Synthesis and characterization of novel phenylindoles as potential probes for imaging of β-amyloid plaques in the brain

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Abstract

We synthesized a novel series of phenylindole (PI) derivatives and evaluated their biological activities as probes for imaging Aβ plaques *in vivo*. The affinity for Aβ plaques was assessed by an *in vitro*-binding assay using pre-formed synthetic Aβ aggregates. 2-Phenyl-1*H*-indole (2-PI) derivatives showed high affinity for Aβ42 aggregates with K_i values ranging from 4 to 32 nM. 2-PI derivatives clearly stained Aβ plaques in an animal model of AD. In biodistribution experiments using normal mice, 2-PI derivatives displayed sufficient uptake for imaging, ranging from 1.1 to 2.6% ID/g. Although additional modifications are necessary to improve uptake by and clearance from the brain, 2-PI derivatives may be useful as a backbone structure to develop novel Aβ imaging agents.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder of the elderly and is characterized clinically by dementia, cognitive impairment, and memory loss. Postmortem brains of AD patients reveal neuropathological features: the presence of senile plaques and neurofibrillary tangles, which contain β-amyloid (Aβ) peptides and highly phosphorylated tau proteins.[1,2] The formation and deposition of Aβ plaques is considered one of the most significant factors in AD. Currently, the only definitive diagnosis of AD is by pathological examination of postmortem staining of affected brain tissue. Therefore, non-invasive techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) are useful for the diagnosis of AD and new anti-amyloid therapies.[3-5]

Many radiolabeled probes based on the core structure of Congo Red (CR) and thioflavin T (ThT) have been developed as imaging agents for Aβ plaques. Although CR has large molecular size, some truncated CR type molecules such as stilbene and styrylpyridine derivatives have been reported.[6-8] Because ThT has a lower molecular weight than CR, implying greater blood-brain penetration and easier organic synthesis,

a number of groups have worked to develop probes for PET/SPECT derived from ThT including [^{11}C]PIB,[9,10] [^{11}C]AZD2184,[11,12] TZDM,[13] IBOX,[14] [^{123}I]IMPY,[15,16] phenylbenzofuran derivatives,[17,18] phenylbezothiophene derivatives[19], imidazopyridine derivatives[20,21] and imidazopyridazine derivatives[22]. (Figure 1) Clinical trials in AD patients have been conducted with [^{11}C]PIB and [^{11}C]AZD2184, and indicated the PET-based imaging of Aβ plaques in the living human brain to be useful for the diagnosis of AD. Radioiodinated probes for SPECT such as TZDM, IBOX and phenylbenzofuran derivatives have shown high affinity for Aβ aggregates *in vitro* and high initial uptake, but a slow washout from the brain.[13,14,17] Since the slow washout leads to a low signal/noise ratio in the imaging of Aβ plaques *in vivo*, a molecular design that facilitates the clearance of the radiolabeled probes from normal areas of the brain is needed. Several reports have shown the lipophilicity of probes to play an important role in uptake by and clearance from brain tissue.[9,23] As this may partly explain the slow washout from the brain, we planned to select a ThT-derived scaffold with less lipophilicity. In the search for such a scaffold, we focused on phenylindole, never before applied to the development of Aβ imaging probes, and calculated its log D

value to be 3.97, lower than that of phenylbenzofuran (4.34) or phenylbenzothiophene (4.94) (calculated with the Sparc On-Line Calculator).

In the present study, we synthesized a novel series of 2-phenyl-1*H*-indole (2-PI) and 1-phenyl-1*H*-indole (1-PI) derivatives and evaluated their potential as probes for imaging Aβ *in vivo*. This is the first time that PI derivatives have been used for SPECT to detect Aβ plaques.

2. Results and discussion

The synthesis of PI derivatives is outlined in Schemes 1-4. We used a one-pot method producing 2-PI.[24] Compounds **3**, **6**, and **12** were prepared from **2** and terminal alkynes (**1**, 4-ethynyl-*N,N*-dimethylaniline and *p*-ethynylanisole) using a palladium catalyst in the presence of tetrabutylammonium fluoride (TBAF) (27.2 – 49.5% yields). Trimethyltin derivatives (**4**, **7** and **13**) were prepared from the corresponding bromo compounds (**3**, **6** and **12**) using a bromo-to-trimethyltin exchange reaction catalyzed by Pd(0). Trimethyltin derivatives (**4** and **13**) were readily reacted with iodine in ethyl acetate at room temperature to give the iodo derivatives, **5** and **14**. The tributyltin

derivative **9** was prepared from the corresponding bromo compound by protecting the tert-butoxycarbonyl (Boc) group. **9** was readily reacted with iodine in ethyl acetate at room temperature to give the iodo derivative **10** and deprotected by TFA. Compounds **12** and **14** were converted to **17** and **15** by demethylation with BBr_3 in CH_2Cl_2 (13.0 and 19.5% yields), respectively. Direct alkylation of **17** and **15** with ethylene chlorohydrin and potassium carbonate in DMF resulted in **18** and **16**, respectively. The synthesis of 1-PI derivatives is outlined in Scheme 5. **20** was prepared by the copper-mediated coupling of a substituted indole with 4-(dimethylamino)-phenylboronic acid in a yield of 44.2%.[25] The tributyltin derivative **21** was readily reacted with iodine in ethyl acetate at room temperature to give the iodo derivative **22**. The trimethyltin derivatives were used as the starting materials for radioiodination in the preparation of [^{125}I]**5**, [^{125}I]**11,** [^{125}I]**14** and [^{125}I]**16**. Novel radioiodinated 2-PI derivatives were obtained by iododestannylation reactions using NCS as an oxidant (Scheme 6). It was anticipated that not adding a carrier would result in a final product bearing a theoretical specific activity similar to that of ^{125}I (2200 Ci/mmol). The radiochemical identity of the radioiodinated ligands was verified by co-injection with non-radioiodinated compounds

from HPLC profiles. The HPLC retention times are shown in the supplementary data. [^{125}I]**5**, [^{125}I]**11,** [^{125}I]**14** and [^{125}I]**16** were each obtained in a radiochemical yield of 14 - 56 % with a radiochemical purity of >95% after purification by HPLC.

