Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

A novel ratiometric fluorescent probe for detection of mitochondrial pH dynamics during cell damage

Gaoqing Fu [a, 1], Guoxing Yin [b, 1] Tingting Niu [a], Wei Wu [a], Hui Han [a], Haimin Chen*[a] and Peng Yin *[b]

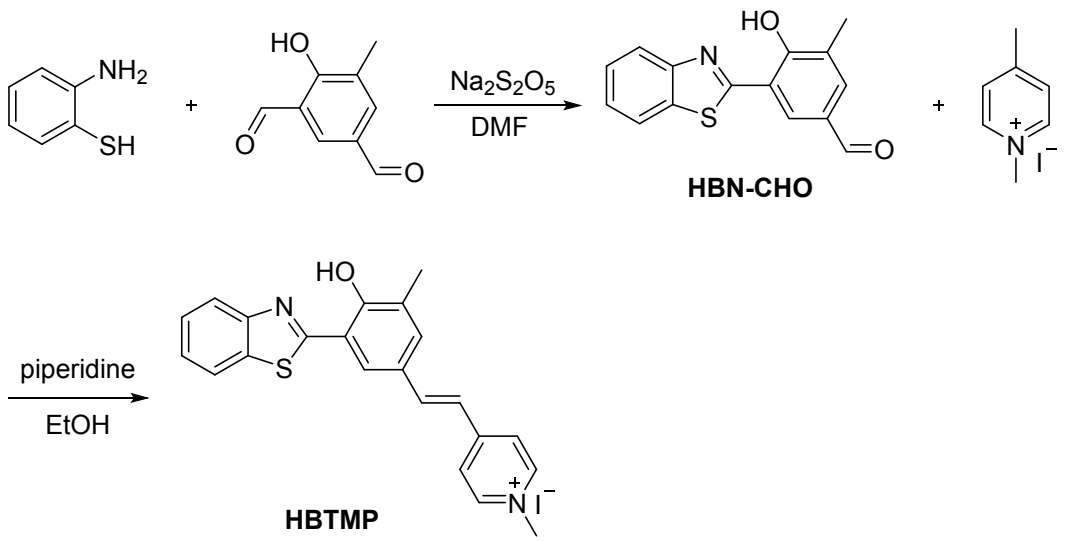
[a]*State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-products, Ningbo University, Ningbo, Zhejiang 315211, China*

[b]*Key Laboratory of the Assembly and Application of Organic Functional Molecules of Hunan Province, Hunan Normal University, Changsha 410081, China.*

* Corresponding author. Tel.: (+86) 57487609575; fax: (+86) 57487609575.

E-mail: chenhaimin@nbu.edu.cn; yinpeng@hunnu.edu.cn

[1] G. Fu and G. Yin contributed equally to this work.



Scheme S1. Synthetic scheme of probe **HBTMP**.

