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Which Metabolites Circulate?

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Non-standard Abbreviations:

ADME: Absorption, distribution, metabolism, excretion

AUC: Area-under-the concentration time curve

AUC_m: Area-under-the concentration time curve for a metabolite

BSEP: Bile salt export pump

CL_f : Formation clearance of metabolite

CL_m : Elimination clearance of metabolite

CL_{parent} : Clearance of parent drug

CLs = Total systemic clearance

$CL_{R, U}$ = Unbound renal clearance

$CL_{NR, U}$ = Unbound non-renal (hepato-biliary) clearance

CYP: Cytochrome P450

DDI: Drug-drug interactions

EM: Extensive metabolizer

EMA: European Medicines Agency

F_a : Fraction of dose absorbed after oral administration

FDA: Food and Drug Administration

$F_{h,m}$: Systemic availability of the metabolite

f_m : Fraction of dose converted to a given metabolite from the parent

f_p : Lipoidal permeability

MIST: Drug metabolites in safety testing

M/P ratio: Relative abundance of metabolite to parent based on the ratio of plasma/serum AUC values

MRP: Multidrug resistance protein

MRP2: Multidrug resistance protein 2

MRP3: Multidrug resistance protein 3

PAPS: 3'-phosphoadenosine 5'-phosphosulfate

PM: Poor metabolizer

tPSA: Topological polar surface area

UDPGA: Uridine 5'-diphospho-glucuronic acid

UGT: Uridine diphosphate glucuronosyltransferase

UM: Ultra rapid metabolizer

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Abstract

Characterization of the circulating metabolites for a new chemical entity in humans is essential for safety assessment, an understanding of their contributions to pharmacologic activities, and their potential involvement in drug-drug interactions (DDI). This review examines the abundance of metabolites relative to the total parent drug (M/P ratio) from 125 drugs in relation to their structural and physicochemical characteristics, lipoidal permeability, protein binding, and fractional formation from parent (f_m). Our analysis suggests that f_m is the major determinant of total drug M/P ratio for amine, alcohol, N- and S-oxide, and carboxylic acid metabolites. Passage from the hepatocyte to systemic circulation does not appear to be limiting owing to the vast majority of metabolites formed being relatively lipid permeable. In some cases, active transport plays an important role in this process (e.g., carboxylic acid metabolites). Differences in total parent drug clearance and metabolite clearance are attenuated by the reduction in lipophilicty introduced by the metabolic step and resultant compensatory changes in unbound clearance and protein binding. A small sub-class of these drugs (e.g. terfenadine) are unintentional pro-drugs with very high parent drug clearance resulting in very high M/P ratios. In contrast, arenol metabolites show a more complex relationship with f_m due largely to the new metabolic routes (conjugation) available to the metabolite compared to the parent drug molecule. For these metabolites, a more thorough understanding of the elimination clearance of the metabolite is critical to discern the likelihood of whether the phenol will constitute a major circulating metabolite.

1. **Introduction**

An early understanding of the key metabolites for a new chemical entity is important in drug discovery and development. At the lead optimization stage, this can help to support medicinal chemistry efforts in designing more metabolically stable candidates via identification of soft spots in lead candidates that are rapidly cleared (Lin and Lu, 1997; Baillie, 2008). From a development perspective, characterization of metabolites provides a systematic understanding of the metabolic pathways of a drug and therefore the enzymes that are involved in their formation. This can aide in the proper design of drug-drug interaction (DDI) studies to evaluate the impact of inhibition or induction of the metabolic enzyme(s) contributing to the major pathway of clearance on systemic drug exposure. Assessment of circulating metabolites in humans also contributes to an overall understanding of their potential impact on the safety and efficacy of a drug. From a safety point of view, an effort towards gathering knowledge of the *in vivo* metabolic profile of a drug in humans has gained more importance since the publication of position papers on drug metabolites in safety testing (MIST) as well as recently issued ICH Guidance M3(R2) on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals (FDA guidance; http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM073246.pdf); (Davis-Bruno and Atrakchi, 2006; Nedderman et al., 2011). This guidance recommends that nonclinical safety evaluation is warranted if the exposure of a human circulating metabolite is observed at greater than 10% of the total drug-related exposure and at significantly greater levels in humans than the maximum exposure seen in the toxicity studies. Assessment of circulating metabolites in humans is also essential for a comprehensive understanding of their contributions to pharmacologic activities. Structural alteration of drugs

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via metabolism can sometimes lead to products that contribute to the therapeutic effect of the drug (on target activity) or cause an undesirable effect as a consequence of off-target activity (Fura, 2006). More recently, efforts have been directed in evaluating the potential involvement of metabolites in inhibitory DDI. Several clinically important drugs (especially clinically recognized CYP inhibitors) have been shown to have circulating metabolites that act as potent inhibitors of CYP enzymes and therefore contribute to in vivo DDIs (Isoherranen et al., 2009; Yeung et al., 2011). The most recent guidance by the European Medicines Agency (EMA) and US Food and Drug Administration (FDA) also suggest evaluation of potential inhibitory effect of metabolites on the common drug metabolizing enzymes. These guidances recommend that circulating metabolite with an AUC of ≥25% relative to parent AUC (Draft FDA Guidance; http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362.pdf) and phase 1 metabolites with an AUC both larger than one fourth of the AUC of parent drug and larger than 10% of the drug-related exposure (EMA Guidance; http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf) should be investigated for their inhibitory potential. These recommendations necessitate characterization and quantification of the metabolites in plasma early on to allow a full assessment of their contributions to the pharmacological or toxicological effects and DDI potential.

In most cases, principal metabolites are identified by performing radiolabeled ADME studies where ^{14}C or ^{3}H analogs of the drugs are administered to humans (Roffey et al., 2007; Isin et al., 2012; Penner et al., 2012). The biological matrices collected from this study are then analyzed to yield a quantitative profile of metabolites in circulation or in the excreta. While the

abundance of metabolites in the excreta provides a means of assessing the clearance pathways and the fraction of the drug metabolized via a given pathway (f_m), the exposure of metabolites in plasma obtained from this study serves as a starting point for prioritizing their further evaluation. The major metabolites are then synthesized and evaluated for pharmacological on-target and off-target interactions that may result in unpredicted toxicity or DDI. However, radiolabeled ADME studies in humans are expensive and resource-intensive and hence, not routinely feasible in early development. This can hinder the knowledge about relevant circulating plasma metabolites and hence their evaluation in a timely manner. Hence, until definitive data are obtained from human studies, one may need to rely on the in vitro systems for an assessment of potential metabolites that are generated. Human-derived reagents such as the liver microsomes, hepatocytes or S9 fractions, are the commonly used *in vitro* systems to study human drug metabolism and identify the principal metabolites, preferably prior to the human radiolabel ADME study (Dalvie et al., 2009). A comprehensive analysis of these in vitro systems by Dalvie and co-workers provide sufficient confidence in using these systems to reliably produce primary human metabolites (Dalvie et al., 2009). Although primary human metabolites can be predicted readily by in vitro systems, it is important to understand which metabolites formed in these systems are likely to circulate *in vivo*. This can help in prioritizing the synthesis of important metabolites for further characterization with respect to their pharmacological activity and/or their role as inhibitors or inducers of important drug metabolizing enzymes.

Smith and Dalvie have recently attempted to analyze the influence of physicochemical properties on the metabolites that circulate. This review showed that metabolites with high lipoidal permeability will passively diffuse from liver to plasma and circulate (Smith and Dalvie,

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2012). The aim of the current analysis was to extend this investigation and explore how the structures of the metabolite (e.g., amines, alcohols, etc.) and their associated physicochemical properties, as well as the fraction of dose converted to a given metabolite from the parent (f_m) may influence their abundance in circulation.

We conducted a literature review to collect data on the plasma or serum area-under-the concentration time curve (AUC) values from 125 drugs and their metabolites after oral administration of single or multiple doses of the parent, and then determine the relative abundance of metabolite to parent based on the ratio of plasma/serum AUC values (M/P ratio). Although several secondary and tertiary metabolites were detected circulating for several drugs, no attempt was made to classify and categorize these metabolites separately. In the vast majority of cases, the ratio of plasma or serum AUC of the metabolite to the corresponding parent was used, although in a few cases (as indicated by the asterisk in Tables 1-6), single time-point concentrations were used due to paucity of the overall AUC data. In a few cases (clozapine, dextromethorphan, dothiepin, flecainide, and zolpiclone) where plasma concentration-time profiles were presented in the publication but the AUC values were not calculated, the data were captured using the software DigitizeIt version 1.5 (Eden Prairie, MN) and the AUC values were calculated by noncompartmental analysis using WinNonlin version 5.2 (Pharsight Corp., Cary, NC). The M/P ratio for these metabolites was then matched with the structure and physicochemical properties of the corresponding parent and metabolite pair to discern any possible relationship between the two. The physicochemical properties for the parent and metabolites were calculated using the ACD software version 12.01 (ACD Labs, Advanced Chemistry Development Inc., Toronto, Ontario, Canada), and the chemical structures of the

parent and corresponding metabolites are shown in the Supplementary file (SuppTable 1-6). In addition, where data exists, information on the f_m value was also estimated from the published human radiolabeled ADME studies. The fraction of dose for the metabolite of interest and its sequential metabolic products recovered in excreta (both urine and feces) was assumed to be representative of the in vivo f_m value. An analysis was conducted in an attempt to identify parameter values for the physicochemical properties and f_m that are associated with metabolites considered to have met the criteria for follow-up DDI evaluations according to the regulatory guidance.

2. **Theoretical Considerations of Determining In Vivo Metabolite Exposure**

Theoretical models related to metabolite kinetics after oral or intravenous administration of the parent drug have been described extensively (Houston, 1982; Lutz et al., 2010) and will only be discussed briefly in this review. In vivo, a metabolite can display either a formation-rate limited or an elimination-rate limited kinetics. In the former case, the half-life of the terminal elimination phase of a metabolite plasma/serum concentration versus time curve will be equal to that of the parent drug; while in the latter case, the half-life of the terminal elimination phase of the metabolite will be greater than that of the parent. Most assessment of circulating concentrations of drugs and their metabolites are made without reference to protein binding, certainly historically and also at the initial profiling and identification stage of drug discovery and development. Regulatory guidelines reflect this in that the identification of “major” metabolite rely on total concentration compared to parent or drug-derived moieties, although there is recognition that certain metabolites may have very different binding characteristics to their parent. In order to understand the dynamics of metabolites, protein binding must be

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measured to derive unbound concentrations. This is reinforced by the tendency for a reduced lipophilicity for the metabolite, which may increase the fraction unbound and lowers unbound clearance leading to disproportionate concentrations of unbound metabolite compared to unbound concentrations of parent. Unfortunately, protein binding data is lacking in many cases to provide more sophisticated analysis. In terms of the effects of changes in chemical structures and how they may impact the ratio of total drug to metabolite, the influence of protein binding may be attenuated. Most metabolic steps typically result in reduced lipophilicity, which will tend to decrease protein binding (or increase fraction unbound) and intrinsic metabolic clearance (unless the metabolic step introduces a route by which the parent cannot be metabolized), thereby attenuating the effect on the difference between parent and metabolite clearance (See Section 9.3). Similarly, the lowered lipophilicity will tend to decrease tissue or membrane affinity, which will be compensated for by the increased free fraction in plasma, thereby attenuating the effects on total volume of distribution. Thus, the ratio of total parent to metabolite is probably more sensitive to other factors as detailed below.

The systemic exposure (total, bound plus free), as represented by the AUC of a metabolite (AUC_m), will be dependent on the dose of the parent drug (D), the fraction of dose absorbed (after oral administration, F_a), the fraction of the absorbed drug dose that is converted to the metabolite (f_m), the systemic availability of the metabolite ($F_{h,m}$), and the clearance of the metabolite (CL_m), i.e., $AUC_m = (f_m \bullet F_{h,m} \bullet F_a \bullet D) / CL_m$ (Houston, 1982). As illustrated in Figure 1, the systemic availability of a metabolite can be conceptualized as the net fraction of the formed metabolite partitioning back into the circulation. Physiologically, this reflects the permeability rate of the metabolite across the sinusoid for passive diffusion (lipoidal

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permeability, f_p) and the effect of sinusoidal efflux transporters (active transport) compared to the rate of further processing of the metabolite by metabolism within the hepatocyte or clearance directly from the hepatocyte or by canalicular transporters (biliary excretion). Lipoidal permeability decreases with decreasing lipophilicity and increasing polar surface area (tPSA). Unless the metabolite is a substrate for sinusoidal transporters, it is possible that its residence time in the liver will be increased by metabolism favoring further processing and attenuating systemic exposure.

In vivo, after oral dosing, the ratio of AUC for the metabolite to parent (M/P ratio) will be determined by $f_m \bullet F_{h,m} \bullet CL_{parent} / CL_m \bullet F_h$ (where F_h is the fraction of absorbed parent drug dose that escape the liver) since dose (parent) and F_a are the same. For a drug cleared by metabolism to a single metabolite which diffuses completely from the hepatocyte to systemic circulation (e.g., a metabolite with high lipoidal permeability), this simplifies to $CL_{parent} / CL_m \bullet F_h$, assuming linear processes. For a drug that undergoes metabolism to more than one metabolite and assuming complete diffusion of the metabolite(s) to systemic circulation, the M/P ratio is expressed as $f_m \bullet CL_{parent} / CL_m \bullet F_h$, which indicates that the formation and elimination clearance (CL_f and CL_m, respectively) of the metabolite are major determinants of its M/P ratio. The differences between CL_{parent} and CL_m likely reflect actual chemical-structural change(s) as a result of the metabolism of a more labile functional group to a more stable functional group rather than simple reduction in lipophilicity (see above discussion on protein binding). These principles for highly diffusable metabolites have been established for some time (Levy et al. 1983), and provide a useful conceptual framework to guide the assessment and interpretation of our analysis regarding the potential relationship between structural and physicochemical

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properties of the metabolites and their abundance in circulation relative to the parent drug based on ratio of AUC.

