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A novel ^{18}F-labeled pyridyl benzofuran derivative for imaging of β-amyloid plaques in Alzheimer's brains

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ABSTRACT

A potential probe for PET targeting β-amyloid plaques in Alzheimer's disease (AD) brain, FPYBF-1 (5-(5-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)benzofuran-2-yl)-*N*,*N*-dimethylpyridin-2-amine), was synthesized and evaluated. In experiments in vitro, FPYBF-1 displayed high affinity for Aβ(1–42) aggregates (K_i = 0.9 nM), and substantial labeling of β-amyloid plaques in sections of postmortem AD brains but not control brains. In experiments in vivo, [^{18}F]FPYBF-1 displayed good initial uptake (5.16%ID/g at 2 min postinjection) and rapid washout from the brain (2.44%ID/g at 60 min postinjection) in normal mice, and excellent binding to β-amyloid plaques in a murine model of AD. Furthermore, the specific labeling of plaques labeling was observed in autoradiographs of autopsied AD brain sections. [^{18}F]FPYBF-1 may be a useful probe for imaging β-amyloid plaques in living brain tissue.

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, irreversible memory loss, disorientation, and language impairment. The presence of β-amyloid (Aβ) aggregates in the brain is generally accepted as a hallmark of AD.[1,2] Since the only definitive diagnosis of AD is by pathological examination of autopsied brain tissue, the development of techniques which enable the imaging of β-amyloid plaques in vivo has been strongly desired.[3–5]

Preliminary studies with positron emission tomography (PET) suggested that [^{11}C]4-*N*-methylamino-4′-hydroxystilbene (SB-13),[6,7] [^{11}C] 2-(4′-(methylaminophenyl)-6-hydroxybenzothiazole (PIB),[8,9] [^{11}C]2-(2-[2-dimethylaminothiazol-5-yl]ethenyl)-6-(2-[fluoro]ethoxy)benzoxazole (BF-227),[10] and [^{11}C]-2-[6-(methylamino)pyridin-3-yl]-1,3-benzothiazol-6-ol (AZD2184)[11] differed in their uptake and retention in the brain between AD patients and controls (Fig. 1). Success in using ^{11}C-labeled tracers to image β-amyloid plaques in the brain in cases of suspected AD has provided considerable impetus for further refinement of this technique. However, the short half-life of ^{11}C ($t_{1/2}$: 20 min) limits its potential as a diagnostic tool. Since ^{18}F with a longer half-life isotope ($t_{1/2}$: 110 min) would be more useful for this purpose, recent efforts have focused on the development of comparable agents labeled with ^{18}F. Preliminary studies with [^{18}F]-2-(1-(2-(*N*-(2-fluoroethyl)-*N*-methylamino)naphthalene-6-yl)ethylidene)malononitrile (FDDNP)[12,13] showed differential uptake and retention in the brain of AD patients for the first time. More recently, a stilbene derivative, (*E*)-4-(*N*-methylamino)-4′-(2-(2-(2-[^{18}F]-fluoroethoxy)ethoxy)-ethoxy)-stilbene (BAY94-9172),[14,15] a styryl pyridine derivative, (*E*)-4-(2-(6-(2-(2-(2-([^{18}F]-fluoroethoxy)ethoxy)ethoxy)pyridyn-3-ylvinyl)-*N*-methyl benzenamine (AV-45),[16–18] and a PIB analogue, 2-(3-[^{18}F]-fluoro-4-methyamino-phenyl)benzothiazol-6-ol (GE-067),[19] have been shown to be useful for the imaging of β-amyloid plaques in living brain tissue in phase II or III clinical trials (Fig. 1).[20]

