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Unsaturated Fatty Alcohol Derivatives of Olive Oil Phenolic Compounds with Potential Low-Density Lipoprotein (LDL) Antioxidant and Antiobesity Properties⊗

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Supporting Information

ABSTRACT: A new route for the synthesis of fatty alcohol derivatives of hydroxytyrosol and other olive oil phenolic compounds was developed to allow the preparation of unsaturated derivatives. The biological activity of synthesized compounds was evaluated. Most of the compounds presented a significant antioxidant activity on low-density lipoprotein (LDL) particles. The activity of the tested products was significantly influenced by the number and position of unsaturations as well as modifications on the polar head of the synthesized compounds. Some of them presented modulation of food intake in rats and, due to their molecular similarity with CB_1 endogenous ligands, the endocannabinoid system and PPAR-α were also evaluated as potential targets. The pharmacodynamics could not be totally explained by CB_1 and PPAR-α receptor interactions because only two of the four compounds with biological activity showed a CB_1 activity and all of them presented low PPAR-α affinity, not justifying its whole in vivo activity. The hydroxytyrosol linoleylether (7) increased LDL resistance to oxidation with a capacity similar to that of hydroxytyrosol and was the most active in vivo compound with a hypophagic effect comparable to that of oleoylethanolamine. We consider that this compound could be a good lead compound for future drug development in obesity treatments.

KEYWORDS: *CB_1, PPAR-α, obesity, ether, fatty alcohol*

INTRODUCTION

Low-density lipoprotein (LDL) oxidation is involved in the earliest events of atherosclerosis,[1] although its role in the latter stages of plaque evolution is nowadays questioned.[2] Hydroxytyrosol (2-(3,4-dihydroxyphenyl)ethanol), a phenolic compound found in olive oil, is a potent in vitro inhibitor of LDL oxidation, being capable of disrupting peroxidative chain reactions.[3] Hydroxytyrosol is also a natural metabolite of dopamine, with which it shares a structural similarity (Figure 1).[4,5]

The poor bioavailability of hydroxytyrosol in humans[6] and the interest of the food industry in new lipophilic antioxidants make the development of long-chain hydroxytyrosol ethers and esters attractive.[7,8]

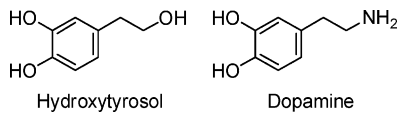

Figure 1. Molecular structures of hydroxytyrosol and dopamine.

A series of saturated alkyl chain hydroxytyrosol ethers[8] assessed with the rancimat test already displayed good antioxidant

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stabilizer properties for food products, having activity equal to or even higher than that of hydroxytyrosol.[9] They have also shown a cell protective activity against oxidation and better bioavailability than hydroxytyrosol.[10−12] It is worth noting that the synthetic route for the hydroxytyrosol ether derivatives previously described in the literature does not allow the synthesis of unsaturated derivatives because a hydrogenation is needed in the last step for catechol deprotection. Thus, only saturated ether derivatives were synthesized.[8]

Obesity is certainly one of the most important challenges for public health in the 21st century, because excessive weight is associated with an increasing risk of mortality, among other unhealthy effects.[13,14] Loss of weight can have a great impact in different scopes of public health. Bearing in mind the structural similarity of hydroxytyrosol with dopamine and the structure of the endocannabinoids *N*-oleoyldopamine and *N*-arachidonoyldopamine (Figure 2), we hypothesize that ether derivatives of

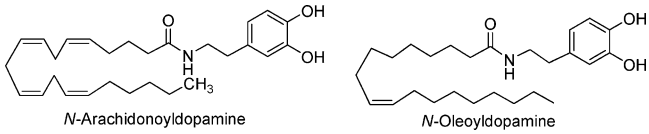


Figure 2. Structures of the endocannabinoids *N*-oleoyldopamine and *N*-arachidonoyldopamine.

unsaturated fatty alcohols and hydroxytyrosol could also interact with the cannabinoid system and display some modulatory activity on food intake, as the role of this receptor on appetite is well-known.[15] Moreover, we hypothesize an affinity of this new class of compounds for PPAR-α, a nuclear receptor involved in lipid metabolism, which is often activated by CB_1 ligands.[16,17] Our team has previously described a group of fatty acid amides with this dual CB_1/PPAR-α interaction. We consider that the ether derivatives of hydroxytyrosol could have a significant importance in obesity management because appetite and lipid metabolism could be simultaneously regulated.[18]

A new route for the synthesis of hydroxytyrosol ether derivatives (Figure 3) with natural unsaturated derived fatty alcohols

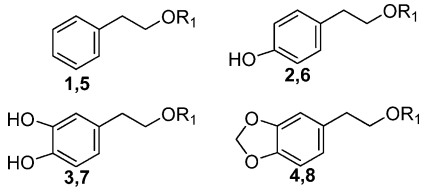


Figure 3. Proposed structures for fatty alcohol ethers. R_1 = oleyl, (*Z*)-1-(2-phenylethoxy)octadec-9-ene (**1**), (*Z*)-1-(2-(4-hydroxyphenyl)-ethoxy)octadec-9-ene (**2**), (*Z*)-1-(2-(3,4-dihydroxyphenyl)ethoxy)octadec-9-ene (**3**), (*Z*)-1-(2-(3,4-methylenedioxyphenyl)ethoxy)octadec-9-ene (**4**); R_1 = linoleyl (9*Z*,12*Z*)-1-(2-phenylethoxy)octadeca-9,12-diene (**5**), (9*Z*,12*Z*)-1-(2-(4-hydroxyphenyl)ethoxy)octadeca-9,12-diene (**6**), (9*Z*,12*Z*)-1-(2-(3,4-dihydroxyphenyl)ethoxy)octadeca-9,12-diene (**7**), (9*Z*,12*Z*)-1-(2-(3,4-methylendioxyphenyl)ethoxy)octadeca-9,12-diene (**8**).

such as oleic and linoleic alcohol is proposed. It is postulated that newly synthesized compounds may have LDL antioxidant activity with a potency similar to that of hydroxytyrosol. In addition, this class of compounds, which shares a structural similarity with known endocannabinoids and PPAR-α ligands, can modulate food intake through interaction with those receptors.

MATERIALS AND METHODS

Chemistry. All reagents and solvents used were commercially available and were employed without further purification unless specifically indicated. The infrared spectrophotometer was a Nicolet Avatar 360. ^{1}H and ^{13}C NMR analyses were carried out with Varian Anova 500 and Varian Mercury 400 spectrometers. The ultracentrifuge used was a Sigma 3K30. The progress of all reactions was monitored by TLC on aluminum sheets precoated with silica gel 60 (HF-254, Merck), film thickness of 0.25 mm. Elemental analyses (C, H, N) were performed on a Thermo Finnigan Elemental Analyzer Flash 1112 series and were within ±0.3% of theoretical values.

*Oleyl Iodide (**15a**).* In a round-bottom flask equipped with a magnetic stirrer, iodine (11.8 mmol), PPh_3 (12.6 mmol), imidazole (26.5 mmol), and THF (20 mL) were added at 4 °C. Oleyl alcohol (**14a**) (11.2 mmol) in THF (5 mL) was slowly added, and the reaction was maintained at room temperature for 48 h. The THF was removed under reduced pressure, and the product was purified by flash chromatography using hexane. The product was obtained as a colorless oil in 83% yield: IR (KBr), ν = 3003, 2924, 2853, 1464, 722 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), δ 0.88 (t, J = 6.84 Hz, 3H), 1.19−1.46 (m, 22H), 1.76−1.88 (m, 2H), 1.97−2.05 (m, 4H), 3.19 (t, J = 7.05 Hz, 2H), 5.29−5.41 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$), δ 7.35, 14.13, 22.69, 27.15, 27.21, 28.51, 29.17, 29.31, 29.32, 29.52, 29.69, 29.76, 30.49, 31.90, 33.54, 129.74, 129.99. Anal. Calcd for $C_{18}H_{35}I$: C, 57.14; H, 9.32. Found: C, 57.01; H, 9.30.

