Title Page

Comprehensive predictions of cytochrome P450 (CYP)-mediated *in vivo* cannabinoid-drug interactions based on reversible and time-dependent CYP inhibition in human liver microsomes

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Running Title Page

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d) Abbreviations: AUC, area under the plasma concentration vs. time curve; AUCR, ratio of AUC of object drug in the presence to absence of inhibitor; BSA, bovine serum albumin; CBD, cannabidiol; CBD-d3, deuterated CBD; $C_{\max}$, maximum systemic plasma concentration; CYP, cytochrome P450; $C_{\max, \text{hepatic inlet, u}}$, unbound maximum hepatic inlet concentration; $C_{\max, \text{sys, u}}$, unbound maximum systemic concentration; $C_{\text{ave, sys, u}}$, unbound average systemic concentration; $C_{\text{ave, portal, u}}$, unbound average portal vein concentration; DMSO, dimethyl sulfoxide; $f_{\text{u, inc}}$, fraction unbound in incubation containing HLM and BSA; $f_{\text{u, p}}$, fraction unbound in plasma; G6P, D-glucose 6- phosphate; FDA, US Food and Drug Administration; G6PDH, glucose-6-phosphate dehydrogenase; HLMs, human liver microsomes; IC_{50} , half-maximal inhibitory concentration; $IC_{50, \text{u}}$, unbound IC_{50} ; K_i , inhibitory constant for the enzyme-inhibitor complex; K_I , half-maximal inactivation concentration; $K_{I, \text{u}}$, unbound $K_{I, \text{u}}$; K_{inact} , maximum

inactivation rate constant; K_{obs} , first-order rate constant for inactivation, LC-MS/MS, liquid chromatography-tandem mass spectrometry; $MgCl_2$, magnesium chloride; MRM, multiple reaction monitoring; $NADP^+$, β -nicotinamide adenine dinucleotide phosphate; NDA, new drug application; OTC, over-the-counter; PBPK, physiologically-based pharmacokinetic; TDI, time-dependent inhibition; THC, *(-)-trans- Δ^9 -tetrahydrocannabinol*; THC-d3, deuterated THC; UPLC, ultra-high performance liquid chromatography; 7-OH CBD, 7-hydroxy CBD; 7-COOH CBD, 7-carboxy CBD; 11-OH THC, 11-hydroxy THC; 11-COOH THC, 11-nor-9-carboxy- Δ^9 -THC.

Abstract

We previously reported the unbound reversible ($IC_{50,u}$) and time-dependent ($K_{I,u}$) inhibition potencies of cannabidiol (CBD), delta-9-tetrahydrocannabinol (THC), and THC metabolites (11-OH THC, 11-COOH THC) against the major cytochrome P450 (CYP) enzymes (1A2, 2C9, 2C19, 2D6, 3A). Here, using human liver microsomes, we determined the CYP2A6, 2B6, and 2C8 $IC_{50,u}$ values of the aforementioned cannabinoids and the $IC_{50,u}$ and $K_{I,u}$ of the circulating CBD metabolites, 7-OH CBD and 7-COOH CBD, against all the CYPs listed above. The $IC_{50,u}$ of CBD, 7-OH CBD, THC, and 11-OH THC against CYP2B6 was 0.05, 0.34, 0.40, and 0.38 μ M, respectively and against CYP2C8 was 0.28, 1.02, 0.67, and 4.37 μ M, respectively. 7-COOH CBD, but not 11-COOH THC, was a weak inhibitor of CYP2B6 and 2C8. All tested cannabinoids except 11-COOH THC were weak inhibitors of CYP2A6. 7-OH CBD inhibited all CYPs examined ($IC_{50,u} < 2.5 \mu$ M) except CYP1A2 and inactivated CYP2C19 and CYP3A, with inactivation efficiencies ($k_{inact}/K_{I,u}$) of 0.10 and 0.14 $\text{min}^{-1} \mu\text{M}^{-1}$, respectively. Using several different static models, we predicted the following maximum pharmacokinetic interactions (affected CYP probe drug and AUC ratio) between oral CBD (700 mg) and drugs predominantly metabolized by CYP3A (midazolam, 14.8) > 2C9 (diclofenac, 9.6) > 2C19 (omeprazole, 7.3) > 1A2 (theophylline, 4.0) > 2B6 (ticlopidine, 2.2) > 2D6 (dextromethorphan, 2.1) > 2C8 (repaglinide, 1.6). Oral (130 mg) or inhaled (75 mg) THC was predicted to precipitate interactions with drugs predominately metabolized by CYP2C9 (diclofenac, 6.6 or 2.3, respectively) > 3A (midazolam, 1.8) > 1A2 (theophylline, 1.4). *In vivo* drug interaction studies are warranted to verify these predictions.

Significance statement

This study, combined with our previous findings, provides for the first time a comprehensive analysis of the potential for cannabidiol, delta-9-tetrahydrocannabinol, and their metabolites to inhibit cytochrome P450 enzymes in a reversible or time-dependent manner. These analyses enabled us to predict the potential of these cannabinoids to produce drug interactions *in vivo* at clinical or recreational doses.

Introduction

Cannabis (*Cannabis sativa*) is used worldwide for both recreational and medicinal purposes. The major phytocannabinoids in cannabis are the non-psychoactive cannabidiol (CBD) and the psychoactive delta-9-tetrahydrocannabinol (THC) (Fig. 1). CBD is approved to treat childhood epilepsy, namely the Lennox-Gastaut and the Dravet syndrome. THC is used as an antiemetic, analgesic, antispasmodic, and appetite stimulant (Amaral Silva *et al.*, 2020). To date, cannabis has been legalized in 36 states for medicinal use and in 18 states for recreational use (State Medical Marijuana Laws, 2021). By 2025, the estimated number of cannabis users in the United States is expected to reach 46.6 million (Conway, 2019). Therefore, it is crucial to evaluate the risk of potential pharmacokinetic interactions between cannabinoids and pharmaceutical drugs. Indeed, two case reports have reported cannabinoid-drug interaction related adverse effects. In the first, a cannabis-warfarin (a CYP2C9 substrate) interaction was observed where the anticoagulant effect of warfarin was considerably increased (INR of 12) to outside the therapeutic range (INR 2-3) in a patient who smoked cannabis frequently (Grayson *et al.*, 2018). In the second, a CBD-tacrolimus (a CYP3A4 substrate) interaction resulted in increased serum creatinine concentration (a marker of tacrolimus renal toxicity) in a patient who consumed a high dose of CBD (1000 mg twice daily, po) with tacrolimus (Leino *et al.*, 2019).

CBD is metabolized to the pharmacologically active circulating metabolite, 7-OH CBD, which is further metabolized to the inactive circulating metabolite, 7-COOH CBD (Fig 1.) (Epidiolex® Package Insert). THC is metabolized to the psychoactive circulating metabolite, 11-OH THC, which is further metabolized to an inactive circulating metabolite, 11-COOH THC (Fig 1). The maximum systemic plasma concentration (C_{max}) of these metabolites is reported to exceed that of CBD or THC except 7-OH CBD, for which the C_{max} is approximately 50% of that of CBD (Frytak *et al.*, 1984; Nadulski *et al.*, 2005; Taylor *et al.*, 2018). Therefore, as recommended by the United States Food and Drug Administration (FDA), the effect of cytochrome P450 (CYP) inhibition by these metabolites on the predicted magnitude of pharmacokinetic CBD- or THC-drug interactions should be evaluated (FDA Drug Interactions Guidance, 2020).

