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Synthesis and CB1 receptor activities of novel arachidonyl alcohol derivatives

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Abstract—Novel derivatives of arachidonyl alcohol were synthesized and evaluated for their CB1 receptor activity by [^{35}S]GTP$_\gamma$S assay using rat cerebellar membranes.

1. Introduction

Since the discovery of Δ^9-THC, remarkable progress has been made in the area of CB1 receptor ligands. CB1 receptor agonists are endogenous, exogenous or synthetic compounds which are traditionally divided into four major groups according to their chemical structure: (1) classical cannabinoids, like Δ^9-THC, (2) nonclassical cannabinoids developed by Pfizer (e.g., CP 55,940), (3) compounds similar to endogenous cannabinoids called eicosanoids and (4) finally aminoalkylindoles developed by the Sterling Winthrop research team (e.g., WIN55, 212-2).

The most potent CB1 receptor ligands are synthetic and belong to the group of classical cannabinois. The endogenous cannabinoid, 2-arachidonoyl glycerol (2-AG), is not as potent, but according to the [^{35}S]GTP$_\gamma$S activation studies, it is the most efficient CB1 agonist so far.[1] The high efficacy of 2-AG provides interest in the molecular structure of eicosanoids, which was also the starting point of this study.

The endogenous cannabinoids, arachidonoyl ethanol amide (AEA) and 2-AG, are metabolically labile molecules, and several attempts have been made to stabilize the linkage between the polar head and the lipophilic tail. With AEA some progress has been made by methylating postitions C-1′[2,3] and C-2,[4] and using reversed amide linkage.[2] The replacement of an ester linkage of 2-AG with an ether linkage (HU-310, hereafter defined as third endogenous cannabinoid,[5] although this has been recently disputed[6]), or with a ketogroup,[7] has improved stability, but efficacy decreases considerably compared to 2-AG.[1]

The aim of the study was to synthesize ester, carbonate and carbamate derivatives of arachidonyl alcohol in an effort to achieve three main goals; firstly, the CB1 receptor activities for derivatives of arachidonyl alcohol have not been previously investigated, secondly, carbamate, carbonate and reversed ester linkages were developed for possible improved enzymatic stability, and finally, to achieve the liberation of arachidonyl alcohol instead of arachidonic acid after enzymatic or chemical hydrolysis of the derivative. In the present study we describe the syntheses of these compounds and their CB1 receptor activities.

2. Synthesis

The syntheses of the arachidonyl alcohol esters (Fig. 1, compounds **1**–**7**) are outlined in Scheme 1. Preparation of the esters of arachidonyl alcohol turned out to be difficult due to self-degradation and polymerization. Compounds **1**–**5** were synthesized as follows (Scheme 1).

Keywords: Cannabinoid; CB1 receptor agonist; Synthesis; [^{35}S]GTP$_\gamma$S activation.
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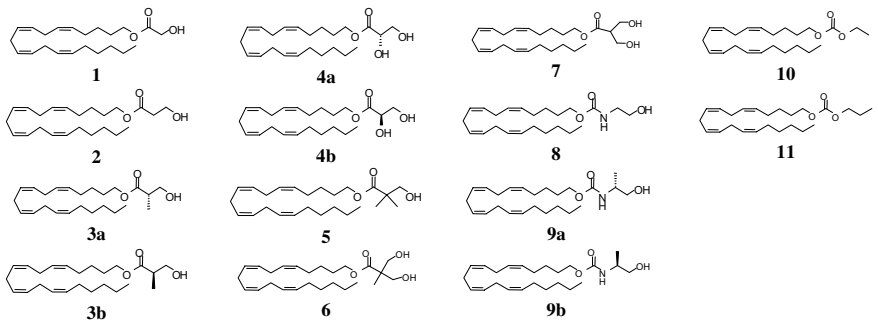
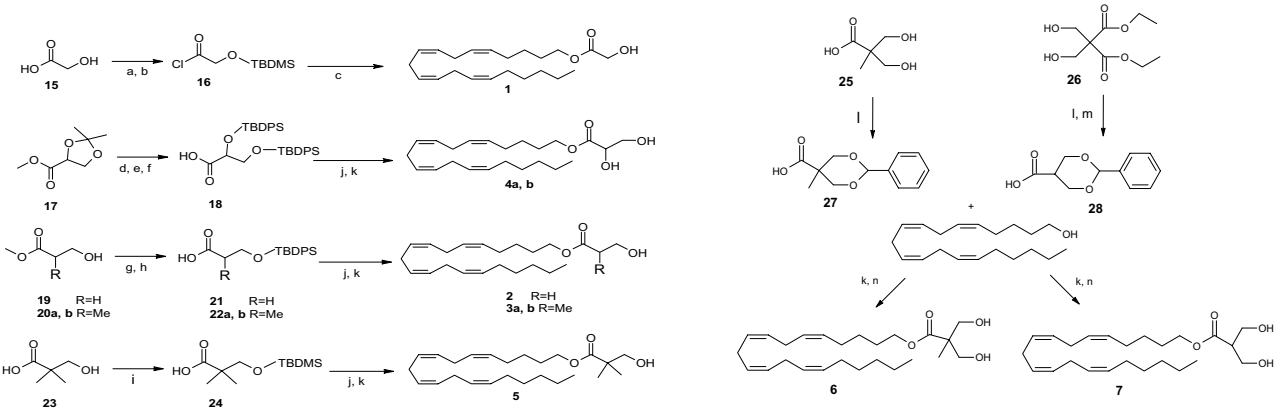


