Special Section on Prediction of Human Pharmacokinetic Parameters from In Vitro Systems

Drug Metabolites as Cytochrome P450 Inhibitors: A Retrospective Analysis and Proposed Algorithm for Evaluation of the Pharmacokinetic Interaction Potential of Metabolites in Drug Discovery and Development

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ABSTRACT

Understanding drug-drug interactions (DDIs) is a key component of clinical practice ensuring patient safety and efficacy of medicines. The role of drug metabolites in DDIs is a developing area of science, and has been recently highlighted in a draft regulatory guidance. The guidance states that metabolites representing $\geq$25% of the parent drug’s area under the plasma concentration/time curve and/or $>10%$ of exposure of total drug-related material should trigger in vitro characterization of metabolites for cytochrome P450 inhibition and propensity for DDIs. The relationship between in vitro cytochrome P450 inhibitory potency, systemic exposure, and DDI potential of drug metabolites was examined using the Pfizer development database to identify compounds with pre-existing in vivo bio-transformation data, where circulating metabolites were identified in humans. The database yielded 33 structurally diverse compounds with collectively 115 distinct circulating metabolites. Of these, 52% (60/115) achieved exposures $>25%$ of parent drug levels as judged from mass balance/metabolite identification studies. It was noted that 14 metabolite standards for 12 parent drugs had been synthesized, monitored in clinical studies, and examined for cytochrome P450 inhibition. For the 14 metabolite/parent drug pairs, no clinically relevant DDIs were expected to occur against the major human cytochrome P450 isoforms.

Introduction

When patients are administered a medication, they are exposed to not only the parent drug itself, but also drug metabolites, which are generated in situ as a natural process of xenobiotic/drug metabolism. The safety of drug metabolites (metabolites in safety testing) has been a topic of recent interest and considerable debate in the scientific literature (Leclercq et al., 2009; Smith and Obach, 2009, 2010; Vishwanathan et al., 2009; Anderson et al., 2010). Most of this debate has centered on the potential for drug metabolites to elicit toxicities that would not be caused by the parent drug itself. Thus, it is desirable that the human metabolites are also generated in preclinical species, such that the risk assessment for a metabolite can be made via administration of the parent drug during standard toxicology studies.

A subset of safety concerns around human drug metabolites is the potential for these molecules to cause pharmacokinetic drug-drug interactions (DDIs) that would not be caused by the parent drug itself. DDIs wherein one drug (the perpetrator) causes a change in the activity(ies) of a drug-metabolizing enzyme(s), thereby affecting the pharmacokinetics of a second drug (the victim), have been a focus of research for several decades. The study of cytochrome P450 DDIs has advanced such that in vivo DDI can be quantitatively predicted from in vitro data using physiologically-based pharmacokinetic (PBPK) modeling (Chenel et al., 2008; Fenneteau et al., 2010; Perdaems et al., 2010; Perdaems et al., 2010; Jones et al., 2012). Although there are a few examples in which metabolite contributions to DDIs have been retrospectively simulated

ABBREVIATIONS: ADME, absorption, distribution, metabolism, and excretion; AUC, area under the plasma concentration/time curve; AUCi, area under the plasma concentration/time curve in the presence of inhibitor; DDI, drug-drug interaction; EMA, European Medicines Agency; FDA, Food and Drug Administration; I, inhibitor (parent or metabolite) concentration in vivo; PBPK, physiologically-based pharmacokinetic; $R_{\text{Met}}$, circulating metabolite concentration ($K_{\text{Met}}$) divided by the in vitro metabolite $K_{s}$ ($K_{\text{Met}}$) value for inhibition of cytochrome P450 isozymes.
using PBPK models (Rowland Yeo et al., 2010; Kudo et al., 2013; Varma et al., 2013), our understanding of and capabilities to predict DDIs from in vitro data have been mostly restricted to cases where the parent drug is the perpetrator. The potential for a drug metabolite(s) to be the perpetrator(s) responsible for DDI has been highlighted in recent publications (Isoherranen et al., 2009; Yu and Tweedie, 2013). Likewise, the European Medicines Agency (EMA) guideline on the investigation of drug interactions (www.emea.europa.eu/pdfs/human/ich/028695en.pdf) and the draft guidance on drug interaction studies from the Food and Drug Administration (FDA; www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidance-ucm079266.pdf) have proposed that metabolites which are present at >25% of the parent area under the plasma concentration/time curve (AUC) and/or >10% of total drug-related exposure should trigger further in vitro characterization of the metabolites as a possible contributor to DDIs arising from cytochrome P450 inhibition.

