Supporting information

Pyrrolopyrrole Aza-BODIPY Near-infrared Photosensitizer for Dual-mode Imaging-Guided Photothermal Cancer Therapy

Chaolong Wu,[a] Xiaoyu Huang,[a] Yunyun Tang,[a] Wanyue Xiao,[a] Liguo Sun,[b] Jinjun Shao,[a*] Xiaochen Dong[a*]*

[a]Key Laboratory of Flexible Electronics (KLOFE) and Institute of Advanced Materials (IAM), Nanjing Tech University (NanjingTech), Nanjing 211800, China.

E-mail: iamxcdong@njtech.edu.cn; iamjjshao@njtech.edu.cn

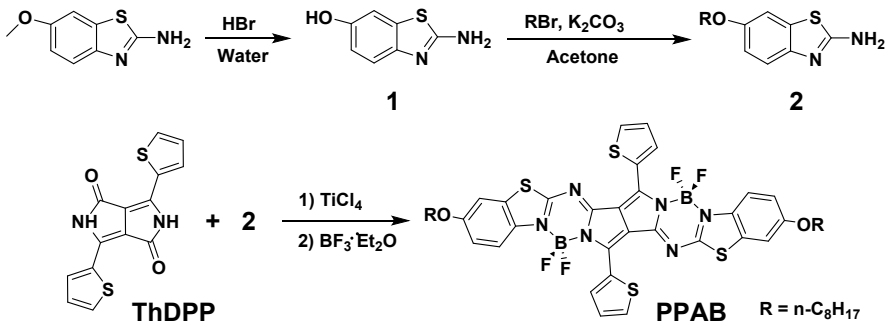
[b]Department of Radiology, Binzhou Medical University Hospital, Yantai, Shandong, 264100, China.

E-mail: zisetasong@sina.com

MATERIALS AND METHODS

Materials and apparatus

General chemicals were purchased from Sigma (Shanghai, Co. td) and used without further purification. 3,6-Di-thiophen-2-yl-2,5-dihydro-pyrrolo[3,4-c]pyrrole-1,4-dione (Th-DPP), potassium carbonate, hydrobromic acid (AR, 40% in water), toluene and acetone were purchased from Sigma-Aldrich. 1-bromooctane and 2-Amino-6-Methoxybenzothiazole were purchased from Adamas-beta. Titanium tetrachloride and boron trifluoride diethyl ether were purchased from Aladdin. The ^{1}H NMR was recorded on Bruker DRX NMR spectrometer (400 MHz) in CD_2Cl_2, $CDCl_3$ or DMSO-d_6 with tetramethylsilane (TMS) as internal standard. Absorption spectra were recorded on an UV-3600 UV-vis spectrophotometer (Shimadzu, Japan). Fluorescence spectra were performed on F-4600 HITACHI spectrometer from Japan. The size of NPs was revealed by using a 90 Plus particle size analyzer (DLS, Brookhaven Instruments, USA). Thermal images were taken by an E50 infrared camera (FLIR, Arlington, VA). TEM of the nanoparticles were measured on JEOL JEM-2100 equipment. The fluorescence bioimaging of tumor and the main organs (heart, liver, spleen, lung and kidney) were recorded on the PerkinElmer IVIS Lumina K.



Scheme S1. Synthetic route of **PPAB**.

Synthesis of compound 1

2-Amino-6-hydroxybenzothiazole was synthesized according to the previous literature. 2-Amino-6-hydroxybenzothiazole (3.66 g, 20.31 mmol) was added to 40 wt% hydrobromic acid in aqueous solution (40 mL), and the resulting solution was refluxed overnight. After cooling to room temperature, neutralization with saturated aqueous $NaHCO_3$ provided a white precipitate, which was collected by filtration (2.69 g, 80%). ^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 9.05 (s, 1 H), 7.04-7.01 (m, 3 H), 6.65 (d, J = 7.5 Hz, 1 H). ^{13}C NMR (500 MHz, DMSO-d_6): δ [ppm] = 163.87, 152.09, 145.63, 131.83, 118.09, 113.56, 106.92.

Synthesis of 2-Amino-6-octyloxybenzothiazole **2**

Compound **1** (2.50 g, 15 mmol), 1-bromooctane (2.90 g, 15 mmol), and K_2CO_3 (8.43 g, 61 mmol) were added to 50 mL acetone, and the resulting mixture was refluxed for 20 hours. After removal of solvent under vacuum, the residue was added to water (250 mL). The mixture solution was stirred for 30 min and the remaining residue was dispersed into hexane to afford compound **2** as a white precipitate (3.59 g, 86%). ^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 7.44 (d, J = 8.5 Hz, 1H), 7.13 (s, 1H) 6.95 (d, J = 9.0 Hz, 1 H), 5.00 (br, 2 H), 3.96 (t, J = 6.5 Hz, 2 H), 1.78 (t, J = 6.0 2 H), 1.58-1.29 (m, 10 H), 0.89 (t, J = 6.0 Hz, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 164.01, 155.22, 146.12, 132.60, 119.59, 114.33, 106.20, 77.42, 77.00, 76.57, 68.87, 31.79, 29.33, 29.21, 26.04, 22.62, 14.04.

