Research Article

Benzyl-1,2,4-triazoles as CB$_1$ Cannabinoid Receptor Ligands: Preparation and In Vitro Pharmacological Evaluation

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1. Introduction

Due to the potential therapeutic effects of cannabinoids that include antiemetic, analgesic, antiglaucoma, obesity treatment, alcoholism, bronchodilatation, and inflammation, a considerable number of cannabinoid ligands have been reported in recent years [1]. Their effects are mediated through G-protein coupled cannabinoid receptors, which are part of the endocannabinoid system (ECS) [2]. So far, two types of cannabinoid receptors, designated as CB$_1$R and CB$_2$R, have been well characterized, and three putative cannabinoid receptors, GPR55, GPR18, and GPR119, have been also proposed [3]. CB$_1$R has been found in the peripheral and central nervous system, and CB$_2$R is mainly present in the immune system. Cannabinoid ligands belong to families of diverse structural classes such as eicosanoids, classical and nonclassical ligands related to $\Delta^8$-tetrahydrocannabinol (THC), and heterocycles. Among the heterocycles family, pyrazoles [4] and aminoalkylindoles [5] are the most representative ligands.

In our early research program, it was found that triazole motif was an attractive scaffold for cannabinoid activity [6]. We reported that the CB$_1$R antagonist 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-3-hexyl-1H-1,2,4-triazole (LH21) exhibited antiobesity activity in vivo assays (Figure 1) [7–9]. Pyrazole [10] and pyrrole [11] cannabinoid ligands bearing a benzyl substituent on position N1 have been reported in the literature as CB$_2$R antagonists (Figure 1). This prompted us to extend our previous investigation by synthesizing a series of 3-alkyl-5-aryl-1H-1,2,4-triazoles in order to establish structure-activity relationships.

2. Materials and Methods

2.1. Chemistry

2.1.1. General. All reagents and solvents were used as commercially received. EtOH was dried over magnesium. TLC
was carried out by precoated silica-gel 60 F254 plates (Merck) and detection by UV light (254 nm). Flash-column chromatography was carried out by Kieselgel 60 (230–400 mesh; Merck). Medium pressure chromatography (MPLC) was carried out by Flash Master Personal system with prepacked silica-gel cartridges. The purity of the final compounds was determined by elemental analysis or analytical HPLC. Elemental analysis was performed on a Heraeus CHN-O rapid analyzer. Analyses indicated by the symbols of the values, except compound 6. Analytical HPLC was run on a Waters 6000 with Delta Pak C18.5 mm, 300 Å (3.9 × 150 mm) column, using an eluent Acetonitrile/H₂O (0.05% H₃PO₄ + 0.04% TEA) in the proportion indicated in each case; flow rate used was 1 mL/min and the UV absorption was detected at a wavelength of 254 nm. HPLC analyses were within ±0.4% of purity, except compound 11b (81% purity). The mass spectra (electrospray positive mode) were determined on a MSD-Series 1100 Hewlett Packard instrument. Melting points (uncorrected) were determined with a Reichert Jung Thermovar apparatus. 1H and 13C NMR spectra were recorded on a Gemini 200, Varian 300 and 400 unity spectrometers using TMS as the internal standard. All chemical shifts are reported in ppm. For the assignment of the protons and carbons of the aromatic rings Scheme 1 is used.

4-Chlorobenzimidamide Hydrochloride (I). Compound 1 was prepared from 4-chlorobenzonitrile (10.00 g, 72.7 mmol), NaOMe (393 mg, 7.3 mmol), and ammonium chloride (3.90 g, 72.7 mmol). Yield: 4.34 g of I (31%) as a white solid. Mp = 246-247 °C. [13] 1H-NMR (CDCl₃) δ: 8.93 (d, 2H, J = 6.2 Hz, Hm); 7.81 (d, 2H, J = 3.8 Hz, Hp). 13C-NMR (CDCl₃) δ: 167.6 C=NH; 141.4 Cpy; 130.8 and 130.7 Co and Cm; 128.2 Cipso. MS (ES⁺) m/z: 122 (100%) [M+H]⁺.

4-Amidinopyridinium Hydrochloride (2). Compound 2 was prepared from 4-cyanopyridine (2.50 g, 24.0 mmol), NaOMe (130 mg, 2.4 mmol), and ammonium chloride (1.28 g, 24.0 mmol). Yield: 3.30 g of 2 (87%) as a white solid. Mp = 248-249 °C. 1H-NMR (CDCl₃) δ: 8.93 (d, 2H, J = 6.2 Hz, Hm); 7.81 (d, 2H, J = 3.8 Hz, Hp). 13C-NMR (CDCl₃) δ: 166.9 C=NH; 151.7 Cm; 138.1 Cipso; 123.2 Co. MS (ES⁺) m/z: 122 (100%) [M+H]⁺.