We have evaluated a series of fluorinated benzofuran derivatives as potential ^{18}F-labeled tracers for the imaging of β-amyloid plaques by PET.[21] These derivatives displayed excellent affinity for Aβ aggregates in vitro and in vivo. The penetration of brain tissues by 4-(5-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)benzofuran-2-yl)-*N*,*N*-dimethylbenzenamine (FPHBF-1, Fig. 2) was particularly encouraging. However, the slow washout of this probe from the normal mouse brain made it unsuitable for imaging in vivo. Therefore, a critical need to fine-tune the kinetics of the uptake and washout of benzofuran derivatives exists. Previous results regarding uptake into and clearance from the brain point to high lipophilicity as one of the reasons for a slow washout from the brain.[8,22–24]

We planned to develop a novel fluorinated pyridyl benzofuran derivative with less lipophilicity by displacing of the phenyl group in phenyl benzofuran with a pyridyl group. Kung and co-workers exploited a novel approach, fluoro-pegylation (FPEG) of the core structure, to label derivatives with ^{18}F.[25] Since this approach offers

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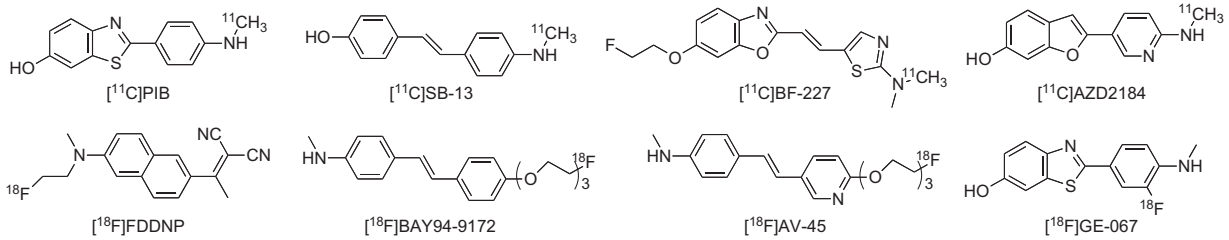


Figure 1. Chemical structure of PET imaging agents targeting β-amyloid plaques in AD patients.

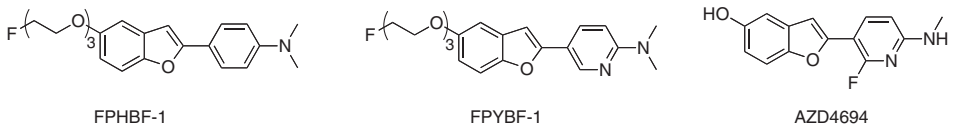


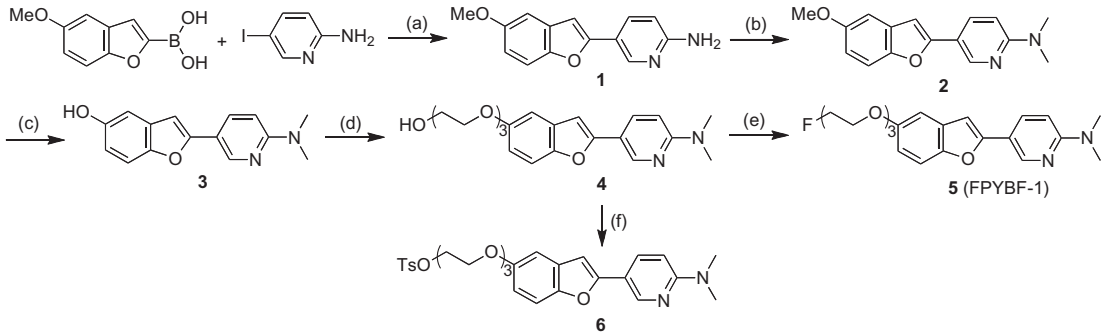
Figure 2. Chemical structure of benzofuran derivatives, FPHBF-1, FPYBF-1, and AZD4694.

a simple and easy way to incorporate ^{18}F into a target without an appreciable increase in lipophilicity, we selected FPEG for the labeling of pyridyl benzofuran derivatives. We designed a novel fluorinated ligand, 5-(5-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)benzofuran-2-yl)-*N*,*N*-dimethylpyridin-2-amine (FPYBF-1, Fig. 2) with a fluoropolyethylene glycol side chain and a dimethylamino pyridyl group. Another group recently reported a different fluorinated pyridyl benzofuran derivative, 2-(2-fluoro-6-(methylamino)pyridin-3-yl)benzofuran-5-ol (AZD4694, Fig. 2) to have potential for the imaging of cerebral β-amyloid plaques in living brain tissue.[26] However, they did not report the ^{18}F-labeling or in vivo characteristics of [^{18}F]AZD4694. This is the first time that a pyridyl benzofuran derivative has been successfully radiolabeled with ^{18}F and evaluated for the imaging of β-amyloid plaques in vivo.

