Pharmacologically Active Drug Metabolites: Impact on Drug Discovery and Pharmacotherapy

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dx.doi.org/10.1124/pr.111.005439.
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ABBREVIATIONS: CNS, central nervous system; COPD, chronic obstructive pulmonary disease; CSF, cerebrospinal fluid; EM, extensive metabolizer; fu, fraction unbound in plasma; HMGCoA, 3-hydroxy-3-methylglutaryl-coenzyme A; HPLC-MS, high pressure liquid chromatography-mass spectrometry; 5-HT, serotonin; IC50, ligand concentration yielding 50% of the maximum response; Kg, ratio of unbound drug concentration within a tissue to unbound plasma concentration; mCPP, m-chlorophenyl piperazine; MEGX, monoethylglycine xylidide; MS, mass spectrometry; NaV, voltage-gated sodium channel; PDE, phosphodiesterase; PK/PD, pharmacokinetic-pharmacodynamic; PM, poor metabolizer; 1-PP, 1-(2-pyrimidyl)piperazine; PSA, polar surface area; SAR, structure-activity relationship.
Abstract—Metabolism represents the most prevalent mechanism for drug clearance. Many drugs are converted to metabolites that can retain the intrinsic affinity of the parent drug for the pharmacological target. Drug metabolism redox reactions such as heteroatom dealkylations, hydroxylations, heteroatom oxygenations, reductions, and dehydrogenations can yield active metabolites, and in rare cases even conjugation reactions can yield an active metabolite. To understand the contribution of an active metabolite to efficacy relative to the contribution of the parent drug, the target affinity, functional activity, plasma protein binding, membrane permeability, and pharmacokinetics of the active metabolite and parent drug must be known. Underlying pharmacokinetic principles and clearance concepts are used to describe the dispositional behavior of metabolites in vivo. A method to rapidly identify active metabolites in drug research is described. Finally, over 100 examples of drugs with active metabolites are discussed with regard to the importance of the metabolite(s) in efficacy and safety.

I. Introduction

Drugs are cleared from the human body by three main mechanisms: renal excretion of unchanged drug, biliary excretion of unchanged drug followed by egestion in feces, and metabolism (there are also other rarer mechanisms of clearance). In the case of metabolic clearance, a drug is converted to new chemicals, i.e., metabolites. In the vast majority of cases, metabolites have undergone enough chemical change from the parent drug that their capabilities to bind to the target macromolecule are greatly diminished or abolished altogether. However, in some cases a metabolite can retain enough intrinsic activity at the target receptor such that it can contribute to the in vivo pharmacological effect(s) to a meaningful extent. (The term “intrinsic” is used throughout this paper to define the binding to the target in the absence of any of the other factors that occur in a more complex system such as a whole organism, tissue, or in vitro cellular assay.) Since active metabolites contribute to effect, they must be understood to the
same extent as the parent drug with regard to their dispositional properties (pharmacokinetics, distribution, and clearance mechanisms). A metabolite that possesses intrinsic activity at the target receptor in an in vitro assay may or may not contribute to activity in vivo. The exposure of the target receptor to the metabolite, which is dictated by several factors, such as the rate and extent of its generation, rate of its subsequent clearance, target tissue penetration, and free fraction, must be understood for the metabolite as much as it is for the parent drug to ascertain whether the metabolite is important. This is critical in defining the proper dose needed for efficacy and the causes of interindividual variability in the dose-concentration-effect relationship.

This is distinct from the case of prodrugs. In the case of prodrugs, the parent drug is not pharmacologically active itself, but it is converted into a drug, which essentially is an “active metabolite” when administered in this fashion. The metabolite possesses all the pharmacological activity, the parent none. Prodrugs are usually designed to address some dispositional flaw of an intrinsically active compound (e.g., poor absorption, short half-life). Some drugs that have active metabolites could be considered “accidental prodrugs.” Several older drugs that were discovered through the use of animal models, and not through the use of biochemical screens, showed activity by virtue of a biochemically inert dosed compound being converted into an active metabolite that exhibited all the effect. Minoxidil could be considered an example of this, wherein its hypotensive effect was described in

Fig. 1. Schematic illustrating drug and metabolite binding to a target receptor. In case (A), the drug, represented as possessing a hydrophobic region, a hydrogen bonding region, and a solvent-exposed region, is shown binding to the receptor with points of interaction between atoms on each. (HA and HD refer to hydrogen bond acceptor and donors, respectively.) In case (B), the metabolic modification, represented by the triangle, does not affect these interactions, so the metabolite would be active. In case (C) the metabolic modification occurs on a position not involved in binding, so the metabolite would be active. In (D), the metabolic modification is on a position that disrupts interaction, so the metabolite would be inactive. Such disruptions can include introduction of a polar or ionic substituent, or a substituent that provides steric bulk.

Fig. 2. Example illustrating formation rate–limiting and elimination rate–limiting kinetics for metabolites. In this example, plasma concentrations of the parent drug and two metabolites are measured following intravenous administration of parent drug. Metabolite 1 demonstrates formation rate–limiting kinetics since its $t_{1/2}$ is the same as the parent drug. Metabolite 2 demonstrates elimination rate–limiting kinetics because its $t_{1/2}$ is longer than that of the parent drug.
animals (Pluss et al., 1972) but the effect in humans would be due entirely to the generation of minoxidil sulfate, a potent ligand of the ATP-dependent potassium channel (Buhl, et al., 1990; Messenger and Rundegren, 2004). A discussion of prodrugs and their active metabolites is beyond the scope of this article.

Over the past decade a great deal of attention has been focused on the assessment of the safety of drug metabolites (the so-called “MIST” or Metabolites in Safety Testing issue; Baillie et al., 2002). In this issue, the focus was on whether laboratory animals used in safety assessments of new drug candidates are

<table>
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adequately exposed to metabolites to which humans are exposed, irrespective of known target receptor activity. Following this, regulatory guidance was issued stating that metabolites of potential interest need to be present in humans at a predefined threshold either relative to the total drug-related material or relative to the parent drug [Food and Drug Administration, 2012 (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM079266.pdf); International Conference on Harmonization, 2012 (http://www.ich.org/products/guidelines/multidisciplinary/article/multidisciplinary-guidelines.html)]. If such a threshold is exceeded, then it must be demonstrated that the species used in toxicology tests were also exposed to the same metabolites to an extent greater than humans. The focus of the guidance is on stable human metabolites present in circulation, presumably because all organs would be exposed to such metabolites via circulation. (This is in spite of the fact that many metabolites that are responsible for toxicity are chemically reactive and are not necessarily stable enough to be present in circulation.) Off-target pharmacological activity that yields safety concerns is also beyond the scope of this article, with the exception of those metabolites that can contribute to safety issues arising from exacerbated target pharmacology. For example, while the right level of activity at opioid receptors yields the beneficial effect of pain reduction, excessive activity can cause respiratory depression. Thus, if there is a metabolite active at this receptor, it must be considered when relating the exposure to the safety effect.

In this review, pharmacologically active metabolites will be discussed with regard to drug research and development. Some pharmacokinetic concepts will be reviewed as they relate to the special case of metabolites (Houston, 1981; Pang, 1985). The types of biotransformation reactions that can give rise to pharmacologically active metabolites will be described in the context of the impact that various chemical modifications can have on receptor/enzyme binding. Experimental approaches that can be applied at different stages of new drug research to detect active metabolites and understand their impact will be discussed. Finally, these principles will be illustrated using examples, along with a description of drugs with well-established active metabolites in humans. The drugs/metabolites described were identified in a SciFinder search on the term "active metabolite," and following exclusion of prodrugs and drugs of abuse, the literature for the remaining set was scrutinized for some activity is observed at ~25 minutes. Structures for these metabolites can be proposed from the MS data. Authentic standards of the active metabolites can then be prepared by synthetic or biosynthetic methods for determination of potency and other pharmacological activities.
Fig. 4. Application of the activity-gram approach to finding active metabolites in humans. In this example, pooled human plasma samples from an early phase 1 clinical study were extracted and analyzed by HPLC-UV-MS. Fractions were collected and tested for binding in an in vitro pharmacological binding assay. In addition to the binding activity expected at the retention time for the parent drug (25–26 minutes), there was strong binding activity at 21–23 minutes and some activity at 19 minutes too. Thus, in human plasma, target activity is caused by not only the parent drug, but metabolites as well. The structure of the major metabolite at 21 minutes was proposed from the mass spectral data, and an authentic standard prepared for determination of intrinsic potency. Potency was comparable to the parent drug.
specific information on the putative active metabolites. It should be noted that our level of thoroughness and sophistication with regard to the identification and characterization of active metabolites is much greater than it was in the past. Thus, the depth of our knowledge regarding active metabolites for older drugs may not be as developed as for more recently introduced drugs. For example, in a review on this topic from over 30 years ago (that had a special emphasis on the increased exposure and effect of active metabolites in renal insufficiency), a list of over 50 drugs with active metabolites was included (Drayer, 1976), although some were prodrugs. Considerable knowledge has been gained since that time. In fact, active metabolites have been proposed to be potential new material for new drug discovery and development (Kang et al., 2010). Certainly the presence of drugs used clinically that were once metabolites (e.g., fexofenadine) or are prodrugs of these metabolites (fesoterodine) is a testament to this notion (Table 2).

II. Absorption, Distribution, Metabolism, and Excretion Aspects of Active Metabolites

To appreciate the potential impact that pharmacologically active metabolites can have on clinical efficacy, some basic concepts regarding the dispositional aspects of metabolites must be understood. In this section, the metabolic reactions that can give rise to active metabolites are discussed followed by discussions of distributional aspects and pharmacokinetic phenomena.

A. Metabolic Reactions That Can Yield Active Metabolites

In order for a metabolite of a drug to retain binding activity to the same receptor to which the drug binds, the chemical modification must be as follows: 1) so minor as to not disrupt the atom-to-atom interactions that occur between the drug and the receptor, or 2) occur on a position on the drug that is not oriented toward the receptor when binding occurs. This is illustrated in the schematic in Fig. 1. Most metabolic transformations provide enough of a chemical change that receptor potency is lost or greatly diminished. Furthermore, many metabolic reactions introduce enough hydrophilicity (by increasing the number of hydrogen bonding substituents, charged entities, and/or size) and increased polar surface area that metabolites are pharmacologically inactive by virtue of being unable to penetrate the membranes of target tissues. However, in some cases, pharmacological activity can be retained in a metabolite, while in other cases affinity for the target can be diminished but pharmacological activity retained due to high concentrations of the metabolite.

Among xenobiotic metabolizing enzymes, the cytochrome P450 family is the most important. These enzymes can catalyze a wide array of reaction types, and the specific biotransformation reactions that occur
for any given drug depend on the substituents present in that drug (Guengerich, 2001). These include aliphatic and aromatic hydroxylations, heteroatom dealkylations, N- and S-oxygenations, dehydrogenations, and epoxidations. In the vast majority of examples, active metabolites arise by one of these reactions. For example, among drugs active at neurotransmitter targets that contain a basic 2°- or 3°-aliphatic nitrogen, it is frequently the case that N-demethylated and deethylated metabolites will also possess activity. The basicity of the amine, an important attribute for these drugs, is retained or even somewhat increased. Thus, drugs such as fluoxetine, amitriptyline, imipramine, fenfluramine, citalpram, ketamine, mianserin, and many others have active N-dealkyl metabolites. Whether activity is exhibited in vivo depends on the pharmacokinetic and dispositional attributes of the metabolite.
In the case of hydroxylations, the likelihood that a metabolite is pharmacologically active is less predictable. The addition of a single alcohol oxygen adds approximately 20 Å² in polar surface area (PSA) as well as hydrogen-bond–donating potential to a position where none existed in the parent structure. Such an alteration can frequently yield an inactive metabolite if the target binding site cannot tolerate such a substituent. However, in some cases the addition of –OH can occur at a position that is not critical for target binding, or faces the solvent in the protein-ligand complex. In those cases, the metabolite can retain intrinsic activity, and its potential to contribute to in vivo activity resides with other properties, such as in vivo exposure and target tissue penetrability. It is not uncommon that alcohol and phenol metabolites are rapidly cleared by conjugation reactions. It is also frequently the case that alcohol and phenol metabolites do not as readily penetrate target tissues, due to a decrease in membrane penetrability caused by the

![Fig. 11. Metabolism of clomiphene to its hydroxylated and demethylated metabolites.](image)

![Fig. 12. Metabolism of codeine to morphine.](image)
increased PSA. Nevertheless, there are several good examples of active hydroxyl metabolites and these are described in Section IV (e.g., risperidone/paliperidone, saxagliptin/5-hydroxsaxagliptan, flutamide/2-hydroxyflutamide, etc). Other reactions shown to yield active metabolites include epoxidation (e.g., carbamazepine/carbamazepine-10,11-epoxide), reduction (dolasetron/hydrodolasetron, buproprion/dihydrobupropion stereoisomers, others), and heteroatom oxygenation (thioridazine/mesoridazine, roflumilast/roflumilast N-oxide).

Other types of drug metabolism reactions usually do not yield active metabolites, with some exceptions. Heteroatom dealkylations that result in loss of a large substituent usually inactivate a drug but there are exceptions [buspirone/1-(2-pyrimidyl)-piperazine (1-PP); nefazodone/m-chlorophenyl piperazine (mCPP)]. However in such cases the pharmacological properties of the metabolite may be different from the parent (e.g., binds to a different member of the same receptor family). Conjugation reactions that add glucuronic acid, sulfuric acid, amino acid, and glutathione generally do not yield active metabolites (Mulder, 1992a). The most well known exception to this is the case of morphine-6-glucuronide, putatively an active metabolite of morphine (Mulder, 1992b; Kilpatrick and Smith, 2005). 4-Hydroxytriamterene sulfate represents an active sulfate conjugate metabolite of triamterene. The metabolite has nominally lower intrinsic potency and a lower free fraction; however, its plasma concentrations are well in excess of parent and thus it contributes to effect (Busch et al., 1996).

B. Pharmacokinetic Aspects of Active Metabolites

When compounds are administered directly, the description of pharmacokinetics is relatively straightforward. However, when a compound arises from within the organism, such as an active metabolite, the picture can be more complex (Pang, 1985; St. Pierre et al., 1988). This is primarily due to the fact that the metabolite represents an unknown fraction of the dose of the parent (i.e., dose is not truly known which is needed for calculation of fundamental pharmacokinetic parameters) and the rate of introduction into the body is not easily discerned.

From a pharmacokinetic standpoint, metabolites (active and inactive) are frequently referred to as to having formation rate–limiting kinetics or elimination rate–limiting kinetics (Houston, 1981). A metabolite cannot have an elimination rate that exceeds the elimination rate of the parent drug from which it is formed. This is referred to as formation rate–limiting
kinetics (Fig. 2). If an experiment is done wherein the metabolite is administered directly, then its $t_{1/2}$ may be shorter than when it is generated after administration of the parent drug. Many metabolites exhibit this type of behavior by virtue of having higher clearances and/or lower volumes of distribution than the parent drug. In elimination rate–limiting kinetics, the $t_{1/2}$ of the metabolite is longer than that of the parent drug (Fig. 2), and this value will be the same irrespective of whether the metabolite arises after administration of the parent drug or whether it is administered directly. If a metabolite exhibits elimination rate–limiting kinetics, then with repeated administration of parent drug the metabolite has the potential to accumulate to a greater extent than the parent drug at steady state.

The factors that drive the total exposure to the metabolite include the clearance rate of the parent drug, the fraction of the dose of the parent drug that is converted to the metabolite, and the subsequent clearance rate of the metabolite. The latter term can also impact the amount of the metabolite formed that escapes from the tissue from which it is formed and gets into the systemic circulation. In the vast majority of cases, the focus can be on the liver as the main site of metabolite formation. When considering an active metabolite, an important metric in understanding the potential contribution of the metabolite to effect relative to the parent drug is the area-under-the-curve (AUC) ratio. This can be defined as:

$$\frac{AUC_{\text{metabolite}}}{AUC_{\text{parent}}} = \frac{f_{CL,m} \cdot F_m \cdot CL_{\text{parent}}}{CL_{\text{metabolite}}}$$

The terms are defined as follows: $f_{CL,m}$ is the fraction of the clearance of the parent drug that yields the metabolite, $F_m$ is the portion of the total metabolite generated within an organ that is released into the systemic circulation before it is either further metabolized or released into bile (in the case of the liver) or urine (in the case of the kidney), $CL_{\text{parent}}$ is the total clearance of the parent drug, and $CL_{\text{metabolite}}$ is the total clearance of the metabolite. It is rarely the case that these individual parameters are measured, especially in humans, since they require the intravenous administration of the metabolite directly. However, they offer conceptual insight as to the determinants of metabolite exposure. Prediction of metabolite exposure is a challenging undertaking when considering the number of contributing variables. Yet the importance of active metabolites (as well as those that could cause deleterious off-target effects) in drug research and clinical practice makes research into methods that can predict human metabolite exposures from in vitro and/or animal data an important endeavor (Lutz et al., 2010; Yeung et al., 2011; Lutz and Isoherranen, 2012; Smith and Dalvie, 2012).