*Linoleyl Iodide (**15b**).* This compound was synthesized as described above for oleyl iodide but starting with linoleyl alcohol (**14b**). The product was obtained as a colorless oil in 71% yield: IR (KBr), ν = 3008, 2926, 2854, 1464, 1179, 730, 631 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), δ 0.88 (t, J = 6.53 Hz, 3H), 1.20−1.47 (m, 16H), 1.74−1.88 (m, 2H) 1.98−2.12 (m, 4H), 2.77 (t, J = 6.36 Hz, 2H), 3.19 (t, J = 7.05 Hz, 2H), 5.27−5.44 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$), δ 7.36, 14.09, 22.58, 25.62, 27.18, 28.50, 29.17, 29.30, 29.34, 29.59, 30.48, 31.53, 33.53, 127.88, 128.02, 130.05, 130.21. Anal. Calcd for $C_{18}H_{33}I$: C, 57.44; H, 8.84. Found: C, 57.56; H, 8.86.

*3,4-(Dimethylmethylenedioxy)phenylacetate Methyl Ester (**10**).* In a round-bottom flask equipped with a Dean−Stark apparatus and a magnetic stirrer, 3,4-dihydroxyphenylacetic acid (**9**) (11.9 mmol), *p*-toluenesulfonic acid (1.2 mmol), 2,2-dimethoxypropane (69.2 mmol), and toluene (30 mL) were added. The reaction was heated under reflux for 24 h. The organic phase was washed with distilled water (two times), then brine, and evaporated until dryness. The product was purified by flash chromatography using ethyl acetate/hexane as eluent. The product was obtained as a colorless oil in 94% yield: IR (KBr), ν = 2990, 2952, 1740, 1498, 1255, 981, 838 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), δ 1.66 (s, 6H), 3.52 (s, 2H), 3.69 (s, 3H), 6.64−6.71 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$), δ 25.85, 40.80, 52.01, 108.04, 109.44, 117.94, 121.70, 146.51, 147.55, 172.25. Anal. Calcd for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81. Found: C, 63.56; H, 5.80.

*2-(3,4-(Dimethylmethylenedioxy)phenyl)ethanol (**11**).* In a round-bottom flask equipped with a magnetic stirrer, compound **10** (5.7 mmol), $NaBH_4$ (13.22 mmol) and THF (100 mL) were added at 4 °C. Iodine (5.6 mmol) in THF was slowly added, and the reaction was heated to reflux for 24 h. The THF was removed under reduced pressure and the residue dissolved in ethyl acetate. The organic phase was washed with distilled water (two times), then brine, and evaporated until dryness. The product was purified by flash chromatography using ethyl acetate/hexane as eluent. The product was obtained as a yellow oil in 72% yield: IR (KBr), ν = 3370, 2989, 2937, 2872, 1739, 1498, 1445, 1255, 1046, 981, 839, 807 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$), δ 1.66 (s, 6H), 2.77 (t, J = 6.47 Hz, 2H), 3.81 (t, J = 6.07 Hz, 2H), 6.62−6.74 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$), δ 25.83, 38.85, 63.75, 108.08, 109.06, 117.71, 121.23, 131.36, 145.99, 147.57. Anal. Calcd for $C_{11}H_{14}O_3$: C, 68.02; H, 7.27. Found: C, 67.85; H, 7.29.

*2-(4-(Methoxymethyleneoxy)phenyl)ethanol (**13**).* In a round-bottom flask equipped with a magnetic stirrer, tyrosol (**12**) (0.26 mmol), MOMCl (3.51 mmol), tetrabutylamonium bromide (0.15 mmol), CH_2Cl_2 (6 mL), and aqueous NaOH (30% w/v, 6 mL) were added at room temperature, and the reaction was stirred for 24 h. The organic

phase was separated and washed with distilled water (two times), then brine, and evaporated until dryness. The product was purified by flash chromatography using ethyl acetate/hexane as eluent. The product was obtained as a white solid in 65% yield: IR (KBr), ν = 3404, 2937, 2827, 1612, 1513, 1233, 1199, 1152, 1110, 1079, 1008, 922, 825 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ 1.79 (s, 1H), 2.80 (t, J = 6.57 Hz, 2H), 3.47 (s, 3H), 3.80 (t, J = 6.60 Hz, 2H), 5.15 (s, 2H), 6.99 (d, J = 8.48 Hz, 2H), 7.14 (d, J = 8.40 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$), δ 38.24, 55.85, 63.66, 94.39, 116.33, 129.92, 131.77, 155.75. Anal. Calcd for $C_{10}H_{14}O_3$: C, 65.91; H, 7.74. Found: C, 66.02; H, 7.75.

*General Method for the Synthesis of Ethers (**1**, **4**, **5**, **8**, and **17a**–**d**).* In a round-bottom flask equipped with a magnetic stirrer, phenylethanol derivatives (**11**, **13**, **16a**, or **16b**) (0.60 mmol), iodoalkenes **15a** or **15b** (1.32 mmol), tetrabutylammonium bromide (0.16 mmol), aqueous KOH (30% w/v, 10 mL), and toluene (10 mL) were added, and the reaction was heated under reflux for 48 h. The organic phase was separated, washed with distilled water (two times), then brine, and evaporated until dryness. The product was purified by flash chromatography using ethyl acetate/hexane as eluent.

*(Z)-1-(2-Phenylethoxy)octadec-9-ene (**1**).* This compound was synthesized following the general procedure using phenylethanol (**16a**) and **15a** as starting materials. The product was obtained as a colorless oil in 20.0% yield: IR (KBr), ν = 2924, 2854, 2358, 1699, 1113, 747, 639 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ 0.89 (t, J = 6.09 Hz, 3H), 1.21−1.40 (m, 22H), 1.51−1.64 (m, 2H), 1.95−2.13 (m, 4H), 2.89 (t, J = 7.28 Hz, 2H), 3.43 (t, J = 6.68 Hz, 2H), 3.63 (t, J = 7.34 Hz, 2H), 5.29−5.42 (m, 2H) 7.15−7.36 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$), δ 14.12, 22.68, 26.15, 27.19, 29.24, 29.32, 29.45, 29.49, 29.52, 29.71, 29.76, 31.90, 36.37, 71.07, 71.79, 126.09, 128.27, 128.87, 129.82, 129.90, 139.03. Anal. Calcd for $C_{26}H_{44}O$: C, 83.80; H, 11.90. Found: C, 83.92; H, 11.91.

*(Z)-1-(2-(3,4-Methylenedioxyphenyl)ethoxy)octadec-9-ene (**4**).* This compound was synthesized following the general procedure using 2-(3,4-methylendioxyphenyl)ethanol (**16b**) and **15a** as starting materials. The product was obtained as a colorless oil in 35.0% yield: IR (KBr), ν = 2924, 2854, 1506, 1490, 1246, 1113, 1042, 940, 639 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ 0.88 (t, J = 5.79 Hz, 3H), 1.19−1.40 (m, 22H), 1.49−1.65 (m, 2H), 1.95−2.07 (m, 4H), 2.80 (t, J = 7.12 Hz, 2H), 3.42 (t, J = 6.63 Hz, 2H), 3.57 (t, J = 7.16 Hz, 2H), 5.27−5.42 (m, 2H), 5.92 (s, 2H), 6.66 (d, J = 7.99 Hz, 1H), 6.70−6.77 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$), δ 14.12, 22.68, 26.16, 29.19, 29.25, 29.32, 29.45, 29.49, 29.52, 29.70, 29.76, 31.90, 36.04, 71.08, 71.93, 100.74, 108.10, 109.35, 121.66, 129.83, 129.91, 132.88, 145.81, 147.45. Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.64. Found: C, 78.01; H, 10.65.

