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Synthesis of long chain lipid amides and their analgesic properties

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Abstract
Long chain lipid amides isolated from animal tissues are known to have analgesic and anti-inflammatory properties. The synthesis of analogues of such lipid amides of biological origin was designed and carried out. The analgesic properties of some of the synthesized molecules were also carried out. Condensation reaction involving palmitoyl chloride and primary amino compounds was carried out to produce the desired compounds. Analgesic properties were investigated using acetic acid induced writhing and hot plate models. The synthetic procedure yielded the desired products in good quantity. There was a dose dependent analgesic activity of the products tested. These simple organic molecules, which show promising analgesic properties, could be developed into useful drugs that could be used as effective pain relievers.

Keywords: Benzyl palmitoylamide, propyl palmitoylamide, acetic acid induced writhing, hot plate model

INTRODUCTION

The search for potent and efficacious anti-inflammatory/analgesic drugs with fewer side effects has been on the increase since acetyl salicylic acid was introduced to the market in late 19th century. The existing drugs, even the selective cyclooxygenase-2 (COX-2) are not free of side effects with debilitating impacts on the patient after a prolonged therapy [1]. Investigation of animal tissue extracts has led to the discovery of fatty acid amides with potent anti-inflammatory and analgesic properties. Prominent among them is anandamide (N-arachidonylethanolamide) which is the endogenous ligand for the cannabinoid receptors [2,3]. Also, Palmitoyl ethanolamide was shown to reduce allergic reaction and inflammation. It has been reported [4] that the chain length of the amino alcohol moiety of N- oleoyl- ethanolamine dramatically affected its ability to interact with the amidohydrolase present in rat liver. This study clearly indicates that the ethyl head chain is a target for structure-activity studies. We earlier reported the anti-inflammatory and anti-nociceptive properties of palmitoyl glycine, palmityol alanine and palmitoyl GABA synthesized in our laboratory [5]. In this study, we undertook the condensation of

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palmitoyl chloride with propyl amine, butyl amine, cyclohexyl amine and benzyl amine. The analgesic properties of palmitoyl propylamide and palmitoyl benzamide were carried out.

EXPERIMENTAL

Materials. The starting materials used were purchased from commercial sources and used without further purification. Palmitoyl chloride, benzylamine, cyclohexylamine, propylamine and butylamine were procured from Sigma-Aldrich, Germany. Acetylsalicylic acid and dioxane were obtained from BDH Chemical Ltd. England. The precoated Thin Layer Chromatography (TLC), silica Gel 60 F_{254} plates used to monitor the reaction, were obtained from Merck (Darmstadt, Germany). Melting points were determined with an electro thermal melting point apparatus and were uncorrected. Infrared (IR) spectra were measured on a Buck scientific IR M500 instrument. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Varian Gemini 200 (250 MHz). Chemical shifts are reported in part per million (ppm) relative to tetramethylsilane (TMS). Mass spectra (MS) were recorded on a Varian MAT 44S mass spectrometer operating at 70eV.

General synthetic procedure. To the amine in dioxane in a conical flask was added palmitoylchloride (0.55, 1.80 mmol.) and swirled continuously until emulsion began to form. The conical flask was allowed to stand for 30 minutes; the resulting precipitate was washed with water (3x10 mL). The product was filtered, air-dried and recrystallized from methanol/water (1:1).

Preparation of propylpalmitoylamide. To a stirring mixture of propylamine (0.3 mL, 3.64 mmoles) in dried dioxane (10 mL) was added palmitoyl chloride (1.1 mL, 3.64 mmoles) drop-wisely. The mixture was further stirred at room temperature and left to stand for 30 minutes. The resultant product was filtered and the residue was recrystallized from water, dried and weighed.

Yield: 0.7716 g (71.4%);

IR (KBr): 3458 (N-H), 2956 (C-H), 2857 (C-H), 1642 (C=O), 1571cm^{-1}.

