

## Visible light mediated killing of multidrug-resistant bacteria using photoacids

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Increasing acidity is a promising method for bacterial inactivation by inhibiting the synthesis of intracellular proteins at low pH. However, conventional ways of pH control are not reversible, which can cause continuous changes in cellular and biological behaviours and are harmful to the host. Utilizing a photoacid that can reversibly alter pH over two units, we demonstrated a strong bacterial inhibition assisted by visible light. The pH value of the solution reverts back to the original level immediately after the irradiation is stopped. If a photoacid is combined with colistin, the minimum inhibitory concentration (MIC) of colistin on multidrug-resistant (MDR) *Pseudomonas aeruginosa* can be improved ~32 times (from 8 to 0.25  $\mu\text{g mL}^{-1}$ ), which significantly decreases the toxicity of colistin in clinics. Evidenced by the extremely low toxicity of the photoacid, this strategy is promising in MDR bacteria killing.

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### 1 Introduction

Global morbidity and mortality induced by infectious diseases are exaggerated with the prevalence of multidrug-resistant (MDR) bacterial pathogens, primarily due to over or misuse of antibiotics. Most of the existing antibiotics fail to deal with MDR bacteria such as methicillin-resistant *Staphylococcus aureus*, extended-spectrum  $\beta$ -lactamase-producing enteric bacteria, and *Mycobacterium tuberculosis*. Thus, improving efficacy of antibiotics and reducing resistance, together with nephrotoxicity and neurotoxicity, are of utmost importance in fighting against MDR bacteria.<sup>1</sup>

*Pseudomonas aeruginosa* (PA), a leading cause of hospital infections, is difficult to treat because of the wide spread MDR strain. Colistin (polymyxin E) is highly effective in fighting against MDR PA by destroying the bacterial outer membrane and allowing small molecules to flow out of the cytoplasm.<sup>2-5</sup> However, colistin can only be considered as the last-line treatment option for MDR organisms due to its serious nephrotoxicity and neurotoxicity,<sup>6-10</sup> together with the appearance of resistance to colistin among these organisms.<sup>11</sup> To reduce the colistin induced damage to the human body, a combination of therapies is increasingly being considered.<sup>12,13</sup> Among all possibilities, increasing acidity is an effective method for bacterial inactivation by inhibiting the synthesis of intracellular

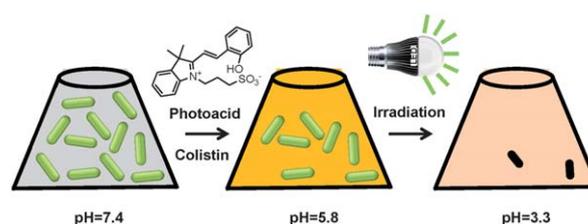


Fig. 1 Scheme of the visible light enabled antibacterial strategy.

proteins at low pH.<sup>14,15</sup> But the conventional ways of pH control are not reversible, which can cause continuous changes in cellular and biological behaviors. Ideally, the pH of a bacterial suspension can be reduced to kill bacteria, and recover back to normal after that. Photoswitchable chemicals can be used to tune pH based on illumination with light of different wavelengths, mostly in the ultraviolet range.<sup>16-19</sup>

Recently, we developed a type of photoacid that can reversibly alter the pH by ~2 pH units using visible light irradiation.<sup>20</sup> Here we report a novel photoswitchable antibacterial strategy *via* controllable adjustment of bacterial intercellular pH, by irradiating the photoacid using a visible light, to increase the antibacterial activity of colistin (Fig. 1).