*(9Z,12Z)-1-(2-Phenylethoxy)octadeca-9,12-diene (**5**).* This compound was synthesized following the general procedure using **16a** and **15b** as starting materials. The product was obtained as a colorless oil in 27.0% yield: IR (KBr), ν = 3009, 2927, 2855, 2361, 1738, 1455, 1115, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ 0.89 (t, J = 6.80 Hz, 3H), 1.15−1.44 (m, 20H), 1.49−1.67 (m, 2H), 1.99−2.10 (m, 4H), 2.78 (t, J = 6.10 Hz, 2H), 2.89 (t, J = 7.30 Hz, 2H), 3.34−3.52 (m, 2H), 3.62 (t, J = 7.30 Hz, 2H), 5.27−5.46 (m, 4H), 7.14−7.41 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$), δ 14.06, 22.57, 25.61, 26.17, 27.19, 27.22, 29.25, 29.34, 29.44, 29.49, 29.66, 29.71, 31.52, 36.37, 71.07, 71.80, 126.10, 127.90, 127.94, 128.28, 128.87, 130.13, 130.18, 139.03. Anal. Calcd for $C_{26}H_{42}O$: C, 84.26; H, 11.42. Found: C, 84.08; H, 11.41.

*(9Z,12Z)-1-(2-(3,4-Methylenedioxyphenyl)ethoxy)octadeca-9,12-diene (**8**).* This compound was synthesized following the general procedure using compounds **16b** and **15b** as starting materials. The product was obtained as a colorless oil in 23.0% yield: IR (KBr), ν = 2927, 2855, 1741, 1489, 1246, 1113, 1043 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$), δ 0.89 (t, J = 6.42 Hz, 3H), 1.22−1.42 (m, 20H), 1.49−1.64 (m, 2H), 198−2.12 (m, 2H), 2.72−2.85 (m, 4H), 3.42 (t, J = 6.65 Hz, 2H), 3.57 (t, J = 7.21 Hz, 2H), 5.26−5.46 (m, 4H), 5.92 (s, 2H), 6.62−6.78 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$), δ 14.08, 22.57, 25.61, 26.15, 27.19, 28.58, 29.25, 29.34, 29.45, 29.49, 29.66, 31.52, 36.04, 38.34, 71.08, 71.93, 100.74, 108.10, 109.35, 121.66, 127.90, 127.94, 130.13, 130.18, 132.88, 145.81, 147.57. Anal. Calcd for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.35; H, 10.22.

*(Z)-1-(2-(4-(Methoxymethyleneoxy)phenyl)ethoxy)octadec-9-ene (**17a**).* This compound was synthesized following the general procedure using **15a** and **13** as starting materials. The product was obtained as a colorless oil in 30.0% yield: IR (KBr), ν = 2925, 2853, 1613, 1511, 1465, 1233, 1153, 1114, 1080, 1010, 924 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ 0.88 (t, J = 6.45 Hz, 3H), 1.20−1.41 (m, 22H), 1.48−1.68 (m, 2H), 1.93−2.11 (m, 4H), 2.83 (t, J = 7.27 Hz, 2H), 3.42 (t, J = 6.70 Hz, 2H), 3.47 (s, 3H), 3.58 (t, J = 7.34 Hz, 2H), 5.15 (s, 2H), 5.27−5.43 (m, 2H), 6.96 (d, J = 8.56 Hz, 2H), 7.14 (d, J = 8.46 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$), δ 14.11, 22.67, 26.15, 27.19, 29.24, 29.31, 29.45, 29.48, 29.51, 29.71, 29.74, 31.89, 35.49, 55.88, 71.06, 71.95, 94.50, 116.14, 129.82, 129.90, 132.44, 155.60. Anal. Calcd for $C_{28}H_{48}O_3$: C, 77.72; H, 11.18. Found: C, 77.65; H, 11.20.

*(9Z,12Z)-1-(2-(4-(Methoxymethyleneoxy)phenyl)ethoxy)-octadeca-9,12-diene (**17b**).* This compound was synthesized following the general procedure using compounds **13** and **15b** as starting materials. The product was obtained as a colorless oil in 20.0% yield: IR (KBr), ν = 3008, 2927, 2854, 1511, 1233, 1153, 1113, 1080, 1010 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ 0.88 (t, J = 6.53 Hz, 3H), 1.18−1.44 (m, 20H), 1.50−1.62 (m, 2H), 1.98−2.11 (m, 4H), 2.77 (t, J = 6.19 Hz, 2H), 2.83 (t, J = 7.26 Hz, 2H), 3.42 (t, J = 6.67 Hz, 2H), 3.47 (s, 3H), 3.58 (t, J = 7.32 Hz, 2H), 5.15 (s, 2H), 5.27−5.46 (m, 4H), 6.96 (d, J = 8.48 Hz, 2H), 7.14 (d, J = 8.23 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$), δ 14.08, 22.57, 25.61, 26.16, 27.19, 27.22, 29.25, 29.34, 29.45, 29.49, 29.65, 29.72, 31.52, 35.50, 55.91, 71.06, 71.96, 94.51, 116.15, 127.90, 127.93, 129.83, 130.13, 130.18, 132.44, 155.60. Anal. Calcd for $C_{28}H_{46}O_3$: C, 78.09; H, 10.77. Found: C, 77.97; H, 10.78.

*(Z)-1-(2-(3,4-(Dimethylmethylendioxy)phenyl)ethoxy)octadec-9-ene (**17c**).* This compound was synthesized following the general procedure using **15a** and **11** as starting materials. The product was obtained as a colorless oil in 30.0% yield: IR (KBr), ν = 2927, 2854, 1736, 1653, 1498, 1445, 1375, 1253, 1234, 1113 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ 0.88 (t, J = 6.21 Hz, 3H), 1.20−1.39 (m, 22H), 1.47−1.62 (m, 2H), 1.66 (s, 6H), 1.96−2.07 (m, 4H), 2.79 (t, J = 7.28 Hz, 2H), 3.42 (t, J = 6.72 Hz, 2H), 3.57 (t, J = 7.32 Hz, 2H), 5.30−540 (m, 2H), 6.57−6.68 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$), δ 14.12, 22.66, 25.84, 26.16, 27.21, 28.98, 29.25, 29.46, 29.49 29.73 31.79, 36.05, 71.06, 72.06, 107.90, 109.14, 117.52, 121.02, 129.84, 129.91, 132.09, 145.69, 147.30. Anal. Calcd for $C_{29}H_{48}O_3$: C, 78.33; H, 10.88. Found: C, 78.10; H, 10.90.

*(9Z,12Z)-1-(2-(3,4-(Dimethylmethylenedioxy)phenyl)ethoxy)-octadeca-9,12-diene (**17d**).* This compound was synthesized following the general procedure using compounds **15b** and **11** as starting materials. The product was obtained as a colorless oil in 53.0% yield: IR (KBr), ν = 3009, 2927, 2855, 1499, 1253, 1234, 1113, 980 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ 0.89 (t, J = 6.51 Hz, 3H), 1.22−1.42 (m, 20H), 1.50−1.62 (m, 2H), 1.66 (s, 6H), 2.00−2.11 (m, 4H), 2.72−2.85 (m, 4H), 3.42 (t, J = 6.51 Hz, 2H), 3.57 (t, J = 7.31 Hz, 2H) 5.26−5.48 (m, 4H), 6.58−6.67 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$), δ 14.07, 22.57, 25.61, 25.82, 26.16, 26.18, 27.18, 27.21, 29.25, 29.34, 29.45, 29.49, 29.65, 29.71, 29.77, 31.51, 36.04, 70.95, 71.04, 72.05, 107.89, 109.13, 117.49, 121.00, 127.93, 130.11, 130.16, 132.08, 145.69, 147.29. Anal. Calcd for $C_{29}H_{48}O_3$: C, 78.33; H, 10.88. Found: C, 78.52; H, 10.85.

