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Antiobesity designed multiple ligands: Synthesis of pyrazole fatty acid amides and evaluation as hypophagic agents

Mario Alvarado [a], Pilar Goya [a,*], Manuel Macías-González [b], Francisco Javier Pavón [d], Antonia Serrano [d], Nadine Jagerovic [a], Jose Elguero [a], Angel Gutiérrez-Rodríguez [c], Santiago García-Granda [c], Margarita Suardíaz [d], Fernando Rodríguez de Fonseca [d,*]

[a] Instituto de Química Médica, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain
[b] CIBER Fisiopatología de la Obesidad y Nutrición, CB06/03, Instituto de Salud Carlos III, Madrid, Spain
[c] Departamento de Química Física y Analítica, Facultad de Química, Universidad de Oviedo, 33006 Oviedo, Spain
[d] Fundación IMABIS, Hospital Carlos Haya, Avda. Carlos Haya 82, 29010 Málaga, Spain

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ABSTRACT

Searching for new antiobesity agents, a new series of fatty acid amide derivatives of 1,5-diarylpyrazole have been synthesized as dual peroxisome proliferator activated receptor alpha (PPARα)/cannabinoid receptor ligands. The compounds have been evaluated in vivo and in vitro as PPARα activators and as cannabinoids in two tests of the mouse tetrad. In vivo, food intake studies have been performed with all the compounds. No significant cannabinoid activity has been found but some compounds behaved as potent PPARα activators. Several compounds showed anorexigenic properties reducing food intake in rats.

1. Introduction

Obesity has significantly increased in the last decades affecting an important part of the adult and children population of western countries.[1] It is a complex disease involving many physiological signaling systems and different factors, and it is linked to serious health problems.[2] Nowadays, only three classes of drug treatments are available, representatives are Orlistat (Xenical)™ a gastrointestinal lipase inhibitor, Sibutramine (Meridia)™ a dual serotonin-norepinephrine reuptake inhibitor, and Rimonabant (Acomplia)™, the first cannabinoid CB_1 receptor antagonist approved for clinical use in humans. Although effective in producing weight loss, current therapies against obesity may have some tolerability and/or safety concerns.[3] Moreover, since obesity is a complex disease often associated with high cardiovascular risk, type II diabetes and dyslipemia, appetite cannot be considered the only target for medicines designed to fight obesity. Therefore, the development of novel antiobesity drugs is a priority and a challenge for medicinal chemists.

The fact that there are different targets for antiobesity therapy together with a growing interest in multiple ligands[4] prompted us to apply this strategy to design potential hypophagic agents capable to target not only appetite, but also lipid and carbohydrate metabolism. Taking in consideration the convergent mechanisms of peroxisome proliferator activated receptors alpha (PPARα) and cannabinoid receptor antagonists as modulators of appetite, lipid metabolism and carbohydrate management by the liver and adipose tissue, we decided to explore a dual cannabinoid/PPARα ligand (Fig. 1). The rationale was to link, in the same molecule, a cannabinoid antagonist motif with a group capable of activating PPARα receptors,[5] since the combination of both drugs seems to be additive in terms of controlling appetite.[6] The cannabinoid part was the 1,5-diarylpyrazole structure present in Rimonabant and other well established cannabinoid ligands[7] and the amide chosen was oleylethanolamide, OEA, a lipid mediator that regulates feeding and lipid metabolism by activating PPARα receptors.[8] This idea was further supported by a recent finding in our group in which we have proved that Rimonabant enhances the metabolic benefits of long term treatment with OEA in Zucker rats, a genetic model of obesity, dyslipemia and diabetes due to the lack of leptin signaling.[9]

2. Chemistry

The synthetic route used for the preparation of compounds **4**–**11** is depicted in Scheme 1 and starts from the 2,4-dicarbonyl esters obtained through condensation of the corresponding ketone

* Corresponding authors. Tel.: +34 914113155; fax: +34 915644853 (P.G.); tel.: +34 669426548; fax: +34 951291447 (F.R.F.).
E-mail addresses: iqmg310@iqm.csic.es (P. Goya), fernando.rodriguez@fundacionimabis.org (F. Rodríguez de Fonseca).

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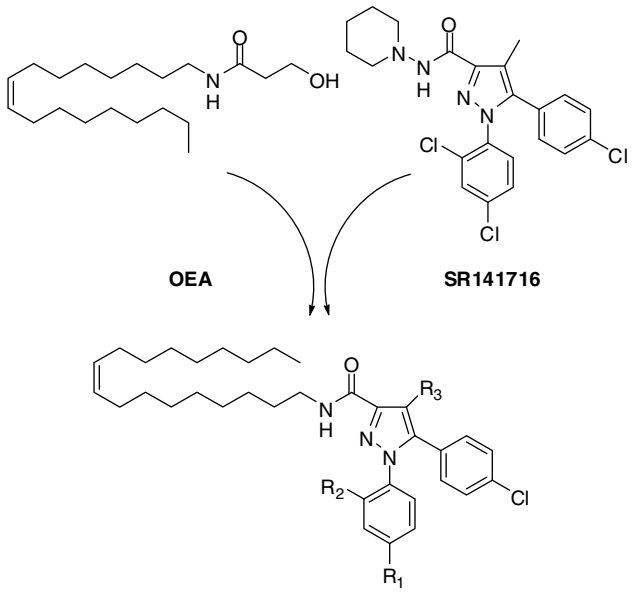


Figure 1. Structures of OEA, SR141716 and designed multiple ligands.

and diethyl oxalate in basic medium.[10] The pyrazole esters **1a** and **1b** used for the synthesis of compounds **8**–**11** have previously been reported.[11,12] The new pyrazole esters **2**–**3** were prepared from ethyl 4-(4-chlorophenyl)-2,4-dioxobutanoate and the corresponding arylhydrazine following the usual procedure.[13] In general, this reaction has been described to provide the 1*H*-pyrazole-3-carboxylate isomer.[14]

For the preparation of the pyrazole carboxamides, we followed a procedure which we had previously used for 1,2,4-triazole-3-carboxamides.[15] Thus, compounds **4–11** were obtained in high yields in a one-step synthesis by treatment of the carboxylates with an aluminum complex prepared in situ reacting trimethylaluminum with the corresponding *N*-oleyl and *N*-hexadecylamine.

The structures of the newly synthesized compounds were established according to analytical and spectroscopic data. The X-ray crystal structures of compounds **2** and **7** have been deposited in the Cambridge Crystallographic Data Centre (Deposition nos.: CCDC 664037 and 664038).

