**Supporting Information for**

# Original article

# Harnessing antimicrobial peptide-coupled photosensitizer to combat drug-resistant biofilm infections through enhanced photodynamic therapy

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## 1. Materials and Experiments

*1.1 Materials and Chemicals.*

The antimicrobial peptide (CysHHC10, sequence: Cys-Lys-Arg-Trp-Trp-Lys-Trp-Ile-Arg-Trp-$NH_2$, HHC10, sequence: Lys-Arg-Trp-Trp-Lys-Trp-Ile-Arg-Trp-$NH_2$) were purchased from China Peptides Co., Ltd. (Shanghai, China). Pyridine-4-boronic acid and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) were purchased from Aladdin (Shanghai, China). 4,7-dibromo-2,1,3-benzothiadiazole, 4-(diphenylamino)phenylboronic acid, aminobenzenethiol, N-hydroxysuccinimide and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride were purchased from Energy Chemical (Shanghai, China). 1,4-dioxane, DMSO, trifluoroacetic acid, methanol, ethanol, acetonitrile, ethanol, THF, ethyl ether and paraformaldehyde were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Polymyxin B, Nutrient broth and LB agar were purchased from Solarbio (Beijing, China). Vancomycin was purchased from Yuanye Bio-Technology (Shanghai, China). MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide), Hoechst 33342 and YO-PRO-1 were purchased from Beyotime Biotechnology Co. (China). All other chemicals were purchased and purified using standard procedures.

*1.2 Characterization*

The $^{1}H$ and $^{13}C$ NMR spectra were measured on a Bruker AV 400MHz and 500MHz spectrometer (Billerica, USA). The HPLC profiles were acquired on an Agilent 1260 II Infinity LC (California, USA) with 0.1% TFA/acetonitrile and 0.1% TFA/$H_2O$ as the eluent. The high-resolution mass spectra (HRMS) were measured on an Orbitrap Velos Pro LC-MS spectrometer (Thermo Scientific, American). UV‑vis absorption spectra were recorded on a Shimadzu UV-2450 UV-Visible spectrophotometer (Kyoto, Japan). Photoluminescence (PL) spectra were taken on a Hitachi F-2700 spectrofluorometer (Hitachi, Japan). SEM images were acquired by a Quanta 250 FEG (FEI make).

*1.3 Synthesis of **TPI-CysHHC10***

Synthesis of **TPI-Br**

A mixture of 4-(diphenylamino)phenylboronicacid (578 mg, 2.00 mmol), 4,7-dibromo-2,1,3-

Benzothiadiazole (588 mg, 2.00 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) (20 mg) and $K_2CO_3$ (200 mg) in a solution of THF (5 mL) and MeOH (10 mL) were refluxed under nitrogen for 8 h. After cooling down to room temperature, the mixture was poured into water and then extracted with DCM. The organic layer was dried over anhydrous $Na_2SO_4$ and evaporated. The residue was purified by column chromatography over silica gel using petroleum ether and DCM. **TPI-Br** was obtained as an orange powder (594 mg, yield: 65%).$^1$H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, $J$ = 7.6 Hz, 1H), 7.83 (d, $J$ = 8.8 Hz, 2H), 7.56 (d, $J$ = 7.6 Hz, 1H), 7.37-7.29 (m, 4H), 7.25-7.17 (m, 6H), 7.11 (dd, $J$ = 11.5, 4.2 Hz, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 153.96, 153.15, 148.44, 147.33, 133.55, 132.39, 129.92, 129.83, 129.43, 127.34, 125.05, 123.53, 122.63, 112.19.

Synthesis of **TPIP**

A mixture of **TPI-Br** (914 mg, 2.00 mmol), pyridine-4-boronic acid (369 mg, 3.00 mmol), $K_2CO_3$ (200 mg), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) (20 mg) and THF (5 mL) was refluxed under nitrogen for 20 h. After cooling down to room temperature, the mixture was poured into water and then extracted with DCM. The organic layer was dried over anhydrous $Na_2SO_4$ and evaporated. The residue was purified by column chromatography over silica gel using petroleum ether and DCM. **TPIP** was yielded as an orange powder (565 mg, yield: 62%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.79 (d, $J$ = 5.1 Hz, 2H), 7.94 (d, $J$ = 5.1 Hz, 2H), 7.90 (d, $J$ = 8.3 Hz, 2H), 7.87-7.75 (m, 2H), 7.31 (t, $J$ = 7.7 Hz, 4H), 7.22 (t, $J$ = 8.7 Hz, 6H), 7.10 (t, $J$ = 7.4 Hz, 2H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 154.03, 153.60, 150.17, 148.49, 147.32, 144.70, 134.65, 130.13, 130.10, 129.46, 129.17, 128.98, 126.87, 125.10, 123.58, 123.50, 122.56.

Synthesis of **TPI-PN**

**TPIP** (456 mg,1.00 mmol) and 2-bromoethylamine hydrobromide (307 mg, 1.50 mmol) were dissolved in $CH_3CN$ (4 mL) and stirred for 6 h at 90$^o$C. After cooling down to room temperature, the $CH_3CN$ was removed under a vacuum. The mixture was separated by HPLC (solvent A: water with 0.1% TFA, solvent B: $CH_3CN$) and lyophilized under vacuum to obtain the desired product **TPI-PN** as a purple solid (250 mg, yield: 43%). $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 9.08 (dd, $J$ = 102.2, 6.6 Hz, 4H), 8.52 (d, $J$ = 7.6 Hz, 1H), 8.11 (d, $J$ = 7.6 Hz, 1H), 8.06 (d, $J$ = 8.7 Hz, 2H), 7.39 (t, $J$ = 7.8 Hz, 4H), 7.13 (dd, $J$ = 18.2, 8.3 Hz, 8H), 4.92 (t, $J$ = 5.2 Hz, 3H), 3.61 (t, $J$ = 5.3 Hz, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 153.71, 153.13, 152.67, 148.92, 148.88, 147.02, 145.72, 136.95, 132.83, 131.15, 130.28, 129.40, 127.23, 126.89, 125.46, 124.56, 121.88, 57.88, 55.37. HRMS (EI):

calculated for $C_{31}H_{26}BrN_5S$ $[M\text{-}Br]^+$: 500.1904; found: 500.2321.

Synthesis of **TPI-PA**

**TPI-PN** (290 mg, 0.50 mmol) and triethylamine (115 μL, 1.00 mmol) were dissolved in a dry DCM (10 mL) solution. After the mixed system was cooled to 0°C, acryloyl chloride (80 μL, 1.0 mmol) was added into the system and stirred evenly. The reaction system was taken out and reacted at room temperature for 10 h. After the reaction was completely monitored by TLC, the solvent was evaporated under reduced pressure, and **TPI-PA** was purified as a purple solid by silica gel column chromatography (184 mg, yield 58%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.19 (d, $J$ = 6.7 Hz, 2H), 8.92 (d, $J$ = 6.5 Hz, 2H), 8.66 (t, 1H), 8.51 (d, $J$ = 7.6 Hz, 1H), 8.14-8.01 (m, 3H), 7.46-7.34 (m, 4H), 7.22-7.08 (m, 8H), 6.26-6.14 (m, 1H), 6.04 (dd, $J$ = 17.1, 2.1 Hz, 1H), 5.61 (dd, $J$ = 10.1, 2.1 Hz, 1H), 4.77 (t, $J$ = 5.4 Hz, 2H), 3.81 (q, $J$ = 5.6 Hz, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 165.82, 153.71, 153.12, 152.15, 148.90, 147.05, 145.45, 136.79, 132.79, 131.54, 131.13, 130.26, 129.45, 127.19, 126.50, 125.44, 124.53, 121.94, 60.22, 40.89. HRMS (EI): calculated for $C_{34}H_{28}BrN_5OS$ $[M\text{-}Br]^+$: 554.2010; found: 554.1982.

Synthesis of **TPI-CysHHC10**

CysHHC10 (30 mg, 0.02 mmol) was dissolved in 3 mL HEPES buffer ($H_2O$: DMSO = 1:1, 10 mM, pH = 8.4). 2 mL HEPES buffer containing **TPI-PA** (19 mg, 0.03 mmol) was added to the reaction system and stirred for 10 h at room temperature. After the reaction was completed, it was lyophilized under vacuum, purified by HPLC (solvent A: water containing 0.1%TFA, solvent B: $CH_3CN$), and lyophilized under vacuum to obtain purple **TPI-CysHHC10** powder (13 mg, yield 32%). $^1$H NMR (500 MHz, DMSO-$d_6$) δ 10.90-10.61 (m, 4H), 9.08 (d, $J$ = 6.3 Hz, 2H), 8.10-8.01 (m, 6H), 7.87-7.65 (m, 6H), 7.61-6.83 (m, 46H), 4.79-3.39 (m, 16H), 3.23-2.84 (m, 16H), 2.73-2.65 (m, 6H), 2.04-1.96 (m, 1H), 1.53-1.18 (m, 26H), 0.80-0.72 (m, 6H). HRMS (EI): calculated for $C_{111}H_{135}BrN_{28}O_{11}S_2$ $[M\text{-}Br]^+$: 2101.0335; found: $[M\text{-}Br]^+$: 2101.0049, $[M\text{-}Br+H]^{2+}$: 1051.0133, $[M\text{-}Br+2H]^{3+}$: 701.0117, $[M\text{-}Br+3H]^{4+}$: 526.0113, $[M\text{-}Br+4H]^{5+}$: 421.0105.

*1.4 AIE property*

The AIE property of **TPI-CysHHC10** was demonstrated by studying its fluorescence behavior in a mixture of $H_2O$ and 1,4-dioxane with different volume ratios. Each group contained 10 μM AIEgens and the FL spectra were collected under 500 nm excitation.

*1.5 Bacteria culturing*

Gram-positive bacteria including *Staphylococcus aureus* (*S. aureus*), and *methicillin-resistant Staphylococcus aureus* (*MRSA*) and gram-negative bacteria including *Escherichia coli* (*E. coli*), *multidrug-resistant Escherichia coli* (*MDR E. coli*), and *Pseudomonas aeruginosa* (*P. aeruginosa*) were used for the antimicrobial tests. Different bacteria were grown on an agar plate overnight based on the streaking method to afford a single colony. Then, 3-5 colonies isolated from the agar plate were cultured in an appropriate volume of broth culture for several hours at 37°C with a shaking speed (180 rpm), respectively. The optical density (OD) at 600 nm was used to monitor the concentration of bacteria. $OD_{600}$ of the bacteria solution was adjusted to 1.0, and then the bacterial experiments were performed.

*1.6 SEM assay*

The bacterial suspensions (*E. coli* and *MRSA*) of $OD_{600}$ = 1.0 were taken from 1 mL and 10 μL **TPI-CysHHC10** (1 mM) was added to it. The Light(–) group was incubated with 37°C under the condition of dark for 2 hours, and the Light(+) group was incubated with 30 min under the dark condition of 37°C, then 15 min was irradiated with white light, and was incubated for another 75 min under the dark condition of 37°C. Subsequently, the bacterial samples were fixed with 2.5% glutaraldehyde, dehydrated, sputtered and observed by scanning electron microscope.

*1.7 Zeta potential measurement*

The bacterial suspensions (*E. coli* and *S. aureus*) with $OD_{600}$ = 1.0 of 1 mL were added with 10 μL **TPI-CysHHC10** (1 mM) and incubated in 37°C for 10 min. Untreated bacteria were also incubated under the same conditions. Then the zeta potential was measured by Zeta potential analyzer.

*1.8 Antibiofilm assay*

*MRSA* in logarithmic growth phase was diluted to $3 \times 10^6$ CFU/mL in LB medium as working suspension, and then added to confocal dishes (2 mL for each dish) or 24-well plate. Then divided into control group, non-light group and light group. 20 μL PBS was added to the control group, and 20 μL **TPI-CysHHC10** (1 mM) was added to each dish in the non-light group and light group. Then the light group was incubated in the dark for 1 h and then irradiated with 15min under white light.