### 2 Experimental procedure

#### Materials

Sodium chloride (NaCl), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), RPMI 1640 culture media, penicillin, streptomycin, fetal bovine serum (FBS), and Dulbecco's phosphate-buffered saline (D-PBS) were from Sigma-Aldrich (St Louis, MO). Yeast extract, bacteriological agar, and

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BD falcon multi-well flat-bottom plate were from VWR (West Chester, PA). The LIVE/DEAD BacLight bacterial viability kit was from Invitrogen (Carlsbad, CA). Trypton powder was obtained from MO BIO (Carlsbad, CA). Ultrapure water ( $18.2 \text{ M}\Omega \text{ cm}^{-1}$ ) from Nanopure System (Barnstead, Kirkland, WA) was used throughout. The photoreactions were performed on a 120 blue LED high-output array with an emission wavelength of 470 nm. Products of the esterification reaction were analyzed by PerkinElmer series 200 High-performance liquid chromatography (HPLC). A Fisher Scientific Accument AR15 benchtop meter and pH combination electrodes were used to measure the pH change. A Varian Cary 50 Scan UV-Vis spectrophotometer was used to measure the UV absorption spectra.

A PA reference strain (ATCC 27853) and multi-drug resistant PA (ATCC 15442), a HeLa (CLL-2) cell line, and a HEK-293 (CRL-1573) cell line were obtained from American Type Culture Collection (ATCC, Manassas, VA). All chemicals used in this study were of analytical grade and used without further purification.

### Synthesis of photoacids

A photoacid was synthesized following a literature method.<sup>20</sup> Mainly, starting material 2,3,3-trimethyl-1-(3-sulfonatepropyl)-3H-indolium was synthesized at the beginning. Then 2,3,3-trimethyl-1-(3-sulfonatepropyl)-3H indolium (100 mg, 0.36 mmol) and 2-hydroxybenzaldehyde (48 mg, 0.39 mmol) were added into anhydrous ethanol (2 mL), followed by refluxing overnight. The photoacid was obtained by filtration (110 mg, 80% yield).

### Cytotoxicity assay

The cytotoxicity of photoacid on bacteria was performed using a bacterial viability assay. For dual staining the bacteria, the bacteria in the water were diluted to a density of  $10^5 \text{ CFU mL}^{-1}$  according to the absorbance at 600 nm. 3  $\mu\text{L}$  of the dye mixture (containing an equal volume of green-fluorescent SYTO® 9 stain and red-fluorescent propidium iodide stain) was mixed with 1 mL bacterial suspension and incubated at 37 °C in the dark for 15 min, followed by observation under a fluorescence microscope. For live or dead cell calibration, 0.6  $\mu\text{L}$  of the dye mixture and 200  $\mu\text{L}$  of the bacterial suspension were added into each well of the 96-well microplate. After incubation for 15 min, the fluorescent signals were measured at 530 nm/630 nm (dual fluorescence). A cell-free control was used to remove the fluorescent backgrounds.

The cytotoxicities of photoacid on mammal cells were tested using MTT assay after incubating with various concentrations of photoacid (5, 50, and 500  $\mu\text{M}$ ). Firstly, the HeLa cells or HEK-293 cells were grown in the microwell of the 96-well plate till 80% confluence. Then the photoacid (combined or not combined with colistin) was incubated with the cells for 3 days, followed by absorbance measurement.

### Bactericidal activity

The bactericidal activity of the photoacid (with/without combined with colistin) was determined by monitoring the growth of PA in the presence of the photoacid. Briefly, PA

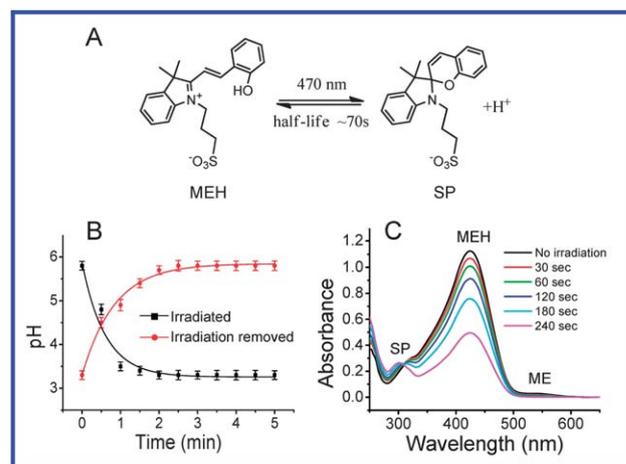
was grown in deionized (DI) water (pH 7.4) until a density of  $\sim 10^6 \text{ mL}^{-1}$  was reached. Then the protonated merocyanine (MEH) was introduced into the bacterial suspension at the final concentration of 500  $\mu\text{M}$ . After different times (1, 2, 3, 4 day respectively), 100  $\mu\text{L}$  of the bacterial suspension was inoculated and cultured on a LB agar plate for 24 h, followed by counting the colony-forming units (CFUs) of PA in the plate.