3. **Secondary or Primary Amines as Metabolites**

N-Dealkylation represents one of the most common biotransformation pathways for drugs containing secondary and tertiary amines. This oxidative pathway results in the formation of metabolites that contain primary or secondary amines or in some cases cleaved products as primary metabolites. In this dataset, 52 out of a total of 125 drugs (42%) were *N*-dealkylated as part of the overall clearance process. Of these 52 drugs, 40 (77%) had an amine metabolite with an abundance, based on the M/P ratio, of >0.25 in human plasma (Table 1). This trend is not surprising since these metabolites retain lipophilicity, as represented by cLogD, which is within the range of -1 to 5 and is optimal for passive diffusion (Figure 2). The physicochemical changes associated with *N*-dealkylation usually showed a small increase in tPSA of ~9Å and a reduction in cLogD of up to 1.0 log unit when simple alkyl substituents were cleaved (Table 1). However, when *N*-dealkylation reaction cleaved a molecule into smaller fragments, more pronounced changes in cLogD and tPSA were observed. For instance, cleavage of nefadozone to *m*-chlorophenylpiperazine (Figure 3) was accompanied by a reduction in cLogD of ~3 log units along with a decrease in tPSA of 36 Å. Interestingly, some drugs in this dataset were exceptions to this trend in that high abundance of amine metabolites were present in circulation despite their high polarity (i.e., cLogD < -1). For example, metabolites of ranolazine, tramadol, zolmitriptan, and sildenafil (the cleaved piperazine metabolite) displayed cLogD values ranging from -1.11 to -2.97 (Table 1). Further, many cleaved metabolites such as 1-pyrimidinylpiperazine metabolite of buspirone and D617 or D620 metabolites of verapamil were also found circulating in human

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plasma with M/P ratios of >0.25 despite their high polarity. While the reason for this is not known, one possibility for the high circulating concentrations of these metabolites relative to the parent could be attributed to transporter-mediated uptake into the blood. These hypotheses will require further experimental evaluation for confirmation.

Our analysis also identified some amine metabolites that circulated in relatively low abundance despite having optimal cLogD values of between -1 and 5 (Table 1). Based on the conceptual framework discussed in Section 2, this was ascribed to either a low CL_f and/or a high CL_m for the metabolite. For instance, low circulating concentrations of *N*-desalkyl metabolites of azimilide (Riley et al., 2005), elzasonan (Kamel et al., 2010), imatinib (Gschwind et al., 2005), itraconazole (Isoherranen et al., 2004), olanzapine (Kassahun et al., 1997), propafenone (Kroemer et al., 1989), and repaglinide (van Heiningen et al., 1999) can be attributed to the relatively minor contribution of *N*-dealkylation to the overall clearance of these drugs. On the other hand, low circulating concentrations of *m*-chlorophenylpiperazine (Figure 3) may be ascribed to a high metabolic rate of this metabolite to the corresponding hydroxylated chlorophenylpiperazine (i.e., high CL_m of this metabolite) (von Moltke et al., 1999). Ranitidine represents an interesting example in that its *N*-desmethyl metabolite showed both high polarity (cLogD of -2.22) and low f_m (0.01) for this metabolic pathway (Table 1). Thus, it is not surprising that the abundance of this metabolite in circulation is negligible.

The differences in the M/P ratio of *N*-desisopropyldelavirdine (Figure 3) following single and multiple dose administration of delavirdine are also informative to our analysis. After oral administration of a single dose, the M/P ratio of *N*-deisopropyldelavirdine to parent drug was ~1.1 (Morse et al., 1997). However, after multiple dosing, this ratio was decreased to ~0.12 to

0.15 (Borin et al., 1997a; Borin et al., 1997b). This disconnect in M/P ratios after single and multiple doses could be possibly attributed to decreased CYP3A-mediated formation of *N*-desisopropyl metabolite of delavirdine leading to the lower concentration in the systemic circulation. *In vitro*, formation of the N-desisopropyldelavirdine is mediated by CYP3A (Voorman et al., 1998a; Voorman et al., 1998b). However, delavirdine has also been shown to inactivate CYP3A (Voorman et al., 1998b), which may account for the reduction in oral clearance of delavirdine and hence, a decrease in formation of *N*-deisopropyldelavirdine with repeated dose administration.

Even though most aliphatic tertiary amines can undergo secondary metabolism to primary amines via the corresponding secondary amines, only two drugs, namely sibutramine and tamoxifen, yielded primary amines (didesethylsibutramine and didesethyltamoxifen, respectively, Figure 3) that were circulating in greater than 25% of the parent AUC (Kim et al., 2009; Yeung et al., 2011). Moreover, other drugs (Table 1) that theoretically could be converted to primary amines (based on their structures and lipophilicity) showed very little or no didesalkyl metabolites in circulation. For instance, the M/P ratios for the didesalkyl metabolite of amiodarone (McDonald et al., 2012) or citalopram (Herrlin et al., 2003) were less than 0.1 despite having similar cLogD values to that of the secondary amine metabolites. It is likely that this disconnect can be ascribed to a low fraction of the secondary amine being metabolized to the corresponding primary amine and is indicative of increased metabolic stability of the secondary amine with respect to the subsequent N-demethylation/N-dealkylation pathway, compared to the parent tertiary amine. Alternatively, the structural change from a tertiary amine to a secondary amine may impart a different biotransformation pathway such as hydroxylation on the secondary

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amine (e.g., desipramine to hydroxydesipramine and nortriptyline to hydroxynortriptyline, Figure 3) rather than formation of a primary amine.

Overall, this dataset suggests that the amine metabolites can be present in circulation that exceeds the threshold of 25% parent AUC over a wide range of cLogD values (Figure 2). Thus, the f_m or CL_m appear to be the more important factors in determining the abundance of the amine metabolites in circulation. For those amine metabolites that have cLogD values below -1, they may still be present in circulation in abundance that exceeds the M/P ratio of 0.25, possibly related to transport-mediated processes. Hence, assessment of f_m, CL_m, cLogD, and transport properties will provide a useful guide to estimate the potential for the amine metabolites to circulate in abundance that may exceed the threshold of 0.25 relative to that for the parent AUC.

4. **Alcohols and Ketones as Metabolites**

In this dataset, oxygenation of compounds constitutes the second most common biotransformation process. The corresponding hydroxylated metabolites could be formed either by oxygen insertion into the alkyl or alicyclic and aromatic groups or via *O*-dealkylation of aliphatic or aromatic ethers. Analysis of the data set for hydroxylation of aliphatic or alicyclic moieties revealed that of the 125 drugs examined, 40 drugs were shown to yield metabolites with this modification (Table 2A), some of which were further oxidized to ketones (Table 2B). Examination of physicochemical properties of these metabolites suggested that formation of an aliphatic or alicyclic alcohol was associated with an increase in tPSA of up to ~20Å and a reduction of cLogD by ~1 to 2 log units, thus increasing their polarity as expected. Although a general trend of a decrease in the lipophilicity for these metabolites relative to the parent drug

was observed, the vast majority of these metabolites retained favorable lipophilicity (cLogD values between -1 and 5) for diffusion into the systemic circulation.

Most of the hydroxylated metabolites in this dataset were found circulating as free aglycones rather than their glucuronide or sulfate conjugates. This was possibly attributed to high pKa values of the hydroxyl groups, which were in the range of ~12 to 16 (Table 2A). Chemically, high pKa value could render conjugation of the hydroxyl group with the glucuronosyl moiety from uridine 5'-diphospho-glucuronic acid (UDPGA) or the sulfate group from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) unfavorable (if the alkoxy anion RO^- is considered the reactive species in the conjugation reaction), with a net effect of reducing CL_m of alcohol metabolites via conjugation reactions. The optimal cLogD and pKa properties impart favorable ADME characteristics to these metabolites and therefore enable their entry into the systemic circulation as free aglycones and maintain a sufficiently high exposure (>25% parent AUC). The contribution of the metabolic pathway also constituted an important parameter in determining if the metabolite circulated in the system in high concentrations, as in the case of amine metabolites. For instance, in the case of erlotinib (Figure 4), its *O*-desmethyl metabolite was found in low levels in systemic circulation despite its high pKa (13.9) and high cLogD (2.41). Assessment of the contribution of this metabolic pathway revealed that *O*-demethylation is a relatively minor route of metabolism for this drug and only accounts for 13% of the dose in humans (Ling et al., 2006). Hence, low CL_f most likely accounted for the low abundance of this metabolite (OSI-420, 5% of the parent) in human plasma (Frohna et al., 2006).

Secondary alcohols as metabolites

Drugs that yielded secondary alcohols as metabolites such as flutamide, itraconazole, metoprolol (5α-hydroxylation), pioglitazone, taranabant or buspirone also circulated as the aglycones in humans (Table 2A). Although this observation could be explained on the basis of their high pKa and cLogD values, an additional consideration to this observation could be that these metabolites are readily oxidized to their corresponding ketones, as exemplified by pioglitazone or itraconazole for which the ketone metabolite was also primarily detected in plasma (Table 2B) (Eckland and Danhof, 2000; Budde et al., 2003). Although this was not observed in all cases the possible reductase and dehydrogenase catalyzed redox cycle between the alcohol → ketone → alcohol (Oppermann and Maser, 2000), cannot be ruled out for these drugs. Taken together, these factors may result in reducing CL_m for the secondary alcohols, enabling these metabolites to circulate as free aglycones.

Primary alcohols as metabolites

It is well recognized that many drugs or metabolites containing a primary alcohol tend to generally undergo further oxidation to a carboxylic acid. Consequently, the alcohol metabolite may appear in low abundance, if at all, in circulation with the carboxylic acid as a major component in plasma (See Section 7 below). For example terfenadine (Figure 4) is converted to the t-butyl alcohol metabolite by CYP3A4 followed by subsequent oxidation to the corresponding carboxylic acid (fexofenadine), which is the primary metabolite found in human plasma (Abernethy et al., 2001). Other drugs that exhibit similar behavior include celecoxib, metoprolol and montelukast (Table 5). However there are exceptions where metabolites could circulate as alcohols instead of the corresponding carboxylic acids. For instance, oxidation of the tertiary butyl functionality of bupropion yields hydroxybupropion (Hesse et al., 2000). The

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plasma exposure of this hydroxylated metabolite, unlike those of metoprolol, montelukast, and terfenadine, is 7 to 41 times higher than that of parent. It can be postulated that the lack of subsequent oxidation to the carboxylic acid may be related to the spatial arrangement of the hydroxy group relative to the carbonyl group in the molecule. Intra-molecular cyclization of the hydroxyl metabolite leads to the formation of the corresponding cyclic *R,R*-hydroxybupropion and *S,S*-hydroxybupropion isomers (Figure 4) and possibly reduces its chance of undergoing further oxidation to the carboxylic acid. Additionally, the CL_m of the hydroxyl metabolite via the carboxylic acid pathway is probably low relative to its CL_f.

Midazolam and valdecoxib (Figure 4) represent an example of drugs in which the hydroxylated metabolite is primarily detected as glucuronide conjugate and not as an aglycone or carboxylic acid. Glucuronide conjugate of 1′-hydroxymidazolam was the major metabolite excreted in urine and circulating in plasma even though the plasma AUC of 1′-hydroxymidazolam was ~0.48 of parent after oral administration of midazolam (Heizmann and Ziegler, 1981; Eap et al., 2004). Similarly, although valdecoxib was oxidized to a hydroxymethyl metabolite (Yuan et al., 2002), the abundance of this metabolite relative to parent in plasma was low (~0.1) (Sarapa et al., 2005). Results from a human ADME study indicated that this metabolite appears to favor glucuronidation as evidenced by a recovery of 23% of dose in urine as the glucuronide conjugate of this metabolite (Yuan et al., 2002). Interestingly, in both cases, the methyl group undergoing hydroxylation is attached to 5-membered heterocyclic rings (the isoxazole in the case of valdecoxib and imidazole in the case of midazolam). It is possible that the heterocyclic rings may influence the propensity for these alcohol metabolites to undergo glucuronidation. In fact the pKa values of the hydroxymethyl metabolites suggest that the

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hydroxyl groups are relatively more acidic (13.09 and 13.59, respectively) compared to the primary alcohols formed via oxidation of other drugs (Table 2A). Chemically, decreased pKa could potentially increase the susceptibility of these metabolites to undergo further conjugation reaction relative to other hydroxymethyl derivatives. Additionally, potential intra-molecular interactions of the hydroxyl group with the nitrogen or oxygen atom in the imidazole or isoxazole rings of the two drugs could possibly increase the propensity of conjugation by rendering the hydroxyl group more nucleophilic. One caveat involves the limitation of our current understanding of the interaction between alcohol metabolites and the conjugation enzymes such as UGT to allow *a priori* prediction of how well the metabolites with this structural motif may undergo conjugation (i.e., assessment of CL_m). This parameter still requires further in vitro experimentation to ascertain, such as the approach used by Lutz and Isoherranen (Lutz and Isoherranen, 2012).

Bosentan represents another example of the exception to the trend that a hydroxyl metabolite on the primary carbon will undergo further oxidation to a carboxylic acid. In humans, the major metabolic pathway for bosentan involves oxidation of the t-butyl group to yield the hydroxyl metabolite Ro 48-5033. This pathway constitutes at least 35% of the overall biotransformation for bosentan (Weber et al., 1999). However, after oral administration, plasma AUC of t-butyl hydroxybosentan to parent was only ~0.13, and the corresponding carboxylic acid was not reported. Based on the pharmacokinetic framework described in Section 2, it can be postulated that the elimination clearance of this metabolite (by pathways other than further oxidation to carboxylic acid) is more likely favored relative to its formation. Bosentan is a substrate for efflux transporter such as BSEP (Hartman et al., 2010). It may be hypothesized that

despite the structural modification, the t-butyl hydroxyl metabolite of bosentan may still retain the affinity to the efflux transporters as with the parent so that biliary elimination rather than further oxidation is favored. If this hypothesis holds true, it will illustrate the importance of understanding the key factors (e.g., structural motifs, physicochemical properties) that impacts the affinity of a metabolite to interact with transporters vs metabolic enzyme(s), which will determine the elimination characteristics of the metabolite. Overall, the learnings from these empiric observations can be taken into consideration when formulating a rational strategy to anticipate and prioritize which alcohol metabolite(s) should be considered for synthesis and quantitation early on to address the potential MIST or DDI issues.

