Supplementary Information

Modulation of intramolecular freedom of movement for tuning fluorescence imaging and photooxidation of amyloid-β aggregates

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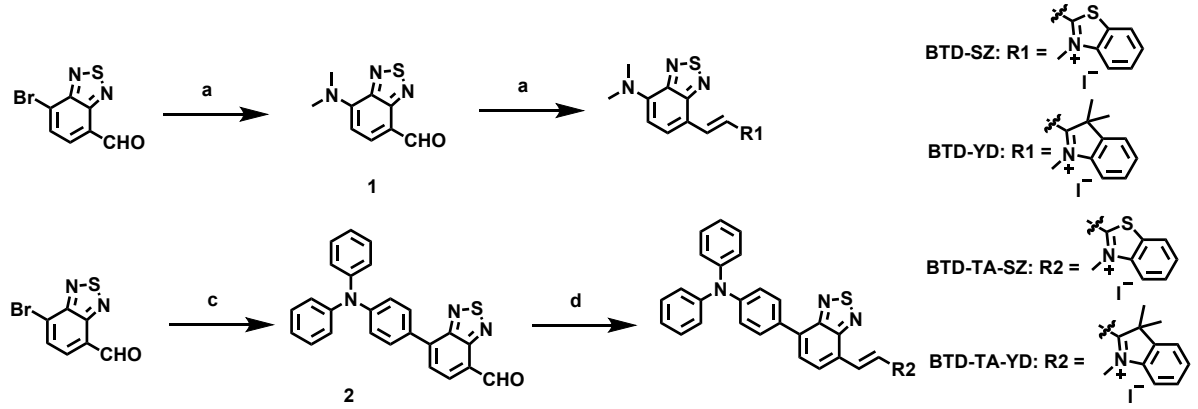
1. Materials and instruments

Unless otherwise stated, all materials were purchased and used without further purification. The synthesized $A\beta_{1-42}$ peptide was from Qiangyao Biotechnology Co., Ltd. (China). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker BioSpin GmbH spectrometer at 400 MHz and 101 MHz. UV-visible absorption spectra and fluorescence spectra were recorded using a UV-2450 spectrophotometer (Shimadzu, Japan).

2. Synthetic procedures



2.1 Synthesis of compound 1: Dimethylamine solution (9 mL) added 7-bromo-2,1,3-benzothiadiazole-4-carbaldehyde (200 mg), and the reaction was refluxed under argon protection and 55 °C. TLC confirms the reaction was completed, stopped the reaction and stand to room temperature. Finally separate and purify by silica gel column chromatography 1 (silica gel 100-200 mesh; Dichloromethane: petroleum ether = 1:2, v/v) to obtain a orange solid (1), with a yield of 43.76%.^{1}H NMR (400 MHz, DMSO-d_6) δ 10.25 (s, 1H), 8.06 (d, J = 8.5 Hz, 1H), 6.63 (d, J = 8.6 Hz, 1H), 3.55 (s, 6H).

2.2 Synthesis of compound 2: 7-bromo-2,1,3-benzothiadiazole-4-carboxaldehyde (200 mg) was

dissolved in tetrahydrofuran/toluene (30 mL/50 mL, v/v), added 4-boronic acid triphenylamine (237 mg). Then, added an aqueous 2M K_2CO_3 solution (1.5 mL) and catalyst $Pd(PPh_3)_4$ (30 mg) to be mixed homogeneously. The reaction was sealed at 105 ℃ for 12 h. The reaction solvent was removed by rotary evaporation under low pressure, and then purified by silica gel column chromatography (silica gel powder 200-300 mesh; dichloromethane: petroleum ether = 1:3, v/v) to obtain compound **2** as an red solid, yield = 61.21%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (s, 1H), 8.35 (d, J = 7.5 Hz, 1H), 8.05 (dd, J = 8.0, 4.7 Hz, 3H), 7.39 (t, J = 7.9 Hz, 5H), 7.27 – 7.04 (m, 10H), 6.96 – 6.88 (m, 2H).

2.3 Synthesis of BTD-SZ: compound 1 (50 mg, 0.24 mM), 2,3-dimethylbenzo[d]thiazol-3-ium (100 mg, 0.34 mM) was dissolved in ethanol (20 mL), then appropriate amount of piperidine was ad ded dropwise. The mixture was heated to reflux and stirred for 12 h. After the reaction was complet ed, leaved to room temperate and solid compounds are precipitated. The filter cake was washed with anhydrous ethanol and dried to obtain pure **BTD-SZ**. Purple solid; Yield: 62.32%; ^{1}H NMR (4 00 MHz, DMSO-*d6*): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (m, J = 8.2 Hz, 3H), 8.22 (d, J = 8.6 Hz, 1H), 8.12 (d, J = 8.4 Hz, 1H), 7.78 (t, J = 7.9 Hz, 1H), 7.68 (t, J = 7.7 Hz, 1H), 6.76 (d, J = 8.8 Hz, 1H), 4.21 (s, 3H), 3.63 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.68, 155.65, 147.62, 147.38, 145.78, 142.34, 140.62, 129.35, 127.81, 127.16, 124.24, 11 6.26, 113.57, 108.05, 107.17, 43.58, 35.87. Mass spectrometry (ESI-MS, m/z): calcd. for $[C_{18}H_{17}N_4S_2]^+$ 353.0889; found 353.0895.

2.4 Synthesis of BTD-YD: compound 1 (50 mg, 0.24 mM), 1,2,3,3-tetramethyl-3H-indol-1-iu m (101 mg, 0.33 mM) was dissolved in ethanol (20 mL), then appropriate amount of piperidine was added dropwise. The mixture was heated to reflux and stirred for 12 h. After the reaction was compl eted, leaved to room temperate and solid compounds are precipitated. The filter cake was washed wit h anhydrous ethanol and dried to obtain pure **BTD-YD**. Purple solid; Yield: 60.12%; ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.56 (d, J = 15.3 Hz, 1H), 8.32 (d, J = 15.4 Hz, 1H), 8.23 (d, J = 8.8 Hz, 1H), 7.69 (dd, J = 7.3, 1.2 Hz, 1H), 7.64 (d, J = 7.9 Hz, 1H), 7.58 (td, J = 7.7, 1.3 Hz, 1H), 7.50 (td, J = 7.4, 1.2 Hz, 1H), 6.83 (d, J = 8.8 Hz, 1H), 4.01 (s, 3H), 3.7 8 (s, 6H), 1.88 (s, 6H).

2.3 Synthesis of BTD-TA-YD: compound 2 (50 mg, 0.12 mM), 1,2,3,3-tetramethyl-3H-indol-1-ium (68 mg, 0.23 mM) was dissolved in ethanol (20 mL), then appropriate amount of piperidine w as added dropwise. The mixture was heated to reflux and stirred for 12 h. After the reaction was co mpleted, leaved to room temperate and solid compounds are precipitated. The filter cake was washed with anhydrous ethanol and dried to obtain pure **BTD-TA-YD**. Purple solid; Yield: 58.10%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.69 (m, 3H), 8.13 (m, 3H), 7.98 (m, 2H), 7.66 (m, 2H), 7. 41 (m, 4H), 7.17 (m, 8H), 4.19 (s, 3H), 1.88 (s, 6H).

2.3 Synthesis of BTD-TA-SZ: compound 2 (50 mg, 0.12 mM), 2,3-dimethylbenzo[d]thiazol-3 -ium (68 mg, 0.22 mM) was dissolved in ethanol (20 mL), then appropriate amount of piperidine was added dropwise. The mixture was heated to reflux and stirred for 12 h. After the reaction was compl eted, leaved to room temperate and solid compounds are precipitated. The filter cake was washed wit h anhydrous ethanol and dried to obtain pure **BTD-TA-SZ**. Purple solid; Yield: 62.12%; ^{1}H N MR (400 MHz, DMSO-d_6) δ 8.78 (d, J = 15.8 Hz, 1H), 8.56 (d, J = 15.8 Hz, 1H), 8.53 – 8.44 (m, 2H), 8.34 (d, J = 8.4 Hz, 1H), 8.10 (dd, J = 8.3, 2.4 Hz, 3H), 7.92 (ddd, J = 8. 5, 7.1, 1.3 Hz, 1H), 7.84 (t, J = 7.7 Hz, 1H), 7.40 (dd, J = 8.8, 6.9 Hz, 4H), 7.20 – 7.09 (m, 9H), 4.41 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.02, 153.67, 153.52, 148.97, 1 46.98, 143.41, 142.58, 136.76, 135.17, 131.17, 130.28, 130.09, 129.40, 129.09, 128.53, 127.2 3, 125.52, 125.06, 124.87, 124.63, 121.73, 117.48, 117.10, 37.00. Mass spectrometry (ESI-M S, m/z): calcd. for $[C_{34}H_{25}IN_4S_2 + H]^+$ 681.0565; found 681.0646.

