

Electronic Supplementary Information

Small molecule-mediated co-assembly of amyloid- β oligomers

reduces neurotoxicity through promoting non-fibrillar aggregation

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1. Experimental section

1.1. Reagents and materials. All reagents used in this study were purchased from commercial suppliers and used as received unless otherwise stated. Human A β 40 and A β 42 was purchased from GL Biochem Ltd. (Shanghai, China). Stock solution of A β was prepared according to the literature method¹. The concentration of A β was immediately measured with a BCA Protein Assay Kit (Pierce). Stock solution of EC was obtained by dissolving the compound in DMSO and filtered using a 0.22 μ M filter (organic system). All solutions and buffers were obtained using Milli-Q water, and filtered through a 0.22 μ M filter (Millipore) before used. C57BL/6J mice were purchased from Model Animal Research Center of Nanjing University (MARC). All animal experiments were conducted in accordance with the National Institutes of Health “Guide for the Care and Use of Laboratory Animals” and were approved by the Institutional Animal Care and Use Committee of MARC.

Caenorhabditis elegans (*C. elegans*) strains CL4176, CL802, CL212, CL2355 were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis, MN, USA). A transgenic strain CL4176 (dvIs27 [myo-3p::A β ₁₋₄₂::let-851 3'UTR + rol-6(su1006)]) expresses A β gene in muscle tissue. CL802 (smg-1 (cc546ts)I; rol-6 (su1006)II) as a control strain of CL4176 does not express A β gene. CL2355 (dvIs50 [pCL45 (snb-1::A β ₁₋₄₂::3' UTR(long) + mtl-2::GFP]) is a neuronal A β -expressing transgenic strain and CL2122 (dvIs15 [(pPD30.38) unc-54(vector) + (pCL26) mtl-2::GFP]) is control strain. The strains were cultured on Nematode Growth Media (NGM) plates with *E.coli* strain OP50 as food sources in Luria Broth (LB) medium. CaCl₂ (0.6 mL, 1 M), cholesterol (0.6 mL, 5 g/L in ethanol), MgSO₄ (0.6 mL, 1 M), and KPO₄ buffer (15 mL, 1 M; 17.8 g KH₂PO₄, 54.2 g K₂HPO₄, 500 mL, pH 6.0) were added to the autoclaved NGM (12.0 g agar, 1.5 g peptone, 1.8 g NaCl, 590 mL H₂O). Aliquot 5 mL of liquid NGM into each Petri Dish and allow them to solidify overnight at room temperature. After autoclavation of LB medium (10.0 g NaCl, 5.0 g yeast extract, 10.0 g tryptone, 1.0 L H₂O), *E.coli* strain OP50 were grown in LB medium. Each NGM plate was spotted with 100 μ L of OP50 and allowed to dry for 6 h, then transferred to 37 °C overnight.

1.2. Methods. ^1H and ^{13}C NMR spectra were performed on a Bruker DRX-400 spectrometer. Electrospray ionization mass spectra (ESI-MS) were carried out with LCQ Fleet electrospray mass spectrometer. Elemental analysis was recorded on a CHNO-Rapid analyzer (Heraeus, Germany). Circular dichroism (CD) spectra were measured by using a JASCO J-810 automatic recording spectropolarimeter (Tokyo, Japan). UV-vis spectra were acquired on a Shimadzu UV-3600 UV-VIS-NIR spectrophotometer. Fluorescence spectra were obtained on a PerkinElmer FL6500 fluorescence spectrometer. Turbidity, ThT, and BCA assays were determined on a Varioskan Flash microplate reader (Thermo Scientific). Transmission electron microscopy (TEM) images were recorded on a transmission electron microscope (JEOL, JEM-2100 LaB6).

1.3. Synthesis of EC. *Synthesis of 2-(4-nitrophenyl)imidazo[1,2-a]pyridine (1).* A mixture of 2-aminopyridine (0.376 g, 4 mmol) and α -bromo-*p*-nitroacetophenone (0.486 g, 2 mmol) was dissolved in 20 mL of dry CH_3CN and then refluxed at 80 °C under stirring for 3 h. The precipitate was collected by filtration and dried to give compound **1** as an orange solid (0.431 g, yield: 90%). ^1H NMR (CDCl_3 , 400 MHz, δ , ppm): 8.31 (d, 2H, $J = 9.2$, Ph), 8.19 (d, $J = 6.8$ Hz, 1H, imidazo[1,2-a]pyridine), 8.14 (d, 2H, Ph), 8.01 (s, 1H, imidazo[1,2-a]pyridine), 7.74 (d, $J = 9.2$ Hz, 1H, imidazo[1,2-a]pyridine), 7.30 (t, 1H, $J = 7.8$ Hz, imidazo[1,2-a]pyridine), 6.90 (t, $J = 6.6$ Hz, 1H, imidazo[1,2-a]pyridine). ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz, δ , ppm): 112.17, 113.42, 117.44, 124.66, 126.44, 126.78, 127.75, 140.95, 142.42, 145.70, 146.96. ESI-MS found (calcd) for $\text{C}_{13}\text{H}_9\text{N}_3\text{O}_2$ (m/z): 240.42 (240.07) $[\text{M} + \text{H}]^+$. Elemental analysis found (calcd) for $\text{C}_{13}\text{H}_9\text{N}_3\text{O}_2$ (%): C, 64.36 (65.27); H, 4.01 (3.79); N, 17.40 (17.56). IR (ν_{max} , cm^{-1}): 3106, 1635, 1599, 1512, 1336, 1109, 855, 759, 744, 718.

Synthesis of 4-(imidazo[1,2-a]pyridin-2-yl)aniline (2). A solution of **1** (0.120g, 0.5 mmol) in the ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate [bmin][BF_4] (2 mL) was sonicated for 15 min to make it uniformly dispersed. $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (0.564 g, 2.5 mmol) was added and the mixture was sonicated overnight to form a pale yellow viscous solution. Water (200 mL) were added to the solution and saturated aqueous solution of Na_2CO_3 was used to adjust the pH of the solution as 8~9. The reaction

mixture was extracted with diethyl ether. The combined ether extracts was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford a pale yellow solid (0.056 g, yield: 54%). ¹H NMR (DMSO-d₆, 400 MHz, δ, ppm): 8.45 (d, *J* = 6.8 Hz, 1H, imidazo[1,2-a]pyridine), 8.11 (s, 1H, imidazo[1,2-a]pyridine), 7.62 (d, *J* = 8.4 Hz, 2H, Ph), 7.48 (d, *J* = 8.8 Hz, 1H, imidazo[1,2-a]pyridine), 7.17 (t, *J* = 8 Hz, 1H, imidazo[1,2-a]pyridine), 6.82 (t, *J* = 6 Hz, 1H, imidazo[1,2-a]pyridine), 6.61 (d, *J* = 8.8 Hz, 2H, Ph). ¹³C NMR (DMSO-d₆, 100 MHz, δ, ppm): 107.12, 112.13, 114.35, 116.52, 122.08, 124.55, 126.85, 127.08, 145.04, 146.20, 149.09. ESI-MS found (calcd) for C₁₃H₁₁N₃ (*m/z*): 210.33 (210.10) [M + H]⁺. Elemental analysis found (calcd) for C₁₃H₁₁N₃ (%): C, 73.54 (74.62); H, 5.57 (5.30); N, 19.18 (20.08). IR (ν_{max}, cm⁻¹): 3394, 3306, 3197, 3135, 1630, 1609, 1487, 1283, 1267, 1179, 1078, 834, 756, 748, 636.

