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Importance of multi-P450 inhibition in drug-drug interactions: evaluation of incidence, inhibition magnitude and prediction from *in vitro* data

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Abstract

Drugs that are mainly cleared by a single enzyme are considered more sensitive to drug-drug interactions (DDIs) than drugs cleared by multiple pathways. However, whether this is true when a drug cleared by multiple pathways is co-administered with an inhibitor of multiple P450 enzymes (multi-P450 inhibition) is not known. Mathematically, simultaneous equipotent inhibition of two elimination pathways that each contributes half of the drug clearance is equal to equipotent inhibition of a single pathway that clears the drug. However, simultaneous strong or moderate inhibition of two pathways by a single inhibitor is perceived as an unlikely scenario. The aim of this study was (i) to identify P450 inhibitors currently in clinical use that can inhibit more than one clearance pathway of an object drug *in vivo*, and (ii) to evaluate the magnitude and predictability of DDIs caused by these multi-P450 inhibitors. Multi-P450 inhibitors were identified using the Metabolism and Transport Drug Interaction Database™. A total of 38 multi-P450 inhibitors, defined as inhibitors that increased the AUC or decreased the clearance of probes of two or more P450's, were identified. Seventeen (45 %) multi-P450 inhibitors were strong inhibitors of at least one P450 and an additional 12 (32 %) were moderate inhibitors of one or more P450s. Only one inhibitor (fluvoxamine) was a strong inhibitor of more than one enzyme. Fifteen of the multi-P450 inhibitors also inhibit drug transporters *in vivo*, but such data are lacking on many of the inhibitors. Inhibition of multiple P450 enzymes by a single inhibitor resulted in significant (>2-fold) clinical DDIs with drugs that are cleared by multiple pathways such as imipramine and diazepam while strong P450 inhibitors resulted in only weak DDIs with these object drugs. The magnitude of the DDIs between multi-P450 inhibitors and diazepam, imipramine and omeprazole could be predicted using *in vitro* data with similar accuracy as probe substrate studies with the same inhibitors. The results of this study suggest that inhibition of multiple clearance pathways *in vivo* is clinically relevant and the risk of DDIs with object drugs may be best evaluated in studies using multi-P450 inhibitors.

1. Introduction

Theory of inhibition drug-drug interactions (DDI) suggests that drugs that are mainly cleared by a single enzyme are more sensitive to DDIs than drugs cleared by multiple pathways. The effect of the fraction metabolized (f_m) by the inhibited enzyme to magnitude of observed DDIs has been well described, and the buffering effect of uninhibited elimination pathways on the magnitude of the *in vivo* DDI shown.^{1,2} As an extrapolation, it is often assumed that significant DDIs do not occur with drugs that have several elimination pathways because it is unlikely that an inhibitor will have a great impact on both or all of the

elimination pathways of the object drug. The theory of simultaneous inhibition of multiple elimination pathways by a single inhibitor has, however, been established, and the theoretical effect of simultaneous inhibition of multiple pathways shown.³ The theory shows that inhibition of multiple P450s simultaneously by a single inhibitor (multi-P450 inhibition) or inhibition of multiple P450s by concurrently administered selective P450 inhibitors may result in clinically important interactions, even when the object drug is cleared by multiple P450 enzymes. While several groups have evaluated *in vitro* to *in vivo* predictions of simultaneous inhibition of drug transporters and multiple P450 enzymes,^{4, 5} the incidence and severity of DDIs involving impairment of multiple pathways have not been examined.

At present, the *in vivo* DDI risk of new chemical entities (NCEs) is predicted using a sequential *in vitro*-to-*in vivo* approach that addresses both the likelihood of the NCE to be an *in vivo* inhibitor and the susceptibility of the NCE to DDIs. The inhibitory potency of drug candidates is tested using specific probes in microsomal or hepatocyte systems and *in vivo* DDI risk predicted from an I/K_i ratio for the given inhibitor-P450 enzyme pair and also by using a simulation and modeling approach (<<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362.pdf>>). When an NCE inhibits more than one P450 enzyme, *in vivo* DDI studies are often prioritized according to the “rank-order” approach in which the most potent interaction is tested first *in vivo* and subsequent interactions are tested only if the first interaction study turns out to be positive.⁶ All of these studies are usually conducted with specific probe substrates that assess the inhibition of a single P450 enzyme, and the ability of the NCE to inhibit multiple P450s simultaneously is not addressed in a systematic fashion. On the other hand, if the clearance of an NCE is >25 % by a single pathway, the susceptibility of the NCE to DDIs is tested using strong inhibitors of a given pathway. It is possible that simultaneous inhibition of multiple elimination pathways is not adequately reflected by this approach, and the susceptibility of a drug cleared by multiple pathways to DDIs caused by multi-P450 inhibitors needs to be addressed in a systematic manner. The recent draft guidance by the FDA recommends considering co-administration of several P450 inhibitors with the NCE to address the susceptibility and worst-case scenario for a magnitude of a DDI for an NCE for which any clearance pathway accounts for >25 % of the total body clearance. However, a multi-P450 inhibitor would be expected to cause a similar magnitude of DDI as multiple co-administered inhibitors.

The increased DDI risk in multiple impairment scenarios is illustrated in the study of repaglinide exposure after simultaneous administration of gemfibrozil and itraconazole.⁷ Gemfibrozil glucuronide is an irreversible inhibitor of CYP2C8 and an inhibitor of OATP, and itraconazole is a CYP3A4 and P-gp inhibitor. When administered alone, itraconazole caused a 1.4-fold increase in repaglinide AUC and gemfibrozil caused an 8.1-fold increase in repaglinide AUC. However, when the two selective inhibitors were administered together, a 19.4-fold increase in repaglinide AUC was observed. In a subsequent similar study, the effect of the combination of itraconazole and gemfibrozil on loperamide clearance was evaluated. While itraconazole alone and gemfibrozil alone increased loperamide AUC by 3.8-fold and 2.2-fold, respectively, the combination of the two resulted in a 12.6-fold increase in loperamide AUC.⁸ More recently, the *in vivo* effect of specific inhibition versus multi-P450 inhibition on ramelteon, a drug metabolized by multiple pathways including CYP1A2, CYP2C19, and CYP3A4, was predicted using *in vitro* metabolism data.⁵ For these predictions, the inhibition of a single elimination pathway of ramelteon or multiple elimination pathways simultaneously was considered. The changes in exposure caused by ketoconazole (CYP3A4 inhibition) and fluconazole (CYP2C19 and CYP3A4 inhibition) were reliably estimated from *in vitro* data. However, the effect of fluvoxamine, an inhibitor of the three enzymes clearing ramelteon, was significantly underestimated (11.4-fold predicted versus 128-fold actual), despite the fact that the prediction of the DDI magnitude

included inhibition of all three elimination pathways.⁵ These studies have raised concerns about whether simultaneous inhibition of multiple elimination pathways of drugs causes interactions greater than what would be predicted by methods adopted for predicting *in vivo* interactions of specific probes.

The aim of this study was to identify the multi-P450 inhibitors currently in clinical use and establish the effect of multi-P450 inhibition on the DDI magnitude with probes as well as substrates of multiple P450 enzymes. Using the extracted literature and reported *in vitro* and *in vivo* DDI studies, *in vitro*-to-*in vivo* predictions were performed to determine whether static *in vitro*-to-*in vivo* extrapolation (IVIVE) methods are useful in predicting the DDI risk of substrates of multiple P450s with a multi-P450 inhibitor.

2. Experimental

2.1. Literature search strategy

The University of Washington Metabolism and Transport Drug Interaction Database™ (MTDI database: <http://www.druginteractioninfo.org>)⁹ was queried to retrieve all *in vivo* pharmacokinetic interactions (defined as resulting in a ≥ 25 % increase in the AUC or decrease in clearance of the object drug) reported with FDA recommended probe drugs and sensitive P450 markers (Table 1). Individual case reports were not considered for the analysis. From the resulting list of *in vivo* inhibition studies, specific P450 enzymes inhibited by each precipitant were identified based on the P450 probes/sensitive markers studied. Inhibitors that demonstrated *in vivo* inhibition of probes of two or more enzymes were classified as multi-P450 inhibitors. Inhibitors that are not currently available in the US market and combination therapies were excluded from the analysis. AUC or CL changes of the marker substrates were used to classify inhibitors according to the FDA recommended system (www.fda.gov/cder/drug/drugInteractions/) as strong (≥ 5-fold increase in AUC), moderate (≥ 2 but <5-fold increase in AUC), or weak (≥ 1.25 but <2-fold increase in AUC) inhibitors, based on the largest interactions observed *in vivo* with a probe drug. For all drugs identified as multi-P450 inhibitors, all negative studies reporting AUC or clearance changes with marker substrates were also collected and the possible inhibition of known transporter systems was evaluated. *In vivo* transporter interactions were characterized based on DDI studies with recognized *in vivo* markers.¹⁰ Finally, the overall inhibition profiles (all DDI studies in the MTDI database that reported CL or AUC change of the substrate) of the identified multi-P450 inhibitors were collected. These inhibition profiles were evaluated for identification of studies with drugs that are not probes or sensitive substrates of given P450's, but are cleared by multiple known P450s. Using the clinical studies available, diazepam, imipramine and omeprazole were selected as examples of object drugs metabolized by multiple pathways for further evaluation.

2.2. *In vitro*-to-*in vivo* predictions of multi-P450 inhibition

The magnitude of *in vivo* DDIs with multi-P450 inhibition was predicted for diazepam, imipramine and omeprazole as objects metabolized by multiple P450s. For comparison, the magnitude of DDIs for the probe drugs desipramine, midazolam and (S)-mephenytoin with the same inhibitors was predicted and the prediction accuracy compared between the multiple and single impairment scenarios. For the predictions, the f_m values for diazepam, imipramine and omeprazole were calculated using data of the effect of genetic polymorphisms (for CYP2D6 and CYP2C19) and strong single P450 inhibitors (itraconazole for CYP3A4) on their clearance as described previously.³ *In vitro* metabolism data was used to support the calculated f_m values. Diazepam, imipramine and omeprazole are extensively metabolized *in vivo* and the fraction of each of these drugs excreted unchanged in the urine (f_e) is insignificant.¹¹⁻¹³ The fraction of drug that escapes intestinal

first pass metabolism (F_g) was calculated as described previously^{14, 15} from grapefruit juice studies for imipramine and omeprazole. CYP3A was considered the only enzyme contributing to first pass intestinal metabolism. Resulting F_g values were 1 and 0.8 for imipramine and omeprazole, respectively.^{16, 17} Based on the absolute bioavailability of 100% of diazepam,¹⁸ the F_g value of diazepam was assumed to be 1. In all cases the inhibitor or PM genotype was assumed to completely eliminate the clearance pathway (or gut CYP3A4 activity) of interest.

For diazepam, the f_m values obtained were 0.46 for CYP2C19 and 0.24 for CYP3A4. This was calculated from the 1.8-fold greater AUC of diazepam in CYP2C19 PMs versus EMs and from the 1.3-fold increase in diazepam AUC after itraconazole.^{19, 20} Since only CYP2C19, CYP3A4 and CYP2B6 metabolized diazepam *in vitro*, CYP2B6 was assumed to be responsible for the remaining f_m of 0.30.²¹ Based on *in vitro* data, omeprazole is metabolized by CYP2C19 and CYP3A4.²² Based on the 4.7-fold greater omeprazole AUC in CYP2C19 poor metabolizers (PMs) compared to extensive metabolizers (EMs),²³ CYP2C19 f_m of 0.78 was calculated. Based on the *in vitro* data, the remaining f_m (0.22) was assigned for CYP3A4. Imipramine's f_m by CYP2C19 and CYP2D6 were calculated from the 2.4-fold and 1.9-fold lower oral clearance of imipramine in CYP2C19 and CYP2D6 PMs compared to EMs, respectively,^{24, 25} resulting in f_m values of 0.55 (CYP2C19) and 0.45 (CYP2D6). Although both CYP3A4 and CYP1A2 have been shown to metabolize imipramine, these enzymes are predicted to contribute to imipramine clearance only in CYP2C19 and/or CYP2D6 PMs.²⁶ Literature f_m values were used for probe substrates: 1.0 for (S)-mephenytoin (CYP2C19), 0.88 for desipramine (CYP2D6) and 0.94 for midazolam (CYP3A4).^{2, 27, 28} The F_g value of 0.57 was used for midazolam.²⁹

The inhibitor specific values, including the K_i values measured in human liver microsomes (HLMs) and inhibitor concentrations *in vivo* were collected from the literature. Unbound K_i values were used when available as described below. The unbound fraction of voriconazole in HLMs was predicted using previously described methods.³⁰ For fluvoxamine the unbound fraction at different HLM protein concentrations (0.1 and 0.3 mg/mL) was predicted by extrapolation from the measured unbound fraction of 0.33 at 0.5 mg/mL³¹ as previously described.³² The *in vitro* K_i values used were: For fluconazole 2.1 μ M for CYP2C19 and 10.7 μ M for CYP3A4;^{33, 34} for fluvoxamine 0.078 μ M for CYP2C19, 1.8 μ M for CYP2D6 and 2.6 μ M for CYP3A4;^{31, 35, 36} for ketoconazole 12 μ M, for CYP2D6, 6.9 μ M for CYP2C19 and 0.059 μ M for CYP3A4;^{37, 38} and for voriconazole 0.34 μ M for CYP2B6, 5.1 μ M for CYP2C19 and 2.97 μ M for CYP3A4.³⁹ The unbound fractions in HLMs were 1.0 for fluconazole, 0.71 for ketoconazole³⁸ and 0.89 for voriconazole. For fluvoxamine the HLM unbound fraction used was 0.33 for CYP2C19,³¹ and 0.45 for CYP2D6 and 0.71 for CYP3A4 based on the different protein concentrations used in the K_i experiments. The inhibitor concentrations were collected from either the same *in vivo* DDI study that was predicted or a study with identical or similar inhibitor dose and dosing schedule. The average steady state plasma concentration was used for all predictions ($[I]$ in equation 1). The average inhibitor concentration was calculated from the steady state dosing interval AUC divided by the dosing interval. The unbound fractions in plasma were 0.89 for fluconazole, 0.23 for fluvoxamine, 0.01 for ketoconazole and 0.42 for voriconazole as previously reported.¹⁸

The *in vivo* ratio between inhibited and uninhibited AUCs (AUC'/AUC) for the object-inhibitor pair was predicted using equation 1, as previously reported:⁴⁰⁻⁴³

$$\frac{AUC'}{AUC} = \frac{F_g'}{F_g} \cdot \frac{1}{\sum_i^n \frac{f_{m,P450i}}{1 + \frac{[I]_j}{K_{i,j}}} + (1 - \sum_i^n f_{m,P450i})}$$

where $[I]_j$ is the inhibitor concentration, $K_{i,j}$ is the inhibition constant for reversible inhibitor for each of the P450 enzymes inhibited by the multi-P450 inhibitor *in vitro*, $f_{m,P450i}$ refers to fraction of object drug metabolized by the inhibited P450 pathways and F_g is the fraction of the object drug escaping gut metabolism following oral administration. The F_g'/F_g ratio was predicted according to equation 2.

$$\frac{F_g'}{F_g} = \frac{1}{F_g + \left(\frac{1 - F_g}{1 + \frac{[I]_j}{K_i}} \right)}$$

All interactions were predicted both using unbound and total plasma concentrations (*in vivo* $[I]$) and *in vitro* K_i values. The potential contribution of inhibition of gut CYP3A4 was predicted when object drug had an intestinal CYP3A4 mediated clearance component (midazolam and omeprazole) using two different methods. In the first method the average steady state plasma concentration was used as the inhibitor concentration in the enterocytes reflecting the effect of inhibitor on gut CYP3A4 when object drug is administered after inhibitor t_{max} . In the second method, the inhibitor concentration in the gut lumen was predicted according to the current FDA draft guidance using inhibitor dose divided by 250 mL, with the adjustment that the obtained concentration was multiplied by the fraction of inhibitor dose absorbed (F_a). The second method was used to obtain the worst-case maximum DDI risk prediction. The F_a values used were 1 for fluconazole, 0.53 for fluvoxamine, 0.75 for ketoconazole and 0.96 for voriconazole. The predicted inhibition magnitude was then compared to the observed *in vivo* increase in AUC or decrease in object clearance.

