



# Potent inhibition of human cytochrome P450 3A isoforms by cannabidiol: Role of phenolic hydroxyl groups in the resorcinol moiety

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## ABSTRACT

**Aims:** In this study, we examined the inhibitory effects of  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), cannabidiol (CBD), and cannabinol (CBN), the three major cannabinoids, on the activity of human cytochrome P450 (CYP) 3A enzymes. Furthermore, we investigated the kinetics and structural requirement for the inhibitory effect of CBD on the CYP3A activity.

**Main methods:** Diltiazem *N*-demethylase activity of recombinant CYP3A4, CYP3A5, CYP3A7, and human liver microsomes (HLMs) in the presence of cannabinoids was determined.

**Key findings:** Among the three major cannabinoids, CBD most potently inhibited CYP3A4 and CYP3A5 ( $IC_{50}$  = 11.7 and 1.65  $\mu$ M, respectively). The  $IC_{50}$  values of  $\Delta^9$ -THC and CBN for CYP3A4 and CYP3A5 were higher than 35  $\mu$ M. For CYP3A7,  $\Delta^9$ -THC, CBD, and CBN inhibited the activity to a similar extent ( $IC_{50}$  = 23–31  $\mu$ M). CBD competitively inhibited the activity of CYP3A4, CYP3A5, and HLMs ( $K_i$  = 1.00, 0.195, and 6.14  $\mu$ M, respectively). On the other hand, CBD inhibited the CYP3A7 activity in a mixed manner ( $K_i$  = 12.3  $\mu$ M). Olivetol partially inhibited all the CYP3A isoforms tested, whereas *d*-limonene showed lack of inhibition. The lesser inhibitory effects of monomethyl and dimethyl ethers of CBD indicated that the ability of CYP3A inhibition by the cannabinoid attenuated with the number of methylation on the phenolic hydroxyl groups in the resorcinol moiety.

**Significance:** This study indicated that CBD most potently inhibited catalytic activity of human CYP3A enzymes, especially CYP3A4 and CYP3A5. These results suggest that two phenolic hydroxyl groups in the resorcinol moiety of CBD may play an important role in the CYP3A inhibition.

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## Introduction

Marijuana is one of the most widely abused drugs in the world. Marijuana leaves contain at least 70 cannabinoids (ElSohly and Slade, 2005). Among them,  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), cannabidiol (CBD), and cannabinol (CBN) are the three major constituents (Fig. 1).  $\Delta^9$ -THC is a primary psychoactive component of marijuana (Pertwee, 2008). CBD is not psychoactive but has several pharmacological effects such as drug-induced sleep prolongation, antiepileptic, anxiolytic, and antiemetic actions (Mechoulam et al., 2002). CBN is thought to exert minimal pharmacological effects in the central nervous system.

These major cannabinoids are known to be extensively metabolized by hepatic microsomal cytochrome P450 (CYP) (Huestis, 2005; Watanabe et al., 2007). Bornheim et al. and we previously demonstrated that the hepatic metabolism of  $\Delta^9$ -THC, CBD, and CBN is predominantly catalyzed by CYP2C and CYP3A (Bornheim and Correia,

1991; Bornheim et al., 1992; Watanabe et al., 1995, 2007). On the other hand, these major cannabinoids have been reported to inhibit CYP-mediated drug metabolism in livers from experimental animals (Fernandes et al., 1973; Chan and Tse, 1978; Watanabe et al., 1981; Hamajima et al., 1983). Furthermore, a previous clinical study has shown that the administration of CBD decreases the systemic clearance of hexobarbital, which is metabolized by CYP2C9, in human subjects (Benowitz et al., 1980). Jaeger et al. (1996) have reported that CBD inhibits cyclosporine and  $\Delta^9$ -THC oxidations catalyzed by CYP3A in human liver microsomes (HLMs). More recently, we have shown that  $\Delta^9$ -THC, CBD, and CBN inhibit catalytic activities of human CYP1 enzymes (Yamaori et al., 2010). These findings suggest that the administration of cannabinoids or marijuana may lead to drug interactions with drugs or toxicants metabolized by these CYP enzymes.

Human CYP3A subfamily is involved in the metabolism of more than 50% of drugs clinically used. The human CYP3A subfamily expressed in the liver consists of at least three members: CYP3A4 (Guengerich et al., 1986; Molowa et al., 1986), CYP3A5 (Aoyama et al., 1989; Wrighton et al., 1989), and CYP3A7 (Kitada et al., 1985). CYP3A4, which is the most abundant isoform expressed in adult

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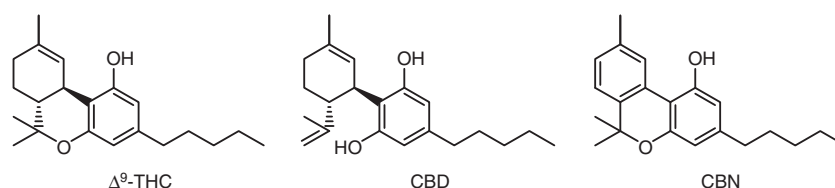


Fig. 1. Structures of three major cannabinoids.

human livers (Shimada et al., 1994), is capable of metabolizing structurally diverse compounds, such as diltiazem, midazolam, erythromycin, and cyclosporine (Rendic, 2002). CYP3A5, which is polymorphically expressed in the liver (Kuehl et al., 2001), also can catalyze oxidations of a variety of drugs (Aoyama et al., 1989; Williams et al., 2002; Yamaori et al., 2003, 2004). The catalytic activity of CYP3A5 is equal or lower as compared with that of CYP3A4, although some drugs, such as diltiazem and midazolam, are more efficiently metabolized by CYP3A5 than by CYP3A4 (Gorski et al., 1994; Yamaori et al., 2004). CYP3A7, which is a major isoform expressed in fetal human livers (Kitada et al., 1985; Komori et al., 1990), is suggested to play a key role in the metabolism of endogenous compounds and xenobiotics during the fetal period (Kitada et al., 1985, 1987; Chen et al., 2000). These findings reveal that human CYP3A subfamily is one of the most important enzyme groups in the hepatic drug metabolism. Furthermore, these findings indicate the possibility of frequent occurrence of drug interactions caused by CYP3A enzymes. Thus, it is very important to elucidate possible interactions of marijuana or its components including cannabinoids with various compounds metabolized by CYP3A enzymes. However, the potency and mechanism of cannabinoid inhibition against individual isoforms of human CYP3A subfamily remain to be clarified.

