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Synthesis of [^{18}F] SR144385: A Selective Radioligand for Positron Emission Tomographic Studies of Brain Cannabinoid Receptors

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Summary

A cannabinoid receptor antagonist, SR144385, has been labeled with fluorine-18. [^{18}F] SR144385 was synthesized in a multi-step reaction in which fluorine-18 was introduced by nucleophilic halogen displacement on a bromo precursor. The fluorine-18 labeled intermediate was deprotected and coupled with 1-aminopiperidine to give [^{18}F] SR144385. The time for radiosynthesis, HPLC purification, and formulation was 2 hours from end-of-bombardment. [^{18}F] SR144385 of high radiochemical purity was obtained at end-of-synthesis with an average (n = 11) specific radioactivity of 1852 mCi/μmol and an average isolated, non-decay corrected radiochemical yield of 4% from potassium [^{18}F] fluoride.

Key Words: Cannabinoid, Fluorine-18, Radiotracer, Positron Emission Tomography

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Introduction

Products derived from *Cannabis sativa* are some of the oldest and most widely used drugs in the world. Potential therapeutic applications of Cannabis preparations are numerous (1,2). Unfortunately, published clinical data have accentuated negative effects of cannabis compounds, including memory impairment, EEG modifications (decreasing in particular REM sleep time), and decreased performance in cognitive or psychomotor tasks (3). Alterations in mood or cognition are dose dependent.

Recent reports have shown that Δ^9-THC, the major psychoactive component of cannabis, as well as the putative endogenous ligand for the cannabinoid receptor anandamide (4), mediate their cellular effects through a specific G protein-coupled receptor in the brain (5). This receptor, designated CB1, recently cloned both in rat (6) and human (7) is also found but in lower abundance in some peripheral tissues (8). A novel type of cannabinoid receptor, designated CB2, has been recently described (9). The CB2 subtype seems to be expressed in immune tissue and may be involved in cannabinoid-mediated immune response, but appears not to be expressed in the brain (8).

(-)-5′-^{18}F-Δ^8-THC, a fluoro derivative of THC, was developed some years ago as a tracer for PET studies (10). However, the concentration of the radiotracer in primate brain was low, and there was no regional specificity of uptake. SR141716A is the first potent and selective antagonist for CB1 receptors (11). This compound displays nanomolar affinity for the central cannabinoid receptor. It antagonizes the inhibitory effects of cannabinoid receptor agonists on both mouse vas deferens contractions and adenyl cyclase activity in rat brain membranes. [^{3}H] SR141716A has been used to demonstrate binding to CB1 brain cannabinoid receptors (12). Recently, an iodo analog of SR141716 has been labeled with ^{123}I (13). This tracer, [^{123}I] AM251, and its morpholine derivative, [^{123}I] AM281, have been used to image brain cannabinoid receptors with SPECT (14).

Using the parent structure of SR141716 which exhibited high affinity and excellent selectivity for CB1 sites as a template for further tracer development, N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-fluoromethyl-1H-pyrazole-3-carboxamide (SR144385) was synthesized and shown to retain

much of the parent ligand's affinity and selectivity for the CB1 site. Here we provide the details of the synthetic route to [^{18}F] SR144385 (see Figure 1).

