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Research Article

Synthesis and *in vivo* evaluation of [*O*-methyl-^{11}C] 2-(4-methoxyphenyl)-*N*-(4-methylbenzyl)-*N*-(1-methyl-piperidin-4-yl)acetamide as an imaging probe for 5-HT_{2A} receptors

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Summary

2-(4-Methoxyphenyl)-*N*-(4-methylbenzyl)-*N*-(1-methylpiperidin-4-yl)acetamide (AC90179, **4**), a highly potent and selective competitive 5-HT_{2A} antagonist, was labeled by [^{11}C]-methylation of the corresponding desmethyl analogue **5** with [^{11}C]methyl triflate. The precursor molecule **5** for radiolabeling was synthesized from *p*-tolylmethylamine in three steps with 46% overall yield. [^{11}C]AC90179 was synthesized in 30 min (30 $\pm$ 5% yield, EOS) with a specific activity of 4500 $\pm$ 500 Ci/mmol and $>$99% chemical and radiochemical purities. Positron emission tomography studies in anesthetized baboon revealed that [^{11}C]**4** Penetrates the blood–brain barrier (BBB) with a rapid influx and efflux of the tracer in all brain regions. Due to lack of tracer retention or specific binding, [^{11}C]**4** cannot be used as PET ligand for imaging 5-HT_{2A} receptors. Copyright © 2006 John Wiley & Sons, Ltd.

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Key Words: radiotracer; methyl triflate; positron emission tomography; 5-HT_{2A} receptors

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Introduction

Alteration of serotonergic 5-HT_{2A} receptors (5-HT_{2A}R) has been reported in schizophrenia, stress, aggression, suicide and major depression.[1–3] These receptors are also targets for atypical antipsychotic drugs (APDs).[4–6] *In vivo* imaging of 5-HT_{2A}R with positron emission tomography (PET) may further our understanding of the pathophysiology of diseases associated with the changes in this receptor and allow occupancy studies to assist new drug development. The utility of currently available radiotracers for *in vivo* imaging of 5-HT_{2A}R are limited by high non-specific binding, lipophilic metabolites or slow kinetics (Figure 1).[7–12] [^{18}F]altanserin has lipophilic radiometabolites that cross the blood-brain barrier (BBB) and slow kinetics.[10,11] Determining a suitable reference region for [^{18}F]setoperone is difficult.[13] [^{11}C]M100907 is a selective 5-HT_{2A} PET tracer, but has the disadvantage of slow kinetics making quantification less reliable.[12] Given the limitations of the existing tracers, we sought to develop 5-HT_{2A} receptor imaging agents that have faster washout to facilitate modeling within the window of time allowed by the half-life of carbon-11, without radiolabeled nonpolar metabolites, and with high selectivity for the 5-HT_{2A} receptor. We describe the radiosynthesis and *in vivo* evaluation of [^{11}C] 2-(4-methoxyphenyl)-*N*-(4-methylbenzyl)-*N*-(1-methylpiperidin-4-yl)acetamide ([^{11}C]AC-90179 or [^{11}C]**4**) in baboon using PET. AC-90179, is a selective, competitive 5-HT_{2A}R antagonist (K_i = 2.5 nM) and a 5-HT_{2A} receptor inverse agonist (K_i = 2.1 nM) and with antipsychotic properties indicated by behavior pharmacology.[14,15] Functional high-throughput screening of many 5-HT_{2A} antagonists indicates they are also inverse agonist activity at 5-HT_{2A}R.[14] Thus the favorable *in vitro* profile, lipophilicity of 2.8 (clog*P*, calculated with ACD/ log *P* DB program) indicating the ability to penetrate BBB, and the presence of a potential site for [^{11}C]-labeling prompted us to develop [^{11}C] AC-90179 as a candidate ligand for *in vivo* quantification of 5-HT_{2A}R binding.

Results and discussion

The synthesis of [^{11}C]AC-90179 ([^{11}C]**4**) was achieved from the precursor molecule, 2-(4-hydroxyphenyl)-*N*-(4-methylbenzyl)-*N*-(1-methylpiperidin-4-yl) acetamide (**5**). The phenolic alcohol **5** was synthesized from *p*-tolylmethylamine

