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Novel sterically hindered cannabinoid CB_1 receptor ligands

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ABSTRACT

In the present study, 11 novel *N*-(3,3-diphenyl)propyl-2,2-diphenylacetamide derivatives (**4a–d** and **9a–g**) and six triphenylacetamides (**10a–c** and **11a–c**) were synthesized and tested as ligands of cannabinoid CB_1 and CB_2 receptors. All compounds exhibited affinity for CB_1 and CB_2 receptors. Four compounds (**4b**, **9a**, **9b**, and **11a**) showed selectivity for CB_1 versus CB_2 receptors, although only the *N*-(3,3-diphenyl)propyl-2,2-diphenylacetamide (**4b**) can be considered a potent CB_1 ligand (K_i = 58 nM). It was 140-fold selective over CB_2 receptors (K_i = 7800 nM) and behaved as an inverse agonist by stimulating forskolin-induced cAMP formation in mouse N18TG2 neuroblastoma cells. This compound is the first of a novel class of tetraphenyl CB_1 ligands that, in view of its easy synthesis and high affinity for CB_1 receptors and despite its sterical hindrance, will be useful for the design of new blockers of this therapeutically exploitable receptor type.

1. Introduction

Mammalian tissues contain at least two types of cannabinoid receptors, CB_1 and CB_2,[1,2] that are coupled to G-proteins of $G_{i/o}$ type. CB_1 receptors, cloned in 1992, are mostly expressed in the central nervous system, but also in peripheral tissues including immune cells, the reproductive system, the gastrointestinal tract and the lung, while CB_2 receptors, cloned in 1993, are most abundant in the immune system, that is, in tonsils, spleen, macrophages, and lymphocytes (B-cells and natural killer cells).[3–5] It has been widely shown that there are several pathophysiological conditions, including pain,[6] inflammation,[7] liver diseases,[8] and obesity,[9] in which blocking the cannabinoid receptors might be beneficial. In fact, many CB_1 receptor antagonists have been developed so far[10,11] and some of them are in clinical trials for the treatment of several disorders. One of these drugs, the CB_1 receptor antagonist/inverse agonist rimonabant (SR141716A) (Fig. 1) (**1**) belongs to the class of diarylpyrazole antagonists, including also other widely used pharmacological tools, such as AM-251 (**2**) and AM-281 (**3**). Rimonabant has been recently approved for marketing in the EU as an adjunct to exercise and diet for the treatment of obesity and metabolic syndrome, and has proved useful to reduce body weight, low HDL-cholesterol and high triglyceride levels, as well as high glycemia, in obese patients, but also hallmarks of type-2 diabetes in treated and untreated patients.[12–14] More recently, two other CB_1 receptor antagonists/inverse agonists have undergone clinical trials for the treatment of obesity: SLV319 (**4**), whose structure still resembles that of rimonabant, and MK-0364 (**5**), which instead belongs to a different class of acyclic compounds and exhibits higher affinity at CB_1 receptors, and higher selectivity versus CB_2 receptors, than rimonabant.[15]

The chemical structures of these previously developed compounds (Fig. 1) show a striking difference from those of both Δ^9-tetrahydrocannabinol (**6**) (Fig. 2), the *Cannabis sativa* natural component from which the cannabinoid receptors were discovered, and the endo cannabinoids anandamide (**7**) and 2-arachidonoylglycerol (**8**). These two naturally occurring classes of CB_1 receptor ligands, in fact, although containing pharmacophores found also in the various synthetic antagonists, are much less sterically hindered than rimonabant, SLV319, and MK-0364.

On the basis of this background, we wondered if it would be possible to obtain new CB_1 receptor ligands with even higher sterical hindrance, and for this purpose we have synthesized eleven novel *N*-(3,3-diphenyl)propyl-2,2-diphenylacetamide derivatives (**4a–d** and **9a–g**) and six triphenylacetamides (**10a–c** and **11a–c**). Using the very simple synthetic procedure shown in Scheme 1, we obtained α-substituted acetamide derivatives **4a–d**.

The synthesis of the 2-(4-substituted phenyl)-2-phenyl-*N*-(3,3-diphenylpropyl)acetamide **9a–g** proceeds from a monosubstituted

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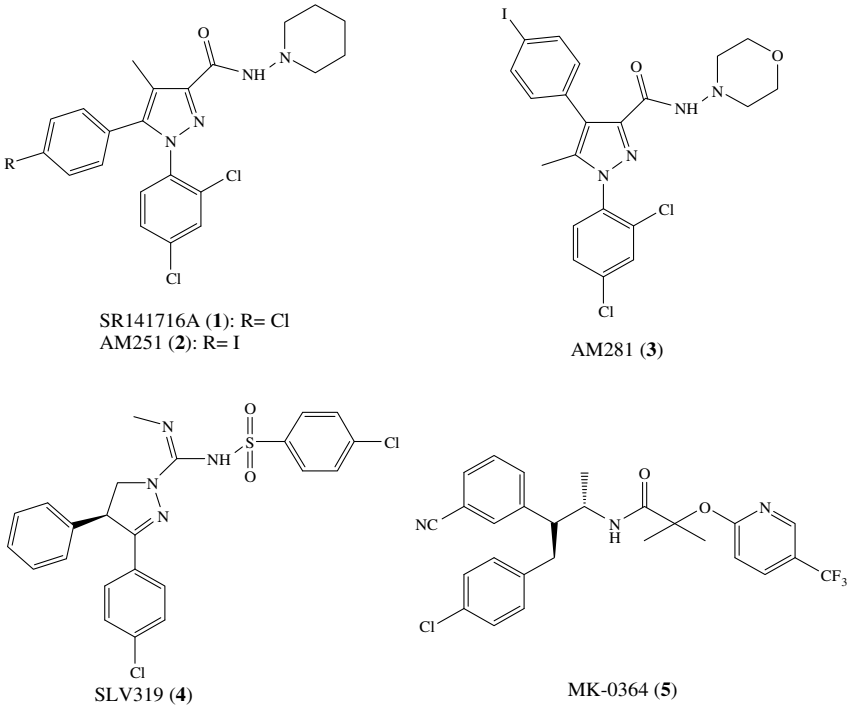