^{1}H NMR ($CDCl_3$) δppm: 0.75-0.84 (q, J=7.5, 3H, CH_3), 1.15 (br s, 26H, CH_2 residue). 1.34-1.45 (q, J = 7.3, 2H, CH_2), 2.03-2.08 (t, J = 7.5, 2H, CH_2), 3.02-3.10 (q, J = 6.5, 2H, CH_2), 6.58 (br s, 1H, NH).

^{13}C NMR ($CDCl_3$) δppm: 16.15, 18.82, 27.31, 29.64, 30.61, 33.84, 34.05, 36.54, 38.91, 41.25, 43.78, 44.78, 45.74, 178.10, 180.55 (C=O).

MS: 298(M^{+}+1) (100%), 254(10), 239(10), 170(10), 156(10), 114(22), 101(60), 86(12), 43(26), 30(10).

Preparation of butylpalmitoylamide. To a stirring mixture of butylamine (0.266 g, 3.64 mmoles) in dried dioxane (10 mL) was added palmitoyl chloride (1.1 mL, 3.64 mmoles) drop-wisely. The mixture was further stirred at room temperature and left to stand for 30 minutes. The resultant product was filtered and the residue was recrystallized from water, dried and weighed.

Yield: 0.868 g (76.5%).

IR (KBr): 3300 (N-H), 2990 (C-H), 2887 (C-H), 1750 (C=O), 1665 (C=O), 1500, 1450, 1350cm^{-1}.

^{1}H NMR ($CDCl_3$) δppm: 0.74-0.84 (q, J = 7.3, 3H,CH_3), 1.17 (br s, 26H, CH_2, residue), 1.24-1.28 (d, J = 7.5, 2H, CH_2), 1.31-1.40 (dd, J = 7.25, 7.5, 2H, CH_2), 2.01-2.07 (t, J = 7.5, 2H, CH_2), 2.13-2.20 (t, J = 7.5, 2H, CH_2), 3.06-3.14 (q, J = 6.5, 2H, CH_2), 6.19 (br s, 1H, NH).

^{13}C NMR ($CDCl_3$) δppm: 29.63, 30.56, 33.83, 34.03, 34.12, 36.36, 36.54, 38.89, 41.37, 43.75, 44.33, 44.67, 45.34, 45.68, 178.03, 180.69 (C=O).

MS: 312 (M^{+}+1) (100%), 282(8), 268(16), 256(26), 128(36), 115(100).

Preparation of benzylpalmitoylamide. To benzylamine (0.2 mL, 1.86 mmole) in dried dioxane (8 mL) in a conical flask was added palmitoyl chloride (0.55 mL, 1.80 mmole) and swirled continuously until an emulsion began to form; then the conical flask was allowed to stand for 30 minutes. The resulting

precipitate was washed with water (3x10 mL), then recrystallized from water and dried.

Yield: 489 g (77.8%); melting point: 89-91^{o}C.

IR (KBr): 3286 (N-H), 2929 (C-H), 2857 (C-H), 1714 (C=O), 1642 (C=O), 1571, 1463 cm^{-1}.

^{1}HNMR ($CDCl_3$) δppm: 0.86-0.91 (t, J = 6.5, 3H, CH_3), 1,26 (br s, 26H, CH_2 residue), 2.18-2.24 (t, J = 7.5, 2H, CH_2), 2.30-2.36 (t, J = 7.5, 2H, CH_2), 4.5 (d, J = 5.5, 2H, CH_2), 5.84 (br s, 1H, NH), 7.26-7.33 (q, J = 5.25, 5H, Ar-H).

MS: 346 (M^{+}+1)(24%), 302 (6), 260 (5), 204 (10), 162(26), 149 (100).

Preparation of cyclohexylpalmitoylamide. To cyclohexylamine (0.2 mL, 1.8 mmole) in dried dioxane (8 mL) in a conical flask was added palmitoyl chloride (0.55 mL, 1.80 mmole) and swirled continuously until an emulsion began to form; then the conical flask was allowed to stand for 30 minutes. The resulting precipitate was washed with water (3x10 mL), then recrystallized from water and dried.

