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Radiosynthesis and preliminary evaluation of 4-[^{18}F]fluoro-*N*-[4-[6-(isopropylamino)pyrimidin-4-yl]-1,3-thiazol-2-yl]-*N*-methylbenzamide as a new positron emission tomography ligand for metabotropic glutamate receptor subtype 1

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ABSTRACT

The purpose of this study was to develop 4-[^{18}F]fluoro-*N*-[4-[6-(isopropylamino)pyrimidin-4-yl]-1,3-thiazol-2-yl]-*N*-methylbenzamide ([^{18}F]FITM, [^{18}F]**4**) as a new PET ligand for imaging metabotropic glutamate receptor subtype 1 (mGluR1). [^{18}F]**4** was synthesized by [^{18}F]fluorination of a novel nitro precursor **3** with [^{18}F]KF in the presence of Kryptofix 222. At the end of synthesis, 429–936 MBq (n = 8) of [^{18}F]**4** was obtained with >99% radiochemical purity and 204–559 GBq/μmol specific activity starting from 6.7 to 13.0 GBq of [^{18}F]F^-. The brain distribution of [^{18}F]**4** was determined by the in vitro and ex vivo autoradiography using rat brain sections. The in vitro and in vivo specific binding of [^{18}F]**4** to mGluR1 was detected in the cerebellum, thalamus, hippocampus, and striatum. These results suggest that [^{18}F]**4** is a promising PET ligand for the in vivo evaluation of mGluR1.

Glutamate (Glu) is a major neurotransmitter in the central nervous system (CNS) in mammals and is involved in over one-half of synapses. The Glu receptors have distinctive metabotropic and iontropic types (mGluRs and iGluRs). The mGluRs are G-protein-coupled receptors and are classified into three groups with eight subtypes based on their pharmacology, signal transduction mechanism, and sequence homology. Among the eight subtypes of mGluRs, mGluR1 and mGluR5 belonging to group I, are located at the postsynapse, and regulate acute neuroexcitation with the efflux of Ca^{2+} from the smooth endoplasmic reticulum. Thus, mGluR1 and mGluR5 are involved in brain development, mechanisms of learning, and neuroprotection, and have been implicated in the pathophysiology of several neurological and psychiatric disorders, such as Parkinson's disease, multiple sclerosis, motor dysfunction, epilepsy, and stroke.[1–5] The development of mGluRs of group I as a biomarker should lead to better understanding of the role of these receptors in pathophysiological and biological processes.

Positron emission tomography (PET) ligands for mGluR5 have been developed and successfully applied in the in vivo evaluation of mGluR5.[6–8] At the same time, potent and selective ligands for mGluR1 have been developed, such as CPCCOEt,[9,10] BAY36-7620,[11] R214127,[12] JNJ16259685,[13] FTIDC,[14] and YM-202074.[15] Subsequently, several radiolabeled ligands for PET study were synthesized to investigate the in vivo localization and biological characteristics of mGluR1, such as [^{11}C]JNJ-16567083,[16] [^{18}F]FTIDC,[17] [^{18}F]MK-1312,[18] [^{11}C]MMTP,[19] and [^{11}C]YM-202074 (Scheme 1).[20] Of these, [^{18}F]MK-1312 and [^{11}C]MMTP have progressed to preclinical evaluation using PET studies involving primates. [^{18}F]MK-1312 demonstrated to have specific binding to mGluR1 in the cerebellum of rhesus monkey by displacement of an mGluR1 allosteric antagonist MK-5435.[21] The uptake of [^{18}F]MK-1312 in the thalamus and striatum was low, and included non-specific signals,[18] although expression of mGluR1 was detected in not only the cerebellum but also the hippocampus, and most of the thalamic nuclei.[22,23] [^{11}C]MMTP showed specific binding to mGluR1 in the cerebellum, hippocampus, frontal cortex, and striatum in the in vitro autoradiography using human brain sections. However, in PET studies with baboons, sufficient uptake of [^{11}C]MMTP was detected in the cerebellum only.[19]

4-Fluoro-*N*-[4-[6-(isopropylamino)pyrimidin-4-yl]-1,3-thiazol-2-yl]-*N*-methylbenzamide (FITM, **4**) was developed as a novel allosteric antagonist for mGluR1.[24] This compound was shown to have potent antagonistic activity against human mGluR1 with an IC_{50} value of 5.1 nM. Excellent selectivity over other subtypes was

