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^{18}F-labeled flavones for in vivo imaging of β-amyloid plaques in Alzheimer's brains

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ABSTRACT

In vivo imaging of β-amyloid (Aβ) aggregates in the brain may lead to early detection of Alzheimer's disease (AD) and monitoring of the progression and effectiveness of treatment. The purpose of this study was to develop novel ^{18}F-labeled amyloid-imaging probes based on flavones as a core structure. Fluoropegylated (FPEG) flavone derivatives were designed and synthesized. The affinity of the derivatives for Aβ aggregates varied from 5 to 321 nM. In brain sections of AD model mice, FPEG flavones with the dimethylamino group intensely stained β-amyloid plaques. In biodistrubution experiments using normal mice, they displayed high uptake in the brain ranging from 2.9 to 4.2%ID/g at 2 min postinjection. The radioactivity washed out from the brain rapidly (1.3–2.0%ID/g at 30 min), which is highly desirable for β-amyloid imaging agents. FPEG flavones may be potential PET imaging agents for β-amyloid plaques in Alzheimer's brains.

1. Introduction

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 postmortem staining of affected brain tissues, the development of techniques which enable one to image β-amyloid plaques in vivo has been strongly desired.[3–5]

Recent success in developing radiolabeled agents targeting Aβ aggregates has provided a window of opportunity to improve the diagnosis of AD. Preliminary reports of positron emission tomography (PET) imaging suggested that [^{11}C]4-*N*-methylamino-4′-hydroxystilbene (SB-13),[6,7] [^{11}C] 2-(4′-(methylaminophenyl)-6-hydroxy benzothiazole (PIB),[8,9] and [^{11}C]2-(2-[2-dimethylaminothiazol-5-yl]ethenyl)-6-(2-[fluoro]ethoxy)benzoxazole (BF-227)[10] showed differential uptake and retention in the brain of AD patients as compared to controls. But ^{11}C is a positron-emitting isotope with a short $t_{1/2}$ (20 min), which limits its clinical application. Recent efforts have focused on the development of comparable agents labeled with a longer half-life isotope, ^{18}F ($t_{1/2}$: 110 min). Preliminary studies with [^{18}F]-2-(1-(2-(*N*-(2-fluoroethyl)-*N*-methylamino)-naphthalen-6-yl)ethylidene)malononitrile ([^{18}F]FDDNP)[11,12] showed differential uptake and retention in the brain of AD patients for the first time. More recently, a stilbene derivative, [^{18}F]BAY94-9172, has been shown to be useful for the imaging of β-amyloid plaques in living human brain tissue in clinical trials.[13]

To search for more candidates for ^{18}F-labeled β-amyloid imaging agents for PET, we planned to evaluate a new series of flavone derivatives previously reported as useful for imaging β-amyloid by single photon emission computed tomography (SPECT).[14] The derivatives showed good affinity for Aβ aggregates in vitro in binding experiments using synthetic Aβ aggregates and neuropathological staining of AD brain sections, suggesting these classes of radioiodinated flavones to be potential imaging agents.

Recently, Kung et al. exploited a novel approach by using fluoro-pegylation (FPEG) of the core structure for ^{18}F labeling of derivatives.[15] Since this approach offers a simple and easy way to incorporate ^{18}F into the target without an appreciable increase in lipophilicity, we planned to apply it to the labeling of flavone derivatives. In addition to the structural characteristics of flavone as the pharmacophore, it has been shown that electron-donating groups such as amino, methylamino, dimethylamino, methoxy, or hydroxy groups play a critical role in the binding of Aβ aggregates.[6,8,16,17] With these considerations, we designed 12 fluorinated flavones with a fluorine or FPEGylation at position 4 and an electron-donating group at position 4′ (Fig. 1).

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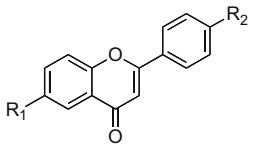


Compound	R_1	R_2
8a	FCH_2CH_2O	$N(CH_3)_2$
8b	$F(CH_2CH_2O)_2$	$N(CH_3)_2$
8c	$F(CH_2CH_2O)_3$	$N(CH_3)_2$
12	FCH_2CH_2O	NH_2
13	FCH_2CH_2O	$NHCH_3$
15b	$F(CH_2CH_2O)_2$	NH_2
15c	$F(CH_2CH_2O)_3$	NH_2
17b	$F(CH_2CH_2O)_2$	$NHCH_3$
17c	$F(CH_2CH_2O)_3$	$NHCH_3$
21	F	NH_2
22	F	$NHCH_3$
23	F	$N(CH_3)_2$

Figure 1. Chemical structure of FPEG-flavone derivatives.

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

2. Experimental

All reagents were commercial products and 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 is defined by s (singlet), d (doublet), t (triplet), br (broad), and m (multiplet). Mass spectra were obtained on a JEOL IMS-DX instrument.

2.1. Chemistry

2.1.1. 4-Nitrobenzoic acid 2-acetyl-4-methoxyphenyl ester (1)

To a stirring solution of 4-nitrobenzoyl chloride (1.8 g, 10 mmol) in pyridine (20 mL) was added 2-hydroxy-5-methoxyacetophenone (1.6 g, 10 mmol). The reaction mixture was stirred at room temperature for 3 h, and poured into a 1 N aqueous HCl/ice solution with vigorous stirring. The precipitate that formed was filtered and washed with water to yield acetophenone **1** (2.9 g, 90.4% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.54 (s, 3H), 3.89 (s, 3H), 7.15–7.16 (m, 2H), 7.38 (d, J = 2.0 Hz, 1H), 8.37 (s, 4H).

2.1.2. 1-(5-Methoxy-2-hydroxyphenyl)-3-(4-nitrophenyl)propane-1,3-dione (2)

A solution of acetophenone **1** (2.9 g, 9.0 mmol) and pyridine (50 mL) was heated to 50 °C, and to it was added pulverized potassium hydroxide (2.5 g, 45.2 mmol). The reaction mixture was stirred for 90 min, and when it had cooled, 30 mL of 10% aqueous acetic acid solution was added. The yellow precipitate that formed was filtered to yield **2** (2.5 g, 88.1% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.85 (s, 3H), 6.84 (s, 1H), 6.99 (d, J = 6.9 Hz, 1H), 7.15–7.18 (m, 1H), 7.22 (d, J = 2.2 Hz, 1H), 8.10 (d, J = 6.6 Hz, 2H), 8.35 (d, J = 6.6 Hz, 2H), 11.5 (s, 1H).

2.1.3. 6-Methoxy-4′-nitroflavone (3)

A mixture of the diketone **2** (2.5 g, 8.0 mmol), concentrated sulfuric acid (2 mL), and glacial acetic acid (40 mL) was heated at 100 °C for 2 h and cooled to room temperature. The mixture was poured onto crushed ice, and the resulting precipitate was filtered to yield **3** (1.5 g, 63.5%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.93 (s, 3H), 6.92 (s, 1H), 7.31–7.37 (m, 1H), 7.54–7.61 (m, 2H), 8.11 (d, J = 9.3 Hz, 2H), 8.39 (d, J = 9.3 Hz, 2H).

