Supplemental Information

Curcumin-based Molecular Probe for Near-Infrared Fluorescence Imaging of Tau Fibrils in Alzheimer’s Disease

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1. Synthetic procedures and characterization of newly synthesized compounds

**Materials and reagents.** All chemical reagents, including heparin sodium salt and thioflavin
S were purchased from Sigma-Aldrich, TCI, or Alfa. Dulbecco’s modified Eagle medium
(DMEM), penicillin, streptomycin, and fetal bovine serum (FBS) were purchased from
Invitrogen. TransIT-LT1 for transfection was purchased from Mirus. Nuclear magnetic
resonance spectra were recorded on a Bruker 400 AMX spectrometer (Karlsruhe, Germany) at
400 MHz for \(^1\)H NMR and 75 MHz (or 100 MHz or 125 MHz) for \(^{13}\)C NMR with
tetramethylsilane as an internal standard. High resolution FAB mass spectrometric data
(HRMS-FAB) were obtained at Korea Basic Science Institute (Daegu, Korea) and reported in
the form of \(m/z\) (intensity relative to base peak=100). Fluorescence was recorded using a
SpectraMax M2e (Molecular Devices, USA). Fluorescence images were obtained through
confocal microscopy (Zeiss LSM701 confocal, Carl Zeiss).

3,5-Dibromoaniline (3a):  

![3a](image)

To a solution of 2,6-dibromo-4-nitroaniline (2) (1.0 g, 3.4 mmol) in EtOH (5 mL) were added
NaNO\(_2\) (0.3 g, 4.0 mmol) and 5 drops of H\(_2\)SO\(_4\). The resulting mixture was stirred for 4 h at
80 °C. After cooling to rt, the reaction mixture was washed with saturated aqueous NaHCO\(_3\)
solution, the organic layer was dried over MgSO\(_4\) and concentrated under reduced pressure.
The residue was purified by column chromatography on silica gel (16:1 = hexanes:EtOAc) to
give 1,3-dibromo-5-nitrobenzene (0.4 g, 1.5 mmol, 44% yield) as yellow powder: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.31 (d, \(J = 1.6\) Hz, 2H), 7.99 (t, \(J = 1.6\) Hz, 1H). To a solution of reduced iron powder (0.6 g, 10.7 mmol) and NH\(_4\)Cl (0.6 g, 10.7 mmol) in H\(_2\)O (5 mL) was added a solution of 1,3-dibromo-5-nitrobenzene (1.0 g, 3.56 mmol) in acetone (15 mL). The resulting mixture was stirred for 6 h at 80 °C, and then cooled to rt. After washing the mixture with saturated NaHCO\(_3\) solution, the organic layer was dried over MgSO\(_4\) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (8:1 = hexanes:EtOAc) to give the desired, 3,5-dibromoaniline (3a) (0.68g, 2.7 mmol, 76% yield) as yellow powder: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.07 (s, 2H), 6.77 (s, 1H).

**3,5-Dibromo-N,N-dimethylaniline (4a):**

\[
\text{Br} \quad \text{Me} \quad \text{N} \quad \text{Me} \\
\text{Br} \quad \text{Me} \quad \text{N} \quad \text{Me} \\
\text{4a}
\]

To a solution of 3a (0.5 g, 2.0 mmol) in CH\(_3\)CN (10 mL) were added K\(_2\)CO\(_3\) (0.5 g, 3.9 mmol) and CH\(_3\)I (0.2 mL, 3.9 mmol). The resulting mixture was stirred for 12 h at 65 °C, cooled to rt, and then filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (16:1 = hexanes:EtOAc) to give 3,5-dibromo-N,N-dimethylaniline (4a) (0.3 g, 1.1 mmol, 53% yield) as yellow powder: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 6.88 (s, 1H), 6.72 (d, \(J = 1.0\) Hz, 2H), 2.94 (s, 6H); \(^{13}\)C NMR (125 MHz, DMSO-d\(_6\)) \(\delta\) 152.2, 123.0, 119.6, 113.2, 39.7; HRMS (FAB) calcd for C\(_8\)H\(_9\)Br\(_2\)N [M]\(^+\) 276.9102, found 276.9099.
3,5-Dichloro-\(N,N\)-dimethylaniline (4b):

\[
\begin{align*}
\text{Me} & \quad \text{N} \\
\text{Cl} & \quad \text{Cl}
\end{align*}
\]

