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CYP2C19 Inhibition: The Impact of Substrate Probe Selection on In Vitro Inhibition Profiles

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Abstract

Understanding the potential for P450-mediated drug-drug interactions is a critical part of the drug discovery process. Factors such as nonspecific binding, atypical kinetics, poor effector solubility and varying ratios of accessory proteins may alter an enzyme's kinetic behavior and subsequently confound the extrapolation of in vitro data to the human situation. The architecture of the P450 active site and the presence of multiple binding regions within the active site may also confound in vitro-in vivo extrapolation, as inhibition profiles may be dependent on a specific inhibitor-substrate interaction. In these studies, the inhibition profiles of a set of twenty-four inhibitors were paneled against the CYP2C19 substrate probes (S)-mephenytoin, (R)-omeprazole, (S)-omeprazole and (S)-fluoxetine, based on their inclusion in recent FDA guidance for in vitro drug-drug interactions with CYP2C19. (S)-mephenytoin was inhibited an average of 5.6-fold more potently than (R)- or (S)-omeprazole and 9.2-fold more potently than (S)-fluoxetine. Hierarchical clustering of the inhibition data suggested three substrate probe groupings, with (S)-mephenytoin exhibiting the largest difference from the rest of the substrate probes, (S)-fluoxetine exhibiting less difference from (S)-mephenytoin and the omeprazoles and (R)- and (S)-omeprazole exhibiting minimal differences from each other. Predictions of in vivo inhibition potency based on the in vitro data suggest that most drug-drug interactions will be identified by either (S)-mephenytoin or omeprazole, although the expected magnitude of the interaction may vary depending on the chosen substrate probe.

Introduction

The cytochrome P450 superfamily of drug metabolizing enzymes is involved in the metabolism of the majority of currently prescribed drugs and new chemical entities. Within the P450 superfamily, the human CYP2C family (CYP2C8, CYP2C9, CYP2C18 and CYP2C19) are responsible for the metabolism of approximately 20% of marketed drugs (Goldstein, 2001). Of the four clinically relevant CYP2C isoforms noted above, CYP2C9 and CYP2C19 are the most highly conserved, with approximately 91% structural similarity between the two enzymes (Romkes et al., 1991). There is roughly 80% sequence identity among all four CYP2C isoforms (Ridderstrom et al., 2001). Crystal structures have been published for CYP2C8 and CYP2C9 (Williams et al., 2003; Schoch et al., 2004), though currently only homology models exist for CYP2C18 and CYP2C19 (Ridderstrom et al., 2001; Suzuki et al., 2004).

CYP2C19 is a polymorphic enzyme that accounts for less than 5% of hepatic P450 (Ring et al., 2001) and 2-3% of intestinal P450 content (Paine et al., 2006). The CYP2C19 poor metabolizer phenotype is found in approximately 25% of Asians and only 3-5% of Caucasians (Rodrigues and Rushmore, 2002). Commonly used drugs that are metabolized by CYP2C19 include proton pump inhibitors such as omeprazole and lansoprazole, psychotropic drugs including diazepam and imipramine and anticonvulsants such as phenobarbital and mephentyoin. Unlike CYP2D6, another polymorphic drug metabolizing enzyme, CYP2C19 has also shown to be inducible by both rifampicin and dexamethasone.

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In drug discovery, phenotyping new chemical entities for CYP2C19-mediated metabolism as well as for their potential to inhibit CYP2C19 is a common practice due to the potential liabilities of the CYP2C19 polymorphism. In vitro assays to examine drug-drug interactions (DDIs) commonly use (S)-mephenytoin as a probe substrate for CYP2C19 catalyzed reactions, though difficulties can arise from the relatively low rate of turnover to its 4'-hydroxy metabolite. According to recent FDA guidance (www.fda.gov/cder/drug/interactions), alternative metabolic pathways that can be used as probes of CYP2C19 activity in vitro include omeprazole metabolism to 5-hydroxyomeprazole and the formation of trifluoromethylphenol via O-dealkylation of fluoxetine. In clinical studies, (S)-mephenytoin and omeprazole are both commonly used as in vivo probes of CYP2C19 activity. Recently, concerns about material availability, metabolite stability and the potential for adverse events have been raised when using (S)-mephenytoin as an in vivo probe for CYP2C19 activity (Streetman et al., 2000). Omeprazole has the disadvantage of being a CYP1A2 inhibitor and inducer in vivo (Fuhr et al., 2007). Differences in probe selection have also arisen for in vivo cocktail protocols, such as the Cooperstown (omeprazole containing) or Pittsburgh ((S)-mephenytoin containing) cocktails. These cocktails have been validated at low doses to be selective for individual P450s without metabolic interactions (Frye et al., 1997; Streetman et al., 2000). With the increasing use of omeprazole for in vitro and in vivo CYP2C19 studies, assessment of the selection of substrate probe for the in vitro experiment and possible implications for the human situation becomes more important.

