Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 29 August 2007, Revised 20 November 2007, Accepted 28 November 2007 Published online 15 February 2008 in Wiley Interscience

(www.interscience.wiley.com) DOI: 10.1002/jlcr.1491

Radiolabeling of a high potency cannabinoid subtype-1 receptor ligand, *N*-(4-fluoro-benzyl)-4-(3-(piperidin-1-yl)-indole-1-sulfonyl) benzamide (PipISB), with carbon-11 or fluorine-18

Sean R. Donohue,[a,b]* Christer Halldin,[b] Magnus Schou,[b] Jinsoo Hong,[a] Lee Phebus,[c] Eyassu Chernet,[c] Stephen A. Hitchcock,[c,d] Kevin M. Gardinier,[c] Kevin M. Ruley,[c] Joseph H. Krushinski,[c] John Schaus,[c] and Victor W. Pike[a]

PipISB [*N*-(4-fluoro-benzyl)-4-(3-(piperidin-1-yl)-indole-1-sulfonyl)benzamide, 9] was identified as a selective high potency CB_1 receptor ligand. Here we describe the labeling of 9 with positron-emitters to provide candidate radioligands for imaging brain CB_1 receptors with positron emission tomography (PET). The radiolabeling of 9 was achieved by two methods, method A with carbon-11 and method B with fluorine-18. In method A, [^{11}C]9 was prepared in one step from [^{11}C]carbon monoxide, itself prepared from cyclotron-produced [^{11}C]carbon dioxide. In method B, [^{18}F]9 was prepared from cyclotron-produced [^{18}F]fluoride ion in a two-stage, four-step synthesis with [^{18}F]4-fluoro-benzyl bromide as a labeling agent. The radiosynthesis time for method A was 44 min; decay-corrected radiochemical yields (RCYs) from [^{11}C]carbon monoxide ranged from 3.1 to 11.6% and specific radioactivities ranged from 21 to 67 GBq/μmol. The radiosynthesis time for method B was 115 min; RCYs from [^{18}F]fluoride ion ranged from 1.5 to 5.6% and specific radioactivities ranged from 200 to 348 GBq/μmol. With these methods, [^{11}C]9 and [^{18}F]9 may be prepared in adequate activity and quality for future evaluation as PET radioligands.

Keywords: CB_1 receptor; PET; carbon-11; fluorine-18; arylsulfonyl-substituted indole; PipISB

Introduction

Cannabinoid type-1 (CB_1) receptors are implicated in a range of neuropsychiatric disorders and hence modulation of these receptors is considered to have broad therapeutic potential. Most notably, rimonabant (Acomplia® or Zimulti®; SR 141716A) (**1**, Figure 1),[1] a potent and selective CB_1 receptor inverse agonist, has demonstrated efficacy for the treatment of obesity[2] and smoking abatement.[3] When CB_1 receptor inverse agonists are given alone or in combination with other receptor ligands, the treatment potential may extend to memory deficits,[4] neuroinflammatory disorders,[4] depression,[5] anxiety,[6] stress[5] and schizophrenia.[7]

Based on the use of *in vitro* techniques, substantial progress has been made in understanding CB_1 receptor activation, inactivation and inverse activation. Nevertheless, how changes in CB_1 receptor populations and occupancy activate a patho-physiological or therapeutic response in living subjects is not well understood. Also, greater clarity is needed to delineate which neurobiological disorders are likely related to CB_1 receptor abnormalities. The development of brain CB_1 receptor imaging with positron emission tomography (PET), as a 'higher-order' *in vivo* assay, is therefore of importance; there is a strong need for radioligands for use with PET for effective imaging of CB_1 receptors *in vivo*. Considerable progress has been made recently in the development of promising PET radioligands for CB_1 receptors. These include inverse agonists from the 1,5-diarylpyrazole platform of rimonabant (e.g. [$^{123/124}$I]AM281 (**2**),[8,9] [^{11}C]JHU75528 (**3**)[10,11] and [^{11}C]JHU75575 (**4**),[11] Figure 1) and from two other structurally distinct platforms (i.e. [^{11}C]MePPEP (**5**)[12] and [^{18}F]MK-9470 (**6**), Figure 1).[13,14] Earlier attempts at radioligand development had focused on the agonist, Δ^8-THC

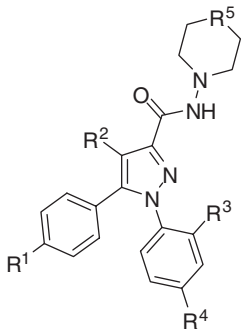
[a]*Molecular Imaging Branch, National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892, USA*

[b] *Karolinska Institutet, Department of Clinical Neuroscience, Psychiatry Section, Karolinska Hospital, S-17176 Stockholm, Sweden*

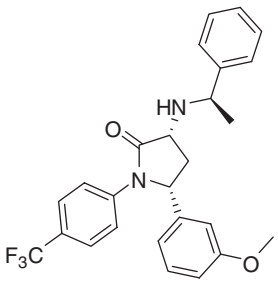
[c]*Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285, USA*

[d]*Present address: Amgen, One Amgen Way, Thousand Oaks, CA 91320, USA*

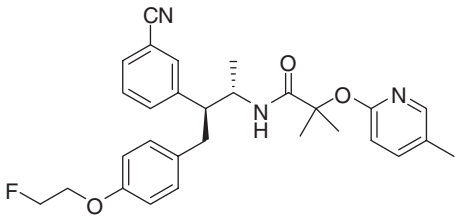
**Correspondence to: Sean R. Donohue, Molecular Imaging Branch, National Institute of Mental Health, National Institutes of Health, Building 10, Rm B3 C346A, 10 Center Drive, Bethesda, MD 20892-1003, USA.*
E-mail: donohues@intra.nimh.nih.gov



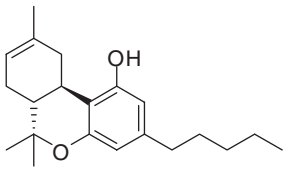
1, R^1 = Cl, R^2 = Me, R^3 = Cl, R^4 = Cl, R^5 = CH_2
2, R^1 = I, R^2 = Me, R^3 = Cl, R^4 = Cl, R^5 = O
3, R^1 = OMe, R^2 = CN, R^3 = Cl, R^4 = Cl, R^5 = CH_2
4, R^1 = OMe, R^2 = CN, R^3 = Br, R^4 = H, R^5 = CH_2