In the present study, we investigated the inhibitory effects of the three major cannabinoids ( $\Delta^9$ -THC, CBD, and CBN) on human CYP3A-mediated oxidation. We report herein that CBD is a potent inhibitor against human CYP3A enzymes. In addition, our study suggests that two phenolic hydroxyl groups in the resorcinol moiety of CBD may have structurally important role in the CYP3A inhibition.

## Materials and methods

### Materials

$\Delta^9$ -THC, CBD, and CBN were isolated from cannabis leaves using the method previously reported (Aramaki et al., 1968). CBD-2'-monomethyl ether (CBDM) and CBD-2',6'-dimethyl ether (CBDD) were prepared as described previously (Gohda et al., 1990). *N*-Desmethyldiltiazem was a gift from Tanabe Seiyaku (Osaka, Japan). Diltiazem was obtained from Wako Pure Chemical Ind. (Osaka, Japan). Olivetol and *d*-limonene were purchased from Sigma-Aldrich Corp. (St. Louis, MO). NADP, glucose 6-phosphate, and glucose 6-phosphate dehydrogenase were obtained from Oriental Yeast Co. Ltd. (Tokyo, Japan). Other chemicals and solvents used were of the highest quality commercially available.

### Enzyme sources

Microsomes from baculovirus-infected insect cells expressing CYP3A4, CYP3A5, and CYP3A7 each with NADPH-CYP reductase and cytochrome *b*<sub>5</sub> (Supersomes™) were purchased from BD Gentest (Woburn, MA). A human liver sample was obtained from a 57-year-old Japanese female who died in a traffic accident. The use of the human liver for these studies was approved by the Ethics Committee of Kanazawa University, Graduate School of Medical Science. HLMs were prepared as reported previously (Watanabe et al., 1995). The protein concentration was estimated by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

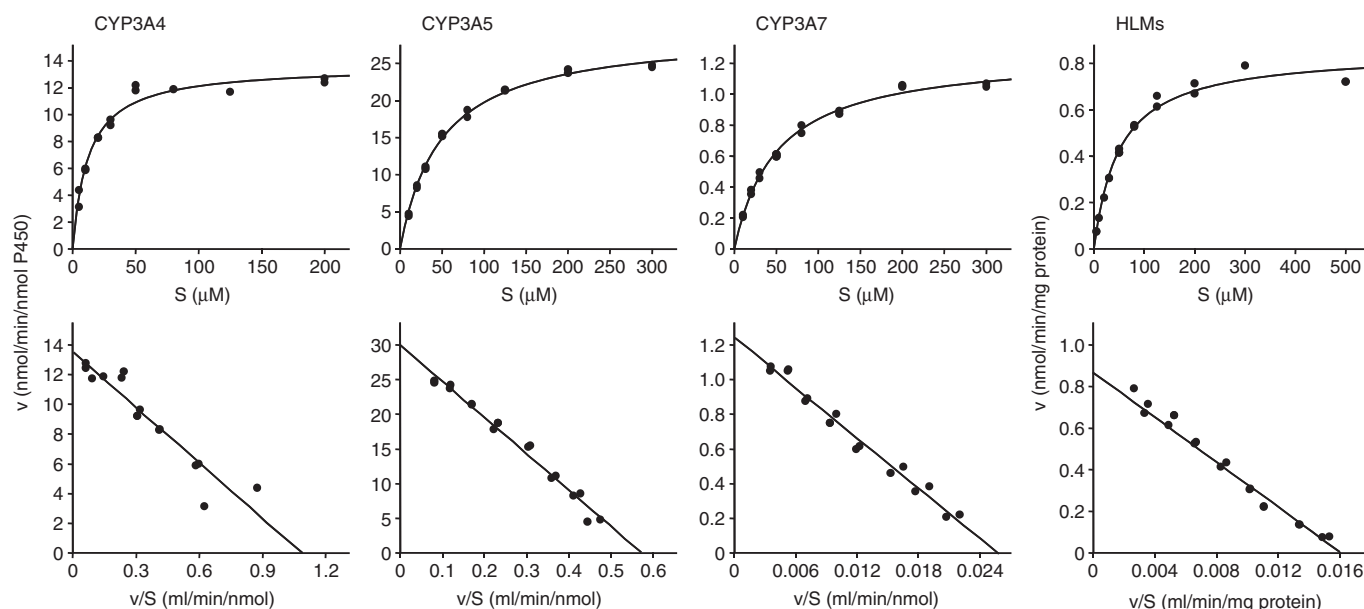


Fig. 2. Kinetic analyses for diltiazem *N*-demethylation catalyzed by human CYP3A isoforms and liver microsomes. Recombinant CYP3A4, CYP3A5, CYP3A7, and HLMs were incubated with diltiazem (5–500  $\mu$ M). *S* and *v* indicate diltiazem and catalytic activity, respectively. Each point indicates an individual value.

**Table 1**

Kinetic parameters for diltiazem *N*-demethylation catalyzed by recombinant human CYP3A isoforms and HLMs.

Enzymes	$K_m$ ( $\mu\text{M}$ )	$V_{\text{max}}$ (nmol/min/nmol P450)
CYP3A4	12.5	13.6
CYP3A5	48.4	29.3
CYP3A7	50.8	1.27 (nmol/min/mg protein)
HLMs	50.2	0.852

Data are derived from two replicates.

