

Carvone derived cannabidiol enantiomers as novel anticonvulsants

Supplementary Material

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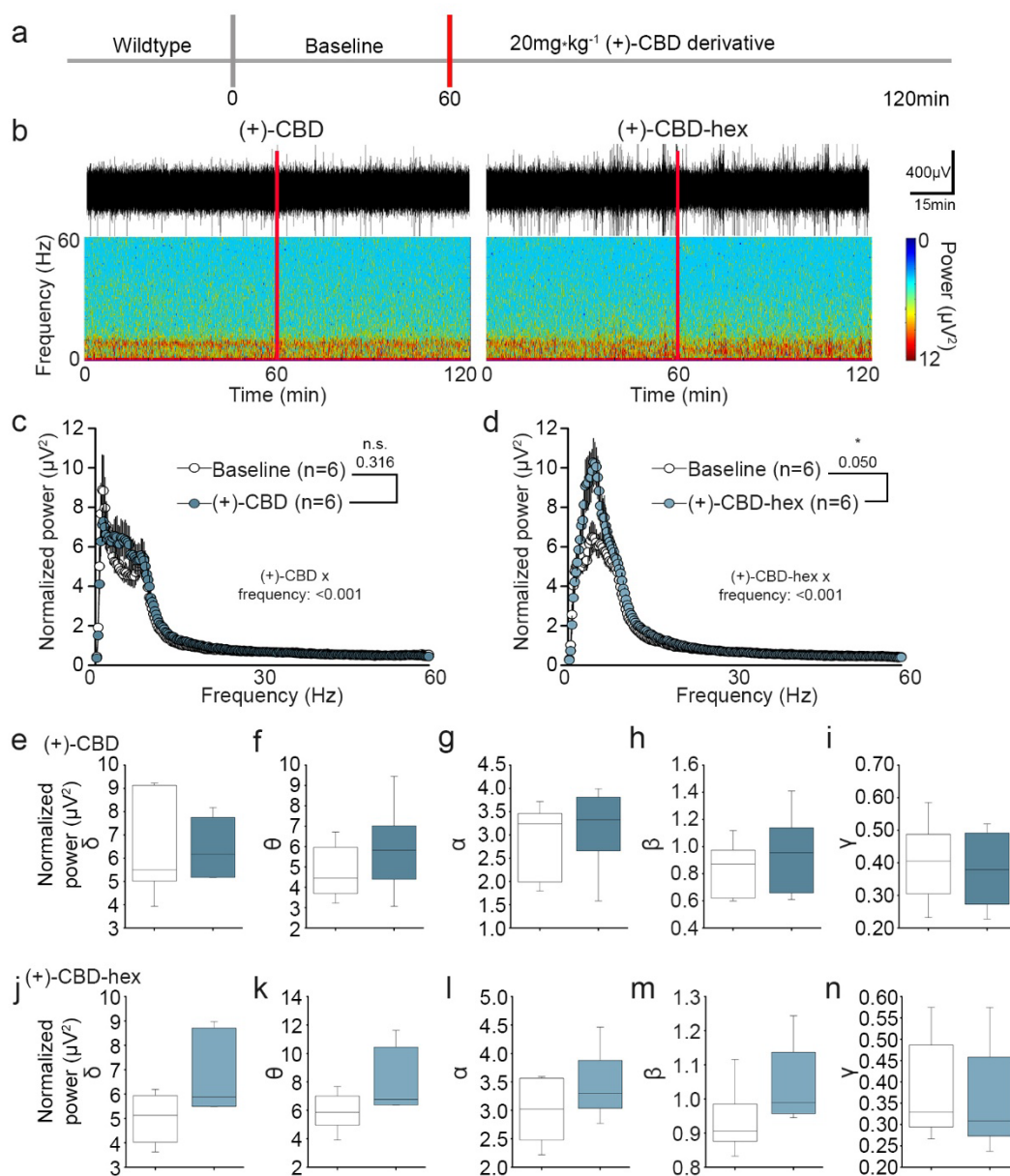
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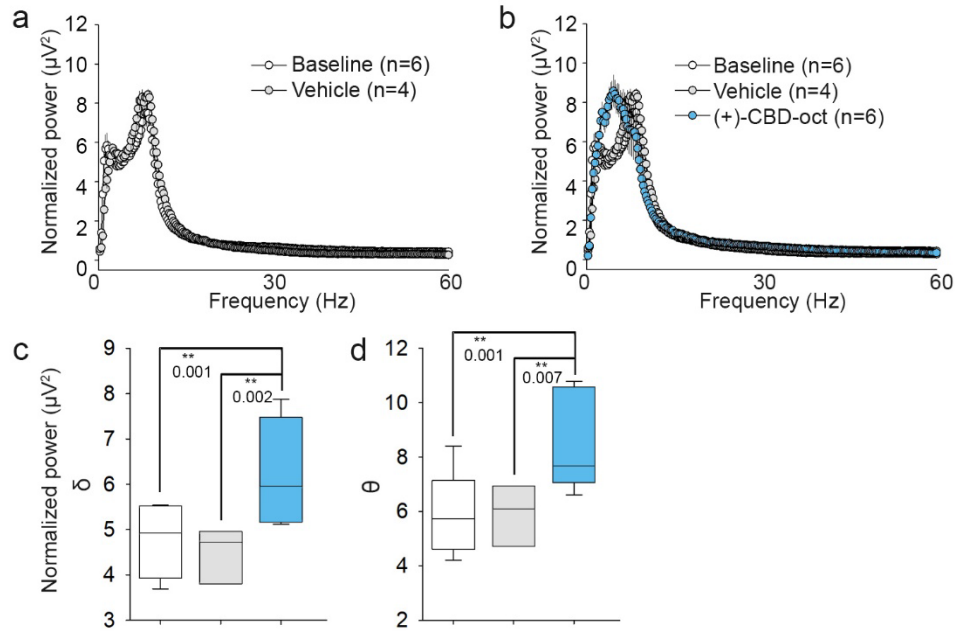
Keywords: cannabidiol, (+)-enantiomer, developmental epilepsy syndrome, refractory epilepsy