### Statistical analysis

Six independent duplicates were performed for each test and the data were shown as mean  $\pm$  standard error. All the figures were plotted using the Origin 8.5 software (OriginLab, Northampton, MA) and statistical analyses were performed using SPSS 16.0 (SPSS Inc, Chicago, Illinois). One-way ANOVA and LSD tests were applied to compare the difference between the numbers of bacterial colony after different treatments.  $p < 0.05$  was considered statistically significant.

## 3 Results and discussion

Fig. 2 shows that the synthesized photoacid is a visible-light activatable merocyanine derivative that has a long proton-dissociation state lifetime (70 s) due to a sequential intramolecular reaction. Fig. 2A illustrates the structural changes of the photoacid with or without irradiation. In the dark, the photoacid exists mainly as a MEH with a propyl sulfonate group on the nitrogen of the indoline moiety. The absorption of deprotonated merocyanine (ME) can also be observed, indicating that MEH is weakly acidic. A 600  $\mu\text{M}$  MEH solution has a pH value of 5.8, from which the  $\text{p}K_{\text{a}}$  of MEH can be calculated to be 7.8. After irradiation by a visible light with a wavelength within its charge transfer band (Fig. 2B), the pH drops by  $\sim 2$  units to 3.3 (close to the theoretical value of the complete proton dissociation of 3.2). When the light was turned off, the pH value increases quickly to  $\sim 4.7$  in 70 s, and gradually returns to its original level of 5.8 in  $\sim 5$  min. The characteristics of visible

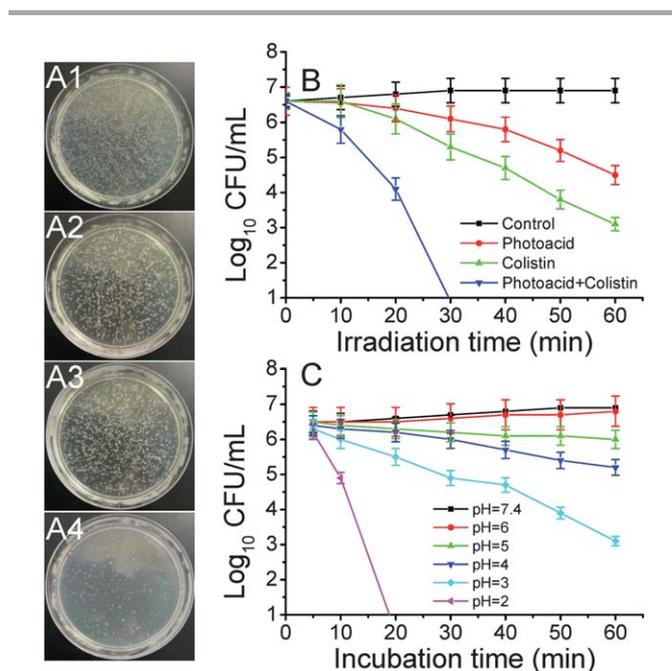


**Fig. 2** Chemical characteristics of photoacid. (A) pH change with or without visible light illumination. (B) Absorbance of MEH after irradiation. (C) Illustration of the reaction between MEH and SP.

light excitation and reversibility make it more attractive in biological applications than conventional photoacids, because no UV light is involved.

