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Are Circulating Metabolites Important in Drug-Drug Interactions? Quantitative Analysis of Risk Prediction and Inhibitory Potency

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Abstract

The potential of metabolites to contribute to drug-drug interactions is not well defined. The aim of this study was to determine the quantitative role of circulating metabolites in inhibitory drug-drug interactions *in vivo*. The AUC data for at least one circulating metabolite was available for 71% of the 102 inhibitors identified. 78% of the 80 metabolites characterized at steady state had AUCs greater than 10% of the parent drug. A comparison of the metabolite's and parent's $[I]/K_i$ showed that 17 of the 21 (80%) reversible inhibitors studied had metabolites that are likely to contribute to *in vivo* drug-drug interactions, with some metabolites predicted to be the major inhibitors. The *in vivo* drug interaction risk of amiodarone, bupropion and sertraline could only be identified from *in vitro* data when metabolites were included into predictions. In conclusion, circulating metabolites are common with CYP inhibitors and do contribute to clinically observed CYP inhibition.

INTRODUCTION

It has long been known that metabolites may contribute to drug action, toxicity, act as inhibitors of drug elimination or displace the drug from plasma or tissue binding sites. However, the quantitative importance of metabolites in drug therapy is not well characterized. Classical examples of drugs that have pharmacologically important (active) metabolites include for example morphine as a metabolite of codeine, phenobarbital as a metabolite of primidone, enalaprilat as a metabolite of enalapril and desipramine as a metabolite of imipramine.

In some cases, metabolites might be a risk factor for drug toxicity. To acknowledge this, the US Food and Drug Administration (FDA) issued the so called MIST (metabolites in safety testing) guidelines (www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm079266.pdf) that establish a strategy for toxicology evaluation of circulating metabolites. Attention is drawn to metabolites that circulate in concentrations greater than 10% of the parent at steady state. The recently issued ICH M3 guideline by the European Medicines Agency, on the other hand, focuses on metabolites that circulate at 10% of total drug related material. The MIST and ICH guidelines are, however, specific for toxicity testing and do not recommend study of the metabolites as inhibitors of metabolic enzymes or as binding displacers.

Despite the fact that metabolites have received consistent attention regarding their role in pharmacological action and in drug toxicity, their role in inhibitory drug-drug interactions

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Conflict of Interest/Disclosure

None

has not been well characterized. This is perhaps due to the fact that there are no established examples of clinical interactions which are caused solely by an inhibitory metabolite with a lack of any inhibitory effect from the parent drug. Several clinically important inhibitors have recently been recognized to have circulating metabolites that are inhibitory. For example, hydroxy-itraconazole as a metabolite of itraconazole, N-desmethyldiltiazem as a metabolite of diltiazem and norfluoxetine as a metabolite of fluoxetine appear to contribute to *in vivo* DDIs [1–4]. Whether these three drugs are the exceptions or examples of more prevalent phenomena of multiple inhibitors being responsible for a single interaction is currently unknown. To evaluate the role of inhibitory metabolites in clinically important drug-drug interactions, we recently determined that majority (82%) of clinically used cytochrome P450 inhibitors possessed circulating metabolites, and that all known potent P450 inhibitors had circulating metabolites [5]. The current work was undertaken to quantitatively analyze the exposures to these metabolites after administration of a parent compound and to analyse the relative importance of the circulating inhibitors in clinical drug-drug interactions. The University of Washington Metabolism and Transport Drug Interaction Database™ was used to collect the relevant *in vitro* enzyme inhibition data and clinical interaction data, and *in vivo* interaction potential was analyzed in accordance to FDA guidelines.

RESULTS

Prevalence of circulating metabolites

The metabolite to parent AUC ratios were calculated for 72 of the 102 inhibitors previously identified [5] based on availability of data. As some of the inhibitors have multiple circulating metabolites, this resulted in 120 datasets of AUC_M/AUC_P -ratios (Figure 1). Within these data, 37% of the metabolites had an AUC that was greater than the AUC of the parent and an additional 46% had an AUC that was between 10% and 100% of the parent AUC. Only 17% of all the metabolites were determined to be insignificant in terms of their AUC in relation to the parent drug ($AUC_M/AUC_P < 0.1$). Figure 1 depicts the distribution of the inhibitors based on metabolite to parent AUC ratio with steady state and single dose data. The individual metabolite to parent pairs and the AUC values used for the analysis are summarized in Appendix 1 (steady state data) and 2 (single dose data). Appendix 3 shows the list of circulating metabolites for which no pharmacokinetic characterization was available. When the data was analyzed separately for weak, moderate and potent inhibitors the distribution of AUC_M/AUC_P ratios was similar as that shown for all the inhibitors together.