The affinity of PI derivatives (**5**, **11**, **14, 15**, **16** and **22**) was evaluated based on inhibition of the binding of [^{125}I]IMPY to Aβ42 aggregates. The 2-PI derivatives (**5**, **11**, **14, 15** and **16**) showed inhibitory activity toward Aβ aggregates, while the 1-PI derivative **22** did not (Table 1 and Figure 2). The K_i values of **5**, **11**, **14, 15** and **16** were 27, 4, 20, 33 and 26 nM, respectively, suggesting high affinity for Aβ(1-42) aggregates and considerable tolerance of structural modifications. They also suggested that the position of the substituted phenyl group in the PI molecule plays an important role in the affinity for Aβ aggregates.

To confirm the affinity of the 2-PI derivatives for Aβ plaques in the brain, fluorescent staining of sections of brain tissue from an animal model of AD was carried out with **11** (Figure 3). Many specks of fluorescence were observed in brain sections of *Tg2576* transgenic mice (female, 28 months old) (Figure 3A), while none were observed in wild-type mice (female, 22 months old) (Figure 3B). The pattern of labeling was

consistent with that observed with thioflavin S (Figure 3C). **11** should therefore show specific binding to Aβ plaques in the mouse brain. **14** also clearly stained Aβ plaques in the *Tg2576* mouse brain (data not shown). A slight difference in K_i of **11** and **14** did not significantly affect the staining in mouse brain sections.

To evaluate the uptake into the brain of the PI derivatives, biodistribution experiments were performed in normal mice with four radioiodinated PI derivatives; [^{125}I]**5**, [^{125}I]**11**, [^{125}I]**14** and [^{125}I]**16** (Table 2). Radioactivity penetrated the blood-brain barrier with the rate of uptake ranging from 1.2 % to 2.6 % ID/g brain at 2-10 min postinjection. But the washout of these probes from the brain in normal mice appears to be relatively slow. The uptake of [^{125}I]**11** was higher in the stomach than in any other organ, possibly due to deiodination. The brain uptake and clearance is similar to that of radioiodinated ThT analogues such as TZDM and IBOX.[13,14] More recently, we have developed ^{11}C-labeled phenylbenzofuran derivative, which are less lipophilic by replacing the iodine with a hydroxy group.[18] [^{11}C]Phenybenzofuran, which has less lipophilicity than [^{125}I]phenylbenzofuran, showed a higher and faster peak of brain uptake and faster washout in normal mice. The improved properties of [^{11}C]phenylbenzofuran derivatives

could make them a better candidate for the imaging of Aβ plaques. Similar to [^{125}I]phenylbenzofuran, the 2-PI derivatives had unfavorable *in vivo* pharmacokinetics in normal mice, despite their good affinity for Aβ aggregates. Additional structural changes, that is, reducing the lipophilicity by introducing a hydrophilic group, are necessary to improve the properties of 2-PI derivatives.

3. Conclusion

We developed PI derivatives as novel SPECT ligands for imaging Aβ plaques in the AD brain. 2-PI derivatives (**5, 11, 14, 15** and **16**) displayed excellent affinity for Aβ in binding experiments *in vitro*. They clearly stained Aβ plaques in *Tg2576* mouse brain, reflecting their affinity for Aβ aggregates *in vitro*. The degree to which the 2-PI derivatives penetrated the brain was very encouraging. However, non-specific binding *in vivo* reflected by a slow washout from the normal mouse brain may make them unsuitable for the imaging of Aβ plaques. In *in vivo* biodistribution results in normal mice indicate that there is a critical need to fine-tune the kinetics of brain uptake and washout. Additional changes to 2-PI may lead to useful probes for detecting Aβ plaques

in the AD brain.

4. Experimental

4.1. General

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 was defined by s (singlet), d (doublet), t (triplet), and m (multiplet). Mass spectra were obtained on a JEOL IMS-DX.

4.1.1. 4-Ethynyl-*N*-methylbenzenamine (1).

To a solution of 4-ethynylaniline (819 mg, 7 mmol) in DMSO were added methyl iodide (1.3 mL, 21 mmol) and anhydrous K_2CO_3 (4.8 g, 35 mmol). The reaction mixture was stirred at room temperature for 3 h. After it was poured into water, the mixture was extracted with ethyl acetate. The organic layers were dried over Na_2SO_4. The solvent was removed and the residue was purified by silica gel chromatography (hexane : ethyl

acetate = 2 : 1) to give 313 mg of **1** (34.1%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.49 (s, 1H), 2.83 (s, 3H), (s, 1H), 6.50 (d, *J* = 8.7 Hz, 2H), 7.32 (d, *J* = 8.7 Hz, 2H).

4.1.2. *N*-(4-Bromo-2-iodophenyl)acetamide (2).

A mixture of 4-bromo-2-iodoaniline (586 mg, 2 mmol) and acetic anhydride (0.19 mL, 2 mmol) in toluene (5 mL) was stirred at room temperature for 3.5 h. The solid that formed was filtered and washed with hexane to give 464 mg of **2** (69.4%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.24 (s, 3H), 7.39 (s, 1H), 7.46 (dd, *J* = 2.4, 2.1 Hz, 1H), 7.90 (d, *J* = 2.1 Hz, 1H), 8.13 (d, *J* = 8.7 Hz, 1H).

