Brief Articles

Novel Sulfamide Analogs of Oleoylethanolamide Showing In Vivo Satiety Inducing Actions and PPARα Activation

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Long chain saturated and unsaturated alkyl sulfamide and propyl sulfamide derivatives, analogs of oleoylethanolamide, have been synthesized and evaluated *in vivo* and *in vitro* as peroxisome proliferator activated receptor alpha (PPARα) activators. Additionally, the anorexic effects of the new compounds have been studied *in vivo* in food-deprived rats. Among the active compounds *N*-octadecyl-*N*′-propylsulfamide (**7**) has been identified as a potent hypolipidemic compound, a potent feeding suppressant, and a concentration-dependent activator of PPARα.

Introduction

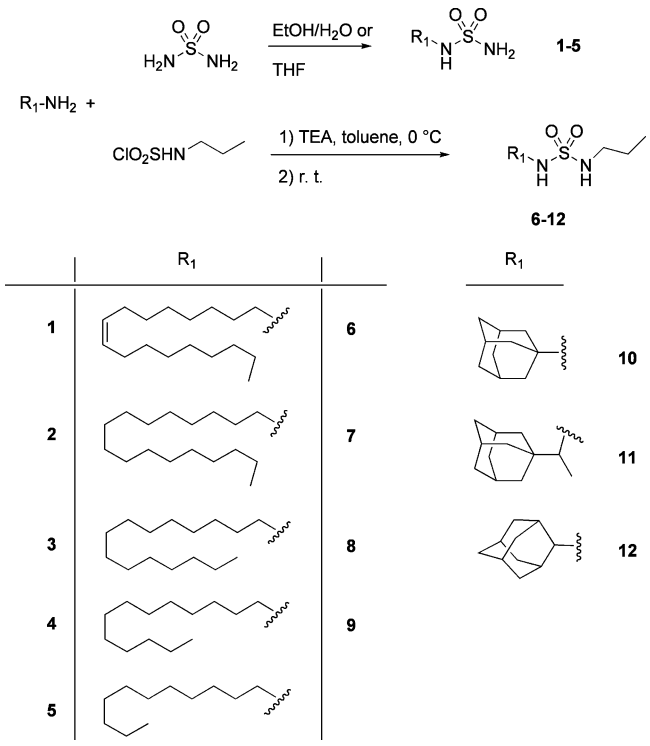
Peroxisome proliferator-activated receptors (PPARs) are members of a superfamily of nuclear hormone receptors for which three isoforms (PPARα, PPARγ, and PPARδ) have been described.[1] These ligand-activated transcription factors play a major role in the regulation of lipid and glucose metabolism and are, therefore, interesting medicinal chemistry targets useful in diseases related to altered metabolism such as dyslipidemia, diabetes, and obesity.[2]

Concerning the PPARα subtype, substantial research effort has focused on the design and synthesis of ligands, including fibrates used clinically as hypolipidemic agents,[3] WY 14643, the PPARα/γ dual agonists potentially useful for type 2 diabetes,[2] and the newer potent selective PPAR agonists such as phenylpropionic acid derivatives[4] or the triazolone LY518674.[5] The natural ligands for PPARs, which was originally cloned as an orphan receptor, include fatty acids, eicosanoids,[6] and more recently *N*-(2-hydroxyethyl)oleamide, commonly known as oleoylethanolamide (OEA), a naturally occurring lipid that exerts its action through PPAR activation.[7−9]

In this paper, we wish to report the first sulfamide derived analogs of OEA, with potent, selective binding affinity for PPARα, which induce satiety, reduce food intake in rats, reduce body weight, and act as lipid-lowering agents.[10] Based on the structure of OEA, ethanolamide of oleic acid, we decided to substitute the amide by sulfamide (N−SO_2−N). To study SAR relationships, the length and saturation of the fatty acid chain were varied, and to mimic the carbon fragment of the hydroxyethyl group of the amide, some propyl derivatives were also prepared.

