Supplementary Information

Superoxide-responsive fluorogenic molecular probes for optical bioimaging of neurodegenerative events in Alzheimer's disease

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Section S1. Methods

Synthesis of BSR1 (BSR-H). Resorufin (100 mg, 0.47 mmol), benzenesulfonyl chloride (330 mg, 1.88 mmol), and triethylamine (190 mg, 1.88 mmol) were dissolved in a mixture of dichloromethane and tetrahydrofuran (1/1 by volume) and stirred overnight at room temperature under argon atmosphere. The product was extracted with dichloromethane and washed with brine three times. The extract was dried over anhydrous $MgSO_4$, concentrated, and purified by column chromatography on a silica gel with ethyl acetate/n-hexane (1/2 by volume). Yield: 80 mg (48.27%). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 7.91-7.88 (d, 2H), 7.75-7.71 (dd, 2H), 7.61-7.57 (t, 2H), 7.43-7.40 (d, 1H), 7.04-7.03 (d, 1H), 7.02-7.00 (dd, 1H), 6.88-6.85 (dd, 1H), 6.311-6.306 (d, 1H). MS m/z: 354.044 $[M+H]^+$. EA, calculated for $C_{18}H_{11}NO_5S$: C 61.19, H 3.14, N 3.96, S 9.07, O 22.64.; found: C 63.27, H 4.36, N 3.67, S 8.23, O 19.10.

Synthesis of BSR2 (BSR-CH_3). Resorufin (100 mg, 0.47 mmol) and triethylamine (190 mg, 1.88 mmol) were dissolved in a mixture of dichloromethane and tetrahydrofuran (3/1 by volume), and a p-toluenesulfonyl chloride (447.2 mg, 2.35 mmol) solution in 3 mL of dichloromethane was added gradually at 0℃. The reaction mixture was stirred overnight at room temperature under argon atmosphere. The product was obtained and purified by following the same procedure as described above for BSR1. Yield: 120 mg (69.64%). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 7.78-7.75 (d, 2H), 7.72-7.70 (d, 1H), 7.43-7.40 (d, 1H), 7.37-7.35 (d, 2H), 7.05-7.04 (d, 1H), 7.03-7.00 (dd, 1H), 6.88-6.85 (dd, 1H), 6.314-6.309 (d, 1H). MS m/z: 368.059 $[M+H]^+$. EA, calculated for $C_{19}H_{13}NO_5S$: C 62.12, H 3.57, N 3.81, S 8.73, O 21.77.; found: C 60.87, H 3.80, N 3.92, S 8.73, O 20.10.

Synthesis of BSR3 (BSR-OCH_3). Resorufin (100 mg, 0.47 mmol), 4-methoxybenzenesulfonyl chloride (388 mg, 1.88 mmol), and triethylamine (190 mg, 1.88 mmol) were dissolved in a mixture of dichloromethane and tetrahydrofuran (1/1 by volume) and stirred overnight at room temperature under argon atmosphere. The product was obtained by following the same procedure as described above for BSR1 and purified by column chromatography on a silica gel with ethyl acetate/n-hexane (1/1 by volume). Yield: 100 mg (55.61 %). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 7.82-7.79 (dd,

2H), 7.73-7.71 (d, 1H), 7.43-7.40 (d, 1H), 7.052-7.046 (d, 1H), 7.03-6.99 (m, 3H), 6.88-6.85 (dd, 1H), 6.32-6.31 (d, 1H). MS m/z: 384.054 $[M+H]^+$. EA, calculated for $C_{19}H_{13}NO_6S$: C 59.53, H 3.42, N 3.65, S 8.36, O 25.04.; found: C 59.11, H 3.84, N 3.76, S 8.24, O 23.47.

Synthesis of BSR4 (RBS-F). Resorufin (100 mg, 0.47 mmol), 4-fluorobenzenesulfonyl chloride (365 mg, 1.88 mmol), and triethylamine (190 mg, 1.88 mmol) were dissolved in a mixture of dichloromethane and tetrahydrofuran and stirred overnight at room temperature under argon atmosphere. The product was obtained and purified by following the same procedure as described above for BSR3. Yield: 90 mg (51.67%). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 7.94-7.90 (m, 2H), 7.75-7.72 (d, 1H), 7.43-7.40 (d, 1H), 7.28-7.24 (m, 2H), 7.06-7.05 (d, 1H), 7.02-7.00 (dd, 1H), 6.88-6.85 (dd, 1H), 6.322-6.317 (d, 1H). MS m/z: 372.034 $[M+H]^+$. EA, calculated for $C_{18}H_{10}FNO_5S$: C 58.22, H 2.71, F 5.12, N 3.77, S 8.63, O 21.54.; found: C 57.79, H 3.18, N 3.61, S 9.05, O 21.18.

