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Research Article

Cannabinoid Interactions with Cytochrome P450 Drug Metabolism: a Full-Spectrum Characterization

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Abstract. Medicinal cannabis use has increased exponentially with widespread legalization around the world. Cannabis-based products are being used for numerous health conditions, often in conjunction with prescribed medications. The risk of clinically significant drug-drug interactions (DDIs) increases in this setting of polypharmacy, prompting concern among health care providers. Serious adverse events can result from DDIs, specifically those affecting CYP-mediated drug metabolism. Both cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (Δ^9-THC), major constituents of cannabis, potently inhibit CYPs. Cannabis-based products contain an array of cannabinoids, many of which have limited data available regarding potential DDIs. This study assessed the inhibitory potential of 12 cannabinoids against CYP-mediated drug metabolism to predict the likelihood of clinically significant DDIs between cannabis-based therapies and conventional medications. Supersomes™ were used to screen the inhibitory potential of cannabinoids *in vitro*. Twelve cannabinoids were evaluated at the predominant drug-metabolizing isoforms: CYP3A4, CYP2D6, CYP2C9, CYP1A2, CYP2B6, and CYP2C19. The cannabinoids exhibited varied effects and potencies across the CYP isoforms. CYP2C9-mediated metabolism was inhibited by nearly all the cannabinoids with estimated K_i values of 0.2–3.2 μM. Most of the cannabinoids inhibited CYP2C19, whereas CYP2D6, CYP3A4, and CYP2B6 were either not affected or only partially inhibited by the cannabinoids. Effects of the cannabinoids on CYP2D6, CYP1A2, CYP2B6, and CYP3A4 metabolism were limited so *in vivo* DDIs mediated by these isoforms would not be predicted. CYP2C9-mediated metabolism was inhibited by cannabinoids at clinically relevant concentrations. *In vivo* DDI studies may be justified for CYP2C9 substrates with a narrow therapeutic index.

KEY WORDS: cannabidiol; cannabinoids; CYP450; drug-drug interactions; drug metabolism.

INTRODUCTION

Over the last decade, there has been increased interest in the use of cannabis and cannabis-based products to treat numerous health conditions. Often, these cannabis-based products are used in conjunction with conventional drug treatments. The potential for serious drug-drug interactions (DDIs) increases in this polypharmacy setting. DDIs are one of the primary causes of adverse drug reactions, which account an estimated burden of $528 billion on health care systems annually [1, 2]. In an effort to avoid adverse drug reactions, drug regulatory agencies mandate that investigational drugs be evaluated for DDI potential. However, due to legislation in many jurisdictions allowing a direct pathway to market and strong consumer demand, cannabis-based products have not followed the standard drug development pipeline. Consequently, data regarding the DDI potential of cannabis-based products is lacking. Indeed, this dearth of knowledge around DDI potential of cannabis-based pharmacotherapies is a major concern among health care providers.

Drug metabolism and cytochrome P450 (CYP) enzymes tend to be the primary focus when considering DDIs since approximately 75% of clinically important drugs are metabolized by the CYP superfamily of proteins. The predominant CYP isoforms responsible for drug metabolism are CYP3A4 (~ 30%), CYP2D6 (~ 20%), CYP2C9 (~ 13%), CYP1A2 (~ 9%), CYP2B6 (~ 7%), CYP2C19 (~ 7%), CYP2C8 (~ 5%), CYP2A6 (~ 4%), CYP2E1 (~ 3%), and CYP2J2 (~ 3%) [3]. Competition between two drugs for the same metabolizing pathway is a common mechanism for DDIs that can lead to serious adverse events. Both cannabidiol (CBD) and Δ^9-

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tetrahydrocannabinol (Δ^9-THC), the major constituents of cannabis, potently inhibit CYPs [4–8]. Indeed, clinically significant DDIs believed to be CYP-mediated have been reported for CBD co-medication with clobazam, warfarin, tacrolimus, and methadone [9–13].

The cannabis plant is a complex mixture of hundreds of bioactive molecules, approximately 120 of which are terpenophenolic compounds known as cannabinoids. Commercial and artisanal cannabis-based products often contain an array of cannabinoids that can be present at concentrations higher than those of CBD and Δ^9-THC [14, 15]. The use of purified CBD products for a myriad of conditions has led many in the medicinal cannabis industry to speculate on what might be the "next big cannabinoid" [16]. Accordingly, purified cannabinoids referred to as isolates, including cannabigerol (CBG) and cannabichromene (CBC), are entering the marketplace on the suggestion they be included in food, supplements, and medicines. Given the high degree of structural similarity between CBD, Δ^9-THC, and other cannabinoids, it is highly probable that they too inhibit CYPs (Fig. 1). Therefore, this study aimed to characterize the reversible inhibitory potential of 12 cannabinoids on the metabolic activity of the six major drug-metabolizing CYP isoforms using FDA-recommended substrates. The cannabinoids screened here represent some of the most abundant cannabinoids across various cannabis strains and have been detected in cannabis-based products [14–18].

