

Novel chalcones as probes for in vivo imaging of β -amyloid plaques in Alzheimer's brains

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Received 17 July 2007; accepted 28 July 2007

Available online 21 August 2007

Abstract—A novel series of chalcone derivatives for in vivo imaging β -amyloid plaques in the brain of Alzheimer's disease (AD) were synthesized and characterized. When in vitro binding studies using A β aggregates were carried out with chalcone derivatives, the binding affinities for A β aggregate varied from 3 to 105 nM. The radioiodinated chalcones were successfully prepared through an iododestannylation reaction from the corresponding tributyltin derivatives using hydrogen peroxide as the oxidant in high yields and with high radiochemical purities. Biodistribution studies in normal mice after iv injection of the radioiodinated chalcones displayed high brain uptake (2.0–4.7%ID/g at 2 min) and rapid clearance from the brain (0.2–0.6%ID/g at 30 min), which is highly desirable for amyloid imaging agents. The results in this study suggest that the novel radioiodinated chalcones may be useful amyloid imaging agents for detecting β -amyloid plaques in the brain of AD.

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1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by cognitive decline, irreversible memory loss, disorientation, and language impairment. The formation and deposition of β -amyloid (A β) plaques consisting mainly of A β peptides in the brain is now considered one of the most significant factors in AD.^{1–4} Currently, the definitive diagnosis of AD is dependent on only the histopathological examination of A β plaques in the postmortem brain. Therefore, in vivo imaging of β -amyloid plaques in the living brain may lead to early detection of AD or monitoring the progression and effectiveness of novel treatments that are currently being investigated.^{5–7} Many radiolabeled probes based on the core structure of Congo Red (CR) and thioflavin T (ThT) have been developed as imaging agents specific for β -amyloid plaques. Several agents including [¹¹C]-4-*N*-methylamino-4'-hydroxystilbene ([¹¹C]SB-13),^{8,9} [¹¹C]-2-(4-(methylamino)phenyl)-6-hydroxybenzothiazole ([¹¹C]6-OH-BTA-1),^{10,11} and

[¹⁸F]-2-(1-(2-(*N*-(2-fluoroethyl)-*N*-methylamino)naphthalene-6-yl)ethylidene)malononitrile ([¹⁸F]FDDNP)^{12,13} have been reported for positron emission tomography (PET) imaging of amyloid plaques in AD patients. More recently, Kung et al. reported that I-123 labeled 6-iodo-2-(4'-dimethylamino)phenyl-imidazo[1,2-*a*]pyridine (IMPY), which is a novel amyloid imaging agent for single photon emission computed tomography (SPECT), demonstrated a good ability to differentiate AD patients from controls.^{14–17} These recent results reported in clinical studies with AD patients encourage the development of more useful imaging agents for detecting β -amyloid plaques in the living human brain by both PET and SPECT.

According to previous studies describing the effects of polyhydroxy flavones on the formation (Fig. 1), extension and destabilization of A β aggregates in vitro, flavones dose-dependently inhibit the formation of A β aggregates, as well as destabilizing preformed A β aggregates, indicating that these molecules could directly interact with A β aggregates.¹⁸ Based on the findings of these previous reports, we synthesized radioiodinated flavones as new amyloid imaging agents and reported their usefulness.¹⁸ They showed high binding affinity to A β aggregates in in vitro binding studies using

Keywords: Alzheimer's disease; β -Amyloid; PET; SPECT; Imaging.

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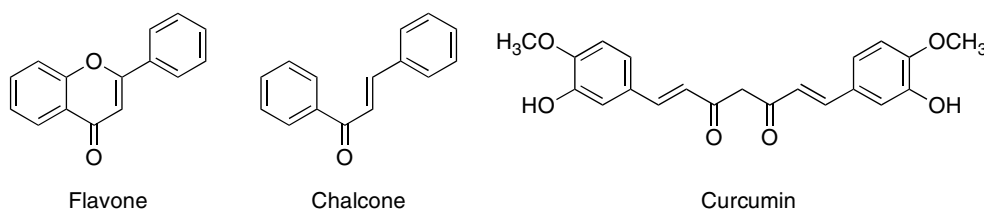


Figure 1. Chemical structures of flavone, chalcone, and curcumin.

synthetic A β aggregates and neuropathological staining on AD brain sections, suggesting that these classes of radioiodinated flavones may be useful candidates as potential imaging agents. This report also suggested that the pharmacophores except thioflavin T and Congo Red can be applied for the development of novel β -amyloid imaging agents.

To search for more useful candidates in the development of in vivo β -amyloid imaging agents, we have devised adequate chemical modification of the flavone structure, and selected the chalcone structure as a novel core for β -amyloid imaging agents (Fig. 1). Chalcone is categorized as a member of flavonoids containing flavone, and has a chemical structure where the ether linkage is removed from the flavone structure. Also, chalcone contains a structurally similar moiety to curcumin (Fig. 1), which

has been reported to have sufficient brain permeability and favorable binding affinity to β -amyloid plaques after iv administration in APPsw transgenic mice.^{19,20} Recently, radiolabeled curcumin derivatives have also been reported as amyloid imaging probes.²¹ In addition to the structural characteristics of chalcone as the pharmacophore, some recent studies have shown that electron-donating groups such as amino, methylamino, dimethylamino, methoxy or hydroxy groups play a critical role in the binding affinity to A β aggregates.^{8,22–24} With these considerations, we designed five radioiodinated chalcones with a radioiodine at the 4' position and an electron-donating group at the 4 position (Fig. 2).

In the present study, we synthesized a series of chalcone derivatives and evaluated their biological activities as in vivo amyloid imaging agents. To our knowledge, this is the first time that chalcone derivatives have been applied as amyloid imaging agents for detecting AD.