Prediction of relative contribution of metabolites to *in vivo* drug-drug interactions

Quantitative prediction of the importance of metabolites in *in vivo* drug-drug interactions was conducted by calculating the IP_M/IP_P -ratios (inhibitory potency of the metabolite as $[I]/K_i$, over the inhibitory potency of the parent as $[I]/K_i$) for all inhibitors for which circulating concentrations of the metabolite and parent were available, and *in vitro* K_i -values had been determined. Table 1 shows a summary of the calculated IP_M/IP_P -ratios. The metabolites were predicted to be most significant *in vivo* inhibitors for 11 of the 21 inhibitors (52%) analyzed whereas 5 metabolites belonging to 4 inhibitors (19%) were predicted not to contribute to the *in vivo* interaction (IP_M/IP_P -ratio < 0.1). Notably, the two inhibitors that are commonly recognized to have inhibitory metabolites, itraconazole and fluoxetine, had IP_M/IP_P -ratios less than 1. For three analyzed potent or moderate inhibitors, amiodarone, amodiaquine and bupropion, the IP_M/IP_P -ratios ranged from 5 to 25 demonstrating that the metabolites are predicted to be the main contributors to interactions. On the other hand, omeprazole sulfone, quinidine N-oxide and 3-hydroxy quinidine had IP_M/IP_P -ratios < 0.01 resulting in a prediction of insignificant contribution of metabolites to *in vivo* interactions.

Role of circulating metabolites in risk analysis of inhibitory interactions

As metabolites were predicted to quantitatively contribute to selected *in vivo* drug-drug interactions, the effect of circulating metabolites on preclinical risk analysis of potential drug-drug interactions was determined. Only inhibitors that had *in vivo* interaction data with accepted probes, allowing establishment of *in vivo* interaction risk were included in the analysis and the $[I]/K_i$ ratios for the inhibitors and metabolites that had sufficient data available were calculated. Six (54%) of the eleven inhibitors included in the analysis were classified into “possible” or “likely” interactions based on parent drug only (Table 2). Metabolites of six of the eleven compounds were qualitatively predicted to have equal or greater risk to cause drug-drug interactions than the parent inhibitor. Five compounds, amiodarone, bupropion, ranolazine, sertraline and venlafaxine, were classified as “remote risk” for *in vivo* interactions when only parent drug was considered. Two of these *in vivo* inhibitors, amiodarone (CYP 2C9) and sertraline (CYP3A4), ended as “possible” and one, bupropion (CYP2D6), as “likely” risk when inhibition by known circulating metabolites was considered, whereas the *in vivo* interaction caused by ranolazine (CYP3A4) and venlafaxine (CYP2D6) was not predicted with the available *in vitro* inhibition data. Figure 2 shows the correlation between *in vitro* risk assessment and the magnitude of *in vivo* drug-drug interactions when only parent drug is accounted for and when parent and metabolites are analyzed together. As shown in Figure 2, the moderate inhibitor amiodarone and potent inhibitor bupropion, were predicted to be *in vivo* inhibitors only when metabolites as well as parent drug were accounted for. It is noteworthy that for sertraline, the interaction with CYP3A4 could be predicted with the metabolite taken into account whereas the CYP2D6 interaction could not.