CBD or THC is known to reversibly inhibit cytochrome P450 (CYP) 1A, 2A6, 1B1, 2B6, 2C8, 2C9, 2C19, 2D6, 2J2, and 3A (Yamaori *et al.*, 2010; Yamaori, Ebisawa, *et al.*, 2011; Arnold *et al.*, 2018; Cox *et al.*, 2019; Epidiolex® Package Insert). In addition, THC has been shown to be a time-dependent inhibitor (TDI) of CYP2A6 (Yamaori, Maeda, *et al.*, 2011). However, these studies did not consider the binding of CBD or THC to microsomal proteins or labware. We recently reported the unbound inhibition potency ($IC_{50,u}$) of CBD, THC, 11-OH-THC, and 11-COOH THC against CYP1A2, 2C9, 2C19, 2D6, and 3A (Bansal *et al.*, 2020). In addition, we showed that CBD was a time-dependent inhibitor of CYP1A2, 2C19, and 3A. However, none of these studies determined the $IC_{50,u}$ of CBD, THC, 11-OH THC, or 11-COOH THC against CYP2A6, 2B6, and 2C8. Moreover, whether 7-OH CBD and 7-COOH CBD inhibits any of these CYPs is not known.

The overall goal of the current study was to predict the magnitude of pharmacokinetic interactions between CBD or THC and drugs predominantly metabolized by CYP2A6, 2B6, or 2C8 and to determine the ability of the circulating CBD metabolites to inhibit the above mentioned CYPs. Specifically, our aims were to (1) determine the fraction unbound in the plasma ($f_{u,p}$) and in the incubation mixture ($f_{u,inc}$) of CBD, THC, and their metabolites; (2) determine the unbound inhibition or inactivation potency of CBD, THC, and their metabolites against CYP2A6, 2B6, or 2C8; (3) determine the potential of circulating CBD metabolites to inhibit or inactivate all the CYPs listed above; and (4) predict, using a mechanistic static model, the magnitude of CYP-mediated interactions between oral CBD or inhaled and oral THC and CYP probe drugs.

Materials and Methods

Biological Materials, Chemicals, and Reagents

Pooled human liver microsomes (HLMs) (mixed gender; pool of 200 donors) and human plasma from a single healthy male donor were purchased from SEKISUI Xenotech, LLC (Kansas City, KS) and Bloodworks (Seattle, WA), respectively. THC, CBD, omeprazole, 5-hydroxy omeprazole, testosterone, 6 β -hydroxy testosterone, dextromethorphan, dextrorphan, phenacetin, acetaminophen, diclofenac, and 4'-hydroxy diclofenac were purchased from Cayman Chemical (Ann Arbor, MI). 7-COOH CBD and 7-OH CBD were procured from BDG Synthesis (Lower Hutt, New Zealand). Methanolic solutions of 11-COOH THC, 11-OH THC, THC-d₃, and CBD-d₃ were purchased from Cerilliant (Round Rock, TX). Acetonitrile, Bovine serum albumin (BSA), and formic acid were bought from Fisher Scientific (Hampton, NH). Coumarin, 7-hydroxycoumarin, bupropion, 4-hydroxy bupropion, amodiaquine, *N*-desethylamodiaquine, tolbutamide, D-glucose 6-phosphate (G6P), β -nicotinamide adenine dinucleotide phosphate (NADP⁺), and glucose-6-phosphate dehydrogenase (G6PDH) were purchased from Sigma-Aldrich (St. Louis, MO). Dulbecco's phosphate-buffered saline was procured from Thermo Scientific (Asheville, NC). Micro ultracentrifuge polycarbonate tubes were purchased from Beckman Coulter (Brea, CA) and ultralow-binding microcentrifuge tubes were purchased from Genesee Scientific (EI Cajon, CA). All other chemicals and experimental reagents were obtained from reputable commercial sources.

Reversible Inhibition of CYPs by Cannabinoids

As per our previous study (Bansal *et al.*, 2020), low-binding microcentrifuge tubes were used to reduce the non-specific binding and adsorption of cannabinoids to labware and we included BSA (0.2%) in all incubations to increase cannabinoid solubility and reduce non-specific binding.

The inhibition of CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A by each cannabinoid was simultaneously evaluated using a previously validated CYP cocktail assay (Dinger *et al.*, 2014). A metabolite of each probe substrate selectively formed by a single CYP was quantified using LC-MS/MS (described below). The probe substrate/metabolite pair and probe substrate concentration were as follows:

phenacetin ($K_m = 1.7\text{-}152\ \mu\text{M}$) /acetaminophen and $50\ \mu\text{M}$ (CYP1A2); coumarin ($K_m = 0.3\text{-}2.3\ \mu\text{M}$) /7-hydroxycoumarin and $2\ \mu\text{M}$ (CYP2A6); bupropion ($K_m = 67\text{-}168\ \mu\text{M}$) /4-hydroxy-bupropion and $5\ \mu\text{M}$ (CYP2B6); amodiaquine ($K_m = 1.9\text{-}2.4\ \mu\text{M}$) /N-desethylamodiaquine and $2\ \mu\text{M}$ (CYP2C8); diclofenac ($K_m = 3.4\text{-}52\ \mu\text{M}$) /4'-hydroxydiclofenac and $5\ \mu\text{M}$ (CYP2C9), omeprazole ($K_m = 17\text{-}26\ \mu\text{M}$) /5-hydroxyomeprazole and $10\ \mu\text{M}$ (CYP2C19), dextromethorphan ($K_m = 0.44\text{-}8.5\ \mu\text{M}$) /dextrorphan and $5\ \mu\text{M}$ (CYP2D6); and testosterone ($K_m = 52\text{-}94\ \mu\text{M}$) /6 β -hydroxytestosterone and $10\ \mu\text{M}$ (CYP3A) (Dinger *et al.*, 2014; Spaggiari *et al.*, 2014; Dahlinger *et al.*, 2016). The probe substrates showed minimal interactions with each other (data not shown).

As an initial screen, each cannabinoid ($10\ \mu\text{M}$) was added to an incubation mixture ($200\ \mu\text{l}$) containing potassium phosphate buffer ($100\ \text{mM}$; pH 7.4), HLMs ($0.1\ \text{mg/ml}$), CYP cocktail, and BSA (0.2%). Stock solutions of probe substrates and cannabinoids were prepared in 100% DMSO. The final concentration of DMSO (v/v) in the incubation mixture was $0.2\text{-}0.5\%$. Each incubation mixture was pre-warmed at 37°C with constant stirring for $10\ \text{min}$ and then the reaction was initiated with a NADPH regenerating system ($1.3\ \text{mM}$ NADP $^+$, $3.3\ \text{mM}$ G6P, $3.3\ \text{mM}$ MgCl $_2$, and $0.4\ \text{units/ml}$ G6PDH). After $15\ \text{min}$, the reaction was terminated with $200\ \mu\text{l}$ ice-cold acetonitrile containing tolbutamide (internal standard; $25\ \text{nM}$ final concentration) to precipitate the proteins. After vortexing, each sample was centrifuged at $18,000g$ for $10\ \text{min}$. Then, an aliquot of the supernatant ($50\ \mu\text{l}$) was stored at -20°C until analysis by LC-MS/MS (described below). Three independent experiments were conducted, each in duplicate.

Because $10\ \mu\text{M}$ is much greater than circulating cannabinoid unbound plasma concentrations observed when cannabis or CBD products are used for medicinal or recreational purposes, IC_{50} values were determined only for those cannabinoids that likely inhibit CYPs (in the screening assay) at pharmacologically relevant concentrations. Therefore, varying concentrations of CBD ($0.003\text{-}100\ \mu\text{M}$), 7-OH CBD ($0.003\text{-}50\ \mu\text{M}$), THC ($0.003\text{-}100\ \mu\text{M}$), or 11-OH THC ($0.01\text{-}100\ \mu\text{M}$) were used to determine their CYP-inhibitory potencies. CYP inhibition assays were conducted as described above. Three independent experiments were conducted, each in duplicate. IC_{50} was determined by nonlinear least

squares regression analysis (GraphPad Prism 6.01; Graphpad Software Inc., San Diego, CA, U.S.A.)

using the following equation:

$$\% \text{ CYP Activity} = A_0 + \frac{A_{\max} - A_0}{1 + 10^{[(\log IC_{50} - \log[I]) \times \text{Hill Slope}]}} \quad (1)$$

where I represents the inhibitor concentration, A_0 and $A_{\max}$ represent minimum and maximum % CYP activity, respectively, and Hill Slope describes the steepness of the curve. The F-test and the Akaike information criterion were used as measures of goodness of fit. Residual plots were used to decide among standard, Poisson (1/y), or relative (1/y²) weighting.

