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Synthesis of [*O*-methyl-^{11}C]1-(2-chlorophenyl)-5-(4-methoxyphenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid piperidin-1-ylamide: a potential PET ligand for CB_1 receptors

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Abstract—Synthesis and in vitro evaluation of [*O*-methyl-^{11}C]1-(2-chlorophenyl)-5-(4-methoxyphenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid piperidin-1-ylamide ([^{11}C]-**1**), a potential imaging agent for CB_1 receptors using PET is described. 1-(2-Chlorophenyl)-5-(4-hydroxyphenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid piperidin-1-ylamide (**5**), the precursor for radiolabeling, was synthesized from 4-OTBDPS-propiophenone (**2**) in five steps with 30% overall yield. The reaction of alcohol **5** with [^{11}C]MeOTf at 60 °C afforded [^{11}C]-**1** with an average radiochemical yield of 14.5% (EOS) and >2000 Ci/mmol specific activity. The radiotracer was found to selectively label CB_1 receptors in slide-mounted sections of postmortem human brain containing prefrontal cortex as demonstrated by in vitro autoradiography using phosphor imaging.

Cannabis has been used for recreational and medicinal purposes for centuries and is the third most commonly used drug after tobacco and alcohol.[1] The potential therapeutic effects of cannabinoids are broad and include antiemetic, bronchodilation, antiinflammatory, antiglaucoma, analgesic, anticonvulsant, as well as treatment of obesity and alcoholism.[2] Also therapeutic benefit for pain is reported. However, the pharmacological mechanisms of these actions are still unclear. Cannabinoid actions are thought to be mediated through two types of cannabinoid receptors designated as CB_1 and CB_2.[3] The CB_1 receptor is the most abundant CB receptor subtype found in the CNS and is also expressed in peripheral neurons and tissues.[3,4] CB_2 receptors are not present in CNS, but are found in immune system cells, especially in B-cells, monocytes, and T-cells.[3] Extensive preclinical as well as clinical studies of CB_1 agonists and antagonists suggest that altered levels of CB_1 receptor binding may underlie the pathogenesis of a diverse range of neuropsychiatric disorders, neurodegenerative diseases, stroke, pain disorders some cancers, diabetes, glaucoma, emesis, asthma, diarrhea, and cardiovascular diseases.[5] We recently demonstrated an upregulation of CB_1 receptor density in the dorsolateral prefrontal cortex (DLPFC) of depressed suicides (DS) and alcoholic suicides (AS) compared with age-matched controls.[6,7] To better understand the role of CB_1 receptors in disease and therapeutics, it would be of great benefit to be able to measure CB_1 receptor binding in vivo, noninvasively and repeatedly over time. Highly selective and high affinity CB_1 antagonists capable of penetrating the blood brain barrier are candidates for PET imaging probes for CB_1 receptors. To date, most efforts at in vivo imaging of human brain CB_1 receptors have been disappointing.[8] We chose 1-(2-chlorophenyl)-5-(4-methoxyphenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid piperidin-1-ylamide (**1**) as our candidate PET ligand for CB_1 receptors due to its high affinity for CB_1 receptors (8 nM) and log*P* value 3.45, which allows sufficient lipophilicity for **1** to enter into brain.[9]

