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Cytochrome P450–catalyzed hydroxylation of Δ 8-tetrahydrocannabinol and implications for pharmacogenetics

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Abstract

The use of $\Delta 8$ -tetrahydrocannabinol ($\Delta 8$ -THC) has become increasingly prevalent in the United States but its metabolic pathways remain poorly characterized. This study aimed to identify the key enzymes responsible for the 11'-hydroxylation of $\Delta 8$ -THC, its primary metabolic pathway, and assess the effect of metabolizing enzyme genotype on this activity. The intrinsic clearance of recombinant (r) CYP2C9 was >6,200-fold higher than that observed for rCYP2C19, the only other tested CYP450 with detectable activity against $\Delta 8$ -THC. The $K_{m,u}$ of rCYP2C9 ($0.013 \pm 0.0032 \mu\text{M}$) was comparable to that observed in human intestinal microsomes ($0.0076 \pm 0.00067 \mu\text{M}$) but it was substantially lower than that observed for human liver microsomes ($0.73 \pm 0.065 \mu\text{M}$). Inhibition of 11-OH- $\Delta 8$ -THC formation in rCYP overexpressing microsomes was observed when using CYP probe inhibitors (sulfaphenazole for CYP2C9 and tranlycypromine for CYP2C19). The intrinsic clearance was markedly reduced in CYP2C9 variant microsomes, with 20- to 60-fold decreases observed for CYP2C9*2 ($59 \pm 7.9 \mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein), CYP2C9*3 ($34 \pm 3.6 \mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein), CYP2C9*8 ($96 \pm 13 \mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein), and CYP2C9*9 ($34 \pm 11 \mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein) as compared to wild-type CYP2C9*1 ($6,028 \pm 266 \mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein). Given that the expression of CYP2C9 is much higher than CYP2C19 in both human intestine and liver, these data suggest that CYP2C9 is the primary enzyme responsible for intestinal 11'-hydroxylation of $\Delta 8$ -THC and that other untested CYPs may be important in hepatic $\Delta 8$ -THC hydroxylation. In addition, the reduced 11-OH- $\Delta 8$ -THC formation activity observed

with CYP2C9 variants (*2, *3, *8, *9) suggests interindividual variability in Δ 8-THC disposition.

Key words: cannabinoid, THC, CYP450, CYP2C9, CYP2C19

Significance Statement

This study represents the first in vitro characterization of CYP2C9 as the primary enzyme mediating 11'-hydroxylation of Δ^8 -THC and that this activity is significantly modified affected by CYP2C9 genotype, suggesting potential interindividual variability in Δ^8 -THC disposition.

Background

Cannabis is one of the most widely used natural products worldwide and has been employed for centuries for both medicinal and recreational purposes.¹ Δ 8-Tetrahydrocannabinol (THC) is a minor naturally occurring cannabinoid whose use has increased substantially in recent years, particularly in regions where the more psychoactive Δ 9-THC remains legally restricted.^{2,3} A recent U.S. survey reported that approximately 11% of 12th-grade students used Δ 8-THC in the past year.⁴ Despite its increasing use, the safety, pharmacology, and pharmacokinetics of Δ 8-THC remain poorly understood.^{5,6} Structurally, Δ 8-THC is an isomer of the more psychoactive Δ 9-THC, differing only in the position of a double bond within the cyclohexene ring.⁷ Recent studies have shown that, similar to that observed for other cannabinoids including Δ 9-THC and cannabidiol (CBD), Δ 8-THC can modulate xenobiotic metabolism.⁸⁻¹⁰ In vitro studies showed that Δ 8-THC can reversibly inhibit the metabolism of warfarin and diclofenac mediated by cytochrome P450 (CYP450) 2C9 and midazolam by CYP3A4 with static modeling predicting a high potential for drug–drug interactions.¹¹ Furthermore, Δ 8-THC has been identified as an irreversible inhibitor of nicotine 5'-oxidation to cotinine, the primary pathway for nicotine clearance.¹² These findings suggest that Δ 8-THC may pose toxicological risks through inhibition of drug metabolizing enzymes, while also offering potential therapeutic effects via modulation of nicotine pharmacokinetics that could reduce smoking behavior. However, the hepatic metabolic fate of Δ 8-THC and its implications for hepatic retention, clearance, and systemic exposure remain unclear.

The metabolism and pharmacokinetics of other important cannabinoids including cannabidiol (CBD) and Δ 9-THC have been well characterized.^{13,14} CBD is primarily metabolized by CYP2C19 and CYP3A4 to form 7-hydroxy-CBD (7-OH-CBD), which is further oxidized to 7-carboxy-CBD which is subsequently glucuronidated.^{15,16} Δ 9-THC undergoes hydroxylation primarily by CYP2C9, with minor contributions from CYP2C19, to form the active metabolite 11-hydroxy- Δ 9-THC, which is further oxidized to the inactive metabolite 11-nor-9-carboxy- Δ 9-THC.¹⁷ The metabolism of Δ 8-THC has been less extensively investigated, with available evidence suggesting that, similar to that observed for Δ 9-THC, 11'-hydroxylation represents the primary metabolic pathway of Δ 8-THC (**Figure 1**).¹⁸ A clinical study reported that following a 20 mg oral dose, Δ 8-THC reaches a similar time to peak concentration (t_{max}) as Δ 9-THC, but with a higher maximum plasma concentration (C_{max}). Meanwhile, the formation of 11-hydroxy- Δ 8-THC (11-OH- Δ 8-THC) occurs later and at a lower C_{max} as compared with that of Δ 9-THC.¹⁹ These findings suggest that Δ 8-THC may be metabolized more slowly than Δ 9-THC. While CYPs 2C9 and 2C19 were implicated in 11-OH- Δ 8-THC formation,²⁰ direct kinetic data supporting this are lacking.

Hepatic metabolism is the primary determinant of drug clearance and oral bioavailability, with intestinal metabolism serving as an additional barrier to systemic exposure.^{21,22} CYP450 enzymes play a central role in Phase I metabolism in both liver and intestine,^{23,24} and major hepatic isoforms—including CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4—account for the metabolism of most clinically used drugs.²⁵ The present study aimed to identify the major cytochrome P450 enzymes responsible for the

11'-hydroxylation of Δ^8 -THC, to characterize the associated enzyme kinetics, and assess the effects of metabolizing enzyme genotypes on this activity.

Methods

Chemicals and reagents. $\Delta 8$ -THC, 11-OH- $\Delta 8$ -THC, and the deuterated internal standard 11-OH- $\Delta 8$ -THC- d_3 were obtained from Cayman Chemical (Ann Arbor, MI, USA). The NADPH-regenerating system was purchased from Corning (Bedford, MA, USA). LC-MS–grade solvents, microcentrifuge tubes, and bicinchoninic acid (BCA) assay kits were acquired from Fisher Scientific (Waltham, MA, USA). All other chemicals used were of analytical grade or higher. The anti-V5 tag monoclonal antibody conjugated to horseradish peroxidase (HRP) (Catalog No. R96125) and the SuperSignal™ Femto Maximum Sensitivity Substrate were purchased from Novex, Fisher Scientific (Waltham, MA, USA). The anti-calnexin polyclonal rabbit antibody (Catalog No. 2433) and the HRP-linked anti-rabbit IgG antibody (Catalog No. 7044S) were purchased from Cell Signaling Technology (Danvers, MA, USA).

