Evaluation of Six Proton Pump Inhibitors as Inhibitors of Various Human Cytochromes P450: Focus on Cytochrome P450 2C19

Tatyana Zvyaga, Shu-Ying Chang, Cliff Chen, Zheng Yang, Ragini Vuppugalla, Jeremy Hurley, Denise Thorndike, Andrew Wagner, Anjaneya Chimalakonda, and A. David Rodrigues

Abbreviations: P450, cytochrome P450; IC₅₀, concentration of inhibitor that decreases activity by 50% (not corrected for f_uninc); IC₅₀(u), IC₅₀ corrected for f_uninc; IC₅₀(t), concentration of inhibitor that decreases activity by 50% after a preincubation time (t); [I], concentration of inhibitor; Kᵢ, inhibition constant; Kᵢ,u, Kᵢ corrected for f_uninc; AUC, area under the plasma concentration versus time curve in poor metabolizers (AUC₉M), extensive metabolizers (AUC₉M), in the presence of inhibitor (AUCᵢ) and in the absence of inhibitor (AUCᵢ); [S], substrate concentration; Kₘ, Michaelis constant; HLM, human
liver microsomes; $f_{a,inc}$, free fraction in the incubation; rCYP2C19, recombinant cytochrome P450 2C19; CEC, 3-cyano-7-ethoxy-coumarin; SPE, solid phase extraction; omeprazole, 5-methoxy-2-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole; esomeprazole, (S)-5-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methylsulfinyl]-3H-benzimidazole; lansoprazole, 2-[[3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methylsulfinyl]-1H-benzimidazole; dexlansoprazole, (R)-2-[[3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methylsulfinyl]-1H-benzimidazole; rabeprazole, 2-[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methylsulfinyl]benzimidazol-1-ide; pantoprazole, 5-(difluoromethoxy)-2-[(3,4-dimethoxypyridin-2-yl)methylsulfinyl]benzimidazol-1-ide; $C_{max}$, maximal concentration in plasma; $C_{max,u}$, maximal concentration in plasma corrected for free fraction in plasma; $f_a$, fraction absorbed; $k_a$, absorption rate constant; $C_{max,portal}$, maximal concentration in portal vein; $C_{max,portal,u}$, maximal concentration in portal vein corrected for plasma protein binding; $T_{max}$, time at which maximal plasma concentration is reached; $K_i$, inhibitor concentration that supports half the maximal rate of inactivation; $k_{inact}$, maximal rate of inactivation; $k_{deg}$, rate of P450 (CYP2C19) holoenzyme degradation in the absence of inhibitor; $K_{i,u}$, $K_i$ corrected for $f_{a,inc}$; FR, fractional activity remaining; CI, confidence interval; $k_{obs}$, rate constant for inactivation at a given concentration of inhibitor; $f_{m,2C19}$, fraction cleared via CYP2C19.
Abstract

Six proton pump inhibitors (PPIs), omeprazole, lansoprazole, esomeprazole, dexlansoprazole, pantoprazole, and rabeprazole, were shown to be weak inhibitors of cytochromes P450 (CYP3A4, 2B6, 2D6, 2C9, 2C8 and 1A2) in human liver microsomes. In most cases, IC$_{50}$ values were greater than 40 μM, except for dexlansoprazole and lansoprazole with CYP1A2 (IC$_{50}$ ~8 μM) and esomeprazole with CYP2C8 (IC$_{50}$ = 31 μM). With the exception of CYP2C19 inhibition by omeprazole and esomeprazole (IC$_{50}$ ratio 2.5 to 5.9), there was no evidence for a marked time-dependent shift in IC$_{50}$ (IC$_{50}$ ratio ≤ 2) following a 30-minute preincubation with NADPH. In the absence of preincubation, lansoprazole (IC$_{50}$ = 0.73 μM) and esomeprazole (IC$_{50}$ = 3.7 μM) were the most potent CYP2C19 inhibitors, followed by dexlansoprazole and omeprazole (IC$_{50}$ ~7.0 μM). Rabeprazole and pantoprazole (IC$_{50}$ ≥ 25 μM) were the weakest. A similar ranking was obtained with recombinant CYP2C19. Despite the IC$_{50}$ ranking, after consideration of plasma levels (static and dynamic), protein binding, and metabolism-dependent inhibition, it is concluded that omeprazole and esomeprazole are the most potent CYP2C19 inhibitors. This was confirmed following the incubation of the individual PPIs with human primary hepatocytes (in the presence of human serum), and by monitoring their impact on diazepam N-demethylase activity at a low concentration of diazepam (2 μM). Data described herein are consistent with reports that PPIs are mostly weak inhibitors of cytochrome P450s in vivo. However, two members of the PPI class (esomeprazole and omeprazole) are more likely to serve as clinically relevant inhibitors of CYP2C19.
Introduction

Proton pump inhibitors (PPIs) inhibit the gastric (parietal cell) H⁺/K⁺ATPase that is involved in the final step of hydrochloric acid secretion. Consequently, such agents are used to treat acid-related conditions like peptic ulcers and their complications (e.g., bleeding), gastroesophageal reflux disease, nonsteroidal anti-inflammatory drug-induced gastrointestinal lesions, Zollinger-Ellison syndrome, and dyspepsia. In combination with antibiotics, PPIs are used to treat *Helicobacter pylori* infections also (Shi and Klotz, 2008; Blume et al., 2006).

Since the introduction of omeprazole in 1989, the market has expanded to encompass newer PPIs, such as lansoprazole, pantoprazole, and rabeprazole, and now also includes the (S)-enantiomer of omeprazole (esomeprazole) and the (R)-enantiomer of lansoprazole (dexlansoprazole) (Andersson et al, 2001; Vakily et al., 2009; Shi and Klotz, 2008). As a drug class, the PPIs are well characterized in terms of their pharmacokinetics, absorption, distribution, metabolism, and excretion properties. For example, it is known that PPIs undergo extensive metabolism by P450s, and that CYP2C19 phenotype substantially influences pharmacokinetics, pharmacodynamics, and clinical outcomes (e.g., speed and degree of gastric acid suppression) (Shi and Klotz, 2008; Hunfeld et al., 2008; Baldwin et al., 2008; Li et al., 2004). In a crowded market, therefore, a considerable effort has been made to differentiate the various PPI class members on the basis of their pharmacokinetics, efficacy and drug-drug interaction profile (Blume et al., 2006; Andersson et al., 2001; Shi and Klotz, 2008; Vakily et al., 2009; Ogawa and Echizen, 2010).
Recently, PPI-associated drug interactions have garnered the attention of various regulatory agencies and researchers. The growing interest has been fueled by reports of drug interactions in subjects receiving the combination of a PPI with clopidogrel, despite the acceptance by many that such an interaction only leads to a slight increase in cardiovascular risk (Zhang et al., 2009; Liu and Jackevicius, 2010; Oyetayo and Talbert, 2010; Furuta et al., 2010; Shmulevich et al., 2011; Ohbuchi et al., 2012; Shah et al., 2012; Rassen et al., 2009; Ray et al., 2010). Clopidogrel, a P2Y₁₂ adenosine diphosphate receptor antagonist, undergoes extensive metabolism to both inactive and active (thiol) metabolites. Although a number of P450s have been shown to catalyze the formation of the active thiol (H4), CYP2C19 has received the most attention; CYP2C19-catalyzed metabolism of clopidogrel is a low $K_m$ process in vitro (Kazui et al., 2010; Hagihara et al., 2009) and CYP2C19 genotype is associated with anti-platelet activity and circulating levels of H4 (Shuldiner et al., 2009; Furuta et al., 2010; Boulenc et al., 2012). Consequently, the prescribing information for clopidogrel includes a boxed warning regarding the effectiveness of clopidogrel in CYP2C19 poor metabolizers. It is also recommended to avoid concomitant use of clopidogrel with omeprazole and esomeprazole.

To date, no attempt has been made to systematically evaluate six marketed PPIs as inhibitors of multiple human P450s under the same experimental conditions. Moreover, data for P450s such as CYP2C8, CYP1A2 and CYP2B6 are limited and there are no reports of various PPIs as time-dependent (metabolism-dependent) inhibitors of P450s (Ko et al., 1997; Li et al., 2004; Liu et al., 2005; Walsky et al., 2005, 2006; VandenBranden et al., 1996). In fact, there are only two reports describing time-
dependent inhibition, with focus on CYP2C19 (multiple PPIs) or multiple P450s (omeprazole only) (Ogilvie et al., 2011; Boulenc et al., 2012).

Evaluation of six PPIs is important because it affords ranking of a single compound across different P450s, as well as the different compounds against a single P450, and because time-dependent inhibition has been reported for numerous P450s beyond CYP3A4 (Venkatakrishnan and Obach, 2007). Therefore, omeprazole, lansoprazole, esomeprazole [(S)-isomer of omeprazole], dexlansoprazole [(R)-isomer of lansoprazole], pantoprazole, and rabeprazole were assessed as reversible and time-dependent inhibitors of P450 activities in HLM (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and CYP3A4). During the course of the study it was evident that some of the PPIs behaved as relatively potent inhibitors of CYP2C19 (versus other P450s) and two of them (omeprazole and esomeprazole) behaved as time-dependent inhibitors also. The latter supports the findings of Ogilvie et al (2011) and Boulenc et al (2012), and the more recent report of Ohbuchi et al (2012) employing 2-oxo-clopidogrel as substrate. Additional studies were conducted with rCYP2C19 employing three different substrates (CEC, (S)-mephénytoin and diazepam) and with serum-co-incubated human primary hepatocytes employing diazepam as substrate. Where appropriate, the determined IC$_{50}$ was corrected for f$_{u,inc}$ in order to generate IC$_{50(u)}$ and support a comparison of HLM with rCYP2C19. Finally, the in vitro CYP2C19 inhibition parameters ($K_{i,u}$, $k_{inact}$ and $K_{I,u}$) were used to predict % inhibition in vivo (% inhibition$_{predicted}$) based on static ($C_{max}$, $C_{max,u}$, $C_{max,portal}$, $C_{max,portal,u}$) and dynamic (time-dependent) concentrations of each PPI. The approach enabled assessment of CYP2C19 inhibition for various substrates
(irrespective of f_{m,2C19}) and the comparison of % inhibition_{predicted} versus % inhibition observed in vivo (% inhibition_{in vivo}).
Materials and Methods

Materials. Omeprazole, esomeprazole [(S)-isomer of omeprazole], lansoprazole, phenacetin, N-desmethyl-diazepam, diazepam, and [\(^2\text{H}_3\)]N-desmethyl-diazepam were obtained from Sigma-Aldrich (St Louis, MO). Dexlansoprazole [(R)-isomer of lansoprazole] and pantoprazole were obtained from Synfine Research Inc. (Ontario, Canada). Rabeprazole, bupropion, [\(^2\text{H}_6\)]hydroxy-bupropion, [\(^2\text{H}_3\)]desethyl-amodiaquine, [\(^2\text{H}_4\)]-acetaminophen, and (S)-mephenytoin were purchased from Toronto Research Chemicals Inc. (North York, Ontario, Canada). Pooled HLM (150 different organ donors), and Supersomes™ containing rCYP2C19 (co-expressed with P450 reductase), were purchased from BD Biosciences (Woburn, MA). CEC, [\(^{13}\text{C}_6\)]4’-hydroxy-diclofenac, [\(^2\text{H}_3\)]4’-hydroxy-mephenytoin, [\(^2\text{H}_3\)]dextrorphan and [\(^{13}\text{C}_3\)]1’-hydroxy-midazolam were also obtained from BD Biosciences (Woburn, MA). AlgiMatrix™ firming buffer and 24-well plates (AlgiMatrix™ 3D culture system) were ordered from Invitrogen Corp. (Carlsbad, CA). Two preparations of cryopreserved human primary hepatocytes were obtained from Celsis In Vitro Technologies (Baltimore, MA). Both preparations had greater than 80% post-thaw viability as determined by trypan blue exclusion. The first represented a single organ donor with relatively high CYP2C19 activity [(S)-mephenytoin 4’-hydroxylase = 95 pmol/min per 10^6 cells]. The second was a pool of twenty different organ donors with medium CYP2C19 activity [(S)-mephenytoin 4’-hydroxylase = 15 pmol/min per 10^6 cells]. Human serum was obtained from Bioreclamation LLC (Westbury, NY); purchased frozen, stored at -80°C and thawed only once before each experiment. Hepatocyte culture medium [Hepatozyme-SFM, L-glutamine (200 mM) and penicillin/streptomycin (10000 IU/mL, 10000 μg/mL)] was
ordered from Invitrogen Corp. (Carlsbad, CA). Prior to cell culture, the medium was
diluted and adjusted to a final concentration of 2 mM (glutamine), 50 IU/mL (penicillin)
and 50 μg/mL (streptomycin). All other reagents and chemicals were of analytical grade
and of the highest quality available commercially.

