Letter

A Positron Emission Tomography Radioligand for the in Vivo Labeling of Metabotropic Glutamate 1 Receptor: (3-Ethyl-2-[C]methyl-6-quinolinyl)(*cis*- 4-methoxycyclohexyl)methanone

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A Positron Emission Tomography Radioligand for the in Vivo Labeling of Metabotropic Glutamate 1 Receptor: (3-Ethyl-2-[^{11}C]methyl-6-quinolinyl)(*cis*-4-methoxycyclohexyl)methanone

Yiyun Huang,*[,†,‡] Raj Narendran,[†] François Bischoff,[§] Ningning Guo,[†] Zhihong Zhu,[†] Sung-A Bae,[†] Anne S. Lesage,[#] and Marc Laruelle[†,‡]

Departments of Psychiatry and Radiology, Columbia University College of Physicians and Surgeons, New York, New York 10032, and Department of Medicinal Chemistry and CNS Discovery Research, Johnson & Johnson Pharmaceutical Research and Development, A Division of Janssen Pharmaceutica, N.V., Beerse, Belgium

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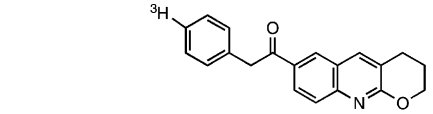
Abstract: A selective metabotropic glutamate 1 receptor (mGlu1) antagonist was labeled with the positron-emitting radioisotope carbon-11 and evaluated in ex vivo biodistribution studies and micro-positron emission tomography (micro-PET) imaging experiments in rats. Results from animal experiments demonstrate that the radioligand [^{11}C]**2** is the first PET tracer capable of labeling the rat mGlu1 receptor in vivo.

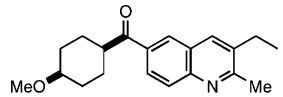
The metabotropic glutamate (mGlu) receptors are G-protein-coupled receptors in the central nervous system that regulate cell excitability and synaptic transmission.[1] They have been classified into three major groups with eight subtypes (group I, mGlu1 and 5; group II, mGlu2 and 3; group III, mGlu4, 6, 7, and 8) based on their sequence homology, signal transduction mechanism, and pharmacology.[2] The group I metabotropic receptors, which encompass the mGlu1 and 5 subtypes, are mainly postsynaptic receptors and have been implicated in disorders such as ischemia, epilepsy, neuropathic pain, anxiety, and schizophrenia.[3,4] As a result, the group I metabotropic glutamate receptors have been the targets of intensive drug development effort.[5]

Over the past few years understanding of the mGlu5 receptor and investigation of its involvement in neurological and psychiatric conditions have been greatly advanced by the discovery of 2-methyl-6-(phenylethynyl)-pyridine (MPEP) as a selective mGlu5 antagonist.[6,7] And the recent emergence of selective radioligands such as [^{3}H]M-MPEP, [^{3}H]methoxymethyl-MTEP (MTEP = 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine), and [^{3}H]methoxy-PEPy (PEPy = 5-(pyridin-2-ylethynyl)-pyridine) should further aid in the elucidation of mGlu5 functions.[8−10] On the other hand, a detailed study of the mGlu1 receptor has heretofore been hindered by the lack of high affinity, selective ligands, and radioligands for this receptor subtype. Lavreysen et al. recently reported the characterization of radioligand **1**, [^{3}H]1-(3,4-dihydro-2*H*-pyrano[2.3-*b*]quinolin-7-yl)-2-phenylethanone ([^{3}H]R214127, Chart 1), as a selective antagonist radioligand for mGlu1 receptor and demonstrated that it can be used to label the mGlu1 receptor in vitro and ex vivo,[11,12] while Mabire et al. disclosed a large series of substituted quinolines, including **2** and **3**, as selective mGlu1 antagonists.[13,14] Still lacking are radioligands suitable for the in vivo labeling of mGlu1 receptor using the noninvasive imaging technology positron emission tomopraphy (PET). In this paper we report on the synthesis and characterization of a ^{11}C-labeled mGlu1 antagonist, (3-ethyl-2-[^{11}C]methyl-6-quinolinyl)(*cis*-4-methoxycyclohexyl)methanone ([^{11}C]**2**, or [^{11}C]JNJ-16567083), as the first selective PET radioligand for in vivo labeling of the mGlu1 receptor.

