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ORIGINAL ARTICLE

A Fluorescent Visual Proton Donor and Photoacid Sterilant Based on Sulfonate-conjugated BODIPY

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Abstract

Increasing acidity is an effective method for bacterial inactivation by inhibiting the synthesis of intracellular proteins at low pH. Photo-driven proton release probe can be used for the measurement of proton in hydrophobic condition. To develop fluorescent proton donor, two boron dipyrromethene derivatives (BDP-S and BDP-S2) were characterized by spectroscopic methods. Irradiation of BDP-S by white LED light resulted in efficient generation of acidic species with changes of fluorescence emission. The linear relationship between the pH value and the fluorescence intensity of BDP-S was obtained, indicating that BDP-S is a fluorescent visual proton donor. Light-induced antibacterial results indicate that BDP-S can significantly inhibit the growth of *E. coli*. The results prove that BDP-S is a very promising photoacid sterilant.

Keywords BODIPY · Antibacterial · Proton donor · Photoacid generator

Introduction

The effectiveness of traditional antibiotics is decreasing due to variations of pathogens and enhancement of drug resistance in recent years. This has given rise to the search for a more effective way of bacterial inactivation. Light-driven bacterial inactivation has become the main treatment method in the fields of biomedicine, environmental science and materials chemistry because of its non-invasiveness, less toxicity and low drug resistance [1–4]. Photo-driven antibacterial by photoacid is a promising approach in photo-driven bacteria killing [5]. Photoacid generators (PAGs) are a class of compounds that undergo dissociation or rearrangement reactions upon illumination, resulting in acidic species eventually [6–10]. Proton-sensitive reactions can be controlled in space and time by PAGs which also provide a new method for converting light energy into chemical energy [11, 12]. PAGs can generally be activated by LED or visible light either in solution or the solid state [13], which makes PAGs applicable to controlling various proton transfer processes, such as acid-catalyzed reactions [14–16], enzyme activity [17], biological probing [18], bacteria killing [19], etc.. Although the acid release properties of PAGs have been studied, there have been few PAGs with fluorescence response. Therefore, the development of new PAGs with fluorescence response is still a work of great significance.

Boron dipyrromethene (BODIPY) dyes have characteristics such as large molar absorption coefficient, high fluorescence quantum yield and easy modification [20–24]. If boron dipyrromethene (BODIPY) dyes could be potential photoacids, a sensitive method for the measurement of in-site produced proton will be developed. In this work, a BODIPY conjugated sulfonate (BDP-S) was reported as a fluorescence responsive photoacid. The light-induced emission changes, photoacid generation and antibacterial activity were studied.

Experimental

Chemicals and Measurements

All chemicals were purchased from commercial suppliers and were used without further purification. 2,4-dimethylpyrrole, *p*-hydroxybenzaldehyde, trifluoroacetic acid (TFA), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), *p*-nitrobenzenesulfonyl chloride, 4-nitro-α-toluenesulfonyl

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chloride, rhodamine B base and other chemicals were purchased from Energy Chemical Reagent Co., Ltd. 4-(5,5-Difluoro-1,3,7,9-tetramethyl-5H-4λ^4,5λ^4- dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)phenol (BDP-OH) was synthesized according to the reported procedure [25].

The electronic absorption spectra were measured at room temperature by a UV-2450 UV–vis spectrophotometer. Fluorescence measurements were carried out on a fluorescence spectrofluorometer Model CARY Eclipse (VARIAN, USA), a 1.0 cm quartz cell (slit width = 5 nm). The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data was recorded on a Bruker AVANCE II 400 MHz spectrometer using $CDCl_3$ as a solvent. The chemical shifts (δ) were reported in ppm and coupling constants (J) in Hz. The pH of solution was measured using a Fisher Scientific Accumet AR15 benchtop meter and a pH combination electrode.

