Supporting information

Activatable near-infrared fluorescent probe for methylglyoxal imaging in Alzheimer's disease mice

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Experimental section

Materials. Methylglyoxal (MGO), 4',6-diamidino-2-phenylindole (DAPI), fetal bovine serum (FBS) and Dulbecco’s modified Eagle’s media Nutrient Mixture F-12 (DMEM/F-12) were purchased from Thermo Fisher Scientific Co. Ltd (Shanghai, China). Benzaldehyde (BA), formaldehyde (FA), glyoxal (GO) and o-phthalaldehyde (OPA) were purchased from Aladdin Industrial Corporation (Shanghai, China). 1,1,3,3-Tetraethoxypropane (TEP) was purchased from Shanghai Macklin Biochemical Co., Ltd. GLO1 inhibitor (ethyl N\(^5\)-((S)-3-(((4-bromophenyl)(methyl)carbamoyl)thio)-1-((2-ethoxy-2-oxoethyl) amino)-1-oxopropan-2-yl)-L-glutamate hydrochloride, BHGD) was purchased from MedChemExpress (San Francisco, North America). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2h-tetrazoliubromide (MTT) was purchased from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China).

Instruments. UV-vis spectra and fluorescence spectrum were acquired with Cary 60 spectrophotometer and Cary Eclipse Spectrofluorophotometer, respectively (Agilent Technologies, Palo. Alto, CA, USA). MTT assays and fluorescence imaging of frozen sections were acquired with a Cytation 3 Cell Imaging Multi-Mode Reader (BioTek Instruments, Inc., VT, USA). Confocal images were acquired with a Leica TCS-SP8 confocal scanning microscope (Leica Microsystem Inc., Wetzlar, Germany). HPLC data was acquired with an Agilent 1260 HPLC system (Agilent Technologies, Palo. Alto, CA, USA). \(^1\)H and \(^{13}\)C NMR spectrum were acquired with a Bruker Advance 500 MHz NMR spectrometer at 25 °C. HR-ESI mass spectra were acquired with
Agilent 1290-6545 UHPLC-QTOF mass spectrometer. Fourier transform infrared (FT-IR) spectra were performed on a Thermo Nicolet 380 FTIR spectrometer (Thermo Nicolet Co., USA).

**Synthesis of**

3,3’-(((5,6-dinitrobenzo[c][1,2,5]thiadiazole-4,7-diyl)bis(4,1-phenylene))bis(oxy))bis(propan-1-ol) (2)

To a stirred solution of compound 1 (100 mg, 0.236 mmol) and K$_2$CO$_3$ in DMF (4 mL) at 60°C for 1 h, 3-bromo-1-propanol (132 mg, 0.944 mmol) was added, the reaction mixture was then stirred for over 2 h. The mixture was extracted with ethyl acetate. The combined organic layer was washed with water and brine and dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated under vacuum. The residue was purified by silica gel column chromatography (DCM:MeOH = 30:1, v/v) to give compound 2 as a yellow solid (87 mg, 70%). $^1$H NMR (500 MHz, Chloroform-d) δ 7.44 (s, 4H), 7.02 (s, 4H), 4.24 – 4.06 (m, 4H), 3.89 – 3.70 (m, 4H), 2.15 – 1.96 (m, 4H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 160.54, 153.11, 142.25, 130.65, 128.21, 122.44, 114.98, 65.24, 59.07, 31.84. HR-ESI-MS: [M-H]$^+$ m/z 549.1050.