4.1.3. 4-(5-Bromo-1*H*-indol-2-yl)-*N*-methylbenzenamine (3)

A mixture of **1** (313 mg, 2.4 mmol), **2** (396 mg, 1.2 mmol), $PdCl_2(PPh_3)$ (60 mg, 0.06 mmol), CuI (50 mg, 0.22 mmol), THF (5 mL) and TBAF (1 M solution in THF, 5 mL) was stirred under reflux for 5 h. After removal of the THF, the residue was diluted with H_2O and extracted with ethyl acetate, and the organic phase was dried over Na_2SO_4. The solvent was removed and the residue was purified by silica gel chromatography

(hexane : ethyl acetate = 2 : 1) to give 179 mg of **3** (49.5 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.90 (s, 3H), 6.57 (d, *J* = 2.4 Hz, 1H), 6.67 (d, *J* = 8.7 Hz, 2H), 7.21 (s, 2H), 7.49 (d, *J* = 8.7 Hz, 2H), 7.69 (s, 1H) 8.25 (s, 1H).

4.1.4. *N*-Methyl-4-(5-(trimethylstannyl)-1*H*-indol-2-yl)benzenamine (4).

A mixture of **3** (179 mg, 0.59 mmol), $Pd(PPh_3)_4$ (88 mg, 0.077 mmol) and $(Me_3Sn)_2$ (198 mg, 0.6 mmol) in 1,4-dioxane (5 mL) was stirred under reflux for 3.5 h. The solvent was removed and the residue was purified by silica gel chromatography (hexane : ethyl acetate = 3 : 1) to give 6 mg of **4** (2.1 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.30 (s, 9H), 2.88 (s, 3H), 3.84 (s, 1H), 6.62 (s, 1H), 6.67 (d, *J* = 8.7 Hz, 2H), 7.22 (d, *J* = 9.0 Hz, 1H), 7.37 (d, *J* = 7.8 Hz, 1H), 7.50 (d, *J* = 9.0 Hz, 2H), 7.70 (s, 1H), 8.18 (s, 1H).

4.1.5. 4-(5-Iodo-1*H*-indol-2-yl)-*N*-methylbenzenamine (5).

To a solution of **4** (9 mg, 0.019 mmol) in ethyl acetate (1 mL) was added a solution of iodine in ethyl acetate (1 mL, 0.25 M) at room temperature. The mixture was stirred for

15 s, and $NaHSO_3$ solution (1 mL) was added. The organic phase was separated and dried over Na_2SO_4. The solvent was removed and the residue was purified by silica gel chromatography (hexane : ethyl acetate = 3 : 1) to give 5 mg of **5** (77.4%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.89 (s, 3H), 6.56 (s, 1H), 6.67 (d, *J* = 8.7 Hz, 2H), 7.13 (d, *J* = 8.1 Hz, 1H), 7.37 (dd, *J* = 1.8, 1.8Hz, 1H), 7.48 (d, *J* = 9.0 Hz, 1H) 7.90 (s, 1H), 8.23 (s, 1H). HRMS m/z $C_{15}H_{13}N_2I$ found 348.0109/ calcd 348.0123 (M^+).

4.1.6. 4-(5-Bromo-1*H*-indol-2-yl)-*N*,*N*-dimethylbenzenamine (6).

The same reaction as described above to prepare **3** was used, and 25 mg of **6** was obtained in a 27.2 % yield from **2** (99 mg, 0.3 mmol) and 4-ethynyl-*N*,*N*-dimethylaniline (65 mg, 0.45 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.02 (s, 6H), 6.59 (d, *J* = 1.8 Hz, 1H), 6.78 (d, *J* = 9.0 Hz, 2H), 7.21-7.22 (m, 2H), 7.53 (d, *J* = 9.0 Hz, 2H), 7.68 (s, 1H) 8.27 (s, 1H).

4.1.7. *N*,*N*-Dimethyl-4-(5-(trimethylstannyl)-1*H*-indol-2-yl)benzenamine (7).

The same reaction as described above to prepare **4** was used, and 2 mg of **7** was

obtained in a 7.1% yield from **6** (22 mg, 0.07 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.31 (s, 9H), 3.01 (s, 6H), 6.64 (s, 1H), 6.79 (d, *J* = 9.3 Hz, 2H), 7.23 (d, *J* = 6.9 Hz, 1H), 7.38 (d, *J* = 8.1 Hz, 1H), 7.55 (d, *J* = 9.0 Hz, 2H), 7.71 (s, 1H), 8.21 (s, 1H).

4.1.8. *tert*-Butyl 5-bromo-2-(4-(dimethylamino)phenyl)-1*H*-indole-1-carboxylate (8).

$(Boc)_2O$ (159 mg, 0.73 mmol) was added to a solution of **7** (41 mg, 0.13 mmol) and 4-(*N,N*-dimethylamino)pyridine (DMAP) (4.8 mg, 0.004 mmol) in acetonitrile. The reaction mixture was stirred at room temperature for 3 h. After it was poured into water, the mixture was extracted with ethyl acetate. The organic layers were dried over Na_2SO_4. The solvent was removed and the residue was purified by silica gel chromatography (hexane : ethyl acetate = 4 : 1) to give 54 mg of **8** (100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (s, 9H), 3.00 (s, 6H), 6.41 (s, 1H), 6.75 (d, *J* = 8.4 Hz, 2H), 7.29 (s, 2H), 7.36 (d, *J* = 9.0 Hz, 1H), 7.63 (s, 1H) 8.02 (d, *J* = 8.7 Hz, 1H).

