Discovery of 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-3-hexyl-1*H*-1,2,4-triazole, a Novel in Vivo Cannabinoid Antagonist Containing a 1,2,4-Triazole Motif

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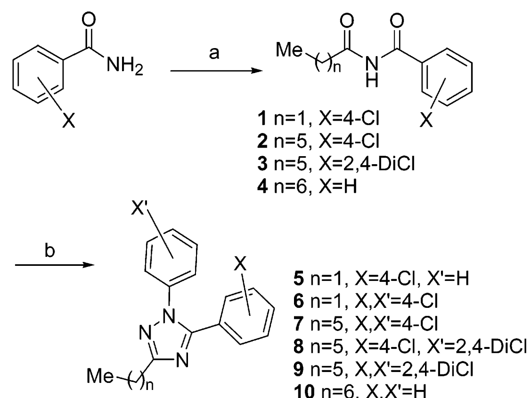
A new series of 1,2,4-triazoles have been prepared and the evaluation of their cannabinoid properties have been carried out. Compound **8** showed cannabinoid silent antagonist activity in mouse vas deferens and guinea pig ileum preparations and in vivo assays (cannabinoid tetrad) in mouse. It did not have intrinsic activity in these bioassays, and therefore, it did not behave as a partial agonist or an inverse agonist.

Introduction

Recognition and evidence of at least two types of cannabinoid receptors CB_1 and CB_2 and the discovery of endogenous cannabinoid ligands have prompted the research of novel cannabinoid receptor agonists and antagonists.[1−3] A wide range of CB_1 and CB_2 ligands with diverse chemical structures are now available.[4,5] We report a silent cannabinoid antagonist derived from a 1,2,4-triazole that represents a novel entry in cannabinoid chemistry.[6] Although diverse heterocyclic structures such as the pyrazoles SR141716 and SR144528[7,8] or the aminoalkylindole AM630[9] or, more recently, imidazolinediones[10] have been shown to be cannabinoid receptor antagonists, triazoles have not yet been explored within this context. Thus, we describe the synthesis of a series of 1,2,4-triazoles, their in vitro and in vivo evaluation, and the identification of **8** as a silent antagonist.

Scheme 1[a]



[a] Reagents and conditions: (a) $(Me(CH_2)_n(CO)_2O$, dry toluene, concentrated H_2SO_4, reflux; (b) phenylhydrazine for **5** and **10**; 4-chlorophenylhydrazine for **6** and **7**; 2,4-dichlorophenylhydrazine for **8** and **9**; AcOH, NaOAc, reflux.

Results and Discussion

1. Chemistry. Triazoles **5**−**10** were prepared from the corresponding *N*-acylbenzamides **1**−**4** as described in Scheme 1.[11] These *N*-acylbenzamides **1**−**4** were obtained by treatment of benzamides with anhydrides in toluene in the presence of concentrated H_2SO_4. Then condensation with the corresponding phenylhydrazines in acetic acid led to triazoles **5**−**10**.

2. Pharmacology. To evaluate the biological activity of the new compounds **5**−**10**, three sets of experiments were carried out. First, their effect in isolated tissues in order to determine whether they behave as cannabinoid agonists or antagonists was tested. Then for those compounds showing interesting profiles, their affinity for cannabinoid receptors was determined in rat cerebellar membranes. Finally, they were tested in vivo using the classic cannabinoid tetrad of behavioral tests.

2.1. Isolated Tissues Assays. The mouse vas deferens and the myenteric plexus-longitudinal muscle (MP-LM) strips of guinea pig ileum are two isolated tissues widely used to easily characterize cannabinoid agonists and antagonists.[12] In guinea pig ileum the cannabinoid agonists acting at prejunctional cannabinoid receptors reduce acetylcholine release[13] and inhibit the electrically evoked contractions. This effect is accepted to be mediated through CB_1 receptors,[14] and the inhibition is selectively reversed by the CB_1 cannabinoid antagonist SR141716. Inhibition of the electrically induced contractions by cannabinoid agonists have also been described in mouse vas deferens. CB_1[15] and CB_2-like[16] cannabinoid receptors seem to be involved in this effect.