MATERIALS AND METHODS

Drugs and Reagents. CBD, CBDV, CBG, CBN, Δ^9-THC, Δ^9-THCA, and Δ^9-THCV were purchased from THC Pharm GmbH (Frankfurt, GER). CBGA was purchased from Cayman Chemical (Ann Arbor, USA). CBDA was generously provided by Medropharm GmbH (Schönenberg, CHE). CBDVA, Δ^9-THCVA (Professor Michael Kassiou), and CBC (Dr. Jia Lin Luo) were synthesized at the University of Sydney. Caffeine, (S)-mephenytoin, dextromethorphan, ticlopidine, nifedipine, testosterone, and diazepam were purchased from Sigma-Aldrich Co. (St. Louis, USA). Bupropion, tolbutamide, α-naphthoflavone, sulfaphenazole, quinidine, and itraconazole were purchased from MedChemExpress (Monmouth Junction, USA). Triazolam, hydroxybuproprion, 4-hydroxytolbutamide, 4-hydroxymephenytoin, dextrorphan, dehydronifedipine, and hydroxytriazolam were purchased from Cayman Chemical. Paraxanthine and 6β-hydroxytestosterone were purchased from Toronto Research Chemicals (North York, CAN) and Cerilliant Corp. (Round Rock, USA), respectively. All cannabinoids and CYP substrates and inhibitors were prepared as 30 or 100 mM stocks in DMSO, with the exception of dextromethorphan and caffeine, which were prepared in water. Final solvent concentrations in CYP-mediated reactions were ≤ 0.13%.

CYP Reactions. CYP-mediated reactions were performed in 40 μL reaction mixtures consisting of substrate, 10 μM inhibitor (cannabinoids or positive control), 100 mM Tris buffer, pH 7.4, and 50 nM Supersomes™ (Corning Inc., Corning, USA) containing a single CYP isoform and NADPH-P450 reductase. Supersome™ concentration for CYP2D6 reactions was 12.5 nM. Mixtures were preincubated at 37°C for 5 min, and reactions were initiated by the addition of an NADPH-regenerating system (1.3 mM $NADP^+$, 3.3 mM glucose-6-phosphate, 3.3 mM magnesium chloride, and 0.4 U/mL glucose-6-phosphate dehydrogenase). Reactions were incubated at 37°C and terminated with the addition of 40 μL ice-cold acetonitrile containing diazepam (5 μg/mL) as an internal standard. Reactions were then centrifuged at 14,000*g* for 30 min, and supernatants were analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). Details regarding experimental conditions for each CYP isoform are provided in Table I. Substrates were screened at concentrations equivalent to K_M values reported in the literature [19–31]. Incubation times and protein concentrations used were within the linear phase of metabolite formation for each CYP probe reaction, with linearity confirmed where incubation conditions were different [19–31].

Analytical Analysis. Metabolite concentrations were assayed by LC-MS/MS using a Shimadzu Nexera HPLC coupled to a Shimadzu 8040 triple quadrupole mass spectrometer (Shimadzu Corp.; Kyoto, JPN). Chromatography separation was achieved on a Zorbax XDB-C_{18} column (2.1 × 50 mm; 3.5 μm) reverse-phase column with a Zorbax C_8 guard column (Agilent; Santa Clara, USA) using a gradient elution. The mass spectrometer was operated in positive electrospray ionization mode (negative for 4-hydroxytolbutamide) with multiple reaction monitoring. Details regarding chromatographic and mass spectrometer conditions, including limits of quantification, are presented in Table II.

Data Analysis. The amount of metabolite formed was quantified by integrating the area under the curve of the chromatographic peaks for the metabolite and diazepam. A ratio of metabolite to the internal standard diazepam was then calculated for each sample. The activity (metabolite to diazepam ratio) of the vehicle was set at 100%, and the percent activity for each cannabinoid was then calculated as a percentage of the vehicle control. Cannabinoids that reduced CYP activity by > 50% were subsequently screened at 1 and 3 μM. The relative activity was determined for each inhibitor and plotted as 1/activity as a percentage of control in GraphPad Prism 8.2 (La Jolla, USA). IC_{50} values were calculated by linear regression and the Cheng-Prusoff equation, where the IC_{50} value equals 1/slope [32]. Because substrates were screened at a concentration equivalent to K_M values and incubation times were within the linear phase of metabolite formation, according to the Cheng-Prusoff equation, the calculated IC_{50} values are approximately 2 times the K_i values. No corrections were made for cannabinoid binding to labware, which could be as great as 80%; hence, the determined IC_{50} values represent the apparent values [33].

