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Synthesis and evaluation of [*O-methyl*-^{11}C]4-[3-[4-(2-methoxyphenyl)- piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione as a 5-HT$_{1A}$ receptor agonist PET ligand

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4-[3-[4-(2-Methoxyphenyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (4), a potent and selective 5-HT$_{1A}$ agonist, was labeled by ^{11}C-methylation of the corresponding desmethyl analogue 3 with ^{11}C-methyl triflate. The precursor molecule 3 was synthesized from commercially available *endo-N*-hydroxy-5-norbornene-2,3-dicarboximide in two steps with an overall yield of 40%. Radiosynthesis of ^{11}C-4 was achieved in 35 min in 20 ± 5% yield (n = 6) at the end of synthesis with a specific activity of 2600 ± 250 Ci/mmol. *In vivo* positron emission tomography (PET) studies in baboon revealed rapid uptake of the tracer into the brain. However, lack of specific binding indicates that ^{11}C-4 is not useful as a 5-HT$_{1A}$ agonist PET ligand for clinical studies.

Keywords: radiotracer; carbon-11; positron emission tomography; 5-HT$_{1A}$; agonist

Introduction

The serotonin 1A receptor (5-HT$_{1A}$R) belongs to the superfamily of G-protein-coupled receptors and is negatively coupled to adenylyl cyclase.[1] Preclinical and clinical studies suggest that 5-HT$_{1A}$R is involved in the pathophysiology and treatment of major neuropsychiatric disorders, including depression, schizophrenia and anxiety.[2–4] Brain 5-HT$_{1A}$Rs are located as autoreceptors in the raphe nuclei and postsynaptically in the terminal fields in many brain regions including limbic regions, prefrontal and entorhinal cortices.[5,6] Positron emission tomography (PET) allows measurement of 5-HT$_{1A}$R binding in living human brain, and the antagonist radiotracers such as [^{11}C]WAY-100635 and [^{18}F]MPPF are the most commonly used 5-HT$_{1A}$ ligands for *in vivo* studies.[7,8] However, antagonist ligands bind both to the high-affinity (HA) and low-affinity conformation of 5-HT$_{1A}$R with equal affinity.[9] In contrast, an agonist ligand would provide a functional measure of the 5-HT$_{1A}$R by preferentially binding to the HA state of the receptor which is coupled to G-protein.[10] Moreover, a sensitive agonist radioligand is expected to provide a better tool for (i) the estimation of occupancy of agonist drugs at the 5-HT$_{1A}$R; (ii) the estimation of the intrasynaptic levels of endogenous serotonin; and (iii) imaging rapid desensitization (internalization) of 5-HT$_{1A}$ autoreceptors after the administration of a selective serotonin reuptake inhibitor as a measure of its treatment efficacy.[11–13] In spite of efforts by several groups, no successful 5-HT$_{1A}$ agonist radioligand is currently available for *in vivo* PET imaging in human.[14,15] We have recently shown that ^{11}C-MPT, a 5-HT$_{1A}$ agonist PET ligand, demonstrates specific binding in preliminary *in vivo* studies in baboon.[15] However, the lack of favorable kinetics of this ligand prompted us to evaluate 4-[3-[4-(2-methoxyphenyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (**4**), a potent and selective 5-HT$_{1A}$ agonist.

Results and discussion

4-[3-[4-(2-Methoxyphenyl)piperazin-1-yl]propoxy]-4-aza-tricyclo [5.2.1.02,6]dec-8-ene-3,5-dione (**4**) is a selective HA 5-HT$_{1A}$R ligand (K_i = 0.021 nM).[16] The syntheses of the standard (**4**) and precursor (**3**) for radiolabeling were achieved by the sequence of reactions demonstrated in Scheme 1.

