Electronic Supplementary Material (ESI) for Biomaterials Science.

Electronic Supplementary Information

Multifunctional BODIPY for effective inactivation of Gram-positive bacteria and promotion of wound healing

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Materials

N(alpha)-boc-L-arginine (L-Arg) was bought from Shanghai Aladdin Biochemical Technology Co., Ltd.. N-(3-dimethylaminopropyl)-N-ethylcarbodiimide (EDC HCl) was bought from Beijing Innochem Co., Ltd.. 4-Dimethylaminopyridine (DMAP) was purchased from Energy Chemical Co., Ltd.. 1,3-Diphenylisobenzofuran (DPBF) was bought from TCI (Shanghai) Development Co., Ltd.. 9,10-Anthracenediyl-bis(methylene)-dimalonic acid (ABDA) was purchased from sigma-aldrich (Shanghai) Co., Ltd.. 2,7-Dichlorodihydrofluorescein diacetate (DCFH-DA) and Griess Reagent were obtained from Shanghai Beyotime Biotechnology Co., Ltd.. Living cell nucleic acid dyes (SYTO green), propidium iodide (PI) and 3-amino,4-aminomethyl-2',7'-difluoresceindiacetate (DAF-FM DA) were purchased from Jiangsu KeyGEN Biotechnology Co., Ltd.. Streptomycin sulfate, broth media and (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Shanghai yuanye Bio-Technology Co., Ltd..

Characterizations

^{1}H NMR spectra were measured in $CDCl_3$ at room temperature by an AV-400 NMR spectrometer from Bruker. Analytical balance (XS105DU) and Rainin Pipettes from METTLER TOLEDO were used to quantify solid and liquid respectively. Confocal light scanning microscopy (CLSM) images were taken using a Zeiss LSM 700 (Zurich, Switzerland).

Experiments

Synthesis of LIBDP. IBDP was synthesized according to our previous work.[1] For the synthesis of LIBDP, IBDP (0.165 mmol) and N(alpha)-boc-L-arginine (0.197 mmol) were added in dry CH_2Cl_2 (20 mL), then EDC HCl (0.33 mmol) and DMAP (0.165 mmol) were added in the mixed solution. After 24 h of stirring at room temperature, the

solution was concentrated. The final product was purified by a silica gel column.

Detection of ROS and NO *in vitro*. The ability of LIBDP in N, N-dimethylformamide (DMF) to produce ROS was detected by the change of absorption intensity of DPBF at 415 nm. DPBF solution was added to LIBDP solution (0.1 μg mL^{-1}) and the absorption spectra of DPBF were measured after a specific time of illumination with green LED light at 12 mW cm^{-2}. The detection interval was 20 s. The ability of LIBDP in water to produce ROS was obtained by the change of absorption intensity of ABDA at 380 nm. ABDA solution was added to LIBDP solution (10 μg mL^{-1}) and the absorption spectra of ABDA were measured after a specific time of illumination with green LED light (12 mW cm^{-2}). The detection interval was 2 min. In the experiment of comparing LIBDP and RB ROS generation efficiency, the solvent was ethanol, and the other steps were as described above.

Bacteria culture. The single colony on the solid agar was transferred to 3 mL broth medium, and the bacteria were in exponential growth stage after shaking at 37 °C for 6 h. The bacteria were centrifuged at 5000 rpm for 5 min and washed twice with phosphate buffer solution (PBS). After discarding the supernatant, the bacteria were suspended in PBS and diluted to an optical density (OD) of 1.0 at 600 nm (OD_{600}=1.0 with about 10^9 CFU mL^{-1}).

Antibacterial effect. The exponential growth bacteria were co-cultured with different concentrations of LIBDP at 37 °C, and the final bacterial concentration was 10^5 CFU mL^{-1}. After 24 h, the optical density at 600 nm was detected. MIC was defined as the minimum concentration of the drug needed to inhibit the visible growth of bacteria after 24 h at 37 °C.

Time-kill efficiency test. The bacteria and LIBDP were co-cultured at 37 °C, and the bacterial solution was diluted and placed on the solid medium at a fixed time point. After 24 h of culture at 37 °C, the plate was photographed and the colony number

was quantified by Image J software.

