Cannabinoid Metabolites as Inhibitors of Major Hepatic CYP450 Enzymes, with Implications for Cannabis-Drug Interactions

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ABSTRACT

The legalization of cannabis in many parts of the United States and other countries has led to a need for a more comprehensive understanding of cannabis constituents and their potential for drug-drug interactions. Although (−)-trans-Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN) are the most abundant cannabinoids present in cannabis, THC metabolites are found in plasma at higher concentrations and for a longer duration than that of the parent cannabinoids. To understand the potential for drug-drug interactions, the inhibition potential of major cannabinoids and their metabolites on major hepatic cytochrome P450 (P450) enzymes was examined. In vitro assays with P450-overexpressing cell microsomes demonstrated that the major THC metabolites 11-hydroxy-Δ9-tetrahydrocannabinol and 11-nor-9-carboxy-Δ9-THC-glucuronide competitively inhibited several major P450 enzymes, including CYP2B6, CYP2C9, and CYP2D6 (apparent K<sub>i</sub> values = 0.086 ± 0.066 μM and 0.90 ± 0.54 μM, 0.057 ± 0.044 μM and 2.1 ± 0.81 μM, 0.15 ± 0.067 μM and 2.3 ± 0.54 μM, respectively). 11-Nor-9-carboxy-Δ9-tetrahydrocannabinol exhibited no inhibitory activity against any CYP450 tested. THC competitively inhibited CYP1A2, CYP2B6, CYP2C9, and CYP2D6; CBD competitively inhibited CYP3A4, CYP2B6, CYP2C9, and CYP2D6, and CYP2E1; and CBN competitively inhibited CYP2B6, CYP2C9, and CYP2E1. THC and CBD showed mixed-type inhibition for CYP2C19 and CYP1A2, respectively. These data suggest that cannabinoids and major THC metabolites are able to inhibit the activities of multiple P450 enzymes, and basic static modeling of these data suggest the possibility of pharmacokinetic interactions between these cannabinoids and xenobiotics extensively metabolized by CYP2B6, CYP2C9, and CYP2D6.

SIGNIFICANCE STATEMENT

Major cannabinoids and their metabolites found in the plasma of cannabis users inhibit several P450 enzymes, including CYP2B6, CYP2C9, and CYP2D6. This study is the first to show the inhibition potential of the most abundant plasma cannabinoid metabolite, THC-COO-Gluc, and suggests that circulating metabolites of cannabinoids play an essential role in CYP450 enzyme inhibition as well as drug-drug interactions.

Introduction

Cannabis and cannabis-derived substances are the most widely consumed psychopharmaceuticals in the world (Atakan, 2012). The legal and ethical implications of their use continue to be the subject of intense global debate because of potential medical applications of cannabis-derived products and the progressive legaliziation of its use as a recreational drug. Modern clinical applications include treatment of multiple sclerosis, epilepsy, neuropathic pain, arthritis, nausea and vomiting due to chemotherapy, appetite stimulation in HIV/AIDS, depression, anxiety disorders, sleep disorders, psychosis, glaucoma, and Tourette syndrome among others (Solinimi et al., 2017). However, concurrent usage of cannabis products (both recreational and medicinal) with conventional medications, nonprescription remedies, and other psychoactive substances can often lead to substance-use disorders (National Institute on Drug Abuse report, July 2020), suggesting that more information is needed to fully understand the effects of cannabis as a psychopharmaceutical (Weinberger et al., 2016).

More than 500 phytochemicals have been detected in commercial strains of the Cannabis sativa plant, with (−)-trans-Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN) being the three main active constituents (Aizpurua-Oliazola et al., 2016). THC is the most well described psychoactive constituent and interacts with the cannabinoid receptor type 1 (CB1) receptor and the complex network of neurologic transmitters to induce psychopharmacological effects (Pertwee, 2008). Guidelines from the Food and Drug Administration (FDA) classify any cannabis-derived substance that contains more than 0.3% THC as a schedule I controlled substance, whereas products with less than 0.3% THC are classified as the agricultural product hemp. CBD does not bind to the CB1 receptor and does not produce the same
psychoactive responses. In fact, when CBD and THC are administered simultaneously, CBD appears to block some of the effects of THC by acting as an antagonist at the cannabinoid receptors (Pertwee, 2008). CBD administered without THC has been shown to reduce subjective anxiety in both animals and humans and is thought to produce a positive effect on conditions such as inflammation, diabetes, cancer, and neurodegenerative diseases (Maroon and Bost, 2018). CBN is only weakly psychoactive and appears to be formed primarily from the degradation of THC within the Cannabis plant itself (Russo and Marcu, 2017).

Upon consumption, the first step of metabolism of THC is hydroxylation mediated by CYP2C9, resulting in the active metabolite 11-hydroxy-Δ²-tetrahydrocannabinol (11-OH-THC; see Fig. 1). Although 11-OH-THC predominates as the first oxidation product, small amounts of 8β-hydroxy-THC (8β-OH-THC) and 8α-hydroxy-THC (8α-OH-THC) are also formed (Matsunaga et al., 1995). Much lower plasma 11-OH-THC concentrations (approximately 10% of THC concentrations) are found after cannabis smoking than after oral administration (Wall et al., 1983). Further hydroxylation by CYP2C9 leads to the inactive carboxylic acid derivative 11-nor-9-carboxy-Δ²-tetrahydrocannabinol (THC-COOH), which does not interact with the CB1 receptor. THC-COOH is a substrate for UDP-glucuronosyltransferase (UGT) enzymes that add a glucuronide moiety to form 11-nor-9-carboxy-Δ²-tetrahydrocannabinol-glucuronide (THC-COO-Gluc), the major end product of THC biotransformation in most species, including humans (Huestis, 2007). CBN is the nonenzymatic oxidation byproduct of THC and is most commonly found after prolonged storage, especially at higher temperatures.