Inhibition Studies with HLM. The HLM panel consisted of assays for seven
different P450s (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4). The assays utilized
pooled HLM and employed well-established substrates (final substrate conc. ~K_m) that
produce P450 isoform-selective metabolites (Walsky and Obach, 2004). Each assay was
performed in a time-dependent format to assess both reversible IC₅₀ and time
(metabolism)-dependent shifts in IC₅₀. test-compounds were pre-incubated at 37°C with
HLM in the presence of NADPH (1mM) for 0 and 30 min. Assays were performed in
384-well microplates in a total volume of 30 μL. Automated liquid handling equipment
was used in the various steps of the assay process: Genesis 150 (Tecan Group Ltd,
Mannedorf, Switzerland), BenchCel System (Velocity 11 Inc., Menlo Park, CA), ECHO
550 (LabCyte Inc., Sunnyvale, CA), and Multidrop Combi (Thermo Electron
Corporation, Vantaa, Finland). Each drug substance was tested as a single point at each
of 10 concentrations ranging from 2 nM to 40 μM, final DMSO concentration in the
reaction mixture was 0.2%. The IC₅₀ value for each compound was determined using a
four-parameter logistic regression model (see Data Analysis).

Sample Preparation. See supplemental data.

Assay. See supplemental data.

RapidFire™-Mass Spectrometry (RF-MS/MS). See supplemental data.
LC-MS/MS Analysis of CYP1A2 (HLM–Phenacetin) Reaction Samples. See supplemental data.

Inhibition Studies with rCYP2C19. CEC O-deethylase. See supplemental data.

(S)-Mephenytoin 4’-Hydroxylase. See supplemental data

Diazepam N-Demethylase. See supplemental data.

Incubations with Human Primary Hepatocytes in the Presence of Human Serum. Cryopreserved human primary hepatocytes were thawed rapidly at 37 °C. The cells were washed with fresh culture medium (50 mL) and then centrifuged (120 x g) for 3 minutes. After removal of the supernatant, the cells were resuspended in hepatocyte culture medium containing 10% (v/v) AlgiMatrix™ firming buffer to yield a final density of 4.2 x 10⁶ cells/mL. An aliquot (300 µL) of the suspension was transferred into the middle of each well of a 24-well AlgiMatrix™ 3D culture plate. After gentle horizontal shaking, the plate was placed in an incubator (37°C; humidified atmosphere of 5% CO₂) for 10 minutes to allow the hepatocytes to seed on the AlgiMatrix™ sponge substratum. Finally, human serum (700 µL) was added to each well, so that the final volume was 1 mL and the cell density was 1.25 x 10⁶ cells/mL. For each of the six PPIs, a stock solution was prepared in culture medium containing 40% DMSO and 5 µL aliquots were added to the specific wells in the assay plate. The final PPI concentration was based on the calculated C_{max,portal} (see legend to Table 3) and was 2.5 µM (omeprazole), 18.7 µM (esomeprazole), 2.9 µM (lansoprazole and dexlansoprazole), 6.7 µM (pantoprazole), and 1.4 µM (rabeprazole). DMSO alone (0.2%) served as a control. Diazepam was prepared as a stock solution in 100% DMSO (at 1 or 2 mM final concentration) and was added (1 µL) to each well. Therefore, the final concentrations of DMSO and serum in the reaction
mixtures were 0.3% (v/v) and 70% (v/v), respectively. The final concentration of diazepam (1-2 μM) approximated the calculated C_max,portal following an intravenous dose (data not shown), and is below the K_m reported for CYP2C19 (Yasumori et al., 1994). At the specific time points of the incubation time course (0, 2, 4, 7, 24 and 30 hrs), an aliquot (50 μL) of the incubate (serum layer) was removed and added to acetonitrile (500 μL) containing 0.3 μM [2H5]N-desmethyl-diazepam (internal standard). The sample was vortexed for 3 minutes, centrifuged and the supernatant (10 μL) subjected to LC-MS/MS analysis (see supplemental data). For the purposes of quantitation, a calibration curve of N-desmethyl-diazepam was prepared in human serum matrix at final concentrations of 0, 1, 2, 4, 8, 16, 32, 64 and 128 nM. The rate of reaction was calculated using the concentration of N-desmethyl-diazepam at each time point (see Data Analysis).

**Determination of f_u,inc.** See supplemental data.

**Data Analysis.** **Determination of IC_{50}.** The end-point of the RF-MS/MS readout for the assays employing HLM was the signal intensity of the metabolite, which was then normalized to the signal of internal standard in the same sample. Therefore, the sample signal intensity was expressed as a signal ratio. The end point readout for the CEC O-deethylase assay was the fluorescence intensity of the metabolite. For both the LC/MS-based and fluorescence assays the sample readout (signal ratio or fluorescence intensity) was then normalized to the signal ratio or fluorescence intensity of the reactions performed in the absence of the test substance (total signal, 0% inhibition), and the reactions performed in the presence of the inhibitor cocktail (background signal, 100% inhibition). These normalized results were expressed as percent inhibition calculated as shown in equation 1:
% Inhibition = \left[ 1 - \left( \frac{S - B}{T - B} \right) \right] \times 100 \tag{1}

where S = Sample, T = Average Total, B = Average Background.

The results were then imported into in-house curve fitting software (CurveMaster), which utilizes MathIQ package (ID Business Solutions, Ltd., Guilford, England), to determine the IC\textsubscript{50} values for each test compound. The IC\textsubscript{50} was defined as the concentration corresponding to 50% inhibition derived from the fitted 10-point curve using a four-parameter logistic regression model (equation 2):

\[
Y = A + \left[ \frac{B - A}{1 + \left( \frac{C}{X} \right)^D} \right]
\tag{2}
\]

where Y = response at a given concentration of inhibitor (X), A=minimum response, B=maximum response, D=Hill coefficient (slope), and C=X at which Y=A+[(B-A)/2].

**Determination of K\textsubscript{I} and k\textsubscript{inact}**. Inhibition parameters (k\textsubscript{inact} and K\textsubscript{I}) were determined by non-linear fitting of the k\textsubscript{obs} at each concentration of PPI versus PPI concentration ([I]) (equation 3; GraFit version 6; Erithacus Software Ltd, Surrey, UK);

\[
k_{\text{obs}} = \frac{k_{\text{inact}} \times [I]}{K_I + [I]} \tag{3}
\]

**CYP2C19 Inhibition in the Presence of Human Primary Hepatocytes**. For the human hepatocyte studies, the rate of N-desmethyl-diazepam formation was determined in the presence of DMSO alone or in the presence of each individual PPI at its respective (predicted) C\textsubscript{max,portal}. Formation of N-desmethyl-diazepam was linear up to 7 hours, so it was possible to determine the rate of reaction. In this instance, % inhibition was calculated as follows (equation 4):
Prediction of CYP2C19 % Inhibition In Vivo Based on In Vitro Data (Employing Static Concentration of Each PPI). The in vitro-derived parameters (HLM and rCYP2C19) were used to predict the % inhibition in vivo as follows (equation 5):

\[
\% \text{ Inhibition}_{\text{predicted}} = [1 - FR_{\text{predicted}}] \times 100
\]  
(5).

In this instance, \( FR_{\text{predicted}} = \delta \times \gamma \)

\[
\gamma = \frac{1}{1 + \frac{[I]}{K_{i,u}}}, \quad \delta = \frac{k_{\text{deg}}}{k_{\text{deg}} + k_{\text{inact}} \times \frac{[I]}{K_{i,u} + [I]}}
\]

For reversible inhibition (\( \gamma \)), \( K_{i,u} \) was obtained by simply dividing \( IC_{50(u)} \) by 2 (assuming competitive inhibition; substrate concentration ~\( K_m \) for each assay). When time-dependent (mechanism-based) inhibition was evident (\( \delta \)), the experimentally-derived estimate of \( K_i \) was corrected for \( f_{u,inc} \) and \( k_{\text{deg}} \) was set at 0.0005 min\(^{-1}\) (half life of CYP2C19 is ~24 h) (Nishiya et al., 2009). For the determination of both \( \gamma \) and \( \delta \), [I] is the inhibitor concentration and is based on \( C_{\text{max}}, C_{\text{max,u}}, C_{\text{max,portal}} \) and \( C_{\text{max,portal,u}} \) (see below). Some of the PPIs did not exhibit time-dependent inhibition, so \( \delta = 1 \).

Where possible, the \( C_{\text{max,portal}} \) for each PPI was calculated as shown in equation 6 (Vuppugalla et al., 2010, references therein):

\[
C_{\text{max,portal}} = C_{\text{max}} + \left( \frac{\text{Dose} \times k_a \times f_a}{Q} \right)
\]

(6)
where Q is the hepatic blood flow (1500 mL/min) and $f_a$ approaches unity for each PPI. The $C_{max}$ and $C_{max,portal}$ were corrected for plasma $f_u$ to generate $C_{max,u}$ and $C_{max,portal,u}$, respectively. For each PPI, the estimated $k_a$ (deconvoluted oral data based on published IV data) was obtained from the literature as follows: 0.037 (omeprazole), 0.178 (esomeprazole), 0.013 (lansoprazole), 0.011 (pantoprazole), and 0.005 (rabeprazole) min⁻¹ (Andersson et al., 2001; Gerloff et al., 1996; Landahl et al., 1992; Pue et al., 1993; Setoyama et al., 2005). Unfortunately, in the absence of published IV data, it was not possible to obtain estimates of $C_{max,portal}$ for dexlansoprazole. For omeprazole, esomeprazole, lansoprazole, dexlansoprazole, pantoprazole and rabeprazole, plasma $f_u$ was 0.05, 0.03, 0.03, 0.02, 0.02 and 0.04, respectively. Unless otherwise indicated, plasma $C_{max}$ ($C_{max,u}$) values for each PPI were based on those reported for a specific diazepam drug interaction; 0.3 (0.02) µM following 20 mg oral omeprazole, 5.2 (0.16) µM following 30 mg oral esomeprazole, 3.4 (0.11) µM following 60 mg oral lansoprazole, 4.0 (0.08) µM following 90 mg oral dexlansoprazole, 57 (1.1) µM following 240 mg IV pantoprazole, and 0.45 (0.02) µM following 20 mg oral rabeprazole (see supplement Table S2) (Anderson et al., 1990, 2001; Lefebre et al., 1992; Gugler et al., 1996; Ishizaki et al., 1995; Vakily et al., 2009). Equation 6 yielded the following estimates for $C_{max,portal}$: 1.7 µM (omeprazole), 15.5 µM (esomeprazole), 4.8 µM (lansoprazole), 57 µM (pantoprazole), and 0.8 µM (rabeprazole). Corresponding $C_{max,portal,u}$ values were: 0.1 µM (omeprazole), 0.47 µM (esomeprazole), 0.14 µM (lansoprazole), 1.1 µM (pantoprazole), and 0.03 µM (rabeprazole).

