

Novel Water-Soluble Photosensitizer for Photodynamic Inactivation of Gram-Positive Bacteria Using Nontoxic Dose of UV Light

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Research

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Abstract

Background Antimicrobial photodynamic therapy (APDT) is a promising alternative to traditional antibiotics for bacterial infections, which inactivates a broad spectrum of bacteria. However, it has some disadvantages including poor water solubility and easy aggregation of hydrophobic photosensitizers (PS), and poor tissue penetration and cytotoxicity when using UV as the light source, leading to undesired photodynamic therapy efficacy.

Results In this study, we develop a novel water-soluble natural PS (sorbicillinoids) obtained by microbial fermentation using recombinant filamentous fungus *Trichoderma reesei* (*T. reesei*). Sorbicillinoids could effectively generate singlet oxygen (1O_2) under ultraviolet (UV) light irradiation, and ultimately display photoinactivation activity on Gram-positive bacteria, but not Gram-negative ones. *Staphylococcus aureus* (*S. aureus*) treated with sorbicillinoids and UV light displayed high levels of intracellular reactive oxygen species (ROS), notable DNA photocleavage, and compromised cell semi-permeability without overt cell membrane disruption. Moreover, the dark toxicity, phototoxicity or hemolysis activity of sorbicillinoids is negligible, showing its excellent biocompatibility.

Conclusion Sorbicillinoids obtained from *T. reesei* display photoinactivation activity on Gram-positive bacteria using nontoxic dose of UV light irradiation and have an excellent biocompatibility. Therefore, sorbicillinoids, a type of secondary metabolite from fungus, has a promising future as a new PS for APDT.

Background

Diseases caused by bacterial infections, such as pneumonia and sepsis, threaten the lives of millions of people every year [1, 2]. The most widely used strategy for coping with bacterial infections at present is antibiotics [3]. However, the abuse of antibiotics in recent decades has led to an increase in the resistance of bacteria [4], which causes the emergence of multi-drug resistant (MDR) bacteria [5, 6]. Thus, it is urgent to develop new antibiotics and efficient antibacterial therapies to fight MDR bacteria. Many novel antibiotics have been explored to successfully combat MDR bacterial infection, including carbon dots nanoparticles (NP) [7, 8] and cationic antibacterial peptides [9, 10]. In addition, new efficient antibacterial therapies have been also applied in treating bacterial infections, such as photodynamic therapy (PDT) [11, 12] and photothermal therapy (PTT) [13, 14]. Antimicrobial photodynamic therapy (APDT) has been widely reported as a promising alternative to traditional antibiotics in the past few years [15, 16]. APDT generally involves molecular oxygen, light source, and photosensitizer (PS). With light irradiation of a suitable wavelength, PS converts molecular oxygen into reactive oxygen species (ROS) [12], primarily singlet oxygen (1O_2). The highly active ROS can cause damage to important biomolecules such as lipids [17], proteins [18], and DNA [19], ultimately leading to bacterial cell death. Unlike conventional antibiotics, APDT only irradiates the lesion location and does not cause damage to other parts [11], thereby achieving selective bacterium-killing. More importantly, APDT can inactivate a broad spectrum of bacteria, opening new avenues for the development of new antibacterial therapies [12].

Most currently available PSs for APDT have poor solubility in water, showing a strong tendency to aggregate, ultimately leading to poor photodynamic therapy efficacy [20]. To improve their hydrophilicity, a variety of modification methods were applied by physical encapsulation or chemical covalent conjugation of PSs to nanocarriers such as peptide nanoparticles [21], polymer nanoparticles [22], and liposomes [23]. However, these surface modification methods are time-consuming and usually bring some unexpected consequences such as low biocompatibility, which may compromise the clinical application of APDT. Another issue that plagues APDT was the need of UV light for some PSs like metal oxide NPs zinc oxide (ZnO) NPs and titanium dioxide (TiO₂) NPs [24], for the reason that UV light is cytotoxic to human cells and has poor tissue penetration [25]. Therefore, it is urgent to find new PSs for the APDT system to overcome these issues. Natural products from fungus and plants have been reported as a promising alternative to conventional PSs such as hypericin [26], curcumin [27], hypocrellin [28] and cationic riboflavin [29]. The compounds are usually derived from secondary metabolism. Natural products as PSs have the advantages of good biocompatibility, wide source, and high ¹O₂ yield [30].

Sorbicillinoids are hexaketide metabolites isolated from both marine and terrestrial ascomycetes, such as *Trichoderma*, *Acremonium*, *Aspergillus*, *Emericella*, *Penicillium*, *Phaeoacremonium*, and *Scytalidium* [31]. Most of these compounds have characteristic structures, including bicyclic or tricyclic structures and C1'–C6' sorbyl sidechain. Sorbicillinoids were divided into four classes based on their structure [32]: monomeric sorbicillinoids, bisorbicillinoids, trisorbicillinoids, and hybrid sorbicillinoids. In recent years, various potential applications of sorbicillinoids have been exploited, such as antioxidants [33, 34], antibiotics [35, 36], and anticancer drugs [37, 38]. However, research on sorbicillinoids as a PS for the APDT system has not been reported.

In this study, we discovered a new type of natural material as a water-soluble PS for APDT (Scheme 1). Sorbicillinoids produced by *Trichoderma reesei* converts oxygen molecules into ¹O₂ effectively under mild ultraviolet (UV) irradiation. As a result, sorbicillinoids exhibited great APDT effect toward Gram-positive bacteria. The underlying mechanism of the light-activated antibacterial activity of sorbicillinoids was explored by monitoring intracellular ROS, DNA photocleavage and cell membrane damage. Furthermore, the biocompatibility of sorbicillinoids-mediated APDT was evaluated using MTT assay and hemolysis assay.

