Supporting Information

Regioisomeric manipulation of AIE-active photosensitizers towards multidrug-resistant bacterial eradication

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Materials and Instruments

Unless otherwise stated, all solvents and chemicals are commercially available, analytically pure and do required no further purification. $[Cp^*RhCl_2]_2$, $Cu(OAc)_2$, AgOTf, TSA broth, TSA agar, etc., were purchased from Aladdin, Sigma-Aldrich, Adamas, Bide, and Macklin reagent companies. HUVECs, *E. coli* and *S. aureus* were obtained from ATCC. MRSA, MDR VRE, MRSE, MDR E. coli were from Southern Medical University. The ^1H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra, operating at 400 MHz, were acquired utilizing a Bruker AV 400M spectrometer. These spectra employed chloroform-d as the solvent and tetramethylsilane as the internal standard for calibration. The coupling constants (J-values) were reported in Hertz (Hz). High-resolution mass spectrometry (HRMS) analyses were performed on an integrated Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) system, comprising a Thermo Scientific Ultimate 3000 RSLC HPLC unit and a Q Exactive Orbitrap mass spectrometer. For optical characterizations, UV-visible (UV-vis) absorption spectra and photoluminescence (PL) spectra were recorded employing a Shimadzu UV-2600 spectrophotometer and a Hitachi F-4700 spectrofluorometer, respectively. The PL quantum yields were quantitatively determined using an absolute method with a Hamamatsu C11347-11 Quantaurus-QY Analyzer.The quantum chemistry calculations were performed using the Gaussian 09 (B3LYP/6–31G (d,p) basis set) software package. Morphological examinations of the bacteria were conducted through Scanning Electron Microscopy (SEM, JSM 7800F) and (TEM, Hitachi HT7700). The bacterial zeta potential was precisely measured using a Delsa Nzno C zeta potential analyzer. Additionally, bacterial fluorescence imaging was captured utilizing a laser confocal scanning microscope from Olympus (Spin 10).

Synthesis of TPA-BQZ-1[1]

1-Phenylpyridinium trifluoromethanesulfonate (0.22 mmol, 1.1eq.), 4,4'-(ethylene-1,2-diyl)bis(N,N-diphenylaniline) (0.2 mmol, 1.0 eq.), $[Cp^*RhCl_2]_2$ (0.01 mmol, 5 mol%), and $Cu(OAc)_2$ (0.4 mmol, 2.0 eq.) were placed in a Schlenk tube. After applying vacuum, anhydrous acetonitrile (2.0 mL) was added under nitrogen protection, and the mixture was stirred at 100 °C for 12 h. After cooling to room

temperature, the reactants were diluted with dichloromethane (2 mL) and methanol (2 mL), and the solvent mixture was evaporated under reduced pressure. Using MeOH/CH_2Cl_2 (1:20) as the eluent, the pure product was obtained by silica gel column chromatography (isolated yield: 80%, 130.5 mg). ^{1}H NMR (400 MHz, Chloroform-d) δ 10.92 (d, J = 7.0 Hz, 1H), 9.48 (d, J = 9.0 Hz, 1H), 8.44 (t, J = 6.2 Hz, 1H), 8.39 – 8.34 (m, 1H), 8.27 – 8.20 (m, 2H), 8.06 (d, J = 8.3 Hz, 1H), 7.91 (t, J = 7.6 Hz, 1H), 7.31 (t, J = 7.2 Hz, 8H), 7.14 (d, J = 8.5 Hz, 8H), 7.07 (dd, J = 15.9, 7.2 Hz, 8H), 6.98 (d, J = 7.1 Hz, 4H). ^{13}C NMR (100 MHz, Chloroform-d) δ 148.45, 148.39, 148.18, 147.05, 143.34, 139.80, 136.69, 134.30, 133.88, 133.04, 131.55, 130.97, 130.87, 129.62, 129.41, 127.18, 126.86, 126.52, 125.13 (d, J = 2.7 Hz), 123.96, 122.18, 121.56, 119.57. HRMS (ESI), m/z: calcd for $C_{49}H_{36}N_3^+$: 666.29037, found: 666.28967.

Synthesis of TPA-BQZ-2[2]

4,4'-(ethylene-1,2-diyl)bis(N,N-diphenylaniline) (0.2 mmol, 1.0 eq.), $[Cp^*RhCl_2]_2$ (0.002 mmol, 1 mol%), and AgOTf (0.2 mmol, 1.0 eq.) were placed in a Schlenk tube. After applying vacuum, ultra-dry methanol (2.0 mL) and 2-phenylpyridine (0.2 mmol, 1.0 eq.) were added under nitrogen protection, and the mixture was stirred at 120 °C for 22 h. After cooling to room temperature, the reactants were diluted with dichloromethane (2 mL) and methanol (2 mL), and the mixed solvent was evaporated under reduced pressure. Using MeOH/CH_2Cl_2 (1:20) as the eluent, the pure product was obtained by silica gel column chromatography (isolated yield: 75%, 122.4 mg).^{1}H NMR (400 MHz, Chloroform-d) δ 9.62 (d, J = 8.9 Hz, 1H), 9.13 (dd, J = 14.5, 7.4 Hz, 2H), 8.72 (t, J = 7.9 Hz, 1H), 8.12 – 8.04 (m, 2H), 7.99 (t, J = 7.5 Hz, 1H), 7.89 (d, J = 7.4 Hz, 1H), 7.35 – 7.28 (m, 8H), 7.18 – 7.08 (m, 16H), 7.07 (d, J = 3.2 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H). ^{13}C NMR (100 MHz, Chloroform-d) δ 149.67, 148.06, 147.20, 146.63, 143.62, 140.32, 138.48, 137.43, 135.85, 134.19, 132.57, 131.85, 131.39, 131.05, 129.75, 129.57, 127.67, 127.01, 126.22, 125.61, 124.93, 124.63, 124.48, 123.76, 122.27, 122.10, 121.69. HRMS (ESI), m/z: calcd for $C_{49}H_{36}N_3^+$: 666.29037, found: 666.28967.

