**Special Section on Emerging Novel Enzyme Pathways in Drug Metabolism**

**Prevalence of Non–Cytochrome P450–Mediated Metabolism in Food and Drug Administration–Approved Oral and Intravenous Drugs: 2006–2015**

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**ABSTRACT**

In recent years, claims of increased involvement of non–cytochrome P450 (non-P450) enzymes in the metabolism of drugs have appeared in the literature. However, no temporal summaries of the contribution of non-P450 enzymes to the metabolism of drugs have been published. Using data from human radiolabeled absorption, distribution, metabolism, and excretion studies available for a set of 125 orally or intravenously administered small-molecule drugs approved by the United States Food and Drug Administration from 2006 to 2015, the contributions of P450 and non-P450 enzymes to the formation of major metabolites (≥10% of dose) were assessed and tabulated. Over this time frame, the involvement of P450 versus non-P450 enzymes in the formation of major metabolites is compared, and the individual non-P450 enzymes responsible are described. This analysis indicates that non-P450 enzymes contribute significantly to the metabolism of the 125 drugs analyzed. Approximately 30% of the metabolism of these drugs is carried out by non-P450 enzymes, with the predominant non-P450 enzymes being gluturorosyltransferases (11.7%), hydrolases (10.8%), carbonyl reductases (2.4%), and aldol hydroxide (1.1%). Although significant, the relative contribution of non-P450 enzymes to drug metabolism does not appear to have increased dramatically over the last 10 years. As the current evaluation involves drugs which emerged from the discovery phase >10 years ago, this evaluation may not reflect the current or evolving situation in some research organizations; therefore, additional monitoring and assessment of the involvement of non-P450 enzymes in the metabolism of drugs will be conducted in the future.

**ABBREVIATIONS:** ADME, absorption, distribution, metabolism, and excretion; AO, aldehyde oxidase; CL, clearance; DDI, drug-drug interaction; FDA, Food and Drug Administration; GDC-0834, N-[3-[6-[4-(2R)-1,4-dimethyl-3-oxopiperazin-2-yl]anilino]-4-methyl-5-oxopyrazin-2-yl]-2-methylphenyl]-4,5,6,7-tetrahydro-1-benzothiophene-2-carboxamide; P450, cytochrome P450; UGT, uridine 5’-diphospho-glucuronyltransferase.

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**Introduction**

A major focus of drug discovery and development is to understand the metabolic biotransformations and enzymes which contribute to drug clearance (CL) and impact oral bioavailability (F). An appreciation of these metabolic reactions and enzymes may impact the design of analogs with longer half-lives or higher F and may inform on the likelihood of drug-drug interactions (DDIs). Assays such as metabolic stability using liver or other tissue subcellular fractions or hepatocytes, enzyme reaction phenotyping using expressed enzymes or with selective inhibitors, metabolite identification studies, as well as radiolabeled absorption, distribution, metabolism, and excretion (ADME) studies in humans and other preclinical species (Penner et al., 2012b; Beaumont et al., 2014) can provide screening and definitive data for these purposes. Data from these assays and studies allow one to better understand the extent and rate of metabolism, identify metabolites, and define the individual enzymes responsible for the CL of a drug. Furthermore, human ADME studies provide quantitative data for the amounts of metabolites formed and, along with reaction phenotyping studies, result in a definitive assignment of the fraction metabolized (fm) by enzymes contributing to the metabolism of the drug (Rodrigues et al., 2001; Zientek and Youdim, 2015).

Cytochrome P450s (P450s) are a superfamily of membrane-associated heme-containing enzymes, for which the human genome contains 57 distinct P450 genes (Ortiz de Montellano, 1995; Guengerich and Cheng, 2011). P450 enzymes represent a well studied and relatively well understood family of enzymes that have been reported as the most important enzymes responsible for the majority of oxidative drug metabolism. In humans, P450s 1A2, 2B6, 2C9, 2C19, 2D6, 3A4, and 3A5 are considered the major P450 isoforms involved in the metabolism of drugs (Walsky and Obach, 2004; Wienkers and Heath, 2005).

