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Structure–activity relationship of chalcones and related derivatives as ligands for detecting of β-amyloid plaques in the brain

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Abstract—A series of novel chalcones and their related derivatives were synthesized and evaluated as β-amyloid imaging probes. In the structure–activity relationship of binding affinities to synthetic Aβ(1–42) aggregates, compound **14** displayed the highest binding affinity in vitro. β-Amyloid plaques in the Alzheimer's model mouse brain were visualized with **14**. In biodistribution studies using normal mice, [^{125}I]**14** showed good brain uptake (2.56% ID/g, 2 min postinjection) and rapid washout from the brain (0.21% ID/g, 60 min postinjection). These results suggest that [^{125}I]**14** should be further investigated as a potentially useful β-amyloid imaging probe.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder pathologically characterized by deposition of β-amyloid (Aβ) peptides as senile plaques in the brain.[1,2] Since the deposition of β-amyloid plaques is an early event in the development of AD, a validated biomarker of β-amyloid deposition in the brain would likely prove useful to identify and follow individuals at risk for AD and to assist in the evaluation of new anti-amyloid therapies currently under development.[3–5]

A number of groups have reported radiolabeled β-amyloid imaging agents for positron emission tomography (PET) and single photon emission computed tomography (SPECT) such as [^{18}F]FDDNP,[6–8] [^{11}C]PIB,[9,10] [^{11}C]SB-13,[11,12] [^{123}I]IMPY,[13–15] and [^{11}C]BF-227.[16] Recent reports using these amyloid imaging agents have indicated that detecting β-amyloid plaques in the living human brain by PET and SPECT may lead to differentiation between AD patients and healthy human.

Recently, in an attempt to search for novel amyloid imaging probes, we found that radioiodinated flavone[17] and chalcone[18] derivatives, which are categorized as flavonoids, showed excellent characteristics as new amyloid imaging agents, such as high binding affinity to Aβ aggregates and high uptake into the brain and rapid clearance from the brain (Fig. 1). Especially, the chalcone backbone structure is considered to be a useful core in the development of new amyloid imaging probes because it can easily be formed by one-pot condensation reaction.

In the present study, we designed and synthesized novel chalcone derivatives and related chalcone-like compounds, and evaluated their structure–activity relationship on the binding affinity to β-amyloid aggregates and in vivo biodistribution using a compound with high binding affinity.

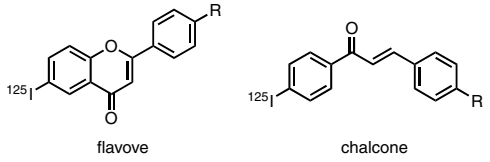

Figure 1. Chemical structures of radioiodinated flavones and chalcones (R = NH_2, NHMe, NMe_2).

Keywords: Alzheimer's disease; β-Amyloid plaque; PET; SPECT; Imaging.
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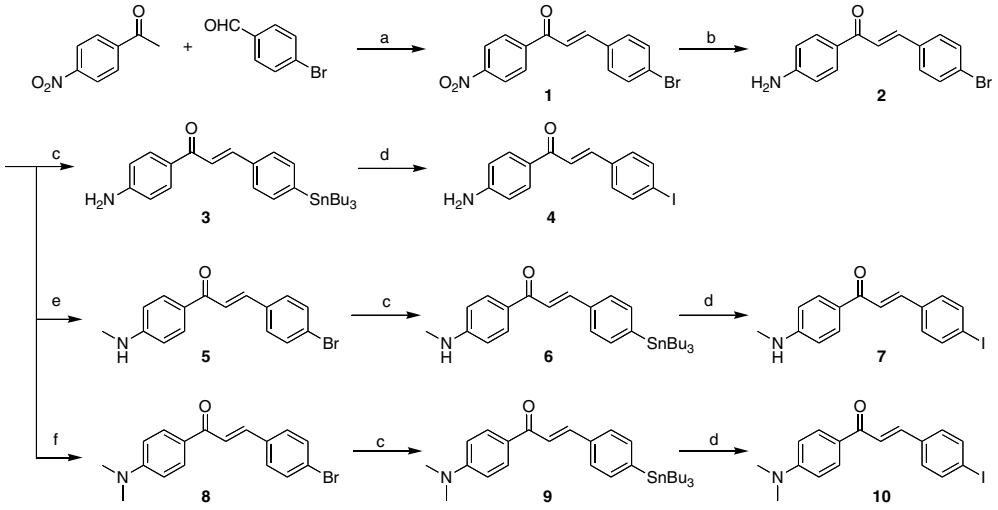
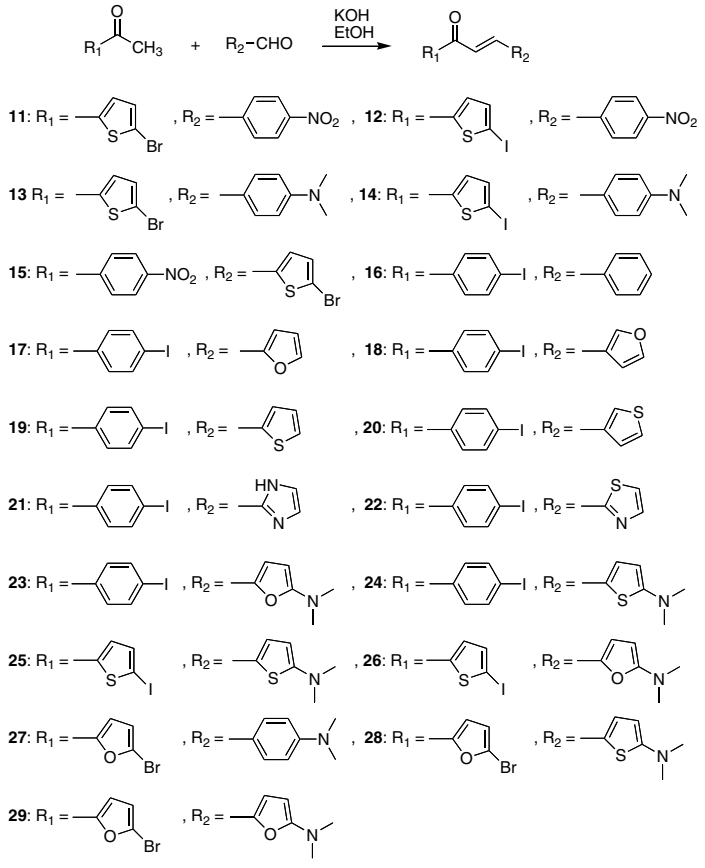
2. Results and discussion