Total Reactive Oxygen Species Assay

0.5 mL of 1 mM DCFH-DA, 2 mL of 10 mM sodium hydroxide solution, and 10 mL of PBS (pH = 7.4) were mixed and oscillated in the dark for 30 min at room temperature. Then, 87.5 μL of TPA-BQZ-1, TPA-BQZ-2, Rose Bengal (RB), and phosphate-buffered saline (PBS) were mixed with 12.5 μL of 40 μM DCFH hydrolysate and transferred to 96-well plates, ensuring that the final concentration of all molecules was 1 μM. The plates were irradiated with white light (16 mW/cm^2) at different time intervals and detected using multifunctional enzyme marker.

Singlet Oxygen Detection

20 μL of TPA-BQZ-1, TPA-BQZ-2, RB, and DMSO were mixed with 1.8 mL of PBS and placed in a quartz cuvette, ensuring that the final concentration of all molecules was 1 μM. The plates were irradiated with white light (16 mW/cm^2) at different time intervals and detected by ultraviolet-visible spectrophotometer.

Hydroxyl Radical and Superoxide Detection

The detection reagents used are HPF (Hydroxyl Radical) and DHR123 (Superoxide), and the specific steps refer to the Total Reactive Oxygen Species Assay.

ESR analyses

Two spin-trapping agents, DMPO and TEMP, were utilized to rigorously substantiate the proficiency of both TPA-BQZ-1 and TPA-BQZ-2 in generating hydroxyl radicals, superoxide anions, and singlet oxygen under specified conditions. Precise mixed solutions were formulated, consisting of either 50 mM DMPO (tailored for hydroxyl radical capture) or methanol (tailored for superoxide anion capture), each of which was blended with 1 μM of either TPA-BQZ-1 or TPA-BQZ-2. These solutions were then quantitatively and accurately dispensed into quartz capillaries. Following this, the samples were subjected to white light illumination at an intensity of 100 mW/cm², and their rotational spectra were meticulously recorded and analyzed both prior to and after one minute of continuous illumination. For the detection of singlet oxygen specifically, a solution composed of 25 mM TEMP in water (designed for singlet oxygen trapping) mixed with 1 μM of either TPA-BQZ-1 or TPA-BQZ-2 was employed. This solution too was quantitatively introduced into a quartz capillary,

and the spin spectra of the samples were thoroughly monitored and analyzed before and after illumination with white light.

Detection of zeta potentials

The zeta potentials of TPA-BQZ-1 and TPA-BQZ-2 dots were precisely quantified utilizing a Zetasizer instrument from Malvern Instruments. For this analysis, solutions of TPA-BQZ-1 and TPA-BQZ-2, each at a concentration of 5 mM, were prepared by diluting them in PBS containing bacterial suspensions at a density of 1×10^8 CFU/mL. Following thorough homogenization to ensure uniform dispersion, all samples were incubated at ambient temperature for a period of 30 min prior to undergoing the analytical process.

Antibacterial experiment

Various single colonies on TSA agar were individually transferred into test tubes containing 2 mL of TSA liquid medium, followed by overnight incubation at 37 °C with shaking at 200 rpm. The culture broth was centrifuged at 4000 rpm for 3 minutes and washed three times with PBS buffer (pH 7.4) to remove impurities. Next, the washed bacterial suspension was diluted to a concentration of approximately 10^8 CFU/mL, and different concentrations (0, 1, 2, 5, 10 μM) of TPA-BQZ-1 and TPA-BQZ-2 solution were added to it. The mixture was further incubated at 37 °C with shaking at 200 rpm for 30 min, then divided into two groups for treatment: one group was exposed to white light (16 mW/cm^2) for 10 min, while the other served as a dark control group and was kept in complete darkness for the same duration.After treatment, both bacterial suspensions were serially diluted to 10^4 CFU/mL, and the diluted solutions were evenly spread onto TSA agar plates. The plates were incubated in a 37 °C incubator until colonies formed, followed by photographing, recording, and counting the colony numbers. Each experimental group was repeated three times.

Membrane permeabilization assay

This assay measures the uptake of propidium iodide (PI) to assess membrane permeabilization by molecules. For the evaluation of bacterial membrane permeabilization, S. aureus bacteria are harvested during mid-logarithmic growth phase, washed, and resuspended in a 1:1 mixture of 5 mM glucose and 5 mM HEPES

buffer (pH 7.2). The bacterial suspension is then mixed with PI at a concentration of 20 μg/mL. A 100 μL aliquot of this bacterial solution is placed in a 96-well plate, followed by the addition of 5 μM TPA-BQZ-1 and TPA-BQZ-2. Fluorescence is monitored at an excitation wavelength of 580 nm and an emission wavelength of 620 nm over a period of 2 hours, with measurements taken at 10-minute intervals.