5. **Arenol Metabolites**

Metabolites formed via hydroxylation of aromatic rings or *O*-dealkylation of aromatic ethers (arenols) represented very different characteristics when compared to their alcoholic counterparts. Thirty six drugs in this dataset revealed arenol formation as a part of their biotransformation pathway (Table 3). The physicochemical properties of the arenol metabolites were moderately changed compared to the parent drug, with increase in tPSA by ~11 to 20Å and decrease in cLogD by ~0.5 to 1 log unit associated with these metabolic steps (Table 3). Although the cLogD values of arenols, which typically ranged from -0.6 to 4, showed considerable overlap with those for the alcohol metabolites, the pKa value of the hydroxyl group in these metabolites ranged from ~4 to 11 (Table 2) and were more acidic compared to the alcohol functionality (pKa of 12-16). As seen in Table 3, in contrast to alcohol metabolites, many of the arenol metabolites circulated as glucuronide or sulfate conjugates in humans. Chemically, lower pKa of arenols would make these metabolites more ionizable to yield a

phenoxy anion and hence favor the nucleophilic addition to UDPGA or PAPS. Thus, it is not surprising that these metabolites are susceptible to further conjugation and circulated either as glucuronide (e.g., apremilast, atomoxetine, chlorpromazine, codeine to morphine, desloratadine, desipramine, dextromethorphan, duloxetine, efavirenz, imipramine, loxapine, and propranolol) or sulfate (e.g., apixaban, pantoprazole, and rosiglitazone) conjugates (Table 3). While in vivo data is lacking, in vitro data suggests that 7-OH S-warfarin likely undergoes extensive glucuronide conjugation as well (Bratton et al., 2012).

Empirically, our meta-analysis suggested that conjugates of *para*-hydroxylated arenols were major circulating metabolites rather than the aglycones while the *ortho* and possibly *meta*-hydroxylated metabolites were primarily found as aglycones in circulation. For instance, the hydroxylated metabolites of atorvastatin, ranolazine, gefitinib and tramadol (Figure 5) primarily circulated as the aglycones with an M/P ratio of 0.25 or greater (Table 3). One reason for this could be limited formation of the conjugates of *ortho*-hydroxyarenols. It is also possible that the position of the hydroxy group on the aromatic ring may influence the efficiency of conjugation. Alternatively, the substrate affinity of the *ortho*-hydroxylated metabolites for various efflux transporters (e.g., MRP2 vs MRP3) may alter the disposition of these metabolites (i.e., being transported to the bile vs to the systemic circulation). The validity of these hypotheses will need to be confirmed with further research.

Some examples from this dataset illustrate the utility of the pharmacokinetic framework discussed in Section 2 in rationalizing the relative abundance of the arenol metabolite to parent. For instance, the ratio of *O*-dealkylamiodarone to amiodarone is low (0.1) (McDonald et al., 2012), suggesting that the CL_f is low relative to the CL_m of this metabolite. It can be postulated

that since N-deethylation and 3′-hydroxy monodeethylation (Figure 5) constitutes the major metabolic pathways rather than O-dealkylation of amiodarone, the low ratio may possibly reflect a low CL_f for this metabolite. Another example may include the O-desmethyl metabolite for bosentan (Ro 47-8634) (Figure 5), which also showed low abundance relative to the parent in plasma (~0.05) (van Giersbergen et al., 2002). The relatively low fraction of dose attributable to the O-demethylation pathway in the excreta for this drug (~6.4% of dose) is consistent with this interpretation (Weber et al., 1999).

Another drug that displayed an exception to the general trend was venlafaxine. *O*-dealkylation of venlafaxine resulted in the formation of desvenlafaxine (Figure 5) (Howell et al., 1993). The systemic exposure of this metabolite was 4 to 6-fold higher than that of parent in CYP2D6 extensive metabolizers (EM) (Patat et al., 1998; Hynninen et al., 2008). Further, while a significant portion of the dose (26%) was excreted in urine as the glucuronide conjugate of desvenlafaxine, 29% of the dose was excreted as the aglycone (Howell et al., 1993). Also, following oral administration of desvenlafaxine, renal clearance constitutes a major clearance mechanism for the parent (DeMaio et al., 2011). This example illustrates that a change in physicochemical properties associated with the metabolite may alter its clearance mechanism, in this case, to renal elimination. This can result in a significant impact on the elimination clearance of the metabolite and thus affecting the anticipated AUC ratio of metabolite to parent. Overall this analysis suggested the complexity of predicting the exact nature of the metabolites that undergo aromatic hydroxylation or O-dealkylation and emphasized the importance to understand the formation and elimination (e.g., conjugation) characteristics of the metabolites. It

also highlighted the importance of obtaining good estimates of both CL_f and CL_m in order to yield a more accurate assessment of the AUC ratio of metabolite (aglycone) to parent.

6. **S- and N-Oxides Metabolites**

S-Oxides

Twelve drugs in this dataset were metabolized to the corresponding sulfoxide and/or sulfone metabolites (Table 4). Formation of a sulfoxide via oxidation of the sulfur moiety lowered the cLogD by ~1 to 2 log units and increased tPSA by ~11Å relative to the parent drug. Despite this, most of the metabolic products from sulfoxidation retained sufficient lipophilicity, as reflected by the cLogD values, in the range of -1 to 3 (Table 4). This favorable physicochemical property probably enabled these entities to reach systemic circulation since most drugs (6 out 8) that were converted to the sulfoxide metabolite had an M/P ratio exceeding 0.25. On the other hand, relatively low amounts of the circulating sulfoxide metabolite of montelukast and ranitidine were detected in human plasma. Montelukast is primarily metabolized in humans via hydroxylation and subsequent conversion to the carboxylic acid while S-oxidation constitutes a minor pathway of metabolism for this drug. Low levels of the montelukast sulfoxide in human plasma despite the favorable cLogD and tPSA values (1.74 and 107, respectively) were therefore likely attributed to a low CL_f, while the sulfoxide metabolite of ranitidine exhibited both low f_m (0.01) and high polarity (cLogD of -3.43).

Drugs containing a sulfoxide moiety can generally undergo further oxidation to a sulfone, which can also circulate as the major metabolite. For instance, flosequinan, omeprazole, lansoprazole in CYP2C19 PM, and thioridazine were converted to their corresponding sulfone

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metabolites and had M/P ratios >0.25 (Table 4). This was ascribed to their favorable cLog D values since in most cases the conversion of a sulfoxide to a sulfone metabolite increased its cLogD by up to ~1 log unit and decreased tPSA by ~6Å (Table 4). The only outlier to this trend was pantoprazole which showed an M/P ratio < 0.25. While the reason for this is not known, the discrepancy could also be ascribed to relatively less contribution of this metabolic pathway towards clearance of this drug since the major pathway appears to be *O*-dealkylation of the methoxy ether to the corresponding arenol metabolite. Of interest, in this analysis, for the parent drugs that contain sulfide in its structure, oxidative metabolism yields sulfoxide as the major circulating metabolite (e.g., albendazole, axitinib, chlorpromazine, dothiepin, thioridazine). However, the corresponding sulfone tends to be either not present as major circulating metabolite, or is present in abundance considerably lower than the corresponding sulfoxide (e.g., thioridazine). Like the amines (Section 3), this may also be attributed to the low CL_f for this sequential oxidation step.

N-Oxides

In this analysis, 11 drugs were converted to aliphatic or aromatic *N*-oxides (Table 4). Three drugs showed the formation of aromatic N-oxide, two of which are pyridine N-oxide (roflumilast and regorafenib), and one is a pyrimidine *N*-oxide (voriconazole). The remaining eight drugs formed aliphatic or cyclic *N*-oxide. Changes in physicochemical properties from *N*-oxidation are of a similar order to S-oxidation to a sulfoxide with an increase of tPSA of 14Å and a lowering of lipophilicity of ~0.5 to 2 log units. All except ranitidine *N*-oxide and zolmitriptan *N*-oxide showed a cLogD value of greater than zero (Table 4), which conferred a favorable lipophilic property to enable these metabolites to reach the systemic circulation provided that a

sufficient amount was formed. A notable feature of *N*-oxide metabolites is their apparent rapid interconversion with parent leading to formation of both parent drug and other primary and secondary metabolites. Interconversion after administration of the metabolite to man and animals has been shown for chlorpromazine (Jaworski et al., 1990), clozapine (Chang et al., 1998) and voriconazole (Roffey et al., 2003). The interconversion will tend to lower the M/P ratio of this type of metabolite.

7. **Carboxylic Acid Metabolites**

Eleven drugs in this dataset were oxidized to a carboxylic acid metabolite via various metabolic reactions (Table 5). Seven of these drugs including celecoxib, losartan, metoprolol (O-demethylation), montelukast, quetiapine, N-dealkyl product of ranolazine (CVT-2534), and terfenadine underwent oxidation of either the parent drug or the intermediate alcohol metabolite to the corresponding carboxylic acid (see Section 4). As observed from the M/P ratios, in all cases, the carboxylic acid was the major circulating metabolic product possibly due to its small volume of distribution and high plasma protein binding (Obach et al., 2008). The data from these seven drugs/metabolites was consistent with this hypothesis in that the AUC of the carboxylic acid metabolite was considerably higher compared to their corresponding alcohol metabolite. Another factor that may also influence the high M/P ratio for the carboxylic acid metabolites is related to the new functional group being introduced to the metabolite, which is metabolically more stable than the parent (i.e., reduced CL_m). This is illustrated by the example of losartan. A comparison of the pharmacokinetics of losartan to its carboxylic acid metabolite indicates that the volume of distribution (~0.45 L/kg) and plasma clearance (~9 mL/min/kg) of losartan was greater than that of carboxylosartan (plasma clearance ~0.7 mL/min/kg and volume

of distribution ~0.14 L/Kg) (Christ, 1995; Lo et al., 1995). Additionally, the renal clearance (~1 mL/min/kg) of losartan accounted for 11% of its total plasma clearance while the renal clearance of carboxylosartan (~0.4 mL/min/kg) accounted for 55% of its total plasma clearance. The free fraction for the metabolite (~0.0035) showed ~4-fold decrease compared to the parent (~0.0135), suggesting that the effect of the additional acidic function in the metabolite has little effect on unbound volume of distribution or renal clearance. However, there was a 7-fold reduction (593 mL/min/kg for losartan and 86 mL/min/kg for carboxylosartan) in unbound non-renal clearance, which can be postulated to be mainly due to the oxidation of the more labile hydroxyl group in losartan to a substantially more metabolically stable carboxyl function. Besides losartan, metabolic conversion of terfenadine to fexofenadine likely represents another example in support of this postulate.

In this dataset, 8 of 11 carboxylic acid metabolites showed negative cLogD values ranging from -0.06 (carboxylosartan) to -3.4 (zolmitriptan indoleacetic acid), suggesting that these metabolites have high polarity (Table 5). However, 7 out of 8 of these metabolites had an M/P ratio of 0.25 or higher, with repaglinide carboxylic acid (Figure 6) as the only exception. Although the mechanism(s) by which passage of these polar carboxylic acid metabolites into systemic circulation is not well understood, one possible explanation may involve transporter-mediated processes. For instance, fexofenadine is a substrate for hMRP3 (Matsushima et al., 2008), which mediates the sinusoidal efflux of a variety of organic anions. This transporter therefore may play a role in mediating the efflux of fexofenadine from the liver to the systemic circulation, as demonstrated in the Mrp3 (-/-) mouse model (Matsushima et al., 2008). Moreover, the example from the carboxylic acid metabolite of repaglinide illustrates the

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potential complexity of these active processes. In humans, the M/P ratio is low (0.05) despite a large fraction of the repaglinide dose being metabolized to this metabolite as evidenced by the recovery of ~60% of the dose as this metabolite in feces (presumably via biliary excretion since the formation of this metabolite involves oxidative metabolic process) and ~2% of the dose in urine (van Heiningen et al., 1999, Honkalammi et al., 2011). This suggests that the CL_m for this metabolite likely is considerably higher relative to its CL_f. Thus, it is possible that the structural motif, physicochemical and/or ADME properties for repaglinide carboxylic acid metabolite favor an interaction with the transporters involved in biliary excretion, while those for the other 7 carboxylic acid metabolites may have properties that favor an interaction with transporters that enables the passage of these metabolites into the systemic circulation. Interestingly, repaglinide and its carboxylic acid metabolite also showed one of the largest physicochemical change amongst the parent/metabolites reviewed in this analysis, with an increase in tPSA from ~80 to 125 Å and a ~4 log unit reduction in cLogD to ~-2 (Table 5). It is possible that another contributing factor to the low plasma concentration of repaglinide carboxylic acid metabolite may be attributed to low f_p, which, in this case, may become limiting for partitioning from the hepatocytes to systemic circulation. As our knowledge in transporter-mediated processes in relation to structural and physicochemical properties is relatively nascent, further research into this area is needed to elucidate these mechanisms.

8. **Novel Metabolites**

We have included in Table 6 novel metabolites that are formed in a “unique” manner, although in fact they are still formed mainly via oxidations and reductions. In most cases, the changes in physicochemical properties of metabolites are relatively small compared to the parent

drug (Table 6). Most metabolites exhibited cLogD values within the range of -1 to 5, which conferred favorable lipophilic characteristics for their passage from the liver to the systemic circulation. For most of these metabolites, unless the structural modifications render the CL_m to be substantially different than CL_{parent}, the M/P ratios will mostly be determined by CL_f. The exception to this was methadone. Conversion of methadone to EDDP metabolite (Figure 6) showed a reduction in cLogD value by almost 6 log units from 2.29 to -3.51 and the tPSA of this metabolite is also very small (~3 Å). Thus low M/P ratio of this metabolite (~0.16), could be ascribed, at least in part, to the high polarity and hence, low lipoidal permeability for diffusion into the systemic circulation.

