Annu. Rev. Pharmacol. Toxicol. 2004. 44:1–25
doi: 10.1146/annurev.pharmtox.44.101802.121546

First published online as a Review in Advance on July 30, 2003

# PREDICTING HUMAN DRUG GLUCURONIDATION PARAMETERS: Application of In Vitro and In Silico Modeling Approaches

John O. Miners,[1] Paul A. Smith,[1] Michael J. Sorich,[2] Ross A. McKinnon,[2] and Peter I. Mackenzie[1]

*[1]Department of Clinical Pharmacology, Flinders University and Flinders Medical Center, Bedford Park, Adelaide, SA 5042, Australia, and [2]School of Pharmaceutical, Molecular, and Biomedical Sciences, University of South Australia, Adelaide, SA 5000, Australia; email: john.miners@flinders.edu.au, paul.smith@flinders.edu.au, michael.sorich@postgrads.unisa.edu.au, ross.mckinnon@unisa.edu.au, peter.mackenzie@flinders.edu.au*

**Key Words** in vitro–in vivo correlation, in silico (computational) modeling, drug glucuronidation, UDP-glucuronosyltransferase, ADMET prediction

■ **Abstract** Cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT), which both exist as enzyme "superfamilies," are together responsible for the metabolism of most hepatically cleared drugs. There is currently intense interest in the development of techniques that permit identification of the CYP and UGT isoform(s) involved in the metabolism of a newly discovered drug, and hence prediction of factors likely to alter elimination in vivo. In addition, the quantitative scaling of kinetic parameters for a metabolic pathway assumes importance for identifying newly discovered drugs with undesirable in vivo pharmacokinetic properties. Although qualitative and quantitative in vitro–in vivo correlation based on data generated using human liver tissue or recombinant enzymes have been applied successfully to many drugs eliminated by CYP, these strategies have proved less definitive for glucuronidated compounds. Computational (in silico) modeling techniques that potentially provide a facile and economic alternative to the in vitro methods are now emerging. This review assesses the utility of in vitro and in silico approaches for the qualitative and quantitative prediction of drug glucuronidation parameters and the challenges facing the development of generalizable models.

## INTRODUCTION

Hepatic metabolism is the principal elimination mechanism for the majority of drugs in humans. Hence, knowledge of hepatic extraction ratio ($E_H$), hepatic clearance ($CL_H$), and factors that modulate these parameters is of fundamental importance given their impact on the efficacy and safety of drug treatment. Impairment of drug metabolism may result in toxicity as a result of decreased $CL_H$

and/or increased bioavailability. Conversely, induction of metabolism may result in loss of efficacy owing to increased $CL_H$ and /or decreased bioavailability. Given these characteristics of hepatically cleared drugs, the development of in vitro techniques to predict aspects of human drug metabolism and pharmacokinetics in vivo (in vitro–in vivo correlation) has attracted enormous interest over the past decade. Indeed, there is now widespread acceptance of in vitro–in vivo correlation, particularly for compounds eliminated by cytochrome P450 (CYP)-catalyzed hepatic biotransformation. More recently, computational (in silico) approaches have also been applied to prediction of the involvement of specific enzymes in the metabolism of particular drugs.

The economic imperatives associated with drug discovery and greater promotion of the rational use of drugs (rational therapeutics) have been the principal factors driving the development of in vitro and in silico methods to predict drug metabolism parameters. Drug discovery and development is immensely expensive and time-consuming. The success rate of new chemical entities selected for clinical development is approximately 20% (1), with most failures attributed to unacceptable pharmacokinetic properties (2). Undesirable properties, such as poor absorption, high $E_H$ and $CL_H$ (leading to low and variable bioavailability), drug interactions, and metabolism by a polymorphic enzyme, may be predicted from in vitro (and possibly in silico) data, thus facilitating selection of the most appropriate lead compound and decreasing attrition during clinical development. Moreover, selecting for development those compounds that are affected to the least extent by genetic polymorphism and drug interactions optimizes the clinical utility and market success of newly approved drugs. Knowledge of genetic polymorphism, drug interactions, and other factors altering $CL_H$ is similarly important for rationalizing and optimizing dosage regimens of established drugs, thereby improving therapeutic outcome.

In vitro–in vivo correlation allows the prediction of drug metabolism parameters at both the qualitative and quantitative levels. The principal drug metabolizing enzymes CYP and UDP-glucuronosyltransferase (UGT) exist as gene "superfamilies" (3, 4). The individual CYP and UGT proteins (isoforms) tend to differ in terms of substrate and inhibitor selectivities, regulation, and patterns of drug interactions. Thus, identification of the isoform(s) responsible for the metabolism of any given drug, a process referred to as reaction phenotyping, together with an understanding of isoform regulation and drug interactions allows prediction of those factors likely to alter $CL_H$ and clinical response. Procedures for CYP reaction phenotyping are well established and normally involve the integration of data from human liver microsomes (although hepatocyte suspensions may also be used) and recombinant human CYP isoforms (5–9). An alternative approach involves normalizing kinetic data generated with recombinant enzymes for relative isoform expression in human liver or by use of the relative activity factor (6, 7, 9).

Quantitative prediction most commonly involves the scaling of in vitro $CL_{int}$, calculated from the kinetic parameters ($K_m$, $V_{max}$) for the formation of a metabolite by human liver microsomes or hepatocytes, to in vivo $CL_H$ and $E_H$ using a mathematical model of hepatic clearance (10–15). Typically, this requires correction

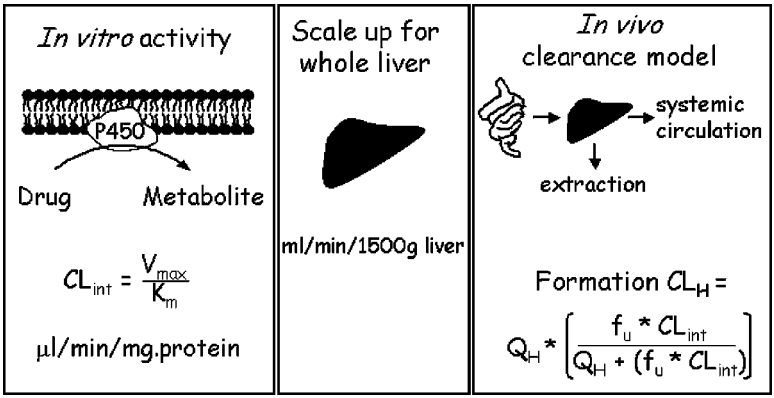


**Figure 1** Approach for the calculation of in vivo hepatic clearance ($CL_H$) from kinetic constants determined for metabolite formation by human liver microsomes using the expression for the well-stirred model of hepatic clearance. Abbreviations: $K_m$, Michaelis constant; $V_{max}$, maximal velocity; $CL_{int}$, intrinsic clearance; $Q_H$, liver blood flow; $f_u$, fraction unbound in blood. Reprinted with permission from Miners JO. 2002. Annual Scientific Meeting of ASCEPT, 2001. Special article: The evolution of drug metabolism: hitchhiking the technology bandwagon. *Clin. Exp. Pharmacol. Physiol.* 29:1040–44

of human liver microsomal $CL_{int}$ for microsome yield (milligrams per gram of human liver) and liver weight (normally assumed as 1.5 kg) to obtain a "whole organ" $CL_{int}$, which subsequently is substituted in the expression for the well-stirred, parallel-tube, or dispersion models (Figure 1). In general, this approach has proved valuable for predicting the in vivo $CL_H$ and $E_H$ of drugs metabolized by CYP (14, 15). The accuracy of prediction may be improved further by correcting the $K_m$ for nonspecific binding of substrate to the microsomal membrane where this occurs (16, 17) or by applying scaling factors derived from animal data to the in vitro $CL_{int}$ (18). An inhibitor constant ($K_i$) calculated for inhibition of metabolite formation in vitro may also be used to forecast in vivo decrement in $CL_H$ arising from coadministration of an inhibitor (15, 19, 20). Although in vitro kinetic data have provided reasonably accurate prediction of some interactions involving CYP substrates, further refinement and validation is necessary before this approach may be applied more widely.

More recently, in silico approaches have been adopted to predict the absorption, distribution, metabolism, excretion, and toxicity (ADMET) parameters of new drug entities (21). Potentially, in silico modeling may be used to evaluate millions of compounds for their ADMET and pharmacological properties, thereby minimizing the need to experimentally characterize large numbers of molecules. In particular, homology- and pharmacophore-models and two- and three-dimensional quantitative structure activity relationships (2D- and 3D-QSAR) have been generated for substrates and inhibitors of CYP isoforms (22). Such quantitative structure

metabolism relationships (QSMR) provide insights into the structural features of drugs that confer isoform selectivity together with an estimate of binding affinity ($K_m$ or $K_i$) (in silico–in vitro correlation).

As alluded to previously, CYP and UGT are quantitatively the most important drug metabolizing enzymes. Together, these enzymes are responsible for the elimination of more than 90% of hepatically cleared drugs. Despite the acceptance of in vitro–in vivo correlation and the promise of in silico–in vitro correlation for drugs eliminated by CYP, the application of these approaches to drug glucuronidation has proved challenging. Here we explore the utility of extrapolating in silico and in vitro metabolism data for drugs and other compounds cleared by UGT.

## UDP-GLUCURONOSYLTRANSFERASE

### Glucuronidation

UGT catalyzes the covalent linkage (conjugation) of glucuronic acid, derived from the cofactor UDP-glucuronic acid (UDPGA), to a substrate bearing a suitable functional group according to a second-order nucleophilic substitution mechanism (4). Glucuronides form via conjugation of a carboxyl, hydroxyl (phenol or aliphatic alcohol), amino, acidic carbon, or sulfuryl moiety present on a typically lipophilic substrate. It is therefore not surprising that glucuronidation serves as an essential clearance mechanism for a myriad of compounds, including drugs from all therapeutic classes, dietary chemicals, environmental pollutants, and endogenous compounds (e.g., bilirubin, bile acids, hydroxysteroids) (4, 23–25). In addition, glucuronidation facilitates excretion of these compounds and the products of phase I metabolism in urine and bile as their hydrophilic conjugates and generally results in detoxification, although a limited number of glucuronides possess biological activity (26).