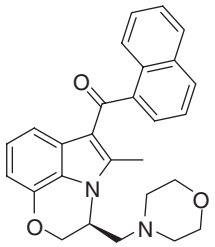
5



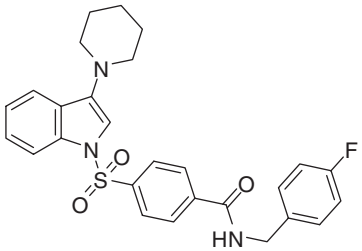
6



7



8



9

Figure 1. Structures of rimonabant (**1**), AM281 (**2**), JHU75528 (**3**), JHU75575 (**4**), MePPEP (**5**), MK-9470 (**6**), Δ^8-THC (**7**), WIN-55212-8 (**8**) and PipISB (**9**).

(**7**, Figure 1)[15] and the aminoalkylindole core of WIN55212-2 (**8**, Figure 1),[16,17] but were unsuccessful.

Recently, a series of arylsulfonyl-substituted indoles was disclosed as a new class of CB_1 receptor inverse agonists with promising therapeutic potential.[18] We sought to explore this class of CB_1 receptor ligand to develop a radioligand for neuroreceptor imaging with PET.

We considered that one of these ligands, PipISB [*N*-(4-fluoro-benzyl)-4-(3-(piperidin-1-yl)-indole-1-sulfonyl)benzamide; **9**] might compare well with previously tested imaging radioligands with regard to critical parameters,[19–21] such as high potency and selectivity. Furthermore, we considered **9** to be amenable to labeling with carbon-11 (β^+, $t_{1/2}$ = 20.4 min) or fluorine-18 (β^+, $t_{1/2}$ = 109.7 min). The opportunity to label with either positron emitter may be advantageous for avoiding troublesome metabolites *in vivo* and for matching the half-life of the radiolabel to the pharmacokinetics of the radioligand. Here, we report the potency and selectivity of PipISB (**9**) for binding to CB_1 receptors and also its radiolabeling by two different pathways (methods A and B). In method A, [^{11}C]PipISB ([^{11}C]**9**) was prepared within a commercially available Synthia apparatus[22] from [^{11}C]carbon monoxide. In method B, [^{18}F]PipISB ([^{18}F]**9**) was prepared within a custom-made remotely controlled apparatus[23] using [^{18}F]4-fluoro-benzyl bromide as the labeling agent.

Results and discussion

The computed lipophilicity of PipISB (**9**) (*c Log* $D_{7.4}$ = 5.10) is high but is lower than those of all other radioligands reported as being promising for imaging CB_1 receptors, with only one exception (Table 1). Ligand **9** was prepared from 3-pyridine-1-yl-1*H*-indole (**10**), as previously described,[18] and showed both high potency at CB_1 receptors (K_b = 1.5 nM) and high selectivity versus CB_2 receptors (> 4667) (Table 1). Overall, these properties of **9** compared well with other tested imaging radioligands (Table 1) and encouraged us to pursue the labeling of **9** with positron-emitters to provide candidate radioligands for PET imaging of brain CB_1 receptors. The precursors (**11**, **13**) that we required for use in radiolabeling were synthesized from **10** in few steps and in moderate yields (Figure 2).

[^{11}C]**9** was prepared in one step from [^{11}C]carbon monoxide through palladium-catalyzed coupling between the aryl halide **11** and 4-fluoro-benzylamine (**14**) (Figure 3) based on a previously reported general methodology.[24] The radiosynthesis was performed in a lead-shielded automated Synthia radiochemistry apparatus[22] that had been equipped with an autoclave for performing carbonylations with [^{11}C]carbon monoxide.[25] The required [^{11}C]carbon monoxide was obtained by passing cyclotron-produced [^{11}C]carbon dioxide over heated molybdenum.[26] [^{11}C]**9** was purified with reverse-phase high-performance liquid chromatography (HPLC) (Figure 4). The fraction containing [^{11}C]**9** (t_R = 10 min) was evaporated to dryness and formulated for intravenous injection first by dissolution in sterile saline containing hydroxypropyl-β-cyclodextrin (to avoid sticking of the radioligand to the glass vessel) and then final sterile filtration. The overall radiosynthesis time was 44 min. Non-optimized decay-corrected radiochemical yields (RCYs) of formulated [^{11}C]**9** from [^{11}C]carbon monoxide ranged from 3.1 to 11.6%. [^{11}C]**9** was obtained in acceptable radiochemical purity (> 98%) and chemical purity (> 90%) and was free of labeling precursors. Specific radioactivities at the end of synthesis (EOS) ranged between 21 and 67 GBq/μmol.

Table 1. Properties of CB_1 receptors ligands **1–6** and PipISB (**9**)

Ligand	Potency at CB_1 receptors (K_B[a] or IC_{50}) (nM)	Potency at CB_2 receptors (K_B[a] or IC_{50}) (nM)	CB_1 vs CB_2 selectivity	*c Log D*$_{7.4}$
1(Rimonabant)	6.1 K_B[b]	>6776 K_B[b]	>1111[b]	6.95[b]
2 (AM281)	8.9 K_B[b]	1069 K_B[b]	120[b]	5.66[b]
3 (JHU75528)	31.3 K_B	1710 K_B	54	5.34
4 (JHU75575)	21.3 K_B	1640 K_B	84.2	4.80
5 (MePPEP)	0.6 K_B[c]	363 K_B[c]	610	5.60
6 (MK-9470)	0.7 IC_{50}[d]	44 IC_{50}[d]	63	5.76
9 (PipISB)	1.5 K_B	>7000 K_B	>4667	5.10

[a]*In vitro* potencies determined through GTP$\gamma^{35}S$ assay.
[b]Data from reference [33].
[c]Data from reference [12].
[d]Data from reference [13].

