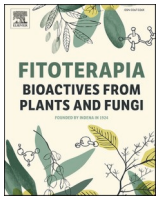
Fitoterapia 181 (2025) 106354

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote



Biomimetic synthesis of rare cannabigerol-type cannabinoids and evaluation of their cytotoxic effect on human glioblastoma cell lines

Martina Giangrossi [a], Stefano Salamone [b], Aurora Camola [b], Orazio Taglialatela-Scafati [c,1], Giuseppina Chianese [c], Ernesto Gargiulo [c], Massimo Nabissi [a,*], Federica Pollastro [b,*]

[a] School of Pharmacy, Department of Experimental Medicine, University of Camerino, Via Madonna delle carceri, 9, 62032 Camerino, MC, Italy
[b] Department of Pharmaceutical Sciences, University of Piemonte Orientale, Largo Donegani 3, 28100 Novara, NO, Italy
[c] Department of Pharmacy, University of Naples Federico II, via Domenico Montesano 49, 80131 Naples, Na, Italy

ARTICLE INFO

Keywords:
Enzymatic promiscuity
Cannabis sativa
Minor cannabinoids
Phytocannabinoids
Human glioblastoma

ABSTRACT

Although *Cannabis sativa* L. is well known for being prolific in phytocannabinoids, their biosynthetic modular mechanism is ruled by a main enzyme: the geranyltransferase able to pursue the *C*-isoprenylation of olivetolic acid with the geranyldiphosphate. However, the existence of more than 160 meroterpenoids can be partially explained by a side degree of promiscuity of the geranyltransferase itself, able to recognise different substrate than the ordinary ones. This biological process led to the identification of several unconventional phytocannabinoids with a limited distribution in the plant both for occurrence and concentration. Taking advance of the existing synthetic protocols and using as example the enzymatic promiscuity, we propose a bio-inspired synthesis of naturally occurring minor cannabinoids related to the cannabigerol-type and their preliminary biological inspection in U87, U251 and T98 human glioblastoma cell lines to investigate their potential contribute as supplement in anticancer therapy.

1. Introduction

The relevance of *Cannabis sativa* L. is rising at exponential grade fostered by the recent progress in academic and clinical field [1]. Pharmacologically, research is undoubtedly focused on cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (Δ^9-THC). On the other side, the requirement of pure CBD and Δ^9-THC along with the deep phytochemical investigation of different chemotypes of the plant, led to discovery several minor phytocannabinoids which interest is rapidly growing together with their medicinal evaluation [2].

The modular mechanism ruling the phytocannabinoids metabolism consists in the *C*-isoprenylation of the polyketide core olivetolic acid with the geranyldiphosfate by the enzyme geranyldiphosphate:olivetolate geranyltransferase identifying cannabigerolic acid (CBGA) as the first phytocannabinoid with the lowest degree of oxidation. The secondary action of oxidative cyclase on the linear terpenyl moiety leads to cannabichromenic acid (CBCA), cannabidiolic acid (CBDA) and tetrahydrocannabinolic acid (Δ^9-THCA) from CBGA (Fig. 1) [3]. However, the astonishing ability of cannabis to biosynthesise more than 160 meroterpenoids can be partially endowed by the enzymatic promiscuity of the geraniltransferase able to recognise different starting moieties composing the primary phytocannabinoid scaffolds: the *para*-oriented isoprenyl residue, the resorcinoid core with different aliphatic side chains (Fig. 1) [3,4].

It has been reported that the enzyme geranyltransferase, in addition to gernyldiphosphate, can recognise its oligomers neryldiphosphate and the (2*E*, 6*E*)-farnesyldiphosphate as isoprenyl substrate with the resulting cannabinerol **1** (CBNR) [5,6] and sesqui-CBG **2** [7]. Moreover, the characterization of viridines, the propyl-cannabinoids, demonstrated that these compounds are the result of the *C*-isoprenylation by the geranyltransferase with another polyketide precursor: the divarinolic acid [8,9]. Same metabolic approach can be assumed for the CBG **3** butyl homologues cannabigerobutol **4** (CBGB) [10], Δ^9-tetrahydrocannabutol, and cannabidibutol [11]. Finally, the recent identification of the cannabidiphorol and Δ^9-tetrahydrocannabiphorol with a *n*-heptyl side chain suppose the existence of cannabigerophorol **5** (CBGP)

* Corresponding authors.
E-mail addresses: massimo.nabissi@unicam.it (M. Nabissi), Federica.pollastro@uniupo.it (F. Pollastro).

[1] Given his role as Editor-in-Chief, Orazio Taglialatela Scafati had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Xue Qiao.

https://doi.org/10.1016/j.fitote.2024.106354
Received 29 July 2024; Received in revised form 11 November 2024; Accepted 13 December 2024
Available online 22 December 2024

[12].

The chemical diversity of these unusual phytocannabinoids could be a useful tool to investigate a biological vast area still unexplored. Anyway, these unconventional compounds have a limited distribution in *Cannabis sativa* both for occurrence and concentration [2], and an isolation process from vegetable material is not convenient. Taking advance of the synthesis proposed by Jentsch and colleagues [13] and using as example the enzymatic promiscuity, we propose a bio-inspired synthesis of rare cannabinoids (Fig. 2) related to the CBG-type as alternative strategy to provide sufficient material to support biological inspection. The synthetized cannabinoids have been tested in U87, U251 and T98 human glioblastoma (GBM) cell lines to investigate their potential contribute as supplement in anticancer therapy, as suggested by the results obtained from recent clinical trials on glioblastoma treatment with a combination of Δ^9-THC and CBD [14].

2. Materials and methods

2.1. General experimental procedures

Silica gel 60 (0.063–0.200 mm) and Celite® 545 (0.02–0.1 mm), used for low-pressure liquid chromatography (LPC), were purchased from Macherey-Nagel (Düren, Germany), acidic alumina SepaFlash® 50–70 μm was purchased from Sepachrom srl (Rho, Italy). Purifications were monitored by TLC 60 F254 (0.25 mm) plates purchased from Merck (Darmstadt, Germany) and visualized by staining with 5 % H_2SO_4 in EtOH and heating. Organic solvents and reagents were purchased from Sigma-Aldrich (Milan, Italy). A HPLC JASCO Hichrom silica (250 × 25 mm), UV–vis detector-2075 plus (Oklahoma, Japan) was used. ^{1}H (400 and 600 MHz) and ^{13}C NMR (100 MHz) spectra were measured on Bruker 400 spectrometers (Bruker, Billerica, MA, USA). Chemical shifts were referenced to the residual solvent signal ($CDCl_3$: $\delta_H = 7.26$, $\delta_C = 77.0$).

2.2. MTT assay

3 × 104 cells/mL were seeded in a 96-well plate. The following day, compounds or vehicles were added, and six replicates were used for each treatment. At 24 h post-treatment, cell viability was analyzed, by MTT assay. The absorbance of the sample was measured at 570 nm using an ELISA reader microliter plate (BioTek Instruments, Winooski, VT, USA).

Statistical Analysis. The data presented represent the mean and standard deviation (SD) of at least 3 independent experiments. The statistical significance was determined by two-way Anova followed by Dunnett's multicomparison test using the GraphPad 9.0.1 software.

3. Synthesis of synthons

3.1. Synthesis of 5-butyl resorcinol **17**

1-iodo-3,5-dimethoxybenzene ***12***. To a solution of 3,5-dimethoxyaniline (**11**, 6.0 g, 1 M equival) in water (60 mL), conc. H_2SO_4 (6.0 mL) and $NaNO_2$ (4.05 g, 1.5 M equival) were added at 0 °C and stirred for 20 min before dilution with diethyl ether and addition of KI (20 g, 117.5 mmol). The reaction is then stirred at RT for 12 h, followed by TLC (silica, Pe/EtOAc 90:10) monitoring the disappearance of **11** and quenched by dilution with 25 % $Na_2S_2O_3$ solution, HCl 1 M and NaOH 2 M and extracted with diethyl ether. The organic phase is dried (Na_2SO_4), filtered and evaporated at reduced pressure. The residue is purified by LPC on silica (200 g, petroleum ether-EtOAc gradient from 100:0 to 80:20) to afford 6.80 g of 1-iodo-3,5-dimethoxybenzene (**12**, 65 % based on conversion of **11** as white powder).

1-iodo-3,5-dimethoxybenzene ***12***: white powder; formula $C_8H_9IO_2$; MW = 264.06; ^{1}H NMR (400 MHz; $CDCl_3$): 6.88 (2H, d, $J = 2.7$ Hz, H-2, H-6); 6.43 (1H, t, $J = 2.1$ Hz, H-4); 3.79 (6H, s, OCH_3).