Synthesis of PPAB

To a toluene solution (20 mL) of ThDPP (199 mg, 0.66 mmol) and compound **2** (1.0 g, 3.6 mmol) heated at reflux were added $TiCl_4$ (0.50 mL, 4.6 mmol) and triethylamine (2.5 mL, 18 mmol). After consumption of ThDPP, which was confirmed by UV-Vis spectra, $BF_3{\cdot}OEt_2$

(2.0 mL, 16 mmol) was added, and resulting solution was heated at reflux for another 4 h. The reaction mixture was poured into 300 mL of water, and the organic phase was extracted with $CHCl_3$. After dried over sodium sulfate, the solvent was removed, and the crude product was purified by silica gel column chromatography using DCM as an eluent. Recrystallization from chloroform and methanol to provide **PPAB** as a green powder (242 mg, 40%). ^{1}H NMR (300 MHz, CD_2Cl_2): δ [ppm] = 9.05 (d, J = 3.6 Hz 2 H), 7.93-7.88 (m, 4 H), 7.36 (t, J = 4.5 Hz, 2 H), 7.24 (s 2 H), 7.15-7.12 (m, 2 H), 4.05 (t, J = 6.0 Hz, 4 H), 2.03 (br, 4 H), 1.63-1.28 (m, 20 H), 0.90 (br, 6 H); MALDI-TOF-MS(m/z): calcd for $C_{44}H_{46}B_2F_4N_6O_2S_4$ ([M^+]): 916.268, found: 916.233.

Preparation of PPAB NPs

0.200 mL tetrahydrofuran (THF) solution of **PPAB** (5.00 mg/mL) was added dropwise into 10 mL DI-water at room temperature with vigorous stirring. 5 min latter, THF in the mixture was removed by air blowing, and the PPAB NPs in the solution was obtained by centrifugation. The size of **PPAB NPs** was determined by TEM and DLS.

***In vitro* photo-thermal effect**

PPAB NPs (0, 25, 50, 75, 100 ppm, respectively) aqueous solutions were introduced into 1mL tubes and irradiated with a 730 nm NIR laser (0.75 W/cm^2). The change of temperature was recorded by an infrared camera.

Cell line

Hela cell line was obtained from the Institute of Biochemistry and Cell Biology, SIBS, CAS (China) and was cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) maintained with 10% fetal bovine serum (FBS) at 37 °C under 85% humidifed atmosphere

of 5% CO_2.

Cytotoxicity assay

Hela cells were divided into 2 groups in 96-well cell-culture plates and washed with PBS, then refilled with culture medium (200 μL). **PPAB NPs** in PBS solution diluted to various concentrations with DMEM were added in the plate wells with same volume of PBS in control. One group of cells was kept in the darkness and another was irradiated by a NIR laser (730 nm, 0.75 W/cm^2, 5 min). All the groups were incubated for another 48 h, then the cells were incubated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (20 μL) in 5% CO2 at 37 °C for 4 h, and treated with 200 μL of DMSO. The absorbance was measured at 570 nm by a Bio-Tek microplate reader.

Animal models

Animal experiments were performed in compliance with the relevant laws and institutional guidelines, and the institutional ethics committee has approved the experiments. Hela cells were subcutaneously injected into the left or right flank of 6-week-old athymic nude mice. The mice were used for fluorescence imaging, photo-thermal imaging and photo-thermal therapy when the volumes of the tumors were about 100 mm^3.

***In vivo* fluorescence and photoacoustic imaging**

100 μL of **PPAB NPs** (50 ppm) PBS solution was tail vein injected into tumor-bearing mice. Fluorescence imaging of tumors at different periods (0, 2, 4, 6, 8, 10, 12 and 24 h) was performed at 710 nm with live animal fluorescence imaging system. Photo-thermal imaging was also monitored by an infrared camera when the tumors were administrated with 730 nm laser with power density of 0.75 W/cm^2 at different period (0, 2, 4, 6, 8 min). Photoacoustic

imaging of tumors at different periods (0, 2, 4, 6, 10, 24 h) was performed at 740 nm with Nexus128 small animal photoacoustic imaging system.

***In vivo* photo-thermal therapy**

20 Hela tumor-bearing mice were divided into four groups randomly (Control, laser only, **PPAB NPs** only and **PPAB NPs** with laser). For treatment groups, mice were injected ***via*** tail vein with 100 μL of **PPAB NPs** (50 ppm) solution and PBS for control group. 6 h later, mice were irradiated with 730 nm laser (0.75 W/cm^2) for 5 min. The treatment was carried out every other day. Mice body weights as well as tumor volumes were recorded every other day before the treatments. Tumor volumes were calculated by the equation: V = width^2·length/2.

