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Laboratory note

Synthesis of [^{11}C]FEDAA1106 as a new PET imaging probe of peripheral benzodiazepine receptor expression

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ABSTRACT

Peripheral benzodiazepine receptor (PBR) is associated with neuroinflammation and tumor progression. [^{11}C]DAA1106 and [^{18}F]FEDAA1106 are two promising radioligands for positron emission tomography (PET) imaging of PBR. This study was designed to develop a new radiolabeled analog of [^{11}C]DAA1106 and [^{18}F]FEDAA1106, [^{11}C]FEDAA1106, for PET imaging of PBR expression in brain and cancer. Precursor *N*-(5-fluoro-2-phenoxyphenyl)-*N*-(2-(2-fluoroethoxy)-5-hydroxybenzyl)acetamide (**9**) was synthesized in multiple steps with moderate to high chemical yields. Precursor **9** was labeled by [^{11}C]CH_3OTf and isolated by high pressure liquid chromatography (HPLC) purification to provide target radioligand *N*-(5-fluoro-2-phenoxyphenyl)-*N*-(2-(2-fluoroethoxy)-5-[^{11}C]methoxybenzyl)acetamide ([^{11}C]FEDAA1106, [^{11}C]**10**) in 60–70% radiochemical yields, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2. The specific activity of the target radiotracer [^{11}C]**10** was in a range of 111–185 GBq/μmol at the end of synthesis (EOS).

1. Introduction

The peripheral benzodiazepine receptor (PBR) is a protein found in lung, liver, heart, spleen, kidney, adrenals, brain, glial cells, masts cell and macrophages and implicated in numerous nervous system disorders such as epilepsy, cerebral ischemia, nerve injury and neurodegenerative diseases, and immune system diseases such as cancer [1]. PBR is an attractive target for molecular imaging of neuroinflammation and tumor progression. There is great interest in imaging of PBR expression in human diseases such as Alzheimer's disease and neurofibromas [2–18]. The high-affinity PBR ligands include new compounds with structural classes of isoquinoline carboxamides, quinoline carboxamides, benzothiazepines, benzoxazepines, indoleacetamides, pyrazolopyrimidines, vinca alkaloids, and aryloxyanilides [1]. These small molecule ligands may have high binding affinity but still lack specificity for the receptor due to non-specific interactions. Recently two acetamide parent compounds DAA1106 (*N*-(2,5-dimethoxybenzyl)-*N*-(5-fluoro-2-phenoxyphenyl)acetamide) and its derivative FEDAA1106 (*N*-(5-fluoro-2-phenoxyphenyl)-*N*-(2-(2-fluoroethoxy)-5-methoxybenzyl)acetamide) were reported as potent and selective ligands for PBR and displayed high PBR binding affinities ($K_i = 0.16$ nM and 0.078 nM in rat brain sections, respectively) [19–22]. Both ligands had more potent *in vitro* binding affinities for PBR than PK11195 (1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-isoquinoline-3-carboxamide), a standard ligand for PBR [23,24]. Consequently, carbon-11 labeled analog of DAA1106, [^{11}C]DAA1106 (*N*-(2-[^{11}C]methoxy-5-methoxybenzyl)-*N*-(5-fluoro-2-phenoxyphenyl) acetamide) and fluorine-18 labeled analog of FEDAA1106, [^{18}F]FEDAA1106 (*N*-(5-fluoro-2-phenoxyphenyl)-*N*-(2-(2-[^{18}F]fluoroethoxy)-5-methoxybenzyl)acetamide), have been developed as two promising radioligands for positron emission tomography (PET) [22,25–27], and the *in vivo* biological evaluation displayed high specific binding of both radioligands to PBR, which suggested both compounds have high specificity [25]. Previous results indicated [^{18}F]FEDAA1106 has higher PBR affinity and specificity than [^{11}C]DAA1106, but requires more complex radiosynthesis. Compared to fluorine-18 tracers (half-life 110 min), carbon-11 tracers (half-life 20 min) have some advantages in back-to-back same-day PET studies, such as avoiding movement of the subject from the PET scanner and performing another study within 2–3 h to explore drug effects at the first study. These advantages become very valuable in studying pharmacological or behavioral changes [28]. We are interested in the development of new PET PBR radioligands. This study was designed to develop a new analog radioligand of [^{11}C]DAA1106 and [^{18}F]FEDAA1106, [^{11}C]FEDAA1106 (*N*-(5-fluoro-2-phenoxyphenyl)-*N*-(2-(2-fluoroethoxy)-5-[^{11}C]methoxybenzyl) acetamide, [^{11}C]**10**), a carbon-11 labeled form of FEDAA1106, which

Abbreviations: PBR, peripheral benzodiazepine receptor; PET, positron emission tomography; HPLC, high pressure liquid chromatography; EOB, end of bombardment; EOS, end of synthesis; TMS, tetramethylsilane; HRMS, high resolution mass spectra; TLC, thin-layer chromatography; rt, room temperature; RDS, radionuclide delivery system; INGEN, Indiana Genomics Initiative.

