Supporting Information

Mitochondria-target NIR AIEgens with Cationic Amphiphilic Character for Imaging and Efficient Photodynamic Therapy

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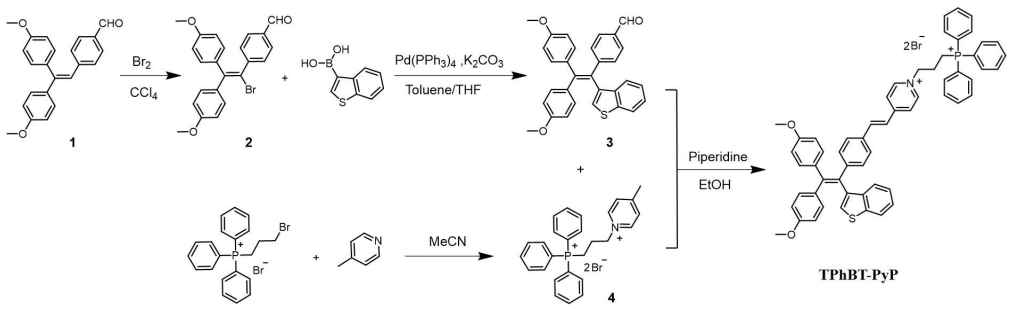
Materials and instruments

All chemical reagents and solvents were obtained from commercial source and used without further purification. ROS assay kit (S0033S), Calcein AM, and propidium iodide (PI) were obtained from Beyotime. 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA) was obtained from Sigma-Aldrich. JC-1 Kit, Mito-Tracker Green FM, ER-Tracker Green (1μM), and Lyso-Tracker Green were purchased from Yeasen. ^{1}H, ^{13}C and ^{31}P NMR spectra were charactered on Bruker Avance 400 NMR spectrometer. High resolution mass spectra (HRMS) were confirmed with Thermo Fisher Scientific LTQ Orbitrap Elite (Thermo Scientific, USA). UV–vis absorption and fluorescence spectra were recorded by UV-2550 spectrophotometer (Shimadzu Corporation, Japan) and Hitachi F-4600 fluorescence *(Hitachi*, Japan), respectively. HPLC was carried out on SHIMADZU CBM-20A. Dynamic light scattering (DLS) was measured with Zetasizer Nano-ZS90 (Malvern, UK). Cell fluorescence images were taken on a confocal microscope (Lecia, TCS SP8). The white light was provided by a PLS-LED100C High power LED light source system (400-780 nm) produced by Beijing Perfect Light Technology Co. Ltd.

Synthesis routes



Scheme. S1. Synthesis routes for **TPhBT-PyP**.

Synthesis of compound 2. 4-(1-bromo-2,2-bis(4-methoxyphenyl)vinyl)benzaldehyde The synthesis of compounds **1** followed the previously reported procedure[1]. To a solution of compound **1** (1.1 g, 3.2 mmol) in tetrachloromethane (20 mL) was cooled at 0 ℃, bromine (1.1 mL, 3.5 mmol) in 10 ml of tetrachloromethane was slowly added and stirred at the same temperature for 2 h. Adding Et_3N (5 mL) to terminate reaction and extract the reaction mixture with ethyl acetate (10 mL x 3), wash combine organic layer with brine (15 mL) and dry over Na_2SO_4. Evaporate the solvent and purify the residue by silica gel column chromatography with hexane/ethyl acetate ((v/v) = 15/1) to obtain compound **2** as yellow solid (1.3 g, 95% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.93 (s, 1H), 7.77 (d, J = 8.3 Hz, 2H), 7.49 (d, J = 8.1 Hz, 2H), 7.26 (d, J = 8.7 Hz, 2H), 6.97 (d, J = 8.8 Hz, 2H), 6.85 (d, J = 8.8 Hz, 2H), 6.69 (d, J = 8.9 Hz, 2H), 3.79 (s, 3H), 3.64 (s, 3H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 192.9, 159.2, 158.9, 147.5, 144.9, 135.7, 135.4, 133.2, 131.8, 131.3, 131.1, 129.8, 118.2, 114.1, 114.0, 55.6, 55.5. ESI-HRMS: calcd. for $C_{23}H_{20}BrO_3^+$ $[M+H]^+$: 423.0590, found: 423.0580.

Synthesis of compounds 3. 4-(1-(benzo[b]thiophen-3-yl)-2,2-bis(4-methoxyphenyl)-vinyl)-benzaldehyde

4-(1-bromo-2,2-bis(4-methoxyphenyl)vinyl)benzaldehyde (1.3g, 3.0 mmol), 3-benzothienylboronic acid (0.8 g, 4.5 mmol), and potassium carbonate (0.8 g, 4.5 mmol) were dissolved in a mixed solution of toluene (60 mL) and tetrahydrofuran (30 mL) under argon atmosphere. The reaction solution was stirred at room temperature for 30 min, then Pd $(PPh_3)_4$ (50 mg, 0.043 mmol) was added and refluxed for 18 h. After the reaction was cooled to the room temperature, the mixture was poured into water and extracted 3 times with dichloromethane. The combined organic layer was dried over anhydrous sodium sulfate and evaporated in vacuum. The crude product was purified by column chromatography on silica gel with hexane/ethyl acetate ((v/v) = 15/1) to obtain compound **3** as a saffron yellow solid powder (0.8 g, 55% yield). ^{1}H NMR (400 MHz, DMSO- d_6) δ 9.84 (s, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.63 (d, J = 8.3 Hz, 2H), 7.43 – 7.34 (m, 2H), 7.27 (t, J = 8.1 Hz, 1H), 7.23 – 7.16 (m, 3H), 6.97 (d, J

= 8.8 Hz, 2H), 6.87 (d, *J* = 8.9 Hz, 2H), 6.77 (d, *J* = 8.8 Hz, 2H), 6.61 (d, *J* = 8.8 Hz, 2H), 3.70 (s, 3H), 3.60 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 192.9, 158.9, 158.6, 149.4, 145.2, 139.8, 138.8, 138.5, 135.8, 134.9, 134.4, 132.8, 131.5, 131.4, 131.1, 129.7, 128.0, 124.7, 124.6, 123.4, 123.1, 114.0, 113.6, 55.5, 55.4. ESI-HRMS: calcd. for $C_{31}H_{25}O_3S^+$ $[M+H]^+$: 477.1519, found: 477.1509.

Synthesis of compounds 4. 4-methyl-1-(3-(triphenylphosphonio)propyl)pyridin-1-ium bromide

(3-bromomethyl) triphenyl phosphine bromide (0.95 g, 2.0 mmol) and 4-methylpyridine (0.19 g, 2.0 mmol) were dissolved in acetonitrile (20 mL), the reaction mixture was refluxed overnight. The crude product was purified by column chromatography on silica gel with DCM/methanol ((v/v) = 10/1) to obtain compound **4** as a white solid powder (1.1 g, 88% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 8.93 (d, *J* = 6.8 Hz, 2H), 7.96 – 7.82 (m, 11H), 7.80 – 7.34 (m, 6H), 4.90 (t, *J* = 7.5 Hz, 2H), 3.79 – 3.66 (m, 2H), 2.67 (s, 3H), 2.49 – 2.35 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 160.5, 143.7, 135.1, 135.1, 133.7, 133.6, 130.4, 130.2, 128.7, 118.2, 117.4, 59.7, 59.5, 24.2, 24.2, 20.7, 19.2, 18.7. ESI-HRMS: calcd. for $C_{27}H_{28}NP^{2+}$ $[M-2Br]^{2+}$: 198.5974, found: 198.5967.

Synthesis of compounds TPhBT-PyP

Compound **3** (476 mg, 1 mmol), pyridinium-triphenylphosphine salt **4** (570 mg, 0.9 mmol) and the catalytic amount of piperidine were dissolved in 15 mL dry ethyl alcohol under argon atmosphere. The reaction mixture was refluxed at 80 ℃ overnight and cooled to room temperature. Then the solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography on silica gel with DCM/methanol ((v/v) = 15/1) to obtain compound **TPhBT-PyP** as an orange-red solid powder (810 mg, 89% yield). The compound was further purified by HPLC for subsequent experiments. ^{1}H NMR (400 MHz, CD_3OD) δ 8.71 (d, *J* = 7.1 Hz, 2H), 8.10 (d, *J* = 7.1 Hz, 2H), 7.94 – 7.88 (m, 3H), 7.85 – 7.74 (m, 14H), 7.47 (d, *J* = 8.6 Hz, 2H), 7.40 – 7.21 (m, 3H), 7.18 – 7.11 (m, 4H), 7.11 – 6.99 (m, 2H), 6.94 – 6.87 (m, 2H), 6.78 – 6.71 (m, 2H), 6.61 – 6.53 (m, 2H), 4.70 (t, *J* = 7.5 Hz, 2H), 3.75

(s, 3H), 3.66 (s, 3H), 3.61 – 3.50 (m, 2H), 2.38 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 159.0, 158.8, 145.9, 144.6, 143.9, 141.7, 140.0, 138.8, 136.0, 135.2, 135.1, 133.7, 133.6, 133.0, 132.5, 131.7, 131.1, 131.1, 130.4, 130.3, 127.7, 126.8, 123.9, 123.8, 123.6, 122.9, 122.3, 122.0, 118.2, 117.4, 113.1, 112.7, 59.4, 59.2, 54.3, 54.2. ESI-HRMS: calcd. for $C_{58}H_{50}NO_2PS^{2+}$ $[M-2Br]^{2+}$: 427.6645, found: 427.6634.