Quantitative *in vitro* to *in vivo* extrapolation of drug-drug interactions

In vivo drug-drug interactions are commonly rationalized by the use of static or dynamic models that allow extrapolation of *in vitro* inhibition potency to *in vivo* interactions. The existence of circulating inhibitory metabolites could cause a significant underprediction of the existing interactions and, if metabolites are not accounted for, lead to poor understanding of the characteristics of specific interactions. To determine if circulating metabolites lead to significant underpredictions in known, well characterized drug-drug interactions, the effect of inhibitory metabolites in the accuracy of *in vitro* to *in vivo* predictions of interaction magnitude was tested using classic static prediction methods. Table 3 shows the predicted and observed interactions for the 10 inhibitors analyzed. Interactions were included in the analysis, if *in vivo* data with a well characterized probe was available and $[I]/K_i$ ratios could be calculated for both the parent drug and metabolite. Both steady state C_{max} and steady state C_{av} were used for the analysis and no apparent differences were observed between the predictions with these two values. This might be because at steady state there is minimum fluctuation in the inhibitor concentrations over the dosing interval and hence C_{max} and C_{av} are similar. For amiodarone, bupropion, fluoxetine and sulfinpyrazone, an improved quantitative prediction of the *in vivo* interaction was obtained when metabolites were accounted for whereas for the remaining six inhibitors no significant differences in prediction accuracy were observed. The fact that quantitative predictions were possible only after accounting for the metabolites for four of ten analyzed inhibitors, suggests that knowledge of circulating metabolites and their inhibitory potency should be obtained prior to quantitative modeling of the observed interaction.

DISCUSSION

This study aimed at determining to what extent circulating metabolites contribute to *in vivo* drug-drug interactions in context of preclinical risk assessment and of development of quantitative methods of predicting *in vivo* drug-drug interactions. The analysis shows that a

majority of the clinically important P450 inhibitors have metabolites that circulate at appreciable concentrations and a third of the known metabolites are more abundant than the parent drug. However, the P450 inhibition potential has been published only for a fraction of the metabolites. It is possible that unpublished negative inhibition data exists but was not available for this analysis.

The existing metabolite data was evaluated to determine whether the known metabolites would alter the preclinical risk assessment of known clinical P450 inhibitors. Surprisingly, for three of the 11 inhibitors evaluated, the risk assessment changed from remote likelihood of DDIs to possible or likely with the inclusion of metabolites. This suggests that determination of K_i values for quantitatively important metabolites can be useful when interactions are not predicted or only weak interactions are predicted with parent drug. On the other hand, testing for inhibitory potency for metabolites might be of limited usefulness when parent drug on its own has an identified risk at the likely category, since the true magnitude of the *in vivo* interaction will be determined in relevant *in vivo* DDI studies. Interestingly, all the compounds that were “missed” in the risk analysis (amiodarone, bupropion, ranolazine, sertraline and venlafaxine) when only parent inhibitor data was included, had metabolites with IP_M/IP_P -ratios >0.1 , suggesting that metabolites of these compounds may be critical in *in vivo* drug-drug interactions. On the other hand omeprazole sulfone, quinidine N-oxide and 3-hydroxy quinidine which were predicted in the risk analysis to only have remote potential to cause drug-drug interactions had IP_M/IP_P -ratios <0.1 .

A possible explanation for the incorrect assignment of risk for the ranolazine-simvastatin interaction could be the involvement of transporters such as P-glycoprotein (P-gp) in the *in vivo* interaction. Ranolazine has been shown to cause in a 1.5-fold increase in *in vivo* digoxin concentrations, suggesting that it is a P-gp inhibitor (http://www.accessdata.fda.gov/drugsatfda_docs/nda/2006/021526_s000_Ranexa.cfm).

Since simvastatin has been shown to be a P-gp substrate [6], it is possible that the *in vivo* interaction is due to P-gp inhibition during simvastatin absorption. Although data for the other probes, inhibitors and metabolites is not available, it is possible that transporter mediated interactions contribute to the other underpredictions as well. In addition, metabolites may be sequestered in the hepatocytes and hence plasma concentrations do not accurately reflect the intracellular free inhibitor concentrations. Such sequestration is not accounted for in the current analysis.

A special consideration may be warranted with compounds that are multi-CYP inhibitors *in vitro*. These compounds are typically analyzed using a rank-order approach [7]. As inhibitor depletion will prevent formation of significant amounts of metabolites in *in vitro* inhibition assays, rank order analyses *in vitro* are likely to reflect the potency of the parent drug. If metabolites do not follow the same rank order, or inhibit only a subset of the CYPs that the parent compound inhibits, rank order approach may fail. For the three multi-CYP inhibitors included in this analysis, the rank order for the metabolite was similar to that observed for the parent drug when reversible inhibition was considered, but the dataset is not representative of the overall multi-CYP inhibitors.