1-[2-(trimethylsilyl)ethinyl]-3,5-dimethoxybenzene ***13***. To a solution of 1-iodo-3,5-dimethoxybenzene (**12**, 6.80 g, 1 M equival) in toluene (100 mL), in the following order are added: CuI (489 mg, 0.1 M equival), TEA (75 mL, 22 M equival), TMSA (10.79 mL, 3 M equival) and $Pd(PPh_4)_3$ (893 mg, 0.03 M equival). The reaction is stirred at 60 °C, followed by TLC (silica, petroleum ether-EtOAc 90:10) monitoring the disappearance of **12**. After 24 h, the reaction is quenched by dilution with sat. NH_4Cl, the organic phase is dried (Na_2SO_4), filtered and evaporated at reduce pressure. The residue is purified by LPC on silica (160 g,

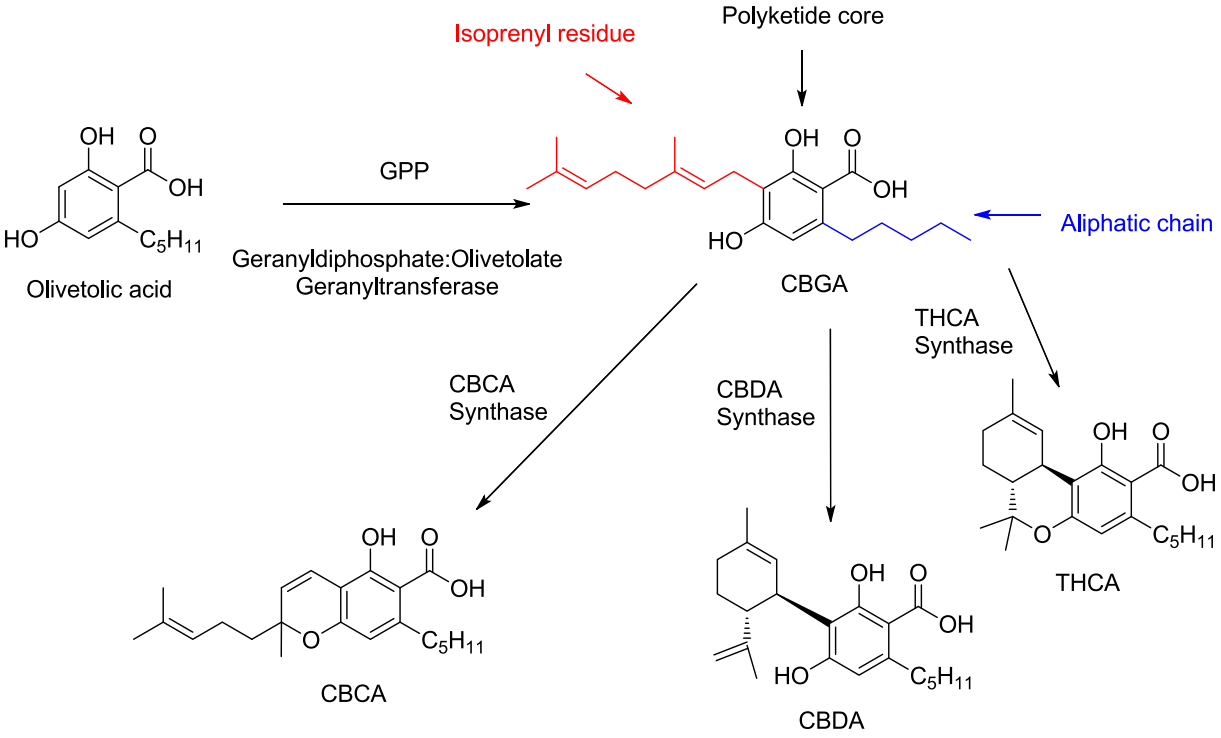


Fig. 1. Biosynthesis of CBGA, CBCA, CBDA and D9-THCA.

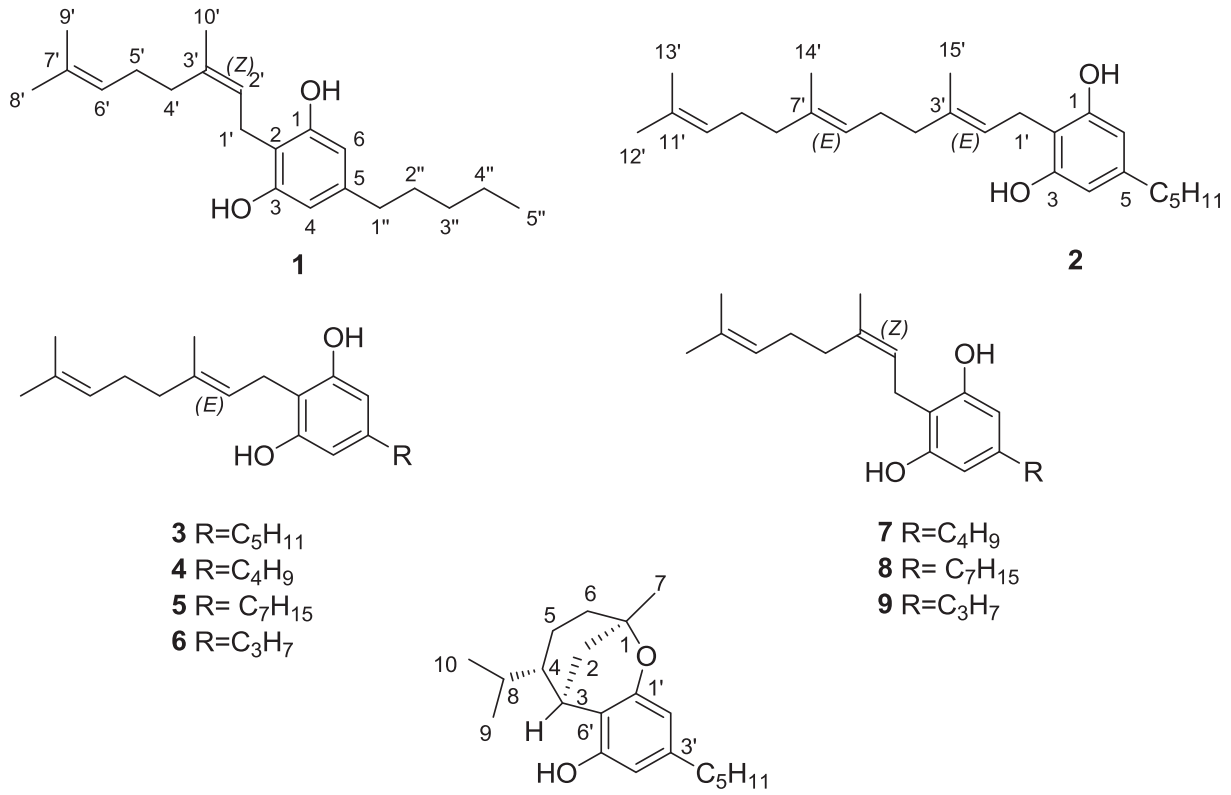


Fig. 2. Synthetic phytocabbaninoids: CBNR 1, sesqui-CBG 2, CBG 3, CBGB 4, CBGP 5, CBGV 6, CBNRB 7, CBNRP 8, CBNRV 9, isohexahydrocannabinol 10.

petroleum ether-EtOAc gradient from 100:0 to 80:20) to afford 4.65 g of 1-[2-(trimethylsilyl) ethinyl]-3,5-dimethoxybenzene (**13**, 77 % based on the conversion of 12 g).

*1-[2-(trimethylsilyl)ethinyl]-3,5-dimethoxybenzene **13**:* white powder; formula $C_{13}H_{18}O_2Si$; MW = 234.37; ^{1}H NMR (400 MHz; $CDCl_3$): 6.65 (2H, d, *J* = 2.9 Hz, H-2, H-6); 6.47 (1H, t, *J* = 2.7 Hz, H-4); 3.80 (6H, s, OCH_3); 0.28 (9H, s, $Si(CH_3)_3$).

*1-(2-ethinyl)-3,5-dimethoxybenzene **14***. To a solution of 1-[2-(trimethylsilyl) ethinyl]-3,5-dimethoxybenzene (**13**, 4.65 g, 1 M equival) in anhydrous MeOH (100 mL), 3.55 g of K_2CO_3 (3.55 g, 1.3 M equival) is added. The reaction is stirred at RT, followed by TLC (silica, petroleum ether-EtOAc 90:10) monitoring the disappearance of **13**. After 72 h, the reaction is quenched by dilution with sat. $NaHCO_3$. The organic phase is dried (Na_2SO_4), filtered and evaporated at reduce pressure. The residue is crystallized with petroleum ether to afford 2.73 g of 1-(2-ethinyl)-3,5-dimethoxybenzene (**14**, 85 % based on the conversion of **13**).

*1-(2-ethinyl]-3,5-dimethoxybenzene **14***: white powder; formula: $C_{10}H_{10}O_2$; MW = 162.19; ^{1}H NMR (400 MHz; $CDCl_3$): 6.68 (2H, s, H-2, H-6), 6.49 (1H, s, H-4), 3.78 (6H, s, OCH_3), 3.10 (1H, s, H-2′).

