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Novel sterically hindered cannabinoid CB_1 receptor ligands

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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 synthesied and tested as ligands of cannabinoid CB_1 and CB_2 receptors. All compounds exhibited afnity 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 ($_i$ 58 nM). It was 140-fold selective over CB_2 receptors ($_i$ 7800 nM) and behaved as an inverse agonist by stimulating forskolin-induced cAMP formation in mouse N18T2 neuroblastoma cells. This compound is the rst of a novel class of tetraphenyl CB_1 ligands that, in view of its easy synthesis and high afnity 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 -proteins of $_{io}$ 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] inammation, [7] liver diseases,[8] and obesity,[9] in which blocking the cannabinoid receptors might be benecial. 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. ne of these drugs, the CB $_1$ receptor antagonistinverse agonist rimonabant (SR141716A) (Fig. 1) (1) belongs to the class of diarylpyraole 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 Eas an aduncto exercise and diet for the treatment of obesity and metabolic syndrome, and has proved useful to reduce body weight, low HD-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 antagonistsinverse agonists have undergone clinical trials for the treatment of obesityS319 (4), whose structure still resembles that of rimonabant, and M-0364 (5), which instead belongs to a different class of acyclic compounds and exhibits higher afnity 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 D^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, S319, and M-0364.

n 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 synthesied eleven novel N-(3,3-diphenyl)propyl-2,2-diphenylacetamide derivatives (4a–d and 9a–g) and six triphenylacetamides (10a–c and 11a–c). sing the very simple synthetic procedure shown in Scheme 1, we obtained a-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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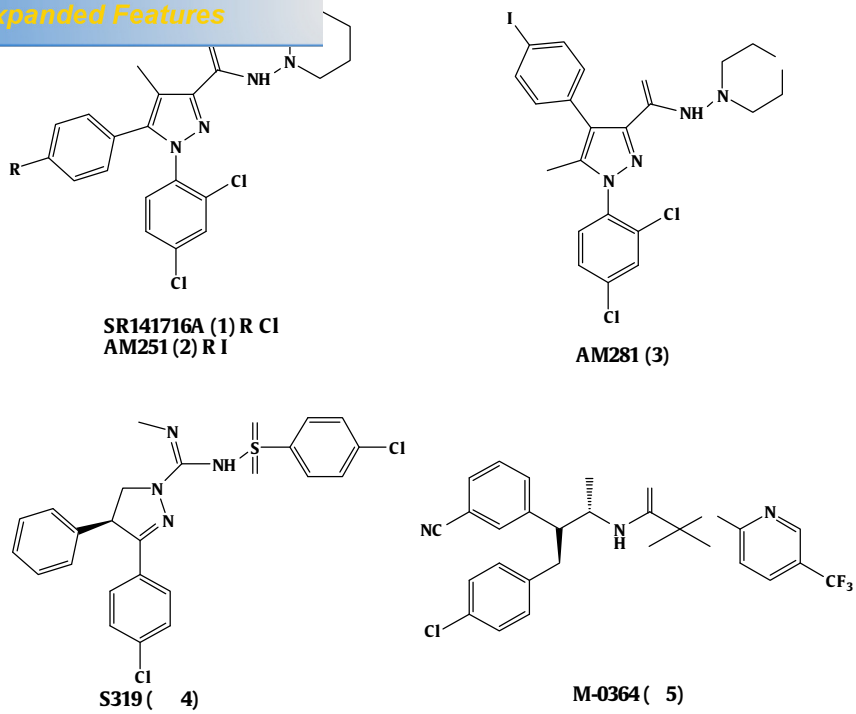


Figure 1. Chemical structures of some CB_1 receptor antagonistsinverse agonists.