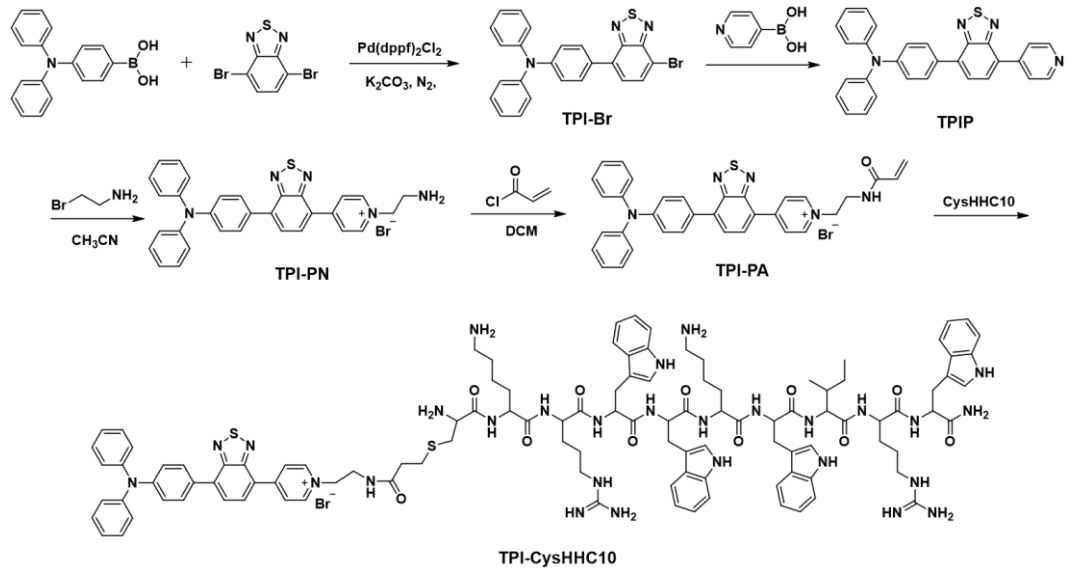
All the confocal was incubated dishes under 37°C for 24 h, 48 h and 72 h, then the culture medium was gently removed and washed with PBS, and the *MRSA* biofilm was stained with Hoechst 33342 and YO-PRO-1. After 10 min, the biofilm was imaged by CLSM.

In the mature biofilm destruction experiment, *MRSA* in logarithmic growth phase was diluted with LB medium to $3 \times 10^6$ CFU/mL and added to confocal dishes (2 mL for each dish). All confocal dishes were incubated under 37°C for 24 h, 48 h and 72 h to form mature biofilms at different stages. They were divided into control group, non-light group and light group. 20 μL PBS was added to the control group, and 20 μL **TPI-CysHHC10** (1mM) was added to each dish in non-light group and light group. The non-light group was incubated in the dark for 2 h, and then the light group was exposed to white light for 1 h and then incubated in the dark group. The culture medium was gently removed and washed with PBS, and the *MRSA* biofilm was stained with Hoechst33342 and YO-PRO-1. After 10min, the biofilm was imaged at different wavelengths by CLSM.

*1.9 Aggregation experiments*

Bacterial suspensions (1.5 mL) of (*E. coli*, *MDR E. coli*, *P. aeruginosa*, *S. aureus*, and *MRSA*) with $OD_{600} \approx 2.0$ was added to an ultraviolet cuvette, and measure and record $OD_{600}$. Then, added **TPI-CysHHC10**, HHC10, or TPI-PA (30 μL, 1 mM) to each bacterial suspension. The $OD_{600}$ quickly recorded (t = 0) and the change in $OD_{600}$ was monitored with time.