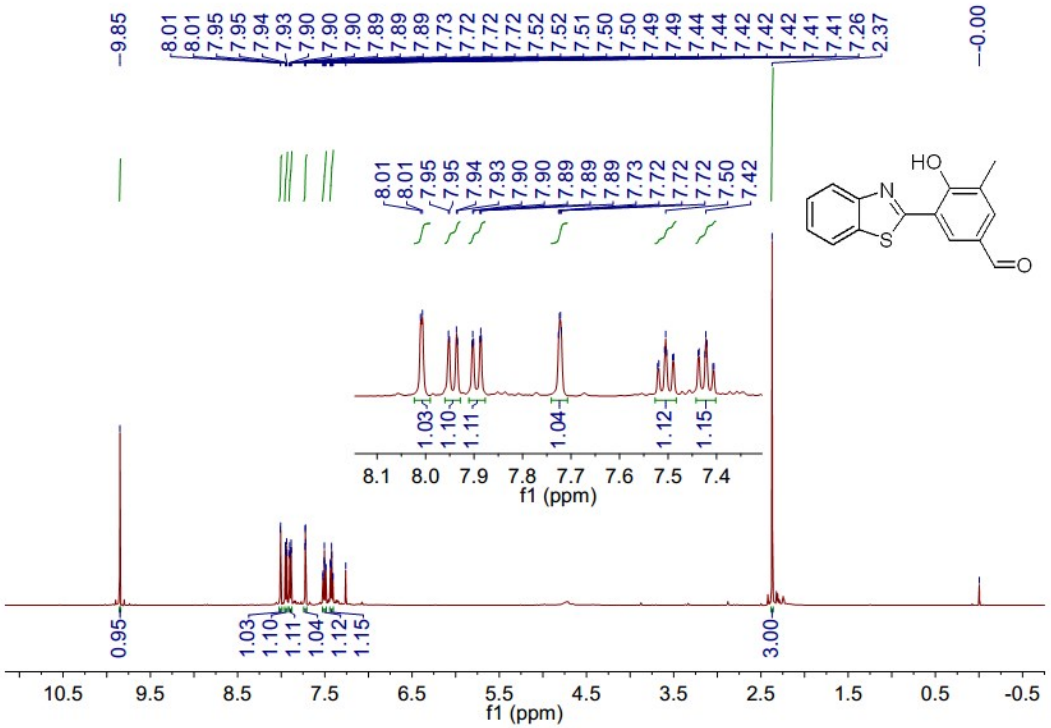


Fig. S1. ^{1}H-NMR spectrum of HBN-CHO in $CDCl_3$.

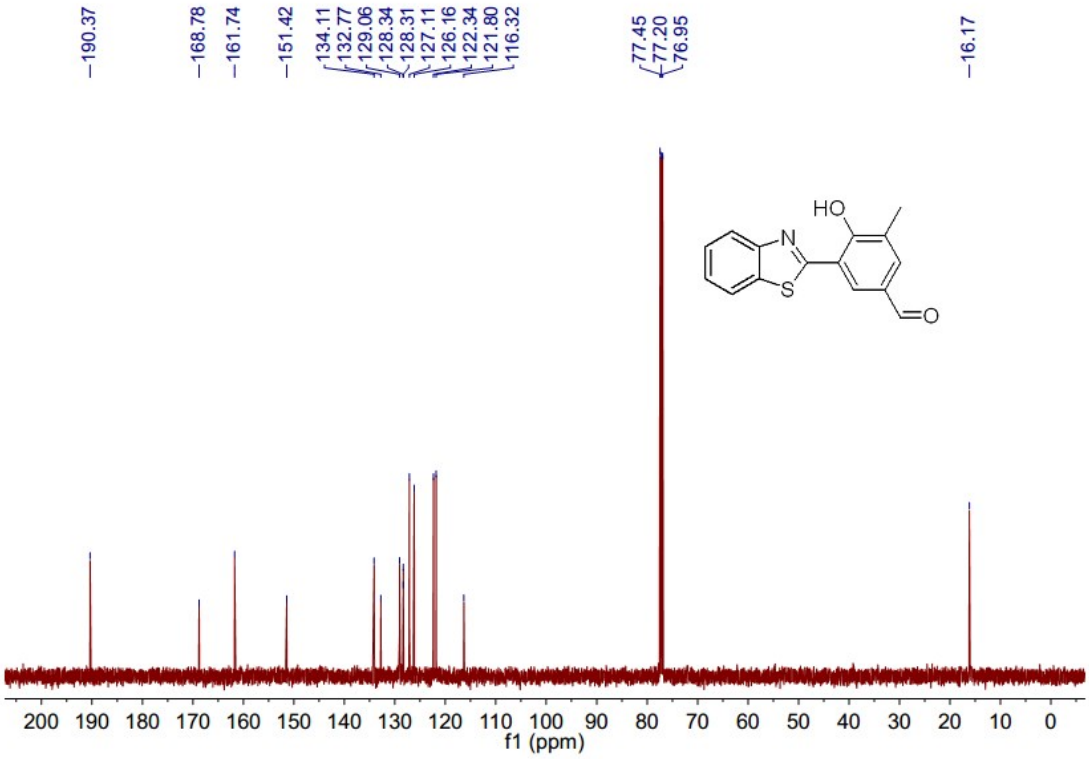


Fig. S2. ^{13}C-NMR spectrum of HBN-CHO in $CDCl_3$.

