A perspective on the contribution of metabolites to DDI potential: the need to consider both circulating levels and inhibition potency

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ABBREVIATIONS:

DDI, drug-drug interaction; AUC, area under time-concentration curve; CYP450, cytochrome P450; NCE, new chemical entities; MS, mass spectrometry; NMR, nuclear magnetic resonance; NTR, narrow therapeutic range; C_{max}, maximum concentration in human plasma; K_i, inhibition potency; K_{ip}, inhibition potency of the parent drug; K_{im}, inhibition potency of the metabolite; [Ip], total plasma concentration of parent as an inhibitor; [Im], total plasma concentration of metabolite as an inhibitor; [Im]/[Ip], metabolite to parent ratio; [Ip]/K_{ip}, [I]/K_i ratio for the parent drug; [Im]/K_{im}, [I]/K_i ratio for the metabolite; clogP, calculated logarithm of partition coefficient; HPLC, high-performance liquid chromatography; MBI, mechanism-based inhibition; K_{i}, dissociation constant for the enzyme-inactivator complex; k_{inact}, maximum rate for the inactivation.
Abstract

The 2012 DDI Guidance from EMA and the draft DDI Guidance from the FDA have proposed that metabolites present at > 25% of parent AUC (EMA and FDA) and >10% of total drug-related exposure (EMA) should be investigated in vitro for their DDI potential. This commentary attempts to rationalize the clinically relevant levels of metabolite(s) that contribute to DDI by considering not only the abundance but also inhibition potency, physicochemical properties and structural alerts of the metabolite. A decision tree is proposed for levels of metabolites which could trigger in vitro DDI assessment. When the parent is an inhibitor of CYP450s, clinical DDI studies will assess the in vivo DDI effect of the combination of parent and metabolite(s). When the parent is not a CYP450 inhibitor, it is important to assess the inhibition potential of abundant metabolites in vitro. The proposal is to apply a default cut-off value of metabolite level which is 100% of parent AUC. It is important to note that exceptions can occur and different metabolite levels may be considered depending on the physiochemical properties of metabolites (e.g. increased lipophilicity) and whether the metabolite contains structural alerts for DDI (e.g. MBI). A key objective of this commentary is to stimulate discussions among the scientific community on this important topic so that appropriate in vitro metabolism studies are conducted for metabolites to ensure safety of drugs in development balanced with the desire to avoid creating unnecessary studies which will add little to no value in ensuring patient safety.
Introduction

The recent EMA Guideline on Investigation of Drug Interactions (European Medicines Agency, 2012) and the Draft Guidance on Drug Interaction Studies from the FDA (The US FDA, 2012) have both proposed that metabolites which are present at >25% of parent AUC (EMA and FDA) and >10% of total drug-related exposure (EMA), should trigger further in vitro characterization of the metabolite as a possible contributor to drug-drug interactions (DDI) as a consequence of inhibition or induction. There is already an appreciation that metabolites can contribute the main component of the pharmacology of a parent molecule (e.g. morphine 6-glucuronide (Hanna, et al., 1990)), or be partial contributors (e.g. N-desmethylsertraline (Rudorfer and Potter, 1997)). Metabolites have also been implicated in adverse effects (e.g. the quinone-imine metabolite of acetaminophen (Manyike, et al., 2000) and trifluoroacetyl chloride of halothane (Satoh, et al., 1989)).

In cases where the parent compound is an inhibitor or an inducer of drug metabolizing enzyme(s), based on in vitro data, in vivo DDI studies are generally conducted to confirm their DDI potential in vivo. Metabolites which contribute to inhibition or induction, in combination with the parent molecule, will, by default, be accounted for in clinical studies at relevant levels. In those situations, the question to ask is whether there are populations where the metabolite could disproportionately contribute to the overall DDI effect by being present at higher levels.

