Supporting Information

A light-triggered nitric oxide-photothermal nanoplatform for combating bacterial infections and promoting wound healing

Dan Qing,[†a] Weihao Sun,[†b] Hanyang Wang,[a] Jiabao Zhang,[a] Hao An,[a] Xurui Li,[a] Xiuyan Wei,[*b] and Zhihong Bao[*a]

[a] School of Pharmacy, Shenyang Key Laboratory of Functional Drug Carrier Materials, Shenyang Pharmaceutical University, Shenyang 110016, PR China

[b] School of Life Sciences and Biopharmaceutical Science, Department of Pharmacology, Shenyang Pharmaceutical University, Shenyang 110016, PR China

Corresponding authors:

Zhihong Bao (zhbao@syphu.edu.cn)

Xiuyan Wei (Wei_xiuyan@163.com)

1. Experiment Section

1.1. Materials

Hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$), 2-acetyl pyridine, furfural and ruthenium(III) chloride trihydrate ($RuCl_3 \cdot 3H_2O$) were acquired from Beijing Innochem Science&Technology Co., Ltd. Sinopharm Chemical Reagent Co., Ltd provided nickel nitrate [$Ni(NO_3)_2 \cdot 6H_2O$] as well as Cetyltrimethylammonium bromide (CTAB). Methyl 3,4-diaminobenzoate (MDAB) was purchased from Alfa Co., Ltd. Thiobenzoic acid (PhCOSH) and ammonium hexafluorophosphate (NH_4PF_6) were purchased from Aladdin Industrial Corporation. Nickel thiobenzoate [$(PhCOS)_2Ni$] was synthesized following a previously established protocol. Beyotime Biotechnology Institute provided dichlorodihydrofluorescein diacetate (DAF-FM DA) and the Griess Assay Kit. *S.aureus* (ATCC 29123) and *E.coli* (BL21) were obtained from Shanghai Beinuo Biotechnology Co., Ltd. Liaoning Changsheng Biotechnology Co., Ltd provided the female KM mice approximately 6 weeks of age. All animal experiments were conducted following the guidelines and approved by the ethics committee of Shenyang Pharmaceutical University (SYPU-IACUC-G2R2021-22).

1.2. Synthesis of 4'-(furan-2-yl)-2,2':6',2''-terpyridine

1.68 g of KOH and 2.25 mL of 2-acetylpyridine were added to 45 mL of ethanol under magnetic stirring. Then, 0.828 mL of furfural and 30 mL of ammonia water (25-28%) were added to the above solution. The solution was stirred for 8 h at room temperature. The yellow-green solid was obtained by filtration and washed with cold ethanol. The white crystalline solid was obtained by recrystallization from acetone and then dried at 50°C in a vacuum.

1.3. Synthesis of [2,2':6',2''-terpyridine]-4'-carboxylic acid

0.45 g of 4'-(Furan-2-yl)-2,2':6',2''-terpyridine was added to aqueous KOH solution (pH 10). Then, 0.95 g KMnO4 (4.00 equiv.) was added to the mixture and refluxed for 3 h at 120℃. After cooling to the temperature, the generated manganese dioxide (MnO_2) was removed via filtration over Celite. The pH of the filtrate was adjusted to pH 5 by the addition of concentrated HCl and the emerging white precipitate was isolated by filtration. The precipitate was allowed to settle for 30 min at room temperature, and the white powder was washed with a large amount of water and then dried at 50°C in a vacuum.

1.4. Synthesis of $Ru(TPY)^{COOH}Cl_3$

0.327 g RuCl3.3H2O and 0.347 g [2,2':6',2''-terpyridine]-4'-carboxylic acid were added to 60 mL ethanol. The solution was heated to reflux at 80 ℃ for 1 h under an argon atmosphere. The mixture solution was cooled to room temperature and filtered. Then, the filtration was washed successively with absolute ethanol and anhydrous ether. The brown solid was

dried under a vacuum.