The biological assays of the new compounds consisted in the determination of *in vivo* and *in vitro* ability for activating transcription mediated by PPARα, the ability to suppress feeding in food-deprived animals and the ability to reduce body weight and plasma triglyceride content. This analysis led to the characterization of *N*-octadecyl-*N*′-propylsulfamide (**7**) as a potent hypolipidemic agent, a potent feeding suppressant, and a concentration-dependent activator of PPARα.

Scheme 1. Synthesis of Monosubstituted **1**−**5** and Disubstituted Sulfamides **6**−**12**



Chemistry

The synthesis of the new compounds can be achieved starting from the corresponding long chain alkyl amines either by a transamination reaction with sulfamide[11] or by reaction with a sulfamoyl chloride.[12] Thus, reaction of *N*-oleylamine, *N*-

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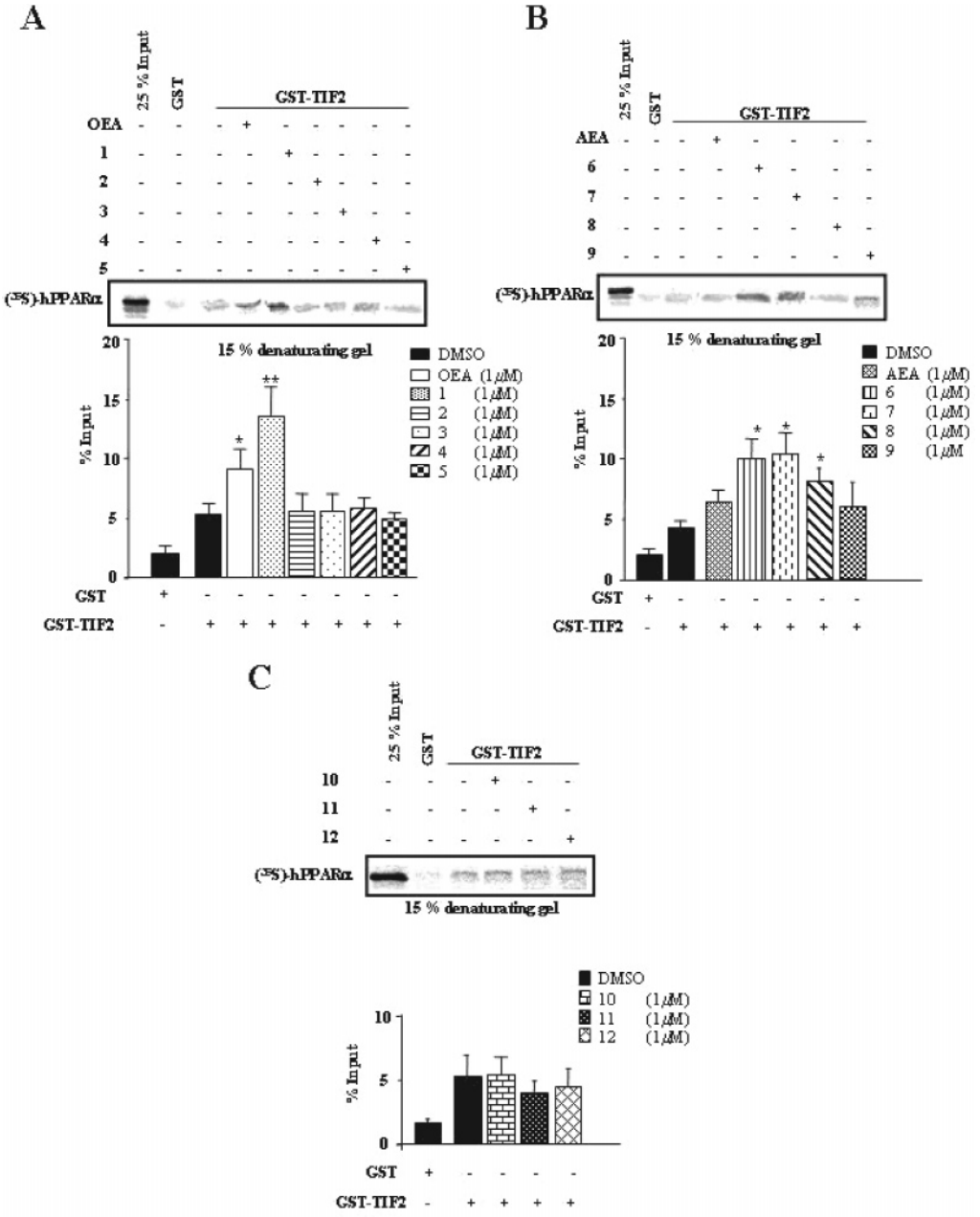