Supplementary Figure 1.

**Structure activity relationship (SAR) study of carvone (+)-CBD derivatives with intermediary alkyl chain lengths. a.**

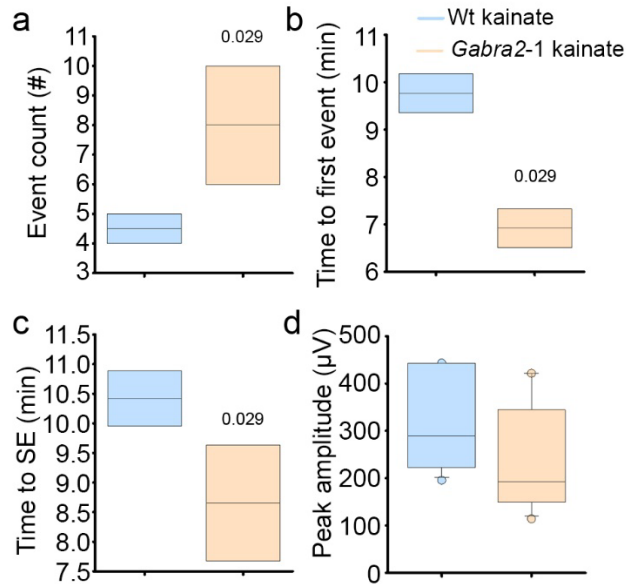
Timeline of experiment, red line denotes administration of (+)-CBD derivatives. **b.** Representative traces (top) and spectrograms (bottom) of EEG recordings following treatment with (+)-CBD derivatives. **c,d.** Fast Fourier transform analysis of the EEG signal comparing (+)-CBD (Baseline: 0.996 ± 0.0365 , (+)-CBD: 1.054 ± 0.0365) and (+)-CBD-hex (Baseline: 0.991 ± 0.0467 , (+)-CBD-hex: 1.160 ± 0.0467) to vehicle baseline. **e-i.** Spectral analysis of (+)-CBD (All bands n.s.). **j-n.** Spectral analysis of (+)-CBD-hex (All bands n.s). All plots shown and all values listed are mean (or LS mean) \pm standard error, *p* values from Two Way Repeated Measures ANOVA with Bonferroni post hoc; or t-test.

Supplementary Figure 2.