Determination of light-triggered $^1O_2$ generation

ABDA was used to detect $^1O_2$ generation by TPhBT-PyP and the commercial photosensitizer. In the experiments, the sample mixture of ABDA (50 μM) and photosensitizer compounds (10 μM) in PBS was irradiated with LED white light (10 mW cm^{-2}). The absorbance spectrum (320 to 450 nm) of ABDA was measured at various irradiation times by Shimadzu UV-2550 spectrophotometer.

ROS generation measurement

The general ROS generation was detected by fluorescent indicator 2,7-dichlorodihydrofluorescein (DCFH). 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA, 0.5 mL, 1 mM) was treatment with aqueous solution of NaOH (2 mL, 1.0 mM) for 30 min at room temperature. PBS buffer (7.5 mL) was added to neutralize the hydrolysate. The activated DCFH (10 μM) and TPhBT-PyP (10 μM) or Ce6 (10 μM), Rose Bengal (10 μM) were mixed in PBS solution in the dark, then the mixure was exposed to white light irradiation (50 mW cm^{-2}). The fluorescence intensity at 525 nm was measured at different time points.

Cell culture

The human cancer cells (HeLa, MCF-7) and normal cells (NIH-3T3, CHO) were obtained from the Laboratory Animal Service Center of Wuhan University (Wuhan, China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in

humidified incubator containing 5% CO_2.

Co-localization fluorescence Imaging

HeLa cells were seeded in 35 mm perti dishes at an approximate density of 5 × 10^4 cells/mL and cultured at 37ºC overnight. The culture medium was discarded, fresh DMEM medium containing the TPhBT-PyP (10 μM) was added and further cultured at 37ºC for 2 h. Afterward the cells were washed with PBS, and incubated with Mito-Tracker Green (100 nM), ER-Tracker Green (1μM), and Lyso-Tracker Green (50 nM), respectively. Hela cells were washed thrice with PBS and imaged by confocal laser scanning microscope (PerkinElmer UltraVIEW VoX, USA) with different combinations of excitation and emission filters. For TPhBT-PyP, the excitation channel was 405 nm and the emission filter was 580-660 nm. For commerical tracker dye, the excitation was 488 nm and the emission filter was 500-550 nm.

Detection of intracellular ROS by DCFH-DA

MCF-7 cells were cultured in 35 mm perti dishes overnight and then incubated with TPhBT-PyP (10 μM) at 37ºC for 2 h. DCFH-DA (10 μM) was added into the medium and incubated for 30 min. Next, MCF-7 cells were washed thrice with PBS, and irradiated with white light (50 mW cm^{-2}) for 0, 1, 5, and 10 min. Cells only were incubated with DCFH-DA as control group. The cells were then imaged by Lecia TCS SP8 with excitation at 488 nm and emission from 500 to 550 nm.

Live/dead cell imaging

HeLa cells were plated onto a six-well plate and cultivated for 24 h. Subsequently, cells were classified into the following different treatments: group 1

and 2 as control were irradiated with white light for 10 min and 30 min, respectively; group 3 was incubated with TPhBT-PyP (10 μM) for 2 h; group 4 was incubated with TPhBT-PyP (10 μM) for 2 h followed with white light irradiation (50 mW cm^{-2}) for 10 min; group 5 was incubated with TPhBT-PyP (10 μM) for 2 h and irradiated with white light (50 mW cm^{-2}) for 30 min. Next, all groups were incubated with Calcein-AM (0.5 μM) and PI (20 ug/mL) at 37ºC for 30 min. Fluorescence images of Hela cells were photographed with confocal microscope. The excitation wavelength for Calcein-AM and PI were 488 nm and 561 nm, the emissions were collected in 500-550 nm and 580-650 nm, respectively.

Mitochondria membrane potential (MMP) analysis

MCF-7 cells were plated onto 35 mm cell dishes at 37ºC in humidified incubator containing 5% CO_2 for 24 h. After that, different groups were prepared according to their respective treatments: control group, no treatment; AIEgens group, incubated with TPhBT-PyP (10 μM) for 2 h; light group, white light irradiation (50 mW cm^{-2}) for 30 min; PDT group, incubated with TPhBT-PyP (10 μM) for 2 h and subsequently irradiated with white light (50 mW cm^{-2}) for 30 min. The JC-1 probe kit was used to the cells stain according to the manufacturer's instructions. Finally, the fluorescence of J-monomer (Ex: 488 nm, Em: 500–550 nm) and J-aggregate (Ex: 561 nm, Em: 580-650 nm) was imaged on confocal microscope.

Bacterial culturing and imaging

A single bacterial colony was added into LB culture medium (10 mL) and grew overnight at 37°C in the orbital shaker. The bacteria were collected by centrifugation

for 2 min and washed twice. Subsequently, the bacteria were prepared into an OD_{600} = 1.0 stock suspension. 500 μL of bacteria (OD_{600} = 1.0) were collected by centrifugation, removed the supernatant and again dispersed in 500 μL PBS solution of TPhBT-PyP (2 μM). The mixture was incubated for 20 min at 37℃, and 2 μL of this mixture was dropped onto glass slide and immobilized to image using confocal microscope (Lecia, TCS SP8). The excitation for TPhBT-PyP was 405 nm, the emission was from 600 to 700 nm.

Photodynamic antibacterial activity study

The suspension of *E. coli* or *S. aureus* (OD_{600} = 0.2) was incubated with different concentrations of TPhBT-PyP for 20 min in dark conditions. To test the photodynamic antibacterial activity of AIEgens , the bacteria were exposed to white light irradiation (50 mW cm^{-2}) for 20 min. Then, the bacteria suspensions were serially diluted 1×10^4 fold with PBS. The diluted suspension (100 μL) was spread onto the solid LB agar plate and the photograph of monoclonal bacterial plate was imaged after grew for 16 h at 37°C. The bacteriostatic effect of TPhBT-PyP was evaluated from the reduced numbers of colonies. The bacteria without the AIEgens treatment and white light irradiation were regarded as the blank group, and the bacteria treated with AIEgens but not irradiated as the negative control group.

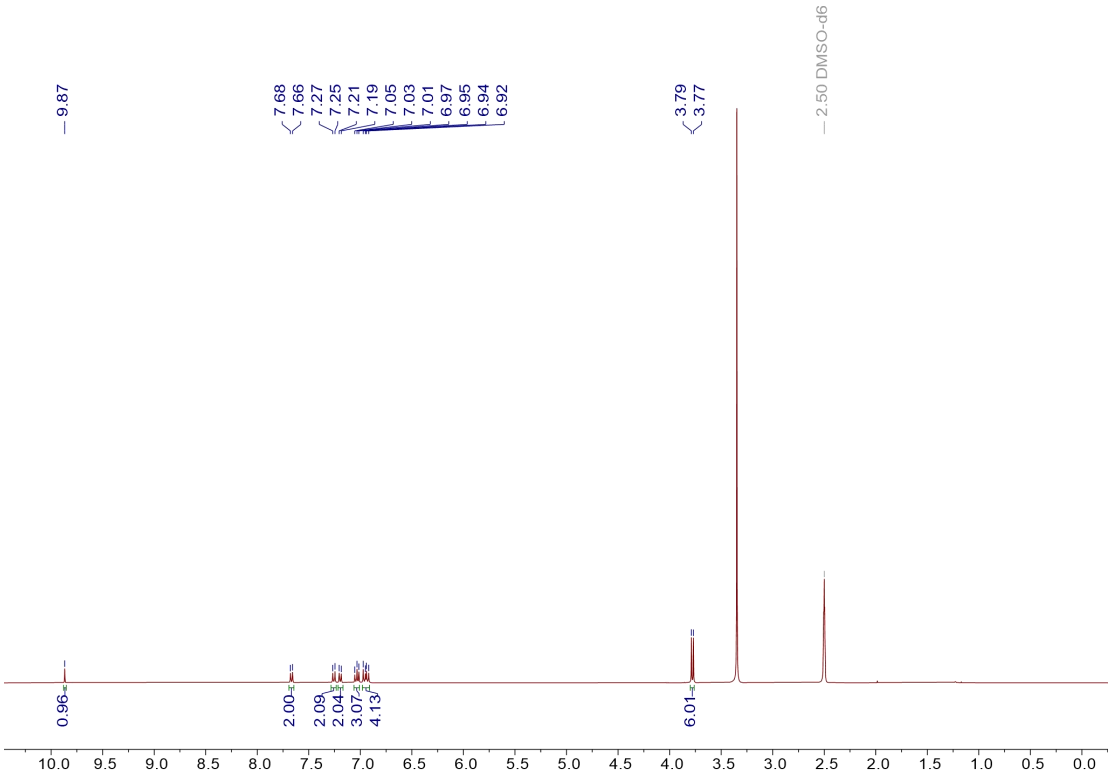


Fig. S1. ^{1}H NMR spectrum of compound **1**.