On the surface, this may be counterintuitive to our basic understanding of drug metabolism and drug-metabolizing enzymes; that is, metabolism of xenobiotics usually results in compounds that are more polar than the parent drug (reduction in logD), which tends to decrease the affinity for drug-metabolizing enzymes. Thus, it would be anticipated that a metabolite will be less likely to cause a DDI through inhibition of drug-metabolizing enzymes than the parent drug, and that testing of the parent drug in vitro assays would be sufficient to mitigate DDI risks. Nevertheless, there are known exceptions to this notion. For example, gemfibrozil is metabolized to an acyl glucuronide metabolite, which causes mechanism-based inactivation of CYP2C8 (Ogilvie et al., 2006; Baer et al., 2009; Honkalanni et al., 2011), resulting in a serious DDI with cerivastatin (Backman et al., 2002) and repaglinide (Tornio et al., 2008). An analysis by Isoherranen et al. (2009) showed that, although drug metabolites are almost always inactive or less potent than their respective parent drug at inhibiting cytochrome P450 enzymes, there are enough exceptions to raise concerns that some metabolites may be responsible for a DDI when the parent lacks such an activity.

To gain a better understanding of this issue, we have undertaken a retrospective analysis of an internal data set comprised of drug candidates in which available radiolabeled human metabolism data and the metabolite profiles are quantitatively and comprehensively established. In this analysis, drug metabolites were classified using criteria proposed by the FDA and EMA to determine how frequently circulating metabolites would require further study for DDI potential. The cytochrome P450 inhibition potency of metabolites relative to the parent was reviewed, and a quantitative method to rule out metabolites was developed an algorithm that can be prospectively used to assess the potential risk of metabolites causing DDIs in a development setting so that excessive effort would not be expended on metabolites that have minimal potential to cause clinically significant DDIs.

Materials and Methods

The database of Pfizer clinical development candidates was queried to identify compounds with measured plasma concentrations of both the parent and metabolites (using authentic metabolite standards). These compounds were denoted as PF-01 to PF-12. Circulating metabolite and parent levels are reported from the highest dose group following multiple-dose administration. The metabolites were assessed for competitive cytochrome P450 inhibition using in vitro studies that were validated for prediction of cytochrome P450–mediated DDIs (Walsky and Obach, 2004). The reported cytochrome P450 inhibition constant (Ki) values are listed in micromolars, and when IC50 values for inhibition of major human cytochrome P450 isoforms are reported, in vitro cytochrome P450 Ki values were calculated using the Cheng-Prusoff equation (Cheng and Prusoff, 1973) (i.e., K __ = IC50(2)). A static model of competitive cytochrome P450 inhibition was used to predict the potential of the parent or metabolite to cause a DDI [R = IKi, where I is inhibitor (parent or metabolite) concentration in vivo] (Shardlow et al., 2011).

Literature Cytochrome P450 Inhibitors. The University of Washington Metabolism and Transport Drug Interaction Database (http://www.druginteractioninfo.org) was queried to identify clinically relevant cytochrome P450 inhibitors, with parent and metabolite data that are currently available on the U.S. market. Publications cited in the database were reviewed to compile in vitro K __ values for cytochrome P450 inhibition by the metabolite and parent for comparison with available clinical DDIs studies.

Metabolite and Parent Cytochrome P450 Inhibition Potency Comparison. For both clinical candidates and cytochrome P450 inhibitors described in the literature, the measured in vitro cytochrome P450 K __ values of metabolites and respective parent compounds were compared. Based on the relative potency between the metabolite and parent, compounds were placed in the following categories: 1) metabolite <1× potent than the parent, 2) metabolite 1–4× more potent than the parent, and 3) metabolite >4× more potent than the parent. For each metabolite/parent pair, fold differences in Ki, metabolic modification, and the relevant cytochrome P450s were reported.

