Novel Benzofuran Derivatives for PET Imaging of β-Amyloid Plaques in Alzheimer's Disease Brains

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A novel series of benzofuran derivatives as potential positron emission tomography (PET) tracers targeting amyloid plaques in Alzheimer's disease (AD) were synthesized and evaluated. The syntheses of benzofurans were successfully achieved by an intramolecular Wittig reaction between triphenylphosphonium salt and 4-nitrobenzoyl chloride. When in vitro binding studies using AD brain gray matter homogenates were carried out with a series of benzofuran derivatives, all the derivatives examined displayed high binding affinities with K_i values in the subnanomolar range. Among these benzofuran derivatives, compound **8**, 5-hydroxy-2-(4-methyaminophenyl)benzofuran, showed the lowest K_i value (0.7 nM). In vitro fluorescent labeling of AD sections with compound **8** intensely stained not only amyloid plaques, but also neurofibrillary tangles. The ^{11}C labeled compound **8**, [^{11}C]**8**, was prepared by reacting the normethyl precursor, 5-hydroxy-2-(4-aminophenyl)benzofuran, with [^{11}C]methyl triflate. The [^{11}C]**8** displayed moderate lipophilicity (log P = 2.36), very good brain penetration (4.8%ID/g at 2 min after iv injection in mice), and rapid washout from normal brains (0.4 and 0.2%ID/g at 30 and 60 min, respectively). In addition, this PET tracer showed in vivo amyloid plaque labeling in APP transgenic mice. Taken together, the data suggest that a relatively simple benzofuran derivative, [^{11}C]**8**, may be a useful candidate PET tracer for detecting amyloid plaques in the brains of patients with Alzheimer's disease.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease characterized by dementia, cognitive impairment, and memory loss. Postmortem brains of AD patients reveal neuropathological features: the presence of senile plaques (SPs) and neurofibrillary tangles (NFTs), which contain β-amyloid (Aβ) peptides and highly phosphorylated tau proteins.[1,2] Although the precise mechanism of neuronal death in AD is still unknown, it is widely accepted that SPs and NFTs play a central role in its development. Thus, quantitative evaluation of SPs and/or NFTs in the brain with noninvasive techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) would allow presymptomatic identification of patients and monitoring of putative neuroprotective effects of novel treatments that are currently being investigated.[3−5]

A number of groups have worked to develop radiolabeled amyloid specific imaging agents, and clinical trials in AD patients have been reported with several agents including [^{18}F]-2-(1-(2-(*N*-(2-fluoroethyl)-*N*-methylamino)naphthalene-6-yl)ethylidene)malononitrile ([^{18}F]FDDNP),[6,7] [^{11}C]-2-(4-(methylamino)-phenyl)-6-hydroxybenzothiazole ([^{11}C]6-OH-BTA-1),[8,9] and [^{11}C]-4-*N*-methylamino-4′-hydroxystilbene ([^{11}C]SB-13)[10,11] (Figure 1), indicating that detecting amyloid plaques in the living human brain with amyloid imaging agents is potentially feasible.

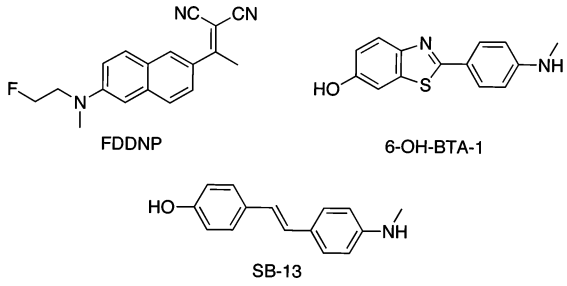

Figure 1. Chemical structures of amyloid imaging agents previously reported.

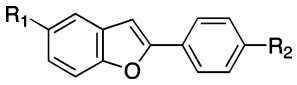

Figure 2. Structure of benzofuran derivatives. Compounds reported here include the following: R_1 = OCH_3, OH; R_2 = NH_2, $NHCH_3$, $N(CH_3)_2$.

In an attempt to develop more useful amyloid specific imaging agents, we chose to investigate a novel series of benzofuran derivatives, designed to be isosteric analogues of thioflavin-T (Figure 2). We reported previously that iodinated benzofuran derivatives displayed excellent binding affinities for Aβ(1−40) aggregates (K_i = 0.4−9.0 nM) and good brain penetration (>1.1%ID after an iv injection in normal mice). However, the in vivo nonspecific binding of these probes, reflected by their slow washout from the normal mouse brain, made them unsuitable for in vivo plaque imaging.[12] These previous findings suggested that additional structural changes to these benzofurans may lead to the development of useful amyloid specific imaging agents.