Ex vivo histology examination

Hela tumor-bearing mice were sacrificed at the end of 16 days treatment. The hearts, livers, spleens, lungs, kidneys and tumors were collected and fixed in 4% formaldehyde solution for the histology analysis. The tissues were embedded in paraffin cassettes after dehydration and stained with hematoxylin and eosin (H & E). The images were viewed by microscope.

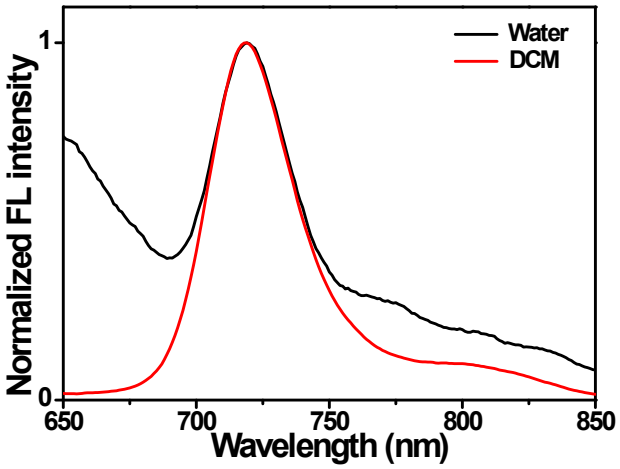


Figure S1. Photoluminescence spectra of **PPAB** in DCM and **PPAB NPs** in water.

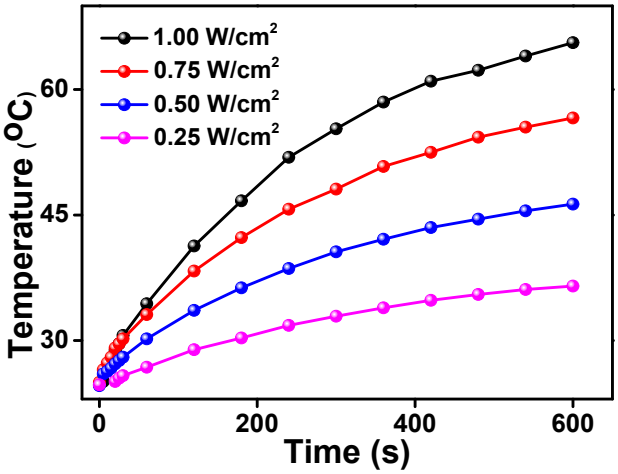


Figure S2. Temperature elevation curves of **PPAB** NPs (50 ppm) under 730 nm laser irradiation at different power density.

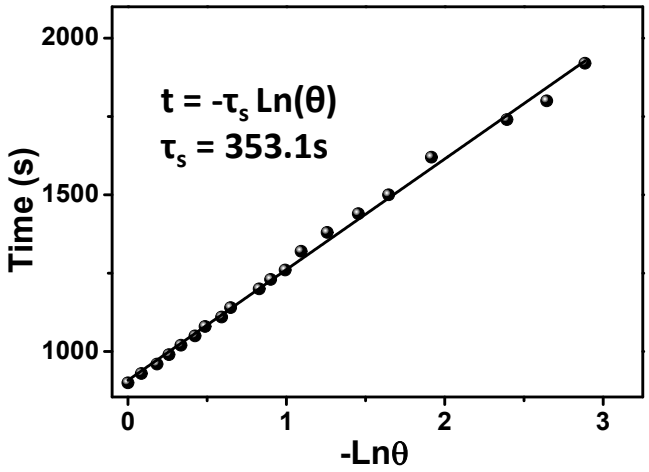


Figure S3. Linear time data from the cooling period *versus* negative natural logarithm of driving force temperature.

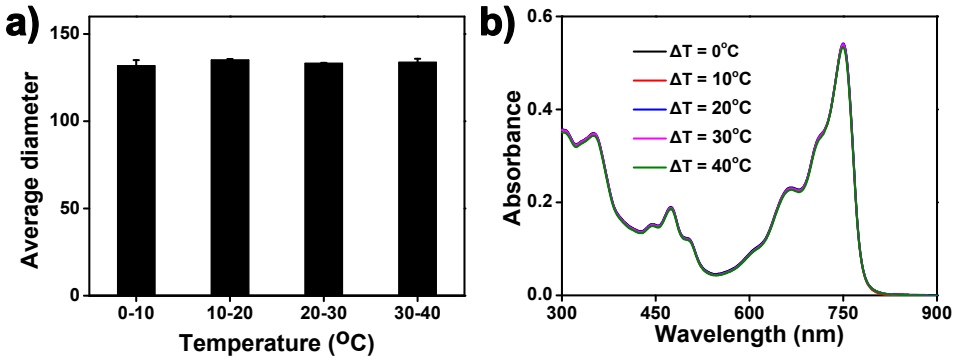


Figure S4. (a) Size change and (b) UV−vis absorption spectra of PPAB NPs during temperature increase.