On the other hand, when the parent compound is not an inhibitor or inducer, based on in vitro data, in vivo DDI studies are generally not conducted. In those cases, how do we identify metabolites which are inhibitors or inducers of drug metabolizing enzyme(s)? There are a few examples of metabolites being the main contributor to clinically relevant DDI reported in the literature. For example, threohydrobupropion and erythrohydrobupropion have 4- and 12-fold...
lower $K_i$ values for CYP2D6, respectively, compared to the parent bupropion and are also circulating at higher concentrations than bupropion (Reese, et al., 2008). Gemfibrozil glucuronide was identified as an unusual example of a conjugated metabolite which was considerably more potent an inhibitor for CYP2C8 compared to the parent molecule (Ogilvie, et al., 2006). This commentary attempts to rationalize the clinically relevant levels of a metabolite that could contribute to DDI by considering abundance, inhibition potency, physicochemical properties and structural alerts of metabolites in addition to the available in vitro/in vivo DDI data for the parent drug.

Proposed strategy for assessing the contribution of metabolites to DDI

Pharmaceutical companies frequently conduct in vitro studies to assess DDI potential (e.g. CYP450 inhibition and induction) for new chemical entities (NCEs) in preclinical and early-stage clinical development. An early readout of clinical relevance of DDI potential can be achieved through targeted studies (e.g. midazolam clearance for CYP3A or cocktail studies). A common strategy is to rank order the likelihood of in vivo DDI and conduct a probe substrate interaction in the clinic targeting the most potent interaction, based on the in vitro DDI data (Obach, et al., 2005). Alternatively a clinical study employing a cocktail of probe substrates has been used to assess DDI potential ((Chainuvati, et al., 2003;Streetman, et al., 2000)). Both approaches have their merits (Bjornsson, et al., 2003;The US FDA, 2006). By this point in the development of the drug, many companies have evaluated circulating metabolite profiles in humans and can assess the relative plasma levels of parent to metabolites. Thus, data from in vitro and early clinical studies, including DDI characterizations of the parent drug with a consideration of metabolites can, and should be, utilized in determining whether separate inhibition or induction studies for the metabolites are warranted. An important consideration in
these early analyses is whether metabolites (or even parent molecule) will accumulate with multiple dosing and so conducting the DDI assessment at steady-state becomes even more important. If inhibition or induction is not observed in these clinical studies, further in vitro characterization of the metabolites for DDI potential is generally not warranted, regardless of their abundance.

A more interesting challenge to this strategy is the situation where the parent drug does not inhibit or induce based on in vitro data, and so in vivo DDI studies for the parent are generally not conducted. Under what circumstances should efforts be extended to evaluate metabolites for their potential to inhibit or induce? There can be significant challenges in trying to synthesize metabolites. The efforts required to synthesize metabolites should not be minimized or dismissed when debating whether such studies should be conducted. The problem is often exacerbated by not having early definitive identification of metabolites (e.g. MS fragmentation patterns suggesting structures rather than definitive NMR). Enantiomers and other structural analogs can also complicate identification and synthesis. Separation of these isomers is important as differences in inhibition potency for isomers have been observed (Reese, et al., 2008). Based on internal experience, the authors have come across a number of clear examples of extensive time and resource commitments in synthesizing metabolites. Approaches to biological generation of metabolites (through recombinant enzymes, tissue fractions, etc) are often restricted to making small amounts of material.

