Inhibition of UDP-glucuronosyltransferase enzymes by major cannabinoids and their metabolites

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Abbreviations: Area under the plasma concentration vs. time curve, AUC; azithromycin, AZT; bicinchoninic acid, BCA; cannabidiol, CBD; cannabinol, CBN;
cytochrome P450, CYP; drug-drug interaction, DDI; human embryonic kidney, HEK; human kidney microsomes, HKM; human liver microsomes, HLM; half-maximal inhibitory concentration, IC₅₀; binding-corrected IC₅₀; IC₅₀, u; liquid chromatography-tandem mass spectrometry, LC-MS/MS; multiple reaction monitoring, MRM; (−)-trans-Δ⁹-tetrahydrocannabinol, THC; UDP glucuronic acid, UDPGA; ultra-high-performance liquid chromatography, UPLC; 11-hydroxy-Δ⁹-tetrahydrocannabinol, 11-OH-THC; 11-nor-9-carboxy-Δ⁹-tetrahydrocannabinol, THC-COOH; 11-nor-9-carboxy-Δ⁹-tetrahydrocannabinol glucuronide, THC-COO-Gluc.
Abstract

The UDP-glucuronosyltransferase (UGT) family of enzymes play a central role in the metabolism and detoxification of a wide range of endogenous and exogenous compounds. UGTs exhibit a high degree of structural similarity and display overlapping substrate specificity, often making estimations of potential drug-drug interactions difficult to fully elucidate. One such interaction yet to be examined may be occurring between UGTs and cannabinoids, as the legalization of recreational and medicinal cannabis and subsequent co-usage of cannabis and therapeutic drugs increases in the U.S. and internationally. In the present study, the inhibition potential of the major cannabinoids Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN), as well as their major metabolites, was determined in microsomes isolated from HEK293 cells over-expressing individual recombinant UGTs and in microsomes from human liver and kidney specimens. The highest inhibition was seen by CBD against the glucuronidation activity of UGTs 1A9, 2B4, 1A6 and 2B7, with binding-corrected IC\textsubscript{50,u} values of 0.12 ± 0.020 μM, 0.22 ± 0.045 μM, 0.40 ± 0.10 μM and 0.82 ± 0.15 μM, respectively. Strong inhibition of UGT1A9 was also demonstrated by THC and CBN, with IC\textsubscript{50,u} values of 0.45 ± 0.12 μM and 0.51 ± 0.063 μM, respectively. Strong inhibition of UGT2B7 was also observed for THC and CBN; no or weak inhibition was observed with cannabinoid metabolites. This inhibition of UGT activity suggests that in addition to playing an important role in drug-drug interactions, cannabinoid exposure may have important implications in patients with impaired hepatic or kidney function.
Significance Statement

Major cannabinoids found in the plasma of cannabis users inhibit several UGT enzymes, including UGT1A6, UGT1A9, UGT2B4, and UGT2B7. This study is the first to show the potential of cannabinoids and their metabolites to inhibit all the major kidney UGTs as well as the two most abundant UGTs present in liver. This study suggests that as all three major kidney UGTs are inhibited by cannabinoids, greater drug-drug interaction effects might be observed from co-use of cannabinoids and therapeutics that are cleared renally.
Introduction

UDP-glucuronosyltransferases (UGTs) are an important family of phase II metabolizing enzymes that facilitate the detoxification of a wide variety of endogenous and exogenous compounds, including steroid hormones, drugs and environmental carcinogens (Meech et al., 2019). Mammalian UGTs are classified based on structural and amino acid sequence homology into two main families, the UGT1 and UGT2 families, which are further divided into three subfamilies UGT1A, UGT2A and UGT2B, and catalyze the transfer of glucuronic acid from UDP-glucuronic acid (UDPGA) to an electrophilic moiety of a given substrate, resulting in a more polar conjugate that is more easily excreted from the body in the urine or bile (Bushey and Lazarus, 2012). An additional subfamily, the UGT3A sub-family, contains two members, UGT3A1 and UGT3A2, which use alternative sugar donors UDP-N-acetylglucosamine, UDP-glucose and UDP-xylose as co-substrates (MacKenzie et al., 2011). Mammalian UGTs are membrane-bound enzymes localized in the endoplasmic reticulum (ER) and expressed with a high degree of tissue specificity (Meech et al., 2019). While many UGTs are highly expressed in the liver, some are also expressed in extrahepatic tissues, including kidney and tissues of the aerodigestive tract (Meech et al., 2019; Vergara et al., 2020). The UGTs that exhibit the highest level of hepatic expression are 2B7 (17% of total hepatic UGT expression), 2B4 (16.1%), UGTs 2B15 (11.2%) and UGT1A1 (11%) (Kasteel et al., 2020). A number of UGTs are also expressed in the kidney, including UGT1A9 (45% of total renal UGT expression), UGT2B7 (41%), and UGT1A6 (7%) (Rowland et al., 2013).
UGTs account for the metabolism of 15% of pharmaceuticals, and one-seventh of the drugs prescribed in the United States in 2002 are cleared by the UGTs (Williams et al., 2004). While studies of drug-drug interactions (DDIs) are a major emphasis of research for phase I metabolizing enzymes including the cytochromes P450 enzyme family, UGT enzymes have historically received less scrutiny for their DDI potential, even though drug interactions via the inhibition of glucuronidation have been increasingly identified. Impaired glucuronidation activity can cause undesired effects resulting from the slow elimination of endogenous substances such as bilirubin (Sun et al., 2017) as well the buildup of toxic drug metabolites, as has been documented in studies correlating individuals with UGT1A1-deficient phenotypes and irinotecan toxicity (Iyer et al., 1998; Tallman et al., 2007). DDIs between therapeutics and UGT inhibitors have also been observed in the case of UGT2B7 inhibition by both valproic acid and probenecid (Cimoch et al., 1998; Rowland et al., 2006).

