Supporting Information

A Light-Activated Nickel(II)-Antibiotic Conjugate for Synergistic Antibacterial Therapy

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1. EXPERIMENTAL SECTION

Materials and instruments

All reagents were purchased from commercial suppliers and used as received unless otherwise indicated. Column chromatography was carried out on silica gel (200-300 mesh, Qingdao Ocean Chemicals) using the indicated eluents. All other chemicals used in this work were of analytical grade and used without further purification unless indicated. *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Methicillin-resistant staphylococcus aureus* (MRSA) were obtained from the American Type Culture Collection (ATCC, USA). *S. aureus*, *E. coli*, *and P. aeruginosa* were cultured in Luria Bertani (LB) broth, whereas MRSA was cultured in Tryptic Soy Broth (TSB). All bacterial were incubated at 37 °C with shaking at 150 rpm. Dulbecco's modified Eagle's medium (DMEM), penicillin-streptomycin solution, phosphate buffered saline (PBS), and fetal bovine serum (FBS) were purchased from Gibco Life Technologies (Gaithersburg, MD, USA). Cell Counting Kit-8 (CCK8) was provided by Beyotime Biotechnology.

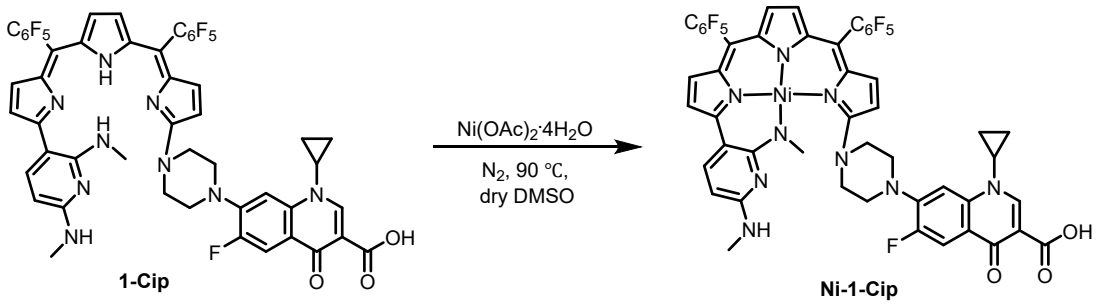
UV-vis spectra were recorded on an Agilent 8453 UV-vis spectrometer equipped with an Agilent 89090A thermostat (± 0.1 °C). MS spectra were recorded on Bruker APEX IV Fourier Transform Ion Cyclotron Resonance Mass Spectrometer using electrospray ionization. ^{1}H NMR spectra were recorded on a Bruker-400 MHz instrument. Liquid chromatography-mass spectrometry (LC-MS) were recorded on Quadrupole-TOF LC-MS/MS System (Vion, Waters). Photothermal effects were determined using a UNI-T UT325 thermometer. Infrared (IR) thermal imaging was performed using a FLIR E53 thermal imaging camera.

Synthesis and characterization

1-Cip

Compound **1** (76.7 mg, 1.0 equiv), ciprofloxacin (Cip, 33.1 mg, 1.0 equiv), and degassed triethylamine (TEA, 13.9 mL, 1.0 equiv) were dissolved in degassed DMSO (20 mL) in a 50 mL Schlenk flask under a nitrogen atmosphere. The reaction mixture was stirred for 4 h under N_2. After completion, the mixture was extracted with H_2O and dichloromethane, and the organic phase was concentrated under reduced pressure. The crude product was purified by recrystallization from methanol to afford **1-Cip** as a green solid (33.57 mg, 33.0%).

Ni-1-Cip



A mixture of **1-Cip** (50.0 mg, 1.0 equiv) and nickel(II) acetate ($Ni(OAc)_2{\cdot}4H_2O$, 157.5 mg, 6.3 equiv) in degassed DMSO (20 mL) was stirred under nitrogen in a 50 mL Schlenk flask. The reaction was monitored until the solution turned brown. The mixture was then extracted with dichloromethane, and the organic layer was concentrated in vacuo. Purification by recrystallization (methanol) yielded **Ni-1-Cip** as a brown solid (31.5 mg, 59.7 %).

