Radiosynthesis of a carbon-11-labeled AMPAR allosteric modulator as a new PET radioligand candidate for imaging of Alzheimer's disease

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Abstract—To develop PET tracers for imaging of Alzheimer's disease, a new carbon-11-labeled AMPAR allosteric modulator 4-cyclopropyl-7-(3-[^{11}C]methoxyphenoxy)-3,4-dihydro-2*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide ([^{11}C]**8**) has been synthesized. The reference standard 4-cyclopropyl-7-(3-methoxyphenoxy)-3,4-dihydro-2*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (**8**) and its corresponding desmethylated precursor 4-cyclopropyl-7-(3-hydroxyphenoxy)-3,4-dihydro-2*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (**9**) were synthesized from 4-methoxyabiline and chlorosulfonyl isocyanate in eight and nine steps with 3% and 1% overall chemical yield, respectively. The target tracer [^{11}C]**8** was prepared from the precursor **9** with [^{11}C]CH_3OTf through *O*-[^{11}C]methylation and isolated by HPLC combined with SPE in 10-15% radiochemical yield, based on [^{11}C]CO_2 and decay corrected to end of bombardment (EOB). The radiochemical purity was >99%, and the molar activity (A_M) at EOB was 370-740 GBq/μmol with a total synthesis time of 35-40-minutes from EOB.

Keywords: α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR); Carbon-11-labeled AMPAR allosteric modulator; Radiosynthesis; Positron emission tomography (PET); Alzheimer's disease (AD).

Imaging has played a variety of important roles in the study of Alzheimer's disease (AD).[1] Neuroimaging of AD becomes one of the particularly active as well as most challenging areas in neuroscience.[2] Advanced biomedical imaging technique positron emission tomography (PET) is a promising modality for AD, and significant advances have occurred in this field of molecular imaging.[3] The development of PET imaging probes for *in vivo* detection of Alzheimer's brains is critical for early and accurate diagnosis and for the successful discovery of AD therapies.[4] Previous PET AD imaging agent development is based on cholinergic hypothesis, amyloid hypothesis, and tau hypothesis. The representative PET tracers in clinical evaluations such as carbon-11-labeled AChEIs [^{11}C]AMP ([^{11}C]MP4A), [^{11}C]PMP ([^{11}C]MP4P), and [^{11}C]Donepezil;[5-7] β-amyloid plaques (Aβ) tracers [^{11}C]PIB and [^{18}F]Amyvid ([^{18}F]AV-45);[8,9] and tau tracers [^{11}C]PBB3 and

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[^{18}F]T807 ([^{18}F]AV-1451)[10,11] are listed in Figure 1. The success and limitations of Aβ imaging and tau imaging have spurred efforts worldwide to develop new selective PET tracers for different imaging targets. Our efforts toward the development of PET agents for AD diagnosis have been ongoing quite some time, and a series of enzyme- or receptor-based PET agents has been developed in this laboratory. For example, we have targeted the enzyme glycogen synthase kinase-3 (GSK-3) and developed carbon-11-labeled GSK-3 inhibitors[12,13] as PET AD imaging agents (Figure 1).

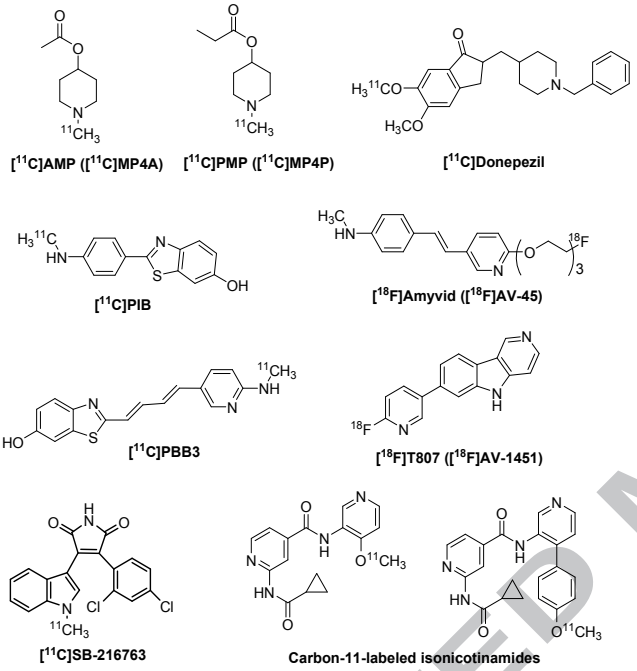


Figure 1. PET radiotracers for imaging of AD.

