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Cannabidiol Is a Potent Inhibitor of the Catalytic Activity of Cytochrome P450 2C19

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Summary: The present study investigated the inhibitory effect of cannabidiol (CBD), a major constituent of marijuana, on the catalytic activity of cytochrome P450 2C19 (CYP2C19). (*S*)-Mephenytoin 4′-hydroxylase activities of human liver microsomes (HLMs) and recombinant CYP2C19 were inhibited by CBD in a concentration-dependent manner (IC_{50} = 8.70 and 2.51 μM, respectively). Omeprazole 5-hydroxylase and 3-*O*-methylfluorescein *O*-demethylase activities in recombinant CYP2C19 were also strongly inhibited by CBD (IC_{50} = 1.55 and 1.79 μM, respectively). Kinetic analysis for inhibition revealed that CBD showed a mixed-type inhibition against (*S*)-mephenytoin 4′-hydroxylation by recombinant CYP2C19. To clarify the structural requirements for CBD-mediated CYP2C19 inhibition, the effects of CBD-related compounds on CYP2C19 activity were examined. Olivetol inhibited the (*S*)-mephenytoin 4′-hydroxylase activity of recombinant CYP2C19 with the IC_{50} value of 15.3 μM, whereas *d*-limonene slightly inhibited the activity (IC_{50} > 50 μM). The inhibitory effect of CBD-2′-monomethyl ether (IC_{50} = 1.88 μM) on CYP2C19 was comparable to that of CBD, although the inhibitory potency of CBD-2′,6′-dimethyl ether (IC_{50} = 14.8 μM) was lower than that of CBD. Cannabidivarin, possessing a propyl side chain, showed slightly less potent inhibition (IC_{50} = 3.45 μM) as compared with CBD, whereas orcinol and resorcinol did not inhibit CYP2C19 activity at all. These results indicate that CBD caused potent CYP2C19 inhibition, in which one free phenolic hydroxyl group and the pentyl side chain of CBD may play important roles.

Keywords: cannabidiol; CYP2C19; inhibition; structural requirement

Introduction

Cannabidiol (CBD), one of the major constituents of marijuana, is not psychoactive in contrast to Δ^9-tetrahydrocannabinol (Δ^9-THC), and has several pharmacological effects such as drug-induced prolongation of sleep, antiepileptic, anxiolytic, and antiemetic actions.[1)] Some of these effects may be of therapeutic importance. Recent reports have also shown that CBD has great therapeutic potential in the treatment of diabetic complications[2)] and liver fibrosis.[3)] Sativex®, a medicine from marijuana extracts containing CBD, has been clinically used for the treatment of neuropathic pain and spasticity in multiple sclerosis.[4–7)]

CBD is usually consumed by smoking or by sublingual or oral ingestion. After its absorption, CBD is mainly eliminated from the body by a metabolic process that occurs primarily in the liver.[8)] Among the metabolic pathways of CBD in humans, the major one is considered to be oxidation on a carbon atom at the C-7 position followed by further hydroxylation in the pentyl side chain and terpene moiety.[9)] This finding has been partly supported by our recent *in vitro* work indicating that CBD is primarily oxidized at the 7-position as well as at the 6- and 4″-positions by human liver microsomes (HLMs).[10)] Furthermore, we demonstrated that CBD 7-hydroxylation is predominantly catalyzed by cytochrome P450 2C19 (CYP2C19).[10)]

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CYP2C19 is known to be a highly polymorphic enzyme in the liver, and is involved in the metabolism of various clinically important drugs such as (*S*)-mephenytoin,[11] omeprazole,[12] lansoprazole,[13] phenytoin,[14] diazepam,[15] and escitalopram.[16] It has been also reported that an antiplatelet agent clopidogrel is metabolized to a pharmacologically active metabolite in part by CYP2C19.[17] A loss of function of polymorphic CYP2C19 is associated with a marked decrease in platelet responsiveness to clopidogrel.[18,19] The U. S. Food and Drug Administration warned that CYP2C19 inhibitors like omeprazole cannot be used concomitantly with clopidogrel.

