



# Synthesis and Evaluation of [<sup>11</sup>C]7-Halogen-2-Phenyl Isoindolone Derivatives: Potential PET Radioligands for *in vivo* Imaging of 5-HT<sub>2C</sub> Receptors

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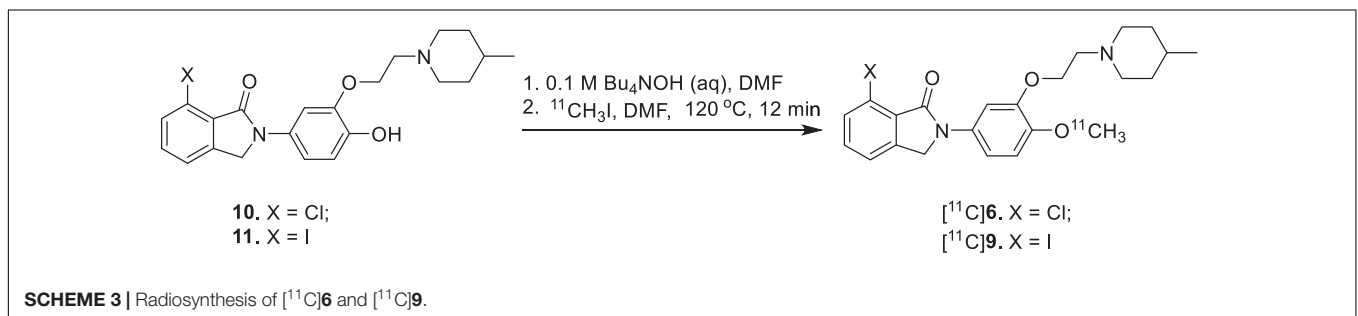
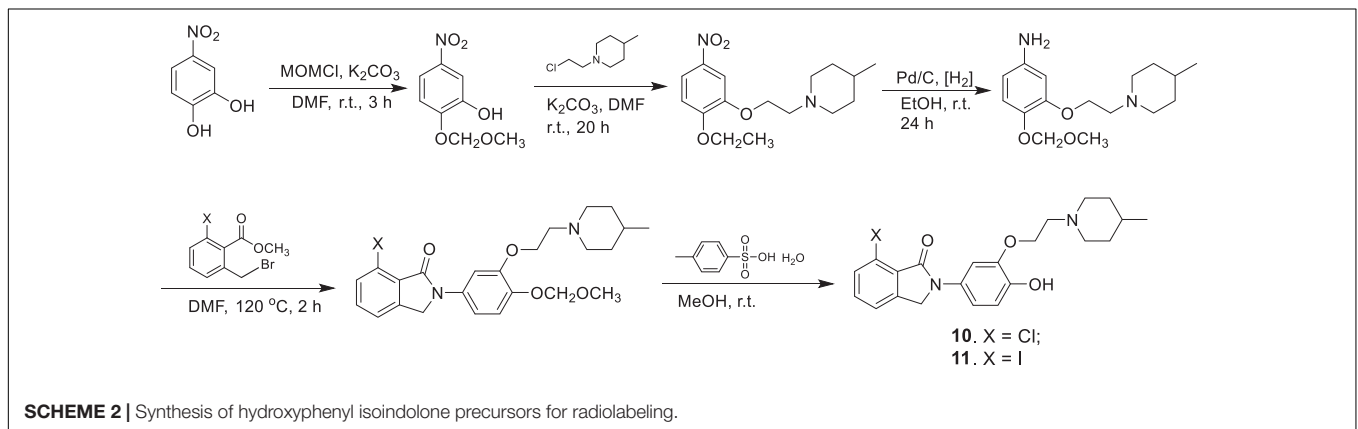
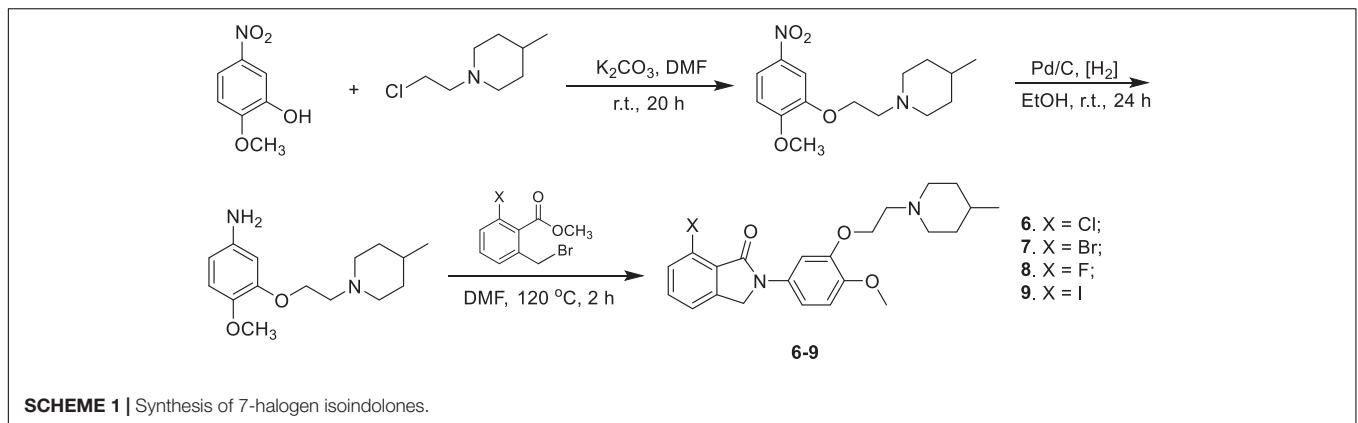
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The serotonin 5-HT<sub>2C</sub> receptor (5-HT<sub>2C</sub>R) is abundantly expressed throughout the central nervous system, and involved in a variety of neuroendocrine and neurobehavioral processes. The development of a selective radioligand that will enable *in vivo* imaging and quantification of 5-HT<sub>2C</sub>R densities represents a significant technological advancement in understanding both the normal function and pathophysiology of the 5-HT<sub>2C</sub>R. Four 7-halogen-2-phenyl isoindolones (7-F, Cl, Br, I) were synthesized and displayed high affinities for 5-HT<sub>2C</sub>R and high selectivity over 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub>. [<sup>11</sup>C]7-Chloro-2-[4-methoxy-3-[2-(4-methylpiperidin-1-yl)ethoxy]phenyl]isoindolin-1-one (**6**) and [<sup>11</sup>C]7-iodo-2-[4-methoxy-3-[2-(4-methylpiperidin-1-yl)ethoxy]phenyl]isoindolin-1-one (**9**) were synthesized in high radiochemical yield of 37–44% [*n* = 10, decay corrected from end of (<sup>11</sup>C)CH<sub>3</sub>I synthesis] with high radiochemical purity *via* *O*-methylation with [<sup>11</sup>C]CH<sub>3</sub>I, respectively. MicroPET imaging studies in male rats with or without 5-HT<sub>2C</sub> antagonist SB-242084 showed that [<sup>11</sup>C]**6** and [<sup>11</sup>C]**9** display specific bindings to 5-HT<sub>2C</sub>R in the choroid plexus and hippocampus. *In vivo* microPET brain imaging studies in rhesus monkeys demonstrated that [<sup>11</sup>C]**6** and [<sup>11</sup>C]**9** exhibit excellent blood-brain barrier penetration. The contrast of bindings to the choroid plexus and hippocampus compared to the cerebellum peaked at 2.7 and 1.6, respectively, for [<sup>11</sup>C]**6**, and 3.7 and 2.7, respectively, for [<sup>11</sup>C]**9**, which were reduced by administration of a dose of SB-242084. Our results support the candidacy of [<sup>11</sup>C]**6** and [<sup>11</sup>C]**9** for further study as radioligands for *in vivo* quantitation of 5-HT<sub>2C</sub> sites by PET.