CBD follows the same metabolic scheme as THC, with hydroxylation on the homologous 7 carbon via CYP2C19 and CYP3A4 to form 7-hydroxy-CBD (7-OH-CBD) and 7-carboxy-CBD (CBD-COOH), which is followed by glucuronidation to form 7-carboxy-CBD-glucuronide. However, 7-OH-CBD is only a minor biotransformation product 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).

Cannabinoids are highly lipophilic, eventually concentrating in adipose tissue, liver, lung, and spleen, with slow release back into the bloodstream. This leads to varying plasma concentrations of active and inactive metabolites that persist in the bloodstream for a much wider timeframe than that of THC. Plasma concentrations of THC and its active metabolite 11-OH-THC change rapidly over time and vary widely depending on the mode of consumption and expertise of the user (occasional vs. frequent user, depth of inhalation, puff duration, and breath-hold) (Sharma et al., 2012). In contrast, the inactive metabolites THC-COOH and THC-COO-Gluc are present at relatively high levels regardless of the mode of consumption. Inhaled and ingested THC produces the same approximate level of THC-COOH. Once reaching maximal levels at approximately 80 minutes and 2 hours postinhalation, the level of THC-COOH and its glucuronide THC-COO-Gluc remain very stable in the bloodstream over many days, whereas THC is rapidly metabolized and cleared from the circulation (Huestis, 2007; Schwwope et al., 2011; Sharma et al., 2012).

Although previous studies have shown that the cannabinoids THC, CBD, and CBN can inhibit several major hepatic P450s (Yamaori et al., 2010, 2011a,b,c; Jiang et al., 2013; Cox et al., 2019), no studies have focused on the inhibitory potential of the major abundant metabolites of THC. The present study is the first to perform a comprehensive analysis of the inhibition activities of major cannabinoids and THC metabolites against major human hepatic CYP450 enzymes.

**Material and Methods**

**Chemicals and Reagents.** The purchase of THC, 11-OH-THC, THC-COOH, THC-COO-Gluc, CBD, and CBN was approved by the Drug Enforcement Agency, and they were obtained from Cayman Chemicals (Ann Arbor, MI) or Sigma-Aldrich (St. Louis, MO). The internal standards (ISs) D₃-THC and D₃-THC-COO-Gluc were purchased from Sigma-Aldrich. Pooled human liver microsomes (HLMs; n = 50 subjects, mixed sex) were obtained from Sekisui Xentech, LLC (Lenexa, Kansas). NADPH-regenerating system (1.3 mM NADP, 3.3 mM glucose 6-phosphate, and 0.4 U/ml glucose 6-phosphate dehydrogenase) was obtained from Corning (Bedford, MA). CYP450 probe substrates (phenacetin,

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**Fig. 1.** Major metabolic pathways and metabolic structures of cannabinoids. (A) Metabolic pathways for THC and CBD. (B) Structure of cannabinoids and major THC metabolites.
In Vitro CYP450 Activity Assays and Metabolite Detection. Human embryonic kidney (HEK) 293 cells individually overexpressing V5-tagged P450 isoforms (1A2, 3A4, 2B6, 2C8, 2C9, 2C19, 2D6, or 2E1) were developed and described previously (Peterson et al., 2017b). Microsomal membrane fractions of P450-overexpressing cell lines were prepared by differential centrifugation as previously described, with protein concentrations estimated using the bicinchoninic acid assay as per the manufacturer’s recommendations (Dellinger et al., 2006; Peterson et al., 2017a,b). An initial screen of the inhibition potential of all reactions. Assays were preincubated for 5 minutes at 37°C, initiated by the addition of NADPH-regenerating system, and incubated for 5–30 minutes (Supplemental Table 1) at 37°C. Reactions were terminated by the addition of 30 μl of ice-cold acetonitrile/methanol (1:1). Samples were mixed on a vortex mixer and centrifuged at 17,000 × g for 15 minutes. The supernatant (~50 μl) was transferred to an ultra-high-performance liquid chromatograph (UPLC) sample vial, and the specific probe substrate 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 analysis (Supplemental Table 1). As a positive control for every inhibition experiment, 1 μM and 10 μM probe inhibitors (see Supplemental Table 1) were added instead of the cannabinoid compounds. As a negative control, only vehicle (3% methanol) was added (without inhibitor) to the reaction. Probe substrate without any inhibitor was used as a positive control for 100% activity. All analyses were performed in three independent experiments. Incubation conditions were optimized by incubations of the individual probe substrates with microsomes from overexpressing cell lines at 10, 20, 30, and 50 μg microsomal protein for different times (5, 15, 25, and 30 minutes). Remaining substrate and formed metabolite peaks for each reaction were analyzed, and conditions were chosen according to 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) all metabolites could be easily detected by LC-MS/MS methods. For UPLC-MS/MS, the mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (100% methanol). Samples (2–5 μl) were injected onto an Acquity UPLC column (BEH C18, 1.7 μM, 2.1 × 100 mm; Waters Corp). A 6-minute gradient elution was used as follows: 2 minutes at 95% A:5% B followed by a linear gradient for 2 minutes to 5% A:95% B, 1 minute at 5% A:95% B, and re-equilibration for 1 minute at 95% A:5% B. The flow rate was 0.4 ml/min, and the column temperature was 40°C.