Scanning electron microscopy (SEM). SEM was applied to observe the morphologies of bacteria treated without or with LIBDP. After the treatment described in the antibacterial experiments above, bacteria were immediately fixed with 4% glutaraldehyde for 30 min. Then the bacteria were centrifuged (5000 rpm for 5 min), followed by removing the supernatant and resuspending the remaining bacteria in sterile PBS. The samples were dehydrated with a series of graded ethanol solutions (30%, 50%, 70%, 85% and 100%, each for 5 min). After 12 h of drying, the samples were coated with gold and observed by SEM.

Detection of ROS and NO in the biofilm. The generation of ROS and NO in the biofilm were investigated by staining the biofilm with a ROS indicator (DCFH-DA) and NO indicator (DAF-FM DA). In short, the biofilms in 24 well plates were harvested first, then PBS and LIBDP (5 μg mL^{-1}) were added, and the light groups were exposed to irradiation (green LED light, 12 mW cm^{-2}, 5 min) after incubation for 30 min. The bacteria in the dark group were not irradiated. Staining was carried out according to the instructions of ROS and NO detection kit, and imaging was performed with CLSM.

Photodynamic antibacterial effect. The bacteria in exponential growth were co-cultured with different concentrations of LIBDP (0-5 μg mL^{-1}) at 37 ^{o}C, and the final bacterial concentration was 10^5 CFU mL^{-1}. The bacteria were irradiated with green LED light for 5 min after being cultured at 37 ^{o}C for 30 min. After 24 h, the optical density at 600 nm was measured.

Evaluation of drug resistance. *S. aureus* was used to study the development of resistance to antibiotics (streptomycin sulfate), LIBDP and LIBDP+L at sublethal doses. The free bacteria were treated with different concentrations of antibiotics, LIBDP and LIBDP+L respectively and inoculated in 96 well plates. Among them, the bacteria in LIBDP+L group were incubated with LIBDP for 30 min and then exposed to

irradiation for 5 min (green LED light, 12 mW cm^{-2}). After incubation for 24 h, OD_{600} in each well was detected to determine the MIC. The bacteria cultured with streptomycin sulfate or LIBDP at half of the MIC were used for the next passage. MIC of each passage was recorded and compared with the initial MIC to determine the drug resistance.

Photodynamic destruction of biofilms. First, the biofilms in the 24 well plates were harvested, and then LIBDP with different concentrations was added. After incubation for 2 h, the light group was exposed to irradiation. After 4 h, the bacteria in the biofilm were stained with SYTO and PI. Finally, the biofilm was observed by CLSM.

Wound infection healing *in vivo*. All animal experiments have been approved by the Ethics Committee of Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, and carried out according to the NIH guidelines for the care and use of laboratory animals (NIH publication No. 85-23 Rev. 1985). The female Kunming mice were randomly divided into 3 groups with 5 mice in each group. A round wound (1 cm in diameter) was formed on the back skin of each mouse. 30 μL of the suspended *S. aureus* solution was inoculated on the wound to form an infection model. After infection, 30 μL of PBS or LIBDP was added to the wound. The mice in the light group were incubated with LIBDP for 30 min and then exposed to light. In the process of wound healing, the wounds were photographed and monitored every day, and the wound areas were measured by Image J software. On the 6th day after treatment, the wound tissue was harvested and the number of bacteria in the wound was measured by plate counting method. The tissues were immersed in 2 mL of sterile PBS, and the bacterial suspension was obtained by ultrasonic treatment. 20 μL of the suspension was placed on agar plate for bacterial growth. Finally, hematoxylin-eosin (H&E) staining was employed to evaluate the therapeutic effect.

Biosafety evaluation of LIBDP *in vitro* and *in vivo*. For the *in vitro* biosafety evaluation, mouse fibroblasts (L929 and NIH 3T3 cells) were inoculated into 96 well

plates and cultured until the cells adhered to the wall. Different concentrations of LIBDP were added into the plates, and the cells were cultured for 24 h, then MTT was added. After 4 h, DMSO was added, and the plate was shaken for 3 min. Finally, the absorbance at 490 nm was measured via a microplate reader. Three parallel experiments were performed for each concentration. Hemolysis assay on LIBDP was detected with reported method.[2] For the *in vivo* biosafety evaluation, the mice were injected with LIBDP intravenously for seven days, and the blood samples were taken for biochemical tests.