Enzyme sources. Pooled human liver microsomes (HLM; mixed genders, n = 50 donors) and human intestinal microsomes (HIM; mixed genders, n = 50 donors) were obtained from BioiVT (Lenexa, KS, USA). Microsomes from HEK293 cells individually overexpressing recombinant CYP450s 1A2, 2B6, 2C8, 2C9, 2C19, 2E1, 2D6, 3A4, and CYP2C9 *2, *3, *8, and *9 (Arg144Cys, Ile359Leu, Arg150His, and His251Arg, respectively) were prepared as previously described with all enzymes containing an N-terminal V5 tag for relative quantification analysis.²⁶ Protein concentrations for all microsomal samples were determined using the bicinchoninic acid (BCA) assay. The relative expression of the recombinant CYP450 enzymes were assessed by Western blot analysis.¹⁰ Briefly, 20 μ g of microsomal protein was separated on 10% SDS–

polyacrylamide gels and transferred to polyvinylidene difluoride (PVDF) membranes using standard protocols. Membranes were blocked with 5% nonfat milk and incubated with an HRP-conjugated mouse monoclonal anti-V5 tag antibody (1:1000 dilution in 5% nonfat milk). To verify equal protein loading across lanes, membranes were also probed with an anti-calnexin polyclonal rabbit antibody (1:1000 dilution in 5% nonfat milk), followed by incubation with an HRP-linked anti-rabbit IgG secondary antibody for 1 hour at room temperature. Protein bands were visualized using the SuperSignal™ Femto Maximum Sensitivity Substrate, and densitometric analysis was performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).²⁶ Relative expression factors (REFs) were determined by normalizing the V5-HRP band intensities to calnexin, with the expression level of CYP2C19 set as the reference (1.0; see **Supplemental Figure 1**).

Enzyme activity assays. Due to its limited solubility in aqueous solutions, $\Delta 8$ -THC was dissolved in water containing 3% methanol and added into a 30- μ L reaction assay containing 3 mM MgCl_2 , 100 mM potassium phosphate buffer (pH 7.4), and either recombinant P450 microsomes (50 μ g), HIM (20 μ g) or HLM (20 μ g) in a total volume of 30 μ L. Reactions were pre-warmed at 37°C for 3 min and initiated by addition of an NADPH-regenerating system (1.3 mM NADP, 3.3 mM glucose 6-phosphate, and 0.4 U/mL glucose 6-phosphate dehydrogenase). Incubations were carried out at 37°C for 15 min with HLM and HIM, and for 30 or 120 min with recombinant P450 microsomes. Reactions were terminated by the addition of 30 μ L of ice-cold acetonitrile containing 0.2 μ g/mL 11-OH- $\Delta 8$ -THC- d_3 , followed by vortexing and centrifugation at $17,000 \times g$ for 15 min. Approximately 30 μ L of the supernatant was transferred to UPLC–MS/MS vials,

and 4 μ L was then injected into the ultra high performance liquid chromatography (UHPLC)–tandem mass spectrometry system for analysis. To reduce nonspecific binding of cannabinoids, all incubations were performed in low-binding 0.6-mL centrifuge tubes. All experiments were performed in triplicate.²⁷

Quantification of 11-OH- Δ 8-THC. To measure 11-OH- Δ 8-THC levels, reaction supernatants were injected into a Waters Acquity UHPLC system coupled to a tandem Xevo TQD (triple quadrupole) mass spectrometer (Waters, Milford, MA, USA). Chromatographic separation was achieved on an Acquity UPLC BEH C₁₈ column (2.1 $\times$ 100 mm, 1.7 μ m) maintained at 40 °C, with a mobile phase consisting of, **(A)** water containing 10 mM ammonium acetate, and **(B)** acetonitrile with 0.1% formic acid. A 10-minute gradient was applied as follows: 95% A for 2 min, a linear ramp to 95% B over 4 min, a 2-min hold at 95% B, and a 2 min re-equilibration at 95% A, at a flow rate of 0.4 mL/min. Detection was performed in positive electrospray ionization mode (ESI⁺) using multiple reaction monitoring (MRM) with the following mass-to-charge (m/z) transitions: Δ 8-THC, 315.1 $\rightarrow$ 135.1; 11-OH- Δ 8-THC, 331.2 $\rightarrow$ 193.1; and 11-OH- Δ 8-THC-d₃, 334.1 $\rightarrow$ 196.1. Metabolite formation was quantified with a standard curve generated from serial dilutions (0.019 – 5 μ g/mL) of 11-OH- Δ 8-THC reference standard, with 11-OH- Δ 8-THC-d₃ serving as the internal standard. The calibration curves exhibited strong linearity, with an r² value of 0.9993. Data were processed using TargetLynx software (Waters) and further analyzed in Microsoft Excel and GraphPad Prism version 10.0 (GraphPad Software, San Diego, CA, USA).

Kinetic analysis of 11-OH- Δ 8-THC formation in HLM, HIM, and recombinant CYP450 microsomes. The enzyme kinetic parameters (Δ 8-THC concentration at half-

maximal velocity [K_m] or [S_{50}] , and maximum 11-OH- Δ 8-THC formation rate [V_{max}]) for the 11'-hydroxylation of Δ 8-THC in HLM, HIM, and recombinant P450 microsomes were determined using at least seven Δ 8-THC concentrations ranging from 0.1 to 100 μ M.²⁸ Three different kinetic models were fitted to the in vitro data (11-OH- Δ 8-THC formation rates vs Δ 8-THC concentrations) by nonlinear regression analysis including Michaelis–Menten (*eq. 1*), allosteric sigmoidal (Hill) (*eq. 2*), and substrate inhibition (*eq. 3*) using GraphPad Prism version 10.0.^{26,29-31}

$$v = \frac{V_{max} \times S}{K_m + S} \quad eq. 1$$

$$v = \frac{V_{max} \times S^h}{S_{50}^h + S^h} \quad eq. 2$$

$$v = \frac{V_{max} \times S}{K_m + S \times (1 + \frac{S}{K_i})} \quad eq. 3$$

v indicates the metabolite (11-OH- Δ 8-THC) formation rate, V_{max} is the maximum rate of metabolite formation, S is the substrate concentration, K_m is the Michaelis-Menten constant, S_{50} is substrate concentration resulting in 50% of V_{max} (analogous to K_m in the Michaelis-Menten model), h is the Hill coefficient. When $h > 1.0$, the binding of the first ligand increases the affinity of the protein for subsequent ligands, creating a steep sigmoidal (S-shaped) binding curve. When $h = 1.0$, the binding of a ligand is independent. The probability of ligand binding does not change, regardless of how many other ligands are already bound. K_i is the inhibition constant, describing the equilibrium dissociation constant for the enzyme-inhibitor complex and can be calculated using *eq. 3*.³² K_m , S_{50} , and K_i values were corrected for abundant nonspecific

binding of $\Delta 8$ -THC to the labware and protein, multiplying by the unbound fraction (f_u ;) (eq. 4, eq. 5, and eq. 6). Given their highly similar chemical structures and properties, the f_u of $\Delta 9$ -THC was used (0.043 in recombinant P450 microsomes and 0.051 in HLM; 0.051 was used as a surrogate f_u for incubations with HIM) as reported previously.⁸

$$K_{m,u} = K_m \times f_u \quad \text{eq. 4}$$

$$S_{50,u} = S_{50} \times f_u \quad \text{eq. 5}$$

$$K_{i,u} = K_i \times f_u \quad \text{eq. 6}$$

Intrinsic clearance [CL_{int}] (for Michaelis Menten model) and maximal clearance [CL_{max}] (for Hill model) for 11'-hydroxylation of $\Delta 8$ -THC in HLM, HIM, and recombinant P450 microsomes were calculated as described in eq. 7 and eq. 8, respectively,³²

$$CL_{int} = \frac{V_{max}}{K_{m,u}} \quad \text{eq. 7}$$

$$CL_{max} = \frac{V_{max}}{S_{50,u}} \times \frac{h - 1}{h \times (h - 1)^{\frac{1}{h}}} \quad \text{eq. 8}$$

where h is the Hill coefficient which describes the extent of cooperativity of ligand binding to multi-subunit proteins or enzymes.

