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# 8,9-Dihydrocannabidiol, an Alternative of Cannabidiol, Its Preparation, Antibacterial and Antioxidant Ability

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**Abstract:** Cannabidiol (CBD) from *Cannabis sativa* is used in cosmetics in North America due to its antibacterial and antioxidant properties, but has been prohibited in many countries except recently; so, finding a non-intoxicating CBD alternative and elucidating the structure–function relationship of CBD analogues is becoming increasingly relevant. Herein, a set of CBD analogues including 8,9-dihydrocannabidiol ($H_2CBD$) was synthesized, and their antibacterial, bactericidal, and antioxidant activity, as well as their structure–function relationship, were studied. The results present a catalytic selectivity near 100% towards $H_2CBD$ with a production yield of 85%. Each CBD analogue presented different antibacterial and antioxidant activity. It is revealed that the phenolic hydroxyl moiety is an essential group for CBD analogues to perform antibacterial and antioxidant activities. Among them, $H_2CBD$ presented much stronger antibacterial activity than the assayed popular antibiotics. $H_2CBD$ and Compound 4 presented very similar radical scavenging activity and inhibition on lipid oxidation to vitamin C, but better thermostability. Moreover, $H_2CBD$ presented lower toxicity to human skin fibroblasts at concentrations up to 64-fold higher than its MIC value (1.25 µg/mL) against *S. aureus*. Above all, in all property experiments, $H_2CBD$ presented extremely similar performance to CBD ($p < 0.05$), including similar time–kill kinetics curves. This research finds $H_2CBD$ to be an alternative for CBD with very high potential in the aspects of antibacterial, bactericidal, and antioxidant activity, as well as lower toxicity to human skin fibroblasts.

**Keywords:** 8,9-dihydrocannabidiol; cannabidiol; antimicrobial; antioxidation; fibroblasts

## 1. Introduction

Cannabidiol (CBD) is a non-psychoactive cannabinoid extracted from *Cannabis sativa* [1–3], which can be easily transformed to psychoactive $\Delta^9$-tetrahydrocannabinol (THC) (Scheme 1), making CBD, a banned chemical in many countries [4].
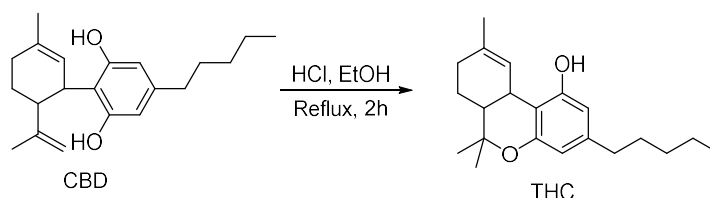


**Scheme 1.** Conversion of CBD to THC [1–3].

In addition to its anticonvulsant activity, CBD is also famous for its antimicrobial and antioxidant activities [5–7], with potential therapeutic effects on cutaneous conditions [8–10]; it has thereby been used in cosmetics and for other antimicrobial applications [9,11,12]. Meanwhile, in addition to CBD, CBD analogues are also expected to form a new class of antibiotics [13–15], but research on their structure–function relationship is still lacking.

Hence, many efforts have been made to discover CBD alternatives either through plant extraction or synthetic approaches. Since plant extraction is costly and time consuming [16] due to the enormous cannabinoid levels in the plant [17–19], the synthetic approach has come a long way, from the hydrogenation of CBD [20] to synthesis without the use of CBD as a starting material [21,22].

The hydrogenation of CBD results in two products [20], including 8,9-dihydrocannabidiol ($H_2CBD$), which was found to be non-intoxicating and demonstrates a similar anticonvulsant effect on rats as CBD [21]. Not derived from CBD, $H_2CBD$ was first synthesized from $\alpha$-phellandrene and olivetol in 1988 [22] (Scheme 2). Recently, Mascal and coworkers optimized the reaction to increase the $H_2CBD$ yield from 26% [22] to 71% with *p*-toluenesulfonic acid monohydrate [21].
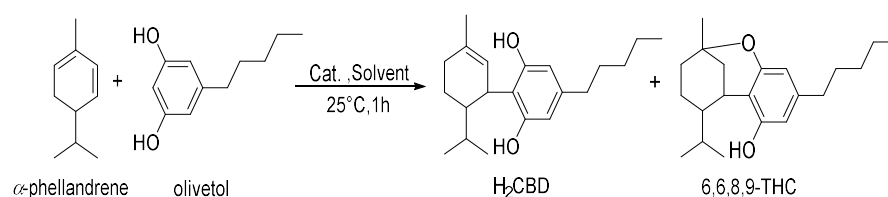


**Scheme 2.** Synthetic route of $H_2CBD$ and 6,6,8,9-THC [22].

However, the reported synthesis of $H_2CBD$ still consumes a huge amount of catalyst with low catalytic specificity [21]. Meanwhile, if $H_2CBD$ is expected to become an alternative to CBD in cosmetics, its skin toxicity and other bioactive properties must be better understood. The properties of the byproduct 6,6,8,9-THC also need to be elucidated.

Therefore, to examine the prospect of $H_2CBD$ as a CBD alternative in cosmetics, it is necessary to investigate whether $H_2CBD$ possesses other biological properties such as antioxidant activity, bactericidal activity, synergistic or antagonistic effects with popular antibiotics, and skin toxicity, as CBD does. Meanwhile, more information about the structure–performance of CBD analogues is needed in order to develop synthetic molecules and determine their properties.

