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Short communication

Structural–activity relationship study on C-4 carbon atom of the CB_1 antagonist SR141716: synthesis and pharmacological evaluation of 1,2,4-triazole-3-carboxamides

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Abstract

A series of 1,2,4-triazole-3-carboxamides has been prepared from alkyl-1,2,4-triazole-3-carboxylates under mild conditions. The ability of these triazoles to displace [^{3}H]-CP55940 from CB_1 cannabinoid receptor was measured. However, they showed only poor to moderate binding affinities, indicating that substitution of the C-4 pyrazole atom of the CB_1 reference compound SR141716 by a nitrogen atom results in loss of affinity. Further investigations for functionality indicated that the compound **6a** exhibited significant cannabinoid antagonistic properties in the mouse vas deferens functional assay. This leads us to the conclusion that **6a** binds at a different CB_1 binding site or at a new cannabinoid receptor subtype.

Keywords: 1,2,4-Triazole; Cannabinoid; Mouse vas deferens; SR141716

Mots clés: 1,2,4-Triazole; Cannabiroide; Canal déférent de souris; SR141716

1. Introduction

Cannabinoid receptors belong to the large superfamily of seven transmembrane domains containing receptors, the G-protein coupled receptors. They include the CB_1 and CB_2 subtypes [1–4] and various classes of compounds act as cannabinoid ligands [5,6]. Their structure–activity relationship (SAR) properties have been of considerable interest these last years [7]. In 1994, the first high-affinity antagonist of the brain, CB_1 cannabinoid receptor, was identified: *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (SR141716) [8]. Currently, SR141716 is undergoing advanced clinical trials as an appetite suppressant [9,10]. As a potent antagonist for the CB_1 receptor, SR141716 was the lead compound for studies designed to examine the SAR of related compounds. Structural modifications around the aminopiperidine region, 2,4-dichlorophenyl and chlorophenyl substituents of the parent pyrazole SR141716 gave insights into the CB_1 pharmacophore [11–16]. Conformationally restricted SR141716 analogues have also been reported [17]. Among various studies dedicated to SAR for SR141716, only a few have been devoted to the C-4 pyrazole position.

As part of our ongoing studies dealing with triazoles as cannabinoids [18,19] we synthesized and tested in isolated tissues 1,5-diaryl-1,2,4-triazole-3-carboxamides. Reports have been

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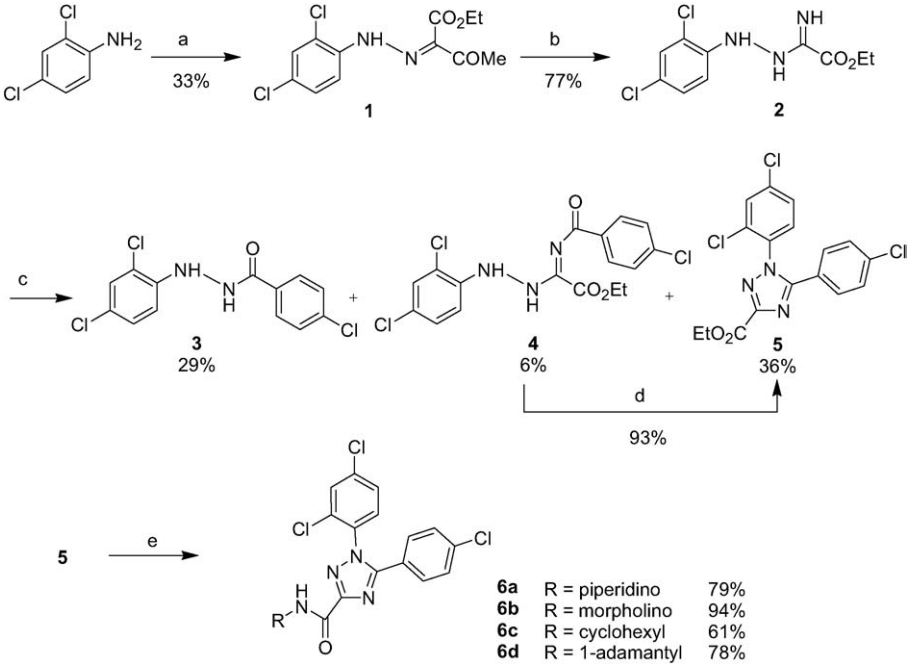
published by Dyck et al. [20] and by Lange et al. [21,22] on 1,2,4-triazole-3-carboxamides. These triazoles were less potent ligands of CB_1 receptors than the corresponding pyrazole SR141716. These studies have prompted us to report here our own results on 1,2,4-triazole-3-carboxamides. This report describes a convenient alternative synthesis for triazolecarboxamides with improved yields. In an effort to study the pharmacological properties of these compounds and in addition to their abilities to displace the radioligand [^{3}H]-CP55940 in CHO cells expressing the human CB_1 receptor, their cannabinoid functional activity has been studied in the mouse vas deferens.