Figure 1. Chemical structures of synthesized molecules.



Scheme 1. (a) TBDMSCl, imidazole, DMF, 63%; (b) 2 M oxalyl chloride, DCM, 84%; (c) arachidonyl alcohol, Et_3N, DCM, 84%; (d) 1. NaOH, 2. HCl, 3. BnBr, 60%; (e) TBDPSCl, imidazole, THF, 100%; (f) Pd/C, H_2, 88%; (g) TBDPSCl, THF, 76%; (h) LiOH, 83%; (i) 1. TBDMSCl, imidazole, THF, 71%, 2. THF/H_2O, 48%; (j) arachidonyl alcohol, DCC, DMAP, 64–95%; (k) $Bu_4N^+F^-$, 30–66%; (l) benzaldehyde, toluene, *p*-TSA, 51–82%; (m) 1. KOH, 2. HCl, 3. Et_3N, Δ, 31%; (n) HCl/MeOH, 74%.

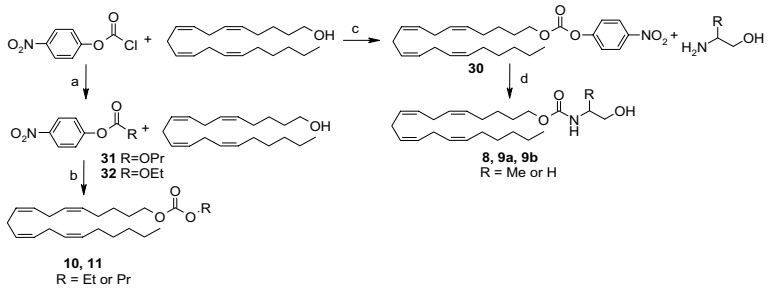
Compound **15** was first treated with *t*-butyldimethylsilyl chloride (TBDMSCl), followed by acid chloride formation using oxalyl chloride.[8] Activated and protected **16** was attached to arachidonyl alcohol under basic conditions.[9] Preparation of **4a** and **4b** began with enantiomerically pure starting materials.[10–12] Compound **17** was first unprotected under basic conditions, followed by protection of carboxylic acid group with benzyl (Bn)[13] and then hydroxyl groups with *t*-butyldiphenylsilyl group (TBDPS).[14] Benzyl group was removed by catalytic hydrogenation,[13] and compound **18** was coupled with arachidonyl alcohol. The syntheses of compounds **3a** and **3b** also began from enantiomerically pure compounds. Compounds **19** and **20** were protected with TBDPS and then hydrolyzed under basic conditions.[14]

Compounds **21** and **22** were coupled with arachidonyl alcohol using *N*,*N*′-dicyclohexylcarbodiimide (DCC) coupling. All silyl protected intermediates were deprotected using $Bu_4N^+F^-$ in THF, leading to the final products **1**–**5**.[15] The synthetic pathways for compounds **6** and **7** are presented in Scheme 1. The hydroxyl groups of **25** and **26** were protected with benzylidene acetal. After protection, **26** was decarboxylated, leading to **28**.[16] Compounds **27** and **28** were attached to arachidonyl alcohol with DCC coupling and deprotected with concentrated HCl/MeOH (1:1) solution, leading to the final products **6** and **7**.

The syntheses of **8**–**11** are outlined in Scheme 2. Carbamates **8**, **9a** and **9b** were prepared by attaching *p*-nitrophenylchlorofomate activated arachidonyl alcohol to aminoalcohol under basic condition.[17,18] The *p*-nitrophenylchloroformate activation was also used in and the preparation of carbonates **10** and **11**. However, in this case the carboxylic acid residue was activated and attached to arachidonyl alcohol.