Currently we are not aware of any reported cases where a metabolite is a CYP450 inducer while the parent drug is not. As such, assessment of the parent induction potential (in vitro and/or in
vivo) should cover both the parent and its metabolites and so separate evaluation of the induction potential of metabolites is generally not warranted. The strategy to cover metabolites, when the parent molecule has no potential DDI liabilities, should focus on inhibition. A proposed strategy for assessing the possible contribution of metabolites to CYP450 inhibition is outlined in Figure 1. The IC_{50} and/or K_i values of the parent drug are generally determined in preclinical development. The plasma concentrations and exposure (total and/or free) of the parent drug and possibly the major metabolites are obtained in early clinical development (Phase Ia & Ib). The [I]/K_i ratio and/or AUCR value for the parent can be calculated following the equations outlined in Figure 4 of the 2012 FDA Draft DDI Guidance (The US FDA, 2012). If [I]/K_i is > 0.1 and AUCR is > 1.25, for one or more CYP450s, the parent is determined to be a possible inhibitor of these CYP450s in vivo. In this case, a dedicated clinical DDI study can be conducted to assess the in vivo DDI potential of the parent and its metabolites. If [I]/K_i is ≤ 0.1, the parent drug is not likely to cause inhibition in vivo. In this case, a dedicated clinical DDI study is generally not conducted, with the caveat that metabolite(s) could be a more potent CYP450 inhibitor than the parent drug. Therefore it is important to assess the DDI potential of the abundant circulating metabolites in vitro to obtain a more complete assessment of the DDI potential of drug-related material. If the metabolite(s) is likely to inhibit CYP450s, based on the [I]/K_i ratio, there will now be a need to conduct clinical DDI studies of the parent at steady state to confirm the in vitro results.

Consideration of clinically relevant levels and inhibition potency of metabolites that contribute to enzyme inhibition
Based on literature data, the risk of DDI as a result of inhibition by metabolites alone (parent is not an inhibitor) appears to be low. Two recent publications indicated that the majority (90%) of drugs (including their metabolites) are not CYP450 inhibitors in vivo (with a survey of 1323 drugs from the US market) (Isoherranen, et al., 2009; Yeung, et al., 2011). Of the remaining 10% of drugs (129) that are CYP450 inhibitors, inhibition data for the individual metabolites were only available for 21 drugs (1.6% of total or 16% of drugs showing DDI). The lack of data certainly questions whether the 16% of drugs truly represent the overall potential. In only 3 out of 21 drugs were the metabolite(s) a CYP450 inhibitor while the parent was not. These 3 drugs (amiodarone, bupropion and sertraline) represent 0.2% of all drugs; 2.3% of drugs exhibiting DDI; and 14% of drugs where metabolites were evaluated. Therefore, for the vast majority of drugs, assessing the DDI potential of the parent drug is adequate to assess the DDI potential of both the parent drug and its metabolites. There is certainly a valid argument that a false negative should be viewed with much greater concern than a false positive, particularly as we strive to ensure patient safety. To minimize further the low risk of DDI caused by metabolites, there are additional considerations. Some structural alerts can be used as a trigger to conduct in vitro DDI assessments for inhibition and inactivation. For example, metabolites derived from N-dealkylation (VandenBrink and Isoherranen, 2010) or containing an epoxide moiety (Brown and Ford-Hutchinson, 1982) have the potential to inactivate CYP450s and should be further evaluated.

In assessing the contribution of metabolites to clinically relevant enzyme inhibition, it is important to consider both the levels of a metabolite(s) (individually or summed) and the inhibition potency of these metabolites. Several literature reports have demonstrated that
hydrophobicity is one of the most important factors in determining the inhibition potency of CYP450 inhibitors. For example, QSAR studies by Lewis et al. showed that a linear relationship existed between lipophilicity and potency for 15 inhibitors/substrates of CYP2C9 including NSAIDS (Lewis, et al., 2006). Modeling by Didziapetris et al. demonstrated that an increase in the size of the molecule with the incorporation of hydrophobic aliphatic or aromatic residues resulted in a higher probability for the compound to inhibit CYP3A4 (Didziapetris, et al., 2010). Roy and Roy also demonstrated that logP appeared to be the most important factor affecting the inhibition potency of CYP3A4 inhibitors (Roy and Pratim Roy, 2009). From an evolutionary perspective, biotransformation of xenobiotics by host enzymes is a protective mechanism, resulting in the generation of (generally) more polar compounds which will then be more readily excreted compared to parent. The decreased hydrophobicity of metabolites generally leads to a lower affinity for the drug metabolizing enzymes. As such, metabolites are expected to be less potent inhibitors than the parent drug. Clearly there are some exceptions. However, we believe this generalization that metabolites are less potent inhibitors than the parent is applicable and is appropriate in defining the relative contribution to inhibition by parent and metabolite(s). Thus calculations of relevant concentrations of metabolites, when considering their contribution to inhibition, cannot be simple arithmetic summations of metabolites and parent molecule levels. Adjustments for expected lower inhibition potency of metabolites should be considered.