To a solution of commercially available 3,5-dichloroaniline 3b (0.3 g, 2.0 mmol) in \(\text{CH}_3\text{CN}\) (10 mL) were added \(\text{K}_2\text{CO}_3\) (0.5 g, 3.9 mmol) and \(\text{CH}_3\text{I}\) (0.2 mL, 3.9 mmol). The resulting mixture was stirred for 12 h at 65 °C, cooled to rt, and then filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (8:1 = hexanes:EtOAc) to give 3,5-dichloro-\(N,N\)-dimethylaniline (4b) (0.2 g, 1.00 mmol, 52% yield) as pale yellow syrup: \(^1\text{H NMR (400 MHz, CDCl}_3) \delta 6.65 \ (t, J = 1.5\text{ Hz, 1H}), 6.51 \ (d, J = 1.6\text{ Hz, 1H}), 2.92 \ (s, 6\text{H}); \(^{13}\text{C NMR (125 MHz, DMSO-d}_6) \delta 151.8, 134.5, 114.3, 110.0, 39.7; \text{HRMS (FAB) calcd for C}_{8}\text{H}_{9}\text{Cl}_2\text{N} [M]^+ 189.0112, found 189.0114.}

3,5-Dimethoxy-\(N,N\)-dimethylaniline (4c):

\[
\begin{align*}
\text{Me} & \quad \text{N} \\
\text{OMe} & \quad \text{OMe}
\end{align*}
\]

To a solution of commercially available 3,5-dimethoxyaniline 3c (0.29 g, 1.9 mmol) in \(\text{CH}_3\text{CN}\) (5 mL) were added HCHO (37% in \(\text{H}_2\text{O}\), 1 mL) and \(\text{NaBH}_3\text{CN}\) (0.34 g, 5.9 mmol) followed by HOAc (0.1 mL). The resulting mixture was stirred for 1 h and then HOAc (0.1 mL) was added again. After washing with saturated \(\text{NaHCO}_3\) solution, the organic layer was dried over \(\text{MgSO}_4\) and concentrated under reduced pressure. The residue was purified by
column chromatography on silica gel (16:1 = hexane:EtOAc) to give 3,5-dimethoxy-\(N,N\)-dimethylaniline (4c) (0.2 g, 1.2 mmol, 62% yield) as pale yellow powder. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 5.91 (s, 3H), 3.78 (s, 6H), 2.92 (s, 6H); \(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)) \(\delta\) 152.1, 91.4, 88.6, 54.7, 40.1; HRMS (FAB) calcd for C\(_{10}\)H\(_{16}\)NO \([M + H]^+\) 182.1176, found 182.1178.

2,6-Dibromo-4-(dimethylamino)benzaldehyde (5a):

![Image of compound 5a]

To a solution of POCl\(_3\) (0.09 mL, 1.0 mmol) and DMF (0.08 mL, 1.0 mmol) in CH\(_2\)Cl\(_2\) (5 mL) was added 4a (0.25 g, 0.9 mmol) at 0 °C. The resulting mixture was stirred for 12 h at rt, and then water was added. The reaction mixture was neutralized by 2N NaOH and extracted with EtOAc. The combined organic layers was dried over MgSO\(_4\) and concentrated under reduced pressure. The residue was purified by column chromatography (2:1 = hexanes:acetone) on silica gel to afford 2,6-dibromo-4-(dimethylamino)benzaldehyde (5a) (0.09 g, 0.3 mmol, 32% yield) as yellow syrup. \(^1\)H NMR (400 MHz, Acetone-\(d_6\)) \(\delta\) 10.07 (s, 1H), 6.98 (s, 2H), 3.13 (s, 6H); \(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)) \(\delta\) 188.6, 153.2, 127.2, 117.0, 115.7, 39.6; HRMS (FAB) calcd for C\(_9\)H\(_9\)Br\(_2\)NO \([M]^+\) 304.9051, found 304.9053.