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will combine the advantages of both [^{11}C]DAA1106 and [^{18}F]FEDAA1106, for PET imaging of PBR expression in brain and tumor (Fig. 1). Here, we report the synthesis of [^{11}C]FEDAA1106.

2. Results and discussion

2.1. Chemistry

The chemistry is straightforward. Synthetic approach for phenolic precursor and standard compound FEDAA1106 is shown in Schemes 1–3.

As depicted in Scheme 1, the key intermediate **4** was prepared from commercially available 2,5-dihydroxybenzaldehyde. 2,5-Dihydroxybenzaldehyde was benzylated selectively using benzyl bromide in the presence of $NaHCO_3$ and KI in CH_3CN to obtain 5-monobenzylated compound **1** in 32% yield [29,30], the slight selectivity is normally attributed to the intramolecular H-bonding that exists between the 2-hydroxyl and the carbonyl group preventing the 2-position from being alkylated, which resulted in low chemical yield. The mixture of both 2- and 5-hydroxyl benzylation products was purified by flash column chromatography. Alkylation of the hydroxyl group of **1** with $BrCH_2CH_2F$ in the presence of NaH in DMF gave 2-fluoroethyl ether **2** in 21% yield. Reduction of aldehyde **2** with $NaBH_4$ in MeOH provided the alcohol **3** in 97% yield, which was treated with PBr_3 in Et_2O to afford benzyl bromide **4** in 83% yield.

As indicated in Scheme 2, another key intermediate acetanilide **7** was obtained efficiently from commercial materials based on the literature procedures [21,23,27]. 1,4-Difluoro-2-nitrobenzene was treated with phenol under basic condition to afford compound **5** in 99% yield. The reaction is highly selective and 1-fluoro position is preferred over 4-fluoro position of the ring system due to the effect of the electron-withdrawal group 2-nitro, which resulted in very high chemical yield. Reduction of **5** with powdered Fe and NH_4Cl in EtOH and H_2O formed anilide **6** in 97% yield, which was acetylated with acetyl chloride in the presence of Et_3N in CH_2Cl_2 to afford acetanilide **7** in 93% yield.

In our approach as shown in Scheme 3, acetanilide **7** was alkylated with benzyl bromide **4** using NaH as a base in DMF to give compound **8** in 71% yield. The benzyl groups of compound **8** were removed by catalytic hydrogenation with 10% Pd–C in THF and MeOH to provide precursor **9** in 66% yield. *O*-Methylation of the phenol precursor **9** with CH_3I in the presence of NaH in DMSO afforded standard FEDAA1106 (**10**) in 70% yield.

2.2. Radiochemistry

Synthesis of target radioligand [^{11}C]FEDAA1106 ([^{11}C]**10**) is indicated in Scheme 4. Precursor **9** was labeled by a reactive [^{11}C] methylating agent, [^{11}C]methyl triflate ([^{11}C]CH_3OTf) [31,32] prepared from [^{11}C]CO_2, in the presence of NaH in acetonitrile through the *O*-[^{11}C]methylation and isolated by semi-preparative high pressure liquid chromatography (HPLC) method to provide target tracer [^{11}C]**10** in 60–70% radiochemical yields, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2. The synthesis was performed in an automated multi-purpose ^{11}C-radiosynthesis module, allowing measurement of specific activity during synthesis [33,34]. The specific activity of [^{11}C]FEDAA1106 was in a range of 222–370 GBq/μmol at EOB measured by the on-the-fly technique using semi-preparative HPLC method during synthesis [34] and 111–185 GBq/μmol at the end of synthesis (EOS) determined by analytical HPLC method [35], respectively. Chemical purity and radiochemical purity were determined by analytical HPLC method [35]. The chemical purity of the precursor **9** and reference standard **10** was >97%. The radiochemical purity of the target tracer [^{11}C]**10** was >99% determined by radio-HPLC through γ-ray (NaI) flow detector, and the chemical purity of the target tracer [^{11}C]**10** was >96% determined by reversed-phase HPLC through UV flow detector.