**Synthesis of**

3,3’-(((5,6-diaminobenzo[c][1,2,5]thiadiazole-4,7-diyl)bis(4,1-phenylene))bis(oxy))bis(propan-1-ol) (DBTPP)

To a stirred solution of compound 2 (50 mg, 0.095 mmol), ammonium chloride (25 mg, 0.475 mmol) and iron in 2 mL solvent (CH$_3$CH$_2$OH: H$_2$O = 5:2 v/v) at 80 °C for
3 h under N₂, water and sodium bicarbonate were added to the above mixture until pH 7. The mixture was then extracted with ethyl acetate. The combined organic layer was washed with water and brine and dried over anhydrous sodium sulfate. The dried solution was filtered and the filtrate was concentrated under vacuum. The residue was purified by silica gel column chromatography (DCM:MeOH = 30:1, v/v) to give **DBTPP** as an orange-yellow solid (20.2 mg, 46%). IR: 3422, 2929, 1609, 1443, 1245, 1057, 816, 536 cm⁻¹; ¹H NMR (500 MHz, Chloroform- d) δ 7.40 (d, J = 8.0 Hz, 4H), 7.03 (d, J = 8.1 Hz, 4H), 4.15 – 4.05 (m, 4H), 3.80 – 3.70 (m, 4H), 2.05 – 1.93 (m, 4H); ¹³C NMR (125 MHz, CDCl₃) δ 158.54, 151.25, 138.28, 131.41, 126.99, 115.20, 112.66, 65.23, 59.33, 31.96. HR-ESI-MS: [M-H]⁺ m/z 489.1565.

**Synthesis of**

3,3'-(((6-methyl-[1,2,5]thiadiazolo[3,4-g]quinoxaline-4,9-diyl)bis(4,1-phenylene))bis(oxy))bis(propan-1-ol) (MTQPP)

To a stirred solution of **DBTPP** (10 mg, 0.021 mmol) in 1 mL solvent (DCM:MeOH = 1:1, v/v), 0.5 mL MGO aqueous solution was added. The mixture was stirred at room temperature for 4 h. Then the reaction mixture was concentrated under vacuum. The residue was purified by preparative thin layer chromatography (DCM:MeOH = 40:1, v/v) to give **MTQPP** as a red solid (4.5 mg, 43%). IR: 3431, 2930, 1726, 1606, 1248, 1032, 830, 598 cm⁻¹; ¹H NMR (500 MHz, Chloroform- d) δ 8.74 (s, 1H), 7.88 (d, J = 8.7 Hz, 2H), 7.82 (d, J = 8.7 Hz, 2H), 7.18 (dd, J = 8.8, 3.1 Hz, 4H), 4.30 (q, J = 6.0 Hz, 4H), 3.95 (q, J = 6.0 Hz, 4H), 2.75 (s, 3H), 2.20 – 2.11 (m, 4H). ¹³C NMR (125 MHz, CDCl₃) δ 159.06, 158.88, 154.61, 153.13, 152.63, 147.62, 137.34, 136.70,
HR-ESI-MS: [M-H]$^+$ m/z 503.1752.

**Selectivity of DBTPP.** OH, ONOO$^-$, NO, H$_2$O$_2$, O$_2^-$, $^1$O$_2$, Fe$^{3+}$, Fe$^{2+}$, Cu$^{2+}$, Cys, Lys, GSH, BA, FA, GO, MDA and OPA were pre-prepared to 1 mM fresh stock solution. A standard solution of MDA was synthesised by hydrolysis of TEP.$^2$ The concentration of MGO stock solution was 500 μM in 100 mM pH 7.4 PBS. DBTPP was dissolved into DMAC and diluted into 62.5 μM. Finally, all above stock solutions were mixed together (v: v: v = 5: 1: 4). The control group repeated the same procedures except without adding MGO.

**Cell culture.** SH-SY5Y cells were grown in DMEM/F-12 (10% FBS, 1% Penicillin-Streptomycin solution) at the environment of 37°C, 5% CO$_2$. The cells were used for confocal imaging after reaching 90% confluence.

