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Radiosyntheses and *in vivo* evaluation of carbon-11 PET tracers for PDE10A in the brain of rodent and nonhuman primate

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Abstract

The radiosyntheses and *in vivo* evaluation of four carbon-11 labeled quinoline group-containing radioligands are reported here. Radiolabeling of [¹¹C]**1–4** was achieved by alkylation of their corresponding desmethyl precursors with [¹¹C]CH₃I. Preliminary biodistribution evaluation in Sprague–Dawley rats demonstrated that [¹¹C]**1** and [¹¹C]**2** had high striatal accumulation: striatum: cerebellum ratios (at peak time) for [¹¹C]**1** and [¹¹C]**2** were 6.0-fold (at 60 min) and 4.5-fold (at 30 min) respectively. Following MP-10 pretreatment, striatal uptake in rats of [¹¹C]**1** and [¹¹C]**2** was reduced, suggesting that the tracers bind specifically to PDE10A. MicroPET studies of [¹¹C]**1** and [¹¹C]**2** in nonhuman primates (NHP) also showed good tracer retention in the striatum with rapid clearance from non-target brain regions. Striatal uptake of [¹¹C]**1** reached equilibrium at 30 min with a 3.5-fold striatum: cerebellum ratio. In addition, HPLC analysis of solvent extracts from NHP plasma samples suggested a very stable metabolic profile for [¹¹C]**1**. Our preclinical investigations suggest that [¹¹C]**1** is a promising candidate for quantification of PDE10A in clinical PET studies.

Keywords

PDE10A; carbon-11; PET imaging; MP-10; CNS

1. Introduction

Phosphodiesterase 10A (PDE10A) is an enzyme that hydrolyzes the phosphodiester bond in both cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), resulting in deactivation of intracellular secondary messengers which regulate a wide variety of biological processes in the brain.^{1, 2} PDE10A has low expression in

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peripheral tissues and uniquely limited distribution in the brain, especially in the striatal medium spiny neurons (MSN),^{3–5} which are the principal input sites of the basal ganglia, and are involved in the regulation of motor, appetitive, and cognitive processes.^{6–8} Numerous studies have shown that PDE10A plays a central role in striatal signaling and is implicated in several neuropsychiatric disorders including schizophrenia, Huntington's disease, Parkinson's disease, obsessive-compulsive disorder, and addiction.^{9–12} Since the enzyme structure was first reported in 1999,^{2, 13, 14} tremendous effort has been dedicated to developing PDE10A inhibitors for the treatment of the aforementioned diseases and other disorders characterized by reduced MSN activity.

Positron emission tomography (PET) allows noninvasive measurement of drug disposition, localization, and quantification in animal models used for preclinical studies and in humans. PET methodology is useful for the evaluation of drugs that target the central nervous system (CNS). The efforts in developing therapeutic agents for PDE10A inhibition has been accompanied by a growing interest in PET imaging agents for PDE10A. Our group reported the first ¹¹C-labeled PET tracer for PDE10A: [¹¹C]papaverine.¹⁵ Despite promising *in vitro* results, *in vivo* imaging in NHP showed rapid washout after high initial uptake; low tracer retention was observed in both rat and NHP striatum. Although papaverine is behaviorally active, kinetics did not allow PDE10A visualization with PET. The fast washout from the CNS was attributed to the relative low affinity of papaverine for PDE10A (IC₅₀ = 36 nM). Subsequent efforts in developing a PET imaging agent focused on more potent inhibitors including MP-10 (IC₅₀ = 1.26 nM).^{16, 17} Several research groups, including ours, independently reported the synthesis and *in vivo* evaluation [¹¹C]MP-10.^{18, 19} Our preliminary evaluation in rats demonstrated that the tracer bind specifically to rodent striatum, with washout from non-target brain regions.¹⁹ Specific striatal binding of [¹¹C]MP-10 was also observed by Plisson et al. in pig and baboon using two different blocking strategies.¹⁸ Our tissue time-activity curve (TAC) analysis of the NHP imaging studies showed continuously increasing brain accumulation. Radioactive metabolism studies in NHP plasma extracts and rodent brain homogenates identified a major radiometabolite generated by the breakdown of the phenol-ether linkage on MP-10 in both species. The resulting tricyclic radiometabolite crossed the blood brain barrier (BBB) and accumulated in CNS tissue. Polar metabolites which accumulate in rodent brain have also been reported for ¹⁸F-labeled PDE10A ligands.²⁰

