New Oleamide Analogues with Potential Food - intake Regulator Effect. II

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Oleamide analogues were synthesized and fully characterized by IR, MS, ^{1}H- and ^{13}C-NMR spectra. Oleamide analogues were synthesized from oleic acid, activated by carbonyldiimidazole to oleoylimidazole, and amines. The compounds were investigated for their influence on bodyweight and food intake effects on mice. Two of the oleamide analogues are new compounds.

Keywords: Oleamide analogues, IR, MS, ^{1}H- and ^{13}C-NMR spectra, food intake, bodyweight decrease

Nowadays, obesity and overweight became at a rate of epidemic proportions worldwide [1]. Both are considered two of the most important medical problems of current day, due to the effect on general health of population and due to the diseases associated or further developing like metabolic syndrome, cardiovascular disease and type II diabetes mellitus (T2D) [2]. Food intake is one of the most incriminated factors generating overweight and is influenced greatly by appetite. This is governed by two main processes: an increased appetite due to a decrease in energy availability which appears in homeostatic food intake and the influence of endogenous or exogenous stimuli in hedonic food intake [2]. In both cases the endocannabinoid system activates stimuli to increase the hunger sensation [3]. Anandamide (AEA) and 2-arachidoyl glycerol (2-AG) are lipid mediators that bind to the two main receptors, cannabinoid receptor 1 (CB_1) and cannabinoid receptor 2 (CB_2) and activate the systems involved in food intake: limbic, hypothalamus and hindbrain [2,4]. AEA, 2-AG and also endogenous oleamide increase the food intake mediated by CB_1 receptor [5]. It is also to be mentioned that between the most studied biological activities of oleamide are the sleeping modulator effect, the decreasing of pain perception and body temperature, the regulator effects on cardiovascular system and lipid metabolism [6]. N-Oleoylethanolamide (OEA) is an endogenous amide which plays important role acting like a sensor on food intake and moreover may have some potential as an anti-obesity drug. Recent research demonstrated that OEA suppress food intake [7]. It is biosynthesized from oleic acid and phosphatidylethanolamine mainly in brain, liver, adipocytes and small intestine [8]. Usually, the OEA biosynthesis occurs in epithelial cells of small intestine in response to fat diet [9]. OEA acts on peroxisome proliferator-activated receptor alpha (PPAR-α) and regulates feeding by recruiting vagal sensors afferents in the gut [9]. Both endogenous and oral/parenteral administration of OEA induced a satiety signal leading to the decrease of food intake and body weight loss [10].

Some inhibitors of fatty acid amide hydrolase (FAAH) (arachidonoylserotonine, AA5HT) [11] and of anandamide cellular uptake [(*R*)-N-oleoyl-(1'-hydroxybenzyl)-2'-ethanolamine, OMDM-1] [12] were realized in search to obtain drugs for reducing food intake and treating obesity. Also a few drugs with different structures as those above were marketed for reducing food uptake like sibutramine [13], rimonabat [14] and orlistat [15] (fig. 1). Now, sibutramine has been withdrawn from the market in almost all countries especially due to increased cardiovascular events and strokes. The same is the situation with rimonabant, which has been withdrawn from US market, but still approved in EU and other countries, due to the same serious side effects. Other studies are made by replacing piperidine fragment with an alkyl residue mimicking those existing in oleic acid, but without carboxyl function [16], but the results were not those expected. Orlistat inhibits the absorption of fats from human diet,

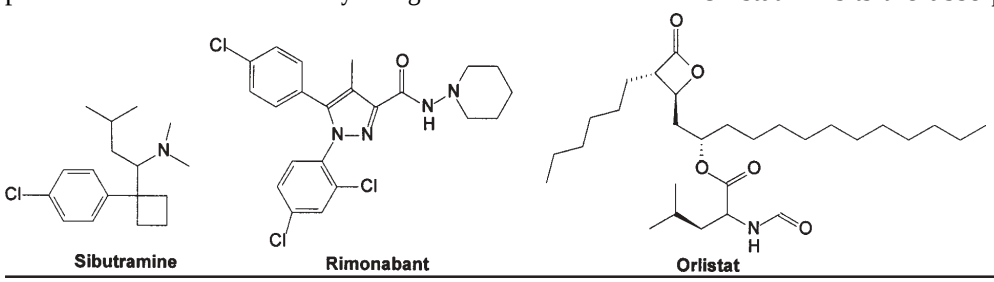

Fig. 1. Commercial drugs marketed for obesity treatment

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thus reducing caloric intake and acts as a lipase inhibitor [15].

Some food supplements were also realized from Maca plant [17] and Schisandra chinensis [18] and the composition of the Maca plant shows that the majority of the isolated identified substances are (un)substituted benzyl or pyridyl oleamides [19].

The importance of oleamide analogues determined us to explore their synthesis by substitution of the amide moiety with different structures in hope to obtain favorable activity for the compounds.