Photothermal effects

The photothermal conversion promoted by the **Ni-1-Cip** of this study was examined under conditions of 880 nm laser irradiation while recording the temperature of the samples as a function of irradiation time. At any given time point, a aqueous solution sample was used as a negative control. The following studies were made:(1) 2.0 mL **Ni-1-Cip** with various concentrations and DMSO irradiated with an 880 nm laser at a power density of 1.5 W cm^{-2} for 15 min. (2) 2.0 mL **Ni-1-Cip** at a concentration of 150 μM was subject to laser irradiation at various power densities for 15 min. (3) 2.0 mL **Ni-1-Cip** was irradiated in iterative fashion four times at a power density of 1.5 W cm^{-2}. The cycles involved irradiation for a period of 15 min and allowing a cooling period sufficient to allow the suspension temperature to decrease to room temperature. The photothermal conversion efficiencies (η) were calculated according to the following equations (1-4): The cycles involved irradiation for a period of 10 min and allowing a cooling period sufficient to allow the suspension temperature to decrease to room temperature. The photothermal conversion efficiencies (η) were calculated according to the following equations (1-4):

$$\eta = \frac{hS(T_{max} - T_{surr}) - Q_s}{I(1 - 10^{-A})} \quad (1)$$

$$\theta = \frac{\Delta T}{\Delta T_{max}} = \frac{T - T_{surr}}{T_{max} - T_{surr}} \quad (2)$$

$$t = -\tau \ln\theta \quad (3)$$

$$hS = \frac{mC_p}{\tau} \quad (4)$$

where h is the heat transfer coefficient, S is the surface area of the container. The T_{max} and T_{surr} are the maximum temperature of the solution and the ambient temperature, respectively, I is the laser power, A is the absorbance of the sample at 880 nm and Q_s expresses the heat associated with light absorption by the solvent. m and C_p are the mass and heat capacities of the system, respectively; τ is the heat transfer time constant, which can be determined by the linear relationship of t versus $-ln\theta$ through the natural cooling curve of the sample.

***In vitro* cytotoxicity**

The biosafety of **Ni-1-Cip** against HaCaT (Human Immortalized Keratinocyte Cell Line) cells were investigated by CCK-8 assay. For the dark cytotoxicity assay, HeLa cells were seeded in a 96-well plate at 1×10^4 cells per well and incubated for 24 h. Cells were then treated with different concentrations of **Ni-1-Cip** in the dark for 24 h at 37 °C. Cell viability was quantified using the CCK-8 assay.

Preparation of bacteria cultures

E. coli, *S. aureus, and P. aeruginosa* and MSRA were used as the model organism to evaluate the antibacterial efficiency of **Ni-1-Cip**. The single colony of bacteria on a solid medium agar plate was transferred to 10 mL of fresh liquid medium culture medium alone with shaking (150 rpm) and cultured at 37 °C overnight until logarithmic growth. The bacteria were then harvested by centrifuging for 3 min and washed with PBS. Then bacteria were suspended in PBS and diluted to an optical density of 0.4 at 600 nm ($OD_{600} = 0.4$) for further antibacterial experiment.

Photothermal imaging

The photothermal performance of **Ni-1-Cip** was evaluated under 880 nm laser irradiation by monitoring the temperature change over time. Pure DMSO was used as a negative control. A 1.0 mL solution of **Ni-1-Cip** (150 μM) was irradiated at a power density of 1.5 W cm^{-2} for 15 min. Infrared thermal images were captured using a handheld NIR camera at 0, 0.5, 1, 2, 4, 6, 8, and 10 min during the irradiation process.

***In vitro* antibacterial effects**

The coated plates and colony count method were used to determine the number of colonies forming units to check the combined antibacterial ability of **Ni-1-Cip** under NIR illumination. The collaborative experiment was divided into six groups: (I) PBS, (II) PBS + NIR-*hv*, (III) **Ni-1**, (IV) **Ni-1** + NIR-*hv*, (V) **Ni-1-Cip**, (VI) **Ni-1-Cip** +

NIR-*hv*. Briefly, gram-positive MRSA bacteria were separately cultured in fresh TSB in a shaking incubator at 37 °C. *S. aureus* bacteria, *E. coli*, *P. aeruginosa*c bacteria were separately cultured in fresh LB. Cells in mid-log phase were diluted to $OD_{600}\approx0.4$, and 100 μL of bacterial culture was added to equal volumes of PBS, **Ni-1**, or **Ni-1-Cip** solution. The mixture was continuously shaken at 37 °C for 24 h. Subsequently, bacterial cells from the PBS + *hv* group, **Ni-1** + *hv* group and **Ni-1-Cip** + *hv* group were further exposed to an NIR laser (880 nm, 1.0 W cm^{-2}, 10 min). After an additional 1 h incubation, the bacterial suspensions of each group were 10^5-fold serially diluted, MRSA spread onto TSA plates, while *S. aureus*, *E. coli*, and *P. aeruginosa*c spread onto LB plates, and incubated at 37 °C overnight. Colonies of visible bacteria on the plates were counted and imaged. All experiments were repeated thrice.