**Prediction of CYP2C19 % Inhibition In Vivo Based on In Vitro Data (Employing Time-Dependent Concentrations of Each PPI).** Simulations were
performed to project the interaction of each PPI with diazepam using a semi-mechanistic compartment model (supplement Fig. S1). Briefly, the observed plasma concentration-time data of each PPI, digitized from literature reports (supplement Table S2), were fitted using a one- or two- compartment model (vide infra). Similarly, the observed concentration-time profiles of diazepam without PPI, digitized from literature reports (supplement Table S2), were fitted using a two-compartmental model (vide infra). The elimination of diazepam was assumed to occur via two pathways, with CYP2C19-mediated metabolism fixed at $f_{m,2C19} = 0.57$ (vide infra; equation 16). Subsequently, the interaction of each PPI with diazepam was simulated by incorporating in vitro inhibition parameters. The simulated profiles of diazepam following PPI administration were compared to the observed data obtained from the literature. All PPI-diazepam interaction modeling was performed using WinNonlin® (Enterprise v5.0, Pharsight Corp., Mountain View, CA) and Berkeley Madonna (Berkeley Madonna Inc, University of California, Berkeley, CA).

**Modeling the Pharmacokinetics of Each PPI.** Briefly, the plasma concentration-time profile of each PPI following single dose oral administration was fitted to a one- or two-compartment model (supplement Fig. S2), wherein $k_a$ and $k_{el}$ are the first order absorption and elimination rate constants and $k_{cp}$ and $k_{pc}$ are the distribution rate constants from the central and the peripheral compartments. In case of pantoprazole, which was administered intravenously, the absorption compartment was not used and $k_a$ was set to zero. The differential equations are shown below (equations 7, 8 and 9):

\[
d(GI)/dt = \text{Input} - k_a \cdot GI
\]  

(7)
\[
d\text{PPI}/dt = (k_a^\text{GI} - k_c^\text{el} \cdot \text{PPI} - k_c^\text{cp} \cdot \text{PeripheralPPI})/\text{Vol} \quad (8)
\]

\[
d(\text{PeripheralPPI})/dt = k_c^\text{cp} \cdot \text{PPI} - k_p^\text{pc} \cdot \text{PeripheralPPI} \quad (9)
\]

wherein “GI”, “PPI”, and “PeripheralPPI” are the amounts of inhibitor (PPI) in gastrointestinal tract, plasma, and peripheral compartment, respectively. The single dose pharmacokinetics of omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole were obtained from the literature (Anderson et al., 1990, 2001; Lefebre et al., 1992; Pue et al., 1993; Setoyama et al., 2005). In the case of rabeprazole, the absorption was modeled using a Weibull function, as a first order process could not adequately capture the time course of absorption. For dexlansoprazole, two first order absorption rate constants (\(k_a^1\) and \(k_a^2\)) were used to model the absorption kinetics following administration of the modified release tablet (Vakily et al., 2009). Values for \(k_c^\text{cp}\) and \(k_p^\text{pc}\) were fixed to zero in all cases except for esomeprazole, where they were estimated due to the bi-exponential nature of the profile.

**Modeling the Pharmacokinetics of Diazepam.** The pharmacokinetics of IV diazepam in the absence of PPI was modeled (fitted) using a two-compartment model with the elimination occurring from the central compartment as shown in Equations 10-12 (supplement Fig. S1). Because diazepam was administered orally (5 mg) in the drug-drug interaction study with dexlansoprazole (Vakily et al., 2009), an absorption compartment with a first order absorption rate constant of \(k_a^\text{ad}\) (3.0 hr\(^{-1}\)) was used to model diazepam kinetics. In addition, because of the low clearance of diazepam relative to hepatic blood flow (<10%), the oral bioavailability was assumed to be complete (Klotz et al., 1975). The elimination was assumed to occur via two pathways, with CYP2C19-
mediated metabolism fixed at $f_{\text{m,2C19}} = 0.57$ (see below; equation 16). The clearance of diazepam via the CYP2C19 mediated component was modeled using well-stirred model equation (equation 12):

$$\frac{d(\text{Diazepam})}{dt} = \frac{-(\text{CL}_1 + \text{CL}_2) \times \text{Diazepam} - k_{12} \times \text{Diazepam} \times \text{Vc} + k_{21} \times \text{PeripheralDZ}}{\text{Vc}}$$  
(10)

$$\frac{d(\text{PeripheralDZ})}{dt} = k_{12} \times \text{Diazepam} \times \text{Vc} \times k_{21} \times \text{PeripheralDZ}$$  
(11)

$$\text{CL}_1 = \frac{Q \times \text{CL}_{\text{int}}}{Q + \text{CL}_{\text{int}}}$$  
(12)

Wherein “Diazepam” is the plasma concentration of diazepam, “PeripheralDZ” is the amount of diazepam in the peripheral compartment, and CL1 and CL2 are the two clearance pathways for diazepam; with CL1 assumed to be CYP2C19-mediated. Vc refers to the volume of central compartment (300 mL/kg) and $k_{12}$ (0.3 hr$^{-1}$) and $k_{21}$ (0.13 hr$^{-1}$) are the distribution rate constants from the central and peripheral compartments. Q is the hepatic blood flow in man (20.5 mL/min per kg) and CL$_{\text{int}}$ is the intrinsic clearance of diazepam in man via the CYP2C19 mediated pathway. Based on data digitized from literature studies (Anderson et al., 1990, 2001), the pharmacokinetic profile of diazepam in the placebo leg of the study was fitted to the above equations to estimate CL$_{\text{int}}$, CL2, $k_{12}$, $k_{21}$, and Vc (above).

**Simulating the Effect of Each PPI on the Pharmacokinetics of Diazepam.** Simulations were performed to predict the effect of each PPI on the pharmacokinetics of diazepam. The results of these simulations were compared to diazepam concentration-time profiles and AUC ratios obtained from drug-drug interaction studies reported in the literature. The dose and dosing regimen used in these simulations were similar to literature reports (see supplement Fig. S1 and Table S2). Briefly, using the semi-mechanistic compartment
model, concentration-time profiles were simulated following “administration” of diazepam as a short intravenous infusion (0.1 mg/kg) or a single oral dose (5 mg) following PPI dosing (supplement Fig. S1 and Table S2). The intrinsic clearance of diazepam in the presence of PPI (CL\textsubscript{int'}) was assumed to be inhibited as a function of [I] as follows (equation 13);

\[
\text{CL'}_{\text{int}} = \text{CL}_{\text{int}} \cdot \delta \cdot \gamma
\]

Wherein, \(k_{\text{deg}}, k_{\text{inact}}\) (determined in vitro), \(K_{i,u}\) (determined in vitro) and \(K_{i,u}\) (determined in vitro) have been defined previously (above) and were obtained using rCYP2C19 with diazepam as substrate. For PPIs that inhibit CYP2C19 only by a reversible mechanism (lansoprazole, pantoprazole, dexlansoprazole, and rabeprazole), \(k_{\text{deg}}, k_{\text{inact}},\) and \(K_{i,u}\) were fixed at zero. Finally, to evaluate the influence of protein binding, drug-drug interaction predictions were performed under two scenarios using time-dependent free plasma PPI concentrations and total PPI plasma concentrations as surrogates for [I]. Data were reported as the predicted ratio of diazepam AUC (PPI vs placebo), and it was possible to determine \% inhibition\textsubscript{predicted} for each PPI (equation 14):

\[
\% \text{ Inhibition}_{\text{predicted}} = (1 - \text{FR}_{\text{predicted}}) \times 100
\]

In equation 14, \(\text{FR}_{\text{predicted}}\) was determined using equation 15 (\(f_{m,2C19} = 0.57;\) see below);

\[
\text{FR}_{\text{predicted}} = \left[ \frac{1 - \frac{\text{AUC}_i}{\text{AUC}_c}}{\frac{\text{AUC}_i}{\text{AUC}_c}} \right]_{\text{predicted}} + 1 \times f_{m,2C19}
\]
**Calculation of \( f_{m,2C19} \) for Diazepam.** The AUC of diazepam is increased 1.8- to 2.3-fold in CYP2C19 poor metabolizer (PM) versus extensive metabolizer (EM) subjects, so the drug can serve as a CYP2C19 probe (Ishizaki et al., 1995; Andersson et al., 1990). Moreover, diazepam is a low clearance compound (0.29 to 0.46 mL/min per kg), with good oral bioavailability, and undergoes extensive metabolism (> 99% of the dose) (Klotz et al., 1975). Therefore, the fraction of the diazepam dose metabolized by CYP2C19 can be calculated based on the clinically determined \( AUC_{PM}/AUC_{EM} \) ratio (equation 16) (Ito et al., 2005). Based on the \( AUC_{PM}/AUC_{EM} \) ratio of 2.3, \( f_{m,2C19} \) for diazepam is calculated to be 0.57.

\[
f_{m,2C19} = 1 - \left( \frac{1}{AUC_{PM} / AUC_{EM}} \right)
\]

(16)

**Determination of CYP2C19 % Inhibition In Vivo For Each PPI.** In vivo inhibition of CYP2C19 was assessed based on the impact of each PPI on the pharmacokinetics of diazepam; diazepam is one of the few well-characterized probes that has been studied with all six PPIs described. For each PPI with diazepam, the % inhibition\(_{in \, vivo}\) was then determined as follows (equation 17):

\[
% \, Inhibition_{in \, vivo} = (1 - FR_{in \, vivo}) \times 100
\]

(17)

In equation 17, \( FR_{in \, vivo} \) was determined using equation 18 (\( f_{m,2C19} = 0.57 \));
The diazepam AUC/AUCₜ ratio (observed) with each PPI (AUC in the presence of PPI versus placebo) has been reported (90% CI); for omeprazole 1.28 (1.19, 1.39; Ishizaki et al., 1995) and 1.36 (1.19, 1.53; Andersson et al., 1990); 1.12 (0.99, 1.23) for lansoprazole (Lefebvre et al., 1992); 1.81 (1.42, 2.31) for esomeprazole (Andersson et al., 2001); 1.06 (1.01, 1.12) for dexlansoprazole (Vakily et al., 2009); 0.99 (0.87, 1.13) for pantoprazole (Gugler et al., 1996); and 0.91 (0.81, 1.02) for rabeprazole (Ishizaki et al., 1995). The CI (90%) was reported or estimated from the mean ± SD data (ratio variance approximated by the Taylor expansion).
Results

Reversible Inhibition of P450s in the Absence of Preincubation with NADPH.