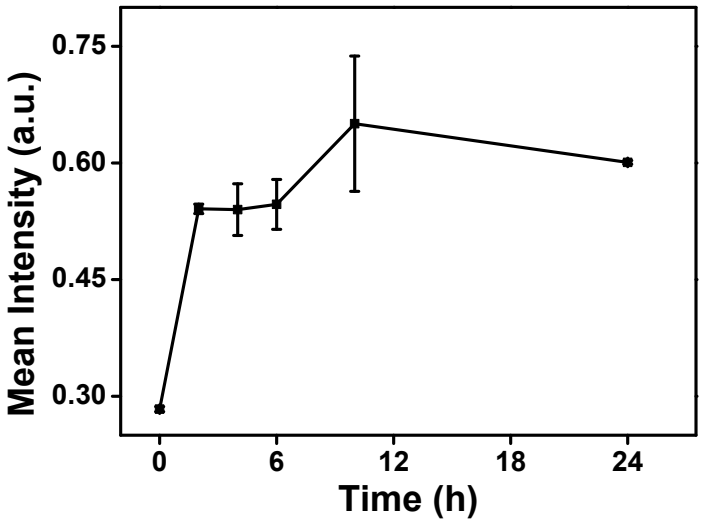


Figure S5. Corresponding PA intensity at different time *in vivo*.

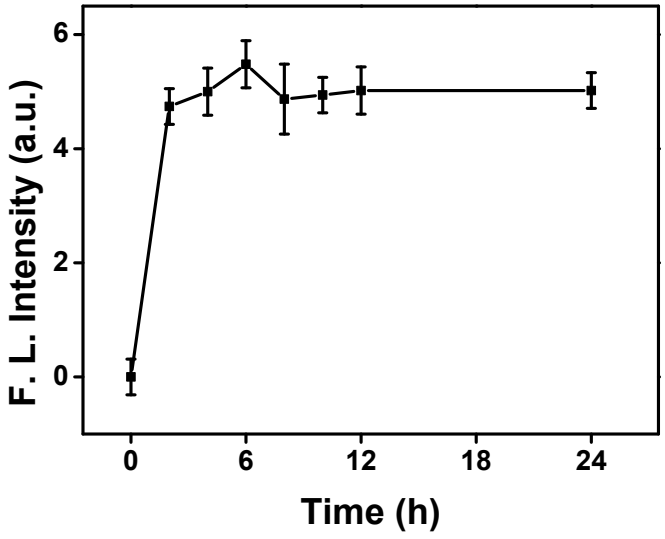


Figure S6. The fluorescence intensity of PS in tumor at various time.

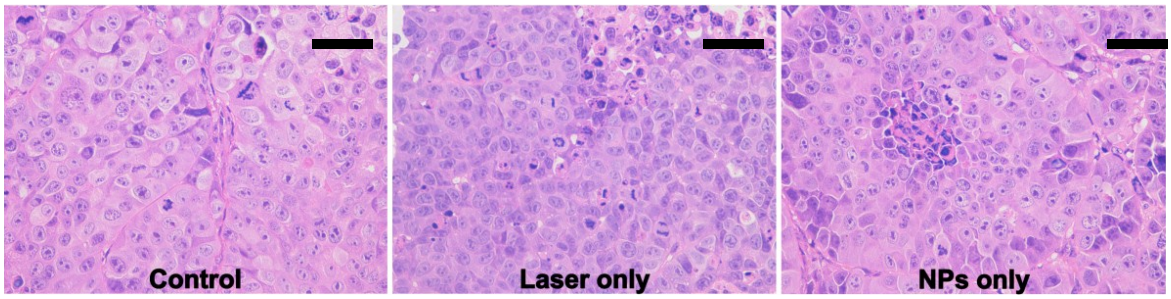


Figure S7. H&E staining of tumor tissues after different treatments (Scale bar: 100 μm).