## 2. Supplemental Figures



**Scheme S1**. The synthesis of **TPI-CysHHC10**.

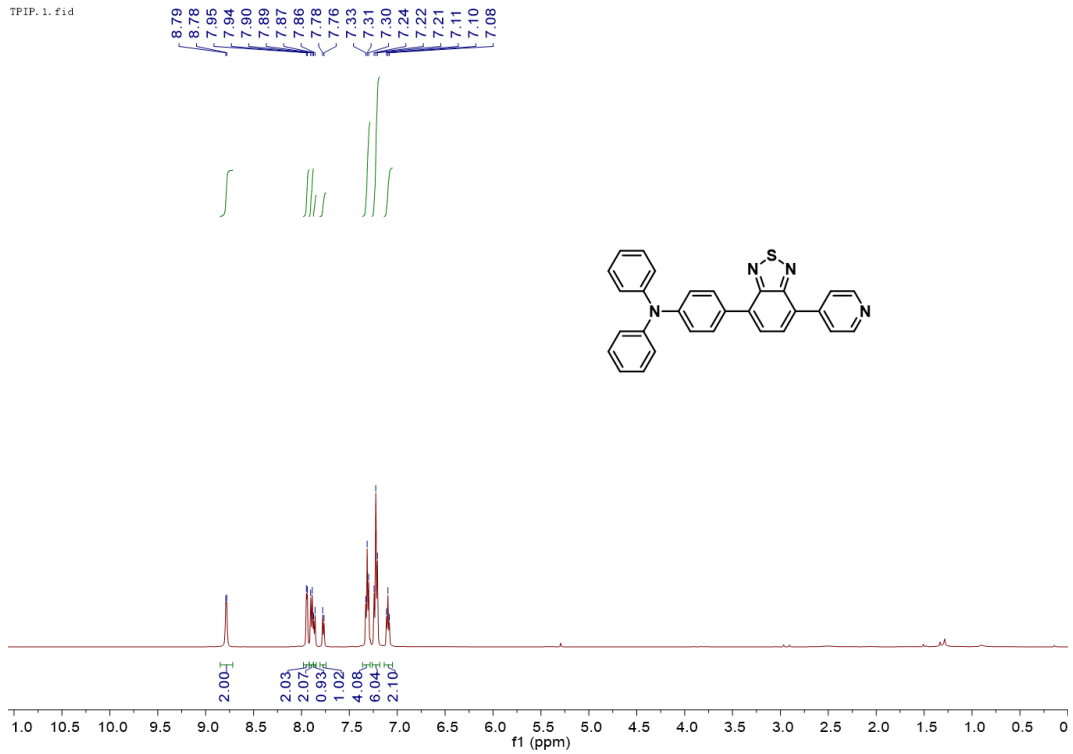



**Figure S1**. $^{1}$H-NMR spectrum of **TPIP**.

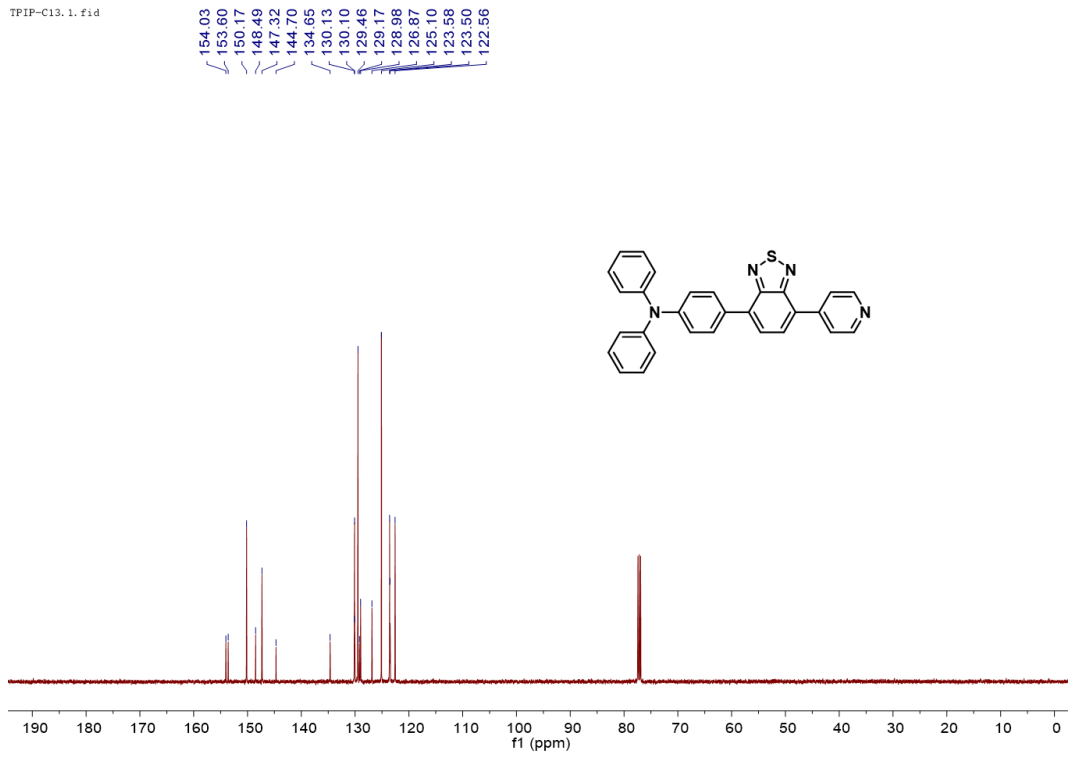



**Figure S2.** $^{13}$C-NMR spectrum of **TPIP**.

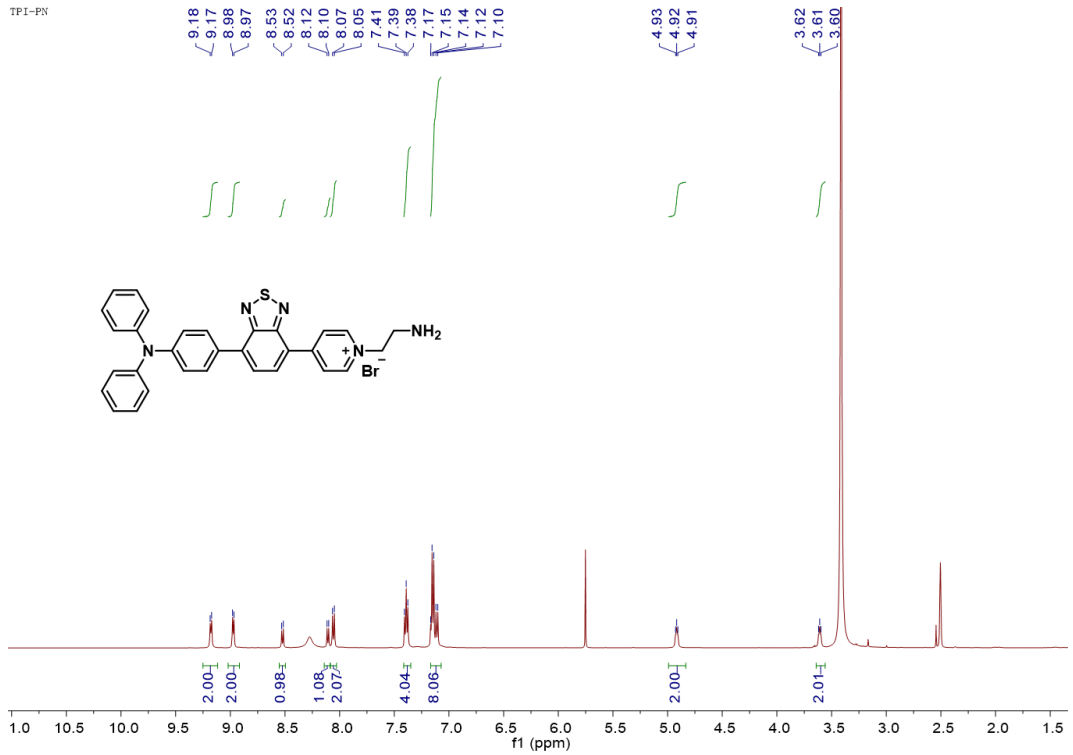



**Figure S3.** [1]H-NMR spectrum of **TPI-PN**.

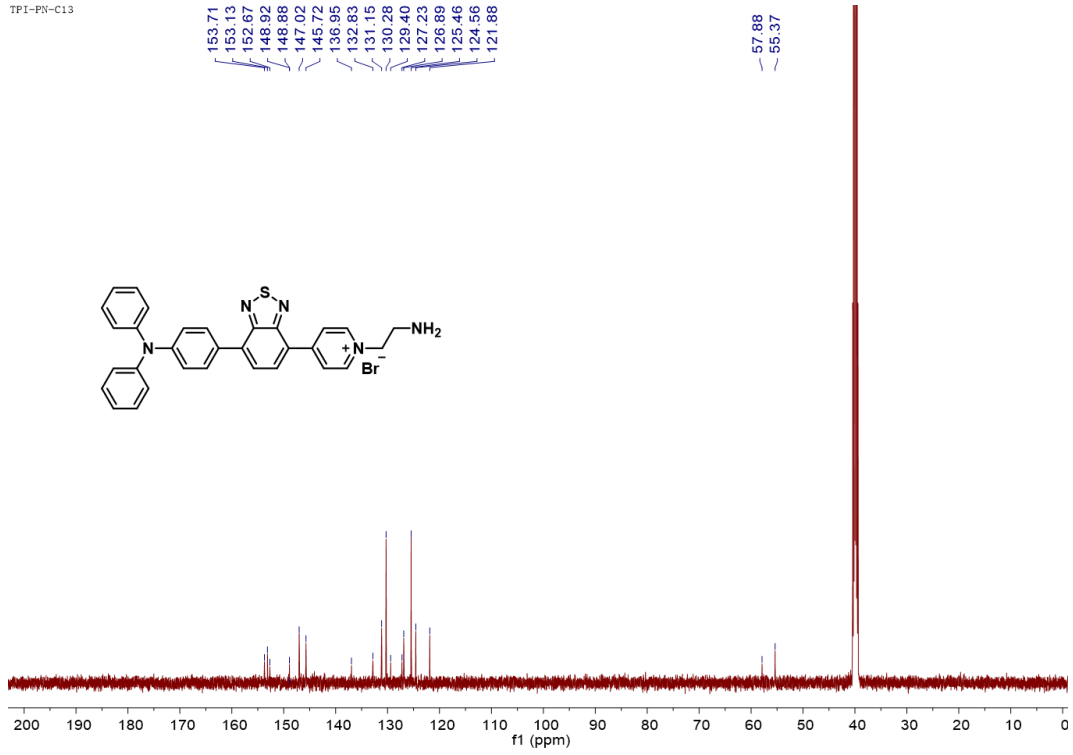



**Figure S4.** [13]C-NMR spectrum of **TPI-PN**.

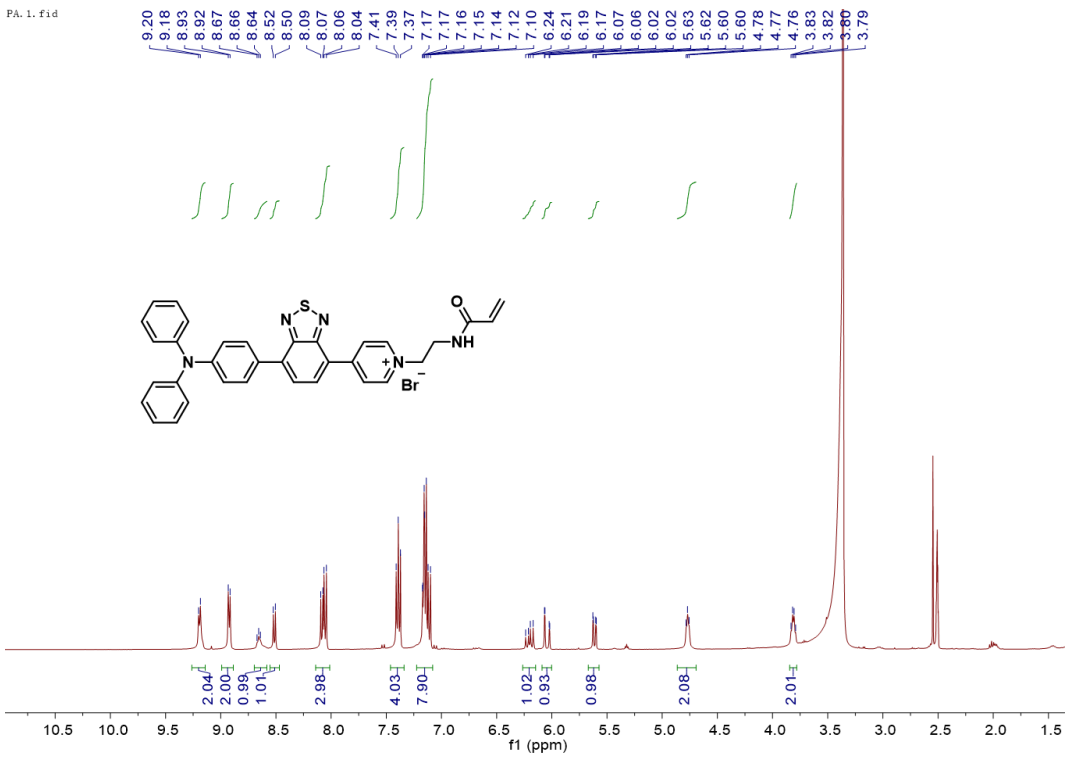



**Figure S5.** $^{1}$H-NMR spectrum of **TPI-PA**.

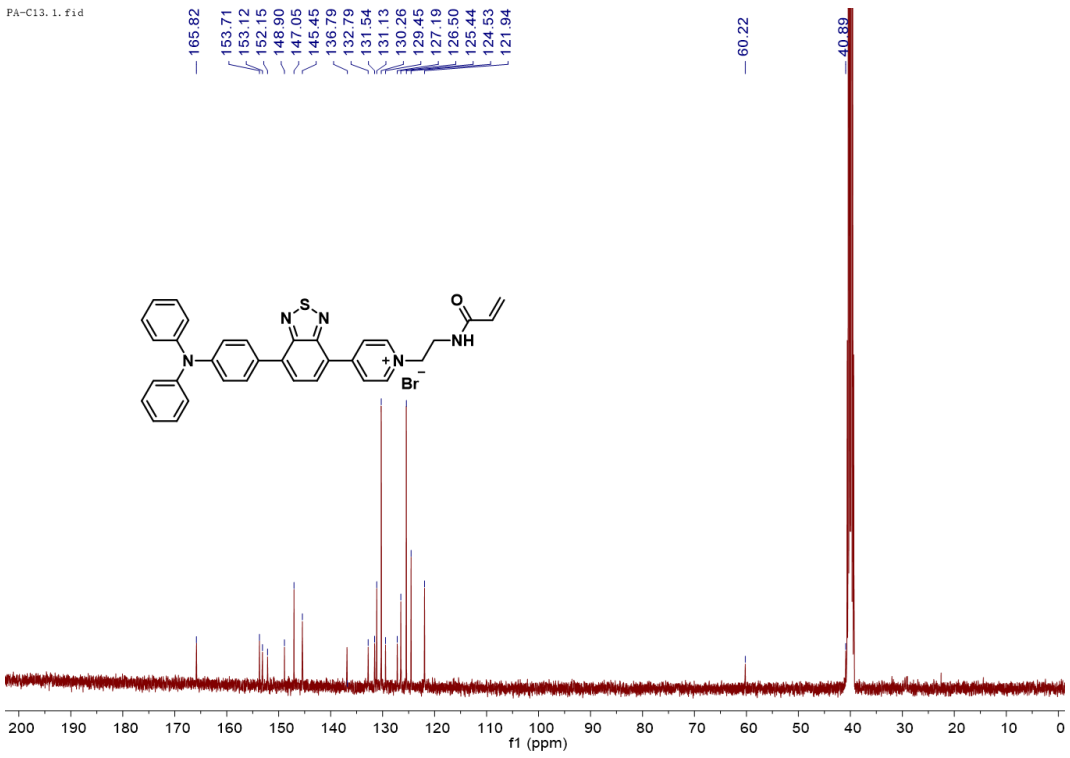



**Figure S6.** $^{13}$C-NMR spectrum of **TPI-PA**.

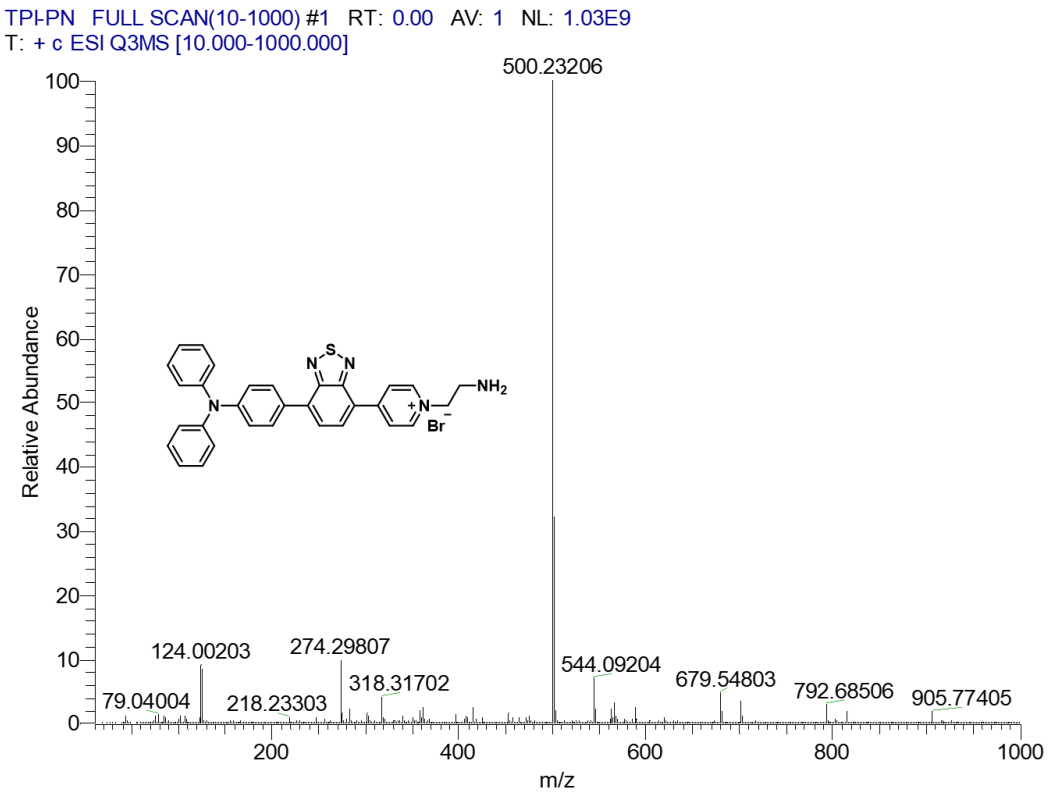



**Figure S7.** HR-MS spectrum of **TPI-PN**.

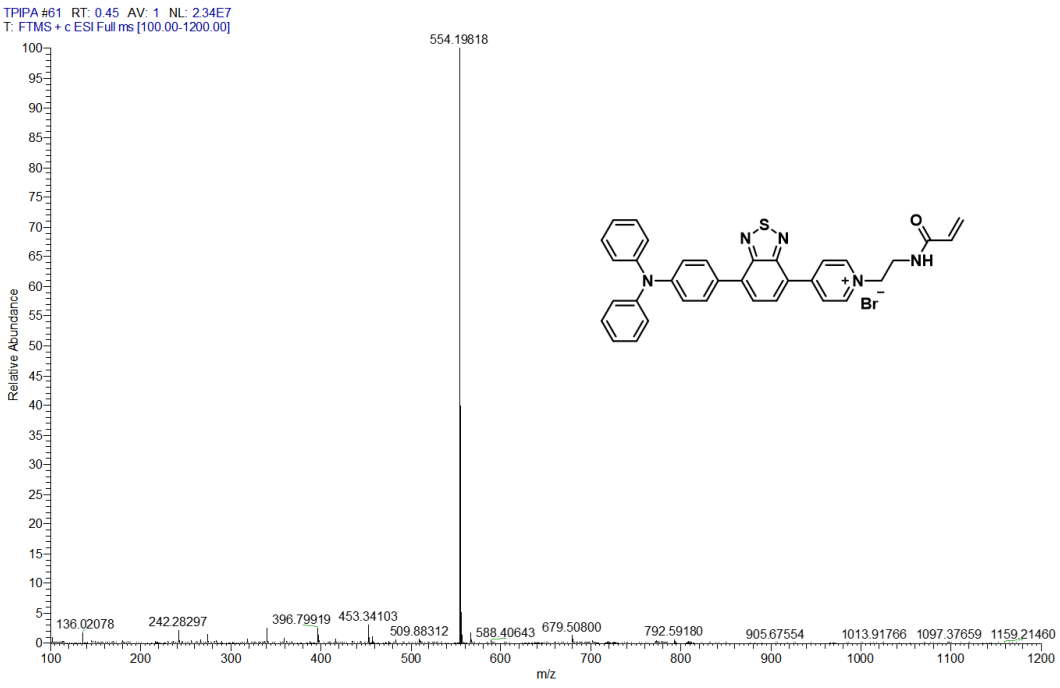



**Figure S8.** HR-MS spectrum of **TPI-PA**.

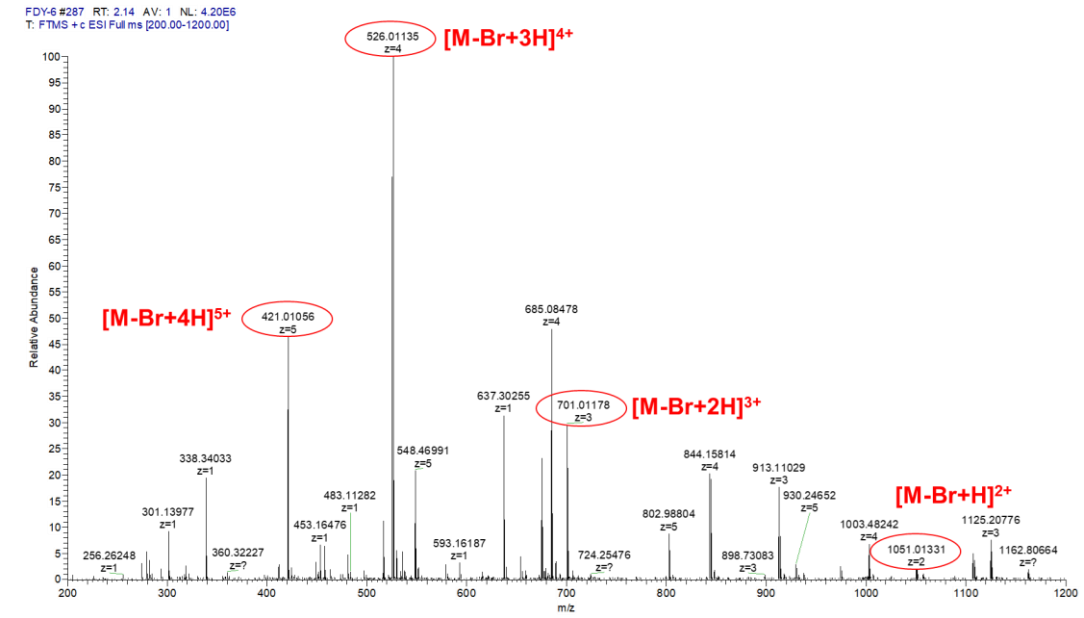



**Figure S9.** HR-MS spectrum of **TPI-CysHHC10**.

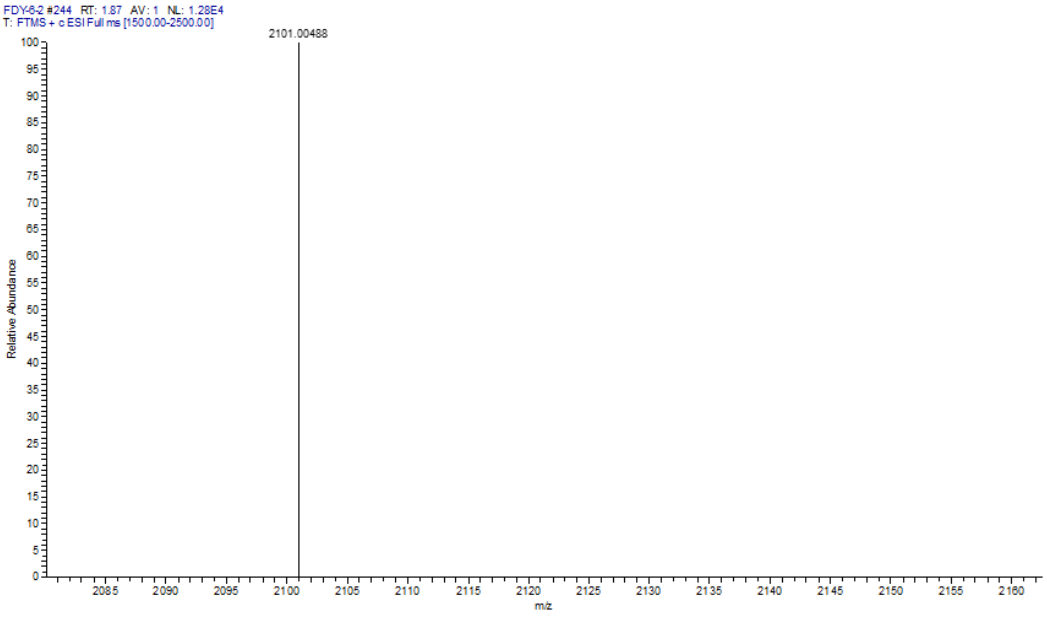



