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# ANALOGS OF 2-ARACHIDONOYLGLYCERIN CONTAINING THE NO-DONOR GROUP

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*1,3-Dinitroglyceryl esters of fatty acids, analogs of endocannabinoid 2-arachidonoylglycerin, were synthesized. Various methods for esterifying fatty acids with glycerine dinitrate were developed.*

**Keywords:** endocannabinoids, 2-arachidonoylglycerin, analogs, nitroesters.

Amide and ester derivatives of arachidonic acid and several other polyunsaturated fatty acids are considered to be neurolipins, a constantly growing family of low-molecular-weight lipid-type bioregulators. The most well studied of these are *N*-arachidonoylethanolamine (anandamide) [1] and 2-arachidonoylglycerin (2-AG) [2], which are ligands of type 1 and type 2 cannabinoid receptors and are therefore called endocannabinoids. These neurolipins exhibit a broad spectrum of biological effects that are not always mediated by binding only to these receptors. 2-AG is formed upon activation of cells by various stimuli and is the product of hydrolysis by diacylglycerinlipase of diacylglycerins containing arachidonic acid in the sn-2 position [3]. 2-AG is found in the central and peripheral nervous systems and in many other organs and tissues including the heart, liver, spleen, lungs, kidneys, plasma, small and large intestines, and breast milk [3]. Like arachidonic acid, 2-AG can activate human platelets containing cannabinoid receptors. However, the activation mechanism remains to this day a subject of discussion.

The broad spectrum of biological effects of 2-AG is attractive for both discovering the structural elements responsible for one type of activity or another and creating prototypes of new drugs based on this molecule. Several new structural analogs of 2-AG were proposed. Both the fatty acid and the glycerin moieties and the chemical bond joining them were modified (e.g., the ester bond was replaced by an ether bond) [4].

In developing the concept promoted by us of hybrid multifunctional compounds containing an NO-generating group as one of the components [5], we synthesized dinitro derivatives of 2-AG and its analogs with various fatty-acid chain lengths. 1,3-Dinitroglycerin esters of fatty acids were prepared via esterification of fatty acids by glycerin 1,3-dinitrate using activation of the carboxylic group of the starting acid (Scheme 1). The carboxylic group was activated by forming a mixed anhydride with arylsulfonic acids (method 1), by using acid halides (methods 2 and 3), and by preparing dinitroglycerin esters with short-chain carboxylic acids, primarily with the use of diisopropylcarbodiimide (method 4).

The acid (**1**) was first converted to the mixed anhydride (**2**) by reaction with arylsulfonylchloride [*p*-toluenesulfonylchloride (TsCl) or 2,4,6-tri-*iso*-propylbenzenesulfonylchloride (TPSCl)] in the presence of $Et_3N$ (Scheme 1) if the mixed-anhydride method was used in order to avoid possible reactions of the arylsulfonylchloride with glycerin 1,3-dinitrate. After completing this step, glycerin 1,3-dinitrate and a catalytic amount of dimethylaminopyridine (DMAP) were added. This produced the desired 1,3-dinitroglycerin esters of the fatty acid (**6**).

The lack of hydroxyls in arachidonic and the other fatty acids enabled the acid-halide method for activating the carboxylic group to be used. We used both the fluorides and chlorides of these acids. The acid chlorides (**3**) were prepared via reaction with an excess of thionylchloride in benzene at room temperature (method 2). After forming the acid chloride (~2 h), the excess of thionylchloride was removed in vacuo. The acid fluorides (**4**) were prepared by treating a $CH_3CN$ solution of the acid with an excess of cyanuric fluoride in the presence of Py at room temperature (method 3). The acid fluoride was formed in ~1 h and used without isolation. The synthesized acid halides were condensed with the alcohol in the presence of DMAP as a catalyst to obtain the desired esters.