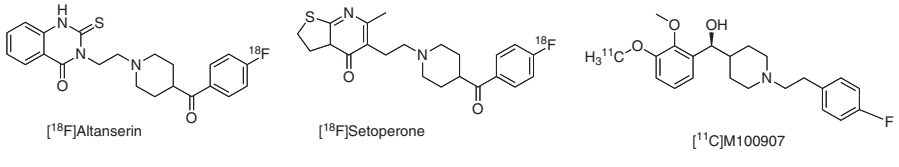

Figure 1. 5-HT_{2A} receptor PET tracers in human use

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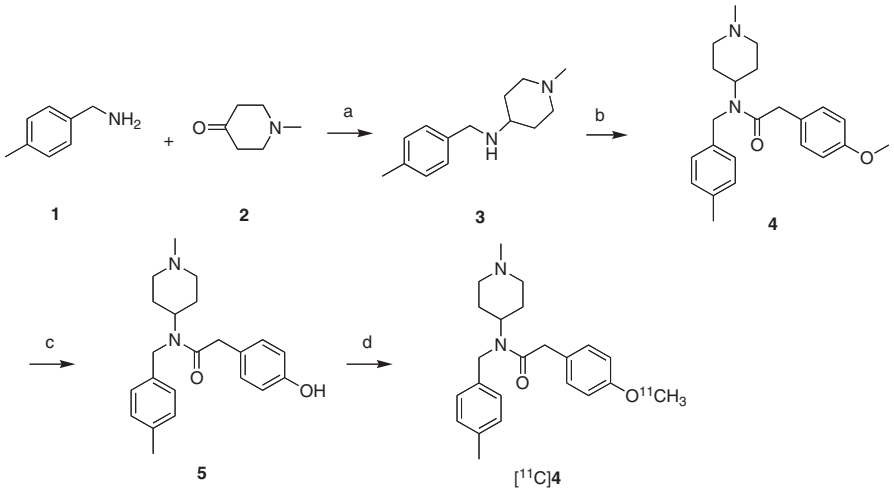

Scheme 1. Synthesis and radiosynthesis of [^{11}C]4: (a) $NaBH_4$, methanol, 74%; (b) 4-methoxyphenylacetyl chloride, triethylamine, THF, 85%; (c) BBr_3, CH_2Cl_2, 80%; (d) i. NaOH, [^{11}C]CH_3OTf, 5 min, acetone, ii. HPLC purification (30% EOS)

(**1**) in three steps (Scheme 1). Reductive amination of **1** with 1-methylpiperidone (**2**) afforded 1-methyl-*N*-(4-methylbenzyl)piperidin-4-amine (**3**) in 74% yield. Condensation of compound **3** with 4-methoxyphenylacetyl chloride in presence of triethylamine provided the standard compound **4** in 85% yield. The radiolabeling precursor **5** was synthesized by a facile demethylation of the methoxy group in compound **4** with boron tribromide (BBr_3) in 80% yield.

Precursor **5** was subjected to radiomethylation using [^{11}C]methyl triflate (MeOTf) in presence of aqueous NaOH in acetone to provide [^{11}C]**4**. The radiolabeled product was separated from the reaction mixture by reverse phase high performance liquid chromatography (RP-HPLC) with an average yield of $30 \pm 5\%$ (EOS, $n = 6$). The chemical identity of [^{11}C]**4** was confirmed by co-injection with an authentic sample of standard **4** on analytical RP-HPLC. Chemical and radiochemical purities of [^{11}C]**4** were found to be $>99\%$ with a specific activity 4500 ± 500 Ci/mmol (EOB, $n = 4$). Average time required for the [^{11}C]-labeling was 30 min (EOB). Partition coefficient ($\log P_{o/w}$) of [^{11}C]**4** was determined by the standard shake flask method[16] and found to be 2.5.

PET imaging studies in baboon show that [^{11}C]**4** penetrates BBB and accumulates in brain (Figure 2). The time activity curves of several brain regions were examined and demonstrated an uptake of [^{11}C]**4** that peaked in most regions within the first 10 min and then declined rapidly. Only slight retention of [^{11}C]**4** was seen in anterior cingulate, thalamus, putamen and