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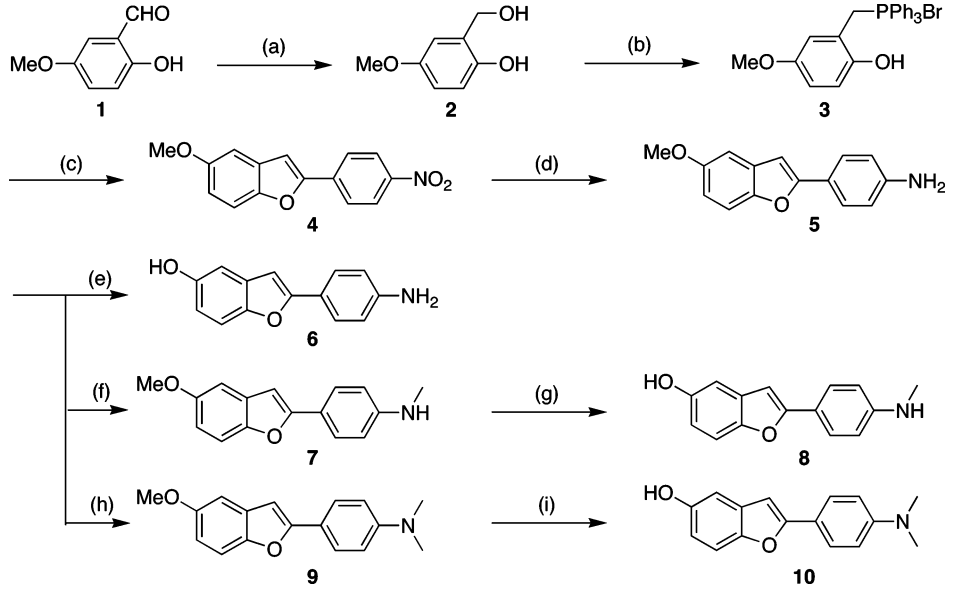

Figure 3. Synthesis of benzofuran derivatives. (a) EtOH, $NaBH_4$; (b) acetonitrile, PPh_3·HBr; (c) toluene, 4-nitrobenzoyl chloride, NEt_3; (d) EtOH, $SnCl_2$; (e) CH_2Cl_2, BBr_3; (f) MeOH, NaOMe, $(CH_2O)_n$, $NaBH_4$; (g) CH_2Cl_2, BBr_3; (h) AcOH, $(CH_2O)_n$, $NaCNBH_3$; (i) CH_2Cl_2, BBr_3.

Figure 4. Radiosynthesis of [^{11}C]**8**.

We report here the in vitro and in vivo evaluation of a novel series of benzofuran derivatives as β-amyloid imaging agents for PET.

Results and Discussion

Chemistry. The synthesis of the benzofuran derivatives is outlined in Figure 3. The key step for the formation of the benzofuran backbone was achieved by an intramolecular Wittig reaction between triphenylphosphonium salt and 4-nitrobenzoyl chloride (Figure 3). The desired Wittig reagent, **3**, was readily prepared from 2-methoxy-5-hydroxybenzyl alcohol, **2**, and triphenylphosphine hydrobromide (yield 84%). Wittig reactions gave the desired benzofuran backbone, **4**, at a yield of 33%. Conversion of **5** to the monomethylamino derivative, **7**, was achieved by first reducing the nitro group to an amino group with $SnCl_2$, and the subsequent monomethylation of the amino group was achieved by using a method previously reported.[13] Compound **5** was also converted to a dimethylamino derivative, **9**, by an efficient method[14] with paraformaldehyde, sodium cyanoborohydride, and acetic acid (yield 39%). The *O*-methyl groups of compounds **5**, **7**, and **9** were removed by reacting with BBr_3 to give **6**, **8**, and **10** at yields of 47, 39, and 7%, respectively.

