Electronic Supplementary Information

An AIE-active theranostic probe for light-up detection of Aβ aggregates and protection of neuronal cells

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Materials and Chemicals

Thioflavin T (ThT), curcumin, hexafluoroisopropanol (HFIP), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich. β-Amyloid Antibody was purchased from Cell Signaling Technology and goat anti-rabbit IgG Rhodamine was purchased from Jackson ImmunoResearch. Anti-Tubulin antibody was purchased from Abcam. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin streptomycin, neurobasal medium and N2 supplement were obtained from Gibco (New York, USA). THF, hexane, dichloromethane were purchased from Guangzhou Chemical Reagent Factory. All chemicals and reagents were purchased from commercial sources and used as received without further purification. Milli-Q water was supplied by a Milli-Q Plus System (Millipore Corp., Breford, USA).

Equipment and Methods

UV-vis absorption spectra were measured on a Shimadzu UV-2600 spectrophotometer, medium scanning rate, and quartz cuvettes of 1 cm path length. Photoluminescence spectra were recorded on a Horiba Fluoromax-4 spectrofluorometer. The absolute fluorescence quantum yield was measured using a Hamamatsu quantum yield spectrometer C11347 Quantaurus_QY. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were measured on a Bruker AV 500 NMR spectrometer. High resolution mass spectra (HRMS) were performed on a Bruker MaXis impact mass spectrometer. Confocal lasing scanning microscopic (CLSM) images were obtained on the Zeiss Laser Scanning Confocal Microscope (LSM710). Transmission electron microscope (TEM)

was obtained on the Tecnai G2 Spririt Twin. The circular dichroism (CD) spectra were recorded using a spectrometer (Applied Photophysics, U.K.). The MTT assay was measured with SUNRISE Absorbance Reader (TECAN). The fluorescence monitoring of $A\beta_{1-42}$ fibrillation process was measured with microplate reader (SpectraMax M5). The bright field images of HT22 cells were obtained with automatic inverted fluorescence microscope (Leica DMI8). All procedures involving animals were approved by the Animal Experimentation Ethics Committee of Sun Yat-sen University.

Circular Dichroism

The circular dichroic (CD) spectra were recorded using a Chirascan circular dichroism spectrometer (Applied Photophysics, U.K.) under nitrogen atmosphere. Peptides were dissolved in 10 mM PBS buffer at pH 7.4 at concentrations of 20 mM. A 0.5 mm path length was used for the measurements, with a step interval 0.5, 1 nm bandwidth, and a scanning speed of 50 nm/min. Three scans were acquired from 190 to 260 nm.

Cell culture

HT22 cell, a mouse hippocampal neuronal cell line,[1] was kindly provided by Professor Jun Liu at the Second Affiliated Hospital of Sun Yat-Sen University. Human neuroblastoma SHSY5Y cell line and rat pheochromocytoma PC12 cell line were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The HT22 cells were cultured in DMEM containing 1% penicillin-streptomycin and 10% FBS at 37 °C in a humidity atmosphere with 5% CO_2 and differentiated in neurobasal medium containing 2 mM/L glutamine and 1 × N2 supplement for 24 h.[2] The SHSY5Y cells and PC12 cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin in a humidity atmosphere with 5% CO_2 at 37 °C.

Cytotoxicity

The cytotoxicity experiment was conducted with MTT assay. HT22 cells were seeded in a 96-well plate at a density of 1×10^4 cells/well. After incubation at 37 ^oC for 24 h, different concentrations of Cur-N-BF_2, Cur-O-BF_2, curcumin, and ThT were added. After further incubation for 24 h, the culture media was removed and washed twice with PBS buffer. The MTT solution (0.5 mg/mL, 100 μL) was added into each well and the plate was incubated at 37 ^oC for 4 h. The MTT solution was then removed and the cells were washed twice with PBS buffer. DMSO (150 μL) was then added into each well and the plate was gently shaken for 10 min at room temperature to dissolve all the precipitates formed. The absorbance at 570 nm was then measured by a microplate reader and the cell viability was calculated by the ratio of the absorbance of sample wells to control wells.