Figure 1. Chemical structures of some CB_1 receptor antagonists/inverse agonists.

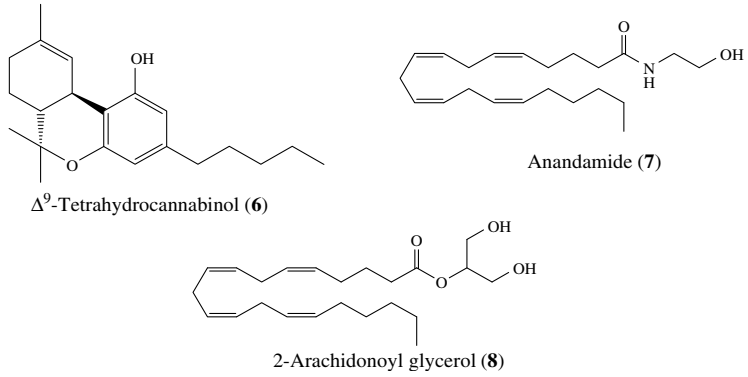
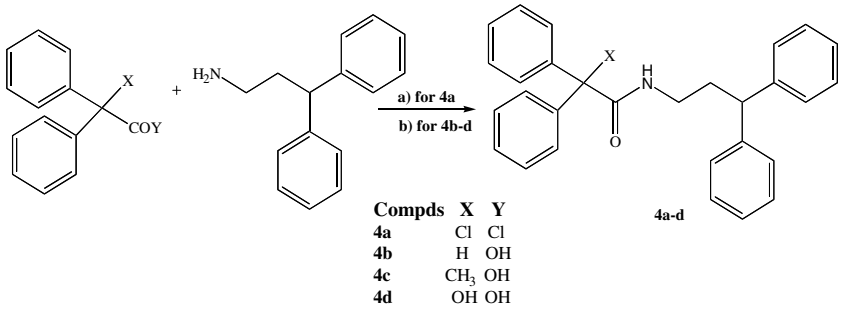
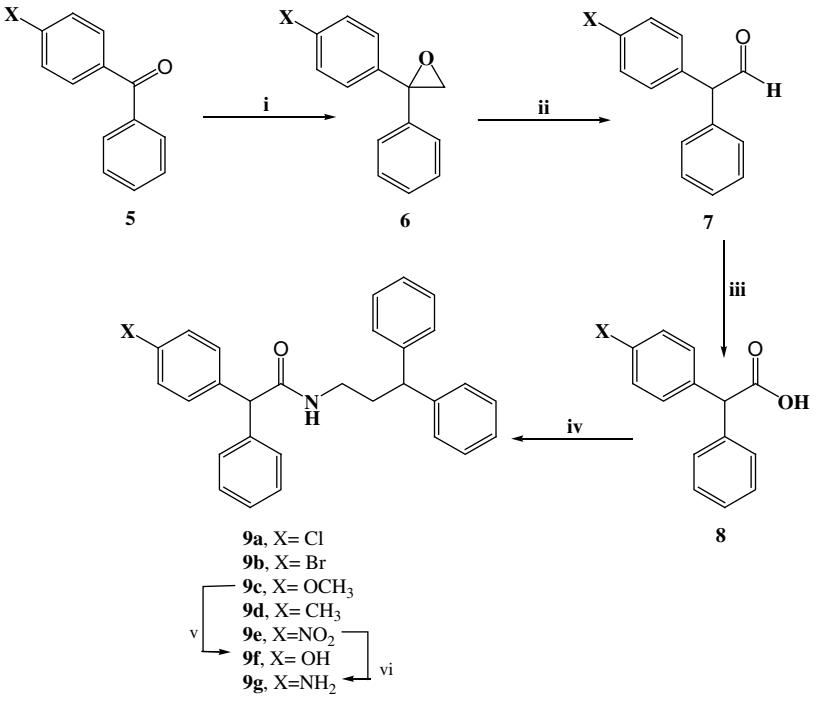


Figure 2. Chemical structures of Δ^9-tetrahydrocannabinol and the endocannabinoids anandamide and 2-arachidonoylglycerol.