1.5. Synthesis of [Ru(TPY)COOH(MDAB)(Cl)]

300 mg $Ru(TPY)^{COOH}Cl_3$, 100 mg MDAB, 170 mg LiCl, and 0.8 mL Et_3N were added to 80 mL EtOH-H_2O (3:1, v: v). The mixture solution was heated to reflux at 85 °C for 8 h under N_2 atmosphere. The hot suspension was filtered through Celite and the filtrate was collected. Then the filtrate was concentrated to about 10 mL and saturated aqueous NH4PF6 (20 mL) was added. Then, the solution was stored overnight at 4 ℃. The solution was then filtered and washed with cold ethanol and anhydrous ether, respectively. The obtained solid was dried in vacuo.

1.6. Synthesis of Au nanorods

A seed induction method was used to synthesize Au NRs. In a seed solution procedure, 0.25 mL of $HAuCl_4 \cdot 3H_2O$ (0.01M) was added to 9.75 mL of CTAB (0.1 M), and then 0.6 mL of fresh and cold $NaBH_4$ solution (0.01 M) was quickly added to the CTAB solution. After gently shaking, the seed solution was incubated at 30 °C for 2 h. To grow desirable Au NRs, 2.0 mL $HAuCl_4 \cdot 3H_2O$ (0.01 M) and 0.4 mL $AgNO_3$ (0.01 M) were first mixed with 40 mL CTAB (0.1 M) in a 50-mL plastic tube. 0.8 mL HCl (1 M) was then added to the growth solution to adjust the pH to 1~2. Then, 0.32 mL of ascorbic acid (0.1 M) was added and mixed well. 0.025 mL CTAB-stabilized seed solution was rapidly injected into the growth solution. The solution was kept at a constant temperature of 30 °C overnight to obtain Au NRs with an absorbance of about 780 nm.

1.7. Characterization of Ru-NO complex and ACRN nanoplatform

The ACRN nanoplatform was evaluated using different testing systems. Ru-NO was determined using a nuclear magnetic resonance (^{1}H NMR, Bruker, Germany). The structures of Au NRs, AC, and ACRN were investigated utilizing transmission electron microscopy (TEM, JEOL, Japan). The chemical absorbance of the nanoplatform was determined using ultraviolet-visible spectra (UV-vis, 756PC, Hengping, China). Fourier transform infrared spectroscopy (FT-IR) analyzes chemical structure. Zeta potential using a Malvern ZetaSizer ZS90 (Malvern Panalytical, England). Elemental analysis utilizing energy dispersive X-ray spectroscopy (EDS), alongside scanning electron microscope (SEM, Thermo Scientific, America) was used to investigate the chemical composition.

1.8. Analysis of inflammation and neovascularization during wound healing in mice

The wound and visceral tissues were collected and placed in 4% paraformaldehyde for histological analysis. These samples were embedded in paraffin and were stained with hematoxylin and eosin (H&E), Masson and via immunohistochemistry. To assess the inflammatory status of the mice, blood was collected on the 10th day, and

concentrations of key inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-1 beta (IL-1β), were quantified using enzyme-linked immunosorbent assay (ELISA). The semi-quantitative analysis of epidermal growth factor (EGF), transforming growth factor-β (TGF-β1), and vascular endothelial growth factor A (VEGFA) was performed using standard Western blot (WB) procedures. To assess neovascularization, IHC staining of platelet endothelial cell adhesion molecule- 1 (CD31) and α-smooth muscle actin (α-SMA) was performed.

1.9. *In Vivo* biochemistry analysis

To assess the biosafety of the drug, blood was collected from the eyeballs, allowed to stand at 4 °C for 30 minutes, and then serum was separated by centrifugation at 3000 rpm for 15 minutes. The serum was stored at -80 °C for later analysis. The concentrations of serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum creatinine (SCR) and blood urea nitrogen (BUN) were measured with assay kits.