2. Chemistry

1,2,4-Triazole-3-carboxamides used to be synthesized from 4-arylazo-2-aryl-2-oxazolin-5-ones reacting with the appropriate amine [23–27]. Dyck et al. [20] and Lange et al. [21] prepared 1,2,4-triazole-3-carboxylates as described by Tsuji et al. [28] and Kudo et al. [29], respectively. They then converted these esters to the corresponding amides by saponification, to give carboxylic acid, followed by amination. The 1,2,4-triazole-3-carboxamides **6a**–**d** described here were prepared in a different synthetic pathway as outlined in Scheme 1. Diazotization of commercially available 2,4-dichloroaniline in presence of tetrafluoroboric acid gave the diazonium salt [30], which was directly coupled with ethyl acetoacetate to afford the oxobutanoate **1**. Subsequent treatment with bromine, then with an aqueous solution of ammonium hydroxide led to the iminoacetate **2**. The cyclization [31] of **2** with 4-chlorobenzoyl chloride was achieved in the presence of pyridine. Three reaction products have been isolated: 4-chloro-*N'*-(2,4-dichlorophenyl)benzohydrazide (**3**), ethyl 2-(4-chlorobenzamido)-2-(2-(2,4-dichlorophenyl)hydrazinyl)acetate (**4**) and the desired ethyl 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-1,2,4-triazolecarboxylate (**5**) in a 5:1:6 ratio. The addition product **4** was allowed to cyclize to give triazole **5** by refluxing in 1,4-dioxane with excess of pyridine for 2 weeks. The preparation of the final 1,2,4-triazole-3-carboxamides **6a**–**d** was achieved by adopting a simple one-pot procedure. In fact, the most common procedure to prepare carboxamides of heterocycles involves the three classical steps: hydrolysis of the ester to acid followed by acid chloride formation, and finally, reaction with the appropriate amine or hydrazine to give the corresponding carboxamide or carboxylic acid hydrazide [15–17]. Conversion of ethyl esters to carboxylic acid hydrazides under mild reaction conditions, via organoaluminum reagents, have been described by Benderly and Stavchansky [32]. Applying this procedure to our 1,2,4-triazole series, the 1,2,4-triazole-3-carboxamides **6a**–**d** were obtained in good yield in a one-step synthesis by treatment of the triazolecarboxylate **5** with 5 equiv. of an aluminum complex which was previously prepared in situ reacting trimethylaluminum with the corresponding amine or hydrazine.

3. Pharmacology

The biological activities of compounds **6a**–**d** were evaluated for their ability to displace the radioligand [^{3}H]-CP55940 in CHO cells expressing the human CB_1 receptor. Their cannabinoid functional activity has been studied in the mouse vas deferens. The effect of compounds **6a**–**d** in isolated tissue was



Scheme 1. (a) i) $NaNO_2$, aq. HBF_4, 0 °C.; ii) ethyl acetoacetate, NaOAc, EtOH, H_2O, 0 °C; (b) i) Br_2, NaOAc, AcOH; ii) NH_4OH 30%, acetone; (c) 4-chlorobenzoylchloride, pyridine, 1,4-dioxane, reflux; (d) pyridine, 1,4-dioxane, reflux; (e) 1-aminopiperidine for **6a**, 4-aminomorpholine for **6b**, cyclohexylamine for **6c** and 1-aminoadamantane for **6d**, $Al(Me)_3$, N_2 atmosphere, dry CH_2Cl_2, 40 °C.

tested in order to determine whether they behave as cannabinoid receptor agonists or antagonists.

4. Results and discussion

4.1. Binding assays

Affinities at CB_1 receptors for triazoles **6a**–**d** were determined by measuring their ability to displace the radioligand [^{3}H]-CP55940. These data are reported in Table 1 including K_i values of the reference pyrazole SR141617. Compound **6a**, triazole SR141716 analogue, possesses low CB_1 receptor affinity ($K_i > 1$ μM). The value reported here is higher than the K_i value (382 nM) reported by Lange et al. [21]. In this compound, the C-4 methyl of SR141716 was replaced by a nitrogen. For pyrazole SR141716 analogues, it has been shown [13,15,33] that replacing the methyl group at the C-4 position by bromine, iodine, hydrogen, or *n*-pentyl maintained the affinity value in a nanomolar range. The 1,2-diarylimidazolecarboxamide analogue to the pyrazole SR141716 has been reported to have similar CB_1 receptor affinity as compared with the pyrazole parent [21]. Based on these data, it seems reasonable to conclude that the low recognition of **6a** for CB_1 receptor can be attributed to a different geometry of the molecule **6a** compared to the pyrazole SR141716. A conformational analysis of **6a** and SR141716 was made and is described below in the present article.

Concerning the *N*-carboxamide substitution of derivatives of 1,2,4-triazole-3-carboxamide, it is interesting to note that at 1 μM, piperidino (**6a**, 31.7%) and morpholino (**6b**, 28.3%) carboxamides have modest affinity while cyclohexyl carboxamide (**6c**, 55.9%) exhibits slightly improved CB_1 receptor binding and adamantyl carboxamide (**6d**, 65.8%) shows the highest affinity. However, the affinity constant determined for the latter compound is still moderate ($K_i = 498.2$ nM). The lipophilicity of the *N*-carboxamide substituents seems to be an important parameter in cannabinoid receptor recognition. The lipophilic properties of **6a**–**d** have been predicted using the software LogP of Interactive Analysis [34]. In general, the displacement values of **6a**–**d** increase with the lipophilic properties (logP = 4.62 (**6a**); logP = 3.72 (**6b**); logP = 5.25 (**6c**); logP = 6.42 (**6d**)).

