Electronic Supplementary Information for:

One-step *In-vivo* Metabolic Labeling as a Theranostic Approach for Overcoming Drug-Resistant Bacterial Infections

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Materials:

Chemicals were all purchased from Sigma-Aldrich and used as received without further purification. Deuterated solvents were purchased from Cambridge Isotope Laboratories Inc. The HPLC grade solvents methanol and acetonitrile were purchased from Aldrich. Tetrahydrofuran (THF) and dichloromethane were dried by distillation using sodium as drying agent and benzophenone as indicator. All non-aqueous reactions were carried out under nitrogen atmosphere in oven-dried glassware. Dulbecco's modified essential medium (DMEM), Penicillin, streptomycin, fetal bovine serum (FBS) and trypsin were obtained from Invitrogen. Milli-Q water (18.2 MΩ) was used to prepare the buffer solutions from 10 × phosphate buffered saline (PBS) stock buffer (1st Base, Singapore). *Staphylococcus aureus* (*S. aureus*, ATCC 25923) and Raw 264.7 mouse macrophage cell were purchased from American Type Culture Collection (ATCC, Manassas, VA).

Instrument:

NMR spectra were measured on a Bruker ARX 400 NMR spectrometer. Chemical shifts are reported in parts per million referenced with respect to residual solvent for ^{1}H NMR and for ^{13}C NMR. The extent of reaction was monitored by thin layer chromatography (TLC) using Merck 60 F254 pre-coated silica gel plates with fluorescent indicator UV254. After the plates were subjected to elution in the TLC chamber, flash column chromatography was carried out using Merck silica gel (0.040-0.063). TPACN-D-Ala was purified by HPLC on a Waters Prep 150 LC System. Mass spectra were recorded on AmaZon X LC-MS for electrospray ionization (ESI). UV-vis absorption spectra were taken on a Shimadzu Model UV-1700 spectrometer.

Photoluminescence (PL) spectra were measured on a Perkin-Elmer LS 55 spectrofluorometer. All UV and PL spectra were collected at 24 ± 1 °C. Hydrodynamic size distributions were measured on a Zetasizer Nano ZS ZEN3600 analyzer (Malvern Instruments Ltd, UK) at 25 °C. CLSM imaging was carried out on confocal laser scanning microscopy platform Leica TCS SP8. Cold Light Illuminator L-150A was used for white light irradiation.

Synthesis of (4-(diphenylamino)phenyl)(4-methoxyphenyl)methanone (compound 3):

4-(*N,N*-diphenylamino)-1-phenylboronic acid (433 mg, 1.5 mmol) and 4-methoxybenzoyl chloride (255 mg, 1.5 mmol) were mixed with cesium carbonate (717 mg, 2.2 mmol) and tetrakis(triphenylphosphine)palladium(0) (17 mg, 0.015 mmol) in 30 mL of toluene. The mixture was stirred and heated at 80 ℃ for 12 h under argon atmosphere. After cooling to room temperature and evaporation of toluene, the residue was dissolved in dichloromethane (50 mL) and washed with water (50 mL × 3). The organic layer was collected and dried with sodium sulfate and the solvent was removed under reduced pressure. The obtained residue was purified with chromatography (hexane/dichloromethane = 2/1, v/v) to give compound 3 (426 mg, 75% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, J = 8.7 Hz, 2H), 7.70 (d, J = 8.7 Hz, 2H), 7.35 (t, J = 7.9 Hz, 4H), 7.20 (d, J = 7.6 Hz, 4H), 7.15 (t, J = 7.3 Hz, 2H), 7.05 (d, J = 8.7 Hz, 2H), 6.98 (d, J = 8.8 Hz, 2H), 3.90 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.18, 162.74, 151.55, 146.69, 132.18, 131.68, 130.92, 130.39, 129.60, 125.86, 124.46, 119.89, 113.43, 55.47.

Synthesis of 2-((4-(diphenylamino) phenyl) (4-methoxyphenyl) methylene) malononitrile (TPACN):

Compound 3 (214 mg, 0.50 mmol) and malononitrile (66 mg, 1.0 mmol) were dissolved in anhydrous dichloromethane (15 mL). Titanium(IV) chloride (0.22 mL, 2.0 mmol) was injected dropwise at 0 ℃ under argon atmosphere. The mixture was further stirred for 15 min at 0 °C. Pyridine (0.16 mL, 2 mmol) was injected dropwise with another 15 min stirring at 0 °C under argon atmosphere. After warming up to room temperature,

the mixture was further stirred for 30 min. The reaction was quenched by adding water (10 mL). The residue was dissolved in dichloromethane (50 mL) and washed with water (50 mL × 3). The organic layer was collected and dried with sodium sulfate and the solvent was removed under reduced pressure. The obtained residue was purified with chromatography (hexane/dichloromethane = 1/1, v/v) to give TPACN (190 mg, 89% yield) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, J = 8.8 Hz, 2H), 7.37 (t, J = 7.8 Hz, 4H), 7.32 (d, J = 8.9 Hz, 2H), 7.22 (d, J = 8.4 Hz, 6H), 7.02-6.94 (m, 4H), 3.90 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.38, 163.10, 152.21, 145.81, 132.99, 132.77, 129.81, 128.63, 127.08, 126.48, 125.41, 118.50, 115.62, 114.13, 75.13, 55.58.

Synthesis of tert-butyl 2-(4-(2,2-dicyano-1-(4-(diphenylamino)phenyl)vinyl)phenoxy)acetate (compound 4):

TPACN (100 mg, 0.23 mmol) was dissolved in anhydrous dichloromethane (20 mL) and treated with boron tribromide (0.11 mL, 1.15 mmol) at 0 ℃. After warming up to room temperature, the mixture was stirred for 12 h. The reaction was quenched by adding water (10 mL) and washed with water (50 mL × 3). The organic layer was collected and dried with sodium sulfate and the solvent was removed under reduced pressure to obtain the crude demethoxy-product. The demethoxy-product was dissolved in DMF (5 mL) with addition of cesium carbonate (90 mg, 0.28 mmol) and tert-butyl bromoacetate (55 mg, 0.28 mmol). The mixture was stirred for 30 min at room temperature. The reaction was then poured into ethyl acetate (50 mL) and washed with water (50 mL × 6). The organic layer was collected and dried with sodium sulfate and the solvent was removed under reduced pressure. The obtained residue was purified with chromatography (hexane/dichloromethane = 1/1, v/v) to give compound 4 (103 mg, 85% yield) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49-7.43 (m, 4H), 7.39-7.28 (m, 5H), 7.23-7.17 (m, 3H), 7.10-7.03 (m, 2H), 7.01-6.93 (m, 4H), 4.60 (s, 2H), 1.51 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.07, 172.92, 167.27, 161.51, 161.33, 152.26, 151.70, 151.15, 145.76, 144.73, 133.04, 132.92, 132.75, 129.83, 127.58, 126.49, 125.46, 119.62, 119.03, 118.47, 118.41, 114.83, 114.74, 82.97, 65.68,

28.07.