CBD is known to inhibit CYP-mediated drug metabolism. There is a report that the metabolism of Δ^9-THC is affected by CBD.[20] A previous clinical study has shown that CBD decreases the systemic clearance of hexobarbital in human subjects.[21] Jaeger *et al.*[22] have reported that CBD inhibited cyclosporine and Δ^9-THC oxidations catalyzed by hepatic microsomal CYP3A enzymes. More recently, we demonstrated that CBD is a potent inhibitor of human CYP1A1, CYP2B6, CYP2C9, CYP2D6, CYP3A4, and CYP3A5.[23–27] However, the inhibitory effect of CBD on CYP2C19 is not clear at present.

In the present study, we investigated the inhibitory effect of CBD on CYP2C19 activity and the structural requirement for this inhibition. We report herein that CBD is a potent inhibitor against human CYP2C19. Our study suggests that one free phenolic hydroxyl group and the pentyl side chain in the resorcinol moiety of CBD may have structurally important roles in the CYP2C19 inhibition.

Materials and Methods

Materials: CBD and Δ^9-THC were isolated and purified from cannabis leaves using a method previously reported.[28] CBD-2′-monomethyl ether (CBDM), CBD-2′,6′-dimethyl ether (CBDD), and 8,9-dihydro-CBD (2H-CBD) were prepared by the previous methods.[29,30] The purities of these cannabinoids were determined to be greater than 97% by gas chromatography, except for 2H-CBD, of which the purity was 93%.[30] Cannabidivarin (CBDV) was generously provided by Dr. Yukihiro Shoyama at Nagasaki International University (Sasebo, Japan). Microsomes from baculovirus-infected insect cells expressing CYP2C19 with NADPH-P450 reductase (Supersomes™), and 4′-hydroxymephenytoin were purchased from BD Gentest (Woburn, MA). Pooled HLMs (CPH-08-069/Lot No. 09004), mixed gender with 15 pool (8 male and 7 female), were purchased from XenoTech, LLC (Lenexa, KS). (*S*)-Mephenytoin was obtained from BIOMOL (Plymouth Meeting, PA). 3-*O*-Methylfluorescein (OMF) and (−)-*N*-3-benzyl-phenobarbital (*N*-3-BPB) were purchased from Cypex (Dundee, UK). 5-Hydroxyomeprazole was purchased from SPI-Bio, Bertin Pharma (Montigny Le Bretoneux, France). Olivetol, *d*-limonene, and fluorescein sodium salt were purchased from Sigma-Aldrich (St. Louis, MO). Orcinol was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Resorcinol and omeprazole were obtained from Wako Pure Chemicals (Osaka, Japan). 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 assays: (*S*)-Mephenytoin 4′-hydroxylase and omeprazole 5-hydroxylase activities were measured as reported previously[31] with minor modifications. Briefly, an incubation mixture consisted of HLMs (50 μg protein) or recombinant CYP2C19 (2 pmol), (*S*)-mephenytoin or omeprazole, 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), and 100 mM potassium phosphate buffer (pH 7.4) in a final volume of 250 μl. Following preincubation at 37°C for 5 min, reactions were initiated by the addition of the NADPH-generating system. Incubations for (*S*)-mephenytoin and omeprazole were carried out at 37°C for 40 and 20 min, respectively. Reactions were stopped by adding 100 μl of ice-cold acetonitrile. After centrifugation, the supernatant was subjected to a high-performance liquid chromatography (HPLC) (D-7500 integrator, L-7100 pump, L-2200 autosampler, and L-7420 UV detector, Hitachi, Tokyo, Japan) equipped with a Mightysil RP-18 GP column (4.6 mm × 250 mm, 5 μm, Kanto Chemical, Tokyo, Japan). The mobile phase, which was delivered at a flow rate of 0.8 ml/min, consisted of acetonitrile: 50 mM sodium phosphate buffer (pH 4.0) with phosphoric acid (26:74, v/v) for (*S*)-mephenytoin, or acetonitrile: 50 mM sodium phosphate buffer (pH 8.5) (25:75, v/v) for omeprazole. The formation of 4′-hydroxymephenytoin and 5-hydroxyomeprazole was monitored at wavelengths of 204 and 302 nm, respectively.

The OMF *O*-demethylase activity, which has been used as a fluorescent marker of CYP2C19,[32] was determined by using a 96-well microtiter plate as described below. An incubation mixture consisted of recombinant CYP2C19 (8 pmol), OMF, 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), and 100 mM potassium phosphate buffer (pH 7.4) in a final volume of 200 μl. After preincubation at 37°C for 10 min, reactions were initiated by the addition of the NADPH-generating system. Fluorescence derived from fluorescein formation was recorded every 3 min for 30 min using FLUOstar OPTIMA® (BMG Labtech, Offenburg, Germany) with excitation and emission filters at 480 and 555 nm, respectively.