2. Supplementary figures

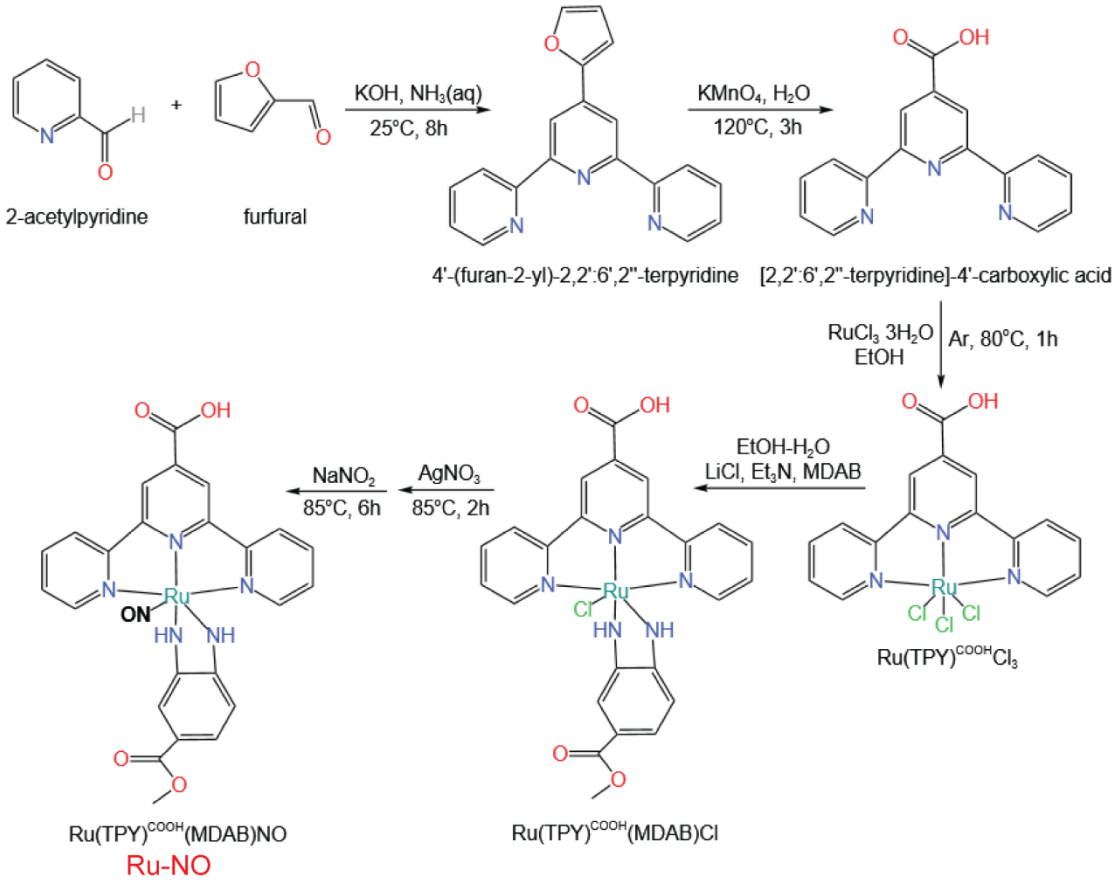

Fig. S1 The synthetic route for the Ru-NO complex.