Scheme 1. Schematic procedure for the one-step synthesis of **4a–d**. Reagents and conditions: (a) dry DCM, dry TEA, rt, 4 h; (b) DCC, HOBT, DCM, rt, 16 h.

diphenylacetic acid[16] (Scheme 2). Homologation of commercially available 4-X-benzophenones **5** (X = Cl, Br, OCH_3, CH_3, and NO_2) by sodium hydride and trimethylsulfoxonium iodide gave the unstable epoxides **6**, which were immediately converted into the aldehydes **7** by the action of BF_3etherate.[16,17] Then oxidation with Jones' reagent[18] converted the aldehydes **7** into the corresponding acids **8** which, after amidation with 3,3-diphenylpropylamine, yielded the target compounds **9a–e** (Scheme 2). The phenol derivative **9f** was produced from the reaction of the methoxyphenyl derivative **9c** with trimethylsilyl chloride/sodium iodide[19] (Scheme 2). Reduction of the nitro group of **9e** by Zn/HCl produced the amine derivative **9g**[20] (Scheme 2).

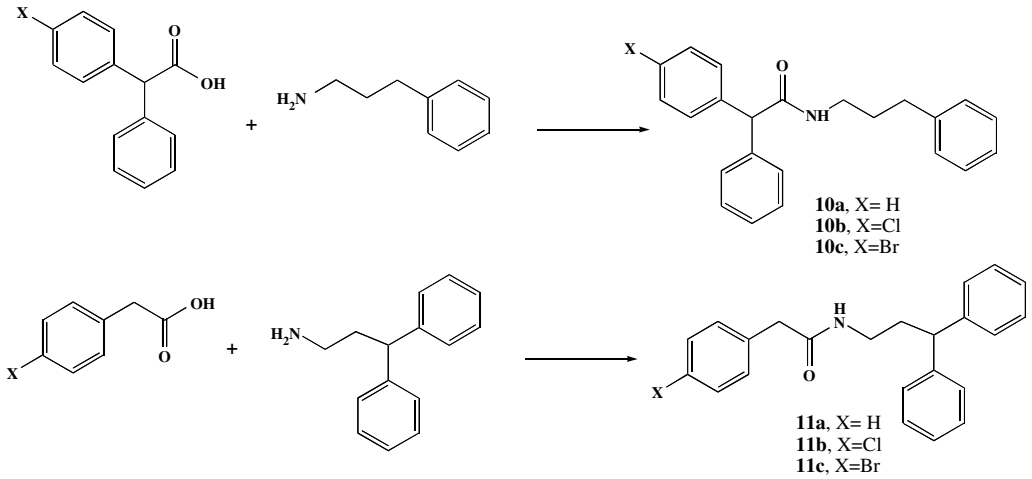


Scheme 2. Procedure for the multi-step synthesis of compounds **9a–g**. Reagents and conditions: (i) NaH, TMS-I, THF; (ii) BF_3–Et_2O, benzene; (iii) Jones' reagent, isopropyl alcohol, acetone; (iv) 3,3-diphenylpropylamine, CDI, DMAP; (v) NaI, TMS–Cl, CH_3CN; (vi) Zn dust, HCl concd, EtOH absolute.

Finally, we synthesized the six triphenylacetamides: *N*-(3-phenyl)propyl-2,2-diphenylacetamide (**10a**), *N*-(3-phenyl)propyl-2-(4-chlorophenyl)-2-phenylacetamide (**10b**), *N*-(3-phenyl)propyl-2-(4-bromophenyl)-2-phenylacetamide (**10c**), *N*-(3,3-diphenyl)propyl-2-phenylacetamide (**11a**), *N*-(3,3-diphenyl)propyl-2-(4-chlorophenyl)acetamide (**11b**), and *N*-(3,3-diphenyl)propyl-2-(4-bromophenyl)acetamide (**11c**) by the following simple one-step synthetic procedure (Scheme 3).

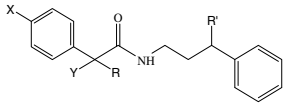
The new compounds were tested for their affinities for human recombinant cannabinoid receptors CB_1 and CB_2, and the corresponding observed K_i (μM) values are shown in Table 1. The functional activity of the most potent CB_1 ligand (**4b**) was also assessed at CB_1 receptors by studying its effect on forskolin-induced cAMP formation in mouse N18TG2 neuroblastoma cells (Fig. 3). The most potent CB_1 cannabinoid receptor ligand in the present study was the *N*-(3,3-diphenyl)propyl-2,2-diphenylacetamide (**4b**), whose affinity for CB_1 receptors (K_i = 58 nM) was higher than that of anandamide (K_i = 89 nM). This compound also showed high (~140-fold) selectivity versus CB_2 receptors (K_i = 7900 nM). Compounds (**4a**, **4c–d**) obtained from **4b**, by introducing various substituents (Cl, CH_3, and OH, respectively) in position α to the amide group, although showing still good affinity for CB_1, lost the selectivity versus CB_2 cannabinoid receptors.

The introduction of various substituents (Cl, Br, OCH_3, CH_3, NO_2, OH, and NH_2) in *para* on one of the two aromatic rings closer to the amide functionality of the parent compound **4b** led to more cannabinoid receptor ligands (**9c–g**), some of which (**9a–b**) were less selective (~16-fold) for CB_1 over CB_2 receptors. In addition, to test the effect of sterical hindrance on the binding to the cannabinoid receptor site, we eliminated first one of the two aromatic rings on the acyclic portion, and then one of those closer to the amide group of the selective compounds **4b**, **9a**, and **9b**, thereby obtaining the six tri-



Scheme 3. Schematic procedure for the one-step synthesis of **10a–c** and **11a–c**. Reagents and conditions: dry DCM, CDI, DMAP, rt, 4 h.