*(Z)-1-(2-(4-Hydroxyphenyl)ethoxy)octadec-9-ene (**2**).* In a round-bottom flask equipped with a magnetic stirrer, compound **17a** (0.17 mmol), isopropanol (5 mL), and aqueous HCl 6 N (2 mL) were added at room temperature, and the reaction was stirred for 24 h. The organic phase was separated and washed with distilled water (two times), then brine, and evaporated until dryness. The product was purified by flash chromatography using ethyl acetate/hexane as eluent. The product was obtained as a colorless oil in a 97.8% yield: IR (KBr), ν = 3373, 2924, 2854, 1614, 1516, 1464, 1236, 1190, 829 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ 0.88 (t, J = 6.13 Hz, 3H), 1.20−1.42 (m, 22H), 1.50−1.65 (m, 2H), 1.94−2.09 (m, 4H), 2.82 (t, J = 7.24 Hz, 2H), 3.45 (t, J = 6.75 Hz, 2H), 3.60 (t, J = 7.32 Hz, 2H), 5.29−5.38 (m, 2H), 5.40 (bs, 1H), 6.73 (d, J = 8.45 Hz, 2H), 7.07 (d, J = 8.32 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$), δ 14.12, 22.67, 26.10, 27.19, 29.23, 29.31, 29.42, 29.47, 29.51, 29.60, 29.74, 29.75, 31.89, 35.33,

71.12, 72.06, 115.16, 129.83, 129.93, 130.80, 154.05. Anal. Calcd for $C_{26}H_{44}O_2$: C, 80.35; H, 11.41. Found: C, 80.19; H, 11.43.

*(Z)-1-(2-(3,4-Dihydroxyphenyl)ethoxy)octadec-9-ene (**3**).* In a round-bottom flask equipped with a magnetic stirrer, compound **17c** (0.26 mmol) and aqueous HCl 6 N (5 mL) were added, and the reaction was heated under reflux for 24 h. The organic phase was separated and washed with distilled water (two times), then brine, and evaporated until dryness. The product was purified by flash chromatography using ethyl acetate/hexane as eluent. The product was obtained as a colorless oil in 50.7% yield: IR (KBr), ν = 3394, 2922, 2854, 1606, 1520, 1465, 1446, 1279, 1193, 1113, 1092 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$), δ 0.88 (t, J = 6.91 Hz, 3H), 1.18−1.38 (m, 20H), 1.51−1.62 (m, 2H), 1.97−2.04 (m, 4H), 2.76 (t, J = 7.14 Hz, 2H), 3.46 (t, J = 6.83 Hz, 2H), 3.62 (t, J = 7.16 Hz, 2H), 5.30−5.41 (m, 2H), 5.63 (bs, 1H), 5.86 (bs, 1H), 6.59−6.63 (m, 1H), 6.68 (d, J = 1.66 Hz, 1H), 6.72 (d, J = 8.03 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$), δ 14.12, 22.68, 26.07, 27.19, 29.23, 29.31, 29.42, 29.48, 29.51, 29.74, 29.75, 31.89, 35.41, 71.18, 71.93, 115.20, 115.85, 121.00, 129.82, 129.93, 131.57, 142.03, 143.58. Anal. Calcd for $C_{26}H_{44}O_3$: C, 77.18; H, 10.96. Found: C, 77.15; H, 10.94.

*(9Z,12Z)-1-(2-(4-Hydroxyphenyl)ethoxy)octadeca-9,12-diene (**6**).* This compound was synthesized as already described for compound **2** starting with **17b**. The product was obtained as a colorless oil in 80.0% yield: IR (KBr), ν = 3361, 2927, 2855, 1516, 1111, 829 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ 0.89 (t, J = 6.30 Hz, 3H), 1.20−1.42 (m, 20H), 1.51−1.63 (m, 2H), 1.99−2.09 (m, 4H), 2.73−2.87 (m, 4H), 3.44 (t, J = 6.75 Hz, 2H), 3.59 (t, J = 7.33 Hz, 2H), 5.26−5.46 (m, Hz, 4H), 6.74 (d, J = 8.46 Hz, 2H), 7.07 (d, J = 8.43 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$), δ 14.08, 22.56, 25.60, 26.10, 27.18, 27.21, 29.23, 29.33, 29.42, 29.47, 29.62, 29.64, 31.51, 35.35, 71.09, 72.06, 115.15, 127.89, 127.93, 129.93, 130.12, 130.18, 130.83,154.05. Anal. Calcd. for $C_{26}H_{42}O_2$: C, 80.77; H, 10.95. Found: C, 80.90; H, 10.94.

*(9Z,12Z)-1-(2-(3,4-Dihydroxyphenyl)ethoxy)octadeca-9,12-diene (**7**).* This compound was synthesized as already described for compound **3** starting with **17d**. The product was obtained as a colorless oil in 27.0% yield: IR (KBr), ν = 3386, 3009, 2935, 2856, 1606, 1520, 1446, 1375, 1279, 1113, 811, 723 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$), δ 0.89 (t, J = 6.96 Hz, 3H), 1.22−1.42 (m, 20H), 1.50−1.62 (m, 2H), 1.96−2.12 (m, 4H), 2.72−2.82 (m, 4H), 3.42 (t, J = 6.71 Hz, 2H), 3.58 (t, J = 7.21 Hz, 2H), 4.99 (bs, 1H), 5.13 (bs, 1H), 5.27−5.45 (m, 4H), 6.61−6.70 (m, 1H), 6.71−6.81 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$), δ 14.07, 22.56, 25.60, 26.04, 27.17, 27.20, 29.23, 29.33, 29.40, 29.47, 29.63, 31.50, 35.37, 71.17, 71.93, 115.19, 115.84, 120.94, 127.89, 127.95, 130.12, 130.19, 131.44, 142.09, 143.63. Anal. Calcd for $C_{26}H_{42}O_3$: C, 77.56; H, 10.51. Found: C, 77.50; H, 10.52.

Biology. *Feeding Experiments.* Feeding experiments were carried out using Wistar male rats with a 200−450 g weight range. Animals were housed in individual cages in a room with controlled temperature (23 °C) and relative humidity (50%) with a 12/12 h light and dark cycle. Water and food were available ad libitum except in specific experimental proceedings. The animals were handled twice in the days prior to the experimental sessions. All products were dissolved in a mixture of DMSO 5%/Tween 60 5%/saline 90% and administered intraperitoneally. The experiments performed in this study are in compliance with Spanish regulations concerning the protection of experimental animals (Real Decreto 1201/2005, October 21, 2005, BOE no. 252), as well as with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

The feeding experiments were carried out using animals that had been deprived of food for 24 h but with free access to water. Thirty minutes after the injection, the previously weighed food was placed in the cage. The food was weighed 30, 60, 120, and 240 min after initiation of the test. All feeding experiments were performed with groups of eight animals (n = 8).

In Vitro Experiments: Dienes Conjugation Experiment. *LDL Isolation.* Blood from healthy volunteers was collected after an overnight fast in tubes containing 1 g/L EDTA. Plasma was separated by centrifugation at 1000g at 4 °C for 15 min. LDL isolation was performed by sequential flotation ultracentrifugation. Native LDL was dialyzed by molecular size exclusion chromatography in a G25 Sephadex column (Pharmacia, Uppsala, Sweden), with 2.7 mL of phosphate-buffered saline (PBS) 0.01 mol, pH 7.4, under gravity feed at 4 °C. Apolipoprotein B100 content was determined by immunoturbidimetry (ABX Diagnostics, Montpellier, France).

