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Structure−Activity Relationships of Cannabigerol and Cannabigerolic Acid Derivatives as Antibacterial Agents against Gram-Positive Bacteria

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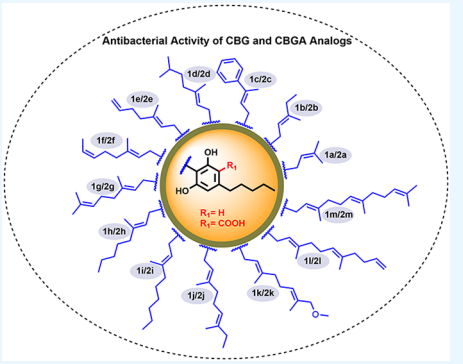
ABSTRACT: Antimicrobial resistance has emerged as a critical global health challenge, necessitating the discovery of new antibiotics. Cannabigerol (CBG) and cannabigerolic acid (CBGA) from *Cannabis sativa* have shown promising activity as antibacterial agents. In this work, a total of 26 CBG and CBGA derivatives (13 of each) featuring varied terpene chain lengths and substitution patterns were synthesized and characterized; of these, 20 are novel analogs. To determine their structure−activity relationships (SAR), we tested their antibacterial activity against Gram-positive bacterial strains, including *Bacillus subtilis*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), and vancomycin-resistant *Enterococcus faecalis* (VRE). Our results reveal that terpene chain lengths between 6 and 13 carbons show potent antibacterial activity with no detectable cytotoxicity toward mammalian cells. In addition, several CBG analogs exhibited minimum inhibitory concentrations (MICs) similar to the FDA-approved drug, daptomycin, against multiple Gram-positive strains. Comparing the antibacterial activities of different CBG and CBGA derivatives establishes the terpene moiety as a critical structural determinant for antibacterial potency in CBG and CBGA scaffolds and provides strong evidence that rational modification of this moiety can significantly enhance bioactivity.



INTRODUCTION

Antimicrobial resistance (AMR) is one of the most forefront threats to global public health. An estimated 4.71 million deaths were associated with bacterial AMR in 2021, and recent projections suggest that this number could rise to 8.22 million deaths annually by 2050.[2] Methicillin-resistant *Staphylococcus aureus* (MRSA) exemplifies this crisis, accounting for the greatest increase in attributable deaths among drug-resistant bacteria from 1990 to 2021.[2] *S. aureus* ranks among the leading bacterial causes of mortality worldwide, demonstrating the capacity to develop resistance to available antibiotic classes and evade host immune defenses.[3] Increasing prevalence of vancomycin-resistant (VRSA), vancomycin-intermediate (VISA), and heterogeneous VISA (hVISA) *S. aureus* strains further complicates treatment options.[4] This urgent clinical challenge necessitates the discovery of new antibacterial drugs.

Natural products have historically provided a rich source of antibacterial agents, with almost 70% of current FDA-approved antibiotics derived from natural product scaffolds.[5] Many phenolic natural products have demonstrated broad-spectrum antibacterial activity through membrane-targeting mechanisms.[6,7] Specifically, flavonoids achieve bacterial membrane disruption through lipid interactions, while stilbenes can induce membrane depolarization and permeabilization.[8,9] Within this context, cannabinoids represent an underexplored class of phenolic natural products with documented antibacterial properties.[10−12]

Among the many compounds produced by the *Cannabis sativa* plant, the major cannabinoids—such as cannabigerolic acid (CBGA), Δ^9-tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), and cannabichromenic acid (CBCA)—and their corresponding decarboxylated derivatives [cannabigerol (CBG), Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabichromene (CBC) respectively] exhibit diverse pharmacological profiles (Figure 1).[13−20] An early study reported the bactericidal effects of THC and CBD against staphylococci and streptococci strains.[21] Notably, this activity was substantially reduced in the presence of serum proteins, with MIC values increasing 10-fold, and no activity was observed against Gram-negative bacteria. Foundational structure−activity relationships have been established for major cannabinoid natural products. In 2008, Appendino and

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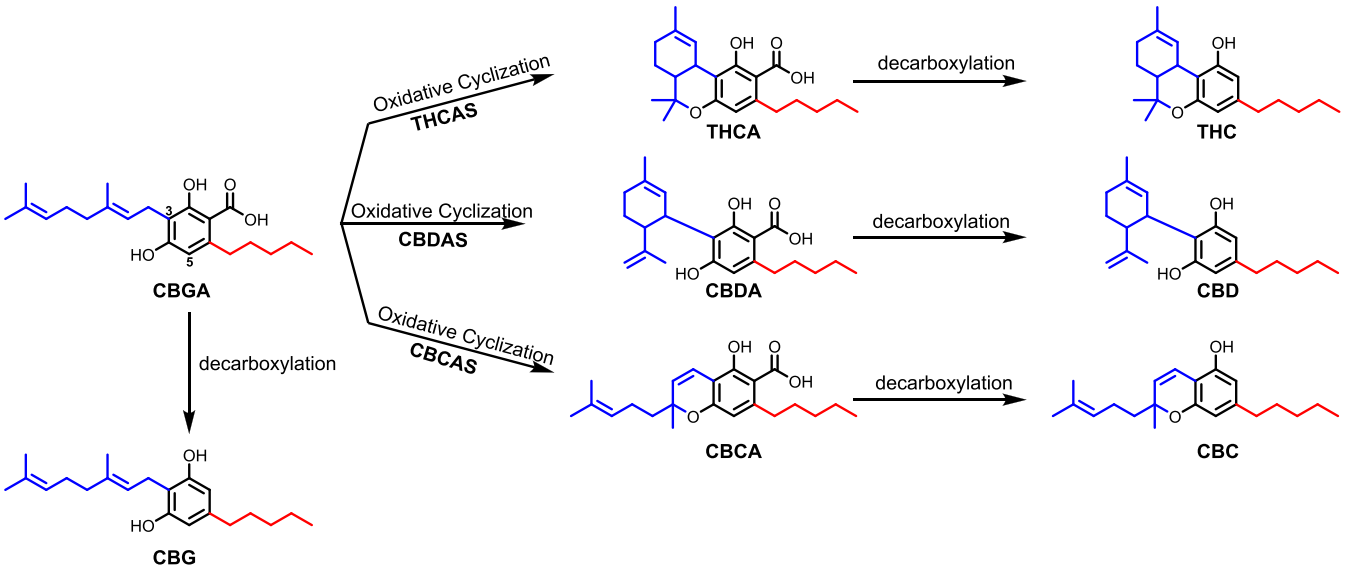
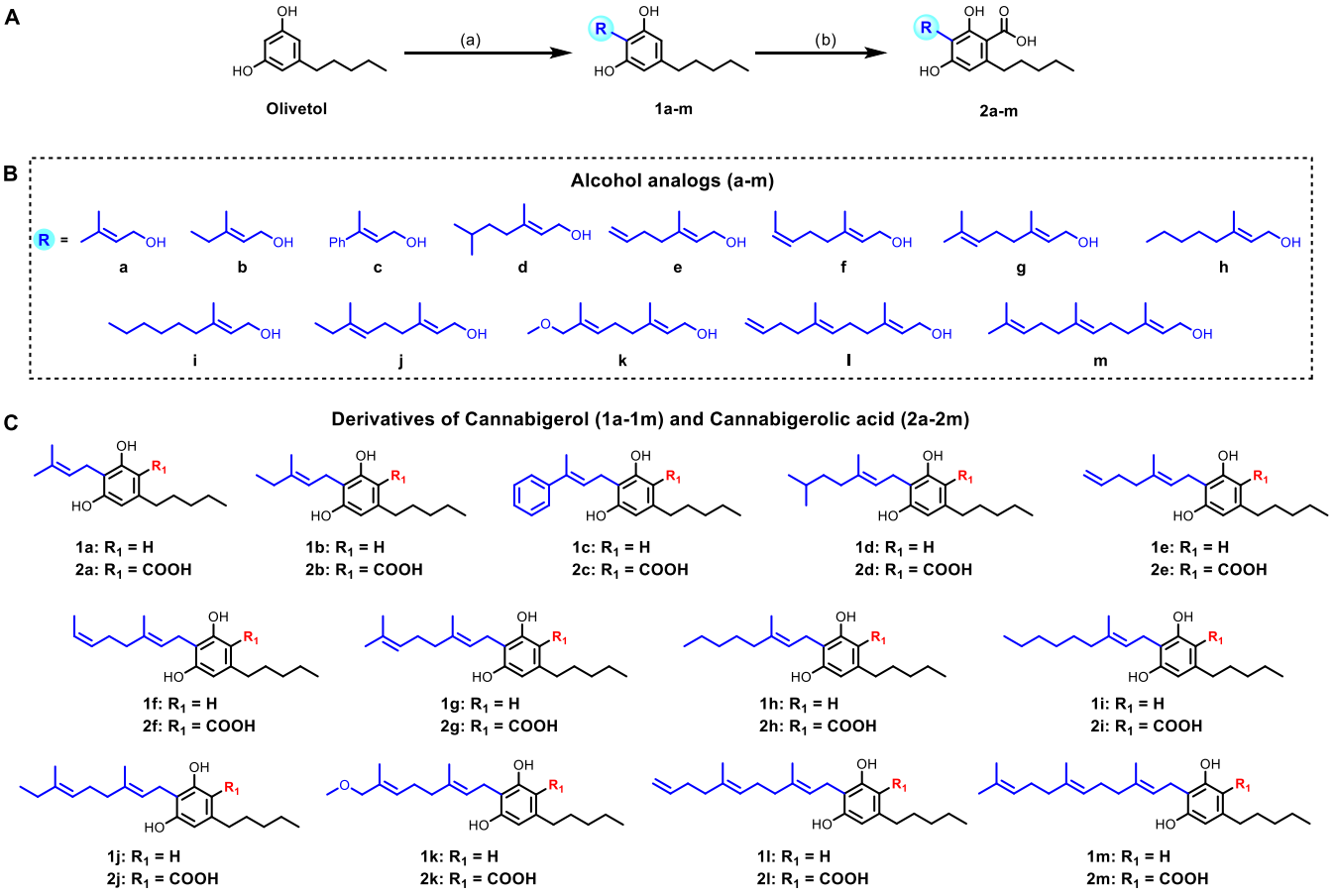