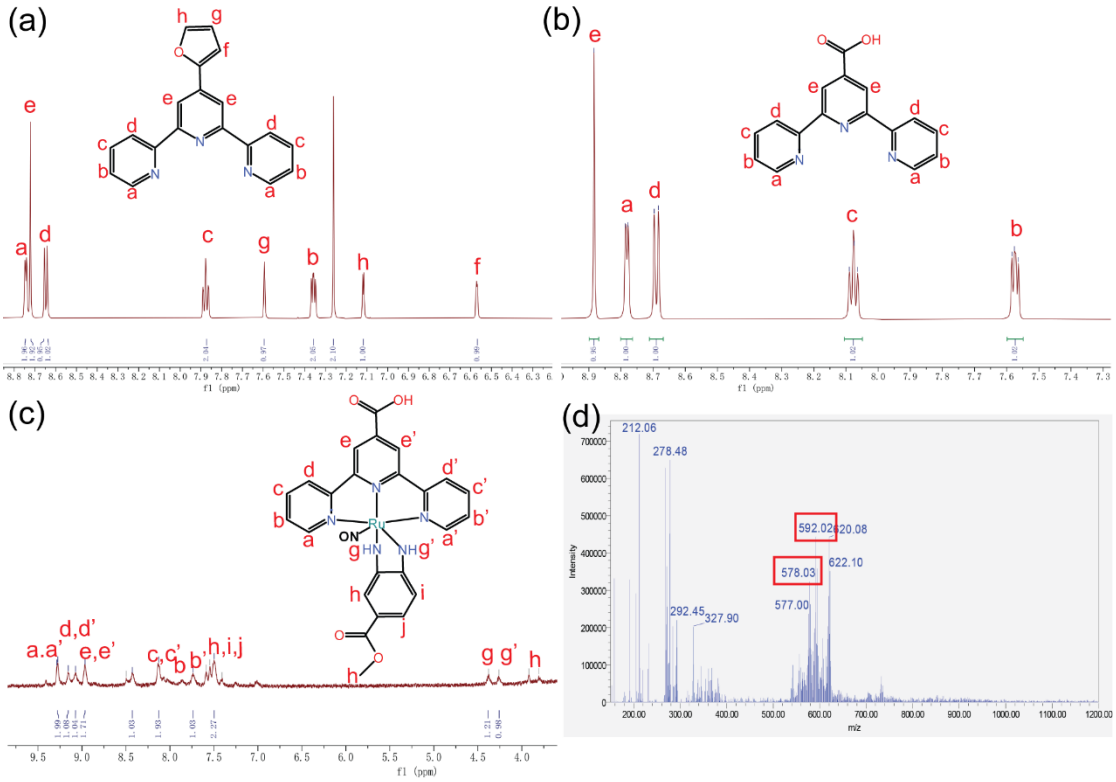

Fig. S2. ^{1}H NMR spectra of (a) 4’-(Furan-2-yl)-2,2’:6’,2’’-terpyridine, (b) [2,2’:6’,2’’-Terpyridine]-4’-carboxylic acid and (c) Ru-NO complex. (d) ESI-MS spectra analysis of Ru-NO complex.

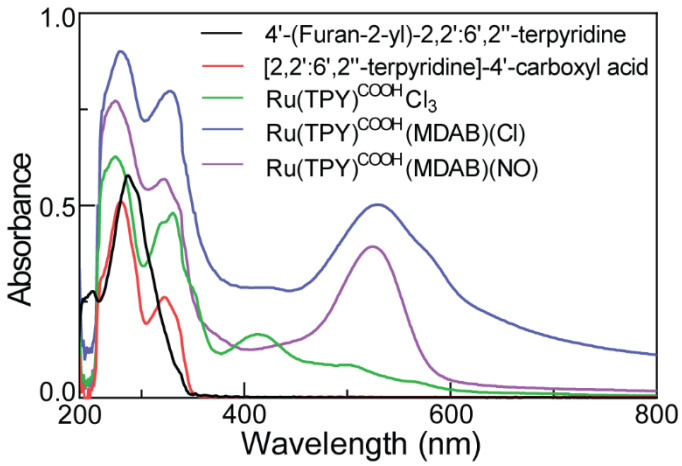

Fig. S3. UV-vis spectra of different ligands and the Ru complex.

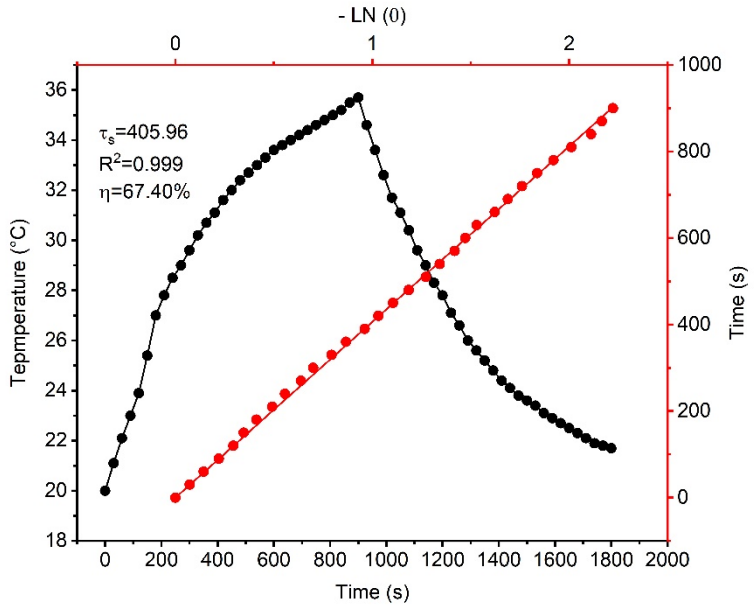

Fig. S4. The photothermal conversion efficiency (*η*) of ACRN (200 μg/mL).

