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


Received March 7, 2012; accepted May 30, 2012

ABSTRACT:

Six proton pump inhibitors (PPIs), omeprazole, lansoprazole, esomprazole, 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, IC50 values were greater than 40 μM, except for dexlansoprazole and lansoprazole with CYP1A2 (IC50 = −8 μM) and esomeprazole with CYP2C8 (IC50 = 31 μM). With the exception of CYP2C19 inhibition by omeprazole and esomeprazole (IC50 ratio, 2.5 to 5.9), there was no evidence for a marked time-dependent shift in IC50 (IC50 ratio, ≤2) after a 30-min preincubation with NADPH. In the absence of preincubation, lansoprazole (IC50 = 0.73 μM) and esomeprazole (IC50 = 3.7 μM) were the most potent CYP2C19 inhibitors, followed by dexlansoprazole and omeprazole (IC50 = −7.0 μM). Rabeprazole and pantoprazole (IC50 = ≥25 μM) were the weakest. A similar ranking was obtained with recombinant CYP2C19. Despite the IC50 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 after the incubation of the individual PPIs with human primary hepatocytes (in the presence of human serum) and by monitoring their impact on diazepam N-demethylation activity at a low concentration of diazepam (2 μM). Data described herein are consistent with reports that PPIs are mostly weak inhibitors of cytochromes P450 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 such as 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 also used to treat Helicobacter pylori infections (Blume et al., 2006; Shi and Klotz, 2008).

Since the introduction of omeprazole (5-methoxy-2-[[4-methoxy-3,5-dimethyl-2-pyridinyl]methyl][sulfynil]-1H-benzimidazol-2-yl)-5-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl][sulfynil]-1H-benzimidazole), pantoprazole (5-(difluoromethyl)-2-[[3,4-dimethoxy-pyridin-2-yl]methyl][sulfynil]benzimidazol-1-ide), and rabeprazole (2-[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl][sulfynil]benzimidazol-1-ide), and now also includes the (S)-enantiomer of omeprazole (esomeprazole; (S)-5-methoxy-2-[[4-methoxy-3,5-dimethylpyridin-2-yl][sulfynil]-1H-benzimidazol-2-yl)-5-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl][sulfynil]-1H-benzimidazole) and the (R)-enantiomer of lansoprazole (dexlansoprazole; (R)-2-[[3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl][sulfynil]-1H-benzimidazole) (Andersson et al., 2001; Shi and Klotz, 2008; Vakily et al., 2009). 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 cytochromes P450 (P450s), and that...
CYP2C19 phenotype substantially influences pharmacokinetics, pharmacodynamics, and clinical outcomes (e.g., speed and degree of gastric acid suppression) (Li et al., 2004; Baldwin et al., 2008; Hunfeld et al., 2008; Shi and Klotz, 2008). 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 (Andersson et al., 2001; Blume et al., 2006; Shi and Klotz, 2008; Vakili 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 who received 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 (Rassen et al., 2009; Zhang et al., 2009; Furuta et al., 2010; Liu and Jackevicius, 2010; Oyetayo and Talbert, 2010; Ray et al., 2010; Shmulevich et al., 2011; Ohbuchi et al., 2012; Shah et al., 2012). 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 Km process in vitro (Hagihara et al., 2009; Kazui et al., 2010), and CYP2C19 genotype is associated with antiplatelet 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 (PMs). 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 (VandenBranden et al., 1996; Ko et al., 1997; Li et al., 2004; Liu et al., 2005; Walsky et al., 2005, 2006). In fact, there are only two reports describing time-dependent inhibition, with a 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 (Venkatarkshnan 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 human liver microsomes (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) also behaved as time-dependent inhibitors. The latter result supports the findings of Ogilvie et al. (2011) and Boulenc et al. (2012), and the more recent findings of Ohbuchi et al. (2012) using 2-oxo-clopidogrel as substrate. Additional studies were conducted with recombinant CYP2C19 (rCYP2C19) using three different substrates [3-cyano-7-ethoxy-coumarin (CEC), (S)-mephentoin, and diazepam] and with serum-coincubated human primary hepatocytes using diazepam as substrate. Where appropriate, the determined IC50 was corrected for f u,inc, to generate IC50 (predicted) and support a comparison of HLM with rCYP2C19. Finally, the in vitro CYP2C19 inhibition parameters (K i,u, K i,thiol, and K i,thiol) were used to predict % inhibition in vivo (% inhibitionpredicted) based on static (Cmax, Cmax, Cmax,portal, and Cmax,portal) and dynamic (time-dependent) concentrations of each PPI. The approach enabled assessment of CYP2C19 inhibition for various substrates (irrespective of f u,inc) and the comparison of % inhibitionpredicted versus % inhibition observed in vivo (% inhibitionin vivo).

