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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 A β plaques in vivo. The affinity for A β plaques was assessed by an in vitro-binding assay using pre-formed synthetic A β aggregates. 2-Phenyl-1H-indole (2-PI) derivatives showed high affinity for A β 42 aggregates with K_i values ranging from 4 to 32 nM. 2-PI derivatives clearly stained A β 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 A β imaging agents.

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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 (A β) peptides and highly phosphorylated tau proteins.^{1,2} The formation and deposition of A β 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 A β 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

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develop probes for PET/SPECT derived from ThT including [¹¹C]PIB,^{9,10} [¹¹C]AZD2184,^{11,12} TZDM,¹³ IBOX,¹⁴ [¹²³I]IMPY,^{15,16} phenylbenzofuran derivatives,^{17,18} phenylbenzothiophene derivatives,¹⁹ imidazopyridine derivatives,^{20,21} and imidazopyridazine derivatives²² (Fig. 1). Clinical trials in AD patients have been conducted with [¹¹C]PIB and [¹¹C]AZD2184, and indicated the PET-based imaging of A β 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 A β 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 A β 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 A β 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-1H-indole (2-PI) and 1-phenyl-1H-indole (1-PI) derivatives and evaluated their potential as probes for imaging A β in vivo. This is the first time that PI derivatives have been used for SPECT to detect A β plaques.

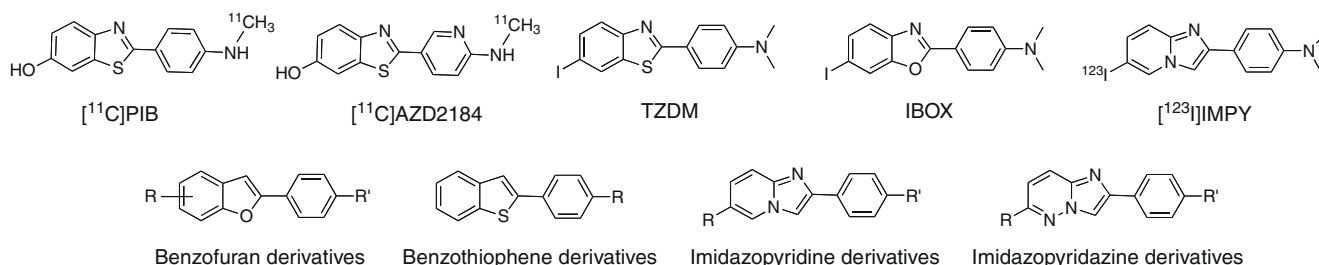
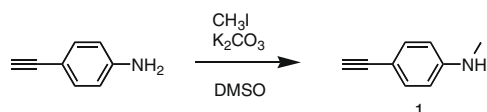


Figure 1. Chemical structure of thioflavin T analogs.

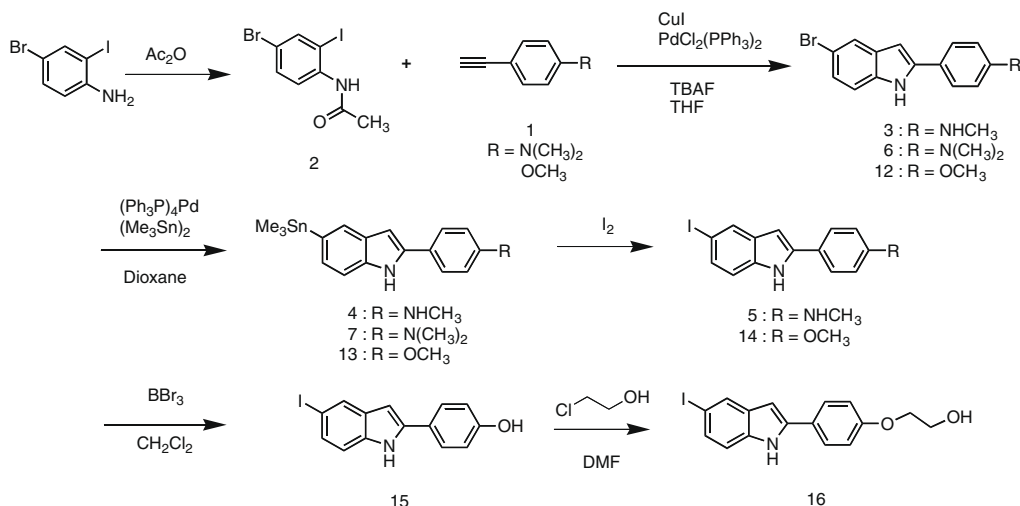


Scheme 1.

