Supporting Information

Development of alkoxy styrylchromone derivatives for imaging of cerebral amyloid-*β* plaques with SPECT

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Materials and Methods

All reagents were commercial products and used without further purification unless otherwise indicated. [^{125}I]NaI was obtained by MP biomedicals (Costa Mesa, CA, USA). ^{1}H NMR spectra were obtained on a Varian Gemini 300 spectrometer with TMS as an internal standard.Mass spectra were obtained on JMS－700N or JMS-T100TDinstruments (JEOL Ltd., Japan). HPLC analysis was performed on a Shimadzu HPLC system (a LC-10AT pump with a SPD-10A UV detector, λ= 254 nm). An automated gamma counter with a NaI(Tl) detector

(PerkinElmer, 2470 WIZARD^2) was used to measure radioactivity. (*E*)-6-Iodo-2-(4-(methylamino)styryl)-chromone **20** and [^{125}I]**20** were prepared by the method in the literature[1]. All final compounds were determined to be ≥95% pure by HPLC analysis (Shimadzu HPLC system, a LC-10AT pump with a SPD-10A UV detector, λ= 254 nm). All animals were supplied by Kyudo, Co., Ltd (Japan). The experiments with animals were conducted in accordance with our institutional guidelines and were approved by Nagasaki University Animal Care Committee.

Chamistry

(*E*)-2-Acetyl-bromophenyl cinnamate (1)

To a mixture of cinnamic acid (740 mg, 5.0 mmol) and thionyl chloride (1.0 mL, 12.9 mmol) was added one drop of DMF and stirred at 80 ˚C for 1 h. The mixture was evaporated then the residue was added a solution of 5'-bromo-2'-hydroxyacetophenone (1.08 g, 5.0 mmol) in pyridine (10 mL) and stirred under reflux for 2.5 h. The reaction mixture was added to 1 M HCl and the precipitated solid was filtrated to give **1** (1.43 g, 88%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.55 (s, 3H), 6.65 (d, *J* = 15.9 Hz, 1H), 7.10 (d, *J* = 8.4 Hz, 1H), 7.43 (dd, *J* = 5.0, 1.7 Hz, 3H), 7.61 (dd, *J* = 7.5, 3.0 Hz, 1H), 7.66 (dd, *J* = 8.7, 2.7 Hz, 2H), 7.88 (s, 1H), 7.93 (d, *J* = 2.4 Hz, 1H). MS (DART) *m/z* 345, 347 [M^+].

(*E*)-2-Acetyl-bromophenyl-3-(4-methoxyphenyl)acrylate (2)

Using the above procedure for **1** starting from*trans*-4-methoxycinnamic acid, the title compound **2** (696 mg, 75%) was obtained as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.54 (s, 3H), 3.86 (s, 3H), 6.51 (d, *J* = 15.6 Hz, 1H), 6.94 (d, *J* = 8.7 Hz, 2H), 7.09 (d, *J* = 8.7 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.89 (d, *J* = 15.9 Hz, 1H), 7.93 (s, 1H). MS (DART) *m/z* 375, 377 [M^+].

(*E*)-2-Acetyl-bromophenyl-3-(3,4-dimethoxyphenyl)acrylate (3)

Using the above procedure for **1** starting from *trans*-3,4-dimethoxycinnamic acid, the title compound **3** (1.08 g, 57%) was obtained as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.56 (s, 3H), 3.92 (d, *J* = 11.4 Hz, 6H), 6.51 (d, *J* = 15.6 Hz, 1H), 6.90 (d, *J* = 8.1Hz, 1H), 7.07-7.20 (m, 3H), 7.66 (d, *J* = 8.7 Hz, 1H), 7.87 (t, *J* = 15.9 Hz, 2H). MS (DART) *m/z* 405, 407 [M^+].

(*E*)-1-(5-Bromo-2-hydroxylphenyl)-3-phenylpent-4-ene-1,3-dione (4)

To a solution of acetophenone **1** (1.43 g, 4.38 mmol) in pyridine (15 mL) was added powdery KOH (220 mg, 3.84 mmol). After stirring for 1 h at 50 ºC, the mixture was treated with ice-cooled 10% aqueous CH_3CO_2H (20 mL). The resulting precipitate was collected by filtration and washed with water, giving **4** (1.43 g, 99%) as a yellow powder. ^{1}H NMR (300

MHz, $CDCl_3$) δ 6.27 (s, 1H), 6.62 (dd, *J* = 15.6, 0.9 Hz, 1H), 6.90 (d, *J* = 9.0 Hz, 1H), 7.25 (d, *J* = 0.6 Hz, 1H), 7.42 (dd, *J* = 5.1, 2.4 Hz, 3H), 7.52 (dd, *J* = 9.0, 2.7 Hz, 1H), 7.56-7.59 (m, 2H), 7.70 (d, *J* = 15.6 Hz, 1H), 7.81 (d, *J* = 2.4 Hz, 1H). MS (DART) *m/z* 345, 347 [M^+].