Results And Discussions

Singlet oxygen generation

Sorbicillinoids was produced from the fermentation of the recombinant *Trichoderma reesei* (*T. reesei*) strain ZC121 with an excellent sorbicillinoids production ability⁴⁰. The obtained sorbicillinoids mainly included sorbicillinol, bisvertinolone and oxosorbicillinol as identified by LC-MS [40]. Given that sorbicillinoids has great absorbance at 370 nm [41], there is a possibility that sorbicillinoids can generate ¹O₂ under light irradiation. To prove this, the ¹O₂ generation ability of sorbicillinoids under the irradiation

of both UV and white light was measured using a SOSG kit (Figure 1). Sorbicillinoids produced significant $^1\text{O}_2$ when irradiated with UV light. The produced $^1\text{O}_2$ was increased noticeably with the irradiation time increasing, being 300% that of UV alone at 30 min. In contrast, when sorbicillinoids was exposed to white light for 30 min, insignificant $^1\text{O}_2$ production was observed. No $^1\text{O}_2$ generation was observed for sorbicillinoids alone. This result confirmed our hypothesis that sorbicillinoids can synthesize $^1\text{O}_2$ in the presence of UV light, but not visible light. It is worth noting that $^1\text{O}_2$ is highly toxic to the microorganisms, serving as one major factor for APDT's antibacterial effect [15,42]. Therefore, sorbicillinoids from *T. reesei* might be explored as a new PS for APDT to kill microorganisms.

Antibacterial activity

Inspired by their excellent $^1\text{O}_2$ production ability under UV irradiation, we investigated the APDT ability of sorbicillinoids. *S. aureus* and *E. coli* representing Gram-positive and Gram-negative bacteria respectively, were incubated with different concentrations of sorbicillinoids in 1.5 mL sterilized centrifuge tubes, followed by UV light irradiation for 30 min (Figure 2). When irradiated under UV light, *S. aureus* was killed by 60.3%, 68.5%, 90.5%, 98.1%, and 99.5% in the presence of 50, 100, 200, 250, and 300 mg/mL sorbicillinoids, respectively (Figure 2a and 2b). Conversely, no antibacterial effect of sorbicillinoids was observed on *E. coli*, even when the administrated concentration was increased up to 1000 mg/mL (Figure 2c and 2d). Sorbicillinoids or UV alone was non-toxic to both *S. aureus* and *E. coli* (Figure 2). The undetected antibacterial effect of UV light in this study is probably ascribed to the poor penetration ability of UV light through the plastic centrifuge tube [43]. It appears that while the plastic is mitigating the intensity of incident UV light to the point where there is no cell death in the negative control, there is still enough incident UV light to sensitize sorbicillinoids to generate drastic $^1\text{O}_2$, leading to the killing of *S. aureus*.

Moreover, the UV-mediated APDT ability of sorbicillinoids was tested on another two Gram-positive bacteria *B. subtilis* and *M. luteus*, and another Gram-negative bacterium *P. vulgaris*. In the presence of 25, 50, 75, 100, 200 and 300 mg/mL sorbicillinoids and under UV light irradiation, *B. subtilis* was killed by 36.7%, 40.4%, 61.4%, 89.3% and 98.5% and 98.9%, respectively, whereas *M. luteus* was killed by 29.5%, 80.8%, 98.0%, 99.7% and 99.9%, respectively in the presence of 50, 100, 200, 250 and 300 mg/mL sorbicillinoids (Figure S1). By contrast, no antibacterial ability was observed against *P. vulgaris* (Figure S2) as it is in the case of *E. coli*. Obviously, sorbicillinoids with the irradiation of nontoxic UV light can successfully kill Gram-positive bacteria, but not Gram-negative ones. This Gram-selective antibacterial ability of sorbicillinoids may be attributed to the different cell wall structures of Gram-positive bacteria and Gram-negative bacteria. It is well-known that compared to Gram-positive bacteria, Gram-negative bacteria have an outer membrane that lies outside of the plasma membrane, serving as a penetration barrier and protecting Gram-negative bacteria from antibiotics and PSs [10,44]. This outer membrane may prevent sorbicillinoids from entering Gram-negative cells to realize APDT. The localization of PSs largely determines the efficiency of PDT, as the half-life of $^1\text{O}_2$ is very short (< 0.04 ms) and its action

radius is only shorter than 0.02 mm in the biological system [45]. Therefore, despite it could produce abundant $^1\text{O}_2$ in the solution under UV light irradiation, the extracellular sorbicillinoids exhibited no bactericidal activity against Gram-negative bacteria. For Gram-positive bacteria without the outer membrane, the water-soluble sorbicillinoids with small molecular weight might readily diffuse through cell surface into the cytosol to exert APDT.

Intracellular ROS generation and agarose gel electrophoresis of genomic DNA

To evidence our speculation that sorbicillinoids enters Gram-positive bacteria to realize APDT, the generation of intracellular ROS was tested employing 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) probe. DCFH-DA freely passes through the cell membrane into cells where it is hydrolyzed by intracellular esterase to form 2',7'-dichlorodihydrofluorescein (DCFH) that cannot penetrate the cell membrane and stay inside cells. DCFH is subsequently oxidized by the intracellular ROS into 2',7'-dichlorofluorescein (DCF) that has fluorescence. Neither DCFH-DA nor DCFH has fluorescence. Therefore, the level of ROS in the cells can be measured by detecting the fluorescence of DCF. After treated with sorbicillinoids and irradiated under UV light, the fluorescence intensity of *S. aureus* increased significantly by 5 times as compared to the untreated *S. aureus*, whereas no increasing fluorescence intensity was observed for *E. coli* (Figure 3a). This suggested that the treatment of sorbicillinoids with the irradiation of UV induced *S. aureus* to generate large sums of intracellular ROS, which might further damage biomacromolecules like proteins, DNA and lipids. This result matched well with the selective photoinactivation of sorbicillinoids against Gram-positive bacteria as we observed above.

Inspired by its ultra-high intracellular ROS level, DNA photocleavage of *S. aureus* with the treatment of sorbicillinoids and UV irradiation was detected by agarose gel electrophoresis (Figure 3b). There was a marked reduction of DNA for *S. aureus* treated with 100 $\mu\text{g}/\text{mL}$ sorbicillinoids under UV light irradiation, indicating nucleic acid photocleavage took place in the treated bacterial cells. *S. aureus* treated with sorbicillinoids or UV light alone did not display a reduction of DNA. On the contrary, sorbicillinoids did not result in observable DNA reduction in *E. coli* with or without UV light. Obviously, the unusually high intracellular ROS induced by sorbicillinoids upon UV irradiation caused cellular DNA damage in *S. aureus*.