Synthesis of BSR5 (RBS-NO_2). Resorufin (100 mg, 0.47 mmol), 4-nitrobenzenesulfonyl chloride (416 mg, 1.88 mmol), and triethylamine (190 mg, 1.88 mmol) were dissolved in a mixture of dichloromethane and tetrahydrofuran (3/1 by volume) solution and stirred at room temperature under argon atmosphere. The product was obtained and purified by following the same procedure as described above for BSR1. Yield: 80 mg (42.81%). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 8.44-8.42 (dd, 2H), 8.13-8.10 (dd, 2H), 7.77-7.74 (d, 1H), 7.43-7.41 (d, 1H), 7.087-7.081 (d, 1H), 7.03-7.00 (dd, 1H), 6.89-6.86 (dd, 1H), 6.323-6.318 (d, 1H) ppm. MS m/z: 399.029 $[M+H]^+$. EA, calculated for $C_{18}H_{10}N_2O_7S$: C 54.27, H 2.53, N 7.03, S 8.05, O 28.11.; found: C 52.39, H 3.11, N 6.24, S 7.40, O 24.66.

Method for preparation of ROS. The stock solution of H_2O_2, TBHP, and OCl^- were delivered from 30 wt%, 70 wt%, and 5 wt% aqueous solution, respectively. $NO^\bullet$ were prepared from a solution of 3-(aminopropyl)-1-hydroxy-3-isopropyl-2-oxo-1-triazene (NOC-5) in DMSO. $O_2^{\bullet-}$ was prepared from KO_2 solution in DMSO containing 0.2 M 18-crown-6 ether. $^\bullet$OH and $^\bullet$OtBu were prepared by Fenton reaction of Fe^{2+} (1 mM) with H_2O_2 (100 μM) or TBHP (100 μM), respectively. RSSs were prepared in water.