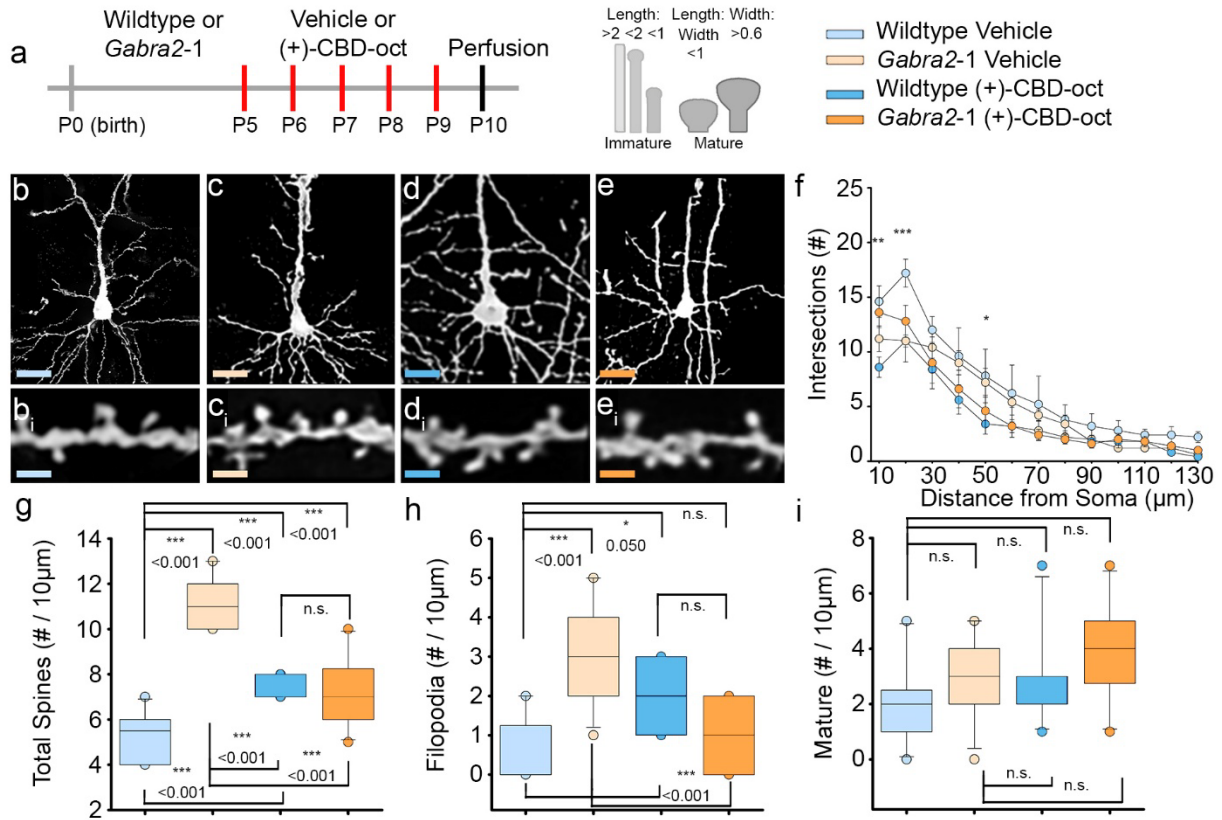
Baseline control and vehicle control comparison. a. Fast Fourier transform analysis of the EEG signal comparing Baseline (0.984 ± 0.0210) and Vehicle control ($n=4$; 0.970 ± 0.0522). **b.** Fast Fourier transform analysis of the EEG signal comparing Baseline (0.984 ± 0.0210) and Vehicle control (0.970 ± 0.0522) to (+)-CBD-oct (1.188 ± 0.0210). **c,d.** Spectral analysis of (+)-CBD-oct compared to Baseline and Vehicle Control for both δ (Baseline: 4.764 ± 0.226 ; Vehicle control: 4.493 ± 0.222 ; (+)-CBD-oct: 6.242 ± 0.313) and θ (Baseline: 5.918 ± 0.418 ; Vehicle control: 5.915 ± 0.407 ; (+)-CBD-oct: 8.407 ± 0.490). All plots shown and all values listed are mean (or LS mean) \pm standard error, p values from Two Way Repeated Measures ANOVA, or One Way ANOVA, with Bonferroni post hoc.

Supplementary Figure 3.



Seizure severity comparison between wildtype and *Gabra2-1* mice. **a.** Quantification of event count (Wildtype: 4.500 ± 0.577 ; *Gabra2-1*: 8.000 ± 2.309). **b.** Time to first seizure event (Wildtype: 9.764 ± 0.476 ; *Gabra2-1* Vehicle: 6.920 ± 0.475). **c.** Time to status epilepticus (SE) (Wildtype: 10.418 ± 0.539 ; *Gabra2-1*: 8.653 ± 1.130). **d.** Quantification of peak amplitude following kainate injection (Vehicle: 315.222 ± 98.307 .; *Gabra2-1* Vehicle: 239.470 ± 111.917). All plots shown and all values listed are mean \pm standard error, *p* values from t-test.

Supplementary Figure 4.