3. Biology

The compounds reported in this study were first evaluated for in vitro GST pull down to see which compounds could induce interaction between PPARα and coactivator into MCF-7 cells. The compounds were also screened in the mouse tetrad for cannabinoid activity. Finally, the compounds were evaluated in vivo on food intake and pharmacology studies in rats.

3.1. Plasmids

Full length cDNAs for human PPARα[16] were subcloned into the T_7/SV40 promoter-driven pSG5 expression vector (Stratagene, Heildelberg, Germany). The same constructs were used both for T_7 RNA polymerase-driven in vitro transcription/translation of the respective cDNAs and for viral promoter-driven overexpression of the respective proteins in mammalian cells. The nuclear receptor interaction domains of human TIF2 (spanning from a 646 to 926)[17] were subcloned into the GST-fusion protein vector pGEX (Amersham-Pharmacia, Uppsala, Sweden). For reporter gene assays in MCF-7 cells, the luciferase gene was driven by three copies of the PPAR response element (PPRE) of the human acyl-CoA oxidase gene promoter[18] fused with the thymidine kinase (tk) minimal promoter using vector pGL-2 Basic (Promega, Mannheim, Germany).

3.2. In vitro translation and bacterial overexpression of proteins

In vitro translated wild-type human PPARα were generated by coupled in vitro transcription/translation (TNT® system) using rabbit reticulocyte lysate as recommended by the supplier (Promega, Mannheim, Germany). Part of PPARα were translated in presence of [^{35}S]-methionine and taking the individual numbers of methionine residues per receptor into account, the specific concentration of the receptor proteins was adjusted to approximately 4 ng/μL after taking the individual number of methionine residues per protein into account. Bacterial overexpression of GST-TIF2 and GST alone was obtained from the *Escherichia coli* BL21(DE3)pLysS strain (Stratagene, Heildelberg, Germany). GST-TIF2 and GST-fusion protein expression were stimulated with 0.25 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 3 h at 37 °C. The fusion proteins were purified and immobilized by glutathione-Sepharose 4B beads (Amersham-Pharmacia, Uppsala, Sweden) according to manufacturer's protocol.

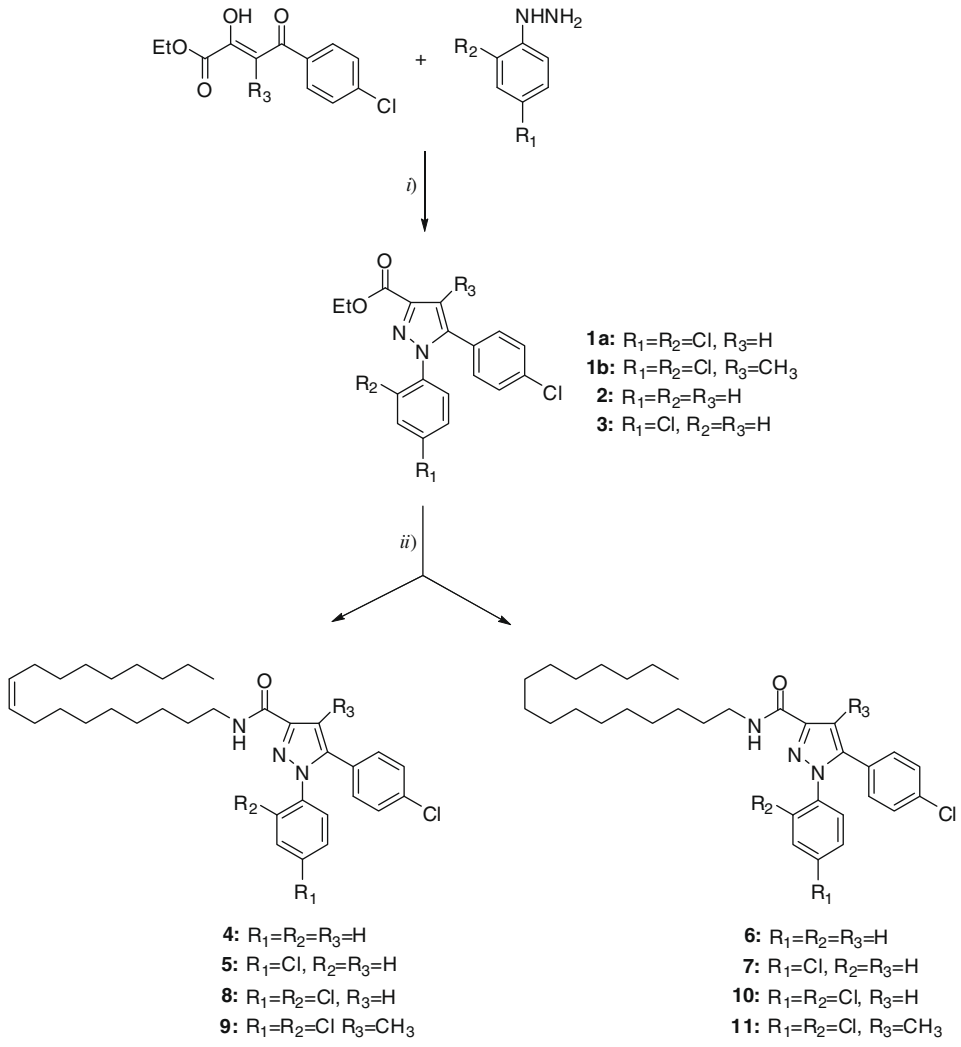
3.3. GST pull down assays

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.[19] Proteins were incubated in immunoprecipitation buffer [20 mM Hepes (pH 7.9), 200 mM KCl, 1 mM EDTA, 4 mM $MgCl_2$, 1 mM dithiothretiol (DTT), 0.1% Nonidet P-40 and 10% glycerol] for 20 min at 30 °C. 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 nuclear receptors were resolved by electrophoresis through 10% SDS–polyacrylamide gels and quantified on a Fuji FLA3000 reader (Tokyo, Japan) using Image Gauge software (Fuji Photo Film Co., Tokyo, Japan).

3.4. Cellular transfection and luciferase reporter gene assays

Human breast cancer cells MCF-7 were seeded onto 6-well plates (105 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, cells were treated for 16 h with solvent (DMSO) and different concentrations (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M) of GW7647, OEA and compounds **4**–**11** as indicated. 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).