*1-(butin-1-yl)-3,5-dimethoxybenzene **15***. To a solution of $NH(i\text{-}Pr)_2$ (3.80 mL, 1.6 M equival) in dry THF (25 mL), BuLi 2 M (13.45 mL, 1.6 M equival) is added. The solution is stirred at RT and after 30 min 1-(2-ethinyl)-3,5-dimethoxybenzene (**14**, 2.73 g, 1 M equival) and EtI (3.66 mL, 2.7 M equival) are added dropwise. The reaction is stirred at RT, followed by TLC (silica, petroleum ether-EtOAc 95:5) monitoring the disappearance of **14**. After 72 h, the reaction is quenched by dilution with H_2SO_4 2 N. The organic phase is dried (Na_2SO_4), filtered and evaporated at reduce pressure. The residue is purified by LPC on silica (95 g, petroleum ether-EtOAc gradient from 100:0 to 95:5) to afford 2.40 g of 1-(butin-1-yl)-3,5-dimethoxybenzene (**15**, 76 % based on the conversion of **14**).

*1-(butin-1-yl)-3,5-dimethoxybenzene **15***: yellow oil; formula: $C_{12}H_{14}O_2$; MW = 90.24; ^{1}H NMR (400 MHz; $CDCl_3$): 6.60 (2H, s, H-2, H-6), 6.40 (1H, s, H-4), 3.70 (6H, s, OCH_3), 2.42 (2H, dd, *J* = 7.6 Hz, H-3′), 1.26 (3H, t, *J* = 7.0 Hz, H-4′).

*1-butyl-3,5-dimethoxybenzene **16***. To a solution of 1-(butin-1-yl)-3,5-dimethoxybenzene (**15**, 2.40 g, 1 M equival) in MeOH/EtOAc 1:1 (140 mL), 10 % Pd/C 55–65 % wet is added in catalytic and the reaction is stirred under hydrogen. After 72 h the reaction is filtered on Celite® and evaporated under reduced pressure to afford 2.15 g of 1-butyl-3,5-dimethoxybenzene (**16**, 88 % based on the conversion of **15**).

*1-butyl-3,5-dimethoxybenzene **16***: yellow oil; formula: $C_{12}H_{18}O_2$; MW = 194.27; ^{1}H NMR (400 MHz; $CDCl_3$): 6.36 (2H, s, H-2, H-6); 6.32 (1H, s, H-4); 3.81 (6H, s, OCH_3); 2.57 (2H, t, *J* = 7.8 Hz, H-1′); 1.61 (2H, td, *J* = 7.6, 15.2 Hz, H-2′); 1.37 (2H, tdt, *J* = 7.4, 7.4, 7.4 Hz, H-3′); 0.96 (3H, t, *J* = 7.6 Hz, H-4′).

*5-butyl resorcinol **17***. To a solution of 1-butyl-3,5-dimethoxybenzene (**16**, 2.15 g, 1 M equival) in dry dichloromethane (70 mL), BBr_3 1 M (33 mL, 3 M equival) is added dropwise. The reaction is stirred at RT, followed by TLC (silica, petroleum ether-EtOAc 80:20) monitoring the disappearance of **16**. After 12 h, the reaction is quenched by dilution with 1 M $NaHCO_3$. The organic phase is washed with HCl 1 M, dried (Na_2SO_4), filtered and evaporated at reduce pressure. The residue is purified by LPC on silica (60 g, petroleum ether-EtOAc gradient from 80:0 to 50:50) to afford 880 mg of 5-butyl resorcinol (**17**, 48 % based on the conversion of **16**).

*5-butyl resorcinol **17**:* brown solid; formula: $C_{10}H_{14}O_2$; MW = 166.22; ^{1}H NMR (400 MHz; $CDCl_3$): 6.38 (2H, s, H-4, H-6); 6.35 (1H, s, H-2); 2.47 (2H, t, *J* = 7.6 Hz, H-1′); 1.57–1.46 (2H, m, H-2′); 1.40–1.27 (2H, m, H-3′); 0.92 (3H, t, *J* = 7.4 Hz, H-4′).

3.2. Synthesis of 5-propyl resorcinol **20**

1-(1-propin-1-yl)-3,5-dimethoxybenzene ***18***. To a solution of $NH(i\text{-}Pr)_2$ (2.78 mL 1.6 M equival) in dry THF (50 mL), BuLi 2 M (9.86 mL, 1.6 M equival) is added. The reaction is stirred at 0 °C and, after 30 min, 1-(2-ethinyl)-3,5-dimethoxybenzene (**14**, 2.0 g, 1 M equival) and CH_3I (2.68 mL, 2.7 M equival) are added. The reaction followed the same procedure of **15** to afford 2.05 g of 1-(1-propin-1-yl)-3,5-dimethoxybenzene (**18**, 94 % based on the conversion of **14**).

1-(1-propin-1-yl)-3,5-dimethoxybenzene ***18:*** yellow oil; formula: $C_{11}H_{12}O_2$; MW = 176.21; ^{1}H NMR (400 MHz; $CDCl_3$): 6.67 (2H, s, H-2, H-6); 6.49 (1H, s, H-4); 3.78 (6H, s, OCH_3); 2.12 (3H, s, H-3′).

1-propyl-3,5-dimethoxybenzene ***19***. To a solution of 1-(propin-1-yl)-3,5-dimethoxybenzene (**18**, 2.05 g, 1 M equival) in MeOH-EtOAc 1:1 (50 mL), 10 % Pd/C 55–65 % is added catalytic under anhydrous condition. The reaction followed the same procedure of **16** to afford 1.95 g of 1-propyl-3,5-dimethoxybenzene, (**19**, 92 % based on the conversion of **18**).

1-propyl-3,5-dimethoxybenzene ***19:*** yellow oil; formula: $C_{11}H_{16}O_2$; MW = 180.24; ^{1}H NMR (400 MHz; $CDCl_3$): 6.27 (2H, s, H-2, H-6); 6.20 (1H, s, H-4); 4.83 (2H, s, OH); 3.80 (6H, s, OCH_3); 2.49 (2H, t, J = 8.8 Hz, H-1′); 1.69–1.58 (2H, m, H-2′); 0.95 (3H, t, J = 7.0 Hz, H-3′).

5-propyl resorcinol ***20***. To a solution of 1-propyl-3,5-dimethoxybenzene (**19**, 1.95 g, 1 M equival) in dry dichloromethane (7 mL), BBr_3 1 M (26.32 mL, 3 M equival) is added dropwise. The reaction followed the same procedure of **17** to afford 1.60 g of 5-propyl resorcinol (**20**, 97 % based on the conversion of **19**).

5-propyl resorcinol **20**: brown powder; formula: $C_9H_{12}O_2$; MW = 152.19; ^{1}H NMR (400 MHz; $CDCl_3$): 6.27 (2H, s, H-4, H-6); 6.20 (1H, s, H-2); 4.83 (2H, s, OH); 2.49 (2H, t, J = 8.8 Hz, H-1′); 1.69–1.58 (2H, m, H-2′); 0.95 (3H, t, J = 7.0 Hz, H-3′).

3.3. Synthesis of 5-heptyl resorcinol **23**

1-(1-heptyn-1-yl)-3,5-dimethoxybenzene ***21***. To a solution of 1-iodo-3,5-dimethoxybenzene (**12**, 2 g, 1 M equival) in toluene (50 mL), CuI (144 mg, 0.1 M equival), 1-heptyn (2.97 mL, 3 M equival), $Pd(PPh_4)_3$ (263 mg, 0.03 M equival) and TEA (22 mL, 22 M equival) are added under anhydrous condition. The reaction followed the same procedure of **13** to afford 1.60 g of 1-(1-heptyn-1-yl)-3,5-dimethoxybenzene (**21**, 90 % based on the conversion of **12**).

1-(1-heptyn-1-yl)-3,5-dimethoxybenzene ***21***: yellow oil; formula: $C_{15}H_{20}O_2$, MW = 232.32; ^{1}H NMR (400 MHz; $CDCl_3$): 6.60 (2H, d, J = 2.4 Hz, H-2, H-6); 6.43 (1H, t, J = 2.4 Hz, H-1); 3.79 (6H, s, OCH_3); 2.43 (2H, t, J = 7.3 Hz, H-3′); 1.64 (2H, td, J = 7.2, 14.4 Hz, H-4′); 1.50–1.38 (4H, m, H-5′, H-6′); 0.96 (3H, t, J = 7.3 Hz, H-7′).

1-heptyl-3,5-dimethoxybenzene ***22***. To a solution of 1-(eptin-1-yl)-3,5-dimethoxybenzene (**21**, 1.60 g, 1 M equival) in MeOH-EtOAc 1:1 (50 mL), 10 % Pd/C 55–65 % wet is added catalytic under anhydrous condition. The reaction followed the same procedure of **16** to afford 1.50 g of 1-heptyl-3,5-dimethoxybenzene (**22**, 92 % based on the conversion of **21**).