### UGT Heterogeneity

Consistent with its broad substrate profile, UGT exists as an enzyme superfamily (4, 25). UGTs are evolutionarily related to enzyme families in bacteria and plants, which also use activated sugar nucleotides to donate monosaccharides to a substrate [(24); see also UGT homepage http://som.flinders.edu.au/FUSA/ClinPharm/UGT)]. The UGTs all contain a conserved 29-residue carboxy terminus "signature sequence," which probably contributes to the recognition and binding of the UDP-sugar (27). To date, nucleotide sequences encoding 18 UGT proteins of approximately 530 amino acids have been identified (Figure 2) (http://som.flinders.edu.au/FUSA/ClinPharm/UGT). Based on sequence identity, UGTs can be divided into two gene families: *UGT1* on human chromosome 2q37 and *UGT2* on human chromosome 4q13. The human UGT1 protein family consists of nine members with different amino-terminal domains but identical carboxyl termini (28). Each UGT1 protein is encoded by a transcript that is formed by the splicing of a distinct first

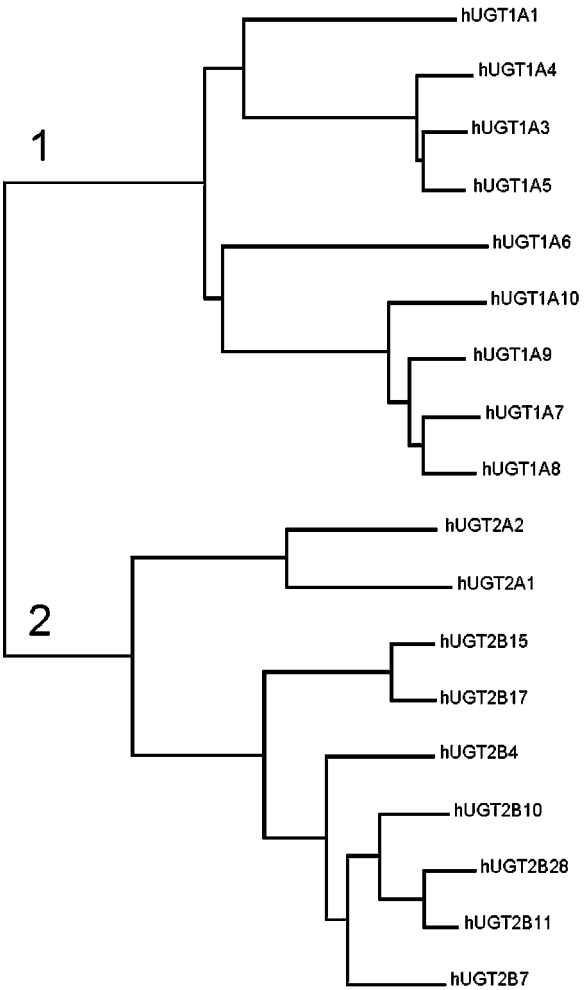


**Figure 2** Phylogram of human UGT proteins.

exon (A1, A3, A4, A5, A6, A7, A8, A9, and A10) to a set of four downstream exons, designated 2–5. Four other first exons on the *UGT1A* locus (A2, A11, A12, and A13) lack open reading frames and are designated as pseudogenes (29). In contrast to the UGT1 family, the UGT2 enzymes do not share a common carboxyl-terminal domain and are encoded by separate genes comprising six exons (27). The human UGT2 family is further subdivided into two subfamilies; UGT2A and UGT2B, which contain two (2A1, 2A2) and seven (2B4, 2B7, 2B10, 2B11, 2B15, 2B17, and 2B28) members, respectively. Five *UGT2B* pseudogenes (*24P–28P*) have additionally been identified.

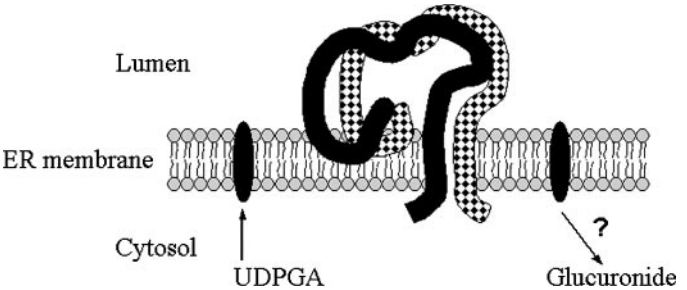


**Figure 3** Hypothetical UGT topology model depicting UGT as a dimer, with the active site localized on the lumenal aspect of the endoplasmic reticulum, and a UDP-glucuronic acid (UDPGA) transporter. Adapted from Reference 4.

## UGT Membrane Localization

The UGTs are resident in the endoplasmic reticulum (ER) and nuclear compartment of cells (4). Each enzyme is synthesized as a precursor of approximately 530 residues containing an amino terminal signal peptide that is removed during insertion of the protein into the ER membrane (Figure 3) (30). The mature protein of approximately 500 residues is oriented on the lumenal aspect of the ER membrane and contains an amino-terminal domain responsible for substrate selection and a carboxyl-terminal domain that binds UDPGA. Experimental evidence in support of this topology has been provided by studies with antibodies, proteases, detergents, and photoaffinity probes on microsomal UGTs and on UGTs synthesized in vitro in the presence and absence of membranes (reviewed in Reference 4).

Accumulating evidence strongly suggests that the active UGT enzyme consists of dimers of two UGT polypeptide chains (Figure 3). Catalytically active homodimers of the rat UGT2B1 that appear to interact through their amino-terminal regions have been detected (31). Homodimers of rat UGT1A6 (32), human UGT1A1 (33), and human UGT1A9 (34), and heterodimers of UGT2B1 and UGT1A6 (32) have also been detected by chemical cross-linking and coimmunopurification. Furthermore, it has been postulated that the stability of the interaction or the rates of dimerization may be governed by the specific UGT monomers involved and/or by interaction with substrate (4). It is possible that dimerization may impact on the kinetics of UGT-catalyzed reactions (discussed below), and incorporation of these possibilities into predictive models metabolism may prove necessary.

## Regulation of UGT Expression

The majority of UGT isoforms (including 1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, 2B15 2B17, and 2B28) are expressed in the liver. However, UGT1A and UGT2B isoforms are also differentially expressed in a range of other tissues, including the kidney, olfactory epithelium, ovary, lung, small intestine, mammary gland,

testis, and prostate (reviewed in Reference 25). Indeed, UGT1A8 and UGT1A10 are expressed exclusively in the gastrointestinal tract (35). In addition, current evidence suggests that the UGT2A forms are predominantly expressed in the nasal epithelium and may be involved in odorant signal termination (36). All UGTs investigated to date display marked interindividual variation in levels within a tissue. The mechanisms that determine UGT tissue distribution and content are largely unknown, although factors that regulate their levels in the liver are beginning to be elucidated (37, 38).

## UGT Isoform Substrate Selectivity

The majority of UGT isoforms exhibit distinct, but overlapping, substrate selectivities (4, 23, 25). For example, only UGT1A3 and UGT1A4 apparently conjugate tertiary amines, although selectivity towards other chemical classes differs. The exception appears to be UGT2B4, which glucuronidates the same substrates as UGT2B7 but with markedly lower activity (39). Despite considerable effort, substrates for UGT2B10 and UGT2B11 have not been identified to date. Although UGTs have been classified in the past according to the chemical characteristics of substrates, this approach is simplistic because most isoforms have the capacity to glucuronidate structurally diverse compounds. Studies of UGT isoform substrate selectivity have also tended to utilize the same "pool" of substrates (e.g., commercially available phenols and aliphatic alcohols), and only recently has the diversity of chemical space screened been increased to allow identification of isoform selective substrates.

## Factors Affecting UGT Activity In Vivo

Numerous factors are known to alter human UGT activity in vivo, including age (particularly the neonatal period), diet, disease states, drug-drug interactions (induction and inhibition), ethnicity, genetic polymorphism, and hormonal effects (23, 40). Apart from genetic polymorphism, however, the isoform selectivity of these influences is generally poorly understood owing to the unavailability of isoform-selective substrates for the investigation of drug glucuronidation in vivo. Nevertheless, available evidence (see below) and experience with CYP (8) suggests selectivity is highly likely. Genetic polymorphism has been reported for *UGT1A1*, *-1A6*, *-1A7*, *-1A8*, *-2B4*, *-2B7*, and *-2B15* (25, 40–45), although there is evidence for allelic variation in most *UGTs* (http://som.flinders.edu.au/FUSA/ClinPharm/UGT). Mutations in *UGT1A1*, *-1A7*, *-1A8*, and *-2B15* have been implicated as risk factors for certain cancers (41, 44, 46, 47). More than 50 mutations in *UGT1A1* may give rise to inherited disorders of bilirubin glucuronidation (Gilbert and Crigler-Najjar syndromes type I and II) and, in some instances, impaired elimination of xenobiotics (25, 44, 48). Patients with variant *UGT1A1* genotypes are overrepresented among those experiencing severe toxicity to the anticancer drug irinotecan owing to impaired glucuronidation of the active metabolite SN-38 (49). In addition, indinavir, which appears to be a UGT1A1 substrate, may precipitate jaundice in

patients with Gilbert syndrome variant alleles as a result of competitive inhibition of bilirubin conjugation (50). Thus, available evidence suggests that knowledge of the UGT isoform selectivity (substrate and inhibitor), particularly involvement of UGT1A1, is likely to be of benefit in assessing the potential effects of genetic polymorphism and drug-drug/endobiotic interactions on the elimination and toxicity of new chemical entities.