Table 1
Affinity constants (K_i, μM) of the new compounds for human recombinant CB_1 and CB_2 receptors



4a-d, 9a-g, 10a-c and 11a-c

	X	Y	R	R′	hCB$_1$ (K_i, μM)	hCB$_2$ (K_i, μM)
4a	H	Cl	Phe	Phe	0.28 ± 0.02	1.4 ± 0.2
4b*	H	H	Phe	Phe	0.058 ± 0.01	7.9 ± 0.3
4c	H	CH_3	Phe	Phe	0.56 ± 0.02	0.29 ± 0.03
4d	H	OH	Phe	Phe	2.2 ± 0.15	2.3 ± 0.2
9a	Cl	H	Phe	Phe	0.56 ± 0.03	>7.9
9b	Br	H	Phe	Phe	0.22 ± 0.02	>7.9
9c	OCH_3	H	Phe	Phe	0.56 ± 0.04	0.79 ± 0.04
9d	CH_3	H	Phe	Phe	0.56 ± 0.03	0.65 ± 0.03
9e	NO_2	H	Phe	Phe	0.56 ± 0.05	0.79 ± 0.05
9f	OH	H	Phe	Phe	0.56 ± 0.02	1.1 ± 0.2
9g	NH_2	H	Phe	Phe	0.9 ± 0.1	1.2 ± 0.1
10a*	H	H	H	Phe	0.56 ± 0.05	>7.9
	H	H	Phe	H	3.4 ± 0.2	>7.9
10b	Cl	H	Phe	H	2.2 ± 0.2	2.4 ± 0.2
10c	Br	H	Phe	H	1.9 ± 0.1	1.8 ± 0.2
11a*	H	H	H	Phe	0.56 ± 0.05	>7.9
11b*	Cl	H	H	Phe	1.6 ± 0.1	2.4 ± 0.3
11c*	Br	H	H	Phe	0.8 ± 0.02	1.3 ± 0.1
AM251					0.0023 ± 0.001	0.11 ± 0.02
Rimonabant					0.008 ± 0.001	0.79 ± 0.1
SR144528					>5.6	0.0054 ± 0.001

Data represent mean values ± SEM for at least three separate experiments performed in duplicate and expressed as K_i, (μM). AM251, CB_1 reference compound; SR144528, CB_2 reference compound. *Note:* *Products commercially available. Registration numbers: (**4b**) 339283-58-8, (**10a**) 353471-19-9, (**11a**) 543711-37-1, (**11b**) 560080-39-9, and (**11c**)749904-13-0.

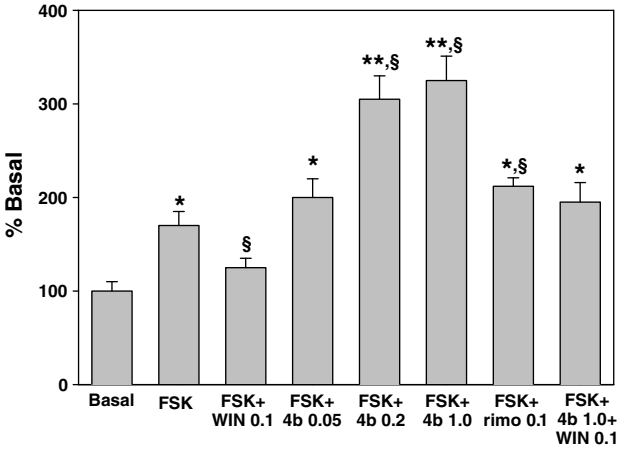


Figure 3. Effect of **4b** (0.05, 0.2, and 1.0 μM) on forskolin (FSK, 1 μM)-induced cAMP formation in intact N18TG2 cells ($^*p < 0.05$ vs basal). The effects of WIN55,212-2 (0.1 μM) and rimonabant (rimo, 0.1 μM) are shown as a comparison. $^{*,**}p < 0.05$, 0.01 versus Basal. $^§p < 0.05$ versus FSK.

phenylacetamides **10a**–**c** and **11a**–**c**, respectively. This led to a significant reduction of the affinity and, with the exception of **10a** and **11a**, to the loss of the selectivity for the CB_1 receptors. It must be emphasized, however, that compounds **9a**–**g** and **10b**–**c** all contain an asymmetric center. Since we only determined the binding activity of the enantiomeric mixtures, the pure enantiomers might have exhibited different K_i values in the binding assays.

Finally, in order to establish the functional activity of the most potent and selective CB_1 ligand (**4b**), we tested its effect on forskolin-induced cAMP formation in intact N18TG2 neuroblastoma cells, which constitutively and selectively express the CB_1 receptor.[21] As shown in Figure 3, the compound was found to stimulate cAMP formation in the presence of forskolin, as would be expected from an inverse agonist in this assay. However, the compound (0.05–1 μM) did not significantly elevate cAMP levels in the absence of forskolin (not shown). As expected, in the same assay, the CB_1/CB_2 agonist WIN55,212-2 (0.1 μM) inhibited forskolin-stimulated cAMP formation, whereas the CB_1 inverse agonist, rimonabant (0.1 μM), produced a stimulation of forskolin effect. WIN55,212-2 (0.1 μM) also blocked the effect of **4b** (1 μM) on cAMP formation (Fig. 3), thus suggesting that the effect of **4b** was mediated by CB_1 receptors.