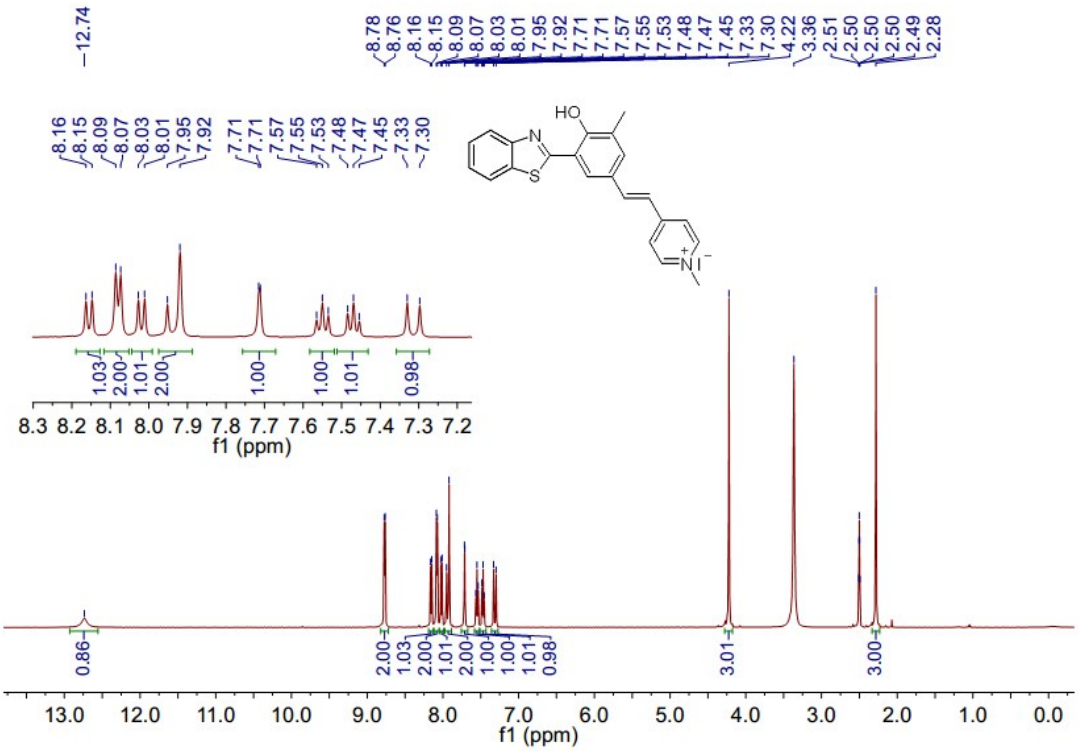


Fig. S3. ^{1}H-NMR spectrum of probe **HBTMP** in DMSO-d_6.

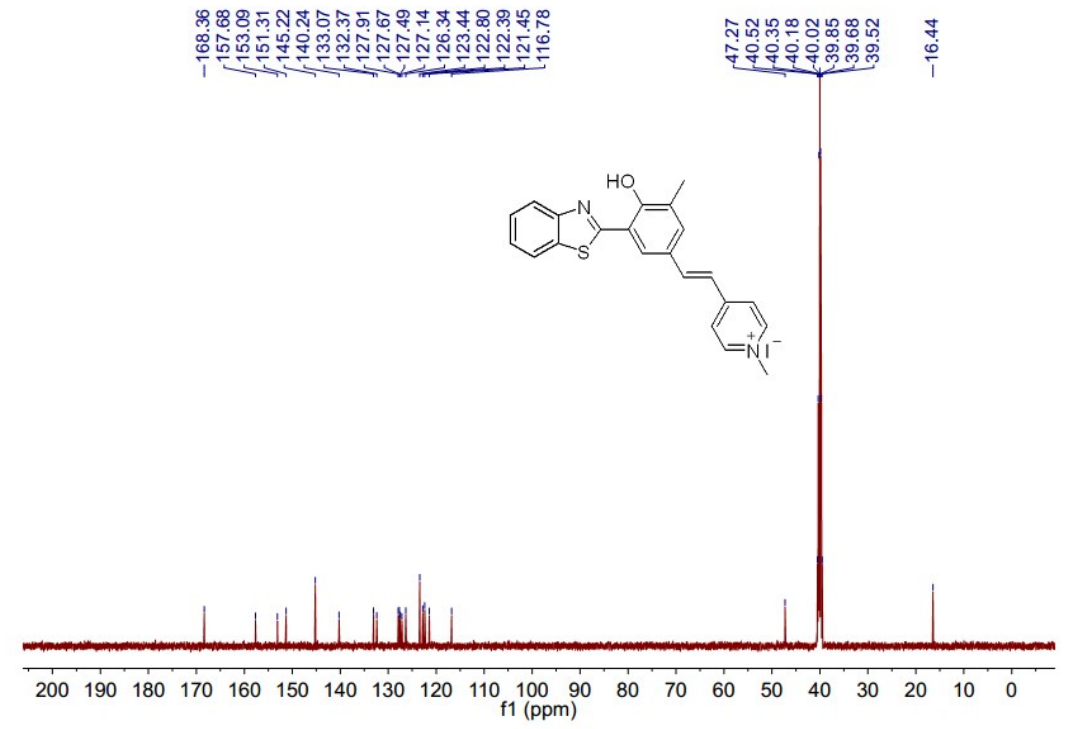


Fig. S4. ^{13}C-NMR spectrum of probe **HBTMP** in DMSO-d_6.