The commercially available *endo-N*-hydroxy-5-norbornene-2,3-dicarboximide (**1**) was tethered with 3-chloropropyl moiety by treatment with 1-bromo-3-chloropropane to afford compound **2** in 83% yield. The chloride **2** was then refluxed with

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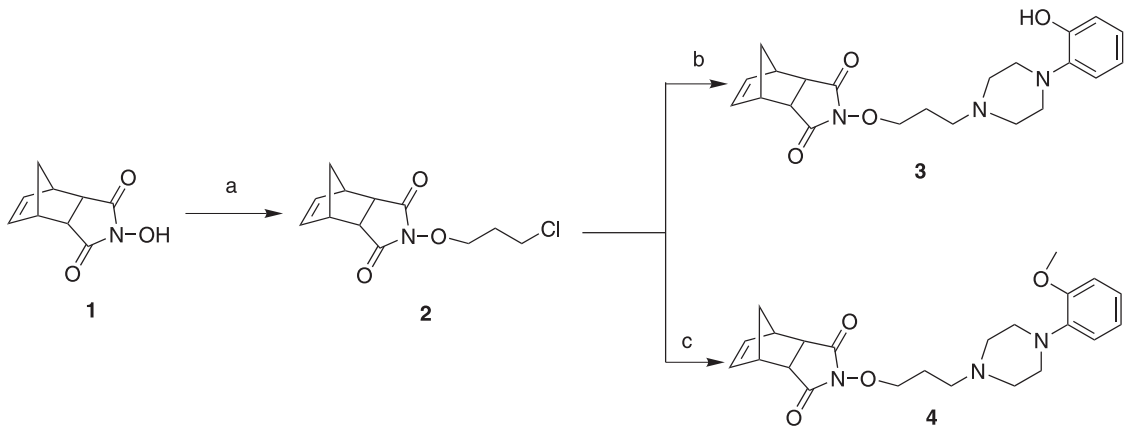
sodium iodide (NaI) in acetonitrile and coupled with 2-(piperazinyl)phenol (**2**) in the presence of excess K_2CO_3 to obtain precursor **3** for radiolabeling in 47% yield. In an analogous way, the standard **4** was synthesized in 52% yield by coupling the chloride **2** with 2-(methoxyphenyl)piperazine hydrochloride after preactivation with NaI. To determine whether **4** is an agonist, we have examined its effect upon GTP-γ, and found that it is a full agonist at 5-HT_{1A}R meaning its maximum response was comparable to serotonin ($E_{max} = 95\%$, $EC_{50} = 1.2$ nM) (Figure 1).

The synthesis of [*O-methyl*-^{11}C]4-[3-[4-(2-methoxyphenyl) piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (^{11}C-**4**) was achieved from the corresponding desmethyl precursor **3** (Scheme 2). The precursor **3** was dissolved in acetone and subjected to radiomethylation using ^{11}C-MeOTf in the presence of aqueous sodium hydroxide to provide ^{11}C-**4**. The radiolabeled product was isolated by reverse phase high-performance liquid chromatography (RP-HPLC) with an average yield of 20 ± 5% (encl of synthesis, EOS, $n = 6$). The average time required for the radiosynthesis was 35 min from the end of bombardment (EOB). The chemical identity of ^{11}C-**4** was established by co-injection with an authentic sample of standard **4** by analytical RP-HPLC on two different columns using different aqueous buffers. Chemical and radiochemical purities of ^{11}C-**4** were found to be 95 and 98, respectively, with an average specific activity 2600 ± 250 Ci/mmol (EOS, $n = 6$). The precursor was not observed as an impurity in the isolated product fraction. The determination of ^{11}C-**4** partition coefficient ($\log P_{o/w}$) was performed in 1-octanol and 0.02 M phosphate buffer at a pH of 7.4 by the modified procedure of Wilson *et al.*[17] and was found to be 2.8.