4.1.9. *tert*-Butyl 5-(tributylstannyl)-2-(4-(dimethylamino)phenyl)-1*H*-indole-1-carboxylate (9).

A mixture of **8** (54 mg, 0.13 mmol), $(Bu_3Sn)_2$ (0.3 mL) and $(Ph_3P)_4Pd$ (16 mg, 0.01 mmol) in a mixed solvent (6 mL, 1 : 1 = dioxane : Et_3N) was stirred under reflux for 11 h. The solvent was removed, and the residue was purified by silica gel chromatography (hexane : ethyl acetate = 9:1) to give 20 mg of **9** (12.4%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84-1.61 (m, 36H), 2.99 (s, 6H), 6.46 (s, 1H), 6.75 (d, *J* = 8.7 Hz, 2H), 7.29 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.61 (s, 1H), 8.11 (d, *J* = 7.5 Hz, 1H).

4.1.10. *tert*-Butyl 2-(4-(dimethylamino)phenyl)-5-iodo-1*H*-indole-1-carboxylate (10).

The same reaction as described above to prepare **5** was used, and 10 mg of **10** was obtained in a 67.6% yield from **9** (20 mg, 0.03 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (s, 9H), 3.00 (s, 3H), 6.39 (s, 1H), 6.74 (d, *J* = 8.7 Hz, 2H), 7.29 (s, 2H), 7.53 (dd, *J* = 1.8, 1.8 Hz, 1H) 7.84 (d, *J* = 2.1 Hz, 1H), 7.91 (d, *J* = 9.0 Hz, 1H).

4.1.11. 4-(5-Iodo-1*H*-indol-2-yl)-*N*,*N*-dimethylbenzenamine (**11**).

To a solution of **10** (71 mg, 0.15 mmol) in CH_2Cl_2 (2 mL) was added TFA (300 μL) at room temperature. After the mixture was stirred for 3 h, the solvent was removed. The residue was purified by preparative TLC (hexane : ethyl acetate = 3:1) to give 13 mg of **11** (27.0%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.02 (s, 6H), 6.57 (s, 1H), 6.79 (d, *J* = 8.7

Hz, 2H), 7.14 (d, *J* = 8.7 Hz, 1H), 7.36 (dd, *J* = 1.5, 1.5 Hz, 1H), 7.53 (d, *J* = 9.0 Hz, 1H), 7.89 (s, 1H), 8.28 (s, 1H). HRMS m/z $C_{16}H_{15}N_2I$ found 362.0278/ calcd 362.0280 (M^+).

4.1.12. 5-Bromo-2-(4-methoxyphenyl)-1*H*-indole (12).

The same reaction as described above to prepare **3** was used, and 54 mg of **12** was obtained in a 30.7 % yield from **2** (198 mg, 0.6 mmol) and *p*-ethynylanisole (116 μL, 0.9 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.87 (s, 3H), 6.50 (s, 1H), 6.99 (d, *J* = 8.7 Hz, 2H), 7.25 (s, 2H), 7.59 (d, *J* = 8.7 Hz, 2H), 7.12 (s, 1H), 8.30 (s, 1H).

4.1.13. 2-(4-Methoxyphenyl)-5-(trimethylstannyl)-1*H*-indole (13).

The same reaction as described above to prepare **4** was used, and 15 mg of **13** was obtained in a 23.9% yield from **12** (49 mg, 0.16 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.30 (s, 9H), 3.86 (s, 3H), 6.68 (s, 1H), 6.98 (d, *J* = 9.0 Hz, 2H), 7.26 (d, *J* = 7.2 Hz, 1H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.59 (d, *J* = 9.0 Hz, 2H), 7.73 (s, 1H), 8.22 (s, 1H).

4.1.14. 5-Iodo-2-(4-methoxyphenyl)-1*H*-indole (14).

The same reaction as described above to prepare **5** was used, and 9 mg of **14** was obtained in a 66.3% yield from **13** (15 mg, 0.039 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.86 (s, 3H), 6.63 (s, 1H), 6.99 (d, *J* = 9.0 Hz, 2H), 7.16 (d, *J* = 8.4 Hz, 1H), 7.41 (dd, *J* = 1.8, 1.5 Hz, 1H), 7.58 (d, *J* = 8.7 Hz, 2H), 7.93 (s, 1H), 8.29 (s, 1H). HRMS m/z $C_{15}H_{12}NOI$ found 348.9962/ calcd 348.9964 (M^+).

4.1.15. 4-(5-Iodo-1*H*-indol-2-yl)phenol (15).

BBr_3 (0.7 mL, 1 M solution in CH_2Cl_2) was added to a solution of **14** (80 mg, 0.23 mmol) in CH_2Cl_2 (5 mL) dropwise in an ice bath. The mixture was allowed to warm to room temperature and stirred for 24 h. Water was added while the reaction mixture was cooled in an ice bath. The mixture was extracted with $CHCl_3$ and the water layer was extracted with ethyl acetate. 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 = 4 : 1) to give 15 mg of **15** (19.5 %). ^{1}H NMR (300 MHz, CD_3OD) δ 6.57 (s, 1H), 6.76 (d, *J* = 8.7 Hz, 2H), 7.16 (d, *J* = 8.7 Hz, 1H), 7.29 (dd, *J*

= 1.5, 1.5 Hz, 1H), 7.60 (d, *J* = 8.7 Hz, 2H), 7.81(s, 1H). HRMS m/z $C_{14}H_{10}NOI$ found 334.9808/ calcd 334.9807 (M^+).

4.1.16. 2-(4-(5-iodo-1*H*-indol-2-yl)phenoxy)ethanol (16).