Both [^{11}C]DAA1106 and [^{18}F]FEDAA1106 have been used as PET PBR imaging probes to study neuroinflammation such as Alzheimer's disease and tumor progression such as neurofibromas in animals [2–18] and humans [36]. New PET PBR imaging probe [^{11}C]FEDAA1106 has higher PBR binding affinity and specificity than its parent compound [^{11}C]DAA1106, easier radiosynthesis

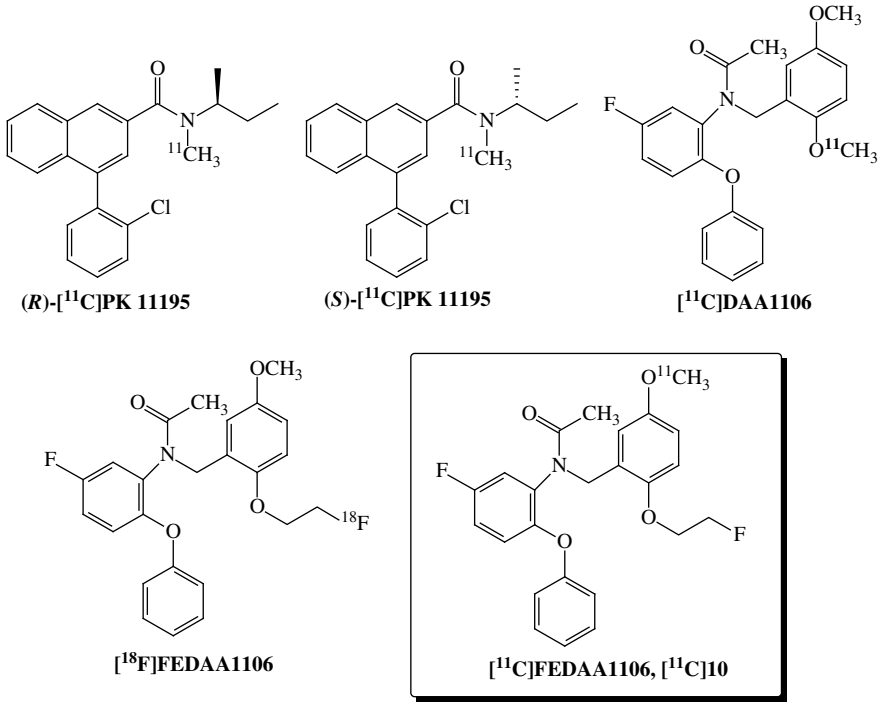
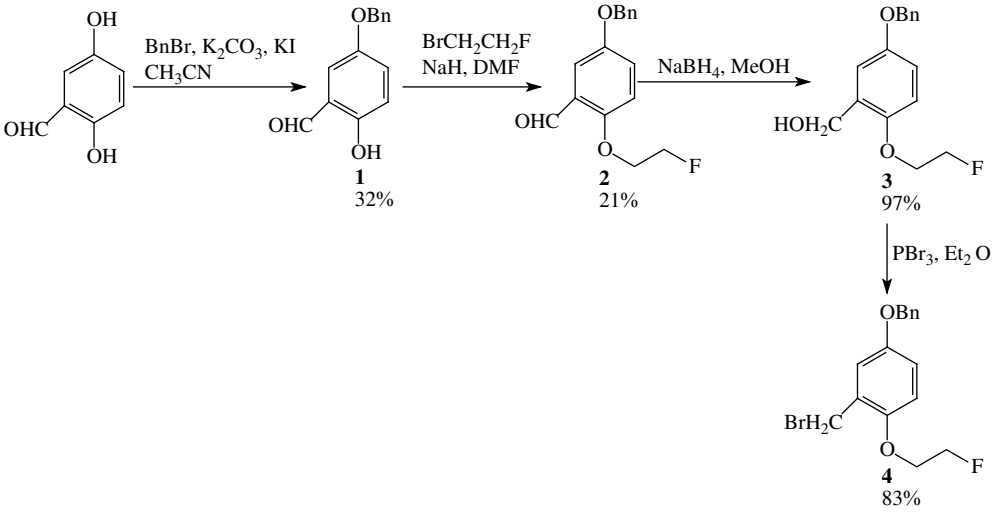


Fig. 1. The structures of (*R*)-[^{11}C]PK11195, (*S*)-[^{11}C]PK11195, [^{11}C]DAA1106, [^{18}F]FEDAA1106 and [^{11}C]FEDAA1106.