Experimental part

Melting points were determined on OptiMelt. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Gemini 300 BB instrument, operating at 300 MHz for ^{1}H- NMR and at 75 MHz for ^{13}C-NMR, using $CDCl_3$ as solvent and TMS as internal standard. The IR spectra (ATR) were recorded on a Vertex 70 Brucker instrument. Mass spectra were recorded at 100000 resolution (m/z 400), in positive mode on an LTQ Orbitrap Velos Pro (Thermo Fisher Scientific), by injecting a solution of 100 pmol/μL in 0.1 % formic acid in MeOH using a Nanospray Flex Ion Source (Proxeon Biosystems). All fragmentations were performed using CID (Collision Induced Dissociation) and the fragments were recorded in the linear ion trap. TLC was performed on Merck silicagel 60 or 60F_{254} plates and spots were developed under UV light, with iodine and/or 15% H_2SO_4 in methanol and heating at 120-140°C.

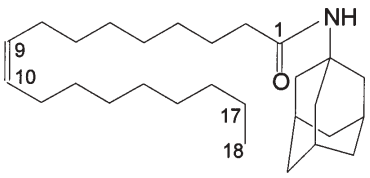
Chemistry

General procedure for synthesis of oleoylamides **7-12**

Oleamide compounds **7-12**, were synthesized from oleic acid (12 mmoles) by reaction with carbonyl diimidazole (CDI) (12.8 mmoles) in dichloromethane (100 mL) for 2 h and then the activated oleoylimidazole solution added dropwise to a stirred solution of 20 mmoles amine and 1.11 mmoles 4-dimethylaminopyridine in CH_2Cl_2 (50 mL); the reaction mixture was stirred for 48 h at r.t., monitoring the reaction by TLC. This procedure was previously succesfully applied for synthesis of 1-naphtyloleamide and cyclohexyloleamide [20] by a published procedure for obtaining oleamide analogues presented in the macamides [19].

The crude product was purified by pressure chromatography (PC) with eluent systems presented for each compound.

Synthesis of N-adamantyloleamide, **7**



TLC (Silicagel, eluent: ethyl acetate-methanol, 90:13, $R_{f\,7}$ = 0.65); PC (eluent: hexane-ethyl acetate, 10:0.5 than 5:1); 2.25 g (44%) pure product as waxy,

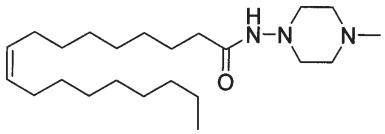
IR: 3304br m (νNH), 3006w ($\nu_{=C-H}$), 2910vs, ($\nu CH_{2\ asim}$); 2853s ($\nu CH_{2\ sim}$), 1644s (νC=ONHR), 1547ms (νC=O sec. amide, band II), 1457m, 1362m,

^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 5.35 (m, 2H, H-9, H-10), 5.10 (s, 1H, NH), 2.07 (t, 2H, H-2, 7.4), 2.00 (m, 10H, 2H-8, 2H-11, $3CH_2$-Ad), 1.98 (s, 3H, CH-Ad), 1.67 (t, 8H, $3CH_2$-Ad, 2H-), 1.35-1.22 (m, 20H), 0.88 (t, 3H, H-18, 6.4),

^{13}C-NMR ($CDCl_3$, δm): 172.46 (C-1), 130.09, 129.91 (C-9, C-10), 51.84 (C_q-Ad), 41.82 ($3CH_2$, $C_á$ to CNH-Ad), 37.96 (C-2), 36.49 (the other $3CH_2$-C, C-Ad), 32.03, 29.90, 29.85 ($3CH_2$), 29.65(3CH-Ad), 29.56, 29.45 (CH_2), 29.31, 29.28, 27.34, 27.30, 25.94 ($7CH_2$), 22.80 (C-17); 14.26 (C-18).

HR-MS, Calc. for $C_{28}H_{49}NO$, $[M+H]^+$: 416.38869, Found: 416.38776, Fragments: 135 ($C_{10}H_{15}$) [adamantyl fragment]+, 152 ($C_{10}H_{18}N$) [adamantylamine fragment + H]$^+$ and minor consecutive fragments of the olefinic backbone.

Synthesis of N-(4-methylpiperazin-1-yl)oleamide, **8**



TLC (Silicagel, eluent: dioxane-tetrahydrofuran-25% amonia, 60:20:30, $R_{f\,8}$ = 0.71); The crude product was crystalized from hexane, resulting 2.585 g pure product, m.p. 80.5-81.8°C. From column chromatography purification of the mother liquors resulted another 0.64g (total yield: 70.9%).

