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Novel antiobesity agents: Synthesis and pharmacological evaluation of analogues of Rimonabant and of LH21

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ABSTRACT

Searching for novel antiobesity agents, a series of cannabinoid LH21 and of Rimonabant-fatty acid amide analogues have been prepared. Synthesis of pyrazoles **2a**–**2c** was achieved by a two steps simple methodology via α,β-unsaturated ketones. Carboxamides **8a**–**8h** were obtained in good yields from esters **7a**–**7c** by a one-pot procedure which takes place under mild conditions. New compounds have been evaluated in vivo as anorectic agents. Some of them showed interesting properties reducing food intake in rats by a mechanism which does not involve the endocannabinoid system.

1. Introduction

Obesity has significantly increased in the last decades affecting an important part of the adult[1] and children[2] population of western countries. It is a complex disease involving many physiological systems and different factors, and it is linked to serious health problems. Although effective in producing weight loss, current therapies against obesity may have some tolerance and/or safety concerns.[3,4] Moreover, since obesity is a complex disease often associated with high cardiovascular risk, type II diabetes and dyslipidemia, the control of appetite cannot be considered the only target for medicines designed to fight obesity.

Till now, only one monotherapy for a long-term treatment was available, Orlistat (Xenical™) a gastrointestinal lipase inhibitor, and two more drugs were approved for short-term use, Diethylpropion (Tenuate™) and Phentermine (Adipex-P™), both amphetamine-like drugs (Fig. 1). Very recently, in June 2012, FDA just approved Lorcaserin (Lorquess™, Arena Pharmaceuticals) for the treatment of obese adults or over-weighted ones with high blood pressure, type II diabetes or high cholesterol levels (Fig. 1).[5] It is a serotonin (5-HT2c) agonist not free of side effects such as headache, dizziness and nausea. Moreover, in July 2012, a combination therapy of Phentermine and the anticonvulsant Topiramate (Qsymia™, Vivus) have also been commercialized (Fig. 1).[6] Topiramate has several mechanisms of action and little is known about where and how it works. It has considerable side effects, which are mainly related to brain function, and has possible teratogenic effects. Therefore, although new medicines are available, most of them have been associated to severe side effects, highlighting the need for alternative therapies.[7]

Sibutramine (Meridia™) a dual serotonin-norepinephrine reuptake inhibitor, and Rimonabant (Acomplia™, Fig. 2), the first cannabinoid CB_1 receptor inverse agonist/antagonist approved for clinical use in humans, have been withdrawn in 2010 and 2009 respectively, due to severe side effects. Other drugs, also based

Abbreviations: LH21, 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-3-hexyl-1*H*-1,2,4-triazole; Rimonabant, 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide; CB_1, cannabinoid receptor 1; CB_2, cannabinoid receptor 2; PPARα, peroxisome proliferator activated receptor α; LHMDS, lithium *bis*(trimethylsilyl)amide; HBF_4, tetrafluoroboric acid; SAR, structure–activity relationships.

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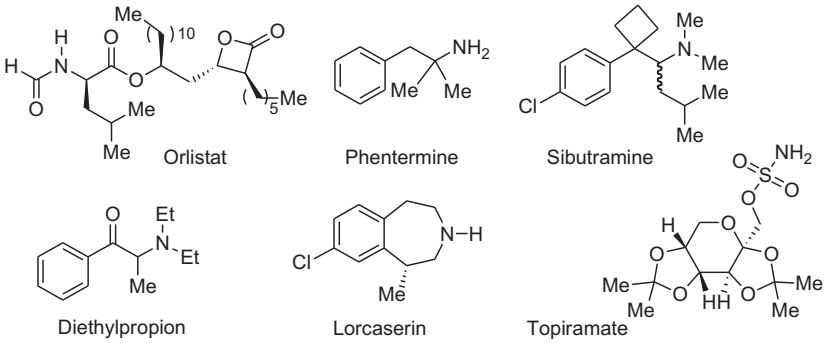


Figure 1. Structures of commercial anti-obesity drugs.

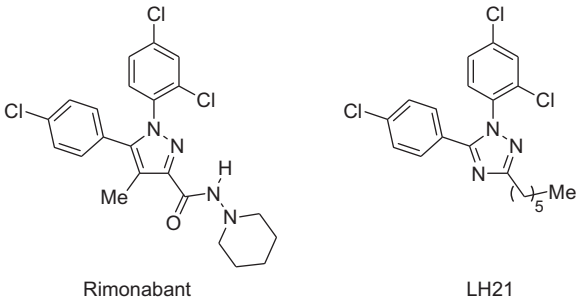


Figure 2. Structures of LH21 and Rimonabant.

on the selective blockade of the CB_1 receptor, which have demonstrated positive weight loss potential, such as Taranabant,[8,9] have been abandoned too during late phase clinical trials due to unacceptable adverse events. However, given that the development of novel antiobesity drugs is a priority and a challenge for medicinal chemists, novel efforts are being done to modify the chemical structure of Rimonabant and related-CB_1 receptor antagonists,[10–13] in order to improve the pharmacodynamic and/or pharmacokinetic properties of these compounds, that is, development of neutral or peripheral antagonists, thus reducing their adverse effects.

In our early research program, we reported LH21[14] and fatty acid amides related to Rimonabant[15] as antiobesity drugs. On the one hand, LH21 (Fig. 2) is a peripheral cannabinoid antagonist whose acute administration to food-deprived rats resulted in a dose-dependent inhibition of feeding and an enhancement of the anorectic actions of oleoylethanolamide, a feeding suppressant lipid that acts on peripheral sensory terminals.[16] Subchronic administration of LH21 reduced food intake and body weight gain in obese Zucker rats, but did not improve hypertriglyceridaemia or hypercholesterolaemia, nor did it reduce liver fat deposits.[17] Moreover, it causes food intake reduction also in diet-induced obese rats without resulting in hepatic, cardiac and renal toxicity.[18] On the other hand, our fatty acid amides related to Rimonabant were designed as hypophagic agents trying to target not only appetite, but also lipid and carbohydrate metabolism.[15,19] As far as we know, only another series of long-chain amide analogues of Rimonabant were reported, but they bore only saturated aliphatic chains.[20]

On the basis of these previous findings, and in order to establish structure–activity relationships (SAR), we now wish to report new analogues of both leads, replacement of the triazole of LH21 by a pyrazole (Fig 3, I), and preparation of new Rimonabant-alkylamide derivatives with different substituents in the aromatic rings (Fig. 3, II).

2. Results and discussion

2.1. Chemistry

Two series of compounds have been prepared: alkyl-diarylpyrazoles **2a**–**2c** and diaryl-pyrazole/triazolecarboxamides **8a**–**8h**.

Synthesis of alkyl-diarylpyrazoles is outlined in Scheme 1. It was performed by a simple two steps procedure. Benzaldehyde and an alkylketone reacted in the presence of piperidine to afford α,β-unsaturated ketones **1a**–**1c**. Then, condensation with the corresponding arylhydrazine also in basic medium led to pyrazoles **2a**–**2c**.

Preparation of diaryl-pyrazole/triazolecarboxamides was carried out as described in Scheme 2. Alkyl-pyrazolecarboxylates **7a** and **7b**, and alkyl-triazolecarboxylate **7c** were synthesized by different routes.