Materials and Methods

Materials. Omeprazole, esomeprazole ([S]-isomer of omeprazole), lansoprazole, phenacetin, N-desmethyl-diazepam, diazepam, and [3H]-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, [3H]-hydroxy-bupropion, [3H]-desethyl-amodiaquine, [3H]-acetaminophen, and (S)-mephentoin were purchased from Toronto Research Chemicals Inc. (North York, Ontario, Canada). Pooled HLM (150 different organ donors), and Supersomones containing rCYP2C19 (coexpressed with P450 3A4) were purchased from BDU Biosciences (Woburn, MA). CES, [3H]-hydroxy-diclofenac, [3H]-hydroxy-mephenytoin, [3H]-dextrophan, and [3H]-hydroxy-midazolam were also obtained from BDU Biosciences (Woburn, MA). Algimatrix firming buffer and 24-well plates (Algimatrix 3D culture system) were purchased from Invitrogen (Carlsbad, CA). Two preparations of cryopreserved human primary hepatocytes were obtained from Celsis In Vitro Technologies (Baltimore, MD). 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]-mephentoin 4'-hydroxylase = 95 pmol/min per 10 6 cells). The second was a pool of 20 different organ donors with medium CYP2C19 activity ([S]-mephentoin 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, 1-glutamine (200 mM), and penicillin/streptomycin (10,000 IU/ml, 10,000 μg/ml)] was ordered from Invitrogen. Before 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 used pooled HLM and well established substrates (final substrate concentration ~ Km) that produce P450 isoform-selective metabolites (Walsky and Obach, 2004). Each assay was performed in a time-dependent format to assess both reversible IC50 (time (metabolism)-dependent shifts in IC50, test compounds were preincubated at 37°C with HLM in the presence of NADPH (1 mM) 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: Genesys 150 (Tecan Group Ltd., Mannedorf, Switzerland), BenchCel System (Velocity 11 Inc., Menlo Park, CA), ECHO 550 (LabCye 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 dimethyl sulfoxide (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). See supplemental data for Sample Preparation, Assay, RapidFire-Mass Spectrometry (RF-MSMS), LC-MS/MS Analysis of CYP1A2 (HLM-Phenacetin) Reaction Samples, Inhibition Studies with rCYP2C19; CES O-deethylation, (S)-Mephentoin 4'-Hydroxylase, Diazepam N-Demethylation, and Determination of f u,inc.

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 (120g) for 3 min. 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 × 10 6 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 min.
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 × 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 Cmax,porta (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 (% μM) to each well. Therefore, the final concentrations of DMSO and serum in the reaction mixtures were 0.3 and 70% (v/v), respectively. The final concentration of diazepam (1–2 μM) approximated the calculated Cmax,porta after an intravenous (IV) dose (data not shown) and is below the KI reported for CYP2C19 (Yasumori et al., 1994). At the specific time points of the incubation time course (0, 2, 4, 7, 24, and 30 h), 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 min, centrifuged, and the supernatant (10 μl) subjected to liquid chromatography/tandem mass spectrometry analysis (see supplemental data). For the purposes of quantitation, a calibration curve of N-desmethyl-diazepam was prepared in human serum 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).

Data Analysis. Determination of IC50. The endpoint of the RapidFire-mass spectrometry readout for the assays using 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 endpoint readout for the CEC O-deethylation assay was the fluorescence intensity of the metabolite. For both the liquid chromatography/mass spectrometry-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 percentage of inhibition calculated as shown in eq. 1:

\[
\% \text{Inhibition} = \left(1 - \frac{S - B}{T - B}\right) \times 100
\]

where S = sample, T = average total, B = average background.

The results were then imported into custom curve fitting software, which uses MathIQ package (ID Business Solutions, Ltd., Guilford, England), to determine the IC50 values for each test compound. The IC50 was defined as the rate of reaction was calculated using the concentration of the substance (total signal, 0% inhibition), and the reactions performed in the absence of the test substance (total signal, 0% inhibition). The IC50 was corrected for the liver blood flow (1500 ml/min) and CYP2C19 (1.0 ml/min) in the liver. The IC50 was based on the calculated KI,u was obtained by simply dividing IC50,iv by 2 (assuming competitive inhibition; substrate concentration – KI,u) for each PPI. The IC50 and Cmax,porta were based on those reported for a specific diazepam drug interaction; 0.3 (0.02) μM after 24 h of oral omeprazole, 5.2 (0.16) μM after 30 mg of oral esomeprazole, 3.4 (0.11) μM after 60 mg of oral lansoprazole, 4.0 (0.08) μM after 90 mg of oral dexlansoprazole, 57 (1.1) μM after 240 mg of IV pantoprazole, and 45 (0.02) μM after 20 mg of oral rabeprazole (see Supplemental Table S2) (Andersen et al., 1999, 2001; Lefebvre et al., 1992; Ishizaki et al., 1995; Gugler et al., 1996; Vakily et al., 2009). Equation 6 yielded the following estimates for Cmax,porta: 1.7 μM (omeprazole), 15.5 μM (esomeprazole), 4.8 μM (lan- soprazole), 57 μM (pantoprazole), and 0.8 μM (rabeprazole). Corresponding Cmax,porta,u values were as follows: 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 (Using Time-Dependent Concentrations of Each PPI). Simulations were performed to predict the interaction of each PPI with diazepam using a semimechanistic compartment model (Supplemental Fig. S1). In brief, the observed plasma concentration-time data of each PPI, digitized from literature reports (Supplemental Table S2), were fitted using a one- or two-compartment model (vide infra). Likewise, the observed concentration-time profiles of diazepam without PPI, digitized from literature reports (Supplemental Table S2), were fitted using a two-compartment model (vide infra). The elimination of diazepam was assumed to occur via two pathways, with CYP2C19-mediated metabolism fixed at fau,2CV = 0.57 (vide infra, eq. 16). Subsequently, the interaction of each PPI with diazepam was simulated by incorporating in vitro inhibition parameters. The simulated profiles of diazepam after PPI adminis-
Modeling the pharmacokinetics of each PPI. In brief, the plasma concentration-time profile of each PPI after single-dose oral administration was fitted to a one- or two-compartment model (Supplemental Fig. S2), wherein $k_{a}$ and $k_{e}$ are the first-order absorption and elimination rate constants and $V_{c}$ and $Q$ are the distribution rate constants from the central and the peripheral compartments. In the 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 (eqs. 7, 8, and 9):