The recently published MIST guidance recommends toxicology review of metabolites that circulate in excess of 10% of the parent drug. Although the MIST guidance does not address metabolites in drug-drug interactions, the current data was analyzed in light of the 10% cutoff value of the MIST guidance. Based on the $[I]/K_i$ ratios of the metabolites in relation to the parent compounds, with the exception of 4-hydroxypropranolol, all the metabolites that were predicted to contribute to *in vivo* interactions had an AUC greater than 10% of the

parent at steady state. Interestingly, there was no correlation between the contribution of the metabolites to interactions (IP_M/IP_P -ratio) and the relative abundance of the metabolites in plasma, as many metabolites were more potent inhibitors than the parent drug while they circulated at lower concentrations than the parent drug. Hence, the 10% cut-off AUC value appears appropriate for inhibition testing of metabolites. Based on these analyses, predicting the $[I]/K_i$ -ratio for abundant metabolites (as well as for the parent inhibitor) can be helpful in drug discovery and development process even when the parent compound is not inhibitory. This also requires that the methodology predicting $[I]$ not only for parent but also metabolite should be established.

The inhibitory metabolites included in this analysis may not be the sole circulating metabolites of that given inhibitor but were the only ones with published *in vitro* inhibition data and *in vivo* AUC values. For example 5-hydroxyomeprazole is more abundant than omeprazole sulfone after omeprazole administration. However, no *in vitro* inhibition data was available for 5-hydroxyomeprazole and hence this metabolite could not be analyzed. Similarly, atorvastatin has several metabolites that circulate at similar concentrations as atorvastatin itself but the inhibition potency of these metabolites has not been determined. On the other hand, itraconazole has two other metabolites, keto-itraconazole and N-desalkyl-itraconazole that inhibit CYP3A4 potently but their steady state concentrations have not been determined. The existence of multiple inhibitory metabolites would further increase the relative contribution of metabolites as a whole in comparison to the parent drug.

The role of inhibitory metabolites in quantitatively predicting the magnitude of drug-drug interactions may be less than would be expected from the abundance of the metabolites. In the analysis of quantitative predictions, inclusion of metabolites did not always improve the accuracy of the prediction and the increase in average predictive accuracy was only modest. However, for some of the inhibitors significant improvement in predictions was observed. As plasma and microsomal unbound fractions are not available for majority of these compounds, the quantitative prediction may not accurately reflect the true relative importance of all of the metabolites analyzed. Despite this, the fact that so many inhibitors have circulating inhibitory metabolites may have implications to overall correlation analyses of *in vitro* to *in vivo* extrapolations, as an accurate model should incorporate all inhibitory species.

In conclusion, these results suggest that metabolites or candidates for major circulating metabolites *in vivo*, should be investigated in addition to parent drug for inhibition potential *in vitro* unless clinical interaction studies are already warranted due to the parent compound. Although the available literature data does not allow for comprehensive evaluation, these results suggest that circulating metabolites contribute to *in vivo* drug-drug interactions and further study will be required for in-depth understanding of their overall clinical significance.

METHODS

Identification of the inhibitors and their metabolites

The University of Washington Metabolism and Transport Drug Interaction DatabaseTM (MTDI database: <http://www.druginteractioninfo.org>) was searched to identify known P450 inhibitors. All inhibitors analyzed had to be drugs currently available in the US market, and herbal products, combination therapies and oral contraceptives were excluded from the analysis. The complete list of identified inhibitors and their P450 inhibition characteristics was previously published [5].

The circulating metabolites and the AUC values for the parent drug and metabolites of these inhibitors were extracted from the MTDI database by searching these compounds as objects in DDI studies in which their metabolites were also measured. If no metabolite data was available in the MTDI database, a Pubmed search was conducted to extract circulating metabolite data. Finally, the product labels and Micromedex were used as a reference for data on circulating metabolites. To calculate metabolite to parent AUC ratios the following criteria were used:

1. The metabolite and the parent must have been measured in the same study and complete (dosing interval at steady state or 0-infinity after single dose) AUC's for both had to be available
2. Steady state values for the parent drug and metabolite were used if available. If no steady state pharmacokinetic data was available single dose kinetics were accepted. The ratio between the metabolite and parent AUC was calculated either by using the steady state dosing interval AUC or by using the AUC to infinity after single dose.
3. For compounds that had data from multiple dosing levels the highest AUC_M/AUC_P -ratio was used.

All AUC values were converted to $\mu\text{mol}\cdot\text{h}/\text{mL}$ units using the molecular weight of the parent drug and the metabolite, and the ratio between the metabolite and parent drug AUCs were calculated.