Preparation of [^{11}C]**8** was done as in Figure 4. [^{11}C]**8** was readily synthesized from its *N*-normethyl precursor **6** and [^{11}C]-methyl triflate. Radiochemical yields of the final formulated product were 21%, decay corrected to end of bombardment (EOB). Radiochemical purities were >99% with specific activities of 37 GBq/μmol. The identity of the radiolabeled [^{11}C]-**8** was further confirmed by a comparison of its HPLC retention time with two other possible isomers, O-methylated, **5**, and N,O-dimethylated, **7**, which were independently prepared. On the basis of different retention times, it was concluded that the desired N-monomethylated, [^{11}C]**8**, was successfully prepared.

Biological Studies. An in vitro binding assay using AD brain homogenates demonstrated that substituted benzofuran derivatives competed with [^{125}I]IMPY binding to amyloid plaques with excellent binding affinities (Table 1). Comparing compounds **5**, **7**, **9** with compounds **6**, **8**, **10**, substitutions of the methoxy group at 5 position with the hydroxy group resulted in only small changes in binding affinity. Compounds **7** and **8** with a monomethylaminophenyl moiety on the phenylbenzofuran molecule displayed slightly lower (higher binding affinity) as compared to compounds **5** and **6** with the aminophenyl moiety or compounds **9** and **10** with the dimethylaminophenyl moiety. However, all of the benzofuran derivatives evaluated maintained good binding affinities in the nanomolar range of K_i values. The results of the binding study strongly support our previous report that benzofuran derivatives have considerable tolerance for structural modification.[12] Some reports have shown that preserving the binding affinity for amyloid plaques and providing compounds with moderate lipophilicity are prerequisites for successful amyloid imaging agents. Thus, we selected compound **8**, with moderate lipophilicity and the highest binding affinity for amyloid plaques in AD brain homogenates, for C-11 labeling and additional studies.

Table 1. Inhibition Constants of Benzofuran Derivatives Using [^{125}I]IMPY as the Ligand in AD Brain Gray Matter Homogenates

compound	K_i (nM)[a]
5	2.3 ± 0.1
6	11.5 ± 2.5
7	1.3 ± 0.2
8	0.7 ± 0.2
9	12.0 ± 2.0
10	2.8 ± 0.5

[a] Values are means ± standard error of the mean of three independent experiments.

Next, compound **8** was investigated for its neuropathological staining of senile plaques in human AD brain sections (Figure 5). Compound **8** stained neuritic plaques, as well as cerebrovascular amyloids (Figure 5, parts A and B). Since it is commonly assumed that neuritic plaques are formed even in very mild AD subjects, and that the density of the neuritic plaques is associated with the severity of dementia and the number of neurons,[15,16] clear staining of amyloid plaques with compound **8** demonstrates that it is a promising compound and deserves further investigation as a potential tool for early diagnosis. Furthermore, compound **8** also displayed high binding affinity for NFTs in

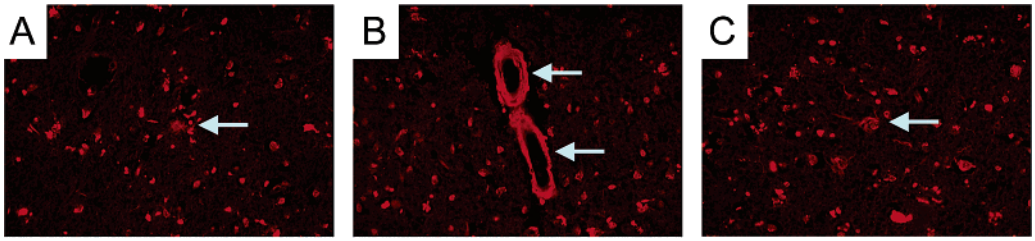

Figure 5. Neuropathological staining of compound **8** on 5 μm AD brain sections from the temporal cortex. (A) Amyloid plaques are stained with compound **8** (× 40 magnification). (B) Many cerebrovascular amyloids are intensely stained with compound **8** (× 40 magnification). (C) Compound **8** also stained neurofibrillary tangles (NFTs) (× 40 magnification).