Yield: 0.547 g (89.1%); melting point: 66-67^{o}C.

IR (KBr): 3500-3300 (N-H), 3100-3000 (C-H), 2750 (Ar-H), 1665 (C=O), 1571, 1463 cm^{-1}. ^{1}HNMR ($CDCl_3$) δppm: 0.85-0.90 (t, J = 6.75, 3H, CH_3), 1.04-1.25 (m, 26H, CH_2 residue), 1.60-1.66 (t, J = 5.75, 10H, cyclohex.-H), 1.88-1.92 (d, J = 10, 2H, CH_2), 2.11-2.17 (t, J = 7.5, 2H, CH_2), 2.31 -2.36 (t, J = 7.25, 2H, CH_2), 5.31-5.34 (d, J = 8, 1H, NH).

^{13}CNMR ($CDCl_3$) δppm: 24.73, 24.83, 25.53, 25.88, 29.07, 37.08, 48.04, 172.40, 178.91 (C=O).

MS: 338 (M^{+}+1)(86%), 308(6), 294(6), 266(6), 256(100), 154(21), 141(100).

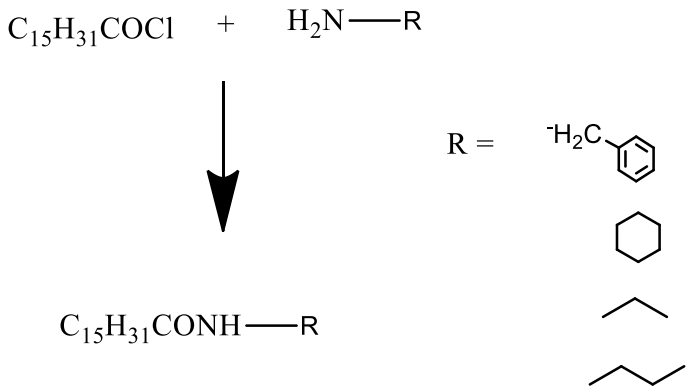
Effect of propylpalmitoylamide on acetic acid induced writhing in mice. The method first described by Koster *et al.* [6] was used. Mice, five per group were placed randomly into four groups. The animals in group one received normal saline (10 ml/kg, *i.p.*), while groups two, three and four received propylpalmitoylamide (50 mg/kg and 100 mg/kg i.p) and acetylsalicylic acid (100mg/kg, subcutaneously, suspended in 5% tragacanth in normal saline) respectively. Acetylsalicylic acid or propylpalmitoylamide was administered to the animals 30 and 60 min, respectively, before intraperitoneal injection of acetic acid (10 ml/kg, 1% v/v in normal saline). The animals were observed for manifestation of pain in the form of writhes, which consisted of constriction of the abdominal muscles together with stretching of the hind limbs. The writhes were cumulatively counted for 30 min following acetic acid injection and the analgesic effect determined as described by Koster *et al*. [6].

Effect of Benzylpalmitoylamide on the hot plate latency time in mice. The method previously described by Woolfe and MacDonald [7] with modification by Zimer *et al*. [8] was used. The responsiveness to nociceptive stimulus was measured with the hot plate analgesimeter (Harvard Apparatus Ltd., UK). The hot plate temperature was maintained at 55 ± 10^{o}C. The licking, biting of the hind paw or jumping was taken as an indication of pain perception. Mice screened 24 h previously for suitable reaction time was used. A cut-off time of 60 s was adopted to prevent tissue damage. Animals were placed on the hot plate surface in a glass cylinder of about 20 cm in diameter. The time in seconds between the placement and licking, biting of the hind paw or jumping was recorded as the index of response latency. Each animal served as its own control, thus one hour before pre-treatment, its basal latency was taken twice at 15 min intervals. The mean of these two values constituted the basal response latency prior to treatment. Mice were randomly divided into four groups of five mice each. The animals in group one were administered normal saline (10 mL/kg *i.p.*), while groups two and three received benzylpalmitoylamide at 50mg/kg and 100mg/kg, with group four administered morphine (10 mg/kg, *i.p.*) 30 min before placement on the hot plate. Response latencies were taken at 30, 60, 90 and 120 min and analgesic effect determined.