TEM measurement

The TEM samples were respectively prepared by incubation of $A\beta_{1-42}$ peptide (20 μM) at 37 °C for 2 days, 6 days, and 6 days in the presence of Cur-N-BF_2 (10 μM). Then, 5 μL of each sample was applied on a 200-mesh carbon-coated formvar grid for 1 min. After washing with distilled water, the samples were negatively stained with 2% aqueous uranyl acetate for 30 s and the excess liquid was blotted with filter paper. The samples were then dried at room temperature and observed under transmission electron microscope (Tecnai G2 Spririt Twin, Czech) at the voltage of 80 kV.

Hemolysis test

Fresh blood from C57BL/6 mice was collected in heparinized tubes and washed three times with 0.9% NaCl by centrifugation at 3000 rpm for 15min and then suspended in

0.9% NaCl solution (5% v/v) for hemolytic test. The erythrocytes suspension (0.5 mL) was added to different concentrations of Cur-N-BF_2, 0.9% NaCl solution (negative control group with 0% hemolysis) and distilled water (positive control group with 100% hemolysis), respectively. After incubation at 37 °C for 2 h in a shaker incubator and centrifugation at 5000 rpm for 5 min, the absorbance of the supernatants at 545 nm was measured with microplate reader. The degree of hemolysis was calculated by the following equation: Hemolysis (%) = $(Abs_{sample}–Abs_{negative})/(Abs_{positive}–Abs_{negative})$x 100%, where Abs_{sample}, $Abs_{negative}$, and $Abs_{positive}$ are the absorbance values of the Cur-N-BF_2 samples, negative, and positive controls, respectively.

Statistical analysis

The assays were formed in triplicate on separate occasions. All data are presented as mean ±S.D. Data were subjected to statistical analysis using SPSS software, version 17.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was evaluated using Student's t test or one-way ANOVA followed by Tukey's multiple comparison test and $P < 0.05$ was considered statistically significant.

