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Aromatic radiofluorination and biological evaluation of 2-aryl-6-[^{18}F] fluorobenzothiazoles as a potential positron emission tomography imaging probe for β-amyloid plaques

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ABSTRACT

To develop agents for radionuclide imaging Aβ plaques in vivo, we prepared three fluorine-substituted analogs of arylbenzothiazole class; compound **2** has a high affinity for Aβ (K_i = 5.5 nM) and the specific binding to Aβ in fluorescent staining. In preparation for the synthesis of these arylbenzothiazole analogs in radiolabeled form as an Aβ plaques-specific positron emission tomography (PET) imaging probe, we investigated synthetic route suitable for its labeling with the short-lived PET radionuclide fluorine-18 ($t_{1/2}$ = 110 min) and diaryliodonium tosylate precursors (**12**, **13a–e** and **14**). 2-Aryl-6-[^{18}F]fluorobenzothiazoles ([^{18}F]**1**–**3**) were synthesized in efficiently short reaction times (40–60 min) with high radiochemical yields (19–40%), purities (>95%) and specific activities (85–118 GBq/μmol). Tissue distribution studies showed that high radioactivity of [^{18}F]**2** accumulated in the brain with rapid clearance in healthy mice. Radioactive metabolites were analyzed in brain samples of mice and corresponded to 81% of parent remained by 30 min after a tail-vein injection. These results suggest that [^{18}F]**2** is a promising probe for evaluation of Aβ plaques imaging in brain using PET.

1. Introduction

AD is a progressive and fatal neurodegenerative disease that leads to brain disorder; half of the affected patients suffer from dementia, cognitive impairment, and memory loss. The most serious risk factor of AD is that it becomes more pronounced with increasing age of the patient, and this is a concern because of the increasing life expectancy of populations worldwide; consequently, there is an increasingly high incidence of AD in the older population. Approximately 5% of those over 65 years are affected, while over 20–30% of those over 80 years show signs of dementia.[1] It has been reported that the main event in the pathogenesis of AD is the formation and accumulation of aggregates of Aβ peptides in the brain.[2,3] Therefore, Aβ plaque, which seems to play an important neuropathological role in AD, is mainly comprises an aggregation of the Aβ peptide.[4] An Aβ plaques-specific imaging probe would be useful for the diagnosis of AD at an early to moderate stage, as well as the diagnosis of MCI and the monitoring of the therapeutic efficacy AD treatment. There has been an ongoing worldwide search for a clinically useful radiolabeled Aβ plaque probe in order to develop noninvasive in vivo PET imaging for AD. To this end, many specific ligands have been developed and evaluated for the imaging of Aβ plaques. One of the lead structural motifs, 2-(4′-([^{11}C]methylaminophenyl)-6-hydroxybenzothiazole (known as [^{11}C]PIB, Fig. 1) has already shown promising results in clinical trial.[5–7] Despite the promising clinical results in AD patients, PIB remains suboptimal on account of its labeling with the short-lived carbon-11 (half life = 20 min), which limits its availability to centers equipped with an on-site cyclotron. To overcome this limitation of [^{11}C]PIB, due to the short half life of carbon-11, many research groups tried to introduce fluorine-18 (half life = 110 min) into their target compound. Thus far, the compounds reported to have a high affinity for Aβ plaque and which contained fluorine-18 were fluorine-18 labeled BTA analogs (GE-067 and 6-OH-4′-FP-BTA),[8–10] FDDNP, BAY94–9174, and AV-45 (Fig. 1).[11–13]

Among these radioligands, FDDNP was known to bind to both Aβ plaques at different binding sites and in intracellular neurofibrillary tangles; it does not selectively measure a specific pathologic component of AD patients.[11] The recently reported fluoropegylated stilbene derivatives ([^{18}F]BAY94-9172 and AV-45) are currently under a phase II and phase III, respectively. The most commonly studied and most promising of the reported

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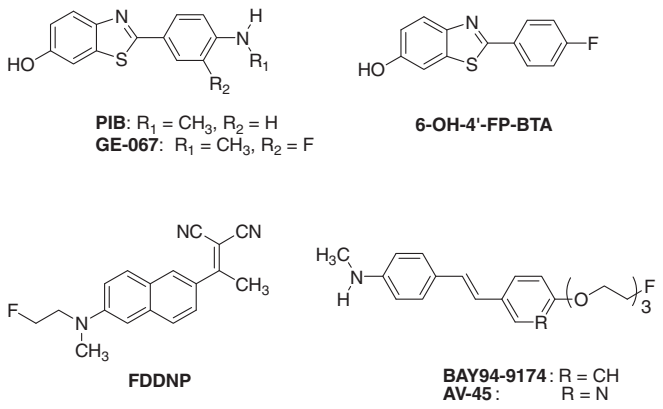


Figure 1. Chemical structures of previously reported Aβ plaque PET imaging probes.

compounds, members of the novel 2-aryl-6-hydroxybenzothiazole class ([^{18}F]GE-067, including PIB), showed a high initial brain uptake, and a particularly high binding affinity to Aβ plaque, and are currently being used in clinical trials (phase III).

Tracing the history of the studies for the fluorine-18 labeled compounds as an Aβ plaque imaging probe, it is believed that 2-(4-aminophenyl)-benzothiazole (BTA) may have potential as a parental compound and aromatic fluorine-18 labeling into the benzothiazole moiety or aniline in BTA is essential for in vivo stability and retaining its biological activities as PET imaging probe for Aβ plaque.

In order to achieve this, preliminary reports on fluorine-18 introduced at the *ortho*- or *para*-position in the right aromatic ring of BTA analogs ([^{18}F]GE-067 and 6-hydroxy-2-(4′-[^{18}F]fluorophenyl)-benzothiazoles) were developed.[10] In addition to, a previous article by Henriksen, et al., described the preparation of a ^{11}C-labeled 5-fluorine substituted benzothiazole analog as an imaging probe and mentioned the fluorine at 5-position metabolically stabilized BTA analog compare to [^{11}C]PIB is known to be rapidly metabolized to the C6-sulfonated derivatives.[14,15] Recently, Zheng et al. also described the preparation of 6-fluroine substituted benzothiazole analog as an imaging probe and showed their compounds have higher binding affinities to Aβ aggregates than PIB.[16] It was interesting to note that fluorine effectively replaced hydroxyl group while continuing to manifest comparable activities, albeit with different properties, in numerous examples.[17,18] Although 6-fluroine substituted benzothiazole analog showed high binding affinity to Aβ plaque, they failed fluorine-18 labeling in the benzothiazole moiety of their compounds because the introduction of fluorine-18 into benzothiazole ring was impossible by general aromatic fluorination.

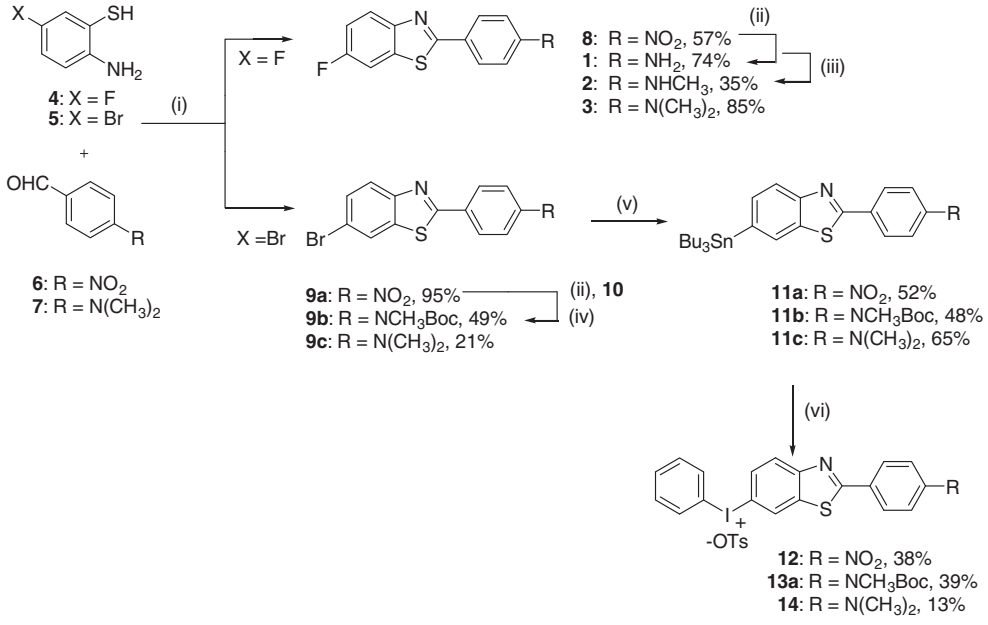
Gratifying, diaryliodonium salt precursors can be useful to label fluorine-18 at aromatic position in aryl compounds, which have proven difficult to label using the general aromatic fluorine-18 labeling methods in the previous works of our group[19,20] and the Pike group.[21]

Therefore, we designed three 6-[^{18}F]fluorine labeled BTA analogs instead of the 6-hydroxy group in [^{11}C]PIB and synthesized various diaryliodonium tosylate precursors to allow aromatic fluorine-18 labeling into the benzothiazole ring. In this investigation, we prepared compounds **1–3** and [^{18}F]**1–3** using two approaches: the first (Scheme 2) involved the preparation of authentic samples of these 6-fluoro-substituted BTA analogs; the second was the synthesis of precursors (diaryliodonium tosylate) and the radio-synthetic procedure for BTA analogs in which fluorine-18 could be introduced to the precursor at a very late step. Reported herein are in vitro and in vivo evaluations of novel three 6-[^{18}F]fluoro labeled BTA analogs for use as a prospective PET probe for Aβ plaque imaging in the brain.

