New Oleamide Analogues with Potential Foodintake Regulator Effect

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This paper presents the synthesis, physico-chemical characterization and preliminary pharmacological study of some oleamide analogues. Their synthesis was realized by basic amidation of methyl oleate with phenylalaninol and phenethylamine and by direct amidation of oleic acid with 1-naphthylamine and cyclohexylamine in the presence of 1, 1' - carbonildiimidazole. The oleamide analogues were fully characterized by IR, MS, ^{1}H- and ^{13}C-NMR spectra. The compounds were investigated for the influence on bodyweight and food intake effects.

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

According to the World Health Organization, obesity is one of the leading preventable causes of death worldwide, with a high prevalence in the modern world [1, 2]. Nowadays many researches are dealing with elucidating the molecular mechanism of obesity and developing new potent therapeutic agents with less secondary effects.

Oleamide and some several structural analogues are fatty acid amides naturally present in food. These compounds occur also endogenous as signaling molecules with various biological effects depending on tissue type.

Oleamide was first discovered in the cerebral spinal fluid. Many researches are focused on its endocannabinoid-*like* effects and the transient activity on TRPV1 vaniloid receptors. The most studied biological activities of oleamide are the sleeping modulator effect, the decreasing of pain perception, the decreasing of body temperature, the regulator effects on cardiovascular system and lipid metabolism.

An important analogue of oleamide is oleoyl-ethanolamide (OEA) **1**, a fatty acid amide biosynthesised from oleic acid and phosphatidylethanol-amine mainly in brain, liver, adipocytes and small intestine [3, 4]. In spite of the structural similarity with oleamide, OEA acts on peroxisome proliferator-activated receptor alpha (PPAR-α) and less on cannabinoid receptors [5, 6]. Both endogenous and oral/parenteral administration of OEA induced a satiety signal leading to the decrease of food intake and body weight loss [5, 7].

We already synthesized oleamides **1** and **4** and studied the electrochemical profile of the last compound [8]. In this study we present the synthesis and characterization of a serie of other oleamide analogues and their biological evaluation together with that of compounds **1** and **4**. The present study aims to develop new molecules of the oleamide class similar to the natural active compounds useful for antiobesity therapy.

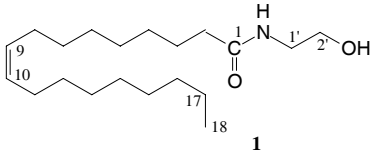
Experimental part

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 on LTQ Orbitrap Velos Pro, by injecting a solution of 100 fmol/µl in 0.1 % formic acid in MeOH. 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 2 and 3 from methyl oleate and phenylalaninol, respectively phenethylamine

The procedure, published by us for N-[2-(methoxy-phenyl)ethyl]oleamide [8], is as follows: 5 g (33.0 mmoles) amine, 9.4 g (31.0 mmoles) methyl oleate in 100 mL anhydrous methanol and 10 mL 29% (w/w) sodium methoxide in methanol as basic catalyst were refluxed for the time mentioned for each reaction, monitoring the reaction by TLC (Merck silica gel plates, eluent I: ethyl acetate-methanol, 90:13). The reaction mixture was then cooled to room temperature, acidulated with 22 mL 10% HCl solution, methanol was distilled under reduced pressure, 75 mL water and 200 mL CH_2Cl_2 added, phases



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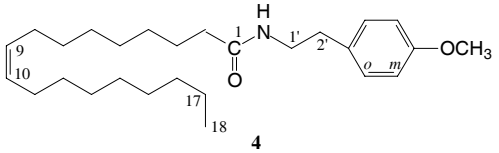

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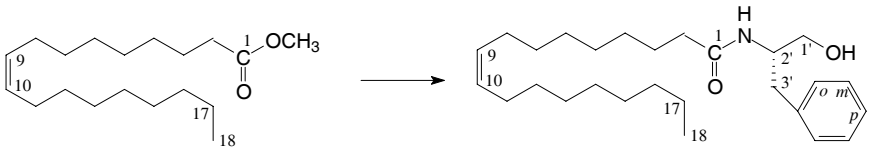
separated, organic phase washed with 50 mL saturated solution $NaHCO_3$, 50 mL brine, dried (Na_2SO_4) and concentrated (Aqueous solutions were extracted with 50 mL CH_2Cl_2 and organic extract unified with organic phase). The crude product was purified by pressure chromatography on a silica gel column prepared in benzine and eluted with the system: benzine-ethyl acetate (5:1).

