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Supporting Information

Ionized Water-Soluble Organic Nanosheets with Light/Ultrasound Dual Excitation Channels for Efficient Killing of Multidrug-Resistant Bacteria

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1. Materials and character

^{1}H NMR and ^{1}C NMR spectra were collected on a Bruker Ascend TM 400 MHz spectrometer with TMS as the internal standard. UV–Vis spectra were recorded on a PerkinElmer LAMBDA 750. The fluorescence data and lifetimes were measured using an Edinburgh Instruments FLS980 or Lengguang Tech F97PrO spectrophotometer. Transmission Electron Microscopy (TEM), The samples were prepared by drop casting 5 μL of the assembly solution onto the carbon-coated copper grids, and then suck up the liquid with a straw. TEM images were obtained on a JEM-2100 high resolution transmission electron microscope operating at 120 KV. The size of each sample was obtained by analyzing 2D rectangular micelles from TEM images using Digital Micrograph software (US Gatan company). Precisely measure the length of each nanosheet. From this data, the average number area A_n and the average weight area Aw of the sample were calculated (L = object area, N = quantity).

$$A_n = \frac{\Sigma_{i=1}^{N} N_i A_i}{\Sigma_{i=1}^{N} N_i} \qquad (1)$$

$$A_w = \frac{\Sigma_{i=1}^{N} N_i A_i^2}{\Sigma_{i=1}^{N} N_i A_i} \qquad (2)$$

For a Gaussian distribution of areas, the standard deviations (σ) of the measured areas are related to area dispersity (A_w/A_n) through the following expression:

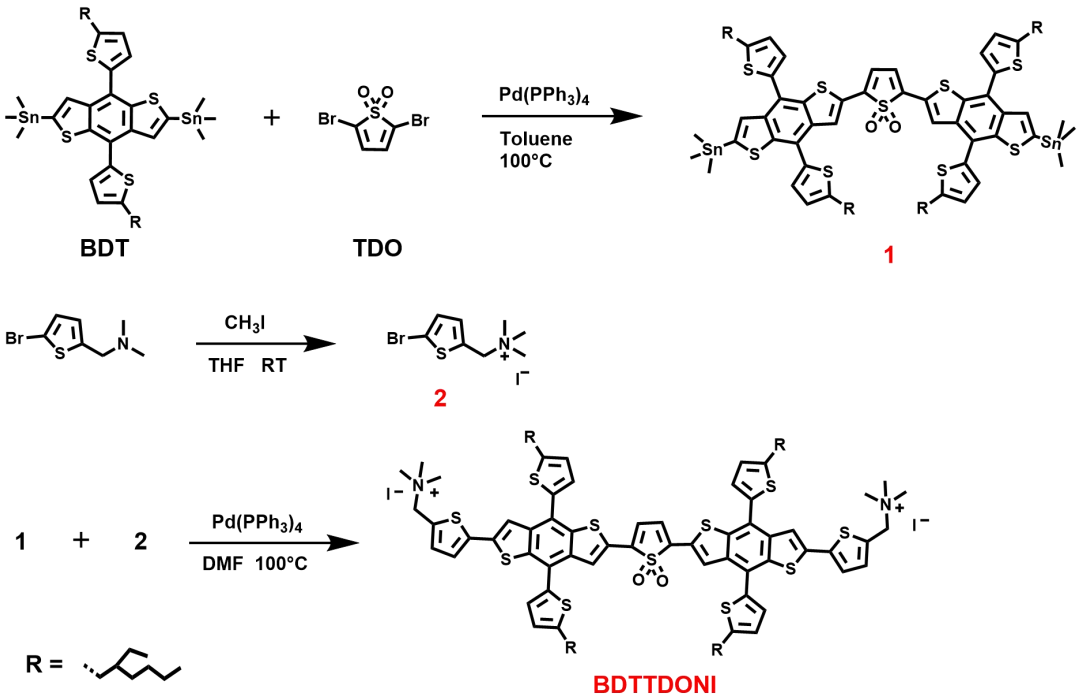
$$\frac{A_w}{A_n} - 1 = \left(\frac{\sigma}{A_n}\right)^2 \qquad (3)$$

Materials: All of the reagents and solvents used for the syntheses were purchased without further purification. 2,6-Bis(triMethyltin)-4,8-bis(5-(2-ethylhexyl)thiophen-2-yl)benzo [1,2-b:4,5-b']dithiophene (BDT), 2,5-dibromothiophene, 1,1-dioxide, (4-Bromobenzyl)dimethylamine, Iodomethane (CH_3I), 1,3-Diphenylisobenzofuran (DPBF), 2',7'-Dichlorofluorescein (DCFH), 9,10-dimethylanthracene (DMA), 2,2,6,6-tetramethylpiperidine (TEMP), 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), Tetrakis(triphenylphosphine)palladium ($Pd(PPh_3)_4$), Toluene, Tetrahydrofuran (THF), Methanol, Deionized water.

2. Synthesis

Preparation and characterization of BDTTDONI.

The synthesis details of Scheme S1 are described in the following.



Scheme. S1 Synthetic routes to the monomer of **BDTTDONI**.