Synthesis of 4-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ^4,5λ^4- Dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)phenyl 4-nitrobenzenesulfonate (BDP-S)

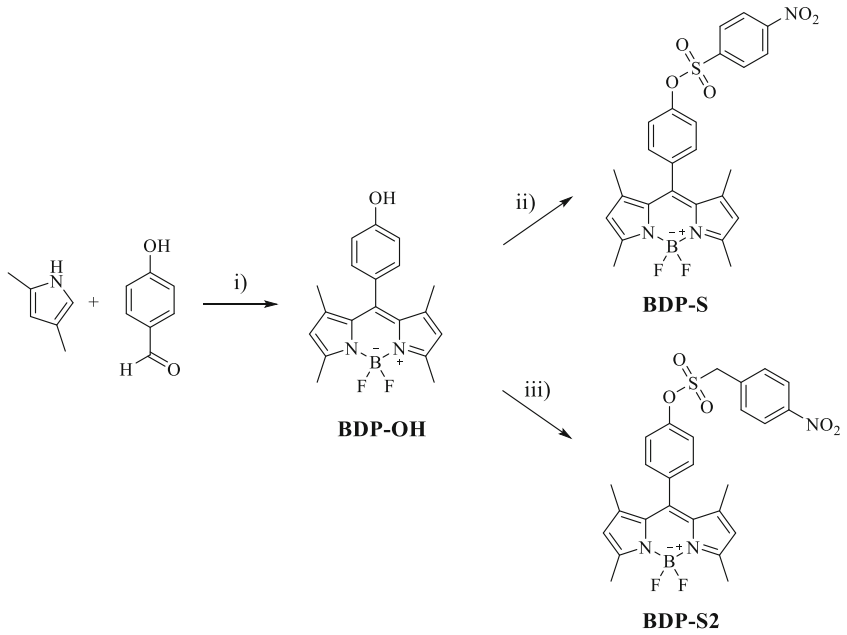
To a solution of BDP-OH (170 mg, 0.5 mmol) and triethylamine (140 μL, 1 mmol) in dry DCM (10 mL) was added *p*-nitrobenzenesulfonyl chloride (133 mg, 0.6 mmol) dropwise at 0 °C under nitrogen. The reaction mixture was stirred for 4 h at room temperature. The solvent was then washed with saturated sodium chloride (3 × 10 mL), dried with sodium sulfate and concentrated *in vacuo*. The residue was loaded onto a column of silica gel to give BDP-S (219 mg, 83.5% yield) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, J = 8.8 Hz, 2H), 8.05 (d, J = 8.8 Hz, 2H), 7.28 (d, J = 8.5 Hz, 2H), 7.19 (d, J = 8.5 Hz, 2H), 6.01 (s, 2H), 2.55 (s, 6H), 1.33 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.22, 151.14, 149.67, 142.44, 140.42, 139.24, 134.75, 131.12, 130.03, 129.97, 124.37, 123.05, 121.66, 14.60, 14.49.

Synthesis of 4-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f] [1,3,2]diazaborinin-10-yl)phenyl (4-nitrophenyl)methanesulfonate (BDP-S2)