Determination of IC50 and Ki Values. For those cannabinoids/THC metabolites that exhibited a >50% decrease in relative P450 activity for any given probe substrate at ≤10 μM, IC50 determinations were performed in both microsomes from HEK293 CYP450-overexpressing cell lines (described above) and in HLMs using multiple concentrations (ranging between 0.5 and 100 μM) of cannabinoid/metabolite in incubations as described above, with all determinations performed for three independent experiments with three replicates per experiment.

To identify possible metabolism-dependent inhibition of the potential cannabinoid/THC metabolite with a fold-IC50 shift of ≥1.5 was considered a time-dependent inhibitor as recommended by the FDA guidelines.

IC50 data were used as a guide to generate appropriate probe substrate and test inhibitor concentrations for the determination of the Ki values for each isoform. The P450 enzyme-specific probe substrate concentrations used were 1–25 μM phenacetin for CYP1A2, 12.5–100 μM bupropion for CYP2B6, 2.5–30 μM diclofenac for CYP2C9, 0.5–5 μM omeprazole for CYP2C19, 1–10 μM dextromethorphan for CYP2D6, 12.5–100 μM chloroxazone for CYP2E1, and 1–25 μM midazolam for CYP3A4.

Data were exported and analyzed using an Excel spreadsheet (Microsoft). The amount of metabolite formed at each concentration relative to the control (percent relative activity) of specific enzyme in the presence and absence of probe inhibitor or test compound was calculated as:

\[ \% \text{Relative activity} = \left( \frac{\text{Peak area with inhibitor}}{\text{Peak area without inhibitor}} \right) \times 100\% \]

Percent Inhibition = [(Peak area without inhibitor – peak area with inhibitor)/peak area without inhibitor] × 100

The IC50 values were calculated by plotting the percent inhibition of P450 enzyme activities versus the log concentration of the test inhibitors using GraphPad Prism 7.04 software (GraphPad Software Inc., San Diego, CA). To calculate Ki values, inhibition data were fit to different models of enzyme inhibition (competitive, uncompetitive, or noncompetitive) by nonlinear least-squares regression analysis using the GraphPad Prism 7.04 software. Ki values were calculated with the use of nonlinear regression according to the equations:

\[ v = \left( V_{\text{max}} \text{S}/(K_{\text{m}}(1 + IK_{\text{s}}) + S) \right) \text{ for competitive inhibition,} \]

\[ v = \left( V_{\text{max}} S/(K_{\text{m}} + S)(1 + IK_{\text{s}}) \right) \text{ for mixed inhibitionin} \]

where I is compound concentration, Ks is the inhibition constant, S is the substrate concentration, and Km is the substrate concentration at half of the Vmax of the reaction (Cornish-Bowden, 1974).

Bound versus Unbound Cannabinoid Determinations. Cannabinoid binding to proteins and tube walls in the incubation mixture (Ibound) was measured using the tube adsorption method as described previously (Isoherranen et al., 2004; Patilea-Viana et al., 2019). Briefly, in separate incubations, 50 μl of 30 μM THC, 11-OH-THC, THC-COO-Gluc, CBD, or CBN was added to each of two 0.6-mL low-binding centrifuge tubes containing buffer without protein, two 0.6-mL low-binding centrifuge tubes containing buffer with HLMs (25 μg), or two 0.6-mL low-binding centrifuge tubes containing buffer with HEK293 microsomal protein (30 μg). This was performed for each cannabinoid separately. Mixtures were incubated for 30 minutes at 37°C with occasional shaking. For one tube of the three incubation conditions (buffer alone, buffer + HLMs, and buffer + HEK293 microsomes), ice-cold acetonitrile (100 μl) containing D3-labeled cannabinoid IS was added (subgroup A). For the other tube of each condition, the entire 50-μl mixture was removed and added to a fresh tube that contained 100 μl ice-cold acetonitrile with D3-labeled cannabinoid IS (subgroup B). Samples were processed for UPLC-
MS/MS as described above for the in vitro assay experiments. All analyses were performed in three independent experiments with four replicates per experiment.

The separation and detection of individual cannabinoids was achieved using the same ACQUITY XEVO TQD (Waters Corporation, Milford, MA) chromatographic system described above using a Waters BEH C18 column 1.7 mm, 2.1 × 50 mm, at 40 °C. UPLC-MS/MS was performed with a gradient elution using mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile) at a flow rate of 0.4 ml/min under the following mass transitions: 5% B for 1.0 minute, increasing to 95% B from 1.0 to 4.0 minutes, 95% B held for 2 minutes, which was followed by a return to initial conditions for 1.5 minutes for a total time of 7.5 minutes. Detection of cannabinoids was performed in multiple reaction monitoring (MRM) mode using the following mass transitions: m/z 315.1 → 193.2 for THC, m/z 331.3 → 313.1 for 11-OH-THC, m/z 315.2 → 181.1 for CBD, m/z 311.2 → 223.0 for CBDA, and m/z 521.2 → 345.0 for THC-COO-Gluc. Cone voltages (V) of 40, 40, 50, and 30, respectively, and collision energies of 25, 18, 20, and 27, respectively, were used for THC, 11-OH-THC, CBD, CBDA, and THC-COO-Gluc. Because of the nonavailability of deuterated CBD or CBDA at the time of this study and because of the similar molecular weights and UPLC retention times between these cannabinoids, THC-D3 was used as the IS for the quantification of THC, CBDA, and 11-OH-THC-D3 was used to quantify 11-OH-THC and THC-COO-Gluc-D3 was used to quantify THC-COO-Gluc. The internal standards were detected using the calculated using the following equations:

\[ \text{Q}_{\text{hep}} = \frac{\text{C}_{\text{BDA}}}{\text{Q}_{\text{NO}}} \]

where \( \text{Q}_{\text{hep}} \) is the hepatic or systemic area under the curve (AUC) ratio.