In recent years, claims have appeared in the literature indicating that metabolism by P450 enzymes is no longer as prominent as it once was (Pryde et al., 2010; Oda et al., 2015). These reports assert that other non-P450 enzymes play a more significant role in the metabolism of recent drugs and drug candidates. Despite these claims, supporting data are sparse. Furthermore, no evaluation exists that looks at the changes in the contribution of P450 versus non-P450 enzymes to the metabolism of drugs over time. As such, one of the main goals of this manuscript is to...
provide a starting data set which can be used to provide a temporal summary of the involvement of non-P450 enzymes in the metabolism of drugs. The current evaluation is intended to provide evidence to support or refute claims of increased involvement of non-P450 enzymes versus P450 enzymes in the metabolism of drugs approved in the last 10 years. Additionally, future expansion of the current data set will provide an ongoing assessment of the involvement of P450 and non-P450 enzymes in the metabolism of emerging drugs.

The involvement of non-P450 enzymes in the metabolism of drugs and endogenous substances is well preceded (Penner et al., 2012a; Bohnert et al., 2016). The more commonly encountered enzymes involved in oxidation, reduction, and hydrolysis (phase I metabolism) include molybdenum cofactor–containing enzymes [aldehyde oxidase (AO) (Hutzler et al., 2013) and xanthine oxidase (Pryde et al., 2010)], flavin-containing monoxygenases (Hines et al., 1994), monoamine oxidase (Edmondson et al., 2009), reductases [carbonyl reductases (Forrest and Gonzalez, 2000) and aldo-keto reductases (Jin and Penning, 2007; Oppermann, 2007; Penning, 2015)], dehydrogenases [alcohol dehydrogenase (Bohnert et al., 2016); alcohol dehydrogenase (Marchitti et al., 2008; Koppaka et al., 2012)] and hydrolitic enzymes (esterases, proteases/peptidases, amidases, etc.) (Testa and Mayer, 2006; Uetrecht and Trager, 2007; Long and Cravatt, 2011; Yang et al., 2011; Bachovchin and Cravatt, 2012; Oda et al., 2015). Additionally, metabolites arising from conjugative enzymes (phase II metabolism), including uridine 5′-diphospho-glucuronosyltransferases (UGT) (Tukey and Strassburg, 2000; Oda et al., 2015), N-acetyl transferases (Hein et al., 2006), glutathione S-transferases (Armstrong, 1997; Eaton and Bannmiller, 1999; Salinas and Wong, 1999; Sheehan et al., 2001), sulfotransferases (Strott, 2002; Riches et al., 2009), and methyltransferases (Petrossian and Clarke, 2011), are also frequently identified.

An appreciation of the metabolic reactions and the totality of enzymes involved in the metabolism of a drug is important for a number of reasons. Properly accounting for both P450 and non-P450 contributions to metabolism provides a more accurate assignment of the fm value for P450 enzymes and, therefore, results in a more accurate determination of the drug’s likelihood of being a P450 DVI victim. Clinically relevant DDIs for a number of phase I and phase II non-P450 enzymes have also been reported. Non-P450 enzymes for which DDIs have been observed clinically include AO (Lake et al., 2002; Renwick et al., 2002), xanthine oxidase (Coffey et al., 1972; Zimm et al., 1983), monoamine oxidase (Livingston and Livingston, 1996; Rolan, 1997; Van Haarst et al., 1999), alcohol dehydrogenase (Roine et al., 1990; Jacobsen et al., 1996), carboxylesterases (Xiao et al., 2013; Wang et al., 2015), UGTs (Kiang et al., 2005; Uchaipichat et al., 2006; Nivoix et al., 2008; Li et al., 2015), sulfotransferases (Schwartz et al., 2009), and methyltransferase (Jorga et al., 1997; Lewis et al., 1997; Relling et al., 1999). Therefore, an early understanding of the routes of metabolism and the contributing enzymes provides insights into potential DDIs as well as time for implementation of mitigation strategies.