Rmax Strategy. Rmax is defined as the circulating metabolite concentration (C_max) divided by the in vitro metabolite Ki (K__max) value for inhibition of cytochrome P450 isozymes (eq. 1). In cases where the metabolite standard was prepared and plasma exposure information was available from clinical studies, measured metabolite C_max and Ki were used in Rmax analysis. In other cases, metabolite concentrations were estimated using percentage values measured in high-pressure liquid chromatography–radioisetric analysis of human plasma samples from the human radiolabel study, as defined earlier. Draft regulatory guidance recommends further evaluation of metabolites with exposures >25% of the parent for metabolite-mediated cytochrome P450 inhibition. In typical parent DDI assessments, an IKi ratio <0.1 is considered low potential for a clinical DDI. Translating to metabolites, if a metabolite is 25% of the parent, a subsequent 4-fold increase in potency would yield Rmax > 0.1, leading to further evaluation for metabolite-mediated DDI. Therefore, in cases where in vitro metabolite inhibitory potency for cytochrome P450 isoforms was not available, the metabolite Ki was assumed to be 4-fold more potent than the parent Ki as a fairly conservative scenario.

\[
R_{\text{max}} = \frac{I_{\text{max}}}{K_{\text{i,met}}} = \frac{C_{\text{max,Parent}} \times \left[\frac{\text{Metabolite}}{(\text{Parent})^{0.25}}\right]}{K_{\text{i,Parent}}^{0.25}}
\]  

(1)

Testing Rmax Strategy. Compounds from the literature and Pfizer database with clinical DDI data, measured systemic exposures, and Ki values for both the parent and metabolite(s) were compiled and served as the reference data set. The University of Washington Metabolism and Transport Drug Interaction Database (http://www.druginteractioninfo.org) was queried to identify clinically relevant cytochrome P450 inhibitors, with parent and metabolite data that are currently available on the U.S. market. Publications cited in the database were reviewed to compile in vitro Ki values for cytochrome P450 inhibition by the metabolite and parent for comparison with available clinical DDIs studies.

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\]  

(1)

Testing Rmax Strategy. Compounds from the literature and Pfizer database with clinical DDI data, measured systemic exposures, and Ki values for both the parent and metabolite(s) were compiled and served as the reference data set.
Using the measured I and Ki values, Ik values were calculated for both the parent and metabolite. For this analysis, Rmax used measured metabolite concentrations, and the metabolite Ki was estimated by dividing the parent Ki by four and is listed in the estimated metabolite Ki (Parent K4) column of Table 4.

**Results**

**Circulating Human Metabolites in the Pfizer Database.** Circulating metabolites from 33 clinical 14C human ADME studies are categorized based on the metabolite/parent ratio (percentage) and depicted in Fig. 1. The 33 compounds had a total of 115 circulating metabolites, which is consistent with the industry average of ~4 metabolites per compound (Smith and Obach, 2009). Analysis of the physicochemical attributes of the parent drugs (n = 33) revealed 3 carboxylic acids, 25 basic amines, and 5 neutral compounds. The drugs span a broad range of molecular weights (142–1030, median = 413), log P values (−1.84 to 7.55, average = 3.26), and topological polar surface areas (12.0–242, average = 83.8 Å2), which is consistent with the diversity of their pharmacological targets. Analysis of the metabolite structures revealed that 23% (26/115) of metabolites were products of phase I reactions, whereas 76% (87/115) of metabolites were products of phase II conjugation pathways [1.7% (2/115) were not assigned]. A significant proportion of metabolites (55 out of 115) represented less than 25% of the parent AUC, 20 out of 115 were
ADME studies.

Overview of the 115 circulating metabolites (from a set of 33 clinical development candidates) with respect to metabolite/parent (percentage) from human ADME studies.

25–49% of the parent AUC, 14 out of 115 were 50–99% of the parent, and 26 out of 115 were ≥100% of the parent AUC. Collectively, 59% (68/115) of metabolites are <10% of the total circulating radioactivity and 41% (47/115) are >10% of the total circulating radioactivity. The FDA draft criteria on metabolite DDIs, requiring evaluation when metabolites are >25% of the parent, identify 52% (60/115) of metabolites requiring further evaluation. These 60 metabolites are from 25 parent compounds, thus 76% (25/33) of parent compounds would require further analysis for metabolite-mediated DDIs using the FDA draft criteria, with each parent having, on average, ~2 metabolites (60/25) meeting the criteria. The EMA guidance of >10% of total drug-related exposure and >25% of the parent AUC identifies 36% (41/115) of metabolites that would require further analysis for potential metabolite-mediated DDI. These 41 metabolites are from 22 parent compounds. Therefore, 67% (22/33) of parent compounds would require further analysis for metabolite-mediated DDI, with each parent having ~2 (41/22) metabolites, which meets the current regulatory criteria.