Diene Conjugates Monitoring. Using a 96-well ELISA plate, dialyzed LDL (final concentration = 0.06 g of Apo-B/L) in PBS at a final volume of 150 μL was incubated with 10 μL of methanol in the presence or absence (control) of the dissolved analytes. Afterward, 10 μL of a 100 μM cupric sulfate (final concentration = 0.67 μmol) solution was added to a 96-well half-area flat-bottom UV-transparent microplate (Corning). To minimize evaporation during prolonged incubation time, 10 μL of mineral oil (Sigma-Aldrich) was layered over the reaction mixture and the plate was covered with adhesive optical transparent film. Absorbance at 234 nm was continuously monitored at 15 min intervals for 24 h at 36 °C in an Infinite M200 lector (Tecan Iberica, Männedorf, Switzerland).

Controls and samples with the concentration of all tested compounds at 0.5, 1, and 3 μM were evaluated in the same run in duplicate, and each experiment was repeated three times. For data presentation, the x-axis value, corresponding to the intercept of the propagation phase tangent with the extrapolated line for the slow propagation reaction, was calculated (lag time).

Binding and PPAR-α Affinity Assay. *Rat Cerebellum and Cortex Membranes Homogenization.* The rats were anesthetized with Dolethal (pentobarbital) and killed with a guillotine. The cortex and cerebellum were extracted and stored separately in dry ice. The cerebellum and cortex were homogenized with a buffer of Tris 50 mmol, pH 7.4, using an Ultraturrax. The homogenate was centrifuged at 25000g for 15 min at 4 °C. The supernatant was discarded. The pellet was again washed with 5 mL of buffer and centrifuged. The supernatant was again discarded. The protein concentration of the pellet was measured using the Bradford test.

Binding Assay. The binding assay was performed using the labeled CB_1 antagonist [^{3}H]-SR141716. In each tube were added 450 μL of buffer A (50 mmol of Tris, pH 7.4, with 0.5% bovine serum albumin (BSA)), 100−200 μg of rat brain membranes (cerebellum), the diluted product, and the tracer [^{3}H]-SR141716. The mixture was incubated with shaking at 37 °C for 60 min, and the reaction was stopped with 1 mL of buffer A. The resultant mixture was centrifuged at 5000 rpm for 5 min. The supernatant was discarded and the pellet washed with 1 mL of buffer A and centrifuged, and again the supernatant was discarded. Scintillation liquid was added, and the samples were read at a beta scintillator (liquid scintillation analyzer, Tri-Carb 2100 TR, Packard, a Packard Bioscience Company). All products were diluted in buffer B (50 mmol of Tris, pH 7.4, with 0.5% BSA and 0.3% DMSO) at the concentrations of 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, and 10^{-11} M. All data points were performed in triplicate.

PPAR-α Affinity Assay. (a) Biology. The compounds reported in this study were first evaluated for in vivo Reporter Gene Assay to assess which compounds could induce interaction between PPAR-α and the coactivator (SRC-1) into MCF-7 cells.

(b) Drugs. Oleoylethanolamide (OEA), GW7647, and WY14643 were purchased from Tocris Biosciences (Cookson Ltd., Bristol, U.K.). For in vitro cell culture experiments, all compounds were dissolved and diluted in DMSO (Sigma-Aldrich Spain).

(c) DNA Constructs. Full-length cDNAs for human PPAR-α, human RXRα, and human SRC-1 were subcloned into the T7/SV40 promoter-driven pSG5 expression vector (Qiagen). Those constructs were used for viral promoter-driven overexpression of the respective proteins in mammalian cells.

(d) Reporter Gene Constructs. Four copies of the human CPTI gene DR1-type RE (core sequence GTAGGGAAAAGGTCA) were individually fused with the thymidine kinase (tk) minimal promoter driving the firefly luciferase reporter gene.

Transient Transfection and Luciferase Reporter Assays[19−21]. MCF-7 human breast cancer cells were seeded into 6-well plates (200 000 cells/well) and grown overnight in phenol red-free DMEM supplemented with 5% charcoal-stripped fetal bovine serum. Plasmid DNA containing liposomes were formed by incubating 1 μg of an expression vector for wild type PPAR-α, RXRα, SRC-1, and 1 μg of

reporter plasmid with 10 μg of *N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammoniummethylsulfate (DOTAP, Roche) for 15 min at room temperature in a total volume of 100 μL. After dilution with 900 μL of phenol red-free DMEM, the liposomes were added to the cells. Phenol red-free DMEM supplemented with 500 μL of 15% charcoal-stripped fetal bovine serum was added 4 h after transfection. At this time, cells were treated for 16 h with solvent (DMSO) at different concentrations (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} mol) of OEA, GW7647, AEA, oleic acid, and the different compounds under evaluation as indicated. The cells were lysed for 16 h after the onset of stimulation using the reporter gene lysis buffer (Roche). Both the constant light signal luciferase reporter gene assay and the constant light signal luciferase reporter gene assay were performed as recommended by the suppliers (Roche and Roche Diagnostics, respectively). Stimulation of normalized luciferase activity was calculated in comparison with solvent-induced cells that did not overexpress any protein. Data were used to calculate the EC_{50} (nmol) for each compound.

RESULTS AND DISCUSSION

Chemistry. The free phenolic hydroxyl groups of the required phenylalcohols were protected (Figure 4). The synthesis

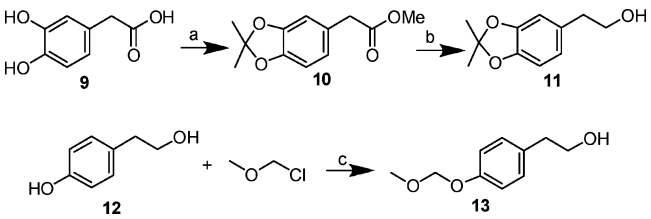
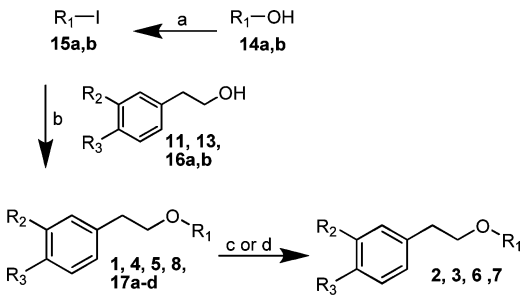


Figure 4. Protection of phenolic hydroxyl groups of tyrosol and hydroxytyrosol: (a) 2,2-dimethoxypropane, TsOH, Dean–Stark, 100 °C, toluene; (b) $NaBH_4$, I_2, 66 °C, THF; (c) CH_2Cl_2, H_2O, $(nBu)_4NBr$, 25 °C.

of protected hydroxytyrosol was performed as described by Gambacorta et al.[22] Thus, intermediate **10**, synthesized by the reaction of 3,4-dihydroxyphenylacetic acid (**9**) with 2,2-dimethoxypropane, was reduced with $NaBH_4/I_2$ to give compound **11**. The protection of the phenol group of tyrosol (**12**) was carried out by reaction with MOMCl in the biphasic system CH_2Cl_2/NaOH 30% aq using $(nBu)_4NBr$ as a phase transfer catalyst,[23] yielding compound **13**.

Iodoalkenes **15a** and **15b** (Figure 5) were obtained by the reaction of oleic and linoleic alcohol (**14a,b**) with I_2/PPh_3/imidazole at room temperature.[24] The fatty alcohol ethers (**17a–d**, **1**, **4**, **5**, and **8**) were prepared by conjugation of the iodine derivatives **15a** and **15b** with the corresponding alcohols, that is, 2-phenylethanol (**16a**), 2-(3,4-methylenedioxyphenyl)-ethanol (**16b**), 2-(3,4-(dimethylmethylendioxy)phenyl)ethanol (**11**), and 2-(4-(methoxymethylenoxy)phenyl)ethanol (**13**).[25,26] Deprotection of compounds **17a–d** with aq HCl yielded the final compounds **2**, **3**, **6**, and **7**.