**Keywords:** PET radioligand, 5-HT<sub>2C</sub> receptor, *in vivo*, brain imaging, carbon-11

## INTRODUCTION

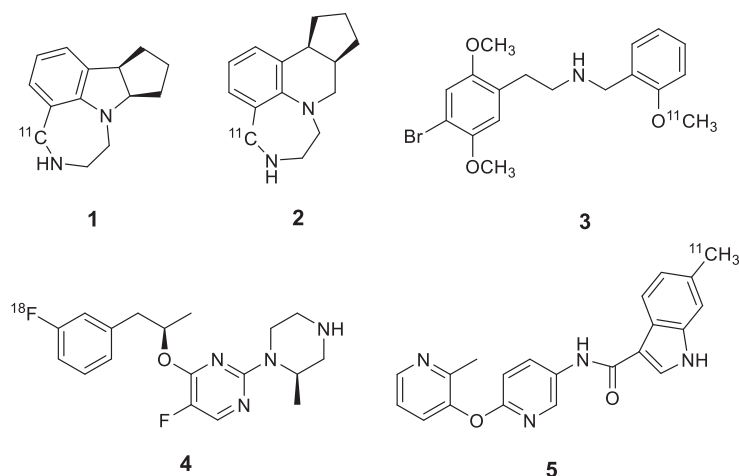
The serotonin (5-HT) system in the brain is directly involved in regulating various physiological actions and mental states as an important neurotransmission network which control behaviors and physical functions (Jacobs and Azmitia, 1992; Barnes and Sharp, 1999; Carhart-Harris and Nutt, 2017). Dysregulation of serotonergic function and significant alteration in serotonin receptor



binding has been increasingly implicated in the pathology of physiological and psychiatric disorders (Messing and Lytle, 1977; Coccaro et al., 1989; Naughton et al., 2000; Stockmeier, 2003). The 5-HT<sub>2C</sub> receptor (5-HT<sub>2C</sub>R) is one of 14 5-HT receptor subtypes that binds the endogenous neurotransmitter serotonin. Expression of the 5-HT<sub>2C</sub>R is widely distributed in the mammalian brain with the highest concentration in the choroid plexus. Sufficient densities for imaging are also identified in the hippocampus, amygdala and hypothalamus, while low densities of 5-HT<sub>2C</sub>R are found in the cortex and cerebellum (Hoyer et al., 1986; Clemett et al., 2000). The 5-HT<sub>2C</sub>R has been implicated in mediating the interaction between serotonergic and dopaminergic systems. Substantial preclinical and clinical findings on 5-HT<sub>2C</sub>R agonists and antagonists demonstrated 5-HT<sub>2C</sub>R as a potential therapeutic target for the treatment of

schizophrenia, anxiety, depression, drug abuse, and Parkinson's disease (Millan, 2005; Heisler et al., 2007; Rosenzweig-Lipson et al., 2007; Jensen et al., 2010). Its involvement in feeding regulation and energy balance led to the FDA approval of the 5-HT<sub>2C</sub>R agonist lorcaserin for treatment of obesity.

Although PET imaging of 5-HTRs has been progressing for almost three decades, the successful radiotracers developed so far for human studies are limited to 5-HT<sub>1A</sub>R, 5-HT<sub>1B</sub>R, 5-HT<sub>2A</sub>R, 5-HT<sub>4</sub>R and 5-HT<sub>6</sub>R. Several attempts for the development of 5-HT<sub>2C</sub>R PET tracers were recently made with limited success (**Figure 1**). Two potent benzazepine 5-HT<sub>2C</sub>R agonists, WAY-163909 and Vabicaserin, were labeled with carbon-11. PET imaging evaluation indicated that both [<sup>11</sup>C]WAY-163909 (Neelamegam et al., 2014) (**1**) and [<sup>11</sup>C]Vabicaserin (Neelamegam et al., 2014) (**2**) exhibited high non-specific