The levels of 11-OH- $\Delta 8$ -THC formation were corrected by the REF of individual CYP450s in either HLM or HIM as determined by Western blot analysis as described above (eq. 9 for different CYP450 enzymes, and eq. 10 for different CYP2C9 variants).

$$Formation_{11-OH-\Delta 8-THC} = Formation_{11-OH-\Delta 8-THC, \text{ detected}} \times REF \quad \text{eq. 9}$$

$$Formation_{11-OH-\Delta 8-THC} = \frac{Formation_{11-OH-\Delta 8-THC, \text{ detected}}}{REF} \quad \text{eq. 10}$$

CYP450 inhibition studies. To verify the involvement of major hepatic CYP enzymes in the 11'-hydroxylation of $\Delta 8$ -THC, chemical inhibition assays were performed using recombinant human P450 microsomes. Probe inhibitor (1 or 10 μ M; sulfaphenazole for CYP2C9 and tranlycypromine for CYP2C19) was added to 30- μ L reaction assays containing $\Delta 8$ -THC (10 μ M) or probe control substrates (10 μ M diclofenac for CYP2C9, or 1 μ M omeprazole for CYP2C19), 3 mM MgCl_2 , 100 mM potassium phosphate buffer (pH 7.4), and 50 μ g recombinant CYP2C9 or CYP2C19 microsomal protein.¹¹ Probe substrates were used at concentrations approximating their known K_m 's for their respective CYP enzyme to minimize off-target interactions.^{33,34} Reaction mixtures were pre-incubated at 37°C for 3 min before being initiated with the NADPH-regenerating system. Assays were incubated at 37 °C for 15 min, terminated by the addition of 30 μ L ice-cold acetonitrile containing the internal standard (11-OH- $\Delta 8$ -THC- d_3) and processed as described above. Detection of diclofenac and omeprazole metabolites and %*Activity* calculations were performed as described previously.³⁵

Statistical analysis. Individual comparisons were assessed using a two-sided Student's t-test, with all tests considered statistically significant at $P < 0.05$. Statistical analyses were performed for all comparisons with all experiments performed in triplicate. Statistical analyses were performed using GraphPad Prism 10.0 (GraphPad Software, San Diego, CA).

Results

As shown in a representative chromatogram of a reference sample mixture containing 0.1 $\mu\text{g/mL}$ of each compound, UHPLC-MS/MS peaks for $\Delta 8$ -THC (retention time = 5.55 min), 11-OH- $\Delta 8$ -THC (retention time = 4.77 min), and 11-OH- $\Delta 8$ -THC- d_3 (retention time = 4.77 min) were clearly identified (**Figure 2**). To determine the major hepatic cytochrome P450 enzymes responsible for the 11'-hydroxylation of $\Delta 8$ -THC, enzyme activity assays were conducted by incubating 50 μM or 100 μM $\Delta 8$ -THC with microsomes from recombinant CYP-overexpressing HEK293 cells (or parental untransfected HEK293 cells as a negative control) for 30 or 120 min, respectively. Recombinant CYP2C9 microsomes produced the highest levels of 11-OH- $\Delta 8$ -THC, while recombinant CYP2C19 microsomes yielded substantially lower amounts (**Figure 3A**). The formation of 11-OH- $\Delta 8$ -THC by CYP2C9 was approximately 10-fold higher than that observed for CYP2C19 under both incubation conditions. No detectable formation of 11-OH- $\Delta 8$ -THC was observed in microsomes from other recombinant CYP enzymes (i.e., CYPs 1A2, 2B6, 2C8, 2D6, 2E1, 3A4) or from parental HEK293 cells.

The roles of CYP2C9 and CYP2C19 were validated through chemical inhibition studies using probe inhibitors in incubations with HLM - sulfaphenazole for CYP2C9 and tranylcypromine for CYP2C19. 11-OH- $\Delta 8$ -THC formation was significantly reduced in incubations containing recombinant CYP2C9-overexpressing microsomes and either 10 μM (27% reduction, $p = 0.0031$) or 100 μM (85% reduction, $p < 0.0001$) sulfaphenazole. Sulfaphenazole also significantly inhibited recombinant CYP2C9 activity against its probe substrate, diclofenac, in a concentration-dependent manner (40% at 1 μM

sulfaphenazole, $p = 0.006$; 85% at 10 μM sulfaphenazole, $p = 0.0003$; and 99% at 100 μM sulfaphenazole, $p = 0.0013$; **Figure 3B**). A similar pattern was observed with tranylcypromine as the probe inhibitor for recombinant CYP2C19 microsomes, with a significant reduction in 11-OH- $\Delta 8$ -THC formation observed at 100 μM $\Delta 8$ -THC (99%, $p = 0.0072$; **Figure 3C**).

To confirm the contributions from CYP2C9 and CYP2C19 in $\Delta 8$ -THC 11'-hydroxylation, the kinetics of this pathway was further characterized using microsomes from HEK293 cells overexpressing recombinant CYP2C9 or CYP2C19, human liver microsomes (HLM), or human intestinal microsomes (HIM). CYP activity assay data were fitted to Michaelis–Menten and Hill models to estimate the V_{max} and $K_{m,u}$. Nonlinear regression of metabolite formation versus substrate concentration revealed similar kinetic behavior between HIM and recombinant CYP2C9 microsomes (**Figure 4**). In both systems, a best fit was achieved using the Michaelis–Menten model, with the reaction velocity increasing rapidly and then reaching a plateau at lower concentrations (below 1 μM). Different kinetic profiles were observed for HLM and recombinant CYP2C19 microsomes, with the Hill model providing a slightly better fit than the Michaelis-Menten model and both showing a more gradual increase in metabolite formation, approaching a plateau only at higher substrate concentrations ($\sim 100 \mu\text{M}$). The Hill coefficients were 2.0 ± 0.11 for recombinant CYP2C19 and 1.6 ± 0.20 for HLM, with values greater than 1 suggesting potential cooperative substrate binding or multiple active-site interactions.^{26,36} The Eadie-Hofstee plot for HLM exhibited two distinct slopes ($R_1^2 = 0.99$; $R_2^2 = 0.61$), suggesting the presence of two active-site interactions, which can be explained by the observation that more than one CYP enzyme is involved in

hepatic 11-OH- Δ 8-THC formation (**Supplemental Figure 3**). In contrast, single linear regression of the Eadie-Hofstee plots for HIM ($R^2 = 0.80$), recombinant CYP2C9 ($R^2 = 0.89$) and CYP2C19 microsomes ($R^2 = 0.69$) revealed a single slope suggesting a single active-site interaction.

Kinetic analysis of the 11'-hydroxylation of Δ 8-THC with recombinant CYP-overexpressing microsomes showed much smaller $K_{m,u}$ and higher V_{max} values for CYP2C9 as compared to CYP2C19, resulting in an intrinsic clearance for recombinant CYP2C9 ($1985 \pm 339 \mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein) that was >2,000-fold that observed for CYP2C19 ($0.97 \pm 0.093 \mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein; **Table 1**). In addition, the $K_{m,u}$ of CYP2C9 ($0.013 \pm 0.0032 \mu\text{M}$) was similar to that observed for HIM ($0.0076 \pm 0.00067 \mu\text{M}$), again consistent with the high relative levels of expression of CYP2C9 vs CYP2C19 in human intestine and the possibility that CYP2C9 is the major enzyme involved in the 11'-hydroxylation of Δ 8-THC in human intestine.³⁷ The intrinsic clearance for HIM ($1405 \pm 68 \mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein) was 6.2-fold higher than that observed for HLM ($225 \pm 23 \mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein), a pattern again consistent with the high relative expression of CYP2C9 vs CYP2C19 in human intestine.³⁸

While the $K_{m,u}$ for HLM ($0.73 \pm 0.065 \mu\text{M}$) was much higher than that observed for recombinant CYP2C9 microsomes, it matched well with that observed for recombinant CYP2C19 microsomes. However, this contrasts with the higher levels of expression of CYP2C9 vs CYP2C19 in liver, suggesting that other CYP enzymes in addition to CYP2C9 and perhaps CYP2C19 may also play an important role in the hepatic 11'-hydroxylation of Δ 8-THC. This is consistent with the fact that no inhibition of 11'-hydroxylation of Δ 8-THC was observed in HLM with sulfaphenazole or

tranylcypromine, probe inhibitors for CYP2C9 or CYP2C19, respectively (**Supplemental Figure 2**).