PI staining and morphological characterization of bacteria

Both DNA and cytoplasmic membrane are two crucial cellular components targeted by ROS in APDT [46]. Therefore, the effect of sorbicillinoids on the cell membrane of *S. aureus* and *E. coli* was investigated using both propidium iodide (PI) staining and SEM imaging (Figure 4 and Figure S3). PI cannot penetrate into viable bacteria cells with complete membrane. Yet it can enter bacterial cells with damaged membrane to intercalate between the base pairs of the accessible double-stranded DNA and emit red fluorescence with the excitation at 580 nm. *S. aureus* were dyed red by PI after the treatment of sorbicillinoids and UV irradiation (Figure 4a), demonstrating their membrane permeability was compromised. Nevertheless, no red fluorescence was observed in the irradiated *E. coli* cells in the

presence of sorbicillinoids, showing their cell membrane was not affected (Figure S3a). This finding was consistent with that sorbicillinoids exhibited no APDT activity against *E. coli*. Similar to most classical PSs [46], sorbicillinoids induces oxidative damage to the cell membrane of *S. aureus*, contributing to its overall antimicrobial activity together with the high intracellular ROS level and the notable DNA photocleavage.

Furthermore, SEM experiments were carried out to monitor the impact of sorbicillinoids on the morphology of bacterial surface. No noticeable morphology change was found in the SEM pictures of the irradiated *S. aureus* (Figure 4b) and *E. coli* (Figure S3b), including wrinkles, holes or leaked intracellular substances as reported previously for some photodynamic antibacterial materials [15,47]. Sorbicillinoids killed *S. aureus* without overt cell membrane disruption, as in the case of other PSs like EPS-RB NPs [48]. Taken together, sorbicillinoids can readily diffuse through the cell wall of Gram-positive bacteria into the cells. When irradiated under UV light, it produced large sums of intracellular ROS, thereby targeting and destroying both cytoplasmic membrane and DNA to achieve APDT effect. This does not happen to Gram-negative bacteria due to their distinctive outer membrane that is an extra effective penetration barrier to block sorbicillinoids out of cells.

Cytotoxicity evaluation assay and hemolysis activity

Last but not least, the biocompatibility of sorbicillinoids was assessed by the cytotoxicity measurement and hemolysis assay (Figure 5). Sorbicillinoids showed a little cytotoxicity to AT-II cells at 400 mg/mL (Figure 5a) that was higher than its bactericidal concentrations for *S. aureus* (Figure 4a), *B. subtilis* (Figure S1a) and *M. luteus* (Figure S1b). When the concentration of sorbicillinoids was increased to 450 mg/mL, more than 85% of AT-II cells remained alive, demonstrating a high safety of sorbicillinoids (Figure 5a). More importantly, even under the irradiation of UV light, only a slight decrease of the cell viability was found with > 75.66% AT-II cells alive when the concentration of sorbicillinoids was increased up to 450 mg/mL. Thus, sorbicillinoids did not induce marked dark toxicity or phototoxicity to AT-II cells. On the other hand, only 0.89% hemolysis was observed with the treatment of 300 mg/mL sorbicillinoids (Figure 5b and Figure S4). Sorbicillinoids show excellent biocompatibility, which is of benefit to their future practical applications in clinic.

Conclusions

We discovered a novel water-soluble PS (sorbicillinoids) from filamentous fungus *Trichoderma reesei* by microbial fermentation. The obtained sorbicillinoids could transform oxygen molecules to $^1\text{O}_2$ effectively under nontoxic dose of UV irradiation. As a result, the irradiated sorbicillinoids by nontoxic dose of UV light displayed photoinactivation activity against Gram-positive bacteria including *S. aureus*, *B. subtilis* and *M. luteus*. *S. aureus* treated with sorbicillinoids and UV light irradiation exhibited high level production of intracellular ROS, marked DNA reduction, and damaged cell membrane without observable cell morphology change, leading to the cell death of Gram-positive bacteria. Sorbicillinoids can be

obtained on a large scale through microbial fermentation by *T. reesei* or other microorganisms, which is sustainable, environmentally-friendly and low cost. This newly-discovered PS has good water solubility. Furthermore, they displayed good biocompatibility. Therefore, sorbicillinoids-based APDT presents a potential platform for combating pathogen-related diseases.

Methods

Materials

Luria Bertani (LB) broth, LB agar and potato dextrose agar (PDA) were purchased from Beijing Land Bridge. Singlet oxygen sensor green (SOSG) kit was acquired from Molecular Probes Inc. (Eugene, Oregon, USA). Propidium iodide (PI) was obtained from KeyGen Biotech (Nanjing, China). Glutaraldehyde, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-*H*-tetrazolium bromide (MTT), methanol, ethanol, dimethyl sulfoxide (DMSO), and triton X-100 were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Reactive oxygen species assay kit was bought from Beyotime Biotech Inc. (Shanghai, China). All other chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solutions were prepared with deionized water (18.2 MΩ cm) purified by a Milli-Q water purification system (Milli-Q, Millipore, USA).

Sorbicillinoids production

The conidia produced by *Trichoderma reesei* strain ZC121 grown on PDA plates for 7 days at 28 °C, were inoculated into 10 mL sabouraud dextrose broth (SDB) and incubated for 48 h with 200 rpm at 28 °C. Pre-grown mycelia were inoculated with an inoculation ratio of 10% (v/v) into 50 mL *Trichoderma* minimal media [39] (TMM) with 2% glucose, and then incubated for 120 h with 200 rpm at 28 °C. The suspension was centrifuged at 14000 rpm for 15 min at 4 °C to remove *Trichoderma reesei* cells and other solid materials, and the supernatant was dried at 120 °C in an oven. The obtained powder was dissolved in methanol to remove inorganic salt. Methanol was evaporated at 60 °C under a nitrogen atmosphere to obtain sorbicillinoids. TMM medium was composed of the following chemicals (all concentration unit is g/L unless otherwise noted): (NH₄)₂SO₄, 4.0; KH₂PO₄, 6.5; Tween-80, 0.0186% (v/v); Yeast extract, 0.75; Tryptone, 0.25; Maleic acid, 11.6; FeSO₄·7H₂O, 0.005; MnSO₄·H₂O, 0.0016; ZnSO₄·7H₂O, 0.0014; CoCl₂·6H₂O, 0.002; MgSO₄, 0.60; CaCl₂, 0.60; urea, 1.0. Then the pH of TMM was adjusted to 5.8 by NaOH.