Global inspection of the PET imaging studies of ^{11}C-**4** in baboon revealed that the tracer penetrates the blood brain barrier (BBB) rapidly, but the distribution of radioactivity is homogenous across the brain. The time–activity curves (TACs) of the distribution of radioactivity in 5-HT_{1A}R-rich regions of interest demonstrated that initial rapid uptake is followed by fast clearance of the radioactivity (Figure 2). The maximum



a) 1-bromo-3-chloropropane, NaOH, EtOH, 70 °C, 24h; b)NaI, CH_3CN, anhyd. K_2CO_3, 2-(piperazin-1-yl)phenol, 70 °C, 24h; c)NaI, CH_3CN, anhyd. K_2CO_3, 2-methoxyphenylpiperazine hydrochloride, 70 °C, 24h

Scheme 1

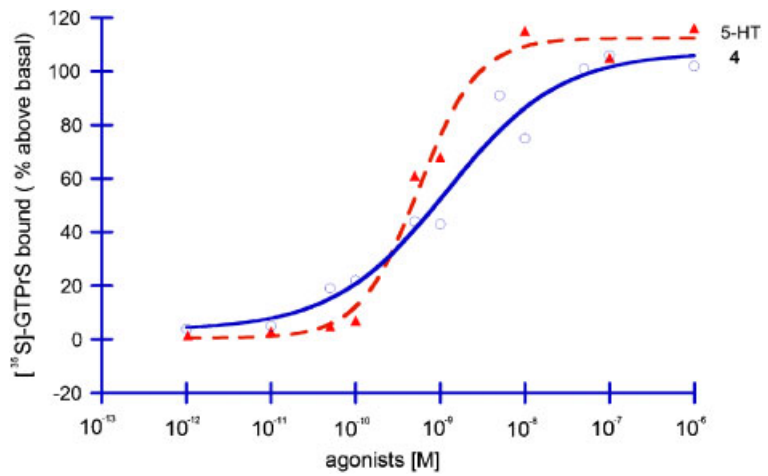
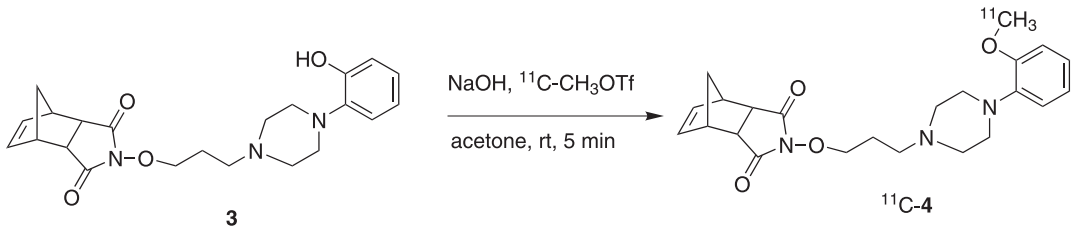


Figure 1. Effect of 5-HT_{1A}R concentration on the stimulation of [^{35}S]GTPγS binding in CHO cells. Values are expressed as a percentage above basal which is the binding of [^{35}S]GTPγS in the absence of agonists. Data points are means of duplicate determinations from representative experiments repeated on at least three independent occasions with similar results. This figure is available in colour online at www.interscience.wiley.com/journal/jlcr.



Scheme 2

uptake is 2–3 min after injection across all brain regions of interest. Highest cerebellum to target ratios are observed at 16 min after the administration of ^{11}C-**4** and are 1.6, 1.5, 1.45, 1.33 and 1.1 for hippocampus, anterior cingulate, cingulate and prefrontal cortex, respectively. The cerebellum to target ratios are ~1 in all regions within 45 min after injection. Metabolite analysis was performed by RC-HPLC (Phenomenex, Prodigy ODS(3) 4.6 × 250 mm, 10 μm, mobile phase: acetonitrile/25 mM disodium hydrogen phosphate solution 50:50, flow rate: 2 mL/min, retention time: 6–7 min). Metabolite studies in baboon show that ^{11}C-**4** undergoes rapid metabolism to give polar metabolites at 2–3 min and the unmetabolized ^{11}C-**4** comprised 86 ± 4% of total plasma radioactivity at 2 min after injection and 28 ± 1% after 60 min. The amount of ^{11}C-**4** unbound to proteins in plasma (free fraction) is 50 ± 4% ($n = 6$), and 31 ± 3% ($n = 6$) for baboon and human plasma, respectively. Despite the excellent *in vitro* profile, the cause of *in vivo* failure of ^{11}C-**4** is not clear. Even though compound **4** shows 50% free fraction in baboon plasma, self-blocking by the carrier can be ruled out taking into account the low injected mass (0.04 ± 0.001 μg/kg baboon weight) and high specific activity (2600 ± 250 Ci/mmol). The possibility of compound **4** as a *p*-glycoprotein (*p*-gp) substrate is more likely to contribute to the low retention of radiotracer in brain.