2.1. Chemistry

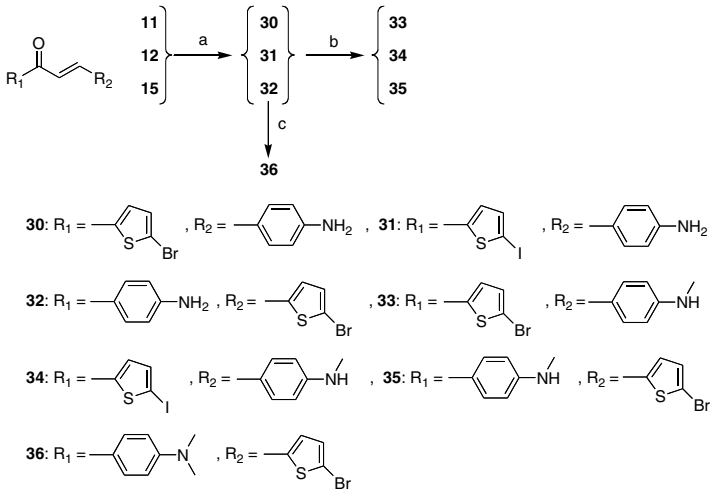
The syntheses of chalcone and chalcone-like compounds are outlined in Schemes 1–3. Chalcone and chalcone-like compounds were prepared by base-catalyzed condensation of appropriately substituted ketones with substituted benzaldehydes or heterocyclic aldehydes. In this process, the substituted ketones were reacted with the substituted benzaldehyde or heterocyclic aldehydes in the presence of 10% aqueous KOH and ethanol at room temperature to form the target chalcone and chalcone-like compounds (**1** and **11**–**29**). The nitro derivatives (**1**, **11**, **12**, and **15**) were converted to amino derivatives (**2**, **30**, **31**, and **32**) by reduction with $SnCl_2$. Conversion



Scheme 1. Reagents: (a) EtOH, KOH; (b) EtOH, $SnCl_2$; (c) dioxane, $(Bu_3Sn)_2$, $(Ph_3P)_4Pd$, Et_3N; (d) $CHCl_3$, I_2; (e) CH_3I, K_2CO_3, DMSO; (f) AcOH, $(CH_2O)_n$, $NaCNBH_3$.



Scheme 2.

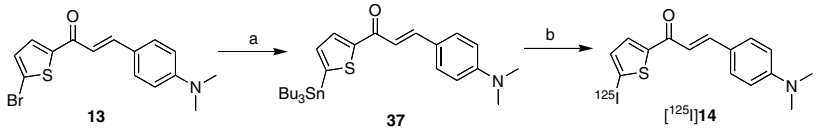


Scheme 3. Reagents: (a)EtOH, $SnCl_2$; (b) CH_3I, K_2CO_3, DMSO; (c) AcOH, $(CH_2O)_n$, $NaCNBH_3$.

of the amino derivatives (**2**, **30**, **31**, and **32**) to the monomethylamino derivatives (**5**, **33**, **34**, and **35**) was achieved by a methylation with CH_3I under alkaline conditions. The amino derivatives (**2** and **32**) were also converted to the dimethylamino derivatives (**8** and **36**) by an efficient method[19] with paraformaldehyde, sodium cyanoborohydride, and acetic acid. The tin compounds (**3**, **6**, **9**, and **37**) were prepared from the corresponding bromo compounds (**2**, **5**, **8**, and **13**) using a bromo-to-tributyltin exchange reaction catalyzed by Pd(0). The tributyltin derivatives (**3**, **6**, and **9**) were readily reacted with iodine in $CHCl_3$ at room temperature to give the iodo derivatives (**4**, **7**, and **10**). The tributyltin derivative **37** was used as the starting materials for radioiodination in preparation of [^{125}I]**14**. Novel radioiodinated ligand was achieved by an iododestannylation reaction using hydrogen peroxide as the oxidant, which produced the desired radioiodinated ligand (Scheme 4). 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 identity of [^{125}I]**14** was verified by co-injection with nonradioactive compound by its HPLC profiles. The [^{125}I]**14** showed a single radioactivity peak at the retention time of 18.2 min. The radioiodinated ligand was obtained in 60–70% radiochemical yield with radiochemical purities of >95% after purification by HPLC.

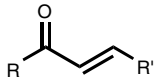
2.2. Binding studies in vitro

(*E*)-4-Dimethylamino-4′-[^{125}I]iodo-chalcone ([^{125}I]DMIC) was synthesized and used as the radioligand for competition binding experiments (K_d value of [^{125}I]DMIC is 4.2 nM).[18] Binding affinities of chalcone and chalcone-like compounds were evaluated with inhibition assays against [^{125}I]DMIC binding on Aβ(1–42) aggregates (Table 1). These K_i values suggested that the new series of chalcone and chalcone-like compounds had high binding affinity for Aβ(1–42) aggregates in the order of *N*,*N*-dimethylated derivatives (**10** and **14**) > *N*-monomethylated derivatives (**7** and **34**) > primary amino derivatives (**4** and **31**) when comparing in the similar core structure. Compounds **4**, **7** and **10** with the substituted group at 4′ position and iodine at 4 position displayed higher K_i values (lower binding affinities) as compared to compounds, 4-amino-4′-iodo-chalcone (AIC), 4′-iodo-4-methylamino-chalcone (IMC) and DMIC, which have the substituted group and iodine at the inverse position against compounds **4**, **7**, and **10**. The K_i values of **32**, **35**, and **36** with the thienyl group at R position and phenyl group at R′ position were higher than those of **31**, **34**, and **14** with the phenyl group at R position and the thienyl group at R′ position, indicating that the binding affinities depended on the combination of heterocycles introduced at R and R′ position, not on the position of the substituted group or iodine (bromine) group. When comparing the K_i values of heterocyclic compounds with the same substituted group, the binding affinities increased in the order of phenyl > thienyl > furanyl at R position and phenyl = thienyl > furanyl at R′ position. The K_i values of compounds without the substituted group on the ring at R′ position (**16**–**22**) were varied by altering the kind of heterocycles. Furthermore, in order to obtain information on the binding site of the new chalcone-like compounds, inhibition studies were carried out using Congo Red (CR) and thioflavin T (ThT), which are well



Scheme 4. Reagents: (a) dioxane, $(Bu_3Sn)_2$, $(Ph_3P)_4Pd$, Et_3N; (b) [^{125}I]NaI, HCl, H_2O_2.