Synthesis of 2,2'-((((carboxymethyl)azanediyl)bis(ethane-2,1-diyl))bis((2-((4-(imidazo[1,2-a]pyridin-2-yl)phenyl)amino)-2-oxoethyl)azanediyl))diacetic acid (L-3H). It was synthesized according to the literature². To a solution of **2** (0.167 g, 0.8 mmol) in DMF (6 mL, anhydrous treatment) were added dropwise triethylamine (1 mL, anhydrous) and DTPA dianhydride (0.143 g, 0.4 mmol) in 8 mL DMF under stirring in nitrogen at 0 °C. Then the ice bath was removed after 2 h and the reaction mixture was stirred at room temperature for 5 h. The reaction was quenched by H₂O (10 ml) and the solvent was evaporated to 1 mL. Acetone (50 ml) was added to the residue and the resulting precipitate was filtered, washed with chloroform and ether, and dried under vacuum to give L-3H as a pale powder (0.143 g, yield: 46%). ¹H NMR (DMSO-d₆, 400 MHz, δ, ppm): 10.19 (s, 2H, -NH-), 8.49 (dd, *J* = 6.8, 17.2 Hz, 2H, imidazo[1,2-a]pyridine), 8.25 (s, 2H, imidazo[1,2-a]pyridine), 7.90–7.85 (m, 4H, Ph), 7.76 (dd, *J* = 8.4, 15.6 Hz, 4H, Ph), 7.55 (d, *J* = 9.2, 2H, imidazo[1,2-a]pyridine), 7.21 (t, *J* = 8 Hz, 2H, imidazo[1,2-a]pyridine), 6.85 (t, *J* = 6 Hz, 2H, imidazo[1,2-a]pyridine), 3.45–3.44 (m, 4H, -CH₂-), 3.06–2.97 (m, 8H, -CH₂-), 2.54 (s, 6H, -CH₂-). ¹³C NMR (DMSO-d₆, 100 MHz, δ, ppm): 55.25, 58.32, 108.44, 112.12, 116.36, 119.49, 124.80, 125.89, 126.69, 128.84, 138.31, 144.18, 144.69, 169.37, 172.57, 173.11. Elemental analysis found (calcd) for C₄₀H₄₁N₉O₈ (%): C, 63.21 (61.93); H, 5.39 (5.33); N, 15.44 (16.25).

IR (ν_{\max} , cm^{-1}): 3410, 1632, 1529, 1486, 1396, 1318, 1251, 1082, 844, 758, 706.

Synthesis of Eu(L)(H₂O) (EC). A solution of Eu(NO₃)₃·6H₂O (0.0892 g, 0.2 mmol) in water (5 mL) was slowly added to the aqueous solution (10 mL) of the ligand L-3H (0.155 g, 0.2 mmol). The pH of the mixture was maintained at 6 by adding aliquots of NaOH (5 M). The mixture was stirred at 45 °C overnight and the precipitate was formed. The product was collected by filtration and washed with water. Then, the obtained light yellow solid was dried in vacuum (0.072 g yield: 38%). ESI-MS found (calcd) for C₄₀H₄₀N₉O₉Eu (m/z): 948.25 (948.22) [M – H₂O + Na]⁺. Elemental analysis found (calcd) for C₄₀H₄₀N₉O₉Eu (%): C, 51.23 (50.96); H, 4.39 (4.28); N, 15.44 (13.37). IR (ν_{\max} , cm^{-1}): 3415, 1604, 1561, 1509, 1487, 1384, 1328, 1254, 1093, 964, 927, 850, 758.

1.4. Time-resolved luminescence of EC. A β 40 (20 μM) was co-incubated with ThT (2 μM) in Tris-HCl buffer (20 mM Tris-HCl/150 mM NaCl, pH 7.4). The ThT fluorescence was recorded over time at 484 nm with excitation wavelength of 440 nm. The obtained kinetics curve was used to estimate A β aggregation states. A β samples (20 μM) within the lag phase were prepared in buffer (20 mM Tris-HCl, 150 mM NaCl, pH 7.4) at 37 °C for 0 h, 6 h, 12 h, and 24 h, respectively. EC (20 μM) was added to the above samples and then the time-resolved luminescence spectra of the resulting solutions were measured on a PerkinElmer FL6500 fluorescence spectrometer with the following settings: delay time, 50 μs ; gate time, 2.00 ms; excitation slit, 20 nm; and emission slit 20 nm.

1.5. Docking studies. The geometry optimization for EC was first performed at the B3LYP/6-31G* level using the program Gaussian 09. The molecular docking program Autodock 4.2.3 and Autodock tools 1.5.4 were employed to dock EC to A β .³ The molecular structures of A β monomer (PDB ID: 1BA4), trimer (PDB ID: 5HOX), and fibrils (PDB ID: 2LMO) were downloaded from the Protein Data Bank (www.rcsb.org/pdb). During the calculation, all the water molecules and ligands were removed from the proteins. The Gasteiger charges and polar hydrogen bonds were added prior to docking. A grid of 126, 100, and 60 points in x, y, and z directions

centered on fibrils, trimer, and monomer, respectively was constructed with a grid spacing of 0.375 Å. A Lamarckian Genetic Algorithm method was used in the Autodocking. The GA population size, number of GA runs, maximum number of energy evaluation, and maximum number of generations were set to 150, 100, 2.5×10^7 , and 2700, respectively. All other docking parameters were kept as default values. The lowest energy conformations were selected and figures of EC docked with trimers were depicted using Pymol. The lowest energies were analyzed by Autodock tools 1.5.4.

1.6. Aggregation kinetics by turbidity assay. A β in buffer solution (20 μ M, 20 mM Tris-HCl/150 mM NaCl, 4% v/v DMSO, pH 7.4) was incubated with or without different concentrations of EC (5, 10, 20, and 40 μ M) at 37 °C. Each incubated solution was assayed by turbidity at wavelength of 405 nm to evaluate the aggregation degree at different incubation time. Aliquots of Tris buffer solution (4% v/v DMSO) were used to blank the spectrophotometer. Each curve was fit to the sigmoidal equation:⁴

$$y = y_0 + A/(1 + \exp(-k(t - t_{50}))) \quad (S1)$$

where y is the turbidity at time t , y_0 is the turbidity at time zero, A represents the maximum turbidity at equilibrium, t_{50} corresponds to the time at half completion of the aggregation process, and k is the apparent first-order rate constant for elongation. The lag time, t_{lag} , was calculated from the fitted parameters as $t_{50} - 2/k$.^{4,5}

1.7. Western blotting. The sample solutions were divided into two groups. In the first group, A β 40 in buffer solution (20 μ M, 20 mM Tris-HCl/150 mM NaCl, 4% v/v DMSO, pH 7.4) was incubated in the absence and presence of EC with different concentrations (5, 10, and 20 μ M) at 37 °C for 24 h. In another group, A β 40 (20 μ M) in the same buffer solution with that of first group was incubated with or without EC (20 μ M) at 37 °C for different time (0, 12, 48, and 72 h). The samples were dissolved in loading buffer containing a small amount of DL-dithiothreitol. Each sample was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes. The membranes were blocked for 1 h at room temperature with non-fat milk powder (5%) and incubated at 4 °C overnight with monoclonal anti-A β antibody 6E10 (1:1000 dilution in PBS buffer with 0.1% Tween 20 (PBST)). The membranes were then washed ($\times 3$) with PBST buffer and incubated

with the HRP-conjugated goat anti-mouse antibody (1: 1000 dilution in PBST) for 1 h at room temperature. Membranes were washed ($\times 3$) with PBST buffer and bands were visualized using ClineX ChemiScope 6000EXP chemiluminescence imaging system.

1.8. Dot blotting. Samples were prepared as described in the Western blot assay. 4 μL aliquots of each sample were spotted onto polyvinylidene difluoride (PVDF) membranes and dried at room temperature. The membranes were blocked for 1 h at room temperature with non-fat milk powder (5%) and incubated at 4 $^{\circ}\text{C}$ overnight with monoclonal anti-A β antibody 6E10 or anti-oligomer antibody A11 (1: 1000 dilution in PBST). The membranes were then washed ($\times 3$) with PBST buffer and incubated with the HRP-conjugated goat anti-mouse IgG or the HRP-conjugated anti-rabbit IgG (1: 1000 dilution in PBST) for 1 h at room temperature. Membranes were washed ($\times 3$) with PBST buffer and were visualized using ClineX ChemiScope 6000EXP chemiluminescence imaging system.

1.9. Transmission electron microscopy (TEM). Samples were incubated as described in the Western blot assay and vortexed for use. An aliquot of each solution (10 μL) was spotted on the 300-mesh carbon-coated copper grids for 2 min at room temperature and excess sample was removed. Each grid was stained with uranyl acetate (1%, w/v) for 1 min. The excess uranyl acetate was removed and water (10 μL) was spotted on each grid to wash the sample and then was removed. Finally, each grid was examined on a JEOL JEM-2100 LaB $_6$ (HR) transmission electron microscope.

1.10. Circular dichroism (CD) measurements. Samples were prepared in the same way as in the TEM experiments. CD characteristic spectra of these sample solutions were then scanned from 170 to 360 nm with 1 nm bandwidth by Chirascan Circular Dichroism Spectropolarimeter.

