Electronic Supplementary Information (ESI)

Rapid Membrane-Specific AIEgen Featuring with Wash-free Imaging and Sensitive Light-excited Killing of Cells, Bacteria and Fungi

1. Materials and Measurements

All chemicals and reagents were purchased from commercial sources and used as received without further purification unless otherwise specified. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), E3 embryo media were purchased from Termo Fisher Scientific (Shanghai, China), 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) was purchased from Tiangen Biotech (Beijing, China). Milli-Q water was supplied by Milli-Q Plus System (Millipore Corporation, United States). Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under nitrogen immediately prior to use.

^{1}H NMR (400 MHz) spectra were measured on a Bruker 500 spectrometer in CD_3OD at room temperature. UV-vis absorption spectra were measured on a Shimadzu UV-2600 spectrophotometer. Photoluminescence spectra were recorded on a Horiba Fluoromax-4 spectrofluorometer. Solution fluorescence quantum yields were measured using a Hamamatsu absolute PL quantum yield spectrometer C11347 Quantaurus_QY. Confocal laser scanning microscope (CLSM) characterization was conducted with a confocal laser scanning biological microscope (LSM 710, Zeiss, Germany). The absorbance for MTT analysis was recorded on a microplate reader (Thermofisher, USA) at a wavelength of 570 nm. Automated cell counter (Countess II, Invitrogen) was employed for cell counting.

HeLa cells were obtained from cell culture center of Institute of Basic Medical Sciences, Chinese Academy of Medical Science (Beijing, China). Candida albicans (*C. albicans*) ATCC 10231, Pseudomonas aeruginosa (*P. aeruginosa*) JCM 5962, Enterococcus faecalis (*E. faecalis*) JCM 5803, Saccharomyces cerevisiae (*S. cerevisiae*) P11 and *S. aureus* ATCC 6538 were obtained from China General Microbiological Culture Collection Center. The Amp Escherichia coli (*E. coli*) TOP 10 was purchased from Beijing Bio-Med Technology Development Co., Ltd.

2. Methods

2.1 Synthetic routes

2.1.1 Synthesis of Compound 1

To a solution of 4-hydroxybenzaldehyde (2.440 g, 20.0 mmol) and K_2CO_3 (5.530g, 40.0 mmol) in 50 mL of acetone, 1,6-dibromopentane (9.64 g, 40.0 mmol) in 10 ml of acetone was added dropwise at room temperature and the resulting mixture was refluxed for 12 h. The solvent removed via rotary evaporation, and the residue was dissolved in CH_2Cl_2 (50 ml) and washed several times with water. After drying over $MgSO_4$, the solvent was evaporated and the residue purified by column chromatography on silica gel, using a solvent gradient from hexane/CH_2Cl_2 (2:8, v/v) to give pure Compound 1 (yield 90%).

2.1.2 Synthesis of Compound 2

Compound 1 (568 mg, 2.0 mmol) was dissolved in pyridine (3 mL), and the mixture was stirred at

reflux for 12 h. After completion of the reaction, the mixture was cooled to room temperature. Cyclohexane was added in the solvent and then removed by evaporation under pressure. Repeat the above steps several times until the excess pyridine was removed and Compound 2 was obtained as a tawny grease (yield 93%).

2.1.3 Synthesis of Compound 3

Compound 2 (284 mg, 1 mmol) was dissolved in absolute ethanol (10 mL), followed by addition of hydrazine monohydrate (500 mg, 10 mmol). The mixture was refluxed for 4 h. The solvent removed by evaporation under pressure, and the residue purified by RP-HPLC, using methanol to give pure Compound 3 (yield 65%).

2.1.4 Synthesis of C6-BD

The Compounds 3 (298mg, 1 mmol) and 1-hydroxy-2-naphthaldehyde (172mg, 1 mmol) were dissolved with THF, ethanol and 2 drops of glacial acetic acid. The reaction was carried out for 4 hours under reflux. The residual solvent was removed by vacuum distillation and then purified by RP-HPLC with methanol as eluent to afford C6-BD with nearly 60% yields. 1H NMR (CD_3OD, 500M Hz), δ(TMS, ppm): 14.32 (s, 1H), 8.89 (d, 2H), 8.74 (t, 1H), 8.57 (s, 1H), 8.29 (s, 1H), 8.32 (d, 1H), 8.24 (t, 2H), 7.83-7.78 (m, 3H), 7.68 (t, 1H), 7.54-7.51 (m, 2H), 7.32 (d, 1H), 7.03 (d, 2H), 5.01 (t, 2H), 4.06 (t, 2H), 8.89 (d, 2H), 2.01 (m, 2H), 1.76 (m, 2H), 1.43 (m, 2H), 1.29 (m, 2H)。

2.3 Cell imaging

The HeLa cells were grown overnight on a 35 mm petri dish with a cover slip and then incubated with different concentrations of C6-BD for different times,and then the cells were imaged under a CLSM. For co-localization assay, the cells were washed with PBS three times, stained with C6-BD and DiD, and the samples were then observed under CLSM. For wash-free imaging, the cells were incubated with C6-BD for 5 min in culture medium, and then observed directly under the confocal microscope without the washing procedures.

