Synthesis of carbon-11-labeled CK1 inhibitors as new potential PET radiotracers for imaging of Alzheimer’s disease

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Abstract—The reference standards methyl 3-(2,2-difluoro-5H-[1,3]dioxolo[4',5':4,5]benzo[1,2-d]imidazol-6-yl)carbamoyl)benzoate (5a) and N-(2,2-difluoro-5H-[1,3]dioxolo[4',5':4,5]benzo[1,2-d]imidazol-6-yl)-3-methoxybenzamide (5c), and their corresponding desmethylated precursors 3-(2,2-difluoro-5H-[1,3]dioxolo[4',5':4,5]benzo[1,2-d]imidazol-6-yl)carbamoyl)benzoic acid (6a) and N-(2,2-difluoro-5H-[1,3]dioxolo[4',5':4,5]benzo[1,2-d]imidazol-6-yl)-3-hydroxybenzamide (6b), were synthesized from 5-amino-2,2-difluoro-1,3-benzodioxole and 3-substituted benzoic acids in 5 and 6 steps with 33% and 11%, 30% and 7% overall chemical yield, respectively. Carbon-11-labeled casein kinase 1 (CK1) inhibitors, [11C]methyl 3-(2,2-difluoro-5H-[1,3]dioxolo[4',5':4,5]benzo[1,2-d]imidazol-6-yl)carbamoyl)benzoate ([11C]5a) and N-(2,2-difluoro-5H-[1,3]dioxolo[4',5':4,5]benzo[1,2-d]imidazol-6-yl)-3-[11C]methoxybenzamide ([11C]5c), were prepared from their O-desmethylated precursor 6a or 6b with [11C]CH3OTf through O-[11C]methylation and isolated by HPLC combined with SPE in 40-45% radiochemical yield, based on [11C]CO2 and decay corrected to end of bombardment (EOB). The radiochemical purity was >99%, and the molar activity (MA) at EOB was 370-740 GBq/µmol with a total synthesis time of ~40-minutes from EOB.

Keywords: Casein kinase 1 (CK1); Carbon-11-labeled CK1 inhibitors; Radiosynthesis; Positron emission tomography (PET); Alzheimer’s disease (AD).

Casein kinases (CK) are serine/threonine-specific enzymes and can be divided two subtypes: casein kinase 1 (CK1) and casein kinase 2 (CK2).1 CK1 contains at least seven isoforms (α, β, γ1, γ2, γ3, δ and ε) expressed in eukaryotic organisms, CK1 is involved in various cellular processes including membrane trafficking, circadian rhythm, cell cycle progression, chromosome segregation, apoptosis and cellular differentiation, and deregulation of CK1 activity is linked to several pathological disorders and diseases like cancer, neurodegenerative diseases such as Alzheimer’s disease (AD), Parkinson’s disease (PD) and amyotrophic lateral sclerosis (ALS), and inflammatory disorders.2,3 The overexpression of CK1 has been described in the human AD brain, since CK1 leads to an increase in amyloid-β (Aβ) peptide production, and also participates in the tau fibrillation reaction pathway through phosphorylation of tau.4,5 CK1 has become an interesting therapeutic target for AD where an urgent need for effective treatment exists, because it opens the door for the use of CK1 inhibitors as novel therapeutic approaches for AD.6 CK1 inhibitors can prevent Aβ formation and reverse tau hyperphosphorylation in AD, and have been used to treat neurodegenerative disorders including AD.4,5,7 Recently a new series of difluoro-dioxolo-benzoimidazol-benzamides have been developed as potent CK1 inhibitors with nanomolar inhibitory activity, these compounds exhibited significant inhibitory effects on several tumor cell lines, and their in vitro biological data suggested they can be as therapeutics for AD as well.8

