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A coumarin based fluorescent probe for NIR imaging guided photodynamic therapy against *S. aureus*-induced infection in mice models

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# J. Li, L. Tu, Q. Ouyang and S. Yang contributed equally to this work.

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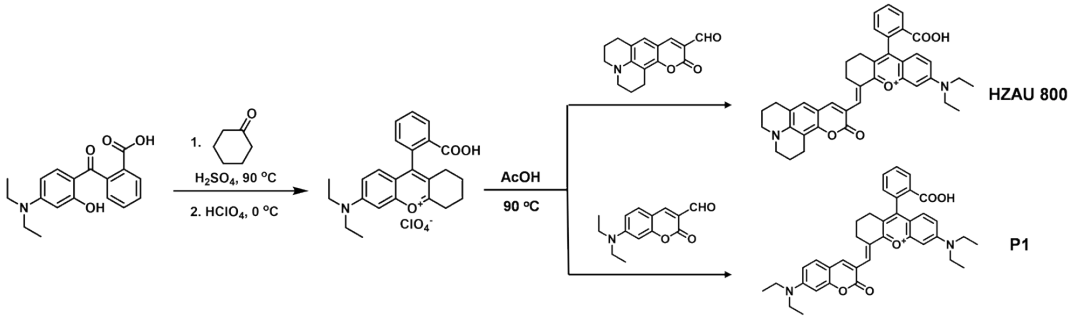
1. Synthesis and characterization of probe HZAU800 and P1



General synthetic procedure. Rhodamine analog 1[1] (1.0 eq) and coumarin derivatives (1.0 eq) [2,3] were dissolved in AcOH (8 mL). The mixture was heated at 90 °C for 5 h. After completing the reaction, the solvent was removed under reduced pressure, the residue was subjected to column chromatography (CH_2Cl_2: MeOH=15:1) to obtain the final product as green solid.

HZAU800: yield: 16.5%. ^{1}H NMR (400 MHz, CD_3CN) δ 8.26 (d, *J* = 8.0 Hz, 1H), 8.13 (s, 1H), 7.89 (s, 1H), 7.83 (t, *J* = 7.6 Hz, 1H), 7.74 (t, *J* = 7.6 Hz, 1H), 7.26 (d, *J* = 7.6 Hz, 1H), 7.07-6.98 (m, 4H), 3.65-3.59 (m, 4H), 3.35 (t, *J* = 5.2 Hz, 4H), 2.90 (t, *J* = 6.0 Hz, 2H), 2.78 (t, *J* = 6.4 Hz, 2H), 2.74 (t, *J* = 6.0 Hz, 2H), 2.33 (m, t, *J* = 5.6 Hz,4H), 1.82-1.74 (m, 4H), 1.26 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6): 166.73, 161.14, 151.19, 147.59, 144.12, 133.40, 130.29, 129.52, 128.57, 127.64, 126.99, 121.95, 119.37, 113.18, 108.35, 105.35, 95.64, 65.00, 59.85, 54.99, 49.75, 49.20, 45.35, 27.20, 26.87, 25.37, 21.05, 20.74, 19.79, 19.66, 15.25, 14.17, 12.59. HRMS: calcd. for [M-ClO_4]$^+$: 627.28535; found: 627.28540.

P1: yield: 13%. ^{1}H NMR (600 MHz, $CDCl_3$): 7.94 (d, *J* = 11.4 Hz, 1H), 7.64-7.62 (m, 1H), 7.58-7.52 (m, 2H), 7.38 (s, 1H), 7.28 (s, 1H), 7.19 (d, *J* = 11.4 Hz, 1H), 6.61-6.58 (dd, *J* = 3.0, 13.2 Hz, 1H), 6.53 (d, *J* = 3.0 Hz, 1H), 6.49-6.45 (m, 2H), 6.35-6.33 (dd, *J* = 3.6, 13.8 Hz, 1H) , 3.43 (q, *J* = 10.8 Hz, 4H), 3.35 (q, *J* = 10.8 Hz, 4H), 2.71-2.69 (m, 1H), 2.15-2.09 (m, 1H), 1.72-1.58 (m, 4H), 1.22 (t, *J* = 10.2 Hz, 6H), 1.17 (t, *J* = 10.2 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-d6): 169.08, 161.33, 155.51, 150.66, 141.56, 135.38, 130.67, 130.49, 130.00, 129.84, 129.47, 128.38, 126.55, 123.28, 115.71, 109.32, 108.12, 96.59, 96.48, 44.21, 43.80, 40.14, 27.09, 31.83, 12.44. HRMS: calcd for [M-ClO_4^-+H]$^+$: 604.2926; found: 604.2884.