The unbound fraction of cannabinoid in incubations with either HLMs or HEK293 microsomes was calculated as the total (adsorbed and unadsorbed) test compound in buffer only, \( \text{CPA} \), minus the test compound adsorbed to the tube wall, \( \text{CPB} \), divided by the total (adsorbed and unadsorbed) test compound in the incubation mixture, CPA. The unbound test compound relative to the test compound adsorbed to the wall, CPA, is the ratio of unbound test compound adsorbed to the tube wall and CPA, the test compound adsorbed to the tube wall, and CPA, the ratio of unbound test compound relative to the test compound adsorbed to the wall. CPA is the total (adsorbed and unadsorbed) test compound in the incubation mixture, and CPA is the unbound test compound in presence of protein source (incubation mixture). CPA, the test compound adsorbed to the tube wall when mixed with microsomes. Calculations of \( f_{u, \text{inc}} \) were performed in three independent experiments with four replicates per group. IC50,u and \( K_{u} \) values were calculated using the following equations:

\[ \text{IC}_{50,u} = f_{u, \text{inc}} \times \text{IC}_{50} \]

\[ \text{IC}_{50,u} = f_{u, \text{inc}} \times \text{IC}_{50} \]

Prediction of Potential In Vivo Drug-Drug Interactions by Static Modeling. To predict the risk of a clinical drug-drug interaction, basic static models of reversible inhibition were used following FDA recommended guidelines. The predicted hepatic and systemic area under the curve ratios (AUCRhep and AUCRsys, respectively) were calculated to determine the overall effect of cannabinoids and their metabolites on a given probe substrate. The basic static model is described by the following equations:

\[ \text{AUCR}_{\text{hep}} = 1 + \frac{\text{IC}_{50,u}}{\text{K}_{u}} \]

where IC50,u was calculated using eq. 1, in which IC50,u is the unbound maximum hepatic inhibition concentration, and the K,u is calculated for each inhibitor as described above.

The IC50,u is calculated using eq. 1, in which IC50,u is the unbound maximum hepatic inhibition concentration, and the K,u is calculated for each inhibitor as described above.

The unbound fraction of drug in plasma and was set at 0.03 based on the plasma protein binding of THC (Garrett and Hunt, 1974), which was suggested to be accurate for 11-OH-THC, CBD, and CBDA as well as THC-COO-Gluc in previous studies (Skopp et al., 2002). Cmax is the maximum concentration of total drug in plasma (parameters described below), \( F_a \) is the fraction of dose absorbed from the gut lumen [set to 1 as recommended by the FDA (Food and Drug Administration, 2020)], \( R_h \) is the rate of intestinal absorption time [set to 0.02; (Cox et al., 2019)], \( R_h \) is the ratio of drug concentration in blood (CBB) to drug concentration in plasma [set to 0.4 as recommended by Schwilke et al. (2009)], and Qhep is the hepatic blood flow = 1500 ml/min (Food and Drug Administration, 2020). For THC, the Cmax was set to 65 nM for a 40-μg oral dose of THC and 254 nM for a 54-μg oral dose of THC (Schwope et al., 2011; Cox et al., 2019). For 11-OH-THC, the Cmax was set to 69.7 nM for a 20-μg oral dose of THC and 50 nM for a 46-μg oral dose of THC (Ménétrey et al., 2005; Hunault et al., 2008). For THC-COO-Gluc, the Cmax was set to 405 nM for an oral dose of 50 mg of THC and to 38.5 nM for a 54-μg oral dose of THC (Spindle et al., 2008). For CBD, the Cmax was set to 703 nM for an oral dose of 800 mg of CBD and to 103 nM for a 20-μg oral dose of CBD (Manini et al., 2015; Cox et al., 2019).

The AUCRsys is calculated using eq. 3, in which I\( _{50,u} \) is equal to Cmax,u, which is the maximum concentration of total unbound drug in plasma (Cmax/I\( _{50,u} \)).

An AUCRsys ≥ 1.25 or an AUCRsys ≥ 1.02 indicates a strong presystemic hepatic or systemic drug interaction potential.

Results

Prescreening of THC, 11-OH-THC, THC-COOH, THC-COO-Gluc, CBD, and CBDA as potential CYP450 inhibitors showed that 10 μM THC decreased the relative activity of several P450 isoforms to approximately 50%, including CYP1A2 (35%), CYP2B6 (50%), CYP2C9 (48%), CYP2D6 (48%), and CYP2C19 (51%), as compared with reactions without added cannabinoid (Fig. 2A). Similar levels of inhibition were observed for 11-OH-THC against CYP2B6, CYP2C9, and CYP2D6, with 10 μM 11-OH-THC resulting in relative activity levels between 27% and 48% for the three enzymes (Fig. 2B). Although no significant inhibition was observed using up to 10 μM THC-COOH against any of the P450 enzymes tested (Fig. 2C), 10 μM THC-COO-Gluc resulted in 25%–49% relative activity for CYP2B6, CYP2C9, and CYP2D6 (Fig. 2D). In addition, decreases in the relative activity to less than 50% was observed for 10 μM CBD against several P450 enzymes (CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) and for 1 μM CBD against CYP1A2 (45%), CYP2B6 (50%), CYP2E1 (49%), and CYP3A4 (47%; Fig. 2E). Similar decreases in relative activity were observed for CBNA against CYP2B6, CYP2C9, and CYP2E1, with 75%, 55%, and 80% decreases in activity observed for 10 μM CBNA against all three enzymes and a 77% decrease observed for 1 μM CBNA against CYP2E1 (Fig. 2F). Initial screening results were validated in assays using commercially prepared pooled HLMs (unpublished data).