**Effect of (+)-CBD-oct in normalizing aberrant dendritic arborization, spine density, and morphology in the hippocampus.**

a. Timeline of experiment, schematic of dendritic spine morphology and classification, and legend. **b-e.** Representative hippocampal pyramidal cells impregnated with Golgi-Cox stain. **b_i-e_i.** Dendritic spine segments (10 μ m in length). **f.** Sholl analysis of dendritic arbor in hippocampal pyramidal cells (Wildtype vehicle: 6.877 ± 0.324 ; *Gabra2-1* vehicle: 4.154 ± 0.324 ; Wildtype (+)-CBD-oct: 5.062 ± 0.324 ; *Gabra2-1* (+)-CBD-oct: 4.769 ± 0.324). **g.** Total spine density per 10 μ m (Wildtype vehicle: 5.300 ± 0.335 ; *Gabra2-1* vehicle: 11.273 ± 0.333 ; Wildtype (+)-CBD-oct: 7.700 ± 0.153 ; *Gabra2-1* (+)-CBD-oct: 7.300 ± 0.473). **h.** Quantification of immature filopodia (Wildtype vehicle: 0.600 ± 0.267 ; *Gabra2-1* vehicle: 3.091 ± 0.415 ; Wildtype (+)-CBD-oct: 1.900 ± 0.277 ; *Gabra2-1* (+)-CBD-oct: 1.000 ± 0.298). **i.** Quantification of mature dendritic spines (Wildtype vehicle: 2.100 ± 0.458 ; *Gabra2-1* vehicle: 3.000 ± 0.467 ; Wildtype (+)-CBD-oct: 2.900 ± 0.504 ; *Gabra2-1* (+)-CBD-oct: 3.800 ± 0.533). All plots shown and all values listed are mean (or LS mean) \pm standard error, *p* values from Two Way Repeated Measures ANOVA or Two way ANOVA, with Bonferroni post hoc.

Supplementary Table 1. Comparison of the established effects of (-)-CBD to (+)-CBD and (+)-CBD-oct.

Domain	(-)-CBD (~20mgkg ⁻¹)	References	(+)-CBD (20mgkg ⁻¹)	(+)-CBD-oct (20 mgkg ⁻¹)
EEG – δ Band (0.5–4 Hz)	Mild to moderate increase	Nicholson et al., 2004; Devinsky et al., 2014	No significant effect	Moderate increase
EEG – θ Band (4–8 Hz)	Variable effects; generally neutral to slight increase	Gomes et al., 2011; Campos et al., 2012	No significant effect	Moderate increase
EEG – β Band (13–30 Hz)	Inconsistent; possible slight region dependent increase	Boggs et al., 2018; Laprairie et al., 2015	No significant effect	No significant effect
EEG – γ Band (30–100 Hz)	Inconsistent; possible reduction in hyperexcitable states	Taffe et al., 2015; Silva et al., 2017	No significant effect	No significant effect
Open Field – Mobility (Distance Traveled)	No significant effect	Kaplan et al., 2017; Boggs et al., 2018	Not tested	No significant effect
Seizure Suppression in Animal Models	Effective in MES, PTZ, Scn1a+/-	Devinsky et al., 2014; Kaplan et al., 2017	Not tested	Effective in Kainate, including in a susceptible model (<i>Gabra2-1</i>)
Dendritic Arborization	Normalizes reduced dendritic complexity	Kaplan et al., 2017; Silva et al., 2020	Not tested	Normalizes reduced dendritic complexity
Dendritic Spine Morphology	Reduces immature filopodia	Silva et al., 2020; Gobira et al., 2020	Not tested	Reduces immature filopodia

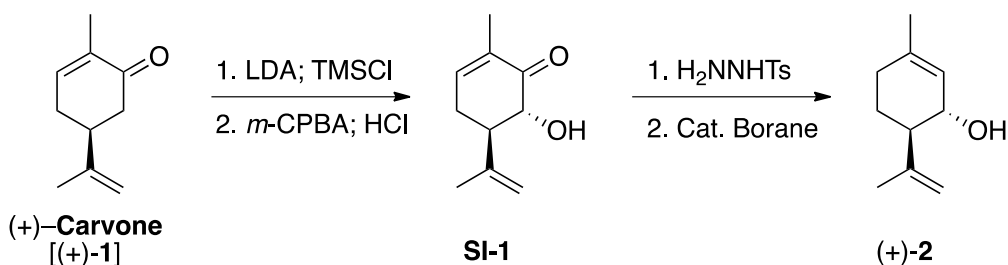
Supplementary Table 2. Summary of Statistics, see enclosed excel spreadsheet.