Synthesis of pyrazolecarboxylates started from the 2,4-dicarbonyl esters obtained through coupling of the corresponding ketone and diethyl oxalate by two basic conditions: **3a** was prepared in presence of sodium ethoxide (A method)[21] and **3b** was prepared in presence of lithium *bis*(trimethylsilyl)amide (LHMDS) (B method).[22] Ethyl 4-phenyl-2,4-dioxobutanoates **3a** and **3b** were then reacted with phenylhydrazine in acidic medium (glacial acetic acid for **7a**[23] and sulfuric acid for **7b**[22]). In both cases, not only 1,5-diphenyl-3-carboxylate was obtained, but also the low yield isomer 1,3-diphenyl-5-carboxylate, which were easily separated by chromatographic methods.[24] Synthesis of triazole carboxylate **7c** was achieved as follows. Diazotation of 4-chloroaniline

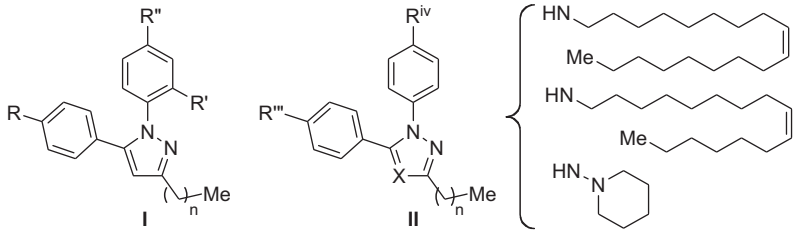
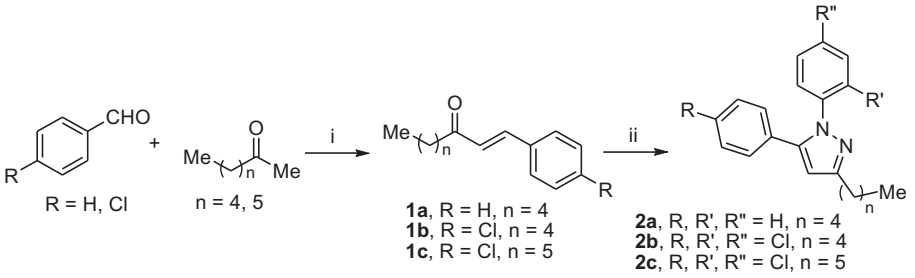
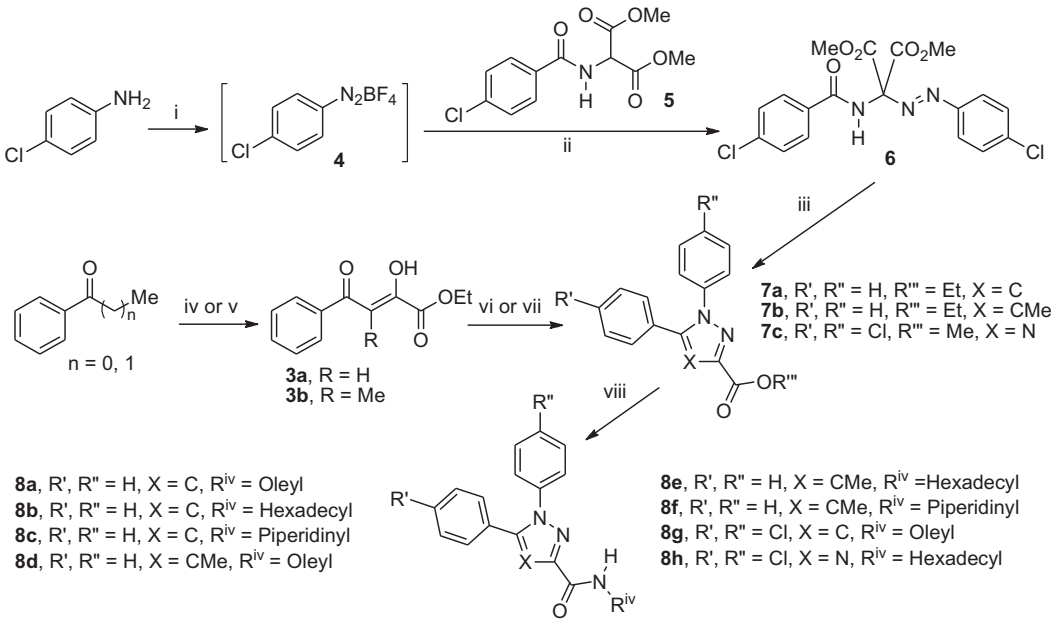


Figure 3. General structures of the synthesized compounds.



Scheme 1. Synthesis of pyrazoles **2a**–**2c**. Reagents and conditions: (i) piperidine, EtOH, δ; (ii) corresponding phenylhidrazine, piperidine, EtOH, Δ.



Scheme 2. Synthesis of pyrazoles **8a**–**8f** and of triazoles **8g** and **8h**. Reagents and conditions: (i) $NaNO_2$ aq, HBF_4, 0 °C; (ii) NaOAc, MeOH, rt; (iii) NaOMe, MeOH, rt (for **7c**); (iv) $(COOEt)_2$, NaOEt, EtOH, rt (for **3a**); (v) $(COOEt)_2$, LHMDS, cyclohexane, rt (for **3b**); (vi) $PhNHNH_2$, AcOH, Δ (for **7a**); (vii) $PhNHNH_2$, 50% aq H_2SO_4, EtOH, Δ for (**7b**); (viii) $R^{iv}NH_2$, $Al(Me)_3$, DCM, Δ.

in presence of tetrafluoroboric acid (HBF_4) gave the diazonium salt **4**,[25] which was coupled to **5** to afford **6**. Compound **5** was synthesized as previously reported in the literature.[26] Diazo intermediate **6** was then cyclized in basic conditions (sodium methoxide) to afford triazole **7c**.

From esters **7a**–**7c**, carboxamides were obtained by a one-pot procedure which we have previously used.[27] It involved the treatment of one equivalent of the ester with 5 equiv of an aluminium complex which was previously prepared in situ by reacting trimethylaluminium with the corresponding amine or hydrazine. Thus, carboxamides **8a**–**8h** were prepared in moderate to good yields under mild conditions.

2.2. Pharmacology

2.2.1. Food intake studies

The synthesized compounds **2a**–**2c** and **8a**–**8h** were structurally very related to our previously reported food intake inhibitors LH21[18] and pyrazole fatty acid amides,[15] respectively. Thus, they were expected to affect the feeding behavior of rodents. Therefore, they were evaluated by feeding experiments performed in food-deprived rats. Some representative results are shown in Figure 4.

Regarding the alkyl-diarylpyrazoles, halogenated derivative **2b** showed some activity as a short-term appetite suppressant at a 3 mg/kg dose. In the series of diaryl-pyrazole/triazolecarboxamides, oleyl derivatives **8a** and **8d** were devoid of activity, whereas triazole **8g** was not active and triazole **8h** was a weak food intake inhibitor. This fact suggests that the triazolecarboxamide scaffold and the oleyl chain were not pharmacophoric requirements for feeding inhibition. Interestingly, diphenylpyrazoles **8b**, **8c** and **8e** exhibited anorectic properties at a 3 mg/kg dose. In the case of **8b** and **8e**, bearing an hexadecyl chain, they showed a significant effect in the food intake experiments at 30, 60, 120 and 240 min after their administration. With regard to **8c**, with a piperidinyl chain, it inhibited food intake at 60, 120 and 240 min after its administration, but not at 30 min. These effects might derive either of the pharmacokinetics of the compound, or to the appearance of an active metabolite.

Surprisingly, dehalogenation of Rimonabant yielded a compound (**8f**) that enhances feeding. Despite a certain capacity of the compound to bind to the CB_1 receptor that cannot be discarded with the data currently generated, dehalogenation might clearly modify the pharmacodynamic properties of the Rimomabant scaffold, thus making possible the interaction with alternative unknown targets.