9. **Theoretical Considerations: Which Factors Appear Dominant?**

9.1 *Lipoidal Permeability*

The liver is the principal site of metabolism and receives approximately 25% of the cardiac output. This equates to a blood flow of around 0.8 mL/min/g in human and 3-4 mL/min/g in rat. The capillaries supplying the hepatocytes are sinusoidal with a broken basement membrane and very leaky endothelial openings of 100-300 nm in diameter (Balaz, 2009). Drug and metabolite molecules are free to access or leave the surface of the hepatocyte without regard to physicochemistry. The membrane permeability of the hepatocyte to drugs and their metabolites has been explored in a series of studies by Chou et al. (Chou et al., 1995). The authors' analysis of the data concludes that the lower limit of PS is 0.3-0.5 mL/min/g liver for compounds with logD values below -3. Permeability then increases dramatically with increases of logD from -3 to 1.5; and at still greater logD values a plateau rate of PS of 200-300 mL/min/g

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liver is reached. In our data set, we have ascribed a lower cLogD value of -1 as a threshold at or above which free passive diffusion of drug and metabolites across the membrane is likely to occur and will not be a limiting factor in determining the presence of circulating metabolites. Around this value PS exceeds liver blood flow, suggesting blood perfusion rate limitations (rather than membrane permeability limitations) on influx and efflux of metabolites.

We have also considered the abundance of circulating concentrations of metabolite relative to the parent drug from the viewpoint of f_m and f_p. The term f_p was thought to be important as a low permeability rate from the hepatocyte would attenuate appearance in the circulation if further metabolism or canalicular biliary excretion occurs. It appears that whether considered from in vitro or in vivo excretion data, f_m is a much more dominant feature in determining the circulating M/P ratios relative to f_p. This is further substantiated by an additional analysis showing that there was no apparent relationship between M/P ratio and change in cLogD or tPSA values from parent drug to its corresponding metabolites (data not shown). This partly reflects the observation that most metabolites still possess favorable lipoidal permeability characteristics due to a limited change in lipophilicity and tPSA. As illustrated by the drugs evaluated in this dataset, in most cases the metabolic steps associated with circulating metabolites lower lipophilicity (cLogD) by up to ~2 log units and raise tPSA by up to ~20Å. While these metabolites typically have lower lipoidal permeability than their parent drugs, the changes are unlikely to be significant enough to render the permeability rate from the hepatocytes be a limiting factor in restricting the passage of metabolites from the liver to the systemic circulation. It is also reasonable to assume that lowered lipophilicity will result in lowered rates of metabolism and canalicular biliary excretion since the binding sites of most drug

metabolizing enzymes and transporters are hydrophobic in nature. Thus, it can be conceived that the net effect of these changes associated with lower lipophilicity for the metabolites would render f_m to be a more dominant determinant relative to f_p.

9.2. *Metabolite to Parent Ratio in Systemic Circulation for High Clearance Drugs*

In most cases, in vivo data is lacking for CL_m so that the ratio of CL_{parent}/CL_m typically cannot be directly calculated. As discussed previously (Section 2), CL_m is an important parameter in determining the M/P ratio; thus, it represents an opportunity for further investigations to develop better predictive models for this parameter. Some of the drugs examined in this dataset are very rapidly and extensively metabolized in humans, and act virtually as pro-drugs. These include flutamide (oral clearance value of >600 mL/min/kg), buspirone (intravenous clearance value of 28 mL/min/kg and 4% bioavailability), and terfenadine (oral clearance value of >800 mL/min/kg) (Anjum et al., 1999; Mahmood and Sahajwalla, 1999; Abernethy et al., 2001). These drugs all have very high M/P ratios in systemic circulation. Although characterization of the in vivo CL_f and CL_m values for these metabolites are incomplete, the high M/P ratio after oral administration of the parent drug suggests that the fraction of the parent drug escaping the liver is low, and/or that the CL_f far exceeds the CL_m for these metabolites (see Section 2). Such drugs probably will not be a major factor in current drug discovery programs due to the emphasis on bioavailability and metabolic stability in compound design.

9.3 *Protein Binding*

In Section 2, it was postulated that overall the reduction in lipophilicity seen in most cases of metabolism would generally result in a higher free fraction and a lower intrinsic (unbound) metabolic clearance, thus attenuating the effects on total drug clearance versus total metabolite clearance. Data are available for several drugs that undergo different metabolic routes to their corresponding metabolites to illustrate this hypothesis (Table 7). In all cases, metabolism lowered lipophilicity and increased free fraction. For those metabolites which are cleared predominantly by metabolism (5/6, desmethylclozapine being predominantly renal), their total clearances are within 3-fold of that for the parent even though their unbound metabolic or hepato-biliary (non-renal) clearance differences can be much larger. This attenuation of differences in clearance is exemplified by darifenacin and its hydroxyl metabolite. The total clearance of the parent and metabolite is similar but there is a 10-fold difference in unbound clearance and fraction unbound. Similar trends are observed for chlorpromazine, diltiazem, and tolterodine (Table 7). These examples illustrate that the effects of the reduction in lipophilicity seen with metabolism does not have pronounced effects on the ratio of CL_{parent}/CL_{m}.

Although the effect of reduction in lipophicity generally tends to attenuate the influence on total drug clearance versus total metabolite clearance, an exception to this has been observed and is related to the new functional group being introduced into the metabolite, which is itself highly labile to a metabolic route unavailable to the parent. In this case, the metabolite may display a higher clearance compared to the parent. This exception is illustrated by the metabolic conversion of propranolol to hydroxypropranolol, which is cleared predominantly by conjugation of the newly introduced phenolic group. The clearance of this metabolite is higher than its parent (Table 7), consistent with its very low concentration after systemic administration and its presence being observed only after the rapid first-pass effects seen for parent after oral

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administration. This reinforces the observation that besides protein binding, additional factors such as the new structural motif(s) introduced into the metabolite, should be taken into consideration when determining the potential influence of structural-chemical changes on the CL_m of the metabolite.

9.4 *Pivotal role of f_m*

Given the pivotal role that f_m plays in determining the AUC of a given metabolite (see Section 2), and hence its relative abundance in relation to the parent drug, an additional analysis was conducted to ascertain if there is a threshold value that can be identified to be associated with a M/P ratio of >25%. The data (M/P ratios and f_m values) used in this analysis are listed in Tables 1-5. The f_m values were estimated from data obtained in human ADME studies with radiolabeled drugs. The fraction of dose for the metabolite of interest and its sequential metabolic products recovered in excreta (both urine and feces) was assumed to be representative of the in vivo f_m value. Data were available for a total of 60 metabolites in this dataset, and the results are presented in Figure 7. Visual inspection of this data suggests that f_m value of 0.15 appears to be a reasonable threshold value to distinguish the metabolites that may circulate with abundance exceeding the threshold of 25% of parent AUC compared to those that do not. Using this threshold f_m value, 4 metabolites have been identified as "false negative" in that while the f_m values are below 0.15, the M/P ratios exceed the threshold of 0.25. These 4 metabolites are cyclized indole product of elzasonan, 2-hydroxycyclopentyl ruxolitinib, zolmitriptan N-oxide, and N-desmethylzolmitriptan. The reason(s) for this is not readily apparent, but it could be postulated that underestimation of f_m (possibly related to incomplete collection of radioactive dose and/or characterization of the metabolite profiles for elzasonan (79% of dose recovered),

and potential back-conversion of N-oxide to parent in feces for zolmitriptan) may have contributed to this discordance.

When the M/P ratio for the aglycone of the arenol metabolites were used in this analysis, there were 14 "false positives" identified (i.e., f_m of >0.15 but M/P of <0.25). Amongst these 14 "false positives", the most common structures are the arenol metabolites where the circulating level of the aglycone is negligible. However, when the M/P values for the conjugates of these arenol metabolites were plotted instead (Figure 7), the number of "false positive" is reduced to 6. Three of these 6 metabolites appear to be conjugated (5-hydroxyelzasonan, O-desalkyl flecainide, and 5-methylhydroxy valdecoxib) and excreted; however, the conjugate was not reported to be present in plasma. Two additional metabolites (t-butyl hydroxybosentan and repaglinide carboxylic acid) may also display similar properties in that once formed, these metabolites likely favors excretion into the bile rather than being transported to the systemic circulation. N-desmethylzopiclone represents a marginal case in that the f_m value is estimated to be 0.17 while the M/P ratio is 0.2.

Using the threshold f_m value of 0.15, the false negative and the false positive rates (when the M/P ratios of the conjugates of the arenol metabolites are considered) are 10% (4 out of 40 metabolites) and 30% (6 out of 20 metabolites), respectively, which represent a reasonable trade-off since further reduction in the false negative rate will increase the false positive rate considerably. Moreover, this suggested threshold value may be further refined as more data become available in the future.

10. **Special Considerations**

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10.1 *Genetic Polymorphisms*

Genetic polymorphisms of the drug metabolizing enzymes play an important role in influencing the disposition of many drugs and metabolites. In this analysis, a few examples illustrate the important influence of genetic polymorphisms on the abundance of major circulating metabolites. The first set of examples involves the differences in the abundance of circulating metabolites between extensive (EM) and poor metabolizers (PM) of CYP2C19 and CYP2D6. In the former case, the M/P ratio of lansoprazole sulfone in CYP2C19 PM (0.88) was considerably higher than those in the CYP2C19 homozygous EM and heterozygous EM (<0.1). Another example that showed a similar pattern was atomoxetine, which is metabolized by CYP2D6 (aromatic 4-hydroxylation, major pathway in CYP2D6 EM) and CYP2C19 (N-demethylation) (Sauer et al., 2005). In CYP2D6 EM subjects, 4-OH atomoxetine glucuronide constitutes the major circulating moiety, while the plasma AUC ratios of N-desmethyl atomoxetine and 4-hydroxyatomoxetine to parent were low (<0.1) (Sauer et al., 2003). Moreover, in CYP2D6 PM, the AUC ratio of N-desmethyl atomoxetine to parent drug was 0.33 to 0.42 (Sauer et al., 2003); FDA Clinical Pharmacology Review:

http://www.accessdata.fda.gov/drugsatfda_docs/nda/2002/21-411_Strattera_biopharmr_P2.pdf), thus constituting a moiety that needs to be considered for assessment for potential DDI under the current EMA and draft FDA Guidance. These examples highlight the need to evaluate the abundance of circulating metabolites in relation to the genotype and phenotype of the metabolizing enzymes, if genetic polymorphisms are known to exist for the particular enzyme of interest.

Another set of examples that illustrate the importance of gene dose on the abundance of the circulating metabolites include nortriptyline and codeine. In humans, nortriptyline undergoes CYP2D6-mediated hydroxylation to form the cyclic E-10-hydroxynortriptyline (major form of this metabolite), while the formation of Z-10-hydroxynortriptyline (minor form of this metabolite) is not related to CYP2D6 polymorphism. Dalen and co-workers demonstrated that the ratio of AUC of 10-hydroxynortriptyline to parent increased with the number of functional CYP2D6 genes, ranging from 0.36 in subjects with no functional CYP2D6 genes to 13 in subjects with 13 functional CYP2D6 genes (Dalen et al., 1998). Likewise, in humans, codeine undergoes CYP2D6-mediated demethylation to form morphine, which is glucuronidated to morphine 3-glucuronide and morphine 6-glucuronide. In subjects who are CYP2D6 PM, ratio of plasma AUC for morphine to codeine was minimal (0.008), whereas this ratio was higher in subjects who are CYP2D6 EM (0.06) and CYP2D6 UM (0.08) (Kirchheiner et al., 2007). Furthermore, in these same subjects, the systemic exposure (plasma AUC) of morphine 3-glucuronide and morphine 6-glucuronide (pharmacologically active) in CYP2D6 EM and UM were substantially higher than in CYP2D6 PM. Thus, gene dose can exert a significant influence on the systemic exposure to the circulating metabolites and should be taken into consideration when attempting to project the abundance of circulating metabolites.

10.2. *Caveats to this Analysis*

This analysis is intended to assess general trends regarding possible relationship of structural motif and the associated physicochemical properties of the metabolites to their relative abundance in systemic circulation. Attempts to rationalize some of the observed trends were made based on existing known biologic and/or chemistry principles. However, there are some

significant knowledge gaps in certain areas where mechanistic insights are lacking to fully understand the observed results. Plausible hypotheses for further testing are postulated for subsequent confirmation when feasible. Not surprisingly, results of this analysis point to the need for: (1) further understanding of the scaling for conjugation reactions (e.g., glucuronidation and sulfation) to provide a more accurate estimate of CL_m for the alcohol and arenol metabolites in order to better predict the ratio of AUC of metabolite to parent, (2) an integrated approach to combine metabolism and transport data for quantitative prediction, particularly the transport processes in the liver that mediate biliary excretion vs passage into systemic circulation, and (3) a more complete understanding of the interplay between physicochemical properties for passive processes with spatial arrangement of the compound for its affinity to enzymatic/active transport processes.

Another caveat to our results involves the complexity of the metabolic pathways. In this dataset, the vast majority of the circulating metabolites are formed from primary biotransformation pathways. However, in humans, there are examples where the major circulating metabolites are derived from secondary or tertiary biotransformation steps, e.g., torceptrapib (Dalvie et al., 2008). Since the ability for the in vitro systems to predict these secondary or tertiary metabolites is poor (Dalvie et al., 2009), there is still the necessity to confirm the major circulating metabolites in a human in vivo ADME study with radiolabeled drug. In addition, this analysis only addressed the relative abundance of the metabolite to parent, and there is no attempt to assess structure-physicochemical properties in relation to pharmacologic activity. Clearly, in drug discovery and development programs, an understanding of both aspects is essential.