2,6-Dichloro-4-(dimethylamino)benzaldehyde (5b):
To a solution of POCl₃ (0.09 mL, 1.0 mmol) and DMF (0.08 mL, 1.0 mmol) in CH₂Cl₂ (5 mL) was added 4b (0.17 g, 0.9 mmol) at 0 °C. The resulting mixture was stirred for 12 h at rt, and then water was added. The reaction mixture was neutralized by 2N NaOH and extracted with EtOAc. The combined organic layers was dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (4:1 = hexanes:EtOAc) on silica gel to afford 2,6-dichloro-4-(dimethylamino)benzaldehyde (5b) (0.08 g, 0.4 mmol, 43% yield) as yellow syrup: ¹H NMR (400 MHz, CDCl₃) δ 10.30 (s, 1H), 6.53 (s, 2H), 3.05 (s, 6H); ¹³C NMR (125 MHz, DMSO-d₆) δ 185.9, 152.9, 138.2, 115.6, 111.8, 39.6; HRMS (FAB) calcd for C₉H₁₀Cl₂NO [M + H]⁺ 218.0134, found 218.0135.

4-(Dimethylamino)-2,6-dimethoxybenzaldehyde (5c):

To a solution of POCl₃ (0.09 mL, 1.0 mmol) and DMF (0.08 mL, 1.0 mmol) in CH₂Cl₂ (5 mL) was added 4c (0.16 g, 0.9 mmol) at 0 °C. The resulting mixture was stirred for 12 h at rt, and then water was added. The reaction mixture was neutralized by 2N NaOH and extracted with EtOAc. The combined organic layers was dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (6:1 = hexanes:EtOAc) on
silica gel to afford 4-(dimethylamino)-2,6-dimethoxybenzaldehyde (5c) (0.1 g, 0.5 mmol, 60%) yield as pale yellow powder: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 10.22 (s, 1H), 5.73 (s, 2H), 3.88 (s, 6H), 3.09 (s, 6H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 183.9, 163.3, 155.6, 104.0, 87.2, 55.5, 39.7; HRMS (FAB) calcd for C$_{11}$H$_{16}$NO $[M + H]^+$ 210.1125, found 210.1132.

4-(Dimethylamino)-2,6-dimethylbenzaldehyde (5d):

To a solution of POCl$_3$ (0.09 mL, 1.0 mmol) and DMF (0.08 mL, 1.0 mmol) in CH$_2$Cl$_2$ (5 mL) was added commercially available N,N-3,5-tetramethylaniline 4d (0.13 g, 0.9 mmol) at 0 °C. The resulting mixture was stirred for 12 h at rt, and then water was added. The reaction mixture was neutralized by 2N NaOH and extracted with EtOAc. The combined organic layers was dried over MgSO$_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (2:1 = hexane:acetone) on silica gel to afford 4-(dimethylamino)-2,6-dimethylbenzaldehyde (5d) (0.06 g, 0.4 mmol, 40% yield) as pale yellow powder: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 10.28 (s, 1H), 6.23 (s, 2H), 2.97 (s, 6H), 2.52 (s, 6H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 189.7, 152.6, 143.2, 120.7, 111.7, 39.3, 21.0; HRMS (FAB) calcd for C$_{11}$H$_{16}$NO $[M + H]^+$ 178.1226, found 178.1230.

General procedure for the synthesis of the curcumin derivatives 1a ~ 1e. Synthesis of (1E,4Z,6E)-1,7-bis(2,6-dibromo-4-(dimethylamino)phenyl)-5-hydroxyhepta-1,4,6-trien-3-one (1a) is representative:
To a solution of acetylacetone (0.05 mL, 0.45 mmol) in DMF (1 mL) was added boron oxide (0.03 g, 0.45 mmol) in a sealed tube. After stirring the reaction mixture for 30 min at 65 °C, a solution of 5a (0.28 g, 0.90 mmol) in DMF (1 mL), tributyl borate (0.25 mL, 0.90 mmol), and nBuNH₂ (0.01 mL, 0.09 mmol) were added. The mixture was stirred for another 4 h at 90 °C in the sealed tube. After cooling to rt, the reaction mixture was treated with 10% acetic acid in water (10 mL) and stirred for 1 h at 75 °C. The reaction mixture was cooled to rt and extracted with EtOAc three times. The combined organic layers were dried over MgSO₄ and, after filtering, the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (Hexane:Acetone:Ether = 8:1:1) to give the desired compound 1a (0.17 g, 0.25 mmol, 56% yield) as orange solid: ¹H NMR (400 MHz, CDCl₃) δ 7.74 (d, J = 16.0 Hz, 2H), 6.89 (s, 4H), 6.74 (d, J = 16.0 Hz, 2H), 5.82 (s, 1H), 2.98 (s, 12H); ¹³C NMR (100 MHz, DMSO-d₆) δ 184.4, 152.1, 140.1, 130.5, 126.9, 123.3, 117.5, 103.6, 41.4; HRMS (FAB) calcd for C₂₃H₂₂Br₄N₂O₂ [M + H]+ 674.8488, found 674.8493.