2.2.2. CB_1/CB_2 binding studies

Among others, one well-accepted mechanism of feeding suppression is the blocking of cannabinoid CB_1 receptors.[28] Thus, cannabinoid binding assays of compounds here reported have been

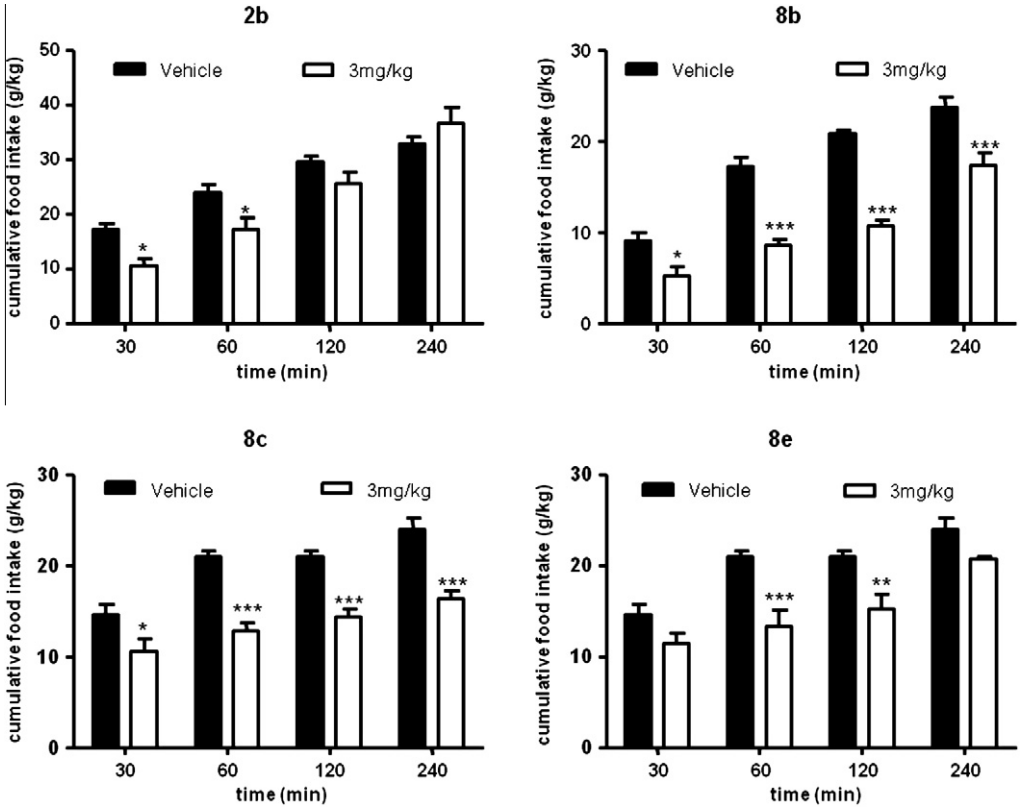


Figure 4. Intake grams per kg of the rats in food-deprived animals was tested 30, 60, 120 and 240 min after the ip injection of **2b** and **8b**–**8e** at a 3 mg/kg dose. Results are mean ± SEM of eight animals per group; $^{*}p$ <0.05, $^{**}p$ <0.01 and $^{***}p$ <0.001 versus vehicle (ANOVA analysis).

achieved to check if their mechanism of feeding reduction is related to the endocannabinoid system.

Radioligand assays have been used to evaluate the affinity of the new compounds to cannabinoid receptors expressed in membrane fractions of human CB_1 or CB_2 transfected cells. They were first subjected to a preliminary screen at a concentration of 40 μM (except for hexadecyl carboxamides **8b**, **8e** and **8h**, which was performed at 10 μM due to solubility reasons). A complete dose–response curve was generated for compounds that displaced the radioligand by >60% in the preliminary screen in at least one of the two receptors analyzed. Therefore, Table 1 lists the experimental binding affinities (K_i values) from the respective displacement curves.

Table 1
Binding affinity of the compounds for the CB_1 and CB_2 cannabinoid receptors

Compound	K_i CB_1 (nM)	K_i CB_2 (nM)
Rimonabant	7.3 ± 0.68	n.d.
WIN55212-2	45.59 ± 8.58	3.73 ± 0.20
LH21	829.4 ± 92.4	3082 ± 824
2a	6157 ± 139	2943 ± 872
2b	>40,000	458.2 ± 25.62
2c	>40,000	>40,000
8a	>40,000	>40,000
8b	>40,000	>10,000
8c	4585 ± 2537	27870 ± 5658
8d	>40,000	>40,000
8e	>10,000	>10,000
8f	9690 ± 6002	18220 ± 3245
8g	>40,000	>40,000
8h	>10,000	>10,000

n.d. = not determined. K_i values were obtained from competition curves using [^{3}H]-CP55940 as radioligand for CB_1 and CB_2 cannabinoid receptors and are expressed as the mean ± SEM of at least three experiments.

The tested compounds showed less affinity for CB_1 and CB_2 receptors than reference Rimonabant and WIN55212-2. In the series of alkyl-diarylpyrazoles, **2b** was the most interesting compound showing a significant CB_2 affinity in the submicromolar range and high CB_2 selectivity. This is the compound with the best affinity for the CB_2 receptor of all the series tested, although significantly lower (K_i >100-fold) than classic CB_2 receptor ligands (i.e., WIN55212-2), and with a significant activity in the in vivo experiments as short-term feeding inhibitor. Despite its accepted key role as modulators of the immune system, there are growing evidences implicating CB_2 receptor in energy expenditure, appetite and glucose homeostasis. Thus, deletion of these receptors produces age-dependent obesity,[29] whereas overexpression of them in the brain results in diabetic and lean animals.[30] Moreover, the administration of different types of cannabinoid CB_2 antagonists has been found to produce alterations (either inhibition or enhancement) of feeding behavior that depends on the species studied, the dose used and the nutritional status of the animal.[31–33] Therefore, it would be possible that the appetite suppressant effect of **2b** may be related in part to its ability to bind CB_2 receptors.

Regarding the diaryl-pyrazole/triazolecarboxamides, only pyrazoles **8c** and **8f** showed weak CB_1 and CB_2 binding. Therefore, feeding modulation of **8b** and **8e** should not take place via cannabinoid receptors as they did not show any relevant binding capacity to CB_1 or CB_2 receptors. This fact is in agreement with our previous results[15] in which Rimonabant-hexadecylamide derivatives showed anorectic activity without cannabinoid binding. Hence, feeding suppression of **8b**, **8c** and **8e** takes place through a mechanism in which the the endocannabinoid system should not be involved. Thus, other possible mechanisms of feeding inhibition, such as blocking pancreas lipases, increasing the brain levels of serotonin, norepinephrine or dopamine, or activation of PPARα receptors should be the subject of further research.

3. Conclusions

Two new series of potential antiobesity compounds have been synthesized, one corresponding to the pyrazole analogues of triazole LH21, and the other to novel carboxamides incorporating diaryl-pyrazole/triazole moieties. The first one (compounds **2a**–**2c**) showed certain affinity for CB_1 and CB_2 receptors, the most important being **2b** which had CB_2 affinity in the submicromolar range and high CB_2 selectivity. In general this series was devoid of anorectic activity except for **2b** which moderately reduced food intake at a 3 mg/kg dose. Thus, this preliminary SAR indicates that replacement of triazole by pyrazole, and shortening of one carbon unit of the alkyl chain of LH21 displaces selectivity towards CB_2 receptor, and reduces the anorectic activity, in line with the fact that cannabinoid CB_2 antagonists have shown to increase orexigenic properties in food-deprived rodents.[31–33]

In the carboxamide series, hexadecyl pyrazole derivatives without chloro substituents reduced food intake significantly (**8b** and **8e**). No cannabinoid activity was found for pyrazole nor for triazole derivatives, so the mechanism of feeding inhibition of hexadecyl pyrazolecarboxamides will be the subject of further investigation. Moreover, dehalogenation of Rimonabant (**8f**) enhanced feeding, an unexpected finding that needs further research.

4. Experimental protocols

4.1. Chemistry

4.1.1. General methods

All reagents and solvents were used as commercially received with exception of DCM which was distilled from P_2O_5 prior to use. LHMDS was provided by Aldrich as a 1.0 M solution in hexane. Tetrafluoroboric acid (HBF_4) was commercially available as a 35% aqueous solution. TLC: precoated silica-gel 60 F254 plates (Merck), detection by UV light (254 nm). Flash-column Chromatography: Kieselgel 60 (230–400 mesh; Merck). Melting points were determined in open capillaries with a Gallenkamp capillary melting points apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a 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. Infra-red spectra were recorded on a FTIR Spectrum One Perkin Elmer spectrometer in the region of 4000–450 cm^{-1}. The mass spectra (EI-MS; 70 eV) were determined on a MSD 5973 Hewlett Packard instrument; when chlorine atoms were present, the *m*/*z* of the peaks corresponds to the ^{35}Cl isotope. Elemental analysis was performed on a Heraeus CHN–O rapid analyzer. Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values.

4.1.2. General procedure for the synthesis of 1a–1c

To a solution of ketone (1 equiv) in EtOH (50 mL), piperidine in excess (2 mL) was added. The mixture was stirred at rt for 2 h and aldehyde (2 equiv) was added. The reaction mixture was stirred at reflux for 72 h. Afterwards, solvent was removed in vacuo and the residue was purified by flash chromatography (eluent *n*-hexane/EtOAc 95:5).