Figure 1. Representative structures of biologically active major cannabinoids from *C. sativa*, where pentyl side chains and terpene moieties are colored red and blue, respectively.[1] Abbreviations: CBGA = cannabigerolic acid, CBG = cannabigerol, THCA = Δ^9-Tetrahydrocannabinolic acid, THC = Δ^9-Tetrahydrocannabinol, THCAS = THCA synthase, CBDA = cannabidiolic acid, CBD = cannabidiol, CBDAS = CBDA synthase, CBCA = cannabichromenic acid, CBC = cannabichromene, CBCAS = CBCA synthase.

Scheme 1. (A) General Synthesis Route for the Preparation of CBG and CBGA Analogs, Reagent and Conditions: (a) Olivetol (1.0 equiv), Alcohol Analog (1.0 equiv), Al_2O_3 (Acidic) (10.0 equiv), DCE, 80 °C, 8 h, N_2 atm; (b) CBG Analogs (1.0 equiv), Methyl Magnesium Carbonate (10.0 equiv) DMF, 120 °C, 3 h, N_2 atm; (B) Structures of Alcohol Analogs (C) Structures of CBG and CBGA Derivatives



colleagues evaluated CBD, CBG, CBC, THC, and cannabinol (CBN) against a panel of six MRSA clinical isolates and found that all cannabinoids demonstrated potent activity (MIC 0.5–2 μg/mL) against MRSA strains.[22] This previous study

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revealed that methylation or acetylation of phenolic hydroxyl groups and esterification of the carboxylic acid was detrimental to activity. The prenyl moiety, however, was highly tolerant to structural modifications, suggesting it may modulate lipophilicity.[22]

More recent investigations have provided mechanistic insights and expanded the scope of cannabinoid antibacterial characterization. Blaskovich et al. found that CBD displayed potent antibacterial activity against diverse bacterial strains and confirmed membrane depolarization and permeabilization as the primary mechanism.[23] SAR analysis of 25 synthetic CBD analogs revealed that extending the pentyl side chain (colored red in Figure 1) from C_5 to C_7 showed similar or marginally improved activity against MRSA.[23] While CBD has been extensively studied, CBG has emerged as a particularly promising scaffold due to its combined antibacterial, antibiofilm, and antipersister activities. Farha et al. demonstrated that CBG exhibits bactericidal activity against MRSA USA300 with an MIC of 2 μg/mL and that this activity could be attributed to membrane disruption.[24] CBG was also shown to inhibit biofilm formation at concentrations as low as 0.5 μg/mL and eradicate persister cells in a dose-dependent manner. Importantly, CBG demonstrated in vivo efficacy in a murine systemic MRSA infection model comparable to vancomycin, validating its therapeutic potential beyond in vitro activity.[24]

Cannabinoid acids display similar antibacterial activity compared to their decarboxylated counterparts and offer distinct advantages as synthetic scaffolds. CBGA is the central cannabinoid precursor and thus represents a synthetically accessible entry point to both acid and neutral cannabinoid series.[25,26] A recent study investigated the antibacterial activity of CBGA derivatives bearing dimethylallyl (C_5), geranyl (C_{10}), and farnesyl (C_{15}) terpene moieties and found that a compound bearing a farnesyl chain at the C_5 position, rather than C_3 (see Figure 1), improved antibacterial activity against Gram-positive strains.[27] Another study demonstrated that increasing the length of the pentyl side chain of CBGA (colored red in Figure 1) enhanced antibacterial activity against *Bacillus subtilis*.[28] These works validate CBGA derivatives as antibacterial agents with potential for medicinal chemistry optimization. Specifically, the terpene moiety (colored blue in Figure 1) offers a tunable structural element for accessing diverse analogs with unexplored antibacterial potential.[29]

Despite findings establishing both CBG and CBGA as promising scaffolds for antibacterial development, structure−activity relationships of CBG and CBGA derivatives with modifications to terpene moiety (colored blue in Figure 1) remain underexplored. Herein, we report the synthesis and characterization of 13 CBG and 13 CBGA derivatives featuring structural modifications of the terpene moiety. Of these 26 compounds, 20 represent novel analogs with diverse terpene chain lengths and functional substitutions (Scheme 1). To produce nonnatural derivatives of both CBG and CBGA, we modified the terpene carbon chain length from C_5 to C_{15} and incorporated lipophilic functional groups. After confirming their structures using NMR spectroscopy, their antibacterial activity was determined against a panel of five Gram-positive bacterial strains. These results demonstrated that many CBG analogs had similar or improved activity compared to CBG, while CBGA analogs demonstrated moderate activity. In addition, these results revealed the CBG analogs were more potent compared to their corresponding CBGA analogs. Overall, we demonstrate that diversification of the terpene moiety of cannabinoids is a promising strategy to achieve potent activities against Gram-positive bacterial strains.

RESULTS AND DISCUSSION

Chemical Synthesis

Utilizing a previously developed and optimized synthetic approach, we started with geraniol (1.0 equiv), olivetol in excess (1.5 equiv), and acidic alumina (2 g/mmol with respect to geraniol) in DCE at reflux temperature for 6 h to afford the analog CBG (**1g**).[30] During this process, we observed that purification of the CBG compound resulted in olivetol recovery, which was attributed to excessive olivetol use. Consequently, we reoptimized the reaction conditions by changing the equivalent of olivetol, geraniol and acidic alumina. Initially, we set up the reaction of olivetol (1.0 equiv), geraniol (1.0 equiv) and varying amounts of acidic alumina (from 5,7, and 10 equiv) in DCE solvent at reflux temperature for 8 h. Monitoring by TLC indicated the complete consumption of starting materials at 10 equiv of acidic alumina (See Supporting Information Figure S1). Therefore, we tested various substituted alcohols for their reactivity with olivetol under optimized conditions of olivetol (1.0 equiv), geraniol (1.0 equiv), acidic alumina (10 equiv), in DCE at reflux for 8 h (Scheme 1A).

To establish structure−activity relationships for terpene chain length and functionality, CBG and CBGA analogs bearing terpene moieties with varying chain lengths and terminal groups were prepared via *C*-alkylation of olivetol. Dimethylallyl alcohol (**a**), Geraniol (**g**) and Farnesol (**m**) are commercially available, and comprehensive syntheses of all other alcohol derivatives have been reported in previous work.[31−33] The synthetic pathway for the CBG and CBGA analogs is shown in Scheme 1. Olivetol and each alcohol analog (**a-m**, Scheme 1B) were converted to the corresponding CBG derivatives **1a−1m** (Scheme 1C) with acidic alumina in DCE solvent under reflux conditions for 8 h. After completion of the reaction, the mixture was filtered and the crude residue was purified by column chromatography with 5 to 7% EtOAc/Hexane (Scheme 1C) to obtain the derivatives in yields ranging from 31 to 52%. After synthesizing the substituted CBG analogs, these compounds were subsequently converted into their corresponding CBGA derivatives (**2a−2m**, Scheme 1C). Each CBG analog was reacted with dry DMF and methyl magnesium carbonate, commonly known as Stile's reagent, at 120 °C for 3 h. The reaction mixture was cooled to 0 °C, quenched, and extracted with CH_2Cl_2. After washing with brine, the crude residue was purified by column chromatography with 15 to 20% EtOAc/Hexane. These reactions resulted in the formation of the respective CBGA derivatives **2a−2m** obtained in yields ranging from 29 to 41%.

Inspection of the 1D and 2D NMR data confirmed the addition of the terpene moiety onto the C_3 carbon of olivetolic acid for **2g** (Figure 2). Briefly, the 1D-^{1}H NMR spectrum shows a single singlet peak in the aromatic region,

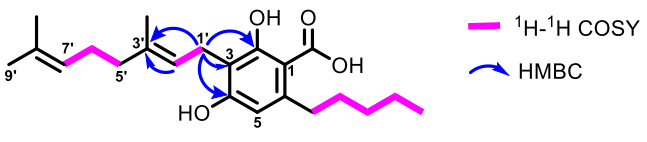

Figure 2. Key ^{1}H−^{1}H and HMBC correlations of CBGA (**2g**).