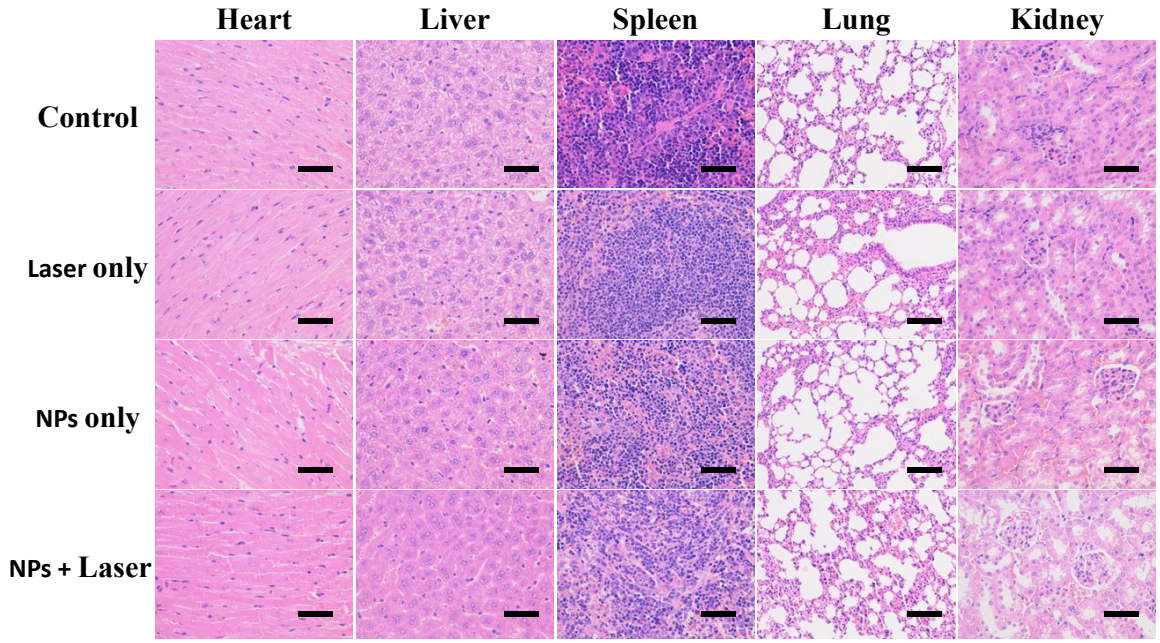


Figure S8. H&E stained images of major organs (heart, liver, spleen, lung, and kidney) for different groups after 16 days treatment (Scale bar: 100 μm).