Singlet oxygen generation

The generation of ¹O₂ was measured with singlet oxygen sensor green (SOSG). 1 mL SOSG dissolved in methanol was mixed with 2 mL 0.9% NaCl and 50 mg/mL sorbicillinoids in 0.9% NaCl, followed by UV light (2 mW/cm²) or white light irradiation (8 mW/cm²). 0.9% NaCl without any light irradiation was set

as control. Then, the fluorescence intensity of the samples at 528 nm was measured by a spectrofluorophotometer (RF-5301PC, Shimadzu, Japan).

Antibacterial activity

Escherichia coli (*E. coli*), and *Staphylococcus aureus* (*S. aureus*) were selected to present Gram-negative and Gram-positive bacteria, respectively. *E. coli* or *S. aureus* were cultivated overnight in LB medium at 37 °C with 200 rpm. Bacteria were obtained from bacteria suspension overnight by centrifugation at 8000 rpm for 5 min and then resuspended in 0.9% NaCl. The optical density of the resuspension at 600 nm (OD_{600}) was measured using a UV-vis spectrophotometer (UV-2600, Shimadzu, Japan). Then, the resuspension was diluted to $OD_{600} = 0.5$ with 0.9% NaCl. After the dilution, 100 mL bacteria suspension was mixed with 900 mL sorbicillinoids dissolved in 0.9% NaCl at different concentrations. The final concentrations of sorbicillinoids were 0, 50, 100, 200, 250, and 300 mg/mL. for *S. aureus* (0, 200, 400, 600, 800, and 1000 mg/mL. for *E. coli*). After irradiation under UV light (2 mW/cm^2) for 30 min, 100 mL solution was plated on LB agar plates. The plates were placed in an incubator at 37 °C for 24 h, followed by colony counting. Antibacterial activity of sorbicillinoids against Gram-negative bacterium *Proteus vulgaris* (*P. vulgaris*), and Gram-positive bacteria *Bacillus subtilis* (*B. subtilis*) and *Micrococcus luteus* (*M. luteus*), were also tested in the same way.

Intracellular ROS generation

2,7-dichlorofluorescein diacetate (DCFH-DA) was applied to measure the generation of intracellular ROS. 0.5 mL DCFH-DA was added into 500 mL bacteria suspension ($OD_{600} = 0.05$), followed by incubation at 37 °C for 10 min. After centrifugation at 8000 rpm for 5 min, bacteria were resuspended in 500 mL water, supplemented with 5 mL sorbicillinoids (10 mg/mL for *S. aureus* and 40 mg/mL for *E. coli*) dissolved in water. After incubation at 37 °C for 30 min, the suspension was irradiated under UV light (2 mW/cm^2) for 30 min. The fluorescence intensity was measured by a flow cytometer (NovoCyte™ 2060, ACEA, USA). Channel used for analyses was FITC with the excitation at 488 nm.

Agarose gel electrophoresis of bacterial genomic DNA

The genomic DNA of bacteria was extracted with the Bacterial DNA kit which was bought from TIAGEN Biotech Co., Ltd. (Beijing, China). The bacteria cell (1×10^6 CFU/mL) with different treatments as indicated in the antibacterial activity assay were collected by centrifugation at 8000 rpm for 5 min and then washed twice with 0.9% NaCl. The genomic DNA was extracted according to manufacturer's instruction. Briefly, the bacterial cells were separately resuspended in 200 μL GA buffer, 20 μL proteinase K solution and 220 μL GB buffer. After incubation at 70 °C for 10 min, 220 μL ethanol was added into each sample. Then the solution was transferred to a spin column CB3 and washed with GD buffer and PW washing solution. After that, the genomic DNA was bound onto the spin column and then eluted with

TE buffer. The extracted DNA was analyzed by electrophoresis in Tris Acetate-EDTA (TAE) buffer containing 0.8% agarose (w/v) for 50 min at 100 V. The electrophoretic profiles were acquired with a Gel imaging system (Tanon 3500R, Shanghai, China).

PI Staining

The bacterial cells (1×10^6 CFU/mL) with different treatments as indicated in the antibacterial activity assay were incubated at 37 °C for 2 h and then collected by centrifugation at 8000 rpm for 5 min. After the centrifugation, the bacteria were stained with red-fluorescent nucleic acid stain (PI) for 30 min. The bacteria samples were imaged using a confocal microscope (TCS SP8, Leica, Germany).

Morphological Characterization of Bacteria

Morphological characterization of bacteria was carried out with a scanning electron microscope (SEM, ULTRA Plus, Zeiss, Germany). The bacterial cells (1×10^6 CFU/mL) with different treatments as indicated in the antibacterial activity assay were collected by centrifugation at 8000 rpm for 5 min and then resuspended in 0.9% NaCl. After washing with 0.9% NaCl three times, the bacteria cells were mixed with 2.5% (v/v) glutaraldehyde for 12 h to fix the cell morphology, followed by dehydration using graded ethanol (30%, 50%, 70%, 90%, and 95%). Then the bacterial cells were resuspended in 100% ethanol and dripped in a silicon slide for SEM imaging.

Cytotoxicity Evaluation

The toxicity of sorbicillinoids towards AT-II (normal human lung cell) by MTT assay. AT-II cells were cultivated in Dulbecco's modified eagle medium (DMEM), containing 10% fetal bovine serum, 100 U/mL of penicillin, and 100 µg/mL streptomycin in an incubator with 5% CO₂ at 37 °C. The cells were seeded in a 96-well plate at a density of 5000 cells per well. After 24 h of culture, the cells were mixed with sorbicillinoids dissolved in DMEM at the final concentrations of 0, 50, 100, 150, 200, 250, 300, 350, 400 and 450 mg/mL, followed by 24 h of culture. Then, 10 mL of MTT (5 mg/mL) was added into each well. After incubation for 4 h, the solution was removed and 150 µL DMSO was added into each cell. Then, the absorbance at 492 nm was measured with a microplate photometer (Multiskan FC, Thermo Fisher Scientific, USA).