(1E,4Z,6E)-1,7-bis(2,6-dichloro-4-(dimethylamino)phenyl)-5-hydroxyhepta-1,4,6-trien-3-one (1b):
The desired compound was obtained as orange solid (0.09 g, 0.18 mmol, 41% yield) starting from 5b: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.89 (d, $J = 16.1$ Hz, 2H), 6.90 (d, $J = 16.1$ Hz, 2H), 6.65 (s, 4H), 5.81 (s, 1H), 3.00 (s, 12H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 183.2, 151.1, 136.5, 134.3, 134.1, 127.0, 116.8, 112.6, 111.2, 103.4, 40.1; HRMS (FAB) calcd for C$_{23}$H$_{23}$Cl$_4$N$_2$O$_2$ [M + H]$^+$ 499.0508, found 499.0487.

$(1E,4Z,6E)$-1,7-bis(4-(dimethylamino)-2,6-dimethoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one (1c):

The desired compound was obtained as bright orange (0.15 g, 0.31 mmol, 68% yield) starting from 5c: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.08 (d, $J = 16.0$ Hz, 2H), 7.26 (s, 4H), 6.91 (d, $J = 16.0$ Hz, 2H), 5.83 (s, 4H), 5.74 (s, 1H), 3.88 (s, 12H), 3.04 (s, 12H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 184.2, 161.5, 153.5, 131.7, 120.4, 101.8, 101.6, 88.6, 56.0, 31.2; HRMS (FAB) calcd for C$_{27}$H$_{35}$N$_2$O$_6$ [M + H]$^+$ 483.2490, found 483.2483.

$(1E,4Z,6E)$-1,7-bis(4-(dimethylamino)-2,6-dimethylphenyl)-5-hydroxyhepta-1,4,6-trien-3-one (1d):
The desired compound was obtained as bright orange powder (0.12 g, 0.28 mmol, 62% yield) starting from 5d: $^1$H NMR (400 MHz, Acetone-$d_6$) $\delta$ 7.92 (d, $J$ = 16.1 Hz, 2H), 6.51 (S, 4H), 6.33 (d, $J$ = 16.1 Hz, 2H), 5.93 (s, 1H), 2.99 (s, 12H), 2.42 (s, 12H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 190.4, 153.3, 150.8, 143.7, 139.8, 121.3, 112.3, 40.1, 39.9, 21.5 (keto form), 183.5, 153.3, 143.7, 139.8, 138.2, 124.5, 112.8, 40.1, 22.7 (enol form); HRMS (FAB) calcd for C$_{27}$H$_{35}$N$_2$O$_2$ [M + H]$^+$ 419.2693, found 419.2701.

(1$E$,4$Z$,6$E$)-1,7-bis(4-(dimethylamino)phenyl)-5-hydroxyhepta-1,4,6-trien-3-one (1e):

![Chemical Structure](image)

The desired compound was obtained as orange powder (0.11 g, 0.31 mmol, 68% yield) starting from 5e: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.59 (d, $J$ = 15.7 Hz, 2H), 7.44 (d, $J$ = 8.8 Hz, 4H), 6.67 (d, $J$ = 8.8 Hz, 4H), 6.43 (d, $J$ = 15.7 Hz, 2H), 5.72 (s, 1H), 3.01 (s, 12H); HRMS (FAB) calcd for C$_{23}$H$_{27}$N$_2$O$_2$ [M + H]$^+$ 363.2067, found 363.2070.