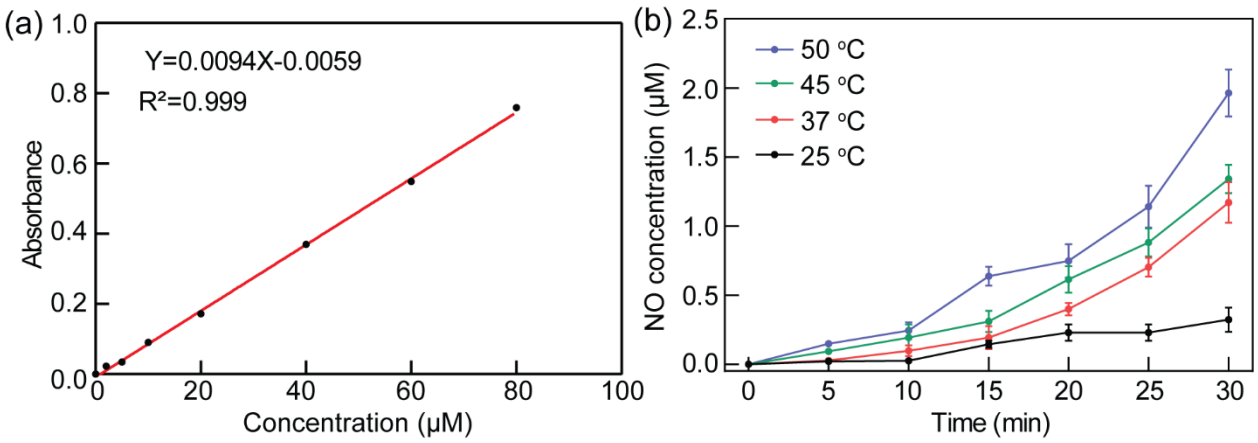

Fig. S5. (a) The standard curve of NO release in the Griess assay. (b) The continuous release behavior curve of ACRN (200 μg/mL) under different temperatures (n=4).

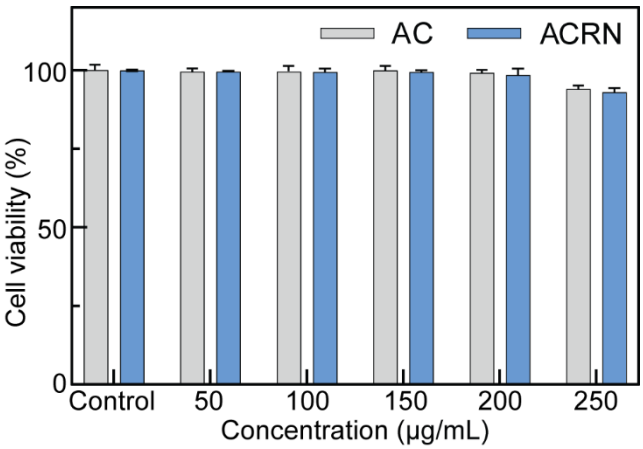

Fig. S6. Cytotoxicity for L929 cells after incubation with AC and ACRN (n=4).