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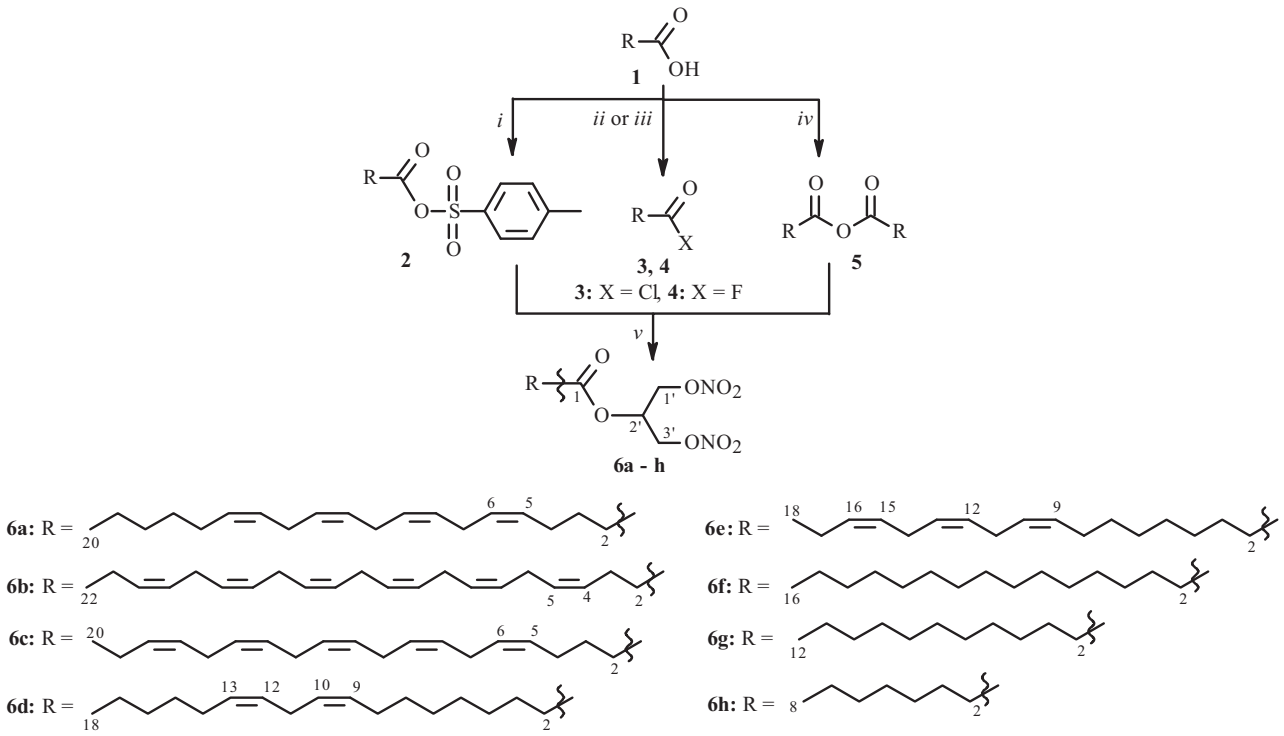


*i*. acetone, TsCl, $Et_3N$, RT, 10 min, Ar; *ii*. benzene, $SOCl_2$, RT, 2 h; *iii*. $CH_3CN$, Py, cyanuric fluoride, RT, 1 h; *iv*. $CH_2Cl_2$, diisopropylcarbodiimide, RT; *v*. glycerin 1,3-dinitrate, DMAP, RT, 1 h.

Scheme 1

Treatment of the fatty acids with diisopropylcarbodiimide formed the symmetric anhydride (**5**) (method 4), which reacted with glycerin 1,3-dinitrate in the presence of DMAP to form the desired esters.

The developed synthetic methods were used to prepare 1,3-dinitroglycerin esters of several fatty acids, i.e., arachidonic (**6a**), docosahexaenoic (**6b**), eicosapentaenoic (**6c**), linoleic (**6d**), linolenic (**6e**), palmitic (**6f**), lauric (**6g**), and caprylic (**6h**). The products were obtained in moderate (up to 64%) yield if methods 1 and 2 were used to synthesize the 1,3-dinitroglycerin esters. Use of the arylsulfonylchlorides (method 1) gave lower yields because of a side reaction involving chlorination of glycerin dinitrate by the excess of the reagent, which could not be avoided. However, the acid-chloride method (method 2) required removal of the excess of thionylchloride, which led to partial loss of the compound during evaporation of the reaction mixture because of the formation of side products, especially for the more highly unsaturated fatty acids. Method 3 turned out to be the most practical. It was based on the use of cyanuric fluoride. The yields of these reactions were 75% and more with practically no side products. Apparently a side product of the reaction with diisopropylcarbodiimide was the acylurea, which had a negative effect on the yield of the desired product. Furthermore, only half of the used acid reacted because only one acid in the intermediate anhydride **5** reacted with glycerin 1,3-dinitrate.

We compared the effects of 2-AG and its 1,3-dinitroglycerin ester on the ability of human platelets to aggregate. As shown above, 2-AG and arachidonic acid itself are inductors of platelet aggregation. However, introduction of the two nitro groups into 2-AG turned this neurolipin into an inhibitor of platelet aggregation that effectively suppressed aggregation induced by both free arachidonic acid and adenosine diphosphate at a concentration of 0.1 mg/mL (for preliminary experimental results, see [6]).