### Enzyme assays

Diltiazem *N*-demethylase activity was determined as reported previously (Yamaori et al., 2004) with minor modifications. Recombinant CYP3A4 (2 pmol), CYP3A5 (2 pmol), CYP3A7 (10 pmol), and HLMs (50  $\mu\text{g}$  protein) were used as enzyme sources. An incubation mixture consisted of an enzyme source, diltiazem, an NADPH-generating system (0.5 mM NADP, 10 mM glucose 6-phosphate, 10 mM magnesium chloride, and 1 unit/ml glucose 6-phosphate dehydrogenase), 100 mM sodium potassium phosphate buffer (pH 7.4), and 50  $\mu\text{M}$  EDTA in a final volume of 200  $\mu\text{L}$ . Incubations were carried out at 37 °C for 15 min (30 min for CYP3A7) and terminated by adding 10  $\mu\text{L}$  of 70% (v/v) perchloric acid. After the removal of protein by centrifugation, 90  $\mu\text{L}$  of the supernatant was subjected to a high-performance liquid chromatography (Hitachi L-2130 pump, L-2200 autosampler, and L-2400 UV detector, Hitachi, Tokyo, Japan) equipped with a Mightysil RP-18 GP column (4.6  $\times$  150 mm, 5  $\mu\text{m}$ , Kanto Chemical, Tokyo, Japan). The mobile phase was 50 mM potassium phosphate buffer (pH 6.0) containing 28% (v/v) acetonitrile. Elution was performed at a flow rate of 1.5 ml/min. The formation of *N*-desmethyl-diltiazem was monitored at a wavelength of 238 nm.

To determine the kinetic parameters for the diltiazem *N*-demethylation in recombinant CYP3A isoforms and HLMs, 5 to 500  $\mu\text{M}$  diltiazem

was incubated with either of these enzyme sources under the same conditions as mentioned above. In preliminary experiments, these reaction conditions were confirmed to ensure linear initial rates for the formation of *N*-desmethyl-diltiazem. Data points were fitted to the Michaelis–Menten equation by nonlinear least-squares regression analysis with Origin 7.5J software (OriginLab, Northampton, MA).

### Inhibition studies

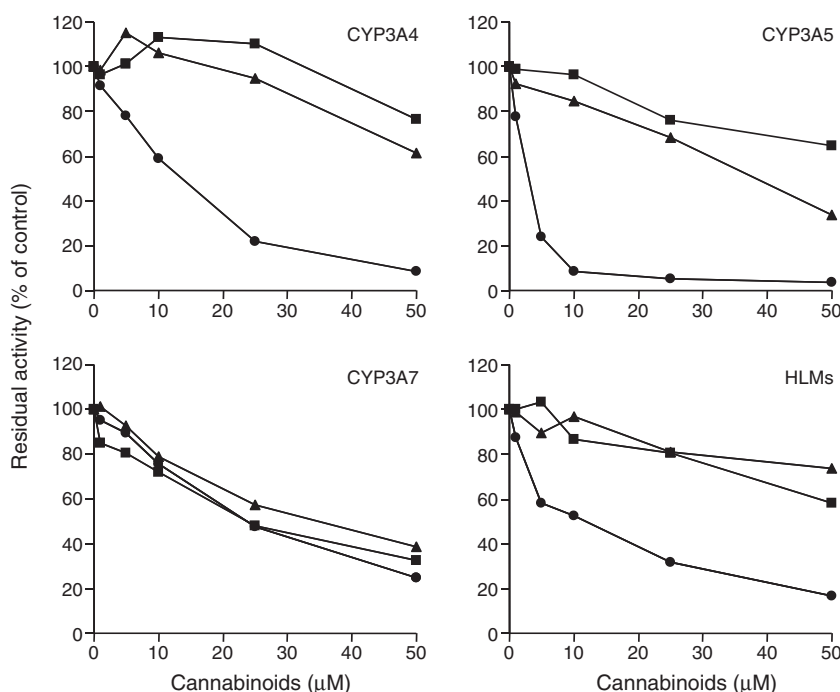
Recombinant CYP3A isoforms and HLMs were incubated with diltiazem (50  $\mu\text{M}$ ) in the presence of  $\Delta^9$ -THC, CBD, CBN, olivetol, *d*-limonene, CBDM, or CBDD (0 to 50  $\mu\text{M}$ ) under the same manner as described in the enzyme assays. All compounds were dissolved in dimethylsulfoxide and added to the incubation mixture at a final dimethylsulfoxide concentration of 0.5%. The  $\text{IC}_{50}$  value was calculated by nonlinear least-squares regression analysis with Origin 7.5J software (OriginLab).

To characterize the enzyme kinetics for the inhibition of human CYP3A enzymes by CBD, the effects of three different concentrations of the cannabinoid on the formation of *N*-desmethyl-diltiazem were examined at five diltiazem concentrations. The apparent  $K_i$  value (inhibition constant) was determined from the *x*-intercept of a plot of apparent  $K_m/V_{\text{max}}$  (obtained from the slope of the Lineweaver–Burk plots) versus inhibitor concentration. The *x*-intercept, which is equal to  $-K_i$ , was calculated by linear regression using the Origin 7.5J software (OriginLab). Lineweaver–Burk plots of the enzyme kinetic data were generated to determine the mode of inhibition.

## Results

### Kinetic analysis for diltiazem *N*-demethylation by human CYP3A enzymes

To clarify enzymatic characteristics of recombinant CYP3A4, CYP3A5, CYP3A7, and HLMs used in this study toward diltiazem *N*-demethylase activity, kinetic analysis was conducted with these enzyme sources. All the reactions examined followed the Michaelis–



**Fig. 3.** Effects of major cannabinoids on diltiazem *N*-demethylase activity of human CYP3A isoforms and liver microsomes. Recombinants CYP3A4, CYP3A5, CYP3A7, and HLMs were incubated with diltiazem (50  $\mu\text{M}$ ) in the presence or absence of  $\Delta^9$ -THC (triangles), CBD (circles), and CBN (squares). Each point indicates a mean value of duplicate determinations.

**Table 2**

IC<sub>50</sub> values of major cannabinoids for inhibition of diltiazem *N*-demethylase activity of human CYP3A enzymes.