Section S2. Supporting characterization of BSRs

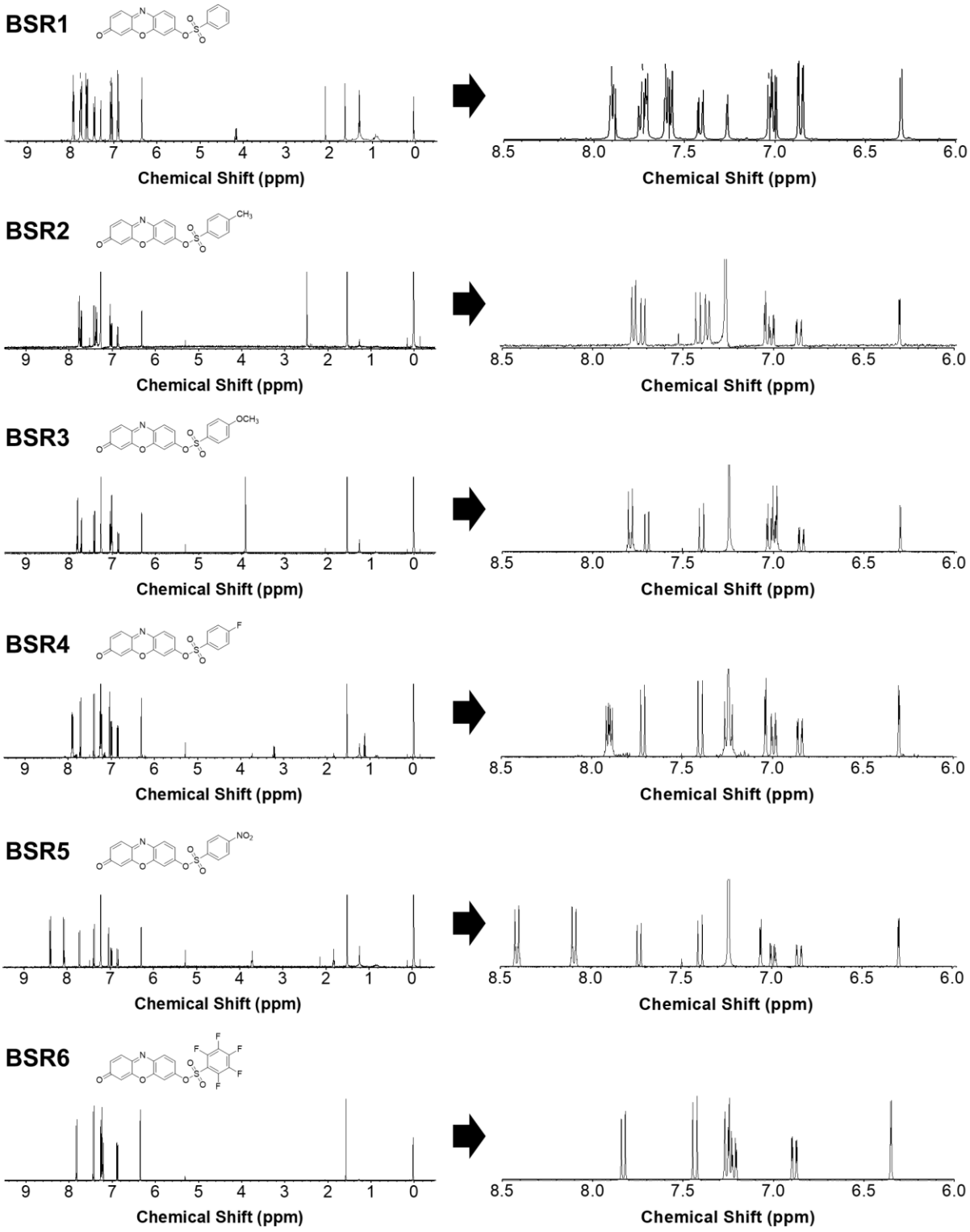

Fig. S1 ^{1}H-NMR spectra of BSRs in $CDCl_3$.

Table. S1 Liquid chromatography-mass spectrometry data for BSRs in MeOH.

Probes	Formula	Calculated m/z	Found m/z
RBS1	$C_{18}H_{11}NO_5S$	353.036	354.044 ($[M+H]^+$)
RBS2	$C_{19}H_{13}NO_5S$	367.051	368.059 ($[M+H]^+$)
RBS3	$C_{19}H_{13}NO_6S$	383.046	384.054 ($[M+H]^+$)
RBS4	$C_{18}H_{10}FNO_5S$	371.026	372.034 ($[M+H]^+$)
RBS5	$C_{18}H_{10}N_2O_7S$	398.021	399.029 ($[M+H]^+$)
RBS6	$C_{18}H_6F_5NO_5S$	442.989	443.997 ($[M+H]^+$)

Table. S2 Elemental analysis data (C, H, N, S, O) for BSRs.

Probes	Formula	Elemental analysis (wt%) calcd/found					
		C	H	N	S	O	F
RBS1	$C_{18}H_{11}NO_5S$	61.19/63.27	3.14/4.36	3.96/3.67	9.07/8.23	22.64/19.10	
RBS2	$C_{19}H_{13}NO_5S$	62.12/60.87	3.57/3.80	3.81/3.92	8.73/8.73	21.77/20.10	
RBS3	$C_{19}H_{13}NO_6S$	59.53/59.11	3.42/3.84	3.65/3.76	8.36/8.24	25.04/23.47	
RBS4	$C_{18}H_{10}FNO_5S$	58.22/57.79	2.71/3.18	3.77/3.61	8.63/9.05	21.54/21.18	5.12/
RBS5	$C_{18}H_{10}N_2O_7S$	54.27/52.39	2.53/3.11	7.03/6.24	8.05/7.40	28.11/24.66	
RBS6	$C_{18}H_6F_5NO_5S$	48.77/49.25	1.36/1.57	3.16/3.21	7.23/7.37	18.05/24.73	21.43/