Table 1. Inhibition constants of chalcone and chalcone-like derivatives on ligand binding to Aβ(1–42) aggregates



Compound	R	R′	K_i[a] (nM)
4	4-Aminophenyl	4-Iodophenyl	248 ± 56
7	4-Methylaminophenyl	4-Iodophenyl	23.9 ± 3.6
10	4-Dimethylaminophenyl	4-Iodophenyl	13.3 ± 1.9
14	5-Iodo-2-thienyl	4-Dimethylaminophenyl	3.9 ± 0.4
16	4-Iodophenyl	Phenyl	151 ± 16
17	4-Iodophenyl	2-Furanyl	908 ± 212
18	4-Iodophenyl	3-Furanyl	125 ± 9.2
19	4-Iodophenyl	2-Thienyl	102 ± 16
20	4-Iodophenyl	3-Thienyl	93 ± 11
21	4-Iodophenyl	2-Imidazoyl	797 ± 316
22	4-Iodophenyl	2-Thiazoyl	>10,000
23	4-Iodophenyl	5-Dimethylamino-2-furanyl	1132 ± 344
24	4-Iodophenyl	5-Dimethylamino-2-thienyl	113 ± 10
25	5-Iodo-2-thienyl	5-Dimethylamino-2-thienyl	137 ± 3.4
26	5-Iodo-2-thienyl	5-Dimethylamino-2-furanyl	1608 ± 85
27	5-Bromo-2-furanyl	4-Dimethylaminophenyl	126 ± 13
28	5-Bromo-2-furanyl	5-Dimethylamino-2-thienyl	2648 ± 222
29	5-Bromo-2-furanyl	5-Dimethylamino-2-furanyl	>10,000
31	5-Iodo-2-thienyl	4-Aminophenyl	121 ± 40
32	4-Aminophenyl	5-Bromo-2-thienyl	476 ± 48
34	5-Iodo-2-thienyl	4-Methylaminophenyl	14.1 ± 0.6
35	4-Methylaminophenyl	5-Bromo-2-thienyl	198 ± 49
36	4-Dimethylaminophenyl	5-Bromo-2-thienyl	106 ± 7.1
CR	—	—	>10,000
ThT	—	—	>10,000
AIC[b]	4-Iodophenyl	4-Aminophenyl	105 ± 12
IMC[c]	4-Iodophenyl	4-Methylaminophenyl	6.3 ± 1.6
DMIC[d]	4-Iodophenyl	4-Dimethylaminophenyl	2.9 ± 0.3

[b,c,d]Data from Ref. 18.

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

[b] 4-Amino-4′-iodo-chalcone.

[c] 4′-Iodo-4-methylamino-chalcone.

[d] 4-Dimethylamino-4′-iodo-chalcone.

known as prototypes of amyloid imaging probes.[4,5] While compound **14** competed for [^{125}I]DMIC binding to Aβ(1–42) aggregates, CR and ThT did not exhibit a dose-dependent decrease in the specific binding of [^{125}I]DMIC (Fig. 2). This result suggests that the binding site of a series of chalcone-like compounds on Aβ(1–42) aggregates may be different from that of CR and ThT.

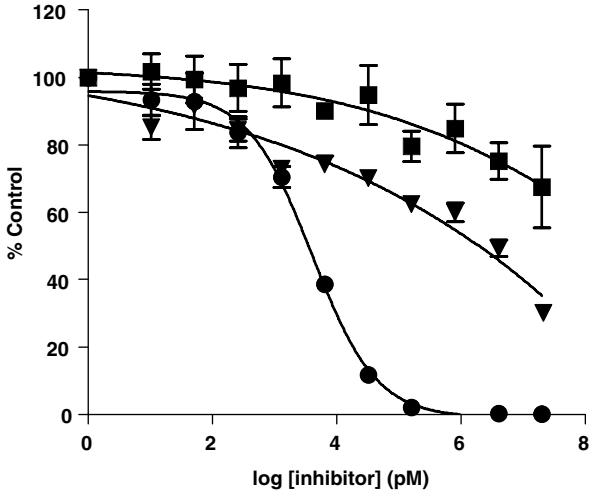


Figure 2. Competition curves of [^{125}I]DMIC against compound **14** (closed circle), Congo Red (closed square), and thioflavin T (closed triangle).

2.3. Neuropathological staining on AD model mouse brain sections

In order to confirm the binding affinity to β-amyloid plaques in the AD brain, fluorescent staining on AD model mouse brain sections was carried out using the fluorescence of compound **14** (Fig. 3). Compound **14** intensely stained β-amyloid plaques in the brain sections. Also, clear staining of cerebrovascular amyloids was observed. This result suggests that compound **14** should detect β-amyloid plaques in the AD brains.

2.4. Biodistribution studies

The radioiodinated compound [^{125}I]**14** was evaluated for its in vivo biodistribution in normal mice (Table 2). A biodistribution study provides important information on brain uptake. The ideal β-amyloid imaging probe should have good blood–brain barrier penetration to de-

Table 2. Biodistribution of radioactivity after intravenous administration of [^{125}I]**14** in mice[a]

Time after Injection (min)			
2	10	30	60
2.46 (0.30)	0.75 (0.31)	0.31 (0.04)	0.21 (0.02)

[a] Expressed as % injected dose per gram. Each value represents the mean (SD) for five mice at each interval.

liver a sufficient dose into the brain while achieving rapid clearance from the normal regions to result in a higher signal to noise ratio in the AD brain. Initial brain uptake of [^{125}I]**14** was 2.46% of injected dose/gram at 2 min post iv injection, whereas the radioactivity accumulated in the brain was rapidly eliminated (0.21% of injected dose/gram, 60 min post iv injection), indicating highly desirable properties for β-amyloid imaging agents.