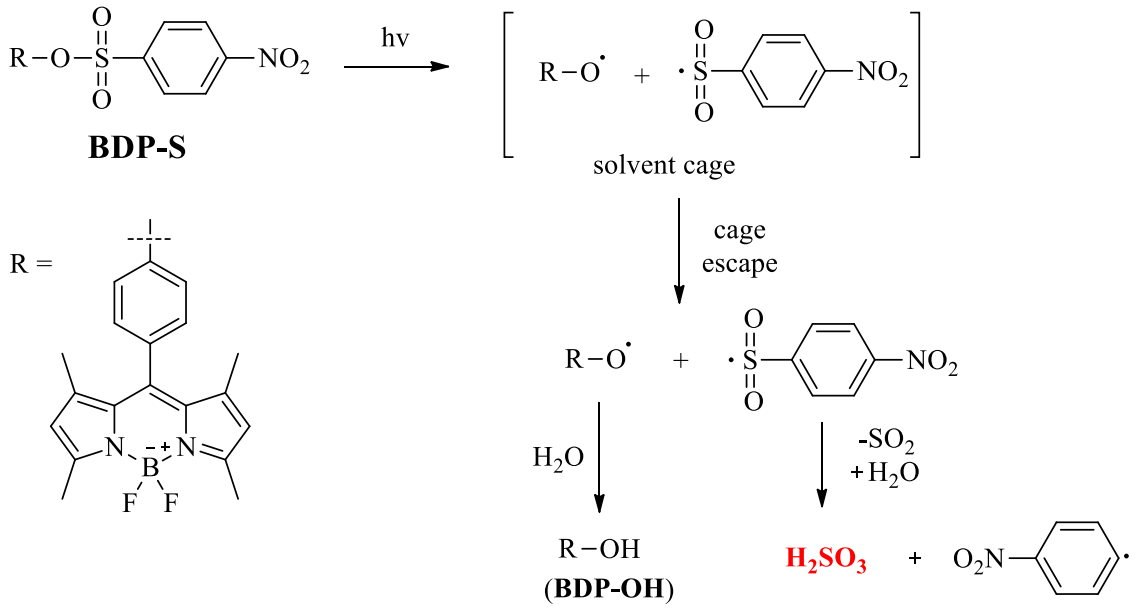
To a solution of BDP-OH (170 mg, 0.5 mmol) and triethylamine (140 μL, 1 mmol) in dry DCM (10 mL) was added 4-nitro-α-toluenesulfonyl chloride (141 mg, 0.6 mmol) dropwise at 0 °C under nitrogen. The reaction mixture was stirred for 10 h at room temperature. The solvent was washed with saturated sodium chloride (3 × 10 mL), dried with sodium sulfate and concentrated *in vacuo*. The residue was loaded onto a column of silica gel to give BDP-S2 (235 mg, 87.1% yield) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, J = 8.7 Hz, 2H), 7.75 (d, J = 8.7 Hz, 2H), 7.37 (q, J = 8.8 Hz, 4H), 6.04 (s, 2H), 4.72 (s, 2H), 2.60 (s, 6H), 1.42 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.13, 149.06, 148.63, 142.77, 139.48, 134.44, 134.12, 131.88, 131.22, 130.04, 124.20, 122.76, 121.60, 56.43, 14.60, 14.52.

Detection of H$^+$ Formed By Photolysis

The photogenerated acid (H$^+$) was captured by the added rhodamine B base whose lactone ring will open after accepting a proton, with a new absorption peak rising at 555 nm [26]. A solution of BDP-S or BDP-S2 (1 mM, MeCN) and rhodamine B base (20 μM, MeCN) was degassed with nitrogen for 15 min and irradiated with an LED lamp (365 nm,



Scheme 1 Schematic illustration of antibacterial mechanism for photoacid



Scheme 2 The mechanism for the photolysis of BDP-S

10 mW/cm^2) for 2 h. The UV-Vis spectrum of the solution was recorded every 20 min. A rhodamine B base solution without BODIPY was used as a negative control.

Fluorescence Emission and PH Changes Under Irradiation

A BDP-S or BDP-S2 stock solution (70 μM, MeCN) was nitrogen saturated for about 15 min firstly and then irradiated with white LED lamps (350–500 nm, 4 mW/cm^2) for 2 h. Photolysis process of BDP-S and BDP-S2 was monitored by fluorescence spectra (λex: 480 nm, time interval: 20 min). The pH of the solution after different exposure times were measured by a Fisher Scientific Accumet AR15 benchtop meter and a pH combination electrode. For determining the changes of emission and pH, three independent experiments were carried out.