2.1.3. General Procedure for the Synthesis of 3-5. To a solution of the corresponding amindiam salt (1.5 equiv) in dry EtOH (10–45 mL), NaOMe (1 equiv) in 10 mL of dry EtOH was added. The suspension was stirred at room temperature for 1 h. Then, the solid formed was filtered on Celite. Octanoic hydrazide (2 equiv) was added to the liquid layer and the mixture was stirred under reflux for 46–49 h. After cooling the reaction mixture, solvent was removed in vacuo. The residue was dissolved in CH₂Cl₂ and washed with water (3 × 20 mL). The organic layer was dried over anhydrous Na₂SO₄ and the solvent was removed in vacuo. The obtained residue was purified by MPLC using cyclohexane/EtOAc (3:1) as eluent, except for compound 5 where cyclohexane/EtOAc (3:1 to 4:1) was used.

3-Heptyl-5-phenyl-1H-1,2,4-triazole (3). Compound 3 was prepared from benzamidine hydrochloride hydrate (857 mg, 5.5 mmol), octanoic hydrazide (581 mg, 3.6 mmol), and NaOMe (394 mg, 73 mmol). Yield: 683 mg of 3 (78%) as a transparent oil. Mp = 129–132 °C oxalate (to a solution of the free base in Et₂O, a solution of oxalic acid in EtOAc was added; the white solid was filtered off, washed with EtOAc, and dried). 1H-NMR (CDCl₃) δ: 10.65 (bs, 1H, NH); 7.96 (m, 2H, Ho); 7.34 (m, 3H, Hm and Hp); 2.69 (t, 2H, J = 7.7 Hz, CH₂CH₂CH₂CH₃CH₂CH₂CH₃); 1.65
5-(4-Chlorophenyl)-3-heptyl-1H,1,2,4-triazole (4). Compound 4 was prepared from 1 (1.00 g, 5.2 mmol), anilinic hydrazide (549 mg, 3.5 mmol), and NaOMe (375 mg, 7.0 mmol). Yield 392 mg of 4 (40%) as a white solid. Mp = 108–110°C. 1H-NMR (CDCl3) δ: 7.91 (d, 2H, J = 8.6 Hz, Ho); 7.34 (d, 2H, J = 8.6 Hz, Hm); 2.72 (t, 2H, J = 6.7 Hz, CH2CH2); 2.87 (s, 6H, CH2CH2); 1.68 (p, 6H, J = 7.6 Hz, CH2CH2); 2.45 (m, 8H, CH2CH2); 1.20 (m, 8H, CH2CH2); 0.82 (t, 3H, J = 6.5 Hz, CH3). 13C-NMR (CDCl3) δ: 160.4 and 159.5 C and 135.5 Cisop; 128.9 Cm; 128.7 Cp; 127.7 Co; 31.6 CH2CH2CH2CH2CH2CH2; 29.2 CH2CH2CH2CH2CH2CH2; 28.8 CH2CH2CH2CH2CH2CH2; 28.6 CH2CH2CH2CH2CH2CH2; 28.1 CH2CH2CH2CH2CH2CH2; 27.0 CH2CH2CH2CH2CH2CH2; 22.5 CH2CH2CH2CH2CH2CH2; 13.9 CH3. MS (ES+) m/z: 244 (100%) [M+H]+. Anal (C22H19N3) % calculated (% found) C: 79.24 (79.10); H: 7.95 (7.95); N: 12.60 (12.60).

2.1.4. General Procedure for the Synthesis of 7a–15a and 7b–15b. To a solution of the 3,5-disubstituted triazole (1 equiv) in 40% NaOH aq solution (3–5 ml) and toluene (7–10 ml) (Bu3NBr (0.05 equiv) were first added, and later the corresponding alkylation agent (1 equiv) was added. The reaction mixture was stirred at 80–90°C (bath temperature) for the reaction time indicated. Afterwards, organic layer was separated and the aqueous layer was extracted with CH2Cl2 (3 × 25 ml). The combined organic layers were dried over anhydrous Na2SO4 and the solvent was removed in vacuo. The residue was purified by MPLC [cyclohexane/EtOAc (9:1)], except for compound 13a, which was purified by flash chromatography [CH2Cl2 → CH2Cl2/MeOH (60:1)].