In this continued effort, we revisit α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), which is a novel and attractive molecular target for treatment and PET imaging of AD.[14,15] We and other groups have developed several AMPAR PET tracers,[15-21] and representative radioligands are shown in Figure 2. However, preclinical and clinical evaluation indicated these radioligands have significant drawbacks like not potent enough *in vitro* IC_{50} and/or K_i values, low specific binding, high non-specific binding, poor brain entry, inconsistent brain uptake compared to known AMPAR distribution, and small dynamic range and metabolite in the brain.[15-21] Thus an ideal AMPAR radioligand that can be used in the clinical setting to study AMPAR expression levels in AD remains to be discovered. Recently a novel series of 7-phenoxy-substituted 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides have been developed as positive allosteric modulators of AMPARs with nanomolar potency for potential treatment of AD, and the lead compound, 4-cyclopropyl-7-(3-methoxyphenoxy)-3,4-dihydro-2*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (**8**), exhibited high potency with EC_{50} = 2.0 nM.[14] This compound has the combination of favorable *in vitro* activity to AMPAR, and *O*- or *N*-methyl positions amenable to labeling with carbon-11, therefore, its carbon-11-labeled radioligand is expected to have high specific binding. Here, we report the design, synthesis and labeling of a carbon-11-labeled AMPAR allosteric modulator, 4-cyclopropyl-7-(3-[^{11}C]methoxyphenoxy)-3,4-dihydro-2*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide ([^{11}C]**8**) (Figure 2), as a new candidate PET radioligand for imaging of AD.

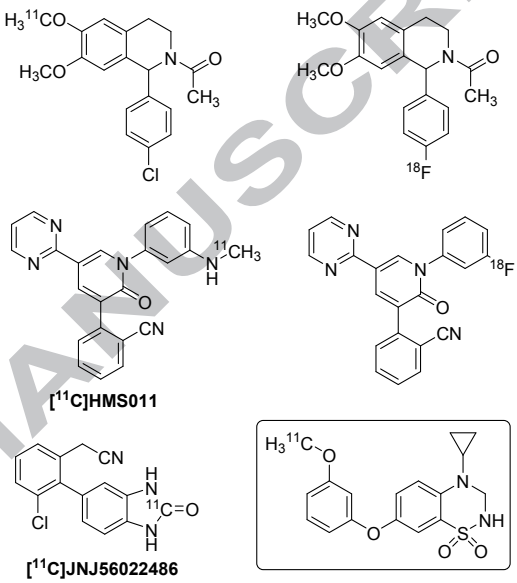
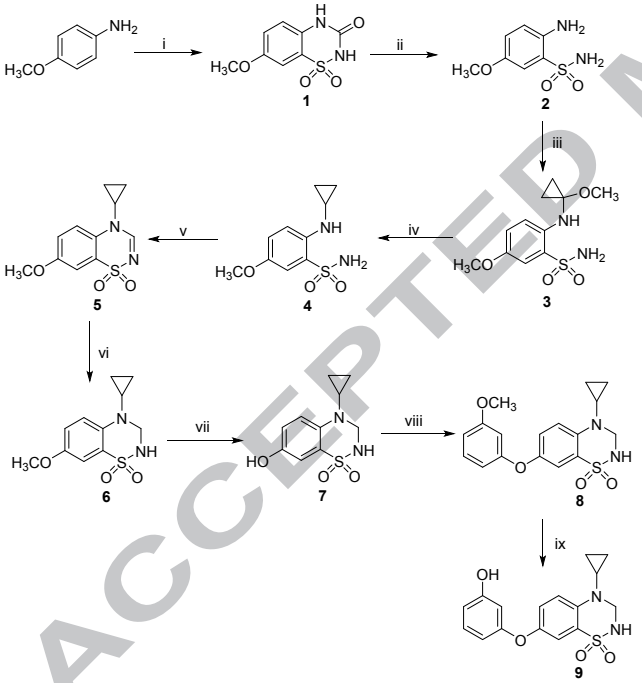


Figure 2. AMPAR PET radioligands.