Herein, to examine the structure–performance characteristics of CBD analogues in their antimicrobial and antioxidant aspects, and to verify whether the CBD analogues, and $H_2CBD$ in particular, could act as CBD alternatives, $H_2CBD$ (Scheme 2) and seven other CBD analogues (Scheme 3) were synthesized, and their antibacterial and bactericidal activity was studied, with a comparison between CBD and four popular antibiotics; the skin toxicity towards human skin fibroblasts was also evaluated. A possible synergistic effect between $H_2CBD$ and the antibiotics was also investigated. The antioxidant activity of CBD and the CBD analogues was compared with vitamin C, in terms of radical scavenging activity and inhibition on lipid oxidation, as well as the thermostability of their antioxidant activity.
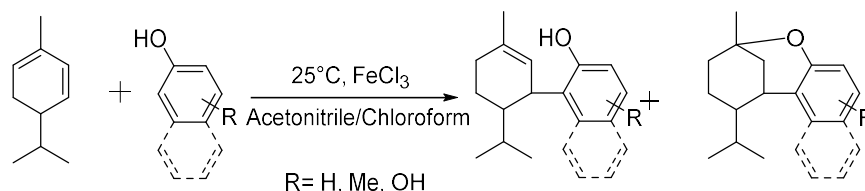


**Scheme 3.** Synthesis of other cannabinoid analogues (this work).

## 2. Results

### 2.1. Synthesis of CBD Analogues and Improvement on $H_2CBD$ Production

The reaction between olivetol and $\alpha$-phellandrene is a typical Friedel–Crafts alkylation, which can be catalyzed by Lewis acid in solvent, resulting a diastereoisomer and accompanied by an intramolecular electrophilic addition as a by-reaction [22]. The reported synthesis of $H_2CBD$ still requires a huge amount of catalyst (*p*-toluenesulfonic acid monohydrate, 30% eq of the starting material) [21], obstructing the scale-up of production. To

improve the synthesis of $H_2CBD$ based on previous studies [21,22], as previously mentioned, the catalysts and solvents were screened based on their reaction specificity and their production yield of $H_2CBD$ (Table 1) first.
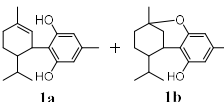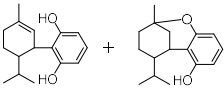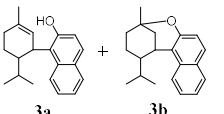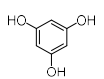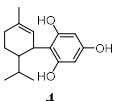
**Table 1.** Reaction condition screening for $H_2CBD$ production.

| Run | Catalyst | Catalyst: Olivetol (mol %) | Solvent | $H_2CBD$ Production Yield (%) | 6,6,8,9-THC Production Yield (%) |
|---|---|---|---|---|---|
| 1 | $FeCl_3$ | 30 | benzene | 73.4 | 10.5 |
| 2 | $FeCl_3$ | 30 | toluene | 70.0 | 12.5 |
| 3 | $FeCl_3$ | 30 | chloroform | 85.3 | Trace |
| 4 | $FeCl_3$ | 10 | chloroform | 85.2 | Trace |
| 5 | $FeCl_3$ | 5 | chloroform | 80.7 | Trace |
| 6 | $AlCl_3$ | 30 | benzene | 69.2 | 12.6 |
| 7 | $BF_3 \cdot H_2O$ | 30 | benzene | 48.2 | 8.7 |
| 8 | $CuCl_2 \cdot 2H_2O$ | 30 | benzene | 32.7 | Trace |
| 9 | TsOH | 30 | benzene | 70.5 | 14.7 |

As presented in Table 1, $FeCl_3$ (Run 1) exhibited slightly better catalytic activity than $AlCl_3$ (Run 6), and presented the best catalysis in chloroform (Run 3–5). The production yields of $H_2CBD$ in different solvents corresponded to the polarity sequence of the solvents (Run 1–3): chloroform > benzene > toluene. With 5%–10% (eq.) of $FeCl_3$, a production yield of $H_2CBD$ of 80.7–85.2% was achieved with almost 100% selectivity (Run 4–5). Therefore, $FeCl_3$ was chosen as the catalyst for the preparation of the remaining cannabinoid analogues.

Subsequently, the remaining cannabinoid analogues were prepared for the analysis of their structure–function relationships. The production yields of the other cannabinoid analogues are presented in Table 2, wherein the reactions were conducted with $\alpha$-phellandrene and other phenols or naphthols under the catalysis of $FeCl_3$ in chloroform and acetonitrile (1:1, *v/v*).

**Table 2.** Production yields of the synthetic cannabinoid analogues other than $H_2CBD$.

| Entry | Phenol/ Naphthol | Product | Compound A Yield (%) | Compound B Yield (%) |
|---|---|---|---|---|
| 1 |  |  | 55 | 25 |
| 2 |  |  | 44 | 30 |
| 3 |  |  | 65 | 20 |
| 4 |  |  | 72 | / |

In Table 2, Compounds **1b**, **2b**, and **3b** were produced from the intramolecular electrophile addition of Compounds **1a**, **2a**, and **3a**, respectively. The yield of the electrophile addition depends on the hydrogen withdrawal from the hydroxyl and the steric hindrance, which is concordant with the structural features of the carbocations.

The NMR and MS profiles (Figures S1–S9) and the data of the as-prepared CBD analogues including $H_2CBD$ are provided in the supporting material.

Hence, the as-prepared CBD analogues are used for the following tests on antibacterial and antioxidant activities.

### 2.2. H₂BCD and CBD Present Similar and Stronger Antibacterial and Bactericidal Activity Than Popular Antibiotics

The most attractive or characteristic properties for the use of CBD in cosmetics are antibacterial activity and antioxidant activity. Hence, the antibacterial and bactericidal activity of $H_2$CBD was evaluated against *S. aureus* and *E. coli*, with the comparison of CBD, 6,6,8,9-THC, and other CBD analogues, as well as four popular antibiotics (tetracycline hydrochloride, gentamicin, ofloxacin and chloramphenicol), presented as the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), respectively (Figure 1).
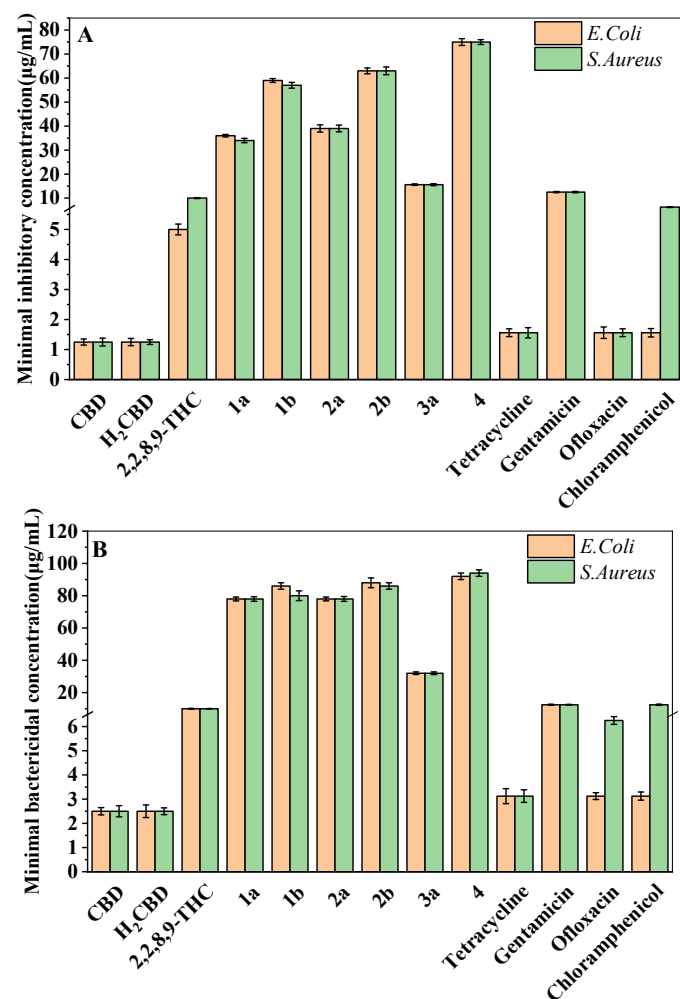