Hemolysis activity

Fresh blood was collected from a healthy male mouse and restored in an anticoagulation tube to avoid the blood coagulation. Red blood cells (RBCs) were obtained from the blood by centrifugation at 2000

rpm for 5 min and resuspended in 0.9% NaCl. The obtained RBCs were treated with different concentrations of sorbicillinoids (50, 100, 200, 250, and 300 mg/mL) for 2 h at 37 °C, and centrifuged at 2000 rpm for 5 min. Then the resultant supernatants were transferred to a 96-well plate for the absorbance measurement at 492 nm with a microplate photometer (Multiskan FC, Thermo Fisher Scientific, USA). RBCs treated with phosphate-buffered saline (PBS) and triton X-100 were set as the negative control and positive control, respectively. The hemolysis percentage was calculated by the following formula:

Hemolysis% = [(absorbance of samples – absorbance of negative control) / (absorbance of positive control – absorbance of negative control)] × 100%.

Declarations

Authors' contributions

YZHY and LFM designed this study; YZHY, LJY and QY performed the experiments; YZHY and LJY analyzed the data. YZHY wrote the manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Figures

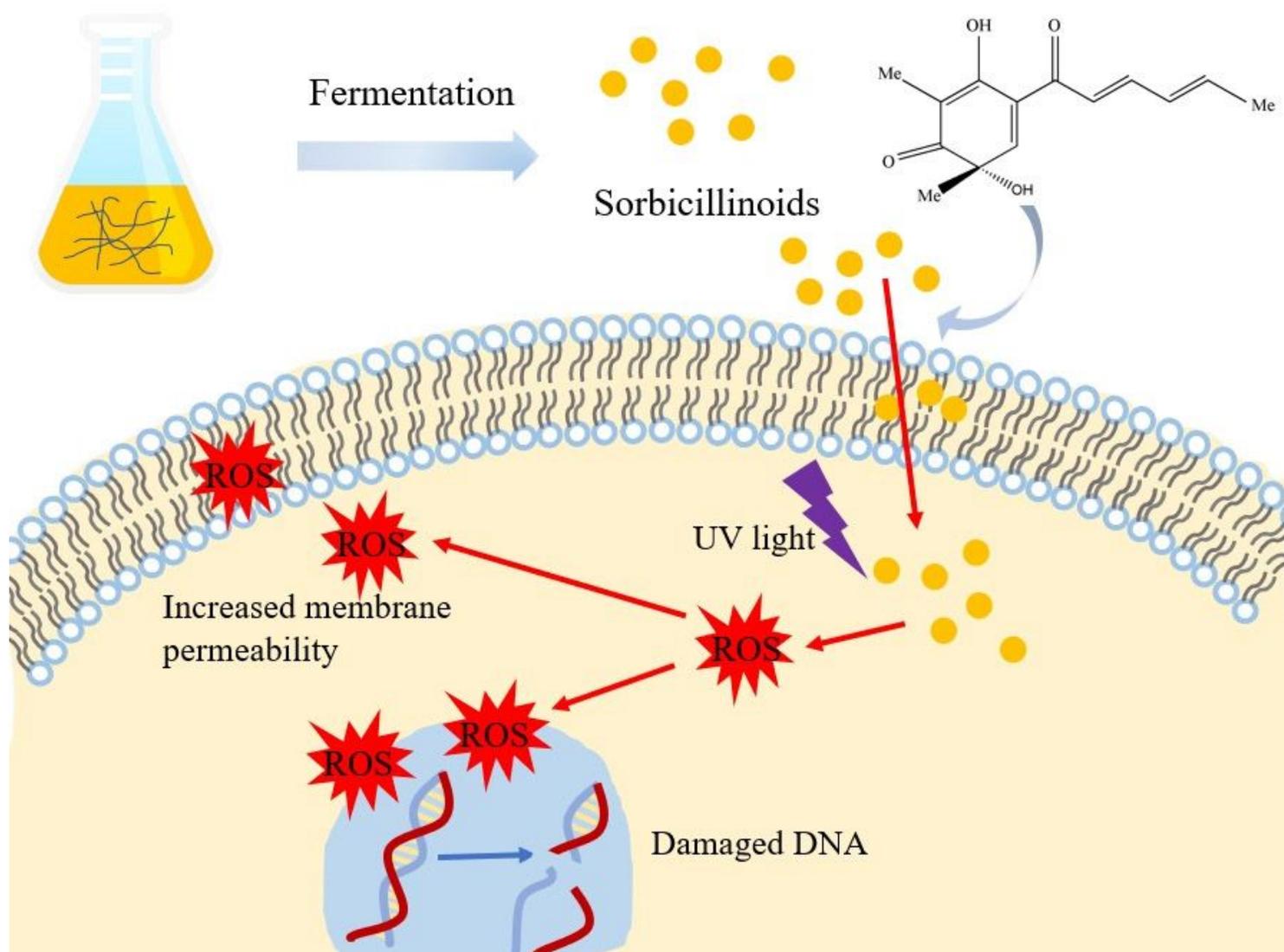


Figure 1

Schematic Illustration of Photodynamic Inactivation against *S. aureus* by Sorbicillinoids under UV Light Irradiation.