Thus, introducing nitro groups into endogenous 2-AG produced a fundamental change of its biological activity. This presents new possibilities for developing hybrid NO-donor compounds based on natural neurolipins.

## EXPERIMENTAL

Arachidonic, eicosapentaenoic, and docosahexaenoic acids (Poliprost, Russia) and the other fatty acids (Aldrich, USA) were purchased. PMR spectra were recorded in $CDCl_3$ on a Bruker WM500 spectrometer (Bruker, Germany). Chemical

shifts (δ, ppm) are given relative to $Me_4Si$. TLC was performed on Silufol UV 254 plates (Kavalier, Czechoslovakia) with detection by phosphomolybdic acid (5%) in alcohol. Column chromatography was carried out over silica gel L (100–250 μm) (Chemapol, Czech Rep.). The separation was monitored by TLC. Solutions were evaporated in a rotary evaporator in vacuo (water aspirator) at bath temperature <30°C. All reactions involving polyenoic fatty acids were carried out under Ar.

**Standard Work up of Reaction Mixture.** After the reaction was finished, the reaction mixture was diluted with $H_2O$ and extracted with EtOAc (3 × 5 mL). The combined organic extract was washed with $H_2O$ and saturated NaCl solution and dried over anhydrous $Na_2SO_4$. The desiccant was filtered off. The filtrate was evaporated in vacuo. The residue was purified by column chromatography over silica gel G 60 (Merck, Germany) using a gradient of hexane:benzene or benzene:EtOAc. Fractions containing the product were combined. The solvent was evaporated in vacuo. The purity of the products was >95% according to HPLC.

**Synthesis of 1,3-Dinitroglycerin Esters of Fatty Acids. Method 1.** A solution of the acid (0.33 mmol) in acetone (3 mL) was treated with $Et_3N$ (0.7 mmol, 100 μL) and *p*-toluenesulfonylchloride (100 mg, 0.53 mmol), stirred for 10 min, treated with DMAP (20 mg) and glycerin 1,3-dinitrate (100 mg, 0.55 mmol), and stirred for another hour at room temperature. The mixture underwent the standard work up.

**Method 2.** A solution of the acid (0.33 mmol) in benzene (2 mL) was treated with thionylchloride (1 mL, 13.8 mmol), stirred for 2 h at room temperature, and evaporated. The residue was dissolved in benzene (2 mL), treated with glycerin 1,3-dinitrate (75 mg, 0.41 mmol) and $Et_3N$ (70 μL, 0.49 mmol), and stirred for 12 h at room temperature. The mixture underwent the standard work up.

**Method 3.** A solution of the acid (0.33 mmol) in $CH_3CN$ (2 mL) was treated with Py (100 μL) and cyanuric fluoride (100 μL), stirred for 1 h at room temperature, treated with glycerin 1,3-dinitrate (75 mg, 0.41 mmol) and DMAP (50 mg), and stirred for 12 h at 23°C. The mixture underwent the standard work up.

**Method 4.** A solution of caprylic acid (180 mg, 1.26 mmol) in $CH_2Cl_2$ (4 mL) was treated successively with diisopropylcarbodiimide (234 μL, 1.47 mmol), glycerin 1,3-dinitrate (255 mg, 1.34 mmol), and DMAP (153 mg, 1.25 mmol), and stirred for 1.5 h at room temperature. The mixture underwent the standard work up.

**1,3-Dinitroglycerin Ester of Arachidonic Acid (6a).** Light-yellow viscous oil, $R_f$ 0.37 (benzene:hexane, 1:1). PMR spectrum (δ, ppm, J/Hz): 5.36 (9H, m, H-5,6,8,9,11,12,14,15,2′), 4.57 (2H, dd, J = 4.3, 12.4, H-1′ or H-3′), 4.73 (2H, dd, J = 5.5, 12.4, H-1′ or H-3′), 2.81 (6H, m, H-7,10,13), 2.34 (2H, t, J = 8, H-2), 2.07 (4H, m, H-4,16), 1.6 (2H, m, H-3), 1.36 (2H, m, H-17), 1.30 (4H, m, H-18,19), 0.9 (3H, t, J = 7, H-20).

**1,3-Dinitroglycerin Ester of Docosahexaenoic Acid (6b).** Colorless viscous oil, $R_f$ 0.38 (benzene:hexane, 1:1). PMR spectrum (δ, ppm, J/Hz): 5.31 (13H, m, H-4,5,7,8,10,11,13,14,16,17,19,20,2′), 4.74 (2H, dd, J = 5.5, 12.4, H-1′ or H-3′), 4.58 (2H, dd, J = 4.3, 12.4, H-1′ or H-3′), 2.78 (10H, m, H-6,9,12,15,18), 2.38 (4H, m, H-2,21), 2.04 (2H, t, J = 7.4, H-3), 0.96 (3H, t, J = 7.5, H-22).