***In vivo* antibacterial efficacy against localized bacterial infection**

All animal procedures were approved by the Institutional Animal Care and Use Committee of Sinoresearch (Beijing) Biotechnology Co., Ltd. (Protocol number: ZYZC202511009J) and carried out in accordance with the requirements of the National Act on the Use of Experimental Animals (People's Republic of China). The BALB/c nude mice (5-6 weeks old, ≈20 g) were purchased and maintained in a sterile environment and allowed free access to food and water.

First, to construct the animal model with an infected wound, the mouse was first anesthetized by intraperitoneal injection of 10% chloral hydrate (4 mL per 1 kg body weight). An oval wound (about 7.0 mm in diameter) was made on the skin of BALB/c mice, and 20 μL of MRSA suspension (3.0×10^7 CFU/mL) was immediately applied to the wound on -1 day. Erythema or purulent exudate were observed the next day (0 day), after which the animals were randomly allocated to groups and received the treatment.

The mice infected with MRSA were randomly divided into four groups randomly (6 mice per group) as follows: PBS, Cip, **Ni-1** + NIR-*hv*, **Ni-1-Cip** + NIR-*hv*. **Ni-1-Cip** was dropped onto the wound site, using a dose volume of 100 μL at a concentration of 50 μM, and then subjected to laser irradiation. For the "*hv*" groups, 880 nm laser irradiation was applied at a powder density of 1.0 W cm^{-2} for 10 min. After treatment on the first day, wounds growth was measured by vernier caliper every two days for 2 weeks and the wounds sizes. The body weight of each mouse was monitored every other day using a digital balance during the treatment.

2、 SUPPORTING FIGURES

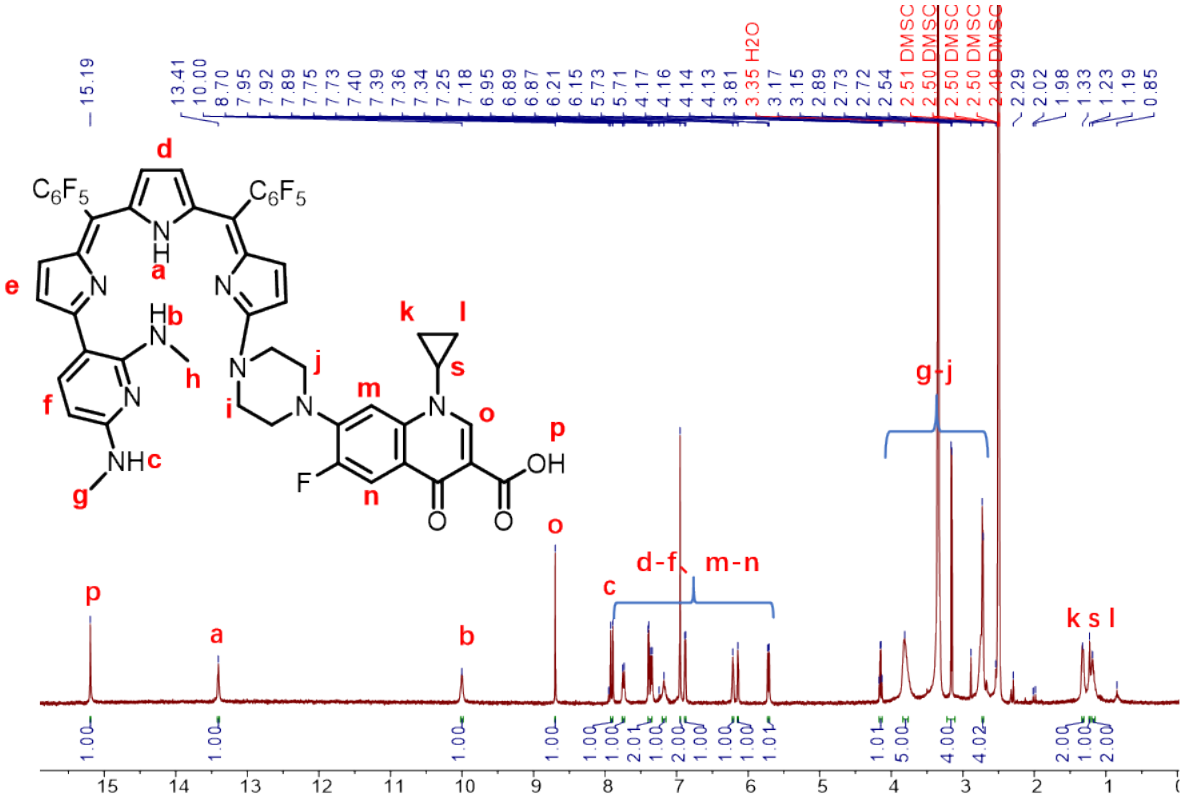


Figure S1. ^{1}H (DMSO-d6) spectra of **1-Cip**.