RESULTS

CBD and CBDV Partially Inhibited CYP3A4-Mediated Metabolism

The FDA strongly recommends that at least two structurally unrelated substrates be used for CYP3A4 studies

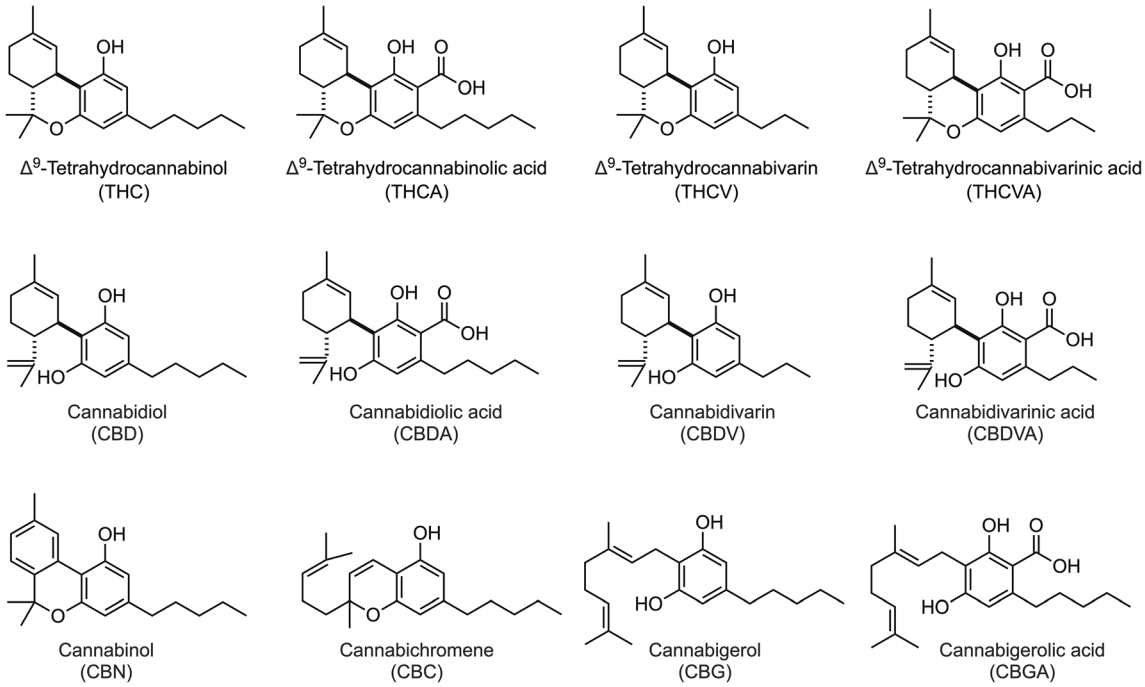

Fig. 1. Chemical structures of cannabinoids used in this study

to account for the presence of multiple binding sites. Here, CYP3A4-mediated metabolism of triazolam, testosterone, and nifedipine was measured in the presence of cannabinoids (10 μM). Most cannabinoids had no effect on CYP3A4 function (Fig. 2). Partial inhibition of triazolam metabolism was observed with CBD and cannabidivarin (CBDV) (Fig. 2a). When nifedipine was used as a substrate, partial inhibition was again observed for CBDV (Fig. 2b). However, CBD had no effect and THCV partially inhibited nifedipine metabolism. When testosterone was used as a substrate, partial inhibition was again observed for CBD and CBDV, and additionally, cannabidivarinic acid (CBDVA) and Δ^9-tetrahydrocannabinvarinic acid (Δ^9-THCVA) partially inhibited testosterone metabolism (Fig. 2c). By comparison, the positive control itraconazole potently inhibited CYP3A4 activity regardless of substrate.

Cannabinoids Had No Effect on CYP2D6 Metabolic Activity

The cannabinoids screened here had little effect on CYP2D6-mediated metabolism of dextromethorphan (Fig. 3). Partial inhibition of dextromethorphan metabolism was observed with CBDVA. However, CYP2D6 activity was completely inhibited by the positive control inhibitor, quinidine.

Cannabinoids Inhibited CYP2C9-Mediated Metabolism

The cannabinoids tended to inhibit CYP2C9-mediated metabolism of tolbutamide (Fig. 4). Cannabinol (CBN) was the only cannabinoid that did not affect CYP2C9 activity. CBC, Δ^9-THC, and Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA) exerted minimal inhibition of tolbutamide metabolism at 10 μM. The remaining cannabinoids inhibited CYP2C9-mediated metabolism of tolbutamide by > 50% so were characterized further. IC_{50} values of CBD, CBDV, cannabigerolic acid (CBGA), Δ^9-THCA, and Δ^9-THCVA ranged from 2.5 to 6.4 μM (Table III). More potent inhibition of CYP2C9 metabolic activity was observed for CBDA, CBDVA, and CBG, with IC_{50} values of 0.4 ± 0.0 μM, 1.3 ± 0.4 μM, and 1.0 ± 0.2 μM, respectively. Notably,