2.4 Preparation of bacterial solutions

A single colony of S. aureus (Gram-postive) on a solid Nutrient Broth (NB) agar plate was transferred to 10 mL of liquid LB culture medium and grown at 37 °C for 12 h. Bacteria were harvested by centrifuging (7100 g for 1 min) and washed by phosphate buffer saline (PBS, 10 mM, pH=7.4) for three times. The supernatant was discarded and the remaining S. aureus was resuspended in PBS, and diluted to an optical density of 1.0 at 600 nm (OD600 = 1.0). As for F. faecalis (Gram-positive), P. aeruginosa (Gram-negative), E. coli (Gram-negative), S. cerecisiae (Fungi), and C. albicans (Fungi), except that the culture medium was replaced by Beef-extract Peptone Yeast-extract (BPY), Trypticase Soy (TS), Luria-Bertani (LB with 10 μM Amp), Yeast Extract Peptone Dextrose (YPD), respectively, other experimental conditions and operations were totally the same as that of S. aureus.

2.5 Bacterial Imaging

Bacterial and fungal suspensions (OD600 = 1.0) were added into phosphate bu□ered saline (PBS) solution containing C6-BD (10×10^{-6} M) before incubating for 20 min at 37 °C. The bacteria were harvested by centrifuging for 3 min at 7100 g, and the supernatant solution was discarded. The

fluorescence images of F. faecalis (Gram-positive), P. aeruginosa (Gram-negative), E. coli (Gram-negative), S. cerecisiae (Fungi), and C. albicans (Fungi), were observed using CLSM.

2.6 Cytotoxicity assay

HeLa cells were seeded in 96-well plate with 8 × 103 cells per well, and incubated with M1-DPAN in DMEM (10% FBS) with the final concentration ranging from 0 μM to 32 μM (100 μL /well). After 24 h, 100 μL of MTT (10 μM) in DMEM (10% FBS) was added, and incubated for additional 4 h. After removing the culture medium, 100 μL of DMSO was added to every well. The absorption was recorded by a microplate reader at 570 nm after shaking for 2 min. The cell viability ratio (VR) was evaluated according to following equation:

$$VR = \frac{A}{A_0} \times 100\%$$

Where A0 is the absorbance of cells without any drugs, and A is the absorbance of cells incubated with C6-BD.

2.7 Antibacterial Activity

The dark toxicity of C6-BD was determined by incubation with S. aureus and C. albicans suspensions at 37 °C for 20 min, respectively. These microbes suspensions were serially diluted 5 × 10^5 fold with PBS. A 100 μL portion of the diluted microbes was spread on the solid agar plate, and the colonies formed after 24 h incubation at 37 °C were counted. The survival fraction was determined by dividing the number of colony-forming units of the specimens incubated with C6-BD by the number of cfu of the control that was carried out in the absence of C6-BD. The diameter of the solid agar plates was 90 mm. The bacterial inhibition ratio (IR) was calculated according to following equation:

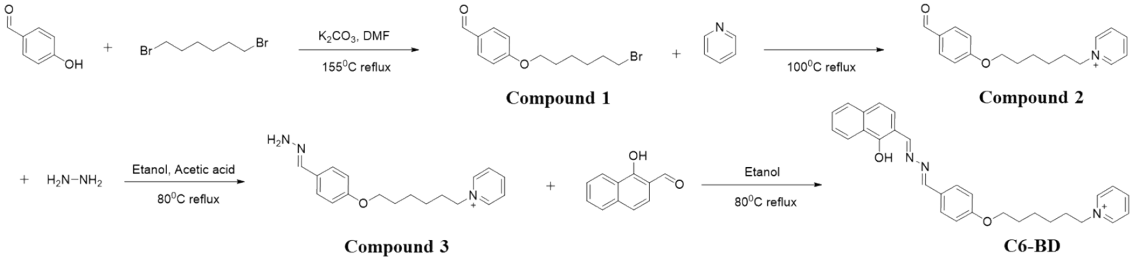
$$IR = \frac{C_0 - C}{C_0} \times 100\%$$

Where C is the cfu of the experimental group treated by C6-BD and C_0 is the cfu of the control group without incubation with C6-BD.

The dark toxicity of C6-BD was determined by incubation with S. aureus and C. albicans suspensions at 37 °C for 10 min, followed by exposing to white light irradiation for 10 min , respectively. other experimental conditions and operations were totally the same as the control groups which were incubated in the dark.