Preparation procedure for 1

BDT (500 mg, 0.55 mmol), 2,5-dibromothiophene 1,1-dioxide (50.5 mg, 0.18 mmol) and $Pd(PPh_3)_4$ (10.4 mg, 0.009 mmol) were dissolved in toluene (20 mL) under a nitrogen atmosphere. The mixture was stirred at 100 ºC for 8 h. After cooling to room temperature, the reaction mixture was poured into a large amount of deionized water. The product was extracted with dichloromethane, and the combined organic layer was dried over anhydrous $MgSO_4$. After evaporation, the product was purified by column chromatography (Hexane/DCM=5:1, v/v) to get the (**1**) as a red solid (193 mg, yield: 68%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.62 (s, 2H), 7.68 (s, 2H), 7.36 (d, 2H), 7.30 (d, 2H), 6.94 (dd, J = 7.2, 2.7 Hz, 4H), 6.84 (s, 2H), 2.91 (m, 8H), 1.72 (m, 4H), 1.55-0.93 (56H), 0.44 (s, 18H);

^{13}C NMR (75 MHz, $CDCl_3$): δ 145.2, 144.9, 143.8, 143.0, 138.4, 137.4, 136.3, 136.0, 134.8, 130.0, 129.0, 127.2, 126.7, 124.7, 124.4, 121.5, 119.5, 40.4, 33.3, 31.54, 27.9, 24.8, 22.0, 13.2, 9.9, -9.3;

HRMS (ESI): Calculated for **1** $[M+H]^+$: 1594.3248, found: 1594.1756.

Preparation procedure for 2

5-bromo-N,N-dimethyl-2-Thiophenemethan amine (100 mg, 0.45 mmol), CH_3I (141.94 mg, 1.0 mmol) were dissolved in THF (20 mL) under a nitrogen atmosphere. The mixture was stirred at RT for 8 h. Centrifuge to collect the white solid precipitate and wash it three times with THF. Discard the THF by centrifugation to obtain white solid product (**2**) (94 mg, yield: 58%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.02 (s, 1H), 6.76 (s, 1H), 4.75 (s, 2H), 3.16 (s, 9H);

^{13}C NMR (75 MHz, $CDCl_3$): δ 130.2, 127.4, 127.3, 112.7, 66.0, 51.14;

HRMS (ESI): Calculated for **1** $[M+H]^+$: 359.8919, found: 359.3123.

Preparation procedure for BDTTDONI

(**1**) (100 mg, 0.063 mmol), (**2**) (45.6 mg, 0.126 mmol) and $Pd(PPh_3)_4$ (10.9 mg, 0.0094 mmol) were dissolved in DMF (10 mL) under a nitrogen atmosphere. The mixture was stirred at 100ºC for 8 h. After cooling to room temperature, Remove the DMF solvent through a vacuum pump. Then the product was purified by column chromatography (DCM/MeOH/Et_3N= 20/1/2, v/v/v) to get the **BDTTDONI** as a red solid (56.8 mg, yield: 49%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65-7.05 (18H), 4.61 (d, 6H), 3.24 (d, 18H), 2.88 (m, 8H), 1.37 (d, 6H);

^{13}C NMR (75 MHz, $CDCl_3$): δ 146.0, 145.4, 145.2, 143.9, 143.0, 138.4, 137.4, 137.0, 136.3, 136.0, 135.6, 134.8, 130.0, 129.0, 128.7, 127.8, 127.2, 126.7, 124.7, 124.4, 122.0, 121.5, 119.8, 119.5, 65.2, 50.1, 41.4, 41.1, 33.3, 31.5, 27.9, 24.8, 22.0, 13.2, 9.9;

HRMS (ESI): Calculated for **1** $[M+H]^+$: 1830.3428, found: 1830.4851.

Preparation of 2D Nanosheets

BDTTDONI were dissolved in THF (0.2 mg/mL) (0.1 mL), and the solution was then added into deionized water (10 mL) under sonication. After that, the mixture was placed on a RT for 12 hours. After that, the mixture was placed on a rotary evaporator for two hours to slowly remove the organic solvents. The remaining solution of **BDTTDONI** 2D nanosheets (**BDTTDONI Ns**) was stored for further tests.

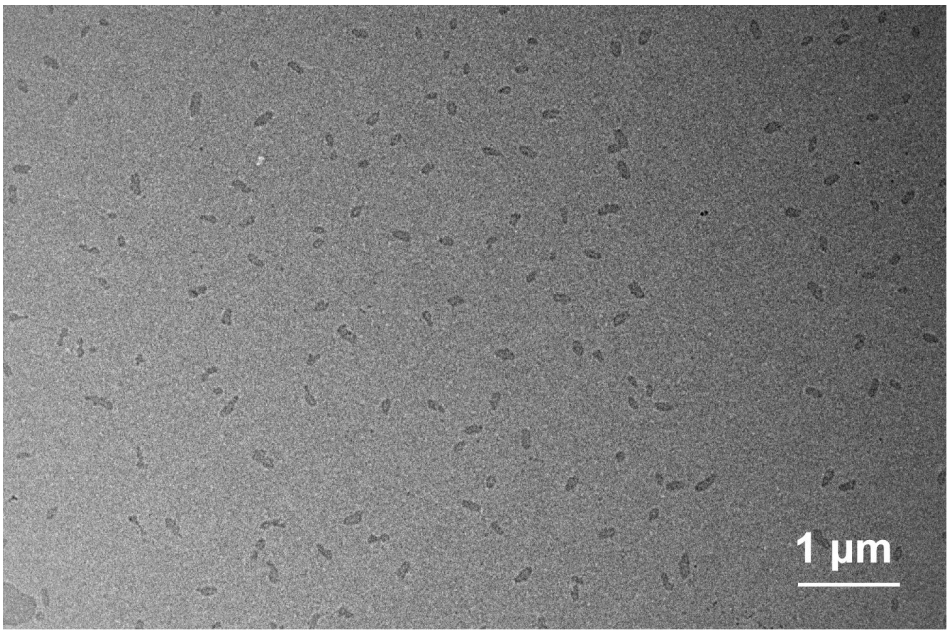


Fig. S1 TEM images of **BDTTDONI Ns**.