1-Benzyl-5-heptyl-3-phenyl-1H,1,2,4-triazole (7a) and 1-Benzyl-3-heptyl-5-phenyl-1H,1,2,4-triazole (7b). Compounds 7a and 7b were prepared from 3 (100 mg, 0.4 mmol), benzyl bromide (52 μL, 0.4 mmol), and (Bu3N)Br (6 mg, 0.02 mmol); reaction time: 1.5 h. Yield: 106 mg of 7a (78%) as a transparent oil, and 13 mg of 7b (9%) as a transparent oil. 1H-NMR (CDCl3) δ: 8.08 (m, 2H, Ph); 7.40 (m, 3H, Ph); 7.31 (m, 3H, Bn); 7.18 (m, 2H, Bn); 5.34 (s, 2H, CH2Ph); 2.69 (t, 2H, J = 7.8 Hz, CH2CH2CH2CH2CH2CH2); 1.64 (p, 2H, J = 7.5 Hz, CH2CH2CH2CH2CH2CH2); 1.22 (m, 8H, CH2CH2CH2CH2CH2CH2CH2); 0.85 (bt, 3H, J = 6.1 Hz, CH3). 13C-NMR (CDCl3) δ: 159.4 and 159.2 C and C5; 149.4 Cm; 139.5 Cisop; 121.0 Co; 31.5 CH2CH2CH2CH2CH2CH2; 29.6 CH2CH2CH2CH2CH2CH2; 28.9 CH2CH2CH2CH2CH2CH2; 28.8 CH2CH2CH2CH2CH2CH2; 28.3 CH2CH2CH2CH2CH2CH2; 27.1 CH2CH2CH2CH2CH2CH2; 14.4 CH3. MS (ES+) m/z: 263 (100%) [M+H]+. Anal (C14H22N2O1/2HCl) % calculated (% found) C: 59.93 (59.10); H: 8.08 (8.11); N: 19.97 (20.45).

Intermediate 6 (1.00 g, 3.6 mmol) in dry EtOH (20 mL) was reacted by refluxing with NaOMe (1.03 g, 19.0 mmol) for 4 days. Under this procedure, 5 was obtained in 78% yield (676 mg).
1-(4-Chlorobenzyl)-5-heptyl-3-phenyl-1H-1,2,4-triazole (8a) and 1-(4-Chlorobenzyl)-5-heptyl-phenyl-1H-1,2,4-triazole (8b). Compounds 8a and 8b were prepared from 3 (73 mg, 0.3 mmol), 4-chlorobenzyl chloride (48 mg, 0.3 mmol), and (Bu)4NBr (6 mg, 0.02 mmol); reaction time: 11 h. Yield: 96 mg of 8a (87%) as a yellow solid and 10 mg of 8b (9%) as a transparent oil. 8a: 1H-NMR (CDCl3) δ: 8.07 (m, 2H, Ho Ph); 7.37 (m, 3H, Hm Ph and Hp Ph); 7.29 (d, 2H, J = 8.4 Hz, Hm Br); 7.11 (d, 2H, J = 8.4 Hz, Ho Br); 5.27 (s, 2H, CH2 Ar); 2.67 (t, 2H, J = 7.7 Hz, CH2CH2CH2CH2CH2CH2); 1.69 (p, 2H, J = 7.7 Hz, CH2CH2CH2CH2CH2CH2); 1.24 (m, 8H, CH2CH2CH2CH2CH2CH2); 0.85 (t, 3H, J = 6.9 Hz, CH3). T3-C-NMR (CDCl3) δ: 161.0 C3; 156.9 C5; 134.2 Cipso Br; 134.0 Cp Br; 131.1 Cipso Ph; 129.0 and 128.3 Co Br, Cm Ph and Cp Ph; 128.5 Cm Ph; 126.2 Co Ph; 51.3 CH2 Ar; 31.5 CH2CH2CH2CH2CH2CH2; 29.2 CH2CH2CH2CH2CH2CH2; 28.9 CH2CH2CH2CH2CH2CH2; 27.7 CH2CH2CH2CH2CH2; 26.1 CH2CH2CH2CH2CH2; 22.5 CH2CH2CH2CH2CH2; 14.0 CH2 Ms (ES+) m/z: 368 (100%) [M+H]+. 8b: 1H-NMR (CDCl3) δ: 7.50 (m, 2H, Ho Ph); 7.43 (m, 3H, Ph); 7.29 (d, 2H, J = 8.4 Hz, Hm Br); 7.06 (d, 2H, J = 8.4 Hz, Ho Br); 5.30 (s, 2H, CH2 Ar); 2.75 (t, 2H, J = 7.5 Hz, CH2CH2CH2CH2CH2); 1.77 (p, 2H, J = 7.5 Hz, CH2CH2CH2CH2CH2); 1.23 (m, 8H, CH2CH2CH2CH2CH2CH2); 0.85 (t, 3H, J = 6.2 Hz, CH3). T3-C-NMR (CDCl3) δ: 164.6 C3; 155.5 C5; 134.7 Cipso Br; 133.8 Cp Br; 130.1 Cp Ph; 129.0 Cm Br; 128.8 Co Br; 128.6 Cm C; 128.1 Co Ph; 128.0 Cipso Ph; 51.7 CH2 Ar; 31.8 CH2CH2CH2CH2CH2CH2; 29.4 CH2CH2CH2CH2CH2CH2; 29.0 CH2CH2CH2CH2CH2CH2; 28.5 CH2CH2CH2CH2CH2CH2; 22.6 CH2CH2CH2CH2CH2CH2; 14.1 CH2 Ms (ES+) m/z: 368 (100%) [M+H]+. HPLC: Acetonitrile/H2O 95:5; tR = 28.5 min (99% purity).