Scheme 1. Synthesis of key intermediate **4**.

procedures than its fluorine-18 labeled form [^{18}F]FEDAA1106. These advantages encourage further biological evaluation of [^{11}C]FEDAA1106. The *in vivo* biodistribution and PET imaging of PBR transgenic mice (brain Alzheimer's disease) and neurofibromatosis type 1 (NF1) gene knockout tumor mice with [^{11}C]FEDAA1106 are currently underway, and the results will be reported in due course.

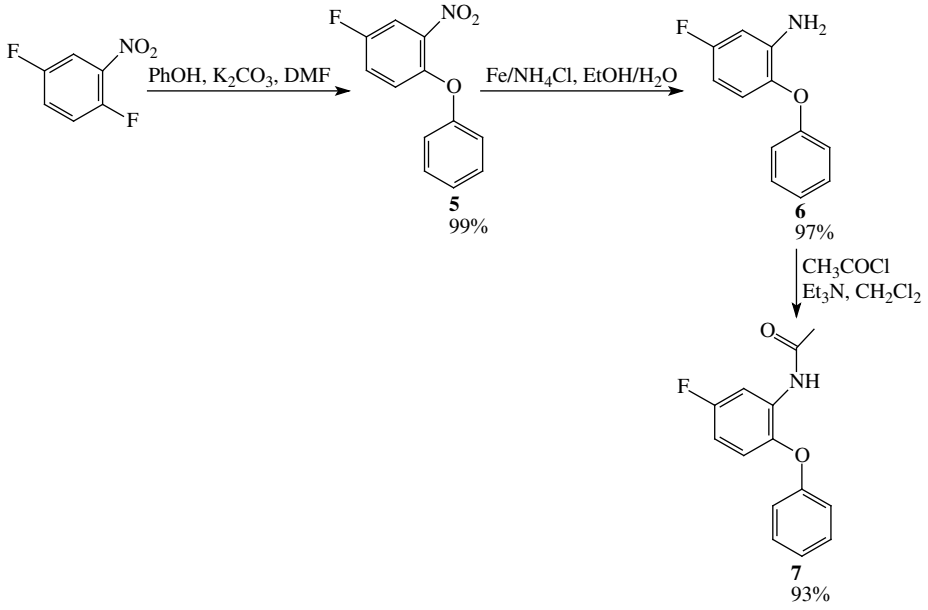
3. Materials and methods

3.1. General

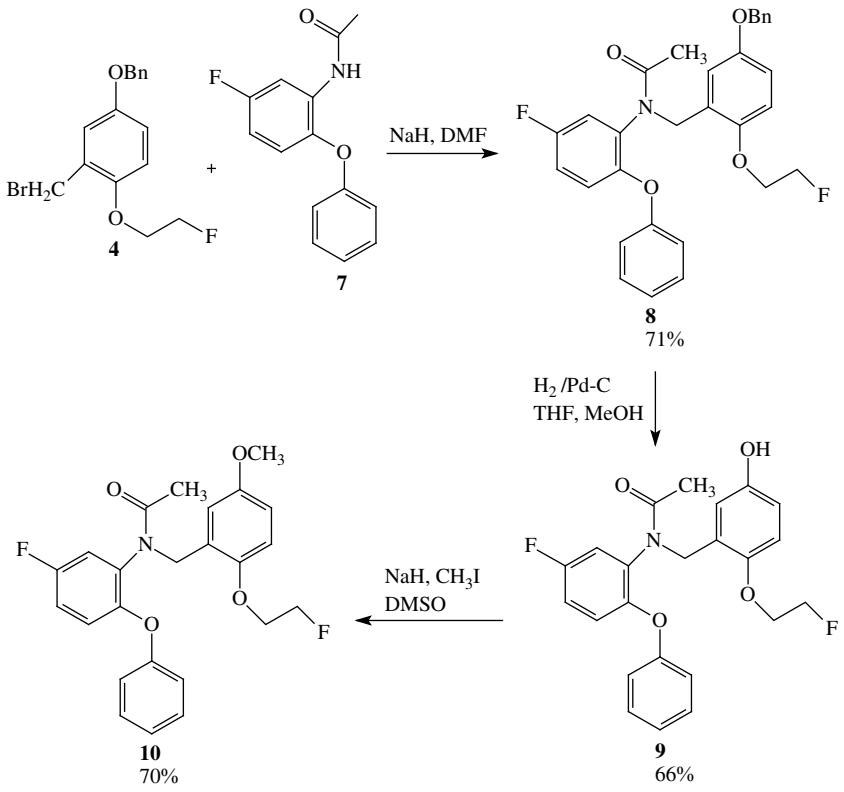
All commercial reagents and solvents from Aldrich and Sigma were used without further purification. [^{11}C]CH_3OTf was prepared according to a literature procedure [31]. Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a Varian Gemini 2000 200 MHz FT-NMR and Bruker Avance II 500 MHz NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shift data for the proton resonances were reported in parts per million (ppm, δ scale) relative to internal standard TMS (δ 0.0), and coupling constants (J) were reported in hertz (Hz). The high resolution mass spectra (HRMS) were obtained using a Thermo MAT 95XP-Trap spectrometer. Thin-layer chromatography (TLC) was run using Analtech silica gel GF uniplates (5×10 cm^2). Plates were visualized under UV light. Preparative TLC was run using Analtech silica gel UV 254 plates (20×20 cm^2). Flash column chromatography was run using EM Science, silica gel 60 (230–400 mesh). Chromatographic solvent proportions are indicated in a volume:volume ratio. Sterile Millex-GS 0.22 μm vented filter unit was obtained from Millipore Corporation, Bedford, MA. All moisture- and/or air-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source.

