Research Article

Benzyl-1,2,4-triazoles as CB₁ 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 CB₁R and CB₂R, have been well characterized, and three putative cannabinoid receptors, GPR55, GPR18, and GPR119, have been also proposed [3]. CB₁R has been found in the peripheral and central nervous system, and CB₂R 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 Δ⁹-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 CB₁R antagonist 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-3-hexyl-1H-1,2,4-triazole (LH21) exhibited antiobesity activity in 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 CB₂R antagonists (Figure 1). This prompted us to extend our previous investigation by synthesizing a series of 3-alkyl-1,5-diaryl-1H-1,2,4-triazoles in order to establish structure-activity relationships.

We describe herein the synthesis of new benzyl-1,2,4-triazoles [12] and present initial results from radioligand binding assays as part of our investigation on cannabinoid active compounds.

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 elements or functions were within ±0.4% of the theoretical 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 ≥90% of purity, except compound 11b (81% purity). The mass spectra (electrospray positive mode) were determined on a MALDI-TOF 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.

2.1.2. General Procedure for the Synthesis of 1 and 2. To a suspension of the corresponding nitrile (10 equiv) in dry EtOH (30–75 mL) NaOMe (1 equiv) was added. It was stirred at room temperature under N₂ atmosphere for 48 h. Afterwards, ammonium chloride (10 equiv) was added, and the stirring was maintained for 24 more hours. Then, unreacted ammonium chloride was filtered off and the solvent was evaporated from the liquid layer. The white solid obtained was washed with Et₂O, dried, and used in the next step without further purification.

4-Chlorobenzimidamide Hydrochloride (1). 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 (236–240°C (EtOH)). [13] ¹H-NMR (CD₃OD): δ 8.02 (d, 2H, J = 9.0 Hz, H₀); 7.84 (d, 2H, J = 9.0 Hz, Hₘ). ¹³C-NMR (CD₃OD): δ: 167.6 C=NH; 141.4 Cₚ; 130.8 and 130.7 C₀ and Cₘ; 128.2 Cipso. MS (ES⁺) m/z: 155 (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 (CD₃OD): δ: 8.93 (d, 2H, J = 6.2 Hz, H₀); 7.87 (d, 2H, J = 6.2 Hz, Hₘ). ¹³C-NMR (CD₃OD): δ: 166.9 C=NH; 151.7 Cₚ; 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 amidinium 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, 7.3 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, H₀); 7.34 (m, 3H, Hₘ and Hₚ); 2.69 (t, 2H, J = 7.7 Hz, CH₃CH₂CH₂CH₂CH₂CH₃); 1.65
(p, 2H, J = 7.7 Hz, CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂; 1.16 (m, 8H, CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂; 0.79 (bt, 3H, J = 6.3 Hz, CH₃). ¹³C-NMR (CDCl₃) δ: 160.4 and 160.0 C3 and C5; 129.8 Cips; 129.5 Cs; 128.6 Cm; 126.4 Co; 31.5 CH₂CH₂CH₂CH₂CH₂CH₂CH₂; 29.1 CH₂CH₂CH₂CH₂CH₂CH₂CH₂; 28.8 CH₂CH₂CH₂CH₂CH₂CH₂CH₂; 28.1 CH₂CH₂CH₂CH₂CH₂CH₂CH₂; 27.0 CH₂CH₂CH₂CH₂CH₂CH₂CH₂; 13.9 CH₃. MS (ES⁺) m/z: 244 (100%) [M+H]⁺. Anal (C₇H₅N₂O₂).H₂O) % calculated (% found) C: 61.25 (61.41); H: 6.95 (712); N: 12.60 (12.62).

5-(4-Chlorophenyl)-3-heptyl-IH-1,2,4-triazole (4). Compound 4 was prepared from 1 (1.00 g, 5.2 mmol), octanoic hydrazide (549 mg, 3.5 mmol), and NaOMe (375 mg, 7.0 mmol). Yield 392 mg of 4 (40%) as a white solid. Mp = 108–112°C. ¹H-NMR (CDCl₃) δ: 7.91 (d, 2H, J = 8.6 Hz, Ho); 7.30 (d, 2H, J = 6.9 Hz, Hm); 2.18 (m, 4H, C₆H₄Ph); 1.86 (m, 2H, J = 7.6 Hz, CH₂CH₂); 1.26 (m, 4H, C₆H₄Ph); 1.09 (m, 2H, J = 7.3 Hz, CH₂CH₂); 0.96 (m, 3H, CH₃). C₂₆H₃₃N₃O₃ (352.5) % calculated (% found) C: 79.24 (79.35); H: 6.62 (6.59); N: 13.13 (13.55).