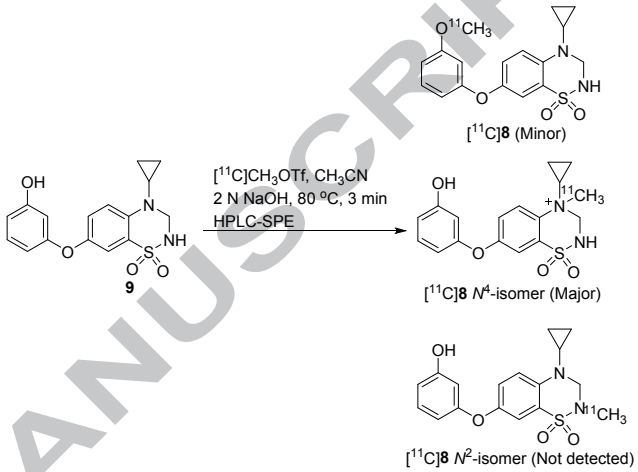
Synthesis of the reference standard **8** and its desmethylated precursor 4-cyclopropyl-7-(3-hydroxyphenoxy)-3,4-dihydro-2*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (**9**) is outlined in Scheme 1 according to the reported procedures.[14, 22-27] The commercially available starting material 4-methoxyaniline was reacted with sulfurisocyanatidic chloride following a modified procedure[22] by using 1-nitropropane as solvent to give 7-methoxy-2*H*-benzo[*e*][1,2,4]thiadiazin-3(4*H*)-one 1,1-dioxide (**1**), which resulted in a better yield (82%) and safer operation. An easy and efficient preparation for hydrolysis of compound **1** in 50% aqueous H_2SO_4 under heating afforded 2-amino-5-methoxybenzenesulfonamide (**2**) in 46% yield. Compound **2** was reacted with 1-ethoxycyclopropyloxy trimethylsilane in methanol through a transacetalization reaction in the presence of glacial acetic acid (AcOH) to generate 5-methoxy-2-((1-methoxycyclopropyl)amino)benzenesulfonamide (**3**) in 61% yield. After the reduction of $NaBH_4$ with boron trifluoride diethyl etherate, methoxyl group of compound **3** was removed and further converted to 2-

(cyclopropylamino)-5-methoxybenzenesulfonamide (**4**) in 63% yield. 4-Cyclopropyl-7-methoxy-4*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (**5**) was formed by a cyclization of compound **4** in triethyl orthoformate at 150 ºC in 75% yield. Reduction of compound **5** with $NaBH_4$ in isopropanol provided 4-cyclopropyl-7-methoxy-3, 4-dihydro-2*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (**6**) in 76% yield. Desmethylation of compound **6** with boron tribromide in dichloromethane gave 4-cyclopropyl-7-hydroxy-3, 4-dihydro-2*H*-benzo[*e*][1,2,4]thiadiazine 1,1-dioxide (**7**) in 73% yield. Compound **7** was reacted with 3-methoxybenzeneboronic acid in the presence of copper (II) acetate in dichloromethane through a Chan-Lam coupling reaction[27] to produce the reference standard compound **8** in 47% yield. The desmethylated precursor **9** was obtained by desmethylation of compound **8** with BBr_3 in dichloromethane in 50% yield. The overall chemical yield for the standard **8** and precursor **9** in eight and nine steps was 3% and 1%, respectively. There was no much room to significantly improve the overall yield in reported multiple step synthetic approach. The potential strategy will be redesign of the synthetic route to decrease the reaction steps in the future work.



Scheme 1. Synthesis of reference standard (**8**) and precursor (**9**). Conditions: (i) (a) $ClSO_2NCO$, 1-nitro-propane, (b) $AlCl_3$, 100 ºC; (ii) H_2SO_4 (50% wt), 130 ºC; (iii) 1-ethoxycyclopropyloxy trimethylsilane, AcOH, CH_3OH, room temperature (RT); (iv) $NaBH_4$, boron trifluoride diethyl etherate, THF, reflux; (v) triethyl orthoformate, 150 ºC; (vi) $NaBH_4$, isopropanol, 50 ºC; (vii) BBr_3, CH_2Cl_2, 0 ºC; (viii) 3-methoxybenzeneboronic acid, copper (II) acetate, molecular sieves, pyridine, CH_2Cl_2, 40 ºC; (ix) BBr_3, CH_2Cl_2, 0 ºC.

Synthesis of the target tracer [^{11}C]**8** is presented in Scheme 2. The desmethylated precursor **9** underwent *O*-[^{11}C]methylation[15] using the reactive [^{11}C]methylating agent [^{11}C]methyl triflate ([^{11}C]CH_3OTf)[28,29] in acetonitrile at 80 °C under basic conditions (2 N NaOH). The product was isolated by semi-preparative reverse-phase (RP) high performance liquid chromatography (HPLC) with a C-18 column, and then concentrated by solid-phase extraction (SPE) with a disposable C-18 Plus Sep-Pak cartridge to produce the corresponding pure radiolabeled compound [^{11}C]**8** in 10-15% radiochemical yield, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2.



Scheme 2. [^{11}C]Methylation reaction of the precursor **9** for the synthesis of target tracer [^{11}C]**8**.