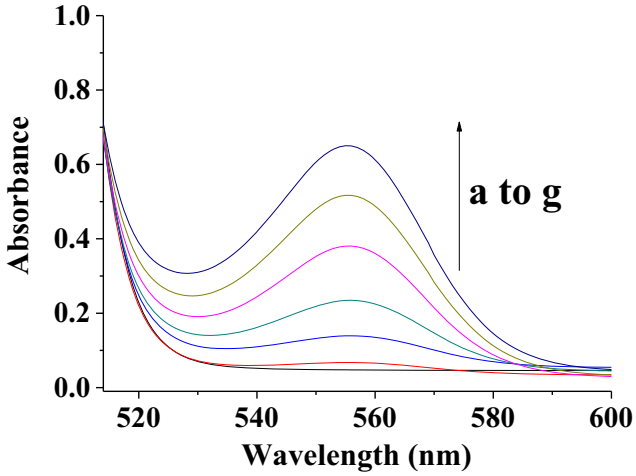


Fig. 1 UV-Vis spectra changes of BDP-S (500 μM, MeCN) and RB Base (10 μM, MeCN) mixture irradiated under 365 nm (a to g: 0 to 120 min, time interval: 20 min)

Antibacterial Assays

Gram-negative Escherichia coli (*E. coli*, ATCC 25,922) strain was chosen as a standard bacterial model. The antibacterial activity of BDP-S was evaluated in the dark condition or LED light irradiation. All assays were conducted in 96-well, flat bottom, sterile plates. Bacterial suspension was adjusted to contain 1×10^6CFU/mL in fresh sterile PBS solution. The as-prepared BDP-S solution (50, 40, 30, 20, 10 and 0 μM) was mixed with bacterial suspension (1×10^6 CFU/mL) and irradiated using a white LED lamp (35–500 nm, 4 mW/cm^2) for 2 h. For each test, the sample in a dark was set as a control group. All tests were performed in triplicate. Survivors were quantified using the viable count technique. Plates were incubated at 37 °C for 24 h.

Results and Discussion

Syntheses and Characterization of Compounds

Two new BODIPY derivatives (BDP-S and BDP-S2) were synthesized by simply coupling hydroxyl-conjugated BODIPY (BDP-OH) with *p*-nitrobenzenesulfonyl chloride or 4-nitro-α-toluenesulfonyl chloride (Scheme 1). The ^{1}H NMR and ^{13}C NMR spectra of the compounds confirm the compounds with exact structures and high purity (Figure S1-S6). BDP-S was found to decompose upon irradiation between the two compounds, whilst the pH of the solution was changed. A mechanism for the photolysis of BDP-S is