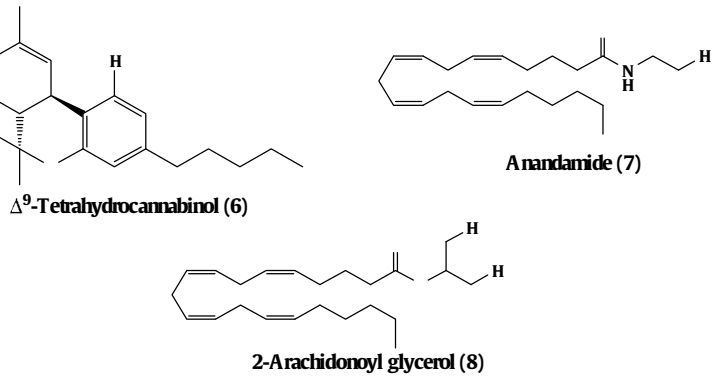
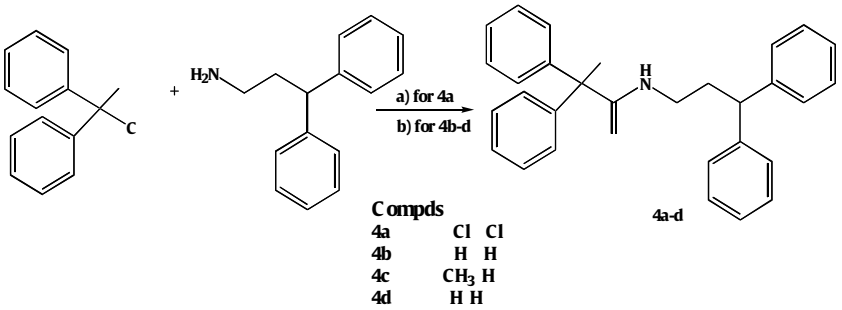
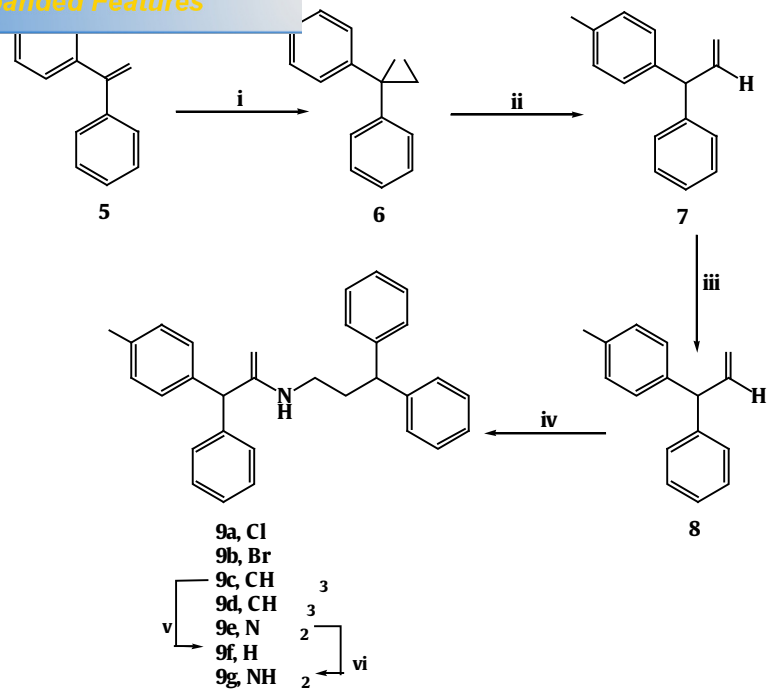


Figure 2. Chemical structures of D^9-tetrahydrocannabinol and the endocannabinoids anandamide and 2-arachidonoylglycerol.



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, HBT, DCM, rt, 16 h.

diphenylacetic acid[16] (Scheme 2). Homologation of commercially available 4--benophenones 5 (Cl, Br, CH_3, CH_3, and N_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 onesreagent [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 chloridesodium iodide [19] (Scheme 2). Reduction of the nitro group of 9e by nHCl produced the amine derivative 9g[20] (Scheme 2).