Chart 1. Metabotropic Glutamate 1 (mGlu1) Antagonists



1, [^{3}H]R214127



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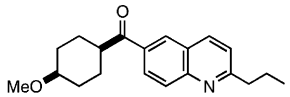

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The synthesis of compound **2** and its radiolabeled form [^{11}C]**2** is presented in Scheme 1.[14] *N*-(4-Bromophenyl)butanamide (**4**) was reacted with DMF and $POCl_3$ to produce 6-bromo-2-chloro-3-ethylquinoline (**5**) in 84% yield. Lithiodebromination of compound **5** with *n*-BuLi and subsequent reaction of the resulting aromatic anion with the Weinreb amide *N*,4-dimethoxy-*N*-methyl-*cis*/*trans*-cyclohexanecarboxamide (**6**) afforded a 2:1 mixture of *cis*/*trans* ketones, which were separated by column chromatography on silica gel to provide **7** in 29% yield. Stille coupling of **7** with tetramethyltin afforded **2** in 56% yield. Similarly, reaction of **7** with hexamethylditin under Pd-catalyzed conditions gave the ^{11}C-labeling precursor **8** in 41% yield. Incorporation of a [^{11}C]methyl group to form the labeled compound [^{11}C]**2** was accomplished by reaction of the trimethyltin precursor **8** with [^{11}C]methyl iodide under the catalysis of $Pd_2(dba)_3$ and $(o\text{-tolyl})_3P$.[15] The crude product was purified by semipreparative HPLC (Phenomenex Prodigy ODS-prep C18 column, 10 μm, 10 mm × 250 mm; mobile phase, 50:50 mixture of MeCN and 0.1 M ammonium acetate; flow rate of 8 mL/min). Compound [^{11}C]**2**, eluting at 12−13 min from the HPLC column, was obtained with a decay-corrected radiochemical yield of 47 ± 17% (n = 10, based on [^{11}C]MeI) and specific activity of 607 ± 228 Ci/mmol at end of synthesis (EOS,

* To whom correspondence should be addressed. Address: Department of Psychiatry, Columbia University College of Physicians and Surgeons, 1051 Riverside Drive, Box #31, New York, NY 10032. Telephone: 212-543-6629. Fax: 212-568-6171. E-mail: hh285@columbia.edu.

† Department of Psychiatry, Columbia University.

‡ Department of Radiology, Columbia University.

§ Department of Medicinal Chemistry, Johnson & Johnson Pharmaceutical Research and Development, A Division of Janssen Pharmaceutica, N.V.

CNS Discovery Research, Johnson & Johnson Pharmaceutical Research and Development, A Division of Janssen Pharmaceutica, N.V.

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Scheme 1

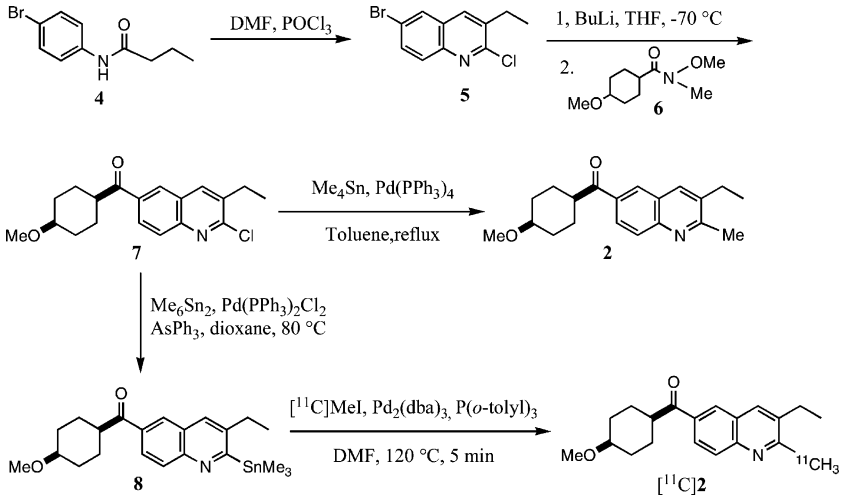

Table 1. Uptake of the Radioligand [^{11}C]**2** in Male Sprague-Dawley Rats[a]

time (min)	blood	medulla	frontal cortex	cerebellum	striatum	hippocampus
10	0.42 ± 0.02	0.47 ± 0.00	0.52 ± 0.06	1.24 ± 0.06	0.73 ± 0.04	0.58 ± 0.05
30	0.26 ± 0.08	0.13 ± 0.03	0.13 ± 0.00	0.61 ± 0.03	0.17 ± 0.01	0.17 ± 0.02
60	0.20 ± 0.06	0.07 ± 0.01	0.07 ± 0.00	0.27 ± 0.06	0.10 ± 0.01	0.10 ± 0.01

[a] Values are expressed as %ID/g. Data are the mean ± SD (n = 3 animals in each group).

n = 10). Total synthesis time was about 40 min. Radiochemical purity of the final product was >99%. Identity of the labeled compound was confirmed by co-injection of the product [^{11}C]**2** with the cold compound **2** and observation of a single UV peak by an analytical HPLC method (Phenomenex Prodigy ODS-3 C18 column, 5 μm, 5 mm × 250 mm; mobile phase, 60:40 mixture of MeCN and 0.1 M ammonium acetate; flow rate of 2 mL/min). The retention time for **2** was 7.8 min.