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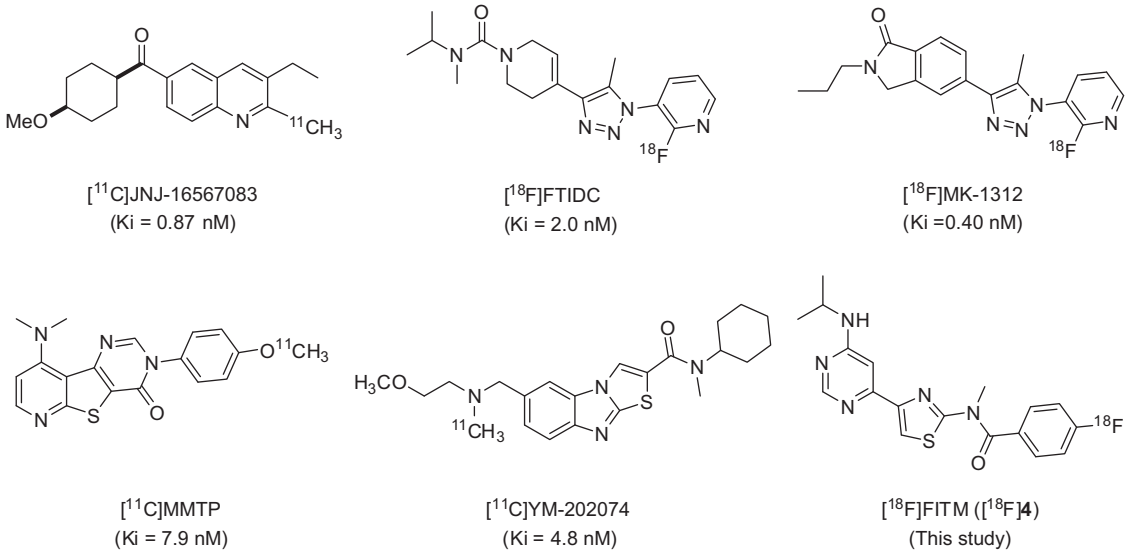
exhibited: IC_{50} values were 7000 nM (for human mGluR5), >10,000 nM (mGluR2), and >10,000 nM (mGluR8).[24]

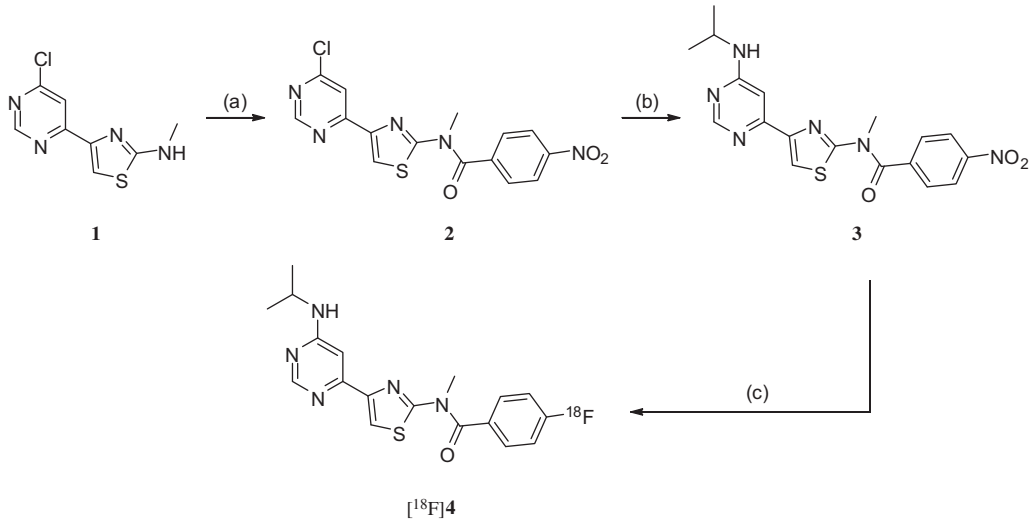
In this Letter, we developed ^{18}F-labeled FITM (**4**) ([^{18}F]FITM, [^{18}F]**4**; Scheme 1) as a new PET ligand and performed preliminary evaluation using in vitro and ex vivo autoradiography on rat brain sections.