Given these findings, we proposed to introduce the [¹¹C]methoxy group at the quinoline fragment of the structures. Our hypothesis was that by changing the labeling position on MP-10 analogues, we would avoid generation of radiometabolites capable of crossing the BBB and thus improve quality of the brain imaging. Structure-activity analysis (SAR) of 28 newly synthesized analogues of MP-10, showed that derivatives with a methoxy group on 3-, 4- and 6-positions of the quinoline fragment and their corresponding analogues with *N*-methyl group on the 2-position of the pyrazole ring exhibited high potency and selectivity over other PDEs.²¹ Compounds **1**, **2**, **3**, and **4** have IC₅₀ values of 0.40 ± 0.02, 0.28 ± 0.06, 1.82 ± 0.25, and 0.36 ± 0.03 nM respectively (Figure 1). In this study we report the radiosynthesis of [¹¹C]**1–4**, their biodistribution in SD rats, microPET imaging studies in NHP, as well as the metabolite analysis in NHP plasma.

2. Experimental

2.1. General procedure of the radiosynthesis

Production of [^{11}C]CH₃I was via a [$^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$] nuclear reaction following the reported procedure.^{22, 23} Briefly, [^{11}C]CH₃I was produced on-site from [^{11}C]CO₂ using a GE PETtrace CH₃I Microlab. Up to 51.8 GBq of [^{11}C]CO₂ was produced from the JSW BC-16/8 cyclotron by irradiating a gas target of 0.5% O₂ in N₂ for 15 – 30 min with a 40 μA beam of 16 MeV protons in the Barnard Cyclotron Facility of Washington University School of Medicine. After the GE PETtrace CH₃I Microlab system converted the [^{11}C]CO₂ to [^{11}C]CH₄ using a nickel catalyst [Shimalite-Ni (reduced), Shimadzu, Japan P.N. 221-27719] in the presence of hydrogen gas at 360 °C; the [^{11}C]CH₄ was further converted to [^{11}C]CH₃I by reaction with iodine in the gas phase at 690 °C. Approximately 12 min following the end-of-bombardment (EOB), several hundred millicuries of [^{11}C]CH₃I were delivered in the gas phase to the hot cell where the radiosynthesis was accomplished.

[^{11}C]CH₃I was bubbled for a period of 2–3 min into a solution of the corresponding precursor²⁴ (1–2 mg) in DMSO (0.20 mL) containing aqueous sodium hydroxide (NaOH) solution (5 N, 3 μL) at room temperature. When the trapping of radioactivity was complete, the sealed reaction vessel was heated at 85 °C for 5 min. After the heat source was removed, 1.8 mL high performance liquid chromatography (HPLC) mobile phase was added to the reaction vessel. The mixture was purified by reversed phase HPLC (Agilent Zorbax, SB-C18 column, 250 $\times$ 10 mm, flow rate: 4.0 mL/min, UV detection 254 nm). Inline radioactive detector signals were used to identify product fractions which were collected in a vial containing 50 mL sterile water. The diluted eluent was passed through a C-18 Plus Sep-Pak cartridge to remove the mobile phase; the radioactive product was then rinsed with 10 mL sterile water; and then, the product was eluted with 0.6 mL ethanol followed by 5.4 mL saline, which was efficient to elute 80–95% of the radioactive product from the cartridge. The formulated solution was passed through a 0.22 μm syringe filter into a sterile glass vial. The total synthesis time was 50–55 min. Quality control was conducted on an analytical HPLC system (Agilent Zorbax, SB-C18 column, 250 $\times$ 4.6 mm, UV detection 254 nm).