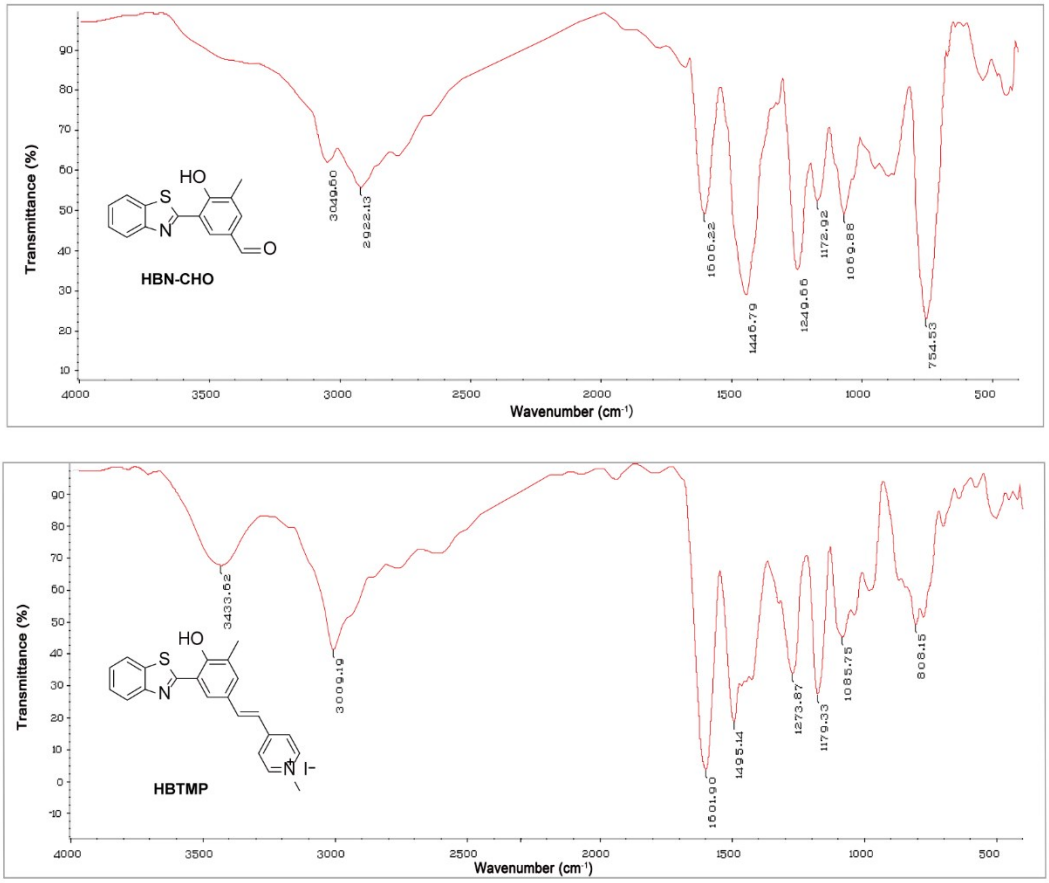


Fig. S5 IR spectrumof HBN-CHO and **HBTMP**.