The recent legalization of cannabis has caused a dramatic increase in the use of cannabis-derived products in both recreational and medicinal situations, where cannabis is frequently used or targeted for more chronic diseases like cancer, arthritis, depression and often concurrently used with important groups of conventional medications including anticancer agents, antidepressants and pain medications (Bridgeman and Abazia, 2017). Situations in which polypharmacy is occurring within a patient could result in deleterious DDIs between cannabinoids and any number of therapeutic agents. Δ⁹-tetrahydrocannabinol (THC) is the most well-described psychoactive constituent of cannabis, and plasma concentrations of THC and its active metabolite, 11-hydroxy (OH)-THC, quickly peak following usage and decrease rapidly.
over a short duration, dependent on the specific mode of consumption (see Figure 1) (Sharma et al., 2012). In contrast, the inactive metabolites, 11-nor-9-carboxy-Δ⁹-tetrahydrocannabinol (THC-COOH) and 11-COO-Δ⁹-tetrahydrocannabinol-glucuronide (THC-COO-Gluc), peak much more slowly, to a lower level than the active cannabinoids, and remain present in plasma over a much longer duration of time (Huestis, 2007). Actual plasma levels of active and inactive cannabinoids are highly variable (in the micromolar to sub-micromolar range) and will vary widely depending on the user, dose and method of ingestion. Cannabinol (CBN) appears to be a degradation product of THC within the Cannabis plant (Russo and Marcu, 2017) and has been shown to be only weakly psychoactive. Cannabidiol (CBD) is often termed as medical marijuana and interacts with the CB₁ and CB₂ receptors in the brain with a much lower affinity as compared to THC and 11-OH-THC, resulting in extremely low psychoactive effects (Pertwee, 2008). However, CBD usage is rapidly expanding among many patient populations due in part to its good safety profile (Larsen and Shahinas, 2020). Recent clinical and pre-clinical trials have shown that CBD has a broad range of potential applications, displaying anti-inflammatory properties, anti-psychotic and anti-epileptic effects; as well as modulation of the immune system and the central nervous system (Esposito et al., 2013; Boychuk et al., 2015; Campos et al., 2016; Devinsky et al., 2016). Similarly, CBD and its metabolites, 7-hydroxy-cannabidiol (7-OH-CBD) and 7-carboxy-cannabidiol (7-COO-CBD), are present in the plasma after cannabis inhalation, with unchanged CBD and, to a lesser extent, glucuronidated CBD, as the main excretion products in urine (Harvey and Mechoulam, 1990; Huestis, 2007). All cannabinoids are highly lipophilic, concentrating in tissues, with slow release back into the bloodstream
(Huestis, 2007). This leads to varying plasma concentrations of active and inactive cannabinoids that persist in the bloodstream, potentially incurring deleterious DDIs over a much wider timeframe than that of the initial cannabis consumption.

Previous studies have shown that THC, CBD, and CBN can strongly inhibit several major hepatic CYPs (Yamaori et al., 2010; Yamaori et al., 2011a; Yamaori et al., 2011b; Yamaori et al., 2011c; Jiang et al., 2013; Cox et al., 2019). In addition, the major active metabolite of THC, 11-OH-THC, and two major inactive metabolites, THC-COOH and THC-COO-Gluc, also exhibited strong inhibition of a number of hepatic CYP enzymes (Nasrin et al., 2020; Nasrin et al., 2021). In the present study, the inhibition potential of major cannabinoids and their metabolites against major hepatic and renal human UGT enzymes were evaluated.
Material and Methods

Chemicals and Reagents

THC, 11-OH-THC, THC-COOH, THC-COO-Gluc, CBD, 7-OH-CBD and CBN were purchased from Cayman Chemicals (Ann Arbor, MI) or Sigma-Aldrich (St. Louis, MO) as certified reference standards; the purchase of methanolic stock solutions of cannabinoids and its metabolites were deemed exempt by the Drug Enforcement Administration. Pooled human liver microsomes (HLM) [n=50, mixed gender (21 female and 29 male), race (42 Caucasian, 4 Hispanic, 2 African American, and 2 Asian), and age (5-77 y)] and pooled human kidney microsomes (HKM) [n=8, mixed gender (50% each), race (3 African American, 3 Caucasian, and 2 Hispanic) and age (42-70 years)] were obtained from Sekisui Xenotech, LLC (Lenexa, Kansas). β-estradiol, chenodeoxycholic acid (CDCA), trifluoperazine (TFP), serotonin, propofol, codeine, zidovudine (AZT), nicotine, oxazepam, dihydroexemestane (DHE), ketoconazole, diclofenac, acetaminophen and furosemide were all purchased from Sigma-Aldrich. Optima grade methanol, acetonitrile, and formic acid were obtained from Fisher Scientific (Waltham, MA). Ultra-low-binding microcentrifuge tubes, Dulbecco’s Modified Eagles Medium, Dulbecco’s phosphate-buffered saline, UDP glucuronic acid (UDPGA), alamethacin, MgCl₂ and geneticin (G418) were purchased from VWR (Radnor, PA). BCA protein assays were purchased from Pierce (Rockford, Illinois, USA), premium grade fetal bovine serum (FBS) was purchased from Seradigm (Radnor, PA), and ChromatoPur bovine serum albumin (BSA) was purchased from MB Biomedicals (Santa Ana, CA).
Inhibition assays

HEK293 cells individually overexpressing UGT isoforms UGTs 1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, 2B10, 2B15 and 2B17 were developed and described previously (Dellinger et al., 2006). Microsomal membrane fractions of UGT-overexpressing cell lines were prepared by differential centrifugation as previously described, with total microsomal protein concentrations determined using the BCA assay as per the manufacturer’s recommendations. An initial screen, performed in duplicate, of the inhibition potential of individual cannabinoids and their metabolites against UGTs 1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, 2B10, 2B15 and 2B17 were determined using microsomes (50 – 100 µg) from UGT-overexpressing HEK293 cell lines in reactions containing either 10 µM or 100 µM of cannabinoid or metabolite, probe substrate (see Supplemental Table 1), 50 mM Tris–HCl buffer (pH 7.4), MgCl₂ (5 mM), 2% BSA, and 4 mM UDPGA in a final reaction volume of 25 µL. All substrates were used at concentrations near their respective Michaelis–Menten constant (Kₘ; see Supplemental Table 1). As cannabinoids exhibit extensive non-specific binding (70-90%) to protein and labware (Garrett and Hunt, 1974), microsomal incubation conditions were optimized to prevent underestimation of inhibitory potency (IC₅₀). To reduce non-specific binding and adsorption to labware, low-binding microcentrifuge tubes were used for all reactions, with BSA added to increase the solubility of cannabinoids as well as to sequester inhibitory long-chain unsaturated fatty acids (Rowland et al., 2008; Patilea-Vrana et al., 2019).