The photochemical reaction after irradiation is monitored by UV-Vis absorption spectroscopy. Fig. 2C shows that the absorption of MEH ( $\lambda_{\max} = 424$  nm) decreases upon irradiation and an absorption peak of SP ( $\lambda_{\max} = 325$  nm) increases accordingly.

To assess the antibacterial activity of the photoacid, two strains of PA (reference strain and MDR strain) are tested. Bacteria are inoculated in DI water until they reach a value of  $10^6$  CFU mL<sup>-1</sup>. DI water is taken as solvent to eliminate the buffering ability against the pH change. Fig. 3A shows the illuminated photoacid or colistin alone can only inhibit the growth of MDR PA, but the combination of illuminated photoacid and colistin kills most of the MDR PA. Fig. 3B shows that in the presence of MEH, illumination with visible light (470 nm) for 1 h kills  $\sim 99\%$  ( $\sim 2 \log_{10}$  CFU mL<sup>-1</sup> decrease) of MDR PA, whereas MDR PA population increases  $\sim 20\%$  in the absence of MEH. The 20% increase of the bacterial population is mainly due to the natural growth of bacteria in DI water. Meanwhile,  $1 \mu\text{g mL}^{-1}$  colistin alone kills  $\sim 3.5 \log_{10}$  CFU mL<sup>-1</sup> MDR PA within 1 h. Interestingly, in the presence of MEH and colistin, the antibacterial activity of colistin is significantly improved by decreasing  $\sim 5.5 \log_{10}$  CFU mL<sup>-1</sup> bacteria number after 30 min illumination. The results clear show that the combination of photoacid and colistin can significantly improve the antibacterial activity. The following experiments are carried out to determine whether this enhanced capability is due to visible light illumination or from the cytotoxicity of the photoacid.

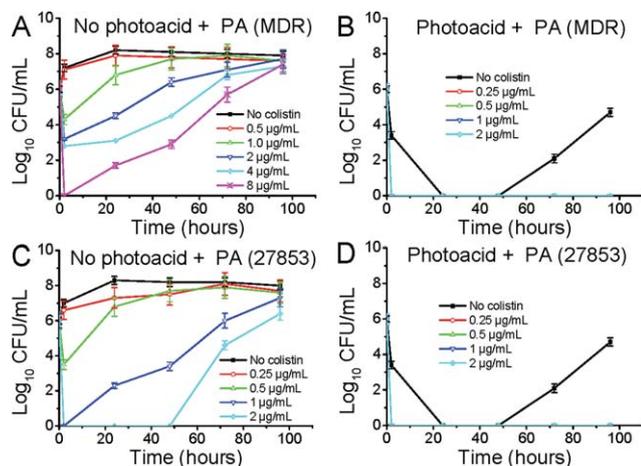


**Fig. 3** Antibacterial activity of the illuminated photoacid. (A) Cultured MDR PA after treating with PBS (A1), colistin (A2), illuminated photoacid (A3), and colistin together with the illuminated photoacid (A4); (B) effect of irradiation time on the bacterial population; (C) effect of different pH values on the bacterial population. The error bars represent standard errors of 6 independent tests.

Firstly, the bactericidal activity of visible light is tested by measuring the bacterial population after exposure at 470 nm for a long time. Results show that there is no significant difference between bacteria numbers in the illuminated sample and the control sample (non-illuminated) even after 24 h of incubation ( $p > 0.05$ ), indicating that the exposure to visible light (470 nm) has no direct bactericidal effect. Meanwhile, the cytotoxicities of the photoacid on bacteria before and after illumination are tested with MTT assay. Results show that  $500 \mu\text{M}$  MEH kills only 25.7% of MDR PA cells in 24 h of incubation without illumination, revealing its low cytotoxicity on bacterial cells. Considering that the illuminated photoacid kills  $5 \log_{10}$  CFU mL<sup>-1</sup> MDR PA within 2 h, we consider the killing effect is mainly due to the pH change of the solution.