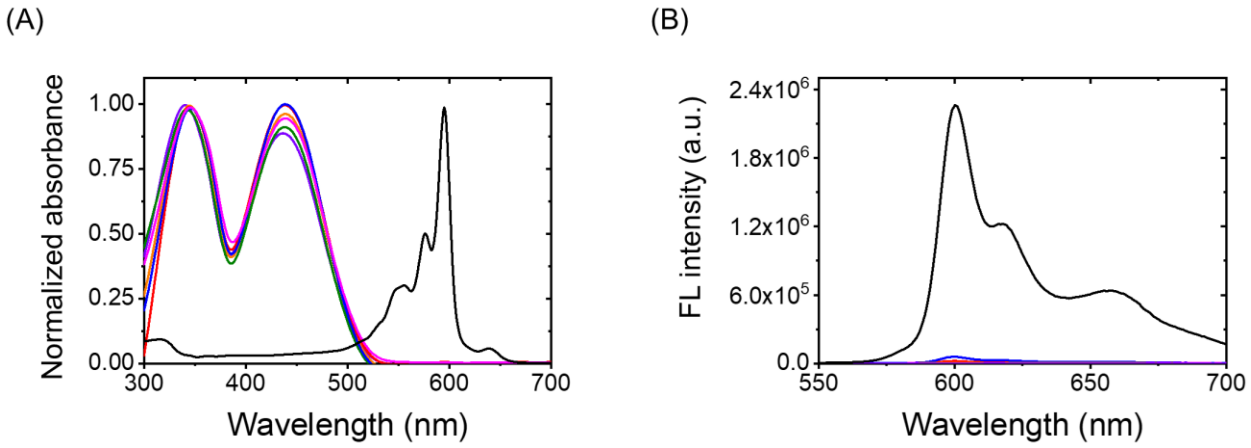

Fig. S2 (A) Absorption and (B) fluorescence spectra of resorufin and BSRs in DMSO. Resorufin (Black); BSR1 (Red); BSR2 (Blue); BSR3 (Magenta); BSR4 (Orange); BSR5 (Green); BSR6 (Violet).

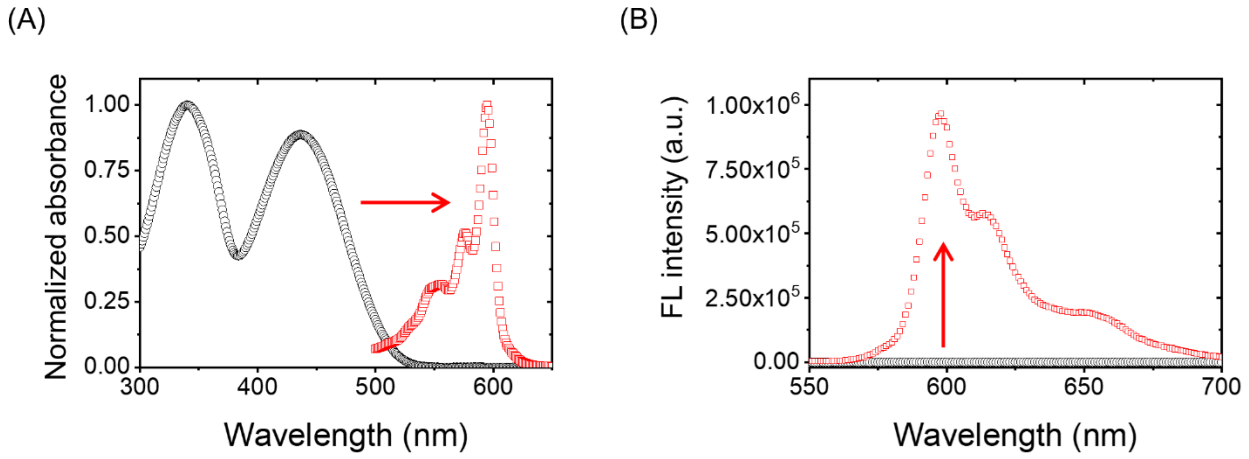

Fig. S3 (A) Absorption and (B) fluorescence spectral change of a BSR6 solution (100 μM in DMSO) upon exposure to superoxide.

