Supporting Information

Dual-response mitochondria-targeted NIR fluorescent probe with large Stokes shift for monitoring viscosity and HOCl in living cells and zebrafish

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Table of contents

Instruments

Ultrapure water was prepared by Milli Q Advantage A10 ultrapure water system and was used to prepare all solutions. The ultraviolet-visible absorption spectrum and fluorescence spectrum were measured by Shimadzu UV 3600 ultraviolet-visible spectrometer and PerkinElmer LS 55 fluorescence spectrometer, respectively. Fluorescence quantum yield and fluorescence lifetime were tested by Edinburgh FLS980 steady-state transient fluorescence spectrometer. All NMR spectra were measured by Bruker AVANCE III 400 MHz with tetramethylsilane (TMS) as the internal standard. All mass spectra were obtained by Thermo Fisher Q Exactive high resolution mass spectrometer. All cell viability was measured by MTT method using Thermo Scientific Varioskan R Flash full-wavelength scanning multi-function reader. Biological imaging experiments were performed by Zeiss LSM 710 laser confocal fluorescence microscope.

Preparation for the experiment

The stock solution of HBTN (10 mM) was prepared using DMF. All analytical substances (HOCl, Cys, Hcy, GSH, Asp, Glu, Gly, His, Leu, Lys, Pro, Ser, Thr, Trp, Tyr, Val, Cu^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}, Fe^{3+}, Ac^{-}, SO_3^{2-}, SO_4^{2-}, $S_2O_3^{2-}$, S^{2-}, CO_3^{-}, Br^{-}, I^{-}, Cl^{-}, $ONOO^{-}$, H_2O_2, ·OH, $^1O_2$, O_2^{-}, NO_2^{-}, NO_3^{-}) were prepared in ultrapure water. Probe HBTN was used at a working concentration of 10 μM (0.1% DMF) for spectroscopic testing and bioimaging.

Synthesis of compound HBTOH

2-Hydroxy-5-methylbenzaldehyde (2.72 g, 20.0 mmol), 2-aminobenzenethiol (2.14 g, 20.0 mmol), sodium metabisulfite (3.80 g, 20.0 mmol) and 30 mL DMF were added to 100 mL single-necked flask, then refluxed for 1.5 h. After the reaction was completed, the reaction solution was cooled to room temperature. It was then added to 100 mL of secondary water, resulting in an obvious solid, and the crude product was obtained by suction filtration. Purification using column chromatography (eluent: petroleum ether/ethyl acetate = 1:1) gave compound HBT-OH (3.88 g, 80%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.37 (s, 1H), 8.12 (d, J = 7.9 Hz, 1H), 8.05 (d, J = 8.1 Hz, 1H), 7.96 (d, J = 1.2 Hz, 1H), 7.53 (ddd, J = 8.3, 7.2, 1.3 Hz, 1H), 7.43 (ddd, J = 8.2, 7.2, 1.2 Hz, 1H), 7.22 (dd, J = 8.4, 2.2 Hz, 1H), 6.98 (d, J = 8.3 Hz, 1H), 2.31 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.34, 154.19, 151.45, 134.22, 133.22, 128.39, 128.26, 126.42, 125.01, 122.03, 121.96, 117.89, 116.87, 20.02. HRMS (ESI): calcd for $C_{14}H_{10}NOS^-$ $[M - H]^-$ m/z, 240.0478; found, 240.0485.