Biology. *LDL Antioxidant Activity.* We tested the antioxidant LDL activity of the synthesized compounds **1–8** using hydroxytyrosol (a well-known natural antioxidant[27]), tyrosol, and homovanillyl alcohol as reference compounds. We monitored the oxidation kinetics of LDL by $CuSO_4$ using a spectrophotometric technique previously standardized by our group.[27] The length of the lag phase was determined as the intercept of the propagation phase tangent with the extrapolated line for the slow reaction. Lag-time data were calculated as the ratio between the observed value for each compound versus those corresponding to the native LDL (with no added compound)



Product	R_1	R_2	R_3
1	Oleyl	H	H
2	Oleyl	H	OH
3	Oleyl	OH	OH
4	Oleyl	$-OCH_2O-$	
5	Linoleyl	H	H
6	Linoleyl	H	OH
7	Linoleyl	OH	OH
8	Linoleyl	$-OCH_2O-$	
11	-	$-OC(CH_3)_2O-$	
13	-	H	OCH_2OCH_3
16a	-	H	H
16b	-	$-OCH_2O-$	
17a	Oleyl	H	OCH_2OCH_3
17b	Linoleyl	H	OCH_2OCH_3
17c	Oleyl	$-OC(CH_3)_2O-$	
17d	Linoleyl	$-OC(CH_3)_2O-$	

Figure 5. General synthesis of fatty alcohol ether derivatives: (a) I_2, PPh_3, imidazole, 25 °C, THF; (b) toluene, KOH 30% aq, $(nBu)_4NBr$, 100 °C; (c) HCl 6 N aq, 100 °C; (d) HCl 6 N aq, iPrOH, 80 °C.

oxidation reaction. Variables were log-transformed for statistical analyses (Figure 6).

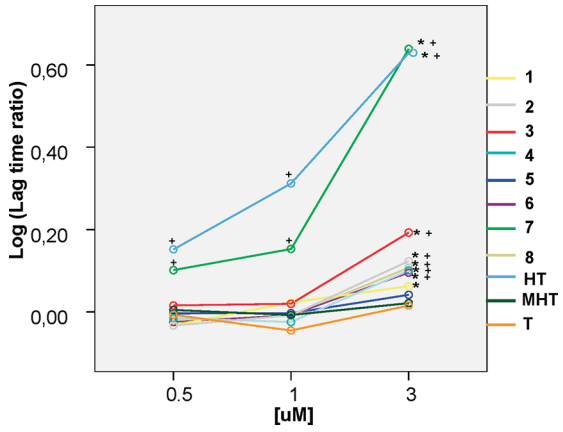


Figure 6. Log (lag time ratio) of LDL oxidation in the presence of the tested compounds at the 0.5, 1, and 3 μM concentrations. HT, hydroxytyrosol; MHT, homovanillyl alcohol; T, tyrosol. *, *P* trend value <0.05; +, *P* value versus native LDL oxidation <0.05.

Compound 7 was the only compound that displayed an activity similar to that of hydroxytyrosol at all three concentrations

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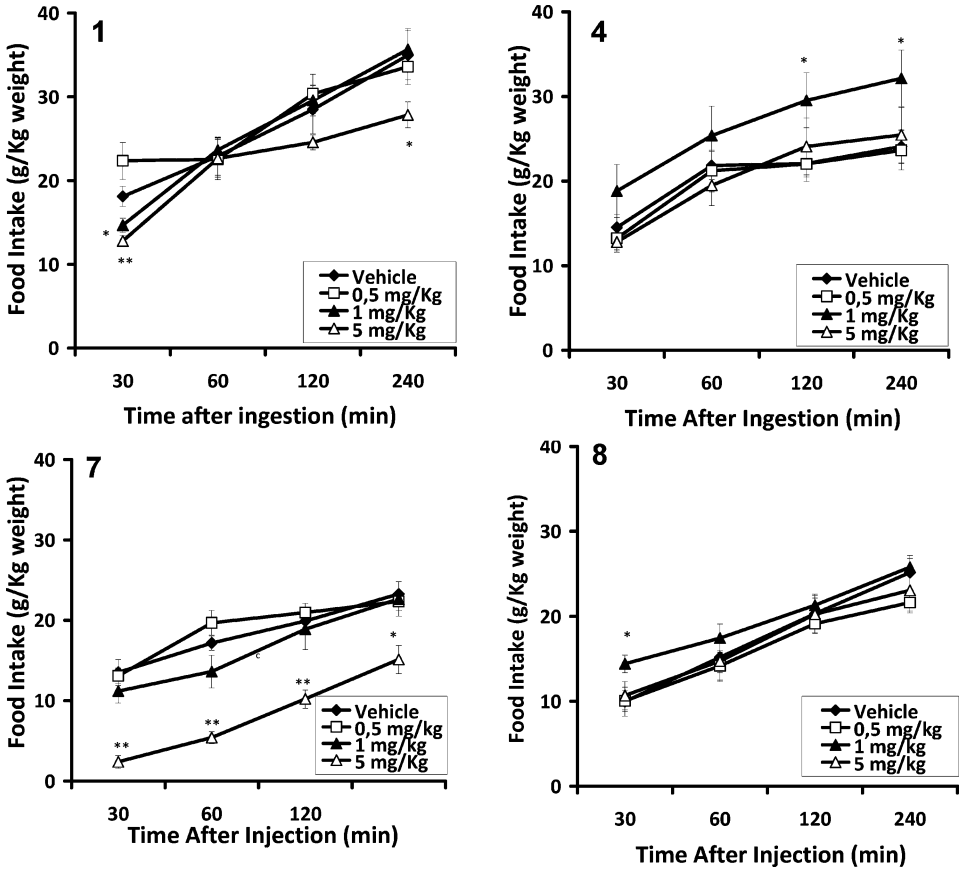

Figure 7. Relative food intake (g of food per kg of animal weight) of 24 h food-deprived male Wistar rats. The food was weighed at 30, 60, 120, and 240 min after the injection. The results are shown as the mean ± SEM of a group of eight animals. (*) $p < 0.05$; (**) $p < 0.01$; (***) $p < 0.001$ (ANOVA analysis).

tested. The rest of the compounds had no significant activity at 0.5 and 1 μM. Nevertheless, at 3 μM compounds **2**−**4**, **6**, and **8** also had significant antioxidant activity compared with native LDL.

Pereira-Caro et al., in their work with saturated alkyl chain hydroxytyrosol ethers,[9] observed that these derivatives had antioxidant activity similar to that of free hydroxytyrosol in the rancimat test and that the longer the alkyl chain, the lower the activity. Our results show that the number and position of unsaturations in the alkyl chain could also influence the LDL antioxidant activity, with the linoleic derivative (**7**) having a higher activity than the oleic derivative (**3**). We also observed that those compounds that lack the free catechol group had less activity than the catechol derivatives.

We hypothesized that the antioxidant activity of the tested compounds is derived from their catechol/phenol groups, in a similar way to the naturally occurring phenol antioxidants, resveratrol and hydroxytyrosol.

Feeding Experiments. The effect of acute food intake of all the products was tested in 24 h food-deprived rats. Compounds **1**, **4**, **7**, and **8** had significant activity on food intake modulation (Figure 7; the rest of the compounds are shown in the Supporting Information. Compounds **1** and **7** presented a hypophagic effect. Compound **7** was the most active; it presented an acute food intake modulation profile and potency similar to that of oleoyl ethanolamine (OEA, a known anorectic endogenous compound that acts through PPAR-α selective activation; data shown in the Supporting Information) and to recently developed PPAR-α/CB_1 amide ligands.[18,28,29] In contrast, the two compounds that carried a methylenedioxy group (**4** and **8**) presented a hyperphagic effect at a 1 mg/kg dose. Compound **4** had a longer lasting activity than compound **8**, as the effect of compound **8** was seen only in the first 30 min. Using the same methodology, tyrosol and hydroxytyrosol did not present any food modulation activity (see the Supporting Information).

