Supporting Information

A Novel Furo[3,2-c]pyridine Based AIE Photosensitizers for Specific Imaging and Photodynamic Ablation of Gram-Positive Bacteria

Ming-Yu Wu,*[a] Yun Wang,[a] Li-Juan Wang,[a] Jia-Li Wang,[a] Feng-Wei Xia[a] and Shun Feng*[a]

[a]*Sichuan Engineering Research Center for Biomimetic Synthesis of Natural Drugs, School of Life Science and Engineering, Southwest Jiaotong University, Chengdu 610031, China. E-mail: wumy1050hx@swjtu.edu.cn; fengshun@swjtu.edu.cn.*

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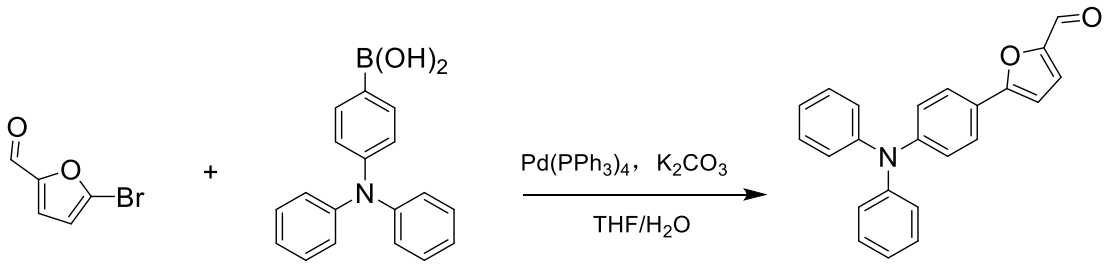
1.Experimental section

1.1Materials and general instruments

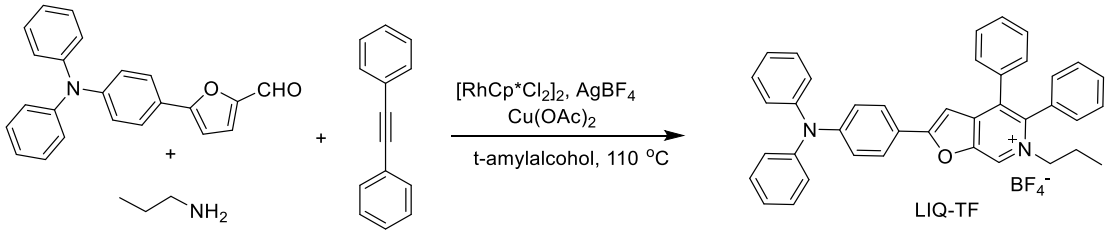
All chemical reagents were obtained from J&K Scientific and were used without further purification. H2DCF-DA (2`,7`-dichlorofluorescein diacetate) Detection Kit, Sytox Green, 9,10-Anthracenediylbis-(methylene)-dimalonic acid (ABDA) and Rose Bengal (RB) were purchased from Sigma-Aldrich. 2-[6-(4′-Hydroxy)phenoxy-3H-xanthen-3-on-9-yl]benzoic acid (HPF) was purchased from aladdin. TLC analysis was performed on silica gel GF 254. Column chromatography purification was carried out on silica gel (200-300 mesh). NMR spectra were recorded using a Bruker AMX-400. Chemical shifts were given in ppm relative to the internal reference TMS as the internal standard. The following abbreviations were used in 1H NMR: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet. High resolution mass spectra were recorded on a Bruker Daltonics Bio TOF mass spectrometer. Fluorescence spectra were obtained using a Horiba Duetta spectrofluorimeter with a 10 mm quartz cuvette. UV-Vis absorption spectra were recorded on a Hitachi PharmaSpec UV-1900 UV-Visible spectrophotometer. Confocal fluorescence images were recorded using a Nikon A1R+ confocal laser scanning microscope. Zeta-potential and average particle size were recorded using Nano ZS (ZEN3600). The light source was a white LED illuminant from Opple Lighting Co., LTD with the power of 5 W, diameter of 1 cm and intensity of 20 mW/cm^2.

1.2 Synthesis and characterization of LIQ-TF



5-(4-(diphenylamino)phenyl)furan-2-carbaldehyde was synthesized via Suzuki

coupling. 5-bromofuran-2-carbaldehyde (525.0 mg, 3.0 mmol), (4-(diphenylamino)phenyl)boronic acid (1029.6 mg, 3.6 mmol), K_2CO_3 (4140.0 mg, 30 mmol) and $Pd(PPh_3)_4$ (34.6 mg, 0.03 mmol) in 70 mL THF/water (6:1 v/v) was heated to reflux overnight under nitrogen. After cooling to room temperature, the mixture was extracted with DCM for three times. The organic phase was collected, washed with saturated salt solution and dried over with anhydrous sodium sulfate. After removed the solvent, the crude product was purified by silica gel column chromatography unsing DCM/EtOAc (10:1 *v/v*) as eluent to obtain 813.6 mg yellow solid with 82% yield.



LIQ-TF: The mixture solution of 5-(4-(diphenylamino)phenyl)furan-2-carbaldehyde (407.3 mg, 1.2 mmol), propylamine (88.1 mg, 1.5 mmol) and diphenylacetylene (178.2 mg, 1.0 mmol), $AgBF_4$ (194.7 mg, 1.0 mmol), $[RhCp^*Cl_2]_2$ (12.4 mg, 0.02 mmol) and $Cu(OAc)_2$ (181.6 mg, 1.0 mmol) in 7.5 mL *t*-amylalcohol was heated to 110 ℃ for 3 h under the N_2. After removed the solvent, the residents were purified with silica gel column chromatography using CH_2Cl_2/MeOH (100:5 *v/v*) as eluent to obtain 496 mg orange solids with 77% yield. ^1H NMR (400 MHz, $CDCl_3$), δ. 9.25 (s, 1H), 7.75 (dd, 2H, *J* = 1.6 Hz, *J* = 5.6 Hz), 7.41 – 7.33 (m, 7H), 7.30 – 7.28 (m, 5H), 7.18 – 7.16 (m, 6H), 7.14-7.12 (m, 2H), 7.04 (d, 2H, *J*= 6.8 Hz), 6.76 (s, 1H), 4.48 (t, 2H, *J* =6.0 Hz), 1.87 – 1.80 (m, 2H), 0.84 (t, 3H, *J* = 6.0 Hz). ^{13}C NMR (150 MHz, $CDCl_3$), δ = 168.5, 151.6, 149.7, 146.6, 146.1, 143.6, 133.5, 132.0, 130.7, 130.3, 129.8, 129.5, 128.9, 128.7, 128.6, 128.4, 128.0, 126.1, 125.1, 120.3, 118.5, 98.9, 60.6, 25.1, 10.7. HRMS (ESI): m/z $[M - BF_4^-]^+$ calculated for $C_{40}H_{33}N_2O$: 557.2587; found: 557.2599.