Time-Dependent Inhibition of CYPs by Cannabinoids

The TDI potential of each cannabinoid was screened at 10 μ M. The previously reported TDI (Bansal *et al.*, 2020) of CYP1A2, 2C19, and 3A by CBD (10 μ M) was repeated as a positive control. Each primary incubation mixture (200 μ l) consisted of HLMs (0.5 mg/ml protein), potassium phosphate buffer (100 mM, pH 7.4), BSA (0.2%), and cannabinoid. Blank DMSO (0.5 % v/v) was used in place of cannabinoid as a negative control. The mixture was pre-warmed at 37°C with constant stirring for 5 min and then the reaction was initiated with the NADPH regenerating system and incubated at 37°C for 0 or 30 min. An aliquot (5 μ l) of the primary incubation mixture was transferred to a pre-warmed (37°C) secondary incubation mixture (195 μ l) containing potassium phosphate buffer (pH 7.4), CYP cocktail (phenacetin; 50 μ M, coumarin; 2 μ M, bupropion; 5 μ M, amodiaquine; 2 μ M, diclofenac; 5 μ M, omeprazole; 20 μ M, dextromethorphan; 5 μ M, and testosterone; 20 μ M), and the NADPH regenerating system. After 15 min, the reaction was terminated with 200 μ l ice-cold acetonitrile containing tolbutamide (internal standard; 25 nM final concentration) to precipitate the proteins. Reaction mixtures were processed and analyzed as described for the reversible inhibition experiments. Three or four independent experiments were conducted, each singly.

Based on the screening results, cannabinoids that were likely to demonstrate TDI for CYPs at pharmacologically relevant concentrations were studied in detail to determine the TDI kinetics. Briefly,

varying concentrations of 7-OH CBD (1, 5, 10, 15, 20, 25, 30, 40, or 50 μM) or blank DMSO (0.5 % v/v) were preincubated with HLMs (0.5 mg/ml protein) and BSA (0.2%) for 0, 4, 8, 12, or 16 min. The assay proceeded as described above. Four independent experiments were conducted, each in singlicate. The observed first-order rate constants for inactivation (k_{obs}) were estimated as described previously (Bansal *et al.*, 2020). The half-maximal inactivation concentration (K_I) and maximal inactivation rate constant (k_{inact}) were estimated by nonlinear least-squares regression analysis (GraphPad Prism 6.01; Graphpad Software Inc., San Diego, CA, U.S.A.) of the k_{obs} vs. inactivator concentration ($[I]$) data using the following equation:

$$k_{\text{obs}} = \frac{k_{\text{inact}} \times [I]}{K_I + [I]} \quad (2)$$

Cannabinoid Plasma Protein Binding

The high nonspecific binding of cannabinoids to plastic and adsorption to filters or membranes precludes the use of ultrafiltration or equilibrium dialysis to determine their fraction unbound in plasma. Therefore, as previously described, the ultracentrifugation method and diluted plasma were used to measure the extent of plasma protein binding of the cannabinoids (Patilea-Vrana and Unadkat, 2019). Briefly, CBD (0.05, 5, or 50 μM), 7-OH CBD (0.25 or 1 μM), or THC (0.5 or 5 μM) was added to low binding tubes and incubated with diluted plasma (10-fold dilution with Dulbecco's phosphate buffer saline) (total volume = 200 μl) for 20 min at 37°C with shaking to facilitate nonspecific and protein binding. Two sets of these samples were prepared: one set (180 μl /sample) was subjected to ultracentrifugation at 37°C for 90 min, and the other (control) set (180 μl /sample) was incubated in the ultracentrifuge tubes at 37°C for 90 min without centrifugation. Samples undergoing ultracentrifugation were centrifuged at 435,000g for 90 min at 37°C using the Sorval Discovery M150 SE centrifuge and S100-AT3 rotor (Thermo Scientific). The middle (aqueous) layer was collected from the sample underwent centrifugation to determine unbound cannabinoid concentration. The total (bound and unbound) concentration of cannabinoid was quantified in the control incubations. Samples were analyzed

using LC-MS/MS (described below). Because both sets of samples were prepared in the low-binding tubes and transferred into the ultracentrifuge tubes, the same degree of nonspecific binding was assumed for both sample types. The fraction unbound in undiluted plasma ($f_{u,p}$) was estimated using the following equation (Schuhmacher *et al.*, 2000), where $f_{u,d}$ represents the fraction unbound in the diluted plasma and DF represents the dilution factor (0.1).

$$f_{u,p} = \frac{DF \cdot f_{u,d}}{1 - f_{u,d} \cdot (1 - DF)} \quad (3)$$

Cannabinoid Protein Binding in the Incubation Mixtures

Although we and Nasrin and colleagues have previously determined $f_{u,inc}$ of CBD and THC by the tube adsorption method (Bansal *et al.*, 2020; Nasrin *et al.*, 2021), the ultracentrifugation method was used to determine $f_{u,inc}$ of CBD, 7-OH CBD, and THC. This method was used because the tube adsorption method requires determination of the partition ratio of unbound cannabinoid to the walls of the tube in protein-free aqueous buffer. Given the limited solubility of the cannabinoids in protein-free buffer, the ultracentrifugation method avoids this problem. Briefly, CBD (0.1, 1, or 5 μ M), 7-OH CBD (1 or 10 μ M), or THC (0.5 or 5 μ M) was added to low binding tubes and incubated with HLMs (0.1 or 0.5 mg/ml) in 0.1 mM potassium phosphate buffer (total volume, 200 μ l) containing BSA (0.2%) for 15 min at 37°C with shaking to facilitate nonspecific and protein binding. Reaction mixtures were processed and analyzed as described for $f_{u,p}$ determination. The $f_{u,inc}$ of each tested cannabinoid was used to compute their corresponding unbound IC_{50} and K_i values. Because 11-OH THC and 7-OH CBD have similar lipophilicity and have a similar chemical structure (ChemDraw Ultra, CambridgeSoft Corporation, Cambridge, MA), the $f_{u,inc}$ for 11-OH THC was assumed to be same as that for 7-OH CBD.

LC-MS/MS Analysis

Each CYP-mediated metabolite was quantified using an UPLC-MS/MS system which consisted of an ACQUITYTM UPLC system (Waters, Milford, MA, USA) coupled with a SCIEX 6500 QTRAP[®] mass spectrometer (SCIEX, Framingham, MA, USA) equipped with an electrospray ion source. Chromatography was performed on an ACQUITYTM UPLC BEH C₁₈ column (2.1 × 50 mm, 1.7 μm) which was maintained at 45°C and was preceded by an ACQUITYTM UPLC BEH C₁₈ VanGuard pre-column (2.1 × 5 mm, 1.7 μm). The autosampler compartment were set at 4°C to prevent the evaporation of the samples. The sample injection volume was 10 μl. Water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid were used as the mobile phase A and B, respectively, with a flow rate of 0.4 ml/min. The gradient conditions were optimized over 4.5 min as follows: 5% B at 0.0-0.3 min, linear increase from 5% to 60% B at 0.3-3.0 min and from 60% to 90% B at 3.0-4.0 min, linear decrease from 90% to 5% B at 4.0-4.2 min, and 5% B at 4.2-4.5 min. The quantification was performed in the positive multiple reaction monitoring (MRM) mode after optimizing the compound- and ion source-dependent parameters. Acetaminophen, 7-hydroxycoumarin, 4-hydroxy bupropion, *N*-desethylamodiaquine, 4'-hydroxy diclofenac, 5-hydroxy omeprazole, dextrophan, 6β-hydroxy testosterone, and tolbutamide were quantified using the following mass transitions of *m/z*: 152.0 → 110.0, 162.9 → 106.9, 256.2 → 238.1, 328.2 → 283.1, 312.0 → 231.0, 362.0 → 214.0, 258.1 → 157.0, 305.3 → 269.1, and 271.3 → 155.0, respectively. The optimized spray voltage, ion source temperature, curtain gas, ion source gas 1, and ion source gas 2 were 5500 V, 600 °C, 30 psi, 50 psi, and 50 psi, respectively.

