Supplementary Information

Insights into eradication of drug resistant *Staphylococcus aureus* via compound 6-nitrobenzo[cd]indole-2(1H)-ketone

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Materials and Methods

Compound synthesis

The compounds were synthesized via the pathway shown in Figure S1. The synthesis methods are as follows.

C2: Benzo[cd]indole-2(1H)-ketone (1.7 g, 10 mmol) was dissolved in glacial acetic acid. Nitric acid (2.1 mL, 50 mmol) was added slowly. Then the solution was stirred at room temperature and reacted for 24 h. After the reaction, the solution was poured into 300 mL of ice water, and a large amount of precipitation was obtained. The precipitation was filtered, washed and dried by vacuum to obtain brown solid, namely 6-nitrobenzo[cd]indole-2(1H)-ketone. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.38 (s, 1H), 8.84 (d, J = 8.4 Hz, 1H), 8.60 (d, J = 8.0 Hz, 1H), 8.15 (d, J = 7.0 Hz, 1H), 8.03 (dd, J = 8.4, 7.1 Hz, 1H), 7.09 (d, J = 8.0 Hz, 1H).^{13}C NMR (400 MHz, DMSO-d_6) δ 169.37, 145.90, 138.19, 132.98, 131.37, 129.53, 126.93, 126.10, 125.77, 122.46, 105.42.$[M-H]^-$ = 213.0305, found: 213.0311.

C1: 6-nitrobenzo[cd]indole-2(1H)-ketone (1.71 g, 8 mmol) and potassium hydroxide (0.8 g, 14 mmol) were added into a round-bottom flask containing DMF. After stirring in an ice bath for 1 h, iodoethane (1.3 mL, 16 mmol) was added and refluxed at 90ºC for 12 h. After cooling to room temperature, the solution was poured into ice water to obtain yellow precipitation. The precipitation was filtered, washed and dried by vacuum to obtain 1-ethyl-6-nitrobenzo[cd]indole-2(1H)-ketone. ^{1}H NMR (400 MHz, Chloroform-d) δ 9.01 (d, J = 8.7 Hz, 1H), 8.63 (d, J = 8.0 Hz, 1H), 8.14 (d, J = 7.0 Hz, 1H), 7.93 (dd, J = 8.6, 7.0 Hz, 1H), 6.95 (d, J = 8.0 Hz, 1H), 4.00 (q, J = 7.3 Hz, 2H),

1.40 (t, J = 7.3 Hz, 3H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 167.78, 145.73, 139.16, 132.04, 130.18, 130.03, 126.18, 125.81, 125.55, 122.71, 102.91, 35.26, 13.89. $[M+H]^+$ = 243.0764, found: 243.0760.

C4: 1-ethyl-6-nitrobenzo[cd]indole-2(1H)-ketone (1.45 g, 6 mmol) was dissolved in anhydrous ethanol and 1 g palladium on carbon was added. After H2 replacement, the solution was refluxed at 90ºC for 5 h. After cooling to room temperature, the solution was poured into 2-3 cm high silica gel column, eluted with anhydrous ethanol to remove palladium on carbon. The product was subjected to rotary evaporateion and vacuum drying to obtain bright red-brown solid, namely 6-amino-1-ethylbenzo[cd]indole-2(1H)-ketone. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.07 (d, J = 7.1 Hz, 1H), 8.00 (d, J = 8.2 Hz, 1H), 7.67 (dd, J = 8.2, 7.0 Hz, 1H), 6.73 (d, J = 7.4 Hz, 1H), 6.64 (d, J = 7.5 Hz, 1H), 4.11 (s, 2H), 3.94 (q, J = 7.2 Hz, 2H), 1.36 (t, J = 7.2 Hz, 3H). ^{13}C NMR (400 MHz, Chloroform-*d*) δ 167.27, 138.39, 131.10, 127.58, 127.41, 125.59, 125.26, 124.33, 121.52, 109.70, 106.44, 34.93, 14.13. $[M+H]^+$ = 213.1022, found: 213.1025.

C3: 4-nitrobenzo[cd]indole-2(1H)-ketone (1.71 g, 8 mmol) and potassium hydroxide (0.8 g, 14 mmol) were added into a round-bottom flask containing DMF. After stirring in an ice bath for 1 h, iodobutane (1.8 mL, 16 mmol) was added and refluxed at 90ºC for 12 h. After cooling to room temperature, the solution was poured into ice water to precipitate yellow precipitation. The precipitation was filtered, washed and dried by vacuum to obtain 1-butyl-6-nitrobenzo[cd]indole-2(1H)-ketone. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (d, J = 8.4 Hz, 1H), 8.64 (d, J = 8.0 Hz, 1H), 8.18 (d, J = 7.0 Hz, 1H), 8.05 – 7.99 (m, 1H), 7.35 (d, J = 8.1 Hz, 1H), 3.92 (t, J = 7.1 Hz, 2H), 1.72 – 1.63 (m,

2H), 1.40 – 1.27 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR (400 MHz, DMSO-d_6) δ 167.73, 146.18, 138.62, 132.89, 131.13, 129.72, 126.15, 125.93, 124.88, 122.12, 104.93, 30.56, 19.96, 14.03. $[M+H]^+$ = 271.1077, found:271.1078.

Reagents

2,7-dichlorofluorescein diacetate (DCFH-DA, R272916) and DiBAC4(3) (D141133, Aladdin, China) were purchased from Aladdin (Shanghai, China). Fluorescein diacetate (FDA, F8040), crystal violet (C8470) and ATP Content Assay Kit (BC0305) were obtained from Solarbio (Beijing, China). Methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC43300) strain was purchased from Chuanxiang Biotechnology (Shanghai, China). *Staphylococcus aureus* (ATCC27664) was purchased from SHBCC (Shanghai, China). SYTO9/PI Live/Dead Bacterial Double Stain Kit were purchased from MaokangBio (MX4234, Shanghai, China). The PerfectStart Green qPCR SuperMix was obtained from TransGen Biotech (AQ601, Beijing China).

MIC and MBC assay

Minimum inhibitory concentration (MIC) of the compounds were determined by microdilution method.[1] The bacterial suspension was diluted to 10^6 cfu mL^{-1}, which was used for determining antibacterial efficacy. The stock solutions of compounds were prepared with DMSO. Then the stock solutions were diluted to different concentration (2048 μg/mL, 1024 μg/mL, 512 μg/mL, 256 μg/mL, 128 μg/mL, 64 μg/mL, 32 μg/mL, 16 μg/mL, 8 μg/mL, 4 μg/mL, 2 μg/mL, 1 μg/mL, 0.5 μg/mL and 0.25 μg/mL) by using media. 100 μL of compound dilutions were added to the wells of 96 well plate followed by the addition of 100 μL of bacterial suspension (10^6 cfu mL^{-1}). Two controls were

made: one containing 200 μL of media and the other containing 200 μL of bacterial suspension. The plates were then incubated at 37°C for 20 h. After the incubation, read the results. Each concentration was determined in triplicate and the whole experiment was repeated at least three times. The results were expressed as minimum inhibitory concentration.

To determine the minimum bactericidal concentration (MBC) of compounds, the bacterial suspension that appeared to have less/little turbidity in the MIC experiment was plated (10 μL) and the agar plates were incubated for 24 h at 37°C. Concentration at which no bacterial growth (no bacterial colony) was observed was taken as the MBC of compounds.

Time-kill kinetic assay

To determine the rapidity of the bactericidal effect of compounds against MRSA, a time-kill kinetic methodology was used.[2] The log-phase MRSA was diluted to approximately 10^6 cfu mL^{-1}, and 5 mL of the bacterial suspension was transferred into glass flasks with different concentrations of compounds (0.5, 1, 2 and 4 × MIC). The suspensions were incubated at 37°C and 180 rpm for 0, 2, 4, 8, 12, 18 and 24 h. After incubation, 200 μL samples were collected and serially diluted, followed by plating of 10 μL in triplicate on agar plates. After overnight incubation at 37°C, the colonies were counted, and average values were calculated. A growth control without compounds, as well as a sterile control, were used in each experiment. The time-kill curves were performed in triplicate.

Live/dead assay (SYTO9/PI staining)

After treatment, the bacteria cells were collected, stained with propidium iodide (PI, 20 μM) and SYTO9 (3.34 μM) for 15 min in the dark, and imaged using a confocal microscopy (Nikon C2, Japan).