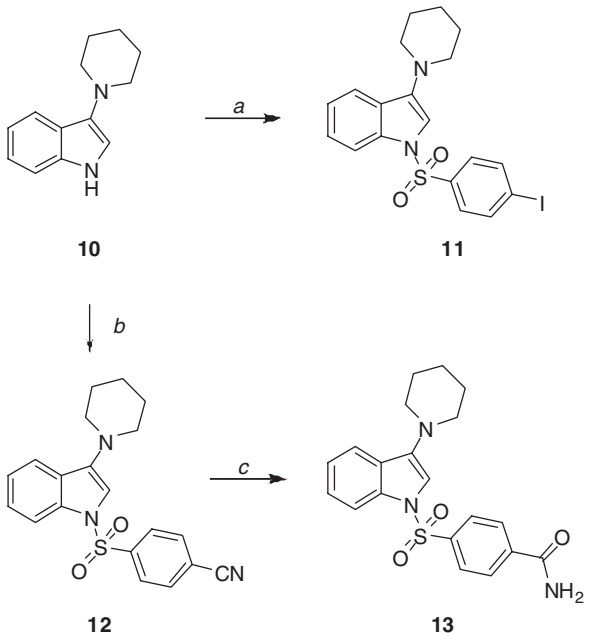


Figure 2. Synthesis of precursors **11** and **13**. Conditions and yields: a) 4-iodobenzenesulfonyl chloride, *t*-BuOK, dioxane, RT, 2h, yield 41%; b) 4-cyanobenzenesulfonyl chloride, *t*-BuOK, dioxane, RT, 4h, yield 27%; c) NaOH, EtOH-H_2O, 30% H_2O_2, RT, 18h, yield 52%.

Product identity was confirmed with liquid chromatography-mass spectrometry (LC-MS) of the carrier and observation of co-elution of radioligand with reference compound **9** in analytical HPLC.

[^{18}F]**9** was prepared by treating the carboxamide **13** with [^{18}F]4-fluoro-benzyl bromide ([^{18}F]**17**) in an overall two-stage, four-step synthesis. In the first stage (Figure 5), [^{18}F]**17** was synthesized in three steps from cyclotron-produced [^{18}F] fluoride ion, as described previously,[27,28,29] but within a custom-made remotely controlled apparatus[23].

In the second stage, brief treatment of the carboxamide **13** with [^{18}F]**17** in the presence of sodium hydride gave [^{18}F]**9** in about 36% RCY ($n=2$) (Figure 5). The dichloromethane was evaporated off, leaving a dimethylformamide (DMF) solution of the crude reaction product. [^{18}F]**9** was purified from the DMF solution with reversed phase HPLC (Figure 6). The fraction containing [^{18}F]**9** ($t_R=15.8$ min) was evaporated to dryness. The radioactive residue was dissolved in a mixture of ethanol–propylene glycol with sterile sodium phosphate buffer solution and then sterile filtered.[30] The overall radiosynthesis time was 115 min. Non-optimized RCYs of formulated [^{18}F]**9** ranged from 1.5 to 5.6% from [^{18}F]fluoride ion. [^{18}F]**9** was obtained in high radiochemical purity (>98%) and chemical purity (>90%) and was free of labeling precursors. Specific radioactivity (EOS) ranged from 200 to 348 GBq/μmol. Product identity was confirmed by its co-elution with reference **9** in analytical HPLC.

Experimental

Materials

All solvents and reagents were purchased from commercial sources and used without further purification. Compounds **3** and **4** were prepared at NIMH using previously published procedures.[11] PipISB (**9**) and 3-piperidine-1-yl-1*H*-indole (**10**) were prepared according to Smith *et al.*[18] 4-(Trimethylamino)-benzaldehyde trifluoromethanesulfonate was prepared as described previously.[31]

General methods

Column chromatography was performed on silica gel columns (35–65 μm; Isco Inc., Lincoln, NE, USA). ^{1}H NMR spectra (400 MHz) were measured on an INOVA spectrometer (Varian, Palo Alto, CA, USA). Abbreviations s, d, dd, m and br denote singlet, doublet, double doublet, multiplet and broad, respectively. Mass detection was performed with an 1100 Series LC/MSD single quadrupole spectrometer (Agilent, Santa Clara, CA, USA) with ESI interface. LC-MS was performed with a reversed-phase column (Luna C18: 150 × 2 mm, 5 μm; Phenomenex, Torrance, CA, USA) eluted with a mixture of aq. 10 mM ammonium formate and acetonitrile (25:75 v/v) at 150 μL/min. After electrospray ionization of the eluted test sample, ions between *m/z* 150 and 750 were acquired. LC analyses, unless otherwise stated, were performed with a heated (50 ± 10°C) reversed phase column (Xterra C18:2.1 × 50 mm, 3.5 μm; Waters Corp., Milford, MA, USA) eluted at 1 mL/min with a gradient of MeCN–MeOH (50:50 v/v) (A) and 0.2% aq. HCO_2NH_4 (B), with A increased linearly from 5 to 100% v/v over 7 min and then held for 1 min. The chemical purities of **11** and **13** were assessed with an identical chromatographic system eluted with a mixture of A and B, with A held at 1% for 2 min, then increased linearly to 30% over

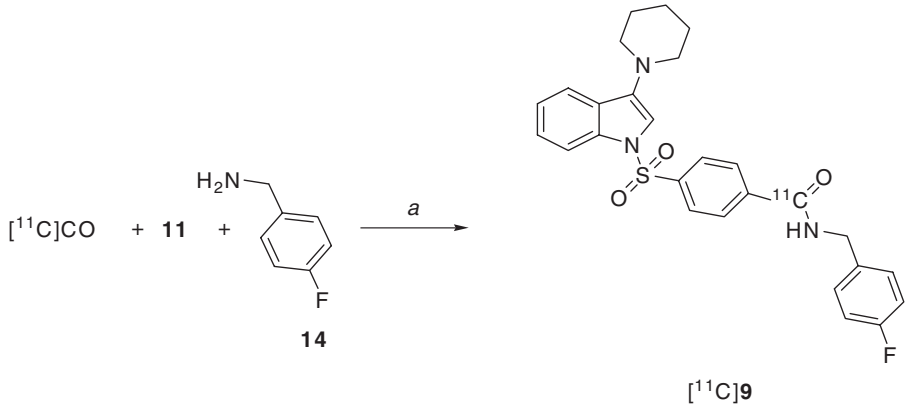


Figure 3. Radiosynthesis of [^{11}C]PipISB ([^{11}C]**9**). Conditions and isolated decay-corrected radiochemical yield; a) $Pd(PPh_3)_4$, THF, 6000 psi, 150 °C, 5 min, 3.1–11.6% RCY.