1-heptyl-3,5-dimethoxybenzene ***22***: yellow oil; formula: $C_{15}H_{24}O_2$; MW = 236.35; ^{1}H NMR (400 MHz; $CDCl_3$): 6.37 (2H, d, J = 2.4 Hz, H-2, H-6); 6.32 (1H, d, J = 2.0 Hz, H-4); 3.81 (6H, s, OCH_3), 2.57 (2H, t, J = 7.6 Hz, H-1′); 1.63 (2H, t, J = 7.6 Hz, H-2′); 1.39–1.25 (8H, m, H-3′, H-4′, H-5′, H-6′), 0.90 (1H, d, J = 7.6 Hz, H-7′).

5-heptyl-resorcinol ***23***. To a solution of 1-heptyl-3,5-dimethoxybenzene (**22**, 1.50 g, 1 M equival) in dry dichloromethane (50 mL), BBr_3 1 M (20.25 mL, 3 M equival) is added dropwise. The reaction followed the same procedure of **17** to afford 1.12 g of 5-heptyl-resorcinol (**23**, 85 % based on the conversion of **22**).

5-heptyl-resorcinol ***23:*** brown solid; formula: $C_{13}H_{20}O_2$; MW = 208.30; ^{1}H NMR (400 MHz; $CDCl_3$): 7.20 (2H, s, OH); 6.34 (2H, s, H-4, H-6); 6.31 (1H, s, H-2); 2.46 (2H, t, J = 8.0 Hz, H-1′); 1.57 (2H, td, J = 7.3, 14.6 Hz, H-2′); 1.35–1.26 (8H, m, H-3′,H-4′, H-5′,H-6′); 0.92 (3H, t, J = 7.1 Hz, H-7′).

3.4. Synthesis of minor cannabinoids

To a solution of each different synthons (1.5 M equival) in DCE (5 mL/mmol isoprenyl alcohols) isoprenyl alcohols (1 M equival) and acidic alumina (2 g/mmol isoprenyl alcohol), previously dried at 200 °C for 6 h, were added (Fig. 4). The reactions were stirred at 80 °C followed by TLC (silica, petroleum ether-EtOAc 90:10) and finally filtered in a sintered funnel on a bed of Celite® and evaporated at reduced pressure. Each residue is purified by LPC on silica (5 g, petroleum ether-EtOAc gradient from 100:0 to 90:10) and then on HPLC (silica, petroleum ether-EOAc isocratic elution 90:10) to afford the cannabinoids reported in Table 1.

3.5. Synthesis of isohexahydrocannabinol ***10***

Iso-hexahydrocannabinol **10**. To a solution of olivetol (**27**, 603.8 mg, 1 M equival) in dicholoromethane (50 mL), *p*-TSA (125 mg, 0.23 M equival) and nerol (**25**, 628 μL, 1.2 M equival) were added (Fig. 5). The reaction is stirred at RT in dark condition followed by TLC (silica, petroleum ether-EtOAc 90:10). After 72 h the reaction is quenched by dilution with sat. $NaHCO_3$. The organic phase is dried (Na_2SO_4), filtered and evaporated at reduce pressure. The residue is purified by LPC on silica (50 g, petroleum ether-EtOAc gradient from 100:0 to 90:10) and then on HPLC (silica, petroleum ether-EtOAc isocratic 90:10) to afford 220 mg of isohexahydrocannabinol (**10**, 20 % based on the conversion of **24**) [16,17]. Olivetol **27** with geraniol **24** in the same condition, lead to afford the same compound **10** (yield 20 %).

The stereostructure has been assigned based on the full network of COSY, HSQC, HMBC and NOE 2D NMR correlations. The ^{1}H and ^{13}C

Table 1
Synthetic protocol of cannabinoids.

Cannabinoid	Synthon	Prenyl alcohol moiety	Acidic alumina	DCE	Time (h)	Yield %
CBNR 1	Olivetol **24**, 1.5 M equivale	Nerol **25**, 1 M equival	2 g/mmol	5 mL/mmol	6	73
Sesqui-CBG 2	Olivetol **24**, 1.5 M equivale	(2*E*, 6*E*)-farnesol **26**, 1 M equival	2 g/mmol	5 mL/mmol	6	77
CBGB 4	5-butyl resorcinol **17**, 1.5 M equivale	Geraniol **24**, 1 M equival	2 g/mmol	5 mL/mmol	6	77
CBNRB 7	5-butyl resorcinol **17**, 1.5 M equivale	Nerol **25**, 1 M equival	2 g/mmol	5 mL/mmol	6	60
CBNRV 9	5-propyl resorcinol **20**, 1.5 M equivale	Nerol **25**, 1 M equival	2 g/mmol	5 mL/mmol	4	50
CBNRP 8	5-heptyl resorcinol **23**, 1.5 M equivale	Nerol **25**, 1 M equival	2 g/mmol	5 mL/mmol	4	55
CBGP 5	5-heptyl resorcinol **23**, 1.5 M equivale	Geraniol **24**, 1 M equival	2 g/mmol	5 mL/mmol	6	79

NMR resonances were in perfect agreement with the literature data of the synthetic (1*S*,3*S*,4*R*)-8,9-dihydro-*p*-isoTHC [16,17].

CBNR 1: yellow oil; formula: $C_{21}H_{32}O_2$; MW = 316.49; ^{1}H NMR (400 MHz; $CDCl_3$): 6.27 (2H, s, H-4, H-6); 5.31 (1H, t, *J* = 7.0 Hz, H-2′); 5.19 (1H, m, H-6′); 3.41 (2H, d, *J* = 6.1 Hz, H-1′); 2.45 (2H, t, *J* = 7.4 Hz, H-1″); 2.30 (2H, t, *J* = 7.8 Hz, H-4′); 2.21 (2H, td, *J* = 7.0, 14.0 Hz, H-5′); 1.75 (3H, s, H-9′); 1.72 (3H, s, H-8′); 1.67 (3H, s, H-10′); 1.59 (2H, td, *J* = 7.5, 15.0 Hz, H-2″); 1.34 (4H, m, H-3″, H-4″); 0.90 (3H, t, *J* = 6.8 Hz, H-5″); ^{13}C NMR (100 MHz; $CDCl_3$): 154.7 (C-1, C-3); 142.7 (C-5); 138.9 (C-3′); 132.4 (C-7′), 123.8 (C-6′); 122.6 (C-2′); 110.7 (C-2); 108.3 (C-4, C-6); 35.5 (C-1″); 32.0 (C-4′); 31.5 (C-3″); 30.8 (C-2″); 26.3 (C-5′); 25.7 (C-9′); 23.4 (C-10′); 22.6 (C-1′); 22.0 (C-4″); 17.7 (C-8′); 14.0 (C-5″).

Sesqui-CBG 2: yellow oil; formula: $C_{26}H_{40}O_2$; MW = 384.60; ^{1}H NMR (400 MHz; $CDCl_3$): 6.26 (2H, s, H-4, H-6); 5.31 (1H, bt, *J* = 6.2 Hz, H-2′); 5.10 (1H, m, H-6′); 5.08 (1H, m, H-10′); 3.40 (2H, d, *J* = 6.3 Hz, H-1′); 2.45 (2H, t, *J* = 7.8 Hz, H-1″); 2.07 (8H, overlapped m, H-4′, H-5′, H-8′, H-9′); 1.99 (2H, m, H-8′); 1.84 (3H, bs, H-15′); 1.70 (3H, bs, H-12′); 1.63 (3H, s, H-14′); 1.61 (3H, s, H-13′); 1.56 (2H, m, H-2″); 1.37–1.31 (4H, m, H-3″, H-4″); 0.92 (3H, t, *J* = 6.8 Hz, H-5″); ^{13}C NMR (100 MHz; $CDCl_3$): 154.8 (C-1, C-3); 142.7 (C-5); 138.9 (C-3′); 135.6 (C-7′); 131.3 (C-11′); 124.4 (C-6′); 123.6 (C-10′); 121.8 (C-2′); 110.6 (C-2); 108.2 (C-4, C-6); 40.0 (C-4′, C-8′), 35.8 (C-1″), 31.6 (C-3″), 31.1 (C-2″), 27.1 (C-5′), 27.0 (C-9′), 26.0 (C-12′), 23.0 (C-1′), 22.5 (C-4″), 18.1 (C-13′), 16.6 (C-15′), 16.4 (C-14′), 14.0 (C-5″).