3. Spectroscopic determinations

The UV-Vis and fluorescence spectra in different solvents were measured by previously reported methods [1]. The calculation method of fluorescence quantum yield is shown in Equation：

$$\Phi_{probe} = \Phi_{standard} \times \frac{OD_{probe} \times A_{standard} \times \eta_{probe}^2}{OD_{standard} \times A_{probe} \times \eta_{standard}^2}$$

The abbreviations meaning in the equation are list as follows, Φ serves as the fluorescence quantum

yield, OD is the area under the fluorescence curve; A, the ultraviolet of the probe:η, is the refractive index of the solution (the same refractive index of the same solvent). The standard for fluorescence quantum yield is Rhodamine 6G, its quantum yield in ethanol is 0.95.

4. Determination of singlet oxygen production efficiency

The 9,10-anthracenediylbis(methylene)dimalonic acid (ABDA) was used as an indicator to specifically detect singlet oxygen production. The mixture dissolving ABDA (100 μM) and different PSs (10 μM) was exposed to white light irradiation (10 mW/cm^2). The ultraviolet spectrum decrease of ABDA was recorded at various irradiation times. The $^1O_2$ quantum yield (Φ_O) of the PSs was obtained by the following calculation formula:

$$\Phi_O = \Phi_{RB} * K_{PS} * A_{RB} / (K_{RB} * A_{PS})$$

Rose Bengal (RB) serves as the standard photosensitizer and Φ_{RB} is the singlet oxygen quantum yield of RB (75%) in water. K_{PS} and K_{RB} are the absorbance decomposition rate of ABDA at 400 nm in the presence of PSs and RB. A_{PS} and A_{RB} represent the integration of the UV-visible absorption bands covering the wavelength range of 400-700 nm of PSs and RB.

5. Fluorescence response of the molecules towards Aβ Aggregates

The PSs and $A\beta_{1-42}$ aggregates were mixed with a final concentration of 1 μM and 10 μM respectively, and then incubated for 10 min at room temperature. The fluorescent intensity was measured by a multifunction microplate reader and the measurements of selectivity and stability were conducted according to our previous reports.[1]

6. Procedures of slice staining *in vitro*

Paraffin brain sections of *in vitro* mice were obtained from transgenic mice. Mice slices were

soaked 5 min × 2 with m-xylene for dewaxing, and then soaked with anhydrous ethanol for 5 min × 2, 90 % ethanol solution for 5 min × 2, 80 % ethanol solution for 5 min, 70 % ethanol solution, and PBS × 1 (pH 7.4) solution, respectively. Then, **BTD-SZ** (1 μM) was soaked for 30 min, washed three times with PBS (pH 7.4) solution and then re-stained with commercial dye Th-S (20 μM) for 30 min, which was washed three times with PBS (pH 7.4). A small amount of blocking solution was dropped on the brain slices and observed with a coverslip. Stained sections were observed under laser confocal microscope.

7. Animals

The National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) has been followed in all animal experiments. Tg mice (C57BL6, APPsw/PSEN1, 11 months old, 6 months old, male) and age-matched WT mice (C57BL6, 11 months old, 6 months old, male) were purchased from the Huafukang Biotechnology Co., Ltd. (Beijing, China). The mice were acclimatized for 1 week in the laboratory animal center house under a 12 h light/dark cycle at 23 °C with 60-70% humidity and provided with food and water. All experiments were strictly executed in accordance with the guide for the care and use of laboratory animals and were approved by the Animal experimentation ethics committee of zunyi medical university.

8. Procedures of imaging AD mice *in vivo*

Tg mice (n = 3, C57BL6, APPsw/PSEN1, 11 months old, male) and age-matched Wt mice (n = 3, C57BL6, 11 months old, male) were used for *in vivo* imaging. The mice with scalp hair removed was i.v. injected with solution of **BTD-SZ** (0.4 mg/Kg, 10% DMSO and 90% propylene glycol, 50 μL). The imaging paradigm was performed on small animal in vivo imaging system (Cypris) with proper filter sets (ex. at 520 nm), and the fluorescence signals of the brain were recorded at various

time points with the mice anesthetized (2.5% isoflurane in oxygen flow, 1.5 L/min). The acquired images were analyzed by drawing ROI with the area around the brain region using the Living Image software 4.5.2. Semiquantitative results were calculated from radiance efficiency.

9. Ex Vivo Fluorescence Staining of BTD-SZ in the Brain Slices of Transgenic Mice.

APP/PS1 and wild-type (WT) mice, both aged 11 months, were anesthetized 30 minutes after intravenous administration of BTD-SZ (0.4 mg/kg). After perfusion with normal saline, their brains were extracted and fixed. The fixed brains were then dehydrated and sectioned into 20 μm thick frozen sections. These sections were stained for Aβ plaques with thioflavin S (ThS; 0.125%) for 10 minutes, followed by washing in 60% ethanol solution for 1 minute, repeated three times. An anti-fluorescence quenching sealing solution was applied, and a coverslip was placed. Imaging was performed using an Olympus inverted fluorescence microscope.

10. CCK8 assay

PC12 cells derived from rat pheochromocytoma were cultured in DMEM medium, 10 % horse serum, 5 % fetal bovine serum and 1 % antibiotic at 37 ° C under 5 % CO_2 conditions. Cells were loaded into 96-well plates (2×10^4 cells / well, 100 μl) and incubated at 37 ° C and 5 % CO_2 for 24 h. Aβ sample solution (5 μl) incubated with or without **BTD-TA-SZ** in dark or light conditions was added and incubated for 24 hours under the same conditions. After incubation, the remaining medium was removed, and a mixture of 10 μL CCK8 solution. The absorbance at 450 nm was measured by microplate reader.

11. The uptake and degradation of $A\beta_{1-42}$ by BV2 cells

Microglia (BV2) can improve the pathological features of AD by engulfing amyloid fibrils. BV2 cells were seeded into plates (5,000 cells/well) and left to culture for 24 h. These cells were then co-incubated with fluorescein labeled $A\beta_{1-42}$ (FAM-$A\beta_{1-42}$, 5 μM) and **BTD-TA-SZ** (1 μM), either in the dark or under 520 light irradiation (1000 mW/cm^2, 10 min) for 12 h. Thereafter, the cells were washed three times with PBS, and co-incubated with 10 μL Destination Access Point Identifier (DAPI, commercial DAN dye) for 30 min, and washed with PBS. Finally, the microglia uptake of $A\beta_{1-42}$ was analyzed by confocal microscopy.

12. The acute toxicology of BTD-TA-SZ

KM mice (18-22 g, male) were utilized in an Up-and-Down procedure following the guidelines of the China Food and Drug Administration for the acute toxicology of BTD-TA-SZ. The mice were intravenously injected with a solution of BTD-TA-SZ (10% DMSO and 90% propylene glycol, 10 mL/kg). The technical guidelines for acute toxicity testing of chemical drugs specify a slope factor sigma of 0.5. The dosing interval was 48 hours, with a concentration gradient of 2000, 550, 175, 55, 17.5, 5.5, and 1.75 mg/kg. Dose escalation was halted when one of the three stopping rules in the AOT425 StatPgm program (USEPA) was met. The intravenous LD50 of BTD-TA-SZ was determined to be 103.6 mg/kg, with a 95% confidence interval ranging from 55 to 175 mg/kg.

13. Drug treatments protocol

The mice were divided into the following groups: (1) Wt: Wild-type (Wt) mice (6 months old) were intravenously injected with a solvent blank (10% DMSO and 90% propylene glycol); (2) AD: Transgenic (Tg) mice (6 months old) were intravenously injected with a solvent blank; (3) AD + Light: Tg mice were intravenously injected with a solvent blank and then exposed to light (520 nm, 1000

mW) for 10 minutes 30 minutes post-injection; (4) AD + BTD-TA-SZ: Tg mice were intravenously injected with BTD-TA-SZ; (5) AD + BTD-TA-SZ + Light: Tg mice were intravenously injected with BTD-TA-SZ and subsequently exposed to light (520 nm, 1000 mW) for 10 minutes 30 minutes post-injection. BTD-TA-SZ was dissolved in 10% DMSO and 90% propylene glycol and administered intravenously at 2 mg/kg every other day for 30 days. Local head temperature was monitored using a thermal infrared imaging camera before and after irradiation.