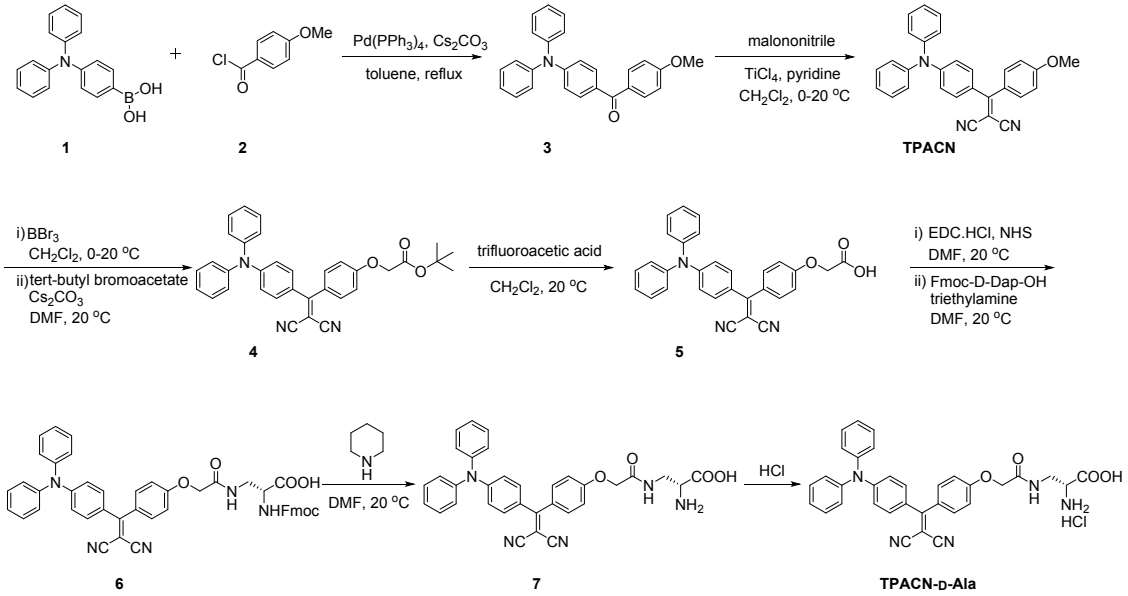
Synthesis of 2-(4-(2,2-dicyano-1-(4-(diphenylamino)phenyl)vinyl)phenoxy)acetic acid (compound 5):

Compound 4 (100 mg, 0.19 mmol) was dissolved in anhydrous dichloromethane (20 mL). Trifluoroacetic acid (0.1 mL, 1.3 mmol) was added dropwise into the solution. The mixture was stirred at room temperature for 12 h. The solvent was removed under reduced pressure. The obtained residue was purified with chromatography (hexane/ethyl acetate = 1/1, v/v) to give compound 5 (64 mg, 72% yield) as an orange solid. ^{1}H NMR (400 MHz, DMSO) δ 13.15 (s, 1H), 7.59 (dd, J = 8.8, 2.5 Hz, 2H), 7.48-7.42 (m, 4H), 7.38-7.32 (m, 2H), 7.29-7.20 (m, 4H), 7.15 (d, J = 8.6 Hz, 2H), 7.09 (d, J = 8.7 Hz, 2H), 6.98-6.83 (m, 2H), 4.82 (s, 2H). ^{13}C NMR (100 MHz, DMSO) δ 173.16, 170.16, 161.77, 152.20, 151.65, 145.78, 145.11, 133.45, 133.33, 130.68, 130.57, 128.53, 128.44, 127.02, 126.19, 118.83, 118.12, 115.23, 110.88, 84.78, 65.08.

Synthesis of (R)-2-amino-3-(2-(4-(2,2-dicyano-1-(4-(diphenylamino)phenyl)vinyl)phenoxy)acetamido)propanoic acid (TPACN-D-Ala):

Compound 5 (60 mg, 0.13 mmol), *N*-ethyl-*N*'-(3-dimethylaminopropyl)carbodiimide hydrochloride (38mg, 0.20 mmol) and *N*-hydroxysuccinimide (23 mg, 0.20 mmol) were dissolved in DMF (5 mL) and stirred at room temperature for 30 min. Ethyl acetate (30 mL) was added into the solution and the mixture was washed with water (30 mL × 6). After solvent evaporation under reduced pressure, the residue was dissolved in DMF (5 mL) with *N*-alpha-Fmoc-D-2,3-diaminopropionic acid monohydrochloride (18 mg, 0.13 mmol) and trimethylamine (0.18 mL, 1.3 mmol). The mixture was stirred at room temperature for 30 min. Ethyl acetate (30 mL) was added into the solution and the mixture was washed with water (30 mL × 6). After solvent evaporation under reduced pressure, crude compound 6 was obtained, which was dissolved in DMF (5 mL) and treated with piperidine (0.13 mL, 1.3 mmol) for 12 h. After evaporation of DMF and piperidine under reduced pressure, crude compound 7 was obtained, which was dissolved in dilute hydrochloric acid (10 mL) and washed with ethyl acetate (30 mL × 3). The aqueous layer was collected and freeze-dried to yield crude TPACN-D-Ala, which was further purified with high-performance liquid chromatography (HPLC).

HPLC conditions: column: Waters XBridge Prep OBDTM C18 5.0 μm (19×150 mm); gradient: 0–5–10 min, 40%–100%–100% B (A: H_2O, B: acetonitrile); flow rate is 8.0 mL min^{-1}; UV-Vis detector: 254 nm, 450 nm. TPACN-D-Ala was obtained as orange powder (13.2 mg, 17.1% yield). ^{1}H NMR (400 MHz, MeOD) δ 7.49 (d, J = 8.7 Hz, 2H), 7.42-7.31 (m, 6H), 7.24-7.13 (m, 8H), 6.92 (d, J = 8.9 Hz, 2H), 4.67 (s, 2H), 4.15-4.07 (m, 1H), 3.92-3.75 (m, 2H). ^{13}C NMR (100 MHz, MeOD) δ 173.34, 170.78, 160.99, 152.49, 145.91, 132.75, 132.52, 129.86, 129.59, 126.98, 126.26, 125.28, 118.11, 114.94, 114.64, 66.74, 38.88, 34.61.



Scheme S1. The synthetic route to TPACN-D-Ala probe.

$^1O_2$ quantum yield measurement

The $^1O_2$ generation quantum yield was calculated using the following equation:

$$\Phi_{SG,\ TPACN\text{-}D\text{-}Ala} = \frac{K_{TPACN\text{-}D\text{-}Ala}\ Area_{Rose\ Bengal}}{K_{Rose\ Bengal}\ Area_{TPACN\text{-}D\text{-}Ala}} \times \Phi_{SG,\ TPACN\text{-}D\text{-}Ala}$$

where $\Phi_{SG,Rose\ Bengal}$ is 0.75; $Area_{Rose\ Bengal}$ and $Area_{TPACN\text{-}D\text{-}Ala}$ represent the light absorbed by Rose Bengal and TPACN-D-Ala, respectively, which were determined by integration of the areas under the absorption bands in the wavelength range of 400-700 nm; $K_{Rose\ Bengal}$ and $K_{TPACN\text{-}D\text{-}Ala}$ are the decomposition rate constants of ABDA by

Rose Bengal and TPACN-D-Ala, respectively. The decomposition rate constant is determined as the slope by plotting $\ln(A_0/A)$ as the Y axis and time as the X axis, in which A_0 and A are the absorbance of ABDA at 378 nm by photosensitizers under white light irradiation at t = 0 and t = n s (n = 60, 120, 180, 240, 300).

Bacterial culture

Two multidrug-resistant staphylococcal strains were employed in this study: green-fluorescent *S. aureus* WH^{GFP} and *methicillin-resistant S. aureus* (*MRSA*). Both strains were cultured in LB medium at 37 °C. When the OD_{600} of the incubated cells reached 0.4-0.5, the cells were diluted to OD_{600}~0.05, which were again incubated in the shaker at 37 °C to reach OD_{600}~0.5, the start of log phase. The obtained bacterial cells were further collected and re-suspended in PBS to concentrations required, as determined in a Bürker–Türk counting chamber, for labeling, killing and infection experiments.