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Table 1. MIC and MBC Values (μg/mL) of CBG and CBGA Derivatives[f]

	B. subtilis[a]		*S. epidermidis*[b]		*S. aureus*[c]		*S. aureus*, MRSA[d]		*E. Faecalis*, VRE[e]	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
daptomycin	1.0	1.0	0.2	1.0	1.0	1.0	0.5	1.0	1.0	1.0
olivetol	>21.6	>86.4	>21.6	>86.4	>21.6	>86.4	>21.6	>86.4	>21.6	>86.4
olivetolic acid	>21.6	>86.4	>21.6	>86.4	>21.6	>86.4	>21.6	>86.4	>21.6	>86.4
CBG (**1g**)	1.0	>4.0	1.0	>4.0	1.0	4.0	2.0	4.0	2.0	>8.0
CBGA (**2g**)	2.3	9.2	2.3	9.2	4.6	18.4	4.6	>18.2	8.0	8.0
1a	>21.6	>86.4	>21.6	>86.4	>21.6	>86.4	>21.6	>86.4	>21.6	>86.4
2a	>21.6	>86.4	>21.6	>86.4	>21.6	>86.4	>21.6	>86.4	>21.6	>86.4
1b	3.3	13.2	3.3	13.2	1.7	6.8	1.7	>6.8	6.6	13.2
2b	6.7	26.8	6.7	26.8	6.7	26.8	6.7	>26.8	6.7	>26.8
1c	2.0	8.0	2.0	8.0	2.0	8.0	2.0	8.0	3.9	7.8
2c	7.9	18.0	4.5	18.0	4.5	18.0	4.5	>18.0	7.9	31.6
1d	3.8	15.2	3.8	15.2	3.8	7.6	3.8	15.2	3.8	>15.2
2d	17.6	70.4	17.6	70.4	17.6	70.4	17.6	>70.4	17.6	70.4
1e	1.8	7.2	1.8	7.2	1.8	7.2	1.8	7.2	1.8	>7.2
2e	2.8	>11.2	2.8	>11.2	4.2	16.8	4.2	>16.8	8.4	32.0
1f	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	7.7	7.6
2f	7.7	7.7	7.7	7.7	7.7	7.7	15.4	15.4	15.4	30.8
1h	1.0	>4.0	1.0	>4.0	1.0	2.0	1.0	2.0	1.9	>7.6
2h	3.9	3.9	3.9	3.9	7.7	30.8	7.7	30.8	7.7	7.7
1i	2.0	4.0	2.0	4.0	2.0	4.0	2.0	8.0	4.0	>16.0
2i	2.3	4.6	2.3	4.6	8.0	16.0	8.0	32.0	4.6	4.6
1j	1.0	2.0	2.0	2.0	2.0	8.0	2.0	2.0	4.2	4.1
2j	4.2	4.1	4.2	4.1	8.4	8.3	16.7	16.7	8.4	8.3
1k	4.4	8.8	4.4	8.8	4.4	4.4	4.4	8.8	17.5	17.5
2k	9.9	39.6	9.9	39.6	9.9	39.6	9.9	>39.6	19.7	19.7
1l	1.1	>4.4	1.1	>4.4	1.1	4.6	2.3	9.2	1.1	>4.4
2l	2.5	2.5	2.5	2.5	5.1	10.2	5.1	10.2	5.1	5.1
1m	19.4	>4.8	1.2	>4.8	9.7	>25.6	19.4	>77.6	4.9	>19.6
2m	3.2	3.2	3.2	3.2	6.4	25.6	6.4	25.6	6.4	6.4

[a]*B. subtilis*, ATCC 6051. [b]*Staphylococcus epidermidis*, ATCC 12228. [c]*Staphylococcus aureus*, ATCC 25923. [d]Methicilin-resistant *Staphylococcus aureus*, ATCC 700787. [e]Vancomycin-resistant *Enterococcus faecalis*, ATCC 700802. [f]Three biological replicates were determined in triplicate (at a minimum) according to CLSI guidelines.[36]

demonstrating that the remaining aromatic carbons are substituted. The chemical shift values in 1D-^{13}C NMR spectrum define the number and type of carbon atoms in the molecule. To further characterize the structure and determine the position of the terpene moiety, we analyzed the 2D-^{1}H−^{1}H COSY, 2D-^{1}H−^{13}C HSQC and 2D-^{1}H−^{13}C HMBC spectra. The pentyl side chain was unambiguously identified by ^{1}H−^{1}H COSY correlations between the neighboring protons of the −CH_2 groups. The correlations between H1′/H2′ and H5′/H6′/H7′ of the terpene moiety along with chemical shift values in the ^{1}H−^{13}C HSQC define the terpene moiety. The HMBC spectrum shows the chemical shift value of H1′/C1′ of the terpene moiety is 3.42/22.1 ppm, clearly indicating that the terpene is attached to a carbon atom on the aromatic ring and not an oxygen. In addition, the HMBC correlations between H1′ of the terpene moiety and phenolic carbons C_2 and C_4 of the aromatic ring, as well as to the quaternary carbon C_3′ provide evidence for the attachment of the terpene moiety to the C_3 carbon of the aromatic ring. The structure of all other compounds was confirmed in an analogous manner, and the spectral analyses of all compounds are included in the Supporting Information (See Supporting Information Figures S2−79).

Structure−Activity Relationship (SAR)

The antibacterial activities of parent compounds **1g** and **2g** against Gram-positive bacteria have been well studied.[22,24,28,34] Antibacterial activities of CBGA compounds containing a prenyl (**2a**) or farnesyl (**2m**) terpene moiety have also been reported against *S. aureus*, *B. subtilis*, and *Micrococcus luterus*.[27] The structures of corresponding CBG analogs with a prenyl (**1a**) and farnesyl (**2a**) moiety have been reported, but no antibacterial activity has been reported for these analogs.[35] Therefore, we sought to identify the potential activities of CBG and CBGA derivatives (**1a-f, h-m, 2b-f, h-l**) as antibacterial agents against Gram-positive bacteria and compare them to previously known compounds. In the preliminary investigation, all analogs were screened against five different Gram-positive bacterial strains, including *B. subtilis*, *S. epidermidis*, *S. aureus*, methicillin-resistant *S. aureus* (MRSA), and vancomycin-resistant *E. faecalis* (VRE) (Table 1). MIC and Minimum Bactericidal Concentration (MBC) values were recorded in triplicate (at a minimum) according to CLSI guidelines (See Supporting Information Tables S1 and S2).[36] *Escherichia coli* K12 was used as a control to compare activity of the analogs against Gram-negative bacteria. Daptomycin is an established antibiotic used as a positive control, while olivetol and olivetolic acid, as precursors to CBG and CBGA, served as additional controls. The positive control, daptomycin, exhibited potent activity, with MIC values ranging from 0.2

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to 1.0 μg/mL, against all tested strains. Conversely, olivetol and olivetolic acid did not show activity even at concentrations of 21.6 μg/mL. Consistent with previous studies, none of the compounds showed antibacterial activity against the Gram-negative bacteria *E. coli* K12 at concentrations of 50.0 μg/mL.[22−24]

All evaluated CBG and CBGA analogs tested were found to have good to moderate activity against Gram-positive bacteria. The analogs exhibited a range of antimicrobial potencies. The natural product CBG (**1g**) was used as the standard and showed the most potent activity, with MIC values of 1.0−2.0 μg/mL against all Gram-positive strains tested. CBGA (**2g**) showed lower activity (MIC 2.3−8.0 μg/mL). This pattern of enhanced activity with CBG persisted across most structural modifications.

Investigation of the antibacterial activity of the CBG and CBGA derivatives showed well maintenance of activity with increasing carbon chain length ($\geq C_6$) of the terpene moiety. The C_5 compounds **1a** and **2a** did not show antibacterial activity up to the maximum concentration tested of 21.6 μg/mL. Increased activity was observed with C_6 compounds **1b** and **2b**. Potent activity was observed for compounds having C_8−C_{13} chain lengths. Specifically, compounds **1e** (C_8), **1h** (C_9), **1i** (C_{10}), and **1l** (C_{13}) exhibited MICs of 1.0−2.0 μg/mL against most tested strains. The corresponding CBGA compounds **2e**, **2h**, **2i**, **2l**, showed similar activity compared to natural CBGA (**2g**) with MICs between 2.3 and 8.4 μg/mL. Compound **1m**, with a farnesyl moiety, displayed markedly reduced activity with all strains except for *S. epidermidis*. The acid form (**2m**) however, showed similar or slightly improved activity to **2g** (MIC 3.2−6.4 μg/mL). This observation aligns with prior findings by Yan et al., who reported moderate activity (EC_{50} 9.62−17.62 μM) for the farnesylated CBGA analog against *S. aureus*, *B. subtilis*, and *M. luteus*.[27]

Addition of a phenyl group to the terpene moiety (**1c**) maintained potent activity (MIC 2.0−3.9 μg/mL). In contrast, introduction of a polar methoxy group (**1k**, **2k**) reduced activity compared to the parent compounds. Optimal antibacterial activity against the Gram-positive strains tested was observed for terpene chain lengths of C_6−C_{13}, with both shorter (C_5) and longer (C_{15}) derivatives showing reduced potency. Compounds **1e** and **1l** with terminal alkene groups both displayed potent activity (MIC 1.1−2.3 μg/mL) against Gram-positive strains. Compared to the parent compounds containing two methyl groups at the prenyl end, these terpene moieties contain only hydrogens. Compounds with saturated alkyl chains, **1h** and **1i**, achieved potent activity (MIC 1.0−2.0 μg/mL) against most strains. The most potent CBGA analogs **2i** and **2l** achieved slight improvement over CBGA (**2g**) for *E. faecalis* VRE and maintained similar activity against *B. subtilis* and *S. epidermidis*. Compound **1h** emerged as a promising antibacterial agent, with MIC values similar to that of daptomycin for *B. subtilis* and *S. aureus*. However, further studies on serum stability and in vivo efficacy will be necessary to fully evaluate its therapeutic potential. Compound **1l** displayed the highest activity against *E. faecalis* VRE, with an MIC value lower than that of CBG (**1g**). Finally, we evaluated the cytotoxicity of all analogs using HEK293 cell toxicity assays (See Supporting Information Figure S80). None of the compounds exhibited cytotoxic effects at concentrations of 25 and 50 μM which suggests that the compounds are likely biocompatible and safe for further pharmacological evaluation.