Enzymes	IC <sub>50</sub> (μM)		
	Δ <sup>9</sup> -THC	CBD	CBN
CYP3A4	>50	11.7	>50
CYP3A5	35.6	1.65	>50
CYP3A7	30.3	24.7	23.8
HLMs	>50	9.18	>50

Menten kinetics based on the Eadie–Hofstee plots (Fig. 2). Therefore, kinetic parameters were determined from the Michaelis–Menten equation. The *V*<sub>max</sub> values for CYP3A4, CYP3A5, and CYP3A7 were 13.6, 29.3, and 1.27 nmol/min/nmol P450, respectively, whereas the *V*<sub>max</sub> value for HLMs was 0.852 nmol/min/mg protein (Table 1). The *K*<sub>m</sub> value of diltiazem for CYP3A4 was 12.5 μM although those for the other enzyme sources were approximately 50 μM (Table 1).

#### Effects of major cannabinoids on diltiazem *N*-demethylation by human CYP3A enzymes

Effects of Δ<sup>9</sup>-THC, CBD, and CBN on diltiazem *N*-demethylase activity of recombinant CYP3A isoforms and HLMs were investigated at a substrate concentration of 50 μM. All the cannabinoids tested inhibited the activity of recombinant CYP3A4, CYP3A5, and CYP3A7 in a concentration-dependent manner (Fig. 3). CYP3A4 and CYP3A5 were most potently inhibited by CBD with the IC<sub>50</sub> values of 11.7 and 1.65 μM, respectively, among the cannabinoids tested (Table 2). The IC<sub>50</sub> values of Δ<sup>9</sup>-THC and CBN for CYP3A4 and CYP3A5 were higher than 35 μM. CYP3A7 was inhibited by Δ<sup>9</sup>-THC, CBD, and CBN to a similar extent (Fig. 3). The IC<sub>50</sub> values of these three cannabinoids were between 23 and 31 μM (Table 2). When HLMs were used as an enzyme source, the inhibitory effects of the cannabinoids were similar to those on recombinants CYP3A4 and CYP3A5 (Fig. 3).

**Table 3**

Kinetic parameters for inhibition of diltiazem *N*-demethylase activity of human CYP3A enzymes by CBD.

Enzymes	<i>K</i> <sub>i</sub> (μM)	Mode of inhibition
CYP3A4	1.00	Competitive
CYP3A5	0.195	Competitive
CYP3A7	12.3	Mixed
HLMs	6.14	Competitive

Data are derived from two replicates.

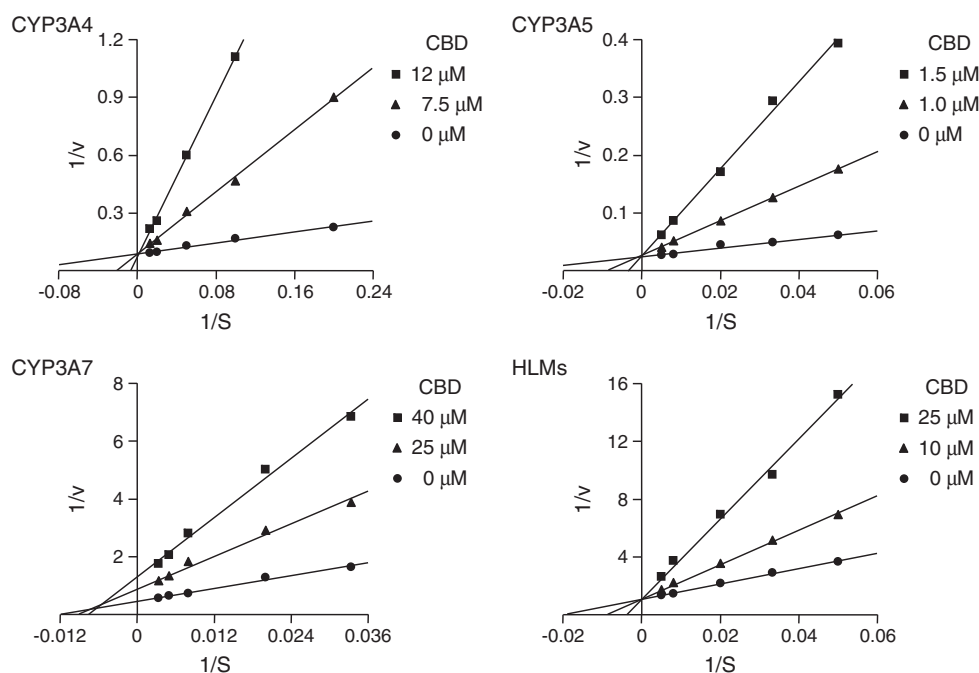
#### Kinetic analysis for cannabidiol-mediated inhibition of human CYP3A enzymes

To determine the mode of inhibition of human CYP3A enzymes by CBD, kinetic analysis for the inhibition was examined with recombinant CYP3A isoforms and HLMs. The enzyme activities of CYP3A4, CYP3A5, and HLMs were competitively inhibited by CBD, whereas the CYP3A7 activity was inhibited by the cannabinoid in a mixed fashion (Fig. 4). The lowest apparent *K*<sub>i</sub> value of the cannabinoid was seen in the inhibition of CYP3A5, followed by that of CYP3A4 (Table 3). The *K*<sub>i</sub> value for CYP3A7 was 12- and 63-fold higher than those for CYP3A4 and CYP3A5, respectively.