**FIGURE 1** | Chemical structures of reported PET imaging agents for 5-HT<sub>2C</sub>R.

binding in baboon brains. [<sup>11</sup>C]Cimbi-36 (Finnema et al., 2014) (**3**) was reported as the first agonist suitable for imaging 5-HT<sub>2C</sub>R in the choroid plexus of the primate brain. However, [<sup>11</sup>C]Cimbi-36 is also a potent 5-HT<sub>2A</sub>R agonist with  $K_i = 0.5$  nM for 5HT<sub>2A</sub>R vs.  $K_i = 1.7$  nM for 5HT<sub>2C</sub>R. This major shortcoming necessitates blocking 5HT<sub>2A</sub>R prior to administration of [<sup>11</sup>C]Cimbi-36 in the quantitative assessment of 5-HT<sub>2C</sub>R binding in CNS disorders. [<sup>18</sup>F]4-(3-Fluorophenoxy)pyrimidine (**4**) was synthesized and evaluated in the rat brain (Kim et al., 2017). Although [<sup>18</sup>F]**4** exhibited specific binding to 5-HT<sub>2C</sub>R, its low to moderate ratios of uptake in regions of interest to cerebellum and fast washout from choroid plexus limited its utility as a PET radiotracer for 5-HT<sub>2C</sub>R. Recently, we evaluated a C-11 labeled pyridyloxy-pyridyl indole carboxamide derivative, 6-methyl-N-[6-[(2-methyl-3-pyridinyl)oxy]-3-pyridinyl]1H-indole-3-carboxamide (**5**) as a 5-HT<sub>2C</sub>R PET imaging agent (Zeng et al., 2018). MicroPET studies in rhesus monkeys demonstrated that [<sup>11</sup>C]**5** displays a high level of specific binding in the choroid plexus, however, the overall brain uptake and retention of the tracer was low.

As our continuous effort to develop 5-HT<sub>2C</sub>R-specific PET imaging agents, we selected halogen substituted isoindolones (represented by **6** and **7**) as potential target molecules based on the reported excellent *in vitro* binding profiles (Hamprecht et al., 2007). Chloro- and bromo- isoindolone derivatives (**6** and **7**) exhibited high affinity for 5-HT<sub>2C</sub>R and high selectivity over 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> (pK<sub>i</sub> of **6** for 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2C</sub> = 6.1, 6.9, and 8.8; pK<sub>i</sub> of **7** for 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>2C</sub> = 6.2, 6.8, and 9.1, respectively). In addition, **6** and **7** showed great brain permeability as measured by brain to blood plasma ratio. We synthesized compounds **6** and **7**, and fluoro- and iodo-isoindolone derivatives, **8** and **9**, and compared their binding affinity and selectivity at 5-HT<sub>2C</sub>R. In addition, compounds **6** and **9** were radiolabeled with carbon-11 and PET imaging studies in rats and non-human primates were performed for evaluation as 5-HT<sub>2C</sub>R PET radioligands.