1.11. Dynamic light scattering. A β 40 in buffer solution (20 μM , 20 mM Tris-HCl/150 mM NaCl, 4% v/v DMSO, pH 7.4) was incubated with or without EC (20 μM) at 37 $^{\circ}\text{C}$ for 84 h to make sure that all samples enter their plateau phases of the kinetics experiments. The samples were diluted 10 times with Milli-Q water and measured by Zetasizer Nano S90 ZEN1690 (Malvern Instruments, Ltd., United Kingdom) to get hydrodynamic radii.

1.12. Seed-catalyzed kinetic assay. Seeds were prepared in vitro by incubating 200 μ M of A β 40 with or without an equimolar EC for 24 h or 48 h in Tris buffer (20 mM Tris-HCl/150 mM NaCl, 4% v/v DMSO, pH 7.4) at 37 °C, respectively. The seeds were sonicated for 2 min before the use. 10% (based on the monomeric A β 40, v/v) seeds were added in Tris buffer containing 20 μ M monomeric A β 40. The kinetics was monitored by the turbidity assay. A β 40 in buffer solution (20 μ M, 20 mM Tris-HCl/150 mM NaCl, 2% v/v DMSO, pH 7.4) was used as the control.

1.13. Cytotoxicity assay. The cytotoxicity was performed by MTT assay with human neuroblastoma SH-SY5Y cell line, *via* the cleavage of MTT to purple formazan crystals by cell mitochondrial dehydrogenases. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and incubated in a humidified incubator at 37 °C with 5% CO₂. The SH-SY5Y cells were seeded in a 96-well flat bottomed microplate at 6000 cells per well and incubated for 24 h at 37 °C in a humidified atmosphere with 5% CO₂. A β 40 (40 μ M) was incubated with or without different concentrations (5, 10, 20 μ M, final DMSO concentration 4%) of EC in DMEM with 10% FBS for 12 h to prepare EC-generated aggregates and oligomers, respectively, and then were added to wells. The samples were incubated for 24h at 37 °C in a humidified atmosphere with 5% CO₂. Then the supernatants were removed, and the formazan crystals were dissolved in 300 μ L DMSO. The absorbance at 490 nm was determined using a Varioskan Flash microplate reader. The data were normalized and calculated as a percentage of untreated cells only containing 4% DMSO as control.

1.14. Immunofluorescence staining. The murine microglial BV-2 cells were cultured in DMEM supplemented with 10% FBS, incubated in a humidified incubator at 37 °C with 5% CO₂. The cells were seeded in 20 mm glass bottom cell culture dishes (Nest) and incubated for 24 h at 37 °C in a humidified atmosphere with 5% CO₂. The samples of A β 40 (10 μ M) incubated with or without EC (10 μ M, final DMSO concentration 1%) in DMEM with 10% FBS for 72 h were added to dishes. The samples were incubated for 4h at 37 °C in a humidified atmosphere with 5% CO₂. Then the culture medium were removed. The cells were washed three times for 15 min with cold PBS and fixed

with 4% paraformaldehyde for 15 min. After washing three times with cold PBS, cells were permeabilized with 0.5% Triton X-100 in PBS for 30 min. Then, the cells were washed by cold PBS for 5 min, blocked for 15 min at room temperature with 0.5% BSA dissolved in PBS. Cells were subsequently incubated with primary antibody 6E10 (dilution, 1:200) at 4 °C overnight. After washing with PBS three times, they were incubated with the Alexa 488 conjugated goat anti-mouse IgG secondary antibody (dilution, 1:200) for 1 h at room temperature. Finally, cells were washed three times with PBS and observed under Zeiss LSM 710 laser scanning microscope. The images were analyzed off-line using ZEN software. In addition, NP40 lysate and various enzyme inhibitors (1% 1 M DTT, 1% 0.1 M NaF, 1% 100 mM NaNO₃, 1% 1M PMST, 1% 100×Pro) were added to the treated BV-2 cells. The proteins of the samples were extracted by centrifugation at 12000 rpm for 15 min at 4 °C. The total concentration of proteins in each sample was measured by the BCA Protein Assay Kit (Pierce) and was adjusted to be the same with the Milli-Q water. Then the samples were analyzed by dot blot assay.

1.15. Acridine orange (AO) staining/Autophagy detection. The procedure of cell culture and treatment with A β samples was the same as that of immunofluorescence staining. The treated cells were stained with 1 μ g/mL AO in PBS for 15 min at 37 °C in the dark. Then, cells were washed three times with PBS and observed under Zeiss LSM 710 laser scanning microscope (excitation: 488 nm band-pass filter; emission: 500 to 560 nm band-pass filter or 600 to 700 nm long-pass filter). The images were analyzed off-line using ZEN software.

1.16. Worm paralysis assay. Strains CL4176 and CL802 were age-synchronized onto new NGM plates spotted with OP50 containing either a vehicle or EC. The worms were incubated at 15 °C until they reached L2 larval stage, then up-shifted to 25 °C. The paralysis of worms was estimated at the L4 larval stage. The number of paralyzed worm was scored at two hours intervals until all worms became paralyzed. The worm was considered paralyzed if it only moved head or did not move at all when gently touched with a platinum loop.

1.17. Chemotaxis behavior assay. The synchronized transgenic strain CL2355 and its

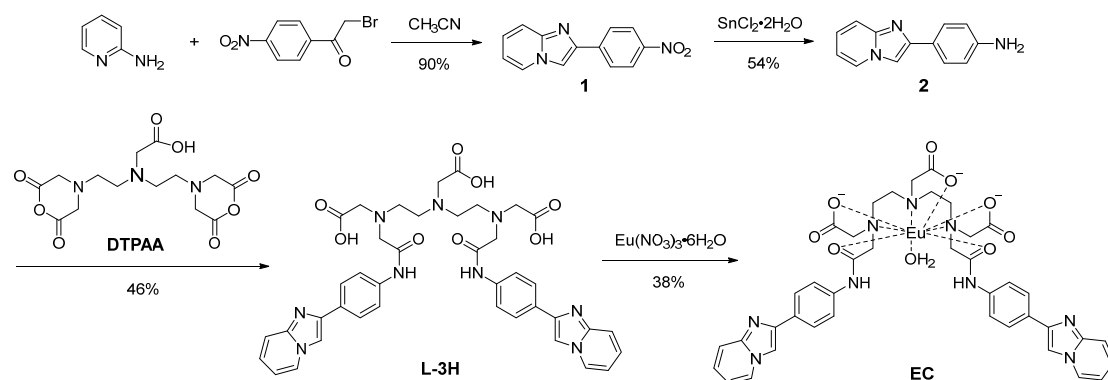
control strain CL2122 were incubated in 16 °C for 36 h and then in 25 °C for 36 h. The worms were collected, washed with M9 buffer (3.0 g KH₂PO₄, 6.0 g Na₂HPO₄, 5.0 g NaCl, 1.0 ml MgSO₄ (1 M), 1.0 L H₂O) three times, and transferred to the center of 60 × 10 mm agar plates. Immediately, 1 µL attractant (0.5% benzaldehyde in ethanol) and control odorant (100% ethanol) was pipetted onto the two polar ends of the plate in the opposite direction with equal distance from the center, respectively, along with 1 µL sodium azide (1 M). Sodium azide was used as anesthetic to arrest the worms upon reaching the solutions. After incubation of the plates at 25 °C for 1 h, the number of worms in different areas and their total number were recorded. A chemotaxis index (CI) with values from -1.0 to +1.0 was calculated to indicate the chemotaxis behavior of worms. An index of +1.0 indicates maximal attraction toward the attractant. An index of -1.0 is evidence of maximal repulsion. Values are averages from at least 3 independent experiments (n >30 worms) for each condition.

1.18. Western blot analysis of A β from *C. elegans*. The worms CL4176 were treated in the same way as in the paralysis assay. The worms were collected and washed with M9 buffer to remove *E. coli*. After quickly frozen in liquid nitrogen, the worms were lysed in M9 buffer by SCIENTZ JY92 Ultrasonic Homogenizer. The supernatant was collected by centrifugation, quantified by the BCA Protein Assay Kit (Pierce), and then dissolved by loading buffer and heated at 95°C for 5 min. The final samples were detected by Western blotting as described in the Western blot assay mentioned above. β -actin was used as internal reference to ensure equal protein loading, which was detected with anti- β -actin antibody (1:2000, Abcam).