3.2. 5-Benzyloxy-2-hydroxybenzaldehyde (**1**)

A mixture of 2,5-dihydroxybenzaldehyde (4.0 g, 29.0 mmol), $NaHCO_3$ (2.77 g, 33.0 mmol) and KI (0.48 g, 2.90 mmol) in CH_3CN



Scheme 2. Synthesis of key intermediate **7**.



Scheme 3. Synthesis of phenolic precursor **9** and standard FEDAA1106, **10**.

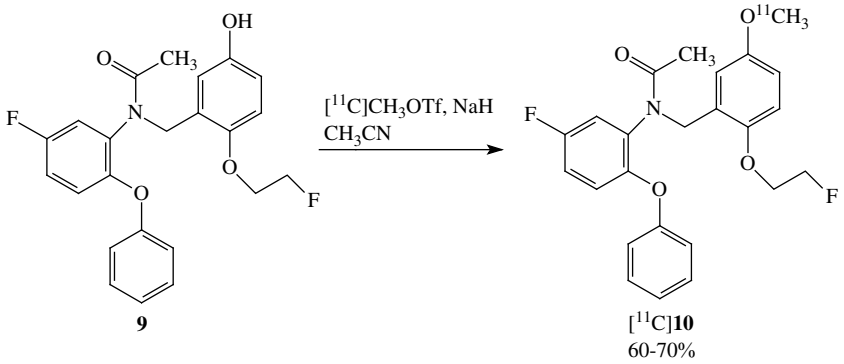
(50 mL) was heated to 60 °C, followed by addition of benzyl bromide (4.47 mL, 37.6 mmol). The reaction mixture was stirred and refluxed under nitrogen overnight. The solvent was evaporated, and the residue was dissolved in EtOAc and washed with 1 N HCl, water, brine and dried over anhydrous Na_2SO_4. The crude product was purified by column chromatography with EtOAc–hexanes (1:4) as eluent to afford **1** (2.13 g, 32%) as a pale yellow solid, mp 88–89 °C (lit. [29] 91–92 °C). ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.69 (1H, s, CHO), 9.85 (1H, s, OH), 7.46–7.35 (5H, m, Ar-H), 7.24 (1H, dd, $J = 9.0$, 3.0 Hz, Ar-H), 7.09 (1H, d, $J = 3.0$ Hz, Ar-H), 6.96 (1H, d, $J = 9.0$ Hz, Ar-H), 5.08 (2H, s, $PhCH_2$).

3.3. 5-Benzyloxy-2-(2-fluoroethoxy)benzaldehyde (*2*)

To a solution of compound **1** (1.39 g, 6.09 mmol) in DMF (5 mL) at 0 °C was added NaH (60% dispersion in mineral oil, 487 mg, 12.2 mmol) portionwise at 0 °C. The mixture was stirred at 0 °C for 20 min, followed by addition of $BrCH_2CH_2F$ (1.55 g, 12.2 mmol). After stirring at room temperature (rt) for 48 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with water and brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude product was purified by column chromatography with EtOAc–hexanes (1:4) as eluent to afford **2** (354 mg, 21%) as a yellow solid, mp 39–40 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.51(1H, s, CHO), 7.46–7.33 (6H, m, Ar-H), 7.21 (1H, dd, $J = 9.0$, 3.0 Hz, Ar-H), 6.91 (1H, d, $J = 9.0$ Hz, Ar-H), 5.07 (2H, s, $PhCH_2$), 4.86–4.84 (1H, m, OC*H*HCH_2F), 4.77–4.75 (1H, m, OCH*H*CH_2F), 4.36–4.34 (1H, m, OCH_2C*H*HF), 4.30–4.28 (1H, m, OCH_2CH*H*F). HRMS (CI) *m*/*z* calculated for $C_{16}H_{15}FO_3$ ($[M]^+$), 274.1000; found, 274.1005.