Experimental section

General Methods

All commercial reagents and solvents were used without further purification unless otherwise specified. ^{11}C-Methyl iodide was synthesized using the reaction of ^{11}C-carbon dioxide with lithium aluminum hydride, followed by hydroiodic acid and was then converted to ^{11}C-methyl triflate at 200°C by passing through a glass column impregnated with silver triflate.[18] Melting points were determined on a Fisher Scientific Melting point apparatus. High-resolution mass spectra were acquired under fast atom bombardment (FAB+) mode using a JKS-HX 11UHF/HX110 HF Tandem Mass Spectrometer. The ^{1}H NMR spectra of all the compounds were recorded on a Bruker PPX 300 or 400 MHz spectrometers. The spectra were recorded in $CDCl_3$ or CD_3OD and chemical shift (δ) data for the proton resonances were reported in parts per million (ppm) relative to internal standard TMS. Thin-layer chromatography was performed using Silica Gel 60 F254 plates from EM Science and visualized by UV light. Flash column chromatography was carried out using silica gel 60 (Fisher Scientific, 230–400 mesh). Analytical HPLC was performed using a Phenomenex Prodigy reverse phase column (ODS 4.6 × 250 mm, 5 μm) eluted with acetonitrile:aqueous buffer (25 mM Na_2HPO_4 solution) and Waters μBondapak RP-HPLC column (4.6 × 300 mm, 10 μm) eluted with acetonitrile:aqueous buffer (0.1 M ammonium formate solution). Semipreparative HPLC was performed using a Phenomenex Prodigy ODS (10 × 250 mm, 10 μm) reverse phase column eluted with an acetonitrile:aqueous buffer (25 mM Na_2HPO_4 solution) mobile phase mixture. For detection of radiolabeled compounds, γ-ray detector (Bioscan Flow-Count fitted with a NaI detector) was used in series with the UV detector (Waters Model 996 set at 254 nm). Data acquisition for both the analytical and preparative systems was accomplished using a Waters Empower Chromatography System. Metabolite analyses were performed using Phenomenex Prodigy reverse phase column (ODS 4.6 × 250 mm, 10 μm) eluted with acetonitrile:aqueous buffer (25 mM Na_2HPO_4 solution). The free fractions and metabolites weremeasured using Packard Instruments Gamma Counter (Model E5005).

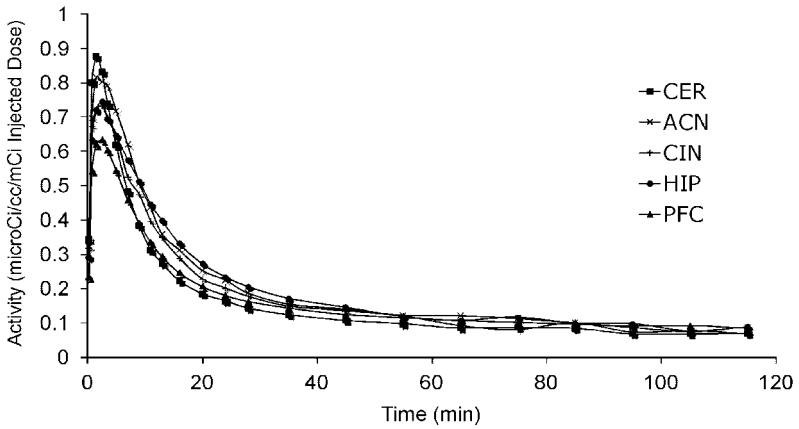


Figure 2. Time–activity curves of the distribution of radioactivity in baboon brain after the injection of ^{11}C-**4** (ACN, anterior cingulate; CER, cerebellum; CIN, cingulate; HIP, hippocampus; PFC, prefrontal cortex).