$$\frac{d(GI)}{dt} = \text{Input} - k_{a} \times GI \quad (7)$$

$$d(\text{PPI})dt = (k_{a} \times GI - k_{a} \times PPI - k_{e} \times PPI + k_{e} \times \text{Peripheral}_{\text{PPI}})/\text{Vol} \quad (8)$$

$$d(\text{Peripheral}_{\text{PPI}})dt = k_{a} \times PPI - k_{e} \times \text{Peripheral}_{\text{PPI}} \quad (9)$$

wherein "GI," "PPI," and "Peripheral$_{\text{PPI}}$" 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 (Andersson et al., 1990, 2001; Lefebvre et al., 1992; Pue et al., 1993; Setoyama et al., 2005). In the case of rabeprazole, the absorption was modeled using a Weibull function, because a first-order process could not adequately capture the time course of absorption. For dexlansoprazole, two first-order absorption rate constants ($k_{a}$ and $k_{e}$) were used to model the absorption kinetics after administration of the modified release tablet (Vakily et al., 2009). Values for $k_{a}$ and $k_{e}$ 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 eqs. 10 to 12 (Supplemental 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 $k_{a}$ (3.0 h$^{-1}$) 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 $f_{m,2C19} = 0.57$ (see below; eq. 16). The clearance of diazepam via the CYP2C19-mediated component was modeled using a well stirred model equation (eq. 12):

$$d(\text{Diazepam})/dt = (-CL_{1} + CL_{2}) \times \text{Diazepam} - k_{12} \times \text{Diazepam} \times V_{c}$$

$$+ k_{21} \times \text{Peripheral}_{\text{Diaz}})/V_{c} \quad (10)$$

$$d(\text{Peripheral}_{\text{Diaz}})dt = k_{a} \times \text{Diazepam} \times V_{c} \times k_{21} \times \text{Peripheral}_{\text{Diaz}} \quad (11)$$

$$CL_{1} = Q \times CL_{\text{un}} (Q + CL_{\text{un}}) \quad (12)$$

Wherein "Diazepam" is the plasma concentration of diazepam, “Peripheral$_{\text{Diaz}}$” is the amount of diazepam in the peripheral compartment, and $CL_{1}$ and $CL_{2}$ are the two clearance pathways for diazepam; with $CL_{i}$ assumed to be CYP2C19-mediated. $VC$ refers to the volume of the central compartment (300 ml/kg), and $k_{a}$ (0.3 h$^{-1}$) and $k_{e}$ (0.13 h$^{-1}$) 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 $CL_{\text{un}}$ is the intrinsic clearance of diazepam in man via the CYP2C19-mediated pathway. Based on data digitized from literature studies (Andersson et al., 1990, 2001), the pharmacokinetic profile of diazepam in the placebo leg of the study was fitted to the above equations to estimate $CL_{\text{un}}, CL_{2}, k_{12}, k_{21},$ and $V_{c}$ (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 with diazepam concentration-time profiles and area under the curve (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 Supplemental Fig. S1 and Table S2). In brief, using the semimechanistic compartment model, concentration-time profiles were simulated after an administration of diazepam as a short intravenous infusion (0.1 mg/kg) or a single oral dose (5 mg) after PPI dosing (Supplemental Fig. S1 and Table S2). The intrinsic clearance of diazepam in the presence of PPI (CL$_{\text{un}}'$) was assumed to be inhibited as a function of [I] as follows (eq. 13):

$$CL_{\text{un}}' = CL_{\text{un}} \times \delta \times \gamma \quad (13)$$

wherein $k_{deg}, k_{max}$ (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 CYP2C19 with diazepam as substrate. For PPIs that inhibit CYP2C19 only by a reversible mechanism (lansoprazole, pantoprazole, dexlansoprazole, and rabeprazole), $k_{deg}$, $K_{I,u}$, 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 versus placebo), and it was possible to determine % inhibitionpredicted for each PPI (eq. 14):

$$% \text{Inhibition}_{\text{predicted}} = (1 - FR_{\text{predicted}}) \times 100 \quad (14)$$