CYP2C9 is a polymorphic enzyme with several variants that exhibit a high minor allele frequency (MAF; > 0.02) in at least one major population group (European, African, East Asian, or South Asian; see **Supplemental Table 1**) and contain non-synonymous single nucleotide polymorphisms (the CYP2C9 *2, *3, *8, and *9 variants).³⁹⁻⁴² The kinetics of 11-OH- Δ 8-THC formation for CYP2C9 variants was assessed using recombinant microsomes from HEK293 cells overexpressing each CYP2C9 variant, with 11-OH- Δ 8-THC formation normalized based on their relative levels of expression in the different CYP2C9 variant-overexpressing cell lines with CYP2C9*1 as the reference (REF = 1.0; see **Figure 5A**). Activity assays were conducted using at least seven concentrations of Δ 8-THC (0.1 – 100 μ M) and the resulting data were fitted to multiple kinetic models (Michaelis-Menten, substrate inhibition, and Hill) to estimate the V_{max} and $K_{m,u}$ (or $S_{50,u}$). Unlike that observed for microsomes from wild-type CYP2C9*1-overexpressing cells, microsomes from both the CYP2C9 *2 and *8 variants exhibited substrate inhibition (**Figure 5B**), a pattern also observed in Eadie-Hofstee plots (**Supplemental Figure 3**), where the data points formed a semi-circular distribution. The estimated $K_{i,u}$ from the substrate inhibition model were 6.7 ± 3.2 μ M for recombinant CYP2C9*2 and 1.5 ± 0.68 μ M for recombinant CYP2C9*8. The metabolic kinetics of the CYP2C9 *3 and *9 variants were better described by Michaelis-Menten and Hill models, respectively (**Figure 5B**). The Hill coefficient was 1.3 ± 0.14 for recombinant CYP2C9*9, with a value greater than 1 suggesting potential cooperative substrate binding or multiple active-site

interactions.^{26,36} Single linear regression of Eadie-Hofstee plots (**Supplemental Figure 3**) showed a single slope for the recombinant CYP2C9 *3 ($R^2 = 0.78$) and *9 microsomes ($R^2 = 0.96$), suggesting Michaelis–Menten or Hill models with a single active-site interaction and potential cooperative substrate binding for CYP2C9*9.

Significant reductions in intrinsic clearance (CL_{int} or CL_{max}) were observed for all tested CYP2C9 variants (**Table 2**). Compared to microsomes from wild-type CYP2C9*1-overexpressing cells ($1985 \pm 339 \mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein), CYP2C9*2, CYP2C9*3, CYP2C9*8, and CYP2C9*9 microsomes exhibited 34- (59 $\pm$ 7.9 $\mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein; $p = 0.010$), 58- ($34 \pm 3.6 \mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein; $p = 0.0099$), 21- ($96 \pm 13 \mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein; $p = 0.011$), and 58- ($34 \pm 11 \mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$; $p = 0.0099$) fold significant decreases in intrinsic clearance, respectively. The significant decreases in catalytic efficiency of CYP2C9 variants were mainly caused by increases in $K_{m,u}$ (or $S_{50,u}$) (**Table 2**). Specifically, microsomes from the CYP2C9*2, CYP2C9*3, and CYP2C9*9 variants displayed $K_{m,u}$ values ($0.35 \pm 0.10 \mu\text{M}$, $p = 0.028$; $0.76 \pm 0.074 \mu\text{M}$, $p = 0.0032$; and $0.21 \pm 0.033 \mu\text{M}$, $p = 0.0091$; respectively) that were 13-45 fold significantly lower than that observed for CYP2C9*1 ($0.013 \pm 0.0032 \mu\text{M}$). CYP2C9*8 microsomes exhibited an even greater increase in $K_{m,u}$ ($3.2 \pm 1.3 \mu\text{M}$), which was 246-fold ($p = 0.051$) greater than that observed for CYP2C9*1 microsomes. Compared to CYP2C9*1 microsomes ($26 \pm 2.9 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein), no significant change in the V_{max} value of CYP2C9 *2 and *3 was observed. A significant 2-fold reduction and an 11-fold increase in V_{max} was observed for CYP2C9*9 ($12 \pm 1.3 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$, $p = 0.032$) and CYP2C9*8 microsomes ($292 \pm 85 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$, $p = 0.0059$), respectively, as compared to CYP2C9*1 microsomes. Together, these findings suggest that the

Arg144Cys, Ile359Leu, Arg150His, and His251Arg amino acid substitutions corresponding to the *2, *3, *8, and *9 variants, respectively, significantly impair the catalytic efficiency of CYP2C9 toward, mainly by altering the binding efficiency of Δ^8 -THC to CYP2C9.

Discussion

Previous studies had implicated CYPs 2C9 and 2C19 as the enzymes involved in $\Delta 8$ -THC metabolism.²⁰ Results from the present study suggests that CYP2C9 appears to be the major enzyme involved in the 11'-hydroxylation of $\Delta 8$ -THC in human intestine. While CYP2C19 also showed detectable levels of 11-OH- $\Delta 8$ -THC formation activity, the intrinsic clearance for CYP2C19 was over 2,000-fold lower than that observed for CYP2C9. In addition, the $K_{m,u}$ observed for CYP2C9 (0.013 μM) was similar to that observed for HIM (0.0076 μM), further implicating CYP2C9 as the main intestinal enzyme involved in 11-OH- $\Delta 8$ -THC formation. Given that CYP2C19 exhibits between 3.8- and 11-fold lower levels of expression as compared to CYP2C9 in various regions of the intestine,³⁷ this suggests that CYP2C19 plays a minor role at best in intestinal 11-OH- $\Delta 8$ -THC formation.

Interestingly, the $K_{m,u}$ observed for CYP2C9 was approximately 56-fold lower than that observed for HLM (0.73 μM). Since CYP2C19 microsomes exhibited a $K_{m,u}$ (0.83 μM) similar to that observed for HLM, this enzyme may be playing a role in the hepatic clearance of $\Delta 8$ -THC. However, the expression of CYP2C19 was shown to represent only 1% of total hepatic CYP protein and is 27-fold lower than that observed for CYP2C9,^{37,38} which exhibited a much higher intrinsic clearance for $\Delta 8$ -THC than CYP2C19. In addition, probe inhibitors of CYPs 2C9 and 2C19 exhibited little inhibition of HLM-mediated 11-OH- $\Delta 8$ -THC formation. Taken together, this implicates other CYP enzymes that were not screened in the present study in hepatic 11-OH- $\Delta 8$ -THC formation.

The metabolic efficiency of CYP2C9 is significantly reduced (by 21- to 58-fold) in recombinant microsomes expressing the CYP2C9 *2 (Arg144Cys), *3 (Ile359Leu), *8 (Arg150His), and *9 (His251Arg) variants. These findings underscore the critical role of these residues in the 11'-hydroxylation of Δ 8-THC and suggest that individuals with any combination of these reduced-function CYP2C9 alleles may require dosage adjustments to avoid adverse effects, especially for orally-consumed Δ 8-THC where first pass metabolism in the intestine is important.

The K_m of CYP2C9 for the 11'-hydroxylation of Δ 8-THC ($0.31 \pm 0.0074 \mu\text{M}$) observed in the present study is lower than that observed for Δ 9-THC ($2.13 \pm 0.41 \mu\text{M}$) in previous studies,⁴³. However, the V_{max} for CYP2C9 for 11-OH- Δ 8-THC formation ($26 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{nmol}^{-1} \text{ protein}$) observed in the present study was comparable to that observed in another study ($19.2 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{nmol}^{-1} \text{ protein}$).²⁰ While differences in kinetics could be due to the recombinant systems used in the different studies (i.e., overexpressed HEK293 cells vs baculosomes), the data suggest a higher catalytic affinity for CYP2C9 for Δ 8-THC vs. Δ 9-THC.