HLM. Data describing the reversible inhibition of P450 activities in HLM by omeprazole, lansoprazole, esomeprazole, dexlansoprazole, pantoprazole, and rabeprazole are presented in Table 1. The PPI concentration range tested (2 nM to 40 μM) afforded coverage of known clinically relevant plasma concentrations and differentiation of the inhibitory potency across the various P450s. Overall, relatively minimal inhibition (IC₅₀ > 40 μM) of CYP3A4, CYP2B6, CYP2D6, CYP2C9, CYP2C8 and CYP1A2 activity was observed. However, it was possible to obtain an IC₅₀ (~31 μM) for esomeprazole with CYP2C8 and an IC₅₀ (~8 μM) for both lansoprazole and dexlansoprazole with CYP1A2. Of the P450s tested, the most potent inhibition was observed with CYP2C19-catalyzed (S)-mephenytoin 4′-hydroxylase activity (Table 2). In this instance, lansoprazole (IC₅₀ = 0.73 μM) was found to be the most potent inhibitor, followed by esomeprazole (IC₅₀ = 3.7 μM), dexlansoprazole (IC₅₀ = 6.0 μM), and omeprazole (IC₅₀ = 7.4 μM). Both rabeprazole and pantoprazole were relatively weak inhibitors of CYP2C19 (IC₅₀ ≥ 25 μM).

rCYP2C19. In agreement with HLM data, lansoprazole and esomeprazole were the most potent inhibitors of rCYP2C19-catalyzed CEC O-deethylase activity (IC₅₀ = 0.4 μM), followed by omeprazole and dexlansoprazole (IC₅₀ = 1.2 and 2.2 μM, respectively), and then by pantoprazole and rabeprazole (IC₅₀ ~ 4.0 μM). Relatively potent inhibition with lansoprazole and esomeprazole was observed also with rCYP2C19-catalyzed diazepam (IC₅₀ = 2.4 and 4.6 μM, respectively) and (S)-mephenytoin (IC₅₀ = 1.1 and 1.3 μM, respectively) metabolism (Table 2). This meant that the difference in CYP2C19
inhibitory potency observed in HLM for omeprazole and its (S)-isomer, as well as lansoprazole and its (R)-isomer, was also evident with rCYP2C19 employing three different substrates; omeprazole was less potent than esomeprazole (~2-fold with HLM and 1.7 - 9.6-fold with rCYP2C19), whereas lansoprazole was more potent than dexamethasone (~8-fold with HLM and ~5-fold with rCYP2C19).

**Time-Dependent Inhibition of P450s in HLM after Preincubation with NADPH.** The six PPIs were also assessed as time-dependent inhibitors of various P450 activities in HLM (Table 1 and 2), involving a 30-minute preincubation of the PPI with NADPH-fortified HLM, and the IC_{50(t)} was compared to IC_{50} obtained without preincubation with NADPH. In most cases, a relatively minimal time-dependent shift in IC_{50} (IC_{50}/IC_{50(t)} ratio < 2.0) was observed in comparison to positive controls such as tienilic acid (CYP2C9), paroxetine (CYP2D6), verapamil (CYP3A4), ticlopidine (CYP2C19; CYP2B6), and furafylline (CYP1A2) (legend to supplement Table S1). However, a time-dependent shift in IC_{50} was evident with omeprazole and CYP1A2 activity (IC_{50} > 40 μM, IC_{50(t)} = 20.6 μM); esomeprazole and CYP2D6 (IC_{50} > 40 μM, IC_{50(t)} = 20.9 μM); and with rabeprazole and CYP1A2 (IC_{50} > 40 μM, IC_{50(t)} = 18.1 μM), CYP2C8 (IC_{50} > 40 μM, IC_{50(t)} = 13.9 μM), and CYP2D6 (IC_{50} > 40 μM, IC_{50(t)} = 28.5 μM) (Table 1). Only omeprazole (IC_{50}/IC_{50(t)} ratio = 2.5) and esomeprazole (IC_{50}/IC_{50(t)} ratio = 4.9) exhibited time-dependent inhibition of CYP2C19-catalyzed (S)-mephenytoin 4’-hydroxylation (Table 2). The higher IC_{50}/IC_{50(t)} ratio for esomeprazole (~9.5) versus omeprazole (~2.9) was confirmed with rCYP2C19 employing diazepam as substrate (data not shown).
**Determination of $k_{\text{inact}}$ and $K_I$ for CYP2C19 (HLM and rCYP2C19).** The time-dependent shift in IC$_{50}$ with (S)-mephenytoin described above is consistent with the observations of Ogilvie et al (2011) and Boulenc et al (2012). In fact, both groups have reported omeprazole (up to 100 µM) as a mechanism-based inhibitor of CYP2C19 in HLM and reported $K_I$ values ranging from 1.7 to 9.1 µM and $k_{\text{inact}}$ values ranging from 0.016 to 0.046 min$^{-1}$. Likewise, a ~2-fold shift in IC$_{50}$ has also been reported by Ohbuchi et al (2012) employing 2-oxo-clopidogrel as substrate of rCYP2C19. Therefore, we also sought to determine $K_I$ and $k_{\text{inact}}$ with HLM employing (S)-mephenytoin as substrate, but wanted to confirm the parameters for both omeprazole and esomeprazole with rCYP2C19 also.

Following a 30-min preincubation at a higher protein concentration by a 10-fold dilution of incubate with the assay buffer containing substrate (~10-fold higher than $K_m$), it was possible to verify that the $k_{\text{inact}}/K_I$ ratio is higher for esomeprazole ($k_{\text{inact}}/K_I$ ratio = 0.056 min$^{-1}$µM$^{-1}$) than for omeprazole ($k_{\text{inact}}/K_I$ ratio = 0.027 min$^{-1}$µM$^{-1}$) in HLM (Fig. 1). Ticlopidine was tested as a positive control under the same assay conditions and similar parameters to those of Nishiya et al (2009) were obtained ($k_{\text{inact}}/K_I$ ratio = 0.068 min$^{-1}$µM$^{-1}$; $K_I = 1.53 \pm 0.03$ µM; $k_{\text{inact}} = 0.105 \pm 0.011$ min$^{-1}$) (data not shown). Likewise, rCYP2C19 with (S)-mephenytoin rendered a higher $k_{\text{inact}}/K_I$ ratio for esomeprazole ($k_{\text{inact}}/K_I$ ratio = 0.085 min$^{-1}$µM$^{-1}$) when compared to omeprazole ($k_{\text{inact}}/K_I$ ratio = 0.018 min$^{-1}$µM$^{-1}$). The results obtained with (S)-mephenytoin were confirmed with diazepam as rCYP2C19 substrate; esomeprazole $k_{\text{inact}}/K_I$ ratio = 0.042 min$^{-1}$µM$^{-1}$ and omeprazole $k_{\text{inact}}/K_I$ ratio = 0.01 min$^{-1}$µM$^{-1}$ (Fig. 1). No attempt was made to determine $k_{\text{inact}}$ and $K_I$ employing HLM with diazepam as substrate, because at higher diazepam concentrations
 (> CYP2C19 K_m) additional P450s are involved in metabolism and the data would be difficult to interpret. In contrast, (S)-mephenytoin is relatively more CYP2C19-selective over a wide concentration range. Overall, the k_inact (0.030 to 0.048 min^{-1}) and K_i (1.1 to 3.8 µM) values for omeprazole described herein fell within the range reported by Ogilvie et al (2011). However, both Ogilvie et al (2011) and Boulenc et al (2012) reported higher K_i values employing HLM (~9 µM) when higher concentrations (>30 µM) of omeprazole are used (e.g., 40 to 100 µM). Although 30 µM was the highest concentration of omeprazole tested in the present study, the use of seven to ten different omeprazole concentrations enabled robust fitting of the data and parameter determination (Fig. 1).

**Various PPIs as Inhibitors of Diazepam N-Demethylation Catalyzed by Human Primary Hepatocytes Co-Incubated with Human Serum.** As shown in Table 3, the six PPIs were evaluated as inhibitors of diazepam N-demethylase activity in the presence of human primary hepatocytes employing the AlgiMarix™ 3D culture system. Two preparations of cells were used, both co-incubated with human serum (70% v/v). Under the incubation conditions described, the formation of N-desmethyl diazepam was linear with time and the scaled diazepam clearance (~1.0 mL/min per kg) was similar to that observed clinically following an IV dose (0.29 - 0.46 mL/min per kg) (Klotz et al., 1975). Moreover, use of low diazepam concentrations (~2 µM) ensured that N-demethylase activity was largely reflective of CYP2C19 (Yasumori et al., 1994).

Each individual PPI was added at a single concentration based on its estimated C_{max,portal} (see Materials and Methods) and the serum was added to account for the differences in f_u, in addition to hepatocyte uptake and binding. Consistent with the estimates of % inhibition in vivo, the greatest inhibition was observed with esomeprazole.
and omeprazole. In comparison, relatively minimal inhibition (≤ 13.5%) was evident with lansoprazole, dexlansoprazole, rabeprazole and pantoprazole (Table 3).

**Prediction of CYP2C19 Inhibition In Vivo Based on In Vitro-Derived Inhibition Data and a Static Concentration of PPI (C\textsubscript{max}, C\textsubscript{max,\textit{u}}, C\textsubscript{\textit{max,portal}}, and C\textsubscript{\textit{max,portal,\textit{u}}}).** HLM [(\textit{S})-mephenytoin] and rCYP2C19 (diazepam, (\textit{S})-mephenytoin and CEC as substrate) IC\textsubscript{50(\textit{u})} data were used to derive K\textsubscript{i,\textit{u}} values (Table 4 and 5). In turn, the K\textsubscript{i,\textit{u}} values were used to estimate the degree of CYP2C19 inhibition in vivo based on published data for each compound (e.g., C\textsubscript{\textit{max}}, dose, f\textsubscript{\textit{u}}, f\textsubscript{\textit{a}}). For omeprazole and esomeprazole, the experimentally-derived parameters for metabolism-dependent inhibition (K\textsubscript{i,\textit{u}} and k\textsubscript{\textit{inact}}) were considered also. Where possible, the k\textsubscript{\textit{a}} for each individual PPI was calculated by leveraging published human oral and IV pharmacokinetic data (Andersson et al., 2001, Gerloff et al., 1996; Landahl et al., 1992; Pue et al., 1993; Setoyama et al., 2005). With the exception of dexlansoprazole, it was possible to calculate C\textsubscript{\textit{max,portal}} and C\textsubscript{\textit{max,portal,\textit{u}}} (see Materials and Methods).