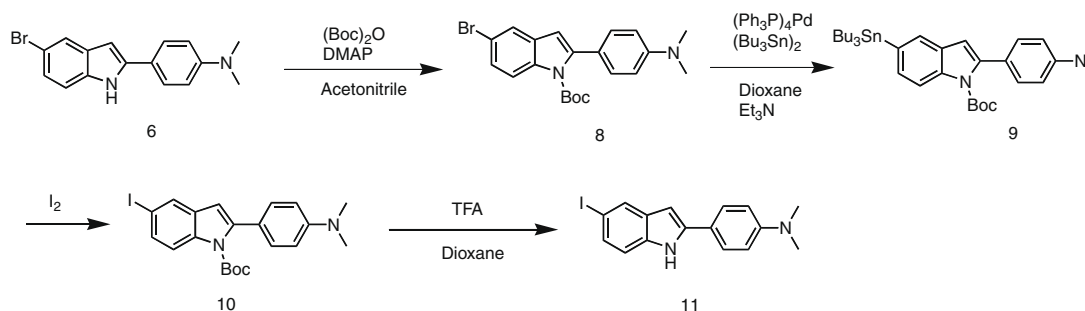
2. Results and discussion

The synthesis of 2-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

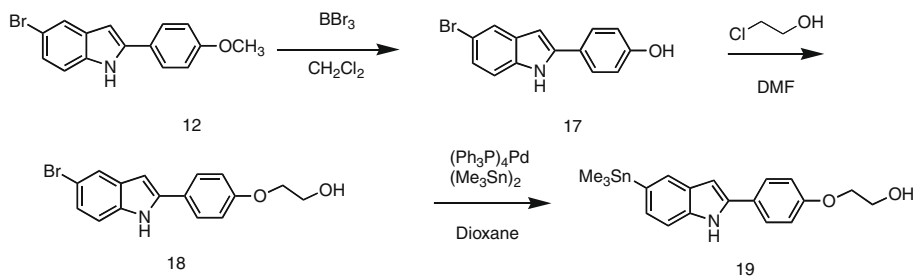
bromo-to-trimethyltin exchange reaction catalyzed by Pd(0). Trimethyltin derivatives (**4** and **13**) were readily reacted with iodine in ethyl acetate at room temperature to give the iodo derivatives, **5** and **14**. The tributyltin derivative **9** was prepared from the corresponding bromo compound by protecting the *tert*-butoxycarbonyl (Boc) group. Compound **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. Compound **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



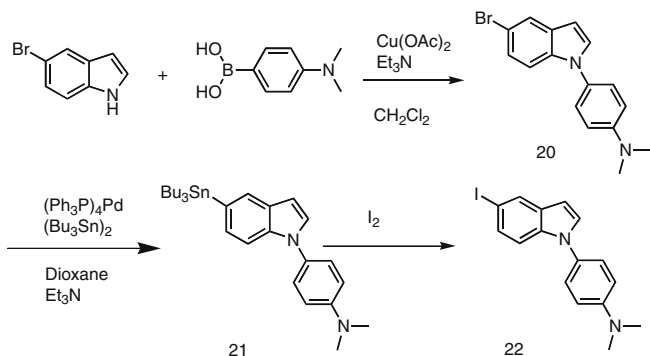
Scheme 2.



Scheme 3.



Scheme 4.



Scheme 5.

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 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 A β 42 aggregates. The 2-PI derivatives (**5**, **11**, **14**, **15**, and **16**) showed inhibitory activity toward A β aggregates, while the 1-PI derivative **22** did not (Table 1 and Fig. 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 A β (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 A β aggregates.