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

$$FR_{\text{predicted}} = \frac{\text{AUC}_{\text{predicted}}}{\text{AUC}_{\text{observed}}} \times f_{m,2C19} + 1 \quad (15)$$

Calculation of $f_{m,2C19}$ for Diazepam. The AUC of diazepam is increased 1.8- to 2.3-fold in CYP2C19 PM versus extensive metabolizer (EM) subjects, so the drug can serve as a CYP2C19 probe (Andersson et al., 1990; Ishizaki et al., 1995). Moreover, diazepam is a low clearance compound (0.29–0.46 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$_{\text{P450}}$/AUC$_{\text{EM}}$ ratio (eq. 16) (Ito et al., 2005). Based on the AUC$_{\text{P450}}$/AUC$_{\text{EM}}$ ratio of 2.3, $f_{m,2C19}$ for diazepam is calculated to be 0.57.

$$f_{m,2C19} = \frac{1}{\text{AUC}_{\text{P450}}/\text{AUC}_{\text{EM}}} \quad (16)$$

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$_{\text{vivo}}$ was then determined as follows (eq. 17):

$$% \text{Inhibition}_{\text{vivo}} = (1 - FR_{\text{vivo}}) \times 100 \quad (17)$$

In eq. 17, FR$_{\text{vivo}}$ was determined using eq. 18 ($f_{m,2C19} = 0.57$):

$$FR_{\text{vivo}} = \frac{1}{\text{AUC}_{\text{observed}}/\text{AUC}_{\text{observed}}} \times f_{m,2C19} + 1 \quad (18)$$

The diazepam AUC/AUC$_{c}$ ratio (observed with each PPI (AUC in the presence of PPI versus placebo) has been reported (90% confidence interval (CI)): 1.28 (1.19, 1.39; Ishizaki et al., 1995) and 1.36 (1.19, 1.53; Andersson et al., 1990) for omeprazole; 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 panto-
CYP2C9, CYP2C8, and CYP1A2 activity was observed. However, it inhibition with lansoprazole and esomeprazole was observed also with P450 activities in HLM by omeprazole, lansoprazole, esomeprazole, -deethylase activity (IC50 known clinically relevant plasma concentrations and differentiation of and (R). In this instance, t = 30 min.

**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–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 using three different substrates; omeprazole was less potent than esomeprazole (~2-fold with HLM and 1.7 to 9.6-fold with rCYP2C19), whereas lansoprazole was more potent than dexlansoprazole (~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 (Tables 1 and 2), involving a 30-min preincubation of the PPI with NADPH-fortified HLM, and the IC50 was compared with IC50 obtained without preincubation with NADPH. In most cases, a relatively minimal time-dependent shift in IC50 (IC50/IC50(t) ratio < 2.0) was observed in comparison to positive controls such as tiencilic acid (CYP2C9), paroxetine (CYP2D6), venlafaxine (CYP3A4), ticlopidine (CYP2C19,

**Table 1**

<table>
<thead>
<tr>
<th>PPI</th>
<th>CYP1A2 IC50 (μM)</th>
<th>CYP2B6 IC50 (μM)</th>
<th>CYP2C8 IC50 (μM)</th>
<th>CYP2C9 IC50 (μM)</th>
<th>CYP2D6 IC50 (μM)</th>
<th>CYP3A4 IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omeprazole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC50(t)</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>
</tr>
<tr>
<td>IC50</td>
<td>20.6 ± 4.2</td>
<td>&gt;40</td>
<td>&gt;40 (38 ± 4)</td>
<td>&gt;40</td>
<td>&gt;40 (40 ± 4)</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Lansoprazole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC50(t)</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>
</tr>
<tr>
<td>IC50</td>
<td>&gt;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>
</tr>
<tr>
<td>Pantoprazole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC50(t)</td>
<td>8.1 ± 2.0</td>
<td>&gt;40</td>
<td>&gt;40 (26 ± 14)</td>
<td>&gt;40 (16 ± 2)</td>
<td>&gt;40</td>
<td>&gt;40 (16 ± 2)</td>
</tr>
<tr>
<td>IC50</td>
<td>18.5 ± 1.9</td>
<td>&gt;40 (13 ± 7)</td>
<td>&gt;40 (24 ± 6)</td>
<td>&gt;40 (11 ± 6)</td>
<td>&gt;40 (24 ± 6)</td>
<td>&gt;40 (24 ± 6)</td>
</tr>
<tr>
<td>Rabeprazole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC50(t)</td>
<td>&gt;40 (37 ± 2)</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>
</tr>
<tr>
<td>IC50</td>
<td>31.8 ± 2.1</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>
</tr>
<tr>
<td>Dexlansoprazole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC50(t)</td>
<td>7.1 ± 1.4</td>
<td>&gt;40</td>
<td>&gt;40 (28 ± 9)</td>
<td>&gt;40 (31 ± 4)</td>
<td>&gt;40</td>
<td>&gt;40 (31 ± 4)</td>
</tr>
<tr>
<td>IC50</td>
<td>20.0 ± 0.8</td>
<td>&gt;40 (12 ± 10)</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Esomeprazole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC50(t)</td>
<td>&gt;40 (40 ± 4)</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>
</tr>
<tr>
<td>IC50</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>
</tr>
</tbody>
</table>

IC50 concentration of inhibitor required to decrease activity by 50% (not corrected for fAUC0-3h); IC50(t) concentration of inhibitor required to decrease activity by 50% after a preincubation time (t). In this instance, t = 30 min.