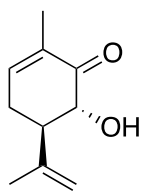
Supplementary Methods

Library Synthesis

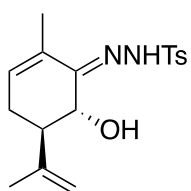
Unless otherwise noted, reactions were performed in flame-dried glassware under an atmosphere of dry nitrogen. Reaction solvents (CH₂Cl₂, THF, and Et₂O) were purified before use in a Glass Contour Solvent Purification System under a flow of dry nitrogen. All other solvents and reagents were purchased from Sigma-Aldrich and used as received, unless otherwise specified. Thin-layer chromatography (TLC) was performed using plates precoated with silica gel 60 Å F- 254 (250 μm) purchased from Silicycle and visualized by UV light, KMnO₄ or anisaldehyde stains, followed by heating. Silicycle SilicaFlash[®] P60 silica gel (particle size 40-63 μm) or Silicycle Brand disposable columns were used for flash chromatography. ¹H and ¹³C NMR spectra were recorded on a Bruker Advance 500 (operating at 500 MHz and 125 MHz, respectively), and are reported relative to residual solvent peak (δ 7.26 for ¹H and δ 77.0 for ¹³C in CDCl₃). Data for ¹H NMR spectra are reported as follows: chemical shift (δ ppm) [multiplicity, coupling constant (Hz), integration]. Spectra obtained are described using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. IR spectra were recorded on a Perkin Elmer Spectrum One FTIR Spectrometer and samples were prepared by evaporation from CHCl₃ or CH₂Cl₂ on NaCl plates. High-resolution mass spectra were obtained through positive electrospray ionization on a Q-ToF Micromass Spectrometer coupled with a Waters Acquity Ultra Performance LC.

Synthesis of Isopiperitenol:



**SI-1**

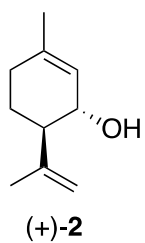
6*R*- α -Hydroxy Carvone [**SI-1**]. Was prepared according to the procedure found in *J. Med. Chem.* **2011**, *54*, 3866–3874. ^1H NMR (500 MHz, CDCl_3) δ = 6.75 (d, J = 6 Hz, 1H), 4.94 (s, 1H), 4.92 (s, 1H), 4.15 (d, J = 13 Hz, 1H), 2.70 (dt, J = 5, 11 Hz, 1H), 2.51-2.45 (m, 1H), 2.40-2.34 (m, 1H), 1.84 (s, 6H). All other data matches known literature values (*Chem. Eur. J.* **2019** *25* 2983–2988).

**SI-2**

Tosylhydrazone **SI-2**. Hydroxycarvone **SI-1** (0.743 g, 4.47 mmol, 1.0 equiv.) was added to a 250 mL round bottom flask and dissolved in CH_2Cl_2 (55 mL). To this solution, tosylhydrazide (0.998 g, 5.36 mmol, 1.2 equiv.) was added in a single portion followed by the drop wise addition of acetic acid (0.17 mL, 3.13

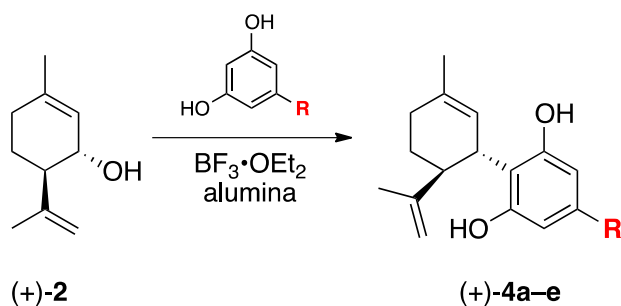
mmol, 0.7 equiv.) and concentrated hydrochloric acid (0.1 mL, 4.08 mmol, 0.9 equiv.). The solution was then heated to reflux (40 °C) and allowed to stir for 48h. After the disappearance of starting material was noted *via* TLC analysis, the reaction mixture was quenched by the addition of a 1N aqueous solution of HCl (50 mL) and transferred to a separatory funnel where the aqueous layer was washed with CH_2Cl_2 (ca. 3x 50 mL). The combined organic layers were then washed with a saturated aqueous solution of NaHCO_3 (ca. 50 mL), followed by brine (ca. 50 mL) before being dried over MgSO_4 , filtered, and concentrated *in vacuo*. The crude product was purified *via* flash column chromatography (15% EtOAc / 85% Hexanes to 30% EtOAc / 70% Hexanes) to afford the desired product **SI-2** (1.100 g, 74% yield) as a thick yellow foam:

$[\alpha]_D^{23}$ = -90.9 (c = 0.4, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ = 10.30 (br. s, 1H), 7.84 (d, J = 8z Hz, 2H), 7.29 (d, J = 8 Hz), 5.97 (br. d, 1H), 5.02 (s, 1H), 4.91 (s, 1H), 4.44 (d, J = 11 Hz, 1H), 2.43-2.51 (m, 1H), 2.42 (s, 3H), 2.13-2.17 (m, 2H), 1.79 (s, 3H), 1.72 (s, 3H); ^{13}C NMR (125 MHz, CDCl_3) 149.5, 143.6, 143.3, 135.8, 133.1, 131.0, 129.4, 127.9, 115.5, 70.5, 51.1, 28.3, 21.6, 18.8, 18.1; IR (neat, thin film) 3470, 3194, 2922, 2857, 1646, 1597, 1450, 1328, 1169, 1030 cm^{-1} ; HRMS (ESI) m/z : $[\text{M}+\text{Na}]^+$ calc'd for $\text{C}_{17}\text{H}_{22}\text{O}_3\text{N}_2\text{SNa}$ 357.1248; found 357.1241 m/z .