As shown in Table 4, for lansoprazole, dexlansoprazole and rabeprazole it was possible to rationalize the inhibition in vivo by considering either f\textsubscript{\textit{u}-corrected C\textsubscript{\textit{max}} or f\textsubscript{\textit{u}-corrected C\textsubscript{\textit{max,portal}}. Moreover, it was possible to show that inhibition of CYP2C19 with rabeprazole was negligible and inhibition with lansoprazole (8-34%) was greater than that observed with dexlansoprazole (1-7%). With the exception of rabeprazole, the % inhibition in vivo was greatly overestimated by not correcting for plasma protein binding. Based on the plasma C\textsubscript{\textit{max}} (57 µM) following a 240 mg IV dose of pantoprazole (Gugler et al., 1996), the % inhibition of CYP2C19 was over-estimated even with f\textsubscript{\textit{u}-correction.
Only HLM-derived (S)-mephenytoin $K_i$ data (> 20 µM) rendered a % inhibition\textsubscript{predicted} value close to that in vivo (< 5%).

In the case of omeprazole, $f_u$-correction was needed in order to avoid overestimation of % inhibition (Table 5). Furthermore, it was also evident that one had to consider metabolism-dependent inhibition. In this regard, rCYP2C19-derived values for % inhibition\textsubscript{predicted} employing (S)-mephenytoin (38-77%) and diazepam (26-66%), and $f_u$-corrected $C_{\text{max}}$, were closest to the % inhibition\textsubscript{in vivo} range reported for diazepam. Unfortunately, data for the inhibition of (S)-mephenytoin 4'-hydroxylase in vivo are not available, so an attempt was made to determine % inhibition\textsubscript{in vivo} with two additional CYP2C19 substrates (moclobemide, $f_m$\textsubscript{CYP2C19} = 0.72; escitalopram, $f_m$\textsubscript{CYP2C19} = 0.44). Just like diazepam, the AUC of moclobemide (1.3 - 2.2-fold) and escitalopram (1.4 - 1.6-fold) is increased in CYP2C19 extensive metabolizer subjects co-dosed with omeprazole (Yu et al., 2001; Malling et al., 2005). Therefore, the % inhibition\textsubscript{in vivo} with moclobemide (32-76%) is closer to that observed with diazepam (28-61%) versus escitalopram (64-89%). Interestingly, HLM (S)-mephenytoin 4'-hydroxylase-derived values for % inhibition\textsubscript{predicted} were higher (51-85%) even after incorporation of plasma $f_u$ (Table 5). Whether or not the degree of CYP2C19 inhibition by omeprazole is substrate-dependent requires further investigation; at least in our hands the $k_{\text{inact}}/K_i$ ratio (rCYP2C19) with diazepam (0.01 min\textsuperscript{-1}µM\textsuperscript{-1}) and (S)-mephenytoin (0.018 min\textsuperscript{-1}µM\textsuperscript{-1}) were comparable (Fig. 1).

As presented in Table 5, esomeprazole has been shown to elicit a relatively marked effect on the AUC of diazepam and % inhibition\textsubscript{in vivo} ranged from 52 to 99%. Therefore, the extent of CYP2C19 inhibition with esomeprazole was greater than that
observed with any other PPI. Although the predictions correctly presented esomeprazole as the most potent CYP2C19 inhibitor, % inhibition was overestimated (% inhibition\textsubscript{predicted} ≥ 95%) when using C\textsubscript{max} or C\textsubscript{max,portal} (Fig. 4). Furthermore, correction for plasma f\textsubscript{u}, consideration of portal concentration, use of HLM versus rCYP2C19, and substrate type, did not improve the prediction. It should be noted that the rate constant for CYP2C19 degradation (k\textsubscript{deg}) is a key parameter and if incorrectly applied can lead to erroneous estimates of % inhibition in vivo. Unfortunately, the k\textsubscript{deg} for CYP2C19 has not been determined in vivo, and so the value used in the current analysis (0.0005 min\textsuperscript{-1}) has been derived in vitro and used by others (Ogilvie et al., 2011; Nishiya et al, 2009).

**Prediction of CYP2C19 Inhibition In Vivo Based on In Vitro-Derived Inhibition Parameters and a Semi-Mechanistic Compartment Model Describing the Pharmacokinetics of Both PPI and Diazepam.** The analyses described above focused on the use of static PPI concentrations. Therefore, the impact of time-dependent PPI concentrations was considered also. Towards this end a semi-mechanistic compartment model was developed to describe each PPI-diazepam drug interaction and leverage in vitro-derived inhibition parameters (rCYP2C19-catalyzed diazepam N-demethylation) (see Materials and Methods). The plots of the observed in vivo and predicted plasma concentration-time profiles are shown for each of the six PPIs (supplement Fig. S2), following oral dosing of omeprazole, esomeprazole, lansoprazole, rabeprazole and dexlansoprazole at 20, 30, 60, 20 and 90 mg, respectively, and pantoprazole IV dose at 240 mg (supplement Table S2). In all cases, the observed plasma concentrations were adequately captured using a one- or two-compartment model (supplement Fig. S1). The subsequent plots of observed and model-simulated plasma concentration-time profiles of
diazepam following PPI dosing are shown in Figure 2 (incorporating plasma total PPI concentration as [I]) and in Figure 3 (incorporating plasma free PPI concentration as [I]). As shown in Figure 2, the semi-mechanistic compartment model incorporating total PPI concentrations was able to reasonably capture the diazepam time course of placebo and PPI-treated subjects and % inhibition predicted was 50% (esomeprazole), 29% (omeprazole), 16% (lansoprazole), 16% (dexlansoprazole), 29% (pantoprazole) and <1% (rabeprazole). On the other hand, incorporation of free [I] resulted in the under-prediction of the diazepam AUC ratio compared to the observed geometric mean ratio (< 1% inhibition predicted) (Fig. 3). Pantoprazole behaved as an outlier, because fu-adjusted plasma concentrations rendered a % inhibition predicted value (16%) that fell within the in vivo range (< 1%, 20%) as defined by the 90% CI. Interestingly, based on total PPI concentration in plasma, the model predicted that only a partial decrease in inhibition is possible when diazepam is dosed 12 h after omeprazole (29 vs 16%) and esomeprazole (50 vs 29%) (supplement Fig S3). At least for these two PPIs, it may not be possible to successfully mitigate CYP2C19 inhibition in a clinical setting by separating the dose of PPI from that of the victim drug.

**Summary Comparison of the Six PPIs as CYP2C19 Inhibitors.** In order to facilitate a comparison across the seven different modeling methods used, summary data are presented for the six PPIs (Fig. 4). In all cases, esomeprazole was predicted to be the most potent CYP2C19 inhibitor, whereas rabeprazole was predicted to be the weakest inhibitor. It was possible to differentiate esomeprazole from omeprazole, especially when fu-corrected static PPI concentrations or when time-dependent changes in total PPI concentration were considered. Consistent with in vivo data, both lansoprazole and
dextansoprazole were predicted to be weaker inhibitors versus esomeprazole and omeprazole. However, these two PPIs could only be differentiated from each other (lansoprazole > dextansoprazole) only when $f_u$-corrected $C_{\text{max}}$ was considered (8 vs 1% inhibition$_{\text{predicted}}$). In the case of pantoprazole, only hepatocyte data correctly predicted minimal inhibition ($\leq 5\%$) of diazepam metabolism (Fig. 4).
Discussion

As described herein, it was possible to evaluate six PPIs as reversible and time-dependent inhibitors of seven different human P450s under the same assay conditions in vitro. To our knowledge, this has not been reported previously. In the absence of preincubation, the IC$_{50}$s obtained (Table 1 and 2) more or less complimented the results of others who have reported IC$_{50}$ data for different PPI-P450 combinations (Ko et al., 1997; Li et al., 2004; Liu et al., 2005; Walsky et al., 2005, 2006; VandenBranden et al., 1996; Ogilvie et al., 2011). For the first time, it is possible to report that the PPIs studied do not exhibit marked time-dependent inhibition of six P450s (CYP1A2, 2B6, 3A4, 2C9, 2C8 and 2D6) in HLM (IC$_{50}$/IC$_{50(t)}$ ratio < 2.0). Only with omeprazole (CYP1A2), esomeprazole (CYP2D6) and rabeprazole (CYP1A2, 2C8 and 2D6) did the IC$_{50}$ shift to any measurable extent. In part, this may reflect the generation of metabolites that serve as reversible inhibitors. For example, the thioether metabolite of rabeprazole is known to be a more potent inhibitor of CYP2D6 in HLM (Li et al., 2004). Based on parent PPI pharmacokinetics, however, inhibition of these six P450s is predicted to be minimal assuming that C$_{max,u}$ (≤ 5% inhibition) or C$_{max,portal,u}$ (≤ 12% inhibition) governs the interaction (data not shown). This is consistent with the minimal effect of various PPIs on the pharmacokinetics of probes such as (S)-warfarin (CYP2C9), theophylline (CYP1A2), and metoprolol (CYP2D6) (Blume et al., 2006; Vakily et al., 2009; Andersson et al., 2001; Ogawa and Echizen, 2010; Uno et al., 2008). In the absence of appreciable CYP3A4 inhibition in vitro, the only drug interactions that could not be rationalized were the ~1.3-fold increase in AUCs of cisapride with esomeprazole,
nifedipine with omeprazole, and tacrolimus with lansoprazole, pantoprazole, and rabeprazole (Andersson et al., 2001; Ogawa and Echizen, 2010). It should be noted that the various metabolites of each PPI were not studied as CYP3A inhibitors. In addition, the effect of CYP2C19 phenotype on the pharmacokinetics of each PPI, and its impact on the [I]/Kᵢ ratio for CYP3A4, was not considered in each case.

With the exception of pantoprazole, the inhibition of CYP2C19 activity in HLM rendered the lowest IC₅₀ values (Tables 1 and 2). Such a result was not unexpected, given that PPIs are known to mostly serve as low Kᵦ CYP2C19 substrates (Li et al., 2005; Karam et al., 1996; Abelo et al., 2000). With all three rCYP2C19 substrates chosen, rabeprazole and pantoprazole were weaker than lansoprazole. This observation is in accord with the findings of Li et al (2004), Zhang et al (2009), and Ohbuchi et al (2012). Liu et al (2005) have determined that lansoprazole is more potent (~3-fold) than dextansoprazole with (S)-mephenytoin as substrate. We report an 8-fold (HLM) and 6-fold (rCYP2C19) greater potency with the same substrate. Li et al (2004), Liu et al (2005) and Ohbuchi et al (2012) have documented esomeprazole and omeprazole as more or less equipotent inhibitors of CYP2C19. In our hands, esomeprazole was more potent when incubated with HLM (~2-fold) and recombinant CYP2C19 (1.7- to 10-fold). This is in contrast to Ogilvie et al (2011), who reported a lower IC₅₀ for omeprazole (6.9 ± 0.7 vs 15 ± 1 μM).