Stimulation of normalized luciferase activity was calculated in comparison with solvent-induced cells that do not overexpress any protein. GW7647 (Tocris Bioscience) was included as positive control because selectively activates PPARα.[20] Data were used to calculate the EC_{50} [nM] for each compound.



Scheme 1. Synthesis of pyrazole fatty acid amides 4–11. Reagents and conditions: (i) AcOH, reflux; (ii) RNH_2, $Al(CH_3)_3$, CH_2Cl_2, reflux.

3.5. Feeding experiments

All experiments were performed in male Wistar rats, weighing 250–450 g from Animal Resources Centre, University of Málaga (Spain). Animals were housed in groups of two in standard plexiglas cages in a temperature and humidity controlled room (23 °C and 50% relative humidity) with a 12:12-h yellow light/dark cycle. Water and standard chow pellets (Prolab. RMH 2500) were available ad libitum. Animals were handled daily for a week before starting the experiments. All animal procedures met the National Institutes of Health guidelines for the care and use of laboratory animals, and the European Communities directive 86/609/EEC regulating animal research.

The acute effects of drugs on feeding behavior were analyzed in 24 h food-deprived Wistar rats, which had been habituated to handling.[6,21] To this end, 48 h before testing, the bedding material was removed from the cage and a small can containing food pellets was placed inside the cage for 4 h. The animals were then food-deprived for 24 h, with free access to water. The drugs were suspended with 2–3 drops of Tween 80 in saline as vehicle and administered intraperitoneally (ip) at doses of 0.3, 3 mg/kg and/or 10 mg/kg. 15 min after drug administration, the animals were returned to their home cage, where a can with a measured amount of food (usually 30–40 g) and a bottle containing 250 mL of fresh water were placed. Food pellets and food spillage were weighed at 30, 60, 120 and 240 min after starting the test, and the amount of food eaten was recorded. At the end of the test, the amount of water consumed was also measured.

3.6. Cannabinoid activity

3.6.1. Hypothermia

Core temperatures in mice were measured using a P6 thermometer and a lubricated rectal probe (CIBERTEC, Spain) inserted into the rectum to a constant depth of 3 cm. Data were recorded before drugs administration and 15 min after ip injection.

3.6.2. Locomotor activity

Although initial studies with hypothermia indicated that the compounds did not exhibit cannabinoid receptor agonist/antagonist properties, we decided to further evaluate exploratory and locomotor activities in mice treated with the drugs. Mice were moved into the behavioral testing room at least 1 h prior to testing. The open field consisted of a 40×40 cm arena divided in 25 squares by lines drawn on the floor of the apparatus. The 9 squares not bounded by the walls of the test were referred to as center squares. The following parameters were recorded during the 20 min session: distance traveled (cm), number of squares crossed, number of center squares entered, time in the center of the field and immobility time. The apparatus was cleaned between mice

with a weak acetic acid solution. Illumination of the test room was the same as the mouse colony room (100 lux). They were monitored by a video-tracking system equipped with a camera (Smart, Panlab, Barcelona) that records the animal's horizontal activity.[22] Drugs were injected ip 15 min before placing the animal in the open field. After drug injection, each mouse was placed into the central square of the arena and allowed to freely explore the field for 20 min.

3.6.3. Animals

Adult (25–30 g) male wild-type 129S1/SvImJ mice (WT) (Jackson Laboratories, USA) were used in all experiments. Experiments were conducted between 9:00 and 15:00 h.

3.7. Statistics

Statistical significance of behavioral studies was assessed by analysis of variance (ANOVA). Following a significant *F* value, post hoc analysis (Student–Newman–Keuls) was performed to assess specific comparisons between dose groups.

4. Results and discussion

As can be seen from Figure 2 and Table 1, oleyl derivatives **4**, **5**, **8** and **9** are capable of activating PPARα receptors, promoting both, its binding to DNA and transcriptional activity. While compound **4** and **8** activated transcription with a similar potency than that of either the reference agonist GW7647 or the endogenous ligand OEA, compounds **5** and **9** were 2–3-fold less potent and the corresponding hexadecyl derivatives **6**, **7, 10** and **11** were deprived of PPARα receptor agonist activity. Therefore, it seems clear that the ability to activate PPARα receptor is linked to the presence of an oleylamide rest, all the saturated derivatives being inactive.

Cannabinoid activity was first studied in the temperature assay of the mouse tetrad.[23] As shown in Figure 3A none of the compounds were able to modify the body temperature except compound **5** which slightly increased body temperature in relation to the control. The study of the antagonist effect of these compounds is shown in Figure 3B. As expected for a cannabinoid agonist WIN 55,292-2 (WIN) reduces body temperature significantly. Compounds **4**, **6** and **7** do not modify the hypothermia induced by WIN. Oleyl derivative **5** significantly inhibits hypothermia induced by the agonist although the temperature does not reach the control values. In the locomotor test only hexadecyl derivative **6** could prevent the decrease in locomotor activity induced by WIN. The lack of consistent cannabinoid agonist/antagonist effects suggests that the addition of the long acyl group to the carboxamide restrains cannabinoid activity in vivo of the diaryl pyrazole structure. However, the compounds displayed a very interesting ability to activate PPARα receptors that has not been described for diaryl pyrazole-based cannabinoid antagonists already reported in the literature.

All the synthesized compounds were tested in feeding experiments in food-deprived rats and some representative results are shown in Figure 4. Compounds **8**, **9** and **10** were weak short acting food inhibitors whereas **5** and **11** were devoid of activity. Oleyl derivative **4** is both a potent PPARα agonist and shows interesting activity as feeding suppressant. On the other hand, hexadecyl derivatives **6** and **7** show anorectic activity but do not activate PPARα receptors. This profile is extremely interesting because it is very similar to that of sulfamoyl derivatives synthesized as PPARα agonists in our laboratory.[24] These compounds also exhibited a range of activation of PPARα receptors and induction of satiety that were often dissociated. This indicates the existence of multiple targets for regulating feeding and PPARα activation that share structural determinants with those exhibited by acylethanolamides such as oleoylethanolamide and anandamide. The identification of these targets may be linked to the growing evidence for the existence of multiple receptors for these lipid transmitters that are potentially involved in feeding and metabolic regulation. The pharmacological characterization of these new anorectic compounds will be the subject of further studies.