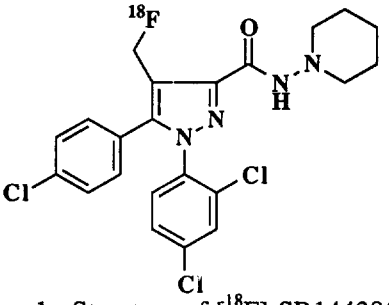


Figure 1. Structure of [^{18}F] SR144385

IC_{50} (nM) or % Inhibition of [^{3}H] CP55940 Binding at 1 μM

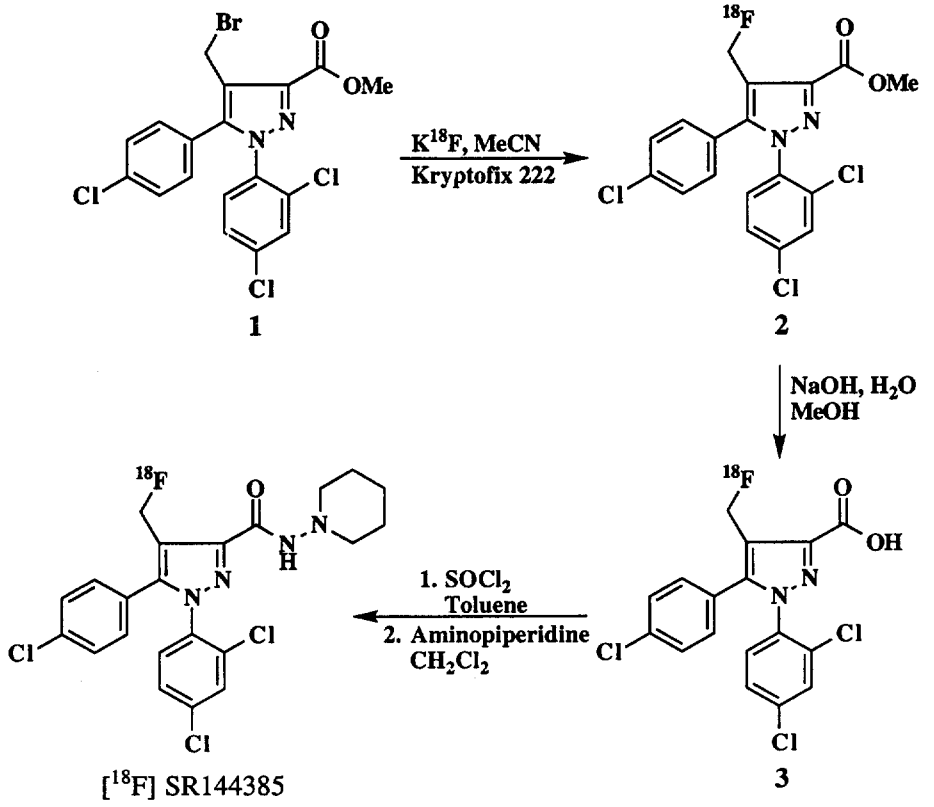
	hCB1	hCB2	Rat Brain	Rat Spleen
SR141716	6.5 ± 0.7	38.7%	2.31 ± 0.42	38.6%
SR144385	20 ± 7	49%	2.90 ± 0.65	50.0%

Discussion and Results

The strategy for the production of [^{18}F] SR144385 is based on a nucleophilic halogen exchange of fluorine-18 for bromine. The radiosynthesis of [^{18}F] SR144385 is outlined in scheme 1. Radiolabeling was achieved by reacting the 4-bromomethyl precursor **1** with activated [^{18}F] potassium fluoride (15) in acetonitrile at 110°C. The methyl ester **2** was saponified with methanolic NaOH at 80°C. The carboxylic acid **3** was converted to the acid chloride with thionyl chloride. The acid chloride was then coupled with 1-aminopiperidine.

The product was purified by semi-preparative reverse phase HPLC (see Figure 2). The radioactive peak corresponding to [^{18}F] SR144385 was collected and evaporated to dryness under reduced pressure in the presence of one drop of Tween-80. The product was redissolved in a 1:300 (v:v) solution of ethanol and sterile saline. The average (n=11) time for radiosynthesis, HPLC purification, and formulation was 2 hours from end-of-bombardment.

Chemical purity, radiochemical purity, and specific radioactivity were all determined by analytical HPLC. While the isolated product did show more than



Scheme 1. Radiosynthesis of [^{18}F] SR144385

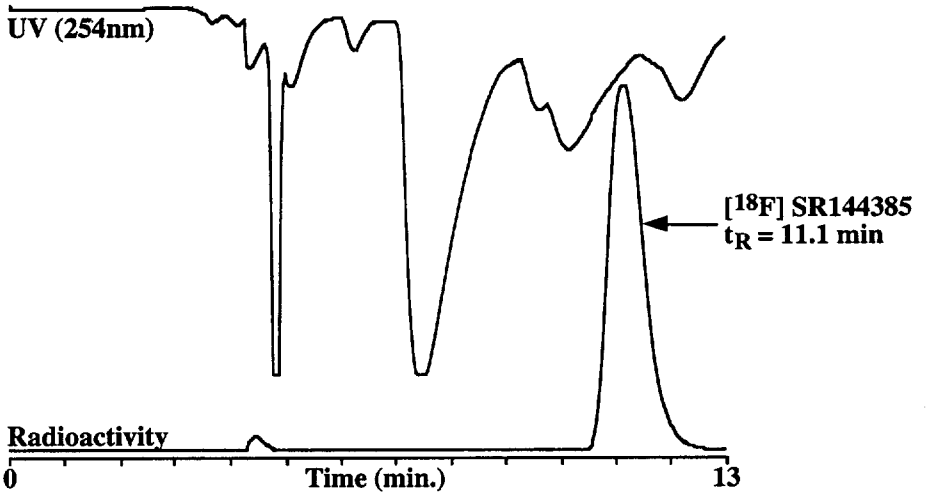


Figure 2. Semi-preparative HPLC chromatograms for [^{18}F] SR144385.

one UV absorbance peak, the peaks were sufficiently separated so as not to interfere with the determination of specific radioactivity. There was no radioactivity associated with the contaminant UV peak, and its identity has not been determined. Specific radioactivity was calculated by relating the area of the UV absorbance peak of carrier SR144385 in an aliquot of known radioactivity to the area of a standard sample of SR144385. [^{18}F] SR144385 of >99% radiochemical purity was obtained at end-of-synthesis with an average specific radioactivity of 1852 mCi/μmol (n = 11; range between 670 and 3900 mCi/μmole) and an average non-decay corrected radiochemical yield of 4%, based on [^{18}F] potassium fluoride (range between 2 and 7%).