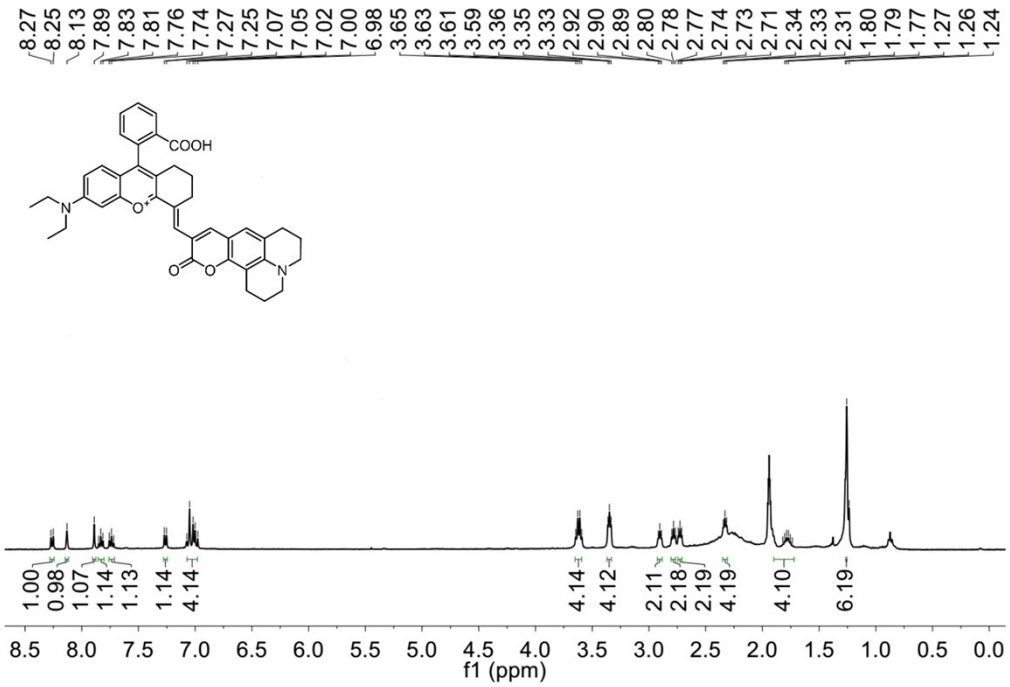


Figure S1. ^{1}H NMR of **HZAU800**

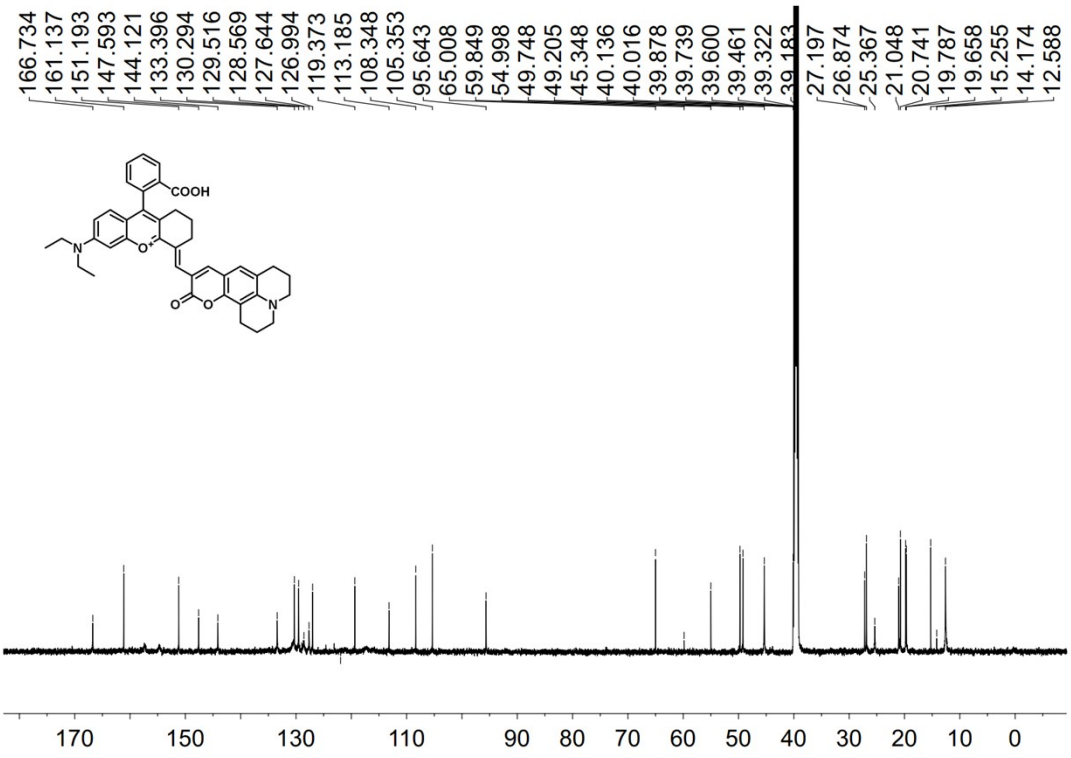


Figure S2. ^{13}C NMR of **HZAU800**

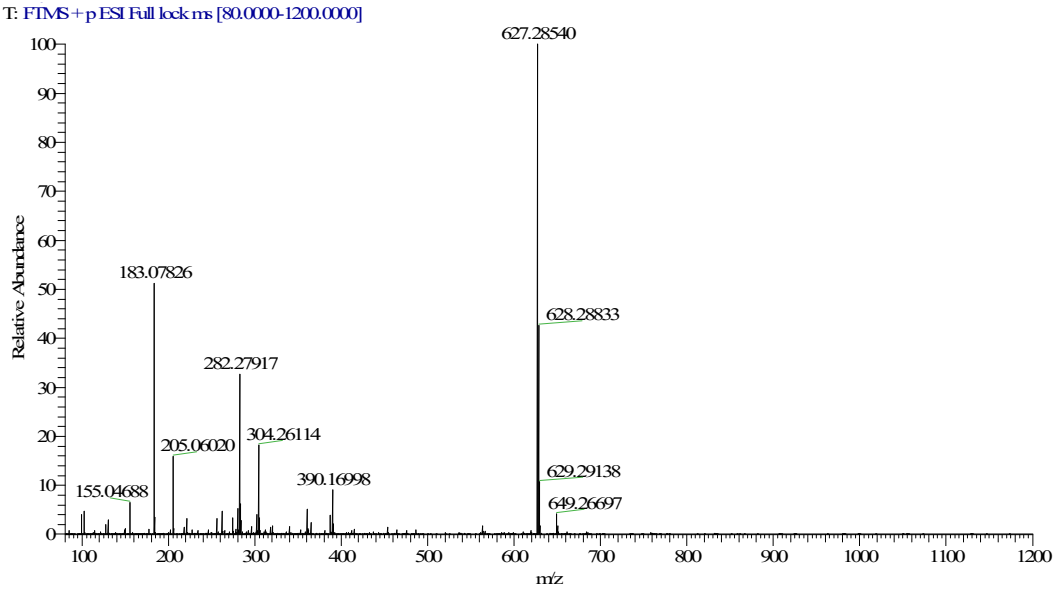


Figure S3. HRMS of **HZAU800**

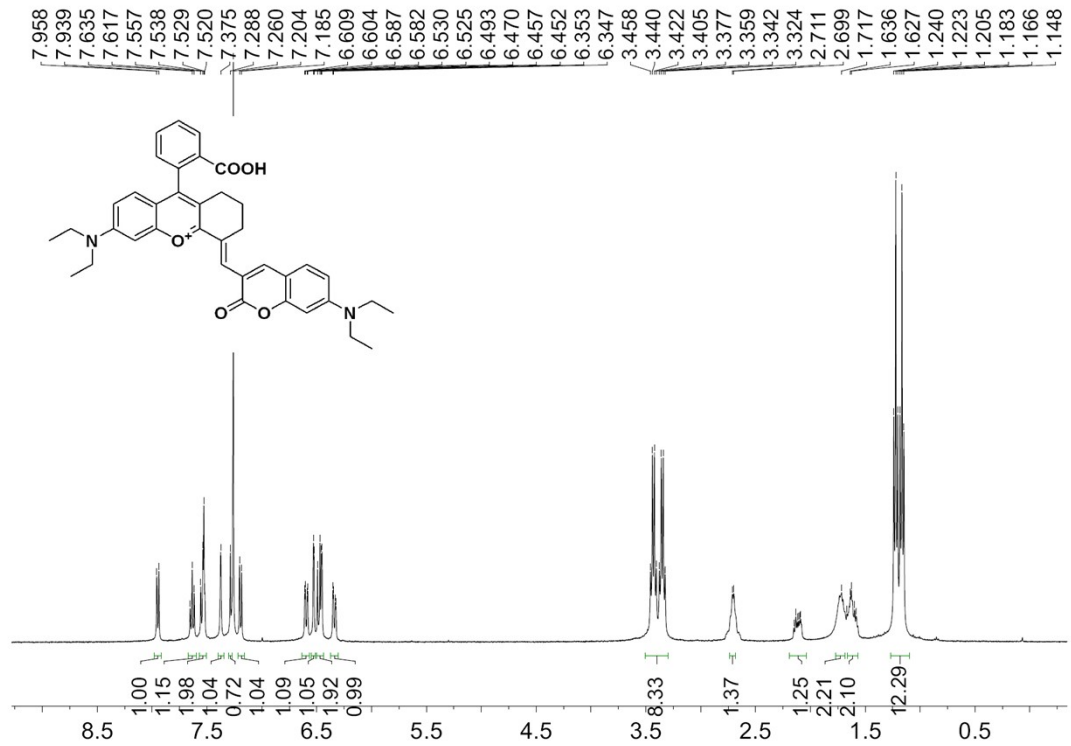