The major aim of this manuscript is to summarize and compare the contributions of P450 and non-P450 routes of metabolism to the formation of major metabolites (≥10% of dose) using data from human radiolabeled ADME studies available for small-molecule drugs approved by the United States Food and Drug Administration (FDA) from 2006 to 2015. Additionally, data comparing the involvement of P450 versus non-P450 metabolism for each year have been compiled to assess the change in involvement of P450 versus non-P450 over time. Last, the individual non-P450 enzymes that are involved in the formation of major metabolites of these drugs are also described. A goal of this analysis was to determine if there were perceptible trends in a changing role of non-P450 enzymes, and if so, which enzymes.

As such, the results of this evaluation serve as a database for recurring analyses of the involvement of non-P450 enzymes in the metabolism of emerging drugs. This work also affords specific examples of substrates for non-P450 enzymes and may result in a better understanding and appreciation of the contribution of non-450 enzymes to drug metabolism. The data provided highlight specific non-P450 enzymes which require greater characterization. Greater knowledge of the involvement and importance of non-P450 enzymes can result in consideration of other factors for these enzymes, such as species differences, hepatic versus extrahepatic expression and metabolism, enzyme polymorphisms (Brian et al., 2016), likely DDIs, non-P450–mediated bioactivation (Grillo and Lyubimov, 2011; Hutzler and Cerny, 2012), as well as the development of in vitro assays to improve extrapolation to predict and understand in vivo CL for these non-P450 enzymes. Improving our understanding of these metabolic enzymes and biotransformations should result in fewer surprises being encountered during the drug discovery and development process, leading to safer drugs in the future.

### Materials and Methods

**Sources of Data and Data Restriction Criteria.** All medications considered for evaluation were approved by the FDA between 2006 and 2015 (Supplemental Table 1). New Drug Application and Biologic License Application approval packages, including the drug labels, are available at the FDA website, Drugs@FDA (http://www.accessdata.fda.gov/scripts/cder/drugsatfda/). The focus of this evaluation is the metabolism of small-molecule drugs. Drugs that fall outside of this category were removed, such as protein drugs (i.e., enzymes, antibodies, antibody drug conjugates), peptide drugs, antisense drugs, elemental/inorganic drugs, and botanical drugs (Table 1). For the subset of small-molecule drugs, only drugs administered intravenously or orally were considered for further evaluation. For the remaining drugs, human radiolabeled ADME data were obtained from sources which included the FDA New Drug Application approval package, specifically the clinical pharmacology and biopharmaceutics reviews, pharmacology reviews, and drug labels. Additionally, if data were available from the European Medicines Agency Application for Marketing Authorization (http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/landing/epar_search.jsp&mid=WCOB010ac0850010124) or from the scientific literature, these data were also used for this evaluation. References describing the sources of data used are presented in Supplemental Table 1.

For medications which are combination therapies, the metabolism of each component (drug) was considered individually. For combination therapies where one or more new chemical entities are combined with one or more previously approved drugs, only the newly approved drugs were included in the evaluation

### Table 1

**Compilation of metrics for FDA-approved drugs during 2006–2015**

<table>
<thead>
<tr>
<th>Year</th>
<th>2006</th>
<th>2007</th>
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<td>31</td>
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</tr>
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</table>

*aNumber of active ingredients contained within mono or combination small-molecule therapies approved for the designated year.*
set. A summary of the total number of drug-substance approvals by year is presented in Table 1.

For intravenous drugs, agents used as imaging agents, generally with extremely short half-lives, were removed from the set. Last, prodrugs were also removed from the main evaluation set. These drugs were compiled and evaluated as a separate grouping. A summary of metrics for small-molecule drugs each year between 2006 and 2015 is presented in Table 2.