Following inhibition testing, the lethal potential of the synthesized analogs was evaluated through MBC analysis (Table 1). Most analogs were found to be bactericidal, with the majority of compounds achieving a 3-log reduction in cell viability compared to untreated controls. This distinction indicates that the analogs primarily function by killing the bacterial cells rather than merely inhibiting new growth. When evaluating the SAR across the entire library, the CBG structures appeared more consistently effective than their CBGA counterparts, often exhibiting lower MBC values across all tested strains. However, both CBG and CBGA analogs exhibited potent bactericidal activity against clinically challenging, multidrug resistant (MDR) pathogens. Significant effects were observed against MRSA and *E. faecalis* VRE, warranting future investigations into these scaffolds to overcome existing resistance mechanisms and induce cell death in resilient bacterial targets.

These SAR patterns parallel trends observed in related cannabinoid antibacterials. Appendino et al. demonstrated that the cannabinoid antibacterial chemotype tolerates substantial structural modification of lipophilic moieties, which were proposed to function as modulators of lipid affinity and influence cellular bioavailability. Notably, they observed that increased hydrophilicity via additional hydroxyl groups substantially reduced antibacterial potency, which they attributed to decreased cellular bioavailability through reduced membrane permeability.[22] Our finding that polar-substituted analogs (**1k, 2k**) exhibit reduced activity is consistent with this pattern. Additionally, Lee et al. identified optimal pentyl chain lengths for CBG and CBGA derivatives against Gram-positive bacteria, with activity diminishing beyond n-undecyl substitution.[28] The enhanced activity of analogs with saturated terminal chains may relate to conformational flexibility afforded by sp^3 bond rotation, which warrants further investigation. Based on structural similarities to characterized cannabinoid antibacterials, we hypothesize that membrane interactions likely contribute to the observed activity.[24,34] While direct mechanistic validation requires future experimental studies, these preliminary findings establish a foundation for lead optimization of CBG and CBGA derivatives as antibacterials.

Although CBGA analogs generally exhibited reduced activity compared to CBG counterparts, CBGA is the common substrate for cannabinoid synthases which can cyclize it into major cannabinoids including THCA, CBDA, and CBCA. The CBGA derivatives reported here not only represent potential antibacterial agents themselves but have the possibility to serve as substrates for cannabinoid synthases, enabling enzymatic diversification to novel cannabinoid analogs with unexplored antibacterial properties.

CONCLUSION

The natural forms of CBG and CBGA exhibit potent antimicrobial activity against various bacterial strains. In this study, we demonstrate a synthetic approach to produce 20 novel CBG and CBGA derivatives, expanding the chemical space of novel CBG and CBGA analogs. Antibacterial activity assays identified that the CBG analogs with terpene chain lengths between 6−13 carbons (**1b**, **1c**, **1e**, **1h**, **1i**, **1l**) displayed potent antibacterial activity (MIC 1.0−2.0 μg/mL) against two or more Gram-positive strains tested, including MRSA. Additionally, the CBGA derivatives maintained antibacterial activity across the tested strains compared to

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the parent compound. Further, none of the analogs showed detectable cytotoxicity toward mammalian cells, highlighting translational significance of these findings for antibacterial drug discovery. Several limitations require further optimization prior to clinical advancement. The pharmacokinetic properties, serum protein binding, and in vivo efficacy remain to be established. Further medicinal chemistry optimization, including exploration of hybrid scaffolds, prodrug strategies, and synergistic combinations, could advance these cannabinoid derivatives toward preclinical studies. Moreover, the CBGA scaffold provides a platform for enzymatic cyclization to CBCA-, CBDA- and THCA-type scaffolds, accessing unexplored chemical space with diverse biological properties and potentially distinct mechanisms of actions.

EXPERIMENTAL SECTION

Chemical Synthesis

Chemicals and Instruments. All chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA), Acros Organics (Fair Lawn, NJ, USA), Alfa-Aesar (Ward Hill, MA, USA), TCI (Portland, OR, USA), Ambeed (Arlington Heights, IL, USA), or AK Scientific (Union City, CA, USA) and were reagent grade or better. Unless otherwise noted, all synthetic reactions were conducted in oven-dried glassware under a nitrogen atmosphere with anhydrous solvents. Reactions were monitored by thin-layer chromatography (TLC) (EMD Millipore Corp, Billerica, MA, USA), and visualization was accomplished with UV light (254 nm) followed by (1) staining with the phosphomolybdic acid solution or anisaldehyde solution and heating, and (2) exposure to iodine and cerium ammonium molybdate with no heating. Flash column chromatography was performed using ACS-grade solvents and silica gel (SiliCycle Inc., P60, particle size 40−63 μm). NMR spectra were obtained on Varian VNMRS 400, 500, and 600 MHz and 400 and 500 JEOL instruments at the NMR facility of the Department of Chemistry and Biochemistry of the University of Oklahoma. ^{1}H and ^{13}C chemical shifts were referenced to internal solvent resonances. Multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), m (multiplet), and br (broad). Chemical shifts are reported in parts per million (ppm), and J's coupling constants are given in Hz. All NMR spectra were recorded at ambient temperature and processed using MestReNova software.

Purity Check Using High Pressure Liquid Chromatography (HPLC) and Liquid Chromatography Coupled with Mass Spectrometry (LCMS) Methods. HPLC experiments were conducted using a Gemini-5U, C-18 110 Å, (5 μm 4.6 mm × 250 mm) reversed-phase column (Phenomenex, Torrance, California, USA) [gradient of 10% to 35% B over 5 min, 100% B over 17 min, 100% B isocratic for 3 min, 100% to 10% B over 5 min (A = ddH_2O with 0.1% TFA; B = acetonitrile); flow rate = 1 mL min^{-1}; A_{260}]. LCMS analyses were performed on a Shimadzu 2020 EV LCMS with a reversed-phase column Kinetex, 1.8 μm, PS C18, 100 Å, 100 mm × 2.1 mm (Phenomenex, Torrance, California, USA) using positive and negative mode electrospray ionization with a gradient [gradient of 40% B to 60% B over 1 min, 60% B to 85% B over 3.5 min, 85% to 100% B for 5 min, 100% B for 3 min, 100% to 40% B over 0.01 min (A = ddH_2O with 0.1% formic acid; B = acetonitrile) flow rate = 0.2 mL min-1; A_{264}].

General Procedure for Synthesis of CBG Compounds. *2-(3-Methylbut-2-en-1-yl)-5-pentylbenzene-1,3-diol (**1a**).* To a round-bottomed flask equipped with a stir bar olivetol (2.77 mmol, 1.0 equiv), Al_2O_3-acidic (36.06 mmol, 10 equiv) and alcohols (2.77 mmol, 1.0 equiv) were added. Dichloroethane (15 mL) was added, and the reaction mixture was heated for 8 h at 80 °C. After completion of the reaction, as monitored by TLC, the reaction mixture was cooled to room temperature and filtered through Celite, and the residue was washed with EtOAc (3 × 25 mL), the filtrate was concentrated in vacuo to obtain a viscous oily compound. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (5:95) to furnish the pure compound **1a** (300 mg, 36%) as an off-yellow solid. The compound was stored at −20 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.23 (s, 2H), 5.26 (tdt, J = 7.2, 2.9, 1.5 Hz, 1H), 5.04 (s, 2H), 3.37 (d, J = 7.1 Hz, 2H), 2.44 (dd, J = 8.8, 6.8 Hz, 2H), 1.81 (d, J = 1.4 Hz, 3H), 1.74 (q, J = 1.5 Hz, 3H), 1.59−1.51 (m, 2H), 1.29 (dt, J = 7.8, 3.3 Hz, 4H), 0.87 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.79, 142.84, 135.25, 121.92, 110.63, 108.39, 35.59, 31.57, 30.90, 25.89, 22.64, 22.39, 17.95, 14.13. LCMS (ESI): m/z calc'd for $C_{16}H_{25}O_2$ [M + H] 249.1854; Found 249.1500.