## RESULTS AND DISCUSSION

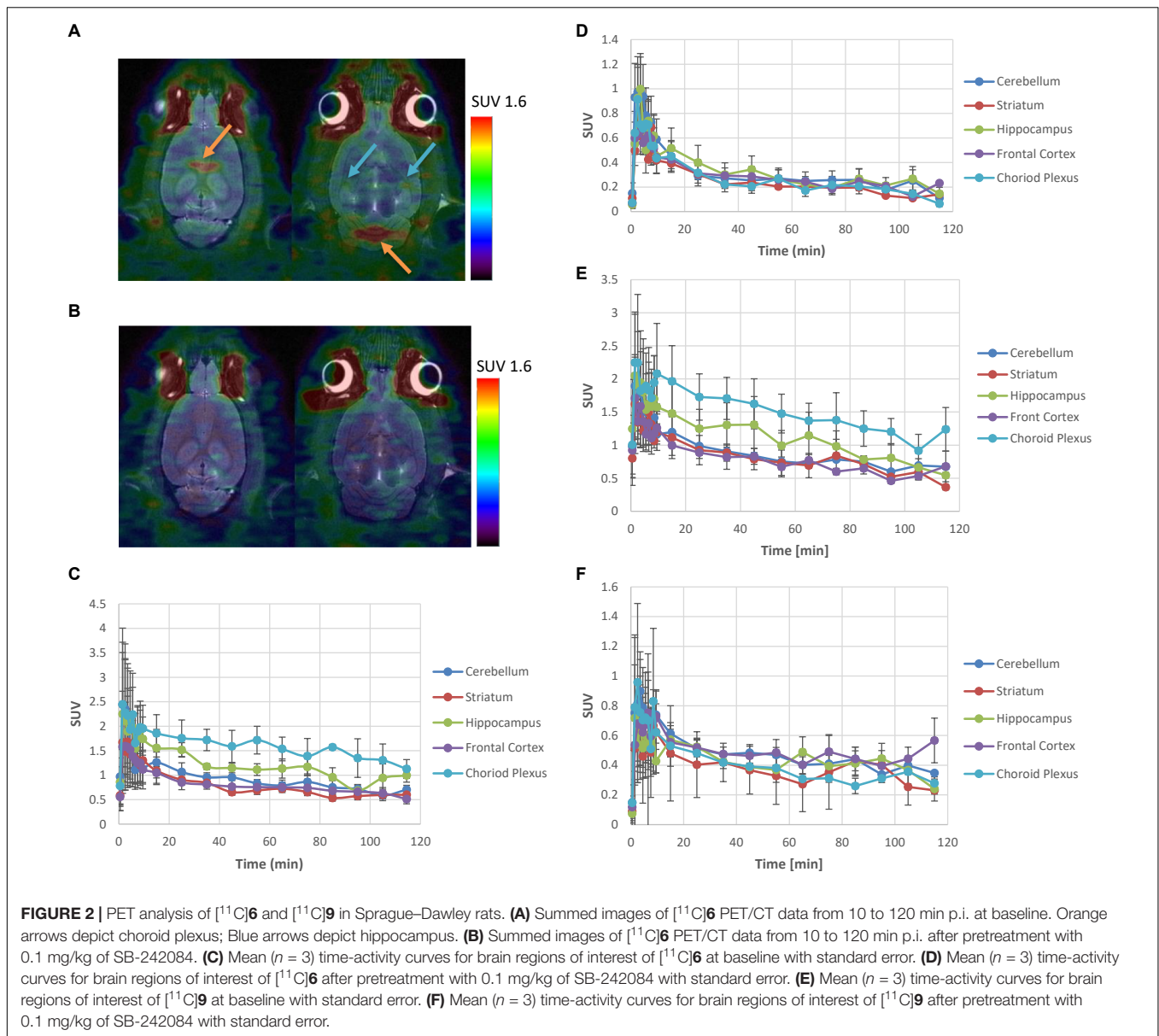
Compounds **6–9** were synthesized in three steps (**Scheme 1**), which include alkylation of the phenols with *N*-(2-chloroethyl)-4-methylpiperidine, reduction of the nitro compounds using Pd/C, and condensation of the resulting phenylamines with the corresponding 6-halogen-2-bromomethylbenzoate. The synthesis of the precursors **10** and **11** followed the same procedure, except that 2-(methoxymethoxy)-5-nitrophenol was used in the alkylation reaction. The MOM protection group was then cleaved with excess *p*-toluenesulfonic acid to afford the hydroxyphenylisoindolones as the radiolabeling precursors (**Scheme 2**).

All compounds **6–9** displayed high binding affinities to 5-HT<sub>2C</sub> with >90% binding at a concentration of 10 μM. The 5-HT<sub>2C</sub> competition assay with [<sup>3</sup>H]mesulergine showed that iodo derivative **9** has the highest binding of 1.1 nM, while chloro and bromo derivatives **6** and **7** exhibit similar bindings of 2.2 and 2.9 nM, respectively, which is 2–3 times less than that for **9**, and fluoro derivative **8** has the lowest affinity of 28 nM. Compounds **6**, **7**, and **9** exhibit high binding selectivity over 5-HT<sub>2B</sub> (80–100 times) and <50% primary bindings at 5-HT<sub>2A</sub>. In addition, no significant binding was observed in other 5-HT subtypes and D<sub>1</sub>-D<sub>5</sub> receptors (**Table 1**). We selected **6** and **9** for carbon-11 radiolabeling and PET imaging studies.