CBD, 7-OH CBD, CBD-d3 (internal standard for CBD and 7-OH CBD assay), THC, and THC-d3 (internal standard for THC assay) were quantified using the same LC-MS/MS system, column, and solvents as mentioned above. The flow rate was optimized to be 0.3 ml/min. The gradient conditions were optimized over 4.5 min as follows: 10% B at 0.0-0.5 min, linear increase from 10% to 95% B at 0.5-2.0 min, 95% B at 2.0-4.0 min, linear decrease from 95% to 10% B at 4.0-4.1 min, and 10% B at 4.1-4.5 min. CBD (or THC), 7-OH CBD, and CBD-d3, (or THC-d3) were quantified using the following mass transitions of *m/z*: 315.2 → 193.3, 331.2 → 313.1, and 318.3 → 196.3, respectively. The optimized spray

voltage, ion source temperature, curtain gas, ion source gas 1, and ion source gas 2 were 4000 V, 300 °C, 30 psi, 50 psi, and 50 psi, respectively. After method development, the LC-MS/MS methods were validated. The calibration curves were linear over the calibrator concentration range used ($r^2 > 0.97$) and the accuracy and precision of the quality control samples (low, middle, and high end of the calibration curves) was within 14% of the nominal concentrations and coefficient of variation of <12.5%, respectively.

Prediction of the Potential of the Cannabinoids to Precipitate Pharmacokinetic Drug Interactions

Unbound reversible inhibition ($IC_{50,u}$) and inactivation ($K_{i,u}$ and k_{inact}) kinetics of CBD, 7-OH CBD, and 11-COOH CBD or THC, 11-OH THC, and 11-COOH THC were incorporated into a previously described mechanistic static model (Fahmi *et al.*, 2008; Cheong *et al.*, 2017) to predict the magnitude of drug-drug interaction (DDI) (Eq 4). Assuming competitive inhibition, the $k_{i,u}$ of the cannabinoids was assumed to be equal to $IC_{50,u}$ when the CYP inhibition experiments were performed at probe concentration $< K_m$ (CYP2B6, 2C19, and 3A) or half the $IC_{50,u}$ when the CYP inhibition experiments were performed at probe concentration $\cong K_m$ (CYP1A2, 2C8, 2C9, and 2D6) (Cheng and Prusoff, 1973). AUCR (the magnitude of DDI) represents the ratio of area under the plasma concentration-time curve of the object (probe) drug in the presence (AUC'_{PO}) of the cannabinoid CYP inactivator/inhibitor to that of the probe drug AUC in the absence of the inactivator/inhibitor.

$$AUCR = \frac{AUC'_{PO}}{AUC_{PO}} = \left(\frac{1}{[A \times B] \times f_m + (1 - f_m)} \right) \times \left(\frac{1}{[X \times Y] \times (1 - F_G) + F_G} \right) \quad (4)$$

where A is the term that describes TDI of a CYP enzyme in the liver:

$$A = \sum_{i=1}^n \frac{k_{deg,H}}{k_{deg,H} + \frac{[I]_{H,i} \times k_{inact,i}}{[I]_{H,i} + K_{I,i}}} \quad (5)$$

B is the term that describes reversible inhibition of a CYP enzyme in the liver:

$$B = \sum_{i=1}^n \frac{1}{1 + \frac{[I]_{H,i}}{k_{i,i}}} \quad (6)$$

X is the term that describes TDI of a CYP enzyme in the intestine:

$$X = \sum_{i=1}^n \frac{k_{deg,G}}{k_{deg,G} + \frac{[I]_{G,i} \times k_{inact,i}}{[I]_{G,i} + K_{I,i}}} \quad (7)$$

Y is the term that describes reversible inhibition of a CYP enzyme in the intestine:

$$Y = \sum_{i=1}^n \frac{1}{1 + \frac{[I]_{G,i}}{k_{i,i}}} \quad (8)$$

and n is the number of inactivators/inhibitors (parent and metabolites) present. $[I]_H$ and $[I]_G$ are the inhibitor/inactivator concentrations in the liver and intestine, respectively. $k_{deg,H}$ and $k_{deg,G}$ represent the degradation rates of the CYP in the liver and intestine, respectively. The fraction of the object drug metabolized by a given CYP is represented by f_m , and the fraction of the object drug escaping intestinal metabolism is represented by F_G . The f_m and F_G values of object drugs and k_{deg} values of CYP enzymes are provided in Supp. Table 1.

A variation of the four different models (Models A-D) described by Tseng *et al.* (Tseng *et al.*, 2021) was used to predict the magnitude of a pharmacokinetic cannabinoid-drug interaction in the liver or intestine after a single (THC) or multiple (CBD) doses of oral or inhaled cannabinoid (liver only) administration (Bansal *et al.*, 2020). AUCR values were predicted using the estimated $[I]_H$ and $[I]_G$ concentrations (Supp. Table 2). $[I]_H$ was estimated to be either the unbound maximum hepatic inlet concentration ($C_{max,hepatic\ inlet,u}$; Model A), the unbound maximum systemic concentration ($C_{max,sys,u}$; Model B) or, the unbound average systemic concentration ($C_{ave,sys,u}$; Model C & D) (Tseng *et al.*, 2021). $[I]_G$ of the cannabinoids, due to their poor aqueous solubility, was estimated as their maximum intestinal fluid solubility (Models A-C) (Bansal *et al.*, 2020) or the unbound average portal vein concentration ($C_{ave,portal,u}$; Model D) to predict any interaction in the intestine (Tseng *et al.*, 2021).

To predict drug interactions precipitated by the cannabinoid metabolites, 7-OH CBD, 7-COOH CBD, 11-OH THC, or 11-COOH THC, the $C_{\max, \text{sys}, u}$ and $C_{\text{ave}, \text{sys}, u}$ of each was set equal to $[I]_H$. The doses (and justification) of CBD and THC to predict *in vivo* hepatic and intestinal CYP-mediated drug interactions were the same as described previously (Bansal *et al.*, 2020). Briefly, the average low, average high, and maximum doses of CBD or THC used for recreational or medicinal purposes were estimated from those reported in clinical trials, case reports, social media, cannabinoid vendor websites, and newspapers (Bansal *et al.*, 2020). The oral doses for CBD were 70 mg, 700 mg, and 2,000 mg and for THC were 20 mg, 130 mg, and 160 mg. The inhaled doses of THC were 25 mg, 70 mg, and 100 mg.

Results

Binding of CBD, 7-OH CBD, or THC to plasma proteins or in microsomal incubations

Because the $f_{u,p}$ of CBD, 7-OH CBD, and THC was concentration-independent, the data at all concentrations tested were pooled (Supp. Table 3). The cannabinoids were highly bound to plasma proteins (>98%). THC demonstrated higher degree of binding to plasma proteins as compared to CBD or 7-OH CBD; the latter two had comparable protein binding (Supp. Table 3). The $f_{u,inc}$ of CBD or 7-OH CBD was similar ($p > 0.05$) despite using different HLM concentrations (0.1 or 0.5 mg/ml) but the same BSA concentration (0.2%). THC behaved in a similar manner (Patilea-Vrana and Unadkat, 2019), indicating that the binding of these cannabinoids in the incubation mixture is driven by the BSA concentration (Supp. Table 4). Because the $f_{u,inc}$ of CBD, 7-OH CBD, or THC was concentration-independent, values at all concentrations (for each cannabinoid), for a given HLM concentration, were pooled (Supp. Table 4). THC and CBD had similar degree of binding to proteins in the incubations, whereas that of 7-OH CBD was lower (Supp. Table 4).