Membrane potential

The change of the membrane potential was investigated by using a membrane potential sensitive fluorochrome, DiBAC4(3).[3, 4] After treatment with C2 and C1 for 6 h, the bacteria cells were collected. Then, 100 μL of collected cell suspensions were placed in black 96-well plates for 30 min at 37°C. 1 μL of the fluorescent probe DiBAC4(3) was added and incubated 30 min at 37°C. Finally, fluorescence was measured at the excitation and emission wavelengths of 492 and 515 nm, using a fluorescence microplate reader (Varioskan LUX, Thermo Fisher, USA).

ROS detection

ROS generation in bacterial cells was measured using an oxidation sensitive dye 2,7-dichlorofluorescein diacetate (DCFH-DA, R272916, Aladdin, China).[5] Bacterial cells were treated with C2 and C1 at the indicated concentrations for 2 h, then the bacteria were incubated with DCFH-DA (10 μM) for 20 min in the dark. The generation of ROS was detected by flow cytometry (NovoCyte, Agilent, USA) and fluorescence microscope (Axio Scope5, ZEISS, Germany).

ATP content assay

The level of cellular ATP is an important parameter for evaluating the available energy in a microorganism. MRSA was incubated with C2 and C1 (0, 2, 4, and 8 μg mL^{-1}) for 6 h. The ATP levels were detected using the ATP Content Assay Kit (BC0305,

Solarbio).

RNA extraction and qPCR

Total RNA was extracted from MRSA using the Total RNA Extraction Kit (R1200, Solarbio, China) following the manufacturer's guideline. 1 μg of the total RNA was reverse transcribed using the All-in-One First-Strand cDNA Synthesis SuperMix (AU341, TransGen Biotech, China). The quantitative real-time PCR (qPCR) amplification mixture contained cDNA, 2×PerfectStart Green qPCR SuperMix (AQ601, TransGen Biotech), and forward and reverse primers (0.2 μM each). Primers for amplification are shown in Table S3.[6] qPCR was carried out with the PIKOREAL 96 Real Time PCR System (Thermo, Finland). Every assay was performed in triplicate and all experiments included analysis of 16S rRNA mRNA levels as internal standard. Relative expression was determined by the Ct method and levels were expressed as percentage relative to the 16S rRNA mRNA levels.

Biofilm formation and crystal violet staining assay

MRSA were cultured in liquid LB medium and grown overnight at 37°C and 180 rpm. A diluted MRSA solution was plated in 96-well SensoPlatesTM (Greiner Bio-One, USA) with glass flat bottom and allowed to culture for 48 h at 37°C for biofilm formation. Removed the medium and added fresh medium every 24 h. To eliminate unbound bacteria, each well was washed with aseptic PBS buffer. 100 μL of crystal violet solution (0.1%) was added to the biofilms and incubated for 10 min. Removed the solution and washed with PBS buffer. The images were acquired using optical microscope.

To quantify the biofilm, the biofilm grew in 24 well plate with glass flat bottom was washed with PBS for 3 times. 500 μL of glutaraldehyde solution (2.5%) was added for fixation for10 min. Then glutaraldehyde solution was discarded, and the biofilm was washed with PBS. 500 μL of 0.1% crystal violet solution was added and incubated for 10 min. Crystal violet solution was removed, washed with PBS and air-dried. Then 30% acetic acid (500 μL) was added to dissolve dye for 30 min. 100 μL of the dye solution was tested by optical density at 590 nm using a plate reader (Epoch2, BioTeK, Germany).

Fluorescein diacetate (FDA) staining assay

The biofilms grew in 24 well plate was washed three times with PBS. 500 μL 20 μg/mL FDA solution was added and incubated in the dark for 30 min. After washed with PBS for two times, the biofilms were observed using confocal microscopy (Nikon C2, Japan).

Quantification of cfu in biofilms

The biofilms grew in 24 well plate was washed with PBS. After washing, 200 μL PBS was added to wells and biofilm was detached by scraping wells with a 200 μL pipette tip 15 times across the well. The scraped biofilm was then homogenized in solution by repeatedly pipetting the solution five times. Serial dilutions were then created by adding 100 μL to 900 μL PBS. Finally, 100 μL of homogenized biofilm suspension was spread out onto agar plates at different dilutions. Plates were incubated for 24 h under static conditions at 37°C. Colonies on plates were enumerated and expressed as cfu/mL.[7]

Animal models

Male ICR mice weighted about 20.0 g were purchased from Shanxi Medical University (Shanxi, China). The mice were housed in a temperature-controlled, ventilated and standardized disinfected animal room, and allowed to acclimatize for 1 week before the start of experiments. All animal procedures were in accord with the guidelines of the Institutional Animal Care and Use Committee. All in vivo experiments were approved by the Animal Ethics Committee of Shanxi Medical University. To assess the effects of C2 and C1 on wound healing, a wound (about 1 cm) was created on the back of the mice, and 10^7 methicillin-resistant *Staphylococcus aureus* (MRSA) were injected to construct an infected wound model. The mice were divided into four groups (n = 6). Then the mice were subcutaneously administrated by PBS, C2, C1 or vancomycin (50 μL, 1 mg kg^{-1}) every day during a 3 days administration period. The wounds of the mice were recorded by camera every day. At day 3, the wound tissues were removed from mice, and the numbers of bacteria in the wounds were determined by plate count methods.[8]

To evaluate biofilm elimination effects of C2 in vivo, the wounds on the back of the mice were infected with MRSA (1×10^8 cfu) for 2 days to form initial abscesses. The mice were divided into four groups (n = 6). Then the mice were subcutaneously administrated by PBS, C2, C1 or vancomycin (50 μL, 1 mg kg^{-1}) every day during an 8 days administration period. The wounds of the mice were recorded by camera every day. At day 8, the wound tissues were removed from mice, and the numbers of bacteria in the wounds were determined by plate count methods.[9]

Enzyme-linked immunosorbent assay (ELISA)

TNF-α and IL-6 levels around the wounds were detected by ELISA. ELISA assay kits (EM0183 and EM0121) were purchased from Fine Biotech (Wuhan, China), and all procedures followed the manufacturer's instructions.

Haematoxylin and eosin (H&E) staining analysis

Immediately after surgical removal, wound tissues and organs (liver, spleen, kidney, heart and lung) were fixed overnight in 4% paraformaldehyde, embedded in paraffin, sectioned (4 μm thick) and stained with hematoxylin and eosin (H&E). The histological sections were observed under an optical microscope.

Statistical analysis

All experiments were repeated at least three times. SPSS 17.0 software was used to calculate the statistical significance of the experimental results. The significance level was set as $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ and $****P < 0.0001$. Error bars denote the standard deviation (SD).

Tables and Figures

Table S1. MIC and MBC values for compounds against MRSA

Compound	MIC (μg mL^{-1})	MBC (μg mL^{-1})	MBC/MIC
C1	8	16	2
C2	4	8	2
C3	32	256	8
C4	64	512	8
Vancomycin	1	2	2
Methicillin	64	128	2

Table S2. MIC and MBC values for compounds against *S. aureus*

Compound	MIC (μg mL^{-1})	MBC (μg mL^{-1})	MBC/MIC
C1	2	4	2
C2	1	2	2
C3	8	32	4
C4	32	256	8

Table S3. Primer sequences for qPCR experiments

Gene	**Sequence (5'-3')**
α-hemolysin	Sense Strand: TTGGTGCAAATGTTTC
	Antisense Strand: TCACTTTCCAGCCTACT
agrA	Sense Strand: TGATAATCCTTATGAGGTGCTT
	Antisense Strand: CACTGTGACTCGTAACGAAAA
16S rRNA	Sense Strand: GCTGCCCTTTGTATTGTC
	Antisense Strand: AGATGTTGGGTTAAGTCCC