**TABLE 1** | Binding affinities of 7-halogen isoindolones at serotonin (5-HT<sub>1A</sub>-5-HT<sub>7</sub>) and dopamine (D<sub>1</sub>-D<sub>5</sub>) receptors ( $K_i$ , nM)<sup>a</sup>.

Compound	5-HT <sub>2C</sub>	5-HT <sub>2A</sub>	5-HT <sub>2B</sub>	5-HT <sub>1A</sub>	5-HT <sub>5–7</sub>	D <sub>1</sub> -D <sub>5</sub>
6	2.2 ± 0.3	> 1,000	194 ± 74	> 1,000	> 1,000	> 1,000
7	2.9 ± 0.7	> 1,000	177 ± 75	> 1,000	> 1,000	> 1,000
8	28 ± 14	> 1,000	434 ± 204	> 1,000	> 1,000	> 1,000
9	1.1 ± 0.3	> 1,000	126 ± 28	> 1,000	> 1,000	> 1,000

<sup>a</sup>Competition binding assays were conducted by NIMH Psychoactive Drug Screening Program (PDSP). Data are reported as means of three separate competitive experiments ± standard deviation.

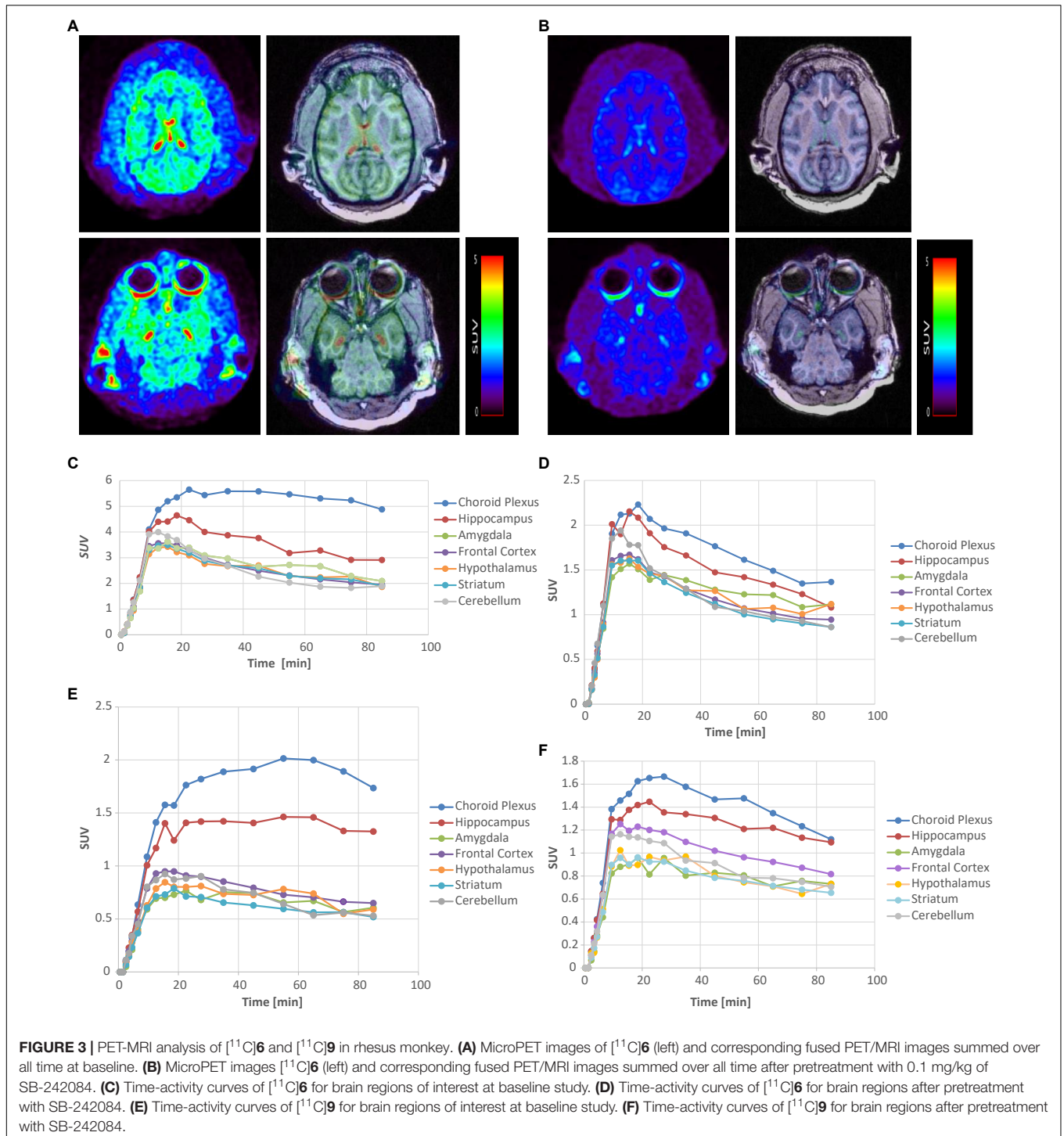


[<sup>11</sup>C]6 and [<sup>11</sup>C]9 were prepared *via* *O*-methylation of **10** and **11** with [<sup>11</sup>C]CH<sub>3</sub>I in the presence of 0.1 M Bu<sub>4</sub>NOH in DMF followed by HPLC purification, respectively. [<sup>11</sup>C]6 and [<sup>11</sup>C]9 were obtained in an average radiochemical yield of 37–44 ± 4% (*n* = 10, decay corrected) with ≥97% radiochemical and chemical purity. The total synthesis time was 50 ± 5 min end of bombardment and the specific activity were in the range of 0.5–1.2 Ci/μmol at time of injection. The lipophilicities of **6** and **9** (octanol/phosphate buffer partition) were measured. The log *P*<sub>7.4</sub> values of [<sup>11</sup>C]6 and [<sup>11</sup>C]9 are 2.81 and 2.89, respectively, which are in the optimal range (1.0–3.0) for compounds expected to enter the brain readily.

[<sup>11</sup>C]6 and [<sup>11</sup>C]9 were intravenously administered to Sprague–Dawley rats and brain images were acquired with a Siemens Inveon microPET/CT imaging system, respectively (**Figure 2**). The regional uptake of [<sup>11</sup>C]6 and [<sup>11</sup>C]9 within