**Table 2**

<table>
<thead>
<tr>
<th>PPI</th>
<th>(S)-Mephenytoin IC50 (μM)</th>
<th>(R)-Mephenytoin IC50 (μM)</th>
<th>CEC IC50 (μM)</th>
<th>Diazepam IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omeprazole</td>
<td>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>IC50</td>
<td>3.0 ± 0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lansoprazole</td>
<td>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>IC50</td>
<td>0.76 ± 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pantoprazole</td>
<td>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>IC50</td>
<td>1.0 ± 0.07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabeprazole</td>
<td>&gt;40 (38)</td>
<td>2.2 ± 0.5</td>
<td>4.1 ± 0.6</td>
<td>7.0 ± 0.9</td>
</tr>
<tr>
<td>IC50</td>
<td>5.7 ± 0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dexlansoprazole</td>
<td>&gt;40 (38)</td>
<td>2.2 ± 0.5</td>
<td>4.1 ± 0.6</td>
<td>7.0 ± 0.9</td>
</tr>
<tr>
<td>IC50</td>
<td>25 ± 4.1</td>
<td>11.4 ± 1.3</td>
<td>4.2 ± 1.3</td>
<td>&gt;40 (38)</td>
</tr>
<tr>
<td>IC50(t)</td>
<td>21 ± 0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IC50 concentration of inhibitor required to decrease activity by 50% (not corrected for fAUC0-3h); IC50(t) concentration of inhibitor required to decrease activity by 50% after a preincubation time (t). In this instance, t = 30 min.

(1) Data obtained with HLM as the enzyme source.
(2) Data obtained with rCYP2C19. IC50 was not determined.
(3) IC50 was greater than the highest tested concentration of PPI (40 μM); the percentage of inhibition observed at 40 μM was less than 10%.
CYP2D6), and furafylline (CYP1A2) (see legend to Supplemental Table S1). However, a time-dependent shift in $IC_{50}$ was evident with omeprazole and CYP1A2 activity ($IC_{50} > 40 \, \mu M, \, IC_{50(0)} = 20.6 \, \mu M$); esomeprazole and CYP2D6 ($IC_{50} > 40 \, \mu M, \, IC_{50(0)} = 20.9 \, \mu M$); and with rabeprazole and CYP1A2 ($IC_{50} > 40 \, \mu M, \, IC_{50(0)} = 18.1 \, \mu M$), CYP2C8 ($IC_{50} > 40 \, \mu M, \, IC_{50(0)} = 13.9 \, \mu M$), and CYP2D6 ($IC_{50} > 40 \, \mu M, \, IC_{50(0)} = 28.5 \, \mu M$) (Table 1). Only omeprazole ($IC_{50}/IC_{50(0)}$ ratio = 2.5) and esomeprazole ($IC_{50}/IC_{50(0)}$ ratio = 4.9) exhibited time-dependent inhibition of CYP2C19-catalyzed (S)-mephenytoin 4′-hydroxylation (Table 2). The higher $IC_{50}/ IC_{50(0)}$ ratio for esomeprazole (~9.5) versus omeprazole (~2.9) was confirmed with rCYP2C19 using 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 reconfirmed above is consistent with the observations of Ogilvie et al. (2011) and Boulenc et al. (2012). In both groups, we sought to determine $K_i$ and $k_{\text{inact}}$ for both omeprazole and esomeprazole with HLM-catalyzed ($S$)-mephenytoin 4′-hydroxylation (Table 2). The higher $IC_{50}/ IC_{50(0)}$ ratio for esomeprazole (~9.5) versus omeprazole (~2.9) was confirmed with rCYP2C19 using diazepam as substrate (data not shown).