11. **Summary**

To our knowledge, this is one of the first attempts to assess the relative abundance of circulating metabolites using an integrated approach of principles of metabolite kinetics along with structure-physicochemical properties of the parent/metabolites. The analysis was conducted in a dataset comprising 125 drugs with their circulating metabolites. The major factors identified in this analysis that will favor a given circulating metabolite to exceed the threshold of 25% of parent appears be: a f_m value of >15%, a clogD value of > -1, and structural motif that minimizes the CL_m relative to its CL_f. Potential hypotheses are proposed where exceptions to these general trends are observed, which provides opportunities for further scientific investigations to refine these recommendations.

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Legends for Figures

Figure 1 Scheme depicting potential routes of metabolite disposition

Figure 2 Relationship between M/P ratio and cLog D values for amine metabolites (A, all metabolites and B, expanded view).

Figure 3 Structures of selected drugs that form amine metabolites.

Figure 4 Structures of selected drugs that form alcohol metabolites.

Figure 5 Structures of selected drugs that form arenol metabolites.

Figure 6 Scheme showing conversion of repaglinide to repaglinide carboxylic acid and methadone to EDDP metabolite.

Figure 7 Relationship of metabolite to parent AUC ratio as a function of f_m (A, all metabolites and B, expanded view).

Table 1: Physicochemical properties, abundance and estimated fm of amine metabolites[1,2].

Drug	cLogD	tPSA	Metabolite	cLogD	tPSA	M/P	fm	REF
Amiodarone	5.87	42.7	N-Desethylamiodarone	5.2	51.5	1*		(McDonald et al., 2012)
			Didesethylamiodarone	5.25	65.5	0.05*		
Amitriptyline	2.64	3.24	Nortriptyline	1.49	12	(CYP2C19) EM: 0.43-0.99; PM: 0.13		(Burch and Hullin, 1981; Venkatakrishnan et al., 2001; Jiang et al., 2002)
Atomoxetine	0.77	21.3	Desmethylatomoxetine	1.39	35.3	(CYP2D6) EM:0.06; PM:0.38	0.03	(Sauer et al., 2003; Sauer et al., 2005); FDA Clinical Pharmacology Review[a]
Azelastine	1.57	35.9	Desmethylazelastine	1.63	44.7	0.41		Product Label[b]
Azimilide	2.66	72.6	N-desmethylazilimide	1.13	81.39	NR	0.04	(Riley et al., 2005)
Buspirone	1.24	69.6	1-Pyrimidinylpiperazine	-0.46	41.1	44 to 57		(Jajoo et al., 1989; Dockens et al., 2006)
Citalopram	1.35	36.3	Desmethylcitalopram	0.014	45.1	(CYP2C19) PM:0.24; EM:0.49		(Herrlin et al., 2003; Yu et al., 2003)
			Didesmethylcitalopram	0.003	45.1	(CYP2C19) EM:0.07; PM:0.05		
Clobazam	1.22	40.6	Norclobazam	1.42	49.4	2.3		(Levy et al., 1983)

Clomipramine	2.95	6.48	Desmethylclomipramine	1.87	15.3	0.37		(Nagy and Johansson, 1977)
Clozapine	3.5	30.9	Desmethylclozapine	3.27	39.7	0.32	0.17	(Chung et al., 1993)
Delavirdine	1.77	119	Desisopropyldelavirdine	1.59	133	SD:1.1; MD:0.14		(Borin et al., 1997a; Borin et al., 1997b; Morse et al., 1997);
Diazepam	2.8	32.7	Desmethyldiazepam	2.78	41.5	0.35 - 1.1		(Perucca et al., 1994; Kosuge et al., 2001; Saari et al., 2007)
Diltiazem	3.36	84.4	N-Desmethyldiltiazem	2.07	93.2	0.35	0.49	(Sugihara et al., 1984; Yeung et al., 1990)
Dothiepin	2.54	28.5	N-Desmethyldothiepin	1.39	37.3	0.54		(Maguire et al., 1981)
Doxepin	2.06	12.5	Desmethyldoxepin	0.91	21.3	0.6 - 1.4		(Kirchheiner et al., 2005)
Enzalutamide	3.17	99.74	Desmethylenzalutamide	2.98	109	1.60		Product Label[c]
Elzasonan	3.24	52.1	Desmethyl elzasonan	2.19	60.9	0.19	NR	(Kamel et al., 2010)
Fenfluramine	0.91	12	Desethylfenfluramine	0.33	26	0.36 - 0.37		(Caccia et al., 1985)
Fentanyl	2.61	23.5	Norfentanyl	-0.44	32.3	2.9		(Yeung et al., 2011)
Fluoxetine	1.41	21.3	Norfluoxetine	2.12	35.3	0.4 - 9.4		(Hamelin et al., 1996; Ouellet et al., 1998; Fjordside et al., 1999; Moraes et al., 1999; Gupta et al., 2004)
Halofantrine	6.93	23.5	Desbutylhalofantrine	3.79	32.3	1.3		(Yeung et al., 2011)
Imatinib	2.49	86.3	Desmethylimatinib	1	95.1	0.16	0.15	(Gschwind et al., 2005)

Imipramine	2.34	6.48	Desipramine	1.27	15.3	1.1		(Sutfin et al., 1984; Wells et al., 1986; Sutfin et al., 1988; Spina et al., 1997)
Itraconazole	4.93	101	Dealkylitraconazole	3.51	110	0.05		(Templeton et al., 2008)
Ivabradine	3.32	60.1	Desmethylivabradine	2.37	69.3	0.38 - 0.49		(Portoles et al., 2006a; Portoles et al., 2006b)
Loratadine	3.9	42.4	Desloratidine	0.84	24.3	2.3	0.55	(Brannan et al., 1995) (Ramanathan et al., 2007a)
Loxapine	2.23	28.1	Desmethylloxapine	1.79	36.7	0.24		(Simpson et al., 1978; Cheung et al., 1991)
Lumefantrine	7.42	23.5	Desbutyllumefantrine	4.76	32.3	0.05		(McGready et al., 2006)
Mianserin	0.76	6.48	Desmethylmianserin	0.49	15.3	0.61		(Pinder and Van Delft, 1983b; Pinder and van Delft, 1983a)
Mirtazapine	0.28	19.4	Desmethylmirtazapine	-0.35	28.2	0.47 - 0.75	0.25	(Timmer et al., 2000)
Nefazodone	3.89	51.6	m-Chlorophenylpiperazine	0.56	15.3	0.09 – 0.19		(Barbhaiya et al., 1995; Barbhaiya et al., 1996a; Barbhaiya et al., 1996b)
Olanzapine	2.35	59.1	Desmethylolanzapine	2.21	67.9	NR	0.04	(Kassahun et al., 1997)
Otenabant (CP-945598)	4.71	102	N-desethylotenabant	3.63	116	4.6		(Miao et al., 2012)
Oxybutynin	4.15	49.8	Desethyloxybutynin	4.39	58.6	R:21.5; S:8.3		(Zobrist et al., 2001)
Propafenone	1.37	58.6	Despropylprofenanone	0.68	72.6	0.06 - 0.18		(Kates et al., 1985; Dilger et al., 1999)

Ranitidine	-1.07	112	Desmethylranitidine	-2.22	120	0.06	0.01	(Prueksaritanont et al., 1989); Product Label[d]
Ranolazine	-1.28	74.3	Desalkylranolazine	-2.16	53.9	0.33 - 0.37		(Abdallah and Jerling, 2005; Jerling and Abdallah, 2005; Jerling et al., 2005)
Regalinide	1.99	78.87	N-dealkylregaplinide (M1)	-0.58	101.65	0.06	0.04	(Honkalammi et al., 2011)
Rosiglitazone	1.93	96.8	Desmethylrosiglitazone	1.39	106	0.58 - 1.2	0.21	(Cox et al., 2000; Kirchheiner et al., 2006)
Sertraline	3.04	12	Desmethylsertraline	3.17	26	1.2 - 1.7		(Hamelin et al., 1996)
Sibutramine	3.24	3.24	Desmethylsibutramine	2.21	12	3.8		(Kim et al., 2009; Bae et al., 2011)
			Didesmethylsibutramine	1.89	26	12		
Sildenafil	2.45	118	Desmethylsildenafil	0.97	126	0.5	0.22	(Muirhead et al., 2002)
			Cleaved piperazine ring	-1.24	135	0.27	0.22	
Sunitinib	0.44	77.2	Desethylsunitinib	-0.91	86	0.4	0.32	(Speed et al., 2012)
Tamoxifen	3.83	12.5	Desmethyltamoxifen	3.19	21.3	1.8		(Yeung et al., 2011)
			Didesmethyltamoxifen	3.11	35.3	0.37		
Terbinafine	5.46	3.24	Desmethylterbinafine	5.1	12	0.9		(Robbins et al., 1996)
Tramadol	0.29	32.7	Desmethyltramadol	-1.11	41.5	0.27		(Ardakani et al., 2008; de Moraes et al., 2012)

Udenafil	-1.21	126	Desalkyludenafil	0.77	137	1.2 - 1.7		(Shin et al., 2010)
Vardenafil	3.45	118	Desethylvardenafil	1.64	126	0.73		(Ku et al., 2009)
Verapamil	2.46	63.9	Norverapamil	1.35	72.7	1.0 - 1.3		(Abernethy et al., 2000)
			D617	-0.69	54.3	0.85 - 1.2		
			D620	-1.29	30.5	0.32 - 0.46		
Zimelidine	2.44	16.1	Desmethylzimelidine	1.38	24.9	3		(Brown et al., 1980)
Zolmitriptan	-1.47	57.4	Desmethylzolmitriptan	-2.97	66.2	0.6	0.04	(Dixon et al., 1997; Seaber et al., 1997)
Zopiclone	2.63	91.8	Desmethylzopiclone	1.57	101	0.2	0.17	(Fernandez et al., 1995; Mistri et al., 2008)

1 Structures of drugs and its metabolites are provided in SupplTable 1

2 References provided in Supplementary File

PM, Poor metabolizers; EM, Extensive metabolizers; SD, Single Dose; MD, Multiple dose; R, R-enantiomer; S, S-enantiomer; NR, Not reported.

* Asterisk denotes M/P ratio from single time point concentration

[a] http://www.accessdata.fda.gov/drugsatfda_docs/nda/2002/21-411_Strattera_biopharmr_P2.pdf

Table 2A: Physicochemical properties, abundance and estimated fm of alcohol metabolites[1, 2].

Drug	cLogD	tPSA	Metabolite	pKa	cLogD	tPSA	M/P	Conjugate	fm	Ref
Amiodarone	5.87	42.7	3'-OH-Desethyl amiodarone	14.97	3.69	71.7	0.39			(McDonald et al., 2012)
Bosentan	2.36	154	Hydroxybosentan	14.63	0.65	174	0.11 to 0.13		0.3	(Weber et al., 1999; van Giersbergen et al., 2002)
Bromhexine	3.69	29.3	Hydroxybromhexine	15.11	2.38	49.5	E4-OH:0.42; E3-OH:0.17			(Liu et al., 2010)
Bufuralol	1.46	45.4	1'-Hydroxybufuralol	14.3	0.066	65.6	1			(Pringle et al., 1986)
Bupropion	2.13	29.1	Hydroxybupropion	15.43	1.28	49.3	7 to 41			(Kirchheiner et al., 2003a; Loboz et al., 2006)
			Dihydrobupropion	13.67	0.94	32.1	Threo: 6.4; Erthro:1.8			(Yeung et al., 2011)
Buspirone	1.24	69.6	Hydroxybuspirone	12.5	-0.4	89.9	34 to 44			(Jajoo et al., 1989; Dockens et al., 2006)
Celecoxib	2.59	83.4	Hydroxycelecoxib	14.16	0.93	107	0.05		0.73	(Paulson et al., 2000; Kirchheiner et al., 2003b)
Cerivastatin	0.071	99.9	Hydroxycerivastatin	14.4	-1.36	120	0.24 to 0.37			(Muck, 2000)

			O-Desmethyl cerivastatin	13.38	-0.7	111	0.08 to 0.11		
Clarithromycin	2.06	183	Hydroxyclarithromycin	14.1	0.99	203	0.67 (SD); 0.42 (MD)	0.2	(Ferrero et al., 1990; Cheng et al., 1998)
Dolansetron	2.54	62.4	Dehydrodolasetron	14.59	1.23	65.6	>1	0.32	(Reith et al., 1995; Lerman et al., 1996)
Darifenacin	1.95	55.6	Hydroxydarifenacin	13.52	0.86	75.6	1 to 1.3	0.32	(Beaumont et al., 1998; Skerjanec, 2006)
Erlotinib	3	74.7	Desmethylerlotinib	13.9	2.41	85.7	0.05	0.04	(Frohna et al., 2006; Ling et al., 2006)
Flutamide	3.52	74.9	Hydroxyflutamide	12.87	2.67	95.2	73		(Anjum et al., 1999)
Indacaterol	2.33	61.4	Hydroxyindacaterol	14.58	0.39	81.6	0.39	0.2	(Kagan et al., 2012)
Haloperidol	2.93	40.5	Dihydrohaloperidol	14.29	1.56	43.7	0.88		(Chakraborty et al., 1989)
Itraconazole	4.93	102	Hydroxyitraconazole	14.98	3.46	121	1.4		(Templeton et al., 2008)
Ketanserine	3.05	69.7	Ketanserinol	14.06	2.07	72.9	3.2 to 3.5		(Lebrec et al., 1990)
Metoprolol	-0.47	50.7	α-Hydroxymetoprolol	13.74	-1.68	70.6	0.56		(Cerqueira et al., 2005)
			O-Desmethylmetoprolol	14.9	-1.09	61.7	ND		