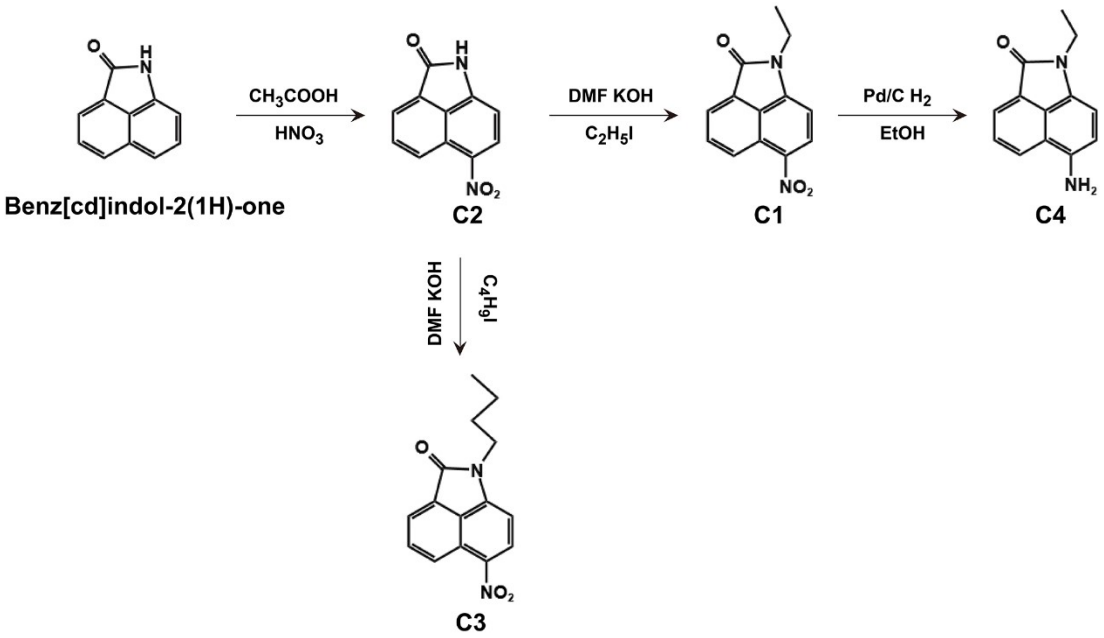

Fig. S1 Synthetic routes of compounds.

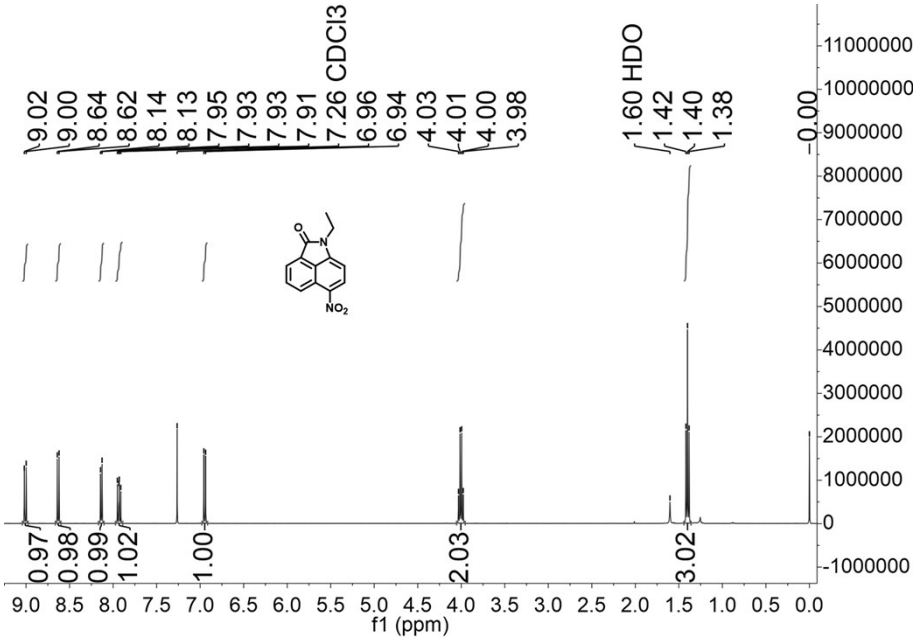

Fig. S2 ^{1}H NMR spectra of compound C1.

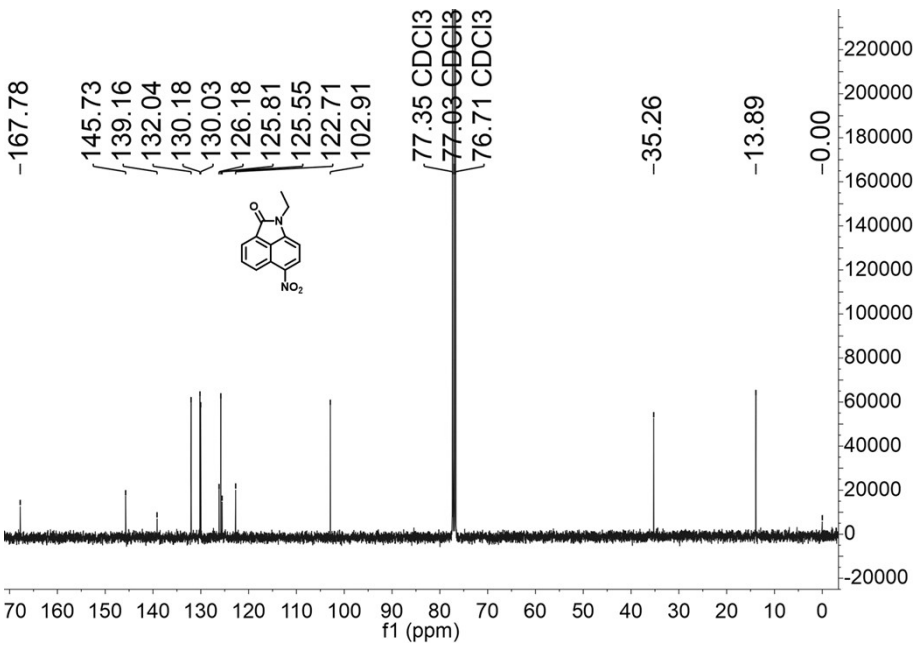

Fig. S3 ^{13}C NMR spectra of compound C1.

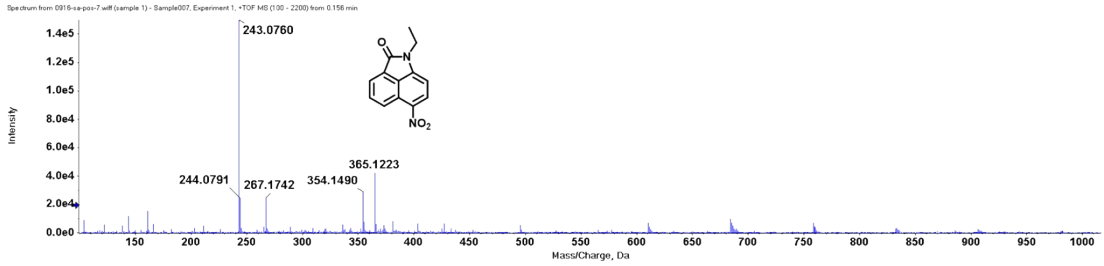

Fig. S4 Mass spectrum of compound C1.

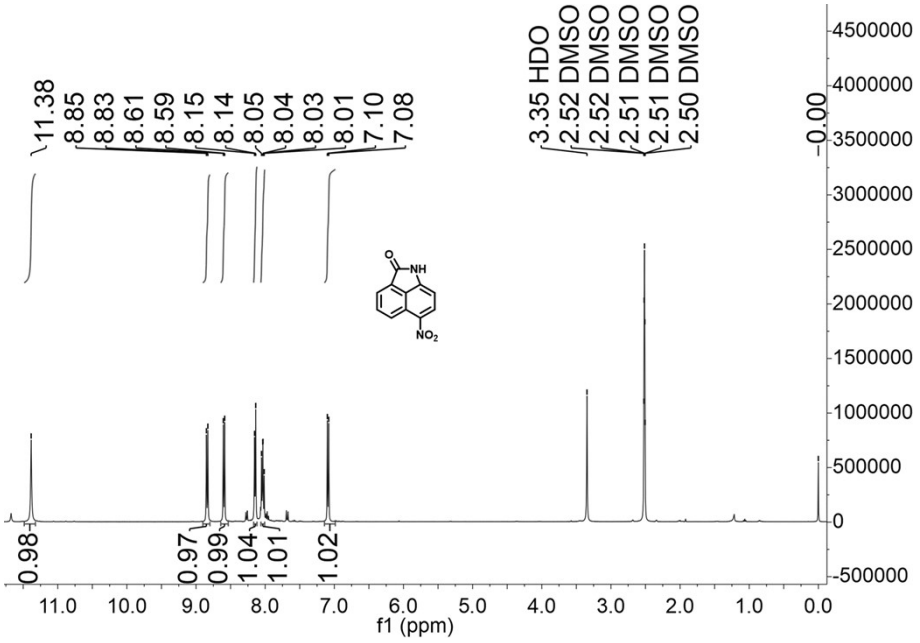

Fig. S5 ^{1}H NMR spectra of compound C2.