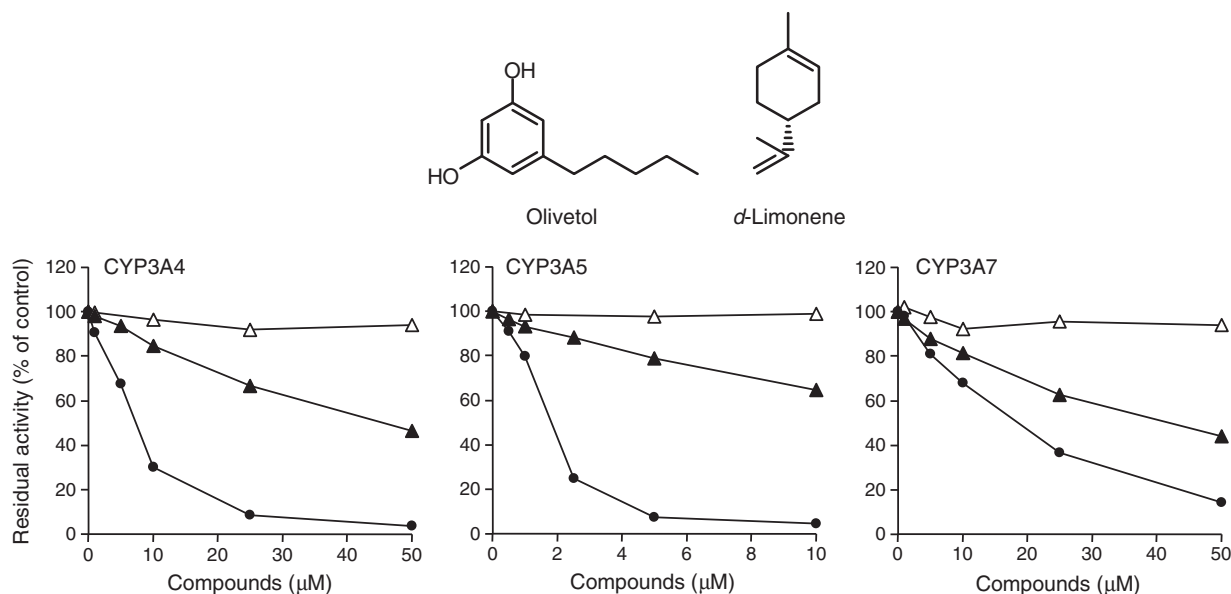
#### Structural requirement for inhibitory effects of CBD and its derivatives on CYP3A-mediated activity

CBD consists of the resorcinol and terpene moieties, as shown in Fig. 1. To determine which moiety is important in the inhibition of human CYP3A enzymes, inhibitory effects of olivetol and *d*-limonene were examined. Olivetol, which corresponds to the resorcinol moiety of CBD, inhibited the activity of CYP3A4, CYP3A5, and CYP3A7, although its inhibitory effect was less potent than that of CBD (Fig. 5). On the other hand, *d*-limonene, which corresponds to the terpene moiety of CBD, did not exert appreciable inhibition of these three CYP3A isoforms under the present conditions (Fig. 5).

Next, to elucidate whether phenolic hydroxyl group(s) of CBD contributes to the inhibition of human CYP3A enzymes, inhibitory



**Fig. 4.** Lineweaver–Burk plots for inhibition of human CYP3A isoforms and liver microsomes by CBD. Recombinant CYP3A4, CYP3A5, CYP3A7, and HLMs were incubated with diltiazem in the presence of various amounts of CBD. *S* and *v* indicate diltiazem (μM) and catalytic activity (nmol/min/nmol P450 or mg protein), respectively. Each point indicates a mean value of duplicate determinations.



**Fig. 5.** Effects of olivetol and *d*-limonene on diltiazem *N*-demethylase activity of human CYP3A isoforms. Recombinant CYP3A4, CYP3A5, and CYP3A7 were incubated with diltiazem (50 μM) in the presence or absence of CBD (closed circles), olivetol (closed triangles), and *d*-limonene (open triangles). Each point indicates a mean value of duplicate determinations.

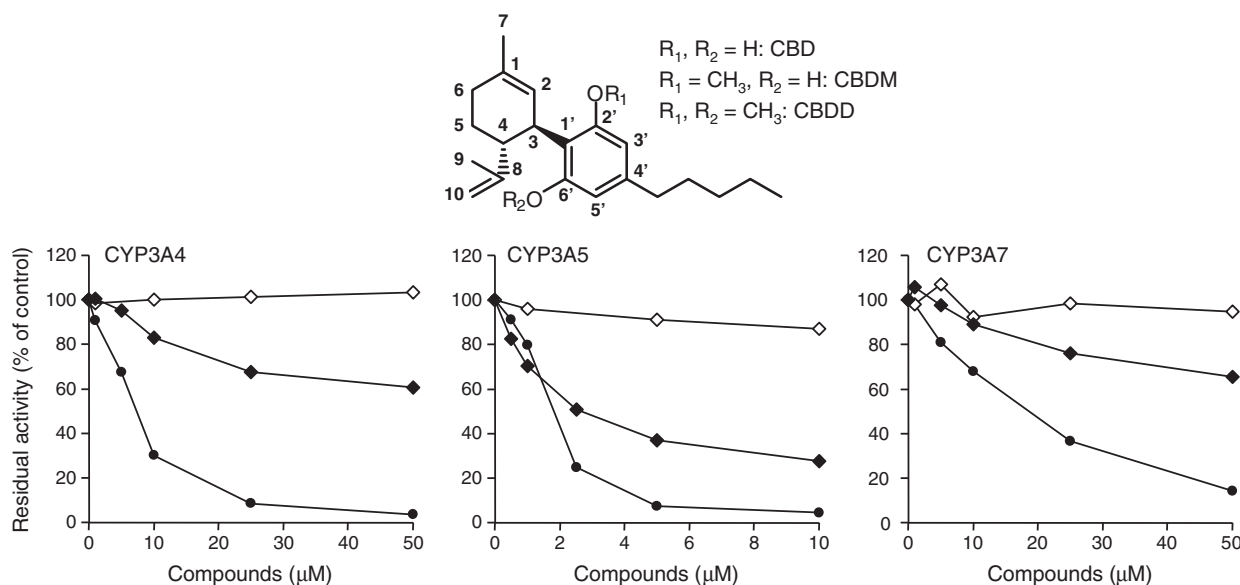
effects of two methylated derivatives of CBD, CBDM and CBDD, were investigated. The inhibitory effect of CBDM on the activity of CYP3A4, CYP3A5, and CYP3A7 was smaller than that of CBD (Fig. 6). Furthermore, CBDD exhibited no or less inhibitory effect on these CYP3A activities (Fig. 6).