Midazolam	3.78	30.2	1'-Hydroxymidozalam	13.59	2.5	50.4	0.48	Yes	0.65	(Heizmann and Ziegler, 1981; Eap et al., 2004)
Montelukast	3.2	95.7	Hydroxymontelukast (M1)	15.86	2.2	116	0.06			(Karonen et al., 2012)
			Hydroxymontelukast (M5a/5b)	13.98	1.82	116	0.77			
Nalidixic Acid	-1.54	70.5	Hydroxynalidixic acid	13.02	-2.3	90.7	0.69			(Cuisinaud et al., 1982)
Naltrexone	1.6	70	6-β-Naltrexol	14.42	0.24	73	29			(Yun et al., 2007)
Nebivolol	2.75	70.6	Hydroxynebivolol	13.98	1.23	91.2	21			(Kamali et al., 1997)
Nefazadone	3.89	51.6	Hydroxynefazadone	13.8	3.55	71.9	0.35 to 0.55			(Barbhaiya et al., 1995; Barbhaiya et al., 1996a; Barbhaiya et al., 1996b)
Nelfinavir	7.26	127	Hydroxynelfinavir	16.11	6.1	147	0.37			(Zhang et al., 2001)
Nortryptyline	1.49	12	Hydroxynortryptyline	13.9	-0.25	32.3	0.36 -13			(Dalen et al., 1998)
Omeprazole	2.35	96.3	Hydroxyomeprazole	13.15	1.2	117	0.42 to 0.7		0.25	(Renberg et al., 1989; Andersson et al., 1990; Lutz and Isoherranen, 2012)
Pagoclone	3.31	65.2	Hydroxypagoclone	14.98	1.64	83.4	65% of total plasma radioactivity			(Dalvie et al., 2009)

Pioglitazone	2.45	93.6	Hydroxypioglitazone	13.74	0.89	114	SD: 3.8; MD:1.8		(Eckland and Danhof, 2000; Budde et al., 2003)
Praziquantel	2.66	40.6	Hydroxypraziquantel	15.02	1.02	60.9	(+)S:9.0; (-) R: 33-38		(Westhoff and Blaschke, 1992; Lima et al., 2009)
Quinidine	0.98	45.6	Hydroxyquinidine	13.93	0.88	65.8	0.26 to 0.29		(Rakhit et al., 1984; Schellens et al., 1991)
Repaglinide	1.99	78.9	Hydroxyrepaglinide	14.79	0.42	99.1	0.02*	0.01	(van Heiningen et al., 1999; Honkalammi et al., 2011)
Risperidone	1.89	61.9	Hydroxyrisperidone	13	0.61	82.2	3 to 6	0.25	(Mannens et al., 1993; Zhou et al., 2006; Mahatthanatrakul et al., 2012)
Ruzolitinib	2.07	83.2	Hydroxyruzolitinib	14.94	0.56	103	0.24 to 0.3	0.05	(Shilling et al., 2010; Shi et al., 2012)
Taranabant	6.29	75	Hydroxytaranabant	15.14	5.58	95.2	2.5 to 3.3		(Addy et al., 2008; Karanam et al., 2010)
Terfenadine	3.6	43.7	Hydroxyterfenadine	14.96	1.88	63.9	ND		(Abernethy et al., 2001)
Tibolone	3.52	37.3	3α-Hydroxytibolone	15	3.96	40.5	0.44	0.16	(Vos et al., 2002)
Ticagrelor	2.02	164	O-Desalkylticagrelor	14.28	2.02	155	0.4	0.22	(Teng et al.,

										2010)
Tolterodine	2.37	23.5	Hydroxytolteridine	14.71	1.93	43.7	0.82			(Brynne et al., 1999)
Valdecoxib	3.56	94.6	Hydroxyvaldecoxib	13.09	2.36	115	0.08 to 0.1	Yes	0.23	(Yuan et al., 2002; Sarapa et al., 2005)

1. Structures of drugs and its metabolites are provided in SupplTable 2A
2. References provided in Supplementary File

* Asterisk denotes M/P ratio from single time point concentration

Table 2B: Physicochemical properties, abundance and estimated fm of ketone metabolites[1, 2].

Drug	cLogD	tPSA	Metabolite	cLogD	tPSA	M/P	fm	Ref
Nefazadone	3.89	51.6	triazolodione	1.07	68.4	2.1		(Barbhaiya et al., 1995; Barbhaiya et al., 1996a; Barbhaiya et al., 1996b)
Itraconazole	3.77	118	Ketoitraconazole	4.93	101	0.04		(Templeton et al., 2008)
Otenabant	4.71	102	Ketone Metabolite	4.55	63.9	1.1		(Miao et al., 2012)
Pioglitazone	2.45	93.6	Ketopioglitazone	0.73	111	0.9 to 2.8 (SD); 0.5 (MD)		(Eckland and Danhof, 2000; Budde et al., 2003)
Bufuralol	1.46	45.4	1'-Oxobufuralol	0.19	62.5	0.1 to 0.2		(Pringle et al., 1986)

1. Structures of drugs and its metabolites are provided in SupplTable 2B
2. References provided in Supplementary File

SD, Single Dose; MD, Multiple Dose

Table 3: Physicochemical properties, abundance and estimated fm of arenol metabolites[1, 2].

Drug	cLogD	tPSA	Metabolite	pKa	cLogD	tPSA	M/P (Aglycone)	Conjugate[3]	fm	Ref
Amoxapine	1.79	36.9	7-Hydroxyamoxapine	11.16	1.68	57.1	0.22 to 0.44	Yes	0.25	(Jue et al., 1982; Kobayashi et al., 1985; Takeuchi et al., 1993)
			8-Hydroxyamoxapine	10.35	1.02	57.1	2.4 to 4.9	Yes	0.33	
Atomoxetine	0.77	21.3	Hydroxyatomoxetine	10.33	-0.13	41.5	CYP2D6 EM: 0.01	Yes (CYP2D6 EM- 2.5 & PM - 0.11)	CYP2D6 EM:0.87	(Sauer et al., 2003; Sauer et al., 2005); FDA Clinical Pharmacology Review[a]
Atorvastatin	0.74	112	*o*-Hydroxyatorvastatin	9.34	1	132	1.4	NR		(Whitfield et al., 2011)
			p-Hydroxyatorvastatin	10	0.1	132	0.04	NR		
Chlorpromazine	3.24	31.8	Hydroxychlorpromazine	9.19	2.65	52	0.58 to 1.8	Yes (2.2 to 4.2)		(Yeung et al., 1993)

Desipramine	1.21	15.3	Hydroxydesipramine	10	-0.19	35.5	0.22 to 0.79	Yes (3.3)		(DeVane et al., 1981; Spina et al., 1997; Patroneva et al., 2008)
Desloratadine	0.84	24.9	Hydroxydesloratadine	9.27	0.49	45.2	0.5	Yes	0.51	(Gupta et al., 2004; Ramanathan et al., 2007b)
Duloxetine	2.31	49.5	Hydroxyduloxetine	9.97	1.51	69.7		Yes (12)	0.22	(Lantz et al., 2003; Knadler et al., 2011)
			5-Hydroxy-6-methoxy duloxetine	7.67	1.49	78.9		Yes (5.6)	0.19	
Efavirenz	3.03	38.3	HydroxyEfavirenz	7.18	2.38	58.6	0.04	Yes (1.1)	0.24	(Mutlib et al., 1999; Cho et al., 2011); FDA Clinical Pharmacology Review[b]
Elzasonan	3.24	52.1	5-HydroxyElsazonan	11.5	3.16	72.3	0	Yes	0.34	(Kamel et al., 2010)
Granisetron	0.0166	50.2	Hydroxygranisetron	8.9	-0.59	70.4	0.11*	0.26*	0.25	(Clarke et al., 1994)
Imipramine	2.34	6.48	Hydroxyimipramine	10.2	1.71	26.7	0.37 to 0.89	Yes (1.9)		(Sutfin et al., 1984; Wells et al., 1986; Sutfin et al., 1988; Spina

										et al., 1997)
Lansoprazole	2.58	87.1	Hydroxylansoprazole	11.16	1.9	10.7	CYP2C19 EM:0.1 - 0.19; PM:0.02			(Miura et al., 2004; Zhang et al., 2011)
Loxapine	2.23	28.1	7-Hydroxyloxapine	11.16	2.1	48.3	0.68			(Simpson et al., 1978; Cheung et al., 1991)
			8-Hydroxyloxapine	10.35	1.45	48.3	3			
Nelfinavir	7.26	127	Methoxycathecol	8.57	7.28	136	0.05			(Zhang et al., 2011)
Propafenone	1.37	58.6	Hydroxypropafenone	9.48	1.21	78.8	0.23 - 0.5			(Kates et al., 1985; Dilger et al., 1999)
Propranolol	0.79	41.5	Hydroxypropanolol	10.17	0.25	61.7	0.01 - 0.09 (without hydrolysis) 2.8 (with hydrolysis)	Yes		(Raghuram et al., 1984; Partani et al., 2009)
Quetiapine	2.51	73.6	Hydroxyquetiapine	11.18	1.91	93.8	0.1			(Gefvert et al., 1998; DeVane and Nemeroff, 2001)
Rosiglitazone	1.93	96.8	Hydroxyrosiglitazone	10.3	1.33	117		Yes (2.8)	0.29	(Cox et al., 2000; Kirchheiner et

										al., 2006)
Tamoxifen	3.83	12.5	Hydroxytamoxifen	10.35	3.63	32.7	0.035			(Yeung et al., 2011)
S-Warfarin	0.33	63.6	Hydroxywarfarin	8.51	0.36	83.8	0.15			(Uno et al., 2008)
Amiodarone	5.87	42.7	O-Desalkylamiodarone	4.84	3.33	50.4	0.1	No		(McDonald et al., 2012)
Ampremilast	0.48	127	Demethoxyampremilast	9.8	-0.032	138	ND	Yes (1.24)	0.39	(Hoffmann et al., 2011)
Apixaban	4.66	111	Demethoxyapixaban	8.56	4.02	122	ND	Yes (0.25)	0.18	(Raghavan et al., 2009)
Astemizole	4.12	42.3	Demethoxyastemizole	10.13	3.53	53.3	6.8			(Lefebvre et al., 1997)
Bosentan	2.36	154	Demethoxybosentan	8.36	2.34	165	0.02 – 0.05		0.07	(Weber et al., 1999; van Giersbergen et al., 2002)
Codeine	0.46	41.9	Morphine	9.48	-0.043	52.9	0.003 – 0.008	Yes (M6G:0.33-0.45; M3G:2 - 2.6)		(Kirchheiner et al., 2007)
Dextromethorphan	2.17	12.5	Demethyl-dextromethorphan	10.07	1.71	23.5	CYP2D6 EM: 0.38 (without	Yes		(Bolden et al., 2002; Pope et

							hydrolysis) 69 (with hydrolysis)			al., 2004)
Encainide	1.62	41.57	O-Demethyencanide	8.34	1.2	52.6	CYP2D6 EM: 1.2			(Funck-Brentano et al., 1989)
Flecainide	1.05	59.6	Dealkylflecainide	9.28	-0.14	70.6	0.04	Yes	0.23	(Munafo et al., 1990)
Gefitinib	2.56	68.7	Demethylgefitinib	5.5	1.79	79.7	0.7		0.23	(McKillop et al., 2004); FDA Clinical Pharmacology Review[c]
Pantoprazole	1.54	106	Demethylpantoprazole	7.69	0.49	117	ND	Yes (0.2 – 0.5)		(Radhofer-Welte, 1999)
Paroxetine	1.48	39.7	4-Hydroxy-3-Methoxy Paroxetine	10.34	1.04	50.7	NR	Yes		(Segura et al., 2003)
Ranolazine	1.28	74.3	Demethylranolazine	9.59	0.94	85.3	0.36	Yes		(Abdallah and Jerling, 2005; Jerling and Abdallah, 2005; Jerling et al., 2005)
Tadalafil	2.71	74.9	4-Hydroxy-3-Methoxy Tadalafil	9.87	2.85	85.9	SD - 0.58 MD - 1.3	Yes		(Forgue et al., 2006; Forgue et al., 2007)

Tramadol	0.29	32.7	Demethyltramadol	10	-0.25	43.7	0.35 - 0.95	No		(Ardakani et al., 2008; de Moraes et al., 2012)
Venlafaxine	0.76	32.7	Desvenlafaxine	10	-0.0015	43.7	EM - 4.0 - 6.0 PM - 0.35	Yes	0.56	(Howell et al., 1993; Patat et al., 1998; Hynninen et al., 2008)

1. Structures of drugs and its metabolites are provided in SupplTable 3
2. References provided in Supplementary File
3. Yes: denotes presence of conjugate of the metabolite in circulation; NR: Not reported; the number represents M/P ratio of the metabolite conjugate to parent

EM, Extensive metabolizers; PM, Poor Metabolizers; SD, Single Dose; MD, Multiple Dose; ND, Not Detected; * Asterik denotes M/P ratio from single time point concentration

[a] http://www.accessdata.fda.gov/drugsatfda_docs/nda/2002/21-411_Strattera_biopharmr_P2.pdf

[b] http://www.accessdata.fda.gov/drugsatfda_docs/nda/98/20972biopharm_review.pdf

[c] http://www.accessdata.fda.gov/drugsatfda_docs/nda/2003/21-399_IRESSA_Clinr.pdf

Table 4: Physicochemical properties, abundance and estimated fm of N and S-oxide metabolites [1, 2].