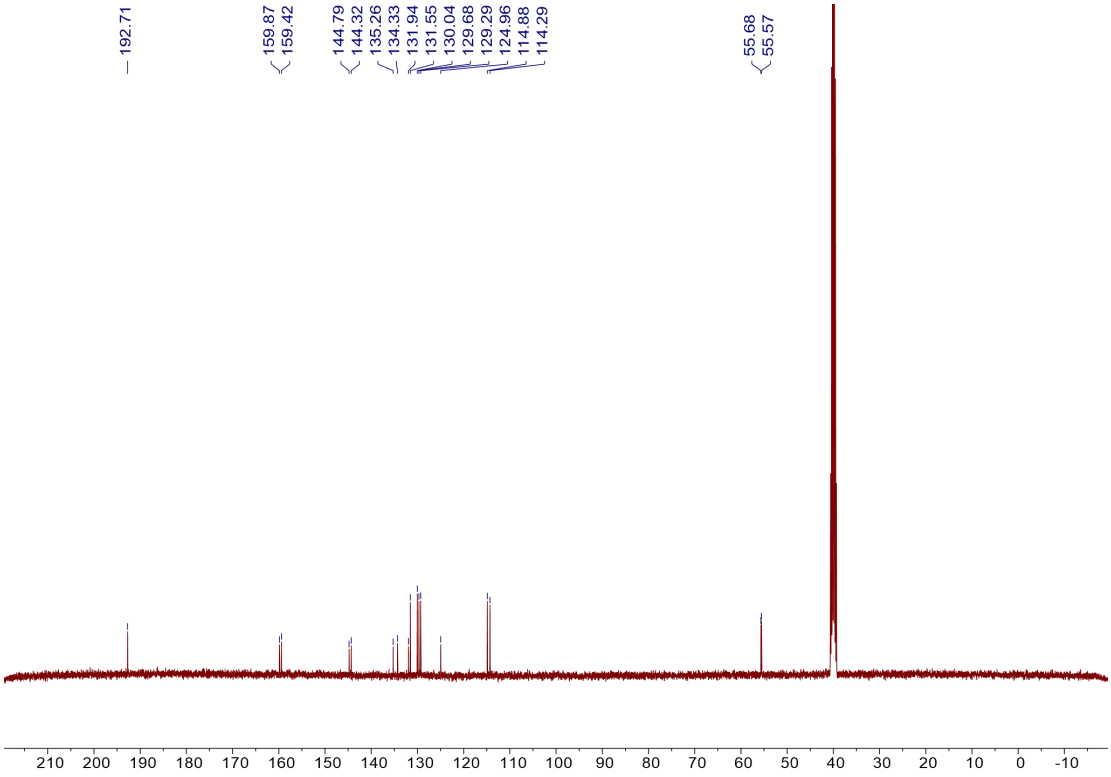


Fig. S2. ^{13}C NMR spectrum of compound **1**.

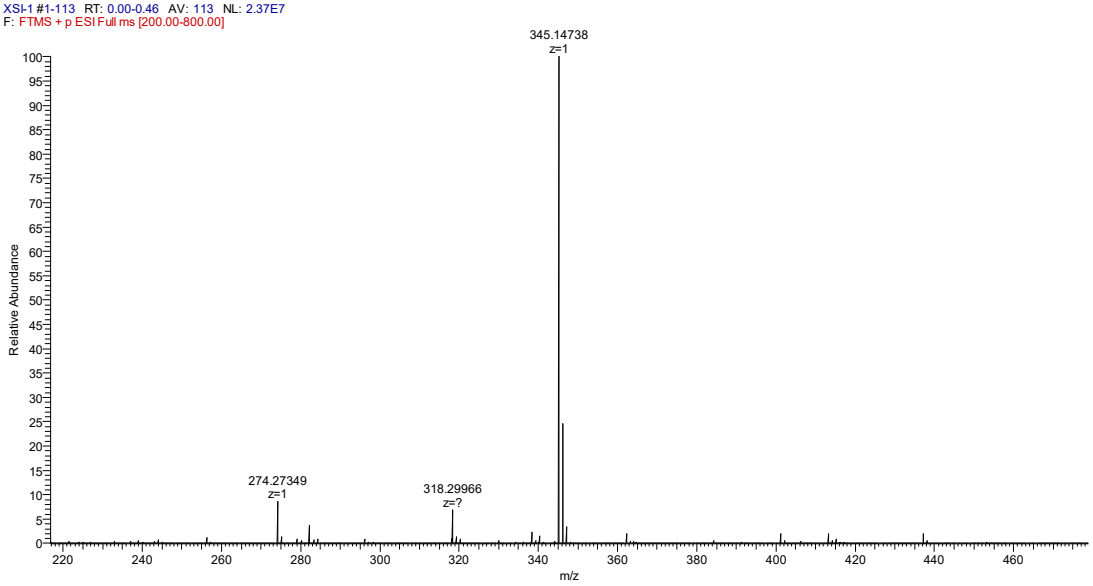


Fig. S3. ESI-HRMS of compound **1**.

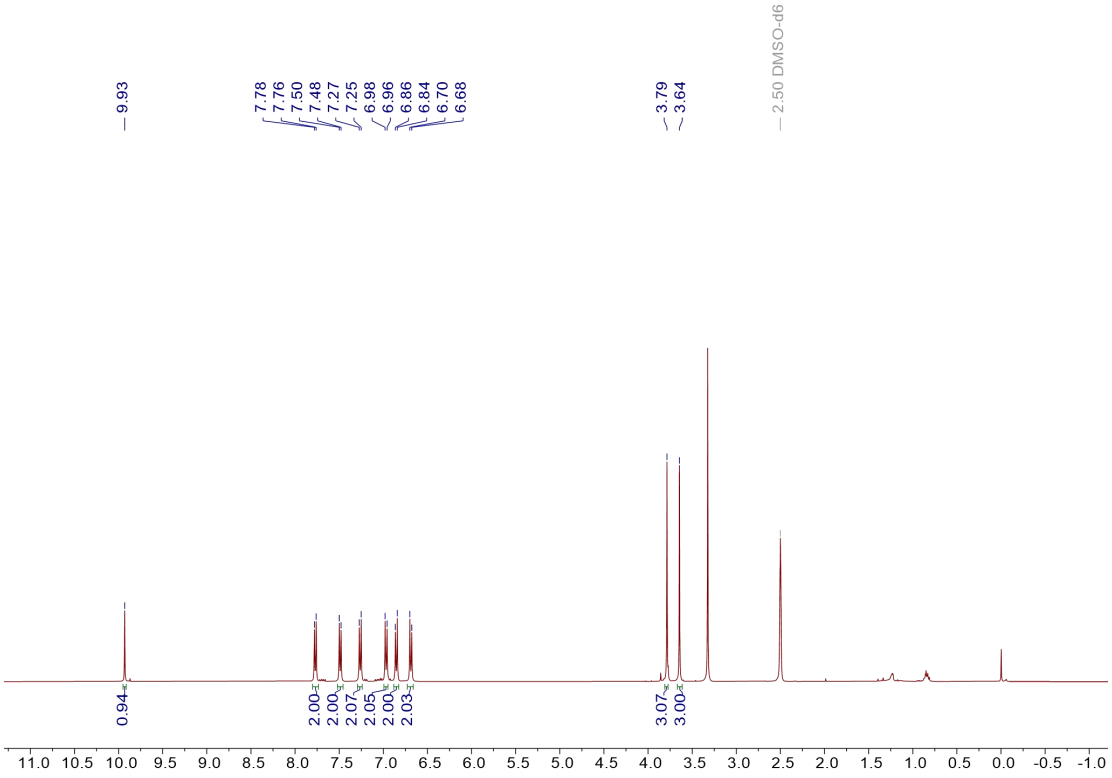


Fig. S4. ^{1}H NMR spectrum of compound **2**.

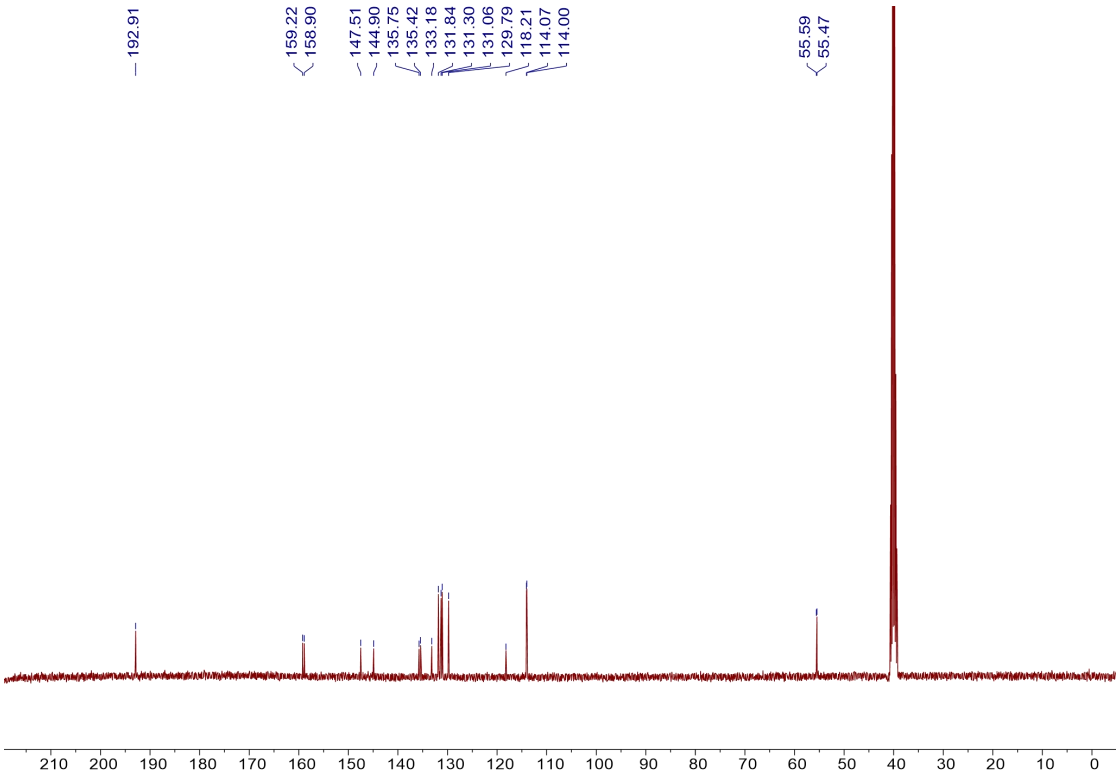


Fig. S5. ^{13}C NMR spectrum of compound **2**.