4-(3-Chloropropoxy)-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (2)

Following the procedure of Fiorino *et al.*,[16] commercially available *endo-N*-hydroxy-5-norbornene-2,3-dicarboximide (**1**) (3.22 g, 18 mmol) was treated with 1-bromo-3-chloropropane (2.83 g, 18 mmol) in ethanol (50 mL) in the presence of sodium hydroxide (0.72 g, 18 mmol). The reaction mixture was heated at 70°C for 24 h. After the completion of the reaction, the mixture was processed and the product was purified by column chromatography (hexane:ethyl acetate 70:30 (v/v) to yield the product as a colorless solid (3.8 g, 83%).

M.p.: 58°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.52 (t, 1H, $J = 9.2$), 1.77 (dt, 1H, $J = 8.8$, 1.6 Hz), 2.10 (pentet, 2H, $J = 6.0$ Hz), 3.19 (dd, 2H, $J = 1.6$, 2.8 Hz), 3.44 (s, 2H), 3.74 (t, 2H, $J = 6.4$ Hz), 4.11 (t, 2H, $J = 5.8$), 6.16 (d, 2H, $J = 1.6$ Hz), HRMS calculated for $C_{12}H_{15}ClNO_3$ (MH^+): 256.0740, Found: 256.0751.

4-[3-[4-(2-Hydroxyphenyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (3)

A mixture of 4-(3-chloropropoxy)-4-aza-tricyclo-[5.2.1.02,6]dec-8-ene-3,5-dione (**2**) (767 mg, 3 mmol) and NaI (749 mg, 5 mmol) in acetonitrile (10 mL) was stirred under reflux for 30 min. Then, 2-(piperazin-1-yl)phenol (2.7 g, 15 mmol) and anhydrous K_2CO_3 (830 mg, 6 mmol) were added. The reaction mixture was stirred under reflux for 48 h. After cooling, the mixture was filtered to remove unreacted K_2CO_3 and concentrated to dryness, and the residue was dissolved in water (50 mL). The solution was extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and the solvent was removed under vacuum. The crude mixtures were purified by silica gel column chromatography using chloroform/methanol 96:4 (v/v) as the eluent. The crude products were triturated with chloroform:diethyl ether to yield the product as a colorless solid (556 mg, 47%).

M.p.: 151–152°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.51 (d, 1H, $J = 8.8$ Hz); 1.77 (m, 1H); 1.88 (pentet, 2H, $–CH_2–$, $J = 7.2$ Hz); 2.59 (t, 2H, $N_1–CH_2$, $J = 7.4$ Hz); 2.63 (brs, 4H, $2CH_2$ piperazine); 2.89 (t, 4H, $2CH_2$ piperazine, $J = 4.8$ Hz); 3.19 (m, 2H); 3.44 (s, 2H); 4.05 (t, 2H, $O–CH_2$, $J = 6.4$ Hz); 6.17 (d, 2H, $J = 1.6$ Hz); 6.86 (dt, 1H, Ar–H, $J = 7.6$, 1.6 Hz); 6.94 (dd, 1H, Ar–H, $J = 8.0$, 1.6 Hz); 7.06 (dt, 1H, Ar–H, $J = 7.6$, 1.6 Hz); 7.16 (dd, 1H, Ar–H, $J = 7.6$, 1.6 Hz). HRMS Calculated for $C_{22}H_{28}N_3O_4$(MH^+): 398.2080; Found: 398.2092.