Using microsomes from both specific recombinant CYP450 (rCYP) overexpressing HEK-293 cell lines and HLMs, the inhibitory effects of THC, 11-OH-THC, THC-COOH, THC-COO-Gluc, CBD, and CBDA were extended to establish IC50 values for each cannabinoid against the P450 enzymes shown to be inhibited in the rCYP screening assays (described above). The unbound fraction (\( f_{u,\text{inc}} \)) of THC, 11-OH-THC, THC-COO-Gluc, CBD, and CBDA in incubation mixtures (\( f_{u,\text{inc}} \)) was calculated as 0.051 ± 0.033 and 0.043 ± 0.012, 0.094 ± 0.014 and 0.078 ± 0.042, 0.43 ± 0.071 and 0.45 ± 0.061, 0.498 ± 0.023 and 0.062 ± 0.011, and 0.052 ± 0.081 and 0.030 ± 0.052 in mixtures containing HLMs or microsomes from HEK293 cells, respectively. The range in \( f_{u,\text{inc}} \) was from 0.030 (CBN) to 0.45 (THC-COO-Gluc) for HEK293 microsomes and 0.051 (THC) to 0.43 (THC-COO-Gluc) for HLMs. The values for HLMs were similar to those obtained for HEK293 microsomes for all cannabinoids tested. Interestingly, THC-COO-Gluc exhibited a \( f_{u,\text{inc}} \) that was approximately 10-fold higher than the other cannabinoids, indicating less adsorption to the components of the incubation mixture.

CBD exhibited strong inhibition against every CYP450 tested, with a range in IC50,u values of 0.037–0.22 μM, with the strongest inhibition
exhibited slightly stronger inhibition against rCYP2D6 (IC50 values of 1.49 µM and 0.47 ± 0.077 µM, respectively). The THC metabolites 11-OH-THC and THC-COO-Gluc both exhibited strong inhibition against rCYP2C9 microsomes (IC50 values of 0.12 ± 0.030 µM, 0.15 ± 0.030 µM, and 0.47 ± 0.077 µM, respectively). The THC metabolites 11-OH-THC and THCCOO-gluc both exhibited slightly stronger inhibition against rCYP2D6 (IC50 values of 0.34 ± 0.12 µM and 3.5 ± 0.72 µM, respectively) and rCYP2B6 (IC50 values of 0.55 ± 0.26 µM and 2.5 ± 0.72 µM, respectively) than THC, while exhibiting similar or less effective inhibition against rCYP2C9 microsomes (IC50 values of 0.30 ± 0.086 µM and 6.8 ± 2.0 µM, respectively). CBN exhibited strong inhibition against rCYP2B6 (IC50 = 0.63 ± 0.26 µM), rCYP2C9 (IC50 = 0.42 ± 0.13 µM), and rCYP2E1 (IC50 = 0.28 ± 0.030 µM) microsomes. Similar IC50 values were obtained for HLMs using specific probe substrates for all CYP450s examined (Table 1).

To confirm the high level of inhibitory activity observed with cannabinoids against CYP2B6, CYP2C9, and CYP2D6, IC50 determinations were also performed for three additional probe substrates. Strong inhibition of activity against efavirenz, bufuralol, and S-warfarin in microsomes from rCYP2B6, rCYP2C6, and rCYP2C9 cell lines, respectively, was observed with THC, 11-OH-THC, THC-COO-gluc, CBD, and CBN (Supplemental Table 2). Consistent with that observed for CBN with dextromethorphan as a probe substrate for CYP2D6, no inhibition was observed with this cannabinoid for bufuralol. These data suggest that the inhibition observed with these cannabinoids against these three enzymes is seen across multiple substrates.

IC50 shift assays were performed for microsomes from each CYP450-overexpressing cell line with the goal of evaluating the time-dependent inhibition (TDI) potencies of cannabinoid metabolites (Table 2). Using a value of 1.5 as the cutoff, none of the cannabinoids tested demonstrated TDI, with the possible exception of CBD for CYP2D6 with an exhibited shift of 1.5.

Additional experiments to determine Ki values were performed to further understand the strength and type of inhibition interactions occurring between the cannabinoids and the P450 enzymes. As shown in Lineweaver-Burk plots, the CYP2B6-catalyzed 4-hydroxylation of bupropion in microsomes from the rCYP2B6-overexpressing cell line was shown to be inhibited in a competitive manner by the THC metabolites 11-OH-THC and THC-COO-Gluc (Fig. 3) and the cannabinoids THC, CBD, and CBN (Supplemental Fig. 2), with Ki values similar for all inhibitors ranging from 0.068 ± 0.015 µM for CBD to 0.90 ± 0.054 µM for THC-COO-Gluc (Table 3). Slightly higher Ki values were observed for inhibition in HLMs, ranging from 0.22 ± 0.052 µM for CBD to 1.1 ± 0.47 µM for THC-COO-Gluc (Table 3). Ki values for the inhibition of CYP2C9 were determined by monitoring the hydroxylation of the CYP2C9 probe substrate diclofenac. Lineweaver-Burk plots for the inhibition of rCYP2C9 (Fig. 3; Supplemental Fig. 2) indicated a competitive type of inhibition for all cannabinoids tested in rCYP2C9-overexpressing cell microsomes, with Ki values ranging from 0.057 ± 0.044 µM for 11-OH-THC to 2.1 ± 0.081 µM for THC-COO-Gluc (Table 3). The inhibition of diclofenac hydroxylation in HLMs was similar, ranging from 0.17 ± 0.046 µM for THC to 3.4 ± 0.28 µM for THC-COO-Gluc. Slightly higher Ki values were observed for inhibition in HLMs, with THC and THC-COO-Gluc exhibiting mixed-type inhibition, whereas 11-OH-THC, CBD, and CBN exhibited competitive inhibition (Table 3).