CBGB 4: yellow oil; formula: $C_{20}H_{30}O_2$; MW = 302.46; ^{1}H NMR (400 MHz; $CDCl_3$): 6.27 (2H, s, H-4, H-6); 5.30 (1H, t, *J* = 6.6 Hz, H-2′); 5.06 (1H, t, *J* = 6.6 Hz, H-6′); 3.42 (2H, d, *J* = 7.5 Hz, H-1′); 2.48 (2H, t, *J* = 7.5 Hz, H-1″); 2.09 (4H, m, H-4′, H-5′); 1.83 (3H, s, H-9′); 1.71 (3H, s, H-8′); 1.57 (3H, s, H-10′); 1.53 (2H, q, *J* = 8.4, 15.4 Hz, H-2″); 1.37 (2H, m, H-3″); 0.94 (3H, t, *J* = 7.3 Hz, H-4″); ^{13}C NMR (100 MHz; $CDCl_3$): 154.8 (C-1, C-3); 142.7 (C-5); 138.9 (C-3′); 132.0 (C-7′); 123.8 (C-6′); 121.8 (C-2′), 110.6 (C-2), 108.3 (C-6, C-4), 39.7 (C-4′), 35.2 (C-1″), 33.2 (C-2″); 26.4 (C-5′); 25.6 (C-9′); 22.3 (C-1′, C-3″); 17.6 (C-8′); 16.1 (C-10′); 13.9 (C-4″).

CBGP 5: yellow oil; formula: $C_{23}H_{36}O_2$; MW = 344.54; ^{1}H NMR (400 MHz; $CDCl_3$): 6.27 (2H, s, H-4, H-6); 5.30 (1H, t, *J* = 6.6 Hz, H-2′); 5.08 (1H, t, *J* = 6.6 Hz, H-6′); 3.42 (2H, d, *J* = 7.1 Hz, H-1′); 2.45 (2H, t, *J* = 8.0 Hz, H-1″); 2.09 (4H, dt, *J* = 9.5, 9.6 Hz, H-4′, H-5′); 1.83 (3H, s, H-9′); 1.70 (3H, s, H-8′); 1.61 (3H, s, H-10′); 1.40 (2H, m, H-2″); 1.32–1.29 (8H, m, H-3″, H-4″, H-5″, H-6″); 0.91 (3H, t, *J* = 7.1 Hz, H-7″); ^{13}C NMR (100 MHz; $CDCl_3$): 154.8 (C-1, C-3); 142.7 (C-5); 138.9 (C-7′); 132.0 (C-3′); 123.7 (C-6′); 121.7 (C-2′); 110.5 (C-2); 108.3 (C-4, C-6); 39.7 (C-4′); 35.5 (C-1″); 31.8 (C-3″); 31.1 (C-2″); 29.2 (C-5″); 29.1 (C-6″); 26.3 (C-5′); 25.6 (C-9′); 22.6 (C-1′); 22.3 (C-4″); 17.7 (C-8′); 16.1 (C-10′); 14.1 (C-7″).

CBNRB 7: yellow oil; formula: $C_{20}H_{30}O_2$; MW = 302.46; ^{1}H NMR (400 MHz; $CDCl_3$): 6.27 (2H, s, H-4, H-6); 5.29 (1H, t, *J* = 7.2 Hz, H-2′); 5.19 (1H, t, *J* = 7.2 Hz, H-6′); 3.40 (2H, d, *J* = 6.4 Hz, H-1′); 2.48 (2H, t, *J* = 7.2 Hz, H-1″); 2.30 (2H, m, H-5′); 2.17 (2H, m, H-4′); 1.78 (3H, s, H-9′); 1.73 (3H, s, H-8′); 1.66 (3H, s, H-10′); 1.57 (2H, m, H-2″); 1.37 (2H, m, H-3″); 0.93 (3H, t, *J* = 7.8 Hz, H-4″); ^{13}C NMR (100 MHz; $CDCl_3$): 154.7 (C-1, C-3); 142.7 (C-5); 138.9 (C-3′); 132.4 (C-7′); 123.8 (C-6′); 122.6 (C-2′); 110.7 (C-2); 108.3 (C-4, C-6); 35.2 (C-1″); 33.3 (C-2″); 32.0 (C-4′); 26.3 (C-5′); 25.7 (C-9′); 23.4 (C-8′); 22.3 (C-1′); 22.0 (C-3″); 17.7 (C-10′); 13.9 (C-4″).

CBNRP 8: yellow oil; formula: $C_{23}H_{36}O_2$, MW = 344.54; ^{1}H NMR (400 MHz; $CDCl^3$): 6.26 (2H, s, H-4, H-6); 5.27 (1H, t, *J* = 6.2 Hz, H-2′); 5.18 (1H, t, *J* = 6.6 Hz, H-6′); 3.40 (2H, d, *J* = 7.2 Hz, H-1′); 2.45 (2H, t, *J* = 7.9 Hz, H-1″); 2.28 (2H, m, H-4′); 2.15 (2H, m, H-5′); 1.77 (3H, s, H-9′); 1.73 (3H, s, H-8′); 1.67 (3H, s, H-10′); 1.57 (2H, m, H-2″); 1.40–1.30 (8H, m, H-3″, H-4″, H-5″, H-6″); 0.88 (3H, t, *J* = 6.9 Hz, H-7″); ^{13}C NMR (100 MHz; $CDCl_3$): 154.7 (C-1, C-3); 142.8 (C-5); 138.9 (C-3′); 132.4 (C-7′); 123.8 (C-6′); 122.5 (C-2′); 110.7 (C-2); 108.3 (C-4, C-6); 35.6 (C-1″); 32.0 (C-4′); 31.8 (C-3″); 31.1 (C-2″); 29.2 (C-5″); 29.1 (C-6″); 26.3 (C-5′); 25.7 (C-9′); 23.4 (C-8′); 22.5 (C-1′); 21.9 (C-4″); 17.6 (C-10′); 14.1 (C-7″).

CBNRV 9: yellow oil; formula: $C_{19}H_{28}O_2$; MW = 288.43; ^{1}H NMR (400 MHz; $CDCl_3$): 6.26 (2H, s, H-4, H-6); 5.29 (1H, t, *J* = 7.3 Hz, H-2′); 5.19 (1H, t, *J* = 6.6 Hz, H-6′); 3.43 (2H, d, *J* = 7.0 Hz, H-1′); 2.46 (2H, t, *J* = 7.9 Hz, H-1″); 2.29 (2H, m, H-4′); 2.19 (2H, m, H-5′); 1.78 (3H, s, H-9′); 1.74 (3H, s, H-8′); 1.66 (3H, s, H-10′); 1.61 (2H, m, H-2″); 0.95 (3H, t, *J* = 7.3 Hz, H-3″); ^{13}C NMR (100 MHz; $CDCl_3$): 154.8 (C-1, C-3); 142.4 (C-5); 138.8 (C-3′); 132.4 (C-7′); 123.8 (C-6′); 122.5 (C-2′); 110.7 (C-2); 108.2 (C-4, C-6); 37.6 (C-1″); 32.0 (C-4′); 26.3 (C-5′); 25.7 (C-9′); 24.1 (C-2″); 23.4 (C-8′); 21.9 (C-1′); 17.7 (C-10′); 13.8 (C-3″).

***Iso*-hexahydrocannabinol 10**: brown oil; formula: $C_{21}H_{32}O_2$, MW = 316.49; ^{1}H NMR (600 MHz; $CDCl_3$): 6.27 (1H, s, H-4′); 6.11 (1H, s, H-2′); 3.32 (1H, bdd, *J* = 2.6 Hz H-3); 2.45 (2H, t, *J* = 7.5 Hz, H-1″); 1.88 (1H, dd, *J* = 13.3, 2.6 Hz, H-2a); 1.81 (1H, m, H-8); 1.71 (1H, dt, *J* = 14.0, 3.4 Hz, H-6b); 1.59 (2H, overlapped, H-2″); 1.56 (1H, overlapped, H-6a); 1.55 (1H, overlapped, H-2b); 1.50 (2H, overlapped, H-5); 1.32 (2H, overlapped, H-4″); 1.30 (2H, overlapped, H-3″); 1.26 (1H, overlapped, H-4); 1.33 (3H, s, H-7); 1.09 (3H, d, *J* = 6.6 Hz, H-10); 0.95 (3H, d, *J* = 6.6 Hz, H-9); 0.88 (3H, t, *J* = 7.1 Hz, H-5″); ^{13}C NMR (150 MHz; $CDCl_3$): 157.3 (C-5′); 152.2 (C-1′); 142.4 (C-3′); 111.7 (C-6′); 107.9 (C-4′); 105.9 (C-2′); 74.5 (C-1); 44.2 (C-4); 35.6 (C-1″); 35.0 (C-6); 31.7 (C-3″); 30.8 (C-2″); 30.5 (C-2); 29.4 (C-7); 27.8 (C-3); 26.2 (C-8); 22.5 (C-4″); 22.0 (C-9); 21.1 (C-10); 20.6 (C-5); 14.0 (C-5″).