The affinities of **2** for the mGlu1 and mGlu5 receptors were determined in radioligand binding experiments in vitro using cloned rat mGlu1 and mGlu5 receptors stably expressed on Chinese hamster ovary cells, according to the published procedures.[11] Compound **2** was found to possess high affinity for the rat mGlu1 receptor (K_i = 0.87 ± 0.43) and low affinity for the mGlu5 receptor (K_i = 2366 ± 628 nM).

Ex vivo binding of the radioligand [^{11}C]**2** was evaluated in animal experiments. Male Sprague-Dawley rats were injected with the radioligand and sacrificed at different time points. The brain parts were dissected, and radioactivity was counted to determine the uptake of the radioligand in each of the dissected brain regions. The results from this ex vivo biodistribution study are listed in Table 1. Initial uptake of [^{11}C]**2** in the brain was high, with the percent injected dose per gram of brain tissue (%ID/g) in various brain regions ranging from 0.47% in the medulla to 1.24% in the cerebellum at 10 min after radioligand injection, thus indicating an excellent entry of the radioligand into the brain. Over time, radioactivity was highly concentrated in the cerebellum, where the level of mGlu1 receptor is high.[12,16] The ratio of radioactivity in the cerebellum to that in the medulla, which can be used as a measure of specific binding, was 2.63 ± 0.11, 4.72 ± 0.77, and 4.06 ± 0.52 at 10, 30, and 60 min, respectively, after intravenous injection of the radioligand [^{11}C]**2** (Figure 1, top). There is some indication of specific binding in other brain regions as well (ratio greater than 1), including the striatum and hippocampus. In a second set of experiment, the binding specificity and selectivity of the radioligand were evaluated by pretreating the animals with different pharmacological agents. When the rats were treated with the cold compound **2** (2 mg/kg, iv) 10 min before the injection of radioligand [^{11}C]**2** and sacrificed 30 min after, specific binding of the radioligand in the cerebellum was reduced by 81% when compared with the control group (Figure 1, bottom). Similarly, when the rats were treated with compound **3**, another selective mGlu1 antagonist,[14] at a dose of 2 mg/kg, binding of the radioligand [^{11}C]**2** in the cerebellum was also reduced by 81%. On the other hand, when the animals were treated with the selective mGlu5 antagonist MPEP, there was no significant change in the specific binding of [^{11}C]**2** (Figure 1, bottom). Taken together, results from ex vivo studies in rats indicate that the radioligand [^{11}C]**2** has excellent brain uptake and its binding in the brain is saturable, specific, and selective to the mGlu1 receptor.

To further evaluate the capability of the radioligand's binding to the mGlu1 receptor in vivo, we conducted micro-PET imaging experiments in a living rat. The rat was first injected with high specific activity [^{11}C]**2**, scanned for 90 min, then injected with a low specific activity dose of [^{11}C]**2**, and scanned again in a second 90 min session. In the high specific activity scan (injected dose of 118 μCi; injected mass of 0.15 μg), radioactivity entered the brain rapidly and over time was localized in brain regions with high densities of mGlu1 receptor, such as the cerebellum and striatum (Figure 2, top). Activity in the cerebellum peaked at ~10 min after the injection of [^{11}C]**2** and decreased thereafter (Figure 2, top). Radioactivity uptake was the highest in the cerebellum, followed by striatum and hippocampus, with the lowest uptake in the cortex

