The glucuronidation of R- and S- lorazepam: Human liver microsomal kinetics, UDP-glucuronosyltransferase enzyme selectivity, and inhibition by drugs

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Abbreviations:

AUC, area under the plasma drug concentration – time curve; BSA, bovine serum albumin; DDI, drug-drug interaction; HLM, human liver microsomes; HPLC, high performance liquid chromatography; IVIVE, in vitro – in vivo extrapolation; LZPG1, R-lorazepam glucuronide; LZPG2, S-lorazepam glucuronide; UDPGA, UDP-glucuronic acid; UGT, UDP-glucuronosyltransferase.
Abstract

The widely used hypnosedative-anxiolytic agent R,S-lorazepam is cleared predominantly by conjugation with glucuronic acid in humans, but the enantioselective glucuronidation of lorazepam has received little attention. The present study characterized the kinetics of the separate R- and S- enantiomers of lorazepam by human liver microsomes (HLM) and by a panel of recombinant human UDP-glucuronosyltransferase (UGT) enzymes. Respective mean $K_m$ and $V_{max}$ values for R- and S-lorazepam by HLM were 29 ± 8.9 and 36 ± 10 µM, and 7.4 ± 1.9 and 10 ± 3.8 pmol/min.mg. Microsomal intrinsic clearances were not significantly different, suggesting the in vivo clearances of R- and S- lorazepam are likely to be similar. Both R- and S- lorazepam were glucuronidated by UGT 2B4, 2B7, and 2B15, while R-lorazepam was additionally metabolized by the extra-hepatic enzymes UGT 1A7 and 1A10. Based on in vitro clearances and consideration of available in vivo and in vitro data, UGT2B15 is likely to play an important role in the glucuronidation of R- and S-lorazepam. However, the possible contribution of other enzymes and the low activities observed in vitro indicate that the lorazepam enantiomers are of limited use as substrate probes for UGT2B15. In order to identify potential drug-drug interactions, codeine, fluconazole, ketamine, ketoconazole, methadone, morphine, valproic acid, and zidovudine were screened as inhibitors of R- and S- lorazepam glucuronidation by HLM. Of these drugs, in vitro – in vivo extrapolation suggested that only ketoconazole had the potential to inhibit lorazepam clearance to a clinically significant extent.
Introduction

The UDP-glucuronosyltransferases comprise a superfamily of enzymes that catalyze the transfer of glucuronic acid from the cofactor UDP-glucuronic acid (UDPGA) to a nucleophilic acceptor group on the substrate. Nineteen human UGTs that primarily utilize UDPGA as cofactor have been identified to date and these have been classified in three subfamilies, UGT1A, UGT2A and UGT2B, based on amino acid sequence identity (Mackenzie et al., 2005). The individual UGT enzymes exhibit characteristic but not uncommonly overlapping substrate selectivities, especially in relation to low molecular weight phenols (Court 2005; Kiang et al., 2005; Miners et al., 2006 and 2010a). In addition, UGTs differ in terms of drug-drug interaction profile, and UGT enzyme activities are variably affected by age, dietary and environmental chemicals, disease states, epigenetic regulation, ethnicity, genetic polymorphism and hormonal factors (Miners and Mackenzie 1991; Miners et al., 2002 and 2004; Guillemette 2003; Guillemette et al., 2010; Court 2010).

The majority of UGT1 and UGT2 enzymes are expressed in liver, although expression of UGT 1A5, 1A7, 1A8, 1A10, 2B11, and 2B28 appears to be minor or negligible in this tissue (Ohno and Nakajin, 2009; Court 2010). UGT 1A5, 1A7, 1A8 and 1A10 are preferentially expressed in the gastrointestinal tract and may therefore contribute to the presystemic glucuronidation of xenobiotics.

UGTs metabolize a diverse range of compounds, including drugs from all therapeutic classes (Kiang et al., 2005; Miners and Mackenzie, 1991; Miners et al., 2010a). With respect to the hepatically expressed enzymes, UGT 1A1, 1A3, 1A4, 1A6, 1A9, 2B7 and 2B15 appear to be of greatest significance in drug and xenobiotic metabolism and clearance, although there is also evidence demonstrating that UGT 2B4, 2B10 and 2B17 may contribute to drug glucuronidation. Recognition of the importance of glucuronidation as a metabolic pathway for drugs and other xenobiotics has stimulated interest in the application of reaction
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phenotyping approaches to drug glucuronidation (Miners et al., 2006 and 2010). In particular, the availability of UGT enzyme selective substrates allows investigation of relationships between the rates of glucuronidation of a ‘test’ compound and enzyme selective ‘probes’ in a panel of human liver microsomes (HLM) or hepatocytes, and provides a valuable experimental tool for investigating the UGT enzyme inhibition selectivity of the ‘test’ drug. Furthermore, enzyme selective substrates have been applied to investigation of the variability in the activities of UGTs in human liver and for the mechanisms responsible for interindividual variability (Court 2010). At present, highly selective substrates have been identified for UGT 1A1, 1A4, 1A6, 1A9, and 2B7 (Miners et al., 2010a).

Lorazepam is used clinically as a hypnosedative-anxiolytic agent (including anticipatory nausea and vomiting during cancer chemotherapy and premedication for surgery) and status epilepticus. Lorazepam is a 3-hydroxy-1,4-benzodiazepine. Like the structurally related benzodiazepines oxazepam and temazepam, lorazepam is eliminated in humans primarily via glucuronidation of the 3-hydroxy group (Schwarz 1979; Greenblatt 1981). Approximately 75% of orally administered lorazepam is recovered in urine as the glucuronide (Greenblatt et al., 1976). Owing to the presence of the 3-hydroxy group, the carbon atom at position-3 is asymmetric and hence lorazepam, oxazepam and temazepam exist as pairs of enantiomers. Stereoselective glucuronidation of oxazepam has been reported; S-oxazepam is primarily glucuronidated by UGT2B15 whereas UGT1A9 and UGT2B7 glucuronidate R-oxazepam (Court et al., 2002). S-Lorazepam and S-oxazepam activities were shown to be highly correlated in a panel of human liver microsomes (HLM) (Court 2005). S- and R- lorazepam were proposed as selective substrate probes for UGT2B15 and UGT1A3, respectively (Court 2005), but apart from the activity correlation data referred to above supporting data were not provided.
In order to clarify the enantioselective glucuronidation of lorazepam, we characterized the kinetics of R- and S-lorazepam glucuronidation by HLM and by a panel of recombinant human UGT enzymes. Since altered lorazepam clearance may impact on efficacy and safety, further studies were performed to investigate potential drug-drug interactions (DDI) arising from inhibition of lorazepam glucuronidation. Inhibition of human liver microsomal S- and R- lorazepam glucuronidation by known substrates and/or inhibitors of UGT2B enzymes, including codeine, fluconazole, ketamine, ketoconazole, methadone, morphine, valproic acid and zidovudine, was characterized and in vitro – in vivo extrapolation (IVIVE) was employed to assess the likely significance of DDIs.
MATERIALS AND METHODS