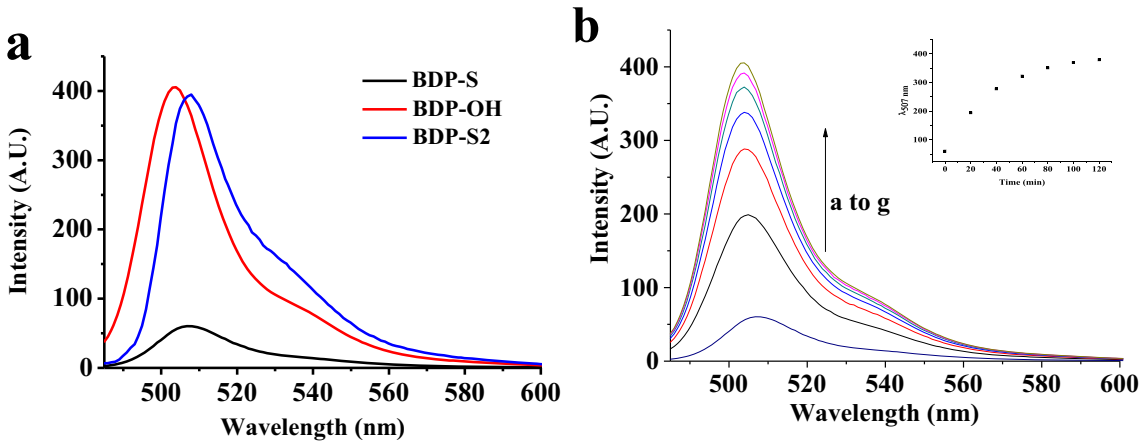


Fig. 2 **a** Fluorescence emission spectra of BDP-OH (70 μM, MeCN), BDP-S (70 μM, MeCN) and BDP-S2 (70 μM, MeCN); **b** Fluorescence emission spectra changes of BDP-S (70 μM, MeCN) irradiated with white LED light (a to g: 0 to 120 min. Time interval: 20 min. Insert: changes of intensity at 507 nm). Excitation wavelength: 480 nm, Voltage: 500V

outlined in Scheme 2. After initial excitation, BDP-S undergoes homolytic O-S bond cleavage to generate phenoxyl and sulfonyl radical pairs [11]. The generated radical pairs, escape from the cage and subsequently abstract trace water or releases SO_2 to produce the corresponding BDP-OH and H_2SO_3.

Detection of H^+ Formed By Photolysis

The generated H^+ was captured by rhodamine B base [25]. Protonation of rhodamine B base leads to a new peak arising at 555 nm. As shown in Fig. 1, a new peak rose gradually at 555 nm in the mixed solution of BDP-S and rhodamine B base while BDP-S2 and the control group showed no significant changes, indicating that an acid species was produced after BDP-S was illuminated.

In addition, with the extension of irradiation time, the color of the mixed solution of BDP-S and rhodamine B base gradually changed from light yellow to a final light orange colour (Fig. S7). When the rhodamine B base was dissolved in acetonitrile, it is colorless, whilst its protonated form is red. The result also showed that rhodamine B base captured protons during LED irradiation and indirectly proved the release of acid species by BDP-S.

Fluorescence Emission and pH Changes of BDP-S After Illumination

BODIPY dyes have the characteristics of large molar absorption coefficient and high photostability of the core while the nitrosulfonate-conjugated one is generally not stable. As shown in Fig. 2a, the integrated area of BDP-OH in the range of 485–600 nm is 7.12 ± 0.02 times higher than BDP-S and 0.98 ± 0.01 times higher than BDP-S2 under the same condition. BDP-S2 shows no significant changes in emission compared with BDP-OH while the emission of BDP-S is greatly

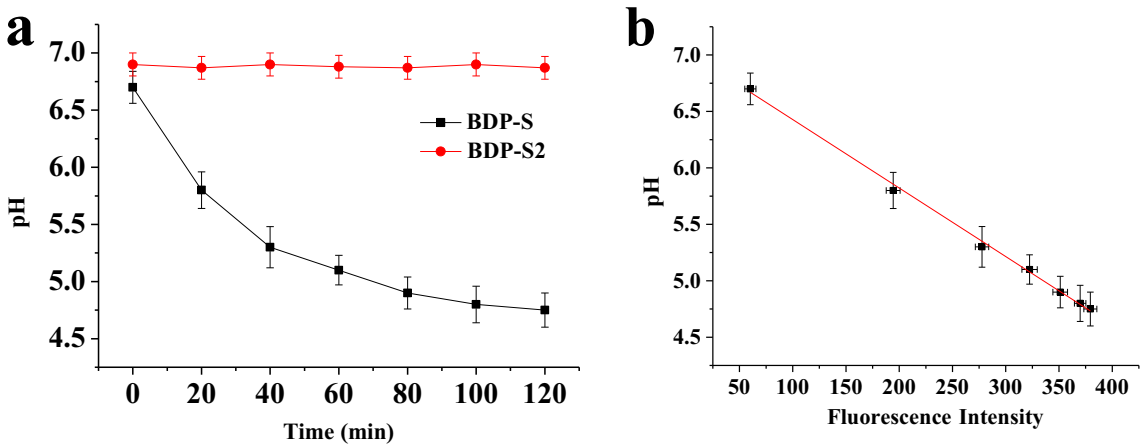
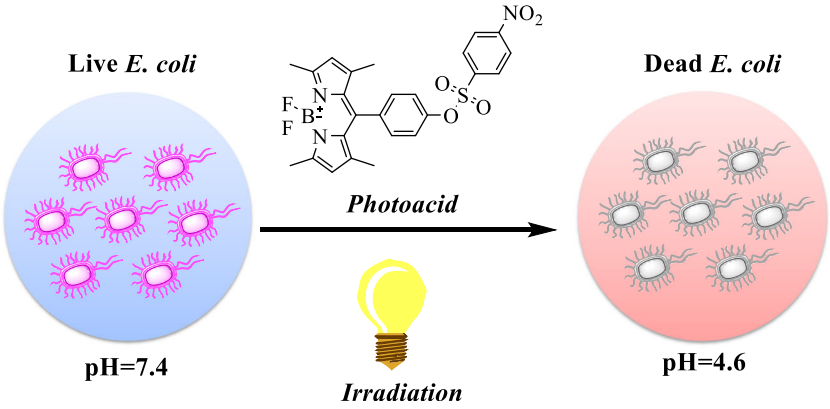