The Δ 8-THC K_m in HLM ($14 \pm 1.3 \mu\text{M}$) observed in the present study was substantially higher than that observed for Δ 9-THC ($K_m = 0.0014 \pm 0.2 \mu\text{M}$) in one study¹⁷ and marginally higher than that observed for Δ 9-THC ($K_m = 5.2 \pm 2.0 \mu\text{M}$) in another study.⁴³ Interestingly, the V_{max} for Δ 8-THC observed for HLM ($318 \pm 20 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \text{ protein}$) in the present study was comparable to that reported previously for Δ 9-THC ($521 \pm 53 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \text{ protein}$) in previous studies.^{17,20} This suggests that the liver may exhibit a lower catalytic affinity for Δ 8-THC than Δ 9-THC. Unfortunately, no previous studies have been performed examining Δ 9-THC in HIM.

The impact of CYP2C9 genetic variants on the metabolism of $\Delta 8$ -THC, $\Delta 9$ -THC and other drugs varies significantly and was more pronounced for $\Delta 8$ -THC in the present study as compared to that observed for $\Delta 9$ -THC in previous studies. For example, the CYP2C9*2 variant exhibited a 27-fold increase in K_m or S_{50} for the 11'-hydroxylation of $\Delta 8$ -THC as compared to wild-type CYP2C9*1 in the present study, which is markedly higher than the 5-fold increase observed for $\Delta 9$ -THC.⁴³ In terms of intrinsic clearance, a previous study reported that CYP2C9*2 exhibited a 2.5-fold reduction in $\Delta 9$ -THC 11'-hydroxylation as compared to wild-type CYP2C9*1,⁴⁴ whereas a 34-fold reduction was observed for $\Delta 8$ -THC in the present study. A similar pattern is observed for the CYP2C9*3 variant, with 58-fold increases in both the K_m and intrinsic clearance observed for 11-OH- $\Delta 8$ -THC formation as compared to the wild-type CYP2C9*1 in the present study vs 3-fold increases observed for $\Delta 9$ -THC in previous studies.^{43,44} Clinically, individuals with the CYP2C9 (*3/*3) genotype exhibited a 2-fold higher systemic exposure (e.g., C_{max}) for $\Delta 9$ -THC as compared to those with the wild-type genotype (CYP2C9 *1/*1).⁴⁵ Therefore, larger effects would be predicted for $\Delta 8$ -THC. These differences indicate that the functional effects of CYP2C9 genetic variation are highly substrate dependent and that clinical investigations are required to better examine the potential impact of CYP2C9 variants on $\Delta 8$ -THC metabolism and pharmacokinetics.

Some minor limitations should be noted. The aqueous solubility of $\Delta 8$ -THC is very limited,⁴⁶ with visible precipitation observed at concentrations as low as 1 mM, restricting our ability to evaluate metabolic kinetics at higher substrate concentrations (> 100 μ M), which may have contributed to uncertainty in the estimation of K_i values

derived from substrate inhibition models. Also, the fraction unbound (f_u) value for $\Delta 8$ -THC was not characterized in this or previous studies, with the (f_u) values observed for $\Delta 9$ -THC used as a surrogate. This concern is mitigated to a large extent by the similar chemical structures, inhibition profiles and kinetics of the two THC isomers.¹¹ Finally, as described above, other CYP enzymes not examined in the present study may be active against $\Delta 8$ -THC in the liver. A larger assessment of additional CYP enzymes will be necessary to further characterize the hepatic clearance of $\Delta 8$ -THC.

Conclusions

In summary, of the eight major CYP enzymes examined in this study, CYP2C9 appears to be the primary enzyme involved in the 11'-hydroxylation of $\Delta 8$ -THC in intestine. While CYPs 2C9 and 2C19 may be involved in hepatic 11-OH- $\Delta 8$ -THC formation, it appears that other enzymes may also be involved. In addition, genetic variations in CYP2C9 appear to exert a significant effect on the metabolism of $\Delta 8$ -THC, an effect that may be greater than that observed for $\Delta 9$ -THC. This is especially pertinent in the intestine and first pass metabolism for orally administered THC where CYP2C9 is playing a major role in 11-OH- $\Delta 8$ -THC formation. Future clinical studies will be needed to confirm the predicted impact of CYP2C9 genetic variants on $\Delta 8$ -THC metabolism.

Abbreviations

CBD, cannabidiol; $\Delta 9$ -THC, $\Delta 9$ -tetrahydrocannabinol; $\Delta 8$ -THC, $\Delta 8$ -tetrahydrocannabinol; 11-OH- $\Delta 8$ -tetrahydrocannabinol, 11-hydroxy- $\Delta 8$ -

tetrahydrocannabinol; Δ^8 -THC-COOH, 11-nor- Δ^8 -tetrahydrocannabinol-9-carboxylic acid; FDA, Food and Drug Administration; HLM, human liver microsomes; HIM, human intestinal microsomes; f_u , unbound fraction of cannabinoid in in vitro assays; HEK, human embryonic kidney; LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem MS; CYP450, cytochrome P450; UHPLC, ultra-high pressure liquid chromatography.

Ethics and Consent to Participate Declaration

Not applicable

Consent to Publish Declaration

Not applicable

Data availability

All data supporting these findings are contained within the paper.

Competing Interest Declaration

The authors declare that they have no competing interests.

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Author Contributions

Mengqi Zhao: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, review and editing

Philip Lazarus: Supervision, Project Administration, Funding acquisition, Resources, Formal analysis, Writing (review and editing), Validation

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Figure legends

Figure 1. Schematic illustration of the enzymatic 11'-hydroxylation of Δ 8-tetrahydrocannabinol (Δ 8-THC) catalyzed by CYP2C9 and CYP2C19, leading to the formation of 11-hydroxy- Δ 8-THC (11-OH- Δ 8-THC).

Figure 2. Representative UHPLC-MS/MS chromatograms of Δ 8-tetrahydrocannabinol (Δ 8-THC, black), 11-hydroxy- Δ 8-THC (11-OH- Δ 8-THC, red), and the internal standard 11-OH- Δ 8-THC- d_3 (green).

Figure 3. Screening of CYP enzymes responsible for the 11'-hydroxylation of Δ 8-THC. (A) Major hepatic cytochrome P450 enzymes were incubated with Δ 8-THC for 30 min (50 μ M, red) or 120 min (100 μ M, black); enzyme activities are indicated by levels of 11-OH- Δ 8-THC formation. (B) Chemical inhibition of recombinant CYP2C9 microsomes incubated with diclofenac (red) or Δ 8-THC (black) by 0, 1, 10 or 100 μ M sulfaphenazole. (C) Chemical inhibition of recombinant CYP2C19 microsomes incubated with omeprazole (orange) or Δ 8-THC (black) by 0, 1, 10 or 100 μ M tranylcypromine. Comparisons were assessed using a two-sided Student's t-test, with all tests considered statistically significant at $P < 0.05$ (significant values shown in bold). All experiments were performed in triplicate. Statistical analysis was conducted using GraphPad Prism 10.0 (GraphPad Software, San Diego, CA).

Figure 4. Representative CYP enzyme kinetic curves of 11'-hydroxylation of Δ 8-THC. (A) human liver microsomes (HLM) fitted with the Hill model, (B) human intestine microsomes (HIM) fitted with the Michaelis-Menten (MM) model, (C) recombinant CYP2C9 microsomes fitted with the MM model, and (D) recombinant CYP2C19 microsomes fitted with the Hill model. Each point represents the mean $\pm$ standard deviation of three individual experiments.

Figure 5. The impact of CYP2C9 variants on the 11'-hydroxylation of Δ 8-THC. (A) Western blot analysis showing the expression of V5-tagged recombinant CYP2C9 variants in overexpressing HEK293 microsomes. REF indicates their relative expression factors, with the wild-type CYP2C9*1 designated as 1.0. (B) Representative enzyme kinetic curves of the 11'-hydroxylation of Δ 8-THC for recombinant CYP2C9*2 fitted with the substrate inhibition (SI) model, recombinant CYP2C9*3 fitted with the Michaelis-Menten (MM) model, recombinant CYP2C9*8 fitted with the SI model, and recombinant CYP2C9*9 fitted with the Hill model.