A mixture of **15** (13 mg, 0.039 mmol), potassium carbonate (48 mg, 0.12 mmol) and ethylene chlorohydrin (4 μL, 0.06 mmol) in anhydrous DMF (3 mL) was stirred under reflux for 10.5 h. After cooling to room temperature, water was added, and the reaction mixture was extracted with $CHCl_3$. The organic layer was separated, dried over Na_2SO_4 and evaporated. The resulting residue was purified by preparative TLC (hexane : ethyl acetate = 1 : 1) to give 3 mg of **16** (20.4%). ^{1}H NMR (300 MHz, CD_3OD) δ 3.89 (t, *J* = 9.6 Hz, 2H), 4.09 (t, *J* = 9.3 Hz, 2H), 6.62 (s, 1H), 7.02 (d, *J* = 8.7 Hz, 2H), 7.17 (d, *J* = 8.1 Hz, 1H), 7.30 (dd, *J* = 1.8, 1.5 Hz, 1H), 7.70 (d, *J* = 9.0 Hz, 2H), 7.82 (s, 1H), 11.0 (s, 1H). HRMS m/z $C_{16}H_{14}NO_2I$ found 379.0078/ calcd 379.0069 (M^+).

4.1.17. 4-(5-bromo-1*H*-indol-2-yl)phenol (17).

The same reaction as described above to prepare **15** was used, and 13 mg of **17** was

obtained in a 13.0% yield from **13** (105 mg, 0.347 mmol). ^{1}H NMR (300 MHz, CD_3OD) δ 6.58 (s, 1H), 6.84 (d, *J* = 9.0 Hz, 2H), 7.11 (dd, *J* = 1.8, 1.5 Hz, 1H), 7.25 (d, *J* = 8.7 Hz, 1H), 7.60 (d, *J* = 9.0 Hz, 3H).

4.1.18. 2-(4-(5-bromo-1*H*-indol-2-yl)phenoxy)ethanol (18).

The same reaction as described above to prepare **16** was used, and 1.8 mg of **18** was obtained in a 14.2% yield from **17** (11 mg, 0.038 mmol). ^{1}H NMR (300 MHz, CD_3OD) δ 3.89 (t, *J* = 8.7 Hz, 2H), 4.09 (t, *J* = 9.6 Hz, 2H), 6.64 (s, 1H), 7.02 (d, *J* = 8.7 Hz, 2H), 7.13 (dd, *J* = 1.5, 1.5 Hz, 1H), 7.26 (d, *J* = 8.7 Hz, 1H), 7.61 (s, 1H), 7.70 (d, J = 8.7 Hz, 2H).

4.1.19. 2-(4-(5-(Trimethylstannyl)-1*H*-indol-2-yl)phenoxy)ethanol) (19).

The same reaction as described above to prepare **4** was used, and 9 mg of **19** was obtained in a 89.8% yield from **18** (8 mg, 0.02 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.31 (s, 9H), 3.99 (t, *J* = 8.7 Hz, 2H), 4.14 (t, *J* = 9.3 Hz, 2H), 6.71 (d, *J* = 11.1 Hz, 1H), 6.76 (d, *J* = 9.3 Hz, 1H), 6.98-7.08 (m, 2H), 7.39 (s, 1H), 7.57-7,62 (m, 2H), 7.88 (d, *J*

= 9.3 Hz, 1H), 8.25 (s, 1H).

4.1.20. 4-(5-Bromo-1*H*-indol-1-yl)-*N*,*N*-dimethylbenzenamine (20).

A mixture of 5-bromoindole (100 mg, 0.51 mmol), 4-(dimethylamino)-phenylboronic acid (84 mg, 0.51 mmol), $Cu(OAc)_2$ (200 mg, 1.00 mmol), triethylamine (0.18 mL), and powdered molecular sieves 3 Å was suspended in CH_2Cl_2 (10 mL), and stirred for 1 h. The solvent was removed, and the residue was purified by silica gel chromatography (hexane : ethyl acetate = 9:1) to give 71 mg of **20** (44.2%). ^{1}H NMR (300MHz, $CDCl_3$) δ 3.02 (s, 6H), 6.55 (d, *J* = 2.7 Hz, 1H), 6.82 (d, *J* = 9.0 Hz, 2H), 7.28 (d, *J* = 1.8 Hz, 1H) 7.78 (d, *J* = 1.2 Hz, 1H).

4.1.21. 4-(5-(Tributylstannyl)-1*H*-indol-1-yl)-*N*,*N*-dimethylbenzenamine (21).

The same reaction as described above to prepare **9** was used, and 21 mg of **21** was obtained in a 12.4% yield from **20** (102 mg, 0.32 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87-1.56 (m, 27H), 3.02 (s, 6H), 6.61 (d, *J* = 3.3 Hz, 1H), 6.82 (d, *J* = 9.0 Hz, 2H), 7.24 (d, *J* = 3.0 Hz, 2H), 7.34 (d, *J* = 8.7 Hz, 2H), 7.45 (d, *J* = 3.0 Hz, 1H), 7.77 (s, 1H).

4.1.22. 4-(5-Iodo-1*H*-indol-1-yl)-*N*,*N*-dimethylbenzenamine (22).

The same reaction as described above to prepare **5** was used, and 8 mg of **22** was obtained in a 55.3% yield from **21** (21 mg, 0.04 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.02 (s, 6H), 6.54 (d, *J* = 3.3 Hz, 1H), 6.81 (d, *J* = 6.6 Hz, 2H), 7.20 (d, *J* = 7.8 Hz, 2H), 7.29 (d, *J* = 9.0 Hz, 2H), 7.41 (dd, *J* = 1.5, 1.8 Hz, 1H), 7.99 (d, *J* = 1.2 Hz, 1H). HRMS m/z $C_{16}H_{15}N_2I$ found 362.0287/ calcd 362.0280 (M^+).