3. Conclusion

In conclusion, we successfully designed and synthesized a series of chalcone and related compounds to evaluate their structure–activity relationship of the binding affinity to Aβ aggregates. In in vitro binding studies with Aβ aggregates, a variety of K_i values were found to be inherent to their structure. Compound **14** with the highest binding affinity to Aβ aggregates clearly stained β-amyloid plaques and cerebrovascular amyloids as reflected in in vitro binding studies. Taken together, the data suggest that the new radioiodinated compound **14** should be further investigated as a potentially useful β-amyloid imaging probe.

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

4.1.1. (*E*)-3-(4-Bromophenyl)-1-(4-nitrophenyl)-2-propen-1-one (1). Equimolar portions of 4-nitroacetophenone (1.67 g, 10.1 mmol) and 4-bromobenzaldehyde (1.86 g, 10.0 mmol) were dissolved in ethanol (10 mL). A 6 mL aliquot of 10% aqueous potassium hydroxide solution was then slowly added dropwise to the reaction mixture. The mixture was allowed to stir for 30 min at room temperature. A precipitate was collected and washed with ethyl acetate to give 1.98 g of **1** (59.4%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (d, J = 15.6 Hz, 1H), 7.52 (d, J = 8.7 Hz, 2H), 7.59 (d, J = 8.7 Hz, 2H), 7.79 (d, J = 15.6 Hz, 1H), 8.15 (d, J = 9 Hz, 2H), 8.36 (d, J = 9 Hz, 2H).

4.1.2. (*E*)-1-(4-Aminophenyl)-3-(4-bromophenyl)-2-propen-1-one (2). A mixture of **1** (372 mg, 1.12 mmol), $SnCl_2$ (1.05 g, 5.55 mmol), and ethanol (5 mL) was stirred under reflux for 1 h. After the mixture was cooled to room temperature, 1 M NaOH (10 mL) was added and extracted with ethyl acetate (10 mL). The organic phase was dried over Na_2SO_4 and filtered. The filtrate was concentrated to give 190 mg of **2** (56.1%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.19 (s, 2H), 6.70 (d, J = 8.7 Hz, 2H), 7.47–7.55 (m, 5H), 7.71 (d, J = 15.6 Hz, 1H), 7.93 (d, J = 8.7 Hz, 2H).

4.1.3. (*E*)-1-(4-Aminophenyl)-3-(4-tributylstannylphenyl)-2-propen-1-one (3). A mixture of **2** (200 mg, 0.66 mmol), $(Bu_3Sn)_2$ (0.4 mL), and $(Ph_3P)_4Pd$ (35 mg, 0.03 mmol) in a mixed solvent (16 mL, 5:3 dioxane/triethylamine mixture) was stirred under reflux for 8 h. The solvent was removed, and the residue was purified by preparative TLC (1:1 hexane/ethyl acetate) to give 165 mg of **3** (48.7%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87–1.52 (m, 27H), 4.23 (s,2H), 6.68 (d, J = 8.7 Hz, 2H), 7.50–7.58 (m, 5H), 7.76 (d, 15.3 Hz, 1H), 7.92 (d, J = 8.4 Hz, 2H). MS m/z 513 (MH^+).

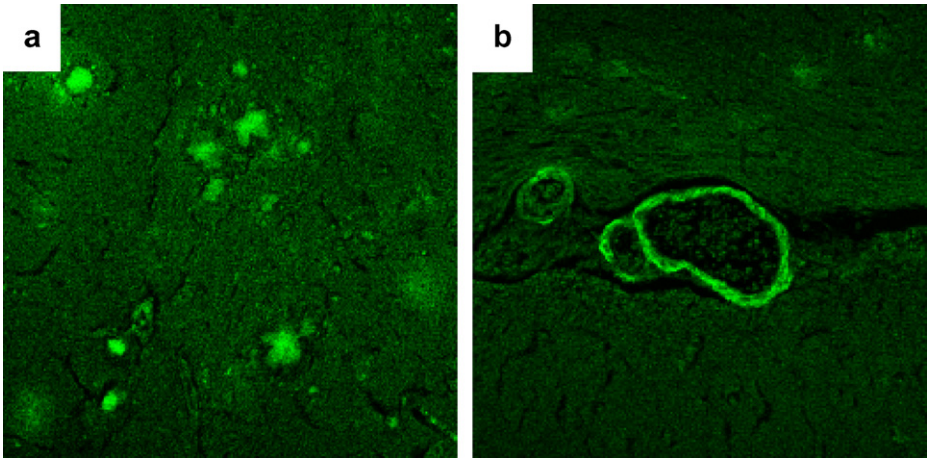


Figure 3. Neuropathological fluorescent staining of compound **14** on AD model mouse brain sections. (a) Compound **14** intensely stained β-amyloid plaques. (b) Clear staining of cerebrovascular amyloids was also observed.

4.1.4. (*E*)-1-(4-Aminophenyl)-3-(4-iodophenyl)-2-propen-1-one (4). To a solution of **3** (90 mg, 0.18 mmol) in $CHCl_3$ (5 mL) was added a solution of iodine in $CHCl_3$ (2 mL, 0.25 M) at room temperature. The mixture was stirred at room temperature for 20 min, and saturated $NaHSO_3$ solution was added. After the organic phase was separated, dried over Na_2SO_4, and filtered, the solvent was removed, and the residue was purified by preparative TLC (1:1 hexane/ethyl acetate) to give 39 mg of **4** (63.6%). ^1H NMR (300 MHz, $CDCl_3$) δ 4.17 (s, 2H), 6.70 (d, 8.4 Hz, 2H), 7,36 (d, J = 8.7 Hz, 2H), 7.54 (d, J = 15.6 Hz, 1H), 7.69 (d, J = 15.6 Hz, 1H), 7.74 (d, J = 8.4 Hz, 2H), 7.92 (d, J = 8.4 Hz, 2H). MS m/z 349 (M^+).

