0.2 mg/ml HLM with an incubation time of 15 min, and those for CYP2C19 activity were 0.3 mg/ml HLM (or 10 pmol rCYP2C19/200 mg/ml HLM with an incubation time of 20 min. The reactions were started by the addition of NADPH after a preincubation of 5 min at 37°C. A 100-µl aliquot of ice-cold acetonitrile (containing 1.5 µM internal standard) was added to stop the reaction. After centrifugation at 4,500 g for 20 min, 20 µl of supernatant was injected into the liquid chromatography/tandem mass spectrometry system. Samples were quantified by monitoring the ratio between the hydroxylated metabolite of the marker substance and the internal standard in each sample and in calibration curves. All the incubations throughout the study were carried out in duplicate. For each incubation, the test compound was dissolved in methanol and sequentially diluted with 40% methanol in 0.1 M Tris-hydrochloride buffer (pH 9) [except rabeprazole thioether, which was diluted with 70% methanol in 0.1 M Tris-hydrochloride buffer (pH 9)].

The final pH value of the incubation mixture was kept at 7.4, and the methanol concentration was less than 1%.

Before full Ki determination was done, the concentration of the PPIs that caused 50% inhibition of the marker reaction (IC50) was determined at a substrate concentration close to KM. The inhibition mechanism was determined, and 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 × KM, KM, 3 KM, and 5 KM) with regard to their Michaelis-Menton kinetics (KM and Vmax) (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.

**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 values, were calculated using the inhibition model, which provided the best fit, and the final results are summarized in Table 3.

**CYP2C9 Activity.** Diclofenac 4'-hydroxylation was used as a marker reaction for CYP2C9 activity in HLM. All PPIs showed inhibitory effects on the formation of the 4'-hydroxylated metabolite of diclofenac. The mechanism of inhibition for all PPIs seemed to be competitive except for esomeprazole, which was best fit in a mixed inhibition model. The respective Ki values were 6, 16, 21, and 51 µM for pantoprazole, omeprazole, lansoprazole, and rabeprazole, respec-
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 rabeprazole thioether was about 9-fold stronger ($K_i = 6 \mu M$) 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, lansoprazole, rabeprazole, and its thioether metabolite was also studied. The inhibition kinetics could best be described by applying a competitive 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 omeprazole all showed $K_i$ values between 2 and 9 μM, whereas rabeprazole showed $K_i$ values between 17 and 21 μM. Again, the inhibitory effects of rabeprazole thioether ($K_i = 2–8 \mu M$) on CYP2C19-mediated 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 substrate 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 ($IC_{50} > 200 \mu M$). However, rabeprazole thioether inhibited CYP2D6 activity with a $K_i$ of 12 μM in HLM. Pantoprazole showed a stronger inhibitory effect ($K_i = 22 \mu M$) on midazolam 1'-hydroxylation than omeprazole ($K_i = 42 \mu M$), esomeprazole ($K_i = 47 \mu M$), and rabeprazole; ($K_i = 51 \mu M$). The $IC_{50}$ of lansoprazole was over 200 μM. The pattern of inhibition was established 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 midazolam. 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, lansoprazole, pantoprazole, and rabeprazole) on four human P450 enzymes (CYP2C9, 2C19, 2D6, and 3A4) using human liver microsomal preparations to assess their potential of interaction with coadministered drugs that are metabolized by these enzymes. Several pharmacokinetic 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 et al., 1996; Steinjans et al., 1997; Jungnickel, 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 diazepam. In a study on genotyped subjects (Andersson et al., 1990), the diazepam-omeprazole drug interaction could be attributed to...
TABLE 3

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<th>Microsomes</th>
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K_i (μM) values of omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole for human P450 isoforms

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</table>

*All the K_i values are calculated with the competitive inhibition model except esomeprazole to CYP3A-mediated diazepam 4-hydroxylation, which showed the best fit to the mixed inhibition model.

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 DDI studies in vivo for, for example, typical CYP2C19 substrates, such as diazepam, phenytoin, and S-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 (Abelö et al., 2000).

Observations that lansoprazole had no significant effect on diazepam (Lefebvre 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 (Zumbrunnen 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.
Our results showed that this thioether product by itself exhibited inhibitory potency to all the four major P450 enzymes \( K_i = 2–8 \, \mu M \) for CYP2C9, 6 \, \mu M for CYP2D6, and 15 \, \mu M for CYP3A4. Now that this information on the affinity and thus inhibitory potential of the thioether metabolite is available, this further accentuates the need for DDI studies with rabeprazole and substrates for CYP2D6 as well as CYP3A4, which are available. This further accentuates the need for DDI studies with rabeprazole and omeprazole on diazepam metabolism in CYP2C19 and CYP3A4, which are available. This further accentuates the need for DDI studies with rabeprazole and omeprazole on diazepam metabolism in CYP2C19 and CYP3A4, which are available. Thus, further studies are required to fully assess whether this in vitro inhibition of P450 enzymes could be a cause of concern in the case of concurrent treatment.

**References**


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