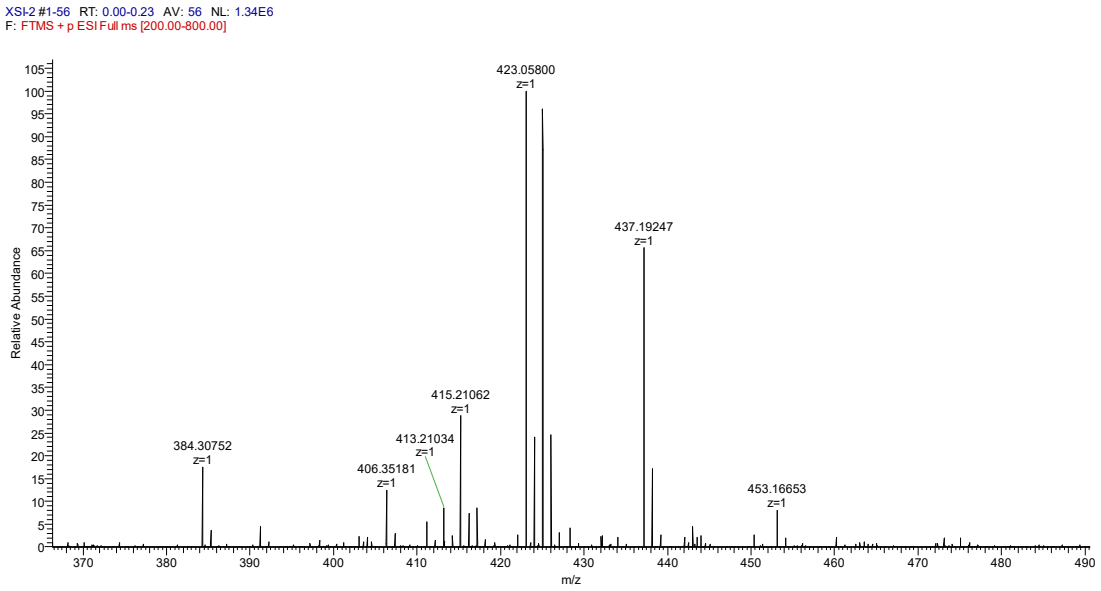


Fig. S6. ESI-HRMS of compound **2**.

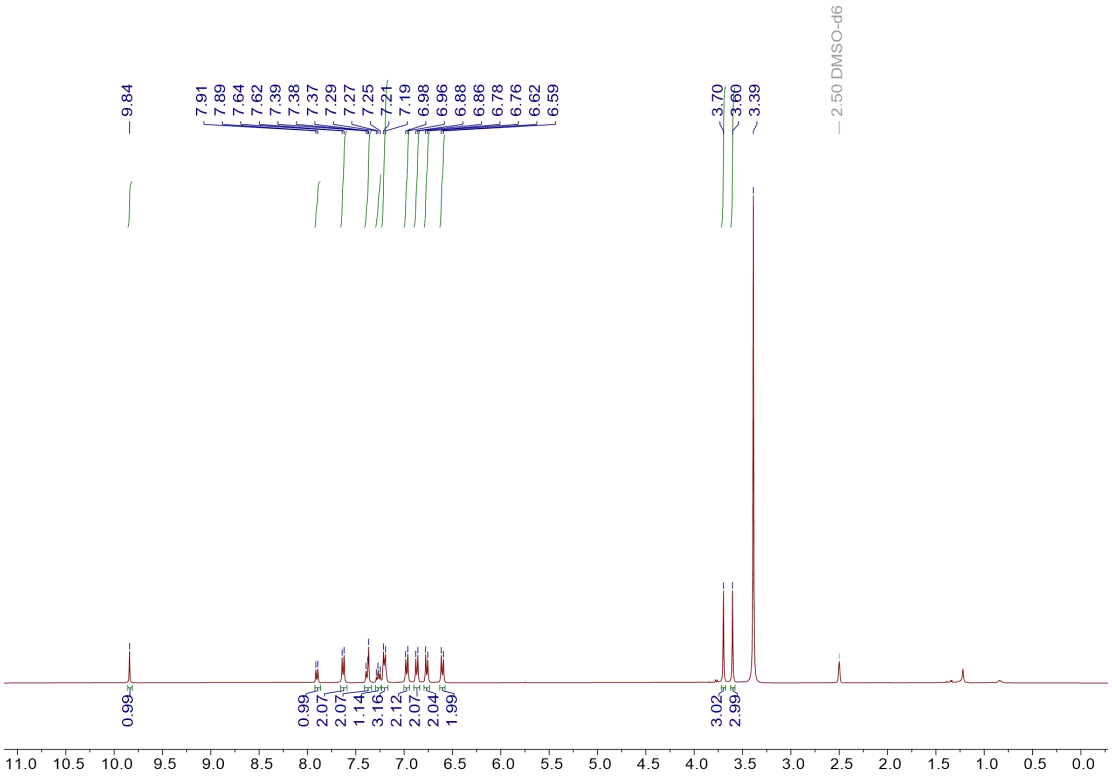


Fig. S7. ^{1}H NMR spectrum of compound **3**.

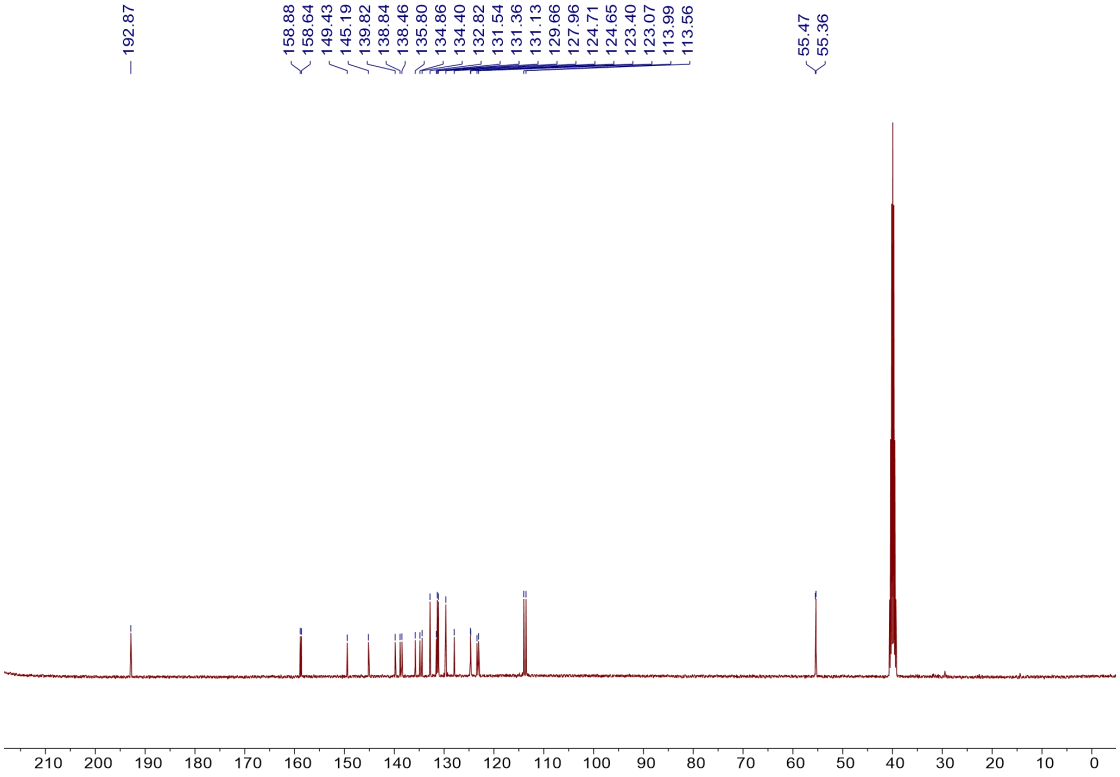


Fig. S8. ^{13}C NMR spectrum of compound **3**.