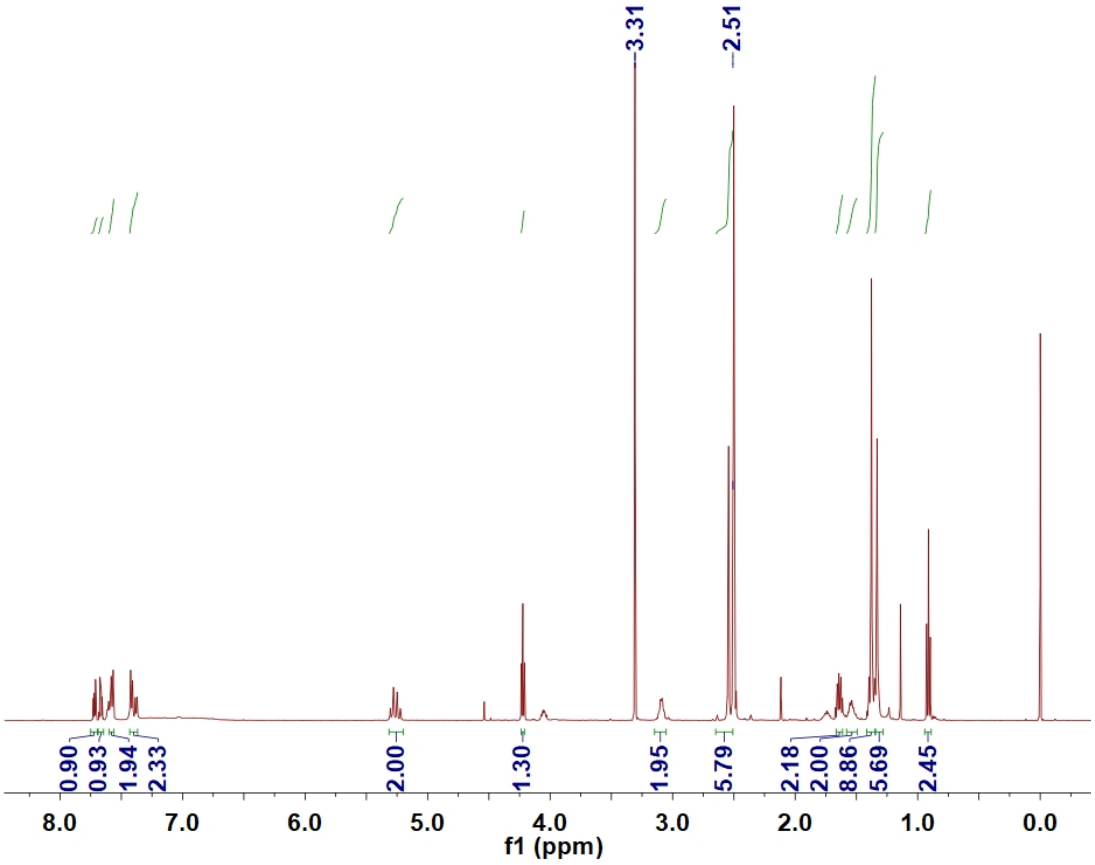

Fig.S1 The ^{1}H NMR spectrum of LIBDP.