Estimates of Metabolite Concentrations. In our analysis of the 33 candidates with human ADME data, there were 12 clinical candidates with 14 metabolites that have circulating metabolite concentrations using authentic standards. Preliminary analysis showed a good concordance (within 2-fold) between the metabolite/parent ratio from measured radiometric analysis in the single-dose ADME studies and the metabolite/parent ratio measured with validated bioanalytical methods at steady state in multiple-dose clinical studies. Therefore, metabolite concentrations were estimated by multiplying the metabolite/parent ratio from the human ADME study by the steady-state plasma parent \( C_{\text{max}} \) following multiple dosing in phase 2 or 3 clinical studies. Estimated metabolite concentrations are depicted in Fig. 2. The number of metabolites versus metabolite/parent (percentage) was plotted, and metabolites were further categorized based on estimated metabolite concentration (<1, 1–10, and >10 \( \mu \text{M} \)). In general, the percentage of metabolites below 1 \( \mu \text{M} \) across the three categories (metabolite/parent <25%, 25–49%, 50–99%) were consistent, ranging from 75–100% of metabolites. When the metabolite/parent ratio was ≥100%, only 54% of metabolites were <1 \( \mu \text{M} \). Of the total 115 metabolites from the human ADME studies, 21 were greater than 1 \( \mu \text{M} \) and 5 were greater than 10 \( \mu \text{M} \). The majority of metabolites [82% (94/115)] had estimated circulating concentrations <1 \( \mu \text{M} \), a concentration below which cytochrome P450 inhibition is usually not of great concern (Wrighton et al., 2000; Obach, et al., 2005).

Pfizer Clinical Development Candidates \( I/K_i \) Values. For a subset of 12 clinical candidates, with 14 synthesized metabolites, circulating concentrations of both the parent and metabolite(s) and in vitro cytochrome P450 inhibition data were also available (Table 1). These 14 metabolites were representative of common biotransformation pathways encountered in drug metabolism. Thus, 13 metabolites were derived from phase I reactions (dealkylation, hydrolysis, and/or hydroxylation) and one was obtained from a phase II glucuronidation pathway. Of the 12 parent drugs, 75% (9/12; PF-01, PF-02, PF-03, PF-04, PF-06, PF-09, PF-10, PF-11, PF-12) had \( I/K_i \) values <0.1 against major human cytochrome P450 isozymes tested, and the corresponding metabolites had \( I/K_i \) values less than or equal to that for the respective parent drugs from which they were derived. Although 25% of the parent drugs demonstrated \( I/K_i \) values >0.1 (3/12: PF-05, PF-07, PF-08), their corresponding metabolites were either devoid of cytochrome P450 inhibition [\( K_i > 30 \mu \text{M} \), PF-07 cleaved acid, PF-08 cleaved acid (a) and (b)] or the \( I/K_i \) value was ~8-fold lower than for the parent compound (PF-05 methylated catechol). Clinical pharmacokinetic interaction studies were conducted with CYP2D6 or CYP3A4 substrates and five (PF-01, CYP2D6; PF-03, CYP3A4; PF-06, CYP3A4; PF-07, CYP2D6; and PF-08, CYP3A4) of the 12 parent drugs. Changes in AUC were <1.2-fold (PF-03 and PF-06), 1.3- to 1.4-fold (PF-01 and PF-08), and 2.2-fold...
relative inhibition potencies of metabolites and parent drugs (Table 2). No metabolites displayed a cytochrome P450 inhibition potency greater than 4-fold relative to the parent compounds as judged from the $K_i$ values. Compared with the parent drug, 29% (4/14) of metabolites were 1- to 4-fold more potent against a single cytochrome P450 isoform, whereas 71% (10/14) were less potent than the parent. There were four examples in which a metabolite had modest increase potency at a specific cytochrome P450 (PF-01, 1A2; PF-04, 1A2; PF-11, 2C9; PF-12, 2C9) that was not inhibited by the parent. In three of these cases, the increased potency was modest, and the parent was an overall more potent cytochrome P450 inhibitor than the metabolite. A search of The University of Washington Metabolism and Transport Drug Interaction Database identified 31 parent and metabolite pairs of known cytochrome P450 inhibitors with measured $K_i$ values, which are listed in Table 3. Within this segment, 19% (6/31) of metabolites were >4-fold more potent than the parent, 42% (13/31) of metabolites were 1- to 4-fold more potent than the parent, and 39% (12/31) of metabolites were less potent than the parent.