Determination of *in vivo* drug-drug interaction magnitude and the inhibitory potency for parent and metabolites

The MTDI database was queried to retrieve all reported *in vivo* interactions (defined as resulting in a 20% increase in the AUC or decrease in clearance of the victim drug) for the inhibitors that were previously identified to have circulating metabolites. From the resulting list of *in vivo* interaction studies, the ones conducted with a known marker substrates (www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072101.pdf) were kept for further analysis. All data for *in vivo* AUC change using marker substrates were extracted and the change in object AUC was recorded for analysis. The inhibitor and its metabolite concentrations measured in the interaction study were recorded if available. For studies that did not measure the plasma concentrations of the inhibitor and its metabolites, literature data using the same dosing regimen was used to obtain steady state C_{max} and C_{av} values for the inhibitor and its metabolites. If data for the inhibitor and its metabolite was not available at the dose level used in the *in vivo* interaction studies, the concentrations were dose normalized to allow the risk analysis.

The MTDI database was queried to extract the *in vitro* K_i -values for the identified metabolites and the parent inhibitors. Inhibitors that were known inactivators of a given enzyme were excluded from further analysis for the enzyme that was irreversibly inactivated *in vitro* by the parent drug or the metabolite. In case there were multiple reversible K_i -values for the parent inhibitor or its metabolites, the K_i -value used for analysis was chosen according to the following criteria: 1) The metabolite and the parent drug were tested in the same study against the same P450 and same probe; 2) if the metabolite and parent were not available in the same study two studies that used the same probe were chosen; 3) in the absence of such data two studies that used the same microsomal protein concentration were used. For equivalent data, the study with the lowest microsomal protein concentration was always used.

Relative importance of circulating metabolites

To predict the relative contribution of the metabolites in *in vivo* drug-drug interactions, the $[I]/K_i$ ratios were calculated for all inhibitors and metabolites that had sufficient *in vitro* data. The clinically relevant steady state average total concentrations of the drug and its metabolite (measured in the same study and dose level) were used as $[I]$ and the K_i values were collected as described above. The $[I]/K_i$ ratio was calculated for the metabolite and parent drug separately, and then the ratio between the $[I]/K_i$ of the metabolite to parent (IP_M/IP_P ratio) was calculated. As the $[I]/K_i$ ratio for the metabolite (IP_M) and for the parent (IP_P) is indicative of their *in vivo* inhibitory potential (IP), the ratio of the two can be used as a predictor of the relative *in vivo* contribution of metabolites to CYP inhibition. The predicted contribution of the metabolites was classified as follows: 1) If the IP_M/IP_P ratio was ≥ 0.1 the metabolites were not predicted to contribute to *in vivo* interactions; 2) if IP_M/IP_P ratio was < 0.1 the metabolite was predicted to be the main inhibitory species causing the *in vivo* interaction; and 3) if $0.1 < IP_M/IP_P$ -ratio < 1 metabolite contribution was predicted to be likely in the interaction but the parent drug was predicted to be the main inhibitor causing the interaction.

Risk analysis using metabolite and parent drug data

A risk analysis for inhibitory interactions was conducted according to the FDA guidance for testing parent drugs (www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm079266.pdf). Only inhibitors for which *in vivo* interaction data with a known probe was available were included in this analysis. The steady state inhibitor concentrations (total C_{max}) and *in vitro* K_i values were used to compute the $[I]/K_i$ ratio for the inhibitors and their metabolites. In order to evaluate the highest risk, the *in vivo* studies with the most sensitive probes for the inhibited P450 and highest *in vivo* interactions were included in the analysis. Based on the $[I]/K_i$ ratio, the potential of the drugs to cause *in vivo* interactions was classified as likely ($[I]/K_i > 1$), possible ($1 > [I]/K_i > 0.1$) or remote ($0.1 > [I]/K_i$) for the parent drug and the metabolite separately. To predict the combined risk of metabolites and parent drug, the $[I]/K_i$ ratios were summed together and the sum was ranked similar to the individual compounds.