14. Morris water maze test

Behavioral studies were conducted on mice (n = 6 per group) using the Morris Water Maze (MWM) test, during routine administration. The experimental setup comprised a circular water tank (120 cm in diameter, 45 cm in height) filled with water at 20 ± 1°C to a depth of 32 cm. The water was made opaque by adding non-toxic white titanium dioxide (TiO_2). A platform (9 cm in diameter, 30 cm in height) was submerged 1.5 cm below the water surface and positioned at the midpoint of the first quadrant. Each mouse underwent daily training for 5 consecutive days, with a single hidden platform located in one of four quadrants, and the starting point rotated between quadrants. Mice were given 60 seconds to locate the hidden platform; if they failed, they were guided to it and the escape latency was recorded as 60 seconds. Latency to escape from the maze was tracked using a computerized video-tracking system (Shanghai Xinruan Information Technology Co., Ltd., Shanghai, China) for each trial. After 24 hours following the fifth day of training, a probe test was conducted with the platform removed. During the probe test, mice were allowed to swim freely for 60 seconds. The time spent crossing the former platform location, average swimming speed, time spent, and path length in the target quadrant, as well as the virtual platform, were recorded using video-tracking software to assess memory consolidation.

15. Aβ plaques Fluorescence Staining in the Brain Slices

After the Morris Water Maze (MWM) test, all mice were anesthetized 30 minutes following intravenous administration of BTD-SZ (0.4 mg/kg). The brains were then extracted after perfusion with normal saline and fixed. Following fixation, the brains were dehydrated and sectioned into 20 μm thick frozen sections. These sections were stained for Aβ plaques with thioflavin S (ThS; 0.125%) for 10 minutes, followed by washing with a 60% ethanol solution for 1 minute, repeated three times. An anti-fluorescence quenching sealing solution was applied, and a coverslip was placed. Imaging was performed using an Olympus inverted fluorescence microscope.

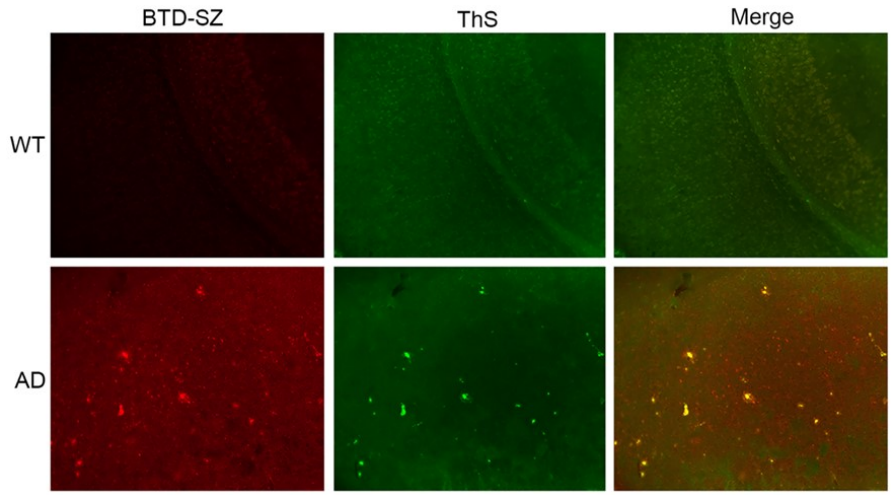


Figure S1.The histological staining of brain tissue sections from mice after imaging.

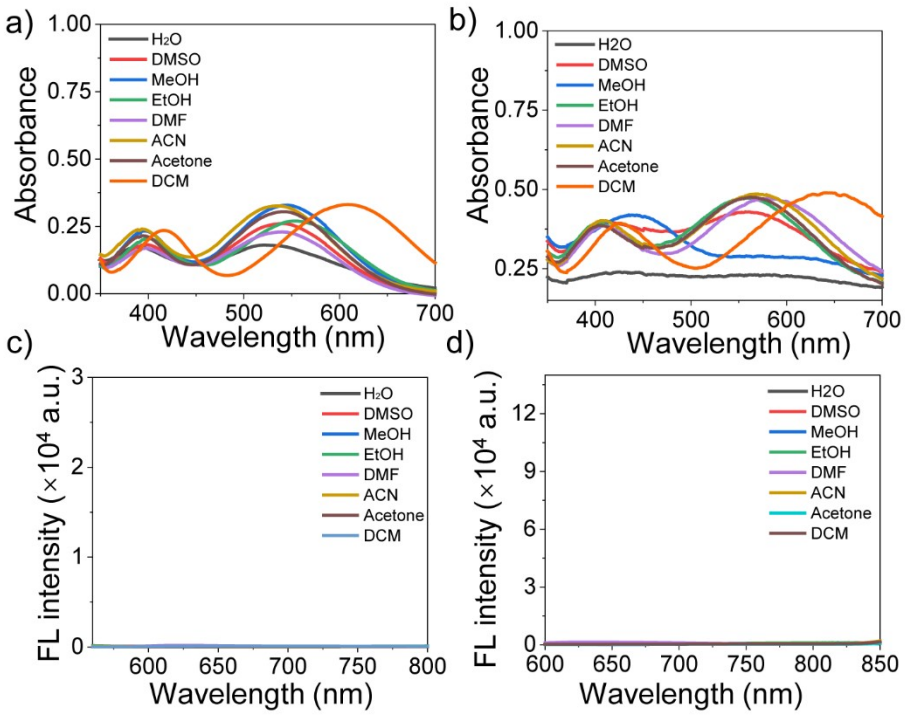


Figure S2. a)b) The ultraviolet spectrum and c)d) fluorescence spectrum of the BTD-TA-SZ and BTD-TA-YD in different solvents (10 μM).

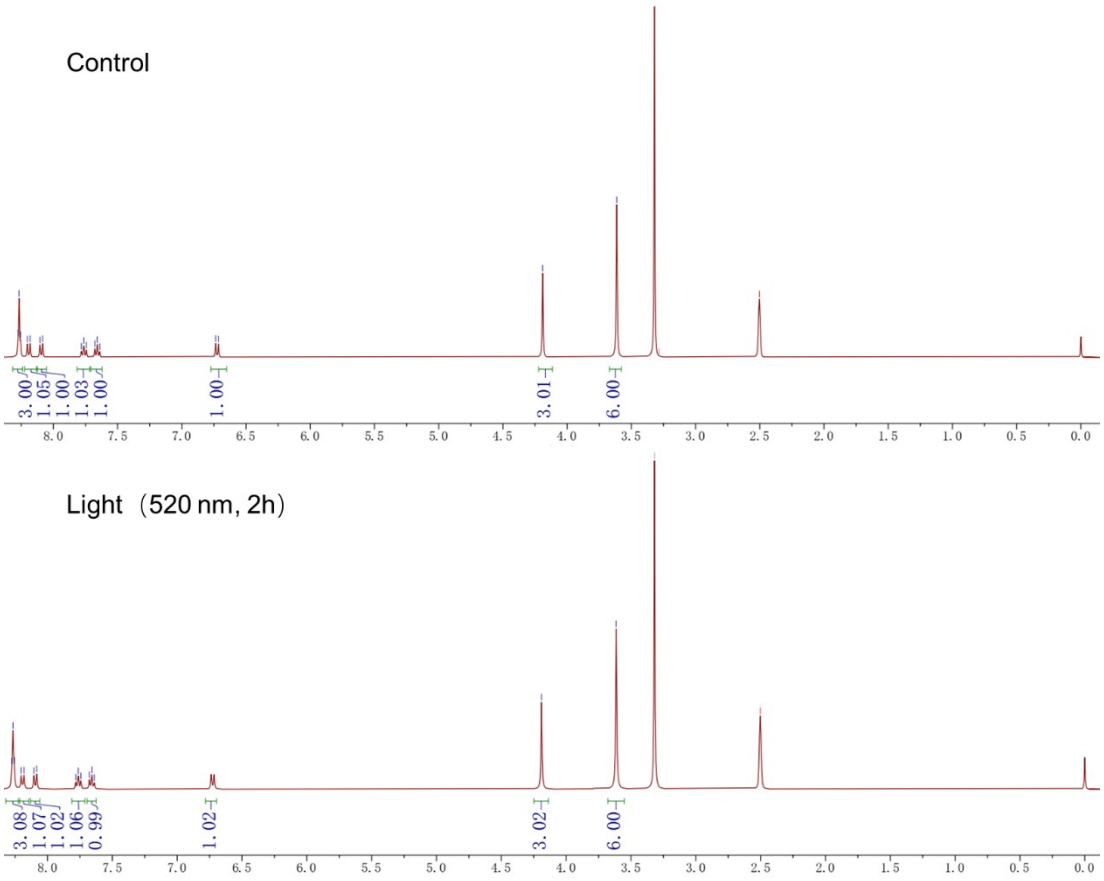


Figure S3. The photostability of BTD-SZ (10 μM) in DMSO solutions (520 nm).