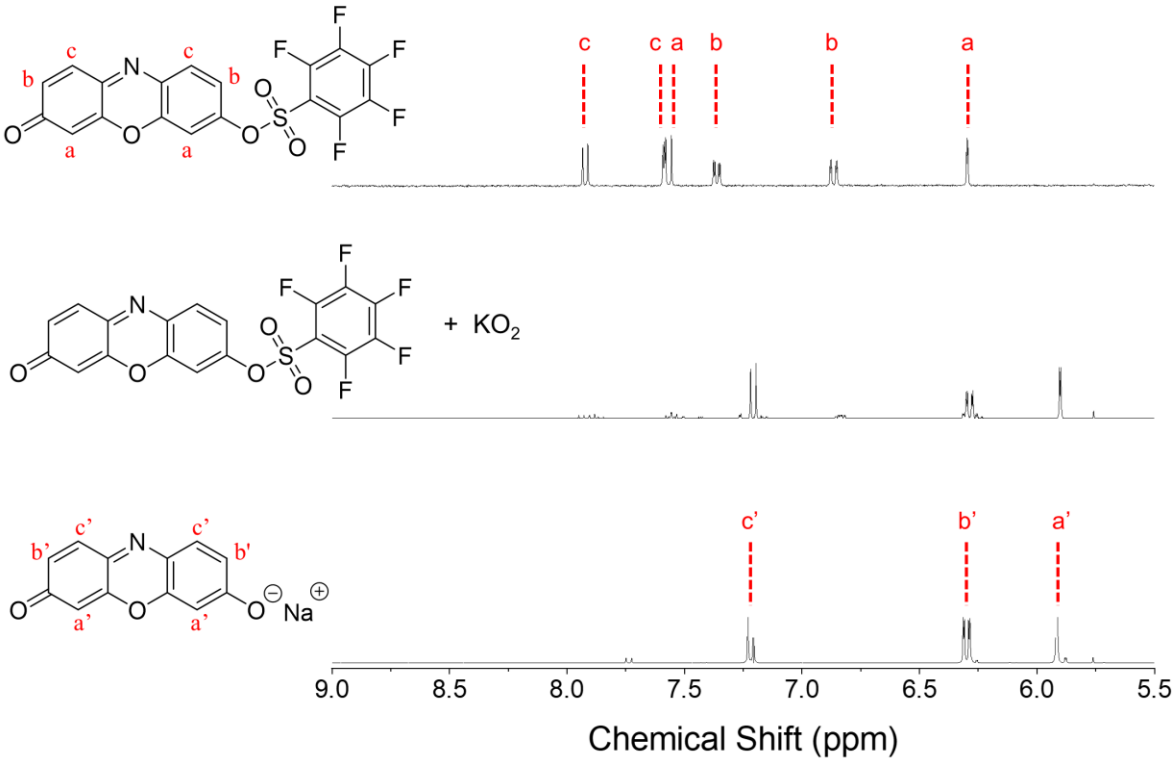

Fig. S4 ^{1}H-NMR spectra of resorufin and BSR6 upon exposure to superoxide in DMSO-d6

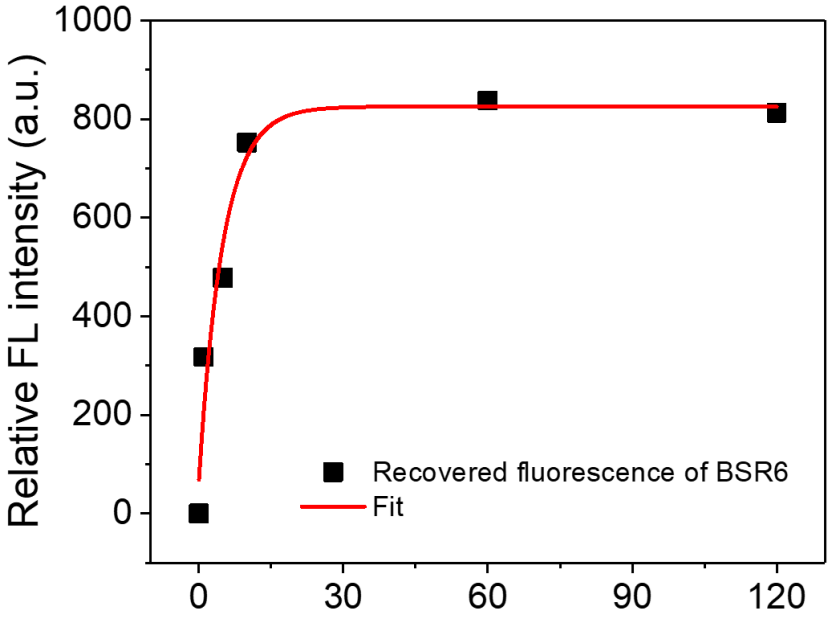

Fig. S5 Reaction kinetics of the BSR6 (100 μM in DMSO) in the presence of superoxide (100 μM) by measuring the fluorescence at 580 nm.