C. Distributional Aspects of Active Metabolites in Relation to Contribution to Pharmacological Effect

The potential for the active metabolite to penetrate the target tissue relative to the parent drug is also necessary to understand whether the metabolite is truly an active metabolite. Even if the metabolite possesses intrinsic potency that is equivalent to the parent, if the free concentration within the tissue is lower than that of the parent, the metabolite will not contribute as much as the parent. The main drivers behind the target-tissue free concentrations include plasma protein binding, membrane penetrability, and the potential for active transporters to alter the free tissue–to–free plasma concentration ratio ($K_{p,uu}$).

Distributional phenomena are largely driven by high capacity, low affinity nonspecific binding interactions between drugs and tissue macromolecules, and macromolecular structures (e.g., phospholipid membranes). Such interactions are mostly a function of physicochemical properties of the drug, especially lipophilicity and ionization state. Greater lipophilicity and greater cationic character are properties that tend to increase nonspecific binding to tissue macromolecular structures. Since a majority of biotransformation reactions result in decreases in lipophilicity, metabolites tend not to partition into tissues as well as their parent drugs. An example of an exception to this is ketoconazole, which undergoes deacetylation thereby converting a neutral amide to a basic amine, and in this case it is the cationic metabolite that is better at binding to membranes (Whitehouse et al., 1994).
Membrane penetrability is also frequently a function of PSA, increases in which tend to debilitate penetrability. Since almost all biotransformation reaction types result in increases in PSA, metabolites will tend to be less membrane permeable than their respective parent drugs. If enough hydrophilicity is introduced, the metabolite could be completely membrane impermeable. If a metabolite is still membrane permeable, but of lower permeability than the parent drug, then the result is that it will take longer for the metabolite to achieve a steady-state exposure in the tissues than the parent drug. Increased hydrophilicity also renders metabolites better substrates for several of the drug efflux transport proteins, which also serves to decrease the free tissue concentrations of metabolites relative to parent drugs.

The effects of decreased tissue partitioning and penetration can be offset by differences in plasma protein binding between parent drugs and their metabolites. Metabolites are frequently less protein-bound in plasma than their respective parent drugs, presumably due to their decreased hydrophobicity (Table 1). Thus, the potential decreases in membrane permeability and \( K_{pu,uu} \) of metabolites relative to their parent drugs can be offset by increases in free fraction. A potentially challenging situation for understanding the relative importance of metabolites to pharmacological effect occurs when the metabolite is generated within the target tissue. For example, the hydroxyl metabolites of atorvastatin have potency at the pharmacological target. They are generated by CYP3A within the liver, which is the target tissue for inhibition of cholesterol biosynthesis. Due to this, the free concentrations within hepatocytes may not be reflected by systemic free plasma concentrations, and thus a true knowledge of the contribution of the metabolites to clinical effect would be unattainable. Estimates would need to be made from in vitro data or data from laboratory animals.

### III. Active Metabolites in Drug Research

For the purposes of this discussion, the process by which new drugs are created can be generally broken down into two major portions: 1) the preclinical phase, in which medicinal chemists and pharmacologists collaborate, along with other related discipline scientists, to identify a new molecular target and design ligands that can bind and affect the activity of that target (termed “drug discovery”), and 2) the clinical
phase, wherein the selected molecule is studied first in healthy volunteers (phase 1) and then in patients for efficacy and safety (“drug development”). The impact of pharmacologically active metabolites in these two phases is different. However, it is critical to account for active metabolites in drug research as early as possible, as the insights that can be gleaned regarding target structure-activity relationship (SAR), pharmacokinetic-pharmacodynamic (PK/PD) relationships, and toxicity are invaluable (Fura, 2006).

A. Research Tools in Metabolite Identification

A lengthy discussion of how drug metabolites are identified is beyond the scope of this review and the interested reader is referred to other descriptions of this topic for greater depth (Prakash et al., 2007; Zhang and Comezoglu, 2007). However, to understand the issue of pharmacologically active metabolites, a very brief description of how metabolites are identified is necessary.

In the early efforts (preclinical), the identification of metabolites of new compounds is done using incubates from in vitro liver-derived systems such as hepatocytes and microsomes, as well as various biologic fluids (e.g., plasma, urine, bile) obtained from laboratory animal species following administration of the compound of interest. Identification of metabolites in the drug discovery phase is done for a variety of reasons, including 1) the identification of specific sites of modification on a rapidly metabolized molecule so that drug design efforts can slow down metabolism, 2) identification of the main routes of metabolism and enzymes involved so that the overall clearance mechanism in humans can be understood and predicted, 3) identification of the possibility of generation of chemically reactive metabolite types associated with toxicity, and, relevant to this article, 4) the identification of pharmacologically active metabolites. In the early stages of drug research, considerable effort is expended through the use of animal models of disease to understand the biologic effects of manipulating the underlying pharmacological target of interest. Developing the relationship between the drug concentration and the effect (i.e., the pharmacokinetic/pharmacodynamic relationship) will offer insight into the duration and degree to which the target receptor needs to be occupied for effect. The presence of an active metabolite will alter that relationship, and if an active metabolite is present but unaccounted for, the pharmacokinetic/pharmacodynamic relationship will be misinterpreted.

In the clinical stage, the same questions regarding establishing the PK/PD relationship exist, albeit in human patients rather than animal disease models, and unlike the earlier work the efforts are focused on one or maybe two compounds for any given target. In early phase 1 studies, plasma and urine samples from human study subjects can be used to seek metabolites. Later in clinical development, it is typical practice to carry out a study wherein humans are administered a radiolabeled drug and excreta and plasma are thoroughly interrogated for a complete and comprehensive picture of the metabolism of the new drug (Penner et al., 2012).

The main tool used by the drug metabolism scientist in metabolite identification is high-pressure liquid chromatography-mass spectrometry (HPLC-MS). HPLC-MS provides information on the molecular weight of the metabolite (so it can be compared with the molecular weight of the parent compound) to discern the type of metabolic modification, and the fragmentation of the metabolite in the MS offers insight into the structure of the metabolite. While MS data can sometimes yield enough information to assign the chemical structure of a metabolite, it is frequently the case that the information is not enough to propose a precise structure. In those cases, additional data using complementary methods is required, such as NMR spectroscopy, chemical derivatization, and/or synthesis of an authentic standard of the metabolite for comparison.

B. Identification of Active Metabolites in Preclinical Research

As stated above, knowledge of pharmacologically active metabolites in animal models of disease is important. Furthermore, the prediction that humans may also be exposed to pharmacologically active
metabolites is important in predicting the efficacious dose prior to human studies. In the preclinical stage, efforts are ongoing to develop knowledge of the SAR for receptor binding potency, and the identification of an active metabolite can offer the medicinal chemist greater insight as to the types of substituents that can be tolerated in the ligand-receptor interaction. Finally, an active metabolite could be a better compound to develop than the drug itself (e.g., terfenadine versus fexofenadine).

A sequential approach to identifying metabolites in biologic matrices—proposing chemical structures of these metabolites, followed by organic synthesis and testing of the metabolite at the target receptor—represents one strategy for identifying active metabolites in preclinical drug research. Some SAR insight may already be known for a chemical series such that proposed metabolite structures could be ruled out from having pharmacological activity in the absence of data. For instance, N-demethylation of a tertiary amine drug to a secondary amine metabolite is frequently anticipated to have minimal impact on target receptor activity, thus it is prudent to establish this potential activity experimentally. However, N-deamination of an amine drug followed by oxidation to the carboxylic acid metabolite is much less likely to yield an active metabolite, due to the marked difference in structure and charge.

An approach we have employed in our laboratories to address the possibility of pharmacologically active metabolites involves a close collaboration between the drug metabolism scientist and pharmacologist (see Fig. 3 and Fura et al., 2004). The drug metabolism scientist carries out an analysis of a biologic sample (in vivo or in vitro) for metabolite identification on HPLC-MS as usual. However, the HPLC eluent is split prior to introduction into the MS. A small portion is introduced to the MS to yield spectral data. The remaining portion is diverted to a fraction collector. The fractions are delivered to the pharmacologist for testing activity at the target receptor. The HPLC-MS chromatogram is compared with the receptor activity profile of the fractions to create an “activitygram.” If receptor activity is detected in fractions corresponding to where a metabolite eluted, then the metabolite is potentially an active metabolite. Since the amount of the metabolite in the fraction is unknown, an absolute potency value cannot be determined. This merely offers an early signal regarding whether an active metabolite is even possible, and if so, then synthesis of an authentic standard of that metabolite for testing can be undertaken. If the metabolite poses a synthetic challenge, it can still be addressed by generating a biologic sample containing the metabolite, purifying it by HPLC, determining the concentration of the metabolite using proton NMR (Walker et al., 2011; Vishwanathan et al., 2009), and using that solution as a concentrated stock solution from which to dilute the metabolite into the activity assay.

C. Identification of Active Metabolites in Clinical Research

As humans are the target species, knowledge of active metabolites in human is essential to proper dose selection and understanding determinants of interpatient variability in drug response. By the time of administration to humans in phase 1 studies, the presence (or not) of active metabolites in laboratory
animals will have already been determined. In addition, human in vitro systems will have been used to predict the metabolites that will be present in vivo. Thus, a good understanding of the potential for active metabolites in humans should already have been obtained. Nevertheless, it is still possible for an active metabolite to be revealed for the first time in a clinical study.

From animal models, a prediction of the human concentration-effect relationship can be made (either for a biomarker or an effect on disease). If that relationship does not hold upon first examination in human (either the magnitude or duration of effect, or both), then the possibility of pharmacologically active metabolites must be entertained. The same type of approach described above can be performed using human plasma samples. An example of this is illustrated in Fig. 4 (Hagen et al., 2008). In this instance, the parent drug was targeting a subtype of the serotonin receptor family. During the phase 1 dose escalation study, suprapharmacological effects were observed at parent drug concentrations that were far lower than anticipated. Plasma samples that had already been used for quantitative analysis of the

![Fig. 26. Metabolism of remacemide to desglycylremacemide.](image)

![Fig. 27. Metabolism of risperidone to paliperidone.](image)

![Fig. 28. Metabolism of roflumilast to roflumilast-N-oxide.](image)

![Fig. 29. Metabolism of sibutramine to its N-demethylated metabolites.](image)
parent drug were subsequently used in the “activitygram” approach, and the presence of two peaks of high receptor binding activity was observed, with one activity peak corresponding to the retention time of the parent drug and an earlier eluting peak corresponding to where a hydroxylated metabolite eluted. This showed the likelihood of a circulating active metabolite that could explain the clinical observations. Subsequent structure elucidation of the metabolite was done, and synthesis of an authentic standard showed that the metabolite had intrinsic binding potency equivalent to the parent drug.

In addition to those instances wherein pharmacodynamic observations trigger an aggressive search for pharmacologically active metabolites, the determination of the metabolites present in human plasma and excreta is done for other purposes, such as gaining an understanding of the important clearance pathways and determining whether human metabolites are also observed in laboratory animal species that are used to explore the toxicology of the drug. As those metabolites are structurally identified, a judgment can be made regarding whether they would potentially be active, based on the known structure-activity relationship for the target receptor. Even if not contributing substantially to the efficacy of the drug, discovering a new active metabolite in clinical samples can potentially offer a new lead for a second-generation drug.

D. Data Needed for Active Metabolites

Once a metabolite is identified that has intrinsic binding potency to the target receptor, the following information needs to be gathered to properly characterize it:

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Fig. 30. Metabolism of tamoxifen to its hydroxylated and N-demethylated metabolites.
1. In addition to target binding potency, functional activity measurement is needed (antagonist, agonist, partial agonist, inhibitor, activator, etc.).
2. Plasma protein binding in humans and laboratory animal species.
3. Penetration into the target tissue(s) and prediction of the free tissue–to–free plasma concentration ratio (i.e., $K_{p,uu}$).
4. Pharmacokinetics in humans and laboratory animal species, and the underlying clearance pathways for the active metabolite in humans.

Essentially, this set of information reflects the same set that is needed to understand the pharmacology of the parent drug. For example, if an active metabolite is responsible for the majority of the pharmacological effect, knowledge of what can cause a change in the exposure to the metabolite (to either increase or decrease it) must be known. Drug interactions that alter the exposure to an active metabolite must be known. For example, tamoxifen is converted to the more potent metabolite endoxifen by CYP2D6, so the concomitant use of potent CYP2D6 inhibitors in patients receiving tamoxifen should be avoided (Mannheimer and Eliasson, 2010).

### IV. Drugs with Active Metabolites

In this section, a description of numerous examples of drugs with active metabolites is included. The molecular targets, indications, and metabolic reaction types span a broad array, indicating that there is no single complement of circumstances that favor or disfavor the possibility of an active metabolite phenomenon. As described in section III.D, the information needed to truly assess the contribution of an active metabolite to clinical effect is challenging and requires knowledge of intrinsic receptor potency, free fraction, target tissue penetrability, and plasma pharmacokinetics. It is rare that all of these properties are known for metabolites (especially plasma protein binding and target tissue penetrability), so in many cases inferences must be made and assumptions accepted when making such projections. Additionally, it is not uncommon that the reported intrinsic potency values measured for active metabolites may have been...
made using nonhuman-derived reagents and tissues, so it must be assumed that relative potency values between parent and metabolite(s) are similar across species. From the assessments of these examples, it is clear that in many cases there is considerable data gathering that needs to be done to fully understand the action of active metabolites. Many of the estimates of contributions of metabolites to efficacy relative to the parent drug described below were made using the properties listed above that were obtained from the scientific literature.

The examples have been divided into four categories: 1) active metabolites wherein the metabolite contributes the majority of the activity, even though the parent drug has intrinsic target potency; 2) active metabolites that contribute to target activity at a level comparable to the parent drug; 3) active metabolites that possess target affinity but would be estimated to contribute little to in vivo effect, relative to the parent; and 4) metabolites that have activity at alternate pharmacological targets closely related to the intended target that the parent drug interacts with, and putatively contribute to clinical effect via this alternate target. Section IV.E describes a few examples wherein the delineation of metabolite and parent contributions is highly ambiguous.

A. Drugs with Active Metabolites That Dominate the Activity

In some instances, while the parent drug possesses affinity for the intended target protein, a metabolite can be present that actually dominates the in vivo effect. Such cases are not truly prodrugs, since the parent drug has target activity, but their behavior can resemble those of prodrugs.

1. Albendazole. Albendazole offers an interesting example of site-dependent effects of parent drug versus its S-oxide metabolite (Fig. 5). Albendazole is an anthelmintic agent used in the treatment of various parasitic diseases and its mechanism of action is through the binding of tubulin in nematodes. The parent is about 6-fold more intrinsically potent at tubulin binding than the S-oxide metabolite (Lubega and Prichard, 1991). For systemic parasitic infection it is the metabolite that is believed to exhibit the effect because metabolite plasma concentrations achieved are in the range of 3 \mu M while the parent drug is below the limit of quantitation of plasma assays (Jung et al., 1992; Medina et al., 1999; Mirfazaelian et al., 2003). It can be used in the treatment of neurocysticercosis because of the brain penetrability of the S-oxide metabolite. However, for parasitic infections of the gastrointestinal tract, it is likely the parent drug has the effect since the S-oxide is generated postabsorption.

2. Allopurinol. Allopurinol is oxidized to oxypurinol by aldehyde oxidase (Fig. 6). Oxypurinol is approximately 10-times less potent an inhibitor of the target enzyme, xanthine oxidase (Tamta et al., 2006); however, the circulating concentrations of oxypurinol in humans following allopurinol administration are far greater and sustained for a longer time. Maximal concentrations of oxypurinol are about 4- to 5-fold greater, and the half-life of parent and metabolite are 1 and 20 hours, respectively (Turnheim et al., 1999; Day et al., 2007). Thus, the contribution of oxypurinol to xanthine oxidase inhibition activity dominates at later timepoints.
3. Artesunate. Artesunate is an ester-containing derivative containing a hydroperoxide that is believed to form toxic adducts with heme as hemoglobin is being digested by the *Plasmodium* species that causes malaria (Creek et al., 2008). It is more water-soluble than other artemisinin derivatives and thus offers multiple dosing approaches. Upon oral administration it is entirely cleaved to dihydroartemisinin, an active metabolite (Fig. 7), but after intravenous administration both parent drug and the active metabolite are present in circulation (Nealon et al., 2002; Morris et al., 2011). The intrinsic potency of the metabolite is approximately 4-fold greater than the parent (Creek et al., 2008), and after correction for differences in plasma free fraction and plasma concentrations, it can be estimated that the metabolite contributes about 4-fold greater activity than the parent.