In rationalizing clinically relevant levels of metabolites, an important consideration is the change in AUC of the victim drug that warrants a concern for DDI. The FDA Draft Guidance on Drug Interaction Studies proposes a 25% increase or 20% decrease of the AUC of the victim drug which, while in line with defining bioequivalence (80-125% (The US FDA, 2012) ), should not
merit full consideration for clinically relevant DDI. Additional safety concerns are warranted for drugs with narrow therapeutic ranges (NTR) such as theophylline, warfarin, and paclitaxel and these can be, and should be, treated as a special case. This conservative value (80-125%) should be addressed through scholarly discussion but is not the focus of this commentary. As such we will use the conservative value of 25% increase of victim drug AUC as the basis for discussion.

The magnitude of DDI caused by a metabolite depends on its concentration and inhibition. As stated, it is important to consider both concentration ($C_{max}$) and inhibition potency ($K_i$) values of parent and metabolites in rationalizing a cut-off value to investigate the inhibition potential of metabolites. For the purpose of illustrating this principle, competitive inhibition and a static model (often most conservative) are used and the focus is on cases where $[I_P]/K_{IP} \leq 0.1$ (i.e. parent is not an inhibitor, see Figure 1). The following set of equations (equations 1-6) outline the rationale for setting 100% of parent AUC as a reasonable level of metabolite which would trigger additional in vitro evaluation of DDI potential. $[I_m]$ and $[I_p]$ are the total systemic $C_{max}$ of the metabolite and the parent, respectively. Although using total $C_{max}$ may generally lead to over-prediction of in vivo DDI, it was demonstrated to predict in vivo DDI almost as well as using unbound hepatic inlet $C_{max}$ and superior than the unbound systemic $C_{max}$ (Obach R.S., et al., 2006) $K_{im}$ and $K_{ip}$ are the inhibition potency of the metabolite and the parent, respectively. $[I_m]/[I_p]$ is the cut-off value of metabolite (a surrogate for metabolite as % of parent AUC).

Using the conservative value of 25% increase in the AUC of a victim drug ($AUC_i/AUC = 1.25$), equations 1 and 2 are used to calculate clinically relevant metabolite levels. Rearranging equation 2, with equations 3 and 4 as the intermediate steps, yields equation 5, which provides a
ratio of inhibitor concentrations of metabolite to parent ([Im]/[Ip]). Applying [Ip]/Kip of 0.1 (no DDI are anticipated for the parent) to equation 5 results in equation 6. Equation 6 clearly illustrates the importance of considering the inhibition potency ratio between metabolite and parent (K_{im}/K_{ip}) in proposing a cut-off value for metabolites ([Im]/[Ip]). The relationship between [Im]/[Ip] and K_{im}/K_{ip} is illustrated in Figure 2.

\[
\frac{\text{AUC}_{Ci}}{\text{AUC}} = 1 + \frac{[\text{Ip}]}{K_{ip}} + \frac{[\text{Im}]}{K_{im}} = 1.25 
\]

(equation 1)

\[
\frac{[\text{Ip}]}{K_{ip}} + \frac{[\text{Im}]}{K_{im}} = 0.25 
\]