1.3 ROS detection

A commonly used ROS indicator H2DCF-DA was utilized to detect the ROS generation of **LIQ-TF** in solution under white light irradiation (20 mW/cm^2). Briefly, 4 mL H2DCF-DA in ethanol (1 m M) was added to 16 mL NaOH solution (10 mM) and stirred at room temperature for 30 min. Then the hydrolysate was diluent with 80 mL of PBS and kept in dark at 4 °C for use. H2DCF-DA (10 μM) was mixed with probe (10 μM) in PBS under dark and then exposed to spectrofluorimeter for dynamic scans from 0-62 s. The fluorescent intensity at 525 nm was monitored by the fluorescence spectrum with the excitation of 488 nm.

The $^1O_2$ generation was measured using ABDA as an indicator, and RB was employed as the standard photosensitizer. ABDA (50 μM) was mixed with the **LIQ-TF** or RB (5 μM) in DMSO/water (1:99, *v*/*v*) and exposed to white light illumination for 0-5 min (20 mW/cm^2). The absorbance of ABDA at 378 nm was recorded at different illumination time to obtain the decay rate of the photosensitizing process.

The •OH generation measurements were conducted using hydroxyphenyl fluorescein (HPF) as the indicator. PBS buffer solution containing 5 μM HPF (stock solution: 5 mM in DMF) and **LIQ-TF** (10 μM) was irradiated with white light irradiation for 0-12 min. The fluorescence intensity at 515 nm was recorded with the excitation wavelength at 492 nm.

1.4 Preparation of pathogen suspension

Culture medium: Luria Broth (LB) for Escherichia coli (*E. coli*), Staphylococcus aureus (*S. aureus*), Methicillin-resistant Staphylococcus aureus (MRSA), and *S. typhimurium*, Nutrient Broth (NB) for Bacillus subtilis (*B. subtilis*), Tryptic Soy Broth (TSB) for Enterococcus faecalis (*E. faecalis*). A single bacterial colony on solid agar plate was added to 10 mL corresponding liquid culture medium, and then incubated for about 6-8 h at 37 °C under the shaking of 200 rpm. After harvested by centrifuging

at 8000 r/min for 5 min, the bacteria were washed with PBS three times and re-suspended with PBS, and diluted to an optical density (about 10^8 CFU/mL). The concentrations of the bacterial suspension were determined via optical density measurements of absorbance at 600 nm using a cell density meter.

1.5 Zeta-potential measurements

1 mL bacteria (about 10^8 CFU/mL) were incubated with **LIQ-TF** (10 μM) or PBS for 10 min at room temperature, and then harvested by centrifuging (8000 r /min, 3 min). Subsequently, the bacteria were re-suspended in PBS for Zeta potential analysis with Nano ZS (ZEN3600).

1.6 Bacteria Staining and Imaging

1000 μL bacteria (OD_{600} = 1.0) in a 1.5 mL centrifuge tube were harvested by centrifuging (8000 r/min, 5 min). The supernatant was removed and wash with PBS for 3 times. 1000 μL of **LIQ-TF** (10 μM) in PBS solutions were added the bacteria, and incubated for 10 min at ambient temperature. After washed with PBS for 3 times, the bacteria were dispersed with PBS and exposed to white light or kept under dark for 30 min. Then, the bacteria were stained with Sytox Green (1 μM) at 37 °C for 10 min, washed with PBS for 3 times and disperse evenly in PBS. The confocal fluorescence images were recorded with Nikon A1R+ laser scanning confocal microscope (Nikon A1R+). The 445nm laser and 500–600 nm emission filter were used for **LIQ-TF**, and 488-nm laser and 500–600 nm emission filter were used for Sytox Green, respectively.

1.7 Photodynamic Antibacterial therapy

The antibacterial activities of **LIQ-TF** was evaluated by plate counting method. 1000 μL bacteria (OD_{600} = 1.0) in a 1.5 mL centrifuge tube were harvested by centrifuging (8000 r/min, 5 min). After removed the supernatant, the bacteria were wash with PBS for 3 times and then incubated with different concentration of **LIQ-TF** for 10 min at

ambient temperature. After washed with PBS the bacteria were dispersed in PBS, and then kept under dark or exposed under white light (20 mW/cm^2) for 30 min. 10 μL bacterial suspension were taken from each sample, serially diluted for 5-fold with PBS, spread on LB agar and incubated at 37 ℃ for 12 h for the determination of colony forming unit (CFU). The bacteria viability was then determined and quantified by the plate count method. Each experiment was performed independently at least three times.

1.8 Scanning Electron Microscopy (SEM) analysis

1000 μL (10^6 CFU/mL) bacteria (*S. aureus* or *E. coil*) in a 1.5 mL centrifuge tube were harvested by centrifuging (8000 r/min, 5 min). The bacteria were treated with **LIQ-TF** (10 μM) or PBS at 37 °C for 10 min, and then kept under dark or exposed under white light for 30 min. The bacteria were fixed with 2.5% glutaraldehyde and dehydrated successively by 30%, 50%, 70%, 80%, 90%, 95% and 100% ethanol solution (*v*/*v*) over 10 min. Finally, the samples were disposed with metal spraying for SEM analysis (COXEM EM-30).

1.9 In *Vivo* Anti-Infection Assay

All animal procedures were carried out under the guidelines set of the Institutional Animal Care and Use Committee of Sichuan province, and the overall project protocols were approved by the Animal Ethics Committee of Southwest Jiaotong University.