4-(3-Heptyl-1H,1,2,4-triazol-3-yl)-pyridine (5) and N'-limino(pyridin-4-yl)methyl-octanoic hydrazide (6). Compound 5 was prepared from 2 (2.00 g, 12.7 mmol), octanoic hydrazide (1.35 g, 8.5 mmol), and NaOMe (918 mg, 17.0 mmol). Yield: 459 mg of 5 (23%) as a white solid and 1.61 g of 6 (45%) as a white solid. 5: Mp = 109–112°C. ¹H-NMR (CDCl₃) δ: 8.69 (d, 2H, J = 6.1 Hz, Hm); 8.06 (d, 2H, J = 6.1 Hz, Ho); 2.95 (t, 2H, J = 7.7 Hz, CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂; 1.78 (p, 2H, J = 7.7 Hz, CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂; 1.21 (m, 8H, CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂; 0.81 (bt, 3H, J = 6.7 Hz, CH₃). ¹³C-NMR (CDCl₃) δ: 159.4 and 159.2 C3 and C5; 149.4 Cm; 139.5 Cips; 121.0 Co; 31.5 CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂; 29.1 CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂; 28.8 CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂; 28.1 CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂; 26.7 CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂; 22.5 CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂; 19.3 CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂CH₂; 13.9 CH₃. MS (ES⁺) m/z: 245 (100%) [M+H]⁺. Anal (C₁₇H₂₇N₃) % calculated (% found) C: 79.24 (79.35); H: 8.16 (8.40); N: 12.60 (12.64). 6: ¹H-NMR (CDCl₃) δ: 7.53 (m, 2H, Ph); 7.41 (m, 3H, Ph); 7.30 (m, 3H, Ph); 7.14 (m, 2H, Ph); 5.35 (2H, J = 6.9 Hz, CH₂CH₂). ¹³C-NMR (CDCl₃) δ: 163.1 C3; 159.3 C5; 136.1 Cips Bo; 120.3 Cips M; 128.5 Co and Cm; 126.7 Co; 126.5 Co; 124.5 Co; 123.0 Co; 120.2 Cps; 121.0 Cps; 119.7 Cps; 118.8 Cps; 118.3 Cps; 117.9 Cps; 117.5 Cps; 115.5 Cps; 114.0 Cps; 109.4 Cps; 108.6 Cps; 108.5 Cps; 107.4 Cps; 105.0 Cps; 87.7 Cps; 83.0 Cps; 79.0 Cps; 77.7 Cps; 76.7 Cps; 75.5 Cps; 74.5 Cps; 73.3 Cps; 72.2 Cps; 71.1 Cps; 69.9 Cps; 66.8 Cps; 63.7 Cps; 60.5 Cps; 57.6 Cps; 54.5 Cps; 51.4 Cps; 48.3 Cps; 45.2 Cps; 42.1 Cps; 39.0 Cps; 35.9 Cps; 32.8 Cps; 30.4 Cps; 28.3 Cps; 26.2 Cps; 24.1 Cps; 22.0 Cps; 20.9 Cps; 18.8 Cps; 16.7 Cps; 14.6 Cps; 12.5 Cps; MS (ES⁺) m/z: 334 (100%) [M+H]⁺. Anal (C₁₇H₂₇N₃) % calculated (% found) C: 79.24 (79.35); H: 8.16 (8.40); N: 12.60 (12.64).