Microsomes were pre-incubated with alamethicin (50 µg/mg of microsomal protein) on ice for 20 min prior to incubation. The reaction was initiated by the addition
of UDPGA and incubated for 60 - 120 min (see Supplemental Table 1) at 37°C.

Reactions were terminated and proteins precipitated by the addition of an equal volume (25 µL) of ice-cold stop solution (acetonitrile/methanol; 1:1). Samples were mixed on a vortex mixer and centrifuged at 17,000 x g for 15 min. The supernatant (~50 µL) was transferred to an ultra-performance liquid chromatograph (UPLC) sample vial, and the probe metabolite was detected using a UPLC (Waters Acquity; Waters Corp, Milford, MA) coupled to a triple-quadrupole mass spectrometer (Waters Xevo TQD; Waters Corp) by multiple reaction monitoring (MRM) analysis. As a positive control for every inhibition experiment, 1 µM or 10 µM probe inhibitors (ketoconazole/diclofenac) were added instead of the cannabinoid compounds. Reactions only containing vehicle (3% methanol) and without any inhibitor were used as an indicator of 100% activity for each substrate/enzyme combination. All analyses were performed in triplicate. Incubation conditions were optimized for HLM, HKM and overexpressing cell lines for both microsomal protein and reaction time, with optimal conditions chosen based on the following criteria: (1) metabolite formation was linear with time and enzyme concentration, (2) substrate consumption was no more than 20% of the initial amount, and (3) metabolite formation was reliably and reproducibly detected by the UPLC–MS/MS method utilized.

For UPLC-MS/MS, samples (2-5 µL) were injected onto an Acquity UPLC column (BEH C18, 1.7 µM, 2.1 X 100 mm; Waters Corp). A 9 min gradient elution was used with mobile phases A (0.1% formic acid in water) and B (100% methanol) as follows: 1 min at 95% A:5% B followed by a linear gradient for 7 min to 5% A:95% B, 1 min at 5% A:95% B and re-equilibration for 1 min at 95% A:5% B. The flow rate was 0.4 mL/min,
and the column temperature was 40°C. Analytes were detected using a Waters Xevo TQD tandem mass spectrometer equipped with a Zspray electrospray ionization interface operated in the positive ion mode for all the UGT metabolites tested in this study except furosemide glucuronide, which was analyzed in negative ion mode, with the capillary voltage at 0.6 kV. Nitrogen was used as both the cone and desolvation gas at 50 and 800 L/h, respectively. Ultrapure argon was used for collision-induced dissociation. The desolvation temperature was 500°C. For detection of the metabolite peaks, the mass spectrometer was operated in multiple reaction-monitoring mode (MRM) using the ion-related parameters for each transitions. The transition for detection of β-estradiol-3-glucuronide (m/z 447 > 271), acyl CDCA-24-glucuronide (m/z 567.5 > 391.5), trifluoperazine N-glucuronide (m/z 584 >408.2), serotonin-glucuronide (m/z 352 > 160.02), propofol-O-glucuronide (m/z 354 > 177.02), codeine-6-glucoronide (m/z 476.2 > 300.2), AZT-5'-glucuronide (m/z 442 > 125.05), nicotine-N-glucuronide (m/z 339.15 > 163.124), S-oxazepam-glucuronide (m/z 463.3 > 269.1), exemestane-17-O-glucuronide (m/z 475.23 > 281.19).

\textit{Determination of IC}_{50} \textit{values}

For those cannabinoids or metabolites that inhibited UGT activity ≥50% at cannabinoid concentrations ≤100 µM, IC}_{50} determinations were performed in HLM, HKM, and microsomes from HEK293 UGT-overexpressing cell lines, using multiple concentrations of cannabinoid inhibitor ranging between 0.5 - 120 µM.

Experiments were performed to determine non-specific binding constants (f_{u,inc}) for the individual cannabinoids in HEK293 microsomes, HLM, and HKM as previously described (Nasrin et al., 2021).
Statistical Analysis

Data were exported and analyzed using an Excel spreadsheet (Microsoft, USA). The amount of metabolite formed at each concentration of inhibitor relative to the control (percent relative activity) was calculated as:

\[
\text{% Relative activity} = \frac{\text{Peak area of metabolite with inhibitor}}{\text{Peak area of metabolite without probe inhibitor}} \times 100\%.
\]

\(IC_{50}\) values were calculated by plotting the relative activity of UGT enzymes versus the log concentration of the test inhibitors using GraphPad Prism 7.04 software (GraphPad Software Inc., San Diego, CA).
Results

Glucuronide metabolite peaks were detected by LC-MS/MS in incubations of each probe substrate analyzed in these studies (Figure 2). Using recombinant UGT (rUGT)-overexpressing cell microsomes and probe UGT substrates, preliminary screening studies demonstrated that 100 µM THC decreased the relative activity of microsomes from rUGT 1A9, 2B4 and 2B7 overexpressing cells by 74%, 79%, and 69%, respectively, as compared to control reactions without added cannabinoids (Figure 3). A similar pattern was observed for CBD, with 10 µM CBD exhibiting 25%, 91%, 66% and 58% inhibition, and 100 uM CBD exhibiting 54%, 98%, 94% and 96% inhibition, against microsomes from rUGTs 1A6, 1A9, 2B4, and 2B7 overexpressing cells, respectively, as compared to control reactions without added cannabinoid (Figure 3). Similar to that observed for THC and CBD, CBN exhibited significant inhibition against rUGT1A9 and rUGT2B7 microsomes. Unlike that observed for THC and CBD, significant inhibition was not observed for rUGT2B4 microsomes with CBN. While no significant inhibition was observed in rUGT1A1, rUGT1A3, rUGT1A4, and rUGT2B15 microsomes by THC, CBD, or CBN, marginal inhibition was observed for 100 uM CBD and CBN against rUGT2B17 microsomes (43% and 34%, respectively). Marginal inhibition (43% and 47%, respectively) was also observed for 100 uM CBN against rUGT1A6 and rUGT2B10 microsomes.

For THC and CBD metabolites, no significant inhibition was observed using up to 100 µM THC-COOH or THC-COO-Gluc against any of the UGT enzymes tested. However, 100 µM 11-OH-THC resulted in marginal inhibition of the activities of
rUGT1A9 (41%), rUGT2B4 (40%), and rUGT2B7 (53%) microsomes while 100 µM 7-OH-CBD resulted in marginal decreases in the activities of rUGT1A9 (40%) and rUGT2B7 (45%) microsomes (Figure 3).