The precursor **9** contains phenol hydroxyl, 4- and 2-thiadiazine nitrogen positions that can be *O*- and *N*-[^{11}C]methylated to form [^{11}C]**8**, [^{11}C]**8** N^4-isomer, and [^{11}C]**8** N^2-isomer, respectively, as indicated in Scheme 2. Based on the Log P and calculated Log P (CLog P) values of [^{11}C]**8** and its isomers obtained from ChemDraw Professional 18.0 (ChemOffice) as listed in Table 1, we can predict their retention time (t_R) sequence in semi-preparative RP-HPLC system would be t_R [^{11}C]**8** N^2-isomer > t_R [^{11}C]**8** > t_R [^{11}C]**8** N^4-isomer. Base effect on the radiosynthesis of [^{11}C]**8** was investigated to maximize the radiochemical yield of desired radiolabeled product. The different bases including solid base sodium hydride (NaH) powder, sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium carbonate (Na_2CO_3), potassium carbonate (K_2CO_3), NaOH-Na_2CO_3 (mixed); and liquid base aqueous NaOH, KOH, Na_2CO_3, K_2CO_3, sodium bicarbonate ($NaHCO_3$) were tested for the radiosynthesis, and 2 N NaOH was identified as the best base. The reaction conditions including the reaction solvent, temperature and time were optimized as well. The optimized conditions (CH_3CN, 2 N NaOH, 80 ºC, 3 min) are listed and the results are summarized in Scheme 2. [^{11}C]**8** N^2-isomer was not detected, [^{11}C]**8** N^4-isomer was a major radiolabeled product, and [^{11}C]**8** was a minor radiolabeled product. These results suggested that 4-thiadiazine nitrogen position of **9** is

more easily methylated than phenol hydroxyl oxygen position to yield undesired radiolabeled by-product. The radiochemical yield of [^{11}C]**8** was optimized to 10-15%. The radiochemical yield of [^{11}C]**8** *N*^{4}-isomer was 45-50%. Likewise, there was no much room to improve the labeling reaction in one-step radiosynthesis of the desired product [^{11}C]**8** using non-protected desmethylated precursor due to its chemistry nature. To make the target compound [^{11}C]**8** as a major product from radiochemistry and synthetic chemistry perspectives, the potential strategy will be redesign of the synthetic route to prepare *N*-protected desmethylated precursor for two-step radiosynthesis of [^{11}C]**8** in the future work.

The radiosynthesis was performed in a home-built automated multi-purpose [^{11}C]-radiosynthesis module.[30-32] Our radiosynthesis module facilitated the overall design of the reaction, purification and reformulation capabilities in a fashion suitable for adaptation to preparation of human doses. The radiosynthesis includes three stages: 1) labeling reaction; 2) purification; and 3) formulation. The overall synthesis time was 35-40 min from EOB. Our module is also designed to allow in-process measurement of [^{11}C]-tracer molar activity (A_M, GBq/μmol at EOB) using a radiation detector with a UV detector at the outlet of the HPLC-portion of the system. At the end of synthesis (EOS), the A_M of [^{11}C]-tracer was determined again by analytical RP-HPLC, calculated, decay corrected to EOB, and based on [^{11}C]CO_2, which was in agreement with the 'on line' determined value. The A_M of [^{11}C]**8** at EOB was 370-740 GBq/μmol.

Chemical purity and radiochemical purity were determined by analytical HPLC.[33] The chemical purity of the precursor and reference standard was >93% determined by RP-HPLC through UV flow detector. The radiochemical purity of the target tracer was >99% determined by radio-HPLC through γ-ray (PIN diode) flow detector.

The octanol-water partition coefficient (commonly expressed as Log P) is an important physical parameter directly correlated with the biological activities of a wide variety of organic compounds.[34] Log P provides an assessment of lipophilicity that often correlates with a compound's ability to penetrate the blood brain barrier (BBB). Table 1 gives Log P and CLog P values of [^{11}C]**8** and its isomers in comparison with [^{11}C]PIB, [^{18}F]Amyvid, [^{11}C]PBB3 and [^{18}F]T807 (Figure 1), which are obtained from ChemDraw Professional 18.0 (ChemOffice). Log P data of [^{11}C]**8** (3.04) is in the range of those of [^{11}C]PIB, [^{18}F]Amyvid, [^{11}C]PBB3 and [^{18}F]T807 (2.25 – 4.09), which are PET AD imaging agents in clinical evaluation. These data suggest [^{11}C]**8** has an appropriate lipophilicity for brain uptake.

Table 1. Log P and CLog P values of [^{11}C]**8** and its isomers in comparison with [^{11}C]PIB, [^{18}F]Amyvid, [^{11}C]PBB3 and [^{18}F]T807 obtained from ChemDraw Professional 18.0 (ChemOffice).

Compound	Log P	CLog P
[^{11}C]8	3.04	3.12
[^{11}C]8 *N*^{4}-isomer	N/A	0.023
[^{11}C]8 *N*^{2}-isomer	3.07	3.27
[^{11}C]PIB	3.41	3.99
[^{18}F]Amyvid	3.16	3.91
[^{11}C]PBB3	4.09	4.05
[^{18}F]T807	2.25	3.18

The stability of the labeled tracer [^{11}C]**8** was evaluated by analytical HPLC from EOS up to 3 h, one injection of the tracer solution in EtOH/saline onto HPLC column per hour. The HPLC chromatograms showed [^{11}C]**8** was stable without decomposition.