AD is a complicated neurodegenerative disease in the central nervous system (CNS), the cause of AD remains unclear, and so far no any effective treatment strategy is approved for preventing, curing and slowing the progress of AD.9 To discover more effective treatments, more accurate diagnostic tools are crucial to reveal new
therapeutic targets. New noninvasive diagnostic imaging modalities for AD are really needed, both to detect and monitor the evolution of this disease, and to evaluate the efficacy of treatments. Advanced biomedical imaging technique positron emission tomography (PET) is one of the most widespread imaging techniques for AD, and significant progress has been made to develop PET agents for two key neuropathological substrates of AD: Aβ plaques and tau neurofibrillary tangles (NFTs). The representative PET Aβ and tau tracers [11C]PIB and [18F]Amyvid ([18F]AV-45), [11C]PBB3 and [18F]T807 ([(18F)AV-1451] are listed in Figure 1. 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. The success and limitations of Aβ imaging and tau imaging have spurred efforts worldwide to develop new selective PET tracers for different imaging targets. CK1 has emerged as a new molecular imaging target of AD, but so far no any carbon-11 or fluorine-18 labeled CK1 inhibitors as PET radiotracers for imaging of CK1 were reported. We are interested in the development of new PET AD imaging agents, and a series of enzyme- or receptor-based PET agents have been developed in this laboratory. In our previous work, we have targeted the enzyme glycogen synthase kinase-3 (GSK-3) and developed carbon-11-labeled GSK-3 inhibitors; and we have also targeted serotonin (5-hydroxytryptamine) 6 receptor (5-HT6,R) and developed carbon-11-labeled 5-HT6,R antagonists, PET radiotracers for AD imaging (Figure 1). In this ongoing study, we first target CK1, which is a novel and attractive molecular target for treatment and PET imaging of AD. Here, we report the design, synthesis and labeling of carbon-11-labeled CK1 inhibitors, [11C]methyl 3-((2,2-difluoro-5H-1,3)dicarboxyl(4,5,5)benzo[1,2-d]imidazol-6-yl)carbamoyl)benzoate ([11C]5a) and N-(2,2-difluoro-5H-[1,3]dicarboxyl(4,5,5)benzo[1,2-d]imidazol-6-yl)3-methoxybenzamide (5c), as new potential PET radiotracers for imaging of AD, for the first time. The basic evaluations of the radiotracers including lipophilicity and stability are presented as well.

The reference standards methyl 3-(2,2-difluoro-5H-1,3)dicarboxyl(4,5,5)benzo[1,2-d]imidazol-6-yl)carbamoyl)benzoate (5a) and N-(2,2-difluoro-5H-[1,3]dicarboxyl(4,5,5)benzo[1,2-d]imidazol-6-yl)3-methoxybenzamide (5c), and their corresponding desmethylated precursors 3-(2,2-difluoro-5H-[1,3]dicarboxyl(4,5,5)benzo[1,2-d]imidazol-6-yl)carbamoyl)benzoic acid (6a) and N-(2,2-difluoro-5H-[1,3]dicarboxyl(4,5,5)benzo[1,2-d]imidazol-6-yl)3-hydroxybenzamide (6b), were synthesized as shown in Scheme 1, according to the literature method with some modifications. The commercially available starting material 5-amino-2,2-difluoro-1,3-benzoxadiazole was treated with acetic anhydride in toluene to obtain acetamide 1 in 92% yield. Compound 1 was then converted to the intermediate 2 through a concurrent nitration and deprotection with nitronium tetrafluoroborate (NO2BF4) in 69% yield. In comparison with the reported method, the use of the nitration reagent NO2BF4 simplified the reaction steps, combining nitration reaction and deprotecting reaction into one step, and improved the reaction yield. The nitro compound 2 was reduced through hydrogenation using H2 and Pd/C as catalyst instead of Raney Nickel reported in the literature to give the intermediate 3 containing two amino groups, which was subsequently reacted with cyanogen bromide to provide the key intermediate amino 4 in 82% yield. The catalyst change in hydrogenation also improved the yield. Then the amino 4 was reacted with several 3-substituted benzoic acids under the catalysis of N,N,N’,N’-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) and N,N-disopropylethylamine (DIPEA) to afford the standard compounds 5a and 5c in 63% and 21% yield, respectively. A protected benzamide 5b was also synthesized in 18% yield. Compound 5a was hydrolyzed in methanol solution of KOH to yield its acid precursor 6a in 93% yield. Compound 5b was converted to O-desmethylated precursor 6b for compound 5c through the deprotecting reaction of benzyl group employing boron trifluoride diethyl etherate (BF3·Et2O) and dimethyl sulfide (Me2S) in 65% yield. This deprotective reagent system was found to be better than other deprotective reagent system like H2 and Pd/C, which is easy to result in byproduct formation and lower yield.