2.1.4. 6-Methoxy-4′-aminoflavone (4)

A mixture of **3** (3.0 g, 10.2 mmol), $SnCl_2$ (7.6 g, 39.8 mmol), and EtOH (30 mL) was stirred under reflux for 40 min. After the mixture had cooled to room temperature, 1 M NaOH (50 mL) was added until the mixture became alkaline. After extraction with ethyl acetate, the combined organic layers were washed with brine, dried over Na_2SO_4, and filtered. The solvent was removed, and the residue was purified by silica gel chromatography (hexane/ethyl acetate = 1:2) to give 1.3 g of **4** (65.3% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.91 (s, 3H), 4.11 (s, broad, 2H), 6.69 (s, 1H), 6.75 (d, J = 6.6 Hz, 2H), 7.24–7.27 (m, 1H), 7.47 (d, J = 6.6 Hz, 1H), 7.59 (d, J = 2.2 Hz, 1H), 7.75 (d, J = 6.6 Hz, 2H).

2.1.5. 6-Methoxy-4′-dimethylaminoflavone (5)

To a mixture of **4** (401 mg, 1.5 mmol) and paraformaldehyde (450 mg, 15 mmol) in AcOH (10 mL) was added $NaCNBH_3$ (471 mg, 7.5 mmol) in one portion at room temperature. The resulting mixture was stirred at room temperature for 1.5 h, and the addition of 1 M NaOH was followed by extraction with CH_3Cl. The organic phase was dried over Na_2SO_4. The solvent was removed, and the residue was purified by silica gel chromatography ($CHCl_3$/MeOH = 20:1) to give 371 mg of **5** (83.7% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.07 (s, 6H), 3.91 (s, 3H), 6.70 (s, 1H), 6.75 (d, J = 9.0 Hz, 2H), 7.24–7.26 (m, 1H), 7.47 (d, J = 9.0 Hz, 1H), 7.59 (d, J = 2.2 Hz, 1H), 7.81 (d, J = 9.0 Hz, 2H).

2.1.6. 6-Hydroxy-4′-dimethylaminoflavone (6)

To a solution of **5** (371 mg, 1.5 mmol) in CH_2Cl_2 (10 mL) at 10 °C was added BBr_3 (7.5 mL, 1 M solution in CH_2Cl_2) dropwise in an ice bath. The mixture was allowed to warm to room temperature and was stirred for 12 h. Water was added while the reaction mixture was cooled in an ice bath to keep the reaction temperature at 0 °C. After extraction with CH_2Cl_2, the combined organic phase was dried over Na_2SO_4. The filtrate was concentrated and the residue was purified by silica gel chromatography ($CHCl_3$/MeOH = 20:1) to give 303 mg of **6** (71.8% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.05 (s, 6H), 6.74 (d, J = 9.3 Hz, 2H), 7.26 (d, J = 3.0 Hz, 1H), 7.45 (d, J = 9.0 Hz, 1H), 7.58 (d, J = 3.0 Hz, 1H), 7.77 (d, J = 9.0 Hz, 2H).

2.1.7. 6-Hydroxyethoxy-4′-dimethylaminoflavone (7a)

To a solution of **6** (85 mg, 0.30 mmol) and ethylene chlorohydrin (100 μL, 1.50 mmol) in DMSO (3 mL) was added anhydrous K_2CO_3 (41 mg, 0.90 mmol). The reaction mixture was stirred at 120 °C for 48 h, then poured into water. After extraction with chloroform, the organic layers were combined and dried over Na_2SO_4. Evaporation of the solvent afforded a residue, which was purified by silica gel chromatography ($CHCl_3$/MeOH = 33:1) to give 30 mg of **7a** (30.5%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.06 (s, 6H), 3.98–4.05 (m, 2H), 4.17–4.23 (m, 2H), 6.69 (s, 1H), 6.76 (d, J = 9.3 Hz, 2H), 7.28 (d, J = 3.0 Hz, 1H), 7.47 (d, J = 9.0 Hz, 1H), 7.60 (d, J = 3.0 Hz, 1H), 7.80 (d, J = 9.0 Hz, 2H).

2.1.8. 6-(2-(2-Hydroxy-ethoxy)-ethoxy)-4′-dimethylaminoflavone (7b)

To a solution of **6** (82 mg, 0.29 mmol) and ethylene glycol mono-2-chloroethyl ether (37 μL, 0.35 mmol) in DMF (3 mL) was added anhydrous K_2CO_3 (120 mg, 0.87 mmol). The reaction mixture was stirred at 120 °C for 11 h, then poured into water. After extraction with chloroform, the organic layers were combined and dried over Na_2SO_4. Evaporation of the solvent afforded a residue, which was purified by silica gel chromatography (hexane/ethyl acetate = 10:1) to give 107 mg of **7b** (99.3%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.07 (s, 6H), 3.69 (t, J = 5.1 Hz, 2H), 3.79 (s, 2H), 3.91 (t, J = 4.5 Hz, 2H), 4.26 (t, J = 4.5 Hz, 2H), 6.69 (s, 1H), 6.75 (d, J = 9.0 Hz, 2H), 7.27–7.30 (m, 1H), 7.47 (d, J = 9.0 Hz, 1H), 7.64 (d, J = 2.2, 1H), 7.81 (d, J = 9.0 Hz, 2H).

2.1.9. 6-(2-(2-(2-Hydroxy-ethoxy)-ethoxy)ethoxy)-4′-dimethylaminoflavone (7c)

To a solution of **6** (100 mg, 0.36 mmol) and 2-[2-(2-chloroethoxy)ethoxy]ethanol (62 μL, 0.43 mmol) in DMF (3 mL) was added anhydrous K_2CO_3 (148 mg, 1.07 mmol). The reaction mixture was stirred at 120 °C for 17 h, then poured into water. After extraction with chloroform, the organic layers were combined and dried over Na_2SO_4. Evaporation of the solvent afforded a residue, which was purified by preparative TLC ($CHCl_3$/MeOH = 20:1) to give 76 mg of **7c** (51.1%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.07 (s, 6H), 3.62–3.76 (m, 8H), 3.91 (t, J = 4.5 Hz, 2H), 4.26 (t, J = 4.5 Hz, 2H), 6.69 (s, 1H), 6.77 (d, J = 9.3 Hz, 2H), 7.28–7.33 (m, 1H), 7.47 (d, J = 9.0 Hz, 1H), 7.60 (d, J = 2.2, 1H), 7.81 (d, J = 9.0 Hz, 2H).