To verify this hypothesis, the growth rate of MDR PA at different pHs (ranging from 2 to 7.4) is determined. Fig. 3C shows that the bacterial population increases  $\sim 18\%$  within 24 h at pH 7.4 due to natural growth. No significant difference is observed in the bacterial population between pH 6 and 7.4 ( $p > 0.05$ ), showing that MDR PA can grow well under a pH range of 6–7.4. But the bacterial population begins to decrease significantly when the pH is lower than 5. The bacterial population decreases to 1.5 and  $3 \log_{10}$  CFU mL<sup>-1</sup> at pH 4 and 3 within 1 h, respectively. At pH 2, all bacteria are killed ( $5.5 \log_{10}$  CFU mL<sup>-1</sup> decrease) within 20 min, indicating that the pH value has a significant influence on the bacterial growth, which is consistent with previous reports that the optimal pH range for the growth of PA is 5.6–8.5.<sup>21</sup> The antibacterial activity of the illuminated photoacid ( $2 \log_{10}$  CFU mL<sup>-1</sup> decrease) is within the range of 1.5 and  $3 \log_{10}$  CFU mL<sup>-1</sup> at pH 4 and 3. Considering that the illuminated photoacid (pH 3.3) is also within the range mentioned above, it can be concluded that the antibacterial activity of the illuminated photoacid is mainly due to the pH decrease after illumination. These results show that visible light illuminated MEH can induce a pH drop of 4 to 3 in solution, which can significantly inhibit the growth of MDR PA. Regrowth of bacteria is observed after a few hours when the pH returns to 7.4, indicating that inactivated bacteria are still alive and can grow into colonies. The bacterial regrowth after acid treatment is likely due to the adaption of MDR PA on environmental stress, which means that lowering the pH alone is not a powerful antibacterial strategy (Fig. 4A and B).

Colistin demonstrates a concentration dependent killing of MDR PA at pH 7.4. Our results show that the minimal inhibition concentration (MIC) of colistin on MDR PA is  $8 \mu\text{g mL}^{-1}$ , meaning that  $8.0 \mu\text{g mL}^{-1}$  colistin can completely inhibit the growth ( $\sim 6 \log_{10}$  CFU mL<sup>-1</sup> decrease) of MDR PA in 1 h (Fig. 4A). Lower colistin concentrations can only partially inhibit the growth of MDR PA. Colistin at 4.0, 2.0,  $1.0 \mu\text{g mL}^{-1}$  can decrease 4.5, 3.0,  $1.5 \log_{10}$  CFU mL<sup>-1</sup> of MDR PA, respectively. No significant antibacterial activity is observed for  $0.5 \mu\text{g mL}^{-1}$  colistin when compared with the negative control ( $p > 0.05$ ). Meanwhile, MDR PA regrows 2–3 days later after being treated by colistin ( $< 8.0 \mu\text{g mL}^{-1}$ ), which is consistent with our previous reports that high dose of colistin is required to kill MDR PA due to increased resistance by accelerating the synthesis of intracellular proteins to reduce the effect of



**Fig. 4** Synergistic antibacterial activity of colistin with photoacid. (A) Antibacterial activity of different colistin concentrations on MDR PA; (B) synergistic antibacterial activity of different colistin concentrations with 500 mM MEH on MDR PA; (C) antibacterial activity of different colistin concentrations on reference PA (27853); (D) synergistic antibacterial activity of different colistin concentrations with 500  $\mu$ M MEH on reference PA strain (27853);  $n = 6$ .

extracellular pH change on inner organelles.<sup>22–24</sup> We are wondering whether the illuminated photoacid can decrease the antibacterial resistance by inhibiting the protein synthesis.