## REACTION PHENOTYPING IN VITRO

### Reaction Phenotyping Approaches

Approaches for CYP reaction phenotyping are based largely on the use of human liver microsomes as the enzyme source, although hepatocyte suspensions have also been used and include (5–9): (*a*) characterization of the effects of CYP isoform-selective inhibitors on the metabolism of the compound; (*b*) investigation of correlations between rates of metabolism of the compound and immunoreactive CYP isoform contents or prototypic isoform-selective activities in a "panel" of human liver microsomes; (*c*) competitive inhibition of the metabolism of isoform-selective substrates by the drug, with $K_m$ matching $K_i$; and (*d*) comparative metabolism by recombinant human CYP isoforms. Taken together, these procedures allow identification of the CYP isoform(s) responsible for the metabolism of a drug with a high degree of certainty, although inhibition by isoform-selective inhibitors alone is often considered diagnostic, providing experimental conditions for inhibitor selectivity are well established (5, 51). Thus, the availability of isoform selective substrates and inhibitors is pivotal to reaction phenotyping in vitro. Examples of CYP isoform selective substrates and inhibitors include phenacetin and furafylline for CYP1A2 and tolbutamide and sulfaphenazole for CYP2C9 (5). Moreover, substrates that may be safely administered to humans are necessary for the characterization of isoform regulation and drug-drug interactions in vivo. Thus, such probes are most commonly clinically used drugs.

### Reaction Phenotyping of UGT Substrates

Although numerous compounds are known to inhibit human UGT activity in vitro and in vivo (52), no UGT isoform selective inhibitors have been identified to date. However, reasonably selective substrates are now available for a number of isoforms, including the more important drug metabolizing hepatic UGTs. Clearly, these may also be used as isoform selective inhibitors in vitro.

It is well established that bilirubin is glucuronidated predominantly, if not solely, by UGT1A1, with a $K_m$ of 24 $\mu$M (48, 53). Impaired UGT1A1 activity owing to genetic polymorphism or drug interactions may give rise to clinically significant hyperbilirubinemia (48–50). In addition, it has been proposed that ethinylestradiol and estradiol are selective substrates for UGT1A1 (54–56). Although estradiol 3-glucuronidation appears to be mainly catalyzed by UGT1A1, other isoforms

(e.g., UGT1A3, -1A9, and -2B7) may also metabolize these compounds, and the absolute contribution of UGT1A1 to human hepatic ethinylestradiol and estradiol glucuronidation requires further characterization. As noted previously, both UGT1A3 and UGT1A4 have the capacity to glucuronidate tertiary amines. However, only UGT1A4 appears to catalyze the N-glucuronidation of imipramine and trifluoperazine (57, 58). A later study further demonstrated that imipramine is not a substrate for other UGT1A family isoforms or for UGT2B7 and UGT2B15 (59), but a role for additional isoforms in trifluoperazine glucuronidation remains to be discounted. Although acetaminophen (paracetamol) glucuronidation has been used as a probe for UGT1A6 activity, multiple isoforms probably contribute to this pathway (60). More recently, it has been shown that serotonin (5-hydroxytryptamine) is glucuronidated only by UGT1A6, and hence, this compound represents a selective, albeit low-affinity ($K_m$ approximately 5 mM), substrate for this isoform (61). Early studies established that propofol was a substrate of UGT1A9 ($K_m$ 170 $\mu$M), but not UGT1A1, -1A4, or -1A6 (54). Subsequent studies have also excluded UGT1A3, -1A10, and -2B15 as catalysts of propofol glucuronidation. Although propofol is a good substrate for UGT1A8 ($K_m$ 412 $\mu$M) (62), this isoform is not expressed in human liver.

Within the UGT2B subfamily, UGT2B7 is the only isoform for which selective substrates have been identified. UGT2B7 is apparently the only member of this subfamily that glucuronidates zidovudine ($K_m$ 82 to 91 $\mu$M) (63). Recent studies in this laboratory have also excluded metabolism of zidovudine by UGT1A family enzymes and indicate that the $K_m$ for glucuronidation by UGT2B7 is higher (i.e., 400 $\mu$M) than originally reported (J.O. Miners & P.I. Mackenzie, unpublished data). UGT2B7 also appears to be the principal hepatically expressed UGT involved in the glucuronidation of epirubicin (4-epi-doxorubicin) (64), although the involvement of UGT1A8 and -1A10 cannot be excluded in other tissues. Although commonly used as a UGT2B7 selective substrate, morphine is glucuronidated in the 3-position by other isoforms, including UGT1A1, -1A3, -1A6, -1A9, and -1A10, and there is some evidence to suggest the involvement of multiple UGTs in human liver microsomal morphine 3-glucuronidation (65). However, morphine 6-glucuronidation is catalyzed solely by UGT2B7 (65). Hyodeoxycholic acid has been proposed as a selective substrate for UGT2B4, but this compound is glucuronidated more efficiently by UGT2B7 (66). Indeed, UGT2B4 and UGT2B7 have similar substrate selectivities, but turnover by the latter is generally at least one to two orders of magnitude higher (39, 66). Similarly, recent experience suggests that many reported UGT2B15 substrates (67) are also effectively glucuronidated by UGT2B7 [(68); J.O. Miners & P.I. Mackenzie, unpublished data].

It is apparent that the experimental tools necessary for reaction phenotyping UGT substrates are becoming increasingly available in parallel with increasing awareness of the importance of this enzyme in drug metabolism. However, significant problems remain. The use of alternate substrates as inhibitors requires careful selection of the concentration used in incubations of human liver microsomes or

other tissue preparations because the degree of inhibition will be dependent on the concentrations of substrate and inhibitor relative to their respective $K_m$ values. Indeed, the successful reaction phenotyping of CYP substrates is critically dependent on the careful selection of substrate/inhibitor concentration and incubation conditions (5). Furthermore, it is not possible to discount at this stage that the selective substrates identified to date act as inhibitors of other UGTs, as is the case with quinidine (a CYP3A substrate) inhibition of CYP2D6. The lack of a convenient substrate/inhibitor probe for UGT1A1, which appears to have an important role in drug metabolism, remains a problem. Bilirubin has only moderate stability, and assays for the measurement of bilirubin glucuronidation in vitro are difficult. It should also be recognized that many isoform "selective" substrates have not been screened with all known UGTs. In particular, the xenobiotic substrate selectivity of UGT2B28 is largely unknown (68) and the involvement of UGT1A5, -1A7, -1A8, -1A10, -2B4, and -2B17 in drug and xenobiotic metabolism is not commonly investigated. With the exception of UGT2B17 and -2B28, however, these forms are expressed in extrahepatic tissues and/or exhibit low activity toward xenobiotics, and a significant role in hepatic drug clearance can generally be discounted. It is also apparent that the selective substrates referred to previously are of limited value for characterizing isoform regulation in vivo. These compounds are either endobiotics or they are drugs that are unsuitable for administration to healthy volunteers (owing to their inherent pharmacological properties and/or toxicity) and which have undesirable pharmacokinetic properties or low clearance by glucuronidation in vivo (e.g., imipramine).

Given the limited availability of isoform selective substrates and inhibitors, reaction phenotyping of UGT substrates has most commonly involved screening for activity by recombinant isoforms, for example, References 69, 70. Although this may yield useful results where only a single isoform is identified, interpretation is difficult where multiple UGTs are involved (71). Whereas CYP expression in vitro may be quantified spectrophotometrically, assessment of the relative levels of UGTs in expression systems remains problematic (being dependent on the interpretation of Western blots). In addition, the relative expression of UGT isoforms in vivo is currently unknown. Hence, the use of a relative activity factor or scaling for isoform expression in human liver, approaches used for CYP-catalyzed reactions (6, 7), are currently not feasible for UGT reaction phenotyping.

## IN VITRO–IN VIVO CORRELATION

### The Predicability of In Vitro Drug Glucuronidation Kinetic Data

As described previously, in vitro–in vivo correlation most commonly involves scaling of the $CL_{int}$ value determined for a reaction in human liver microsomes to in vivo $E_H$ and $CL_H$ (Figure 1). Although this approach has proved useful for many CYP-catalyzed reactions, recent studies have demonstrated that extrapolated $CL_H$

consistently underestimates known clearance by glucuronidation in vivo (72–74). The relationship between in vivo blood clearance by glucuronidation and predicted $CL_H$ (from the scaling of human liver microsomal $CL_{int}$ using the dispersion model) for 14 glucuronidated drugs is shown in Figure 4. Predicted and known in vivo $CL_H$ are significantly correlated ($r^2 = 0.53$, $p < 0.01$), but predicted $CL_H$ underestimates known glucuronidation clearance by a factor of 11.2. Exclusion of the data for propofol and naloxone (predicted $CL_H$ values 1.4 and 3.4 l/h, respectively) improves the correlation markedly (slope $= 0.136$, $r^2 = 0.95$, $p < 0.001$). Mistry & Houston previously reported similar findings for glucuronidated opioids in rat, where hepatic microsomal $CL_{int}$ values were 20- to 30-fold lower than their in vivo counterparts (75). The data shown in Figure 4 suggest that it may be possible to apply a scaling factor to the extrapolated $CL_H$ in order to predict in vivo glucuronidation clearance, but further validation of this approach is clearly required. Interestingly, a linear relationship was reported for the rate of glucuronidation of a series of 5-lipoxygenase inhibitors by cynomolgus monkey microsomes and

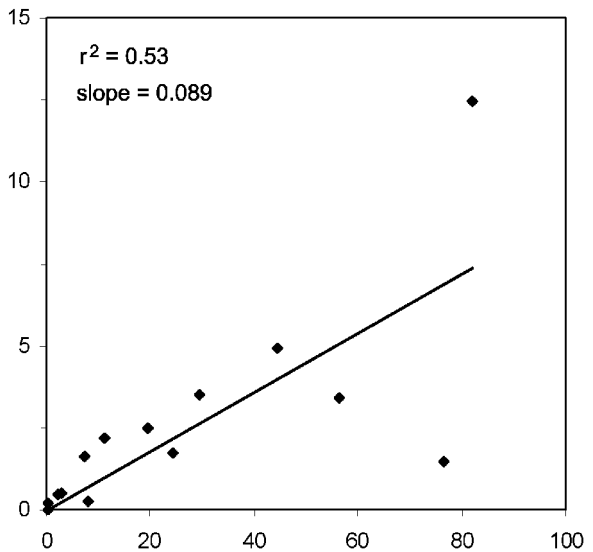


**Figure 4** Correlation between the in vivo blood clearances by glucuronidation and hepatic clearances predicted from published human liver microsomal $CL_{int}$ values (using the dispersion model) for amitriptyline, clofibric acid, 5,6-dimethylxanthenone-4-acetic acid, lamotrigine, morphine, naloxone, naproxen, propofol, valproic acid, zidovudine (as reported in Reference 72), codeine, ethinylestradiol, gemfibrozil (as reported in Reference 73), and dihydroartemisinin (reported in Reference 74). $K_m$ values used for the calculation of the $CL_{int}$ values for amitriptyline, ethinylestradiol, gemfibrozil, and propofol were corrected for nonspecific binding to human liver microsomes using literature values of $fu_{inc}$ (16, 73).

in vivo plasma clearance in this species (76), which provided the basis for selection of an analogue with improved metabolic stability.