Keywords: CB_1 receptors; Autoradiography; Positron emission tomography; Biological imaging; Human postmortem.
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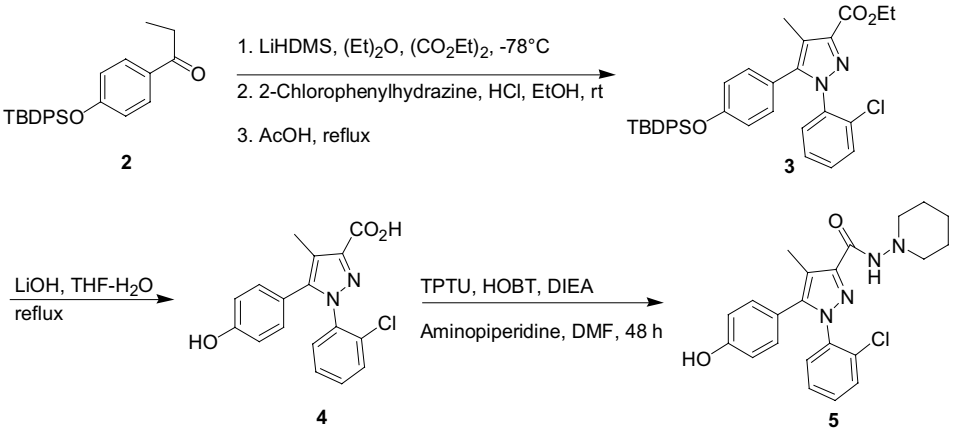
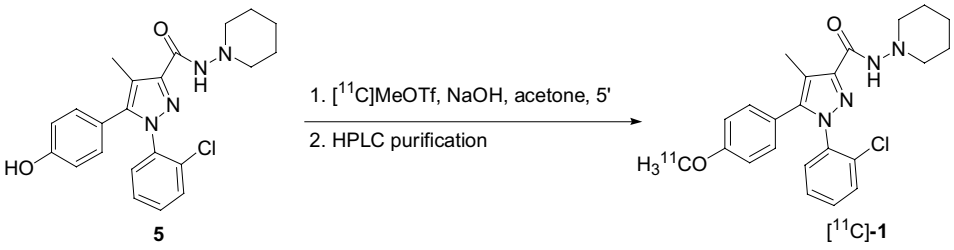
The synthesis of 1-(2-chlorophenyl)-5-(4-hydroxyphenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid piperidin-1-ylamide (**5**), the precursor for radiolabeling, has been achieved in five steps from the OTBDPS protected propiophenone (**2**) (Scheme 1). Compound **2** was initially treated with LiHMDS and diethyloxalate followed by coupling the enolate with 2-chlorophenylhydrazine hydrochloride and cyclization of the imine derivative obtained with acetic acid to afford the pyrazole ester (**3**). The ester **3** upon hydrolysis followed by condensation with 1-aminopiperidine in presence of HOBT, TPTU, and diisopropylethylamine in acetonitrile provided the precursor **5** in 30% overall yield.[10] Alternatively, synthesis of the standard **1** was achieved from 4′-methoxyacetophenone using a reported procedure.[9]

Even though, under optimized reaction conditions, the methylation of **5** was achieved using methyl iodide in presence of tetrabutylammonium hydroxide (TBAOH) or NaOH as base, our labeling experiments under these conditions with $[^{11}C]CH_3I$ did not provide $[^{11}C]$-**1** in sufficient yield and purity. However, using $[^{11}C]$MeOTf in the presence of 5 N NaOH in acetone at room temperature provided $[^{11}C]$-**1** with a 14.5% radiochemical yield (EOS, Scheme 2, Fig. 1).[11] The chemical identity of $[^{11}C]$-**1** was confirmed by co-injection with an authentic



Scheme 1. Synthesis of radiolabeling precursor **5**.



Scheme 2. Radiosynthesis of $[^{11}C]$-**1**.