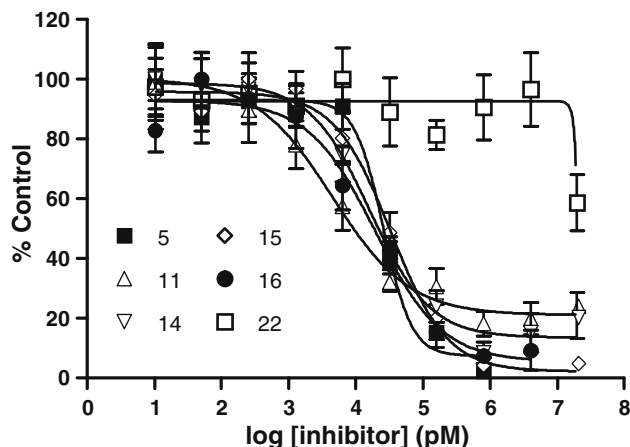
To confirm the affinity of the 2-PI derivatives for A β plaques in the brain, fluorescent staining of sections of brain tissue from an animal model of AD was carried out with **11** (Fig. 3). Many specks of fluorescence were observed in brain sections of Tg2576

Table 1

Inhibition constants (K_i) for binding of PI derivatives determined using [125 I]IMPY as the ligand in A β (1–42) aggregates

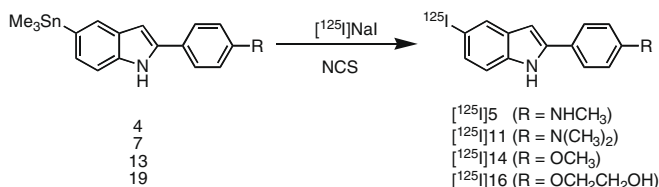
Compound	K_i (nM) ^a
5	27.0 \pm 0.18
11	4.24 \pm 0.71
14	20.2 \pm 5.15
15	32.9 \pm 2.93
16	25.9 \pm 5.13
22	>10,000

^a Values are means \pm standard error of the mean for 3–6 independent experiments.

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

transgenic mice (female, 28 months old) (Fig. 3A), while none were observed in wild-type mice (female, 22 months old) (Fig. 3B). The pattern of labeling was consistent with that observed with thioflavin S (Fig. 3C). Compound **11** should therefore show specific binding to A β plaques in the mouse brain. Compound **14** also clearly stained A β 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, bio-distribution 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 analogs 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



Scheme 6.

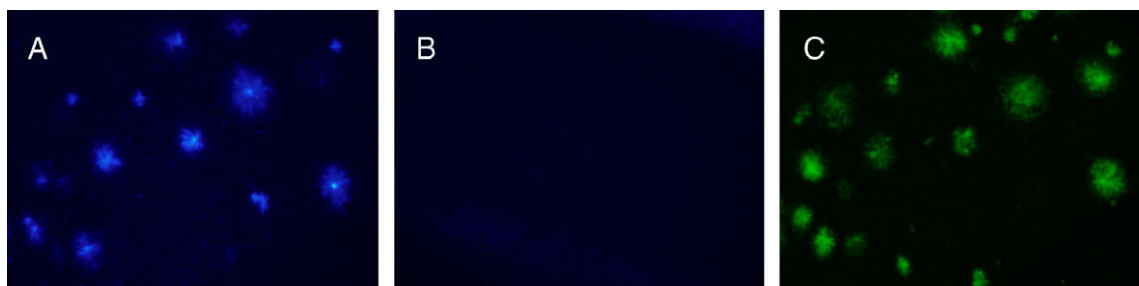


Figure 3. Neuropathological staining of **11** in 10-μm AD model mouse sections (A) and wild-type mouse sections (B). Labeled plaques were confirmed by staining of the adjacent sections with thioflavin S (C).