Synthesis of compound N-[2-(3-phenyl)-1-propanol]oleamide 2



Methyl oleate Compound 2

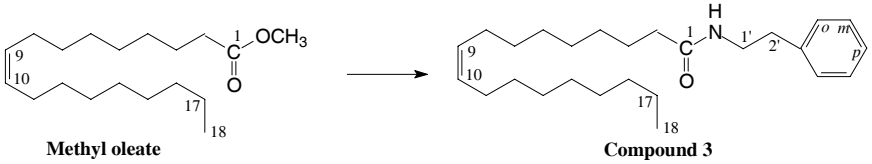
20 h, TLC ($R_{f\,methyl\,oleate}$ = 0.80, $R_{f\,amine}$ = 0.06, $R_{f\,compound\,2}$ = 0.63), 9.91g (77 %), wax, $[\alpha]_D$ = -13.8°(c=1% in $CHCl_3$) {lit.[13] -12° (2% in $CHCl_3$)},

IR: 3292s (νOH; αNH), 3006w ($\nu_{=C-H}$), 2922vs, ($\nu CH_{2\ asim}$); 2852s ($\nu CH_{2\ sim}$), 1640s (νC=ONHR sec. amide), 1548s (νC=O sec. amide, band II), 1465m, 1455m, 1182w, 1144w,

^{1}H-NMR ($CDCl_3$ δ ppm, *J* Hz): 7.33-7.20 (m, 5H, 2H-*o*, 2H-*m*, H-*p*); 5.75 (d, 1H, NH, 7.1); 5.40-5.29 (m, 2H, H-9, H-10); 4.17 (m, 1H, H-2'', 7.4); 3.68 (dd, 1H, H-1', 3.8, 11.0); 3.65 (dd, 1H, H-1', 5.2, 11.0); 2.89 (dd, 1H, H-3', 7.1, 14.0); 2.83 (dd, 1H, H-3', 7.4, 14.0); 2.16-1.94 (2m, 6H); 1.59-1.49 (m, 2H); 1.40-1.20 (m, 20H); 0.88 (t, 3H, H-18, 6.3), ^{13}C-NMR ($CDCl_3$, δ ppm): 174.00 (C-1); 137.64 (C_q); 130.03; 129.74 (2C, C-9, C-10); 129.19 (2C-*o*); 128.66 (2CH, C-*m*); 126.70 (C-*p*); 64.44 (CH_2, C-1''); 52.85 (CH, C-2''); 37.01 (C-3'); 36.83 (C-2); 31.91 (CH_2); 29.78; 29.73; 29.67; 29.54; 29.33; 29.26; 29.14; 29.13; 27.24; 27.19; 25.19 ($11CH_2$); 22.69 (C-17); 14.12 (C-18). IR and NMR are in agreement with published data [13].

MS for $C_{27}H_{45}NO_2$, presents the peaks at m/z 416.35, m/z 417.35 and m/z 418.35, corresponding to isotope peaks at $[M+H]^+$, $[M+1+H]^+$ and $[M+2+H]^+$. Fragments: 398.34 $[M-OH+H]^+$, 282 [oleoylamide +H]$^+$, 265 [oleoyl fragment]$^+$, 247 and minor fragments of consecutive olefinic backbone fragmentation.

Synthesis of compound N-[2-phenylethyl]oleamide 3



Methyl oleate Compound 3

20 h , TLC ($R_{f\,methyl\,oleate}$ = 0.80, $R_{f\,amine}$ = 0.05, $R_{f\,compound\,3}$ = 0.58), 8.36 g (70 %), wax,

IR: 3305s (nNH), 3003w ($\nu_{=C-H}$), 2918vs, ($\nu CH_{2\ asim}$); 2872m, 2850s ($\nu CH_{2\ sim}$), 1639s (νC=ONHR sec. amide), 1550s (νC=O sec. amide, band II), 1495w, 1466m, 1259w, 722w, 698w,