RESULTS AND DISCUSSION

Antinociception. The effect of propylpalmitoylamide on peripheral pain model is presented in Table 1. The results indicate that it reduced the writhes in mice caused by the acetic acid. The percentage inhibitions of writhes were 31.2, 39.2 and 52.5 for propylpalmitoylamide 50 mg/kg, 100 mg/kg and acetylsalicylic acid 100 mg/kg respectively. Acetic acid causes inflammatory pain by increasing capillary permeability. Writhes induced by noxious chemicals injected intraperitoneally is due to sensitization of nociceptors by prostaglandins. This test is useful for evaluation of mild analgesic nonsteroidal anti-inflammatory compounds. The inhibition of writhes by propylpalmitoylamide suggests that it possesses analgesic potentials for mild pain.

In Table 2 is presented the results of benzylpalmitoylamide on the hot plate latency time in mice.



Scheme 1. General synthetic procedure

Table 1: Effect of propylpalmitoylamide on acetic acid induced writhing in mice

Treatment	Number of writhes per 30 minutes	% Inhibition of writhes
Normal Saline (10 ml/kg)	75.0 ± 7.01	-
PPA (50 mg/kg)	51.6 ± 1.81	31.2
PPA (100 mg/kg)	45.6 ± 2.89	39.2
Acetylsalicylic acid (100 mg/kg)	35.6 ± 1.54	52.5

Values are expressed as Mean ± SEM. Percentage inhibition of writhes is presented as simple percentage in relation to the normal saline treated animals. PPA represents propylpalmitoylamide.

Table 2: Effect of Benzylpalmitoylamide on the hot plate latency time in mice

Treatment	Latency time (minutes) at time post-treatment				
	(0 min)	30 min	60 min	90 min	120 min
Normal saline (10 ml/kg)	24.06 ±.50	24.60 ±2.16	23.3 ±0.83	23.64 ±0.9	23.20 ±1.53
BPA (50mg/kg)	25.71 ±5.02	32.60 ±1.94	38.0 ±3.27* (47.8 %)	38.40 ±2.04* (49.4 %)	34.80 ± 2.48* (35.4 %)
BPA (100mg/kg)	24.32 ±1.63	34.40 ±2.11	42.0 ±1.52* (72.7 %)	39.20 ±2.80* (61.2 %)	34.80 ±2.27* (43.1 %)
Morphine (10mg/kg)	25.30 ±1.32	41.60 ±1.94	49.40 ±1.69* (95.3 %)	50.40 ±0.25* (99.2 %)	48.40 ±2.23* (91.3 %)

Values are the mean + S.E.M. *p < 0.05; significantly different from the normal saline treated group. Student's t-test (n = 5 per group). Values in parentheses are percentage pain inhibition. BPA = Benzylpalmitotylamide

The synthesized benzylpalmitoylamide caused a dose dependent increased in the hot plate latency time of the treated mice. The latency time of the treated mice were significantly, $p<0.05$ higher than that of normal saline shame treated mice (control) at

60min, 90 min and 120 min post treatment. The reference drug, morphine significantly, $p<0.05$ increased the hot plate latency time in the mice compared with the control. The hot plate model is an analgesic model that is used to demonstrate central analgesic mechanism while the acetic acid induced mouse writhing assay demonstrates peripheral pain. That propylpalmitoylamide markedly reduced the writhes in the mice, suggests that its analgesic effect is through peripheral mechanism while benzylpalmitoylamide inhibits pain through central mechanisms.

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