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
[125I]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)
[125I]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)
[125I]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)
[125I]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.

hydroxy group.¹⁸ [11 C]Phenylbenzofuran, 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. ^1H 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 **1** (34.1%). ^1H 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 **2** (69.4%). ^1H 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-1H-indol-2-yl)-N-methylbenzenamine (**3**)

A mixture of **1** (313 mg, 2.4 mmol), **2** (396 mg, 1.2 mmol), $\text{PdCl}_2(\text{PPh}_3)$ (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 water and extracted with ethyl acetate, and the organic phase was dried over Na₂SO₄. 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%). ¹H NMR (300 MHz, CDCl₃) 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(PPh₃)₄ (88 mg, 0.077 mmol) and (Me₃Sn)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%). ¹H NMR (300 MHz, CDCl₃) 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 NaHSO₃ solution (1 mL) was added. The organic phase was separated and dried over Na₂SO₄. 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%). ¹H NMR (300 MHz, CDCl₃) 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, 1.8 Hz, 1H), 7.48 (d, *J* = 9.0 Hz, 1H) 7.90 (s, 1H), 8.23 (s, 1H). HRMS *m/z* C₁₅H₁₃N₂I 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). ¹H NMR (300 MHz, CDCl₃) 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). ¹H NMR (300 MHz, CDCl₃) 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 Na₂SO₄. 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%). ¹H NMR (300 MHz, CDCl₃) 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), (Bu₃Sn)₂ (0.3 mL) and (Ph₃P)₄Pd (16 mg, 0.01 mmol) in a mixed solvent (6 mL, 1:1 =

1,4-dioxane/Et₃N) 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%). ¹H NMR (300 MHz, CDCl₃) 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). ¹H NMR (300 MHz, CDCl₃) 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 CH₂Cl₂ (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%). ¹H NMR (300 MHz, CDCl₃) 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* C₁₆H₁₅N₂I 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). ¹H NMR (300 MHz, CDCl₃) 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). ¹H NMR (300 MHz, CDCl₃) 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). ¹H NMR (300 MHz, CDCl₃) 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* C₁₅H₁₂NOI found 348.9962/calcd 348.9964 (M⁺).

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

BBr₃ (0.7 mL, 1 M solution in CH₂Cl₂) was added to a solution of **14** (80 mg, 0.23 mmol) in CH₂Cl₂ (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 CHCl₃ and the water layer was extracted with ethyl acetate. The organic phase was dried over Na₂SO₄ 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%). ¹H NMR (300 MHz, CD₃OD) 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* C₁₄H₁₀NOI found 334.9808/calcd 334.9807 (M⁺).

4.1.16. 2-(4-(5-Iodo-1H-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 μ L, 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%). ^1H 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 $\text{C}_{16}\text{H}_{14}\text{NO}_2\text{I}$ found 379.0078/calcd 379.0069 (M^+).

4.1.17. 4-(5-Bromo-1H-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). ^1H 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-1H-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). ^1H 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)-1H-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). ^1H 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-1H-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), $\text{Cu}(\text{OAc})_2$ (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%). ^1H NMR (300 MHz, 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)-1H-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). ^1H 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-1H-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). ^1H 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 $\text{C}_{16}\text{H}_{15}\text{N}_2\text{I}$ 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 μ L solution of **5**, **11**, **14**, and **16** in methanol containing 1% acetic acid (0.56 mg/mL) was mixed with 20 μ L 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 β peptide in solution

A solid form of A β 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 °C 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 \times 75 mm borosilicate glass tubes. A mixture containing 50 μ L of test compound (0.2 pM–400 nM in 10% EtOH), 50 μ L of [^{125}I]IMPY (0.02 nM diluted in 50% EtOH), 50 μ L of A β 42 aggregates, and 850 μ L 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. Tg2576 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 μ m thick. Each slide was incubated with a 50% EtOH solution (100 μ M) 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; dichromatic mirror, 400 nm; longpass filter, 460 nm). Thereafter, the sections were also stained with thioflavin S, a pathological dye commonly used for staining A β plaques in the brain, and examined using a microscope (KEYENCE BZ-8100) equipped with a GFP-BP filter set (excitation, 470 nm; dichromatic mirror, 495 nm; longpass filter, 535 nm).

4.5. In vivo biodistribution in normal mice

A saline solution (100 μ L) of radiolabeled agents (0.2–0.4 μ Ci) containing ethanol (10 μ L) 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 wizard2).

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Supplementary data

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