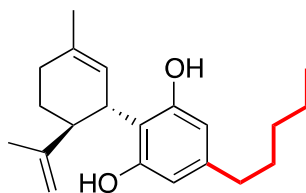


1S-(-)-Isopiperitenol **[2]**. Tosylhydrazone **SI-2** (0.053 g, 0.158 mmol, 1.0 equiv.) was added to a flame dried 25 mL round bottom flask and dissolved in CH_2Cl_2 (3 mL). To this solution at 0 °C was added catecholborane (0.037 mL, 0.347 mmol, 2.2 equiv.) drop wise over the course of 2 min. After the addition was complete, the reaction mixture was allowed to stir for 1 h before $\text{NaOAc}\cdot\text{XH}_2\text{O}$ (0.078 g, 0.573 mmol, 3.6 equiv.) was added in a single portion. After an additional 1 h of stirring, a reflux condenser was attached and the mixture was heated to reflux (40 °C) overnight. After the disappearance of starting material was noted *via* TLC analysis, the reaction mixture was passed over a pad a celite and concentrated *in vacuo*. The crude product was purified *via* flash column chromatography (5% EtOAc / 95% Hexanes to 15% EtOAc / 85% Hexanes) to afford the desired product **2** (0.021 g, 88% yield) as a light yellow oil: ^1H NMR (500 MHz, CDCl_3) δ = 5.44 (s, 1H), 4.89 (s, 1H), 4.85 (s, 1H), 4.11 (d, J = 8.5 Hz, 1H), 2.05-2.09 (m, 2H), 1.93 (dd, J = 4.5, 17.5 Hz, 1H), 1.73 (s, 3H), 1.69 (s, 3H), 1.56-1.64 (ddd, J = 5.5, 12, 24.5 Hz, 2H). All other data matches known literature values (*J. Nat. Prod.* **2018** *81*, 1546–1552).

Synthesis of CBD analogs:



CBD-2 =

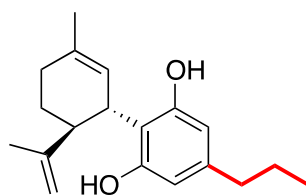


(+)-4b [ent-(+)-CBD]

CBD-2 / (+)-*ent*-CBD [(+)-**4b**] = **(+)-CBD**. Basic alumina (0.920 g, 9.032 mmol, 25.0 equiv.) was added to a flame dried 10 mL roundbottom flask and suspended with CH₂Cl₂ (1.5 mL). A reflux condenser was attached to the flask before BF₃•OEt₂ (0.133 mL, 1.083 mmol, 3.0 equiv.) was added drop wise through the top of the condenser. After being allowed to stir 15 min, the flask was lowered into a pre-heated oil bath and the contents of the flask were boiled for ca.

1 min. To this solution, was added a solution of olivetol (0.078 g, 0.433 mmol, 1.2 equiv.) and 1S-(–)-Isopiperitenol [**2**, 0.055 g, 0.361 mmol, 1.0 equiv.] in CH₂Cl₂ (1.5 mL) as quickly as possible *via* cannula. After 10 sec, the reaction was quenched by the addition of 2 mL of a saturated aqueous solution of NaHCO₃. After cooling to room temperature, the contents of the flask were passed over a pad of celite before being added to a separatory funnel and extracted using CH₂Cl₂ (ca. 10 mL). The combined organic layers were then washed with brine (ca. 20 mL) before being dried over MgSO₄, filtered, and concentrated *in vacuo*. The crude product was purified *via* flash column chromatography (10% EtOAc / 90% Hexanes to 30% EtOAc / 70% Hexanes) to afford the desired product **4b** (0.0245 g, 22% yield) as an amorphous white solid. Characterization data for (+)-**4b** (known): ¹H NMR (500 MHz, CDCl₃) δ = 6.27 (br. s, 1H), 6.17 (br. s, 1H), 5.97 (br. s, 1H), 5.57 (s, 1H), 4.69 (br. s, 1H), 4.66 (s, 1H), 4.56 (s, 1H), 3.85 (br. d, *J* = 8.5 Hz), 2.44 (t, *J* = 8 Hz, 2H), 2.40 (dt, *J* = 3, 11 Hz, 1H), 2.20-2.26 (m, 1H), 2.07-2.12 (m, 1H), 1.79 (s, 3H), 1.76-1.84 (m, 2H), 1.65 (s, 3H), 1.53-1.59 (m, 2H), 1.25-1.32 (m, 4H), 0.88 (t, *J* = 7 Hz, 3H). All other data matches known literature values (*Org. Biomol. Chem.* **2005**, 3, 1116–1123).