The primary aim of this study was to examine the impact of probe selection on the in vitro inhibition profiles of the CYP2C19 substrate probes (S)-mephenytoin, (R)- and

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(S)-omeprazole, and (S)-fluoxetine. K_i values were determined for twenty-four effectors with wide structural diversity and expected inhibition potency against the substrate probes. The secondary aim was to determine whether the substrate probes would identify similar compounds for potential clinical DDI studies.

Materials and Methods

Chemicals. CYP2C19*1 + b5 BD Supersomes™ and (S)-(+)-(N)-(3)-benzylrivanol were purchased from BD Gentest (Woburn, MA). (S)-mephenytoin and raloxifene were obtained from Biomol International (Plymouth Meeting, PA). NADPH was purchased from EMD Biosciences (San Diego, CA). Ammonium formate, HPLC-grade acetonitrile and HPLC-grade methanol were obtained from Alfa Aesar (Ward Hill, MA). Racemic omeprazole was purchased from Sigma-Aldrich (St. Louis, MO). Separation of the omeprazole enantiomers was performed according to published methods (Raju et al., 2006). All other chemicals used were purchased from Sigma-Aldrich (St. Louis, MO) and were of the highest purity available.

K_i determination: The incubation times and protein concentrations used were within the linear range of metabolite formation of each assay. Incubations were carried out using four substrate probes of CYP2C19 [(S)-mephenytoin, (S)-omeprazole, (R)-omeprazole, and (S)-fluoxetine]. (R)-fluoxetine was not used as a substrate probe due to potent time dependent inhibition of CYP2C19. Prior to assessing inhibitor potency, the K_m and V_{max} values for the four substrate probes were determined in the current lot of enzyme. Twenty-four known inhibitors exhibiting a wide range of inhibition potencies were selected for study. Stock solutions of all the inhibitors were made in dimethylsulphoxide (DMSO) and then diluted 10-fold with acetonitrile prior to addition to the incubation mixtures to minimize DMSO content. Four concentrations of each substrate [approximately 0.5K_m to 5K_m: 80, 40, 20 and 10 μM for (S)-mephenytoin; 25, 12.5, 6.25 and 3.13 μM for (R)-omeprazole; 50, 25, 12.5 and 6.25 μM for (S)-omeprazole

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and 250, 100, 50 and 25 μM for (S)-fluoxetine] and five concentrations of each inhibitor (spanning a ten-fold range of the expected K_i) were used for determination of K_i in a 96 well plate format. Briefly, each reaction was carried out in duplicate and one pmol of CYP2C19 enzyme (2 pmol when (S)-fluoxetine was the substrate) was used per incubation. Each incubation reaction mixture (200 μl) contained enzyme, substrate and inhibitor suspended in phosphate buffer (100 mM, pH 7.4) containing 3 mM MgCl_2 and was preincubated for three minutes in an incubator-shaker at 37 °C. The reactions were initiated by the addition of NADPH (1 mM final concentration). Organic solvent concentrations did not exceed 0.5% v/v. Solvent concentrations were the same for all experiments and turnover rates did not differ significantly from minimal solvent controls. The reactions were terminated with 100 μl of acetonitrile containing 0.1 μM of tolbutamide (internal standard) and centrifuged at 3000 rpm for 10 min. Length of the incubations for (S)-mephenytoin, (R)-omeprazole and (S)-omeprazole was 20 min. For (S)-fluoxetine the incubations were carried out for 30 min.