2.3. Simulation of multi-P450 inhibition

To rationalize the effect of simultaneous inhibition of multiple elimination pathways on object clearance, the effect of multi-P450 inhibition on the magnitude of AUC fold-increase was simulated using equation 1. Theoretical inhibitor-substrate combinations, with varying f_m values, number of enzymes inhibited, intestinal metabolism and inhibitor potencies were considered. In the first simulation, the clearance of the object drug was mediated by two P450 enzymes, with $f_{m,1} = 0.87$ and $f_{m,2} = 0.13$. Gut metabolism by enzyme 2 (P450₂) was set to result in $F_g = 0.8$. The AUC ratio was simulated at increasing inhibitor concentrations ($[I]$) for an inhibitor that inhibited both enzymes with K_{i1}/K_{i2} ratios of 0.01, 0.1, 1, 10, 100 and 1000. In a second simulation, the effect of an inhibitor on the AUC of a drug cleared by three P450 enzymes ($f_m = 0.32$ for each P450, fraction excreted unchanged (f_e) = 0.04 and unaffected) and with an $F_g = 0.66$ due to intestinal metabolism by enzyme 2 was simulated using inhibitor parameters in which the $K_{i,1} = K_{i,2} = 10 \cdot K_{i,3}$ i.e. the inhibitor is a potent inhibitor of enzyme 3 and a 10-fold weaker inhibitor of enzymes 1 and 2. A situation in which the inhibitor only inhibits enzymes 1 and 2, 1 and 3 or just 1 was also simulated.

3. Results and Discussion

3.1. Identification of multi-P450 inhibitors and the incidence of multi-P450 inhibition

The recently released FDA draft guidance on drug interaction studies recognizes the fact that complex DDIs occur as a result of simultaneous inhibition of multiple elimination pathways of the object drug by the inhibitor or its metabolites. However, at present, there is no comprehensive analysis available that would establish the prevalence of such complex DDIs with clinically relevant P450 inhibitors. The first aim of this study was to identify clinically available P450 inhibitors that inhibit *in vivo* more than one enzyme, as determined by probe substrate studies. Based on the probe data, a total of 38 multi-P450 inhibitors were identified (Table 2). Of the 38 multi-P450 inhibitors, 45 % (17 of the 38) were strong inhibitors of at least one P450 and an additional 29 % (11 of the 38) were moderate inhibitors of one or more P450s. Nine inhibitors were strong inhibitors of CYP3A4, two were strong inhibitors of CYP1A2, three were strong inhibitors of CYP2C19 and three were strong inhibitors of CYP2D6 (Table 2). Thirty-two percent (12 of 38) of the multi-P450 inhibitors were moderate or strong inhibitors of more than one enzyme. One of these twelve inhibitors, fluvoxamine, was a strong inhibitor of two enzymes (CYP1A2 and CYP2C19).

It is important to recognize that our results are dependent on the availability of *in vivo* studies with probe substrates. To address this, negative DDI studies with marker substrates for all the identified multi-P450 inhibitors were collected with probes of P450's for which positive studies were not available (Table 2). Despite this, as shown by the lack of data for many inhibitors with probes of several P450 enzymes, the collected data does not provide a complete profile of the enzymes inhibited *in vivo* for these inhibitors. Only a few of the multi-P450 inhibitors have been comprehensively characterized *in vivo* (Table 2). While 88 % of the inhibitors were tested against CYP3A4 inhibition and about 70 % were tested against CYP1A2 and CYP2C9 inhibition, only about 50 % were tested against CYP2D6 and CYP2C19 and only 20-30 % were tested for CYP2C8 and CYP2B6 inhibition. To some degree, this reflects the relatively recent implementation of systematic DDI evaluation into new drug development. As such, a thorough *in vitro* characterization of the inhibition profile of the known *in vivo* P450 inhibitors together with a targeted *in vivo* DDI risk analysis with selected probes is warranted.

In addition to incomplete *in vivo* P450 inhibition data, *in vivo* information of the effect of P450 inhibitors on drug transporters is sparse. It is widely recognized that *in vivo* inhibition of transport processes should also be considered in multiple impairment scenarios of DDIs. Therefore, the effect of all of the 38 inhibitors on the most specific *in vivo* markers of transport was evaluated and is summarized in Table 3. Sixteen inhibitors were found to have at least one positive pharmacokinetic DDI study with a transporter probe. Of note, 18 of the 38 inhibitors (47 %) had no available data with acceptable probe substrates regarding their possible impact *in vivo* on known transporters, highlighting the fairly recent focus on *in vivo* transporter-mediated interaction risk assessment during drug development. The highest AUC changes (i.e., 7.1- and 9.9-fold) were observed when cyclosporine, which inhibits OATP1B1 and OATP1B3 in the liver, was co-administered with either pravastatin or rosuvastatin. All DDIs involving the efflux transporter P-gp had AUC ratios under 3, with quinidine and dronedarone showing the highest extent of inhibition. The AUC ratios for OCT2 inhibition by cimetidine and ranitidine were below 2.1. Inhibition of intestinal OATPs by grapefruit juice resulted in decreases in AUCs.

3.2. Confounding factors in defining multi-P450 inhibition

Many of the strong CYP3A4 inhibitors were classified as moderate or weak inhibitors of a second enzyme. This classification may be due (at least in part) to strong inhibition of a

minor CYP3A4 pathway of the probes used rather than moderate or weak inhibition of a second enzyme. For example, several of the compounds that are usually considered to be selective inhibitors of CYP3A4 (clarithromycin, grapefruit juice, itraconazole, erythromycin, ritonavir, troleandomycin, ketoconazole) or CYP2D6 (quinidine and paroxetine) were identified as multi-P450 inhibitors. The classification of these inhibitors as multi-P450 inhibitors is equivocal.

The confounding effect of inhibition of a minor elimination pathway was probed by simulations of a scenario in which only the minor pathway and gut metabolism by the minor pathway was inhibited strongly, with or without weak inhibition of the major pathway (Figure 1A). The simulation shows that strong inhibition of the minor pathway alone when associated with an F_g increase can cause a greater than 1.25-fold increase in probe AUC even when the inhibited pathway contributes <20 % to the systemic clearance of the probe. This can be used to rationalize observed weak inhibition of repaglinide (CYP2C8 probe) by clarithromycin and itraconazole. Neither itraconazole nor clarithromycin has been shown to inhibit CYP2C8 *in vitro* suggesting they are selective inhibitors of CYP3A4. Based on the inhibition of repaglinide clearance by the CYP2C8 inhibitor gemfibrozil, the CYP3A4 pathway contributes approximately 15 % to repaglinide clearance. However, it is possible that CYP3A4 contribution in repaglinide clearance is larger if OATP mediated uptake to hepatocytes is rate limiting for repaglinide clearance. Although it is possible that itraconazole and clarithromycin are weak inhibitors of CYP2C8 (100- to 1000-fold less potent than for CYP3A4) *in vivo*, it is likely that the interactions are caused by CYP3A4 inhibition.

Similarly, CYP3A4 contributes 13-22 % to omeprazole clearance in CYP2C19 extensive metabolizers (EMs) based on comparison to poor metabolizers (PMs). CYP3A4 is also responsible for an F_g of 0.8 for omeprazole. Hence, strong inhibition of CYP3A4 by troleandomycin likely explains the observed weak interaction with omeprazole. However, weak inhibition of CYP2C19 cannot be excluded based on this data. Due to the high potency of troleandomycin towards CYP3A4, it is not possible to differentiate between 100-fold weaker inhibition of CYP2C19, or no inhibition of CYP2C19 together with strong CYP3A4 inhibition *in vivo*.

Most P450 probe substrates have minor P450 mediated elimination pathways that contribute to their clearance. Therefore, it is expected that a multi-P450 inhibitor that affects the minor and major elimination pathways of the probe, will be classified as a stronger inhibitor *in vivo* than an inhibitor of a single P450, even if they have equal potency towards the major elimination pathway. This concept is illustrated by simulations in Figure 1B using three multi-P450 inhibition scenarios for an object drug that has an f_m of 0.87 for the major pathway. As shown in Figure 1B, the magnitude of the observed DDI increases as the potency of inhibition towards the minor pathway increases relative to the major pathway. The simulation shows that when the f_m for the major pathway of probe clearance is 0.87, a selective inhibitor cannot result in >5-fold interaction unless there is a significant effect on F_g , or the minor elimination pathway is also inhibited. However, if active uptake to hepatocytes is rate limiting and inhibited, greater interactions could be observed regardless of the P450 mediated f_m values. On the other hand, the interaction magnitude is increased to >5-fold when the minor elimination pathway is also inhibited, even if this interaction is weak. As such, multi-P450 inhibitors that are strong inhibitors of one P450 (usually CYP3A4) will be more likely to result in a detectable DDI with probe substrates. These simulations may explain the magnitude of interactions observed between ketoconazole and tolbutamide (1.8-fold) and omeprazole (2.1-fold). While ketoconazole inhibits CYP2C9 and CYP2C19 *in vitro*, it is a weak inhibitor of these enzymes *in vitro* and large *in vivo* DDIs are not expected based on specific P450 enzyme inhibition data. However, if ketoconazole

inhibits the minor CYP3A4 mediated elimination pathway of tolbutamide and omeprazole as well as the CYP2C mediated major elimination pathways of these probes, a greater *in vivo* interaction is expected. This analysis suggests that the inhibition of CYP2C9 and CYP2C19 by ketoconazole *in vivo* can mainly be detected due to the simultaneous inhibition of CYP3A4. Similarly based on the data available and as shown by the simulation (Figure 1), the moderate interaction between clarithromycin and omeprazole in CYP2C19 EMs is likely due to simultaneous inhibition of both CYP3A4 and CYP2C19. Unfortunately, no *in vitro* data to compare the potency of clarithromycin towards CYP3A4 and CYP2C19 is available. Due to the confounding effect of minor pathway inhibition, these data suggest that the use of the rank order approach for *in vivo* inhibitors of P450s will be probe dependent and cannot be considered absolute.

The evaluation of multi-P450 inhibition can also be complicated by the possible simultaneous inhibition of an uptake or efflux transporter. For example, cyclosporine was classified in our analysis as a strong CYP3A4 inhibitor based on the interactions with simvastatin and lovastatin (8- and 5-fold increase in AUC, respectively)^{44, 45} and as a moderate CYP2C8 inhibitor based on the interaction with repaglinide (Table 2). However, these interactions are likely to be due, at least in part, to inhibition of OATPs by cyclosporine.⁴⁶ Cyclosporine appears to be a weak inhibitor of CYP2C8 *in vitro* suggesting it will not inhibit CYP2C8 *in vivo* unless its circulating metabolites contribute to CYP2C8 inhibition. Based on the interactions of cyclosporine with the CYP3A4 specific substrates felodipine (AUC ratio of 1.6)⁴⁷ and oral midazolam (AUC ratio 1.5-2.2)⁴⁸ cyclosporine is classified only as a weak to moderate inhibitor of CYP3A4.⁴⁸ Similarly, the large observed extent of interaction between and itraconazole-gemfibrozil combination and loperamide as well as repaglinide, can be, at least partly, explained by the concurrent inhibition of P-glycoprotein and CYP3A4 by itraconazole and OATP and CYP2C8 by gemfibrozil.^{4, 8} Finally, the classification of quinidine as CYP3A4 inhibitor is based on its effect on fentanyl AUC. Since quinidine is a P-glycoprotein inhibitor this classification may be confounded by inhibition of P-glycoprotein as has been suggested. These examples of overlapping P450 and transporter inhibitors emphasize the importance of characterizing the possible role of transport in the overall disposition of drugs used as probe markers of metabolic enzymes.

3.3. Effect of multi-P450 inhibitors versus selective inhibitors on drug clearance

Current practice of testing *in vivo* DDIs focuses on testing for *in vivo* susceptibility of DDIs when the object drug or NCE has 25 % or more of its clearance mediated by a single enzyme. *In vivo* studies are recommended using a strong inhibitor of the given P450 because this is viewed as the worst-case scenario of *in vivo* DDIs. Whether this approach provides the worst case scenario of the substrates susceptibility to inhibition in a multiple impairment situation was first evaluated via simulation of DDI magnitude with a multi-P450 inhibitor affecting single or multiple elimination pathways of a drug cleared by three enzymes that each contribute a third to the clearance of the drug (Figure 2). The simulation suggests that a strong inhibitor of only one of these pathways will never provide the true susceptibility of the substrate to DDIs. As seen in Figure 2, a weak-to-moderate inhibitor of two of the three pathways will result in a greater interaction than what is observed with a strong inhibitor of one pathway. Although the result of this simulation is theoretically valid, its relevance to clinical situations is not well established. To evaluate this aspect, the overall magnitude of DDIs precipitated by multi-P450 inhibitors was compared to the magnitude of DDIs precipitated by single-P450 inhibitors for a set of drugs cleared by multiple P450's (diazepam, imipramine and omeprazole).