(equation 2)

\[
\frac{[\text{Im}]}{[\text{Ip}]} = 0.25 \times \frac{K_{im}}{I_{p}} - \frac{K_{im}}{K_{ip}} 
\]

(equation 3)

\[
\frac{[\text{Im}]}{[\text{Ip}]} = 0.25 \times \frac{K_{im}}{K_{ip}} - \frac{K_{im}}{K_{ip}} 
\]

(equation 4)

\[
\frac{[\text{Im}]}{[\text{Ip}]} = \frac{K_{im}}{K_{ip}} \left( \frac{0.25}{I_{p}} - 1 \right) 
\]

(equation 5)

\[
\frac{[\text{Im}]}{[\text{Ip}]} = 1.5 \times \frac{K_{im}}{K_{ip}} \text{ when } \frac{[\text{Ip}]}{K_{ip}} = 0.1 
\]

(equation 6)

As demonstrated in equation 6 and also in Figure 2, the more potent the metabolite as an inhibitor, relative to the parent, the lower the level of metabolite which would trigger in vitro DDI studies. If the metabolite and the parent have the same inhibition potency (K_{im}/K_{ip} =1), the metabolite level to trigger in vitro inhibition studies would be 150% of parent AUC ([Im]/[Ip] = 1.5, see Table 1 and Figure 2). A metabolite present at 25% of parent AUC, as suggested by the Draft FDA Guidance, would need to be 6-fold more potent in inhibition than parent to cause an
increase of 25% of AUC of the victim drug ([Im]/[Ip] = 0.25 and K_{im}/K_{ip} =1/6, see Table 1 and Figure 2). Since metabolites are generally expected to be more polar and have a lower affinity for CYP450 and thus be less potent inhibitors than the parent drug (K_{im}/K_{ip} \geq 1)(Backes, et al., 1993;Johnston, et al., 1991), we propose that metabolite levels approaching 100% of parent AUC is a more reasonable default value to trigger in vitro inhibition studies when the parent drug is not an in vitro inhibitor of CYP450s (i.e. [Ip]/K_{ip} \leq 0.1).