3.6. Isolation of CBG ***3*** and CBGV ***6***

Plant material: Non-psychoactive *Cannabis sativa* L. plant material, belonging to a CBGV-rich chemotype, was purchased from Canvasalus Srl (Monselice, Italy). A voucher specimen (Cs-CBGV12/2023) of the vegetal material is stored in Novara Laboratories.

Extraction and Isolation: nonwoody *C. sativa* aerial parts (100 g) is extracted with acetone (2 × 5 L) in a vertical percolator at room temperature, affording 6.9 g (6.9 %) of a dark green syrup. This is later dissolved at 45 °C in 70 mL of MeOH (raw extract/MeOH ratio 1:10 *w/v*) and left at 8 °C to condense fatty acids and waxes. After 12 h, the solution is vacuum-filtered with cold MeOH in a sintered funnel protected by a bed of stratified Celite®, obtaining a residual methanolic fraction. This latter portion is filtered through solid-phase extraction on C-18 silica gel (35 g) to remove pigments, unsaturated fatty acids, and poly isoprenoids to finally obtain 4.3 g of the purified fraction after evaporation of MeOH under reduced pressure.

Once the fraction is dried, it is heated at 130 °C under stirring for 45 °C in a paraffin bath to achieve the decarboxylation. This latter decarboxylated fraction is fractionated by LPC on silica gel (100 g, PE–EtOAc gradient from 90:10 to 20:80 *v*/v) to afford three fractions (I, II, and III). Fraction I (1.3 g) is further purified with LPC on silica gel (50 g, PE–EtOAc gradient from 90:10 to 80:20 v/v) to afford 332 mg of CBG **3** [18] as a white powder. Fraction II (2.5 g) is fractionated with LPC on silica gel (100 g, PE–EtOAc gradient from 80:20 to 70:30 v/v) to afford 400 mg of CBGV **7** [8] as a white powder. All of the isolated compounds are identified according to ^{1}H NMR previously described in the literature. NMR data of the isolated compounds are shown in **Fig. S5, S6** and **S11, 12** of the Supporting Information.

CBG 3: white powder; formula: $C_{21}H_{32}O_2$; MW = 316.49; ^{1}H NMR (400 MHz; $CDCl_3$): 6.27 (2H, s, H-4, H-6), 5.30 (1H, t, *J* = 6.9 Hz, H-2′), 5.08 (1H, t, *J* = 6.2 Hz, H-6′), 5.04 (2H, s, OH), 3.42 (2H, d, *J* = 7.1 Hz, H-1′), 2.48 (2H, t, *J* = 7.6 Hz, H-1″), 2.18–2.04 (4H, m, H-4′, H-5′), 1.83 (3H, s, H-9′), 1.70 (3H, s, H-8′), 1.58 (5H, t, *J* = 7.6 Hz, H-10′, H-2″), 1.38–1.29 (4H, m, H-3″, H-4″), 0.92 (3H, t, *J* = 7.1 Hz, H-5″); ^{13}C NMR (100 MHz; $CDCl_3$): 154.8(C-1, C-3), 142.8 (C-5), 138.9 (C-3′), 132.0 (C-7′), 123.8 (C-6′), 121.7 (C-2′), 110.6 (C-2), 108.4 (C-6, C-4), 39.7 (C-4′), 35.5 (C-1″), 31.5 (C-2″), 30.8 (C-4″), 26.4 (C-5′), 25.7 (C-9′), 22.6C-1′), 22.3 (C-3″), 17.7 (C-8′), 16.2 (C-10′), 14.0 (C-5″).

CBGV 6: white powder; formula: $C_{19}H_{28}O_2$; MW = 288.43; ^{1}H NMR (400 MHz; CDCl3): 6.28 (2H, s, H-4, H-6), 5.30 (1H, t, *J* = 7.1 Hz, H-2′), 5.08 (1H, t, *J* = 5.7 Hz, H-6′), 5.04 (2H, s, OH), 3.42 (2H, d, *J* = 7.1 Hz, H-1′), 2.46 (2H, t, *J* = 7.6 Hz, H-1″), 2.18–2.04 (4H, m, H-4′, H-5′), 1.84 (3H, s, H-9′), 1.70 (3H, s, H-8′), 1.62 (2H, t, *J* = 3.7 Hz, H-2″), 1.62 (3H, s, H-10′), 0.95 (3H, t, *J* = 7.3 Hz, H-3″); ^{13}C NMR (100 MHz; $CDCl_3$): 154.8

(C-1, C-3), 142.5 (C-5), 139.0 (C-3′), 132.1 (C-7′), 123.8 (C-6′), 121.7 (C-2′), 110.7 (C-2), 108.4 (C-6, C-4), 39.7 (C-4′), 37.6 (C-1″), 26.4 (C-5′), 25.7 (C-9′), 24.2 (C-2″), 22.3 (C-1′), 17.7 (C-8′), 16.2 (C-10′), 13.9 (C-3″).

4. Results and discussion

All the CBG-like cannabinoids have been achieved in appreciable yields through the *C*-allylic alkylation of resorcinoid-synthons with isoprenyl alcohols. 5-Butyl resorcinol **17** was generated with a Sand-Meyer reaction on 3,5-dimethoxyaniline **11** to the 1-iodo-3,5-dimethoxybenzene **12**. The reaction was carried out with a Sonogashira coupling to obtain the alkyne 1-(2-ethinyl)-3,5-dimethoxybenzene **14** after deprotection. The desired elongation of the alkyl chain was reached through an alkylation with lithium diisopropylamide (LDA), obtaining respectively the 4-C alkyne **15** and the C-3 alkyne **18**, which underwent a reduction and demethylation to generate 5-butyl resorcinol **17** and 5-propyl resorcinol **20**.

The lengthening of 1-iodo-3,5-dimethoxybenzene **12** was carried out in a single step involving 1-heptyn. The remaining reactions to complete the synthesis of 5-heptyl resorcinol **23** were carried out with the same

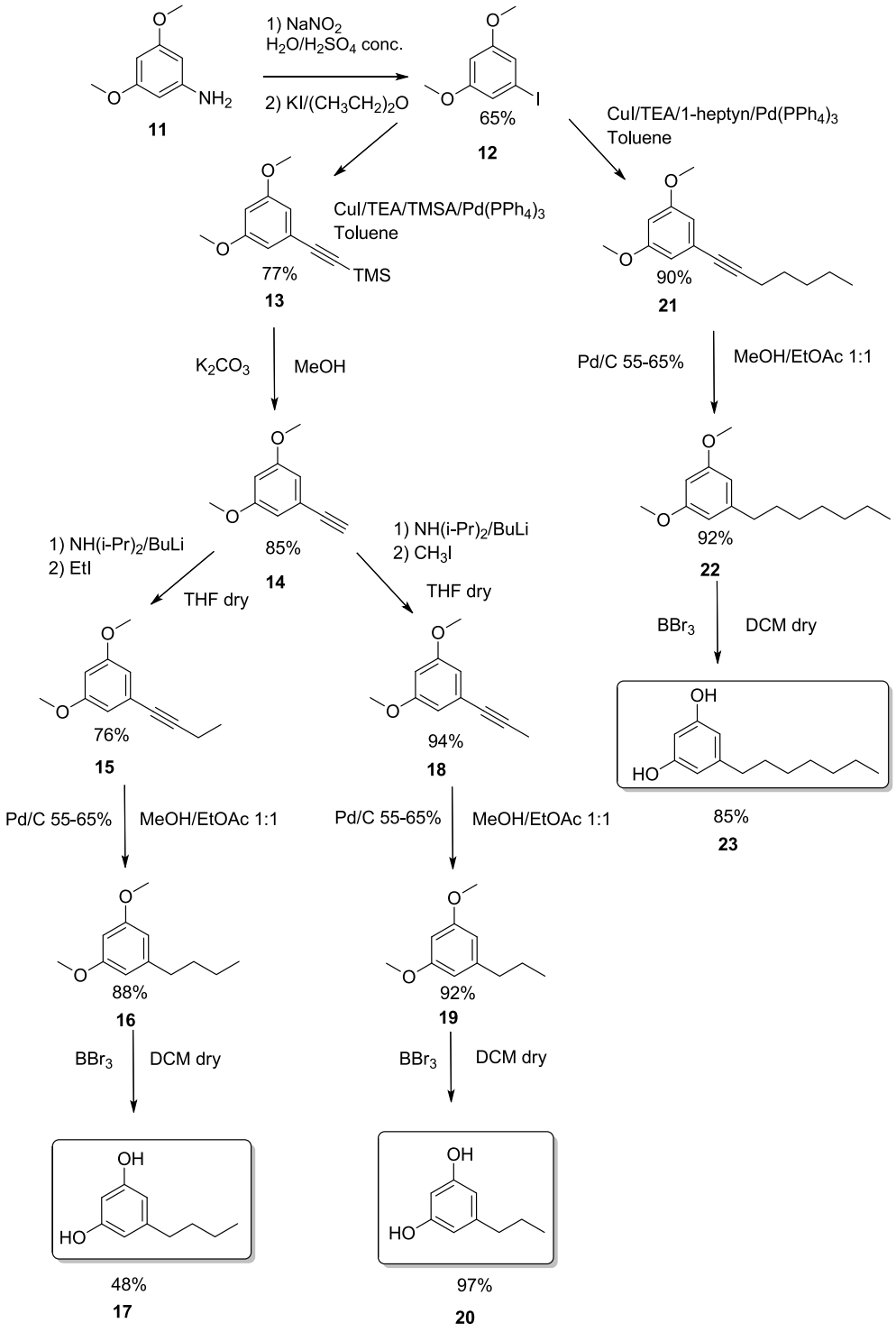


Fig. 3. Scheme of the synthesis of 5-butyl resorcinol 17, 5-propyl resorcinol 20 and 5-heptyl resorcinol 23.

steps (Fig. 3).