References


2. Expression and purification of tau protein. To express the recombinant tau K18 protein, the cDNA that contained the His-tagged K18-tau protein was transformed in the Escherichia coli strain BL21(DE3). The transformed E. coli strain BL21(DE3) in LB medium containing ampicillin was inoculated with a stationary overnight culture. The culture was grown at 37 °C to an OD600 of 0.8–1.0, and protein expression was induced by the addition of 1 mM of isopropyl β-D-1-thiogalactopyranoside for 4 h. The cells were pelleted and sonicated. Recombinant tau was purified through a succession of Ni-Sepharose chromatography [equilibrated in 20 mM NaH₂PO₄, 500 mM NaCl, and 20 mM imidazole (pH 7.4), elution with 200 mM imidazole buffer]. The purity of the protein was verified on a Coomassie Brilliant Blue-stained sodium dodecyl sulfate-polyacrylamide gel. The elution buffer was changed to storage buffer (phosphate-buffered saline, PBS). The protein was concentrated and stored at -20 °C until use. The concentration of the purified tau was determined with the extinction coefficient at 280 nm (1,490 M⁻¹ cm⁻¹).
3. Preparation of aggregated tau protein. Monomeric tau protein was prepared by incubating 50 μM of the purified K18-tau protein in 25 mM Tris-HCl (pH 7.0), 50 mM NaCl, and 1 mM dithiothreitol (DTT) at 37 °C for 1 h in a LoBind tube (Eppendorf AG, Hamburg, Germany). After treatment with heparin (5 μM), the resulting mixture was incubated for 72 h at 37 °C in a shaking incubator. The tau aggregate that was formed was confirmed by a ThS-binding assay and atomic force microscopy (AFM).
4. Thioflavin S binding assay. K18-tau fibril formation was confirmed by ThS fluorescence. Reactions comprising K18-tau fibrils (50 μM) and various concentrations of ThS (0, 0.01, 0.1, 1, 10, and 100 μM) in PBS (pH 7.4) were analyzed at 440 nm (excitation) and 521 nm (emission), with an integration time of 1 s. Measurements were recorded with a SpectraMax spectrophotometer (Molecular Devices LLC) (Figure S1).

Figure S1. Thioflavin S binding assay of tau aggregates

Figure S2. Fluorescence intensity of ThS (20 μM) with or without tau aggregates
5. **Atomic Force Microscopy.** Fibril formation of K18-tau was further confirmed by AFM. K18-tau protein (50 μM) was incubated in 25 mM Tris-HCl (pH 7.0), 50 mM NaCl, and 1 mM DTT at 37 °C for 1 h in a LoBind tube (Eppendorf AG). After treatment with heparin (5 μM), the resulting mixture was incubated for 72 h at 37 °C in a shaking incubator. The aggregated tau protein was immobilized onto freshly cleaved mica. Excess protein was removed by washing with distilled water. AFM imaging was performed in noncontact mode in XE-100 (Park Systems, Suwon, Korea) with NCHR cantilevers (Park Systems) exhibiting a frequency of 6.39 KHz. The drive amplitude was set to 19.47 nm, and the amplitude set point was adjusted to 14.6 nm. (Figure S2).

**Figure S3.** An atomic force microscopic image confirming a tau aggregate (scale bar: 1 μm)
6. Fluorescence of the curcumin derivatives (1a–1e) with aggregated tau. Fluorescence of the curcumin derivatives (1a–1e) (50 μM) were examined in the absence and presence of the preaggregated tau proteins (50 μM) with optimized excitations and emissions for each of the compounds. Fluorescence was measured by SpectraMax (Molecular Devices LLC) in various solvents (Figures 2 and S4).
**Figure S4.** Solvent-dependent fluorescence of (a) curcumin and (b ~ f) its derivatives (1a ~ 1e) in the absence of the preaggregated tau proteins.
7. **In vitro tau-binding assay of 1c.** K18-tau fibrils (50 μM) were incubated with increasing concentrations of 1c (0, 0.01, 0.1, 1, 10, and 100 μM). The binding reactions were incubated for 10 min at rt in 100 μL of fibril incubation buffer [25 mM Tris-HCl (pH 7.0), 50 mM NaCl, and 1 mM DTT]. The fluorescence of each sample was measured by SpectraMax (Molecular Devices LLC) at an excitation of 520 nm and emission of 620 nm (Figure S3). The binding data were analyzed with curve fitting software that calculated the $K_d$ with a nonlinear regression (Sigmaplot; Systat Software, Inc., San Jose, CA, USA). All of the experiments were conducted in triplicate. The quantum yield of 1c was calculated from the dose-response curve obtained above with the following equation.\(^1\)\(^2\)

$$\Phi(X) = \Phi(ST) \left( \frac{\text{Grad}(X)}{\text{Grad}(ST)} \right) \left( \frac{\eta(X)^2}{\eta(ST)^2} \right)$$