6-8 week Wistar mice purchased from Chengdu Dashou Experimental Animal Co.Ltd were randomly divided into four groups: (1) MRSA infected wounds with PBS treatment; (2) MRSA infected wounds with **LIQ-TF** treatment; (3) MRSA infected wounds with vancomycin (Van) treatment; (4) MRSA infected wounds with **LIQ-TF** and white light irradiation treatment. The mice were anesthetized by intraperitoneal injection of 10% chloral hydrate sodium. Then, two about10 mm diameter full-thickness injuries were cut on the both sides of the spine. The

bacteria-infected wound models were established by inoculating 50 μL of MRSA suspension (10^8 CFU/mL) over each wound and covered with sterile nonwoven fabrics for 24-hour. For the mice treated with **LIQ-TF** or vancomycin, 50 μL **LIQ-TF** or vancomycin (10 μM) was smeared on the wounds, while 50 μL PBS was used as control. Subsequently, the mice were irradiated with white light or kept under dark 30 min. Wound healing of all mice were recorded every day. Meanwhile, on the Day 1, Day 3, Day 7 and the Day 14 after surgery, one side of the entire wound with adjacent normal skin were obtained. The infectious tissues were separated and homogenized in normal saline. The homogenates were diluted 1000 times with normal saline. 20 μL of the bacteria solution was sprayed onto LB agar plate and cultured at 37 °C for 24 h. The bacterial colonies on the plate were counted for analysis. The other sides of tissues were fixed in 4% paraformaldehyde for the histological analysis.

1.10 Histological analysis

The collected tissues were fixed in 4% paraformaldehyde, and then embedded in paraffin. The fixed tissues were cut into slices with a thickness of 4 mm. Next, hematoxylin-eosin (H&E) staining was carried out according to the standard protocol.

2. Supplementary Table and Figures

Table 1 Optical properties of **LIQ-TF** in different solvents

Solvent	λ_{abs}(nm)	ε (M^{-1} cm^{-1})	s_{eem}(nm)	Φ_F(%)	△SS[b]/nm
Toluene	444	4.12×10^4	542	9.78	98
THF	446	4.24×10^4	651	12.28	205
EtOH	449	4.21×10^4	646	6.22	197
DMSO	443	4.53×10^4	661	3.06	218
PBS	448	2.41×10^4	566	66.00	118

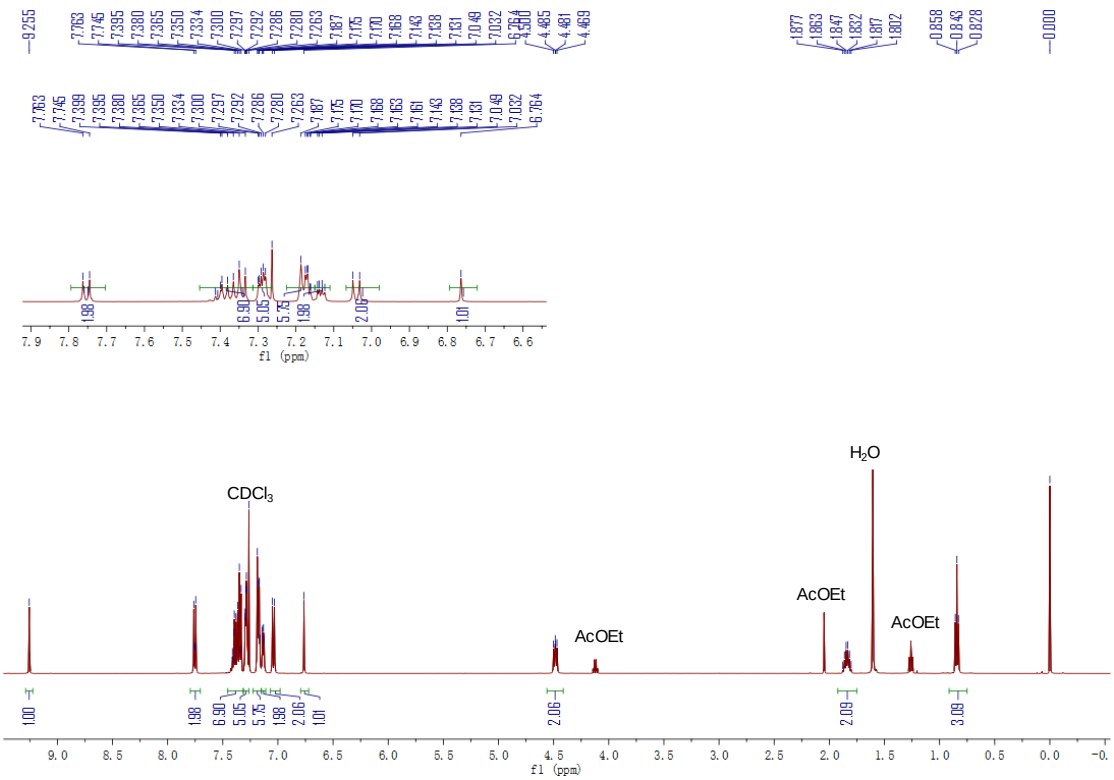


Fig. S1 ^{1}H NMR of **LIQ-TF** in $CDCl_3$.