**Figure 1.** MIC (**A**) and MBC (**B**) of $H_2$CBD, CBD, 6,6,8,9-THC, Compounds **1a**, **1b**, **2a**, **2b**, and **3a**, and the antibiotics against *S. aureus* and *E. coli*.

Figure 1 indicates that both CBD and $H_2$CBD possess very similar and much stronger antibacterial activity than the popular commercial antibiotics, and both presented the lowest MICs and MBCs in the assayed samples. The MIC of CBD was $1.25 \pm 0.10$ μg/mL to *E. coli* and $1.25 \pm 0.13$ μg/mL to *S. aureus*, whereas the MIC of $H_2$CBD was $1.25 \pm 0.12$ μg/mL to *E. coli* and $1.25 \pm 0.08$ μg/mL to *S. aureus*.

In addition to $H_2$CBD, 6,6,8,9-THC also demonstrated stronger antibacterial activity than the other assayed CBD analogues, providing similar MIC/MBC to that of gentamicin and chloramphenicol (Figure 1). All phenols (Compounds **1a**, **2a**, and **3a**) exhibited lower MICs/MBCs than their addition products (Compounds **1b**, **2b**, and **3b**); Compound 3b did not present an obvious antibacterial effect. Compound **4** had the most hydroxyl groups but presented the highest MIC/MBC, which implies that the number of hydroxyl groups is not

a significant factor in the antibacterial activity of phenolic compounds, which somehow does not fit expectations.

Thus, $H_2CBD$ may be a potential alternative to CBD in the antimicrobial aspect, and as an antibacterial agent, 6,6,8,9-THC could be employed together with $H_2CBD$ to save the purification process after the synthesis.

### 2.3. $H_2CBD$ Does Not Present Synergistic or Antagonistic Effect on Bacteria with the Popular Antibiotics

Based on the superior antibacterial and bactericidal activities of $H_2CBD$, we became interested in whether $H_2CBD$ would present a synergistic or antagonistic effect towards the bacteria with the assayed antibiotics. Hence, the synergistic effects of $H_2CBD$ and 6,6,8,9-THC with tetracycline hydrochloride, gentamicin, chloramphenicol, and ofloxacin against *S. aureus* were verified on the antibiotic resistance of drug combinations. However, Figure 2 presents the findings that all the FIC indexes were in the range of 1–2, indicating that there was no obvious synergistic or antagonistic effect between $H_2CBD$ and its analogues or other antibiotics.
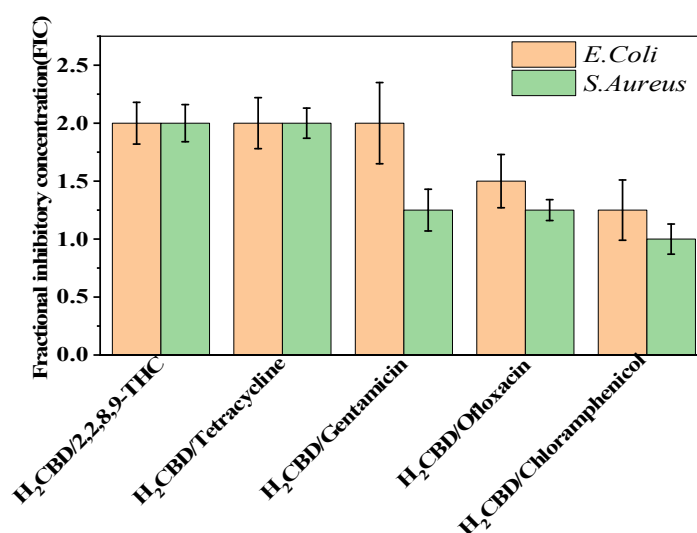


**Figure 2.** Synergy test on *S. aureus* and *E. coli*.

Therefore, $H_2CBD$ and CBD may serve as alternatives to tetracycline hydrochloride, gentamicin, chloramphenicol, and ofloxacin against *S. aureus*, making them synthetic or botanic antibiotics, respectively.

### 2.4. $H_2CBD$ Follows CBD in the Time–Kill Kinetics Curves

To compare the kinetic bactericidal effect of $H_2CBD$ and CBD, a time–kill assay towards the growth of *S. aureus* was conducted over time in the presence of MIC, $2 \times MIC$, $4 \times MIC$, and $8 \times MIC$ of $H_2CBD$ and CBD (Figure 3); tetracycline (at the concentration of its MIC) was set as the positive control.