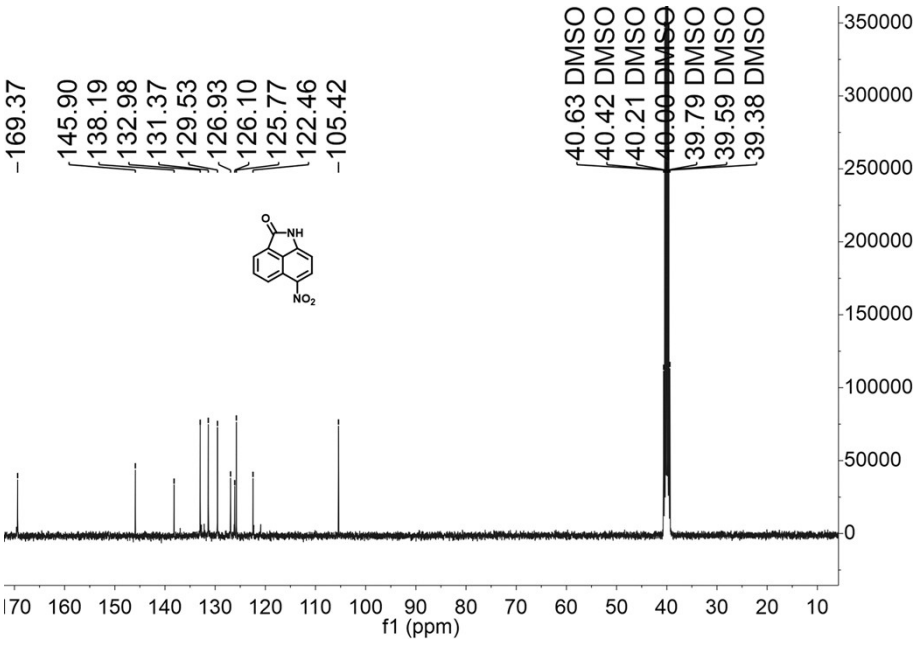

Fig. S6 ^{13}C NMR spectra of compound C2.

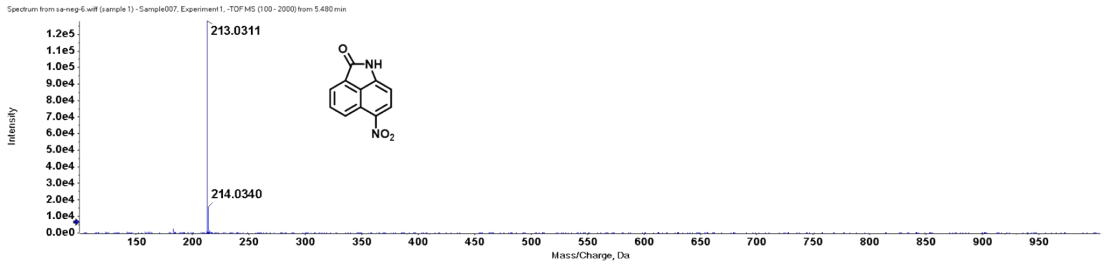

Fig. S7 Mass spectrum of compound C2.

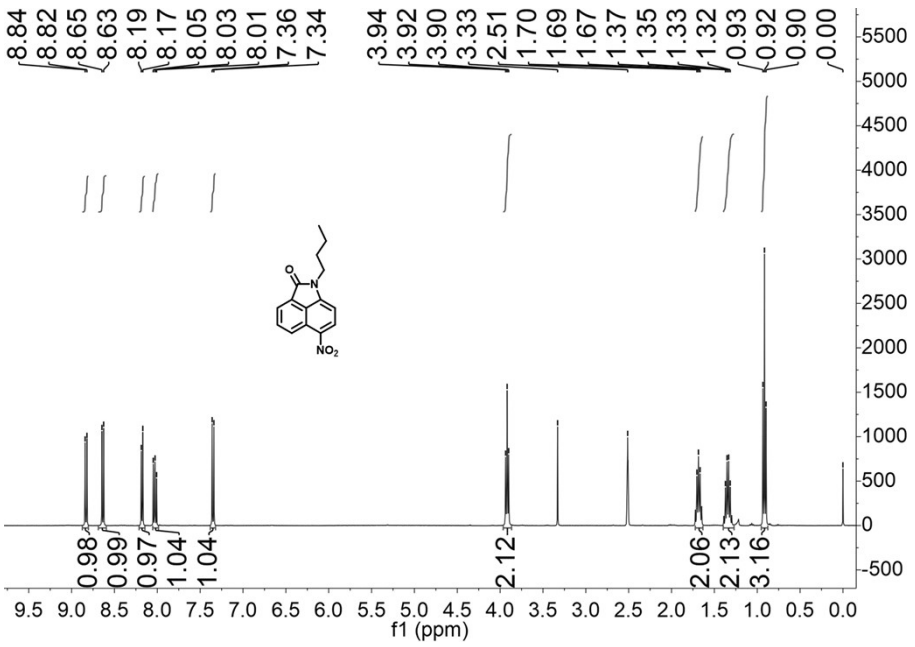

Fig. S8 ^{1}H NMR spectra of compound C3.

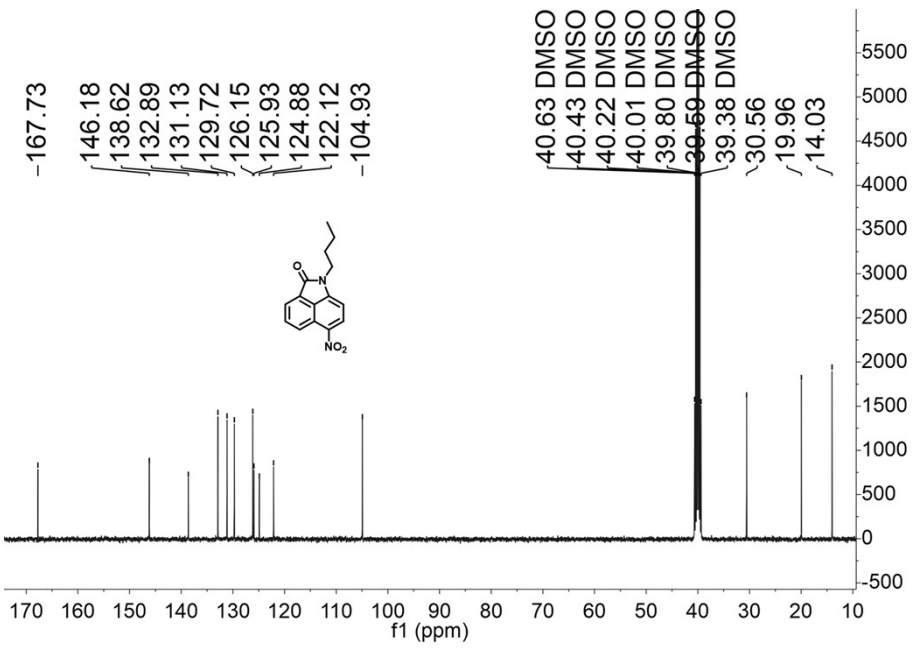

Fig. S9 ^{13}C NMR spectra of compound C3.

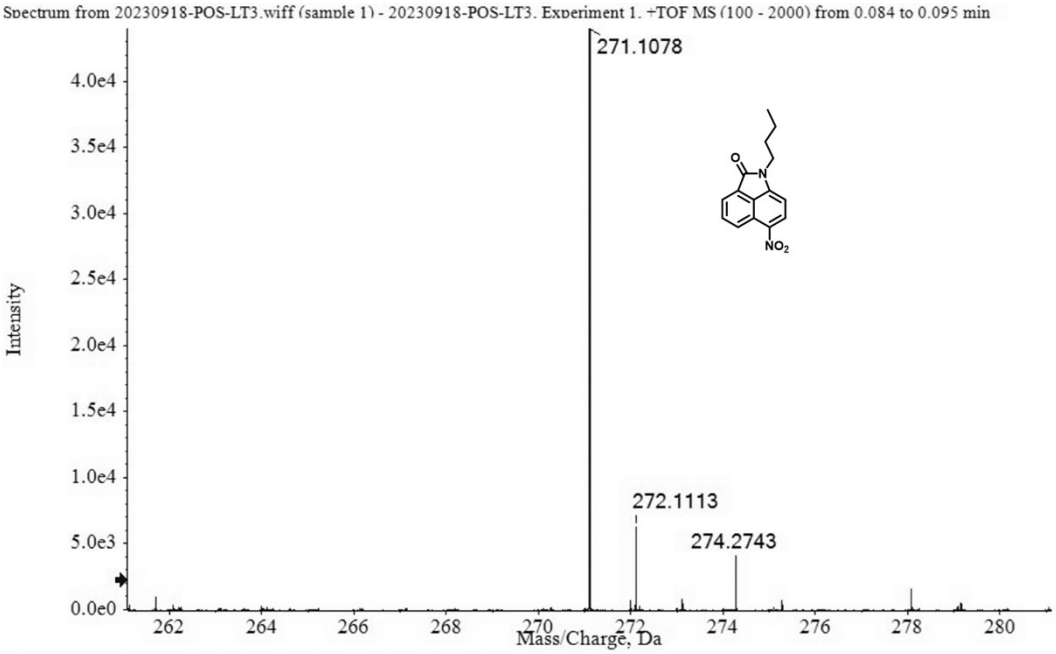

Fig. S10 Mass spectrum of compound C3.