## Discussion

In this study, we demonstrated that  $\Delta^9$ -THC, CBD, and CBN inhibited human CYP3A activity. Among these major cannabinoids, CBD most potently inhibited the activity of human CYP3A enzymes, especially CYP3A4 and CYP3A5. Recently, we have reported that the CBD-mediated inhibition of 7-ethoxyresorufin *O*-deethylation by recombinant human CYP1A1 showed the lowest  $K_i$  value (0.155 μM) among the values of CBD for microsomal CYPs reported so far

(Yamaori et al., 2010). This study indicates that the inhibition of CYP3A5 by CBD is comparable to that of CYP1A1. In addition, CBD caused a preferential inhibition against CYP3A5 among the human CYP3A isoforms examined. Many CYP3A inhibitors preferentially inhibit CYP3A4 activity (McConn et al., 2004; Granfors et al., 2006; Liu et al., 2007), although the selectivity of ritonavir, nelfinavir, and saquinavir to the inhibition of CYP3A4 and CYP3A5 is equivalent (Granfors et al., 2006). Therefore, CBD may be a useful prototype as a CYP3A5-selective inhibitor. The  $K_i$  value of CBD for CYP3A5 is comparable to that of ketoconazole ( $K_i = 0.219$  μM) (McConn et al., 2004).

The result that the kinetics for diltiazem *N*-demethylation by recombinant CYP3A4, CYP3A5, and CYP3A7 followed the Michaelis–Menten equation indicates that there is a site of diltiazem binding within these CYP3A active sites. Since CBD competitively inhibited the





activity of CYP3A4 and CYP3A5, it is suggested that CBD may bind to the catalytic sites of these CYP3A isoforms. On the other hand, CBD is suggested to bind to the catalytic site and a site different from the position of diltiazem binding within the CYP3A7 active site, because the cannabinoid inhibited the CYP3A7 activity in a mixed manner.

Further investigations were conducted to characterize the structural requirement for the inhibitory effect of CBD on human CYP3A activity. The partial inhibition of CYP3A4, CYP3A5, and CYP3A7 by olivetol but not by *d*-limonene suggests that the resorcinol structure in CBD is essential for the inhibition of these CYP3A isoforms, although the whole structure of CBD is required for the overall inhibition of the CYP3A activity. Inhibition studies with CBDM and CBDD indicate that the degree of inhibitory effect of CBD on the CYP3A activity depends on the number of free phenolic hydroxyl groups in the resorcinol moiety of CBD. These results suggest that both phenolic hydroxyl groups of CBD play an important role in the inhibition of CYP3A4, CYP3A5, and CYP3A7.

In contrast to CYP3A4 and CYP3A5, inhibitory effects of  $\Delta^9$ -THC, CBD, and CBN on the CYP3A7 activity were almost equivalent.  $\Delta^9$ -THC and CBN are structurally constrained because one of the two hydroxyl groups in the resorcinol moiety is utilized for the formation of the pyran ring, whereas CBD has free rotatable structure between the resorcinol and terpene moieties. These findings suggest that CYP3A7 may recognize these three cannabinoids as nearly planar structures.

In general, marijuana is consumed by smoking. The average contents of  $\Delta^9$ -THC, CBD, and CBN in dried plant preparations of marijuana are 3.1, 0.3, and 0.3%, respectively although these contents vary widely (ElSohly et al., 2000). In marijuana resin, which is commonly referred to as hashish, the mean contents of  $\Delta^9$ -THC, CBD, and CBN are 5.2, 4.2, and 1.7%, respectively (ElSohly et al., 2000). It has been previously reported that smoking a single marijuana cigarette containing 34 mg  $\Delta^9$ -THC (the content of 3.55%) shows a plasma peak level of  $\Delta^9$ -THC at 162 ng/ml (0.516  $\mu$ M) (Huestis et al., 1992). A peak level of plasma concentration of CBD in human subjects has been reported to be 114 ng/ml (0.363  $\mu$ M) after smoking a placebo marijuana cigarette spiked with 20 mg of the cannabinoid (Ohlsson et al., 1984). The  $K_i$  value of CBD for CYP3A5 is lower than the plasma concentration after marijuana smoking, although the values for CYP3A4 and CYP3A7 are higher than the blood level. Since cannabinoids are readily distributed in various tissues due to a high lipophilicity (Leighly, 1973), tissue concentrations of CBD may be even higher than the blood concentration. Thus, it is suggested that the inhibition of human CYP3A isoforms by CBD might be caused during and/or after marijuana smoking in some cases.

A lot of recent studies provide the evidence that the hepatic expression of polymorphic CYP3A5 affects pharmacokinetics of several CYP3A substrates (Thervet et al., 2003; Wong et al., 2004; Anglicheau et al., 2005). Furthermore, CYP3A5 is a major isoform of CYP3A expressed in extrahepatic tissues and is thought to play an important role in the metabolism of endogenous and exogenous compounds in these tissues (Koch et al., 2002). Thus, the inhibition of CYP3A5 by CBD may not only cause its interactions with other drugs but may also disturb normal metabolic pathways of endogenous compounds.

CYP3A7 is involved in the metabolism of dehydroepiandrosterone 3-sulfate and all-*trans*-retinoic acid in fetal livers (Kitada et al., 1987; Chen et al., 2000). These endogenous compounds are essential for normal growth and development of the fetus (Schuetz et al., 1993; Means and Gudas, 1995). It has been previously reported that marijuana use during pregnancy affects fetal growth (Zuckerman et al., 1989; Hurd et al., 2005). In addition, it has been demonstrated that cannabinoids are detected in animal fetuses after maternal exposure to cannabinoids (Harbison and Mantilla-Plata, 1972; Bailey et al., 1987), although such an investigation has not been conducted with humans. Taken together with these findings, it is possible that cannabinoids transferred to the fetus by marijuana use in pregnancy

might influence the activity of CYP3A7. Further studies are necessary to clarify the effect of cannabinoid-mediated CYP3A7 inhibition on fetal growth.

## Conclusions

We demonstrated that CBD is a potent inhibitor against human CYP3A enzymes. This study indicates that two hydroxyl groups in the resorcinol moiety of CBD play an important role in the CYP3A inhibition. Our study will provide useful information to understand precise mechanism(s) underlying the CBD-mediated CYP3A inhibition.