*(E)-2-(3-Methylpent-2-en-1-yl)-5-pentylbenzene-1,3-diol (**1b**).* The synthetic procedure followed that of **1a**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (5:95) to furnish the pure compound **1b** (280 mg, 38%) as an off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.24 (s, 2H), 5.26 (tq, J = 7.1, 1.4 Hz, 1H), 5.07 (s, 2H), 3.39 (d, J = 6.8 Hz, 2H), 2.44 (d, J = 2.1 Hz, 2H), 2.06−2.03 (m, 2H), 1.81 (s, 3H), 1.57−1.54 (m, 2H), 1.29 (dt, J = 4.3, 2.9 Hz, 4H), 1.00 (t, J = 7.5 Hz, 3H), 0.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.88, 154.82, 142.86, 140.99, 121.42, 120.29, 110.59, 108.38, 108.37, 35.60, 32.48, 31.58, 31.56, 30.89, 22.64, 22.31, 16.29, 14.12, 12.65. LCMS (ESI): m/z calc'd for $C_{17}H_{27}O_2$ [M + H] 263.2010; Found 263.1500.

*(E)-5-Pentyl-2-(3-phenylbut-2-en-1-yl)benzene-1,3-diol (**1c**).* The synthetic procedure followed that of **1a**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (8:92) to furnish the pure compound **1c** (450 mg, 52%) as an off-yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: δ 7.37 (d, J = 7.7 Hz, 2H), 7.29 (d, J = 7.3 Hz, 2H), 7.21 (t, J = 7.3 Hz, 1H), 6.24 (s, 2H), 5.89−5.84 (m, 1H), 4.90 (s, 2H), 3.58 (t, J = 7.9 Hz, 2H), 2.49−2.42 (m, 2H), 2.22 (s, 3H), 1.59−1.53 (m, 2H), 1.30 (tt, J = 8.1, 4.6 Hz, 4H), 0.88 (t, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: δ 154.73, 143.27, 142.96, 136.92, 128.29, 128.26, 126.98, 125.81, 125.80, 125.66, 110.67, 108.41, 35.60, 31.57, 30.90, 23.05, 22.65, 16.08, 14.14. LC-HRMS (ESI): m/z calc'd for $C_{21}H_{26}O_2$ [M + H] 311.2010; Found 311.2500.

*(E)-2-(3,6-Dimethylhept-2-en-1-yl)-5-pentylbenzene-1,3-diol (**1d**).* The synthetic procedure followed that of **1a**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (5:95) to furnish the pure compound **1d** (290 mg, 36%) as an off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.24 (d, J = 3.1 Hz, 2H), 5.26 (tdd, J = 6.0, 2.9, 1.5 Hz, 1H), 5.07 (s, 2H), 3.39 (d, J = 7.1 Hz, 2H), 2.47−2.42 (m, 2H), 2.02 (td, J = 7.1, 3.0 Hz, 2H), 1.80 (s, 3H), 1.55 (ddd, J = 10.1, 5.3, 2.1 Hz, 2H), 1.52−1.46 (m, 1H), 1.33−1.27 (m, 6H), 0.90−0.83 (m, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.87, 154.83, 142.88, 142.86, 140.29, 139.96, 121.76, 121.18, 110.63, 110.57, 108.40, 37.68, 37.25, 37.16, 35.60, 31.59, 31.57, 30.89, 30.02, 28.36, 27.93, 23.72, 22.64, 22.36, 22.08, 16.41, 14.13. LCMS (ESI): m/z calc'd for $C_{20}H_{33}O_2$ [M + H] 305.24804; Found 305.2000.

*(E)-2-(3-Methylhepta-2,6-dien-1-yl)-5-pentylbenzene-1,3-diol (**1e**).* The synthetic procedure followed that of **1a**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (5:95) to furnish the pure compound **1e** (250 mg, 31%) as an off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.23 (d, J = 4.3 Hz, 2H), 5.89−5.71 (m, 1H), 5.27 (ddt, J = 8.4, 7.0, 1.3 Hz, 1H), 5.06−4.92 (m, 4H), 3.42−3.36 (m, 2H), 2.44 (td, J = 7.7, 1.8 Hz, 2H), 2.24−2.09 (m, 4H), 1.80 (d, J = 1.3 Hz, 3H), 1.54 (td, J = 7.8, 3.9 Hz, 2H), 1.33−1.28 (m, 4H), 0.87 (t, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.83, 154.75, 142.87, 138.44, 138.27, 138.26, 122.87, 122.15, 115.21, 114.93, 110.61, 108.43, 108.37, 39.06, 35.60, 32.16, 31.58, 30.89, 22.64, 22.28, 16.28, 14.13. LCMS (ESI): m/z calc'd for $C_{19}H_{29}O_2$ [M + H] 289.2167; Found 289.1500.

*2-((2E,6Z)-3-Methylocta-2,6-dien-1-yl)-5-pentylbenzene-1,3-diol (**1f**).* The synthetic procedure followed that of **1a**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (5:95) to furnish the pure compound **1f** (320 mg, 38%) as an off-yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.26 (s, 2H), 5.47 (m, 1H), 5.40−5.33 (m, 1H), 5.30 (m, 1H), 4.99 (s, 2H), 3.41 (d, J = 6.7 Hz, 2H), 2.46 (dd, J = 8.7, 6.8 Hz, 2H), 2.23−2.15 (m, 2H), 2.13−2.07 (m, 2H), 1.83 (s, 3H), 1.63−1.51 (m, 5H),

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1.37−1.26 (m, 4H), 0.90 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 154.68, 142.69, 138.64, 130.46, 129.62, 125.34, 124.29, 121.75, 110.44, 108.26, 39.26, 35.42, 31.40, 30.69, 25.10, 22.45, 22.14, 16.08, 13.93, 12.69. LCMS (ESI): m/z calc'd for $C_{20}H_{31}O_2$ [M + H] 303.4655; Found 303.0000.

*(E)-2-(3,7-Dimethylocta-2,6-dien-1-yl)-5-pentylbenzene-1,3-diol (CBG **1g**).* The synthetic procedure followed that of **1a**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (5:95) to furnish the pure compound CBG **1g** (350 mg, 40%) as an off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.24 (s, 2H), 5.26 (tq, J = 7.1, 1.3 Hz, 1H), 5.09 (s, 2H), 5.05 (ddt, J = 8.3, 6.8, 1.5 Hz, 1H), 3.39 (d, J = 7.1 Hz, 2H), 2.44 (dd, J = 8.8, 6.8 Hz, 2H), 2.13−2.03 (m, 4H), 1.80 (d, J = 1.4 Hz, 3H), 1.67 (d, J = 1.5 Hz, 3H), 1.58 (d, J = 1.4 Hz, 3H), 1.55 (td, J = 8.3, 4.6 Hz, 2H), 1.30 (ddt, J = 10.8, 7.5, 3.0 Hz, 4H), 0.88 (t, J = 6.9 Hz, 3H).^{13}C NMR (126 MHz, $CDCl_3$) δ: 154.88, 142.84, 139.09, 132.16, 123.84, 121.79, 110.66, 108.45, 39.78, 35.61, 31.59, 30.90, 26.46, 25.77, 22.64, 22.35, 17.79, 16.28, 14.13. LCMS m/z calc'd for $C_{21}H_{32}O_2$ [M + H] 317.2474; Found 317.3000.

*(E)-2-(3-Methyloct-2-en-1-yl)-5-pentylbenzene-1,3-diol (**1h**).* The synthetic procedure followed that of **1a**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (5:95) to furnish the pure compound CBG **1h** (275 mg, 33%) as an off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.23 (d, J = 3.1 Hz, 2H), 5.26 (tq, J = 7.2, 1.4 Hz, 1H), 5.01 (d, J = 6.3 Hz, 2H), 3.38 (d, J = 6.9 Hz, 2H), 2.43 (dd, J = 8.0, 2.0 Hz, 2H), 2.04−1.98 (m, 2H), 1.80 (d, J = 1.4 Hz, 3H), 1.56 (d, J = 7.5 Hz, 2H), 1.43−1.37 (m, 2H), 1.31−1.27 (m, 8H), 0.88−0.86 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.87, 142.86, 139.74, 121.32, 110.55, 108.38, 39.78, 35.73, 35.59, 31.64, 31.58, 30.89, 27.65, 22.64, 22.33, 16.30, 14.14, 14.13. LCMS (ESI): m/z calc'd for $C_{20}H_{33}O_2$ [M + H] 305.2480; Found 305.2500.