Table I. Experimental Conditions for CY450 Reactions

CYP450 isoform	Substrate	Substrate concentration (μM)	Inhibitor	Incubation time (min)
CYP1A2	Caffeine	660 [19]	α-Naphthoflavone	15 [19]
CYP2B6	Bupropion	50 [28]	Ticlopidine	20 [28]
CYP2C9	Tolbutamide	100 [25–27, 53]	Sulfaphenazole	45 [25–27, 53]
CYP2C19	(S)-Mephenytoin	30 [27, 28]	Omeprazole	15 [27, 28]
CYP2D6	Dextromethorphan	5 [28, 29]	Quinidine	15 [28, 29]
CYP3A4	Triazolam	50 [20, 28]	Itraconazole	20 [20, 28]
CYP3A4	Nifedipine	10 [20, 30, 31]	Itraconazole	10 [20, 30, 31]
CYP3A4	Testosterone	25 [21–23]	Itraconazole	10 [21–23]

Table II. Experimental Conditions for LC-MS/MS Analysis

Analyte	Mobile phase A	Mobile phase B	Molecular weight	Parent > daughter ions (*m/z*)	LOQ (ng/mL)
Paraxantine	0.1% formic acid in water	Methanol	180.16	181.00 > 124.1 181.00 > 96.20	0.5
Hydroxybupropion	0.1% formic acid in water	Methanol	255.74	256.05 > 258.15 256.05 > 139.1	0.1
4-Hydroxytolbutamide	Deionized water	Acetonitrile	286.35	285.00 > 186.00	5
4-Hydroxymephenytoin	0.1% formic acid in water	Methanol	234.25	235.20 > 150.05 235.20 > 133.20	0.5
Dextrorphan	0.1% formic acid in water	Acetonitrile	257.37	258.90 > 239.00 259.90 > 176.05	0.1
Hydroxytriazolam	0.1% formic acid in water	Methanol	359.2	358.90 > 331.10 358.90 > 239.00	0.5
Dehydronifedipine	0.1% formic acid in water	Methanol	344.3	345.05 > 284.10 345.05 > 268.20	0.1
6β-Hydroxytestosterone	0.1% formic acid in water	Methanol	304.4	305.15 > 269.30 305.15 > 287.25	2
Diazepam (internal standard)	n/a	n/a	284.7	284.60 > 257.00 284.60 > 220.00	-

sulfaphenazole inhibited CYP2C9 with an IC_{50} value of 635 ± 80 nM so these three cannabinoids were as potent as the positive control inhibitor (Supp. Figure 1).

CBN Potently Inhibited CYP1A2-Mediated Metabolism

CYP1A2-mediated metabolism of caffeine was measured in the presence of cannabinoids. Most cannabinoids had no effect on caffeine metabolism (Fig. 5). CBC and CBG inhibited CYP1A2 activity minimally. CYP1A2 metabolism of caffeine in the presence of 10 μM CBN was reduced to ~ 11%, and an IC_{50} value of 1.0 ± 0.1 μM was calculated for CBN (Table III). For comparison, the IC_{50} value of the positive control inhibitor α-naphthoflavone was 2.8 ± 1.0 μM (Supp. Figure 2).

CYP2B6-Mediated Metabolism Inhibited by CBD

Metabolism of bupropion by CYP2B6 in the presence of cannabinoids was measured here. The majority of the cannabinoids examined had no effect on CYP2B6 activity (Fig. 6). CBDV and CBG partially inhibited CYP2B6 activity. CBD inhibited CYP2B6-mediated metabolism of bupropion with an IC_{50} value of 6.2 ± 1.7 μM (Table III). Ticlopidine, the positive control inhibitor, had an IC_{50} value of 142 ± 30 nM (Supp. Figure 3).

Cannabinoids Inhibited CYP2C19 Metabolic Activity

Lastly, CYP2C19-mediated metabolism of (S)-mephenytoin was examined in the presence of cannabinoids. CBDVA, CBG, CBN, THCV, and THCVA were partial inhibitors of CYP2C19 activity (Fig. 7). CBDV modestly inhibited CYP2C19 (IC_{50} value 6.4 ± 1.3 μM; Table III). CYP2C19-mediated metabolism of (S)-mephenytoin was potently inhibited by CBD, with an IC_{50} value of 2.1 ± 0.5 μM (Table III). The IC_{50} value of the positive control inhibitor ticlopidine was 330 ± 10 nM (Supp. Figure 4).

DISCUSSION

Medicinal cannabis use has skyrocketed, prompting concern among health care providers over potential DDIs with prescribed medications. Serious adverse events can result from DDIs, specifically interactions involving CYP drug metabolism. The present study explored the inhibitory potential of 12 cannabinoids commonly present in medicinal cannabis-based products on the six major drug-metabolizing CYP isoforms. The cannabinoids exhibited varied effects and inhibitory potencies across each of the CYP isoforms (Fig. 8).