## Conflict of interest statement

The authors have declared that no conflict of interest exists.

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## References

- Anglicheau D, Le Corre D, Lechaton S, Laurent-Puig P, Kreis H, Beaune P, et al. Consequences of genetic polymorphisms for sirolimus requirements after renal transplant in patients on primary sirolimus therapy. *Am J Transplant* 2005;5:595–603.
- Aoyama T, Yamano S, Waxman DJ, Lapenson DP, Meyer UA, Fischer V, et al. Cytochrome P-450 hPCN3, a novel cytochrome P-450 IIIA gene product that is differentially expressed in adult human liver. cDNA and deduced amino acid sequence and distinct specificities of cDNA-expressed hPCN1 and hPCN3 for the metabolism of steroid hormones and cyclosporine. *J Biol Chem* 1989;264:10388–95.
- Aramaki H, Tomiyasu N, Yoshimura H, Tsukamoto H. Forensic chemical study on marihuana. I. A detection method of the principal constituents by thin-layer and gas chromatographies. *Chem Pharm Bull (Tokyo)* 1968;16:822–6.
- Bailey JR, Cunneen HC, Paule MG, Slikker Jr W. Fetal disposition of  $\Delta^9$ -tetrahydrocannabinol (THC) during late pregnancy in the rhesus monkey. *Toxicol Appl Pharmacol* 1987;90:315–21.
- Benowitz NL, Nguyen TL, Jones RT, Herning RI, Bachman J. Metabolic and psychophysiologic studies of cannabidiol–hexobarbital interaction. *Clin Pharmacol Ther* 1980;28:115–20.
- Bornheim LM, Correia MA. Purification and characterization of the major hepatic cannabinoid hydroxylase in the mouse: a possible member of the cytochrome P-450IIC subfamily. *Mol Pharmacol* 1991;40:228–34.
- Bornheim LM, Lasker JM, Raucy JL. Human hepatic microsomal metabolism of  $\Delta^1$ -tetrahydrocannabinol. *Drug Metab Dispos* 1992;20:241–6.
- Chan MY, Tse A. The effect of cannabinoids ( $\Delta^9$ -THC and  $\Delta^8$ -THC) on hepatic microsomal metabolism of testosterone in vitro. *Biochem Pharmacol* 1978;27:1725–8.
- Chen H, Fantel AG, Juchau MR. Catalysis of the 4-hydroxylation of retinoic acids by CYP3A7 in human fetal hepatic tissues. *Drug Metab Dispos* 2000;28:1051–7.
- ElSohly MA, Slade D. Chemical constituents of marijuana: the complex mixture of natural cannabinoids. *Life Sci* 2005;78:539–48.
- ElSohly MA, Ross SA, Mehmedic Z, Ararat R, Yi B, Banahan BF. Potency trends of  $\Delta^9$ -THC and other cannabinoids in confiscated marijuana from 1980–1997. *J Forensic Sci* 2000;45:24–30.
- Fernandes M, Warning N, Christ W, Hill R. Interactions of several cannabinoids with the hepatic drug metabolizing system. *Biochem Pharmacol* 1973;22:2981–7.
- Gohda H, Narimatsu S, Yamamoto I, Yoshimura H. In vivo and in vitro metabolism of cannabidiol monomethyl ether and cannabidiol dimethyl ether in the guinea pig: on the formation mechanism of cannabidiol-type metabolite from cannabidiol. *Chem Pharm Bull (Tokyo)* 1990;38:1697–701.
- Gorski JC, Hall SD, Jones DR, VandenBranden M, Wrighton SA. Regioselective biotransformation of midazolam by members of the human cytochrome P450 3A (CYP3A) subfamily. *Biochem Pharmacol* 1994;47:1643–53.
- Granfors MT, Wang JS, Kajosaari LI, Laitila J, Neuvonen PJ, Backman JT. Differential inhibition of cytochrome P450 3A4, 3A5 and 3A7 by five human immunodeficiency virus (HIV) protease inhibitors in vitro. *Basic Clin Pharmacol Toxicol* 2006;98:79–85.
- Guengerich FP, Martin MV, Beaune PH, Kremers P, Wolff T, Waxman DJ. Characterization of rat and human liver microsomal cytochrome P-450 forms involved in