To determine the kinetic parameters for (*S*)-mephenytoin 4′-hydroxylation, omeprazole 5-hydroxylation, and OMF *O*-demethylation by HLMs and/or recombinant CYP2C19, (*S*)-mephenytoin (16 to 1,000 μM), omeprazole (0.25 to 32 μM), or OMF (1 to 10 μM) was incubated with each enzyme source under the same conditions as mentioned above. In preliminary experiments, these reaction conditions were confirmed to ensure linear initial rates for the formation of 4′-hydroxymephenytoin, 5-hydroxyomeprazole, and fluorescein. 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: HLMs and recombinant CYP2C19 were incubated with (*S*)-mephenytoin (60 μM), omeprazole (2 μM), or OMF (4 μM) in the presence of test compounds including cannabinoids (up to 50 μM) in the same manner as described for the enzyme assays. All compounds were dissolved in dimethylsulfoxide and added to the incubation mixture at a final dimethylsulfoxide concentration of ≤0.8%. The IC_{50} values were calculated by nonlinear least-squares regression analysis with Origin 7.5J software (OriginLab).

To characterize the enzyme kinetics for the inhibition of CYP2C19 by CBD and its structurally related compounds, the effects of three or four different inhibitor concentrations on (*S*)-mephenytoin 4′-hydroxylation were examined at five substrate concentrations. The apparent K_i value (inhibition constant) was determined from the *x*-intercept of a plot of apparent K_m/V_{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 Origin 7.5J software (OriginLab). Lineweaver-Burk plots of enzyme kinetic data were generated to determine the mode of inhibition.

Estimation of *in vivo* inhibition potency: An estimate of *in vivo* inhibition potency was determined using previously described methods.[33] The maximum unbound hepatic input concentration, $C_{max,u,inlet}$, was determined using the following equation[34]:

$$C_{max,u,inlet} = f_u \cdot \left(C_{max} + \frac{K_a \cdot F_a \cdot D}{Q_h} \right)$$

C_{max} is defined as the maximum systemic concentration, D is the oral dose, K_a is the first-order absorption rate constant, F_a is the fraction of the oral dose absorbed, f_u is the fraction of the unbound compound in the blood, and Q_h is the hepatic blood flow. Values of 0.03 min^{-1},[33] 1.45 l/min, and unity[33] were used for K_a, Q_h, and F_a, respectively. C_{max} and the oral dose were obtained from the data of Fusar-Poli *et al.*[35] The f_u for cannabinoids is at most 0.05 because 95 to 99% of plasma cannabinoids is bound to plasma proteins, mainly lipoproteins.[36] $C_{max,u,inlet}$ had been tested as the best value for the concentration of inhibitor *in vivo* ($[I]_{in\ vivo}$).[33] With the $[I]_{in\ vivo}$ parameter determined, a ratio of AUC with inhibitor to control AUC could be estimated using the following equation:

$$\frac{CL_{control}}{CL_{inhibited}} = \frac{AUC_I}{AUC} = \frac{1}{\left(\frac{f_{m(CYP2C19)}}{1 + \frac{[I]_{in\ vivo}}{K_i}} \right) + (1 - f_{m(CYP2C19)})}$$

AUC_I is the area under the curve value for a given substrate probe in the presence of an inhibitor and AUC is the area under the curve for the same probe substrate without the inhibitor. The fraction of the metabolism of the substrate by a given P450 is represented by $f_{m(CYP)}$ and the magnitude of the potency of the inhibitor by K_i. The value of 0.95 was used for the $f_{m(CYP2C19)}$ value of (*S*)-mephenytoin.[33]

Results

Inhibition of CYP2C19 activity by CBD: To clarify enzymatic characteristics of HLMs and recombinant CYP2C19 used in this study toward (*S*)-mephenytoin 4′-hydroxylase, omeprazole 5-hydroxylase, and OMF *O*-demethylase activities, kinetic analyses were conducted with these enzyme sources. All of the reactions tested followed the Michaelis-Menten kinetics based on the Eadie-Hofstee plots (data not shown). The apparent K_m values for (*S*)-mephenytoin 4′-hydroxylation by HLMs and recombinant CYP2C19 were 70.2 and 50.8 μM, respectively (**Table 1**). The K_m values for omeprazole 5-hydroxylation and OMF *O*-demethylation by recombinant CYP2C19 were 1.26 and 2.32 μM, respectively (**Table 1**).