Synthesis of compound HBTOF

A mixture of HBT-OH (3.52 g, 14.6 mmol), HMTA (5.12 g, 36.5 mmol) and trifluoroacetic acid (20 mL) was added to a 100 mL single-neck flask, and the mixture was refluxed at 80 °C for 24 h. After cooling to room temperature, a pale-yellow solid precipitated out as the pH was adjusted to neutrality with NaOH solution. The crude product was obtained by suction filtration. We purified it by column chromatography (eluent: dichloromethane) to obtain compound HBTOF (3.29 g, 84%) as a pale-yellow

solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.74 (s, 1H), 10.33 (s, 1H), 8.24 - 8.17 (m, 2H), 8.12 (dt, J = 8.1, 1.0 Hz, 1H), 7.73 (dd, J = 2.3, 0.9 Hz, 1H), 7.60 (ddd, J = 8.2, 7.2, 1.3 Hz, 1H), 7.51 (ddd, J = 8.3, 7.2, 1.2 Hz, 1H), 2.39 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 191.82, 165.17, 157.03, 150.99, 135.28, 133.56, 133.10, 129.31, 126.95, 125.84, 123.24, 122.32, 122.28, 119.12, 19.75. HRMS (ESI): calcd for $C_{15}H_{10}NO_2S^-$ [M - H]$^-$ m/z, 268.0427; found, 268.0439.

Synthesis of compound HBTDF

The HBTOF (1.00 g, 3.72 mmol) was dissolved in toluene (30 mL) and then (triphenylphosphoranylidene)acetaldehyde (1.25 g, 4.10 mmol) was added. After the mixture was reacted at 60 °C for 7 h, the solvent was removed. The solid was purified using column chromatography (eluent: dichloromethane) to give compound HBTDF (1.06 g, 96%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.09 (s, 1H), 9.72 (d, J = 7.8 Hz, 1H), 8.22 (d, J = 8.1 Hz, 1H), 8.13 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 16.1 Hz, 1H), 7.80 (dd, J = 13.4, 1.6 Hz, 2H), 7.61 (ddd, J = 8.3, 7.2, 1.3 Hz, 1H), 7.53 (ddd, J = 8.3, 7.2, 1.2 Hz, 1H), 6.97 (dd, J = 16.0, 7.8 Hz, 1H), 2.37 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.84, 168.27, 154.01, 150.82, 146.97, 132.78, 132.45, 131.65, 129.40, 129.19, 127.18, 126.12, 122.53, 122.43, 122.05, 117.11, 19.82. HRMS (ESI): calcd for $C_{17}H_{12}NO_2S^-$ [M - H]$^-$ m/z, 294.0583; found, 294.0595.