As an expected cannabinoid agonist used as a reference compound, WIN 55,212-2 was able to induce dose-dependent inhibition of the electrically induced contrac-

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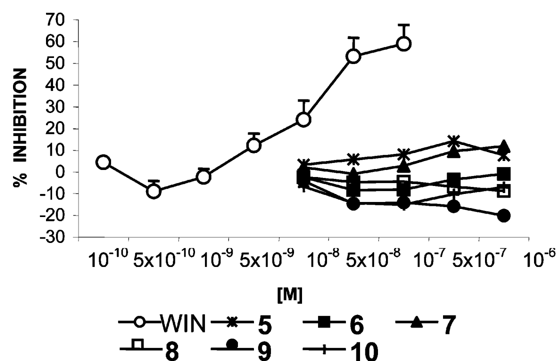

Figure 1. Lines show the mean % ($n = 7$) of modification of the electrically induced contraction of the mouse vas deferens by cumulative addition of WIN 55,212-2 (WIN) or the new compounds. SEM was not included in the figure to clarify the presentation (table in Supporting Information).

tions in mouse vas deferens and in guinea pig ileum. This effect was also dose-dependently antagonized by the CB_1 cannabinoid antagonist SR141716. When the effect of **5**−**10** was tested, they did not induce significant modification of the contractile responses in the mouse vas deferens (10^{-8}−10^{-6} M) (see Figure 1). Similar results were found when their effect was tested in the guinea pig ileum (10^{-8} to 2.4×10^{-6} M). These data eliminate the possibility that the new compounds act as cannabinoid agonists. It could also be established that they lack both direct (activation) or indirect (induction of the neurotransmitter release) agonist activity on a large number of well-known receptors that play a role in the electrically induced contraction in mouse vas deferens (adrenergic, purinergic, cannabinoid, δ, μ, and κ opioid receptors) or in guinea pig ileum (cholinergic, histaminergic, serotonergic, adrenergic, susbstance P, μ and κ opioids, cannabinoid, CGRP, VIP, purinergic receptors). Compound **5** induced inhibition of the contractile activity in the guinea pig ileum. This effect was not antagonized by SR141716 disregarding cannabinoid agonistic activity, and more studies are required to determine the mechanism underlying this inhibition. No statistical differences were found between tissues incubated with **6**−**10** and control or vehicle-treated tissues (see Supporting Information). To evaluate if **5**−**10** can act as cannabinoid antagonists, the effect of WIN 55,-212-2 was tested in tissues incubated with the triazoles. Compound **8** induced a significant and dose-dependent decrease of the inhibition evoked by cannabinoid agonists in both tissues. Figure 2 shows the inhibition of the WIN effect induced by SR141716 and **8** in mouse vas deferens. From these data, it could be suggested that **8** is a cannabinoid antagonist lacking agonistic activity.

2.2. Binding Assay. Radioligand displacement assays have been used to evaluate the affinity of **8** to CB_1 receptors in rat cerebellar membranes. They have been performed with [^{3}H]-SR141716A and [^{3}H]-WIN 55,212-2 as labeled ligands. Compound **8** retained a moderate affinity ($K_i = 855.6 \pm 296$ nM and $K_i = 748 \pm 193$ nM, respectively) for CB_1 receptors. Although **8** was shown in our functional studies to have antagonistic efficacy in vitro and in vivo similar to that of SR141716A, it has a reduced affinity for CB_1 receptors compared to SR141716 ($K_i = 4$ nM).

Binding results do not corroborate the results obtained in isolated tissues. Differences in binding affini-

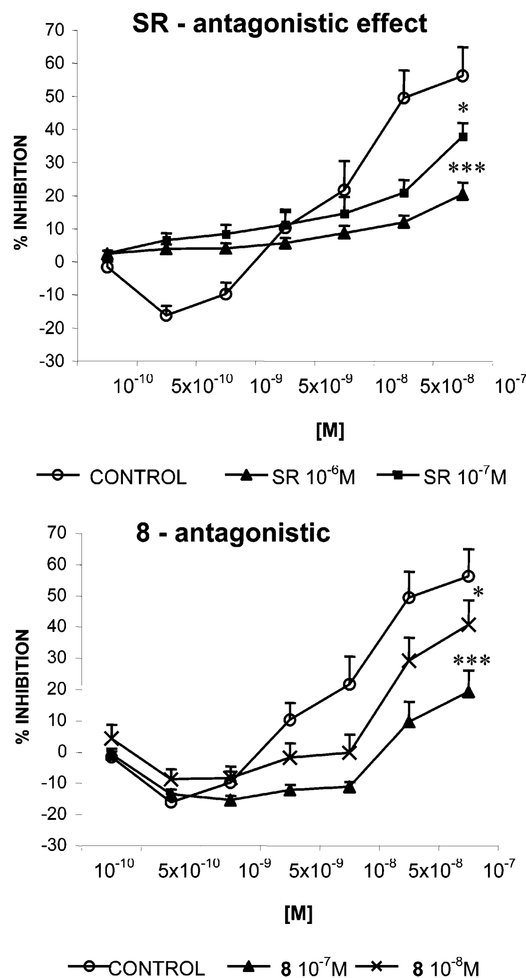

Figure 2. Lines show the mean % ± SEM ($n = 7$) of modification of the electrically induced contraction of the mouse vas deferens by cumulative addition of WIN 55,212-2 (WIN) in control tissues or in tissues incubated with SR141716A (SR) in the top panel or of **8** in the bottom panel. The asterisk represents the significant difference vs control tissues: (*) $p < 0.05$; (***) $p < 0.001$.