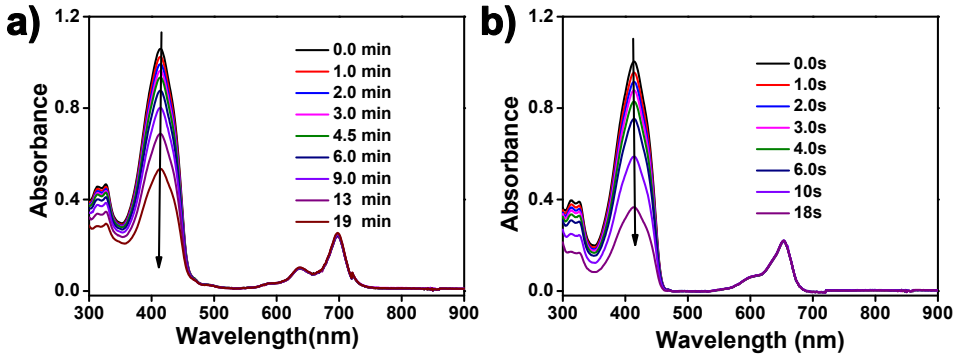
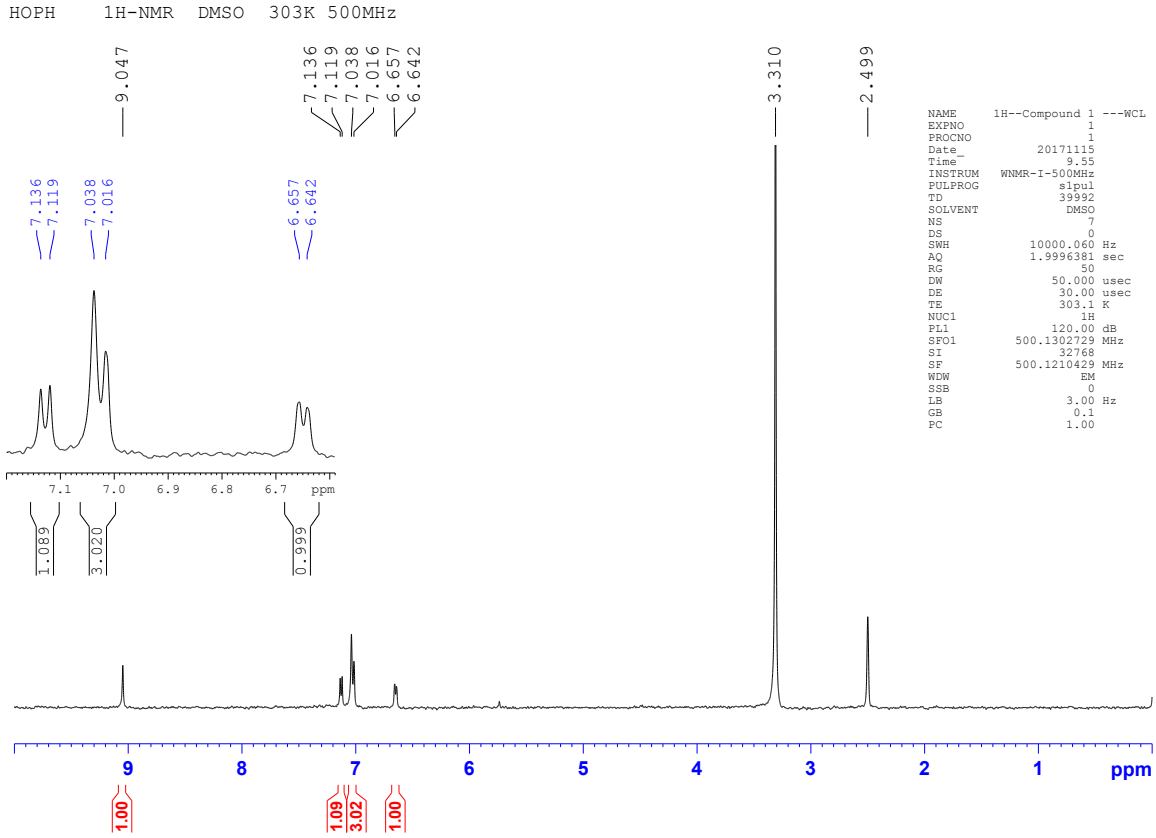
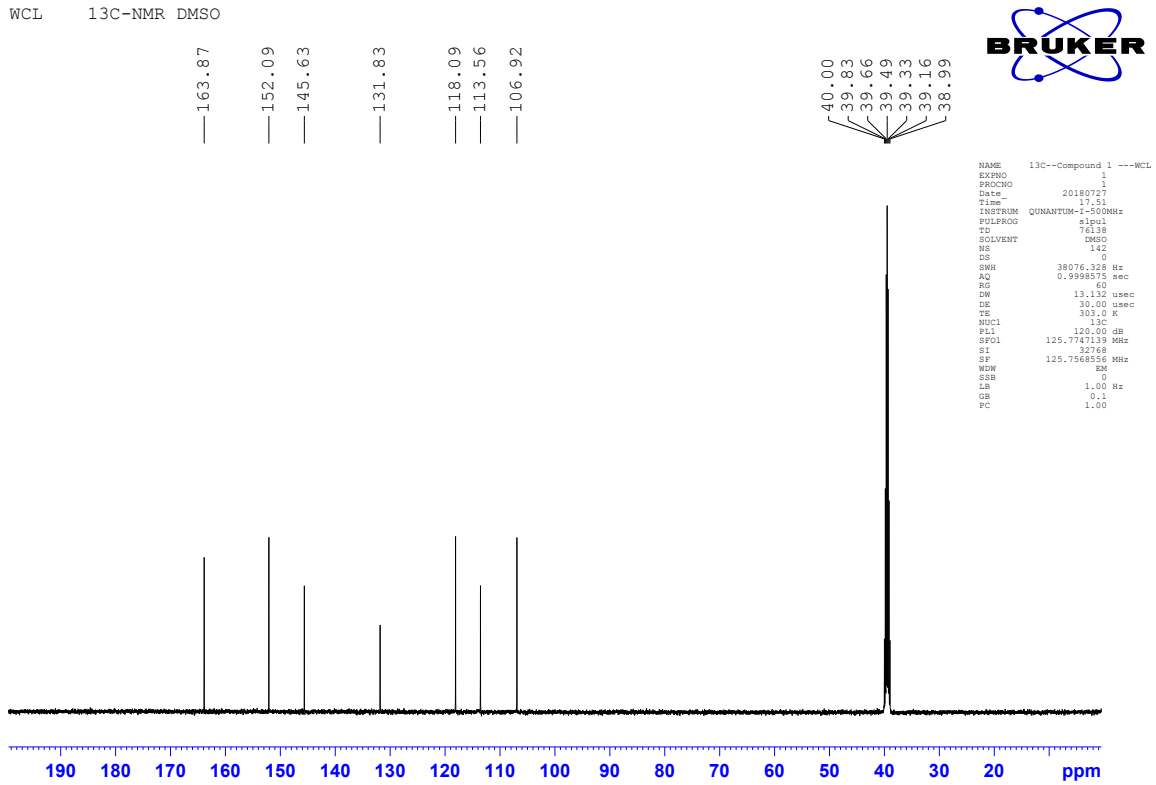


Figure S9. Absorbance decrease of ROS probe (DPBF) in the presence of (a) PPAB and (b) Methylene blue under the same exposure conditions.