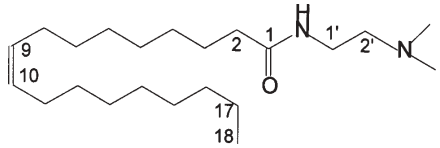
IR: 3233m (νNH), 3006m ($\nu_{=C-H}$), 2922vs, ($\nu CH_{2\ asim}$); 2852s ($\nu CH_{2\ sim}$), 2791m, 1651s (νC=ONHR), 1553m (νC=O sec. amide, band II), 1455m, 1385w, 1287w, 729w,

^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 6.24 (br s, 1H, NH), 5.32 (m, 2H, H-9, H-10), 2.90-2.50 (m, 4H, H-1', H-2'), 2.30 (s, 3H, CH_3), 2.10 (m, 2H, H-2), 2.08-1.92 (m, 4H, 2H-8, 2H-11), 1.65-1.58 (m, 2H, H-3), 1.23-1.20 (m, 20H), 0.81 (t, 3H, H-18, 6.6),

^{13}C-NMR ($CDCl_3$, δ ppm): 176.51 (C-1), 129.96, 129.77 (C-9, C-10), 56.51 (CH_2 C-2'or 1'), 55.54 (CH_2 C-2' or 1'), 54.54 (CH_2 C-1' or 2'), 54.24 (CH_2 C-1' or 2'), 45.66 (CH_3N), 32.28 (CH_2), 32.11, 31.88, 29.74, 29.68, 29.50, 29.42, 29.29, 29.15 ($8CH_2$), 27.20, 24.87, (nCH_2); 22.65 (C-17), 14.08 (C-18).

HR-MS, Calc. for. $C_{23}H_{45}N_3O$, $[M+H]^+$: 380.36354, Found: 380.36281, Fragments: 265 ($C_{18}H_{33}O$) [oleoyl fragment]$^+$, 284, 247, 116 ($C_5H_{14}N_3$), 99 ($C_5H_{11}N_2$) and minor fragments of consecutive olefinic backbone fragmentation.

Synthesis of N-(2-(dimethylamino)ethyl)oleamide, **9**



TLC (Silicagel, eluent: dichlorometane-methanol, 9:1 double eluted, $R_{f\,9}$ = 0.25); PC (eluent: dichloromethane-methanol, 9:1), resulting a pure fraction of 1.82 g (43.0%) oleamide **9**, as oil [21], used for analysis and for biological testing on mice; (another fraction of 1.6g, containing >90 oleamide was kept in the fridge for further purification).

IR: 3292br m (νNH), 3005w ($\nu_{=C-H}$), 2923vs, ($\nu CH_{2\ asim}$); 2853s ($\nu CH_{2\ sim}$), 2819m, 2768m, 1644s (νC=ONHR sec. amide), 1559m (νC=O sec. amide, band II), 1460m, 1257w,

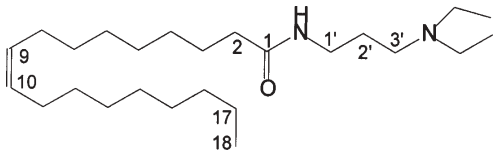
^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 6.00 (br s, 1H, NH), 5.27 (m, 2H, H-9, H-10), 3.26 (dt, 2H, H-1', 5.5, 6.0), 2.34 (t, 2H, H-2', 6.0), 5.7), 2.17 (s, 6H, NCH_3), 2.10 (t, 2H, H-2, 7.4), 2.00-1.90 (m, 4H, 2H-8, 2H-11), 1.62-1.53 (m, 2H, H-3), 1.23-1.20 (m, 20H), 0.81 (t, 3H, H-18, 6.6),

^{13}C-NMR ($CDCl_3$, δ ppm): 173.40 (C-1), 130.10, 129.89 (C-9, C-10); 58.01 (C-1'), 45.23 (CH_3N), 36.88, 36.75 (C-2, C-2'), 32.03, 29.89, 29.85; 29.65, 29.42, 29.29, 27.34, 27.31 (12 CH_2), 25.92 (CH_2), 22.81 (C-17), 14.25 (C-18).

HR-MS, Calc. for. $C_{22}H_{44}N_2O$, $[M+H]^+$: 353.35264, Found: 353.35194 [308 ($C_{20}H_{38}NO$)] and fragments of consecutive olefinic backbone fragmentation in MS^3 of the main fragment.

Synthesis of N-(3-(diethylamino)propyl)oleamide, **10**

TLC (Silicagel, eluent: dichlorometane-methanol, 9:1, double eluted, $R_{f\,10}$ = 0.08); PC (eluent: dichloromethane-



methanol, 9:1), resulting 3.64 g (76.9%) pure product as oil [21],

IR: 3291br m (νNH), 3006w ($\nu_{=C-H}$), 2925vs, ($\nu CH_{2\ asim}$); 2850s ($\nu CH_{2\ sim}$), 1644s (νC=ONHR sec. amide), 1553m (νC=O sec. amide, band II), 1466m, 1379w,

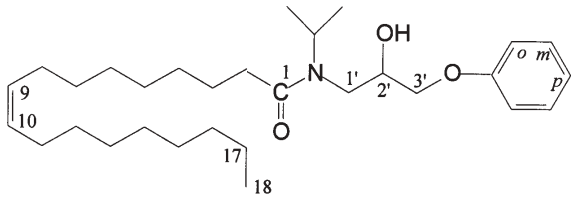
^{1}H-NMR ($CDCl_3$, δ ppm, J Hz): 7.35 (br s, 1H, NH), 5.27 (m, 2H, H-9, H-10), 3.27 (dt, 4H, H-1', 5.1, 7.1), 2.49 (dd, 2H, CH_3CH_2N, 3.3, 7.1), 2.47 (m, 2H, H-3'), 2.45 (dd, 2H, CH_3CH_2N, 5.0, 7.1), 5.7), 2.06 (t, 2H, H-2, 7.7), 1.93-1.90 (m, 4H, 2H-8, 2H-11), 1.62-1.53 (m, 2H, H-3), 1.35-1.22 (m, 20H), 0.98 (t, 6H, CH_3CH_2, 7.1), 0.88 (t, 3H, H-18, 6.4),