As stated in the previous section, the risk of a metabolite being the sole contributor to CYP450 inhibition is very small (3 drugs out of 1323 drugs) (Isoherranen, et al., 2009;Yeung, et al., 2011). The 3 drugs are bupropion, amiodarone and sertraline. Bupropion metabolites (threohydrobupropion and erythrohydrobupropion) are present at 3.5- to 6-fold higher levels compared to parent (Reese, et al., 2008) and thus warrant further analysis by the criteria proposed herein (100% of parent AUC). N-desethylamiodarone would also be covered since the AUC ratio between N-desethylamiodarone and amiodarone is 1.5 (Marchiset, et al., 1985). The same is true for the N-desmethyl metabolite of sertraline, which is present at \sim 3 fold higher in AUC than sertraline (Patel, et al., 2009). Gemfibrozil glucuronide is often quoted as an example of a metabolite which causes significant DDI through inhibition of CYP2C8. This is certainly an exceptional case and the presence of a mechanism based inhibitor which is formed through further metabolism of a glucuronide is quite unique (Ogilvie, et al., 2006) (Jenkins S.M., et al., 2011). However, gemfibrozil is itself an inhibitor of CYP2C8 in vitro. Additionally the glucuronide metabolite is present at \sim 100% of AUC of gemfibrozil (Tornio, et al., 2008). As such, it would also be covered by the criteria proposed herein (i.e. metabolites = 100% of parent AUC as the cut-off value to trigger in vitro DDI studies for metabolites).
We propose metabolite levels approaching 100% of parent AUC as a default value to trigger in vitro inhibition studies of metabolites (see the proposed decision tree in Figure 3). There are two notable exceptions where this default value needs to be adjusted: 1. Metabolites are less polar than the parent drug; and 2. Metabolites contain a structural alert(s) for mechanism-based inactivation (MBI) regardless of their change of polarity. Formation of less polar metabolites can occur through biochemical or chemical reactions (e.g. converting carboxylic acid to lactone as in the case of atorvastatin (Jacobsen, et al., 2000)). These metabolites should be readily recognized from their structures, clogP calculations, and most practically, from the relative elution order on reverse phase HPLC columns (e.g. threohydrobupropion and erythrophobupropion eluted later than bupropion (Petsalo, et al., 2007)). Examples of less polar metabolites that are associated with increased inhibition potency include the lactone of atorvastatin (Jacobsen, et al., 2000) and threohydrobupropion and erythrophobupropion (Reese, et al., 2008). It is worth noting that not all metabolites with decreased polarity would lead to more potent inhibition of CYP450s. The authors have compared the CYP450 inhibition potential between an internal compound and one of its less polar lactam metabolite. In this case, the lactam metabolite was a less potent inhibitor for major CYP450s than the parent (data not shown). Nonetheless, a lower cut-off value should be considered to trigger in vitro DDI studies for the less polar metabolites as we strive to predict the DDI potential of drug related material (parent and metabolites) more accurately using in vitro studies. Based on the $K_{in}/K_{ip}$ ratios between metabolites and parent of 21 CYP450 inhibitors (Yeung, et al., 2011), 80% of the $K_{in}/K_{ip}$ ratios are $< 1/6$. As shown in Table 1 and Figure 2, when $K_{in}/K_{ip} = 1/6$, the cut-off value $[Im]/[Ip]$ is equal to 0.25. Therefore, it appears to be appropriate to propose a cut-off value of 25% of parent AUC for metabolites that are less polar and can be more potent in inhibition than
the parent drug. As mentioned above, attention should be paid to metabolites containing structural alerts for MBI, which are absent in the parent molecule. Currently a well-characterized structural alert for MBI is the formation of a primary amine through N-dealkylation of the secondary and tertiary amines. The primary amine metabolites can cause MBI through the formation of the nitroso intermediate by metabolic activation (Orr, et al., 2012). For these N-dealkylated primary amine metabolites, since their inactivation parameters ($K_I$ and $k_{\text{inact}}$) cannot be related to the parent, it would be prudent to conduct in vitro DDI studies of these metabolites, even if they are present at low but appreciable concentrations. For currently known drugs, it is interesting to note that these N-dealkylated metabolites tended to circulate at a concentration that was close to or higher than that of the parent (VandenBrink and Isoherranen, 2010).

As shown in Figure 3, polarity and structural alerts of metabolites are two key considerations in this decision tree. There will be exceptions. Since it is challenging to include all possible scenarios and exceptions, we intend to use this decision tree as a basis to stimulate discussions in the scientific community. The default cut-off value to trigger in vitro studies for metabolites is 100% of parent AUC based on the premise that metabolites are generally more polar and less potent inhibitors of CYP450s than the parent. A lower cut-off value of 25% of parent AUC is proposed for metabolites that are less polar than the parent drug. These less polar metabolites can be more potent inhibitors than the parent drug. A lower cut-off value should also be applied to metabolites carrying structural alerts for MBI that are absent from the parent drug on a case-by-case basis by considering multiple factors. These factors include: 1. Difficulty to predict the inactivation parameters of metabolites from those of the parent drug; 2. More severe safety
consequences due to enzyme inactivation; 3. The extent of formation of the metabolite with structural alerts in the inactivation experiment of the parent drug.