Materials

Alamethicin (from *Trichoderma viride*), codeine, ketamine hydrochloride, R,S-lorazepam, UDP-glucuronic acid (UDPGA; trisodium salt), β-glucuronidase (from *Escherichia coli*), valproic acid and zidovudine were purchased from Sigma Aldrich (Sydney, Australia). Supersomes® expressing UGT 2B4, 2B7, 2B15 and 2B17 were purchased from BD Biosciences (Wembley, Australia). Fluconazole was obtained from Pfizer Australia (Sydney, Australia); ketoconazole from Janssen-Cilag Pty Ltd (Sydney, Australia); methadone hydrochloride from Professor AA Somogyi (University of Adelaide, Australia); and morphine hydrochloride from GlaxoSmithKline (Melbourne, Australia). Solvents and other reagents were of analytical reagent grade.

Enzyme sources and preparation

Human livers (H7, H10, H40 – female donors; H12 and H13 – male donors) were sourced from the human liver ‘bank’ of the Department of Clinical Pharmacology of the Flinders University School of Medicine. Approval was obtained from the Flinders Medical Centre Research Ethics Committee for the use of human liver tissue in xenobiotic metabolism studies. Human liver microsomes (HLM) were prepared by differential centrifugation, as described by Bowalgaha et al. (2005). HLM were activated with the pore forming peptide alamethicin (50 µg/mg microsomal protein) by pre-incubation on ice for 30 min according to Boase and Miners (2002).

UGT1A cDNAs (viz. 1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, and 1A10) were stably expressed in a human embryonic kidney cell line (HEK293) as described previously (Stone et al. 2003; Uchaipichat et al. 2004). After growth to at least 80% confluency, cells were harvested and washed twice in phosphate-buffered saline (0.1M, pH 7.4). The harvested cells
were lysed by sonication (Heat Systems Ultrasonics, Plainsview, NY) set at microtip limit of 4 and sonicated with 4 x 1-sec ‘bursts’, each separated by 3 min cooling on ice. Lysed cells were then centrifuged at 12,000g for 1 min at 4°C. The supernatant fraction was collected and kept at -80°C until use. Due to the relatively low activities of UGT 2B4, 2B7, 2B15 and 2B17 expressed in HEK293 cells, UGT2B enzymes expressed in insect cells (Supersomes®) were employed in activity and inhibition studies.

The expression of UGT proteins was demonstrated by immunoblotting using a nonselective UGT antibody (raised against purified mouse Ugt; see Uchaipichat et al., 2004), or an antibody that recognizes UGT2B7 (Kerdpin et al., 2009). UGT1A1, 1A3, 1A6, 1A7, 1A8, 1A9, 1A10, 2B7, 2B15, and 2B17 activities were confirmed using the nonselective substrate 4-methylumbelliferone (Uchaipichat et al., 2004; Lewis et al., 2007), while UGT1A4 and UGT2B4 activities were demonstrated by measurement of lamotrigine N2-glucuronidation and codeine 6-glucuronidation formation, respectively (Kubota et al., 2007; Raungrut et al., 2010; Rowland et al., 2006).

**Lorazepam glucuronidation assay**

Incubations, in a total volume 0.2 ml, contained phosphate buffer (0.1 M, pH 7.4), MgCl₂ (4 mM), UDP-glucuronic acid (5 mM), R,S-lorazepam (10-500 µM), and alamethicin-activated HLM (1 mg/ml) or recombinant UGT protein (2 mg/ml). Reactions were initiated by the addition of UDP-glucuronic acid and performed at 37°C in a shaking water bath for 150 and 180 min for HLM and the recombinant UGTs, respectively. Reactions were terminated with 70% HClO₄ (2 µl) and then centrifuged at 5000 g for 10 min at 10°C. A 0.12 ml aliquot of the supernatant fraction was transferred to a 1.5 ml eppendorf tube containing 4 M KOH (2 µl), mixed and centrifuged at 14 000g for 5 min. A 20 µl aliquot of the supernatant fraction was injected into the HPLC column. Rates of R- and S-lorazepam
glucuronide formation were optimized for linearity with respect to incubation time and protein concentration.

As indicated in the Introduction, lorazepam exists as a pair of enantiomers; R- and S-lorazepam. The rate of lorazepam racemization at pH 7.4 and 37°C is relatively fast (Lu and Yang, 1990; Nishikawa et al., 1997). The half-life of lorazepam racemization under these conditions is ≤5 min, which is short relative to the incubation times (150 – 180 min) employed here. Thus, it can be assumed that the concentration of each of R- and S-lorazepam is half of the total R,S-lorazepam concentration added to incubations. In the latter regard, substrate utilization was < 5% in all experiments. It should be noted that substitution of the 3-hydroxy group of lorazepam to form an ester or ether (as occurs in glucuronidation) forms a derivative that is stable to racemization (Lu and Yang, 1990; Baldacci and Thormann, 2006).