For each small-molecule drug, a summary of the metabolites identified in plasma, expired air, urine, and feces from the human ADME study was compiled. Metabolites that accounted for ≥10% of circulating radioactivity or ≥10% of dose in expired air or excreta as a component of urine, feces, or a compilation of plasma, expired air, urine, and feces from the human ADME study was compiled. Drugs which showed sparse formation of metabolites or which produced multiple low-abundance (<10%) metabolites were specified as drugs with “no major metabolites” and were accounted for as such.

Data Analyses. The current evaluation is focused on defining the contribution of P450 and non-P450 enzymes to metabolism, not in terms of contribution to CL. The metabolism of the drugs included in the evaluation set focused on the primary biotransformation step, as this transformation and the enzyme system(s) responsible for the reaction(s) are of greatest interest due to their relationship to CL. Assignment of the contribution of a specific enzyme to CL of a drug is, of course, of utmost interest. However, this is not possible for the current evaluation for several reasons. Human ADME metabolite data are reported as static percentages of circulating radioactivity or dose, not in terms of rates of formation. Again, owing to the static nature of the data, other processes, such as reversible metabolism, reabsorption, and transporter-mediated uptake and efflux, are not accounted for. Additionally, for metabolites that are formed by multiple enzymes, the fractional clearance through one particular enzyme cannot be defined using human ADME data alone. Furthermore, an evaluation of metabolism after the initial biotransformation (subsequent metabolism) was considered. However, accounting for these transformations was complicated and, therefore, is not included in this evaluation.

For drugs where one or more major metabolites were observed in plasma or excreta, each metabolite was assigned a designation of “P450” or “non-P450” and assigned 1 point based on the enzyme responsible for the primary step in its formation. For major metabolites where either a P450 or a non-P450 enzyme may be responsible for the primary biotransformation, the major metabolite was divided between P450 and non-P450 (i.e., 0.5 points each). The point totals for P450 and non-P450 were then divided by the total number of major metabolites so that the P450 and non-P450 reactions were fractions that would sum to 1.0. For example, if three major metabolites were determined for drug X, and two were P450-mediated and one was non-P450-mediated, the P450 component of drug X would be 0.67, whereas the non-P450 component would be 0.33, with the total of P450 and non-P450 equaling 1.00.

Using the profile for each drug from the aforementioned analyses, the data were examined in two ways. For the first method (method 1), the drugs were categorized by the enzyme systems involved in their metabolism without fractionation. More specifically, each drug was defined as having no major metabolites or as being metabolized by P450 enzymes, non-P450 enzymes, or metabolized by both P450 and non-P450 enzymes (mixed). A secondary assessment (method 2) used the fractional assignments for the contribution of P450 or non-P450 to each drug. The results of the method 2 assessment were summed for each year from 2006 to 2015, for two 5-year periods (2006–2010 and 2011–2015), and for 2006–2015. Therefore, the relative contributions of P450 and non-P450 enzymes for drugs which fell into the mixed category for method 1 were assigned a fractional assignment, thereby providing greater granularity to the data set.

As a part of this secondary analysis, for metabolites whose primary bio-transformation was determined to be partially or entirely mediated by non-P450 enzymes, the enzyme(s) responsible were assigned and tabulated. Similar to what is described earlier, if multiple enzymes were possibly responsible for a major metabolite, the metabolite was divided equally between the two enzymes. For the sake of simplicity, esterases, amidases, or other hydrolytic enzymes that carried out hydrolytic biotransformations were combined and designated as “hydrolases.” Similarly, biotransformations resulting in reduction of a carbonyl are referred to as “carbonyl reductases” and may be carried out by carbonyl reductase, aldo-keto reductases, or other enzymes which carry out similar biotransformations.