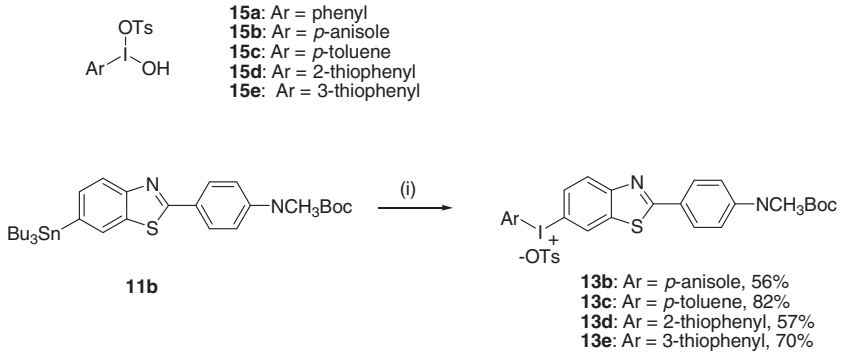
2. Results and discussion

2.1. Chemical synthesis

We considered a number of approaches that would be suitable for the preparation of 6-fluoro-substituted BTA analogs in fluorine-18 labeled form that would have the high specific activity required for a PET imaging probe. In our system, it was not clear whether the benzothiazole substituent at the 6-position would provide



Scheme 1. Reagents and conditions: (i) DMSO, 180 °C, 30 min; (ii) $SnCl_2$, EtOH, 90 °C, 2 h; (iii) CH_3I, K_2CO_3, DMSO, 100 °C, 16 h; (iv) (a) formaldehyde, MeOH, reflux, 2 h; (b) $NaCNBH_3$, AcOH, room temperature, 1.5 h; (c) Boc_2O, THF, 85 °C, 12 h; (v) Sn_2Bu_6, Pd(0), THF, 85 °C, 8 h; (iv) Koser's reagent (**15a**), CH_3CN, room temperature, 12 h.



Scheme 2. Reagents and conditions: (i) **15b–e**, CH_2Cl_2, CH_3CN, room temperature, 12 h.

Table 1
Reaction of aromatic radiofluorination with **12**, **13a**, and **14**

Entry	Precursor[a]	[^{18}F]F$^-$X$^+$	Solvent[c]	M_W or temp	Time	Yield[e]
1	**14**[b]	[^{18}F]F$^-$Cs$^+$	DMF	130 °C	10 min	NP[g]
2	**14**	[^{18}F]F$^-$Cs$^+$	MeCN	130 °C	10 min	2.4%
3	**14**	[^{18}F]F$^-$Cs$^+$	MeCN	100 W	3 min	26.7%
4	**14**	[^{18}F]F$^-$K$^+$Kryptofix	MeCN	130 °C	10 min	5%
5	**14**	[^{18}F]F$^-$K$^+$Kryptofix	DMF	100 W	3 min	NP[g]
6	**14**	[^{18}F]TBAF	MeCN[d]	100 W	3 min	18.7%[f]
7	**14**	[^{18}F]TBAF	MeCN	100 W	3 min	29%[f]
8	**14**	[^{18}F]TBAF	MeCN	100 W	6 min	35.6 ± 3.5% (n = 2)[f]
9	**12**	[^{18}F]F$^-$K$^+$Kryptofix	MeCN	100 W	3 min	5%
10	**12**	[^{18}F]TBAF	MeCN	100 W	6 min	35.3 ± 5.7% (n = 4)[f]
11	**13a**	[^{18}F]TBAF	MeCN	130 °C	10 min	9%
12	**13a**	[^{18}F]TBAF	MeCN[d]	130 °C	10 min	19.3 ± 7.7% (n = 5)[f]
13	**13a**	[^{18}F]F$^-$K$^+$Kryptofix	MeCN[d]	130 °C	10 min	6.4%
14	**13a**	[^{18}F]F$^-$K$^+$Kryptofix	MeCN[d]	130 °C	15 min	13.3%
15	**13a**	[^{18}F]TBAF	MeCN	100 W	6 min	24.4 ± 5.5% (n = 14)[f]

[a] Radiolabeling was carried out with a radical scavenger (TEMPO, 1 mg).
[b] No TEMPO use.
[c] Solvent (DMF-*N*′,*N*′-dimethylformamide or MeCN–acetonitrile, 300 μL) was used with H_2O (10 μL).
[d] No water use.
[e] Progress of the reaction (as shown Scheme 3) and yields were analyzed by radio-TLC (developing solvent: ethyl acetate/hexane = 40:60, v/v).
[f] Yield of isolated pure compounds ([^{18}F]**1–3**) by semipreparative column using HPLC (55:45 acetonitrile–water, 254 nm, 3 mL/min).
[g] No product.

sufficient activation for fluoride ion substitution with general nucleophilic aromatic substitution of a suitable leaving group.[29,30] We were unable to effect fluoride ion substitution at the 6-position with a nitro group. Curiously, we were also unable to prepare the more reactive *p*-trimethylammonium precursor because of the side compound (*N*-methylbenzothiazole) in the final methylation step from the dimethylaniline precursor. The limitation of general aromatic fluorine-18 labeling in our target compounds led us to propose an alternative approach to aromatic fluorination. It is known that diaryliodonium salts can be used as precursors for fluoroarenes,[31,32] and their value corresponds well with our reported fluorine-18 labeled PPARgamma ligand by iodonium salts in a previous work.[20]

The three 6-fluoro-substituted BTA analogs **1–3** were simply synthesized from 2-amino-5-fluorobenzenethiol **4** using a little modification of known methods (Scheme 1).[23,33] 6-Fluoro-substituted benzothiazol rings, **8** and **3** were synthesized directly with 2-amino-5-fluorobenzenethiol **4** and 4-nitrobenzaldehyde **6** or 4-(dimethylamino)-benzaldehyde **7**, respectively, followed by reduction of the nitro compound **8** with tin chloride to produce the corresponding amine **1**. This amine compound proceeded N-monomethylation with iodomethane to produce the corresponding monomethyl amine **2**. A different approach was needed for the synthesis of target compounds **1–3** in radiolabeled form. The tributyltin analogs **11a–c** can be readily prepared from the bromo compounds **9a–c** in the presence of bis(tributyl)tin and a catalytic quantity of tetrakis(triphenylphosphine)palladium(0). At a late stage in the synthesis, the fluorine-18 labeling step can rapidly and efficiently introduce fluorine-18 into appropriate precursors that are available in radiolabeled form at high specific activity because the fluorine-18 ion has a relatively short life (half life = 110 min). Therefore, we designed a different radiolabeling pathway in which one is a single step with direct aromatic fluorine-18 labeling in the synthesis of [^{18}F]**3** and the other is a one-pot two step with aromatic fluorine-18 labeling and reduction or deprotection in the syntheses of [^{18}F]**1** and [^{18}F]**2**. Therefore, the tributyltin analogs were prepared in different functional groups: dimethyl amine **14**, nitro **12** and Boc-protected monomethyl amine **13a–e** on the right aromatic ring.

To prepare iodonium tosylate precursors suitable for the introduction of fluorine-18 ion into the BTA analogs, we reacted the tributyltin compounds **11a–c** with a commercially available Koser's reagent **15a** and various hydroxy(tosyloxy)iodoarenes **15b–e**.

The electron-rich hydroxy(tosyloxy)iodoarenes are known to be unstable and to decompose violently at room temperature. Thus, we prepared **15b–e** according to the literature and use it immediately to prepare the diaryliodnium tosylate precursors **13b–e** under nitrogen atmosphere. Generally, the fluoride anion is

Table 2
The radiofluorination with diaryliodonium tosylates **13a–e** and hydrolysis[a]

Entry	Precursor	Yield[b] (decay-corrected yields)[c]
1	**13a**	23.5 ± 7.7% (19.3%)[c]
2	**13b**	18.9 ± 8.3%
3	**13c**	26.5 ± 8.1%
4	**13d**	34.3 ± 6.2% (30.1%)[c]
5	**13e**	60.4 ± 5.6% (40.5%)[c]

[a] Radiolabeling of precursors (2 mg) were carried out with a radical scavenger (TEMPO, 1 mg), acetonitrile solvent without H_2O at 130 °C for 10 min using [^{18}F]TBAF. Deprotection was carried out using 3 M HCl in ethyl acetate (200 μL) at 75 °C for 10 min.
[b] Progress of the reaction (as shown scheme 3) and yields were analyzed by radio-TLC (developing solvent: ethyl acetate/hexane = 40:60, v/v) (n = 5).
[c] Yields of isolated pure compound [^{18}F]**2** by semipreparative column using HPLC (55:45 acetonitrile–water, 254 nm, 3 mL/min).