4.1.5. (*E*)-3-(4-Bromophenyl)-1-(4-methylaminophenyl)-2-propen-1-one (5). To a solution of **2** (230 mg, 0.76 mmol) in DMSO (5 mL) were added methyl iodide (0.2 mL) and anhydrous K_2CO_3 (526 mg, 3.81 mmol). The reaction mixture was stirred at room temperature for 5 h. After it was poured into water (50 mL), the mixture was extracted with ethyl acetate (50 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 = 3:1) to give 78 mg of **5** (32.4%). ^1H NMR (300 MHz, $CDCl_3$) δ 2.89–2.94 (m, 3H), 4.36 (s, 1H), 6.61 (d, J = 8.7 Hz, 2H), 7.50–7.57 (m, 3H), 7.70 (d, J = 15.6 Hz, 1H), 7.84 (d, J = 8.4 Hz, 2H), 7.96 (d, J = 8.7 Hz, 2H).

4.1.6. (*E*)-1-(4-Methylaminophenyl)-3-(4-tributylstannylphenyl)-2-propen-1-one (6). The same reaction as described above to prepare **3** was used, and 70 mg of **6** was obtained in a 38.2% yield from **5**. ^1H NMR (300 MHz, $CDCl_3$) δ 0.87–1.59 (m, 27H), 2.93 (d, J = 5.1 Hz, 3H), 4.31 (s, 1H), 6.61 (d, J = 8.7 Hz, 2H), 7.50–7.60 (m, 5H), 7.77 (d, J = 15.6 Hz, 1H), 7.97 (d, J = 9 Hz, 2H). MS m/z 527 (MH^+).

4.1.7. (*E*)-3-(4-Iodophenyl)-1-(4-methylaminophenyl)-2-propen-1-one (7). The same reaction as described above to prepare **4** was used, and 16 mg of **7** was obtained in a 38.2% yield from **6**. ^1H NMR (300 MHz, $CDCl_3$) δ 2.93 (d, J = 4.5 Hz, 3H), 4.35 (s, 1H), 6.61 (d, J = 9 Hz, 2H), 7.36 (d, J = 8.4 Hz, 2H), 7.56 (d, J = 15.6 Hz, 1H), 7.68 (d, J = 16.2 Hz, 1H), 7.74 (d, J = 8.1 Hz, 2H), 7.96 (d, J = 9 Hz, 2H). MS m/z 363 (M^+).

4.1.8. (*E*)-3-(4-Bromophenyl)-1-(4-dimethylaminophenyl)-2-propen-1-one (8). To a stirred mixture of **2** (300 mg, 0.99 mmol) and paraformaldehyde (315 mg, 10.5 mmol) in AcOH (15 mL) was added in one portion $NaCNBH_3$ (300 mg, 4.77 mmol) at room temperature. The resulting mixture was stirred at room temperature for 4 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 = 4:1) to give 150 mg of **8** (45.7%). ^1H NMR (300 MHz, $CDCl_3$) δ 3.09 (s, 6H), 6.71 (d, J = 9 Hz, 2H), 7.36 (d, J = 8.4 Hz 2H), 7.59 (d, J = 15.3 Hz, 1H), 7.69 (d, J = 15.9 Hz, 1H), 7.74 (d, J = 8.4 Hz, 2H), 8.00 (d, J = 9 Hz, 2H). MS m/z 377 (M^+).

4.1.9. (*E*)-1-(4-Dimethylaminophenyl)-3-(4-tributylstannylphenyl)-2-propen-1-one (9). The same reaction as described above to prepare **3** was used, and 36 mg of **9** was obtained in a 31.4% yield from **8**. ^1H NMR (300 MHz, $CDCl_3$) δ 0.87–1.66 (m, 27H), 3.09 (s, 6H), 6.71 (d, J = 8.7 Hz, 2 H), 7.34–7.62 (m, 5H), 7.77(d, J = 15.9 Hz, 1H), 8.01(d, J = 9 Hz, 2H). MS m/z 541 (MH^+).

4.1.10. (*E*)-1-(4-Dimethylaminophenyl)-3-(4-iodophenyl)-2-propen-1-one (10). The same reaction as described above to prepare **4** was used, and 11 mg of **10** was obtained in a 38.2% yield from **9**. ^1H NMR (300 MHz, $CDCl_3$) δ 3.09 (s, 6H), 6.71 (d, J = 9 Hz, 2H), 7.36 (d, J = 8.4 Hz, 2H), 7.56 (d, J = 15.6 Hz, 1H), 7.68 (d, J = 16.2 Hz, 1H), 7.74 (d, J = 8.1 Hz, 2H), 7.96 (d, J = 9.0 Hz, 2H). MS m/z 363 (M^+).

4.1.11. (*E*)-1-(5-Bromo-2-thienyl)-3-(4-nitrophenyl)-2-propen-1-one (11). The same reaction as described above to prepare **1** was used, and 686 mg of **11** was obtained in a 66.8% yield from 2-acetyl-5-bromothiophene and 4-nitrobenzaldehyde. ^1H NMR (300 MHz, $CDCl_3$) δ 7.19 (d, J = 3.9 Hz, 1H), 7.48 (d, J = 15.6 Hz, 1H), 7.63 (d, J = 3.9 Hz, 1H), 7.78 (d, J = 8.4 Hz, 2H), 7.85 (d, J = 15.6 Hz, 1H), 8.29 (d, J = 8.7 Hz, 2H).

4.1.12. (*E*)-1-(5-Iodo-2-thienyl)-3-(4-nitrophenyl)-2-propen-1-one (12). The same reaction as described above to prepare **1** was used, and 625 mg of **12** was obtained in a 84.1% yield from 2-acetyl-5-iodothiophene and 4-nitrobenzaldehyde. ^1H NMR (300 MHz, $CDCl_3$) δ 7.38–7.43 (m, 2H), 7.51 (d, J = 4.2 Hz, 1H), 7.78 (d, J = 8.4 Hz, 2H), 7.84 (d, J = 15.6 Hz, 1H), 8.29 (d, J = 9 Hz, 2H).