**Testing the $R_{Met}$ Strategy.** Sixteen drugs from the literature and five clinical development candidates with clinical DDI studies that include measured circulating parent and metabolite exposures and $K_i$ values for cytochrome P450 inhibition are listed in Table 4. When parent and metabolite $I/K_i$ or $R_{Met}$ values were >0.1, the respective parent or metabolite was identified as requiring further analysis for assessment of clinical DDI potential, and is listed as “Y” in Table 4. If either the parent or metabolite $I/K_i$ or $R_{Met}$ were listed as “Y,” then the overall parent/metabolite pair was also listed as “Y.” When the parent and metabolite $I/K_i$ or $R_{Met}$ value was <0.1, the respective parent or metabolite was identified as not requiring further analysis, and is listed as “N” in Table 4. Literature compounds were then categorized based on the $I/K_i$ analysis. Compounds were listed as category P, for parent-mediated DDI, when parent $I/K_i$ values were >0.1. Compounds were listed as category M, for metabolite-mediated DDI, when parent $I/K_i$ values were <0.1 and metabolite $I/K_i$ values were >0.1. Gemfibrozil was categorized as M, since the DDI is due to the glucuronide metabolite (Ogilvie et al., 2006; Baer et al., 2009). Compounds were listed as category U, for unexplained DDI, when both parent and metabolite $I/K_i$ values were <0.1.

In the literature data set, there were six, six, and four parent/metabolite pairs in the P, M, and U categories, respectively. Within category P, all of the parent drugs contributed to the DDI, and based on the measured $I/K_i$ values, it is possible that three of the six metabolites may also contribute further toward the observed DDI. The $R_{Met}$ analysis predicted that six of six metabolites may contribute to the DDI phenomenon for the compounds in the P category. Overall, all of the category P compounds will require further analysis based on the parent $I/K_i$ analysis. For category M, there are four parent compounds and six metabolites with bupropion having three metabolites: M1 (threo-hydro), M2 (hydroxy), and M3 (erythro-hydro). Based on parent $I/K_i$ values, one (gemfibrozil) of the four parent drugs contributed to the in vivo DDI. The measured metabolite $I/K_i$ and $R_{Met}$ identified six of six and five of six metabolites, respectively, in the M category that would require further evaluation. Interestingly, the $R_{Met}$ analysis did not identify bupropion metabolite M3 as a DDI perpetrator, as the $R_{Met}$ value of 0.08 was below the 0.1 criterion. However, the parent compound, bupropion, will still be evaluated for metabolite-mediated DDI due to the identification of M1 and M2 by the $R_{Met}$ analysis. Both the measured $I/K_i$ and $R_{Met}$ analysis identified all compounds as possessing the potential for a metabolite-mediated DDI. For category U, neither parent nor metabolite $I/K_i$ or $R_{Met}$ would identify candidates requiring further evaluation for cytochrome P450 inhibition. It is, however, important to realize that the magnitude of the DDI was low (1.3-1.9) for these compounds. For the clinical development candidates, two (PF-07 and PF-08) were predicted to have parent-mediated DDIs, three (PF-01, PF-07, and PF-08) were identified as potential metabolite-mediated DDIs based on $R_{Met}$ analysis, and two (PF-03 and PF-06) of the compounds were predicted to have neither parent- nor metabolite-mediated DDIs.

**Discussion**

The potential for metabolite-mediated DDIs has been highlighted in recent regulatory guidance by both the EMA and FDA, and criteria have been provided for when metabolites require further evaluation. To determine the impact on drug development, the regulatory guidance was applied to the Pfizer clinical development compounds. Based on the current EMA metabolite DDI criteria (metabolite >10% of total drug-related material and metabolite >25% of parent AUC), 67% (22/33) of parent compounds would require evaluation for a metabolite-mediated DDI and 36% (41/115) of all metabolites would be expected to contribute significantly to reversible cytochrome P450-mediated DDIs following administration of the parent drug.