It is of note that to assure validity of the results and to allow comparison of inhibition profiles from different sets of experiments, a number of precautions were taken. To avoid batch to batch variability in enzyme, all samples were taken from the same batch provided by the manufacturer. The experiments were planned to minimize the amount of enzyme in each incubation to reduce the potential impact of non-specific binding of both substrate and inhibitor, and incubation times were limited to thirty minutes or less to avoid substrate or inhibitor depletion.

Liquid Chromatography/Tandem Mass Spectral Analysis. All analytical methods were conducted using HPLC-MS/MS technology. In brief, the LC-MS/MS system was

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comprised of an Applied Biosystems 4000 Q-Trap (operated in triple quadrupole mode) equipped with an electrospray ionization source (Applied Biosystems, Foster City, CA). The MS/MS system was coupled to two LC-20AD pumps with an in-line CBM-20A controller and DGU-20A₅ solvent degasser (Shimadzu, Columbia, MD) and a LEAP CTC HTS PAL autosampler equipped with a dual-solvent self-washing system (CTC Analytics, Carrboro, NC). The injection volume was 20 μ L for each analyte. For all assays except fluoxetine *O*-dealkylation to trifluoromethylphenol (TFMP), HPLC separation was achieved using a Gemini C18 2.0 x 30 mm 5 μ m column (Phenomenex, Torrance, CA). Gradient elution (flow rate = 500 μ L/min) was carried out using a mobile phase system consisting of (A) 5 mM ammonium formate with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. The gradient conditions were 5% B for 0.5 min, increasing to 100% B from 0.5 to 1.0 min, holding at 100% B from 1.0 to 1.75 min and returning to 5% B from 1.75 to 2.5 min. HPLC flow was diverted from the MS/MS system for the first 20 seconds to remove any non-volatile salts. For TFMP, the HPLC column was a Synergi Polar-RP (4 μ m, 30 x 2.0 mm; Phenomenex, Torrance, CA). Gradient conditions utilized the same mobile phase components as noted above, though the maximum percentage of acetonitrile in the mobile phase was limited to 70%. MS/MS conditions were optimized for individual analytes accordingly. Generic MS parameters included the curtain gas (10 arbitrary units), CAD gas (medium), ionspray voltage (4500 V), source temperature (450 °C) and ion source gas 1 and gas 2 (40 arbitrary units, each). Interface heaters were kept on for all analytes. Analysis masses were: 4'-hydroxymephenytoin, m/z 232.9 \rightarrow 190.0, negative ion mode; 5-hydroxymeprazole, m/z

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362.2→214.1, positive mode; tolbutamide, m/z 268.9→169.7, negative mode; m/z 271.2→91.1, positive mode; and TFMP, m/z 160.8, SIM, negative mode.

Statistical Analysis. Standard curve fitting was performed using Analyst (version 1.4; Applied Biosystems, Foster City, CA). In general, standard curves were weighted using $1/x$. Average values of inhibition potency were calculated for each substrate probe by summing and averaging the inhibition data collected versus the panel of inhibitors. Substrate saturation curves and inhibition data were plotted and analyzed using GraphPad Prism (version 4.01; GraphPad Software Inc., San Diego, CA). Visual inspection of the Dixon ($[I]$ vs $1/v$) and Lineweaver-Burke ($1/[S]$ vs $1/v$) plots as well as inspection of the residuals and use of Akaike's Information Criteria was used to determine the mechanism of inhibition and model selection. Data was the fitted to either a competitive (Equation 1) or mixed inhibition model (Equation 2):

$$(1) \quad v = \frac{V_{\max} \bullet [S]}{K_m (1 + \frac{[I]}{K_i}) + [S]}$$

$$(2) \quad v = \frac{V_{\max} \bullet [S]}{K_m (1 + \frac{[I]}{K_i}) + [S] (1 + \frac{[I]}{K_i'})}$$

In the preceding equations, K_m is equal to half the substrate concentration at maximal reaction velocity, $[I]$ is the concentration of inhibitor in the system, K_i is the dissociation constant for the enzyme-inhibitor complex and K_i' is the dissociation constant for the enzyme-substrate-inhibitor complex. Note that in the above equations, K_m , K_i and V_{\max} were treated as global parameters. The goodness of the fit was determined by visual

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inspection of the data with the Dixon and Lineweaver-Burke plots and global r^2 values. Linear regression was used to determine the correlation between the K_i values of pairs of substrates using GraphPad Prism 4.