The experimental details and characterization data for compounds **1-9** and for the tracer [^{11}C]**8** are given.[35]

In summary, facile synthetic routes with moderate to excellent yields have been developed to produce the precursor **9**, the reference standard **8**, and the target PET radiotracer [^{11}C]**8.** The radiosynthesis employed [^{11}C]CH_3OTf for *O*-[^{11}C]methylation at the phenyl hydroxyl position of the precursor, followed by product purification and isolation by a semi-preparative RP-HPLC combined with SPE. The base effect on the radiotracer production of [^{11}C]**8** has been investigated. The desired [^{11}C]**8** was obtained in reasonable radiochemical yield, and high radiochemical purity, with a reasonably short overall synthesis time, and high molar activity. A new AMPAR radioligand has been successfully radiosynthesized. This will facilitate studies to evaluate the carbon-11-labeled AMPAR allosteric modulator as a new candidate PET radioligand for imaging of AD.

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35. *(a). General:* All commercial reagents and solvents were purchased from Sigma-Aldrich and Fisher Scientific, and used without further purification. [^{11}C]CH_3OTf was prepared according to a literature procedure.[29] Melting points were determined on WRR apparatus and were uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II 400 or 600 MHz NMR Fourier transform spectrometer at 400, 600 or 100 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) relative to an internal standard tetramethylsilane (TMS, δ 0.0) (^{1}H NMR) and to the solvent signal (^{13}C NMR), and coupling constants (*J*) are reported in hertz (Hz). Liquid chromatography-mass spectra (LC-MS) analysis was performed on AB Sciex 4000Q Trap instrument, consisting of an 1100 series HPLC connected to a diode array detector and a 1946D mass spectrometer configured for positive-ion/negative-ion electrospray ionization (ESI). The high resolution mass spectra (HRMS) were obtained using a Waters/Micromass LCT Classic spectrometer. Chromatographic solvent proportions are indicated as volume : volume ratio. Thin-layer chromatography (TLC) was run using HS silica gel GF254 uniplates (5 × 10 cm^2). Plates were visualized under UV light. Normal phase flash column chromatography was carried out on Combiflash Rf 150 silica gel 60 (300-400 mesh) with a forced flow of the indicated solvent system in the proportions described below. All moisture- and air-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source. Analytical RP HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6 × 250 mm, mobile phase 65% CH_3CN/35% 4.0 mM CH_3COONa, flow rate 1.0 mL/min; UV (254 nm) and γ-ray (PIN diode) flow detectors. Semi-preparative RP HPLC column was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 10 × 250 mm; 70% CH_3CN:30% H_2O mobile phase; 6 mL/min flow rate; UV (254 nm) and γ-ray (PIN diode) flow detectors. C18 Plus Sep-Pak cartridges were obtained from Waters Corporation (Milford, MA). Sterile Millex-FG 0.2 μm filter units were obtained from Millipore Corporation (Bedford, MA).

*(b). 7-Methoxy-2H-benzo[e][1,2,4]thiadiazin-3(4H)-one 1,1-dioxide (**1**):* To a stirred solution of chlorosulfonyl isocyanate (1.79g, 12.30 mmol) in 1-nitropropane (10 mL), 4-methoxyaniline (1.23 g, 10.0 mmol) in 1-nitropropane (20.0 mL) was added at 0 ºC during 5 min. The purple chlorosulfonylurea intermediate was formed after the reaction mixture was stirred at 0 ºC for 30 min. Then, the anhydrous aluminum chloride ($AlCl_3$) (1.68 g, 12.7 mmol) was added quickly, a clear solution was formed. The reaction mixture was heated, and stirred at 100 ºC for 1 h, then cooled to room temperature (RT), and poured into stirred ice water. The precipitation was filtered, washed with water, and purified by recrystallization in ethanol to afford **1** as a pale yellow solid (1.87 g, 82%), mp 298.7-301.2 ºC. ^{1}H NMR (400 MHz, DMSO-d6): δ 9.22 (s, 1H), 6.89 (t, J = 2.4 Hz, 1H), 6.95 (d, J = 2.4 Hz, 1H), 6.92 (d, J = 8.8 Hz, 1H), 3.75 (s, 3H). LC-MS (ESI, *m/z*): Calcd for $C_8H_9N_2O_4S$ ($[M+H]^+$) 229.03, found: 229.03.