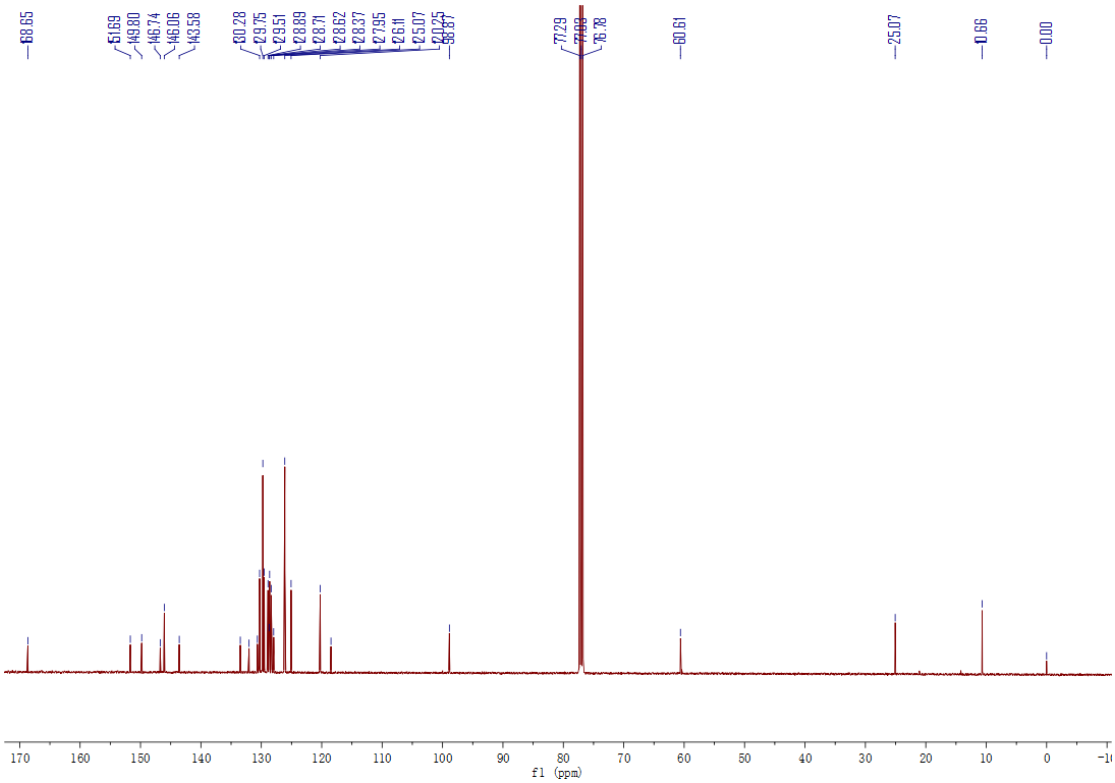


Fig. S2 ^{13}C NMR of **LIQ-TF** in $CDCl_3$.

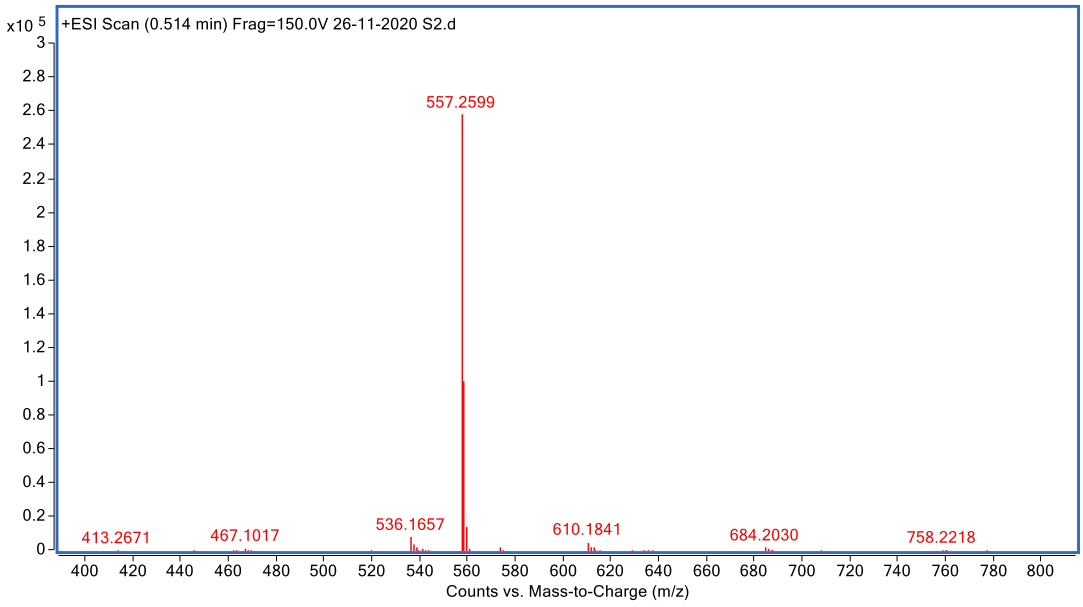


Fig. S3 HRMS spectrum of **LIQ-TF**.

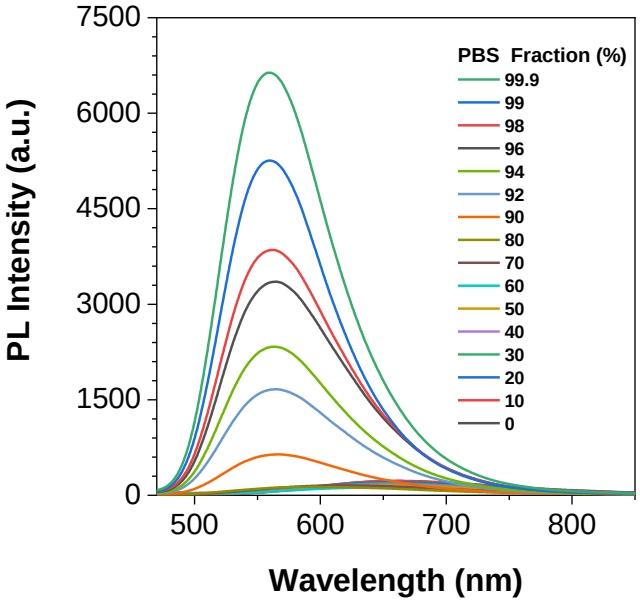


Fig. S4 PL spectra of **LIQ-TF** (10 μM) in mixtures of DMSO and PBS with different PBS contents.