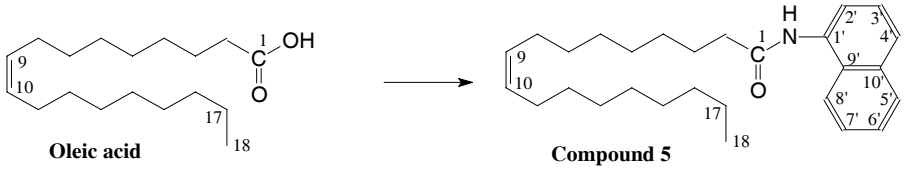
^{1}H-NMR ($CDCl_3$ δ ppm, *J* Hz): 7.33-7.17 (m, 5H, 2H-*o*, 2H-*m*, H-*p*); 5.59 (br s, 1H, NH); 5.34 (m, 2H, H-9, H-10); 3.51 (q, 2H, H-1', 6.9); 2.81 (t, 2H, H-2', 6.9); 2.11 (dd, 2H, H-2, 7.1, 8.0); 2.04-1.97 (m, 4H, 2H-8, 2H-11); 1.63-1.3 (m, 2H, H-3); 1.40-1.20 (m, 20H); 0.88 (t, 3H, H-18, 6.0), ^{13}C-NMR ($CDCl_3$, δ ppm): 173.33 (C-1); 139.04 (C_q); 130.09; 129.83 (2C, C-9, C-10); 128.85 (2C-*o*); 128.70 (2CH, C-*m*); 126.57 (C-*p*); 40.62 (CH_2, C-1''); 36.89 (CH, C-2''); 35.80 (C-2); 32.00 (CH_2); 29.86; 29.81; 29.62; 29.46; 29.41; 29.36; 29.34; 29.23; 27.32; 27.28; 25.85 ($11CH_2$); 22.78 (C-17); 14.21 (C-18).

MS for $C_{26}H_{43}NO$, presents the peaks at m/z 386.34, m/z 387.35 and m/z 388.35, corresponding to isotope peaks at $[M+H]^+$, $[M+1+H]^+$ and $[M+2+H]^+$. Fragments: 282 [oleoylamide +H]$^+$, 265 [oleoyl fragment]$^+$, 247 and minor fragments of consecutive olefinic backbone fragmentation.

General procedure for synthesis of oleoylamides 5 and 6 from oleic acid, activated by 1,1'-carbonyl-diimidazole, and 1-naphthylamine, respectively cyclohexylamine.

The published procedure [9], proceeded as follows: 3.45 g (12 mmoles) oleic acid were dissolved in 100 mL CH_2Cl_2, 2.08g (12.8 mmoles) 1,1'-carbonyldiimidazole (CDI) were added and the mixture was stirred 2 h at room temperature (r.t.). Then this solution was added dropwise to a stirred solution of 2.08 g (20 mmoles) cyclohexylamine and 0.136 g (1.11 mmoles) 4-dimethylaminopyridine in 50 mL CH_2Cl_2 and stirred for 48 h at r.t., monitoring the reaction by TLC (Merck silica gel plates, eluent I: ethyl acetate-methanol, 90:13, or eluent II: ethyl acetate-hexane-acetic acid, 5:4:0.1). The mixture is washed with 2x100 mL sat. soln. $NaHCO_3$, 2x100 mL 3.5% oxalic acid soln., 100 mL sat. soln. $NaHCO_3$, 100 mL brine, dried (Na_2SO_4) and concentrated (Aqueous solutions were extracted with 50 mL CH_2Cl_2 and organic extract unified with organic phase). The products were crystalized from hexane and the remaining product from mother liquor was purified by pressure chromatography on a silica gel column prepared in benzine and eluted with the system: benzine-ethyl acetate (5:1).