As Figure 3 indicates, at all assayed concentrations and times, the performance of $H_2CBD$ mirrored that of CBD; in another words, $H_2CBD$ possessed similar kinetic bactericidal effect to CBD. In addition, after 24 h, the plates inoculated with the above drug-containing bacteria liquid showed no colonies, further confirming the bactericidal effect of $H_2CBD$ and its potential as an alternative to CBD in respect to its bactericidal properties.
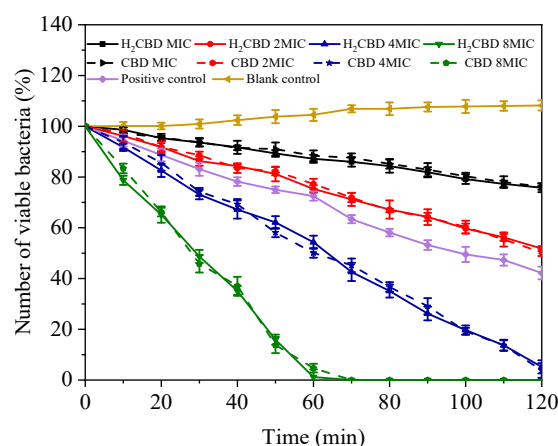
**Figure 3.** Time–kill kinetics curves of *S. aureus* under treatment of minimum inhibitory concentration (MIC), 2 × MIC, 4 × MIC, and 8 × MIC of H$_2$CBD, CBD.

### 2.5. H$_2$CBD Possess Similar Toxicity towards to Human Skin Fibroblasts as CBD

H$_2$CBD presented similar antibacterial effects to CBD, yet its application in cosmetics also raises concerns regarding its toxicity towards human skin. So, the safety of H$_2$CBD on human skin fibroblasts was evaluated with MTT assay, including a comparison with CBD (Figure 4). The cells were treated with 1.25–320 µg/mL of H$_2$CBD and CBD for 24 h, respectively; and the percentage of cell survival was analyzed.
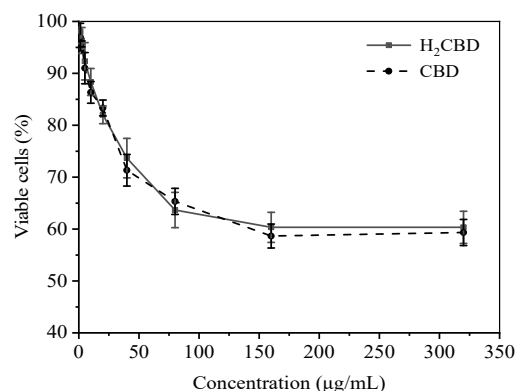


**Figure 4.** Toxicity of H$_2$CBD or CBD towards human skin fibroblasts.

Figure 4 indicates that the H$_2$CBD showed a dose-dependent toxicity in the range of 1.25 µg/mL to 80 µg/mL, and the viable cells stabilized at around 60% at a H$_2$CBD concentration of up to 320 µg/mL. Both CBD and H$_2$CBD presented lower toxicity to human skin fibroblasts at concentrations up to 64-fold higher than their MIC values (1.25 µg/mL) against *S. aureus*.

In view of the similar antibacterial and bactericidal effects, as well as the toxicity of CBD and H$_2$CBD towards human skin fibroblasts, H$_2$CBD appears to be a promising alternative to CBD in the relative fields. Next, we present the analysis of the antioxidant activity of these CBD analogues.

### 2.6. H$_2$CBD Possesses Similar Antioxidant Activity to CBD and Is Comparable with VC

Antioxidant activity, especially radical scavenging ability and inhibition on lipid oxidation, plays an important role in cosmetics. Most phenolic compounds demonstrate antioxidant activity, so do CBD and its phenolic analogues (Figure 5). They all exhibited concentration-dependent antioxidant activity with respect to the radical scavenging ability (Figure 5A,B), antioxidant capacity for the reduction of ferric ions (Figure 5C), and the inhibition of lipid oxidation (Figure 5D).
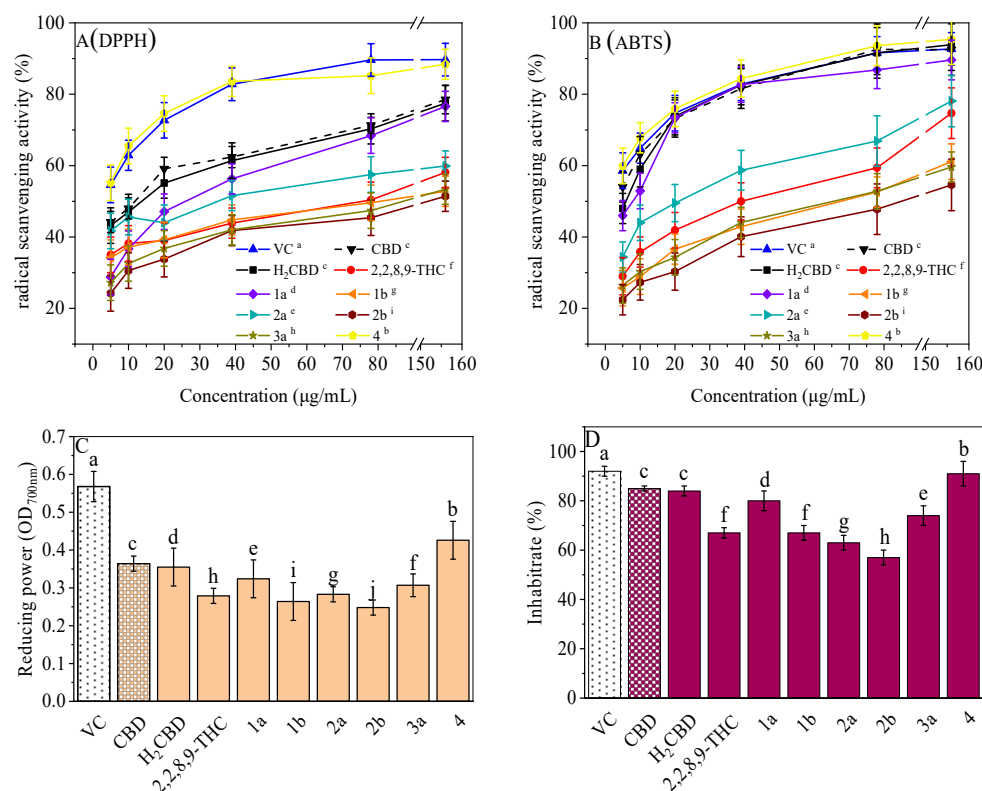
**Figure 5.** Antioxidant activities of the $H_2CBD$ with other antioxidants. DPPH (**A**), ABTS (**B**), FRAP (**C**), Lipid oxidation inhibition (**D**). Data are expressed as mean SD (n = 3). Different letters indicate statistically significant difference according to Duncan's test at the significance level (*p* < 0.05).