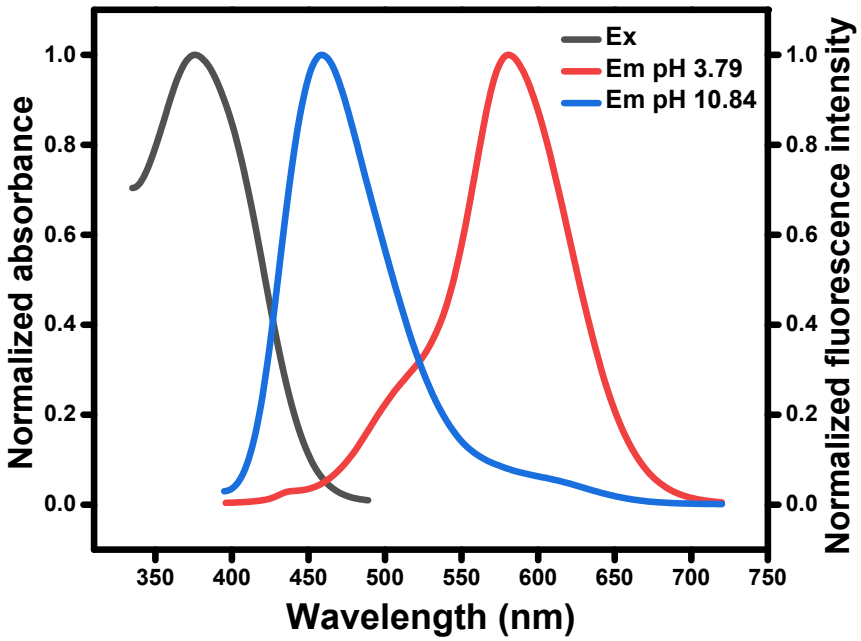


Fig. S6. Normalized fluorescence emission spectra of probe **HBTMP** (10 μM) in two different Britton-Robinson buffer (0.04 M, pH = 3.79 (red line), 10.84 (blue line)), respectively, and normalized excited spectra of probe **HBTMP** (black line). λ_{ex} = 375 nm, λ_{em} = 470 nm, 580 nm, silt: 5 nm/5 nm.

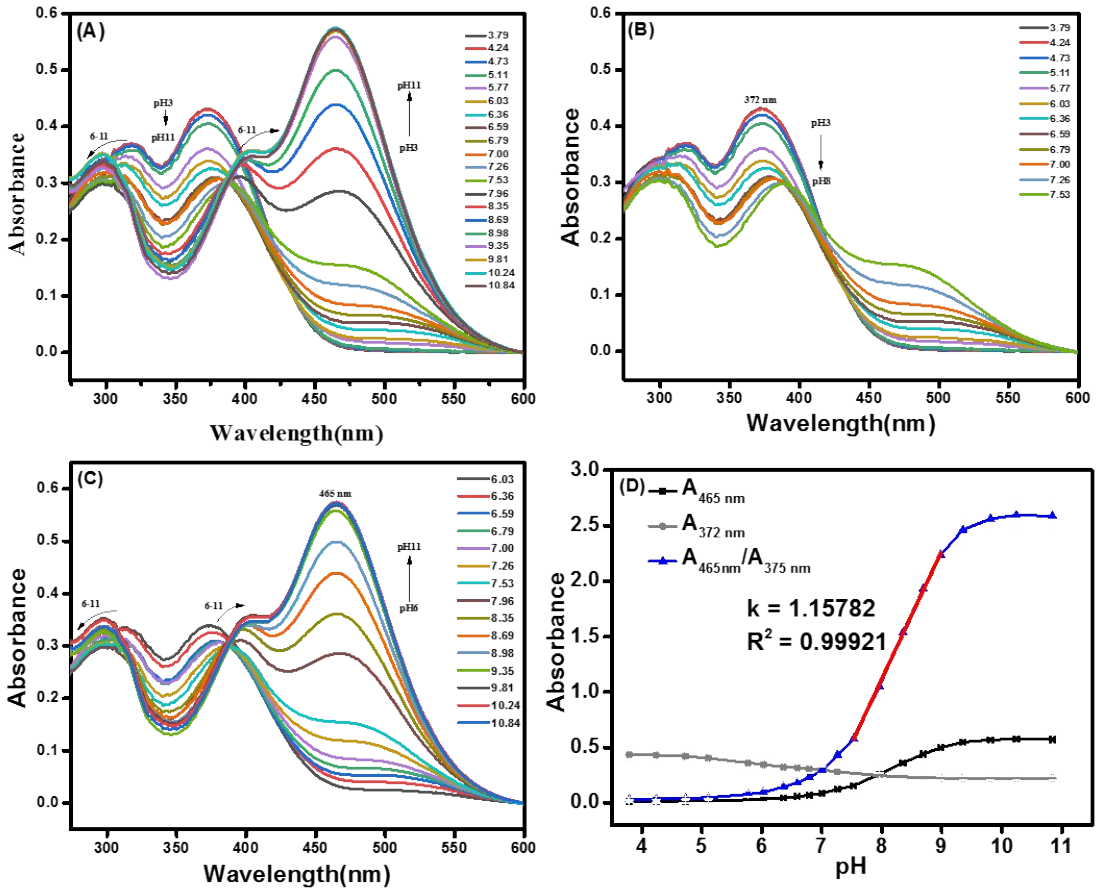


Fig. S7. Absorption spectra of probe **HBTMP** (20 μM) in the (A) B-R buffer (0.04 M), (B) acid environment and (C) base environment. (D) pH-dependent absorption profiles of probe **HBTMP** at 465 nm (black line), 372 nm (pink blue), and 465 nm/375 nm (blue line) in B-R buffer solutions. The Britton-Robinson buffer's pHs were 3.79, 4.24, 4.73, 5.11, 5.77, 6.03, 6.36, 6.59, 6.79, 7.00, 7.26, 7.53, 7.96, 8.35, 8.69, 8.98, 9.35, 9.81, 10.24, and 10.84.