**Materials and Methods.** Each data point represents the mean ± S.D. of $n = 3$ to $5$ determinations. Inhibition parameters $k_{\text{inact}}$ and $K_i$ (mean ± S.E. of the parameter estimate) were determined for omeprazole with HLM-catalyzed (S)-mephenytoin 4′-hydroxylation activity (A), rCYP2C19-catalyzed (S)-mephenytoin 4′-hydroxylation activity (B), rCYP2C19-catalyzed diazepam N-demethylation activity (C); and for esomeprazole with HLM-catalyzed (S)-mephenytoin 4′-hydroxylation activity (D), rCYP2C19-catalyzed (S)-mephenytoin 4′-hydroxylation activity (E), and rCYP2C19-catalyzed diazepam N-demethylation activity (F).
Various PPIs as Inhibitors of Diazepam N-Demethylation Catalyzed by Human Primary Hepatocytes Coincubated with Human Serum. As shown in Table 3, the six PPIs were evaluated as inhibitors of diazepam N-demethylation activity in the presence of human primary hepatocytes using the AlgiMarx 3D culture system. Two preparations of cells were used, both coincubated 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 mg) was similar to that observed clinically after an IV dose (0.29–0.46 ml/min per mg) (Klotz et al., 1975). Moreover, use of low diazepam concentrations (≤2 μM) ensured that N-demethylation activity was largely reflective of CYP2C19 (Yasumori et al., 1994).

Each individual PPI was added at a single concentration based on its estimated Cmax,portal (see Materials and Methods), and the serum was added to account for the differences in \( f_{d,p} \), 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 (Cmax, Cmax,u, Cmax,portal, and Cmax,portal,u). HLM [(S)-mephenytoin] and rCYP2C19 (diazepam, (S)-mephenytoin, and CEC as substrate) IC50,u data were used to derive \( K_{i,u} \) values (Tables 4 and 5). In turn, the \( K_{i,u} \) values were used to estimate the degree of CYP2C19 inhibition in vivo based on published data for each compound (e.g., Cmax, dose, \( f_{d,p} \), \( f_{a} \)). For omeprazole and esomeprazole, the experimentally derived parameters for metabolism-dependent inhibition (\( K_{i,a} \) and \( k_{max} \)) were also considered. Where possible, the \( k_{c} \) for each individual PPI was calculated by leveraging published human oral and IV pharmacokinetic data (Landahl et al., 1992; Pue et al., 1993; Gerloff et al., 1996; Andersson et al., 2001; Setoyama et al., 2005). With the exception of dexlansoprazole, it was possible to calculate Cmax,portal and Cmax,portal,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 \( f_{d,p} \)-corrected Cmax or \( f_{a} \)-corrected Cmax,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 Cmax (57 μM) after a 240-mg IV dose of pantoprazole (Gugler et al., 1996), the % inhibition of CYP2C19 was overestimated even with \( f_{a} \) correction. Only HLM-derived (S)-mephenytoin \( K_{i} \) data (>20 μM) rendered a % inhibitionpredicted value close to that in vivo (<5%).

In the case of omeprazole, \( f_{d,p} \) correction was needed to avoid overestimation of % inhibition (Table 5). Furthermore, it was also evident that one had to consider metabolism-dependent inhibition. In this regard, CYP2C19-driven values for % inhibitionpredicted using (S)-mephenytoin (38–77%) and diazepam (26–66%), and \( f_{a} \)-corrected Cmax were closest to the % inhibitionin 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 % inhibitionin vivo with two additional CYP2C19 substrates (moclobemide, \( f_{a},CYP2C19 = 0.72 \); escitalopram, \( f_{a},CYP2C19 = 0.44 \)). 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 coosed with omeprazole (Yu et al., 2001; Malling et al., 2005). Therefore, the % inhibitionin vivo with moclobemide (32–76%) is closer to that observed with diazepam (28–61%) versus escitalopram (64–89%). It is interesting to note that HLM (S)-mephenytoin 4′-hydroxylation-driven values for % inhibitionpredicted were higher (51–85%) even after incorporation of plasma \( f_{a} \) (Table 5). Whether or not the degree of CYP2C19 inhibition by omeprazole is substrate-dependent requires further investigation; in our study, the \( k_{max}/K_{i} \) ratio (rCYP2C19) with diazepam (0.01 min−1 μM−1) and (S)-mephenytoin (0.018 min−1 μM−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 % inhibitionin 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 (% inhibitionpredicted ≥95%) when using Cmax or Cmax,portal (Fig. 4). Furthermore, correction for plasma \( f_{a} \) 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
with HLM only, the rCYP2C19-derived parameters (\(K_i\), \(\Delta C_{max, portal}\)) were used to estimate the contribution of metabolism-dependent inhibition. Toward this end, a semi-empirical model was used to predict the effect of each PPI on the AUC of diazepam (see Materials and Methods, eqs. 17 and 18).

**TABLE 4**

Comparison of lansoprazole, dexlansoprazole, pantoprazole, and rabeprazole as CYP2C19 inhibitors