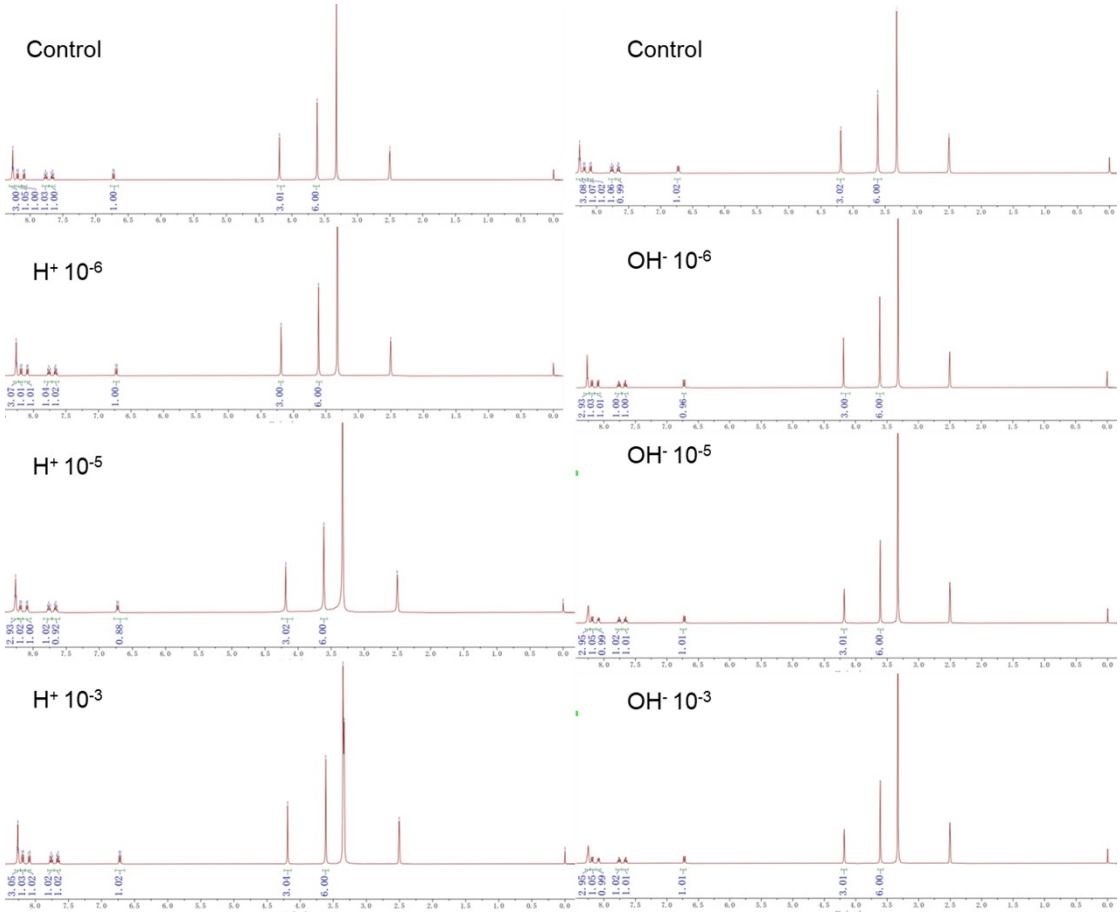


Figure S4. The pH stability of BTD-SZ (10 μM) in DMSO solutions.

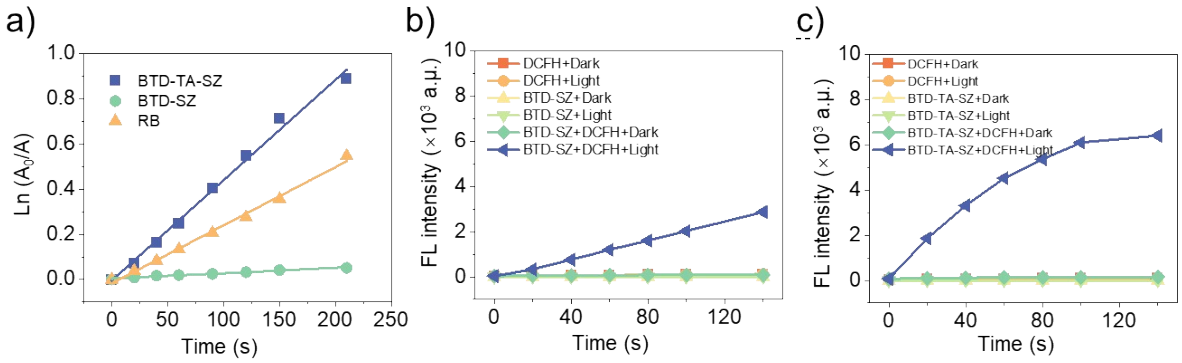


Figure S5. a) Comparison of singlet oxygen generation rate between BTD-SZ, BTD-TA-SZ and RB under white light irradiation; b) c) ROS generation by BTD-SZ and BTD-TA-SZ, using DCFH-DA as the probe under the irradiation of white light.

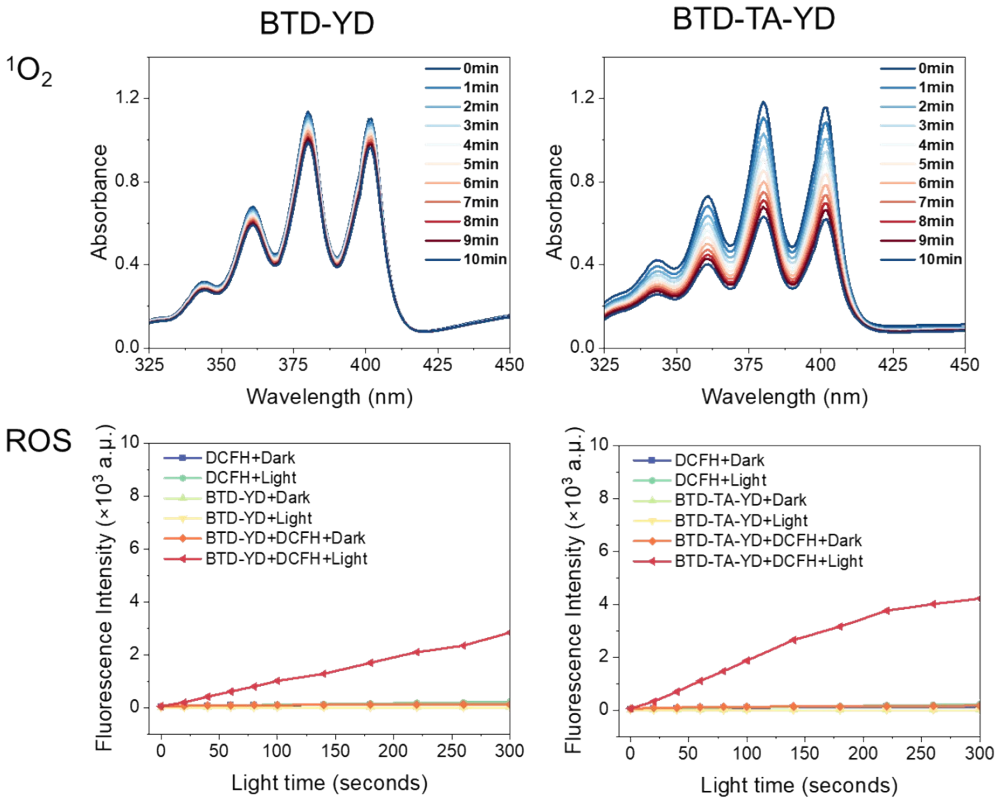


Figure S6. Comparison of singlet oxygen and ROS generation generation rate between BTD-YD, BTD-TA-YD under white light irradiation

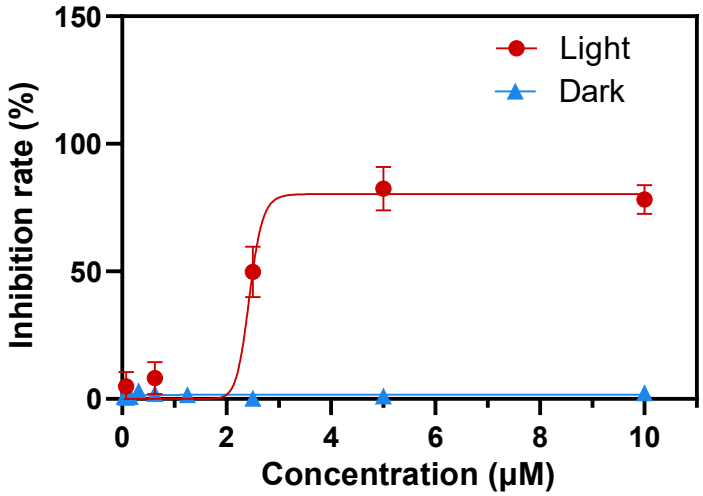


Figure S7. Dark toxicity and phototoxicity of BTD-TA-SZ.