The radiosynthesis was performed in a home-built automated multi-purpose [11C]-radiosynthesis module. 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. More reactive $[^{11}]^{}$C-methyl iodide ($[^{11}]^{}$CCH$_4$I),$^{32}$ was used in $[^{11}]^{}$Cmethylation to improve radiochemical yield of $[^{11}]^{}$C$^5$a and $[^{11}]^{}$C$^5$c. The Eckert & Ziegler Modular Lab C-11 Methyl Iodide/Triflate module in our facility can produce $[^{11}]^{}$Cmethyllating agent either $[^{11}]^{}$CCH$_4$OTf or $[^{11}]^{}$CCH$_4$I ($[^{11}]^{}$CCH$_4$Br passed through a NaI column).

The direct comparison between $[^{11}]^{}$CCH$_4$OTf and $[^{11}]^{}$CCH$_4$I confirmed the result that the labeling yield was improved from 30-35% to 40-45%. The labeling reaction was conducted using a V-vial method. Addition of aqueous NaHCO$_3$ to quench the radiolabeling reaction and to dilute the radiolabeling mixture prior to the injection onto the semi-preparative HPLC column for purification gave better separation of $[^{11}]^{}$C$^5$a or $[^{11}]^{}$C$^5$c from its $O$-desmethylated precursor $6$a or $6$b. Both Sep-Pak trap/release and rotatory evaporation are available for formulation in our multi-purpose $[^{11}]^{}$C-radiosynthesis module, and we used Sep-Pak method instead of rotatory evaporation for formulation to improve the chemical purity of radiolabeled product $[^{11}]^{}$C$^5$a or $[^{11}]^{}$C$^5$c. The direct comparison between Sep-Pak method and rotatory evaporation confirmed the result that the chemical purity of radiolabeled product was improved from <90% to >90%. In addition, a C18 Light Sep-Pak to replace a C18 Plus Sep-Pak allowed final product formulation with ≤5% ethanol.$^{33}$ Overall, it took ~40 min for synthesis, purification, and dose formulation.

Our module is designed to allow in-process measurement of $[^{11}]^{}$C-tracer molar activity (MA, GBq/μmol at EOB) using a radiation detector with a UV detector at the outlet of the HPLC-portion of the system.$^{31}$ In the HPLC chromatogram, peak analysis of the chromatographic data utilized PeakSimple software (SRI Instruments, Las Vegas, NV). Immediately following elution of the product peak, the chromatographic data are exported to PeakSimple readable files, and the area of the radioactivity peak is converted to GBq - mCi at EOB by comparison to a reference calibration curve previously constructed using the same detector, loop column, mobile phase and flow rate. The mass peak from the UV chromatogram (without decay correction) is similarly compared to a standard curve made at the same UV wavelength, mobile phase and flow rate. Simple division of the total EOB radioactivity peak (in GBq - mCi) by the total mass peak (in nmoles) gives specific activity at EOB in GBq - Ci/μmol. For the reported syntheses, product MA was in a range of 370-740 GBq/μmol at EOB. The factors that affect the EOB MA significantly to lead to such a wide range have been discussed in our previous works.$^{34-36}$ The general methods to increase SA have been described as well, and the SA of our $[^{11}]^{}$C-tracers is significantly improved.$^{34-36}$ The ‘wide range’ of MA we reported is for the same $[^{11}]^{}$C-tracer produced in different days, because very different $[^{11}]^{}$C-target and $[^{11}]^{}$C-radiosynthesis unit situations would make MA in a wide range. For a $[^{11}]^{}$C-tracer produced in the same day, the MA of the same tracer in different production runs will be in a small range, because $[^{11}]^{}$C-target and $[^{11}]^{}$C-radiosynthesis unit would not be much different in the same day. Likewise, the methods to minimize such wide range of MA from practice perspective have been provided in our previous works.$^{34-36}$ At the end of synthesis (EOS), the MA of $[^{11}]^{}$C-tracer was determined again by analytical RP HPLC, calculated, decay corrected to EOB, and based on $[^{11}]^{}$C$^4$O$_2$, which was in agreement with the ‘on line’ determined value. In this work, semi-preparative HPLC was used for purification, thus the MA of $[^{11}]^{}$C-tracer was assessed by both semi-preparative HPLC (during synthesis) and analytical HPLC (EOS).