Table 1
Displacement of specific [^{3}H]-CP55940[b] binding (at 1 μM) in CHO cells stably transfected with human CB_1 receptor, expressed as percentage (%)

Compound	CB_1: displacements (%) at 1 μM
SR141716	($K_i = 11.5$ nM) [13]
6a	31.7[a]
6b	28.3 ± 5.4
6c	55.9[a]
6d	65.8 ± 11.7 ($K_i = 498.2$ nM)[c]

[a] Values expressed as mean of three determinations with same internal standard.
[b] $K_d = 0.52$.
[c] K_i value was determined using the method of Cheng and Prusoff [47].

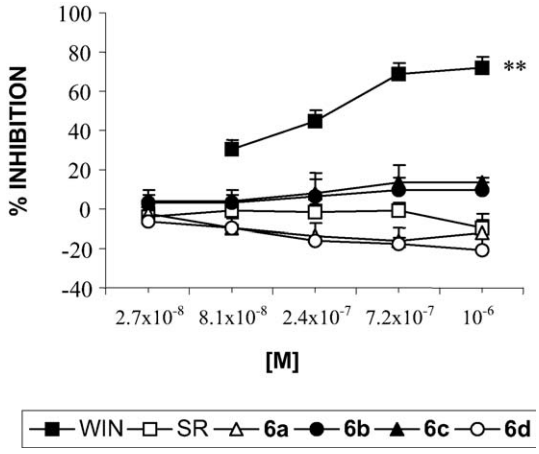


Fig. 1. Effect of WIN55212-2 (WIN), SR141716 (SR), **6a**–**d** in mouse isolated vas deferens. Lines show the mean % ($n = 6$, $n = 8$ for WIN) (± S.E.M.) of modification of the electrically induced contraction of the mouse vas deferens by cumulative addition of WIN55212-2, SR141716, and **6a**–**d**.
** $P < 0.01$ One-way ANOVA Post-test Bonferroni's multiple comparison test.

4.2. Isolated tissues assays

The cannabinoid activity of target compounds **6a**–**d** was functionally determined by in vitro assays using the mouse vas deferens. The mouse vas deferens is a tissue widely used to characterize cannabinoid agonists and antagonists [35–37]. CB_1 cannabinoid agonists inhibit electrically evoked contractions of the vas deferens by activating the CB_1 receptors expressed in this tissue. As shown in Fig. 1, the agonist WIN55212-2 induces dose-dependent inhibition of electrically induced contractions in this tissue. This effect was antagonized by the CB_1 cannabinoid antagonist SR141716 when added to the organ bath 15 min before WIN55212-2 addition (Fig. 3). Experiments with SR141716 and with the triazoles **6a**–**d** indicated that they did not induce significant modification of contractile responses at concentrations of 10^{-8}–10^{-6} M. We can conclude from these data that **6a**–**d** do not behave as cannabi-

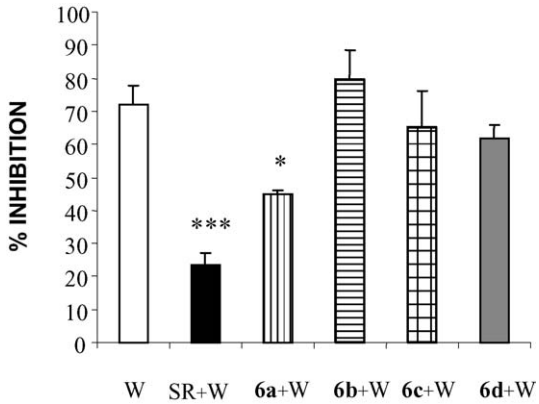


Fig. 2. Antagonistic effect of SR141716 (SR) and **6a**–**d** in mouse isolated vas deferens. Columns represent mean values ± S.E.M. ($n = 6$) of modifications induced by WIN55212-2 (W) at 10^{-6} M for 15 min after treatment with SR141716 and **6a**–**d** at 10^{-6} M.
*** $P < 0.001$; * $P < 0.05$, Student's *t*-test. One-way ANOVA Post-test Bonferroni's multiple comparison test.

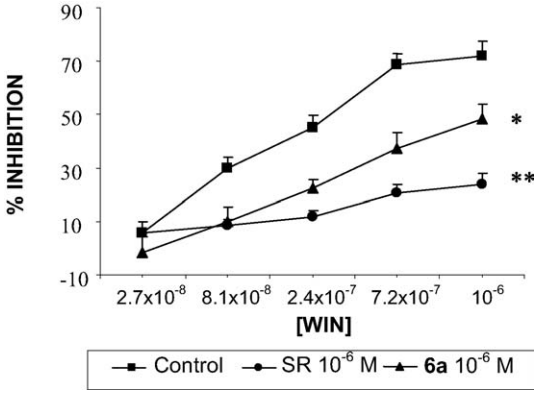


Fig. 3. Lines show the mean % ($n = 6$, $n = 8$ for control) (± S.E.M.) of modification of the electrically induced contraction of the mouse vas deferens by cumulative addition of WIN55212-2 in control tissues or tissues incubated at 15 min with SR141716 (SR) or **6a**.
*$P < 0.05$; **$P < 0.01$ One-way ANOVA Post-test Bonferroni's multiple comparison test.