Bacterial Imaging

Bacterial solution is prepared using a bacterial turbidimeter to achieve a concentration of 5×10^8 CFU/mL. Subsequently, this working bacterial solution is transferred into a centrifuge tube, TPA-BQZ-1, TPA-BQZ-2 ,and Hoechst 33342 are added to it until each reaches a final concentration of 5 μM. Following this, the mixture is thoroughly vortexed and then incubated with shaking at 37 °C for 10 min. After incubation, the bacteria are collected by centrifugation and washed three times with PBS buffer to remove excess reagents. Next, 3 μL of the stained bacterial solution is carefully pipetted onto a clean slide, and immediately covered with a coverslip for immobilization. Finally, the bacteria on the slide are observed and analyzed using a laser scanning confocal microscope.

SEM measurement

TPA-BQZ-1 and TPA-BQZ-2 were added to a bacterial suspension containing 1×10^8 CFU/mL, and the mixture was then incubated in a shaker at 37 °C and 200 rpm for 30 minutes. Following incubation, the bacterial samples were divided into two groups: one group was kept in the dark, while the other was exposed to white light (16 mW/cm^2), both for a duration of 15 min. Subsequently, both groups of samples underwent three rounds of centrifugation. After centrifugation, the bacterial samples were fixed with 2.5% glutaraldehyde and stored in a refrigerator at 4 °C overnight. The next day, the fixed bacterial samples were removed, washed five times with PBS (phosphate-buffered saline). Subsequently, the bacterial samples were subjected to a gradient dehydration process using 30%, 50%, 70%, 80%, 90%, 95%, and 100% ethanol solutions in sequence. After 15 minutes, the dehydrated bacterial samples were dripped onto a microporous filter membrane at the critical point to allow for

drying. A layer of gold was then coated onto the surface of the bacterial samples, and the samples on the silicon slides were measured and observed using a scanning electron microscope.

TEM measurement

TPA-BQZ-1 and TPA-BQZ-2 were added to a bacterial suspension containing 1×10^8 CFU/mL, and the mixture was then incubated in a shaker at 37 °C and 200 rpm for 30 minutes. Following incubation, the bacterial samples were divided into two groups: one group was kept in the dark, while the other was exposed to white light (16 mW/cm^2), both for a duration of 15 minutes. Subsequently, both groups of samples underwent three rounds of centrifugation. After centrifugation, the bacterial samples were fixed with 2.5% The subsequent processes for the samples, including agarose pre-embedding, post-fixation, dehydration, resin infiltration and embedding, polymerization, ultrathin sectioning, and staining, were carried out by Wuhan Servicebio Technology Co., Ltd.Finally, the treated samples were characterized by transmission electron microscopy.

Cytotoxicity Assay

A cell suspension containing HUVEC cells (5×10^3 cells/ well) is seeded into a 96-well plate. The cells are then cultured for 24 h under conditions of 37 °C and 5% CO_2. After the initial culture period, the medium is replaced with fresh medium containing varying concentrations of either TPA-BQZ-1 or TPA-BQZ-2, and the cells are further incubated in the incubator for 1 h. Following this, the cells are exposed to white light (16 mW/cm^2) or kept in darkness for 10 min, after which the medium is replaced with new medium. Subsequently, the cells are incubated for an additional 24 h. Cytotoxicity was assessed using the MTT assay, and the relative toxicity was evaluated by measuring the absorbance at 490 nm using a multifunctional enzyme marker.

Cell Imaging

HUVEC cells (1×10^5 cells/mL) were seeded in a confocal petri dish and incubated at 37 °C in a 5% CO2 incubator for 24 h. The medium was replaced with 5 μM TPA-BQZ-1 and TPA-BQZ-2, and the cells were incubated for 20 min.

Subsequently, the cells were washed with PBS and imaged using a confocal laser scanning microscope (CLSM).

Statistical Analysis

During the experimental process, each analysis was independently repeated at least three times. All collected research data were analyzed using GraphPad Prism 9 statistical software, and the results were presented in the form of mean ± standard deviation (SD).

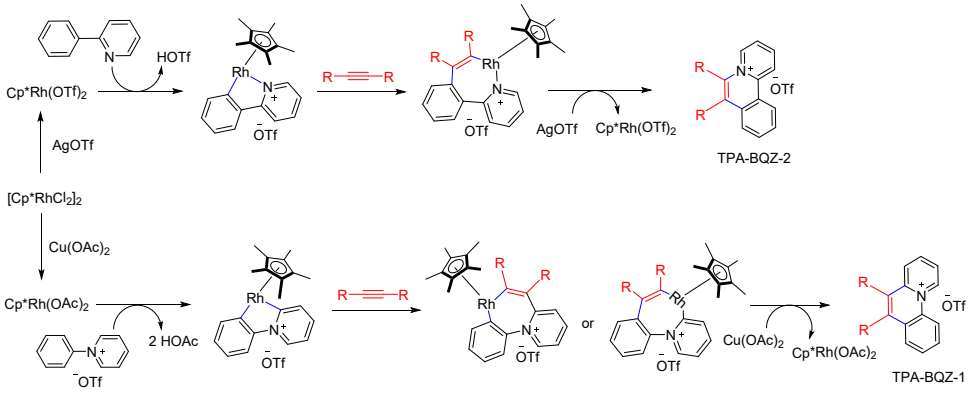

Figure S1. The reaction mechanisms of TPA-BQZ-1 and TPA-BQZ-2.