Reversible Inhibition of CYPs by CBD, THC, and their metabolites

CBD and 7-OH CBD inhibited CYP2B6, 2C8, 2C9, and 2D6 by similar extents, whereas CBD was a more potent inhibitor of CYP2C19 and 3A than 7-OH CBD. 7-COOH CBD was a weak inhibitor of CYP2B6, 2C8, 2C9, and 2D6 (Supp. Fig. 1). CBD and its metabolites were weak inhibitors of CYP2A6. THC and 11-OH THC (10 μ M each) inhibited CYP1A2, 2B6, 2C8, 2C9, 2C19, and 2D6 but only THC inhibited CYP3A. Like CBD and its metabolites, THC and 11-OH THC were weak inhibitors of CYP2A6. 11-COOH THC did not inhibit any of the CYPs tested.

CBD, 7-OH CBD, THC, and 11-OH THC inhibited CYP2B6 and 2C8 in a concentration-dependent manner (Fig. 2). The coefficient of variation (CV%) for IC_{50} estimates was <25%. Regarding the narrative below, potency refers to the unbound IC_{50} values ($IC_{50,u}$) which were calculated from IC_{50} values (Supp. Table 5) using $f_{u,inc}$ (Supp. Table 4). Among the cannabinoids tested, CBD was the most

potent inhibitor of CYP2B6 (Fig. 2, Table 1). CBD inhibited CYP2B6 with ~8-fold greater potency than 7-OH CBD, THC, or 11-OH THC; the latter three inhibited CYP2B6 with comparable potency (Table 1). CBD was also the most potent inhibitor of CYP2C8, being 3.6- and 2.3-fold more potent than 7-OH CBD and THC, respectively; THC was a 6.5 times more potent inhibitor of CYP2C8 than 11-OH THC (Table 1).

7-OH CBD inhibited CYP2C9, 2C19, 2D6, and 3A with the order of potency $\text{CYP2C9} > 2\text{C19} \approx 3\text{A} > 2\text{D6}$ (Table 1, Fig. 2C). The potency of 7-OH CBD to inhibit these enzymes was $1/10^{\text{th}}$ that of CBD (Table 1). Like CBD, 7-OH CBD inhibited CYP2C9 with 50% less potency against CYP2C19 or 3A and inhibited 2C9 and 2B6 with similar potency (Table 1). The IC_{50} of 7-OH CBD against CYP1A2 and 7-COOH CBD against all the CYPs could not be determined at the concentration range tested.

TDI of CYPs by CBD, THC, and their metabolites

Of the CYPs tested, 7-OH CBD (10 μM) demonstrated TDI of only CYP2C19 and 3A (Supp. Fig. 2). Unlike CBD (Bansal *et al.* 2020), 7-OH CBD (10 μM) did not show TDI of CYP1A2 (Supp. Fig. 2). TDI of CYPs 1A2, 2C19 and 3A by CBD was repeated in the current study, and the data were consistent with our previously published findings (Bansal *et al.*, 2020). CBD was not a time-dependent inhibitor of CYP2A6, 2B6, and 2C8 (data not shown). 7-COOH CBD was not a time-dependent inhibitor of any of the CYPs tested (data not shown).

The inhibition of CYP2C19 and 3A activities by 7-OH CBD was concentration- and time-dependent (Supp. Fig. 3). The K_i for 7-OH CBD (Fig. 3, Supp. Table 6) and the previously reported K_i of CBD (Bansal *et al.*, 2020) were converted to unbound K_i ($K_{i,u}$) using $f_{u,inc}$ (Supp. Table 4). The CV% for K_i or k_{inact} estimates was $<28\%$. 7-OH CBD inactivated CYP2C19 with similar efficiency ($k_{inact}/K_{i,u}$) as CYP3A, which was $\sim 1/6^{\text{th}}$ that of CBD (Table 2).

Prediction of *In Vivo* CYP-Mediated Cannabinoid-Drug Interactions

The estimated maximum plasma concentrations of the cannabinoids and/or metabolites or the calculated maximum intestinal fluid solubility at various doses of the cannabinoids consumed for recreational or medicinal purposes were used to predict the magnitude of *in vivo* cannabinoid-drug interactions (Supp. Table 2). Based on the predicted AUCR values using the $C_{\max, \text{hepatic inlet, u}}$ (Model A), CBD at high oral doses (700 and 2000 mg) was predicted to precipitate pharmacokinetic interactions with drugs that are predominantly metabolized by CYP2B6 (ticlopidine AUCR > 2.1) or 2C8 (repaglinide AUCR > 1.5) (Table 3). However, except for a modest interaction with ticlopidine at the maximum dose (2000 mg), CBD was predicted not to produce these drug interactions using its unbound systemic plasma concentration (Models B, C, & D) (Table 3). The predicted AUCR for CBD-ticlopidine or -repaglinide interactions was not affected by the inclusion of 7-OH CBD formed *in vivo* after CBD administration (data not shown). THC (oral or inhaled) and 11-OH THC were not predicted to precipitate interactions with ticlopidine or repaglinide, even at the highest doses consumed (Table 3).

Although the AUCR for CBD or THC (oral or inhaled) interactions with prototypic CYP substrates metabolized by CYP1A2 (theophylline), 2C9 (diclofenac), 2C19 (omeprazole), 2D6 (dextromethorphan), or 3A (midazolam) were predicted based on the protein binding data obtained from the current study (therefore revised $IC_{50, u}$), these values were not different from those in our previous publication (Bansal *et al.*, 2020) when predicted using the $C_{\max, \text{hepatic inlet, u}}$ (Model A). That is, at the higher doses oral CBD was predicted to precipitate drug interactions with drugs metabolized extensively by CYP1A2, 2C9, 2C19, 2D6, and 3A (Table 3). Oral THC was predicted to precipitate modest drug interactions with drugs metabolized extensively by CYP1A2 and 3A, and much larger interaction with those extensively metabolized by CYP2C9. Inhaled THC was predicted to precipitate interactions with only CYP2C9-metabolized drugs (Table 3). However, compared to Model A, lower AUCR was predicted using Model B, C, or D. Moreover, Models C & D (using $C_{\text{ave, sys, u}}$) did not predict any interaction between CBD and CYP2D6 substrate. Oral THC was predicted not to interact with CYP3A substrate when using $C_{\text{ave, portal, u}}$ as the enterocyte concentration (Model D vs Model C) (Table 3).

Discussion

Using HLMs, we previously determined the unbound inhibition potency ($IC_{50,u}$ or $K_{I,u}$) of CBD, THC, 11-OH THC, and 11-COOH THC towards CYP1A2, 2C9, 2C19, 2D6, or 3A (Bansal *et al.*, 2020). To complete the assessment of the potential for CBD or THC (and their *in vivo* circulating metabolites) to precipitate CYP-mediated cannabinoid-drug interactions, we determined for the first time the ability of CBD, THC, or 7-OH CBD, 7-COOH CBD, 11-OH THC, and 11-COOH THC to reversibly and irreversibly inhibit (TDI) CYP2A6, 2B6, and 2C8 activities in HLM. In addition, we determined the ability of the *in vivo* circulating metabolites of CBD to reversibly and irreversibly inhibit CYP1A2, 2C9, 2C19, 2D6, and 3A activity in HLM. To mitigate cannabinoid non-specific binding to labware and to increase their aqueous solubility, BSA (0.2%) was added to the incubation mixture. Incubations were conducted in low binding Eppendorf tubes to further reduce cannabinoid non-specific binding. In addition to determining unbound inhibition potency ($IC_{50,u}$ or $K_{I,u}$), the $f_{u,inc}$ and $f_{u,p}$ of the cannabinoids were determined by ultracentrifugation, a method best used to determine the fraction unbound of highly bound drugs. The $f_{u,inc}$ of CBD and THC was 25% and 40%, respectively, of that determined by tube adsorption method (Bansal *et al.*, 2020), and the $f_{u,p}$ of THC was 50% of that determined previously (Patilea-Vrana and Unadkat, 2019).