4.1.13. (*E*)-1-(5-Bromo-2-thienyl)-3-(4-dimethylaminophenyl)-2-propen-1-one (13). The same reaction as described above to prepare **1** was used, and 565 mg of **13** was obtained in a 82.8% yield from 2-acetyl-5-bromothiophene and 4-dimethylaminobenzaldehyde. ^1H NMR (300 MHz, $CDCl_3$) δ 3.05 (s, 6H), 6.69 (d, J = 8.7 Hz, 2H), 7.12 (d, J = 15.3 Hz, 1H), 7.13 (d, J = 3.9 Hz, 1H), 7.55–7.57 (m, 3H), 7.18 (d, J = 15.3 Hz, 1H). MS m/z 337 (MH^+).

4.1.14. (*E*)-1-(5-Iodo-2-thienyl)-3-(4-dimethylaminophenyl)-2-propen-1-one (14). The same reaction as described above to prepare **1** was used, and 274 mg of **14** was obtained in a 69.3% yield from 2-acetyl-5-iodothiophene and 4-dimethylaminobenzaldehyde. ^1H NMR (300 MHz, $CDCl_3$) δ 3.05 (s, 6H), 6.79 (d, J = 8.7 Hz, 2H), 7.11 (d, J = 15.3 Hz, 1H), 7.32 (d, J = 3.9 Hz, 1H), 7.45 (d, J = 3.9 Hz, 1H), 7.54 (d, J = 8.7 Hz, 2H), 7.81 (d, J = 15 Hz, 1H). MS m/z 383 (M^+).

4.1.15. (*E*)-3-(5-Bromo-2-thienyl)-1-(4-nitrophenyl)-2-propen-1-one (15). The same reaction as described above to prepare **1** was used, and 422 mg of **15** was obtained in a 41.2% yield from 4-nitroacetophenone and 5-bromothi-

ophene-2-carboxaldehyde. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.09 (d, J = 3.9 Hz, 1H), 7.14–7.20 (m, 2H), 7.86 (d, J = 15.6 Hz, 1H), 8.12 (d, J = 8.7 Hz, 2H), 8.35 (d, J = 8.7 Hz, 2H).

4.1.16. (*E*)-4′-Iodochalcone (16). The same reaction as described above to prepare **1** was used, 293 mg of **16** was obtained in a 87.4% yield from 4′-iodoacetophenone and benzaldehyde. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42–7.44 (m, 3H), 7.47 (d, J = 15.6 Hz, 1H), 7.63–7.66 (m, 2H), 7.74 (d, J = 8.4 Hz, 2H), 7.82 (d, J = 15.9 Hz, 1H), 7.88 (d, J = 8.7 Hz, 2H). MS m/z 334 (M^+).

4.1.17. (*E*)-3-(2-Furanyl)-1-(4-iodophenyl)-2-propen-1-one (17). The same reaction described above to prepare **1** was used, and 212 mg of **17** was obtained in a 65.4% yield from 4-iodoacetophene and 2-furaldehyde. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.52–6.54 (m, 1H), 6.74 (d, J = 3.6 Hz, 1H), 7.39 (d, J = 15.0 Hz, 1H), 7.54 (d, J = 1.8 Hz, 1H), 7.60 (d, J = 15.6 Hz, 1H), 7.74 (d, J = 8.4 Hz, 2H), 7.86 (d, J = 8.4 Hz, 2H). MS m/z 324 (M^+).

4.1.18. (*E*)-3-(3-Furanyl)-1-(4-iodophenyl)-2-propen-1-one (18). The same reaction as described above to prepare **1** was used, and 279 mg of **18** was obtained in a 86.1% yield from 4-iodoacetophenone and 3-furaldehyde. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.70 (d, J = 1.8 Hz, 1H), 7.17 (d, J = 15.3 Hz, 1H), 7.48 (s, 1H), 7.69–7.75 (m, 4H), 7.86 (d, J = 9.0 Hz, 2H). MS m/z 324 (M^+).

4.1.19. (*E*)-1-(4-Iodophenyl)-3-(2-thienyl)-2-propen-1-one (19). The same reaction as described above to prepare **1** was used, and 546 mg of **19** was obtained in a 79.1% yield from 4-iodoacetophenone and 2-thiophenecarboxaldehyde. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.09–7.12 (m, 1H), 7.26 (d, J = 15.0 Hz, 1H), 7.38 (d, J = 3.6 Hz, 1H), 7.44 (d, J = 5.4 Hz, 1H), 7.18 (d, J = 8.7 Hz, 2H), 7.87 (d, J = 8.4 Hz, 2H), 7.95 (d, J = 15.3 Hz, 1H). MS m/z 340 (M^+).

4.1.20. (*E*)-1-(4-Iodophenyl)-3-(3-thienyl)-2-propen-1-one (20). The same reaction as described above to prepare **1** was used, and 295 mg of **20** was obtained in a 86.7% yield from 4-iodoacetophenone and thiophene-3-carboxaldehyde. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 (d, J = 15.6 Hz, 1H), 7.38–7.43 (m, 2H), 7.62–7.65 (m, 1H), 7.71 (d, J = 8.7 Hz, 2H), 7.80 (d, J = 15.6 Hz, 1H), 7.87 (d, J = 8.4 Hz, 1H). MS m/z 340 (M^+).

4.1.21. (*E*)-1-(4-Iodophenyl)-3-(1H-imidazol-2-yl)-2-propen-1-one (21). The same reaction as described above to prepare **1** was used, and after purification by silica gel chromatography (1:1 hexane/ethyl acetate), 142 mg of **21** was obtained in a 43.8% yield from 4-iodoacetophenone and 2-imidazolecarboxaldehyde. MS m/z 324 (M^+).

4.1.22. (*E*)-1-(4-Iodophenyl)-3-(thiazol-2-yl)-2-propen-1-one (22). The same reaction as described above to prepare **1** was used, and 210 mg of **22** was obtained in a 61.6% yield from 4-iodoacetophenone and 2-thiazolecarboxaldehyde. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, J = 3.0 Hz, 1H), 7.75–7.79 (m, 3H), 7.85–7.92 (m, 3H), 7.99 (d, J = 3.6 Hz, 1H). MS m/z 341 (M^+).