CBD-1 =



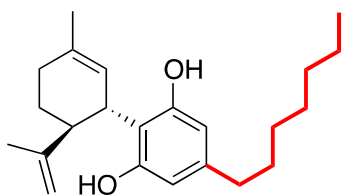
(+)-4a [ent-(+)-CBDV]

CBD-1 / (+)-*ent*-CBDV [(+)-**4a**] = **(+)-CBDV**. Was prepared in a similar manner to (+)-*ent*-CBD [(+)-**4b**] by substituting olivetol with 5-propylbenzene-1,3-diol (0.059 g, 0.394 mmol, 1.2 equiv.). The crude product was purified *via* flash column chromatography (10% EtOAc / 90% Hexanes to 30% EtOAc / 70% Hexanes) to afford the desired product (+)-**4a** (0.0349 g, 37% yield) as an amorphous white solid. Characterization data for (+)-**4a**: [α]_D²³ = +64.0 (c = 1.9,

CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ = 6.28 (br. s, 1H), 6.16 (br. s, 1H), 5.99 (br. s, 1H), 5.57 (s, 1H), 4.72 (br. s, 1H), 4.65 (s, 1H), 4.55 (s, 1H), 3.85 (br. d, *J* = 10 Hz, 1H), 2.42 (t, *J* = 7.5 Hz, 2H), 2.36-2.38 (m, 1H), 2.10-2.26 (m, 1H), 2.07-2.11 (m, 1H),

1.79 (s, 3H), 1.73-1.83 (m, 2H), 1.65 (s, 3H), 1.58 (q, $J = 7.5$ Hz, 2H), 0.90 (t, $J = 7$ Hz, 3H) ; ^{13}C NMR (125 MHz, CDCl_3) $\delta =$ 156.2, 154.0, 149.5, 142.8, 140.2, 124.2, 123.8, 113.9, 110.9, 107.7, 46.2, 37.7, 37.3, 30.5, 28.5, 24.1, 23.8, 20.6, 13.9 ; IR (neat, thin film) 3435 (br.), 2865-2965, 1629, 1582, 1448 cm^{-1} ; HRMS (ESI) m/z : $[\text{M}+\text{K}]^+$ calc'd for $\text{C}_{19}\text{H}_{26}\text{O}_2\text{K}$ 325.2916; found 325.1598 m/z .

CBD-4 =

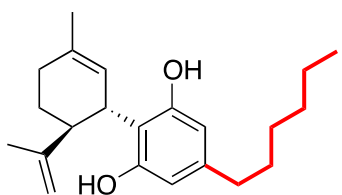


(+)-4d [ent-(+)-CBDP]

CBD-4 / (+)-*ent*-CBDP [(+)-**4d**] = **(+)-CBDP**. Was prepared in a similar manner to (+)-*ent*-CBD [(+)-**4a**] by substituting olivetol with 5-heptylbenzene-1,3-diol (0.0782 g, 0.375 mmol, 1.2 equiv.). The crude product was purified *via* flash column chromatography (10% EtOAc / 90% Hexanes to 30% EtOAc / 70% Hexanes) to afford the desired product (+)-**4d** (0.0232 g, 22% yield) as an amorphous white solid. Characterization data for (+)-**4b**: $[\alpha]_D^{23} = +126.0$

($c = 0.02$, MeCN); ^1H NMR (500 MHz, CDCl_3) $\delta =$ 6.28 (br. s, 1H), 6.16 (br. s, 1H), 5.97 (br. s, 1H), 5.56 (s, 1H), 4.66 (s, 1H), 4.59 (br. s, 1H), 4.55 (s, 1H), 3.84 (br. d, $J = 8.5$ Hz, 1H), 2.43 (t, $J = 8$ Hz, 2H), 2.39 (dt, $J = 5, 10.5$ Hz, 1H), 2.19-2.26 (m, 1H), 2.06-2.12 (m, 1H), 1.79 (s, 3H), 1.72-1.83 (m, 2H), 1.65 (s, 3H), 1.52-1.57 (m, 3H), 1.24-1.29 (m, 7H), 0.87 (t, $J = 7$ Hz, 3H) ; ^{13}C NMR (125 MHz, CDCl_3) $\delta =$ 156.2, 153.9, 149.5, 143.1, 140.2, 124.2, 113.8, 110.9, 109.9, 108.1, 46.2, 37.4, 35.6, 31.9, 31.1, 30.5, 29.4, 29.3, 28.5, 23.8, 22.8, 20.6, 14.2 ; IR (neat, thin film) 3433 (br.), 2855-2958, 1629, 1584, 1443 cm^{-1} ; HRMS (ESI) m/z : $[\text{M}]^+$ calc'd for $\text{C}_{23}\text{H}_{35}\text{O}_2$ 343.2639; found 343.2639 m/z .

