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Radiosynthesis and evaluation of 5-methyl-*N*-(4-[^{11}C]methylpyrimidin-2-yl)-4-(1*H*-pyrazol-4-yl)thiazol-2-amine ([^{11}C]ADX88178) as a novel radioligand for imaging of metabotropic glutamate receptor subtype 4 (mGluR4)

Masayuki Fujinaga [a,†], Tomoteru Yamasaki [a,†], Nobuki Nengaki [a,b], Masanao Ogawa [a,b], Katsushi Kumata [a], Yoko Shimoda [a], Joji Yui [a], Lin Xie [a], Yiding Zhang [a], Kazunori Kawamura [a], Ming-Rong Zhang [a,*]

[a] Molecular Probe Program, Molecular Imaging Center, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan
[b] SHI Accelerator Service Co. Ltd, 1-17-6 Osaki, Shinagawa-ku, Tokyo 141-0032, Japan

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ABSTRACT

ADX88178 (**1**) has been recently developed as a potent positive allosteric modulator for metabotropic glutamate receptor 4 (mGluR4). The aim of this study was to develop [^{11}C]**1** as a novel positron emission tomography ligand and to evaluate its binding ability for mGluR4. Using stannyl precursor **3**, [^{11}C]**1** was efficiently synthesized by introducing an [^{11}C]methyl group into a pyrimidine ring via C–^{11}C coupling and deprotection reactions, in 16 ± 6% radiochemical yield (n = 10). At the end of synthesis, 0.54–1.10 GBq of [^{11}C]**1** was acquired with >98% radiochemical purity and 90–120 GBq/μmol of specific activity. In vitro autoradiography and ex vivo biodistribution study in rat brains showed specific binding of [^{11}C]**1** in the cerebellum, striatum, thalamus, cerebral cortex, and medulla oblongata, which showed dose-dependent decreases by administration with multi-dose of unlabeled **1**.

Metabotropic glutamate receptors (mGluRs) are G-protein coupled receptors that can activate excitatory neurotransmission via stimulation of secondary messengers in the central nervous system (CNS).[1] MGluRs are classified into three groups including eight subtypes based on sequence homology, intracellular transduction pathways, and pharmacological properties.[2] Of these, mGluR4, mGluR6, mGluR7, and mGluR8 belonging to group III are identified primarily on presynaptic terminals of GABAergic and glutamatergic neurons. These receptors interact with the Gi protein negatively coupled with adenylate cyclase to inhibit the cAMP-dependent signaling pathway, which is responsible for regulation of synaptic transmission via inhibition of voltage-gate calcium flow across the cell membrane within the basal ganglia circuitry of the brain.[3]

Based on their physiological backgrounds, the therapeutic potential of group III mGluRs is rapidly expanding. Of these, mGluR4 has received particular attention because of the potential benefits of mGluR4 activation in several CNS disorders. In particular, several pharmaceuticals for mGluR4 were reported to show neuroprotective activity in models of Parkinson's disease, a degenerative disorder of dopaminergic neurons in the basal ganglia.[4]

The development of radioligands for positron emission tomography (PET) imaging of mGluR4 in brain has become of increasing interest. Kil et al. first developed two PET ligands for mGluR4, such as [^{11}C]ML128 and [^{18}F]KALB001 (Fig. 1).[5] However, the binding of these radioligands did not correspond to the regional distribution of mGluR4 in rat and monkey brains, likely because of low affinity (ML128[5]: EC_{50} = 240 nM; KALB001: EC_{50} = 50 nM) for mGluR4. More recently, 5-methyl-*N*-(4-methylpyrimidin-2-yl)-4-(1*H*-pyrazol-4-yl)thiazol-2-amine (ADX88178, **1**) (Fig. 2) was developed as a potent and selective mGluR4 positive allosteric modulator.[6] Compound **1** potentiated glutamate-mediated activation of human mGluR4 with EC_{50} values of 4 nM without significant effects on other mGluRs (EC_{50} > 30 μM). In addition, compound **1** showed neuroprotection in rodent models of anxiety, obsessive–compulsive disorder, fear, depression, and psychosis.[6]

In present study, we developed [^{11}C]ADX88178 ([^{11}C]**1**, Fig. 2) as a novel PET ligand for mGluR4. Compound **1** contains methyl groups in the pyrimidine and thiazole ring. We labeled the methyl group at the 4-position in pyrimidine to synthesize [^{11}C]**1** by a C–^{11}C coupling reaction with [^{11}C]CH_3I, using stannyl precursor **2** or **3** (Fig. 2). Although introduction of a [^{11}C]methyl group into

* Corresponding author. Tel.: +81 43 382 3079; fax: +81 43 206 3261.
E-mail address: zhang@nirs.go.jp (M.-R. Zhang).
† Equal contributions.