CBD, CBN, and Δ^9-THC have previously been shown to inhibit CYP2C9-mediated metabolism of (S)-warfarin and diclofenac [6]. Here, CBD and Δ^9-THC also inhibited CYP2C9-mediated metabolism of tolbutamide; however, CBN had no effect. Consistent with previous reports, CBD

Table III. Summary of IC_{50} values

Cannabinoid	Apparent IC_{50} value (μM)
CYP2C9	
CBD	2.5 ± 0.9
CBDA	0.4 ± 0.0
CBDV	4.3 ± 1.3
CBDVA	1.3 ± 0.4
CBG	1.0 ± 0.2
CBGA	6.4 ± 5.3
THCA	5.1 ± 2.0
THCVA	4.0 ± 1.3
CYP1A2	
CBN	1.0 ± 0.1
CYP2B6	
CBD	6.2 ± 1.7
CYP2C19	
CBD	2.1 ± 0.5
CBDV	6.4 ± 1.3

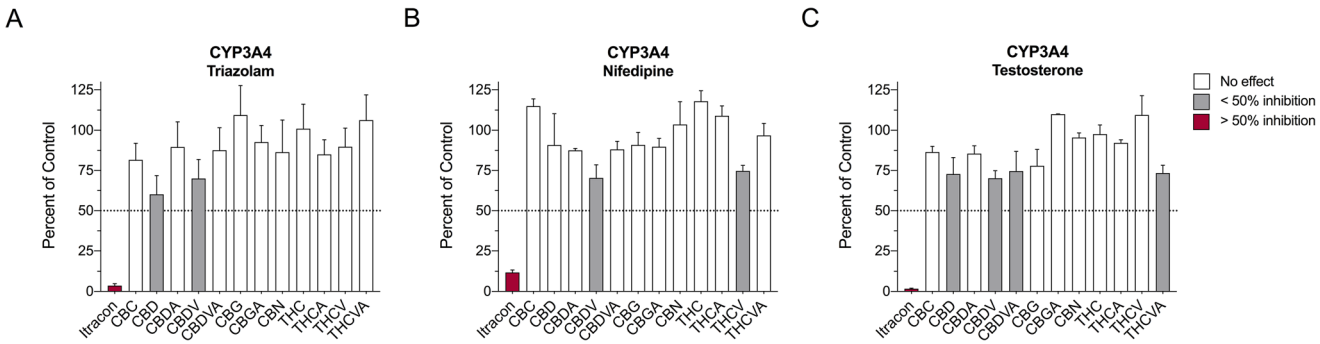

Fig. 2. CYP3A4-mediated metabolism affected minimally by cannabinoids. Relative CYP3A4-mediated metabolism of **A** triazolam, **B** nifedipine, or **C** testosterone in the presence of cannabinoids at 10 μM. Activity is represented as a percentage of vehicle control, with the dashed line representing 50% activity. Intracon, Itraconazole. Error bars represent SEM, with n = 3–5 per treatment. The reaction velocities (pmol/min/pmol CYP3A4) for the vehicle-treated conditions were triazolam, 1.3 ± 0.1; nifedipine, 8.3 ± 0.9; and testosterone, 7.8 ± 2.1

inhibited CYP2C19 and CYP3A4 activity and CBN potently inhibited CYP1A2 [5, 8, 34]. A previous report showed that CBD inhibited CYP2D6-mediated metabolism of dextromethorphan with a K_i value of 1 μM but no inhibition was observed in the present study [7]. A limitation of this study is that binding of the cannabinoids to the labware was not measured. As such, the IC_{50} values are apparent values so the potency of the cannabinoids could be underestimated here. A study by Bansal *et al.* accounted for binding to labware and found that in human liver microsomes, CBD and Δ^9-THC inhibited CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A with K_i values ranging from 0.012 to 1.3 μM [33].

All of the cannabinoids, except CBN, inhibited CYP2C9-mediated metabolism of tolbutamide. CYP2C9 is integral for the metabolism of many medications with a narrow therapeutic index including amitriptyline, cyclophosphamide, phenytoin, tamoxifen, and warfarin so identifying inhibitors is essential to avoid DDIs and serious adverse events [35]. Indeed, clinically significant DDIs have been reported between warfarin and CBD or smoked cannabis [9, 11]. CBDA was the most potent inhibitor of CYP2C9 with an estimated K_i value of 200 nM (Fig. 8). Serum concentrations of CBDA achieved following vaporization of a medicinal cannabis preparation containing 0.8 mg CBDA were 10.2 ng/mL (34 nM) [36]. The CBDA dose administered in artisanal cannabis-based products used by childhood epilepsy patients is 0.5 mg/kg/day on average [15]. Assuming an average patient weight of 30 kg, it is conceivable that CBDA serum concentrations could be ~ 600 nM. Thus, CBDA inhibits CYP2C9 activity with a K_i value around physiologically relevant concentrations. The widespread inhibition of CYP2C9-mediated metabolism observed with the cannabinoids warrants caution. Therapeutic drug monitoring may be advised for patients treated with cannabis-based products concomitant with CYP2C9 substrates, especially those drugs with narrow therapeutic windows.