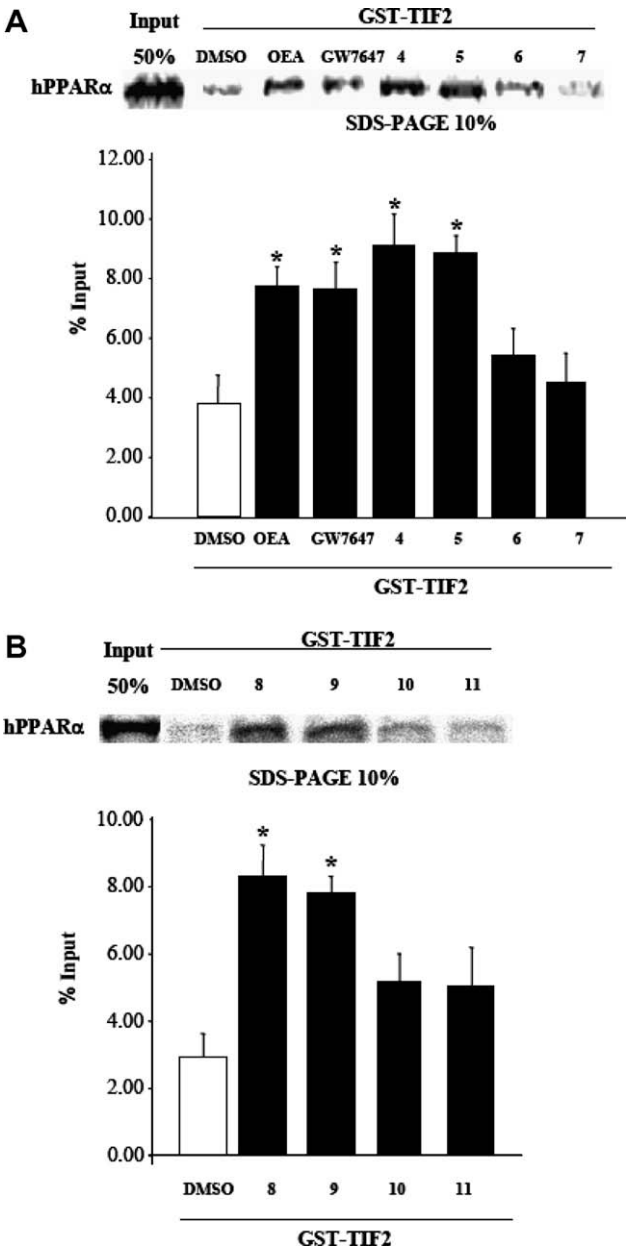


Figure 2. Individual ligand-triggered interaction profiles of hPPARα with coactivator (TIF2) in solution. GST pull down assays were performed with bacterially expressed GST-TIF2 and full-length in vitro translated (^{35}S)-labeled human PPARα, in the absence and presence of different compounds: **4**, **5**, **6**, **7**, GW7647 and OEA (A) and compounds **8**, **9**, **10** and **11** (B). Percentage of precipitated PPARα was quantified in respect to input (mean ± SEM, *n*: 3–6). (∗) $p < 0.05$ (Student's *t*-test) vs DMSO solvent.

5. Conclusions

Dual compounds incorporating cannabinoid and PPARα features in their structure have been synthesized and evaluated. None of the compounds exhibited significant cannabinoid properties. All

Table 1
Data of activation PPARα (EC_{50} [nM]) of pyrazole fatty acid amides **4**–**11** calculated from estimation of normalized luciferase activity

Compounds	PPARα activation EC_{50} [nM][a]
4	183 ± 43
5	524 ± 69
6	>10,000
7	>10,000
8	223 ± 52
9	467 ± 79
10	>10,000
11	>10,000
OEA	121 ± 26
GW7647	178 ± 31

[a] EC_{50} values calculated in the presence of the different compounds by GraphPad Prism 4. Results are mean ± SEM of eight experiments.

the chlorophenylpyrazoles bearing oleylamides were capable of activating the PPARα receptors whereas the corresponding hexadecyl derivatives were inactive. Concerning the pyrazole part, the introduction of a methyl at position 4 (as in Rimonabant) does not improve PPARα activity, being the Rimonabant analog **9** slightly less active than the 4H derivative **8**. Some compounds reduced food intake at doses of 3 mg/Kg. Oleyl derivative **4** is both a potent PPARα agonist and a feeding suppressant. Hexadecylamides **6** and **7** also reduced food intake through some other mechanism which will be the subject of further investigation.

6. Experimental

6.1. General methods

All reagents and solvents were used as commercially received with exception of CH_2Cl_2 which was distilled from P_2O_5 prior to use. TLC: precoated silica-gel *60* F_{254} plates (Merck), detection by UV light (254 nm). Flash-column Chromatography (FC): *Kieselgel 60* (230–400 mesh; *Merck*). Melting points (mp) were determined in open capillaries with a *Gallenkamp* capillary melting-points apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on Bruker Advance 300 spectrometer operating at 300.13 and 75.47 MHz, respectively, in $CDCl_3$ as solvent and Me_4Si as the internal standard. Chemical shifts are reported in ppm on the δ scale. The mass spectra (EI-MS; 70 eV) were determined on a MSD 5973 Hewlett Packard instrument. Elemental analyses were performed on a Heraeus CHN-O Rapid Analysis in our Analytical Service at Centro de Química Orgánica "Manuel Lora Tamayo" (CSIC). The X-ray crystallographic determination of **2** and **7** was performed using a Nonius Kappa CCD diffractometer with copper K_α (λ = 1.54184 Å) radiation. The structure was solved by Direct Methods using SIR92, an absorption correction was applied and the refinements were carried out with SHELXL97.

6.2. General procedure for the preparation of ethyl 1,5-diaryl-1*H*-pyrazole-3-carboxylate (2–3)

To a solution of ethyl 4-(4-chlorophenyl)-2,4-dioxobutanoate in glacial acetic acid was added an amount equimolar of the arylhydrazine. The reaction mixture was refluxed and later poured into water. The yellowish oil was separated by extraction with ether (3 × 50 mL) and the organic extract was washed with $NaHCO_3$ solution (10%) (3 × 15 mL) and water (3 × 15 mL). The extract was dried (Na_2SO_4) and the solvent was removed under reduced pressure to give predominantly 1*H*-pyrazole-3-carboxylate isomer as a viscous yellow oil. The residue was purified by flash chromatography (FC) and crystallization.