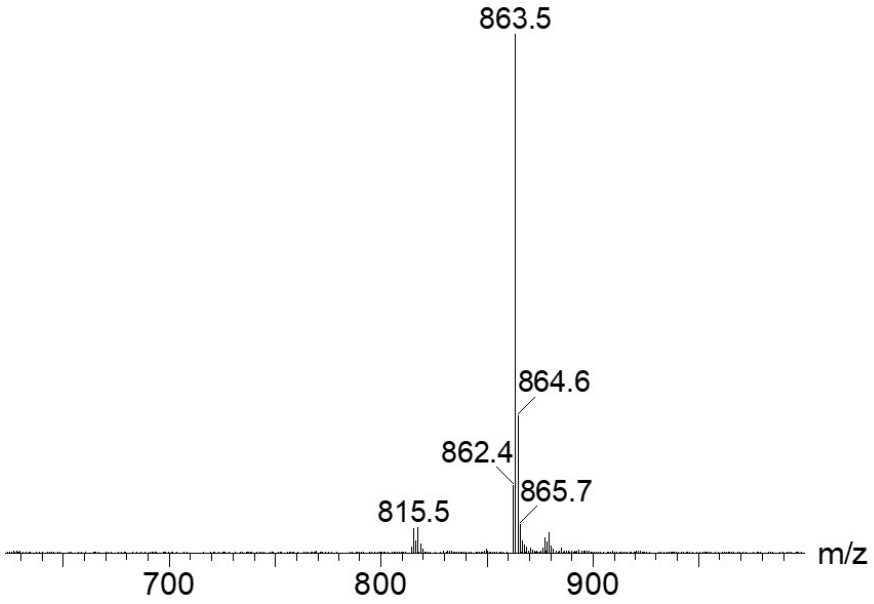

Fig. S2 The ESI-MS spectrum of LIBDP.

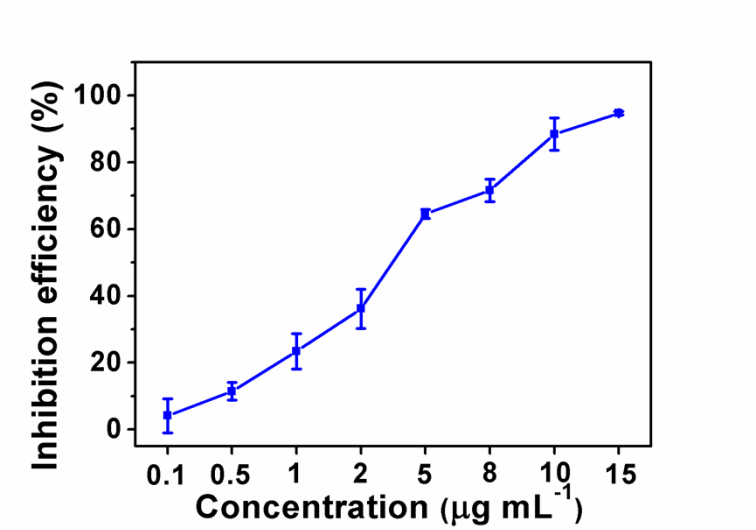

Fig. S3 The inhibition efficiency of LIBDP for *S. aureus* according to the MIC assay.