CYP2C19 metabolic function was also inhibited by a majority of the cannabinoids. Consistent with previous reports, CBD inhibited CYP2C19 activity with an estimated K_i value of 1.1 μM, which is a physiologically relevant concentration (Fig. 8) [5]. Clinical doses of CBD typically range from 300 to 1500 mg/day resulting in plasma CBD concentrations that range from 0.3 to 3.1 μM [10, 37]. Because CBD inhibits CYP2C19 at clinically relevant concentrations,

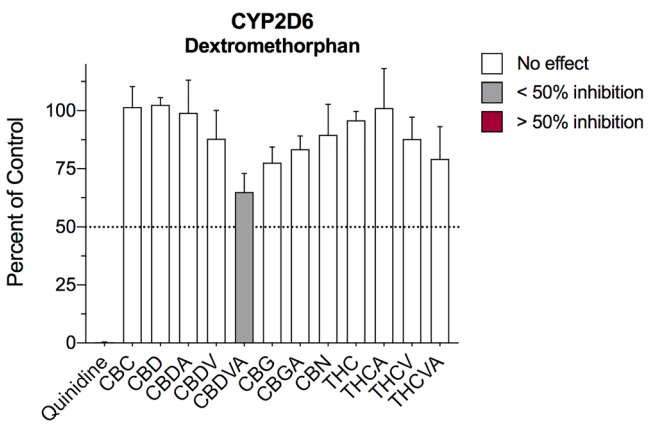

Fig. 3. Cannabinoids did not inhibit CYP2D6-mediated metabolism of dextromethorphan. Relative CYP2D6 activity in the presence of cannabinoids at 10 μM. Activity is represented as a percentage of vehicle control, with the dashed line representing 50% activity. Cannabinoids had little effect on the metabolism of dextromethorphan by CYP2D6. Error bars represent SEM, with n = 5 per treatment. The reaction velocity for the vehicle-treated condition was 4.8 ± 0.6 pmol/min/pmol CYP2D6

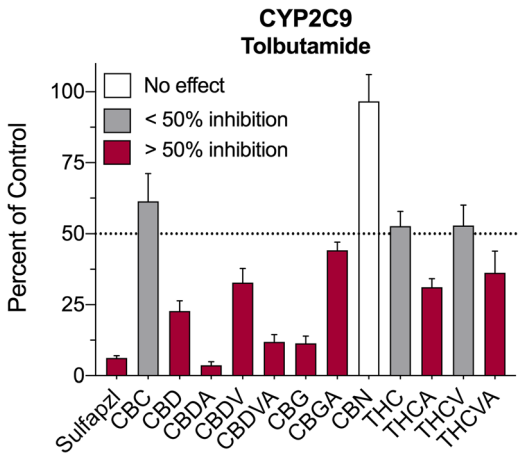

Fig. 4. CYP2C9-mediated metabolism of tolbutamide inhibited by cannabiniods. Relative CYP2C9 activity in the presence of cannabinoids at 10 μM. Activity is represented as a percentage of vehicle control, with the dashed line representing 50% activity. Sulfapzl, sulfaphenazole. Error bars represent SEM, with n = 5 per condition. The reaction velocity for the vehicle-treated condition was 0.7 ± 0.1 pmol/min/pmol CYP2C9

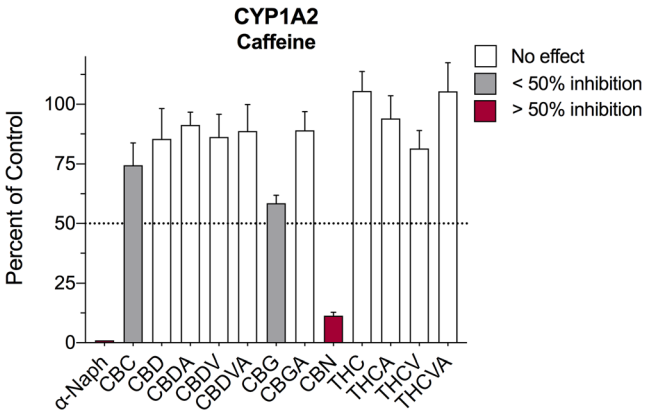

Fig. 5. CBN inhibited CYP1A2-mediated metabolism of caffeine. Relative CYP1A2 activity in the presence of cannabinoids at 10 μM. Activity is represented as a percentage of vehicle control, with the dashed line representing 50% activity. CBN inhibited CYP1A2 by > 50% (red bar). α-Naph, α-naphthoflavone. Error bars represent SEM, with n = 5 per condition. The reaction velocity for the vehicle-treated condition was 0.7 ± 0.1 pmol/min/pmol CYP1A2