Table 2. Biodistribution of Radioactivity after Intravenous Administration of [^{11}C]**8** in Mice[a]

time (min)	brain	blood
2	4.78 (1.10)	3.86 (0.36)
5	2.80 (0.63)	4.04 (1.14)
15	0.80 (0.31)	2.49 (0.67)
30	0.35 (0.08)	1.32 (0.41)
60	0.19 (0.04)	1.17 (0.14)

[a] Expressed as % injected dose per gram. Each value represents the mean (SD) for four animals at each interval.

the AD sections (Figure 5C). A previous study reported that a marked increase in the amount of NFTs accumulation in the hippocampus and entorhinal cortex was observed in the preclinical AD stage.[17] Because compound **8** intensely stained NFTs in human AD sections, it could detect increased NFT accumulation in the hippocampus and entorhinal cortex of the AD brain. These findings from the neuropathological staining of human AD sections suggest that compound **8** can bind amyloid plaques and NFTs with almost the same pattern of FDDNP[6] or X-34[18] previously reported, and quantitative evaluation of radiolabeled compound **8** in the brain may provide useful information on Aβ and tau pathology.

To predict the permeability of the blood−brain barrier, a 1-octanol/phosphate buffer (pH 7.4) partition coefficient of [^{11}C]-**8** was examined. The log P of [^{11}C]**8** was 2.36 at pH 7.4. Previous studies suggest that the optimal lipophilicity range for brain entry is observed for compounds with log P values between 1 and 3.[19] Below that range, passive diffusion through the BBB is poor, and above that range, binding of any radiotracers to blood components (e.g., red blood cells and albumin) is so great as to limit the amount available for brain entry. Since this ligand displayed moderate lipophilicity for BBB penetration, it was expected to show adequate brain uptake to detect amyloid plaques following systemic injection.

A biodistribution study in normal mice after iv injection showed that [^{11}C]**8** exhibited excellent brain uptake (4.8%ID/g of the brain at 2 min) and rapid washout (0.4 and 0.2%ID/g of the brain at 30 and 60 min, respectively), while the blood levels were relatively low at all time points measured (Table 2). When [^{11}C]**8** with high binding affinity to amyloid plaques is delivered into brain regions containing amyloid plaques, [^{11}C]**8** is expected to be trapped in amyloid plaque regions longer due to its high binding affinity. A biodistribution study of [^{11}C]**8** was carried out using normal mice. Since the normal brain has no Aβ plaques to trap [^{11}C]**8** in the brain, the radioactivity should wash out from the brain rapidly. Therefore, the rapid clearance of [^{11}C]**8** from the normal brain (over 90% washout of the radioactivity from the brain in 30 min or less) is an appropriate pharmacokinetic property for early detection of amyloid plaques in the AD brain. Our previous study demonstrated that a radioiodinated benzofuran derivative, 5-iodo-2-(4-methylaminophenyl)benzofuran, ([^{125}I]MABF) showed slow brain washout in normal mice, which prevented this ligand from being useful for in vivo imaging.[12] The unfavorable in vivo pharmacokinetics of [^{125}I]MABF were improved by changing iodine to a hydroxyl group at 5 position on its benzofuran molecule. Although many factors such as molecular size, ionic charge, and lipophilicity affect the brain clearance of compounds,[19−21] the precise mechanisms responsible for the rapid clearance of [^{11}C]**8** from the normal brain still remain unknown.

[^{11}C]PIB is currently the most widely utilized for detecting amyloid plaques in AD patients.[9,22,23] More recently, it has been reported that [^{11}C]SB-13 also showed similar performance to the more established tracer, [^{11}C]PIB.[11] Both tracers displayed appropriate properties as amyloid imaging agents: high binding affinity for amyloid plaques at 4.3 and 6.0 nM of K_i values for PIB and SB-13, respectively, in the in vitro binding assay, and high brain uptake and rapid clearance from the normal brain in animal studies. [^{11}C]PIB entered the brain rapidly (7.0%ID/g at 2 min after iv injection in mice) and cleared rapidly from normal mice brains (0.6%ID/g at 30 min after iv injection in mice). Since the ratio of 2-to-30 min mouse brain uptake after iv injection of [^{11}C]**8** was 0.07, which was comparable to that of [^{11}C]PIB, [^{11}C]**8** is also expected to have suitable in vivo pharmacokinetic properties for amyloid imaging in AD patients, similar to [^{11}C]PIB.