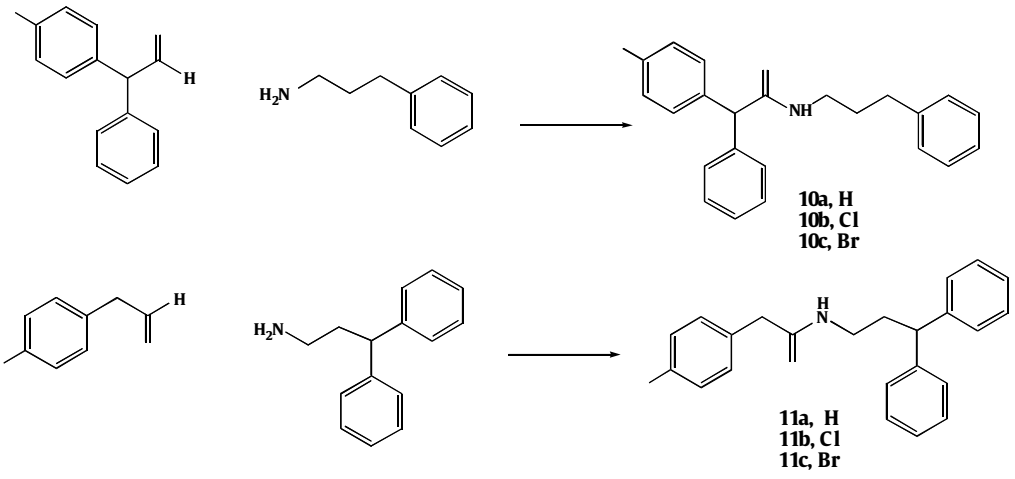


Scheme 2. Procedure for the multi-step synthesis of compounds 9a–g. Reagents and conditions(i) NaH, TMS-I, THF(ii) BF 3–Et2, benene(iii) onesreagent, isopropyl alcohol, acetone(iv) 3,3-diphenylpropylamine, CDI, DMAP(v) Nal, TMS–Cl, CH 3CN(vi) n dust, HCl concd, EtH absolute.

Finally, we synthesied 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).

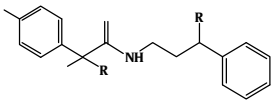
The new compounds were tested for their afnities for human recombinant cannabinoid receptors CB_1 and CB_2, and the corresponding observed i (l M) values are shown in Table 1. The functional activity of the most potent CB_1 ligand (4b) was also assessed at CB_1 receptors by studying its effect on forskolin-induced cAMP formation in mouse N18T2 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 afnity for CB 1 receptors (i 58 nM) was higher than that of anandamide (i 89 nM). This compound also showed high (~140-fold) selectivity versus CB_2 receptors (i 7900 nM). Compounds (4a, 4c–d) obtained from 4b, by introducing various substituents (Cl, CH_3, and H, respectively) in position a to the amide group, although showing still good afnity for CB 1, lost the selectivity versus CB_2 cannabinoid receptors.

The introduction of various substituents (Cl, Br, CH 3, CH_3, N 2, H, 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 rst 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 conditionsdry DCM, CDI, DMAP, rt, 4 h.

nt CB_1 and CB_2 receptors



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

	R	R		′	hCB$_1$ ($_i$, l M)	hCB$_2$ ($_i$, l M)
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	H	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	CH $_3$	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	N $_2$	H	Phe	Phe	0.56 0.05	0.79 0.05
9f	H	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 $_i$, (l M). 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.

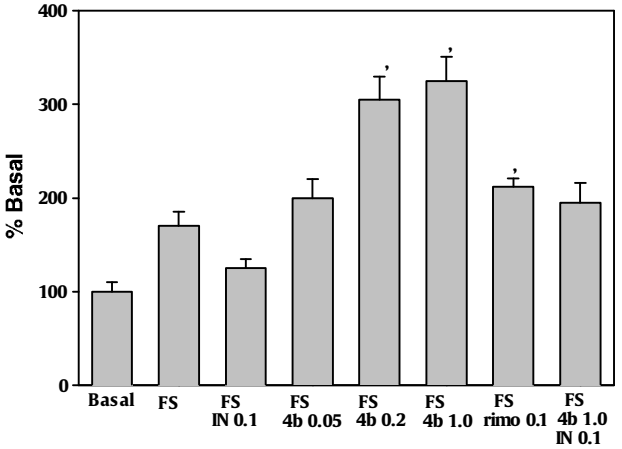


Figure 3. Effect of 4b (0.05, 0.2, and 1.0 l M) on forskolin (FS, 1 l M)-induced cAMP formation in intact N18T2 cells (p 0.05 vs basal). The effects of IN55,212-2 (0.1 l M) and rimonabant (rimo, 0.1 l M) are shown as a comparison. ' p 0.05, 0.01 versus Basal. p 0.05 versus FS.

phenylacetamides 10a–c and 11a–c, respectively. This led to a signicant reduction of the afnity and, with the exception of 10a and 11a, to the loss of the selectivity for the CB_1 receptors. It must be emphasied, 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 $_i$ 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 N18T2 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 l M) did not signicantly elevate cAMP levels in the absence of forskolin (not shown). As expected, in the same assay, the CB_1CB_2 agonist IN55,212-2 (0.1 l M) inhibited forskolin-stimulated cAMP formation, whereas the CB_1 inverse agonist, rimonabant (0.1 l M), produced a stimulation of forskolin effect. IN55,212-2 (0.1 l M) also blocked the effect of 4b (1 l M) on cAMP formation (Fig. 3), thus suggesting that the effect of 4b was mediated by CB_1 receptors.