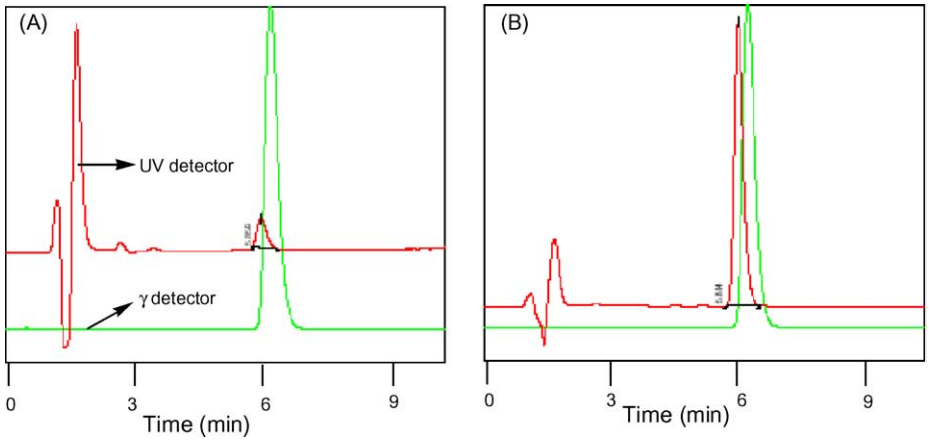


Figure 1. (A) Radiosynthesis of $[^{11}C]$-**1**; (B) co-injection with cold **1**.

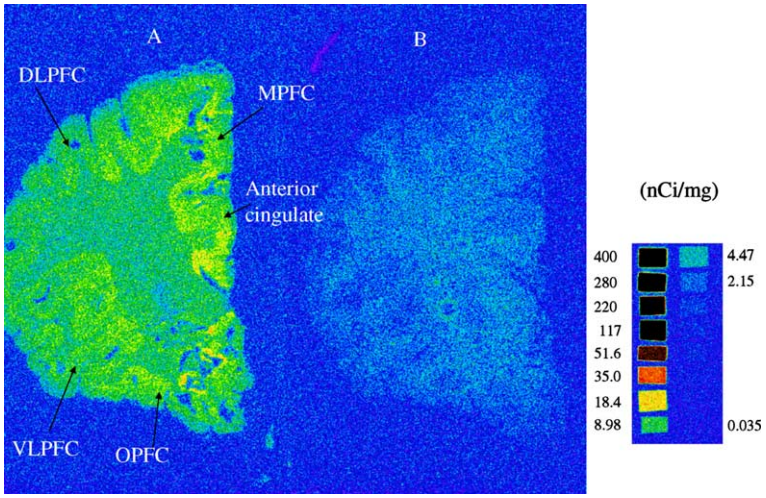


Figure 2. Phosphor image of PFC brain sections with [^{11}C]-**1**: (A) total; (B) nonspecific VLPFC (ventral lateral prefrontal cortex); OPFC (orbital prefrontal cortex); MPFC (medial prefrontal cortex).

sample of **1** on analytical reverse-phase HPLC (Fig. 1). The chemical and radiochemical purity of [^{11}C]-**1** was found to be >99% with a specific activity >2000 Ci/mmol (EOB). The average time required for the [^{11}C] labeling was 30 min (EOB).

After successful synthesis and specific activity determination of [^{11}C]-**1**, phosphor image autoradiographic studies were undertaken to determine the distribution of CB_1 binding sites in the prefrontal cortex (PFC) postmortem.[12] Figure 2 illustrates the total and nonspecific binding of 10 nM [^{11}C]-**1** incubated for 60′. Nonspecific binding was determined by co-incubating with 1 μM SR141716A, a known CB_1 antagonist.[13] As is evident from Figure 2, specific binding was observed in the gray matter of DLPFC, ventral lateral prefrontal cortex (VLPFC), orbital prefrontal cortex (OLPFC), medial prefrontal cortex (MPFC), and anterior cingulate cortex with [^{11}C]-**1**. The distribution of CB1 binding is consistent with what is reported in other in vitro studies.[4,6,7]

In summary, we successfully synthesized [^{11}C]-**1**, a potential imaging agent for CB_1 receptors. The total time required for the synthesis of [^{11}C]-**1** is 30 min from EOB using [^{11}C]methyl triflate in acetone, with a 14.5% yield at EOS based on [^{11}C]MeOTf. The chemical and radiochemical purities are >99% with a specific activity >2000 Ci/mmol. The radiotracer binds to PFC postmortem human brain as visualized by phosphor imaging suggesting specific affinity for quantifying CB_1 receptors. The phosphor image studies indicate that the newly developed [^{11}C] labeled CB_1 ligand has the potential for the quantification of CB_1 receptors in brain, and further detailed in vitro and in vivo studies are under progress.