Diazepam is eliminated mainly by CYP3A4, CYP2C19 and CYP2B6.¹⁹⁻²¹ The strong CYP3A4 inhibitor itraconazole caused a 1.3-fold increase in diazepam AUC in a population with unknown genotypes.^{20, 49} In debrisoquine (CYP2D6) and mephenytoin (CYP2C19)

EMs, the moderate CYP2C19 inhibitor omeprazole also caused a 1.3-fold increase in diazepam AUC.^{20, 49} Together, these studies suggest that diazepam is not susceptible to clinically significant DDIs. However, the multi-P450 inhibitors fluconazole, voriconazole and fluvoxamine that inhibit both CYP3A4 and CYP2C19 resulted in 2.7-fold, 2.2-fold and 2.8-fold increases in diazepam AUC (subjects with unknown CYP2C19 genotype).^{50, 51} Interestingly, the interactions between the multi-P450 inhibitors and diazepam were also much greater in magnitude than the 25 % increase in diazepam AUC observed in CYP2C19 PMs with the moderate CYP3A4 inhibitor diltiazem⁵² suggesting that (i) CYP2B6 could play a significant role in diazepam clearance and (ii) the diltiazem DDI study in PM subjects failed to identify the maximal susceptibility of diazepam to multi-P450 inhibition.

Imipramine is eliminated by CYP2D6 (2-hydroxylation), as well as CYP2C19.^{24, 25} The strong inhibitors of CYP2D6, paroxetine (population with unknown CYP2D6 genotype) and quinidine (debrisoquine EMs) caused a 1.7-fold increase in imipramine AUC each^{53, 54} suggesting that the risks of CYP2D6-mediated DDIs with imipramine are modest. However, the multi-P450 inhibitors fluvoxamine, fluoxetine, cimetidine and oral contraceptives caused a 3.6 (debrisoquine EMs⁵⁵), 3.3 (genotype not known⁵⁶), 2.7 (genotype not known⁵⁷) and 2.0-fold (genotype not known⁵⁸) increase in imipramine AUC, respectively. This demonstrates that the maximal susceptibility of imipramine to *in vivo* DDIs is only established in studies with multi-P450 inhibitors.

Omeprazole is used as a CYP2C19 probe but it is also cleared by CYP3A4²³ and CYP3A4 contributes to the first pass elimination of omeprazole. The strong CYP3A4 inhibitor ketoconazole increases omeprazole AUC up to 2-fold in CYP2C19 PMs who were also debrisoquine EMs, and 1.4-fold in CYP2C19 EMs.²³ The selective moderate inhibitors of CYP2C19, etravirine and armodafinil, increased omeprazole AUC by 1.4- and 2-fold, respectively, in non-genotyped populations.^{59, 60} The moderate CYP2C19 (and CYP2D6) inhibitor moclobemide also increased omeprazole AUC by 2-fold in CYP2C19 EMs.⁶¹ However the multi-P450 inhibitors fluvoxamine and fluconazole, that inhibit both CYP3A4 and CYP2C19, resulted in 5.6-fold (CYP2C19 EMs) and 6.3-fold (genotype not known) increase in omeprazole AUC, respectively,^{62, 63} showing again that the susceptibility of omeprazole to DDIs is best evaluated with a multi-P450 inhibitor. These results are also in agreement with the simulations shown in Figure 2, which show the difference in *in vivo* DDI magnitude when multiple elimination pathways are inhibited.

Overall these data demonstrate the increased risk of interaction magnitude with simultaneous inhibition of multiple elimination pathways and support the validity of the simulations shown in Figure 2. These data show that existence of multiple P450-mediated elimination pathways does not make a drug immune to DDIs because many P450 inhibitors are inhibitors of multiple P450's *in vivo*. The data suggest that for drugs that are cleared by multiple pathways, an *in vivo* DDI study with a multi-P450 inhibitor may be more justifiable than a study with a strong single P450 inhibitor. They also suggest that more sophisticated methods are needed to assess the overall DDI risk associated with multi-P450 inhibitors. To establish the sensitivity of an object drug to DDIs, the rational selection of a multi-P450 inhibitor for clinical DDI studies may provide a more appropriate "worst-case" scenario than a strong single-P450 inhibitor. This requires a reliable identification of the quantitatively important elimination pathways of an NCE. An *in vivo* inhibitor can then be chosen to match a simultaneous inhibition of at least two elimination pathways based on the broad inhibition spectrum of the inhibitor, even if it is only weak to moderate; such an approach is preferable to the practice of selecting a strong, selective P450 inhibitor.

While it appears that the susceptibility of an NCE to DDIs (NCE as victim) is best evaluated using a multi-P450 inhibitor, the worst-case scenario for the NCE as an inhibitor (greatest

magnitude of DDIs caused by the NCE) is detected using probe drugs as substrates rather than substrates of multiple elimination pathways, despite the multi-P450 inhibition. For example all DDIs with diazepam were only moderate by classification, while all three multi-P450 inhibitors, voriconazole, fluconazole and fluvoxamine, are strong inhibitors of at least one probe substrate (Table 2). Similarly, the DDIs with imipramine were generally smaller in magnitude (2- to 3.6-fold) than what is observed with selective probes with fluvoxamine, fluconazole and voriconazole demonstrating that the worst-case scenario of inhibitor potency is better identified with well-characterized probe substrates.

3.4. Predicting *in vivo* multi-P450 inhibition from *in vitro* data

Based on the prevalence of multi-P450 inhibitors, a key question is whether the magnitude and risk of multiple impairment DDIs can be accurately predicted using static IVIVE methods or PBPK modeling. One would expect that prediction of multi-P450 interactions is more difficult than prediction of single P450 interactions since determination of multiple f_m and K_i values is required and is often challenging. To determine whether the magnitude of *in vivo* interactions with multi-P450 inhibitors could be predicted from *in vitro* data, the *in vitro* inhibitory potency and *in vivo* circulating concentrations for voriconazole, fluconazole, fluvoxamine and ketoconazole were collected and the *in vivo* interactions were predicted with the probe substrates midazolam (CYP3A4), (S)-mephenytoin (CYP2C19) and desipramine (CYP2D6), as well as with diazepam (eliminated by CYP2C19, CYP3A4 and CYP2B6), imipramine (CYP2C19, and CYP2D6) and omeprazole (CYP2C19 and CYP3A4). Predicted and observed AUC ratios are summarized in Table 4. The predictions were conducted using total and unbound inhibitor concentrations and K_i values but, with the exception of ketoconazole, incorporation of unbound fractions to the predictions did not significantly change the predicted DDI magnitude (Table 4). Similarly, using the predicted inhibitor concentrations in the gut lumen during absorption phase instead of using circulating concentrations, had a modest effect on the predicted magnitude of DDIs with midazolam and omeprazole as substrates (Table 4), perhaps due to the relatively high baseline F_g (0.57 and 0.8 for omeprazole and midazolam respectively) of these drugs. The use of the gut lumen concentrations of inhibitors resulted in predicted increase of F_g to 1 for all object drugs demonstrating that this method will likely provide the worst-case scenario for intestinal interaction and is not expected to be quantitatively accurate. Using circulating inhibitor concentrations, fluvoxamine was predicted to have no effect on midazolam and omeprazole F_g , fluconazole was predicted to increase both F_g 's by 50% and voriconazole to increase midazolam F_g by 25-35%. For ketoconazole, use of total circulating concentrations predicted an F_g increase to 1 while unbound concentrations predicted only 50% increase in the F_g of midazolam and omeprazole. This data demonstrates the need to evaluate the effect of inhibitors on F_g values even when CYP3A4 is not the major or only elimination pathway. The predictions also emphasize the need to carefully assess the dosing interval between the inhibitor and the object drug and the appropriate inhibitor concentration to use for the specific interaction when quantitatively accurate DDI predictions are required.

Overall, the DDI risk of fluconazole was correctly predicted from *in vitro* data for probe substrate (midazolam) as well as for substrates metabolized by multiple pathways (omeprazole and diazepam). Based on the predictions, fluconazole was predicted to be a moderate inhibitor of CYP3A4 and cause a moderate and strong interaction with diazepam and omeprazole, respectively. In the relevant *in vivo* studies, the same classification was observed. In the *in vivo* studies, midazolam was administered 2 hours after fluconazole, which likely explains why incorporation of predicted gut lumen concentrations during inhibitor's absorption phase overpredicted the fluconazole-midazolam interaction. Similarly, omeprazole was administered simultaneously with fluconazole partially explaining why this interaction was more accurately predicted using absorption phase gut lumen concentrations

of inhibitor (Table 4). Quantitatively, the magnitude of interactions between fluconazole and omeprazole or fluconazole and diazepam were predicted within 8-24 % of the observed interactions, demonstrating excellent prediction accuracy regardless of the number of elimination pathways inhibited.

The DDI risk of ketoconazole towards midazolam was predicted within 30% accuracy using total concentrations but 74% underpredicted using unbound inhibitor concentrations, unbound K_i values and predicted gut lumen inhibitor concentrations (Table 4). In contrast, use of unbound concentrations resulted in accurate predictions towards inhibition of omeprazole (within 3%), desipramine (within 2%) and imipramine elimination (within 20%). Although a possible interaction was predicted with imipramine (1.3-fold) using total concentrations, no true interaction was observed (1.2-fold), in agreement with predictions using unbound concentrations and K_i values. Interestingly, in the *in vivo* studies predicted, only the ketoconazole-midazolam study used 400 mg ketoconazole dosing while other studies used 200 mg q.d. suggesting a ketoconazole dose specific prediction error.

Interestingly, the interaction between voriconazole and midazolam was also underpredicted, regardless of the method used for predictions. At the same time, the interaction between voriconazole and diazepam was predicted accurately. This substrate and isoform specific discrepancy may be due to inhibitory metabolites of voriconazole contributing to CYP3A4 inhibition but not to CYP2B6 and CYP2C19 inhibition. Unfortunately, no studies with CYP2B6 and CYP2C19 probes have been reported with voriconazole to help assess the P450 enzyme specific prediction errors.

Finally, the DDI risk caused by fluvoxamine was also identified for P450 probe substrates (midazolam and (S)-mephenytoin) and for drugs cleared by multiple pathways (imipramine, omeprazole and diazepam). However, a considerable quantitative gap was observed in the prediction of the fold change in the AUC for all compounds (1.5- to 4.7-fold). The largest gap was observed in cases where CYP2C19 contribution to the substrate clearance was significant (4.7-fold and 4-fold for (S)-mephenytoin and omeprazole, respectively) and the gap decreased when other inhibited pathways contributed to the substrate clearance (2.4-fold with imipramine and 2.2-fold with diazepam). The gap in *in vitro*-to-*in vivo* predictions of CYP1A2 and CYP2C19 inhibition by fluvoxamine is well documented.^{31, 64} As reported before, the greater gap with CYP2C19 than other P450 enzymes is likely due to circulating metabolites of fluvoxamine that inhibit CYP2C19.³¹ If active uptake of fluvoxamine into hepatocytes occurs, it could also contribute to the general under-prediction.

Overall, the magnitude of inhibition by a multi-P450 inhibitor towards a substrate with multiple elimination pathways was predicted with similar or better accuracy than the inhibition of probe substrates. The data suggest that application of current static methods for predicting specific P450 inhibition from *in vitro* data is adequate for identifying potential *in vivo* inhibitors and the risk of inhibition of multiple elimination pathways simultaneously *in vivo*. Although some gaps in predictions were observed, they were probably due to unaccounted but testable mechanisms. Thus, it is encouraging that probe studies and *in vitro*-to-*in vivo* prediction methods can be applied to assess more complex prediction scenarios.

4. Concluding remarks

The aim of this study was to determine whether complex DDIs resulting from simultaneous inhibition of multiple elimination pathways of the object drug are a frequent phenomenon and need further attention in DDI risk assessment strategies and in the design of DDI studies. Based on the 38 multi-P450 inhibitors identified in the present analysis and the common use of these drugs in clinical practice, the possibility of simultaneous inhibition of

multiple elimination pathways of a drug should not be ignored. The DDI sensitivity of drugs cleared by multiple elimination pathways is likely underestimated if DDI studies are only conducted with selective P450 inhibitors. This is well illustrated in the studies of administration of gemfibrozil and itraconazole as multiple P450 inhibitors with repaglinide and loperamide.^{7, 8} Based on the inhibitors characterized here, administration of a single inhibitor may have similar effects via inhibition of multiple elimination pathways as was shown with multiple simultaneously administered inhibitors. In addition, the simulations shown here help explain the magnitude of interactions observed following co-administration of selective P450 inhibitors. It is worthwhile noting that many *in vivo* P450 inhibitors have been shown to inhibit a much broader spectrum of P450 enzymes *in vitro* than what has been studied *in vivo* and hence their overall *in vivo* interaction profile may not be adequately characterized. In addition, metabolites of the inhibitors may simultaneously inhibit additional P450s. Although this may not be important for interactions with probe substrates, it may play a role in interactions with object drugs with multiple elimination pathways.

Our analysis provides convincing *in vivo* evidence that the magnitude of DDIs is increased with multi-P450 inhibitors when co-administered with probe drugs that have a minor secondary elimination pathway by an inhibited P450, as well as with substrates of multiple P450 enzymes. This may warrant the conduct of clinical studies of nonspecific substrates with multi-P450 inhibitors to evaluate the susceptibility of a substrate to P450 inhibition. Based on the *in vitro*-to-*in vivo* predictions shown here, it appears plausible to conduct more studies that aim to predict the overall interaction magnitude *in vivo* of drugs eliminated by multiple pathways. At present, such studies are sparse and often not mechanistically driven. More comprehensive *in vitro*-to-*in vivo* predictions that account for multiple elimination pathways being inhibited may be useful in new drug development to prioritize DDI studies for the true worst-case scenario of the given substrate. Finally, the data presented suggest that for DDI risk analysis, characterizing the f_m and fraction excreted unchanged (renal clearance) of the substrate, as well as the *in vivo* DDI potency of the inhibitor using selective probes, will allow extrapolation of DDI risk to more complex multi-P450 interactions. The main limiting factors for this approach are at present the lack of reliable f_m data for many clinically used drugs and the potential inhibition of minor elimination pathways of probe substrates or concurrent inhibition of drug transporters and P450s, skewing the estimation of true P450 specific inhibition. In addition, in the absence of grapefruit juice studies and determination of absolute bioavailability, incorporation of F_g values for object drugs in predictions is difficult.