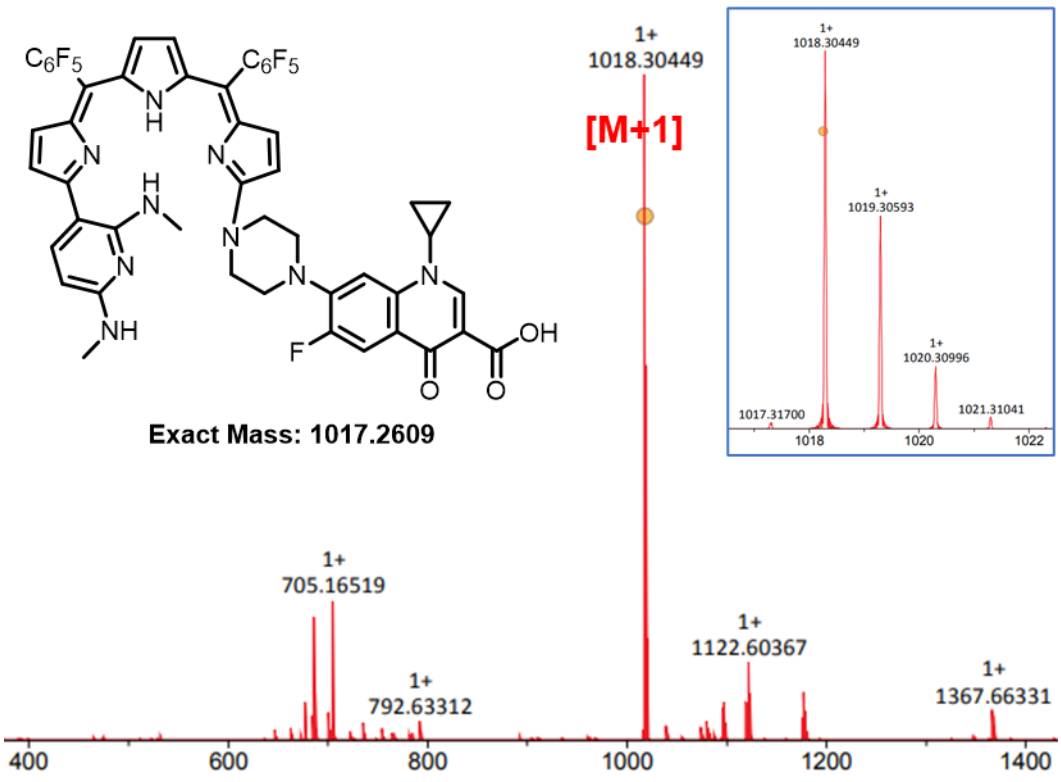


Figure S2. MS spectra of **1-Cip**.