**Quantification of R- and S-lorazepam glucuronides by HPLC**

The analytical conditions for measurement of the R- and S-lorazepam glucuronides were modified from the method of Franzelius and Besserer (1993). HPLC was performed using an Agilent 1100 series instrument (Agilent Technologies, Sydney, NSW, Australia) fitted with a Zorbax Eclipse XBD-C8 analytical column (4.6 x 150 mm, 5 µm; Agilent Technologies). Analytes were separated using a linear gradient with a mobile phase flow rate of 1 ml/min. Initial conditions were 10 mM sodium phosphate buffer (pH 7) – acetonitrile (87.5:12.5) held for 5 min. The proportion of acetonitrile was then increased to 30% over 20 min and then continued for 7 min. Column eluant was monitored by UV absorbance at 230 nm. Under these conditions, retention times of the lorazepam glucuronides, designated LZPG1 and LZPG2, and R,S-lorazepam were 12.3, 13.4 and 26.3 min, respectively (Figure 1). LZPG1 and LZPG2 were quantified by comparison of peak areas to those of a R,S-lorazepam external standard curve prepared over the concentration range 0.5-5 µM since authentic

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standards of the glucuronides were unavailable. Thus, V\text{max} values should be considered ‘apparent’. Within-day overall assay reproducibility was assessed by measuring LZPPG1 and LZPG2 formation in 9 separate incubations of the same batch of pooled HLM (from HL 7, 10, 12, 13 and 40). Coefficients of variation were less than 2% for added R,S-lorazepam concentrations of 25 and 400 µM. The lower limit of quantification of the lorazepam glucuronides, defined as 5-times background noise, was 0.005 µM, which translates to a rate of 0.015 pmol/min.mg.

**Identification of the R- and S-lorazepam glucuronides**

The identity of the lorazepam glucuronides (LZPG1 and LZPG2) was confirmed by enzymatic hydrolysis. It has been reported that the glucuronides of the R- diastereoisomers of benzodiazepines are more resistant to hydrolysis by β-glucuronidase from *E. coli* compared to the S- diastereoisomers (Ruelius et al., 1979). Thus, identification of the R-and S-lorazepam glucuronides may be inferred from differences in the rates of enzymatic hydrolysis. An incubation of R,S-lorazepam and UDPGA with HLM as the enzyme source (see above) was terminated with 70% HClO\textsubscript{4} (2 µl) and centrifuged. The aqueous sample was decanted and mixed with 2.2 µl of 4M KOH (to raise the pH to 6.5) and 25 units of β-glucuronidase (to provide a final concentration of 125 units/ml) from *E. coli* was added. A control reaction was performed in the same manner, except distilled water was added instead of β-glucuronidase. The sample was incubated at 37°C for 0, 2, 4, 10, and 120 min. After incubation, a 150 µl aliquot was separated and treated with 70% HClO\textsubscript{4} (1.5 µl). Following centrifugation (5000 g for 10 min), 10 µl of the supernatant fraction from each reaction was injected into the HPLC column. The hydrolysis reaction resulted in a 50% reduction in the area of the peak corresponding to LZPG2 within 10 min, and this peak was undetectable after 2 hr. In contrast, the peak corresponding to LZPG1 was resistant to hydrolysis by β-
glucuronidase (125 units /ml) from *E. coli*. LZPG1 and LZPG2 peak areas were unchanged in the control incubations. Thus, the LZPG1 and LZPG2 peaks were assigned as R-lorazepam glucuronide and S-lorazepam glucuronide, respectively.

**Binding of R,S-lorazepam to human liver microsomes**

The binding of R,S-lorazepam to HLM was characterized by equilibrium dialysis as described by McLure et al. (2000). One side of dialysis cell contained R,S-lorazepam (10, 50, 100, 300 or 500 µM) in phosphate buffer (0.1M, pH 7.4), while the other side contained a suspension of pooled HLM (1 mg/ml). The dialysis cell assembly was immersed in a water bath at 37°C and rotated at 12 rpm for 4 hr. Control experiments were performed with phosphate buffer or HLM on both sides of the cell, at low and high R,S-lorazepam concentrations, to ensure that equilibrium was attained. A 200 µl aliquot was collected from each cell and treated with 200 µl of ice-cold methanol containing 4% glacial acid. Samples were chilled on ice for 20 min and subsequently centrifuged at 5000 g for 10 min at 10°C. A 10 µl aliquot of the supernatant fraction was analyzed by HPLC. The HPLC system and conditions were essentially as described previously for the measurement of the R- and S-lorazepam glucuronides, except that the mobile phase (60% phosphate buffer / acetonitrile) was delivered isocratically at a flow rate of 1 ml/min. Under these conditions, R,S-lorazepam eluted at 4.5 min. R,S-Lorazepam standards in the concentration range 1-250 µM were prepared in phosphate buffer (100 mM, pH 7.4), alone or in a suspension of pooled HLM in phosphate buffer, and treated in the same manner as the dialysis samples. The R,S-lorazepam concentrations of dialysis samples were determined by comparison of peak areas with those of a standard curve. The fraction unbound of R,S-lorazepam to HLM (f_{unb}) was determined by dividing the drug concentration in the buffer compartment by the drug concentration in the protein compartment.
Inhibition of R- and S-lorazepam glucuronidation was investigated using pooled HLM, prepared by mixing equal protein amounts of microsomes from the five livers used in the lorazepam glucuronidation kinetic study. Inhibition screening studies were performed using a R,S-lorazepam concentration of 100 µM (i.e. 50 µM of each of separate enantiomers), which is corresponds to the approximate apparent K_m (viz. 37 µM) for both R- and S-lorazepam glucuronidation by pooled HLM (see Results). Concentrations of codeine, fluconazole, ketamine, ketoconazole, methadone, morphine, and zidovudine added to incubations (i.e. total drug concentration) in the inhibition screening studies were in the range 10-1000 µM, which was considered sufficient to detect potentially clinically significant inhibition. The concentration range of valproic acid was 100-7500 µM, which spanned the expected IC_50 (determined in preliminary inhibition experiments). All inhibitors were dissolved in distilled water, except for ketoconazole and valproic acid, which were dissolved in methanol such that the final concentration of solvent in the incubation mixture was 1% v/v. It has been shown that 1% methanol (v/v) has a negligible effect on UGT enzyme activities (Uchaipichat et al., 2004). Further experiments to determine the inhibitor constants (K_i) for ketoconazole and valproic acid included four inhibitor concentrations at each of three R,S-lorazepam concentrations (50, 100, and 200 µM).