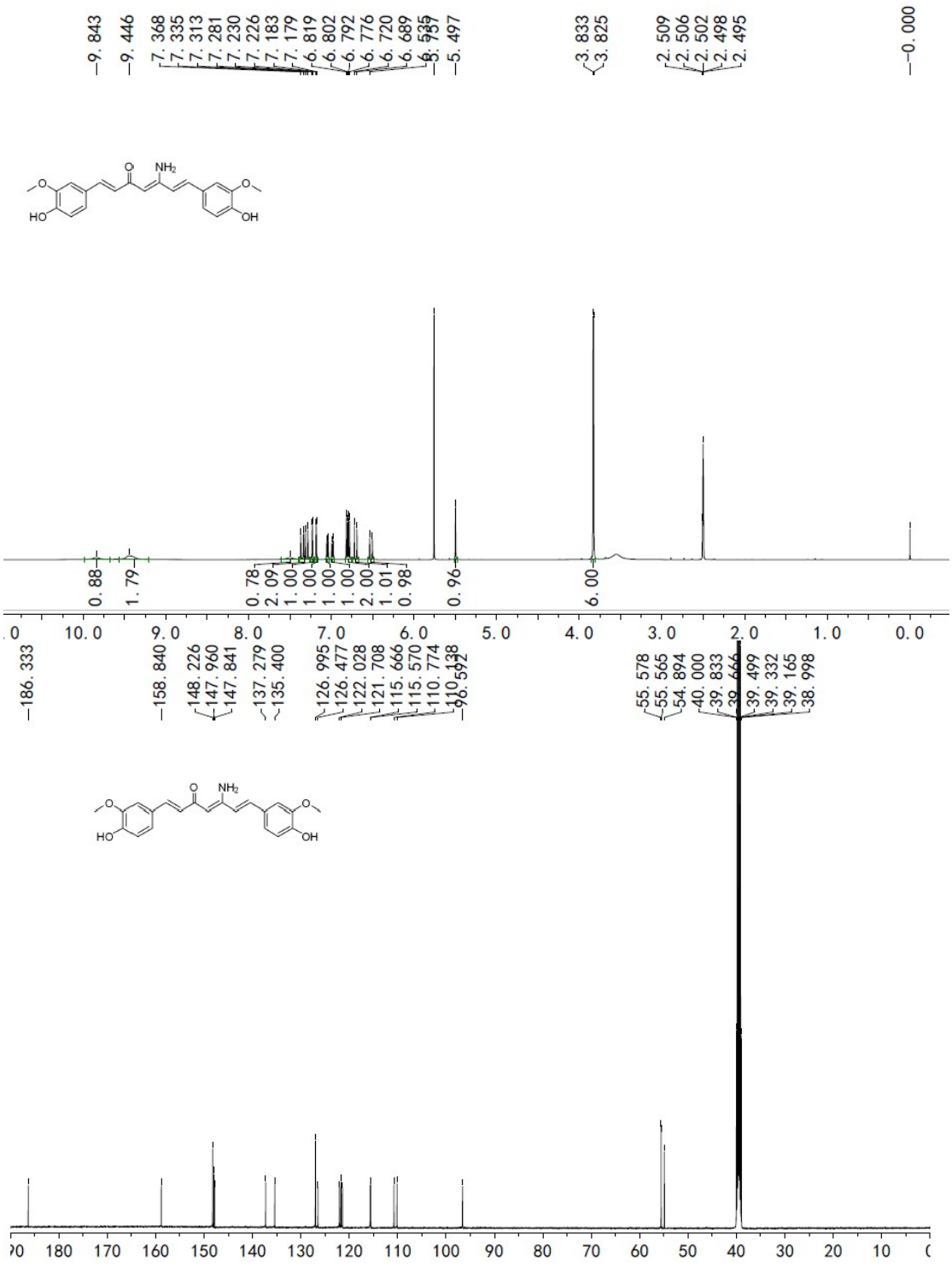

Fig. S1 ^{1}H and ^{13}C NMR spectra of compound Cur-NH_2 in DMSO-d_6.