1-(2,4-Dichlorobenzyl)-5-heptyl-3-phenyl-1H-1,2,4-triazole (9a) and 1-(2,4-Dichlorobenzyl)-5-heptyl-phenyl-1H-1,2,4-triazole (9b). Compounds 9a and 9b were prepared from 3 (100 mg, 0.4 mmol), 2,4-dichlorobenzyl chloride (57 μL, 0.4 mmol), and (Bu)4NBr (6 mg, 0.02 mmol); reaction time: 5 h. Yield: 123 mg of 9a (74%) as a white solid and 12 mg of 9b (7%) as a transparent oil. 9a: 1H-NMR (CDCl3) δ: 8.11 (m, 2H, Ho Ph); 7.42 (m, 4H, Hm Br, Hm and Hp Ph); 7.19 (dd, 1H, J = 8.3 Hz and 1.6 Hz, Ho Br); 6.85 (d, 1H, J = 8.3 Hz, Ho Br); 5.41 (s, 2H, CH2 Ar); 2.73 (t, 2H, J = 7.7 Hz, CH2CH2CH2CH2CH2CH2); 1.73 (p, 2H, J = 7.7 Hz, CH2CH2CH2CH2CH2CH2); 1.27 (m, 8H, CH2CH2CH2CH2CH2CH2); 0.88 (bt, 3H, J = 6.4 Hz, CH3). T3-C-NMR (CDCl3) δ: 161.4 C3; 157.4 C5; 134.5 Cipso Br; 132.8 Cp Br; 132.2 Co Br; 131.0 Cipso Ph; 129.3 Co Br; 129.2 Cm Br; 129.1 Cp Ph; 128.5 Cm Ph; 127.7 Cm Br; 126.3 Co Ph; 48.6 CH2 Ar; 31.5 CH2CH2CH2CH2CH2CH2; 29.1 CH2CH2CH2CH2CH2CH2; 28.8 CH2CH2CH2CH2CH2CH2; 27.8 CH2CH2CH2CH2CH2CH2; 26.0 CH2CH2CH2CH2CH2CH2; 22.5 CH2CH2CH2CH2CH2CH2; 14.0 CH2 Ms (ES+) m/z: 402 (100%) [M+H]+. Anal (C22H25Cl2N3) % calculated (% found) C: 56.67 (65.42); H: 6.26 (6.50); N: 10.44 (10.35). 9b: 1H-NMR (CDCl3) δ: 7.50–7.43 (m, 5H, Ph); 7.40 (d, 1H, J = 1.7 Hz, Hm Br); 7.29 (dd, 1H, J = 8.6 Hz and 1.7 Hz, Hm Br); 6.84 (d, 1H, J = 8.6 Hz, Ho Br); 5.39 (s, 2H, CH2 Ar); 2.76 (t, 2H, J = 7.7 Hz, CH2CH2CH2CH2CH2CH2); 1.79 (p, 2H, J = 7.7 Hz, CH2CH2CH2CH2CH2CH2); 1.23 (m, 8H, CH2CH2CH2CH2CH2CH2); 0.85 (bt, 3H, J = 6.1 Hz, CH3). T3-C-NMR (CDCl3) δ: 165.2 C3; 156.2 C5; 134.6 Cipso Br; 133.0 Cp Br; 132.9 Co Br; 130.5 Co Br; 129.7 Cm Br; 129.2 Cm Ph; 129.0 Cp Ph; 128.6 Co Ph; 127.9 Cm Br; 127.8 Cipso Ph; 50.1 CH2 Ar; 32.0 CH2CH2CH2CH2CH2CH2; 29.6 CH2CH2CH2CH2CH2CH2; 29.2 CH2CH2CH2CH2CH2CH2; 28.7 CH2CH2CH2CH2CH2CH2; 22.9 CH2CH2CH2CH2CH2CH2; 14.3 CH2 Ms (ES+) m/z: 402 (100%) [M+H]+. Anal (C22H25Cl2N3-C4H9) % calculated (% found) C: 69.12 (69.42); H: 7.67 (7.79); N: 8.64 (8.24).
reaction time: 6 h. Yield: 132 mg of 11a (91%) as a white solid and 2 mg of 11b (1%) as a transparent oil. 11a: Mp = 58–60°C. 1H-NMR (CDCl₃) δ: 8.00 (d, 2H, J = 8.8 Hz, Ho Ar); 7.37 (d, 2H, J = 8.6 Hz, Hm Ar); 7.18 (dd, 1H, J = 8.4 and 2.0 Hz, Hm' CH₂Ar); 6.84 (d, 1H, J = 8.0 Hz, Hm' CH₃Ar); 5.37 (s, 2H, CH₂Ar); 2.70 (t, 2H, J = 7.6 Hz, CH₂CH₂CH₂CH₂CH₂CH₂CH₃); 1.69 (p, 2H, J = 7.6 Hz, CH₂CH₂CH₂CH₂CH₂CH₂CH₃); 1.22 (m, 8H, CH₂CH₂CH₂CH₂CH₂CH₂CH₃); 0.85 (bt, 3H, J = 7.1 Hz, CH₃). 13C-NMR (CDCl₃) δ: 160.5 C₃; 157.6 C₅; 135.0 Cipso Ar; 134.6 Cipso CH₂Ar; 132.9 C₂ CH₂Ar; 132.0 C' CH₂Ar; 129.5 Cp Ar; 129.4 Co CH₂Ar; 129.3 Cm' CH₂Ar; 128.7 Cm Ar; 127.7 Cm CH₃Ar; 127.6 Co Ar; 48.7 CH₂Ar; 31.5 CH₂CH₂CH₂CH₂CH₂CH₃; 29.1 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 28.8 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 27.7 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 26.0 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 22.5 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 14.0 CH₃. MS (ES⁺) m/z: 436 (100%) [M+H]⁺. Anal (C₂₂H₂₄Cl₃N₃) % calculated (% found) C: 60.49 (60.42); H: 5.54 (5.74); N: 9.62 (9.42).