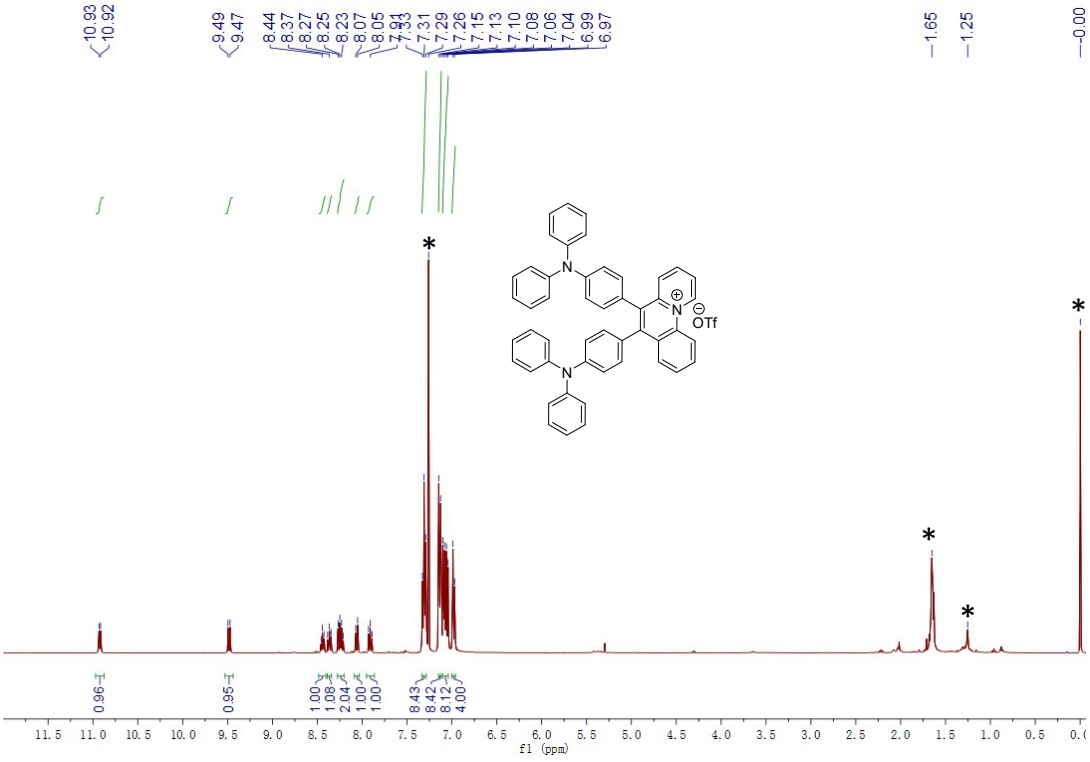

Figure S2. ^{1}H NMR spectrum of TPA-BQZ-1. The solvent peaks are marked with asterisks.

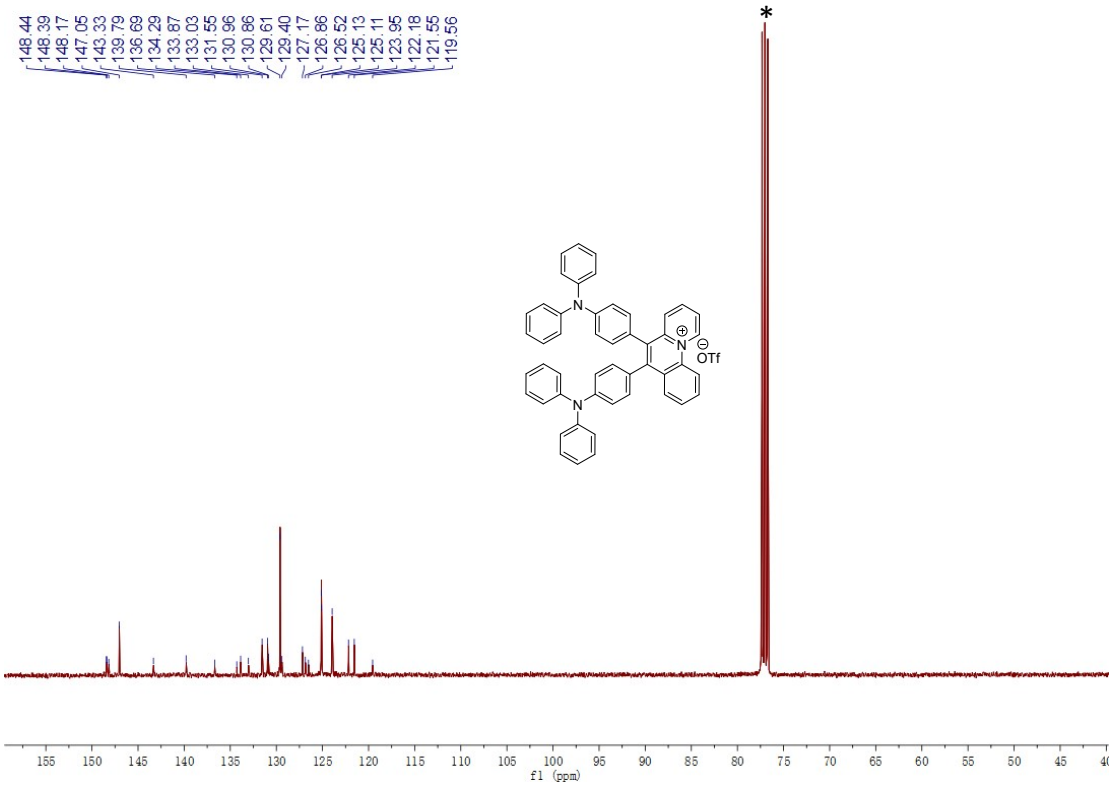

Figure S3. ^{13}C NMR spectrum of TPA-BQZ-1. The solvent peaks are marked with asterisks.