The nding of 4b demonstrates that it is still possible to obtain high afnity and selective CB $_1$ receptor ligands by making compounds that are even more sterically hindered than rimonabant, S319, and M-0364. However, it is clear from our data that, although 4b maintains strong selectivity toward CB_2 receptors and functional activity as an antagonistinverse agonist, it shows at least a 10-fold lower afnity 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 nding of 4b will be useful for future studies exploring further the structural reuirements of the CB $_1$ receptor binding site. Furthermore, 4b will serve as a template for the development of new CB_1 inverse agonists, by capitaliing on the four phenyl groups present in this new molecule, which can be variedly derivatied as previously demonstrated by the several derivatives of rimonabant available to date.

2. Experimental

Melting points were taken on a allenkamp melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Bruker Avance 300 MHspectrometer. Mass spectra of compounds 4a–d, 9a–g, 10a–c, and 11a–c were obtained by C–MSMS analysis carried out via liuid chromatography-electrospray-ion trap-time of ight (C-ESI-IT-ToF) by using an IT-ToF mass spectrometer (Shimadu) in conunction with an C-20AB (Shimadu). The ToF analyser allowed the determination of the molecular mass with high resolution. Chromatographic separations were performed on silica gel column (ieselgel 40, 0.040–0.063 mm, Merck). Reactions and product mixtures were routinely monitored by thin-layer chromatography (TC) on silica gel precoated F $_{254}$ Merck plates. All new compounds were ~98pure.

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 m) at room temperature. After 4 h, the solvent was removed under reduced pressure, and the residue was taken up in EtAc and washed with brine. The organic portion was dried (Na_2S $_4$), solvent was evaporated and resulting residue was puried by silica gel column chromatography (n-hexane EtAc, 73) to give the title compound (273 mg, 58) as a white solidmp 125 °C ^{1}H NMR ($CDCl_3$), d 2.38 (, 2H, 7.5 H), 3.37 (, 2H, 6.6 H), 3.95 (t, 1H, 7.9 H), 7.02 (br s, 1H), 7.23–7.46 (m, 20H). HR m 462.1595 correlates with the chemical formula $C_{29}H_{26}NClNa$ within ⩽1 ppm.

N,N′-Dicyclohexylcarbodiimide (500 mg, 2.42 mmol) was added to a stirred mixture of diphenylacetic acid (460 mg, 2.17 mmol), HBT (327 mg, 2.42 mmol), and 3,3-diphenylpropylamine (511 mg, 2.42 mmol) in dry dichloromethane (15 m) at room temperature. After 16 h, the reaction mixture was ltered through a pad of Celite, and the ltrate was evaporated under reduced pressure. The resulting residue was taken up with EtAc (15 m) and washed with 2 N NaH solution (2 × 15 m), 2 N HCl solution (2× 15 m) and brine (2 × 15 m), then the organic phase was dried (Na_2S_4), and evaporated under reduced pressure. The resulting residue was puried by silica gel column chromatography (n-hexaneEtAc, 73) to give the title compound (764 mg, 87) as a white solidmp 130 °C ^{1}H NMR ($CDCl_3$) d 2.28 (, 2H, 7.2 H), 3.27 (, 2H, 6.5 H), 3.86 (t, 1H, 7.8 H), 4.88 (s, 1H), 5.57 (br s, 1H), 7.19–7.38 (m, 20H). HR m 428.2007 correlates with the chemical formula $C_{29}H_{27}NNa$ 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 solidmp 133 °C ^{1}H NMR ($CDCl_3$) d 1.99 (s, 3H), 2.25 (, 2H, 7.4 H), 3.22 (, 2H, 6.6 H), 3.77 (t, 1H, 7.9 H), 5.48 (br s, 1H), 7.13–7.36 (m, 20H). HR m 442.2156 correlates with the chemical formula $C_{30}H_{29}NNa$ within ⩽3 ppm.