4.2. Iododestannylation reaction.

The radioiodinated forms of compounds **5**, **11**, **14** and **16** were prepared from the corresponding trimethyltin derivatives by iododestannylation using the previously described *N*-chlorosuccinimide (NCS) method, with some modifications.[26] Briefly, a 80 μL solution of **5**, **11**, **14** and **16** in methanol containing 1% acetic acid (0.56 mg/mL) was mixed with 20 μL of NCS in methanol (0.5 mg/mL) in a sealed vial, and [^{125}I]NaI (0.1-0.2 mCi, specific activity 2200 Ci/mmol) was added. The reaction was allowed to proceed at room temperature for 20 s and terminated by addition of $NaHSO_3$. After extraction with ethyl acetate, the extract was dried by passing through an anhydrous

Na_2SO_4 column and blown dry with a stream of nitrogen gas. The radioiodinated ligand was purified by HPLC on a Cosmosil C_{18} column with an isocratic solvent of H_2O/acetonitrile (4 : 6 – 1 : 1) at a flow rate of 1.0 mL/min.

4.3. Binding assays using the aggregated A® peptide in solution.

A solid form of A®42 was purchased from Peptide Institute (Osaka, Japan). Aggregation 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 solution was incubated at 37 ℃ for 42 h with gentle and constant shaking. Binding assays were carried out as described previously.[27] [^{125}I]IMPY (6-iodo-2-(4'-dimethylamino)phenyl-imidazo[1,2]pyridine) with 2200 Ci/mmol specific activity and greater than 95% radiochemical purity was prepared using the standard iododestannylation reaction as described previously.[15] Binding assays were carried out in 12 × 75 mm borosilicate glass tubes. A mixture containing 50 μL of test compound (0.2 pM - 400 nM in 10%EtOH), 50 μL of [^{125}I]IMPY (0.02 nM diluted in 50% EtOH), 50 μL of Aβ42 aggregates, and 850 μL of 10% ethanol 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 filters containing the bound ^{125}I ligand were placed 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.[28]

4.4. Neuropathological staining of mouse brain sections.

The experiments with animals were conducted in accordance with our institutional guidelines and approved by the Nagasaki University Animal Care Committee. *Tg*2576 transgenic mice (female, 28 months old) and wild-type mice (female, 22 months old) were used as the Alzheimer's model and control, respectively. After the mice were sacrificed by decapitation, the brain was immediately removed and frozen in powdered dry ice. The frozen blocks were sliced into serial sections, 10 μm thick. Each slide was incubated with a 50% EtOH solution (100 μM) of compounds **11** and **14** for 10 min. The sections were washed in 50% EtOH for 1 min two times, and examined using a

microscope (KEYENCE BZ-8100) equipped with a DAPI-BP filter set (excitation, 360 nm; diachromic mirror, 400 nm; longpass filter, 460 nm). Thereafter, the sections were also stained with thioflavin S, a pathological dye commonly used for staining A® plaques in the brain, and examined using a microscope (KEYENCE BZ-8100) equipped with a GFP-BP filter set (excitation, 470 nm; diachromic mirror, 495 nm; longpass filter, 535 nm).

4.5. *In vivo* biodistribution in normal mice.

A saline solution (100 μL) of radiolabeled agents (0.2–0.4 μCi) containing ethanol (10 μL) was injected intravenously directly into the tail of ddY mice (5 weeks old, 25-30 g). The mice were sacrificed at various time points postinjection. The organs of interest were removed and weighed, and the radioactivity was measured with an automatic gamma counter (Aloka, ARC-380 or Perkin Elmer 2470 wizared^2).

Supplementary Data Available

HPLC retention times and partition coefficients of compounds **5**, **11**, **14**, **15**, and **16**.

Acknowledgments

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Kung, H. P.; Zhang, W.; Kung, M. P.; Skovronsky, D.; Dyrks, T.; Holl, G.; Krause, S.; Friebe, M.; Lehman, L.; Lindemann, S.; Dinkelborg, L. M.; Masters, C. L.; Villemagne, V. L. *Lancet Neurol.* **2008**, *7*, 129.

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Table 1. Inhibition constants (K_i) for binding of PI derivatives determined using [^{125}I]IMPY as the ligand in Aβ(1-42) aggregates.

Compound	K_i (nM)[a]
5	27.0 ± 0.18
11	4.24 ± 0.71
14	20.2 ± 5.15
15	32.9 ± 2.93
16	25.9 ± 5.13
22	>10000

[a]Values are the mean ± standard error of the mean for 3-6 independent experiments.

Table 2. Biodistribution of radioactivity after injection of [^{125}I]2-PI derivatives in normal mice[a].