3. In vitro studies

Maximal responses (E_{max}, % basal) to all compounds, as well as their CB_1 receptor-dependent activity, were determined by [^{35}S]GTP$_\gamma$S-binding studies. These studies were conducted using four-week-old male Wistar rats. All animal experiments were approved by the local ethics committee. The animals lived in a 12-h light/12-h dark cycle (lights on at 07:00 h) with water and food available ad libitum. The rats were decapitated 8 h after lights on (15:00 h), whole brains were removed, dipped in isopentane on dry ice and stored at −80 °C. Rat



Scheme 2. (a) Ethanol/propanol, pyridine, THF, 77–88%; (b) DMAP, DCM, 77–91%; (c) pyridine, THF, 87%; (d) Et_3N, THF, 80–100%.

cerebellar membranes were prepared as previously described.[1] Incubations using PMSF-pretreated (1 mM) rat cerebellar membranes were carried out essentially as previously described.[1] For agonist dose–response and antagonist experiments, results are presented as mean ± SEM of at least three independent experiments, performed in duplicate. Data analysis for dose–response curves were calculated as nonlinear regressions by GraphPad Prism 3.0 for Windows.

4. Results and discussion

Among all the ligands synthesized, only compounds **4a** and **4b** showed dose-dependent CB1 activity (Table 1 and Fig. 2). For both of these compounds, responses at 5×10^{-5} M were reversed by CB1 receptor antagonist, AM251 (1 μM), to 109 ± 2 and 115 ± 6 (% basal ± SEM, $n = 2$), respectively. However, both efficacy and potency values of **4a** and **4b** were weaker than those of AEA (Table 1). Other compounds did not stimulate [^{35}S]GTP$_\gamma$S binding, or the activity achieved was not attenuated by 1 μM AM251 (data not shown).

Table 1. Comparison of efficacy (E_{max}) and potency ($-\log EC_{50}$) of **4a** and **4b** and AEA

Compound	CB1 activation	
	E_{max} (% basal ± SE)	$-\text{Log}EC_{50}$ ± SE
4a	278 ± 21	4.4 ± 0.2
4b	301 ± 8	4.6 ± 0.1
AEA	380 ± 6	4.8 ± 0.0

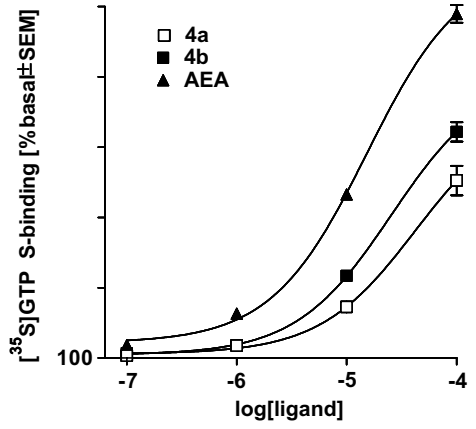


Figure 2. Dose–response curves for **4a** and **4b** and AEA for G-protein binding in rat cerebellar membranes (mean ± SEM, $n = 3$).