Drug	cLogD	tPSA	Metabolite	cLogD	tPSA	M/P	fm	Ref
			S- Oxidation					
Albendazole	2.99	92.3	Albendazole sulfoxide	0.68	103	>1		(Marriner et al., 1986; Delatour et al., 1991)
Axitinib	2.39	96	Axitinib sulfoxide	1.89	107	1		Product label
Chlorpromazine	3.24	31.78	Chlorpromazine sulfoxide	2.32	0.37	0.95 to 2.4		(Yeung et al., 1993)
Dothiepin	2.54	28.5	Dothiepin sulfoxide	0.98	39.52	2.4		(Maguire et al., 1981)
Flosequinan	-1.43	56.6	Flosequinan sulfone	-1.18	62.83	14		(Wynne et al., 1985)
Lansoprazole	2.58	87.1	Lansoprazole sulfone	3.55	93.3	CYP2C19 EM:0.05; PM:0.33 - 0.88		(Miura et al., 2004; Zhang et al., 2011)
Montelukast	3.2	95.7	Montelukast sulfoxide	1.74	107	NR		(Karonen et al., 2012)
Omeprazole	2.35	96.3	Omeprazole sulfone	3.31	103	0.6 – 0.89		(Renberg et al., 1989; Andersson et al., 1990; Lutz and Isoherranen, 2012)

Pantoprazole	1.54	106	Pantoprazole sulfone	2.46	112	Low (<0.25)		(Radhofer-Welte, 1999)
Quetiapine	2.51	73.6	Quetiapine sulfoxide	-0.76	84.6	0.65		(Gefvert et al., 1998; DeVane and Nemeroff, 2001)
Ranitidine	-2.47	117	Ranitidine sulfoxide	-3.43	128	0.09	0.01	(Prueksaritanont et al., 1989); Product label
Thioridazine	3.49	57.1	Thioridazine sulfoxide (Mesoridazine)	0.76	68.1	4.3 – 6.5		(Hartigan-Go et al., 1996)
			Thioridazine sulfoxide-2	1	68.1	3.9 – 4.2		
			Mesoridazine sulfone	2.15	74.3	0.95 to 1.6		
			N-Oxidation					
Azimilide	2.66	72.6	Azimilide N-oxide	2.22	86.43	0.01	0.07	(Riley et al., 2005)
Chlorpromazine	3.24	31.8	Chlorpromazine N-oxide	2.39	45.6	0.8 – 1.8		(Yeung et al., 1993)
Dothiepin	2.54	28.5	Dothiepin N-oxide	1.61	42.4	Not known		(Maguire et al., 1981)
Elzasonan	3.24	52.1	Elzasonan N-oxide	2.47	65.9	0.27		(Kamel et al., 2010)

Quinidine	0.98	45.6	Quinidine N-oxide	1.26	59.4	0.14 – 0.39		(Rakhit et al., 1984; Schellens et al., 1991)
Ranitidine	-2.47	117	Ranitidine N-oxide	-4.56	131	0.19	0.04	(Prueksaritanont et al., 1989); Product label
Regorafinib	4.2	92.4	Regorafinib N-oxide	1.55	105	0.96 – 1.1		(Mross et al., 2012; Strumberg et al., 2012)
			N-Desmethylregorafinib N-oxide	1.64	119	1.3 – 1.8		
Roflumilast	2.3	60.5	Roflumilast N-oxide	0.21	73	13	0.21	(Lahu et al., 2008); FDA Clinical Pharmacology Review[a]
Voriconazole	1.21	77	Voriconazole N-oxide	0.45	89.3	1.6	0.21	(Roffey et al., 2003; Damle et al., 2008)
Zolmitriptan	-1.47	57	Zolmitriptan N-oxide	-2.43	71.2	0.38 – 0.48	0.07	(Dixon et al., 1997; Seaber et al., 1997)
Zopiclone	2.63	91.8	Zopiclone N-oxide	1.24	106	0.18	0.14	(Mistri et al., 2008)

1. Structures of drugs and its metabolites are provided in SupplTable 4
2. References provided in Supplementary File

[a] http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/022522Orig1s000ClinPharmR.pdf

Table 5: Physicochemical properties, abundance and estimated fm of carboxylic acid metabolites[1, 2].

Drug	cLogD	tPSA	Metabolite	cLogD	tPSA	M/P	fm	Ref
Azimilide	2.66	72.6	Carboxylic acid	1.05	50.4	4	0.35	(Riley et al., 2005)
Celecoxib	2.59	83.4	Carboxylic acid Metabolite	-1.77	124	1	0.73	(Paulson et al., 2000; Kirchheiner et al., 2003a)
Losartan	1.48	92.5	Carboxylosartan	-0.06	110	4.4	0.14	(Lo et al., 1995; Neves et al., 2008)
Metoprolol	-0.47	50.7	O-Desmethylmetoprolol Carboxylic acid	-1.65	78.8	6.5		(Cerqueira et al., 2005)
Montelukast	3.2	95.7	Montelukast carboxylic acid	0.21	133	0.24		(Karonen et al., 2012)
Nabumetone	3.14	26.3	6-Methoxynapthyl carboxylic acid	-0.45	46.5	>1		(Nobilis et al., 2003)
Quetiapine	2.51	73.6	Quetiapine carboxylic acid	-1.13	90.7	0.63		(Gefvert et al., 1998; DeVane and Nemeroff, 2001)
Ranolazine	1.28	74.3	Cleaved Carboxylic acid	-3.12	76	0.25		(Abdallah and Jerling, 2005; Jerling and Abdallah, 2005; Jerling et al., 2005)
Regaplinide	1.99	78.9	Carboxylic Acid Metabolite	-1.86	125	0.05	0.66	(van Heiningen et al., 1999; Honkalammi et al., 2011)
Terfenadine	3.6	43.7	Fexofenadine	1.23	81	98		(Abernethy et al., 2001)
Zolmitriptan	-1.47	57.4	Zolmitriptan indoleacetic acid	-3.4	91.4	1.3	0.31	(Dixon et al., 1997; Seaber et al., 1997)

1. Structures of drugs and its metabolites are provided in SupplTable 5
2. References provided in Supplementary File

Table 6: Physicochemical properties, abundance and estimated fm of novel metabolites[1, 2].

Drug	cLogD	tPSA	Metabolite	cLogD	tPSA	M/P	fm	Ref
Aripiprazole	3.54	44.8	Dehydroapripiprazole	3.32	44.8	0.5	0.38	(Caccia, 2011); FDA Clinical Pharmacology Review[a]
Carbamazepine	1.89	46.3	Epoxycarbamazepine	0.16	58.9	0.25		(Ragueneau-Majlessi et al., 2004)
Elzasonan	3.24	52.1	Indole Derivative of Elzasonan	3.39	53.8	3.2	0.06	(Kamel et al., 2010)
Indapamide	1.96	101	Dehydroindapamide	2.65	103	Major		(Yeung et al., 2011)
Methadone	2.29	20.3	EDDP Metabolite	-3.51	3.01	0.16		(Kharasch et al., 2009)
Otenabant	4.71	102	Otenabant oxime	4.64	79.4	1.5		(Miao et al., 2012)
Proguanil	0.77	88.8	Cycloguanil	-0.69	80	0.67		(Funck-Brentano et al., 1997)

1 Structures of drugs and its metabolites are provided in SupplTable 6

2 References provided in Supplementary File

[a] http://www.accessdata.fda.gov/drugsatfda_docs/nda/2002/21-436_Abilify_biopharmr_P2.pdf

Table 7: **Drug and metabolite free fraction and calculated total and free drug clearances for selected drugs shown in Tables 1-6.**

Drug	Metabolite	Fraction unbound	CL_s[1] (mL/min/kg)	$CL_{R,U}$[2] (mL/min/kg)	$CL_{NR,U}$[3] (mL/min/kg)	Ref
Clozapine		0.055	5.4	0.1	98	(Schaber et al., 1998)
	desmethyl-clozapine	0.097	0.6	5	1.2	
Chlorpromazine		0.0013	18.3	1	14000	(Freedberg et al., 1979) (Yeung et al., 1993)
	hydroxy-chlorpromazine	0.015	6.1	6	400	
Darifenacin		0.02	12	6	594	(Kerbusch et al., 2004)
	Hydroxyl-darifenacin	0.13	14	47	60	
Diltiazem		0.25	12		48	(Boyd et al., 1989)
	N-desmethyl diltiazem	0.32	8		25	
Propranolol		0.15	20	0.7	132	(Weiss et al., 1978) (Rochester et al., 1980)
	hydroxy-propranolol	0.26	66	5	248	
Tolterodine		0.037	44	1	1190	(Pahlman and Gozzi, 1999)

	N-dealkyl-tolterodine	0.14				
	hydroxyl-tolterodine	0.36	21	2.9	55	
Thioridazine		0.0016				(Freedberg et al., 1979)
	thioridazine sulfoxide (Mesoridazine)	0.090				

1. CLs = Total systemic clearance
2. $CL_{R, U}$ = Unbound renal clearance
3. $CL_{NR, U}$ = Unbound non-renal (hepato-biliary) clearance

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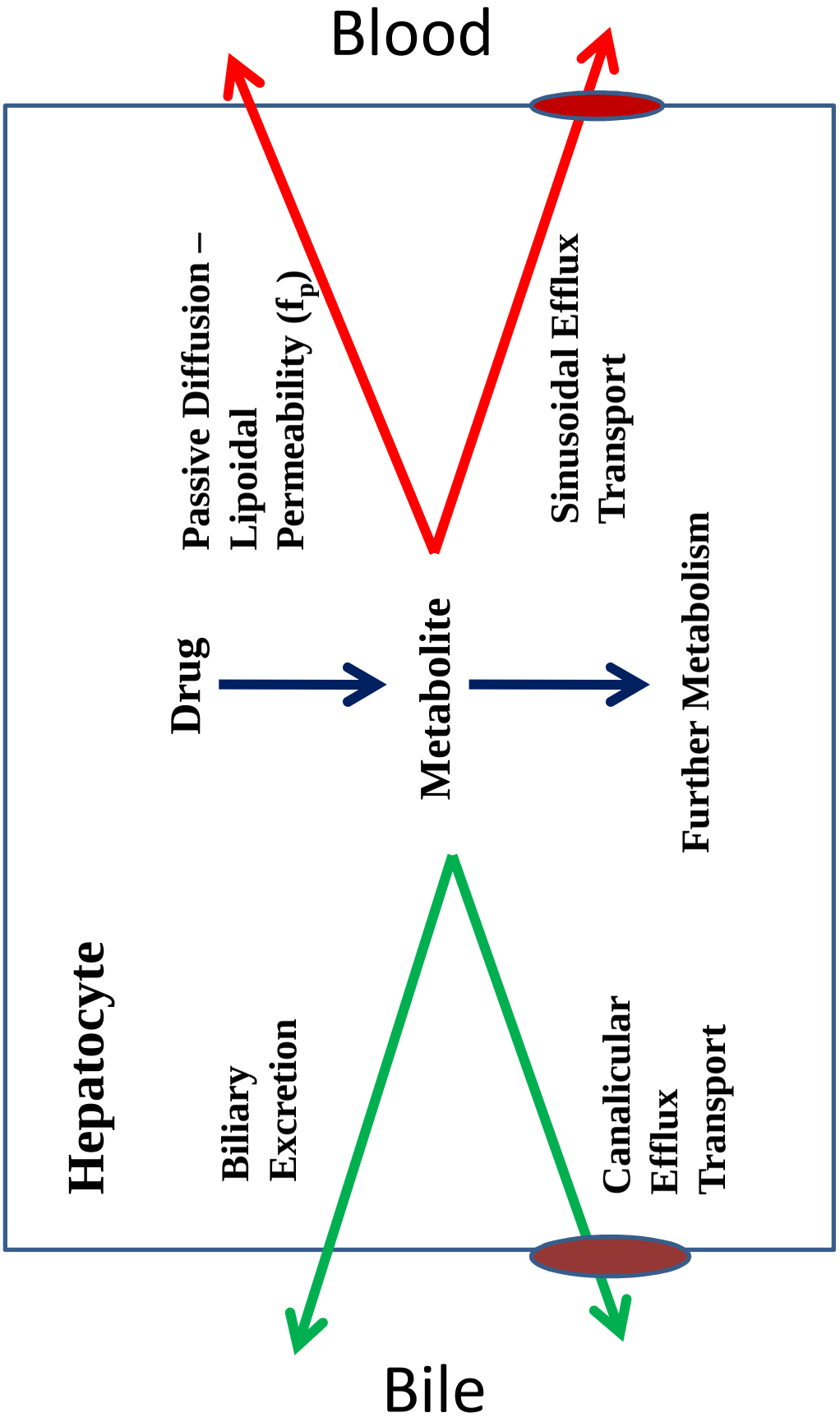