**Figure S10.** HR-MS spectrum of **TPI-CysHHC10**.

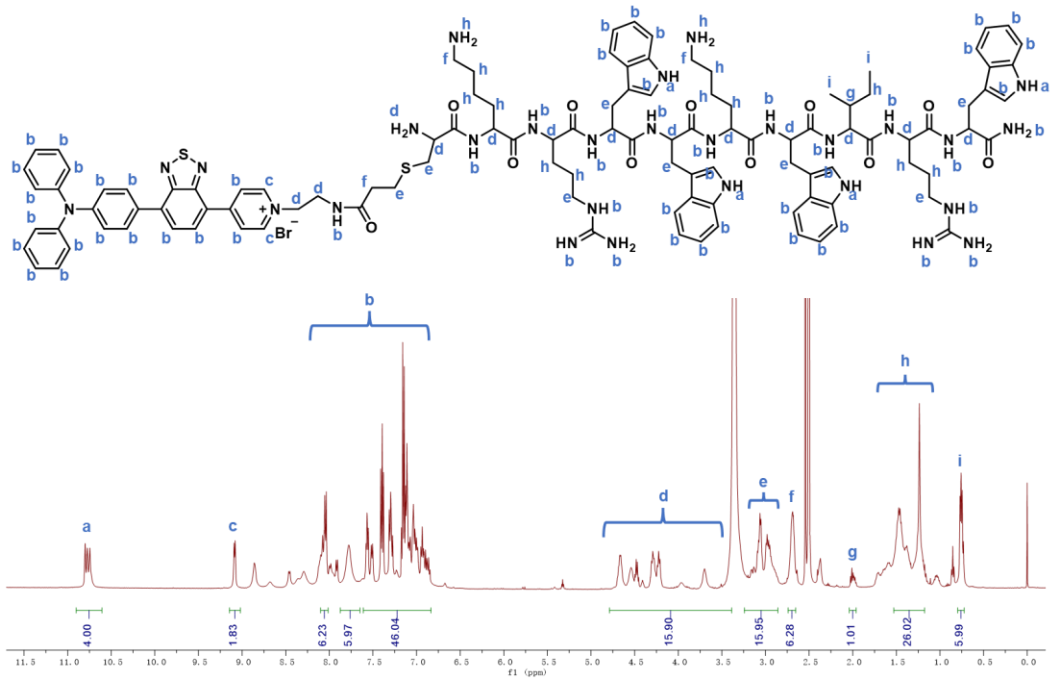


**Figure S11.** [1]H-NMR spectrum of **TPI-CysHHC10**.

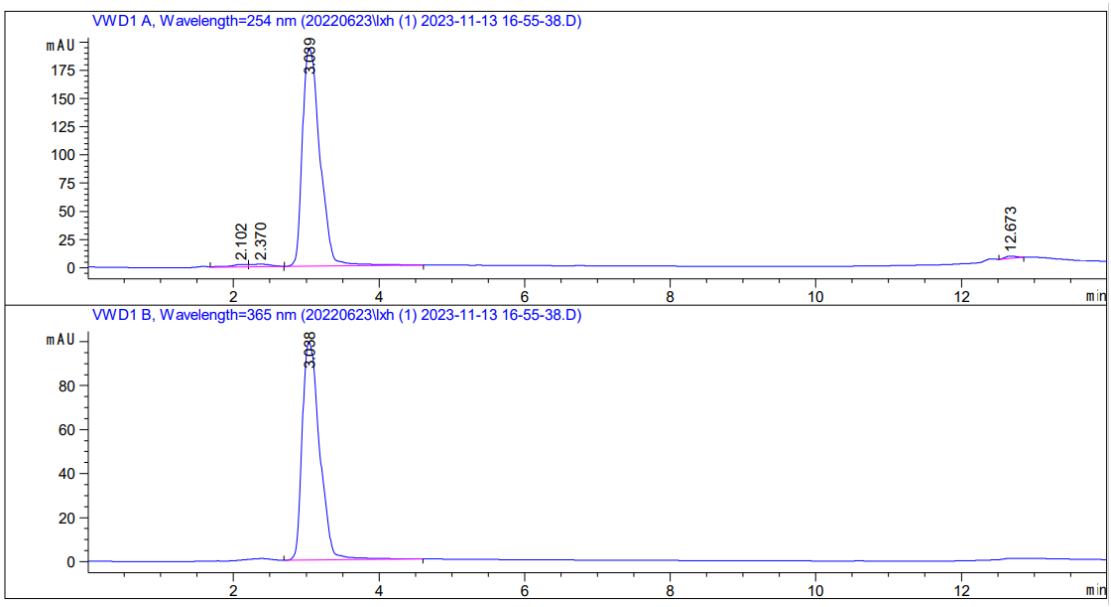



**Figure S12.** HPLC spectrum of purified **TPI-CysHHC10**.

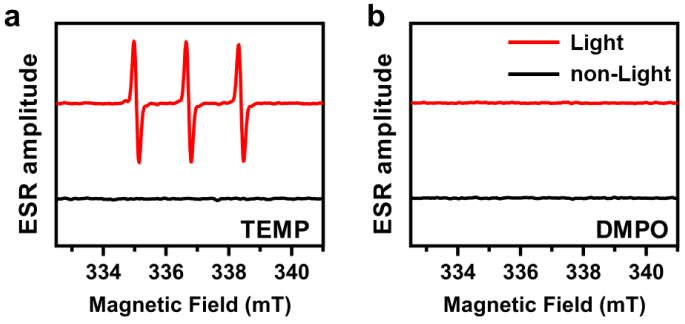



**Figure S13.** (a) ESR spectra of $^1O_2$ generated by **TPI-CysHHC10** before (black line) and after (red line) light irradiation for 15 min. (b) ESR spectra of $O^{2-\cdot}$ and · OH generated by **TPI-CysHHC10** before and after light irradiation for 15 min.

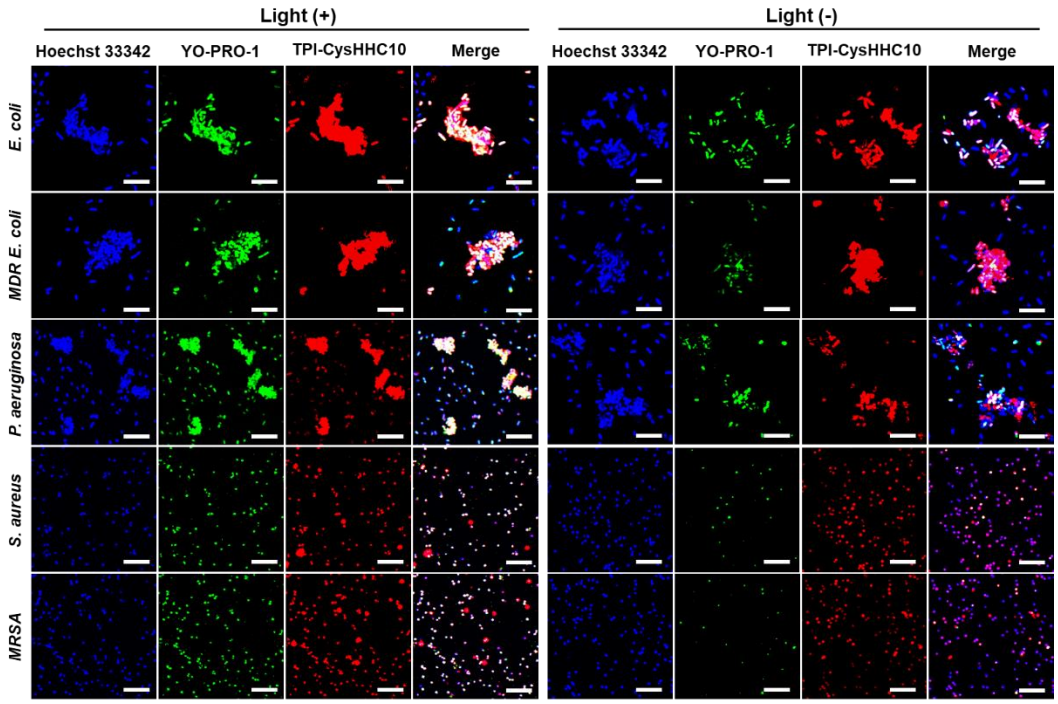



**Figure S14.** Dead-alive staining assays. CLSM images of *E. coli*, *MDR E. coli*, *P. aeruginosa*, *S. aureus*, and *MRSA* after incubation with **TPI-CysHHC10** (5 μM) (red fluorescence) and then without or with light irradiation for 15 min. Then co-staining with Hoechst 33342 (blue fluorescence, a nucleic acid dye for all bacteria) and YO-PRO-1 (green fluorescence, a dye for dead bacteria). Light intensity: 60 mW/cm$^2$. Scale bar: 10 μm.

