PREPARATION OF IODINE-123 LABELED AM251: A POTENTIAL SPECT RADIOLIGAND FOR THE BRAIN CANNABINOID CB1 RECEPTOR

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SUMMARY

We report the synthesis and labeling with iodine-123 of N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251). This compound is an analog of the recently described cannabinoid receptor antagonist, SR141716A, in which a 4-chlorophenyl group is replaced by 4-iodophenyl. Labeling in good yield (62%) and radiochemical purity (> 95%), and high specific activity (> 2500 Ci/mmol) was achieved by an iododestannylation reaction using the tributyltin precursor, no carrier added I-123 iodide, and chloramine-T.

Key Words: cannabinoid receptor antagonist, SR141617A, iodine-123

INTRODUCTION

Marijuana is the most commonly used illicit drug [1]. Its major active ingredient, Δ^9-tetrahydrocannabinol (THC), exerts many of its effects at the brain cannabinoid CB_1 receptor. The high affinity tritiated compounds CP 55,940 and WIN 55,212-2 [2,3] have allowed autoradiographic studies of cannabinoid receptors in slide-mounted sections of animal [4] and human [5] brains. A ligand labeled with a radionuclide suitable for positron emission tomographic (PET) or single-photon emission computed tomographic

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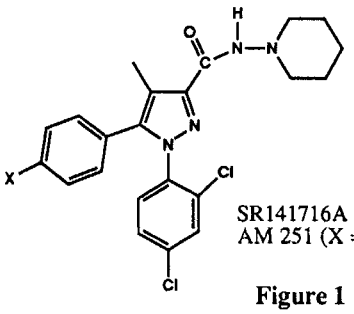


Figure 1

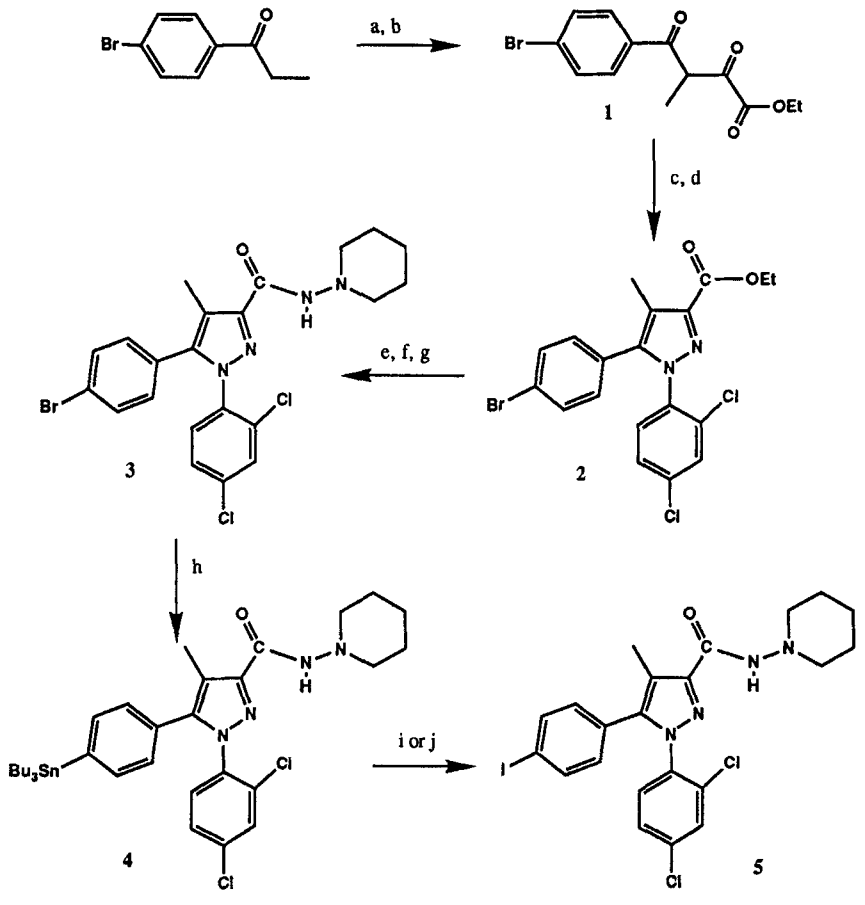
(SPECT) imaging could be used to examine the distribution of cannabinoid receptors in the living human brain. However, previous attempts to study THC binding sites in baboon brain with Δ^8-tetrahydrocannabinol labeled in the omega position of the alkyl side chain was only partially successful, probably because of a combination of high lipophilicity and low affinity [6, 7]. A structurally distinct compound, SR141716A, which is a high affinity antagonist of the cannabinoid CB_1 receptor has recently been described [8]. We report here the synthesis of AM251, an iodine containing analog of SR141716A (Fig. 1), and its labeling with iodine-123. We have previously shown that [^{123}I]AM251 binds in vivo to the cannabinoid CB_1 receptor in mouse brain [9], and is therefore a candidate radioligand for SPECT experiments.