Thus, the effect of CBD on CYP2C19 activity was investigated at substrate concentrations of 60 μM (*S*)-mephenytoin, 2 μM omeprazole, and 4 μM OMF. CBD inhibited (*S*)-mephenytoin 4′-hydroxylase activities of HLMs and recombinant CYP2C19 in a concentration-dependent manner (**Fig. 1A**), showing the IC_{50} values of 8.70 and 2.51 μM, respectively. CBD also efficiently inhibited omeprazole 5-hydroxylase and OMF *O*-demethylase activities of recombinant CYP2C19 with the IC_{50} values of 1.55 and 1.79 μM, respectively (**Figs. 1B** and **1C**).

In addition, the inhibitory effect of *N*-3-BPB, a CYP2C19-selective inhibitor,[37,38] on the catalytic activities of HLMs and recombinant CYP2C19 was investigated. The (*S*)-mephenytoin 4′-hydroxylase activities of HLMs and recombinant CYP2C19 were strongly inhibited by *N*-3-BPB with the IC_{50} values of 0.254 and 0.320 μM, respectively. The omeprazole 5-hydroxylase and OMF *O*-demethylase activities of recombinant CYP2C19 were inhibited by *N*-3-BPB with the IC_{50} values of 0.822 and 0.535 μM, respectively. The inhibitory effects of *N*-3-BPB on CYP2C19 activities in this study were comparable to the previous findings.[37,38]

Structural requirements for inhibition of CYP2C19 activity by CBD: The inhibitory effects of CBD-related compounds on CYP2C19 activity were examined to elucidate the structural requirements for CYP2C19 inhibition by CBD. In these inhibition studies, recombinant CYP2C19 was used as an enzyme source. CBD consists of terpene and pentylresorcinol moieties, as shown in **Fig. 2A**. To determine which moiety of CBD is important in CYP2C19 inhibition, inhibition studies were conducted with *d*-limonene and olivetol, which correspond to the terpene and pentylresorcinol moieties of CBD, respectively. *d*-Limonene exhibited

Table 1. Kinetic parameters for CYP2C19-mediated oxidations by HLMs and recombinant enzymes

Enzymes	Substrates	K_m (μM)	V_{max}
			(pmol/min/mg protein)
HLMs	(*S*)-Mephenytoin	70.2	57.5
			(nmol/min/nmol P450)
CYP2C19	(*S*)-Mephenytoin	50.8	2.76
	Omeprazole	1.26	3.72
	OMF	2.32	0.477

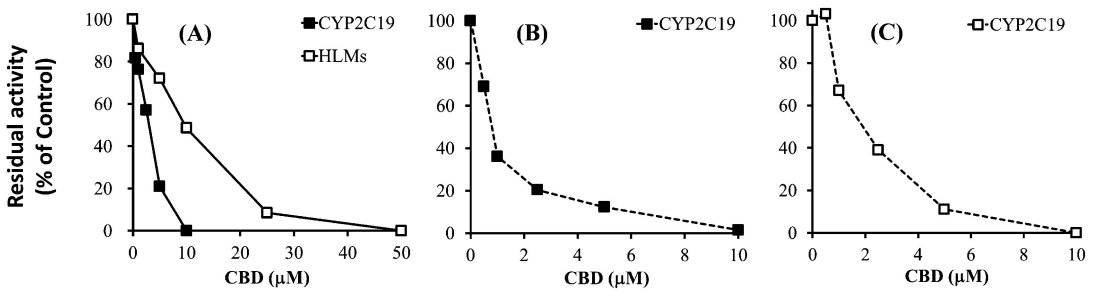

Fig. 1. Effects of CBD on (*S*)-mephenytoin 4′-hydroxylation, omeprazole 5-hydroxylation, and OMF *O*-demethylation by CYP2C19
(A) HLMs (white square) and recombinant CYP2C19 (black square) were incubated with 60 μM (*S*)-mephenytoin in the presence of various amounts of CBD. (B) Recombinant CYP2C19 was incubated with 2 μM omeprazole (black square with broken line) in the presence of various amounts of CBD. (C) Recombinant CYP2C19 was incubated with 4 μM OMF (white square with broken line) in the presence of various amounts of CBD. Each point is the mean of two determinations.