Supplemental Figure 1. Expression of V5-tagged recombinant P450 enzymes in overexpressing HEK293 microsomes. Western blot analysis showing the expression CYP enzymes in microsomes from each of the CYP-overexpressing HEK293 cell lines, probed with anti-V5 and anti-calnexin antibodies. V5-HRP bands indicates the expression of each V5-tagged CYP enzyme, while calnexin bands serve as the loading

control for microsomal protein samples. No V5 tag was detected in microsomes from the parental HEK293 cells. REF denotes their relative expression factors, with CYP2C19 designated as 1.0.

Supplemental Figure 2. Chemical inhibition of recombinant CYP2C9 microsomes.

Recombinant CYP2C9 microsomes were incubated with 25 μ M Δ 8-THC using sulfaphenazole at 1 μ M (black) or 10 μ M (red). Recombinant CYP2C19 microsomes were incubated with 25 μ M Δ 8-THC using tranylcypromine at 1 μ M (black) or 10 μ M (red).

Supplemental Figure 3. Eadie-Hofstee plots for the 11'-hydroxylation of Δ 8-THC in human liver microsomes, human intestine microsomes, recombinant CYP2C9 microsomes, recombinant CYP2C19 microsomes, recombinant CYP2C9*2 microsomes, recombinant CYP2C9*3 microsomes, recombinant CYP2C9*8 microsomes, and recombinant CYP2C9*9 microsomes. Each point represents the mean $\pm$ standard deviation of three individual experiments.

Table 1. Enzyme kinetic parameters for $\Delta 8$ -THC hydroxylation by recombinant P450 microsomes, HLM and HIM.

Enzyme source	K_m or S_{50} (μM) ^b	$K_{m,u}$ or $S_{50,u}$ (μM) ^b	V_{max} ^c ($\text{pmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ total protein)	CL_{int} or CL_{max} ^d ($\mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ total protein)	Model
Rec CYP2C9 ^a	$0.31 \pm$ 0.0074	$0.013 \pm$ 0.0032	26 ± 2.9	1985 ± 339	MM
Rec CYP2C19	19 ± 2.4	0.83 ± 0.10	1.6 ± 0.23	0.97 ± 0.093	Hill
HLM	14 ± 1.3	0.73 ± 0.065	318 ± 20	225 ± 23	Hill
HIM	$0.15 \pm$ 0.013	$0.0076 \pm$ 0.00067	11 ± 0.91	1405 ± 68	MM

^a Rec, recombinant microsomes from recombinant P450 overexpressing-HEK293 cells; HLM, human liver microsomes; HIM, human intestinal microsomes; MM, Michaelis Menton model.

^b S_{50} is the substrate concentration resulting in 50% of V_{max} in the Hill model (analogous to K_m in the Michaelis-Menten model). $K_{m,u}$ or $S_{50,u}$ is the corrected K_m or S_{50} calculated by multiplying the unbound fraction (f_u) in the tube using f_u for $\Delta 9$ -THC (0.051 in HLM, and 0.043 in recombinant P450 microsomes) as a surrogate for $\Delta 8$ -THC.⁸

^c V_{max} values in recombinant P450 microsomes were normalized by relative expression levels as shown in Supplemental Figure 1 (recombinant CYP2C9 = 3.5, recombinant CYP2C19 = 1.0).

^d CL_{int} was calculated as $\frac{V_{max}}{K_{m,u}}$ in the MM model, CL_{max} was calculated as $\frac{V_{max}}{S_{50,u}} \times \frac{h-1}{h \times (h-1)^{\frac{1}{h}}}$ in the Hill model. The Hill coefficients were 2.0 ± 0.11 for recombinant CYP2C19, and 1.6 ± 0.20 for HLM.

Table 2. Enzyme kinetic parameters for $\Delta 8$ -THC hydroxylation by recombinant CYP2C9 variants microsomes.

Rec CYP2C9 a microsomes	K_m or S_{50} (μM) ^b	$K_{m,u}$ or $S_{50,u}$ (μM) ^b	V_{max} ^c ($\text{pmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein)	CL_{int} or CL_{max} ^d ($\mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein)	Model
*1	0.31 $\pm$ 0.0074	0.013 $\pm$ 0.0032	26 $\pm$ 2.9	1985 $\pm$ 339	MM ^e
*2	8.0 $\pm$ 2.3	0.35 $\pm$ 0.10	20 $\pm$ 2.6	59 $\pm$ 7.9	SI ^e
*3	18 $\pm$ 1.7	0.76 $\pm$ 0.074	25 $\pm$ 1.9	34 $\pm$ 3.6	MM
*8	73 $\pm$ 30	3.2 $\pm$ 1.3	292 $\pm$ 85	96 $\pm$ 13	SI ^e
*9	4.9 $\pm$ 0.78	0.21 $\pm$ 0.033	12 $\pm$ 1.3	34 $\pm$ 11 ^d	Hill

^a Rec, recombinant microsomes from recombinant P450 overexpressing-HEK293 cells; MM, Michaelis-Menten model; SI, substrate inhibition model.

^b S_{50} is the substrate concentration resulting in 50% of V_{max} in the Hill model (analogous to the K_m in the Michaelis-Menten model). $K_{m,u}$ or $S_{50,u}$ calculated by multiplying the unbound fraction (f_u) in the tube using f_u for $\Delta 9$ -THC (0.051 in HLM, and 0.043 in recombinant P450 microsomes) as a surrogate for $\Delta 8$ -THC.⁸

^c V_{max} values in recombinant P450 microsomes were normalized by relative expression levels as shown in Figure 5.

^d CL_{int} was calculated as $\frac{V_{max}}{K_{m,u}}$ in the MM and SI models; CL_{max} was calculated as

$\frac{V_{max}}{S_{50,u}} \times \frac{h-1}{h \times (h-1)^{\frac{1}{h}}}$ in the Hill model. The Hill coefficient was 1.3 ± 0.11 for recombinant CYP2C9*9.

^e For the substrate inhibition model, the estimated unbound K_i values ($K_{i,u}$), corrected using the f_u for $\Delta 9$ -THC as described above, were $6.7 \pm 3.2 \mu\text{M}$ for recombinant CYP2C9*2 and $1.5 \pm 0.68 \mu\text{M}$ for recombinant CYP2C9*8.

Supplemental Table 1. Minor allele frequencies of CYP2C9 *2, *3, *8, and *9 in different populations.

CYP2C9 Variant	Genetic Impact	Minor Allele Frequency (MAF)			
		European	African	Eastern Asian	South Asian
*2	Arg144Cys	0.13 ^a	0.02	< 0.01	0.05
*3	Ile359Leu	0.07	0.01	0.03	0.11
*8	Arg150His	< 0.01	0.06	< 0.01	< 0.01
*9	His251Arg	< 0.01	0.08	< 0.01	< 0.01

^a MAF > 0.02 are bolded.

Supplemental Table 2. Impact of CYP2C9 *2, *3, *8, and *9 variants on Δ 8-THC 11'-hydroxylation.

Rec CYP2C9 microsomes ^b	Amino acid change	Fold-difference as compared to CYP2C9*1 ^a		
		$K_{m,u}$ or $S_{50,u}$ (μ M) ^c	V_{max} (pmol·min ⁻¹ ·mg ⁻¹ protein)	CL_{int} or CL_{max} (μ L·min ⁻¹ ·mg ⁻¹ protein)
*2	Arg144Cys	27 (0.028)	1.3 (0.0527)	34 (0.010)
*3	Ile359Leu	58 (0.0032)	1.0 (0.78)	58 (0.0099)
*8	Arg150His	246 (0.051)	11 (0.032)	21 (0.011)
*9	His251Arg	16 (0.0091)	2.2 (0.0059)	58 (0.0099)

^a Comparisons were assessed using a two-sided Student's t-test, with all tests considered statistically significant at $P < 0.05$ (bolded). Values in parenthesis are the P-values vs CYP2C9*1.

^b Rec, microsomes from recombinant P450 overexpressing HEK293 cells.

