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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}\text{I}]\text{NaI}\) was obtained by MP biomedicals (Costa Mesa, CA, USA). \(^{1}\text{H}\) 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-T100TD instruments (JEOL Ltd., Japan). HPLC analysis was performed on a Shimadzu HPLC system (a LC-10AT pump with a SPD-10A UV detector, \(\lambda = 254\) nm). An automated gamma counter with a NaI(Tl) detector
(PerkinElmer, 2470 WIZARD®) was used to measure radioactivity. 

\((E)-6\text{-iodo-2-(4-(methylamino)styryl)}\text{-chromone 20 and [}^{125}\text{I}]20\) were prepared by the method in the literature\(^1\). All final compounds were determined to be \(\geq 95\%\) pure by HPLC analysis (Shimadzu HPLC system, a LC-10AT pump with a SPD-10A UV detector, \(\lambda = 254\) 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.

**Chemistry**

\((E)-2\text{-Acetyl-bromophenyl cinnamate (1)}\)

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.

\(^1\text{H NMR (300 MHz, CDCl}_3\text{)} \delta 2.55 (s, 3H), 6.65 (d, } J = 15.9 \text{ Hz, 1H), 7.10 (d, } J = 8.4 \text{ Hz, 1H), 7.43 (dd, } J = 5.0, 1.7 \text{ Hz, 3H), 7.61 (dd, } J = 7.5, 3.0 \text{ Hz, 1H), 7.66 (dd, } J = 8.7, 2.7 \text{ Hz, 2H), 7.88 (s, 1H), 7.93 (d, } J = 2.4 \text{ Hz, 1H). MS (DART) } m/z 345, 347 [M^+]\).
(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, $J = 8.1$Hz, 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-hydroxyphenyl)-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$_3$CO$_2$H (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, CDCl₃) 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 yellow powder. ¹H NMR (300 MHz, CDCl₃) 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. ¹H NMR (300 MHz, CDCl₃) 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 CH₃CO₂H (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$_3$) $\delta$ 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 Hz, 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$_3$) $\delta$ 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$_3$) $\delta$ 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 Hz, 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 Hz, 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) (0.8 mL, 1.60 mmol), Pd(PPh₃)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. 1H NMR (300 MHz, CDCl₃) 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. 1H NMR (300 MHz, CDCl₃) δ 0.86-1.57 (m, 27H), 3.86 (s, 3H), 6.31 (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 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$_2$SO$_4$. 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, $J = 2.1$Hz, 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$_4$I [M$^+$] 435.0093, found 435.0091.

$(E)$-6-Iodo-2-(4-hydroxystyryl)-chromone (16)

To a solution of 14 (113 mg, 0.28 mmol) in CH$_2$Cl$_2$ (8.0 mL) was added BBr$_3$ in CH$_2$Cl$_2$ (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$_2$SO$_4$ and evaporated to dryness. The crude product was chromatographed
on silica gel with CHCl3/MeOH= 49: 1 to give 16 (49 mg, 45%) as a orange powder.  

\[ ^{1}H \text{NMR} \] (300 MHz, CDCl3) 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 C17H12O3I [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 K2CO3 (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 Na2SO4 and evaporated to dryness. The crude product was chromatographed on silica gel with hexane/EtOAc = 1:1 to give 17 (6.0 mg, 25%) as a yellow ocher solid.  

\[ ^{1}H \text{NMR} \] (300 MHz, CDCl3) 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 C19H16O4I [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$_3$) $\delta$ 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), 6.30 (s, 1H), 6.65 (d, $J = 15.9$ Hz, 1H), 6.85-6.99 (m, 2H), 6.90 (d, $J = 8.8$ Hz, 1H), 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$_3$) $\delta$ 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, $J = 8.4$Hz, 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$_2$O$_2$ (50 $\mu$L) 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 $\mu$L) 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$_2$SO$_4$ 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$_3$CN/H$_2$O (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 Aβ 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 Aβ 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 \textbf{14} and \textbf{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 \textbf{14} and \textbf{15} (excitation, 360 nm; dichroic mirror, 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 Aβ 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 $[^{125}\text{I}]\textbf{14}$ or $[^{125}\text{I}]\textbf{15}$ (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