11b: 1H-NMR (CDCl₃) δ: 7.41 (s, 5H, Ho Ar, Hm Ar and Hm' CH₂Ar); 7.21 (dd, 1H, J = 8.3 Hz and 2.0 Hz, Hm CH₂Ar); 6.84 (d, 1H, J = 8.3 Hz, Hm' CH₃Ar); 5.37 (s, 2H, CH₂Ar); 2.75 (t, 2H, J = 7.7 Hz, CH₂CH₂CH₂CH₂CH₂CH₂CH₃); 1.77 (p, 2H, J = 7.7 Hz, CH₂CH₂CH₂CH₂CH₂CH₂CH₃); 1.23 (m, 8H, CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₃); 0.85 (bt, 3H, J = 6.1 Hz, CH₃). 13C-NMR (CDCl₃) δ: 161.5 C₃; 154.9 C₅; 136.5 Cipso Ar; 134.7 Cipso CH₂Ar; 132.8 C₂ CH₂Ar; 132.5 C' CH₂Ar; 129.7 Cm and Cp Ar; 129.6 Co CH₂Ar; 129.3 Co Ar; 129.0 Cm' CH₂Ar; 127.8 Cm CH₃Ar; 49.9 CH₂Ar; 31.8 CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 29.7 CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 29.3 CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 29.0 CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 28.4 CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 22.6 CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 14.0 CH₃. MS (ES⁺) m/z: 436 (99%) [M+H]⁺. HPLC: Acetonitrile/H₂O 90:10; tᵣ = 66.2 min (99% purity).