For CYP2D6, Ki values were determined by examining the O-demethylation of dextromethorphan to dextrorphan. Lineweaver-Burk plots for the inhibition of CYP2D6 (Fig. 3; Supplemental Fig. 2) suggested a competitive type of inhibition for all inhibitors for both rCYP2D6 microsomes and HLMs. The Ki values for THC, THC-OH,
<table>
<thead>
<tr>
<th>Cannabinoid Metabolites as CYP450 Inhibitors</th>
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**Table 1**

IC₅₀ values of cannabinoids and THC metabolites against major hepatic CYP450 enzymes in microsomes from recombinant CYP450-overexpressing cells or HLMs.

<table>
<thead>
<tr>
<th>Cannabinoid Metabolites</th>
<th>THCA-Gluc</th>
<th>THC-COOH</th>
<th>THC-COO-Gluc</th>
<th>CBD</th>
<th>CBN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>THC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenacetin</td>
<td>rCYP1A2</td>
<td>2.7 ± 2.1</td>
<td>0.12 ± 0.05</td>
<td>0.27 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>HLM</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Midazolam</td>
<td>rCYP3A4</td>
<td>9.5 ± 4.3</td>
<td>0.04 ± 0.08</td>
<td>0.05 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>HLM</td>
<td>2.6 ± 1.0</td>
<td>1.3 ± 0.6</td>
<td>1.3 ± 0.6</td>
<td>1.3 ± 0.6</td>
<td>1.3 ± 0.6</td>
</tr>
<tr>
<td>Bupropion</td>
<td>rCYP2B6</td>
<td>10 ± 6.4</td>
<td>0.43 ± 0.28</td>
<td>0.55 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>HLM</td>
<td>19 ± 2.3</td>
<td>0.97 ± 0.12</td>
<td>0.26 ± 0.10</td>
<td>0.26 ± 0.10</td>
<td>0.26 ± 0.10</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>rCYP2C9</td>
<td>4.5 ± 3.0</td>
<td>0.19 ± 0.13</td>
<td>0.19 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>HLM</td>
<td>7.2 ± 2.6</td>
<td>0.37 ± 0.13</td>
<td>0.37 ± 0.13</td>
<td>0.37 ± 0.13</td>
<td>0.37 ± 0.13</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>rCYP2C19</td>
<td>3.6 ± 0.7</td>
<td>0.15 ± 0.03</td>
<td>0.01 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>HLM</td>
<td>3.6 ± 0.7</td>
<td>0.15 ± 0.03</td>
<td>0.01 ± 0.00</td>
<td>0.01 ± 0.00</td>
<td>0.01 ± 0.00</td>
</tr>
<tr>
<td>Dextromethorphan</td>
<td>rCYP2D6</td>
<td>11 ± 1.8</td>
<td>0.47 ± 0.07</td>
<td>0.47 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>HLM</td>
<td>15 ± 3.3</td>
<td>0.47 ± 0.27</td>
<td>0.47 ± 0.27</td>
<td>0.47 ± 0.27</td>
<td>0.47 ± 0.27</td>
</tr>
</tbody>
</table>

**Discussion**

In the present study, the inhibitory effects of major cannabinoids and THC metabolites on major hepatic CYP450s were investigated. Consistent with previous studies (Jiang et al., 2011, 2013; Yamaori et al., 2011a,b,c; Cox et al., 2019), THC demonstrated reversible inhibition of CYP1A2, CYP2B6, CYP2C9, CYP2C19, and CYP2D6 activity; CBD demonstrated reversible inhibition of CYP1A2, CYP2B6, CYP2C9, CYP2C19, and CYP2D6; and CBN demonstrated reversible inhibition of CYP2B6 and CYP2C9. Although not examined in previous studies, CYP2E1 was also inhibited by the major cannabinoids, with both CBD and CBN demonstrating strong, competitive inhibition of this P450. In ranking the magnitude of inhibition observed in CYP450-overexpressing cell lines, inhibition by THC was shown to be highest for CYP2C19, followed by 2C9 > 1A2 > 2D6 > 2B6; inhibition by CBD was highest for CYP2E1, followed by 2C9 > 2B6 > 2D6 > 3A4 ~ 2C9 > 1A2; and inhibition by CBN was also highest for CYP2E1, followed by 2B6 > 2C9.