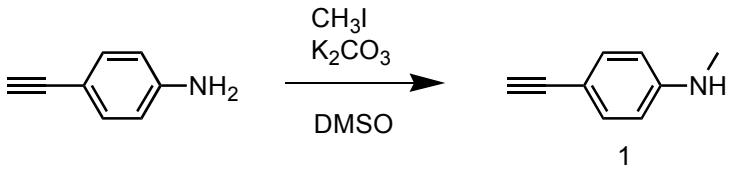
Tissue	Time after injection (min)			
	2	10	30	60
		[^{125}I]**5**		
Blood	3.77 (1.25)	1.71 (0.15)	1.36 (0.15)	0.80 (0.13)
Liver	22.85 (9.60)	26.83 (1.28)	23.07 (2.33)	15.89 (6.13)
Kidney	5.53 (1.77)	4.31 (0.44)	3.84 (0.67)	2.61 (0.86)
Intestine	0.97 (0.33)	3.57 (0.34)	8.54 (1.80)	9.48 (1.88)
Spleen	7.83 (2.24)	19.67 (8.82)	10.34 (1.26)	5.89 (3.27)
Pancreas	2.31 (0.70)	2.87 (0.33)	2.12 (0.32)	1.27 (0.23)
Heart	5.68 (1.27)	3.59 (0.29)	2.96 (0.42)	1.88 (0.57)
Stomach[b]	0.52 (0.03)	1.34 (0.53)	2.55 (2.12)	1.68 (0.53)
Brain	1.10 (0.27)	1.68 (0.13)	1.42 (0.03)	0.83 (0.17)
		[^{125}I]**11**		
Blood	6.18 (0.65)	4.53 (0.37)	3.68 (0.41)	3.29 (1.00)
Liver	10.20 (1.92)	5.14 (1.01)	3.55 (0.62)	3.24 (0.76)
Kidney	9.49 (1.61)	4.46 (0.71)	4.58 (1.24)	4.71 (2.44)
Intestine	1.80 (0.24)	3.09 (0.40)	4.52 (0.58)	6.08 (1.58)
Spleen	4.13 (0.64)	3.28 (0.57)	2.60 (0.41)	2.24 (0.64)
Pancreas	5.21 (2.50)	4.13 (0.67)	3.10 (0.41)	2.30 (0.54)

Heart	8.08 (1.59)	4.82 (0.67)	1.87 (0.21)	1.84 (0.76)
Stomach[b]	4.03 (0.65)	10.65 (2.18)	17.77 (2.04)	16.26 (3.52)
Brain	1.19 (0.34)	1.19 (0.30)	0.96 (0.17)	0.71 (0.19)
		[^{125}I]**14**		
Blood	3.59 (1.41)	1.97 (0.54)	1.38 (0.25)	0.74 (0.37)
Liver	17.81 (5.73)	13.96 (4.21)	10.38 (2.64)	8.22 (1.87)
Kidney	8.76 (2.19)	4.96 (1.44)	2.89 (0.44)	2.05 (0.54)
Intestine	1.90 (0.66)	6.85 (1.86)	12.32 (3.65)	20.35 (5.86)
Spleen	4.22 (0.78)	3.43 (1.15)	2.96 (0.41)	2.02 (0.92)
Pancreas	4.54 (0.44)	3.94 (1.46)	1.95 (0.33)	1.29 (0.37)
Heart	7.72 (1.94)	2.97 (1.26)	1.47 (0.20)	1.06 (0.42)
Stomach[b]	0.72 (0.39)	1.38 (1.42)	2.67 (3.24)	3.06 (1.28)
Brain	2.11 (0.69)	2.07 (0.71)	1.36 (0.37)	1.16 (0.32)
		[^{125}I]**16**		
Blood	4.07 (0.30)	1.60 (0.30)	1.26 (0.26)	0.80 (0.20)
Liver	17.69 (2.64)	16.77 (2.20)	13.42 (1.70)	8.17 (1.28)
Kidney	11.93 (1.83)	9.35 (0.46)	6.77 (0.83)	2.76 (0.45)
Intestine	1.97 (0.22)	5.76 (0.66)	11.32 (1.65)	18.98 (3.21)
Spleen	7.56 (1.24)	7.09 (1.60)	4.75 (0.82)	2.39 (0.52)
Pancreas	5.55 (1.00)	5.59 (0.56)	3.57 (0.40)	2.22 (0.29)
Heart	12.91 (2.16)	5.84 (0.36)	2.97 (0.62)	1.36 (0.17)

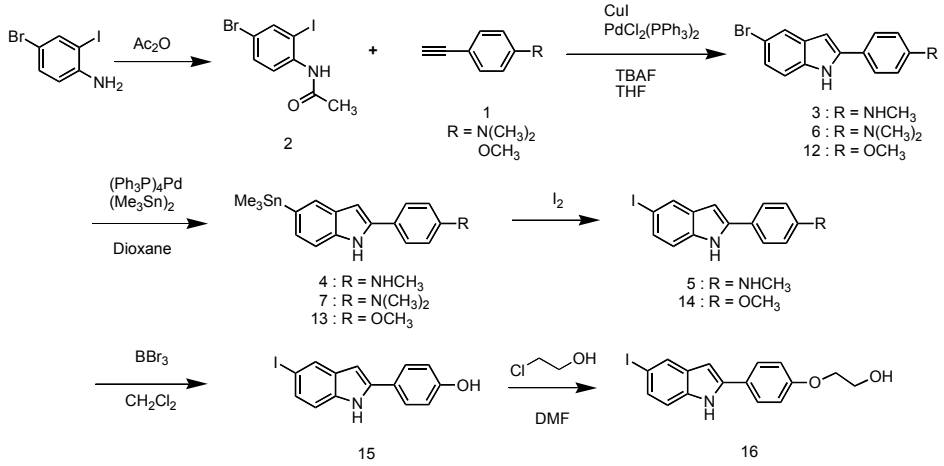
Stomach[b]	0.97 (0.31)	1.97 (1.05)	2.11 (0.26)	1.62 (0.44)
Brain	2.13 (0.54)	2.62 (0.21)	1.93 (0.18)	1.82 (0.35)

[a] Expressed as % injected dose per gram. Each value represents the mean (SD) for 3-5 animals.

[b] Expressed as % injected dose per organ.