The data was fitted by following exponential decay equation:

$$y = y_0 + A*\exp(-x/\tau_c)$$

$y_0 = 825.31$

$A = -756.47$

$\tau_c = 4.97$ min

$R^2 = 0.958$

Section S3. Biological experiments

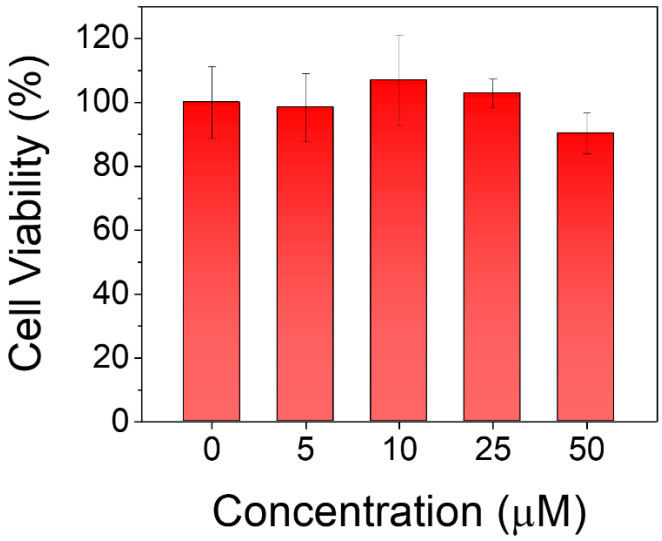

Fig. S6 Cytotoxicity of BSR6 against HeLa cells, evaluated by the colorimetric MTT assay. HeLa cells were treated with various concentrations of BSR6 for 2 h.

The cytotoxicity of BSR6 in HeLa cells was assessed by MTT assay. HeLa cells were seeded in 96-well plates at a density of 0.1×10^5 cells per well and incubated for 24 h. HeLa cells were treated with BSR6 at different concentrations and incubated for 24 h in complete media. After media suction, MTT solution was treated to each well at dilution 1:10 for 1 h. After MTT solution suction, DMSO was added to each well and incubated for 5 min. Absorbance was measured at 570 nm on microplate reader.

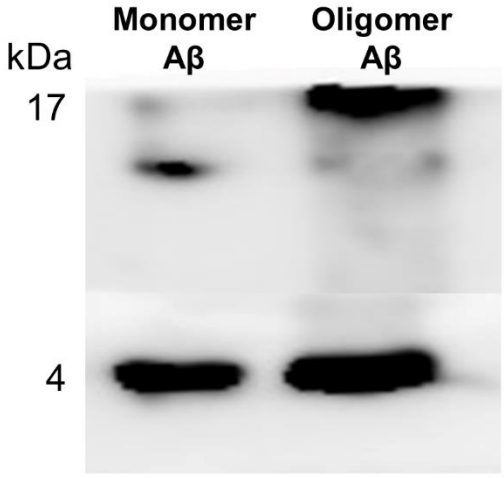

Fig. S7 Characterization of Aβ monomer and oligomer analyzed by SDS-PAGE and Western blot.

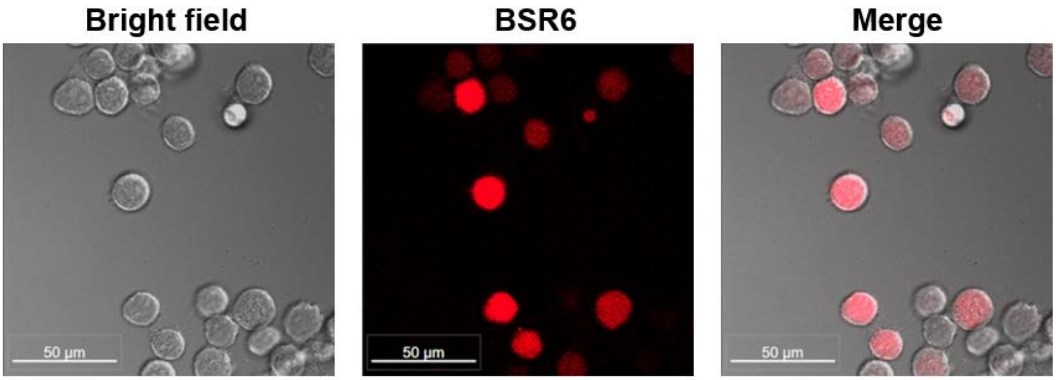

Fig. S8 *In vitro* microscopic images of Bv2 cells treated with BSR6 for 30 min (filter set for fluorescence image: λ_{ex} = 635 nm, λ_{em} = 645-700 nm). Cells were pre-incubated with oligomer Aβ for 24 h.