Figure 1

Figure 2

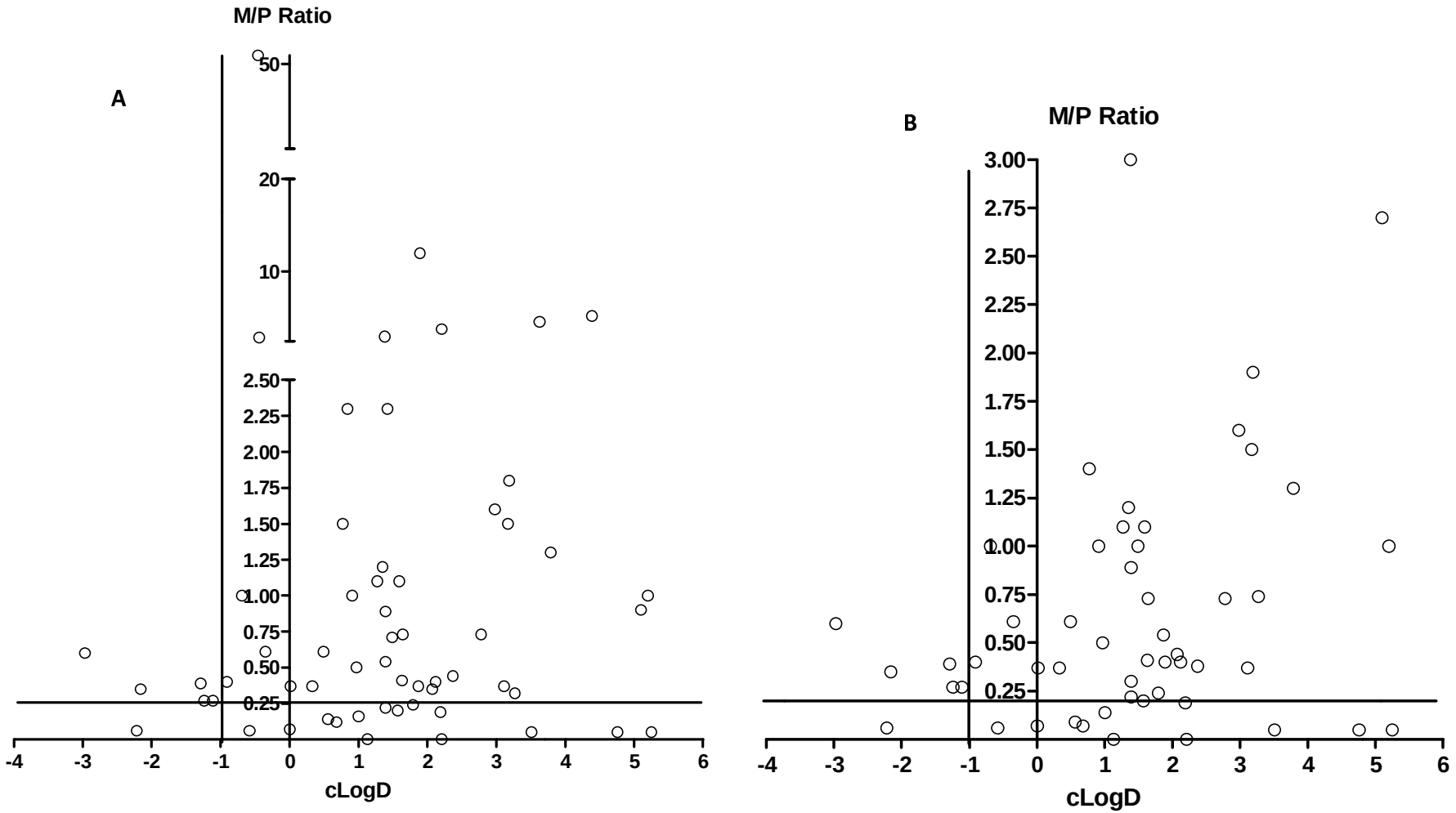
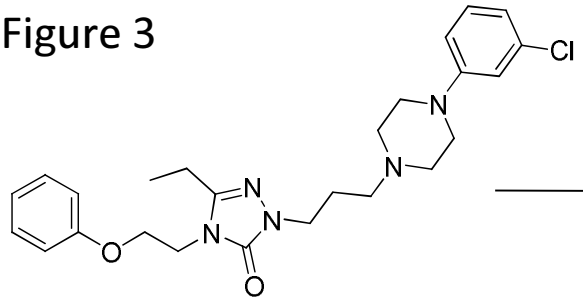
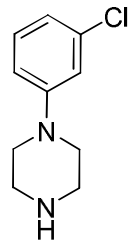


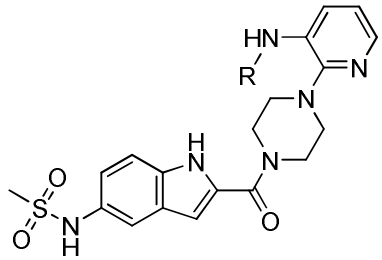
Figure 3



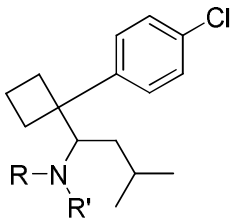
Nefazadone
cLogD = 3.89



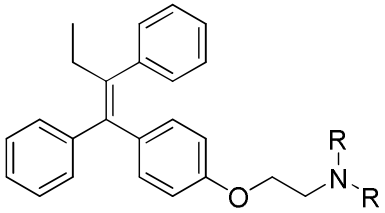
m-Chlorophenylpiperazine
cLogD = 0.56



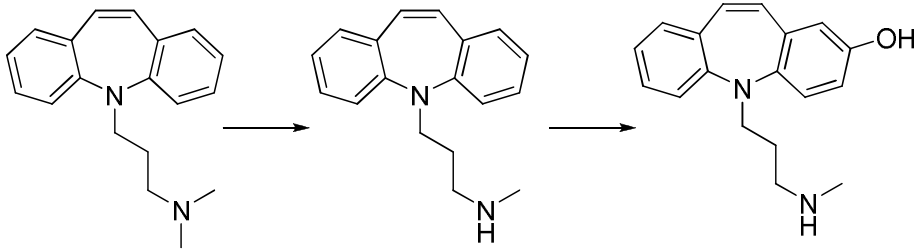
R = $CH(CH_3)_2$, Delavirdine
R = H, *N*-Desisopropyldelavirdine



R & R' = CH_3, Sibutramine
R = H & R' = CH_3, *N*-Desmethylsibutramine
R & R' = H, Didesmethylsibutramine



R & R' = CH_3, Tamoxifen
R = H & R' = CH_3, *N*-Desmethyltamoxifen
R & R' = H, Didesmethyltamoxifen



Imipramine Desipramine Hydroxydesipramine

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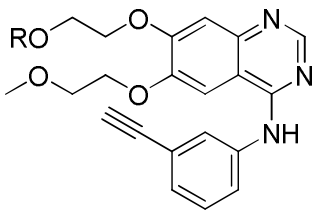
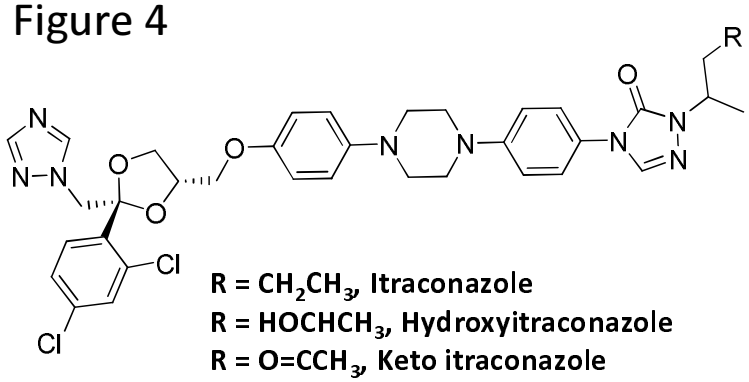
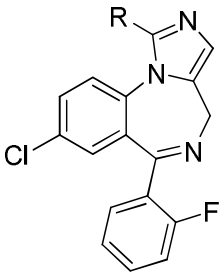
Figure 4



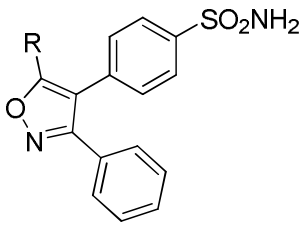
R = CH_3, Erlotinib
R = H, *O*-Desmethylerlotinib



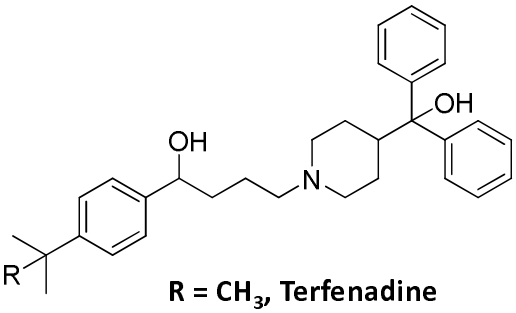
R = CH_2CH_3, Itraconazole
R = $HOCHCH_3$, Hydroxyitraconazole
R = $O{=}CCH_3$, Keto itraconazole



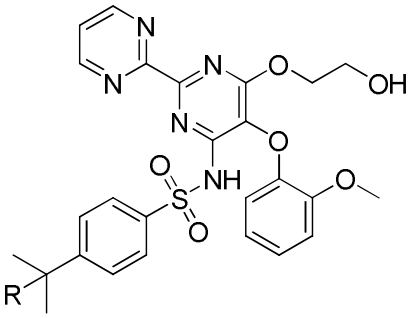
R = CH_3, Midazolam
R = CH_2OH, Hydroxymethylmidazolam
R = CH_2OGlucuronide, Glucuronide conjugate of Hydroxy metabolite



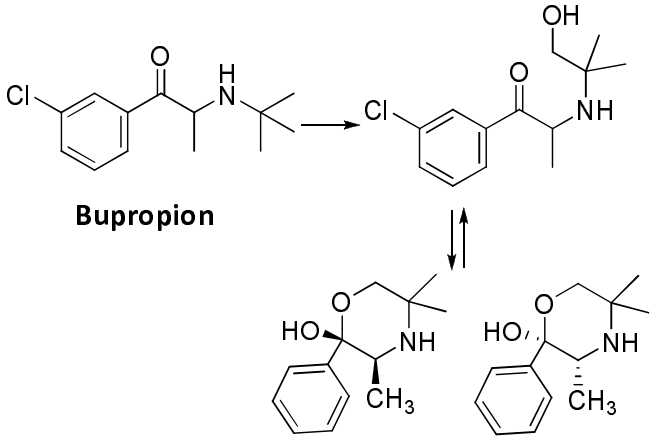
R = CH_3, Valdecoxib
R = CH_2OH, Hydroxymethylvaldecoxib
R = CH_2OGlucuronide, Glucuronide conjugate of Hydroxy metabolite



R = CH_3, Terfenadine
R = CO_2H, Fexofenadine



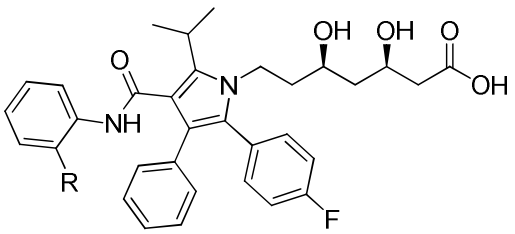
R = CH_3, Bosentan
R = OH, Hydroxybosentan



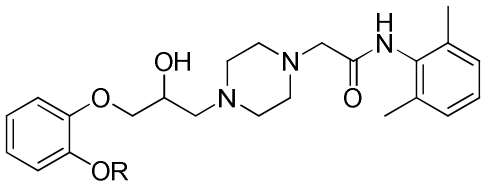
Bupropion

***R, R,* and *S,S*- Hydroxybupropion**

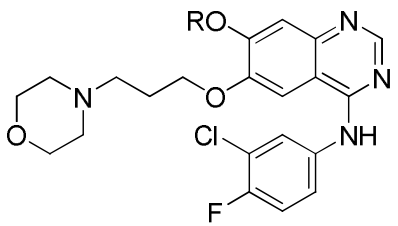
Figure 5



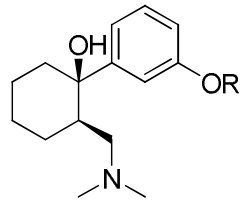
R = H Atorvastatin
R = OH *ortho*-Hydroxyatorvastatin



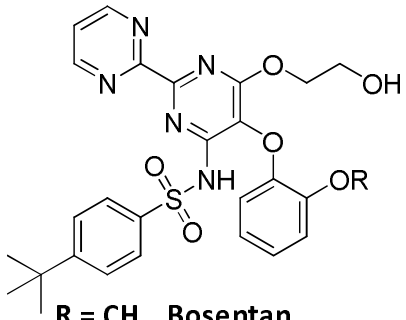
R = CH_3, Ranolazine
R = H, *ortho*-Hydroxy metabolite



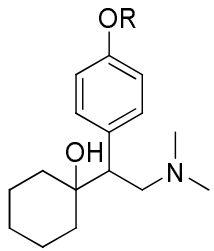
R = CH_3, Gefitinib
R = H, *ortho*-Hydroxy metabolite



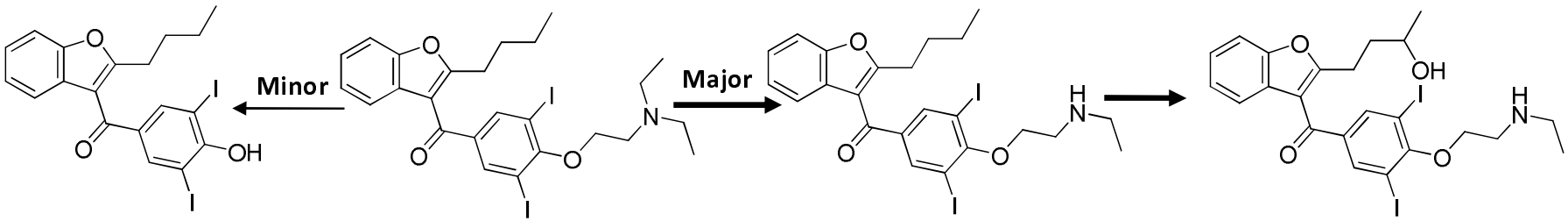
R = CH_3, Tramadol
R = H, *meta*-Hydroxy metabolite



R = CH_3, Bosentan
R = H, Hydroxy metabolite

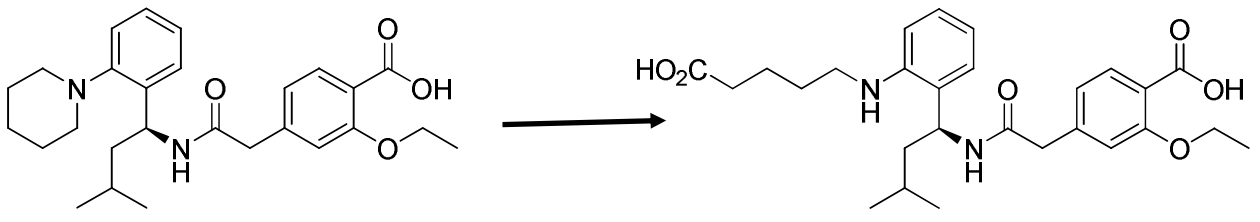


R = CH_3, Venlafaxine
R = H, Desvenlafaxine



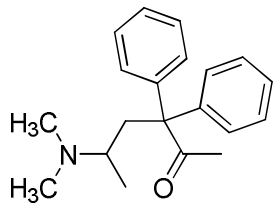
O-Dealkylamiodarone | Amiodarone | *N*-Desethylamiodarone | Hydroxy-*N*-Desethylamiodarone

Figure 6

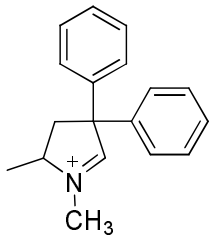


Repaglinide

Repaglinide carboxylic acid



Methadone



EDDP Metabolite

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Figure 7