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Synthesis and characterization of styrylchromone derivatives as β-amyloid imaging agents

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Abstract—Several promising agents have been synthesized and evaluated for in vivo imaging probes of β-amyloid plaques in Alzheimer's disease (AD) brain. Recently, we have developed flavone derivatives, which possess the basic structure of the 2-phenylchromone, as useful candidates for amyloid imaging agents. In an attempt to further develop novel tracers, we synthesized and evaluated a series of 2-styrylchromone derivatives, which replace the 2-phenyl substituent of flavone backbone with the 2-styryl. A series of radioiodinated styrylchromone derivatives were designed and synthesized. The binding affinities for amyloid plaques were assessed by in vitro binding assay using pre-formed synthetic Aβ(1–40) aggregates. The new series of styrylchromone derivatives showed high binding affinity to Aβ aggregates at the K_d values of 32.0, 17.5 and 8.7 nM for [^{125}I]**6**, [^{125}I]**9**, and [^{125}I]**12**, respectively. In biodistribution studies using normal mice, [^{125}I]**6** and [^{125}I]**9** examined in normal mice displayed high brain uptakes with 4.9 and 2.8%ID/g at 2 min post injection. The radioactivity washed out from the brain rapidly (1.6 and 1.0%ID/g at 60 min post injection for [^{125}I]**6** and [^{125}I]**9**, respectively). But [^{125}I]**12** did not show marked brain uptake, and the washout rate from the brain was relatively slow throughout the time course (1.1 and 1.4%ID/g at 2 and 30 min post injection, respectively). Although additional modifications are necessary to improve the brain uptake and rapid clearance of non-specifically bound radiotracer, the styrylchromone backbone may be useful as a backbone structure to develop novel β-amyloid 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. The neuropathological hallmarks of AD include abundant deposits of β-amyloid fibrils in senile plaques (SPs), accumulation of abnormal tau protein filaments in neurofibrillary tangles (NFTs), and extensive neuronal degeneration and loss. Although the precise mechanism of neuronal death in AD still remains unknown, SPs and NFTs have been regarded as a critical event for the pathogenesis of AD.[1,2] Therefore, quantitative evaluation of SPs and/or NFTs in the brain with non-invasive techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) would lead to presymptomatic detection of AD patients and evaluation of new anti-amyloid therapies currently under development.[3–5]

Several promising agents based on the backbone structure of DDNP, thioflavin-T, and Congo Red have been synthesized and evaluated for in vivo imaging probes of β-amyloid plaques in AD brain. Clinical trials in AD patients have been reported with several agents including [^{18}F]-2-(1-(2-(*N*-(2-fluoroethyl)-*N*-methylamino)naphthalene-6-yl)ethylidene)malononitrile ([^{18}F]FDDNP),[6,7] [^{11}C]-2-(4-(methylamino)phenyl)-6-hydroxybenzothiazole ([^{11}C]-6-OH-BTA-1),[8,9] [^{11}C]-4-*N*-methylamino-4′-hydroxystilbene ([^{11}C]SB-13),[10,11] and [^{123}I]-6-iodo-2-(4′-dimethylamino)phenyl-imidazo[1,2-*a*]pyridine ([^{123}I]IMPY),[12,13] (Fig. 1), indicating that detecting amyloid plaques in the living human brain with amyloid imaging agents is potentially feasible by both PET and SPECT.

Recently, we have reported flavone derivatives, which possess the basic structure of the 2-phenylchromone, as useful candidates for β-amyloid imaging agents (Fig. 2).[14] This report also suggested that the backbone except DDNP, thioflavin-T, and Congo Red can be applied for the development of more useful β-amyloid imaging agents. In an attempt to further develop novel ligands for imaging of amyloid plaques in AD, we designed a series of 2-styrylchromone derivatives, which

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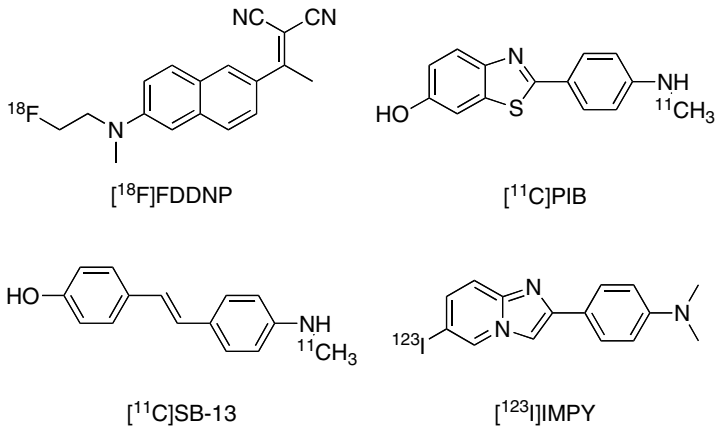


Figure 1. Chemical structures of amyloid imaging agents previously reported.