Synthesis of compound N-[1-naphthyl]oleamide 5



Oleic acid Compound 5

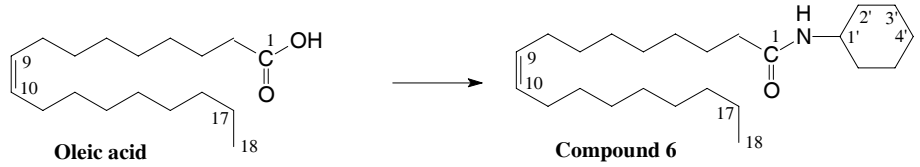
TLC (II, $R_{f\ oleic\ acid}$=0.75, $R_{f\ amine}$=0.02, $R_{f\ compound\ 5}$=0.20), 4.55g (93%), wax,

IR: 3265br m (νNH) 3007w ($\nu_{=C-H}$), 2921vs, ($\nu CH_{2\ asim}$); 2852s ($\nu CH_{2\ sim}$), 1649s (νC=ONHR sec. amide), 1534s (νC=O sec. amide, band II), 1502m, 1463w, 1260w, 793w, 775w, 721w,

^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 7.90-7.40 (m, 8H, 7H-Ar, NH); 5.35 (m, 2H, H-9, H-10); 2.47 (t, 2H, H-2, 7.4); 2.10-1.90 (m, 4H, 2H-8, 2H-11); 1.86-1.70 (m, 2H, H-3); 1.48-1.02 (m, 20H); 0.88 (t, 3H, H-18, 6.0), ^{13}C-NMR ($CDCl_3$, δ ppm): 172.25 (C-1); 134.22; 132.43 ($2C_q$,C-9', C-10'); 130.14; 129.87 (2C, C-9, C-10); 128.82 (C-Ar); 127.45 (C-1'); 126.31 (C-Ar); 126.04 (C-Ar); 125.91 (C-Ar); 125.82 (C-Ar); 121.37 (C-Ar); 120.87 (C-Ar); 37.71 (C-2); 32.03 (CH_2); 29.89; 29.84; 29.66; 29.45(3C); 29.28; 27.34; 27.31; 26.00 ($11CH_2$); 22.71 (C-17); 14.25 (C-18).

MS for $C_{28}H_{41}NO$, presents the peaks at m/z 408.32, m/z 409.33 and m/z 410.33, corresponding to isotope peaks at $[M+H]^+$, $[M+1+H]^+$ and $[M+2+H]^+$. Fragments: 144 [Naphthylamine+H]$^+$, 127 [Naphthyl]+, 282 [oleoylamide +H]$^+$, 265 [oleoyl fragment]$^+$, 247 and minor fragments of consecutive olefinic backbone fragmentation.

Synthesis of compound N-[1-cyclohexyl]oleamide 6



Oleic acid **Compound 6**

TLC (II, $R_{f\ oleic\ acid}$=0.75, $R_{f\ amine}$=0.04, $R_{f\ compound\ 6}$=0.68), 4.24g (97.2%), wax,

IR: 3302m (νNH), 2921vs ($\nu CH_{2\ asim}$), 2851s ($\nu CH_{2\ sim}$), 1636s (νC=ONHR sec. amide), 1543s (νC=O sec. amide, band II), 1467w, 1447m,

^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 5.46 (br d, 1H, NH, 7.7); 5.34 (m, 2H, H-9, H-10); 3.77 (m, 1H, H-1', 4.1, 8.5); 2.13 (dd, 2H, H-2, 7.4, 8.0); 1.80-1.01 (m, 36H); 0.88 (t, 3H, H-18, 6.6), ^{13}C-NMR ($CDCl_3$, δ ppm): 172.33 (C-1); 130.06; 129.83 (2C, C-9, C-10); 48.11 (C-1'); 37.16 (C-2); 33.34 (2C-2'); 32.00 (CH_2); 29.85; 29.80; 29.61; 29.41 (2C); 29.37 (2C); 29.24; 27.30; 27.26; 25.64 ($11CH_2$); 26.00 (C-4'); 24.99 (2C-3'); 22.78 (C-17); 14.21 (C-18).

MS for $C_{24}H_{45}NO$, presents the peaks at m/z 364.36, m/z 365.36 and m/z 366.36, corresponding to isotope peaks at $[M+H]^+$, $[M+1+H]^+$ and $[M+2+H]^+$. Fragments: 144 [Cyclohexylamine+H]$^+$, 282 [oleoylamide +H]$^+$, 265 [oleoyl fragment]$^+$, 247 and minor fragments of consecutive olefinic backbone fragmentation.