(*E*)-1-(5-Bromo-2-hydroxylphenyl)-5-(4-methoxyphenyl)pent-4-ene-1,3-dione (5)

Using the above procedure for **4** starting from aryl acrylate **2**, the title compound **5** (696 mg, 75%) was obtained as a yellowpowder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.87 (s, 3H), 6.23 (s, 1H), 6.49 (d, *J* = 15.6 Hz, 1H), 6.89 (d, *J* = 8.7 Hz, 2H), 7.53 (m, 3H), 7.66 (d, *J* = 15.3 Hz, 1H), 7.80 (d, *J* = 0.9 Hz, 1H). MS (DART) *m/z* 375, 377 [M^+].

(*E*)-1-(5-Bromo-2-hydroxylphenyl)-5-(3,4-dimethoxyphenyl)pent-4-ene-1,3-dione (6)

Using the above procedure for **4** starting from aryl acrylate **3**, the title compound **6** (790 mg, 70%) was obtained as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.95 (d, 6H), 6.24 (s, 1H), 6.49 (d, *J* = 15.6 Hz, 1H), 6.89 (dd, *J* = 8.7, 3.3 Hz, 2H), 7.09 (d, *J* = 1.8 Hz, 1H), 7.16 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.50 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.65(d, *J* = 15.6 Hz, 1H), 7.79 (d, *J* = 2.4 Hz, 1H). MS (DART) *m/z* 405, 407 [M^+].

(*E*)-6-Bromo-2-styrylchromone (7)

To a solution of **4** (1.43 g, 4.14 mmol) in CH_3CO_2H (10 mL) was added concentrated

sulfuric acid (0.8 mL). After stirring under reflux for 1 h, the mixture was stirred further 1 h at room temperature. The reaction mixture was poured into ice-cold water and the resulting precipitate was collected by filtration and washed with water, giving **7** (1.00 g, 70%) as a yellow ocher powder. ^{1}H NMR (300MHz, $CDCl_3$) δ 6.44 (s, 1H), 6.79 (d, *J* = 15.9 Hz, 1H), 7.42-7.44 (m, 2H), 7.46 (s, 1H), 7.59-7.60 (m, 2H), 7.64 (s, 1H), 7.77 (dd, *J* = 8.7, 2.7 Hz, 1H), 8.33 (d, *J* = 2.4 Hz, 1H). MS (DART) *m/z* 327, 329 [M^+].

(*E*)-6-Bromo-2-(4-methoxystyryl)-chromone (8)

Using the above procedure for **7** starting from **5**, the title compound **8** (268 mg, 71%) was obtained as a yellow ocher powder. ^{1}H NMR (300MHz, $CDCl_3$) δ 3.86 (s, 3H), 6.29 (s, 1H), 6.63 (d, *J* = 15.9 Hz, 1H), 6.94 (d, *J* = 9.0 Hz, 2H), 7.41 (d, *J* = 9.0 Hz, 1H), 7.54 (m, 3H), 7.75 (d, *J* = 9.0 Hz, 1H), 8.31 (d, *J* = 2.7 Hz, 1H). MS (DART) *m/z* 357, 359 [M^+].

(*E*)-6-Bromo-2-(3,4-dimethoxystyryl)-chromone (9)

Using the above procedure for **7** starting from **6**, the title compound **9** (762 mg, 99%) was obtained as a yellow ocher powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.96 (d, *J* = 9.0 Hz, 6H), 6.32 (s, 1H), 6.65 (d, *J* = 15.9 Hz, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 7.17 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.43 (d, *J* = 8.7 Hz, 1H), 7.56 (d, *J* = 15.9 Hz, 1H), 7.76 (dd, *J* = 11.4, 0.3 Hz, 1H), 8.32 (d, *J* = 0.2 Hz, 1H). MS (DART) *m/z* 387, 389 [M^+].