4-[3-[4-(2-Methoxyphenyl)piperazin-1-yl]propoxy]-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (4)

The title compound was prepared from 4-(3-chloropropoxy)-4-aza-tricyclo-[5.2.1.02,6]dec-8-ene-3,5-dione (**2**) and 2-methoxyphenylpiperazine hydrochloride (3.4 g, 15 mmol) by following the procedure of Fiorino *et al.*[16] The reaction mixture was stirred under reflux for 24 h. After cooling, the mixture was filtered to remove excess K_2CO_3, and concentrated to dryness. The residue was dissolved in water (50 mL) and the solution was repeatedly extracted with EtOAc. The combined organic layers were dried over anhydrous $MgSO_4$ and the solvent was removed under vacuum. The crude mixture was purified by silica gel column chromatography using chloroform/methanol 97:3 (v/v) as the eluent and was recrystallized fromdiethyl ether to yield the desired product as a colorless solid (640 mg, 52%).

M.p.: 123°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.50 (d, 1H, $J = 9.2$ Hz); 1.76 (d, 1H, $J = 7.2$ Hz); 1.88 (pentet, 2H, $–CH_2–$, $J = 7.2$ Hz); 2.57 (t, 2H, $N_1–CH_2$, $J = 7.2$ Hz); 2.65 (brs, 4H, $2CH_2$ piperazine); 3.08 (brs, 4H, $2CH_2$ piperazine); 3.18 (m, 2H); 3.43 (s, 2H); 3.86 (s, 3H, $–OCH_3$); 4.04 (t, 2H, $O–CH_2$, $J = 6.4$ Hz); 6.17 (s, 2H); 6.85 (d, 1H, Ar–H, $J = 7.6$ Hz); 6.89–6.94 (m, 2H, ArH); 6.96–7.02 (m, 1H, ArH). HRMS Calculated for $C_{23}H_{30}N_3O_4$ (MH^+): 412.2236; Found: 412.2252.

Radiosynthesis of [*O-Methyl*-^{11}C]4-[3-[4-(2-methoxyphenyl)-piperazin-1-yl]propoxy]-4-aza-tricyclo-[5.2.1.02,6]dec-8-ene-3,5-dione (^{11}C-4)

Precursor **3** (1.0 mg) was dissolved in 400 μL of acetone in a capped 1 mL reactivial. Aqueous NaOH (10 μL, 5 M) was then added to the solution and the reaction mixture was vortexed. ^{11}C-methyl triflate was transported by a stream of argon (20–30 mL/min) into the reactivial over a period of 5 min at room temperature. At the end of the trapping, the reaction mixture was diluted with the mobile phase and directly injected into a semipreparative RP-HPLC (Phenomenex C18, 10 × 250 mm, 10 μm) and eluted with acetonitrile:25 mM aqueous disodium hydrogen phosphate solution (35:65) at a flow rate of 10 mL/min. The precursor appeared at 2–3 min during the semipreparative HPLC analysis. The product fraction with a retention time of 13–14 min based on 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 20 ± 5% yield (EOS, $n = 6$). A portion of the ethanol solution was analyzed by analytical HPLC (Phenomenex, Prodigy ODS(3) 4.6 × 250 mm, 5 μm mobile phase: acetonitrile/25 mM disodium hydrogen phosphate solution 40:60, flow rate: 2 mL/min, retention time: 9.1 min) to determine the specific activity and radiochemical purity. The chemical and radiochemical purities of ^{11}C-**4** were reconfirmed by RP-HPLC (Waters μBondapak 4.6 × 300 mm, 10 μm, mobile phase, 50:50 acetonitrile/0.1 M ammonium formate flow rate: 2 mL/min, retention time = 5.8-min). The ethanol solution was then diluted with 9 mL of saline and passed through a sterile filter.