RESULTS AND DISCUSSION

Chemistry. N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251) was synthesized as outlined in Scheme 1. The condensation of 4'-bromopropiophenone with diethyl oxalate gave the lithium salt of ethyl 2,4-dioxo-4-(4-bromophenyl)butanoate **1**, which reacted with 2,4-dichlorophenyl-hydrazine hydrochloride to provide the desired substituted pyrazole compound **2**. After hydrolysis to the carboxylate, the ester **2** was converted to the carboxamide **3** using thionyl chloride followed by 1-aminopiperidine. Compound **3** reacted with hexabutylditin in the presence of catalytic tetrakis(triphenylphosphine)palladium(0) to yield its tributyltin analog, **4**, which was used as a synthetic intermediate to prepare both labeled and unlabeled AM251 (**5**).

Labeling. Radioiododestannylation [10] of the tributyltin starting material **4** with commercially obtained iodine-123 in the presence of chloramine-T yielded [^{123}I]AM251 in about 60% radiochemical yield after direct HPLC purification of the reaction mixture. The mass of AM251 determined from UV absorption associated with the I-123 peak was less than 0.1 μg, corresponding to a specific radioactivity of > 2500 Ci/mmol. The identity of [^{123}I]AM251 was verified by rechromatography after mixing an aliquot with 5 μg of authentic AM251 and demonstrating that radioactive and UV peaks were coincident. A further confirmation of the identity of the labeled product was given by the demonstration that [^{123}I]AM251 competed with the cannabinoid receptor ligands SR141716A, THC, WIN 55,212 or CP 55,940 for binding to high affinity sites in membranes prepared from mouse hippocampus [9].

Scheme I. Preparation of AM251 and [^{123}I]AM251.



a) LiHMDS; b) EtO_2CCO_2Et; c) 2,4-dichlorophenylhydrazine, EtOH; d) AcOH; e) KOH, MeOH; f) $SOCl_2$, toluene; g) 1-aminopiperidine, Et_3N, CH_2Cl_2; h) Bu_6Sn_2, $Pd(PPh_3)_4$, Et_3N; i) NaI, chloramine-T, AcOH; j) $^{123}I^-$, chloramine-T, H_3PO_4, aq EtOH.

In preliminary experiments (not shown) other labeling conditions (acetonitrile rather than ethanol as solvent for the starting material, < 50 μg of starting material, reaction times shorter than 2 min) gave lower radiochemical yields. Also, reactions using hydrogen peroxide or peracetic acid instead of chloramine-T were unsuccessful. Under the standard reaction conditions, a trace of SR141716A, the 4-chloro analog of AM251, was also observed in the HPLC UV traces. This may be produced by chlorodestannylation of the starting material by chloramine-T. The amount of SR141716A under the standard reaction conditions was comparable to that of non-radioactive AM251, but increased with longer reaction times. The HPLC system described in the experimental section separated unreacted tributyltin compound **4** from product **5** (R.T = 40 min). However, it did not

completely separate [^{123}I]AM251 from SR141716A. Therefore in some experiments the [^{123}I]AM251 was subjected to further HPLC purification using acetonitrile/25 mM ammonium acetate (1:2, v/v) at 2 mL/min as mobile phase. The collected [^{123}I]AM251peak was quantitatively extracted into diethylether and evaporated to near dryness (not shown) to remove salts. This system achieved baseline separation of SR141716A (20 min) and [^{123}I]AM251 (25 min). While this second purification step slightly decreased the products radiochemical yield and specific radioactivity, because of physical decay and handling losses, it increased the effective specific radioactivity of the radioligand at least two-fold. If desired, this HPLC system could be used in the primary purification step, provided that the column is washed with 50% acetonitrile between experiments to remove starting material **4**.