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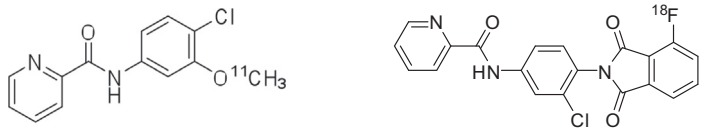
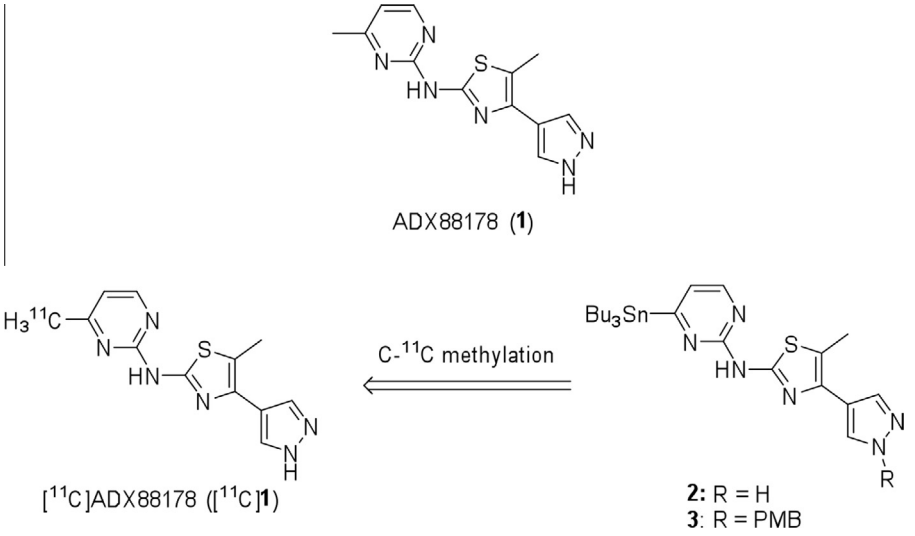


[^{11}C]ML128 [^{18}F]KALB001

Figure 1. Chemical structures of primary two radioligands for mGluR4.



[^{11}C]ADX88178 ([^{11}C]**1**)

Figure 2. Targeted compound and radiolabeling strategy for synthesis of [^{11}C]**1**.

an electron-deficient heteroaromatic ring such as a pyridine or pyrimidine has been reported by Suzuki et al. [7] To our knowledge, no practical PET ligand bearing a [^{11}C]methylated pyrimidine moiety has been reported. Herein, we introduced a [^{11}C]methyl group into pyrimidine ring to synthesize [^{11}C]**1** as a practical PET ligand bearing [^{11}C]methylated pyrimidine moiety for the first time and evaluated the binding ability of [^{11}C]**1** to mGluR4 in brain in vitro and in vivo.

Compound **1**,[8] precursors **2**[9] and **3**[10] were synthesized as shown in Scheme 1. 4-(1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-5-methylthiazol-2-amine (**5**) was prepared as described previously.[11] Reaction of **5** with 2-bromo-4-methyl or (tributylstannyl)pyrimidine derivatives[12] in the presence of $Pd(OAc)_2$ and Xantphos afforded **3** and **4** in moderate yields of 39% and 52%, respectively. Debenzylation of **4** with TFA gave **1** in 38% yield. However, debenzylation of **3** with TFA did not give the desired compound **2** and protodestannylation was mainly observed on the pyrimidine ring in **3**. Other research groups had also reported such protodestannylation of heteroarylstannanes under acidic conditions.[13] Thus, to avoid protodestannylation of **3** in the acid condition, **5** was treated with TFA and then reacted with 2-bromo-4-(tributylstannyl) pyrimidine to successfully give precursor **2**.

Radiosynthesis[14] conditions of [^{11}C]**1** via C–[^{11}C]methylation and results are summarized in Table 1. [^{11}C]CH_3I was obtained by the reduction of cyclotron-produced [^{11}C]CO_2 with $LiAlH_4$, followed by treatment with 48% HI.[15] The resulting [^{11}C]CH_3I was distilled from the reaction mixture and trapped in a DMF solution containing precursor **2** or **3**, $Pd_2(dba)_3$, P(*o*-tol)$_3$, CuCl, and base. When K_2CO_3 was used as a base, only trace [^{11}C]**1** was produced and unreacted [^{11}C]CH_3I was mainly observed (entry 1). Changing the base from K_2CO_3 to CsF did not improve the reaction efficiency for C–^{11}C coupling (entry 2). Based on these data, we considered that the unprotected pyrazole ring in **2** may hinder the C–^{11}C coupling reaction.

Thus, the precursor for synthesis of [^{11}C]**1** was changed from **2** to **3** in which the pyrazole ring was protected with a *p*-methoxybenzyl (PMB) group (entry 3–6). As expected, use of CsF as a base was effective for C–^{11}C coupling, giving [^{11}C]**4** in a high yield. However, the following deletion of the PMB group in [^{11}C]**4** did not proceed by adding TFA to the reaction mixture in DMF (entry 4). When DMF was partly removed after ^{11}C-methylation, the deprotection with TFA produced a mixture of [^{11}C]**1** and [^{11}C]**4** in an approximately 1:1 ratio (entry 5). As shown in entry 6, to accomplish efficient deprotection of PMB in [^{11}C]**4**, DMF was completely removed under reduced pressure after the C–^{11}C coupling reaction, and TFA was then added to the reaction mixture. Under a neat condition, cleavage of the PMB group in [^{11}C]**4** proceeded efficiently at 100 °C for 5 min.

After optimization of conditions for C–^{11}C coupling and deprotection reactions, radiosynthesis of [^{11}C]**1** was achieved according to entry 6 using a home-made automated synthesis system.[16] Reversed-phase HPLC purification of the reaction mixtures gave [^{11}C]**1** in 16 ± 6% (n = 10) radiochemical yield (n = 10, based on [^{11}C]CO_2, corrected for decay). Starting from 22.2 to 24.0 GBq [^{11}C]CO_2, 0.54–1.10 GBq [^{11}C]**1** was produced within 45 min (n = 10) of averaged synthesis time from the end of bombardment. In the final product solution, identity of [^{11}C]**1** was confirmed by co-injection with unlabeled **1** onto analytic HPLC.[14] The radiochemical purity of [^{11}C]**1** was greater than 98% and the specific activity was 90–120 GBq/μmol at the end of synthesis. No significant peaks corresponding to chemical impurities were observed on HPLC charts for the final product solutions. Moreover, [^{11}C]**1** did not show radiolysis at room