Caveats of the Current Analyses. The primary goal of the current analyses was to evaluate the relative contribution of P450 versus non-P450 enzymes in the metabolism of drugs. The current approach is also a consequence of certain limitations of data availability. As such, there are some caveats worth describing:

- For some drugs, only a portion of the mass balance data was reported or an incomplete description or characterization of the metabolism was disclosed by the sponsor. In these cases, only reported metabolism assignment and percentage data could be included.
- Glucuronide conjugates are generally not stable in feces, and therefore, their contribution to the total metabolism may be underappreciated.
- Multistep oxidations could involve both P450 and non-P450 enzymes, e.g., conversion of a benzyl alcohol to a carboxylic acid. Additional

Additional

<table>
<thead>
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<th>Year</th>
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*Lacosamide, tedizolid phosphate, and isavuconazonium sulfate are administered either orally or intravenously. For simplicity, these drugs were only counted in the oral category.

Table 2. Compilation of metrics for FDA-approved small-molecule drugs 2006–2015.

**Fig. 1.** Enzymes responsible for metabolic bioactivation of the 22 prodrugs approved by the FDA between 2006 and 2015.
characterization of non-P450 enzymes involved may not have been explored or reported.

- Multiple minor metabolites (<10%) formed by the same enzyme or enzyme group (i.e., P450 or non-P450) may additively contribute a major metabolic pathway and will not be appropriately accounted for. Similarly, multiple metabolites resulting from subsequent metabolism of a common intermediate may not be properly attributed.

- The contribution of enzymes to the formation of metabolites which are derived from multiple enzymes may be underestimated for one enzyme and overstated for another. There is no correction factor for the contribution percentage of each enzyme (such as an fm value), and therefore, the responsibility for the formation of the metabolite is divided equally between the enzymes (e.g., for an oxidative metabolite formed by four individual P450s and FMO1, the metabolite would be counted as 50% P450 and 50% non-P450).

- The average development time for a drug is >10 years (DiMasi and Hanse, 2014). Thus, the drugs included in the evaluation represent chemical matter that was in the discovery setting 10–20 years prior. The chemical matter currently being created and evaluated by medicinal chemists and drug metabolism scientists within the discovery setting may be significantly different in terms of the enzymes contributing to its metabolism.

- The majority of ADME studies are carried out as single-dose studies, and therefore, do not represent steady-state levels of metabolites. Thus, the relative abundance of parent and metabolites may be different.

Results and Discussion

As medicinal chemists and drug metabolism scientists more routinely mitigate P450-mediated metabolism, there is an expectation that drug metabolism will shift to non-P450 enzymes and processes (Pryde et al., 2010). Currently, only anecdotal data exist, indicating a movement away from P450-mediated metabolism. Evaluation of metabolism in the discovery phase is made difficult by a lack of access to data across the industry and a lack of definitive data. Additionally, metabolism in the discovery phase is often compound- or scaffold-dependent for a particular drug target. With these challenges in mind, attention was focused on data obtained from more definitive human radiolabeled ADME studies.

Because human ADME data are commonly reported as part of drug applications, drugs approved by the U.S. FDA from 2006 to 2015 were used as the source of drugs included in the evaluation set. Over this time period, 293 medications were approved which, due to combination therapies, included 298 active ingredients (Table 1). The list of approved medications include 62 protein drugs, 6 peptide drugs, and 9 drugs that fall into a category of “other,” which comprises elemental/inorganic drugs, botanical drugs, and polymers. Drugs are by far the largest subset of approved drugs, tallying 221 drugs. Of the small molecule drugs approved, administration through the oral route (156 drugs, 71% of small-molecule drugs) represents the largest subset of these drugs (Table 2). Intravenously administered drugs are the next largest category of small-molecule drugs, representing approximately 16% of all small-molecule approvals. The remainder of small-molecule drugs (30 drugs, 14%) fall under the category of “other routes” of administration, which includes inhalation/nasal spray, topical, subcutaneous, ophthalmic, or intramuscular. Drugs administered via these other routes of administration were removed from the analysis for a few reasons. The metabolism of these drugs is likely to involve a greater contribution of extrahepatic metabolism. Furthermore, concentrations of parent drug and metabolites in circulation and excreta are generally low because of the low doses administered and poor absorption encountered for drugs administered by many of these routes. For several of the applications for drugs in this subset, it is noted that levels of parent drug are extremely low or below the limit of quantitation. Although not included in this evaluation, metabolism data for other routes of administration, where available, may be included in future analyses.