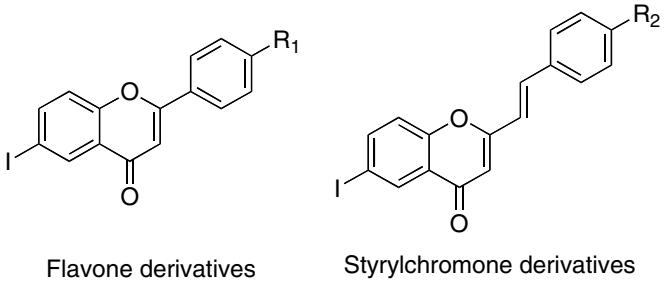


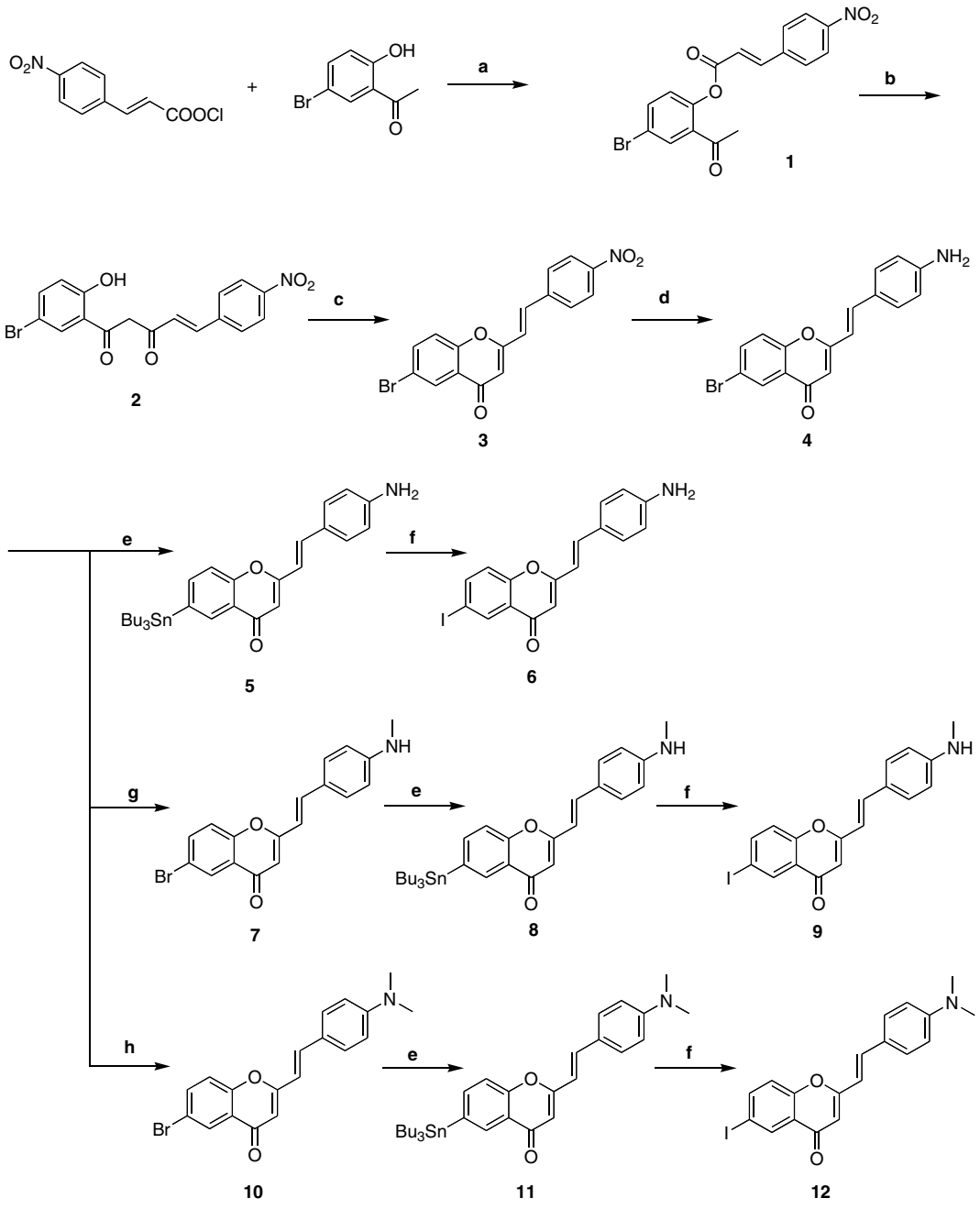
Figure 2. Chemical structures of flavone derivatives reported previously and styrylchromone derivatives reported in this report. R_1 = $NHCH_3$, $N(CH_3)_2$, OCH_3, OH; R_2 = NH_2, $NHCH_3$, $N(CH_3)_2$.

replace the 2-phenyl substituent of flavone backbone with the 2-styryl. In this paper, we report the synthesis of a novel series of styrylchromone derivatives and the characterization as β-amyloid imaging agents.