3.4. (5-Benzyloxy-2-(2-fluoroethoxy)phenyl)methanol (*3*)

To a solution of compound **2** (280 mg, 1.02 mmol) in MeOH (10 mL) was added $NaBH_4$ (77 mg, 2.04 mmol) portionwise at 0 °C. After stirring at rt for 4 h, the solvent was evaporated, and to the residue was added ice water and extracted with EtOAc. The organic layer was washed with water and brine, dried over anhydrous



Scheme 4. Synthesis of [^{11}C]FEDAA1106 ([^{11}C]**10**).

Na_2SO_4. Evaporation of the solvent afforded **3** (273 mg, 97%) as a white solid, mp 64–65 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.43–7.30 (5H, m, Ar-H), 6.99 (1H, d, J = 3.0 Hz, Ar-H), 6.84 (1H, dd, J = 9.0, 3.0 Hz, Ar-H), 6.79 (1H, d, J = 9.0 Hz, Ar-H), 5.11 (2H, s, $PhCH_2$), 4.80–4.78 (1H, m, OCH_2CHHF), 4.70–4.69 (3H, m + s, OCH_2CHHF + $PhCH_2OH$), 4.26–4.24 (1H, m, $OCHHCH_2F$), 4.20–4.18 (1H, m, $OCHHCH_2F$). HRMS (CI) m/z calculated for $C_{16}H_{17}FO_3$ ($[M]^+$), 276.1156; found, 276.1155.

3.5. 4-(Benzyloxy)-2-(bromomethyl)-1-(2-fluoroethoxy) benzene (**4**)

To a solution of compound **3** (250 mg, 0.91 mmol) in anhydrous Et_2O (7 mL) was added a solution of PBr_3 (90 μL, 0.95 mmol) in Et_2O (2 mL) dropwise. After stirring at rt for 3.5 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with water and brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude product was purified by column chromatography with EtOAc–hexanes (1:4) as eluent to afford **4** (254 mg, 83%) as a white solid, mp 65–66 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.43–7.31 (5H, m, Ar-H), 7.00 (1H, d, J = 3.0 Hz, Ar-H), 6.88 (1H, dd, J = 9.0, 3.0 Hz, Ar-H), 6.82 (1H, d, J = 9.0 Hz, Ar-H), 5.02 (2H, s, $PhCH_2$), 4.83–4.81 (1H, m, OCH_2CHHF), 4.74–4.72 (1H, m, OCH_2CHHF), 4.55 (2H, s, $PhCH_2Br$), 4.28–4.27 (1H, m, $OCHHCH_2F$), 4.23–4.21 (1H, m, $OCHHCH_2F$). HRMS (CI) m/z calculated for $C_{16}H_{16}BrFO_2$ ($[M]^+$), 338.0312; found, 338.0324.

3.6. 5-Fluoro-2-phenoxynitrobenzene (**5**)

A mixture of 1,4-difluoro-2-nitrobenzene (20 g, 125.7 mmol), phenol (12.4 g, 131.7 mmol) and K_2CO_3 (19.1 g, 138.2 mmol) in DMF (70 mL) was stirred at 96 °C for 4 h. After cooling to rt, water was added and the mixture was extracted with EtOAc. The combined organic layer was washed with water and brine, and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* to obtain **5** (29.1 g, 99%) as a yellow solid, mp 35–36 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.70 (1H, dd, J = 3.0, 7.8 Hz, Ar-H), 7.38 (2H, t, J = 7.8 Hz, Ar-H), 7.27–7.14 (2H, m, Ar-H), 7.08–6.99 (3H, m, Ar-H).

3.7. 5-Fluoro-2-phenoxyaniline (**6**)

A mixture of compound **5** (29 g, 124.4 mmol), iron (21.7 g, 388.9 mmol), and ammonium chloride (13.8 g, 257.9 mmol) in EtOH (300 mL) and water (100 mL) was stirred under nitrogen overnight at 110 °C. After cooling, the reaction mixture was filtered and extracted with EtOAc. The combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by column chromatography with EtOAc–hexanes (1:10) as eluent to afford **6** (24.4 g, 97%) as an orange oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.34 (2H, t, J = 8.0 Hz, Ar-H), 7.26 (1H, t, J = 3.0 Hz, Ar-H), 6.97–6.80 (3H, m, Ar-H), 6.53 (1H, dd, J = 3.0, 9.8 Hz, Ar-H), 6.43 (1H, dt, J = 2.9, 8.1 Hz, Ar-H), 3.82 (2H, br s, NH_2).