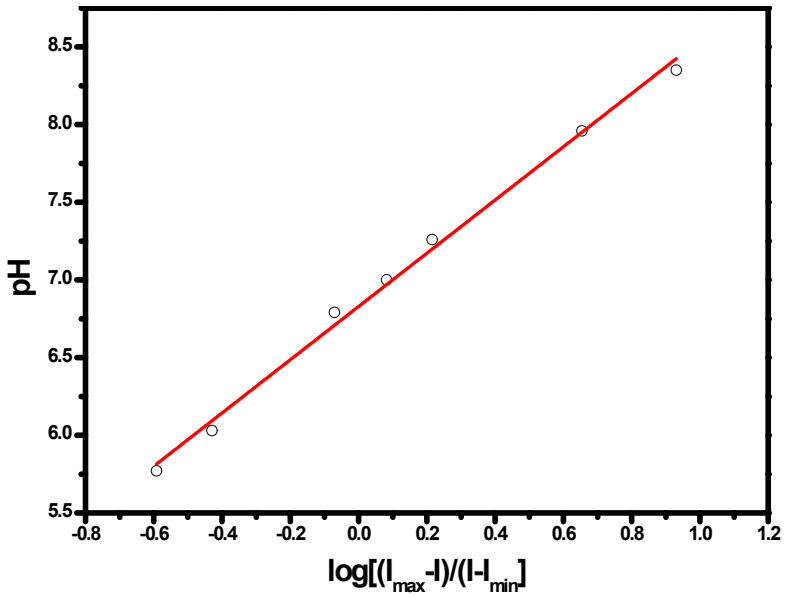


Fig. S8. Plot of pH vs log [(Imax-I) (I-Imin)], where I is the observed normalized fluorescence intensity of **HBTMP** at 580 nm upon excitation at 375 nm. The y-intercept is the pK_a value (6.829 ± 0.02627) of **HBTMP**.

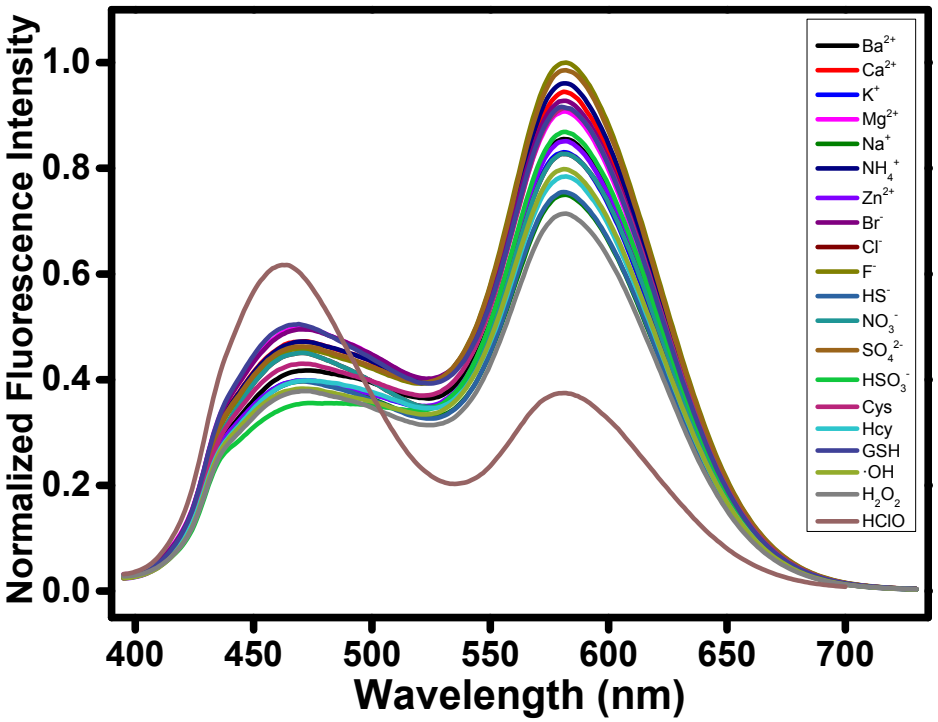


Fig. S9. Normalized fluorescence spectra of probe **HBTMP** (10 μM) in the presence of different analytes in Britton-Robinson buffer (0.04 M, pH = 7.4).