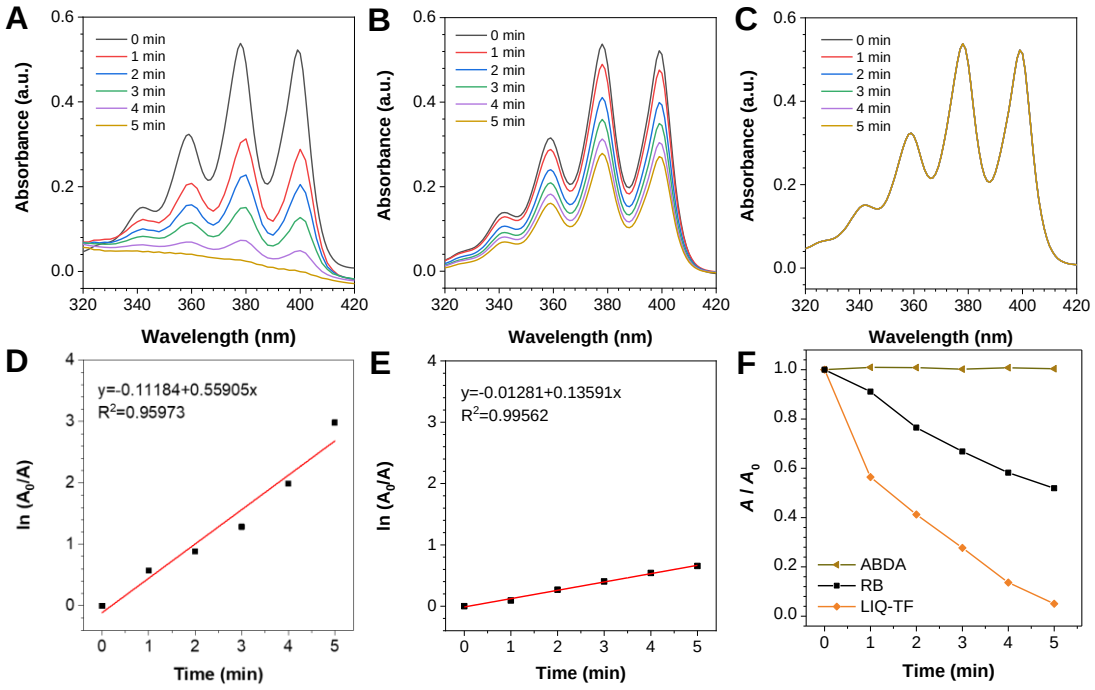


Fig. S5 $^1O_2$ generation efficiency of **LIQ-TF** in water. UV/Vis spectra of ABDA in the presence of **LIQ-TF** (A), RB (B) and blank control (C) under white-light illumination. The decomposition rate constant of ABDA in the presence of **LIQ-TF** (D) and RB (E). (F) Decomposition rates of ABDA in the absence or presence of **LIQ-TF** and RB under light illumination, where A_0 and A are the initial and final absorbances of ABDA at 378 nm, respectively.

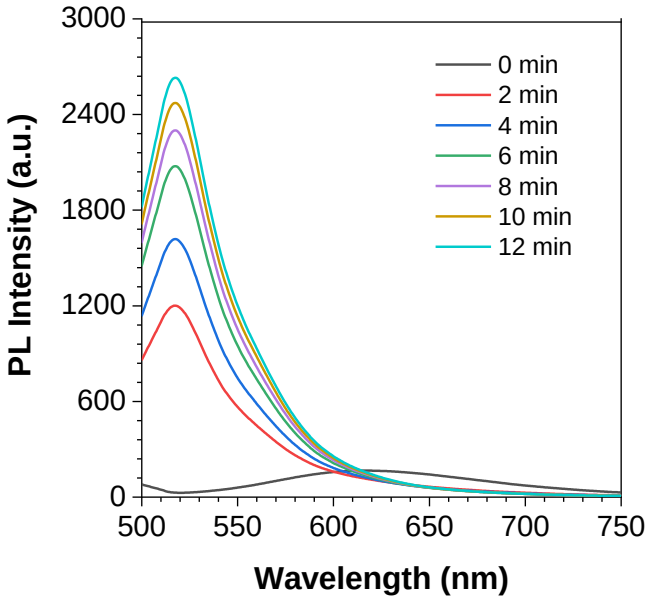


Fig. S6 PL spectra of HPF (5 μM) in the present of **LIQ-TF** (10 μM) under white light irradiation from 0-12 min (λ_{ex} = 492 nm) in PBS solution.

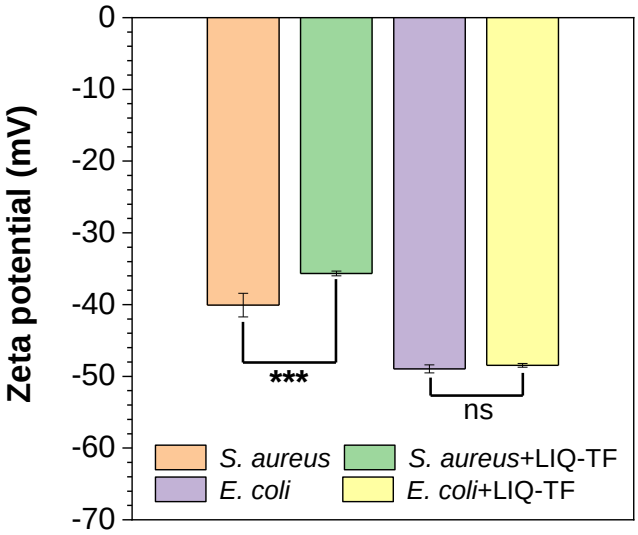


Fig. S7 Zeta potential of bacteria (*S. aureus* and *E. coli*) before and after treated with **LIQ-TF** (10 μM) for 10 min. Data represented the mean ± SD (n = 3). (***P < 0.001).

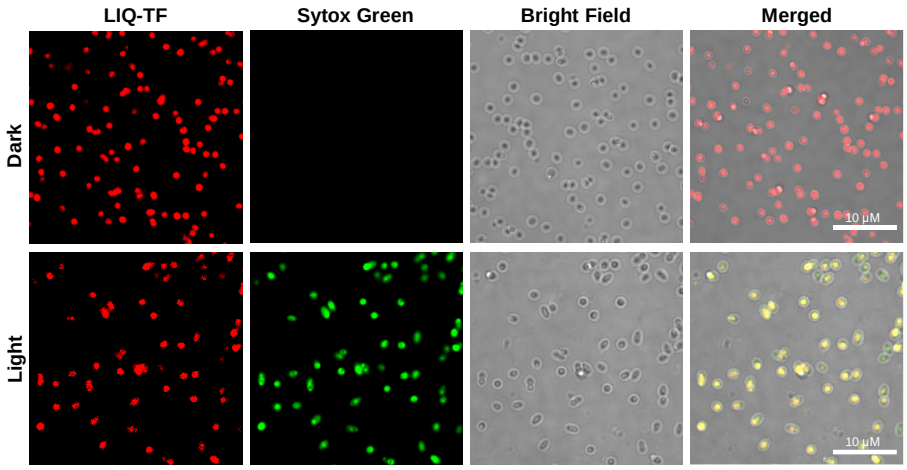


Fig. S8 Fluorescence images of *E. faecalis*. The *E. faecalis* were incubated with 10 μM **LIQ-TF** for 10 min, treated without or with white light for 30 min and then stained with Sytox Green. (**LIQ-TF**: λ_{ex} = 445 nm, λ_{em} = 600-700 nm, Sytox Green: λ_{ex} = 448 nm, λ_{em} = 500-550 nm)