Fig. 4B shows that the combination of colistin and photoacid provides higher antibacterial activity than colistin or photoacid alone. After illuminating (470 nm) the bacterial solution containing photoacid and colistin, the MIC of colistin on MDR PA dropped from 8.0 to 0.25  $\mu$ g mL<sup>-1</sup>. In addition, no regrowth is observed after 4 days culturing, in the presence of 0.25  $\mu$ g mL<sup>-1</sup> colistin and 500  $\mu$ M MEH. These results clearly show that low pH can significantly enhance the antibacterial activity of colistin and prevent the regrowth of MDR PA. To verify the feasibility of this combination in killing bacteria, the killing of a reference PA strain (27538) is also studied (Fig. 4C and D). A MIC of 1  $\mu$ g mL<sup>-1</sup> colistin on PA 27538 is observed without the presence of photoacid, and the regrowth can be observed 2 days after colistin treatment. However, in the presence of 500  $\mu$ M MEH assisted with illumination, the MIC of colistin decreases to 0.25  $\mu$ g mL<sup>-1</sup> (Table 1). Thus the synergistic effect of visible light assisted MEH and an extremely low concentration of colistin (0.25  $\mu$ g mL<sup>-1</sup>) can effectively kill PA (both laboratory and MDR strain), and no regrowth has been observed within 5 days of culture.

The synergistic antibacterial activity of photoacid and colistin can be understood as below. Colistin kills bacteria by destroying the outer membrane of bacterial cells. The polycationic colistin molecules can displace Mg<sup>2+</sup> and Ca<sup>2+</sup> ions that cross-bridge between adjacent negatively charged phosphate groups of

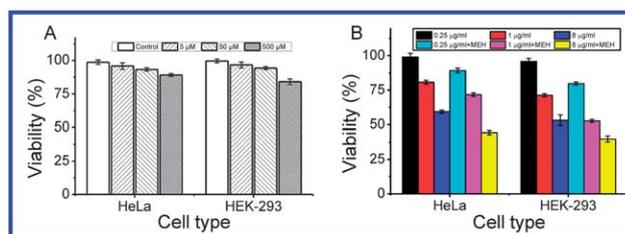
lipopolysaccharides (LPS) in the outer membrane of bacteria. The disruptions of outer and cytoplasmic membranes are followed by the leakage of small intracellular molecules, and bacterial killing occurs within few minutes. Generally, a relatively high concentration of colistin is needed to trigger disruption of the membrane, because bacteria can repair a slight displacement of Mg<sup>2+</sup> and Ca<sup>2+</sup> ions in the membrane at the optimal intracellular pH (pH<sub>i</sub>). But if the cytoplasmic membrane is disrupted seriously, extracellular H<sup>+</sup> (high concentration) can quickly diffuse into the cytoplasm and a considerable number of small intracellular molecules flow out of the membrane, which overwhelmed the ability of bacteria to maintain optimal pH<sub>i</sub> and induced irreversible bacterial death. In addition, the change of pH<sub>i</sub> hinders the ability to compensate the displacement of Mg<sup>2+</sup> and Ca<sup>2+</sup> in the membrane, accelerating bacterial killing. Thus, the antibacterial activity of colistin is significantly enhanced by decreasing the MIC in the case of combination.

The cytotoxicity of MEH on mammalian cells is also studied either with or without colistin. HeLa (cervical cancer cell) and HEK-293 (human renal cell) are used to assess the cytotoxicity of photoacid because the kidney toxicity is frequently reported. Fig. 5A shows that cytotoxicities of the photoacid depend on its concentration: no significant cytotoxicity is observed for 5 and 50  $\mu$ M photoacid up to 72 h when compared with the control ( $p > 0.05$ ). And for 500  $\mu$ M photoacid, only 10% HeLa cells and 16% HEK-293 cells are killed after 72 h treatment, respectively. These results reveal that the photoacid has low toxicity to mammalian cells even at high concentration. Meanwhile, Fig. 5B shows that the cytotoxicity of colistin is highly concentration dependent: an extremely low concentration of colistin (0.25  $\mu$ g mL<sup>-1</sup>) imposes nearly no toxicity on both HeLa and HEK-293 cells, 1  $\mu$ g mL<sup>-1</sup> of colistin kills 19% HeLa cells and 28% HEK-293 cells, while 8  $\mu$ g mL<sup>-1</sup> colistin kills 50% HeLa cells and 84% HEK-293 cells within 72 h.