1.19. BBB penetration assay. Both control and treatment groups contained four mice (C57BL/6J, male, 4 – 6 weeks old). Each mouse was given EC (4.7 mg/kg, dissolved in a 10 : 90 DMSO/H₂O mixture, 200 µL) via the tail vein injection. The mice were sacrificed at different post-injection time (2, 10, 30, and 60 min). The brain tissues were removed, rinsed with cold PBS, and then were homogenized with NP40 lysate (2 mL). The homogenates were mixed with MeOH (1 mL) and centrifuged at 4 °C. The supernatants (500 µL) were digested by concentrated HNO₃ at 95 °C, 30% H₂O₂ and concentrated HCl at 37 °C. The europium amount in the samples was determined by

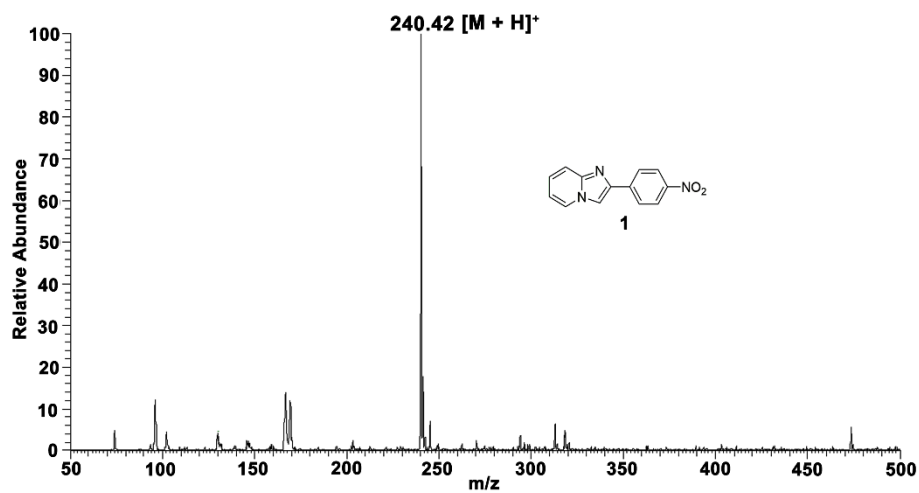
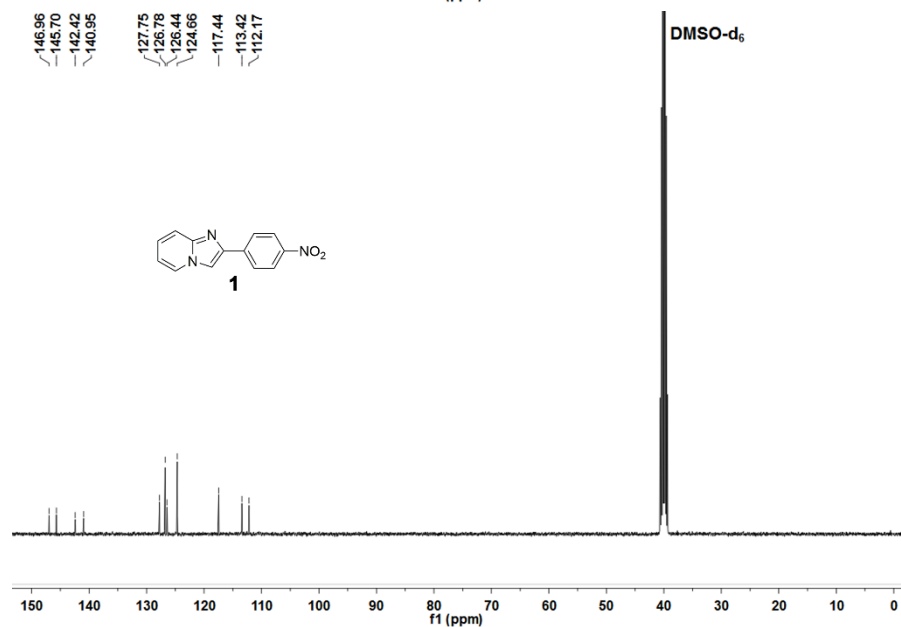
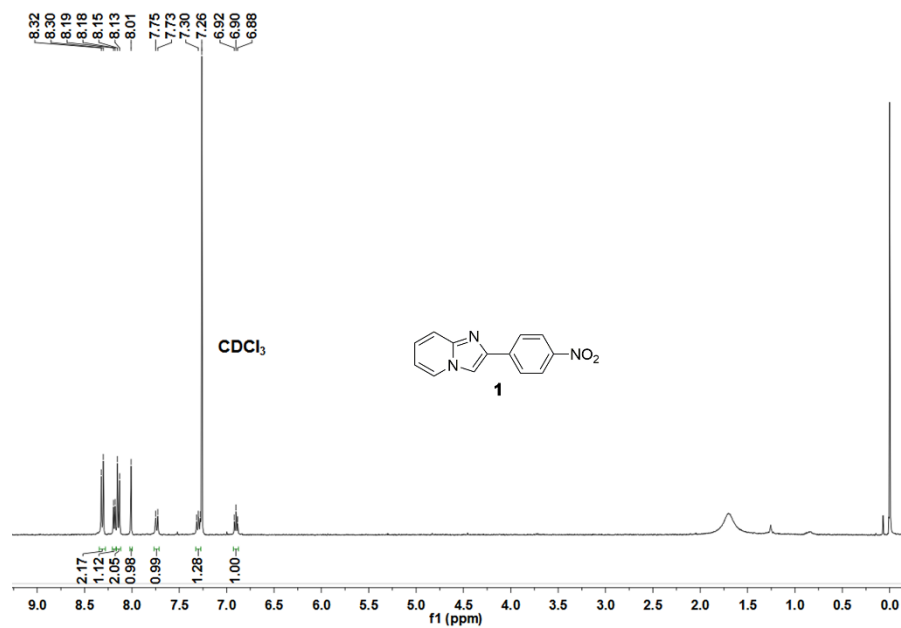
ICP-MS. To determine the wastage of the compound during homogenate, extraction, and centrifugation, the recovery rate was measured based on the control experiment: C57BL/6J mice were sacrificed and the brains were removed. 200 μ L of the solution of EC was directly injected into the brains ($n = 4$) and incubated for 1 h at 37 $^{\circ}$ C, and then treated by the previous procedures.