3-(methoxy- ^{11}C)-2-((4-(1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl)phenoxy)methyl)quinoline ([^{11}C]1): The mobile phase used in purification was acetonitrile/ 0.1 M ammonium formate buffer pH = 4.5 (45/55, v/v); product fractions were collected between 14.5 and 16.1 min; precursor (2-((4-(1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl)phenoxy)methyl)quinolin-3-ol) (**5**) retention time was 6.7 min. The QC mobile phase was acetonitrile/ 0.1 M ammonium formate buffer pH 4.5 (59/41, v/v). For the quality control using the analytical HPLC system, the retention time for [^{11}C]1 was 4.7 min at a flow rate of 1.5 mL/min. The sample was authenticated by co-injecting with the cold standard. Radiochemical purity was > 99%, chemical purity was > 95%, radiochemical yield (RCY) was 55–70% (n > 5, decay corrected to EOB) and the specific activity (SA) was > 425 GBq/ μmol (decay corrected to EOB).

4-(methoxy- ^{11}C)-2-((4-(1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl)phenoxy)methyl)quinoline [^{11}C]2: The mobile phase used in the purification was acetonitrile/ 0.1 M ammonium formate buffer pH = 6.5 (50/50, v/v); product fractions were

collected between 14.6 and 16.5 min; precursor (2-((4-(1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl)phenoxy)methyl)quinolin-4-ol) (**6**) retention time was 5.6 min. The QC mobile phase was acetonitrile/ 0.1 M ammonium formate buffer pH 6.5 (60/40, v/v). The retention time for [^{11}C]**2** was 4.7 min at a flow rate of 1.0 mL/min. The sample was authenticated by co-injecting with the cold standard. Radiochemical purity was > 99%, chemical purity was > 95%, RCY 30–35% ($n > 5$, decay corrected to EOB) and SA > 477 GBq/ μmol (decay corrected to EOB).

6-(methoxy- ^{11}C)-2-((4-(1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl)phenoxy)methyl)quinoline [^{11}C]**3**: The mobile phase used in the purification was acetonitrile/ 0.1 M ammonium formate buffer pH = 4.5 (45/55, v/v); product fractions were collected between 15.6 and 17.5 min; precursor (2-((4-(1-methyl-4-(pyridin-4-yl)-1H-pyrazol-3-yl)phenoxy)methyl)quinolin-6-ol) **7** retention time was 6.1 min. The QC mobile phase was acetonitrile/0.1 M ammonium formate buffer pH 6.5 (60/40, v/v). The retention time for [^{11}C]**3** was 4.5 min at a flow rate of 1.0 mL/min. The sample was authenticated by co-injecting with the cold standard. Radiochemical purity was >99%, chemical purity was > 95%, RCY 20–30% ($n = 4$, decay corrected to EOB) and SA > 480 GBq/ μmol (decay corrected to EOB).

4-(methoxy- ^{11}C)-2-((4-(1-methyl-4-(pyridin-4-yl)-1H-pyrazol-5-yl)phenoxy)methyl)quinoline [^{11}C]**4**: The mobile phase used in the purification was acetonitrile / 0.1 M ammonium formate buffer pH = 6.5 (50/50, v/v); product fractions were collected between 18.4 and 20.5 min; precursor (2-((4-(1-methyl-4-(pyridin-4-yl)-1H-pyrazol-5-yl)phenoxy)methyl)quinolin-4-ol) **8** retention time was 5.6 min. The QC mobile phase was acetonitrile/0.1 M, ammonium formate buffer pH 6.5 (60/40, v/v). The retention time for [^{11}C]**4** was 4.8 min at a flow rate of 1.0 mL/min. The sample was authenticated by co-injecting with the cold standard. Radiochemical purity was > 99%, chemical purity was > 95%, the RCY 20–30% ($n = 4$, decay corrected to EOB) and SA > 318 GBq/ μmol (decay corrected to EOB).