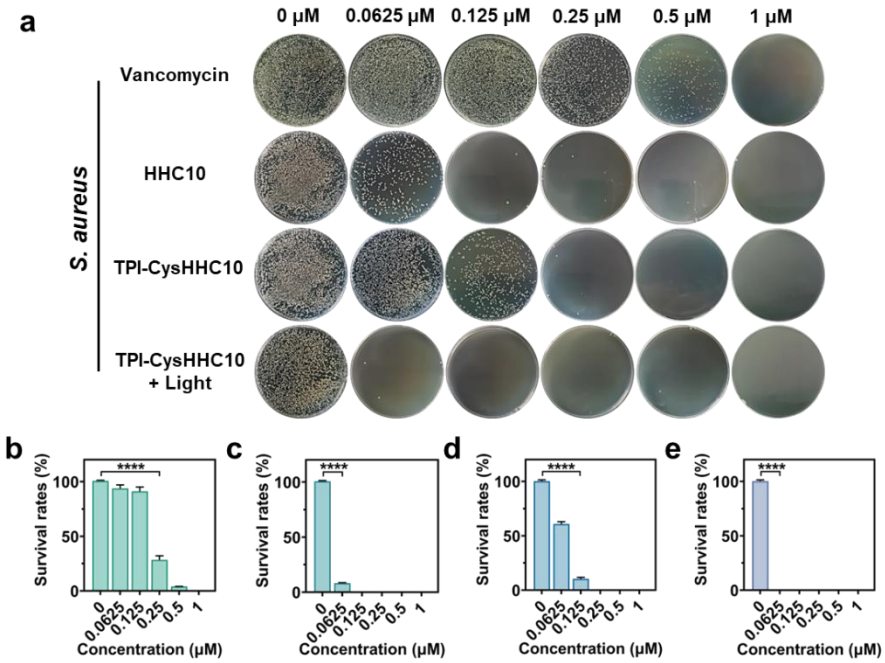



**Figure S15.** (a) Plate photographs of *S. aureus* LB plates treated with vancomycin, HHC10, **TPI-CysHHC10**, and **TPI-CysHHC10** + Light at different concentration. (b-e) Quantized survival rates of *S. aureus via* colony counting on LB agar plates treated with vancomycin (b), HHC10 (c), **TPI-CysHHC10** (d), and **TPI-CysHHC10** + Light (e). Data are mean ± SD (n = 3). ****$P < 0.0001$.

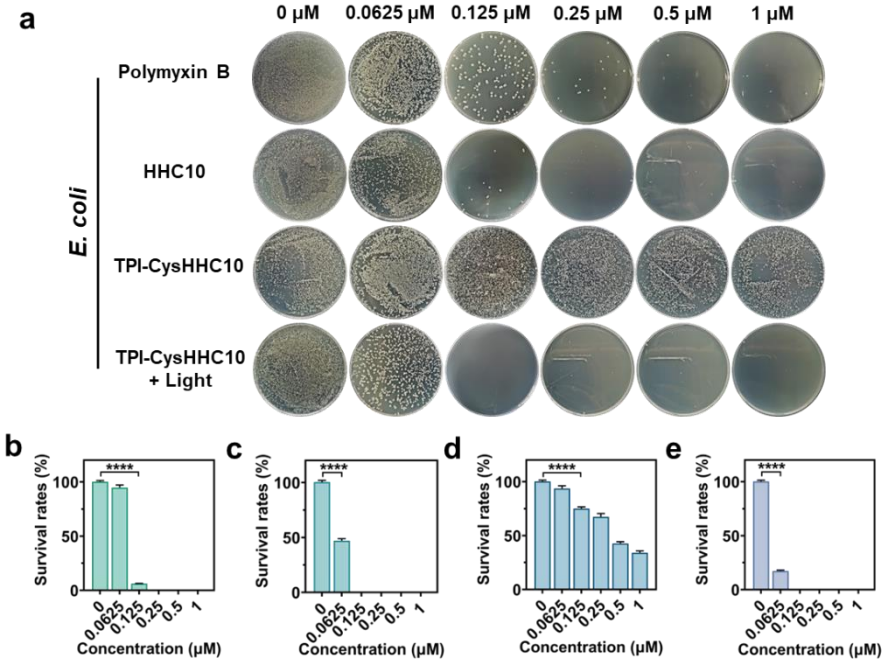



**Figure S16.** (a) Plate photographs of *E. coli* LB plates treated with vancomycin, HHC10, **TPI-CysHHC10**, and **TPI-CysHHC10** + Light at different concentration. (b-e) Quantized survival rates

of *E. coli via* colony counting on LB agar plates treated with polymyxin B (b), HHC10 (c), **TPI-CysHHC10** (d), and **TPI-CysHHC10** + Light (e). Data are mean ± SD (n = 3). ****$P < 0.0001$.

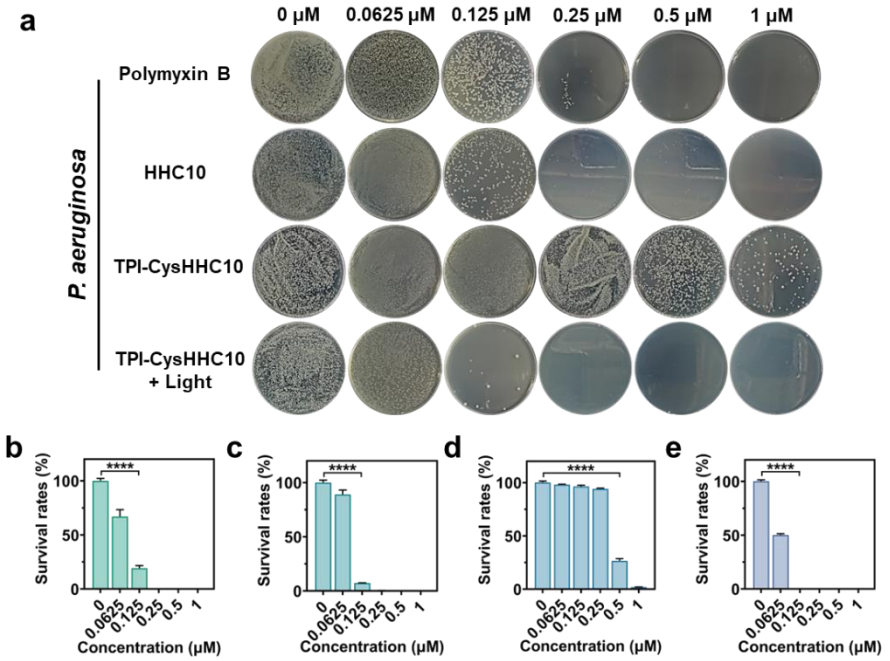



**Figure S17.** (a) Plate photographs of *P. aeruginosa* LB plates treated with vancomycin, HHC10, **TPI-CysHHC10**, and **TPI-CysHHC10** + Light at different concentration. (b-e) Quantized survival rates of *P. aeruginosa via* colony counting on LB agar plates treated with polymyxin B (b), HHC10 (c), **TPI-CysHHC10** (d), and **TPI-CysHHC10** + Light (e). Data are mean ± SD (n = 3). ****$P < 0.0001$.

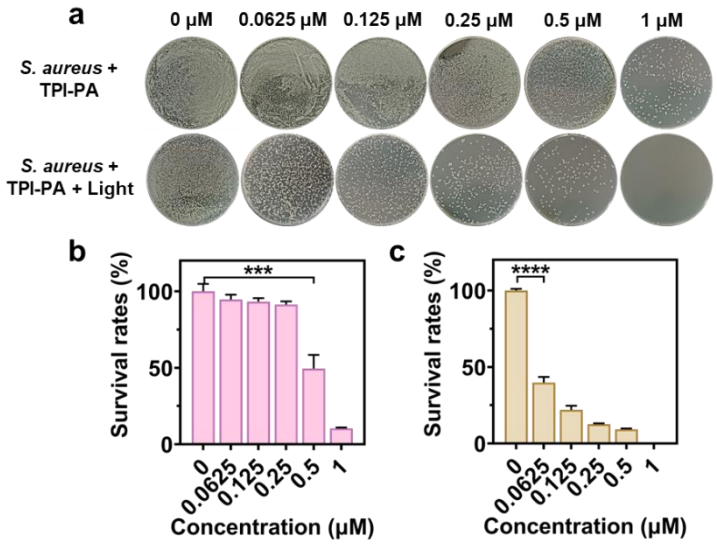



**Figure S18.** (a) Plate photographs of *S. aureus* LB plates treated with **TPI-PA** and **TPI-PA** + Light at different concentration. (b-c) Quantized survival rates of *S. aureus via* colony counting on LB agar plates treated with **TPI-PA** (b) and **TPI-PA** + Light (c). Data are mean ± SD (n = 3). ***$P < 0.001$, ****$P < 0.0001$.

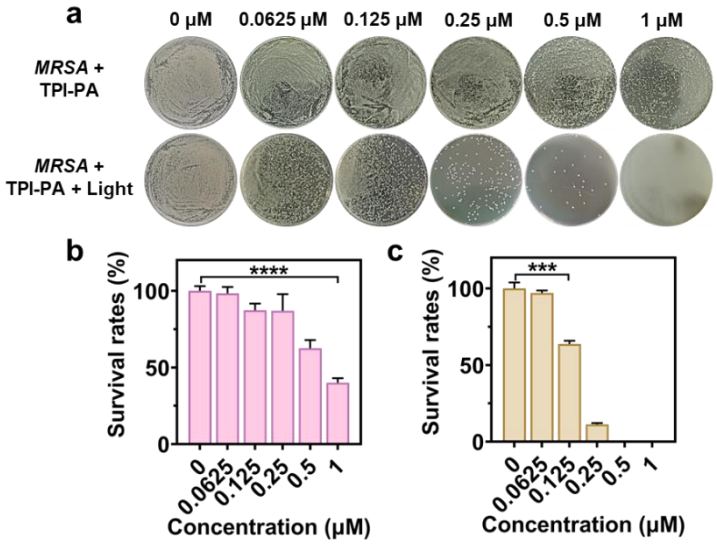



**Figure S19.** (a) Plate photographs of *MRSA* LB plates treated with **TPI-PA** and **TPI-PA** + Light at different concentration. (b-c) Quantized survival rates of *MRSA via* colony counting on LB agar plates treated with **TPI-PA** (b) and **TPI-PA** + Light (c). Data are mean ± SD (n = 3). ***$P < 0.001$, ****$P < 0.0001$.

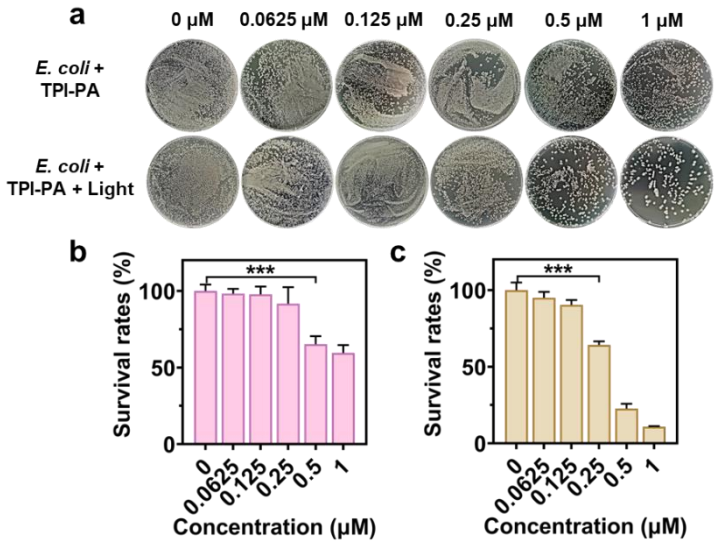



**Figure S20.** (a) Plate photographs of *E. coli* LB plates treated with **TPI-PA** and **TPI-PA** + Light at different concentration. (b-c) Quantized survival rates of *E. coli via* colony counting on LB agar plates treated with **TPI-PA** (b) and **TPI-PA** + Light (c). Data are mean ± SD (n = 3). ****P* < 0.001.