2.1.10. 6-Fluoroethoxy-4′-dimethylaminoflavone (8a)

To a solution of **7a** (30 mg, 0.09 mmol) in ethylene glycol dimethyl ether (3 mL) was added dimethylamino sulfur trifluoride (DAST) (30 μL, 0.23 mmol) in a dry ice-acetone bath. The reaction mixture was stirred for 6 h at room temperature. The mixture was then poured into a saturated $NaHSO_3$ solution and after extraction with chloroform, the organic phase was separated, dried over Na_2SO_4, and filtered. The residue was purified by silica gel chromatography (hexane/ethyl acetate = 2:1) to give 16 mg of **8a** (53.0%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.93 (s, 6H), 4.26–4.40 (m, 2H), 4.70–4.92 (m, 2H), 6.71 (s, 1H), 6.76 (d, J = 9.0 Hz, 2H), 7.29–7.35 (m, 1H), 7.50 (d, J = 9.0 Hz, 1H), 7.59 (d, J = 3.3 Hz, 1H), 7.82 (d, J = 9.3 Hz, 2H). EI-MS m/z 327 (M^+).

2.1.11. 6-(2-(2-Fluoro-ethoxy)-ethoxy)-4′-dimethylaminoflavone (8b)

To a solution of **7b** (29 mg, 0.08 mmol) in 1,2-dimethoxyethane (DME) (5 mL) was added DAST (21 μL, 0.16 mmol) in a dry ice-acetone bath. The reaction mixture was stirred for 1.5 h at room temperature. The mixture was then poured into a saturated $NaHSO_3$ solution and after extraction with chloroform, after the organic phase was separated, dried over Na_2SO_4, and filtered. The residue was purified by preparative TLC ($CHCl_3$/MeOH = 20:1) to give 15 mg of **8b** (51.5%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.07 (s, 6H), 3.79 (t, J = 4.2 Hz, 1H), 3.89–3.96 (m, 3H), 4.26 (t, J = 4.8 Hz, 2H), 4.54 (t, J = 4.2 Hz), 4.69 (t, J = 4.2 Hz), 6.70 (s, 1H), 6.76 (d, J = 9.0 Hz, 2H), 7.27–7.33 (m, 1H), 7.49 (d, J = 9.3 Hz, 1H), 7.59 (d, J = 3.0, 1H), 7.81 (d, J = 9.0 Hz, 2H). EI-MS m/z 371 (M^+).

2.1.12. 6-(2-(2-(2-Fluoro-ethoxy)-ethoxy)ethoxy)-4′-dimethylaminoflavone (8c)

To a solution of **7c** (141 mg, 0.34 mmol) in DME (5 mL) was added DAST (90 μL, 0.68 mmol) in a dry ice-acetone bath. The reaction mixture was stirred for 1 h at room temperature. The mixture was then poured into saturated $NaHSO_3$ solution and extracted with chloroform. After the organic phase was separated, dried over Na_2SO_4 and filtered, the residue was purified by preparative TLC (hexane/ethyl acetate = 1:5) to give 21 mg of **8c** (14.9%). ^{1}H NMR ($CDCl_3$) δ: 3.08 (s, 6H), 3.69–3.81 (m, 6H), 3.91 (t, J = 4.8 Hz, 2H), 4.24 (t, J = 4.8 Hz, 2H), 4.49 (t, J = 4.5 Hz, 1H), 4.65 (t, J = 4.5 Hz, 1H), 6.69 (s, 1H), 6.76 (d, J = 9.0 Hz, 2H), 7.27–7.33 (m, 1H), 7.48 (d, J = 9.0 Hz, 1H), 7.59 (d, J = 2.2, 1H), 7.81 (d, J = 9.0 Hz, 2H). EI-MS m/z 415 (M^+).

2.1.13. 6-Hydroxy-4′-nitroflavone (9)

The same reaction as described above to prepare **6** was used, and 560 mg of **9** was obtained from **3** and BBr_3. EI-MS m/z 283 (M^+).

2.1.14. 6-(2-Hydroxy-ethoxy)-4′-nitroflavone (10a)

The same reaction as described above to prepare **7a** was used, and 40 mg of **10a** was obtained from **9** in a yield of 9.9%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.88–4.02 (m, 2H), 4.13–4.20 (m, 2H), 6.89 (s, 1H), 7.38–7.41 (m, 1H), 7.59 (d, J = 9.3 Hz, 1H), 7.67 (d, J = 3.3 Hz, 1H), 8.12 (d, J = 9.0 Hz, 2H), 8.46 (d, J = 9.0 Hz, 2H).

2.1.15. 6-(2-(2-Hydroxy-ethoxy)-ethoxy)-4′-nitroflavone (10b)

The same reaction as described above to prepare **7b** was used, and 830 mg of **10b** was obtained from **9**. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.70 (t, J = 5.1 Hz, 2H), 3.79 (s, 2H), 3.93 (t, J = 5.0 Hz, 2H), 4.28 (t, J = 4.8 Hz, 2H), 6.90 (s, 1H), 7.37–7.41 (m, 1H), 7.56 (d, J = 9.3 Hz, 1H), 7.66 (d, J = 3.3 Hz, 1H), 8.10 (d, J = 9.0 Hz, 2H), 8.40 (d, J = 9.0 Hz, 2H).

2.1.16. 6-(2-(2-(2-Hydroxy-ethoxy)-ethoxy)ethoxy)-4′-nitroflavone (10c)

The same reaction as described above to prepare **7c** was used, and **10c** was obtained from **9** in a yield of 83.1%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.64 (t, J = 4.5 Hz, 2H), 3.71–3.78 (m, 6H), 3.93 (t, J = 4.8 Hz, 2H), 4.27 (t, J = 4.5 Hz, 2H), 6.90 (s, 1H), 7.38–7.42 (m, 1H), 7.55 (d, J = 9.0 Hz, 1H), 7.61 (d, J = 3.1 Hz, 1H), 8.10 (d, J = 8.7 Hz, 2H), 8.39 (d, J = 8.7 Hz, 2H). EI-MS m/z 415 (M^+).

2.1.17. 6-(2-Fluoro-ethoxy)-4′-nitroflavone (11)

The same reaction as described above to prepare **8a** was used, and 24 mg of **11** was obtained from **10a** in a yield of 41.6%. ^{1}H NMR (300 MHz, $CDCl_3$) 4.26–4.41 (m, 2H), 4.71–4.92 (m, 2H), 6.91 (s, 1H), 7.42–7.44 (m, 1H), 7.56–7.61 (m, 2H), 8.11 (d, J = 9.0 Hz, 2H), 8.39 (d, J = 9.3 Hz, 2H).

2.1.18. 6-(2-Fluoro-ethoxy)-4′-aminoflavone (12)

The same reaction as described above to prepare **4** was used, and 22 mg of **12** was obtained from **11** in a yield of 41.6%. ^{1}H NMR (300 MHz, $CDCl_3$) 4.10 (s, 2H), 4.27–4.39 (m, 2H), 4.71–4.88 (m, 2H), 6.70 (s, 1H), 6.76 (d, J = 9.0 Hz, 2H), 7.29–7.35 (m, 1H), 7.49 (d, J = 9.3 Hz, 1H), 7.58 (s, 1H), 7.75 (d, J = 9.0 Hz, 2H). EI-MS m/z 299 (M^+).