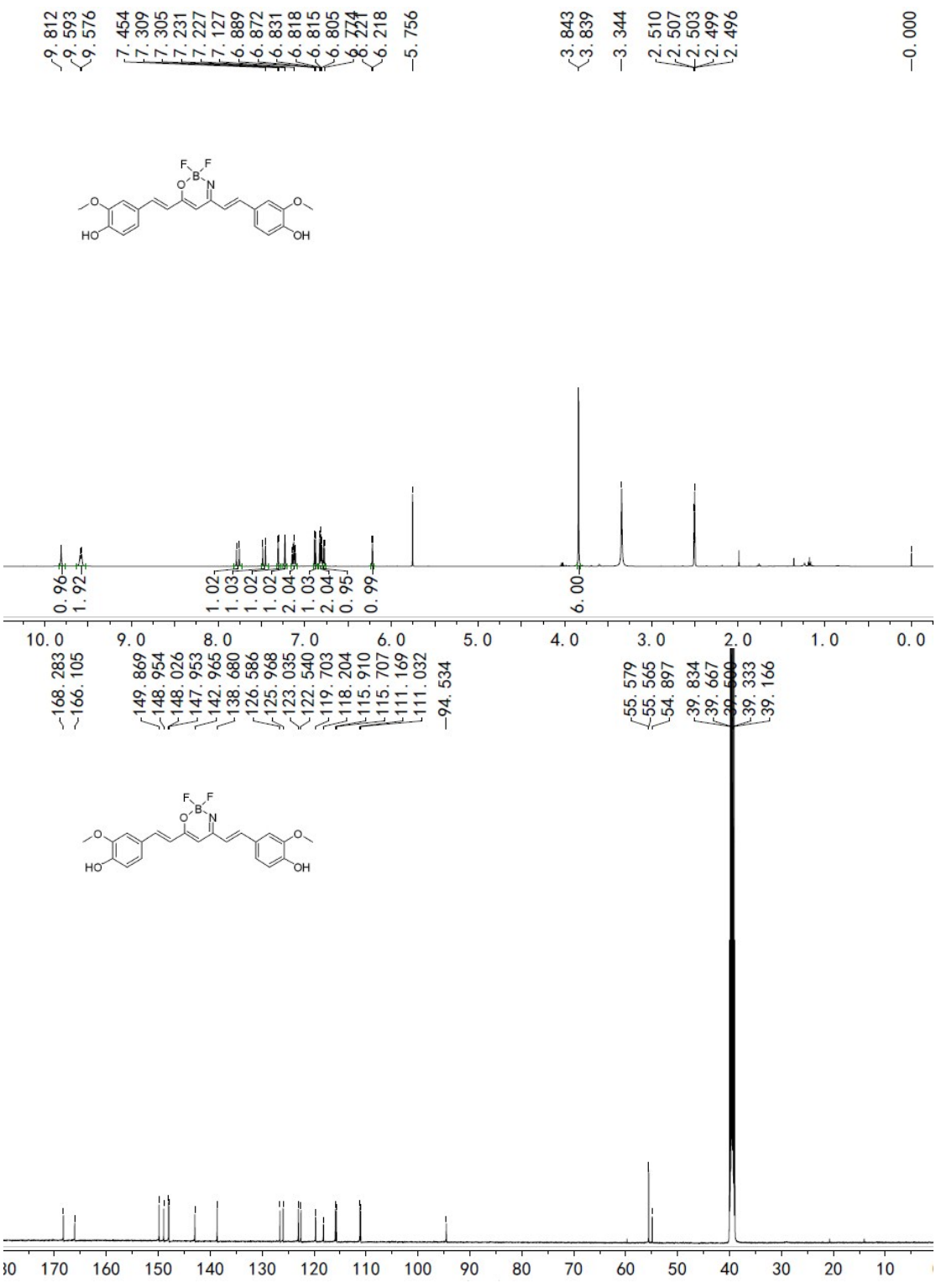

Fig. S2 ^{1}H and ^{13}C NMR spectra of compound Cur-N-BF_2 in DMSO-d_6.