2.2. In vivo biodistribution and regional brain uptake in rats

All animal experiments were conducted in compliance with the Guidelines for the Care and Use of Research Animals approved by Washington University's Animal Studies Committee. For the biodistribution studies, 14.8–18.5 MBq of the ^{11}C -tracer in 200 μL of 10% ethanol/saline solution (v/v) was injected into the tail vein of mature male Sprague–Dawley rats (250–400 g) under anesthesia (2.5% isoflurane in oxygen). At 5, 30, and 60 min post injection ($n = 4$ per study group), rats were anesthetized and euthanized. The whole brain was quickly harvested and dissected into segments comprising hippocampus, striatum, cortex, thalamus, brain stem, and cerebellum. The remainder of the brain was also collected in order to determine total brain uptake. Peripheral tissues including blood, heart, lung, liver, spleen, pancreas, kidney, muscle, fat, and tail were also collected. Samples were counted in an automatic gamma counter (Beckman Gamma 8000 well counter) with a diluted sample of the injectate and the %ID/g was calculated, values are represented as mean $\pm$ SD unless otherwise stated. When appropriate, a 2-tailed paired student t test was used. A p value of 0.05 was considered a threshold criterion of statistical significance.

2.3. In vivo microPET brain imaging studies in male cynomolgus monkeys

A microPET Focus 220 scanner (Concorde/CTI/Siemens Microsystems, Knoxville, TN) was used for the imaging studies of [^{11}C]1–2 in male cynomolgus monkey (4–6 kg). The animals were fasted for 12 h before the study. The animals were initially anesthetized using an intramuscular injection with ketamine (10 mg/kg) and glycopyrulate (0.13 mg/kg), and intubated with an endotracheal tube under anesthesia (maintained at 0.75–2.0% isoflurane in oxygen) throughout the PET scanning procedure. After intubation, a percutaneous venous catheter was placed for radiotracer injection. A 10 min transmission scan was performed to check the positioning; once confirmed, a 45 min transmission scan was obtained for attenuation correction. After that, the animal was administrated 260–370 MBq of the radiotracer via the venous catheter. Subsequently, a 120 min dynamic (three 1 min frames, four 2 min frames, three 3 min frames, and twenty 5 min frames) PET scan was acquired. During the whole procedure, core temperature was kept constant at 37 °C with a heated water blanket. In each microPET scanning session, the head was positioned supine in the adjustable head holder with the brain in the center of the field of view. For each subject, at least three independent PET studies were performed ($n=3$). All animal experiments were conducted in compliance with the Guidelines for the Care and Use of Research Animals established by the Animal Studies Committee at Washington University School of Medicine in St. Louis.

2.4. Metabolite studies on NHP plasma

Arterial blood samples (1.2–1.5 mL) were collected at 5, 15, 30, and 60 min post-iv injection of the radiotracers in a heparinized syringe. Aliquots of whole blood (1 mL) were counted in a well counter, then centrifuged to separate red blood cells from plasma. Aliquots of plasma (400 μL) were solvent deproteinated using 0.92 mL ice-cold methanol and separated by centrifugation. The supernatant was mixed with water, 1:1 (v/v) and the mixture was injected to a HPLC system for radioactive metabolite determination. The HPLC system consisted of an Agilent SB C-18 analytic HPLC column (250 mm $\times$ 4.6 mm, 5 μA) and an UV detector with wavelength set at 254 nm. For [^{11}C]1, the mobile phase was acetonitrile/0.1 M, pH 4.5 ammonium formate buffer (52:48, v/v), and the flow rate was 1.0 mL/min. For [^{11}C]2, the mobile phase was acetonitrile/0.1 M, pH 4.5 ammonium formate buffer (48:52, v/v), and the flow rate was 1.1 mL/min. The HPLC fractions were collected at 1 min intervals for 16 min; each fraction was counted by a well counter to determine the radioactivity of each collection. The results were corrected for background radiation and physical decay.

2.5. MicroPET image processing and analysis

PET image reconstructed resolution was < 2.0mm full width half maximum for all 3 dimensions at the center of the field of view. Emission scans were corrected using individual attenuation and model-based scatter correction and reconstructed using filtered back projection as described previously.²⁵ The first baseline PET image for each animal acted as the target image with the MPRAGE and subsequent PETs coregistered to it using automated image registration program AIR.^{26, 27} All MPRAGE-based volume of interest (VOI) analyses were accomplished by investigators blinded to the clinical status of the monkeys.