3. ROS Generation

ESR measurements of •OH: DMPO was employed as spin-trapping agents to detect •OH. 2D Nanosheets was dissolved in water at a dilution of 100 μM, and then 100 mM DMPO was added into water with US irradiation (1.5 MHz, 1.0 W cm^{-2}, 2 min) or light irradiation (5 W, 3 min). The EPR signal was recorded at room temperature.

ESR measurements of $O_2^{\bullet-}$: 5,5-Dimethyl-1-pyrroline-N-oxide (DMPO) was employed as spin-trapping agents to detect $O_2^{\bullet-}$. 2D nanosheet was dissolved in methanol at a dilution of 100 μM, and then 100 mM DMPO was added into methanol with US irradiation (1.5 MHz, 1.0 W cm^{-2}, 2 min) or light irradiation (5 W, 3 min). The EPR signal was recorded at room temperature.

ESR measurements of $^1O_2$: 2,2,6,6-Tetramethylpiperidine (TEMP) was employed as spin-trapping agents to detect $^1O_2$. 2D nanosheet was dissolved in water at a dilution of 10 μ M, and then 25 mM TEMP was added into water with US irradiation (1.5 MHz, 1.0 W cm^{-2}, 2 min) or light irradiation (5 W, 3 min). The EPR signal was recorded at room temperature.

$^1O_2$ detection by fluorescence analysis: DCFH as a typical molecular probe was used to detect $^1O_2$ production. The mixture was exposed to US (1.5 MHz, 1.0 W cm^{-2}) or light (5 W) . The fluorescence intensity of DCFH at 520 nm was recorded by a fluorescence spectrophotometer.

Evaluation of Singlet Oxygen ($^1O_2$): Absorption spectra of DPBF and DMA were recorded. The degradation rate of DPBF and DMA were obtained from the absorbance changes at the peak of 411 nm and between 300-400 nm.

Evaluation of decomposition rate constant of DPBF**:**

The decomposition rate constant of DPBF could be compared to determine the rate of $^1O_2$ generation for different materials. The decomposition rate formula was as follows:

$$\mathrm{Ln}(\mathrm{A_0}/\mathrm{A_n}) = \mathrm{Kt} + \mathrm{b}$$

where K was the slope of decomposition rate constant of DPBF at 411 nm with the irradiation time. t was the irradiation time, A_0 was the initial absorption intensity of DPBF at 411 nm, and A_n was the absorption intensity of DPBF at 411 nm at different times.

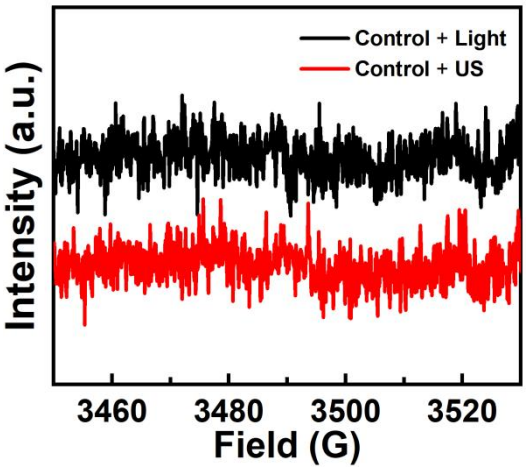


Fig. S2 EPR spectra demonstrating $^1O_2$ generation of control with light (5 W, 3 min) and US irradiation (1.5 W cm^{-2}, 1.0 MHz, 2 min), using TEMP as a spin trapper.

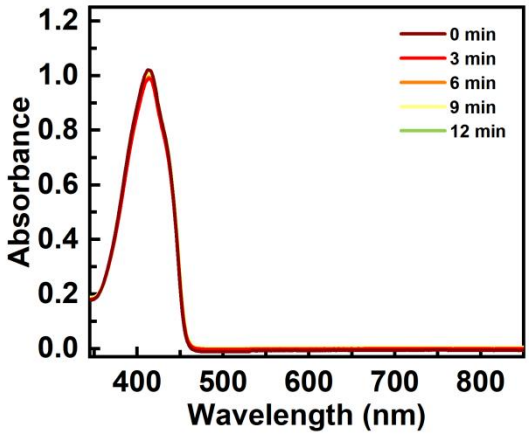


Fig. S3 DPBF absorbance change in MeOH after light irradiation.