1-(4-Chlorobenzyl)-5-heptyl-3-phenyl-1H-1,2,4-triazole (8a) and 1-(4-Chlorobenzyl)-5-heptyl-5-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)_2NBr (6 mg, 0.02 mmol); reaction time: 1 h. Yield: 96% of 8a (87%) as a yellow solid and 10 mg of 8b (9%) as a transparent oil. 8a: 1H-NMR (CDCl_3) δ: 8.07 (m, 2H, Ho Ph); 7.37 (m, 3H, Hm and Hp Ph); 7.29 (d, 2H, J = 8.4 Hz, Hm Ph); 7.11 (d, 2H, J = 8.4 Hz, Ho Ph); 5.27 (s, 2H, CH_2Ar); 2.67 (t, 2H, J = 7.7 Hz, CH_2CH_2CH_2CH_2CH_2CH_2CH_2); 1.69 (p, 2H, J = 7.7 Hz, CH_2CH_2CH_2CH_2CH_2CH_2CH_2); 1.24 (m, 8H, CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2); 0.85 (ta, 3H, J = 6.9 Hz, CH_3). 13C-NMR (CDCl_3) δ: 161.0 C3; 156.9 C5; 134.2 Cipso Bn; 134.0 Cpb Bn; 131.1 Cipso Ph; 129.0 and 128.3 Co Bn; Cm Bn; 128.5 Cm Ph; 126.2 Co Ph; 51.3 CH_2Ar; 31.5 CH_2CH_2CH_2CH_2CH_2CH_2; 29.2 CH_2CH_2CH_2CH_2CH_2CH_2; 28.8 CH_2CH_2CH_2CH_2CH_2CH_2; 27.7 CH_2CH_2CH_2CH_2CH_2; 26.1 CH_2CH_2CH_2CH_2CH_2CH_2; 22.6 CH_2CH_2CH_2CH_2CH_2 CH_2; 14.0 CH_3; MS (ES^+) m/z: 368 (100%) [M+H]^+. Anal (C_{22}H_{25}ClN_2)_% calculated (% found) C: 75.04 (75.04); H: 6.26 (6.50); N: 10.44 (10.35). 8b: 1H-NMR (CDCl_3) δ: 7.50–7.43 (m, 5H, Ph); 7.40 (d, 1H, J = 1.7 Hz, Hm Bn); 7.20 (dd, 1H, J = 8.6 Hz and 1.7 Hz, Hm Bn); 6.84 (d, 1H, J = 8.6 Hz, Ho Bn); 5.39 (s, 2H, CH_2Ar); 2.76 (t, 2H, J = 7.7 Hz, CH_2CH_2CH_2CH_2CH_2CH_2CH_2); 1.79 (p, 2H, J = 7.7 Hz, CH_2CH_2CH_2CH_2CH_2CH_2CH_2); 1.23 (m, 8H, CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2); 0.85 (bt, 3H, J = 6.9 Hz, CH_3). 13C-NMR (CDCl_3) δ: 165.2 C3; 156.2 C5; 134.6 Cipso Bn; 133.0 Cm Bn; 132.9 Co Bn; 130.5 Co Bn; 129.7 Cm Bn; 129.2 Cm Ph; 129.0 Cp Bn; 128.6 Co Ph; 1279 Cm Bn; 1278 Cipso Ph; 50.1 CH_2Ar; 32.0 CH_2CH_2CH_2CH_2CH_2CH_2; 29.6 CH_2CH_2CH_2CH_2CH_2CH_2; 29.2 CH_2CH_2CH_2CH_2CH_2CH_2; 28.7 CH_2CH_2CH_2CH_2CH_2CH_2; 22.9 CH_2CH_2CH_2CH_2CH_2CH_2; 14.3 CH_3; MS (ES^+) m/z: 402 (100%) [M+H]^+. Anal (C_{22}H_{25}ClN_2)_% calculated (% found) C: 69.12 (69.42); H: 7.67 (7.79); N: 8.64 (8.24).