*(E)-2-(3-Methylnon-2-en-1-yl)-5-pentylbenzene-1,3-diol (**1i**).* The synthetic procedure followed that of **1a**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (6:94) to furnish the pure compound **1i** (289 mg, 33%) as an off-yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.25 (d, J = 2.7 Hz, 2H), 5.28 (dddd, J = 8.5, 7.2, 2.8, 1.4 Hz, 1H), 5.04 (dd, J = 5.5, 2.0 Hz, 2H), 3.40 (d, J = 7.1 Hz, 2H), 2.46 (dd, J = 8.8, 7.1 Hz, 2H), 2.03 (ddd, J = 9.1, 6.3, 1.2 Hz, 2H), 1.82 (d, J = 1.3 Hz, 3H), 1.62−1.55 (m, 4H), 1.32 (tdd, J = 13.9, 6.7, 3.8 Hz, 10H), 0.91−0.87 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.87, 142.85, 139.73, 121.34, 110.59, 108.40, 39.83, 35.61, 31.82, 31.59, 30.90, 29.11, 27.94, 22.70, 22.65, 22.34, 16.30, 14.18, 14.13. LCMS (ESI): m/z calc'd for $C_{21}H_{35}O_2$ [M + H] 319.2636; Found 319.2000.

*2-((2E,6E)-3,7-Dimethylnona-2,6-dien-1-yl)-5-pentylbenzene-1,3-diol (**1j**).* The synthetic procedure followed that of **1a**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (5:95) to furnish the pure compound **1j** (320 mg, 35%) as an off-yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 6.25 (s, 2H), 5.27 (tq, J = 7.2, 1.3 Hz, 1H), 5.05 (tq, J = 7.0, 1.4 Hz, 1H), 5.02 (s, 2H), 3.39 (d, J = 7.1 Hz, 2H), 2.48−2.42 (m, 2H), 2.16−2.04 (m, 4H), 2.00−1.94 (m, 2H), 1.82 (s, 3H), 1.60−1.55 (m, 5H), 1.35−1.27 (m, 5H), 0.96 (t, J = 7.5 Hz, 3H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 154.87, 142.84, 139.12, 137.62, 122.19, 121.75, 110.61, 108.43, 39.81, 35.60, 32.39, 31.58, 30.91, 26.33, 22.65, 22.33, 16.32, 16.05, 14.14, 12.79. LCMS (ESI): m/z calc'd for $C_{22}H_{35}O_2$ [M + H] 331.5195; Found 331.1000.

*2-((2E,6E)-8-Methoxy-3,7-dimethylocta-2,6-dien-1-yl)-5-pentylbenzene-1,3-diol (**1k**).* The synthetic procedure followed that of **1a**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (7:93) to furnish the pure compound **1k** (330 mg, 34%) as an off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.23 (s, 2H), 5.33 (t, J = 6.9 Hz, 1H), 5.26 (t, J = 7.1 Hz, 1H), 3.77 (s, 2H), 3.37 (d, J = 7.2 Hz, 2H), 3.28 (s, 3H), 2.47−2.41 (m, 2H), 2.19−2.13 (m, 2H), 2.13−2.06 (m, 2H), 1.80 (s, 3H), 1.62 (s, 3H), 1.57 (d, J = 7.4 Hz, 2H), 1.30 (d, J = 7.7 Hz, 4H), 0.89 (d, J = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.85, 142.72, 137.84, 132.44, 127.88, 122.36, 108.39, 108.33, 78.83, 57.53, 39.25, 35.60, 31.58, 30.90, 25.96, 22.64, 22.27, 16.22, 14.12, 13.91. LCMS (ESI): m/z calc'd for $C_{22}H_{35}O_3$ [M − H] 345.2429; Found 345.6500.

*2-((2E,6E)-3,7-Dimethylundeca-2,6,10-trien-1-yl)-5-pentylbenzene-1,3-diol (**1l**).* The synthetic procedure followed that of **1a**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (7:93) to furnish the pure compound **1l** 500 mg scale reaction (345 mg, 35%) as an off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.24 (s, 2H), 5.79 (ddt, J = 16.8, 10.2, 6.4 Hz, 1H), 5.27 (tq, J = 7.1, 1.3 Hz, 1H), 5.08 (tt, J = 5.5, 1.5 Hz, 1H), 5.02 (q, J = 1.7 Hz, 1H), 4.97 (q, J = 1.7 Hz, 1H), 4.95−4.91 (m, 1H), 3.39 (d, J = 7.1 Hz, 2H), 2.49−2.41 (m, 2H), 2.17−2.04 (m, 8H), 1.81 (d, J = 1.4 Hz, 3H), 1.61−1.53 (m, 5H), 1.37−1.28 (m, 4H), 0.92−0.86 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 176.30, 163.85, 163.72, 160.58, 159.77, 147.53, 146.70, 139.58, 121.02, 112.37, 111.71, 111.30, 107.19, 103.26, 101.89, 39.55, 39.43, 36.65, 36.58, 32.14, 32.09, 31.50, 30.16, 25.89, 24.11, 23.25, 22.59, 22.52, 16.40, 16.32, 14.18, 14.16, 14.08. LCMS (ESI): m/z calc'd for $C_{24}H_{37}O_2$ [M + H] 357.2793; Found 357.3000.

*5-Pentyl-2-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)-benzene-1,3-diol (**1m**).* The synthetic procedure followed that of **1a**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (7:93) to furnish the pure compound **1m** (450 mg, 52%) as an orange viscus oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.23 (t, J = 2.4 Hz, 2H), 5.26 (d, J = 7.2 Hz, 1H), 5.07 (q, J = 10.6 Hz, 2H), 5.01 (d, J = 2.5 Hz, 2H), 3.38 (d, J = 7.0 Hz, 2H), 2.48−2.41 (m, 2H), 2.05 (tt, J = 20.1, 12.2 Hz, 8H), 1.80 (t, J = 3.2 Hz, 3H), 1.67 (s, 5H), 1.63−1.52 (m, 10H), 1.36−1.25 (m, 4H), 0.87 (td, J = 7.0, 2.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.87, 142.83, 139.11, 135.70, 124.51, 124.47, 124.38, 123.66, 121.75, 110.60, 108.42, 39.78, 39.74, 35.60, 31.58, 30.89, 26.75, 26.43, 25.80, 22.64, 22.34, 17.79, 16.33, 16.14, 14.13. LCMS (ESI): m/z calc'd for $C_{26}H_{41}O_2$ [M + H] 385.3106; Found 385.2500.

General Procedure for Synthesis of CBGA Compounds. *2,4-Dihydroxy-3-(3-methylbut-2-en-1-yl)-6-pentylbenzoic Acid (**2a**).* To a round-bottomed flask equipped with a stir bar with **1a** (1.2 mmol, 1.0 equiv) dissolved in anhydrous DMF (5 mL) and methyl magnesium carbonate solution (1.8 M, 12.4 mmol, 10 equiv) were added. The flask was stirred at 120 °C for 3 h or until TLC showed full conversion. The reaction mixture was cooled to room temperature and cooled to 0 °C, and 2 M HCl was added slowly until reaching pH ∼ 2. The resulting solution was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with cold saturated brine solution (5 × 20 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (15:85) to furnish the pure compound **2a** (95 mg, 40%) as an off-orange solid. The compound was stored at −20 °C.^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.88 (s, 1H), 6.26 (s, 1H), 5.93 (s, 1H), 5.26 (t, J = 7.4 Hz, 1H), 3.41 (d, J = 7.2 Hz, 2H), 2.88 (d, J = 8.0 Hz, 2H), 1.81 (s, 3H), 1.74 (s, 3H), 1.57 (d, J = 7.5 Hz, 2H), 1.33 (d, J = 4.1 Hz, 4H), 0.89 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 176.07, 163.75, 160.37, 147.56, 135.39, 121.49, 111.73, 111.28, 36.65, 32.08, 31.50, 25.92, 22.59, 22.21, 18.00, 14.16. LCMS (ESI): m/z calc'd for $C_{17}H_{23}O_4$ [M − H] 291.1596; Found 291.1500.

*(E)-2,4-Dihydroxy-3-(3-methylpent-2-en-1-yl)-6-pentylbenzoic acid (**2b**).* The synthetic procedure followed that of **2a** starting from **1b**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (15:85) to furnish the pure compound **1b** (70 mg, 30%) as an orange solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.91 (s, 1H), 6.27 (d, J = 1.1 Hz, 1H), 6.05 (s, 1H), 5.25 (dt, J = 17.5, 7.1 Hz, 1H), 3.45 (dd, J = 7.2, 1.7 Hz, 2H), 2.87 (d, J = 7.8 Hz, 2H), 2.26−2.20 (m, 2H), 1.59−1.56 (m, 2H), 1.35−1.32 (m, 4H), 0.91−0.87 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.92, 163.70, 160.77, 147.59, 145.83, 120.60, 120.11, 111.38, 103.14, 38.84, 36.66, 32.08, 31.50, 29.77, 22.60, 14.16, 12.76. LCMS (ESI): m/z calc'd for $C_{18}H_{25}O_4$ [M − H] 305.1752; Found 305.2000.