2. Results and discussion

2.1. Chemistry

The target iodo compounds (**4**, **7**, **10**, **14**, and **16**) were prepared as shown in Schemes 1 and 2. The most useful method used to prepare chalcones is the condensa-

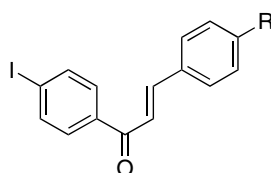
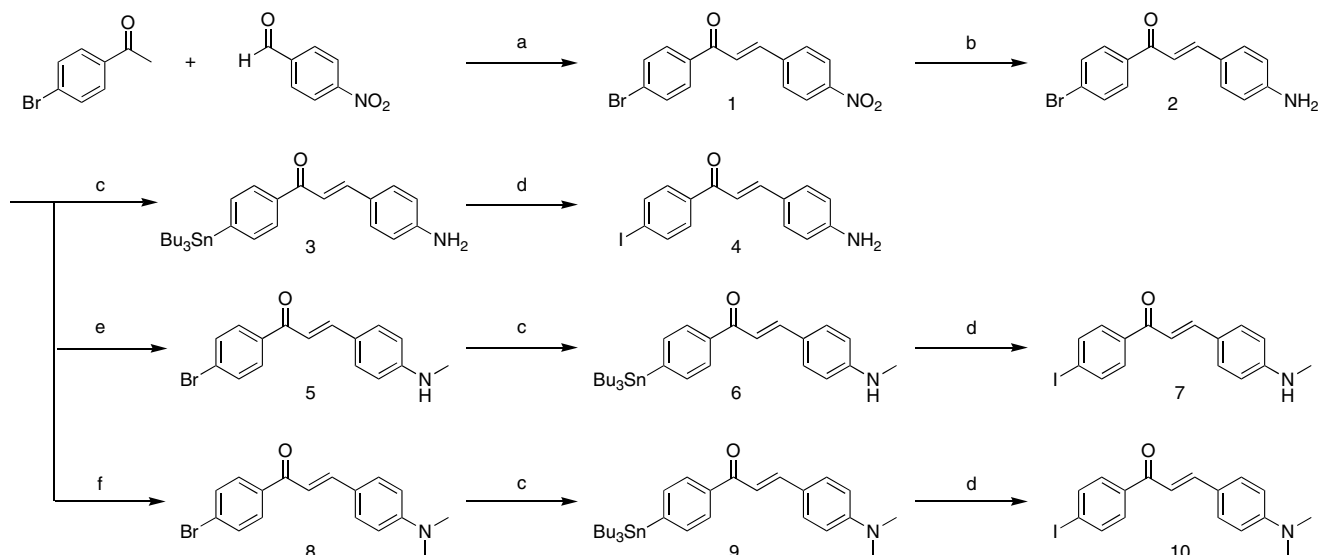
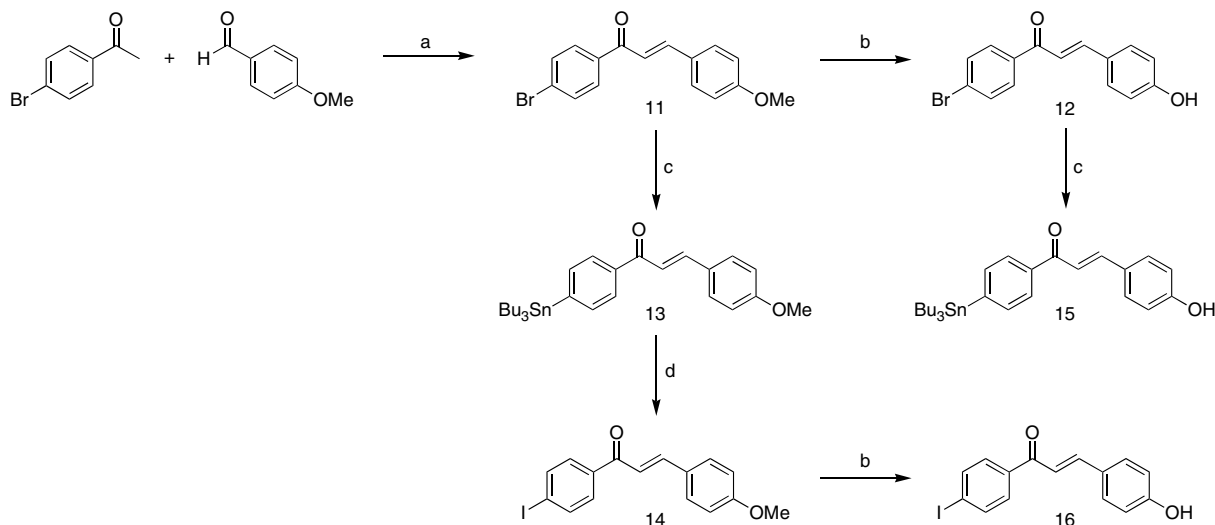


Figure 2. Structure of chalcone derivatives. Compounds reported in the study include the following: R = NH₂, NHCH₃, N(CH₃)₂, OCH₃, and OH.



Scheme 1. Reagents: (a) EtOH, 10% KOH; (b) EtOH, SnCl₂; (c) dioxane, (Bu₃Sn)₂, (Ph₃P)₄Pd, Et₃N; (d) CHCl₃, I₂; (e) DMSO, MeI, K₂CO₃; (f) AcOH, (CH₂O)_m, NaCNBH₃.