1-Benzyl-3-(4-chlorophenyl)-5-heptyl-1H-1,2,4-triazole (10a) and 1-Benzyl-5-(4-chlorophenyl)-3-heptyl-1H,1,2,4-triazole (10b). Compounds 10a and 10b were prepared from 4 (80 mg, 0.3 mmol), benzyl bromide (34 μL, 0.3 mmol), and (Bu)_2NBr (6 mg, 0.02 mmol); reaction time: 20 min. Yield: 94% of 10a (89%) as a white solid and 9 mg of 10b (8%) as a yellow oil. 10a: Mp = 47–50°C. 1H-NMR (CDCl_3) δ: 8.02 (d, 2H, J = 8.8 Hz, Ho Ar); 7.36 (d, 2H, J = 8.8 Hz, Hm Ar); 7.29 (m, 3H, Bn); 7.19 (m, 2H, Bn); 5.31 (s, 3H, CH_2Ph); 2.67 (t, 2H, J = 7.7 Hz, CH_2CH_2CH_2CH_2CH_2CH_2); 1.66 (p, 2H, J = 7.7 Hz, CH_2CH_2CH_2CH_2CH_2CH_2); 1.22 (m, 8H, CH_2CH_2CH_2CH_2CH_2CH_2); 0.85 (bt, 3H, J = 6.9 Hz, CH_3). 13C-NMR (CDCl_3) δ: 159.9 C3; 157.1 C5; 135.5 Cipso Ar; 134.7 Cipso Bn; 129.8 Cp Bn; 128.9 Cm Ar; 128.6 Cm Bn; 124.5 Cm Ph; 126.7 Co Ar; 126.9 Cm Bn; 52.1 (CH_3); 31.5 CH_2CH_2CH_2CH_2CH_2CH_2; 29.2 CH_2CH_2CH_2CH_2CH_2CH_2; 28.8 CH_2CH_2CH_2CH_2CH_2CH_2; 27.7 CH_2CH_2CH_2CH_2CH_2CH_2; 26.1 CH_2CH_2CH_2CH_2CH_2CH_2; 22.5 CH_2CH_2CH_2CH_2CH_2CH_2; 14.0 CH_3; MS (ES^+) m/z: 368 (100%) [M+H]^+. Anal (C_{22}H_{25}ClN_2)_% calculated (% found) C: 71.82 (72.02); H: 7.12 (6.89); N: 11.42 (11.24). 10b: 1H-NMR (CDCl_3) δ: 7.47 (d, 2H, J = 8.5 Hz, Hm Ar); 7.38 (d, 2H, J = 8.5 Hz, Ho Ar); 7.30 (3H, Bn); 7.00 (m, 2H, Bn); 5.33 (s, 2H, CH_2Ph); 2.75 (t, 2H, J = 7.6 Hz, CH_2CH_2CH_2CH_2CH_2CH_2); 1.78 (p, 2H, J = 7.6 Hz, CH_2CH_2CH_2CH_2CH_2CH_2); 1.25 (m, 8H, CH_2CH_2CH_2CH_2CH_2CH_2); 0.85 (bt, 3H, J = 6.4 Hz, CH_3). 13C-NMR (CDCl_3) δ: 164.6 C3; 155.0 C5; 136.3 Cipso Ar; 136.2 Cipso Bn; 130.0 Cm Ar; 129.4 Cp Ar; 129.1 Co Ar; 129.0 Cm Bn; 128.0 Cm Ph; 52.6 (CH_3); 31.8 CH_2CH_2CH_2CH_2CH_2CH_2; 29.4 CH_2CH_2CH_2CH_2CH_2CH_2; 29.0 CH_2CH_2CH_2CH_2CH_2CH_2; 22.5 CH_2CH_2CH_2CH_2CH_2CH_2; 14.0 CH_3; MS (ES^+) m/z: 368 (100%) [M+H]^+. Anal (C_{22}H_{25}ClN_2)_% calculated (% found) C: 65.67 (65.42); H: 6.26 (6.50); N: 10.44 (10.35).
C_{22}H_{23}Cl_{3}N_{3} % calculated (% found) C: 60.49 (60.42); H: 5.54 (5.74); N: 9.62 (9.42). 12b: 1-H-NMR (CDCl$_3$) δ: 7.41 (bs, 5H, Ho Ar, Hm Ar and Hm' CH$_2$- Ar); 7.21 (dd, 1H, J = 8.3 Hz and 2.0 Hz, Hm CH$_3$ Ar); 6.84 (d, 1H, J = 8.3 Hz, Ho CH$_3$ Ar); 5.37 (s, 2H, CH$_2$ Ar); 2.75 (t, 2H, J = 7.7 Hz, CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$ CH$_3$); 1.77 (p, 2H, J = 7.7 Hz, CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$); 1.23 (m, 8H, CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$); 0.85 (bt, 3H, J = 6.1 Hz, CH$_3$).