Hierarchical Clustering Analysis. Statistical and clustering analysis of the inhibition potency data was performed using Spotfire DecisionSite 8.1 (Spotfire, Inc, Somerville, MA). An UPGMA (Unweighted Pair Group Method with Arithmetic mean) clustering algorithm was used to determine similarity between the inhibition data sets and form successively larger clusters using a Euclidean distance similarity measure (Kenworthy et al., 1999). Data were entered as inhibition potency (K_i) values. Compounds that exhibited K_i values above 100 μM were entered as a K_i of 100 μM . Since a complete substrate-inhibitor matrix was necessary for correlation analysis, K_i values for instances where the substrate and inhibitor were the same were obtained by averaging the K_i values obtained using the other three substrate probes.

Estimation of in vivo Inhibition Potency. An estimate of in vivo inhibition potency was determined using previously described methods (Obach et al., 2006). The maximum unbound hepatic input concentration, $C_{\text{max},u,\text{input}}$, was determined using the following equation (Kanamitsu et al., 2000):

$$(3) \quad C_{\text{max},u,\text{inlet}} = f_u * (C_{\text{max}} + \frac{K_a * F_a * D}{Q_h})$$

In the preceding equation $C_{\text{max},u,\text{input}}$ is defined as the maximum systemic concentration, D is the oral dose, K_a is the first-order absorption rate constant, F_a is the fraction of the oral dose absorbed, F_u is the fraction unbound in the blood and Q_h is the hepatic blood flow.

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Values of 0.03 min^{-1} , 1.45 L/min and unity were used for K_a , Q_h and F_a , respectively. C_{\max} , oral dose and f_u values were obtained from Goodman and Gilman's *The Pharmacological Basis of Therapeutics* (Brunton et al., 2006). With the in vivo $[I]_{\text{in vivo}}$ parameter determined, a ratio of AUC with inhibitor to control AUC could be estimated using the following equation (Obach et al., 2006):

$$(4) \quad \frac{CL_{\text{control}}}{CL_{\text{inhibited}}} = \frac{AUC_I}{AUC} = \frac{1}{\left(\frac{f_{m(\text{CYP})}}{1 + \frac{[I]_{\text{in vivo}}}{K_i}} \right) + (1 - f_{m(\text{CYP})})}$$

In the preceding equation AUC_I is the area under-the-curve value for a given substrate probe in the presence of an inhibitor and AUC is the area under-the curve for the same probe substrate without inhibitor. The fraction of the substrate's metabolism by a given P450 is represented by $f_{m(\text{CYP})}$ and the magnitude of the inhibitor's potency by K_i . Values of 0.95 and 0.87 were used for the $f_{m\text{CYP}2\text{C}19}$ values of (S)-mephenytoin and omeprazole, respectively (Obach et al., 2006).

Results

Prior to conducting inhibition studies with the four substrate probes, kinetics were determined using the current batch of enzyme for (S)-mephenytoin ($K_m = 15.5 \pm 1.1 \mu\text{M}$, $V_{\max} = 8.2 \pm 0.2 \text{ nmol/min/nmol}$), (R)-omeprazole ($K_m = 3.7 \pm 0.5 \mu\text{M}$, $V_{\max} = 35.6 \pm 1.0 \text{ nmol/min/nmol}$), (S)-omeprazole ($K_m = 8.2 \pm 0.8 \mu\text{M}$, $V_{\max} = 8.7 \pm 0.2 \text{ nmol/min/nmol}$) and (S)-fluoxetine ($K_m = 98.1 \pm 11.8 \mu\text{M}$, $V_{\max} = 5.2 \pm 0.2 \text{ nmol/min/nmol}$). For each of the substrate probes [(S)-mephenytoin, (R)-omeprazole, (S)-omeprazole and (S)-fluoxetine], inhibition profiles and the resulting inhibition constant (K_i) were determined with a set of twenty-four inhibitors for CYP2C19 (Table 1). (R)-fluoxetine was not included in the selection of probe substrates, as potent time dependent inhibition of CYP2C19 led to low rates of product formation. Potency of inhibition across the twenty-four inhibitors spanned several orders of magnitude for each substrate (Table 1). The compounds were selected to possess wide structural diversity and to exhibit a wide range of inhibition potency based upon literature data. (R)-fluoxetine, (S)-fluoxetine and amitriptyline exhibited mixed inhibition when (S)-mephenytoin was used as a substrate probe, and (S)-mephenytoin exhibited mixed inhibition when (S)-omeprazole was used as a substrate probe; all other substrate-inhibitor combination were fitted to a competitive inhibition model. While no substrates or inhibitors that trended towards mixed inhibition, a number of interesting trends were noted upon examination of the data.