For the radiosynthesis, the novel nitro precursor **3** was prepared according to reaction sequences delineated in Scheme 2.[25] Reaction of 4-pyrimidinyl-2-methylaminothiazole **1** with 4-nitrobenzoyl chloride afforded the benzoylated compound **2**, which was substituted with isopropylamine to give **3**. The authentic product **4** was prepared according to procedures reported previously.[24]

Next, we examined the conditions for [^{18}F]fluorination of **3** using a home-made automated synthesis system.[26] For the [^{18}F]fluorination of **3**, the reaction proceeded via a S_NAr substitution mechanism. In our previous report, 6-[1-(2-[^{18}F]fluoro-3-pyridyl)-5-methyl-1*H*-1,2,3-triazol-4-yl]quinoline was synthesized by heating the corresponding bromo precursor with [^{18}F]F^- in the presence of K_2CO_3/Kryptofix 222 at 150 °C for 10 min, resulted in a radiochemical yield of 69 ± 13% (n = 8).[26] However, the reaction of [^{18}F]F^-, which was eluted by K_2CO_3 (66 mM) and Kryptofix 222 according to a routine procedure,[26] with **3** at 150 °C for 10 min gave [^{18}F]**4** only with a low yield (<1%). Although raising the reaction temperature from 150 to 180 °C slightly improved the reaction efficiency, decomposition of **3** took place in the presence of a large amount of K_2CO_3. Prolonging the reaction time from 10 to 30 min further accelerated the decomposition. To avoid decomposition of **3**, the amount of K_2CO_3 was reduced from 66 to 10 mM and [^{18}F]F^- was dried to remove water completely. Under the optimized reaction condition, [^{18}F]fluorination of **3** proceeded efficiently at 180 °C for 10 min. Purification of the reaction mixtures using a semi-preparative HPLC system (Fluofix 120 N C_{18} column: 10 mm ID × 250 mm, CH_3OH/50 mM $CH_3CO_2NH_4$ = 5:5, 5.0 mL/min) gave [^{18}F]**4** in 14 ± 3% radiochemical yield (n = 8 based on [^{18}F]F^-, corrected for decay). [^{18}F]**4** could be separated from **3** by prolonging their retention times (**3**:



Scheme 1. Chemical structures of PET ligands for mGluR1.



Scheme 2. Chemical synthesis and radiosynthesis. Reagents and conditions: (a) *p*-nitrobenzoyl chloride, Et_3N, toluene, 100 °C, 2 h, 65%; (b) isopropylamine, K_2CO_3, 1,4-dioxane, 80 °C, 8 h, 49%; (c) [^{18}F]KF, Kryptofix 222, DMSO, 180 °C, 10 min, 18% (decay-corrected) based on total [^{18}F]F^-.

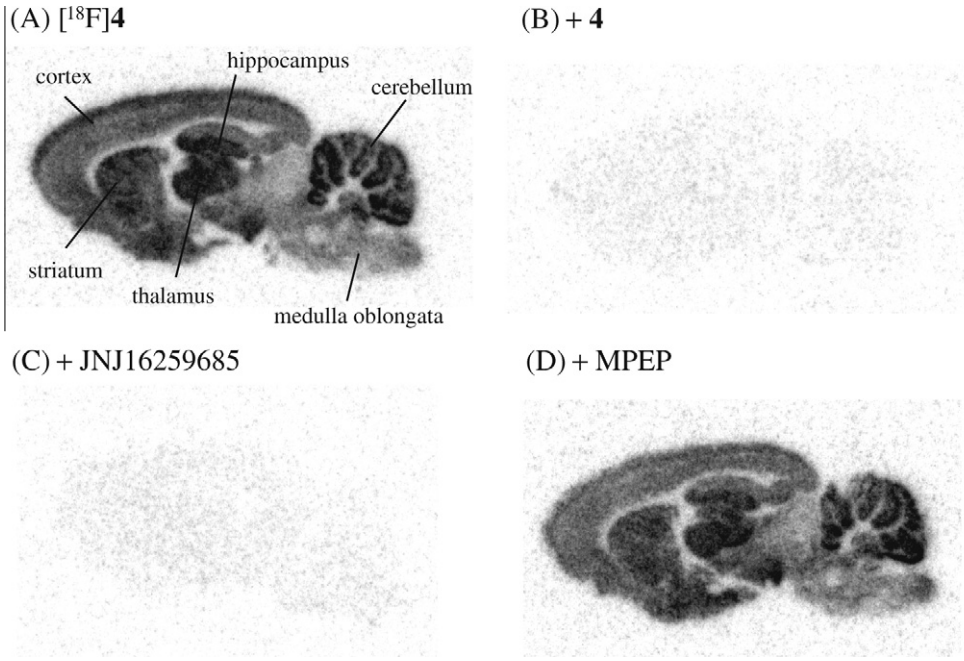


Figure 1. Representative in vitro autoradiographic images of the rat brain with [^{18}F]**4** in the absence (A) or presence of 1 μM **4** (B), 1 μM JNJ16259865 (C), or 1 μM MPEP (D).