Chemical purity and radiochemical purity were determined by analytical HPLC.$^{37}$ The chemical purity of the precursor and reference standard was >93%. The radiochemical purity of the target tracer was >99% determined by radio-HPLC through γ-ray (PIN diode) flow detector, and the chemical purity of the target tracer was >90% determined by reversed-phase HPLC through UV flow detector.

![Figure 1. PET radiotracers for imaging of AD.](image-url)
Scheme 1. Synthesis of reference standards 5a, 5c and O-desmethylated precursors 6a, 6b. Reaction reagents, conditions and yields: (i) acetic anhydride, toluene, 100 °C, 92%. (ii) NOBF₄, CH₂Cl₂, 69%. (iii) hydrogen, Pd/C, methanol; (iv) cyanogen bromide, methanol, room temperature (RT), 40 h, 82%. (v) 3-substituent-benzoic acid, HBTU, DIPEA, 18-65%. (vi) for 6a, KOH, methanol, 93%; for 6b, Me₂S, BF₃·Et₂O, CH₂Cl₂, 65%.

Scheme 2. Synthesis of target tracers [¹¹]C5a and [¹¹]C5c. Reaction reagents, conditions and yields: (i) [³¹]CCH₃OTf, CH₂CN, 2 N NaOH, 80 °C, 3 min; HPLC-SPE, 40-45%.


<table>
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<tr>
<th>Compound</th>
<th>Log P</th>
<th>CLog P</th>
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<tr>
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<td>3.60</td>
<td>5.09</td>
</tr>
<tr>
<td>[¹¹]C5c</td>
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<td>[¹¹]CPBB3</td>
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<td>[¹⁸]FT807</td>
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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. Log P provides an assessment of lipophilicity that often correlates with a compound’s ability to penetrate the blood brain barrier (BBB). Log P can be determined experimentally by liquid-liquid extraction and by HPLC and can also be theoretically calculated from parameters related to the structure of molecules. We obtained Log P and calculated CLog P values of carbon-11-labeled CK1 inhibitors [¹¹]C5a and [¹¹]C5c in comparison with [¹¹]CPIB, [¹⁸]FAmyvid, [¹¹]CPBB3 and [¹⁸]FT807 (Figure 1) from ChemDraw Professional 15.1 (ChemOffice), and the data are listed in Table 1. Log P data of [¹¹]C5a and [¹¹]C5c (3.60 - 3.66) are in the range of Log P data of [¹¹]CPIB, [¹⁸]FAmyvid, [¹¹]CPBB3 and [¹⁸]FT807 (2.25 - 4.09), which are PET AD imaging agents in clinical evaluation. These data suggest [¹¹]C5a and [¹¹]C5c have appropriate lipophilicity to pass the BBB for brain uptake.

The stability of the labeled tracers [¹¹]C5a and [¹¹]C5c 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]C5a and [¹¹]C5c were stable without decomposition.