4.1.2.1. (*E*)-1-Phenyloct-1-en-3-one (1a). (*E*)-1-Phenyloct-1-en-3-one (**1a**) was prepared from heptan-2-one (4.86 mL, 35.0 mmol), and benzaldehyde (1.77 mL, 17.5 mmol). Yield, 2.60 g (73%), as a yellow gummy solid. R_f: (*n*-hexane/EtOAc 9:1) 0.40. Mp = 36–38 °C. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.60–7.54 (m, 3H, H-2 and COC*H*=CH), 7.43–7.38 (m, 3H, H-3 and H-4), 6.73 (d, 1H, *J* = 16.2 Hz, COCH=C*H*), 2.66 (t, 2H, *J* = 7.4 Hz, C$H_2$$CH_2CH_2CH_2CH_3$), 1.74–1.64 (m, 2H, $CH_2CH_2$$CH_2CH_2CH_3$), 1.37–1.32 (m, 4H, $CH_2CH_2CH_2$$CH_2CH_3$), 0.93–0.87 (m, 3H, CH_3). MS/EI: *m*/*z* (%) 146 (47), 131 (100), 103 (38) and 77 (16).

4.1.2.2. (*E*)-1-(4-Chlorophenyl)oct-1-en-3-one (1b). (*E*)-1-(4-Chlorophenyl)oct-1-en-3-one (**1b**) was prepared from heptan-2-one (3.95 mL g, 28.4 mmol) and 4-chlorobenzaldehyde (2.00 g, 14.2 mmol). Yield, 3.36 g (quant), as a yellow oil. R_f: (*n*-hexane/EtOAc 9:1) 0.40. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.40 (d, 1H, *J* = 16.6 Hz, COCH=C*H*), 7.39 (d, 2H, *J* = 8.5 Hz, H-2), 7.28 (d, 2H, *J* = 8.5 Hz, H-3), 6.63 (d, 1H, *J* = 16.6 Hz, COC*H*=CH), 2.59 (t, 2H, *J* = 7.4 Hz, C$H_2CH_2CH_2CH_2CH_3$), 1.65–1.55 (m, 2H, $CH_2CH_2$$CH_2CH_2$ CH_3), 1.28–1.22 (m, 4H, $CH_2CH_2CH_2$$CH_2CH_3$), 0.83 (br t, 3H, *J* = 6.8 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.3 CO, 140.7 COCH=*C*H, 136.2 C-4, 133.1 C-1, 129.3 C-2, 129.2 C-3, 126.6 CO*C*H=CH, 41.1 *C*$H_2CH_2CH_2CH_2CH_3$, 31.4 CH_2CH_2*C*$H_2CH_2CH_3$, 23.9 CH_2*C*$H_2CH_2CH_2CH_3$, 22.4 $CH_2CH_2CH_2$*C*H_2CH_3, 13.8 CH_3. MS (EI): *m*/*z* (%) 180 (31), 165 (100), 145 (53), 137 (25) and 102 (19).

4.1.2.3. (*E*)-1-(4-Chlorophenyl)non-1-en-3-one (1c). (*E*)-1-(4-Chlorophenyl)non-1-en-3-one (**1c**) was prepared from octan-2-one (4.44 mL, 28.4 mmol) and 4-chlorobenzaldehyde (2.00 g, 14.2 mmol). Yield, 3.00 g (84%), as white crystals. R_f: (*n*-hexane/EtOAc 9:1) 0.40. Mp = 57–58 °C. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.43 (d, 1H, *J* = 16.3 Hz, COCH=C*H*), 7.42 (d, 2H, *J* = 8.3 Hz, H-2), 7.29 (d, 2H, *J* = 8.3 Hz, H-3), 6.63 (d, 1H, *J* = 16.3 Hz, COC*H*=CH), 2.58 (t, 2H, *J* = 7.4 Hz, C$H_2CH_2CH_2CH_2CH_2CH_3$), 1.62–1.53 (m, 2H, $CH_2CH_2CH_2CH_2CH_2CH_3$), 1.25–1.18 (m, 6H, $CH_2CH_2CH_2CH_2CH_2$ CH_3), 0.82–0.80 (m, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.4 (CO), 140.7 (COCH=*C*H), 136.2 (C-4), 133.1 (C-1), 129.3 and 129.2 (C-2) and (C-3), 126.6 (CO*C*H=CH), 41.1 (*C*$H_2CH_2CH_2CH_2CH_2CH_3$), 31.6 ($CH_2CH_2CH_2CH_2CH_2CH_3$), 29.0 ($CH_2CH_2CH_2CH_2CH_2CH_3$), 24.2 ($CH_2CH_2CH_2CH_2CH_2CH_3$), 22.5 ($CH_2CH_2CH_2CH_2CH_2CH_3$), 14.0 CH_3. MS (EI): *m*/*z* (%) 208 (100), 165 (46) and 145 (25).

4.1.3. General procedure for the synthesis of 2a–2c

To a solution of α,β-insaturated ketone (1 equiv) in EtOH, piperidine (1 mL) and hydrazine were added dropwise. The mixture was stirred at rt for 15 min, and then refluxed for 24 h. EtOH was removed in vacuo, and the residue was purified by flash chromatography (eluent *n*-hexane/EtOAc).

4.1.3.1. 3-Pentyl-1,5-diphenyl-1*H*-pyrazole (2a). 3-Pentyl-1,5-diphenyl-1H-pyrazole (**2a**) was prepared from **1a** (1.00 g, 4.9 mmol) and phenylhydrazine (480 μL, 4.9 mmol); chromatography *n*-hexane/EtOAc 98:2 Yield, 500 mg (35%), as a red oil. R_f: (*n*-hexane/EtOAc 95:5) 0.35. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.79 (d, 2H, *J* = 8.5 Hz, H-2″), 7.42–7.16 (m, 8H, Ph), 6.46 (s, 1H, H-4), 2.58 (t, 2H, *J* = 7.7 Hz, C$H_2CH_2CH_2CH_2CH_3$), 1.62–1.50 (m, 2H, $CH_2CH_2CH_2CH_2CH_3$), 1.25–1.18 (m, 4H, $CH_2CH_2CH_2CH_2CH_3$), 0.78 (t, 3H, *J* = 6.9 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.4 (C-3), 145.3 (C-5), 140.0 (C-1′), 133.4 (C-1″), 129.1 (C-3″), 128.5 (C-3′), 127.8 (C-4″), 127.7 (C-4′), 125.7 and 125.5 (C-2′ and C-2″), 102.7 (C-4, 31.3 $CH_2CH_2CH_2CH_2CH_3$), 28.5 (*C*$H_2CH_2CH_2CH_2CH_3$), 26.3 ($CH_2CH_2CH_2CH_2CH_3$), 22.3 ($CH_2CH_2CH_2CH_2CH_3$), 13.9 CH_3. IR (film) ν/cm^{-1}: 3061, 2965, 1930, 2859, 1709, 1598, 1501, 765 and 695. MS (EI): *m*/*z* (%) 290 (59), 247 (24), 234 (100), 130 (12) and 77 (11); elemental analysis $C_{20}H_{22}N_2$, Calcd: C, 82.72; H, 7.64; N, 9.65. Found: C, 83.01; H, 7.55; N, 9.73.