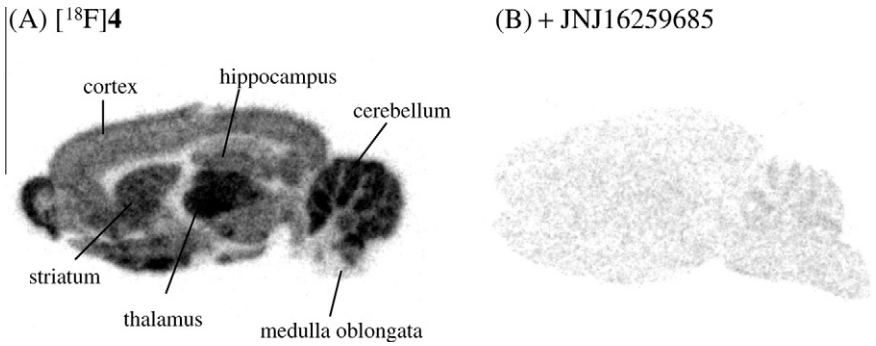


Figure 2. Representative ex vivo autoradiographic images of the rat brain with [^{18}F]**4** in the absence (A) or presence (B) of co-injection with 1 mg/kg JNJ16259865.

14.2 min; [^{18}F]**4**: 18.5 min) during the HPLC purification. Starting with 6.7–13.0 GBq of [^{18}F]F^-, [^{18}F]**4** was reliably obtained as an injectable solution with 429–936 MBq (n = 8) at the end of synthesis (EOS). The identity of [^{18}F]**4** was confirmed by co-injection of non-radioactive **4** on analytic HPLC (CAPCELL PAK C_{18} column: 4.6 mm ID × 250 mm, $CH_3CN/H_2O/Et_3N$ = 6:4:0.1%, 1.0 mL/min, 6.6 min). In the final product solution, no significant peak of **3** was observed in its HPLC chart (see Fig. 1 in Supplementary data). The radiochemical purity of [^{18}F]**4** was higher than 99% and the specific activity was 204–559 GBq/μmol. The radiochemical purity remained >95% after being maintained at 25 °C for 180 min. The radioactivity yield, radiochemical purity and stability, and specific activity of [^{18}F]**4** were sufficient for animal experiments.

The distribution coefficient (Log D) of [^{18}F]**4** was determined by the water/octanol system at pH 7.4 using the shaking method.[27] The Log D value was measured to be 1.46 ± 0.01 (n = 3). Although this value is below the range of suitable values of lipophilicity (Log D = 2–3) as a PET ligand, this lipophilicity may prevent high non-specific binding with protein in the plasma, giving a high penetration into brain.

Subsequently, we performed preliminary evaluation of [^{18}F]**4** by means of in vitro and ex vivo autoradiography using rat brain sections.[28,29]

To confirm the specificity and selectivity of [^{18}F]**4** against mGluR1, we used JNJ16259685[13] (mGluR1 antagonist) and MPEP[30] (mGluR5 antagonist). Figure 1 shows representative images of in vitro autoradiography using [^{18}F]**4** co-incubated with vehicle (A), 1 μM **4** (B), 1 μM JNJ16259685 (C), or 1 μM MPEP (D) (also see Table 1 in Supplementary data). In the section treated [^{18}F]**4** with vehicle, strong signals of radioactivity were found in the cerebellum and thalamus, followed by the hippocampus, striatum, and cerebral cortex, which are known mGluR1-rich regions[31] (Fig. 1A). By co-incubation with **4** or JNJ16259685, radioactivity through the brain section decreased significantly (Fig. 1B and C) compared with those in the control section (Fig. 1A). On the other hand, the signals did not change upon co-incubation with MPEP. The distribution pattern and concentration of radioactivity were similar to those of the control section (Fig. 1D). These results indicated excellent properties in that [^{18}F]**4** had specific and selective binding to mGluR1 on the rat brain section in the in vitro conditions.

Figure 2 shows representative images of ex vivo autoradiography using the brain sections of rat co-injected with [^{18}F]**4** only (A)/plus 1 mg/kg JNJ16259685 (B) (also see Table 2 in Supplementary data). In the control treated with [^{18}F]**4** only, radioactivity was observed at a high level in the cerebellum and thalamus. A modest

radioactive signal was also observed in the hippocampus, striatum, and cerebral cortex (Fig. 2A). This distribution pattern was similar to that in the in vitro conditions. Moreover, co-injection with JNJ16259685 decreased the uptake of radioactivity significantly in the brain section, indicating that [^{18}F]**4** was bound to mGluR1 specifically in the in vivo conditions (Fig. 2B). The signals of radioactivity in the brain sections of rat co-injected with JNJ16259685 decreased by 86–91% in each mGluR1-rich region compared with those of the control.