4. Astemizole. Astemizole is a histamine-1 receptor antagonist that was withdrawn from clinical use due to occurrence of cardiac arrhythmia caused by blockade of the human ether-a-go-go-related gene potassium channel. It has been described to be converted to O-desmethyldastemizole (Fig. 8) which has a much longer $t_{1/2}$ than astemizole (Heykants, 1984). Detailed information on the human H$_1$ receptor potency of this metabolite is not available in the literature, but it has been described as being equipotent (Richards et al., 1984). It can thus be anticipated that the metabolite would contribute the majority of the pharmacological activity, especially upon accumulation at steady state. Other metabolites, such as 6-hydroxydastemizole, have been shown to have activity in guinea pig-derived models of antihistamine, but this may be due to alterations in histamine release (Kamei et al., 1991).
5. Buspirone. Buspirone is an anxiolytic agent purported to act via binding to the 5-HT1A (serotonin) receptor. It has long been known to undergo N-dealkylation to 1-(2-pyrimidyl)piperazine (1-PP), which has weak activity at the 5-HT1A receptor, and that this metabolite could also play a role in the effect of buspirone (Fig. 9). However, in a clinical pharmacokinetic study, it was shown that 6-hydroxybuspirone is present at much greater concentrations than buspirone and may contribute to activity (Dockens et al., 2006; Wong et al., 2007). If it is assumed that the free fraction of this metabolite is equivalent to buspirone itself, then it can be estimated that the metabolite contributes seven times the activity of the parent. Reports in the literature on the process of making 6-hydroxybuspirone on a large scale suggest that there is interest in this metabolite as a potential drug itself. Investigation of 1-PP as an active metabolite has revealed that it also could contribute considerably to the action of buspirone by virtue of higher exposure. In addition, 1-PP has activity at the α2 receptor (Caccia et al., 1986), the role of which is undefined.

6. Chlordiazepoxide. Chlordiazepoxide generates several active metabolites via sequential demethylation, deamination, reduction, and hydroxylation reactions (Fig. 10). Which metabolite contributes the greatest activity cannot be discerned, but it is clear that metabolites, especially desmethyldiazepam, contribute to activity. The affinities of the entities with the N-O bond (chlordiazepoxide, norchlordiazepoxide, and demoxapam) for the benzodiazepine receptor are 10- to 20-fold lower than the desmethyldiazepam and oxazepam metabolites (Richelson et al., 1991). Concentrations of oxazepam after chlordiazepoxide were not available, but unbound concentrations of desmethyldiazepam were comparable to its binding affinity (Dixon et al., 1976; Boxenbaum et al., 1977; Greenblatt et al., 1978; Ito et al., 1997).

7. Clomiphene. Similar to tamoxifen (section IV. A26.), clomiphene is metabolized by hydroxylation at the para position of one of the phenyl rings by CYP2D6 to yield a metabolite, 4-hydroxyclomiphene (Fig. 11), that has two orders of magnitude greater potency at binding to the estrogen receptor (Ruenitz et al., 1983; Mürdter et al., 2012). Thus, CYP2D6 extensive-metabolizer (EM) and poor-metabolizer (PM) subjects exhibit different responses, with the EM subjects expected to enjoy greater efficacy due to 7-fold greater exposure (Mürdter et al., 2012). Clomiphene also undergoes N-deethylation, but N-desethylclomiphene has similarly low activity to the parent drug. The 4-hydroxy metabolite of N-desethylclomiphene also has activity and would be estimated to contribute 50-fold more than the parent drug. Furthermore, the picture is complicated by the fact that the drug is administered as two geometric isomers, with the E-isomers possessing greater activity.

8. Codeine. Codeine is converted to the active O-demethylated metabolite morphine by CYP2D6 (Fig. 12). After oral administration, the exposure to codeine is almost 40-fold greater than that of morphine (Quiding et al., 1986). However, the potency of morphine at the μ-opioid receptor is 600-fold greater (Volpe et al., 2011), thus the pharmacological effects of codeine administration are almost exclusively attributable to the active metabolite morphine (and its subsequent pharmacologically active glucuronide metabolites; see Section IV.B.15.). In CYP2D6 poor metabolizers, morphine concentrations are very low (~50-fold lower than in extensive metabolizers; Kirchheiner et al., 2007), and there is not enough generated to elicit an effect. Codeine still has other clearance pathways besides CYP2D6, and thus exposures are not different between EM and PM subjects. This supports the notion that in EM subjects, codeine itself does not contribute to pharmacological effect.

9. Dihydroergotamine. Dihydroergotamine is a vasodilator effective in the treatment and prophylaxis of migraine, with the serotonin 1A receptor considered as the target. The oral bioavailability of dihydroergotamine itself is very low, yet the drug is effective at low parent drug concentrations. Investigation led to the identification of the metabolite 8'-hydroxydihydroergotamine as the putative active agent (Fig. 13). Its intrinsic potency is about thrice that of the parent (Hanoun et al., 2003), but more importantly, the plasma concentrations of the metabolite are 6-times greater (Wyss et al., 1991; Chen et al., 2002). It can be estimated that the metabolite possesses 20-fold greater in vivo activity than the parent, assuming that free fractions and brain penetrability properties are similar. Considering that the addition of one hydroxyl group to a molecule with a molecular weight of nearly
600 would alter the physicochemical properties to only a very small extent, such a consideration is reasonable.

10. Diphenoxylate. Diphenoxylate is an ethyl ester that is hydrolyzed to difenoxine (Fig. 14). It is used as an antidiarrheal agent that acts by agonizing the opiate receptor. The carboxylic acid metabolite is somewhat more potent than the parent drug (as assessed using a rat brain homogenate binding assay), and systemic concentrations of the metabolite are greater than for the parent (Karim et al., 1972). The target of action is the gut, so it cannot be necessarily stated that the metabolite bears the greater role in activity since the effect could be due to local exposures to parent drug upon oral administration (Wüster and Herz, 1978). In a comparison, dosing the metabolite directly to humans showed that five-times less was needed to achieve a constipatory effect the same as the parent ester (Rubens et al., 1972).

11. Dolasetron. Dolasetron is a 5-HT₃ receptor antagonist used in the treatment of nausea and vomiting caused by emetogenic anticancer agents (Anzemet). It undergoes reduction to hydrodolasetron and this metabolite dominates the contribution to activity (Fig. 15). When used orally, dolasetron is not measurable, thus it has been concluded that the activity is dominated by the alcohol metabolite (Keung et al., 1997). The metabolite molecule has 20- to 60-times greater affinity at the 5-HT₃ receptor than the parent (Gregory and Ettinger, 1998). Even when dolasetron is intravenously administered, hydrodolasetron is generated rapidly and exceeds the parent in concentration by over 10-fold. Thus, even though dolasetron possesses intrinsic affinity for the 5-HT₃ receptor, it offers no contribution to efficacy in vivo; the activity resides with the reduced metabolite.

12. Ebastine. Ebastine is a second-generation antihistamine that contains a t-butyl substituent that undergoes extensive conversion to a carboxylic acid metabolite, carebastine (Fig. 16). After administration of ebastine, carebastine concentrations are 30-fold greater (Sastre, 2008; Shon et al., 2010). Furthermore, carebastine is intrinsically more potent at the H₁ receptor (Anthes et al., 2002). The exact values for plasma protein binding have not been reported as both parent and metabolite are highly bound and merely reported as >95%. On the basis of these measurements, it can be estimated that the metabolite contributes virtually all of the antihistamine activity following oral administration of ebastine.

13. Flutamide. Flutamide is an antiandrogen that is converted to the active metabolite 2-hydroxyflutamide (Fig. 17). The intrinsic potency and plasma protein binding values of the parent and metabolite are not very different (Feau et al., 2009), but what drives estimation of a high contribution of the metabolite to efficacy are the 20- to 40-fold greater

[Fig. 38. Metabolism of venlafaxine to O-desvenlafaxine.]

[Fig. 39. Metabolism of acebutolol to diacetolol.]
plasma concentrations of the metabolite (Belanger et al., 1988).

14. Hydroxyzine. Hydroxyzine is a first-generation antihistamine. Its use in the treatment of allergy has fallen out of favor because it causes sedation, unlike more recently available antihistamines. However, because of this side effect, it has found use as a sedative and potentially as an anxiolytic. Hydroxyzine contains a 1° alcohol that is metabolized by oxidation to the carboxylic acid metabolite cetirizine (Fig. 18). Cetirizine also has intrinsic H₁ potency that is about 3-fold weaker than the parent drug (Chen, 2008), yet this potency was enough to permit introduction of this metabolite as a drug itself. Cetirizine is zwitterionic and therefore not necessarily as good at penetrating the brain as the parent hydroxyzine, and this serves as an advantage by offering antihistimic activity peripherally while causing much less sedation. With hydroxyzine dosing, the contribution of cetirizine to antihistamine activity is likely high, due to higher concentrations of the metabolite (Simons et al., 1989).

15. Irinotecan. Irinotecan is a topoisomerase 1 inhibitor structurally derived from natural products from Camptotheca. It undergoes hydrolysis of the carbamate bond to the active metabolite SN-38 (Fig. 19), which is over 1000-times more potent at inhibition of topoisomerase 1.

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**TABLE 2**

<table>
<thead>
<tr>
<th>Original Drug</th>
<th>Metabolite That Is Used as a Drug</th>
<th>Mechanism/Indication</th>
<th>Rationale for the Usefulness of the Metabolite as a Drug</th>
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<tr>
<td>Amitriptyline</td>
<td>Nortriptyline</td>
<td>Antidepressant</td>
<td>Differential effect at neurotransmitter transport proteins</td>
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<tr>
<td>Bromhexine</td>
<td>Ambroxol</td>
<td>Mucokinetic agent</td>
<td>and topical anesthetic</td>
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<td>Diazepam</td>
<td>Temazepam</td>
<td>Anxiolytic</td>
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<tr>
<td>Diazepam</td>
<td>Oxazepam</td>
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<td>Eretinate</td>
<td>Acetretin</td>
<td>Antipsoriatic</td>
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<td>Hydroxyzine</td>
<td>Cetirizine</td>
<td>Antihistamine</td>
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<td>Phenobarbital</td>
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<td>Risperidone</td>
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<td>Mesoridazine</td>
<td>Antipsychotic</td>
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<td>Tolterodine</td>
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<tr>
<td>Venlafaxine</td>
<td>Desvenlafaxine</td>
<td>Antidepressant</td>
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Fig. 40. Metabolism of acetohexamide to hydroxyhexamide and structures of analogs.
of the enzyme (Kawato et al., 1991). While SN-38 is present in circulation, it is only there at about a 40-fold lower concentration than the parent drug, and its free fraction is about 10-times lower (Wiseman and Markham, 1996; Iyer and Ratain, 1998; Mathijssen et al., 2001; Camptosar, 2012). Combining the inhibition potency and free plasma concentrations, it would be estimated that SN-38 contributes 10-fold more than the parent. However, it is likely that irinotecan is cleaved inside the tumor cell and bioactivated there, and SN-38 would then make a much larger contribution (Kawato et al., 1991).

16. Loratadine. Loratadine was one of the first nonsedating antihistamines and as such was a popular alternative to the older generation antihistamines that readily penetrated the blood-brain barrier and caused sedation. It is extensively metabolized to desloratadine via hydrolysis of the carbamate moiety (Fig. 20). The intrinsic potency of desloratadine far exceeds that of the parent (Anthes et al., 2002), the free fraction is higher by 5-fold, and the circulating concentrations are also higher (Ramanathan et al., 2007). When these three factors are combined, it suggests that loratadine itself is responsible for none of the in vivo antihistaminic activity. Desloratadine is now used as a drug itself. The picture is further complicated by the fact that 3-hydroxydesloratadine is also listed as an active metabolite; however, the intrinsic potency value is not available in the scientific literature. 3-Hydroxydesloratadine has a comparable free fraction and circulates at about half the concentration of desloratadine.

17. Losartan. Losartan is an antihypertensive agent that acts by binding to the angiotensin II receptor. It is a primary alcohol that is oxidized in two steps to a carboxylic acid (EXP3174) by CYP3A and CYP2C9 (Fig. 21). An integrated examination of the relative potency (Le et al., 2003), free fraction in plasma (Cozaar, 2011), and plasma exposures (Lo et al., 1995) yields the projection that the carboxylic acid metabolite would contribute approximately 14-times the activity than the parent drug. This is primarily driven by the approximately 25-fold greater potency of the metabolite. The relevance of the metabolite to efficacy is reinforced by clinical observations. As CYP2C9 has a major role in the generation of EXP3174, subjects that are genotyped as containing CYP2C9*3 have shown lower metabolite concentrations (Yasar et al., 2002). Such subjects have also been shown to have a lower therapeutic response to losartan (Lajer et al., 2007; Joy et al., 2009).

18. Oxcarbazepine. Oxcarbazepine is reduced to 10-hydroxyoxcarbazepine (Fig. 22) and it is the metabolite that has been claimed to carry the majority, if not all, of the antiepileptic activity. Exposure to the active metabolite is considerably greater than the parent drug and it is somewhat less protein-bound (Dickinson

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**Fig. 41.** Metabolism of amiodarone to desethylamiodarone.

**Fig. 42.** Metabolism of aripiprazole to dehydroaripiprazole.

**Fig. 43.** Metabolism of carbamazepine to the epoxide metabolite.
et al., 1989; Patsalos et al., 1990). Although the mechanism of action may not be definitively proven, both compounds block sodium channels in vitro with equivalent potency (Schmutz et al., 1994). Considering the relative potency, plasma exposure, and free fraction values, and assuming equivalent brain penetrability, it can be estimated that the metabolite carries 17-times the in vivo activity of the parent drug.

19. Oxybutynin. Oxybutynin is an antimuscarinic agent used in the treatment of urinary incontinence. It undergoes an N-deethylation reaction to yield a major circulating metabolite present at 8-fold greater concentrations (Fig. 23) (Reiz et al., 2007). Interestingly, this metabolite has shown a higher protein binding (Mizushima et al., 2007), which is counter to most cases wherein secondary amine metabolites have higher free fractions than their corresponding tertiary amine parent drugs. Both parent and metabolite are equally active in vitro (Waldeck et al., 1997). Thus, it can be estimated from the combination of these data that desethyl oxybutynin has about 4-fold the in vivo activity as the parent drug. It should be noted that these estimates are based on data for the racemate, and somewhat different estimates could be possible by considering the stereoisomers independently.

20. Procainamide. Procainamide is a class 1 antiarrythmic agent that blocks the sodium channel. It undergoes acetylation to the active metabolite N-acetylprocainamide (Fig. 24), which also has been shown to bind to this channel (Sheldon et al., 1994b). Combining the circulating concentrations, plasma protein binding (which is low for both), and the sodium channel affinity leads to the conclusion that the acetylated metabolite contributes twice as much to the receptor activity as the parent drug. Despite this estimation, there are other data to suggest that N-acetylprocainamide does not make this contribution and actually works as a class III antiarrythmic via potassium channel block (Harron and Brogden, 1990). Direct intravenous administration showed that N-acetylprocainamide concentrations needed to be greater than what would be expected from findings when the metabolite was measured after administration of procainamide and that the electrophysiological profile qualitatively differed from the parent drug (Roden et al., 1980; Dangman and Hoffman, 1981; Jaillon et al., 1981). To further complicate this picture, acetylation is catalyzed by the polymorphic N-acetyltransferase 2 enzyme, thus the ratio of parent to metabolite in vivo differs between extensive and poor metabolizers (Reidenberg et al., 1975).
21. Propoxyphene. Propoxyphene is a \( \mu \)-opioid agonist that was recently removed from clinical use in the United States due to cardiac toxicity. It undergoes N-demethylation to norpropoxyphene (Fig. 25), which has nearly equivalent intrinsic potency and plasma binding (Giacomini et al., 1978; Neil and Terenius, 1981). However, it is present in nearly 10-fold excess to parent after steady-state dosing, with nonstationary clearance for both parent and metabolite resulting in high accumulation (Inturrisi et al., 1982). This would suggest that norpropxyphene carries the bulk of the target activity. However, in rats it was shown that the norpropoxyphene metabolite does not partition into brain as readily as the parent drug (Way and Schou, 1979) and that propoxyphene itself is a P-glycoprotein substrate in mouse (Doran et al., 2005). Thus, the relative central effects of the two agents are unclear. The cardiotoxicity may be largely due to the active metabolite (Way and Schou, 1979; Ulens et al., 1999).