Circulating metabolites have been shown to contribute to many *in vivo* DDIs and the role of metabolites in the magnitude of observed *in vivo* interactions can be substantial.^{65, 66} However, at present there is no information on whether inhibitory metabolites are expected to share the same inhibition profile as the parent drug. As such, it is possible that P450 specific prediction errors are a result of inhibition of specific P450 enzymes by circulating metabolites and contribution of inhibitory metabolites to multi-P450 inhibition requires more study. Alternatively, it is possible that some minor elimination pathways of object drugs are less susceptible to inhibition *in vivo* than the major elimination pathways. This could be the case for example when minor elimination pathway follows saturation kinetics (high affinity substrate) and hence is less susceptible to competitive inhibition than an elimination pathway that follows linear kinetics. Such prediction errors should, however, be object drug specific.

In conclusion, multi-P450 inhibition situations should be addressed during the development of an NCE that inhibits several enzymes. Also, in most cases, IVIVE is effective in addressing and predicting these situations.

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References

1. Rowland M, Matin SB. Kinetics of drug-drug Interactions. *J Pharmacokinet Biopharm.* 1973; 1:553–567.
2. Brown HS, Ito K, Galetin A, Houston JB. Prediction of in vivo drug-drug interactions from in vitro data: impact of incorporating parallel pathways of drug elimination and inhibitor absorption rate constant. *Br J Clin Pharmacol.* 2005; 60:508–518. [PubMed: 16236041]
3. Ito K, Hallifax D, Obach RS, Houston JB. Impact of parallel pathways of drug elimination and multiple cytochrome P450 involvement on drug-drug interactions: CYP2D6 paradigm. *Drug Metab Dispos.* 2005; 33:837–844. [PubMed: 15897600]
4. Hinton LK, Galetin A, Houston JB. Multiple inhibition mechanisms and prediction of drug-drug interactions: status of metabolism and transporter models as exemplified by gemfibrozil-drug interactions. *Pharm Res.* 2008; 25:1063–1074. [PubMed: 17901929]
5. Obach RS, Ryder TF. Metabolism of ramelteon in human liver microsomes and correlation with the effect of fluvoxamine on ramelteon pharmacokinetics. *Drug Metab Dispos.* 2010; 38:1381–1391. [PubMed: 20478852]
6. Obach RS, Walsky RL, Venkatakrishnan K, Houston JB, Tremaine LM. In vitro cytochrome P450 inhibition data and the prediction of drug-drug interactions: qualitative relationships, quantitative predictions, and the rank-order approach. *Clin Pharmacol Ther.* 2005; 78:582–592. [PubMed: 16338274]
7. Niemi M, Backman JT, Neuvonen M, Neuvonen PJ. Effects of gemfibrozil, itraconazole, and their combination on the pharmacokinetics and pharmacodynamics of repaglinide: potentially hazardous interaction between gemfibrozil and repaglinide. *Diabetologia.* 2003; 46:347–351. [PubMed: 12687332]
8. Niemi M, Tornio A, Pasanen MK, Fredrikson H, Neuvonen PJ, Backman JT. Itraconazole, gemfibrozil and their combination markedly raise the plasma concentrations of loperamide. *Eur J Clin Pharmacol.* 2006; 62:463–472. [PubMed: 16758263]
9. Hachad H, Ragueneau-Majlessi I, Levy RH. A useful tool for drug interaction evaluation: the University of Washington Metabolism and Transport Drug Interaction Database. *Hum Genomics.* 2010; 5:61–72. [PubMed: 21106490]
10. Giacomini KM, Huang SM, Tweedie DJ, Benet LZ, Brouwer KL, Chu X, Dahlin A, Evers R, Fischer V, Hillgren KM, Hoffmaster KA, Ishikawa T, Keppler D, Kim RB, Lee CA, Niemi M, Polli JW, Sugiyama Y, Swaan PW, Ware JA, Wright SH, Yee SW, Zamek-Gliszczynski MJ, Zhang L. Membrane transporters in drug development. *Nature Reviews in Drug Discovery.* 2010; 9:215–236.
11. Renberg L, Simonsson R, Hoffmann KJ. Identification of two main urinary metabolites of [14C]omeprazole in humans. *Drug Metab Dispos.* 1989; 17:69–76. [PubMed: 2566473]
12. Roche. Valium package insert. Nutley, New Jersey: 2008.
13. Gram LF, Christiansen J. First-pass metabolism of imipramine in man. *Clin Pharmacol Ther.* 1975; 17:555–563. [PubMed: 1126113]
14. Nishimuta H, Sato K, Mizuki Y, Yabuki M, Komuro S. Prediction of the intestinal first-pass metabolism of CYP3A substrates in humans using cynomolgus monkeys. *Drug Metab Dispos.* 2010; 38:1967–1975. [PubMed: 20702772]
15. Galetin A, Gertz M, Houston JB. Contribution of intestinal cytochrome p450-mediated metabolism to drug-drug inhibition and induction interactions. *Drug Metab Pharmacokinet.* 2010; 25:28–47. [PubMed: 20208387]
16. Panchagnula R, Bansal T, Varma MV, Kaul CL. Co-treatment with grapefruit juice inhibits while chronic administration activates intestinal P-glycoprotein-mediated drug efflux. *Pharmazie.* 2005; 60:922–927. [PubMed: 16398269]

17. Tassaneeyakul W, Vannaprasaht S, Yamazoe Y. Formation of omeprazole sulphone but not 5-hydroxyomeprazole is inhibited by grapefruit juice. *Br J Clin Pharmacol*. 2000; 49:139–144. [PubMed: 10671908]
18. Thummel, KE.; Isoherranen, N.; Shen, D. Design and Optimization of Dosage Regimens: Pharmacokinetic Data. In: Brunton, LL., editor. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. 12. McGraw Hill; 2011. p. 1891-1990.
19. Ishizaki T, Chiba K, Manabe K, Koyama E, Hayashi M, Yasuda S, Horai Y, Tomono Y, Yamato C, Toyoki T. Comparison of the interaction potential of a new proton pump inhibitor, E3810, versus omeprazole with diazepam in extensive and poor metabolizers of S-mephenytoin 4'-hydroxylation. *Clin Pharmacol Ther*. 1995; 58:155–164. [PubMed: 7648765]
20. Ahonen J, Olkkola KT, Neuvonen PJ. The effect of the antimycotic itraconazole on the pharmacokinetics and pharmacodynamics of diazepam. *Fundam Clin Pharmacol*. 1996; 10:314–318. [PubMed: 8836707]
21. Niwa T, Shiraga T, Ishii I, Kagayama A, Takagi A. Contribution of human hepatic cytochrome p450 isoforms to the metabolism of psychotropic drugs. *Biol Pharm Bull*. 2005; 28:1711–1716. [PubMed: 16141545]
22. Andersson T, Miners JO, Veronese ME, Tassaneeyakul W, Tassaneeyakul W, Meyer UA, Birkett DJ. Identification of human liver cytochrome P450 isoforms mediating omeprazole metabolism. *Br J Clin Pharmacol*. 1993; 36:521–530. [PubMed: 12959268]
23. Bottiger Y, Tybring G, Gotharson E, Bertilsson L. Inhibition of the sulfoxidation of omeprazole by ketoconazole in poor and extensive metabolizers of S-mephenytoin. *Clin Pharmacol Ther*. 1997; 62:384–391. [PubMed: 9357389]
24. Koyama E, Tanaka T, Chiba K, Kawakatsu S, Morinobu S, Totsuka S, Ishizaki T. Steady-state plasma concentrations of imipramine and desipramine in relation to S-mephenytoin 4'-hydroxylation status in Japanese depressive patients. *J Clin Psychopharmacol*. 1996; 16:286–293. [PubMed: 8835703]
25. Brosen K, Klysner R, Gram LF, Otton SV, Bech P, Bertilsson L. Steady-state concentrations of imipramine and its metabolites in relation to the sparteine/debrisoquine polymorphism. *Eur J Clin Pharmacol*. 1986; 30:679–684. [PubMed: 3533565]
26. Koyama E, Chiba K, Tani M, Ishizaki T. Reappraisal of human CYP isoforms involved in imipramine N-demethylation and 2-hydroxylation: a study using microsomes obtained from putative extensive and poor metabolizers of S-mephenytoin and eleven recombinant human CYPs. *J Pharmacol Exp Ther*. 1997; 281:1199–1210. [PubMed: 9190854]
27. Obach RS, Walsky RL, Venkatakrishnan K, Gaman EA, Houston JB, Tremaine LM. The utility of *in vitro* cytochrome P450 inhibition data in the prediction of drug-drug interactions. *J Pharmacol Exp Ther*. 2006; 316:336–348. [PubMed: 16192315]
28. Yu KS, Yim DS, Cho JY, Park SS, Park JY, Lee KH, Jang IJ, Yi SY, Bae KS, Shin SG. Effect of omeprazole on the pharmacokinetics of moclobemide according to the genetic polymorphism of CYP2C19. *Clin Pharmacol Ther*. 2001; 69:266–273. [PubMed: 11309556]
29. Paine MF, Shen DD, Kunze KL, Perkins JD, Marsh CL, McVicar JP, Barr DM, Gillies BS, Thummel KE. First-pass metabolism of midazolam by the human intestine. *Clin Pharmacol Ther*. 1996; 60:14–24. [PubMed: 8689807]
30. Hallifax D, Houston JB. Binding of drugs to hepatic microsomes: comment and assessment of current prediction methodology with recommendation for improvement. *Drug Metab Dispos*. 2006; 34:724–726. [PubMed: 16552024]
31. Yao C, Kunze KL, Trager WF, Kharasch ED, Levy RH. Comparison of *in vitro* and *in vivo* inhibition potencies of fluvoxamine toward CYP2C19. *Drug Metab Dispos*. 2003; 31:565–571. [PubMed: 12695344]
32. Austin RP, Barton P, Cockcroft SL, Wenlock MC, Riley RJ. The influence of nonspecific microsomal binding on apparent intrinsic clearance, and its prediction from physicochemical properties. *Drug Metab Dispos*. 2002; 30:1497–1503. [PubMed: 12433825]
33. Gibbs MA, Thummel KE, Shen DD, Kunze KL. Inhibition of cytochrome P-450 3A (CYP3A) in human intestinal and liver microsomes: comparison of Ki values and impact of CYP3A5 expression. *Drug Metab Dispos*. 1999; 27:180–187. [PubMed: 9929500]

34. Wienkers LC, Wurden CJ, Storch E, Kunze KL, Rettie AE, Trager WF. Formation of (R)-8-hydroxywarfarin in human liver microsomes. A new metabolic marker for the (S)-mephenytoin hydroxylase, P4502C19. *Drug Metab Dispos.* 1996; 24:610–614. [PubMed: 8723744]
35. Otton SV, Ball SE, Cheung SW, Inaba T, Rudolph RL, Sellers EM. Venlafaxine oxidation in vitro is catalysed by CYP2D6. *Br J Clin Pharmacol.* 1996; 41:149–156. [PubMed: 8838442]
36. Foti RS, Rock DA, Wienkers LC, Wahlstrom JL. Selection of alternative CYP3A4 probe substrates for clinical drug interaction studies using in vitro data and in vivo simulation. *Drug Metab Dispos.* 2010; 38:981–987. [PubMed: 20203109]
37. Emoto C, Murase S, Sawada Y, Jones BC, Iwasaki K. In vitro inhibitory effect of 1-aminobenzotriazole on drug oxidations catalyzed by human cytochrome P450 enzymes: a comparison with SKF-525A and ketoconazole. *Drug Metab Pharmacokinet.* 2003; 18:287–295. [PubMed: 15618748]
38. Galetin A, Ito K, Hallifax D, Houston JB. CYP3A4 substrate selection and substitution in the prediction of potential drug-drug interactions. *J Pharmacol Exp Ther.* 2005; 314:180–190. [PubMed: 15784650]
39. Jeong S, Nguyen PD, Desta Z. Comprehensive *in vitro* analysis of voriconazole inhibition of eight cytochrome P450 (CYP) enzymes: major effect on CYPs 2B6, 2C9, 2C19, and 3A. *Antimicrob Agents Chemother.* 2009; 53:541–551. [PubMed: 19029318]
40. Guest EJ, Rowland-Yeo K, Rostami-Hodjegan A, Tucker GT, Houston JB, Galetin A. Assessment of algorithms for predicting drug-drug interactions via inhibition mechanisms: comparison of dynamic and static models. *Br J Clin Pharmacol.* 2011; 71:72–87. [PubMed: 21143503]
41. Rostami-Hodjegan A, Tucker G. 'In silico' simulations to assess the 'in vivo' consequences of 'in vitro' metabolic drug-drug interactions. *Drug Discov Today.* 2004; 1:441–448.
42. Mayhew BS, Jones DR, Hall SD. An in vitro model for predicting in vivo inhibition of cytochrome P450 3A4 by metabolic intermediate complex formation. *Drug Metab Dispos.* 2000; 28:1031–1037. [PubMed: 10950845]
43. Thummel, KE.; Shen, DD. The Role of the Gut Mucosa in Metabolically Based Drug-Drug Interactions. In: Rodrigues, AD., editor. *Drug-Drug Interactions*. Marcel Dekker, Inc; New York: 2001. p. 359–385.
44. Ichimaru N, Takahara S, Kokado Y, Wang JD, Hatori M, Kameoka H, Inoue T, Okuyama A. Changes in lipid metabolism and effect of simvastatin in renal transplant recipients induced by cyclosporine or tacrolimus. *Atherosclerosis.* 2001; 158:417–423. [PubMed: 11583721]
45. Gullestad L, Nordal KP, Berg KJ, Cheng H, Schwartz MS, Simonsen S. Interaction between lovastatin and cyclosporine A after heart and kidney transplantation. *Transplant Proc.* 1999; 31:2163–2165. [PubMed: 10456002]
46. Koenen A, Kroemer HK, Grube M, Meyer Zu Schwabedissen HE. Current understanding of hepatic and intestinal OATP-mediated drug-drug interactions. *Expert Rev Clin Pharmacol.* 2011; 4:729–742. [PubMed: 22111859]
47. Madsen JK, Jensen JD, Jensen LW, Pedersen EB. Pharmacokinetic interaction between cyclosporine and the dihydropyridine calcium antagonist felodipine. *Eur J Clin Pharmacol.* 1996; 50:203–208. [PubMed: 8737760]
48. de Jonge H, de Loor H, Verbeke K, Vanrenterghem Y, Kuypers DR. In vivo CYP3A activity is significantly lower in cyclosporine-treated as compared with tacrolimus-treated renal allograft recipients. *Clin Pharmacol Ther.* 2011; 90:414–422. [PubMed: 21753749]
49. Caraco Y, Tateishi T, Wood AJ. Interethnic difference in omeprazole's inhibition of diazepam metabolism. *Clin Pharmacol Ther.* 1995; 58:62–72. [PubMed: 7628184]
50. Saari TI, Laine K, Bertilsson L, Neuvonen PJ, Olkkola KT. Voriconazole and fluconazole increase the exposure to oral diazepam. *Eur J Clin Pharmacol.* 2007; 63:941–949. [PubMed: 17676319]
51. Perucca E, Gatti G, Cipolla G, Spina E, Barel S, Soback S, Gips M, Bialer M. Inhibition of diazepam metabolism by fluvoxamine: a pharmacokinetic study in normal volunteers. *Clin Pharmacol Ther.* 1994; 56:471–476. [PubMed: 7955810]
52. Kosuge K, Jun Y, Watanabe H, Kimura M, Nishimoto M, Ishizaki T, Ohashi K. Effects of CYP3A4 inhibition by diltiazem on pharmacokinetics and dynamics of diazepam in relation to CYP2C19 genotype status. *Drug Metab Dispos.* 2001; 29:1284–1289. [PubMed: 11560871]