Agonist Stimulated [^{35}S]GTPγS Binding of 4

The experiments were carried out as described previously with some modification.[19] Chinese hamster ovary cell expressing 5-HT_{1A} receptor (CHO-h5-HT_{1A}) membranes (30 μg) were pre-incubated with compound **4** for 5 min at room temperature with indicated concentrations in a buffer containing 20 mM HEPES pH 7.4, 3 mM $MgCl_2$, 100 mM NaCl and 3 μM GDP in a final volume of 0.5 mL. [^{35}S]GTPγS (0.1 nM; 1250 Ci/mmol Perkin Elmer Life Science, Boston, MA) was added and the incubation was continued for 60 min at room temperature. Experiments were terminated by rapid filtration through Whatman GF/B filters followed by three washes with ice-cold 20 mM HEPES buffer, pH 7.4, using a cell harvester (Brandel, M-24R, Gaithersburg, MD). Bound radioactivity was determined by liquid scintillation spectrometry (Beckman, LS9000).

PET Studies in Baboons

PET studies were performed in two male baboons with an ECAT EXACT HR+ scanner (CPS/Knoxville, TN). For each scanning session, the fasted animal was immobilized with ketamine (10 mg/kg, im) 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 intravenous 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 the input function. The head was positioned at centre of the field of view, and a 10 min transmission scan was performed before the tracer injection. For each scan, 5 ± 1 mCi (SA = 2600 ± 250 Ci/mmol) of ^{11}C-**4** was injected as an i.v. bolus and emission data were collected for 120 min in 3-D mode. Blood samples were taken every 10 s for the first 2 min, using an automatic system, and thereafter manually for a total of 34 samples over 2 h. For metabolites analyses, the plasma was separated from cellular elements, and the radiolabeled species in the plasma were analyzed by RP-HPLC at 2, 6, 12, 30, 60 and 90 min after radioactivity injection as previously described.[15]

The PET data were reconstructed with attenuation correction using the transmission data, and scatter correction was done using model-based scatter correction.[20] The reconstructionfilter 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 was5.1 mm FWHM.[21] 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 (ROIs) were drawn on the MRI using the MEDX software (Sensor Systems, Inc., Sterling, VA). ROIs included cerebellum, hippocampus, prefrontalcortex, occipital cortex and cingulate. The PET data were co-registered to the MRI using the software AIR,[22] andTACs were generated for each ROI.

Conclusion

The radiosynthesis of ^{11}C-**4**, a 5-HT$_{1A}$R agonist was achieved in 35 min from EOB using [^{11}C]methyl triflate in 20% yield at EOS, and with an average specific activity $\sim 2600 \pm 250$ Ci/mmol. PET study of ^{11}C-**4** in baboon showed that the tracer penetrated the BBB. However, rapid washout of the radioactivity was observed without any specific uptake in the 5-HT$_{1A}$R-rich regions. Despite the HA and selectivity based on *in vitro* studies, demonstration that it is a full agonist and rapid BBB permeability, our *in vivo* studies show that ^{11}C-**4** is not useful as a 5-HT$_{1A}$R agonist PET tracer for imaging in baboon due to the lack of detectable specific binding.