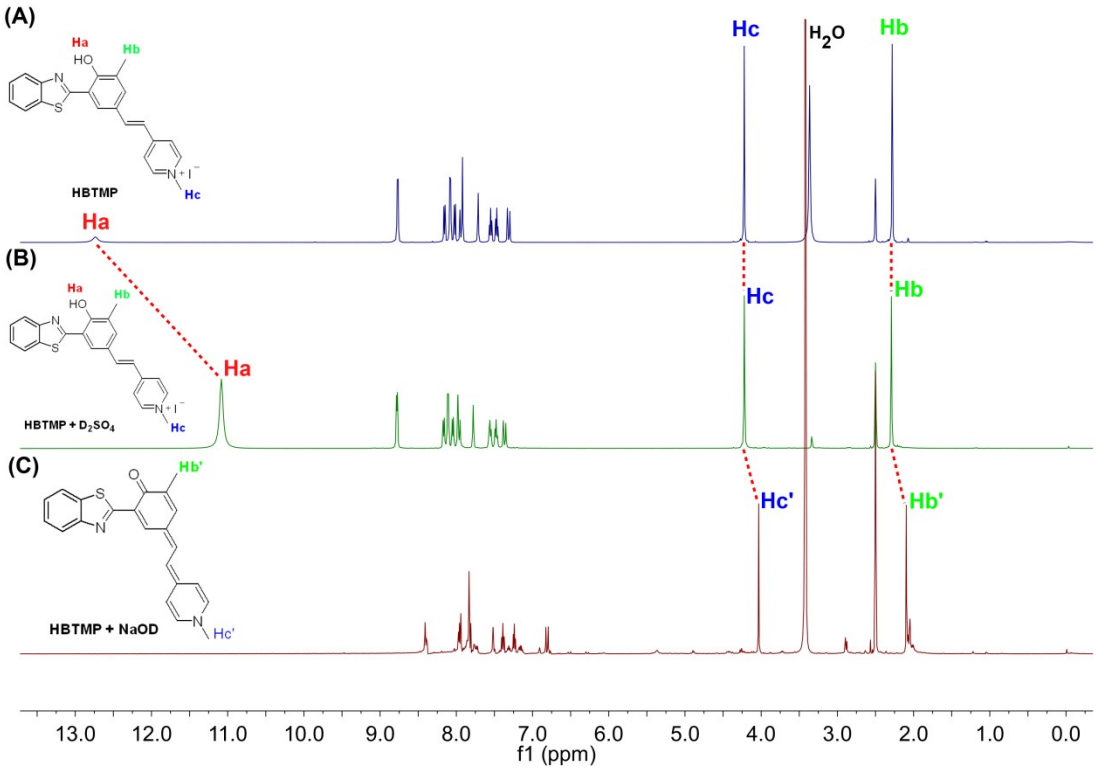


Fig. S10. ^{1}H NMR spectra of (a) probe **HBTMP**, (b) probe **HBTMP** upon addition of D_2SO_4, and (c) probe **HBTMP** upon addition of NaOD in DMSO-d_6.