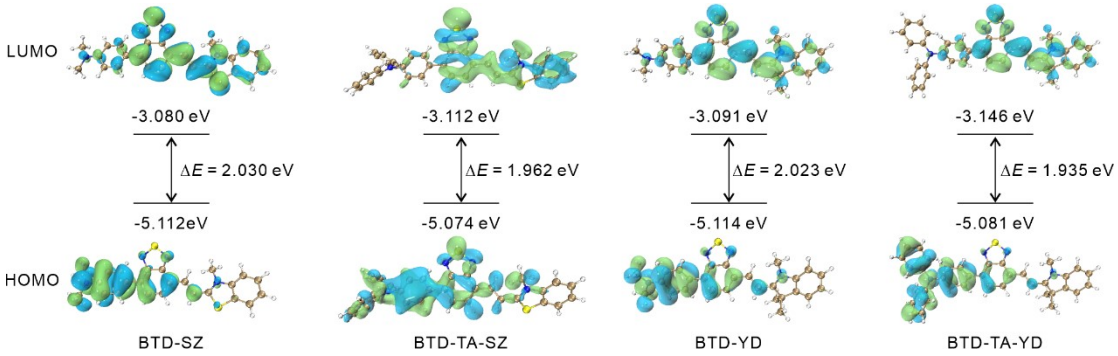


Figure S8. The distributions and energy of HOMO and LUMO of BTD-SZ, BTD-TA-SZ, BTD-TD, and BTD-TA-TD at B3LYP/6-31G(d,p) level.

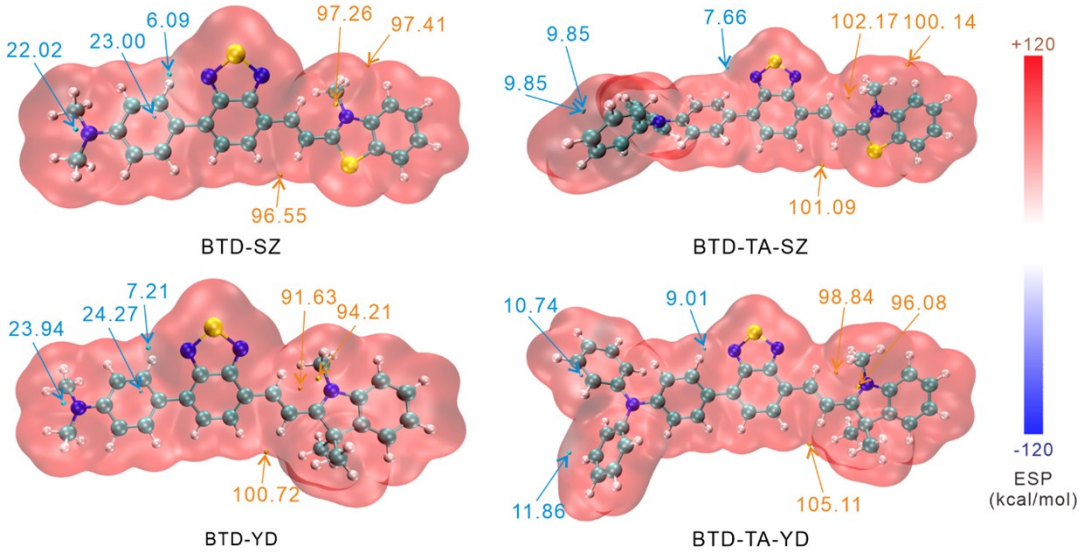


Figure S9. Electrostatic potential on the ρ(r)=0.001 au molecular surface of BTD-SZ, BTD-TA-SZ, BTD-TD, and BTD-TA-TD at B3LYP/6-31G(d,p) level.

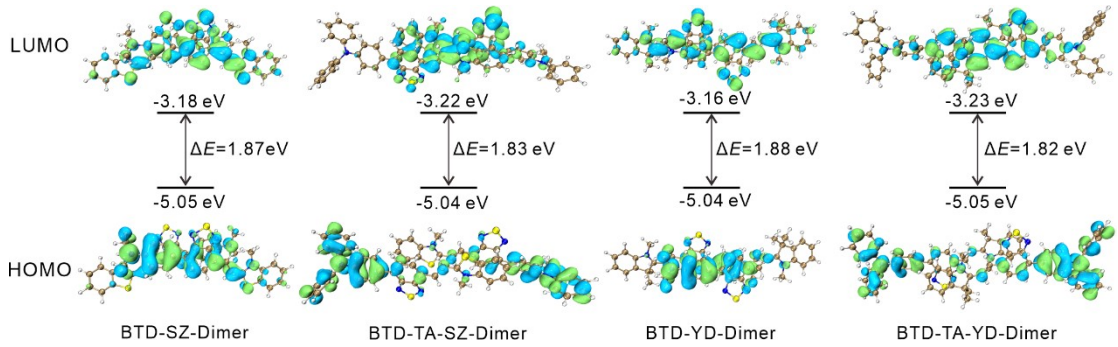


Figure S10. The distributions and energy of HOMO and LUMO of BTD-SZ-Dimer, BTD-TA-SZ-Dimer, BTD-TD-Dimer, and BTD-TA-TD-Dimer at B3LYP/6-31G(d,p) level.

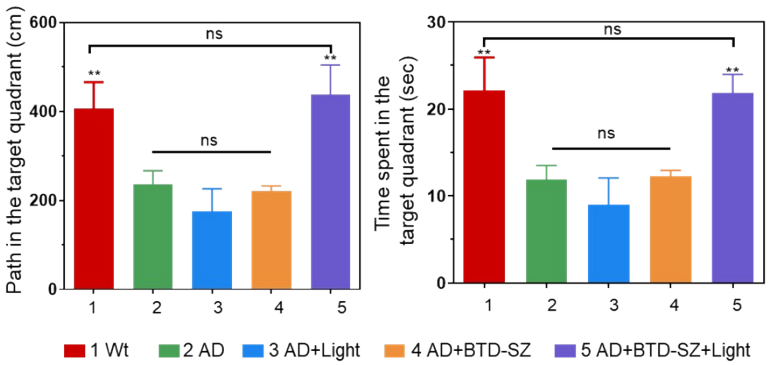


Figure S11. Morris Water Maze Test in different groups (n = 6). The path and time spent in the target quadrant.