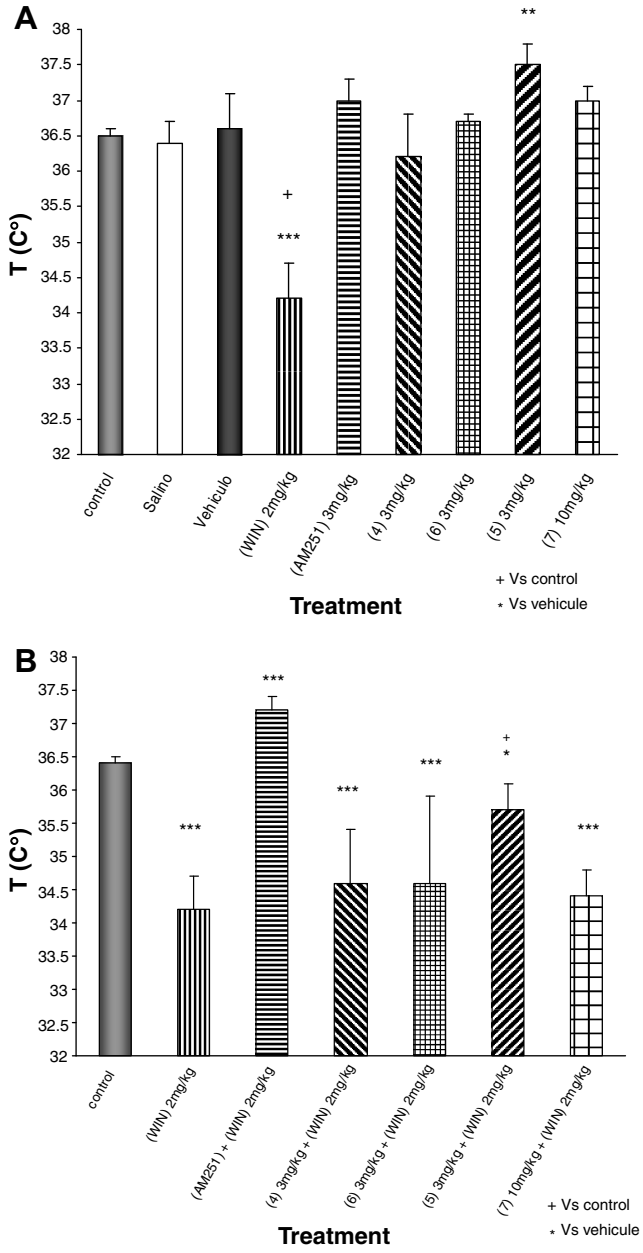


Figure 3. The bars show the averages of the values obtained in the rectal temperature (C°) of the mice (A and B). The bar "control" is the average of the values obtained of all the animals used in this study, before administering no compound to them (T^a). The rest of the bars show the average of the values of the temperature measured after the administrations of different drugs (mg/kg): (A) WIN, 2 mg/kg; AM, 3 mg/kg; **4**, 3 mg/kg; **6**, 3 mg/kg; **5**, 3 mg/kg; **7**, 10 mg/kg. (B) WIN, 2 mg/kg plus 3 mg/kg of AM and WIN, 2 mg/kg plus 3 mg/kg **4**, **6**, **5** and **7**, 10 mg/kg. Each point corresponds to the average ± EEM of the obtained values. ($^*p < 0.05$; $^{***}p < 0.001$ vs control; $+p < 0.05$ vs WIN ; T^a; *T*-Student).

6.2.1. Ethyl 5-(4-chlorophenyl)-1-phenyl-1*H*-pyrazole-3-carboxylate (2)

The title compound was prepared according to the previously described general procedure by refluxing ethyl 4-(4-chlorophenyl)-2,4-dioxobutanoate (3.0 g, 11.8 mmol) and phenylhydrazine (1.27 g, 11.8 mmol) in glacial acetic acid (10 mL) during 24 h. The product was purified by FC (SiO_2; EtOAc/*n*-hexane 1:9) and crystallization (79% yield). Yellowish solid, mp: 94–5 °C (EtOH); MS/EI: *m/z* (%) = 328 $[M+1]^+$ (34), 326 (91), 254 (100); ^1H NMR (300 MHz, $CDCl_3$): δ = 7.29–7.18 (m, 7 H), 7.07 (d, *J* = 8.5 Hz, 2H), 6.95 (s, 1H), 4.38 (q, *J* = 7.0 Hz, 2H), 1,34 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 162.2 (CO), 144.3 (C), 143.3 (C),