13-C-NMR (CDCl$_3$) δ: 165.1 C3; 154.9 C5; 136.6 Cispo Ar; 134.7 Cispo CH$_2$ Ar; 132.5 C' Ar; 129.7 Cm and Cp Ar; 129.6 Co CH$_2$ Ar; 129.0 Cm' CH$_2$ Ar; 127.8 Cm CH$_2$ Ar; 49.9 CH$_2$ Ar; 31.8 CH$_3$CH$_2$ CH$_2$CH$_2$CH$_2$; 29.7 CH$_3$CH$_2$CH$_2$CH$_2$CH$_2$; 29.3 CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$; 29.0 CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$; 22.8 CH$_3$CH$_2$CH$_2$CH$_2$; 14.0 CH$_3$. MS (ES$^+$) m/z: 436 (99%) [M+H]$^+$. HPLC: Acetonitrile/H$_2$O 90:10, t$_R$ = 66.2 min (99% purity).

4-[(1-Benzyl-5-heptyl-1H-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 (Bu)$_2$NBr (6 mg, 0.02 mmol); reaction time: 2.5 h. Yield: 166 mg of 13a (81%) as an orange oil. 1-H-NMR (CDCl$_3$) 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, ArH); 2.63 (t, 2H, J = 7.8 Hz, CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$); 1.65 (p, 2H, J = 7.0 Hz, CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$); 1.21 (m, 8H, CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$): 0.83 (bt, 3H, J = 7.1 Hz, CH$_3$). 13-C-NMR (CDCl$_3$) δ: 158.7 C3; 157.5 C5; 150.1 Cm pyr; 138.5 Cispo pyr; 135.2 Cispo Ph; 128.9 Cm Ph; 128.2 Cpy Ph; 127.0 Co Ph; 120.4 Co pyr; 52.3 CH$_2$ Ph; 31.5 CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$; 29.1 CH$_2$CH$_2$ CH$_2$CH$_2$CH$_2$CH$_2$; 28.8 CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$; 27.6 CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$; 26.1 CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$; 22.5 CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$; 14.0 CH$_3$. MS (ES$^+$) m/z: 335 (100%) [M+H]$^+$. Anal (C$_3$H$_5$N$_4$) % calculated (% found) C: 75.41 (75.71); H: 7.84 (7.79); N: 16.75 (16.54).