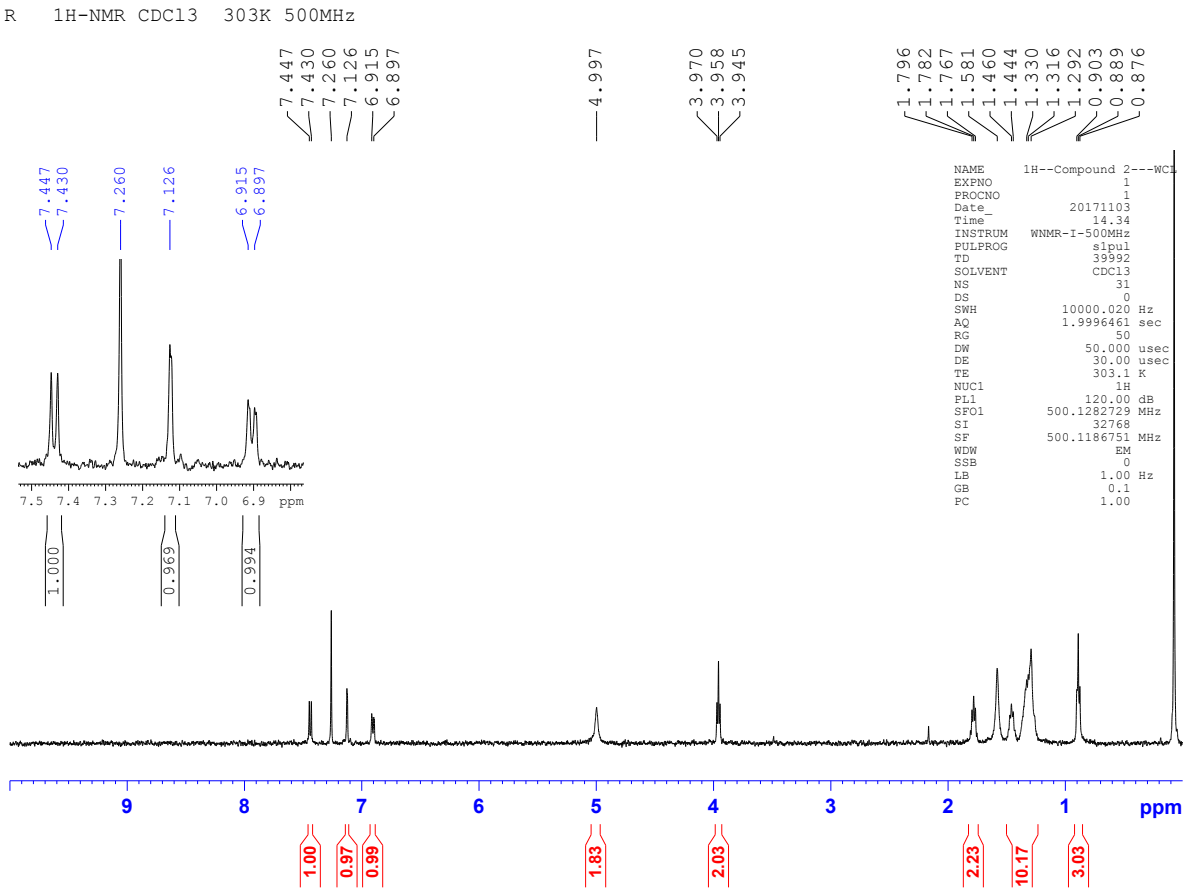
^{1}H NMR spectrum for **1** in DMSO-d_6



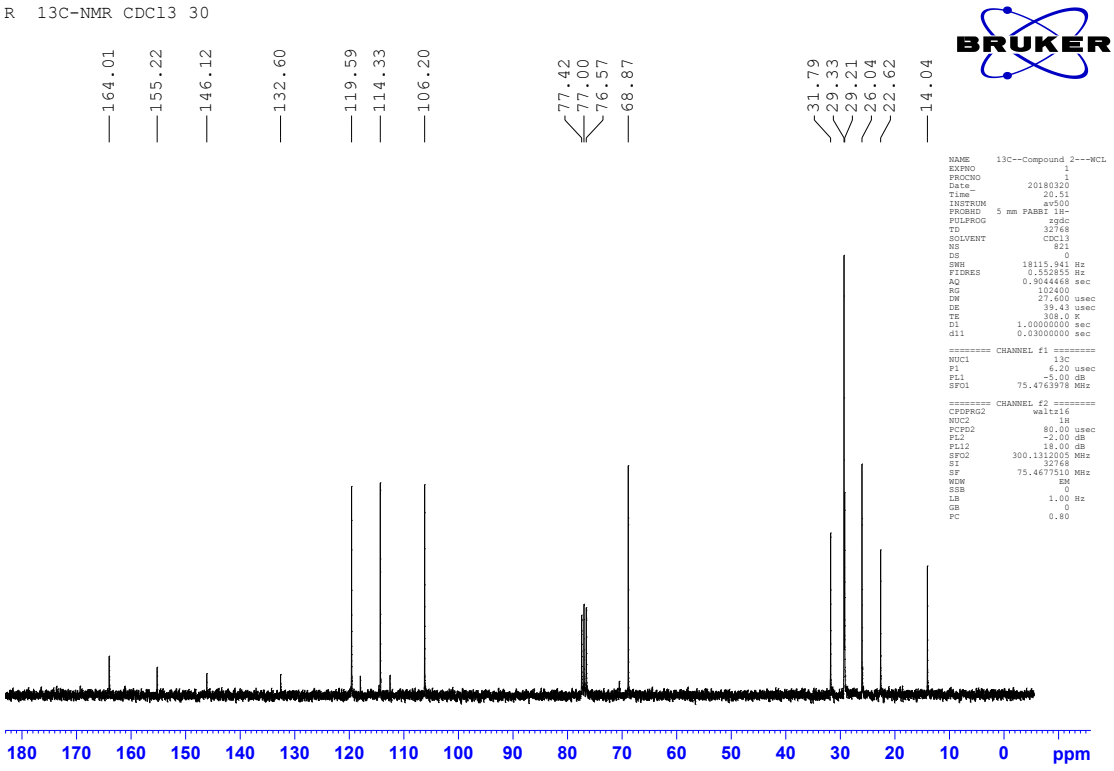
^{13}C NMR spectrum for **1** in DMSO-d_6