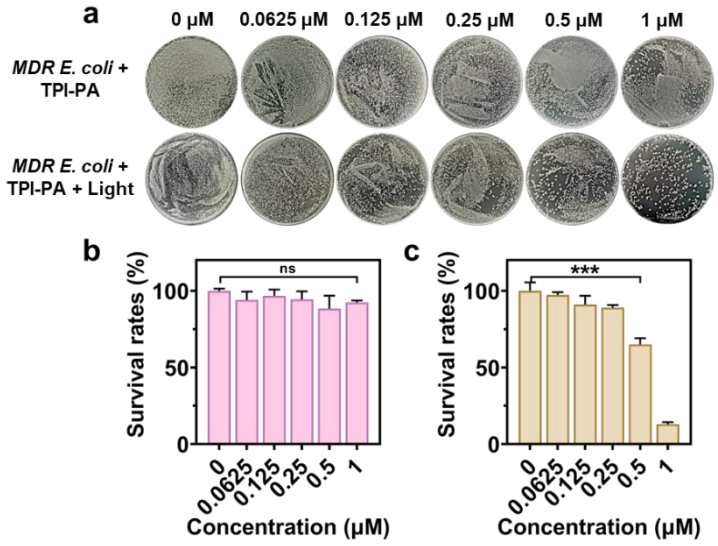



**Figure S21.** (a) Plate photographs of *MDR E. coli* LB plates treated with **TPI-PA** and **TPI-PA** + Light at different concentration. (b-c) Quantized survival rates of *MDR E. coli via* colony counting on LB agar plates treated with **TPI-PA** (b) and **TPI-PA** + Light (c). Data are mean ± SD (n = 3). ****P* < 0.001, ns: no significance.

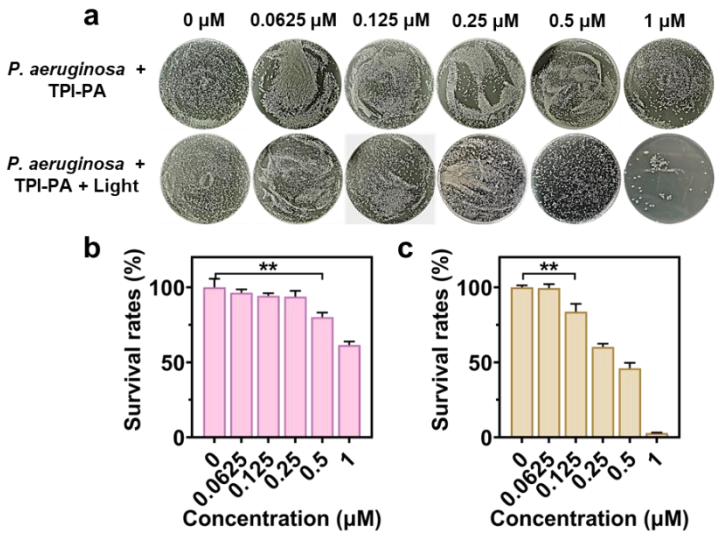



**Figure S22.** (a) Plate photographs of *P. aeruginosa* LB plates treated with **TPI-PA** and **TPI-PA** + Light at different concentration. (b-c) Quantized survival rates of *P. aeruginosa via* colony counting on LB agar plates treated with **TPI-PA** (b) and **TPI-PA** + Light (c). Data are mean ± SD (n = 3). $^{**}P < 0.01$.

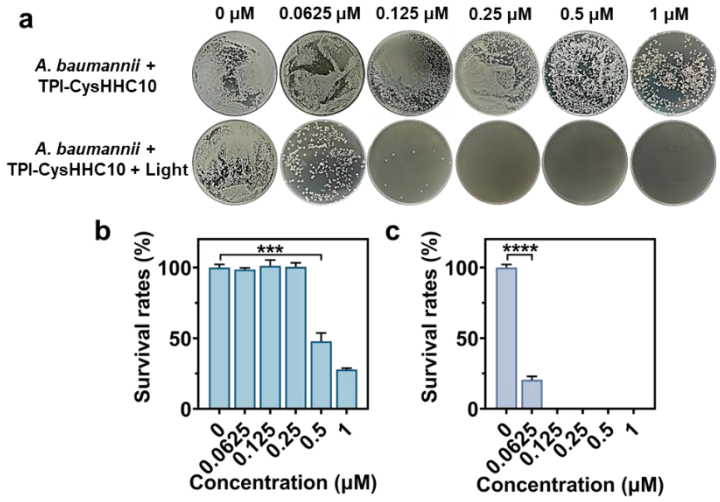



**Figure S23.** (a) Plate photographs of *A. baumannii* LB plates treated with **TPI-CysHHC10** and **TPI-CysHHC10** + Light at different concentration. (b-c) Quantized survival rates of *A. baumannii via* colony counting on LB agar plates treated with **TPI-CysHHC10** (b) and **TPI-CysHHC10** + Light (c). Data are mean ± SD (n = 3). $^{***}P < 0.001$, $^{****}P < 0.0001$.

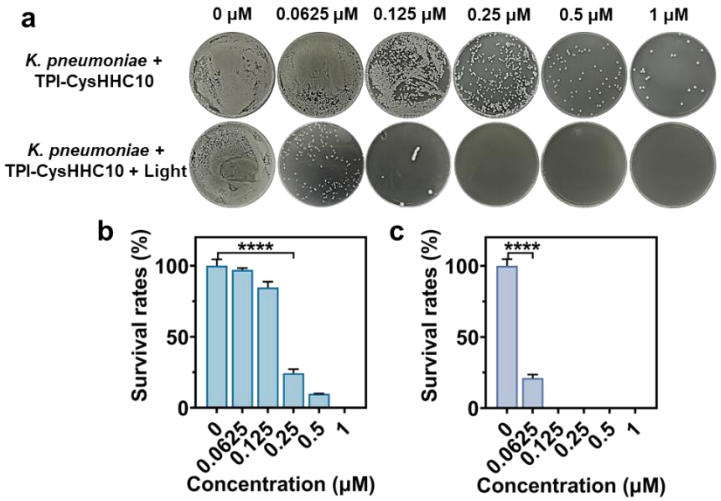



**Figure S24.** (a) Plate photographs of *K. pneumoniae* LB plates treated with **TPI-CysHHC10** and **TPI-CysHHC10** + Light at different concentration. (b-c) Quantized survival rates of *K. pneumoniae via* colony counting on LB agar plates treated with **TPI-CysHHC10** (b) and **TPI-CysHHC10** + Light (c). Data are mean ± SD (n = 3). ****$P < 0.0001$.

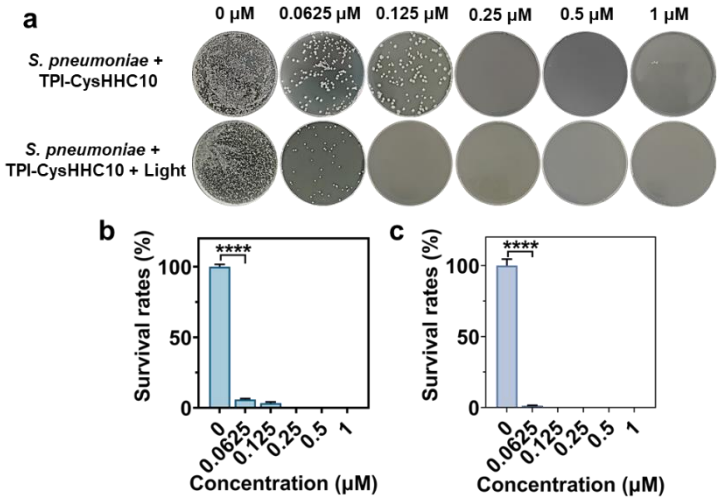



**Figure S25.** (a) Plate photographs of *S. pneumoniae* LB plates treated with **TPI-CysHHC10** and **TPI-CysHHC10** + Light at different concentration. (b-c) Quantized survival rates of *S. pneumoniae via* colony counting on LB agar plates treated with **TPI-CysHHC10** (b) and **TPI-CysHHC10** + Light (c). Data are mean ± SD (n = 3). ****$P < 0.0001$.

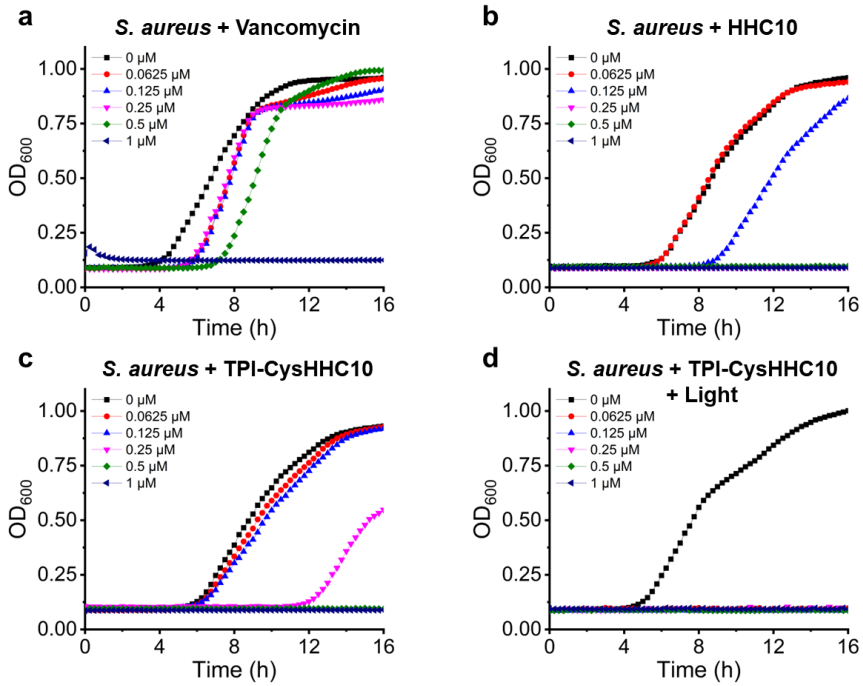



**Figure S26**. Growth inhibition curve of *S. aureus* with the treatment of vancomycin (a), HHC10 (b), **TPI-CysHHC10** (c), and **TPI-CysHHC10** + Light (d) at different concentrations.

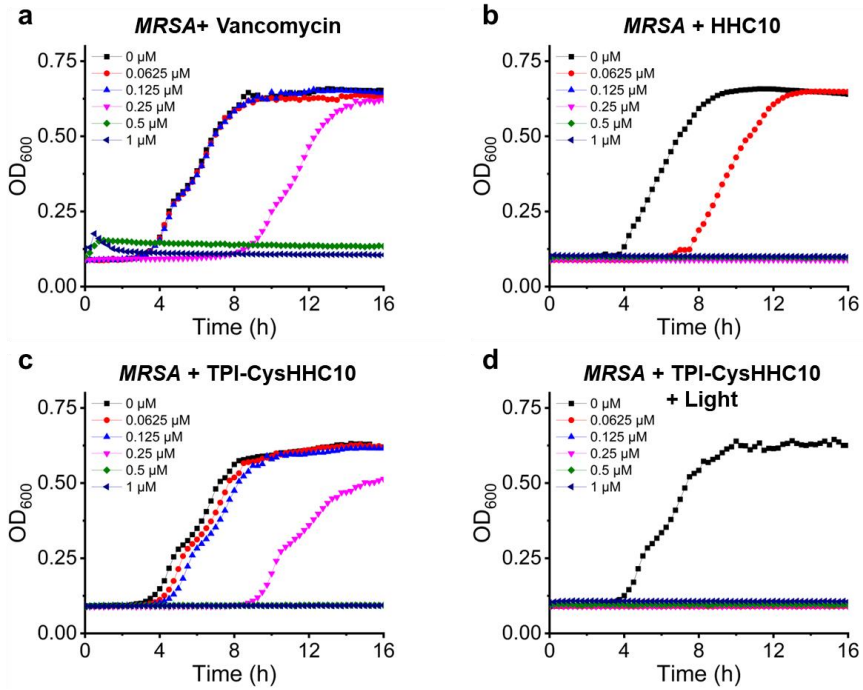



**Figure S27**. Growth inhibition curve of *MRSA* with the treatment of vancomycin (a), HHC10 (b), **TPI-CysHHC10** (c), and **TPI-CysHHC10** + Light (d) at different concentrations.