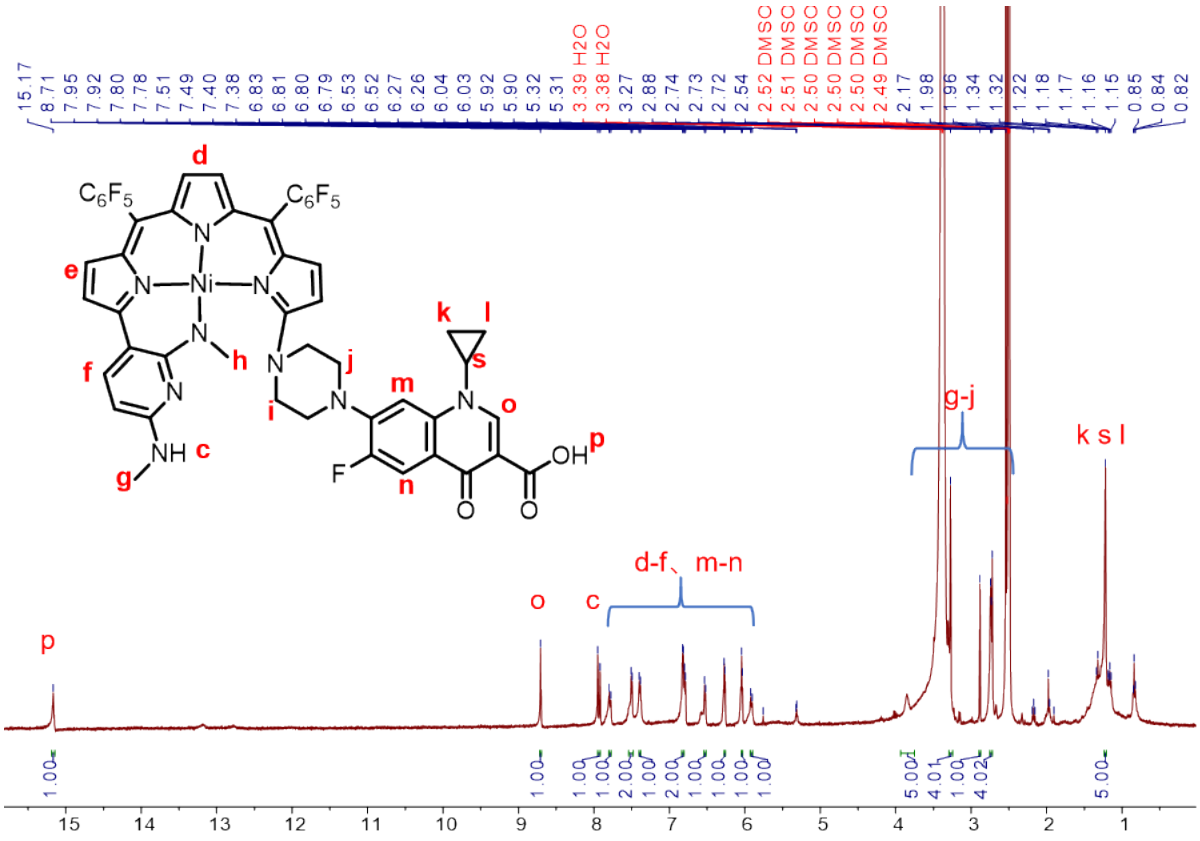


Figure S3. ^{1}H (DMSO-d6) spectra of **Ni-1-Cip**.

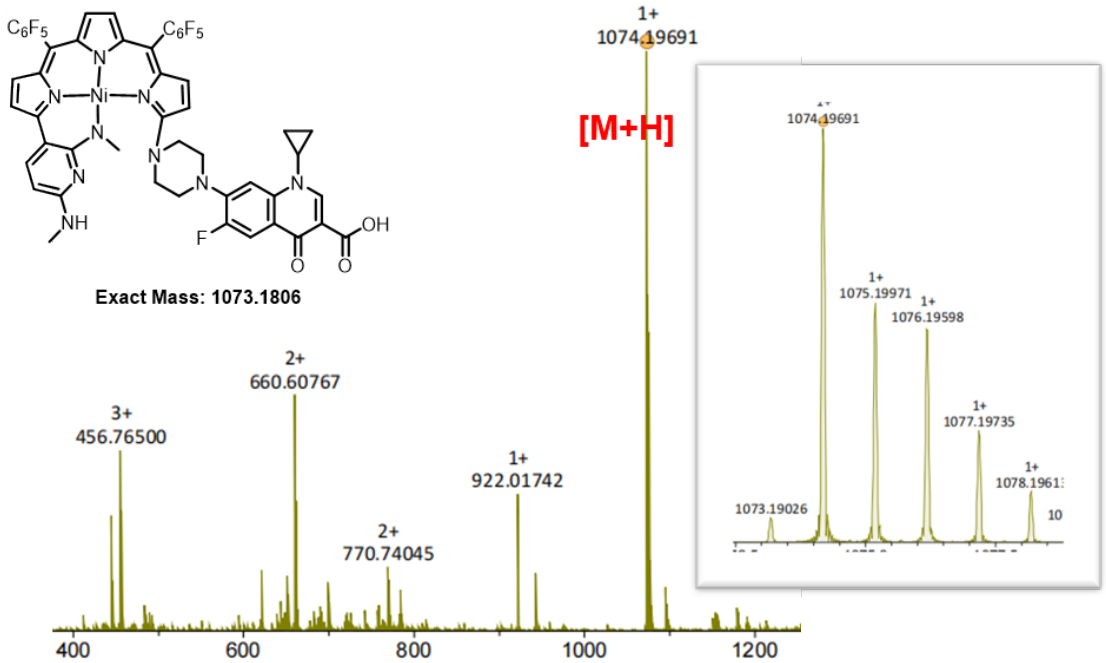


Figure S4. MS spectra of **Ni-1-Cip**.