Table 3
K_i values of 2-aryl-6-fluorobenzothiazole analogs (**1–3**) and log P of 2-aryl-6-[^{18}F]fluorobenzothiazole analogs ([^{18}F]**1–3**)

Compound	K_i[a] (nM)	Log $P_{oct/PBS}$[c]
1	26.2	2.83 ± 0.02
2	5.5 (0.2)[b]	3.20 ± 0.06
3	5.9 (0.3)[b]	3.60 ± 0.09
PIB	5.8 (1.6)[b]	1.2[d]

[a] K_i was measured by [^{125}I]TZDM competition binding studies to a precipitate of synthetic Aβ_{1-42} peptide.
[b] Previously published values[16] shown here for comparison (K_i was measured by radioligand [^{3}H]-PIB competition binding studies in human brain homogenate).
[c] Values are mean ± SD (n = 5).
[d] Ref. 5.

expected to attack the more electron deficient ring in diaryliodonium tosylate precursor, so that we have prepared various diaryliodonium salt precursors with the 'dispensable ring' is phenyl, or the more electron rich rings; *p*-methoxyphenyl, *p*-methylphenyl, and 2- or 3-thiophenyl on 6-position of benzothiazole ring for [^{18}F]**2**, which might be superior to fluorine-18 labeling efficiency.

The aromatic radiofluorination between a diaryliodonium salt and fluoride is dominated by an electron-rich heteroaromatic ring, while the effects of base and solvent also play a role in increasing the florine-18 labeling yield. Various conditions were explored for the preparation of the three BTA analogs [^{18}F]**1**–**3** from different diaryliodonium tosylate precursors **12**, **13a**–**e** and **14**, whose preparation has been previously described (Schemes 1 and 2). When the precursor (**14**, 2 mg scale) was reacted with cesium [^{18}F]fluoride under general anhydrous radiofluorination conditions, aromatic radiofluorination did not work (Table 1, entry 1). In condition of acetonitrile for solvent or potassium [^{18}F]fluoride Kryptofix 2.2.2 for the fluorine-18 salt form, the radiochemical yields were poor (data not shown). Considering that these low radiochemical yields might be due to the instability of iodonium tosylate, McEwen et al. noted that diaryliodonium salts carried out radical-induced decomposition in a base condition and generated aromatic hydrocarbon.[34] Therefore, we added a radical scavenger, 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO) to the solvent (300 μL) and found that the yields were significantly increased, except for DMF solvent (entry 5).[35] Microwave irradiation also successfully improved the yields (entries 2 vs 3 and 11 vs 15), as compared to an oil bath system. In the aqueous environment (addition of 10 μL of water), fluorine-18 labeling yield was increased more than anhydrous condition in case of microwave irradiation condition for 360 s in 18–35% yield (entries 7–10 and 15). On the other hand, an oil bath heating in the anhydrous acetonitrile at 130 °C for 10 min gave higher yield than aqueous environment (entry 11 vs 12).

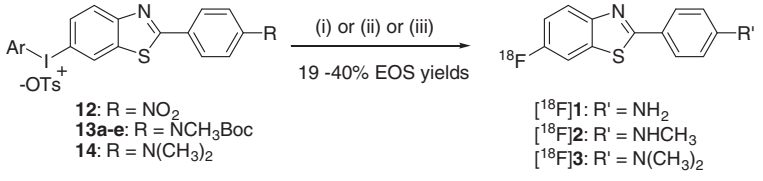
In order to understand the electron-rich heteroaromatic ring effect, we prepared five diaryliodonium salt precursors containing the more electron rich rings; *p*-methoxyphenyl, *p*-methylphenyl, and 2- or 3-thiophenyl, compare to phenyl ring on 6-position of benzothiazole ring for [^{18}F]**2** (Scheme 2) and evaluated their aromatic radiofluorination using an oil bath heating. In general, the fluorine-18 anion attacked the more electron deficient ring, so that when these diaryliodonium salt precursors bearing electron-donating groups (4-MeO and 4-Me) and electron-rich heteroatom aryl (2- or 3-thiophenyl) tend to undergo nucleophilic aromatic fluorination on the contrary benzothiazole ring. These electron-rich heteroaromatic ring effect had an impact on the radiofluorination of benzothiazole ring as shown in Table 2. The results indicate that compounds, **13b** and **13c** did not work effectively compare to compound **13a**, while *para*-electron-donating groups substituted compounds, **13d** and **13e** showed high aromatic radiochemical yields of [^{18}F]**2** (Table 2, entries 4 and 5). Consequently, three fluorine-18 labeled compounds [^{18}F]**1**–**3** were synthesized with desired yields in an environment of radical degeneration, microwave irradiation of 360 s or oil bath heating. On the 2-mg scale of precursors in acetonitrile, the radiochemical yields of [^{18}F]**1**–**3** at end-of-synthesis (EOS) were 19–40%, which included HPLC isolation and a total time of 40–60 min (Scheme 3). These regioselectivity of diaryliodoniim salt precursors in benzothiazole ring will be useful in introducing no-carrier-added fluorine-18 into aromatic region of PET probes.

2.2. Determination of K_i-values

The three prepared 6-fluoro-substituted BTA analogs showed K_i values in the range of 5.5–26.2 nM (PIB = 5.8 nM) on Aβ_{1-42}, indicating that fluorine substituted compounds at the 6-position of benzothiazole bind to the same site with good affinity (Table 3), just as known BTA analogs do on AD homogenates despite the fact that the fluorine ion has decreasing electron-donating capacity as compared to a hydroxy group in PIB.[36] Zheng et al. also synthesized 6-substituted benzothiazole anilines and evaluated their possibilities as an amyloid-imaging probes using AD human brain homogenates (Table 3).[14] Although the affinities of their compounds were shown to be below 1 nM as measured using [^{3}H]-PIB, they failed to label at the 6-position of their compounds with radioisotopes.

2.3. In vitro fluorescent staining assay

The thioflavine-S dye fluorescence is widely used for the identification of amyloid fibrils in vitro. Our target compounds also have



Scheme 3. Reagents and conditions: (i) (a) nBu$_4$N[^{18}F]F, CH$_3$CN, TEMPO, M.W. (100 W), 6 min; (b) SnCl$_2$, EtOH, 90 °C,10 min for [^{18}F]**1**; (ii) (a) nBu$_4$N[^{18}F]F, CH$_3$CN, TEMPO, M.W. (100 W), 6 min or 130 °C, 10 min; (b) EtOAc/HCl (v/v = 3:1), 75 °C, 10 min for [^{18}F]**2**; (iii) nBu$_4$N[^{18}F]F, CH$_3$CN, TEMPO, M.W. (100 W), 6 min for [^{18}F]**3**.

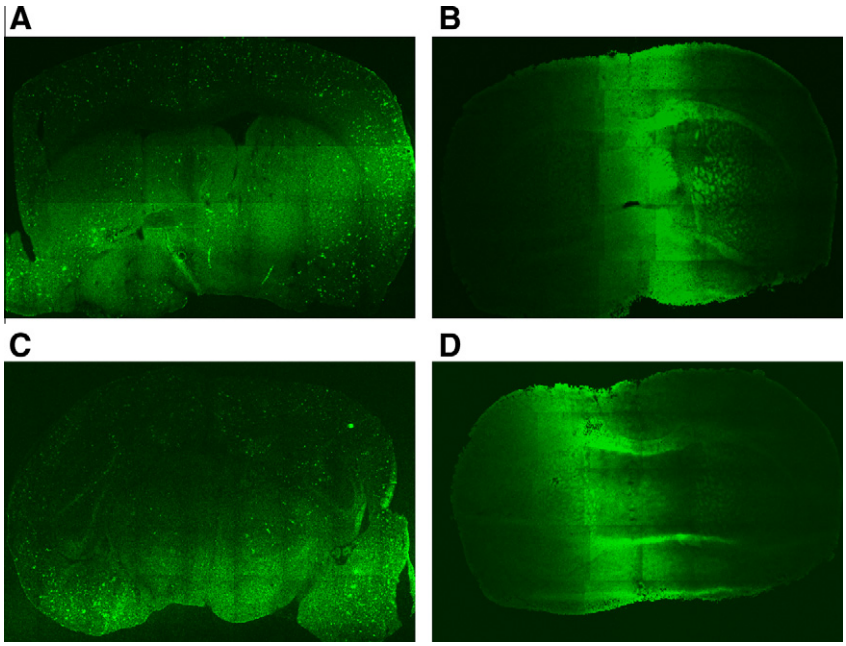


Figure 2. In vitro fluorescent staining of brain sections from double transgenic (APPswe/PS1△E9, 23 months) and the wild-type mouse: confocal tile scan images—(A) Thioflavine-S in a APPswe/PS1△E9 mouse brain; (B) Thioflavine-S in a wild-type mouse brain; (C) compound **2** in a APPswe/PS1△E9 mouse brain; (D) compound **2** in a wild-type mouse brain.

similar structure, benzothiazole analog, to thioflavine-S and fluoresce strongly with excitation and emission. As expected, both thioflavine-S and compound **2** clearly bind plaque using brain sections of double transgenic mouse (Fig. 2 A and C). Other sets of images (Fig. 2 B and D) indicate that compound **2** has specific binding to amyloid plaque in mouse brain.

2.4. Partition coefficient measurement

In order to measure the potential of compounds [^{18}F]**1**–**3** to cross the blood–brain barrier (BBB) by passive diffusion, the log of the partition coefficient (log $P_{oct/PBS}$) values were distributed in the range of 2.83–3.60 (Table 3). The results suggested that the three compounds readily cross the BBB, and that [^{18}F]**1** and [^{18}F]**2**, in particular, are within the optimal range (log *P* between 1 and 3).