For quantitative analyses, three-dimensional regions of interest (ROI) (cerebellum, frontal occipital, striatum, temporal, white matter, midbrain and hippocampus) were transformed to the baseline PET space and then overlaid on all reconstructed PET images to obtain time–activity curves. Activity measures were standardized to body weight and dose of radioactivity injected to yield standardized uptake value.

3. Results and discussion

3.1. Radiochemistry

The radiolabeling of [^{11}C]1–4 was accomplished by employing standard conditions for methylation of an *O*-desmethyl precursor with [^{11}C]CH₃I. Reaction of the corresponding desmethyl precursor 5–8²⁴ with [^{11}C]CH₃I was performed in DMSO and in the presence of the NaOH as outlined in Scheme 1 to give [^{11}C]1–4 with 20–70% RCY after HPLC purification. The radiochemical purity of [^{11}C]1–4 was > 99% and chemical purity was > 95%. [^{11}C]1–4 were identified by co-eluting with the standard. The entire synthetic procedure including the production of [^{11}C]CH₃I, HPLC purification and formulation of the radiotracer for *in vivo* studies, was completed within 50–55 min. [^{11}C]1–4 were obtained with high SA (> 318 GBq/μmol, n > 15, decay corrected to EOB), which was sufficient for *in vivo* validation.

3.2. Biodistribution

The rat biodistribution data of [^{11}C]1–4 are summarized in Table 1. The brain uptake of [^{11}C]1–4 at 5 min ranged from 0.09 to 0.25 (I.D./g), all four of radioligands displayed heterogeneous distribution throughout the brain with the highest accumulation of radioactivity in striatal tissue and gradual washout. [^{11}C]1–2 showed the slowest washout from the striatal region between 5 min and 60 min post injection (p.i.). The target to non-target ratio was calculated using cerebellum as the non-target reference region. The striatum to cerebellum ratio of radioactivity accumulation and washout is shown in Figure 2. A blocking dose of 2 mg/kg MP-10 was injected i.v. 5 min prior to injection of [^{11}C]1 or [^{11}C]2 (Table 2), and resulted in a significant reduction in striatal binding ($p < 0.05$). These observations are consistent with the reported that striatal region has the highest expression of PDE10A compared to other non-target brain regions such as cerebellum and cortex.^{1, 28} [^{11}C]1 displayed a 4.6-fold ratio at 30 min p.i. and a 6.0-fold ratio at 60 min p.i. The ratio for [^{11}C]2 was 3.8-fold at 30 min and 2.5-fold at 60 min p.i. [^{11}C]4 displayed a 3.6-fold ratio at 30 min p.i.; however the absolute brain uptake of [^{11}C]4 was significantly lower than that of the other tracers (Table 1). Based on this promising preliminary rodent evaluation, both [^{11}C]1 and [^{11}C]2 demonstrated potential for further evaluation.

3.3. MicroPET studies in nonhuman primate (NHP)

To confirm the feasibility of using [^{11}C]1 or [^{11}C]2 to measure the levels of PDE10A *in vivo*, PET imaging studies were conducted in an adult male NHP on a microPET Focus 220 scanner. The representative summed images from 0 to 120 min were co-registered with MRI images to accurately identify the regions of interest (Figure 3). The summed images revealed high uptake of both [^{11}C]1 and [^{11}C]2 in striatum (Figure 3A and 3B, top panels), which are regions that have high expression of PDE10A. The TACs (Figure 3A and 3B,

lower panels) also indicated the highest uptake in striatum and washout trend in other brain regions for both [^{11}C]**1** and [^{11}C]**2**. Specifically, for [^{11}C]**1**, striatum uptake peaked at 30 min p.i. with a 3.5-fold striatum to cerebellum uptake. For [^{11}C]**2**, the striatum equilibrium was observed at 25 min p.i. with 2.7-fold striatum to cerebellum uptake. These data indicate: (a) both [^{11}C]**1** and [^{11}C]**2** can readily penetrate the blood–brain-barrier and enter into the NHP brain; (b) the distribution of both tracers is consistent with the distribution of the PDE10A in brain; (c) the data supports our hypothesis that by labeling on the quinoline fragment of the molecule, striatum uptake were equilibrated for both [^{11}C]**1** and [^{11}C]**2**; (d) TACs showed higher PDE10A binding for [^{11}C]**1** than [^{11}C]**2**, which is further supported by the microPET images, in that better signal to background ratio was found for [^{11}C]**1**.