## Factors Influencing Drug Glucuronidation Kinetics In Vitro

The reason(s) for the underestimation of in vivo $CL_H$ from in vitro kinetic data remain unclear. The possibility remains that the assumptions underpinning the mathematical models of $CL_H$ are not applicable to drug glucuronidation by human liver microsomes. As noted earlier, UGT is localized on the lumenal side of the microsomal membrane and this may give rise to "diffusional" barriers. Indeed, Soars et al. reported that $CL_{int}$ values generated using human hepatocytes as the enzyme source predicted glucuronidation clearance in vivo reasonably well and speculated that transport of drug substrates may be more limited in microsomes than in intact hepatocytes (73). It has also been suggested that renal glucuronidation may be a major contributor to drug glucuronidation in vivo, but this seems unlikely (discussed below). Nonspecific microsomal binding presents another confounding factor in the calculation of kinetic constants for human liver microsomal drug metabolism reactions, leading to overestimation of $K_m$ and, hence, underprediction of $CL_{int}$ (16). However, nonspecific binding is minor for most of the drugs reported in Figure 4 and was accounted for when known to be significant (e.g., propofol).

The quality of the in vitro kinetic data used for in vitro–in vivo extrapolation warrants special consideration. The success of predictions of in vivo $CL_H$ is critically dependent on in vitro $CL_{int}$ and how closely the kinetic parameters ($K_m$, $V_{max}$) used to derive this parameter reflect enzyme activity in vivo. Incubation components are known to modulate microsomal UGT activity, and the kinetics of drug glucuronide formation therefore varies with experimental conditions. In particular, we have demonstrated that the kinetics of human liver microsomal zidovudine glucuronidation are dependent on buffer type, pH, and ionic strength, and on the presence of $Mg^{2+}$, detergent, alamethacin, and the endogenous activator UDP-N-acetylglucosamine (72). Depending on incubation conditions, in vitro $CL_{int}$ varied almost sixfold. Because investigations of UGT activity in vitro often employ widely differing reaction conditions (77), comparison of drug glucuronidation kinetic data between laboratories is frequently not meaningful.

## Drug Glucuronidation Kinetics In Vitro

The interpretation and analysis of drug glucuronidation kinetic data further impacts on the reliability and predictive value of in vitro $CL_{int}$. Until recently, kinetic plots of drug glucuronidation by human liver microsomes or recombinant isoforms were published infrequently and data were generally uncritically fitted to the Michaelis-Menten equation to obtain estimates of $K_m$ and $V_{max}$. However, there is increasing evidence demonstrating that drug glucuronidation reactions in vitro commonly exhibit atypical kinetic behavior. Estradiol 3-glucuronidation by human

liver microsomes (believed to involve UGT1A1; see previous discussion) and 1-naphthol glucuronidation by recombinant UGT1A1 follow sigmoidal kinetics characteristic of autoactivation (56, 78, 79). However, UGT1A1-catalyzed 4-methylumbelliferone glucuronidation follows hyperbolic (Michaelis-Menten) kinetics (79). Consistent with these observations, alternate UGT1A1 substrates variably caused activation or inhibition of human liver microsomal estradiol 3-glucuronidation (78). Recent studies in this laboratory have also provided evidence for multiple kinetic mechanisms in UGT2B7-catalyzed xenobiotic glucuronidation. Morphine 3- and 6-glucuronidation by recombinant UGT2B7 exhibit biphasic kinetics suggestive of "negative cooperativity" (65), whereas 4-methylumbelliferone and zidovudine glucuronidation by this enzyme follow sigmoidal and hyperbolic kinetics, respectively [(63); J.O. Miners & P.I. Mackenzie, unpublished data]. However, not only may glucuronidation kinetic models vary between substrates for the same isoform, but kinetic behavior varies between isoforms for the glucuronidation of a common substrate. For example, we have observed hyperbolic (Michaelis-Menten), substrate inhibition and sigmoidal (homotropic positive cooperativity) kinetics for the glucuronidation of the nonselective substrate 4-methylumbelliferone by UGT1A1, UGT1A3, and UGT2B7, respectively (Figure 5) [(79); J.O. Miners & P.I. Mackenzie, unpublished data].

These observations are consistent with the existence of allosteric effector sites or the simultaneous binding of two substrate molecules to the active site, mechanisms that have been proposed for CYP3A4-catalyzed reactions. Alternatively, UGTs may act as cooperative ligand-binding multisubunit enzymes because there is evidence indicating at least some UGT isoforms exist as dimers (see previous discussion). Irrespective of the mechanism, it is clear that model-fitting is essential for the description and kinetic analysis of UGT-catalyzed reactions, and multisite models developed for CYP3A4 (80, 81) are likely to prove useful in this regard. A

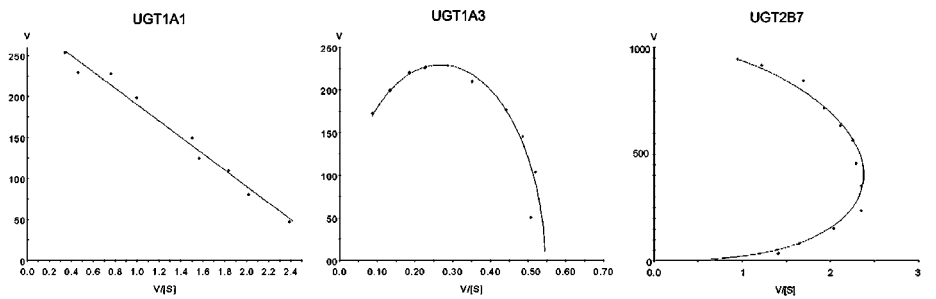


**Figure 5** Eadie-Hofstee plots for 4-methylumbelliferone glucuronidation by UGT1A1, UGT1A3, and UGT2B7. Points show experimentally derived values. Curves of best fit were generated using expressions for hyperbolic (UGT1A1), substrate inhibition (UGT1A3), and homotropic positive cooperativity (UGT2B7) models. Units: rates of reaction (v) as pmol glucuronide formed/min mg and substrate concentration ([S]) as $\mu$M.

typical glucuronidation kinetics further complicate in vitro–in vivo extrapolation as the in vivo correlate of nonhyperbolic kinetics in vitro remains obscure (80).

### Extrahepatic Drug Glucuronidation

UGTs are widely distributed throughout the body. Apart from the liver, UGT1A3, -1A9, and -2B7 are expressed in kidney and multiple isoforms are expressed throughout the gastrointestinal tract (25, 42). Not surprisingly, UGT activity is readily measurable in human kidney and gastrointestinal tract and this has led to the proposal that these organs contribute significantly to the systemic clearance and first-pass extraction, respectively, of glucuronidated drugs (73, 82–84). In particular, the rates of glucuronidation of a number of drugs by human kidney microsomes are at least comparable to those of human liver microsomes (73, 82, 84). However, in proposing a major role for the kidney in systemic drug clearance, such studies have failed to recognize that scaling factors for renal and hepatic kinetic parameters differ, and when relative organ weights and microsome yields are taken into account, metabolic clearance by the kidney appears to be low (85). Interpretation of drug glucuronidation kinetic data by human small intestine similarly requires careful interpretation because UGT activity and microsome yield vary between duodenum, jejunum, and ileum (42, 86). Nevertheless, comparison of $CL_{int}$ values derived using liver, kidney, and intestinal microsomes may provide important insights into the relative contribution of these organs to $CL_H$ and/or first-pass extraction (85) and the role of glucuronidation as a local detoxification mechanism.

## IN SILICO REACTION PHENOTYPING

Computational methods potentially provide a facile and economic alternative to in vitro reaction phenotyping approaches. In silico reaction phenotyping utilizes pattern recognition techniques to elucidate a set of chemical properties (descriptors) associated with the binding and metabolism of substrates by an enzyme. The two- and three-dimensional (2D and 3D) descriptors employed represent chemical features extracted by a well-defined algorithm from a molecular representation of a complex system (87).

### Implicit Classification

If a set of descriptors is common to the known substrates of an enzyme, the presence of these descriptors in other compounds can be used to classify them as substrates of that enzyme by implication. Descriptors used to imply classification are commonly found using pharmacophore elucidation or the development of 2D-QSAR. Pharmacophores, which represent a configuration of common structural features associated with biological activity (in this case, metabolism by an isoform), provide one of the most intuitive 3D descriptors used for reaction

phenotyping. Ideally, a pharmacophore should be unique for a particular isoform. Adoption of in silico reaction phenotyping for compounds metabolized by CYP has progressed in parallel with the increasing availability of isoform-selective substrates and inhibitors, which are necessary for pharmacophore generation, and has additionally been aided by the availability of homology models based on the X-ray crystal structures of bacterial and mammalian P450s (21, 22, 88, 89). As discussed previously, the substrate selectivities of UGT isoforms have begun to approach interpretable levels and, hence, a basis now exists for pharmacophore and QSAR modeling of UGT substrates. Indeed, such approaches assume particular relevance in the absence of a UGT X-ray crystal structure.