2.5. In vitro stability studies

Radiotracers [^{18}F]**1**–**3** were incubated in human serum at 37 °C and analyzed by radio-TLC (data not shown). The results showed that over 90% of the three radiotracers remained intact even after 120 min, demonstrating their high chemical stability.

2.6. Analysis of metabolites

Radiolabeled materials were extracted from ICR mice brain homogenates with over 95% efficiency. When samples of brain were analyzed by HPLC, ratios of peak area under metabolites to [^{18}F]**2** were 5:95 at 5 min, 19:81 at 30 min, and 42:58 at 60 min sample of the brain.

2.7. Biodistribution in normal mice

To measure their in vivo brain penetration and clearance, biodistribution studies of [^{18}F]**1**–**3** were performed in healthy male ICR mice, and their blood, remnant, cortex and cerebellum regional brain tissue uptakes were determined at different points in time (at 2, 30, and 60 min, Table 4). For an ideal in vivo Aβ plaque probe, a high initial brain entry and low nonspecific binding in a normal brain are necessary in order to increase the signal-to-noise ratio. Table 4 lists the tracer uptake in the brain as the most important terms of percent injected dose per gram of organ (% ID/g).

In a comparison of the three radiotracers [^{18}F]**1**–**3**, the initial brain uptake in the cortex of [^{18}F]**2** was the highest of the three compounds (6.62 ± 0.3% ID/g at 2 min post-injection), and its radioactivity was rapidly washed out from the brain at 30 and 60 min post-injection (1.20 ± 0.2 and 0.73 ± 0.1% ID/g, respectively). [^{18}F]**1** and [^{18}F]**3** showed an initial uptake in the cortex of 5.86 ± 0.3 and 4.39 ± 0.3% ID/g, respectively. In the measure of brain clearance for the three radiotracers expressed as the ratio of % ID/g in the cortex at 2 min over % ID in the cortex at 60 min, [^{18}F]**2** showed a higher value (9.1) than [^{18}F]**1** (8.0) and [^{18}F]**3** (2.7). The blood levels of [^{18}F]**1**–**3** were relatively low at all points in time. Figure 3 shows the brain radiouptake of [^{18}F]**1**–**3** in ICR mice in terms of percent injected dose per gram of organ normalized to body weight (% ID-kg/g) to compare across animals of different size.

Table 4
Tissue distribution of 2-aryl-6-[^{18}F]fluorobenzothiazoles ([^{18}F]**1–3**)

Radiotracer	Time	Tissue % ID/g ± SD			
		Blood	Cortex	Cerebellum	Remnant[a]
[^{18}F]**1**	2 min	1.92 ± 0.1	5.86 ± 0.3	5.83 ± 0.2	6.41 ± 0.2
	30 min	1.23 ± 0.5	1.79 ± 0.3	2.19 ± 0.3	2.86 ± 0.4
	60 min	1.21 ± 0.1	0.73 ± 0.1	1.02 ± 0.2	1.37 ± 0.1
[^{18}F]**2**	2 min	2.08 ± 0.2	6.62 ± 0.3	6.24 ± 0.4	6.30 ± 0.5
	30 min	1.13 ± 0.1	1.20 ± 0.2	1.38 ± 0.2	1.91 ± 0.3
	60 min	1.11 ± 0.1	0.73 ± 0.1	0.98 ± 0.1	1.32 ± 0.2
[^{18}F]**3**	2 min	1.55 ± 0.1	4.39 ± 0.2	4.45 ± 0.2	4.26 ± 0.2
	30 min	1.04 ± 0.2	2.17 ± 0.3	2.03 ± 0.3	2.80 ± 0.4
	60 min	1.02 ± 0.1	1.61 ± 0.2	1.16 ± 0.1	2.01 ± 0.2

[a] Remnant means the remaining brain tissues after extraction of cerebral cortex and cerebellum.

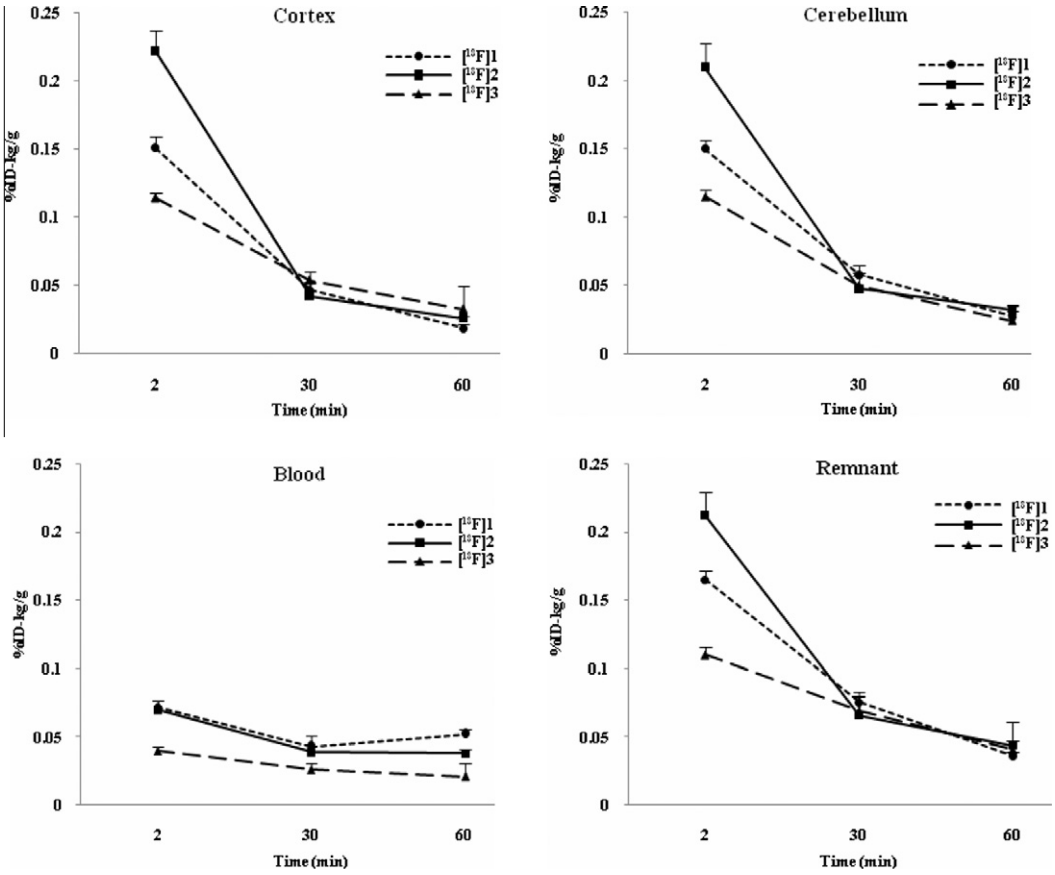


Figure 3. Comparison of biodistribution data of 2-aryl-6-[^{18}F]fluorobenzothiazoles ([^{18}F]**1–3**) in ICR mice. The data are expressed as % ID-kg/g.

The highly lipophilic analog [^{18}F]**3**, however, showed the poorest rate of brain entry and it elimination from the brain was slow, despite its high binding affinity to Aβ plaque.

The tendency toward uptake of [^{18}F]**1** and [^{18}F]**2** in the cerebellum was similar to that they exhibited in the cortex, indicating that these compounds exhibit low non-specific binding in the brain and a rapid washout rate, which is a highly desirable combination of properties for an Aβ plaque-specific imaging probe. These results then, taken together with other in vitro and in vivo results, showed that [^{18}F]**1** and [^{18}F]**3** may be unsuitable for an Aβ plaque imaging probe because of their low binding affinity to Aβ plaque, and the poor rate of brain entry of the former and the slow washout of the latter. On the basis of these results, [^{18}F]**2** stands out as a particularly promising compound because it exhibits good in vitro binding affinity to Aβ plaque, a high rate of brain uptake, and a fast brain washout.

3. Conclusion

In this study, we focused aromatic fluorine-18 labeling on the 6-position of benzothiazole analogs in order to determine the practicality of an diaryliodonium tosylate precursor, lipophilicity, in terms of its binding affinity for Aβ plaque as well as on the in vivo biodistribution. Based on the radiosynthesis results reported in our work, it is reasonable to conclude that the relatively simple BTA analog, 2-(4′-*N*-methylaminophenyl)-6-[^{18}F]fluorobenzothiazole ([^{18}F]**2**), can introduced fluorine-18 in the 6-position with good radiochemical yields obtained with the diaryliodonium tosylate precursor **13e**; furthermore, it also shows a good binding affinity to Aβ aggregates by in vitro binding assay. Tissue distribution studies in healthy mice showed a good rate of binding brain entry and rapid elimination from the brain. Judging from these results, the new fluorine-18 labeled BTA analog, [^{18}F]**2**, may be a useful PET probe for Aβ plaque imaging. Imaging studies in the transgenic mouse model of AD are underway to pursue the further development of [^{18}F]**2** as an amyloid-imaging probe.