CBD and THC demonstrated potent reversible inhibition of CYP2B6 and weak reversible inhibition of 2A6, consistent with a previous report (Yamaori, Maeda, *et al.*, 2011; Nasrin *et al.*, 2021). However, the previously reported CYP2B6 IC_{50} and $IC_{50,u}$ values were approximately 50-200 and 2.4-5 fold, respectively, higher than those determined in the current work, substantiating the necessity to consider the low-aqueous solubility and non-specific binding of cannabinoids when determining their inhibition potency (Bansal *et al.*, 2020). The IC_{50} of CBD was comparable to that for the CYP2B6-selective inhibitor thio-tepa ($IC_{50} = 1.75 \mu M$), and was approximately 20% that of ticlopidine ($IC_{50} = 0.32 \mu M$), which to date is the most potent mixed-type (reversible and irreversible) inhibitor of CYP2B6 (Turpeinen *et al.*, 2004). Ideally, these comparisons should be made using $IC_{50,u}$, but the $IC_{50,u}$ values for

these drugs are not available in the literature. Overall, these results suggest that CBD and THC are potent inhibitors of CYP2B6.

Here, we report for the first time that both CBD and THC are potent inhibitors of CYP2C8 ($IC_{50,u} < 0.7 \mu M$). Nasrin and colleagues did not observe CYP2C8 inhibition by CBD or THC (Nasrin *et al.*, 2021). Low aqueous solubility and non-specific binding of these cannabinoids may be a plausible explanation for this discrepancy. As a comparison, the IC_{50} of CBD ($IC_{50} = 9.42 \mu M$) was approximately 500-times greater than that of montelukast ($IC_{50} = 0.02 \mu M$), the most potent inhibitor of CYP2C8 reported to date. However, this value was comparable to that of nifedipine, lovastatin, clopidogrel, irbesartan, and amlodipine ($IC_{50} \sim 10 \mu M$) (Walsky *et al.*, 2005). Again, $IC_{50,u}$ values of these drugs would provide the best comparison of their potencies, but these values are not available in the literature.

CBD, THC, and their metabolites did not demonstrate TDI of CYP2A6, 2B6, or 2C8 in HLMs. These results, except for the lack of TDI of CYP2A6 by THC, were consistent with a previous report (Yamaori, Maeda, *et al.*, 2011). Yamaori and colleagues showed that preincubation of THC with recombinant CYP2A6 yielded weak to moderately potent inactivation of CYP2A6 ($k_{inact}/K_I = 0.02 \text{ min}^{-1} \mu M^{-1}$) (Yamaori, Maeda, *et al.*, 2011). A plausible explanation for this discrepancy could be the use of HLMs in the current study instead of recombinant CYP2A6. HLMs represent a complete CYP system that includes CYP2C9, which rapidly metabolizes THC (Patilea-Vrana and Unadkat, 2019). 7-OH CBD displayed TDI of CYP2C19 and 3A but showed 17% of the potency of CBD. COOH-CBD did not show TDI of these CYPs. These results suggest that hydroxylation or carboxylation of CBD at the C₇ position (Fig. 1) diminished or abolished the ability of the metabolite to inactivate CYP2C19 or CYP3A.

In this study, drug interaction (as measured by AUCR) was predicted using plasma concentrations observed after multiple doses of CBD or a single dose of THC. This is because CBD accumulates after multiple dosing (Taylor *et al.*, 2018), likely due to auto-inhibition of its metabolism (Bansal *et al.*, 2020). In contrast, THC is predicted not to accumulate to a significant extent as THC is not a TDI of any CYPs and the terminal phase of THC concentration-time curve does not account for a significant portion of its $AUC_{0-\infty}$ (Patilea-Vrana and Unadkat, 2021).

One of the most important steps in accurately predicting drug interaction is to estimate the hepatic and intestinal concentrations of the inhibitor. To do so, several models have been proposed (Tseng *et al.*, 2021; Bansal *et al.*, 2020). Here, we implemented a variation of these models. The magnitude of pharmacokinetic interactions between each cannabinoid and CYP2B6 or CYP2C8 probe substrates was predicted using $C_{\max, \text{hepatic inlet}, u}$, $C_{\max, \text{sys}, u}$ or $C_{\text{ave}, \text{sys}, u}$ as $[I]_H$ and the calculated maximum intestinal fluid solubility or $C_{\text{ave}, \text{portal}, u}$ as $[I]_G$ (Tseng *et al.*, 2021; Bansal *et al.*, 2020). Using $C_{\max, \text{hepatic inlet}, u}$ as $[I]_H$ (Model A) a CBD oral dose of 700 mg was predicted to precipitate moderate ($2 \leq \text{AUCR} < 5$) or weak ($1.2 < \text{AUCR} < 2$) interactions (FDA drug interaction guidance 2020) with drugs extensively metabolized ($f_m \sim 0.75$) by CYP2B6 or CYP2C8, respectively. If $C_{\text{ave}, \text{sys}, u}$ (vs. $C_{\max, \text{hepatic inlet}, u}$) is used as an estimate of $[I]_H$ and $C_{\text{ave}, \text{portal}, u}$ (vs. maximum cannabinoid solubility) as an estimate of $[I]_G$ (Model D), CBD (700 mg) was predicted not to produce CYP2B6- or 2C8-mediated drug interactions. However, based on the *in vitro* data, the label for Epidiolex® (CBD) recommends adjustment in dosage of substrates of CYP2C8, and CYP2B6 be considered, if clinically appropriate, when administered concomitantly with Epidiolex (Epidiolex® Package Insert).

Despite inhibition or inactivation by 7-OH CBD of the CYPs tested, 7-OH CBD formed after oral administration of CBD was not predicted (based on 7-OH CBD $C_{\max, \text{sys}, u}$ or $C_{\text{ave}, \text{sys}, u}$) to enhance CBD-drug interactions. 7-OH CBD may be formed in the intestine by CYP3A (Jiang *et al.*, 2011) and its $C_{\max, \text{hepatic inlet}, u}$ could be higher than $C_{\max, \text{sys}, u}$. However, until the fraction of CBD metabolized to 7-OH CBD by intestinal CYP3A is known, it is not possible to correctly estimate 7-OH CBD $C_{\max, \text{hepatic inlet}, u}$. This fraction could be predicted using physiologically-based pharmacokinetic (PBPK) modeling and simulation. 11-OH THC was also predicted to have no effect on the magnitude of THC-drug interactions after oral or inhalational administration.