Figure S4. ^{1}H NMR of **P1**

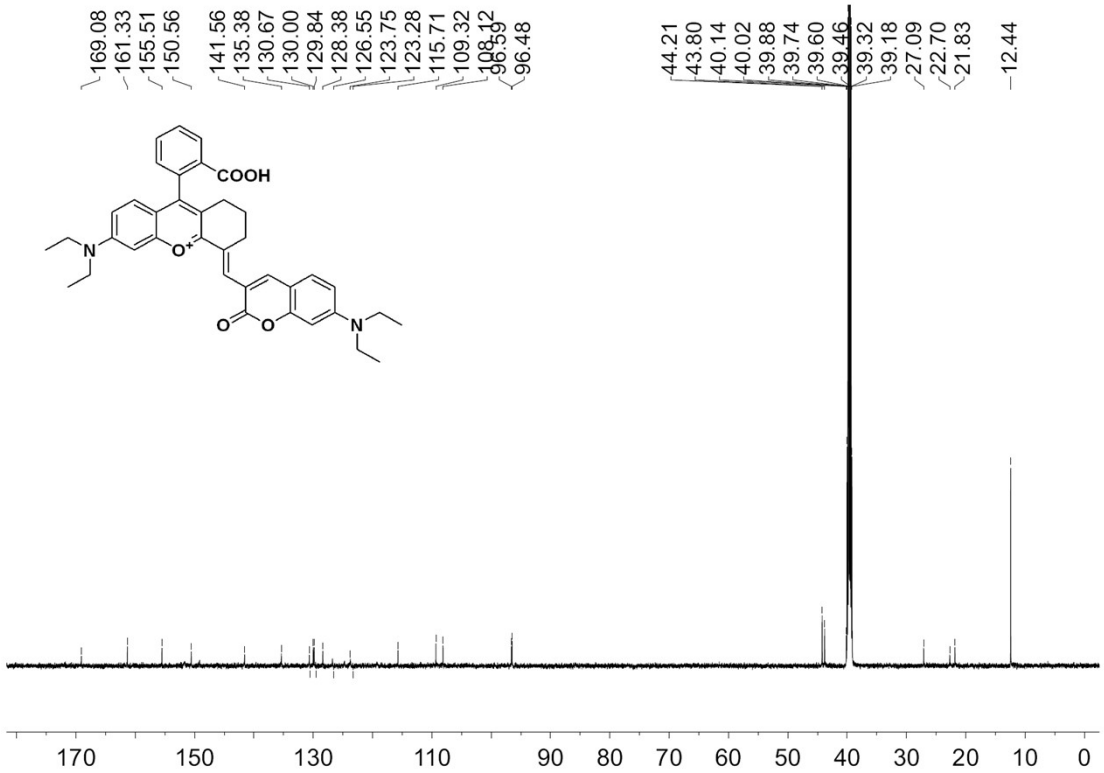


Figure S5. ^{13}C NMR of **P1**

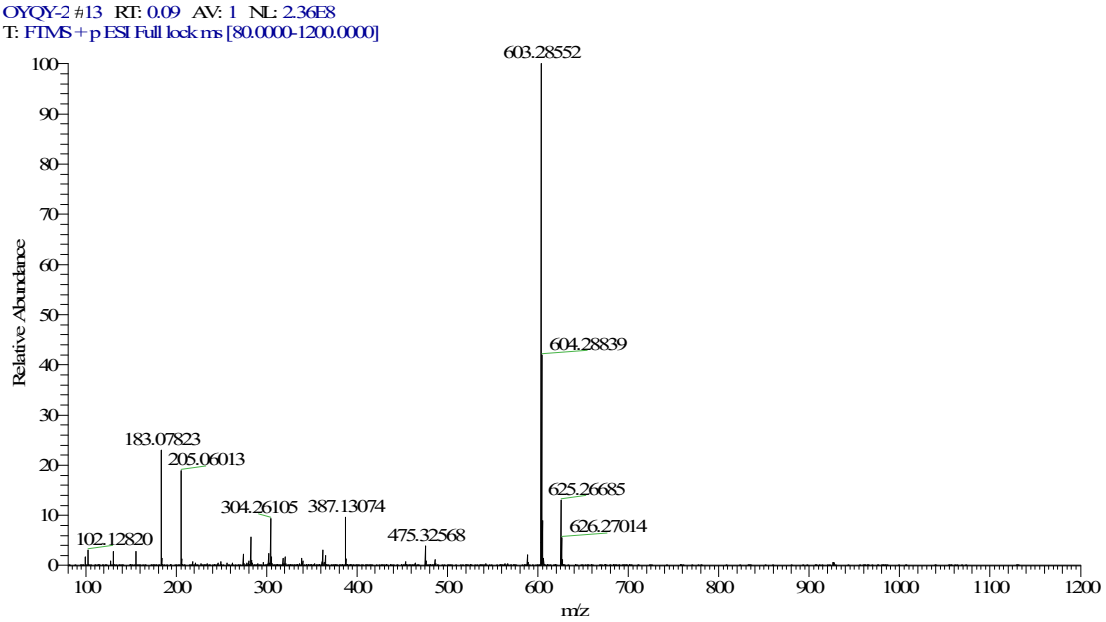


Figure S6. HRMS of **P1**

2. Optical properties of HZAU800

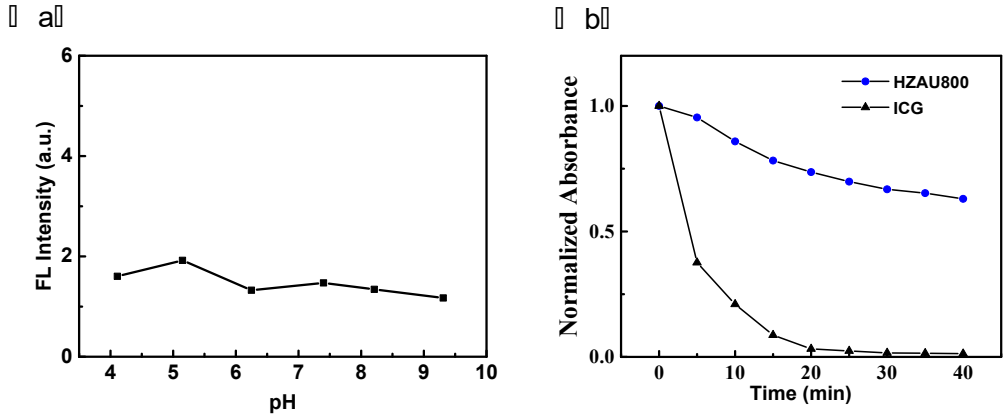