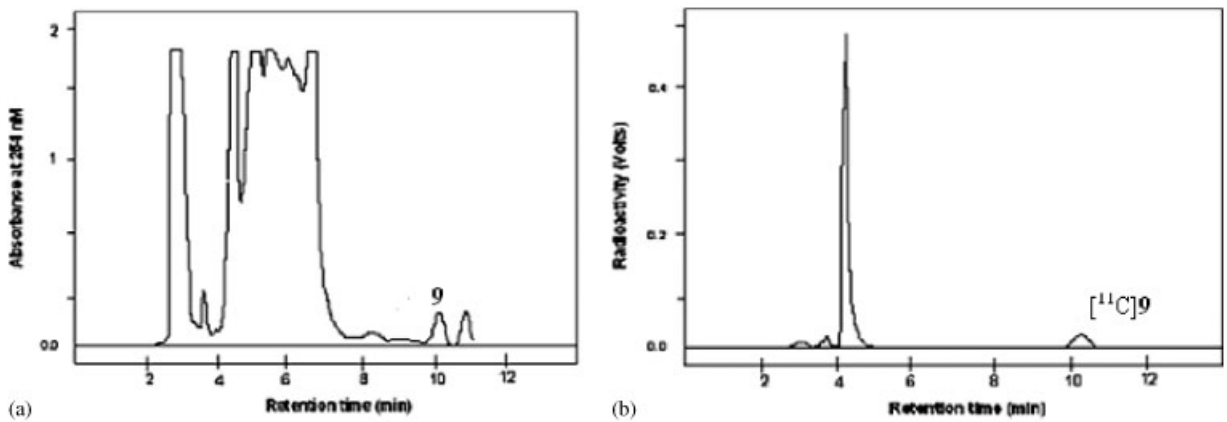


Figure 4. Chromatogram from the HPLC separation of [^{11}C]PipISB ([^{11}C]**9**) (absorbance at 245 nm response, Panel A; radioactivity response, Panel B).

4 min, and finally increased linearly to 100% over 1 min. Radio-thin-layer chromatography (TLC) was performed on silica gel and analyzed with an automatic AR-2000 Imaging Scanner (Bioscan Inc., Washington, DC, USA) with Winscan 2.2 software (LabLogic Inc., Sheffeld, UK). Radioactivity was measured in calibrated ionization chambers (AtomLab 300, Biodex, Shirley, NY, USA) and corrected for background and physical decay.

CB_1 and CB_2 GTPγ^{35}S binding assays

GTPγ35S binding of **9** in Sf9 cell membranes expressing human CB_1 or CB_2 receptors was measured in a 96-well format with a modified antibody capture technique described previously.[32,33] Agonist EC_{50} and antagonist IC_{50} values were calculated with Activity Base software using a four-parameter fit and K_B values (binding constants) calculated as follows:

$$K_B = IC_{50}/[1 + [\text{Agonist}]/EC_{50}]$$

Chemistry

1-(4-Iodo-benzenesulfonyl)-3-piperidin-1-yl-1H-indole (**11**): Potassium *tert*-butoxide (440 mg, 3.9 mmol) was added to a cooled (ice bath) solution of 3-piperidin-1-yl-1*H*-indole (**10**) (665 mg, 3.3 mmol) in dioxane (10 mL). The ice bath was removed and the reaction warmed to room temperature over 40 min. A solution of 4-iodo-benzenesulfonyl chloride (960 mg, 3.2 mmol) in dioxane (6 mL) was added and the reaction mixture stirred at room temperature for 2 h. The reaction mixture was partitioned between water and ethyl acetate. The aqueous layer was extracted with ethyl acetate. The ethyl acetate extracts were combined, washed with water and then brine, dried over sodium sulfate, filtered and concentrated under reduced pressure to give crude product (1.5 g). Silica gel chromatography (eluent: 10% ethyl acetate–hexanes) of this crude product gave pure **11** as a yellow foam (600 mg, 41%). ^{1}H NMR (dimethyl sulfoxide (DMSO)-d_6) δ 7.874 (d, 1H, J = 7.9 Hz), 7.866 (d, 2H, J = 8.8 Hz), 7.59 (d, 2H, J = 8.8 Hz), 7.50 (d, 1H, J = 7.5 Hz), 7.30 (dd, 1H, J = 7.9, 7.9 Hz), 7.20 (dd, 1H, J = 7.6, 7.6 Hz), 6.99 (s, 1H), 2.94–2.90 (m, 4H), 1.66–1.59 (m, 4H), 1.53–1.46 (m, 2H); MS (ESI) m/z 467 ($M^+ + 1$); HPLC, 100%.

4-(3-Piperidin-1-yl-indole-1-sulfonyl)-benzonitrile (**12**)**:** Potassium *tert*-butoxide (420 mg, 3.7 mmol) was added to a solution of **10** (600 mg, 3.0 mmol) in dioxane (30 mL). The mixture was stirred at room temperature for 30 min. A solution of 4-cyanobenzene-sulfonyl chloride (634 mg, 3.2 mmol) in dioxane (2 mL) was added and the reaction mixture stirred at room temperature for 4 h. The reaction mixture was diluted with ethyl acetate and then washed with saturated sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and then evaporated to dryness. The residue was purified with silica gel chromatography (eluent: 10% ethyl acetate–hexanes) to yield **12** as a yellow solid (298 mg, 27%). ^{1}H NMR (DMSO-d_6) δ 8.04 (d, 2H, J = 8.8 Hz), 7.97 (d, 2H, J = 8.8 Hz), 7.91 (d, 1H, J = 8.4 Hz), 7.51 (d, 1H, J = 7.9 Hz), 7.32 (dd, 1H, J = 7.7, 7.7 Hz), 7.21 (dd, 1H, J = 7.7, 7.7 Hz), 7.04 (s, 1H), 2.96–2.91 (m, 4H), 1.66–1.59 (m, 4H), 1.54–1.46 (m, 2H).