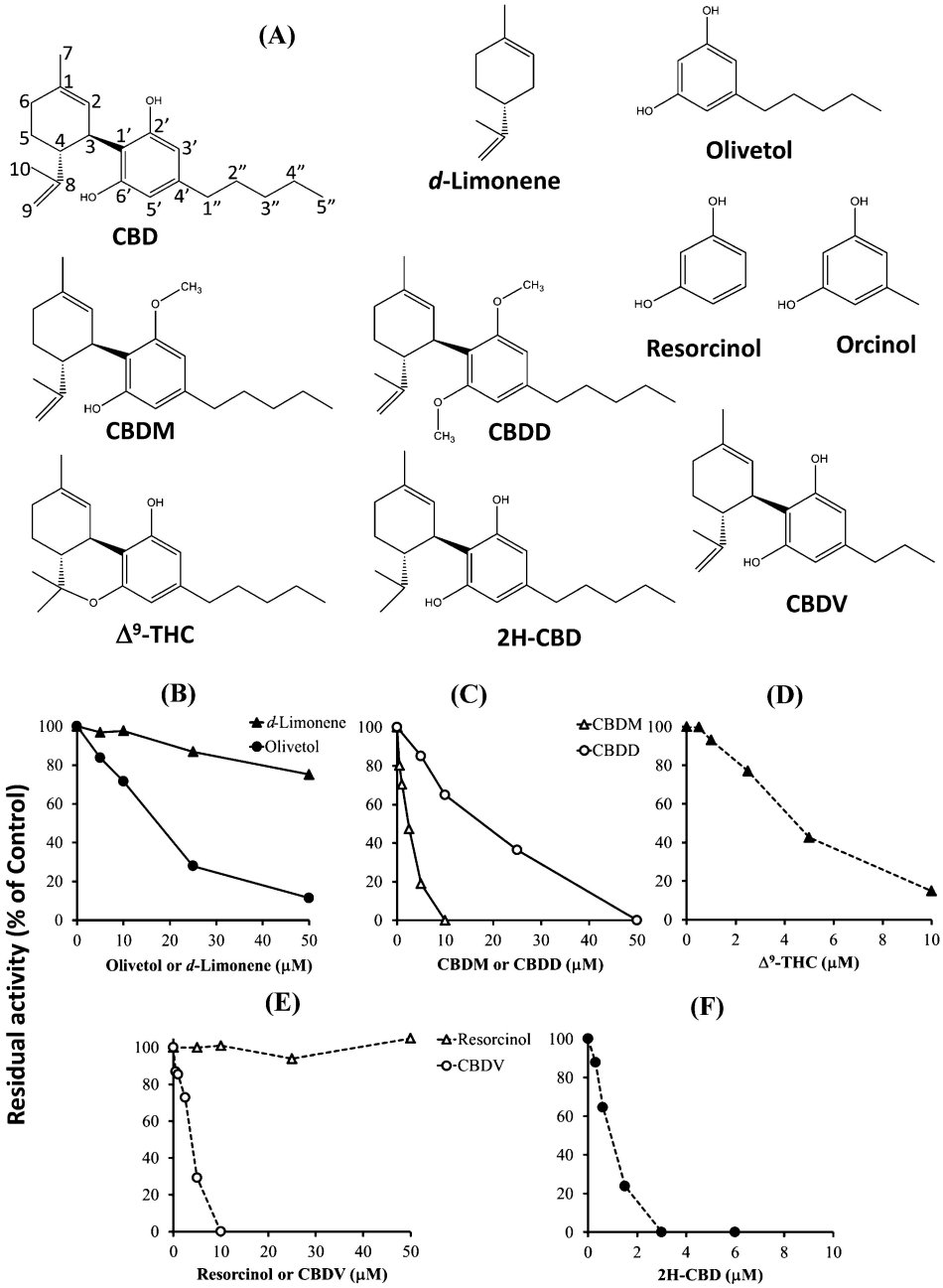

Fig. 2. Effects of CBD-related compounds on (*S*)-mephenytoin 4′-hydroxylase activity of CYP2C19
(A) Structures of CBD and its structurally related compounds. (B–F) Recombinant CYP2C19 was incubated with 60 μM (*S*)-mephenytoin in the presence of various amounts of CBD-related compounds; (B) *d*-limonene and olivetol, (C) CBDM and CBDD, (D) Δ^9-THC, (E) CBDV and resorcinol, and (F) 2H-CBD. Each point is the mean of two determinations.