**Table 2** Comparison of parent and metabolite in vitro cytochrome P450 $K_i$ values for Pfizer clinical development candidates

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Parent $I/K_i$</th>
<th>Metabolite</th>
<th>Parent $I/K_i$</th>
<th>Metabolite</th>
<th>Parent $I/K_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF-01 (2×, O-desmethyl, P at 2D6 M at 1A2)</td>
<td>0.12</td>
<td>PF-02 (&lt;1, N-desmethyl, 3A4)</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF-04 (1×, N-desmethyl, 2B6)</td>
<td>0.1</td>
<td>PF-03 (&lt;0.3, O-glucuronide, 2C9)</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF-04 (1×, N-oxide, 2D6)</td>
<td>0.2</td>
<td>PF-05 (&lt;0.4, methyl catechol, 2D6)</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF-11 (1×, N-desethyl, 2D6)</td>
<td>0.3</td>
<td>PF-06 (&lt;0.3, N-desethyl, 3A4)</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF-07 (&lt;0.003, cleaved acid, 2D6)</td>
<td>0.07</td>
<td>PF-08 (&lt;0.01, cleaved acid (a), 3A4)</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF-08 (&lt;0.01, cleaved acid (b), 3A4)</td>
<td>0.09</td>
<td>PF-09 &lt;P and M inactive, alcohol</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF-10 &lt;P and M inactive, N-desmethyl</td>
<td>0.1</td>
<td>PF-12 &lt;(0.2, hydroxy, 2C19)</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P, parent; M, metabolite; (fold change potency, metabolite structure, cytochrome P450).
82% (94/115) of the metabolites were less than 1. Further analysis. Metabolite concentrations were estimated, using data of parent compounds and 52% (60/115) of metabolites would require further consideration for cytochrome P450 inhibition. Using the FDA draft criteria (metabolite >25% of parent AUC), 76% (25/33) of parent compounds and 52% (60/115) of metabolites would require further analysis. Metabolite concentrations were estimated, using data that are routinely collected in drug development, demonstrating that 82% (94/115) of the metabolites were less than 1 μM in circulation, which is below concentrations that are typically associated with relevant cytochrome P450 inhibition (Wrighton et al., 2000; Obach et al., 2005).

For 12 (out of 33) clinical candidates, metabolite concentrations and in vitro Kᵢ values for cytochrome P450 inhibition were available for both parent and corresponding metabolites. For the 14 metabolites (from 12 clinical candidates), the metabolite I/Kᵢ < 0.1 criterion ruled out any metabolite contributions to clinically relevant DDIs. Although we acknowledge that our data set is limited, the results suggest that metabolite-mediated DDIs are generally uncommon. This is consistent with previous reports from Isoherranen et al. (2009) and Yeung et al.

### TABLE 3
Comparison of parent and metabolite in vitro Kᵢ values for literature cytochrome P450 inhibitors

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>More Potent than Parent (n = 6)</th>
<th>Metabolite 1–4× More Potent than Parent (n = 13)</th>
<th>Metabolite &lt;1× Potent than Parent (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiodarone (6.6×, desethyl, 2C9)</td>
<td>Bupropion (4×, threo, 2D6)</td>
<td>Amiodarone (0.5×, desethyl, 2D6)</td>
<td></td>
</tr>
<tr>
<td>Bupropion (12×, ethyro, 2D6)</td>
<td>Bupropion (1.6×, hydroxy, 2D6)</td>
<td>Clomipramine (0.5×, N-desethyl, 2D6)</td>
<td></td>
</tr>
<tr>
<td>Atorvastatin (100×, lactone, 3A)</td>
<td>Sulfipyrazole (3×, sulfone, 2C9)</td>
<td>Risperidone (0.4×, hydroxy, 2D6)</td>
<td></td>
</tr>
<tr>
<td>Citalopram (11×, desemethyl, 2C19)</td>
<td>Venlafaxine (1.5×, N-desethyl, 2D6)</td>
<td>Risperidone (0.8×, hydroxy, 3A)</td>
<td></td>
</tr>
<tr>
<td>Sulfipyrazole (8×, sulfide, 2C9)</td>
<td>Sertraline (1.4×, N-desethyl, 2D6)</td>
<td>Itraconazole (0.3×, hydroxy, 3A)</td>
<td></td>
</tr>
<tr>
<td>Propranolol (4.5×, 4-hydroxy, 2D6)</td>
<td>Sertraline (1×, N-desethyl, 3A4)</td>
<td>Ranolazine (0.9×, O-desethyl, 3A4)</td>
<td></td>
</tr>
</tbody>
</table>
| Met, = metabolite 