Metabolic labeling of bacteria

MRSA cells were cultured until the start of log phase and then collected by centrifugation at 3,000 rpm for 5 min. *MRSA* were re-suspended in 1 mL of LB medium (10^8 CFU/mL) containing TPACN-D-Ala (20 μM) and were incubated at 37 °C for 20 min. Cells were then collected (3,000 rpm, 3 min) and washed with 1 mL of PBS buffer for three times. Cells were re-suspended in 1 mL of PBS buffer with Hoechst 33342 and incubated at room temperature for 15 min to stain the bacterial nuclei. After being washed three times with PBS, bacteria were re-suspended in 1 mL of PBS and added into confocal dish for imaging. For TPACN-D-Ala imaging, the excitation wavelength was 405 nm, and the emission filter was 580−750 nm. For Hoechst 33342 detection, the excitation wavelength was 405 nm, and the emission filter was 430−470 nm.

Characterization of peptidoglycan fragment from MRSA.

Overnight pre-cultured bacterial cells were added into new LB medium and the bacteria were incubated on shaker until reaching log phase. Then, 5 mL of the bacteria were obtained by centrifugation and re-suspended in 5 mL of LB medium containing

10 μM TPACN-D-Ala and the mixture was incubated for 2 h. After that, the labeled bacteria were obtained by centrifugation at 5,000 g for 10 min and they were quickly cooled on the ice for 15 min and subsequently re-suspended in 4 mL water. The cells were washed once with 5 mL of PBS. The cell pellets were re-suspended in 5 mL of PBS for protecting the acetyl groups in bacteria. Then, the bacterial suspension was added into sodium dodecyl sulphate (SDS, 8% w/v) with the same volume and the mixture was boiled for 4 h under stirring. The samples were equilibrated to room temperature and collected with a tabletop centrifuge at 30,000 g for 15 min at room temperature and washed with water for three times to remove SDS and pellet the insoluble peptidoglycan. The pellet peptidoglycan was dispersed in a Tris buffer (pH = 7.5) with a concentration of 5 mM Tris-HCl, 10 mM NaCl and 100 μg/mL α-amylase and incubated for 2 h at 37 °C. The samples were then collected and dispersed in 10 mM Tris-pH 7.5 with 200 μg/mL pronase and incubated for 2 h at 37 °C under agitation. The samples were obtained and dispersed in 1 mL of water and added into the same volume of 4% SDS and boiled for 30 min with stirring. The samples were equilibrated to room temperature and collected with a tabletop centrifuge at 30,000 g for 15 min at room temperature and was washed with water for three times to remove SDS. The purified peptidoglycan was transferred to 10 mM Tris-pH 7.5 with 2 mg/mL mutanolysin and incubated at 37 °C for 2 h under agitation. Centrifugation at 15,000 g for 15 min was performed to remove the mutanolysin and insoluble peptidoglycan. The supernatant containing soluble peptidoglycan was collected for matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis. 2.5-dihydroxybenzoic acid (DHB) was used as the matrix. DHB was dissolved in water/acetonitrile (v/v = 50/50) at a concentration of 10 mg/ml. 10 μL DHB solution and 10 μL peptidoglycan fragment from MRSA was completely mixed on MALDI plate to run MALDI-TOF MS analysis.

Cell Culture:

Raw 264.7 cells were provided by American Type Culture Collection (ATCC). The cells were cultured in DMEM medium containing 100 μg/mL streptomycin, 10% heat

inactivated FBS, 100 U/mL penicillin, and maintained in a humidified incubator with 5% CO_2 at 37 °C for 5 days. The viabilities of Raw 264.7 cells were determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.

Raw 264.7 cells infection, intracellular bacteria imaging and ablation:

Raw 264.7 cells were plated in an 8-well plate with a density of 1×10^5 cells/well and the cells were incubated overnight at 37 °C with 5% CO_2. Then, the samples were infected with *MRSA* at a ratio of 15-20 bacteria per Raw 264.7 cell. Raw 264.7 cell cultures were maintained in growth medium (DMEM plus 10% FBS) and the plates were placed in the incubator for 1 h to allow the adherence of bacteria to macrophage cells and then the cell culture medium was removed and replaced with bacterial infection medium with different concentrations (10-100 μM) of TPACN-D-Ala. After 2 h incubation at 37 °C, the medium was removed and bacteria pellet was washed with PBS for three times, followed by addition of DMEM cell culture medium for intracellular bacteria imaging. For TPACN-D-Ala detection, the excitation wavelength was 405 nm, and the emission filter was 550−750 nm.

To measure the intracellular bacteria ablation effect, the *MRSA* intruded Raw 264.7 cells after incubation with different concentrations (10-100 μM) of TPACN-D-Ala were irradiated under white light with a power of 60 mW/cm^2 for 10 min. The survival of intracellular bacteria was assessed by using plate colony counting method. Macrophages were lysed with PBS solution supplemented with 1 % Triton-X, and serial dilutions of the lysate were made in PBS. The number of the surviving intracellular bacteria was determined by plating on LB agar plates.

Metabolic labeling and photodynamic therapy of bacterial biofilm:

Biofilms were grown by adding 100 μL of *S. aureus* WH^{GFP} suspension (10^8 CFU/mL) in PBS to 96-wells plates at 37 °C for 1 h to allow bacteria to adhere. Next, bacterial suspensions were removed, and the wells were washed with 100 μL of PBS. Subsequently, 1 mL of Tryptic Soy Broth (TSB) was added and adhering bacteria were grown into a biofilm for 48 h at 37 °C. Then, TPACN-D-Ala was added into TSB medium

and stained for different times. Subsequently, TPACN-D-Ala suspension was removed, and the biofilms were rinsed with PBS twice and were re-suspended in 1 mL of PBS and prepared for imaging. For TPACN-D-Ala detection, the excitation wavelength was 405 nm, and the emission filter was 580−750 nm. For *S. aureus* WH^{GFP} detection, the excitation wavelength was 488 nm, and the emission filter was 500-550 nm. To determine the inhibition effect of TPACN-D-Ala to bacterial biofilm, TPACN-D-Ala probe labeled *MRSA* were exposed to white light with a power of 60 mW/cm^2 for 15 min. After PDT, biofilms were scraped off a well and bacteria were suspended in PBS and serially diluted up to 5×10^6 fold. 10 μL of diluted bacterial solution was spread over a TSB agar plate for 24 h incubation at 37 °C and the CFUs were counted.

***In vitro* bacterial photodynamic therapy:**

MRSA cells (1 mL) were incubated with TPACN-D-Ala (20 μM) for 20 min. After washing twice, cells were re-suspended in PBS. 200 μL of the bacteria cells were dispersed into a 96-well plate. The phototoxicity of TPACN-D-Ala to *MRSA* cells was determined by irradiation under white light with a power of 60 mW/cm^2 for 15 min. To explore the relationship of the inhibition effect of TPACN-D-Ala to infectious bacteria, different concentrations of TPACN-D-Ala were incubated with *MRSA* before light irradiation. After light treatment, bacteria were directly diluted 2000 times in PBS and 100 μL of the diluted bacterial cells were spread on the LB agar plate, and the plates were incubated for 24 h at 37 °C to form the bacteria colonies. The colonies were counted to determine the colony-forming unit (CFU) of bacteria.