3. Supporting figures

3.1 Synthesis Routes of C6-BD



Scheme S1 Synthesis Routes of C6-BD

3.2 NMR spectra of C6-BD

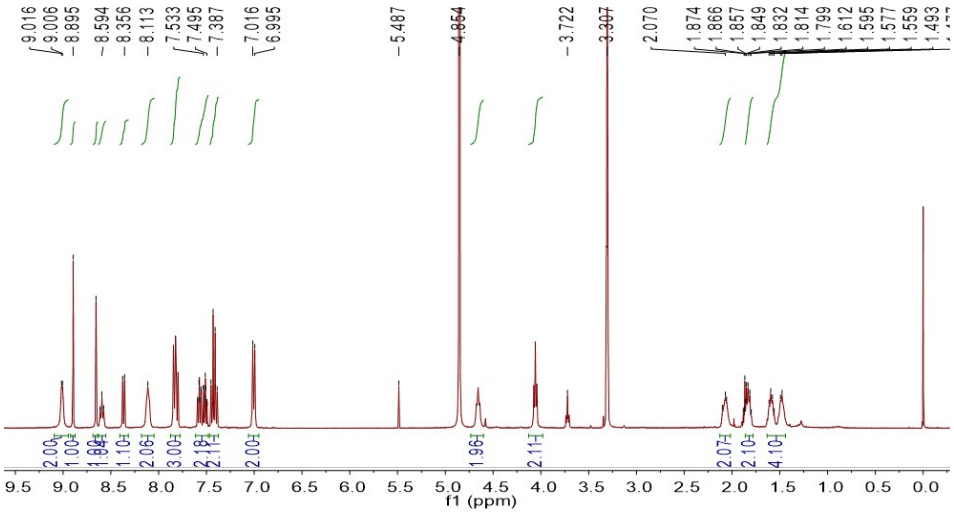


Fig. S1 ^{1}H NMR spectrum of C6-BD in CD_3OD.

3.2 Application in fluorescence imaging

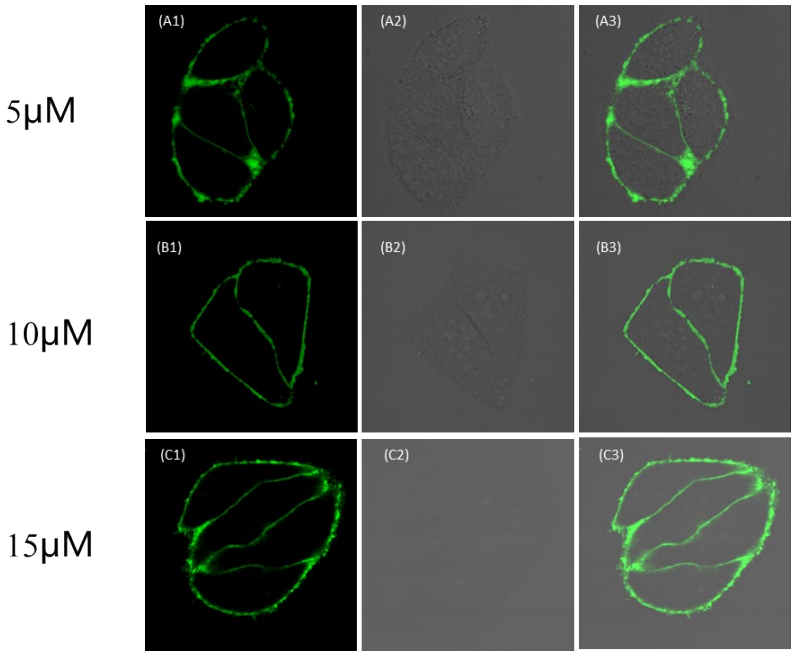


Fig. S2 Confocal images of Hela cells after incubation with C6-BD at di□erent concentration for 5 min observed in the green channel (410-540 nm). Excitation wavelength: 405 nm. All images share the same scale bar, 20 μm.

3.3 Antibacterial experiments.

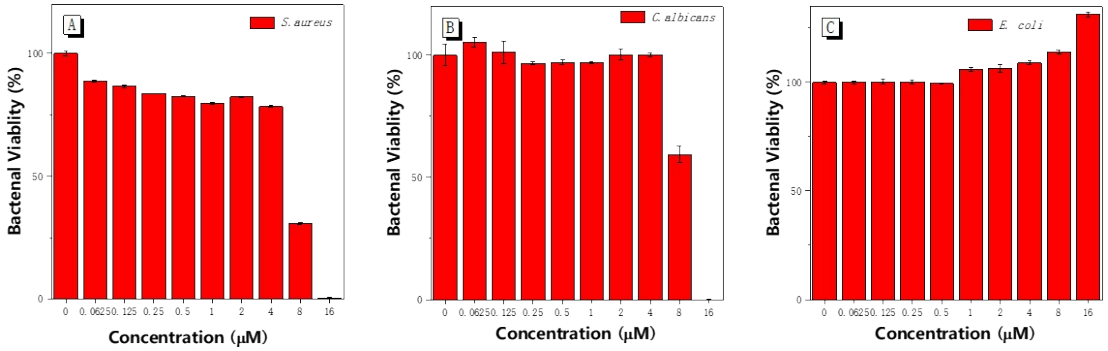


Fig. S3 The bacterial viability of Gram-positive *S. aureus* (A), Fungi *C. albicans* (B), *E. coli* (C) treated with C6-BD at di□erent concentration.