As we predicted earlier (Bansal *et al.*, 2020), CBD was predicted to precipitate drug interaction with CYP1A2, 2C9, 2C19, 2D6, or 3A substrates using Model A (Table 3). Drug interactions predicted with CYP1A2, 2C19, or 3A substrates were mostly due to TDI of these enzymes by CBD. Of the different static models used, as expected, Model A predicted the highest AUCR, whereas Model D

predicted the lowest AUCR. Our predictions are largely consistent with the reported clinical CBD-drug interactions. For example, CBD (5-25 mg/kg/day or 750 mg twice daily orally) increases the AUC and $C_{\max}$ (3- to 5-fold) of *N*-desmethyloclobazam, which is metabolized predominantly by CYP2C19 (Geffrey *et al.*, 2015; Gaston and Szaflarski, 2018; Morrison *et al.*, 2019). CBD (750 mg twice daily) increases the AUC of caffeine, a CYP1A2-metabolized drug, by 2-fold (Thai *et al.*, 2021). CBD (1000 mg twice daily) and CBD (400-800 mg daily) increase the $C_{\max}$ of tacrolimus and citalopram, drugs cleared predominately by CYP3A enzymes, by 3-fold and 2-fold, respectively (Leino *et al.*, 2019; Anderson *et al.*, 2021). Surprisingly, CBD (dose unknown) does not affect midazolam plasma concentrations (Epidiolex® Package Insert) which could be possibly due to CYP3A induction (Epidiolex® New Drug Application). However, it is puzzling as to why such induction would not affect the interaction with tacrolimus or citalopram. Due to the route of administration, inhaled THC-drug interactions were predicted using the only $C_{\max, \text{sys}, u}$ or $C_{\text{ave}, \text{sys}, u}$, and showed only CYP2C9-mediated drug interactions, and not CYP1A2 or 3A interactions predicted for oral THC (Table 3). Except for the warfarin case study described in the introduction, well-controlled THC-drug interaction studies (inhibition or induction) have not been reported in the literature. Although smoked cannabis appears to induce CYP1A2 (Jusko *et al.*, 1978), it is not clear if this is due to THC or other constituents in the smoke (e.g. arylamines) that are known to induced this enzyme.

There are several uncertainties associated with the current static model predictions, which may affect the predicted magnitude of cannabinoid-drug interactions. First, the depletion of CBD, 7-OH CBD, THC, 11-OH THC during incubations could result in the estimated IC_{50} or K_i values that are higher than the true values. Second, the $C_{\max, \text{hepatic inlet}, u}$ of the metabolites was not considered when predicting AUCRs for CBD- or THC-drug interactions after oral administration. Third, the predicted cannabinoid-drug interactions, based on static inhibitor concentrations, varied depending on the model used. Although Tseng *et al.* have proposed that Model D be used to predict such interactions (Tseng *et al.*, 2021), as to whether this model will accurately predict *in vivo* cannabinoid-drug interactions can only be confirmed by conducting such an interaction study. Fourth, static models do not consider the change in CBD, THC, or

their metabolite concentrations with time *in vivo*. Therefore, we are developing a dynamic (PBPK) model to capture these changes and predict the magnitude of drug interactions with CBD, THC, and their metabolites formed after oral or inhalational administration. Lastly, the TDI parameters (K_I and k_{inact}) obtained in HLM might differ from those obtained in human hepatocytes due to proteins present in hepatocytes, but not in HLM, that could neutralize the generated reactive metabolite(s) causing TDI of enzymes. Lastly, inhibition of non-CYP enzymes such as UGT1A9 and 2B7 by CBD or THC (Bansal *et al.*, 2021) should be considered when predicting interactions with drugs metabolized by CYP and/or UGT1A9/2B7.

In conclusion, using mechanistic static models, we predicted modest or no (depending on the model used) pharmacokinetic interactions between CBD (>700 mg, po), but not THC, and drugs predominantly metabolized by CYP2B6 or 2C8. CYP inhibition exhibited by 7-OH CBD or 11-OH THC did not enhance the magnitude of drug interactions with CBD or THC after oral or inhalational administration. Overall, at higher oral doses (>700 mg), and for all the models used, CBD was predicted to precipitate interactions with drugs metabolized by all the CYPs examined except CYP2A6 and for some models, CYP2D6. The order of magnitude of maximum drug interactions predicted at the highest dose (2000 mg) was CYP3A > 2C9 > 2C19 > 2D6 > 1A2 > 2B6 > 2C8. Oral or inhaled THC was predicted to precipitate interactions only with CYP2C9 substrates. *In vivo* drug interaction studies are needed to verify these predictions.

Authorship Contributions

Participated in research design: Bansal and Unadkat.

Conducted experiments: Bansal.

Contributed new reagents or analytic tools: N/A.

Performed data analysis: Bansal.

Wrote or contributed to the writing of the manuscript: Bansal, Unadkat, and Paine.

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Footnotes

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Legends for Figures

Fig. 1. CBD and THC biotransformation pathways. Bolded enzymes are the predominant contributors (Patilea-Vrana and Unadkat, 2019; Beers *et al.*, 2021). CYPs involved in the metabolism of 7-OH CBD to 7-COOH CBD have not been identified.

Fig. 2. Concentration-dependent inhibition of (A) CYP2B6 or (B) 2C8 activity in HLMs by CBD, 7-OH CBD, THC, or 11-OH THC and (C) concentration-dependent inhibition of CYP2C9, 2C19, 2D6, and 3A by 7-OH CBD. Of the cannabinoid tested, CBD was the most potent inhibitor of CYP2B6 and 2C8 (see Table 1). The order of 7-OH CBD inhibition potency was $CYP2B6 \approx 2C9 > 2C19 \approx 3A > 2C8 > 2D6$ (Table 1). Data shown are from a representative experiment conducted in duplicate. Solid lines represent model fit to the data.

Fig. 3. Kinetics of time-dependent inhibition of CYP2C19 and 3A by 7-OH CBD. Estimates of k_{inact} and K_i obtained by fitting appropriate equations to the k_{obs} data by nonlinear least-squares regression, showed that 7-OH CBD inactivated CYP2C19 and 3A with comparable efficiency ($k_{inact}/K_{i,u}$) but with lesser efficiency than CBD (positive control) (Table 2). Data shown are mean $\pm$ SD of four independent experiments, each conducted singly.

Table 1. IC_{50,u} values of CBD, 7-OH CBD, THC, and 11-OH THC for CYPs using HLMs

Enzyme	CBD	7-OH CBD	THC	11-OH THC
	IC _{50,u} ^a (μM)	IC _{50,u} ^a (μM)	IC _{50,u} ^a (μM)	IC _{50,u} ^{a,b} (μM)
CYP2B6	0.05 ± 0.02	0.34 ± 0.04	0.40 ± 0.06	0.38 ± 0.09
CYP2C8	0.28 ± 0.02	1.02 ± 0.15	0.67 ± 0.16	4.37 ± 1.21
CYP1A2	0.11 ± 0.04	>13.4	0.028 ± 0.007	1.81 ± 0.61
CYP2C9	0.04 ± 0.01	0.43 ± 0.09	0.005 ± 0.001	0.36 ± 0.12
CYP2C19	0.08 ± 0.01	0.82 ± 0.21	0.252 ± 0.098	1.76 ± 0.26
CYP2D6	0.24 ± 0.12	2.46 ± 0.62	0.561 ± 0.108	5.25 ± 1.03
CYP3A	0.09 ± 0.03	0.80 ± 0.14	0.574 ± 0.149	>6.7

^aIC_{50,u} values were calculated using the IC₅₀ values provided in Supp. Table 5.

^bf_{u,inc} for 11-OH THC was assumed to be same as 7-OH CBD.

Data shown are mean ± SD of three independent experiments, each conducted in duplicate.

Table 2. Kinetics of time-dependent inhibition of CYP2C19 and 3A by CBD or 7-OH CBD in HLMs

Enzyme	CBD ^a			7-OH CBD ^b		
	$K_{I,u}$ (μM)	k_{inact} (min^{-1})	$k_{\text{inact}}/K_{I,u}$ ($\text{min}^{-1} \mu\text{M}^{-1}$)	$K_{I,u}$ (μM)	k_{inact} (min^{-1})	$k_{\text{inact}}/K_{I,u}$ ($\text{min}^{-1} \mu\text{M}^{-1}$)
CYP2C19	0.07 ± 0.04	0.035 ± 0.006	0.62 ± 0.32	0.67 ± 0.27	0.063 ± 0.007	0.10 ± 0.02
CYP3A	0.11 ± 0.05	0.078 ± 0.022	0.79 ± 0.20	1.01 ± 0.46	0.128 ± 0.018	0.14 ± 0.05

^a $K_{I,u}$ was calculated using the reported K_I of CBD (Bansal *et al.*, 2020).