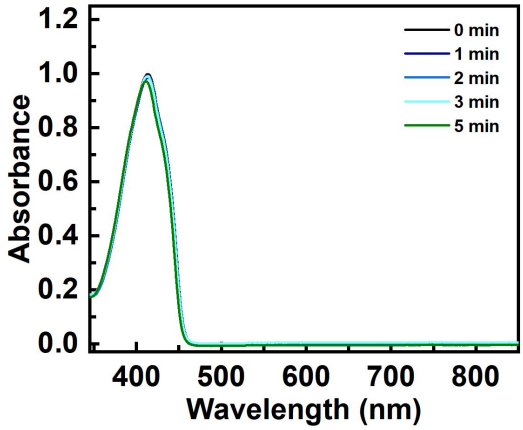


Fig. S4 DPBF absorbance change in MeOH after US irradiation.

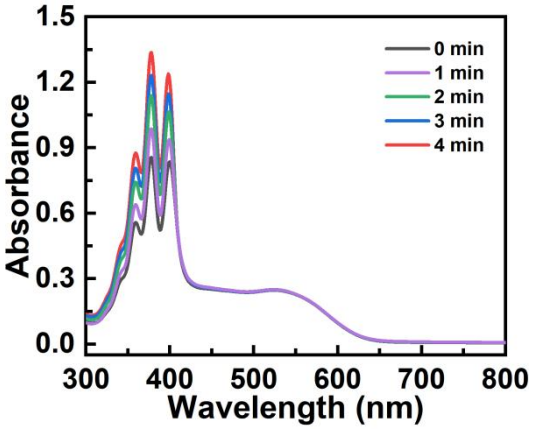


Fig. S5 Photoirradiation of DMA in the presence of **BDTTDONI Ns** in solution followed by UV-Vis spectroscopy within US irradiation (1.5 MHz, 1.0 W cm^{-2}).

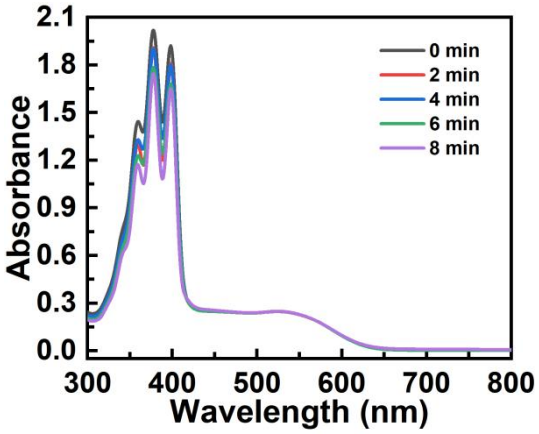


Fig. S6 Photoirradiation of DMA in the presence of **BDTTDONI Ns** in solution followed by UV-Vis spectroscopy within light irradiation (5 W).

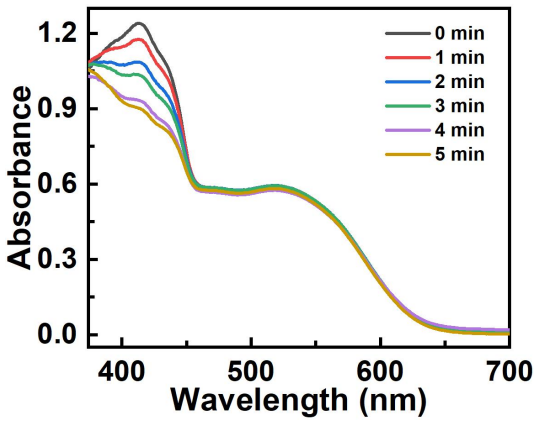


Fig. S7 Photoirradiation of DPBF in the presence of **BDTTDONI** in solution followed by UV-Vis spectroscopy within 5 min US irradiation (1.5 MHz, 1.0 W cm^{-2}).

4. Antibacterial activity in vitro

Drug-resistant bacteria include Gram-negative Escherichia coli (*E. coli*) (*Escherichia coli O25b-ST131*) and Gram-positive Staphylococcus aureus (*S. aureus*) (*Staphylococcus aureus ATCC 33591*), respectively. A monocolony of *E. coli* and *S. aureus* was cultured in a liquid Luria-Bertani (LB) broth and shaken at 37 °C for 12 h under 120 rpm.

Bacterial survival rate was measured by counting the bacterial colony numbers in the agar plates. Appropriate amount of 2D nanosheets in deionized water was mixed with the bacterial suspension. After US irradiation (1.5 W cm^{-2}, 1.0 MHz) and light irradiation (5 W), the mixture was incubated at 37 °C for 6 h before the spread plate operation. Bacteria survival rate was calculated using the following equation: %survival =C/C_0 100%, C represents the percentage of the area occupied by bacteria per unit area in the experimental group, while C_0 represents the percentage of the area occupied by bacteria per unit area in the control group (the control group in the ultrasound experiment consists of bacteria + US, and the control group in the light experiment consists of bacteria + light.