*(c). 2-Amino-5-methoxybenzenesulfonamide (**2**):* Compound **1** (0.51 g, 2.2 mmol) was added to a stirred solution of H_2SO_4 (15 mL, 50% wt) at RT, the resulting reaction mixture was heated to 130 - 140 ºC, and the reaction was continued for 3 h. The reaction mixture was cooled to 25 ºC, and then poured into ice water (100 mL). The solution was neutralized with NaOH (40%), and extracted with EtOAc (3 × 60 mL). The combined organic layer was washed with water, brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated under vacuum. The crude product was purified by silica gel column chromatography with EtOAc/petroleum ether (PE) (1:10 to 1:4) as eluent to afford **2** as a pale yellow solid (0.21 g, 46%), mp 118.3-119.7 ºC. ^{1}H NMR (600 MHz, DMSO-d6): δ 7.23 (s, 2H), 7.11 (d, J = 3.0 Hz, 1H), 6.93 (dd, J = 9.0, 3.0 Hz, 1H), 6.77 (d, J = 9.0 Hz, 1H), 5.43 (s, 2H), 3.67 (s, 3H). LC-MS (ESI, *m/z*): Calcd for $C_7H_{11}N_2O_3S$ ($[M+H]^+$) 203.05, found: 203.01.

*(d). 5-Methoxy-2-((1-methoxycyclopropyl)amino)benzenesulfonamide (**3**):* To a stirred solution of **2** (1.00 g; 4.9 mmol) in methanol (20 mL), (1-ethoxycyclopropyloxy) trimethylsilane (4 mL) and glacial acetic acid (4 mL) were added at RT. After refluxed for 18 h, the reaction mixture was evaporated to dry under reduced pressure. The resulted oily residue was treated with water (30 mL), and extracted with $CHCl_3$ (3 × 30 mL). The combined organic layer was washed with water, brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under vacuum. The crude product was purified by silica gel column chromatography with EtOAc/PE (1:20 to 1:5) as eluent to afford **3** as a white solid (0.80 g, 61%), mp 147.5-149.4 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.41 (d, J = 9.0 Hz, 1H), 7.34 (d, J = 3.0 Hz, 1H), 7.07 (dd, J = 9.0, 3.0 Hz, 1H), 6.48 (s, 1H), 4.86 (s, 2H), 3.78 (s, 3H), 3.28 (s, 3H), 1.15 (t, J = 5.4 Hz, 2H), 0.90 (t, J = 4.8 Hz, 2H). LC-MS (ESI, *m/z*): Calcd for $C_{11}H_{17}N_2O_4S$ ($[M+H]^+$) 273.09, found: 273.10.

*(e). 2-(Cyclopropylamino)-5-methoxybenzenesulfonamide (**4**):* To a stirred solution of **3** (0.80 g, 2.94 mmol) in THF (50 mL), $NaBH_4$ (2.00 g, 52.9 mmol), and boron trifluoride diethyl etherate (2 mL) were added at RT. The reaction mixture was then refluxed for 16 h. The solvent was evaporated under vacuum. The resulted oily residue was treated with water (30 mL) and the pH was adjusted to 4 with aqueous 6 N HCl solution. The mixture was extracted with $CHCl_3$ (3 × 30 mL), and the combined organic layer was washed with water, brine, dried over anhydrous Na_2SO_4 and filtered. The organic mixture was evaporated under vacuum, and the crude product was purified by silica gel column chromatography with EtOAc/PE (1:10 to 1:4) as eluent to afford **4** as a white solid (0.46 g, 63%), mp 116.5-118.7 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.35 (d, J = 3.0 Hz, 1H), 7.23 (d, J = 9.0 Hz, 1H), 7.07 (dd, J = 9.0, 3.0 Hz, 1H), 5.65 (s, 1H), 4.84 (s, 2H), 3.78 (s, 3H), 2.47 - 2.45 (m, 1H), 0.81 - 0.78 (m, 2H), 0.53 - 0.51 (m, 2H). LC-MS (ESI, *m/z*): Calcd for $C_{10}H_{15}N_2O_3S$ ($[M+H]^+$) 243.08, found: 243.20.

*(f). 4-Cyclopropyl-7-methoxy-4H-benzo[e][1,2,4]thiadiazine 1,1-dioxide (**5**):* A stirred solution of **4** (0.45 g, 1.86 mmol) in triethyl orthoformate (10 mL) was heated to 150 ºC, and the reaction mixture was refluxed for 6 h. After cooled on an ice-bath, the solid product was collected by filtration, and washed with diethyl ether. The product was purified by recrystallization in methanol to afford **5** as a white solid (0.35 g, 75%), mp 229.8-230.2 ºC. ^{1}H NMR (600 MHz, DMSO-d6): δ 8.07 (s, 1H), 7.80 (d, J = 9.0 Hz, 1H), 7.41 (dd, J = 9.6, 3.0 Hz, 1H), 7.31 (d, J = 3.0 Hz, 1H), 3.87 (s, 3H), 3.39 - 3.36 (m, 1H), 1.17 - 1.14 (m, 2H), 1.10 - 0.98 (m, 2H). LC-MS (ESI, *m/z*): Calcd for $C_{11}H_{13}N_2O_3S$ ($[M+H]^+$) 253.06, found: 253.07.