53. Albers LJ, Reist C, Helmeste D, Vu R, Tang SW. Paroxetine shifts imipramine metabolism. *Psychiatry Res.* 1996; 59:189–196. [PubMed: 8930024]
54. Brosen K, Gram LF. Quinidine inhibits the 2-hydroxylation of imipramine and desipramine but not the demethylation of imipramine. *Eur J Clin Pharmacol.* 1989; 37:155–160. [PubMed: 2792169]
55. Spina E, Pollicino AM, Avenoso A, Campo GM, Perucca E, Caputi AP. Effect of fluvoxamine on the pharmacokinetics of imipramine and desipramine in healthy subjects. *Ther Drug Monit.* 1993; 15:243–246. [PubMed: 8333005]
56. Abernethy DR, Greenblatt DJ, Shader RI. Imipramine-cimetidine interaction: impairment of clearance and enhanced absolute bioavailability. *J Pharmacol Exp Ther.* 1984; 229:702–705. [PubMed: 6726654]
57. Bergstrom RF, Peyton AL, Lemberger L. Quantification and mechanism of the fluoxetine and tricyclic antidepressant interaction. *Clin Pharmacol Ther.* 1992; 51:239–248. [PubMed: 1544284]
58. Abernethy DR, Greenblatt DJ, Shader RI. Imipramine disposition in users of oral contraceptive steroids. *Clin Pharmacol Ther.* 1984; 35:792–797.
59. Tibotec. Intelence (etravirine) tablets, NDA#022187. Yardley, PA: 2008.
60. Darwish M, Kirby M, Robertson P Jr, Hellriegel ET. Interaction profile of armodafinil with medications metabolized by cytochrome P450 enzymes 1A2, 3A4 and 2C19 in healthy subjects. *Clinical pharmacokinetics.* 2008; 47:61–74. [PubMed: 18076219]
61. Cho JY, Yu KS, Jang IJ, Yang BH, Shin SG, Yim DS. Omeprazole hydroxylation is inhibited by a single dose of moclobemide in homozygotic EM genotype for CYP2C19. *Br J Clin Pharmacol.* 2002; 53:393–397. [PubMed: 11966672]
62. Kang BC, Yang CQ, Cho HK, Suh OK, Shin WG. Influence of fluconazole on the pharmacokinetics of omeprazole in healthy volunteers. *Biopharm Drug Dispos.* 2002; 23:77–81. [PubMed: 11932962]
63. Yasui-Furukori N, Takahata T, Nakagami T, Yoshiya G, Inoue Y, Kaneko S, Tateishi T. Different inhibitory effect of fluvoxamine on omeprazole metabolism between CYP2C19 genotypes. *Br J Clin Pharmacol.* 2004; 57:487–494. [PubMed: 15025747]
64. Yao C, Kunze KL, Kharasch ED, Wang Y, Trager WF, Ragueneau I, Levy RH. Fluvoxamine-theophylline interaction: gap between *in vitro* and *in vivo* inhibition constants toward cytochrome P4501A2. *Clin Pharmacol Ther.* 2001; 70:415–424. [PubMed: 11719727]
65. Isoherranen N, Hachad H, Yeung CK, Levy RH. Qualitative analysis of the role of metabolites in inhibitory drug-drug interactions: literature evaluation based on the metabolism and transport drug interaction database. *Chem Res Toxicol.* 2009; 22:294–298. [PubMed: 19216580]
66. Yeung CK, Fujioka Y, Hachad H, Levy RH, Isoherranen N. Are circulating metabolites important in drug-drug interactions?: Quantitative analysis of risk prediction and inhibitory potency. *Clin Pharmacol Ther.* 2011; 89:105–113. [PubMed: 21124313]
67. Schmider J, Brockmoller J, Arold G, Bauer S, Roots I. Simultaneous assessment of CYP3A4 and CYP1A2 activity *in vivo* with alprazolam and caffeine. *Pharmacogenetics.* 1999; 9:725–734. [PubMed: 10634135]
68. Buch AB, Van Harken DR, Seidehamel RJ, Barbhuiya RH. A study of pharmacokinetic interaction between buspirone and alprazolam at steady state. *J Clin Pharmacol.* 1993; 33:1104–1109. [PubMed: 8300893]
69. Becquemont L, Neuvonen M, Verstuyft C, Jaillon P, Letierce A, Neuvonen PJ, Funck-Brentano C. Amiodarone interacts with simvastatin but not with pravastatin disposition kinetics. *Clin Pharmacol Ther.* 2007; 81:679–684. [PubMed: 17301736]
70. O'Reilly RA, Trager WF, Rettie AE, Goulart DA. Interaction of amiodarone with racemic warfarin and its separated enantiomorphs in humans. *Clin Pharmacol Ther.* 1987; 42:290–294. [PubMed: 3621782]
71. Werner D, Wuttke H, Fromm MF, Schaefer S, Eschenhagen T, Brune K, Daniel WG, Werner U. Effect of amiodarone on the plasma levels of metoprolol. *Am J Cardiol.* 2004; 94:1319–1321. [PubMed: 15541258]
72. Davis RL, Quenzer RW, Kelly HW, Powell JR. Effect of the addition of ciprofloxacin on theophylline pharmacokinetics in subjects inhibited by cimetidine. *Ann Pharmacother.* 1992; 26:11–13. [PubMed: 1606331]

73. Schoerlin MP, Mayersohn M, Hoevels B, Eggers H, Dellenbach M, Pfefen JP. Cimetidine alters the disposition kinetics of the monoamine oxidase-A inhibitor moclobemide. *Clin Pharmacol Ther.* 1991; 49:32–38. [PubMed: 1988238]
74. Iteogu MO, Murphy JE, Shleifer N, Davis R. Effect of cimetidine on single-dose phenytoin kinetics. *Clin Pharm.* 1983; 2:302–304. [PubMed: 6883958]
75. Mutschler E, Spahn H, Kirch W. The interaction between H₂-receptor antagonists and beta-adrenoceptor blockers. *Br J Clin Pharmacol.* 1984; 17(Suppl 1):51S–57S. [PubMed: 6146340]
76. Pourbaix S, Desager JP, Hulhoven R, Smith RB, Harvengt C. Pharmacokinetic consequences of long term coadministration of cimetidine and triazolobenzodiazepines, alprazolam and triazolam, in healthy subjects. *Int J Clin Pharmacol Ther Toxicol.* 1985; 23:447–451. [PubMed: 2864320]
77. Kustra R, Corrigan B, Dunn J, Duncan B, Hsyu PH. Lack of effect of cimetidine on the pharmacokinetics of sustained-release bupropion. *J Clin Pharmacol.* 1999; 39:1184–1188. [PubMed: 10579150]
78. Hatorp V, Thomsen MS. Drug interaction studies with repaglinide: repaglinide on digoxin or theophylline pharmacokinetics and cimetidine on repaglinide pharmacokinetics. *J Clin Pharmacol.* 2000; 40:184–192. [PubMed: 10664925]
79. Granfors MT, Backman JT, Neuvonen M, Neuvonen PJ. Ciprofloxacin greatly increases concentrations and hypotensive effect of tizanidine by inhibiting its cytochrome P450 1A2-mediated presystemic metabolism. *Clin Pharmacol Ther.* 2004; 76:598–606. [PubMed: 15592331]
80. Hedaya MA, El-Afify DR, El-Maghraby GM. The effect of ciprofloxacin and clarithromycin on sildenafil oral bioavailability in human volunteers. *Biopharm Drug Dispos.* 2006; 27:103–110. [PubMed: 16372380]
81. Washington C, Hou SY, Hughes NC, Campanella C, Berner B. Ciprofloxacin prolonged-release tablets do not affect warfarin pharmacokinetics and pharmacodynamics. *J Clin Pharmacol.* 2007; 47:1320–1326. [PubMed: 17724088]
82. Kumar JN, Devi P, Narasu L, Mullangi R. Effect of ciprofloxacin and ibuprofen on the *in vitro* metabolism of rosiglitazone and oral pharmacokinetics of rosiglitazone in healthy human volunteers. *Eur J Drug Metab Pharmacokinet.* 2008; 33:237–242. [PubMed: 19230597]
83. Furuta T, Ohashi K, Kobayashi K, Iida I, Yoshida H, Shirai N, Takashima M, Kosuge K, Hanai H, Chiba K, Ishizaki T, Kaneko E. Effects of clarithromycin on the metabolism of omeprazole in relation to CYP2C19 genotype status in humans. *Clin Pharmacol Ther.* 1999; 66:265–274. [PubMed: 10511062]
84. Niemi M, Neuvonen PJ, Kivisto KT. The cytochrome P4503A4 inhibitor clarithromycin increases the plasma concentrations and effects of repaglinide. *Clin Pharmacol Ther.* 2001; 70:58–65. [PubMed: 11452245]
85. Jayasagar G, Dixit AA, Kishan V, Rao YM. Effect of clarithromycin on the pharmacokinetics of tolbutamide. *Drug Metabol Drug Interact.* 2000; 16:207–215. [PubMed: 11116754]
86. Jacobson TA. Comparative pharmacokinetic interaction profiles of pravastatin, simvastatin, and atorvastatin when coadministered with cytochrome P450 inhibitors. *Am J Cardiol.* 2004; 94:1140–1146. [PubMed: 15518608]
87. Bruce MA, Hall SD, Haehner-Daniels BD, Gorski JC. *In vivo* effect of clarithromycin on multiple cytochrome P450s. *Drug Metab Dispos.* 2001; 29:1023–1028. [PubMed: 11408369]
88. Gillum JG, Israel DS, Scott RB, Climo MW, Polk RE. Effect of combination therapy with ciprofloxacin and clarithromycin on theophylline pharmacokinetics in healthy volunteers. *Antimicrob Agents Chemother.* 1996; 40:1715–1716. [PubMed: 8807068]
89. Turpeinen M, Tolonen A, Uusitalo J, Jalonen J, Pelkonen O, Laine K. Effect of clopidogrel and ticlopidine on cytochrome P450 2B6 activity as measured by bupropion hydroxylation. *Clin Pharmacol Ther.* 2005; 77:553–559. [PubMed: 15961986]
90. Chen BL, Chen Y, Tu JH, Li YL, Zhang W, Li Q, Fan L, Tan ZR, Hu DL, Wang D, Wang LS, Ouyang DS, Zhou HH. Clopidogrel inhibits CYP2C19-dependent hydroxylation of omeprazole related to CYP2C19 genetic polymorphisms. *J Clin Pharmacol.* 2009; 49:574–581. [PubMed: 19398604]
91. Caplain H, Thebault JJ, Necciari J. Clopidogrel does not affect the pharmacokinetics of theophylline. *Semin Thromb Hemost.* 1999; 25(Suppl 2):65–68. [PubMed: 10440427]