4. Experimental

4.1. Chemistry

Solvents and reagents were purchased from Sigma–Aldrich (St. Louis, MO). ^{1}H and ^{13}C NMR were obtained on Varian Gemini-400 (Palo Alto, USA), JEOL Ltd-300 (Tokyo, Japan), and Bruker-300 instrument (Billerica, USA). Chemical shifts were reported in parts per million (ppm, δ units). Electron impact (EI) mass spectra were obtained on a GC/MS QP5050A spectrometer (Shimadzu, Kyoto, Japan), and fast atom bombardment (FAB) mass spectra were obtained on a JMS 700 (Jeol Ltd, Tokyo, Japan). Microwave-assisted reactions were performed with model 520A Resonance Instruments, Inc. (Skokie, IL). HPLC was carried out on a Thermo Separation Products System (Fremont, USA) equipped with a semipreparative column (Waters, Xterra RP-18 column, 10 μ, 7.9 × 300 mm) or an analytical column (Thermo, Hypresil Gold C-18 silica gel 5 μ, 4.6 × 250 mm and Hypersil silica gel 3 μ, 4.6 × 250 mm). Chromatography systems were fitted with a UV detector (SpectraSystem UV3000 set at 254 nm; Thermo, Fremont, USA) and a gamma-ray detector (Bioscan Flow-Count fitted with a NaI(Tl) detector). Thin Layer Chromatography (TLC) was performed

using Merck F_{254} silica plates and analyzed on a Bioscan radio-TLC scanner (Washington DC, USA). $H_2{}^{18}O$ was purchased from Taiyo Nippon Sanso Corporation. ^{18}F-Fluoride was produced at Seoul National University Bundang Hospital by the $^{18}O(p,n)^{18}F$ reaction through proton irradiation using the KOTRAN-13 cyclotron (Samyoung Unitech Co., Ltd, Seoul, Korea). The syntheses of compounds (2-amino-5-fluorobenzenthiol **4** and 2-amino-5-bromobenzenthiol **5**) were prepared according to the literature, respectively.[22,23] The preparation of compound **15b–e** was followed by the reported methods.[19,24,25]

4.2. General procedure for the synthesis of 8, 3, 9a, and 9c

4.2.1. 2-(4′-Nitrophenyl)-6-fluorobenzothiazole (8)

To a solution of 2-amino-5-fluorobenzenethiol **4** (300 mg, 2.09 mmol) in dimethylsulfoxide (3 mL) was added 4-nitrobenzaldehyde **6** (315 mg, 2.09 mmol). The reaction mixture was heated at 180 °C for 30 min. At the end of the reaction, the reaction mixture was cooled to room temperature and poured into ice-water (6 mL). The precipitate was filtered under reduced pressure. The filtrate was purified by recrystallization from tetrahydrofuran (5 mL)-methanol (150 mL) to give 325 mg (57%) of the title compound **8** as a yellow solid; mp = 201.2–201.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (d, J = 8.7 Hz, 2H), 8.21 (d, J = 8.7 Hz, 2H), 8.09–8.04 (m, 1H), 7.62 (dd, J = 8.1 2.4 Hz, 1H), 7.31–7.24 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.00 (d, J = 246.6 Hz), 150.74 (d, J = 1.8 Hz), 149.02, 138.83, 136.50 (d, J = 11.2 Hz), 128.09, 124.97 (d, J = 9.2 Hz), 124.33, 115.78 (d, J = 24.8 Hz), 108.02 (d, J = 26.6 Hz); MS (EI) m/z 274 (M^+); Anal. Calcd for $C_{13}H_7FN_2O_2S$: C, 56.93; H, 2.57; N, 10.21; S, 11.69. Found: C, 56.98; H, 2.60; N, 10.31; S, 11.69. Registry number: 343975–46–2.

4.2.2. 2-(4′-Dimethylaminophenyl)-6-fluorobenzothiazole (3)

A yellow solid; mp = 204.4–205.9 °C; Analytical HPLC: reverse phase (70:30 acetonitrile–H_2O) k' = 2.01, purity 99.8%; normal phase (10:90, 5% IPA in dichloromethane–hexane) k' = 0.06, purity 99.11%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92–7.87 (m, 3H), 7.50 (dd, J = 8.1, 2.4 Hz, 1H), 7.19–7.12 (m, 1H), 6.73 (d, J = 9.0 Hz, 2H), 3.05 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.51 (d, J = 3.2 Hz), 159.92 (d, J = 242.3 Hz), 152.16, 151.04 (d, J = 1.88 Hz), 135.47 (d, J = 11.2 Hz), 128.72, 122.95 (d, J = 9.3 Hz), 121.12, 114.29 (d, J = 24.1 Hz), 111.67, 107.61 (d, J = 26.6 Hz), 40.11; MS (CI) m/z 273 (M^++H); Anal. Calcd for $C_{15}H_{13}FN_2S$: C, 66.15; H, 4.81; N, 10.29; S, 11.77. Found: C, 66.13; H, 4.85; N, 10.27; S, 11.77. Registry number: 1071423–42–1.

4.2.3. 2-(4′-Nitrophenyl)-6-bromobenzothiazole (9a)

A yellow solid; mp = 192.4–193.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.55 (d, J = 2.1 Hz, 1H), 8.38 (dd, J = 9.3 Hz, J = 9.0 Hz, 2H), 8.08 (d, J = 8.7 Hz, 1H), 7.76 (dd, J = 8.7 Hz, J = 2.1, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.87, 152.49, 148.92, 137.92, 137.02, 130.34, 128.51, 125.27, 124.98, 124.64, 123.58, 119.10; MS (EI) m/z 336 (M^+, ^{81}Br), 334 (M^+, ^{79}Br); Anal. Calcd for $C_{13}H_7BrN_2O_2S$: C, 46.58; H, 2.11; N, 8.36; S, 9.57. Found: C, 46.72; H, 2. 06; N, 8.38; S, 9.56. Registry number: 566169-96-8.

4.2.4. 2-(4′-Dimethylaminophenyl)-6-bromobenzothiazole (9c)

A yellow solid; mp = 208.6–208.7 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96–7.91 (m, 3H), 7.81 (d, J = 8.7 Hz, 1H), 7.52 (dd, J = 8.7, 2.0 Hz, 1H), 6.74 (d, J = 9.0 Hz, 2H), 3.06 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.46, 154.36, 153.49, 136.37, 129.52, 129.06, 123.99, 123.42, 120.96, 117.53, 111.80, 40.30; MS (EI) m/z 334 (M^+, ^{81}Br), 332 (M^+, ^{79}Br); Anal. Calcd for $C_{15}H_{13}BrN_2S$: C, 54.06; H, 3.93; N, 8.41; S, 9.62. Found: C, 54.05; H, 3.91; N, 8.45; S, 9.62. Registry number: 346691-88-1.

4.3. General procedure for the synthesis of 1 and 10

4.3.1. 2-(4′-Aminophenyl)-6-fluorobenzothiazole (1)

To a solution of 2-(4′-nitrophenyl)-6-fluorobenzenethiol **8** (1.6 g, 5.84 mmol) in ethanol (150 mL) was added tin(II) chloride (4.92 g, 26.3 mmol). The reaction mixture was heated at 90 °C under nitrogen gas for 2 h. Ethanol was removed by evaporator, and the residue was dissolved in ethyl acetate (100 mL), the organic solution was washed with saturated sodium bicarbonate (30 mL) followed by water (30 mL) and dried over sodium sulfate. The crude product was purified by flash chromatography (silica gel, 70:30 hexane–ethyl acetate) to give 1.05 g (74%) of the title compound **1** as a yellow solid; mp = 201.2–201.5 °C; Analytical HPLC: reverse phase (60:40 acetonitrile–H_2O) k' = 0.46, purity 98.37%; normal phase (10:90 5% IPA in dichloromethane–hexane) k' = 5.39, purity 98.14%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93–7.89 (m, 1H), 7.85 (d, J = 8.4 Hz, 2H), 7.52 (dd, J = 8.1, 2.4 Hz, 1H), 7.20–7.13 (m, 1H), 6.72 (d, J = 8.4 Hz, 2H), 4.01 (br s, 2H, NH_2);^{13}C NMR (75 MHz, $CDCl_3$) δ 168.21 (d, J = 3.8 Hz), 160.06 (d, J = 242.9 Hz), 150.87 (d, J = 1.8 Hz), 149.26, 135.52 (d, J = 11.1 Hz), 129.01, 123.67, 123.22 (d, J = 9.3 Hz), 114.76, 114.46 (d, J = 24.0 Hz), 107.70 (d, J = 26.6 Hz); MS (CI) m/z 245 (M^++H); Anal. Calcd for $C_{13}H_9FN_2S$: C, 63.92; H, 3.71; N, 11.47; S, 13.13. Found: C, 63.82; H, 3.71; N, 11.44; S, 13.24. Registry number: 328087-15-6.

4.3.2. 2-(4′-Aminophenyl)-6-bromobenzothiazole (10)

A yellow solid; mp = 219.3–221.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (d, J = 1.8 Hz, 1H), 7.87 (d, J = 8.7 Hz, 2H), 7.82 (d, J = 8.7 Hz, 1H), 7.53 (dd, J = 8.4, 1.8 Hz, 1H), 6.73 (d, J = 8.7, 2.0 Hz, 2H), 4.03 (br s, 2H, NH_2); MS (CI) m/z 306 (M^++H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.50, 149.48, 136.24, 129.48, 129.19, 123.92, 123.52, 123.43, 117.76, 117.73, 114.76; MS (FAB) m/z 307 (M^++H, ^{81}Br), 305 (M^++H, ^{79}Br) Anal. Calcd for $C_{13}H_9BrN_2S$: C, 51.16; H, 2.97; N, 9.18; S, 10.51. Found: C, 51.04; H, 3.03; N, 9.03; S, 10.69. Registry number: 566169-97-9.