*(E)-2,4-Dihydroxy-6-pentyl-3-(3-phenylbut-2-en-1-yl)benzoic Acid (**2c**).* The synthetic procedure followed that of **2a** starting from **1c**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (15:85) to furnish the pure

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compound **2c** (110 mg, 38%) as an orange solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.90 (s, 1H), 7.39−7.36 (m, 2H), 7.28 (t, J = 7.6 Hz, 2H), 7.22−7.19 (m, 1H), 6.26 (s, 1H), 5.87 (td, J = 7.2, 1.5 Hz, 1H), 3.60 (d, J = 7.2 Hz, 2H), 2.90−2.86 (m, 2H), 2.23 (s, 3H), 1.57 (dt, J = 8.0, 3.6 Hz, 2H), 1.33 (dt, J = 7.0, 3.5 Hz, 4H), 0.90 (q, J = 3.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.82, 164.01, 159.77, 147.66, 143.39, 136.79, 128.26, 126.92, 125.79, 125.28, 111.85, 111.09, 103.45, 36.66, 32.09, 31.52, 22.87, 22.60, 16.08, 14.17. LCMS (ESI): m/z calc'd for $C_{22}H_{25}O_4$ [M − H] 353.4385; Found 353.2000.

*(E)-3-(3,6-Dimethylhept-2-en-1-yl)-2,4-dihydroxy-6-pentylbenzoic Acid (**2d**).* The synthetic procedure followed that of **2a** starting from **1d**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (15:85) to furnish the pure compound **2d** (85 mg, 36%) as an orange solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.89 (s, 1H), 6.27 (d, J = 2.9 Hz, 1H), 6.05 (d, J = 62.6 Hz, 1H), 5.27 (dddt, J = 7.2, 5.4, 2.7, 1.3 Hz, 1H), 3.42 (d, J = 7.2 Hz, 2H), 2.91−2.84 (m, 2H), 2.06−1.98 (m, 2H), 1.77 (dd, J = 33.0, 1.4 Hz, 3H), 1.59−1.55 (m, 2H), 1.49 (dt, J = 13.3, 6.7 Hz, 1H), 1.38−1.25 (m, 8H), 0.90−0.85 (m, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: δ 163.72, 160.63, 147.57, 140.17, 120.74, 111.58, 111.34, 103.17, 37.71, 37.22, 36.66, 32.08, 31.50, 27.93, 22.63, 22.59, 22.18, 16.44, 14.15. LCMS (ESI): m/z calc'd for $C_{20}H_{32}O_4$ [M − H] 305.2474; Found 305.2000.

*(E)-2,4-Dihydroxy-3-(3-methylhepta-2,6-dien-1-yl)-6-pentylbenzoic Acid (**2e**).* The synthetic procedure followed that of **2a** starting from **1e**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (15:85) to furnish the pure compound **2e** (90 mg, 39%) as an orange solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.89 (d, J = 5.6 Hz, 1H), 6.27 (s, 1H), 5.76 (ddt, J = 16.8, 10.1, 6.4 Hz, 1H), 5.31−5.25 (m, 1H), 5.04−4.92 (m, 2H), 3.42 (t, J = 6.8 Hz, 2H), 2.90−2.85 (m, 2H), 2.20−2.09 (m, 4H), 1.85−1.75 (m, 3H), 1.60−1.55 (m, 2H), 1.34 (s, 4H), 0.89 (q, J = 2.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.90, 163.72, 160.47, 147.56, 138.61, 138.26, 121.70, 114.91, 111.61, 111.32, 103.21, 39.08, 36.65, 32.16, 32.09, 31.50, 22.60, 16.32, 14.16. LCMS (ESI): m/z calc'd for $C_{20}H_{27}O_4$ [M − H] 331.1909; Found 331.2000.

*2,4-Dihydroxy-3-((2E,6Z)-3-methylocta-2,6-dien-1-yl)-6-pentylbenzoic Acid (**2f**).* The synthetic procedure followed that of **2a** starting from **1f**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (15:85) to furnish the pure compound **2f** (75 mg, 33%) as an off-orange solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.92 (s, 1H), 6.29 (d, J = 2.2 Hz, 1H), 5.93 (s, 1H), 5.51−5.43 (m, 1H), 5.38−5.28 (m, 1H), 3.45 (d, J = 7.1 Hz, 2H), 2.93−2.86 (m, 2H), 2.23−2.15 (m, 2H), 2.11 (t, J = 7.5 Hz, 2H), 1.84 (d, J = 1.3 Hz, 2H), 1.63−1.57 (m, 5H), 1.36 (m, 4H), 0.95−0.89 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.44, 163.55, 160.37, 147.30, 138.84, 130.45, 129.62, 125.34, 124.28, 121.33, 111.15, 39.29, 36.47, 31.91, 31.33, 25.09, 22.42, 21.97, 16.13, 13.97, 12.69. LCMS (ESI): m/z calc'd for $C_{21}H_{29}O_4$ [M − H] 345.4670; Found 345.3000.

*(E)-3-(3,7-Dimethylocta-2,6-dien-1-yl)-2,4-dihydroxy-6-pentylbenzoic Acid (CBGA **2g**).* The synthetic procedure followed that of **2a** starting from CBG **1g**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (15:85) to furnish the pure compound CBGA **2g** (85 mg, 37%) as an off-orange solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.89 (s, 1H), 6.27 (s, 1H), 5.27 (td, J = 6.7, 3.2 Hz, 1H), 5.04 (ddd, J = 8.3, 4.1, 2.6 Hz, 1H), 3.43 (d, J = 7.1 Hz, 2H), 2.89−2.86 (m, 2H), 2.07 (dq, J = 13.2, 7.0 Hz, 4H), 1.80 (d, J = 1.3 Hz, 3H), 1.66 (d, J = 1.5 Hz, 3H), 1.56 (s, 5H), 1.34 (dd, J = 7.1, 3.7 Hz, 4H), 0.90 (d, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 176.08, 163.70, 160.67, 147.57, 139.35, 132.16, 123.82, 121.35, 111.59, 111.40, 103.19, 39.80, 36.66, 32.09, 31.51, 26.43, 25.78, 22.60, 22.16, 17.80, 16.32, 14.16. LCMS (ESI): m/z calc'd for $C_{26}H_{41}O_2$ [M + H] 385.3106; Found 385.2500.

*(E)-2,4-Dihydroxy-3-(3-methyloct-2-en-1-yl)-6-pentylbenzoic Acid (**2h**).* The synthetic procedure followed that of **2a** starting from **1h**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (15:85) to furnish the pure compound **2h** (80 mg, 35%) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.88 (d, J = 1.8 Hz, 1H), 6.28 (d, J = 2.6 Hz, 1H), 5.96 (s, 1H), 5.33−5.24 (m, 1H), 3.44 (d, J = 7.1 Hz, 2H), 2.93−2.86 (m, 2H), 2.03 (t, J = 7.7 Hz, 2H), 1.78 (dd, J = 25.0, 1.4 Hz, 3H), 1.58 (s, 2H), 1.47−1.38 (m, 2H), 1.35 (tt, J = 5.9, 2.4 Hz, 5H), 1.30−1.21 (m, 3H), 0.95−0.86 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 176.12, 163.72, 160.65, 147.60, 139.99, 120.89, 111.59, 111.37, 103.17, 39.80, 36.66, 32.08, 31.63, 31.50, 27.62, 22.62, 22.59, 22.16, 16.34, 14.16, 14.14. LCMS (ESI): m/z calc'd for $C_{21}H_{31}O_4$ [M − H] 347.4830; Found 347.3500.

*(E)-2,4-Dihydroxy-6-pentyl-3-(3-phenylbut-2-en-1-yl)benzoic Acid (**2i**).* The synthetic procedure followed that of **2a** starting from **1i**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (15:85) to furnish the pure compound **2i** (75 mg, 33%) as an orange solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.90 (s, 1H), 6.27 (d, J = 2.5 Hz, 1H), 5.30−5.24 (m, 1H), 3.43 (d, J = 7.0 Hz, 2H), 2.89−2.85 (m, 2H), 2.11 (dt, J = 98.2, 7.6 Hz, 2H), 1.77 (dd, J = 31.7, 1.4 Hz, 3H), 1.60−1.55 (m, 2H), 1.44−1.28 (m, 12H), 0.90−0.86 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 176.09, 163.71, 160.62, 147.56, 139.88, 120.93, 111.61, 111.34, 103.20, 39.84, 36.65, 32.09, 31.81, 31.50, 29.08, 27.91, 22.70, 22.60, 16.33, 14.17, 14.16. LCMS (ESI): m/z calc'd for $C_{22}H_{33}O_4$ [M − H] 361.2378; Found 361.3000.

*3-((2E,6E)-3,7-Dimethylnona-2,6-dien-1-yl)-2,4-dihydroxy-6-pentylbenzoic Acid (**2j**).* The synthetic procedure followed that of **2a** starting from **1j**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (15:85) to furnish the pure compound **2j** (65 mg, 29%) as an off-orange solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 11.90 (s, 1H), 6.27 (s, 1H), 5.98 (s, 1H), 5.30−5.24 (m, 1H), 5.04 (ddt, J = 6.9, 4.1, 1.3 Hz, 1H), 3.42 (d, J = 7.2 Hz, 2H), 2.90−2.84 (m, 2H), 2.10 (t, J = 7.1 Hz, 2H), 2.08−2.05 (m, 2H), 1.98−1.93 (m, 2H), 1.81 (d, J = 1.3 Hz, 3H), 1.60−1.55 (m, 5H), 1.33 (dt, J = 7.2, 3.7 Hz, 4H), 0.94 (t, J = 7.4 Hz, 3H), 0.91−0.87 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 175.87, 163.71, 160.66, 147.54, 139.39, 137.63, 122.17, 121.33, 111.57, 111.40, 103.12, 39.82, 36.67, 32.38, 32.09, 31.52, 26.28, 22.60, 22.16, 16.36, 16.06, 14.17, 12.78 LCMS (ESI): m/z calc'd for $C_{23}H_{33}O_4$ [M−H] 373.5210; Found 373.4000.