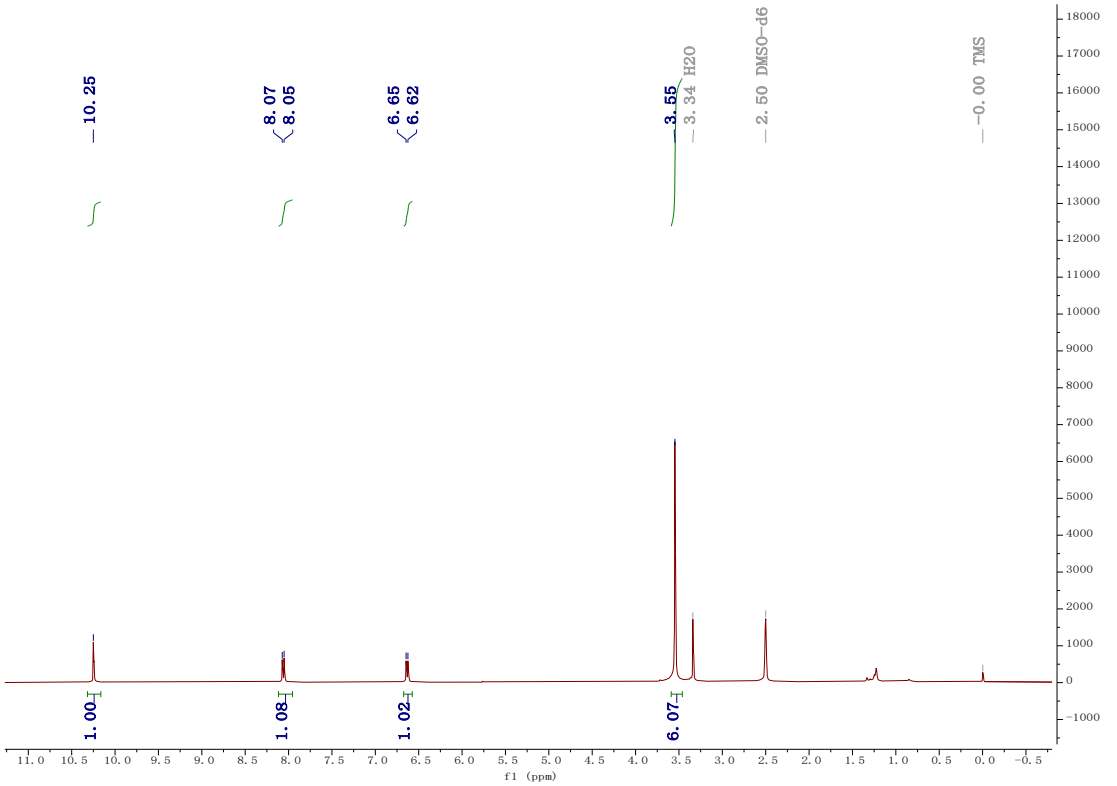


Figure S12 ^{1}H-NMR spectrum of **1**

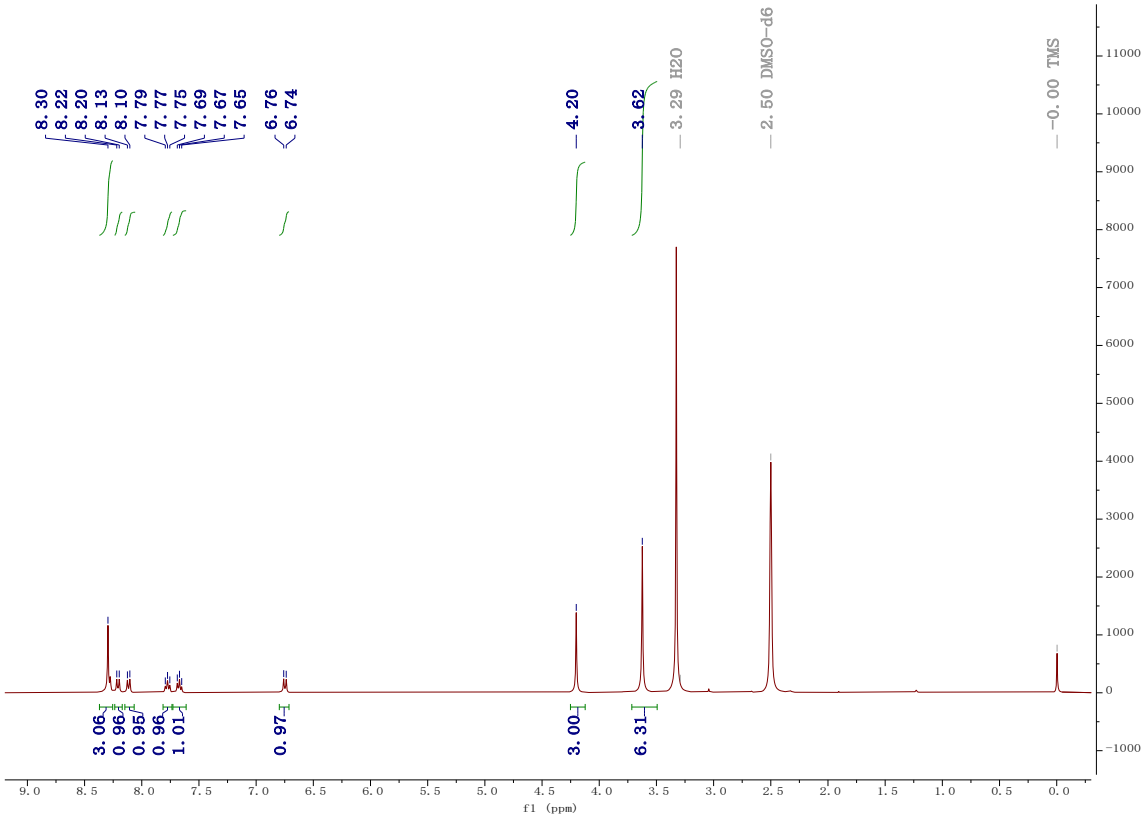


Figure S13 ^{1}H-NMR spectrum of **BTD-SZ**

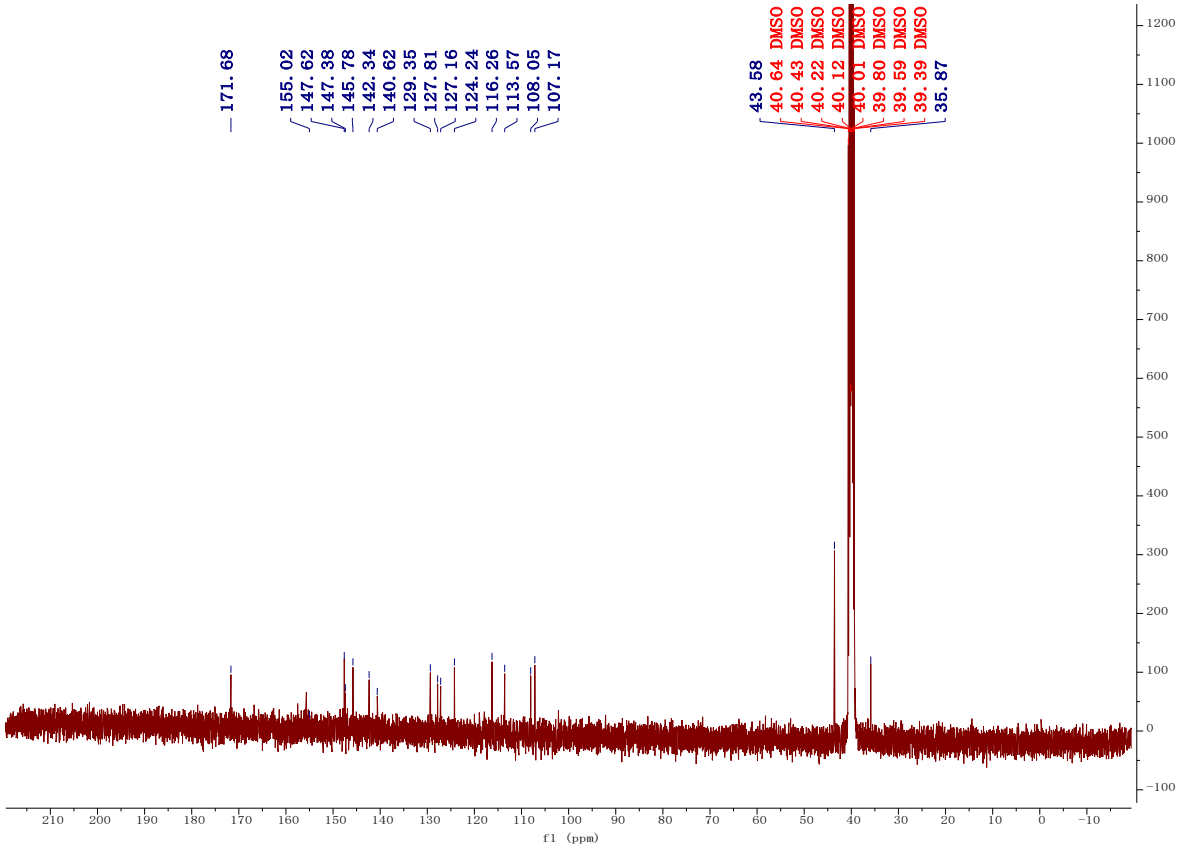