Recent studies in this laboratory resulted in development of the first 2D-QSAR, 3D-QSAR, and pharmacophore models for substrates of human UGT isoforms (79, 90, 91). In generating pharmacophores for UGTs, it became apparent that it was essential to overlay the glucuronidation sites of the individual substrates to obtain catalytically sensible alignments. The benefits of incorporating sites of metabolism have similarly been highlighted in a recent evaluation of pharmacophores for substrates of CYP2B6 (92). Common features pharmacophores, which included an essential "glucuronidation feature," have been developed for UGT1A1, UGT1A4, and UGT1A9 (79, 90, 91), and these are shown in Figure 6. Owing to their similarity, the utility of the pharmacophores for reaction phenotyping substrates of the individual isoforms is probably limited, although the pharmacophores do provide important insights into the binding requirements of UGT1A family enzymes more generally. The site of glucuronidation is invariably adjacent to a hydrophobic region, with another hydrophobic domain located 6 to 8 Å from the site of conjugation. A hydrogen-bond acceptor near the distal hydrophobe differentiates UGT1A9 substrates, but high-affinity substrates for UGT1A4, and possibly UGT1A1, may additionally benefit from hydrogen-bonding interactions (90). The common "core" features associated with each pharmacophore represent a molecular basis for the overlapping substrate selectivities characteristic of UGT1A isoforms.

There are two important limitations of pharmacophores that impact on their application to UGTs. First, it is assumed that the substrates used to define the pharmacophore share the same binding mode (93). The atypical glucuronidation kinetics observed for some UGT substrates and known ability of UGT isoforms to glucuronidate poly-functional substrates at multiple sites [for example, clozapine; (94)] indicate that this may not always be the case. Second, the presence or absence of pharmacophoric features in nonsubstrates is seldom investigated, and, hence, it remains unclear whether this approach can differentiate substrates from nonsubstrates.

Not uncommonly, descriptors characteristic of substrates are extracted from a QSAR, which is a quantitative relationship between descriptors and an activity measurement. Two-dimensional descriptors characteristic of human CYP isoforms have been identified from 2D-QSAR developed using kinetic constants, such as $K_m$ and $K_i$ (95), allowing differentiation of CYP1, -2A, -2B, -2C, -2D, -2E, and

-3 substrates on the basis of physicochemically interpretable descriptors, such as $pK_a$, logP, and collision diameter. Again, this approach does not necessarily aid the distinction of substrates and nonsubstrates.

## Explicit Classification

In combination with known isoform substrate selectivities, carefully selected and relevant nonsubstrate data provide valuable information when classification algorithms are used to probe explicit differences between the two sets, and this represents another approach to in silico reaction phenotyping. Afzelius et al. reported the use of partial least squares discriminant analysis (PLS-DA) to classify inhibitors and noninhibitors of CYP2C9, with an accuracy of 74% (96). Nonlinear classification algorithms potentially provide greater flexibility and generalization performance (97). For example, an artificial neural network (ANN) based on 2-D Unity "fingerprints" has been developed that recognized CYP3A4 inhibitors with 89% accuracy (98). Nonlinear Support Vector Machine (SVM) algorithms, which have been successfully applied to classifying drug CNS-permeability (99), provide another option for reaction phenotyping.

A comprehensive database of all reported substrates and nonsubstrates of human UGT isoforms has been compiled in this laboratory to investigate the utility of various classification techniques for UGT reaction phenotyping. PLS-DA, ANN, and SVM were compared using 2D chemical descriptors generated for substrates and nonsubstrates of twelve human UGT isoforms (100). Using SVM, predictability was excellent (>80% accuracy) for five isoforms and good (63%–80% accuracy) for the other seven, confirming the potential value of this approach. The variability between isoforms probably reflects differences in the size and structural diversity of the datasets.

As noted previously, there is evidence to suggest multiple binding modes for UGT substrates. Multiple pharmacophores have been utilized to characterize molecular recognition by a number of xenobiotic binding proteins (101–103) and recent studies in this laboratory investigated whether an "ensemble" of pharmacophores, which define the chemical features relevant to each of the possible binding modes, would prove useful for UGT substrate reaction phenotyping (M.J. Sorich, J.O. Miners, R.A. McKinnon & P.A. Smith, manuscript in preparation). Employing concepts developed for pharmacophore fingerprinting (104, 105) and using pattern-recognition techniques to select subsets of pharmacophores associated with substrates and nonsubstrates, models that were more intuitive but marginally less predictive than classification using 2D descriptors were generated. In addition, it was observed that a number of the pharmacophores selected as important included simple chemical features. Further analysis demonstrated significant isoform-related differences in the prevalence of nucleophilic functional groups (e.g., phenol, hydroxyl, carboxyl, imidazole, and primary, secondary, and tertiary amine function) in substrates. These simple, intuitive features could be used for classification with equal or better accuracy than approaches using 2D descriptors or pharmacophore fingerprints. This method is likely to prove most valuable when

conjugative regioselectivity is included. In this regard, nucleophile preferences between isoforms may well correlate with quantum chemical descriptors. Importantly, advances in the prediction of CYP regioselectivity using quantum chemical descriptors (106, 107) is in no small part due to the availability of training data derived from thorough characterization of metabolite regioselectivity. Diligence in the characterization of conjugative regioselectivity is required in order to realize similar advances with in silico UGT reaction phenotyping.

## IN SILICO–IN VITRO PREDICTION

As discussed previously, the kinetic parameters $K_m$, $V_{max}$, and $CL_{int}$ underpin the in vitro–in vivo extrapolation of kinetic data for drugs eliminated by hepatic metabolism. Kinetic parameters generated in silico may potentially be used as surrogates for experimentally derived values, thereby precluding the requirement for time-consuming and expensive in vitro studies using human tissues and/or recombinant enzymes. Both 2D- and 3D-QSARs have been generated for numerous CYP isoforms that allow prediction of $K_m$ (or $K_i$ in the case of inhibitors) (21, 22, 95). However, in most instances, predictability varies by up to 1 log order.

UGT QSMR were developed initially from activity data using liver microsomes from various species as the enzyme source, and include the pioneering studies of Bray et al. (108) and Hansch et al. (109) through to later mechanistic (110) and kinetic investigations (111). More recently, studies have been conducted with recombinant UGTs, although these have tended to investigate QSMR for compounds from the same chemical class (for example, References 112, 113). Reports from this laboratory represent the first attempts to develop UGT QSMR from structurally diverse compounds. Linear 2D-QSAR approaches were found to outperform pharmacophore and molecular field-based 3D-QSAR alignment methods for UGT1A1 and UGT1A4 (79, 90), with the 2D-QSAR generally predicting the $K_m$ for substrates of these enzymes within 0.5 log order. Interestingly, although the generation of predictive models was possible with UGT1A1 and UGT1A4, attempts to develop predictive QSMR for substrates of UGT1A9 were unsuccessful (91).

Further development of QSMR capable of predicting the kinetic parameters of glucuronidated compounds is problematic and complicated by several factors. Data sets available for modeling are generally limited in size and vary in quality. As discussed above, kinetic constants published by different laboratories are frequently not comparable owing to the use of differing incubation conditions. The atypical kinetic behavior of many glucuronidation reactions further confounds data generation and selection for QSMR modeling. Indeed, classification of kinetic mechanism would appear to be a necessary precursor to the modeling of $K_m$ and other parameters. Efforts to date have focused solely on prediction of $K_m$, but $V_{max}$ is also required for calculation of $CL_{int}$. Development of a 2D-QSAR for prediction of $V_{max}$ has been reported recently for the CYP2E1-catalyzed metabolism of a series of alkylbenzene derivatives (i.e., a “local” model suitable only for prediction of $V_{max}$ values for structurally related molecules) (114). The limited

UGT kinetic data currently available similarly lends itself only to the development of local models. Increasingly, however, there is a requirement for "global" or generalizable ADMET models (115, 116). Given the inherent complexity of the physiological processes involved and the vastness of the chemical space to be investigated, sophisticated pattern recognition techniques and large, quality data sets will undoubtedly be required to generate globally predictive models in future.

## CONCLUSIONS

The UGT reaction phenotyping of glucuronidated drugs and other compounds using human liver microsomal preparations and possibly isolated hepatocytes is feasible, although currently limited in scope by the relatively few isoform selective "probes" available. However, the number of isoform-selective substrates and inhibitors will undoubtedly increase as increasing numbers of structurally diverse UGT substrates are identified and characterized. Isoform-specific inhibitory antibodies may provide another option in this regard. Quantitative characterization of the expression of isoforms in liver and other tissues will similarly improve the interpretation of relative activity measurements obtained using recombinant UGT isoforms. Available evidence suggests that quantitative prediction of $CL_H$ and $E_H$ from human liver microsomal kinetic data is unlikely to be successful, although the application of a scaling factor may at least differentiate high- and low-$CL_H$ glucuronidated drugs. Further exploration of this option is warranted, as is the utility of isolated human hepatocytes for the generation of kinetic parameters that may be accurately extrapolated. Irrespective of the enzyme source, however, atypical kinetic behavior may confound meaningful in vitro–in vivo correlation for many glucuronidated compounds. Apart from in vitro approaches, in silico modeling shows promise for the reaction phenotyping of UGT substrates. In particular, substrates and nonsubstrates of individual UGTs may be characterized using 2-D descriptors and combinations of pharmacophores, the latter incorporating "site of conjugation" features, which account for multiple, catalytically meaningful binding modes and facilitate model interpretability. Flexible nonlinear classification algorithms, such as SVM, are most suited to delineating the complex relationships between chemical structure and glucuronidation (or absence thereof) by an isoform. The UGT QSMR field is in its infancy. Although the recent generation of 2D- and 3D-QSAR, which predict the $K_m$ values of substrates of UGT1A1 and UGT1A4, demonstrates the potential utility of this approach, the development of global UGT QSMR will ultimately require large data sets that encompass the multiple processes associated with metabolism by UGT.