Figure S7. pH dependence (a) and photostability of **HZAU800** (b)

3. Verification of ROS produced by HZAU800

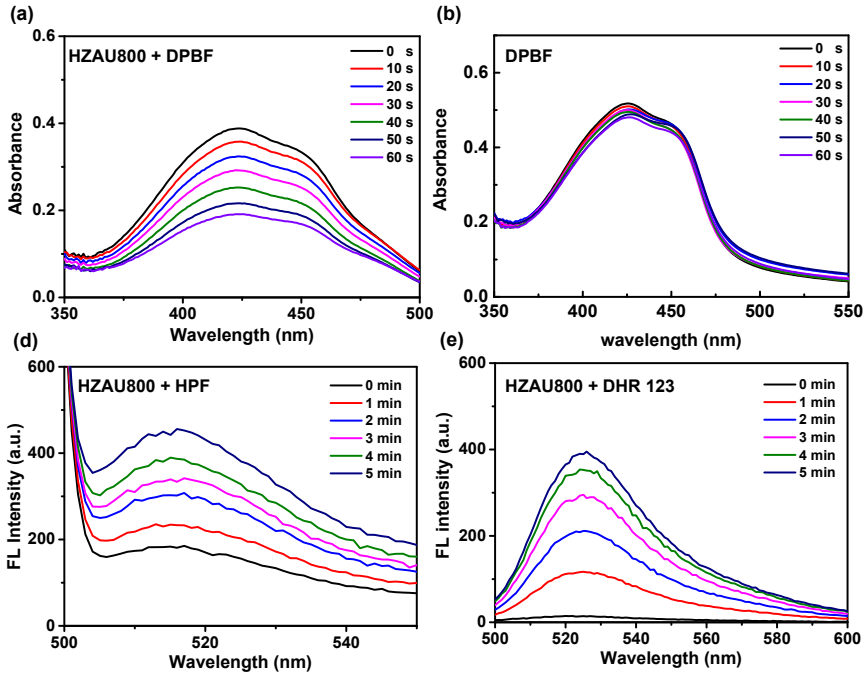


Figure S8. Verification of ROS produced by **HZAU800** using different ROS indicators: (a) UV spectrum