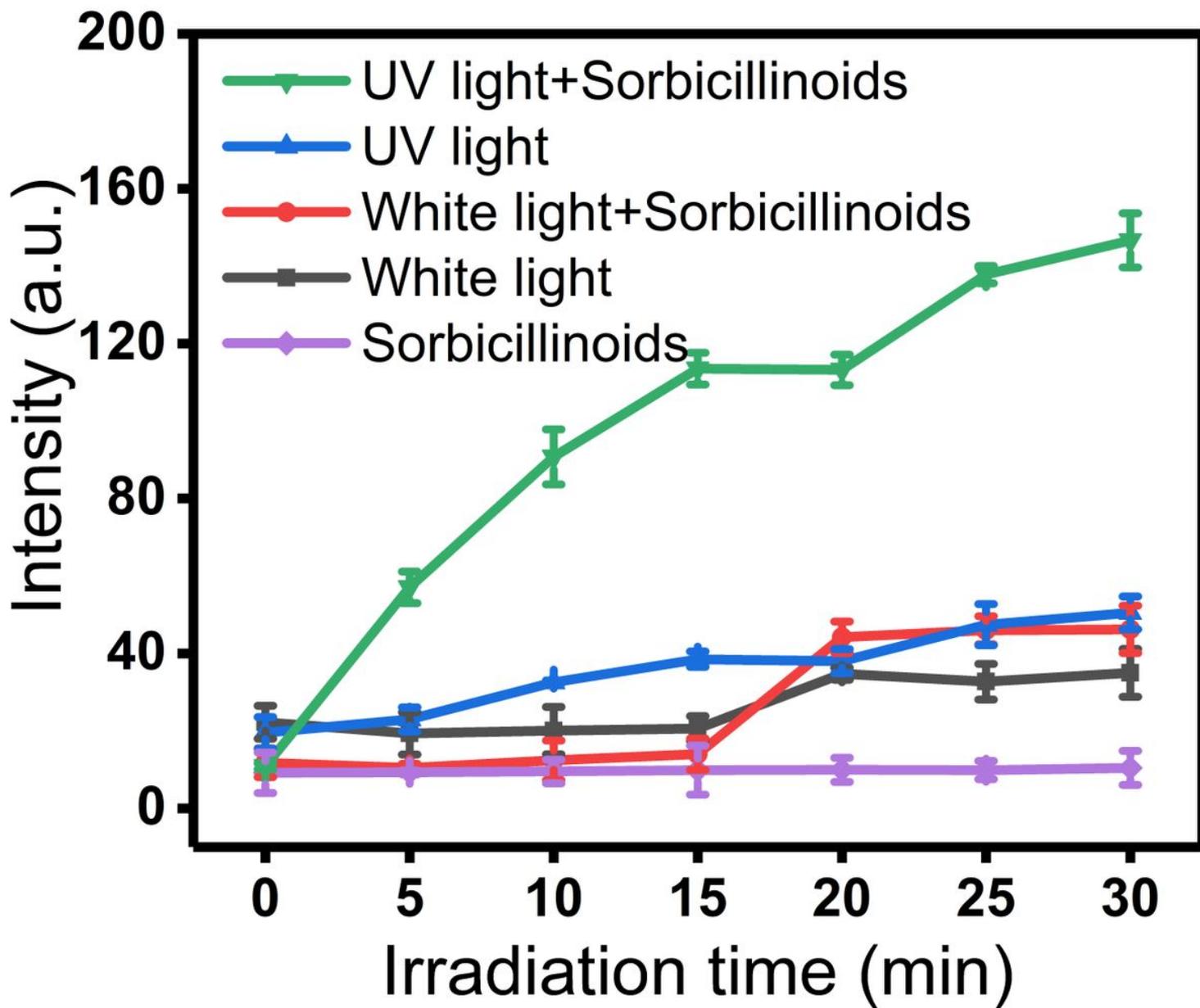


Figure 2

102 generation of sorbicillinoids in 0.9% NaCl as measured by the fluorescence intensity changes of SOSG at 528 nm with the excitation wavelength of 504 nm.

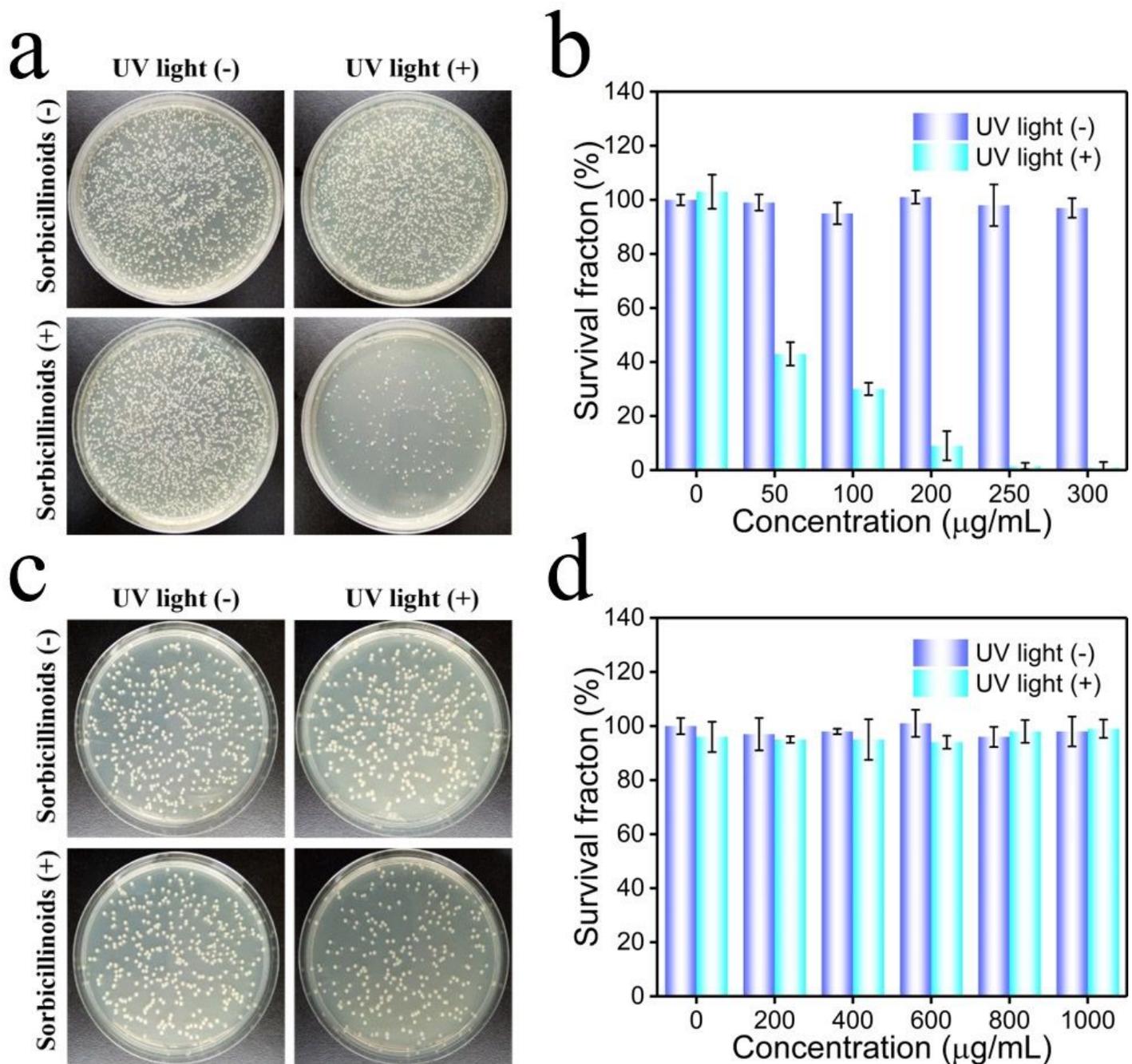


Figure 3

The APDT ability of sorbicillinoids against *S. aureus* (a, b) and *E. coli* (c, d). Agar plate photographs of *S. aureus* (a) and *E. coli* (c) treated with sorbicillinoids under (no) UV light irradiation conditions. The corresponding dependence of bacterial survival fraction on the concentration of sorbicillinoids was measured (b, d).