2.1.19. 6-(2-Fluoro-ethoxy)-4′-methylaminoflavone (13)

To a solution of **12** (22 mg, 0.07 mmol) in DMSO (2 mL) were added methyl iodide (0.14 mL) and K_2CO_3 (50.8 mg, 0.37 mmol). The reaction mixture was stirred at room temperature for 5 h, and poured into water (30 mL). After extraction with ethyl acetate (2 × 30 mL), the organic layers were combined and dried over Na_2SO_4. Evaporation of the solvent afforded a residue, which was purified by reversed phase HPLC (acetonitrile/H_2O = 3:2) to give 10 mg of **13** (43.4% yield). ^{1}H NMR (300 MHz, $CDCl_3$) 2.93 (s, 3H), 4.22 (s, 1H), 4.26–4.40 (m, 2H), 4.70–4.91 (m, 2H), 6.71 (s, 1H), 6.76 (d, J = 9.0 Hz, 2H), 7.29–7.35 (m, 1H), 7.50 (d, J = 9.3 Hz, 1H), 7.58 (s, 1H), 7.78 (d, J = 8.7 Hz, 2H). EI-MS m/z 313 (M^+).

2.1.20. 6-(2-(2-Hydroxy-ethoxy)-ethoxy)-4′-aminoflavone (14b)

The same reaction as described above to prepare **4** was used, and 251 mg of **14b** was obtained from **10b** in a yield of 37.9%. ^{1}H NMR ($CDCl_3$) δ: 3.69 (t, J = 5.1 Hz, 2H), 3.79 (s, 2H), 3.91 (t, J = 4.5 Hz, 2H), 4.09 (s, 2H), 4.27 (t, J = 4.2 Hz, 2H), 6.69 (s, 1H), 6.76 (d, J = 8.7 Hz, 2H), 7.27–7.32 (m, 1H), 7.48 (d, J = 9.3 Hz, 1H), 7.65 (d, J = 3.0 Hz, 1H), 7.75 (d, J = 8.4 Hz, 2H). EI-MS m/z 387 (M^+).

2.1.21. 6-(2-(2-(2-Hydroxy-ethoxy)-ethoxy)ethoxy)-4′-aminoflavone (14c)

The same reaction as described above to prepare **4** was used, and 553 mg of **14c** was obtained from **10c** in a yield of 58.8%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.62–3.65 (m, 2H), 3.71–3.78 (m, 6H), 3.91 (t, J = 4.8 Hz, 2H), 4.11 (s, 2H), 4.25 (t, J = 4.5 Hz, 2H), 6.68 (s, 1H), 6.75 (d, J = 8.7 Hz, 2H), 7.27–7.32 (m, 1H), 7.50 (d, J = 9.0 Hz, 1H), 7.59 (d, J = 2.2 Hz, 1H), 7.74 (d, J = 8.7 Hz, 2H). EI-MS m/z 387 (M^+).

2.1.22. 6-(2-(2-Fluoro-ethoxy)-ethoxy)-4′-aminoflavone (15b)

The same reaction as described above to prepare **8b** was used, and 10 mg of **15b** was obtained from **14b** in a yield of 9.1%. ^{1}H NMR ($CDCl_3$) δ: 3.79 (t, J = 4.2 Hz, 1H), 3.86–3.95 (m, 3H), 4.11 (s, 2H), 4.25 (t, J = 4.5 Hz, 2H), 4.53 (t, J = 4.2 Hz, 1H), 4.70 (t, J = 4.2 Hz, 1H), 6.68 (s, 1H), 6.75 (d, J = 9.0 Hz, 2H), 7.28–7.33 (m, 1H), 7.47 (d, J = 9.0 Hz, 1H), 7.58 (d, J = 3.0 Hz, 1H), 7.74 (d, J = 8.4 Hz, 2H). EI-MS m/z 343 (M^+).

2.1.23. 6-(2-(2-(2-Fluoro-ethoxy)-ethoxy)ethoxy)-4′-aminoflavone (15c)

The same reaction as described above to prepare **8** was used, and 85 mg of **15c** was obtained from **14** in a yield of 81.3%. ^{1}H NMR ($CDCl_3$) δ: 3.62–3.65 (m, 2H), 3.70–3.78 (m, 7H), 3.82 (t, J = 3.9 Hz, 1H), 3.90 (t, J = 4.5 Hz, 2H), 4.22 (t, J = 4.5 Hz, 2H), 4.49 (t, J = 4.2 Hz, 1H), 4.66 (t, J = 4.2 Hz, 1H), 6.68 (s, 1H), 6.75 (d, J = 8.7 Hz, 2H), 7.27–7.32 (m, 1H), 7.46 (d, J = 9.3 Hz, 1H), 7.57 (d, J = 2.2 Hz, 1H), 7.73 (d, J = 8.7 Hz, 2H).

2.1.24. 6-(2-(2-Hydroxy-ethoxy)-ethoxy)-4′-methylaminoflavone (16b)

The same reaction as described above to prepare **13** was used, and 41 mg of **16b** was obtained from **14b** in a yield of 37.9%. ^{1}H NMR ($CDCl_3$) δ: 3.49 (s, 3H), 3.69 (t, J = 3.6 Hz, 2H), 3.77–3.79 (m, 2H), 3.91 (t, J = 4.8 Hz, 2H), 4.27 (t, J = 4.0 Hz, 2H), 6.65 (s, 1H), 6.68–6.69 (m, 2H), 7.29–7.32 (m, 1H), 7.47 (d, J = 9.0 Hz, 1H), 7.65 (d, J = 3.0 Hz, 1H), 7.78 (d, J = 9.0 Hz, 2H). EI-MS m/z 355 (M^+).

2.1.25. 6-(2-(2-(2-Hydroxy-ethoxy)-ethoxy)ethoxy)-4′-methylaminoflavone (16c)

The same reaction as described above to prepare **13** was used, and 145 mg of **16c** was obtained from **14c** in a yield of 64.8%. ^{1}H NMR ($CDCl_3$) δ: 2.92 (d, J = 3.0 Hz, 3H), 3.63 (t, J = 5.4 Hz, 2H) 3.72–3.76 (m, 6H), 3.91 (t, J = 5.1 Hz, 2H), 4.25 (t, J = 4.8 Hz, 3H), 6.65 (s, 1H), 6.68 (s, 2H), 7.28–7.32 (m, 1H), 7.46 (d, J = 9.3 Hz, 1H), 7.59 (d, J = 2.2 Hz, 1H), 7.77 (d, J = 8.7 Hz, 2H).

2.1.26. 6-(2-(2-Fluoro-ethoxy)-ethoxy)-4′-methylaminoflavone (17b)

The same reaction as described above to prepare **8** was used, and 9 mg of **17b** was obtained from **16b** in a yield of 21.9%. ^{1}H NMR ($CDCl_3$) δ: 2.93 (d, J = 5.1 Hz, 3H), 3.79 (t, J = 4.2 Hz, 1H), 3.85–3.95 (m, 3H), 4.26 (t, J = 4.8 Hz, 3H), 4.53 (t, J = 4.2 Hz, 1H), 4.70 (t, J = 4.5 Hz, 1H), 6.65 (s, 1H), 6.68 (s, 2H), 7.28–7.32 (m, 1H), 7.47 (d, J = 9.0 Hz, 1H), 7.59 (d, J = 3.0 Hz, 1H), 7.78 (d, J = 9.0 Hz, 2H). EI-MS m/z 357 (M^+).