(*E*)-6-Tributylstannyl-2-styrylchromone (**10**)

A mixture of **7** (123 mg, 0.38 mmol), bis(tributyltin) (0.8mL, 1.60 mmol), $Pd(PPh_3)_4$ (40 mg, 0.034 mmol) and triethylamine (8.0 mL) in dioxane (12 mL) was stirred for 7 h under reflux. The solvent was removed and the crude product was chromatographed on silica gel with hexane/EtOAc= 4:1 to give **7** (88 mg, 50%) as a yellow ocher oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86-1.55 (m, 27H), 6.35 (s, 1H), 6.80 (s, 1H), 7.42 (d, *J* = 8.1 Hz, 3H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.59-7.62 (m, 1H), 7.64 (s, 1H), 7.76 (dd, *J* = 8.4, 1.2 Hz, 1H), 8.28 (d, *J* = 0.9 Hz, 1H). MS (DART) *m/z* 539 [M^+].

(*E*)-6-Tributylstannyl-2-(4-methoxystyryl)-chromone (11)

Using the above procedure for **10** starting from **8** (128 mg, 0.38 mmol), the title compound **11** (89 mg, 42%) was obtained as a yellow ocher oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86-1.57 (m, 27H), 3.86 (s, 3H), 6.31 (s, 1H), 6.66 (s, *J* = 16.2 Hz, 1H), 6.95 (d, *J* = 8.7 Hz, 2H), 7.49-7.59 (m, 4H), 7.75 (d, *J* = 8.1 Hz, 1H), 8.27 (d, *J* = 1.2 Hz, 1H). MS (DART) *m/z* 569 [M^+].

(*E*)-6-Tributylstannyl-2-(3,4-dimethoxystyryl)-chromone (12)

Using the above procedure for **10** starting from **9** (368 mg, 0.95 mmol), the title

compound **12** (141 mg, 25%) was obtained as a yellow ocher oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87-1.32 (m, 27H), 3.95 (d, *J* = 10.8 Hz, 6H), 6.33 (s, 1H), 6.66 (d, *J* = 16.2 Hz, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 7.11 (d, *J* = 2.1 Hz, 1H), 7.17 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.48 (d, *J* = 8.1 Hz, 1H), 7.56 (d, *J* = 15.9 Hz, 1H), 7.75 (dd, *J* = 9.9, 1.5 Hz, 1H), 8.28 (d, *J* = 1.2 Hz, 1H). MS (DART) *m/z* 599 [M^+].

(*E*)-6-Iodo-2-styrylchromone (13)

To a solution of **10** (88 mg, 0.16 mmol) in $CHCl_3$ (5.0 mL) was added a solution of iodine in $CHCl_3$ (2.0 mL, 0.25 M) at room temperature. The mixture was stirred at room temperature for 30 min and a saturated $NaHSO_3$ solution (10 mL) was added. The mixture was stirred for 5 min and the organic phase was separated. The aqueous layer was extracted with $CHCl_3$ three times. The organic layer was washed successively with saturated aqueous $NaHCO_3$, and brine and then dried over Na_2SO_4. The crude product was chromatographed on silica gel with hexane/EtOAc= 2:1 to give **13** (6 mg, 99%) as a yellow ocher powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.35 (s, 1H), 6.79 (d, *J* = 16.2 Hz, 1H), 7.32 (d, *J* = 8.7 Hz, 1H), 7.43 (d, *J* = 6.6 Hz, 3H), 7.59 (dd, *J* = 16.2, 2.4 Hz, 2H), 7.64 (s, 1H), 7.95 (dd, *J* = 8.7, 2.1 Hz, 1H), 8.53 (d, *J* = 2.1Hz, 1H). MS (FAB) *m/z* 374 [M^+]

(*E*)-6-Iodo-2-(4-methoxystyryl)chromone (14)

Using the above procedure for **13** starting from **11**, the title compound **14** (118 mg, 66%) was obtained as a yellow ocher oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.86 (s, 3H), 6.29 (s,1H), 6.64 (d, *J* = 16.2 Hz, 1H), 6.95 (d, *J* = 8.7 Hz, 2H), 7.29 (d, *J* = 9.0 Hz, 1H), 7.52-7.54 (m, 3H), 7.93 (d, *J* = 8.7 Hz, 1H), 8.51 (d, *J* = 2.1 Hz, 1H). HRMS (EI) *m/z*: calcd for $C_{18}H_{14}IO_3$ [M^+] 404.9988, found 404.9975.