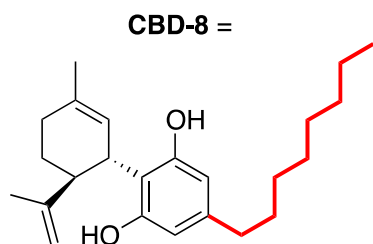
CBD-3 =



(+)-4c [ent-(+)-CBD-Hex]

CBD-3 / (+)-*ent*-CBD-Hex [(+)-**4c**] = **(+)-CBD-hex**. Was prepared in a similar manner to (+)-*ent*-CBD [(+)-**4a**] by substituting olivetol with 5-hexylbenzene-1,3-diol (0.061 g, 0.313 mmol, 1.0 equiv.). The crude product was purified *via* flash column chromatography (10% EtOAc / 90% Hexanes to 30% EtOAc / 70% Hexanes) to afford the desired product (+)-**4c**

(0.036 g, 35% yield) as an amorphous white solid. Characterization data for (+)-**4c**: $[\alpha]_D^{23} = +66.86$ (c = 0.3, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ = 6.28 (br. s, 1H), 6.16 (br. s, 1H), 5.98 (br. s, 1H), 5.57 (s, 1H), 4.69 (br. s, 1H), 4.66 (s, 1H), 4.55 (s, 1H), 3.85 (br. d, *J* = 10 Hz, 1H), 2.43 (t, *J* = 8 Hz, 2H), 2.36-2.40 (m, 1H), 2.07-2.26 (m, 2H), 1.79 (s, 3H), 1.71-1.82 (m, 2H), 1.65 (s, 3H), 1.53-1.57 (m, 2H), 1.25-1.30 (m, 6H), 0.86-0.89 (m, 3H); ¹³C NMR (125 MHz, CDCl₃) δ = 154.7, 154.1, 149.4, 143.0, 140.0, 124.1, 113.7, 110.8, 110.0, 107.5, 46.1, 37.2, 35.5, 31.7, 30.9, 30.4, 28.9, 28.4, 23.7, 22.6, 20.5, 14.1; IR (neat, thin film) 3444 (br.), 2853-2961, 1629, 1582, 1446, 1028 cm⁻¹; HRMS (ESI) m/z: [M+Na]⁺ calc'd for C₂₂H₃₂O₂Na 351.2288 m/z.



(+)-**4e** [*ent*-(+)-**CBD-oct**]

CBD-8 / (+)-*ent*-CBD-Oct [(+)-**4e**] = **(+)-CBD-oct**. Was prepared in a similar manner to (+)-*ent*-CBD [(+)-**4a**] by substituting olivetol with 5-octylbenzene-1,3-diol (0.086 g, 0.394 mmol, 1.2 equiv.). The crude product was purified *via* flash column chromatography (10% EtOAc / 90% Hexanes to 30% EtOAc / 70% Hexanes) to afford the desired product (+)-**4e** (0.0324 g, 28% yield) as an amorphous white solid. Characterization data for (+)-

4e: $[\alpha]_D^{23} = +71.64$ (c = 0.1, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ = 6.27 (br. s, 1H), 6.15 (br. s, 1H), 5.97 (br. s, 1H), 5.56 (s, 1H), 4.65 (s, 1H), 4.55 (s, 1H), 3.84 (br. d, *J* = 8.5 Hz, 1H), 2.43 (t, *J* = 8 Hz, 2H), 2.39 (dt, *J* = 2.5, 11 Hz, 1H), 2.20-2.25 (m, 1H), 2.06-2.12 (m, 1H), 1.79 (s, 3H), 1.75-1.83 (m, 2H), 1.65 (s, 3H), 1.20-1.60 (m, 12H), 0.87 (t, *J* = 6.5 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ = 156.2, 154.0, 149.5, 143.1, 140.2, 124.2, 113.8, 100.9, 109.9, 108.0, 46.2, 37.4, 35.6, 32.0, 31.1, 30.5, 29.6, 29.4, 29.4, 28.5, 23.8, 22.8, 20.6, 14.2; IR (neat, thin film) 3443 (br.), 2855-2955, 1628, 1581, 1445 cm⁻¹; HRMS (ESI) m/z: [M]⁺ calc'd for C₂₄H₃₇O₂ 357.2795; found 357.2785 m/z.