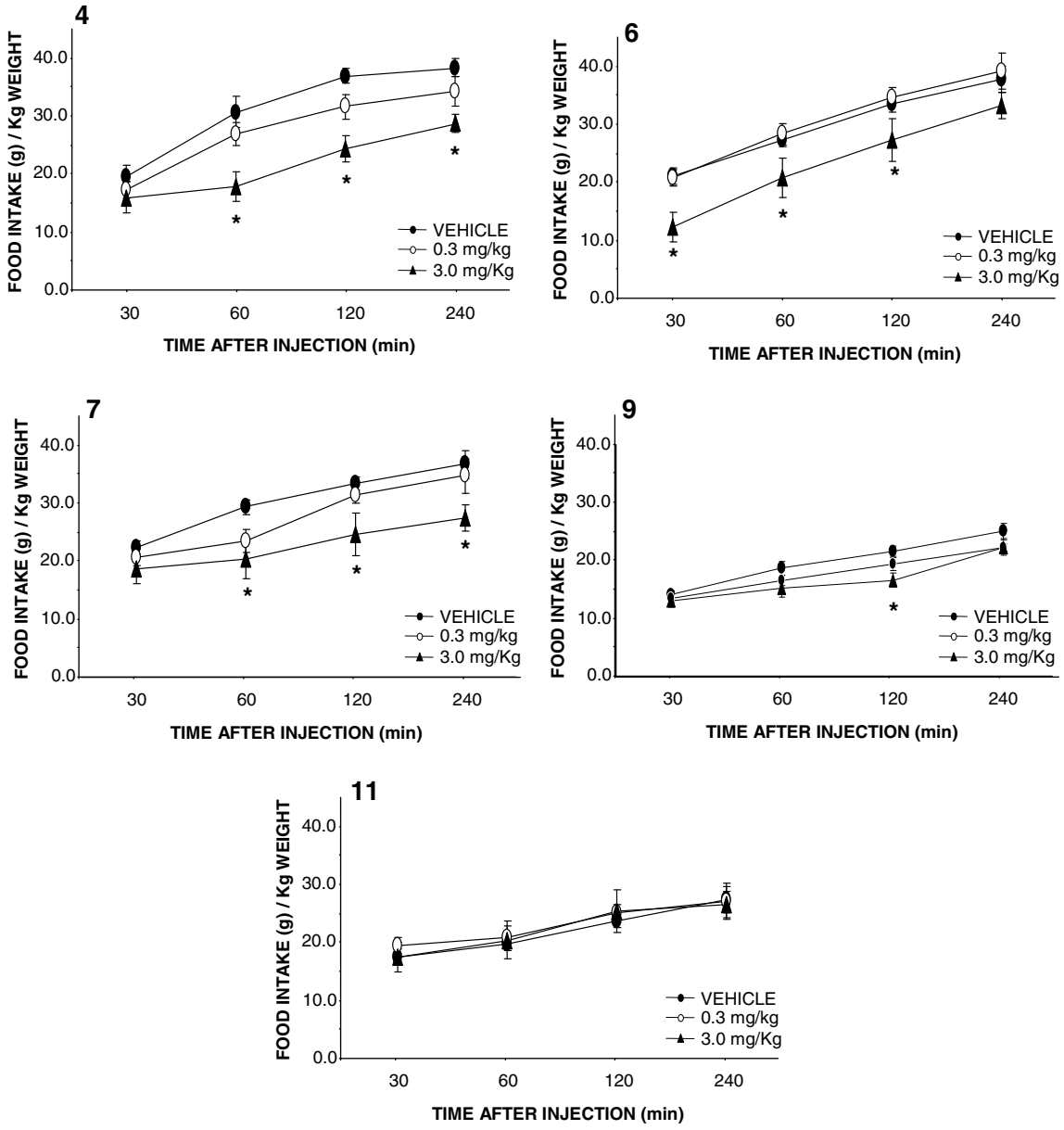


Figure 4. Intake grams per kg weight of the rats in food-deprived animals was tested 30, 60, 120 and 240 min after the ip injection of the different synthesized compounds: **4**, **6**, **7**, **9** and **11** at 0.3 and 3 mg/kg doses, ip. Results are mean ± SEM of at least eight animals per group. (*) $p < 0.05$ vs vehicle, ANOVA.

139.2 (C), 134.8 (C), 129.8 (CH), 129.0 (CH), 128.8 (CH), 128.5 (CH), 127.9 (C), 125.6 (CH), 109.9 (CH), 61.1 (CH_2), 14.3 (CH_3).

6.2.2. Ethyl 1,5-bis(4-chlorophenyl)-1*H*-pyrazole-3-carboxylate (3)

The title compound was prepared according to the previously described general procedure by refluxing ethyl 4-(4-chlorophenyl)-2,4-dioxobutanoate (3.0 g, 11.8 mmol) and 4-chlorophenylhydrazine chlorhydrate (2.1 g, 11.8 mmol) in glacial acetic acid (10 mL) during 48 h. The product was purified by FC (SiO_2; EtOAc/*n*-hexane 1:9) and crystallization (60% yield). Yellowish solid, mp: 120–1 °C (EtOH); MS/EI: *m/z* (%) = 362 $[M+1]^+$ (59), 360 (94), 288 (100); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.29–7.23 (m, 4H), 7.20 (d, *J* = 8.5 Hz, 2H,), 7.08 (d, *J* = 8.3 Hz, 2H), 6.95 (s, 1H), 4.39 (q, *J* = 7.1 Hz, 2H), 1.35 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 162.0 (CO), 144.7 (C), 143.4 (C), 137.7 (C), 135.1 (C), 134.3 (C), 129.9 (CH), 129.3 (CH), 129.0 (CH), 127.6 (C), 126.7 (CH), 110.2 (CH), 61.2 (CH_2), 14.3 (CH_3).

6.3. General procedure for the preparation of 1,5-diaryl-1*H*-pyrazole-3-carboxamide (4–11)

To the respective amine (5 equiv) dissolved in dry CH_2Cl_2 was added dropwise during 5 min a commercial solution 2 M $Al(CH_3)_3$ in heptane (5 equiv). The reaction mixture was stirred during 1 h at room temperature. Then, a solution of the suitable 1*H*-pyrazole-3-carboxylate (1 equiv) dissolved in dry CH_2Cl_2 was added and the mixture was heated at reflux. The reaction was quenched by slow and careful addition of a solution 2 N HCl (50 mL). The organic layer was then separated and washed with a solution 2 N HCl (3 × 15 mL), dried (Na_2SO_4) and evaporated to dryness. The residue was purified by flash chromatography and crystallization.

6.3.1. *N*-(1-Oleyl)-5-(4-chlorophenyl)-1-phenyl-1*H*-pyrazole-3-carboxamide (4)

The title compound was prepared according to the previously described general procedure by refluxing *N*-oleylamine (0.61 g,

2.30 mmol), a commercial solution 2 M $Al(CH_3)_3$ in heptane (1.15 mL, 2.30 mmol) and **2** (0.15 g, 0.45 mmol) dissolved in dry CH_2Cl_2 during 24 h. The product was purified by FC (SiO_2; EtOAc/*n*-hexane 1:4) (90% yield). Yellowish oil; MS/EI: *m/z* (%) = 549 $[M+1]^+$ (20), 547 (42), 281 (100); ^1H NMR (300 MHz, $CDCl_3$): δ = 7.33–7.31 (m, 3H), 7.23–7.19 (m, 4H), 7.07 (d, *J* = 8.5 Hz, 2H,), 6.96 (s, 1H), 5.32–5.25 (m, 2H), 3.38 (q, *J* = 6.8 Hz, 2H), 1.94–1.90 (m, 4H), 1.55–1.49 (m, 2H), 1.22–1.18 (m, 22H), 0.80 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.5 (CO), 147.4 (C), 143.6 (C), 139.3 (C), 134.7 (C), 129.8 (CH), 129.7 (CH), 129.1 (CH), 128.8 (CH), 128.3 (CH), 128.1 (C), 125.3 (CH), 108.1 (CH), 39.2 (CH_2), 32.5 (CH_2), 31.8 (CH_2), 29.7–29.1 (CH_2), 27.1 (CH_2), 26.9 (CH_2), 22.6 (CH_2), 14.0 (CH_3); elemental analysis calcd (%) for $C_{34}H_{46}ClN_3O$ (547.33): C 74.49, H 8.46, N 7.67, found: C: 74.76; H: 8.70; N: 7.91.