^{13}C-NMR ($CDCl_3$, δ ppm): 173.10 (C-1), 130.09, 129.88 (C-9, C-10), 52.87 (CH_2, C-1'), 46.88 (CH_2CH_3), 39.98 (C-3'), 37.15 (C-2), 32.02, 29.88, 29.85, 29.64, 29.50, 29.47 27.43, 27.33, 27.31 (12 CH_2), 26.01 (C-2'), 25.50 (CH_2), 22.80 (C-17), 14.22 (C-18), 11,72 (CH_2CH_3).

HR-MS, Calc. for. $C_{25}H_{50}N_2O$, $[M+H]^+$: 395.399591, Found: 395.39888 [322 ($C_{21}H_{40}NO$)]. MS^3 fragmentation of the 322 peak gave the following ions: 265 ($C_{18}H_{33}O$) [oleoyl fragment]+, 294 ($C_{19}H_{36}NO$) and ions of consecutive fragmentation of the olefinic backbone.

Synthesis of compound N-(2-hydroxy-3-phenoxypropyl)-N-isopropyloleamide, **11**

TLC (Silicagel, eluent: dichloromethane-methanol, 9:1, $R_{f\,11}$ = 0.71); PC (eluent: dichloromethane-methanol,



10:0.1), resulting 3.346 g (58.9%) of pure product as oil, used for analysis and biological testing; another 1.269 g of impure product was also obtained.

IR: 3349brm(νOH), 2923vs($\nu CH_{2\ asim}$), 2853s($\nu CH_{2\ sim}$), 1615s, 1601s, 1495m, 1465m, 1420m, 1370w, 1347w, 1299w, 1245s, 1117w, 1078w, 1042m, 752m, 691w,

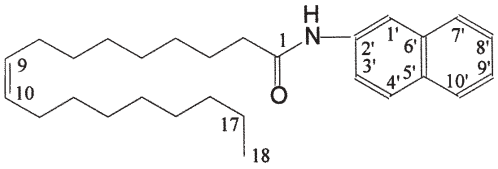
^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 7.31-7.26 (m, 2H, 2H-m), 6.98-6.89 (m, 3H, 2H-o, H-p), 5.62 (s, 1H, OH), 5.40-5.29 (m, 2H, H-9, H-10), 4.13-3.99 (m, 3H, H-2', 2H-3'), 3.82 (t, 1H, H-3', 8.5), 3.61 (dd, 1H, H-1', 8.0, 14.8), 3.42 (dd, 1H, H-1', 1.4, 14.8), 2.43 (dq, 1H, H-2, 7.7, 15.1), 2.36 (dq, 1H, H-2, 7.4, 15.1), 2.01 (m, 4H, 2H-8, 2H-11), 1.65 (m, 2H, H-3), 1.40-1.21 (m, 20H), 0.88 (t, 3H, H-18, 6.3),

^{13}C-NMR ($CDCl_3$, δ ppm): 176.17 (C-1), 158.48 (C_q), 130.01, 129.75 (2C, C-9, C-10), 129.51 (2C-*m*), 120.99 (C-*p*), 114.40 (2CH, C-*o*), 72.41 (CH_2, C-2'), 69.55 (CH, C-3'), 49.17 (CH-^{i}Pr), 46.17 (C-1'), 33.73 (C-2), 31.91, 29.77, 29.72, 29.52, 29.43, 29.33, 29.14, 27.24, 27.23, 25.45 (12CH_2), 22.68 (C-17), 14.10 (C-18).

HR-MS, Calc. for. $C_{30}H_{51}NO_3$, $[M+H]^+$: 474.39417, Found: 474.39306 [456 ($C_{30}H_{50}NO_2$), 415 ($C_{27}H_{45}NO_2$), 380 ($C_{24}H_{46}NO_2$), 210 ($C_{12}H_{20}NO_2$)].

Synthesis of compound N-[2-naphthyl]oleamide, **12**

TLC (Silicagel, eluent: ethyl acetate-methanol, 90:13, $R_{f\,12}$ = 0.74; for comparison, $R_{f\,5}$ = 0.70); The crude product was dissolved in hexane at reflux, decholorized with



charcoal, cooled overnight and filtered, resulting 3.64 g (74.4%) pure oleamide **12**, m.p. 69.7-71.5°C (lit. 68.5-69.0°C, [22]).