Figure 1. Individual ligand-triggered interaction profiles of hPPARα with coactivator (CoA) *in solution*. GST pull down assays were performed with bacterially expressed GST-CoA and full-length *in vitro* translated (^{35}S)-labeled human PPARα, in the absence and presence of their respective ligands. GST alone (−) was used as a control, and the percentage of precipitated PPARα was quantified in respect to input (mean ± SD, $n = 3-6$). *$p < 0.05$ and **$p < 0.01$ (student's t-test) vs DMSO.

octadecylamine, *N*-hexadecylamine, *N*-tetradecylamine, and *N*-dodecylamine with sulfamide in water/ethanol or THF afforded the corresponding *N*-monosubstituted derivatives **1**−**5** (Scheme 1). To avoid the disubstitution products obtained on reaction of these amines with *N*-propylsulfamide, *N*-propylsulfamoyl chloride[13] was treated *in situ* with *N*-oleylamine, *N*-octadecylamine, *N*-hexadecylamine, or *N*-tetradecylamine in toluene with triethylamine as base (Scheme 1), and thus, the corresponding *N*-propylsufamoyl derivatives **6**−**9** were obtained. As a different approach to include highly lipophilic chains, adamantyl derivatives **10**−**12** were also prepared from *N*-1-adamantylamine in the same way (Scheme 1). The structures of the newly synthesized compounds were established according to their analytical and spectroscopic data. Only compound **5** had previously been reported in a patent as a precursor of light sensitive material.[14]

Table 1. Pharmacological Properties of Substituted Sulfamides **1**−**3**, **6**−**8**, and **12**

compound	PPARα **activation** EC_{50}
WY 14643	0.74 μM
OEA	110 nM
1	524 nM
2	>10 000 μM
3	>10 000 μM
6	11 μM
7	100 nM
8	21 μM
12	>10 000 μM

Biology

The compounds reported in this study were first evaluated for *in vitro* GST pull down to see which compound could induce interaction between PPARα and CoA. After that, these com-