In summary, we succeeded in the development of [^{18}F]**4** as a new PET ligand for mGluR1, and praliminary demonstrated the specific binding for mGluR1 in the in vitro and ex vivo autoradiography using rat brain sections. Thus, [^{18}F]**4** is a promising PET ligand to evaluate pathophysiological and biological processes mediated by mGluR1. In further experiments, the determination of specific uptake for mGluR1 in PET study using rodents and primates will be performed to confirm the usefulness of [^{18}F]**4**.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.046.

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25. Synthesis of nitro precursor **3**: 4-nitro-*N*-[4-(6-(isopropylamino)pyrimidin-4-yl)-1,3-thiazol-2-yl]-*N*-methylbenzamide: To a stirred solution of **2** (188 mg, 0.5 mmol) and K_2CO_3 (207 mg, 1.5 mmol) in dioxane (5 mL) was added excess isopropylamine (1 mL) at room temperature. The reaction mixture was stirred at 80 °C for 8 h until the full conversion of **2** (TLC monitoring). The reaction mixture was quenched with water and extracted with AcOEt. The organic layer was washed with water and saturated NaCl, and dried over Na_2SO_4. The solvent was removed to give a residue. Column chromatography of the residue on silica gel under hexane/AcOEt (1:1) with Et_3N (1%) gave the title compound (98 mg, 49%) as a white powder, mp: 208–210 °C. The chemical purity of **3** was higher than 98% (Fluofix 120 N C18 column: 4.6 mm ID × 250 mm, CH_3OH/50 m$MCH_3CO_2NH_4$ = 6:4, 0.8 mL/min, 8.5 min).
^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.29 (d, J = 6.6 Hz, 6H), 3.72 (s, 3H), 4.10 (br, 1H), 4.83 (br, 1H), 7.02 (s, 1H), 7.76 (d, J = 8.8 Hz, 2H), 7.97 (s, 1H), 8.39 (d, J = 8.4 Hz, 2H), 8.57 (s, 1H); GCMS (EI), m/z: 399.
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27. Distribution coefficient values were measured by mixing [^{18}F]**4** with *n*-octanol (3.0 g) and sodium phosphate buffer (PBS, 3.0 g; 0.1 M, pH 7.4) in a test tube. The tube was vortexed for 3 min at room temperature, followed by centrifugation at 3500 rpm for 5 min. An aliquot of 1 mL PBS and 1 mL *n*-octanol was removed, weighed, and counted. Samples from the remaining organic layer were removed and re-partitioned until consistent Log *D* values were obtained. The Log *D* value was calculated by comparing the ratio of the count per minute (cpm)/g of *n*-octanol to that of PBS and expressed as Log *D* = Log[cpm/g (*n*-octanol)/cpm/g(PBS)]. All assays were performed in triplicate.
28. Animal experiments were performed according to the recommendations of the Committee for the Care and Use of Laboratory Animals, National Institute of Radiological Sciences (Chiba, Japan).
29. In vitro autoradiography: Rat brain sections were pre-incubated (3 × 5 min) in Tris–HCl (50 mM, pH 7.4, containing 1.2 mM $MgCl_2$ and 2 mM $CaCl_2$) at room temperature. After pre-incubation, these sections were incubated for 30 min at room temperature in fresh buffer with [^{18}F]**4** (1 MBq/mL in saline with 5% ethanol and Tween80) in the presence or absence of 1 μM **4**, 1 μM JNJ16259685 (ENZO, USA), or 1 μM MPEP (Sigma, Japan). After incubation, the sections were washed (3 × 5 min) in cold buffer, dipped in cold distilled water, and dried with air. These sections were exposed to imaging plate (BAS-MS2025; Fuji Film, Japan). Autoradiograms were obtained using a bio-imaging analyzer (BAS 5000; Fuji Film, Japan).
Ex vivo autoradiography: Male Sprague–Dawley rats were injected with saline including approximately 5 MBq [^{18}F]**4** in the presence or absence of 1 mg/kg JNJ16259685 (ENZO, USA) through the tail vein. At 30 min after the injection of radioactivity, rats were sacrificed by cervical dislocation, and their brains were removed quickly and frozen on powder dry ice. Brain sagittal sections (20 μm) were cut on a cryostat microtone (HM560; Carl Zeiss, Germany) and thaw-mounted on glass slides. Autoradiographic images were obtained by the method described above.
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