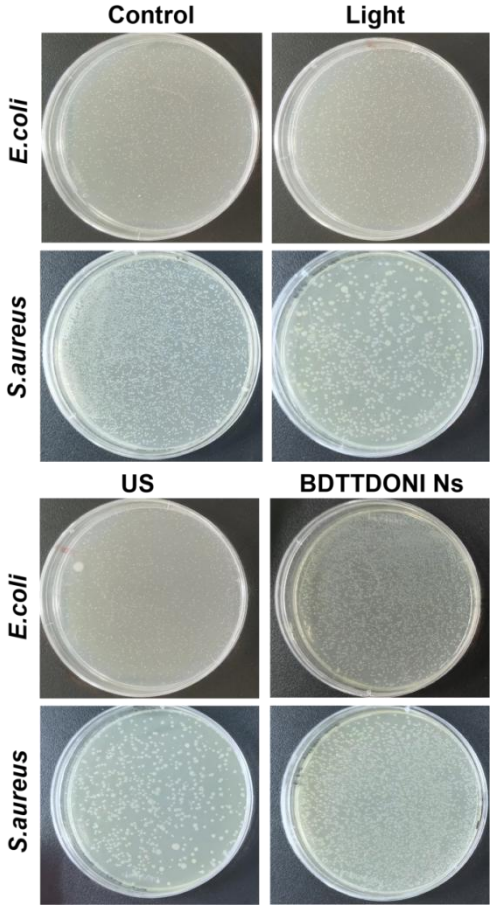


Fig. S8 Growth colony images of *E. coli* and *S. aureus* under blank control condition.

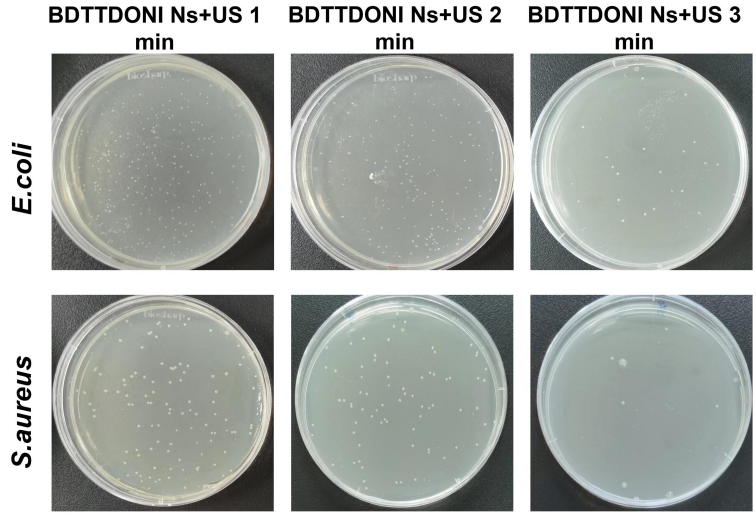


Fig. S9 BDTTDONI Ns against *E. coli* and *S. aureus* under US irradiation for 0-3 min.

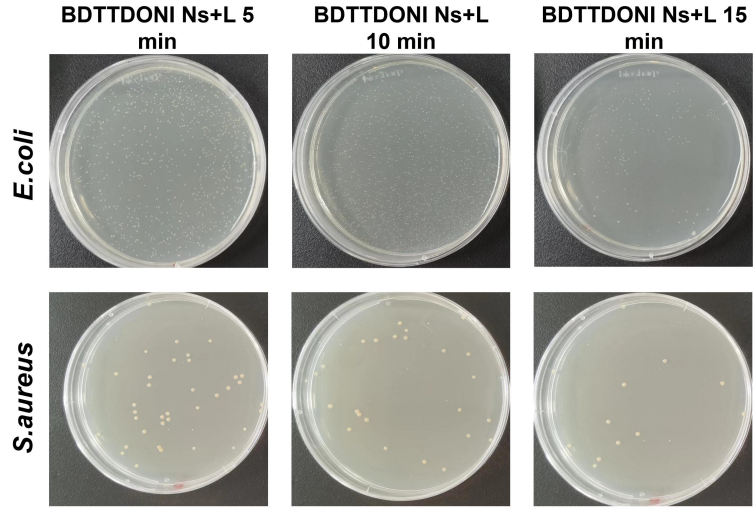


Fig. S10 BDTTDONI Ns against *E. coli* and *S. aureus* under light irradiation for 0-15 min.

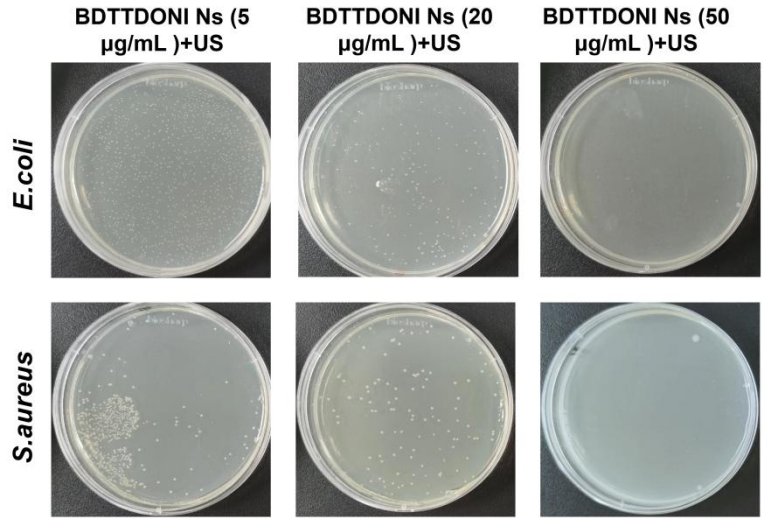


Fig. S11 BDTTDONI Ns against *E. coli* and *S. aureus* under US irradiation at different material concentrations.