**MTT assays.** SH-SY5Y cells were grown in a 96-well plate at the environment of 37°C, 5% CO$_2$ at least 12 h for the appropriate confluence. Then the cells were treated with MGO, DBTPP and MTQPP at different concentration (MGO: 0-50 μM; DBTPP and MTQPP: 0-30 μM) for 12 h. Then freshly prepared MTT aqueous solution (5 mg/mL) was added into each well for another 4 h in the incubator. 100 μL DMSO was added to each well after removing the medium. After shaking for 20 min, the optical density values were detected at 450 nm.

**Exogenous MGO imaging.** SH-SY5Y cells were grown in a 4-Chamber glass bottom dish at the environment of 37°C, 5% CO$_2$ for at least 12 h for the appropriate confluence. After replacing the fresh DMEM/F-12 medium, 2 μL of DBTPP was
added into each chamber to reach a final concentration of 6 μM, then the co-incubation would last 1.5 h. The cells were washed three times with 1 × PBS followed by treated with MGO (0-8 μM) for another 1.5 h. The cells were immobilized by 4% paraformaldehyde for 15 min then were used for confocal imaging in 1 × PBS.

**Endogenous MGO imaging.** SH-SY5Y cells were grown in a 4-Chamber glass bottom dish at the environment of 37°C, 5% CO₂ for at least 12 h for the appropriate confluence. Then different concentrations of GLO1 inhibitor BHGD was added into each chamber then the co-incubation would last at least 5 h. The cells were washed three times with 1 × PBS followed by treated with DBTPP (10 μM) for another 1.5 h. The cells were immobilized by 4% paraformaldehyde for 15 min then were used for confocal imaging in 1 × PBS.

**Animals.** BALB/c-nu-nu mice (male, 4-5 months), C57BL/6 wild-type mice and APP/PS1 (APPsWe and PSEN1D9) double transgenic Alzheimer’s mice (male, 6-7 months) were acquired from Shanghai Model Organisms Center, Inc. All animal studies were performed in accordance with the guidelines approved by the Institutional Animal Care and Use Committee of East China Normal University.

**Fluorescence imaging of AD mice brain.** All mice were sacrificed by cervical dislocation. The whole brains were taken out and cut into 4-μm-thick frozen sections preserved at -30°C. At first, frozen sections were immersed in 4% paraformaldehyde for dehydration. Then frozen section of APP/PS1+GSH group were pre-incubated with 50 μM GSH for 2 h. After washed with 1 × PBS three times, all frozen sections of each group were treated with 10 μM DBTPP (final concentration) for 2 h, next
stained with 5 μg/mL DAPI for 5 min. Before the imaging experiment, all frozen sections were washed with 1 × PBS and covered with antifade mounting medium.

**In vivo fluorescence imaging.** All mice were anesthetized by intraperitoneal injection of 1% pentobarbital sodium and fixed on the stereotaxic apparatus. Five μL probe solution was injected into the right hippocampus of each mouse. Each mouse was given a dosage of 0.2 mg/kg DBTPP or 0.1 mg/kg MTQPP and conglutinated the wound with biomedical glue. The fluorescence imaging was performed with IVIS imaging system (λ_{ex} = 488 nm, λ_{em}= 650-800 nm). After the monitoring, all animals were cervical dislocation and anatomized. In vitro tissues were washed with PBS and preserved in 4% paraformaldehyde at 4°C.
Fig. S1. $^1$H NMR spectrum of DBTPP in CDCl$_3$ and methanol-D4.

Fig. S2. $^{13}$C NMR spectrum of DBTPP in CDCl$_3$ and methanol-D4.
Fig. S3. FT-IR spectrum of DBTPP.

Fig. S4. $^1$H NMR spectrum of MTQPP in CDCl$_3$. 
Fig. S5. $^{13}$C NMR spectrum of MTQPP in CDCl$_3$.

Fig. S6. FT-IR spectrum of MTQPP.
Fig. S7. HR-ESI-MS of (A) DBTPP and (B) MTQPP.