slight inhibition against CYP2C19-mediated (*S*)-mephenytoin 4′-hydroxylation (**Fig. 2B**). In contrast, olivetol inhibited the (*S*)-mephenytoin 4′-hydroxylase activity in a concentration-dependent manner (**Fig. 2B**); its inhibitory potency (IC_{50} = 15.3 μM) was less potent than that of CBD. Similar profiles were also seen when omeprazole and OMF were used as substrates (**Table 2**).

To elucidate whether the two free phenolic hydroxyl groups of CBD have a role in inhibition of CYP2C19, the inhibitory effects of two methylated derivatives of CBD were examined. A monomethylated derivative of CBD, CBDM (**Fig. 2A**), showed strong inhibition against CYP2C19-mediated (*S*)-mephenytoin 4′-hydroxylation (**Fig. 2C**). The inhibitory effect of CBDM (IC_{50} = 1.88 μM) was comparable to that of CBD. A dimethylated derivative of CBD, CBDD (**Fig. 2A**), inhibited CYP2C19 activity in a concentration-dependent manner (**Fig. 2C**), although the inhibitory potency of CBDD (IC_{50} = 14.8 μM) was weaker than that of CBD. These results suggested that either of the two free phenolic hydroxyl groups in the resorcinol moiety of CBD may be required for potent CYP2C19 inhibition. CBDM contains a free phenolic hydroxyl group and is rotatable between terpene and resorcinol

Table 2. IC_{50} values of CBD-related compounds for inhibition of CYP2C19-mediated oxidations

Compounds	IC_{50} (μM)		
	(*S*)-Mephenytoin	Omeprazole	OMF
CBD	2.51	1.55	1.79
d-Limonene	>50	>50	>50
Olivetol	15.3	14.9	11.8
CBDM	1.88	2.64	2.43
CBDD	14.8	14.4	45.1
Δ^9-THC	4.35	2.20	4.64
CBDV	3.45	3.75	3.78
Resorcinol	>50	>50	>50
Orcinol	—	>50	>50
2H-CBD	0.821	0.740	1.08

moieties, while Δ^9-THC has a free phenolic hydroxyl group and is structurally constrained due to the presence of a dibenzopyran structure (**Fig. 2A**). Thus, to determine what configuration of CBD contributes to CYP2C19 inhibition, the inhibitory effect of Δ^9-THC was compared with that of CBDM. Δ^9-THC caused CYP2C19 inhibition (**Fig. 2D**); its inhibitory effect ($IC_{50} = 4.35$ μM) was less potent than that of CBDM. Similar inhibitory effects were observed when omeprazole and OMF were used as substrates, although the inhibition of OMF *O*-demethylation by CBDD was less effective as compared with that of the other CYP2C19-mediated oxidations (**Table 2**).

CBD, as well as olivetol, contains a pentyl side chain in its structure. To determine the importance of the pentyl group of CBD in CYP2C19 inhibition, inhibition experiments were carried out with CBDV, orcinol, and resorcinol. CBDV, having a shorter side chain (**Fig. 2A**), inhibited the (*S*)-mephenytoin 4′-hydroxylase, omeprazole 5-hydroxylase, and OMF *O*-demethylase activities; its inhibitory effect ($IC_{50} = 3.45$, 3.75, and 3.78 μM, respectively) was slightly less potent than that of CBD (**Fig. 2E** and **Table 2**). On the other hand, orcinol, possessing a methyl side chain and resorcinol without the alkyl side chain (**Fig. 2A**), did not inhibit these activities at all (**Fig. 2E** and **Table 2**), although the inhibitory effect of orcinol on (*S*)-mephenytoin 4′-hydroxylation could not be determined due to the partial overlapping of UV spectra of 4′-hydroxymephenytoin and orcinol on a HPLC chromatogram.