^b $K_{I,u}$ was calculated using the K_I of 7-OH CBD provided in Supp. Table 6.

Data are mean $\pm$ SD of four independent experiments, each conducted singly.

Table 3. Predicted magnitude of pharmacokinetic interactions between CBD or THC interaction and drugs extensively metabolized by CYP enzymes using different static models

Precipitant	CYP Enzyme	Object Drug	Predicted AUCR after oral administration of the precipitant (Inhibition of hepatic and gut metabolism)				Predicted AUCR after Inhalation of the precipitant (Inhibition of hepatic metabolism)	
			Model				Model	
			#A ^a	#B ^b	#C ^c	#D ^d	#B ^e	#C or D ^f
CBD (70 mg, Oral)	1A2	Theophylline	3.9	3.2	2.7	2.7	--	--
	2B6	Ticlopidine	1.2	1.0	1.0	1.0	--	--
	2C8	Repaglinide	1.1	1.0	1.0	1.0	--	--
	2C9	Diclofenac	2.4	1.6	1.6	1.1	--	--
	2C19	Omeprazole	5.3	1.9	1.5	1.5	--	--
	2D6	Dextromethorphan	1.1	1.0	1.0	1.0	--	--
	3A	Midazolam	12.3	5.1	3.5	3.2	--	--
CBD (700 mg, Oral)	1A2	Theophylline	4.0	3.9	3.8	3.8	--	--
	2B6	Ticlopidine	2.2	1.2	1.1	1.1	--	--
	2C8	Repaglinide	1.6	1.1	1.0	1.0	--	--
	2C9	Diclofenac	9.6	2.3	1.9	1.6	--	--
	2C19	Omeprazole	7.3	5.1	3.9	3.9	--	--
	2D6	Dextromethorphan	2.1	1.1	1.0	1.0	--	--
	3A	Midazolam	14.8	12.0	9.8	9.7	--	--
CBD (2000 mg, Oral)	1A2	Theophylline	4.0	3.9	3.9	3.9	--	--
	2B6	Ticlopidine	3.1	1.4	1.2	1.2	--	--
	2C8	Repaglinide	2.2	1.2	1.1	1.1	--	--
	2C9	Diclofenac	20.7	3.8	2.6	2.4	--	--
	2C19	Omeprazole	7.6	6.5	5.6	5.6	--	--
	2D6	Dextromethorphan	4.1	1.3	1.1	1.1	--	--
	3A	Midazolam	15.0	13.9	12.6	12.6	--	--
THC (20 mg, Oral)	1A2	Theophylline	1.1	1.0	1.0	1.0	--	--
	2B6	Ticlopidine	1.0	1.0	1.0	1.0	--	--
	2C8	Repaglinide	1.0	1.0	1.0	1.0	--	--
	2C9	Diclofenac	2.1	1.6	1.6	1.1	--	--
	2C19	Omeprazole	1.0	1.0	1.0	1.0	--	--
	2D6	Dextromethorphan	1.0	1.0	1.0	1.0	--	--
	3A	Midazolam	1.8	1.8	1.8	1.0	--	--
THC (130 mg, Oral)	1A2	Theophylline	1.4	1.0	1.0	1.0	--	--
	2B6	Ticlopidine	1.0	1.0	1.0	1.0	--	--
	2C8	Repaglinide	1.0	1.0	1.0	1.0	--	--
	2C9	Diclofenac	6.6	2.1	1.7	1.4	--	--
	2C19	Omeprazole	1.0	1.0	1.0	1.0	--	--

	2D6	Dextromethorphan	1.0	1.0	1.0	1.0	--	--
	3A	Midazolam	1.8	1.8	1.8	1.0	--	--
THC (160 mg, Oral)	1A2	Theophylline	1.5	1.1	1.0	1.0	--	--
	2B6	Ticlopidine	1.0	1.0	1.0	1.0	--	--
	2C8	Repaglinide	1.0	1.0	1.0	1.0	--	--
	2C9	Diclofenac	7.6	2.3	1.7	1.4	--	--
	2C19	Omeprazole	1.0	1.0	1.0	1.0	--	--
	2D6	Dextromethorphan	1.1	1.0	1.0	1.0	--	--
	3A	Midazolam	1.8	1.8	1.8	1.0	--	--
THC (20 mg, Inhaled)	1A2	Theophylline	--	--	--	--	1.1	1.0
	2B6	Ticlopidine	--	--	--	--	1.0	1.0
	2C8	Repaglinide	--	--	--	--	1.0	1.0
	2C9	Diclofenac	--	--	--	--	1.5	1.1
	2C19	Omeprazole	--	--	--	--	1.0	1.0
	2D6	Dextromethorphan	--	--	--	--	1.0	1.0
	3A	Midazolam	--	--	--	--	1.0	1.0
THC (75 mg, Inhaled)	1A2	Theophylline	--	--	--	--	1.2	1.0
	2B6	Ticlopidine	--	--	--	--	1.0	1.0
	2C8	Repaglinide	--	--	--	--	1.0	1.0
	2C9	Diclofenac	--	--	--	--	2.3	1.2
	2C19	Omeprazole	--	--	--	--	1.0	1.0
	2D6	Dextromethorphan	--	--	--	--	1.0	1.0
	3A	Midazolam	--	--	--	--	1.0	1.0
THC (100 mg, Inhaled)	1A2	Theophylline	--	--	--	--	1.2	1.0
	2B6	Ticlopidine	--	--	--	--	1.0	1.0
	2C8	Repaglinide	--	--	--	--	1.0	1.0
	2C9	Diclofenac	--	--	--	--	2.9	1.4
	2C19	Omeprazole	--	--	--	--	1.0	1.0
	2D6	Dextromethorphan	--	--	--	--	1.0	1.0
	3A	Midazolam	--	--	--	--	1.0	1.0

^apredicted AUCR using $[I]_H = C_{\max, \text{hepatic inlet}, u}$ (unbound maximum hepatic inlet concentration) in eq. 5 & 6 and $[I]_G =$ calculated maximum intestinal fluid solubility in eq. 7 & 8.

^bpredicted AUCR using $[I]_H = C_{\max, \text{sys}, u}$ (unbound maximum systemic concentration) in eq. 5 & 6 and $[I]_G =$ calculated maximum intestinal fluid solubility in eq. 7 & 8.

^cpredicted AUCR using $[I]_H = C_{\text{ave}, \text{sys}, u}$ (unbound average systemic concentration) in eq. 5 & 6 and $[I]_G =$ calculated maximum intestinal fluid solubility in eq. 7 & 8.

^dpredicted AUCR using $[I]_H = C_{\text{ave}, \text{sys}, u}$ (unbound average systemic concentration) in eq. 5 & 6 and $[I]_G = C_{\text{ave}, \text{portal}, u}$ (unbound average portal vein concentration) in eq. 7 & 8.

^epredicted AUCR using only $[I]_H = C_{\max, \text{sys}, u}$ (unbound maximum systemic concentration) in eq. 6.

^fpredicted AUCR using only $[I]_H = C_{\text{ave}, \text{sys}, u}$ (unbound average systemic concentration) in eq. 6.

-- not relevant

Bold values represent AUCR > 1.25 (cutoff recommended by the FDA).

$C_{\max, \text{hepatic inlet}, u}$ and $C_{\text{ave}, \text{portal}, u}$ of CBD and THC and $C_{\max, \text{sys}, u}$ and $C_{\text{ave}, \text{sys}, u}$ of CBD, THC, and their metabolites are provided in Supp. Table 2.

Maximum intestinal fluid solubility of CBD and THC is from Bansal *et al.*, 2020.

Fig. 1

Fig. 2

Fig. 3