of 10 μM **HZAU800** + 30 μM DPBF under laser's irradiation at different time; (b) UV spectrum of 30 μM DPBF under laser's irradiation at different time; (c) fluorescence response of 10 μM **HZAU800** + 10 μM HPF under laser's irradiation at different time; (d) fluorescence response of 10 μM **HZAU800** + 10 μM DHR 123 under laser's irradiation at different time.

4. Cytotoxicity assay

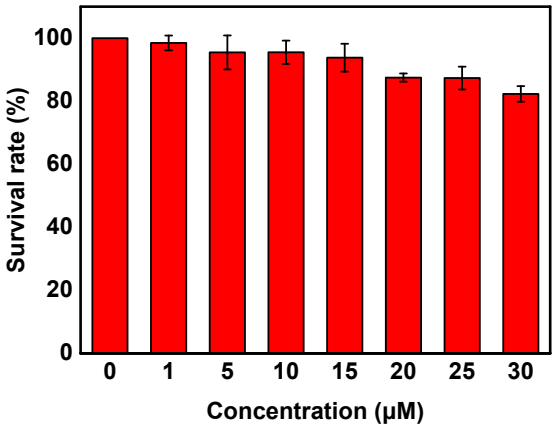


Figure S9. Cytotoxicity data of **HZAU800** (0, 1, 5, 10, 15, 20, 25, 30μM) in Hela cells.