noid agonists and, at the tested doses, they did not show any intrinsic activity. The ability of triazoles **6a**–**d** to oppose the effect of WIN55212-2 in this tissue was investigated. Variations produced by **6a**–**d**, on the inhibition of electrically induced contractions evoked by WIN55212-2, are shown in Fig. 2. Compounds **6b**, **6c** and **6d** were devoid of antagonistic properties when added to the organ bath 15 min before starting the cumulative addition of WIN55212-2. However, compound **6a** antagonized the effect of WIN55212-2 as shown in Fig. 3. These results indicate that in the mouse vas deferens, **6a** behaves as cannabinoid antagonist with moderate potency compared with SR141716. This last result does not corroborate the displacement value of **6a** ($K_i > 1000$ nM) suggesting allosteric CB_1 interactions or new subtype cannabinoid receptor recognition.

4.3. Conformational studies

The importance of spatial orientation of the carboxamide group for CB_1 receptor recognition has been pointed out [33,38,39]. Using AM1 molecular orbital calculations, energy minimized conformers of SR141716 led us to confirm a low-energy *s*-trans conformer defined by the angle (N2=C3–C=O). This result is in agreement with previous studies reported in the literature. We carried out energy minimization of triazoles **6a** and **6d** structures. For **6a** and **6d** the AM1 calculations results indicated an energetically preferred conformation in which the amide oxygen was placed on the same side of triazole N2 (*s*-cis conformation). Based on superimposing the heterocyclic five atoms of the pyrazole and triazole cores, the alignments of SR141716 with **6a** and SR141716 with **6d** were undertaken and are represented in Fig. 4. As shown in these superpositions, the carboxamides of **6a** and **6d** adopt a conformation different from SR141716. This result disagrees with the receptor-based alignment presented by Lange et al. [21] in which the carboxamide moieties of the triazole and the pyrazole overlap. However, the pharmacophoric feature presented here suggests that this difference in carboxamide spatial orientations due to electronic repulsion between nitrogen and oxygen electronic pairs, could contribute to conferring to 1,2,4-triazole-3-carboxamides **6a**–**d** a low affinity for CB_1 receptor.

5. Conclusion

A series of 1,2,4-triazole-3-carboxamides (**6a**–**d**) have been prepared by a new efficient synthesis. Results from AM1 energy minimization and the CB_1 cannabinoid receptor binding assays showed the negative contribution of triazole N4 for recognition of the CB_1 binding site. However, **6a** was found to be CB_1 cannabinoid antagonist in isolated tissues assays. This result did not corroborate with the binding data. These results suggest the possibility for **6a** to behave as an antagonist by acting on a new cannabinoid receptor subtype or by binding to a different CB_1 binding site. These hypotheses are supported by the fact that, in the literature, there is evidence that some ligands induce their cannabinoid activity through an allosteric mechanism [40]. In particular, it has been suggested that the cannabinoid CB_1 receptor antagonist SR141716 A induces inverse agonistic effect by binding to an additional site on the cannabinoid CB_1 receptor [41]. There are also convincing evidences that support the existence of additional cannabinoid re-

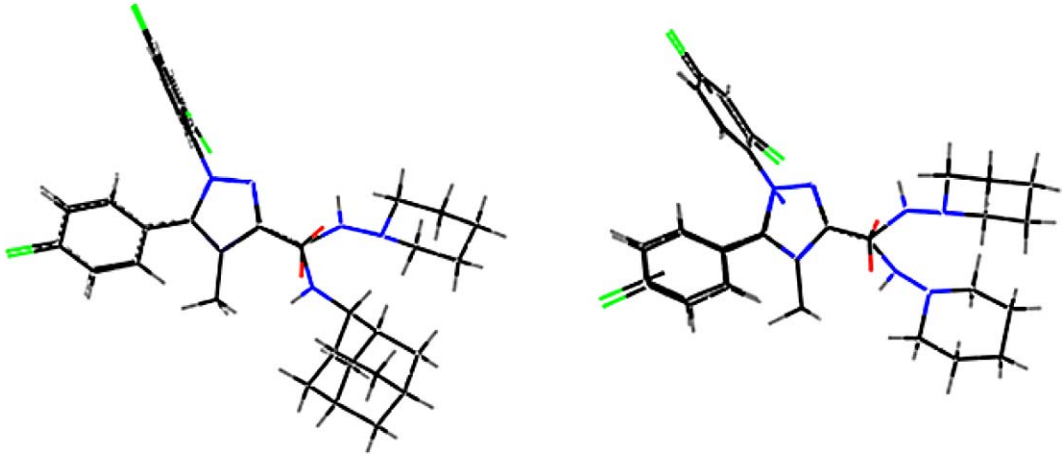


Fig. 4. Superposition of **6a** with SR141716 (right) and of **6d** with SR141716 (left).

ceptor [42–44]. Therefore, there is still a need for additional pharmacological experiments to establish the mechanism underlying the effect of **6a** in the mouse vas deferens.