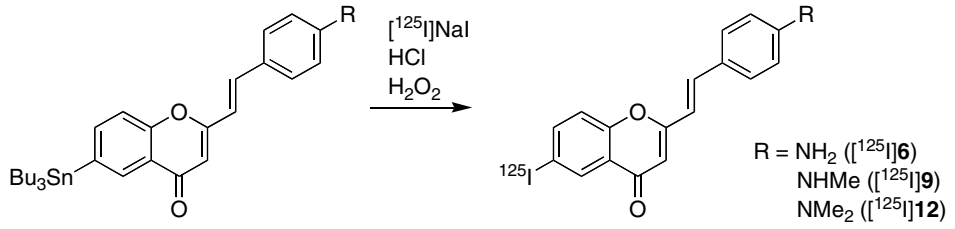
2. Results and discussion

The synthesis of the styrylchromone derivatives is outlined in Scheme 1. Various strategies have been developed for the synthesis of 2-styrylchromone. Among them, the most utilized procedure for building the 2-styrylchromone ring system is the Baker-Vankataraman approach, based on the cyclization of 2-cinnamoyl-*O*-hydroxyacetophenones, obtained by the condensation of 2-hydroxyacetophenone with the cinnamic acid chloride, followed by rearrangement of the intermediate ester. In this process, the 2′-cinnamoyloxyacetophenone **1** has been prepared by O-acylation of the 2-hydroxyacetophenone with a cinnamoyl chloride in the presence of pyridine. The arrangement of ester to give the β-diketone **2** was performed by treatment with KOH in pyridine. The cyclodehydration of 2,4-pentadien-1-one, by refluxing sulfuric acid and acetic acid, gave the corresponding 2-styrylchromone **3**. The amino derivative **4** was readily prepared from **3** by reduction with $SnCl_2$ (63.3% yield). Conversion of **4** to the monomethylamino derivative **7** was achieved by a method previously reported[15] (96.0% yield). Compound **4** was also converted to the dimethylamino derivative **10** by an efficient method[16] with paraformaldehyde and acetic acid (71.5% yield). The tributyltin derivatives (**5**, **8**, and **11**) were prepared from the corresponding bromo compounds (**4**, **7**, and **10**) using a bromo to tributyltin exchange reaction catalyzed by Pd(0) for yields of 56.2%, 42.8%, and 37.4%, respectively. These tributyltin derivatives were readily reacted with iodine in chloroform at room temperature to give the iodo derivatives (**6**, **9**, and **12**). Furthermore, these tributyltin derivatives can be also used as the starting materials for redioiodination in preparation of [^{125}I]**6**, [^{125}I]**9**, and [^{125}I]**12**. Novel radioiodinated styrylchromone derivatives were achieved by an iododestannylation reaction using hydrogen peroxide as the oxidant, which produced the desired radioiodinated ligands (Scheme 2). It was anticipated that the no-carrier-added preparation would result in a final product bearing a theoretical specific activity similar to that of ^{125}I (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 [^{125}I]**6**, [^{125}I]**9**, and [^{125}I]**12** showed a single radioactivity peak at retention times of 5.3, 7.2, and 11.7 min, respectively (Table 1). Three radioiodinated products were obtained in 50–70% radiochemical yields with radiochemical purities of >95% after purification by HPLC.

The binding studies of [^{125}I]**6**, [^{125}I]**9**, and [^{125}I]**12** to aggregates of Aβ(1–40) were carried out (Fig. 3). Transformation of the saturation binding of [^{125}I]**6**, [^{125}I]**9**, and [^{125}I]**12** to Scatchard plots gave linear plots, indicating that the styrylchromone derivatives have one bind-



Scheme 1. Synthetic route for styrylchromone derivatives. Reagents: (a) pyridine; (b) pyridine, KOH; (c) H_2SO_4, AcOH; (d) EtOH, $SnCl_2$; (e) dioxane, $(Bu_3Sn)_2$, $(Ph_3P)_4Pd$, Et_3N; (f) $CHCl_3$, I_2; (g) MeOH, NaOMe, $(CH_2O)_n$, $NaBH_4$; (h) AcOH, $(CH_2O)_n$, $NaCNBH_3$.