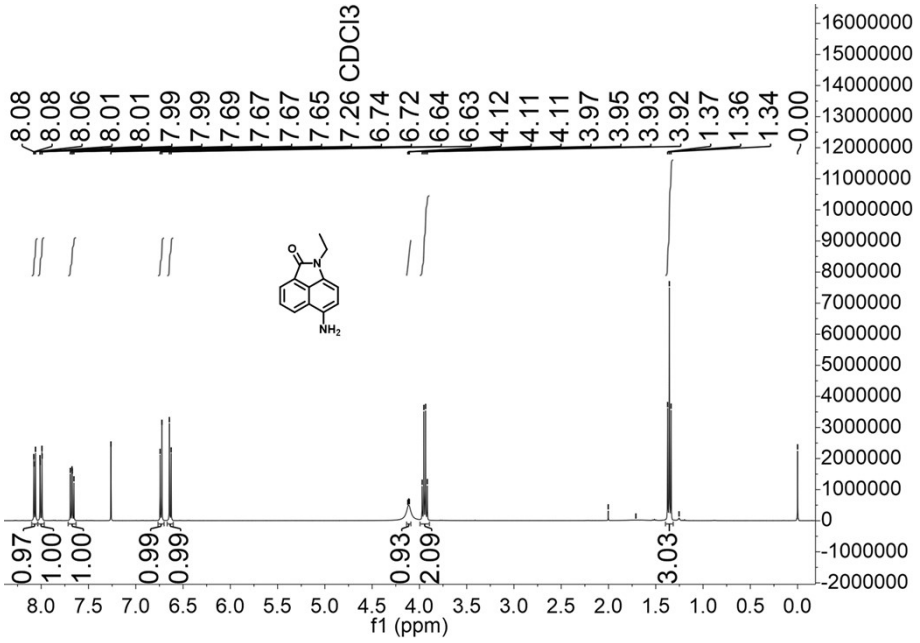

Fig. S11 ^{1}H NMR spectra of compound C4.

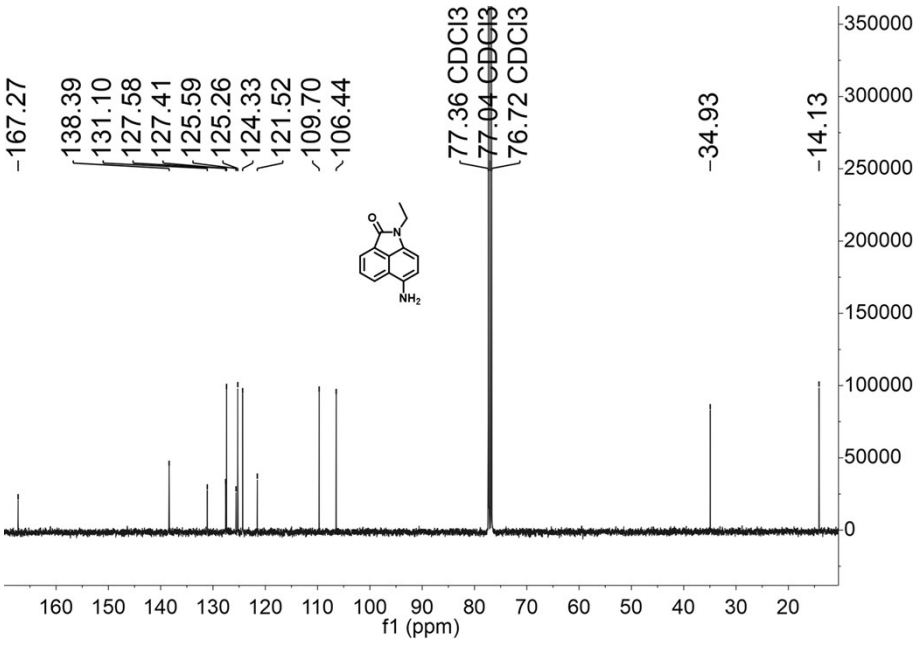

Fig. S12 ^{13}C NMR spectra of compound C4.

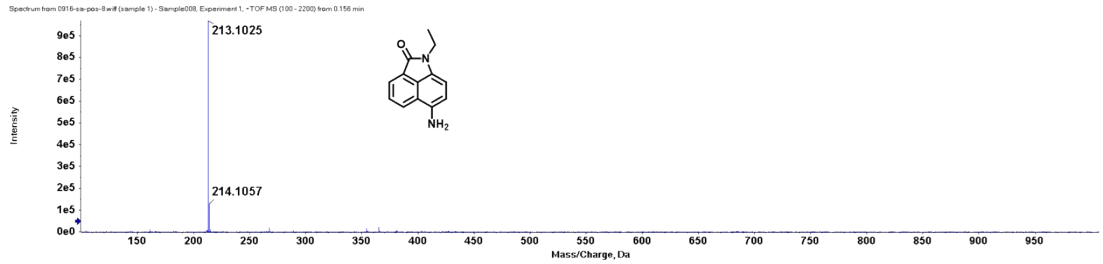

Fig. S13 Mass spectrum of compound C4.

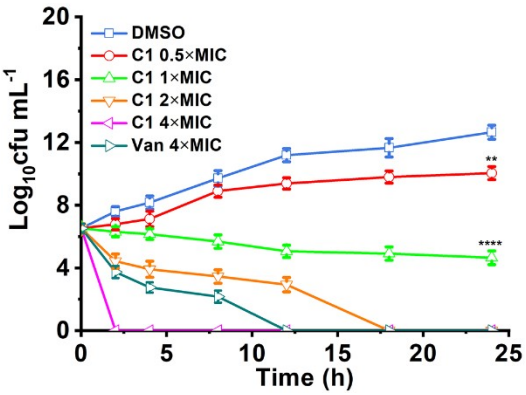

Fig. S14 Time-kill curves of MRSA in the presence of 0.5 ×, 1 ×, 2 × and 4 × MIC of **C1** for 24 h (mean ± SD, n = 3). cfu, colony-forming units. Van, vancomycin. $^{**}P < 0.01$, $^{****}P < 0.0001$ (two-way ANOVA, Dunnett's *posthoc* test).

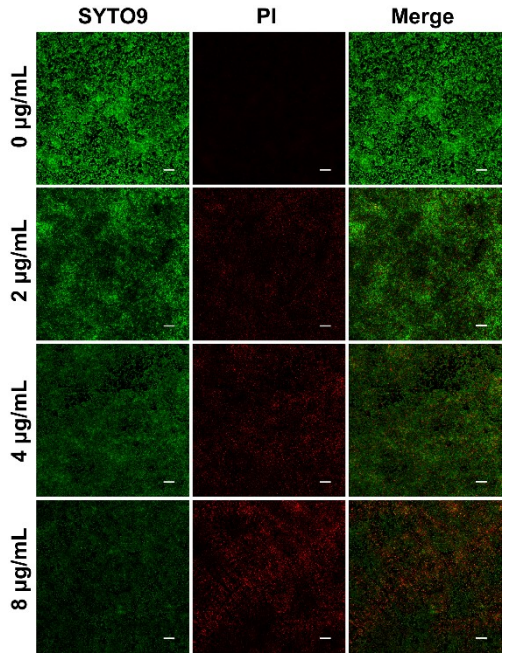

Fig. S15 Live/dead (SYTO9/PI) stain images of MRSA treated with **C1** at the concentrations of 0, 2, 4 and 8 μg mL^{-1} for 6 h using confocal microscopy. Red, dead bacteria. Green signal, living bacteria. PI, propidium iodide. Scale bar = 20 μm.