In terms of the time-dependent inhibition observed with omeprazole and esomeprazole (Fig. 1), the results are in accord with other reports (Boulenc et al., 2012; Ogilvie et al., 2011; Ohbuchi et al., 2012). Ogilvie et al (2011) have shown that the time-dependent inhibition is consistent with mechanism-based inactivation resulting in
irreversible inactivation of CYP2C19 in HLM. This implies that both PPIs undergo P450-mediated oxidation to a product that covalently binds to CYP2C19. Because metabolism is known to be catalyzed by CYP2C19 (low $K_m$) and CYP3A4 (high $K_m$) in HLM (Abelo et al, 2000; Li et al., 2005), we also sought to assess the time-dependent inhibition with preparations of rCYP2C19 employing both $(S)$-mephenytoin and diazepam as substrates. In our hands, it was possible to confirm that the $K_I$ with CYP2C19 ranged from 1.8 to 3.8 µM (Fig. 1) and was consistent with the low $K_m$s reported for both omeprazole and esomeprazole (Abelo et al, 2000; Li et al., 2005). The HLM-derived estimates of $K_I$ (~1.0 µM) reported herein are largely reflective of low $K_m$ CYP2C19-dependent metabolism (Li et al., 2005) and of the PPI concentration range used in the present study ($\leq 30 \mu M$). Therefore, the higher HLM-derived values of $K_I$ (~9 µM) reported by Ogilvie et al (2011) and Boulenc et al (2012) may reflect the use of higher PPI concentrations (> 30 µM) and metabolism by high $K_m$ P450s (e.g., CYP3A4) giving rise to CYP2C19 inhibition also.

Overall, the data presented indicate that lansoprazole is the PPI that serves as the most potent reversible CYP2C19 inhibitor in vitro. As such, one would anticipate drug interactions with known CYP2C19 substrates. That is not the case, however, because lansoprazole interactions with drugs such as diazepam and phenytoin are relatively minimal (Lefebvre et al., 1992; Ogawa and Echizen, 2010). On the other hand, esomeprazole and omeprazole are weaker reversible inhibitors of CYP2C19 in vitro, but their effect on the pharmacokinetics of diazepam and phenytoin is relatively greater. For example, the AUC of phenytoin is increased 25%, 20%, <1%, 3%, and <1%, respectively, by omeprazole, esomeprazole, dexlansoprazole, lansoprazole and
pantoprazole. Likewise, the AUC of diazepam is increased 28%, 81%, 6%, 12%, and <1%, respectively (Andersson et al., 1990; Ishizaki et al., 1995; Andersson et al., 2001; Ogawa and Echizen, 2010). Such clinical data are in agreement with the hepatocyte data described herein (Table 3). Given these differences, an attempt was made to rationalize clinical findings based on in vitro-derived inhibition parameters.

It is accepted that integration of in vitro P450 inhibition data with in vivo data is difficult and one has to consider multiple factors in any modeling exercise (Vuppugalla et al., 2010). Towards this end, inhibition data were obtained using three different model systems. In addition, PPI plasma protein binding was considered when determining % inhibition\textsubscript{predicted}, as were PPI plasma C\textsubscript{max} and C\textsubscript{max,portal} values. Finally, the pharmacokinetic profile of each PPI was considered also (see Materials and Methods). Notably, inhibitory metabolites, possible interactions between (R)- and (S)-forms of each racemic PPI, and accumulation in hepatocytes were not considered (Li et al., 2004, 2005; Ogilvie et al., 2011; Jones et al., 2004).

As summarized in Figure 4, use of diazepam in the modeling exercise was useful, because it is a well established CYP2C19 probe and all six PPIs have been studied clinically as perpetrators. In addition, the degree of inhibition varies across the series of compounds, ranging from 79% (esomeprazole) and 46% (omeprazole) to <1% (pantoprazole and rabeprazole). With such a range it was possible to leverage in vitro inhibition data and compare the seven different modeling approaches in an attempt to differentiate the PPIs and predict % inhibition in vivo. All seven methods presented esomeprazole as the most potent CYP2C19 inhibitor, whereas rabeprazole was predicted to be the weakest inhibitor. Furthermore, it was possible to differentiate esomeprazole
from omeprazole, especially when $f_u$-corrected static PPI concentrations or when time-dependent changes in total PPI concentration were considered (Fig. 4). The same two methods also correctly predicted that both lansoprazole and dexlansoprazole are weaker inhibitors versus esomeprazole and omeprazole. Of the PPIs studied, pantoprazole was the most problematic. The compound is known to be a weak inhibitor of CYP2C19 in vivo (< 1%), even when plasma $C_{\text{max}}$ is high (57 µM) following an IV dose of 240 mg (Gugler et al., 1996). Only the semi-mechanistic model, with correction for $f_u$, rendered a % inhibition predicted value that fell within the 90% CI reported in vivo (Fig. 4). Typically, pantoprazole is dosed orally (40 mg) and the $C_{\text{max,portal}}$ (~7 µM) is calculated to be well below the $C_{\text{max}}$ reported by Gugler et al., (1996). When pantoprazole is added to serum co-incubated hepatocytes, at such a lower concentration, the degree of inhibition ($\leq 5\%$) is more consistent with in vivo data (Table 3). It should be noted that, as reported by other authors (Ogilvie et al., 2011; Li et al., 2004), pantoprazole was shown to be a relatively weak inhibitor of HLM-catalyzed (S)-mephenytoin 4’-hydroxylase activity in our hands (Table 2). At least for pantoprazole, this implies that a rCYP2C19-derived $K_{i,u}$ is problematic and renders overestimates of % inhibition in vivo.

In conclusion, the results of the present study have shown that six PPIs are not potent reversible or metabolism-dependent inhibitors of P450s such as CYP2D6, 2C8, 2C9, 1A2, 2B6, and 3A4 in vitro. Of the P450s tested, the lowest IC$_{50}$ were obtained with CYP2C19. In this regard, lansoprazole was the most potent inhibitor, whereas pantoprazole and rabeprazole were the weakest. Moreover, it was confirmed that two of the six PPIs (esomeprazole and omeprazole) are time-dependent inhibitors of CYP2C19. Despite the CYP2C19 inhibition potency rank in vitro, when one considers plasma
protein binding and exposure following a dose, the integrated data set predicts that pantoprazole and rabeprazole will cause relatively minimal inhibition of CYP2C19. In contrast, it is anticipated that esomeprazole will exhibit the greatest inhibition of CYP2C19, more so than omeprazole, dexlansoprazole and lansoprazole. When it comes to the inhibition of drug-metabolizing P450s, therefore, it may be possible to differentiate the various PPIs in terms of their ability to inhibit CYP2C19 and conclude that drug interactions involving the inhibition of this particular P450 are not a class effect (Shah et al., 2012; Angiolillo et al., 2011).
Acknowledgements

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Authorship contributions:

Participated in study design: Zvyaga, Chang, Chen, and Rodrigues.

Conducted experiments: Zvyaga, Chen, Thorndike, Hurley, and Wagner.

Performed data analysis: Zvyaga, Vuppugalla, Chimalakonda, Chang, Yang, and Rodrigues.

Wrote or contributed to the writing of the manuscript: Zvyaga, Yang, Chimalakonda, Vuppugalla, Chen, and Rodrigues.
References


Karam WG, Goldstein JA, Lasker JM, and Ghanayem BI (1996) Human CYP2C19 is a major omeprazole 5-hydroxylase, as demonstrated with recombinant cytochrome P450 enzymes. *Drug Metab Dispos* **24**:1081-1087.


Figure legends

Figure 1. Omeprazole and esomeprazole as metabolism-dependent inhibitors of CYP2C19 in vitro

Omeprazole and esomeprazole were assessed as metabolism-dependent inhibitors of HLM- and rCYP2C19-catalyzed (S)-mephenytoin 4’-hydroxylase activity, as well as rCYP2C19-catalyzed diazepam N-demethylase activity (see Materials and Methods). Each data point represents the mean ± SD of n = 3 - 5 determinations. Inhibition parameters $k_{\text{inact}}$ and $K_I$ (mean ± SE of the parameter estimate) were determined for omeprazole with (A) HLM-catalyzed (S)-mephenytoin 4’-hydroxylase activity, (B) rCYP2C19-catalyzed (S)-mephenytoin 4’-hydroxylase activity, (C) rCYP2C19-catalyzed diazepam N-demethylase activity; and for esomeprazole with (D) HLM-catalyzed (S)-mephenytoin 4’-hydroxylase activity, (E) rCYP2C19-catalyzed (S)-mephenytoin 4’-hydroxylase activity, (F) rCYP2C19-catalyzed diazepam N-demethylase activity.

Figure 2. Predicting the impact of different PPIs on the pharmacokinetics of diazepam (employing total PPI plasma concentration in the modeling exercise)

For each PPI, in vitro inhibition data (employing rCYP2C19 with diazepam as substrate) were used in conjunction with its modeled pharmacokinetic profile (total plasma concentration versus time) to predict the effect of PPI on the AUC of diazepam. In each case, the diazepam AUC ratio (PPI versus placebo) is shown (predicted versus observed). The data points (symbols) are actual clinical data from the specific PPI-diazepam drug
interaction study reported in the literature. The lines are model-derived diazepam concentration versus time plots (see Materials and Methods and Supplement).

**Figure 3. Predicting the impact of different PPIs on the pharmacokinetics of diazepam (employing free PPI plasma concentration in the modeling exercise)**

For each PPI, in vitro inhibition data (employing rCYP2C19 with diazepam as substrate) were used in conjunction with its modeled pharmacokinetic profile (free plasma concentration versus time) to predict the effect of PPI on the AUC of diazepam (see Materials and Methods). In each case, the diazepam AUC ratio (PPI versus placebo) is shown (predicted versus observed). The data points (symbols) are actual clinical data from the specific PPI-diazepam drug interaction study reported in the literature. The lines are model-derived diazepam concentration versus time plots (see Materials and Methods and Supplement).

**Figure 4. Comparison of seven approaches to predict the inhibition of CYP2C19-catalyzed diazepam metabolism by six different PPIs**

Inhibition of diazepam metabolism was estimated for omeprazole (A), esomeprazole (B), lansoprazole (C), dexlansoprazole (D), pantoprazole (E), and rabeprazole (F), using in vitro-derived inhibition parameters (rCYP2C19 diazepam N-demethylase). Estimates were based on plasma PPI $C_{\text{max}}$, $C_{\text{max,portal}}$, $C_{\text{max,u}}$, $C_{\text{max,portal,u}}$ (Table 4 and 5), the use of human primary hepatocytes co-incubated with human serum (Table 3), and a semi-mechanistic model employing total or free PPI concentration (Fig. 2 and 3). For each PPI, the solid horizontal line indicates the % inhibition observed in vivo (90% CI shown.
as dotted lines) based on the reported AUC/AUCₜ ratio for diazepam (see Materials and Methods).
Table 1