**1,3-Dinitroglycerin Ester of Eicosapentaenoic Acid (6c).** Colorless viscous oil, $R_f$ 0.66 (benzene). PMR spectrum (δ, ppm, J/Hz): 5.35 (11H, m, H-5,6,8,9,11,12,14,15,17,18,2′), 4.74 (2H, dd, J = 4.2, 12.4, H-1′ or H-3′), 4.56 (2H, dd, J = 5.6, 12.4, H-1′ or H-3′), 2.83 (8H, m, H-7,10,13,16), 2.38 (2H, t, J = 7.4, H-2), 2.13 (4H, m, H-4,19), 1.74 (2H, quin, J = 7.3, H-3), 1.01 (3H, t, J = 7.5, H-20).

**1,3-Dinitroglycerin Ester of Linoleic Acid (6d).** Yellow viscous oil, $R_f$ 0.62 (benzene). PMR spectrum (δ, ppm, J/Hz): 5.29 (5H, m, H-9,10,12,13,2′), 4.71 (2H, dd, J = 4.2, 12.4, H-1′ or H-3′), 4.53 (2H, dd, J = 5.6, 12.4, H-1′ or H-3′), 2.73 (2H, m, H-11), 2.33 (2H, t, J = 7.4, H-2), 2.03 (4H, m, H-8,14), 1.62 (2H, m, H-3), 1.32 (14H, m, H-4,5,6,7,15,16,17), 0.90 (3H, t, J = 6.7, H-18).

**1,3-Dinitroglycerin Ester of Linolenic Acid (6e).** Colorless viscous oil, $R_f$ 0.37 (benzene:hexane, 1:1). PMR spectrum (δ, ppm, J/Hz): 5.29 (7H, m, H-9,10,12,13,15,16,2′), 4.71 (2H, dd, J = 4.2, 12.4, H-1′ or H-3′), 4.53 (2H, dd, J = 5.6, 12.4, H-1′ or H-3′), 2.76 (4H, m, H-11,14), 2.33 (2H, t, J = 7.4, H-2), 2.04 (4H, m, H-8,17), 1.62 (2H, m, H-3), 1.32 (8H, m, H-4,5,6,7), 0.98 (3H, t, J = 7.5, H-18).

**1,3-Dinitroglycerin Ester of Palmitic Acid (6f).** Colorless viscous oil, $R_f$ 0.36 (benzene:hexane, 1:1). PMR spectrum (δ, ppm, J/Hz): 5.29 (1H, m, H-1′), 4.70 (2H, dd, J = 4.3, 12.4, H-1′ or H-3′), 4.52 (2H, dd, J = 5.6, 12.4, H-1′ or H-3′), 2.32 (2H, t, J = 7.4, H-2), 1.61 (2H, m, H-3), 1.26 (24H, m, H-4,5,6,7,8,9,10,11,12,13,14,15), 0.87 (3H, t, J = 7.0, H-16).

**1,3-Dinitroglycerin Ester of Lauric Acid (6g).** Colorless viscous oil, $R_f$ 0.41 (benzene:hexane, 1:1). PMR spectrum (δ, ppm, J/Hz): 5.29 (1H, m, H-2′), 4.70 (2H, dd, J = 4.2, 12.4, H-1′ or H-3′), 4.52 (2H, dd, J = 5.6, 12.4, H-1′ or H-3′), 2.31 (1H, t, J = 7.4, Hb-2), 2.30 (1H, t, J = 7.4, Ha-2), 1.60 (2H, m, H-3), 1.26 (16H, m, H-4,5,6,7,8,9,10,11), 0.87 (3H, t, J = 6.9, H-12).

**1,3-Dinitroglycerin Ester of Caprylic Acid (6h).** Colorless viscous oil, $R_f$ 0.59 (benzene). PMR spectrum (δ, ppm, J/Hz): 5.31 (1H, m, H-2′), 4.72 (2H, dd, J = 4.2, 12.4, H-1′ or H-3′), 4.54 (2H, dd, J = 5.6, 12.4, H-1′ or H-3′), 2.33 (2H, t, J = 7.4, H-2), 1.62 (2H, m, H-3), 1.30 (8H, m, H-4,5,6,7), 0.89 (3H, t, J = 6.9, H-8).


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