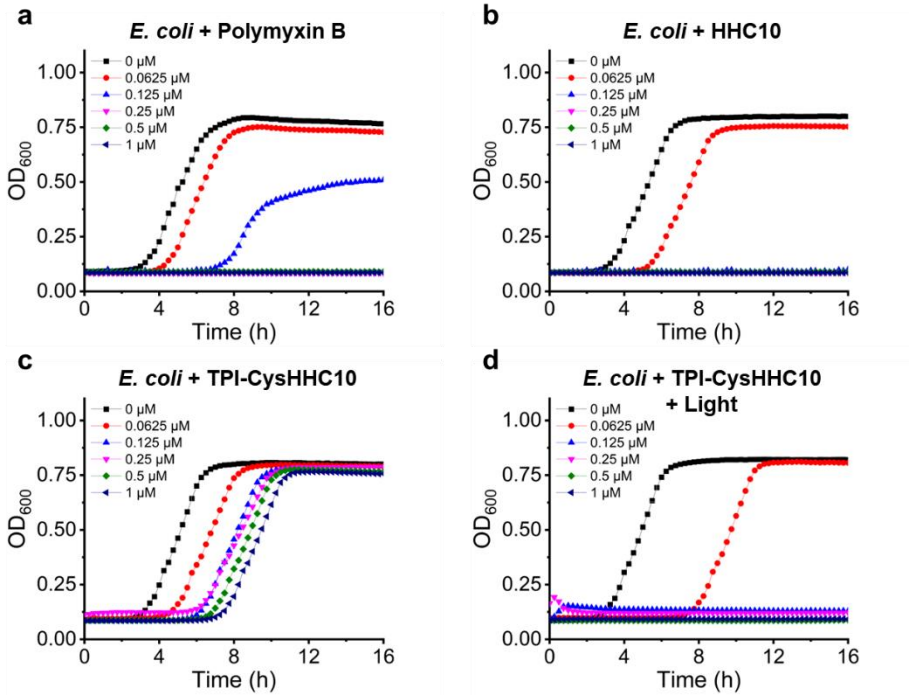



**Figure S28**. Growth inhibition curve of *E. coli* with the treatment of polymyxin B (a), HHC10 (b), **TPI-CysHHC10** (c), and **TPI-CysHHC10** + Light (d) at different concentrations.

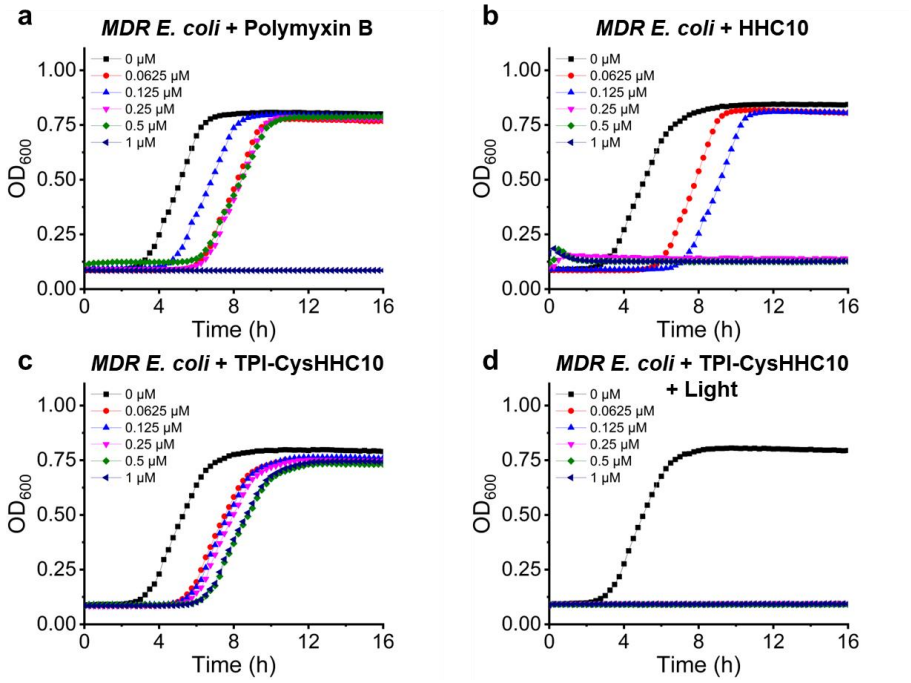



**Figure S29**. Growth inhibition curve of *MDR E. coli* with the treatment of polymyxin B (a), HHC10 (b), **TPI-CysHHC10** (c), and **TPI-CysHHC10** + Light (d) at different concentrations.

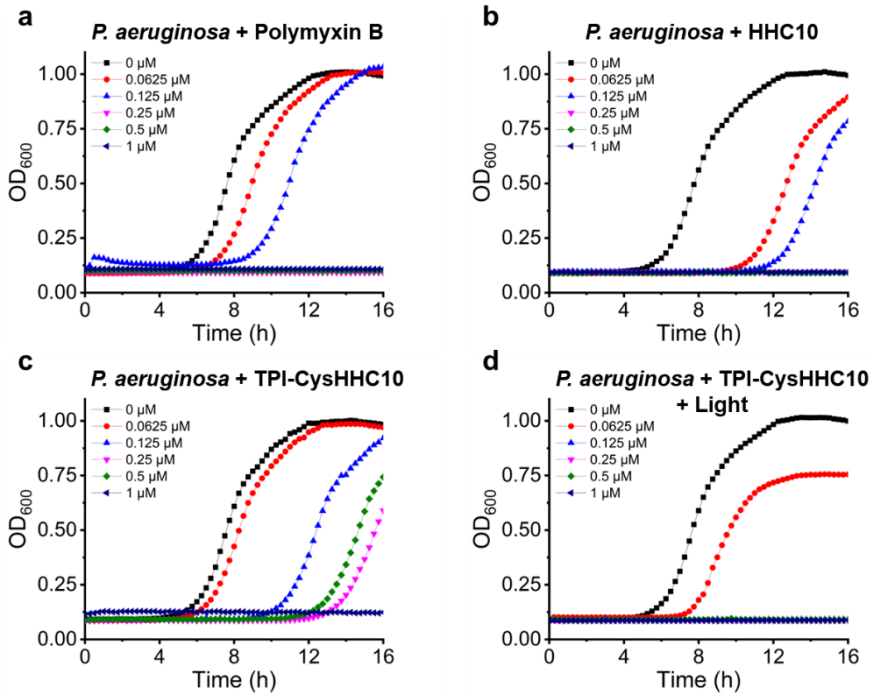



**Figure S30**. Growth inhibition curve of *P. aeruginosa* with the treatment of polymyxin B (a), HHC10 (b), **TPI-CysHHC10** (c), and **TPI-CysHHC10** + Light (d) at different concentrations.

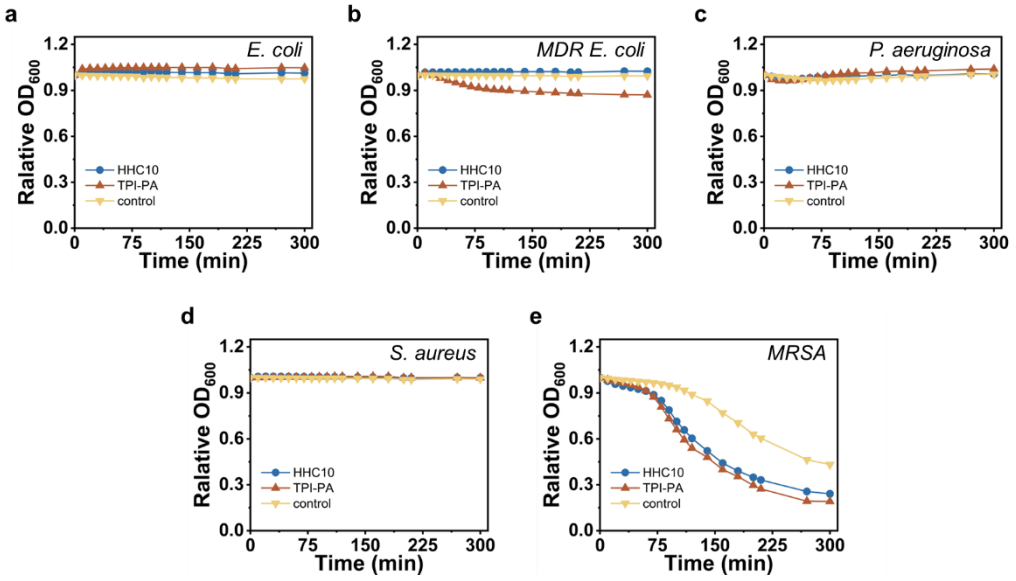



**Figure S31**. Aggregation as measured by turbidimetry for *E. coli, MDR E. coli, P. aeruginosa, S. aureus, and MRSA* in the absence and presence of HHC10 and TPI-PA.

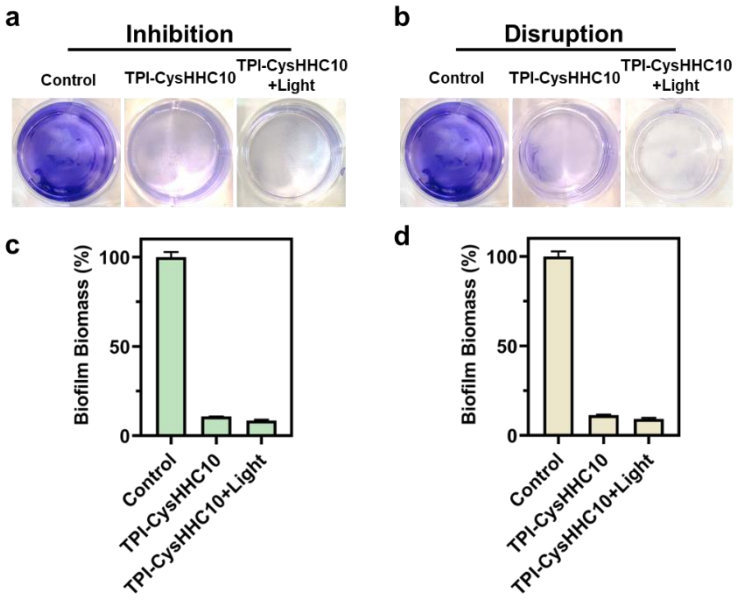



**Figure S32.** Investigation of biofilm (24 h) inhibition/disruption *via* crystal violet staining method. (a-b) Images of *MRSA* stained by crystal violet after different treatments. (c-d) Biofilm biomass of *MRSA* after different treatments.

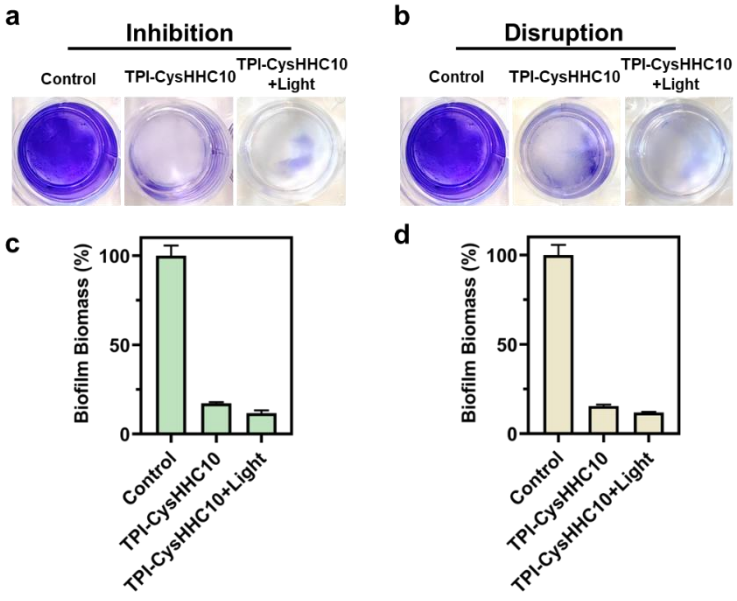



**Figure S33.** Investigation of biofilm (48 h) inhibition/disruption *via* crystal violet staining method. (a-b) Images of *MRSA* stained by crystal violet after different treatments. (c-d) Biofilm biomass of *MRSA* after different treatments.