4-[1-(4-Chlorobenzyl)-5-heptyl-1H-1,2,4-triazol-3-yl]pyridine (14a) and 4-[1-(4-Chlorobenzyl)-3-heptyl-1H-1,2,4-triazol-5-yl]pyridine (14b). Compounds 14a and 14b were prepared from 5 (180 mg, 0.7 mmol), 4-chlorobenzyl chloride (119 mg, 0.7 mmol), and (Bu)$_2$NBr (12 mg, 0.04 mmol); reaction time: 6 h. Yield: 150 mg of 14a (51%) as a brown solid and 10 mg of 14b (4%) as a brown oil. 14a: Mp = 77–80°C. 1-H-NMR (CDCl$_3$) δ: 6.61 (d, 2H, J = 6.0 Hz, Hm pyr); 7.90 (d, 2H, J = 6.0 Hz, Ho pyr); 7.27 (d, 2H, J = 8.6 Hz, Ho Ar); 7.09 (d, 2H, J = 8.6 Hz, Ho Ar); 5.27 (s, 2H, CH$_2$ Ar); 2.66 (t, 2H, J = 7.8 Hz, CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$); 1.65 (p, 2H, J = 7.8 Hz, CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$); 1.20 (m, 8H, CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$): 0.82 (bt, 3H, J = 6.6 Hz, CH$_3$). 13-C-NMR (CDCl$_3$) δ: 158.9 C3; 157.5 C5; 150.2 Cm pyr; 138.4 Cispo pyr; 134.2 Cispo Ar; 133.7 Cp Ar; 129.1 Cm Ar; 128.4 Co Ar; 120.4 Co pyr; 51.8 CH$_2$ Ar; 31.5 CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$; 29.1 CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$; 28.8 CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$;
26.0 CH₂CH₂CH₂CH₂CH₂CH₂CH₂; 22.5 CH₂CH₂CH₂CH₂CH₂; CH₂CH₂CH₂; 14.0 CH₃. MS (ES⁺) m/z: 369 (100%) [M+H]⁺. Anal (C₂₁H₂₃ClN₁) % calculated (% found) C: 68.37 (68.35); H: 6.83 (6.90); N: 15.19 (15.21). 14b: ¹H-NMR (CDCl₃) δ: 8.65 (d, 2H, J = 6.2 Hz, Hm pyr); 7.44 (d, 2H, J = 6.2 Hz, Ho pyr); 7.31 (d, 2H, J = 8.5 Hz, Hm Ar); 7.06 (d, 2H, J = 8.5 Hz, Ho pyr); 5.35 (s, 2H, CH₂Ar); 2.76 (t, 2H, J = 7.6 Hz, CH₂CH₂CH₂CH₂CH₂CH₂); 1.74 (p, 2H, J = 7.6 Hz, CH₂CH₂CH₂CH₂CH₂CH₂); 1.27 (m, 8H, CH₂CH₂CH₂CH₂CH₂CH₂); 0.85 (m, 3H, CH₃). ¹³C-NMR (CDCl₃) δ: 165.1 (C3); 152.8 (C5); 150.5 (Cm pyr); 135.4 (Cipso pyr); 134.2 (Cipso Ar); 134.0 (Cp Ar); 129.3 (Cm Ar); 128.0 (C Ar); 122.5 (Cpyr pyr); 52.1 (CH₂ Ar); 31.7 (CH₂CH₂CH₂CH₂CH₂CH₂); 29.3 (CH₂ CH₂CH₂CH₂CH₂CH₂); 29.2 (CH₂CH₂CH₂CH₂CH₂CH₂); 28.4 and 29.0 (CH₂CH₂CH₂CH₂CH₂CH₂CH₂); 22.6 (CH₂CH₂CH₂CH₂CH₂CH₂CH₂); 14.1 (CH₃, MS (ES⁺) m/z: 369 (100%) [M+H]⁺).

4-[(2,4-Dichlorobenzyl)-5-heptyl-1H,1,2,4-triazole-3-yl]-1-methylpyridinium iodide (16). Compound 16 was prepared from 15a (15 mg, 0.05 mmol) and Mel (4 µL, 0.07 mmol); reaction time: 16 h. Purification: flash chromatography [CH₂Cl₂/MeOH (99:1) → CH₂Cl₂/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 pyr); 8.54 (d, 2H, J = 6.7 Hz, Ho pyr); 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, CH₂CH₂CH₂CH₂CH₂CH₂); 1.67 (p, 2H, J = 7.6 Hz, CH₂CH₂CH₂CH₂CH₂CH₂); 1.23 (m, 8H, CH₂CH₂CH₂CH₂CH₂CH₂); 0.85 (bt, 3H, J = 6.7 Hz, CH₃). ¹³C-NMR (CDCl₃) δ: 159.0 (C3); 155.3 (C5); 146.7 (Cipso pyr); 145.6 (Cm pyr); 143.4 (Cipso Ph); 129.1 (Cm Ph); 128.6 (Cp Ph); 127.3 (Cpyr pyr); 53.0 (CH₂ Ar); 49.1 (NMe); 31.5 (CH₂CH₂CH₂CH₂CH₂CH₂); 29.0 (CH₂CH₂CH₂CH₂CH₂CH₂); 28.8 (CH₂CH₂CH₂CH₂CH₂CH₂); 27.2 (CH₂CH₂CH₂CH₂CH₂CH₂); 26.1 (CH₂CH₂CH₂CH₂CH₂CH₂); 22.5 (CH₂CH₂CH₂CH₂CH₂CH₂); 14.0 (CH₂, MS (ES⁺) m/z: 349 (100%), [M⁺]). Anal (C₉H₁₅N₁I) % calculated (% found) C: 55.47 (55.42); H: 6.14 (6.30); N: 11.76 (11.57).