Materials and methods

Pharmacological activity

Wistar rats weighing 220±20g were used to assess the administration effect of five oleamide analogues **2-6** 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 7 groups of 5 animals as it follows:

- Group 1 treated with oleamide **1** in oral dose 10 mg/Kg bw
- Group 2 treated with oleamide **2** in oral dose 12.5 mg/Kg bw
- Group 3 treated with oleamide **3** in oral dose 11.5 mg/Kg bw
- Group 4 treated with oleamide **4** in oral dose 12.5 mg/Kg bw
- Group 5 treated with oleamide **5** in oral dose 12 mg/Kg bw
- Group 6 treated with oleamide **6** in oral dose 11 mg/Kg bw
- Group 7 = control group treated with physiological serum in oral dose 5mL/Kg bw.

The oleamide analogues concentration used for the study are molar equivalent with the therapeutic dose recommended for oral administration of oleoylethanolamide **1** (10mg/Kg bw) [10, 11].

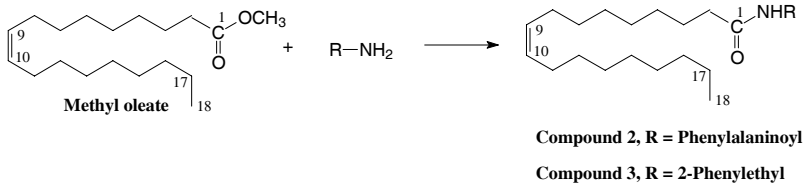
The animals were daily treated for 10 days. The body weight and food intake were daily measured. In the last day of the treatment, the blood was collected in tubes with EDTA as anticoagulant for the determination of the following hematological parameters: red blood cell count (RBC), hemoglobin, hematocrite, red blood cell indices (mean corpuscular volume – MCV, mean corpuscular hemoglobin concentration – MCHC, mean corpuscular hemoglobin – MCH), white blood cell count (WBC), differential white blood cell count (lymphocytes, monocytes, granulocytes), platelets count and indices. A hematological multiparameter analyzer Abacus Junior Vet for veterinary purpose was used for the parameters analyses.

Results and discussions

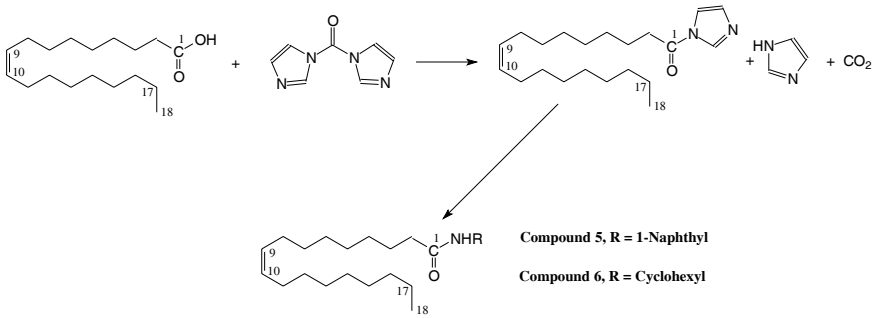
Chemistry

Synthesis of oleamide analogues

Due to their importance as signaling molecules in satiety control, we decided to synthesize and use in our study a series of oleamide analogues, including phenylalaninol, 2-phenylethylamino, 1-naphthylamino and cyclohexylamino in the amide moiety of oleoylamides. Previously [8] we synthesized N-[2-(4-methoxyphenyl) ethyl]oleamide by amidation of methyl oleate with 2-(4-methoxy-phenyl)ethylamine in methanol at reflux, reaction catalyzed by sodium methoxide as base catalyst, a procedure which was previously optimized in the synthesis of oleoyl-(2-ethanol)amide. Following this procedure, we obtained phenylalaninoyl- and phenethyl-oleamide analogues, **2** and **3** (scheme 1). Previously [13], compound **2** were obtained [13] in 66 % (- enantiomer) and 80% (+ enantiomer) from oleic acid and (-)- and (+)-phenylalaninol by carbodiimide (EDC) method. The compounds obtained by this procedure were obtained in 77% and respectively 70% yield, and were used as so in biological evaluation of the compounds.