92. Kajosaari LI, Niemi M, Neuvonen M, Laitila J, Neuvonen PJ, Backman JT. Cyclosporine markedly raises the plasma concentrations of repaglinide. *Clin Pharmacol Ther.* 2005; 78:388–399. [PubMed: 16198658]
93. Park JW, Siekmeier R, Lattke P, Merz M, Mix C, Schuler S, Jaross W. Pharmacokinetics and pharmacodynamics of fluvastatin in heart transplant recipients taking cyclosporine A. *J Cardiovasc Pharmacol Ther.* 2001; 6:351–361. [PubMed: 11907637]
94. Nafziger AN, May JJ, Bertino JS Jr. Inhibition of theophylline elimination by diltiazem therapy. *J Clin Pharmacol.* 1987; 27:862–865. [PubMed: 3429693]
95. Tateishi T, Nakashima H, Shitou T, Kumagai Y, Ohashi K, Hosoda S, Ebihara A. Effect of diltiazem on the pharmacokinetics of propranolol, metoprolol and atenolol. *Eur J Clin Pharmacol.* 1989; 36:67–70. [PubMed: 2917591]
96. Mousa O, Brater DC, Sunblad KJ, Hall SD. The interaction of diltiazem with simvastatin. *Clin Pharmacol Ther.* 2000; 67:267–274. [PubMed: 10741630]
97. Dixit AA, Rao YM. Pharmacokinetic interaction between diltiazem and tolbutamide. *Drug Metabol Drug Interact.* 1999; 15:269–277. [PubMed: 10716041]
98. Abernethy DR, Kaminsky LS, Dickinson TH. Selective inhibition of warfarin metabolism by diltiazem in humans. *J Pharmacol Exp Ther.* 1991; 257:411–415. [PubMed: 2020000]
99. Frye RF, Tammara B, Cowart TD, Bramer SL. Effect of disulfiram-mediated CYP2E1 inhibition on the disposition of vesnarinone. *J Clin Pharmacol.* 1999; 39:1177–1183. [PubMed: 10579149]
100. Loi CM, Day JD, Jue SG, Bush ED, Costello P, Dewey LV, Vestal RE. Dose-dependent inhibition of theophylline metabolism by disulfiram in recovering alcoholics. *Clin Pharmacol Ther.* 1989; 45:476–486. [PubMed: 2721103]
101. Svendsen TL, Kristensen MB, Hansen JM, Skovsted L. The influence of disulfiram on the half life and metabolic clearance rate of diphenylhydantoin and tolbutamide in man. *Eur J Clin Pharmacol.* 1976; 09:439–441. [PubMed: 971708]
102. Ciraulo DA, Barnhill J, Boxenbaum H. Pharmacokinetic interaction of disulfiram and antidepressants. *Am J Psychiatry.* 1985; 142:1373–1374. [PubMed: 4061700]
103. Kharasch ED, Hankins DC, Jubert C, Thummel KE, Taraday JK. Lack of single-dose disulfiram effects on cytochrome P-450 2C9, 2C19, 2D6, and 3A4 activities: evidence for specificity toward P-450 2E1. *Drug Metab Dispos.* 1999; 27:717–723. [PubMed: 10348802]
104. Sanofi-Aventis. Multaq (dronedarone) tablets, NDA#022425. Bridgewater, NJ: 2009.
105. Gorski JC, Huang SM, Pinto A, Hamman MA, Hilligoss JK, Zaheer NA, Desai M, Miller M, Hall SD. The effect of echinacea (*Echinacea purpurea* root) on cytochrome P450 activity *in vivo*. *Clin Pharmacol Ther.* 2004; 75:89–100. [PubMed: 14749695]
106. Prince RA, Wing DS, Weinberger MM, Hendeles LS, Riegelman S. Effect of erythromycin on theophylline kinetics. *J Allergy Clin Immunol.* 1981; 68:427–431. [PubMed: 7031111]
107. Williamson KM, Patterson JH, McQueen RH, Adams KF Jr, Pieper JA. Effects of erythromycin or rifampin on losartan pharmacokinetics in healthy volunteers. *Clin Pharmacol Ther.* 1998; 63:316–323. [PubMed: 9542475]
108. Kantola T, Kivisto KT, Neuvonen PJ. Erythromycin and verapamil considerably increase serum simvastatin and simvastatin acid concentrations. *Clin Pharmacol Ther.* 1998; 64:177–182. [PubMed: 9728898]
109. Black DJ, Kunze KL, Wienkers LC, Gidal BE, Seaton TL, McDonnell ND, Evans JS, Bauwens JE, Trager WF. Warfarin-fluconazole. II. A metabolically based drug interaction *in vivo* studies. *Drug Metab Dispos.* 1996; 24:422–428. [PubMed: 8801057]
110. Kharasch ED, Walker A, Hoffer C, Sheffels P. Sensitivity of intravenous and oral alfentanil and pupillary miosis as minimally invasive and noninvasive probes for hepatic and first-pass CYP3A activity. *J Clin Pharmacol.* 2005; 45:1187–1197. [PubMed: 16172184]
111. Konishi H, Morita K, Yamaji A. Effect of fluconazole on theophylline disposition in humans. *Eur J Clin Pharmacol.* 1994; 46:309–312. [PubMed: 7957514]
112. Dingemans J, Wallnofer A, Gieschke R, Guentert T, Amrein R. Pharmacokinetic and pharmacodynamic interactions between fluoxetine and moclobemide in the investigation of development of the “serotonin syndrome”. *Clin Pharmacol Ther.* 1998; 63:403–413. [PubMed: 9585794]

113. Lam YW, Alfaro CL, Ereshefsky L, Miller M. Pharmacokinetic and pharmacodynamic interactions of oral midazolam with ketoconazole, fluoxetine, fluvoxamine, and nefazodone. *J Clin Pharmacol*. 2003; 43:1274–1282. [PubMed: 14551182]
114. Granfors MT, Backman JT, Neuvonen M, Ahonen J, Neuvonen PJ. Fluvoxamine drastically increases concentrations and effects of tizanidine: a potentially hazardous interaction. *Clin Pharmacol Ther*. 2004; 75:331–341. [PubMed: 15060511]
115. Lamberg TS, Kivisto KT, Laitila J, Martensson K, Neuvonen PJ. The effect of fluvoxamine on the pharmacokinetics and pharmacodynamics of buspirone. *Eur J Clin Pharmacol*. 1998; 54:761–766. [PubMed: 9923581]
116. Madsen H, Enggaard TP, Hansen LL, Klitgaard NA, Brosen K. Fluvoxamine inhibits the CYP2C9 catalyzed biotransformation of tolbutamide. *Clin Pharmacol Ther*. 2001; 69:41–47. [PubMed: 11180037]
117. Pedersen RS, Damkier P, Brosen K. The effects of human CYP2C8 genotype and fluvoxamine on the pharmacokinetics of rosiglitazone in healthy subjects. *Br J Clin Pharmacol*. 2006; 62:682–689. [PubMed: 16856883]
118. Bidstrup TB, Damkier P, Olsen AK, Ekblom M, Karlsson A, Brosen K. The impact of CYP2C8 polymorphism and grapefruit juice on the pharmacokinetics of repaglinide. *Br J Clin Pharmacol*. 2006; 61:49–57. [PubMed: 16390351]
119. Fuhr U, Klittich K, Staib AH. Inhibitory effect of grapefruit juice and its bitter principal, naringenin, on CYP1A2 dependent metabolism of caffeine in man. *Br J Clin Pharmacol*. 1993; 35:431–436. [PubMed: 8485024]
120. Lilja JJ, Kivisto KT, Neuvonen PJ. Grapefruit juice-simvastatin interaction: effect on serum concentrations of simvastatin, simvastatin acid, and HMG-CoA reductase inhibitors. *Clin Pharmacol Ther*. 1998; 64:477–483. [PubMed: 9834039]
121. Uno T, Yasui-Furukori N, Takahata T, Sugawara K, Tateishi T. Lack of significant effect of grapefruit juice on the pharmacokinetics of lansoprazole and its metabolites in subjects with different CYP2C19 genotypes. *J Clin Pharmacol*. 2005; 45:690–694. [PubMed: 15901751]
122. Zaidenstein R, Soback S, Gips M, Avni B, Dishy V, Weissgarten Y, Golik A, Scapa E. Effect of grapefruit juice on the pharmacokinetics of losartan and its active metabolite E3174 in healthy volunteers. *Ther Drug Monit*. 2001; 23:369–373. [PubMed: 11477318]
123. O'Shea D, Kim RB, Wilkinson GR. Modulation of CYP2E1 activity by isoniazid in rapid and slow N-acetylators. *Br J Clin Pharmacol*. 1997; 43:99–103. [PubMed: 9056059]
124. Samigun, Mulyono; Santoso, B. Lowering of theophylline clearance by isoniazid in slow and rapid acetylators. *Br J Clin Pharmacol*. 1990; 29:570–573. [PubMed: 2350534]
125. Ochs HR, Greenblatt DJ, Knuchel M. Differential effect of isoniazid on triazolam oxidation and oxazepam conjugation. *Br J Clin Pharmacol*. 1983; 16:743–746. [PubMed: 6140941]
126. Ducharme MP, Slaughter RL, Warbasse LH, Chandrasekar PH, Van de Velde V, Mannens G, Edwards DJ. Itraconazole and hydroxyitraconazole serum concentrations are reduced more than tenfold by phenytoin. *Clin Pharmacol Ther*. 1995; 58:617–624. [PubMed: 8529326]
127. Neuvonen PJ, Jalava KM. Itraconazole drastically increases plasma concentrations of lovastatin and lovastatin acid. *Clin Pharmacol Ther*. 1996; 60:54–61. [PubMed: 8689812]
128. Park JY, Kim KA, Shin JG, Lee KY. Effect of ketoconazole on the pharmacokinetics of rosiglitazone in healthy subjects. *Br J Clin Pharmacol*. 2004; 58:397–402. [PubMed: 15373932]
129. Krishnaiah YS, Satyanarayana S, Visweswaram D. Interaction between tolbutamide and ketoconazole in healthy subjects. *Br J Clin Pharmacol*. 1994; 37:205–207. [PubMed: 8186066]
130. Varhe A, Olkkola KT, Neuvonen PJ. Oral triazolam is potentially hazardous to patients receiving systemic antimycotics ketoconazole or itraconazole. *Clin Pharmacol Ther*. 1994; 56:601–607. [PubMed: 7995001]
131. Brown MW, Maldonado AL, Meredith CG, Speeg KV Jr. Effect of ketoconazole on hepatic oxidative drug metabolism. *Clin Pharmacol Ther*. 1985; 37:290–297. [PubMed: 3971653]
132. Wahllander A, Paumgartner G. Effect of ketoconazole and terbinafine on the pharmacokinetics of caffeine in healthy volunteers. *Eur J Clin Pharmacol*. 1989; 37:279–283. [PubMed: 2612543]

133. Spina E, Avenoso A, Campo GM, Scordo MG, Caputi AP, Perucca E. Effect of ketoconazole on the pharmacokinetics of imipramine and desipramine in healthy subjects. *Br J Clin Pharmacol*. 1997; 43:315–318. [PubMed: 9088587]
134. Hartter S, Dingemanse J, Baier D, Ziegler G, Hiemke C. Inhibition of dextromethorphan metabolism by moclobemide. *Psychopharmacology (Berl)*. 1998; 135:22–26. [PubMed: 9489930]
135. Prichard PJ, Walt RP, Kitchingman GK, Somerville KW, Langman MJ, Williams J, Richens A. Oral phenytoin pharmacokinetics during omeprazole therapy. *Br J Clin Pharmacol*. 1987; 24:543–545. [PubMed: 3689634]
136. Winston A, Back D, Fletcher C, Robinson L, Unsworth J, Tolowinska I, Schutz M, Pozniak AL, Gazzard B, Boffito M. Effect of omeprazole on the pharmacokinetics of saquinavir-500 mg formulation with ritonavir in healthy male and female volunteers. *AIDS (London, England)*. 2006; 20:1401–1406.
137. Rost KL, Fuhr U, Thomsen T, Zaigler M, Brockmoller J, Bohnemeier H, Roots I. Omeprazole weakly inhibits CYP1A2 activity in man. *Int J Clin Pharmacol Ther*. 1999; 37:567–574. [PubMed: 10584979]
138. Uno T, Sugimoto K, Sugawara K, Tateishi T. The role of cytochrome P2C19 in R-warfarin pharmacokinetics and its interaction with omeprazole. *Ther Drug Monit*. 2008; 30:276–281. [PubMed: 18520598]
139. Hilli J, Korhonen T, Turpeinen M, Hokkanen J, Mattila S, Laine K. The effect of oral contraceptives on the pharmacokinetics of melatonin in healthy subjects with CYP1A2 g.-163C>A polymorphism. *J Clin Pharmacol*. 2008; 48:986–994. [PubMed: 18490497]
140. Palovaara S, Tybring G, Laine K. The effect of ethinylloestradiol and levonorgestrel on the CYP2C19-mediated metabolism of omeprazole in healthy female subjects. *Br J Clin Pharmacol*. 2003; 56:232–237. [PubMed: 12895199]
141. Kendall MJ, Jack DB, Quarterman CP, Smith SR, Zaman R. Beta-adrenoceptor blocker pharmacokinetics and the oral contraceptive pill. *Br J Clin Pharmacol*. 1984; 17(Suppl 1):87S–89S. [PubMed: 6146344]
142. Stoehr GP, Kroboth PD, Juhl RP, Wender DB, Phillips JP, Smith RB. Effect of oral contraceptives on triazolam, temazepam, alprazolam, and lorazepam kinetics. *Clin Pharmacol Ther*. 1984; 36:683–690. [PubMed: 6149030]
143. Palovaara S, Pelkonen O, Uusitalo J, Lundgren S, Laine K. Inhibition of cytochrome P450 2B6 activity by hormone replacement therapy and oral contraceptive as measured by bupropion hydroxylation. *Clin Pharmacol Ther*. 2003; 74:326–333. [PubMed: 14534519]
144. Hatorp V, Hansen KT, Thomsen MS. Influence of drugs interacting with CYP3A4 on the pharmacokinetics, pharmacodynamics, and safety of the prandial glucose regulator repaglinide. *J Clin Pharmacol*. 2003; 43:649–660. [PubMed: 12817528]
145. Skinner MH, Kuan HY, Pan A, Sathirakul K, Knadler MP, Gonzales CR, Yeo KP, Reddy S, Lim M, Ayan-Oshodi M, Wise SD. Duloxetine is both an inhibitor and a substrate of cytochrome P4502D6 in healthy volunteers. *Clin Pharmacol Ther*. 2003; 73:170–177. [PubMed: 12621382]
146. Belle DJ, Ernest CS, Sauer JM, Smith BP, Thomasson HR, Witcher JW. Effect of potent CYP2D6 inhibition by paroxetine on atomoxetine pharmacokinetics. *J Clin Pharmacol*. 2002; 42:1219–1227. [PubMed: 12412820]
147. Martin DE, Zussman BD, Everitt DE, Benincosa LJ, Etheredge RC, Jorkasky DK. Paroxetine does not affect the cardiac safety and pharmacokinetics of terfenadine in healthy adult men. *J Clin Psychopharmacol*. 1997; 17:451–459. [PubMed: 9408807]
148. Bannister SJ, Houser VP, Hulse JD, Kisicki JC, Rasmussen JG. Evaluation of the potential for interactions of paroxetine with diazepam, cimetidine, warfarin, and digoxin. *Acta Psychiatr Scand Suppl*. 1989; 350:102–106. [PubMed: 2530759]
149. Bano G, Raina RK, Zutshi U, Bedi KL, Johri RK, Sharma SC. Effect of piperine on bioavailability and pharmacokinetics of propranolol and theophylline in healthy volunteers. *Eur J Clin Pharmacol*. 1991; 41:615–617. [PubMed: 1815977]