Kinetic analysis of in vitro glucuronidation activity data

Kinetic constants for R- and S-lorazepam glucuronidation by HLM or recombinant human UGTs were derived by fitting equations for empirical kinetic models (viz. Michaelis-Menten, substrate inhibition and Hill equations; see Uchaipichat et al. 2004 for expressions) to untransformed experimental data using Enzfitter (Biosoft, Cambridge, UK). Intrinsic
clearance (CL_{int}) was calculated as $V_{\text{max}}/K_m$ and maximal clearance (CL_{\text{max}}; Uchaipichat et al., 2004) as:

$$\frac{V_{\text{max}}}{S_{50}} \times \frac{(n-1)}{n(n-1)^{1/n}}$$

where $S_{50}$ is the substrate concentration corresponding to half $V_{\text{max}}$ and $n$ is the Hill coefficient (from fitting with the Hill equation). Inhibitor constants (K_i values) were estimated by fitting the expressions for competitive, uncompetitive, noncompetitive and mixed inhibition models to untransformed data using Enzfitter (Biosoft, Cambridge, UK). Goodness of fit to kinetic and inhibition models is assessed from the F statistic, $r^2$ values, parameter standard error estimates, and 95% confidence intervals. Kinetic constants are reported as the value ± standard error of the parameter estimate.

**In vitro – in vivo extrapolation (IVIVE)**

Predicted inhibition of R- and S-lorazepam hepatic clearances via glucuronidation by selected inhibitors in vivo was calculated as the ratio of the areas under the plasma concentration - time curves with (AUC_i) and without (AUC) inhibitor co-administration (Miners et al., 2010a):

$$\frac{AUC_i}{AUC} = \frac{1}{1+\frac{f_m}{1+[I]/K_i}+(1-f_m)}$$

where [I] is the inhibitor concentration; fm is the fraction of R,S-lorazepam hepatic clearance via glucuronidation, which was taken as 75% (Greenblatt et al., 1976), and K_i is the inhibition constant generated in vitro. The inhibitor concentration [I] in vivo was taken as the reported maximum concentration in plasma or the hepatic inlet concentration, calculated as:

$$[I_{\text{inlet}}] = [I_{\text{max}}] + \frac{k_d \times F_a \times Dose}{Q_H}$$
where $[I_{\text{max}}]$, $k_a$, $F_a$ and $Q_H$ are the maximum systemic total inhibitor concentration associated with a given dose, absorption rate constant, fraction absorbed from the gastrointestinal tract, and liver blood flow, respectively. The unbound inhibitor concentration was calculated as the product of total inhibitor concentration and the fraction unbound of inhibitor in blood.
RESULTS

Non-specific binding of R,S-lorazepam to human liver microsomes

The average binding of R,S-lorazepam to HLM, which was concentration independent over the range investigated, was 28% ($f_{unc} = 0.72 \pm 0.01$). The non-specific binding of the putative inhibitors investigated here has been determined previously in this laboratory. Ketoconazole and methadone bind to HLM with mean $f_{unc}$ values of $0.27 \pm 0.005$ and $0.72 \pm 0.039$, respectively, whereas the binding of codeine and valproic acid to HLM is negligible (Raungrut et al., 2010). The binding of fluconazole, ketamine, morphine, and zidovudine to HLM is also minor (Uchaipichat et al., 2006 and 2011). Where binding was observed, $f_{unc}$ was accounted for in the calculation of kinetic parameters ($K_m$, $IC_{50}$ and $K_i$ values).

Identification of the R- and S-lorazepam glucuronides

The separate R- and S-lorazepam glucuronides were identified on the basis of the known relative sensitivity of the diastereoisomeric benzodiazepine glucuronides to hydrolysis by β-glucuronidase (Ruelius et al., 1979). The peak in the high performance liquid chromatogram eluting with a retention time of 12.3 min (designated LZPG1; Figure 1) was resistant to hydrolysis, whereas the peak eluting at 13.4 min was readily hydrolysed by β-glucuronidase. Thus, LZPG1 and LZPG2 were assigned as the glucuronides of R- and S-lorazepam, respectively. LC/MS analysis of each peak gave m/z values of 497 and 495 in positive and negative ion modes, respectively, which correspond to the molecular mass of lorazepam glucuronide. It is further known that, in general, the R-glucuronides of benzodiazepines elute before the S-glucuronides when separated by reversed-phase HPLC (Ruelius et al., 1979; Franzelius and Besserer 1993). As indicated in Methods, substitution of the 3-hydroxy group of lorazepam to form an ester or ether (including lorazepam glucuronide) forms a derivative that is stable to racemization (Lu and Yang, 1990; Baldacci and Thomann, 2006).
Kinetics of R- and S-lorazepam glucuronide formation by human liver microsomes

Representative kinetic plots and derived kinetic parameters for R- and S-lorazepam glucuronide formation by non-genotyped HLM are shown in Figure 2 and Table 1, respectively. R- and S-lorazepam glucuronidation by microsomes from all five livers exhibited weak substrate inhibition ($K_{si} > K_m$). Mean $K_m$, $V_{max}$ and $CL_{int}$ values were similar for each enantiomer, although the $K_m$ value for S-lorazepam was significantly higher (by 24%; $p < 0.05$). R- and S-lorazepam glucuronidation by pooled HLM, prepared by mixing equal protein amounts of microsomes from the 5 separate livers, also exhibited substrate inhibition kinetics. Mean kinetic constants for R- and S-lorazepam glucuronidation by the HLM pool were comparable to the mean data obtained using microsomes from the separate livers (Table 1). Thus, subsequent experiments investigating the magnitude of the interaction between lorazepam and putative inhibitors employed pooled HLM as the enzyme source.