4.1.3.2. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-3-pentyl-1*H*-pyrazole (2b). 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-3-pentyl-1H-pyrazole (**2b**) was prepared from **1b** (2.50 g, 10.6 mmol) and 2,4-dichlorophenylhydrazine hydrochoride (3.38 g, 15.8 mmol); chromatography *n*-hexane/EtOAc 97:3. Yield, 788 mg (19%), as an orange solid. R_f: (*n*-hexane/EtOAc 95:5) 0.35. Mp = 91–93 °C. ^1H NMR

($CDCl_3$, 300 MHz) δ 7.77 (d, 2H, J = 8.3 Hz, H-2″), 7.57 (s, 1H, H-3′), 7.40–7.34 (m, 4H, H3″ and H-5′, H-6′), 6.50 (s, 1H, H-4), 2.44 (t, 2H, J = 7.1 Hz, $CH_2CH_2CH_2CH_2CH_3$), 1.62–1.57 (m, 2H, $CH_2CH_2CH_2CH_2CH_3$), 1.29–1.26 (m, 4H, $CH_2CH_2CH_2CH_2CH_3$), 0.89–0.86 (m, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.2 (C-3), 147.1 (C-5), 136.1 (C-1′), 135.9 (C-2′), 133.7 and 133.6 (C-4′ and C-4″), 131.7 (C-1″), 130.7 (C-6′), 130.2 (C-3′), 128.7 (C-3″), 127.9 (C-5′), 127.0 (C-2″), 102.0 (C-4), 31.2 ($CH_2CH_2CH_2CH_2CH_3$), 28.1 ($CH_2CH_2CH_2CH_2CH_3$), 25.6 ($CH_2CH_2CH_2CH_2CH_3$) 22.3 ($CH_2CH_2CH_2CH_2CH_3$) 13.9 CH_3. IR (KBr) ν/cm^{-1}: 3436, 2960, 2931, 2860, 1493, 1091, 838, 826, 815 and 785. MS (EI): m/z (%) 392 (47), 349 (23), 336 (100), 300 (27), 265 (24) and 163 (22); elemental analysis $C_{20}H_{19}Cl_3N_2$, Calcd: C, 61.01; H, 4.86; N, 7.11. Found: C, 61.24; H, 4.95; N, 6.96.

4.1.3.3. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-3-hexadecyl-1*H*-pyrazole (2c). 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-3-hexadecyl-1H-pyrazole (**2c**) was prepared from **1c** (1.00 g, 4.0 mmol) and 2,4-dichlorophenylhydrazine hydrochoride (1.27 g, 6.0 mmol); chromatography *n*-hexane/EtOAc 99:1. Yield, 426 mg (21%), as a yellow solid. R_f: (*n*-hexane/EtOAc 95:5) 0.35. Mp = 61–62 °C. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.76 (d, 2H, J = 8.6 Hz, H-2″), 7.57 (t, 1H, J = 1.2 Hz, H-3′), 7.40–7.39 (m, 2H, H-5′ and H-6′), 7.35 (d, 2H, J = 8.6 Hz, H-3″), 6.50 (s, 1H, H-4), 2.44 (t, 2H, J = 7.6 Hz, $CH_2CH_2CH_2CH_2CH_2CH_3$), 1.61–1.52 (m, 2H, $CH_2CH_2CH_2CH_2CH_2CH_3$), 1.34–1.25 (m, 6H, $CH_2CH_2CH_2CH_2CH_2CH_3$ CH_3), 0.86 (t, 3H, J = 6.9 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.2 (C-3), 147.1 (C-5), 136.1 (C-1′), 135.9 (C-2′), 133.6 and 133.5 (C-4′ and C-4″), 131.7 (C-1″), 130.7 (C-6′), 130.2 (C-3′), 128.7 (C-3″), 127.9 (C-5′), 127.0 (C-2″), 102.0 (C-4), 31.4 ($CH_2CH_2CH_2CH_2CH_2CH_3$), 28.7 ($CH_2CH_2CH_2CH_2CH_2CH_3$), 28.4 ($CH_2CH_2CH_2CH_2CH_2CH_3$), 25.6 ($CH_2CH_2CH_2CH_2CH_2CH_3$), 22.4 ($CH_2CH_2CH_2CH_2CH_2CH_3$), 14.0 CH_3. IR (KBr) ν/cm^{-1}: 3436, 2950, 2926, 2851, 1493, 1089 and 815.6. MS (EI): m/z (%) 406 (31), 349 (37), 336 (100), 165 (73) and 57 (74); elemental analysis $C_{21}H_{21}Cl_3N_2$, Calcd: C, 61.86; H, 5.19; N, 6.87. Found: C, 62.07; H, 5.40; N, 6.63.

4.1.4. Method A for the synthesis of diaryl-pyrazolecarboxylates: synthesis of 7a

4.1.4.1. (*Z*)-Ethyl 2-hydroxy-4-oxo-4-phenylbut-2-enoate (3a). Sodium (1 equiv, 570 mg, 25.0 mmol) was dissolved in anhydrous EtOH. To this ice-cooled solution of sodium ethoxide, a solution of diethyl oxalate (1 equiv, 3.59 mL, 25.0 mmol) and acetophenone (1 equiv, 2.91 mL, 25.0 mmol) was slowly added. The paste formed was stirred at rt for 15 h and then warmed at 80 °C for 30 min. The mixture was cooled and acidified with H_2SO_4 1 M to pH 2. Organic solvent was removed in vacuo and the mixture was extracted with Et_2O. The organic layer was dried over anhydrous $MgSO_4$, and the solvent was evaporated to give 3.67 g (66%) of **3a** as a red oil. R_f: (*n*-hexane/EtOAc 2:1) 0.80.

4.1.4.2. Ethyl 1,5-diphenyl-1*H*-pyrazole-3-carboxylate (7a). To a solution of **3a** (1 equiv, 3.65 g, 16.6 mmol) in acetic acid glacial (15 mL), phenylhydrazine (1 equiv, 1.62 mL, 16.6 mmol) was added. The mixture was refluxed for 6 h, then poured onto ice and extracted with Et_2O. The organic layer was washed with 10% $NaHCO_3$ aq and water, dried over anhydrous $MgSO_4$ and the solvent was removed in vacuo. The oily residue was purified by flash chromatography (eluent *n*-hexane/EtOAc, 95:5) to give 3.22 g (66%) of **7a** as a red solid. R_f: (*n*-hexane/EtOAc 95:5) 0.30. Mp = 79–80 °C. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.26–7.20 (m, 8H, Ph), 7.15–7.12 (m, 2H, Ph), 6.97 (s, 1H, H-4), 4.38 (q, 2H, J = 7.2 Hz, CH_2), 1.34 (q, 3H, J = 7.2 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.4 (CO), 144.6 and 144.3 (C-3 and C-5), 139.5 (C-1′), 129.5 (C-1″), 129.2 128.9 and 128.6 (C-3′, C-2″ and C-3″), 128.5 and 128.3 (C-4′ and C-4″), 125.7 (C-2′), 109.9 (C-4), 61.1 (CH_2), 14.4 (CH_3). MS (EI): m/z (%) 292 (99), 247 (59), 220 (100), 180 (26), 117 (20), 104 (22) and 77 (22).

4.1.5. Method B for the synthesis of diaryl-pyrazolecarboxylates: synthesis of 7b

4.1.5.1. (*Z*)-Ethyl 2-hydroxy-3-methyl-4-oxo-4-phenylbut-2-enoate (3b). To a solution of LHMDS (1.1 equiv, 4.11 g, 24.6 mmol) in cyclohexane (50 mL) at 15–25 °C under N_2 atm, a solution of propiophenone (1 equiv, 3.00 g, 22.4 mmol) in cyclohexane (10 mL) was added for 30–45 min. Afterwards, the solution was stirred for 2.5 h. Then, diethyl oxalate (1.1 equiv, 3.53 mL, 24.6 mmol) was slowly added, and the mixture was stirred at rt for 17 h. The solid formed (**3b**) was filtered, washed with cyclohexane several times and dried under vacuum. Yield: 4.72 g (90%) as a white solid.

4.1.5.2. Ethyl 4-methyl-1,5-diphenyl-1*H*-pyrazole-3-carboxylate (7b). To a solution of **3b** (1 equiv, 1.60 g, 6.83 mmol) in EtOH (40 mL), phenylhidrazine (1 equiv, 660 μL, 6.83 mmol) and 50% H_2SO_4 aq (20 mL) were added, and the mixture was refluxed for 12 h. Then, the reaction mixture was poured onto ice, extracted with Et_2O. The organic layer was washed with 10% $NaHCO_3$ aq, dried over anhydrous Na_2SO_4. The residue was purified by flash chromatography (eluent *n*-hexane/EtOAc, 3:1) to give 500 mg (24%) of **7b** as a white solid. R_f: (*n*-hexane/EtOAc 3:1) 0.45. Mp = 105–106 °C. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.28–7.28 (m, 2H, Ph), 7.19 (br s, 6H, Ph), 7.10–7.06 (m, 2H, Ph), 4.39 (q, 2H, J = 7.2 Hz, CH_2), 2.25 (s, 3H, CH3), 1.34 (q, 3H, J = 7.2 Hz, CH_2CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.2 (CO), 141.1 (C-3 and C-5), 138.6 (C-1′), 128.6 (C-1″), 129.1, 127.7 and 127.5 (C-3′, C-2″, C-3″ and C-4″), 126.8 (C-4′), 124.4 (C-2′), 118.8 (C-4), 59.8 (CH_2), 13.5 (CH_2CH_3), 8.7 (CH_3). MS (EI): m/z (%) 306 (100), 260 (56), 233 (93), 180 (66) and 77 (32).