Several methods have been developed to compare inhibition profiles for panels of inhibitors with differing substrate probes: binning, average differences, correlation analysis and hierarchical clustering (Kenworthy et al., 1999; Kumar et al., 2006). Comparing the results using a recently proposed system of binning inhibition potency [K_i

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< 1 μ M (high concern), K_i 1-10 μ M (moderate concern), K_i > 10 μ M (low concern)] (Obach et al., 2006), eighteen of the inhibitors would be considered of high concern using (S)-mephenytoin, compared to ten for (R)- and (S)-omeprazole and nine for (S)-fluoxetine. When compared to (R)-omeprazole, (S)-omeprazole and (S)-fluoxetine, (S)-mephenytoin was 5.5-, 5.8- or 9.2-fold more sensitive on average to the set of test inhibitors than the other three probe substrates, respectively (Figure 1). Both the increased protein content and incubation time for (S)-fluoxetine may contribute to the reduction in inhibition potency observed with this substrate probe (Margolis and Obach, 2003). (R)- and (S)-omeprazole exhibited the lowest average difference in inhibition potency (1.4-fold) when compared to each other, while (R)- and (S)-omeprazole exhibited some average differences versus (S)-fluoxetine (3.8- and 2.4- fold differences, respectively). It is of note that even though differences in inhibition potency were exhibited, a high degree of correlation was observed for the test set of inhibitors when log transformed inhibition data was analyzed for correlation between the substrate probes ($r^2 \geq 0.73$, Table 2) for each of the substrate probes. The inhibition data can be highly correlated if the shift of inhibition potency remains relatively constant for the panel of inhibitors.

These differences were also observed in individual cases where one probe substrate was incubated with another probe substrate as the effector. (S)-mephenytoin was the most sensitive to interactions with the other three probes, as K_i values were less than 1 μ M when (S)-mephenytoin was incubated with (R)-omeprazole, (S)-omeprazole and (S)-fluoxetine. Conversely, (S)-mephenytoin exhibited little to moderate inhibition of (S)-omeprazole (K_i > 100 μ M), (R)-omeprazole (K_i = 15.3 μ M) or (S)-fluoxetine (K_i >

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100 μ M). Inhibition of (S)-fluoxetine by either (R)- or (S)-omeprazole resulted in K_i values of 2 to 3 μ M. Slightly higher K_i values were observed for inhibition of either omeprazole enantiomer by (S)-fluoxetine.

Hierarchical clustering analysis was performed on the non-transformed inhibition potency data using an UPGMA clustering algorithm and a Euclidean distance similarity measure. Results from the clustering analysis for the CYP2C19 data were visualized as a dendrogram (Figure 2) where the horizontal axis of the dendrogram represents the distance between substrate clusters. For the panel of effectors, the vertical axis of the dendrogram represents the difference between effector clusters. The clustering analysis for CYP2C19 probe substrate inhibition data suggested three distinct groupings of probe substrate similarities: (R)- and (S)-omeprazole; (S)-fluoxetine; and (S)-mephenytoin.

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Discussion

In vitro screening for potential DDIs is a crucial part of the drug discovery and development paradigm. DDIs represent a large fraction of reported adverse drug events, making such interactions a key hurdle in bringing a new drug to market. Recent examples of drugs withdrawn from the market due to drug interactions include mibefradil (Krayenbuhl et al., 1999), terfenadine (Monahan et al., 1990) and cerivastatin (Sica and Gehr, 2002). With patient safety and product success depending on the ability of drug research groups to detect potential interactions prior to initiating clinical trials, screening for DDIs has seen a prolific rise in importance over the past ten years (Wienkers and Heath, 2005). The aim of this manuscript was to evaluate the effect of probe substrate selection, based upon FDA guidance, on the inhibition profiles of a panel of compounds with CYP2C19.