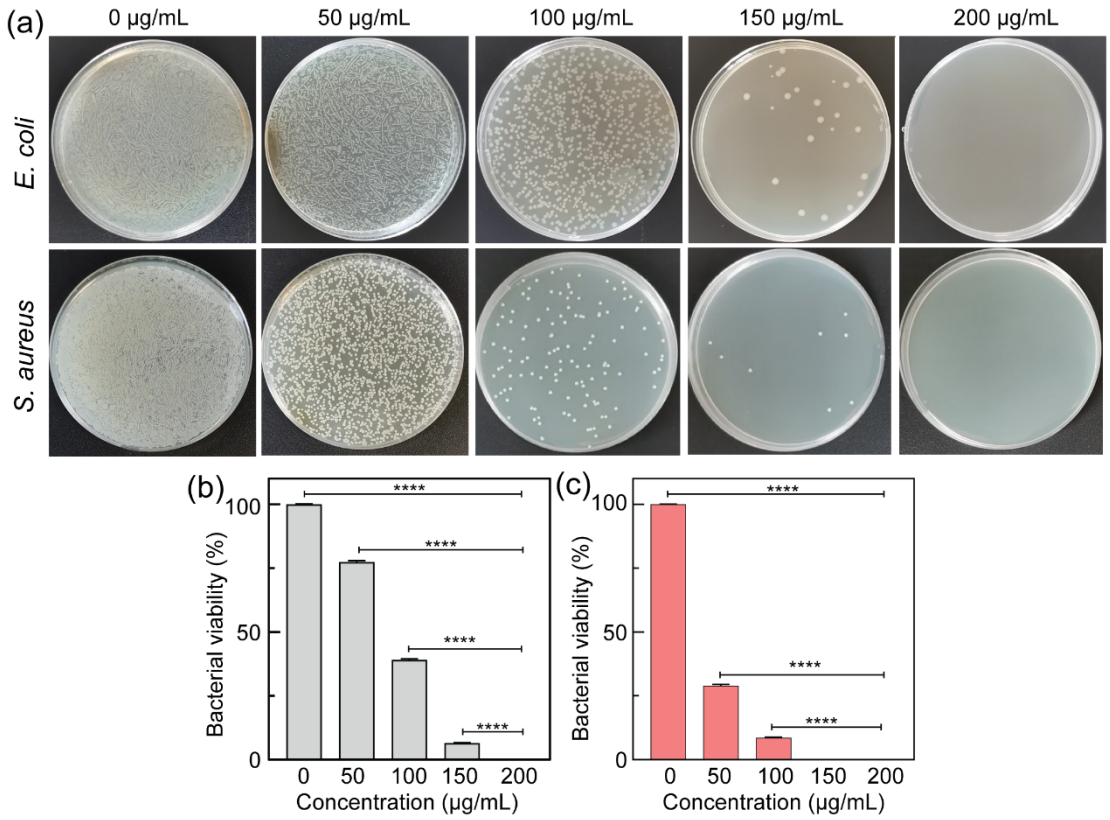

Fig. S7. (a) Representative plates of *E. coli* and *S. aureus* colonies treated by ACRN with different concentrations under the laser illumination (808 nm, 1.0 W/cm^2, 10 min). The corresponding bacterial viability of *E. coli* (b) and *S. aureus* (c) obtained from the spread plate results. $^{****}p<0.0001$, n=3.

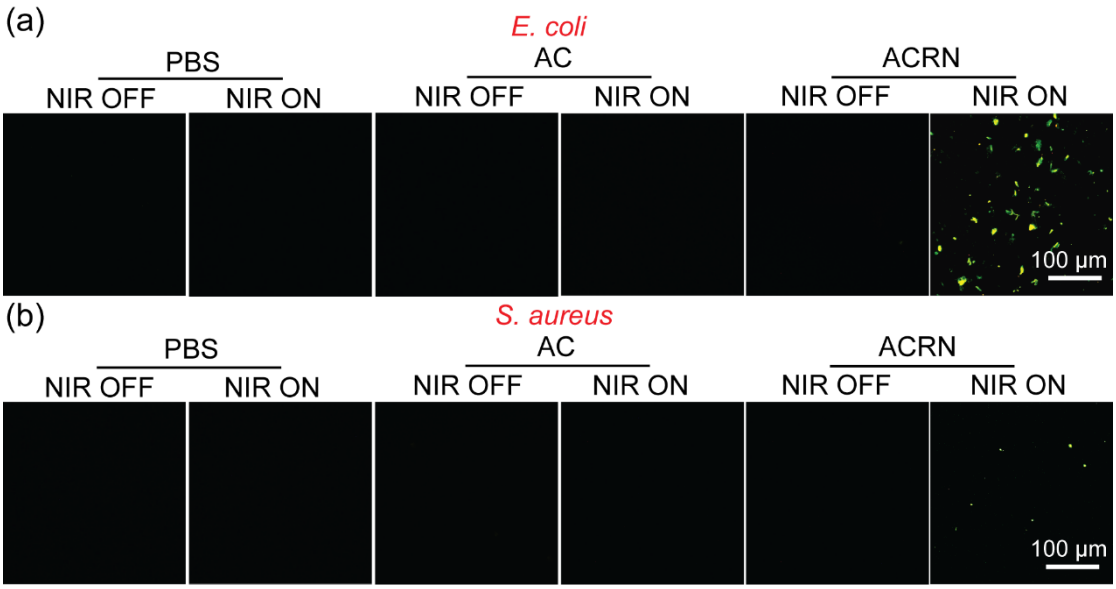

Fig. S8. Fluorescence microscope images of *E.coli* (a) and *S. aureus* (b) that co-stained with DAF-FM DA under different treatments.