- nifedipine oxidation, a prototype for genetic polymorphism in oxidative drug metabolism. *J Biol Chem* 1986;261:5051–60.
- Hamajima K, Watanabe K, Narimatsu S, Tateoka Y, Yamamoto I, Yoshimura H. Sex difference in the effects of  $\Delta^9$ -tetrahydrocannabinol and cannabidiol on pentobarbital-induced sleeping time and hepatic microsomal drug metabolizing enzyme systems in mice. *Yakugaku Zasshi* 1983;103:1289–97.
- Harbison RD, Mantilla-Plata B. Prenatal toxicity, maternal distribution and placental transfer of tetrahydrocannabinol. *J Pharmacol Exp Ther* 1972;180:446–53.
- Huestis MA. Pharmacokinetics and metabolism of the plant cannabinoids,  $\Delta^9$ -tetrahydrocannabinol, cannabidiol and cannabinol. *Handb Exp Pharmacol* 2005;168:657–90.
- Huestis MA, Henningfield JE, Cone EJ. Blood cannabinoids. I. absorption of THC and formation of 11-OH-THC and THCCOOH during and after smoking marijuana. *J Anal Toxicol* 1992;16:276–82.
- Hurd YL, Wang X, Anderson V, Beck O, Minkoff H, Dow-Edwards D. Marijuana impairs growth in mid-gestation fetuses. *Neurotoxicol Teratol* 2005;27:221–9.
- Jaeger W, Benet LZ, Bornheim LM. Inhibition of cyclosporine and tetrahydrocannabinol metabolism by cannabidiol in mouse and human microsomes. *Xenobiotica* 1996;26:275–84.
- Kitada M, Kamataki T, Itahashi K, Rikihisa T, Kato R, Kanakubo Y. Purification and properties of cytochrome P-450 from homogenates of human fetal livers. *Arch Biochem Biophys* 1985;241:275–80.
- Kitada M, Kamataki T, Itahashi K, Rikihisa T, Kanakubo Y. P-450 HFLa, a form of cytochrome P-450 purified from human fetal livers, is the 16 $\alpha$ -hydroxylase of dehydroepiandrosterone 3-sulfate. *J Biol Chem* 1987;262:13534–7.
- Koch I, Weil R, Wolbold R, Brockmüller J, Hustert E, Burk O, et al. Interindividual variability and tissue-specificity in the expression of cytochrome P450 3A mRNA. *Drug Metab Dispos* 2002;30:1108–14.
- Komori M, Nishio K, Kitada M, Shiramatsu K, Muroya K, Soma M, et al. Fetus-specific expression of a form of cytochrome P-450 in human livers. *Biochemistry* 1990;29:4430–3.
- Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* 2001;27:383–91.
- Leighy EG. Metabolism and distribution of cannabinoids in rats after different methods of administration. *Biochem Pharmacol* 1973;22:1613–21.
- Liu YT, Hao HP, Liu CX, Wang GJ, Xie HG. Drugs as CYP3A probes, inducers, and inhibitors. *Drug Metab Rev* 2007;39:699–721.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265–75.
- McConn II DJ, Lin YS, Allen K, Kunze KL, Thummel KE. Differences in the inhibition of cytochromes P450 3A4 and 3A5 by metabolite-inhibitor complex-forming drugs. *Drug Metab Dispos* 2004;32:1083–91.
- Means AL, Gudas LJ. The roles of retinoids in vertebrate development. *Annu Rev Biochem* 1995;64:201–33.
- Mechoulam R, Parker LA, Gallily R. Cannabidiol: an overview of some pharmacological aspects. *J Clin Pharmacol* 2002;42:11S–9S.
- Molowa DT, Schuetz EG, Wrighton SA, Watkins PB, Kremers P, Mendez-Picon G, et al. Complete cDNA sequence of a cytochrome P-450 inducible by glucocorticoids in human liver. *Proc Natl Acad Sci USA* 1986;83:5311–5.
- Ohlsson A, Lindgren JE, Andersson S, Agurell S, Gillespie H, Hollister LE. Single dose kinetics of cannabidiol in man. In: Agurell S, Dewey WL, Willette R, editors. The cannabinoids: chemical, pharmacologic, and therapeutic aspects. New York: Academic Press; 1984. p. 219–25.
- Pertwee RG. The diverse CB<sub>1</sub> and CB<sub>2</sub> receptor pharmacology of three plant cannabinoids:  $\Delta^9$ -tetrahydrocannabinol, cannabidiol and  $\Delta^9$ -tetrahydrocannabinol. *Br J Pharmacol* 2008;153:199–215.
- Rendic S. Summary of information on human CYP enzymes: human P450 metabolism data. *Drug Metab Rev* 2002;34:83–448.
- Schuetz JD, Kauma S, Guzelian PS. Identification of the fetal liver cytochrome CYP3A7 in human endometrium and placenta. *J Clin Invest* 1993;92:1018–24.
- Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* 1994;270:414–23.
- Thervet E, Anglicheau D, King B, Schlageter MH, Cassinat B, Beaune P, et al. Impact of cytochrome P450 3A5 genetic polymorphism on tacrolimus doses and concentration-to-dose ratio in renal transplant recipients. *Transplantation* 2003;76:1233–5.
- Watanabe K, Yamamoto I, Oguri K, Yoshimura H. In vitro and in vivo interactions of  $\Delta^8$ -tetrahydrocannabinol and its metabolites with hepatic microsomal drug metabolizing enzyme systems of mice. *J Pharmacobiodyn* 1981;4:604–11.
- Watanabe K, Matsunaga T, Yamamoto I, Funae Y, Yoshimura H. Involvement of CYP2C in the metabolism of cannabinoids by human hepatic microsomes from an old woman. *Biol Pharm Bull* 1995;18:1138–41.
- Watanabe K, Yamaori S, Funahashi T, Kimura T, Yamamoto I. Cytochrome P450 enzymes involved in the metabolism of tetrahydrocannabinols and cannabinol by human hepatic microsomes. *Life Sci* 2007;80:1415–9.
- Williams JA, Ring BJ, Cantrell VE, Jones DR, Eckstein J, Ruterbories K, et al. Comparative metabolic capabilities of CYP3A4, CYP3A5, and CYP3A7. *Drug Metab Dispos* 2002;30:883–91.
- Wong M, Balleine RL, Collins M, Liddle C, Clarke CL, Gurney H. CYP3A5 genotype and midazolam clearance in Australian patients receiving chemotherapy. *Clin Pharmacol Ther* 2004;75:529–38.
- Wrighton SA, Ring BJ, Watkins PB, VandenBranden M. Identification of a polymorphically expressed member of the human cytochrome P-450III family. *Mol Pharmacol* 1989;36:97–105.
- Yamaori S, Yamazaki H, Suzuki A, Yamada A, Tani H, Kamidate T, et al. Effects of cytochrome b<sub>5</sub> on drug oxidation activities of human cytochrome P450 (CYP) 3A5: similarity of CYP3A5 with CYP3A4 but not CYP3A7. *Biochem Pharmacol* 2003;66:2333–40.
- Yamaori S, Yamazaki H, Iwano S, Kiyotani K, Matsumura K, Honda G, et al. CYP3A5 contributes significantly to CYP3A-mediated drug oxidations in liver microsomes from Japanese subjects. *Drug Metab Pharmacokinet* 2004;19:120–9.
- Yamaori S, Kushihara M, Yamamoto I, Watanabe K. Characterization of major phytocannabinoids, cannabidiol and cannabinol, as isoform-selective and potent inhibitors of human CYP1 enzymes. *Biochem Pharmacol* 2010;79:1691–8.
- Zuckerman B, Frank DA, Hingson R, Amaro H, Levenson SM, Kayne H, et al. Effects of maternal marijuana and cocaine use on fetal growth. *N Engl J Med* 1989;320:762–8.