The inhibitory effects of THC, 11-OH-THC, CBD and CBN were extended to establish IC$_{50}$ values and binding-corrected IC$_{50}$ values (IC$_{50,u}$) for each cannabinoid against the UGT enzymes shown to be inhibited by $\geq$50% using 100 µM cannabinoid in the rUGT screening assays (described above). The unbound fraction in the incubation mixture were 0.042 ± 0.003, 0.038 ± 0.002, and 0.085 ± 0.005 in overexpressing HEK cell lines for THC, CBD and CBN respectively. For HLM, the unbound fraction of THC, CBD and CBN in the incubation mixture were 0.048 ± 0.002, 0.051± 0.008, and 0.092 ± 0.006, respectively, and 0.052 ± 0.005, 0.062 ± 0.009, and 0.12 ± 0.015, respectively, in HKM.

The strongest inhibition was observed by CBD against rUGTs 1A9 and 2B4, with IC$_{50}$ values of 3.2 ± 0.52 µM and 5.8 ± 1.2 µM, and IC$_{50,u}$ values of 0.12 ± 0.020 µM and 0.22 ± 0.045 µM, using propofol and codeine as UGT1A9 and UGT2B4 probe substrates, respectively (Table 1). CBD also exhibited significant inhibition against serotonin (a probe substrate for rUGT1A6) in rUGT1A6 microsomes (IC$_{50}$ = 10 ± 2.6 µM and IC$_{50,u}$ = 0.40 ± 0.10 µM), and AZT glucuronidation as a probe substrate in rUGT2B7 microsomes (IC$_{50}$ = 21 ± 3.9 µM and IC$_{50,u}$ = 0.82 ± 0.15 µM). The IC$_{50}$ values for CBD for propofol glucuronidation were similar in HKM (IC$_{50}$ = 5.5 ± 0.56 µM and IC$_{50,u}$ = 0.34 ± 0.035 µM) but higher in HLM (IC$_{50}$ = 19 ± 4.6 µM and IC$_{50,u}$ = 1.0 ± 0.24 µM) as compared to that observed for rUGT1A9 microsomes (Table 1), a pattern that was reversed in HKM (IC$_{50}$ = 39 ± 5.9 µM and IC$_{50,u}$ = 2.5 ± 0.37 µM) vs HLM (IC$_{50}$ = 8.0 ±
1.1 µM and $IC_{50,u} = 0.40 \pm 0.058 \mu M$) for CBD inhibition of codeine glucuronidation. The decreased level of inhibition of propofol glucuronidation by CBD in HLM vs HKM and the similar inhibition pattern of HKM and rUGT1A9 microsomes is apparent when examining plots of percent glucuronidation activity vs CBD concentrations (Figure 4). Similar to that observed for rUGT2B7 microsomes, more moderate inhibition was observed for CBD of AZT glucuronidation in HLM ($IC_{50} = 30 \pm 4.1 \mu M$ and $IC_{50,u} = 1.5 \pm 0.21 \mu M$) and HKM ($IC_{50} = 35 \pm 3.5 \mu M$ and $IC_{50,u} = 2.2 \pm 0.22 \mu M$), with $IC_{50}$ values that were only slightly higher than that observed for rUGT2B7 microsomes (Table 1 and Figure 4). The $IC_{50}$ values for serotonin glucuronidation of $28 \pm 6.5 \mu M$ ($IC_{50,u} = 1.4 \pm 0.33 \mu M$), and $17 \pm 3.7 \mu M$ ($IC_{50,u} = 1.0 \pm 0.23 \mu M$) in HLM and HKM, respectively, were slightly higher than that observed for rUGT1A9 microsomes (Table 1).

THC exhibited $IC_{50}$ values that were slightly higher than CBD for propofol, codeine, and AZT glucuronidation in rUGT microsomes, HLM, and HKM (Table 1). Similar to that observed for CBD, THC exhibited similar $IC_{50}$ values for propofol in rUGT1A9 microsomes ($IC_{50} = 11 \pm 3.0 \mu M$ and $IC_{50,u} = 0.45 \pm 0.12 \mu M$) and codeine glucuronidation in rUGT2B4 microsomes ($IC_{50} = 11 \pm 2.7 \mu M$ and $IC_{50,u} = 0.47 \pm 0.11 \mu M$), with a higher value observed for AZT glucuronidation in rUGT2B7 microsomes ($IC_{50} = 33 \pm 8.5 \mu M$ and $IC_{50,u} = 1.4 \pm 0.36 \mu M$). Also similar to that observed for CBD, the $IC_{50}$ values for THC for propofol glucuronidation was similar in HKM ($IC_{50} = 12 \pm 3.4 \mu M$ and $IC_{50,u} = 0.64 \pm 0.18 \mu M$) but higher in HLM ($IC_{50} = 30 \pm 6.4 \mu M$ and $IC_{50,u} = 1.4 \pm 0.31 \mu M$) as compared to that observed for rUGT1A9 microsomes, a pattern that was reversed for THC inhibition of codeine glucuronidation in HKM ($IC_{50} = 55 \pm 5.2 \mu M$ and $IC_{50,u} = 2.9 \pm 0.27 \mu M$) vs HLM ($IC_{50} = 13 \pm 2.6 \mu M$ and $IC_{50,u} = 0.61 \pm 0.13 \mu M$). Again
similar to that observed for CBD, more moderate inhibition was observed for THC inhibition of AZT glucuronidation in HLM and HKM, with $IC_{50}$ values that were only slightly higher than that observed for rUGT2B7 microsomes [$IC_{50}$ values = 59 ± 6.6 (IC$_{50,u}$ = 2.8 ± 0.32 $\mu$M) and 51 ± 12 $\mu$M (IC$_{50,u}$ = 2.6 ± 0.65 $\mu$M), respectively].