IR: 3307br m (νNH), 3003w ($\nu_{=C-H}$), 2958w, 2918vs, ($\nu CH_{2\ asim}$); 2850s ($\nu CH_{2\ sim}$), 1657s (νC=ONHR sec. amide), 1585w, 1551ms (νC=O sec. amide, band II), 1526ms, 1503w, 1468w, 1258w; 1226w, 819w, 741w,

^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 8.21 (s, 1H, H-Ar), 7.75-7.73 (m, 3H, H-Ar); 7.64 (br s, 1H, NH), 7.47-7.35 (m, 3H, 2H-Ar), 5.36 (m, 2H, H-9, H-10), 2.38 (t, 2H, H-2, 7.4), 2.10-1.90 (m, 4H, 2H-8, 2H-11), 1.80-1.70 (m, 2H, H-3), 1.44-1.01 (m, 20H), 0.88 (t, 3H, H-18, 6.3),

^{13}C-NMR ($CDCl_3$, δ ppm): 171.95 (C-1), 135.56, 133.96 (2CH, C-Ar), 130.69 (C_q Ar), 130.13, 129.85 (2C, C-9, C-10), 128.78 (C-Ar), 127.74 (C-1'), 127.63 (C-Ar), 126.56 (C-Ar), 125.04 (C-Ar), 120.05 (C-Ar), 116.75 (C-Ar), 37.95 (C-2), 32.02, 29.89, 29.83, 29.65, 29.44, 29.40, 29.26, 27.34, 27.29, 25.79 (12CH_2), 22.80 (C-17), 14.24 (C-18).

HR-MS, Calc. for. $C_{28}H_{41}NO$, $[M+H]^+$: 408.326091, Found: 408.32516 Fragments: 144 ($C_{10}H_{10}N$) [Naphthylamine+H]$^+$, 127 ($C_{10}H_7$) [Naphthyl]+, 265 ($C_{18}H_{33}O$) [oleoyl fragment]$^+$, 282 ($C_{18}H_{36}NO$) [oleoylamide +H]$^+$ and minor fragments of consecutive olefinic backbone fragmentation.

Albino Swiss mice weighing 20±2g were used to assess the administration effect the oleamide analogues **2**, **4**, **5**, **7-12** comparing to a group treated with oleoylethanolamide **1** and a control group treated with physiological solution. The animals were purchased from the Animal Biobase of the University of Medicine and Pharmacy Carol Davila, Bucharest. All animals used in the study were kept in standard laboratory conditions. They received water *ad libitum* and were not fed for 12h before the experiment. All experiments were performed in compliance with European Communities Council Directive 1986 (86/609/EEC) and Ordinance No. 37 of the Romanian Government from 2nd February 2002.

The animals were distributed in 11 groups of 5 animals each, as it follows:

Group 1 – treated with oleamide **1** (i.p. dose 5mg/Kg bw);
Group 2 – treated with oleamide **2** (i.p. dose 6.38mg/Kg bw);
Group 3 – treated with oleamide **4** (i.p. dose 6.38mg/Kg bw);
Group 4 – treated with oleamide **5** (i.p. dose 6.26mg/Kg bw);
Group 5 – treated with oleamide **7** (i.p. dose 6.39mg/Kg bw);
Group 6 – treated with oleamide **9** (i.p. dose 5.42mg/Kg bw);
Group 7 – treated with oleamide **10** (i.p. dose 6.06mg/Kg bw);
Group 8 – treated with oleamide **11** (i.p. dose 7.28mg/Kg bw);
Group 9 – treated with oleamide **12** (i.p. dose 6.26mg/Kg bw);
Group 10 – treated with oleamide **8** (i.p. dose 5.38mg/Kg bw);
Group 11 – control group treated with physiological solution (i.p. dose 5mL/Kg bw).

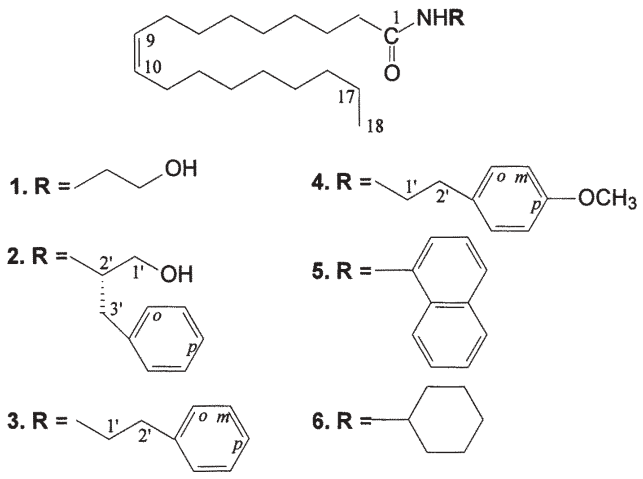
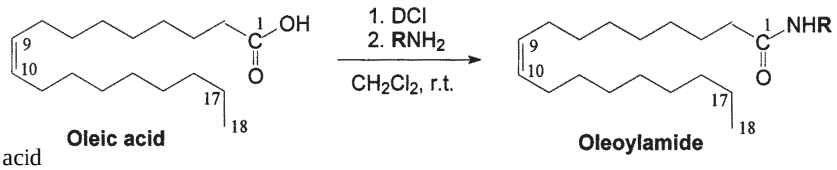
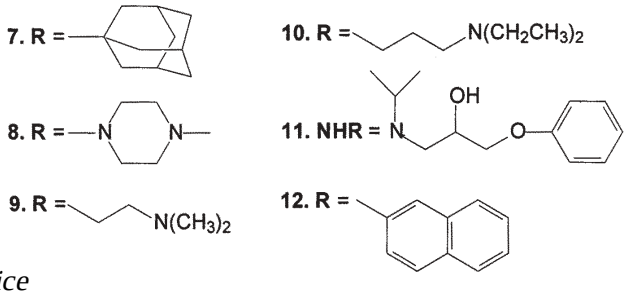