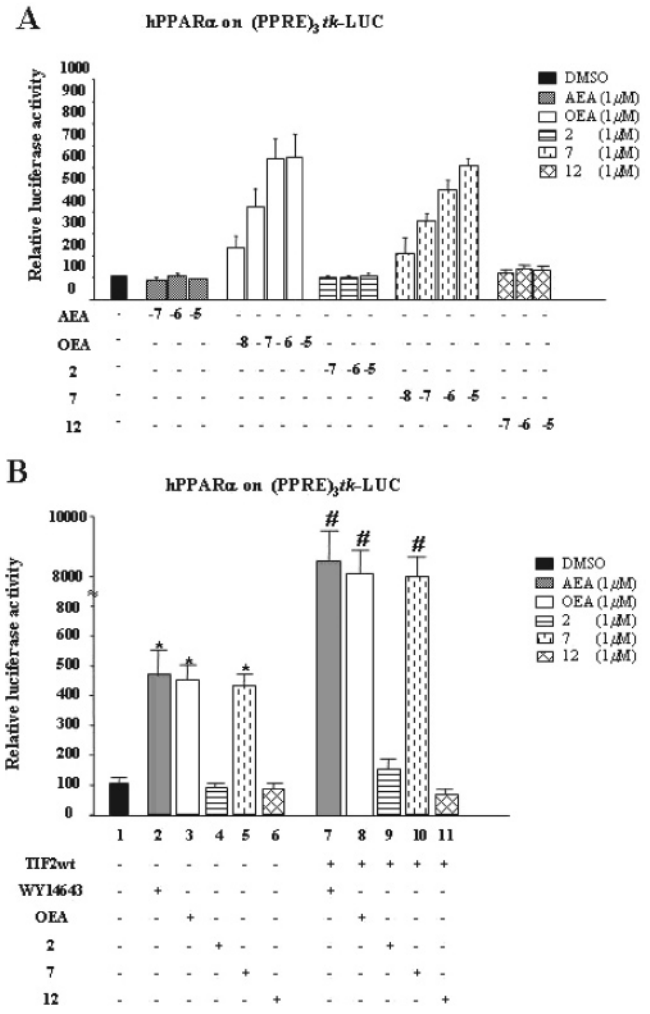

Figure 2. Agonistic action of OEA and analogs. Luciferase reporter gene assays were performed with extracts from MCF-7 cells that were transiently transfected with a luciferase reporter construct of the human ACO of PPRE. (A) Wild-type human PPARα expression vector was also cotransfected as indicated. Cells were treated for 16 h with DMSO, and different concentrations (10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M) of OEA and compound **2** or 10^{-7}, 10^{-6}, and 10^{-5} M of AEA (anandamide), **7** and **12** compounds as indicated. (B) WT hPPARα expression as well as plasmids coding full length TIF2 were also cotransfected as indicated. Cells were treated for 16 h with DMSO, 10 μM WY14643, 1 μM OEA, and its analogs **2**, **7**, and **12**. The luciferase activities were normalized with respect to protein concentration, and relative values were calculated in reference DMSO-induced cells not overexpressing any protein (mean ± SD, $n = 3-6$). *$p < 0.01$ vs DMSO, #$p < 0.01$ vs DMSO and without TIF2.

pounds were evaluated for their agonist activity on the human PPARα transfected into MCF-7 cells. The compounds that exhibited potent and/or selective PPARα subtype transactivation activity were then evaluated further, *in vivo*, on food intake and pharmacology studies in rats.

GST-Pull-Down Assays. GST pull down assays were performed with 50 μL of a 50% Sepharose bead slurry of GST or GST-TIF2 (pre-blocked with 1 μg/μl bovine serum albumin) and 20 ng *in vitro* translated, [^{35}S]-labeled PPARα in the presence or absence of their respective compounds, as previously described.[15] *In vitro* translated proteins that were not bound to GST-fusion proteins were washed away with immunoprecipitation buffer. GST-fusion protein bound [^{35}S]-labeled PPARα were resolved by electrophoresis through 15% SDS−polyacrylamide gels and quantified on a Fuji FLA3000 reader using Image Gauge software (Fuji).