Lorazepam glucuronidation by recombinant UGT enzymes

Twelve recombinant UGTs were screened for R- and S-lorazepam glucuronidation activity at three R,S-lorazepam concentrations, 20, 100 and 500 µM, which correspond to separate R- and S-lorazepam concentrations of 10, 50 and 250 µM. Results are shown in Figure 3. UGT 2B4, 2B7 and 2B15 were the only hepatically expressed enzymes that catalyzed R-lorazepam glucuronidation with rates > 0.1 pmol/min/mg. UGT 2B4, 2B7 and 2B15 also catalyzed S-lorazepam glucuronidation. Highest activity was associated with UGT2B15. At S-lorazepam concentrations of 50 and 250 µM, UGT2B15 activity was approximately 4- to 6- fold higher than the activities observed with UGT2B4 and UGT2B7. Notably, UGT 1A7 and 1A10, which are expressed only in the gastrointestinal tract, also glucuronidated R-lorazepam.
The kinetics of both R- and S-lorazepam glucuronidation by UGT 2B4, 2B7 and 2B15 and R-lorazepam glucuronidation by UGT 1A7 and 1A10 were further investigated. R-Lorazepam glucuronidation by UGT2B4, UGT2B7 and UGT2B15 followed sigmoidal (autoactivation), Michaelis-Menten, and substrate inhibition kinetics, respectively (Figure 4). In contrast, sigmoidal kinetics were observed for S-lorazepam glucuronidation by UGT2B4, UGT2B7 and UGT2B15 (Figure 5). Derived $K_m$ or $S_{50}$ values for UGT 2B4, 2B7 and 2B15 catalyzed R-lorazepam glucuronidation ranged from 13 to 44 µM, and the $S_{50}$ values for S-lorazepam glucuronidation spanned a similar range (17 to 46 µM). The kinetics of UGT1A7 and UGT1A10 catalyzed R-lorazepam glucuronidation were well modeled by the Michaelis-Menten and substrate inhibition equations, respectively. The $K_m$ for R-lorazepam glucuronidation by UGT1A10 was approximately an order of magnitude higher than that for UGT1A7 and the $K_m$ values for both R- and S-glucuronidation by UGT 2B4, 2B7 and 2B15.

**Inhibition of R- and S- lorazepam glucuronidation**

The inhibition of R- and S-lorazepam glucuronidation by codeine, fluconazole, ketamine, ketoconazole, methadone, morphine, valproic acid and zidovudine was investigated using pooled HLM as the enzyme source. Four concentrations of each inhibitor (see Methods) were screened at an R,S-lorazepam concentration of 100 µM, which provides separate enantiomer concentrations close to the $K_m$ (37 µM) for R- and S-lorazepam glucuronide formation by pooled HLM. Non-specific binding of inhibitors was taken into account in the estimation of $IC_{50}$ values. Each compound generally inhibited both R- and S-lorazepam glucuronidation to a similar extent (Table 4). Ketoconazole was the only compound with an $IC_{50} < 100$ µM.

Kinetic studies were undertaken to determine the mechanism and $K_i$ values for ketoconazole inhibition of R- and S-lorazepam glucuronidation, and for valproic acid since a lorazepam – valproic acid interaction has been reported in vivo (see Discussion). As shown in
Figure 6, ketoconazole inhibition of R- and S-lorazepam glucuronide formation was best described by the equations for non-competitive and competitive inhibition, respectively, whereas inhibition of R- and S-lorazepam glucuronidation by valproic acid was modeled well by the equation for non-competitive inhibition. $K_i$ values for inhibition of R- and S-lorazepam glucuronidation by ketoconazole were $15 \pm 0.1 \mu M$ and $4.0 \pm 0.1 \mu M$, respectively, whereas the respective $K_i$ values for valproic acid inhibition of R- and S-lorazepam glucuronidation were $3997 \pm 0.1 \mu M$ and $3286 \pm 0.1 \mu M$.

**IVIVE to predict potential of drug-drug interaction**

The $K_i$ values generated in vitro were substituted in Equation 2 to predict the increases in the AUCs of R- and S-lorazepam when coadministered with ketoconazole or valproic acid. Predictions for inhibition by valproic acid were based on the total and unbound maximum hepatic inlet concentration; $k_a$, $F_a$ and $Q_H$ were taken as 0.1 min$^{-1}$ (Ito et al., 2004), 1.0 (Bressolle et al., 1994), and 1.5 L/min, respectively. However, since the bioavailability (and $F_a$) of ketoconazole is unknown, predictions were undertaken using total and unbound maximum plasma concentration. Maximum plasma concentrations reported for ketoconazole (200 mg once daily) and valproic acid (500 mg twice a day) are 7.9 $\mu M$ (Huang et al., 1986) and 693 $\mu M$ (Dutta et al., 2003), respectively. Fractions unbound of ketoconazole and valproic acid in blood were taken as 0.01 (Daneshmend and Warnock, 1988) and 0.10 (Anderson et al., 1994a), respectively. Thus, the respective maximum unbound concentrations of ketoconazole and valproic acid in plasma are 0.079 $\mu M$ and 69.3 $\mu M$. Based on systemic total concentration, predicted AUC increases of R- and S-lorazepam when coadministered with ketoconazole (200mg) were 35% and 99%, respectively. Predicted increases in AUC due to co-administration of valproic acid (500mg) were in the range of 15-18%. Due to the high plasma protein binding of ketoconazole and valproic acid, no
interaction was predicted when inhibitor concentration was taken as the unbound concentration.
DISCUSSION

The human liver microsomal glucuronidation kinetics and UGT enzyme selectivity of the lorazepam enantiomers have been characterized here for the first time. R- and S-oxazepam glucuronidation by HLM exhibited substrate inhibition kinetics and the derived mean kinetic parameters for each enantiomer were close in value. $CL_{int}$ values varied 2.3- and 4.9-fold for R- and S- lorazepam glucuronidation, respectively. The lack of enatioselectivity observed here for lorazepam glucuronidation by HLM suggests that the in vivo clearances of R- and S-lorazepam are likely to be similar. Studies with recombinant human UGTs demonstrated that both enantiomers were glucuronidated by UGT2B4, UGT2B7 and UGT2B15, although R-lorazepam was additionally metabolized by the extrahepatic enzymes UGT1A7 and UGT1A10. The UGT protein content of the recombinant enzyme systems is unknown and hence it is not possible to unequivocally determine the relative contributions of UGT2B4, UGT2B7 and UGT2B15 to R- and S- lorazepam glucuronidation by HLM. However, the $CL_{int}$ value for S-lorazepam glucuronidation by UGT2B15 was an order of magnitude higher than those of UGT2B4 and UGT2B15, while the $CL_{int}$ values for R-lorazepam glucuronidation by UGT2B7 and UGT2B15 were an order of magnitude higher than that for UGT2B4.