Quantitative prediction of *in vivo* drug-drug interactions

Ten inhibitors with *in vivo* DDI data with a probe substrate with a known f_{mCYP} were used to estimate the role of metabolites in quantitative *in vitro* to *in vivo* extrapolations (IVIVE) of inhibitory interactions. Studies were included in the quantitative analysis if the *in vivo* study was conducted with a reliable probe with a defined f_m and the C_{max} and C_{av} were available in the DDI study or in another study with similar dosing regimen. If multiple DDI studies were available, the study with the highest AUC change was selected to consider possible worst case scenario. Both the total C_{max} and C_{av} values for the inhibitor and its metabolite were used in the predictions as $[I]$. IVIVE was done according to Equation 1:

$$\frac{AUC_i}{AUC} = \frac{1}{\left(\frac{f_{mCYP}}{1 + \sum_j \left([I]_j / K_{ij} \right)} \right) + (1 - f_{mCYP})} \quad (1)$$

in which the f_{mCYP} for each marker substrate was included according to the following list. $f_{mCYP3A4}$: midazolam 0.94, nifedipine 0.71, simvastatin 0.99; $f_{mCYP2D6}$: desipramine 0.877, metoprolol 0.828; $f_{mCYP2C9}$: phenytoin 0.75, (S)-warfarin 0.87 ([8, 9]). The effect of multiple inhibitors was accounted for by summing the $[I]/K_i$ ratios.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CYP	cytochrome P450
C_{max}	maximum plasma concentrations
C_{av}	average steady state plasma concentration
f_m	fraction metabolized
f_{mCYP}	fraction metabolized via specific CYP
AUC	area under plasma concentration time curve
AUC_i	area under plasma concentration time curve in the presence of an inhibitor
DDI	drug-drug interaction
[I]	inhibitor concentration
K_i	inhibition constant

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Figure 1.

Distribution of metabolite to parent AUC ratios amongst all analyzed inhibitors. Panel A shows all the inhibitors analyzed in Appendices 1 and 2 with each bar representing a specific metabolite-parent pair. Blue bars refer to steady-state data and red bars to single dose data. The percentages above the bars indicate the fraction of inhibitors in each group ($AUC_M/AUC_P < 0.1$; $0.1 < AUC_M/AUC_P < 1$; $AUC_M/AUC_P > 1$). Panel B shows the distribution of AUC_M/AUC_P ratios for the inhibitors that had available in vivo interaction data including clearance or AUC data from marker substrates and were classified according to the FDA-recommended system (www.fda.gov/cder/drug/drugInteractions/) as potent (> 5 -fold increase in AUC), moderate (> 2 - but < 5 -fold increase in AUC), or weak (> 1.25 - but < 2 -fold increase in AUC) inhibitors of the target enzyme.

Figure 2.

Evaluation of DDI risk with $[I]/K_i$ data for inhibitors presented in Table 2. Panels A and B show the correlation between *in vitro* risk assessment and the magnitude of *in vivo* DDIs when only parent drug is accounted for (A) and when parent and metabolites are analyzed together (B). Panel C shows the 9 zones for prediction in accordance to panels A and B, including true negative, true positive, false negative, and false positive.

Table 1

Determination of the relative importance of the metabolites in comparison to parent drug using the IP_M/IP_P ratios.