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

Benilic acid (300 mg, 1,31 mmol) and 3,3-diphenylpropylamine (276 mg, 1.31 mmol) were dissolved in DMF (4 m), and the resulting solution was cooled to 0 °C. After 30 min, HBT (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 $NaHC_3$ saturated solution and brine, and extracted with EtAc. rganic phase was dried (Na_2S_4), and solvent was removed under reduced pressure. The resulting residue was puried by silica gel column chromatography using n-hexaneEtAc (73) as eluent to give the title compound (490 mg, 93) as a white solid mp 134–135 °C ^{1}H NMR ($CDCl_3$) d 2.32 (m, 2H), 3.32 (m, 2H), 3.84 (t, 1H, 7.9 H), 3.98 (s, 1H), 6.34 (br s, 1H), 6.92–7.43 (m, 20H). HR m 444.1920 correlates with the chemical formula $C_{29}H_{27}N_2Na$ 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 m), N,N′-carbonyldiimidaol (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 m, 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 EtAc and washed with brine. The organic portion was dried (Na_2S_4), the solvent was evaporated and the resulting residue was puried by silica gel column chromatography (hexaneEtAc, 64) to give the title compound 9a (709 mg, 80yield) as a white solidmp 115–120 °C ^{1}H NMR ($CDCl_3$) d 7.40–7.18 (m, 19H)5.52 (br s, 1H)4.81 (s, 1H)3.87 (t, 1H, 7.9 H)3.29 (, 2H, 6.7 H)2.29 (, 2H, 7.4 H). HR m 462.1592 correlates with the chemical formula $C_{29}H_{26}NClNa$ 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 87yieldmp 109–115 °C ^{1}H NMR ($CDCl_3$) d 7.49 (d, 2H, 8.4 H)7.37–7.12 (m, 17H)5.52 (br s, 1H)4.78 (s, 1H)3.89 (t, 1H, 7.8 H)3.29 (, 2H, 6.7 H) 2.29 (, 2H, 7.4 H). HR m 506.1102 correlates with the chemical formula $C_{29}H_{26}NBrNa$ 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 75yieldmp 100–105 °C ^{1}H NMR ($CDCl_3$) d 7.45–7.18 (m, 17H)6.89 (d, 2H, 8.6 H)5.56 (br s, 1H) 4.83 (s, 1H)3.90–3.83 (m, 4H)3.28 (, 2H, 6.7 H)2.28 (, 2H, 7.3 H). HR m 458.2098 correlates with the chemical formula $C_{30}H_{29}N_2Na$ 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 72yieldmp 125 °C ^{1}H NMR ($CDCl_3$) d 7.38–7.13 (m, 19H)5.55 (br s, 1H)4.86 (s, 1H)3.87 (t, 1H, 7.8 H)3.27 (, 2H, 6.7 H)2.37 (s, 3H), 2.28 (, 2H, 7.5 H). HR m 442.2154 correlates with the chemical formula $C_{30}H_{29}NNa$ 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 72yieldmp 125 °C ^{1}H NMR ($CDCl_3$) d 8.20 (d, 1H, 8.9 H), 7.45–7.18 (m, 18H)5.53 (br s, 1H)4.83 (s, 1H)3.88 (t, 1H, 8.0 H)3.32 (, 2H, 6.6 H)2.30 (, 2H, 7.5 H). HR m 473.1803 correlates with the chemical formula $C_{29}H_{26}N_3Na$ 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 m), chlorotrimethylsilane (0,80 m, 6.07 mmol) was added at 23 °C. After heating under reux for 16 h, the reaction was uenched with water and extracted with ether. The organic layer was washed with saturated $Na_2S_2O_3$ and brine, dried over Na_2S_4, and evaporated. Column chromatography (silica gel, 11 petroleum etherether) gave 9f as a white solid in a 60yieldmp 145–150 °C ^{1}H NMR ($CDCl_3$) d 7.39–7.18 (m, 17H)6.89 (d, 1H, 8.5 H)6.77 (d, 1H, 8.5 H)5.56 (br s, 1H)4.82 (s, 1H)3.89 (t, 1H, 8.0 H)3.27 (, 2H, 6.7 H) 2.28 (, 2H, 7.3 H). HR m 444.1921 correlates with the chemical formula $C_{29}H_{27}N_2Na$ 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), inc dust (2.12 g, 33.2 mmol), absolute ethanol (40 m), and 37HCl (15 m) at −10 °C was mixed under reux for 2 h. After addition of 10NaH (pH ∼10), the organic layer was extracted with ether, washed with brine, dried over Na_2S_4, and evaporated. Column chromatography (silica gel, 11 petroleum etherethyl acetate) gave 90yield of 9g