CB_1 and PPAR-α Affinity Experiments. A study on the affinity of this series of compounds for the CB_1 receptor and PPAR-α was performed to further characterize their biological activity. The CB_1 receptor binding test evaluated the capacity of the synthesized compounds to displace [^{3}H]-SR141716 in rat cerebellum homogenate. Compounds **1**, **4**, **7**, and **8**, the four active compounds in the food ingestion test, were evaluated. The linoleic alcohol derivatives **7** and **8** (Figure 8) showed CB_1 receptor affinity, although with a smaller pK_i when compared with endogenous anandamide and synthetic CB_1 ligands, such as WIN55212-2 and SR141716 (Table 1).[30] Oleic alcohol derivative compounds **1** and **4** did not display activity in this test.

We also evaluated the affinity of compounds **1**, **4**, and **7** against PPAR-α by the transient transfection and luciferase reporter assay (Table 1). We used OEA and WY14643 as positive controls. All tested compounds showed an EC_{50} at the micromolar range (Table 1). They displayed lower PPAR-α affinity than OEA (endogenous PPAR-α ligand) and WY14643 (synthetic PPAR-α ligand), although comparable to that observed in other examples of CB_1/PPAR-α ligands previously described by our group.[18]

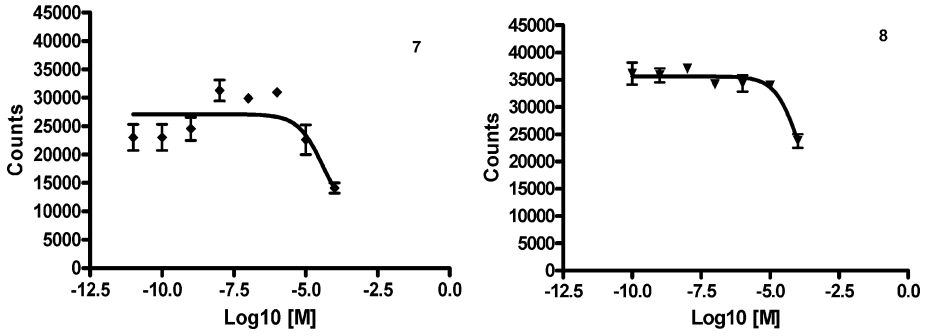

Figure 8. CB_1 binding assay for compounds **7** and **8**.

Table 1. Pharmacological Properties of Fatty Alcohol Ethers

compound	CB_1 K_i (M)	CB_1 pK_i	PPAR-α EC_{50}[a] (nM)
SR141716	1.15×10^{-9}	8.94	
anandamide	1.7×10^{-7} [b]	6.55[b]	>10.000
WIN55212-2			684 ± 47
OEA			148 ± 29
1	no activity	no activity	1165 ± 166
4	no activity	no activity	791 ± 119
7	2.25×10^{-5}	4.65	1934 ± 224
8	7.49×10^{-6}	5.12	

[a]Values calculated in the presence of the different compounds by GraphPad Prism 4. Results for PPAR-α are the mean ± SEM of three experiments. [b]Values from the literature.[27]

In conclusion, in the dienes conjugation experiment we could observe that most of the synthesized compounds had LDL antioxidant activity at the 3 μM concentration. Hydroxytyrosol linoeyl ether (**7**) also had significant antioxidant activity at 0.5 and 1 μM. Both the alkenyl chain and the "head" are important for food intake activity. In addition, depending on structural modifications, the modulation activity could change both in potency and from negative to positive, and vice versa. Possibly, the pharmacodynamic profile of the synthesized compounds operates through mechanisms other than CB_1 and PPAR-α interactions. This is due to the fact that among the four compounds with modulatory food activity, only two displayed CB_1 activity, and their affinity toward PPAR-α was lower than that of OEA. These CB_1 and PPAR-α affinities do not justify their whole in vivo activity.

In summary, the pharmacological profile of these new compounds suggests that they can be further developed as new medications for complicated obesity. They may provide a new way of not only reducing feeding but also ameliorating the negative impact derived of oxidized LDL. However, further research is needed to identify the ADME/TOX profile of these compounds. This a relevant issue because the two only specific therapies against obesity, Sibutramine and Rimonabant,[31] have recently been withdrawn from the market. Although we used the ip route for bypassing the oral route, we do not have information on the absorption of these compounds. However, the natural related compound OEA has been found to be orally active and to cross major barriers, including blood–brain barrier.[32,33] Another important issue is the potential toxic effects of these compounds. Again, initial studies have revealed that not only OEA but also other closely related compounds such amide or sulfonamide derivatives of oleic acid have no effect on liver transaminase levels and cytochrome P450 isoforms, indicating a safe profile.[34,35] However, this needs to be conclusively determined for this present series of compounds. The linoleic hydroxytyrosol linoleyl ether (**7**) was the most active compound in vivo with a hypophagic effect comparable to that of oleoylethanolamine. In addition, it was also the compound that conferred the highest protection of LDL to oxidation in vitro in the same low micromolar range seen for hydroxytyrosol. For all of the above considerations, compound **7** can be regarded as a promising lead for future drug development aiming at concomitantly combating obesity and coronary artery disease, one of its most dangerous related diseases.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra of compounds **15a**, **15b**, **10**, **11**, **13**, **1**, **4**, **5**, **8**, **17a**, **17b**, **17c**, **17d**, **2**, **3**, **6**, and **7**. Graphics of "relative food intake" for tyrosol, hydroxytyrosol, oleoylethanolamine, and compounds **2**, **3**, **5**, and **6**, CB_1 binding assay for compounds SR141716, **1**, and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ DEDICATION

⊗In memory of Prof. Dr. Octavio Augusto Ceva Antunes (Universidade Federal do Rio de Janeiro).

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■ REFERENCES

(1) Glass, C. K.; Witztum, J. L. Atherosclerosis: the road ahead. *Cell* **2001**, *104*, 503–516.

(2) Steinberg, D. The LDL modification hypothesis of atherogenesis: an update. *J. Lipid Res.* **2009**, *50* (Suppl.), S376–S381.