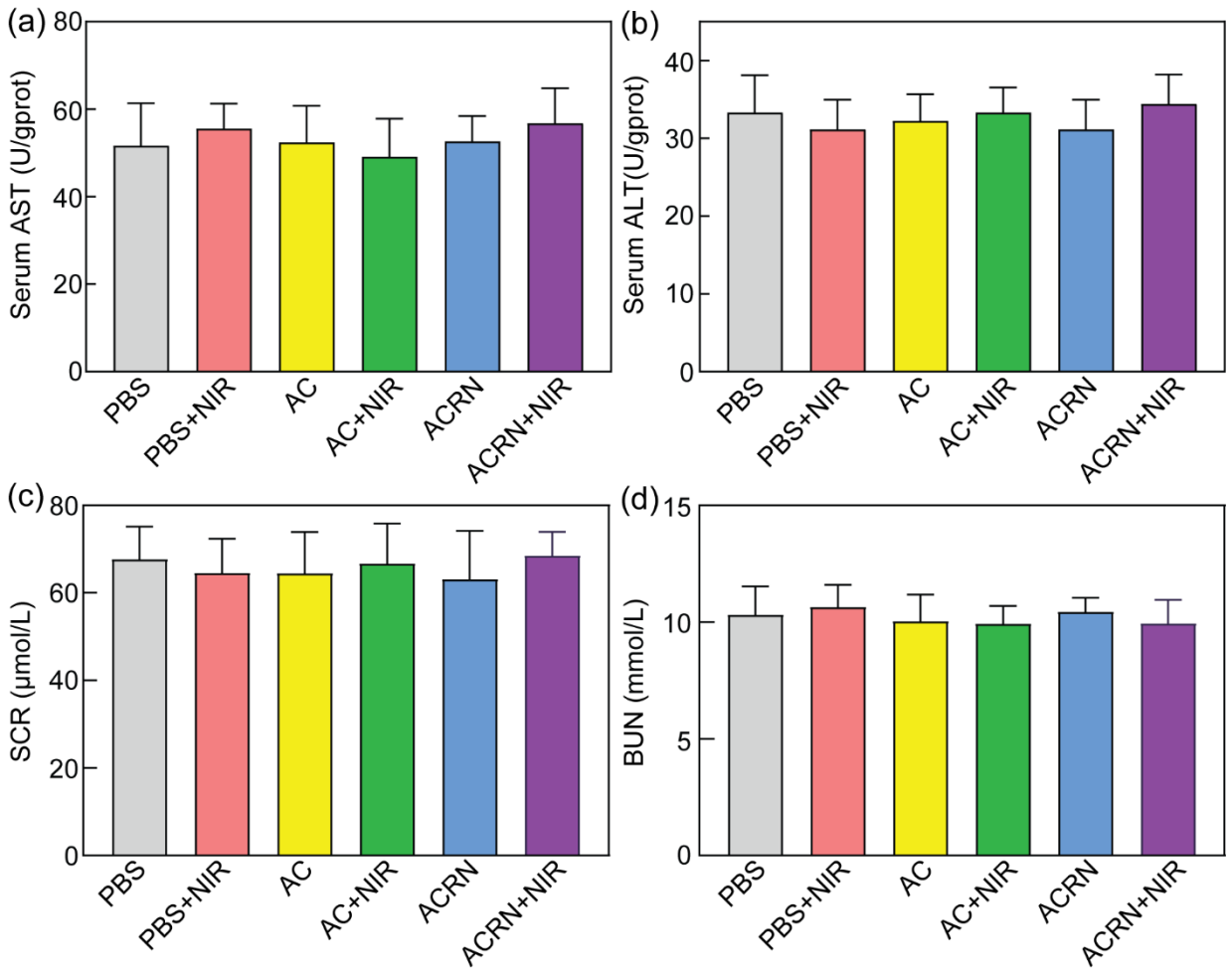

Fig. S9. Biochemical analysis of the serum of mice with various treatments: (a) serum aspartate aminotransferase (AST), (b) serum alanine aminotransferase (ALT), (c) serum creatinine (SCR), and (d) blood urea nitrogen (BUN) (n=3).