4.1.23. (*E*)-1-(4-Iodophenyl)-3-(5-dimethylamino-2-furanyl)-2-propen-1-one (23). The same reaction as described above to prepare **1** was used, and after purification by silica gel chromatography (2:1 hexane/ethyl acetate), 25 mg of **23** was obtained in a 6.8% yield from 4-iodoacetophenone and 5-dimethylamino-2-furaldehyde.[20] ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.04 (s, 6H), 5.22 (d, J = 3.9 Hz, 1H), 6.80 (d, J = 3.6 Hz, 1H), 6.89 (d, J = 15.0 Hz, 1H), 7.46 (d, J = 14.7 Hz, 1H), 7.70 (d, J = 8.1 Hz, 2H), 7.81 (d, J = 8.7 Hz, 2H). MS m/z 367 (M^+).

4.1.24. (*E*)-3-(5-Dimethylamino-2-thienyl)-1-(4-iodophenyl)-2-propen-1-one (24). The same reaction as described above to prepare **1** was used, and after purification by silica gel chromatography (4:1 hexane/ethyl acetate), 18 mg of **24** was obtained in a 4.7% yield from 4-iodoacetophenone and 5-dimethylamino-2-thiophenecarboxaldehyde.[20] ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.04 (s, 6 H), 5.22 (d, J = 3.9 Hz, 1H), 6.80 (d, J = 3.6 Hz, 1H), 6.89 (d, J = 15.0 Hz, 1H), 7.46 (d, J = 14.7 Hz, 1H), 7.70 (d, J = 8.1 Hz, 2H), 7.81 (d, J = 8.7 Hz, 2H). MS m/z 383 (M^+).

4.1.25. (*E*)-3-(5-Dimethylamino-2-thienyl)-1-(5-iodo-2-thienyl)-2-propen-1-one (25). The same reaction as described above to prepare **1** was used, and after purification by silica gel chromatography (4:1 hexane/ethyl acetate), 15 mg of **25** was obtained in 7.9% yield from 2-acetyl-5-iodothiophene and 5-dimethylamino-2-thiophenecarboxaldehyde. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.07 (s, 6H), 5.85 (d, J = 4.2 Hz, 1H), 6.63 (d, J = 14.7 Hz, 1H), 7.15 (d, J = 4.5 Hz, 1H), 7.29 (d, J = 3.9 Hz, 1H), 7.38 (d, J = 4.2 Hz, 1H), 7.87 (d, J = 14.7 Hz, 1H). MS m/z 389 (M^+).

4.1.26. (*E*)-3-(5-Dimethylamino-2-furyl)-1-(5-iodo-2-thienyl)-2-propen-1-one (26). The same reaction as described above to prepare **1** was used, and after purification by silica gel chromatography (3:1 hexane/ethylacetate), 21 mg of **26** was obtained in a 11.5% yield from 2-acetyl-5-iodothiophene and 5-dimethylamino-2-furaldehyde. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.04 (s, 6H), 5.22 (d, J = 3.6 Hz, 1H), 6.73 (d, J = 14.4 Hz, 1H), 6.79 (d, J = 3.9 Hz, 1H), 7.29 (d, J = 3.9 Hz, 1H), 7.50 (d, J = 3.9 Hz, 1H), 7.45 (d, J = 14.7 Hz, 1H). MS m/z 373 (M^+).

4.1.27. (*E*)-1-(5-Bromo-2-furanyl)-3-(4-dimethylaminophenyl)-2-propen-1-one (27). The same reaction as described above to prepare **1** was used, and 470 mg of **27** was obtained in a 55.4% yield from 2-acetyl-5-bromofuran[21] and 4-dimethylaminobenzaldehyde. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.04 (s, 6H), 5.22 (d, J = 3.9 Hz, 1H), 6.80 (d, J = 3.6 Hz, 1H), 6.89 (d, J = 15.0 Hz, 1H), 7.46 (d, J = 14.7 Hz, 1H), 7.70 (d, J = 8.1 Hz, 2H), 7.81 (d, J = 8.7 Hz, 2H). MS m/z 321 (MH^+).

4.1.28. (*E*)-1-(5-Bromo-2-furyl)-3-(5-dimethylamino-2-thienyl)-2-propen-1-one (28). The same reaction as described above to prepare **1** was used, and after purification by silica gel chromatography (7:2 hexane/ethyl acetate), 18 mg of **28** was obtained in a 11.4% yield from 2-acetyl-5-bromofuran and 5-dimethyl-2-thiophenecarboxaldehyde. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.08 (s, 6H), 5.85 (d, J = 3.9 Hz, 1H), 6.48 (d, J = 3.6 Hz, 1H), 6.72 (d, J = 15 Hz, 1H), 7.14–7.16 (m, 2H), 7.91 (d, J = 15.0 Hz, 1H). MS m/z 327 (MH^+).

4.1.29. (*E*)-1-(5-Bromo-2-furyl)-3-(5-dimethylamino-2-furyl)-2-propen-1-one (29). The same reaction as described above to prepare **1** was used, and after purification by silica gel chromatography (4:1 hexane/ethyl acetate), 29 mg of **29** was obtained in a 17.7% yield from 2-acetyl-5-bromofuran and 5-dimethylamino-2-furaldehyde. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.05 (s, 6H), 5.25 (d, J = 3.6 Hz, 1H), 6.48 (d, J = 3.3 Hz, 1H), 6.78–6.83 (m, 2 H), 7.14 (d, J = 3.3 Hz, 1H), 7.49 (d, J = 14.7 Hz, 1H). MS m/z 311 (MH^+).

4.1.30. (*E*)-1-(5-Bromo-2-thienyl)-3-(4-aminophenyl)-2-propen-1-one (30). The same reaction as described above to prepare **2** was used, 626 mg of **30** was obtained in a 43.6% yield from **11**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.03 (s, 2H), 6.68 (d, J = 9 Hz, 2H), 7.11–7.16 (m, 2 H), 7.47 (d, J = 8.7 Hz, 2H), 7.56 (d, J = 3.9 Hz, 1H), 7.78 (d, J = 15.3 Hz, 1H). MS m/z 309 (MH^+).