Scheme 2. Radioiodination reaction of styrylchromone derivatives.

ing site on Aβ aggregates. [^{125}I]**6**, [^{125}I]**9**, and [^{125}I]**12** showed excellent binding affinity for Aβ(1–40) aggregates at the K_d value of 32.0 ± 5.0, 17.5 ± 5.0, and 8.7 ± 2.7 nM, respectively. The binding affinity to Aβ aggregates increased in the order of [^{125}I]**12** > [^{125}I]**9** > [^{125}I]**6**. The result of the binding study was in agreement

Table 1. HPLC data and partition coefficient for [^{125}I]**6**, [^{125}I]**9**, and [^{125}I]**12**

Compound	Retention time[a] (min)	log P[b]
[^{125}I]**6**	5.3	1.65 (0.08)
[^{125}I]**9**	7.2	2.15 (0.08)
[^{125}I]**12**	11.7	2.65 (0.05)

[a] Using a mixture of H_2O/acetonitrile (3:7) as the mobile phase.
[b] Octanol/buffer (0.1 M phosphate-buffered saline, pH 7.4) partition coefficients. Each value represents the mean (SD) of three experiments.

with that of previous reports,[10,14] indicating that the binding affinity increased in the order of N,N-dimethylated derivative > N-monomethylated derivative > primary amino derivative. Comparing compounds **6**, **9**, and **12** with corresponding radioiodinated flavone derivatives, substitutions of the phenyl group on flavone backbone[14] with the styryl group resulted in a 1.5-fold increase in binding affinity. In addition, all of the styrylchromone derivatives evaluated maintained good binding affinities in the nanomolar range of K_d values. The results of the binding study suggest that the styrylchromone derivatives have considerable tolerance for structural modification.

One of the important prerequisites for an in vivo imaging agent of the brain is to penetrate the blood–brain barrier after an iv injection. The log P values of [^{125}I]**6**, [^{125}I]**9**, and [^{125}I]**12** were 1.65, 2.15, and 2.65, respectively (measured by a partition between 1-octanol and pH 7.4 phosphate buffer) (Table 1). Previous studies suggest that the optimal lipophilicity range for brain entry is observed for compounds with log P values between 1 and 3.[17–19] Since these ligands displayed moderate lipophilicity for BBB penetration, it was expected to show adequate brain uptake to detect amyloid plaques following systemic injection. To test the brain uptake and washout from the brain, three styrylchromone derivatives were injected into normal mice (Table 2). Generally, the ideal amyloid imaging agents should have a high initial brain uptake and a fast washout from the normal brain area. Since the normal brain has no amyloid plaques to trap the agent, the radioactivity washout should be fast from the normal brain to obtain a higher signal to noise ratio in the AD brain. In the biodistribution studies, initial brain uptake of [^{125}I]**6** and [^{125}I]**9** at 2 min after iv injection was relatively high (4.88 and 2.84%ID/g, respectively), whereas the brain retention at later time points of [^{125}I]**6** and [^{125}I]**9** was low (1.62 and 0.99%ID/g at 60 min post iv injection, respectively).

Table 2. Biodistribution of radioactivity after intravenous administration of [^{125}I]**6**, [^{125}I]**9**, and [^{125}I]**12** in mice[a]