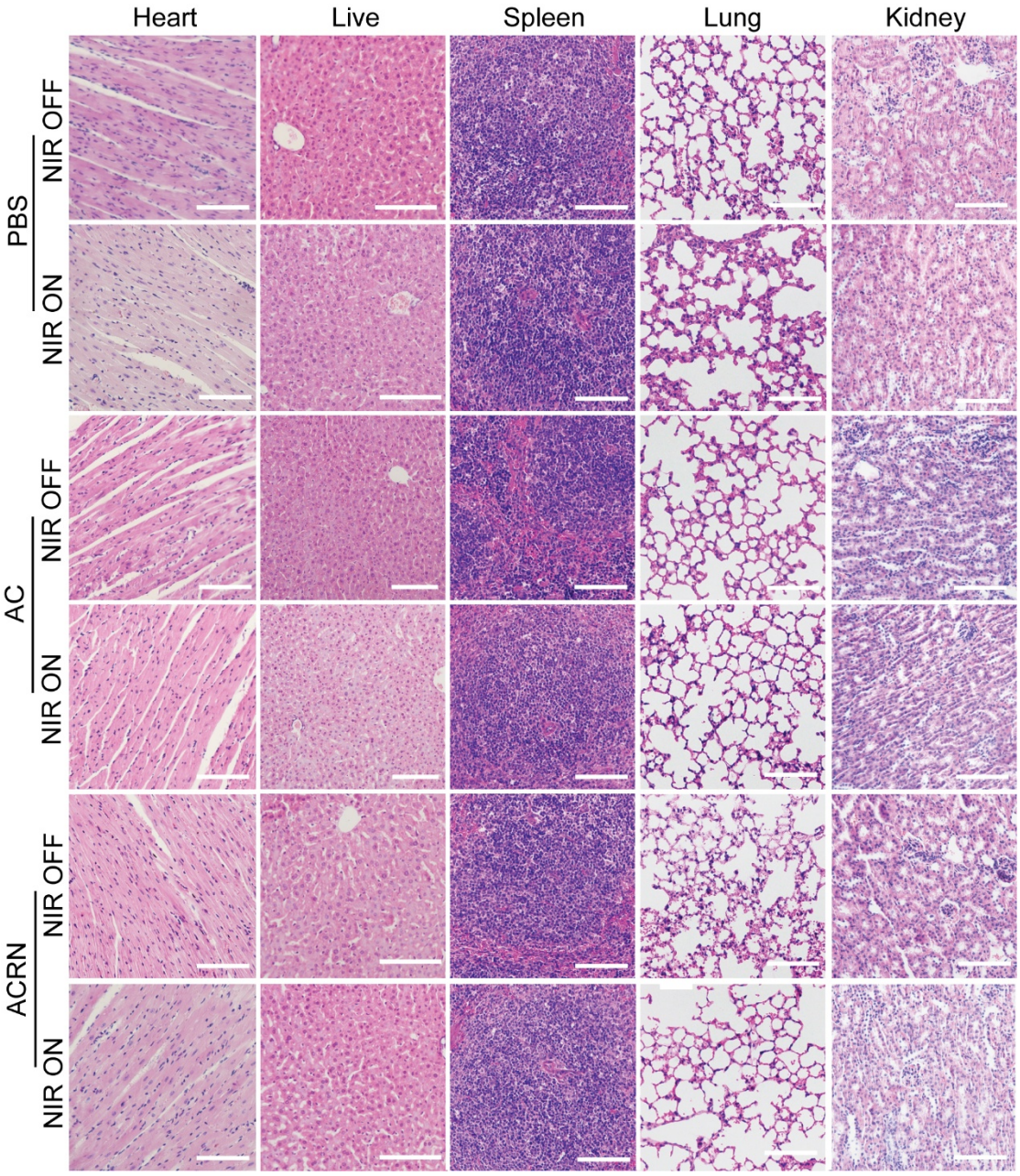

Fig. S10. Histological evaluation of heart, liver, spleen, lung and kidney retrieved from infected mice after treatment with PBS, AC and ACRN with or without NIR light (Scale bar: 100 μm).