To confirm the in vivo labeling of amyloid plaques in a living mouse brain, we evaluated [^{11}C]**8** using a model mouse of AD, Tg2576 mice, which are specifically engineered to overproduce the amyloid plaques in the brain. Autoradiographic images of Tg2576 mouse brain at 30 min after injection of [^{11}C]**8** showed high radioactivity accumulation in the cerebral cortex and hippocampus (Figure 6-A). In contrast, wild-type mouse brain displayed no remarkable accumulation of [^{11}C]**8** in the brain (Figure 6-A). Furthermore, we confirmed that the hot spots of [^{11}C]**8** in Tg2576 brains corresponded with those of in vitro thioflavin-S staining in the same brain section (Figure 6, parts B and C). The specific in vivo labeling for amyloid plaques demonstrates the feasibility of using it as an in vivo PET imaging agent for detecting amyloid plaques in the brains of AD patients.

Conclusion

In summary, we introduced here novel benzofuran derivatives that have high in vitro binding affinity for amyloid plaques as candidate PET probes for imaging amyloid plaques. [^{11}C]**8**, one of these compounds, showed highly desirable properties: fast high initial uptake kinetics and rapid washout from the non-Aβ plaque-containing areas. In addition, ex vivo autoradiograms of brain sections from Tg2576 after injection of [^{11}C]**8** showed selective labeling of amyloid plaques with little nonspecific binding. These findings in the study suggest that [^{11}C]**8** is a promising PET probe for in vivo imaging amyloid plaques in the brain.

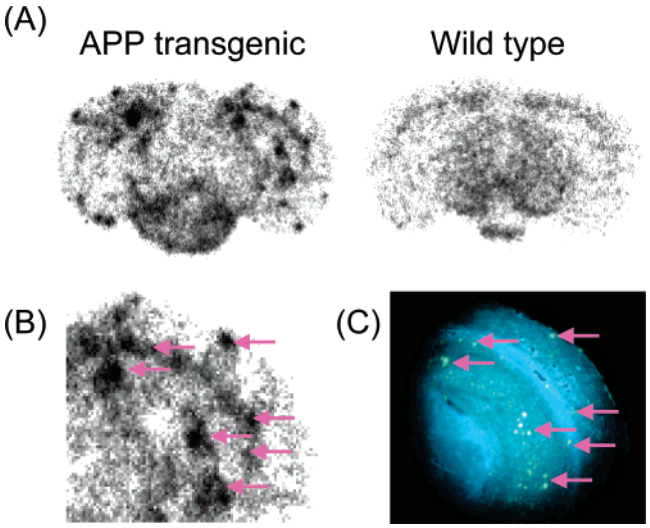

Figure 6. Ex vivo plaque labeling in brain sections from an APP transgenic mouse (A and B) and a wild-type mouse with [^{11}C]**8** (A). Amyloid plaques were confirmed by in vitro staining of the same section with thioflavin-S (C). Arrows show amyloid plaques labeled by both [^{11}C]**8** and thioflavin-S.

Experimental Sections

All reagents used in syntheses were commercial products and were used without further purification unless otherwise indicated. ^{1}H NMR spectra were obtained on a Varian Gemini 300 spectrometer with TMS as an internal standard. Coupling constants are reported in hertz. Multiplicity was defined by s (singlet), d (doublet), t (triplet), br (broad), and m (multiplet). Mass spectra were obtained on a JEOL IMS-DX instrument.

Chemistry. 2-Hydroxy-5-methoxybenzyl Alcohol (2). Sodium borohydride (250 mg, 6.61 mmol) was added to a stirring solution of 2-hydroxy-5-methoxybenzaldehyde (2.0 g, 13.1 mmol) in ethanol (20 mL) in an ice bath. The reaction mixture was stirred at room temperature for 1 h. After the solvent was removed, 1 N aqueous HCl solution (40 mL) was added to the residue and extracted with diethyl ether (40 mL). The organic phase was dried over Na_2SO_4 and filtered. The filtrate was concentrated to give 2.02 g of **2** (99.7%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.72 (s, 3H), 4.72 (s, 2H), 6.59 (s, 1H), 6.73 (m, 2H).

2-Hydroxy-4-methoxybenzyltriphenylphosphonium Bromide (3). A solution of **2** (2.02 g, 13.1 mmol) and triphenylphosphine hydrobromide (4.50 g, 13.1 mmol) in acetonitrile (40 mL) was stirred under reflux for 1 h. The solid that formed was filtered and washed with acetonitrile to give 5.28 g of **3** (84.1%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.38 (s, 3H), 4.87 (d, J = 14.7 Hz, 2H), 6.33 (s, 1H), 6.65−6.71 (m, 2H), 7.67−7.89 (m, 15H), 9.34 (s, 1H).