2.1.27. 6-(2-(2-(2-Fluoro-ethoxy)-ethoxy)ethoxy)-4′-methylaminoflavone (17c)

The same reaction as described above to prepare **8** was used, and 20 mg of **17c** was obtained from **16c** in a yield of 13.8%. ^{1}H NMR ($CDCl_3$) δ: 2.92 (d, J = 4.8 Hz, 3H), 3.69–3.76 (m, 5H), 3.82 (t, J = 4.5 Hz, 1H), 3.91 (t, J = 4.8 Hz, 2H), 4.25 (t, J = 4.2 Hz, 3H), 4.50 (t, J = 4.2 Hz, 1H), 4.66 (t, J = 4.5 Hz, 1H), 6.65 (s, 1H), 6.68 (s, 2H), 7.28–7.31 (m, 1H), 7.46 (d, J = 9.3 Hz, 1H), 7.59 (d, J = 3.0 Hz, 1H), 7.77 (d, J = 8.7 Hz, 2H). EI-MS m/z 401 (M^+).

2.1.28. 4-Nitrobenzoic acid 2-acetyl-4-fluorophenyl ester (18)

The same reaction as described above to prepare **1** was used, and 2.5 g of **18** was obtained from 2-hydroxy-5-fluoroacetophenone and 4-nitrobenzoyl chloride in a yield of 85.6%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.56 (s, 3H), 7.23–7.34 (m, 2H), 7.56–7.60 (m, 1H), 8.37 (s, 4H).

2.1.29. 1-(5-Fluoro-2-hydroxyphenyl)-3-(4-nitrophenyl)propane-1,3-dione (19)

The same reaction as described above to prepare **2** was used, and 2.5 g of **19** was obtained from **18** in a yield of 96.3%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.81 (s, 2H), 7.02 (d, J = 9.0 Hz, 1H), 7.45 (d, J = 9.0 Hz, 1H), 7.68 (s, 1H), 8.11 (d, J = 8.7 Hz, 2H), 8.36 (d, J = 8.7 Hz, 2H), 11.7 (s, 1H).

2.1.30. 6-Fluoro-4′-nitroflavone (20)

The same reaction as described above to prepare **3** was used, and 2.0 g of **20** was obtained from **19** in a yield of 85.3%. EI-MS m/z 285 (M^+).

2.1.31. 6-Fluoro-4′-aminoflavone (21)

The same reaction as described above to prepare **4** was used, and 944 mg of **21** was obtained from **20** in a yield of 67.4%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.13 (s, broad, 2H), 6.74 (s, 1H), 6.76 (d, J = 9.0 Hz, 2H), 7.35–7.42 (m, 1H), 7.51–7.56 (m, 1H), 7.75 (d, J = 8.7 Hz, 2H), 7.82–7.85 (m, 1H).

2.1.32. 6-Fluoro-4′-methylaminoflavone (22)

To a mixture of **21** (300 mg, 1.2 mmol) and paraformaldehyde (179 mg, 5.9 mmol) in MeOH (15 mL) was added a solution of NaOMe (0.34 mL, 28 wt % in MeOH) dropwise at 0 °C. The mixture was stirred under reflux for 1 h. After addition of $NaBH_4$ (246 mg, 6.5 mmol), the solution was heated under reflux for 45 min. To the cold mixture, 1 M NaOH was added followed by extraction with $CHCl_3$. 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 = 5:3) to give 314 mg of **22** (99.2%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.91 (s, 3H), 4.37 (s, broad, 1H), 6.63 (s, 1H), 6.66 (s, 2H), 7.32–7.39 (m, 1H), 7.49–7.53 (m, 1H), 7.74 (d, J = 8.7 Hz, 2H), 7.82–7.85 (m, 1H).

2.1.33. 6-Fluoro-4′-dimethylaminoflavone (23)

The same reaction as described above to prepare **5** was used, and 203 mg of **23** was obtained from **21** in a yield of 61.0%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.08 (s, 6H), 6.69 (s, 1H), 6.76 (d, J = 9.3 Hz, 2H), 7.35–7.41 (m, 1H), 7.51–7.56 (m, 1H), 7.81 (d, J = 9.0 Hz, 2H), 7.83–7.86 (m, 1H).

2.1.34. 6-(2-Tosyloxyethoxy)-4′-dimethylaminoflavone (24a)

To a solution of **8a** (136 mg, 0.28 mmol) in pyridine (4 mL) was added tosyl chloride (122 mg, 0.65 mmol) in an ice bath. The reaction mixture was stirred for 32 h at room temperature following the reaction in an ice bath for 1 h. The organic phase was dried over Na_2SO_4 and filtered. The solvent was removed, and the residue was purified by silica gel chromatography (chloroform/MeOH = 20:1) to give 50 mg of **24a** (36.8%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.45 (s, 3H), 3.07 (s, 6H), 4.23 (t, 2H, J = 4.5 Hz), 4.41 (t, J = 5.1 Hz, 2H), 6.68 (s, 1H), 6.75 (d, J = 9.0 Hz, 2H), 7.12–7.18 (m, 1H), 7.35 (d, J = 8.1 Hz, 2H), 7.43–7.56 (m, 2H), 7.82 (t, J = 9.0 Hz, 4H). EI-MS: m/z 479 [M^+].

2.1.35. 6-(2-(2-Tosyloxyethoxy)ethoxy)-4′-dimethylaminoflavone (24b)

The same reaction as described above to prepare **24a** was used, and 111 mg of **24b** was obtained from **8b** in a yield of 34.1%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.41 (s, 3H), 3.08 (s, 6H), 3.76–3.85 (m, 4H), 4.12 (t, J = 5.1 Hz, 2H), 4.22 (t, J = 5.1 Hz, 2H), 6.70 (s, 1H), 6.76 (d, J = 9.0 Hz, 2H), 7.25–7.33 (m, 3H), 7.47 (d, J = 9.0 Hz, 1H), 7.55 (d, J = 3.0 Hz, 1H), 7.79–7.83 (m, 4H). EI-MS m/z 523 (M^+).