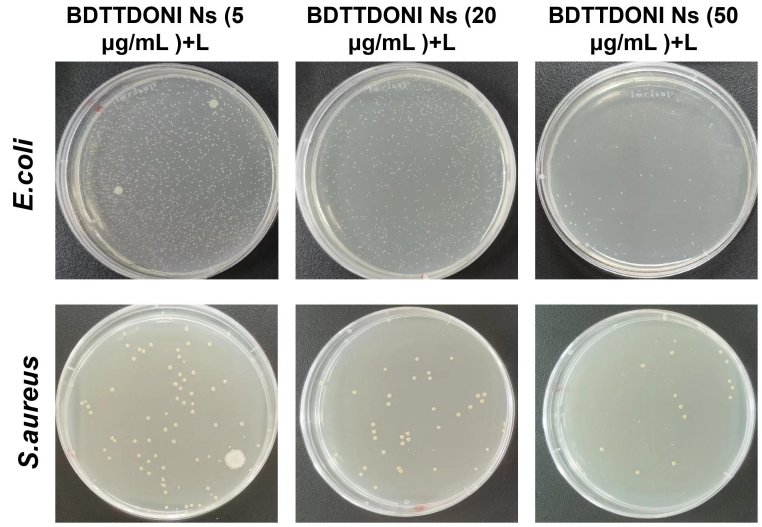


Fig. S12 BDTTDONI Ns against *E. coli* and *S. aureus* under light irradiation at different material concentrations.

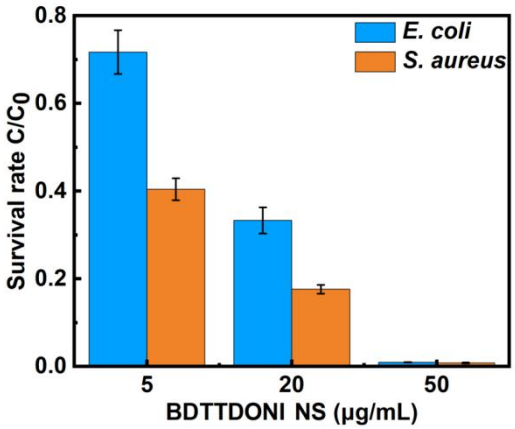


Fig. S13 In vitro antibacterial effect of **BDTTDONI Ns** under US irradiation at concentrations of 5, 20, and 50 μg/mL against *E. coli* and *S. aureus*.

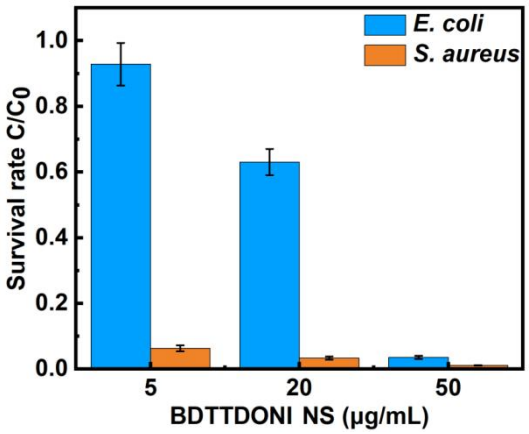


Fig. S14 In vitro antibacterial effect of **BDTTDONI Ns** under light irradiation at concentrations of 5, 20, and 50 μg/mL against *E. coli* and *S. aureus*.

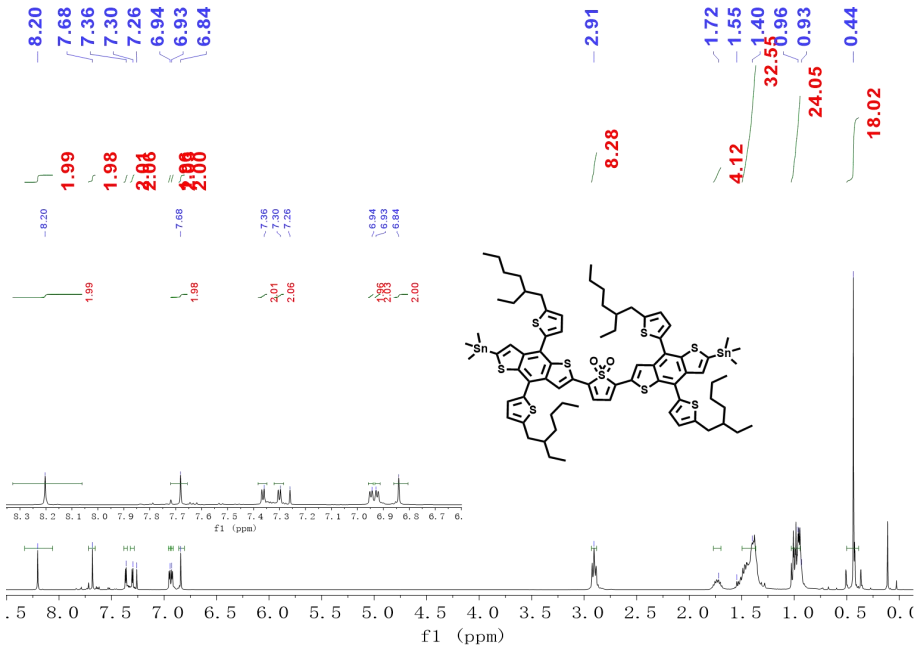


Fig. S15 ^{1}H NMR spectrum of **1** in $CDCl_3$.