### ACKNOWLEDGMENT

Studies conducted in the authors' laboratories were supported by the National Health & Medical Research Council of Australia.

**The *Annual Review of Pharmacology and Toxicology* is online at http://pharmtox.annualreviews.org**

## LITERATURE CITED


1. DiMasi JA. 2001. Risks in new drug development: approval success rates for investigational drugs. *Clin. Pharmacol. Ther.* 69:297–307
2. Prentis RA, Lis Y, Walker SR. 1988. Pharmaceutical innovation by the seven UK-owned drug companies (1964–1985). *Br. J. Clin. Pharmacol.* 25:387–96
3. Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, et al. 1996. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* 6:1–42
4. Radominska-Pandya A, Battaglia E, Mackenzie PI. 1999. Structural studies of UDP-glucuronosyltransferases. *Drug Metab. Rev.* 31:817–99
5. Miners JO, Veronese ME, Birkett DJ. 1994. In vitro approaches for the prediction of human drug metabolism. *Ann. Rep. Med. Chem.* 29:307–16
6. Rodrigues AD. 1999. Integrated cytochrome P450 reaction phenotyping. Attempting to bridge the gap between cDNA-expressed cytochromes P450 and native human liver microsomes. *Biochem. Pharmacol.* 57:465–80
7. Clarke SE. 1998. In vitro assessment of human cytochrome P450. *Xenobiotica* 28:1167–202
8. Miners JO. 2002. Hitchhiking the technology bandwagon: the evolution of drug metabolism. *Clin. Exp. Pharmacol. Physiol.* 29:1040–44
9. Venkatakrishnan K, von Moltke LL, Greenblatt DJ. 2001. Human drug metabolism and the cytochromes P450: application and relevance of in vitro models. *J. Clin. Pharmacol.* 41:1149–79
10. Houston JB. 1994. Utility of in vitro drug metabolism data in predicting in vivo metabolic clearance. *Biochem. Pharmacol.* 47:1469–79
11. Wilkinson GR, Shand DG. 1975. A physiological approach to hepatic drug clearance. *Clin. Pharmacol. Ther.* 18:377–90
12. Pang KS, Rowland M. 1977. Hepatic clearance of drugs. I. Theoretical considerations of a well-stirred model and a parallel-tube model. Influence of hepatic blood flow, plasma and cell binding, and the hepatocellular enzymatic activity on hepatic drug clearance. *J. Pharmacokinet. Biopharm.* 5:625–53
13. Roberts M, Rowland M. 1986. Correlation between in vitro microsomal enzyme activity and whole organ hepatic elimination kinetics: analysis with a dispersion model. *J. Pharm. Pharmacol.* 38:177–81
14. Iwatsubo T, Hirota N, Ooie T, Suzuki H, Shimada N, et al. 1997. Prediction of in vivo drug metabolism in human liver from in vitro metabolism data. *Pharmacol. Ther.* 73:147–71
15. Ito K, Iwatsubo T, Kanamitsu S, Nakajima Y, Sugiyama Y. 1998. Quantitative prediction of in vivo drug clearance and drug interactions from in vitro data on metabolism together with binding and transport. *Annu. Rev. Pharmacol. Toxicol.* 38:461–99
16. McLure JA, Miners JO, Birkett DJ. 2000. Nonspecific binding of drugs to human liver microsomes. *Br. J. Clin. Pharmacol.* 49:453–61
17. Obach RS. 1997. Nonspecific binding to microsomes: impact on scale-up of in vitro intrinsic clearance to hepatic clearance as assessed through examination of warfarin, imipramine and propranolol. *Drug Metab. Dispos.* 25:1359–69
18. Naritomi Y, Terashita S, Kimura S, Suzuki

A, Kagayama A, Sugiyama Y. 2001. Prediction of human hepatic clearance from in vivo animal experiments and in vitro metabolic studies with human liver microsomes from animals and humans. *Drug Metab. Dispos.* 29:1316–24

19. Ito K, Kanamitsu S, Ueda K, Sugiyama Y. 1998. Prediction of pharmacokinetic alterations caused by drug-drug interactions: metabolic interaction in the liver. *Pharmacol. Rev.* 50:387–411
20. von Moltke LL, Greenblatt DJ, Schmider J, Wright CE, Harmatz JS, Shader RI. 1998. In vitro approaches to predicting drug interactions in vivo . *Biochem. Pharmacol.* 55:113–22
21. Ekins S, Boulanger B, Swann PW, Hupcey AZ. 2002. Towards a new age of virtual ADME/TOX and multidimensional drug discovery. *J. Comput.-Aid. Mol. Des.* 16:381–401
22. de Groot MJ, Ekins S. 2002. Pharmacophore modelling of cytochromes P450. *Adv. Drug Deliv. Rev.* 54:367–83
23. Miners JO, Mackenzie PI. 1991. Drug glucuronidation in humans. *Pharmacol. Ther.* 51:347–69
24. Mackenzie PI, Owens IS, Burchell B, Bock KW, Bairoch I, et al. The UDP-glycosyltransferase gene superfamily: recommended nomenclature update based on evolutionary divergence. *Pharmacogenetics* 7:255–69
25. Tukey RH, Strassburg CP. 2000. Human UDP-glucuronosyltransferases: metabolism, expression and disease. *Annu. Rev. Pharmacol. Toxicol.* 40:581–616
26. Ritter JK. 2000. Roles of glucuronidation and UDP-glucuronosyltransferases in xenobiotic bioactivation reactions. *Chem.-Biol. Interact.* 129:171–93
27. Mackenzie PI. 1995. Molecular aspects of UDP glucuronosyltransferases. In *Reviews in Biochemical Toxicology*, ed. E Hodgson, JR Bend, RM Philpot, pp. 29–72. Raleigh, NC: Toxicol. Commun.
28. Owens IS, Ritter JK. 1992. The novel bilirubin/phenol UDP-glucuronosyltransferase UGT1 gene locus: implications for multiple nonhemolytic familial hyperbilirubinemia phenotypes. *Pharmacogenetics* 2:93–108
29. Gong QH, Cho JW, Huang T, Potter C, Gholami N, et al. 2001. Thirteen UDP glucuronosyltransferase genes are encoded at the human UGT1 gene complex locus. *Pharmacogenetics* 11:357–68
30. Mackenzie PI. 1984. Cleavage of nascent UDP glucuronosyltransferase for rat liver by dog pancreatic microsomes. *Biochem. Biophys. Res. Commun.* 122:1441–49
31. Meech R, Mackenzie PI. 1997. UDP-glucuronosyltransferase, the role of the amino terminus in dimerization. *J. Biol. Chem.* 272:26913–17
32. Ikushiro S, Emi Y, Iyanagi T. 1997. Protein-protein interactions between UDP-glucuronosyltransferase isozymes in rat hepatic microsomes. *Biochemistry* 36:7154–61
33. Ghosh SS, Sappal BS, Kalpana GV, Lee SW, Chowdhury JR, Chowdhury NR. 2001. Homodimerization of human bilirubin-uridine-diphosphoglucuronate glucuronosyltransferase-1 (UGT1A1) and its functional implications. *J. Biol. Chem.* 276:42108–15
34. Kurkela M, Garcia-Horsman JA, Luukkanen L, Morsky S, Taskinen J, et al. 2003. Expression and characterization of recombinant human UDP-glucuronosyltransferases (UGTs). *J. Biol. Chem.* 278: 3536–44
35. Mojarrabi B, Mackenzie PI. 1998. Characterization of two UDP-glucuronosyltransferases that are predominantly expressed in human colon. *Biochem. Biophys. Res. Commun.* 247:704–9
36. Heydel JM, Leclerc S, Bernard P, Pelczar H, Gradinaru D, et al. 2001. Rat olfactory bulb and epithelium UDP-glucuronosyltransferase 2A1 (UGT2A1) expression: in situ mRNA localization and quantitative analysis. *Brain Res. Mol. Brain Res.* 90:83–92
37. Ishii Y, Hansen AJ, Mackenzie PI. 2000.