6. Experimental protocols

6.1. Chemistry

Dry dichloromethane was distilled over calcium chloride, and dry pyridine over potassium hydroxide. Melting points (uncorrected) were determined with a Reichert Jung Thermovar apparatus. Mass spectra were recorded using electrospray positive mode. Flash column chromatographies were run on silica gel 60 (230–400 mesh) or on medium pressure flash system with prepacked silica gel cartridge. Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values. ^{1}H and ^{13}C NMR spectra were recorded on a Gemini 200, Varian 300 and 400 unity spectrometers using TMS as the internal standard.

6.1.1. Ethyl 2-[2-(2,4-dichlorophenyl)hydrazono]-3-oxobutanoate (1)

A solution of 2,4-dichloroaniline (8.00 g, 49.4 mmol) and HBF_4 (13 ml, 48% wt.) in H_2O (13 ml) was cooled to 0 °C. $NaNO_2$ (3.41 g, 49.4 mmol) in H_2O (5 ml) previously cooled to 0 °C was added drop-wise. The reaction mixture was stirred at 0 °C for 30 min and then the temperature was allowed to reach r.t. The formed diazonium salt was filtered off, washed with HBF_4 aq., then with EtOH and finally with Et_2O. A solution of dried diazonium salt in EtOH (250 ml) was stirred with ethyl acetoacetate (6.25 ml, 49.4 mmol), NaOAc (11.12 g, 135.5 mmol) and H_2O (25 ml) at 0 °C for 1 h. The precipitate was filtered off, washed with H_2O, and recrystallized from cyclohexane to give 4.89 g of **1** (33%) as an orange solid: m.p. = 118–121 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.93 (d, J = 8.8 Hz, 1H, H_6), 7.54 (brs, 1H, H_3), 7.44 (brd, J = 8.8 Hz, 1H, H_5), 4.50 (q, J = 7.1 Hz, 2H, $\underline{CH_2}CH_3$), 2.76 (s, 3H, CH_3), 1.55 (t, J = 7.1 Hz, 3H, $CH_2\underline{CH_3}$); ^{13}C NMR (200 MHz, $CDCl_3$) δ 197.0 ($\underline{C}OCH_3$), 164.5 ($\underline{C}O_2CH_2CH_3$), 137.4 (C_1), 130.3 (N=C), 129.3 (C_3), 128.4 (C_5), 127.8 (C_4), 122.0 (C_2), 117.5 (C_6), 61.2 ($\underline{CH_2}CH_3$), 30.8 (CH_3), 14.3 ($CH_2\underline{CH_3}$); MS (ES^+) *m/z* (rel. intensity %) 303 (M^++1, 100); Anal. $C_{12}H_{12}Cl_2N_2O_3$ (C, H, N).

6.1.2. Ethyl 2-[2-(2,4-dichlorophenyl)hydrazinyl]-2-iminoacetate (2)

To a solution of **1** (1.44 g, 4.7 mmol) and NaOAc (3.52 g, 42.8 mmol) in AcOH (35 ml) was added drop-wise Br_2 (243 μl, 4.7 mmol). After 30 min of stirring at r.t., the solution was poured into H_2O (100 ml) and extracted with CH_2Cl_2. The organic layers were dried over anhydrous Na_2SO_4. The solvent was then removed under reduced pressure. To the residue dissolved in acetone (50 ml) was added drop-wise 30% aq. NH_4OH (4 ml) diluted in acetone (10 ml). This mixture was stirred 1 h at r.t. Then the solvent was evaporated under reduced pressure. The residue dissolved in 100 ml of 10% aq. HCl was washed with toluene. The aqueous layer was basified with 5 N NaOH to pH 8 precipitating a brown solid, which was then filtered off. This precipitate was then suspended in boiling EtOH and filtered. The liquid layer was treated with activated carbon for 30 min, and filtered over celite. Then, the solvent was evaporated, obtaining **2** (1.76 g, 77%) as a brown solid: m.p. = 82–87 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.42 (d, J = 8.9 Hz, 1H, H_6), 7.21 (brs, 1H, H_3), 7.15 (brd, J = 8.9 Hz, 1H, H_5), 6.77 (brs, 1H, NH), 4.65 (brs, 2H, NH), 4.32 (q, J = 7.1 Hz, 2H, CH_2), 1.34 (t, J = 7.1 Hz, 3H, CH_3); ^{13}C NMR (200 MHz, $CDCl_3$) δ 161.8 ($\underline{C}O_2CH_2CH_3$), 140.2 (C=NH), 138.4 (C_1), 128.4 (C_3), 128.0 (C_5), 125.1 (C_4), 118.8 (C_2), 116.2 (C_6), 62.5 (CH_2), 14.1 (CH_3); MS (ES^+) *m/z* (rel. intensity %) 276 (M^++1, 100); Anal. $C_{10}H_{11}Cl_2N_3O_2$ (C, N, H).