It is interesting that Compound **4** exhibited the best antioxidant activity of all the assays (Figure 5), although it presented the weakest antibacterial effect (Figure 1). The antioxidant activity of Compound **4** was even close to that of VC. Compound **3b** also did not present activity in all antioxidant assays. This may imply that a hydroxyl moiety is an essential group for CBD analogues to carry out their antibacterial and antioxidant activities.

For the other analogues, no obvious performance–structure relationship was observed. Again, $H_2BCD$ possessed similar antioxidant activity to that of CBD, although their activity was weaker than that of Compound **4**.

Moreover, in addition to demonstrating antioxidant activity at room temperature, these three ($H_2BCD$, CBD, and Compound **4**) demonstrated higher and more stable antioxidant activity at 80 °C, overtaking VC (Figure 6).
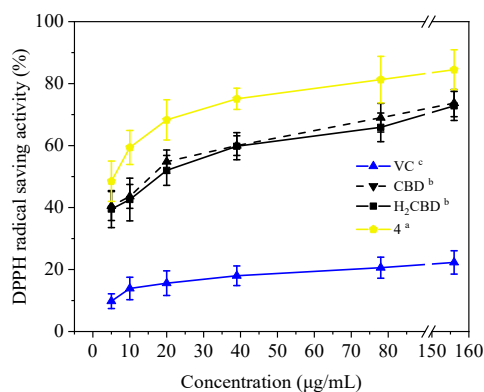


**Figure 6.** DPPH assay of the CBD analogues and VC at 80 °C. Different letters present the data significance.

Therefore, $H_2BCD$ and Compound **4**, which have the most phenolic hydroxyl groups, may be alternatives to CBD in terms of antioxidant activity.

## 3. Discussion

### 3.1. Synthesis of $H_2CBD$

$H_2CBD$ can be obtained together with $H_4CBD$ from the hydrogenation of CBD [13,20]. Without CBD as the starting material, Mascal et al. prepared $H_2CBD$ with olivetol and α-phellandrene under the catalysis of *p*-toluenesulfonic acid monohydrate (30% eq.), resulting in a yield of $H_2CBD$ of 71% [21], which was optimized based on what Crombie reported. The authors did not mention the catalytic selectivity or the yield of 6,6,8,9-THC [21].

In general, the best Lewis acid catalyst for a regular Friedel–Crafts reaction would be $AlCl_3$. As presented in Table 1, $FeCl_3$ (Run 1) demonstrated slightly better catalytic activity than $AlCl_3$ (Run 6). Considering the cost and safety, $FeCl_3$ was selected for the subsequent experiments (Run 1–5).

Table 1 indicates that the catalytic activity of $FeCl_3$ is dramatically influenced by the solvent polarity, which can be explained by the fact that a solvent with a higher polarity would better stabilize the formed electrophilic carbocation generated from olivetol. Hence, $FeCl_3$ presented the best catalysis in chloroform (Run 3–5), and the production yields of $H_2CBD$ in different solvents followed the polarity sequence (Run 1–3): chloroform > benzene > toluene. With 5–10% (eq.) of $FeCl_3$, a production yield of $H_2CBD$ of 80.7–85.2% was achieved with nearly 100% selectivity (Run 4–5).

In addition to the solvent, the intramolecular addition reactivity also varied dramatically depending on the catalyst (Table 1), resulting in remarkable differences in the reaction selectivity. The intramolecular addition of CBD may be driven by acidic conditions that are responsible for further electrophile additions toward the more thermodynamically stable compounds [23]. This may explain why the higher catalyst dosage would yield more additional byproducts, as seen in Table 1.

In this Friedel–Crafts reaction, diastereoisomer and regioisomer can be formed, so the products obtained in Tables 1 and 2 are diastereoisomers [13]. As Blaskovich pointed out, the dihydrocannabidiol regioisomers presented in the NMR data resemble each other [13], and the NMR and MS data in the supporting material confirm their structures.

### 3.2. The Antibacterial Activity of $H_2BCD$ and CBD in Comparison to Popular Antibiotics

Although it is well-known that CBD possess antibacterial properties [6,13,24–27], its efficacy, according to different groups, varies widely. Blaskovich studied the antibacterial activity of CBD against highly resistant strains of *S. aureus*, and confirmed the gram-positive inhibition of CBD [13]. Martinenghi et al. reported that both CBD and its analogue, cannabidiolic acid (CBDA), showed significant antimicrobial effects against the gram-positive *S. aureus* and *Staphylococcus epidermidis*, but no activity was noticed against gram-negative *E. coli* and *P. aeruginosa* [24]. However, a reduction of 11% ($p = 0.0161$) on *E. coli* cell viability was observed with 5 μm of CBD, but no significant effect was observed on *S. aureus* cell viability, as assessed by a disk diffusion test [25].

The results in Figure 1 are differ slightly to those that Martinenghi reported, in which the MIC of CBD is higher than 64 μg/mL to *E.coli* and 1 μg/mL to *S. aureus* [24]. This difference could be due to the strain difference; the *E. coli* (ATCC 25922) they assayed [24] is a recommended reference strain that may be a resistant bacterium, and another study on the performance of CBD against resistant bacteria [28] revealed an MIC value above 128 μg/mL for CBD against *E. coli* (UTI89).

As a CBD analogue, the antibacterial activity of $H_2CBD$ has been reported occasionally. It is interesting to note that although Compounds **1a** and **2a** and $H_2CBD$ are phenols each containing two phenolic hydroxyl groups, their antibacterial activities are starkly different (Figure 1). That might be caused by the only structural difference, the alkyls attached to the benzene ring, together with the phenomena presented by Compound **4**, which might imply that an appropriate amphipathy is needed for antibacterial activity

since H$_2$CBD has a longer alkyl moiety. Appendino et al. studied the structure–activity of antibacterial cannabinoids obtained from *Cannabis sativa*, and their results suggest that the prenyl moieties of cannabinoids serve mainly as modulators of lipid affinity for the olivetol core; the authors claimed that the high potency of cannabinoids suggests a specific yet elusive mechanism of their activity [28]. They also found that the methylation and acetylation of the phenolic hydroxyls and the introduction of a second prenyl moiety were detrimental for antibacterial activity; this is in accordance with our findings from the alkylation of the phenolic hydroxyl (Compounds **1b**, **2b**, and **3b**).