The pattern of inhibition observed for CBN for propofol glucuronidation was virtually identical to that observed for both THC and CBD, with similar $IC_{50}$ values observed for rUGT1A9 microsomes ($IC_{50}$ = 6.0 ± 0.75 $\mu$M and $IC_{50,u}$ = 0.51 ± 0.063 $\mu$M) and HKM ($IC_{50}$ = 7.5 ± 1.7 $\mu$M and $IC_{50,u}$ = 0.90 ± 0.20 $\mu$M) and a higher $IC_{50}$ value observed for HLM ($IC_{50}$ = 31 ± 4.1 $\mu$M and $IC_{50,u}$ = 2.9 ± 0.38 $\mu$M; Table 1). Similar to that observed for both THC and CBD, CBN exhibited more moderate inhibition of AZT glucuronidation, with similar $IC_{50}$ values observed for rUGT2B7 microsomes ($IC_{50}$ = 49 ± 12 $\mu$M and $IC_{50,u}$ = 4.2 ± 1.1 $\mu$M), HLM ($IC_{50}$ = 59 ± 8.6 $\mu$M and $IC_{50,u}$ = 5.5 ± 0.79 $\mu$M) and HKM ($IC_{50}$ = 57 ± 7.5 $\mu$M and $IC_{50,u}$ = 6.9 ± 0.090 $\mu$M). Since CBN did not exhibit inhibitory activity against codeine glucuronidation in the screening assays, $IC_{50}$ values were not determined for CBN against codeine glucuronidation in rUGT2B4 microsomes, HLM or HKM.

The only THC metabolite that exhibited $\geq$50% inhibition for any UGT in the rUGT microsomal screening assays was 11-OH-THC for UGT2B7. This metabolite exhibited weak inhibition of rUGT2B7 microsomal activity ($IC_{50}$ = 79 ± 11 $\mu$M and $IC_{50,u}$ = 4.9 ± .41 $\mu$M, calculated using the 11-OH-THC $f_{u,inc}$ value from previous studies (Nasrin et al., 2021). The $IC_{50}$ values for AZT glucuronidation in HLM and HKM were not determined.
as inhibition did not occur at >50% at the concentration range tested (up to 100 μM AZT).

To better validate the inhibitory effects of cannabinoids on UGT1A9-mediated glucuronidation, two additional UGT1A9 probe substrates, furosemide and acetaminophen, were examined. As shown in Table 1, the glucuronidation of both agents were strongly inhibited by THC, CBD and CBN at levels similar to those observed for propofol glucuronidation in rUGT1A9 microsomes. The highest level of inhibition was again observed with CBD, with \( IC_{50} \) values in rUGT1A9 microsomes of 2.4 ± 0.66 μM (\( IC_{50,u} = 0.090 ± 0.025 \) μM) and 1.9 ± 0.29 μM (\( IC_{50,u} = 0.073 ± 0.011 \) μM) for furosemide and acetaminophen glucuronidation, respectively (Table 1). The \( IC_{50} \) values observed in HKM were very similar to those determined in rUGT1A9 microsomes, with CBD exhibiting the highest level of inhibition at 3.6 ± 0.80 μM (\( IC_{50,u} = 0.22 ± 0.049 \) μM) and 3.8 ± 0.82 μM (\( IC_{50,u} = 0.24 ± 0.05 \) μM) for furosemide and acetaminophen glucuronidation, respectively, and less inhibition in HLM, with \( IC_{50} \) values of 29 ± 4.0 μM (\( IC_{50,u} = 1.5 ± 0.20 \) μM) and 12 ± 3.2 μM (\( IC_{50,u} = 0.64 ± 0.16 \) μM), respectively. The decreased level of inhibition of furosemide and acetaminophen glucuronidation by CBD in HLM vs HKM and the similar inhibition pattern of HKM and rUGT1A9 microsomes is apparent when examining plots of percent glucuronidation activity vs CBD concentrations (Figure 4). THC and CBN were slightly less potent inhibitors in rUGT1A9 microsomes, with \( IC_{50} \) values of 8.0 ± 0.47 μM (\( IC_{50,u} = 0.33 ± 0.020 \) μM) and 9.2 ± 2.1 μM (\( IC_{50,u} = 0.78 ± 0.18 \) μM), respectively, against furosemide, and 12 ± 3.7 μM (\( IC_{50,u} = 0.49± 0.15 \) μM) and 6.9 ± 0.54 μM (\( IC_{50,u} = 0.59 ± 0.046 \) μM), respectively, against acetaminophen (Table 1). However, the same trend observed in
the tissue microsomes was also observed for rUGT1A9 microsomes, with similar $IC_{50}$ values for HKM against furosemide ($IC_{50} = 10 \pm 4.1$ μM and $IC_{50,u} = 0.54 \pm 0.22$ μM for THC; $IC_{50} = 15 \pm 0.8$ μM and $IC_{50,u} = 1.9 \pm 0.092$ μM for CBN) and acetaminophen ($IC_{50} = 15 \pm 3.0$ μM and $IC_{50,u} = 0.79 \pm 0.16$ μM for THC; $IC_{50} = 21 \pm 3.4$ μM and $IC_{50,u} = 2.6 \pm 0.41$ μM for CBN), but somewhat higher for HLM against furosemide ($IC_{50} = 32 \pm 6.3$ μM and $IC_{50,u} = 1.5 \pm 0.30$ μM for THC; $IC_{50} = 30 \pm 4.5$ μM and $IC_{50,u} = 2.8 \pm 0.96$ μM for CBN) and acetaminophen ($IC_{50} = 29 \pm 8.9$ μM and $IC_{50,u} = 1.4 \pm 0.43$ μM for THC; $IC_{50} = 34 \pm 6.3$ μM and $IC_{50,u} = 3.1 \pm 0.58$ μM for CBN; Table 1). The decreased level of inhibition of furosemide and acetaminophen glucuronidation by THC and CBN in HLM vs HKM and the similar inhibition pattern with both THC and CBN of HKM and rUGT1A9 microsomes is apparent when examining plots of percent glucuronidation activity vs CBD concentrations (Supplemental Figure 1).
Discussion

The present study is the first to conduct a comprehensive examination of the inhibitory effects of major cannabinoids (THC, CBD and CBN) on the enzymatic activities of each of the primary hepatic UGT enzymes (UGTs 1A1, 1A3, 1A4, 1A9, 2B4, 2B7, 2B10, 2B15, and 2B17). In addition, the major metabolites of THC and CBD (11-OH-THC, THC-COOH, THC-COO-Gluc, and 7-OH-CBD) were also screened as potential inhibitors. The results from the present study indicate that the parent cannabinoids (THC, CBD and CBN) exhibit strong inhibition of the glucuronidation activities of UGTs 1A6, 1A9, 2B4 and 2B7, and marginal inhibition of a number of additional UGTs including 2B17 by both CBD and CBN, and UGT2B10 by CBN. In contrast to that observed previously for major hepatic CYP450 enzymes (Yamaori et al., 2011a; Yamaori et al., 2011b; Yamaori et al., 2011c; Yamaori et al., 2012; Bansal et al., 2020; Nasrin et al., 2020; Nasrin et al., 2021), major THC metabolites exhibited little inhibition of hepatic UGT enzymes, with only 11-OH-THC exhibiting significant inhibition against a single UGT (UGT2B7). Similar to that observed for THC metabolites, the major CBD metabolite, 7-OH-CBD, exhibited no significant inhibition against the UGTs tested, with only marginal inhibition observed for UGTs 1A9 and 2B7. In a pattern similar to that observed for CYP450 enzymes, THC-COOH exhibited no significant inhibition against any of the UGT enzymes tested in the present study.