22. Remacemide. Remacemide is an antiepileptic agent that undergoes loss of a glycine to an active metabolite (Fig. 26). While a complete examination of all input parameters necessary for assessing the relative contributions to efficacy of parent drug and active metabolite are not available, it has been shown that the metabolite is over 100-fold more potent at the target ion channel and achieves greater free exposure in the brain than the parent drug (Palmer et al., 1992; Schachter and Tarsy, 2000). Assuming equivalent plasma protein binding and target tissue penetration leads to the estimate that the desglycine metabolite actually possesses nearly all of the pharmacological effects, essentially making the parent a prodrug.

23. Risperidone. Risperidone is an effective antipsychotic agent active at several human neurotransmitter receptors, particularly the serotonin-2A and dopamine-2 types (Schotte et al., 1996). It is extensively metabolized to the active metabolite 9-hydroxyrisperidone (paliperidone) by CYP2D6 (Fig. 27) (Huang et al., 1993; Fang et al., 1999). The metabolite possesses intrinsic potency similar to the parent. Deconvolution of the relative contributions of each to efficacy is challenging because of potential differences in brain penetrability caused by P-glycoprotein, for which both parent and metabolite are substrates (Wang et al., 2004; Ejsing et al., 2005). In mouse, risperidone and 9-hydroxyrisperidone brain penetration appear to be hampered 10- and 20-fold, respectively, as shown by comparison of brain/plasma ratios in multidrug resistance knockout mice. Since CYP2D6 is polymorphically expressed, risperidone demonstrates different exposure levels in EM and PM subjects; however, this difference does not appear to manifest itself in different levels of efficacy and this is attributed to the fact that 9-hydroxyrisperidone is also an active antipsychotic agent. In EM subjects, it can be projected that the metabolite carries the large share of
receptor occupancy (maybe as much as 10-fold), even with a potentially greater P-glycoprotein-catalyzed efflux. The efficacy of 9-hydroxyrisperidone has been leveraged in that this compound is now used as a drug itself.

24. Roflumilast. The phosphodiesterase (PDE) 4 inhibitor roflumilast is used for chronic obstructive pulmonary disease (COPD) and it possesses an active N-oxide metabolite (Fig. 28). A thorough analysis of the pharmacokinetics of these two and their variability, as they relate to contribution to activity, has been done (Lahu et al., 2010). In this analysis the following equation was used:

\[
t_{\text{PDE4i}} = \frac{\text{AUC}_p \cdot f_{u,p}}{\text{IC}_{50,p} \cdot \tau} + \frac{\text{AUC}_m \cdot f_{u,m}}{\text{IC}_{50,m} \cdot \tau}
\]

in which \( t_{\text{PDE4i}} \) refers to a unitless value for inhibition of the target enzyme, which is related to the sum of a parent term and a metabolite term, each of which contain values for the total exposure, free fraction, potency, and dosing interval. The contribution of the N-oxide metabolite was modeled to be greater than the parent, and interestingly, based on comparisons of healthy volunteers and COPD patients, this contribution was modeled to be even greater in the patients. Using exposure values from Böhmer et al., (2009) during the control phase of a drug interaction study done at steady-state roflumilast dosing, along with values of 0.8 and 2.0 nM for PDE4 inhibition in vitro (Sanz et al., 2007), with free fractions yields values for the two terms of:

roflumilast contribution:

\[
\frac{\text{AUC}_p \cdot f_{u,p}}{\text{IC}_{50,p} \cdot \tau} = \frac{35.2 \, \mu g \cdot hr/1 \cdot 0.01 \cdot 1000 \, \text{nmol} \cdot \mu \text{mol}}{0.8 \, \text{nM} \cdot 24 \, \text{h} \cdot \mu \text{mol} \cdot \mu \text{mol}^2} = 0.046
\]

roflumilast N-oxide contribution:

\[
\frac{\text{AUC}_m \cdot f_{u,m}}{\text{IC}_{50,m} \cdot \tau} = \frac{417 \, \mu g \cdot hr/1 \cdot 0.03 \cdot 1000 \, \text{nmol} \cdot \mu \text{mol}}{2.0 \, \text{nM} \cdot 24 \, \text{h} \cdot \mu \text{mol} \cdot \mu \text{mol}^2} = 0.62
\]

thus projecting that the N-oxide metabolite contributes 93% of the activity, provided it can penetrate the target tissue equivalently to the parent.

25. Sibutramine. Sibutramine offers an interesting example of two active metabolites and an instance wherein the parent drug probably contributes little effect. It was used as a weight-loss agent, although safety problems led to its removal from clinical practice. Its mechanism of action is purported to be via action on serotonin, norepinephrine, and possibly dopamine transporters as well, although it does not work in models of depression in which other drugs possessing these activities work. The most potent binding is to the norepinephrine transporter, but the desmethyl and didesmethyl metabolites (Fig. 29) possess 100- and 60-times greater intrinsic potency (Cheetham et al., 1996). Furthermore, the metabolites are present in circulation at concentrations in the same range as the parent drug (Kim et al., 2009), and the parent drug is more bound in plasma (\( f_u = 0.03 \) versus 0.06 for both metabolites) (Meridia, 2010). Thus, the metabolites are really driving the in vivo activity. Understanding the relationship among the active entities can be further confounded by the fact that sibutramine is administered as a racemate and that the metabolism can show quantitative differences between the enantiomers.

26. Tamoxifen. Tamoxifen offers a particularly interesting example of active metabolites in that it had been used clinically for many years before it was uncovered that a secondary metabolite, endoxifen, may contribute the majority of the antiestrogenic activity.
Endoxifen arises via sequential hydroxylation and N-demethylation reactions that are catalyzed by CYP2D6 and CYP3A, respectively (Fig. 30). CYP2D6 generates 4-hydroxytamoxifen, which is also pharmacologically active. In CYP2D6 poor metabolizers or patients taking CYP2D6 inhibitors, like paroxetine, the levels of endoxifen will be lower and may reduce efficacy in these patients (Goetz et al., 2005; Jin et al., 2005), although this may not be that clear cut (Nowell et al., 2005; Regan et al., 2012). An examination of the intrinsic potency of the metabolites and circulating concentrations would suggest that endoxifen is important in efficacy. While a side-by-side comparison of tamoxifen, 4-hydroxytamoxifen, N-desmethyltamoxifen, and endoxifen has not been reported, assembling the data from several reports on these compounds suggests that 4-hydroxytamoxifen and endoxifen are two orders of magnitude more potent than their respective non-hydroxylated counterparts (Furr and Jordan, 1984; Johnson et al., 1989, 2004). Endoxifen is present in circulation at approximately 1/10th that of tamoxifen, whereas 4-hydroxytamoxifen is present at about 1/100th that of tamoxifen. Summing these up, and assuming similar free fractions (Bourassa et al., 2011), since affinities for albumin are nearly equivalent, it can be projected that endoxifen contributes approximately 10 times the receptor activity as tamoxifen, whereas the contributions of 4-hydroxytamoxifen and tamoxifen are about equal. N-Desmethyltamoxifen, which quantitatively is the major metabolite present in circulation at concentrations in the same range as tamoxifen and in 20- to 100-fold greater levels than endoxifen and 4-hydroxytamoxifen, would have a contribution similar to tamoxifen itself. One final interesting point regarding tamoxifen active metabolites is that a sophisticated analytical method was developed whereby an estrogen receptor binding assay was placed in-line with an HPLC for the facile detection of active estrogen receptor compounds, and it can be used to detect active metabolites (Oosterkamp et al., 1996; Kool et al., 2006).

27. Terfenadine. Terfenadine was the first of the nonsedating antihistamines; however, it was withdrawn from clinical use due to serious cardiac toxicity, particularly when coadministered with CYP3A inhibitors such as azole antifungals. Terfenadine undergoes a very high first-pass extraction that results in much higher exposures to its major carboxylic acid metabolite, fexofenadine (Fig. 31), while terfenadine itself is barely measurable (Lalonde et al., 1996). Fexofenadine has about 4-fold lower intrinsic potency at the H1 receptor (Anthes et al., 2002); however, due to its very high relative exposure, it contributes extensively to in vivo efficacy. Essentially, terfenadine was a prodrug. When terfenadine was removed from clinical use, it was replaced with fexofenadine. The exposure values for fexofenadine that are efficacious after direct administration are similar to those achieved following administration of efficacious doses of terfenadine, lending support to the notion that terfenadine exhibited its effect primarily via the active metabolite.

28. Thiocolchicoside. Thiocolchicoside (Fig. 32) is an unusual derivative of a natural product that has muscle-relaxant activity believed to be mediated by binding to GABA receptors. It possesses a glucose substituent that undergoes deglycosylation to the phenol intermediate, which is subsequently glucuronidated. After an oral dose that shows efficacy, the glucuronide is present but the glucose-containing parent drug is undetectable (Trellu et al., 2004). Administration of the metabolite directly to rats yielded the same effect on polysynaptic reflex as the
same dose of parent drug. Data comparing the in vitro activity of parent and metabolite was not found, so the importance of the metabolite to effect in humans must be inferred.

29. Thioridazine. Thioridazine is an old antipsychotic agent that is still used, but not as much as the more modern antipsychotic agents. It undergoes S-oxidation reactions on the thiomethyl side chain to form mesoridazine (sulfoxide) and sulforidazine (sulfone) (Fig. 33). Mesoridazine has also been used as a drug itself. Mesoridazine and thioridazine possess affinity for the dopamine D2, D3, and D4 receptors (Richtand et al., 2007), with mesoridazine showing somewhat greater affinity. After administration of thioridazine, total exposure to thioridazine is about 6-fold greater than the mesoridazine; however, it has been shown that the parent drug is vastly more protein-bound than the metabolite. Values for \( f_u \) have been reported to be 0.0016 and 0.09 for parent and mesoridazine, respectively, in one report (Freedberg et al., 1979) and 0.0015 and 0.017 in a second report (Nyberg et al., 1978); the reasons for the difference reported for mesoridazine are not apparent. However, in either case, it can be estimated that mesoridazine contributes virtually all of the receptor occupancy relative to parent. Additionally, the second S-oxidation to mesoridazine yields the other active metabolite, sulforidazine. This metabolite has similar potency and free fraction as mesoridazine, but it circulates at about a third the concentration. Nevertheless, it is estimated that this metabolite also plays a more important role in efficacy than thioridazine.

30. Tibolone. Tibolone is a steroidal agent that has activity at the \( \alpha \)-estrogen receptor and the androgen receptor and is used in osteoporosis and breast cancer prevention. It undergoes reduction of the 3-keto group to two stereoisomeric alcohols and isomerization of the double bond to the 4-position (Vos et al., 2002; Fig. 34). The alcohols are more potent than parent at the estrogen receptor, while the alkene isomer is more potent at the androgen receptor (Escande et al., 2009). The 3\( \alpha \)-hydroxy isomer is present at more than 9-fold greater concentrations than tibolone (Timmer et al., 2002) and thus can be estimated to have a 20-fold greater contribution than tibolone itself, assuming other distributional aspects are similar between parent and metabolite. The 3\( \beta \) isomer is also present at greater concentrations, but not as much as the other isomer. The alkene isomer metabolite is estimated to contribute about twice as much androgen receptor binding as the parent.

31. Tolterodine. Tolterodine, a muscarinic antagonist used to treat urinary incontinence and overactive bladder, offers an interesting example wherein a marked difference in plasma protein binding probably contributes to the metabolite contributing a greater
share to the efficacy. Tolterodine is metabolized to 5-hydroxymethyltolterodine (desfesoterodine; Fig. 35) by CYP2D6, as demonstrated by marked differences in exposure to both the parent and the metabolite between CYP2D6 EM and PM subjects. CYP2D6 EM subjects have high metabolite and low parent; vice-versa for CYP2D6 PM subjects (Brynne et al., 1998). Both parent and metabolite bind to the target with similar potency (Yono et al., 1999) and have similar exposures in EM subjects; however, the metabolite is 10-fold less protein-bound (Pahlman and Gozzi, 1999; Olsson et al., 2001). Thus, it can be extrapolated that in EM subjects, the metabolite contributes the lion’s share of the activity, while in PM subjects the parent drug would be the major contributor. Furthermore, it was shown in animal studies that 5-hydroxymethyltolterodine has a lower capability to penetrate the brain (as assessed using $K_{p,uu}$ values; Callegari et al., 2011), thus it could be proposed that the metabolite would have lower propensity to cause side effects caused by antimuscarinic activity in the brain, such as memory impairment. The lack of an impact of CYP2D6 on the metabolism of 5-hydroxymethyltolterodine served as a rationale for the development of fesoterodine, an ester prodrug of the metabolite (Malhotra et al., 2009).

32. Tramadol. Tramodol is an opiate used in pain treatment. It is dosed as a racemate and undergoes $O$-demethylation to an active metabolite (Fig. 36). The (+)enantiomer of the active metabolite possesses $\mu$-opioid receptor binding that is over 100-fold greater than the parent [as a racemate or the (+)-enantiomer] (Lai et al., 1996). This biotransformation reaction is catalyzed by CYP2D6, thus poor metabolizers do not form appreciable amounts of the metabolite and as a consequence do not experience the same antinociceptive effect (Paulsen et al., 1996). This also suggests that the metabolite may be entirely responsible for action, essentially making tramadol itself a prodrug.

33. Trimethadione. Trimethadione is an old oxazolidinedione antiepileptic agent that undergoes $N$-demethylation to dimethadione (Fig. 37). It offers an interesting example in that $N$-demethylation results in the generation of an acidic 2° imide which substantially alters the physicochemical properties of the molecule. At the neuromuscular junction the effect of trimethadione and dimethadione differ (Alderdice and McMillan, 1982). By use of frog tissue, the parent drug was shown to alter the postjunctural sensitivity, whereas the metabolite acts by altering acetylcholine release. The concentrations at which these effects are demonstrated are relevant to those measured in vivo for the metabolite; however, the potency of the parent is not as great and the antiepileptic effects may be due mostly to the metabolite.

34. Venlafaxine. Venlafaxine is an inhibitor of the serotonin reuptake transporter that has been used as an antidepressant. It has an active metabolite, $O$-desmethylvenlafaxine (Fig. 38) that is also now used as a drug itself (Deecher et al., 2006). Venlafaxine is metabolized to the metabolite by CYP2D6, thus exposure values differ between CYP2D6 EM and PM subjects with the former having higher metabolite...
concentrations and the latter having higher parent drug concentrations (Fukuda et al., 1999). Thus, the contribution of parent versus metabolite to in vivo efficacy will differ between the two populations. Considering the free plasma levels and in vitro affinities (Owens et al., 1997), in EM subjects the metabolite can be calculated to contribute almost 4-times the activity relative to parent, whereas for PM subjects the parent can be estimated to contribute about twice the activity relative to the metabolite. Both parent and metabolite have been shown to have equivalent brain partitioning and impact of P-glycoprotein (in mouse; Karlsson et al., 2010), thus it is not anticipated that different metabolite-versus-parent ratios would alter this relationship. The affinity of both for the norepinephrine transporter is considerably less and a low occupancy of this protein would be predicted from pharmacokinetic data.

B. Drugs with Active Metabolites That Contribute Comparably to the Parent

1. Acebutolol. Acebutolol is an antagonist of the β-adrenergic receptor used in the treatment of hypertension and cardiac arrhythmias. It is metabolized by hydrolysis of a butyl amide group followed by acetylation to diacetolol (Fig. 39), a pharmacologically active metabolite with a longer half-life than the parent (and similarly low plasma protein binding) which permits once-per-day dosing (Coombs et al., 1980; DeBono et al., 1985; Piquette-Miller et al., 1991). Diacetolol has been shown to have activity by direct administration to humans (Ohashi et al., 1981). While data on the in vitro potency at the human receptor are not reported, considerable exploration of the relative effects of acebutolol and diacetolol has been done in animals and using animal tissues. In a guinea pig cardiac preparation, the metabolite was shown to have about 3-fold lower potency (Basil and Jordan, 1982). At early timepoints following oral administration, acebutolol and diacetolol plasma concentrations are

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Fig. 59. Metabolism of spironolactone to canrenone.

Fig. 60. Metabolism of triamterene to 4-hydroxtriamterene sulfate.

Fig. 61. Metabolism of verapamil to norverapamil.

Fig. 62. Metabolism of zolmitriptan to N-desmethylzolmitriptan.
about equal, but at later timepoints diacetolol is present at much greater concentrations. Thus, it is likely that the parent contributes more activity at early timepoints (up to $T_{\text{max}} \approx 4$ hours) and the metabolite contributes at the later timepoints.