<table>
<thead>
<tr>
<th>PPI</th>
<th>Substrate</th>
<th>(K_i^\text{a} )</th>
<th>(C_{max, portal}^b)</th>
<th>% Inhibition\textunderscore pred. (\text{portal}^b)</th>
<th>% Inhibition\textunderscore pred. (\text{portal}^b)</th>
<th>% Inhibition\textunderscore in vivo (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(\mu M)</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>
<td>80</td>
<td>11 (19, 97)</td>
</tr>
<tr>
<td></td>
<td>(S)-Mephenytoin</td>
<td>0.6</td>
<td>86</td>
<td>16</td>
<td>90</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>CEC</td>
<td>0.2</td>
<td>94</td>
<td>34</td>
<td>96</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>(S)-Mephenytoin (HLM)</td>
<td>0.3</td>
<td>91</td>
<td>23</td>
<td>93</td>
<td>30</td>
</tr>
<tr>
<td>Dexlansoprazole</td>
<td>Diazepam</td>
<td>6.1</td>
<td>38</td>
<td>1</td>
<td>10 (2, 19)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(S)-Mephenytoin</td>
<td>3.0</td>
<td>56</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CEC</td>
<td>1.1</td>
<td>78</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(S)-Mephenytoin (HLM)</td>
<td>2.8</td>
<td>59</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pantoprazole</td>
<td>Diazepam</td>
<td>3.6</td>
<td>94</td>
<td>25</td>
<td>94</td>
<td>25 (1, 1)</td>
</tr>
<tr>
<td></td>
<td>(S)-Mephenytoin</td>
<td>6.7</td>
<td>90</td>
<td>15</td>
<td>90</td>
<td>15 (1, 1)</td>
</tr>
<tr>
<td></td>
<td>CEC</td>
<td>2.1</td>
<td>96</td>
<td>35</td>
<td>96</td>
<td>35 (1, 1)</td>
</tr>
<tr>
<td></td>
<td>(S)-Mephenytoin (HLM)</td>
<td>&gt;20</td>
<td>74</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabeprazole</td>
<td>Diazepam</td>
<td>&gt;20</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(S)-Mephenytoin</td>
<td>5.7</td>
<td>7</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CEC</td>
<td>2.1</td>
<td>18</td>
<td>&lt;1</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(S)-Mephenytoin (HLM)</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

\(i\) unless otherwise indicated, the \(K_{IC_{50}}\) 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 \(K_{IC_{50}}\) 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.

\(i\) Percentage of inhibition\(\text{pred.}_\text{portal}\) was determined as described under Materials and Methods. \(C_{max, portal}\) values for each PPI were based on those reported for a specific diazepam drug interaction: 3.4 (0.11) \(\mu M\) after 60 mg of oral lansoprazole, 4.0 (0.08) \(\mu M\) after 90 mg of oral dexlansoprazole, 57 (1.1) \(\mu M\) after 240 mg of IV pantoprazole, and 0.45 (0.02) \(\mu M\) after 20 mg of oral rabeprazole (see Supplement Table S2). \(C_{max, portal}\) values were 4.8 \(\mu M\) (lansoprazole), 57 \(\mu M\) (pantoprazole), and 0.8 \(\mu M\) (rabeprazole). Corresponding \(C_{max, portal}^d\) values were 0.14 \(\mu M\) (lansoprazole), 1.1 \(\mu M\) (pantoprazole), and 0.03 \(\mu M\) (rabeprazole).

\(g\) No clinical IV data available in the literature for dexlansoprazole, so it was not possible to estimate \(C_{max, portal}^d\).

Prediction of CYP2C19 Inhibition In Vivo Based on In Vitro-Derived Inhibition Parameters and a Semimechanistic 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. Toward this end, a semi-empirical model was used to predict the effect of each PPI on the AUC of diazepam (see Materials and Methods, eqs. 17 and 18).
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 (Supplemental Fig. S2), after 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 (Supplemental Table S2). In all cases, the observed plasma concentrations were adequately captured using a one- or two-compartment model (Supplemental Fig. S1). The subsequent plots of observed and model-simulated plasma concentration-time profiles of diazepam after PPI dosing are shown in Fig. 2 (incorporating total PPI plasma concentration as [II]) and in Fig. 3 (incorporating free PPI plasma concentration as [I]). As shown in Fig. 2, the semimechanistic compartment model incorporating total PPI concentrations was able to reasonably capture the diazepam time course of placebo and PPI-treated subjects, and % inhibitionpredicted was 50% (esomeprazole), 29% (omeprazole), 16% (lansoprazole), 16% (dexlansoprazole), 29% (pantoprazole), and <1% (rabeprazole). In contrast, incorporation of free [I] resulted in the underprediction of the diazepam AUC ratio compared with the observed geometric mean ratio (<1% inhibitionpredicted) (Fig. 3). Pantoprazole behaved as an outlier.

![Diazepam Plasma Conc (nM) vs Time after dose (h) for Omeprazole, Esomeprazole, Pantoprazole, Rabeprazole, Lansoprazole, Dexlansoprazole](image)

**FIG. 2.** Predicting the impact of different PPIs on the pharmacokinetics of diazepam (using total PPI plasma concentration in the modeling exercise). For each PPI, in vitro inhibition data (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 supplemental data).
because $f_{u}$-adjusted plasma concentrations rendered a % inhibition predicted value (16%) that fell within the in vivo range (<1%, 20%) as defined by the 90% CI. It is interesting to note that, 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 versus 16%) and esomeprazole (50 versus 29%) (Supplemental Fig S3). 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.** 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 $f_{u}$-corrected static PPI concentration or when time-dependent changes in total PPI concentration were considered. Consistent with in vivo data, both lansoprazole and dexlansoprazole were predicted to be weaker inhibitors versus esomeprazole.