As for the synergistic or antagonistic effects of popular antibiotics towards the bacteria, CBD was reported to potentiate the effect of bacitracin (BAC) against gram-positive bacteria (*Staphylococcus species, Listeria monocytogenes,* and *Enterococcus faecalis*) but appears ineffective against gram-negative bacteria; CBD reduced the MIC of BAC at least 64-fold and the combination yielded an FIC index of 0.5 or below in most gram-positive bacteria tested [25]. Furthermore, the synergistic or antagonistic effects of CBD analogues in combination with popular antibiotics towards bacteria remain unclear [25]. Our findings do indicate that the combination is not synergistic; however, it was close to synergistic.

In addition, the performance of CBD in the time–kill kinetics experiment is in accordance with what has been recently reported [24]. The results also show that both H$_2$CBD and CBD presented a significant inhibitory effect on the growth of bacterial strains over the time. It is notable that in the time–kill profile the data dots of H$_2$CBD and CBD remained close, indicating a very similar kinetic bactericidal performance.

### 3.3. The Toxicity of H$_2$BCD and CBD towards Human Skin Fibroblasts

CBD has demonstrated several beneficial skin properties [11,29–31]. CBD reduced reactive oxygen species levels in human dermal fibroblasts better than vitamin C [31]. As H$_2$BCD possesses similar low toxicity towards human skin fibroblasts as CBD does, H$_2$BCD is a promising option for use in cosmetics, as an alternative to CBD, in terms of the safety inherent with a botanic chemical.

### 3.4. The Antioxidant Activity of H$_2$BCD and CBD in Comparison of VC

The antioxidant activity of CBD [32] as well as its combined regimens with other antioxidants [33] have drawn much attention recently The antioxidant and anti-inflammatory effects of CBD in monocrotaline-induced pulmonary hypertension have been reported [34]. CBD was found to be more effective than vitamin C and E as a neuroprotective antioxidant [35] Herein, H$_2$CBD and Compound **4** presented similar antioxidant activity as VC, but exhibited a much higher thermostability of antioxidant activity than VC.

## 4. Materials and Methods

### 4.1. Chemicals and Reagents

CBD (98%, HPLC purity) was purchased from Guilin Layn Natural Ingredients Corp., Guangxi, China. Olivetol (98%, HPLC purity) was purchased from Shenghong Corporation, Shanghai, China. $\alpha$-Phellandrene (85%, HPLC purity) was purchased from Sigma-Aldrich, Shanghai, China. BF$_3$·2H$_2$O (AR, 99%), CuCl$_2$·2H$_2$O (AR, 97%), AlCl$_3$ (AR, 99%), and FeCl$_3$ (AR, 98%) were purchased from Macklin Biochemical Co., Ltd., Shanghai, China. All other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. All the chemicals were of analytical grade and used as received unless otherwise stated.

*Escherichia coli* (CICC 20549) and *Staphylococcus aureus* (CICC 23656) strains were supplied by the China Center of Industrial Culture Collection (CICC), Beijing, China. Tetracycline hydrochloride, gentamicin, chloramphenicol, and ofloxacin were obtained from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China.

### 4.2. Preparation of 8,9-Dihydrocannabidiol (H$_2$CBD) and NMR Profiles of CBD Analogues

In a typical procedure, 0.081 g of FeCl$_3$ (0.05 mmol) was suspended in the solution of olivetol (1.8 g, 10.0 mmol) and $\alpha$-phellandrene (1.5 g, 11 mmol) in chloroform (10 mL).

The suspension was then stirred at 25 °C for 1 h. The reaction was monitored with TLC (petroleum:ethyl acetate = 40:1, *v/v*). Next, the reaction was completed and the solvent was removed from the reaction mixture under vacuum. The resultant residue was separated with gradient column chromatography, and eluted with the mixture of petroleum and ethyl acetate (100:1 to 40:1, *v/v*) to obtain $H_2$CBD (2.7 g, production yield of 85%) as a yellow oil. The byproduct was confirmed as 6,6,8,9-tetrahydrocannabinol (6,6,8,9-THC) with the analysis of NMR and MS.

$^1$H and $^{13}$C NMR spectra were recorded on a Bruker AV-400 spectrometer at 400 and 100 MHz, respectively, in CDCl$_3$ unless otherwise mentioned. Coupling constants (J) are expressed in hertz (Hz). The chemical shifts (δ) of NMR are reported in parts per million (ppm) units relative to the solvent. EIMS was recorded on a Pegasus BT EI mass spectrometer. HRMS was recorded on a MALDI SYNAPT MS spectrometer.

### 4.3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Assays against S. aureus and E. coli

The antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* was tested according to the literature with slight modifications [36]. The samples were dissolved in 95% ethanol first to make a stock solution, and then diluted with Luria–Bertani broth to the concentration of 1 mg/mL, followed by the dilutions needed and tested against *S. aureus* and *E. coli*, respectively. All strains were cultivated in Luria–Bertani broth at 37 °C. The bacterial inoculum (100 μL, $10^6$ CFU/mL) was added to wells, and the plate was incubated at 37 °C for 24 h. The MIC was determined as the lowest concentration at which no growth was observed. The drug-containing bacteria liquid was inoculated in the corresponding culture medium plate and continued to be cultured at 37 °C for 24 h. The drug concentration with the minimum dilution of less than five colonies on average was recorded as the minimum bactericidal concentration (MBC). A total of 1% ethanol in Luria–Bertani broth was set as the blank. All data were determined in triplicate and represented as the mean ± SD.