ties and biological activities have been described for many compounds including dopamine uptake blockers[17] and cannabinoids.[18] They might be dependent on the nature of receptor−ligand interaction (competitive and noncompetitive antagonism), type of in vitro assay (cations, presence of GDP/GTP analogues), existence of receptor subtypes including constitutively active receptors (described also for CB_1 receptors),[19] or local cellular G-protein concentration. Additionally, inverse agonists bind preferentially to normal receptors with higher affinity than neutral antagonists.[20]

2.3. Cannabinoid Tetrad of Behavioral Tests. The effect of **8** was tested using a well accepted model to evaluate cannabinoid compounds in vivo: the cannabinoid tetrad. Treatment with **8** did not induce significant modifications in any of the tested parameters (nociception, temperature, spontaneous motility, and catalepsy), discarding again agonistic activity, whereas the cannabinoid agonist WIN 55,212-2 induced dose-dependent antinociception, hypothermia, inhibition of spontaneous motility, and catalepsy. Figure 3 shows the effect of the intraperitoneally (ip) administration of 1.5 mg/kg of WIN 55,212-2 in control animals and after the ip administration of 1 mg/kg of **8** or of reference antagonist SR141716. These data are in agreement with

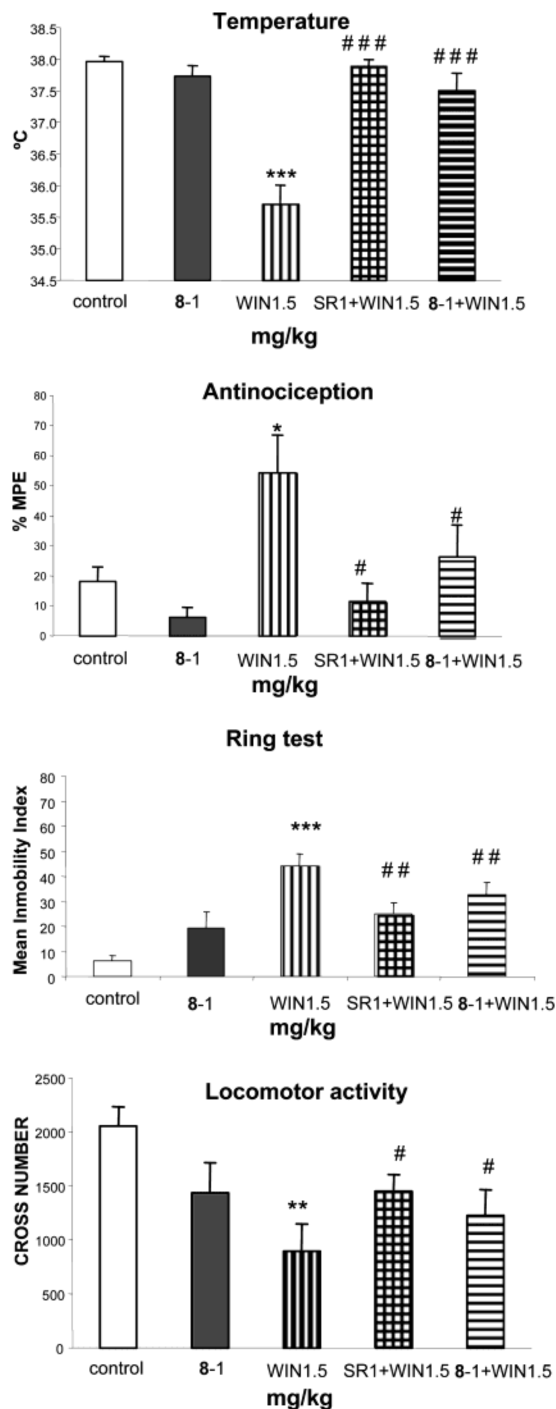

Figure 3. Effects on the cannabinoid tetrad. Bars show modifications induced by treatment with WIN 55,212-2 (WIN), **8**, and WIN after treatment with SR141716A (SR) or **8**. Values show the mean ± SEM ($n = 10$). The asterisk represents the significant difference vs control value: (*) $p < 0.05$; (**) $p < 0.01$; (***) $p < 0.001$). The # represents significant difference vs WIN bar: (#) $p < 0.05$; (##) $p < 0.01$; (###) $p < 0.001$.

those recorded in mouse vas deferens and in guinea pig ileum, and although more experiments are required to better characterize this compound, it can be suggested that **8** behaves as a CB_1 cannabinoid antagonist.