The finding of **4b** demonstrates that it is still possible to obtain high affinity and selective CB_1 receptor ligands by making compounds that are even more sterically hindered than rimonabant, SLV319, and MK-0364. However, it is clear from our data that, although **4b** maintains strong selectivity toward CB_2 receptors and functional activity as an antagonist/inverse agonist, it shows at least a 10-fold lower affinity toward the CB_1 receptor than these previously developed compounds, thus suggesting that the binding site of this receptor will probably not accept ligands with bigger hindrance. Interestingly, our new compounds resemble previously reported non rigid structures that were also shown to be CB_1 receptor inverse agonists.[22]

In summary, we have described here the synthesis and pharmacological activity in vitro of a new class of sterically hindered CB_1 receptor ligands. The finding of **4b** will be useful for future studies exploring further the structural requirements of the CB_1 receptor binding site. Furthermore, **4b** will serve as a template for the development of new CB_1 inverse agonists, by capitalizing on the four phenyl groups present in this new molecule, which can be variedly derivatized as previously demonstrated by the several derivatives of rimonabant available to date.

2. Experimental

Melting points were taken on a Gallenkamp melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Bruker Avance 300 MHz spectrometer. Mass spectra of compounds **4a**–**d**, **9a**–**g**, **10a**–**c**, and **11a**–**c** were obtained by LC–MS/MS analysis carried out via liquid chromatography-electrospray-ion trap-time of flight (LC-ESI-IT-ToF) by using an IT-ToF mass spectrometer (Shimadzu) in conjunction with an LC-20AB (Shimadzu). The ToF analyser allowed the determination of the molecular mass with high resolution. Chromatographic separations were performed on silica gel column (Kieselgel 40, 0.040–0.063 mm, Merck). Reactions and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated F_{254} Merck plates. All new compounds were ~98% pure.

2.1. Synthesis of compounds 4a–d

2.1.1. *N*-(3,3-Diphenyl)propyl-2-chloro-2,2-diphenylacetamide (4a)

Dry triethylamine (130 mg, 1.28 mmol) was added to a stirred solution of 2-chloro-2,2-diphenylacetyl chloride (285 mg, 1.07 mmol), and 3,3-diphenylpropylamine (270 mg, 1.28 mmol) in dry dichloromethane (4 mL) at room temperature. After 4 h, the solvent was removed under reduced pressure, and the residue was taken up in EtOAc and washed with brine. The organic portion was dried (Na_2SO_4), solvent was evaporated and resulting residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 7:3) to give the title compound (273 mg, 58%) as a white solid: mp 125 °C; ^{1}H NMR ($CDCl_3$), δ 2.38 (q, 2H, J = 7.5 Hz), 3.37 (q, 2H, J = 6.6 Hz), 3.95 (t, 1H, J = 7.9 Hz), 7.02 (br s, 1H), 7.23–7.46 (m, 20H). HR m/z 462.1595 correlates with the chemical formula $[C_{29}H_{26}NOCl+Na]^+$ within ⩽1 ppm.

2.1.2. *N*-(3,3-Diphenyl)propyl-2,2-diphenylacetamide (4b)

N,*N*′-Dicyclohexylcarbodiimide (500 mg, 2.42 mmol) was added to a stirred mixture of diphenylacetic acid (460 mg, 2.17 mmol), HOBT (327 mg, 2.42 mmol), and 3,3-diphenylpropylamine (511 mg, 2.42 mmol) in dry dichloromethane (15 mL) at room temperature. After 16 h, the reaction mixture was filtered through a pad of Celite, and the filtrate was evaporated under reduced pressure. The resulting residue was taken up with EtOAc (15 mL) and washed with 2 N NaOH solution (2× 15 mL), 2 N HCl solution (2× 15 mL) and brine (2× 15 mL), then the organic phase was dried (Na_2SO_4), and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 7:3) to give the title compound (764 mg, 87%) as a white solid: mp 130 °C; ^{1}H NMR ($CDCl_3$) δ 2.28 (q, 2H, J = 7.2 Hz), 3.27 (q, 2H, J = 6.5 Hz), 3.86 (t, 1H, J = 7.8 Hz), 4.88 (s, 1H), 5.57 (br s, 1H), 7.19–7.38 (m, 20H). HR m/z 428.2007 correlates with the chemical formula $[C_{29}H_{27}NO+Na]^+$ within ⩽5 ppm.

2.1.3. *N*-(3,3-Diphenyl)propyl-2-methyl-2,2-diphenylacetamide (4c)

Starting from 2,2-diphenylpropionic acid (350 mg, 1.55 mmol), the title compound (530 mg, 82%) was obtained as reported for **4b** as a white solid: mp 133 °C; ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 3H), 2.25 (q, 2H, J = 7.4 Hz), 3.22 (q, 2H, J = 6.6 Hz), 3.77 (t, 1H, J = 7.9 Hz), 5.48 (br s, 1H), 7.13–7.36 (m, 20H). HR m/z 442.2156 correlates with the chemical formula $[C_{30}H_{29}NO+Na]^+$ within ⩽3 ppm.