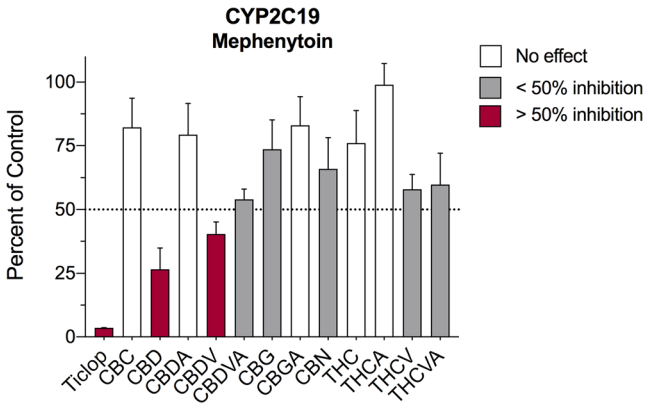

Fig. 7. CYP2C19-mediated metabolism (S)-mephenytoin inhibited by cannabinoids. Relative CYP2C19 activity in the presence of cannabinoids at 10 μM. Activity is represented as a percentage of vehicle control, with the dashed line representing 50% activity. CBDVA, CBG, CBN, Δ^9-THCV, and Δ^9-THCVA partially inhibited CYP2C19 (gray bars). CYP2C19 activity was inhibited by > 50% by CBD and CBDV (red bars). Ticlop, ticolpidine.. Error bars represent SEM, with n = 5 per condition. The reaction velocity for the vehicle-treated condition was 1.3 ± 0.1 pmol/min/pmol CYP2C19

in vivo DDIs would be predicted. As expected, CBD has been shown to interact with CYP2C19 substrates and lead to adverse events *in vivo* [10, 38, 39]. The other cannabinoids CBDV, CBDVA, CBG, CBN, Δ^9-THCV, and Δ^9-THCVA inhibited CYP2C19-mediated metabolism of (S)-mephenytoin less potently (> 3 μM) at concentrations unlikely to be achieved *in vivo* (Fig. 8). However, this does not eliminate the possibility of a clinically significant DDI with alternate CYP2C19 substrates.

It is important to consider substrate specificity when evaluating potential CYP interactions. Because the binding affinities of both the substrate and inhibitor for the CYP affect K_i values, an inhibitor can display a range of K_i values against different substrates at a single CYP isoform [27, 40]. Thus, although these cannabinoids are only moderate to weak inhibitors of CYP2C19 here, the inhibition potency could be more pronounced and clinically relevant with different CYP2C19 substrates. As such, even partial inhibitors identified in the CYP screen should be considered

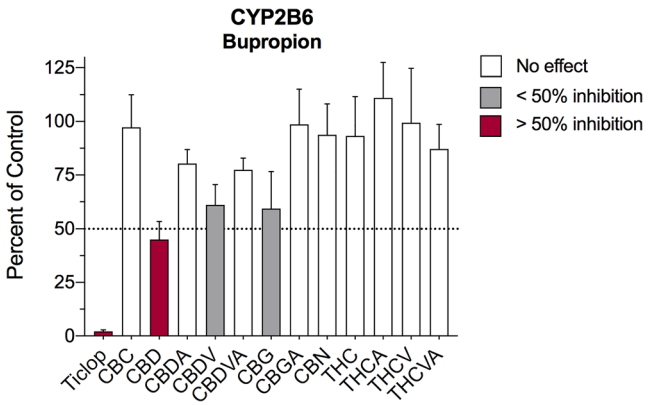

Fig. 6. CBD moderately inhibited CYP2B6-mediated metabolism of bupropion. Relative CYP2B6 activity in the presence of cannabinoids at 10 μM. Activity is represented as a percentage of vehicle control, with the dashed line representing 50% activity. Partial inhibition was observed in the presence of CBDV and CBG (gray bars). CBD inhibited CYP2B6 by > 50% (red bar). Ticlop, ticolpidine. Error bars represent SEM, with n = 5 per condition. The reaction velocity for the vehicle-treated condition was 1.2 ± 0.1 pmol/min/pmol CYP2B6