Prior to the present analysis, a comprehensive inhibition study of hepatic P450 enzymes by all major THC metabolites (11-OH-THC, THC-COOH, and THC-COO-Gluc) had not been previously...
TABLE 2
Effect of preincubation on inhibition of P450 activity by cannabinoids and THC metabolites in microsomes from CYP450-overexpressing cell lines

<table>
<thead>
<tr>
<th>THC</th>
<th>CYP1A2</th>
<th>CYP3A4</th>
<th>CYP2B6</th>
<th>CYP2C9</th>
<th>CYP2C19</th>
<th>CYP2D6</th>
<th>CYP2E1</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-OH-THC</td>
<td>1.2 ± 0.31</td>
<td>NA</td>
<td>3.1 ± 0.26</td>
<td>5.1 ± 0.55</td>
<td>1.2 ± 0.09</td>
<td>NA</td>
<td>1.4 ± 0.6</td>
</tr>
<tr>
<td>THC-COO-Gluc</td>
<td>1.1 ± 0.55</td>
<td>4.1 ± 0.07</td>
<td>11 ± 0.22</td>
<td>14 ± 0.59</td>
<td>14 ± 0.51</td>
<td>14 ± 0.49</td>
<td>6 ± 0.5</td>
</tr>
<tr>
<td>11-THC-OH</td>
<td>1.1 ± 0.36</td>
<td>4.1 ± 0.24</td>
<td>11 ± 0.18</td>
<td>14 ± 0.36</td>
<td>14 ± 0.51</td>
<td>14 ± 0.49</td>
<td>6 ± 0.5</td>
</tr>
<tr>
<td>THC-COOH</td>
<td>1.1 ± 0.04</td>
<td>1.0 ± 0.27</td>
<td>10 ± 0.51</td>
<td>22 ± 0.22</td>
<td>22 ± 0.22</td>
<td>22 ± 0.22</td>
<td>6 ± 0.5</td>
</tr>
<tr>
<td>CBN</td>
<td>1.1 ± 0.4</td>
<td>1.0 ± 0.35</td>
<td>10 ± 0.51</td>
<td>22 ± 0.22</td>
<td>22 ± 0.22</td>
<td>22 ± 0.22</td>
<td>6 ± 0.5</td>
</tr>
<tr>
<td>CBD</td>
<td>1.1 ± 0.4</td>
<td>1.0 ± 0.35</td>
<td>10 ± 0.51</td>
<td>22 ± 0.22</td>
<td>22 ± 0.22</td>
<td>22 ± 0.22</td>
<td>6 ± 0.5</td>
</tr>
</tbody>
</table>

*IC50 ± S.D. (mM) value of three experiments with 30-min preincubation of test cannabinoid with enzyme without NADPH.

**Shift** (IC50 - NADPH/IC50) ± S.D. of three experiments.

† NA, not analyzed.
cannabis interact, co-users tend to report that cannabis increases the ketamine high and can, in some instances, exacerbate effects like drowsiness and dizziness (Garcia-Romeu et al., 2016).

The CYP2C9 hydroxylation of diclofenac was also strongly inhibited by the cannabinoid metabolites 11-THC-OH and THC-COO-Gluc in a reversible manner, with a competitive type in the rCYP and mixed in HLMs. Previous studies have also found potent inhibition of CYP2C9 by THC, CBD, and 11-OH-THC (Bansal et al., 2020). CYP2C9 is one of the most abundant P450 enzymes in the liver (about 20% of the hepatic total P450 content) (Ghodke-Puranik and Lamba, 2017). It metabolizes several important drugs, including anticancer agents, nonsteroidal anti-inflammatory agents, selective cyclooxygenase-2 inhibitors, antibiotics, antiepileptics, antihypertensives, anticoagulants, and antihyperlipidemics (Ghodke-Puranik and Lamba, 2017). Drug-drug interactions have been controlled oral administration of 51 mg THC (Newmeyer et al., 2016). The results from the present study can potentially explain the in vivo mechanism for increased adverse effects or potency of CYP2D6-metabolized agents. For example, dextromethorphan is a widely used antitussive agent metabolized by CYP2D6 into an active metabolite, dextrophan. Using dextromethorphan together with cannabis has been shown to increase side effects, such as dizziness, drowsiness, confusion, and difficulty concentrating (Ziaee et al., 2005). In addition, the widely used anticancer drug and CYP2D6 substrate tamoxifen showed decreased tamoxifen metabolism in vivo, and the level of CYP2D6-mediated active metabolite endoxifen decreased in plasma in subjects also taking cannabinoids (Parih et al., 2020).

For CYP1A2 and CYP2C19, a mixed-type inhibition was observed for CBD and THC, respectively, in both rCYPs and HLMs, suggesting that, in contrast to the competitive type inhibition observed for most of the cannabinoid and THC metabolites analyzed in this study, THC and CBD are not interacting with CYP1A2 and CYP2C19 in a similar fashion. In addition, CYP2C9 and CYP2C19 showed different types of inhibition when analyzing their corresponding rCYP microsomes versus HLMs; with THC, THC-COO-Gluc, and CBD all exhibiting competitive inhibition in the rCYP and mixed inhibition in HLMs, possibly due to a confounding effect of the presence of multiple P450s within HLMs.

Although cannabis can be consumed through oral dosing, smoking cannabis is still the most common way of consumption. These differences become important when analyzing metabolite levels, as the major THC metabolites will differ in plasma concentration based on the route of administration. After smoking a cannabis cigarette containing 10 mg THC, the plasma AUCs of THC, 11-OH-THC, and THC-COO-Gluc are 110 ng·h/l (0.35 µM), 19 ng·h/l (0.06 µM), and 840 ng·h/l (1.6 µM), respectively. In contrast, after oral dosing of a consumable containing 10 mg of THC, the AUCs of THC and 11-OH-THC are even higher at 360 ng·h/l (1.14 µM) and 14,600 ng·h/l (28 µM), respectively (Nadulski et al., 2005). THC-COO-Gluc levels were 139 ug/l (0.27 µM) and 14,600 ug/l (28 µM) after a controlled oral administration of 51 mg THC (Newmeyer et al., 2016). The
### TABLE 3