The results based upon binning, average differences, correlation analysis and hierarchical clustering all suggest three substrate probe clusters: (S)-mephenytoin, (R)- and (S)-omeprazole and (S)-fluoxetine. (S)-mephenytoin stands out as the substrate probe most sensitive to inhibition. (R)- and (S)-omeprazole exhibit intermediate susceptibility to inhibition, while (S)-fluoxetine is the probe least sensitive to inhibition. Substrate dependent inhibition profiles are not a new phenomenon to drugs metabolized by the cytochromes P450. Recently, two groups have noted significant differences in IC₅₀ values for panels of CYP3A4 inhibitors depending on the CYP3A4 probe used (Kenworthy et al., 1999; Stresser et al., 2000). Additionally, CYP2C9 has exhibited this behavior with substrate groupings based on diclofenac, (S)-warfarin and (S)-flurbiprofen (Kumar et al., 2006). While CYP2C19 is not typically associated with atypical kinetics,

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it does share 91% sequence homology with CYP2C9, and residues that convey omeprazole 5-hydroxylation (Ibeanu et al., 1996) and (S)-mephenytoin 4'-hydroxylation (Tsao et al., 2001) activity to the CYP2C9 have been identified, indicating similarities in functional active site architecture.

Stereochemistry has been demonstrated to be a factor in determining inhibition potency and substrate turnover with CYP2C19. Two potent and selective CYP2C19 inhibitors, (S)-(+)-(N)-(3)-benzylrivanol and (R)-(-)-(N)-(3)-benzylphenobarbital, exhibit over an order of magnitude increase in inhibition potency when compared to their corresponding enantiomer (Suzuki et al., 2004). Docking and homology modeling suggested a lipophilic binding region encompassed by residues A103, V113, F114, V208, I362, L366 and F476 was an important component involved in these differences. While (R)- and (S)-omeprazole exhibit differences in enzyme kinetics and inverted regioselectivity (with 5-hydroxylation favored for (R)-omeprazole and 5'-O-demethylation favored for (S)-omeprazole) (Li et al., 2005), differences in inhibition potency versus the other probes was negligible. (R)- and (S)-fluoxetine did exhibit an order of magnitude difference in inhibition potency for the other substrate probes, but this appears to be the result of differential potency in time dependent inactivation, not reversible inhibition (data not shown).

A primary goal of drug interaction screening is to be able to predict the in vivo relevance of the interaction. Recent methodologies based on variations of equation 4 have been used to successfully predict in vivo drug interactions for multiple P450s from in vitro data (Obach et al., 2006). Key factors for the in vivo predictions include the fraction metabolized (f_m) of a substrate probe by a particular P450 and the expected in

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vivo concentration of the inhibitor, as well as the inhibition potency. In vivo predictions for compounds exhibiting K_i values less than 1 μM for (S)-mephenytoin are shown in Table 3. It is of note that many of the compounds which exhibit potent inhibition of CYP2C19 in vitro are not anticipated to exhibit in vivo inhibition due to high plasma protein binding or low expected in vivo concentrations. Two of the three compounds (fluvoxamine and ticlopidine) expected to give clinically relevant DDI in vivo (greater than a two-fold change in AUC due to the presence of an inhibitor) were also identified by (R)- and (S)-omeprazole. (S)-fluoxetine was not included in the in vivo projection due to its low $\text{fm}_{\text{CYP2C19}}$ and resulting low potential for use as an in vivo DDI probe (Ring et al., 2001).

In summary, inhibition of CYP2C19 by a panel of 24 inhibitors appeared to be dependent on choice of probe substrates. Use of (S)-mephenytoin as the CYP2C19 probe substrate in vitro provided the most sensitive measure of inhibitory potency, though complexities may arise in an vivo setting. The use of omeprazole as an in vitro substrate probe may be appropriate as long as the reduced sensitivity to identifying drug interactions at the screening stage is understood. Finally, the results presented within this manuscript strengthen the claim that in addition to the effects of nonspecific binding, co-enzymes, genetics and the source of enzyme, the choice of probe substrates may have a large impact on the drug interaction profile for a given cytochrome P450 inhibitor.

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Figure Legends

Figure 1

CYP2C19 Linear Correlation Graphs of the Log Inhibition Data. A) (S)-mephenytoin vs (S)-omeprazole, (R)-omeprazole and (S)-fluoxetine (flx); B) (R)-omeprazole vs (S)-fluoxetine; (S)-omeprazole vs (R)-omeprazole and (S)-fluoxetine exhibited similar correlations (data not shown for clarity).

