Electronic Supplementary Information for

Fluorination in enhancing photoactivated antibacterial activity of Ru(II) complexes with photo-labile ligands

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Experimental section

Materials

Thiazolylblue tetrazolium bromide(MTT), 9,10-anthracenedipropanoicacid (9,10-ADPA) were purchased from Acros and Alfa Aesar. Hoechst 33342/Propidium Iodide (PI) and Bacterial Genomic DNA Extraction Kitwere purchased from Solarbio and used without further purification unless otherwise noted.Dulbecco's modification of Eagle's medium (DMEM), fetal bovine serum (FBS), phosphate buffer saline (PBS, 10 mM, pH=7.4), penicillin, and streptomycin were purchased from Corning.*Staphylococcus.aureus*(*S. aureus*)and *Escherichia coli*(*E. coli*) were provided by China General Microbiological Culture Collection Center. Methicillin-resistant*Staphylococcus aureus* (MRSA ATCC43300) andVancomycin-resistant *Enterococcus faecalis* (VRE ATCC51299) were purchased from Biofeng.

Instruments

UV-vis absorption spectra were obtained on a Shimadzu UV-4100 spectrophotometer.An LED lamp (470 ± 10 nm) was purchased from Suzhou D&R Instruments Co., Ltd.MTT and hemolysis assays were carried out on a Thermo MK3Multiscanmicroplate reader at 570 nm.ICP-MS was detected on a PerkinElmer ELAN DRC-e inductively coupled plasma mass spectrometer.Zeta potential was measured by a Malvern Zetasizer Nano ZS90. SEM images were taken on a HITACHI S-4800. Confocal laser scanning microscopy results were obtainedwith a Nikon Corporation A1R MP device.

Methods

Bacterial culture

S. aureus, MRSA,and *E.coli*wascultured either in LB medium or on an agar plate medium. Culture medium for VRE was brain heart infusion with Vancomycin (4 μg/mL). Incubation of bacteria was proceeded on a shaker bed of 100 rpm at 37℃for 16 h. Centrifuged and washed by PBS, the bacteria would be ready for following experiments.

Antibacterial activity of the photo-product of complex 4

First, complex 4 in H_2O was irradiated by 470 nm LED (22.5 mW/cm^2) for 20 min, then the antibacterial activity of the photo-product was examined against *E.coli*, *S. aureus* and MRSA without further irradiation.

Bacterial binding/uptake and subcellular distribution

1 mL of bacterial cells (10^8cells/mL) in PBS was incubated with complexes**1-4** (5 μM), respectively, at 37 ℃ for 30 min in the dark.The light groups were irradiated with 470 nm LED (22.5 mW cm^{-2}) for 20 min,and the dark groups remained in the dark. Then the cells were centrifuged and washed with PBS for three times and DNA was isolated with a kit provided by Solarbio. Both DNA and residuals were made into 2% nitric acid solutions separatelyfor ICP-MS measurements to determine the ruthenium concentrations.

Bacterial binding/uptake at 37 ℃ and 4 ℃

Two samples of about 10^8 *S.aureus* were cultured in 3 ml PBS with 10 μM of complex 4 in the dark at 37 ℃ and 4 ℃, respectively. After 30 min, the samples were centrifuged. The absorbance at 429 nm of the supernatant was measured. The binding/uptake levels can be calculated from the reduction of the absorbance at 429 nm. The experiments were carried out independently for 3 times.

ζ -potentialmeasurements

Complexes were addedto suspensions of bacteria (10^9 cells/mL) at a final concentration of 5 μM. Samples were incubated for 30 min , washed and re-suspended in 1 mL of PBSfor ζ-potential measurements.

Cell culture

The human hepatocyte L-O2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C and 5% CO_2

Hemolysis assay

20 μLwashed rabbit erythrocytes, 980 μL of gradient concentrations of Ru(II) complexes (PBS solution) were mixed in microcentrifuge tubes. Negative control (980 μLPBS and 20 μL erythrocytes) and positive control (980 μL H_2O and 20 μL erythrocytes) were employed as quality controls in this assay. Mixtures were incubated at 37 ℃ for 2 h on shaking bed before centrifuged. 200 μL of supernatant was transferred and examined on 96-well plates by reading the absorbance measured at 570 nm via a Thermo MK3 Multiscanmicroplate reader.

Equation for hemolytic activity: H (%) = (A_S - A_N)/(A_P - A_N) × 100%, where A_S, A_N and A_P stand for the optical density (OD_{570}) of the sample, the negative control and positive control, respectively.

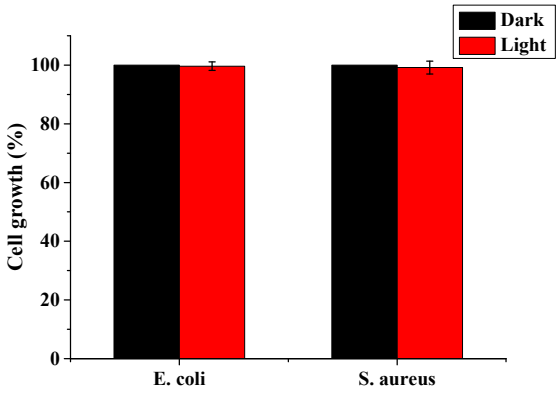


Figure S1. The effect of light irradiation alone (470 nm, 22.5 mW/cm^2, 30 min) on the growth of *E.coli*