4.1.31. (*E*)-3-(4-Aminophenyl)-1-(5-iodo-2-thienyl)-2-propen-1-one (31). The same reaction as described above to prepare **2** was used, and 586 mg of **31** was obtained from **12**. Compound **31** was used in the next reaction without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.03 (s, 2H), 6.68 (d, J = 8.7 Hz, 2H), 7.13 (d, J = 15.3 Hz, 1H), 7.33 (d, J = 3.9 Hz, 1H), 7.44–7.49 (m, 3H), 7.78 (d, J = 15.3 Hz, 2H). MS m/z 355 (M^+).

4.1.32. (*E*)-3-(5-Bromo-2-thienyl)-1-(4-aminophenyl)-2-propen-1-one (32). The same reaction as described above to prepare **2** was used, and 453 mg of **32** was obtained in a 23.5% yield from **15**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.16 (s, 2H), 6.80 (d, J = 8.4 Hz, 2H), 7.03–7.07 (m, 2 H), 7.23 (d, J = 15.6 Hz, 1H), 7.78 (d, J = 15 Hz, 1H), 7.89 (d, J = 8.7 Hz, 2H). MS m/z 309 (MH^+).

4.1.33. (*E*)-1-(5-Bromo-2-thienyl)-3-(4-methylaminophenyl)-2-propen-1-one (33). The same reaction as described above to prepare **5** was used, 40 mg of **33** was obtained in a 18.0% yield from **30**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.91 (s, 3H), 4.19 (s, 1H), 6.60 (d, J = 8.7 Hz, 2H), 7.09–7.14 (m, 2H), 7.50 (d, J = 8.4 Hz, 2H), 7.56 (d, J = 4.2 Hz, 1H), 7.80 (d, J = 15.6 Hz, 1H). MS m/z 323 (MH^+).

4.1.34. (*E*)-1-(5-Iodo-2-thienyl)-3-(4-methylaminophenyl)-2-propen-1-one (34). The same reaction as described above to prepare **5** was used, and 45 mg of **34** was obtained in a 22.8% yield from **31**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.90 (s, 3H), 4.22 (s, 1H), 6.59 (d, J = 8.4 Hz, 2H), 7.11 (d, J = 15.3 Hz, 1H), 7.32 (d, J = 4.2 Hz, 1H), 7.44 (d, J = 3.9 Hz, 1H), 7.50 (d, J = 8.7 Hz, 2H), 7.80 (d, J = 15.3 Hz, 1H). MS m/z 369 (M^+).

4.1.35. (*E*)-3-(5-Bromo-2-thienyl)-1-(4-methylaminophenyl)-2-propen-1-one (35). The same reaction as described above to prepare **5** was used, and after purification by silica gel chromatography (4:1 hexane/ethyl acetate), 228 mg of **35** was obtained in a 25.4% yield from **32**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.92–2.94 (m, 3H), 4.33 (s, 1H), 6.61 (d, J = 8.7 Hz, 2H), 7.02–7.06 (m, 2H), 7.26 (d, J = 15.3 Hz, 1H), 7.78 (d, J = 15.6 Hz, 1H), 7.93 (d, J = 9 Hz, 2H). MS m/z 323 (MH^+).

4.1.36. (*E*)-3-(5-Bromo-2-thienyl)-1-(4-dimethylaminophenyl)-2-propen-1-one (36). The same reaction as described above to prepare **8** was used, and after purification by silica gel chromatography (4:1 hexane/ethyl acetate), 53 mg of **36** was obtained in a 5.7% yield from **32**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.09 (s, 6H), 6.70 (d, J = 9 Hz, 2H), 7.02–7.06 (m, 2H), 7.28 (d, J = 15.3 Hz, 1H), 7.78 (d, J = 15 Hz, 1H), 7.96 (d, J = 9 Hz, 2H). MS m/z 337 (MH^+).

4.1.37. (*E*)-1-(5-Tributylstannyl-2-thienyl)-3-(4-dimethylaminophenyl)-2-propen-1- one (37). The same reaction as described above to prepare **3** was used, and 10 mg of **37** was obtained in a 15.1% yield from **13**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88–1.63 (m, 27H), 3.04 (s, 6H), 6.70 (d, J = 9 Hz, 2H), 7.21–7.27 (m, 2H), 7.55 (d, J = 9 Hz, 2H), 7.81 (d, J = 15.3 Hz, 1H), 7.92 (d, J = 3.6 Hz, 1H). MS m/z 547 (MH^+).

4.2. Iododestannylation reaction

The radioiodinated form of compound **14** was prepared from corresponding tributyltin derivatives by an iododestannylation. Briefly, 50 μL of H_2O_2 (3%) was added to a mixture of a tributyltin derivative (100 μg/50 μL in EtOH), [^{125}I]NaI (3.7–7.4 MBq, specific activity 2200 Ci/mmol), and 100 μL of 1 N HCl in a sealed glass vial. The reaction was allowed to proceed at room temperature for 2 min and terminated by addition of 100 μL of a saturated aqueous $NaHSO_3$. After addition of 100 μL of a saturated aqueous $NaHCO_3$, the reaction mixture was extracted with ethyl acetate (1 mL). 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 C_{18} column with an isocratic solvent of H_2O/acetonitrile (2/3) at a flow rate of 1.0 mL/min.

4.3. Binding assays using the aggregated Aβ peptide in solution

A solid form of Aβ(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 previously.[17] A mixture containing 50 μL of test compounds (8 pM–12.5 μM in 10% ethanol), 50 μL of 0.02 nM [^{125}I]DMIC, 50 μL of Aβ(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 equation[22]: $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the concentration of [^{125}I]DMIC used in the assay, and K_d is the dissociation constant of DMIC (4.2 nM).[18]

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

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 of 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 **14**. 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 **14** 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.[23]

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 [^{125}I]**14** (4.2–6.3 kBq) and 10% ethanol was injected directly into the tail vein of ddY mice (5-week-old, average weight 23–25 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).

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