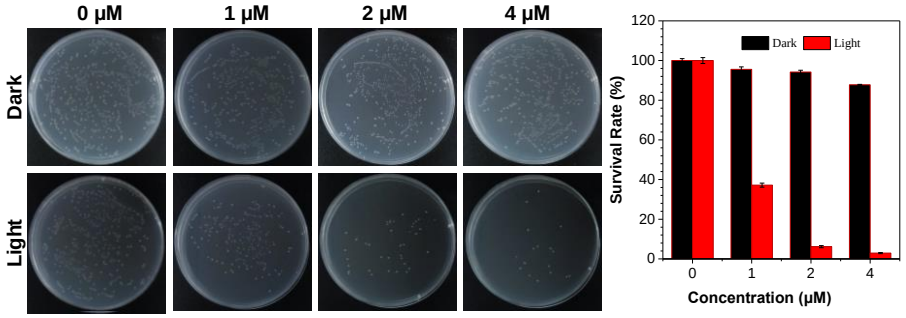


Fig. S9 Plates images and the survival rates of *E. faecalis* after incubated with different concentration of **LIQ-TF** (0, 1, 2, 4 μM) for 10 min and then treated without or with light illumination (20 mW/cm^2) for 30 min.

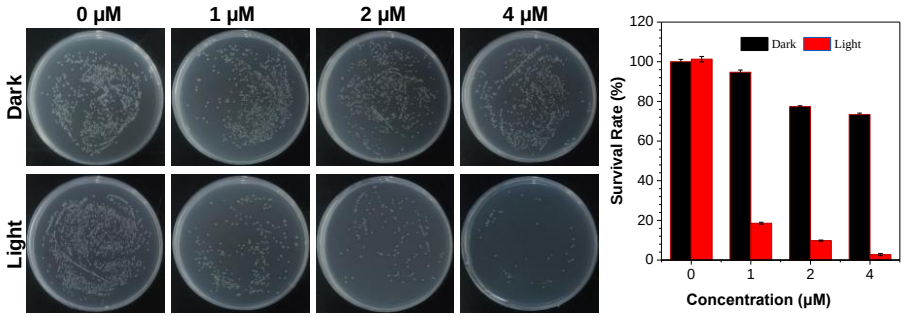


Fig. S10 Plates images and the survival rates of *S. typhimurium* after treatment different concentration of **LIQ-TF** (0, 1, 2, 4 μM) for 10 min and then treated without or with light illumination (20 mW/cm^2) for 30 min.

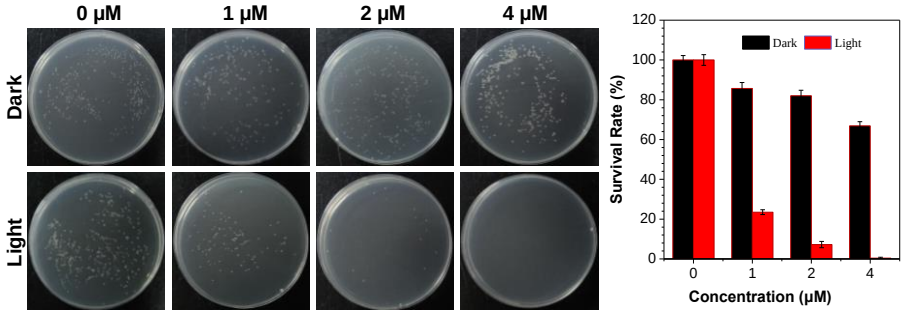


Fig. S11 Plates images and the survival rates of *B. subtilis* after incubated with different concentration of **LIQ-TF** (0, 1, 2, 4 μM) for 10 min and then treated without or with light illumination (20 mW/cm^2) for 30 min.

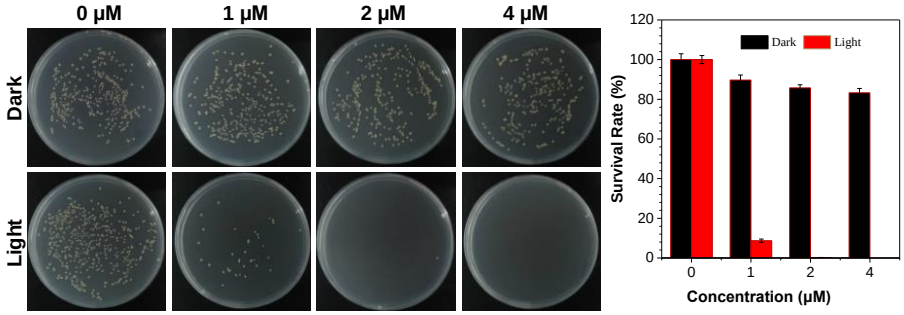


Fig. S12 Plates images and the survival rates of *S. aureus* after incubated with different concentration of **LIQ-TF** (0, 1, 2, 4 μM) for 10 min and then treated without or with light illumination (20 mW/cm^2) for 30 min.

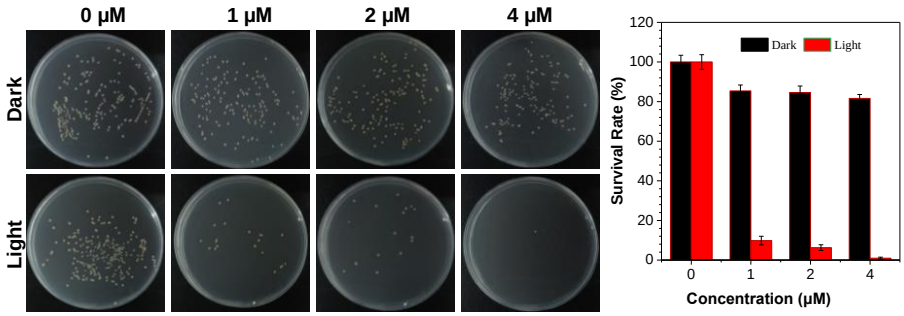


Fig. S13 Plates images and the survival rates of MRSA after incubated with different concentration of **LIQ-TF** (0, 1, 2, 4 μM) for 10 min and then treated without or with light illumination (20 mW/cm^2) for 30 min.