Scheme 1. Synthesis of oleamide analogues **2** and **3** from methyl oleate and amines in the presence of sodium methoxide as catalyst



Scheme 2. Synthesis of oleamide analogues **5** and **6** from oleic acid and amines in the presence of 1,1'-carbonildiimidazole

The oleoylamide analogues **5** and **6** were synthesized by another procedure, not because the above procedure is not applicable for these compounds (in fact, both compounds were later obtained by the procedure mentioned above), but to have in our hands another procedure suitable to be used in the future for usual aromatic and alifatic amines and for expensive ones with more elaborated structure.

We chose a procedure already used in the literature for macamide synthesis [9], fatty acid structurally analogues, and developed the procedure for synthesis of oleamide analogues **5** and **6** (scheme 2). In this procedure oleic acid is activated by reaction with 1, 1'- carbonyldiimidazole to the oleyl-imidazol intermediate and this preformed active compound is added to the dichloromethane solution of amine containing also 4-dimethylaminopyridine (DMAP) as catalyst. The reaction takes place in mild conditions (r.t.) and the yields are high, for example 77% for 1-naphthyloleamide and 92.7% for cyclohexyloleamide. Cyclohexyloleamide was previously obtained by reaction of oleyl chloride and cyclohexylamine [15] in 90% yield and 1-naphthyloleamide by reaction of oleic acid with 1-naphthyl amine at 230°C for 5 h in 79% yield [16].

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

IR spectrum of the compounds presents an intense band at ~ 3300 for νNH, 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 is present a very intense band at 2918-2922 cm^{-1} ($\nu CH_{2\ asim}$), and an intense band at 2850-2852 cm^{-1} ($\nu CH_{2\ sim}$).

In ^{1}H-NMR spectra there are present all signals for the protons characteristic for the introduced amide moiety of the molecule together with the signals characteristic for the oleic acid moiety (See experimental part for details). The same is observed for carbon atoms of both fragments of the molecule.

Mass spectra gives molecular peaks corresponding to isotope peaks of compounds at $[M+H]^+$, $[M+1+H]^+$ and $[M+2+H]^+$. 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).

All above mentioned data are in full agreement with the molecular structure of the oleoylamide compounds synthesized.

Biological activity

The influence of the treatment with oleamide analogues on the body weight variation in rats

The daily administration of the oleamide analogues decrease the body weight of the animals comparing to the control group, except for compounds **4** and **6**, with 26% and respectively 20.4% increase of the body weight after 10 day of oral administration. The most significant effect was noticed in case of compound **2**. The animals treated with compound **2** maintained their weight during the study, registering a decrease of approximately 100% comparing to the control group in the last day of the experiment. Oleoylethanolamide **1** treatment decreased the body weight by 20.6% comparing to control. The data obtained for oleoylethanolamide **1** group are in respect with communicated data in scientific publications [10, 11]. The treatment with compounds **3** and **5** produced a 34% and respectively 11.4% decrease of the rats body weight comparing to control (fig. 1).

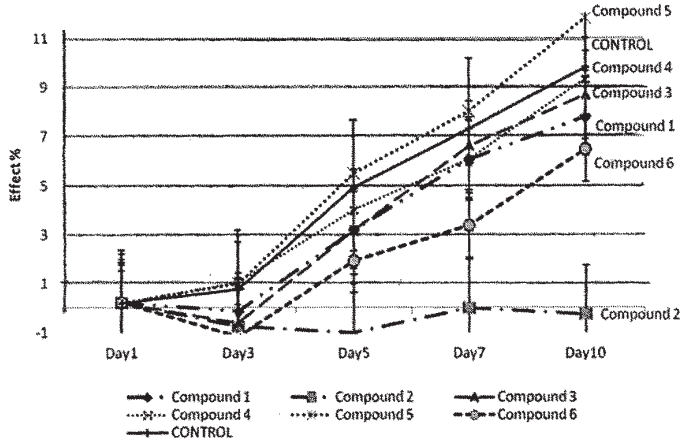


Fig. 1. The effects of the daily administration with the oleamide analogues on the animal body weights during 10 days of treatment. The oleamide analogues: compounds **2-6** were oral administrated in dose molar equivalent with the therapeutic dose of oleoylethanolamide **1** (10mg/Kg bw). The control group was treated with physiological serum in oral dose of 5mL/Kg bw. Standard errors are represented in the figure by bars