6.3.2. *N*-(1-Oleyl)-1,5-bis(4-chlorophenyl)-1*H*-pyrazole-3-carboxamide (5)

The title compound was prepared according to the previously described general procedure by refluxing *N*-oleylamine (0.92 g, 3.46 mmol), a commercial solution 2 M $Al(CH_3)_3$ in heptane (1.73 mL, 3.46 mmol) and **3** (0.25 g, 0.69 mmol) dissolved in dry CH_2Cl_2 during 20 h. The product was purified by FC (SiO_2; EtOAc/*n*-hexane 1:4) (88% yield). Yellowish oil; MS/EI: *m/z* (%) = 583 $[M+1]^+$ (13), 581 (19), 315 (100); ^1H NMR (300 MHz, $CDCl_3$): δ = 7.30–7.22 (m, 4H), 7.17 (d, *J* = 8.4 Hz, 2H), 7.08 (d, *J* = 8.4 Hz, 2H), 6.96 (s, 1H), 5.31–5.25 (m, 2H), 3.38 (q, *J* = 6.7 Hz, 2H), 1.94–1.90 (m, 4H), 1.54–1.49 (m, 2H), 1.22–1.18 (m, 22H), 0.80 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.3 (CO), 147.7 (C), 143.7 (C), 137.8 (C), 135.0 (C), 134.2 (C), 129.9 (CH), 129.7 (CH), 129.3 (CH), 129.0 (CH), 127.9 (C), 126.5 (CH), 108.4 (CH), 39.2 (CH_2), 32.6 (CH_2), 31.8 (CH_2), 29.7–29.2 (CH_2), 27.2 (CH_2), 26.9 (CH_2), 22.6 (CH_2), 14.5 (CH_3); elemental analysis calcd (%) for $C_{34}H_{45}Cl_2N_3O$ (581.29): C 70.09, H 7.78, N 7.21, found: C: 70.23; H: 7.48; N: 7.25.

6.3.3. *N*-(1-Hexadecyl)-5-(4-chlorophenyl)-1-phenyl-1*H*-pyrazole-3-carboxamide (6)

The title compound was prepared according to the previously described general procedure by refluxing of *N*-hexadecylamine (0.92 g, 3.82 mmol), a commercial solution 2 M $Al(CH_3)_3$ in heptane (1.91 mL, 3.82 mmol) and **2** (0.25 g, 0.76 mmol) dissolved in dry CH_2Cl_2 during 48 h. The product was purified by FC (SiO_2; EtOAc/*n*-hexane 1:4) and crystallization (93% yield). White solid, mp: 59–60 °C (*n*-hexane); MS/EI: *m/z* (%) = 523 $[M+1]^+$ (5), 521 (13), 281 (100); ^1H NMR (300 MHz, $CDCl_3$): δ = 7.33–7.31 (m, 3H), 7.23–7.19 (m, 4H), 7.08 (d, *J* = 8.4 Hz, 2H), 6.97 (s, 1H), 3.38 (q, *J* = 6.7 Hz, 2H), 1.56–1.51 (m, 2H), 1.30–1.17 (m, 26H), 0.82 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.5 (CO), 147.4 (C), 143.6 (C), 139.3 (C), 134.7 (C), 129.9 (CH), 129.1 (CH), 128.8 (CH), 128.3 (CH), 128.1 (C), 125.4 (CH), 108.5 (CH), 39.6 (CH_2), 32.3 (CH_2), 30.1–29.9 (CH_2), 29.7 (CH_2), 27.4 (CH_2), 23.0 (CH_2), 14.5 (CH_3); elemental analysis calcd (%) for $C_{32}H_{44}ClN_3O$ (521.32): C 76.31, H 8.49, N 8.05, found: C: 73.90; H: 8.44; N: 7.95.

6.3.4. *N*-(1-Hexadecyl)-1,5-bis(4-chlorophenyl)-1*H*-pyrazole-3-carboxamide (7)

The title compound was prepared according to the previously described general procedure by refluxing *N*-hexadecylamine (0.83 g, 3.46 mmol), a commercial solution 2 M $Al(CH_3)_3$ in heptane (1.73 mL, 3.46 mmol) and **3** (0.25 g, 0.69 mmol) dissolved in dry CH_2Cl_2 (100 mL) during 20 h. The product was purified by FC (SiO_2; EtOAc/*n*-hexane 1:4) and crystallization (90% yield). White solid, mp: 78–9 °C (*n*-hexane); MS/EI: *m/z* (%) = 557 $[M+1]^+$ (10), 555 (15), 315 (100); ^1H NMR (300 MHz, $CDCl_3$): δ = 7.30–7.22 (m, 4H), 7.17 (d, *J* = 8.7 Hz, 2H), 7.07 (d, *J* = 7.0 Hz, 2H), 6.96 (s, 1H), 3.38 (q, *J* = 7.0 Hz, 2H), 1.58–1.49 (m, 2H), 1.29–1.17 (m, 26H), 0.82 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.3 (CO), 147.7 (C), 143.7 (C), 137.8 (C), 135.0 (C), 134.2 (C), 129.9 (CH), 129.3 (CH), 129.0 (CH), 127.9 (C), 126.5 (CH), 108.9 (CH), 39.6 (CH_2), 32.3 (CH_2), 30.1–29.9 (CH_2), 29.7 (CH_2), 27.3 (CH_2), 23.0 (CH_2), 14.5 (CH_3); elemental analysis calcd (%) for $C_{32}H_{43}Cl_2N_3O$ (555.28): C 69.05, H 7.79, N 7.55, found: C: 68.89; H: 7.51; N: 7.73.

6.3.5. *N*-(1-Oleyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazole-3-carboxamide (8)

The title compound was prepared according to the previously described general procedure by refluxing *N*-oleylamine (1.01 g, 3.80 mmol), a commercial solution 2 M $Al(CH_3)_3$ in heptane (1.90 mL, 3.80 mmol) and ethyl 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazole-3-carboxylate (**1a**)[11] (0.30 g, 0.76 mmol) dissolved in dry CH_2Cl_2 during 20 h. The product was purified by FC (SiO_2; EtOAc/*n*-hexane 1:4) (78% yield). Yellowish oil; MS/EI: *m/z* (%) = 617 $[M+1]^+$ (8), 615 (8), 349 (100); ^1H NMR (300 MHz, $CDCl_3$): δ = 7.41 (d, *J* = 1.3 Hz, 1H), 7.29–7.18 (m, 4H), 7.04 (d, *J* = 8.4 Hz, 2H), 6.99 (s, 1H), 5.26–5.21 (m, 2H), 3.34 (q, *J* = 6.4 Hz, 2H), 1.94–1.92 (m, 4H), 1.52–1.50 (m, 2H), 1.21–1.19 (m, 22H), 0.80 (t, *J* = 6.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.3 (CO), 148.3 (C), 145.6 (C), 136.2 (C), 135.9 (C), 135.0 (C), 133.0 (C), 130.5 (CH), 130.4 (CH), 129.9 (CH), 129.7 (CH), 129.1 (CH), 129.0 (CH), 128.1 (CH), 127.5 (C), 107.0 (CH), 39.2 (CH_2), 32.5 (CH_2), 31.7 (CH_2), 29.7-29.2 (CH_2), 27.1 (CH_2), 26.9 (CH_2), 22.6 (CH_2), 14.0 (CH_3); Elemental analysis calcd (%) for $C_{34}H_{44}Cl_3N_3O$ (615.25): C 66.18, H 7.19, N 6.81, found: C: 66.28; H: 7.15; N: 7.02.