To determine the role of the 8,9-double bond of the terpene moiety in CBD for CYP2C19 inhibition, the inhibitory effect of 2H-CBD, a CBD analogue reduced on the 8,9-double bond (**Fig. 2A**), was investigated. 2H-CBD inhibited the (*S*)-mephenytoin 4′-hydroxylase, omeprazole 5-hydroxylase, and OMF *O*-demethylase activities of recombinant CYP2C19 more efficiently than CBD did (**Fig. 2F** and **Table 2**).

Kinetic analyses for inhibition of CYP2C19 activity by CBD and its structurally related compounds: Kinetic analysis for inhibition was conducted to characterize the mode of CYP2C19 inhibition by CBD and its structurally related compounds. CBD inhibited the (*S*)-mephenytoin 4′-hydroxylase activity of CYP2C19 in a mixed manner. The apparent K_i value of CBD was 0.793 μM (**Table 3**). Similarly, olivetol, CBDM, Δ^9-THC, CBDV, and 2H-CBD exhibited a mixed-type inhibition against CYP2C19 (**Table 3**). On the other hand, CBDD competitively inhibited the CYP2C19 activity (**Table 3**). The resulting K_i values of these compounds correlated well with the corresponding IC_{50} values ($r^2 = 0.862$, $p < 0.01$).

Table 3. Kinetic parameters for inhibition of (*S*)-mephenytoin 4′-hydroxylase activity by CBD and its structurally related compounds

Compounds	K_i (μM)	Mode of inhibition
CBD	0.793	Mixed
Olivetol	2.71	Mixed
CBDM	0.454	Mixed
CBDD	3.40	Competitive
Δ^9-THC	1.93	Mixed
CBDV	1.47	Mixed
2H-CBD	0.488	Mixed

Estimation of *in vivo* inhibitory potency of CBD on CYP2C19 catalyzed (*S*)-mephenytoin metabolism: The ratio of AUC_I/AUC calculated from the equation described in Materials and Methods is 3.12 *in vivo* metabolic interaction between CBD and (*S*)-mephenytoin.

Discussion

In the present study, we demonstrated that CBD efficiently inhibits human CYP2C19 activity. The K_i value of CBD for (*S*)-mephenytoin 4′-hydroxylase activity of recombinant CYP2C19 was comparable to those of lansoprazole and (*S*)-fluoxetine.[39,40] The K_i values of CBD for other human CYP enzymes previously reported are listed in **Table 4**. The inhibition of CYP2C19 by CBD is the fourth most potent among 11 CYP enzymes examined. These results indicate that CBD is a potent inhibitor for CYP2C19. The mode of CYP2C19 inhibition by CBD was a mixed type that was similar to those of CYPs 3A7,[24] 2B6[25] and 2C9.[27]

The results that (*S*)-mephenytoin 4′-hydroxylation, omeprazole 5-hydroxylation, and OMF *O*-demethylation by recombinant CYP2C19 followed the Michaelis-Menten kinetics reveal that there is a binding site of these substrates within the CYP2C19 active site. Since CBD inhibited the (*S*)-mephenytoin 4′-hydroxylase activity of CYP2C19 in a mixed manner, it is suggested that CBD may bind to the catalytic site and a site different from the position of (*S*)-mephenytoin binding within the CYP2C19 active site. It has been previously reported that (*R*)-fluoxetine, (*S*)-fluoxetine, and amitriptyline cause a mixed-type inhibition against CYP2C19.[40] However, a structural characteristic showing such a mode of inhibition against CYP2C19 does not appear among these inhibitors including CBD.

In the study of structural requirements for inhibition of CYP2C19 activity, *d*-limonene failed to efficiently inhibit CYP2C19 activity. It has been previously reported that *d*-limonene is mainly oxidized at the 6- and 7-positions by CYP2C19.[41] However, the binding affinity of *d*-limonene for CYP2C19 appears to be relatively low because the apparent K_m values for the CYP2C19-mediated *d*-limonene oxidations are approximately 300 μM.[41] Thus, slight inhibition of CYP2C19 by *d*-limonene is compatible with the previous findings. On the other hand, the inhibitory profile of olivetol suggests that the pentylresorcinol structure in CBD is critical for CYP2C19 inhibition, although the whole structure of CBD is required for the overall inhibition of CYP2C19 activity. The structure of the pentylresorcinol moiety contains two free phenolic hydroxyl groups and a pentyl side chain. Inhibition studies with CBDM and CBDD suggest that one of the phenolic hydroxyl groups in CBD may be required for the interaction with CYP2C19. This is in contrast to the previous findings that both free phenolic hydroxyl groups in CBD contribute to the inhibition of CYP1A1, CYP2B6, CYP2D6, CYP3A4, and