2.1.4. *N*-(3,3-Diphenyl)propyl-2-hydroxy-2,2-diphenylacetamide (4d)

Benzilic acid (300 mg, 1,31 mmol) and 3,3-diphenylpropylamine (276 mg, 1.31 mmol) were dissolved in DMF (4 mL), and the resulting solution was cooled to 0 °C. After 30 min, HOBT (195 mg, 1.44 mmol) and *N*-methylmorpholine (265 mg, 2.62 mmol) were added, and the resulting mixture was stirred at 0 °C for 1 h, then DCC (300 mg, 1.45 mmol) was added. The resulting mixture was stirred at room temperature overnight, then washed with $NaHCO_3$ saturated solution and brine, and extracted with EtOAc. Organic phase was dried (Na_2SO_4), and solvent was removed under reduced pressure. The resulting residue was purified by silica gel column chromatography using *n*-hexane/EtOAc (7:3) as eluent to give the title compound (490 mg, 93%) as a white solid: mp 134–135 °C; ^{1}H NMR ($CDCl_3$) δ 2.32 (m, 2H), 3.32 (m, 2H), 3.84 (t, 1H, J = 7.9 Hz), 3.98 (s, 1H), 6.34 (br s, 1H), 6.92–7.43 (m, 20H). HR m/z 444.1920 correlates with the chemical formula $[C_{29}H_{27}NO_2+Na]^+$ within ⩽3 ppm.

2.2. Synthesis of compounds 9a–g, 10a–c, and 11a–c

2.2.1. 2-(4-Chlorophenyl)-2-phenyl-*N*-(3,3-diphenylpropyl)-acetamide (9a)

To a stirred solution of 2-(4-chlorophenyl)-2-phenylacetic acid[16] (500 mg, 2.03 mmol) in dry dichloromethane (10 mL), *N*,*N*′-carbonyldiimidazol (658 mg, 4.06 mmol) and 4-dimethylaminopyridine (123 mg, 1.01 mmol) were added at room temperature. After 30 min, 3,3-diphenylpropylamine (0.9 mL, 4.06 mmol) was added. The mixture was stirred at room temperature for 4 h, then solvent was removed under reduced pressure, and the resulting residue was taken up in EtOAc and washed with brine. The organic portion was dried (Na_2SO_4), the solvent was evaporated and the resulting residue was purified by silica gel column chromatography (hexane/EtOAc, 6:4) to give the title compound **9a** (709 mg, 80% yield) as a white solid; mp 115–120 °C; ^{1}H NMR ($CDCl_3$) δ 7.40–7.18 (m, 19H); 5.52 (br s, 1H); 4.81 (s, 1H); 3.87 (t, 1H, J = 7.9 Hz); 3.29 (q, 2H, J = 6.7 Hz); 2.29 (q, 2H, J = 7.4 Hz). HR m/z 462.1592 correlates with the chemical formula $[C_{29}H_{26}NOCl+Na]^+$ within ⩽1 ppm.

2.2.2. 2-(4-Bromophenyl)-2-phenyl-*N*-(3,3-diphenylpropyl)-acetamide (9b)

Starting from 2-(4-bromophenyl)-2-phenylacetic acid[16] (710 mg, 2.43 mmol), the title compound was obtained as reported for **9a** as a white solid in a 87% yield; mp 109–115 °C; ^{1}H NMR ($CDCl_3$) δ 7.49 (d, 2H, J = 8.4 Hz); 7.37–7.12 (m, 17H); 5.52 (br s, 1H); 4.78 (s, 1H); 3.89 (t, 1H, J = 7.8 Hz); 3.29 (q, 2H, J = 6.7 Hz); 2.29 (q, 2H, J = 7.4 Hz). HR m/z 506.1102 correlates with the chemical formula $[C_{29}H_{26}NOBr+Na]^+$ within ⩽2 ppm.

2.2.3. 2-(4-Methoxyphenyl)-2-phenyl-*N*-(3,3-diphenylpropyl)-acetamide (9c)

Starting from 2-(4-methoxyphenyl)-2-phenylacetic acid[16] (2 g, 8.54 mmol), the title compound was obtained as reported for **9a** as a white solid in a 75% yield; mp 100–105 °C; ^{1}H NMR ($CDCl_3$) δ 7.45–7.18 (m, 17H); 6.89 (d, 2H, J = 8.6 Hz); 5.56 (br s, 1H); 4.83 (s, 1H); 3.90–3.83 (m, 4H); 3.28 (q, 2H, J = 6.7 Hz); 2.28 (q, 2H, J = 7.3 Hz). HR m/z 458.2098 correlates with the chemical formula $[C_{30}H_{29}NO_2+Na]^+$ within ⩽1 ppm.