2.1.36. 6-(2-(2-(2-Tosyloxyethoxy)ethoxy)ethoxy)-4′-dimethylaminoflavone (24c)

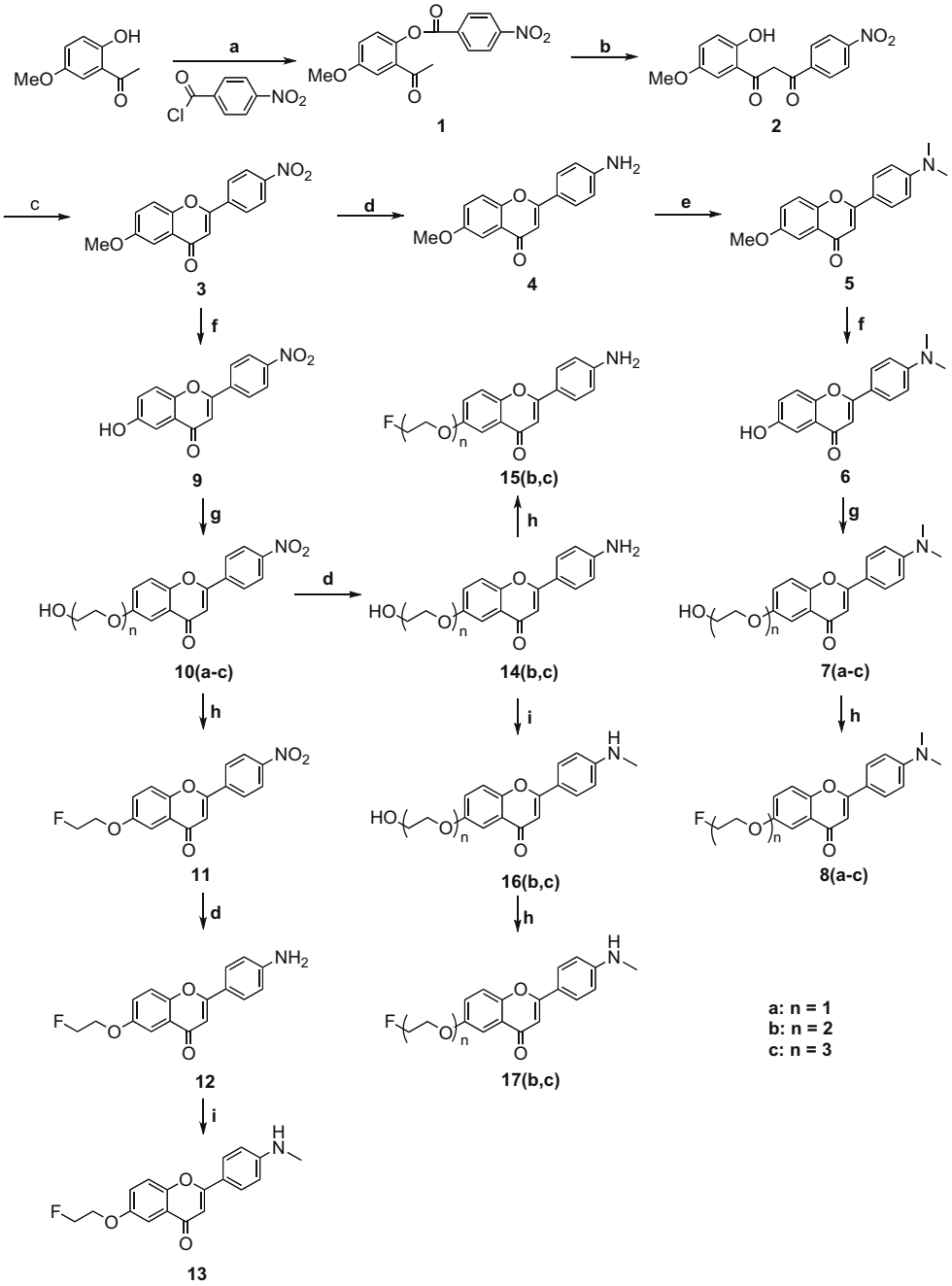
The same reaction as described above to prepare **24a** was used, and 35 mg of **24c** was obtained from **8c** in a yield of 39.9%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.43 (s, 3H), 3.08 (s, 6H), 3.62–3.73 (m, 6H), 3.87 (t, J = 4.8 Hz, 2H), 4.16–4.21 (m, 4H), 6.70 (s, 1H), 6.76 (d, J = 9.0 Hz, 2H), 7.28–7.33 (m, 3H), 7.47 (d, J = 9.0 Hz, 1H), 7.60 (d, J = 2.2 Hz, 1H), 7.79–7.83 (m, 4H). EI-MS m/z 567 (M^+).

2.2. Radiolabeling

[^{18}F]Fluoride produced by an ultracompact cyclotron (CYPRIS model 325R; Sumitomo Heavy Industry Ltd) via an $^{18}O(p,n)^{18}F$ reaction was adsorbed to a strong-base anion exchange resin (Bio-Rad), and was eluted with 500 μL of K_2CO_3 solution (33 mM) into 1 mL of acetonitrile containing Kryptofix 222 (K222) (20 mg). The solvent was removed azeotropically with anhydrous acetonitrile at 120 °C under a nitrogen stream. A solution of tosylate precursor **24(a–c)** (0.2 mg) in 400 μL of DMSO was added to the reaction vessel containing [^{18}F]Fluoride. The mixture was heated at 160 °C for 5 min. The reaction mixture was purified by the reversed phase HPLC system (a Shimadzu LC-6A isocratic pump, a Shimadzu SPD-6A UV detector and an Aloka NDW-351D scitillation detector) on a YMC Hydrosphere C18 column (20 × 150 mm) with acetonitrile/water (70:30) at a flow rate of 9.0 mL/min to obtain [^{18}F]**8(a–c)**. The radiochemical purity and specific activity were determined by analytical HPLC on a YMC Pack Pro C18 column (4.6 × 150 mm, acetonitrile/water (60:40), 1.0 mL/min).

2.3. Binding assays using the aggregated Aβ peptide in solution

A solid form of Aβ(1-42) was purchased from Peptide Institute (Osaka, Japan). Aggregation of peptides was carried out by gently dissolving the peptide (0.25 mg/mL) in a buffer solution (pH 7.4) containing 10 mM sodium phosphate and 1 mM EDTA. The solutions were incubated at 37 °C for 42 h with gentle and constant



Scheme 1. Reagents: (a) pyridine; (b) KOH, pyridine; (c) H_2SO_4, AcOH; (d) EtOH, $SnCl_2$; (e) $(CH_2O)_n$, $NaCNBH_3$, AcOH; (f) CH_2Cl_2, BBr_3; (g) $Cl(CH_2CH_2O)_nH$ (n = 1–3) K_2CO_3, DMF; (h) DAST, DME; (i) DMSO, CH_3I, K_2CO_3.

shaking. Binding experiments were carried out as described previously.[14] [^{125}I]DMFV ([^{125}I]6-iodo-4′-dimethylaminoflavone) with 81.4 TBq/mmol specific activity and greater than 95% radiochemical purity was prepared using the standard iododestannylation reaction.[14] A mixture containing 50 μL of test compounds (0.2 pM-400 μM in 10% EtOH), 50 μL of 0.02 nM [^{125}I]DMFV, 50 μL of Aβ(1-42) aggregates and 850 μL of 10% EtOH was incubated at room temperature for 3 h. The mixture was then filtered through Whatman GF/B filters using a Brandel M-24 cell harvester, and the radioactivity on the filters containing the bound ^{125}I ligand was measured in a gamma counter (Aloka, ARC-380). Values for the half-maximal inhibitory concentration (IC_{50}) were determined from displacement curves of three independent experiments using GraphPad Prism 4.0, and those for the inhibition constant (K_i) were calculated using the Cheng-Prusoff equation:[18] $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the concentration of [^{125}I]DMFV used in the assay, and K_d is the dissociation constant of DMFV (12.3 nM).[14]