*(g). 4-Cyclopropyl-7-methoxy-3, 4-dihydro-2H-benzo[e][1,2,4]thiadiazine 1,1-dioxide (**6**):* To a stirred solution of **5** (0.28 g, 1.11 mmol) in isopropyl alcohol (7 mL), $NaBH_4$ (0.14 g, 3.71 mmol) was added at RT, and the reaction mixture was heated to 50 ºC for 10 min. The reaction mixture was evaporated to dry under vacuum. The resulted oily residue was treated with water (10 mL) and the pH was adjusted to 4 with aqueous 6 N HCl solution. The resulted solution was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layer was washed with water, brine, dried over anhydrous Na_2SO_4 and filtered. The organic mixture was evaporated under vacuum. The resulted crude product was purified by silica gel column chromatography with EtOAc/PE (1:10 to 1:4) as eluent to afford **6** as a white solid (0.21 g, 76%), mp 160.8-161.2 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.27 (s, 1H), 7.15 (d, J = 3.0 Hz, 1H), 7.02 (dd, J = 9.6, 3.0 Hz, 1H), 4.82 (t, J = 8.4 Hz, 1H), 4.69 (d, J = 8.4 Hz, 2H), 3.78 (s, 3H), 2.36 - 2.35 (m,

1H), 0.94 - 0.91 (m, 2H), 0.70 - 0.68 (m, 2H). LC-MS (ESI, *m/z*): Calcd for $C_{11}H_{15}N_2O_3S$ ($[M+H]^+$) 255.08, found: 255.08.

(h). 4-Cyclopropyl-7-hydroxy-3,4-dihydro-2H-benzo[e][1,2,4]thiadiazine 1,1-dioxide (7): To a stirred solution of **6** (0.2 g, 0.79 mmol) in CH_2Cl_2 (12 mL), BBr_3 (0.3 mL) was added slowly at 0 ºC, and the reaction continued at 0 ºC for 20 h. The reaction mixture was poured into ice water (10 mL), and then the dichloromethane was removed under reduced pressure. The resulted aqueous solution was extracted with EtOAc (3 × 40 mL), and the combined organic layer was washed with water, brine, dried over anhydrous Na_2SO_4 and filtered. The organic solution was evaporated under vacuum, and the resulted crude product was purified by silica gel column chromatography with EtOAc/PE (1:10 to 1:2) as eluent to afford **7** as a white solid (0.14 g, 73%), mp 175.5-177.9 ºC. ^{1}H NMR (600 MHz, DMSO-d6): δ 9.32 (s, 1H), 7.82 (t, J = 7.8 Hz, 1H), 0.19 (d, J = 9.0 Hz, 1H), 6.94 (dd, J = 9.0, 3.0 Hz, 1H), 6.92 (d, J = 3.0 Hz, 1H), 4.55 (d, J = 7.8 Hz, 2H), 2.39 - 2.36 (m, 1H), 0.86 - 0.83 (m, 2H), 0.62 - 0.60 (m, 2H). LC-MS (ESI, *m/z*): Calcd for $C_{10}H_{13}N_2O_3S$ ($[M+H]^+$) 241.06, found: 241.06.

*(i). 4-Cyclopropyl-7-(3-methoxyphenoxy)-3,4-dihydro-2H-benzo[e][1,2,4]thiadiazine 1,1-dioxide (**8**):* To a stirred solution of **7** (0.32 g, 1.3 mmol) in CH_2Cl_2 (40 mL), pyridine (8 drops), molecular sieves (4 g), 3-methoxybenzeneboronic acid (0.30 g, 2.0 mmol), and copper (II) acetate (0.63 g, 2.0 mmol) were added at RT. The reaction mixture was stirred at 40 ºC for 5 h, and then CH_2Cl_2 (15 mL) was added to the reaction mixture. The solid was removed from the reaction mixture by filtration, and the resulted organic solution was evaporated under vacuum. The resulted residue was suspended with aqueous HCl (pH = 5-6) solution (50 mL), and then the resulted aqueous solution was extracted with EtOAc (3 × 50 mL). The combined organic layer was washed with water, brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated under vacuum. The crude product was purified by silica gel column chromatography with EtOAc/PE (1:10 to 1:5) as eluent to afford **8** as a white solid (0.22 g, 47%), mp 151.2-152.7 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.26 (d, J = 2.4 Hz, 1H), 7.32 (d, J = 9.0 Hz, 1H), 7.21 (t, J = 8.4 Hz, 1H), 7.14 (dd, J = 9.0, 2.4 Hz, 1H), 6.65 - 6.64 (m, 1H), 6.54 - 6.52 (m, 2H), 4.76 - 4.70 (m, 3H), 3.78 (s, 3H), 2.42 - 2.38 (m, 1H), 0.98 - 0.95 (m, 2H), 0.73 - 0.70 (m, 2H). LC-MS (ESI, *m/z*): Calcd for $C_{17}H_{19}N_2O_4S$ ($[M+H]^+$) 347.11, found: 347.10.