Scheme 2. Reagents: (a) EtOH, 10% KOH; (b) CH₂Cl₂, BBr₃; (c) dioxane, (Bu₃Sn)₂, (Ph₃P)₄Pd, Et₃N; (d) CHCl₃, I₂.

tion of acetophenones with benzaldehydes. In this process, 4-bromoacetophenone was reacted with 4-nitrobenzaldehyde or 4-methoxybenzaldehyde in the presence of basic catalyst (10% KOH) in ethanol at room temperature to form 4'-bromo-4-nitrochalcone **1** and 4'-bromo-4-methoxychalcone **11** in yields of 38.2% and 93.6%, respectively. The amino derivatives **2** were readily prepared from **1** by reduction with SnCl₂ (61.1% yield). Conversion of **2** to the monomethylamino derivative **5** was achieved by a methylation with CH₃I under alkaline conditions (35.5% yield). Compound **2** was also converted to the dimethylamino derivative **8** by an efficient method²⁵ with paraformaldehyde, sodium cyanoborohydride, and acetic acid (86.1% yield). Compound **11** was converted to **12** by demethylation with BBr₃ in CH₂Cl₂ (64.1% yield). The tributyltin derivatives (**3**, **6**, **9**, **13**, and **15**) were prepared from the corresponding bromo compounds (**2**, **5**, **8**, **11**, and **12**) using a bromo-to-tributyltin exchange reaction catalyzed by Pd(0) for yields of 52.1%, 55.0%, 11.9%, 43.9%, and 31.1%, respectively. The tributyltin derivatives (**3**, **6**, **9**, and **13**) were readily reacted with iodine in CHCl₃ at room temperature to give the iodo derivatives, compounds **4**, **7**, **10**, and **14** at yields of 46.5%, 80.6%, 46.6%, and 61.0%, respectively. Compound **16** was obtained in 24.7% yield from **14** by the same reaction used to prepare **12** from **11**. Furthermore, the tributyltin derivatives (**3**, **6**, **9**, **13**, and **15**) can be also used as the starting materials for radioiodination in preparation of [¹²⁵I]**4**, [¹²⁵I]**7**, [¹²⁵I]**10**, [¹²⁵I]**14**, and [¹²⁵I]**16**. Novel radioiodinated chalcones were achieved by an iododestannylation reaction using hydrogen peroxide as the oxidant, which

produced the desired radioiodinated ligands (Fig. 3). It was anticipated that the no-carrier-added preparation would result in a final product bearing a theoretical specific activity similar to that of ¹²⁵I (2200 Ci/mmol). The radiochemical identities of the radioiodinated ligands were verified by co-injection with nonradioactive compounds by their HPLC profiles. The final radioiodinated compounds [¹²⁵I]**4**, [¹²⁵I]**7**, [¹²⁵I]**10**, [¹²⁵I]**14**, and [¹²⁵I]**16** showed a single radioactivity peak at retention times of 7.7, 13.8, 22.0, 17.1, and 6.5 compounds **4**, **7**, **10**, **14**, and **16**. Five radioiodinated products were obtained in 25–85% radiochemical yields with radiochemical purities of >95% after purification by HPLC.

2.2. Binding studies using Aβ aggregates in solution

Binding studies of [¹²⁵I]**10** to aggregates of Aβ(1–42) were carried out. Transformation of the saturation binding of [¹²⁵I]**10** to Scatchard plots gave linear plots, suggesting one binding site. [¹²⁵I]**10** showed excellent binding affinity for Aβ(1–42) aggregates ($K_d = 4.2 \pm 1.1$ nM) (Fig. 4). Binding affinities of nonradioactive chalcones (**4**, **7**, **10**, **14**, and **16**) were also evaluated with inhibition studies against [¹²⁵I]**10** binding on Aβ(1–42) aggregates. The K_i values estimated for **4**, **7**, **10**, **14**, and **16** were 105, 6, 3, 6, and 21 nM for Aβ(1–42) aggregates, respectively (Table 1). These K_i values suggested that the new series of chalcones had a high binding affinity for Aβ(1–42) aggregates and showed considerable tolerance for structural modification. Also, when comparing these K_i values with the radioiodinated flavones reported previously,¹⁸ the K_i values of the radioiodinated chalcones were lower than those of the radioiodin-

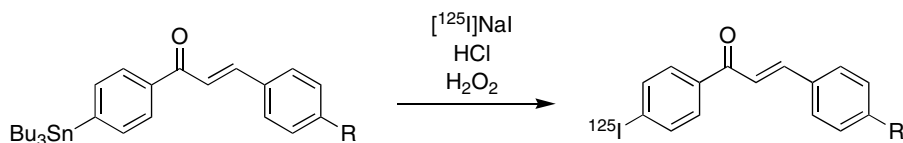


Figure 3. Radioiodination reaction of chalcone derivatives. R = NH₂, NHCH₃, N(CH₃)₂, OCH₃, and OH.

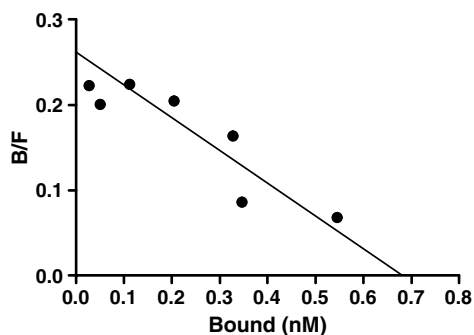


Figure 4. Scatchard plots of [^{125}I]10 binding to $\text{A}\beta(1-42)$ aggregates. [^{125}I]10 showed one-site binding. High binding affinity with a K_d value in a nanomolar range was obtained ($K_d = 4.2 \pm 1.1$ nM).

Table 1. Inhibition constants of chalcone derivatives on ligand binding to $\text{A}\beta(1-42)$ aggregates

Compound	K_i^a (nM)
4	104.7 ± 12.0
7	6.3 ± 1.6
10	2.9 ± 0.3
14	6.3 ± 1.7
16	21.4 ± 1.4
Thioflavin T	$>10,000$
Congo Red	$>10,000$

^a Values are means \pm standard error of the mean of three independent experiments.

ated flavones, indicating that the radioiodinated chalcones had higher binding affinities to β -amyloid plaques than those of the corresponding radioiodinated flavones. It is especially valuable that the radioiodinated chalcones displayed high binding affinities to $\text{A}\beta(1-42)$ aggregates, because we aim to develop novel probes that can detect diffuse plaques mainly composed of $\text{A}\beta(1-42)$. More interestingly, when thioflavin T and Congo Red were evaluated for their competition against [^{125}I]10 binding on $\text{A}\beta(1-42)$ aggregates, high K_i values ($>10,000$ nM) were observed, indicating poor binding competition. This finding suggests that these chalcones may have a binding site on $\text{A}\beta$ aggregates different from that of thioflavin T and Congo Red. We selected compound 10, which showed the highest binding affinity for $\text{A}\beta(1-42)$ aggregates for additional studies.