Evaluation of various PPIs as inhibitors of P450s in HLM

<table>
<thead>
<tr>
<th>PPI</th>
<th>IC₅₀ (μM)</th>
<th>IC₅₀(t) (μM)</th>
<th>IC₅₀ (μM)</th>
<th>IC₅₀(t) (μM)</th>
<th>IC₅₀ (μM)</th>
<th>IC₅₀(t) (μM)</th>
<th>IC₅₀ (μM)</th>
<th>IC₅₀(t) (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CYP1A2</td>
<td>CYP2B6</td>
<td>CYP2C8</td>
<td>CYP2C9</td>
<td>CYP2D6</td>
<td>CYP3A4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omeprazole</td>
<td>&gt; 40 (48 ± 2)</td>
<td>&gt; 40</td>
<td>&gt; 40 (27 ± 5)</td>
<td>&gt; 40</td>
<td>&gt; 40 (27 ± 4)</td>
<td>&gt; 40</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.6 ± 4.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esomeprazole</td>
<td>&gt; 40 (14 ± 7)</td>
<td>&gt; 40</td>
<td>31.0 ± 9.0</td>
<td>&gt; 40</td>
<td>&gt; 40 (45 ± 7)</td>
<td>&gt; 40</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 (22 ± 6)</td>
<td>&gt; 40 (15 ± 4)</td>
<td>31.9 ± 9.1</td>
<td>&gt; 40 (19 ± 5)</td>
<td>20.9 ± 4.8</td>
<td>&gt; 40 (19 ± 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lansoprazole</td>
<td>8.1 ± 2.0</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
<td>&gt; 40 (16 ± 2)</td>
<td>&gt; 40</td>
<td>&gt; 40 (16 ± 2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18.5 ± 1.9</td>
<td>&gt; 40 (13 ± 7)</td>
<td>&gt; 40 (26 ± 14)</td>
<td>&gt; 40 (24 ± 6)</td>
<td>&gt; 40 (11 ± 6)</td>
<td>&gt; 40 (24 ± 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEXLansoprazole</td>
<td>7.1 ± 1.4</td>
<td>&gt; 40</td>
<td>&gt; 40 (17 ± 6)</td>
<td>&gt; 40 (19 ± 5)</td>
<td>&gt; 40</td>
<td>&gt; 40 (19 ± 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.0 ± 0.8</td>
<td>&gt; 40 (14 ± 5)</td>
<td>&gt; 40 (28 ± 9)</td>
<td>&gt; 40 (31 ± 4)</td>
<td>&gt; 40</td>
<td>&gt; 40 (31 ± 4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pantoprazole</td>
<td>&gt; 40 (37 ± 2)</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>31.8 ± 2.1</td>
<td>&gt; 40</td>
<td>&gt; 40 (12 ± 10)</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabeprazole</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
<td>&gt; 40 (27 ± 3)</td>
<td>&gt; 40</td>
<td>&gt; 40 (32 ± 4)</td>
<td>&gt; 40</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18.1 ± 4.8</td>
<td>&gt; 40 (48 ± 4)</td>
<td>13.9 ± 2.0</td>
<td>&gt; 40 (27 ± 7)</td>
<td>28.5 ± 1.1</td>
<td>&gt; 40 (27 ± 7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Legend to Table 1:

$^{a}$ IC$_{50}$, concentration of inhibitor required to decrease activity by 50% (not corrected for f$_{inc}$); IC$_{50(t)}$, concentration of inhibitor required to decrease activity by 50% after a preincubation time (t). In this instance, t = 30 minutes. Data represent mean ± standard deviation of four different experiments performed on different days. Data in parentheses represent percent inhibition at the highest concentration tested (40 μM); when not reported the percent inhibition was less than 10%.
<table>
<thead>
<tr>
<th>PPI</th>
<th>IC₅₀ (μM) for each substrateᵃ</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(S)-Mephenytoinᵇ</td>
<td>(S)-Mephenytoinᶜ</td>
<td>CECᶜ</td>
<td>Diazepamᶜ</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>IC₅₀ 7.4 ± 1.1</td>
<td>12.5 ± 0.4</td>
<td>1.2 ± 0.2</td>
<td>7.9 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>IC₅₀(t) 3.0 ± 0.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Esomeprazole</td>
<td>IC₅₀ 3.7 ± 0.5</td>
<td>1.3 ± 0.3</td>
<td>0.44 ± 0.09</td>
<td>4.6 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>IC₅₀(t) 0.76 ± 0.05</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lansoprazole</td>
<td>IC₅₀ 0.73 ± 0.13</td>
<td>1.1 ± 0.2</td>
<td>0.41 ± 0.04</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>IC₅₀(t) 1.0 ± 0.07</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dexlansoprazole</td>
<td>IC₅₀ 6.0 ± 1.0</td>
<td>6.2 ± 0.7</td>
<td>2.2 ± 0.5</td>
<td>13 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>IC₅₀(t) 5.7 ± 0.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pantoprazole</td>
<td>IC₅₀ &gt; 40ᵈ</td>
<td>13.4 ± 2.0</td>
<td>4.1 ± 0.6</td>
<td>7.0 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>IC₅₀(t) &gt; 40ᵈ</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rabeprazole</td>
<td>IC₅₀ 25 ± 4.1</td>
<td>11.4 ± 1.3</td>
<td>4.2 ± 1.3</td>
<td>&gt; 40ᵈ</td>
</tr>
<tr>
<td></td>
<td>IC₅₀(t) 21 ± 0.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Legend to Table 2:

a IC₅₀, concentration of inhibitor required to decrease activity by 50% (not corrected for f unin) ; IC₅₀(t), concentration of inhibitor required to decrease activity by 50% after a preincubation time (t). In this instance, t = 30 minutes. Data represent mean ± standard deviation of four different experiments performed on different days.

b Data obtained with HLM as the enzyme source.

c Data obtained with rCYP2C19. IC₅₀(t) was not determined.

d IC₅₀ was greater than the highest tested concentration of PPI (40 μM); % inhibition observed at 40 μM was less than 10%.
Table 3

Various PPIs as inhibitors of diazepam N-demethylation in the presence of human primary hepatocytes co-incubated with human serum

<table>
<thead>
<tr>
<th>PPI (Final Concentration)(^a)</th>
<th>% Inhibition(^b)</th>
<th>% Inhibition(_{\text{in vivo}}) (90% CI)(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hepatocyte preparation 1 (n = 1 organ donor)</td>
<td>Hepatocyte preparation 2 (pool of n = 20 organ donors)</td>
</tr>
<tr>
<td>Omeprazole (2.5 μM)</td>
<td>30.2 ± 5.4</td>
<td>23.2 ± 5.2</td>
</tr>
<tr>
<td>Esomeprazole (18.7 μM)</td>
<td>60.1 ± 1.4</td>
<td>23.6 ± 4.4</td>
</tr>
<tr>
<td>Lansoprazole (2.9 μM)</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>Dexlansoprazole (2.9 μM)</td>
<td>8.0 ± 3.8</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>Pantoprazole (6.7 μM)</td>
<td>&lt; 2</td>
<td>4.9 ± 2.8</td>
</tr>
<tr>
<td>Rabeprazole (1.4 μM)</td>
<td>13.5 ± 6.4</td>
<td>2.8 ± 2.9</td>
</tr>
</tbody>
</table>
**Legend to Table 3:**

a Each PPI was added at a single concentration, based on its estimated C\text{max, portal} (see *Materials and Methods*). For dexlansoprazole, it was assumed that its C\text{max, portal} is identical to that of lansoprazole; assumed an oral dose of 20, 30, 30, 40 and 40 mg for omeprazole, esomeprazole, lansoprazole, pantoprazole and rabeprazole, respectively.

b Data are reported as % inhibition, relative to a DMSO alone control, and represent mean ± SD of triplicate determinations. In the presence of DMSO alone, the rate of N\text{-desmethyl-diazepam formation was 28} ± 0.4 pmol/min per 10^6 cells (hepatocyte preparation 1) and 15 ± 0.7 pmol/min per 10^6 cells (hepatocyte preparation 2). The final diazepam concentration was 1 μM (preparation 1) and 2 μM (preparation 2); close to the estimated C\text{max, portal} for diazepam (data not shown). Hepatocyte preparation 1 exhibited higher CYP2C19 activity ((S)-mephenytoin 4′-hydroxylase = 95 pmol/min per 10^6 cells vs 15 pmol/min per 10^6 cells).

c % Inhibition\text{in vivo} was calculated based on the reported effect of each PPI on the AUC of diazepam (see *Materials and Methods*; equations 17 and 18).

d % Inhibition\text{in vivo} was calculated based on the AUC/AUC\text{c} ratio reported by Ishizaki et al (1995).

e % Inhibition\text{in vivo} was calculated based on the AUC/AUC\text{c} ratio reported by Andersson et al (1990).
### Table 4
Comparison of lansoprazole, dexlansoprazole, pantoprazole and rabeprazole as CYP2C19 inhibitors

<table>
<thead>
<tr>
<th>PPI</th>
<th>Substrate</th>
<th>$K_{i,u}$ (μM)$^a$</th>
<th>% Inhibitionpredicted $^b$</th>
<th>% Inhibitionin vivo (90% CI)$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$C_{\text{max}}$</td>
<td>$C_{\text{max,u}}$</td>
<td>$C_{\text{max,portal}}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lansoprazole</td>
<td>Diazepam</td>
<td>1.2</td>
<td>74</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>(S)-Mephenytoin</td>
<td>0.6</td>
<td>86</td>
<td>16</td>
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<tr>
<td></td>
<td>CEC</td>
<td>0.2</td>
<td>94</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>(S)-Mephenytoin (HLM)</td>
<td>0.3</td>
<td>91</td>
<td>23</td>
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<tr>
<td>Dexlansoprazole</td>
<td>Diazepam</td>
<td>6.1</td>
<td>38</td>
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<td>(S)-Mephenytoin</td>
<td>3.0</td>
<td>56</td>
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<td>CEC</td>
<td>1.1</td>
<td>78</td>
<td>7</td>
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<td>(S)-Mephenytoin (HLM)</td>
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<td>59</td>
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<td>Pantoprazole</td>
<td>Diazepam</td>
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<td>(S)-Mephenytoin</td>
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<td>90</td>
<td>15</td>
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<td></td>
<td>CEC</td>
<td>2.1</td>
<td>96</td>
<td>35</td>
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<td></td>
<td>(S)-Mephenytoin (HLM)</td>
<td>&gt; 20</td>
<td>&lt; 74</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Rabeprazole</td>
<td>Diazepam</td>
<td>&gt; 20</td>
<td>&lt; 2</td>
<td>&lt; 1</td>
</tr>
<tr>
<td></td>
<td>(S)-Mephenytoin</td>
<td>5.7</td>
<td>7</td>
<td>&lt; 1</td>
</tr>
<tr>
<td></td>
<td>CEC</td>
<td>2.1</td>
<td>18</td>
<td>&lt; 1</td>
</tr>
<tr>
<td></td>
<td>(S)-Mephenytoin (HLM)</td>
<td>12</td>
<td>1</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>
Legend to Table 4:

a Unless otherwise indicated, the $K_{i,u}$ is the IC$_{50}$ with rCYP2C19 (Table 2) divided by 2 (not corrected for $f_{u,inc}$ since binding to rCYP2C19 was negligible: $f_{u,inc} > 0.97$). For (S)-mephenytoin with HLM only, the $K_{i,u}$ is the IC$_{50}$ obtained with HLM, corrected for $f_{u,inc}$ and then divided by 2. No time-dependent effect was observed after preincubation of these PPIs with HLM, so only reversible inhibition was considered.

b % Inhibition$_{predicted}$ was determined as described in Materials and Methods. $C_{max}$ ($C_{max,u}$) values for each PPI were based on those reported for a specific diazepam drug interaction; 3.4 (0.11) µM following 60 mg oral lansoprazole, 4.0 (0.08) µM following 90 mg oral dexamethasone, 57 (1.1) µM following 240 mg IV pantoprazole, and 0.45 (0.02) µM following 20 mg oral rabeprazole (see supplement Table S2). $C_{max,portal}$ values were 4.8 µM (lansoprazole), 57 µM (pantoprazole), and 0.8 µM (rabeprazole). Corresponding $C_{max,portal,u}$ values were 0.14 µM (lansoprazole), 1.1 µM (pantoprazole), and 0.03 µM (rabeprazole).