A significant contribution of UGT2B15 to lorazepam glucuronidation is supported by the observation that the systemic clearance of lorazepam was 42% lower in subjects homozygous for the $UGT2B15^*2$ allele compared to subjects who were homozygous for wild-type $UGT2B15^*1$ (Chung et al., 2005). Indeed, in the absence of interacting drugs, UGT2B15 genotype was estimated to account for 61% of the interindividual variability in lorazepam clearance (Chung et al., 2005). Further, Court (2005) reported a high correlation between S-lorazepam and S-oxazepam glucuronidation in a panel of HLM; available evidence indicates that S-oxazepam is glucuronidated largely by UGT2B15 (see later
discussion). In the same review, Court (2005) presented data showing lower rates of S-lorazepam glucuronidation by HLM from donors carrying the *UGT2B15* allele, consistent with the in vivo study of Chung et al. (2005). It should be noted, however, that experimental details were not provided by Court (2005). In an investigation of the influence of UGT2B7 polymorphism on lorazepam disposition, lorazepam clearance was not significantly different between subjects with the *UGT2B7* *1* and *2* genotypes (Chung et al., 2008). However, the latter polymorphism appears to minimally affect the glucuronidation of UGT2B7 substrates and hence little can be inferred regarding the role of UGT2B7 in lorazepam glucuronidation in vivo from this observation (Bhasker et al., 2000).

$K_m$ values for the substrates of several UGT enzymes, especially UGT 1A9, 2B4 and 2B7, are over-estimated by as much as an order of magnitude when HLM and recombinant proteins (expressed in HEK293 and insect cells) are used as enzyme sources, presumably due to the presence of long-chain unsaturated fatty acids which act as inhibitors of xenobiotic glucuronidation (Rowland et al., 2007 and 2008; Kilford et al., 2009; Raungrut et al., 2010; Manevski et al., 2011; Walsky et al., 2012). Variable effects on $V_{max}$ have also been reported. Addition of bovine serum albumin (BSA) or fatty acid free human serum albumin sequesters the inhibitory fatty acids released from membranes during the course of an incubation, resulting in a reduction in $K_m$ to a value which presumably reflects true substrate binding affinity. More recently, 0.1% BSA was shown to reduce the $K_m$ values for UGT2B15-catalyzed 17α-estradiol and 4-methylumbelliferone glucuronidation by 45% and 77%, respectively, with variable effects on $V_{max}$ (Manevski et al., 2013). Since lorazepam binds extensively to albumin, addition of BSA to incubations was not feasible in the present study. However, based on the data of Manevski et al. (2013) the $K_m$ values for R- and S-lorazepam reported here may be higher than the ‘true’ parameter.
Despite the structural similarity of lorazepam and oxazepam, marked differences occur in glucuronidation kinetics and enantioselectivity. Court et al. (2002) reported that $K_m$ values for S-oxazepam glucuronidation by 3 livers were lower than those of R-oxazepam (54 vs. 277 µM) and $V_{\max}$ values were higher (303 vs. 101 pmol/min.mg), resulting in an approximately 10-fold higher $CL_{\text{int}}$ for S-oxazepam. Although Patel et al. (1995a) observed similar $K_m$ values for R- and S-oxazepam glucuronidation (250 vs. 219 µM) for ‘typical’ livers in a larger sample of HLM ($n = 33$), the mean $V_{\max}$ was 3.6-fold higher for S-oxazepam (249 vs. 70 pmol/min.mg). Despite the differences between the two reports, the higher $CL_{\text{int}}$ for S-oxazepam glucuronidation by HLM determined by Court et al. (2002) and Patel et al. (1995a) is consistent with the preferential glucuronidation of S-oxazepam in vivo; the S-oxazepam to R-oxazepam glucuronide ratios observed in plasma and urine are 3.5 and 3.9, respectively (Patel et al., 1995a). The $K_m$ values for R- and S- lorazepam found in the present study are similar to those reported by Court et al. (2002) for S-oxazepam, but $V_{\max}$’s are considerably lower than those of both R- and S- oxazepam. Comparative data for S-lorazepam and S-oxazepam glucuronidation activities in a panel of HLM reported by Court (2005) are also consistent with an approximate 10-fold lower rate of S-lorazepam glucuronidation.

In comparison to lorazepam, Court et al. (2002) further reported marked differences in the UGT enzyme selectivity of the oxazepam enantiomers. Whereas S-oxazepam was glucuronidated almost exclusively by UGT2B15 (with minor contributions from UGT 1A1, 1A6 and 2B7), R-oxazepam was glucuronidated by UGT1A9 and UGT2B7. Moreover, recombinant UGT2B15*2 exhibited very low activity with S-oxazepam. UGT1A7, which was observed here to glucuronidate R-lorazepam, also metabolized R-oxazepam. Court and colleagues (Court et al., 2004; He et al., 2009) subsequently showed S-, but not R-, lorazepam glucuronidation activity was reduced in HLM from donors expressing
UGT2B15*2, and the apparent oral clearance of oxazepam was reduced in subjects homozygous for UGT2B15*2. Overall, the UGT2B15*2 allele was estimated to account for 34% of the interindividual variability in oxazepam clearance (He et al., 2009). The differential effects of inhibitors on R- and S-oxazepam glucuronidation by HLM further support the involvement of different UGT enzymes in the glucuronidation of the oxazepam enantiomers (Patel et al., 1995b). In contrast to lorazepam and oxazepam, there appear to be no studies that have investigated the UGT enzymes involved in the glucuronidation of temazepam.

In addition to characterizing the kinetics and UGT enzyme selectivity of lorazepam glucuronidation, this study also aimed to investigate potential DDIs that might arise from inhibition of lorazepam glucuronidation. Inhibition of lorazepam glucuronidation would be expected to prolong the hypnosedative effects of this drug, with potential effects on safety (Lee et al., 2002). Several studies have shown that valproic acid, which appears to be glucuronidated primarily by UGT2B7 (Jin et al., 1993; Argikar and Remmel, 2009), decreases the plasma clearance of lorazepam by approximately 20 - 40% for doses ranging from 500 – 1000 mg/day (Anderson et al., 1994b; Samara et al., 1997; Chung et al., 2005 and 2008). Furthermore, several drugs have been shown to inhibit UGT2B4 and/or UGT2B7 in vitro and/or in vivo, including codeine, fluconazole, ketamine, ketoconazole, methadone, morphine, and zidovudine (Yong et al., 2005; Uchaipichat et al., 2006 and 2011; Takeda et al., 2006; Raungrut et al., 2010).