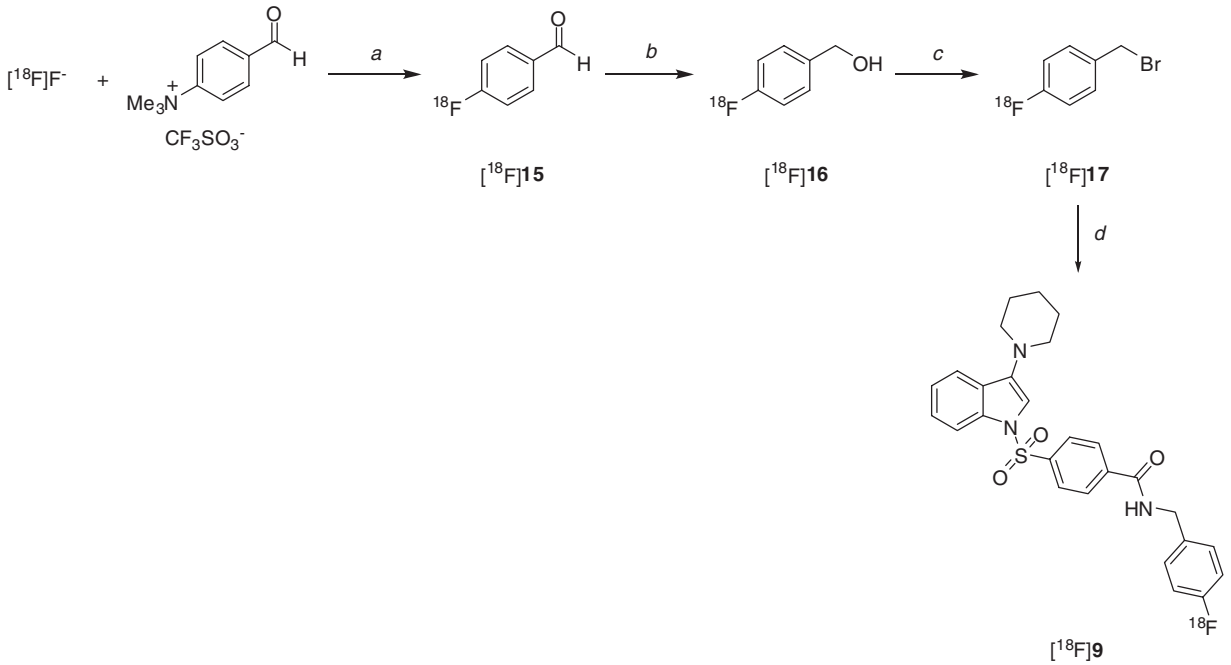


Figure 5. Radiosynthesis of [^{18}F]PipISB ([^{18}F]**9**). Conditions and yields: a) Kryptofix 2.2.2, K_2CO_3, DMSO, 110 °C, 7 min; b) *aq.* $NaBH_4$, RT, 5 min; c) PPh_3Br_2, RT, 5 min; d) NaH, DMF-DCM, RT, 3 min, 1.5–5.6% RCY (from [^{18}F]fluoride ion).

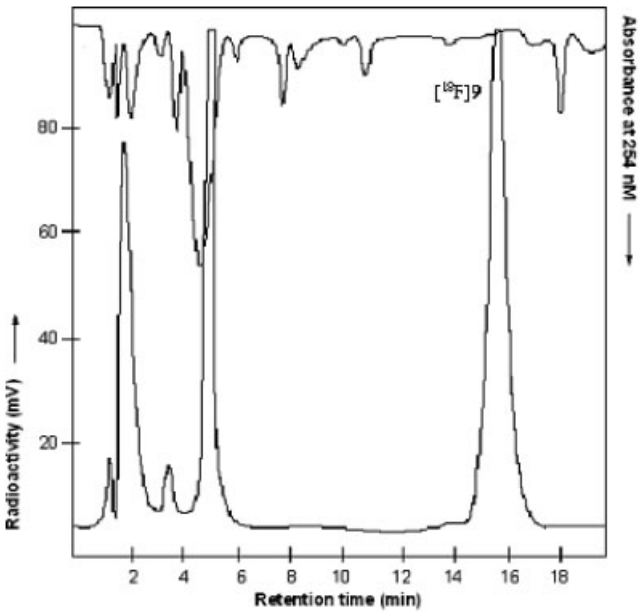


Figure 6. Chromatogram from the HPLC separation of [^{18}F]PipISB ([^{18}F]**9**) (upper trace shows absorbance response and lower trace radioactivity response).

4-(3-Piperidin-1-yl-indole-1-sulfonyl)-benzamide (**13**): Sodium hydroxide pellets (520 mg) were dissolved in water (3.9 mL) and the solution was chilled to −10°C. A solution of **12** (145 mg, 0.4 mmol) in ethanol (7.8 mL) was added to the sodium hydroxide solution. Then hydrogen peroxide solution (30%, 390 μL) was added over 20 min. The mixture was slowly warmed to room temperature, stirred for 18 h, quenched with a sodium bisulfite solution (20% w/v, 3 mL), and then stirred for 30 min at room temperature. The ethanol was removed under vacuum and the resulting aqueous solution partitioned between ethyl acetate and water. The water was removed and the organic layer washed with brine, dried over sodium sulfate and then evaporated to dryness. The residue was purified with silica gel chromatography (eluent: 50% ethyl acetate–hexanes) to yield **13** as a yellow solid (78 mg, 52%). ^{1}H NMR (DMSO-d_6) δ 8.04 (br s, 1H), 7.93 (d, 2H, J = 8.8 Hz), 7.92 (d, 1H, J = 8.2 Hz), 7.87 (d, 2H, J = 8.7 Hz), 7.54 (br s, 1H), 7.49 (d, 1H, J = 7.9 Hz), 7.30 (dd, 1H, J = 7.7, 7.7 Hz), 7.19 (dd, 1H, J = 7.6, 7.6 Hz), 7.03 (s, 1H), 2.95–2.90 (m, 4H), 1.66–1.59 (m, 4H), 1.53–1.46 (m, 2H); MS (ESI) *m/z* 384 (M^+ + 1); HPLC, 100%.