In conclusion, the DDI risk caused by metabolites alone is considered to be low based on current literature. When the parent drug is an inhibitor of one or more CYP450 isoforms, clinical DDI studies are conducted to assess the in vivo inhibition potential for both the parent and metabolites. When the parent drug is not an inhibitor of one or more CYP450s, the proposed default cut-off value to trigger in vitro DDI studies for metabolite(s) is \( \geq 100\% \) of parent AUC. It is recognized that there can be important exceptions to this default cut-off value (e.g. metabolites are less polar or contain structural alerts for MBI). For metabolites which are less polar than parent molecule, a lower cut-off value should be considered (25% of parent AUC). Metabolites that contain structural alerts for MBI should be considered on a case-by-case basis as it is not possible to ascribe a level of expected inhibition based simply on structure (or inhibition by parent molecule). Pharmaceutical companies and regulatory authorities are concerned with patient safety It is in this spirit that we make this proposal that rationally addresses the concern of DDI by metabolites. We welcome comments from the scientific community and hope to move the science on metabolite related DDI forward through open dialogue.
Authorship Contributions
Wrote or contributed to the writing of the manuscript: Hongbin Yu and Donald Tweedie.
References


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Figure legends.

Figure 1. A proposed strategy to assess the inhibition potential of metabolites by taking account into the available in vitro and in vivo DDI data of the parent compound.

Figure 2. The metabolite cut-off value ([Im]/[Ip]) decreases with the increase of the inhibition potency ratio between metabolite and parent (K_{im}/K_{ip}).

Figure 3. Decision tree to trigger in vitro DDI studies for metabolites.
### Table 1. Metabolite cut-off value vs. the metabolite/parent inhibition potency ratio.

<table>
<thead>
<tr>
<th>[Im]/[Ip] (metabolite to parent ratio)</th>
<th>$K_{im}/K_{ip}$ (metabolite/parent inhibition potency ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 (150%)</td>
<td>1/1</td>
</tr>
<tr>
<td>1 (100%)</td>
<td>2/3</td>
</tr>
<tr>
<td>0.5 (50%)</td>
<td>1/3</td>
</tr>
<tr>
<td>0.25 (25%)</td>
<td>1/6</td>
</tr>
</tbody>
</table>

Preclinical development

Determine $K_i$ of parent for major CYP450s

Determine $C_{max}$ & AUC of parent & metabolites

Phase Ia & Phase Ib

Parent:

$[I_p]/K_i$

$[I_p]/K_i > 0.1$ and AUCR$>1.25$ in vivo inhibition by parent possible

$[I_p]/K_i \leq 0.1$ or AUCR $\leq 1.25$: in vivo inhibition by parent remote; assess metabolite inhibition potential in vitro

Conduct clinical DDI studies to assess inhibition potential of parent and metabolites in vivo

If the metabolite is likely to inhibit CYP450s in vivo, conduct clinical DDI studies

Figure 1
\[
\frac{[\text{Im}]}{[\text{Ip}]} = 1.5 \\
\text{when } K_{\text{im}}/K_{\text{ip}} = 1
\]

\[
\frac{[\text{Im}]}{[\text{Ip}]} = 1 \\
\text{when } K_{\text{im}}/K_{\text{ip}} = 2/3
\]

\[
\frac{[\text{Im}]}{[\text{Ip}]} = 0.5 \\
\text{when } K_{\text{im}}/K_{\text{ip}} = 1/3
\]

\[
\frac{[\text{Im}]}{[\text{Ip}]} = 0.25 \\
\text{when } K_{\text{im}}/K_{\text{ip}} = 1/6
\]

Figure 2
\[ \frac{[I_p]}{K_i} \leq 0.1 \text{ or } \text{AUCR} \leq 1.25: \text{ in vivo inhibition by parent remote; assess metabolite inhibition potential in vitro} \]

- metabolites less polar than parent
- metabolites more polar than parent
- Metabolites with structural alerts for MBI

- in vitro DDI studies for metabolites
  if \( \text{AUC}_{\text{metabolite}} \geq 25\% \text{AUC}_{\text{parent}} \)
- in vitro DDI studies for metabolites
  if \( \text{AUC}_{\text{metabolite}} \geq 100\% \text{AUC}_{\text{parent}} \)
- in vitro DDI studies for metabolites at a low cut-off value (case-by-case)

Figure 3