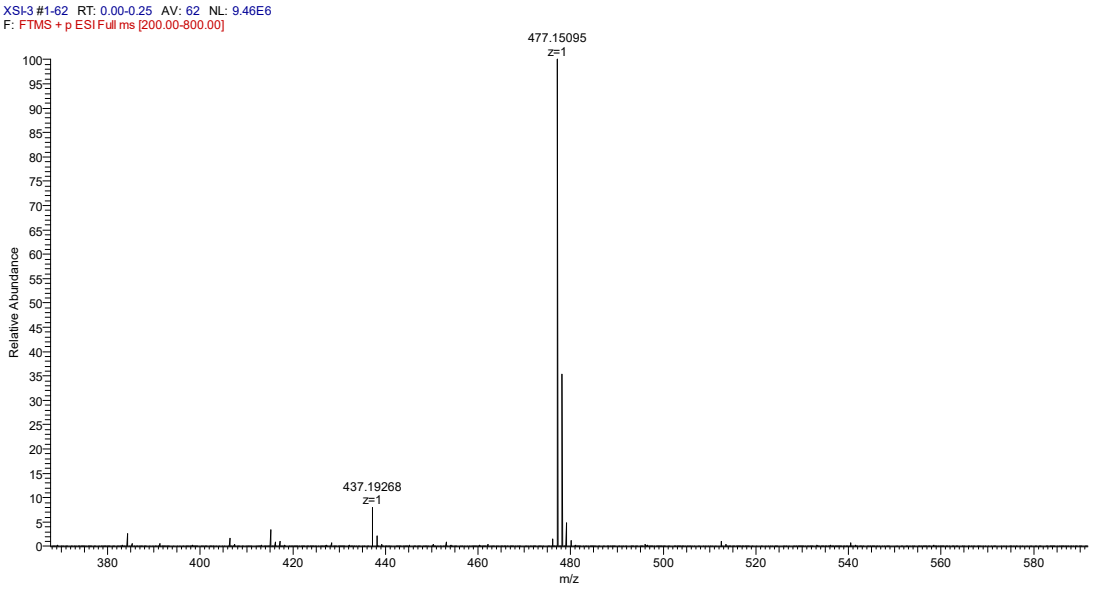


Fig. S9. ESI-HRMS of compound **3**.

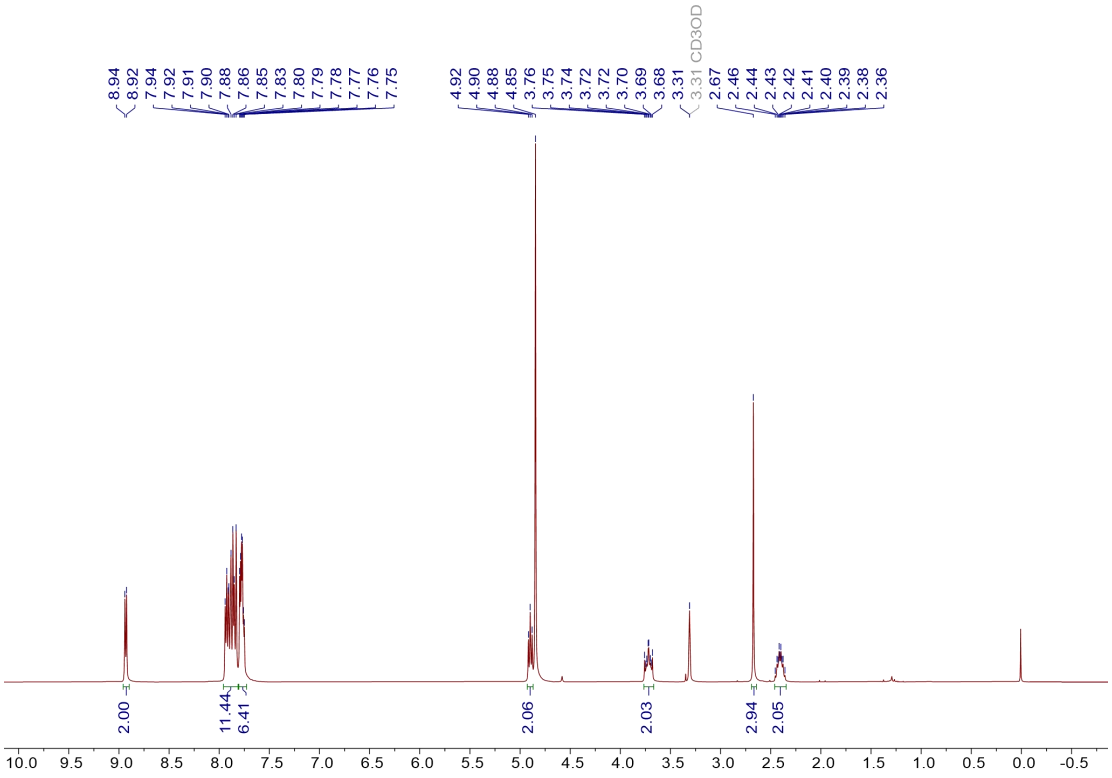


Fig. S10. ^{1}H NMR spectrum of compound **4**.

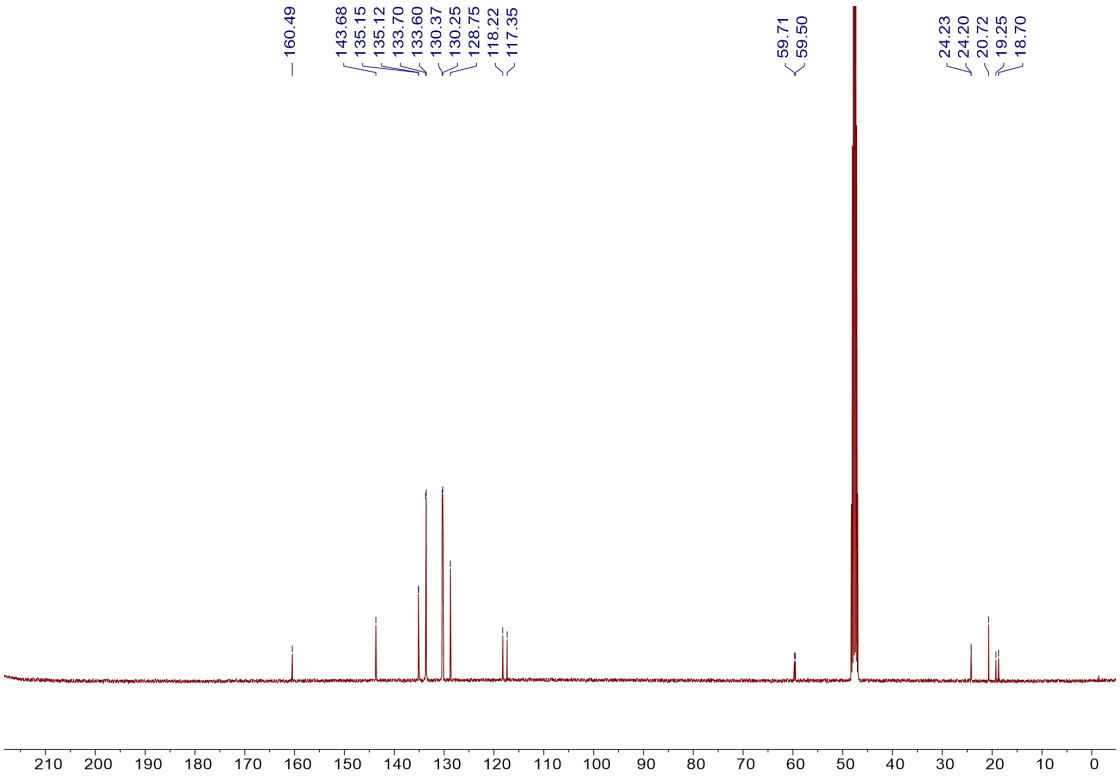


Fig. S11. ^{13}C NMR spectrum of compound **4**.

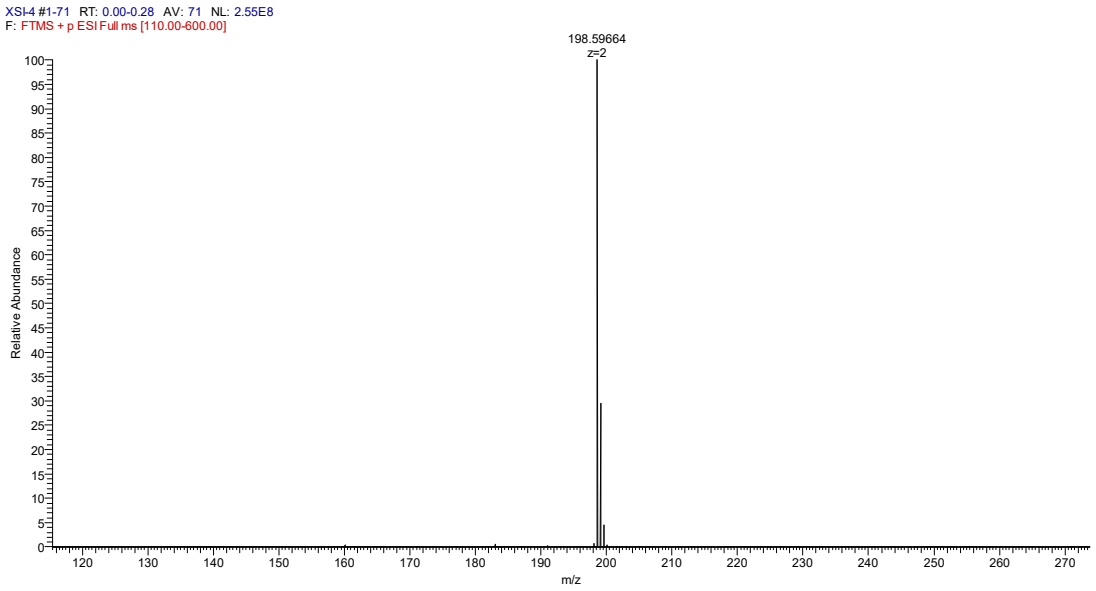


Fig. S12. ESI-HRMS of compound **4**.