Because the main focus of this work is the metabolism of small-molecule drugs, the current evaluation focused on drugs administered via the two most prevalent routes of administration, intravenous and oral. Imaging agents were excluded from the set of intravenously administered drugs, as they generally have short half-lives and typically do not possess “drug-like” properties. Additionally, prodrugs were removed from the sets of intravenously and orally administered drugs, as these drugs possess functionalities to improve ADME or physicochemical properties and generally require metabolic bioactivation to generate the active drug.

Because ester and phosphate prodrugs are commonly used prodrug approaches (Clas et al., 2014; Wiemer and Wiemer, 2015), it was decided to consider these prodrugs as parent drug for the purpose of this evaluation. Data for drugs meeting acceptance criteria by year are presented as a percentage (A) or by number of drugs (B). Values presented on the top of each column (B) are the total number of drugs which meet acceptance criteria for that year. Categorization was carried out using method 2 as described in the Data Analyses section.

![Fig. 2. Fractional assignment of FDA-approved intravenous and orally administered small-molecule drugs meeting acceptance criteria (2006–2015) which have no major metabolites or whose metabolites are formed by P450, non-P450, or a mix of both P450 and non-P450 enzymes. Categorization was carried out using method 1 as described in the Data Analyses section.](image-url)

![Fig. 3. Fraction of metabolism of approved intravenous and orally administered small-molecule drugs meeting acceptance criteria by year characterized as having no major metabolites or metabolites which are formed by P450 or non-P450 enzymes. Data for drugs meeting acceptance criteria by year are presented as a percentage (A) or by number of drugs (B). Values presented on the top of each column (B) are the total number of drugs which meet acceptance criteria for that year. Categorization was carried out using method 2 as described in the Data Analyses section.](image-url)
thought that the data set would be biased toward the enzymes involved in the bioactivation of the prodrugs (Fig. 1), and that these enzymes would likely differ significantly from the major enzymes evaluated. Despite their removal from the larger set of drugs, data for the primary and subsequent metabolism of prodrugs were compiled. Due to the previously stated reasons, the primary metabolism of prodrugs was evaluated separately. Data for metabolites formed after or in addition to the initial prodrug step are available and may be included in future evaluations. As can be seen in Fig. 1, “hydrolase” enzymes represent 86.4% (19 of 22 drugs) of the bioactivation reactions for prodrugs approved between 2006 and 2015. Activation by kinases, glutathione, or chemical transformation was found for one drug each. Clearly, combining prodrugs with non-prodrugs would significantly bias the larger data set toward hydrolytic enzymes.

After removal of drugs based on the aforementioned criteria, the resulting set of 125 drugs evaluated included 10 intravenously administered drugs and 115 drugs administered orally. The 96 small molecules not included in the set of drugs evaluated were removed because they fell into the following categories: other routes of administration (30), imaging agents (11), prodrugs (22), no ADME study performed (22), or insufficient data available (11). Using “major” metabolites as indicators of route(s) of metabolism, the metabolism of the 125 compounds can be divided into the following categories: no major metabolites (22, 17.6%), P450 only (56, 44.8%), non-P450 only (26, 20.8%), or a mixture of P450- and non-P450-mediated metabolism (21, 16.8%). Based on these values (Fig. 2), P450 enzymes account for the largest percentage of the metabolism of drugs receiving recent FDA approval. However, non-P450 enzymes, alone (20.8%) or in combination with compounds with mixed metabolism (37.6%), represent a substantial portion of the metabolism of these drugs.