Fig. 3 **a** pH changes of BDP-S and BDP-S2 under illumination. **b** A fitted curve according to the measured pH and fluorescence intensity of BDP-S (y = -0.00607 * a + 7.03523, R^2 = 0.997). Data are expressed as the means of three independent experiments ± SD



Scheme 3 Schematic illustration of antibacterial mechanism for photoacid

quenched, indicating that the methylene group in BDP-S2 breaks the conjugated structure and blocks the electron-withdrawing effect of the nitro group on BODIPY core. The emission of BDP-S2 shows no obvious change under long-term illumination while emission intensity of BDP-S at 507 nm increases with the extension of the illumination time and the amplitude decreases (Fig. 2b). Furthermore, BDP-S can be a fluorescence-response photo-acid. Besides, there is a slight blue shift (508 nm → 504 nm) observed that further proves that the structure of BDP-S changes under illumination.

Also, the pH of the solution under different exposure times was measured using a Fisher Scientific Accumet AR15 bench-top meter with a pH combination electrode (Fig. 3a). The results showed that the pH value of the BDP-S2 solution remained basically unchanged under illumination, while the photolysis of BDP-S occurred and the pH of the solution decreased significantly with the exposure time. A curve based on the pH value and fluorescence intensity of BDP-S was fitted (linear regression equation: $y = -0.00607 * a + 7.03523$, $R^2 = 0.997$) (Fig. 3b), which allows us to roughly estimate of the pH value of the irradiated solution according to fluorescence emission intensity. Since the pH is the negative logarithm of the hydrogen ion concentration, the number of protons in solution can be easily calculated, which provides a new method for estimating the number of protons in a hydrophobic environment. This makes BDP-S a fluorescent visual proton donor.

Increasing acidity is an effective method for bacterial inactivation by inhibiting the synthesis of intracellular proteins at low pH. Since BDP-S can lower the pH of the solution with light, it can be applied for bacterial inactivation.