5-Methoxy-2-(4-nitrophenyl)benzofuran (4). A mixture of **3** (525 mg, 1.10 mmol) and 4-nitrobenzoyl chloride (206 mg, 1.11 mmol) in a mixed solvent (toluene 20 mL and triethylamine 0.5 mL) was stirred under reflux for 2 h. The precipitate was removed by filtration. The filtrate was concentrated, and the residue was recrystalized with ethyl acetate to give 97 mg of **4** (32.9%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.87 (s, 3H), 6.96−7.00 (m, 1H), 7.08 (d, 1H), 7.18 (d, 1H), 7.45 (d, J = 8.7 Hz, 1H), 7.96−7.99 (m, 2H), 8.31 (d, J = 9.3 Hz, 2H).

5-Methoxy-2-(4-aminophenyl)benzofuran (5). A mixture of **4** (170 mg, 0.63 mmol), $SnCl_2$ (1.50 g, 7.91 mmol) and ethanol (20 mL) was stirred under reflux for 2 h. After the mixture cooled to room temperature, 1 M NaOH (20 mL) was added and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4 and filtered. The filtrate was concentrated and the residue was purified by silica gel chromatography (hexane:ethyl acetate = 3:1) to give 140 mg of **5** (92.9%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.85 (s, 3H), 6.73 (d, J = 8.7 Hz, 2H), 6.84 (m, 2H), 6.99 (s, 1H), 7.36 (d, J = 8.7 Hz, 1H), 7.64 (d, J = 8.1 Hz, 2H). MS m/z 239 (M^+). Anal. $C_{15}H_{13}NO_2$: C, H, N.

5-Hydroxy-2-(4-aminophenyl)benzofuran (6). BBr_3 (2.8 mL, 1 M solution in CH_2Cl_2) was added to a solution of **5** (132 mg, 0.55 mmol) in CH_2Cl_2 (10 mL) dropwise in an ice bath. The mixture was allowed to warm to room temperature and stirred for 30 min. Water (20 mL) was added while the reaction mixture was cooled in an ice bath. The mixture was extracted with ethyl acetate, and the organic phase was dried over Na_2SO_4 and filtered. The filtrate was concentrated, and the residue was purified by HPLC on a C18 column with an isocratic solvent of acetonitrile/H_2O (1/1) at a flow rate of 7.0 mL/min to give 13 mg of **6** (10.5%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.50 (s, 2H), 6.63 (d, J = 8.4 Hz, 2H), 6.85 (s, 2H), 7.28 (d, 1H), 7.52 (d, J = 8.7 Hz, 2H), 9.08 (s, 1H). MS m/z 225 (M^+). Anal. $C_{14}H_{11}NO_2$: C, H, N.

5-Methoxy-2-(4-methylaminophenyl)benzofuran (7). A solution of NaOMe (28 wt % in MeOH, 0.27 mL) was added to a mixture of **5** (40 mg, 0.17 mmol) and paraformaldehyde (18 mg, 0.69 mmol) in methanol (5 mL) dropwise. The mixture was stirred under reflux for 1 h. After adding $NaBH_4$ (15 mg, 0.47 mmol), the solution was heated under reflux for 2 h. 1 M NaOH (30 mL) was added to the cold mixture and extracted with $CHCl_3$ (30 mL). The organic phase was dried over Na_2SO_4 and filtered. The solvent was removed, and the residue was purified by silica gel chromatography (hexane:ethyl acetate = 5:1) to give 20 mg of **7** (47.3%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.87 (s, 3H), 3.84 (s, 3H), 6.64 (d, J = 8.7 Hz, 2H), 6.72 (s, 1H), 6.83 (m, 1H), 7.35 (d, J = 9.0 Hz, 1H), 7.67 (d, J = 8.7 Hz, 2H). MS m/z 253 (M^+). Anal. $C_{16}H_{15}NO_2 \cdot 0.25H_2O$: C, H, N.

5-Hydroxy-2-(4-methylaminophenyl)benzofuran (8). The same reaction as described above to prepare **6** was employed, and **8** was obtained at a 26.8% yield from **7**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.87 (s, 3H), 3.84 (s, 3H), 6.64 (d, J = 8.7 Hz, 2H), 6.72 (s, 1H), 6.83 (m,1H), 7.35 (d, J = 9.0 Hz, 1H), 7.67 (d, J = 8.7 Hz, 2H). MS m/z 239 (M^+). Anal. $C_{15}H_{13}NO_2$: C, H, N.