150. Velpandian T, Jasuja R, Bhardwaj RK, Jaiswal J, Gupta SK. Piperine in food: interference in the pharmacokinetics of phenytoin. *Eur J Drug Metab Pharmacokinet*. 2001; 26:241–247. [PubMed: 11808866]
151. Spinler SA, Gammaitoni A, Charland SL, Hurwitz J. Propafenone-theophylline interaction. *Pharmacotherapy*. 1993; 13:68–71. [PubMed: 8437970]
152. Wagner F, Kalusche D, Trenk D, Jahnchen E, Roskamm H. Drug interaction between propafenone and metoprolol. *Br J Clin Pharmacol*. 1987; 24:213–220. [PubMed: 3620296]
153. Pope LE, Khalil MH, Berg JE, Stiles M, Yakatan GJ, Sellers EM. Pharmacokinetics of dextromethorphan after single or multiple dosing in combination with quinidine in extensive and poor metabolizers. *J Clin Pharmacol*. 2004; 44:1132–1142. [PubMed: 15342614]
154. Kharasch ED, Hoffer C, Altuntas TG, Whittington D. Quinidine as a probe for the role of p-glycoprotein in the intestinal absorption and clinical effects of fentanyl. *J Clin Pharmacol*. 2004; 44:224–233. [PubMed: 14973303]
155. Desmond PV, Mashford ML, Harman PJ, Morphet BJ, Breen KJ, Wang YM. Decreased oral warfarin clearance after ranitidine and cimetidine. *Clin Pharmacol Ther*. 1984; 35:338–341. [PubMed: 6321081]
156. Kelly JG, Salem SA, Kinney CD, Shanks RG, McDevitt DG. Effects of ranitidine on the disposition of metoprolol. *Br J Clin Pharmacol*. 1985; 19:219–224. [PubMed: 3986080]
157. Elliott P, Dundee JW, Elwood RJ, Collier PS. The influence of H₂ receptor antagonists on the plasma concentrations of midazolam and temazepam. *Eur J Anaesthesiol*. 1984; 1:245–251. [PubMed: 6152615]
158. Fraser IM, Buttoo KM, Walker SE, Stewart JH, Babul N. Effects of cimetidine and ranitidine on the pharmacokinetics of a chronotherapeutically formulated once-daily theophylline preparation (Uniphy). *Clin Ther*. 1993; 15:383–393. [PubMed: 8519046]
159. Leucuta A, Vlase L, Farcau D, Nanulescu M. A pharmacokinetic interaction study between omeprazole and the H₂-receptor antagonist ranitidine. *Drug Metabol Drug Interact*. 2004; 20:273–281. [PubMed: 15663296]
160. Aarnoutse RE, Kleinnijenhuis J, Koopmans PP, Touw DJ, Wieling J, Hekster YA, Burger DM. Effect of low-dose ritonavir (100 mg twice daily) on the activity of cytochrome P450 2D6 in healthy volunteers. *Clin Pharmacol Ther*. 2005; 78:664–674. [PubMed: 16338282]
161. Hsu A, Granneman GR, Cao G, Carothers L, el-Shourbagy T, Baroldi P, Erdman K, Brown F, Sun E, Leonard JM. Pharmacokinetic interactions between two human immunodeficiency virus protease inhibitors, ritonavir and saquinavir. *Clin Pharmacol Ther*. 1998; 63:453–464. [PubMed: 9585800]
162. Hesse LM, Greenblatt DJ, von Moltke LL, Court MH. Ritonavir has minimal impact on the pharmacokinetic disposition of a single dose of bupropion administered to human volunteers. *J Clin Pharmacol*. 2006; 46:567–576. [PubMed: 16638740]
163. Ma Q, Forrest A, Rosenkranz SL, Para MF, Yarasheski KE, Reichman RC, Morse GD. Pharmacokinetic interaction between efavirenz and dual protease inhibitors in healthy volunteers. *Biopharm Drug Dispos*. 2008; 29:91–101. [PubMed: 18041735]
164. Kees F, Holstege A, Ittner KP, Zimmermann M, Lock G, Scholmerich J, Grobecker H. Pharmacokinetic interaction between proton pump inhibitors and roxithromycin in volunteers. *Aliment Pharmacol Ther*. 2000; 14:407–412. [PubMed: 10759619]
165. Backman JT, Aranko K, Himberg JJ, Olkkola KT. A pharmacokinetic interaction between roxithromycin and midazolam. *Eur J Clin Pharmacol*. 1994; 46:551–555. [PubMed: 7995324]
166. Saint-Salvi B, Tremblay D, Surjus A, Lefebvre MA. A study of the interaction of roxithromycin with theophylline and carbamazepine. *J Antimicrob Chemother*. 1987; 20(Suppl B):121–129. [PubMed: 3429380]
167. Paulsen O, Nilsson LG, Saint-Salvi B, Manuel C, Lunell E. No effect of roxithromycin on pharmacokinetic or pharmacodynamic properties of warfarin and its enantiomers. *Pharmacol Toxicol*. 1988; 63:215–220. [PubMed: 3194342]
168. Kurtz DL, Bergstrom RF, Goldberg MJ, Cerimele BJ. The effect of sertraline on the pharmacokinetics of desipramine and imipramine. *Clin Pharmacol Ther*. 1997; 62:145–156. [PubMed: 9284850]

169. Alderman J. Coadministration of sertraline with cisapride or pimozone: an open-label, nonrandomized examination of pharmacokinetics and corrected QT intervals in healthy adult volunteers. *Clin Ther*. 2005; 27:1050–1063. [PubMed: 16154484]
170. Tremaine LM, Wilner KD, Preskorn SH. A study of the potential effect of sertraline on the pharmacokinetics and protein binding of tolbutamide. *Clinical pharmacokinetics*. 1997; 32(Suppl 1):31–36. [PubMed: 9068933]
171. Rotger M, Colombo S, Furrer H, Decosterd L, Buclin T, Telenti A. Does tenofovir influence efavirenz pharmacokinetics? *Antivir Ther*. 2007; 12:115–118. [PubMed: 17503755]
172. Chittick GE, Zong J, Blum MR, Sorbel JJ, Begley JA, Adda N, Kearney BP. Pharmacokinetics of tenofovir disoproxil fumarate and ritonavir-boosted saquinavir mesylate administered alone or in combination at steady state. *Antimicrob Agents Chemother*. 2006; 50:1304–1310. [PubMed: 16569845]
173. Ahonen J, Olkkola KT, Neuvonen PJ. Effect of itraconazole and terbinafine on the pharmacokinetics and pharmacodynamics of midazolam in healthy volunteers. *Br J Clin Pharmacol*. 1995; 40:270–272. [PubMed: 8527290]
174. Guerret M, Francheteau P, Hubert M. Evaluation of effects of terbinafine on single oral dose pharmacokinetics and anticoagulant actions of warfarin in healthy volunteers. *Pharmacotherapy*. 1997; 17:767–773. [PubMed: 9250555]
175. Madani S, Barilla D, Cramer J, Wang Y, Paul C. Effect of terbinafine on the pharmacokinetics and pharmacodynamics of desipramine in healthy volunteers identified as cytochrome P450 2D6 (CYP2D6) extensive metabolizers. *J Clin Pharmacol*. 2002; 42:1211–1218. [PubMed: 12412819]
176. Colli A, Buccino G, Cocciolo M, Parravicini R, Elli GM, Scaltrini G. Ticlopidine-theophylline interaction. *Clin Pharmacol Ther*. 1987; 41:358–362. [PubMed: 3816023]
177. Ieiri I, Kimura M, Irie S, Urae A, Otsubo K, Ishizaki T. Interaction magnitude, pharmacokinetics and pharmacodynamics of ticlopidine in relation to CYP2C19 genotypic status. *Pharmacogenet Genomics*. 2005; 15:851–859. [PubMed: 16272957]
178. Niemi M, Kajosaari LI, Neuvonen M, Backman JT, Neuvonen PJ. The CYP2C8 inhibitor trimethoprim increases the plasma concentrations of repaglinide in healthy subjects. *Br J Clin Pharmacol*. 2004; 57:441–447. [PubMed: 15025742]
179. Hansen JM, Kampmann JP, Siersbaek-Nielsen K, Lumholtz IB, Arroe M, Abildgaard U, Skovsted L. The effect of different sulfonamides on phenytoin metabolism in man. *Acta Med Scand Suppl*. 1979; 624:106–110. [PubMed: 284708]
180. Naline E, Sanceaume M, Pays M, Advenier C. Application of theophylline metabolite assays to the exploration of liver microsome oxidative function in man. *Fundam Clin Pharmacol*. 1988; 2:341–351. [PubMed: 3265407]
181. He N, Huang SL, Zhu RH, Tan ZR, Liu J, Zhu B, Zhou HH. Inhibitory effect of troleandomycin on the metabolism of omeprazole is CYP2C19 genotype-dependent. *Xenobiotica*. 2003; 33:211–221. [PubMed: 12623762]
182. Kharasch ED, Walker A, Hoffer C, Sheffels P. Intravenous and oral alfentanil as *in vivo* probes for hepatic and first-pass cytochrome P450 3A activity: noninvasive assessment by use of pupillary miosis. *Clin Pharmacol Ther*. 2004; 76:452–466. [PubMed: 15536460]
183. Abernethy DR, Egan JM, Dickinson TH, Carrum G. Substrate-selective inhibition by verapamil and diltiazem: differential disposition of antipyrine and theophylline in humans. *J Pharmacol Exp Ther*. 1988; 244:994–999. [PubMed: 3252045]
184. McLean AJ, Knight R, Harrison PM, Harper RW. Clearance-based oral drug interaction between verapamil and metoprolol and comparison with atenolol. *Am J Cardiol*. 1985; 55:1628–1629. [PubMed: 4003307]
185. Shimizu M, Uno T, Yasui-Furukori N, Sugawara K, Tateishi T. Effects of clarithromycin and verapamil on rabeprazole pharmacokinetics between CYP2C19 genotypes. *Eur J Clin Pharmacol*. 2006; 62:597–603. [PubMed: 16783561]
186. Liu P, Foster G, LaBadie RR, Gutierrez MJ, Sharma A. Pharmacokinetic interaction between voriconazole and efavirenz at steady state in healthy male subjects. *J Clin Pharmacol*. 2008; 48:73–84. [PubMed: 18025525]

187. Hynninen VV, Olkkola KT, Leino K, Lundgren S, Neuvonen PJ, Rane A, Valtonen M, Laine K. Effect of voriconazole on the pharmacokinetics of diclofenac. *Fundam Clin Pharmacol*. 2007; 21:651–656. [PubMed: 18034666]
188. Saari TI, Laine K, Leino K, Valtonen M, Neuvonen PJ, Olkkola KT. Effect of voriconazole on the pharmacokinetics and pharmacodynamics of intravenous and oral midazolam. *Clin Pharmacol Ther*. 2006; 79:362–370. [PubMed: 16580904]
189. Granneman GR, Braeckman RA, Locke CS, Cavanaugh JH, Dube LM, Awni WM. Effect of zileuton on theophylline pharmacokinetics. *Clinical pharmacokinetics*. 1995; 29(Suppl 2):77–83. [PubMed: 8620674]
190. Awni WM, Cavanaugh JH, Leese P, Kasier J, Cao G, Locke CS, Dube LM. The pharmacokinetic and pharmacodynamic interaction between zileuton and terfenadine. *Eur J Clin Pharmacol*. 1997; 52:49–54. [PubMed: 9143867]
191. Awni WM, Hussein Z, Granneman GR, Patterson KJ, Dube LM, Cavanaugh JH. Pharmacodynamic and stereoselective pharmacokinetic interactions between zileuton and warfarin in humans. *Clinical pharmacokinetics*. 1995; 29(Suppl 2):67–76. [PubMed: 8620673]
192. Santostasi G, Fantin M, Maragno I, Gaion RM, Basadonna O, Dalla-Volta S. Effects of amiodarone on oral and intravenous digoxin kinetics in healthy subjects. *J Cardiovasc Pharmacol*. 1987; 9:385–390. [PubMed: 2438499]
193. Wang ZJ, Yin OQ, Tomlinson B, Chow MS. OCT2 polymorphisms and in-vivo renal functional consequence: studies with metformin and cimetidine, *Pharmacogenet. Genomics*. 2008; 18:637–645.
194. Gurley BJ, Swain A, Williams DK, Barone G, Battu SK. Gauging the clinical significance of P-glycoprotein-mediated herb-drug interactions: comparative effects of St. John's wort, Echinacea, clarithromycin, and rifampin on digoxin pharmacokinetics. *Mol Nutr Food Res*. 2008; 52:772–779. [PubMed: 18214850]
195. Hedman M, Neuvonen PJ, Neuvonen M, Holmberg C, Antikainen M. Pharmacokinetics and pharmacodynamics of pravastatin in pediatric and adolescent cardiac transplant recipients on a regimen of triple immunosuppression. *Clin Pharmacol Ther*. 2004; 75:101–109. [PubMed: 14749696]
196. Simonson SG, Raza A, Martin PD, Mitchell PD, Jarcho JA, Brown CD, Windass AS, Schneck DW. Rosuvastatin pharmacokinetics in heart transplant recipients administered an antirejection regimen including cyclosporine. *Clin Pharmacol Ther*. 2004; 76:167–177. [PubMed: 15289793]
197. Mahgoub AA, El-Medany AH, Abdulatif AS. A comparison between the effects of diltiazem and isosorbide dinitrate on digoxin pharmacodynamics and kinetics in the treatment of patients with chronic ischemic heart failure. *Saudi Med J*. 2002; 23:725–731. [PubMed: 12070557]
198. Schwarz UI, Gramatte T, Krappweis J, Oertel R, Kirch W. P-glycoprotein inhibitor erythromycin increases oral bioavailability of talinolol in humans. *Int J Clin Pharmacol Ther*. 2000; 38:161–167. [PubMed: 10783825]
199. Saruwatari J, Yasui-Furukori N, Niioka T, Akamine Y, Takashima A, Kaneko S, Uno T. Different effects of the selective serotonin reuptake inhibitors fluvoxamine, paroxetine, and sertraline on the pharmacokinetics of fexofenadine in healthy volunteers. *J Clin Psychopharmacol*. 2012; 32:195–199. [PubMed: 22367658]
200. Dresser GK, Bailey DG, Leake BF, Schwarz UI, Dawson PA, Freeman DJ, Kim RB. Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. *Clin Pharmacol Ther*. 2002; 71:11–20. [PubMed: 11823753]
201. Tapaninen T, Neuvonen PJ, Niemi M. Grapefruit juice greatly reduces the plasma concentrations of the OATP2B1 and CYP3A4 substrate aliskiren. *Clin Pharmacol Ther*. 88:339–342. [PubMed: 20664534]
202. Jalava KM, Partanen J, Neuvonen PJ. Itraconazole decreases renal clearance of digoxin. *Ther Drug Monit*. 1997; 19:609–613. [PubMed: 9421099]
203. Nolan PE Jr, Marcus FI, Erstad BL, Hoyer GL, Furman C, Kirsten EB. Effects of coadministration of propafenone on the pharmacokinetics of digoxin in healthy volunteer subjects. *J Clin Pharmacol*. 1989; 29:46–52. [PubMed: 2708548]