Table 4. Summary of K_i values for the *in vitro* inhibition of human CYP enzymes by CBD

CYPs	Substrates	K_i (μM)
1A1[23]	7-Ethoxyresorufin	0.155
3A5[24]	Diltiazem	0.195
2B6[25]	7-Benzoxyresorufin	0.694
2C19	(*S*)-Mephenytoin	0.793
2C9[27]	(*S*)-Warfarin	0.954
3A4[24]	Diltiazem	1.00
2D6[26]	Dextromethorphan	2.69
1A2[23]	7-Ethoxyresorufin	2.69
1B1[23]	7-Ethoxyresorufin	3.63
3A7[24]	Diltiazem	12.3
2A6[25]	Coumarin	55.0

CYP3A5.[24–27] In addition, the inhibitory profiles of Δ^9-THC and CBDM suggest that the bulky structure of CBD may be preferentially recognized by CYP2C19.

The substitution of the pentyl group of CBD to a propyl group showed only a small change in the inhibitory effect on CYP2C19 activity. On the other hand, the further shortening of the side chain in the pentylresorcinol moiety resulted in a loss of CYP2C19 inhibition. Therefore, these results suggest that the pentyl side chain of CBD is also important in potent inhibition of CYP2C19.

It has been previously reported that oral administration of 300 mg or 600 mg CBD exhibits anxiolytic effects on healthy control patients and/or treatment-naïve patients with social anxiety disorder.[35,42,43] To our knowledge, the highest oral dose of CBD in clinical use is 1,500 mg/day for the treatment of schizophrenia.[44] The blood level of CBD 2 h after 600 mg of the cannabinoid was orally administered has been reported to be 17 ng/ml (0.054 μM).[35] When the *in vivo* inhibition potency of CBD against CYP2C19 is determined from the blood concentration of CBD described above and the K_i value of CBD for the (*S*)-mephenytoin 4′-hydroxylase activity of recombinant CYP2C19 by the methods of Obach *et al.*,[33] the ratio of AUC_l/AUC is estimated to be 3.12. The AUC_l/AUC values of CBD for (*S*)-mephenytoin 4′-hydroxylase activity of recombinant CYP2C19 was comparable to that of (*R*)-fluoxetine.[40] Fluoxetine has been reported to be capable of altering CYP2C19 activity at doses of 20 and 40 mg/day in young and elderly volunteers.[45] Thus, it is suggested that *in vivo* inhibition of CYP2C19 by CBD might be caused after oral administration.

It is known that CBD accumulates in the fatty tissue due to high lipophilicity.[46] It has been previously reported that the mean plasma levels of CBD range from 5.9 to 11.2 ng/ml (0.019 to 0.036 μM) over 6 weeks of repetitive oral administration of CBD (700 mg/day).[47] Furthermore, the plasma level of CBD averaged 1.5 ng/ml (0.0048 μM) one week after CBD treatment was discontinued. CBD could be stored for weeks in fatty tissues from which it could slowly release back into the blood and distribute into the liver. Therefore, CBD is eliminated over 2–3 weeks after its oral ingestion.[47] From these studies and the present results, it is suggested that CBD may cause a drug-interaction with other drugs metabolized mainly by CYP2C19 in some cases. Phenytoin, with a narrow therapeutic index, is mainly 4′-hydroxylated by CYP2C9 and to a minor extent by CYP2C19.[14] The relative contribution of CYP2C19 to phenytoin metabolism rises as phenytoin concentrations increase, leading to saturation of CYP2C9.[14] It has been previously reported that drugs that are potent inhibitors of CYP2C19 (but not of CYP2C9) increase plasma concentrations and/or toxicity of phenytoin.[48,49] Thus, much attention should be paid to the drug-drug interaction of CBD and phenytoin.

In conclusion, we demonstrated that CBD is a potent inhibitor of CYP2C19. Our results suggest that one phenolic hydroxyl group and the pentyl side chain of CBD may be required for CYP2C19 inhibition and will provide reliable information for understanding the metabolic interaction of CBD with other drugs metabolized by CYP2C19 in the clinical situation.

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