**FIG. 3.** Predicting the impact of different PPIs on the pharmacokinetics of diazepam (using free PPI plasma concentration in the modeling exercise). For each PPI, in vitro inhibition data (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 supplemental data).
FIG. 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 (tCYP2C19 diazepam N-demethylase). Estimates were based on plasma PPI C<sub>max</sub>, C<sub>max,portal</sub>, C<sub>max,u</sub>, C<sub>max,portal,u</sub> (Tables 4 and 5), the use of human primary hepatocytes coincubated with human serum (Table 3), and a semimechanistic model using total or free PPI plasma concentration (Figs. 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<sub>i</sub>/AUC<sub>c</sub> ratio for diazepam (see Materials and Methods).
and omeprazole. However, these two PPIs could only be differentiated from each other (lansoprazole > dexlansoprazole) only when fR-corrected Cmax was considered (8 versus 1% inhibition predicted). In the case of pantoprazole, only hepatocyte data correctly predicted minimal inhibition (≤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 IC50s obtained (Tables 1 and 2) more or less complimented the results of others who have reported IC50 data for different PPI-P450 combinations (VandenBranden et al., 1996; Ko et al., 1997; Li et al., 2004; Liu et al., 2005; Walsky et al., 2005, 2006; 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 (IC50/IC50(t) ratio <2.0). Only with omeprazole (CYP1A2), esomeprazole (CYP2D6), and rabeprazole (CYP1A2, -2C8, and -2D6) did the IC50 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 Cmax,μ (≤5% inhibition) or Cmax,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) (Andersson et al., 2001; Blume et al., 2006; Uno et al., 2008; Vakily et al., 2009; Ogawa and Echizen, 2010). 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]/K1 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 Km CYP2C19 substrates (Karam et al., 1996; Abelo et al., 2000; Li et al., 2005). 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 dexlansoprazole with (S)-mephentoin 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 (1.7–10-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 (Ogilvie et al., 2011; Boulenc et al., 2012; 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 Km) and CYP3A4 (high Km) in HLM (Abelo et al., 2000; Li et al., 2005), we also sought to assess the time-dependent inhibition with preparations of rCYP2C19 using both (S)-mephentoin and diazepam as substrates. In our study, it was possible to confirm that the Km with CYP2C19 ranged from 1.8 to 3.8 μM (Fig. 1) and was consistent with the low Km reported for both omeprazole and esomeprazole (Abelo et al., 2000; Li et al., 2005). The HLM-derived estimates of Km (~1.0 μM) reported herein are largely reflective of low Km CYP2C19-dependent metabolism (Li et al., 2005) and of the PPI concentration range used in the present study (~30 μM). Therefore, the higher HLM-derived values of Km (~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 Km 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). In contrast, 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% by omeprazole, esomeprazole, dexlansoprazole, lansoprazole, and pantoprazole, respectively. Likewise, the AUC of diazepam is increased 28, 81, 6, 12, and <1%, respectively (Andersson et al., 1990, 2001; Ishizaki et al., 1995; 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 (Vupputigallala et al., 2010). Toward this end, inhibition data were obtained using three different model systems. In addition, PPI plasma protein binding was considered when determining % inhibition predicted as were PPI plasma Cmax and Cmax,portal values. Finally, the pharmacokinetic profile of each PPI was also considered (see Materials and Methods). It is noteworthy that inhibitory metabolites, possible interactions between (R)- and (S)-forms of each racemic PPI, and accumulation in hepatocytes were not considered (Jones et al., 2004; Li et al., 2004, 2005; Ogilvie et al., 2011).

As summarized in Fig. 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 fR-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 CYP2C19 is high (57 μM) after an IV dose of 240 mg (Gugler et al., 1996). Only the semimechanistic model, with correction for \( f_{\text{irr}} \), 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 incubated hepatocytes, at such a low concentration, the degree of inhibition (~5%) is more consistent with in vivo data (Table 3). It should be noted that, as reported by other authors (Li et al., 2004; Ogilvie et al., 2011), pantoprazole was shown to be a relatively weak inhibitor of HLM-catalyzed (S)-methionyn 4'-hydroxylation activity in our study (Table 2). For pantoprazole, this implies that a rCYP2C19-derived \( K_{i,a} \) 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\(_{50}\)s 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 after 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 (Angiolillo et al., 2011; Frelinger et al., 2012; Shah et al., 2012).

Acknowledgments
We thank Dr. Fabrice Hurbin (Sanofi-Aventis, Research and Development, Montpellier, France) for input and suggestions.

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

References
Brouwers F, Mignot A, Barth H, and Heintze K (1996) Pharmacokinetics and absolute bioavail-

Downloaded from jmd.aspetjournals.org at ASPET Journals on June 21, 2017


Address correspondence to: Dr. Tatyana Zvyaga, Bristol-Myers Squibb, 5 Research Parkway, Wallingford, CT 06492. E-mail: tatyana.zvyaga@bms.com