2.2.4. 2-(4-Methylphenyl)-2-phenyl-*N*-(3,3-diphenylpropyl)-acetamide (9d)

Starting from 2-(4-methylphenyl)-2-phenylacetic acid[16] (2.5 g, 11.05 mmol), the title compound was obtained as reported for **9a** as a white solid in a 72% yield; mp 125 °C; ^{1}H NMR ($CDCl_3$) δ 7.38–7.13 (m, 19H); 5.55 (br s, 1H); 4.86 (s, 1H); 3.87 (t, 1H, J = 7.8 Hz); 3.27 (q, 2H, J = 6.7 Hz); 2.37 (s, 3H), 2.28 (q, 2H, J = 7.5 Hz). HR m/z 442.2154 correlates with the chemical formula $[C_{30}H_{29}NO+Na]^+$ within ⩽3 ppm.

2.2.5. 2-(4-Nitrophenyl)-2-phenyl-*N*-(3,3-diphenylpropyl)-acetamide (9e)

Starting from 2-(4-nitrophenyl)-2-phenylacetic acid[16] (2.5 g, 9.72 mmol), the title compound was obtained as reported for **9a** as a white solid in a 72% yield; mp 125 °C; ^{1}H NMR ($CDCl_3$) δ 8.20 (d, 1H, J = 8.9 Hz), 7.45–7.18 (m, 18H); 5.53 (br s, 1H); 4.83 (s, 1H); 3.88 (t, 1H, J = 8.0 Hz); 3.32 (q, 2H, J = 6.6 Hz); 2.30 (q, 2H, J = 7.5 Hz). HR m/z 473.1803 correlates with the chemical formula $[C_{29}H_{26}NO_3+Na]^+$ within ⩽7 ppm.

2.2.6. 2-(4-Hydroxyphenyl)-2-phenyl-*N*-(3,3-diphenylpropyl)-acetamide (9f)

To a solution of sodium iodide (900 mg, 6.07 mmol) and amide **9c** (1.2 g, 2.76 mmol), in CH_3CN (20 mL), chlorotrimethylsilane (0,80 mL, 6.07 mmol) was added at 23 °C. After heating under reflux for 16 h, the reaction was quenched with water and extracted with ether. The organic layer was washed with saturated $Na_2S_2O_3$ and brine, dried over Na_2SO_4, and evaporated. Column chromatography (silica gel, 1:1 petroleum ether/ether) gave **9f** as a white solid in a 60% yield; mp 145–150 °C; ^{1}H NMR ($CDCl_3$) δ 7.39–7.18 (m, 17H); 6.89 (d, 1H, J = 8.5 Hz); 6.77 (d, 1H, J = 8.5 Hz); 5.56 (br s, 1H); 4.82 (s, 1H); 3.89 (t, 1H, J = 8.0 Hz); 3.27 (q, 2H, J = 6.7 Hz); 2.28 (q, 2H, J = 7.3 Hz). HR m/z 444.1921 correlates with the chemical formula $[C_{29}H_{27}NO_2+Na]^+$ within ⩽3 ppm.

2.2.7. 2-(4-Aminophenyl)-2-phenyl-*N*-(3,3-diphenylpropyl)-acetamide (9g)

A solution of amide **9e** (1.5 g, 3.32 mmol), zinc dust (2.12 g, 33.2 mmol), absolute ethanol (40 mL), and 37% HCl (15 mL) at −10 °C was mixed under reflux for 2 h. After addition of 10% NaOH (pH ∼10), the organic layer was extracted with ether, washed with brine, dried over Na_2SO_4, and evaporated. Column chromatography (silica gel, 1:1 petroleum ether/ethyl acetate) gave 90% yield of **9g**

as a yellow solid; mp 135 °C; ^{1}H NMR ($CDCl_3$) δ 7.25–7.14 (m, 15H); 6.98 (d, 2H, J = 6.4 Hz); 6.63 (d, 2H, J = 6.4 Hz); 5.57 (br s, 1H); 4.78 (s, 1H); 3.83 (t, 1H, J = 6.0 Hz); 3.22 (q, 2H, J = 5.0 Hz) 2.17 (q, 2H, J = 5.0 Hz). HR m/z 443.2101 correlates with the chemical formula $[C_{29}H_{28}N_2O+Na]^+$ within ⩽2 ppm.

2.2.8. *N*-(3-Phenyl)propyl-2,2-diphenylacetamide (10a)

Starting from 2,2-diphenylacetic acid (400 mg, 1.89 mmol) and 3-phenylpropylamine (383 mg, 2.83 mmol), the title compound was obtained as reported for **9a** as a white solid in a 63% yield; mp 135 °C; ^{1}H NMR ($CDCl_3$) δ 7.39–6.94 (m, 15H), 5.60 (br s, 1H), 4.88 (s, 1H), 3.40 (q, 2H, J = 6.9 Hz), 2.66 (t, 2H, J = 7.0 Hz), 1.89–1.77 (m, 2H). HR m/z 352.1678 correlates with the chemical formula $[C_{23}H_{23}NO+Na]^+$ within ⩽2 ppm.

2.2.9. *N*-(3-Phenyl)propyl-2-(4-chlorophenyl)-2-phenylacetamide (10b)

Starting from 2-(4-chlorophenyl)2-phenylacetic acid[16] (620 mg, 2.52 mmol), and 3-phenylpropylamine (681 mg, 5.04 mmol) the title compound was obtained as reported for **9a** as a white solid in a 70% yield; mp 125 °C; ^{1}H NMR ($CDCl_3$) δ 7.35–7.05 (m, 14H), 5.57 (br s, 1H), 4.86 (s, 1H), 3.35 (q, 2H, J = 6.7 Hz), 2.62 (t, 2H, J = 7.6 Hz), 1.89–1.80 (m, 2H). HR m/z 386.1250 correlates with the chemical formula $[C_{23}H_{22}NOCl+Na]^+$ within ⩽10 ppm.