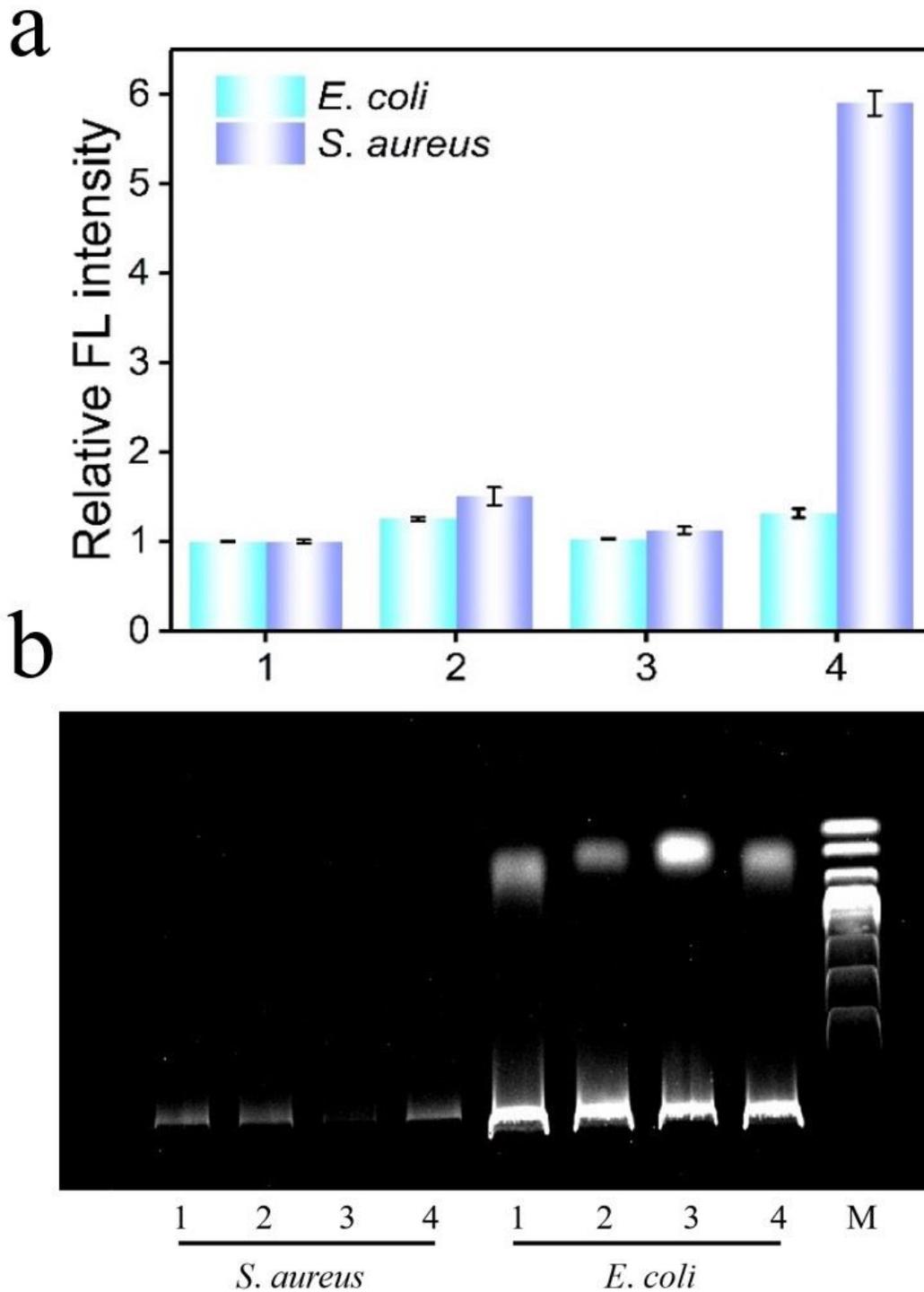


Figure 4

a) Generation of intracellular ROS in *S. aureus* and *E. coli*. b) Electrophoretic profiles of DNA extracted from *S. aureus* and *E. coli*. 1: no treatment; 2: UV light; 3: sorbicillinoids; 4: sorbicillinoids and UV light; M: molecular weight marker.