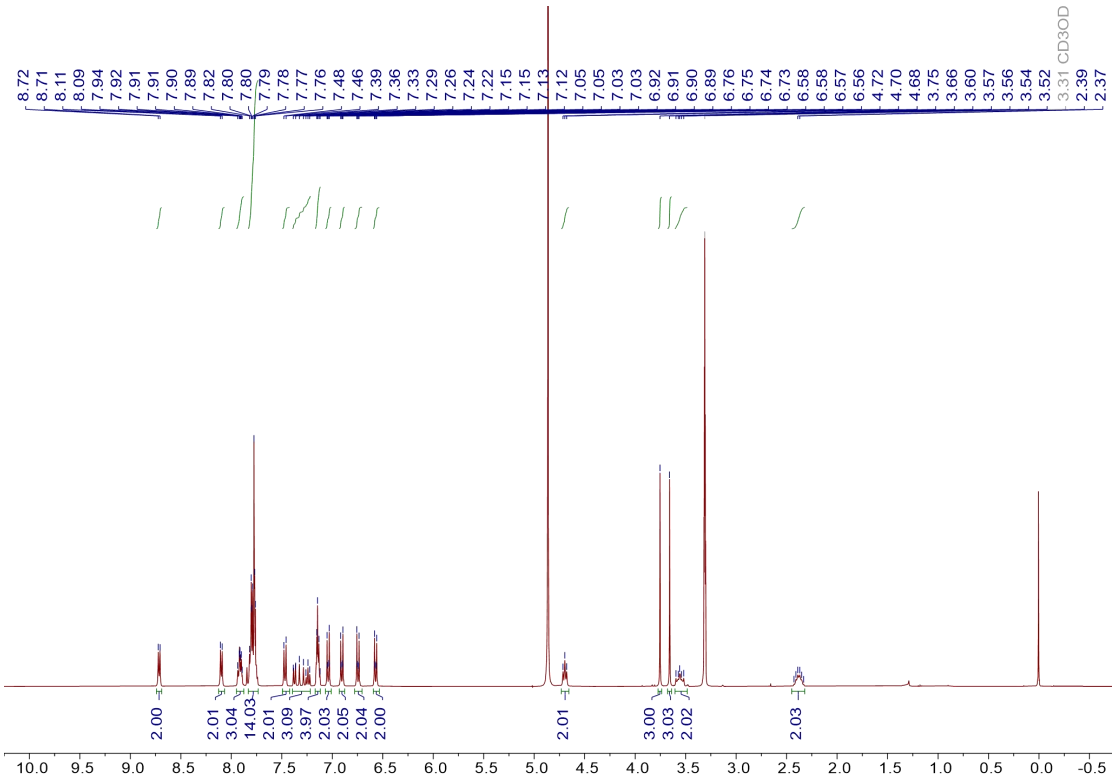


Fig. S13. ^{1}H NMR spectrum of **TPhBT-PyP**.

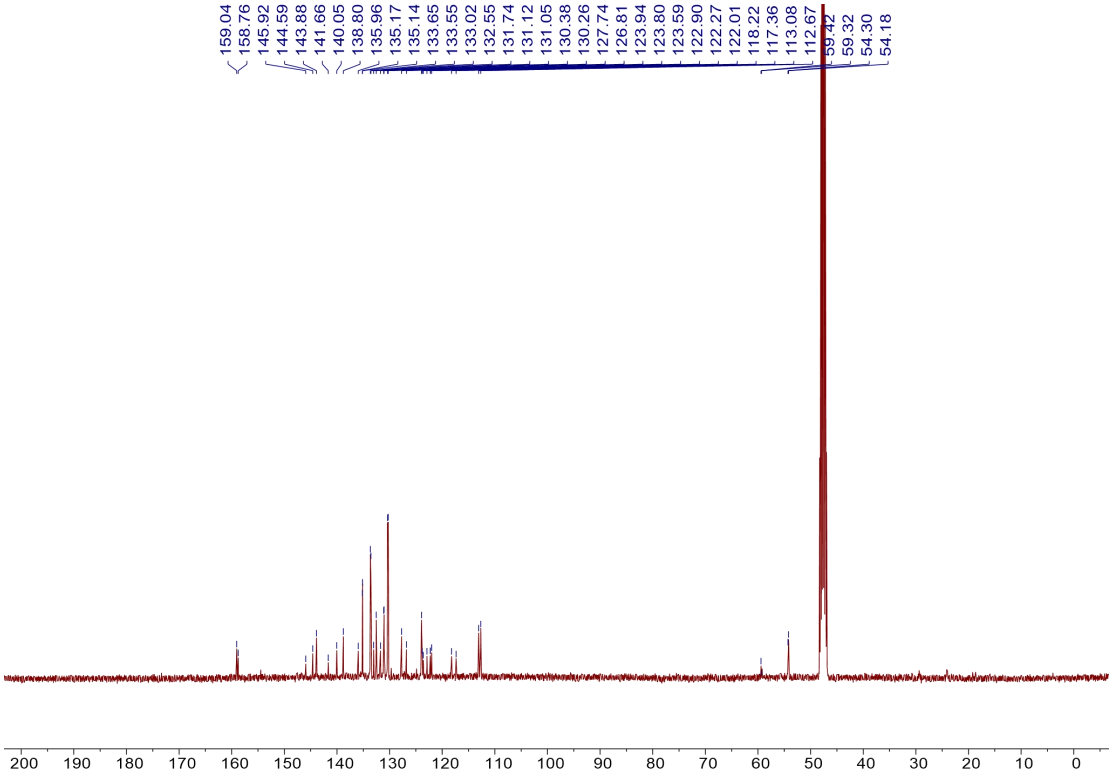


Fig. S14. ^{13}C NMR spectrum of **TPhBT-PyP**.

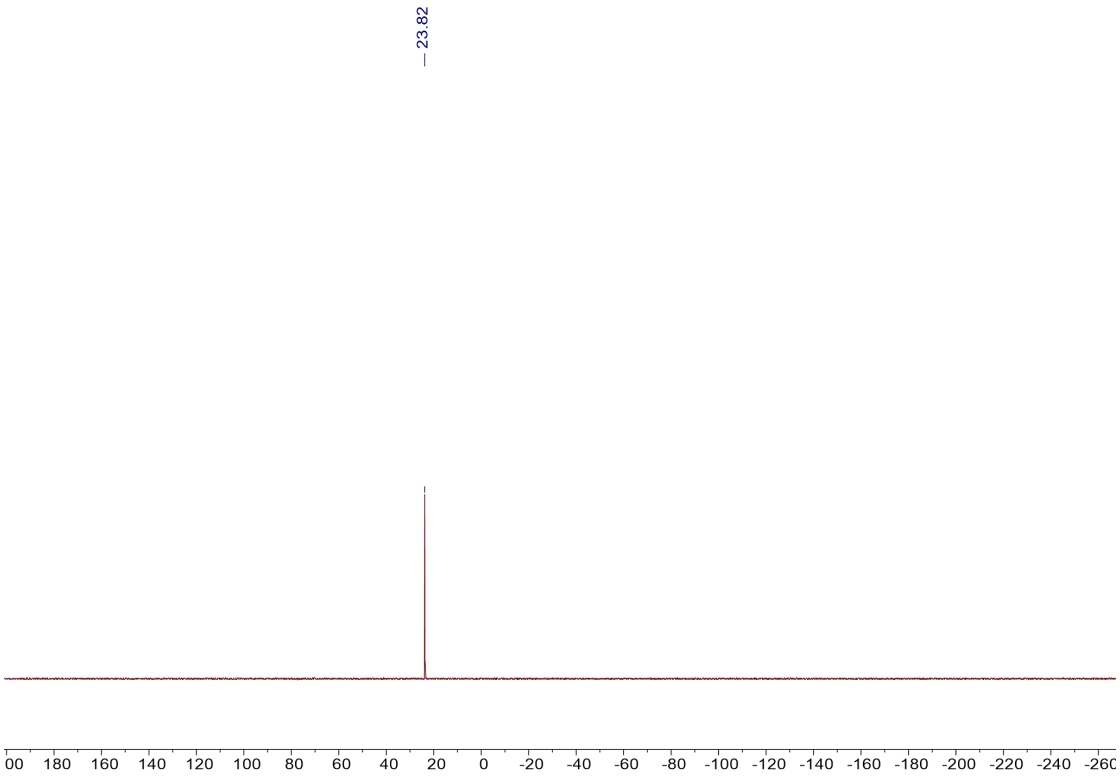


Fig. S15. ^{31}P NMR spectrum of **TPhBT-PyP**.