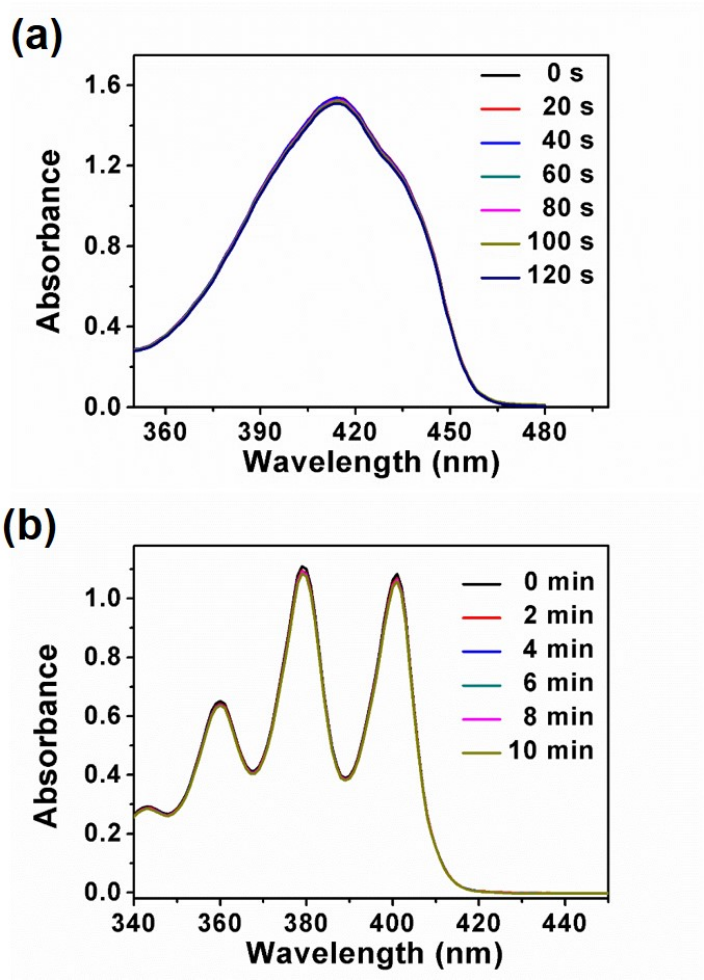

Fig. S4 Absorbance changes of (a) DPBF and (b) ABDA without LIBDP under the irradiation of green LED light.