2. Supplementary Scheme, Figures and Tables

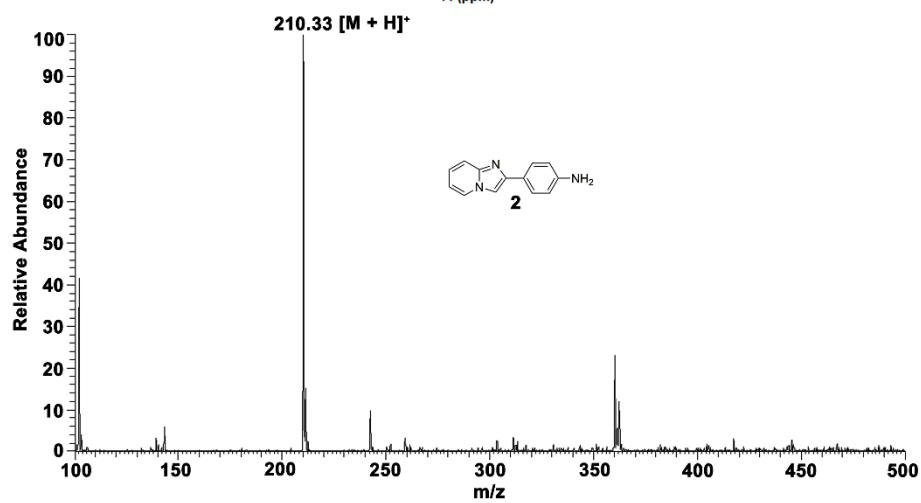
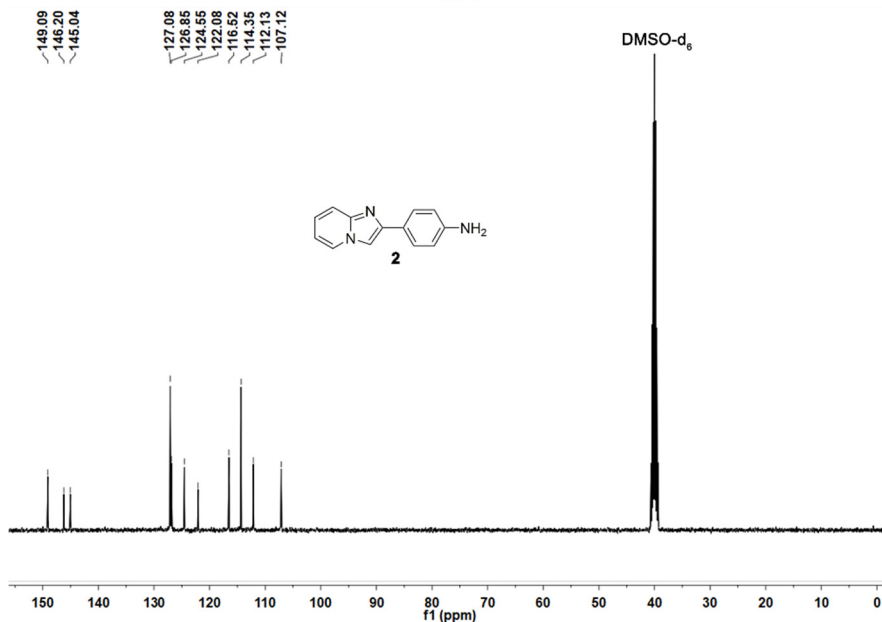
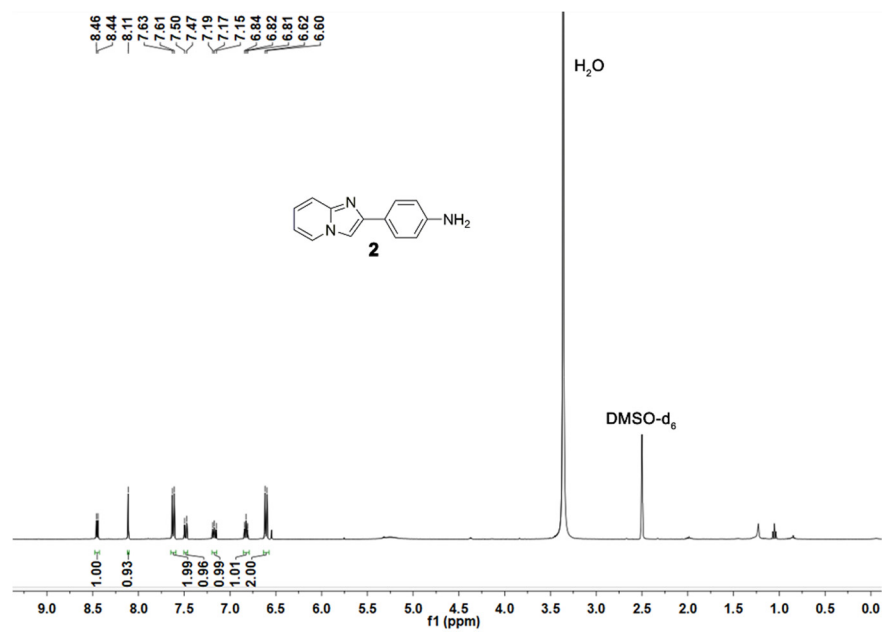


Scheme S1. Synthetic route to EC.

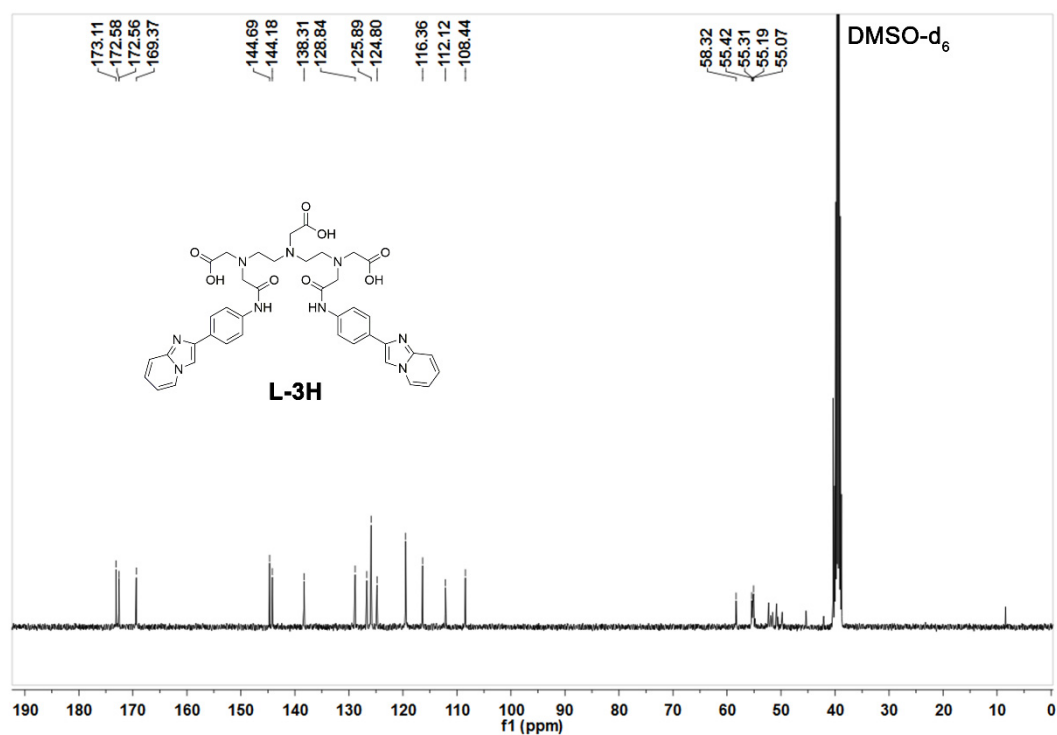
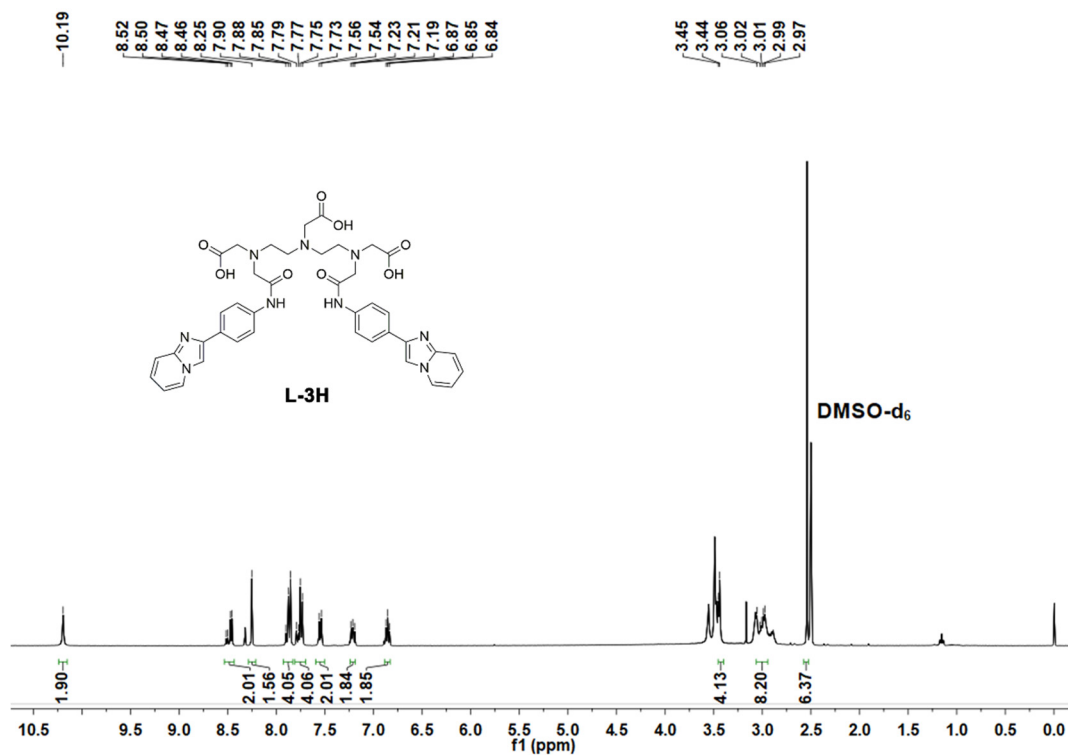
A