Tissue	Time after injection (min)			
	2	10	30	60
[^{125}I]**6**				
Blood	2.34 (0.16)	2.44 (0.19)	2.56 (0.64)	2.27 (0.47)
Liver	13.23 (1.65)	14.49 (1.10)	10.27 (1.84)	8.16 (1.33)
Kidney	11.94 (1.49)	6.99 (0.91)	4.91 (1.07)	3.76 (0.91)
Intestine	2.84 (0.18)	5.51 (0.55)	11.71 (1.27)	16.08 (2.44)
Spleen	4.23 (0.62)	3.64 (0.38)	2.92 (0.73)	2.16 (0.85)
Pancreas	6.58 (0.69)	5.18 (0.54)	3.07 (0.94)	1.11 (0.93)
Heart	8.40 (0.47)	3.78 (0.35)	2.43 (0.70)	1.64 (0.29)
Stomach[b]	1.24 (0.43)	1.81 (0.51)	2.17 (0.40)	3.29 (0.92)
Brain	4.88 (0.47)	4.40 (0.44)	2.78 (0.72)	1.62 (0.40)
[^{125}I]**9**				
Blood	3.21 (0.38)	2.44 (0.08)	2.19 (0.29)	1.79 (0.23)
Liver	14.55 (2.64)	14.41 (2.52)	11.13 (1.63)	7.82 (0.94)
Kidney	10.41 (1.33)	7.05 (0.56)	5.48 (0.97)	3.52 (1.36)
Intestine	2.59 (0.38)	8.55 (1.21)	16.72 (2.58)	19.16 (2.63)
Spleen	3.88 (0.74)	4.42 (1.05)	2.66 (0.47)	1.51 (0.19)
Pancreas	4.92 (0.54)	3.56 (0.96)	2.25 (0.63)	1.32 (0.33)
Heart	6.92 (1.41)	3.31 (0.69)	2.09 (0.39)	1.47 (0.27)
Stomach[b]	1.00 (0.23)	1.89 (0.33)	2.01 (0.53)	1.90 (0.29)
Brain	2.84 (0.35)	2.96 (0.25)	1.86 (0.30)	0.99 (0.09)
[^{125}I]**12**				
Blood	2.62 (0.42)	1.44 (0.57)	1.13 (0.37)	n.d.[c]
Liver	11.17 (2.23)	10.15 (1.43)	7.72 (1.83)	
Kidney	6.70 (0.92)	4.51 (0.62)	3.69 (0.19)	
Intestine	1.45 (0.31)	4.95 (0.96)	12.68 (2.31)	
Spleen	2.43 (0.75)	2.01 (0.39)	1.38 (0.34)	
Pancreas	3.02 (0.57)	2.73 (0.54)	1.34 (0.39)	
Heart	6.25 (1.27)	1.75 (0.73)	1.27 (0.24)	
Stomach[b]	0.70 (0.16)	1.14 (0.62)	1.23 (0.52)	
Brain	1.14 (0.20)	1.76 (0.24)	1.37 (0.36)	

[a] Expressed as % injected dose per gram. Each value represents the mean (SD) for 3–5 animals at each interval.
[b] Expressed as % injected dose per organ.
[c] Not determined.

These in vivo properties observed with [^{125}I]**6** and [^{125}I]**9** (a high initial brain uptake and fast washout from the normal mice brain) suggest that these radioiodinated styrylchromones may be novel candidates of amyloid imaging agents. Compared to the radioiodinated flavones previously reported,[14] [^{125}I]**6** and [^{125}I]**9** showed very similar in vivo pharmacokinetics in normal mice. On the other hand, [^{125}I]**12** did not show marked initial brain uptake in the normal mice after iv injection (1.14%ID/g at 2 min postinjection), whereas the radioactivity level of blood was similar to that of [^{125}I]**6** and

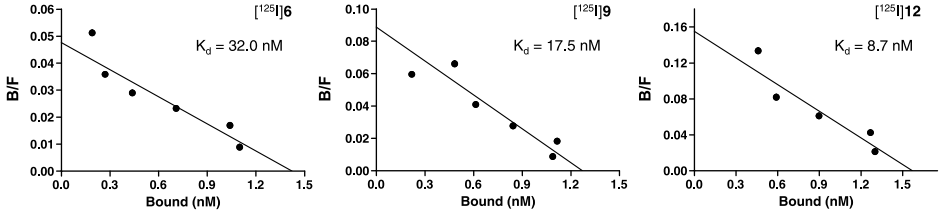


Figure 3. Scatchard plots of [^{125}I]**6**, [^{125}I]**9**, and [^{125}I]**12** binding to Aβ(1–40) aggregates. Three ligands showed one-site binding. High binding affinities with a K_d value in a nanomolar range were obtained (K_d = 32.0 ± 5.0, 17.5 ± 2.5, and 8.7 ± 2.7 nM for [^{125}I]**6**, [^{125}I]**9**, and [^{125}I]**12**, respectively).

[^{125}I]**9**. The brain uptake of [^{125}I]**12** peaked at 10 min with a maximum brain uptake of 1.76%ID/g, and the washout rate from the brain was relatively slow throughout the time course that was evaluated (1.37%ID/g at 30 min postinjection), which is unsuitable for in vivo imaging. Compound [^{125}I]**12** with the highest log *P* displayed the lowest brain uptake among three radioiodinated styrylchromones, and the brain uptake of three ligands increased in order of their decreasing log *P* value. Although many factors such as molecular size, ionic charge, and lipophilicity affect the brain uptake of a compound, the high lipophilicity of [^{125}I]**12** may be one reason for the low brain uptake of [^{125}I]**12**. Furthermore, this result suggests that the optimal log *P* value for brain uptake of styrylchromone derivatives may be approximately 1.7 or less. Despite the highest binding affinity for Aβ aggregates, [^{125}I]**12** cannot serve as in vivo amyloid imaging due to this unfavorable in vivo pharmacokinetics. In addition to in vivo pharmacokinetics, in vivo stability of a compound is one of important prerequisites for β-amyloid imaging agents. Although the radiometabolites derived from radioiodinated styrylchromone derivatives in the brain and blood have not been investigated in this study, little radioactivity was observed in the stomach and blood in biodistribution studies, indicating that at least these styrylchromone derivatives possess high stabilities against in vivo deiodination in mice.