Tissue and plasma concentrations of cannabinoids vary widely by user and are dependent upon a number of factors including dose, cannabis strain, mode of consumption and expertise of the user (Sharma et al., 2012). Average plasma
concentrations of THC from a 10 mg dose by inhalation are 110 ug/L (0.35 µM), which are about 3-fold higher than those observed for oral dosing (360 ug/L; 1.1 µM). The average CBD plasma levels after a 400 mg oral dose is 181 ug/L (0.76 µM) (Manini et al., 2015). The IC50, u values observed in the present study for THC and CBD against several UGT enzymes are in the micromolar to sub-micromolar range, suggesting that unwanted DDIs with xenobiotics metabolized by the same UGT enzymes may occur in co-users of cannabis.

Of the cannabinoids tested, the strongest inhibition in rUGT microsomes was observed by CBD against the glucuronidation of propofol (UGT1A9), serotonin (UGT1A6), codeine (UGT2B4) and AZT (UGT2B7), followed by THC, which also exhibited strong inhibition against the same suite of enzymes. CBN was shown to be similarly effective at inhibiting the glucuronidation of propofol and AZT in rUGT1A9 and rUGT2B7 microsomes, respectively; however, unlike that observed for CBD and THC, no inhibition of rUGT2B4 microsomal activity was observed by CBN using codeine as the probe substrate.

While the liver is considered the most important organ for the metabolism of drugs and other xenobiotics, the kidney also plays an important role, especially when glucuronidation is a primary component of a drug’s metabolism and elimination (Margaillan et al., 2015). UGT protein expression in both the human liver and human kidney has large interindividual variability, however current literature estimates that 13 UGTs are expressed in significant amounts in liver, while only 3 UGTs are appreciably expressed in human kidney, including UGTs 1A9 and 2B7, which are expressed at similar levels, and UGT1A6, which is expressed at a much lower level; UGT2B4 shows
negligible expression in human kidney (Margaillan et al., 2015; Basit et al., 2020). Consistent with the relatively high expression pattern of UGT1A9 in human kidney, the $IC_{50}$ values observed in HKM for propofol, furosemide and acetaminophen, all UGT1A9 substrates, were similar to that observed for each agent in rUGT1A9 microsomes for CBD, THC and CBN. This contrasts with HLM, where the $IC_{50}$ values were higher (approximately 3-fold) than those observed in rUGT1A9 microsomes in all cases. In addition, the 6-fold lower $IC_{50}$ exhibited by UGT1A9 as compared to UGT2B7 in rUGT microsomes by THC, CBD and CBN corresponds with the larger $IC_{50}$ values observed in HKM using a UGT2B7 probe substrate (AZT) vs that observed for UGT1A9 probe substrates, reflecting the relative inhibition of the two enzymes by cannabinoids. These data support the possibility that the major cannabinoids in Cannabis, CBD, THC and CBN, may all act to inhibit the two highly expressed UGT enzymes in human kidney, UGT1A9 and UGT2B7, in vivo.

The relative inhibition of codeine glucuronidation observed in HKM was approximately 5- to 7-fold higher for THC and CBD as compared to rUGT2B4 microsomes, suggesting that UGT2B4 is likely not a major glucuronidating enzyme in kidney. This pattern is consistent with the low relative expression of UGT2B4 in this organ (Basit et al., 2020). This activity pattern contrasts to the very similar $IC_{50}$ values observed in HLM for codeine glucuronidation as compared to those observed in rUGT2B4 microsomes, a pattern consistent with the high expression of UGT2B4 in human liver. While codeine glucuronidation is considered a probe substrate of UGT2B4 activity, UGT2B7 is also likely a major contributor to the hepatic glucuronidation of this agent (Court et al., 2003). When comparing codeine glucuronidation activities for both
THC and CBD in the present study, the $IC_{50}$ values are nearly identical in HLM to those determined for rUGT2B4 microsomes. In addition the $IC_{50}$ values were approximately 3.6-fold lower for rUGT2B4 microsomes than for rUGT2B7 microsomes for both cannibinoids. These data indicate that although these two UGT enzymes are highly homologous, they may have unique binding interactions with cannabinoids, and suggest that CBD, THC and CBN strongly inhibit hepatic UGT2B4 activity.

UGT2B7 is arguably the most important UGT isoform involved in phase II metabolism, as it is expressed at high levels in the liver and is the most commonly listed UGT isoform involved in the biotransformation of the top 200 drugs currently prescribed in the United States (Williams et al., 2004). Inhibition of this enzyme has the potential to impact thousands of patients through unwanted drug-drug interactions, toxicities and off-target effects. As seen from the $IC_{50}$ values of THC, CBD and CBN against the UGT2B7 probe substrate AZT in HLM, the UGT2B7-mediated glucuronidation of AZT is moderately inhibited by these cannabinoids. In all three cases, the $IC_{50}$ determined in HLM is similar to the value determined in rUGT2B7, suggesting that UGT2B7 is inhibited by these cannabinoids and that this inhibition can be translated to the human liver, where the potential for unwanted drug-drug interactions may occur. Indeed, one such interaction has been observed when Epidolex (CBD) is prescribed as an anti-seizure medication concurrently with the sedative midazolam (Patsalos et al., 2020). While midazolam itself is not glucuronidated by UGT2B7, its active metabolite, 1-hydroxymidazolam, is a well documented UGT2B7 substrate (Seo et al., 2010). Administration of midazolam with steady state levels of Epidolex results in increased plasma concentrations of active 1-hydroxymidazolam ($C_{\text{max}} = \uparrow12\%, \ AUC_{0-t} = \uparrow68\%$), as
well as a delay in $t_{\text{max}}$ (difference in median of 2.2 h) and an increase in $t_{1/2}$ of 35%.