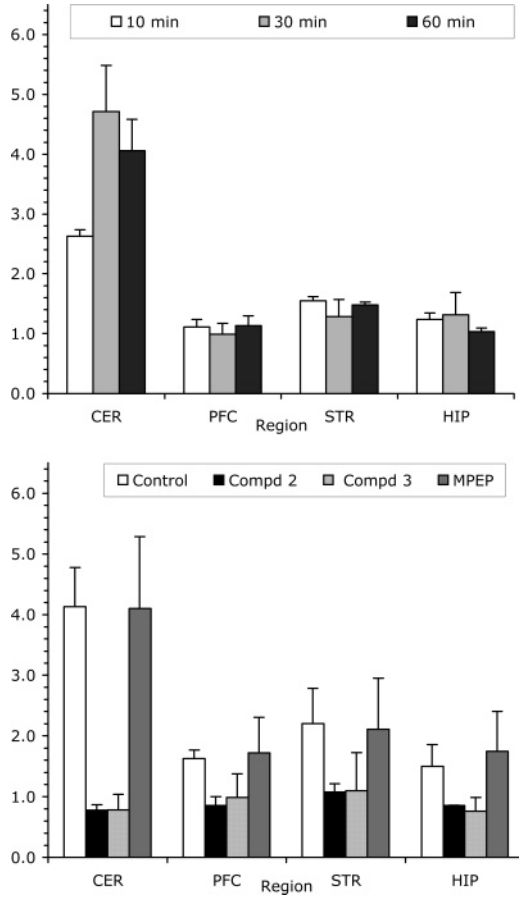

Figure 1. Regional specific binding of the radioligand [^{11}C]**2** in the rat brain in the time-course experiment (top) and in the pretreatment experiment (bottom). In the time-course experiment animals were sacrificed at 10, 30, and 60 min after the adminstration of [^{11}C]**2**. In the pretreatment experiment animals were treated with compound **2**, the mGlu1 antagonist **3**, or the mGlu5 antagonist MPEP (2 mg/kg each, iv) 10 min before the administration of [^{11}C]**2** and sacrificed 30 min after. Brain regions examined are cerebellum (CER), prefrontal cortex (PFC), striatum (STR), and hippocampus (HIP).

(Figure 2, top). In the low specific activity scan (injected dose of 106 μCi; injected mass of 110 μg), distribution of radioactivity in the rat brain was homogeneous (Figure 2, bottom), indicating the displacement of specific uptake in mGlu1 receptor-rich regions by the cold compound **2**, thus demonstrating the binding specificity of the radioligand [^{11}C]**2** to the mGlu1 receptor in vivo.

In conclusion, an mGlu1 antagonist radioligand, [^{11}C]**2**, was successfully prepared. Evaluation of this radioligand for its potential to label mGlu1 receptor was carried out in ex vivo biodistribution studies and in vivo micro-PET imaging experiments in rats. Ligand [^{11}C]**2** displays highly selective and specific binding to the mGlu1 receptor in the cerebellum and, to a lesser degree, in the striatum and hippocampus. Micro-PET imaging experiments confirm the specific binding of [^{11}C]**2** in vivo in a living rat. Taken together, these results demonstrate that the radioligand [^{11}C]**2** represents the first selective PET tracer capable of labeling the mGlu1 receptor in vivo in rat. The emergence of such an in vivo imaging agent should open new avenues for the investigation of the mGlu1 receptor.

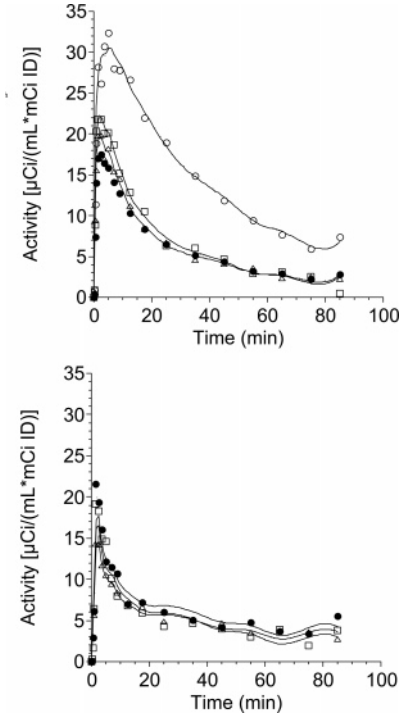

Figure 2. Regional time−activity curves of the radioligand [^{11}C]**2** in a rat brain. The top panel represents time−activity curves of the radioligand [^{11}C]**2** in the high specific activity (HSA) scan. The bottom panel represents time−activity curves in the low specific activity (LSA) scan. Points are activities measured in the cerebellum (open circles), striatum (open squares), hippocampus (open triangles), and cortex (closed circles). Activity data were normalized to the injected doses to allow for comparison on the same scale. Analysis by simplified reference tissue model (SRTM) was then performed on the normalized data. Lines represented the data analyzed by SRTM in both scans. In the HSA scan differential uptake among brain regions is detected, with the highest uptake in the cerebellum. In the LSA scan activity distribution is homogeneous across brain regions.

Supporting Information Available: Reaction procedures and elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

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