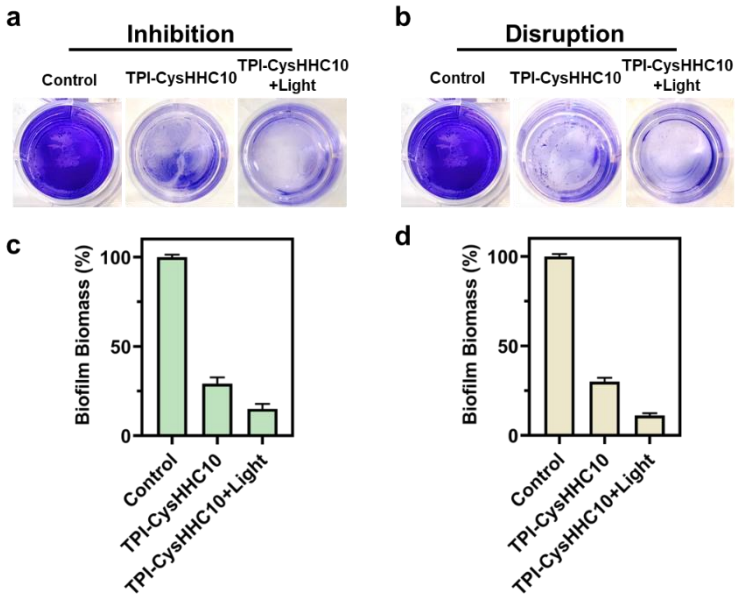



**Figure S34.** Investigation of biofilm (72 h) inhibition/disruption *via* crystal violet staining method. (a-b) Images of *MRSA* stained by crystal violet after different treatments. (c-d) Biofilm biomass of *MRSA* after different treatments.

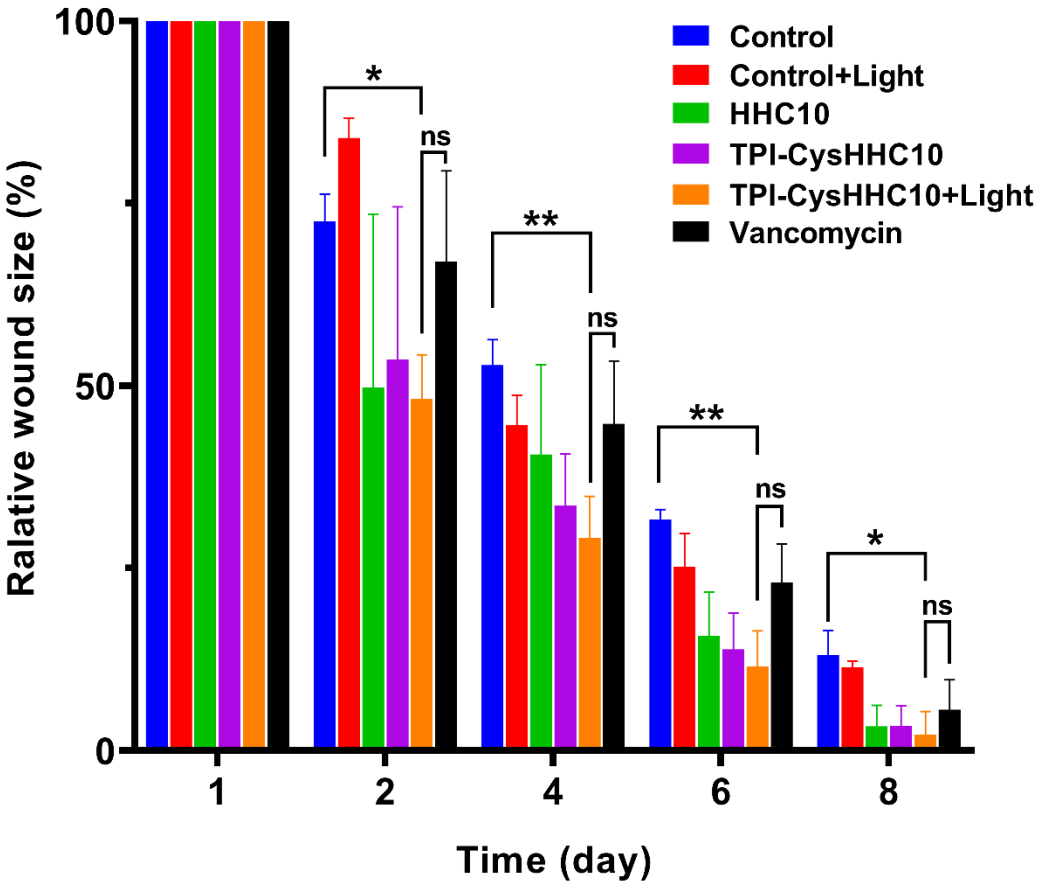



**Figure S35.** Relative wound area of mice with different treatment on day 1, day 2, day 4, day 6, and day 8. Data are mean ± SD (n = 4). $^{**}P < 0.01$, $^{*}P < 0.1$, ns: no significance.

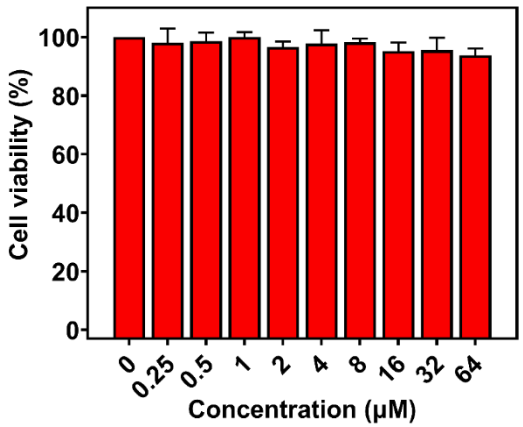



**Figure S36.** Cell viability of HeLa cells treated with **TPI-CysHHC10** for 24 h determined by MTT assay (n = 3).

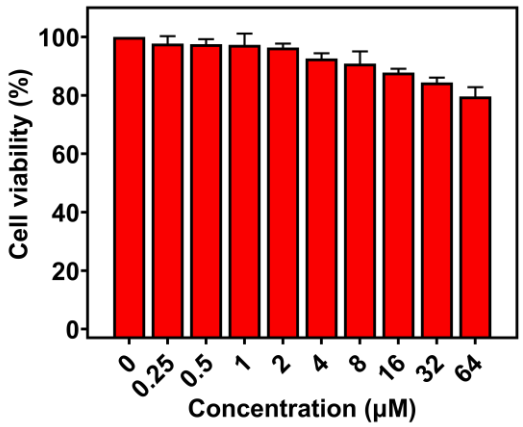



**Figure S37.** Cell viability of HeLa cells treated with **TPI-CysHHC10** for 24 h after 15 min irradiation determined by MTT assay (n = 3).

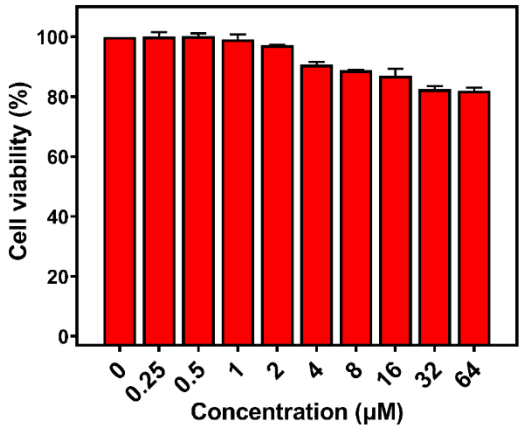



**Figure S38.** Cell viability of HeLa cells treated with **HHC10** for 24 h determined by MTT assay (n = 3).

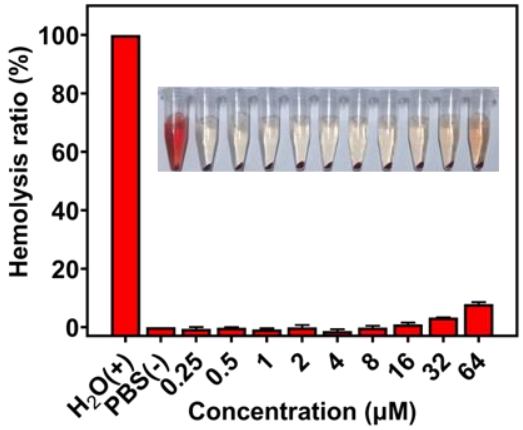



**Figure S39.** Hemolysis ratio of **TPI-CysHHC10** at different concentrations (n = 6).

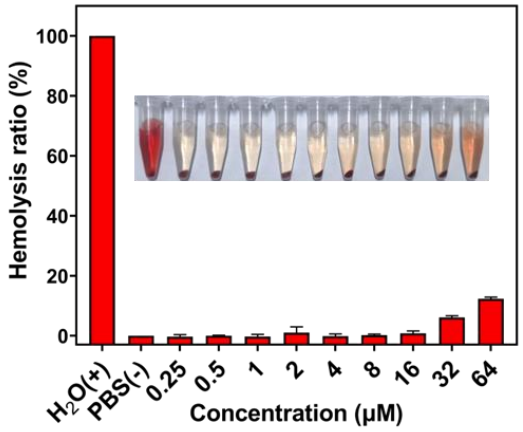



**Figure S40.** Hemolysis ratio of **HHC10** at different concentrations (n = 6).

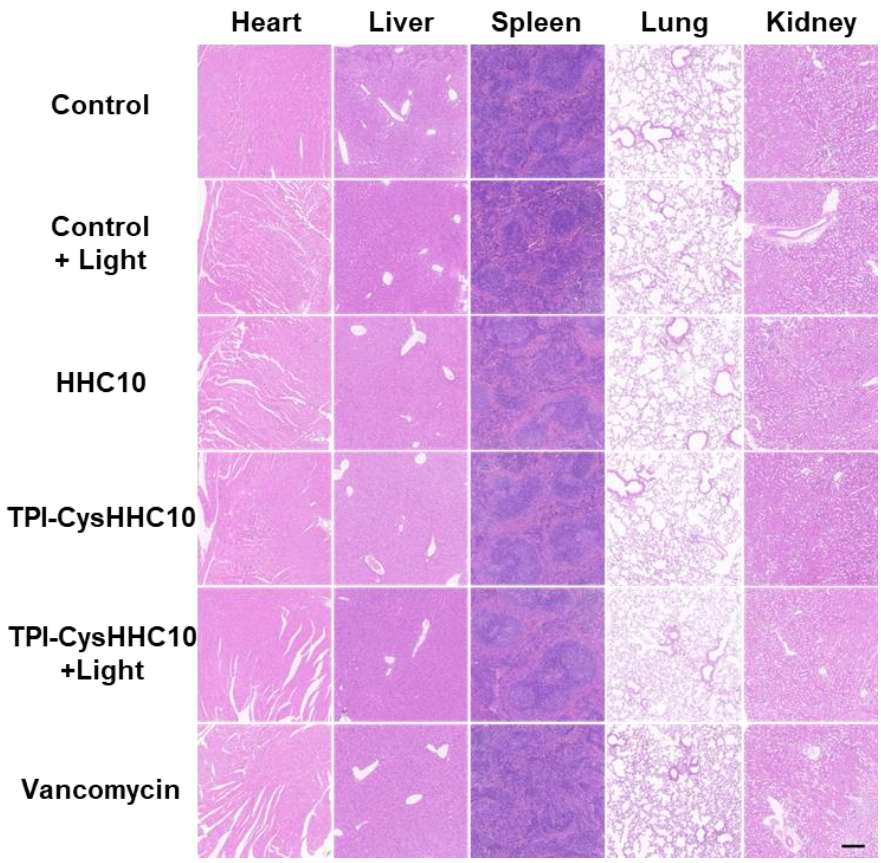



**Figure S41.** Representative images of H&E-stained heart, liver, spleen, lung and kidney slices from mice 8 days post treatment at each group. Scale bar: 200 μm.