2. Acetohexamide. Acetohexamide is reduced to hydroxyhexamide (Fig. 40), which is also active at stimulating insulin secretion and lowering blood glucose. The glucose-lowering effect is better correlated to the pharmacokinetics of the metabolite, which has a longer average half-life (4.6 versus 1.3 hours) and later $T_{\text{max}}$ (4 hours versus 1) (Smith et al., 1965). The importance of knowing that an active metabolite can be responsible for effect is illustrated by the observation that coadministration of phenylbutazone results in an enhancement of the effect of acetohexamide, despite no change in acetohexamide exposure (Field et al., 1967). Hydroxyhexamide is excreted in urine, and phenylbutazone is known to affect renal clearance of anionic drugs; thus it can be proposed that the phenylbutazone-acetohexamide interaction is due to an increase in hydroxyhexamide exposure caused by inhibition of the renal clearance of the metabolite. The activity of hydroxyhexamide can be rationalized by comparison with other members of the sulfonylurea class of drugs. Tolbutamide, chlorpropamide, and tolazemide possess

---

**Fig. 63.** Metabolism of alprazolam to 1′-hydroxyalprazolam.

**Fig. 64.** Metabolism of buprenorphine to $N$-dealkyl and glucuronide metabolites.
similar structures, with a site of structural variation being on the 4-position of the central phenyl ring, which is the site of reduction of acetohexamide to hydroxyhexamide (Gopalakrishnan et al., 2000) (Fig. 40).

3. Amiodarone. Amiodarone is an unusual antiarrhythmic agent that putatively has multiple mechanisms of action, thus delineating the relative roles of the parent drug and its active desethyl metabolite (Fig. 41) is challenging. Multiple cation channels are affected by both parent and metabolite as well as inhibition of the conversion of thyroxine to triiodothyronine (Kodama et al., 1997). Plasma concentrations of amiodarone and desethylamiodarone associated with efficacy are about 2 and 1.5 \( \mu M \), respectively, and due to high lipophilicity the plasma protein binding is high (Stäubli et al., 1985; Ujhelyi et al., 1996). In vivo studies suggest that desethylamiodarone has greater effects in animal models at equivalent total concentrations and that these effects may be due to a higher free fraction (Nattel and Talajic, 1988).

4. Aripiprazole. Aripiprazole is a dopamine-receptor partial agonist used in the treatment of various psychiatric disorders. It undergoes dehydrogenation of a dihydroquinolone ring to generate quinolone (Fig. 42). The metabolite has intrinsic affinity and functional activity at the dopamine receptors equivalent to the parent drug (Tadori et al., 2011) and steady-state concentrations in patients have been shown to be comparable, with somewhat greater concentrations of the metabolite (Molden et al., 2006). Protein binding is very high for both (Abilify, 2012), making a precise estimate challenging, but if assumed to be equal to the parent (which is reasonable considering the very moderate difference in physicochemical properties between the two), it could be estimated that the dehydro metabolite contributes slightly more than the parent. Both are substrates of P-glycoprotein and this may alter estimates of the relative contributions of parent and active metabolite to efficacy in vivo (Kirschbaum et al., 2010).

5. Carbamazepine. Carbamazepine is used in the treatment of epilepsy, as well as some other central nervous system (CNS) disorders. It is oxidized by cytochrome P450 enzymes to the active 10,11-epoxide metabolite (Fig. 43), which subsequently undergoes hydrolysis to the inactive diol. The mechanism of action is believed to arise via blockage of voltage-dependent sodium channels in the brain, and the in vitro activity of carbamazepine and the epoxide are similar (McLean and Macdonald, 1986). Circulating concentrations of the parent exceed those of the metabolite by about 5-fold, while the metabolite has a free fraction about twice that of the parent. Cerebrospinal fluid (CSF) concentrations have been measured in humans for both, and consistent with the total plasma concentrations and free fractions, the metabolite is present at about one-third of the parent (Johannessen et al., 1976), suggesting equilibration of free plasma and CSF levels. Thus, it can be estimated that the epoxide metabolite will contribute about a third of the activity in vivo. Interestingly, in a study in neuralgia patients, blinded replacement of effective carbamazepine therapy with carbamazpine-10,11-epoxide dosing resulted in no alteration in reported effect on pain (Bertilsson and Tomson, 1984).

6. Clarithromycin. Clarithromycin is metabolized by CYP3A to 14-hydroxyclarithromycin, which also has antibacterial activity (Fig. 44). The plasma concentration of the metabolite is about half of that of the parent.
drug (Rodvold, 1999), but its in vitro minimally inhibitable concentration for *Haemophilus influenzae* is also about half that of parent (Hardy et al., 1990). Thus, it can be estimated that the parent and metabolite contribute equally, depending on the potential for differential tissue penetration.

7. Clobazam. Clobazam is a benzodiazepine drug that is used as an anxiolytic and anticonvulsant. It undergoes N-demethylation (Fig. 45) to a metabolite with one-fifth of the potency (Onfi Product Label). The metabolite has a longer *t*\(_{1/2}\) and accumulates to greater exposures than the parent (Ochs et al., 1984; Bun et al., 1986). Taking into consideration a 2-fold difference in free fraction, it can be predicted that the metabolite and parent drug would have approximately equal contributions to efficacy.

8. Disopyramide. Disopyramide is a class I antiarrhythmic agent that blocks the cardiac sodium channel. It possesses two isopropyl groups on an amine and undergoes N-dealkylation of one of these to the active metabolite N-desisopropyl disopyramide (Fig. 46). The metabolite possesses comparable potency for the channel, but the free plasma concentrations of the metabolite are about a third of those of the parent (Hill et al., 1988; Sheldon et al., 1994b). [The parent drug has been shown to be subject to saturable plasma protein binding (Hinderling et al., 1974), so the contribution of the metabolite may increase as the parent drug concentration decreases.] An estimate can be made from these data that the metabolite contributes about a third of the activity of the parent drug (Hinderling and Garrett, 1976).

9. Fluoxetine. Fluoxetine has a major active metabolite norfluoxetine (Fig. 47). The half-life of the metabolite is about a week, as compared with the parent drug, which has a half-life of about 2 days (Aronoff et al., 1984). CYP2D6 has been shown to have a role in the conversion of fluoxetine to norfluoxetine, and poor metabolizers have less of the metabolite in circulation (Llerena et al., 2004; Scordo et al., 2005). Also, after repeated dosing, CYP2D6 in EM subjects becomes inhibited so that CYP2C9, another enzyme involved in fluoxetine metabolism, plays an increasing role in fluoxetine clearance as shown by comparison of pharmacokinetics in subjects containing a defective CYP2C9 allele (Scordo et al., 2005). Affinity for the human serotonin uptake transporter is almost the same between the parent and metabolite, and concentrations of the two are also similar (Owens et al., 1997; Scordo et al., 2005). Initially, fluoxetine was approved as a once-per-day drug—recognition that the norfluoxetine metabolite possesses the same activity while having a longer half-life led to the introduction of a once-per-week formulation. Brain permeation for both parent drug and metabolite was high and the residence time was long with a half-life the same as in plasma (as assessed with \(^{19}\text{F} \) NMR for combined parent and metabolite; Bolo et al., 2000).

10. Itraconazole. The antifungal agent itraconazole undergoes metabolism to hydroxyitraconazole (Fig. 48). In vitro the metabolite possesses similar antifungal activity (Mikami et al., 1994; Odds and Bossche, 2000). It also circulates in plasma at levels similar to parent (Barone et al., 1994; Odds and Bossche, 2000). It also circulates in plasma at levels similar to parent (Barone et al., 1998), so it can be estimated that the metabolite and parent drug will have a similar contribution to antifungal activity.

11. Ketamine. Ketamine is an N-methyl-D-alanine–receptor antagonist used as an intravenous anesthetic. It is converted to an N-demethyl metabolite that also possesses N-methyl-D alanine–receptor binding activity
After oral administration, norketamine exposure values are greater than ketamine values and it could be estimated that the metabolite may contribute almost equally to effect (Hijazi and Boulieu, 2002). However after intravenous administration, exposures are about equal, and the contribution to activity would be mostly due to the parent compound (Clements et al., 1982). Ketamine is thus illustrative of the potential impact the route of administration may have on metabolite exposures and hence contribution of a metabolite to pharmacological effect. For drugs that undergo high first pass metabolism (hepatic and/or intestinal), there can be a large amount of active metabolite generated following oral administration. Metabolite/parent ratios can differ, and this is illustrated by the example of ketamine.

12. Levosimendan. Levosimendan is an intravenously administered inotropic agent for heart failure patients, believed to cause its effect via sensitization of troponin-C to Ca$^{2+}$ binding, although the mechanism is not definitively proven. It possesses a propanedinitrile-substituted hydrazinylidene substituent that is removed via reduction of the N–N bond by gut microflora to yield an intermediate aniline metabolite that subsequently undergoes N-acetylation to the active metabolite OR-1896 (Fig. 50). While the half-life of levosimendan is short (~1 hour), the active metabolite has a half-life of a few days, and the beneficial effect is observed for considerably longer than what the parent half-life would indicate, suggesting an important role for OR-1896 in the extended duration of activity (Antoniades et al., 2007). In contrast to this, no apparent difference in efficacy has been observed between poor and extensive acetylators despite a difference in exposure to OR-1896 between the two groups (Antila et al., 2004; Kivikko et al., 2011). Delineating the relative contributions of OR-1896 to levosimendan is not straightforward; the pharmacokinetics are such that during the intravenous infusion, the parent drug concentrations are far greater than the
metabolite and cardiovascular effect is observed (Kivikko et al., 2002). Upon cessation of the infusion, levosimendan concentrations drop rapidly. OR-1896 concentrations slowly increase to levels about 10% of those that were observed for the parent drug during the infusion phase, presumably due to biliary secretion of parent, reduction in the gut by microflora, absorption of the reduced intermediate, and acetylation to yield OR-1896. OR-1896 concentrations are measurable for days, while levosimendan levels are undetectable. Thus, it may be the case that levosimendan parent is responsible for the hemodynamic effects during infusion and the metabolite takes over postinfusion.

13. Metoprolol. Metoprolol is a β-blocker that is subject to a pharmacokinetic difference in CYP2D6 EM and PM subjects. It undergoes extensive metabolism to mostly inactive metabolites with the exception of the hydroxylation to α-hydroxymetoprolol, an active metabolite by CYP2D6 (Kaila and Isalo, 1993) (Fig. 51). Delineation of the temporal relationship between the pharmacokinetics of metoprolol (short $t_1/2$, 3–4 hours), α-hydroxymetoprolol ($t_1/2$, 7–10 hours), and the effect on tremor measured over time in humans suggests that the metabolite can contribute between 50–100% of the activity relative to parent drug (Quarterman et al., 1981; Gengo et al., 1984). It has also been observed that the pharmacodynamic responses in EM versus PM
subjects do not differ to the extent that the pharmacokinetic differences in parent drug exposure between these two groups would suggest; i.e., the PK/PD relationship for metoprolol differs between EM and PM subjects in that EM subjects have lower metoprolol concentrations but the efficacy is not diminished (Jonkers et al., 1991). This also supports the presence and importance of an active metabolite.

14. Metronidazole. The nitroimidazole drug metronidazole is an antibacterial that is metabolized by hydroxylation of the methyl substituent and this metabolite also has bactericidal activity (Fig. 52). The relative activities depend on the species and strain. For Gardnerella vaginalis, the metabolite has been reported to be 8-fold more efficacious (as determined by comparison of minimum inhibitory concentration; Ralph and Amatnieks, 1980), whereas for Bacteroides fragilis the parent is more potent than metabolite (Ralph and Amatnieks, 1980; Pendland et al., 1994). The parent is present at greater circulating concentrations and neither entity has appreciable plasma protein binding (Easmon et al., 1982). For G. vaginalis, an estimate that the metabolite and parent contribute nearly equally can be made, whereas for B. fragilis it is the parent drug that should dominate the efficacy. Fractional inhibitory concentration values were calculated using in vitro activity and in vivo exposure data and were about 0.7 for the effect of the metabolite for B. fragilis (Pendland et al., 1994).

15. Morphine. Morphine represents a fascinating example of the potential for an active metabolite to contribute to the pharmacological activity of the parent drug because it has been well studied, and the reader interested in a greater level of detail is directed to a thorough review by Kilpatrick and Smith (2005). Morphine is metabolized by glucuronidation to the 3- and 6-glucuronides (Fig. 53). The 3-glucuronide is the major circulating metabolite in humans but does not possess activity at the \( \mu \)-opioid receptor. Morphine-6-glucuronide possesses nearly equivalent in vitro receptor activity (binding and agonism) as the parent drug. Morphine-6-glucuronide is present in human circulation at about the same concentrations as morphine after intravenous administration of the latter, and it has been estimated that about 10% of morphine is cleared by the 6-glucuronidation route (Lotsch et al., 1996). The analgesic effects of morphine-
6-glucuronide have been studied directly in humans following intravenous administration of the metabolite and such data offer the best approach to delineate whether a metabolite can contribute to activity in vivo. (This represents a rather unique situation as it is rare that a metabolite is administered directly to humans, unless the metabolite is being tested as a drug itself.) Morphine-6-glucuronide showed good efficacy at standard endpoints of opioid activity, albeit the central effects occurred later (6–8 hours), relative to morphine itself (2–3 hours) (Lotsch et al., 2001; Murthy et al., 2002; Skarke et al., 2003). This could be due to either a slow uptake into human brain or a delayed egress of the metabolite from the brain. Based on physicochemical attributes, high penetration of glucuronide metabolites into the brain would be unexpected; however in the case of morphine-6-glucuronide it has been suggested that a folded conformation may be adopted that reduces hydration and increases lipophilicity (Gaillard et al., 1994). In later work, the partitioning of unbound drug into brain (Kp,uu), a metric used to assess the potential for free-plasma and free-brain (hence efficacious) concentrations, showed a value for morphine-6-glucuronide that was far lower than that for morphine (in rat) (Friden et al., 2009). Animal studies suggest that drug transport may be involved in morphine-6-glucuronide brain disposition, but data in humans are lacking or inconclusive. Furthermore, interpretation can be confounded by the possibility that morphine is glucuronidated within the brain, as it

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**Fig. 72.** Metabolism of diltiazem to desmethyl and desacetyl metabolites.

**Fig. 73.** Metabolism of donepezil to 6-O-desmethyldonepezil.
has been shown in vitro that human brain tissue is capable of this (Yamada et al., 2003).

16. Pioglitazone. Pioglitazone is a peroxisome proliferator-activated receptor–γ agonist useful in the treatment of diabetes. It is metabolized on the two benzylic positions adjacent to the pyridine to hydroxyl and ketone metabolites (Fig. 54) (Krieter et al., 1994; Actos, 2011). The potencies of these two metabolites have been measured in a cellular lipogenesis assay and mouse glucose lowering assay (Sohda et al., 1995; Tanis et al., 1996; Young et al., 1998) and shown to be equivalent to the activity of pioglitazone itself. Protein binding is comparable and the metabolites are present in circulation at concentrations of 10–50% of parent (Kalliokoski et al., 2008). As such, it can be estimated that these two metabolites combined can make a considerable contribution to the activity of pioglitazone, in the range of 40% of the total activity.

17. Praziquantel. Praziquantel, a drug to treat schistosomiasis, is converted to a hydroxyl metabolite (Fig. 55) that could contribute some effect. The in vitro potency of the metabolite at killing the parasite is 60-fold less than parent (Ronketti et al., 2007); however, it is present at over 20-fold greater exposure (Westhoff and Blaschke, 1992; Cioli et al., 1995). If free fractions are assumed to be nearly equal, it can be estimated that the metabolite may contribute about a third of the effect of the parent drug.

18. Quazepam. Quazepam is a benzodiazepine anxiolytic with an unusual thioamide structure. Metabolism results in the replacement of the =S to =O, the 2-oxoquazepam metabolite (Fig. 56). This metabolite has 2- to 3-fold greater potency than the parent (Sieghart, 1983) and is present in circulation at about two-thirds of the parent (Chung et al., 1984). As such, it can be estimated that 2-oxoquazepam contributes substantially to the total activity.