Bacterial infection mouse models:

All animal studies were performed in compliance with the guidelines set by Tianjin Committee of Use and Care of Laboratory Animals and the overall project protocols were approved by the Animal Ethics Committee of Nankai University. Male BALB/c nude mice (8-12 weeks old) were purchased from the Laboratory Animal Center of the Academy of Military Medical Sciences (Beijing, China). To establish the inflammation-bearing mouse models, *S. aureus* WH^{GFP} were firstly grown in LB media for 12 h in a

bacterial shaking incubator. When mid-log phase (OD_{600} = 0.5) was achieved, the bacteria were pelleted, washed and re-suspended in sterile PBS to a final concentration of 2 × 10^6 CFU/μL at 4 °C. The left back of mice was inoculated subcutaneously with 30 μL of the *S. aureus* WH^{GFP} suspension (2 × 10^6 CFU/μL) using a 27-gauge insulin syringe. The bacterial infection induced inflammation was formed after 24 h, which was confirmed by H&E staining.

***In vivo* bacteria labeling and photodynamic therapy of bacteria with *i.v.* injection of TPACN-D-Ala:**

Bacterial infection model was established in 6-week-old male BALB/c mice by subcutaneous injection of 30 μL of the *S. aureus* WH^{GFP} suspension (2 × 10^6 CFU/μL) into left back of mice. At 12 h after inoculation, TPACN-D-Ala (100 μL, 0.5 mg/mL), and saline (100 μL) were *i.v.* injected into bacteria bearing mice, respectively. The mice were immediately imaged with a Maestro *in vivo* fluorescence imaging system (CRi, Inc., Woburn, USA) (Excitation filter: 480 nm; Emission filter: 670 nm longpass; Acquisition setting: 500 to 900 in 10 nm steps; Exposure time: 500 ms). The autofluorescence was removed by spectral unmixing software. In addition, the bacteria-bearing mice were sacrificed at 6 h post-injection of TPACN-D-Ala and the different tissues including infected skin were collected, imaged and quantified by the Maestro system. All values were expressed as means ± standard deviation (n = 3). For photodynamic therapy, at 6 h post injection of TPACN-D-Ala and saline (n = 5 in each group), the infected sites of BALB/c mice in each group were exposed to white light (300 mW/cm^2) for 15 min. After 7 days, bacteria-bearing mice were sacrificed and infected skin was collected and then homogenized in a tissue grinder with 2 mL of PBS and spread over a LB agar plate. In addition, skin tissues of mice in two groups were collected, dissected and fixed in 4 % paraformaldehyde (PFA). Afterwards, the tissues were embedded in 30% sucrose/PBS overnight and embedded in Optimal Cutting Temperature (OCT) compound (Tissue-Tek). The slices were subsequently stained with DAPI containing mounting solution (DAPI Fluoromount G, Southern Biotech). These slices were finally imaged by CLSM.

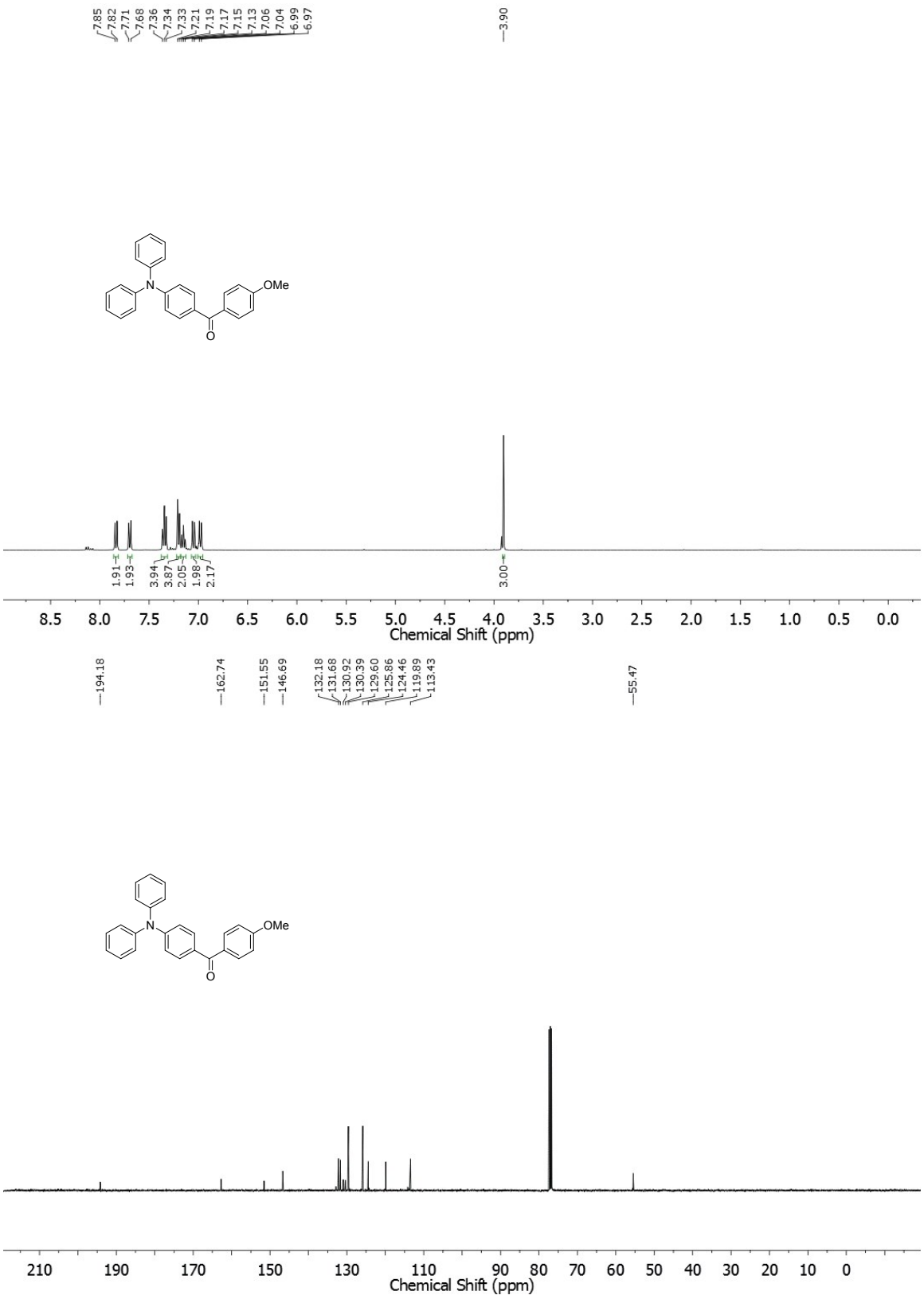


Fig. S1. ^{1}H and ^{13}C NMR of compound 3 in $CDCl_3$.