and *S.aureus.*

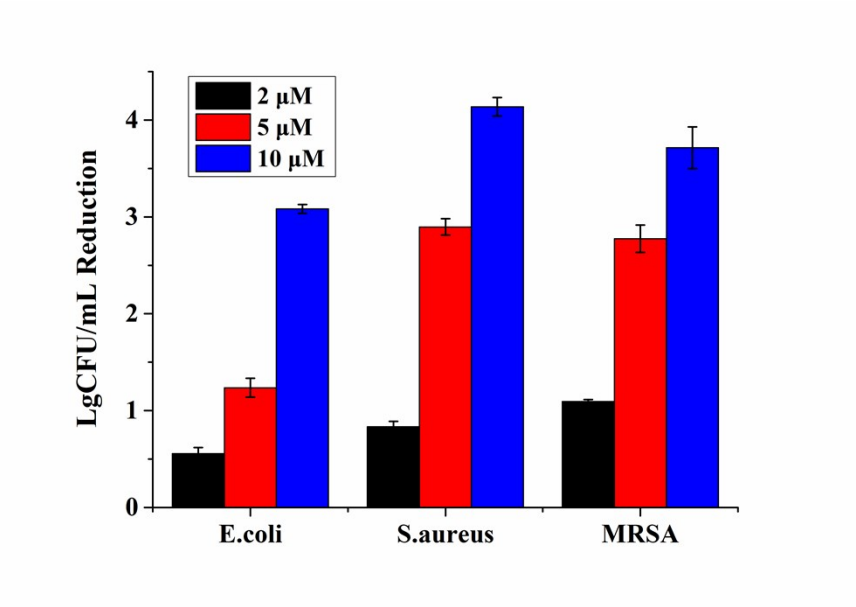
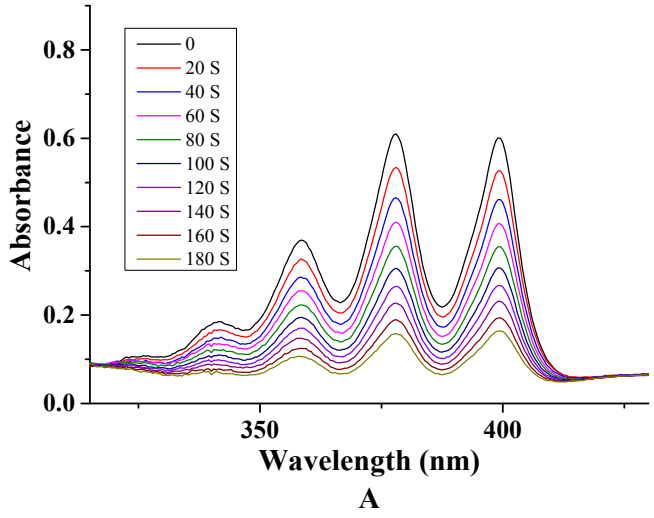


Figure S2. Antibacterial activity of the photo-product of complex **4** against *E. coli*, *S. aureus* and MRSA in the dark.



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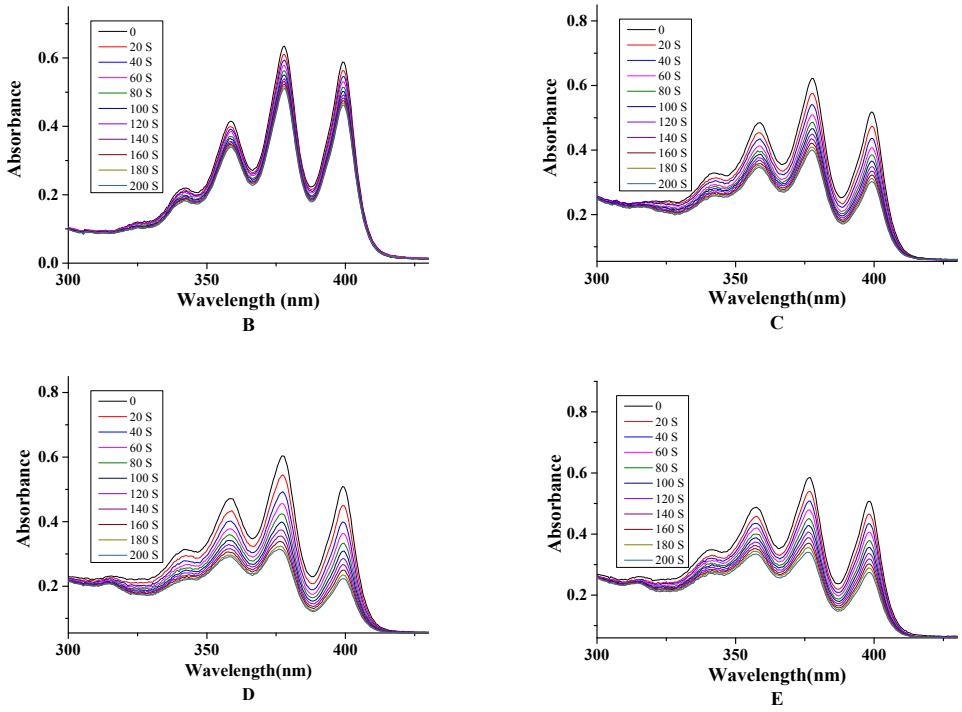

Figure S3. Absorption spectra changes of 9,10-ADPA (50 μM) in the presence of $[Ru(bpy)_3]^{2+}$ (A), **1** (B), **2** (C), **3** (D), 4 (E) upon light irradiation (470 nm LED, 22.5 mW/cm^2) for different times. The absorbance of Ru complexes at 470 nm was adjusted as the same.

Table S1. The measured concentration reduction of complex 4 in the supernatant by *S.aureus* at 37 ℃ and 4 ℃ (data are mean values ± standard deviation of three independent experiments)

	Concentration Reduction (μM)
37 ℃	0.88 ± 0.01
4 ℃	0.82 ± 0.01