[^{123}I]IMPY is currently the most promising radioiodinated tracer for detecting amyloid plaques in AD patients.[12,13,20,21] This tracer displays appropriate properties as amyloid imaging agents: high binding affinity for amyloid plaques at 15 nM of K_i value in the in vitro binding assay, and high brain uptake and rapid clearance from the normal brain in animal studies. [^{123}I]IMPY entered the brain rapidly (2.88%ID/organ at 2 min after iv injection in mice) and cleared rapidly from normal mice brains (0.26%ID/organ at 30 min after iv injection in mice). The in vivo pharmacokinetics of the styrylchromone derivatives reported in this study appears inferior to that of IMPY, although their high binding affinities for amyloid plaques are sufficient for in vivo imaging of β-amyloid plaques. Therefore, additional structural changes are essential to further improve the in vivo properties of the styrylchromone derivatives to be suitable for imaging.

3. Conclusion

In conclusion, we reported the synthesis of a new series of styrylchromone derivatives as amyloid imaging agents. These styrylchromone derivatives displayed excellent binding affinities for in vitro binding assay using synthetic Aβ(1–40) aggregates. In biodistribution studies using normal mice, [^{125}I]**6** and [^{125}I]**9** showed a good initial brain penetration and fast washout from the brain, desirable properties for an amyloid plaque-specific imaging agent. However, the slow brain washout observed with [^{125}I]**12** in normal mice made it unsuitable for in vivo plaque imaging. Since styrylchromone backbone with high binding affinity for amyloid plaques is very useful for the development of new amyloid imaging agents, appropriate structural changes on the styrylchromone backbone may lead to useful amyloid imaging agents.

4. Experimental

4.1. General information

All reagents used in syntheses were commercial products and were used without further purification unless otherwise indicated. ^{1}H NMR spectra were obtained on Varian Gemini 300 spectrometer with TMS as an internal standard. Coupling constants are reported in Hertz. The multiplicity is defined by s (singlet), d (doublet), t (triplet), br (broad), and m (multiplet). Mass spectra were obtained on a JEOL IMS-DX instrument.

4.1.1. (*E*)-2-Acetyl-bromophenyl 3-(4-nitrophenyl)acrylate (1). To a stirring solution of trans-4-nitrocinnamoyl chloride (545 mg, 2.58 mmol) in pyridine (10 mL) in an ice bath was added 5′-bromo-2′-hydroxyacetophenone (500 mg, 2.58 mmol). The reaction mixture was stirred at room temperature for 60 min and poured into 1 N aqueous HCl solution with vigorous stirring. The precipitate which formed was filtered and washed with water to yield acetophenone (**1**) (1.07 g, 99.2% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.80 (d, J = 16.2 Hz, 1H), 7.10 (d, J = 8.4 Hz, 2H), 7.95 (d, J = 2.4 Hz, 1H), 7.62–7.77 (m, 4H), 7.95 (d, J = 2.4 Hz, 1H), 8.29 (d, J = 9.0 Hz, 1H).

4.1.2. 1-(5-Bromo-2-hydroxyphenyl)-3-(4-nitrophenyl)pent-4-ene-1,3-dione (2). A solution of acetophenone **1** (500 mg, 6.34 mmol) and pyridine (12 mL) was heated to 50 °C and to it was added pulverized potassium hydroxide (220 mg, 3.84 mmol). The reaction mixture was stirred for 30 min, and upon cooling, 20 mL of 10% aqueous acetic acid solution was added. The pale yellow precipitate which formed was filtered to yield **2** (410 mg, 82.0% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.34 (s, 1H), 6.77 (d, J = 16.2 Hz, 1H), 6.98 (s, 1H), 7.12 (d, J = 5.7 Hz, 2H), 7.71 (m, 3H), 8.27 (d, J = 9.0 Hz, 2H).

4.1.3. 6-Bromo-2-(4′-nitrostyryl)chromone (3). A mixture of the diketone **2** (860 mg, 2.20 mmol), concentrated sulfuric acid (1.2 mL), and glacial acetic acid (15 mL) was heated at reflux for 1 h and cooled to room temperature. The mixture was poured onto crushed ice, and the resulting precipitate was filtered to yield **3** (790 mg, 94.0% yield).