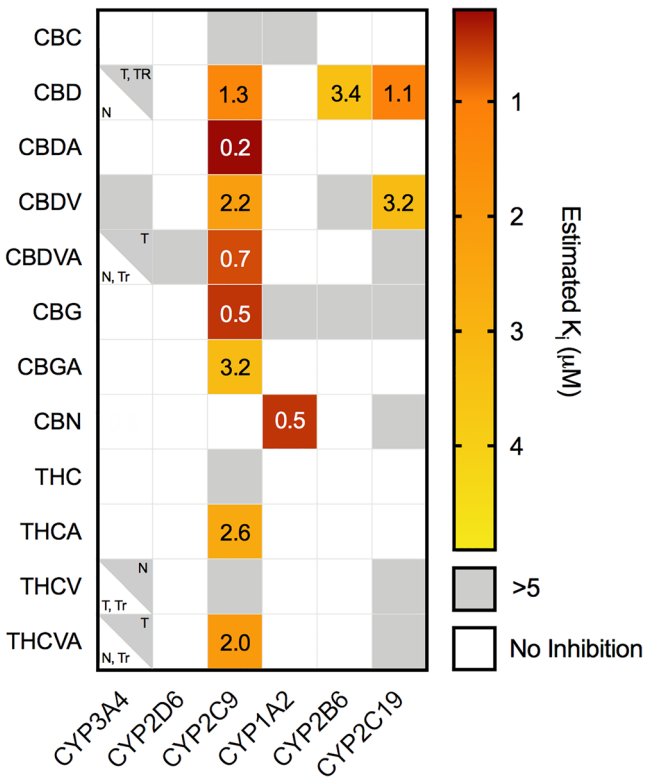

Fig. 8. Summary of cannabinoid-CYP450 interactions. Heat map of cannabinoid inhibition at each CYP450 isoform, including estimated K_i values. Substrates were screened at K_M values and according to the Cheng-Prusoff equation $K_i = IC_{50}/2$. Three substrates (testosterone (T), triazolam (TR), and nifedipine (N)) were all screened at CYP3A4. CBD, CBDVA, THCV, and THCVA all exhibited substrate-specific inhibition at CYP3A4 so the square is split to depict all K_i values with the corresponding substrate abbreviation overlaid

potential perpetrators of substrate-specific DDIs and substrate-specific evaluations and subsequent *in vivo* DDI studies would be required to definitely assess the safety of phytocannabinoid coadministration with a particular therapy.

The importance of considering substrate specificity related to CYP inhibition is further re-enforced with CYP3A4. Here, CYP3A4-mediated metabolism of triazolam, nifedipine, and testosterone was relatively unaffected by the cannabinoids, with a couple cannabinoids exhibiting only partial inhibition. The lack of substantial inhibition by CBD is consistent with package labelling of Epidiolex™ which states that CBD does not affect plasma concentrations of the CYP3A4 probe substrate midazolam [41]. However, a recent study showed that CBD potently inhibited (K_i value 680 nM) CYP3A4-mediated metabolism of citalopram, which correlated with increased plasma citalopram concentrations [42].

CBN potently inhibited (estimated K_i value 0.5 μM) CYP1A2-mediated metabolism of caffeine. Caffeine has a large therapeutic window; thus, inhibition of caffeine metabolism by CBN is likely of little therapeutic concern. However, CYP1A2 substrates theophylline, olanzapine, and clozapine have narrow therapeutic indices so clinically significant DDIs could result from concomitant CBN [43, 44]. Plasma concentrations of CBN achieved from smoking a cannabis cigarette containing 1.6 mg CBN had a median of 2.2 ng/mL (7 nM) so CYP1A2 function is unlikely to be affected by this route of CBN administration [45]. However, media sources are dubbing CBN the "sleepy cannabinoid" so there is an increasing demand for CBN isolates as a sleep aid. The K_i value for CBN inhibition of CYP1A2 may become physiologically relevant when using these purified CBN products, in which case DDIs could be pertinent.

For the most part, the cannabinoids had minimal effects on the metabolic activity of CYP1A2, CYP2B6, and CYP2D6, suggesting that *in vivo* DDIs mediated by these CYP isoforms are unlikely. However, DDIs between cannabinoids and substrates of these CYP enzymes cannot be ruled out completely as DDIs might occur as a result of alternate pharmacokinetic mechanisms or pharmacodynamic interactions. DDIs at drug transporters, as has been reported for CBD and Δ^9-THC, can profoundly affect drug bioavailability and disposition [46–50]. Furthermore, only reversible inhibition of CYP metabolic activity was evaluated here but the cannabinoids could exhibit time-dependent inhibition of CYP function since previous studies have shown time-dependent inactivation for both CBD and Δ^9-THC [7, 8, 33, 51, 52]. It is plausible that these cannabinoids could induce the expression of CYP enzymes, which could also lead to a clinically significant DDI.

CONCLUSION

The inhibitory potential of 12 cannabinoids on the major drug-metabolizing CYPs was assessed to predict the likelihood of clinically significant DDIs between cannabis-based therapies and conventional medications. Largely, the cannabinoids did not affect the metabolic activity of CYP2D6, CYP1A2, CYP2B6, and CYP3A4 so *in vivo* DDIs mediated by these isoforms would not be predicted. A majority of the cannabinoids exhibited some degree of inhibition of CYP2C19. The most potent and extensive inhibition of the cannabinoids was at CYP2C9. The cannabinoids inhibited CYP2C9-mediated metabolism at concentrations likely to be clinically relevant so this inhibition could translate to significant *in vivo* DDIs. Future *in vivo* DDI studies and therapeutic drug monitoring may be justified for CYP2C9 substrates that have a narrow therapeutic index.

SUPPLEMENTARY INFORMATION

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AUTHOR CONTRIBUTION

PTD, JCA, and LLA contributed to conception and design of the study. PTD, LDO, and LLA contributed to the acquisition and analysis of data. PTD and LLA wrote the first draft of the manuscript. All the authors contributed to drafting the manuscript or figures and have approved the final manuscript.

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DECLARATIONS

Conflict of Interest Associate Professor Jonathon Arnold is the Deputy Academic Director of the Lambert Initiative. He has served as an expert witness in various medicolegal cases involving cannabis and recently served as a temporary advisor to the World Health Organization (WHO) on their review of cannabis and the cannabinoids. The remaining authors have no conflicts of interest.

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