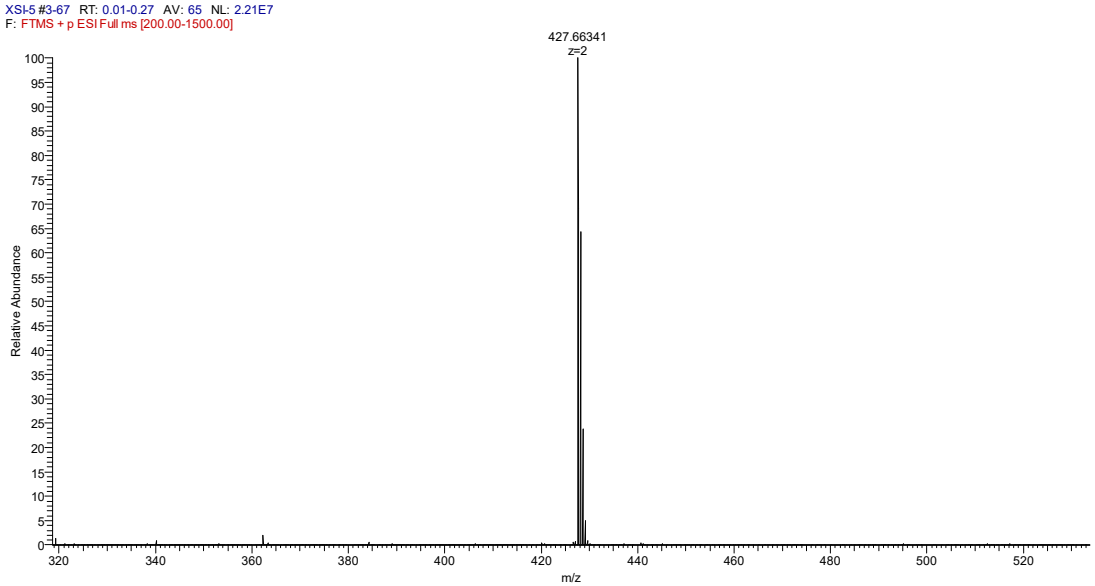


Fig. S16. ESI-HRMS of compound **TPhBT-PyP**.

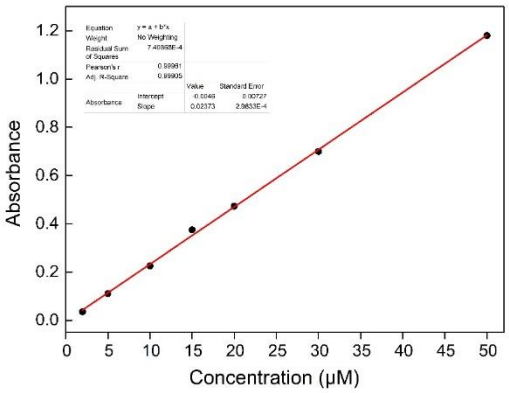


Fig. S17. The mass extinction coefficient of TPhBT-PyP in DMSO solution.

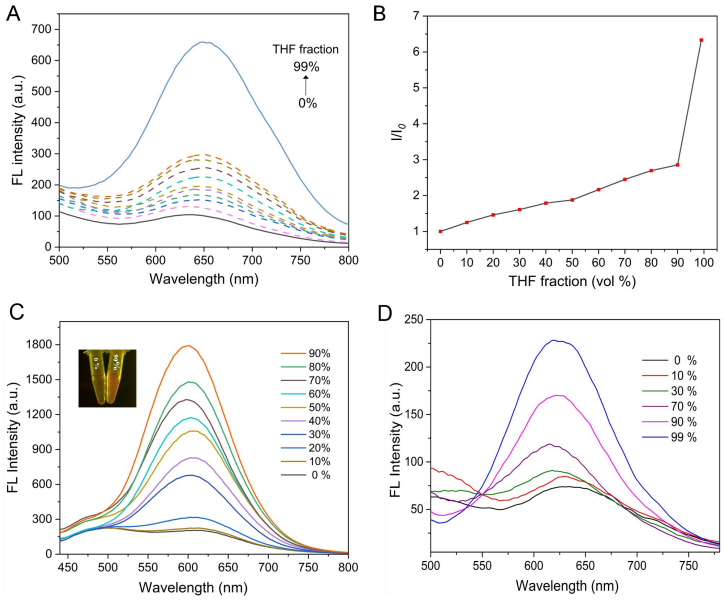


Fig. S18. Fluorescence spectra (A) and I/I_0 plot of intensity changes (B) of TPhBT-PyP (10 μM) in the water/THF mixed solution system. (C) Fluorescence spectra of TPhBT-PyP with the variation of solution viscosity (glycerol/H_2O mixture) and (D) the DMSO/PBS mixed solution system. (λ_{ex}: 420 nm).

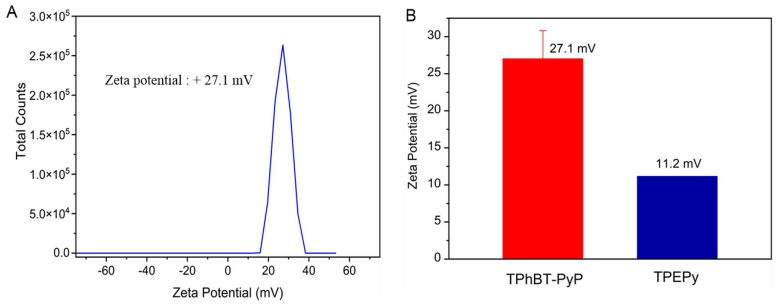


Fig. S19. (A) Zeta potentials of TPhBT-PyP and (B) TPEPy (10 μM) nanocomposites in aqueous media.

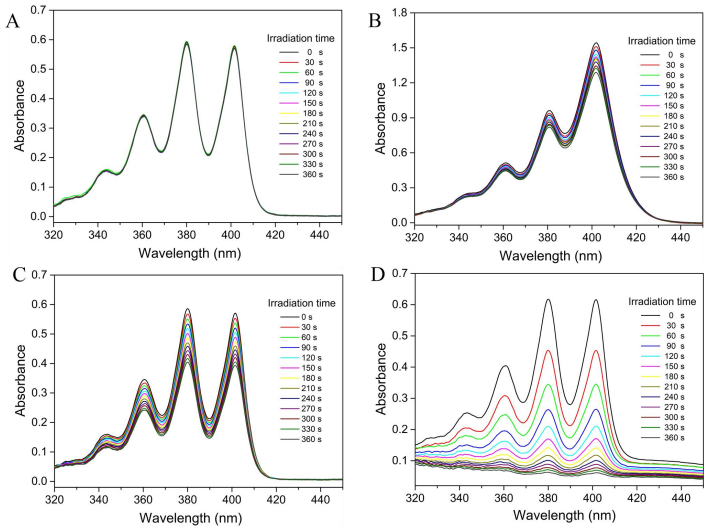


Fig. S20. Absorption spectra changes of (A): ABDA (50 μM), the mixture of ABDA and (B) Ce6 (10 μM), (C) Rose Bengal (10 μM), (D) TPhBT-PyP (10 μM) upon white light irrdiation (10 mW cm^{-2}) for different time (0 - 360 s).

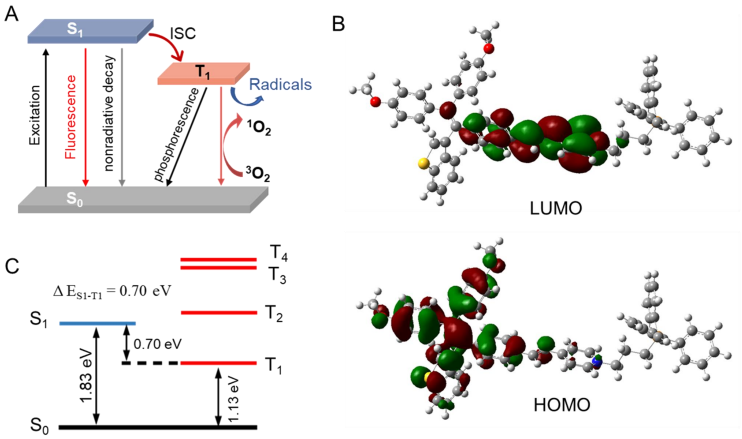


Fig. S21. (A) Schematic illustration of AIEgen-involving ROS generation based on the Jablonski diagram. (B) HOMO and LUMO distribution of and (C) calculated energy level diagram between singlet and triplet states of TPhBT-PyP calculated by TD-DFT at the basis set level of M06-2X/6-31G(d,p) using Gaussian09.