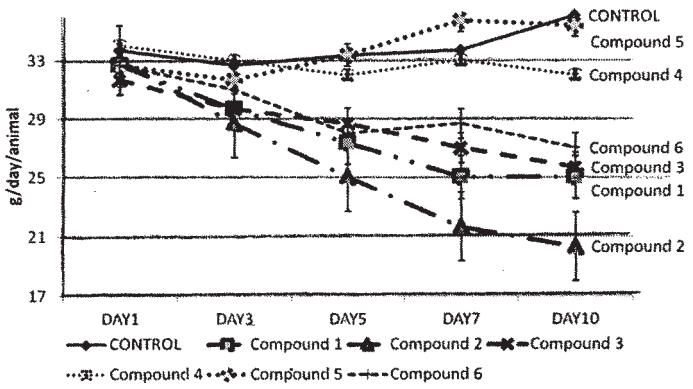


Fig. 2. The effects of the daily administration with the oleamide analogues on the animal body weights during 10 days of treatment. The oleamide analogues: compounds **2-6** were oral administrated in dose molar equivalent with the therapeutic dose of oleoylethanolamide **1** (10mg/Kg bw). The control group was treated with physiological solution in oral dose of 5mL/Kg bw. Standard errors are represented in the figure by bars

The influence of the treatment with oleamide analogues on the food-intake in rats

The daily administration of oleamide analogues decreased the animal food intake in case of **3, 4** and **6** by 28%, 11% and respectively 25% comparing to control (fig.2). The effect of oleamide **3** treatment was much closed to the data obtained for oleoylethanolamide **1** group with a 30% decrease of food intake in rats. The most significant decrease by 38% was noticed in case of oleamide **2** treatment. The administration of oleamide **5** produced no significant variation on food intake on rats comparing to control group.

The influence of the oleamide analogues treatment on the hematological parameters on rats

The hematological parameters determined in this study are part of the preliminary toxicological study regarding the effect of the oleamide analogues administration. Red blood cell (RBC) count, RBC indicies, hematocrit and hemoglobin are the most used biomarkers for diagnosing anemia. The differential white blood cell count is frequently used for the identification of an infectious or inflammatory state. The platelets count and indices are used for the determination of blood clotting problems. The data are presented in tables 1, 2 and 3. No significant variations of any of the hematological parameters were noticed comparing to control group. All the parameters values respect the normal hematological values accepted for Wistar rats [12].

The daily treatment with oleamides **1**, **3** and **6** decreased the body weight and the food intake of the rats after 10 days of oral administration comparing to the not-treated control group. The oleamides **4** and **6** increased significant the body weight and had no influence on the food intake during the administration. A significant effect of body weight decreasing was obtained in case of oleamide **2** administration. The weight loss effect is correlated with a slightly decrease of food intake comparing to oleoylethanolamide **1** group.

The oleamide analogue administration during 10 days of oral treatment had no influence on hematological parameters demonstrating a potential low toxicity of these compounds.

Table 1
THE INFLUENCE OF THE TREATMENT WITH OLEAMIDE ANALOGUES ON THE RED BLOOD CELL COUNT AND INDICES, HEMOGLOBIN AND HEMATOCRIT

	Compound **1** Mean±SD	Compound **2** Mean±SD	Compound **3** Mean±SD	Compound **4** Mean±SD	Compound **5** Mean±SD	Compound **6** Mean±SD	Control Mean±SD
RBC NV 5.3-10 x10^6/µl	7.13 ± 1.34	6.96±2.28	7.79±0.58	8.03±0.1	7.59±0.34	8.06±0.42	7.34±0.6
HGB NV 14-18 g/dl	14.8±2.26	14.30±4.24	16±0.79	16.9±0.1	15.74±.53	16.40±0.26	15.58±1.06
HCT NV 35-52%	40.05±6.72	39.35±11.1	42.3±1.97	44.2±1.1	41.94±2.05	43.37±1.24	40.35±2.98
MCV NV 50-62fl	56.5±0.71	57±2.83	54.33±1.53	55±1	55.44±1	54±1.73	55±0.82
MCH NV 16-23pg	20.85±.78	20.65±0.78	20.57±0.57	21.1±0.35	20.76±0.5	20.37±0.76	21.23±0.45
MCHC NV 31-40 g/dl	36.95±0.64	36.25±0.49	37.77±0.21	38.3±0.7	37.43±0.79	37.73±0.38	38.55±1.1
RDW	19.35±0.78	19.8±1.56	19.9±0.3	19.3±0.2	19.68±0.57	19.63±1.03	19.45±0.79