19. Quinidine. Quinidine has been used for decades in the management of cardiac arrhythmias. Despite this length of use, its molecular mechanism is largely unknown, although it is known to interact with several ion channels (Na+ and K+). It gives rise to the active 3-hydroxyquinidine metabolite (Fig. 57). Following oral quinidine administration, concentrations of the metabolite are not as great as quinidine; however, it has lower plasma protein binding and thus the free concentrations of quinidine and 3-hydroxyquinidine are about the same (Wooding-Scott et al., 1988). 3-Hydroxyquinidine has been administered to humans directly, with measurement of cardiac pharmacodynamic endpoints (Vozeh et al., 1985), which is an excellent way to gather data that can be used in estimating the contribution of the metabolite to the parent drug action. When administered directly, plasma concentrations of 1.5 μM were shown to yield similar effects to those observed when quinidine was administered, and 1.5 μM concentrations of metabolite are similar to those observed after quinidine administration. Thus, it can be concluded that 3-hydroxyquinidine contributes equivalently to quinidine in effect.

20. Saxagliptan. Saxagliptan is an antihyperglycemic dipeptidyl peptidase-4 inhibitor for the treatment of diabetes. It possesses a hydroxyl adamantyl substituent, and metabolic introduction of a second hydroxyl group yields 5-hydroxysaxagliptan (Fig. 58), which retains about half of the intrinsic potency (Augeri et al., 2005). In consideration of the SAR for the parent drug (Augeri et al., 2005), it is not surprising that the addition of a second hydroxyl to this substituent does not abolish activity. The metabolite circulates at concentrations about twice that of the parent and neither compound is bound to plasma proteins (Onglyza, 2011), thus the 2-fold difference in potency and 2-fold difference in exposure essentially cancel each other out and lead to the conclusion that parent
and metabolite could contribute to in vivo efficacy about equally, provided that free target tissue concentrations are similar to free plasma concentrations.

21. Spironolactone. Spironolactone is an aldosterone inhibitor used as a diuretic in hypertension. It possesses a thioacetate group that is converted to an alkene bond in the metabolite canrenone (Fig. 59) (Abshagen et al., 1976). Canrenone itself is used as a drug, as well as an analog in which the lactone ring is open and dosed as the potassium salt (canrenoate). It is not entirely clear regarding all the entities’ contributions to activity and whether activity is better related to plasma or urinary concentrations; however, canrenone has been estimated to contribute to 73% of the activity (Ramsay et al., 1977). While spironolactone and potassium canrenoate yield similar levels of canrenone, spironolactone is more potent, suggesting that other spironolactone metabolites may contribute to activity (Kojima et al., 1985; Peile, 1985). Spironolactone had been shown to generate metabolites that lost the acetyl group but retained the sulfur (Abshagen et al., 1976). A comparison of the concentrations of spironolactone and canrenone (Dong et al., 2006) shows that the metabolite is present at about 3-fold excess at maximum plasma concentration but vastly greater concentrations at later timepoints. When correcting for relative free fractions (Chien et al., 1976) and intrinsic potencies (Funder et al., 1976), it would suggest that canrenone contributes a quarter of the activity of parent. At later timepoints, this contribution would increase.

22. Triamterene. Triamterene is used in the treatment of hypertension as a potassium-sparing antihypertensive acting at sodium channels. It is frequently co-dosed with antihypertensives that cause loss of potassium. A major circulating metabolite in humans arises by sequential hydroxylation and sulfation reactions, 4-hydroxyltrimterene sulfate (Fig. 60), with concentrations in excess of 10-times those of the parent drug (Sörgel et al., 1985). The metabolic reactions are catalyzed by CYP1A2 and platelet phenol sulfotransferase. Accounting for differences in free fraction and estimates of intrinsic potency (Busch et al., 1996), it can be estimated that the metabolite contributes approximately half of the activity of the parent drug. The metabolite has been shown to be active in animal models (Voelger, 1991).

23. Verapamil. Verapamil undergoes extensive metabolism, including a major N-demethylation pathway that yields the active circulating metabolite norverapamil (Fig. 61). The metabolite has been shown to be active in vivo and an estimate of its intrinsic potency at the Ca2+ channel places it approximately 5-fold less active (Ferry et al., 1985; Johnson et al., 1991). It circulates at a greater concentration than parent and is slightly less protein-bound in plasma (Yong et al., 1980; Powell et al., 1988), thus it can be estimated that it contributes about a third of the activity of the parent drug.

24. Zolmitriptan. Zolmitriptan is a serotonin receptor agonist used in the treatment of migraine. It undergoes N-demethylation to yield a secondary amine metabolite that is claimed to be twice as intrinsically potent (Fig. 62) (Jandu et al., 2001). Following oral administration, the metabolite circulates at about half the level of the parent drug (Dixon et al., 1997; Peck et al., 1998), thus it can be projected that the contributions of zolmitriptan and the N-desmethyl metabolite are about equal.

C. Drugs with Metabolites That Possess Target Potency But Contribute Little to In Vivo Effect

In some cases, metabolites are identified in laboratory animals or in vitro systems and are then shown to have some affinity to the receptor targeted by the

![Fig. 76. Metabolism of imatinib to N-desmethylinatinib.](image1)

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parent drug. However, when examined in humans in vivo, it is shown that the metabolite is estimated to actually contribute little effect. This can be due to low in vivo concentrations, higher plasma protein binding, or a lower ability to enter the target tissue.

1. Alprazolam. Alprazolam is a highly used anxiolytic agent that is cleared by oxidative metabolism and renal excretion. Metabolism is on the methyl group on the triazole (1'-hydroxyalprazolam) and on the diazepine (4-hydroxyalprazolam), with the 1'-hydroxyalprazolam possessing intrinsic binding potency that is about 3-fold lower than the parent drug (Richelson et al., 1991; Fig. 63). The metabolite is present at far lower concentrations in plasma (Smith and Kroboth, 1987; Garzone and Kroboth, 1989) and it is thus unlikely to contribute significantly to the pharmacological effects of alprazolam.

2. Buprenorphine. Buprenorphine is an opioid agent that acts as a partial agonist at the $\mu$ receptor and also binds the $\kappa$ subtype as well. Data regarding the contribution to activity of the metabolites norbuprenorphine and buprenorphine 3-glucuronide (Fig. 64) have only recently emerged (Brown et al., 2011), and thus the full picture of data needed to assess the relative contributions of parent and metabolites to in vivo effect in humans has not emerged. The drug is typically administered intravenously, sublingually, or transdermally, and plasma exposure to the metabolites is less than that for the parent drug (Polettini and Huestis, 2001). Protein binding values have not been reported for the metabolites. In postmortem brain samples, it has been shown that buprenorphine is present in the brain, but the desmethyl and glucuronide metabolites were undetectable (Elkader and Sproule, 2005). Furthermore, it has been shown that norbuprenorphine is a substrate of P-glycoprotein, which may diminish brain exposure (Tournier et al., 2010). Thus, while buprenorphine metabolites may show intrinsic receptor activity, in vivo data suggest that only the parent drug contributes to the effect.

3. Carisoprodol. Carisoprodol is a sedative and muscle relaxant that yields an active metabolite meprobamate, which itself was a drug used as an anxiolytic in the 1960s (Fig. 65). The mechanism of action is not entirely clear, although some activity may be mediated through the GABAA receptor (Gonzalez et al., 2009). The conversion of carisprodol to meprobamate is partially catalyzed by CYP2C19 (Dalen et al., 1996), an enzyme subject to genetic polymorphism, and thus concentrations of parent drug and metabolite differ between extensive and poor metabolizers (Olsen et al., 1994). If GABAA is the receptor responsible for the activity, it can be estimated that meprobamate contributes about 10% of the activity; less in CYP2C19 poor metabolizers.

4. Chloroquine. The antimalarial agent chloroquine is converted to an active N-desethyl metabolite (Fig. 66). The metabolite is present in human circulation at about a third of the concentration of the parent drug following a single dose. The mean residence time for the metabolite is longer than for the parent drug, so after repeated administration the metabolite concentrations will increase more than the parent. Incorporating the differences in free fraction and intrinsic potency, it can be estimated that the desethyl
metabolite contributes about 20% to activity (Ofori-Adjei et al., 1986; Augustijns and Verbeke, 1993; Vippagunta et al., 1999).

5. Citalopram. Citalopram is a serotonin selective reuptake inhibitor widely used as an antidepressant in both its racemic forms and as the S-isomer alone (escitalopram). It is metabolized via sequential N-demethylation reactions to yield N-desmethylcitalopram and didesmethylcitalopram (Fig. 67). The metabolites have lower affinity for the reuptake transporter than the parent (6- to 8-fold) (Owens et al., 1997; Deupree et al., 2007) and circulate at lower steady-state concentrations than the parent (Sidhu et al., 1997). Thus, the metabolites contribute very little to the antidepressant effect of citalopram.

6. Cyclosporine. The immunosuppressant cyclosporine undergoes complex metabolism, and considerable attention has been paid to whether it is important to measure metabolites during therapeutic monitoring of this drug (Santori et al., 1997; Ozbay et al., 2007). Among the metabolites identified, two hydroxylated metabolites possess measurable in vitro activity (Copeland et al., 1990; Fig. 68). Combining blood concentrations (Christians et al., 1991) and in vitro potencies, and assuming similar tissue penetrability of the metabolites and cyclosporine which, considering the very minor alteration introduced by metabolism into such a large molecule, along with little difference between plasma binding (Kodobayashi et al., 1995), is likely a reasonable assumption, it can be estimated that the two metabolites probably only contribute 5–15% to efficacy.

7. Dabigatran. Dabigatran is a thrombin inhibitor intended to prevent deep vein thrombosis. It is orally administered as a carbamate ester of an amidine that yields dabigatran, which is the major entity in circulation. Dabigatran undergoes glucuronidation of its carboxylic acid group and the resulting 1-O-acylglucuronide (Fig. 69) as well as its rearrangement products were all shown to cause prolongation of thromboplastin time. It was hypothesized that the COOH group is remote from the amidine group that is essential for target binding, thus alteration of the COOH, even with a change as large as esterification of glucuronic acid, would not affect activity (Ebner et al., 2010). However, in humans the circulating concentrations of glucuronides are less than a tenth of that for dabigatran itself (Bleich et al., 2008; Stangier et al., 2008), thus while this represents an interesting example of a glucuronide conjugate that retains in vitro potency, its relevance to in vivo effect is minimal.

8. Dasatinib. Dasatinib is a second-generation kinase inhibitor affecting the Bcr-Abl Src family of enzymes and is used in the treatment of leukemia. It undergoes extensive metabolism (including an N-dealkylation of an ethanol side chain) to yield a metabolite with equivalent intrinsic potency (including an N-dealkylation of an ethanol side chain) to yield a metabolite with equivalent intrinsic potency (Christopher et al., 2008) (Fig. 70). The free fraction of the metabolite is almost twice as high (Sprycel, 2011); however, the circulating concentrations of the metabolite are far lower than the parent (Furlong et al., 2012), thus the metabolite likely contributes less than 10% to the activity.

9. Diazepam, Temazepam, and Oxazepam. Diazepam is metabolized to several metabolites via oxidation reactions, and three of these metabolites have been used as drugs themselves. Diazepam undergoes N-demethylation and this metabolite is the major circulating metabolite, relative to temazepam (a hydroxyl metabolite) and oxazepam (the corresponding hydroxyl metabolite of desmethyldiazepam) (Ghabrial et al., 1986; Rouini et al., 2008) (Fig. 71). All three metabolites have lower affinity for the benzodiazepine receptor (4- to 7-fold; Richelson et al., 1991) and circulate at concentrations much lower than the parent drug (8- to 50-fold lower). With only small differences in free fraction, it is unlikely that any of these metabolites contribute substantially to the effects of diazepam; even when summed the contributions of the
active metabolites are less than 10% of that of parent drug. The half-lives for temazepam and oxazepam are shorter than diazepam, indicating formation-rate limitation, while the half-life for N-desmethyldiazepam is similar to that of diazepam.

10. **Diltiazem.** Diltiazem represents an interesting example of active metabolites. The N-desmethyl and desacetyl metabolites have been a focus as contributing to activity (Fig. 72). These circulate at concentrations that are lower than the parent (Montamat and Abernethy, 1987) and are comparably protein-bound (Boyd et al., 1989). The in vitro potency values of the metabolites relative to the parent drug are nominally lower (Li et al., 1992), thus overall the contribution of metabolites to the efficacy of diltiazem is probably quite low. It is interesting to note that in a single-dose study a clockwise hysteresis was observed for diltiazem concentrations (i.e., decreased effect at the same concentrations at a later time), and the authors proposed the possibility of metabolites that antagonize the P-R interval–prolongation effect of diltiazem, among other possibilities (Boyd et al., 1989). Such an effect of the desmethyl and desacetyl metabolites would not be consistent with their diminished activity at the Ca\(^{2+}\) channel but would need to be due to an alternate target.

11. **Donepezil.** Donepezil, an acetylcholinesterase inhibitor used in the treatment of dementia, possesses an active metabolite that arises via O-demethylation of the 6-methoxy substituent on the indanone ring (Fig. 73). The metabolite is reported as possessing equivalent intrinsic potency (Sugimoto et al., 1995); however, when plasma concentrations have been measured, they are far below those measured for the parent compound (at least 150-fold), thus it is unlikely that 6-O-desmethyldonepezil actually contributes to in vivo efficacy (Okereke et al., 2004; Patel et al., 2008).

12. **Granisetron.** Granisetron is a serotonin-3 receptor antagonist used as an antiemetic in cancer patients undergoing treatment with emetogenic chemotherapeutics (Fig. 74). It undergoes hydroxylation on the indazole ring at the 7-position (Clarke et al., 1994) to yield a metabolite with slightly greater target potency than the parent drug (Vernekar et al., 2010). After both oral and intravenous administration, the metabolite is present at about 10% of that of the parent (Clarke et al., 1994; Boppana, 1995). Assuming similar free fractions, it can be estimated that the metabolite would contribute about 20% of the activity of the parent.

13. **Halofantrine.** Halofantrine is an antimalarial agent that is not used very much due to QT-interval prolongation. However, it has a major circulating metabolite (Milton et al., 1989; Veenendaal et al., 1991) that possesses some in vitro activity as well, desbutylhalofantrine, in which one of the two butyl substituents are removed (Fig. 75) (Bhattacharjee and Karle, 1998). While protein binding of desbutylhalofantrine has not been reported, if potency and total concentrations are compared, the metabolite could have about a quarter of the activity.

14. **Imatinib.** Imatinib is a protein–tyrosine kinase inhibitor selective for the \(v\)-ABL type as well as potency for the platelet-derived growth factor kinase.
It undergoes \( N \)-demethylation by CYP3A4 (Fig. 76), and the metabolite circulates at approximately 10–15% of levels of the parent drug. Other attributes (free fraction, intrinsic potency) are the same between the parent and metabolite, thus it can be projected that the parent will dominate the contribution to activity by virtue of its greater exposure (Kretz et al., 2004; Le Coutre et al., 2004; Gschwind et al., 2005; Peng et al., 2005; Treiber et al., 2008).

15. **Lidocaine.** Lidocaine is an antiarrhythmic agent that undergoes sequential \( N \)-deethylation reactions to yield putatively active metabolites monoethylglycine xylidide (MEGX) and glycine xylidide (Fig. 77). The metabolites are not as active at sodium channel block as the parent drug using rat in vitro data (Sheldon et al., 1991); however, after comparison to free circulating concentrations it can be estimated that MEGX contributes about a fifth of the activity while glycine xylidide contributes very little. MEGX is very poorly bound to plasma proteins so free concentrations are almost equal to parent and the potency is about one-fourth that of parent. Given earlier animal data, it had been concluded that MEGX might contribute more to the activity (Drayer et al., 1983).

16. **Lumefantrine.** The example of lumefantrine is very similar to that of halofantrine. Similar to halofantrine, it undergoes \( N \)-dealkylation to desbutyl-lumefantrine (Fig. 78). The metabolite has 6-fold greater in vitro potency at killing *Plasmodium falciparum* but is present in circulation of patients undergoing successful treatment of malaria at about 1/20th the concentration of the parent compound (Wong et al., 2011). Plasma binding has not been reported, but if assumed to be equal, it can be predicted that the metabolite carries about 30% of the activity of the parent.

17. **Macitentan.** Macitentan is an endothelin antagonist being developed to treat pulmonary arterial hypertension. It has an \( N \)-propylsulfonyleurea that undergoes \( N \)-depropylation to yield an active metabolite that possesses about an eighth of the intrinsic activity of the parent (Fig. 79) (Iglarz et al., 2008). In humans, the metabolite is present at about 50%
greater plasma concentrations (Sidharta et al., 2011). The free fraction is unreported, so making an estimate of the contribution of the metabolite relative to parent suggests that the metabolite contributes to about 20% of the endothelin inhibition.