2.3. Neuropathological staining on double transgenic mouse sections

Compound 10 was investigated for their neuropathological staining of β -amyloid plaques in double transgenic mouse brain sections (Fig. 5). Many β -amyloid plaques, as well as cerebrovascular amyloid, were stained with compound 10. The results suggest that chalcone derivatives have high binding affinities for amyloid plaques in transgenic mice brain, as reflected by in vitro binding assay using $\text{A}\beta(1-42)$ aggregates. Also, chalcone derivatives with high binding affinity for $\text{A}\beta(1-42)$ aggregates may be more useful for presymptomatic and early detection of AD pathology.

2.4. Biodistribution studies

Five radioiodinated chalcones ([^{125}I]4, [^{125}I]7, [^{125}I]10, [^{125}I]14, and [^{125}I]16) were evaluated for their in vivo biodistribution in normal mice (Table 2). A biodistribution study provides important information on brain penetration. The chalcone ligands displayed high brain uptakes ranging from 2.0 to 4.7%ID/g brain at 2 min postinjection, indicating a level sufficient for brain imaging. In addition, they displayed good clearance from the normal brain with 0.46, 0.61, 0.49, 0.22, and 0.40%ID/g at 30 min postinjection for [^{125}I]4, [^{125}I]7, [^{125}I]10, [^{125}I]14, and [^{125}I]16, respectively. These values were equal to 10.9%, 12.9%, 24.0%, 9.0%, and 11.2% of initial brain uptake peak for [^{125}I]4, [^{125}I]7, [^{125}I]10, [^{125}I]14, and [^{125}I]16, respectively. Since the normal brain has no $\text{A}\beta$ plaques to trap the agent, the washout from the normal brain should be fast. These desirable pharmacokinetics demonstrated by radioiodinated chalcones are critical to obtaining a higher signal-to-noise ratio earlier in the AD brain. We previously reported that radioiodinated flavones showed high brain uptake (3.2–4.1%ID/g at 2 min postinjection) and good clearance from the brain (0.5–1.9%ID/g at 30 min postinjection).¹⁸ However, the ratios of 2–30 min mouse brain uptake of these radioiodinated flavones were 12.8–58.7%, which were higher than those of radioiodinated chalcones (9.0–24.0%). These biodistribution data suggest that novel radioiodinated chalcones may have more suitable in vivo pharmacokinetic properties for amyloid imaging

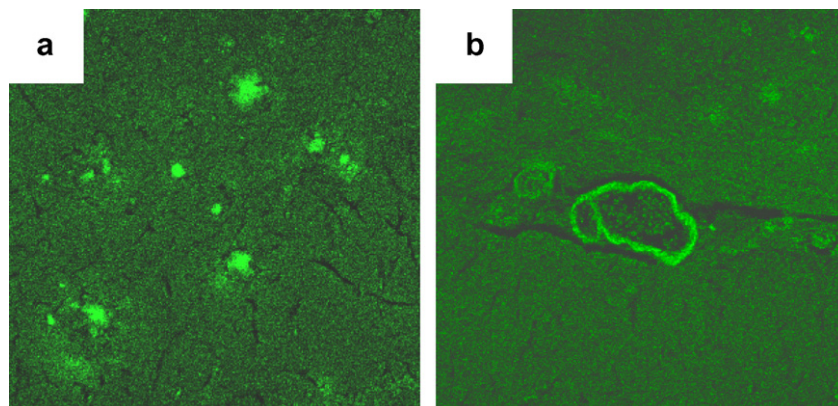


Figure 5. Neuropathological staining of compound 10 on 5- μm double transgenic mouse brain sections from the cortex. (a) Many β -amyloid plaques are clearly stained with compound 10. (b) Clear staining of cerebrovascular amyloids can be also observed.

Table 2. Biodistribution of radioactivity after intravenous administration of [125 I]**4**, [125 I]**7**, [125 I]**10**, [125 I]**14**, and [125 I]**16** in mice^a