3.4. Metabolite studies in NHP plasma

Because MicroPET studies in NHP identified both [^{11}C]**1** and [^{11}C]**2** as potential candidates for imaging PDE10A in the brain, we performed radioactive metabolite analysis of both tracers at 5, 15, 30, and 60 min. After centrifugation of arterial blood samples, 65–75% of the radioactivity was in the plasma and 35–25% of the radioactivity was in the red blood cell (RBC). Following solvent deproteination, 88–95% of the radioactivity in plasma was in the supernatant while 5–12% of the radioactivity in plasma was in the protein pellet. These results suggested that the supernatant contains the majority of the radioactivity. As shown in Figure 4, HPLC analysis of plasma samples revealed only two radioactive peaks for both [^{11}C]**1** and [^{11}C]**2**, a hydrophilic metabolite with retention time at 3–5 min; the retention time for parent compound [^{11}C]**1** was 8–10 min, whereas that of [^{11}C]**2** was 9–12 min. For [^{11}C]**1**, a stable metabolism profile was obtained: at 2 min p.i. 97% of the extracted activity represented the parent compound, at 15 min 87% parent was measured, and by 30 min 82% of the recovered activity was parent, while 60 min p.i., 65% of the recovered radioactivity in the arterial blood sample represented intact [^{11}C]**1** while only 30% of the recovered radioactivity was the radiometabolite. For [^{11}C]**2**, 85% of the extracted activity was parent, which decreased to 75% at 15 min p.i. and 60% parent at 30 min p.i.; by 60 min p.i. equal percentages (42%) of parent [^{11}C]**2** and metabolite were observed. Due to the both the excellent image quality and the superior *in vivo* metabolic stability, we concluded that [^{11}C]**1** is the more promising probe for imaging PDE10A.

4. Conclusions

Previous studies have suggested that [^{11}C]MP-10 has limited utility as a PDE10A PET tracer for human brain imaging due to a brain penetrable radiometabolite. Similar problems were observed in the preclinical evaluation of ^{18}F -labeled PDE10A tracers.²⁰ In a PET tracer recently approved for human studies of PDE10A, although reproducible brain imaging was obtained, significant levels of radiometabolite were also reported: >50% was metabolized with a half-life of 18.7 min.²⁹ To address the challenges involved with radiometabolites, we introduced the [^{11}C]methoxy group onto the quinoline fragment of structures that belongs to this series, unlike [^{11}C]MP-10 which was ^{11}C -labeled on the pyrazole fragment. Here we reported the radiosyntheses and the preclinical evaluation of [^{11}C]**1–4** in rats and the subsequent evaluation of the two promising radioligands, [^{11}C]**1** and [^{11}C]**2**, in cynomolgus macaques. Rat biodistribution studies indicated that [^{11}C]**1** had

the highest brain uptake, good striatal retention and the highest striatum to cerebellum ratio. MicroPET studies in NHP also revealed high accumulation of [¹¹C]**1** in the striatum, which plateaued at 30 min p.i. with a 3.5-fold target to non-target ratio. Although target to non-target ratios were acceptable for [¹¹C]**2**, [¹¹C]**1** had superior metabolic stability. We observed no significant interference from radiometabolites, suggesting the strategy of radiolabeling the quinoline fragment of the structure rather than the pyrazole fragment of the structure improved *in vivo* stability. Further evaluation of [¹¹C]**1** is warranted to investigate its potential as a PDE10A PET tracer in clinical neuroimaging.

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Figure 1.
Structure of MP-10 and compounds **1**, **2**, **3**, and **4**.

Figure 2.

Striatum: cerebellum ratios of [¹¹C]1–4 in rat brain. (A) [¹¹C]1 and [¹¹C]2 displayed higher ratio than [¹¹C]3 and [¹¹C]4. For [¹¹C]1, the ratio reached 6-fold at 60 min p.i., that of [¹¹C]2 reached 4.5-fold. (B) Blocking studies were performed for [¹¹C]1 and [¹¹C]2 by pretreating rats with MP-10 (2 mg/kg, 5 min prior to tracer injection). Significant reduction in striatal binding at 30 min p.i. was obtained for both [¹¹C]1 and [¹¹C]2; striatum: cerebellum ratio was decreased for 50% on [¹¹C]1, and 60% decreased for [¹¹C]2. * $p < 0.05$.