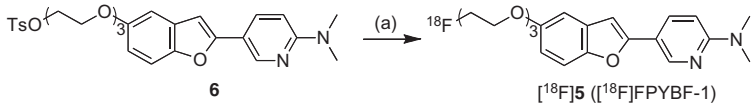
The synthesis of **5** (FPYBF-1) is outlined in Scheme 1. The key step in the formation of the pyridyl benzofuran backbone is accomplished by Suzuki coupling between 5-methoxybenzofuran-2-boronic acid and 2-amino-5-iodopyridine.[27] Suzuki coupling afforded the desired compound **1** in a yield of 52.1%. Conversion of **1** to the corresponding dimethylamino derivative **2** was achieved by dimethylation with paraformaldehyde and sodium cyanoborohydride (yield 62%). A methoxy group of **2** was converted to a hydroxyl group using BBr_3/CH_2Cl_2, which afforded **3** in a yield of 98.9%. The synthesis of **4**–**6** was achieved using conventional methods reported previously.[28] The ^{18}F-labeled **5** ([^{18}F]FPYBF-1) was prepared from a tosyl precursor (**6**) via a nucleophilic displacement reaction with a fluoride anion as shown in Scheme 2. Radiolabeling of the precursor generated [^{18}F]FPYBF-1 with an average radiochemical yield of 52% and radiochemical purity of >99%, and a specific activity of 242 GBq/μmol. The identity of [^{18}F]FPYBF-1 was verified by a comparison of the retention time with the nonradioactive compound.

Experiments in vitro to evaluate the affinity of FPYBF-1 for Aβ aggregates were carried out in solutions with [^{125}I]IMPY as the ligand according to conventional methods.[29,30] FPYBF-1 inhibited the binding of [^{125}I]IMPY in a dose-dependent manner with a K_i value of 0.9 nM, indicating that it has excellent affinity for Aβ(1–42) aggregates (Fig. 3). This K_i value is similar to that of phenyl benzofuran derivatives (K_i = 2.0 nM) reported previously,[21] and the affinity of the pyridyl benzofuran derivative for Aβ(1–42) aggregates remained high despite displacement of the phenyl group with a pyridyl group. This result also shows that the benzofuran scaffold can tolerate extensive structural modification.[21,22,31]

To evaluate the uptake of [^{18}F]FPYBF-1 in the brain, a biodistribution experiment was performed in normal mice (Table 1).



Scheme 1. Reagents and conditions: (a) $Pd(Ph_3P)_4$, Na_2CO_3 (aq)/dioxane, reflux.; (b) paraformaldehyde, sodium cyanoborohydride, acetic acid, rt; (c)BBr_3, CH_2Cl_2, rt; (d) 2-[2-(2-chloroethoxy)ethoxy]ethanol, K_2CO_3, DMF, 100 °C; (e) DAST, DME, 0 °C; (f) tosyl chloride, pyridine, rt.



Scheme 2. Reagents and conditions: (a) Kryptofix222, K_2CO_3, acetonitrile, 120 °C.

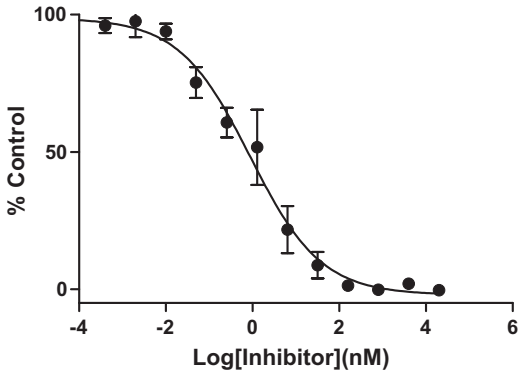


Figure 3. Competition curve of FPYBF-1 against [^{125}I]IMPY.