Biological experiments. Following intravenous injection into mice, peak whole brain concentrations of [^{123}I]AM251 of about 1 % injected radioactivity occurred at about 2 hours [9]; the half-clearance time was approximately 8 hours. Brain areas with higher concentrations of cannabinoid receptors cleared more slowly than receptor-poor areas [9]. [^{123}I]AM251 may therefore be suitable for SPECT studies of cannabinoid CB_1 receptors in the human brain.

EXPERIMENTAL

General. Proton nuclear magnetic resonance spectra were recorded on a Brucker WP-211sy (200-Mhz) instrument. Chemical shifts are expressed in ppm referenced to the internal standard tetramethylsilane. Coupling constants are given in Hz. Mass spectra (MS, electron impact, 70 eV) and high resolution mass spectra (HRMS) were recorded on a KRATOS MS-902 instrument. Merck silica gel 60 (230-400 mesh) was used for flash column chromatography. Diethylether, dichloromethane and triethylamine were dried and purified using general methods, and solvents for column chromatography were distilled before use. Iodine-123 was obtained from Nordion International as 5-20 μL of a solution in 0.01N NaOH. HPLC (both analytical and preparative) was performed using a 4.6 x 250 mm Alltech Econosil column containing a cyanopropyl stationary phase (10 μm). Knauer HPLC pumps and UV detectors were employed. Radioactive peaks were detected by leading the column output through a loop of tubing held near a lead-shielded Geiger or scintillation probe connected to suitable counting electronics. Detector outputs were recorded and displayed using a Vision-4 system (Autochron Inc, College Station, PA). Iodine-123 was assayed using a Capintec ionization chamber. The mass of AM251 in radiochemical reaction mixtures, and thus the specific activity of [^{123}I]AM251, was determined by comparing the area of the UV peak with those obtained after injection of AM251 standards on the chromatograph.

Lithium salt of ethyl 2,4-dioxo-4-(4-bromophenyl)butanoate (1). A solution of 4'-bromopropiophenone (1.06 g, 5 mmole) in dry diethylether (10 mL) was added dropwise to a solution of lithium bis(trimethylsilyl)amide (5.1 mL, 1.0 M in hexane, 5.1 mmole) in

dry diethylether (20 mL) at -78° under a nitrogen atmosphere. After additional stirring for 45 min at the same temperature, diethyl oxalate was added. The resulting mixture was allowed to warm to room temperature and stirred for 16 hours. The precipitate was filtered, washed with diethylether and dried under vacuum to afford the lithium salt **1** as an off-white solid (1.36 g, 86%).

5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxylic acid, ethyl ester (2). 2,4-dichlorophenylhydrazine hydrochloride (958 mg, 4.4 mmole) was added to a solution of the lithium salt **1** (1.27g, 4.0 mmole) in ethanol (20 mL). The resulting mixture was stirred at room temperature for 20 hours. The precipitate was filtered, washed with ethanol followed by diethylether and dried under vacuum (1.71 g). This solid was then dissolved in acetic acid (10 mL) and heated under reflux for 24 hours. The mixture was then poured into cold water (20 mL) and extracted twice with ethyl acetate. The combined organic fractions were washed in turn with water, saturated sodium bicarbonate and brine, dried over anhydrous sodium sulphate and evaporated to dryness. Purification by flash column chromatography with petroleum ether, ethyl acetate (9:1) gave compound **2** (1.15 g, 63%). ^{1}H NMR ($CDCl_3$) δH 7.48-7.27 (5H, m), 7.01 (2H, dd, J = 1.8 and 6.6 Hz), 4.46 (2H, q, J = 7.1 Hz), 2.33 (3H, s), 1.43 (3H, t, J = 7.1 Hz). MS *m/e* 453 (M^+).