The experimental details and characterization data for compounds 1-6 and for the tracers [¹¹]C5a and [¹¹]C5c are given. In summary, synthetic routes with moderate to high yields have been developed to produce difluorodioxolo-benzoimidazol-benzamides including reference standards 5a and 5c, and O-desmethylated precursors 6a and 6b, and carbon-11-labeled difluoro-dioxolo-benzoimidazol-benzamides target tracers [¹¹]C5a and [¹¹]C5c. The radiosynthesis employed [¹¹]CH₂OTf for O-[¹¹]Cmethylation at O-desmethylated precursor, followed by product purification and isolation using a semi-preparative RP HPLC combined with SPE. [¹¹]C5a and [¹¹]C5c were obtained in high radiochemical yield, radiochemical purity and chemical purity, with a reasonable short overall synthesis time, and high molar activity. This will facilitate studies to evaluate carbon-11-labeled CK1 inhibitors [¹¹]C5a and [¹¹]C5c as new candidate PET radiotracers for imaging of AD.

Acknowledgments

This work was partially supported by Indiana University Department of Radiology and Imaging Sciences in the United States. [¹]H NMR and [¹³]C NMR spectra were recorded at 500 and 125 MHz, respectively, on a Bruker Avance II 500 MHz NMR spectrometer in the Department of Chemistry and Chemical Biology at Indiana University Purdue University Indianapolis (IUPUI), which is supported by the United States National Science Foundation (NSF) Major Research Instrumentation Program (MRI) grant CHE-0619254.

References and notes


39. (a) General: All commercial reagents and solvents were purchased from Sigma-Aldrich and Fisher Scientific, and used without further purification. [11C]CH3OTf was prepared according to a literature procedure.

(a) General: All commercial reagents and solvents were purchased from Sigma-Aldrich and Fisher Scientific, and used without further purification. [11C]CH3OTf was prepared according to a literature procedure. Melting points were determined on a WR-4 apparatus and were uncorrected. 1H and 13C NMR spectra were recorded on a Bruker Avance II 500 MHz NMR Fourier transform spectrometer at 500 and 125 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) relative to an internal standard tetramethylsilane (TMS, δ 0.0) (1H NMR) and to the solvent signal (13C NMR), and coupling constants (J) are reported in hertz (Hz). Liquid chromatography-mass spectra (LC-MS) analysis was performed on an Agilent system, consisting of an 1100 series HPLC configured for positive-ion/negative-ion electrospray ionization. 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 Analtech silica gel GF uniplates (5 x 10 cm²). Plates were visualized under UV light. Normal phase flash column chromatography was carried out on EM Science silica gel 60 (230-400 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 x 250 mm; mobile phase 60%CH3CN/40% H2O; flow rate 1.3 mL/min; UV (254 nm) and γ-ray (PIN diode) flow detectors. Semi-preparative RP HPLC was performed using a Prodigy (Phenomenex) 5 µm C-18 column, 10 x 250 mm; mobile phase 60%CH3CN/40%H2O; flow rate 5 mL/min; UV (254 nm) and γ-ray (PIN diode) flow detectors. 18 Light Sep-Pak cartridges were obtained from Waters Corporation (Milford, MA). Sterile Milllex-FG 0.2 µm filter units were obtained from Millipore Corporation (Bedford, MA).

(b) N-(2,2-Difluorobenzoyl)[1,3]dioxol-5-y]acetamide (1): A solution of 5-amino-2,2-difluoro-1,3-benzodioxole (14.4 g, 83.2 mmol) in dry toluene (230 mL) and acetic anhydride (9.76 g, 95.7 mmol, 1.15 equiv) was stirred at 100 °C for 3 h. The solvent was removed under reduced pressure, then the crude product was dissolved in 80 mL of methanol to remove traces of acetic anhydride. The solvent was subsequently evaporated. The crude product was recrystallized from toluene, and the resulting product was filtered off and dried to give a beige crystal 1 (16.5 g, 92%), Rf = 0.25 (1:2 EtOAc/Hexanes), mp 140-142 °C. 1H NMR (DMSO-d6): δ 2.05 (s, 3H, CH3), 7.21 (dd, J = 2.0, 8.5 Hz, 1H, Ph-H), 7.31 (d, J = 8.5 Hz, 1H, Ph-H), 7.75 (d, J = 2.0 Hz, 1H, Ph-H), 10.14 (s, 1H, NH). MS (ESI): 216 ([IM+H]+, 100%); MS (ESI): 214 ([IM]+, 100%).