This high cytotoxicity of colistin can be adopted to explain the serious nephrotoxicity during MDR PA treatment in clinics.<sup>7,8,24,25</sup> The side effect of colistin can be significantly reduced by decreasing its dosage. Fig. 5B shows that the combination of 500  $\mu$ M photoacid and 0.25  $\mu$ g mL<sup>-1</sup> colistin kills 13% HeLa cells and 21% of HEK-293 cells after 72 h treatment, which are much lower than that of 8  $\mu$ g mL<sup>-1</sup> colistin on HeLa cells (74%) and HEK-293 cells (81%) alone ( $p < 0.05$ ).

**Table 1** MICs of colistin on PA

	Reference PA (27853)	MDR PA
No photoacid	1 mg mL <sup>-1</sup>	8 mg mL <sup>-1</sup>
500 mM photoacid	0.25 mg mL <sup>-1</sup>	0.25 mg mL <sup>-1</sup>



**Fig. 5** Cytotoxicity of photoacid and colistin. (A) Concentration dependent cytotoxicity of MEH on HeLa cells and HEK-293 cells. (B) Cytotoxicity of the anti-bacterial combination including MEH and different concentrations of colistin. Error bars represent the standard errors of 6 independent tests.

The cytotoxicity of the illuminated photoacid (SP) is not assessed directly, because the entire monolayer cultured cells die within 30 min in such a low pH (3.3) condition. It is difficult to differentiate whether the cell death is caused by low pH or the cytotoxicity of SP. But, previous studies have shown that 8-methoxy-6-nitro-BIPS (SP derivative) did not affect the cellular survival of THP-1, AGS, and A549 cells for 24 h at concentration ranging from  $10^{-4}$  to  $10^{-9}$  M.<sup>26</sup> Photochromic molecules from the SP family also have no effect on the cellular survival of HEK-293 cells.<sup>27</sup> Compared to these SP derivatives reported before, our SP molecule has a similar structure except for lacking a  $\text{NH}_3^+$  group, indicating a much lower cytotoxicity than those reported SP derivatives.

## 4 Conclusions

A strong bactericidal activity is observed by combining the visible light illumination of photoacid and colistin. The nephrotoxicity of colistin can be drastically decreased by reducing the MIC of colistin when combined with a photoacid, indicating a promising approach in MDR bacteria killing. In the future, photoacids may be incorporated into the structure of a drug carrier. Visible light irradiation will produce a temporary and localized pH drop that increases the bactericidal activity of the drug.