5. NIR imaging of *S. aureus* and *E. colic* using HZAU800

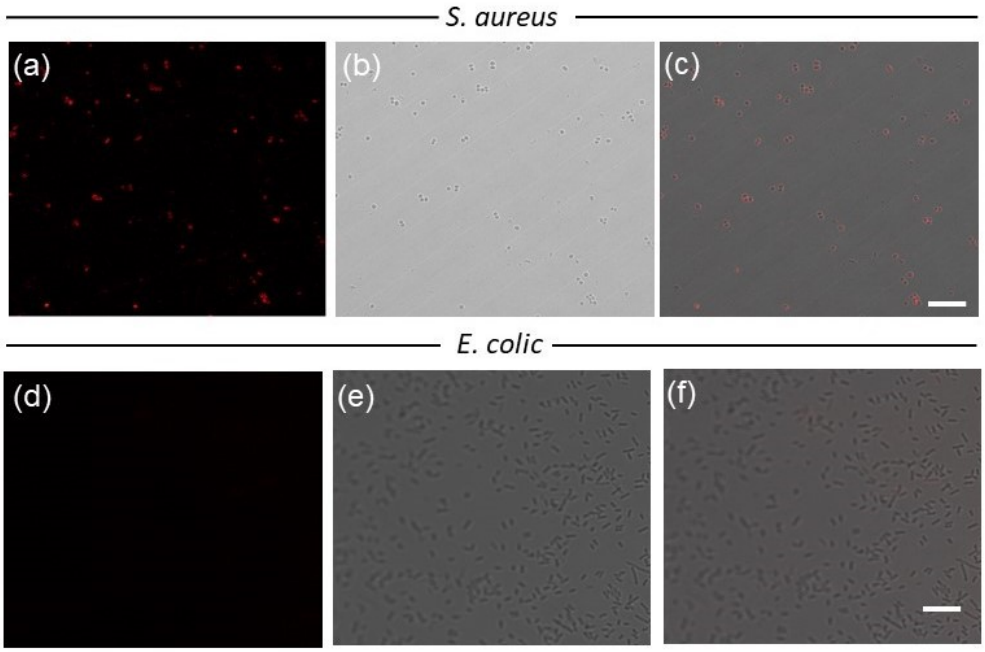


Figure S10. NIR imaging of **HZAU800** (40 μg/mL) in *S. aureus and E. colic* with dark field (a) and (d), bright field (b) and (e), merged field (c) and (f), scale bar: 10 μm.

6. Evaluation of phototherapeutic effects of HZAU800 on *S. aureus* infected tissues

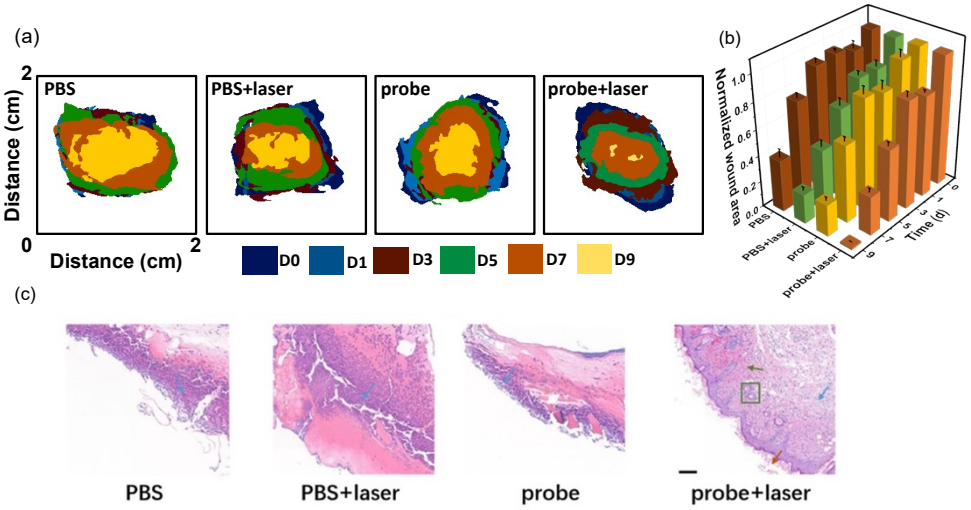


Figure S11. (a) and (b) the distance and area of S. a*ureus*-infected wounds after different treatments; (c) The hematoxylin and eosin (H&E) staining assessment of S. a*ureus*-infected tissues after different treatments, blue arrow: neutrophils, green arrow: fibroblast; green rectangle: granulation tissue. Scale bar: 100 μm.