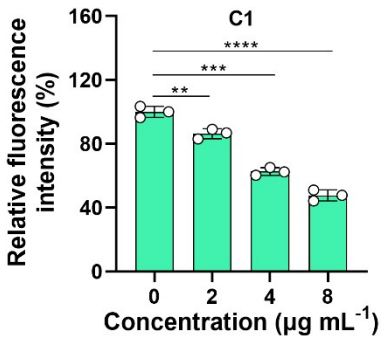

Fig. S16 The membrane potentials of MRSA treated with **C1** at the concentrations of 0, 2, 4 and 8 μg mL^{-1} for 6 h was tested using membrane potential sensitive fluorochrome DiBAC4(3) (mean ± SD, n = 3). $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ (two-way ANOVA, Dunnett's *posthoc* test).

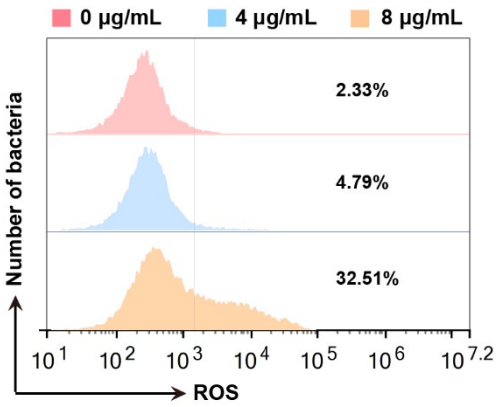

Fig. S17 ROS production in MRSA incubated with **C1** (0, 4 and 8 μg mL^{-1}) for 2 h was tested using 2,7-dichlorofluorescein diacetate (DCFH-DA, 10 μM) as a probe by flow cytometry.

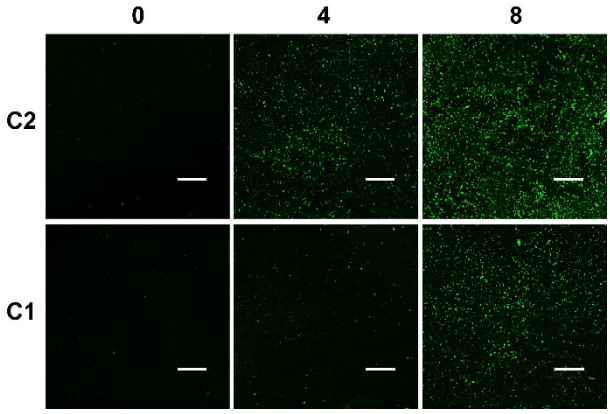

Fig. S18 ROS detection in MRSA under different treatments. MRSA were incubated with **C2** and **C1** (0, 4 and 8 μg mL^{-1}) for 2 h. ROS generation was tested using 2,7-dichlorofluorescein diacetate (DCFH-DA, 10 μM) by fluorescence microscope. Scale bar = 100 μm.

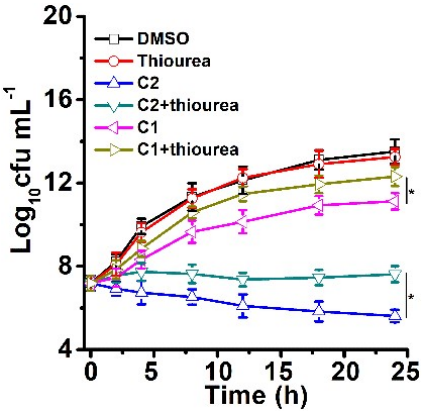

Fig. S19 The effects of ROS scavenger thiourea on the bacteriocidal activity of C2 and C1. MRSA were incubated with C2 (4 μg mL^{-1}), C1 (4 μg mL^{-1}) and thiourea (10 mM) for 24 h. Bacterial survival was estimated by plating and counting of cfu (mean ± SD, n = 3). cfu, colony-forming units. *$P < 0.05$ (two-way ANOVA, Tukey's *posthoc* test).

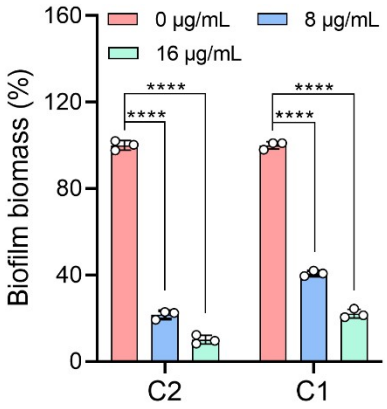

Fig. S20 Quantitative analysis of the crystal violet-stained biofilms with pretreatments compared with PBS-treated control group (mean ± SD, n = 3). ****$P < 0.0001$ (two-way ANOVA, Dunnett's *posthoc* test).

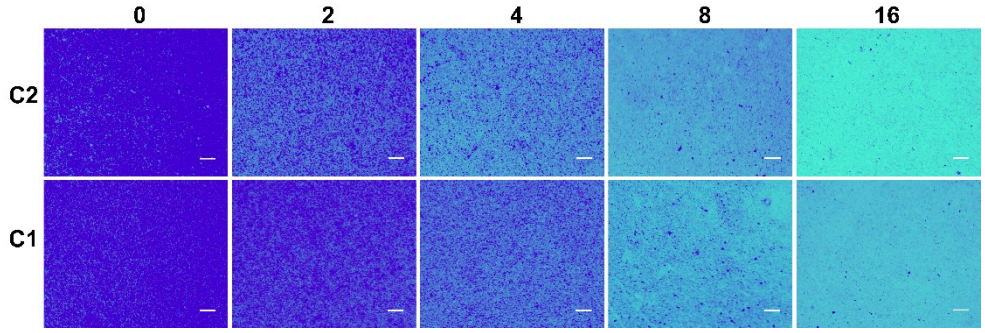

Fig. S21 Biofilm formation tested by crystal violet staining. MRSA were incubated with different concentrations (0, 2, 4, 8 and 16 μg mL^{-1}) of **C2** and **C1** in 96-well plates with glass flat bottom for 48 h at 37°C. Then 0.1% crystal violet was added and incubated for 10 min. The biofilm formation was observed by microscope. Scale bar = 50 μm.

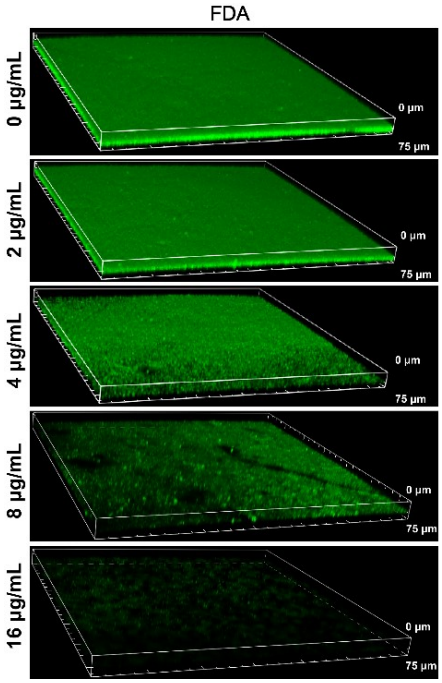

Fig. S22 3D confocal images of the biofilms treated with different concentrations (0, 2, 4, 8 and 16 μg mL^{-1}) of **C1** for 48 h. The biofilms were stained with fluorescein diacetate (FDA).

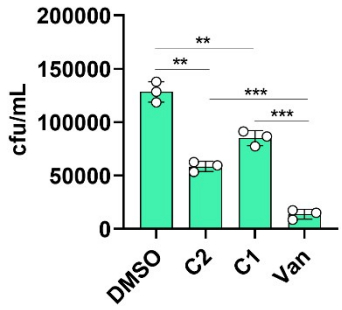

Fig. S23 Quantification of MRSA in biofilms. MRSA were incubated with **C2**, **C1** and Van (4 μg/mL) in 24-well plates for 48 h at 37°C. Then the bacteria in biofilms was quantified by colony counting assay and expressed as cfu/mL. cfu, colony-forming units. Van, vancomycin. $^{**}P < 0.01$, $^{***}P < 0.001$ (two-way ANOVA, Dunnett's *posthoc* test).