*(j). 4-Cyclopropyl-7-(3-hydroxyphenoxy)-3,4-dihydro-2H-benzo[e][1,2,4]thiadiazine 1,1-dioxide (**9**):* Compound **9** was prepared by referring to the synthesis procedure of compound **7** as a white solid (0.13 g, 50%), mp 166.4-168.4 ºC. ^{1}H NMR (400 MHz, CD_3OD): δ 7.43 (d, J = 9.2 Hz, 1H), 7.23 - 7.14 (m, 3H), 6.56 (dd, J = 8.4, 2.4 Hz, 1H), 6.45 - 6.41 (m, 2H), 4.75 (s, 2H), 2.52 - 2.45 (m, 1H), 1.01 - 0.97 (m, 2H), 0.79 - 0.76 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD): δ 158.88, 158.84, 148.23, 140.98, 129.94, 124.83, 123.77, 116.48, 114.54, 110.05, 108.44, 104.90, 61.31, 29.48, 7.86 (overlap, 2C). HRMS (ESI, *m/z*): Calcd for $C_{16}H_{17}N_2O_4S$ ($[M+H]^+$) 333.0904, found: 333.0904.

*(k). 4-Cyclopropyl-7-(3-[^{11}C]methoxyphenoxy)-3,4-dihydro-2H-benzo[e][1,2,4]thiadiazine 1,1-dioxide ([^{11}C]**8**):* $[^{11}C]CO_2$ was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction in the small volume (9.5 cm^3) aluminum gas target provided with the Siemens RDS-111 Eclipse cyclotron. The target gas consisted of 1% oxygen in nitrogen purchased as a specialty gas from Praxair, Indianapolis, IN. Typical irradiations used for the development were 58 μA beam current and 20 min on target. The production run produced approximately 37.0 GBq of $[^{11}C]CO_2$ at EOB. The precursor **9** (0.1-0.3 mg) was dissolved in CH_3CN (500 μL). To this solution was added aqueous NaOH (2 N, 2 μL). The mixture was transferred to a small reaction vial. No-carrier-added (high molar activity) $[^{11}C]CH_3OTf$ that was produced by the gas-phase production method[29] within 12 min from $[^{11}C]CO_2$ through $[^{11}C]CH_4$ and $[^{11}C]CH_3Br$ with AgOTf column was passed into the reaction vial at RT until radioactivity reached a maximum (2 min), and then the reaction vial was isolated and heated at 80 °C for 3 min. The contents of the reaction vial were diluted with aqueous $NaHCO_3$ (0.1 M, 1 mL). The reaction vial was connected to a 3-mL HPLC injection loop. The labeled product mixture solution was injected onto the semi-preparative HPLC column for purification. The product fraction was collected in a recovery vial containing 30 mL water. The diluted tracer solution was then passed through a C-18 Plus Sep-Pak cartridge, and washed with water (3 × 10 mL). The cartridge was eluted with EtOH (3 × 0.4 mL) to release the labeled product, followed by saline (10-11 mL). The eluted product was then sterile-filtered through a Millex-FG 0.2 μm membrane into a sterile vial. Total radioactivity was assayed and total volume (10-11 mL) was noted for tracer dose dispensing. The overall synthesis time including HPLC-SPE purification and reformulation was 35-40 min from EOB. The decay corrected radiochemical yield was 10-15%. Retention times in the analytical RP-HPLC system were: t_R **9** = 4.55 min, t_R **8** = 7.02 min, and t_R [^{11}C]**8** = 7.10 min. Retention times in the preparative RP-HPLC system were: t_R **9** = 4.87 min, t_R **8** = 8.10 min, and t_R [^{11}C]**8** = 8.21 min.

Radiosynthesis of a carbon-11-labeled AMPAR allosteric modulator as a new PET radioligand candidate for imaging of Alzheimer's disease

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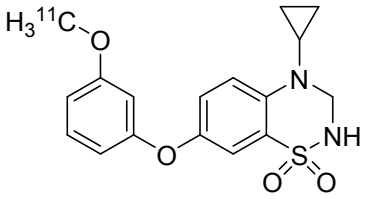

Carbon-11-labeled AMPAR allosteric modulator