B



C



D

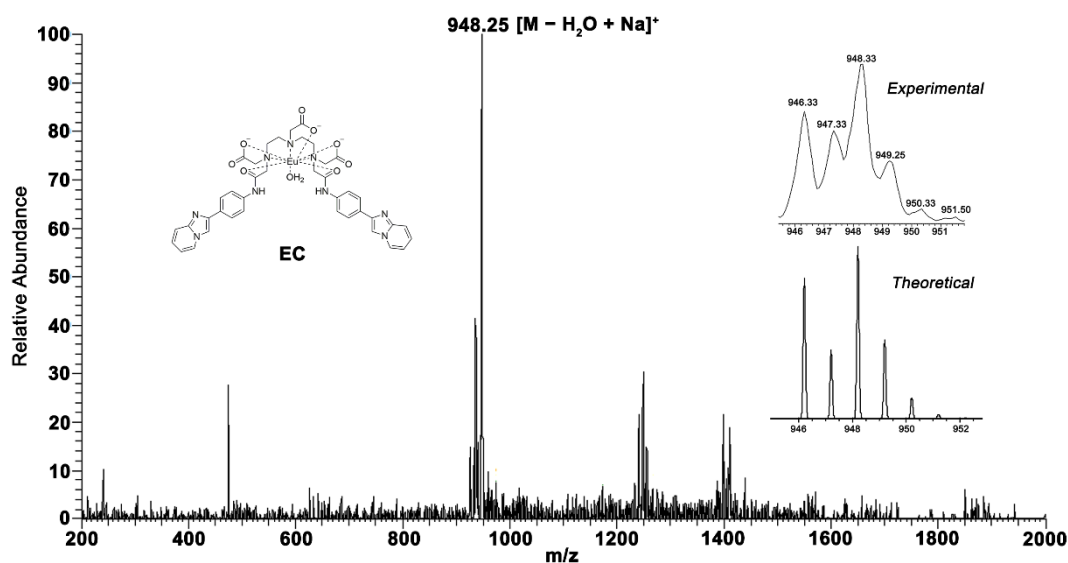


Fig. S1. (A) ^1H NMR (CDCl_3), ^{13}C NMR ($\text{DMSO}-d_6$), and ESI-MS spectra of compound **1**. (B) ^1H NMR ($\text{DMSO}-d_6$), ^{13}C NMR ($\text{DMSO}-d_6$), and ESI-MS spectra of compound **2**. (C) ^1H NMR ($\text{DMSO}-d_6$) and ^{13}C NMR ($\text{DMSO}-d_6$) spectra of compound **L-3H**. (D) ESI-MS spectra of compound **EC**. Insets are the determined isotopic distribution pattern and the corresponding simulated one of the observed peaks.

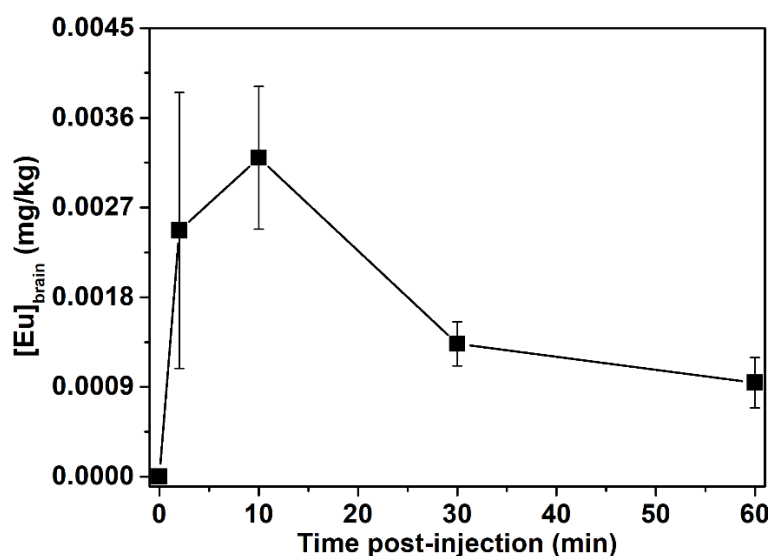


Fig. S2. The levels of Eu in the brain of C57BL/6J mice at different post-injection time after i.v. injection of 4.7 mg EC per kilogram body weight. The brain homogenates were analyzed for the levels of Eu by inductively coupled plasma mass spectrometry (ICP-MS). Error bars indicate mean \pm s.d. ($n = 4$ independent experiments).

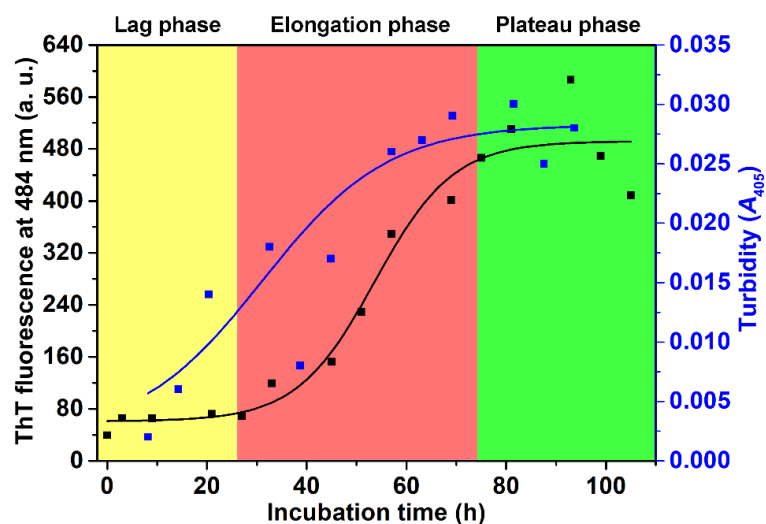


Fig. S3. Kinetics of Aβ40 (20 μM) self-aggregation monitored by ThT fluorescence assay ($\lambda_{\text{ex}} = 440$ nm) and turbidity assay (A_{405}) in Tris-HCl buffer (20 mM Tris-HCl/150 mM NaCl, 4% v/v DMSO, pH 7.4) at 37 °C, respectively. The sigmoidal curve of ThT fluorescence consists of three phases, i.e., lag phase, elongation phase, and plateau phase. The lag phase is relatively slow, in which soluble monomers aggregate into oligomers or nuclei, subsequently transform into protofibrils. This is followed by a fast elongation phase in which the protofibrils grow rapidly to form mature fibrils until reaching the steady state with maximum fibril growth as a plateau phase.

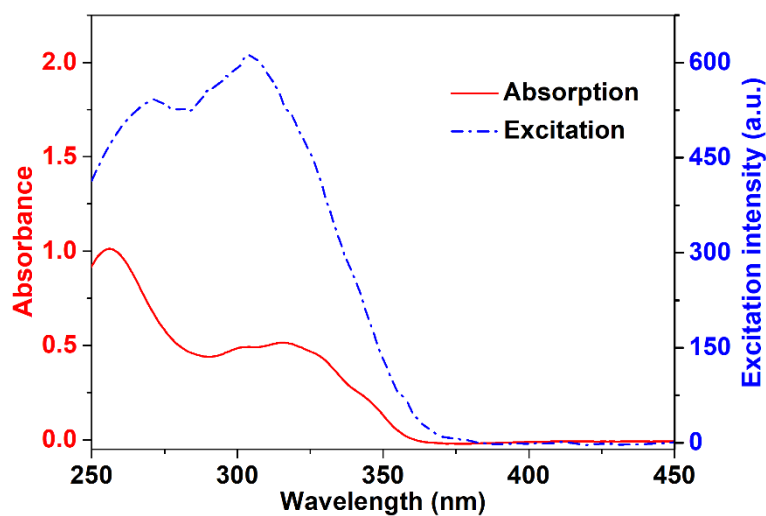


Fig. S4. The absorption and excitation spectra of EC (20 μM) in Tris-HCl buffer (20 mM Tris-HCl/150 mM NaCl, 4% v/v DMSO, pH 7.4).

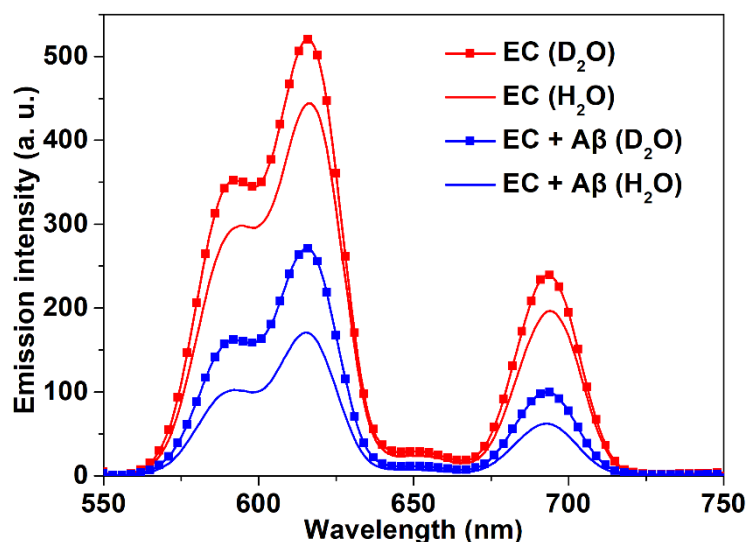


Fig. S5. The time-resolved luminescence spectra of EC (20 μ M, $\lambda_{\text{ex}} = 300$ nm) in the presence and absence of A β 40 (20 μ M) determined in H₂O and D₂O.