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References

[1] D. Hoyer, J. P. Hannon, G. R. Martin, *Pharmacol. Biochem. Behav*. **2002**, *71*, 533–554.
[2] Z. Bhagwagar, F. A. Rabiner, P. A. Sargent, P. M. Grasby, P. J. Cowen, *Mol. Psychiatry* **2004**, *9*, 386–392.
[3] M. Barros, E. L. Mello, R. S. Maior, C. P. Muller, M. A. De Souza Silva, R. J. Carey, J. P. Huston, C. Tomaz, *Eur. J. Pharmacol.* **2003**, *482*, 197–203.
[4] F. Yasuno, T. Suhara, T. Ichimiya, A. Takano, T. Ando, Y. Okubo, *Biol. Psychiatry* **2004**, *55*, 439–444.
[5] K. Varnas, C. Halldin, H. Hall, *Hum. Brain Mapp*. **2004**, *22*, 246–260.
[6] H. Hall, C. Lundkvist, C. Halldin, L. Farde, V. W. Pike, J. A. McCarron, A. Fletcher, I. A. Cliffe, T. Barf, H. Wikstrom, G. Sedvall, *Brain. Res.* **1997**, *745*, 96–108.
[7] R. V. Parsey, M. A. Oquendo, R. T. Ogden, D. M. Olvet, N. Simpson, Y. Y. Huang, R. L. Van Heertum, V. Arango, J. J. Mann, *Biol. Psychiatry* **2006**, *59*, 106–113.
[8] N. Costes, I. Merlet, K. Ostrowsky, I. Faillenot, F. Lavenne, L. Zimmer, P. Ryvlin, D. Le Bars, *J. Nucl. Med.* **2005**, *46*, 1980–1989.
[9] H. Gozlan, S. Thibault, A. M. Laporte, L. Lima, M. Hamon, *Eur. J. Pharmacol*. **1995**, *288*, 173–186.
[10] J. Watson, L. Collin, M. Ho, G. Riley, C. Scott, J. V. Selkirk, G. W. Price, *Br. J. Pharmacol*. **2000**, *130*, 1108–1114.
[11] R. A. Bantick, E. A. Rabiner, E. Hirani, M. H. de Vries, S. P. Hume, P. M. Grasby, *Neuropsychopharmacology* **2004**, *29*, 847–859.
[12] M. Laruelle, *J. Cereb. Blood Flow. Metab*. **2000**, *20*, 423–451.
[13] M. Riad, K. C. Watkins, E. Doucet, M. Hamon, L. Descarries, *J. Neurosci*. **2001**, *21*, 8378–8386.
[14] J. Prabhakaran, R. V. Parsey, V. J. Majo, S. C. Hsiung, M. S. Milak, H. Tamir, N. R. Simpson, R. L. Van Heertum, J. J. Mann, J. S. Dileep Kumar, *Bioorg. Med. Chem. Lett*. **2006**, *16*, 2101–2104.
[15] J. S. D. Kumar, V. J. Majo, S. C. Hsiung, M. S. Millak, K. P. Liu, H. Tamir, J. Prabhakaran, N. R. Simpson, R. L. Van Heertum, J. J. Mann, R. V. Parsey, *J. Med. Chem*. **2006**, *49*, 125–134. (A detailed account of previous unsuccessful attempts for developing 5-HT$_{1A}$R ligands is discussed in this reference.)
[16] F. Fiorino, E. Perissutti, B. Severino, V. Santagada, D. Cirillo, S. Terracciano, P. Massarelli, G. Bruni, E. Collavoli, C. Renner, G. Caliendo, *J. Med. Chem*. **2005**, *48*, 5495–5503.
[17] A. A. Wilson, L. Jin, A. Garcia, J. N. DaSilva, S. Houle, *Appl. Radiat. Isot.* **2001**, *54*, 203–208.
[18] D. M. Jewett, *Int. J. Rad. Appl. Instrum. [A]* **1992**, *43*, 1383–1385.
[19] T. A. Newman, D. Cussac, L. Marini, M. J. Millan, *Mol. Pharmacol*. **2002**, *62*, 590–601.
[20] C. C. Watson, D. Newport, M. E. Casey, *A Single Scatter Simulation Technique for Scatter Correction in 3D PET*. Kluwer Academic Publishers, Dordrecht, The Netherlands, **1996**.
[21] O. Mawlawi, D. Martinez, M. Slifstein, A. Broft, R. Chatterjee, D. R. Hwang, Y. Huang, N. Simpson, K. Ngo, R. Van Heertum, M. Laruelle, *J. Cereb. Blood Flow. Metab*. **2001**, *21*, 1034–1057.
[22] R. P. Woods, S. R. Cherry, J. C. Mazziotta, *J. Comput. Assist. Tomogr*. **1992**, *16*, 620–633.