### TABLE 4
Testing Rᵢₐₜₑ for compounds with clinical drug-drug interaction data

<table>
<thead>
<tr>
<th>Clinical DDIs</th>
<th>Cytochrome P450</th>
<th>Parent</th>
<th>Metabolite</th>
<th>Parent Kᵢ/4</th>
<th>Rᵢₐₜₑ</th>
</tr>
</thead>
<tbody>
<tr>
<td>I, Kᵢ, I/Kᵢ</td>
<td>µM</td>
<td>µM</td>
<td>µM</td>
<td>µM</td>
<td></td>
</tr>
<tr>
<td>Pᵢₐₜₑ</td>
<td>Fluoxetine</td>
<td>7.8</td>
<td>2D6</td>
<td>0.37</td>
<td>0.2</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>36.4</td>
<td>3A4</td>
<td>0.94</td>
<td>8.5</td>
<td>0.11</td>
</tr>
<tr>
<td>Sulfipyrazole</td>
<td>1.9</td>
<td>2C9</td>
<td>51</td>
<td>229</td>
<td>0.22</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>1.5</td>
<td>2C19</td>
<td>1.6</td>
<td>2</td>
<td>0.8</td>
</tr>
<tr>
<td>Quinidine M1</td>
<td>42.6</td>
<td>2D6</td>
<td>1.9</td>
<td>0.3</td>
<td>6.33</td>
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<tr>
<td>Bupropion M1</td>
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<td>2D6</td>
<td>0.67</td>
<td>21</td>
<td>0.03</td>
</tr>
<tr>
<td>Bupropion M2</td>
<td>5.2</td>
<td>2D6</td>
<td>0.67</td>
<td>21</td>
<td>0.03</td>
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* Rᵢₐₜₑ = metabolite I/Kᵢ/4 (parent Kᵢ = 0.25); P, parent-mediated DDI; M, metabolite-mediated DDI; U, unexplained DDI; N, no; Y, yes.
* This table is an expansion of the data presented in Table 3, with a focus on testing Rᵢₐₜₑ for compounds with clinical drug-drug interaction data.

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P, parent; M, metabolite; (fold change potency, metabolite structure, cytochrome P450).
threohydrobupropion and erythrohydrobupropion are present at 3-fold compared with the parent drug, the reductive metabolites bupropion, which undergoes reductive metabolism on its carbonyl (Jenkins et al., 2011). A second example is the antidepressant acid – CYP2C8 by the acyl glucuronide metabolite of gemfibrozil is rare covalently adducts the heme. This mechanism-based inactivation of the parent drug AUC), causes the time-dependent inactivation of metabolites by estimating \( R_{\text{Met}} = I_{\text{Met}}/K_{\text{Met}} \). We propose \( R_{\text{Met}} \) be used as an initial yes/no criterion, similar to the parent \( I/K \) analysis for assessing DDI potential of parent drugs. Thus, a metabolite with \( R_{\text{Met}} > 0.1 \) would require further analysis, whereas a metabolite with \( R_{\text{Met}} < 0.1 \) would not need further analysis with respect to cytochrome P450 inhibition. In cases where authentic standards of metabolites are unavailable for cytochrome P450 inhibition assess-ment, we assume the metabolite(s) to be 4 times more potent than the parent as a cytochrome P450 inhibitor (i.e., parent \( K_i/4 \)). This represents a conservative estimate but is consistent with the stated regulatory guidance. When a metabolite is 25% of the parent AUC, it cannot be ruled out that inhibitory metabolites may be formed in situ. Therefore, compounds with short microsomal half-lives (e.g., \(<5 \) minutes) will require further metabolite profiling to ensure proper interpretation of in vitro microsomal DDI results.