Table S1. Lowest docking energies and inhibition constants (K_i) for the binding between EC and different A β species calculated by the AutoDock program.

| A β species | E_{HVd} (KJ mol ⁻¹) ^a | E_e (KJ mol ⁻¹) | E_{im} (KJ mol ⁻¹) ^b | E_t (KJ mol ⁻¹) | ΔG (KJ mol ⁻¹) ^c | K_i (nM) |
|-------------------------------------|---|-------------------------------|--|-------------------------------|---|------------|
| A β 40 trimer (PDB ID: 5HOX) | -48.83 | -1.46 | -50.29 | +6.90 | -43.39 | 24.85 |
| A β 40 monomer (PDB ID: 1BA4) | -36.99 | -7.57 | -44.56 | +5.73 | -38.83 | 156.97 |
| A β 40 fibrils (PDB ID: 2LMO) | -30.91 | -2.01 | -32.92 | +5.73 | -27.19 | 17320 |

^a $E_{\text{HVd}} = E_{\text{H}}$ (H-bonding energy) + E_{V} (van der Waals energy) + E_{d} (desolvo energy); ^b E_{im} (intermolecular energy) = $E_{\text{HVd}} + E_e$ (electrostatic energy); ^c ΔG (binding free energy) = $E_{\text{im}} + E_t$ (torsional energy).

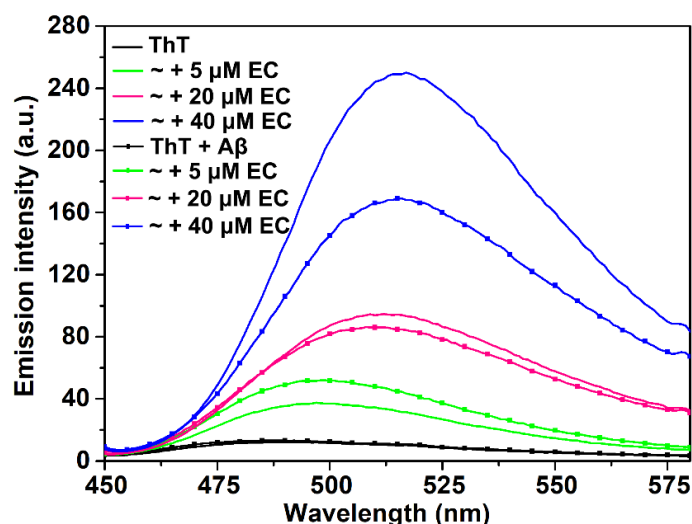


Fig. S6. Effect of EC on ThT fluorescence ($\lambda_{\text{ex}} = 440$ nm) in the absence or presence of A β 40 in Tris-HCl buffer (20 mM Tris-HCl/150 mM NaCl, 4% v/v DMSO, pH 7.4) ([ThT] = [A β 40] = 20 μ M).

Table S2. The aggregation kinetics of 20 μ M A β 40 peptide in the presence of 0, 5, 10, 20, or 40 μ M EC.^a

| Samples | t_{50} (h) | k (h^{-1}) |
|---------------------------|------------------|-------------------------|
| A β | 25.69 ± 4.41 | 0.08 ± 0.01 |
| A β + 5 μ M EC | 30.10 ± 2.04 | 0.13 ± 0.02 |
| A β + 10 μ M EC | 20.05 ± 1.25 | 0.10 ± 0.01 |
| A β + 20 μ M EC | 4.94 ± 0.53 | 0.66 ± 0.14 |
| A β + 40 μ M EC | 10.95 ± 0.82 | 0.44 ± 0.08 |

^aThe t_{50} and k were obtained from fitting eq 1 to turbidity assay.

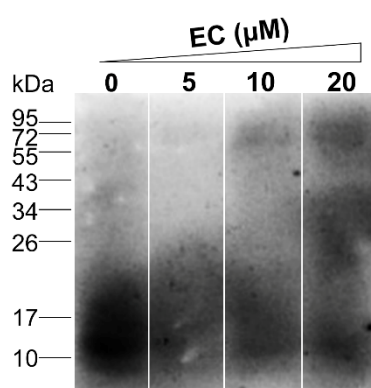


Fig. S7. Western blot analysis of concentration dependence of EC promoting A β 40 (20 μ M) aggregation. The samples were incubated in Tris-HCl buffer (20 mM Tris-HCl/150 mM NaCl, 4% v/v DMSO, pH 7.4) at 37 $^{\circ}$ C for 24 h.

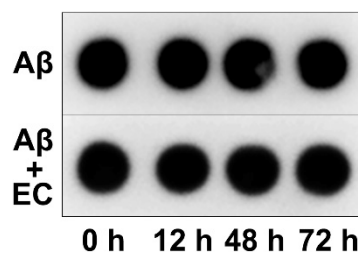


Fig. S8. Dot blot analysis of A β 40 in the absence or presence of EC (20 μ M) using 6E10 antibody.

Table S3. The aggregation kinetics of 20 μ M A β 40 peptide upon addition of EC (20 μ M) at different incubation time points.^a

| Addition time points (h) | t_{50} (h) | k (h^{-1}) | t_{lag} (h) |
|--------------------------|------------------|-------------------------|----------------------|
| 0 | 8.93 ± 0.55 | 0.37 ± 0.07 | 3.43 ± 0.94 |
| 12 | 17.73 ± 1.27 | 0.31 ± 0.02 | 11.19 ± 0.08 |
| 24 | 29.55 ± 6.21 | 0.12 ± 0.01 | 12.93 ± 2.17 |
| 48 | 54.14 ± 5.46 | 0.06 ± 0.01 | 22.82 ± 5.63 |

^aThe t_{50} , k , and t_{lag} were obtained from fitting eq 1 to turbidity assay.

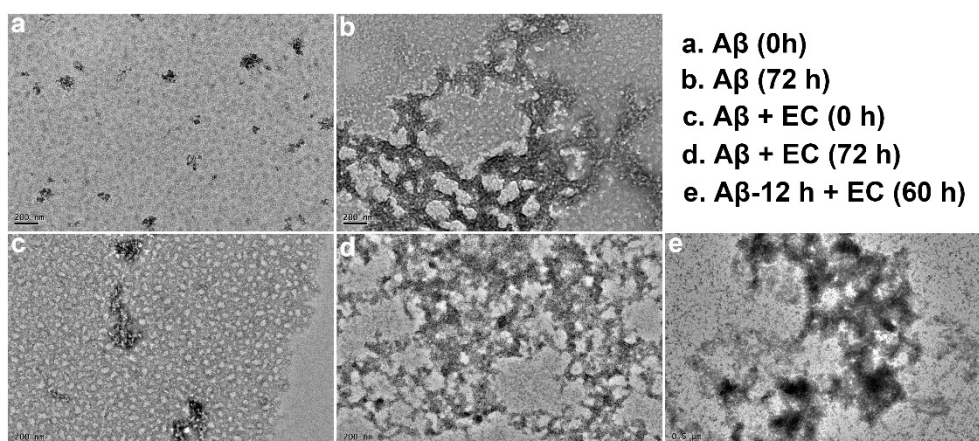


Fig. S9. TEM images of fresh and 12 h-preincubated A β 40 (20 μ M) in the absence or presence of EC (20 μ M) at different incubation time points in Tris-HCl buffer (20 mM Tris-HCl/150 mM NaCl, 4% v/v DMSO, pH 7.4) at 37 $^{\circ}\text{C}$.