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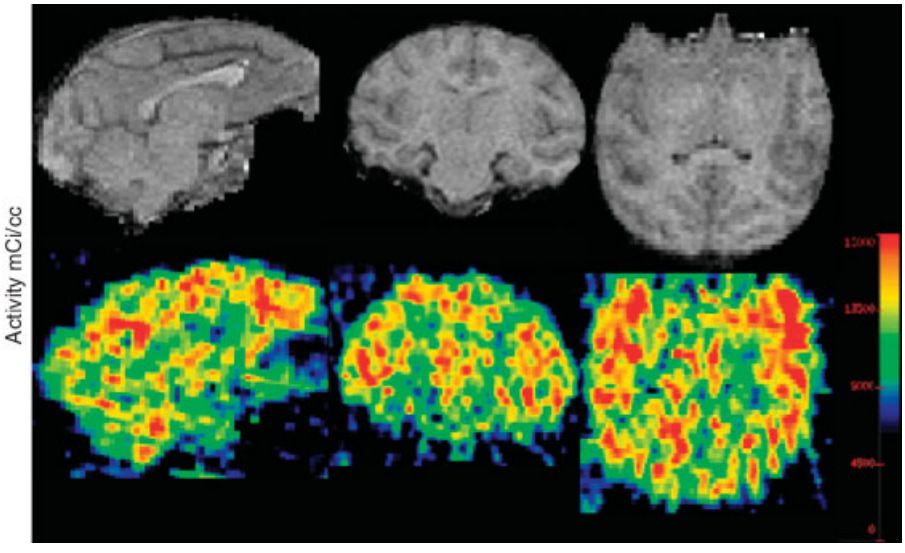

Figure 2. MRI and later time phase PET images of [^{11}C]4 in baboon brain (Top row: MRI; bottom row: PET scan: sum of 14–40 min frames, left column: sagittal, middle column: coronal, right column: axial views)

hippocampus in comparison to cerebellum with binding ratios of 1.24, 1.17, 1.13, 1.12 at 115 min, respectively (Figure 3).

It appears there is no quantifiable specific binding detected by this tracer despite the fact that it has high affinity and selectivity *in vitro* and penetrates the BBB. It is possible that [^{11}C]**4** is a substrate for *p*-glycoproteins and is actively and rapidly extruded from the brain which may contribute to the low retention of radiotracer in brain. The low injected mass of the carrier (0.68 ± 0.02 μg) along with high [^{11}C]**4** specific activity (3400 ± 500 Ci/mmol, at the time of injection) in the range of 0.034 ± 0.001 μg/kg baboon weight, given a B_{max} of 520 fmol/mg/protein in frontal cortex of postmortem human brain,[17] the possibility of self blocking or occupancy of the 5-HT$_{2A}$ receptor sites by **4** is unlikely. A structure affinity relationship study of compound **4** may provide a better PET ligand with *in vivo* kinetics that permit valid and reliable measurement of 5-HT$_{2A}$R binding.

Experimental section

All commercial reagents and solvents were used without further purification unless otherwise specified. ^{1}H NMR spectra were recorded on a Bruker PPX 300 MHz spectrometer. Spectra were recorded in $CDCl_3$ and chemical shifts are reported in ppm relative to TMS, which is used as the internal standard. Mass spectra were recorded on JKS-HX 11UHF/HX110 HF Tandem Mass Spectrometer in the EI+ mode. HPLC analyses were performed using Waters

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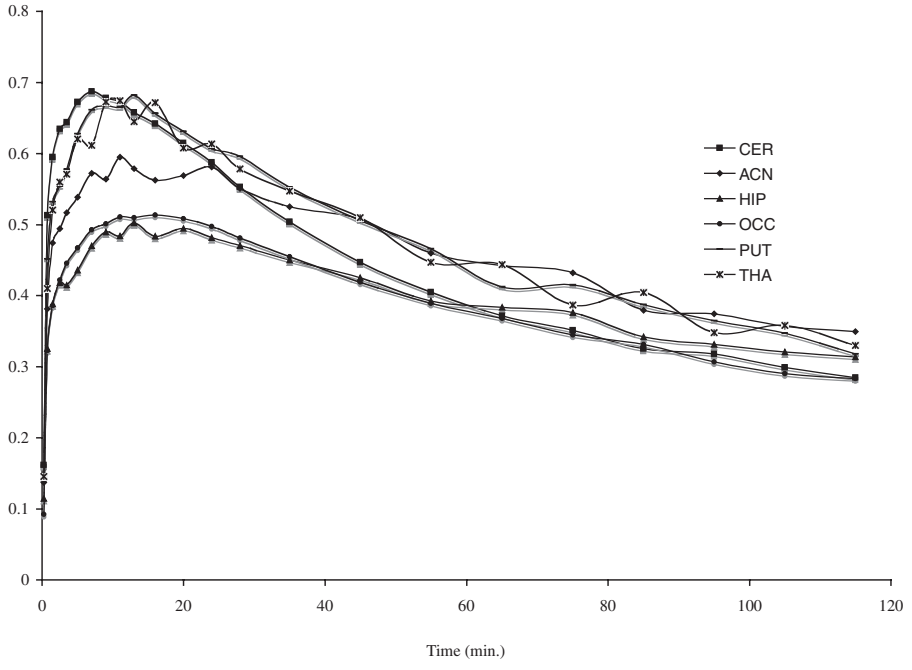