	Organ	Time after injection	
		2 min	30 min
[125 I] 4	Blood	3.62 \pm 0.69	1.46 \pm 0.32
	Liver	7.52 \pm 0.72	8.16 \pm 2.15
	Kidney	6.96 \pm 0.43	6.34 \pm 3.51
	Intestine	1.84 \pm 0.32	11.27 \pm 3.34
	Spleen	1.53 \pm 0.37	0.60 \pm 0.25
	Lung	4.74 \pm 1.05	1.43 \pm 0.23
	Stomach ^b	0.87 \pm 0.16	1.58 \pm 0.44
	Heart	4.59 \pm 0.54	0.84 \pm 0.19
	Brain	4.49 \pm 0.55	0.46 \pm 0.07
[125 I] 7	Blood	1.64 \pm 0.47	1.47 \pm 0.27
	Liver	7.20 \pm 1.72	8.61 \pm 1.13
	Kidney	6.62 \pm 1.10	11.43 \pm 4.02
	Intestine	2.06 \pm 0.35	10.46 \pm 1.71
	Spleen	2.85 \pm 0.81	0.80 \pm 0.28
	Lung	8.30 \pm 3.43	4.01 \pm 0.30
	Stomach ^b	1.02 \pm 0.50	1.28 \pm 0.41
	Heart	4.72 \pm 1.00	1.08 \pm 0.16
	Brain	4.72 \pm 1.50	0.61 \pm 0.11
[125 I] 10	Blood	1.85 \pm 0.40	1.40 \pm 0.24
	Liver	10.02 \pm 0.57	11.04 \pm 2.42
	Kidney	5.32 \pm 0.74	11.62 \pm 2.06
	Intestine	1.31 \pm 0.16	10.38 \pm 2.37
	Spleen	1.37 \pm 0.21	0.60 \pm 0.08
	Lung	3.40 \pm 0.11	1.84 \pm 0.15
	Stomach ^b	0.96 \pm 0.09	2.45 \pm 0.19
	Heart	3.92 \pm 0.36	1.18 \pm 0.30
	Brain	2.04 \pm 0.36	0.49 \pm 0.08
[125 I] 14	Blood	1.34 \pm 0.20	0.46 \pm 0.08
	Liver	5.97 \pm 0.97	4.03 \pm 0.72
	Kidney	4.61 \pm 0.72	3.27 \pm 0.28
	Intestine	1.93 \pm 0.28	10.19 \pm 0.51
	Spleen	1.38 \pm 0.21	0.29 \pm 0.07
	Lung	3.81 \pm 0.87	0.79 \pm 0.13
	Stomach ^b	0.9 \pm 0.37	2.11 \pm 2.38
	Heart	3.74 \pm 0.92	0.63 \pm 0.18
	Brain	2.45 \pm 0.49	0.22 \pm 0.08
[125 I] 16	Blood	1.89 \pm 0.13	0.57 \pm 0.04
	Liver	8.39 \pm 1.49	6.64 \pm 0.46
	Kidney	9.30 \pm 0.84	3.51 \pm 0.28
	Intestine	1.87 \pm 0.32	12.94 \pm 2.43
	Spleen	3.56 \pm 0.99	1.04 \pm 0.25
	Lung	10.04 \pm 2.09	1.04 \pm 0.07
	Stomach ^b	1.22 \pm 0.19	2.16 \pm 0.55
	Heart	5.92 \pm 1.32	0.49 \pm 0.06
	Brain	3.57 \pm 0.39	0.40 \pm 0.02

^a Expressed as % injected dose per gram. Each value represents means \pm SD for four mice at each interval.

^b Expressed as % injected dose per organ.

in AD brains compared with the radioiodinated flavones.

3. Conclusion

In summary, we successfully designed and synthesized a new series of chalcone derivatives as probes for in vivo imaging of β -amyloid plaques in the brain. In the in vitro binding studies, these chalcones showed high binding affinity to A β (1–42) aggregates. In biodistribu-

tion studies using normal mice, they displayed good brain penetration and fast washout from the brain, highly desirable characteristics for in vivo amyloid imaging agents. Taken together, the present results suggest that the novel radioiodinated chalcones may be useful probes for diagnosis of AD. Additional chemical modification on the chalcone structure may lead to more useful β -amyloid imaging agents for both PET and SPECT.

4. Experimental

All reagents used in syntheses were commercial products and were 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), br (broad), and m (multiplet). Mass spectra were obtained on a JEOL IMS-DX instrument.

4.1. Chemistry

4.1.1. (*E*)-1-(4-Bromophenyl)-3-(4-nitrophenyl)prop-2-en-1-one (1**).** Equimolar portions of 4-bromoacetophenone (1.99 g, 10 mmol) and 4-nitroacetophenone (1.52 g, 10 mmol) were dissolved in 15 mL of ethanol. The mixture was allowed to stir for 15 min in an ice bath. A 10 mL aliquot of 10% aqueous potassium hydroxide solution was then slowly added dropwise to the reaction mixture. The reaction solution was allowed to stir at room temperature for 4 h. A precipitate was collected and washed with ethanol to give 1.27 g of **1** (38.2%). ^1H NMR (300 MHz, CDCl_3) δ 7.59 (d, J = 15.9 Hz, 1H), 7.68 (d, J = 9.0 Hz, 2H), 7.79 (d, J = 9.0 Hz, 2H), 7.74 (d, J = 15.3 Hz, 1H), 7.91 (d, J = 8.7 Hz, 2H), 8.29 (d, J = 8.7 Hz, 2H).

4.1.2. (*E*)-3-(4-Aminophenyl)-1-(4-bromophenyl)prop-2-en-1-one (2**).** A mixture of **1** (1.0 g, 3.01 mmol), SnCl_2 (5.0 g, 26.4 mmol), and ethanol (25 mL) was stirred under reflux for 2 h. After the mixture cooled to room temperature, 1 M NaOH (20 mL) was added and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4 and filtered. The filtrate was concentrated to give 556 mg of **2** (61.1%). ^1H NMR (300 MHz, CDCl_3) δ 4.03 (s, 2H), 6.68 (d, J = 8.7 Hz, 2H), 7.29 (d, J = 15.0 Hz, 1H), 7.48 (d, J = 8.7 Hz, 2H), 7.62 (d, J = 8.7 Hz, 2H), 7.76 (d, J = 15.3 Hz, 1H), 7.87 (d, J = 8.7 Hz, 2H).

4.1.3. (*E*)-3-(4-Aminophenyl)-1-(4-(tributylstannyl)phenyl)prop-2-en-1-one (3**).** A mixture of **2** (100 mg, 0.33 mmol) (Bu_3Sn)₂ (1 mL), and (Ph_3P)₄Pd (35 mg) in a mixed solvent (15 mL, 2:1 dioxane/triethylamine mixture) was stirred under reflux for 4 h. The solvent was removed, and the residue was purified by preparative TLC (1:1 hexane/ethyl acetate) to give 88 mg of **3** (52.1%). ^1H NMR (300 MHz, CDCl_3) δ 0.89–1.64 (m, 27H), 4.01 (s, 2H), 6.68 (d, J = 8.7 Hz, 2H), 7.35 (d, J = 15.9 Hz, 1H), 7.48 (d, J = 8.4 Hz, 2H), 7.60 (d, J = 8.1 Hz, 2H), 7.76 (d, J = 15.6 Hz, 1H), 7.92 (d, J = 8.1 Hz, 2H). EI-MS m/z 513 (MH^+).