3-(4-Chlorophenyl)-1-(2,4-dichlorobenzyl)-5-heptyl-1,2,4-triazole (12a) and 5-(4-Chlorophenyl)-1-(2,4-dichlorobenzyl)-3-heptyl-1,2,4-triazole (12b). Compounds 12a and 12b were prepared from 4 (90 mg, 0.3 mmol), 2,4-dichlorobenzyl chloride (45 µL, 0.3 mmol), and (Bu₄N)Br (6 mg, 0.02 mmol); reaction time: 6 h. Yield: 122 mg of 12a (86%) as a white solid and 8 mg of 12b (6%) as a transparent oil. 12a: Mp = 97–99°C. 1H-NMR (CDCl₃) δ: 8.00 (d, 2H, J = 8.6 Hz, Ho Ar); 7.41 (d, 1H, J = 2.0 Hz, Hm' CH₂Ar); 7.37 (d, 2H, J = 8.6 Hz, Hm Ar); 7.18 (dd, 1H, J = 8.4 and 2.0 Hz, Hm CH₂Ar); 6.84 (d, 1H, J = 8.4 Hz, Hm' CH₃Ar); 5.37 (s, 2H, CH₂Ar); 2.70 (t, 2H, J = 7.6 Hz, CH₂CH₂CH₂CH₂CH₂CH₂CH₃); 1.69 (p, 2H, J = 7.6 Hz, CH₂CH₂CH₂CH₂CH₂CH₂CH₃); 1.22 (m, 8H, CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₃); 0.85 (bt, 3H, J = 7.1 Hz, CH₃). 13C-NMR (CDCl₃) δ: 160.5 C₃; 157.6 C₅; 135.0 Cipso Ar; 134.6 Cipso CH₂Ar; 132.9 C₂ CH₂Ar; 132.0 C' CH₂Ar; 129.5 Cp Ar; 129.4 Co CH₂Ar; 129.3 Cm' CH₂Ar; 128.7 Cm Ar; 127.7 Cm CH₃Ar; 127.6 Co Ar; 48.7 CH₂Ar; 31.5 CH₂CH₂CH₂CH₂CH₂CH₃; 29.1 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 28.8 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 27.7 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 26.0 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 22.5 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 14.0 CH₃. MS (ES⁺) m/z: 436 (100%) [M+H]⁺. Anal (C₂₂H₂₄Cl₃N₃) % calculated (% found) C: 60.49 (60.42); H: 5.54 (5.74); N: 9.62 (9.42). 4-(1-Benzyl-5-heptyl-1,2,4-triazol-3-yl)pyridine (13a). Compound 13a was prepared from 5 (150 mg, 0.6 mmol), benzyl chloride (73 µL, 0.6 mmol), and (Bu₄N)Br (6 mg, 0.02 mmol); reaction time: 2.5 h. Yield: 166 mg of 13a (81%) as an orange oil. 1H-NMR (CDCl₃) δ: 8.63 (d, 2H, J = 6.0 Hz, Ho pyr); 7.93 (d, 2H, J = 6.0 Hz, Ho pyr); 7.31 (m, 3H, Ph); 7.16 (m, 2H, Ph); 5.33 (s, 2H, CH₂Ar); 2.68 (t, 2H, J = 7.8 Hz, CH₂CH₂CH₂CH₂CH₂CH₂CH₃); 1.65 (p, 2H, J = 7.0 Hz, CH₂CH₂CH₂CH₂CH₂CH₂CH₃); 1.21 (m, 8H, CH₂CH₂CH₂CH₂CH₂CH₂CH₃); 0.83 (bt, 3H, J = 7.1 Hz, CH₃). 13C-NMR (CDCl₃) δ: 158.7 C₃; 157.5 C₅; 150.1 Cm pyr; 138.5 Cipso pyr; 135.2 Cipso Ph; 128.9 Cm Ph; 128.2 Cp Ph; 127.0 Co Ph; 120.4 Co pyr; 52.3 CH₂Ph; 31.5 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 29.1 CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 28.8 CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 27.6 CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 26.1 CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 22.5 CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 14.0 CH₃. MS (ES⁺) m/z: 335 (100%) [M+H]⁺. Anal (C₂₄H₂₅Cl₂N₂) % calculated (% found) C: 75.41 (75.71); H: 7.84 (7.79); N: 16.75 (16.54).
26.0 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 22.5 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 14.0 CH₃. MS (ES⁺) m/z: 369 (100%) [M+H]⁺. Anal (C₂₁H₂₃Cl₂N₄) % calculated (% found) C: 51.73 (51.58); H: 5.52 (5.41); N: 10.97 (10.97).

2.1.5. General Procedure for the Synthesis of 16-18. To a solution of the corresponding triazole (1 equiv) in dry CH₂Cl₂ (4-10 mL), excess of Mel was added. The reaction mixture was stirred at room temperature for the time indicated. Afterwards, solvent was removed in vacuo and the residue was purified by chromatography or recrystallization from Et₂O/CH₂Cl₂.

4-[(1-Benzyl-5-heptyl)-1H,1,2,4-triazol-3-yl]-1-methylpyridinium iodide (16). Compound 16 was prepared from 13a (15 mg, 0.05 mmol) and Mel (4 μL, 0.07 mmol); reaction time: 16 h. Purification: flash chromatography [CH₂Cl₂/MeOH (99:1) → CH₃Cl/MeOH (9:1)]. Yield: 14 mg of 16 (66%) as a yellow gummy solid. ¹H-NMR (CDCl₃): δ: 9.18 (d, 2H, J = 6.7 Hz, Hm pry); 8.54 (d, 2H, J = 6.7 Hz, Ho pry); 7.35 (m, 3H, Ph); 7.19 (m, 2H, Ph); 5.36 (s, 2H, CH₂Ar); 4.68 (s, 3H, NMe); 2.71 (t, 2H, J = 7.6 Hz, CH₂CH₂CH₂CH₂CH₂CH₃); 1.67 (p, 2H, J = 7.6 Hz, CH₂CH₂CH₂CH₂CH₂CH₃); 1.23 (m, 8H, CH₂CH₂CH₂CH₂CH₂CH₂CH₃); 0.85 (bt, 3H, 3H, J = 7.0 Hz, CH₃). C₁₅-NMR (CDCl₃): δ: 165.0 C₃; 152.8 C₃; 150.6 C₂; 145.7 C₅; 143.4 Cipso pry; 129.1 Cm Pry; 128.6 Cm Pry; 127.3 Co Ph; 123.7 Co Pry; 53.0 C₂H₇; 49.1 NMe; 31.5 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 28.8 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 27.2 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 26.1 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 22.5 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 14.0 CH₃. MS (ES⁺) m/z: 349 (100%), [M⁺]. Anal (C₁₅H₂₂N₃I) % calculated (% found) C: 54.47 (54.42); H: 6.14 (6.30); N: 11.76 (11.57).