Conclusion

We have developed synthetic procedures which provide [^{18}F] SR144385 with high specific radioactivity and a high degree of radiochemical purity. Sufficient radioligand can be produced to allow PET investigations of brain cannabinoid receptors.

Experimental

[^{18}O] Water was obtained from Rotem Industries, Ltd. Chemicals and solvents were reagent grade, and were used as received. The high performance liquid chromatography system consisted of a Waters model 590 pump, Rheodyne model 7126 injector, Waters C_{18} NovaPak radial compression module (25 mm x 10 cm), a Waters model 440 UV detector, and a flow radioactivity detector. Radioactivity measurements were made using a Capintec CRC-12 dose calibrator. An Anotop 25 Plus 0.2 μm sterile filter was used to remove pyrogens.

Radiosynthesis of [^{18}F] SR144385: Two mL of 95% [^{18}O] water were bombarded with 16 MeV protons and transferred under helium pressure to the hot cell through 1/16" Teflon tubing. The aqueous solution of [^{18}F] fluoride was concentrated by passing it through a 2.5 x 50 mm column containing 17.5 mg of DOWEX 1-X8 resin. The [^{18}F] fluoride was eluted off the resin with 300 μL of water containing 2.3 mg of potassium carbonate. This solution was transferred to

a 5 mL crimp-top vial containing 2.3 mg of potassium carbonate and 13 mg of Kryptofix 222. The potassium [^{18}F]fluoride was dried by azeotroping at 110°C with five 200 μL portions of acetonitrile. After drying, heating was continued at 110°C for three minutes.

One mg (2.11 μmol) of the bromo precursor **1**, was dissolved in 300 μL of acetonitrile and added to the vial containing the dried fluoride and Kryptofix. The cap was replaced, and the reaction mixture was heated at 110°C for 5 minutes. The solution was diluted with 300 μL of methanol, treated with 50 μL of 6M sodium hydroxide, and heated at 80°C for five minutes. The solution was then acidified with 200 μL of 2N sulfuric acid and diluted with 4.0 mL of water.

The dilute solution was passed through a Waters C_{18} Sep-Pak plus cartridge which was previously activated with 2 mL of methanol and 5 mL of sterile water. The Sep-Pak was rinsed with 5.0 mL of water and dried by a stream of argon for five minutes. The Sep-Pak was then eluted with 2.0 mL of methanol. The methanol solution was evaporated to dryness under a stream of argon, and the vial was cooled to room temperature. The residue was redissolved in 50 μL of toluene to which 10 μL of thionyl chloride was added. The vial was recapped and heated at 110°C for 10 minutes. After heating, the solution was again evaporated to dryness under a stream of argon and redissolved in 300 μL of methylene chloride. The solution was treated with 50 μL (463 μmol) of 1-aminopiperidine and allowed to react at room temperature for ten minutes.

The solution was evaporated to near dryness with argon and redissolved in 350 μL of 70:30 (v:v) acetonitrile:water containing 0.1% trifluoroacetic acid. The mixture was injected onto the semipreparative HPLC system described above and eluted with 70:30 (v:v) acetonitrile:water containing 0.1% trifluoroacetic acid at a flow rate of 8 mL/min. The radioactive peak corresponding to the desired product (t_R = 11.1 min) was collected in a rotary evaporator modified for remote addition and removal of solutions. One drop (20 μL) of Tween-80 was added to the receiving flask prior to collection of the product. Upon collection, the flask was spun for 10 seconds, and then the HPLC solvent was evaporated at 80°C under reduced pressure.

After evaporation, one drop (20 μL) of ethanol was added to the residue, and the product was dissolved in 6.0 mL of sterile saline. The solution was

filtered through an Anotop 25 plus 0.2 μm sterile filter into a sterile, pyrogen-free bottle.

A 100 μL aliquot of the final product was injected onto an Alltech C_{18} analytical HPLC column (25 mm x 10 cm) and eluted with 70:30 (v:v) acetonitrile:water containing 0.1% trifluoroacetic acid at a flow rate of 6 mL/min. The radioactive peak corresponding to [^{18}F] SR144385 (t_R = 2.1 min) coeluted with a standard sample.

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