Figure S14 ^{13}C-NMR spectrum of **BTD-SZ**

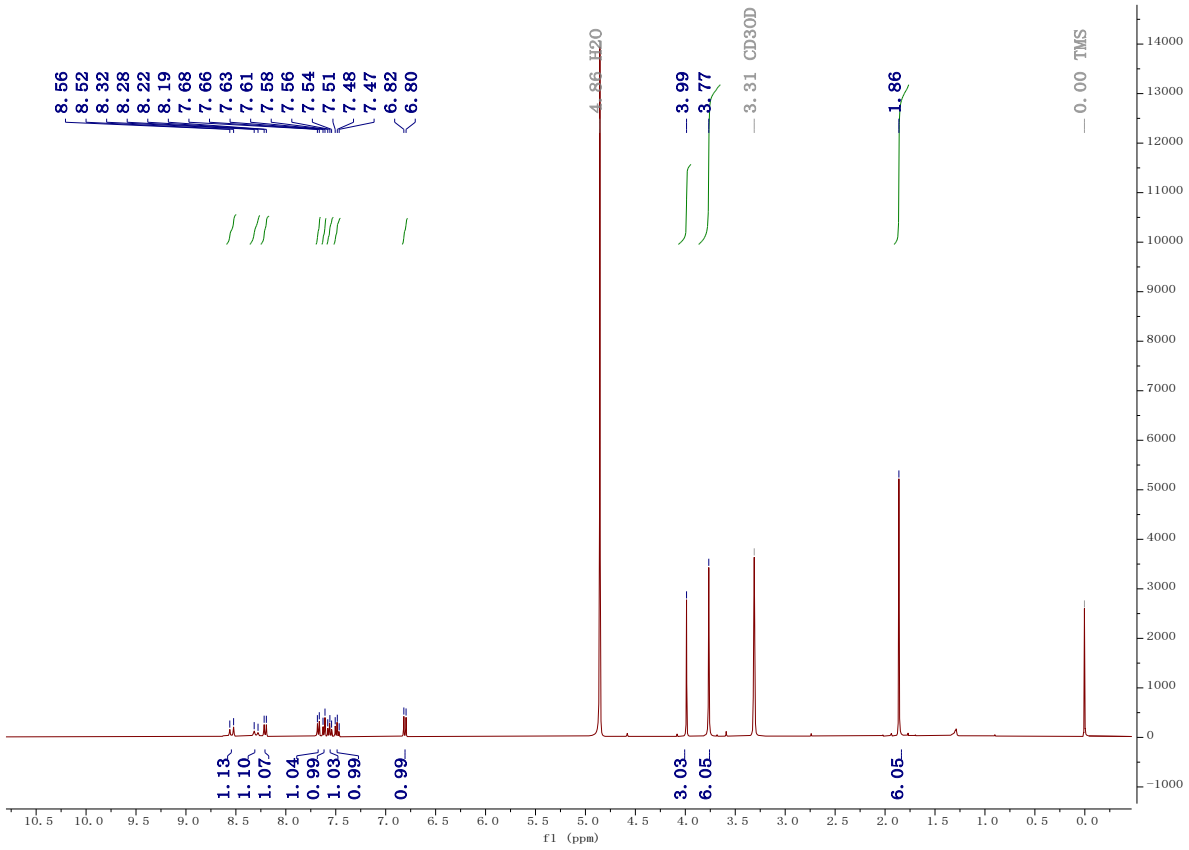


Figure S15 ^{1}H-NMR spectrum of **BTD-YD**

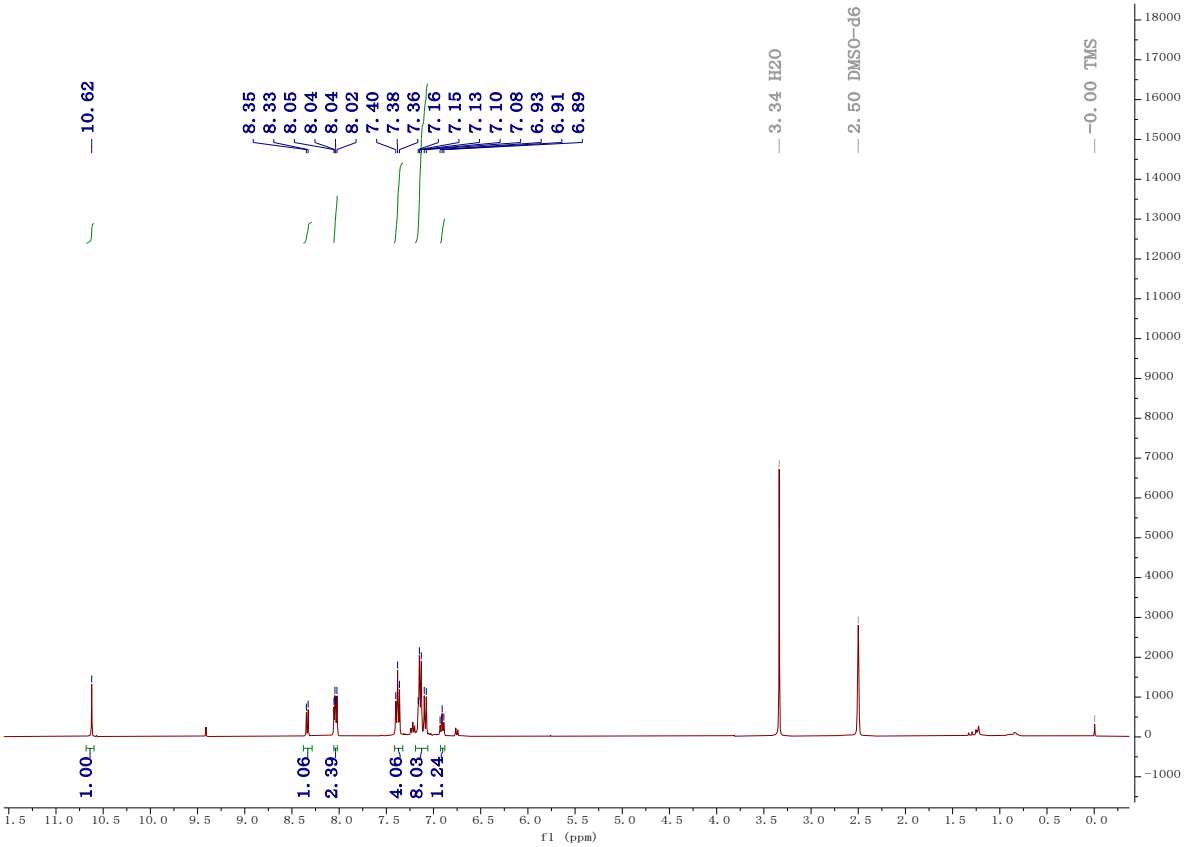


Figure S16 ^{1}H-NMR spectrum of **2**

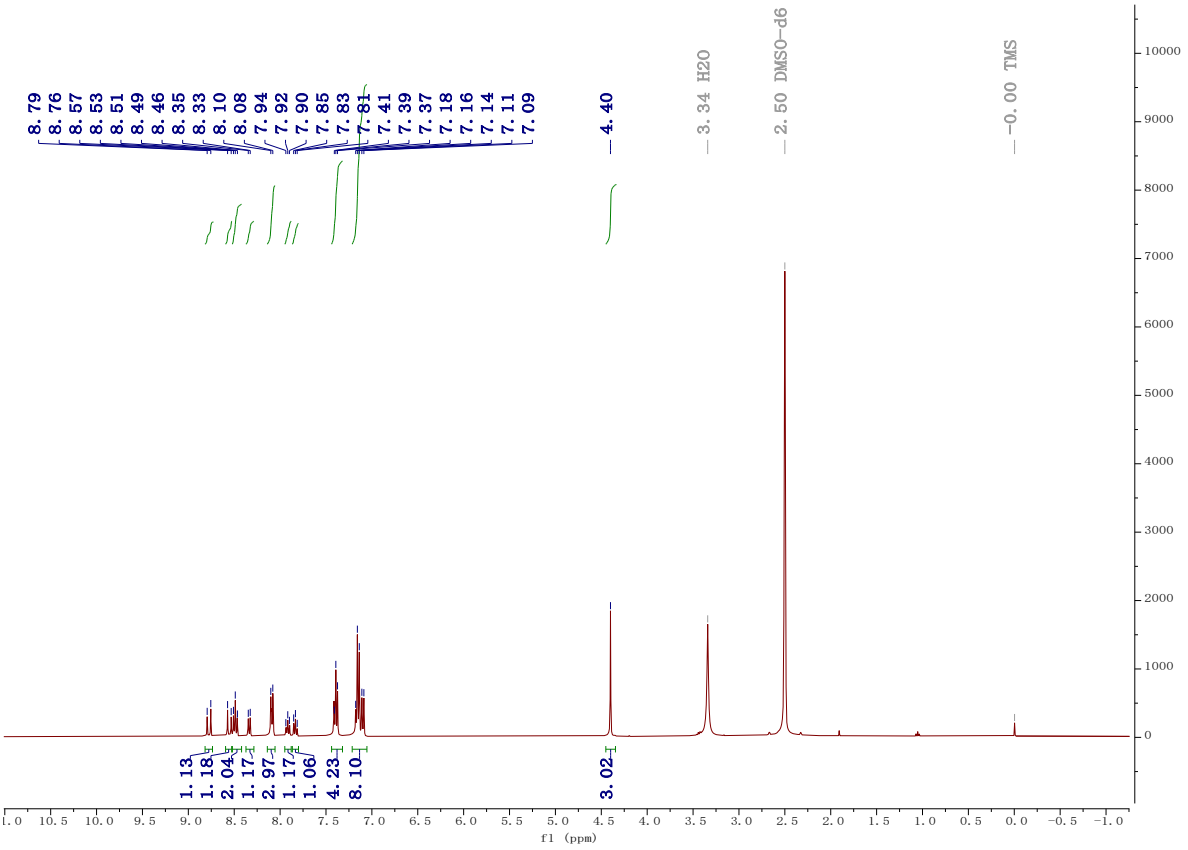