18. Mexiletine. Mexiletine is a sodium channel blocker used as an antiarrhythmic agent. In humans it is converted to several hydroxyl metabolites (Paczkowski et al., 1992), two of which have been shown to have in vitro activity (p-hydroxymexiletine and hydroxymethyl-mexiletine) (Fig. 80) (Catalano et al., 2004; DeBellis et al., 2006). While the free fraction for the parent is known, there is no report of the free fraction for the metabolites, so the comparison on contributions to activity in vivo must be made based on total concentrations. Neither metabolite is projected to contribute more than 10% of activity, and even if they are assumed to be entirely free, their contributions would not exceed 20% of the parent drug.

19. Mianserin. Mianserin is a tetracyclic antidepressant that is metabolized to its N-demethyl analog (Fig. 81). It binds many receptors, the \( \alpha_2 \)-adrenergic being one of the most important. Upon demethylation, the intrinsic affinity for \( \alpha_2 \) decreases by about 3-fold (Nickolson et al., 1982). Circulating concentrations of the metabolite are about 60% of those of parent (Reis et al., 2009). The free fraction of the parent is available (Kristensen et al., 1985), but for the metabolite it is not reported, so estimation of relative contributions to activity can only be made assuming that protein binding and central penetration are equivalent. Under these assumptions, and also assuming that the \( \alpha_2 \)-adrenergic receptor is the most relevant for effect, it is estimated that the metabolite contributes about 25% of the efficacy of the parent.

20. Midazolam. Midazolam is used as an anesthetic agent frequently by the intravenous route. It is initially metabolized to 1'-hydroxy and 4-hydroxy metabolites (Fig. 82), with the former possessing intrinsic binding activity to the benzodiazepine receptor similar to that of the parent drug (Richelson et al., 1991). Furthermore, the glucuronide conjugate of 1'-hydroxymidazolam may also contribute to activity, based on the observation of prolonged activity in renal insufficiency patients who are less able to renally clear this metabolite (Bauer et al., 1995). Plasma protein binding of the glucuronide metabolite is considerably lower than midazolam; however, the ability of the glucuronide metabolite to penetrate the brain relative to the parent is unknown, thus precluding a true assessment of the relative contributions of parent and metabolite to effect. The 1'-hydroxymidazolam metabolite has been shown to have activity in humans following its direct administration, which is a highly valuable approach to understanding the activity of a metabolite. However, it is infrequently carried out (Mandema et al., 1992). This activity was comparable to midazolam itself. However, the clearance of 1'-hydroxymidazolam is high and thus
exposure to this metabolite after administration of midazolam is a lot lower than exposure to midazolam itself. Thus, the contribution of 1'-hydroxymidazolam to the sedative/anesthetic properties of midazolam is likely to be low.

21. Mirtazapine. Mirtazapine, a close analog of mianserin described above, also undergoes N-demethylation to an active metabolite with reported 3- to 4-fold lower intrinsic potency than the parent drug (Fig. 83) (Stimmel et al., 1997). Mirtazapine acts by antagonizing the \( \alpha_2 \)-adrenergic receptor, which results in downstream effects on serotonin and norepinephrine release (de Boer et al., 1988). It also binds to some serotonin receptors and the \( H_1 \) receptor. The metabolite is present at about half the exposure of the parent drug (Shams et al., 2004), but the role that the metabolite can play in all of these activities has not been described in the literature. For the \( \alpha_2 \)-adrenergic receptor activity, if it is assumed that the free fraction does not differ between parent and metabolite and brain penetration is equal, it would be anticipated that the metabolite would contribute less than 20% of the activity. A more accurate assessment would require further investigation.

22. Primidone. Primidone is a relatively old drug that is used in the treatment of epilepsy. It is oxidized to phenobarbital (Fig. 84), a drug itself, but a metabolite when primidone is the parent drug. Affinity for the GABA receptor is approximately 2.5-fold greater for primidone (Ticku and Olsen, 1978), and phenobarbital concentrations following oral primidone are lower than the parent drug (Martines et al., 1990). Plasma protein binding is comparable between the two compounds, so if brain penetration is equivalent it can be estimated that phenobarbital contributes about a quarter of the activity. A second metabolite, phenylethylmalonamide is present in circulation but does not contribute to in vivo activity.

23. Propafenone. Propafenone is a sodium channel blocker used in the treatment of cardiac arrhythmias. It is metabolized by the polymorphic enzyme CYP2D6 to 5-hydroxypropafenone (Fig. 85), which is an active metabolite (Zoble et al., 1989). Propafenone is subject to a supraproportional exposure-dose relationship in CYP2D6 extensive metabolizers that is not shown in poor metabolizers, consistent with saturation of CYP2D6-catalyzed first-pass metabolism (Siddoway...
et al., 1987). At the higher doses, EM and PM differences are about 2-fold and no dose adjustment is made between the two populations. 5-Hydroxypropafenone, which is also subject to nonlinear clearance (Vozeh et al., 1990), has about half the intrinsic potency as the parent and is present at about 4-fold lower unbound exposure. Thus, it does not contribute much to the efficacy of propafenone. The N-despropyl metabolite is also weakly active in vitro (Oti-Amoako et al., 1990) but is present at even lower concentrations in vivo and therefore would not be expected to contribute to in vivo effect.

24. Propranolol. Propranolol is oxidized to 4-hydroxypropranolol (Fig. 86) and this metabolite is present in human circulation following administration of propranolol but at 25- to 150-times lower exposures (depending on CYP2D6 genotype) (Raghuram et al., 1984). The metabolite is less potent at the β-adrenergic receptor (compared using turkey as the source species for receptor; Bilezikian et al., 1978), so it is unlikely that the metabolite has any substantive contribution to activity.

25. Rifampin. Rifampin (rifampicin) is an antibiotic drug that has been used for decades to treat tuberculosis. It undergoes hydrolysis of an acetyl group at the 23-position to yield a metabolite that has similar efficacy against Mycobacterium (Fig. 87; Tencon et al., 1970) and increased potency against many other bacteria (Nakazawa et al., 1970). After oral administration, 23-desacetylrifampicin is present in circulation at about 1/12th the concentrations (Loos et al., 1985; Tenconi et al., 1970). Plasma protein binding for the metabolite is not reported, but if assuming a similar value (i.e., 81% bound; Kochansky et al., 2008), then it can be assumed that the metabolite contributes less than 10% to the antibacterial activity in vivo.

26. Rizatriptan. Rizatriptan is an antimigraine drug that acts by binding serotonergic receptors (5-HT1B and 5-HT1D) with high affinity (Hargreaves, 2000). The mono N-desmethyl metabolite (Fig. 88) has intrinsic potency that is about 3-fold lower. Its contribution to in vivo efficacy is probably very low or negligible, since circulating concentrations are about one-tenth those of the parent drug (Goldberg et al., 2000). Consideration of any potential free-fraction differences are not necessary to understanding the effect of the metabolite since rizatriptan itself is mostly free in plasma (Maxalt, 2011).

27. Rosuvastatin. Rosuvastatin is a widely used lipid-lowering agent that inhibits 3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA) reductase, with an inhibitory potency greater than other clinically used compounds of this class (McTaggart et al., 2001). It undergoes N-demethylation of a sulfonamide nitrogen to yield N-desmethylrosuvastatin, which is claimed to have 2- to 6-fold less potency at inhibiting the enzyme (Crestor, 2012; Fig. 89). It also is present in circulation at concentrations 7-fold less than the parent drug (Schneck et al., 2004). Thus it is projected to have very little contribution to the activity of rosvustatin.

28. Sertraline. Sertraline is a serotonin reuptake inhibitor widely used in the treatment of depression. It undergoes N-demethylation to desmethylsertraline (Fig. 90), which has only about 1/50th the binding affinity of the parent drug (Owens et al., 1997). It circulates at about 1.5-fold greater exposure at steady state (Ronfeld et al., 1997) but the free fraction is unknown. Because of the considerably lower potency, it is doubtful that desmethylsertraline contributes to in vivo efficacy.

29. Triazolam. Similar to midazolam and alprazolam described above, triazolam is also metabolized on the methyl group of the azole ring to the 1’-hydroxy
metabolite (Fig. 91), and also similar to the other two drugs, this metabolite possesses intrinsic potency that is approximately 3- to 5-fold lower than the parent (Richelson et al., 1991). However, less is known about this metabolite regarding pharmacodynamics, and the human free fraction is not reported. Combining the plasma exposure relative to parent (Eberts et al., 1981; O’Connor-Semmes et al., 2001) and the relative potency values, it can be estimated that 1'-hydroxytriazolam would only possess a minor contribution to the efficacy of triazolam. However, if the relationship between the free fractions of triazolam and 1'-hydroxytriazolam follow those for midazolam and 1'-hydroxymidazolam, then the contribution of 1'-hydroxytriazolam to efficacy could be greater.

30. Valproic Acid. Valproic acid is an antiepileptic agent that has been used successfully in the control of seizures for many years. While its molecular target may be ambiguous, studies in systems in vitro of nerve cell firing suggest that its dehydrogenated metabolite trans-2-propyl-2-pentenoic acid (Fig. 92) also possesses this activity at about half of the potency (Löscher, 1981). However, valproic acid concentrations are extremely high (≈0.1 mM) and considerably exceed those of this metabolite (Addison et al., 2000). Furthermore, in a unique study wherein human brain samples were able to be taken from patients on valproic acid therapy who were also undergoing brain surgery, it was shown that the brain concentrations were a lot less than those of valproic acid itself and that the free fraction for the metabolite was lower than that of valproic acid (Adkison et al., 1995). It can be concluded from these data that it is unlikely that valproic acid metabolites actually contribute much to the efficacy in vivo, despite the in vitro potency shown for trans-2-propyl-2-pentenoic acid. This example illustrates the importance of gathering dispositional data for metabolites that are shown to have in vitro activity.

31. Zopiclone. Zopiclone is a sedative agent active at the benzodiazepine receptor family. In one paper it was claimed that the N-oxide metabolite possessed activity and the N-desmethyl did not (Fernandez et al., 1995). However in receptor binding studies (using rat), the N-oxide was at least two orders of magnitude less potent than parent (Trifiletti and Snyder, 1984), and the desmethyl was one order of magnitude less potent (Fig. 93). Subsequently, a closer examination of the S-enantiomer of the N-desmethyl metabolite showed that it possesses pharmacological activity by binding better to GABA<sub>A</sub> receptors possessing the γ2 subunit (Fleck, 2002). Nevertheless, after administration of zopiclone, neither the N-oxide nor desmethyl metabolites are detected in plasma (Fernandez et al., 1993), and
therefore, it is unlikely that any drug-related effect in vivo can be attributed to metabolites.

D. Drugs with Metabolites Possessing Activity at Related Targets

In some instances, changes in structure introduced by metabolism can cause a metabolite to have increased affinity at a receptor other than the one targeted by the parent drug. It is typically the case that the receptor or enzyme that the metabolite binds is one among those in a family that the parent binds (e.g., alternate G-protein coupled receptors). This can cause subtle alterations in clinical effect. Examples of this are below.

1. Amitriptyline and Nortriptyline. Amitriptyline, a tricyclic antidepressant, is metabolized by N-demethylation to nortriptyline, which undergoes further metabolism to several hydroxyl metabolites including E-10-hydroxynortriptyline (Fig. 94). The activity profile contribution of these entities is complex. Amitriptyline is more potent at the serotonin transporter than the norepinephrine transporter, whereas the reverse is true for nortriptyline (Owens et al., 1997). Consideration of the affinity and free plasma concentrations associated with efficacy with amitriptyline dosing suggests that for the serotonin transporter, amitriptyline would contribute to about 50% occupancy and nortriptyline about another 10%. For the norepinephrine transporter, nortriptyline free concentrations are about the same as the potency value, while for amitriptyline the free concentrations are about a fifth of the reported affinity suggesting a similar level of occupancy for the norepinephrine transporter with the metabolite contributing the greater share (Breyer-Pfaff et al., 1982). This is all further complicated by the observation that 10-hydroxynortriptyline also can contribute to effect (Nordin and Bertilsson, 1995). After amitriptyline administration, this metabolite has a concentration that is above its IC50 for the norepinephrine transporter (measured in whole human plasma). Relative to amitriptyline, it can be estimated that 10-hydroxynortriptyline contributes almost twice as much to norepinephrine transport inhibition than the parent drug, but still less than nortriptyline.

When nortriptyline is administered as the parent drug the picture is somewhat simpler because the serotonin transporter is no longer of much importance (since that activity was driven by amitriptyline). Using the respective in vitro potency values of nortriptyline and the 10-hydroxy metabolite (which were generated using human plasma as the matrix) and the in vivo concentrations after repeated administration (Dahl et al., 1996) suggests that the metabolite contributes to about a third of the activity. 10-Hydroxynortriptyline is generated by CYP2D6 and research on the polymorphism in this enzyme has shown that there are differences among CYP2D6 extensive and poor metabolizers, with lower concentrations of the metabolite in the latter. Examination of CSF concentrations have shown that there may be a partial barrier to brain penetration of 10-hydroxynortriptyline, since concentrations are lower than corresponding plasma ultrafiltrate concentrations (Nordin et al., 1985; Bertilsson et al., 1991). Finally, it has been suggested that 10-hydroxynortriptyline could itself be an antidepressant that would have a better side-effect profile because it has less activity at muscarinic receptors.

2. Clomipramine. Clomipramine is a tricyclic antidepressant agent that is metabolized by N-demethylation and hydroxylation to produce three putatively active metabolites: nortriparmine, 8-hydroxycloclomipramine, and 8-hydroxynorcloclomipramine (Fig. 95). Intrinsic potency comparisons have been made for the serotonin and norepinephrine reuptake transporters, as well as the cholinergic receptors (which may be responsible for side effects). N-Demethylation seems to
favor increased norepinephrine transporter potency while retention of the N-methyl favors serotonin transporter potency (Thomas and Jones, 1977; Linnoila et al., 1982; Nunez and Perel, 1995; Agnel et al., 1996; Tatsumi et al., 1997). Plasma concentrations among the four entities are relatively comparable (50–300 ng/ml), but unfortunately the lack of free fraction data on these entities precludes reliable estimations of the relative contributions of each (Bertilsson et al., 1979). If free fractions and CNS penetration are considered comparable, it would suggest that norclomipramine and 8-hydroxyclomipramine contribute 10-times the activity of parent drug at the norepinephrine transporter. For the serotonin transporter, norclomipramine is only projected to contribute less than 20% of the parent, while 8-hydroxyclomipramine would be expected to contribute very substantially (about equally to parent).

3. Clozapine. Clozapine is N-demethylated to norclozapine and while both entities possess activity at the 5-HT2A and 5-HT2C receptors, they appear to possess differing activities at the various dopamine receptors which will help to describe the efficacy profile of the drug as an antipsychotic agent (Fig. 96). Norclozapine and clozapine circulate at about the same free plasma concentrations, albeit the total concentrations and free fractions differ somewhat (Schaber et al., 1998). Clozapine binds most tightly to the D4 receptor and is an antagonist, while it also binds with 10-fold lower affinity to D2 and D3 receptors, where it is an inverse agonist (Burstein et al., 2005). Norclozapine binds with similar affinity to D2 and D3 as clozapine but is a partial agonist at these two receptors. It binds to D4.

Fig. 95. Metabolism of clomipramine by N-demethylation and hydroxylation.

Fig. 96. Metabolism of clozapine to norclozapine.
as well but with ten-fold lower affinity and is an antagonist. This offers a truly confusing picture regarding what may be happening in vivo. For the 5-HT<sub>2</sub> activity, the contribution of the parent and metabolite to occupancy and activity can be considered more straightforward and it is estimated that the norclozapine metabolite contributes about two-thirds of the activity (Kuoppamaki et al., 1993). But for the dopamine receptors, with the varying affinities and functional activities of the two circulating entities, it is much more difficult to claim what is actually occurring.

4. Doxepin.
Doxepin is an unusual agent in that the parent and N-desmethyl metabolite appear to possess very different pharmacological activities (Mundo et al., 1974). Doxepin is a potent antihistamine that readily penetrates the brain and thus causes sedation and is indicated for this purpose at low doses (Silenor, 2010). N-Desmethyldoxepin (Fig. 97) binds to the norepinephrine transporter and thus has antidepressant properties like tricyclic antidepressants. High doses are indicated for antidepressant activity. An estimation of relative norepinephrine transporter occupancies for parent and metabolite suggest that the metabolite has nearly seven-times the activity.