[^{18}F]FPYBF-1 displayed high uptake (5.16%ID/g) at 2 min postinjection, sufficient for PET, and the radioactivity in the brain cleared with time (2.44%ID/g at 60 min postinjection). Since normal brain tissue has no β-amyloid plaques to trap [^{18}F]FPYBF-1, the radioactivity should wash out quite rapidly. Therefore, the rapid clearance of [^{18}F]FPYBF-1 from normal brain is appropriate for the detection of β-amyloid plaques in the AD brain. One way to select a ligand with appropriate kinetics in vivo is to use the brain$_{2\ min}$/brain$_{60\ min}$ ratio as an index to compare the washout rate.[32] Although the brain$_{2\ min}$/brain$_{60\ min}$ ratio of [^{18}F]FPYBF-1 (2.1) was lower than that of [^{18}F]BAY94-9172 (4.8)[14] or [^{18}F]AV-45 (3.8),[16] it was improved as compared to the values for [^{18}F]FPHBF-1 (1.0) reported previously.[21] The favorable in vivo pharmacokinetics of [^{18}F]FPYBF-1 were achieved by changing the phenyl group in [^{18}F]FPHBF-1 to a pyridyl group. In HPLC analyses, [^{18}F]FPYBF-1 and [^{18}F]FPHBF-1 showed retention times of 14.8 and 36.5 min, respectively, indicating that [^{18}F]FPYBF-1 is less lipophilic than [^{18}F]FPHBF-1. Although lipophil-

Table 1
Biodistribution of radioactivity after injection of [^{18}F]FPYBF-1 in normal mice[a]

Organ	2 min	10 min	30 min	60 min
Blood	2.83 ± 0.89	2.13 ± 0.49	1.76 ± 0.09	1.98 ± 0.35
Brain	5.16 ± 0.30	3.75 ± 0.64	2.78 ± 0.22	2.44 ± 0.36
Bone	1.61 ± 0.33	1.33 ± 0.28	1.11 ± 0.13	1.42 ± 0.24

[a] Expressed as % of injected dose per gram. Each value represents the mean ± SD for five mice.

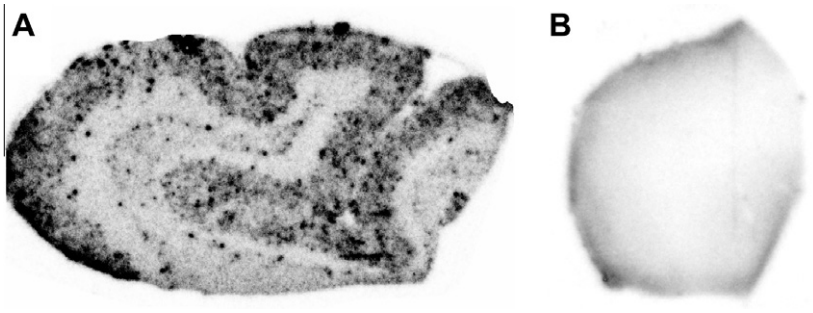


Figure 4. In vitro autoradiograms of sections of AD brain labeled with [^{18}F]FPYBF-1. Intensive labeling of β-amyloid plaques in brain tissue from AD patients (A). The control subject exhibits no labeling by this tracer (B).