Parent (P)	Metabolite (M)	P			M			IP_M/IP_P	Reference(s)
		CYP	[I] (μ M)	K_i (μ M)	[I]/ K_i	[I] (μ M)	K_i (μ M)		
amiodarone	N-desethylamiodarone	2C9	2.5	94.6*	0.026	1.5	2.3*	0.67	25.3 [10], [11]*
bupropion	theobupropion	2D6	0.3	21	0.014	1.9	5.4	0.36	24.8 [12]
	hydroxybupropion	2D6				3.2	13	0.24	17.0 [12]
	erythropropion	2D6				0.37	1.7	0.22	15.3 [12]
sulfinpyrazone	sulfinpyrazone sulphide	2C9	17.8	229	0.031	5.0	27	0.19	6.0 [13]
venlafaxine	N-desmethylenlafaxine	2D6	0.23	30	0.0078	0.93	20	0.047	6.0 [14]
atorvastatin	atorvastatin lactone	3A	0.06*	90	0.0007	0.003*	0.9	0.004	5.5 [15], [16]*
amodiaquine	desethylamodiaquine	2D6	0.08	2.1	0.039	0.82	4.1	0.20	5.1 [17]
clomipramine	N-desmethyldomipramine	2D6	0.24	16	0.014	0.49	7.9	0.062	4.2 [18]
sertraline	N-desmethylertraline	2D6	0.086	23	0.0038	0.14	16	0.009	2.4 [19]
risperidone	paliperidone	3A	0.020	67	0.0003	0.046	80	0.001	2.0 [20]
sertraline	N-desmethylertraline	3A4	0.33	3.5	0.094	0.53	3.5	0.15	1.6 [21], [22]
haloperidol	reduced haloperidol	2D6	0.023	0.89	0.026	0.008	0.24	0.033	1.3 [23]
risperidone	paliperidone	2D6	0.020	6.9	0.0028	0.046	16	0.003	1.0 [20]
fluoxetine	norfluoxetine	2D6	0.37	0.2	2.2	0.38	0.2	2.0	0.92 [24]
citalopram	didesmethylditalopram	2C19	0.20	87.3	0.002	0.015	7.7	0.002	0.85 [25]
	desmethylditalopram	2C19	0.24	87.3	0.003	0.13	56	0.002	0.85 [25]
propranolol	4-hydroxypropranolol	2D6	0.42	4.5*	0.094	0.046	1.0	0.046	0.49 [26], [27]*
sulfinpyrazone	sulfinpyrazone sulfone	2C9	17.8	229	0.078	2.3	73	0.031	0.40 [13]
itraconazole	hydroxyitraconazole	3A4	0.94	8.5	0.11	1.09	26.	0.042	0.38 [3]
ranolazine	O-demethyl ranolazine	3A4	2.2	90.0	0.025	0.89	104.	0.009	0.35 ^a
chloroquine	monodesethylchloroquine	2D6	0.62	15.3	0.040	0.20	15.5	0.013	0.32 [28]
imatinib	N-desmethylinatinib	2C9	8.6*	59.0	0.15	1.4*	40.3	0.034	0.23 ^b , [29]*
		3A		8	1.1		13.7	0.099	0.092 ^b , [29]*

Parent (P)	Metabolite (M)	CYP	P			M			IP _M /IP _P	Reference(s)
			[I] (μ M)	K _i (μ M)	[I]/K _i	[I] (μ M)	K _i (μ M)	[I]/K _i		
		2D6		7.5	1.1		13.5	0.10	0.088	<i>b</i> , [29]*
carbamazepine	carbamazepine-10,11-epoxide	2C19	30.5	29.6	1.0	4.8	53.5	0.089	0.086	[30]
atomoxetine	N-desmethylatomoxetine	3A	0.59	34.0	0.017	0.019	16.0	0.001	0.069	[31]
omeprazole	omeprazole sulfone	2C	1.6	2.0	0.80	0.38	8.2	0.046	0.058	[32]
	omeprazole sulfone	3A		201.0	0.008		188	0.002	0.0025	[33]
quinidine	3-hydroxyquinidine	2D6	1.9	0.03	70	0.30	2.3	0.13	0.0019	[34]
	quinidine N-oxide	2D6				0.60	43.5	0.014	0.0002	[34]

^aU.S. Food and Drug Administration. Clinical Reviews- Ranolazine. Drugs@FDA 2007

^bU.S. Food and Drug Administration. Clinical Review- Imatinib. Drugs@FDA 2002.

Table 2
Evaluation of Potential DDI risk using parent and metabolite [I]/K_i data according to the FDA guidance.