5-Methoxy-2-(4-dimethylaminophenyl)benzofuran (9). To a stirred mixture of **5** (73 mg, 0.31 mmol) and paraformaldehyde (97 mg, 0.31 mmol) in AcOH (4 mL) was added in one portion $NaCNBH_3$ (96 mg, 1.53 mmol) at room temperature. The resulting mixture was stirred at room temperature for 8 h, and 1 M NaOH (30 mL) was added and extracted with CH_3Cl (30 mL). The organic phase was dried over Na_2SO_4 and filtered. The solvent was removed, and the residue was purified by silica gel chromatography (hexane:ethyl acetate = 14:1) to give 32 mg of **9** (39.3%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.08 (s, 6H), 6.70−6.77 (m, 3H), 7.43 (d, J = 9.0 Hz, 1H), 7.72−7.82 (m, 3H), 8.34 (s, 1H). MS m/z 267 (M^+). Anal. $C_{17}H_{17}NO_2$: C, H, N.

5-Hydroxy-2-(4-dimethylaminophenyl)benzofuran (10). The same reaction as described above to prepare **6** was employed, and **10** was obtained in 6.6% yield from **9**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.97 (s, 6H), 6.66 (m, 1H), 6.79 (d, J = 8.7 Hz, 2H), 6.85 (s, 1H), 6.95 (s, 1H), 7.32 (d, J = 9.0 Hz, 1H), 7.67 (d, J = 8.7 Hz, 2H). 9.11 (s, 1H). MS m/z 253 (M^+). Anal. $C_{16}H_{15}NO_2 \cdot 0.25H_2O$: C, H, N.

Radiolabeling. ^{11}C was produced via a ^{14}N(p,α)^{11}C reaction with 16-MeV protons on a target of nitrogen gas with an ultracompact cyclotron (CYPRIS model 325R; Sumitomo Heavy Industry Ltd.). The $^{11}CO_2$ produced was transported to an automated synthesis system of ^{11}C-methyl iodide (CUPID C-100; Sumitomo Heavy Industry Ltd.), and converted sequentially to [^{11}C]methyl triflate ([^{11}C]CH_3OTf) by the previously described method of Jewett.[24] [^{11}C]**8** was produced by reacting [^{11}C]CH_3OTf with the normethyl precursor, 5-hydroxy-2-(4-aminophenyl)benzofuran, **6** (0.4 mg), in 250 μL of methyl ethyl ketone (MEK). After complete transfer of the [^{11}C]CH_3OTf, the reaction solvent was dried with a stream of nitrogen gas. The residue taken up in 200 μL of acetonitrile was purified by a reverse phase HPLC system (a Shimadzu LC-6A isocratic pump, a Shimadzu SPD-6A UV detector and a Aloka NDW-351D scintillation detector) on a Cosmosil C18 column (Nacalai Tesque, 5C18-AR-300, 10 × 250 mm) with an isocratic solvent of 0.1 M $AcONH_4$/acetonitrile (45/55) at a flow rate of 6.0

mL/min. The desired fraction (Rt 7.6 min) was collected in a flask and evaporated to dryness. Radiochemical yields, purities and specific activity of [^{11}C]**8** were further confirmed by analytical reverse phase HPLC on 5C18-AR-300 column (Nacalai Tesque, 4.6 × 150 mm, 0.1 M $AcONH_4$/acetonitrile (50/50), 1.0 mL/min, Rt-precursor 3.0 min; Rt-product 4.4 min).

Binding Studies. Binding studies were carried out according to the method described previously.[25] [^{125}I]IMPY (6-iodo-2-(4′-dimethylamino)phenyl-imidazo[1,2]pyridine) with 81.4 TBq/mmol specific activity and greater than 95% radiochemical purity was prepared using the standard iododestannylation reaction, and purified by a simplified C-4 mini column as described previously.[26] Binding assays were carried out in 12 × 75 mm borosilicate glass tubes. The reaction mixture contained 50 μL of AD brain homogenates (20−50 μg), 50 μL of [^{125}I]IMPY (0.04−0.06 nM diluted in PBS), and 50 μL of inhibitors (10^{-5}−10^{-10} M diluted serially in PBS containing 0.1% bovine serum albumin) in a final volume of 1 mL. Nonspecific binding was defined in the presence of 600 nM IMPY in the same assay tubes. The mixture was incubated at 37 °C for 2 h, and the bound and free radioactivity were separated by vacuum filtration through Whatman GF/B filters using a Brandel M-24R cell harvester followed by 2 × 3 mL washes with PBS at room temperature. Filters containing the bound I-125 ligand were counted in a gamma counter (Packard 5000) with 70% counting efficiency. Under the assay conditions, the specifically bound fraction was less than 15% of the total radioactivity. The results of inhibition experiments were subjected to nonlinear regression analysis using EBDA,[27] from which K_i values were calculated.