4.1.4. (*E*)-3-(4-Aminophenyl)-1-(4-iodophenyl)prop-2-en-1-one (4). To a solution of **3** (80 mg, 0.16 mmol) in CHCl_3 (10 mL) was added a solution of iodine in CHCl_3 (2 mL, 50 mg/mL) at room temperature. The mixture was stirred at room temperature for 30 min, and NaHSO_3 solution (10 mL) was added. The mixture was stirred for 5 min, and the organic phase was separated, dried over Na_2SO_4 , and filtered. The solvent was removed, and the residue was purified by preparative TLC (3:2 hexane/ethyl acetate) to give 26 mg of **4** (46.5%). ^1H NMR (300 MHz, CDCl_3) δ 4.03 (s, 2H), 6.68 (d, $J = 8.7$ Hz, 2H), 7.28 (d, $J = 15.6$ Hz, 1H), 7.48 (d, $J = 8.1$ Hz, 2H), 7.71 (d, $J = 8.7$ Hz, 2H), 7.76 (d, $J = 15.3$ Hz, 1H), 7.85 (d, $J = 8.4$ Hz, 2H). MS m/z 349 (M^+). Anal. Calcd for $\text{C}_{15}\text{H}_{12}\text{INO}$: C, 51.60; H, 3.46; N, 4.01. Found: C, 51.30; H, 3.85; N, 4.26.

4.1.5. (*E*)-1-(4-Bromophenyl)-3-(4-(methylamino)phenyl)prop-2-en-1-one (5). To a solution of **2** (200 mg, 0.66 mmol) in DMSO (anhydrous, 5 mL) were added methyl iodide (228 mg, 1.60 mmol) and anhydrous K_2CO_3 (500 mg, 3.75 mmol). The reaction mixture was stirred at room temperature for 3 h. After it was poured into water (30 mL), the mixture was extracted with ethyl acetate (2×30 mL). The organic layers were combined and dried over Na_2SO_4 . Evaporation of the solvent afforded a residue, which was purified by silica gel chromatography (hexane/ethyl acetate = 6:1) to give 74 mg of **5** (35.5%). ^1H NMR (300 MHz, CDCl_3) δ 2.90 (s, 3H), 4.20 (s, 1H), 6.60 (d, $J = 9.0$ Hz, 2H), 7.26 (d, $J = 15.3$ Hz, 1H), 7.51 (d, $J = 8.7$ Hz, 2H), 7.62 (d, $J = 8.4$ Hz, 2H), 7.78 (d, $J = 15.9$ Hz, 1H), 7.87 (d, $J = 8.7$ Hz, 2H).

4.1.6. (*E*)-1-(4-(Tributylstannyl)phenyl)-3-(4-(methylamino)phenyl)prop-2-en-1-one (6). The same reaction as described above to prepare **3** was used, and 64 mg of **6** was obtained in a 55.0% yield from **5**. ^1H NMR (300 MHz, CDCl_3) δ 0.87–1.61 (m, 27H), 2.90 (s, 3H), 4.16 (s, 1H), 6.60 (d, $J = 8.7$ Hz, 2H), 7.33 (d, $J = 15.6$ Hz, 1H), 7.51 (d, $J = 8.4$ Hz, 2H), 7.59 (d, $J = 8.1$ Hz, 2H), 7.78 (d, $J = 15.6$ Hz, 1H), 7.92 (d, $J = 7.8$ Hz, 2H). MS m/z 527 (MH^+).

4.1.7. (*E*)-1-(4-Iodophenyl)-3-(4-(methylamino)phenyl)prop-2-en-1-one (7). The same reaction as described above to prepare **4** was used, and 41 mg of **7** was obtained in an 80.6% yield from **6**. ^1H NMR (300 MHz, CDCl_3) δ 2.90 (s, 3H), 4.19 (s, 1H), 6.60 (d, $J = 9.0$ Hz, 2H), 7.25 (d, $J = 15.6$ Hz, 1H), 7.51 (d, $J = 8.7$ Hz, 2H), 7.71 (d, $J = 8.7$ Hz, 2H), 7.77 (d, $J = 15.6$ Hz, 1H), 7.84 (d, $J = 8.7$ Hz, 2H). MS m/z 363 (M^+). Anal. Calcd for $\text{C}_{16}\text{H}_{14}\text{INO}$: C, 52.91; H, 3.89; N, 3.86. Found: C, 53.24; H, 4.19; N, 3.44.

4.1.8. (*E*)-1-(4-Bromophenyl)-3-(4-(dimethylamino)phenyl)prop-2-en-1-one (8). To a stirred mixture of **2** (250 mg, 0.83 mmol) and paraformaldehyde (400 mg, 13.4 mmol) in AcOH (15 mL) was added in one portion NaCNBH_3 (250 mg, 3.98 mmol) at room temperature. The resulting mixture was stirred at room temperature for 3 h, 1 M NaOH (50 mL) was added and extracted with CH_3Cl

(50 mL). The organic phase was dried over Na_2SO_4 and filtered. The solvent was removed, and the residue was purified by silica gel chromatography (hexane/ethyl acetate = 12:1) to give 236 mg of **8** (86.1%). ^1H NMR (300 MHz, CDCl_3) δ 3.06 (s, 6H), 6.70 (d, $J = 8.7$ Hz, 2H), 7.27 (d, $J = 15.3$ Hz, 1H), 7.54 (d, $J = 8.7$ Hz, 2H), 7.59 (d, $J = 8.7$ Hz, 2H), 7.74 (d, $J = 15.3$ Hz, 1H), 7.87 (d, $J = 7.8$ Hz, 2H).