Figure 2

CYP2C19 Inhibitor Hierarchical Clustering Dendrogram and Heat Map [(red < 1 μ M); (1 μ M < yellow < 10 μ M); (green > 10 μ M)].

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Table 1. K_i values (μM) obtained using four substrate probes: (S)-mephenytoin, (S)-omeprazole, (R)-omeprazole and (S)-fluoxetine using CYP2C19^a

	(S)- Mephenytoin	(S)- Omeprazole	(R)- Omeprazole	(S)- Fluoxetine
Loratadine	0.006	0.035	0.022	0.025
Fluvoxamine	0.013	0.062	0.014	0.070
Ticlopidine ^b	0.020	0.115	0.061	0.067
(S)-(+)-(N)-(3)-benzyl nirvanol	0.027	0.156	0.225	0.046
Clotrimazole	0.055	0.172	0.736	0.080
(R)-Fluoxetine ^b	0.067	0.631	0.627	0.347
Sertraline	0.089	1.54	0.371	0.375
Oxybutynin	0.209	0.601	0.328	0.536
Ketoconazole	0.239	0.183	0.447	4.32
Raloxifene	0.266	0.795	0.289	0.167
Ethinylestradiol	0.289	1.54	2.14	3.20
Progesterone	0.376	0.995	2.07	29.1
(R)-Omeprazole	0.445	NA	NA	2.99
Estradiol	0.524	2.03	3.93	7.82
(S)-Omeprazole	0.538	NA	NA	2.59
Nifedipine	0.553	1.63	2.1	3.32
(S)-Fluoxetine ^b	0.813	9.69	3.93	NA
Amitriptyline	3.38	1.07	5.81	13.8
Ketoprofen	4.15	>100	>100	>100
Suprofen	5.52	35.1	>100	60.4
Docetaxel	12.28	47.9	>100	>100
Sulfaphenazole	43.7	>100	>100	>100
Verapamil	43.8	100	79.7	8.35
(S)-Mephenytoin	NA	29.9	15.27	>100

^aGlobal standard error for data fitting was less than 15% and $r^2 > 0.9$ for each effector

^bTime dependent inactivator of CYP2C19

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Table 2. r^2 values obtained from the linear regression of the log K_i values for the four substrate probes using CYP2C19

Substrate	(S)-Mephenytoin	(S)-Omeprazole	(R)-Omeprazole	(S)-Fluoxetine
(S)-Mephenytoin	1			
(S)-Omeprazole	0.86	1		
(R)-Omeprazole	0.90	0.90	1	
(S)-Fluoxetine	0.78	0.73	0.81	1

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Table 3 Predicted change in AUC_I/AUC for compounds exhibiting K_i < 1 μM for (S)-mephenytoin

Inhibitor	Predicted AUC _I /AUC			Actual DDI ^a
	(S)-Mephenytoin	(S)-Omeprazole	(R)-Omeprazole	
Loratadine	1.8	1.2	1.2	
Fluvoxamine	13.8	5.3	6.6	5.6 ^b
Ticlopidine ^c	9.4	3.1	3.7	2.4 ^d
Clotrimazole	1.0	1.0	1.0	
(R)-Fluoxetine ^c	3.8	1.3	1.3	
Sertraline	1.9	1.1	1.2	
Oxybutynin	1.1	1.0	1.1	
Ketoconazole	1.3	1.2	1.1	
Raloxifene	1.4	1.2	1.3	
Ethinylestradiol	1.0	1.0	1.0	
(R)-Omeprazole	1.1	1.0	1.0	
(S)-Omeprazole	1.4	1.1	1.1	
Nifedipine	1.1	1.0	1.0	
(S)-Fluoxetine ^c	1.3	1.0	1.1	