Summary of $K_i$ values and inhibition type for major cannabinoids and THCB metabolites against hepatic CYP450 enzymes

<table>
<thead>
<tr>
<th>Microsomal Protein</th>
<th>THC</th>
<th>11-OH-THC</th>
<th>THC-COO-Glu</th>
<th>CBD</th>
<th>CBN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_{ia}$</td>
<td>$K_{ia}$</td>
<td>$T$</td>
<td>$K_{ia}$</td>
<td>$K_{ia}$</td>
</tr>
<tr>
<td>rCYP1A2$^a$</td>
<td>2.1 ± 0.62</td>
<td>0.090 ± 0.027</td>
<td>C</td>
<td>NA$^d$</td>
<td>1.9 ± 1.2</td>
</tr>
<tr>
<td>HLM</td>
<td>2.0 ± 1.1</td>
<td>0.10 ± 0.056</td>
<td>C</td>
<td>NA</td>
<td>2.1 ± 1.1</td>
</tr>
<tr>
<td>rCYP3A4$^c$</td>
<td>NA</td>
<td>NA</td>
<td>C</td>
<td>1.5 ± 0.59</td>
<td>0.093 ± 0.037</td>
</tr>
<tr>
<td>HLM</td>
<td>2.2 ± 0.45</td>
<td>0.22 ± 0.044</td>
<td>C</td>
<td>2.6 ± 1.1</td>
<td>1.1 ± 0.47</td>
</tr>
<tr>
<td>rCYP2B6</td>
<td>5.7 ± 1.0</td>
<td>0.25 ± 0.043</td>
<td>C</td>
<td>1.1 ± 0.85</td>
<td>0.086 ± 0.066</td>
</tr>
<tr>
<td>HLM</td>
<td>7.5 ± 0.56</td>
<td>0.38 ± 0.029</td>
<td>C</td>
<td>2.8 ± 0.44</td>
<td>0.26 ± 0.041</td>
</tr>
<tr>
<td>rCYP2C9</td>
<td>1.7 ± 0.53</td>
<td>0.073 ± 0.023</td>
<td>C</td>
<td>0.73 ± 0.56</td>
<td>0.057 ± 0.044</td>
</tr>
<tr>
<td>HLM</td>
<td>3.4 ± 0.91</td>
<td>0.17 ± 0.046</td>
<td>M</td>
<td>2.2 ± 0.34</td>
<td>0.21 ± 0.032</td>
</tr>
<tr>
<td>rCYP2C19</td>
<td>1.3 ± 0.42</td>
<td>0.056 ± 0.018</td>
<td>M</td>
<td>NA</td>
<td>0.81 ± 0.092</td>
</tr>
<tr>
<td>HLM</td>
<td>4.1 ± 1.6</td>
<td>0.21 ± 0.082</td>
<td>M</td>
<td>NA</td>
<td>0.94 ± 0.063</td>
</tr>
<tr>
<td>rCYP2D6</td>
<td>2.5 ± 0.35</td>
<td>0.11 ± 0.015</td>
<td>C</td>
<td>1.9 ± 0.86</td>
<td>0.15 ± 0.067</td>
</tr>
<tr>
<td>HLM</td>
<td>5.4 ± 0.58</td>
<td>0.28 ± 0.030</td>
<td>C</td>
<td>3.4 ± 2.5</td>
<td>0.32 ± 0.24</td>
</tr>
<tr>
<td>rCYP2E1$^c$</td>
<td>NA</td>
<td>NA</td>
<td>C</td>
<td>0.34 ± 0.098</td>
<td>0.021 ± 0.0061</td>
</tr>
<tr>
<td>HLM</td>
<td>0.59 ± 0.23</td>
<td>0.058 ± 0.023</td>
<td>C</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

$^a$Values are expressed as μM; shown are the mean ± S.D. for three independent experiments performed for the inhibition of each CYP450 enzyme by each cannabinoid.

$^b$T, type of inhibition. C, competitive inhibition; M, mixed inhibition.

$^c$The probe substrate used were phenacetin, midazolam, bupropion, diclofenac, omeprazole, dextromethorphan, and chlorzoxazone for CYP1A2, CYP3A4, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP2E1, respectively.

$^d$NA, not analyzed.

### TABLE 4

Prediction of P450-mediated clinical drug interaction potential after oral or inhaled dose of THC or CBD

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>THC</th>
<th>11-OH-THC</th>
<th>THC-COO-Glu</th>
<th>CBD</th>
<th>CBN</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>1.25</td>
<td>1.02</td>
<td>1.08</td>
<td>NA$^b$</td>
<td>NA</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>NA</td>
<td>NA</td>
<td>1.08</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>1.09</td>
<td>1.01</td>
<td>1.03</td>
<td>1.32</td>
<td>1.03</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>1.31</td>
<td>1.03</td>
<td>1.10</td>
<td>1.44</td>
<td>1.04</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>1.40</td>
<td>1.03</td>
<td>1.13</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>1.20</td>
<td>1.02</td>
<td>1.07</td>
<td>1.17</td>
<td>1.02</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>NA</td>
<td>NA</td>
<td>1.08</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

$^a$The AUCR$_{exp}$ and AUCR$_{sys}$ were calculated for THC, 11-OH-THC and THC-COO-Glu based on oral or inhaled dosing of THC; the AUCR$_{exp}$ and AUCR$_{sys}$ were calculated for CBD based on oral or inhaled dosing of CBD as described in Materials and Methods.

$^b$NA, not analyzed.
Cannabinoid Metabolites as CYP450 Inhibitors


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