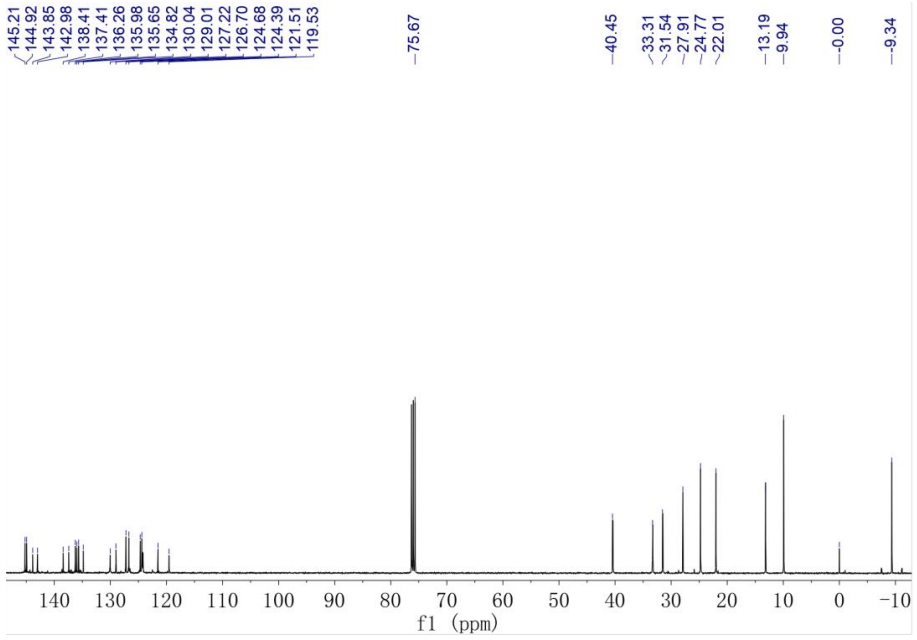


Fig. S16 ^{13}C NMR spectrum of **1** in $CDCl_3$.

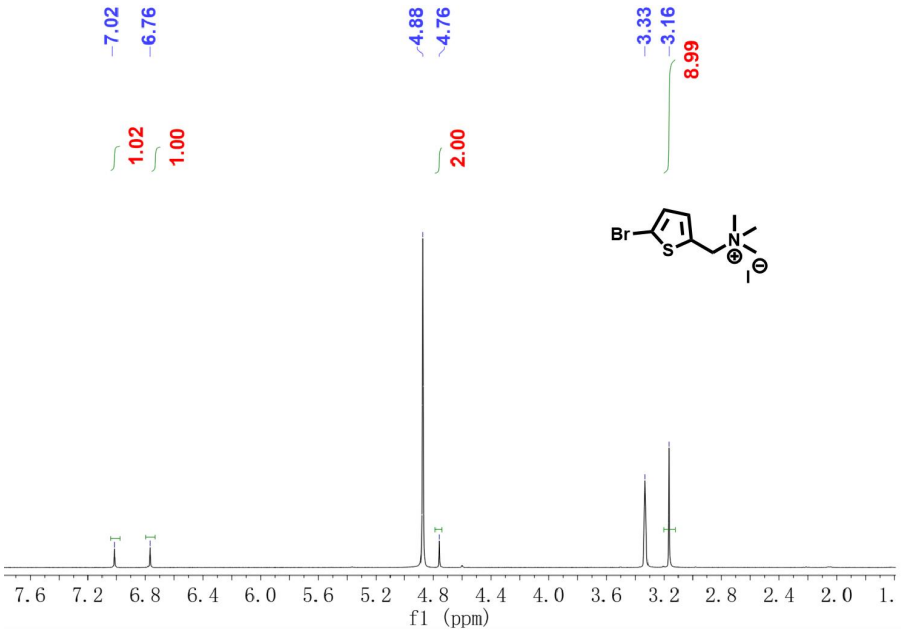


Fig. S17 ^{1}H NMR spectrum of **2** in MeOD.