c % Inhibition$_{in vivo}$ was calculated based on the reported effect of each PPI on the AUC of diazepam (see Materials and Methods; equations 17 and 18).

d No clinical IV data available in the literature for dexamethasone, so it was not possible to estimate $C_{max,portal}$. 
## Table 5
Omeprazole and esomeprazole as CYP2C19 inhibitors

<table>
<thead>
<tr>
<th>PPI</th>
<th>Substrate</th>
<th>Parameter(s)</th>
<th>% Inhibition\textsubscript{predicted}\textsuperscript{b}</th>
<th>% Inhibition\textsubscript{in vivo} (90% CI)\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omeprazole</td>
<td></td>
<td></td>
<td>(C_{\text{max}})</td>
<td>(C_{\text{max,u}})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(K_{\text{iu}}) (μM)</td>
<td>Reversible</td>
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</tr>
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<td>Diazepam</td>
<td></td>
<td>4.0</td>
<td>7</td>
<td>&lt;1</td>
</tr>
<tr>
<td>(S)-Mephenytoin</td>
<td>6.3</td>
<td>5</td>
<td>&lt;1</td>
<td>21</td>
</tr>
<tr>
<td>CEC</td>
<td></td>
<td>0.6</td>
<td>33</td>
<td>2</td>
</tr>
<tr>
<td>(S)-Mephenytoin (HLM)</td>
<td>3.3</td>
<td>8</td>
<td>&lt;1</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(K_{\text{iu}}) (μM), (k_{\text{max}}) (min\textsuperscript{-1})</td>
<td>3.4, 0.039</td>
<td>86</td>
</tr>
<tr>
<td>Diazepam</td>
<td></td>
<td>2.30, 0.048</td>
<td>92</td>
<td>38</td>
</tr>
<tr>
<td>(S)-Mephenytoin (HLM)</td>
<td>0.84, 0.030</td>
<td>94</td>
<td>51</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(K_{\text{iu}}) (μM), (k_{\text{max}}) (min\textsuperscript{-1}), (K_{\text{iu}}) (μM)</td>
<td>3.4, 0.039, 4.0</td>
<td>87</td>
</tr>
<tr>
<td>(S)-Mephenytoin (HLM)</td>
<td>2.30, 0.048, 6.3</td>
<td>92</td>
<td>38</td>
<td>98</td>
</tr>
<tr>
<td>(S)-Mephenytoin (HLM)</td>
<td>0.84, 0.030, 3.3</td>
<td>95</td>
<td>51</td>
<td>98</td>
</tr>
<tr>
<td>Esomeprazole</td>
<td></td>
<td>(K_{\text{iu}}) (μM)</td>
<td>Reversible</td>
<td></td>
</tr>
<tr>
<td>Diazepam</td>
<td></td>
<td>2.3</td>
<td>69</td>
<td>6</td>
</tr>
<tr>
<td>(S)-Mephenytoin</td>
<td>0.6</td>
<td>89</td>
<td>19</td>
<td>96</td>
</tr>
<tr>
<td>CEC</td>
<td></td>
<td>0.3</td>
<td>96</td>
<td>42</td>
</tr>
<tr>
<td>(S)-Mephenytoin (HLM)</td>
<td>1.7</td>
<td>75</td>
<td>8</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(K_{\text{iu}}) (μM), (k_{\text{max}}) (min\textsuperscript{-1})</td>
<td>1.70, 0.076</td>
<td>99</td>
</tr>
<tr>
<td>(S)-Mephenytoin (HLM)</td>
<td>2.30, 0.049</td>
<td>99</td>
<td>96</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(K_{\text{iu}}) (μM), (k_{\text{max}}) (min\textsuperscript{-1}), (K_{\text{iu}}) (μM)</td>
<td>1.70, 0.076, 2.3</td>
<td>100</td>
</tr>
<tr>
<td>(S)-Mephenytoin (HLM)</td>
<td>2.30, 0.049, 6.6</td>
<td>100</td>
<td>97</td>
<td>100</td>
</tr>
<tr>
<td>(S)-Mephenytoin (HLM)</td>
<td>0.72, 0.049, 1.7</td>
<td>100</td>
<td>95</td>
<td>100</td>
</tr>
</tbody>
</table>
Legend to Table 5:

a Unless otherwise indicated, the $K_{i,u}$ is the IC$_{50}$ obtained with rCYP2C19 (Table 2) divided by 2 (not corrected for $f_{u,inc}$ since binding to rCYP2C19 was negligible: $f_{u,inc} > 0.97$). For (S)-mephenytoin with HLM only, the $K_{i,u}$ is the IC$_{50}$ obtained with HLM, corrected for $f_{u,inc}$ and then divided by 2. Because time-dependent shifts in IC$_{50}$ were noted, the HLM- and rCYP2C19-derived parameters ($k_{inact}$ and $K_I$) were used to estimate the contribution of metabolism-dependent inhibition. $K_I$ was also corrected for $f_{u,inc}$.

b % Inhibition$_{predicted}$ was determined as described in Materials and Methods. C$_{max}$ (C$_{max,u}$) values for each PPI were based on those reported for a specific diazepam drug interaction; 0.3 (0.02) µM following 20 mg oral omeprazole, and 5.2 (0.16) µM following 30 mg oral esomeprazole (see supplement Table S2). C$_{max,portal}$ values were 1.7 µM (omeprazole) and 15.5 µM (esomeprazole). Corresponding C$_{max,portal,u}$ values were 0.1 µM (omeprazole) and 0.47 µM (esomeprazole).

c % Inhibition$_{in vivo}$ was calculated based on the reported effect of each PPI on the AUC of diazepam (see Materials and Methods; equations 17 and 18).

d % Inhibition$_{in vivo}$ was calculated based on the AUC$_i$/AUC$_c$ ratio reported by Ishizaki et al (1995).

e % Inhibition$_{in vivo}$ was calculated based on the AUC$_i$/AUC$_c$ ratio reported by Andersson et al (1990).
Figure 1

(A) (S)-Mephenytoin 4'-Hydroxylase (HLM)

\[ K_i = 1.1 \pm 0.23 \, \mu M \]
\[ k_{inact} = 0.030 \pm 0.002 \, \text{min}^{-1} \]

(B) (S)-Mephenytoin 4'-Hydroxylase (rCYP2C19)

\[ K_i = 2.6 \pm 0.60 \, \mu M \]
\[ k_{inact} = 0.048 \pm 0.003 \, \text{min}^{-1} \]

(C) Diazepam N-Demethylase (rCYP2C19)

\[ K_i = 3.8 \pm 1.1 \, \mu M \]
\[ k_{inact} = 0.039 \pm 0.004 \, \text{min}^{-1} \]

(D) (S)-Mephenytoin 4'-Hydroxylase (HLM)

\[ K_i = 0.87 \pm 0.08 \, \mu M \]
\[ k_{inact} = 0.049 \pm 0.001 \, \text{min}^{-1} \]

(E) (S)-Mephenytoin 4'-Hydroxylase (rCYP2C19)

\[ K_i = 2.4 \pm 0.32 \, \mu M \]
\[ k_{inact} = 0.204 \pm 0.008 \, \text{min}^{-1} \]

(F) Diazepam N-Demethylase (rCYP2C19)

\[ K_i = 1.8 \pm 0.18 \, \mu M \]
\[ k_{inact} = 0.076 \pm 0.0021 \, \text{min}^{-1} \]
Figure 2

- **Omeprazole**
  - AUC Ratio<sub>observed</sub> (90% CI) = 1.4 (1.2, 1.5)
  - AUC Ratio<sub>predicted</sub> = 1.2

- **Esomeprazole**
  - AUC Ratio<sub>observed</sub> (90% CI) = 1.8 (1.4, 2.3)
  - AUC Ratio<sub>predicted</sub> = 1.4

- **Pantoprazole**
  - AUC Ratio<sub>observed</sub> (90% CI) = 1.0 (0.9, 1.1)
  - AUC Ratio<sub>predicted</sub> = 1.2

- **Rabeprazole**
  - AUC Ratio<sub>observed</sub> (90% CI) = 0.9 (0.8, 1.0)
  - AUC Ratio<sub>predicted</sub> = 1.0

- **Lansoprazole**
  - AUC Ratio<sub>observed</sub> (90% CI) = 1.1 (1.0, 1.1)
  - AUC Ratio<sub>predicted</sub> = 1.1

- **Dexlansoprazole**
  - AUC Ratio<sub>observed</sub> (90% CI) = 1.1 (1.0, 1.2)
  - AUC Ratio<sub>predicted</sub> = 1.1
Figure 3

- **Omeprazole**
  - AUC Ratio\textsuperscript{observed} (90% CI) = 1.4 (1.2, 1.5)
  - AUC Ratio\textsuperscript{predicted} = 1.0

- **Esomeprazole**
  - AUC Ratio\textsuperscript{observed} (90% CI) = 1.8 (1.4, 2.3)
  - AUC Ratio\textsuperscript{predicted} = 1.1

- **Pantoprazole**
  - AUC Ratio\textsuperscript{observed} (90% CI) = 1.0 (0.9, 1.1)
  - AUC Ratio\textsuperscript{predicted} = 1.1

- **Rabeprazole**
  - AUC Ratio\textsuperscript{observed} (90% CI) = 0.9 (0.8, 1.0)
  - AUC Ratio\textsuperscript{predicted} = 1.0

- **Lansoprazole**
  - AUC Ratio\textsuperscript{observed} (90% CI) = 1.1 (1.0, 1.1)
  - AUC Ratio\textsuperscript{predicted} = 1.0

- **Dexlansoprazole**
  - AUC Ratio\textsuperscript{observed} (90% CI) = 1.1 (1.0, 1.2)
  - AUC Ratio\textsuperscript{predicted} = 1.0

---

Diazepam Plasma Conc (nM)
Figure 4A

Esomeprazole

<table>
<thead>
<tr>
<th>% Inhibition (Diazepam)</th>
<th>Cmax</th>
<th>Cmax,portal</th>
<th>Cmax</th>
<th>Cmax,portal</th>
<th>Hepatocytes</th>
<th>Hepatocytes</th>
<th>Semi-Mech Model</th>
<th>Semi-Mech Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Total)</td>
<td>100%</td>
<td>100%</td>
<td>93%</td>
<td>98%</td>
<td>60%</td>
<td>24%</td>
<td>50%</td>
<td>&lt;1</td>
</tr>
<tr>
<td>(Free)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4B

Omeprazole

<table>
<thead>
<tr>
<th>% Inhibition (Diazepam)</th>
<th>Cmax</th>
<th>Cmax,portal</th>
<th>Cmax</th>
<th>Cmax,portal</th>
<th>Hepatocytes</th>
<th>Hepatocytes</th>
<th>Semi-Mech Model</th>
<th>Semi-Mech Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Total)</td>
<td>87%</td>
<td>97%</td>
<td>26%</td>
<td>66%</td>
<td>30%</td>
<td>23%</td>
<td>29%</td>
<td>&lt;1</td>
</tr>
<tr>
<td>(Free)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
C

Lansoprazole

![Graph showing inhibition of dialepam for different compounds]

Dexlansoprazole

![Graph showing inhibition of dialepam for different compounds]

Figure 4C

Figure 4D
Figure 4E

Pantoprazole

Figure 4F

Rabeprazole