4.3.3. 2-(4′-Methylaminophenyl)-6-fluorobenzothiazole (2)

To a solution of 2-(4′-aminophenyl)-6-fluorobenzenethiol **1** (300 mg, 1.23 mmol) in acetonitrile (15 mL) was added iodomethane (153 μL, 2.46 mmol) and K_2CO_3 (1.7 g, 12.3 mmol. The reaction mixture was refluxed for 4 h. After acetonitrile was removed by evaporator and vacuum, the residue was dissolved in ethyl acetate (50 mL), the organic solution was washed with saturated sodium bicarbonate (30 mL) followed by water (30 mL) and dried over sodium sulfate. The crude product was purified by flash chromatography (silica gel, 70:30 hexane–ethyl acetate) to give 111 mg (35%) of 2-(4′-methylaminophenyl)-6-fluorobenzothiazole **2** as a yellow solid; mp = 153.5–154.5 °C; Analytical HPLC: reverse phase (60:40 acetonitrile–H_2O) k' = 4.38, purity 99.67%; normal phase (10:90 5% IPA in dichloromethane–hexane) k' = 2.25, purity 98.58%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92–7.86 (m, 3H), 7.51 (dd, J = 8.1, 2.4 Hz, 1H), 7.20–7.12 (m, 1H), 6.63 (d, J = 8.4 Hz, 2H), 4.14 (br s, 1H, NH), 2.90 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.49 (d, J = 3.1 Hz), 159.96 (d, J = 242.3 Hz), 151.57, 150.97, 135.46 (d, J = 11.1 Hz), 128.95, 123.02 (d, J = 9.3 Hz), 122.25, 114.34 (d, J = 24.8 Hz), 112.01, 107.64 (d, J = 26.6 Hz), 30.26; MS (CI) m/z 259 (M^++H); Anal. Calcd for $C_{14}H_{11}FN_2S$: C, 65.10; H, 4.29; N, 10.84; S, 12.41. Found: C, 65.13; H, 4.30; N, 10.86; S, 12.43. Registry number: 1071423-41-0.

4.3.4. 2-(4′-*N*-*tert*-Butyloxycarbonyl-methylaminophenyl)-6-bromobenzothiazole (9b)

To a solution of 2-(4′-*N*-aminophenyl)-6-bromobenzenethiol **10** (300 mg, 0.98 mmol) in methanol (20 mL) was added formaldehyde (220 μL, 2.96 mmol). The reaction mixture was refluxed for 1.5 h. Methanol was removed by evaporator and vacuum. The

residue was dissolved in methanol (100 mL) again, and was added sodium cyanoborohydride (247 mg, 3.92 mmol) and acetic acid (6 μL, pH 6) slowly. The reaction mixture was stirred at room temperature for 1.5 h. Methanol was removed by evaporator and the residue was dissolved in ethyl acetate (50 mL), the organic solution was washed with saturated sodium bicarbonate (30 mL) followed by water (30 mL) and dried over sodium sulfate. The crude product was purified by flash chromatography (silica gel, 70:30 hexane–ethyl acetate). To the obtained 2-(4′-methylaminophenyl)-6-bromobenzothiazole in tetrahydrofuran (15 mL) was added di-*tert*-butyl-dicarbonate (217 mg, 0.96 mmol) at 0 °C. The reaction mixture was refluxed for 12 h. At the end of the reaction, the reaction mixture was cooled to room temperature and poured into ice-water (20 mL) and ethyl acetate (40 mL). The organic solution was washed with saturated sodium bicarbonate (30 mL) followed by water (30 mL) and dried over sodium sulfate. The crude product was purified by flash chromatography (silica gel, 80:20 hexane–ethyl acetate) to give 200 mg (49%) of 2-(4′-*N*-*tert*-butyloxycarbonyl-methylaminophenyl)-6-bromobenzothiazole **9b** as a pale yellow solid; mp = 144.5–145.2 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02–8.00 (m, 3H), 7.89 (d, J = 8.4 Hz, 1H), 7.57 (d, J = 8.7 Hz, 1H), 7.40 (d, J = 8.1 Hz, 1H), 3.30 (s, 3H), 1.47 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.88, 154.21, 153.01, 146.48, 136.63, 129.81, 129.57, 127.72, 125.21, 124.14, 118.60, 81.01, 67.95, 36.93, 28.29; MS (FAB) *m/z* 421 (M^++H, ^{81}Br), 419 (M^++H, ^{79}Br); Anal. Calcd for $C_{19}H_{19}BrN_2O_2S$: C, 54.42; H, 4.57; N, 6.68; S, 7.65. Found: C, 54.34; H, 4.56; N, 6.78; S, 7.62.

4.4. General procedure for the synthesis of 11a–c

4.4.1. 2-(4′-Nitrophenyl)-6-tributylstannylbenzothiazole (11a)

To a mixture of 2-(4′-nitrophenyl)-6-bromobenzothiazole **9a** (200 mg, 0.60 mmol) and tetrakis(triphenylphosphine)-palladium(0) (69 mg, 0.06 mmol) in anhydrous tetrahydrofuran (10 mL) was added and bistributyltin (658 μL, 1.31 mmol) in anhydrous tetrahydrofuran (10 mL) under argon gas at room temperature. The reaction mixture was heated at 85 °C for 8 h. At the end of the reaction, the reaction mixture was cooled to room temperature and filtered by Celite. The crude product was purified by flash chromatography (silica gel, 80:20 hexane–ethyl acetate) to give 170 mg (52%) of the title compound **11a** as a pale yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (d, J = 9.0 Hz, 2H), 8.27 (d, J = 9.0 Hz, 2H), 8.09–8.03 (m, 2H), 7.62 (dd, J = 8.1, 0.4 Hz, 1H), 1.57–1.52 (m, 6H), 1.41–1.29 (m, 6H) 1.16–1.10 (m, 6H), 0.92–0.85 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.39, 154.18, 149.17, 141.20, 139.63, 135.84, 134.52, 128.48, 128.42, 124.50, 123.37, 29.28, 27.57, 13.87, 10.13; MS (FAB) *m/z* 547 (M^++H); Anal. Calcd for $C_{25}H_{34}N_2O_2SSn$: C, 55.06; H, 6.28; N, 5.14; S, 5.88. Found: C, 55.05; H, 6.28; N, 5.11; S, 5.88.

4.4.2. 2-(4′-*N*-*tert*-Butyloxycarbonyl-methylaminophenyl)-6-tributylstannylbenzothiazole (11b)

A yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06–7.98 (m, 4H), 7.56 (d, J = 8.0 Hz, 1H), 7.38 (d, J = 8.8 Hz, 2H), 3.32 (s, 3H), 1.59–1.53 (m, 6H), 1.40–1.31 (m, 6H), 1.14–1.10 (m, 6H), 0.94–0.88 (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.81, 154.31, 154.02, 146.11, 139.05, 134.67, 133.78, 130.30, 129.04, 127.72, 125.24, 122.45, 36.98, 30.29, 29.07, 28.30, 27.36, 13.67, 9.82; MS (FAB) *m/z* 631 (M^++H); Anal. Calcd for $C_{31}H_{46}N_2O_2SSn$: C, 59.15; H, 7.37; N, 4.45; S, 5.09. Found: C, 59.13; H, 7.34; N, 4.45; S, 5.04.

4.4.3. 2-(4′-Dimethylaminophenyl)-6-tributylstannylbenzothiazole (11c)

A yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98–7.92 (m, 4H), 7.50 (d, J = 7.6 Hz, 1H), 6.75 (d, J = 8.4 Hz, 2H), 3.06 (s, 6H), 1.58–1.52 (m, 6H), 1.39–1.32 (m, 6H), 1.12–1.08 (m, 6H), 0.91–0.88 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.44, 154.53, 152.36, 137.78, 135.00, 133.71, 129.08, 129.03, 121.97, 121.82, 111.91, 40.40, 29.21, 27.60, 13.89, 10.04; MS (EI) *m/z* 544 (M^+); Anal. Calcd for $C_{27}H_{40}N_2SSn$: C, 59.68; H, 7.42; N, 5.16; S, 5.90. Found: C, 59.65; H, 7.37; N, 5.22; S, 5.94. Registry number: 346691-92-7.