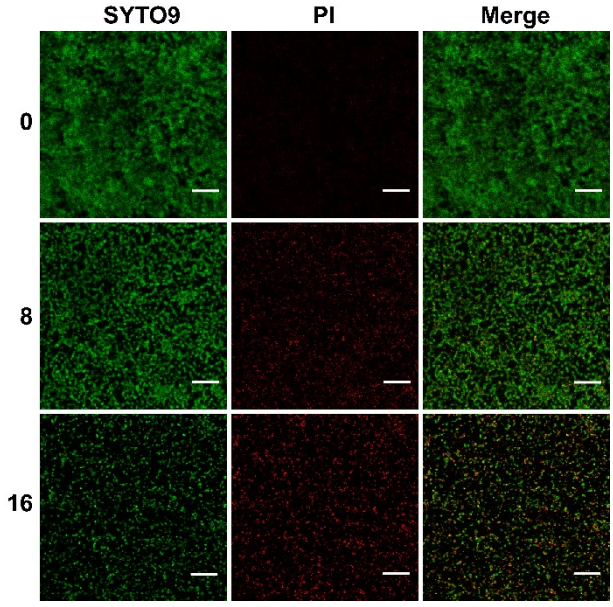

Fig. S24 Live/dead stain images of MRSA in biofilms treated with **C2** at the concentrations of 0, 8 and 16 μg mL^{-1} for 12 h using confocal microscopy. PI, propidium iodide. Scale bar = 20 μm.

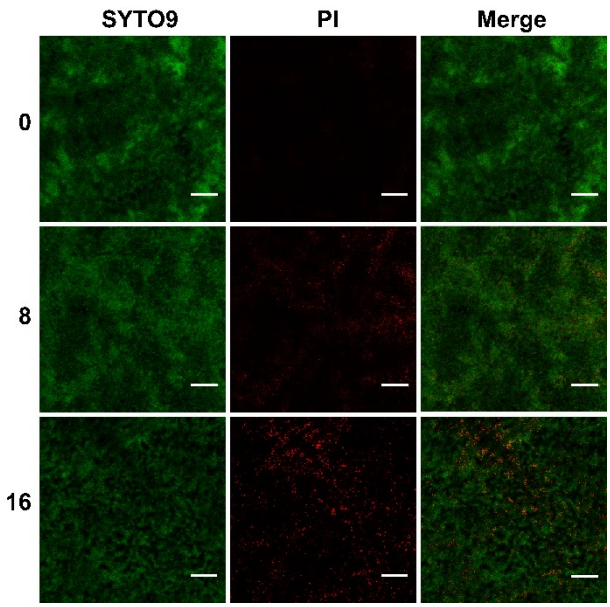

Fig. S25 Live/dead stain images of MRSA in biofilms treated with **C1** at the concentrations of 0, 8 and 16 μg mL^{-1} for 12 h using confocal microscopy. PI, propidium iodide. Scale bar = 20 μm.

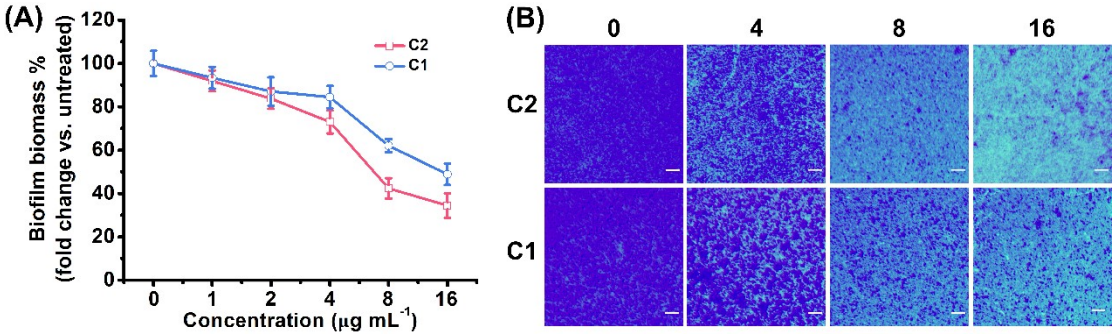

Fig. S26 (A) Quantization of biofilms. 36 h old biofilms were treated with the indicated concentrations of **C2** and **C1** for 12 h. Then the biofilms were stained with crystal violet and quantized. The optical density of the dye solution was read at 590 nm (mean ± SD, n = 3). (B) Microscopic images of the biofilms stained by crystal violet. 36 h old biofilms were treated with different concentrations (0, 4, 8 and 16 μg mL^{-1}) of **C2** and **C1** for 12 h. The biofilms were observed by microscope. Scale bar = 50 μm.

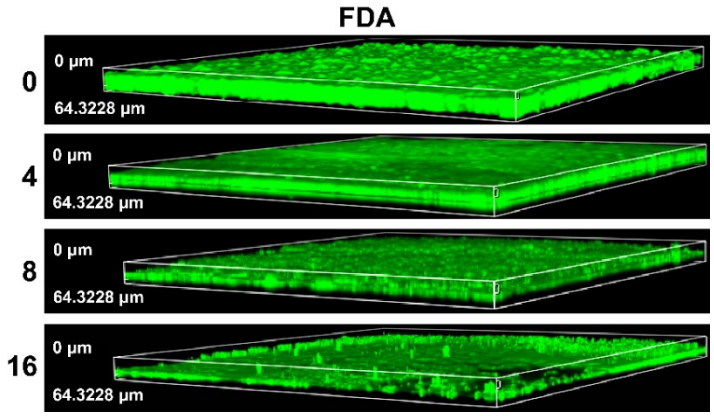

Fig. S27 3D confocal images of the biofilms. 36 h old biofilms were treated with different concentrations (0, 4, 8 and 16 μg mL^{-1}) of **C2** for 12 h. Then the biofilms were stained with fluorescein diacetate (FDA) and observed by confocal microscopy.

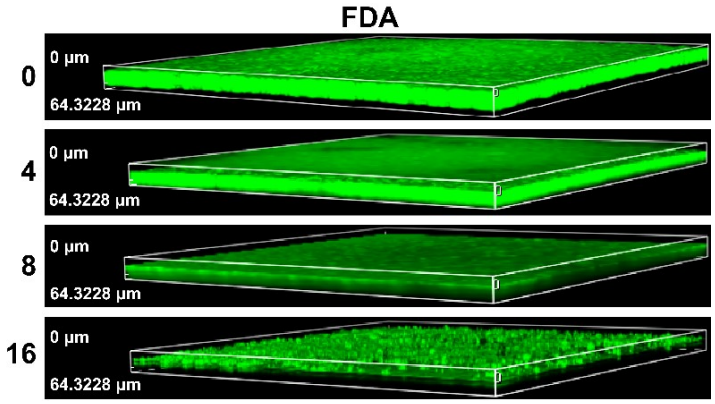

Fig. S28 3D confocal images of the biofilms. 36 h old biofilms were treated with different concentrations (0, 4, 8 and 16 μg mL^{-1}) of **C1** for 12 h. Then the biofilms were stained with fluorescein diacetate (FDA) and observed by confocal microscopy.

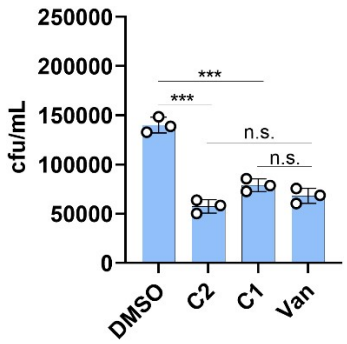

Fig. S29 Quantification of MRSA in biofilms. 24 h old biofilms were treated with C2, C1 and Van (8 μg/mL) for 24 h. Then the bacteria in biofilms was quantified by colony counting assay and expressed as cfu/mL. cfu, colony-forming units. Van, vancomycin. ***$P < 0.001$ (two-way ANOVA, Dunnett's *posthoc* test).

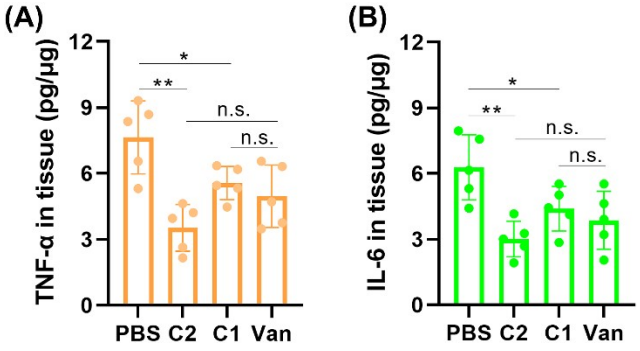

Fig. S30 Secretion levels of TNF-α (A) and IL-6 (B) in the infected skin tissues after treatments with PBS, C2, C1 and Van for 3 days. Van, vancomycin. *$P < 0.05$, **$P < 0.01$ (two-way ANOVA, Dunnett's *posthoc* test).