5. Imipramine and Desipramine.
Like the tricyclic antidepressant amitriptyline and its metabolites nortriptyline and hydroxynortriptyline, a similar situation exists for imipramine and its metabolites desipramine and 2-hydroxydesipramine (Fig. 98). Imipramine has activities at both the serotonin and norepinephrine transporters with greater affinity for the serotonin transporter while desipramine and 2-hydroxydesipramine have greater affinities for the norepinephrine transporter (Owens et al., 1997). Thus, a complex picture emerges regarding the molecular activities occurring over the time course of drug and metabolite exposures as well as among different individuals (Caccia and Garattini, 1990). Following administration of imipramine, it can be estimated that the norepinephrine transporter is almost completely occupied by desipramine (free plasma concentration/IC<sub>50</sub> ratio of ~20) (Borga et al., 1969; Sutfin et al., 1988; Szymura-Oleksiaik et al., 2001). 2-Hydroxydesipramine concentrations are approximately half of those of the parent and is approximately 3-fold more potent at the norepinephrine transporter than imipramine suggesting that it also can contribute more to the occupancy of that protein (Javaid et al., 1979; Nelson et al., 1983). Imipramine would be predicted to drive the serotonin transport inhibition since its potency is far greater at that target relative to the two metabolites; however, because of the higher free concentrations of desipramine it can also contribute appreciably to this activity.

When desipramine itself is dosed as the parent drug, the activity is projected to be mostly at the norepinephrine transporter, with contributions from desipramine and the hydroxyl metabolite. Based on comparative in vitro potency values and free concentration values (Cooke et al., 1984), it can be estimated that 2-hydroxydesipramine has about 40% of the in vivo potency. This must be interpreted with some
caution since it is based on potency values measured in rat brain. Finally, since desipramine is converted to the active 2-hydroxdesipramine metabolite by CYP2D6, there are differences between EM and PM subjects in exposure to desipramine and its metabolite (Shimoda et al., 2000).

6. Loxapine and Amoxapine. The antipsychotic agent loxapine, and its N-desmethyl metabolite amoxapine (which is also used as a drug itself) poses a complex picture of the contribution of parent drug and metabolites to pharmacological activity. Loxapine undergoes N-demethylation to amoxapine as well as hydroxylation to 7-hydroxyloxapine and 8-hydroxyloxapine. Amoxapine also undergoes these same hydroxylations to the 7- and 8-hydroxyamoxapine metabolites (Fig. 99). The drugs and their metabolites bind to 5-HT2A and D2 receptors with varying potencies, as well as other CNS receptors to lesser extents (Ereshefsky, 1999). Following administration of loxapine, concentrations of parent drug, amoxapine, 7-hydroxyloxapine, and 8-hydroxyloxapine are approximately 51, 11, 32, and 180 nM, respectively (Cheung et al., 1991). Among the four entities, 7-hydroxyloxapine has the greatest potency and can be projected to deliver 3-times the 5-HT2A receptor activity as loxapine and 11-times the D2 receptor activity. 8-Hydroxyloxapine is less potent, but has greater concentrations and can be projected to have 70% of the activity at 5-HT2A receptors and 1.5-fold the activity at D2 receptors. Amoxapine has similar potency as loxapine at these two receptors, and as it circulates at about one-third of the levels, it can be estimated to have one-third the activity.

Administration of amoxapine yields the corresponding 7- and 8-hydroxy metabolites. 7-Hydroxyamoxapine
is more potent than amoxapine at both the 5-HT2A and D2 receptors (Ereshefsky, 1999) and is present in circulation at about half the concentration (Takeuchi et al., 1993). From this, it can be estimated that 7-hydroxyamoxapine contributes to 5-HT2A occupancy at about an equal amount as parent but is the dominant contributor to D2 occupancy. 7-Hydroxyamoxapine has been shown to readily partition into brain tissue (in rat), but CSF concentrations of it and parent drug are low (Wong et al., 2012). The 8-hydroxyamoxapine metabolite is present in greater abundance, but is 10- to 20-fold less potent at both receptors and therefore contributes

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**Fig. 100.** Metabolism of nefazodone to hydroxynefazodone, mCPP, and its triazolodione metabolites.

**Fig. 101.** Metabolism of atorvastatin to hydroxy metabolites.
about a third of the activity of parent. This contribution likely increases with multiple dosing, since the t1/2 of 8-hydroxyamoxapine is much longer than that of amoxapine or 7-hydroxyamoxapine (Calvo et al., 1985).

7. Nefazodone. The antidepressant nefazodone is metabolized to three active metabolites, hydroxylation on the ethyl side chain (hydroxynefazodone), which undergoes further oxidative metabolism to lose the ethyl group altogether (triazolodione metabolite), and N-dealkylation to yield mCPP (Fig. 100). Nefazodone and its hydroxyl metabolite have very similar receptor binding profiles with the most potent binding occurring at the 5-HT2A receptor (Taylor et al., 1995; Owens et al., 1997). The triazolodione metabolite appears to have far weaker activity. The mCPP metabolite has a different receptor binding profile, is more potent at 5-HT1A and is an agonist. While plasma protein binding is very high for nefazodone (fu = 0.009), data are not available for the metabolites, so estimations of contribution to activity relative to parent drug must be made assuming that these are similar, which is reasonable for the hydroxynefazodone metabolite, but likely not reasonable for mCPP. For hydroxynefazodone, it can be estimated that it has approximately ~25% of the activity of nefazodone itself. Protein binding data for mCPP is available in rat (fu = 0.7), and if the value in human is similar, it would suggest that mCPP could have profound activity in humans [maximum plasma concentration (Cmax) = 0.5 μM; Barbhaiya et al., 1996] including activity at serotonin and norepinephrine reuptake transporters (Owens et al., 1997).

E. Drugs That Generate Active Metabolites but Assessment of In Vivo Contribution Is Ambiguous

1. Atorvastatin. Atorvastatin is a very commonly used lipid lowering agent for the prevention of atherosclerotic disease. It is metabolized by CYP3A to two metabolites claimed to be active, 2- and 4-hydroxyatorvastatin (Fig. 101); however actual inhibition potencies for the HMGCoA reductase target are not available in the scientific literature. In the product label for atorvastatin, it is claimed that 70% of the activity is attributable to metabolites (Lipitor, 2012). However, examinations of the relationship between atorvastatin efficacy and various CYP3A polymorphisms that could cause alterations in parent and metabolite ratios have yielded mixed results (Kivistö et al., 2004; Gao et al., 2008; Li et al., 2011). It is important to note that since the target tissue for inhibition of HMGCoA reductase is the liver within which the active metabolites are generated and that it has been shown that atorvastatin and its metabolites are substrates for hepatic uptake transporters, it may be difficult and even inappropriate to relate systemic

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**Fig. 102. Metabolism of bromhexine to ambroxol.**

**Fig. 103. Metabolism of bupropion to hydroxybupropion and stereoisomeric dihydrobupropion metabolites.**
circulating concentrations of atorvastatin and its active metabolites to effect.

2. Bromhexine. Bromhexine is a relatively old agent that has been used as an expectorant, to help make mucus less viscous and more easily expectorated. Its active metabolite that arises from N-demethylation and hydroxylation, ambroxol, has also been used in that manner (Fig. 102). These agents could find use in the treatment of serious respiratory disorders such as cystic fibrosis or COPD (Malerba and Ragnoli, 2008). When bromhexine is administered orally, the systemic exposure to ambroxol is much lower (∼8-fold; Liu et al., 2010), but since the true molecular action of these agents is not entirely known, it cannot be ascertained whether bromhexine or ambroxol, or both, contribute to activity. Ambroxol has also been associated with a reduction in throat pain, which has triggered investigation into the activity of it and bromhexine at sodium channels (such as NaV1.8 and the tetrodotoxin sensitive channel; Leffler et al., 2010).

3. Bupropion. Bupropion (Fig. 103) represents an interesting challenge when attempting to discern whether the metabolites are relevant to pharmacological effect. In the product labels for Wellbutrin and Zyban (two products containing bupropion), it is stated that the hydroxyl, and the two reduced metabolites are active (Wellbutrin, 2011). However, even for bupropion itself, it is not entirely clear as to the mechanism of action as an antidepressant or smoking-cessation aid. Bupropion does interact with dopamine and norepinephrine transporters, with somewhat greater affinity to the former (Damaj et al., 2004). The CSF concentration of bupropion is about 40% of that in plasma, which is slightly higher than what would be projected when comparing to plasma protein binding (f_u = 0.16) (Golden et al., 1988). The CSF concentration is, however, far lower than the affinity (by ∼20-fold), casting some doubt on whether bupropion is the active entity and whether the dopamine transporter is the target. The same goes for hydroxybupropion, although the affinity is only 4-fold lower than CSF concentrations. Affinity for the reduced metabolites is not published, but as the concentration of threohydrobupropion in CSF is nearly 1 mM and if its affinity for the dopamine transporter is similar to that of bupropion, it is possible that this metabolite carries the majority of the in vivo activity. However, direct administration to animals has suggested that bupropion itself and perhaps the hydroxyl metabolite are actually more active than the reduced metabolites thr- and

![Fig. 104. Metabolism of etretinate to acitretin.](image)

![Fig. 105. Metabolism ivabradine to N-desmethylvabradine.](image)

![Fig. 106. Metabolism of mycophenolic acid to its glucuronide metabolite.](image)
erythrobupropion (Butz et al., 1982; Martin et al., 1990). Furthermore, plasma concentrations of the metabolites were shown to be higher in nonresponder patients suffering from bipolar disorder (Golden et al., 1988).

4. Etretinate. Etretinate is a drug no longer used in the treatment of psoriasis that had an active metabolite acitretin that arises via hydrolysis of the ethyl ester (Fig. 104). Etretinate had a very long half-life, due presumably to sequestration and slow release from deep tissue compartments. The teratogenic effect of retinoids with this long half-life made etretinate undesirable for therapy. Acitretin shows a formation rate–limiting half-life when generated from etretinate, and a much shorter half-life when given directly. The shorter half-life is more amenable for therapy, in light of the teratogenic effect. The molecular mechanism (i.e., target binding protein) is unknown (Saurat, 1999), so delineating the relative contributions of etretinate and acitretin to activity when etretinate is administered is challenging. With etretinate dosing, acitretin achieves concentrations of ~150 ng/ml, whereas etretinate is present at 4- to 5-fold higher concentrations (Larsen et al., 1988). The free fraction of the ester is also considerably greater (Urien et al., 1992; Soriatane, 2011). When acitretin is dosed directly, higher concentrations are observed (Pilkington and Brogden, 1992) than when generated from etretinate. This suggests that etretinate also likely had some contribution to activity when administered directly. Finally, the generation of etretinate by esterification in vivo has also been observed following acitretin dosing, which even further complicates the understanding of relative contributions to effect (Lambert et al., 1992).

5. Ivabradine. Ivabradine is a drug used in the treatment of angina which undergoes demethylation to an active metabolite (Fig. 105). The delineation of the parent drug and metabolite to effect was made using population PK/PD modeling after oral and intravenous administration, because the pharmacological properties of the metabolite are not published (Ragueneau et al., 1998). The metabolite circulates at much lower concentrations than parent. These investigators proposed an indirect PK/PD model wherein the metabolite contributed to effect at the earlier times following dosing and a sustained effect contributed by the parent drug.

6. Mycophenolic Acid. The immunosuppressive agent mycophenolic acid is used to reduce rejection of transplanted organs. It is metabolized by glucuronidation to a phenol glucuronide and an acyl glucuronide (Fig. 106). The latter had been tested in in vitro assays that model immunosuppression and shown to have potency about half that of the parent drug (Shipkova et al., 2001); however, such a determination is challenged by the observation that the acyl glucuronide is converted partially to the parent drug under the conditions used to measure intrinsic activity. In vivo, the acyl glucuronide circulates at a far lower level than the parent drug (Shipkova et al., 2002). To consider whether this metabolite can contribute to in vivo activity, the free fraction needs to be assessed for parent and metabolite. A protein binding value for the parent is known at approximately 98% bound. However, the acyl glucuronide forms an irreversible covalent bond with albumin, which makes measurement of the free fraction technically challenging to obtain. It is thus not presently possible to ascribe relative participation of the parent drug and acyl glucuronide in the pharmacological effect.

V. Conclusions

In drug research, it is clearly the case that the potential for pharmacologically active metabolites must be considered. Knowledge of the properties of active metabolites, i.e., intrinsic potency, functional activity, pharmacokinetics, clearance mechanism and rate, free fraction, and membrane permeability are necessary to understand concentration-effect relationships in humans as well as interpatient variability in response. The occurrence of active metabolites among drugs is frequent, consistent with the notion that small alterations in chemical structure of a drug will not necessarily alter its biologic properties. In the research of new drug candidates, the early identification of active metabolites is of critical importance in developing optimal clinical study designs and correct data interpretation. Proactively hunting for active metabolites in ex vivo and in vitro samples is a sound strategy in drug research. Finally, active metabolites may possess advantages as drugs themselves (i.e., greater potency/efficacy, superior dispositional properties, improved safety profile), and there are many examples of drugs used in clinical practice that were originally observed as metabolites of other drugs.

Acknowledgments

The author greatly appreciates Dr. Doug Spracklin for critical evaluation of this manuscript.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Obach.

References

Bertilsson L and Tomson T (1986) Clinical pharmacokinetics and pharmacological
Augeri DJ, Robl JA, Betebenner DA, Magnin DR, Khanna A, Robertson JG, Wang A,
Barone JA, Moskovitz BL, Guarnieri J, Hassell AE, Colaizzi JL, Bierman RH,
Boyd RA, Chin SK, Don-Pedro O, Verotta D, Sheiner LB, Williams RL,
Antila S, Pesonen U, Lehtonen L, Tapanainen P, Nikkanen H, Vaahtera K,
Anthes JC, Gilchrest H, Richard C, Eckel S, Hesk D, West RE Jr, Williams SM,
Pharmacol
114: 385–393.
Antila S, Pesonen U, Lehtonen L, Tapanainen P, Nikkanen H, Vaahtera K,
Anthes JC, Gilchrest H, Richard C, Eckel S, Hesk D, West RE Jr, Williams SM,
Barrhiaui RH, Burch AB, and Greene DS (1996) A study of the effect of age and
gender on the pharmacokinetics of nefazodone after single and multiple doses.
Barone JA, Moskovitz BL, Guarnieri J, Hassell AE, Colaizzi JL, Bierman RH,
Boyd RA, Chin SK, Don-Pedro O, Verotta D, Sheiner LB, Williams RL,
Antila S, Pesonen U, Lehtonen L, Tapanainen P, Nikkanen H, Vaahtera K,
Anthes JC, Gilchrest H, Richard C, Eckel S, Hesk D, West RE Jr, Williams SM,
Barrhiaui RH, Burch AB, and Greene DS (1996) A study of the effect of age and
gender on the pharmacokinetics of nefazodone after single and multiple doses.
Barone JA, Moskovitz BL, Guarnieri J, Hassell AE, Colaizzi JL, Bierman RH,
Boyd RA, Chin SK, Don-Pedro O, Verotta D, Sheiner LB, Williams RL,
Antila S, Pesonen U, Lehtonen L, Tapanainen P, Nikkanen H, Vaahtera K,
Anthes JC, Gilchrest H, Richard C, Eckel S, Hesk D, West RE Jr, Williams SM,
Barrhiaui RH, Burch AB, and Greene DS (1996) A study of the effect of age and
gender on the pharmacokinetics of nefazodone after single and multiple doses.
Barone JA, Moskovitz BL, Guarnieri J, Hassell AE, Colaizzi JL, Bierman RH,
Boyd RA, Chin SK, Don-Pedro O, Verotta D, Sheiner LB, Williams RL,
Antila S, Pesonen U, Lehtonen L, Tapanainen P, Nikkanen H, Vaahtera K,
Anthes JC, Gilchrest H, Richard C, Eckel S, Hesk D, West RE Jr, Williams SM,
Barrhiaui RH, Burch AB, and Greene DS (1996) A study of the effect of age and
gender on the pharmacokinetics of nefazodone after single and multiple doses.
Barone JA, Moskovitz BL, Guarnieri J, Hassell AE, Colaizzi JL, Bierman RH,
Boyd RA, Chin SK, Don-Pedro O, Verotta D, Sheiner LB, Williams RL,
Antila S, Pesonen U, Lehtonen L, Tapanainen P, Nikkanen H, Vaahtera K,
Anthes JC, Gilchrest H, Richard C, Eckel S, Hesk D, West RE Jr, Williams SM,
Barrhiaui RH, Burch AB, and Greene DS (1996) A study of the effect of age and
gender on the pharmacokinetics of nefazodone after single and multiple doses.
Barone JA, Moskovitz BL, Guarnieri J, Hassell AE, Colaizzi JL, Bierman RH,
Boyd RA, Chin SK, Don-Pedro O, Verotta D, Sheiner LB, Williams RL,
Antila S, Pesonen U, Lehtonen L, Tapanainen P, Nikkanen H, Vaahtera K,


Tomson T and Bertilsson L (1984) Potent therapeutic effect of carbamazepine-10,11-
edoxycycline in trigeminal neuralgia.