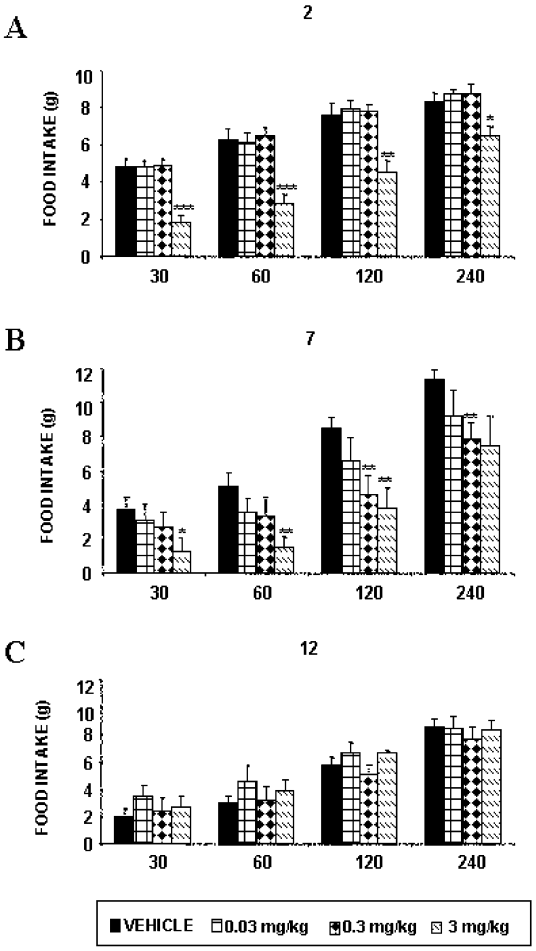

Figure 3. Activity of OEA analogs as feeding suppressants. Food intake in food-deprived animals was tested 30, 60, 120, and 240 min after the i.p. injection of the different compounds synthesized at 0.03, 0.3, and 3 mg/kg doses, i.p. Here we show three typical patterns observed. Compound **2** does not activate PPARα but reduces feeding behavior; **7** is a potent feeding suppressant and a potent activator of PPARα, and finally, **12** neither activates PPARα nor suppresses food intake (means ± SEM, $n = 3-6$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs vehicle, ANOVA.

Cellular Transfection and Luciferase Reporter Gene Assays. Human breast cancer cells (MCF-7) were seeded onto 6-well plates (10^5 cells/mL) and grown overnight in phenol red-free DMEM supplemented with 5% charcoal-treated fetal bovine serum (FBS). Plasmid DNA containing liposomes were formed by incubating 1 μg of the reporter plasmid and expression vector wild-type human PPARα and TIF2 with 10 μg *N*-[1-(2,3-Dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium methylsulfate (DOTAP, Roche) for 15 min at room temperature in a total volume of 100 μL. After dilution with 900 μL phenol red-free DMEM, the liposomes were added to the cells. Phenol red-free DMEM supplemented with 500 μL of 15% charcoal-treated FBS was added 4 h after transfection. At this time, ligands or DMSO were also added.[16] The cells were lysed 16 h after onset of stimulation using the reporter gene lysis buffer (Roche), and the constant light signal luciferase reporter gene assay was performed as recommended by the supplier (Roche Diagnostics).

Food Intake Studies in Animals. The acute effects of drugs on feeding behavior were analyzed in 24-h food-deprived Wistar rats, as previously described.[9] The drugs were administered intraperitoneally (i.p.) at doses of 0.03, 0.3, and 3 mg/kg suspended in Tween 80 and saline as vehicle. After a period of 15 min after drug administration the animals were returned to

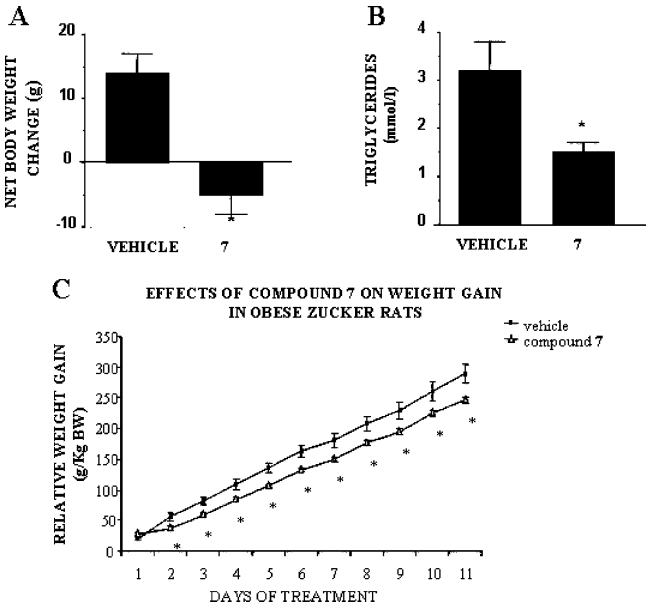

Figure 4. Effects of chronic treatment with **7** (*N*-octadecyl-*N*′-propylsulfamide) on body weight gain and plasma triglycerides. Chronic treatment (8 days, 1 mg/kg, i.p. daily) with *N*-octadecyl-*N*′-propylsulfamide reduces body weight (A) and plasma triglyceride contents (B) when compared to vehicle-treated normal rats. Subchronic (11 days) administration of the compound **7** (1 mg/kg, i.p.) reduces cumulative relative weight gain (C) (g/kg body weight, day 1) in obese Zucker rats (means ± SEM, $n = 3-6$). *$p < 0.05$, versus vehicle, ANOVA.