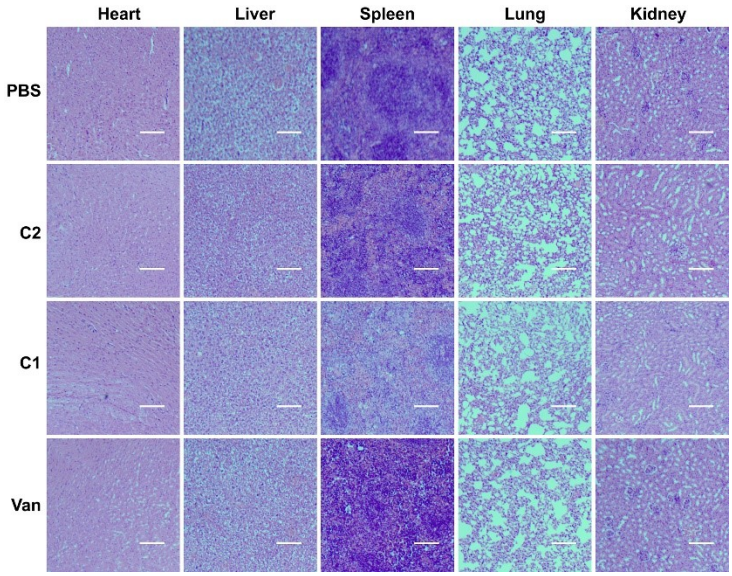

Fig. S31 Representative micrographs of H&E staining of five important organs (heart, liver, spleen, lung and kidney) with different treatments for 3 days.

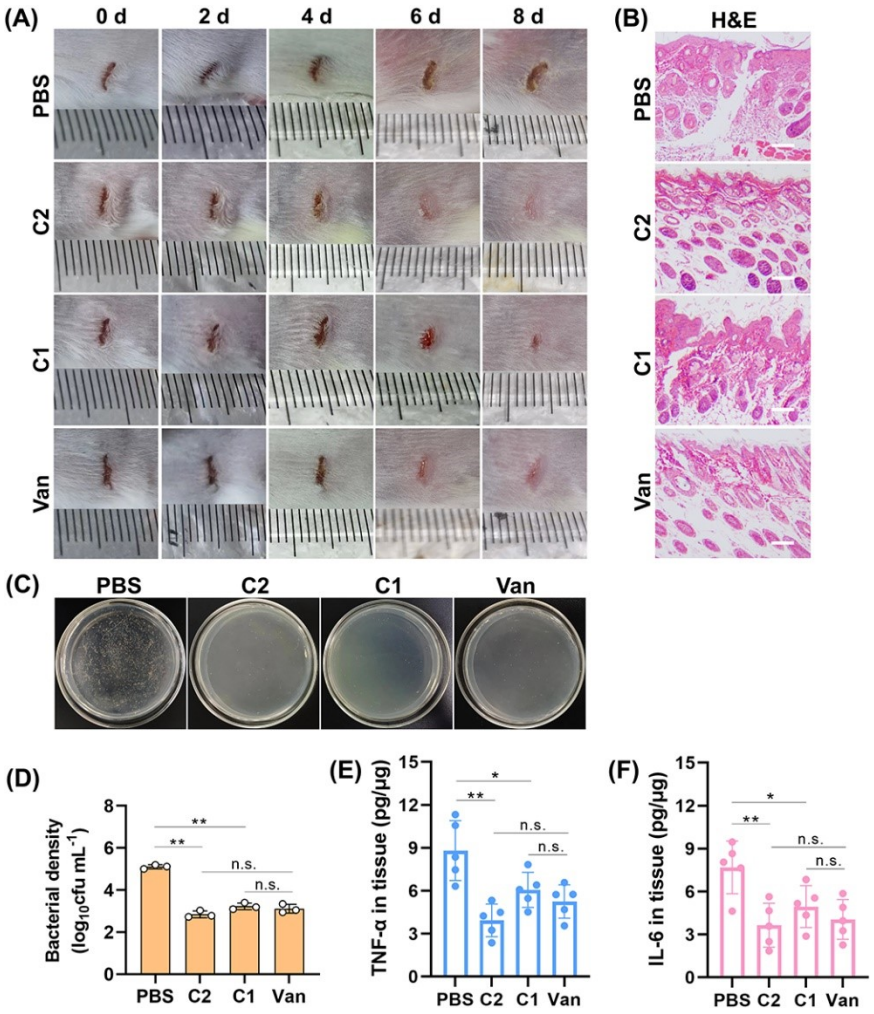

Fig. S32 The effectiveness of C2-triggered bacterial elimination in biofilms in vivo. The wounds were infected with MRSA for 2 days to form initial abscesses. Then the wounds were treated with PBS, C2, C1 or vancomycin for 8 days. (A) Photographs of the infected wounds after treatments for 8 days. (B) H&E-stained images of infected skin tissues at 8 d post-treatment. Scale bar = 100 μm. (C) The bacteria isolated from the wound tissues were cultured on agar plates. (D) Colony numbers of bacteria in infected wounds at 8 d post-treatment. (E, F) Secretion levels of TNF-α and IL-6 in the infected skin tissues after treatments for 8 d. Van, vancomycin. *$P < 0.05$, **$P < 0.01$ (two-way ANOVA, Dunnett's *posthoc* test).

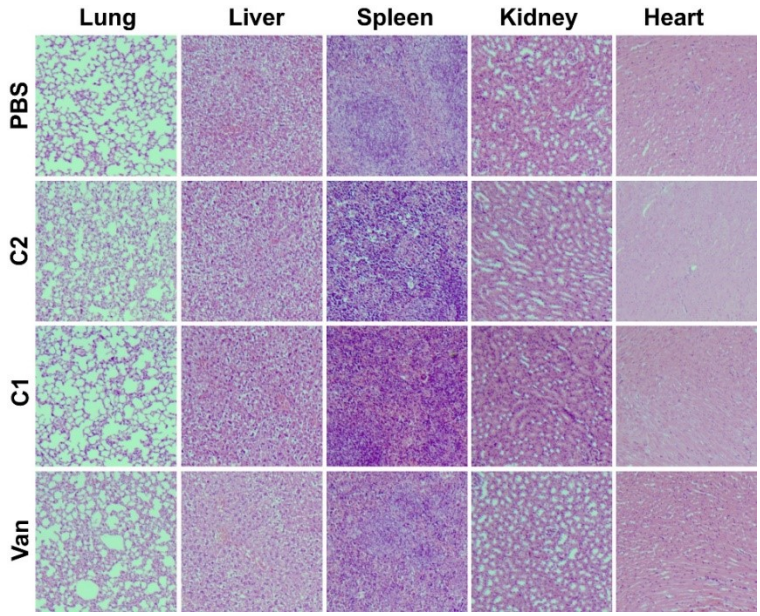

Fig. S33 Representative micrographs of H&E staining of five important organs (lung, liver, spleen, kidney and heart) with different treatments for 8 days.

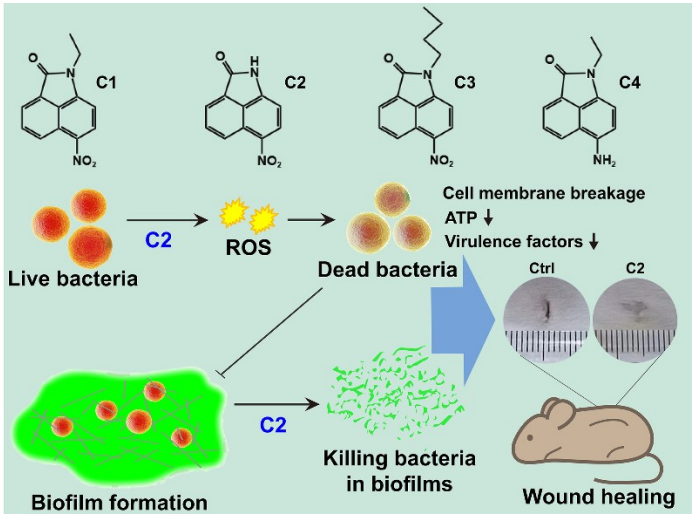

Fig. S34 Compound **C2** induced ROS generation and caused cell membrane injury, ATP content reduction as well as virulence factor down-regulation, resulting in bacterial death and biofilm formation inhibition. Moreover, **C2** could eradicate the bacteria wrapped in biofilms. More importantly, **C2** was able to accelerate wound healing in mouse model.

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