Fig. 2. Oleamide analogues previously synthesized in our laboratory [20, 22]



Scheme 1. Synthesis of oleamides **7-12** from oleic acid and amines using CDI for activating carboxyl group



(i.p. = intraperitoneal administration)

a) The determination of body weight variations on mice treated with oleamide analogues

The animals were i.p. treated daily for 7 days with oleoylethanolamide **1**, oleamide analogues **2**, **4**, **5**, presented in the previous paper [20], **7**, **9**, **10**, **12** and new oleamide analogues **8** and **11**, synthesized and presented in this paper. Control group was i.p. treated daily for 7 days with physiological serum.

Body weight of each animal was measured in days 1, 3, 5 and 7.

The doses of synthetic oleamide analogues used in the study are molecular equivalent with the pharmacological dose of oleoylethanolamide **1** for intraperitoneal administration mentioned in the scientific literature [23].

b) The determination of food-intake variations on mice treated with oleamides

After the first administration, the animal received the food which was measured before and after 3 h. The food consumption was determined for each treated group.

Optimistic results regarding body weight loss and a decreased food-intake were obtained in a previous study using oleamide **2** [20] and this substance was taken into this study to confirm a possible pharmacological effect on mice.

Results and discussions

Chemistry

Synthesis of oleamide analogues

For some time we are interested in synthesis of nucleoside analogues to be used in satiety control, by reducing the food intake and as a consequence, but not only, to reduce body weight and so to prevent the appearance of diabetes and the associate diseases.

So we first synthesized *N*-[2-(4-methoxyphenyl)ethyl] oleamide [24] **4** (fig. 1) by amidation of methyl oleate with 4-methoxyphenethylamine catalyzed by sodium methoxide. By the same procedure we obtained N-[2-(3-phenyl)-1-propanol]oleamide **2** as optically active compound with L-phenylalaninol and N-[2-phenylethyl]oleamide **3**; oleoylethanolamide (OEA) **1** was also obtained by us using the same procedure (fig. 2) [20].

Other two oleamide compounds, 1-naphthyl oleamide **5** and cyclohexyl oleamide **6** were obtained by a second procedure from oleic acid, activated by carbonyldiimidazole, and primary amines [20], a procedure used in the field for synthesis of makamide's oleamide compounds [19]. By this already used procedure, now we synthesized the following oleamide analogues, **7-12**, presented in scheme 1, in which the primary amines are: adamantylamine, 4-N-methyl-1-aminopyperazine, 2-N,N-dimethylamino-ethylamine, 3-N,N-diethylamino-propylamine, 2-naphtylamine, and 1-(isopropylamino)-3-phenoxypropan-2-ol as secondary amine. The compounds were purified by pressure chromatography and obtained as oil, with exception of the compounds **8** and **12**.

The oleamide analogues **7-12**, together with previously obtained oleamide **1-2**, **4-5** were used to study their influence in decreasing body weight and food intake after intraperitoneal administration on mice.

In the same time, some of the oleamide analogues, **1-6** [25] and **7**,**9** [26] have been tested as modifiers for the design of stochastic microsensors based on graphite paste and have proved to be reliable screening tools for pattern recognition of analites in whole blood samples.

IR, ^{1}H, ^{13}C-NMR and MS

As we mentioned in the previous paper [20], IR spectrum of the compounds presents an intense band at ~ 3300 for íNH, a very intense band at 1636-1640 cm^{-1} and a second intense band at 1543-1555 cm^{-1}, characteristics for an amide group. For CH_2 groups of the oleic moiety of the compounds there are present a very intense band at 2918-2922 cm^{-1} ($\nu CH_{2\ asim}$) and an intense band at 2085-2852 cm^{-1} ($\nu CH_{2\ sim}$).