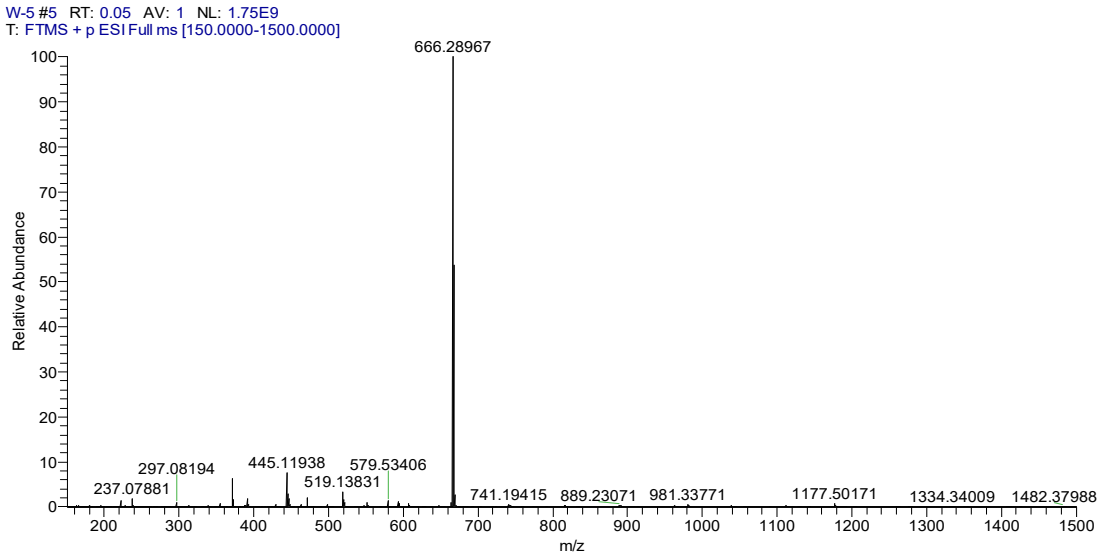

Figure S4. ESI-HRMS of TPA-BQZ-1

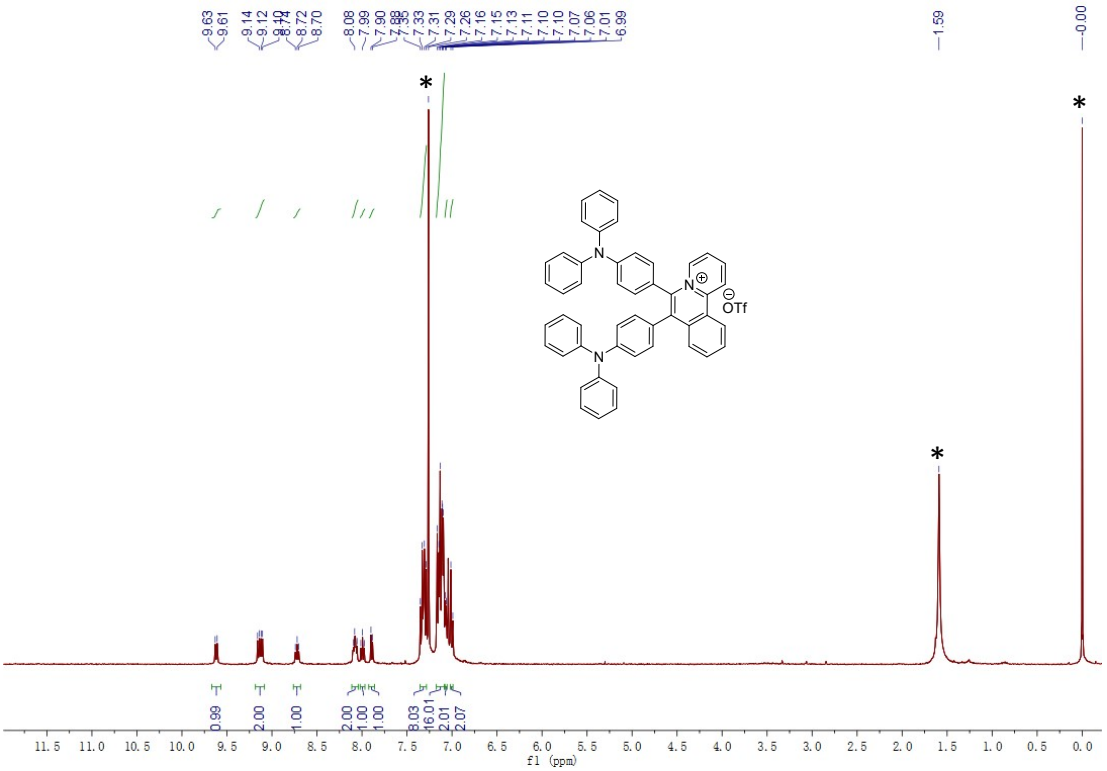

Figure S5. [1]H NMR spectrum of TPA-BQZ-2. The solvent peaks are marked with asterisks.