To proceed with the alkylation of synthons, we followed the procedure reported by Jentsch and colleague [13] who proposed one-step regioselective alumina-promoted allylation reaction, which mechanistic investigations were reported by Zangh and colleagues [15]. Acidic alumina, previously dried at 200 °C for 6 h was added in 1,2-dichloroethane (DCE) and the *C*-alkylation of synthons in presence of the specific isoprenylic alcohol occurred after 4–6 h at 80 °C. Conditions are summarized in Table 1. This procedure provided a relatively clean and selective *ortho*-prenylation with allylic alcohols. The only observable by-products were the di-isoprenylated compounds that could be easily removed by purification with silica and low-pressure chromatography (LPC).

The different elongation of the aliphatic side chains of the synthetized cannabinoids could be identify by ^{1}H NMR resonance taking advantage of the number of aliphatic hydrogens present at δ 1.25–140. In the *n*-heptyl **5**, **8** 8H (**Fig. S9, S15** of the Supporting Information), *n*-pentyl **1**, **2**, **3** 4H (**Fig. S1, S3, S5** of the Supporting Information), *n*-butyl **4**,**7** 2H (**Fig. S7, S13** of the Supporting Information) and absent in the *n*-propyl chain **6**, **9** (**Fig. S11, S17** of the Supporting Information). The elongation of the aliphatic side chain could also be evinced by the number of the sp^2 C in the region δ 35–14 of the ^{13}C NMR data in the Supporting Information.

To taste an alternative procedure of synthon allylic alkylation, we tried the protocol suggested by Morimoto and co-workers [5] who involved *p*-toluenesulfonic acid (*p*-TAS) in dichloromethane (DCM). Originally low yielding (27 % in 12 h), we increased the time of reaction at 72 h. Unfortunately, we achieved the isohexahydrocannabinol **10** [16,17] both with nerol and geraniol (Fig. 5.

Few information has been provided for minor cannabinoids activity in GBM and the only available concerns CBG **3** and a combination of CBD and Δ^9-THC on glioblastoma tumour cells and glioblastoma stem cells, for the evaluation of their cytotoxic, apoptotic, and anti-invasive effects [19]. In light of this, the compounds obtained were tested in order to evaluate their cytotoxic effect in U87, U251 and T98 glioblastoma cell lines. The results showed that all the cannabinoids induced a dose dependent reduction of cell viability, at 24 h post-treatments. In particular, CBNR **1** and sesqui-CBG **2** resulted the most effective in reducing cell lines viability, with IC$_{50}$ being respectively 8,61 μg\mL in T98, 8,37 μg\mL in U87 and 12,31 μg\mL in U251 cell lines for CBNR **1** and IC$_{50}$ 9,35 μg\mL in T98, 9,91 μg\mL in U87 and 13,37 μg\mL in U251 cell lines for sesqui-CBG **2**. The other cannabinoids exerted lower cytotoxic effect. Considering these results, we can speculate that the differences in the aliphatic side chains between the prolyl-, butyl- heptyl- and the pentyl-cannabinoid did not affect the overall viability of the human glioblastoma cell lines. Meanwhile, the difference in the isoprenyl-residue can contribute to cytotoxicity as it is underlined by higher cytotoxicity of the sesqui-CBG **2** compared to CBG **3**. Notably, the most important influence on cell viability is not due to the difference in the prenylation grade (from geranyl to farnesyl), but in the isomerization characterizing CBG **3** and CBNR **1** being this latter cannabinoid the most cytotoxic of all the tested molecules (Table 2).

5. Conclusion

The interest in minor phytocannabinoids, although rapidly growing, is hindered by their limited occurrence in Cannabis [2]. In this work, we isolated and, taking advantage of the existing synthesis, we obtained a series of rare CBG-like cannabinoids in sufficient amount to provide biological investigation. All these cannabinoids have been tested on human glioblastoma cell lines to investigate their potential contribute as supplement in anticancer therapy (Table 2). Results showed that the most relevant influence to cytotoxicity of CBG-like cannabinoids was determined by the isomerization of the isoprenyl-moiety being the CBNR **1** able to reduce cell viability in higher degree than its major isomer CBG **3**. Taken together, these results showed that these minor cannabinoids could be a useful tool to better investigate not only the structure activity relationship of CBG **3**, but also a possibility to further evaluate the biological space of Cannabis.

Funding

This work was supported by the Bando 2022 PNRR under Grant Prot. P2022TXJX8.

CRediT authorship contribution statement

Martina Giangrossi: Investigation, Data curation. **Stefano Salamone:** Formal analysis, Data curation. **Aurora Camola:** Formal analysis, Data curation. **Orazio Taglialatela-Scafati:** Investigation, Formal analysis. **Giuseppina Chianese:** Investigation, Formal analysis. **Ernesto Gargiulo:** Investigation, Formal analysis. **Massimo Nabissi:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Federica Pollastro:** Writing – original draft, Supervision, Data

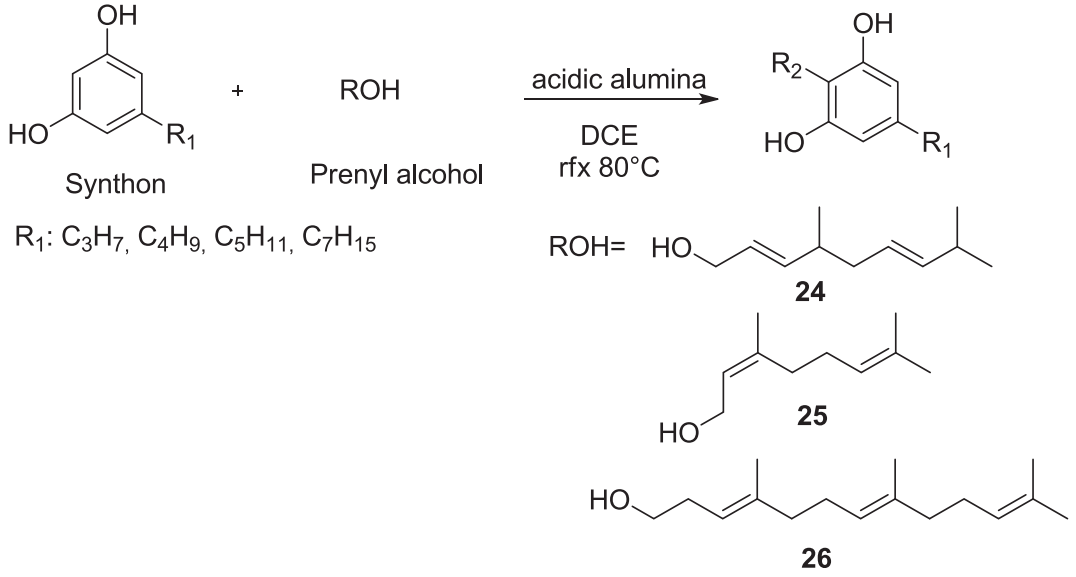


Fig. 4. Scheme of the synthesis of minor cannabinoids.

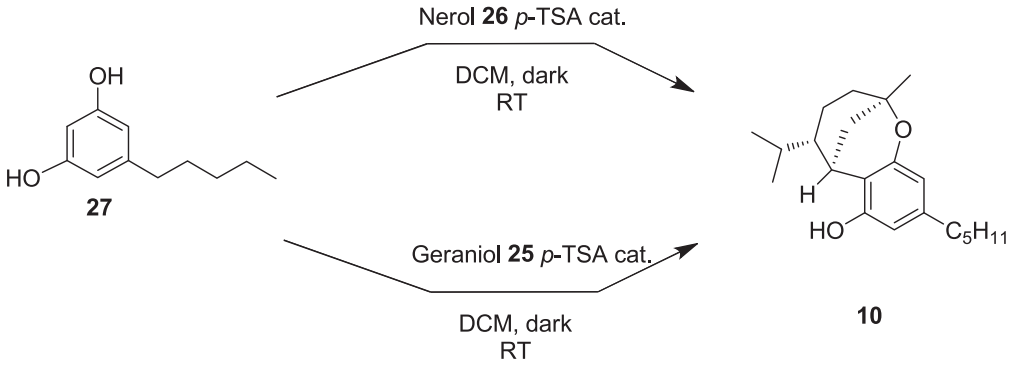


Fig. 5. Synthesis of isohexahydrocannabinol 10 from olivetol 27 with nerol 26 and geraniol 25.