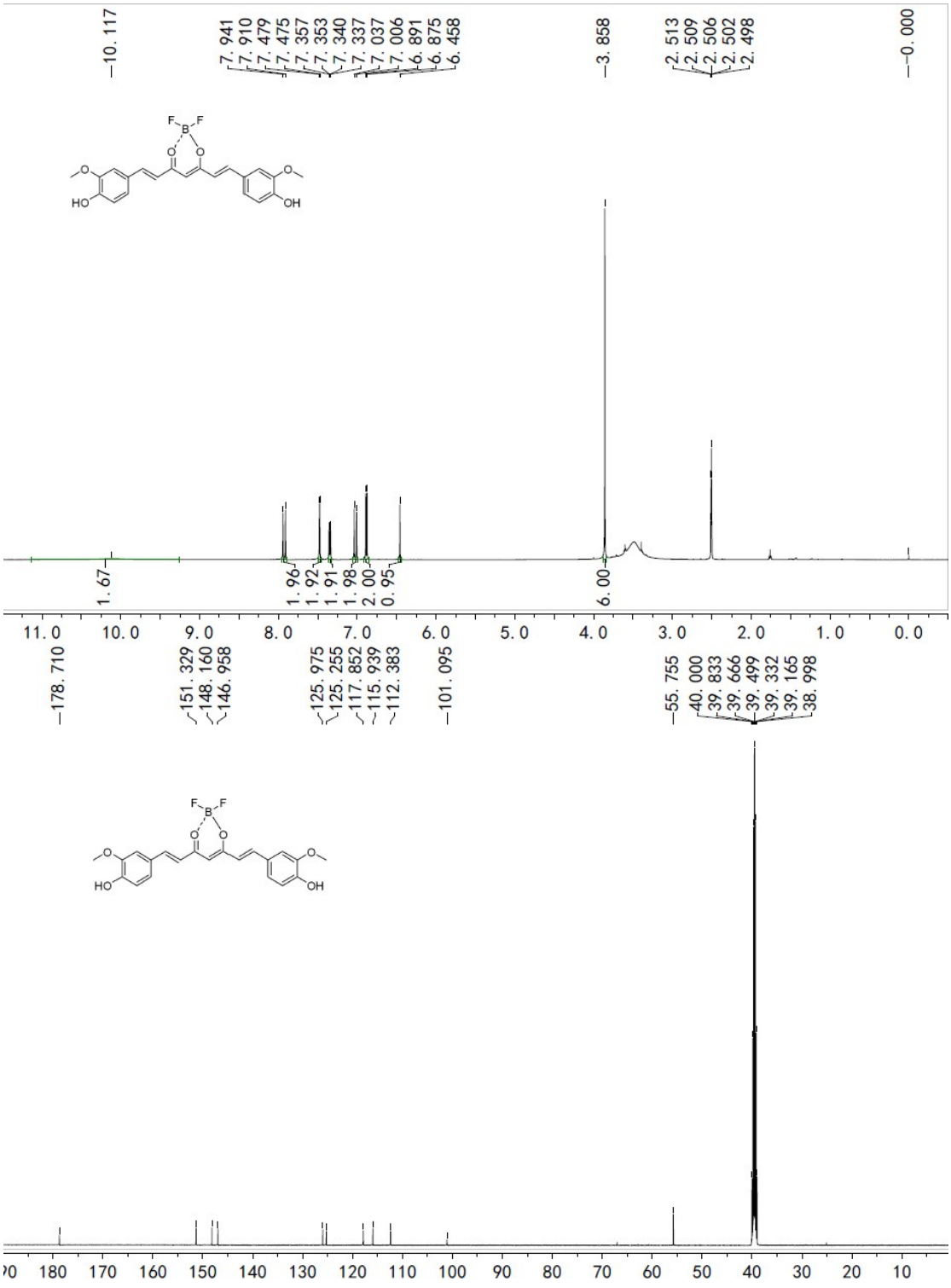

Fig. S3 ^{1}H and ^{13}C NMR spectra of compound Cur-O-BF_2 in DMSO-d_6.

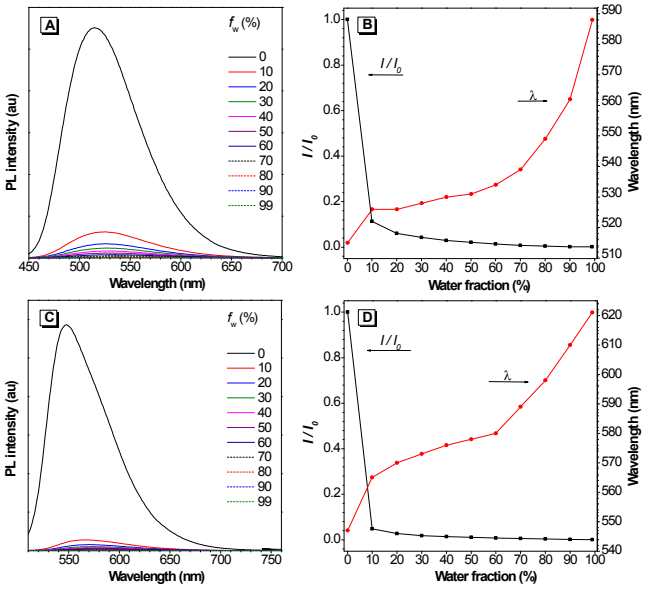

Fig. S4 (A) The PL spectra of Cur-N-BF_2 in THF/H_2O mixture with different fractions of water; λ_{ex} = 426 nm; (B) The plots of relative maximum emission intensity (I/I_0) and wavelength (λ_{em}) of Cur-N-BF_2 in THF/water mixture with different water fractions; (C) The PL spectra of Cur-O-BF_2 in THF/H_2O mixture with different fractions of water; λ_{ex} = 504 nm; (D) The plots of relative maximum emission intensity (I/I_0) and wavelength (λ_{em}) of Cur-O-BF_2 in THF/water mixture with different water fractions; [Cur-N-BF_2] = [Cur-O-BF_2] = 10 μM.