A closer examination of the ADME data for the 22 drugs which fell into the category of no major metabolites shows that, for these drugs, ~50% of the dose or greater is observed in the urine (8 drugs) or feces (12 drugs) as unchanged drug. Only two drugs (darunavir and ixabepilone) exhibited minor levels (≤10%) of unchanged parent drug in the urine and feces. For both of these drugs, multiple, low-level metabolites were observed, indicating that metabolism is likely responsible for a significant portion of the CL of these drugs. Together, these data show that only a small portion of drugs in the “no major metabolites” category are significantly metabolized.

In an effort to obtain more detailed data to describe the initial metabolic step for these drugs, the initial biotransformations leading to major metabolites of these drugs were tabulated based on the relative contribution of P450 or non-P450 enzymes. Metabolism data were then grouped and analyzed by year. Figure 3A presents the data for no major metabolites, P450, and non-P450 by year for 2006–2015. The same data are also presented in Fig. 3B in terms of number of drugs per year. The P450 component for all but 3 years (2006, 2007, and 2008) remains at or above 50%. Non-P450 metabolism is also relatively consistent at ~20–35%, with the exception of 2008 (64.6%) and 2010 (not present), where a spike and dip, respectively, were seen in the non-P450 contribution. The percentage of drugs with no major metabolites generally falls between 10 and 25% for the majority of the years analyzed, which represents one to three compounds per year.

Although the aforementioned assessment provides some chronological data for categorization of the biotransformations for approved drugs which meet the assessment criteria, the number of drugs which meet the criteria for inclusion per year is generally small (7–18 drugs). The low numbers of drugs approved per year may result in a more easily biased data set. For this reason, data sets over 5-year periods were also combined, which offers a larger and likely less variable data set for analysis. Data for the 46 drugs approved between 2006 and 2010 which meet the acceptance criteria are presented in Fig. 4A. Likewise, data for drugs approved between 2011 and 2015 (79 drugs) are presented in Fig. 4B. A summary of all 125 drugs evaluated is presented in Fig. 4C. Whether looking at 5-year time periods or the entire 10-year period, metabolism by P450 enzymes represents the largest percentage of metabolism. Comparing the two 5-year groupings indicates that the non-P450 component appears relatively similar at around 30%, and therefore,
the non-P450 contribution over the 10 years is also similar. However, the percentage representing P450 metabolism and no major metabolites varies by >10% between the two 5-year spans of time. For the period of 2006–2010, P450 metabolism accounts for 43.1%, whereas it increases to 58.0% for 2011–2015. For the same two time periods, the no major metabolites component decreases from 28.3 to 11.4%.

The current assessment of the metabolism of drugs approved by the FDA between 2006 and 2015 indicates that P450-mediated metabolism is still the most common route of metabolism of the drugs evaluated (52.5%). Regardless of the method of assessment, metabolism by non-P450 enzymes contributes to a significant portion (~30%) of the metabolism of these drugs. Although not as prominent as metabolism by P450 and non-P450 enzymes, drugs which exhibit no major metabolites represent a nontrivial, but more variable portion of the total.