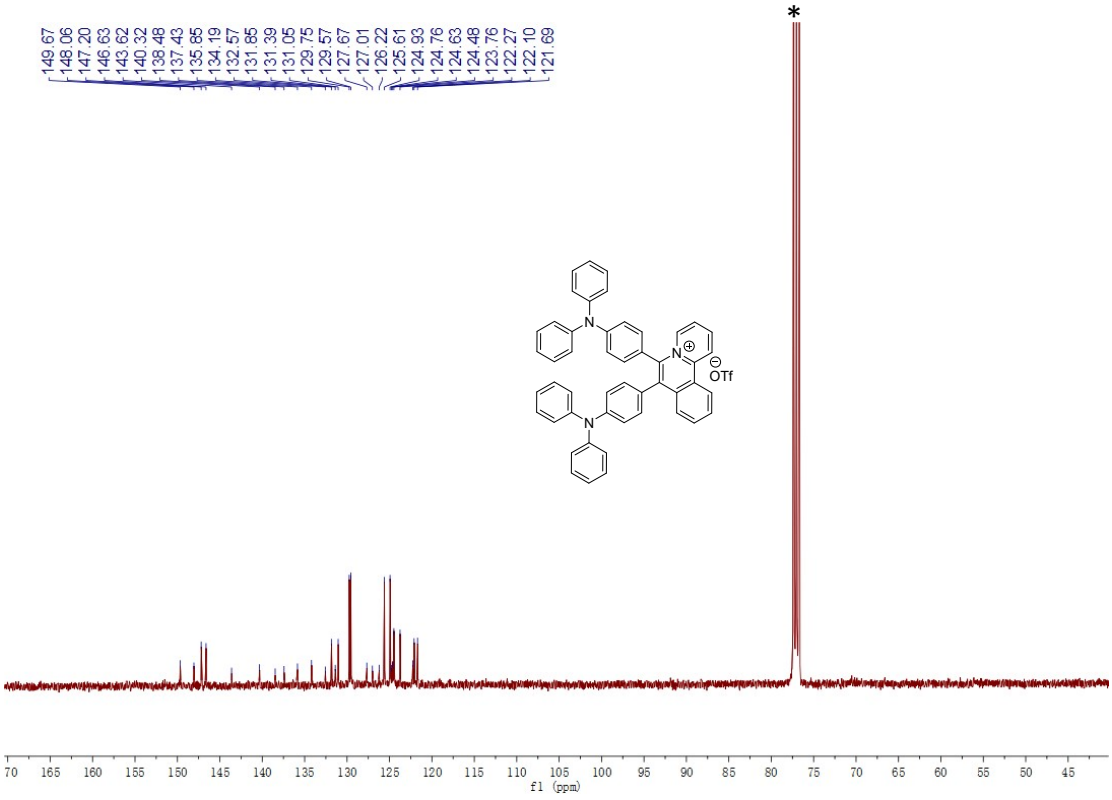

Figure S6. [13]C NMR spectrum of TPA-BQZ-2. The solvent peaks are marked with asterisks.

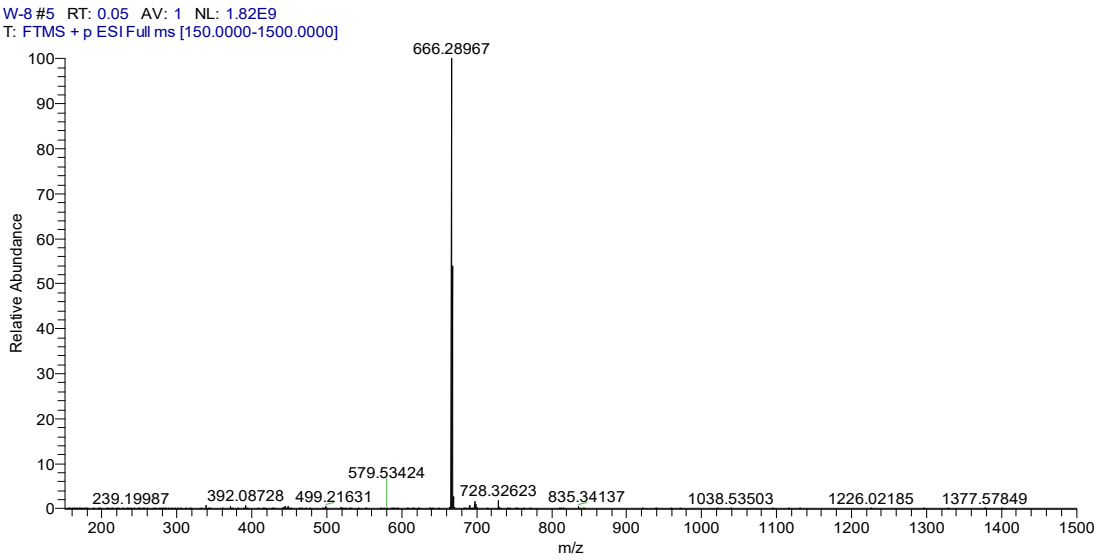

Figure S7. ESI-HRMS of TPA-BQZ-2

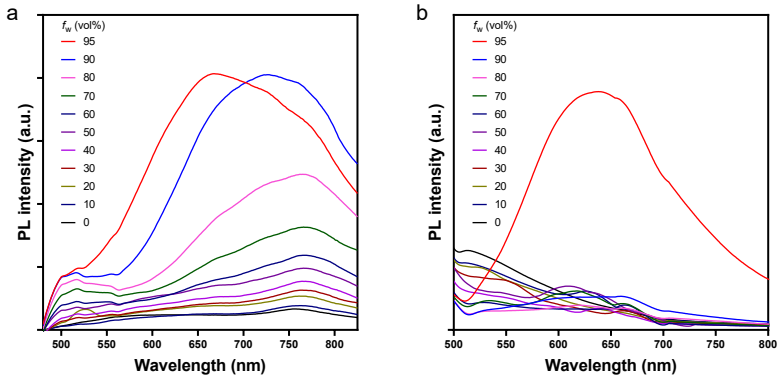

Figure S8. PL spectra of (a) TPA-BQZ-1 and (b) TPA-BQZ-2 in the DMSO/H_2O with different water fractions.