2.4. Staining of amyloid plaques in transgenic mouse brain sections

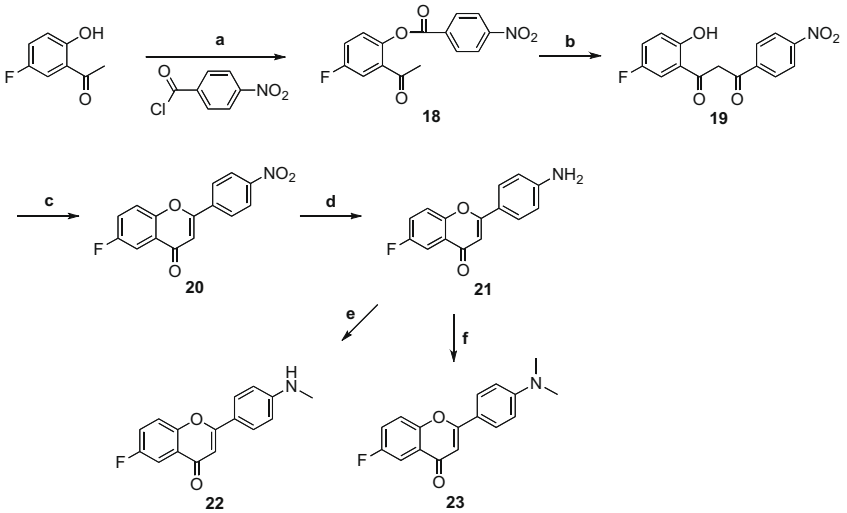
Animal studies were conducted in accordance with institutional guidelines and approved by the Kyoto University Animal Care Committee. Tg2576 transgenic mice (female, 20-month-old) were used as an Alzheimer's model. While under isoflurane anesthesia, the mice were sacrificed by decapitation, and the brains were immediately removed and frozen in powdered dry ice. The frozen blocks were sliced into serial sections 10 μm thick using a cryostat (Leica Instruments, CM1900). Each slide was incubated with a 50% ethanol solution (100 μM) of compound **8a**, **8b**, or **8c**, which have the characteristics to emit fluorescence. The sections were washed in 50% ethanol for 3 min two times, and examined using a microscope (Nikon, Eclipse 80i) equipped with a B-2A filter set (excitation, 450–490 nm; dichronic mirror, 505 nm; longpass filter, 520 nm). Thereafter, the serial sections were also immunostained with DAB as a chromogen using monoclonal antibodies against β-amyloid (Amyloid β-Protein Immunohistostain kit, WAKO).

2.5. In vivo biodistribution in normal mice

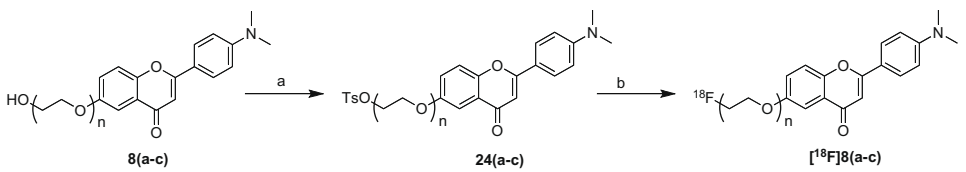
A saline solution (100 μL) containing ethanol (5 μL) of radiolabeled agents (18.5 kBq) was injected directly into a tail vein of ddY mice (5-week-old, 22–25 g). While under isoflurane anesthesia, the mice were sacrificed at various time points postinjection. The organs of interest were removed and weighed, and radioactivity was measured with an automatic gamma counter (Packard Cobra Auto-Gamma Counter 5003).

3. Results and discussion

The target FPEG flavone derivatives were prepared as shown in Scheme 1. The most common method of synthesizing flavones is known as the Baker-Venkataraman transformation.[19] In this process, a hydroxyacetophenone is first converted into a benzoyl ester **1**, and this species is then treated with a base, forming a 1,3-diketone **2**. Treatment of this diketone with acid leads to generation of the desired flavone **3**. In the route for the synthesis of dimethylamino derivatives, the free amino derivative **4** was readily prepared from **3** by reduction with $SnCl_2$. Compound **5** was converted to **6** by demethylation with BBr_3 in CH_2Cl_2. To prepare compounds with 1-3 ethoxy groups as the PEG linkage, commercially available chlorides were coupled with the OH group of **6** to obtain **7(a–c)**, respectively. The fluorinated flavones, **8(a–c)**, were successfully obtained by reacting **7(a–c)** with DAST in DME or ethylene glycol dimethyl ether. In the route for the synthesis of monomethylated derivatives and the primary amino derivatives, the demethylation of **3** with BBr_3 and the introduction of 1-3 ethoxy groups into **9** gave **10(a–c)**. To prepare



Scheme 2. Reagents: (a) pyridine; (b) KOH, pyridine; (c) H_2SO_4, AcOH; (d) EtOH, $SnCl_2$; (e) $(CH_2O)_n$, NaOMe, $NaBH_4$; (f) $(CH_2O)_n$, $NaCNBH_3$, AcOH.



Scheme 3. Reagents: (a) tosyl chloride, pyridine; (b) K_2CO_3, [^{18}F]F$^-$, kryptofix[222], DMSO/acetonitrile.

the FPEG flavone with one ethoxy group (n = 1) (**12** and **13**), the fluorination of **10a** with DAST, the reduction of **11** with $SnCl_2$ and the methylation of **12** were performed. The primary amino derivatives of FPEG flavones (n = 2 and 3) (**15b** and **15c**) were synthesized by the fluorination of **14b** and **14c** with DAST following the reduction of the nitro group in **10b** and **10c**. The monomethylated FPEG flavones (n = 2 and 3) (**17b** and **17c**) were synthesized by the methylation of **16b** and **16c** following the fluorination of **14b** and **14c** with DAST. We successfully synthesized the flavone derivatives (**21**, **22**, and **23**) with fluorine directly bound to the phenyl group according to a procedure reported previously (Scheme 2). To make the desired ^{18}F-labeled FPEG flavones, [^{18}F]**8(a–c)**, the tosylates **24(a–c)** were employed as the precursors. The free OH groups of **8(a–c)** were converted into tosylates by reacting with TsCl in the presence of pyridine to give **24(a–c)** (Scheme 3). Each of the tosylates, **24(a–c)**, was mixed with [^{18}F]fluoride/potassium carbonate and Kryptofix 222 in DMSO and heated at 160 °C for 5 min. The crude product was purified by HPLC (radiochemical purity >99%, radiochemical yield 5–13%, decay corrected). The total synthesis time was 70 min, and the specific activity was estimated to be 33.3–55.5 GBq/mmol at the end of synthesis.

Table 1
Inhibition constants (K_i, nM) of compounds for the binding of [^{125}I]DMFV to Aβ(1-42) aggregates[a]

Compound	K_i (nM)	Compound	K_i (nM)
8a	5.3 ± 0.8	**15c**	234.0 ± 60.6
8b	14.4 ± 2.5	**17b**	54.5 ± 10.3
8c	19.3 ± 4.0	**17c**	45.1 ± 5.8
12	234.3 ± 63.5	**21**	260.5 ± 43.3
13	99.0 ± 11.8	**22**	110.0 ± 47.4
15b	321.1 ± 74.4	**23**	73.9 ± 5.3

[a] Values are the mean ± standard error of the mean for 4–9 experiments.