4-[(1,2-Dichlorobenzyl)-5-heptyl-1H,1,2,4-triazol-3-yl]-1-methylpyridinium iodide (17). Compound 17 was prepared from 14a (70 mg, 0.2 mmol) and Mel (140 μL, 2.3 mmol); reaction time: 8 days. Purification: flash chromatography [CH₂Cl₂/MeOH (95:5)]. Yield: 87 mg of 17 (90%) as a yellow gummy solid. ¹H-NMR (CDCl₃): δ: 9.21 (d, 2H, J = 6.8 Hz, Hm pry); 8.54 (d, 2H, J = 6.8 Hz, Ho pry); 7.33 (d, 2H, J = 8.5 Hz, Hm Ar); 7.15 (d, 2H, J = 8.5 Hz, Ho Ar); 5.33 (s, 2H, CH₂Ar); 4.68 (s, 3H, NMe); 2.71 (t, 2H, J = 7.6 Hz, CH₂CH₂CH₂CH₂CH₂CH₃); 1.69 (p, 2H, J = 7.6 Hz, CH₂CH₂CH₂CH₂CH₂CH₃); 1.23 (m, 8H, CH₂CH₂CH₂CH₂CH₂CH₂CH₃); 0.85 (bt, 3H, 3H, J = 6.5 Hz, CH₃). C₁₅-NMR (CDCl₃): δ: 158.9 C₃; 155.4 C₅; 146.3 Cipso pry; 145.7 Cm Pry; 134.4 Cipso Ar; 129.1 Cm Pry; 128.6 Cm Pry; 127.3 Co Ph; 123.7 Co Pry; 53.0 C₂H₇; 49.1 NMe; 31.5 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 28.8 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 27.2 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 26.1 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 22.5 CH₂CH₂CH₂CH₂CH₂CH₂CH₃; 14.0 CH₃. MS (ES⁺) m/z: 383 (100%), [M⁺]. Anal (C₁₅H₂₂Cl₂N₃I) % calculated (% found) C: 51.73 (51.58); H: 5.52 (5.41); N: 10.97 (10.72).

4-[(1,2-Dichlorobenzyl)-5-heptyl-1H,1,2,4-triazol-3-yl]-1-methylpyridinium iodide (18). Compound 18 was prepared from 15a (50 mg, 0.1 mmol) and Mel (277 μL, 4.45 mmol); reaction time: 8 days. Purification: recrystallization from Et₂O/CH₂Cl₂. Yield: 43 mg of 18 (64%) as a yellow solid. Mp = 136–138°C. ¹H-NMR (CDCl₃): δ: 9.22 (d, 2H, J = 6.7 Hz,
benzyl halide reagents. Preparation of disubstituted triazoles 3–5 is depicted in Scheme 2. In the first step, 4-chlorobenzonitrile and 4-cyanopyridine reacted successively with sodium methoxide and ammonium chloride under inert conditions to afford amidinium hydrochlorides 1 and 2, respectively. Triazoles 3–5 were obtained from 1, 2, and the commercially available benzamidine hydrochloride in moderate yields by refluxing them with octanoic hydrazide under basic conditions. Cyclization of 4-amidinopyridinium hydrochloride (2) was incomplete and the addition intermediate 6 was allowed to be isolated. Acylamidrazone 6 was then cyclized to 5 under the same basic conditions (Scheme 2).

The second step took place with the alkylation of triazoles 3–5 under phase transfer catalysis conditions, using an aqueous sodium hydroxide solution as base and toluene as organic solvent [16]. These conditions were chosen after unsuccessful attempts of alkylation in an organic solvent (tetrahydrofuran) with mild (sodium bicarbonate) or strong (sodium hydride) bases. As depicted in Scheme 3, reaction of 3–5 with different benzyl halides in the presence of tetra-ethylammonium bromide yielded two products by alkylation on N2 (7a–7b, 10a–10b, and 13a) and N1 (7b–15b) of the triazole. Alkylation on N4 of the triazole was not detected, since its formation is not considered [14].

Isomers ratio (a/b) was calculated (% found) for 78% (46.71); H: 4.99 (5.20); N: 10.27 (10.06).