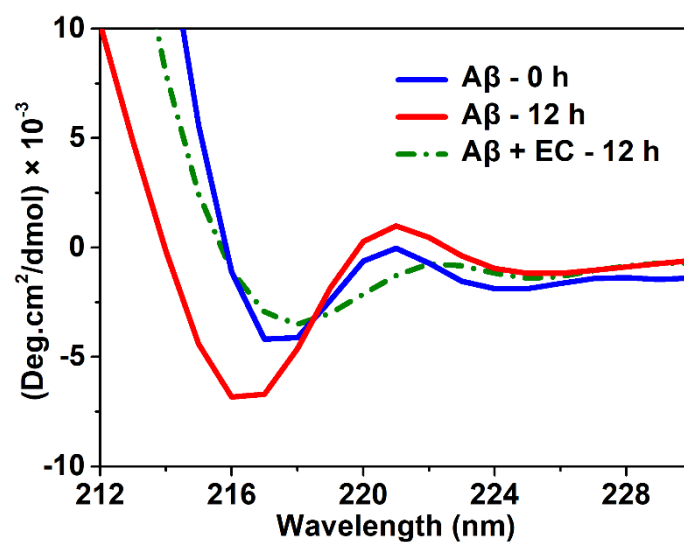


Fig. S10. CD spectra of Aβ40 (20 μM) in the absence or presence of EC (20 μM) at different incubation time points (0 and 12 h) in Tris-HCl buffer (20 mM Tris-HCl/150 mM NaCl, 4% v/v DMSO, pH 7.4) at 37 °C.

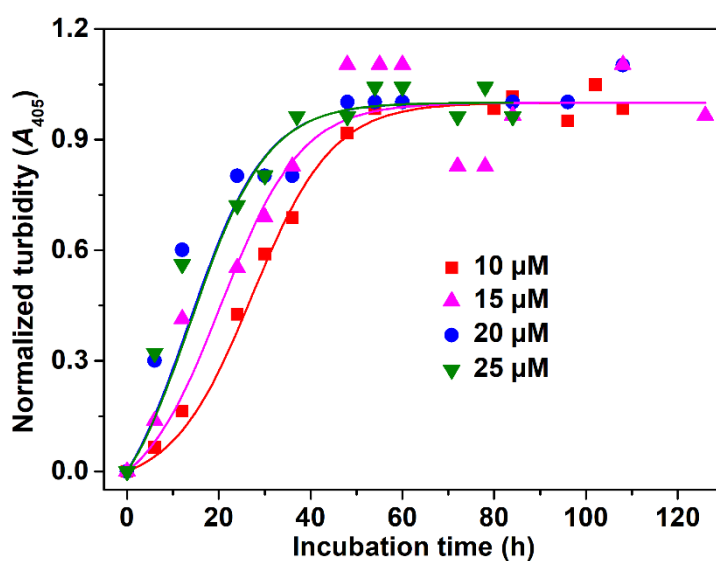


Fig. S11. Turbidity assay-monitored aggregation kinetics of Aβ40 with different initial concentrations in Tris-HCl buffer (20 mM Tris-HCl/150 mM NaCl, 4% v/v DMSO, pH 7.4) at 37 °C.

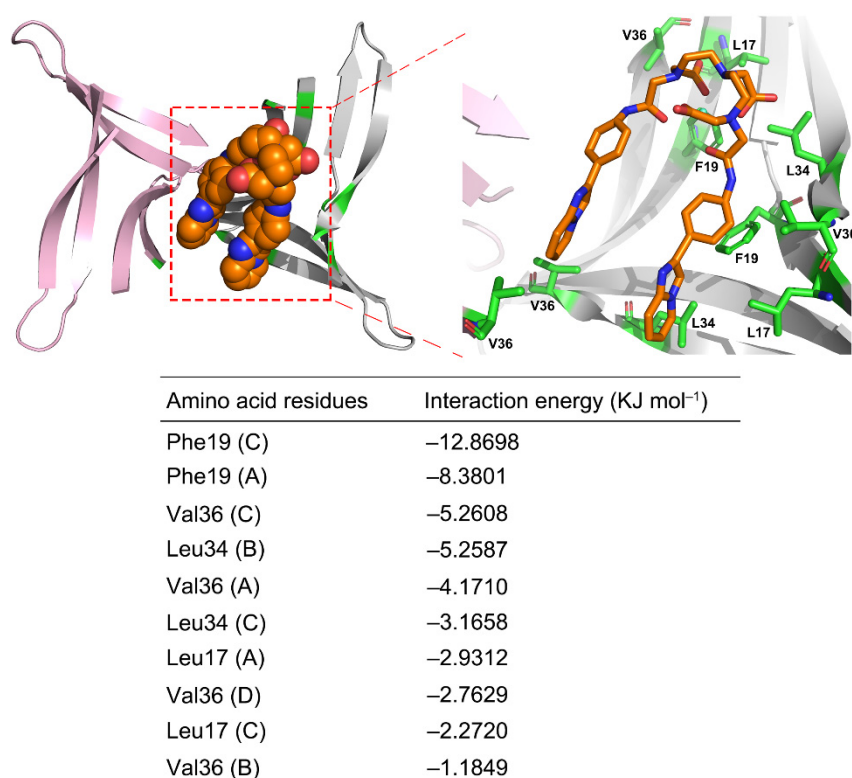


Fig. S12. Lowest energy docking model of EC with two A β trimers (PDB ID: 5HOX) in cartoon representations. Two trimers are depicted in pink and green, respectively. The amino acid residues interacting with EC are highlighted in green.

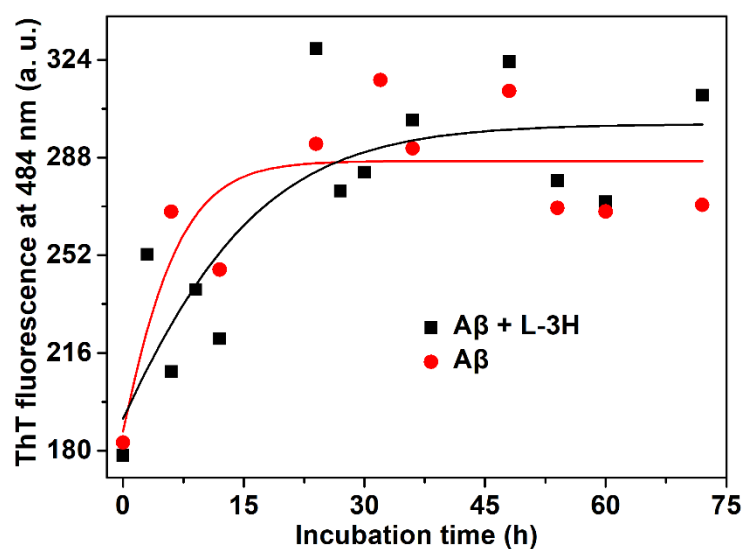


Fig. S13. The aggregation kinetics of A β 40 (20 μ M) in the absence or presence of L-3H (20 μ M) monitored by ThT fluorescence assay in Tris-HCl buffer (20 mM Tris-HCl/150 mM NaCl, 4% v/v DMSO, pH 7.4) at 37 $^{\circ}$ C.

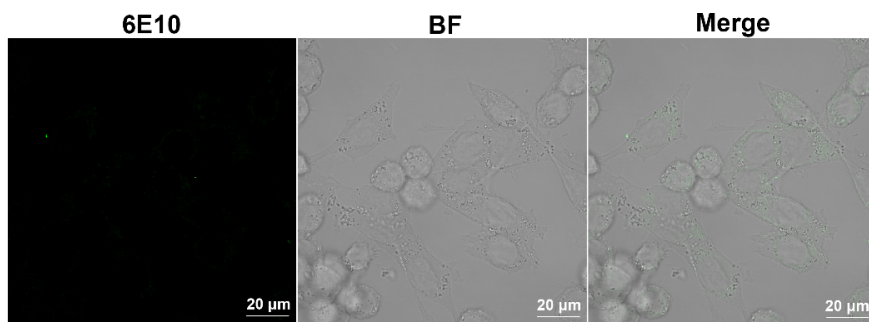


Fig. S14. Immunofluorescence imaging of BV-2 cells treated with EC (10 μ M) for 4 h.

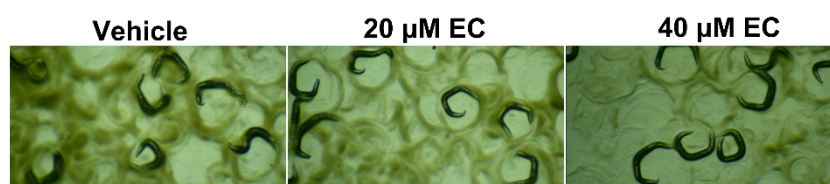


Fig. S15. The motion modalities of wild-type *C. elegans* CL802 upon treatment with DMSO (4%, v/v) or different concentrations of EC after living at 25 $^{\circ}$ C for 44 h.

3. Supplementary references

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