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## Notes and references

- N. Aoki, K. Tateda, Y. Kikuchi, S. Kimura, C. Miyazaki, Y. Ishii, Y. Tanabe, F. Gejyo and K. Yamaguchi, *J. Antimicrob. Chemother.*, 2009, **63**, 534–542.
- V. Pintado, L. G. S. Miguel, F. Grill, B. Mejia, J. Cobo, J. Fortun, P. Martin-Davila and S. Moreno, *J. Infect.*, 2008, **56**, 185–190.
- A. Walkty, M. DeCorby, K. Nichol, J. A. Karlowsky, D. J. Hoban and G. G. Zhanel, *Antimicrob. Agents Chemother.*, 2009, **53**, 4924–4926.
- M. E. Falagas, P. I. Rafailidis, E. Ioannidou, V. G. Alexiou, D. K. Matthaiou, D. E. Karageorgopoulos, A. Kapaskelis, D. Nikita and A. Michalopoulos, *Int. J. Antimicrob. Agents*, 2010, **35**, 194–199.
- J. Li, R. L. Nation, J. D. Turnidge, R. W. Milne, K. Coulthard, C. R. Rayner and D. L. Paterson, *Lancet Infect. Dis.*, 2006, **6**, 589–601.
- C. Y. Cheng, W. H. Sheng, J. T. Wang, Y. C. Chen and S. C. Chang, *Int. J. Antimicrob. Agents*, 2010, **35**, 297–300.
- S. J. Wallace, J. Li, R. L. Nation, C. R. Rayner, D. Taylor, D. Middleton, R. W. Milne, K. Coulthard and J. D. Turnidge, *Antimicrob. Agents Chemother.*, 2008, **52**, 1159–1161.
- M. E. Falagas, P. L. Rafailidis, S. K. Kasiakou, P. Hatzopoulou and A. Michalopoulos, *Clin. Microbiol. Infect.*, 2006, **12**, 1227–1230.
- B. Lin, C. Zhang and X. Xiao, *J. Vet. Pharmacol. Ther.*, 2005, **28**, 349–354.
- M. E. Falagas, M. Rizos, I. A. Bliziotis, K. Rellos, S. K. Kasiakou and A. Michalopoulos, *BMC Infect. Dis.*, 2005, **5**, 1.
- K. R. Peck, M. J. Kim, J. Y. Choi, H. S. Kim, C. I. Kang, Y. K. Cho, D. W. Park, H. J. Lee, M. S. Lee and K. S. Ko, *J. Med. Microbiol.*, 2012, **61**, 353–360.
- M. Hornsey, C. Longshaw, L. Phee and D. W. Wareham, *Antimicrob. Agents Chemother.*, 2012, **56**, 3080–3085.
- P. J. Bergen, B. T. Tsuji, J. B. Bullitta, A. Forrest, J. Jacob, H. E. Sidjabat, D. L. Paterson, R. L. Nation and J. Li, *Antimicrob. Agents Chemother.*, 2011, **55**, 5685–5695.
- Z. Erdogan-Yildirim, A. Burian, M. Manafi and M. Zeitlinger, *Res. Microbiol.*, 2011, **162**, 249–252.
- H. Zhu, C. A. Hart, D. Sales and N. B. Roberts, *J. Med. Microbiol.*, 2006, **55**, 1265–1270.
- F. May, M. Peter, A. Hutten, L. Prodi and J. Mattay, *Chemistry*, 2012, **18**, 814–821.
- R. Tong, H. D. Hemmati, R. Langer and D. S. Kohane, *J. Am. Chem. Soc.*, 2012, **134**, 8848–8855.
- J. Folling, S. Polyakova, V. Belov, A. van Blaaderen, M. L. Bossi and S. W. Hell, *Small*, 2008, **4**, 134–142.
- R. M. Nunes, M. Pineiro and L. G. Arnaut, *J. Am. Chem. Soc.*, 2009, **131**, 9456–9462.
- Z. Shi, P. Peng, D. Strohecker and Y. Liao, *J. Am. Chem. Soc.*, 2011, **133**, 14699–14703.
- D. Emerson and C. Moyer, *Appl. Environ. Microbiol.*, 1997, **63**, 4784–4792.
- H. G. Prentice, I. M. Hann, B. Nazareth, P. Paterson, A. Bhamra and C. C. Kibbler, *Br. J. Haematol.*, 2001, **115**, 46–52.
- P. J. Bergen, J. Li, R. L. Nation, J. D. Turnidge, K. Coulthard and R. W. Milne, *J. Antimicrob. Chemother.*, 2008, **61**, 636–642.
- L. M. Lim, N. Ly, D. Anderson, J. C. Yang, L. Macander, A. Jarkowski, A. Forrest, J. B. Bullitta and B. T. Tsuji, *Pharmacotherapy*, 2010, **30**, 1279–1291.
- J. M. Yousef, G. Chen, P. A. Hill, R. L. Nation and J. Li, *J. Antimicrob. Chemother.*, 2012, **67**, 452–459.
- D. Movia, A. Prina-Mello, Y. Volkov and S. Giordani, *Chem. Res. Toxicol.*, 2010, **23**, 1459–1466.
- J. R. Nilsson, S. M. Li, B. Onfelt and J. Andreasson, *Chem. Commun.*, 2011, **47**, 11020–11022.