3.8. N-(5-Fluoro-2-phenoxyphenyl)acetamide (**7**)

To a stirred solution of compound **6** (0.6 g, 3.0 mmol) and Et_3N (0.5 mL, 3.6 mmol) in CH_2Cl_2 (6 mL) was added acetyl chloride (0.5 mL, 3.3 mmol) dropwise at 0 °C. After stirring at rt for 2.5 h, the reaction mixture was concentrated *in vacuo*. The residue was poured into water and extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$, water and brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude product was purified by column chromatography using EtOAc–hexanes (1:4) as eluent to obtain **6** (0.67 g, 93%) as a white solid, mp 81–82 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.31 (1H, dd, J = 2.9, 9.6 Hz, Ar-H), 7.72 (1H, br s, NH), 7.72–7.32 (2H, m, Ar-H), 7.17–7.10 (1H, m, Ar-H), 7.00–6.96 (2H, m, Ar-H), 6.85–6.65 (2H, m, Ar-H), 2.16 (3H, s, CH_3).

3.9. N-(5-Fluoro-2-phenoxyphenyl)-N-(2-(2-fluoroethoxy)-5-benzyloxybenzyl)acetamide (**8**)

To a suspension of NaH (60% dispersion in mineral oil, 24 mg, 0.60 mmol) in DMF (1 mL) was added compound **7** (128 mg, 0.52 mmol) at 0 °C, and the mixture was stirred at 0 °C for 20 min, followed by addition of compound **4** (200 mg, 0.59 mmol). After stirring at 0 °C for 4 h, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with water and brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude product was purified by column chromatography with EtOAc–hexanes (1:4) as eluent to afford **8** (214 mg, 71%) as a white solid, mp 89–90 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.43–7.30 (7H, m, Ar-H), 7.12 (1H, t, J = 2.5 Hz, Ar-H), 7.01 (1H, d, J = 3.0 Hz, Ar-H), 6.95–6.91 (1H, m, Ar-H), 6.88–6.81 (3H, m, Ar-H), 6.79 (1H, dd, J = 9.0, 6.0 Hz, Ar-H), 6.70–6.68 (2H, m, Ar-H), 5.18 (1H, d, J = 15 Hz, $CHHN$), 4.94 (2H, q, J = 11.5 Hz, $PhCH_2$), 4.66–4.51 (2H, d + m, J = 15 Hz, $CHHN$ + OCH_2CHHF), 4.56–4.49 (1H, m, OCH_2CHHF), 4.07–3.90 (2H, m, OCH_2CH_2F), 1.95 (3H, s, CH_3). HRMS (CI) m/z calculated for $C_{30}H_{27}F_2NO_4$ ($[M]^+$), 503.1903; found, 503.1920.

3.10. N-(5-Fluoro-2-phenoxyphenyl)-N-(2-(2-fluoroethoxy)-5-hydroxybenzyl)acetamide (**9**)

A solution of compound **8** (180 mg, 0.36 mmol) in THF (1.5 mL) and MeOH (3 mL) was hydrogenated at atmospheric pressure over 10% Pd–C (36 mg) overnight. The catalyst was removed by filtration and the solution was evaporated. The product was purified by preparative TLC plate using EtOAc–hexanes (1:2) as eluent to afford **9** (98 mg, 66%) as a white solid, mp 124–125 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.35–7.32 (2H, m, Ar-H), 7.16–7.12 (2H, m, Ar-H), 6.99–6.95 (1H, m, Ar-H), 6.93–6.87 (3H, m, Ar-H), 6.79 (1H, dd, J = 8.5, 3.0 Hz, Ar-H), 6.73 (1H, dd, J = 8.5, 3.0 Hz, Ar-H), 6.66 (1H, d, J = 9.0 Hz, Ar-H), 5.16 (1H, d, J = 14.5 Hz, $CHHN$), 4.63 (1H, d, J = 14.5 Hz, $CHHN$), 4.57–4.37 (2H, m, OCH_2CH_2F), 4.00–3.84 (2H, m, OCH_2CH_2F), 1.95 (3H, s, CH_3). HRMS (CI) m/z calculated for $C_{23}H_{21}F_2NO_4$ ($[M]^+$), 413.1433; found, 413.1422.