Of the drugs screened for inhibition, ketoconazole was the only compound with an IC₅₀ < 100 µM (Table 4). The Kᵢ values generated for ketoconazole inhibition of R- and S-lorazepam glucuronidation predicted AUC increases of 35% and 99%, respectively, based on total inhibitor concentration in blood, whereas no interaction was predicted when unbound inhibitor (ketoconazole) concentration was used for IVIVE. The magnitude of DDIs
involving glucuronidated drugs appear to be predicted more accurately when total rather than unbound inhibitor concentration is employed for IVIVE (Miners et al., 2010 a and b). Likewise, the magnitude of the lorazepam – valproic acid interaction was more accurately predicted (15 – 18% increase in R-/S- lorazepam AUC) using total valproic acid concentration in blood. Differences were observed in the IC₅₀ values for inhibition of the glucuronidation of the separate lorazepam enantiomers by a number of drugs (Table 4), and Kᵢ values and mechanisms of inhibition for ketoconazole differed between R- and S-lorazepam. These observations may arise from differences in the effects of inhibitors of the various enzymes that contribute to R- and S-lorazepam. For example, codeine, morphine and zidovudine are known substrates of UGT2B7 (and possibly UGT2B4) and might therefore be expected to inhibit UGT2B7 to a greater extent than UGT2B15 (Court et al., 2003; Stone et al., 2003; Raungrut et al., 2010).

In summary, kinetic parameters determined here for R- and S- lorazepam glucuronidation by HLM were similar in value, indicating a lack of stereoselectivity in lorazepam glucuronidation. Multiple UGT enzymes, including UGT2B4, 2B7 and 2B15 contribute to the hepatic glucuronidation of R- and S- lorazepam, although UGT2B15 appears to be the major form responsible for S-lorazepam glucuronidation. However, S-lorazepam is not recommended as a substrate ‘probe’ for UGT2B15 given the potential involvement of other UGT enzymes in S-lorazepam glucuronidation and the low activities for this pathway in HLM. Inhibition studies in vitro suggest that co-administration of ketoconazole, a commonly used antifungal agent, may result in inhibition of lorazepam clearance.
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AUTHORSHIP CONTRIBUTIONS

Participated in research design: Uchaipichat, Miners and Suhsisang

Conducted experiments: Uchaipichat

Performed data analysis: Uchaipichat and Miners

Wrote manuscript: Uchaipichat and Miners
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Rowland A, Gaganis P, Elliot DJ, Mackenzie PI, Knights KM, and Miners JO (2007) Binding of inhibitory fatty acids is responsible for the enhancement of UDP-


FOOTNOTES

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FIGURE LEGENDS

Figure 1. Chromatograms of R,S-lorazepam (panel A) and the lorazepam glucuronides LZPG1 and LZPG2 Panel 2). R,S-Lorazepam (400 µM) was incubated with pooled human liver microsomes (1 mg/ml) for 150 min in the absence (panel A) and presence (panel B) of UDPGA (5 mM).

Figure 2. Representative rate versus substrate concentration (panels A and C) and Eadie Hofstee (panels B and D) plots for R- and S- lorazepam glucuronidation by human liver microsomes. Points are experimentally determined values (mean of duplicate estimates) while the solid lines are the computer generated curves of best-fit.

Figure 3. Formation of R- and S- lorazepam glucuronides (panels A and B, respectively) by recombinant human UGT enzymes at R- and S- lorazepam concentrations of 10, 50 and 250 µM. Results are represent the mean of duplicate measurements (< 10% variance).

Figure 4. Eadie-Hofstee plots for R-lorazepam glucuronidation by recombinant UGT1A7 (panel A), UGT1A10 (panel B), UGT2B4 (panel C), UGT2B7 (panel D), and UGT2B15 (panel E). Points are experimentally determined values (mean of duplicate estimates) while the solid lines are the computer generated curves of best-fit.

Figure 5. Eadie-Hofstee plots for S-lorazepam glucuronidation by recombinant UGT2B4 (panel A), UGT2B7 (panel B), and UGT2B15 (panel C). Points are experimentally determined values (mean of duplicate estimates) while the solid lines are the computer generated curves of best-fit.

Figure 6. Dixon plots for the inhibition of R- and S- lorazepam by ketoconazole (panels A and B) and valproic acid (panels C and D). Points are experimentally determined values (mean of duplicate estimates) while the solid lines are the computer generated curves of best-fit.
### Table 1. Derived kinetic parameters of R- and S-lorazepam glucuronidation by human liver microsomes

<table>
<thead>
<tr>
<th>Liver</th>
<th>R-Lorazepam</th>
<th></th>
<th></th>
<th></th>
<th>S-Lorazepam</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_m$ (µM)</td>
<td>$V_{max}$ (pmol/min/mg protein)</td>
<td>$K_{Si}$ (µM)</td>
<td>$CL_{int}^b$ (µl/min/mg)</td>
<td>$K_m$ (µM)</td>
<td>$V_{max}$ (pmol/min/mg protein)</td>
<td>$K_{Si}$ (µM)</td>
<td>$CL_{int}^b$ (µl/min/mg)</td>
</tr>
<tr>
<td>H7</td>
<td>32</td>
<td>5.9</td>
<td>408</td>
<td>0.18</td>
<td>39</td>
<td>5.0</td>
<td>447</td>
<td>0.13</td>
</tr>
<tr>
<td>H10</td>
<td>17</td>
<td>5.0</td>
<td>424</td>
<td>0.29</td>
<td>24</td>
<td>9.3</td>
<td>381</td>
<td>0.39</td>
</tr>
<tr>
<td>H12</td>
<td>38</td>
<td>9.9</td>
<td>395</td>
<td>0.26</td>
<td>43</td>
<td>13</td>
<td>303</td>
<td>0.30</td>
</tr>
<tr>
<td>H13</td>
<td>40</td>
<td>7.1</td>
<td>252</td>
<td>0.18</td>
<td>51</td>
<td>8.7</td>
<td>229</td>
<td>0.17</td>
</tr>
<tr>
<td>H40</td>
<td>22</td>
<td>9.1</td>
<td>358</td>
<td>0.41</td>
<td>25</td>
<td>16</td>
<td>395</td>
<td>0.64</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>29 ± 8.9</td>
<td>7.4 ± 1.9</td>
<td>367 ± 62</td>
<td>0.26 ± 0.08</td>
<td>36 ± 10*</td>
<td>10 ± 3.8</td>
<td>351 ± 76</td>
<td>0.33 ± 0.18</td>
</tr>
<tr>
<td>Pooled HLM</td>
<td>37</td>
<td>7.6</td>
<td>263</td>
<td>0.21</td>
<td>37</td>
<td>10</td>
<td>354</td>
<td>0.27</td>
</tr>
</tbody>
</table>

$^a$ Data modeled using the substrate inhibition equation

$^b$ $CL_{int}$ calculated as $V_{max}/K_m$

*p < 0.05
Table 2. Derived kinetic constants for R-lorazepam glucuronidation by recombinant human UDP-glucuronosyltransferase enzymes.