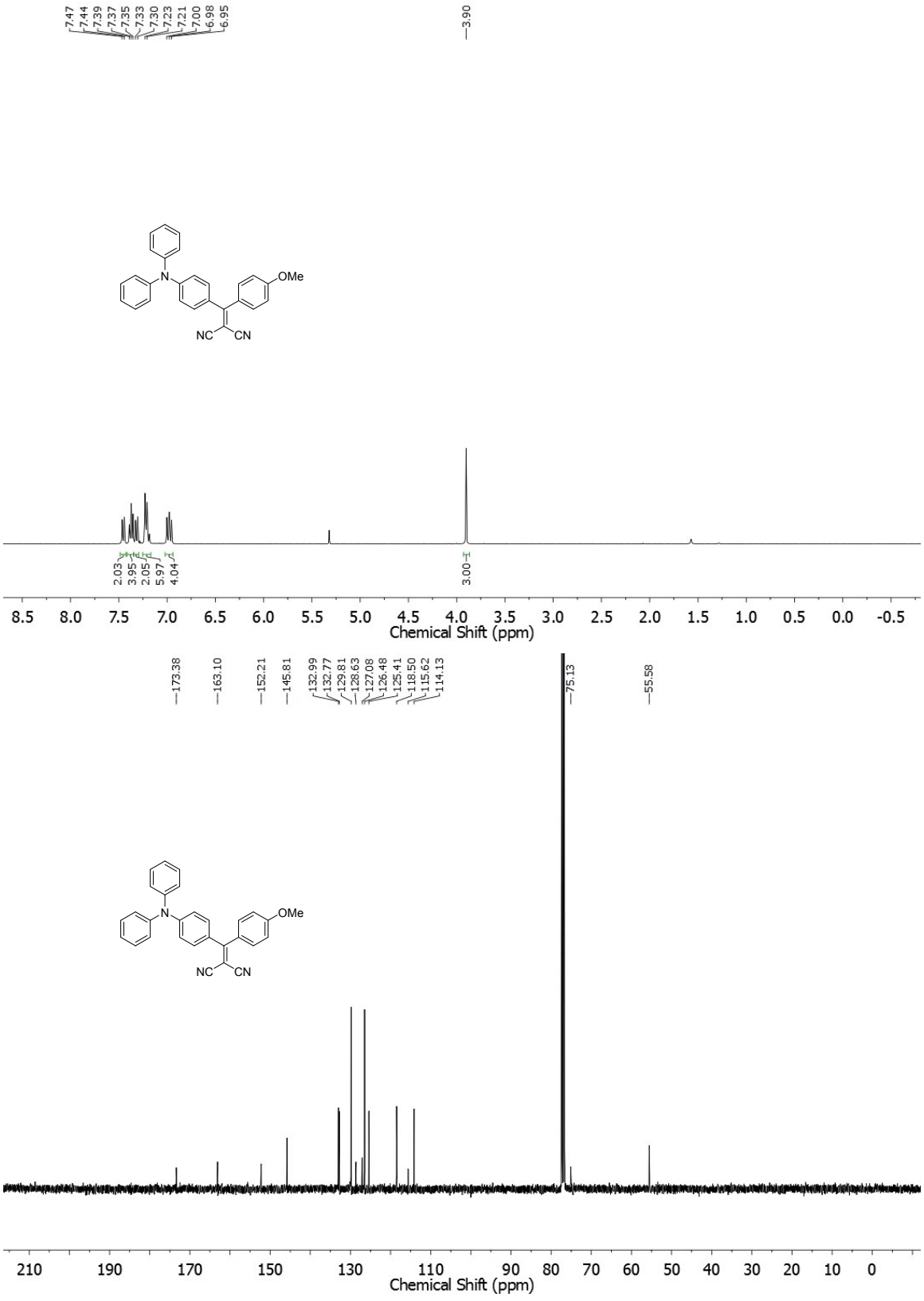


Fig. S2. ^{1}H and ^{13}C NMR of TPACN in $CDCl_3$.

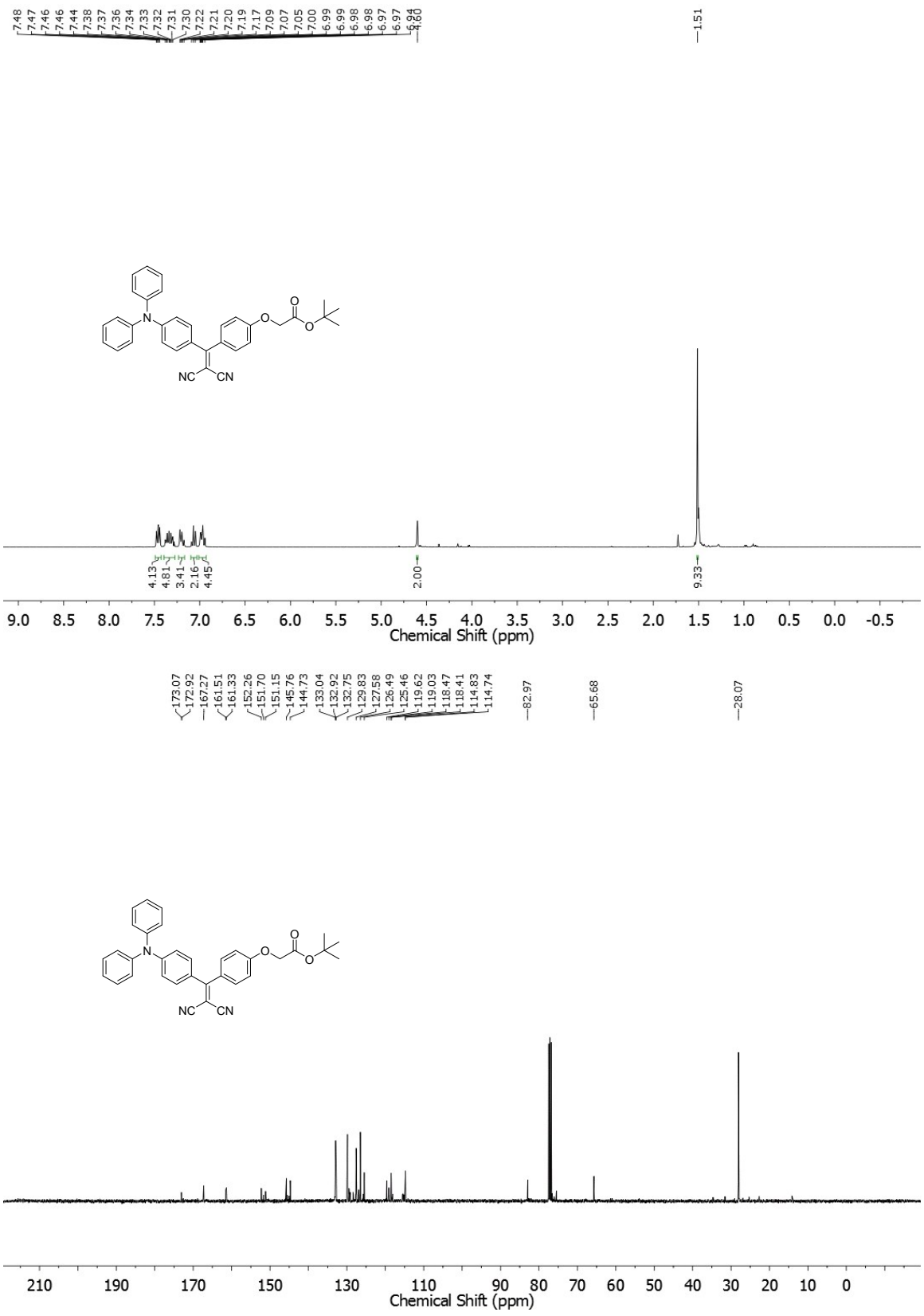


Fig. S3. ^{1}H and ^{13}C NMR of compound 4 in $CDCl_3$.