4.1.4. 6-Bromo-2-(4′-aminostyryl)chromone (4). A mixture of **3** (361 mg, 0.97 mmol), $SnCl_2$ (1.28 g, 4.74 mmol), and EtOH (30 mL) was stirred under reflux for 2 h. After the mixture was cooled to room temperature, 1 M NaOH (100 mL) was added until the mixture became alkaline. After extraction with ethyl acetate (100 mL), the combined organic layers was washed with brine, dried over Na_2SO_4, and evaporated to gave 210 mg of **4** (63.3%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.99 (s, 2H), 6.25 (s, 1H), 6.55 (d, J = 15.9 Hz, 1H),

6.68 (d, J = 8.4 Hz, 2H), 7.38–7.47 (m, 4H), 7.71 (d, J = 8.1 Hz, 1H), 8.30 (d, J = 2.4 Hz, 1H).

4.1.5. 6-Tributylstannyl-2-(4′-aminostyryl)chromone (5). A mixture of **4** (242 mg, 0.71 mmol), bis(tributyltin) (0.55 mL), and $(Ph_3P)_4Pd$ (40 mg, 0.034 mmol) in a mixed solvent (20 mL, 3:2 dioxane/triethylamine mixture) was stirred at 90°C for 6 h. The solvent was removed, and the residue was purified by silica gel chromatography (3:2 hexane/ethyl acetate) to give 220 mg of **5** (56.2%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86–1.54 (m, 27H), 4.07 (s, 2H), 6.27 (s, 1H), 6.57 (d, J = 15.9 Hz, 1H), 6.68 (d, J = 8.4 Hz, 2H), 7.42 (d, J = 9.0 Hz, 1H), 7.48–7.54 (m, 3H), 7.74 (d, J = 8.1 Hz, 1H), 8.28 (s, 1H). MS m/z 496 (M^+).

4.1.6. 6-Iodo-2-(4′-aminostyryl)chromone (6). To a solution of **5** (220 mg, 0.40 mmol) in $CHCl_3$ (5 mL) was added a solution of iodine in $CHCl_3$ (3 mL, 0.25 M) at room temperature. The mixture was stirred at room temperature for 10 min, and $NaHSO_3$ solution (15 mL) was added. The mixture was stirred for 5 min, and the organic phase was separated. The aqueous phase was extracted with CH_3Cl, and the combined organic phase was dried over Na_2SO_4 and filtered. The solvent was removed, and the residue was purified by silica gel chromatography (2:1 hexane/ethyl acetate) to give 60 mg of **6** (38.7%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.99 (s, 2H), 6.56 (d, J = 15.9 Hz, 1H), 6.69 (d, J = 8.4 Hz, 2H), 7.27 (d, J = 8.7 Hz, 1H), 7.39–7.53 (m, 3H), 7.89 (m, 1H), 8.50 (d, J = 9.0 Hz, 1H). MS m/z 389 (M^+).

4.1.7. 6-Bromo-2-(4′-methylaminostyryl)chromone (7). To a mixture of **4** (710 mg, 2.07 mmol) and paraformaldehyde (231 mg, 7.70 mmol) in MeOH (15 mL) was added a solution of NaOMe (28 wt% in MeOH, 0.45 mL) dropwise. The mixture was stirred under reflux for 1 h. After adding $NaBH_4$ (270 mg, 7.13 mmol), the solution was heated under reflux for 2 h. To the cold mixture 1 M NaOH (30 mL) was added followed by extraction with $CHCl_2$ (2× 30 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 using chloroform to give 690 mg of **7** (96.0%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.89 (s, 3H), 4.18 (s, 1H), 6.25 (s, 1H), 6.56–6.61 (m, 3H), 7.29–7.45 (m, 4H), 7.71 (m,1H), 8.30 (d, J = 2.4 Hz, 1H). MS m/z 355 (M^+).

4.1.8. 6-Tributylstannyl-2-(4′-methylaminostyryl)chromone (8). The same reaction as described above to prepare **5** was employed, and 204 mg of **8** was obtained in a 42.8% yield from **7**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86–1.52 (m, 27H), 2.89 (s, 3H), 4.11 (s, 1H), 6.26 (s, 1H), 6.57–6.62 (m, 3H), 7.43–7.54 (m, 4H), 7.74 (d, J = 8.1 Hz, 1H), 8.28 (s, 1H). MS m/z 567 (MH^+).

4.1.9. 6-Iodo-2-(4′-methyaminostyryl)chromone (9). The same reaction as described above to prepare **6** was used, and 32 mg of **9** was obtained in a 22.4% yield from **8**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.94 (s, 3H), 4.21 (s, 1H), 6.25 (s, 1H), 6.54 (d, J = 15.6 Hz, 1H), 6.61 (d, J = 8.7 Hz, 2H), 7.28 (d, J = 7.2 Hz, 1H), 7.42–7.59 (m, 3H), 7.94 (d, J = 15.6 Hz, 1H), 8.50 (d, J = 2.1 Hz, 1H). MS m/z 403 (M^+).