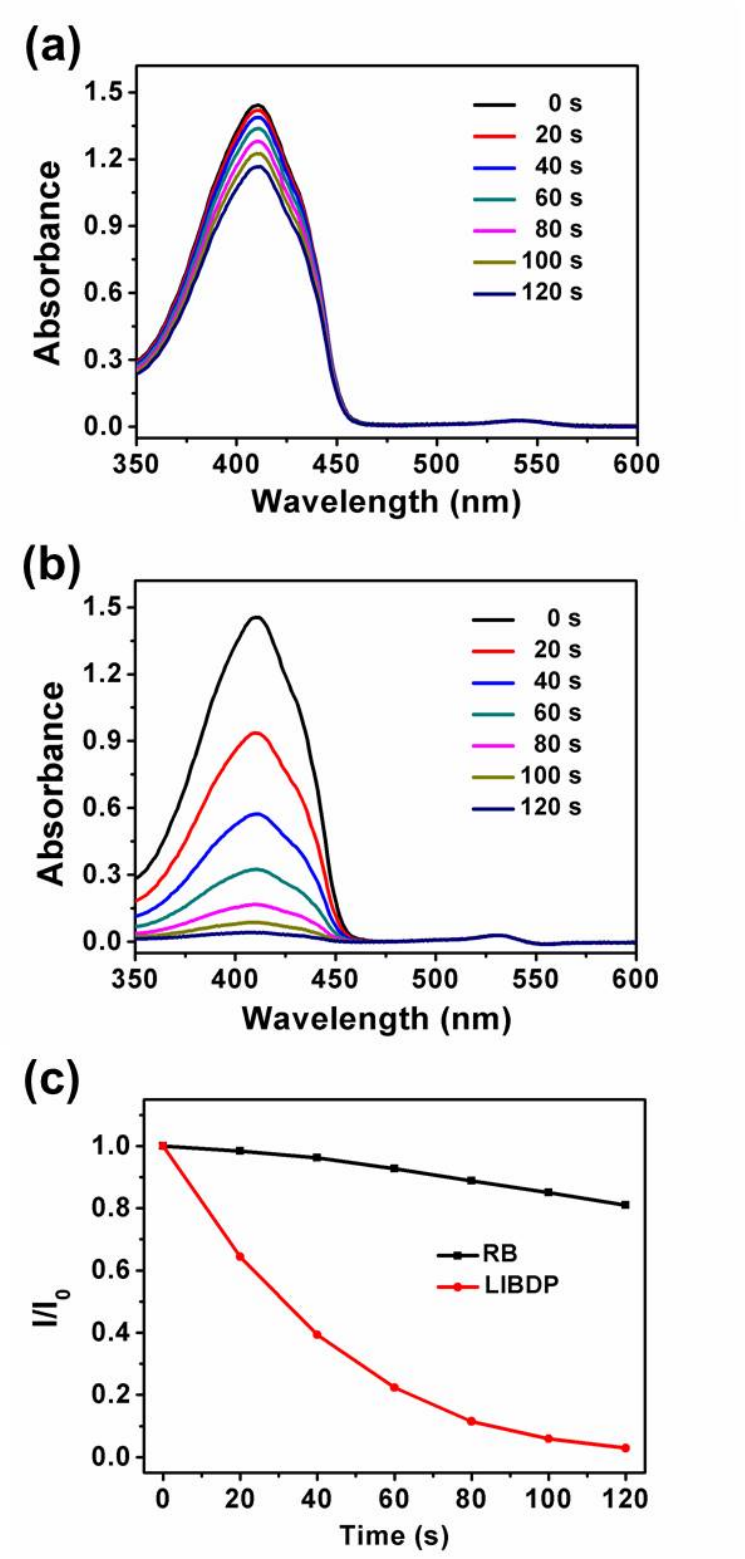

Fig. S5 Absorbance changes of DPBF with (a) Rhodamine B and (b) LIBDP under the irradiation of green LED light (12 mW cm^{-2}). (c) The decay rate of the absorbance of DPBF at 410 nm with different experimental groups under the irradiation of green LED light (12 mW cm^{-2}).

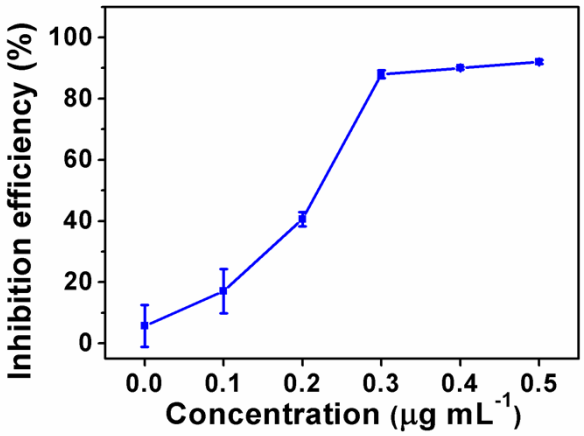

Fig. S6 The inhibition efficiency of LIBDP for *S. aureus* with irradiation according to the MIC assay.

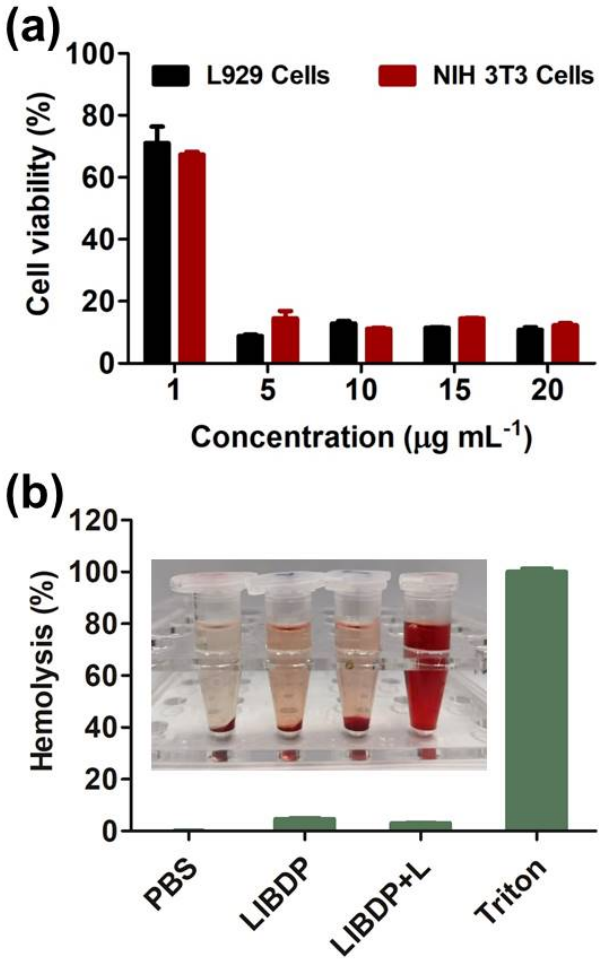

Fig. S7 (a) The effect of LIBDP on the viability of L929 and NIH 3T3 cells upon light irradiation. (b) Hemolysis results of LIBDP with/without light irradiation.

Reference

1. C. Li, W. Lin, S. Liu, W. Zhang and Z. Xie, *J. Mater. Chem. B*, 2019, **7**, 4655-4660.
2. X. Zheng, L. Wang, Y. Guan, Q. Pei, J. Jiang and Z. Xie, *Biomaterials*, 2020, **235**, 119792.