2.2.10. *N*-(3-Phenyl)propyl-2-(4-bromophenylacetamide) (10c)

Starting from 2-(4-bromophenyl)-2-phenylacetic acid[16] (1.85 g, 6.35 mmol) and 3-phenylpropylamine (1.72 g, 12.70 mmol), the title compound was obtained as reported for **9a** as a white solid in a 81% yield; mp 132 °C; ^{1}H NMR ($CDCl_3$) δ 7.50–7.10 (m, 14H), 5.57 (br s, 1H), 4.84 (s, 1H), 3.34 (q, 2H, J = 6.7 Hz), 2.62 (t, 2H, J = 7.6 Hz); 1.96–1.82 (m, 2H). HR m/z 430.0765 correlates with the chemical formula $[C_{23}H_{22}NOBr+Na]^+$ within ⩽3 ppm.

2.2.11. *N*-(3,3-Diphenyl)propyl-2-phenylacetamide (11a)

Starting from phenylacetic acid (300 mg, 2.21 mmol) and 3,3-diphenylpropylamine (700 mg, 3.31 mmol), the title compound was obtained as reported for **9a** as a white solid in a 85% yield; mp 85 °C; ^{1}H NMR ($CDCl_3$) δ 8.0 (s, 1H), 7.41–7.06 (m, 15H), 4.10 (t, 1H, J = 7.2 Hz), 3.44 (s, 2H), 3.33 (q, 2H, J = 6.9 Hz), 2.19 (q, 2H, J = 7.5 Hz). HR m/z 352.1675 correlates with the chemical formula $[C_{23}H_{23}NO+Na]^+$ within ⩽2 ppm.

2.2.12. *N*-(3,3-Diphenyl)propyl-2-(4-chlorophenyl)acetamide (11b)

Starting from 4-chlorophenylacetic acid (500 mg, 2.93 mmol) and 3,3-diphenylpropylamine (1.2 g, 5.86 mmol), the title compound was obtained as reported for **9a** as a white solid in a 71% yield; mp 107 °C; ^{1}H NMR ($CDCl_3$) δ 7.37–7.16 (m, 14H), 5.27 (s, 1H), 3.91 (t, 1H, J = 7.7 Hz), 3.47 (s, 2H), 3.27 (q, 2H, J = 6.6 Hz), 2.25 (q, 2H, J = 7.4 Hz). HR m/z 386.1247 correlates with the chemical formula $[C_{23}H_{22}NOCl+Na]^+$ within ⩽10 ppm.

2.2.13. *N*-(3,3-Diphenyl)propyl-2-(4-bromophenyl) acetamide (11c)

Starting from 4-bromophenylacetic acid (500 mg, 2.32 mmol) and 3,3-diphenylpropylamine (980 mg, 4.64 mmol) the title compound was obtained as reported for **9a** as a white solid in a 75% yield; mp 110 °C; ^{1}H NMR ($CDCl_3$) δ 7.50 (d, 2H, J = 7.4 Hz), 7.32–7.12 (m, 10H), 6.96 (d, 2H, J = 7.5 Hz), 5.33 (br s, 1H), 3.90 (q, 1H, J = 7.5 Hz), 3.46 (s, 2H); 3.24 (q, 2H, J = 6.6 Hz), 2.25 (q, 2H, J = 7.2 Hz). HR m/z 430.0776 correlates with the chemical formula $[C_{23}H_{22}NOBr+Na]^+$ within <1 ppm.

2.3. Binding assay

For CB_1 and CB_2 receptor binding assays, the new compounds were tested using membranes from HEK cells transfected with either the human CB_1 or CB_2 receptor and [^{3}H]-(−)-cis-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxy-propyl)-cyclohexanol ([^{3}H]CP-55,940) (K_d = 0.31 nM for CB_2 and 0.18 nM for CB_1 receptors) as the high affinity ligand as described by the manufacturer (Perkin-Elmer, Italia).[23] Displacement curves were generated by incubating drugs with [^{3}H]CP-55,940 (0.084 for CB_2 and 0.14 nM for CB_1 binding assay). In all cases, K_i values were calculated by applying the Cheng–Prusoff equation to the IC_{50} values (obtained by GraphPad) for the displacement of the bound radioligand by increasing concentrations of the test compounds.

2.4. cAMP assay

Cyclic AMP assays were performed on intact confluent N18TG2 cells plated in six-well dishes and stimulated for 10 min at 37°C with forskolin 1 μM in 400 μL of serum-free Dulbecco's modified Eagle's medium containing 20 mM Hepes, 0.1 mg/mL BSA, 0.1 mM 1-methyl-3-isobutylxanthine.[24] Cells were treated with vehicle (methanol, 0.1%) or compounds (at various concentrations) or WIN-55,212 (100 nM) or WIN-55,212 plus compound **4b** (1 μM). After incubation, 800 μL of ethanol was added, cells were extracted and cyclic AMP was determined by means of a cyclic AMP assay kit (Amersham, UK), as advised by the manufacturer.

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