6.3.6. *N*-(1-Oleyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (9)

The title compound was prepared according to the previously described general procedure by refluxing *N*-oleylamine (0.81 g, 3.06 mmol), a commercial solution 2 M $Al(CH_3)_3$ in heptane (1.53 mL, 3.06 mmol) and ethyl 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylate (**1b**)[12] (0.25 g, 0.61 mmol) dissolved in dry CH_2Cl_2 during 30 h. The product was purified by FC (SiO_2; EtOAc/*n*-hexane 1:4) (47% yield). Yellowish oil; MS/EI: *m/z* (%) = 631 $[M+1]^+$ (8), 629 (8), 363 (100); ^1H NMR (300 MHz, $CDCl_3$): δ = 7.35 (d, *J* = 1.3 Hz, 1H), 7.23–7.20 (m, 4H), 6.99 (d, *J* = 8.5 Hz, 2H), 5.29–5.21 (m, 2H), 3.34 (q, *J* = 6.7 Hz, 2H), 2.30 (s, 3H), 1.96–1.92 (m, 4H), 1.54–1.47 (m, 2H), 1.21–1.17 (m, 22H), 0.80 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 162.5 (CO), 145.1 (C), 142.9 (C), 135.9 (C), 135.8 (C), 134.8 (C), 132.9 (C), 130.7 (CH), 130.5 (CH), 130.2 (CH), 129.9 (CH), 129.7 (CH), 128.8 (CH), 127.8 (CH), 127.2 (C), 117.6 (C), 39.0 (CH_2), 32.5 (CH_2), 31.8 (CH_2), 29.7–29.2 (CH_2), 27.3 (CH_2), 26.9 (CH_2), 22.6 (CH_2),14.0 (CH_3), 9.3 (CH_3); Elemental analysis calcd (%) for $C_{35}H_{46}Cl_3N_3O$ (629.27): C 66.61, H 7.35, N 6.66, found: C: 66.34; H: 7.08; N: 6.86.

6.3.7. *N*-(1-Hexadecyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazole-3-carboxamide (10)

The title compound was prepared according to the previously described general procedure by refluxing *N*-hexadecylamine (0.91 g, 3.80 mmol), a commercial solution 2 M $Al(CH_3)_3$ in heptane (1.91 mL, 3.80 mmol) and ethyl 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazole-3-carboxylate (**1a**)[11] (0.30 g, 0.76 mmol) dissolved in dry CH_2Cl_2 during 24 h. The product was purified by FC (SiO_2; EtOAc/*n*-hexane 1:4) (87% yield). White solid; mp: 61–2 °C (*n*-hexane); MS/EI: *m/z* (%) = 591 $[M+1]^+$ (8), 589 (8), 240 (100); ^1H NMR (300 MHz, $CDCl_3$): δ = 7.41 (d, *J* = 1.3 Hz, 1H), 7.29–7.17 (m, 4H), 7.05–7.00 (d, *J* = 8.6 Hz, 2H), 6.99 (s, 1H), 3.34 (q, *J* = 6.8 Hz, 2H), 1.57–1.47 (m, 2H), 1.25–1.17 (m, 26H), 0.89 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.2 (CO), 148.2 (C), 145.6 (C), 136.2 (C), 135.9 (C), 135.0 (C), 133.0 (C), 130.5

(CH), 130.4 (CH), 129.3 (CH), 129.1 (CH), 128.1 (CH), 127.5 (C), 107.0 (CH), 39.2 (CH_2), 31.8 (CH_2), 29.6–29.2 (CH_2), 26.9 (CH_2), 22.6 (CH_2), 14.0 (CH_3); Elemental analysis calcd (%) for $C_{32}H_{42}Cl_3N_3O$ (589.24): C 65.03, H 7.16, N 7.11, found: C: 65.18; H: 7.35; N: 7.40.

6.3.8. *N*-(1-Hexadecyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (11)

The title compound was prepared according to the previously described general procedure by refluxing *N*-hexadecylamine (0.88 g, 3.67 mmol), a commercial solution 2 M $Al(CH_3)_3$ in heptane (1.83 mL, 3.67 mmol) and ethyl 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylate (**1b**)[12] (0.30 g, 0.73 mmol) dissolved in dry CH_2Cl_2 during 96 h. The product was purified by FC (SiO_2; EtOAc/*n*-hexane 1:4) (79% yield). White solid; mp: 64–5 °C (*n*-hexane); MS/EI: *m/z* (%) = 605 $[M+1]^+$ (8), 603 (8), 240 (100); ^1H NMR (300 MHz, $CDCl_3$): δ = 7.43 (d, *J* = 1.9 Hz, 1H), 7.31–7.26 (m, 4H), 7.06 (d, *J* = 8.4 Hz, 2H), 3.39 (q, *J* = 6.7 Hz, 2H), 2.37 (s, 3H), 1.58–1.54 (m, 2H), 1.34–1.25 (m, 26H), 0.89 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.6 (CO), 144.1 (C), 141.9 (C), 134.9 (C), 134.8 (C), 133.8 (C), 132.0 (C), 129.8 (CH), 129.5 (CH), 129.3 (CH), 127.8 (CH), 126.8 (CH), 126.2 (C), 116.6 (C), 38.0 (CH_2), 30.9 (CH_2), 28.7–28.3 (CH_2), 26.0 (CH_2), 21.6 (CH_2), 13.1 (CH_3), 8.4 (CH_3); Elemental analysis calcd (%) for $C_{33}H_{44}Cl_3N_3O$ (603.25): C 65.50, H 7.33, N 6.94, found: C: 65.24; H: 7.46; N: 6.93.

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