Figure 3.

MicroPET studies of $[^{11}\text{C}]1$ (A) and $[^{11}\text{C}]2$ (B) in an adult male cynomolgus monkey. Upper panel: MicroPET images (left), co-registered images (middle) and MRI images (right). High uptake of $[^{11}\text{C}]1$ and $[^{11}\text{C}]2$ in striatum produced clear visualization of tracer in putamen and caudate; low panel: tissue time activity curves for cerebellum (black), frontal cortex (blue), occipital cortex (magenta), striatum (red), temporal cortex (olive), white matter (navy), middle brain (violet), and hippocampus (brown).

Figure 4.

Radiometabolite analysis of NHP arterial plasma samples post injection of [^{11}C]1 (A) or [^{11}C]2 (B). This black histogram represents the percentage radioactivity from the intact parent compound, while the gray histogram represents the percentage radioactivity from the metabolite at 2, 15, 30 and 60 min postinjection. The stability of radioligand [^{11}C]1(A) are better than that of radioligand [^{11}C]2 (B) *in vivo*.

Scheme 1.

Radiosynthesis of [^{11}C]**1**–**4** through O-methylation of corresponding precursor **5**–**8** with [^{11}C] CH_3I .

Table 1

Biodistribution of [¹¹C]1–4 in male SD rats (%ID/gram)

Organ	5 min	30 min	60 min	5 min	30 min	60 min
	[¹¹ C]1			[¹¹ C]2		
<i>blood</i>	0.679 ± 0.116	0.321 ± 0.021	0.275 ± 0.028	0.268 ± 0.053	0.192 ± 0.075	0.152 ± 0.030
<i>lung</i>	1.602 ± 0.509	0.408 ± 0.083	0.299 ± 0.042	0.890 ± 0.206	0.233 ± 0.045	0.163 ± 0.027
<i>liver</i>	11.005 ± 0.758	7.345 ± 1.472	6.398 ± 0.945	7.080 ± 0.435	10.085 ± 1.972	8.941 ± 1.805
<i>kidney</i>	4.354 ± 0.473	1.647 ± 0.459	1.275 ± 0.141	2.340 ± 0.295	1.294 ± 0.268	0.758 ± 0.112
<i>muscle</i>	0.412 ± 0.064	0.244 ± 0.073	0.142 ± 0.014	0.258 ± 0.035	0.133 ± 0.035	0.084 ± 0.013
<i>fat</i>	0.935 ± 0.462	0.851 ± 0.314	0.500 ± 0.087	0.242 ± 0.065	0.371 ± 0.091	0.278 ± 0.040
<i>heart</i>	0.733 ± 0.039	0.296 ± 0.038	0.199 ± 0.013	0.464 ± 0.023	0.187 ± 0.042	0.121 ± 0.025
<i>cerebellum</i>	0.229 ± 0.039	0.097 ± 0.016	0.064 ± 0.006	0.215 ± 0.031	0.066 ± 0.023	0.046 ± 0.014
<i>Striatum</i>	0.488 ± 0.101 ^{*a}	0.452 ± 0.086 ^{*a}	0.380 ± 0.048 ^{*a}	0.352 ± 0.052 ^{*a}	0.230 ± 0.030 ^{*a}	0.201 ± 0.037 ^{*a}
<i>total brain</i>	0.231 ± 0.038	0.133 ± 0.024	0.095 ± 0.008	0.210 ± 0.035	0.087 ± 0.016	0.057 ± 0.008
	[¹¹ C]3			[¹¹ C]4		
<i>blood</i>	0.282 ± 0.025	0.275 ± 0.041	0.210 ± 0.023	0.099 ± 0.015	0.067 ± 0.006	0.067 ± 0.011
<i>lung</i>	0.744 ± 0.241	0.389 ± 0.082	0.249 ± 0.015	0.643 ± 0.196	0.156 ± 0.020	0.107 ± 0.016
<i>liver</i>	5.357 ± 0.989	3.172 ± 0.606	1.724 ± 0.510	3.312 ± 0.494	4.430 ± 0.481	4.588 ± 0.577
<i>kidney</i>	1.333 ± 0.282	0.883 ± 0.123	0.637 ± 0.052	1.744 ± 0.316	0.645 ± 0.088	0.575 ± 0.075
<i>muscle</i>	0.151 ± 0.027	0.175 ± 0.009	0.129 ± 0.006	0.146 ± 0.027	0.093 ± 0.010	0.052 ± 0.009
<i>fat</i>	0.139 ± 0.038	0.221 ± 0.060	0.135 ± 0.026	0.066 ± 0.016	0.139 ± 0.027	0.139 ± 0.058
<i>heart</i>	0.416 ± 0.026	0.264 ± 0.032	0.182 ± 0.020	0.335 ± 0.026	0.132 ± 0.019	0.103 ± 0.012
<i>cerebellum</i>	0.251 ± 0.031	0.090 ± 0.018	0.066 ± 0.008	0.094 ± 0.017	0.024 ± 0.007	0.015 ± 0.003
<i>striatum</i>	0.293 ± 0.075 ^{*b}	0.160 ± 0.023 ^{*a}	0.089 ± 0.014 ^{*a}	0.151 ± 0.033 ^{*a}	0.083 ± 0.010 ^{*a}	0.037 ± 0.004 ^{*a}
<i>total brain</i>	0.249 ± 0.037	0.105 ± 0.011	0.074 ± 0.005	0.091 ± 0.017	0.025 ± 0.004	0.015 ± 0.002