Where ST and X denote the standard and test sample, respectively; $\Phi$ is the fluorescence quantum yield; Grad is the gradient from the plot of integrated fluorescence intensity versus absorbance; and $\eta$ is the refractive index of the solvent. Fluorescein isothiocyanate was used as the standard.

**Figure S5.** (A) Binding constant measurement of 1c with tau aggregates. (B) Linear concentration dependence of 1c in PBS ($R^2 = 0.9669$) indicates that 1c is not self-quenched with the range tested.
\[ Y = y_0 + 1 + e^{-\left(\frac{x - x_0}{b}\right)} \]
8. Titration of 1c with tau aggregate. Aggregate of tau was prepared by incubating 500 ng/mL of the purified K18-tau protein in 25 mM Tris-HCl (pH 7.0), 50 mM NaCl at 37 °C for 72 h. The prepared tau aggregate was serially diluted (500, 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9, 2 ng/mL) using PBS pH 7.4. Each of the diluted tau aggregate was treated with 1c (50 μM) and then, the fluorescence emission was monitored by SpectraMax (Molecular Devices LLC) after excitation at 520 nm.

Figure S6. Titration of 1c (50 μM) with tau aggregate in PBS (1% DMSO, pH 7.4)
9. Fluorescence of the curcumin derivative 1c with Aβ fibrils. The Aβ fibril was prepared according to our previous publication. Fluorescence of the curcumin derivative 1c (50 μM) was examined in the absence and presence of the preaggregated Aβ (50 μM) (λ<sub>ex</sub> = 500 nm, λ<sub>em</sub> = 620 nm). Fluorescence was measured by SpectraMax (Molecular Devices LLC) in 25 mM Tris-HCl, pH 7.0, 50 mM NaCl (Figure S5).

Figure S7. The fluorescence intensity of 1c upon interaction with Aβ fibrils in Tris-HCl (black solid line: with tau aggregate, black dashed line: with Aβ fibrils, red lines: with neither tau aggregate nor Aβ)
**10. Detection of the tau aggregates by 1c in SHSY-5Y cells.** Human neuroblastoma SH-SY5Y cells were purchased from ATCC (American Type Culture Collection, Manassas, VA, USA). For the fluorescence microscopic analysis, SH-SY5Y cells were seeded at $5 \times 10^5$ in a glass-bottom cell culture dish (Nest Biotechnology Co., Ltd., Wuxi, China) and cultured in Dulbecco’s Modified Eagle’s Medium/F-12 medium with 10% fetal calf serum. Cells at 70% confluence were transfected with pCMV6-htau40-green fluorescent protein (GFP) (OriGene Technologies, Inc., Rockville, MD, USA) with TransIT-LT1 (Mirus Bio LLC). After 72 h, the cells were treated with 1c (5 μM) for 5 min and washed 3 times with PBS. The cells were then monitored by a Zeiss LSM701 confocal microscope (Carl Zeiss Microscopy GmbH) at 520 nm for GFP and 620 nm for compound 1c.
Figure S8. Confocal microscope images of the non-transfected and the tau-GFP-transfected SHSY-5Y cells before or after treatment with 1c. (A ~ B) SHSY-5Y cells without expression of tau were observed by a confocal microscope (at 620 nm, λ_{ex} of 1c) after treatment with 1c: (A) Fluorescence image and (B) differential interference contrast (DIC) image. (C ~ D) Confocal microscope images of tau-GFP-transfected SHSY-5Y cells before treatment with 1c: (C) Fluorescence image and (D) differential interference contrast (DIC) image. White arrows indicate tau aggregates in vacuole compartment.
References