(c) 2,2-Difluoro-6-nitrobenzo[d][1,3]dioxol-5-amine (2): Compound 1 (15.6 g, 72.5 mmol) was dissolved in dichloromethane (250 mL). To the resulting mixture, nitromonium tetrafluoroborate solution (174 mL, 0.5 M in sulfolane) was added dropwise at 0 °C. After addition, the reaction mixture was allowed to RT and stirred for 24 h. Then the reaction mixture was evaporated to remove dichloromethane, and the resulting mixture was added with water, extracted...
with EtOAc, washed with water two times, and dried over Na2SO4. The mixture was filtered, and filtrate was evaporated in vacuo. The resultant residue was purified by column chromatography on silica gel with eluent (2.98 to 20.80 EtOAc/hexanes) to give a light beige solid 2 (10.9 g, 69%). Rf = 0.73 (CH2Cl2), mp 138-140 °C. 1H NMR (DMSO-d6): δ 1.76 (s, 1H, Ph-H), 7.79 (s, 2H, NH2), 7.94 (s, 1H, Ph-H). MS (EI): 219 ([M+H]+, 3%); MS (EI): 217 ([M-H]-, 100%).

(2,2-Difluoro-5H-[1,3]dioxolo[4',5';4,5]benzo[1,2-d]imidazol-6-yl)carboxylic acid (6a): Potassium hydroxide (KOH, 0.5 g, 7.6 mmol) was added into the solution of compound 5a (375 mg, 1.0 mmol) in methanol (20 mL). The reaction mixture was stirred at RT for 15 h. Then the reaction mixture was concentrated in vacuo, and HCl (1 N) was added to adjust pH to 7. The resulting precipitate was filtered, washed with cold water, dried in air to give a white solid 6a (336 mg, 93%). Rf = 0.20 (1:9 MeOH/CH2Cl2, mp > 310 °C). 1H NMR (DMSO-d6): δ 7.48 (s, 2H, Ph-H), 7.63 (t, J = 7.5 Hz, 1H, Ph-H), 8.16 (d, J = 7.5 Hz, 1H, Ph-H), 8.26 (d, J = 7.5 Hz, 1H, Ph-H), 12.55 (br s, 1H, OH). MS (EI): 362 ([M+H]+, 15%); MS (EI): 360 ([M-H]-, 25%). HRMS (EI) calcd for C18H10N3O2F2, 362.0589 ([M+H]+), found 362.0579.

N-(2,2-Difluoro-5H-[1,3]dioxolo[4',5';4,5]benzo[1,2-d]imidazol-6-yl)carboxamoyl)benzoic acid (6b): BF3.OEt2 (0.8 mL) and Me3S (1.0 mL) were added to a solution of compound 5b (212 mg, 0.5 mmol) in dichloromethane (20 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 2 h and at RT for 2 h. Then the reaction mixture was evaporated to dryness in vacuo. The residue was suspended in a mixture of aqueous NaHCO3, and extracted with EtOAc (3 × 80 mL). The combined organic layers were washed with brine, dried over Na2SO4, and concentrated. The residue was purified by column chromatography on silica gel with eluent (1:99 to 5:95 MeOH/CH2Cl2) to afford a white solid 5b.