In our present work, we have applied the static model \( (R = I/K_i) \) used in DDI prediction for parent drugs to predict DDI potential of metabolites by estimating \( R_{\text{Met}} \) values (Reese et al., 2008). Consequently, the alcohol metabolites have been rationalized as the biochemical basis for the observed in vivo DDI between bupropion and the CYP2D6 substrate desipramine (Kotlyar et al., 2005; Reese et al., 2008; Yeung et al., 2011; Lutz and Isoherranen, 2012). Similarly, the DDI arising from cytochrome P450 inhibition by amiodarone and sertraline could only be predicted when the respective N-dealkylated metabolites of amiodarone and sertraline (present at 2- to 3-fold higher AUC than the parent drug) were taken into consideration (Yeung et al., 2011). It is important to note that pharmacokinetic profiling of most alkylamine (and alkylamide) drugs reveals the presence of N-dealkylated metabolites in circulation, often at plasma concentrations significantly higher than the parent drug (Ohyama et al., 2000; Mori et al., 2009; Orr et al., 2012). Thus, a general rule of thumb in research efforts around alkylamine and amide chemotypes should involve the synthesis of the putative N-dealkylated metabolites, especially considering that the N-dealkylated metabolites possess the potential to inhibit cytochrome P450 isoforms distinct from those inhibited by the parent drug. Even though the design of the in vitro cytochrome P450 inhibition experiment is not conducive to the formation of metabolites due to short incubation times, it cannot be ruled out that inhibitory metabolites may be formed in situ. Therefore, compounds with short microsomal half-lives (e.g., \(<5 \) minutes) will require further metabolite profiling to ensure proper interpretation of in vitro microsomal DDI results.
first approximation for $R_{met}$ analysis, and is generally supported in our analysis of measured cytochrome P450 inhibition potency of metabolites and parents. For the 12 clinical candidates, there were no metabolites $>4$-fold more potent than the parent (Table 2). Likewise, in the case of literature cytochrome P450 inhibitors, approximately 80% (25/31) of the metabolites were $<4$-fold more potent than the parent, whereas $20%$ of the metabolites were $>4$-fold more potent than the parent compounds (Table 3). In total, when considering both Pfizer candidates and literature cytochrome P450 inhibitors, only 13% (6/45) of metabolites reviewed were $>4$-fold more potent than the parent. Considering that this is a rapidly evolving area of science, we could anticipate that the “$4$-fold” criterion may be adjusted as more metabolite/parent $K_i$ data become available.

Although the $R_{met}$ analysis can play an important role in ruling out compounds for potential metabolite-mediated DDIs, a systematic approach is needed to identify which metabolites may contribute to or cause metabolite-mediated DDIs. To this end, a metabolite DDI algorithm (Fig. 3) was also developed to assist the $R_{met}$ analysis in a prospective fashion in the course of drug development. Based on our analysis, 67% of clinical development compounds each have, on average, two metabolites, which would require further analysis for metabolite-mediated DDI. In these cases, the metabolite $K_i$ was assumed to be $4$-fold more potent than the parent and the $R_{met}$ calculated. For compounds with $R_{met}$ values $<0.1$, no further studies (e.g., cytochrome P450 inhibition) would be needed. For compounds with an $R_{met}$ value $>0.1$, we propose the use of PBPK modeling using available pharmacokinetic data and relevant in vitro cytochrome P450 inhibition data for the parent drug to define the potential for metabolite-mediated DDI. If PBPK modeling is not available, we recommend using mechanistic static models to estimate metabolite-mediated AUC changes (Fahmi et al., 2009). If the modeling predicts AUC changes (AUC/AUC) $>1.25$, then synthesis of the metabolite(s) is recommended for determination of in vitro $K_i$ values for cytochrome P450 inhibition and subsequent reincorporation into the model to examine synergistic effects [parent plus metabolite(s)]. These data along with project-specific information are then used to decide if a clinical DDI study is warranted. We fully recognize that synthesis (or biosynthesis) of some metabolites (e.g., diastereomeric derivatives) can be challenging, and will require additional diligence to examine metabolite-mediated DDIs.

We have provided a pragmatic method of determining whether a clinical DDI study is needed based on potential for a metabolite, rather than the parent drug, to inhibit cytochrome P450 activity. The $R_{met}$ analysis was tested and successfully identified drugs with reported metabolite-mediated DDIs. Since the occurrence of metabolite-mediated DDIs in drug development is low, a data-driven algorithm was developed as a useful tool to further rule out compounds for metabolite-mediated DDIs. The algorithm should be useful in drug development since it provides a framework enabling productive scientific decisions and discussions with regulators on select compounds to determine whether clinical DDI studies are warranted.

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Participated in research design: Callegari, Kalogtarkar, Leung, Obach, Plowchalk, Tse.
Performed data analysis: Callegari, Kalogtarkar, Leung, Obach, Plowchalk, Tse.

Wrote or contributed to the writing of the manuscript: Callegari, Kalogtarkar, Leung, Obach, Plowchalk, Tse.

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