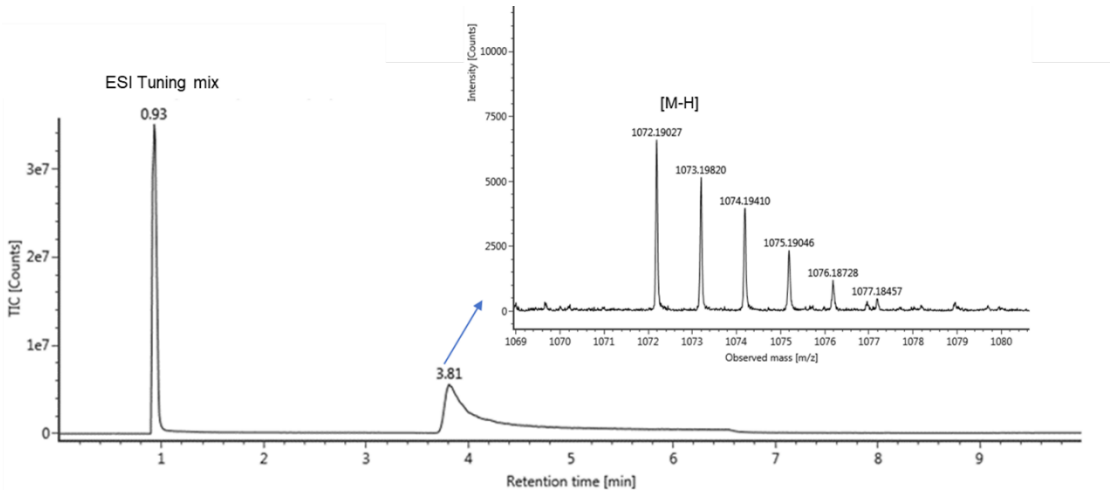


Figure S5. LC-MS of **Ni-1-Cip**. Conditions in the experimental section: Ultimate C18 column (5 µm, 4.6 × 150 mm); gradient 10–90 % DMSO/0.1 % TFA; flow rate 0.2 mL min^{-1}. The peak at 0.93 min corresponds to the internal standard (ESI Tuning mix) peak.

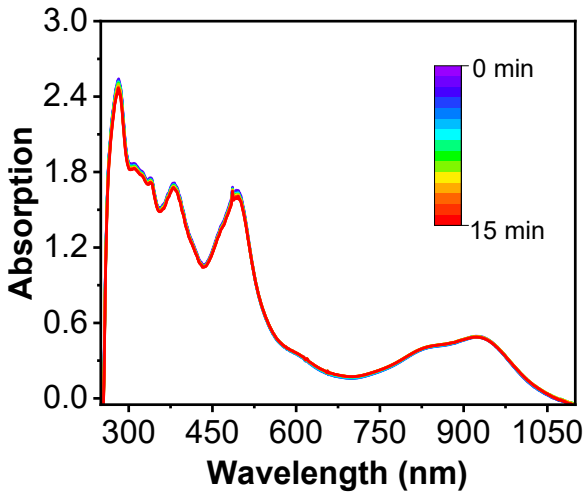


Figure S6. Photostability studies of **Ni-1-Cip** in DMSO with the 880 nm at the intensity of 1.5 W cm^{-2} over 15 min.

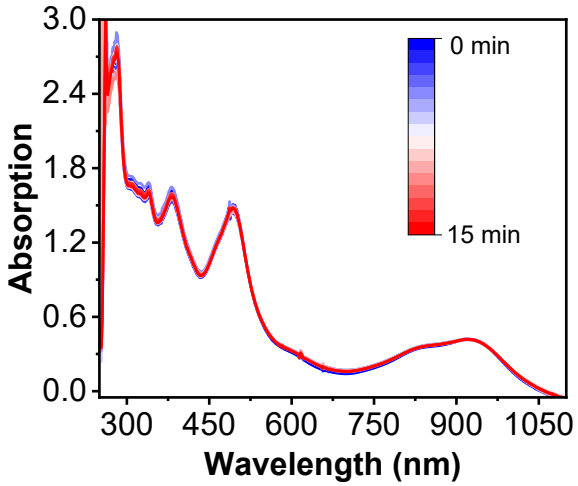


Figure S7. Thermal stability of **Ni-1-Cip** in DMSO at high temperatures heated to 80 ℃.