4.1.9. (*E*)-1-(4-(Tributylstannyl)phenyl)-3-(4-(dimethylamino)phenyl)prop-2-en-1-one (9). The same reaction as described above to prepare **3** was used, and 43 mg of **9** was obtained in a 11.9% yield from **8**. ^1H NMR (300 MHz, CDCl_3) δ 0.87–1.57 (m, 27H), 3.05 (s, 6H), 6.70 (d, $J = 8.7$ Hz, 2H), 7.34 (d, $J = 15.3$ Hz, 1H), 7.55 (d, $J = 8.7$ Hz, 2H), 7.59 (d, $J = 8.7$ Hz, 2H), 7.79 (d, $J = 15.6$ Hz, 1H), 7.92 (d, $J = 7.8$ Hz, 2H). MS m/z 541 (MH^+).

4.1.10. (*E*)-3-(4-(Dimethylamino)phenyl)-1-(4-iodophenyl)prop-2-en-1-one (10). The same reaction as described above to prepare **4** was used, and 13 mg of **10** was obtained in a 46.6% yield from **9**. ^1H NMR (300 MHz, CDCl_3) δ 3.05 (s, 6H), 6.69 (d, $J = 9.0$ Hz, 2H), 7.26 (d, $J = 15.6$ Hz, 1H), 7.54 (d, $J = 9.0$ Hz, 2H), 7.72 (d, $J = 8.7$ Hz, 2H), 7.79 (d, $J = 15.6$ Hz, 1H), 7.84 (d, $J = 8.4$ Hz, 2H). MS m/z 377 (M^+). Anal. Calcd for $\text{C}_{17}\text{H}_{16}\text{INO}$: C, 54.13; H, 4.28; N, 3.71. Found: C, 53.92; H, 4.40; N, 3.53.

4.1.11. (*E*)-1-(4-Bromophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (11). Equimolar portions of 4-bromoacetophenone (1.99 g, 10 mmol) and 4-anisaldehyde (1.36 g, 10 mmol) were dissolved in 20 mL of ethanol. The mixture was allowed to stir for 15 min in an ice bath. A 20 mL aliquot of 10% aqueous potassium hydroxide solution was then slowly added dropwise to the reaction mixture. The reaction solution was allowed to stir at room temperature for 3 h. A precipitate was collected and washed with ethanol to give 2.97 g of **11** (93.6%). ^1H NMR (300 MHz, CDCl_3) δ 3.86 (s, 3H), 6.94 (d, $J = 8.7$ Hz, 2H), 7.36 (d, $J = 15.3$ Hz, 1H), 7.59–7.65 (m, 4H), 7.80 (d, $J = 15.6$ Hz, 1H), 7.84 (d, $J = 8.4$ Hz, 2H).

4.1.12. (*E*)-1-(4-Bromophenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (12). BBr_3 (5 mL, 1 M solution in CH_2Cl_2) was added to a solution of **11** (500 mg, 1.58 mmol) in CH_2Cl_2 (20 mL) dropwise in an ice bath. The mixture was allowed to warm to room temperature and stirred for 30 min. Water (50 mL) was added while the reaction mixture was cooled in an ice bath. The mixture was extracted with chloroform (2×30 mL), and the organic phase was dried over Na_2SO_4 and filtered. The filtrate was concentrated and the residue was purified by silica gel chromatography (hexane/ethyl acetate = 7:2) to give 307 mg of **12** (64.1%). ^1H NMR (300 MHz, CDCl_3) δ 5.59 (s, 1H), 6.90 (d, $J = 8.7$ Hz, 2H), 7.35 (d, $J = 15.3$ Hz, 1H), 7.56 (d, $J = 8.7$ Hz, 2H), 7.64 (d, $J = 8.4$ Hz, 2H), 7.79 (d, $J = 15.6$ Hz, 1H), 7.88 (d, $J = 8.4$ Hz, 2H).

4.1.13. (*E*)-1-(4-(Tributylstannyl)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (13). The same reaction as

described above to prepare **3** was used, and 366 mg of **13** was obtained in a 43.9% yield from **11**. ^1H NMR (300 MHz, CDCl_3) δ 0.87–1.54 (m, 27H), 3.86 (s, 3H), 6.94 (d, $J = 9.0$ Hz, 2H), 7.42 (d, $J = 15.6$ Hz, 1H), 7.59–7.62 (m, 4H), 7.79 (d, $J = 15.6$ Hz, 1H), 7.93 (d, $J = 8.1$ Hz, 2H). EI-MS m/z 528 (MH^+).

4.1.14. (E)-1-(4-Iodophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (14). The same reaction as described above to prepare **4** was used, and 140 mg of **14** was obtained in a 61.0% yield from **13**. ^1H NMR (300 MHz, CDCl_3) δ 3.86 (s, 3H), 6.94 (d, $J = 9.0$ Hz, 2H), 7.34 (d, $J = 15.9$ Hz, 1H), 7.60 (d, $J = 8.7$ Hz, 2H), 7.72 (d, $J = 8.7$ Hz, 2H), 7.79 (d, $J = 15.6$ Hz, 1H), 7.86 (d, $J = 8.7$ Hz, 2H). EI-MS m/z 364 (M^+). Anal. Calcd for $\text{C}_{16}\text{H}_{13}\text{IO}_2$: C, 52.77; H, 3.60. Found: C, 52.65; H, 3.59.

4.1.15. (E)-1-(4-(Tributylstannyl)phenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (15). The same reaction as described above to prepare **3** was used, and 131 mg of **15** was obtained in a 31.1% yield from **12**. ^1H NMR (300 MHz, CDCl_3) δ 0.87–1.58 (m, 27H), 5.30 (s, 1H), 7.41 (d, $J = 15.6$ Hz, 1H), 7.56 (d, $J = 8.7$ Hz, 2H), 7.61 (d, $J = 8.4$ Hz, 2H), 7.78 (d, $J = 15.6$ Hz, 1H), 7.93 (d, $J = 8.4$ Hz, 2H). EI-MS m/z 514 (MH^+).