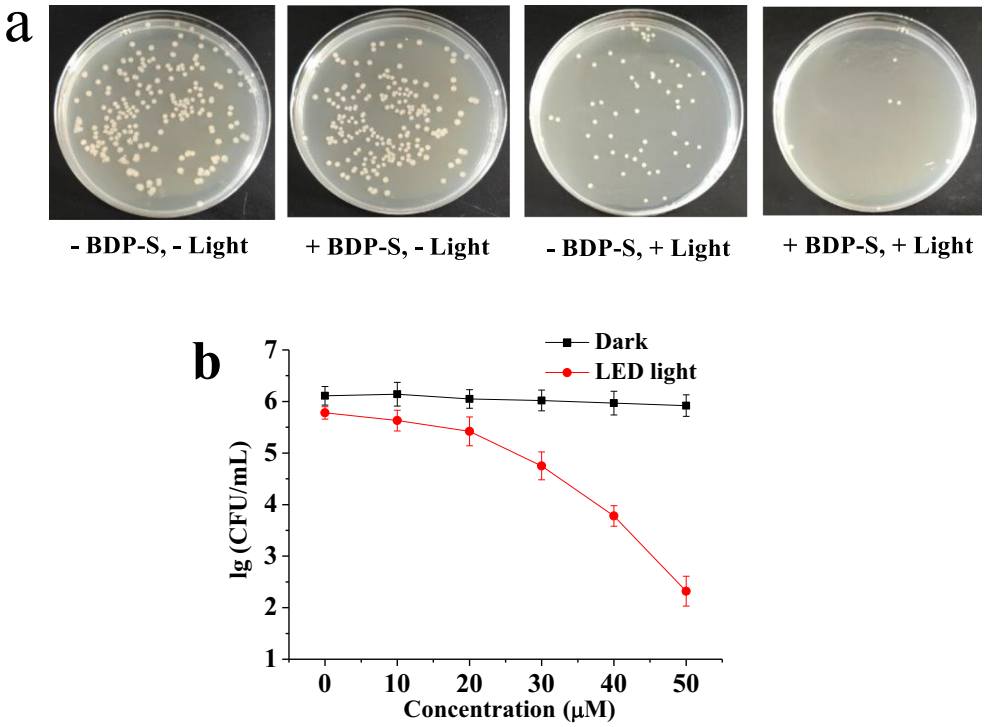


Fig. 4 **a** Growth of *E. coli* under different conditions (concentration of BDP-S: 50 μM); **b** Effects of increasing concentrations of BDP-S on the growth inhibition of *E. coli*. Data are expressed as the means of three independent experiments ± SD. The white light source (350–500 nm, 4 mW/cm^2)

The proposed antibacterial mechanism for photoacid is outlined in Scheme 3.

Antibacterial Assays

The antibacterial activity of BDP-S was evaluated in the dark condition or under white LED light irradiation As shown in Fig. 4a, the bacteria can grow normally in the dark condition (- BDP-S, - light) and the addition of BDP-S has limited effects on its growth (+ BDP-S, - light). The number of survivors drops dramatically after *E. coli* is irradiated with white LED light (- BDP-S, + light) compared to the group without light (- BDP-S, - light). The number of bacteria drops after the addition of BDP-S under irradiation, indicating that the BDP-S caused a reduction in the number of bacteria. The light-driven antibacterial activity of BDP-S depends on its concentration. The number of survivors was reduced by 3 log units when the concentration of BDP-S was increased from 0 to 50 μM (Fig. 4b). The results indicate that BDP-S has the potential to be an excellent photoacid sterilizer.

Conclusions

Two BODIPY sulfonates (BDP-S and BDP-S2) were characterized by spectroscopic methods. The emission changes, acid generation and antibacterial activity on *E. coli* were studied in depth. BDP-S was found to lower the pH of the solution under illumination, which make it a kind of hydrophobic proton donor. Light-induced antibacterial results indicate that BDP-S has a high inhibitory effect on the growth of *E. coli*. Our research results demonstrate that BDP-S is a very promising photoacid sterilant.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10895-021-02682-8.

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Author Contributions All authors contributed to the study conception and design. Conceptualization: Qiu-Yun Chen; Methodology: Ling-Ling Qu; Formal analysis and investigation: Abbas Mohammed Ali, Jian Shao, Jia-Xin Wang and Yang Li; Writing- original draft preparation: Abbas Mohammed Ali; Writing - review and editing: Qiu-Yun Chen and Ling-Ling Qu; Funding acquisition: Ling-Ling Qu; Supervision: Qiu-Yun Chen.

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Data Availability The data used to support the findings of this study are included within the article.

Compliance with Ethical Standards

Conflict of Interest The authors declare that there is no conflict of interest.

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