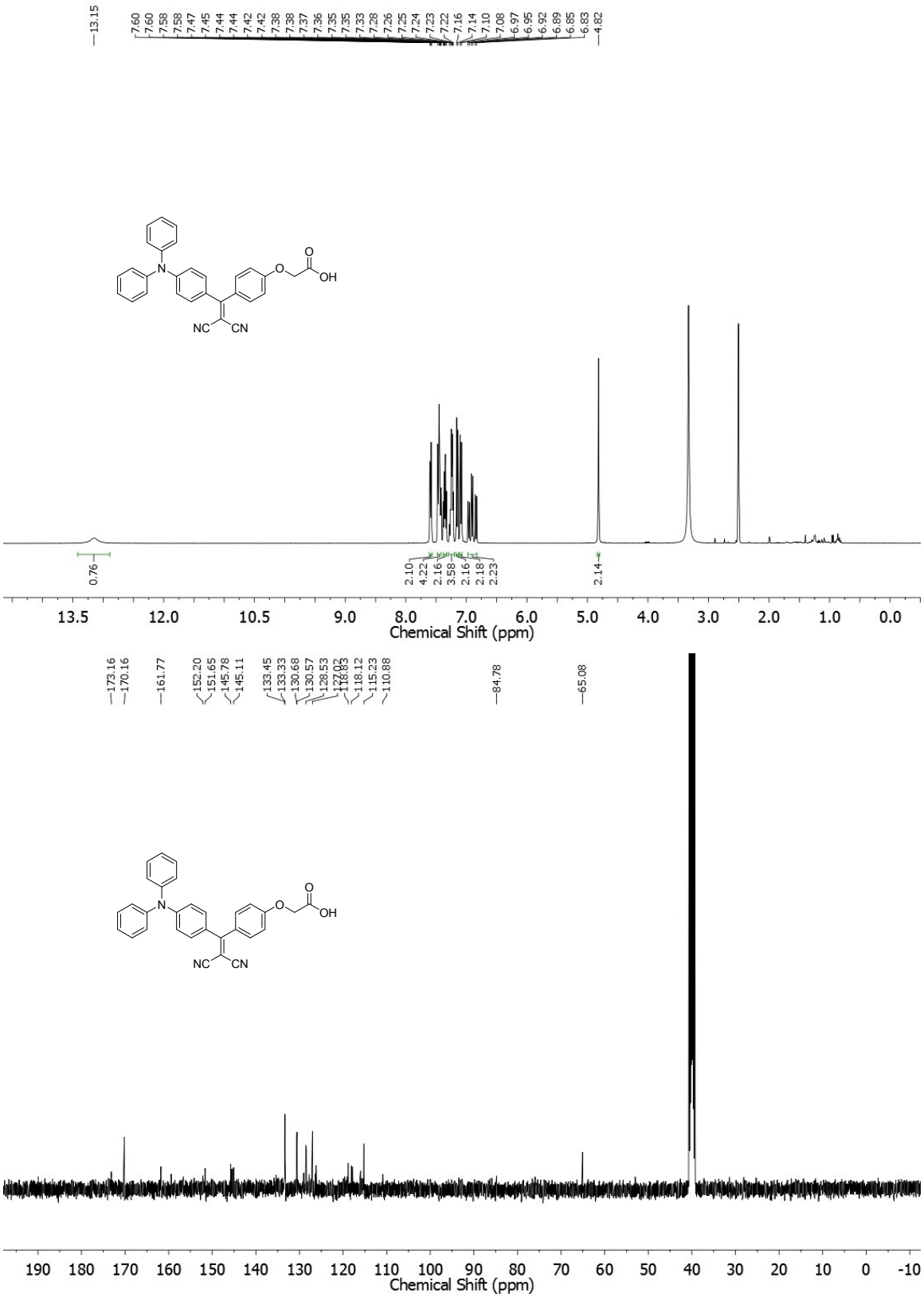


Fig. S4. ^{1}H and ^{13}C NMR of compound 5 in DMSO-d_6.

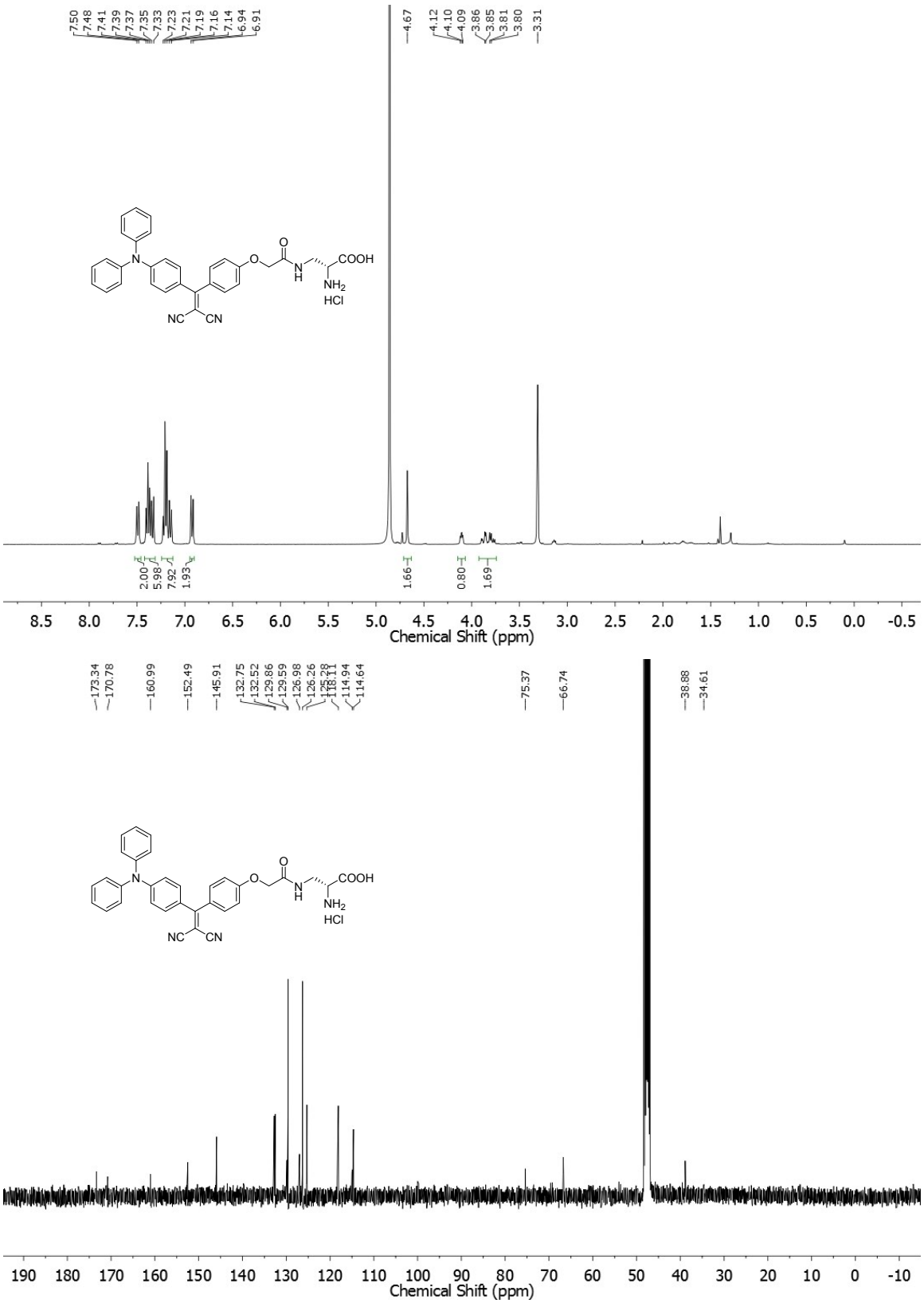


Fig. S5. ^{1}H and ^{13}C NMR of TPACN-D-Ala in DMSO-d_6.