4-[(2,4-Dichlorobenzyl)-5-heptyl-1H,1,2,4-triazole-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 pyr); 8.54 (d, 2H, J = 6.8 Hz, Ho pyr); 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 = 7.5 Hz, CH₂CH₂CH₂CH₂CH₂CH₂); 1.69 (p, 2H, J = 7.5 Hz, CH₂CH₂CH₂CH₂CH₂CH₂); 1.23 (m, 8H, CH₂CH₂CH₂CH₂CH₂CH₂); 0.85 (bt, 3H, J = 6.5 Hz, CH₃). ¹³C-NMR (CDCl₃) δ: 158.9 (C3); 155.4 (C5); 146.3 (Cipso pyr); 145.7 (Cm pyr); 134.4 (Cipso Ar); 132.7 (Cp Ar); 129.1 (Cm Ar); 128.7 (C Ar); 123.5 (Cpyr pyr); 52.1 (CH₂ Ar); 48.9 (NMe); 31.4 (CH₂CH₂CH₂CH₂CH₂CH₂); 28.9 (CH₂CH₂CH₂CH₂CH₂CH₂); 28.6 (CH₂CH₂CH₂CH₂CH₂CH₂); 27.0 (CH₂CH₂CH₂CH₂CH₂CH₂); 25.9 (CH₂CH₂CH₂CH₂CH₂CH₂); 22.4 (CH₂CH₂CH₂CH₂CH₂CH₂); 13.9 (CH₂, MS (ES⁺) m/z: 383 (100%), [M⁺]). Anal (C₁₂H₁₇Cl₂N₂) % calculated (% found) C: 51.73 (51.88); H: 5.52 (5.41); N: 10.97 (10.72).

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.
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 octanolic 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-butylammonium bromide yielded two products by alkylation on N2 (7a–15a) or N1 (7b–15b) of the triazole. Alkylation on N4 of the triazole was not detected, since its formation is hindered by steric reasons. Both alkylated isomers were easily isolated by chromatography, being the N2-benzyl derivatives obtained in greater proportion (≈10:1). The only N1 isomer that could not be isolated and characterized was 13b; however it was detected by HPLC during the synthesis of 13a.
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 1,2,4-triazoles with benzyl halides is governed by steric reasons.

Since compounds 7–15 are very lipophilic, pyridinium salts (16–18, Scheme 4) of some of the triazolopyridines 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, 1 equiv for 14a, and 4 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 synthetized triazoles to CB1R in rat cerebellar membranes. They have been performed with [3H]-SR141716A and [3H]-WIN55522 as labelled ligands. The results of these preliminary assays are reported in Table 1.

Table 1: Affinity of compounds 7a–8a and 10a–12a and the reference cannabinoids SR141716 and LH21 for CB1R determined using rat cerebellar membranes and [3H]-SR141716 or [3H]-WIN5552122 as radioligand. $K_i$ values were obtained from three independent experiments carried out in triplicate and are expressed as mean ± standard error.

| Compound | $K_i$ (nM) CB1R versus [3H]-SR141716 | $K_i$ (nM) CB1R versus [3H]-WIN5552122 |
|----------|-------------------------------------|--------------------------------------|
| SR141716 | $K_i = 0.59$                         | 4                                    |
| LH21     | 855.6 ± 296                         | 748 ± 193                            |
| 7a       | 436 ± 120                           | 477 ± 94                             |
| 8a       | 589 ± 136                           | 561 ± 125                            |
| 10a      | 389.5 ± 180                         | 2437 ± 888                           |
| 11a      | 562 ± 183                           | 720 ± 165                            |
| 12a      | 13.9 ± 2.4                          | 323 ± 60.5                           |

Compound 12a showed high CB1R affinity versus [3H]-SR141716 ($K_i = 13.9$ nM) and moderate affinity versus [3H]-WIN5552122 ($K_i = 323$ nM). These binding data indicate 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., WIN5552122) [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.

Competing Interests

The authors declare that they have no competing interests.
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References

[1] V. K. Vemuri and A. Makriyannis, "Medicinal chemistry of cannabinoids," Clinical Pharmacology & Therapeutics, vol. 97, no. 6, pp. 553–558, 2015.

[2] F. Fezza and M. Maccarrone, "Endocannabinoid biochemistry: what do we know after 50 years?" in Cannabinoids, pp. 53–94, John Wiley & Sons, 2014.

[3] R. G. Pertwee, A. C. Howlett, M. Abood et al., "Cannabinoid receptors, IUPHAR/BPS Guide to Pharmacology," November 2015, http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=13.