Figure S17 ^{1}H-NMR spectrum of **BTD-TA-SZ**

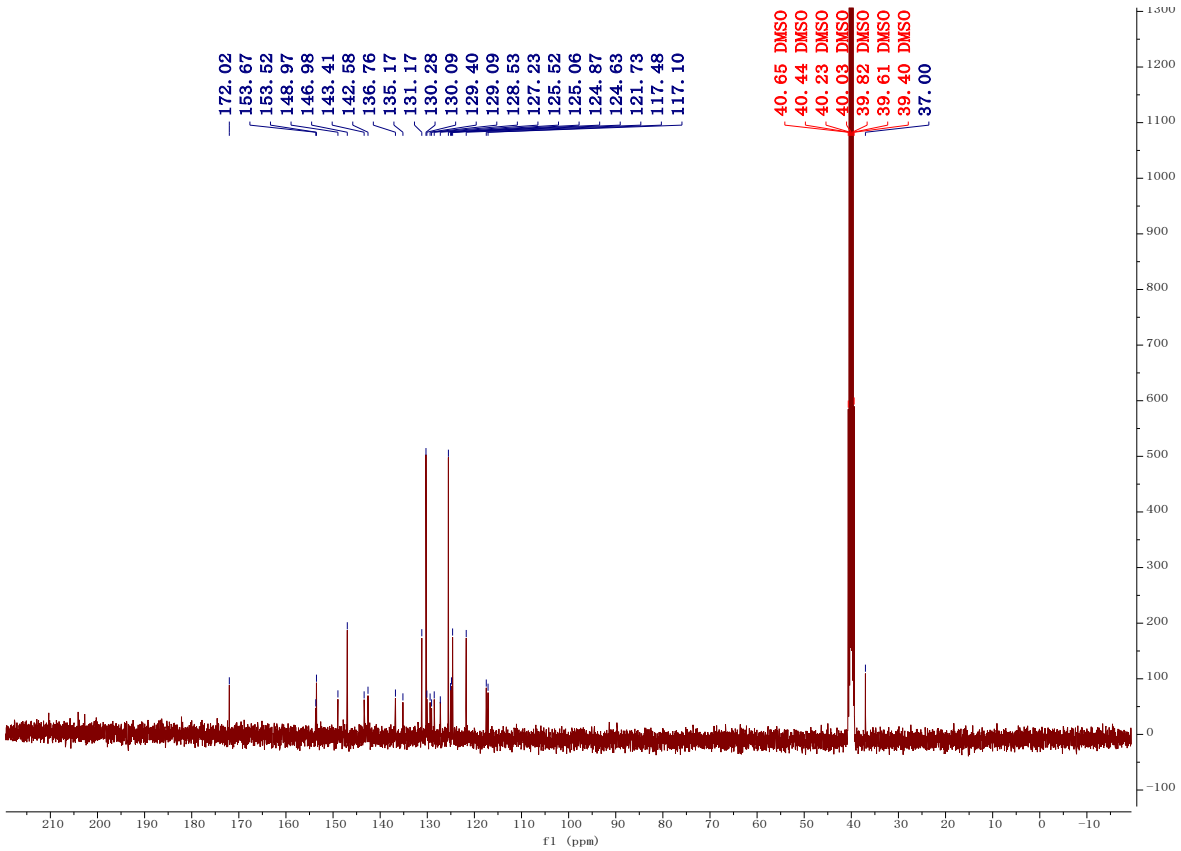


Figure S18 ^{13}C-NMR spectrum of **BTD-TA-SZ**

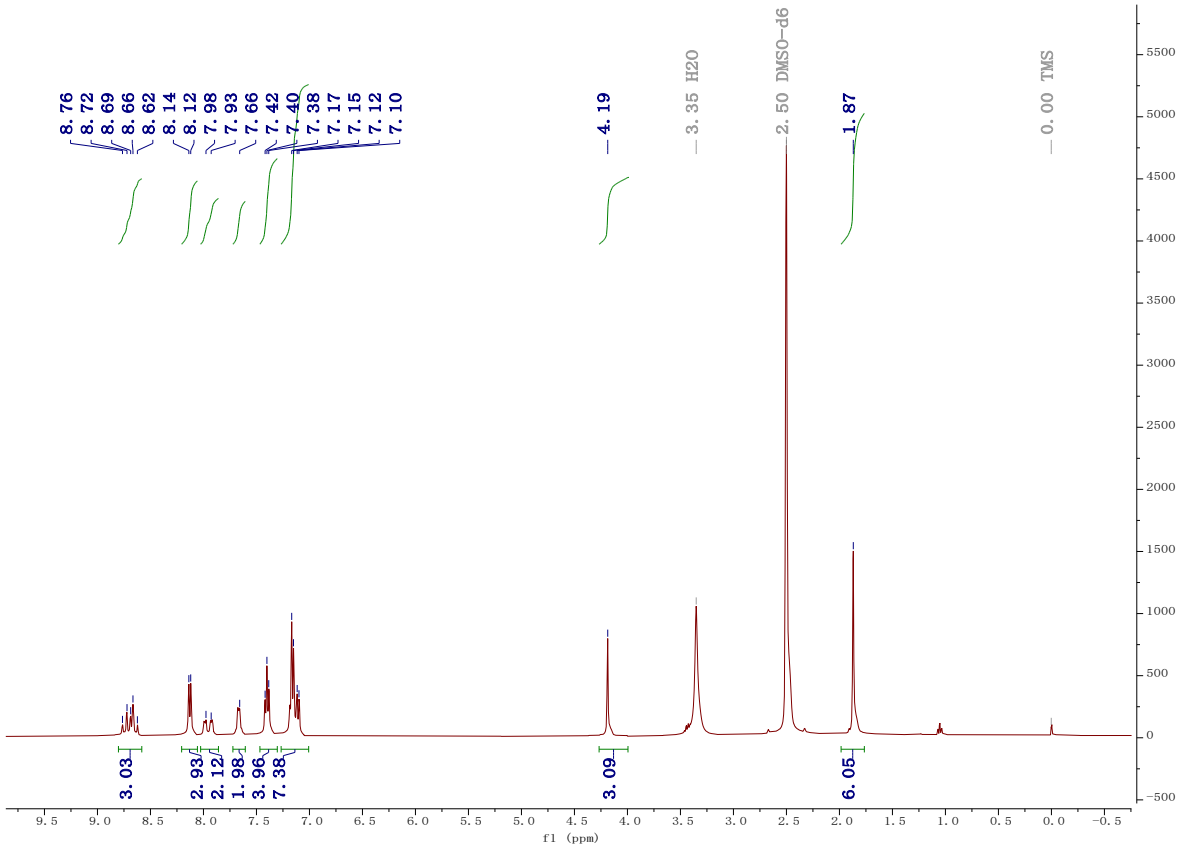


Figure S19 ^{1}H-NMR spectrum of **BTD-TA-YD**