204. Rameis H. Quinidine-digoxin interaction: are the pharmacokinetics of both drugs altered? *Int J Clin Pharmacol Ther Toxicol.* 1985; 23:145–153. [PubMed: 3997300]
205. Somogyi A, Bochner F. Dose and concentration dependent effect of ranitidine on procainamide disposition and renal clearance in man. *Br J Clin Pharmacol.* 1984; 18:175–181. [PubMed: 6091709]
206. Penzak SR, Shen JM, Alfaro RM, Remaley AT, Natarajan V, Falloon J. Ritonavir decreases the nonrenal clearance of digoxin in healthy volunteers with known MDR1 genotypes. *Ther Drug Monit.* 2004; 26:322–330. [PubMed: 15167636]
207. Johnson BF, Wilson J, Marwaha R, Hoch K, Johnson J. The comparative effects of verapamil and a new dihydropyridine calcium channel blocker on digoxin pharmacokinetics. *Clin Pharmacol Ther.* 1987; 42:66–71. [PubMed: 2954736]
208. Peeters PA, Crijns HJ, Tamminga WJ, Jonkman JH, Dickinson JP, Necciari J. Clopidogrel, a novel antiplatelet agent, and digoxin: absence of pharmacodynamic and pharmacokinetic interaction. *Semin Thromb Hemost.* 1999; 25(Suppl 2):51–54. [PubMed: 10440424]
209. Purkins L, Wood N, Kleinermans D, Nichols D. Voriconazole does not affect the steady-state pharmacokinetics of digoxin. *Br J Clin Pharmacol.* 2003; 56(Suppl 1):45–50. [PubMed: 14616413]
210. Olkkola KT, Ahonen J, Neuvonen PJ. The effects of the systemic antimycotics, itraconazole and fluconazole, on the pharmacokinetics and pharmacodynamics of intravenous and oral midazolam. *Anesthesia and analgesia.* 1996; 82:511–516. [PubMed: 8623953]
211. Varhe A, Olkkola KT, Neuvonen PJ. Effect of fluconazole dose on the extent of fluconazole-triazolam interaction. *Br J Clin Pharmacol.* 1996; 42:465–470. [PubMed: 8904618]
212. Bondolfi G, Chautems C, Rochat B, Bertschy G, Baumann P. Non-response to citalopram in depressive patients: pharmacokinetic and clinical consequences of a fluvoxamine augmentation. *Psychopharmacology (Berl).* 1996; 128:421–425. [PubMed: 8986013]
213. Olkkola KT, Backman JT, Neuvonen PJ. Midazolam should be avoided in patients receiving the systemic antimycotics ketoconazole or itraconazole. *Clin Pharmacol Ther.* 1994; 55:481–485. [PubMed: 8181191]

Abbreviations

AUC	area under plasma concentration-time curve
DDI	Drug-Drug Interaction
FDA	Food and Drug Administration
MBI	Mechanism Based Inhibitor
MTDI database	Metabolism and Transport Drug Interaction Database™
NCE	New Chemical Entity
P450	cytochrome P450

Figure 1.

Simulation of the effect of minor pathway inhibition on AUC ratio for a probe drug with an $f_{m,1}$ of 0.87 and $f_{m,2}$ of 0.13. The probe drug was assumed to have an F_g of 0.8 due to metabolism by enzyme 2. Panel A focuses on the situation where the inhibition of the minor elimination pathway and F_g is predominant and a weak inhibition of enzyme 1 is observed. The magnitude of AUC change is shown as a function of $1+[I]/K_i$ for enzyme 2. Panel B focuses on the situation where the main enzyme inhibited is the major elimination pathway (enzyme 1) and weaker or equal inhibition of enzyme 2 and F_g (mediated by enzyme 2) is observed. Panel C shows a 3-dimensional depiction of the relationship between the *in vivo* AUC change, potency of the inhibitor towards enzyme 2 and the relative potency of the inhibitor towards enzyme 1 and enzyme 2.

Figure 2.

Simulation of the effect of a multi-P450 inhibitor in comparison to selective inhibitors on the magnitude of the DDI. An object drug with three equally important clearance pathways with $f_m = 0.32$ and a renal clearance contributing to an $f_e = 0.04$ was considered. For this simulation, $K_{i,1} = K_{i,2} = 10 \cdot K_{i,3}$ and $F_g = 0.66$. Only enzyme 2 is present in the gut. The simulated AUC ratio is shown at increasing $1+I/K_i$ for enzyme 1.

Table 1

List of *in vivo* probes and sensitive P450 markers used in the MTDI database search to identify multi-P450 inhibitors.

P450 enzyme	<i>In vivo</i> probe
CYP1A2	theophylline, caffeine, tizanidine, tacrine, duloxetine, alosetron, melatonin
CYP2B6	efavirenz, bupropion
CYP2C8	repaglinide, rosiglitazone
CYP2E1	chlorzoxazone
CYP2C9	(S)-warfarin, warfarin, tolbutamide, diclofenac, fluvastatin, losartan, phenytoin
CYP2C19	(S)-mephenytoin, mephenytoin, esomeprazole, lansoprazole, moclobemide, omeprazole, rabeprazole, pantoprazole
CYP2D6	(S)-metoprolol, metoprolol, atomoxetine, desipramine, debrisoquine, dextromethorphan, thioridazine
CYP3A	alfentanil, astemizole, budesonide, buspirone, cisapride, cyclosporine, dihydroergotamine, eletriptan, eplerenone, ergotamine, felodipine, fentanyl, fluticasone, lovastatin, midazolam, pimozone, quinidine, saquinavir, sildenafil, simvastatin, sirolimus, tacrolimus, terfenadine, triazolam, vardenafil

Table 2

Characterization of multi-P450 inhibitors based on *in vivo* drug-drug interaction studies. The inhibited P450 enzyme is indicated. Inhibitors were classified as weak (W), moderate (M) or strong (S) according to the classification system adopted by the US FDA based on change in the probe AUC or Cl. Negative *in vivo* interactions (neg) are also noted.

Drug	3A	1A2	2B6	2C8	2C9	2C19	2D6	2E1
alprazolam ^{67, 68}	W	W						
amiodarone ⁶⁹⁻⁷¹	W				M		W	
Cimetidine ⁷²⁻⁷⁸	M	W	neg	neg	W	M	W	
ciprofloxacin ⁷⁹⁻⁸²	M	S		neg	neg			
clarithromycin ⁸³⁻⁸⁸	S	neg		W	W	M		
clopidogrel ⁸⁹⁻⁹¹		neg	W			W		
cyclosporine ^{44, 92-93}	S			M	M			
diltiazem ⁹⁴⁻⁹⁸	M	W			neg		W	
disulfiram ⁹⁹⁻¹⁰³	neg	W			W		W	S
dronedarone ¹⁰⁴	M	neg			neg		M	
echinacea ¹⁰⁵		W			neg		W	
erythromycin ¹⁰⁶⁻¹⁰⁸	S	W			W			
fluconazole ^{62, 109-111}	M	neg			M	S		
fluoxetine ^{57, 112, 113}	neg					M	S	
fluvoxamine ^{31, 35, 114-117}	M	S		W	W	S	neg	
grapefruit juice ¹¹⁸⁻¹²²	S	W		neg	neg			
isoniazid ¹²³⁻¹²⁵	W	W						M
itraconazole ^{7, 126, 127}	S			W	neg			
ketoconazole ^{23, 128-133}	S	neg		W	W	M	neg	
moclobemide ^{61, 134}						M	M	
omeprazole ^{28, 135-138}	W	neg ^d			neg	M		
oral contraceptives ¹³⁹⁻¹⁴⁴	W	M	neg	neg		W	W	
paroxetine ¹⁴⁵⁻¹⁴⁸	neg	W			neg		S	
pipertine ^{149, 150}		W			M			

Drug	3A	1A2	2B6	2C8	2C9	2C19	2D6	2E1
propafenone ^{151, 152}		M					W	
quinidine ^{153, 154}	M						S	
ranitidine ¹⁵⁵⁻¹⁵⁹	W	neg			W	neg	W	
ritonavir ¹⁶⁰⁻¹⁶³	S ^b	b	neg ^b	b	b		W ^b	
roxithromycin ¹⁶⁴⁻¹⁶⁷	W	neg			neg	W		
sertraline ¹⁶⁸⁻¹⁷⁰	W				neg		W	
tenofovir ^{171, 172}	W		W					
terbinafine ^{132, 173-175}	neg	W			neg		M	
ticlopidine ^{89, 176, 177}		W	W			S		
trimethoprim ^{178, 179}				W	W			
troleandomycin ¹⁸⁰⁻¹⁸²	S	M				W		
verapamil ^{108, 183-185}	M	W				neg	W	
voriconazole ¹⁸⁶⁻¹⁸⁸	S		W		W			
zileuton ¹⁸⁹⁻¹⁹¹	W	W			neg			

^a omeprazole is an inducer of CYP1A2

^b ritonavir is an inducer of CYP3A4 and may also be an inducer of CYP1A2, CYP2B6, CYP2C8, CYP2C9 and CYP2D6 based on probe studies but differentiation of in vivo induction of CYP3A4 and other P450s is not possible at present.

Table 3

Profiles of inhibition of drug transporters *in vivo* by inhibitors of multiple P450 enzymes. In parentheses are the transporter probe substrate used and the AUC ratio, respectively. P-gp, P-glycoprotein; OATP, organic anion transport protein; OCT, organic cation transporter. The references for the clinical studies are included after each inhibitors name.

Drug	P-gp	OATPs	OCTs	Others
amiodarone ¹⁹²	Yes (digoxin, 1.5)			
cimetidine ¹⁹³			Yes, OCT2 (metformin, 1.5)	Yes, MATE1 (metformin, 1.5)
clarithromycin ¹⁹⁴	Yes (digoxin, 1.7)			
cyclosporine ^{195, 196}		Yes, OATP1B1/3 (pravastatin, 9.9; rosuvastatin, 7.1)		Yes, BCRP (rosuvastatin, 7.1)
diltiazem ¹⁹⁷	Yes (digoxin, 1.4)			
dronedarone ¹⁰⁴	Yes (digoxin, 2.3)			
erythromycin ¹⁹⁸	Yes (talinalolol, 1.5)			
fluvoxamine ¹⁹⁹	Yes (fexofenadine, 1.8)			
grapefruit juice ^{200, 201}		Yes, OATP1A2 (fexofenadine, 0.4), OATP2B1 (aliskiren, 0.4)		
itraconazole ²⁰²	Yes (digoxin, 1.7)			
paroxetine ¹⁹⁹	Yes (fexofenadine, 1.4)			
propafenone ²⁰³	Yes (digoxin, 1.3)			
quinidine ²⁰⁴	Yes (digoxin, 2.7)			
ranitidine ²⁰⁵			Yes, OCT2 (procainamide, 1.2)	
ritonavir ²⁰⁶	Yes (digoxin, 1.2)			
verapamil ²⁰⁷	Yes (digoxin, 1.2)			

Note: Clopidogrel ²⁰⁸, Echinacea extract ¹⁹⁴, sertraline ¹⁹⁹, and voriconazole ²⁰⁹ had negative studies with the selected probe markers.

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Table 4

In vitro-to-*in vivo* predictions of drug-drug interactions caused by the multi-P450 inhibitors: fluconazole, fluvoxamine, ketoconazole and voriconazole. The P450 enzyme(s) involved in the *in vivo* clearance of the object drug, and the predicted and observed AUC ratio are shown. All object drugs were orally administered. The AUC ratios were predicted according to equation 1 using total [I] and K_i values and the unbound [I] and K_i values as described. The predicted AUC ratios in parentheses are the predicted interactions using gut inhibitor concentration calculated from $F_a \cdot D/250\text{mL}$ as described in the Experimental section. The references for the clinical studies are included in the observed AUC ratio column.

Inhibitor	Object	P450(s) Involved	Predicted AUC Ratio		Observed AUC Ratio
			Total [I]	Unbound [I]	
fluconazole	midazolam	3A4	4.7 (5.6) ^d	4.3 (5.2) ^d	3.32 ²¹⁰
	omeprazole	2C19 and 3A4	5.8 (6.5)	5.3 (5.9)	6.29 ^{62, 211}
	diazepam	2B6, 2C19 and 3A4	2.1	2.1	2.74 ⁵⁰
fluvoxamine	(S)-mephenytoin	2C19	2.6	2.1	9.89 ³¹
	midazolam	3A4	1.2 (2.0) ^d	1.1 (1.8) ^d	1.66 ¹¹³
	omeprazole	2C19 and 3A4	1.6 (1.9)	1.4 (1.7)	5.62 ^{63a}
	imipramine	2C19 and 2D6	2.1	1.8	3.63 ^{55, 212}
	diazepam	2B6, 2C19 and 3A4	1.7	1.6	2.80 ^{51, 113}
ketoconazole	desipramine	2D6	1.2	1.0	1.02 ^{133b}
	midazolam	3A4	22.5 (22.6) ^d	3.3 (4.2) ^d	15.9 ²¹³
	omeprazole	2C19 and 3A4	2.1 (2.1)	1.2 (1.4)	1.36 ^{23c}
	imipramine	2C19 and 2D6	1.3	1.0	1.20 ^{133b}
voriconazole	midazolam	3A4	2.2 (3.1) ^d	1.6 (2.4) ^d	9.40 ¹⁸⁸
	diazepam	2B6, 2C19 and 3A4	2.2	1.7	2.23 ⁵⁰

^a population studied was CYP2C19*1/*1 genotyped subjects,

^b dextromethorphan extensive metabolizers and

^c debrisoquine and (S)-mephenytoin extensive metabolizers,

^d Midazolam was administered at the t_{max} of the inhibitor and hence circulating concentrations are likely a better estimate for enterocyte concentrations than the predicted maximum enterocyte concentration following inhibitor administration.