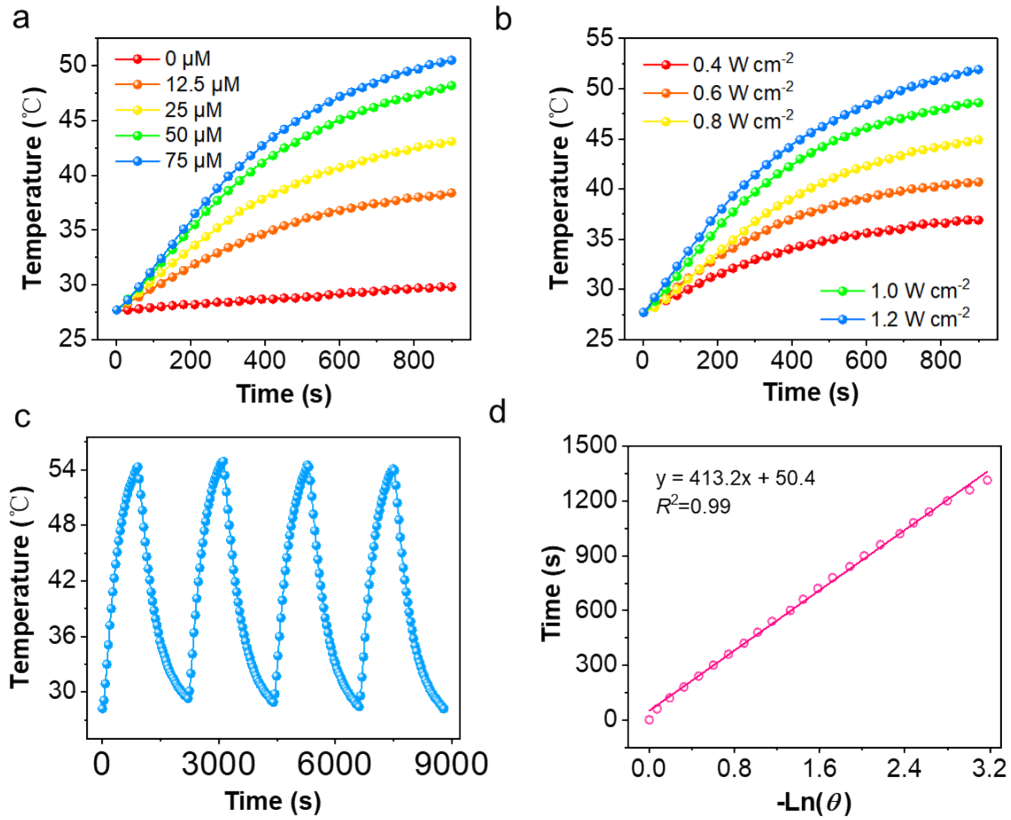


Figure S8. Photothermal properties of **Ni-1** in DMSO. (a) Temperature change curves of **Ni-1** at different concentrations exposed to the 880 nm laser at a power density of 1.5 W cm^{-2}. (b) Temperature change curves of **Ni-1** at concentration of 75 μM with different exposure intensities. (c) Photothermal stability study of **Ni-1** during four cycles of the heating-cooling process. (d) Linear fitting of -Ln(θ) and time of **Ni-1**.

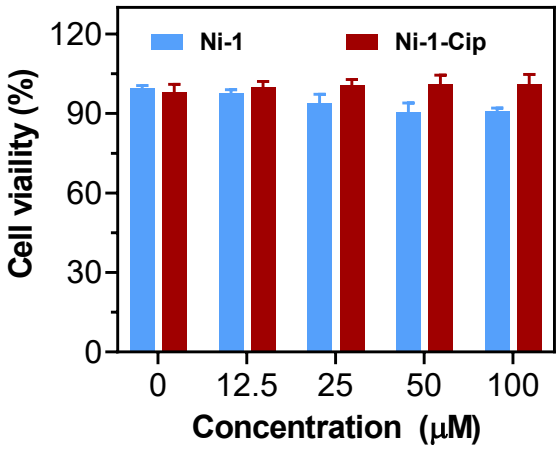


Figure S9. Viability of normal cell level (HaCat cells) incubated with **Ni-1-Cip** and **Ni-1** in the absent of NIR laser irradiation, respectively.