4.1.6. Synthesis of diaryl-triazolecarboxylate 7c

4.1.6.1. (*E*)-Dimethyl 2-(4-chlorobenzamido)-2-[(4-chlorophenyl)diazenyl]malonate (6). A solution of 4-chloroaniline (1 equiv, 440 mg, 3.5 mmol) and HBF_4 aq (2 equiv, 170 μL, 7.0 mmol) in H_2O (1 mL) was cooled to 0 °C. Then, sodium nitrite (1 equiv, 230 mg, 3.5 mmol) in H_2O (0.5 mL) previously cooled to 0 °C, was added dropwise. The reaction mixture was stirred at 0 °C for 30 min, and at rt for 30 more min. The formed diazonium salt was filtered, washed with HBF_4 aq, and was used in the next step without further purification. Afterwards, diazonium salt was coupled to **5**, which was prepared as previously reported.[26] To a solution of **5** (1 equiv, 250 mg, 0.87 mmol) and NaOAc (2.75 equiv, 190 mg, 2.40) in MeOH (10 mL) at 0 °C, diazonium salt solved in MeOH (5 mL) and H_2O (5 mL) was added. Reaction mixture was stirred at rt for 2 h. The solid formed (**6**) was filtered, washed with MeOH and dried under vacuum. Yield 98% (362 mg). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.80–7.75 (m, 2H, ClPhCO), 7.70–7.65 (m, 2H, ClPhCO), 7.60 (s, 1H, NH), 7.40–7.30 (m, 4H, ClPhN=N), 3.85 (s, 6H, $2CH_3$); MS (EI): m/z (%) 139 (100) and 111 (42).

4.1.6.2. Methyl 1,5-bis(4-chlorophenyl)-1*H*-1,2,4-triazole-3-carboxylate (7c). Compound **6** (1 equiv, 430 mg, 1.0 mmol) was dissolved in MeOH (32 mL) and a catalytic amount of NaOMe/MeOH was added. The mixture was stirred at rt for 24 h. Afterwards, MeOH was removed in vacuo, the residue was redissolved in DCM and was washed with H_2O. The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure. The brown solid obtained was purified by flash chromatography (eluent *n*-hexane/EtOAc, 1:3), to give 300 mg (85%) of **7c** as a pink solid. R_f: (*n*-hexane/EtOAc 1:3) 0.30. Mp = 144–145 °C. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.49–7.43 (m, 4H, Ar), 7.37–7.32 (m, 4H, Ar),

4.04 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.5 (CO), 155.9 and 155.1 (C-3 and C-5), 137.7 (C-1″), 136.3 and 136.2 (C-4′ and C-4″), 130.8 130.3, 129.6 and 127.1 (C-2′ C-3′ C-2″ and C-3″), 125.3 (C-1′), 53.4 (CH_3). MS (EI): *m*/*z* (%) 347 (66), 210 (62), 125 (100) and 90 (22); elemental analysis $C_{16}H_{11}C_{l2}N_3O_2$, Calcd: C, 55.19; H, 3.18; N, 12.07. Found: C, 55.21; H, 3.46; N, 12.20.

4.1.7. General procedure for the synthesis of 8a–8h

To a solution of the corresponding amine (5 equiv) in dry DCM was added a solution of $Al(Me)_3$ in heptane (2 M, 5 equiv) under N_2 atmosphere. The mixture was stirred at rt for 1 h. Then, a solution of alkyl carboxylate (1 equiv) in dry DCM was added dropwise. The mixture was refluxed for 14–48 h, and then was carefully poured onto 1 N HCl. The biphasic solution was heated to 40 °C for 30 min and cooled to rt. The organic layer was separated, dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (eluent *n*-hexane/EtOAc, 9:1 for pyrazoles and 1:1 for triazoles), except **8b** which was recrystallized from hexane.

4.1.7.1. *N*-Oleyl-1,5-diphenyl-1*H*-pyrazole-3-carboxamide (8a).

N-Oleyl-1,5-diphenyl-1*H*-pyrazole-3-carboxamide (**8a**) was prepared from oleylamine (1.68 mL, 5.13 mmol), $Al(Me)_3$ (2.56 mL, 5.13 mmol) and **7a** (300 mg, 1.02 mmol) Yield 440 mg (84%) as a yellow oil. R_f: (*n*-hexane/EtOAc 1:9) 0.20. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.40–7.26 (m, 8H, Ph), 7.22–7.19 (m, 2H, Ph), 7.05 (s, 1H, H-4), 7.00 (bt, 1H, J = 5.5 Hz, NH), 5.39–5.39 (m, 2H, CH=CH), 3.44 (q, 2H, J = 6.9 Hz, NHCH_2), 2.04–1.98 (m, 4H, CH_2CH=CHCH_2), 1.63–1.53 (m, 2H, CH_2CH$_3$), 1.30–1.25 (m, 22H, oleyl), 0.87 (t, 3H, J = 6.5 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.7 (CO), 147.4 (C-3), 144.9 (C-5), 139.6 (C-1′), 129.9, 129.8 and 129.7 (C-1″ and CH=CH), 129.0, 128.7, 128.6, 128.5 and 128.2 (C-3′ C-4′ C-2″ C-3″ and C-4″), 125.4 (C-2′), 108.0 (C-4), 39.2 (NHCH_2), 31.9 and 31.8 (CH_2CH$_2$CH$_3$ and NHCH$_2$CH_2), 29.7, 29.6, 29.5, 29.4, 29.3, 29.2 and 29.0 (oleyl), 27.2 and 27.0 (CH_2CH=CHCH_2), 22.7 (CH_2CH$_3$), 14.1 (CH_3). IR (film) ν/cm^{-1}: 3315, 3103, 2923, 2853, 1643, 1557, 1541, 1499, 1430, 1369, 765 and 699. MS (EI): *m*/*z* (%) 513 (27) and 247 (100); elemental analysis $C_{34}H_{47}N_3O$, Calcd: C, 79.49; H, 9.22; N, 8.18. Found: C, 79.28; H, 9.13; N, 7.99.

4.1.7.2. *N*-Hexadecyl-1,5-diphenyl-1*H*-pyrazole-3-carboxamide (8b).

N-Hexadecyl-1,5-diphenyl-1*H*-pyrazole-3-carboxamide (**8b**) was prepared from **7a** (300 mg, 1.02 mmol), hexadecylamine (1.23 g, 5.13 mmol) and $Al(Me)_3$ (2.56 mL, 5.13 mmol). Yield 475 mg (99%) as a white solid. R_f: (*n*-hexane/EtOAc 4:1) 0.43. Mp = 75–76 °C. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.37–7.21 (m, 10 H, Ph), 7.00 (s, 1H, H-4), 6.47–6.43 (m, 1H, NH), 3.44 (q, 2H, J = 6.6 Hz, NHCH_2), 1.63–1.56 (m, 2H, CH_2CH$_3$), 1.25 (br s, 26H, hexadecyl), 0.89–0.85 (m, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.8 (CO), 147.4 (C-3), 144.9 (C-5), 139.6 (C-1′), 129.7 (C-1″), 129.1, 128.7 and 128.5 (C-3′, C-2″ and C-3″), 128.6 (C-4″), 128.2 (C-4′), 125.4 (C-2′), 108.0 (C-4), 39.2 (NHCH_2), 31.9 (CH_2CH$_2$CH$_3$), 29.7, 29.6, 29.5, and 29.3 (hexadecyl), 27.0 (NHCH$_2$CH$_2$CH_2), 22.7 (CH$_2$CH_2CH$_3$), 14.1 (CH_3). IR (KBr) ν/cm^{-1}: 3430, 3317, 2919, 2851, 1642, 1499, 766 and 699. MS (EI): *m*/*z* (%) 247 (100); elemental analysis $C_{32}H_{45}N_3O$, Calcd: C, 78.80; H, 9.30; N, 8.62. Found: C, 78.60; H, 9.16; N, 8.55.