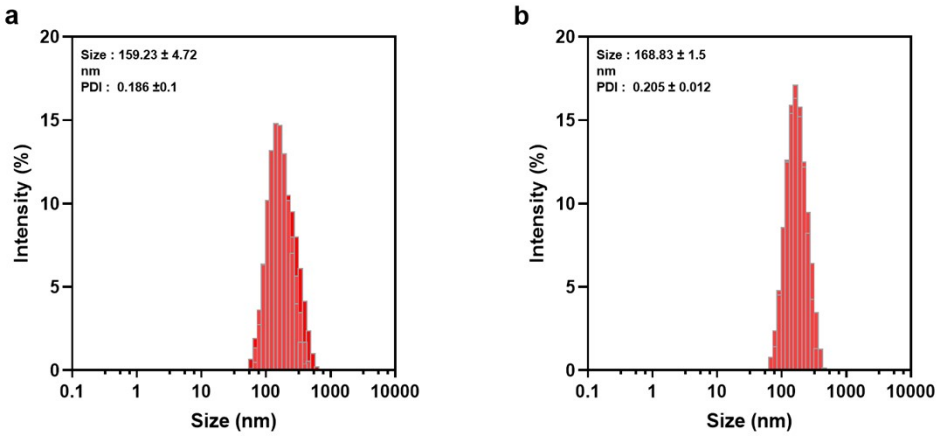

Figure S9. Size distribution of TPA-BQZ-1 (a) and TPA-BQZ-2 (b) aggregates in aqueous solution measured by dynamic light scattering.

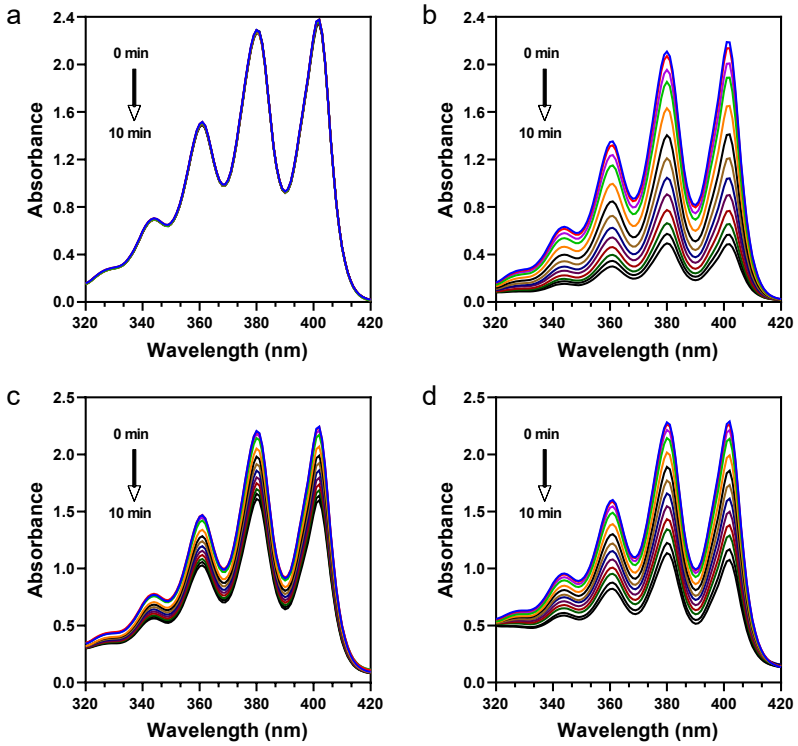

Figure S10. Absorbance spectra changes of (a) ABDA, (b) ABDA + RB, (c) ABDA + TPA-BQZ-1, (d) ABDA + TPA-BQZ-2 under white light irrdiation (16 mW/cm^2) for different time with the concentration of ABDA (10 μM), TPA-BQZ-1 (1 μM) and TPA-BQZ-2 (1 μM).

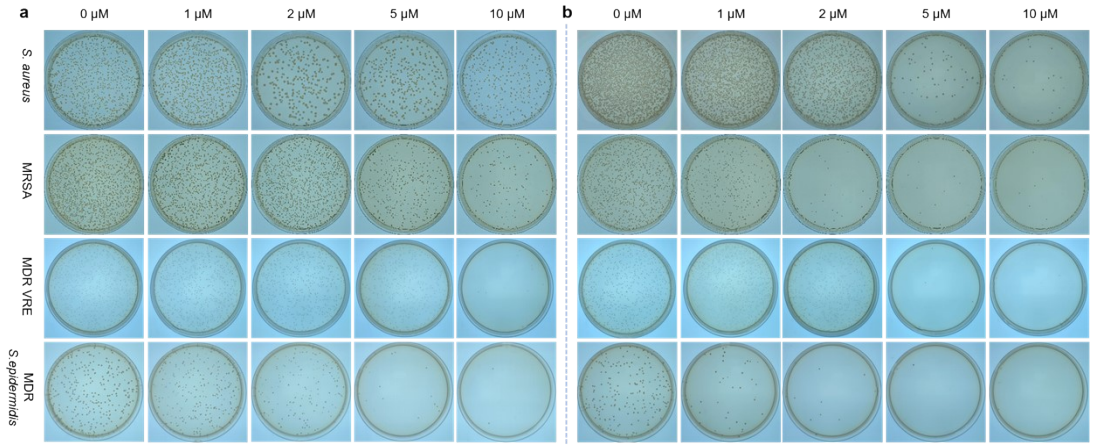

Figure S11. *S. aureus*, MRSA, MDR VRE and MDR *S.epidermidis* grown on agar plates after the treatment with (a) TPA-BQZ-1 and (b) TPA-BQZ-2 in the darkness.