2. Pharmacology

Radioligand Binding Assays. CB1R binding assays in rat cerebellar membranes were performed using [3H]-SR141716A and [3H]-WIN552122 (NEN-Dupont, Boston, MA, 40–60 Ci/mmol) as radioligands, using the previously described methods [14]. Kᵢ were calculated from the equation of Yung-Chi and Prusoff [15], using fixed Kᵢ values for either [3H]-WIN552122 (8 nM) or [3H]-SR141716A (4 nM) obtained from independent experimental assays.

3. Results and Discussion

3.1. Chemistry. 5-Aryl-3-heptyl-1H-1,2,4-triazoles were first synthesized, and then they were alkylated with different...
ratio of N2 isomers was obtained by alkylation of 5 with 4-chlorobenzyl and 2,4-dichlorobenzyl chlorides that led to a mixture of N2/N1 isomers in proportion of 13:1 and 18:1, respectively. These results support the fact that alkylation of chlorobenzyl and 2,4-dichlorobenzyl chlorides that led to a ratio of N2 isomers was obtained by alkylation of 

Since compounds 7–15 are very lipophilic, pyridinium salts (16–18, Scheme 4) of some of the triazolylpyridines previously obtained were synthesized in order to test if they possessed improved aqueous solubility compared to the parent compounds. Increasing the aqueous solubility was important to perform the radioligand binding assays of the series of benzyl triazoles. Therefore, compounds 13a–15a readily reacted with an excess of methyl iodide (1.5 equiv for 13a, II equiv for 14a, and 44 equiv for 15a). Achievement of the triazolyl-1-methyl pyridinium salts needed long reaction times (16 h for 16 and 8 days for 17 and 18), but the products were obtained in good yields.

Qualitative solubility tests of compounds 16–18 did not show any improvement in their solubility in water; therefore they were not assessed by pharmacological assays.

3.2. Radioligand Binding Assays. Competitive radioligand binding assays have been used to evaluate the affinity of selected synthesized triazoles to CB1R in rat cerebellar membranes. They have been performed with [3H]-SR141716A and [3H]-WIN555222 as labelled ligands. The results of these preliminary assays are reported in Table I.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Kᵢ (nM) CB₁R versus [3H]-SR141617</th>
<th>Kᵢ (nM) CB₁R versus [3H]-WIN552122</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR141716</td>
<td>0.59</td>
<td>4</td>
</tr>
<tr>
<td>LH21</td>
<td>85.6 ± 296</td>
<td>748 ± 193</td>
</tr>
<tr>
<td>7a</td>
<td>4.36 ± 1.20</td>
<td>477 ± 94</td>
</tr>
<tr>
<td>8a</td>
<td>589 ± 136</td>
<td>561 ± 125</td>
</tr>
<tr>
<td>10a</td>
<td>389.5 ± 180</td>
<td>2437 ± 888</td>
</tr>
<tr>
<td>11a</td>
<td>562 ± 183</td>
<td>720 ± 165</td>
</tr>
<tr>
<td>12a</td>
<td>13.9 ± 2.4</td>
<td>323 ± 60.5</td>
</tr>
</tbody>
</table>

Compound 12a showed high CB₁R affinity versus [3H]-SR141617 (Kᵢ = 13.9 nM) and moderate affinity versus [3H]-WIN552122 (Kᵢ = 323 nM). These binding data indicate that 12a displaced better SR141617, an inverse agonist of CB₁R, than WIN552122, an agonist of CB₁R. Since both SR141716 and WIN552122 have been reported in the literature to bind to CB₁R in the same binding pocket [17], the results obtained here suggest that 12a binds to the inactive state of CB₁R, as the inverse agonists do (e.g., SR141716), and not to the active state of the receptor, as the agonists do (e.g., WIN552122) [18].

The other tested compounds 7a, 8a, 10a, and 11a showed moderate CB₁R affinity with affinity constant values in the low micromolar range.

In what refers to the binding to CB₂R, none of the compounds showed significant affinity using [3H]-CP55940 as radioligand in membranes purified from cells transfected with human CB₂R (data not shown).

4. Conclusions

In our ongoing program searching for novel cannabinoid ligands, we reported a CB₁R antagonist [5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-3-hexyl-1H-1,2,4-triazole, LH21], which showed an interesting in vitro and in vivo pharmacological profile and was able to reduce food intake and body weight in obese animals with major peripheral components. In the present study, we have explored structural modifications on this 1,2,4-triazole scaffold. A series of new 3(5)-alkyl-5(3)-aryl-1-benzyl-1H-1,2,4-triazoles were synthesized and competitive binding assays of selected compounds were carried out. One of these triazoles (12a) showed high affinity for CB₁R.

Compelling Interests

The authors declare that they have no competing interests.
Acknowledgments

The authors gratefully acknowledge research support from Spanish Grant SAF2012-400075-C02-02 as well as a grant from the "Programa de Biomedicina de la Comunidad de Madrid" (S2010/BMD-2308).

References