6.1.3. Ethyl 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1H-1,2,4-triazole-3-carboxylate (5)

A mixture of **2** (0.60 g, 2.2 mmol), pyridine (0.20 ml, 2.5 mmol), and 4-chlorobenzoyl chloride (0.32 ml, 2.5 mmol) in 1,4-dioxane (50 ml) was heated to reflux for 3 h. The solvent was then evaporated. The residue dissolved in CH_2Cl_2 (120 ml) was washed successively with 1 M HCl, H_2O, sat. aq. K_2CO_3 and finally H_2O. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was chromatographied on silica gel (cyclohexane/EtOAc 8:1) to give ethyl 2-(4-chlorobenzamido)-2-(2-(2,4-dichlorophenyl)hydrazinyl)acetate (**4**; 53 mg, 6%, yellow solid), 4-chloro-*N*′-(2,4-dichlorophenyl)benzohydrazide (**3**; 198 mg, 29%, yellow solid) and the desired product **5** as a white solid (0.31 g, 36%).

3. m.p. = 163–166 °C; ^{1}H NMR (200 MHz, CD_3OD) δ 7.88 (d, J = 8.6 Hz, 2H, H_2), 7.49 (d, J = 8.6 Hz, 2H, H_3), 7.31 (d, J = 2.3 Hz, 1H, $H_{3'}$), 7.14 (dd, J = 8.7 Hz, J = 2.3 Hz, 1H, $H_{5'}$), 6.87 (d, J = 8.7 Hz, 1H, $H_{6'}$); ^{13}C NMR (200 MHz, CD_3COD) δ 169.0 (CONH), 144.9 (C_4), 139.5 ($C_{1'}$), 132.4 (C_1), 130.3 and 130.0 (C_2, C_3 and $C_{3'}$), 128.9 ($C_{5'}$), 125.6 ($C_{4'}$), 120.5 ($C_{2'}$), 115.3 ($C_{6'}$); MS (ES^+) (rel. intensity %) 315 (M^+ + 1, 100); IR (KBr) 3435 cm^{-1} NH amide, 1649 cm^{-1} CO amide.

4. m.p. = 168–171 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 10.10 (brs, 1H, NH), 8.81 (brs, 1H, NH), 7.62 (d, J = 8.5 Hz, 2H, H_2), 7.32 (d, J = 8.8 Hz, 2H, Aromatics), 7.24 (d, J = 8.5 Hz, 2H, H_3), 7.00 (t, J = 8.8 Hz, 1H, Aromatics), 4.15 (q, J = 7.1 Hz, 2H, CH_2), 1.20 (t, J = 7.1 Hz, 3H, CH_3); ^{13}C NMR (200 MHz, $CDCl_3$) δ 165.3 and 163.6 ($\underline{C}O_2CH_2CH_3$, CON), 140.0 (C = N), 139.8 (C_4 and $C_{1'}$), 131.1 (C_1), 129.3 and 129.2 (C_2 and C_3), 129.0 ($C_{3'}$), 127.9 ($C_{5'}$), 126.8 ($C_{4'}$), 122.9 ($C_{2'}$), 116.1 ($C_{6'}$), 63.0 (CH_2), 14.2 (CH_3); MS (ES^+) (rel. intensity %) 414 (M^+ + 1, 80).

5. m.p. = 113–118 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.53 (d, J = 1.9 Hz, 1H, Aromatics), 7.45 (d, J = 8.6 Hz, 2H, $H_{2'}$), 7.44 (m, 2H, Aromatics), 7.31 (d, J = 8.6 Hz, 2H, $H_{3'}$), 4.52 (q, J = 7.2 Hz, 2H, CH_2), 1.44 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (200 MHz, $CDCl_3$) δ 159.4 ($\underline{C}O_2CH_2CH_3$), 155.9 (C_3 triazole), 154.9 (C_5 triazole), 137.3, 137.1, 133.8, and 132.9 ($C_{1'}$, $C_{2'}$, $C_{4'}$ and $C_{4'}$), 130.5 ($C_{6'}$), 129.9 ($C_{3'}$), 129.3 and

129.0 ($C_{2'}$ and $C_{3'}$), 128.4 ($C_{5'}$), 124.6 ($C_{1'}$), 62.1 (CH_2), 14.1 (CH_3); MS (ES^+) *m/z* (rel. intensity %) 296 ($M^+ + 1$, 90).

The adduct product **4** (30 mg, 0.07 mmol) was cyclized into **5** by refluxing in 1,4-dioxane (3 ml) with pyridine (23.4 μl, 0.3 mmol) for 2 weeks. After evaporation, the residue was dissolved in CH_2Cl_2 (20 ml), washed with 1 M HCl, dried over anhydrous Na_2SO_4, and evaporated to give **5** (38 mg, 93%).

6.1.4. General procedure for the synthesis of 1,2,4-triazole-3-carboxamide (**6a–d**)

To a solution of the corresponding hydrazine or amine (5 equiv.) in dry CH_2Cl_2 (3–5 ml) was added a solution of $Al(Me)_3$ in heptane (2 M, 5 equiv.) under N_2 atmosphere. The reaction mixture was stirred at r.t. for 1 h. A solution of **5** (1 equiv.) in dry CH_2Cl_2 (3–6 ml) was then added drop-wise. The mixture was heated to 35–50 °C during the respective time, and then was carefully poured onto 1 N HCl (20–30 ml). The biphasic solution was heated to 40 °C for 30 min and cooled to r.t. The organic layer was separated, dried over anhydrous Na_2SO_4 and evaporated. The crude product was purified by recrystallization or chromatography.