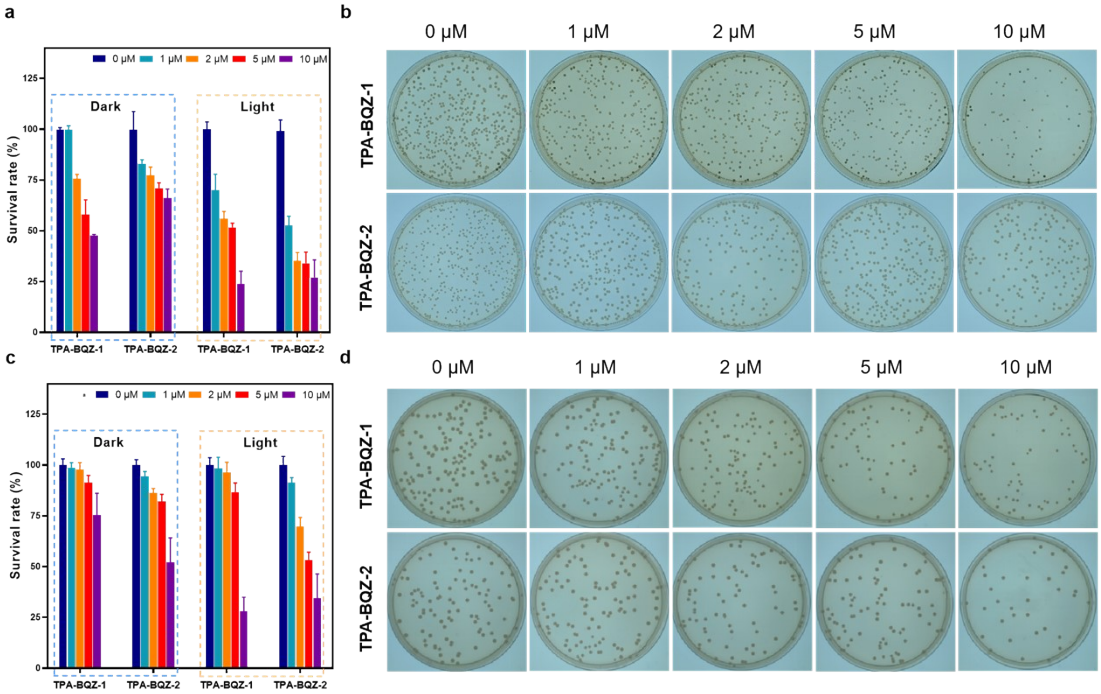

Figure S12. Antibacterial activity of TPA-BQZ-1 and TPA-BQZ-2 against (a) *E.coli* and (c) MDR *E.coli* survival rates after the treatment with different concentrations of TPA-BQZ-1 or TPA-BQZ-2 under white irradiation (16 mW/cm^2) or in the dark. (b) *E.coli* and (d) MDR *E.coli* grown on agar plates after the treatment with TPA-BQZ-1 or TPA-BQZ-2 under light irradiation.

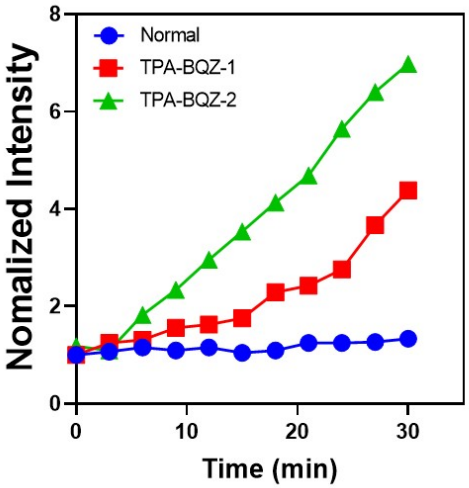

Figure S13. Membrane permeabilization assay with propidium iodide after treatment with TPA-BQZ-1 and PA-BQZ-2 (5 μM). E_x = 580 nm, E_m = 620 nm.

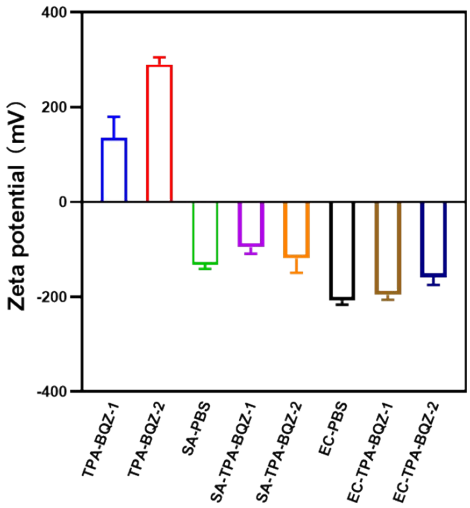

Figure S14. Zeta potential changes of *S. aureus* and *E.coli* after treatment with TPA-BQZ-1 and TPA-BQZ-2.

Reference

(1) Ge, Q.; Hu, Y.; Li, B.; Wang, B. Synthesis of Conjugated Polycyclic Quinoliniums by Rhodium(III)-Catalyzed Multiple C-H Activation and Annulation of Arylpyridiniums with Alkynes. *Org. Lett.* **2016**, *18*, 2483-2486.

(2) Zhang, G.; Yang, L.; Wang, Y.; Xie, Y.; Huang, H. An efficient Rh/O_2 catalytic system for oxidative C-H activation/annulation: evidence for Rh(I) to Rh(III) oxidation by molecular oxygen. *J. Am. Chem. Soc.* **2013**, *135*, 8850-8853.