Octamer transcription factor-1 enhances hepatic nuclear factor-1α mediated activation of the human UDP-glucuronosyltransferase 2B7 promoter. *Mol. Pharmacol.* 57:940–47
38. Gregory PA, Mackenzie PI. 2002. The homeodomain Pbx2-Prep1 complex modulates hepatocyte nuclear factor 1α-mediated activation of the UDP-glucuronosyltransferase 2B17 gene. *Mol. Pharmacol.* 62:154–61
39. Jin C-J, Mackenzie PI, Miners JO. 1997. The regio- and stereo-selectivity of C19 and C-21 steroid glucuronidation by UGT2B7 and UGT2B11. *Arch. Biochem. Biophys.* 341:207–11
40. de Wildt SN, Kearns GL, Leeder JS, van den Anker JN. 1999. Glucuronidation in humans: pharmacogenetic and developmental aspects. *Clin. Pharmacokinet.* 36:439–52
41. Bhasker CR, McKinnon W, Stone A, Lo ACT, Kubota T, et al. 2000. Genetic polymorphism of UDP-glucuronosyltransferase 2B7 (UGT2B7) at amino acid 268: ethnic diversity of alleles and potential clinical significance. *Pharmacogenetics* 10:670–85
42. Tukey RH, Strassburg CP. 2001. Genetic multiplicity of the human UDP-glucuronosyltransferases and regulation in the gastrointestinal tract. *Mol. Pharmacol.* 59:405–14
43. Guillemette C, Ritter JK, Auyeung DJ, Kessler FK, Houseman DE. 2000. Structural heterogeneity at the UDP-glucuronosyltransferase 1 locus: functional consequences of three novel missense mutations in the human UGT1A7 gene. *Pharmacogenetics* 10:629–44
44. Miners JO, McKinnon RA, Mackenzie PI. 2002. Genetic polymorphisms of UDP-glucuronosyltransferases and their functional significance. *Toxicology* 181:453–56
45. Huang Y-H, Galijatovic A, Nguyen N, Geske D, Beaton D, et al. 2002. Identification and functional characterization of UDP-glucuronosyltransferases *UGT1A8*1, UGT1A8*2* and *UGT1A8*3*. *Pharmacogenetics* 12:287–97
46. Guillemette C, Millikan RC, Newman B, Houseman DE. 2000. Genetic polymorphisms in uridine diphospho-glucuronosyltransferase 1A1 and association with breast cancer among African Americans. *Cancer Res.* 60:950–56
47. Strassburg CP, Vogel A, Kneip S, Tukey RH, Manns MP. 2002. Polymorphisms of the human UDP-glucuronosyltrasferase (UGT) 1A7 gene in colorectal cancer. *Gut* 50:851–56
48. Kadakol A, Ghosh SS, Sappal BS, Sharma G, Chowdhury JR, Chowdhury N. 2000. Genetic lesions of bilirubin uridine-diphospho glucuronate glucuronosyltransferase (UGT1A1) causing Crigler-Najjar and Gilbert syndromes: correlation of genotype to phenotype. *Hum. Mutat.* 16:297–306
49. Ando Y, Saka H, Ando M, Sawa T, Muro K, et al. 2000. Polymorphism of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. *Cancer Res.* 60:6921–26
50. Zucker SD, Qin X, Rouster SD, Yu F, Green RM, et al. 2001. Mechanism of indinavir induced hyperbilirubinemia. *Proc. Natl. Acad. Sci. USA* 98:12671–76
51. Lu AYH, Wang RW, Lin JH. 2003. Cytochrome P450 in vitro reaction phenotyping: a re-evaluation of approaches used for P450 isoform detection. *Drug Metab. Dispos.* 31:345–50
52. Grancharov K, Naydenova Z, Lozeva S, Golovinsky E. 2001. Natural and synthetic inhibitors of UDP-glucuronosyltransferase. *Pharmacol. Ther.* 89:171–86
53. Bosma PJ, Seppen J, Goldhoorn B, Bakker C, Oude Elferinck RPJ, et al. 1994. Bilirubin UDP-glucuronosyltransferase 1 is the only relevant bilirubin glucuronidating isoform in man. *J. Biol. Chem.* 269:17960–64
54. Ebner T, Remmel RP, Burchell B.

1993. Human bilirubin UDP-glucuronosyltransferase catalyzes the glucuronidation of ethinylestradiol. *Mol. Pharmacol.* 43:649–54

55. Senafi SB, Clarke DJ, Burchell B. 1994. Investigation of the substrate specificity of a cloned expressed human bilirubin UDP-glucuronosyltransferase: UDP sugar specificity and involvement in steroid and xenobiotic glucuronidation. *Biochem. J.* 303:233–40
56. Fisher MB, VandenBranden M, Findlay K, Burchell B, Thummel K, et al. 2000. Tissue distribution and interindividual variation in human UDP-glucuronosyltransferase activity: relationship between UGT1A1 promoter genotype and variability in a liver bank. *Pharmacogenetics* 10:727–39
57. Green MD, Bishop WP, Tephly TR. 1995. Expressed human UGT1.4 protein catalyzes the formation of quaternary ammonium-linked glucuronides. *Drug Metab. Dispos.* 23:299–302
58. Green MD, King CD, Mojarrabi B, Mackenzie PI, Tephly TR. 1998. Glucuronidation of amines and other xenobiotics by expressed human UDP-glucuronosyltransferase 1A3. *Drug Metab. Dispos.* 26:507–12
59. Nakajima M, Tanaka E, Kobayashi T, Ohashi N, Kume T, Yokoi T. 2002. Imipramine N-glucuronidation in human liver microsomes: biphasic kinetics and characterization of UDP-glucuronosyltransferase isoforms. *Drug Metab. Dispos.* 30:636–42
60. Court MH, Duan SX, Von Moltke LL, Greenblatt DJ, Patten CJ, et al. 2001. Interindividual variability in acetaminophen glucuronidation by human liver microsomes: identification of relevant acetaminophen UDP-glucuronosyltransferase isoforms. *J. Pharmacol. Exp. Ther.* 299:998–1006
61. Krishnaswamy S, Duan SX, Von Moltke LL, Greenblatt DJ, Court MH. 2003. Validation of serotonin (5-hydroxytryptamine) as an in vitro substrate probe for human UDP-glucuronosyltransferase (UGT) 1A6. *Drug Metab. Dispos.* 31: 133–39
62. Cheng Z, Radominska-Pandya A, Tephly TR. 1999. Studies on the specificity of human intestinal UDP-glucuronosyltransferases 1A8 and 1A9. *Drug Metab. Dispos.* 27:1165–70
63. Barbier O, Turgeon D, Girard C, Green MD, Tephly TR, et al. 2000. 3'-Azido-3'-deoxythymidine (AZT) is glucuronidated by human UDP-glucuronosyltransferase 2B7 (UGT2B7). *Drug Metab. Dispos.* 28:497–502
64. Innocenti F, Iyer L, Ramirez J, Green MD, Raitan MJ. 2001. Epirubicin glucuronidation is catalyzed by human UDP-glucuronosyltransferase 2B7. *Drug Metab. Dispos.* 29:686–92
65. Stone AN, Mackenzie PI, Galetin A, Houston JB, Miners JO. 2003. Isoform selectivity and kinetics of morphine 3- and 6-glucuronidation by human UDP-glucuronosyltransferases: evidence of atypical glucuronidation kinetics by UGT-2B7. *Drug Metab. Dispos.* In press
66. Ritter JK, Chen F, Sheen YY, Lubet RA, Owens IS. 1992. Two human liver cDNAs encode UDP-glucuronosyltransferases with 2 log differences in activity towards parallel substrates including hyodeoxycholic acid and certain estrogen derivatives. *Biochemistry* 31:3409–14
67. Green MD, Oturu EM, Tephly TR. 1994. Stable expression of human liver UDP-glucuronosyltransferase (UGT2B15) with activity toward steroid and xenobiotic substrates. *Drug Metab. Dispos.* 22:799–805
68. Turgeon D, Carrier J-B, Levesque E, Hum DW, Belanger A. 2001. Relative enzymatic activity, protein stability, and tissue distribution of human steroid metabolising UGT2B subfamily members. *Endocrinology* 142:778–87
69. Ethell BT, Beaumont K, Rance DJ, Burchell B. 2001. Use of cloned and

expressed human UDP-glucuronosyltransferases for the assessment of human drug conjugation and identification of potential drug interactions. *Drug Metab. Dispos.* 29:48–53
70. Watanabe Y, Nakajima M, Yokoi T. 2002. Troglitazone glucuronidation in human liver and intestine microsomes: high catalytic activity of UGT1A8 and 1A10. *Drug Metab. Dispos.* 30:1462–69
71. Miners JO, Valente L, Lillywhite KJ, Mackenzie PI, Burchell B, et al. 1997. Preclinical prediction of factors influencing the elimination of 5,6-dimethylxanthenone-4-acetic acid, a new anticancer drug. *Cancer Res.* 57:284–89
72. Boase S, Miners JO. 2002. In vitro–in vivo correlations for drugs eliminated by glucuronidation: investigations with the model substrate zidovudine. *Br. J. Clin. Pharmacol.* 54:493–503
73. Soars MG, Burchell B, Riley RJ. 2002. In vitro analysis of human drug glucuronidation. *J. Pharmacol. Exp. Ther.* 301:382–90
74. Ilett KF, Ethell BT, Maggs JL, Davis TME, Batty KT, et al. 2002. Glucuronidation of dihydroartemisinin in vivo and by human liver microsomes and expressed UDP-glucuronosyltransferases. *Drug Metab. Dispos.* 30:1005–12
75. Mistry M, Houston JB. 1987. Glucuronidation in vitro and in vivo: comparison of intestinal and hepatic conjugation of morphine, naloxone and buprenorphine. *Drug Metab. Dispos.* 15:710–17
76. Bouska JJ, Bell RL, Goodfellow CL, Stewart AO, Brooks CDW, Carter GW. 1997. Improving the in vivo duration of 5-lipoxygenase inhibitors. Application of an in vitro glucuronosyltransferase assay. *Drug Metab. Dispos.* 25:1032–38
77. Burchell B, Brierley CH, Rance D. 1995. Specificity of human UDP-glucuronosyltransferases and xenobiotic glucuronidation. *Life Sci.* 57:1819–31
78. Williams JA, Ring BJ, Cantrell VE, Campanale K, Jones DR, et al. 2002. Differential modulation of UDP-glucuronosyltransferase 1A1 (UGT1A1) catalyzed estradiol 3-glucuronidation by the addition of UGT1A1 substrates and other compounds to human liver microsomes. *Drug Metab. Dispos.* 30:1266–73
79. Sorich MJ, Smith PA, McKinnon RA, Miners JO. 2002. Pharmacophore and quantitative structure activity relationship modelling of UDP-glucuronosyltransferase 1A1 (UGT1A1) substrates. *Pharmacogenetics* 12:635–45
80. Houston JB, Kenworthy KE. 2000. In vitro–in vivo scaling of CYP kinetic data not consistent with the classical Michaelis-Menten model. *Drug Metab. Dispos.* 28:246–54
81. Galetin A, Clarke SE, Houston JB. 2002. Quinidine and haloperidol as modifiers of CYP3A4 activity: multisite kinetic approach. *Drug Metab. Dispos.* 30:1512–22
82. Raoof AA, Van Obbergh LJ, Ville de Goyet J, Verbeeck RK. 1996. Extrahepatic glucuronidation of propofol in man: possible contribution of gut wall and kidney. *Eur. J. Clin. Pharmacol.* 50:91–96
83. Shipkova M, Strassburg CP, Braun F, Streit F, Grone HJ, et al. 2001. Glucuronide and glucoside conjugation of mycophenolic acid by human liver, kidney and intestinal microsomes. *Br. J. Pharmacol.* 132:1027–34
84. Watanabe Y, Nakajima M, Yokoi T. 2002. Troglitazone glucuronidation in human liver and intestine microsomes: high catalytic activity of UGT1A8 and UGT1A10. *Drug Metab. Dispos.* 30: 1462–69
85. Bowalgaha K, Miners JO. 2001. The glucuronidation of mycophenolic acid by human liver, kidney and jejunum microsomes. *Br. J. Clin. Pharmacol.* 52:605–9
86. Paine MF, Khalighi M, Fisher JM, Shen DD, Kunze KL, et al. 1997. Characterization of interintestinal and intraintestinal variations in human CYP3A