the brain was observed in the choroid plexus and hippocampus, whereas retention in all other brain regions was low. The time-activity curves generated from imaging data showed that ratios of uptake in choroid plexus and hippocampus to that in cerebellum peaked at 1.8 and 1.2 for [<sup>11</sup>C]6, and 1.9 and 1.3 for [<sup>11</sup>C]9, respectively. Pretreating the rats with a dose of 0.1 mg/kg of SB-242084, a 5-HT<sub>2C</sub>R antagonist (*K*<sub>i</sub> at 5-HT<sub>2C</sub>R = 3.6 nM), prior to injection resulted in a reduction of radioactivity uptake in the choroid plexus and hippocampus to the same level as of the cerebellum, suggesting that the uptake of [<sup>11</sup>C]6 and [<sup>11</sup>C]9 in choroid plexus and hippocampus reflected specific binding.

[<sup>11</sup>C]6 and [<sup>11</sup>C]9 were further evaluated in rhesus monkeys using a Siemens MicroPET Focus 220 scanner (**Figure 3**). Contrary to the relatively low uptakes in rat brain, [<sup>11</sup>C]6 and [<sup>11</sup>C]9 exhibited excellent brain blood barrier penetration in the monkey. The regional uptake of [<sup>11</sup>C]6 showed high uptake in



the choroid plexus and hippocampus with low uptake in the cerebellum. The corresponding time-activity curves (TACs) of [<sup>11</sup>C]6 showed that the radioactivity uptake in the choroid plexus peaked between 20–30 min after injection with a mean SUV value of 5.6, and the peak uptakes in the hippocampus, amygdala, frontal cortex, and cerebellum were achieved at 9.5–18.5 min postinjection. Ratios of uptake in choroid plexus, hippocampus,

amygdala, frontal cortex to that in cerebellum peaked at 2.7, 1.6, 1.3, and 1.1, respectively.