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References and notes

1. Rudgley, R.; Brown, L. http://www.cannabis.net/hist/ (website accessed September 20, 2003).
2. Baker, D.; Pryle, G.; Glovannoni, G.; Thomson, A. J. *Lancet* **2003**, *2*, 291.
3. Howlett, A. C.; Bonner, B. F.; Casellas, C. G.; Devane, W. A.; Felder, C. C.; Herkenham, M.; Martin, M. K.; Mechoulam, R.; Pertwee, R. G. *Pharmacol. Rev.* **2003**, *54*, 161.
4. Glass, M.; Dragunow, M.; Faull, R. L. *Neuroscience* **1997**, *77*, 299.
5. Croxford, J. L. *CNS Drugs* **2003**, *17*, 179; Whittle, B. A.; Guy, G. W.; Robson, P. *J. Cannabis Ther.* **2001**, *1*, 183; Williamson, E. M.; Evans, F. J. *Drugs* **2000**, *60*, 1303; Pertwee, R. G. *Pharm. Sci.* **1997**, *3*, 539; Grundy, R. I. *Expert Opin. Invest. Drugs.* **2002**, *11*, 1365; Seitz, R. *Dtsch. Apoth. Ztg.* **2002**, *142*, 140.
6. Hungund, B. L.; Vinod, K. Y.; Kassir, S. A.; Basavarajappa, B. A.; Yalamanchilli, R.; Cooper, T. B.; Mann, J. J.; Arango, V. A. *Mol. Psychiatry* **2004**, *9*, 184–190.
7. Vinod, K. Y.; Arango, V.; Xie, X.; Kassir, S. A.; Mann, J. J.; Cooper, T. B.; Hungung, B. L. *Am. J. Psychiatry* **2004**, submitted for publication.
8. Marciniak, G.; Charalambous, A.; Shiue, C. Y.; Dowey, S. L.; Schlyer, D. J.; Makriyannis, A.; Wolf, A. P. *J. Label. Compd.* **1991**, *30*, 413; Charalambous, A.; Marciniak, G.; Shiue, C. Y.; Dowey, S. L.; Schlyer, D. J.; Wolf, A. P.; Makriyannis, A. *Pharmacol. Biochem. Behav.* **1991**, *40*, 503; Gifford, A. N.; Makriyannis, A.; Volkow, N. D.; Gatley, S. J. *Chem. Phys. Lipids* **2002**, *121*, 65; Mathews, W. B.; Scheffel, U.; Finley, P.; Ravert, H. T.; Frank, R. A.; Carmona, M. R.; Barth, F.; Dannals, R. F. *Nucl. Med. Biol.* **2000**, *27*, 757; Mathews, W. B.; Scheffel, U.; Rauseo, P. A.; Ravert, H. T.; Frank, R. A.; Ellames, G. J.; Herbert, J. M.; Barth, F.; Rinaldi, C. M.; Dannals, R. F. *Nucl. Med. Biol.* **2002**, *29*, 671; D'Ambra, T. E.; Estep, K. G.; Bell, M. R.; Eissenstat, M. A.; Josef, K. A.; Ward, S. J.; Haycock, D. A.; Baizman, E. R.; Casiano, F. M.; Beglin, N. C. *J. Med. Chem.* **1992**, *35*, 124; Willis, P. G.; Katoch,