their home cage and food intake was monitored 30, 60, 120, and 240 min after starting the test. At the end of the test, the amount of water consumed was also measured. Subchronic effects of compound **7** (1 mg/kg daily injected at the start of the dark period of the light cycle) were analyzed in free-feeding Wistar and Zucker rats.

Results and Discussion

Three main conclusions can be derived from the present experimental design. First, susbstitution of the ethanolamine moiety of OEA by a sulfamide or propylsulfamide group does not modify the ability of C-18 derivatives to activate PPARα (Table 1 and Figure 1). Second, the reduction of the acyl side chain reduces the transcriptional activity, which was not present when adamantyl groups were incorporated to the sulfamide moiety (see Supporting Information). Third, the incorporation of the propyl substitution gives more transcriptional activity at the PPARα, in accordance with the prediction based on the structure of the ethanolamide moiety of OEA (Figure 2A).

To determine the ligand-induced activity of PPARα by OEA and its analogs **2**, **7**, and **12**, we transiently transfected MCF-7 human breast cancer with expression vectors for the respective human PPARα and coactivator TIF2 (Figure 2B). WY14643 was included as positive control because this hypolipidemic fibrate selectively activates PPARα.[6] At endogenous coregulator levels, the basal level of PPARα on the PPRE (lane 1) was induced nearly 6 times higher by OEA, WY14643, and compound **7** (lanes 2, 3, and 5), whereas the application of compounds **2** and **12** did not provide induction (lanes 4 and 6). The overexpression of TIF2 resulted in a significant increase in the basal level (lane 1) and a subsequently high prominent increase of agonist-stimulated values (lane 7, 8, and 10). However, the application of compounds **2** and **12** did not provide significantly higher induction (lanes 9 and 11).

Based on that analysis, compounds were tested for feeding experiments in normal rats (Figure 3). An extended dose−response curve was done using i.p. injections in food-deprived animals. The fact that these sulfamoyl derivatives, structurally related to OEA, showed no cannabimetic properties and did not affect fatty acid amidohydrolase activity (data not shown) made them suitable candidates to study their effects on food intake and to compare them with those of OEA. This naturally occurring lipid has been found to decrease food intake through activation of the nuclear receptor PPARα.[9] However, this was not always true. As an example, compound *N*-octadecylsulfamide (**2**) clearly reduced food intake but did not activate PPAR receptor−mediated transcription. A potential explanation, despite the discrepancies between feeding suppression and activity at the PPARα, may depend on the activation of alternative targets, such as the vanilloid receptors (VR1), which are involved also in OEA-mediated inhibition of feeding.[17,18]

From the analysis of behavioral effects and transcriptional activity, three classes of compounds emerge: (1) compounds active at PPARα and with activity as feeding suppressants (the most potent in both tests is *N*-octadecyl-*N*′-propylsulfamide (**7**); (2) anorectic compounds without activity at PPARα (the paradigmatic is *N*-octadecylsulfamide (**2**)); and (3) inactive compounds such as the adamantyl-substituted ones, that is, **12**.