### 4.4. Synergistic Effect of CBD Analogues with the Classic Antibiotics against S. aureus and E. coli

The fractional inhibitory concentration (FIC) index was applied to evaluate the synergistic effect [37] of the CBD analogues with the classic antibiotics against *S. aureus* and *E. coli*. For this test, 72-fold dilutions with LB from 8 MIC to 1/8 MIC of the $H_2$CBD, 6,6,8,9-THC, and the four antibiotics (tetracycline hydrochloride, gentamicin, chloramphenicol, and ofloxacin) were employed, respectively. The FIC of the compound was calculated as the ratio of the MIC of the compounds in the combination with the MIC of the compound alone. The FIC index was calculated as the sum of the FIC of the antibiotic (a) and the FIC of $H_2$CBD (b).

$$FIC_a = \frac{MIC_{a+b}}{MIC_a}$$

$$FIC_b = \frac{MIC_{b+a}}{MIC_b}$$

$$FIC_{index} = FIC_a + FIC_b \tag{1}$$

The synergistic effect based on the FIC index is defined as follows: an FIC index ≤ 0.5, indicates a synergistic effect; an FIC index 0.5–1 indicates an additive effect; an FIC index 1–4 indicates indifference; an FIC index > 4 indicates antagonism.

### 4.5. Optical Density Measurements for Kinetic Bactericidal Effect of H₂CBD

The kinetic bactericidal effect of $H_2$CBD was determined with the optical density measurement, presented as the time–kill kinetics assay according to the literature [24], with the comparison of CBD. The optical density measurement of the drug-containing bacteria (*S. aureus*) liquid was conducted with a 96-well microtiter plate. $H_2$CBD with concentrations of 8 × MIC, 4 × MIC, 2 × MIC, and MIC were added to the wells with

50 μL of bacterial culture ($1 \times 10^6$ CFU/mL), respectively. The absorbance was measured at 600 nm and every 10 min at 37 °C in 2 h. Sterile control and positive control ($1 \times$ MIC of tetracycline) were used in the experiment, and three replicates were set, respectively. After determination, the above drug-containing bacteria liquid was inoculated into the corresponding culture medium plate and continued to be cultured at 37 °C for another 24 h to test the bactericidal effect of $H_2CBD$.

### 4.6. Cytotoxicity of $H_2CBD$ to Human Skin Fibroblasts

Human skin fibroblasts were inoculated into 96-well plates at a density of 2000/100 μL (three parallels were set in each group) and cultured at 37 °C in 5% $CO_2$ for 24 h to allow the cells to adhere completely to the walls [29]. The medium was removed, then 200 μL each of $H_2CBD$ of different concentrations (dissolved in fresh medium) were added to the wells in the plate, and culturing was continued for 12 h (time can be adjusted according to the specific situation). Each well was pretreated with MTT (5 mg/mL, 20 μL). After incubation for 4 h, the supernatant in the well was carefully absorbed and 150 μL DMSO was added to each well, and the purple crystals were dissolved by vibration for 10 min. The absorbance value of each hole at the wavelength of 490 nm was recorded with a microplate analyzer, and calculated according to the formula:

$$\text{Cell survival rate\%} = \frac{\text{absorbance of the sample}}{\text{absorbance of the blank}} \times 100\% \tag{2}$$

### 4.7. DPPH Radical Scavenging Activity

The DPPH radical scavenging activity was measured according to the literature [38], with some modifications. Briefly, 50 μL of DPPH ethanol solution (0.06 mmol/L) and 50 μL of sample ethanol solution were mixed in a well of a 96-well microtiter plate and incubated at testing temperature for 30 min in dark. The absorbance of the reaction mixture was measured at 517 nm. Ethanol alone in the reaction mixture served as the control. A sample blank was prepared by replacing the DPPH with ethanol. The DPPH radical scavenging activity was calculated according to the following equation:

$$\text{DPPH inhibition \%} = \frac{A_{\text{control}} - \left( A_{\text{sample}} - A_{\text{sample blank}} \right)}{A_{\text{control}}} \times 100\% \tag{3}$$

The DPPH radical scavenging capacity of the samples was plotted against the sample concentrations to determine the IC50 values.

### 4.8. ABTS Radical Scavenging Assay

The ABTS radical scavenging activity was examined according to the literature [38]. The ABTS solution containing ABTS (final concentration of 7 mmol/L) and potassium persulphate (final concentration of 2.45 mmol/L) was incubated in the dark at room temperature for 16 h. The mixture was adjusted with ethanol to obtain an absorbance of $0.700 \pm 0.02$ at 734 nm. The diluted ABTS solution (50 μL) was mixed with 50 μL of sample in each well on a 96-well microtiter plate and stored at room temperature for 10 min. Then, the absorbance of the mixture was recorded at 734 nm. Ethanol alone in the reaction mixture served as control. A sample blank was prepared by replacing the ABTS with ethanol. The ABTS radical scavenging activity is expressed as ABTS (%) and calculated as below:

$$\text{ABTS inhibition \%} = \frac{A_{\text{control}} - \left( A_{\text{sample}} - A_{\text{sample blank}} \right)}{A_{\text{control}}} \times 100 \tag{4}$$

The radical scavenging ability of ABTS was plotted against the sample concentrations to determine the IC50 values.

### 4.9. Ferric Reducing Antioxidant Power Assay

A ferric reducing power was tested using a modified FRAP assay [39]. The sample solutions were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). After incubating the reaction mixture for 20 min at 50 °C, 2.5 mL of trichloroacetic acid (10%) was added to each test tube, and the mixture was centrifuged at 8000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1%). The absorbance of the reaction mixture was recorded at 700 nm.

### 4.10. Lipid Oxidation Inhibition Assay

The thiobarbituric acid reactive substances (TBARS) assay was conducted using the modified method of Murzakhmetova [40] as a measurement of the lipid oxidation. Briefly, 0.15 mL of hydrogen peroxide (2 mg/mL) was mixed with 0.5 mL of olive oil ethanol solution (mass ratio, 1:3) in a test tube, then 1 mL of sample ethanol solution and 2 mL of 2-thiobarbituric acid ethanol solution (0.2 wt%) were pipetted into the tube, respectively, and incubated at 37 °C for 25 min. After that, the mixture was incubated at 90 °C for 30 min with 2 mL of trichloroacetic acid (20%) and then cooled to room temperature. Afterwards, 1 mL of trichloromethane was employed to extract the organic phase, and the absorbance of the supernatant was determined at 532 nm for the calculation of lipid oxidation inhibition. The control sample was prepared by replacing the hydrogen peroxide with ethanol. The inhibition rate of lipid oxidation was calculated as following:

$$\text{Lipid oxidation inhibition \%} = \frac{A_{control} - \left(A_{sample} - A_{blank}\right)}{A_{control}} \times 100\% \tag{5}$$

### 4.11. Statistics

All data obtained in this study were analyzed using IBM SPSS Statistics (version 26; SPSS Inc., Chicago, IL, USA). The results are presented as the mean $\pm$ standard deviation (n = 3). $p < 0.05$ was considered significant (Duncan's test).