Parent	Metabolite	CYP	Inhibitor dose	Victim drug	AUC change of victim drug (observed)	Parent (P)		Metabolite (M)		P+M		FDA guidance ^b			Reference
						[I] (μM)	[I]/K _i	[I] (μM)	[I]/K _i	[I]/K _i	[I]/K _i	P	M	P+M	
amiodarone	N-desethylamiodarone	2C9	200 mg, b.i.d., 10 d	(s)-warfarin	2.1	1.8	0.019	1.2	0.51	0.53		R	P	P	[35] [36]
bupropion	erythrohydrobupropion	2D6	150 mg, b.i.d., 12 d	desipramine	5.2	0.67	0.032	0.43	0.25	1.02		R	P	L	[12] [12]
	hydroxybupropion							4.0	0.30				P		
	threo-hydrobupropion							2.3	0.43				P		
fluoxetine	norfluoxetine	2D6	60 mg, q.d., 8 d	desipramine	7.8	1.4 ^a	7.9 ^a	1.6 ^a	8.1	16		L	L	L	[37] [38]
imatinib	N-desmethylimatinib	2D6	400 mg, b.i.d., 9 d	metoprolol	1.2	8.6	1.1	1.4	0.10	1.2		L	P	L	[29] [29]
		3A	400 mg, q.d., 7 d	simvastatin	2.9	8.6	1.0	1.4	0.099	1.2		L	R	L	c [29]
itraconazole	hydroxyitraconazole	3A4	200 mg, q.d., 4 d	lovastatin	36.4	0.94	60	1.1	29	89		L	L	L	[39] [39]
omeprazole	omeprazole sulfone	2C19	40 mg, q.d., 7 d	proguanil	1.5	1.2 ^a	0.62	0.44 ^a	0.054	0.67		P	R	P	[40] [41]
		3A4	20 mg, q.d., 8 d	nifedipine	1.3	0.62	0.003	0.22	0.001	0.004		R	R	R	[42] [41]
quinidine	quinidine N-oxide	2D6	50 mg, single	dextromethorphan	42.6	0.57 ^a	19	0.18 ^a	0.004	19		L	R	L	[43] [44]
	3-hydroxyquinidine							0.09	0.039				R		
ranolazine	O-desmethylranolazine	3A4	1000 mg, b.i.d., 7 d	simvastatin	1.9	5.0	0.056	2.7	0.026	0.082		R	R	R	d [45]
sertraline	N-desmethylsertraline	2D6	150 mg, q.d., 8 d	imipramine	1.7	0.25 ^a	0.011	0.32 ^a	0.020	0.031		R	R	R	[46] [46]
		3A4	200 mg, q.d., 29 d	pinozide	1.4	0.33	0.095	0.53	0.15	0.25		R	P	P	[22] [22]
sulfapyrazone	sulfapyrazone sulfide	2C9	200 mg, b.i.d., 13 d	(s)-warfarin	1.9	51	0.223	36	2.1	2.4		P	L	L	[47] [48]
	sulfapyrazone sulfone							8.1	0.11				P		
venlafaxine	N-desmethylvenlafaxine	2D6	50 mg, t.i.d., 3 d	imipramine	1.3	0.25	0.008	0.027	0.001	0.010		R	R	R	[49] [50]

^aThe inhibitor and its metabolites concentration were calculated with dose normalization.

^bR; remote, P; possible, L; likely

^cU.S. Food and Drug Administration. Clinical Reviews- Imatinib. Drugs@FDA 2002

^dU.S. Food and Drug Administration. Clinical Review- Ranolazine. Drugs@FDA 2007

The K_i-values used were the same in Table 1.

Table 3

Predicted versus observed AUC values calculated with $C_{\max}$ and C_{av} .

Parent (P) Metabolite (M)	CYP	Inhibitor dose	Victim drug	AUC change of victim drug				
				observed	predicted ($C_{\max}$)		predicted (C_{av})	
				P	P+M	P	P	P+M
amiodarone N-desethylamiodarone	2C9	200 mg, q.d., 6.5 w	phenytoin	1.4	1.01	1.35	1.01	1.31
bupropion erythrohydrobupropion hydroxybupropion threohydrobupropion	2D6	150 mg, b.i.d., 12 d	desipramine	5.2	1.03	1.8	1.01	1.5
fluoxetine norfluoxetine	2D6	20 mg, q.d., 21 d	desipramine	4.4	2.41	3.35	2.47	3.61
imatinib N-desmethylinanib	2D6	400 mg, b.i.d., 9 d	metoprolol	1.2	1.79	1.85	—	—
itraconazole hydroxyitraconazole	3A4	200 mg, q.d., 4 d	midazolam	10.8	10.9	12.5	8.1	10.4
omeprazole omeprazole sulfone	3A4	20 mg, q.d., 8 d	nifedipine	1.3	1.0	1.0	1.0	1.0
quinidine quinidine N-oxide 3-hydroxyquinidine	2D6	50 mg, single	desipramine	3.2	6.0*	6.0*	—	—
ranolazine O-desmethyranolazine	3A4	1000 mg, b.i.d., 7 d	simvastatin	1.9	1.06	1.08	1.07	1.1
sertraline N-desmethylertraline	2D6	50 mg, q.d., 10 d	desipramine	1.4	1.0	1.01	1.0	1.01
sulfinpyrazone sulfinpyrazone sulfide sulfinpyrazone sulfone	2C9	200 mg, b.i.d., 13 d	(s)-warfarin	1.9	1.19	2.61	—	—

— : The C_{av} was not available.

* The inhibitor and its metabolites concentration were calculated with dose normalization.