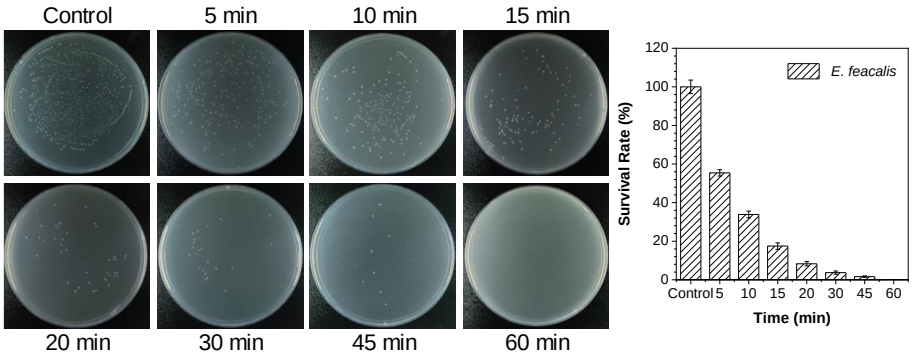


Fig. S14 Plates images and the survival rates of *E. faecalis* after incubated with 4 μM **LIQ-TF** for 10 min and then treated with light illumination (20 mW/cm^2) for different time (5, 10, 15, 20, 30, 45, 60 min). The *E. faecalis* without any treatment was used as control.

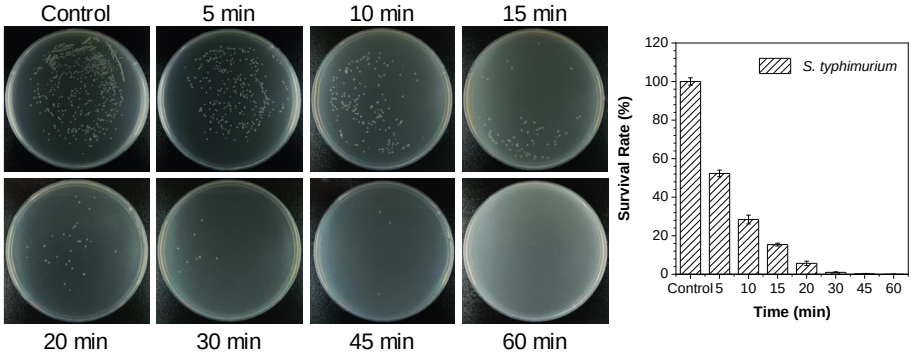


Fig. S15 Plates images and the survival rates of *S. typhimurium* after incubated with 4 μM **LIQ-TF** for 10 min and then treated with light illumination (20 mW/cm^2) for different time (5, 10, 15, 20, 30, 45, 60 min). The *S. typhimurium* without any treatment was used as control.

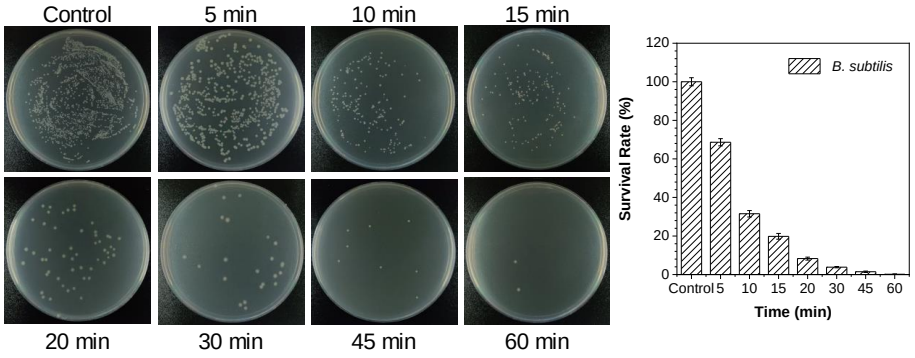


Fig. S16 Plates images and the survival rates of *B. subtilis* after incubated with 4 μM **LIQ-TF** for 10 min and then treated with light illumination (20 mW/cm^2) for different time (5, 10, 15, 20, 30, 45, 60 min). The *B. subtilis* without any treatment was used as control.

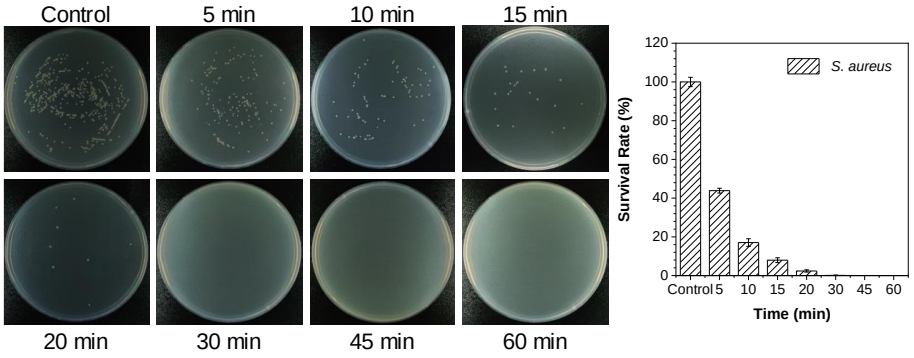


Fig. S17 Plates images and the survival rates of *S. aureus* after incubated with 4 μM **LIQ-TF** for 10 min and then treated with light illumination (20 mW/cm^2) for different time (5, 10, 15, 20, 30, 45, 60 min). The *S. aureus* without any treatment was used as control.

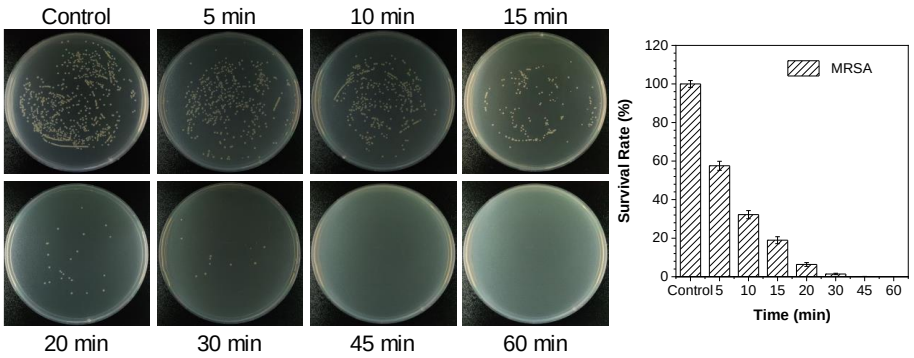


Fig. S18 Plates images and the survival rates of MRSA after incubated with 4 μM **LIQ-TF** for 10 min and then treated with light illumination (20 mW/cm^2) for different time (5, 10, 15, 20, 30, 45, 60 min). The MRSA without any treatment was used as control.