4.5. General procedure for the synthesis of 12, 13a–e and 14

4.5.1. 2-(4′-Nitrophenyl)-6-iodo(phenyl)benzothiazole iodonium tosylate (12)

To a solution of Koser's reagent (69.5 mg, 0.17 mmol) in dichloromethane (10 mL) was added 2-(4′-nitrophenyl)-6-tributylstannylbenzothiazole **11a** (95 mg, 0.17 mmol) under argon atmosphere. The reaction mixture was stirred at room temperature under argon atmosphere for 12 h. The solvent was evaporated using a stream of nitrogen. The crude mixture was dissolved a small amount of methanol (1.5 mL) and transferred to the centrifuge tube to which was added excess diethyl ether (20 mL). After centrifuging, the collected oil was dried in vacuo to give 40 mg (38%) of the title compound **12** as a yellow solid: mp = 216.6–218.5 °C; ^{1}H NMR (400 MHz, MeOH-d_4) δ 9.02 (d, J = 1.6 Hz, 1H), 8.43–8.37 (m, 5H), 8.31 (dd, J = 8.0, 2.0 Hz, 1H), 8.24–8.18 (m, 3H), 7.68 (d, J = 8.0 Hz, 2H), 7.54 (t, J = 8.0 Hz, 2H), 7.21 (d, J = 8.0 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 168.30, 154.33, 148.43, 140.77, 138.88, 136.77, 136.37, 133.74, 131.55, 131.04, 130.50, 128.69, 127.21, 127.03, 125.00, 124.18, 122.77, 113.89, 109.20, 18.54; MS (FAB) *m/z* 459 (M^+−OTs); HRMS calcd for $C_{19}H_{12}IN_2O_2S$ 458.9664, found 458.9671.

4.5.2. 2-(4′-*N*-*tert*-Butyloxycarbonyl-methylaminophenyl)-6-iodo(phenyl)benzothiazole iodonium tosylate (13a)

A yellow solid; mp = 156.2–156.9 °C; ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.92 (d, J = 1.6 Hz, 1H), 8.26–8.20 (m, 3H), 8.13–8.06 (m, 3H), 7.70–7.63 (m, 3H), 7.56–7.47 (m, 4H), 7.19 (d, J = 8.0 Hz, 2H), 3.32 (s, 3H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 173.26, 157.30, 155.92, 148.67, 143.56, 141.63, 139.11, 136.40, 134.08, 133.74, 133.23, 131.13, 130.51, 129.78, 129.28, 127.67, 126.99, 126.95, 116.64, 110.86, 82.46, 37.45, 28.51, 21.30; MS (FAB) *m/z* 543 (M^+−OTs); HRMS calcd for $C_{25}H_{24}IN_2O_2S$ 543.0603, found 543.0599.

4.5.3. 2-(4′-Dimethylaminophenyl)-6-iodo(phenyl)benzothiazole iodonium tosylate (14)

A yellow solid; mp = 172.0–173.8 °C; ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.81 (d, J = 2.0 Hz, 1H), 8.21–8.17 (m, 3H), 7.95–7.92 (m, 3H) 7.70–7.67 (m, 3H), 7.53 (t, J = 7.8 Hz, 2H), 7.20 (d, J = 8.0 Hz, 2H), 6.82 (d, J = 8.8 Hz, 2H), 3.08 (s, 6H), 2.34 (s, 3H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 156.55, 153.76, 150.39, 140.98, 140.48, 135.07, 133.61, 132.78, 132.48, 131.99, 129.42, 129.24, 128.60, 125.75, 124.42, 116.27, 111.77, 108.07, 81.18, 39.10, 16.16; MS (FAB) *m/z* 457 (M^+−OTs); HRMS calcd for $C_{21}H_{18}IN_2S$ 457.0235, found 457.0246.

4.5.4. 2-(4′-*N*-*tert*-Butyloxycarbonyl-methylaminophenyl)-6-iodo(4′-methoxyphenyl)benzothiazole iodonium tosylate (13b)

A white solid; mp = 159.4–159.8 °C; ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.87 (d, J = 1.6 Hz, 1H), 8.21 (dd, J = 8.8, 2.0 Hz, 1H), 8.14–8.10 (m, 4H), 8.08 (d, J = 8.8 Hz, 1H), 7.69 (d, J = 8.0 Hz, 2H), 7.49 (d, J = 8.4 Hz, 2H), 7.21 (d, J = 8.0 Hz, 2H), 7.06 (d, J = 9.2 Hz, 2H), 3.83 (s, 3H), 3.31 (s, 3H), 2.35 (s, 3H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 173.15, 164.60, 157.21, 155.93, 148.66, 141.64, 139.01, 138.53, 133.69, 130.63, 130.53, 129.79, 129.27, 127.00, 126.96, 126.84, 118.90, 111.44, 106.44, 105.10, 82.47, 56.35, 37.45, 28.51, 21.30; MS (FAB) *m/z* 573 (M^+−OTs); HRMS calcd for $C_{26}H_{26}IN_2O_2S$ 573.0709, found 573.0707.

4.5.5. 2-(4′-*N*-*tert*-Butyloxycarbonyl-methylaminophenyl)-6-iodo(4′-methylphenyl)benzothiazole iodonium tosylate (13c)

A white solid; mp = 159.5–159.9 °C; ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.90 (d, J = 1.6 Hz, 1H), 8.23 (dd, J = 8.8, 1.6 Hz, 1H), 8.12–8.07 (m, 5H), 7.68 (d, J = 8.0 Hz, 2H), 7.49 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 7.21 (d, J = 8.0 Hz, 2H), 3.31 (s, 3H), 2.39 (s, 3H), 2.35 (s, 3H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 173.21, 157.26, 155.93, 148.68, 143.56, 141.63, 139.06, 136.39, 133.95, 130.92, 130.88, 130.52, 129.78, 129.28, 127.00, 126.95, 126.88, 112.91, 111.00, 82.47, 37.45, 28.51, 21.35, 21.30; MS (FAB) m/z 557 (M^+−OTs); HRMS calcd for $C_{26}H_{26}IN_2O_2S$ 557.0760, found 557.0762.

4.5.6. 2-(4′-*N*-*tert*-Butyloxycarbonyl-methylaminophenyl)-6-iodo(2′-thiophenyl)benzothiazole iodonium tosylate (13d)

A pale yellow solid; mp = 146.8–147.2 °C; ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.94 (d, J = 2.0 Hz, 1H), 8.27 (dd, J = 8.8, 2.0 Hz, 1H), 8.13–8.09 (m, 3H), 8.06 (dd, J = 4.0, 1.2 Hz, 1H), 7.89 (dd, J = 5.2, 1.2 Hz, 1H), 7.69 (d, J = 8.0 Hz, 2H), 7.49 (d, J = 8.8 Hz, 2H), 7.22 (d, J = 8.0 Hz, 2H), 7.18 (dd, J = 5.2, 4.0 Hz, 1H), 3.31 (s, 3H), 2.36 (s, 3H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 173.38, 157.28, 155.92, 148.69, 143.52, 142.15, 141.64, 138.97, 138.67, 133.47, 130.88, 130.52, 129.79, 129.29, 126.99, 126.94, 126.88, 113.86, 99.77, 82.47, 37.45, 28.51, 21.31; MS (FAB) m/z 549 (M^+−OTs); HRMS calcd for $C_{23}H_{22}IN_2O_2S$ 549.0167, found 549.0167.

4.5.7. 2-(4′-*N*-*tert*-Butyloxycarbonyl-methylaminophenyl)-6-iodo(3′-thiophenyl)benzothiazole iodonium tosylate (13e)

A pale yellow solid; mp = 128.2–130.2 °C; ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.90 (d, J = 2.0 Hz, 1H), 8.54 (dd, J = 2.8, 1.2 Hz, 1H), 8.24 (dd, J = 2.0, 8.8 Hz, 1H), 8.12 (d, J = 8.4 Hz, 2H), 8.08 (d, J = 8.8 Hz, 1H), 7.72–7.66 (m, 4H), 7.49 (d, J = 8.4 Hz, 2H), 7.21(d, J = 8.4 Hz, 2H), 3.31 (s, 3H), 2.35 (s, 3H), 1.48 (s, 9H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 173.21, 157.20, 155.92, 148.66, 143.55, 141.63, 139.02, 137.26, 133.78, 132.48, 131.81, 130.80, 130.52, 129.78, 129.28, 126.99, 126.94, 126.85, 111.80, 100.18, 82.46, 37.45, 28.52, 21.30; MS (FAB) m/z 549 (M^+−OTs); HRMS calcd for $C_{23}H_{22}IN_2O_2S$ 549.0167, found 549.0161.

4.6. The radiosynthesis and purification of [^{18}F]1–3

4.6.1. 2-(4′-Aminophenyl)-6-[18F]fluorobenzothiazole ([^{18}F]1)

[^{18}F]Fluoride was produced in a cyclotron by the ^{18}O(p,n)^{18}F reaction. A volume of 100–200 μL [^{18}F]fluoride (18.5–370 MBq) in water was added to a vacutainer containing n-Bu_4NHCO_3 (40% aq 2.12 μL, 2.76 μmol). The azeotropic distillations were carried out each time with 200 μL aliquots of CH_3CN at 85 °C under a stream of nitrogen. Compound **12** (2 mg, 3.3 μmol) in acetonitrile (300 μL, adding 10 μL of H_2O and 1 mg of TEMPO) was added to the dried tetrabutylammonium fluoride in the reaction vial and reacted in the microwave equipment with 100 W (180 sec. × 2) or heated in oil bath at 130 °C for 10 min. After the reaction, the vial was cooled in an ice bath and the solvent was removed under a gentle stream of nitrogen at 80 °C. The crude reaction mixture was diluted with 2 mL of ethanol–tetrahydrofuran–ethyl acetate (5:47.5:47.5, v/v), loaded into silica Sep-Pak and washed with 2 mL of the same solution again. The obtained organic solution was removed under a gentle stream of nitrogen and added tin(II) chloride (3.35 mg, 13 μmol) and EtOAc (200 μL). The mixture was heated at 80 °C for 10 min. The solvent was removed with a gentle stream of nitrogen. The reaction mixture was purified by HPLC at a flow 3 mL/min using a 30:7:63 mixture of 50 mM $(NH_4)H_2PO_4$–tetrahydrofuran–acetonitrile, and [^{18}F]**1** was eluted at 9.3 min. Radiotracer [^{18}F]**1** collected from HPLC was purified with C-18 Sep Pak cartridge with the help water (12 mL) and ethanol (1 mL), respectively. After the ethanol was evaporated, radiotracer [^{18}F]**1** was used for biological study. For the identification of the radio-product, the collected HPLC fraction was matched with the authentic compound. Specific activity at the end of synthesis was calculated by relating radioactivity to the mass associated with the UV absorbance (254 nm) peak of authentic compound. Specific radioactivity of [^{18}F]**1** (85 GBq/μmol) was obtained after purification on analytic HPLC column.