4.1.7.3. *N*-Piperidinyl-1,5-diphenyl-1*H*-pyrazole-3-carboxamide (8c).

N-Piperidinyl-1,5-diphenyl-1*H*-pyrazole-3-carboxamide (**8c**) was prepared from **7a** (300 mg, 1.02 mmol), 1-aminopiperidine (540 μL, 5.13 mmol) and $Al(Me)_3$ (2.56 mL, 5.13 mmol). Yield 451 mg (91%) as a yellow solid. R_f: (*n*-hexane/EtOAc 1:9) 0.30. Mp = 147–148 °C. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.70 (br s, 1H, NH), 7.40–7.34 (m, 4H, Ph), 7.32–7.27 (m, 4H, Ph), 7.22–7.18 (m, 2H, Ph), 7.09 (s, 1H, H-4), 2.87 (t, 4H, J = 5.1 Hz, CH$_2$CH$_2$CH_2NCH_2CH$_2$), 1.81–1.73 (m, 4H, CH$_2$CH_2CH$_2$NCH$_2$CH_2), 1.48–1.44 (m, 2H, CH_2CH$_2$CH$_2$NCH$_2$CH$_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.0 (CO), 146.7 (C-3), 144.8 (C-5), 139.6 (C-1′), 129.6 (C-1″), 129.1, 128.7 and 128.5 (C-3′, C-2″ and C-3″), 128.6 (C-4″), 128.3 (C-4′), 125.5 (C-2′), 108.6 (C-4), 57.3 (CH$_2$CH$_2$CH_2 NCH_2CH$_2$), 25.3 (CH$_2$CH_2CH$_2$NCH$_2$CH_2), 23.3 (CH_2CH$_2$CH$_2$NCH$_2$ CH$_2$). IR (KBr) ν/cm^{-1}: 3436, 3285, 2927, 2856, 1671, 1498, 768 and 696. MS (EI): *m*/*z* (%) 263 (89), 247 (100), 219 (24), and 84 (40); elemental analysis $C_{21}H_{22}N_4O$, Calcd: C, 72.81; H, 6.40; N, 16.17. Found: C, 72.63; H, 6.48; N, 16.35.

4.1.7.4. *N*-Oleyl-1,5-diphenyl-4-methyl-1*H*-pyrazole-3-carboxamide (8d).

N-Oleyl-1,5-diphenyl-4-Methyl-1*H*-pyrazole-3-carboxamide (**8d**) was prepared from **7b** (300 mg, 0.97 mmol), oleylamine (1.61 mL, 4.89 mmol) and $Al(Me)_3$ (2.44 mL, 4.89 mmol). Yield 347 mg (68%) as a yellow oil. R_f: (*n*-hexane/EtOAc 1:9) 0.25. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.28–7.07 (m, 10H, Ph), 7.02–6.98 (m, 1H, NH), 5.29–5.26 (m, 2H, CH=CH), 3.36 (q, 2H, J = 6.7 Hz, NHCH_2), 2.31 (s, 3H, CH_3), 1.95–1.91 (m, 4H, CH_2CH=CHCH_2), 1.57–1.52 (m, 2H, CH_2CH$_3$), 1.25–1.19 (m, 22H, oleyl), 0.81 (t, 3H, J = 6.8 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.0 (CO), 144.2 (C-3), 142.2 (C-5), 139.6 (C-1′), 129.9, 129.8 and 129.7 (C-1″ and CH=CH), 130.0, 128.8 and 128.5 (C-3′, C-2″ and C-3″), 128.4 (C-4″), 127.5 (C-4′), 124.9 (C-2′), 118.4 (C-4), 38.9 (NHCH_2), 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3 and 29.2 (oleyl), 27.2 and 27.0 (CH_2CH=CHCH_2), 22.6 (CH_2CH$_3$), 14.1 (CH$_2$CH_3), 9.4 (CH_3). IR (film) ν/cm^{-1}: 3421, 3344, 2924, 2853, 1673, 1598, 1538, 1501, 1467, 1457, 1445, 1361, 774 and 700. MS (EI): *m*/*z* (%) 527 (22), 261 (100), 233 (20); elemental analysis $C_{35}H_{49}N_3O$, Calcd: C, 79.65; H, 9.36; N, 7.96. Found: C, 79.40; H, 9.37; N, 7.90.

4.1.7.5. *N*-Hexadecyl-1,5-diphenyl-4-methyl-1*H*-pyrazole-3-carboxamide (8e).

N-Hexadecyl-1,5-diphenyl-4-methyl-1*H*-pyrazole-3-carboxamide (**8e**) was prepared from **7b** (200 mg, 0.65 mmol), hexadecylamine (780 mg, 3.26 mmol) and $Al(Me)_3$ (1.63 mL, 3.26 mmol). Yield 170 mg (53%) as a yellow solid. R_f: (*n*-hexane/EtOAc 1:9) 0.25. Mp = 64–65 °C. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.35–7.20 (m, 8H, Ph), 7.17–7.14 (m, 2H, Ph), 7.08–7.04 (m, 1H, NH), 3.43 (q, 2H, J = 6.6 Hz, NHCH_2), 2.37 (s, 3H, CH_3), 1.64–1.57 (m, 2H, CH_2CH$_3$),1.25 (br s, 26H, hexadecyl), 0.88 (t, 3H, J = 6.8 Hz, CH$_2$CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.0 (CO), 143.3 (C-3), 141.2 (C-5), 138.7 (C-1′), 128.7 (C-1″), 129.0, 127.8 and 127.5 (C-3′ C-2″ and C-3″), 126.7 (C-4″), 126.6 (C-4′), 124.0 (C-2′), 117.4 (C-4), 38.0 (NHCH_2), 30.9 (CH_2CH$_2$CH$_3$), 28.8, 28.7, 28.6 and 28.3 (hexadecyl), 26.0 (NHCH$_2$CH$_2$CH_2), 21.7 (CH$_2$CH$_2$CH$_3$), 13.1 (CH$_2$CH_3), 8.4 (CH_3). IR (KBr) ν/cm^{-1}: 3429, 3317, 2917, 2849, 1647, 1540 and 698. MS (EI): *m*/*z* (%) 501 (20), 261 (100) and 240 (36); elemental analysis $C_{33}H_{47}N_3O$, Calcd: C, 78.99; H, 9.44; N, 8.37. Found: C, 78.75; H, 9.40; N, 8.28.

4.1.7.6. *N*-Piperidinyl-1,5-diphenyl-4-methyl-1*H*-pyrazole-3-carboxamide (8f).

N-Piperidinyl-1,5-diphenyl-4-methyl-1*H*-pyrazole-3-carboxamide (**8f**) was prepared from **7b** (300 mg, 0.97 mmol), 1-aminopiperidine (520 μL, 4.89 mmol) and $Al(Me)_3$ (2.44 mL, 4.89 mmol). Yield 240 mg (68%) as a yellow solid. R_f: (*n*-hexane/EtOAc 1:9) 0.50. Mp = 195–196 °C. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.76 (br s, 1H, NH), 7.35–7.26 (m, 6H, Ph), 7.23–7.20 (m, 2H, Ph), 7.15–7.12 (m, 2H, Ph), 2.88 (t, 4H, J = 5.1 Hz, CH$_2$CH$_2$CH_2NCH_2CH$_2$), 2.36 (s, 3H, CH_3), 1.82–1.72 (m, 4H, CH$_2$CH_2CH$_2$NCH$_2$CH_2), 1.48–1.42 (m, 2H, CH_2CH$_2$CH$_2$NCH$_2$CH$_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.3 (CO), 143.5 (C-3), 142.2 (C-5), 139.6 (C-1′), 129.6 (C-1″), 130.0 128.8 and 128.5 (C-3′, C-2″ and C-3″), 128.4 (C-4″), 127.7 (C-4′), 125.0 (C-2′), 119.0 (C-4), 57.1 (CH$_2$CH$_2$CH$_2$NCH$_2$CH$_2$), 25.5 (CH$_2$CH$_2$CH$_2$NCH$_2$CH$_2$), 23.4 (CH$_2$CH$_2$ CH$_2$NCH$_2$CH$_2$), 9.3 (CH_3). IR (KBr) ν/cm^{-1}: 3435, 2929, 1688,

1528, 1499, 784 and 701. MS (EI): *m*/*z* (%) 360 (21), 277 (26), 261 (100), 233 (48), 180 (39), 84 (45) and 77 (24); elemental analysis $C_{22}H_{24}N_4O$, Calcd: C, 73.31; H, 6.71; N, 15.54. Found: C, 73.21; H, 6.90; N, 15.37.