(*E*)-6-Iodo-2-(3,4-dimethoxystyryl)chromone (15)

Using the above procedure for **13** starting from **12** (141 mg, 0.24 mmol), the title compound **15** (104 mg, 99%) was obtained as a yellow ocher oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.96 (d, *J* = 9.3 Hz, 6H), 6.31 (s,1H), 6.64 (d, *J* = 15.9 Hz, 1H), 6.91 (d, *J* = 8.4 Hz, 1H), 7.11 (d, *J* = 2.1 Hz, 1H), 7.17 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.29 (d, *J* = 8.7 Hz, 1H), 7.55 (d, *J* = 15.9 Hz, 1H) , 7.94 (dd, *J* = 11.1, 2.1 Hz, 1H), 8.52 (d, *J* = 2.4 Hz, 1H). HRMS (FAB) m/z: calcd for $C_{19}H_{16}O_4I$ [M^+] 435.0093, found 435.0091.

(*E*)-6-Iodo-2-(4-hydroxylstyryl)-chromone (16)

To a solution of **14** (113 mg, 0.28 mmol) in CH_2Cl_2 (8.0 mL) was added BBr_3 in CH_2Cl_2 (2.0 mL, 1.0 M). After stirred for 48 h at room temperature, the reaction mixture was quenched with water and then extracted with $CHCl_3$ three times. The combined organic layers were dried with Na_2SO_4 and evaporated to dryness. The crude product was chromatographed

on silica gel with $CHCl_3$/MeOH= 49: 1 to give **16** (49 mg, 45%) as a orange powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.42 (s, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 6.98 (d, *J* = 16.2 Hz, 2H), 7.53-7.66 (m, 4H), 8.08 (d, *J* = 8.7 Hz, 1H), 8.24 (s, 1H). HRMS (FAB) *m/z*: calcd for $C_{17}H_{12}O_3I$ [M^+] 390.9831, found 390.9838.

(*E*)-6-Iodo-2-(4-hydroxyethoxystyryl)-chromone (17)

To a solution of **16** (22 mg, 0.056 mmol) in DMF (5.0 mL) was added K_2CO_3 (387 mg, 2.8 mmol) and 2-chloroethanol (11.4 μL, 0.17 mmol).After stirring under reflux for 24 h, the reaction mixture was quenched with water and then extracted with EtOAc three times. The combined organic layers were dried with Na_2SO_4 and evaporated to dryness.The crude productwas chromatographed on silica gel with hexane/EtOAc= 1:1 to give **17** (6.0 mg, 25%) as a yellow ocher solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.00 (s, *J* = 4.3 Hz, 2H), 4.15 (t, *J* = 4.2 Hz, 2H), 6.30 (s, 1H), 6.65 (d, *J* = 15.9 Hz, 1H), 6.88-6.98 (m, *2* H), 6.97 (d, *J* = 8.4 Hz, 2H), 7.56 (d, *J* = 8.7 Hz, 2H), 7.94 (dd, *J* = 11.1, 2.1 Hz, 1H), 8.52 (d, *J* = 2.1 Hz, 1H). HRMS (FAB) *m/z*: calcd for $C_{19}H_{16}O_4I$ [M^+] 435.0093, found 435.0103.

6-Iodo-2-(4'-(4'-hydroxyethoxy)ethoxy)styryl)chromone (18)

Prepared using the above procedure for **17** from **16** and ethylene glycol mono-2-chloroethyl ether , the title compound **18** (9.0 mg, 50%) was obtained as a yellow

ocher solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.69 (t, *J* = 4.2 Hz, 2H), 3.77 (t, *J* = 4.3 Hz, 2H), 3.90 (t, *J* = 4.7 Hz, 2H), 4.22 (t, *J* = 4.5 Hz, 2H), 4.22 (t, *J* = 4.5 Hz, 2H), 6.30 (s, 1H), 6.65 (d, *J* = 15.9 Hz, 1 H), 6.85-6.99 (m, 2 H), 6.90 (d, J= 8.8 Hz, 1 H), 7.53 (d, *J*= 8.8 Hz, 2H), 7.93 (dd, *J*= 8.7, 2.2 Hz, 1H), 8.52 (d, *J*= 2.1 Hz, 1H). MS (FAB) *m/z*: 479 [M^+]

6-Iodo-2-(4'-(4'-(4'-hydroxyethoxy)ethoxy)ethoxy)styryl)chromone (19)

Prepared using the above procedure for **17** from **16** and 2-[2-(2-Chloroethoxy)ethoxy] ethanol, the title compound **19** (11 mg, 13%) was obtained as a yellow ocher solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.63 (t, *J* = 4.5 Hz, 2H), 3.74 (t, *J* = 4.3 Hz, 6H), 3.89 (t, *J* = 4.8 Hz, 2H), 4.18 (t, *J* = 4.2 Hz, 2H), 6.31 (s, *J* = 6.6 Hz, 1H), 6.64 (d, *J* = 15.9 Hz, 1H), 6.86-6.91 (m, 2H), 6.96 (d, *J* = 9.0 Hz, 2H), 7.52 (d, *J* = 8.7 Hz, 2H), 7.93 (dd, *J* = 8.7, 2.4 Hz, 1H), 8.50 (d, *J* = 8.4Hz, 1H). MS (FAB) *m/z*: 523 [M^+].