Scheme 1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TBAF
THF
Br
R
N
H
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6 : R = N(CH3)2
12 : R = OCH3
(Ph3P)4Pd
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Dioxane
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7 : R = N(CH3)2
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I
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N
H
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14 : R = OCH3
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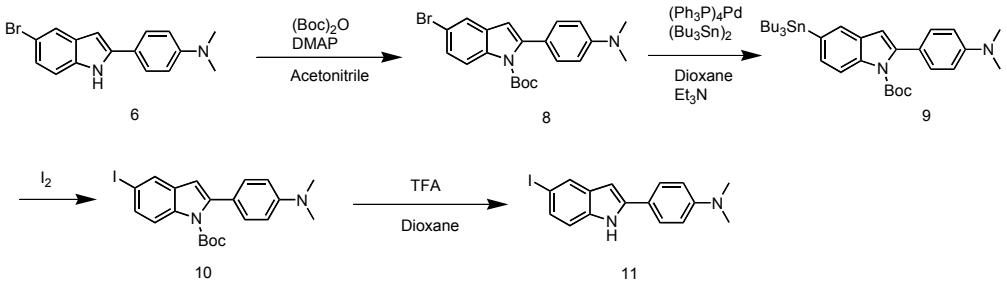
Scheme 2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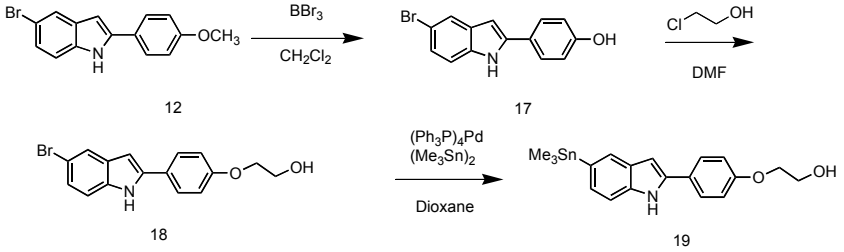
Scheme 3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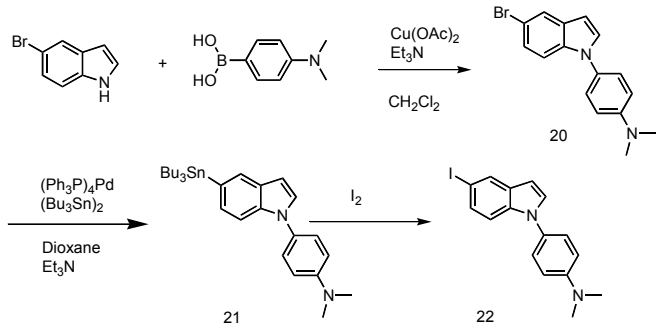
Scheme 4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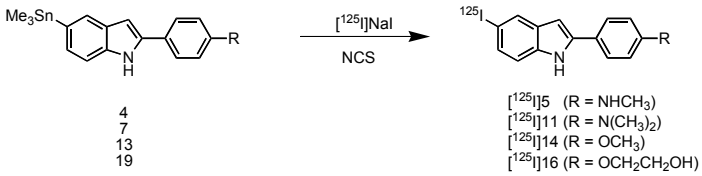
22

Scheme 5



4
7
13
19

[^{125}I]5 (R = $NHCH_3$)
[^{125}I]11 (R = $N(CH_3)_2$)
[^{125}I]14 (R = OCH_3)
[^{125}I]16 (R = OCH_2CH_2OH)

Scheme 6

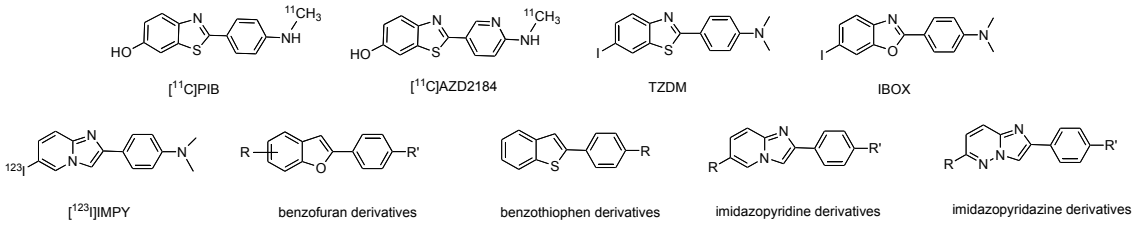


Figure 1. Chemical structure of thioflavin T derivatives.

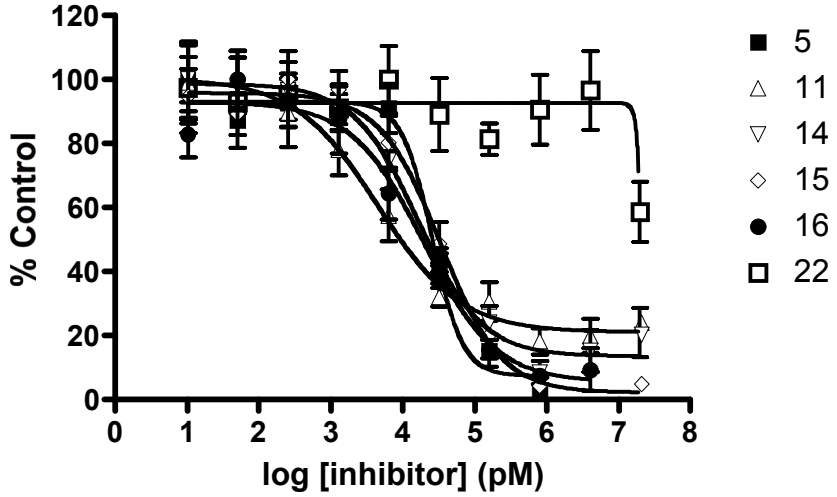


Figure 2. Curves of [^{125}I]IMPY against 2-PI (**5**, **11**, **14**, **15** and **16**) and 1-PI (**22**).

Figure 3. Neuropathological staining of **11** in 10-μm AD model mouse sections (A) and wild-type mouse sections (B). Labeled plaques were confirmed by staining of the adjacent sections with thioflavin S (C).