Mass Spectrum SmartFormula Report

Analysis Info

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Method	YCH-50-500.m	Operator	default user
Sample Name	3	Instrument / Ser#	micrOTOF-Q II 10269
Comment	ChBE Prof Liu Bin		

Acquisition Parameter

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Scan End	800 m/z	Set Collision Cell RF	200.0 Vpp	Set Divert Valve	Waste

Meas. m/z	#	Formula	m/z	err [ppm]	rdb	e¯ Conf	N-Rule
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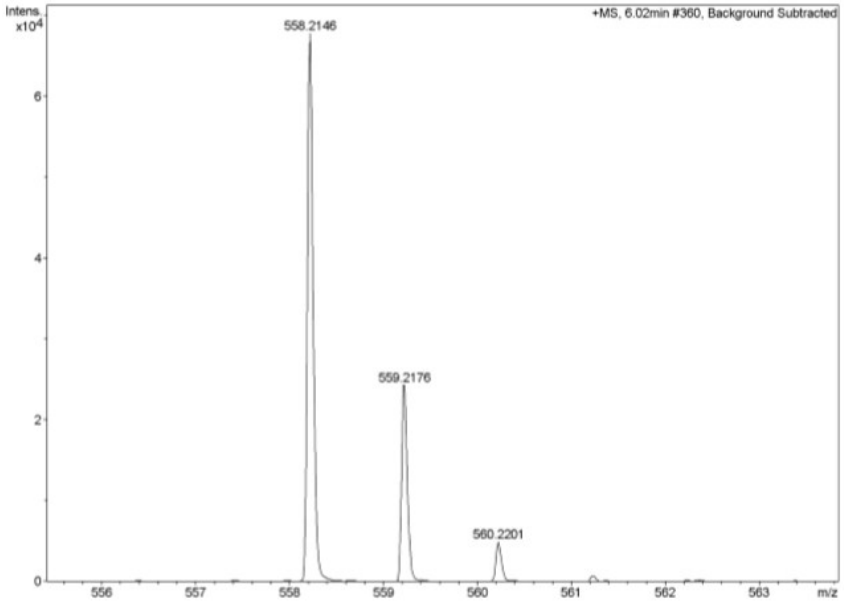

Fig. S6. High-resolution mass spectrum of TPACN-D-Ala.

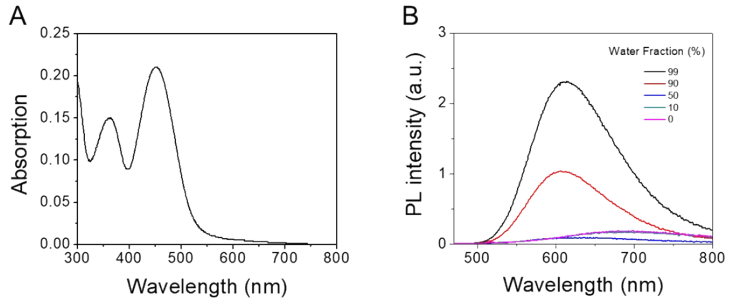

Fig. S7. (A) UV absorption and (B) AIE curve of TPACN in water.

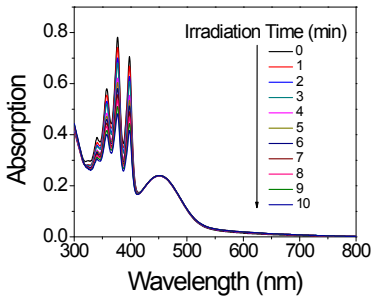

Fig. S8. Absorption spectra of ABDA and TPACN (10 μM) mixture upon white light irradiation (60 mW/cm^2).

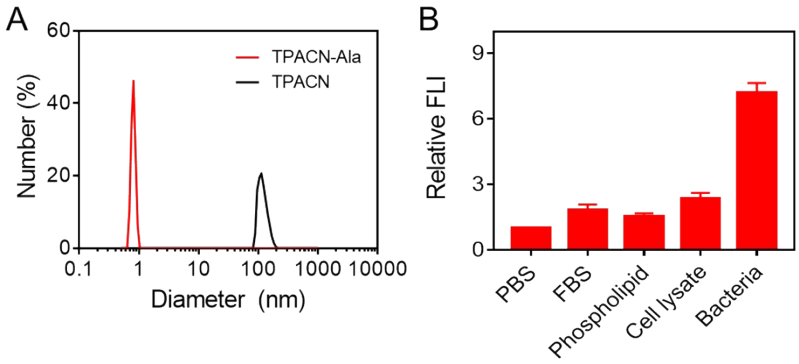

Fig. S9. (A) Size distribution of TPACN-D-Ala as measured by dynamic light scattering (DLS). (B) Relative fluorescence intensities (FLI) of TPACN-D-Ala in PBS or different solutions, including FBS (1% in PBS), phospholipid (0.01 mg/mL), cell lysate, and *MRSA*.

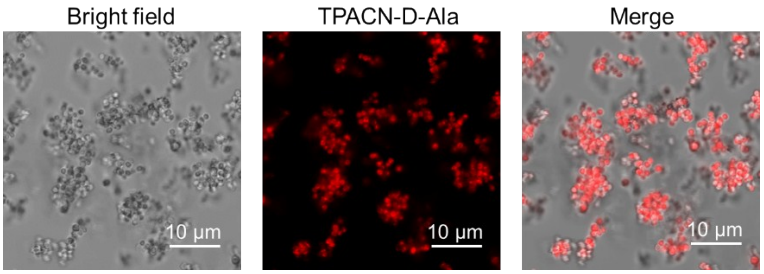

Fig. S10. CLSM images of Van A cells treated with TPACN-D-Ala (10 μM).

Bacterial Peptidoglycan Fragment	Calculated Molecular Weight
GlcNAc-MurNAc-Ala-Glu-DAP	940
GlcNAc-MurNAc-Ala-Glu-DAP-D-Ala-TPACN	1408

Table S1. Calculated molecular weights of TPACN-D-Ala treated bacterial peptidoglycan fragments

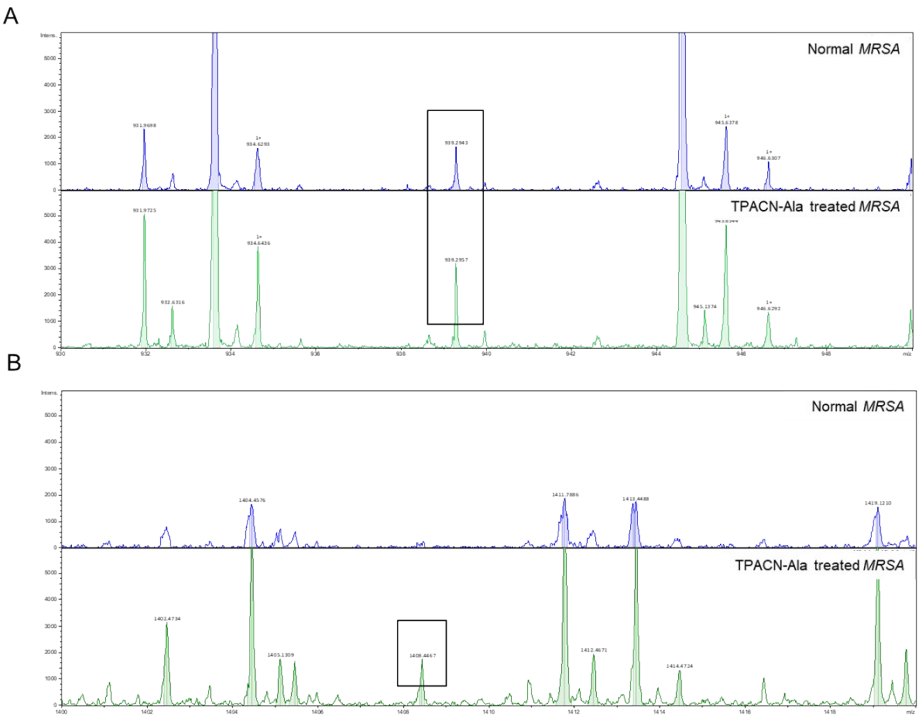

Figure S11. MALDI-TOF MS of normal and TPACN-D-Ala treated MRSA. A. M/Z range is 930-948. B. M/Z range is 1400-1418.