4.6.2. 2-(4′-*N*-Methylaminophenyl)-6-[^{18}F]fluorobenzothiazole ([^{18}F]2)

The preparation of [^{18}F]fluoride and the azeotropic distillations were carried out according to [^{18}F]**1** experiment. Compound **13a–e** (2 mg, 2.9 μmol) in acetonitrile (300 μL, adding 10 μL of H_2O and 1 mg of TEMPO) was added to the dried tetrabuthylammonium fluoride in the reaction vial and reacted in the microwave equipment with 100 W (180 sec. × 2) or heated in oil bath at 130 °C for 10 min. The vial was cooled in an ice bath and the solvent was removed under a gentle stream of nitrogen at 80 °C. 3 N HCl in ethyl acetate (3:1 ethyl acetate–concd HCl, v/v, 250 μL) was added to the crude reaction mixture and reacted at 75 °C for 10 min. After the reaction, the vial was cooled in an ice bath and the solvent was removed under a gentle steam of nitrogen at 75 °C. The reaction mixture was purified by HPLC at a flow 3 mL/min using a 45:55 mixture of H_2O–acetonitrile, and [^{18}F]**2** was eluted at 17.5 min. Radiotracer [^{18}F]**2** collected from HPLC was purified with Sep Pak cartridge with the help water (12 mL) and ethanol (1 mL), respectively. Specific radioactivity of [^{18}F]**2** (110 GBq/μmol) was obtained after purification on analytic HPLC column.

4.6.3. 2-(4′-*N*-Dimethylaminophenyl)-6-[^{18}F]fluorobenzothiazole ([^{18}F]3)

The preparation of [^{18}F]fluoride and the azeotropic distillations were carried out according to [^{18}F]**1** experiment. Compound **14** (2 mg, 3.3 μmol) in acetonitrile (300 μL, adding 10 μL of H_2O and 1 mg of TEMPO) was added to the dried tetrabuthylammonium fluoride in the reaction vial and reacted in the microwave equipment with 100 W (180 sec. × 2) or heated in oil bath at 130 °C for 10 min. The vial was cooled in an ice bath and the solvent was removed under a gentle stream of nitrogen at 80 °C. The reaction mixture was purified by HPLC at a flow 3 mL/min using a 40:3:57 mixture of 50 mM $(NH_4)H_2PO_4$–tetrahydrofuran–acetonitrile, and [^{18}F]**3** was eluted at 15.7 min. Radiotracer [^{18}F]**3** collected from HPLC was purified with Sep-Pak cartridge with the help water (12 mL) and ethanol (1 mL), respectively. Specific radioactivity of [^{18}F]**3** (118 GBq/μmol) was obtained after purification on analytic HPLC column.

4.7. Aβ binding affinity assay

Aggregated Aβ peptides were prepared using $Aβ_{1–42}$, and Aβ binding affinity was evaluated as published elsewhere.[26,27] The solid forms of peptides $Aβ_{1–42}$ was purchased from Bachem (King of Prussia, PA). Aggregation of peptides was carried out by gently dissolving the peptide (0.5 mg/mL for $Aβ_{1–42}$) in a buffer solution (pH 7.4) containing 10 mM sodium phosphate and 1 mM EDTA. The solutions were incubated for 42 h at 37 °C with gently shaking. Aggregated Aβ peptides were stored at −70 °C. For binding assay, aggregated Aβ (27.5 nM in the final assay mixture) were added to the mixture containing 50 μL of radioligands ([^{125}I]TZDM, 0.078–10 nM in 40% ethanol) and 10% ethanol in a final volume of 1 mL for saturation studies. Nonspecific binding assay was defined in the presence of 10 μM thioflavin-T (M.W.: 318.87) per tube. For inhibition studies, 1 mL of the reaction mixture contained 50 μL of inhibitors **1–3** (0.124–10,000 nM in 10% ethanol) and

0.05 nM radioligand ([^{125}I]TZDM) in 40% ethanol was incubated for 3 h at room temperature for the binding assay. The reaction mixture was filtered through Whatman GF/B glass filters and washed twice with 3 mL of 10% ethanol aliquots. The radioactivity retained on the filter was counted by a gamma-counter. Under the assay conditions, the percent of the specific binding fraction was less than 20% of the total ^{125}I radioactivity. The results of inhibition and saturation experiments were subjected to non-linear regression analysis using PRISM software by which K_d (The K_d values of [^{125}I]TZDM in $A\beta_{1-42}$ aggregates were 0.45 ± 0.032 nM) and K_i values of **1–3** were calculated.

$$K_i = IC_{50}/(1 + (\text{ligand})/K_d)$$

4.8. In vitro fluorescent staining assay

Brain sections were obtained from the transgenic mouse (APPswe/PS1△E9, 25 months) and the wild-type mouse.[28] After equilibration to −25 °C, 20 μm thickness of coronal sections were cut on a Cryocut Microtome (CM3050S, LEICA), thaw-mounted onto silane coated glass microscope slides (MUTO PURE CHEMICALS CO.), dried in aeration room, and stored at room temperature until use. To identify of Aβ peptides deposits in the sections, fluorescence staining with Thioflavine-S, and compound **2** were performed by the following steps: Staining with 0.0125% fluorescent dye (Thioflavine-S or compound **2**) in 40% ethanol-100 mM phosphate buffered saline (PBS) for 3 min in the dark at room temperature. After staining samples were rapidly washed in 50% ethanol-PBS for 3 min, PBS for 1 min, and water for 5 min, respectively. Staining samples were dried in the dark at room temperature. Fluorescent image of Aβ deposits brain tissue were obtained by Confocal Microscopy (LSM-700, Carl zeiss) using a FITC cube with an excitation filter of 350–390 nm and emission filter of 530 ± 15 nm.

4.9. Partition coefficient determination

Radioligands, [^{18}F]**1–3** were purified with HPLC, were concentrated under a gentle stream of nitrogen gas and redissolved in ethanol. The radioligands were added to premixed suspensions containing 5 mL of octanol and 5 mL of 0.1 M PBS buffer in a test tube, respectively, and then vortexed vigorously for 5 min and centrifuged at 3000 rpm for 5 min. Two layers separated out, and 20 μL aliquots of the octanol and aqueous layers were drawn and their radioactivity content was determined in a gamma counter. Samples from the octanol and the aqueous layers were repartitioned until consistent values were obtained. The experiments were carried out in triplicate. Log $P_{oct/PBS}$ was expressed as the logarithm of the ratio of the counts per minute from octanol versus that of PBS buffer.

4.10. In vitro stability studies

An aliquot (3.7 MBq) of [^{18}F]**1–3** in 10% ethanol-saline was added to human serum (1 mL, respectively) and incubated at 37 °C. The solution was analyzed at 5, 15, 30, 60, and 120 min by radio-TLC using hexane–ethyl acetate (60:40) as the developing solvents.

4.11. Analysis of metabolites in brain

ICR mice (male, 25 g) were administrated of compounds ([^{18}F]**2**, 37 MBq/mouse) in 0.2 mL of 10% ethanol-saline via a tail vein. Mice were sacrificed by cervical dislocation at 5, 15, 30, and 60 min post-injection and samples of the brain were collected. The brain samples were homogenized in 2 mL of 80% methanol-PBS in a commercial blender for 3 min and centrifuged 3500 rpm for 5 min at 4 °C. The resulting supernatant filtered through a 0.45 μm GH Polypro (GHP) membrane Disc filter. The samples were analyzed by HPLC at a flow rate of 3 mL/min using a 70:30 mixture of acetonitrile and water as the eluants.

4.12. Biodistribution in mice

Brain distribution studies were measured as the followed method: ICR mice (male, 25–28 g, 5 mice per time point) were administrated of compounds ([^{18}F]**1–3**, 1.1 MBq/mouse, respectively) in 0.2 mL of 10% ethanol-saline via a tail vein. Mice were sacrificed by cervical dislocation at 2, 30, and 60 min post-injection. After samples of blood, cortex, cerebellum, and remnant were removed, there are weighed and counted with Wizard®3″ 1480 automatic gamma-counter (PerkinElmer, Turku, Finland). Data are expressed as percentages of injected dose per gram of tissue (% ID/g) and normalized to body weight (% ID-kg)/g. All animal experiments were performed in compliance with the rules of the Seoul National University Bundang Hospital Laboratory Animal Care.

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