7. The H&E staining assessment of HZAU 800 in different tissues

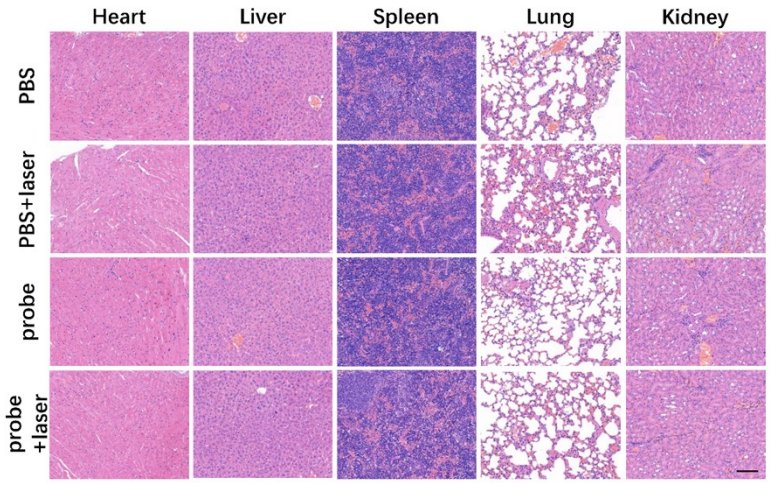


Figure S12. The H&E staining assessment of **HZAU800** in different tissues**.** Scale bar: 100 μm.

8. The dihedral angle and molecular dynamics simulation of HZAU800

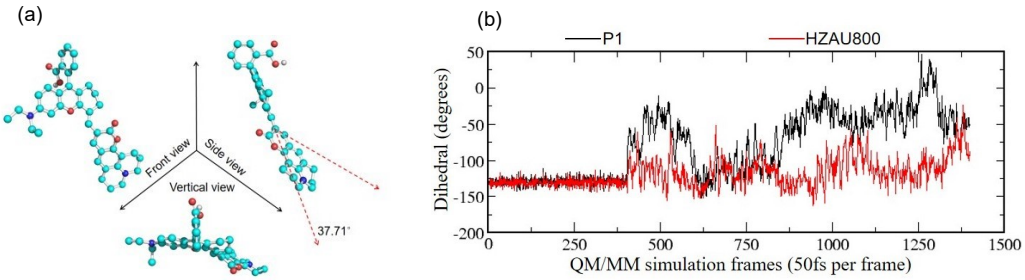


Figure S13. (a) the dihedral angle of **HZAU800**; (b) the molecular dynamics simulation of **HZAU800** and **P1**.

9. Reference

(1) L. Yuan, W. Lin, Y. Yang and H. Chen, A unique class of near-infrared functional fluorescent dyes with carboxylic-acid-modulated fluorescence ON/OFF switching: rational design, synthesis, optical properties, theoretical calculations, and applications for fluorescence imaging in living animals. *J. Am. Chem. Soc.,* 2012, **134**, 1200-1211.

(2) J. Li, Y. Cui, C. Bi, S. Feng, F. Yu, E. Yuan, S. Xu, Z. Hu, Q. Sun, D. Wei and J. Yoon, Oligo (ethylene glycol)-functionalized ratiometric fluorescent probe for the detection of hydrazine in vitro and in vivo. *Anal. Chem.* 2019, **91**, 7360-7365.

(3) Y. Liang, Y. Zhao, C. Lai, X. Zou and W. Lin, Coumarin-based TICT fluorescent probe for real-time fluorescence lifetime imaging of mitochondrial viscosity and systemic inflammation in vivo. *J. Mater. Chem. B*, 2021, **9**, 8067-8073.