Group	Treatment day 1		Treatment day 3		Treatment day 5		Treatment day 7	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
1. Oleamide 1	**28.9**	3.23	**29.99**	1.92	**29.78**	2.09	**31.49**	2.36
2. Oleamide 2	**29.82**	2.12	**29.8**	2.74	**29.82**	2.87	**30.63**	4.23
3. Oleamide 4	**29.34**	2.95	**30.6**	3.18	**30.78**	3.11	**32.46**	3.09
4. Oleamide 5	**25.1**	3.36	**26.4**	2.86	**26.51**	2.20	**29.54**	4.52
5. Oleamide 7	**28.53**	2.50	**29.01**	1.28	**29.96**	1.95	**31.69**	2.04
6. Oleamide 9	**28.43**	1.46	**29.46**	1.10	**29.28**	1.02	**29.5**	1.16
7. Oleamide 10	**28.66**	1.46	**30.02**	2.29	**29.66**	2.05	**30.47**	1.99
8. Oleamide 11	**26.95**	2.04	**28.72**	1.85	**30.1**	1.86	**31.42**	1.99
9. Oleamide 12	**27.85**	1.89	**27.5**	4.15	**30.82**	2.39	**32.44**	2.22
10. Oleamide 8	**26.95**	2.47	**28.28**	3.67	**29.5**	3.42	**30.35**	3.68
11. Control	**29.6**	4.08	**30.15**	2.83	**31.58**	2.58	**34**	2.78

Table 1
THE BODY WEIGHT VARIATIONS AFTER THE I.P. OLEAMIDE ADMINISTRATION ON ALBINO SWISS MICE

SD = Standard Deviation

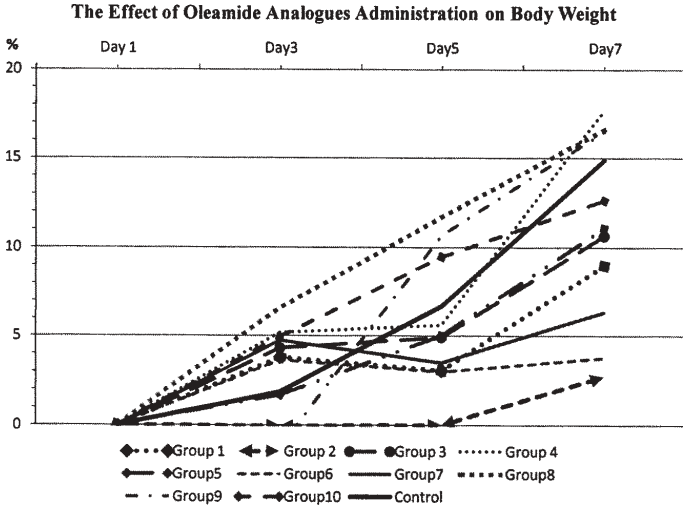


Fig. 3. The effect of oleamide analogues administration for 7 days on Albino Swiss mice

^{1}H- and ^{13}C-NMR spectra presents the signals for the protons and carbon atoms characteristic for the amide moiety of the molecule toghether with the signals characteristic for the oleic acid moiety (See experimental part for details). HR-MS spectra gives molecular peaks corresponding to isotope peaks of compounds at $[M+H]^+$. Fragments for the amide moiety are readily visible for all compounds. In the same time the fragments for oleic moiety are well recognized, beginning with m/z of 282 [oleoylamide $+H]^+$, 265 [oleoyl fragment]$^+$ and ending with small fragments for consecutive olefinic skeleton fragmentation, the corresponding ions being separated by 14 units (CH_2).

These analytical data are in full agreement with the molecular structure of the oleamide compounds synthesized.

Biological activity

The study aims to determine the effect of some new oleamide analogues on food-intake and body weight after the daily intraperitoneal administration on mice.

a) The effect of i.p. oleamide administration on body weight variations on experimental animals

Oleamide analogues were administrated daily for 7 days and the bodyweight was measure for each animal in days 1, 3, 5 and 7. The body weight mean of each group are presented in tabel 1 and the effect comparing to initial are presented in figure 3.

The variation effects are expressed as percent comparing to the body weight mean of each group registered on first day of treatment (initial). Group 1 – treated with oleamide **1** (i.p. dose 5mg/Kg bw); Group 2 – treated with oleamide **2** (i.p. dose 6.38mg/Kg bw); Group 3 – treated with oleamide **4** (i.p. dose 6.38mg/Kg bw); Group 4 – treated with oleamide **5** (i.p. dose 6.26mg/Kg bw); Group 5 – treated with oleamide **7** (i.p. dose 6.39mg/Kg bw); Group 6 – treated with oleamide **9** (i.p. dose 5.42mg/Kg bw); Group 7 – treated with oleamide **10** (i.p. dose 6.06mg/Kg bw); Group 8 – treated with oleamide **11** (i.p. dose 7.28mg/Kg bw); Group 9 – treated with oleamide **12** (i.p. dose 6.26mg/Kg bw); Group 10 – treated with oleamide **8** (i.p. dose 5.38mg/Kg bw); Group 11 – control group treated with physiological solution (i.p. dose 5mL/Kg bw).

The administration of oleamide analogues induced no significant variations of the body weights comparing to control group in the first 3 days of administration.

After 7 days of treatment, the body weight increased more than control group in case of groups 4 (oleamide **5**), 8 (oleamide **11**) and 9 (oleamide **12**) by 17.7%, 16.1% and respectively 15.4%. No significant variations comparing to control (14.9%) were observed in case of group 10 treated with oleamide **8** (13.6%) and group 5 treated with oleamide **7** (11%).