dependent metabolism. *J. Pharmacol. Exp. Ther.* 283:46–58
87. Todeschini R, Consonni V, eds. 2000. *Handbook of Molecular Descriptors*. Weinheim: Wiley-VCH
88. Ekins S, Bravi G, Ring BJ, Gillespie TA, Gillespie JS, et al. 1999. Three-dimensional quantitative structure activity relationship analysis of substrates for CYP2B6. *J. Pharmacol. Exp. Ther.* 288:21–29
89. Ekins S, Bravi G, Binkley S, Gillespie JS, Ring BJ, et al. 1999. Three and four dimensional-quantitative structure activity relationship (3D/4D-QSAR) analyses of CYP2D6 inhibitors. *Pharmacogenetics* 9:477–89
90. Smith PA, Sorich MJ, McKinnon RA, Miners JO. 2003. Pharmacophore and quantitative structure activity relationship (QSAR) modelling: complementary approaches to the rationalisation and prediction of UDP-glucuronosyltransferase 1A4 (UGT1A4) substrate selectivity. *J. Med. Chem.* 46:1617–26
91. Smith PA, Sorich MJ, Low LSC, McKinnon RA, Miners JO. 2003. Modelling metabolism by UDP-glucuronosyltransferases. *J. Mol. Graph. Model.* In press
92. Wang Q, Halpert JR. 2002. Combined three-dimensional quantitative structure-activity relationship analysis of cytochrome P450 2B6 substrates and protein homology modeling. *Drug Metab. Dispos.* 30:86–95
93. Ghose AK, Wendoloski JJ. 1998. Pharmacophore modelling—methods, experimental verification and applications. *Perspect. Drug Discov. Des.* 9–11:253–71
94. Green MD, Tephly TR. 1996. Glucuronidation of amines and hydroxylated xenobiotics and endobiotics catalyzed by expressed human UGT1.4 protein. *Drug Metab. Dispos.* 24:356–63
95. Lewis DFV, Dickins M. 2002. Substrate SARs in human P450s. *Drug Discov. Today* 7:918–25
96. Afzelius L, Masimirembwa CM, Karlen A, Andersson TB, Zamora I. 2002. Discriminant and quantitative PLS analysis of competitive CYP2C9 inhibitors versus non-inhibitors using alignment independent GRIND descriptors. *J. Comput.-Aided Mol. Des.* 16:443–58
97. Winkler D. 2001. The broader applications of neural and genetic modelling methods. *Drug Discov. Today* 6:1198–99
98. Molnar L, Keseru GM. 2002. A neural network based virtual screening of cytochrome P450 3A4 inhibitors. *Bioorg. Med. Chem. Lett.* 12:419–21
99. Doniger S, Hofmann T, Yeh J. 2002. Predicting CNS permeability of drug molecules: comparison of neural network and support vector machine algorithms. *J. Comput. Biol.* 9:849–64
100. Sorich MJ, Miners JO, McKinnon RA, Winkler D, Burden FR, Smith PA. 2003. Comparison of linear and non-linear classification algorithms: prediction of drug metabolism by UDP-glucuronosyltransferase isoforms. *J. Chem. Inf. Comput. Sci.* In press
101. Garrigues A, Loiseau N, Delaforge M, Ferte J, Garrigos M, et al. 2002. Characterization of two pharmacophores on the multidrug transporter P-glycoprotein. *Mol. Pharmacol.* 62:288–98
102. Palma PN, Bonifacio MJ, Loureiro AI, Wright LC, Learmonth DA, Soares-da-Silva P. 2003. Molecular modeling and metabolic studies of the interaction of catechol-O-methyltransferase and a new nitrocatechol inhibitor. *Drug Metab. Dispos.* 31:250–58
103. Watkins RE, Noble SM, Redinbo MR. 2002. Structural insights into the promiscuity and function of the human pregnane X receptor. *Curr. Opin. Drug Discov. Dev.* 5:150–58
104. Mason JS. 2001. 3D-pharmacophores in drug discovery. *Curr. Pharm. Des.* 7:567–97
105. McGregor MJ, Muskal SM. 1999. Pharmacophore fingerprinting. 1. Application to QSAR and focused library design. *J. Chem. Inf. Comput. Sci.* 39:569–74

106. de Groot MJ, Ackland MJ, Horne VA, Alex AA, Jones BC. 1999. A novel approach to predicting P450 mediated drug metabolism. CYP2D6 catalyzed N-dealkylation reactions and qualitative metabolite predictions using a combined protein and pharmacophore model for CYP2D6. *J. Med. Chem.* 42:4062–70
107. Jones JP, Mysinger M, Korzekwa KR. 2002. Computational models for cytochrome P450: a predictive electronic model for aromatic oxidation and hydrogen atom abstraction. *Drug Metab. Dispos.* 30:7–12
108. Bray HG, Humphris BG, Thorpe WV, White K, Wood PB. 1952. Kinetic studies of the metabolism of foreign organic compounds. III. Conjugation of phenols with glucuronic acid. *Biochem. J.* 52:416–19
109. Hansch C, Lien EJ, Helmer F. 1968. Structure-activity correlations in the metabolism of drugs. *Arch. Biochem. Biophys.* 128:319–30
110. Yin HQ, Bennett G, Jones JP. 1994. Mechanistic studies of uridine diphosphate glucuronosyltransferase. *Chem.-Biol. Interact.* 90:47–58
111. Cupid BC, Holmes E, Wilson ID, Lindon JC, Nicholson JK. 1999. Quantitative structure-metabolism relationships (QSMR) using computational chemistry: pattern recognition analysis and statistical prediction of phase II conjugation reactions of substituted benzoic acids in the rat. *Xenobiotica* 29:27–42
112. Vashishtha SC, Hawes EM, McKay G, McCann DJ. 2001. Quaternary ammonium-linked glucuronidation of 1-substituted imidazoles: studies of human UDP-glucuronosyltransferases involved and substrate specificities. *Drug Metab. Dispos.* 29:1290–95
113. Ethell BT, Ekins S, Wang JB, Burchell B. 2002. Quantitative structure activity relationships for the glucuronidation of simple phenols by expressed human UGT1A6 and UGT1A9. *Drug Metab. Dispos.* 30:734–38
114. Lewis DFV, Sams C, Loizou GD. 2003. A quantitative structure-activity relationship analysis on a series of alkyl benzenes metabolized by human cytochrome p450 2E1. *J. Biochem. Mol. Toxicol.* 17:47–52
115. Van de Waterbeemd H. 2002. High-throughput and in silico techniques in drug metabolism and pharmacokinetics. *Curr. Opin. Drug Discov. Dev.* 5:33–43
116. Buchwald P, Bodor N. 2002. Computer-aided drug design: the role of quantitative structure-property, structure-activity and structure-metabolism relationships (QSPR, QSAR, QSMR). *Drugs Future* 27:577–78

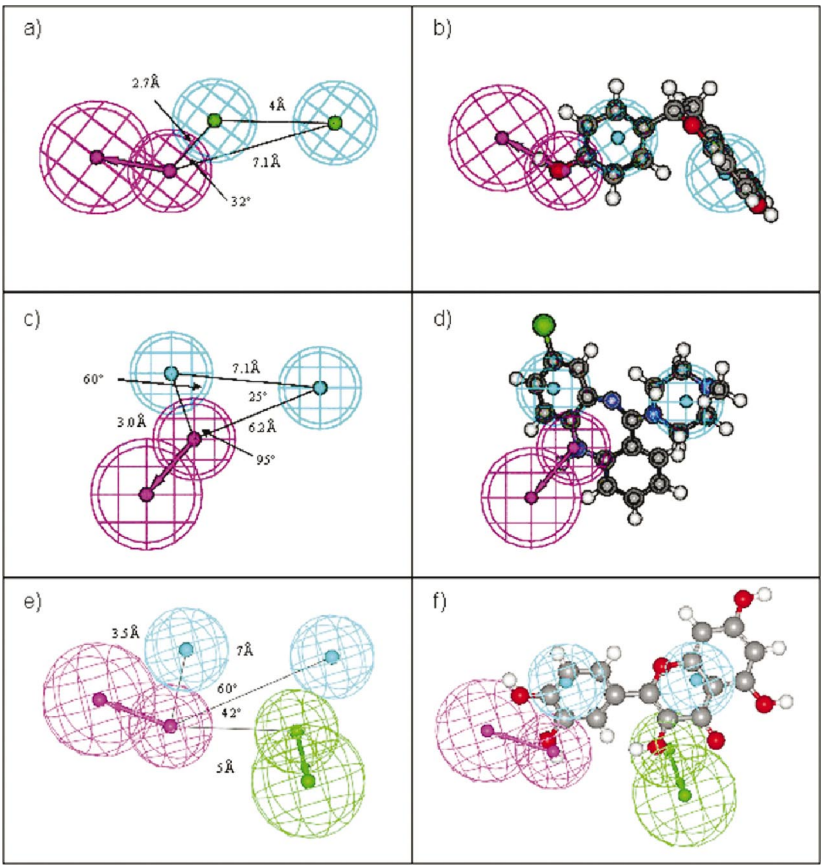


**Figure 6** UGT1A1 (*panel a*), UGT1A4 (*panel c*), and UHT1A9 (*panel e*) common features pharmacophores. The cyan, green, and purple spheres represent a hydrophobic feature, hydrogen bond acceptor, and glucuronidation feature, respectively. Bold arrows show the direction of lone-pair electron donation. Panels *b*, *d*, and *f* show naringenin, clozapine, and quercetin mapped to the respective UGT1A1, UGT1A4, and UGT1A9 pharmacophores.