4.1.7.7. *N*-Oleyl-1,5-bis(4-chlorophenyl)-1*H*-1,2,4-triazole-3-carboxamide (8g). *N*-Oleyl-1,5-bis(4-chlorophenyl)-1*H*-1,2,4-triazole-3-carboxamide (**8g**) was prepared from **7c** (90 mg, 0.25 mmol), oleylamine (410 μL, 1.29 mmol) and $Al(Me)_3$ (640 μL, 1.29 mmol). Yield 80 mg (53%) as a yellow oil. R_f: (*n*-hexane/EtOAc 1:1) 0.30. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.44–7.38 (m, 4H, Ar), 7.35–7.28 (m, 4H, Ar), 7.21 (bt, 1H, J = 5.8 Hz, NH), 5.36–5.26 (m, 2H, CH=CH), 3.47 (q, 2H, J = 6.7 Hz, $NHCH_2$), 1.99–1.95 (m, 4H, CH_2CH=$CHCH_2$), 1.63–1.56 (m, 2H, CH_2CH_3), 1.27–1.23 (br s, 22H, oleyl), 0.85 (t, 3H, J = 6.2 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 158.6 (CO), 156.8 (C-3), 153.7 (C-5), 137.0 (C-1″), 135.9 and 135.5 (C-4′ and C-4″), 130.1, 129.7, 129.9 and 126.6 (C-2′ C-3′ C-2″ and C-3″), 129.8 and 129.7 (CH=CH), 125.1 (C-1′), 39.4 ($NHCH_2$), 31.8, 29.6, 29.5, 29.4, 29.3, 29.2 and 29.1 (oleyl), 27.1 and 26.8 (CH_2CH=$CHCH_2$), 22.6 (CH_2CH_3), 14.0 (CH_2CH_3). IR (KBr) ν/cm^{-1}: 3418, 2921, 2851, 1643, 1557, 1541, 1499, 1430, 1369, 765 and 699. MS (EI): *m*/*z* (%) 316 (100), 248 (35), 151 (30) and 111 (20); elemental analysis $C_{33}H_{44}C_{l2}N_4O$, Calcd: C, 67.91; H, 7.60; N, 9.60. Found: C, 67.99; H, 7.74; N, 9.81.

4.1.7.8. *N*-Hexadecyl-1,5-bis(4-chlorophenyl)-1*H*-1,2,4-triazole-3-carboxamide (8h). *N*-Hexadecyl-1,5-bis(4-chlorophenyl)-1*H*-1,2,4-triazole-3-carboxamide (**8h**) was prepared from **7c** (120 mg, 0.34 mmol), hexadecylamine (410 mg, 1.72 mmol) and $Al(Me)_3$ (860 μL, 1.72 mmol). Yield 135 mg (70%) as a yellow solid. R_f: (*n*-hexane/EtOAc 1:1) 0.70. Mp = 77–78 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.46–7.31 (m, 8H, Ar), 7.21 (m, 1H, NH), 3.49 (q, 2H, J = 6.8 Hz, $NHCH_2$), 1.68–1.59 (m, 2H, CH_2CH_3), 1.38–1.25 (m, 26H, hexadecyl), 0.87 (t, 3H, J = 6.9 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 158.7 (CO), 156.9 (C-3), 153.9 (C-5), 137.1 (C-1″), 135.9 and 135.6 (C-4′ and C-4″), 130.2, 129.8, 129.2 and 126.7 (C-2′ C-3′ C-2″ and C-3″), 125.2 (C-1′), 39.5 ($NHCH_2$), 31.9 ($CH_2CH_2CH_3$), 29.7, 29.3, 29.6, 29.5 and 29.3 (hexadecyl), 26.9 ($NHCH_2CH_2CH_2$), 22.7 ($CH_2CH_2CH_3$), 14.1 (CH_3). IR (KBr) ν/cm^{-1}: 3418, 2921, 2851, 1676 and 1495. MS (EI): *m*/*z* (%) 346 (34), 316 8100), 303 (26), 289 (20), 248 (27) and 151 (21); elemental analysis $C_{31}H_{42}C_{l2}N_4O$, Calcd: C, 66.77; H, 7.59; N, 10.05. Found: C, 66.48; H, 7.65; N, 10.21.

4.2. Pharmacology

4.2.1. Binding studies

The binding assays were performed as previously described.[34] Membranes from transfected cells with human CB_1 or CB_2 expressed cannabinoid receptors (RBHCB1M400UA and RBXCB2M400UA) were supplied by Perkin-Elmer Life and Analytical Sciences (Boston, MA). The protein concentration for the CB_1 receptor membranes was 8.4 mg/mL, whereas for the CB_2 receptor membranes was 3.6 mg/mL. The commercial membrane was diluted (1:20) with the binding buffer (50 mM TrisCl, 5 mM $MgCl_2.H_2O$, 2.5 mM EDTA, 0.5 mg/mL BSA and pH = 7.4 for CB_1 binding buffer; 50 mM TrisCl, 5 mM $MgCl_2{\cdot}H_2O$, 2.5 mM EGTA, 1 mg/mL BSA and pH = 7.5 for CB_2 binding buffer). The radioligand used was [^{3}H]-CP55940 (PerkinElmer) at a concentration of membrane $k_D \times 0.8$ nm, and the final volume was 200 μL for CB_1 binding and was 600 μL for CB_2 binding. 96-Wells plates and the tubes necessary for the experiment were previously siliconized with Sigmacote (Sigma).

Membranes were resuspended in the corresponding buffer and were incubated with the radioligand and each compound (10^{-4} to 10^{-11} M) for 90 min at 30 °C. Non-specific binding was determined with 10 μM WIN55212-2 and 100% binding of the radioligand to the membrane was determined by incubation of it and the membrane without any compound. Filtration was performed by a Harvester® filtermate (Perkin–Elmer) with Filtermat A GF/C filters pretreated with polyethylenimine 0.05%. After filtering, the filter was washed nine times with binding buffer, dried and a melt-on scintillation sheet (Meltilex™ A, Perkin Elmer) was melted onto it. Then, radioactivity was quantified by a liquid scintillation spectrophotometer (Wallac MicroBeta Trilux, Perkin–Elmer). Competition binding data were analyzed by using GraphPad Prism® version 5.01 (GraphPad Software Inc., San Diego, CA, USA) and K_i values are expressed as mean ± SEM of at least three experiments performed in triplicate for each point.

4.2.2. In vivo studies

All experiments were performed in male Wistar rats, weighing 350–450 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 light/dark cycle. Water and standard chow pellets (Prolab. RMH 2500) were available ad libitum. 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 animals deprived of food for 24 h and habituated to handling.[16] To habituate the animals, 72 h before the testing with drugs, animals were food-deprived for 24 h. Then, the bedding material was removed from the cage and a small can containing weighted food pellets was placed inside the cage for 4 h and the amount of food eaten registered. After the initial test, the animals were under a free-feeding period of 48 h. Then, the animals were food-deprived for 24 h again, with access to water *ad libitum*. Drugs were suspended with 2–3 drops of Tween 80 in saline as vehicle. Fifteen minutes before the start of the test, drugs were administered ip (dose of 3 mg/kg) 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 again. Food pellets and food spillage were weighted at 30, 60, 120 and 240 min after starting the test, and the amount of food eaten was recorded.

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

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