^aOmeprazole used as the in vivo substrate probe

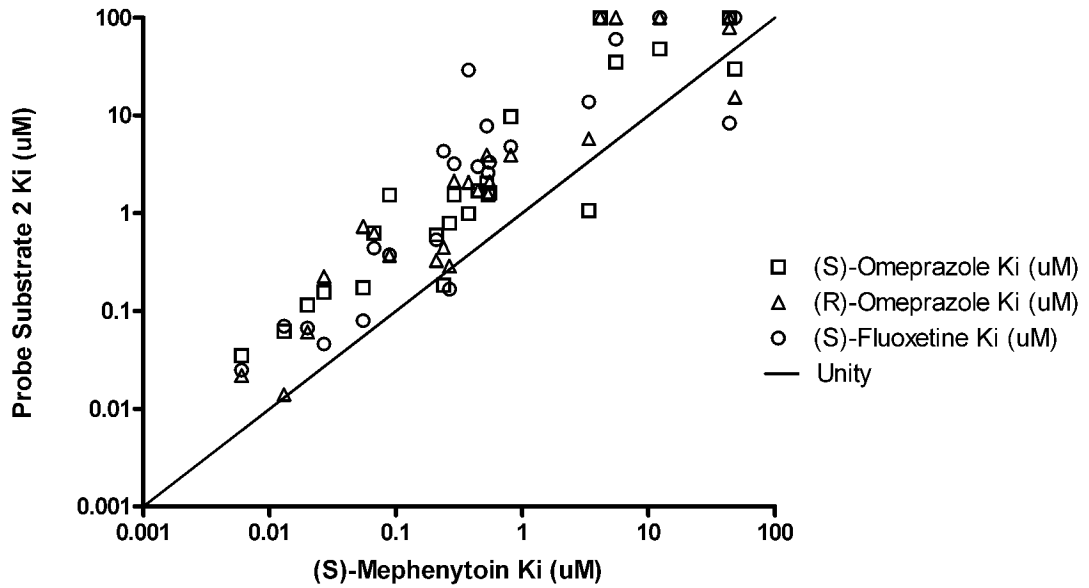
^b(Yasui-Furukori et al., 2004)

^cTime dependent inactivator of CYP2C19

^d(Tateishi et al., 1999)

Figure 1

A



B

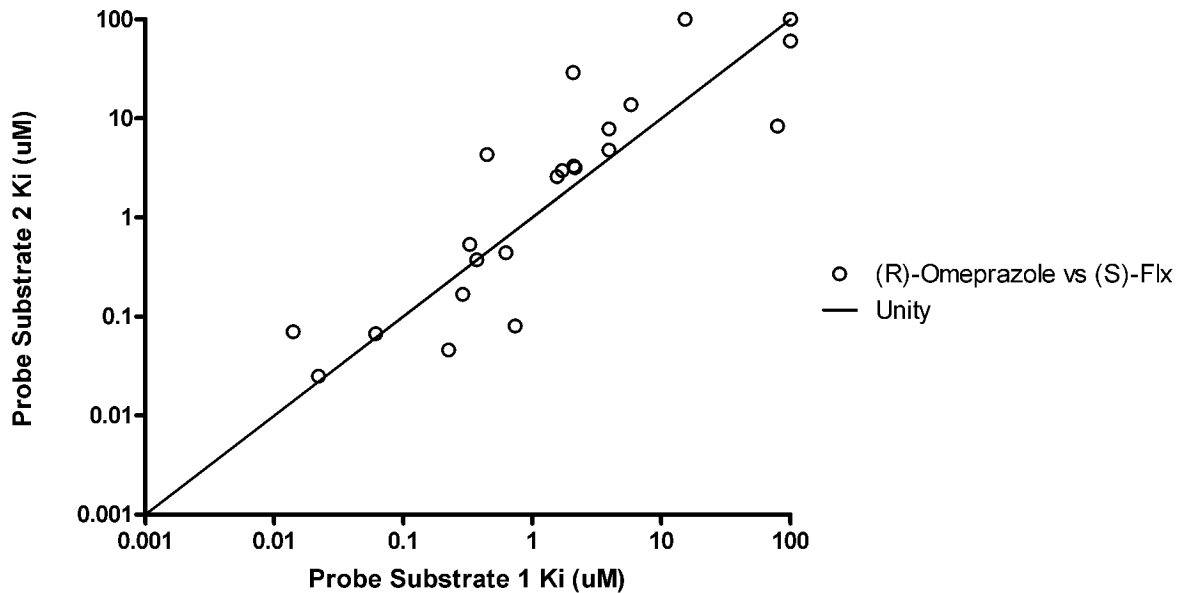


Figure 2