Partition Coefficient Determination. The determination of partition coefficient of [^{11}C]**8** was performed according to the procedure previously reported with some modifications.[28] 1-Octanol (3 mL) and 0.1 M phosphate buffer (pH 7.4, 3 mL) were pipetted into a test tube with 2 μL of [^{11}C]**8** (370 kBq). The test tube was vortexed for 3 min at room temperature, followed by centrifugation for 5 min. Two weighed samples (500 μL each) from the 1-octanol and buffer layers were counted in a well counter. The partition coefficient was determined by calculating the ratio of cpm/500 μL of 1-octanol to that of buffer. Samples from 1-octanol layer were repartitioned until consistent partitions of coefficient values were obtained. The measurement was done in triplicate and repeated four times.

Staining of Senile Plaques in Human AD Brain Sections. Postmortem brain tissues from AD cases (74 and 79 years old) were confirmed by conventional neuropathology, including amyloid and tau staining, in addition to silver staining. Experiments were performed under the regulations of the ethics committee of Osaka City University Medical School. Five-micrometer-thick serial sections of paraffin-embedded blocks from the temporal cortex were used for staining. Paraffin sections were first taken through five 10-min incubations in xylene, five 10-min incubations in 100% ethanol to completely deparaffinize them, followed by two 5-min washes in water and then PBS. Tissue sections were immersed in the compound solution at various concentrations. At more than 0.5 mM concentration, we used 50% ethanol in PBS to ensure the full solubilization of the compound. Finally, the sections were washed in PBS for 15 min. Thereafter, the sections were incubated in ethanol and xylene and embedded in Entellan Neu (Merck, Darmstadt, Germany). Fluorescent sections were viewed using a BX50 Fluoromicroscope with an M-3204C CCD camera (Olympus, Tokyo) equipped with a G-filter. The sections were also immunostained with DAB as a chromogen using antibodies against Aβ[29] and tau[30] protein, as previously described.

In Vivo Biodistribution in Normal Mice. Animal studies were conducted in accordance with our institutional guidelines and were approved by Kyoto University Animal Care Committee. A saline solution (100 μL) containing ascorbic acid (1 mg/mL) of [^{11}C]**8** (3.7 MBq) was injected directly into the tail vein of ddY mice (male, 6-week-old). The mice were sacrificed at various time points postinjection. The organs of interest were removed and weighed, and the radioactivity was counted with an automatic gamma counter (COBRAII, Packard).

Ex Vivo Plaque Labeling with [^{11}C]8 in Transgenic Mice. The ex vivo evaluation was performed using Tg2576 transgenic (female, 22-month-old) and wild type (female, 22-month-old) mice which were kindly provided by Dr. Tooyama (Shiga University of Medical Science, Otsu, Japan). A saline solution (200 μL) containing ascorbic acid (1 mg/mL) of the labeled agent (7.4−9.3 MBq) was injected directly into the tail vein. The mice were sacrificed by decapitation at 30 min after intravenous injection. The brains were immediately removed and frozen in powdered dry ice. Sections of 30 μm were cut and exposed to a BAS imaging plate (Fuji Film, Tokyo, Japan) for 3 h. Ex vivo autoradiographic images were obtained using a BAS2500 scanner system (Fuji Film). After autoradiographic examination, the same sections were stained with thioflavin-S to confirm the presence of amyloid plaques. For the staining with thioflavin-S, sections were immersed in 0.125% thioflavin-S solution containing 50% ethanol for 3 min and washed in 50% ethanol. After drying, the sections were then examined using a TE−300 Fluoromicroscope with a COOLPIX910 CCD camera (Nikon, Tokyo) equipped with an EPI−FL filter block.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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