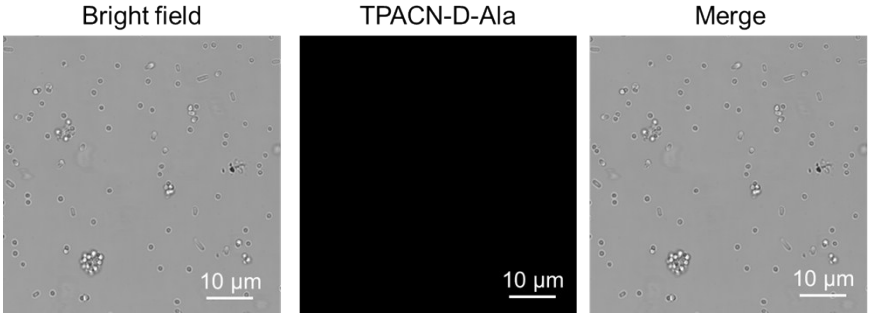

Figure S12. CLSM images of 4 % PFA fixed MRSA treated with TPACN-D-Ala (10 μM).

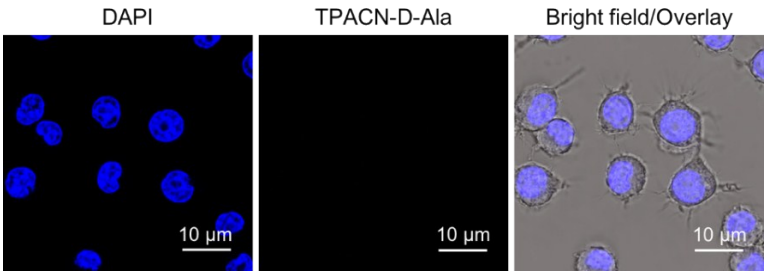

Fig. S13. Confocal fluorescence images of normal RAW 264.7 cells treated with TPACN-D-Ala and Hoechst 33342 (blue color). Scale bar =10 μm.

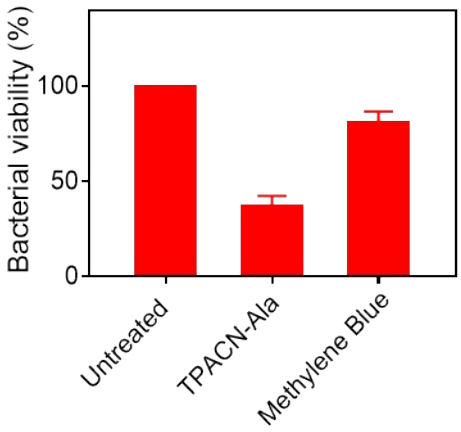

Fig. S14. Intracellular bacterial viability of *MRSA*-infected RAW 264.7 cells incubated with TPACN-D-Ala or Methylene Blue (50 μM) upon light irradiation (60 mW/cm^2).

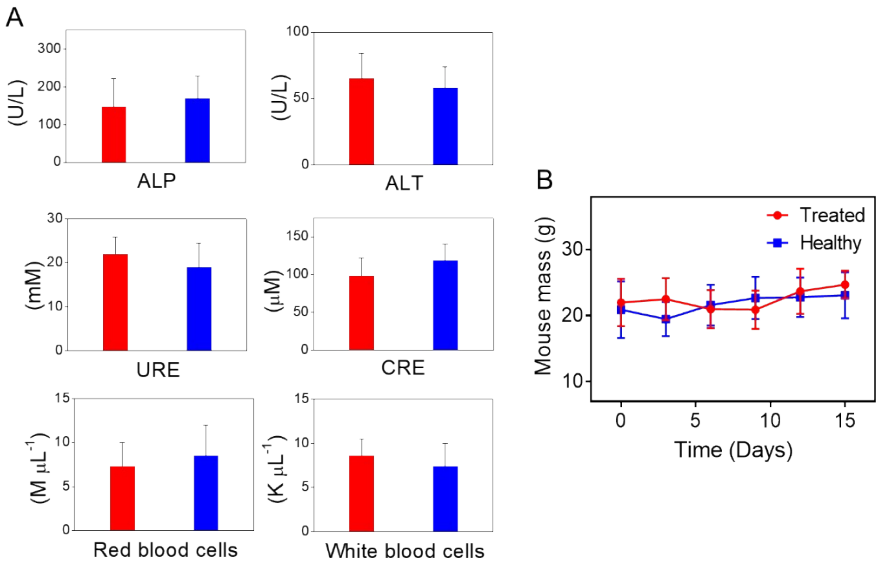

Fig. S15. Blood test parameters about liver and kidney function and body weight measurement of mice with and without injection of TPACN-D-Ala (n = 3 per group).

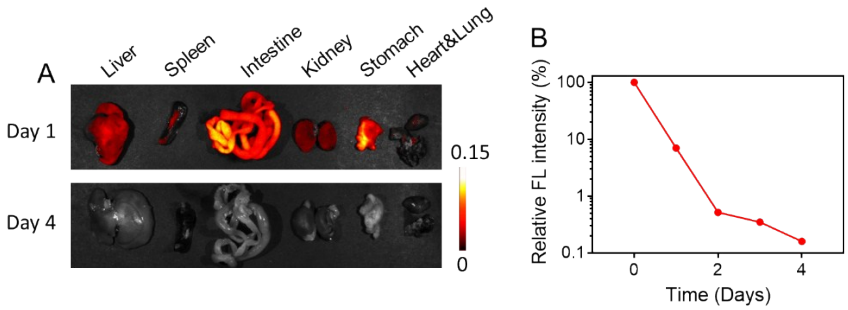

Fig. S16. (A) *Ex vivo* fluorescent images of organs at various time post injection of TPACN-D-Ala. (B) Relative fluorescence intensity of TPACN-D-Ala in the blood at different time points.

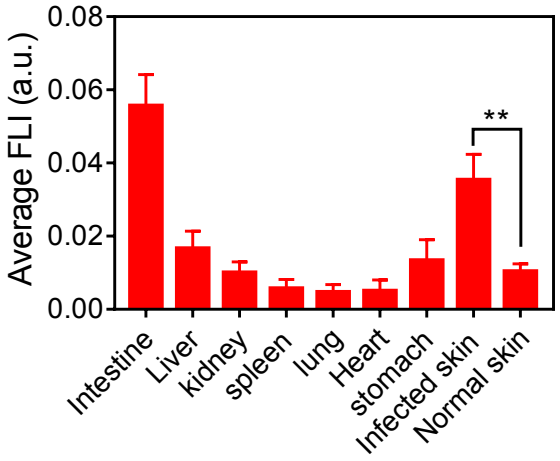

Fig. S17. Relative fluorescence intensity of different organs of mice post-injection of TPACN-D-Ala. (n = 3, **p < 0.01)

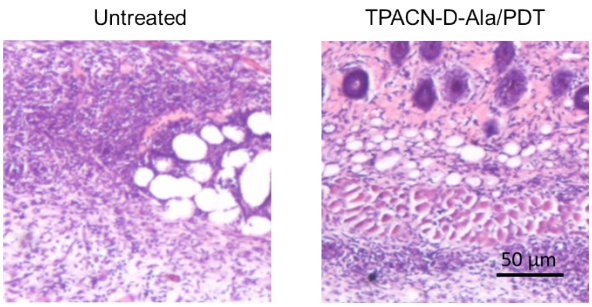

Fig. S18. Enlarged H&E staining images of the infected skin slices of mice subjected to TPACN-D-Ala and PDT treatments.