The daily administration of oleoylethanolamide (group 1) for 7 days increased the body weight by 9% similar to groups 3 (oleamide **4**) and 7 (oleamide **10**).

Optimistic results with no significant variations comparing to initial were obtained after the treatment with oleamide **2** (group 2) and oleamide **9** (group 6) with a body weight increases by 2.7% and respectively 3.7% after 7 days of treatment. The results are correlated with a decrease in food intake for these groups.

b) The effect of the oleamide analogues treatment on food intake

The food consumption was determined after the administration of oleamide analogues. The food quantity was before feeding and after 3 times intervals: 1, 2 and 3

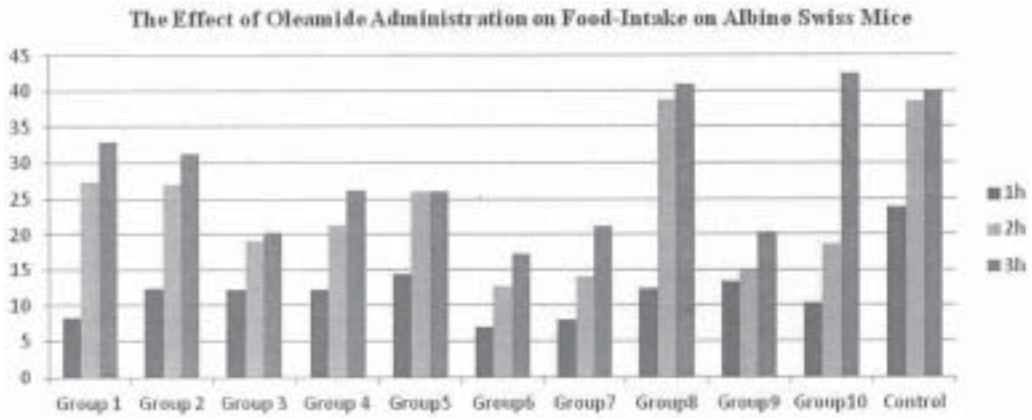


Fig. 4. The effect (%) of the oleamide analogues treatment on food intake measured at 1,2 and 3 h after feeding the Albino Swiss mice

hour. The results regarding the effect on food intake in each group are presented in figure 4.

The effect of food consumption is expressed in percent comparing to initial quantity of food given to experimental animals. Group 1 – treated with oleamide **1** (i.p. dose 5mg/Kg bw); Group 2 – treated with oleamide **2** (i.p. dose 6.38mg/Kg bw); Group 3 – treated with oleamide **4** (i.p. dose 6.38mg/Kg bw); Group 4 – treated with oleamide **5** (i.p. dose 6.26mg/Kg bw); Group 5 – treated with oleamide **7** (i.p. dose 6.39mg/Kg bw); Group 6 – treated with oleamide **9** (i.p. dose 5.42mg/Kg bw); Group 7 – treated with oleamide **10** (i.p. dose 6.06mg/Kg bw); Group 8 – treated with oleamide **11** (i.p. dose 7.28mg/Kg bw); Group 9 – treated with oleamide **1**2 (i.p. dose 6.26mg/Kg bw); Group 10 – treated with oleamide **8** (i.p. dose 5.38mg/Kg bw); Group 11 – control group treated with physiological solution (i.p. dose 5mL/Kg bw).

After the oleamide analogues administration, the food intake is decreased in case of all treated groups comparing to the control. The food consumption is maintained decreased comparing to control in case of the treatment with oleoylethanolamide **1** (group 1) and oleamide analogues **2** (group 2), **4** (group 3), **5** (group 4), **7** (group5), **9** (group 6), **10** (group 7) and **12** (group 9).

Small increases of food intake comparing to control were noticed in case of group 8 (oleamide **11**) and group 10 (oleamide **8**)

A decreased body weight comparing to control correlated with a decreased appetite were observed in case of the treatment with oleoylethanolamide **1**, oleamide **2**, oleamide **4**, oleamide **9** and oleamide **10**.

Conclusions

We synthesized other oleamide analogues for preliminary studying their pharmacological activity regarding bodyweight and food intake effects. The compounds were obtained by direct amidation of oleic acid, activated with carbonyldiimidazole, with amines: adamantylamine, 4-N-methyl-1-aminopyperazine, 2-N,N-dimethylamino-ethylamine,3-N,N-diethylamino-propylamine, 2-naphtylamine, and 1-(isopropylamino)-3-phenoxypropan-2-ol as secondary amine. IR, MS, ^{1}H-, ^{13}C-NMR and complementary APT, COSY and HETCOR spectra confirmed the structure of the oleamide analogues synthesized.

The treatment with oleamide analogues **2**, **4**, **9** and **10** decreased the body weight during the period of one week administration comparing to control group. Results obtained for these groups are correlated with a food intake decrease after 3 h from the administration time. Optimistic results are obtained after the treatment with oleamide **2** and **9** due to a better pharmacological effect on body weight and food intake comparing to oleoyethanolamide **1** treatment.

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