3. Molecular Modeling. Extensive SAR studies have yielded information on structural requirements for cannabinoid receptor affinity, and pharmacophores for the major classes of cannabinoid ligands have been reported.[21] The 1,2,4-triazoles series reported here constitute a new template for cannabinoid activity and in particular for antagonistic properties. However, triazole **8** showed a moderate affinity for CB_1 receptors. To get insight on this discrepancy, on a first approximation, a superposition of the 1,2,4-triazole structure with a common pharmacophore for cannabinoid ligands that was constructed whatever their structure and their activity (agonist, antagonist, or inverse agonist) was attempted. For the modeling of the pharmacophore, the classical cannabinoids Δ^9-THC, nabilone, and cannabinol, pyrazole SR141716, aminoalkylindole JWH018, and aminoalkylpyrrole JWH030, all described in the literature,[1,4] were selected. The alignment of our triazole series with the selected molecules and the template Δ^9-tetrahydrocannabinolic acid B (thcanb)[22] agreed with the following elements involved in the pharmacophore: hydrocarbon area, aromatic ring, heteroatom, and aliphatic chain (see Supporting Information).

In summary, we have identified 1,2,4-triazole as a new heterocyclic core with potential cannabinoid properties. In particular **8** behaves as a CB_1 antagonist both in vivo and in functional assays. However, the affinity of **8** for rat cerebellar CB_1 receptors was revealed to be moderate. Although further investigations are now required to establish the mechanism and interactions that contribute to its in vitro and in vivo pharmacological effects, triazole **8** can be considered as a lead in the search for silent cannabinoid receptor antagonists.

Experimental Section

Chemistry. General Procedure for Preparing *N*-Acylamides 1−4. The preparation of *N*-heptanoyl-4-chlorobenzamide (**2**) is given as a representative example.

***N*-Heptanoyl-4-chlorobenzamide (2).** To a suspension of 4-chlorobenzamide (3.89 g, 25.0 mmol) in dry toluene (40 mL) was added heptanoic anhydride (10.5 mL, 40.0 mmol) and concentrated H_2SO_4 (0.5 mL). The reaction mixture was heated to reflux for 1 h. The resulting dark solution was treated with activated carbon and filtered over Celite. The solvent was evaporated under reduced pressure, and the residue was washed with water. Recrystallization from EtOH gave 2.70 g of **2** (40%) as a white solid: mp 139−142 °C; MS (ES$^+$) *m*/*z* (rel intensity %) 268 (M$^+$ + 1, 100). Anal. ($C_{14}H_{18}ClNO_2$) C, H, N.

***N*-Propanoyl-4-chlorobenzamide (1)** was prepared from 4-chlorobenzamide (1.00 g, 6.4 mmol) and propionic anhydride (1.32 mL, 10.3 mmol) as described for **2**: yield, 451 mg (33%) as a white solid; mp 141−145 °C (lit.[11] 163−165 °C); MS (ES$^+$) *m*/*z* (rel intensity %) 212 (M$^+$ + 1, 95). Anal. ($C_{10}H_{10}ClNO_2$) C, H, N.

***N*-Heptanoyl-2,4-dichlorobenzamide (3)** was prepared from 2,4-dichlorobenzamide (4.75 g, 25.0 mmol) and heptanoic anhydride (10.5 mL, 40.0 mmol) as described for **2**: yield, 4.18 g (55%) as a white solid; mp 65−69 °C; MS (ES$^+$) *m*/*z* (rel intensity %) 302 (M$^+$ + 1, 100). Anal. ($C_{14}H_{17}Cl_2NO_2$) C, H, N.

***N*-Octanoylbenzamide (4).** White solid; mp 62−64 °C; MS (ES$^+$) *m*/*z* (rel intensity %) 248 (M$^+$ + 1, 90). Anal. ($C_{14}H_{17}Cl_2NO_2$) C, H, N.

General Procedure for Preparing 3-Alkyl-1,5-diaryl-1*H*-1,2,4-triazoles 5−10. The preparation of 5-(4-chlorophenyl)-3-ethyl-1-phenyl-1*H*-1,2,4-triazole (**5**) is given as a representative example.