Calculation of *c log D$_{7.4}$*

The *c Log D*$_{7.4}$ values of ligands (Table 1) were calculated with Pallas 3.0 software (Compudrug International Inc., San Francisco, CA, USA).

Radiochemistry instrumentation

Labeling with [^{11}C]carbon monoxide was performed within a lead-shielded automated Synthia apparatus (Uppsala Imanet, Uppsala, Sweden), equipped with an autoclave module.[22] The Synthia consists of a Gilson Aspec AL, XYZ-robot, a Compact PCI running a Visual C++ program, an evaporation unit, specially designed modules (for labeling chemistry, solid-phase extraction, HPLC purification and sterile filtration) and photo-diodes for monitoring radioactivity levels.

Labeling with [^{18}F]fluoro-benzyl bromide was performed within a lead-shielded custom-made remotely controlled apparatus (Laboratech, Stockholm, Sweden).[23] This apparatus is essentially based on a stainless-steel box housing control electronics and supporting on its outside face nine 3-way valves (Bürkert-Contromatic AB, Malmo, Sweden), a heating block with a cooling gun, two reaction vessels with moving needles and a gas manifold.

Radiochemistry

NCA [^{11}C]carbon monoxide: No-carrier-added (NCA) [^{11}C]carbon dioxide was produced at the National Institutes of Health (NIH)

with a PETtrace cyclotron (GE Medical Systems, Milwaukee, WI, USA) to implement the $^{14}N(p,\alpha)^{11}C$ reaction on nitrogen (300 psi) containing a low concentration of oxygen (1%). Typically, proton irradiations (16.5 MeV, 45 μA) for 20 min gave about 52 GBq of [^{11}C]carbon dioxide. [^{11}C]Carbon monoxide was generated by passing the [^{11}C]carbon dioxide in helium carrier gas (20 mL/min) over molybdenum wire (3.5–5.8 g; Strem Chemicals, Newport, MA, USA) contained in a quartz column (7 mm ID × 12 mm OD) while heated in a furnace at 845°C.[31] The resulting [^{11}C]carbon monoxide was trapped on GC-grade silica (19 mg, 100/120, GC grade, Alltech, Raleigh, NC, USA) contained in a stainless-steel tube (1 mm ID × 26 mm) that was cooled in liquid nitrogen.

NCA [^{18}F]fluoride ion: NCA [^{18}F]fluoride ion was produced at the Karolinska Hospital with a PETtrace cyclotron (GE Medical Systems, Uppsala, Sweden) to implement the $^{18}O(p,n)^{18}F$ reaction on ^{18}O-enriched water (95 atom %, 2 mL). Typically, proton bombardments (16.4 MeV, 35 μA) for 20 min gave 27 GBq of [^{18}F]fluoride ion.

*[^{11}C]PipISB ([^{11}C]**9**)*: Trapped [^{11}C]carbon monoxide was released from the silica gel by heating the gel to 60°C. The [^{11}C]carbon monoxide was transferred into a stream of helium (20 mL/min) into the high-pressure stainless-steal micro-autoclave [which had been pre-washed with tetrahydrofuran (THF) (10 mL) and then dried by heating to 150°C for 5 min)]. A THF solution (200 μL) of *tetrakis*triphenylphosphine palladium(0) (1.5 mg, 1.3 μmol), aryl iodide (**11**) (2.1 mg, 4.5 μmol) and 4-fluoro-benzylamine (**14**) (20 μL, 180 μmol) was injected into the loading vessel of the apparatus and then flushed into the micro-autoclave with a steam of helium (90 mL/min). The autoclave was sealed and heated under pressure (41 MPa, 6000 psi) for 5 min in a 150°C bath. The autoclave contents were then emptied into an evacuated V-vial and the THF (0.2 mL plus 0.2 mL rinse) was evaporated off under nitrogen. The crude residue was dissolved in MeCN–aq. HCO_2NH_4 (0.1 M) (65:35 v/v) and injected onto a Luna C-18 column (10 × 250 mm; Phenomenex, Torrance, CA, USA) eluted at 6 mL/min with a gradient of 65% acetonitrile (C) and 35% HCO_2NH_4 (0.1 M) (D) with C increasing linearly to 80% over 10 min. The fraction containing [^{11}C]**9** (t_R = 9.8 min) was collected and the mobile phase was removed by rotary evaporation at 60 °C. The residue of [^{11}C]**9** was reconstituted in a solution of hydroxypropyl-β-cyclodextrin (TCI America, Portland, OR, USA) (10% w/v) in sterile saline for injection (5–10 mL), in readiness for intravenous injection into monkey.

The radiochemical and chemical purities of [^{11}C]**9** were assessed with HPLC on a Luna C-18 column (4.6 × 250 mm, Phenomemex) eluted with MeCN–aq. HCO_2NH_4 (0.1 M) (80:20 v/v) at 2 mL/min (t_R = 4.7 min). The identity of the product was confirmed by its co-elution with reference **9** in the HPLC system and also LC-MS of associated carrier in NCA material.