N-(piperidin-1-yl)-5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (3). A solution of potassium hydroxide (78.4 mg, 1.4 mmole) in methanol (8 mL) was added to a solution of the ester 2 (318 mg, 0.7 mmole) in methanol (7 mL) and the mixture was heated at reflux for 3 hours. The reaction mixture was then poured into cold water (10 mL) and acidified with 10% hydrochloric acid. The resulting precipitate was filtered, washed with water, and dried under vacuum to yield the free acid (298 mg). A solution of the acid (298 mg) and thionyl chloride (153 µL) in toluene (10 mL) was heated at reflux for 4 hours. Solvents were removed, and the residue was twice re-dissolved in toluene (20 mL) and evaporated to dryness to give the acyl chloride (309 mg). The residue was dissolved in dry dichloromethane (5 mL) and added dropwise to a solution of 1-aminopiperidine (116 µL, 1.05 mmole) and triethylamine (146 µL, 1.05 mmole) in dry dichloromethane (5 mL) at 0°C. After stirring at room temperature for a further 3 hours, the reaction mixture was added to brine and extracted 3 times with dichloromethane. The combined organic fractions were washed with brine, dried, and evaporated to dryness. Purification of the residue by flash column chromatography on silica gel with petroleum ether and acetone (4:1) afforded compound **3** (343 mg, 96%). ^{1}H NMR ($CDCl_3$) δH 7.63 (1H, s), 7.47-7.42 (3H, m), 7.30-7.27 (2H, m), 6.98 (2H, dd, J = 2.0 and 6.5 Hz), 2.86 (4H, t, J = 5.2 Hz), 2.36 (3H, s), 1.75 (4H, m), 1.43 (2H, m). MS *m/e* 507 (M^+), 408 ($M^+ - C_5H_{11}N_2$), HRMS *m/e* $C_{22}H_{21}BrCl_2N_4O$. Calcd. 506.0276, found 506.0268.

N-(piperidin-1-yl)-5-(4-tributylphenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (4). To a suspension of compound **3** (406 mg, 0.8 mmole) in

dry triethylamine (20 mL) under a nitrogen atmosphere, was added tetrakis(triphenylphosphine)palladium(0) (92 mg. 80 μmole), followed by hexabutylditin (529 μL, 1.05 mmole). The mixture was heated at reflux for 22 hours. After cooling, the solvent was removed in vacuo and the residue purified by column chromatography on Florisil (200 mesh) using petroleum ether and ethyl acetate (3:1) to afford the organotin derivative **4** (219 mg, 38 %). ^{1}H NMR ($CDCl_3$) δH 7.64 (1H, s), 7.39 (1H, d, J = 8.0 Hz), 7.26-7.25 (3H, m), 7.03 (2H, d, J = 8.0 Hz), 2.87 (4H, t, J = 5.2 Hz), 2.39 (3H, s), 1.76 (4H, m), 1.55-1.42 (6H, m), 1.39-1.24 (8H, m), 1.03 (6H, t, J = 8.0 Hz), 0.86 (9H, t, J = 7.1 Hz). MS *m/e* 716 (M^+), HRMS *m/e* $C_{34}H_{48}Cl_2N_4OSn$. Calcd. 718.2227, found 718.2202.

N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (5). To a solution of compound **4** (13 mg, 18 μmole) in ethanol (1 mL) and acetic acid (0.5 mL), was added sodium iodide (10.8 mg, 72 μmole), followed by chloramine-T (4.5 mg, 20 μmole). The reaction mixture was stirred at room temperature for 6 hours and then quenched by adding 5% sodium metabisulphite (1 mL). The mixture was extracted 3 times with dichloromethane (5 mL). The combined organic fractions were washed in turn with saturated sodium bicarbonate solution and brine, dried, and evaporated to dryness. The crude product was purified by flash column chromatography on silica gel using petroleum ether and ethyl acetate (2:1) to give compound **5** (6.9 mg, 69%). ^{1}H NMR ($CDCl_3$) δH 7.58 (2H, d, J = 8.2 Hz), 7.56 (1H, s), 7.36 (1H, m), 7.27-7.18 (2H, m), 6.68 (2H, d, J = 8.2 Hz), 2.79 (4H, t, J = 5.0 Hz), 2.29 (3H, s), 1.68 (4H, m), 1.37 (2H, m). MS *m/e* 554 (M^+), 455 ($M^+ - C_5H_{11}N_2$), HRMS *m/e* $C_{22}H_{21}ICl_2N_4O$. Calcd. 554.0137, found 554.0121.

[^{123}I]N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (5). To $Na^{123}I$ in dilute NaOH (15 μL, 1 mCi) was added compound **4** (50 μg dissolved in 50 μL ethanol). The mixture was shaken, and 0.5M H_3PO_4 (10 μL) and chloramine -T (10 μL of a freshly prepared 0.1% aqueous solution) were added in turn. The reaction vial was capped and shaken, and after 2 min the reaction mixture was diluted with HPLC mobile phase (200 μL) and applied directly to an HPLC column eluted at 2 mL/min with acetonitrile/water (40:60, v/v) containing 0.1% acetic acid. The major radioactive peak at 20 min was collected and evaporated to near dryness at 60°C under a gentle stream of nitrogen gas (620 μCi, 62 %).

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