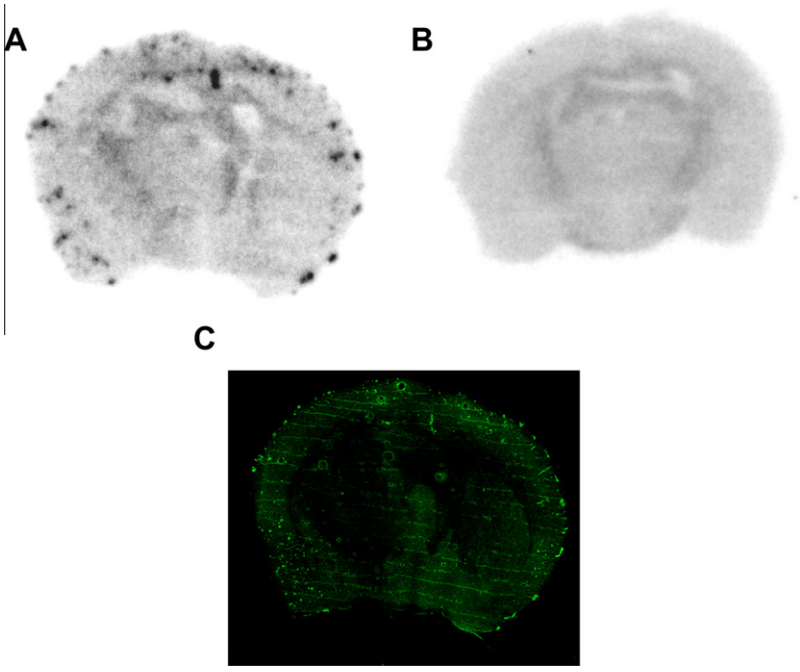


Figure 5. The labeling of β-amyloid plaques in vivo was visualized by autoradiography ex vivo with [^{18}F]FPYBF-1 in sections of Tg2576 mouse brain (A). The same section was also stained with thioflavin-S (C). Wild-type mouse brain showed no β-amyloid plaques (B).

icity is just one of the factors affecting the uptake of a compound into the brain,[4] it may explain the favorable pharmacokinetics of [^{18}F]FPYBF-1 in the brain. Uptake in the bone at 60 min was reduced (1.42%ID/g), suggesting little defluorination in vivo and interference with the imaging is expected to be relatively minor.

Next, sections of brain tissue from AD and control subjects (5 μm) were used to confirm the specific binding of [^{18}F]FPYBF-1 to β-amyloid plaques. Autoradiographic images revealed extensive labeling of β-amyloid plaques in the AD brain (Fig. 4A) but not control brain (Fig. 4B). The results suggest that [^{18}F]FPYBF-1 shows affinity for β-amyloid plaques in addition to synthetic Aβ aggregates.

To further characterize the potential of [^{18}F]FPYBF-1 as a probe for imaging β-amyloid plaques in living brain tissue, we carried out autoradiography ex vivo in Tg2576 mice (36 months, male) and in wild-type mice (36 months, male) as age-matched controls. Tg2576 transgenic mice show marked Aβ deposition in the cingulated cortex, entorhinal cortex, dentate gyrus, and CA1 hippocampal subfield by 11–13 months of age[33] and have been frequently used to evaluate the specific binding of β-amyloid plaques in experiments in vitro and in vivo.[28,34,35] The autoradiography showed clear labeling of β-amyloid plaques in the Tg2576 mouse brain (Fig. 5A). Wild-type mouse brain showed no such labeling (Fig. 5B). β-Amyloid plaques were confirmed present by co-staining the sections with thioflavin-S, a pathological dye commonly used to stain β-amyloid plaques (Fig. 5C). This is consistent with the results in vitro, showing [^{18}F]FPYBF-1 to be highly selective in binding to β-amyloid plaques in the brain.

In conclusion, based on previous results, we designed a novel fluorinated pyridyl benzofuran ligand, FPYBF-1, for the imaging of β-amyloid plaques in the brain. FPYBF-1 showed high binding affinity for Aβ aggregates in vitro and for β-amyloid plaques in sections of autopsied AD brain. It also displayed good uptake in the brain (5.16%ID/g at 2 min postinjection) and excellent binding to β-amyloid plaques ex vivo in transgenic mice. [^{18}F]FPYBF-1 is now under preclinical evaluation for use as a probe in PET. Other pyridyl benzofuran derivatives are also under investigation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.016.

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