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References and notes

1. Savinainen, J. R.; Järvinen, T.; Laine, K.; Laitinen, J. T. *Br. J. Pharmacol.* **2001**, *134*, 664.
2. Abadji, V.; Lin, S.; Taha, G.; Griffin, G.; Stevenson, L. A.; Pertwee, R. G.; Makriyannis, A. *J. Med. Chem.* **1994**, *37*, 1889.
3. Lin, S.; Khanolkar, A. D.; Fan, P.; Goutopoulos, A.; Qin, C.; Papahadjis, D.; Makriyannis, A. *J. Med. Chem.* **1998**, *41*, 5353.
4. Adams, I. B.; Ryan, W.; Singer, M.; Razdan, R. K.; Compton, D. R.; Martin, B. R. *Life Sci.* **1995**, *56*, 2041.
5. Hanuš, L.; Abu-Lafi, S.; Fride, E.; Breuer, A.; Vogel, Z.; Shalev, D. E.; Kustanovich, I.; Mechoulam, R. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3662.
6. Oka, S.; Tsuchie, A.; Tokumura, A.; Muramatsu, M.; Suhara, Y.; Takayama, H.; Waku, K.; Sugiura, T. *J. Neurochem.* **2003**, *85*, 1374.
7. Suhara, Y.; Nakane, S.; Arai, S.; Takayama, H.; Waku, K.; Ishima, Y.; Sugiura, T. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1985.
8. Glabe, A. R.; Sturgeon, K. L.; Ghizzoni, S. B.; Musker, W. K.; Takahashi, J. N. *J. Org. Chem.* **1996**, *61*, 7212.
9. Gu, L.; Dunn, J.; Dvorak, C. *Drug Dev. Ind. Pharm.* **1989**, *15*, 209.
10. (*R*)-2,3-Di-[(*t*-butyldiphenylsilyl)oxy] propionic acid (**18**). Methyl-(*R*)-2,2-dimethyl-1,3-dioxalane-4-carboxylate (**17**) (47 mg, 2.93 mmol) was dissolved in THF/H_2O (150 mL/85 mL), and the solution was cooled on an ice bath. 2 M NaOH (2.6 mL) was added slowly. After the addition, the cooling bath was removed and stirring was continued for 1 h. The reaction mixture was acidified with 2 M HCl and stirred at rt for 2 h. The solvent was evaporated. The residue was dissolved in MeOH/H_2O (5 mL/0.5 mL), and the solution was neutralized with 20% $NaHCO_3$. The solvent was evaporated. The residue was dissolved in dry DMF, and benzylbromide (0.38 mL, 3.22 mmol) was added. The reaction mixture was stirred under Ar at rt for 2 days. The solvent was evaporated, the residue was

dissolved in EtOAc and washed with brine. The organic layer was dried over Na_2SO_4, filtered and evaporated. The crude product was purified by flash chromatography, eluting with EtOAc/petroleum ether 3:1. Yield of the colourless oil was 340 mg (60%). $R_f = 0.46$ (EtOAc/petroleum ether 4:1). ^{1}H NMR ($CDCl_3$): 7.40–7.33 (m, 5H), 5.29–5.24 (m, 2H), 4.31 (t, $J = 3.5$ Hz, 1H), 3.93–3.86 (m, 2H). The mixture of benzyl-((*R*)-2,3-dihydroxy) propionate (340 mg, 1.73 mmol), TBDMSCl (0.98 mL, 3.81 mmol) and imidazole (366 mg, 5.37 mmol) in DMF (7 mL) was stirred under Ar at rt for 5 h. The solvent was evaporated, and the residue was dried in vacuo. The reaction proceeded quantitively. $R_f = 0.70$ (EtOAc/petroleum ether 1:4). ^{1}H NMR ($CDCl_3$): 7.76–7.74 (m, 1H), 7.66–7.57 (m, 8H), 7.41–7.27 (m, 14H), 7.18–7.16 (m, 2H), 4.94 (d, $J = 12.3$ Hz, 1H), 4.90 (d, $J = 12.3$ Hz, 1H), 4.37 (t, $J = 4.3$ Hz, 1H), 3.93 (dd, $J = 4.5$ Hz, 10.2 Hz, 1H), 3.86 (dd, $J = 4.4$ Hz, 10.3 Hz, 1H), 1.07 (s, 9H), 1.00 (s, 9H). Benzyl-{(*R*)-2,3-di-[(*t*-butyldiphenylsilyl)oxy]} propionate (1.16 g, 1.73 mmol) was dissolved in dry THF (10 mL) and Pd/C (941 mg) was added. The reaction mixture was stirred under H_2-balloon for 3 days. The catalyst was filtered, and the solvent was evaporated. The crude product was purified with flash chromatography, eluting with petroleum ether/EtOAc 8:1. Evaporation of solvents yielded colourless oil (890 mg, 88%). $R_f = 0.29$ (petroleum ether/EtOAc 4:1). ^{1}H NMR ($CDCl_3$): 7.74–7.72 (m, 1H), 7.65–7.58 (m, 8H), 7.45–7.28 (m, 11H), 4.32 (t, $J = 4.3$ Hz, 1H), 3.88 (dd, $J = 3.1$ Hz, 10.7 Hz, 1H), 3.64 (dd, $J = 3.1$ Hz, 10.7 Hz, 1H), 1.14 (s, 9H), 1.06 (s, 9H).