^c S_{50} is the substrate concentration resulting in 50% of V_{max} (analogous to K_m in the Michaelis-Menten model). $K_{m,u}$ or $S_{50,u}$ is the corrected K_m or S_{50} by multiplying the unbound fraction (f_u) of Δ 9-THC in the tube (0.051 in HLM, and 0.043 in recombinant P450 microsomes).

Figure 1

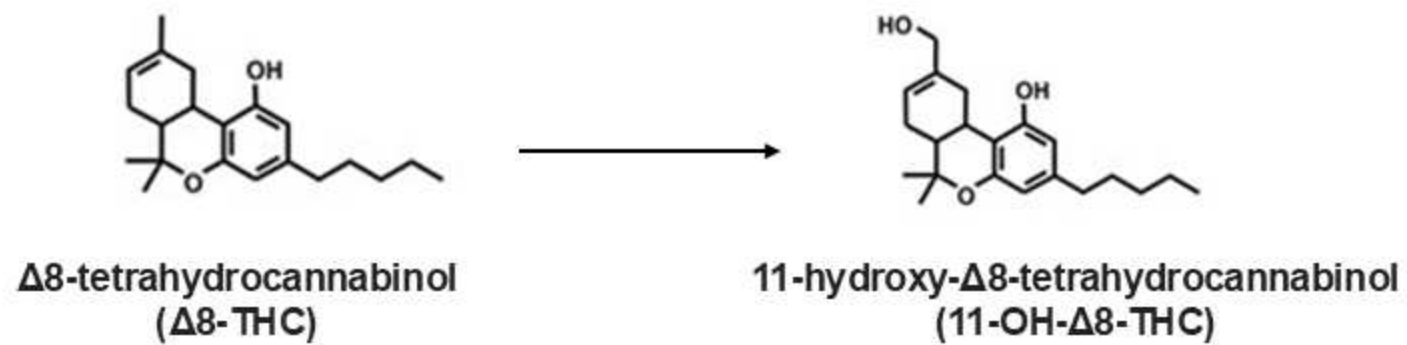


Figure 2

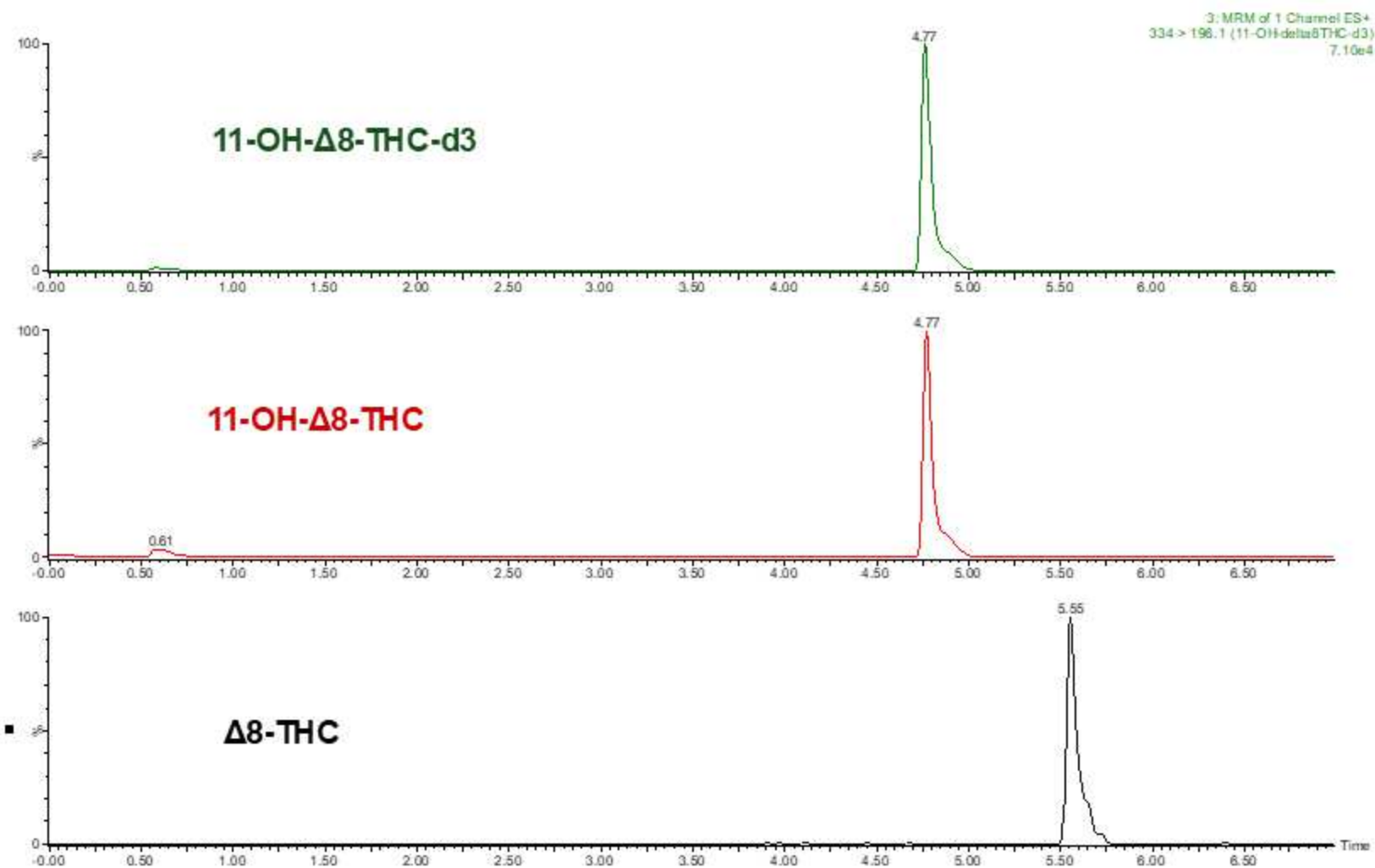