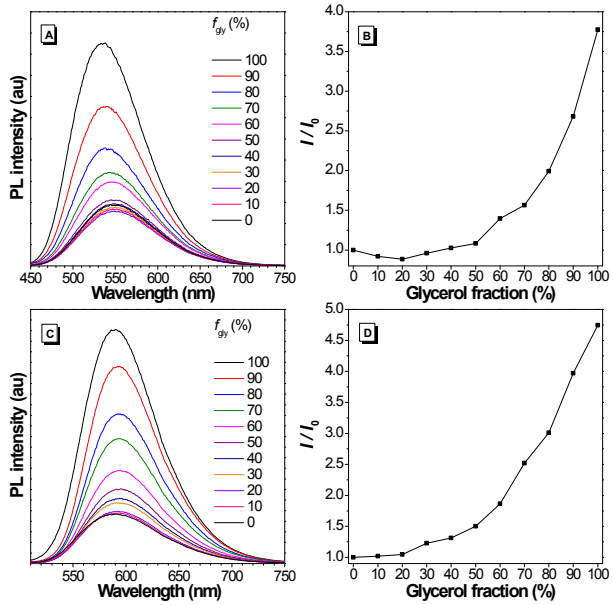

Fig. S5 (A) The PL spectra of Cur-N-BF_2 in methanol/glycerol mixture with increasing fractions of glycerol; (B) The plots of relative emission intensity (I/I_0) of Cur-N-BF_2 at 538 nm in methanol/glycerol mixture with different fractions of glycerol; λ_{ex} = 427 nm; (C) The PL spectra of Cur-O-BF_2 in methanol/glycerol mixture with different fractions of glycerol; (D) The plots of relative emission intensity (I/I_0) of Cur-O-BF_2 at 590 nm in methanol/glycerol mixture with different fractions of glycerol; λ_{ex} = 501 nm; [Cur-N-BF_2] = [Cur-O-BF_2] = 10 μM.

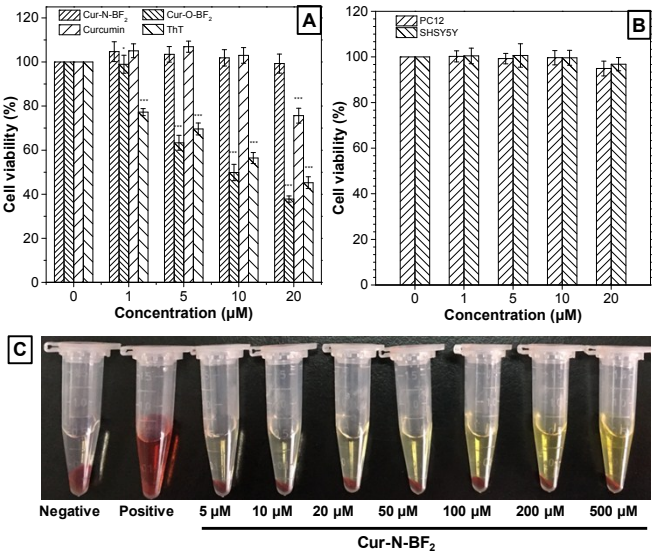

Fig. S6 (A) The cell viabilities of HT22 cells treated with different concentrations of Cur-N-BF_2, Cur-O-BF_2, curcumin, and ThT. (B) The cell viabilities of PC12 cells and SHSY5Y cells treated with different concentrations of Cur-N-BF_2. (C) The hemolysis results treated with Cur-N-BF_2 at different concentrations. *P<0.05; ***P<0.001.

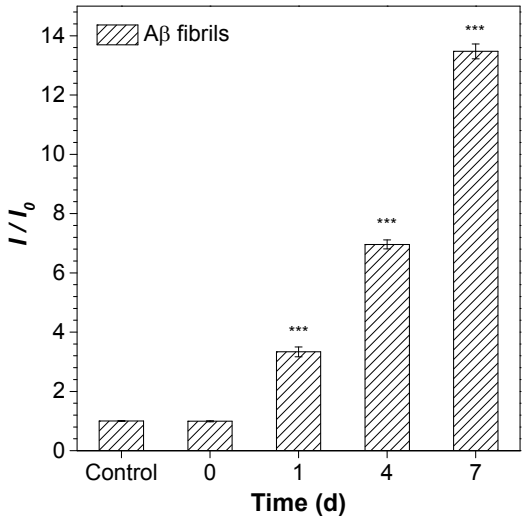

Fig. S7 The PL intensity (I/I_0) changes of ThT at 482 nm for detection of $A\beta_{1\text{-}42}$ fibrils incubated with 0, 1, 4, and 7 days, respectively; λ_{ex} = 450 nm; [ThT] = 10 μM; [$A\beta_{1\text{-}42}$] = 50 μM. ***P<0.001.