<table>
<thead>
<tr>
<th>UGT enzyme</th>
<th>Kinetics</th>
<th>K_m or S_50 (µM)</th>
<th>V_max (pmol/min.mg)</th>
<th>CL_{int} or CL_{max} (µl/min.mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A7</td>
<td>Michaelis-Menten</td>
<td>25 ± 1</td>
<td>0.44 ± 0.01</td>
<td>0.0176</td>
</tr>
<tr>
<td>1A10^d</td>
<td>Substrate inhibition</td>
<td>236 ± 19</td>
<td>2.8 ± 0.5</td>
<td>0.0119</td>
</tr>
<tr>
<td>2B4^e</td>
<td>Hill equation</td>
<td>43 ± 4.2</td>
<td>0.29 ± 0.02</td>
<td>0.0034</td>
</tr>
<tr>
<td>2B7</td>
<td>Michaelis-Menten</td>
<td>13 ± 0.05</td>
<td>0.52 ± 0.01</td>
<td>0.0400</td>
</tr>
<tr>
<td>2B15^f</td>
<td>Substrate inhibition</td>
<td>44 ± 6.5</td>
<td>0.5 ± 0.07</td>
<td>0.0114</td>
</tr>
</tbody>
</table>

^a Data presented as mean ± standard error of parameter fit

^b CL_{int} calculated as V_{max}/K_m

^c CL_{max} calculated as \( \frac{V_{max}}{S_{50}} \times \frac{(n-1)}{n(n-1)^{1/n}} \)

^d K_{si} = 350 ± 121 from substrate inhibition equation

^e n = 1.7 ± 0.2 from Hill equation

^f K_{si} = 65 ± 15 from substrate inhibition equation
Table 3. Derived kinetic constants for S-lorazepam glucuronidation by recombinant human UDP-glucuronosyltransferase enzymes \(^{a,b}\)

<table>
<thead>
<tr>
<th>UGT</th>
<th>(S_{50}) (µM)</th>
<th>(n)</th>
<th>(V_{\text{max}}) (pmol/min.mg)</th>
<th>(\text{CL}_{\text{max}}) (µl/min.mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2B4</td>
<td>46 ± 8</td>
<td>1.6 ± 0.2</td>
<td>0.13 ± 0.01</td>
<td>0.0015</td>
</tr>
<tr>
<td>2B7</td>
<td>17 ± 0.7</td>
<td>1.5 ± 0.06</td>
<td>0.20 ± 0.01</td>
<td>0.0062</td>
</tr>
<tr>
<td>2B15</td>
<td>23 ± 0.8</td>
<td>1.4 ± 0.04</td>
<td>0.83 ± 0.01</td>
<td>0.0198</td>
</tr>
</tbody>
</table>

\(^a\) Data presented as mean ± standard error of parameter fit  
\(^b\) Data modeled using Hill equation  
\(^c\) \(\text{CL}_{\text{max}}\) calculated as \(\frac{V_{\text{max}}}{S_{50}} \times \frac{(n-1)}{n(n-1)^{1/n}}\)
### Table 4. IC₅₀ values for inhibition of human liver microsomal R- and S-lorazepam glucuronidation

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>IC₅₀ (µM)ᵃᵇ</th>
<th>R-Lorazepam</th>
<th>S-Lorazepam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codeine</td>
<td>809 ± 0.4</td>
<td>2562 ± 277</td>
<td></td>
</tr>
<tr>
<td>(10-1000 µM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluconazole</td>
<td>4599 ± 315</td>
<td>4245 ± 19</td>
<td></td>
</tr>
<tr>
<td>(10-1000 µM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketamine</td>
<td>205 ± 2</td>
<td>118 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>(10-1000 µM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>22 ± 0.1ᶜ</td>
<td>18 ± 0.1ᶜ</td>
<td></td>
</tr>
<tr>
<td>(2.7-270 µM)ᶜ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methadone</td>
<td>166 ± 4ᶜ</td>
<td>138 ± 1ᶜ</td>
<td></td>
</tr>
<tr>
<td>(7.2-720 µM)ᶜ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphine</td>
<td>6259 ± 103</td>
<td>10629 ± 887</td>
<td></td>
</tr>
<tr>
<td>(10-1000 µM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valproic acid</td>
<td>3575 ± 32</td>
<td>3200 ± 19</td>
<td></td>
</tr>
<tr>
<td>(100-7500 µM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zidovudine</td>
<td>6471 ± 39</td>
<td>12818 ± 25</td>
<td></td>
</tr>
<tr>
<td>(10-1000 µM)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ᵃ Data given as IC₅₀ ± standard error of parameter fit.
ᵇ Unbound concentration range of each inhibitor (i.e. corrected for non-specific binding to HLM) is shown in parenthesis.
ᶜ IC₅₀ value is the unbound concentration (corrected for non-specific binding to HLM).
Figure 2

(A) *R*-lorazepam glucuronidation

(B) *R*-lorazepam glucuronidation

(C) *S*-lorazepam glucuronidation

(D) *S*-lorazepam glucuronidation
Figure 3

(A) R-lorazepam

(B) S-lorazepam
Figure 5

(A) UGT2B4

Rate (pmol/min.mg protein) vs. Rate (pmol/min.mg)/[S-Lorazepam] (μM)

(B) UGT2B7

Rate (pmol/min.mg protein) vs. Rate (pmol/min.mg)/[S-Lorazepam] (μM)

(C) UGT2B15

Rate (pmol/min.mg protein) vs. Rate (pmol/min.mg)/[S-Lorazepam] (μM)