*6.1.4.1. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-N-(piperidin-1-yl)-1H-1,2,4-triazole-3-carboxamide (**6a**)*. Compound **6a** was prepared from **5** (240 mg, 0.6 mmol), 1-aminopiperidine (0.32 ml, 3.0 mmol), $Al(Me)_3$ (1.50 ml, 3.0 mmol); reaction time: 21 h; medium pressure chromatography [cyclohexane/EtOAc (25:1–3:1)]; yield: 214 mg (79%) as a white solid; m.p. = 115–118 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.82 (brs, 1H, NH), 7.46 (d, $J = 1.9$ Hz, 1H, Aromatics), 7.38 (m, 2H, Aromatics), 7.36 (d, $J = 8.7$ Hz, 2H, $H_{2'}$), 7.26 (d, $J = 8.7$ Hz, $H_{3'}$), 2.84 (t, $J = 5.6$ Hz, 4H, $CH_2CH_2\underline{CH_2}N\underline{CH_2}CH_2$), 1.71 (t, $J = 5.6$ Hz, 4H, $CH_2\underline{CH_2}CH_2NCH_2\underline{CH_2}$), 1.39 (m, 2H, $\underline{CH_2}CH_2CH_2NCH_2CH_2$); ^{13}C NMR (300 MHz, $CDCl_3$) δ 157.0 (CONH), 156.2 and 155.7 (C_3 triazole and C_5 triazole), 137.8, 137.7, 134.5, and 133.0 ($C_{1'}$, $C_{2'}$, $C_{4'}$ and $C_{4'}$), 130.7 ($C_{6'}$), 130.0 ($C_{3'}$), 129.3 and 129.2 ($C_{2'}$ and $C_{3'}$), 128.5 ($C_{5'}$), 125.4 ($C_{1'}$), 57.2 ($CH_2CH_2\underline{CH_2}N\underline{CH_2}CH_2$), 25.2 ($CH_2\underline{CH_2}CH_2NCH_2\underline{CH_2}$), 23.2 ($\underline{CH_2}CH_2CH_2NCH_2CH_2$); MS ($ES^+$) *m/z* (rel. intensity %) 450 ($M^+ + 1$, 99); Anal. $C_{20}H_{18}Cl_3N_5O$ (C, H, N).

*6.1.4.2. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-N-morpholino-1H-1,2,4-triazole-3-carboxamide (**6b**)*. Compound **6b** was prepared from **5** (150 mg, 0.4 mmol), 4-aminomorpholine (0.18 ml, 1.9 mmol), $Al(Me)_3$ (0.95 ml, 1.9 mmol); reaction time: 2.5 h; recrystallization from toluene; yield: 161 mg (94%) as a white solid; m.p. = 193–195 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 8.00 (brs, 1H, NH), 7.51 (d, $J = 1.3$ Hz, 1H, Aromatics), 7.44 (brs, 2H, Aromatics), 7.41 (d, $J = 8.6$ Hz, 2H, $H_{2'}$), 7.31 (d, $J = 8.6$ Hz, $H_{3'}$), 3.87 (t, $J = 4.5$ Hz, 4H, CH_2OCH_2), 3.01 (t, $J = 4.5$ Hz, 4H, CH_2NCH_2); ^{13}C NMR (300 MHz, $CDCl_3$) δ 156.2 (CONH), 156.0 and 155.5 (C_3 triazole and C_5 triazole), 137.5, 137.4, 134.0, and 132.5 ($C_{1'}$, $C_{2'}$, $C_{4'}$ and $C_{4'}$), 130.7 ($C_{6'}$), 130.0 ($C_{3'}$), 129.3 and 129.2 ($C_{2'}$ and $C_{3'}$), 128.6 ($C_{5'}$), 124.8 ($C_{1'}$), 66.2 (CH_2OCH_2), 55.9 (CH_2NCH_2); MS (ES^+) *m/z* (rel. intensity %) 425 ($M^+ + 1$, 100); Anal. $C_{19}H_{16}Cl_3N_5O_2.1/2C_7H_8$ (C, H, N).