Figure 3. Time activity curves of the radioactivity in baboon after the injection of [^{11}C]4. (CER = cerebellum, ACN = anterior cingulate, HIP = hippocampus, OCC = occipital cortex, PUT = putamen, THA = thalamus)

1525 HPLC system (column: Phenomenex, Prodigy ODS(3) 4.6 × 250 mm, 5 μm for analytical and Phenomenex C18, 10 × 250 mm, 10 μm for semi-preparative RP-HPLC). Flash column chromatography was performed on silica gel (Fisher 200–400 mesh) using the solvent system indicated. [^{11}C]MeOTf was synthesized from [^{11}C]CO_2 according to a reported procedure.[17]

*1-methyl-N-(4-methylbenzyl)piperidin-4-amine (**3**)*

Sodium borohydride (1.6 g, 43 mmol) was added in small portions to a solution of benzylamine **1** (3 g, 25 mmol) and 1-methylpiperidone **2** (3.2 g, 28.5 mmol) in methanol (95 ml) and the mixture was stirred at room temperature for 16 h. Water (50 ml) was added to the reaction mixture and the products were extracted into dichloromethane (4 × 30 ml). The dichloromethane layer was separated, washed with brine and dried over anhydrous magnesium sulfate. Solvent was evaporated under reduced pressure to obtain the piperidine amine (**3**) in 83% yield (4.5 g).

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.16 (m, 4 H), 3.64 (s, 2 H), 2.69 (m, 2 H), 2.43 (m, 1 H), 2.38 (s, 3 H), 2.17 (s, 3 H), 2.02-1.99 (m, 4 H), 1.61 (m, 2 H); HRMS (EI$^+$) calculated for: $C_{14}H_{23}N_2$: 219.1861; Found: 219.1852

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*2-(4-methoxyphenyl)-N-(4-methylbenzyl)-N-(1-methylpiperidin-4-yl)acetamide (**4**)*

To a solution of piperidine amine **3** (350 mg, 1.60 mmol) and triethylamine (0.45 ml, 3.2 mmol) in THF (20 ml), 4-(methoxyphenyl)acetyl chloride (325 mg, 3.2 mmol) was added and the solution was stirred at room temperature for 2 h. The reaction was diluted with water and the products were extracted into ethyl acetate. The ethyl acetate layer was washed with water, saturated brine and then dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure and product was purified by silica gel column chromatography using ethyl acetate and hexane (1:1) as the eluent to obtain methoxyacetamide (**4**) in 70% yield (411 mg) as a viscous solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.24-7.04 (m, 6 H), 6.82 (m, 2 H), 4.65 (m, 1 H), 4.43 (m, 2 H), 3.78 (brs, 4 H), 3.55 (s, 1 H), 2.81 (m, 2 H), 2.31-2.22 (m, 6 H), 2.16 (m, 2 H), 1.81-1.43 (m, 4 H); HRMS (EI^+) calculated for: $C_{23}H_{31}O_2N_2$: 367.2386; Found: 367.2375

*2-(4-hydroxyphenyl)-N-(4-methylbenzyl)-N-(1-methylpiperidin-4-yl)acetamide (**5**)*

Methoxyacetamide **4** (127 mg, 0.35 mmol) was dissolved in anhydrous dichloromethane (2 ml) and a 1 M solution of BBr_3 in dichloromethane (1.4 ml) was added to it. The solution was stirred for 1 h at room temperature after which it was quenched by adding methanol drop wise at 0°C. The products were extracted into ethyl acetate, washed with water and brine and dried over anhydrous magnesium sulfate. Solvent was evaporated under reduced pressure and the product was purified using silica gel column chromatography. Upon elution with a mixture of ethyl acetate and hexane (60:40), the phenolicacetamide (**5**) was isolated in 80% yield as a viscous solid (100 mg).

^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.24-6.7 (m, 8 H), 4.67 (m, 1 H), 4.44 (m, 2 H), 3.52 (s, 2 H), 3.15 (brs, 2 H), 2.85 (m, 1 H), 2.40-2.24 (m, 7 H), 1.78-1.49 (m, 4 H); HRMS (EI^+) calculated for: $C_{22}H_{29}O_2N_2$: 353.2229; Found: 353.2204

Radiosynthesis of (O-methyl[^{11}C]2-(4-methoxyphenyl)-N-(4-methylbenzyl)-N-(1-methyl- piperidin-4-yl)acetamide ([^{11}C]4)