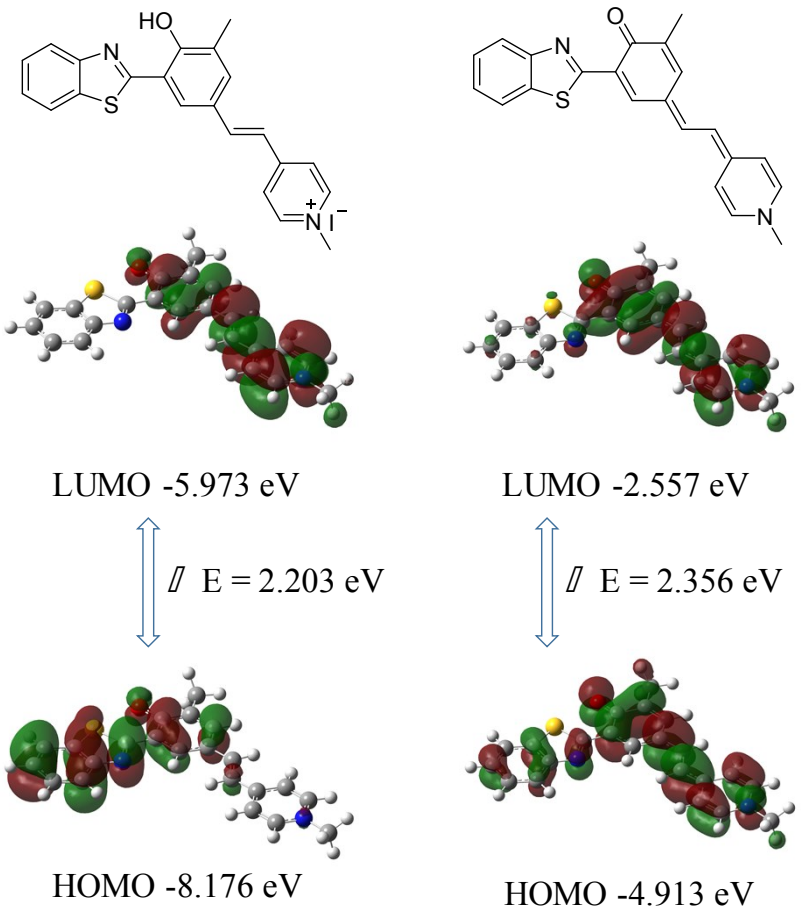


Fig. S11. The frontier molecular orbital energy of probe **HBTMP** at acid and neutral form. Calculations were performed using DFT with the B3LYP exchange functional employing 6-311G (d, p) basis sets using Gaussian 16 programs.

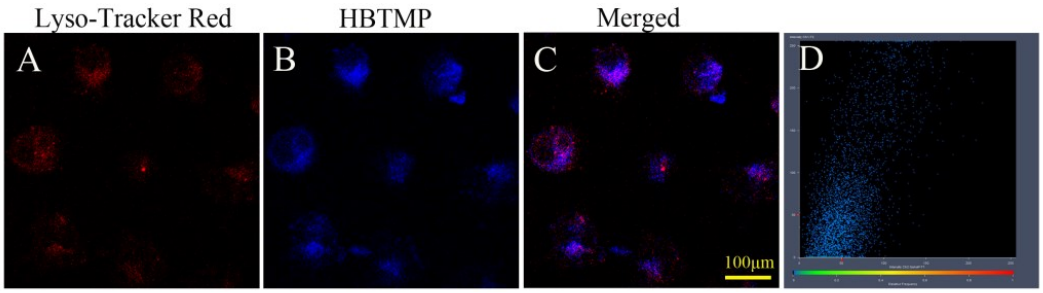


Fig. S12. Colocalization fluorescence images of the probe in the lysosomes of BEL-7402 cells. (A) Red channel of Lyso-Tracker Red (λ_{ex}= 577 nm, λ_{em}= 590 nm); (B) Blue channel of probe **HBTMP** (λ_{ex}= 405 nm, λ_{em} = 430-490 nm); (C) Merged image of A and B; (D) Image of colocalization coefficient. Scale bar: 100 μm.

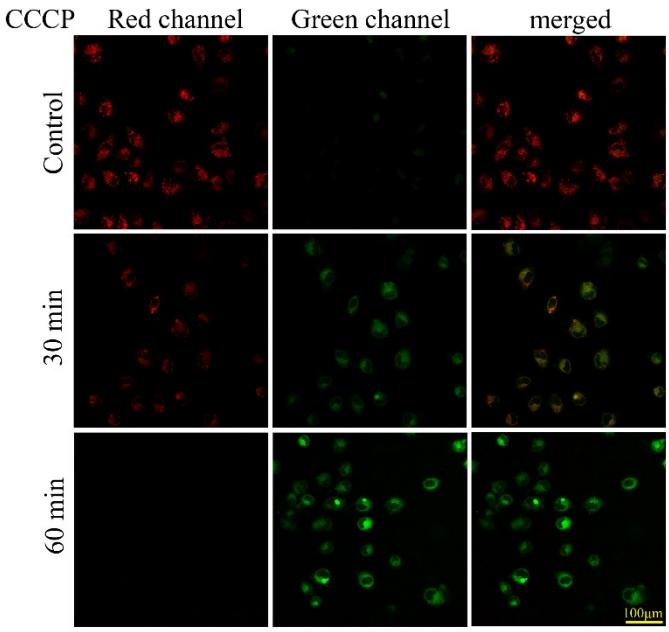


Fig. S13. The fluorescence images of the mitochondrial membrane potential changes of BEL-7402 cells caused by CCCP were detected by JC-1. The fluorescence images were captured in the red channel (λ_{ex} = 585 nm, λ_{em} = 590 nm) and the green channel (λ_{ex} = 514 nm, λ_{em} = 529 nm) for JC-1. Scale bar: 100 μm.