When looking at the contribution of non-P450 metabolism over time, there is no apparent trend toward increasing contributions of non-P450 enzymes to the metabolism of these drugs. In actuality, the contribution of non-P450 enzymes remains relatively constant over time, with 2 of the 10 years displaying higher (2008) or lower (2010) involvement. As stated earlier, based on current industry timelines for drug development (DiMasi and Hansen, 2014), the current set of drugs likely emerged from drug discovery >10 years ago. Despite this fact, the current data set is the first to provide an evaluation of P450 versus non-P450 metabolism for drugs approved over a 10-year period. This evaluation also provides a starting point for comparison for future evaluations. Because an assessment such as this is not possible in the research setting due to the limitations in data type and access to data, future evaluation of this nature with an ever-increasing data set will be needed to determine if the perceived changes are indeed occurring in the discovery space. Therefore, the results of this evaluation will serve as a database for recurring analyses of the involvement of non-P450 enzymes in the metabolism of emerging drugs. Based on a longer duration, a larger data set, and a greater appreciation of non-P450 reactions, it is expected that future analyses will be more informative.

Figure 5 depicts the individual enzymes and enzyme classes responsible for the non-P450-mediated metabolism. Interestingly, metabolism by non-P450 enzymes is carried out by a relatively limited set of enzymes, with UGTs and hydrolyses being the most dominant, representing 11.7 and 10.8%, respectively. Although the predominance of UGT metabolism is not surprising based on a previous analysis (Williams et al., 2004), the high percentage of metabolism by hydrolyses is somewhat unanticipated, especially in light of the fact that prodrugs have been removed from the evaluation set. The hydrolyase-mediated metabolism of 16 of the 19 drugs involves hydrolysis of an amide bond. Therefore, hydrolytic metabolism, specifically of amide bonds, and the enzymes capable of this metabolism may represent underappreciated metabolic routes in need of greater characterization (Testa and Mayer, 2006). This point has been recently illustrated by the report of the unanticipated ability of AO to catalyze amide hydrolysis of GDC-0834 (V-[3-[6-[4-[[2R]-1,4-dimethyl-3-oxopiperazin-2-yl]anilino]-4-methyl-5-oxopyrrol-2-yl]-2-methylphenyl]-4,5,6,7-tetrahydro-1-benzothiophene-2-carboxamide), resulting in no measurable levels of the parent compound in human circulation (Li et al., 2011; Sodhi et al., 2015).

Recent literature (Pryde et al., 2010; Hutzel et al., 2012, 2013, 2014b; Dalvie and Zientek, 2015) indicates that non-P450 enzymes, such as AO, may represent areas of emerging importance. Increased interest in AO may be due, in part, to the enzyme’s absence in some preclinical species, which has contributed to some well documented drug failures (Dittrich et al., 2002; Diamond et al., 2010; Hutzel et al., 2014a; Lolkema et al., 2015). However, the current evaluation indicates that the contribution of AO to the metabolism of drugs in the set is minor at 1.1%. For other enzymes, increased awareness may be the result of focused efforts on diminishing the involvement of P450-mediated metabolism. All other non-P450 enzymes identified in this evaluation were relatively minor (<1%) and included sulfotransferases (0.8%), cytidine deaminase (0.8%), nucleotidases (0.8%), alcohol/aldhyde dehydrogenase (0.4%), flavin-containing monooxygenases (0.3%), glutathione conjugation (0.3%), gut microbes (0.3%), and undefined/unknown (0.3%). A number of these minor contributing enzymes fall outside of the “usual” drug-metabolizing enzymes and may be the result of the drug targets and target space captured in the evaluation set.

The introduction of novel drug targets and drug-targeting approaches to the drug-discovery environment will likely bring innovative approaches to small-molecule drugs. Whether this will result in an increased role in non-P450 metabolism for drugs of the future is yet to be answered. Additionally, one may also question whether the introduction of enzyme families which are not as well characterized or as commonly encountered in drug metabolism as P450 enzymes will provide benefits or greater challenges to drug discovery and development. Remaining in the area of P450-mediated metabolism may afford an easier discovery and development path, as the enzymes and methods for their evaluation are well developed and widely deployed. Whether medicinal chemists and drug-metabolism scientists can exert sufficient control over the design of drug molecules which engage emerging drug targets and which remain in this well characterized space of P450 metabolism will likely only be answered with more time and greater scrutiny of emerging drugs.

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