4.1.16. (E)-3-(4-Hydroxyphenyl)-1-(4-iodophenyl)prop-2-en-1-one (16). The same reaction as described above to prepare **12** was used, and 71 mg of **16** was obtained in a 24.7% yield from **14**. ^1H NMR (300 MHz, CDCl_3) δ 5.26 (s, 1H), 6.88 (d, $J = 8.4$ Hz, 2H), 7.34 (d, $J = 15.3$ Hz, 1H), 7.56 (d, $J = 8.4$ Hz, 2H), 7.72 (d, $J = 9.0$ Hz, 2H), 7.78 (d, $J = 15.9$ Hz, 1H), 7.86 (d, $J = 8.7$ Hz, 2H). EI-MS m/z 350 (M^+). Anal. Calcd for $\text{C}_{15}\text{H}_{11}\text{IO}_2$: C, 51.45; H, 3.17. Found: C, 51.25; H, 3.06.

4.2. Iododestannylation reaction

The radioiodinated forms of compounds **4**, **7**, **10**, **14**, and **16** were prepared from the corresponding tributyltin derivatives by an iododestannylation. Briefly, to initiate the reaction 50 μL of H_2O_2 (3%) was added to a mixture of a tributyltin derivative (100 $\mu\text{g}/50$ μL EtOH), $^{125}\text{I}[\text{NaI}]$ (0.1–0.2 mCi, specific activity 2200 Ci/mmol), and 100 μL of 1 N HCl in a sealed vial. The reaction was allowed to proceed at room temperature for 10 min and was terminated by addition of NaHSO_3 . The reaction, after neutralization with sodium bicarbonate, was extracted with ethyl acetate. The extract was dried by passing through an anhydrous Na_2SO_4 column and was then blown to dryness with a stream of nitrogen gas. The radioiodinated ligand was purified by HPLC on a Cosmosil C18 column with an isocratic solvent of $\text{H}_2\text{O}/\text{acetonitrile}$ (2:3) at a flow rate of 1.0 mL/min. The purified ligand was stored at -20°C for in vitro binding and biodistribution studies.

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

A solid form of A β (1–42) was purchased from Peptide Institute (Osaka, Japan). Aggregation of peptides was

carried out by gently dissolving the peptide (0.25 mg/mL) in a buffer solution (pH 7.4) containing 10 mM sodium phosphate and 1 mM EDTA. The solutions were incubated at 37°C for 42 h with gentle and constant shaking. Binding studies were carried out in 12×75 mm borosilicate glass tubes according to the procedure described before²² with some modification. For saturation studies, a solution of $^{125}\text{I}[\text{10}]$ (final concentration, 0.8–100 nM) was prepared by mixing nonradioactive **10**. Nonspecific binding was defined in the presence of 400 nM nonradioactive **10**. For saturation studies, 1 mL of the reaction mixture contained 50 μL of inhibitors (10^{-5} – 10^{-10} M in 10% EtOH) and 0.05 nM of radiotracer in 10% EtOH. The binding assay was performed by mixing 50 μL of A β (1–42) aggregates (29 nM in the final assay mixture), an appropriate concentration of 50 μL of $^{125}\text{I}[\text{10}]$, and 900 μL of 10% ethanol. After incubation for 3 h at room temperature, the binding mixture was filtered through GF/B filters (Whatman, Kent, UK) using a M-24 cell harvester (Brandel, Gaithersburg, MD). Filters containing the bound ^{125}I ligand were counted in a gamma camera counter. The dissociation constant (K_d) of compound **10** was determined by Scatchard analysis using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA). For inhibition studies, a mixture containing 50 μL of test compounds (8 pM–12.5 μM in 10% ethanol), 50 μL of 0.02 nM $^{125}\text{I}[\text{10}]$, 50 μL of A β (1–42) 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 counted in a gamma counter. 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 equation²⁶: $K_i = \text{IC}_{50}/(1 + [\text{L}]/K_d)$, where $[\text{L}]$ is the concentration of $^{125}\text{I}[\text{10}]$ used in the assay and K_d is the dissociation constant of compound **10**.

4.4. Staining of amyloid plaques in double transgenic mice brain sections

The double transgenic mice (6 months of age) produced by Tg2576 crossed with mutated PS1 (A260V) mice were used as Alzheimer's model mice. Brain tissues were obtained followed by fixation with 10% formaldehyde. Dehydrated tissues with ethanol and xylene were paraffinized and the resultant wax blocks were sliced into serial sections with 5 μm thickness. The tissue slides were deparaffinized with xylene, ethanol, and distilled water. After incubation with PBS for 30 min, each slide was incubated with 50% ethanol solution (100 μM) of compound **10**. Finally, the sections were washed in PBS for 15 min. Thereafter, the sections were incubated in ethanol and xylene, and embedded in Entellan Neu (Merck, Darmstadt, Germany). Fluorescent observation was performed by the Leica TCS SP2 system with DMIRE2 fluorescence microscope. Staining with compound **10** was detected using filter set with 458 nm excitation and 540–580 nm emission. The sections were also

immunostained with DAB as a chromogen using monoclonal antibodies against β -amyloid as previously reported.²⁷

4.5. In vivo biodistribution in normal mice

Animal studies were conducted in accordance with our institutional guidelines and were approved by Nagasaki University Animal Care Committee. A saline solution (100 μ L) containing radiolabeled agents (0.2–0.3 μ Ci) and 10% ethanol was injected directly into the tail vein of ddY mice (5 weeks old, average weight 20–25 g). The mice were sacrificed at various time points postinjection. The organs of interest were removed and weighed, and the radioactivity was counted with an automatic gamma counter (Aloka, ARC-380).

Acknowledgments

This work was supported in part by Industrial Technology Research Grant Program in 2005 from New Energy and Industrial Technology Development Organization (NEDO) of Japan.

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