7.14 (m, 15H)6.58 (d, 2H, 6.4 H)6.65 (d, 2H, 6.4 H)5.57 (br s, 1H)4.78 (s, 1H)3.83 (t, 1H, 6.0 H)3.22 (, 2H, 5.0 H) 2.17 (, 2H, 5.0 H). HR m 443.2101 correlates with the chemical formula C $_{29}H_{28}N_2Na$ 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 63yield mp 135 °C ^{1}H NMR ($CDCl_3$) d 7.39–6.94 (m, 15H), 5.60 (br s, 1H), 4.88 (s, 1H), 3.40 (, 2H, 6.9 H), 2.66 (t, 2H, 7.0 H), 1.89–1.77 (m, 2H). HR m 352.1678 correlates with the chemical formula C $_{23}H_{23}$NNa 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 70yieldmp 125 °C ^{1}H NMR ($CDCl_3$) d 7.35–7.05 (m, 14H), 5.57 (br s, 1H), 4.86 (s, 1H), 3.35 (, 2H, 6.7 H), 2.62 (t, 2H, 7.6 H), 1.89–1.80 (m, 2H). HR m 386.1250 correlates with the chemical formula C $_{23}H_{22}$NClNa 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 81yieldmp 132 °C ^{1}H NMR ($CDCl_3$) d 7.50–7.10 (m, 14H), 5.57 (br s, 1H), 4.84 (s, 1H), 3.34 (, 2H, 6.7 H), 2.62 (t, 2H, 7.6 H)1.96–1.82 (m, 2H). HR m 430.0765 correlates with the chemical formula C $_{23}H_{22}$NBrNa 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 85yield mp 85 °C ^{1}H NMR ($CDCl_3$) d 8.0 (s, 1H), 7.41–7.06 (m, 15H), 4.10 (t, 1H, 7.2 H), 3.44 (s, 2H), 3.33 (, 2H, 6.9 H), 2.19 (, 2H, 7.5 H). HR m 352.1675 correlates with the chemical formula C $_{23}H_{23}$NNa 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 yieldmp 107 °C ^{1}H NMR ($CDCl_3$) d 7.37–7.16 (m, 14H), 5.27 (s, 1H), 3.91 (t, 1H, 7.7 H), 3.47 (s, 2H), 3.27 (, 2H, 6.6 H), 2.25 (, 2H, 7.4 H). HR m 386.1247 correlates with the chemical formula C $_{23}H_{22}$NClNa 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 yieldmp 110 °C ^{1}H NMR ($CDCl_3$) d 7.50 (d, 2H, 7.4 H), 7.32–7.12 (m, 10H), 6.96 (d, 2H, 7.5 H), 5.33 (br s, 1H), 3.90 (, 1H, 7.5 H), 3.46 (s, 2H)3.24 (, 2H, 6.6 H), 2.25 (, 2H, 7.2 H). HR m 430.0776 correlates with the chemical formula C $_{23}H_{22}$NBrNa within 1 ppm.

2.3. Binding assay

For CB_1 and CB_2 receptor binding assays, the new compounds were tested using membranes from HEcells 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}HCP-55,940) ($_d$ 0.31 nM for CB $_2$ and 0.18 nM for CB_1 receptors) as the high afnity ligand as described by the manufacturer (Perkin-Elmer, Italia).[23] Displacement curves were generated by incubating drugs with ^{3}HCP-55,940 (0.084 for CB $_2$ and 0.14 nM for CB_1 binding assay). In all cases, $_i$ values were calculated by applying the Cheng–Prusoff euation to the IC $_{50}$ values (obtained by raphPad) 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 conuent N18T2 cells plated in six-well dishes and stimulated for 10 min at 37°C with forskolin 1 l M in 400 l of serum-free Dulbeccos modied Eagles medium containing 20 mM Hepes, 0.1 mgmBSA, 0.1 mM 1-methyl-3-isobutylxanthine.[24] Cells were treated with vehicle (methanol, 0.1) or compounds (at various concentrations) or IN-55,212 (100 nM) or IN-55,212 plus compound 4b (1 l M). After incubation, 800 l of ethanol was added, cells were extracted and cyclic AMP was determined by means of a cyclic AMP assay kit (Amersham,), as advised by the manufacturer.

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