(NV = normal value, RBC = red blood cell, HGB = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, RDW = red blood cell width)

	Compound 1 Mean±SD	Compound 2 Mean±SD	Compound 3 Mean±SD	Compound 4 Mean±SD	Compound 5 Mean±SD	Compound 6 Mean±SD	Control Mean±SD
WBC NV 2.1-19.5 x10^3cell/µl	11.36±3.7	16.9±3.1	11.8±1.2	11.8±4.8	7.11±0.4	6.6±1.3	16.78±1.6
Lymphocytes NV 2-14 x10^3cell/µl	6.65±0.1	11.94±2.8	8.8±1.2	8.8±3.7	4.76±2	6.81±1.4	10.98±1.2
Monocytes NV 0.1-1 x10^3cell/µl	0.35±0.2	1.01±1.1	0.8±0.3	0.82±0.5	0.33±0.6	0.26±0.5	0.76±0.4
Granulocytes NV 0.1-5.4 x10^3cell/µl	3.4±2.2	3.95±1.3	2.2±0.1	2.18±0.5	2.01±0.7	2.9±1.1	5.07±0.9
Lymphocytes NV 55-97%	68.8±10.5	70.3±4	74.3±2.7	74.3±1.9	65.77±4.6	68.77±4.2	66.07±3.5
Monocytes NV 3-7%%	2.95±0.5	6.65±7.4	6.4±2.6	6.4±2.2	4.93±4.9	2.53±3.6	4.27±3
Granulocytes NV 2-31%	28.15±10	23.1±3.4	19.2±1.5	19.2±2.9	29.27±4.7	28.67±4.4	29.63±3.6

(NV = normal value, WBC = white blood cell count)

Table 2
THE INFLUENCE OF THE TREATMENT WITH OLEAMIDE ANALOGUES ON DIFFERENTIAL WHITE BLOOD CELL COUNT

	Compound 1 Mean±SD	Compound 2 Mean±SD	Compound 3 Mean±SD	Compound 4 Mean±SD	Compound 5 Mean±SD	Compound 6 Mean±SD	Control Mean±SD
Platelets x10^6/m m^3	617±94.7	557.5±0.7	739±47.3	540±40	744±87.4	678±25.3	566±34.3
PCT %	0.25±0.2	0.37±0.01	0.46±0.01	0.48±0.06	0.47±0.04	0.45±0.1	0.37±0.23
MPV (fl)	7.05±1.2	6.6±0.1	6.23±0.2	6.47±0.3	6.3±0.1	6.6±0.2	6.7±0.46
PDWc%	35.4±2.8	33.25±0.7	33±1	32.9±0.6	32.4±0.17	32.7±0.4	33.45±1

(PCT = platelet hematocrit, MPV = mean platelets volume, PDW = platelets distribution width)

Table 3
THE INFLUENCE OF THE OLEAMIDE ANALOGUES TREATMENT ON THE PLATELETS COUNT AND INDICES

Conclusions

Oleamide analogues were synthesized for preliminary pharmacological activity regarding bodyweight and food intake effects. Two of the compounds were obtained by base amidation of methyl oleate with phenylalaninol (compound **2**) and 2-phenylethylamine (compound **3**) and by direct amidation of 1, 1'- carbonyldiimidazole activated oleic acid with amines: 1-naphthylamine (compound **5**) and cyclohexylamine (compound **6**). IR, MS, ^{1}H-, ^{13}C-NMR and complementary APT, COSY and HETCOR spectra confirmed the structure of the oleamide analogues synthesized.

The oleamide analogues treatment, except for compounds **4** and **6**, decreased the body weight and food intake. The most significant effect was obtained in case of oleamide **2** comparing to control group and oleoyl-ethanolamide **1** treated group. The results in this case are very optimistic for developing a new drug for obesity treatment. No hematological parameters were modified after 10 days treatment with oleamide analogues, demonstrating a potential low toxicity of these compounds. Further more elaborated toxicological studies and other potential biological activities of these compounds will be necessary.

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