Table 2
Biodistribution of ^{18}F-labeled flavones in normal mice[a]

Organ	2 min	10 min	30 min	60 min
*[^{18}F]**8a***				
Blood	2.80 ± 0.41	2.71 ± 0.13	2.53 ± 0.17	3.25 ± 0.31
Brain	4.17 ± 0.77	3.62 ± 0.21	1.89 ± 0.13	2.19 ± 0.18
Bone	2.02 ± 0.53	2.83 ± 0.23	4.51 ± 0.55	6.21 ± 0.84
*[^{18}F]**8b***				
Blood	2.09 ± 0.35	2.30 ± 0.07	2.50 ± 0.21	2.94 ± 0.27
Brain	3.54 ± 0.54	2.75 ± 0.21	2.00 ± 0.20	2.13 ± 0.10
Bone	1.13 ± 0.22	1.65 ± 0.10	2.42 ± 0.38	3.74 ± 0.30
*[^{18}F]**8c***				
Blood	2.35 ± 0.54	1.50 ± 0.26	1.40 ± 0.04	1.88 ± 0.08
Brain	2.89 ± 0.74	2.23 ± 0.36	1.31 ± 0.14	1.37 ± 0.11
Bone	1.53 ± 0.52	2.38 ± 0.39	4.06 ± 0.49	5.21 ± 0.98

[a] Expressed as % of injected dose per gram. Each value represents the mean ± SD for 4–5 mice at each interval.

In vitro binding experiments to evaluate the affinity of the FPEG flavones for Aβ aggregates were carried out in solutions with [^{125}I]DMFV as the ligand. The affinity of flavone derivatives for Aβ aggregates varied from 5 to 321 nM (Table 1). The flavone derivatives had affinity for Aβ(1-42) aggregates in the following order: the dimethyamino derivatives (**8a**, **8b**, **8c**, and **23**) > the monomethylamino derivatives (**13**, **17b**, **17c**, and **22**) > the primary amino derivatives (**12**, **15b**, **15c**, and **21**). The results of the binding experiments are consistent with those of previous reports.[14,20,21] The K_i values indicated that the affinity for Aβ(1-42) aggregates was affected by the substituted group at position 4′ in the flavone structure, not by the length of the PEG introduced into the flavone backbone. We selected the dimethylamino derivatives (**8a**, **8b**, and **8c**), which showed greater binding affinity than the monomethylamino derivatives and the primary amino derivatives, for additional study.

Three ^{18}F FPEG flavones ([^{18}F]**8a**, [^{18}F]**8b**, and [^{18}F]**8c**) were examined for their biodistribution in normal mice (Table 2). All three ligands displayed high uptake from the brain 2.89–4.17%ID/g, at 2 min postinjection, indicating a level sufficient for imaging. In addition, they displayed good clearance from the normal brain with 1.89, 2.00, and 1.31%ID/g at 30 min postinjection for [^{18}F]**8a**, [^{18}F]**8b**, and [^{18}F]**8c**, respectively. These values were equal to 45.3%, 56.5%, and 45.3% of the initial uptake peak for [^{18}F]**8a**, [^{18}F]**8b**, and [^{18}F]**8c**, respectively. A rapid initial uptake in normal brain coupled with a fast washout are highly desirable properties for β-amyloid-imaging probes, as they lead to a high signal to background ratio. [^{18}F]**8(a–c)** showed the bone uptake (3.74–6.21%ID/g) at 60 min postinjection, suggesting there may be in vivo defluorination. However, the free fluorine was not taken up by brain tissue; therefore, the interference from this free fluoride is expected to be relatively low for brain imaging.[22]

To confirm the affinity of FPEG chalcone derivatives for β-amyloid plaques in the brain, neuropathological fluorescent staining

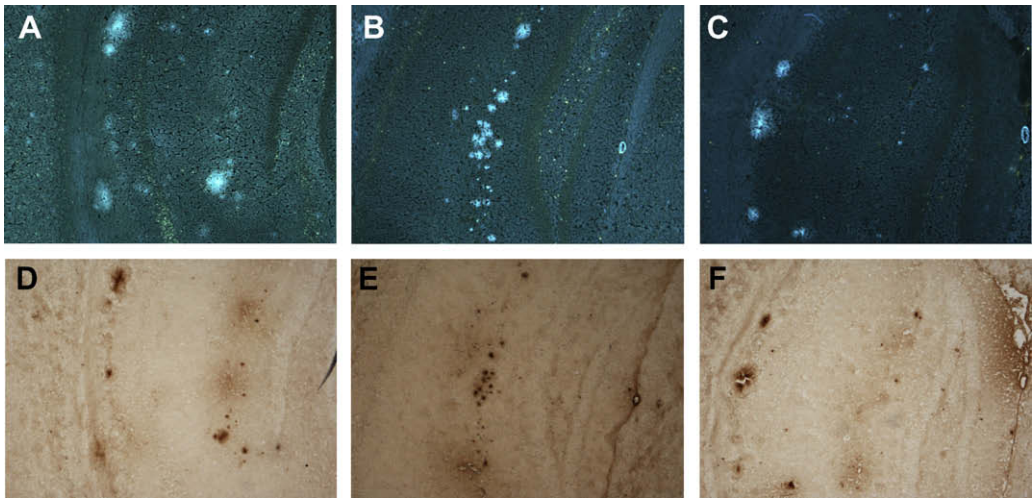


Figure 2. Neuropathological staining of flavone derivatives **8a** (A), **8b** (B), and **8c** (C) in 10-μm brain sections of Tg2576 mice. Immunohistological staining with an antibody against β-amyloid (D, E, and F) in the adjacent sections of A, B, and C, respectively.

with **8a**, **8b**, and **8c** was carried out using the Alzheimer's model (Fig. 2A–C). Many fluorescence spots were observed in the brain sections of Tg2576 transgenic (female, 20-month-old) mice, while no spots were observed in the brain sections of wild-type (female, 22-month-old) mice (data not shown). The fluorescent labeling pattern was consistent with that obtained by immunohistochemical labeling with an antibody specific for Aβ (Fig. 2D–F), indicating that FPEG flavones show specific binding to β-amyloid plaques in the mouse brain.

In conclusion, we successfully designed and synthesized novel ^{18}F labeled flavones with the FPEG strategy for PET imaging of β-amyloid in the brain. The affinity of the derivatives for Aβ aggregates varied from 5 to 321 nM. When in vitro plaque labeling was carried out using sections of brain from Tg2576 mice, FPEG flavones intensely stained β-amyloid plaques. In addition, they displayed good uptake into and a rapid washout from the brain after injection in normal mice. The combination of high binding affinity for β-amyloid plaques, high brain uptake, and good clearance in mice of the FPEG-flavone derivatives may provide a series of promising in vivo amyloid imaging agents for PET.

Acknowledgments

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