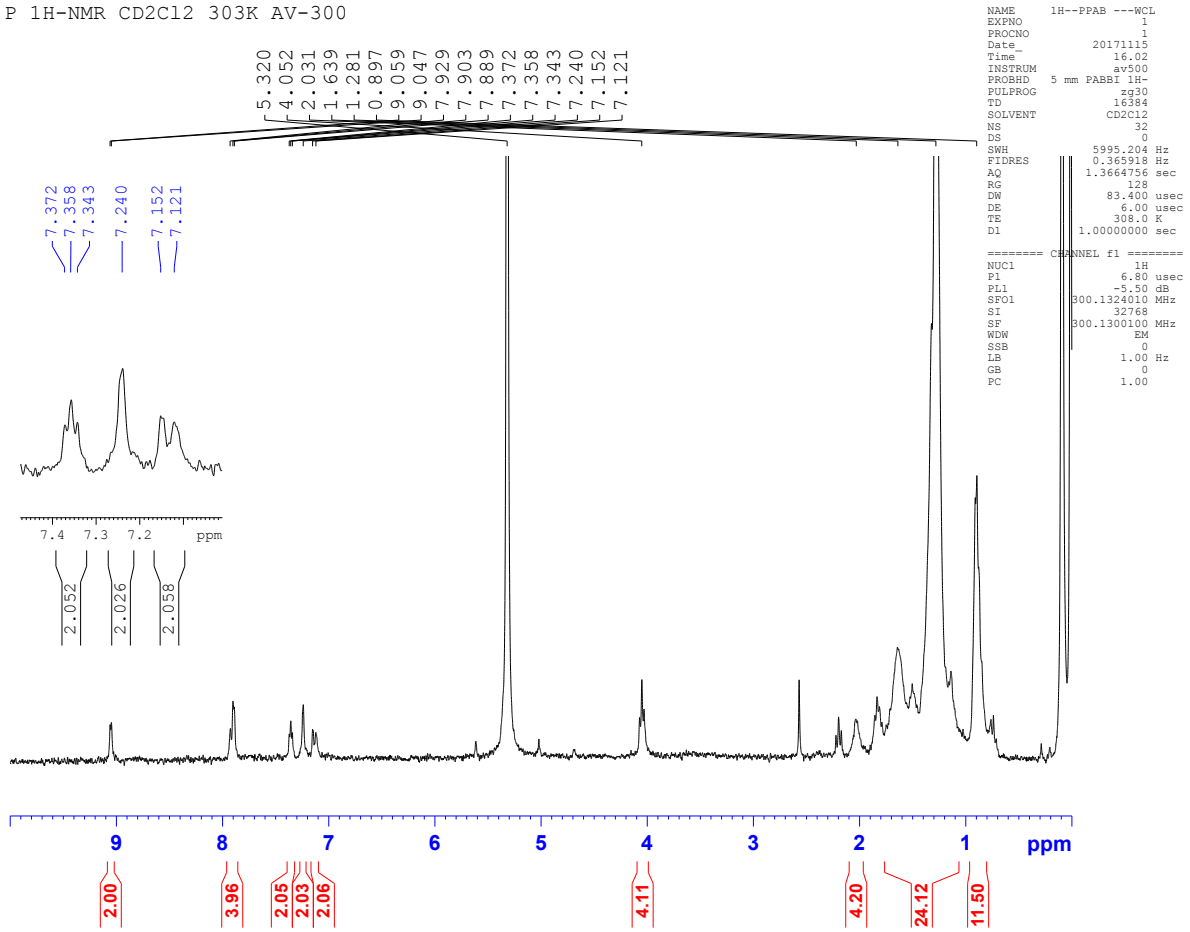
^{1}H NMR spectrum for **2** in $CDCl_3$



^{13}C NMR spectrum for **2** in $CDCl_3$



^{1}H NMR spectrum for **PPAB** in $CDCl_3$



MALDI-TOF-MS spectrum for **PPAB**