4.1.10. 6-Bromo-2-(4′-dimethylaminostyryl)chromone (10). To a stirred mixture of **4** (194 mg, 0.57 mmol) and paraformaldehyde (170 mg, 5.67 mmol) in AcOH (10 mL) was added $NaCNBH_3$ (214 mg, 3.40 mmol) in one portion at room temperature. The resulting mixture was stirred at room temperature for 3 h, 1 M NaOH (50 mL) was added followed by extraction with CH_3Cl (50 mL). The organic phase was dried over Na_2SO_4. The solvent was removed, and the residue was purified by silica gel chromatography using chloroform to give 150 mg of **10** (71.5%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.04 (s, 6H), 6.24 (s, 1H), 6.57 (d, J = 15.9 Hz, 1H), 6.72 (d, J = 2.7 Hz, 2H), 7.39 (d, J = 8.7 Hz, 1H), 7.46–7.56 (m, 3H), 7.71 (d, J = 7.8 Hz, 1H), 8.31 (d, J = 2.4 Hz, 1H). MS m/z 371 (MH^+).

4.1.11. 6-Tributylstannyl-2-(4′-dimethylaminostyryl)chromone (11). The same reaction as described above to prepare **5** was employed, and 204 mg of **11** was obtained in a 37.4% yield from **10**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86–1.74 (m, 27H), 3.04 (s, 6H), 6.27 (s, 1H), 6.54 (d, J = 15.9 Hz, 1H), 6.69 (d, J = 8.7 Hz, 2H), 7.42 (m, 1H), 7.47–7.59 (m, 3H), 7.74 (d, J = 8.1 Hz, 1H), 8.23 (s, 1H). MS m/z 580 (M^+).

4.1.12. 6-Iodo-2-(4′-dimethyaminostyryl)chromone (12). The same reaction as described above to prepare **6** was used, and 18 mg of **12** was obtained in a 71.8% yield from **11**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.04 (s, 6H), 6.25 (s, 1H), 6.56 (d, J = 15.9 Hz, 1H), 6.65 (d, J = 8.7 Hz, 2H), 7.23 (d, J = 8.1 Hz, 1H), 7.44–7.59 (m, 3H), 7.94 (d, J = 8.7 Hz, 1H), 8.45 (s, 1H). MS m/z 417 (MH^+).

4.2. Iododestannylation reaction

The radioiodinated forms of compounds **6**, **9**, and **12** were prepared from the corresponding tributyltin derivatives by an iododestannylation. Briefly, to initiate the reaction 50 μL H_2O_2 (3%) was added to a mixture of a tributyltin derivative (50 μg/50 μL EtOH), [^{125}I]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 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 H_2O/acetonitrile (3:7) 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 Aβ peptide in solution

A solid form of Aβ(1–40) was purchased from Peptide Institute (Osaka, Japan). Aggregation of peptides was carried out by gently dissolving the peptide (0.5 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 before[22] with some modification. For saturation studies, a solution of [^{125}I]**6**, [^{125}I]**9**, and [^{125}I]**12** (final concentration, 1–125 nM) was prepared by mixing nonradioactive **6**, **9**, and**12**. Nonspecific binding was defined in the presence of 1 μM nonradioactive**12**. The binding assay was performed by mixing 50 μL Aβ(1–40) aggregates (57 nM in the final assay mixture), an appropriate concentration of 50 μL of [^{125}I]**6**, [^{125}I]**9**, and [^{125}I]**12**, 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 compounds **6**, **9**, and **12** was determined by Scatchard analysis using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA).

4.4. Partition coefficient determination

Partition coefficients were measured by mixing the radioiodinated tracers with 3 mL each of 1-octanol and buffer (0.1 M phosphate, pH 7.4) in a test tube. The test tube was vortexed for 3 min at room temperature, followed by centrifugation for 5 min. Two weighed samples (0.5 mL each) from the 1-octanol and buffer layers were counted in a well counter. The partition coefficient was determined by calculating the ratio of cpm/0.5 mL of the 1-octanol to that of buffer. A sample from the octanol layer was re-partitioned with the same volume of buffer until consistent partition coefficient values were obtained. The measurement was done in triplicate and repeated three times.

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 μL) containing ethanol (10 μL) of radiolabeled agents (0.5 μCi) was injected directly into the tail vein of ddY mice (5-week-old, 22–25 g). The mice were sacrificed at various time points post injection. The organs of interest were removed and weighed, and the radioactivity was counted with an automatic gamma counter (Aloka 3000).

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