3.11. N-(5-Fluoro-2-phenoxyphenyl)-N-(2-(2-fluoroethoxy)-5-methoxybenzyl)acetamide (FEDAA1106, **10**)

To a stirred solution of compound **9** (10 mg, 0.024 mmol) in DMSO (0.3 mL) was added NaH (60% dispersion in mineral oil, 1.9 mg, 0.048 mmol), and the mixture was stirred at rt for 30 min, followed by addition of CH_3I (5 μL, 0.08 mmol). After stirring at rt for 15 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with water and brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude product was purified by preparative TLC plate using EtOAc–hexanes (1:3) as eluent to afford **10** (7.2 mg, 70%) as a pale yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.35–7.26 (2H, m, Ar-H), 7.13–7.06 (1H, m, Ar-H), 6.92–6.77 (6H, m, Ar-H), 6.78–6.76 (2H, m, Ar-H), 6.68–6.54 (2H, m, Ar-H), 5.16 (1H, d, J = 15 Hz, $CHHN$), 4.68–4.59 (2H, d + m, J = 15 Hz, $CHHN$ + OCH_2CHHF), 4.42 (1H, m, OCH_2CHHF), 4.12–4.01 (1H, m, $OCHHCH_2F$), 3.99–3.84 (1H, m, $OCHHCH_2F$), 3.65 (3H, s, OCH_3), 1.95 (3H, s, CH_3).

3.12. N-(5-Fluoro-2-phenoxyphenyl)-N-(2-(2-fluoroethoxy)-5-[^{11}C]methoxybenzyl)acetamide ([^{11}C]FEDAA1106, [^{11}C]**10**)

[^{11}C]CO_2 was produced by the ^{14}N(p,α)^{11}C nuclear reaction in small volume (9.5 cm^3) aluminum gas target (CTI) from 11 MeV

proton cyclotron on research purity nitrogen (+1% O_2) in a Siemens radionuclide delivery system (Eclipse RDS-111). In a small reaction vial (5 mL), the precursor **9** (0.1 mg) was dissolved in CH_3CN (300 μL). To this solution was added NaH (1 mg). No-carrier-added (high specific activity) [^{11}C]CH_3OTf that was produced by the gas-phase production method [31] from [^{11}C]CO_2 through [^{11}C]CH_4 and [^{11}C]CH_3Br with silver triflate (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 reacted at rt for 5 min. The contents of the reaction vial were diluted with $NaHCO_3$ (1 mL, 0.1 M), and injected onto the semi-preparative HPLC column with 2 mL injection loop. The product fraction was collected, the solvent was removed by rotatory evaporation under vacuum, and the final product, [^{11}C]FEDAA1106 ([^{11}C]**10**), was formulated in saline, sterile-filtered through a sterile vented Millex-GS 0.22 μm cellulose acetate membrane, and collected into a sterile vial. Total radioactivity was assayed and total volume was noted for tracer dose dispensing. The overall synthesis, purification and formulation time was 20–25 min from EOB. Analytical HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6 × 250 mm; 70% CH_3CN–H_2O mobile phase; flow rate 1.5 mL/min; and UV (254 nm) and γ-ray (NaI) flow detectors. Semi-preparative HPLC was performed using a Prodigy (Phenomenex), S-5 μm, 12 nm, 10 × 250 mm i.d. C-18 column; 70% CH_3CN–H_2O mobile phase; flow rate 5.0 mL/min; and UV (254 nm) and γ-ray (NaI) flow detectors. Retention times in the analytical HPLC system were: t_R **9** = 3.20 min, t_R **10** = 5.13 min, t_R [^{11}C]**10** = 5.13 min. Retention times in the semi-preparative HPLC system were: t_R **9** = 5.62 min, t_R **10** = 7.78 min, t_R [^{11}C]**10** = 7.78 min. The radiochemical yields were 60–70% decay corrected to EOB, based on [^{11}C]CO_2.

4. Conclusions

An efficient and convenient synthesis of new PET PBR radioligand [^{11}C]FEDAA1106 has been well developed. The synthetic methodology employed classical organic chemistry such as benzylation, alkylation, reduction, bromination, acetylation, hydrogenation and methylation to prepare phenolic precursor and standard compound FEDAA1106. The target radioligand [^{11}C]FEDAA1106 was prepared by *O*-[^{11}C]methylation of its corresponding phenolic precursor using a reactive [^{11}C]methylating agent, [^{11}C]CH_3OTf, and isolated by HPLC purification procedure in high radiochemical yields, short overall synthesis time, and high specific radioactivities. These chemistry results combined with the reported *in vitro* and *in vivo* biological data [14,15,22] encourage further *in vivo* biological evaluation of new carbon-11 labeled FEDAA1106 analog, [^{11}C]FEDAA1106, as a potential PET radioligand for imaging of PBRs in brain and tumor.

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