*[^{18}F]4-Fluoro-benzyl bromide ([^{18}F]**9**)*: NCA [^{18}F]fluoride ion was separated from irradiated ^{18}O-enriched water on an anion exchange resin (Sep-Pak® Light QMA cartridge, Accell™; Waters Corp.) that had been preconditioned by elution first with potassium carbonate solution (0.5 M, 10 mL) and then deionized with water (10 mL). The [^{18}F]fluoride ion was transferred to a 10-mL vial by elution of the QMA resin with acetonitrile–water (94:4 v/v, 2 mL) containing Kryptofix® 2.2.2 (9.5 mg, 25 μmol; Aldrich Chem. Co.) and potassium carbonate (1.7 mg, 12 μmol). Solvents were evaporated off at 130°C under a nitrogen flow. The resulting [^{18}F]fluoride–K^+–Kryptofix 2.2.2 complex was heated to 110°C for 7 min with a solution of (4-trimethylamino) benzaldehyde trifluoromethanesulfonate (7 mg, 22.4 μmol) in DMSO (500 μL). The reaction mixture was then cooled, diluted with water (2 × 7 mL) and eluted through a short cartridge (Sep-Pak® Plus C-18, Accell™; Waters Corp.) that had been preconditioned by elution first with ethanol (10 mL) and then deionized water (10 mL). [^{18}F]4-Fluorobenzaldehyde ([^{18}F]**15**) (silica gel-TLC: R_f = 0.59, chloroform) was retained on the cartridge. Aqueous sodium borohydride (30 mg/mL) solution was slowly passed through the cartridge and then nitrogen was passed through for 5 min. The generated and retained [^{18}F]4-fluoro-benzyl alcohol ([^{18}F]**16**) (silica gel-TLC: R_f = 0.15, chloroform) was then eluted from the column with dichloromethane (2 mL), passed through an extraction tube (3 mL; Supelco, Stockholm, SE) that had been filled with potassium carbonate (1 g in upper part) and magnesium sulfate (800 mg in lower part), and finally emptied into a vial containing triphenyldibromophosphorane (90 mg, 213 μmol). The reaction mixture was vortexed with remote control at room temperature for 5 min. The crude [^{18}F]4-fluoro-benzyl bromide ([^{18}F]**17**) (silica gel-TLC: R_f = 0.83, chloroform) was then purified by passage through a Sep-Pak® Plus silica cartridge.

*[^{18}F]PipISB ([^{18}F]**9**)*: The purified [^{18}F]4-fluoro-benzyl bromide was immediately reacted with precursor (**13**) (1 mg, 2.6 μmol) dissolved in DMF (300 μL) containing sodium hydride (1 mg, 60% in dispersion) at room temperature for 3 min. The remaining dichloromethane was removed by heat under a nitrogen flow. HPLC mobile phase (700 μL), consisting of MeCN-–aq. HCO_2NH_4 (0.1 M) (55:45 v/v), was added to the reaction vial containing the remaining DMF solution and the resultant solution was transferred to a HPLC loop. The [^{18}F]**9** was purified on a μ-Bondapak C-18 column (300 × 7.8 mm, 10 μm; Waters Corp.) eluted at 7 mL/min. Mobile phase was evaporated off from the fraction containing radioligand [^{18}F]**9** (t_R = 15.8 min). The residue was re-dissolved in a mixture of ethanol–propylene glycol (30:70 v/v, 3 mL) with sterile sodium phosphate buffer solution (0.2 M, pH = 7.4, 5 mL), and filtered through a sterile filter (Millex® GV, 0.22 μm pore size; Millipore Corp., Corrigtwohill, Co. Cork, Ireland).

The radiochemical and chemical purities of [^{18}F]**9** were determined by HPLC on a μ-Bondapak C-18 column (300 × 3.9 mm, 10 μm; Waters corp.) eluted with MeCN–aq. HCO_2NH_4 (0.1 M) (55:45 v/v) at 3 mL/min. The identity of [^{18}F]**9** was confirmed by its co-elution with reference **9** (t_R = 9.95 min) under the same HPLC conditions.

Specific radioactivity determination

Specific radioactivities (GBq/μmol) at EOS were determined with analytical radio-HPLC, calibrated for absorbance (λ = 254 nm) response per mass of ligand, using the same HPLC conditions that were used to determine the radiochemical and chemical purities for [^{11}C]**9** or [^{18}F]**9**, respectively. The radioactivity of the radioligand peak (decay corrected) (GBq) was divided by the mass of the associated carrier peak (μmol).

Conclusion

PipISB (**9**) was identified as a highly potent and selective CB_1 receptor ligand with a calculated lipophilicity similar to other PET radioligands targeting brain CB_1 receptors and was successfully labeled with No-carrier-added (NCA) carbon-11 or fluorine-18, hence permitting future evaluation of the two

generated radioligands for *in vivo* imaging of brain CB_1 receptors with PET.

Acknowledgements

The authors thank other members of the PET group at the Karolinska Institutet and of the Molecular Imaging Branch (NIMH) for assistance. This study was supported in part by the Intramural Research Program of the National Institutes of Health (NIH), specifically the National Institute of Mental Health (NIMH) and was also performed under a CRADA agreement between NIMH, the Karolinska Institutet and Eli Lilly and Co. Mr Sean R. Donohue received support through the National Institutes of Health–Karolinska Institutet Graduate Training Partnership in Neuroscience. We thank the Clinical PET Center (NIH) for performing cyclotron irradiations.