## 5. Conclusions

In this experiment, several CBD analogues were synthesized with a one pot Friedel–Crafts alkylation with phenols and $\alpha$-phellandrene, catalyzed by Lewis acid in solvent. $FeCl_3$ performed best catalytic activity, and its catalytic activity dramatically depends on the solvent polarity. Typically, the production yields of 8,9-dihydrocannabidiol ($H_2CBD$) followed the sequence of solvent polarity: chloroform > benzene > toluene. A nearly 100% of catalytic selectivity towards $H_2CBD$ was achieved with a production yield of 85% in 1 h at room temperature.

Among the synthesized CBD analogues, $H_2CBD$ performed much stronger in terms of inhibitory activity against the bacteria than the four assayed popular antibiotics (tetracycline hydrochloride, gentamicin, chloramphenicol, and ofloxacin). The MIC of CBD is $1.25 \pm 0.10$ μg/mL to *E. coli* and $1.25 \pm 0.13$ μg/mL to *S. aureus*, whereas the MIC of $H_2CBD$ is $1.25 \pm 0.12$ μg/mL to *E. coli* and $1.25 \pm 0.08$ μg/mL to *S. aureus*. The addition product of $H_2CBD$, that is, 6,6,8,9-THC, also demonstrated stronger antibacterial activity than the other CBD analogues did, performing similar MIC/MBC as that of gentamicin and chloramphenicol. $H_2CBD$ does not present synergistic or antagonistic effects with the popular antibiotics towards the bacteria.

The structure–antibacterial effect study shows that an appropriate amphipathy might be necessary for antibacterial activity. Compound **4** had the most hydroxyl groups but presented the highest MIC/MBC and antioxidant activity, which implies that the number of hydroxyl groups is not an essential aspect of antibacterial activity in CBD analogues, but

may benefit to their antioxidant activity. All CBD analogues exhibited lower MICs/MBCs than their addition products. It was found that an appropriate amphipathy might be needed for antibacterial activity since $H_2CBD$ has a longer alkyl moiety. The number of phenolic hydroxyls might not be the essential for antibacterial activity but may be beneficial for antioxidant activity. All of the obtained CBD analogues exhibited lower MICs/MBCs than their intramolecular addition products.

In comparison to CBD, $H_2CBD$ presented extremely similar antibacterial activity against *E. coli* and *S. aureus*, similar antioxidant activity, as well as lower toxicity to human skin fibroblasts. Time–kill assay also confirmed the similar bactericidal effect of $H_2CBD$ and CBD. $H_2BCD$, CBD, and Compound **4** performed higher and more stable antioxidant activity at 80 °C, whereas VC was denatured under the same conditions.

Taken together, $H_2CBD$ may be a potential alternative to CBD in terms of antimicrobial and antioxidant action, and could be a promising functional agent for the production of cosmetics.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules28010445/s1, Figure S1. $^1$H NMR(400 MHz, CDCl$_3$) and $^{13}$C NMR(126 MHz, CDCl$_3$) and MS spectra of 8,9-dihydrocannabidiol (H$_2$CBD); Figure S2: $^1$H NMR(400 MHz, CDCl$_3$) and $^{13}$C NMR(126 MHz, CDCl$_3$) and MS spectra of 6,6,8,9-tetrahydrocannabinol (6,6,8,9-THC); Figure S3: $^1$H NMR(400 MHz, CDCl$_3$) and $^{13}$C NMR(126 MHz, CDCl$_3$) and MS spectra of 2′-Isopropyl-4,5′-dimethyl-1′,2′-dihydro-3′,4′-tetrahydro-[1,1′-biphenyl]-2,6-diol(**1a**); Figure S4: $^1$H NMR(400 MHz, CDCl$_3$) and $^{13}$C NMR(126 MHz, CDCl$_3$) and MS spectra of 2′-Isopropyl-4,5′-dimethyl-1′,2′-dihydro-3′,4′,6′-hexahydro-1′,2-methoxybenzo[b]oxacyclooctatrien-6-ol (**1b**); Figure S5: $^1$H NMR(400 MHz, CDCl$_3$) and $^{13}$C NMR(126 MHz, CDCl$_3$) and MS spectra of 2′-Isopropyl-5′-methyl-1′,2′-dihydro-3′,4′-tetrahydro-[1,1′-biphenyl]-2,6-diol (**2a**); Figure S6: $^1$H NMR(400 MHz, CDCl$_3$) and $^{13}$C NMR(126 MHz, CDCl$_3$) and MS spectra of of 2′-Isopropyl-5′-methyl-1′,2′-dihydro-3′,4′,6′-hexahydro-1′,2-methoxybenzo[b]oxacyclooctatrien-6-ol (**2b**); Figure S7: $^1$H NMR(400 MHz, CDCl$_3$) and $^{13}$C NMR(126 MHz, CDCl$_3$) and MS spectra of 2′-Isopropyl-5′-methyl-1′,2′-dihydro-3′,4′-tetrahydrocyclohexyl-6-en-1-yl)naphthalene-2-ol (**3a**); Figure S8: $^1$H NMR(400 MHz, CDCl$_3$) and $^{13}$C NMR(126 MHz, CDCl$_3$) and MS spectra of 2′-Isopropyl-5′-methyl-1′,2′-dihydro-3′,4′,6′-hexahydro-1,7′-methylnonaphthalene [2,1b] oxoxine (**3b**); Figure S9: $^1$H NMR(400 MHz, CDCl$_3$) and $^{13}$C NMR(126 MHz, CDCl$_3$) and MS spectra of 2′-Isopropyl-5′-methyl-1′,2′-dihydro-3′,4′-tetrahydro-[1,1′-biphenyl]-2,4,6-triol (**4**).

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