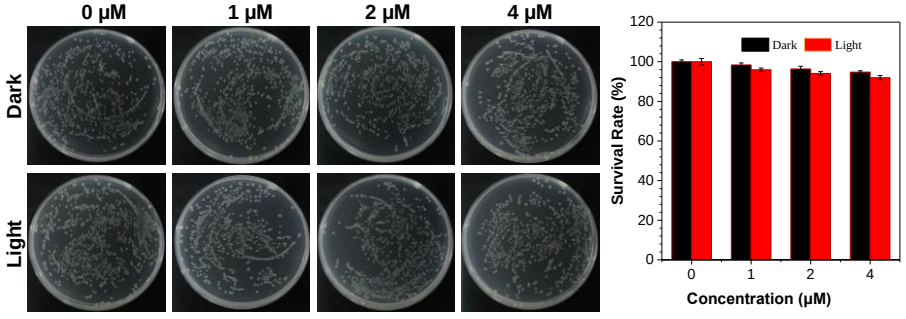


Fig. S19 Plates images and the survival rates of *E. coli* after incubated with different concentration of **LIQ-TF** (0, 1, 2, 4 μM) for 10 min and then treated without or with light illumination (20 mW/cm^2) for 30 min.

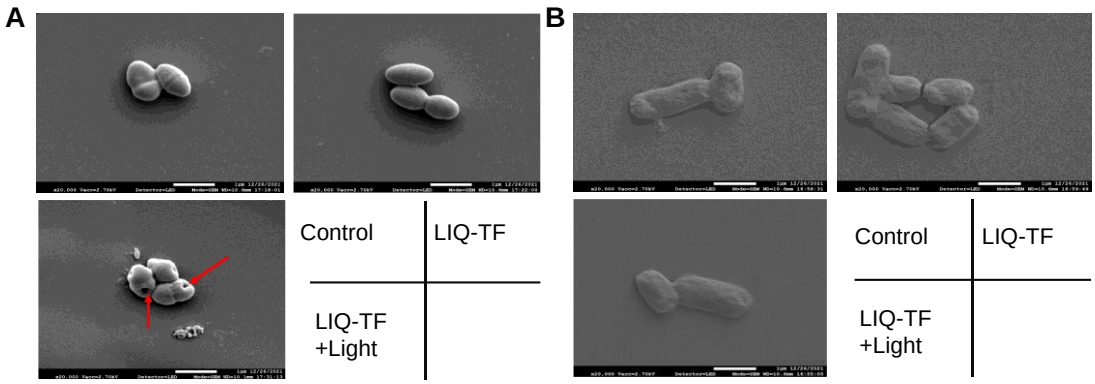


Fig. S20 SEM images of *S. aureus* (A) and *E. coli* (B) after treatment with PBS or **LIQ-TF** in the absence or presence of white light illumination for morphological analysis.

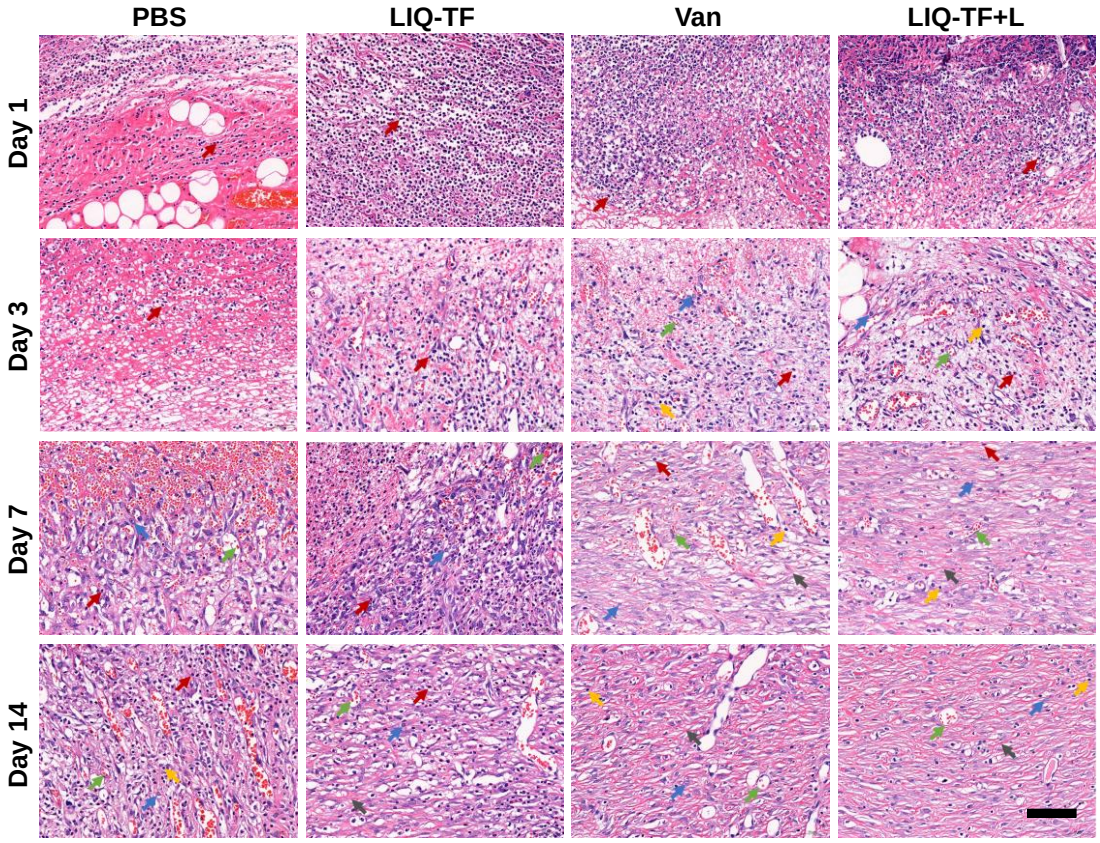


Fig. S21 H&E staining of the wound tissues harvested from different mice with MRSA-infection on Day 1, 3 ,7and 14 post-treatment. The arrows in the images indicate specific cell types and structures in the histological sections. Red arrows: neutrophils; yellow arrows: lymphocytes; blue arrows: fibroblasts; green arrows: blood vessels; gray arrow: collagenous fiber. Black line indicates the scale bar (100 μm).