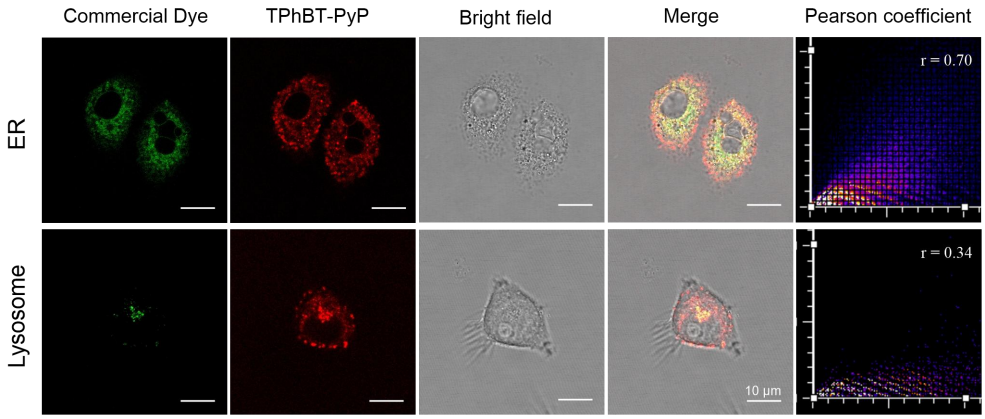


Fig. S22. Subcellular co-localization images of AIEgen TPhBT-PyP with ER-Tracker Green (top) and Lyso-Tracker Green (bottom) in Hela cells, respectively. Commercial dye: λex = 488 nm and λem = 500–550 nm. TPhBT-PyP: λex = 405 nm and λem = 580–660 nm. Scale bar = 10 μm.

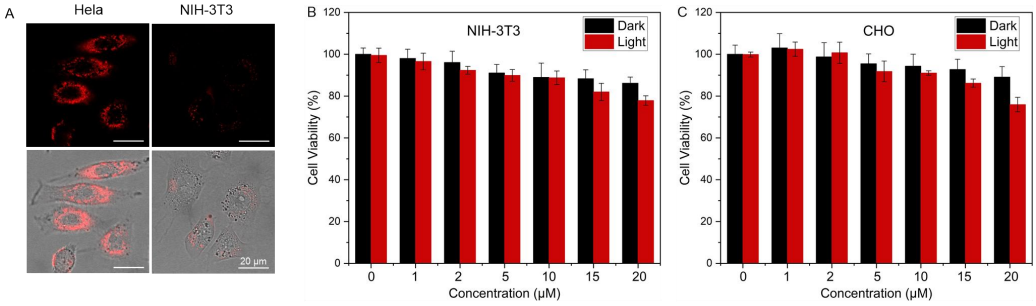


Fig.S23. (A) CLSM images of Hela and NIH-3T3 cells incubated with TPhBT-PyP (10μM) after 2 h. λex = 405 nm and λem = 580–660 nm. The viability of NIH-3T3 (B) and CHO (C) cells upon treatment with different concentrations of TPhBT-PyP without or with white light irradiation (50 mW cm^{-2}, 30 min).

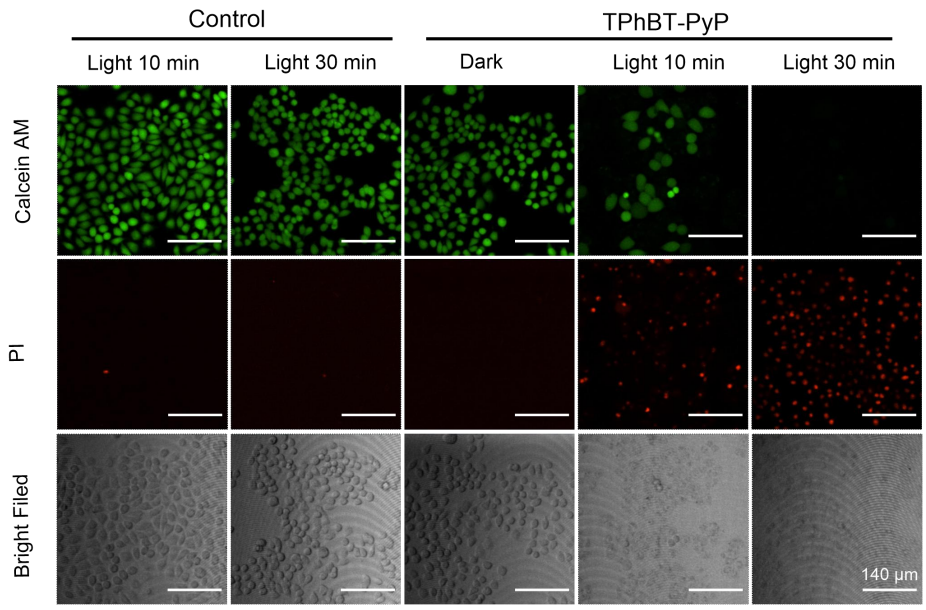


Fig. S24. Live/dead staining of Hela cells incubated with TPhBT-PyP (10 μM) after different white light irradiation times. Scale bar: 140 μm.

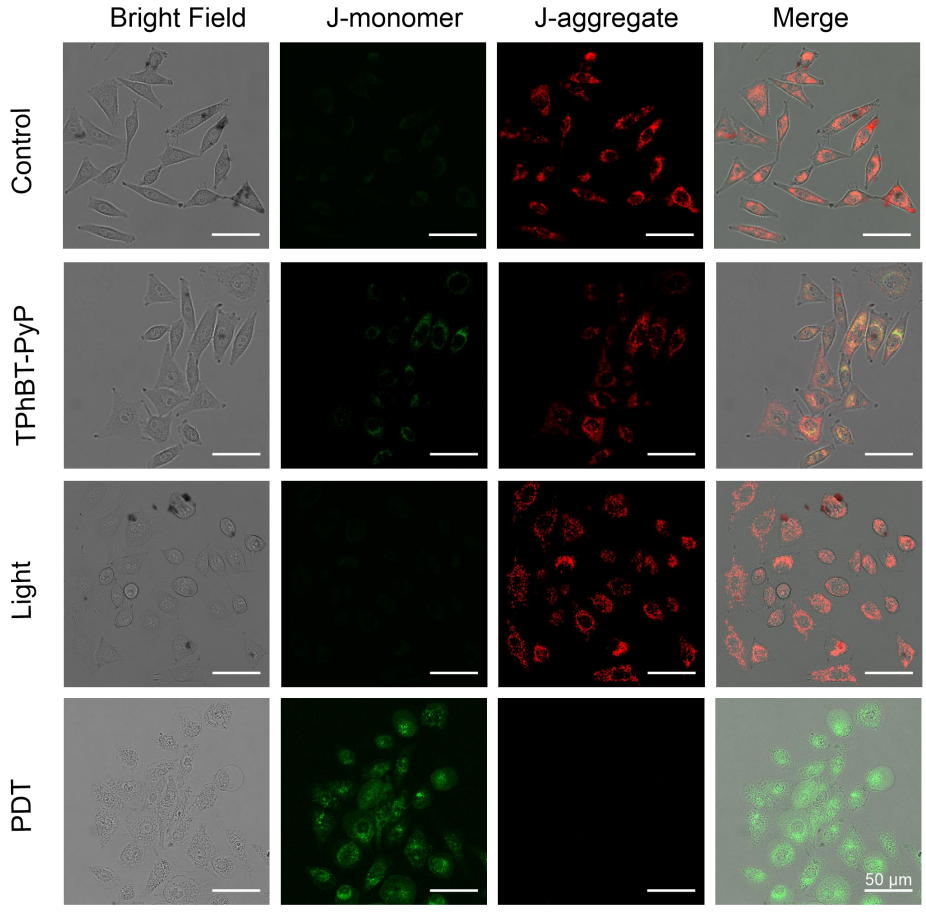


Fig. S25. Mitochondrion membrane potential decrease measurement using JC-1 kits in MCF-7 incubated with TPhBT-PyP (10 μM) and white light irradiation (50 mW

cm^{-2}) for 30 min. For J-monomer: λex = 488 nm and λem = 500-550 nm. For J-aggregate: λex = 561 nm and λem = 580-650 nm. Scale bar = 50 μm.

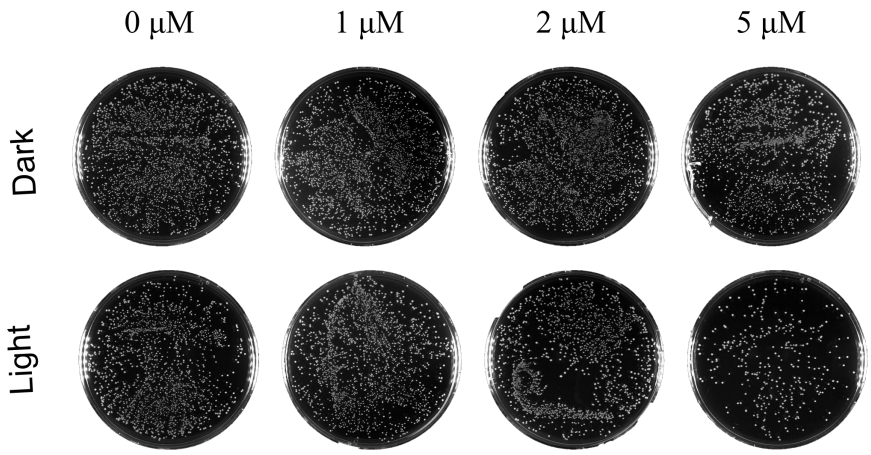


Fig. S26. Plates of Gram-negative E. coli after incubation with different concentrations of TPhBT-PyP for 20 min without or with white light irradiation (50 mW cm^{-2}) treatment for 20 min.

[1] W. Wen, Z.-F. Shi, X.-P. Cao, N.-S. Xu, Triphenylethylene-based fluorophores: Facile preparation and full-color emission in both solution and solid states, Dyes and Pigments, 132(2016) 282-90.