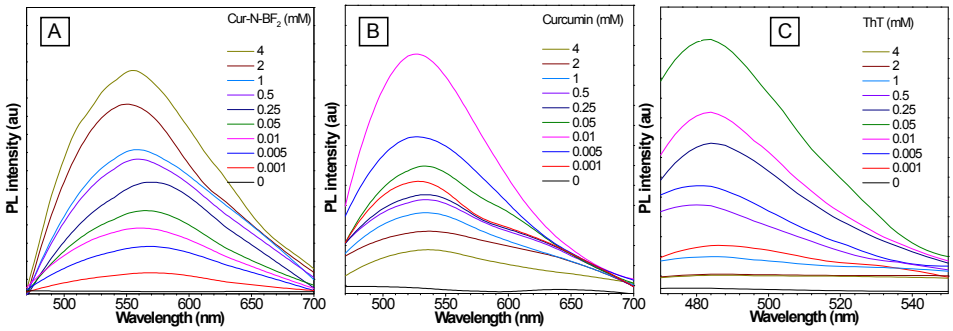

Fig. S8 In the presence of $A\beta_{1-42}$ fibrils (20 μM), the PL spectra changes of (A) Cur-N-BF_2, (B) Curcumin, and (C) ThT with increasing concentrations.

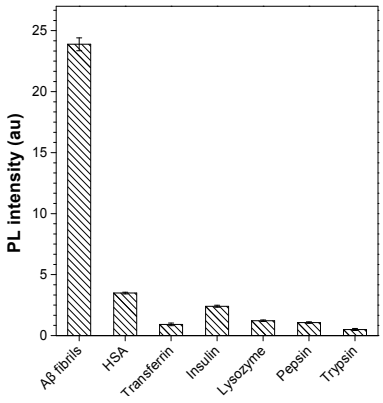

Fig. S9 (A) The PL intensity of Cur-N-BF_2 at 565 nm in the presence of $A\beta_{1-42}$ fibrils and different proteins, respectively; λ_{ex} = 426 nm; [Cur-N-BF_2] = 10 μM; [Protein] = 100 μg/mL.

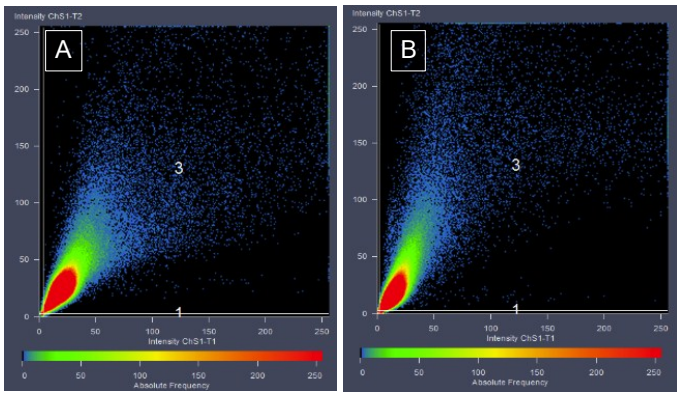

Fig. S10 The intensity correlation plots of Cur-N-BF_2 (X-axis) and β-Amyloid Antibody (Y-axis) for staining of Aβ plaques in (A) hippocampus and (B) cerebral cortex slices obtained from APP/PS1 mice.