*2,4-Dihydroxy-3-((2E,6E)-8-methoxy-3,7-dimethylocta-2,6-dien-1-yl)-6-pentylbenzoic Acid (**2k**).* The synthetic procedure followed that of **2a** starting from **1k**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (15:85) to furnish the pure compound **2k** (80 mg, 35%) as a brown solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.94 (s, 1H), 6.23 (s, 1H), 5.32 (td, J = 6.3, 3.1 Hz, 1H), 5.25 (tt, J = 6.1, 3.2 Hz, 1H), 3.78−3.76 (m, 2H), 3.40 (d, J = 7.1 Hz, 2H), 3.28 (s, 3H), 2.88−2.84 (m, 2H), 2.16 (q, J = 7.3 Hz, 2H), 2.09 (t, J = 7.3 Hz, 2H), 1.78 (d, J = 1.3 Hz, 3H), 1.61 (d, J = 1.4 Hz, 3H), 1.56 (s, 2H), 1.33 (dd, J = 7.2, 3.4 Hz, 4H), 0.89 (q, J = 2.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.15, 171.37, 163.68, 160.45, 147.25, 137.87, 132.32, 128.05, 122.00, 112.00, 111.27, 103.10, 78.89, 60.54, 57.54, 39.25, 36.68, 32.10, 31.55, 25.94, 22.60, 22.06, 21.17, 16.24, 14.29, 14.17, 13.92. LCMS (ESI): m/z calc'd for $C_{23}H_{33}O_5$ [M − H] 389.2327; Found 389.2000.

*3-((2E,6E)-3,7-Dimethylundeca-2,6,10-trien-1-yl)-2,4-dihydroxy-6-pentylbenzoic Acid (**2l**).* The synthetic procedure followed that of **2a** starting from **1l**. The obtained residue was purified by column chromatography over silica gel using EtOAc/Hexane (15:85) to furnish the pure compound **2l** (90 mg, 40%) as an orange solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.88 (s, 1H), 6.28 (s, 1H), 5.93 (s, 1H), 5.79 (ddt, J = 16.9, 10.2, 6.4 Hz, 1H), 5.30−5.26 (m, 1H), 5.08 (tt, J = 5.5, 2.8 Hz, 1H), 4.99 (dq, J = 17.1, 1.7 Hz, 1H), 4.92 (ddt, J = 10.2, 2.3, 1.2 Hz, 1H), 3.43 (d, J = 7.2 Hz, 2H), 2.91−2.86 (m, 2H), 2.14−2.03 (m, 8H), 1.82 (d, J = 1.3 Hz, 3H), 1.61−1.55 (m, 5H), 1.35 (p, J = 3.8 Hz, 4H), 0.91 (q, J = 3.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.87, 142.84, 138.89, 135.19, 123.96, 121.84, 114.30, 110.60, 108.41, 39.75, 39.05, 35.60, 32.37, 31.58, 30.90, 26.32, 22.64, 22.32, 16.30, 16.10, 14.13. LCMS (ESI): m/z calc'd for $C_{25}H_{35}O_4$ [M − H] 399.2535; Found 399.4000.

*2,4-Dihydroxy-6-pentyl-3-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)benzoic Acid (**2m**).* The synthetic procedure followed that of **2a** starting from **1m**. The obtained residue was purified by column chromatography over silica gel using EtOAc/

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Hexane (15:85) to furnish the pure compound **2m** (92 mg, 41%) as an orange solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.91 (s, 1H), 6.28 (d, J = 1.9 Hz, 1H), 5.29 (t, J = 7.3 Hz, 1H), 5.16−5.04 (m, 2H), 3.44 (d, J = 7.2 Hz, 2H), 2.94−2.85 (m, 2H), 2.14−1.95 (m, 8H), 1.82 (d, J = 3.5 Hz, 3H), 1.67 (s, 3H), 1.64−1.52 (m, 8H), 1.35 (p, J = 3.7 Hz, 4H), 0.93−0.87 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.60, 163.71, 160.62, 147.47, 139.36, 135.72, 131.40, 124.50, 124.45, 124.37, 123.64, 121.36, 121.33, 111.56, 111.34, 103.12, 39.79, 39.74, 36.67, 32.10, 32.07, 31.52, 26.74, 26.68, 26.38, 26.22, 25.82, 25.80, 23.46, 22.60, 22.16, 17.79, 17.73, 16.37, 16.34, 16.15, 14.17. LCMS (ESI): m/z calc'd for $C_{27}H_{40}O_4$ [M − H] 427.2853; Found 427.4000.

Antibacterial Activity Assays

All bioactivity assays were conducted in triplicate and values were determined at a minimum according to CLSI guidelines.[36] All bacterial strains were obtained from the American Type Culture Collection (ATCC) or the Biodefense and Emerging Infections Research Resources Repository (BEI Resources). The Gram-positive strains for which minimum inhibitory concentrations (MICs) were determined included *S. aureus* (ATCC 25923); *S. aureus*, MRSA (ATCC 700787); *S. epidermidis* (ATCC 12228); *E. faecalis*, VRE (ATCC 700802); and *B. subtilis* (ATCC 6051). The Gram-negative *E. coli* K12 strain was used as a control in the study. MIC testing against all strains was performed in Mueller-Hinton Broth (MHB) medium supplemented with 50 mg/L of calcium (MHBc) using NCCLS guidelines for broth microdilution methods and inoculum of 1×10^5−5×10^5 CFU/mL (CLSI: Performance Standards for Antimicrobial Susceptibility Testing. 29th ed.). The serial 2-fold dilutions of compounds ranged from 50 μM to 0.3 μM. Briefly, the overnight cultures were grown at 35 °C in MHBc, while the test analogs were serially diluted in MHBc (75 μL). The serially diluted media was then inoculated with 5×10^5 CFU/mL of bacterial cells (~75 μL) from an overnight culture, after which the culture plates were incubated with shaking at 35 °C for 20−24 h. Growth was evaluated using the absorbance at 600 nm. The lowest concentration causing 90% inhibition of microbial growth was defined as the MIC, and the concentration units were converted to μg/mL.

Minimum Bactericidal (MBC) Assay. After determining the MIC values for these CBG analogs, the untreated, 1× MIC well, 2× MIC well, and 4× MIC well were spot-plated onto tryptic soy agar (TSA) in technical triplicate. Then, the TSA plates were placed in a 37 °C incubator overnight before performing colony enumeration. The reduction in viability was calculated by comparing the treated wells to the initial concentration of the untreated control. MBC is defined as the concentration at which a drug causes a 3-log reduction (99.9%) in viability compared to the untreated cells. A drug is considered bactericidal if the MBC/MIC ratio is less than or equal to four, and it is considered bacteriostatic if the MBC/MIC ratio is greater than four.[37−39] Three biological replicates were performed for each compound (n = 3).

Cytotoxicity Assay

HEK293 cells were grown in cultured in DMEM media (Gibco) with 1% Penicillin-streptomycin (Sigma-Aldrich) at 37 °C and 5% CO_2 until 90% confluency in a 6 well culture plate (Greiner Bio-One). Cells were collected via forceful washing of media to dislodge cells. Cells were then seeded at a density of 4.0×10^4 per well in 100 μL of culture medium or PBS (Gibco) and allowed to recover for 24 h. The next day, the test compounds were added to the wells at a concentration of 25 μg/mL and 50 μg/mL and cells were allowed to interact with the test compounds for 24 h. Then 12.5 μL of the 10× resazurin reagent (AmBeed, final concentration 44 μM) was added to each well, and the cells were incubated for 4 h at 37 °C and 5% CO_2.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.6c00126.

1D-^{1}H NMR, 1D-^{13}C NMR, 2D-^{1}H−^{1}H COSY, 2D-^{1}H−^{13}C HSQC, and 2D-^{1}H−^{13}C HMBC spectroscopic data for all compounds, along with HPLC analytical data for representative compounds (PDF)

SMILES (CSV)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

CBC, cannabichromene; CBCA, cannabichromenic acid; CBCAS, CBCA synthase; CBD, cannabidiol; CBDA, cannabi-

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diolic acid; CBDAS, CBDA synthase; CBG, cannabigerol; CBGA, cannabigerolic acid; CBN, cannabinol; HEK293, human embryonic kidney cells; hVISA, heterogeneous vancomycin-intermediate *Staphylococcus aureus*; MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; SAR, structure−activity relationship; TLC, thin-layer chromatography; THC, Δ^9-Tetrahydrocannabinol; THCA, Δ^9-Tetrahydrocannabinolic acid; THCAS, THCA synthase; VISA, vancomycin-intermediate *Staphylococcus aureus*; VRE, vancomycin-resistant *Enterococcus faecalis*; VRSA, vancomycin-resistant *Staphylococcus aureus*

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