Another study examined the disposition kinetics of the opioid morphine with and without concurrent inhaled vaporized cannabis (900 mg, 3.56% THC) (Abrams et al., 2011). Morphine is a well-studied substrate for UGT2B7 (Osborne et al., 1990) and is glucuronidated to both the inactive 3-glucuronide (M3G) and the highly active 6-glucuonide (M6G). A statistically significant decrease in steady state plasma levels of morphine was found when administered with vaporized cannabis (which was attributed to a decrease in the uptake of morphine), and a near significant decrease in the $C_{\text{max}}$ of inactive metabolite M3G was also observed, indicating reduced UGT2B7 glucuronidation activity in the presence of the inhaled vaporized THC.

Although the role of renal metabolism is still an underexplored area compared to hepatic metabolism, mounting evidence from recent publications indicate that the human kidney has significant metabolic capacity. Renal metabolism by UGT enzymes plays a major role in clearance of many drugs including acetaminophen and furosemide (assayed in this study) as well as carbamazepine, codeine, gemfibrozil, morphine, and the commonly used over the counter non-steroidal anti-inflammatory drugs (NSAIDs) ibuprofen, ketoprofen, and S-naproxen (Knights et al., 2013). Preferential inhibition of the renal UGTs may have a larger effect on drugs that are mainly excreted by renal glucuronidation, and interestingly, the two most highly expressed UGTs in human kidney (UGTs 1A9 and 2B7) were inhibited by CBD, THC and CBN in the present study. Therefore, cannabinoids, and especially CBD, may significantly and disproportionately affect the 1.5 million people in the United States (Rein, 2020) who are diagnosed with chronic kidney disease (CKD) and acute kidney injury. One-quarter to one-half of those...
patients also experience chronic symptoms such as pain, nausea, anorexia, sleep disturbance, anxiety, and depression (Rein, 2020), several of which are approved indications for medical cannabis (CBD). Additionally, CKD is associated with decreased activity of drug metabolizing enzymes and transporters (Dreisbach and Lertora, 2008). Moreover, a recent study showed significant reduction in the glucuronidation capacity of drugs metabolized by UGT1A9 and UGT2B7 in patients with kidney tumors (Margaillan et al., 2015). AZT and propofol metabolism were decreased 96- and 7.6-fold, respectively, in a patient with neoplastic kidney when compared with normal kidney, suggesting that the use of Cannabis or CBD in these patients may be deleterious.

In conclusion, the present study is the first to demonstrate that the major cannabinoids present in Cannabis are able to inhibit several of the primary UGT enzymes involved in phase II metabolism. CBD was shown to be the most potent cannabinoid inhibitor, exhibiting $IC_{50}$ values 2-3 fold lower than that observed for THC. While this is the first study to specifically address the inhibition of UGTs by CBD and other cannabinoids, previous reports indicate that CBD, THC and several THC metabolites are potent inhibitors of several major CYP450 enzymes (Yamaori et al., 2011a; Yamaori et al., 2011b; Yamaori et al., 2011c; Bansal et al., 2020; Nasrin et al., 2020; Nasrin et al., 2021). Results from this study now show that two major hepatic UGTs and three of the most highly expressed UGTs present in kidney are strongly inhibited by these cannabinoids, suggesting that deleterious drug-drug interactions may be more likely to occur in patients where reduced hepatic or kidney function and cannabis use are occuring simultaneously. In light of the rising acceptance of cannabis
use in the United States and internationally, further *in vivo* studies examining cannabinoid-drug interactions of both phase I and phase II are warranted.
Conflict of interests

None declared.

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Authorship contributions

Participated in research design: Nasrin, and Lazarus.

Conducted experiments: Nasrin, Watson, Fort and Bardhi

Contributed new reagents or analytic tools: NA

Performed data analysis: Nasrin, Watson, Chen, Lazarus.

Wrote or contributed to the writing of the manuscript: Nasrin, Watson, and Lazarus.
References

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Footnotes

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Figure Legends

Figure 1. Metabolic pathways and structures of major cannabinoids and their metabolites.

Figure 2. Chromatograms of probe metabolites in microsomes from UGT-overexpressing HEK293 cell lines. Probe substrates (at concentrations close to their known $K_m$; see Supplemental Table 1) were incubated in rUGTs for 60-120 min at 37°C, and individual corresponding metabolites were analyzed by UPLC-MS/MS as described in Supplemental Table 1 and in the Materials and Methods.

Figure 3. Screening of cannabinoid inhibition of major hepatic UGTs in microsomes from UGT-overexpressing HEK293 cell lines. Probe substrates were β-estradiol for UGT1A1, chenodeoxycholic acid for UGT1A3, trifluoperazine for UGT1A4, serotonin for UGT1A6, propofol for UGT1A9, codeine for UGT2B4, zidovudine for UGT2B7, nicotine for UGT2B10, oxazepam for UGT2B15 and dihydroexemestane for UGT2B17. Incubations were performed using 10 or 100 μM of cannabinoid, with probe substrate concentrations at or close to their known $K_m$ for their corresponding enzyme (see Supplemental Table 1). Shown are the mean inhibition of two individual experiments performed for each probe substrate. Data are expressed as a percentage.
of metabolite formation formed in assays with cannabinoid compared to assays without cannabinoid.

Figure 4. Inhibitory effects of CBD on the glucuronidation of UGT probe substrates in microsomes from UGT-overexpressing HEK293 cell lines (rUGT), HLM and HKM. Shown are representative plots comparing CBD concentration with the percent glucuronidation activity against probe substrates in rUGT microsomes, HLM and HKM. Incubations were performed for 60-120 min at 37°C using 80-90 µg of rUGT microsomes or 90-200 µg HLM or HKM with the following probe substrates: propofol, acetaminophen, and furosemide for UGT1A9; serotonin for UGT1A6; codeine for UGT2B4; and zidovudine (AZT) for UGT2B7 (see Supplemental Table 1 for concentrations). Individual metabolites were analyzed by UPLC-MS/MS as described in the Materials and Methods.