* p value was determined by using 2-tailed paired student t test for the uptake of striatum vs. that of cerebellum.

^a p < 0.05;

^b p > 0.05.

Table 2

The distribution of [^{11}C]1 and [^{11}C]2 in control rats and rats pretreated using MP-10 (2.0 mg/kg, 5 min prior to tracer injection) at 30 min p.i.(%ID/gram)

<i>Organ</i>	$[^{11}\text{C}]1$		$[^{11}\text{C}]2$	
	Control	MP-10 block	Control	MP-10 block
<i>blood</i>	0.321 $\pm$ 0.021	0.407 $\pm$ 0.026	0.192 $\pm$ 0.075	0.343 $\pm$ 0.007
<i>lung</i>	0.408 $\pm$ 0.083	0.404 $\pm$ 0.024	0.233 $\pm$ 0.045	0.362 $\pm$ 0.021
<i>liver</i>	7.345 $\pm$ 1.472	5.781 $\pm$ 0.696	10.085 $\pm$ 1.972	4.723 $\pm$ 0.489
<i>kidney</i>	1.647 $\pm$ 0.459	1.523 $\pm$ 0.162	1.294 $\pm$ 0.268	1.072 $\pm$ 0.069
<i>muscle</i>	0.244 $\pm$ 0.073	0.257 $\pm$ 0.026	0.133 $\pm$ 0.035	0.224 $\pm$ 0.012
<i>fat</i>	0.851 $\pm$ 0.314	1.353 $\pm$ 0.202	0.371 $\pm$ 0.091	0.697 $\pm$ 0.219
<i>heart</i>	0.296 $\pm$ 0.038	0.340 $\pm$ 0.036	0.187 $\pm$ 0.042	0.310 $\pm$ 0.040
<i>cerebellum</i>	0.097 $\pm$ 0.016	0.119 $\pm$ 0.006	0.066 $\pm$ 0.023	0.112 $\pm$ 0.011
<i>Striatum</i>	0.452 $\pm$ 0.086*	0.294 $\pm$ 0.013*	0.230 $\pm$ 0.030*	0.175 $\pm$ 0.022*
<i>total brain</i>	0.133 $\pm$ 0.024	0.128 $\pm$ 0.003	0.087 $\pm$ 0.016	0.114 $\pm$ 0.007

* p value was determined by using 2-tailed paired student t test for the striatum uptake in control rats vs. rats pretreated using MP-10. $p < 0.05$.