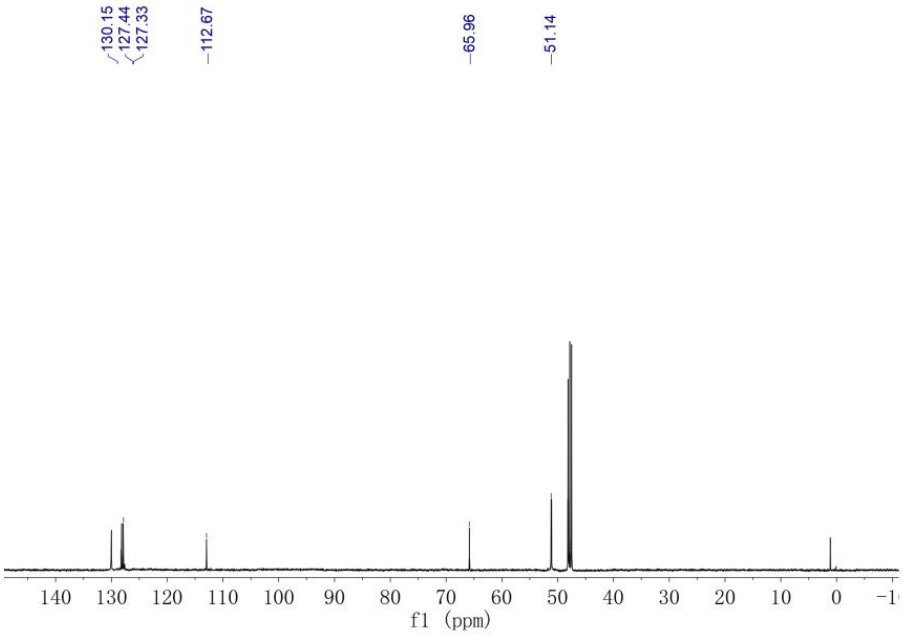


Fig. S18 ^{13}C NMR spectrum of **2** in MeOD.

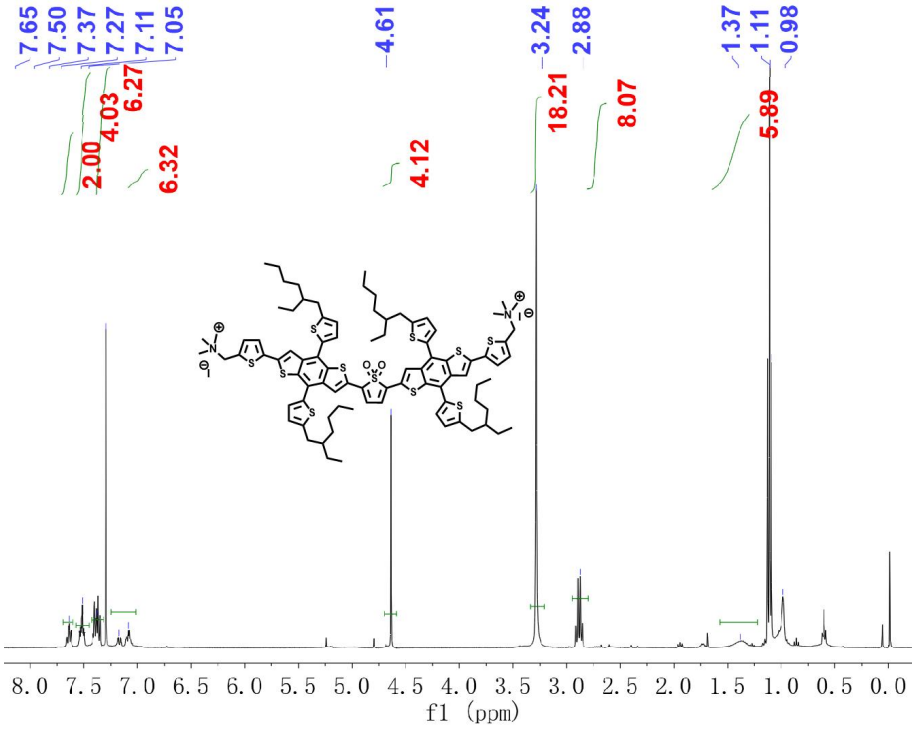


Fig. S19 ^{1}H NMR spectrum of **BDTTDONI** in $CDCl_3$.

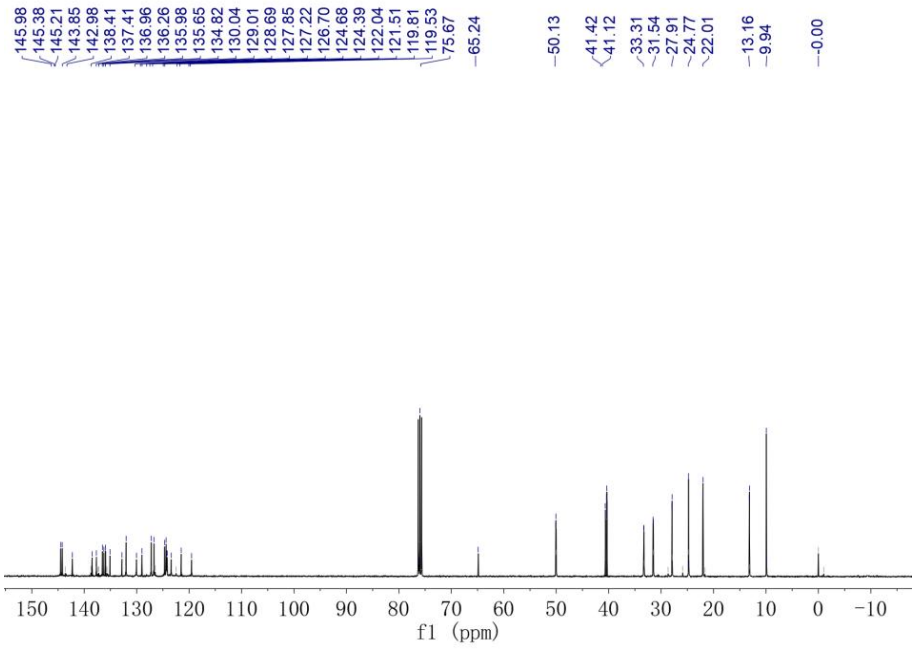


Fig. S20 ^{13}C NMR spectrum of **BDTTDONI** in $CDCl_3$.