Supplemental Figure 1. Inhibitory effects of THC and CBN on the glucuronidation of UGT probe substrates in microsomes from UGT-overexpressing HEK293 cell lines (rUGT), HLM and HKM. Shown are representative plots comparing THC (panel A) and CBN (panel B) concentrations with the percent glucuronidation activity against probe substrates in rUGT microsomes, HLM and HKM. Incubations were performed for 60-120 min at 37°C using 80-90 µg of rUGT microsomes or 90-200 µg HLM or HKM with the following probe substrates: propofol, acetaminophen, and furosemide for
UGT1A9; serotonin for UGT1A6; codeine for UGT2B4; and zidovudine (AZT) for UGT2B7 (see Supplemental Table 1 for concentrations). Individual metabolites were analyzed by UPLC-MS/MS as described in the Materials and Methods.
Table 1. *IC*<sub>50</sub> values<sup>a</sup> (µM) of cannabinoids against major hepatic UGT enzymes in microsomes from recombinant UGT-overexpressing cells, HLM or HKM.

<table>
<thead>
<tr>
<th>Probe substrate</th>
<th>microsomes</th>
<th>THC</th>
<th></th>
<th>THC</th>
<th></th>
<th>CBD</th>
<th></th>
<th>CBN</th>
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<td></td>
<td></td>
<td><em>IC</em>&lt;sub&gt;50&lt;/sub&gt; (µM)</td>
<td><em>IC</em>&lt;sub&gt;50,u&lt;/sub&gt; (µM)</td>
<td><em>IC</em>&lt;sub&gt;50&lt;/sub&gt; (µM)</td>
<td><em>IC</em>&lt;sub&gt;50,u&lt;/sub&gt; (µM)</td>
<td><em>IC</em>&lt;sub&gt;50&lt;/sub&gt; (µM)</td>
<td><em>IC</em>&lt;sub&gt;50,u&lt;/sub&gt; (µM)</td>
<td><em>IC</em>&lt;sub&gt;50&lt;/sub&gt; (µM)</td>
<td><em>IC</em>&lt;sub&gt;50,u&lt;/sub&gt; (µM)</td>
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<td>Serotonin</td>
<td>rUGT1A6</td>
<td></td>
<td>10 ± 2.6</td>
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<tr>
<td></td>
<td>HKM</td>
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<td>17 ± 3.7</td>
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<tr>
<td></td>
<td>HLM</td>
<td></td>
<td>28 ± 6.5</td>
<td></td>
<td>1.4 ± 0.33</td>
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<tr>
<td>Codeine</td>
<td>rUGT2B4</td>
<td>11 ± 2.7</td>
<td>0.47 ± 0.11</td>
<td>5.8 ± 1.2</td>
<td>0.22 ± 0.045</td>
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<td></td>
<td>HKM</td>
<td>55 ± 5.2</td>
<td>2.9 ± 0.27</td>
<td>39 ± 5.9</td>
<td>2.5 ± 0.37</td>
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<td></td>
<td>HLM</td>
<td>13 ± 2.6</td>
<td>0.61 ± 0.13</td>
<td>8.0 ± 1.1</td>
<td>0.40 ± 0.058</td>
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<tr>
<td>Zidovudine (AZT)</td>
<td>rUGT2B7</td>
<td>33 ± 8.5</td>
<td>1.4 ± 0.36</td>
<td>21 ± 3.9</td>
<td>0.82 ± 0.15</td>
<td>49 ± 12</td>
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<td>4.2 ± 1.1</td>
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<td>HKM</td>
<td>51 ± 12</td>
<td>2.6 ± 0.65</td>
<td>35 ± 3.5</td>
<td>2.2 ± 0.22</td>
<td>57 ± 5.5</td>
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<td>6.9 ± 0.90</td>
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<td>HLM</td>
<td>59 ± 6.6</td>
<td>2.8 ± 0.32</td>
<td>30 ± 4.1</td>
<td>1.5 ± 0.21</td>
<td>59 ± 6.6</td>
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<td>5.5 ± 0.79</td>
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<td>Propofol</td>
<td>rUGT1A9</td>
<td>11 ± 3.0</td>
<td>0.45 ± 0.12</td>
<td>3.2 ± 0.52</td>
<td>0.12 ± 0.020</td>
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<td>0.51 ± 0.063</td>
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<td>19 ± 4.6</td>
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<td>2.9 ± 0.38</td>
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<td>Furosemide</td>
<td>rUGT1A9</td>
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<td>0.090 ± 0.025</td>
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<td>HKM</td>
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<td>15 ± 0.77</td>
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<td>1.9 ± 0.092</td>
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<td>HLM</td>
<td>32 ± 6.3</td>
<td>1.5 ± 0.30</td>
<td>29 ± 4.0</td>
<td>1.5 ± 0.20</td>
<td>34 ± 6.3</td>
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<td>3.1 ± 0.58</td>
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<td>Acetaminophen</td>
<td>rUGT1A9</td>
<td>12 ± 3.7</td>
<td>0.49 ± 0.15</td>
<td>1.9 ± 0.29</td>
<td>0.073 ± 0.011</td>
<td>6.9 ± 0.54</td>
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<td>0.59 ± 0.046</td>
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<td>HKM</td>
<td>15 ± 3.0</td>
<td>0.79 ± 0.16</td>
<td>3.8 ± 0.82</td>
<td>0.24 ± 0.05</td>
<td>21 ± 3.4</td>
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<td>29 ± 8.9</td>
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<td>30 ± 4.5</td>
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<sup>a</sup> *IC*<sub>50</sub> values are presented as mean ± SD of three independent experiments. <sup>b</sup> *IC*<sub>50,u</sub>, binding-corrected *IC*<sub>50</sub>. <sup>c</sup> NA, not analyzed.
Figure 2

- β-estradiol-3-glucuronide
  447 > 271
- acyl CDCA-24-glucuronide
  567.5 > 391.5
- trifluoperazine N-glucuronide
  584 > 408.2
- serotonin-glucuronide
  352 > 160.02
- propofol-O-glucuronide
  354 > 177.02
- codeine-6-glucuronide
  476.2 > 300.2
- AZT-5'-glucuronide
  442 > 125.05
- nicotine-N-glucuronide
  339.15 > 163.124
- S-oxazepam-glucuronide
  463.3 > 269.1
- exemestane-17-O-glucuronide
  475.23 > 281.19
Figure 4

- Propofol
- Acetaminophen
- Furosemide
- Serotonin
- Codeine
- Zidovudine (AZT)