[4] F. Barth, Annual Reports in Medicinal Chemistry, Volume 40, Elsevier, 2005.

[5] C. Manera, T. Tuccinardi, and A. Martinelli, “Indoles and related compounds as cannabinoid ligands,” Mini-Reviews in Medicinal Chemistry, vol. 8, no. 4, pp. 370–387, 2008.

[6] N. Jagerovic, L. Hernandez-Folgado, I. Alkorta et al., "Discovery of 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-3-hexyl-1H-1,2,4-triazole, a novel in vivo cannabinoid antagonist containing a 1,2,4-triazole motif," Journal of Medicinal Chemistry, vol. 47, no. 11, pp. 2939–2942, 2004.

[7] F. J. Pauon, A. Bilbao, L. Hernández-Folgado et al., "Antiobesity effects of the novel in vivo neutral cannabinoid receptor antagonist 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-3-hexyl-1H-1,2,4-triazole–LH 21," Neuropharmacology, vol. 51, no. 2, pp. 358–366, 2006.

[8] F. J. Pavón, A. Serrano, V. Pérez-Valero et al., "Central versus peripheral antagonism of cannabinoid CB1 receptor in obesity: effects of LH-21, a peripherally acting neutral cannabinoid receptor antagonist, in Zucker rats," Journal of Neuroendocrinology, vol. 20, no. 1, pp. 116–123, 2008.

[9] M. Alonso, A. Serrano, M. Vida et al., "Anti-obesity efficacy of LH-21, a cannabinoid CB1 receptor antagonist with poor brain penetration, in diet-induced obese rats," British Journal of Pharmacology, vol. 165, no. 7, pp. 2274–2291, 2012.

[10] M. Rinaldi-Carmona, F. Barth, J. Millan et al., “SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor,” Journal of Pharmacology and Experimental Therapeutics, vol. 284, no. 2, pp. 644–650, 1998, http://jpet.aspetjournals.org/cgi/content/long/284/2/644.

[11] G. Ragusa, M. Gómez-Canas, P. Morales et al., “Synthesis, pharmacological evaluation and docking studies of pyrrole structure-based CB1 receptor antagonists,” European Journal of Medicinal Chemistry, vol. 101, pp. 651–667, 2015.

[12] N. Jagerovic, P. Goya, L. Hernández-Folgado, and I. Alcorta, "1,2-4 Triazole derivatives with cannabinoid properties," WO03082833, 2003, http://digital.csic.es/handle/10261/3178.

[13] R. A. Moss, J. Terpinski, D. P. Cox, D. Z. Denney, and R. Krog-Jespersen, "Azide and fluoride exchange reactions of halodiazirines," Journal of the American Chemical Society, vol. 107, no. 9, pp. 2743–2748, 1985.

[14] R. A. Hirat, S. L. Almond, and D. G. Lambert, "Characterisation of the rat cerebella CB1 receptor using SR141716A, a central cannabinoid receptor antagonist," Neuroscience Letters, vol. 220, no. 2, pp. 101–104, 1996.

[15] C. Yung-Chi and W. H. Prusoff, "Relationship between the inhibition constant (KI) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction," Biochemical Pharmacology, vol. 22, no. 23, pp. 3099–3108, 1973.

[16] J. Torres, J. L. Lavandera, P. Cabildo, R. M. Claramunt, and J. Elguero, "Synthesis and physicochemical studies on 1,2-bisazolylethanines," Journal of Heterocyclic Chemistry, vol. 25, no. 3, pp. 771–782, 1988.

[17] S. D. McAllister, G. Rizvi, S. Anavi-Goffer et al., "An aromatic microdomain at the cannabinoid cb1 receptor constitutes an agonist/inverse agonist binding region," Journal of Medicinal Chemistry, vol. 46, no. 24, pp. 5139–5152, 2003.

[18] S. D. McAllister, D. P. Hurst, J. Barnett-Norris, D. Lynch, P. H. Reggio, and M. E. Abood, "Structural mimicry in class A G protein-coupled receptor rotamer toggle switches: the importance of the F3.36(201)/W6.48(357) interaction in cannabinoid CB1 receptor activation," The Journal of Biological Chemistry, vol. 279, no. 46, pp. 48024–48037, 2004.