Methyl 3-((2,2-Difluoro-5H-[1,3]dioxolo[4',5';4,5]benzo[1,2-d]imidazol-6-yl)carboxamido)benzoate (5a): Yield 63%. Rf = 0.52 (1:13 MeOH/CH2Cl2), mp 252-254 °C. 1H NMR (acetone-d6): δ 3.90 (s, 3H, OCH3), 7.35 (s, 2H, Ph-H), 7.71 (t, J = 8.0 Hz, 1H, Ph-H), 8.23 (dt, J = 1.5, 8.0 Hz, 1H, Ph-H), 8.39 (dt, J = 1.5, 8.0 Hz, 1H, Ph-H), 8.72 (t, J = 1.5 Hz, 1H, Ph-H). MS (EI): 376 ([M+H]+, 25%); MS (EI): 374 ([M-H]-, 100%).

3-(Benzyloxy)N-(2,2-Difluoro-5H-[1,3]dioxolo[4',5';4,5]benzo[1,2-d]imidazol-6-yl)benzamide (5b): Yield 18%. Rf = 0.67 (1:9 MeOH/CH2Cl2), mp 221-223 °C. 1H NMR (acetone-d6): δ 7.30-7.32 (m, 3H, Ph-H), 7.33 (dt, J = 2.0, 7.0 Hz, 1H, Ph-H), 7.39 (tt, J = 1.5, 8.5 Hz, 2H, Ph-H), 7.45-7.51 (m, 3H, Ph-H), 7.76 (td, J = 1.0, 8.0 Hz, 1H, Ph-H), 7.80 (t, J = 2.0 Hz, 1H, Ph-H), 11.80 (br s, 1H, NH). MS (ESI): 424 ([M+H]+, 9%); MS (ESI): 422 ([M-H]-, 100%).

N-(2,2-Difluoro-5H-[1,3]dioxolo[4',5';4,5]benzo[1,2-d]imidazol-6-yl)carboxamoyl)benzoate ([13]C)5a and N-(2,2-Difluoro-5H-[1,3]dioxolo[4',5';4,5]benzo[1,2-d]imidazol-6-yl)carboxamoyl)benzoate ([13]C)5b
3-[11C]methoxybenzamide ([11C]5c): [11C]CO₂ was produced by the ¹⁴N(p,α)¹¹C nuclear reaction in the small volume (9.5 cm³) 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 [11C]CO₂ at EOB. The precursor 6a or 6b (0.1-0.3 mg) was dissolved in CH₃CN (300 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) [¹¹C]CH₃OTf that was produced by the gas-phase production method²⁶ within 12 min from [¹¹C]CO₂ through [¹¹C]CH₄ and [¹¹C]CH₃Br 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 NaHCO₃ (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 [¹¹C]5a or [¹¹C]5c fraction was collected in a recovery vial containing 30 mL water. The diluted tracer solution was then passed through a C-18 Light 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 ~40 min from EOB. The decay corrected radiochemical yield was 40-45%. Retention times in the analytical HPLC system were: \( t_R \) 6a = 4.77 min, \( t_R \) 5a = 6.13 min, \( t_R \) [¹¹C]5a = 6.21 min; and \( t_R \) 6b = 4.82 min, \( t_R \) 5c = 6.34 min. \( t_R \) [¹¹C]5c = 6.41 min. Retention times in the preparative HPLC system were: \( t_R \) 6a = 5.85 min, \( t_R \) 5a = 10.02 min, \( t_R \) [¹¹C]5a = 10.18 min; and \( t_R \) 6b = 6.05 min, \( t_R \) 5c = 10.23 min, \( t_R \) [¹¹C]5c = 10.38 min.
Synthesis of carbon-11-labeled CK1 inhibitors as new potential PET radiotracers for imaging of Alzheimer’s disease

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- New carbon-11-labeled CK1 inhibitors were synthesized.
- A fully automated multi-purpose $[^{11}\text{C}]$-radiosynthesis module was built up.
- A semi-preparative RP HPLC-SPE technique was employed in radiosynthesis.
Synthesis of carbon-11-labeled CK1 inhibitors as new potential PET radiotracers for imaging of Alzheimer’s disease
Mingzhang Gao, Min Wang, Qi-Huang Zheng*

Carbon-11-labeled CK1 inhibitors