Radioiodination

The ^{125}I-labeled compounds ([^{125}I]**14**, [^{125}I]**15**) were prepared from the corresponding tributyltin derivatives (**11**, **12**) by iododestannylation. In brief, 3% H_2O_2 (50 μL) was added to a mixture of corresponding tributyltin derivative (1.0 mg/mL-EtOH), [^{125}I]NaI (3.7–7.4 MBq, specific activity 81.4 GBq/μmol), and 1 M HCl (50 μL) in a sealed vial. The reaction was allowed to proceed at room temperature for 3 min and terminated by addition of satd

$NaHSO_3$aq (100 μL). After alkalization with 100 μL of satd. $NaHCO_3$ and extraction with ethyl acetate, the extract was dried by passing it through an anhydrous Na_2SO_4 column and evaporated to dryness. The crude products were purified by HPLC on a Cosmosil C_{18} column (Nacalai Tesque, 5C_{18}-AR-II, 4.6×250 mm) with an isocratic solvent of CH_3CN/H_2O (6:4) at a flow rate of 1.0 mL/min. Because a small amount of *Z*-isomer was found during the radiosynthesis of ^{125}I labeled *E*-isomers of SCs, the separated *E*-isomer of SCs were kept protected from light until the *in vitro* and *in vivo* experiments were performed.

Binding assays using the aggregated A*β* peptide in solution

Binding assays by using filtration techniques were carried out as described previously[2]. Briefly, a mixture containing 50 μL of test compounds (8 pM–12.5 μM in 10% ethanol), 50 μL of 0.02 nM [^{125}I]**20**, 50 μL of the A*β* 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 measured by an automatic gamma counter (PerkinElmer, 2470 WIZARD[2]). 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.

Fluorescence staining on *Tg2576* mice brain sections

The *Tg2576* mice (female, 22-24 months old) and wild-type mice (female, 24 months old) were used as the Alzheimer's model and control mice, respectively. After the mice were euthanized, the brains were immediately removed and frozen in powdered dry ice. The frozen blocks were sliced into serial sections, 10 μm thick. Each slide was incubated with a 50% DMSO solution (100 μM) of **14** and **15** for 10 min. The slices were rinsed twice with 50 % DMSO for 1 min, and subsequently dipped into water for 30 s. The fluorescence images were collected by BZ8100 (Keyence) using a DAPI-BP filter set for **14** and **15** (excitation, 360 nm; dichroicmirror, 400 nm; longpass filter, 460 nm) or a GFP-BP filter set for thioflavin-S (excitation, 470 nm; dichroic mirror, 495 nm; longpass filter, 535 nm). Thereafter, the serial sections were also stained with thioflavin-S, a pathological dye commonly used for staining A*β*plaques in the brain, and examined using the microscope in the same condition with that of styrylchromone.

***In vitro* autoradiography on *Tg2576* mice brain sections**

The brain sections from*Tg2576* transgenic mice (female, 31 months old) were incubated in the 50 % DMSO solution containing [^{125}I]**14** or [^{125}I]**15** (20 kBq/150 μL) for 2 h. The slices were rinsed twice with 80 % DMSO solution for 2 min, and subsequently dipped into water for 1 min. The sections were dried under a stream of cold air and placed in contact

with the imaging plates (BAS-MS 2040; Fuji Film) for 2 h. The distribution of the radioactivity on the plates were analyzed by a Fluoro Image Analyzer (FLA5100;Fuji Film). Then, the adjacent sections were stained with thioflavin S.The fluorescence images were collected by an ECLIPSE 80i microscope (Nicon Corp., Japan) using a B-2A filter set (excitation, 450-490 nm; dichroic mirror, 505 nm; long pass filter, 520 nm).

In vivo biodistribution in normal mice

Each ^{125}I labeled tracer (7.4–14.8 kBq) was injected intravenously *via* the tail vein into ddY mice (male, 5 weeks old, 25–30 g). The mice were euthanized at 2, 30, 60, 120 and 180 min after injection. The tissues were dissected, weighed and the radioactivity was measured by automated gamma counting. Data were calculated as the percentage of the injected dose per gram (% ID/g).

References

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