Similarly, [<sup>11</sup>C]9 showed high uptake in the choroid plexus and hippocampus, with low uptake in the cerebellum in the baseline study. The TACs of [<sup>11</sup>C]9 indicated that the peak uptake of radioactivity in the choroid plexus and hippocampus were achieved 25–55 min after injection, and slowly washed out during

the 90 min-course of PET study. The slower binding kinetics exhibited by [<sup>11</sup>C]9 in comparison to [<sup>11</sup>C]6 may reflect a higher binding affinity of [<sup>11</sup>C]9 to the 5-HT<sub>2C</sub>R. Although [<sup>11</sup>C]9 showed lower uptakes in the choroid plexus and hippocampus than [<sup>11</sup>C]6, with SUV values of 2.01 and 1.46 vs. 5.6 and 4.6, respectively, the ratio of uptake in the choroid plexus and hippocampus compared to the cerebellum peaked higher than [<sup>11</sup>C]6 at 3.7 and 2.7, respectively.

To test specific binding of [<sup>11</sup>C]6 and [<sup>11</sup>C]9 to the choroid plexus and hippocampus, an *in vivo* microPET blocking study was performed with 0.1 mg/kg SB-242084 30 min prior to injection, respectively. Unfortunately, due to the unexpected low solubility of SB-242084 in the injection form (10% ethanol in saline), the administered SB-242084 in soluble form to the monkeys was likely much less than the target 0.1 mg/kg. As a result, the uptake of [<sup>11</sup>C]6 and [<sup>11</sup>C]9 in the choroid plexus and hippocampus was still present. However, a reduction in the uptake and faster washout from the choroid plexus and hippocampus was observed compared to the baseline studies shown in **Figures 3D,F**. Combined with the blocking study results on rats, these results strongly suggest that PET images acquired with [<sup>11</sup>C]6 and [<sup>11</sup>C]9 represent the specific binding to 5-HT<sub>2C</sub>R.

In summary, two 5-HT<sub>2C</sub> ligands, 7-chloro-2-[4-methoxy-3-[2-(4-methylpiperidin-1-yl)ethoxy]phenyl]isoindolin-1-one (**6**) and 7-iodo-2-[4-methoxy-3-[2-(4-methylpiperidin-1-yl)ethoxy]phenyl]isoindolin-1-one (**9**) have been synthesized, radiolabeled with carbon-11, and evaluated in rats and non-human primates. Competition binding assays demonstrated that (**6**) and (**9**) possess high 5-HT<sub>2C</sub> binding affinity and high selectivity over 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub>. *In vivo* microPET imaging studies in rats showed that [<sup>11</sup>C]6 and [<sup>11</sup>C]9 display specific binding to 5-HT<sub>2C</sub>R in the choroid plexus and hippocampus. *In vivo* microPET imaging studies in

monkeys demonstrated that [<sup>11</sup>C]6 and [<sup>11</sup>C]9 exhibited high brain uptake with specific binding to the choroid plexus and hippocampus. The high uptakes in the choroid plexus and hippocampus to cerebellar ratios in non-human primates strongly support the candidacy of [<sup>11</sup>C]6 and [<sup>11</sup>C]9 for further study as radioligands for *in vivo* quantitation of 5-HT<sub>2C</sub> sites by PET.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author/s.

## ETHICS STATEMENT

The animal study was reviewed and approved by Institutional Animal Care and Use Committee (IACUC), Emory University.

## AUTHOR CONTRIBUTIONS

FZ and MG contributed to the design of the study. FZ performed the synthesis and radiolabelling. RV and JM contributed to the radiolabeling. JN performed the imaging process. All authors contributed to manuscript revision, read, and approved the submitted version.

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