References

[1] M. Rinaldi-Carmona, F. Barth, M. Heaulme, D. Shire, B. Calandra, C. Congy, S. Martinez, J. Maruani, G. Neliat, D. Caput, P. Ferrara, P. Soubrie, J. C. Breliere, G. Le Fur, *FEBS Lett.* **1994**, *350*, 240–244.
[2] S. P. Vickers, G. A. Kennett, *Curr. Drug Targets* **2005**, *6*, 215–223.
[3] C. Cohen, E. Kodas, G. Griebel, *Pharmacol. Biochem. Behav.* **2005**, *81*, 387–395.
[4] R. N. Takahashi, F. A. Pamplona, M. S. Fernandes, *Neurosci. Lett.* **2005**, *380*, 270–275.
[5] J. M. Witkin, E. T. Tzavara, R. J. Davis, X. Li, G. G. Nomikos, *Trends Pharmacol. Sci.* **2005**, *26*, 609–617.
[6] B. Costa, A. E. Trovato, M. Colleoni, G. Giagnoni, E. Zarini, T. Croci, *Pain* **2005**, *116*, 52–61.
[7] G. Griebel, J. Stemmelin, B. Scatton, *Biol. Psychiatry* **2005**, *57*, 261–267.
[8] S. J. Gatley, R. Lan, N. D. Volkow, N. Pappas, P. King, C. T. Wong, A. N. Gifford, B. Pyatt, S. L. Dewey, A. Makriyannis, *J. Neurochem.* **1998**, *70*, 417–423.
[9] G. Berding, U. Schneider, P. Gielow, R. Buchert, F. Donnerstag, W. Brandau, W. H. Knapp, H. M. Emrich, K. Muller-Vahl, *Psychiatry Res.* **2006**, *147*, 249–256.
[10] A. G. Horti, H. Fan, H. Kuwabara, J. Hilton, H. T. Ravert, D. P. Holt, M. Alexander, A. Kumar, A. Rahmim, U. Scheffel, D. F. Wong, R. F. Dannals, *J. Nucl. Med.* **2006**, *47*, 1689–1696.
[11] H. Fan, H. T. Ravert, D. P. Holt, R. F. Dannals, A. G. Horti, *J. Label. Compds. Radiopharm.* **2006**, *49*, 1021–1036.
[12] F. Yasuno, A. K. Brown, S. S. Zoghbi, J. H. Krushinski, E. Chernet, J. Tauscher, J. M. Schaus, L. A. Phebus, A. K. Chesterfield, C. C. Felder, R. L. Gladding, J. Hong, C. Halldin, V. W. Pike, R. B. Innis, *Neuropsychopharmacology* **2007** (DOI: 10.1038/sj.npp.1301402).
[13] H. D. Burns, K. Van Laere, S. Sanabria-Bohorquez, T. G. Hamill, G. Bormans, W. S. Eng, R. Gibson, C. Ryan, B. Connolly, S. Patel, S. Krause, A. Vanko, A. Van Hecken, P. Dupont, I. De Lepeleire, P. Rothenberg, S. A. Stoch, J. Cote, W. K. Hagmann, J. P. Jewell, L. S. Lin, P. Liu, M. T. Goulet, K. Gottesdiener, J. A. Wagner, J. de Hoon, L. Mortelmans, T. M. Fong, R. J. Hargreaves, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 9800–9805.
[14] P. Liu, L. S. Lin, T. G. Hamill, J. P. Jewell, T. J. Lanza Jr, R. E. Gibson, S. M. Krause, C. Ryan, W. Eng, S. Sanabria, X. Tong, J. Wang, D. A. Levorse, K. A. Owens, T. M. Fong, C. P. Shen, J. Lao, S. Kumar, W. Yin, J. F. Payack, S. A. Springfield, R. Hargreaves, H. D. Burns, M. T. Goulet, W. K. Hagmann, *J. Med. Chem.* **2007**, *50*, 3427–3430.
[15] A. Charalambous, G. Marciniak, C. Y. Shiue, S. L. Dewey, D. J. Schlyer, A. P. Wolf, A. Makriyannis, *Pharmacol. Biochem. Behav.* **1991**, *40*, 503–507.
[16] P. G. Willis, O. A. Pavlova, S. I. Chefer, D. B. Vaupel, A. G. Mukhin, A. G. Horti, *J. Med. Chem.* **2005**, *48*, 5813–5822.
[17] J. Dhawan, H. Deng, S. J. Gatley, A. Makriyannis, T. Akinfeleye, M. Bruneus, A. A. Dimaio, A. N. Gifford, *Synapse* **2006**, *60*, 93–101.
[18] J. B. Allen, A. K. Amegadzie, K. M. Gardinier, G. S. Gregory, S. A. Hitchcock, P. J. Hoogstraat, W. J. Jones Jr, D. L. Smith, PCT Int. App. WO2005066126, **2005**.
[19] C. Halldin, B. Gulyas, O. Langer, L. Farde, *Q. J. Nucl. Med.* **2001**, *45*, 130–152.
[20] V. W. Pike, *J. Psychopharm.* **1993**, *7*, 139–158.
[21] M. Laruelle, M. Slifstein, Y. Huang, *Mol. Imaging Biol.* **2003**, *5*, 363–375.
[22] P. Bjurling, R. Reineck, G. Westerberg, A. D. Gee, J. Sutcliffe, B. Långström, *Proceedings of the VIth Workshop on Targetry and Target Chemistry*, TRIUMF **1995**, 282–284.
[23] R. N. Krasikova, V. V. Zaitsev, P. Truong, M. Schou, C. Halldin, *Eur. J. Nucl. Med. Mol. Imaging* **2002**, *29*, S376.
[24] T. Kihlberg, B. Långström, *J. Org. Chem.* **1999**, *64*, 9201–9205.
[25] T. Kihlberg, B. Långström, Patent, US 2004/0197257 A1, **2004**.
[26] S. K. Zeisler, M. Nader, A. Theobald, F. Oberdorfer, *Appl. Radiat. Isot.* **1997**, *48*, 1091–1095.
[27] C. Lemaire, P. Damhaut, A. Plenevaux, R. Cantineau, L. Christiaens, M. Guillaume, *Appl. Radiat. Isot.* **1992**, *43*, 485–494.
[28] K. Hatano, T. Ido, R. Iwata, *J. Label. Compd. Radiopharm.* **1991**, *29*, 373–380.
[29] R. N. Krasikova, V. V. Zaitsev, S. M. Ametamey, O. F. Kuznetsova, O. S. Fedorova, I. K. Mosevich, Y. N. Belokon, S. Vyskocil, S. V. Shatik, M. Nader, P. A. Schubiger, *Nucl. Med. Biol.* **2004**, *31*, 597–603.
[30] C. Foged, C. Halldin, J. Hiltunen, C. Braestrup, C. Thomsen, H. C. Hansen, T. Suhara, S. Pauli, C. G. Swahn, P. Karlsson, S. Larsson, L. Farde, *Nucl. Med. Biol.* **1996**, *23*, 201–209.
[31] O. Langer, F. Dollé, H. Valette, C. Halldin, F. Vaufrey, C. Fuseau, C. Coulon, M. Ottaviani, K. Någren, M. Bottlaender, B. Mazière, C. Crouzel, *Bioorg. Med. Chem.* **2001**, *9*, 677–694.
[32] N. W. DeLapp, J. H. McKinzie, B. D. Sawyer, A. Vandergriff, J. Falcone, D. McClure, C. C. Felder, *J. Pharmacol. Exp. Ther.* **1999**, *289*, 946–955.
[33] S. R. Donohue, C. Halldin, V. W. Pike, *Bioorg. Med. Chem.* **2006**, *14*, 3712–3720.