(3) Visioli, F.; Bellomo, G.; Montedoro, G.; Galli, C. Low density lipoprotein oxidation is inhibited in vitro by olive oil constituents. *Atherosclerosis* **1995**, *117*, 25−32.
(4) Gordon, M. H.; Martins, F. P.; Almeida, M. Antioxidant activity of hydroxytyrosol acetate compared with that of other olive oil polyphenols. *J. Agric. Food Chem.* **2001**, *49*, 2480−2485.
(5) Mateos, R.; Domínguez, M. M.; Espartero, J. L.; Cert, A. Antioxidant effect of phenolic compounds, α-tocopherol, and other minor components in virgin olive oil. *J. Agric. Food Chem.* **2003**, *51*, 7170−7175.
(6) Casas, E. M.; Covas, M. I.; Farre, M.; Fito, M.; Ortuño, J.; Weinbrenner, T.; Roset, P.; de la Torre, R. Hydroxytyrosol disposition in humans. *Clin. Chem.* **2003**, *49*, 945−952.
(7) Trujillo, M.; Mateos, R.; de Teran, L. C.; Espartero, J. L.; Cert, R.; Jover, M.; Alcudia, F.; Bautista, J.; Cert, A.; Parrado, J. Lipophilic hydroxytyrosyl esters. Antioxidant activity in lipid matrices and biological systems. *J. Agric. Food Chem.* **2006**, *54*, 3779−3785.
(8) Madrona, A.; Pereira-Caro, G.; Mateos, R.; Rodríguez, G.; Trujillo, M.; Fernández-Bolaños, J.; Espartero, J. L. Synthesis of hydroxytyrosyl alkyl ethers from olive oil waste waters. *Molecules* **2009**, *14*, 1762−1772.
(9) Pereira-Caro, G.; Madrona, A.; Bravo, L.; Espartero, J. L.; Alcudia, F.; Cert, A.; Mateos, R. Antioxidant activity evaluation of alkyl hydroxytyrosyl ethers, a new class of hydroxytyrosol derivatives. *Food Chem.* **2009**, *115*, 86−91.
(10) Pereira-Caro, G.; Sarri, B.; Madrona, A.; Espartero, J. L.; Goya, L.; Bravo, L.; Mateos, R. Alkyl hydroxytyrosyl ethers show protective effects against oxidative stress in HepG2 cells. *J. Agric. Food Chem.* **2011**, *59*, 5964−5976.
(11) Pereira-Caro, G.; Bravo, L.; Madrona, A.; Espartero, J. L.; Mateos, R. Uptake and metabolism of new synthetic lipophilic derivatives, hydroxytyrosyl ethers, by Hhuman hepatoma HepG2 cells. *J. Agric. Food Chem.* **2010**, *58*, 798−806.
(12) Pereira-Caro, G.; Mateos, R.; Saha, S; Madrona, A.; Espartero, J. L.; Bravo, L.; Kroon, P. A. Transepithelial transport and metabolism of new lipophilic ether derivatives of hydroxytyrosol by enterocyte-like Caco-2/TC7 cells. *J. Agric. Food Chem.* **2010**, *58*, 11501−11509.
(13) Haslam, D. W.; James, W. P. Obesity. *Lancet* **2005**, *366*, 1197−1209.
(14) Calle, E. E.; Rodriguez, C.; Walker-Thurmond, K; Thun, M. J. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N. Engl. J. Med.* **2003**, *348*, 1625−1638.
(15) Mackie, K. Cannabinoid receptors as therapeutic targets. *Annu. Rev. Pharmacol.* **2006**, *46*, 101−122.
(16) Kota, B. P.; Huang, T. H.; Roufogalis, B. D. An overview on biological mechanisms of PPARs. *Pharmacol. Res.* **2005**, *51*, 85−94.
(17) O'Sullivan, S. Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. *Br. J. Pharmacol.* **2007**, *152*, 576−582.
(18) Almeida, B.; Joglar, J.; Luque-Rojas, M. J.; Decara, J. M.; Bermúdez-Silva, F. J.; Macías-Gonzalesz, M.; Fitó, M.; Romero, M.; Farré, M.; Covas, M.; de Fonseca, F. R.; de la Torre, R. Synthesis of fatty acid amides of catechol metabolites that exhibit antiobesity properties. *ChemMedChem* **2010**, *5*, 1781−1787.
(19) Lempiaien, H.; Molnar, H.; Gonzalez, M. M.; Perakyla, M.; Carlberg, C. Antagonist and inverse agonist-driven interactions of the vitamin D receptor and the constitutive androstane receptor with corepressor protein. *Mol. Endocrinol.* **2005**, *19*, 2258−2272.
(20) Gonzalez, M. M.; Samenfeld, P.; Perakyla, M.; Carlberg, C. Corepressor excess shifts the two-side chain vitamin D analog gemini from an agonist to an inverse agonist of the vitamin D receptor. *Mol. Endocrinol.* **2003**, *17*, 2028−2038.
(21) Alvarado, M.; Goya, P.; Macias-Gonzalez, M.; Pavon, F. J.; Serrano, A.; Jagerovic, N.; Elguero, J.; Gutierrez-Rodriguez, A.; García-Granda, S.; Suardiaz, M.; Rodríguez de Fonseca, F. Antiobesity designed multiple ligands: synthesis of pyrazole fatty acid amides and evaluation as hypophagic agents. *Bioorg. Med. Chem.* **2008**, *18*, 10098−10105.
(22) Gambacorta, A.; Tofani, D.; Bernini, R.; Migliorini, A. High-yielding preparation of a stable precursor of hydroxytyrosol by total synthesis and from the natural glycoside poleuropein. *J. Agric. Food Chem.* **2007**, *55*, 3386−3391.
(23) van Heerden, F. R.; van Zyl, J. J.; Rall, G. J. H.; Brandt, E. V.; Roux, D. G. Phase-transfer catalysis: a general method of methoxymethylation of the hydroxyl function. *Tetrahedron Lett.* **1978**, *19*, 661−662.
(24) Harcken, C.; Brückner, R. Elucidation of the stereostructure of the annonaceous acetogenin (+)-montecristin through total synthesis. *New J. Chem.* **2001**, *25*, 40−54.
(25) Freedman, H. H.; Dubois, R. A. An improved Williamson ether synthesis using phase transfer catalysis. *Tetrahedron Lett.* **1975**, *16*, 3251−3254.
(26) Rosenthal, A. F.; Vargas, L. A.; Dixon, J. F. Synthesis of long-chain ethers by phase-transfer. Improved preparation of D-mannitol 1,2,5,6-tetraoctadecyl ether. *Chem. Phys. Lipids* **1977**, *20*, 205−209.
(27) Fitó, M.; Covas, M. I.; Raventós, R. M. L.; Vila, J.; de la Torre, C.; Marrugat, J. Aceite de oliva e inhibición de la oxidación de las lipoproteínas de baja densidad. Importancia de los compuestos fenólicos. *Med. Clin. (Barcelona)* **2000**, *115*, 166−169.
(28) de Fonseca, F. R.; Navarro, M.; Gómez, R.; Escuredo, L.; Nava, F.; Fu, J.; Murillo-Rodríguez, E.; Giuffrida, A.; LoVerme, J.; Gaetani, S.; Kathuria, S.; Galk, C.; Piomelli, D. An anorexic lipid mediator regulated by feeding. *Nature* **2001**, *414*, 209−212.
(29) Fu, J.; Gaetani, S.; Oveisi, F.; LoVerme, J.; Serrano, A.; de Fonseca, F. R.; Rosengarth, A.; Lueke, H.; Giacomo, B. D.; Tarzia, G.; Piomelli, D. Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-α. *Nature* **2003**, *425*, 90−93.
(30) Griffin, G.; Wray, E. J.; Martin, B. R.; Abood, M. E. Cannabinoid agonists and antagonists discriminated by receptor binding in rat cerebellum. *Br. J. Pharmacol.* **1999**, *128*, 684−688.
(31) Rodríguez de Fonseca, F. Should we ever treat obesity? The case of Rimonabant. *Obes. Metab.−Milan* **2008**, *4*, 192.
(32) Oveisi, F.; Gaetani, S.; Eng, K. T.; Piomelli, D. Oleoylethanolamide inhibits food intake in free-feeding rats after oral administration. *Pharmacol. Res.* **2004**, *49*, 461−466.
(33) Plaza-Zabala, A.; Berrendero, F.; Suarez, J.; Bermudez-Silva, F. J.; Fernandez-Espejo, E.; Serrano, A.; Pavon, F. J.; Parsons, L. H.; De Fonseca, F. R.; Maldonado, R.; Robledo, P. Effects of the endogenous PPAR-α agonist, oleoylethanolamide on MDMA-induced cognitive deficits in mice. *Synapse* **2010**, *64*, 379−389.
(34) Serrano, A.; Del Arco, I.; Javier Pavón, F.; Macías, M.; Perez-Valero, V.; Rodríguez de Fonseca, F. The cannabinoid CB_1 receptor antagonist SR141716A (Rimonabant) enhances the metabolic benefits of long-term treatment with oleoylethanolamide in Zucker rats. *Neuropharmacology* **2008**, *54*, 226−234.
(35) Rodríguez de Fonseca, F.; de la Torre, R.; Joglar, J. et al. Unpublished observation.