[^{11}C]MeOTf[18] was trapped into an acetone (400 µl) solution containing 0.5 mg of precursor **5** and 10 µl of 5 N NaOH at room temperature for 5 min. At the end of the trapping, the reaction mixture was heated on a water bath at 60°C for 4 min and then directly injected onto a semi-preparative RP-HPLC (Phenomenex C18, 10 × 250 mm, 10 µm) and eluted with acetonitrile: 0.1 M ammonium formate: acetic acid solution (30:69.5:0.5 v/v/v) at a flow rate of 12.5 ml/min. The product fraction with a retention time of 9–10 min based on

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γ-detector was collected, diluted with 100 ml of deionized water, and passed through a classic C-18 Sep-Pak® cartridge. Reconstitution of the product in 1 ml of absolute ethanol afforded [^{11}C]**4** in 30% yield, based on [^{11}C]CO_2 at EOS). A portion of the ethanol solution was analyzed by analytical HPLC (Phenomenex C18; mobile phase: acetonitrile/0.1 M ammonium formate/acetic acid solution, 30:69.5: 0.5 v/v/v, flow rate: 2 ml/min, retention time: 9 min) to determine the specific activity and radiochemical purity.

Measurement of partition coefficient (log $P_{o/w}$)

Partition coefficient of [^{11}C]**4** was measured by mixing 0.1 ml of the radioligand formulation with 5 g each of 1-octanol and freshly prepared PBS buffer (pH = 7.4) in a culture tube.[16] The culture tube was shaken mechanically for 5 min followed by centrifugation (5 min). Radioactivity per 0.5 g each of 1-octanol and aqueous layer was measured using a well counter. The partition coefficient was determined by calculating the ratio of counts/g of 1-octanol to that of buffer layers. 1-Octanol fractions were repeatedly portioned with fresh buffer to get consistent values for partition coefficient. All the experimental measurements were performed in triplicate.

PET studies in baboons

PET studies were done in two baboons (22 and 20 kg) (*Papio anubis*) with an ECAT EXACT HR+ scanner (Siemens, Knoxville, TN). For each scanning session, the fasted animal was immobilized with ketamine (10 mg/kg, i.m.), and anesthetized with 1.5–2.0% isoflurane via an endotracheal tube. Core temperature was kept constant at 37°C with a heated water blanket. An i.v. infusion line with 0.9% NaCl was maintained during the experiment and used for hydration and radiotracer injection. An arterial line was placed for obtaining arterial samples for determination of the input function for quantification purposes. In each scanning session, the head was positioned so that the brain was in the center of the field of view, and a 10 min transmission scan was performed before the first tracer injection. For baboon scans 5.0 ± 0.5 mCi (mass = 0.68 ± 0.02 μg, specific activity 3300 ± 500 Ci/mmol) of [^{11}C]**4** were injected as an i.v. bolus and emission data were collected for 120 min in 3D mode over the following time frames: 2 × 0.5, 3 × 1, 5 × 2, 4 × 4, 9 × 10 min. Plasma samples were taken at every 10 s for the first 2 min, using an automatic system, and thereafter manually for a total of 34 samples over 2 h.

Image processing and analysis

The PET data was reconstructed with attenuation correction using the transmission data, and scatter correction was done using model-based scatter

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correction.[19] The reconstruction filter and estimated image filter were Shepp 0.5, the axial (Z) filter was all pass 0.4, and the zoom factor was 4.0. The final image resolution at the center of the field of view was 5.1 mm FWHM.[20] A T1-weighted magnetic resonance image (MRI) of the animal's head was acquired on a GE 1.5-T Signa Advantage system. Regions of interests were drawn on the MRI using the MEDX software (Sensor Systems, Inc., Sterling, VA). ROIs included cerebellum, hippocampus, occipital cortex, anterior cinulate cortex, putamen, and thalamus. The PET data were co-registered to the MRI using the software AIR,[21] and time-activity curves (TACs) were generated for each ROI.

Conclusion

The radiosynthesis of [^{11}C]AC-90179, a 5-HT$_{2A}$R competitive antagonist has been achieved. Total time required for the synthesis of [^{11}C]**4** is 30 min from EOB using [^{11}C]MeOTf in acetone, with a 30% yield at EOS based on [^{11}C]MeOTf. The chemical and radiochemical purities are $>99\%$ and specific activity is >4000 Ci/mmol. PET studies of [^{11}C]**4** in baboon showed that the tracer penetrates the BBB, but tracer washout from the brain is rapid and was not retained to a quantifiable degree in any brain region. Thus, [^{11}C]**4** is not a useful PET tracer for quantifying 5-HT$_{2A}$R binding in baboon.

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