R. R.; Horti, A. G. *J. Label. Compd. Radiopharm.* **2003**, *46*, 799.
9. LaVerne, B. L.; Kulkarni, S.; Pavlova, O. A.; Koren, A. O.; Mukhin, A. G.; Newman, A. H.; Horti, A. G. *J. Med. Chem.* **2003**, *46*, 642.
10. 1-(2-Chlorophenyl)-5-(4-hydroxyphenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid piperidin-1-ylamide (**5**): (2-Chlorophenyl)-5-(4-hydroxyphenyl)-4-methyl-1*H*-pyrazol-3-yl]-oxo-acetic acid (657.50 mg, 2.0 mmol) was dissolved in 15 mL acetonitrile. To the above solution 1-aminopiperidine (0.30 mL, 2.6 mmol), HOBT (540.50 mg, 4 mmol), TPTU (713.0 mg, 2.4 mmol), and diisopropylethylamine (1.1 mL, 6.0 mmol) were successively added and stirred for 2 days. The reaction mixture was diluted with water and the products were extracted into ethyl acetate. The ethyl acetate layer was washed with water (2 × 30 mL), saturated $NaHCO_3$ solution (2 × 30 mL), and then with brine. The organic layer was dried over anhydrous $MgSO_4$ and the solvent was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography using 60–70% ethyl acetate in hexane as the eluent. The product was obtained as colorless crystals (600 mg, 73%), **5**: mp > 270 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.58 (m, 6H), 2.49 (s, 3H), 2.69 (m, 4H), 6.67 (d, $J = 8$ Hz, 2H), 6.95 (d, $J = 7.8$ Hz, 2H), 7.45–7.52 (m, 3H), 7.59 (d, $J = 7.5$ Hz, 1H), 8.95 and 9.80 (2s, 1H), HRMS calcd for $C_{22}H_{24}O_2N_4Cl$ 411.1588; found 411.1601.
11. Radiosynthesis of [*O*-Methyl-^{11}C]1-(2-chlorophenyl)-5-(4-methoxyphenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid piperidin-1-ylamide ([^{11}C]-**1**): [^{11}C]MeOTf was trapped into an acetone (400 μL) solution containing 0.5 mg of **5** and 10 μL of 5 N NaOH at rt for 5′. The resulting mixture was loaded into a semipreparative HPLC (Phenomenex C18) column and the product fraction, between 7 and 8 min was collected based on a γ-detector using 50:49:1 (acetonitrile–water–acetic acid), 12 mL/min. The collected fraction was then diluted with deionized water (100 mL), passed through a C-18 Sep-Pak and washed with water (2 × 20 mL). Following a water rinse, the product [^{11}C]-**1** was eluted from the Sep-Pak with 1 mL of ethanol. A small portion of [^{11}C]-**1** was analyzed with analytical HPLC for chemical identity, chemical and radiochemical purities, and specific activity. The average yield was found to be 14.5% at EOS with a specific activity >2000 Ci/mmol.
12. In vitro phosphor imaging: Slide-mounted prefrontal cortical sections from the right hemisphere (20 μm) were brought to 22 °C and pre-incubated in 50 mM Tris hydrochloride buffer (pH 7.4) containing 1% BSA for 45 min (22 °C). This was followed by incubation in 10 nM of [^{11}C]-**1** for 60 min at room temperature. Adjacent sections were incubated in parallel in with [^{11}C]-**1** and 1 μM SR141716A to determine nonspecific binding. Sections were washed in 50 mM Tris buffer (pH 7.4) at 4 °C and briefly dipped in ice-cold water to remove salts. Slides were quickly dried under a stream of cold air and exposed to ST-phosphor-imaging screen (Packard, wrapped in Mylar film) with high- and low-activity [^{14}C] standards (American Radiolabeled Chemicals) for 90 min. Screens were scanned with a Packard Cyclone phosphor-imaging system and analyzed with OptiQuant Acquisition and Analysis software (Packard).
13. Rinaldi, C. M.; Barth, F.; Heaulme, M.; Shire, D.; Calandra, B.; Congy, C.; Martinez, S.; Maruani, J.; Neliat, G.; Caput, D. *FEBS Lett.* **1994**, *350*, 240.