11. (*R*)-2,3-Dihydroxy-propionic acid icosa-5,8,11,14-tetraenyl ester (**4a**). (*R*)-2,3-Di-[(*t*-butyldiphenyl-silyl)oxy] propionic acid (**18**) (408 mg, 0.71 mmol), was dissolved in dry DCM (10 mL) and arachidonyl alcohol (172 mg, 0.59 mmol), DCC (244 mg, 1.18 mmol) and DMAP (43 mg, 0.35 mmol) were added. The reaction mixture was stirred under Ar at rt over night. The white precipitate was filtered, and the filtrate was washed with 10% citric acid, 5% $NaHCO_3$ and water. The combined organic layers were dried over Na_2SO_4, filtered and evaporated. The crude product was purified by flash chromatography, eluting with 0–4% EtOAc in petroleum ether. Evaporation of solvents yielded a colourless oil (320 mg, 57%). The solution of (*R*)-2,3-di-[(*t*-butyldiphenyl-silyl)oxy] propionic acid icosa-5,8,11,14-tetraenyl ester (170 mg, 0.20 mmol) and 1 M $Bu_4N^+F^-$ (300 μL) in dry THF (3 mL) was stirred under Ar at rt for 2 h. THF was evaporated, and the crude product was purified by flash chromatography, eluting with petroleum ether/EtOAC 3:1. Evaporation of solvents yielded 59 mg of an oily product (78%). ^{1}H NMR ($CDCl_3$): δ 5.43–5.31 (m, 8H), 4.26 (br, 1H), 4.23 (t, $J = 6.6$ Hz, 2H), 3.87 (dd, 2H), 3.23 (br, 1H), 2.85–2.80 (m, 6H), 2.25 (br, 1H), 2.11 (q, $J = 7.1$ Hz, 2H), 2.06 (q, $J = 7.1$ Hz, 2H), 1.70 (qui, $J = 6.8$ Hz, 2H), 1.44 (qui, $J = 7.7$ Hz, 2H), 1.39–1.26 (m, 6H), 0.89 (t, $J = 6.8$ Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 173.1, 130.5, 129.4, 128.6, 128.5, 128.2, 128.2, 127.9, 127.5, 71.5, 66.1, 64.1, 31.5, 29.3, 28.1, 27.2, 26.7, 26.6, 25.7 (3C), 22.6, 14.1. $R_f = 0.11$ (petroleum ether/EtOAc 3:1). Elemental analysis. Calculated for $C_{23}H_{38}O_4$*1/10H_2O: C 72.63%; H 10.12%. Found C 72.49%, H 10.18%.

12. (*S*)-2,3-Dihydroxy-propionic acid icosa-5,8,11,14-tetraenyl ester (**4b**). The synthesis was similar to the synthesis of compound **4a**. ^{1}H NMR ($CDCl_3$): δ 5.43–5.31 (m, 8H), 4.26 (br, 1H), 4.23 (t, $J = 6.6$ Hz, 2H), 3.86 (dd, 2H), 2.85–2.80 (m, 6H), 2.25 (br, 1H), 2.11 (q, $J = 7.2$ Hz, 2H), 2.06 (q, $J = 7.2$ Hz, 2H), 1.70 (qui, $J = 6.7$ Hz, 2H), 1.44 (qui, $J = 7.6$ Hz, 2H), 1.39–1.26 (m, 6H), 0.89 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 173.1, 130.5, 129.4, 128.6, 128.5, 128.2, 128.2, 127.9, 127.5, 71.6, 66.1, 64.1, 31.5, 29.3, 28.1, 27.2, 26.7, 26.6, 25.8, 25.7 (2C), 22.6, 14.1. $R_f = 0.11$ (petroleum ether/EtOAc 3:1). ESI-MS 400.3 (M+Na). Elemental analysis. Calculated for $C_{23}H_{38}O_4$*1/10H_2O: C 72.63%; H 10.12%. Found C 72.30%, H 10.30%.

13. Shin, I.; Lee, M.-R.; Lee, J.; Jung, M.; Lee, W.; Yoon, J. *J. Org. Chem.* **2000**, *65*, 7667.
14. Oliver, J. E.; Doss, R. P.; Williamson, T. R.; Carney, J. R.; DeVilbiss, E. D. *Tetrahedron* **2000**, *56*, 7633.
15. Lal, B.; Gangopadhyay, A. K.; Rajagopalan, R.; Ghate, A. V. *Bioorg. Med. Chem.* **1998**, *6*, 2061.
16. Scriba, G. K. E. *Arch. Pharm.* **1993**, *326*, 477.
17. Andrews, D. M.; Cherry, P. C.; Humber, D. C.; Jones, P. S.; Keeling, S. P.; Martin, P. F.; Shaw, C. D.; Swanson, S. *Eur. J. Med. Chem.* **1999**, *34*, 563.
18. Hay, M. P.; Wilson, W. R.; Denny, W. A. *Tetrahedron* **2000**, *56*, 645.