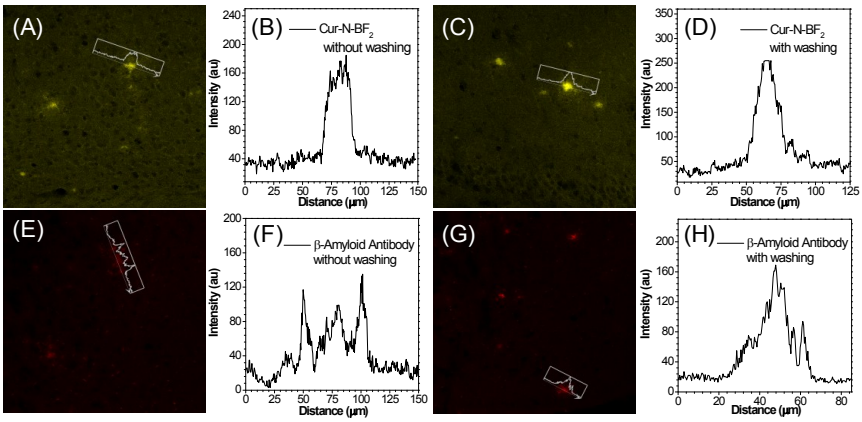

Fig. S11 The CLSM images of brain slices obtained from APP/PS1 mouse. (A) Fluorescence image and (B) intensity profile of ROI line from Cur-N-BF_2 without washing; (C) Fluorescence image and (D) intensity profile of ROI line from Cur-N-BF_2 with repeated washing; (E) Fluorescence image and (F) intensity profile of ROI line from β-Amyloid Antibody (Cell Signaling Technology, 1:200) without washing; (G) Fluorescence image and (H) intensity profile of ROI line from β-Amyloid Antibody (Cell Signaling Technology, 1:200) by washing with PBS for three times; [Cur-N-BF_2] = 100 μM.

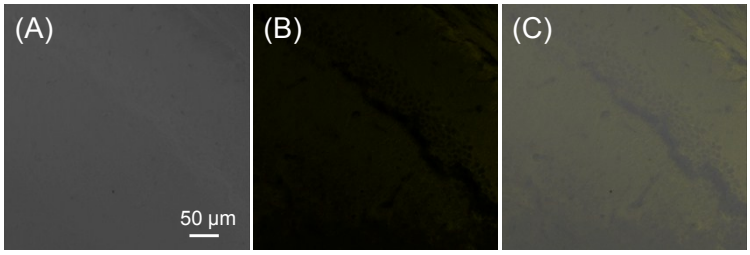

Fig. S12 The CLSM images of brain slices from wild-type mouse by staining with Cur-N-BF_2 (100 μM): (A) Bright field image; (B) Fluorescence image; (C) The merged image. Scale bar = 50 μm.

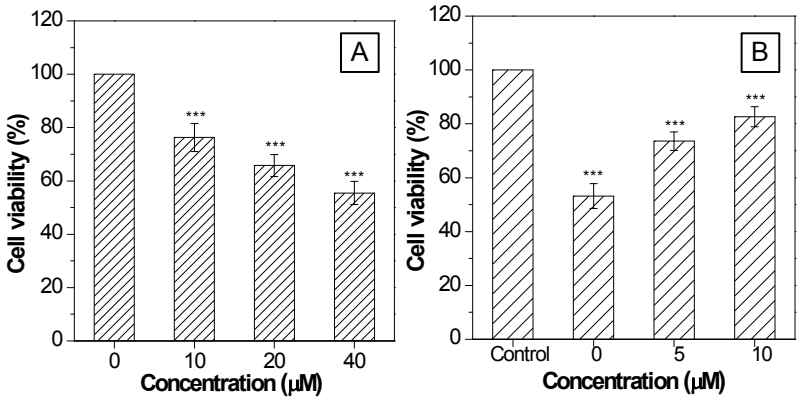

Fig. S13 (A) The cell viabilities of HT22 cells after incubation with different concentrations of $A\beta_{1-42}$ fibrils for 36 h. (B) The cell viabilities of HT22 cells under control and treated with 40 μM $A\beta_{1-42}$ fibrils and Cur-N-BF_2 (0, 5, 10 μM). ***P<0.001.

References

[1] a) W. B. Stine, L. Jungbauer, C. Yu and M. J. LaDu, *Methods Mol. Biol.,* 2011, **670**, 13-32; b) M. Wu, J. Jia, C. Lei, L. Ji, X. Chen, H. Sang and L. Xiong, *J. Mol. Neurosci.*, 2015, **55**, 778-787.

[2] a) M. He, J. Liu, S. Cheng, Y. Xing, and W. Z. Suo, *Neural Regen. Res.,* 2013, **8**, 1297; b) J. Liu, L. Li and W. Z. Suo, *Life Sci.*, 2009, **84**, 267-271.