Based on these findings, we studied whether *N*-octadecyl-*N*′-propylsulfamide (**7**; 1 mg/kg, i.p. for 8 days) may mimic the effects of OEA on body weight gain and plasma triglycerides.[7,8] As expected for a PPAR receptor agonist, *N*-octadecyl-*N*′-propylsulfamide (**7**), reduced body weight (Figure 4A) after 8 days of treatment and produced a marked reduction in plasma triglycerides (Figure 4B). To further explore the anti-obesity properties of this compound, we used an additional animal model of obesity. Similar effects were observed in genetically obese Zucker (*fa*/*fa*) rats, and the daily administration of compound **7** (1 mg/kg, i.p.) for 11 days reduced body weight gain (Figure 4C) and food intake. The potency of this new analog of OEA is greater than that of the natural ligand of PPARα, because it has been described that OEA hypolipemiant effects are present only at doses greater than 5 mg/kg,[8−10] whereas *N*-octadecyl-*N*′-propylsulfamide (**7**) was found to be active at lower doses (1 mg/kg).

Conclusions

Sulfamoyl analogues of OEA resulted in compounds with the same biological activity as OEA, some of them with greater potency than the natural PPAR receptor ligand. Some of the compounds had biological activity as feeding suppressants, but were inactive at the PPARα, suggesting the existence of alternative targets for OEA and related compounds for feeding inhibition, as suggested recently.[17,18] Taken all together, these new compounds offer a new opportunity for a simple pharmacological approach to the treatment of obesity and feeding disorders.

Experimental Section

Chemistry. General Procedure for the Preparation of Monosubstituted Sulfamides 1−5. The amine dissolved in EtOH was added dropwise to a solution of sulfamide in H_2O. The reaction mixture was refluxed and evaporated to dryness; the crude was purified by column chromatography on silica gel with CH_2Cl_2/MeOH+NH_3 9:1, giving the expected product.

The preparation of *N*-oleylsulfamide (**1**) is given as representative example.

***N*-Oleylsulfamide (1).** From *N*-oleylamine (0.1 mL, 2.1 mmol), sulfamide (0.200 g, 2.1 mmol), H_2O (20 mL), and EtOH (10 mL); reaction time, 6 h; yield = 0.522 g of white solid, 72%; mp = 68−70 °C; ^{1}H NMR (300 MHz, $CDCl_3$) 5.32 (m, 2H), 4.59 (br s, 2H), 4.38 (br s, 1H), 3.09 (q, $J = 7.0$ Hz, 2H), 1.97 (m, 4H), 1.55

(m, 2H), 1.24 (m, 22H), 0.85 (t, $J = 6.7$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) : 129.9, 129.7, 43.6, 31.8, 29.7−26.6, 27.1, 27.2, 22.6, 14.1. MS (ES^+) $[M + H]^+$ 347 (100%). Anal. ($C_{18}H_{38}N_2SO_2$, 346.57): C, H, N, S.

General Procedure for the Preparation of Disubstituted Sulfamides 6−12. Freshly distilled *N*-propylsulfamoyl chloride was added dropwise to a solution of the amine and TEA in dry toluene at 0 °C under a nitrogen atmosphere. The reaction mixture was stirred at room temperature, the white solid formed was filtered, the solvent was evaporated to dryness, and the crude was purified by column chromatography on silica gel with CH_2Cl_2/MeOH+NH_3 9:1, giving the desired product.

The preparation of *N*-octadecy-*N*′-propylsulfamide **(7)** is given as representative example.

***N*-Octadecyl-*N*′-propylsulfamide (7).** From *N*-octadecylamine (0.270 g, 1.0 mmol), *N*-propylsulfamoyl chloride (0.157 g, 1.0 mmol), TEA (0.300 g, 3.0 mmol), and toluene (30 mL); reaction time, 24 h; yield = 0.122 g of white solid, 31%; mp = 110−112 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 3.01 (m, 4H), 1.53 (m, 4H), 1.24 (br m, 30H), 0.94 (t, $J = 7.6$ Hz, 3H), 0.86 (t, $J = 6.8$ Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) 45.0, 43.3, 31.9, 29.7−29.2, 26.7, 22.9, 22.6, 14.1, 11.2; MS (ES^+) $[M + H]^+$ 391 (100%). Anal. ($C_{21}H_{46}N_2SO_2$, 390.67): C, H, N, S.

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Supporting Information Available: Experimental details and CHNS analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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