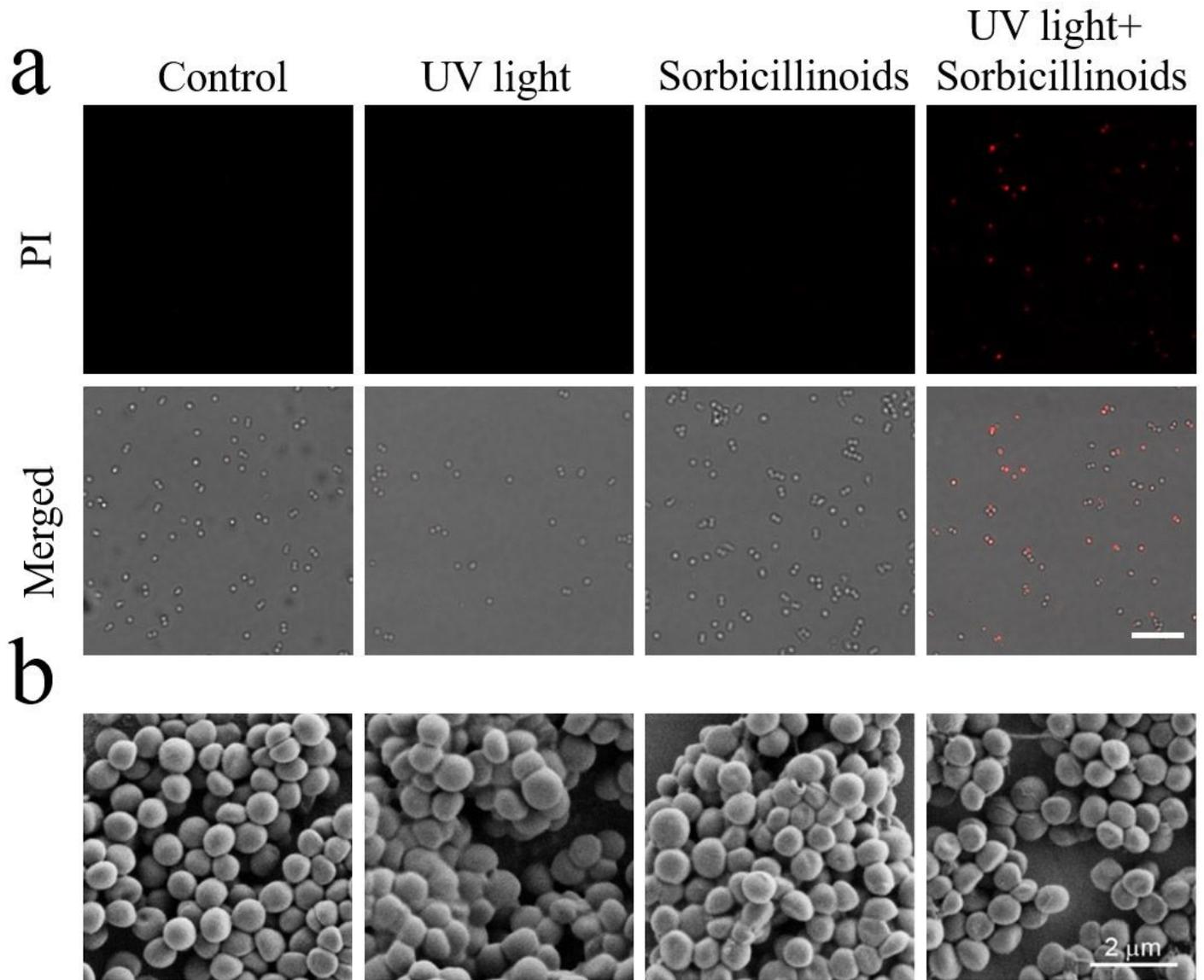


Figure 5

a) Confocal fluorescence images of *S. aureus* that was incubated with PI after different treatments as indicated in the figure. Bacteria with damaged cell membrane would be stained by PI, showing red fluorescence. (scale bar = 10 μ m). b) the corresponding SEM images were also taken.

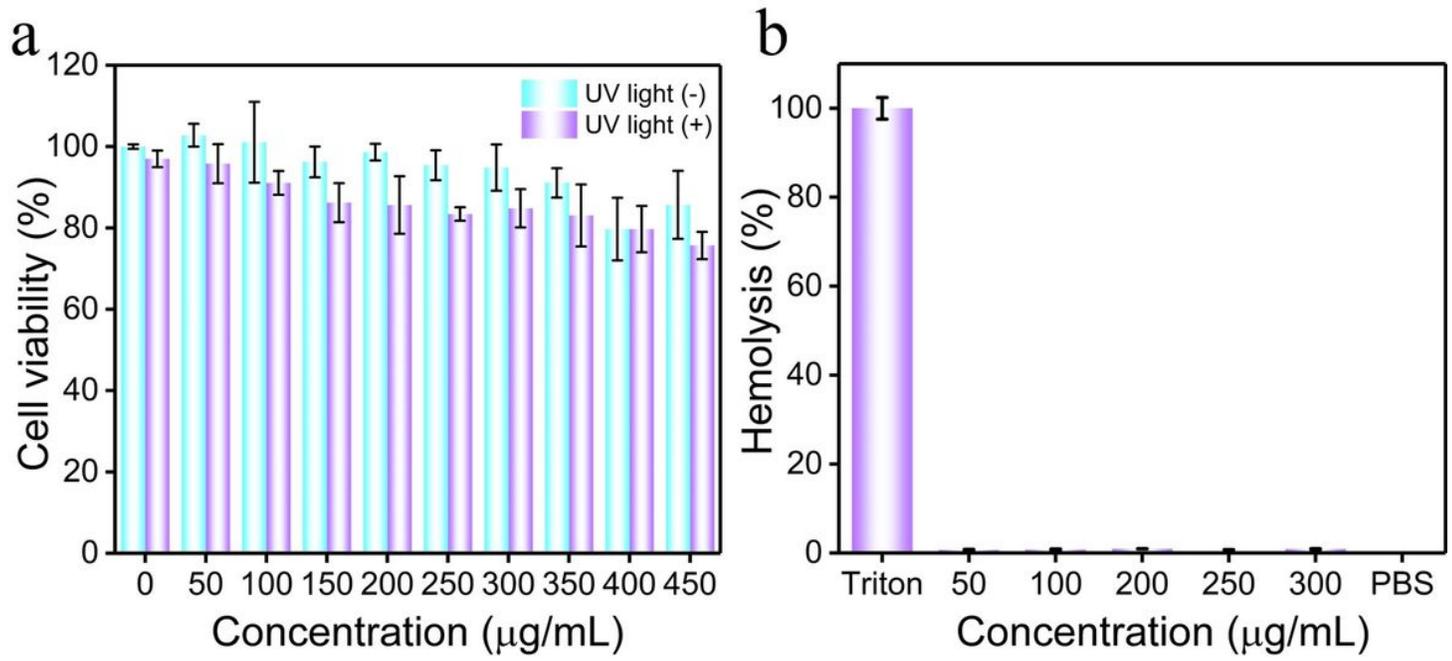


Figure 6

Biocompatibility evaluation of sorbicillinoids. a) Viabilities of AT-II cells in the presence of different concentrations of sorbicillinoids with and without UV light. b) Hemolysis rates of RBCs after incubation with various concentrations of sorbicillinoids. RBCs in Triton X-100 and PBS were set as the positive and negative controls, respectively.

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