Fig. S8. Measurement of fluorescence quantum yield of DBTPP. (A-B) The absorbance and fluorescence spectra of DBTPP in DMAC. (C-D) The absorbance and fluorescence spectra of reference probe quinine sulfate in 0.1 M H$_2$SO$_4$. Both probes were tested with exactly the same excitation wavelength, slit width and voltage.
Fig. S9. Measurement of fluorescence quantum yield of MTQPP. (A-B) The absorbance and fluorescence spectra of MTQPP in DMAC. (C-D) The absorbance and fluorescence spectra of reference probe carboxyl fluorescein in 0.1 M NaOH. Both probes were tested with exactly the same excitation wavelength, slit width and voltage.

Fig. S10. (A) The fitting linearity of integrated fluorescence and absorbance of DBTPP and quinine sulfate (QY= 54%, in 0.1 M H₂SO₄). (B) The fitting linearity of integrated fluorescence and absorbance of MTQPP and carboxyl fluorescein (QY= 79%, in 0.1 M NaOH).
**Fig. S11.** Measurement of molar extinction coefficient of DBTPP and MTQPP. (A) The absorbance spectra of DBTPP in DMAC. (B) Linear fitting diagram of concentration and absorbance of DBTPP at 375 nm. (C) The absorbance spectra of MTQPP in DMAC. (B) Linear fitting diagram of concentration and absorbance of MTQPP at 500 nm.

**Fig. S12.** HPLC chromatograms of 25 μM DBTPP (yellow line, recorded at 375 nm), DBTPP+MGO (blue line, recorded at 500 nm), MTQPP (red line, recorded at 500 nm). Buffer: 10 mM PBS (pH = 7.4, 40% DMAC).
**Fig. S13.** Fluorescence changes as a function of pH at 650 nm of 25 μM DBTPP (yellow dot), DBTPP+MGO (blue dot), MTQPP (red dot). Buffer: 10 mM PBS (pH = 7.4, 40% DMAC).

**Fig. S14.** Photostability investigation of DBTPP (A) and MTQPP (B). Fluorescein isothiocyanate isomer (FITC) and Nile red (NR) were chosen as controls. Buffer: 10 mM PBS pH 7.4, 40% DMAC. All solutions were continuously irradiated under 365 nm UV lamp (8 W), then tested under the same slit width and voltage by a fluorescence spectrometer.
**Fig. S15.** Cell viability of SH-SY5Y cells with different concentrations of (A) DBTPP and (B) MTQPP by using MTT assay.

**Fig. S16.** Relative red channel fluorescence intensity of 6 μM DBTPP treated with MGO in SH-SY5Y cells.
**Fig. S17.** Confocal images of SH-SY5Y cells co-incubated with 10 μM DBTPP at different concentrations of GLO1 inhibitor BHGD (0, 15, 30, 60 μM). Red channel: $\lambda_{ex} = 488$ nm, $\lambda_{em} = 600$-800 nm. Scale bar = 25 μm.
**Fig. S18.** In vivo fluorescence images of normal nude mice at 3, 6, 9, 12, and 24 h after intracranial injection of MTQPP. Ex vivo fluorescence images of organs after intracranial injection of MTQPP for 12, 24 and 48 h. (From left to right: heart, liver, spleen, lung, brain, kidney and muscle). \( \lambda_{\text{ex}} = 488 \text{ nm}, \lambda_{\text{em}} = 650-800 \text{ nm}. \)
Fig. S19. In vivo fluorescence images of AD mice and wild-type mice at 1.5, 4.5, and 7.5 h after intracranial injection of DBTPP.
Table S1. Quantum yield (QY) and molar extinction coefficient (ε) of DBTPP and MTQPP in DMAC.

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<th>QY (%)</th>
<th>ε (L·mol⁻¹·cm⁻¹)</th>
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<tr>
<td>DBTPP</td>
<td>6.8</td>
<td>14280 (375 nm)</td>
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<tr>
<td>MTQPP</td>
<td>12.4</td>
<td>5462 (500 nm)</td>
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References