*6.1.4.3. 5-(4-Chlorophenyl)-N-cyclohexyl-1-(2,4-dichlorophenyl)-1H-1,2,4-triazole-3-carboxamide (**6c**)*. Compound **6c** was prepared from **5** (120 mg, 0.3 mmol), cyclohexylamine (0.18 ml, 1.5 mmol), $Al(Me)_3$ (0.76 ml, 1.5 mmol); reaction time: 4.5 h; recrystallization from toluene and flash chromatography [cyclohexane/EtOAc (2:1)]; yield: 83 mg (61%) as a white solid; m.p. = 181–185 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, $J = 1.7$ Hz, 1H, Aromatics), 7.40–7.30 (m, 4H, $H_{2'}$ and aromatics), 7.26 (d, $J = 8.7$ Hz, 2H, $H_{3'}$), 7.02 (d, $J = 8.7$ Hz, 1H, NH), 4.15–3.85 (m, 1H, NHCH), 2.20–1.11 (m, 10H, $CH_2CH_2CH_2CH_2CH_2$); ^{13}C NMR (300 MHz, $CDCl_3$) δ 157.7 (CONH), 157.5 (C_3 triazole), 155.4 (C_5 triazole), 137.4, 137.2, 134.3, and 132.7 ($C_{1'}$, $C_{2'}$, $C_{4'}$ and $C_{4'}$), 130.7 ($C_{6'}$), 130.1 ($C_{3'}$), 129.3 and 129.2 ($C_{2'}$ and $C_{3'}$), 128.5 ($C_{5'}$), 125.2 ($C_{1'}$), 48.3 (NHCH), 33.1 ($\underline{CH_2}CH(NH)\underline{CH_2}$), 25.5 ($CH_2CH_2CH_2CH(NH)CH_2CH_2$), 24.8 ($CH_2\underline{CH_2}CH_2CH(NH)CH_2\underline{CH_2}$); MS ($ES^+$) *m/z* (rel. intensity %) 449 ($M^+ + 1$, 100); Anal. $C_{21}H_{19}Cl_3N_4O$ (C, H, N).

*6.1.4.4. 5-(4-Chlorophenyl)-N-(1-adamantyl)-1-(2,4-dichlorophenyl)-1H-1,2,4-triazole-3-carboxamide (**6d**)*. Compound **6d** was prepared from **5** (76 mg, 0.2 mmol), 1-aminoadamantane (145 mg, 1.0 mmol), $Al(Me)_3$ (0.48 ml, 1.0 mmol); reaction time: 26 h; flash chromatography [cyclohexane/EtOAc (4:1)]; yield: 75 mg (78%) as a white solid; m.p. = 185–189 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.50 (d, $J = 1.7$ Hz, 1H, Aromatics), 7.47–7.44 (m, 2H, Aromatics), 7.42 (d, $J = 8.9$ Hz, 2H, $H_{2'}$), 7.30 (d, $J = 8.9$ Hz, 2H, $H_{3'}$), 6.91 (s, 1H, NH), 2.14 (brs, 6H, H_2 adamantyl), 2.11 (brs, 3H, H_3 adamantyl), 1.75–1.66 (m, 6H, H_4 adamantyl); ^{13}C NMR (300 MHz, $CDCl_3$) δ 157.9 (CONH), 157.3 (C_3 triazole), 155.1 (C_5 triazole), 137.2, 137.0, 134.2 and 132.5 ($C_{1'}$, $C_{2'}$, $C_{4'}$ and $C_{4'}$), 130.6 ($C_{6'}$), 130.0 ($C_{3'}$), 129.2 and 129.1 ($C_{2'}$ and $C_{3'}$), 128.5 ($C_{5'}$), 125.1 ($C_{1'}$), 52.3 (C_1 adamantyl), 41.4 (C_2 adamantyl), 36.3 (C_4 adamantyl), 29.4 (C_3 adamantyl); MS (ES^+) *m/z* (rel. intensity %) 501 ($M^+ + 1$, 100); Anal. $C_{25}H_{23}Cl_3N_4O$ (C, H, N).

6.2. Pharmacological materials and methods

6.2.1. Binding assays

Membranes from HEK-293 EBNA cells with human CB_1 cannabinoid receptor expressed were supplied by PerkinElmer. The receptor concentration was 3.5 pmol mg^{-1} protein and the protein concentration was 6.4 mg ml^{-1}. The binding assays were performed as described by Ross et al. [45], with modifications. The commercial membrane was diluted (1:60) with the binding buffer (50 mM Tris Cl, 5 mM $MgCl_2$, 2.5 mM EDTA, 0,5 mg ml^{-1} BSA, pH = 7.4). The radioligand used was [^3H]-CP55940 (PerkinElmer) at 0.135 nM and the final volume was 200 μl. The incubation was initiated with the addition of 160 μl of membrane and the incubation time was 90 min at 30 °C. After incubation, the membrane was collected onto pretreated glass fiber filters (Schleicher and Schnell 3362), with polyethy-

lenimine 0.5%. The filter was washed four times each with 1 ml of washing buffer (50 mM Tris Cl, pH 7.4) and then the filter sections were transferred to vials and 5 ml of Ecoscint H liquid scintillation cocktail was added to each vial. The vials were allowed to settle for several hours and then quantified by liquid scintillation spectrophotometry (Wallac Winspectral 1414). Nonspecific binding was determined with 10 μM WIN55212-2. Competition binding data were analyzed by using the LIGAND program [46] and assays were performed in triplicate determinations for each point.

6.2.2. Isolated tissues assays

Male CD-1 mice weighing 25–30 g each were used. Inhibition of electrically evoked contractions of mouse vas deferens was determined following previously described procedures [19]. WIN55212-2 and SR141716 were obtained, respectively, from TOCRIS (Biogen Científica S.L., Madrid, Spain) and from SANOFI AVENTIS (Paris, France). For experiments, drugs were dissolved in ethanol/Tween80.

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