Table 2
Effects on viability expressed as mean ± SD of IC_{50} (μg/mL) in glioblastoma T98, U87 and U251 cell lines after 24 h of incubation with cannabinoids 1–10.

IC_{50} μg/mL	T98 cell line	U87 cell line	U251 cell line
CBNR **1**	8,61 ± 0,35	8,37 ± 0,33	12,31 ± 0,62
Sesqui-CBG **2**	9,35 ± 0,37	9,91 ± 0,4	13,37 ± 0,54
CBG **3**	13,19 ± 0,66	12,38 ± 0,49	12,12 ± 0,61
CBGB **4**	14,3 ± 0,57	14,6 ± 0,58	14,76 ± 0,59
CBGP **5**	15,18 ±0,61	14,01 ±0,56	19,74 ±1,38
CBGV **6**	15,29 ±0,62	17,15 ± 0,69	19,93 ± 1,19
CBNRB **7**	7,81 ± 0,47	10,84 ±0,54	16,17 ± 0,81
CBNRP **8**	15,41 ± 0,62	17,96 ± 0,72	20,96 ± 1,26
CBNRV **9**	11,38 ± 0,57	16,64 ± 0,67	19,33 ± 0,97
Iso-hexahydrocannabinol **10**	10,59 ± 0,74	11,48 ± 0,69	13,37 ± 0,54

curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Federica Pollastro reports financial support was provided by University of Eastern Piedmont Amedeo Avogadro Department of Pharmaceutical Sciences. Prof. Orazio Taglialatela-Scafati, the editor on chief, is listed between the authors If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

We are grateful to the Financial support for this work from Bando 2022 PNRR under Grant Prot. P2022TXJX8.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2024.106354.

Data availability

Data will be made available on request.

References

[1] M. Antonelli, B. Benedetti, G. Cannazza, A. Cerrato, C. Citti, C.M. Montone, S. Piovesana, A. Laganà, New insights in hemp chemical composition: a comprehensive polar lipidome characterization by combining solid phase enrichment, high-resolution mass spectrometry, and cheminformatics, Anal. Bioanal. Chem. 412 (2020) 413–423, https://doi.org/10.1007/s00216-019-02247-6.
[2] M.T. Welling, M.A. Deseo, A. Bacic, M.S. Doblin, Biosynthetic origins of unusual cannabimimetic phytocannabinoids in *Cannabis sativa* L: a review, Phytochemistry 201 (2022) 113282, https://doi.org/10.1016/j.phytochem.2022.113282.
[3] L.O. Hanuš, G. Appendino, Natural Product Report 33, 2016, pp. 1357–1392, https://doi.org/10.1039/C6NP00074F.
[4] P. Romero, A. Peris, K. Vergara, J.T. Matus, Comprehending and improving cannabis specialized metabolism in the systems biology era, Plant Sci. 298 (2020) 110571, https://doi.org/10.1016/j.plantsci.2020.110571.
[5] F. Taura, S. Morimoto, Y. Shoyama, Cannabinerolic acid, a cannabinoid from Cannabis sativa, Phytochemistry 39 (2) (1995) 457–458, https://doi.org/10.1016/0031-9422(94)00887-Y.
[6] M. Fellermeier, M.H. Zenk, Prenylation of olivetolate by a hemp transferase yields cannabigerolic acid, the precursor of tetrahydrocannabinol, FEBS Lett. 427 (2) (1998) 283–285, https://doi.org/10.1016/S0014-5793(98)00450-5.
[7] F. Pollastro, O. Taglialatela-Scafati, M. Allarà, E. Muñoz, V. Di Marzo, L. De Petrocellis, G. Appendino, Bioactive prenylogous cannabinoid from fiber hemp (Cannabis sativa), J. Nat. Prod. 74 (9) (2011) 2019–2022, https://doi.org/10.1021/np200500p.
[8] Y. Shoyama, H. Hirano, H. Makino, N. Umekita, I. Nishioka, Cannabis. X. The isolation and structures of four new propyl cannabinoid acids, tetrahydrocannabivarinic acid, cannabidivarinic acid, cannabichromevarini c acid and cannabigerovarinic acid, from Thai Cannabis, 'Meao variant', Chem. Pharm. Bull. 25 (9) (1977) 2306–2311, https://doi.org/10.1248/cpb.25.2306.
[9] M. Novotny, M.L. Lee, C.E. Low, A. Raymond, Analysis of marijuana samples from different origins by high-resolution gas-liquid chromatography for forensic application, Anal. Chem. 48 (1) (1976) 24–29.
[10] F. Tolomeo, F. Russo, M.A. Vandelli, G. Biagini, A.L. Capriotti, A. Laganà, L. Carbone, G. Gigli, C. Citti, HPLC-UV-HRMS analysis of cannabigerovarin and cannabigerobutol, the two impurities of cannabigerol extracted from hemp, J. Pharm. Biomed. Anal. 203 (2021) 114215, https://doi.org/10.1016/j.jpba.2021.114215.
[11] P. Linciano, C. Citti, L. Luongo, C. Belardo, S. Maione, M.A. Vandelli, F. Forni, G. Gigli, A. Lagnanà, M.C. Mantone, G. Cannazza, Isolation of a high-affinity cannabinoid for the human CB1 receptor from a medicinal Cannabis sativa variety: Δ9-tetrahydrocannabutol, the butyl homologue of Δ9-tetrahydrocannabinol, J. Nat. Prod. 83 (1) (2019) 88–98, https://doi.org/10.1021/acs.jnatprod.9b00876.
[12] C. Citti, P. Linciano, F. Russo, L. Luongo, M. Iannotta, S. Maione, A. Laganà, A. L. Capriotti, M.A. Vandelli, G. Gigli, G. Cannazza, A novel phytocannabinoid isolated from Cannabis sativa L. with an in vivo cannabimimetic activity higher than Δ9-tetrahydrocannabinol: Δ9-Tetrahydrocannabiphorol, Sci. Rep. 9 (1) (2019) 1–13, https://doi.org/10.1038/s41598-019-56785-1.
[13] N.G. Jentsch, X. Zhang, J. Magolan, Efficient synthesis of cannabigerol, grifolin, and piperogalin via alumina-promoted allylation, J. Nat. Prod. 83 (9) (2020) 2587–2591, https://doi.org/10.1021/acs.jnatprod.0c00131.
[14] C. Twelves, M. Sabel, D. Checketts, S. Miller, B. Tayo, M. Jove, L. Brazil, S. Short, C., A phase 1b randomised, placebo-controlled trial of nabiximols cannabinoid oromucosal spray with temozolomide in patients with recurrent glioblastoma, Br. J. Cancer 124 (8) (2021) 1379–1387, https://doi.org/10.1038/s41416-021-01259-3.
[15] X. Zhang, Alumina Directed Ortho Allylation of Phenols (Doctoral Dissertation), 2020.
[16] R. Tanaka, R. Kikura-Hanajiri, Identification of hexahydrocannabinol (HHC), dihydro-iso-tetrahydrocannabinol (dihydro-iso-THC) and hexahydrocannabiphorol (HHCP) in electronic cigarette cartridge products, Foren. Toxicol. 42 (1) (2024) 71–81, https://doi.org/10.1007/s11419-023-00667-9.
[17] L. Crombie, W.M.L. Crombie, D.F. Firth, Terpenylations using (R)-(−)-α-phellandrene. Synthesis of the (3 S, 4 R)-8, 9-dihydro-o-and-p-cannabidiols, their iso-THC's, and the natural dihydrochalcone (3 S, 4 R)-(+)-linderatin,

J. Chem. Soc. Perkin Trans. (5) (1988) 1251–1253, https://doi.org/10.1039/P19880001251.

[18] Y.H. Choi, A. Hazekamp, A.M. Peltenburg-Looman, M. Frédérich, C. Erkelens, A. W. Lefeber, R. Verpoorte, NMR assignments of the major cannabinoids and cannabiflavonoids isolated from flowers of Cannabis sativa, Phytochem. Anal. 15 (6) (2004) 345–354, https://doi.org/10.1002/pca.787.

[19] T.T. Lah, M. Novak, M.A. Pena Almidon, O. Marinelli, B. Žvar Baškovič, B. Majc, M. Mlinar, R. Bosnjak, B. Breznik, R. Zomer, M. Nabissi, Cannabigerol is a potential therapeutic agent in a novel combined therapy for glioblastoma, Cells 10 (2) (2021) 340, https://doi.org/10.3390/cells10020340.