5-(4-Chlorophenyl)-3-ethyl-1-phenyl-1*H*-1,2,4-triazole (5). Phenylhydrazine (0.24 mL, 1.4 mmol) and NaOAc (153 mg, 1.9 mmol) were added to a solution of **1** (300 mg, 1.4 mmol) dissolved in glacial acetic acid (10 mL). The reaction mixture was heated to reflux for 22 h. The solvent was evaporated. The residue was then dissolved in CH_2Cl_2 and washed with H_2O. The organic layer was then dried over $MgSO_4$, and the solvent was evaporated. The orange residue was purified on flash chromatography (EtOAc/*n*-hexane 1:2

to 2:1) to give **5** as a yellowish solid (10%): mp 76−79 °C; MS (ES^+) *m*/*z* (rel intensity %) 284 (M^+ + 1, 100). Anal. ($C_{16}H_{14}ClN_3$) C, H, N.

1,5-Bis(4-chlorophenyl)-3-ethyl-1*H*-1,2,4-triazole (6) was prepared from **1** (300 mg, 1.4 mmol), 4-chlorophenylhydrazine (202 mg, 1.4 mmol), and NaOAc (153 mg, 1.9 mmol) as described for **5** (chromatography, CH_2Cl_2/*n*-hexane 1:1 to 1:0): yield, 29 mg (6%) as a brownish solid; mp 94−96 °C (lit.[11] 94−95 °C); MS (ES^+) *m*/*z* (rel intensity %) 318 (M^+ + 1, 100). Anal. ($C_{16}H_{13}Cl_2N_3{\cdot}2.5H_2O$): C, calcd, 52.90; found, 52.60. H, calcd, 4.99; found, 4.19. N, calcd, 11.57; found, 10.99.

1,5-Bis(4-chlorophenyl)-3-hexyl-1*H*-1,2,4-triazole (7) was prepared from **2** (700 mg, 2.6 mmol), 4-chlorophenylhydrazine (544 mg, 3.8 mmol), and NaOAc (284 mg, 3.5 mmol) as described for **5** (chromatography, EtOAc/*n*-hexane 1:7 to 1:1): yield, 113 mg (12%) as a yellow oil; MS (ES^+) *m*/*z* (rel intensity %) 374 (M^+ + 1, 100). Anal. ($C_{20}H_{21}Cl_2N_3$) C, H, N.

5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-3-hexyl-1*H*-1,2,4-triazole (8) was prepared from **2** (700 mg, 2.6 mmol), 2,4-dichlorophenylhydrazine (442 mg, 2.6 mmol), and NaOAc (284 mg, 3.5 mmol) as described for **5** (chromatography, CH_2Cl_2/*n*-hexane 1:1 to 2:1): yield, 94 mg (9%) as a white solid; mp 60−63 °C; MS (ES^+) *m*/*z* (rel intensity %) 408 (M^+ + 1, 76). Anal. ($C_{20}H_{20}Cl_3N_3$) C, H, N.

1,5-Bis(2,4-dichlorophenyl)-3-hexyl-1*H*-1,2,4-triazole (9) was prepared from **3** (1.0 g, 3.3 mmol), 2,4-dichlorophenylhydrazine (595 mg, 3.5 mmol), and NaOAc (433 mg, 5.3 mmol) as described for **5** (chromatography, EtOAc/*n*-hexane 1:9 to 1:1): yield, 84 mg (6%) as a yellow oil; MS (ES^+) *m*/*z* (rel intensity %) 442 (M^+ + 1, 78). Anal. ($C_{20}H_{19}Cl_4N_3$) C, H, N.

1,5-Diphenyl-3-heptyl-1*H*-1,2,4-triazole (10) was prepared from **4** (100 mg, 0.4 mmol), phenylhydrazine (44 mg, 0.4 mmol), and NaOAc (44 mg, 0.5 mmol) as described for **5** (chromatography, CH_2Cl_2/*n*-hexane 8:2, CH_2Cl_2/MeOH 9:1): yield, 13 mg (10%) as a yellow oil; MS (ES^+) *m*/*z* (rel intensity %) 320 (M^+ + 1, 100).

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Supporting Information Available: ^1H and ^{13}C NMR data and assignments for **1**−**10**, CHN analysis, experimental details with references and a figure from molecular modeling, protocols of biological assays in isolated tissues, data of the guinea pig ileum assays, statistical analysis, protocols of cannabinoid tetrad in vivo, and binding tests. This material is available free of charge via the Internet at http://pubs.acs.org.

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