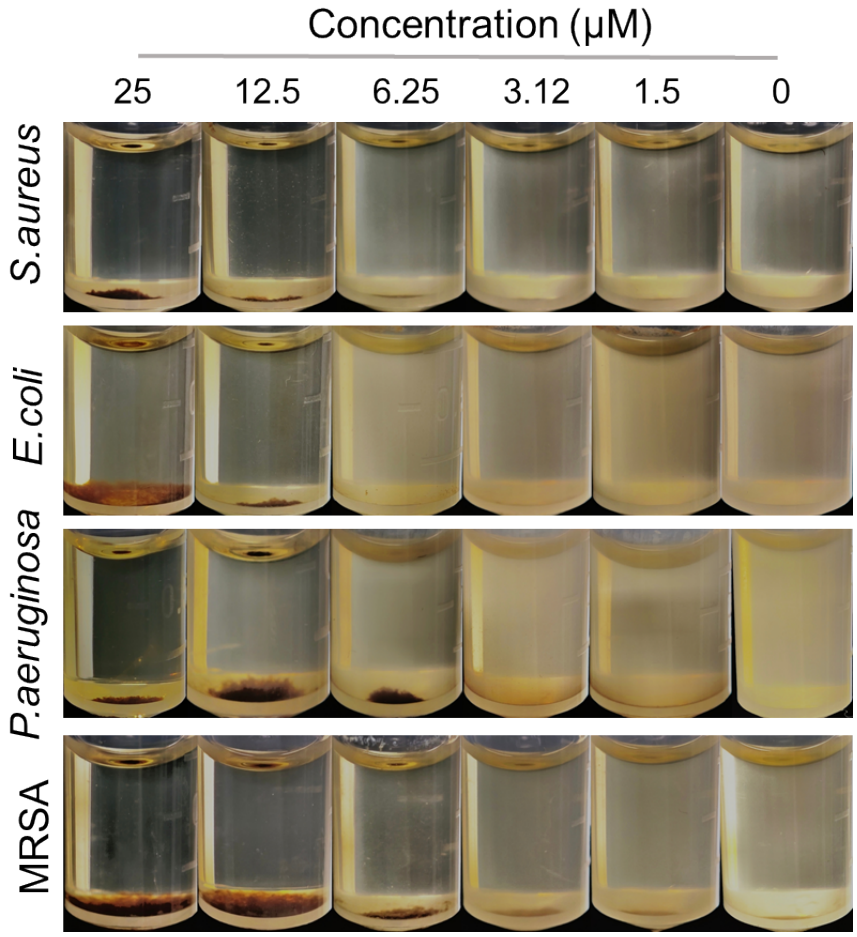


Figure S10. Bacterial survival status of *S. aureus*, *E. coli*, *P. aeruginosa* and MRSA treated with **Ni-1-Cip** at different concentrations with laser exposure.

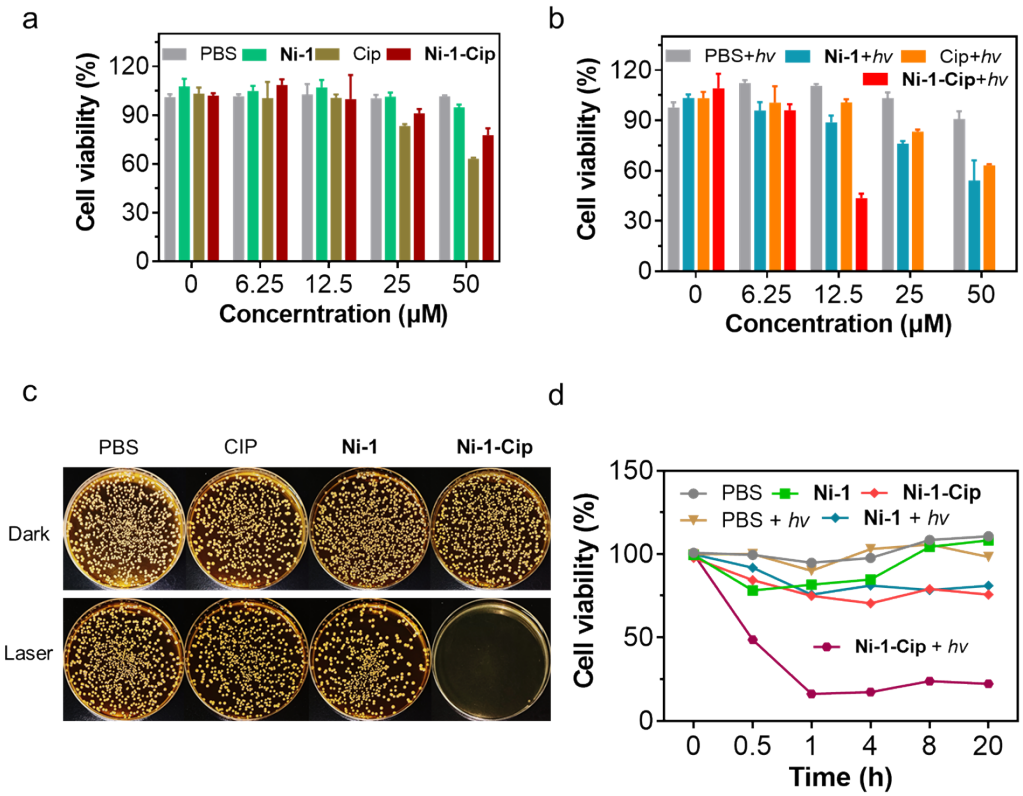


Figure S11. Antibacterial activity of Cip, **Ni-1** and **Ni-1-Cip** toward MRSA in the (a) dark and (b) under 880 nm laser irradiation. (c) Photographs of MRSA-plated bacterial colonies obtained after treatment with Cip, **Ni-1** and **Ni-1-Cip** without or with laser irradiation, respectively. (d) Antibacterial activity over time of MRSA after different treatments determined by the plate counting method.