Figure 3

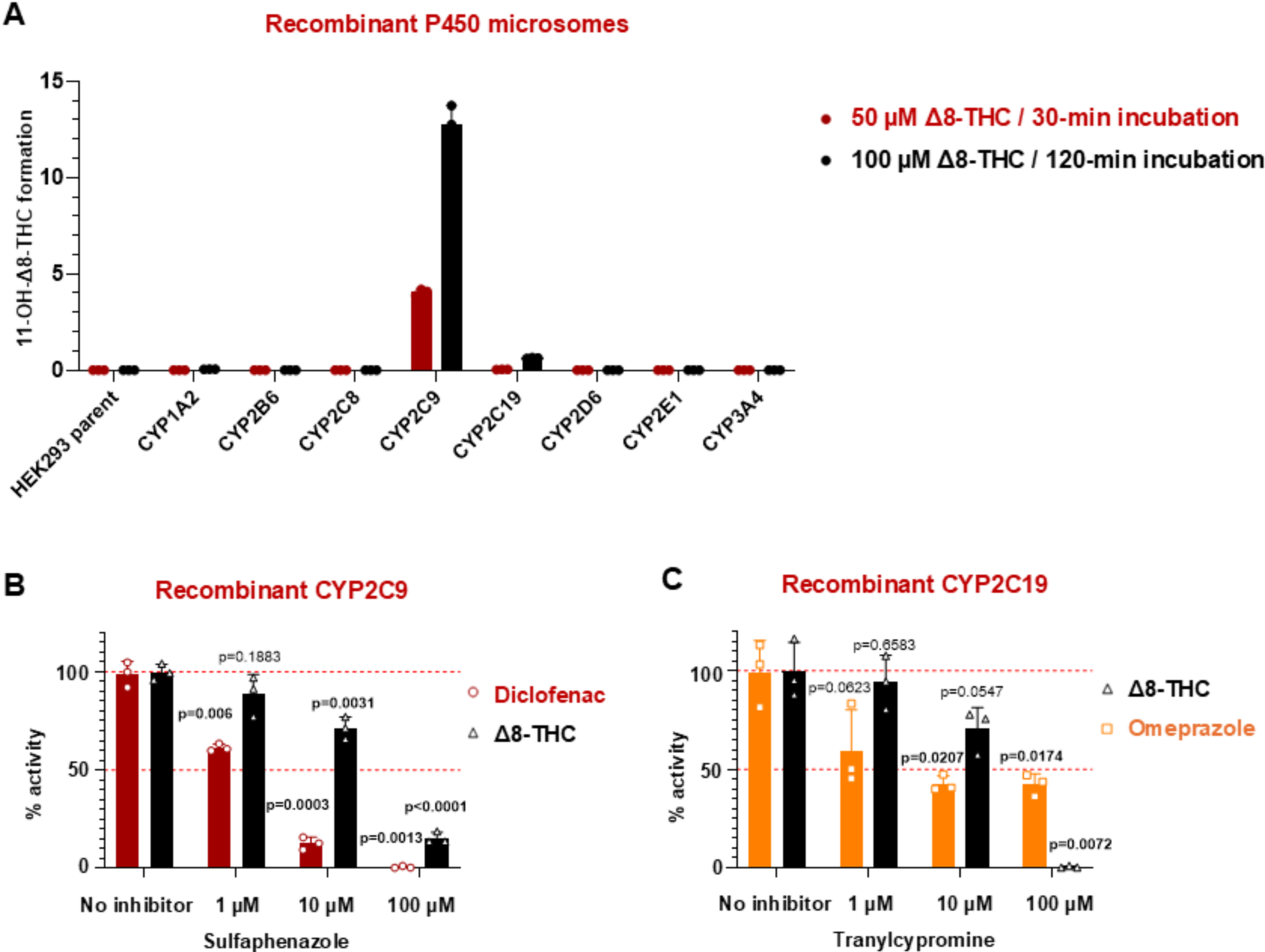


Figure 4

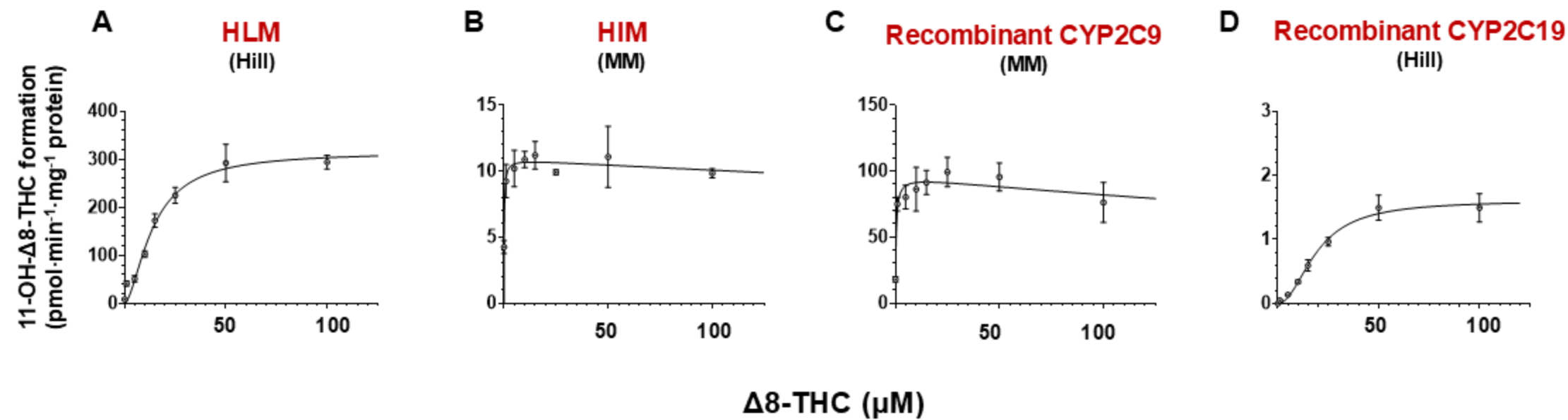
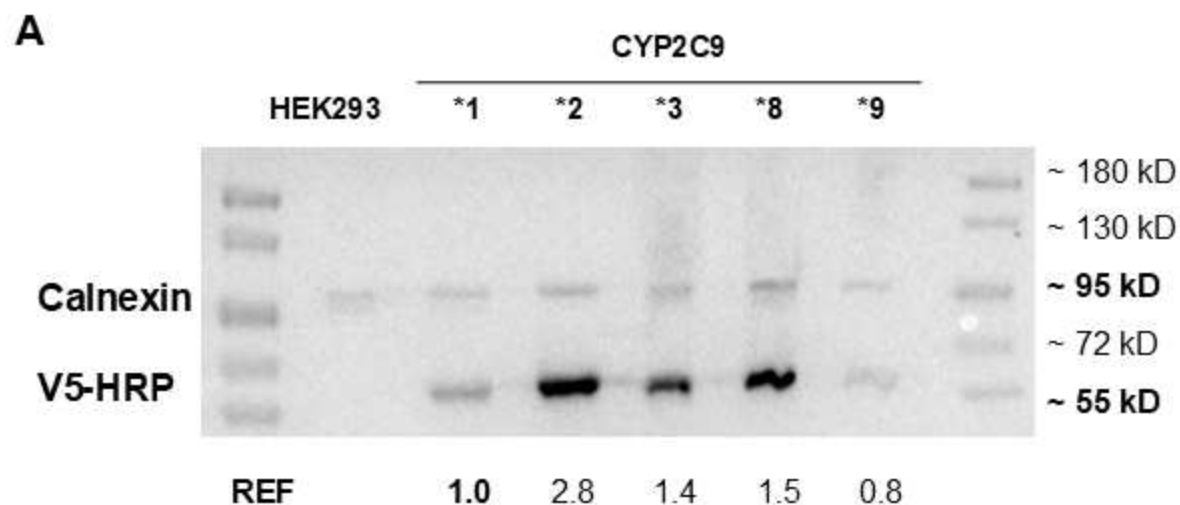
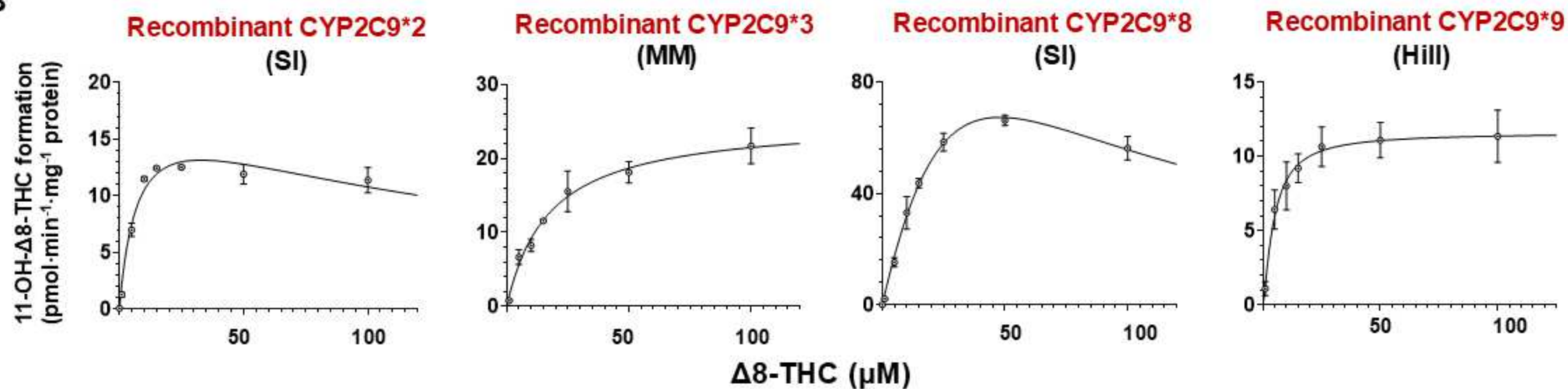


Figure 5



B



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- [ZhaoandLazarus8THCSupplFig1.tif](#)
- [ZhaoandLazarus8THCSupplFig2.tif](#)
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