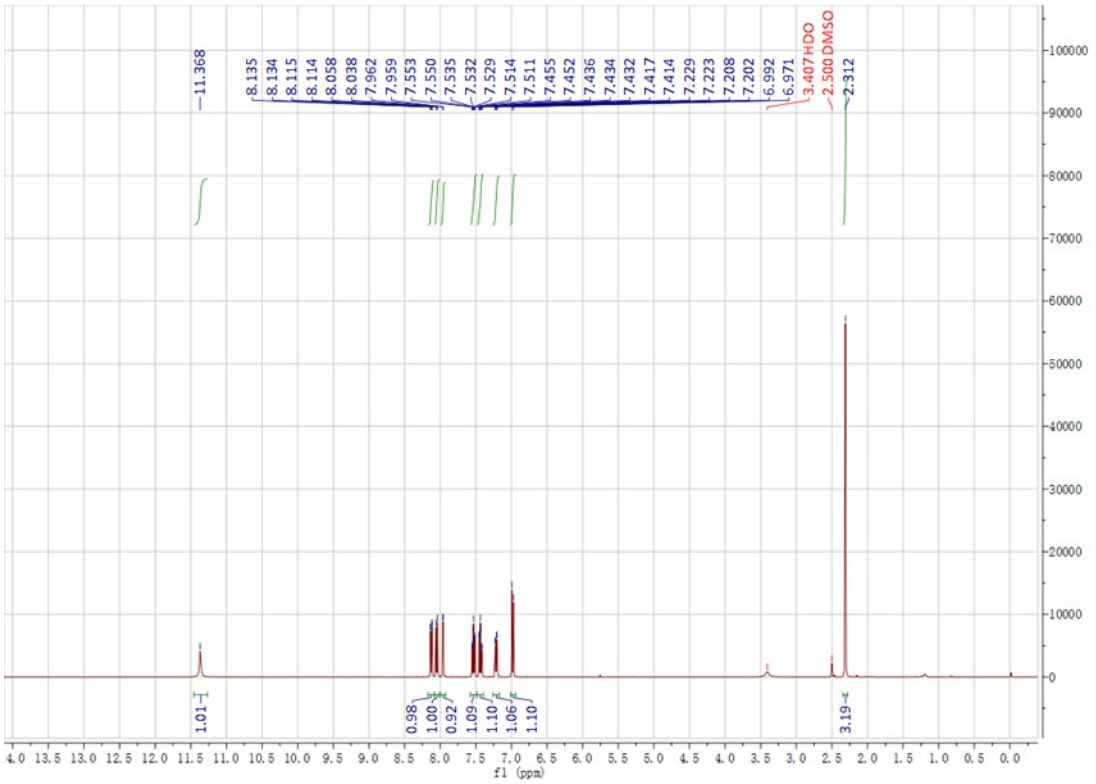

Figure S1. ^{1}H NMR (400 MHz) spectra of HBTOH in DMSO-d_6.

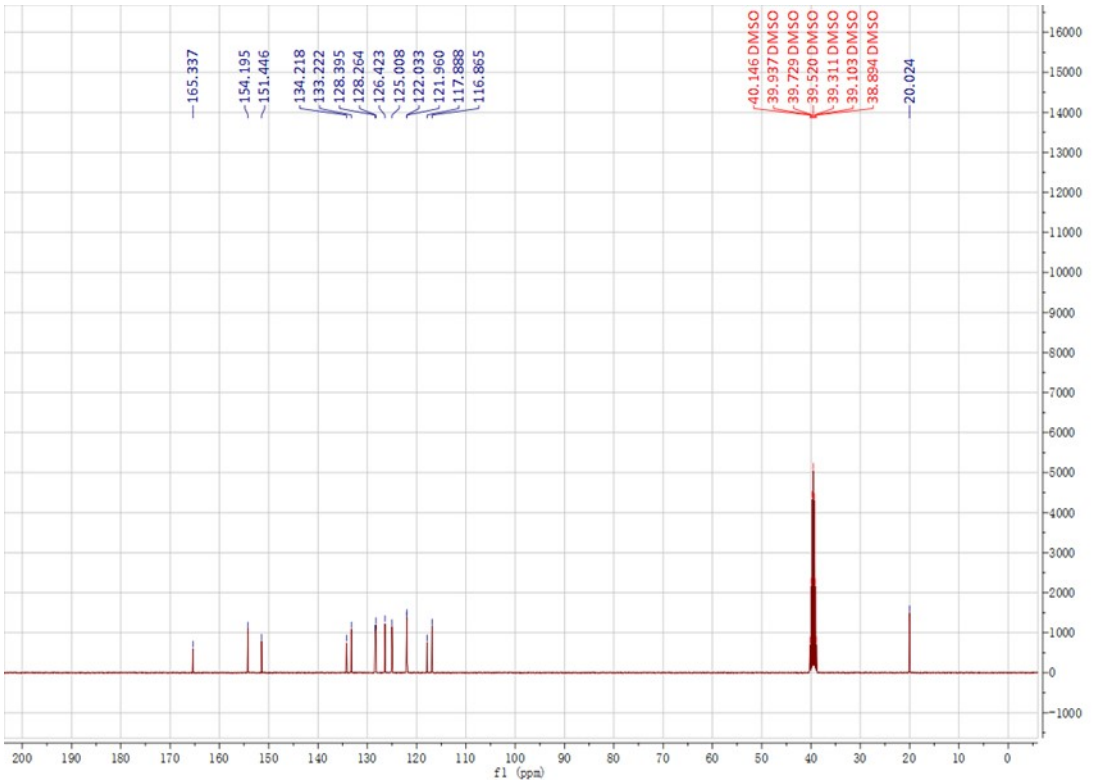

Figure S2. ^{13}C NMR (100 MHz) spectra of HBTOH in DMSO-d_6.

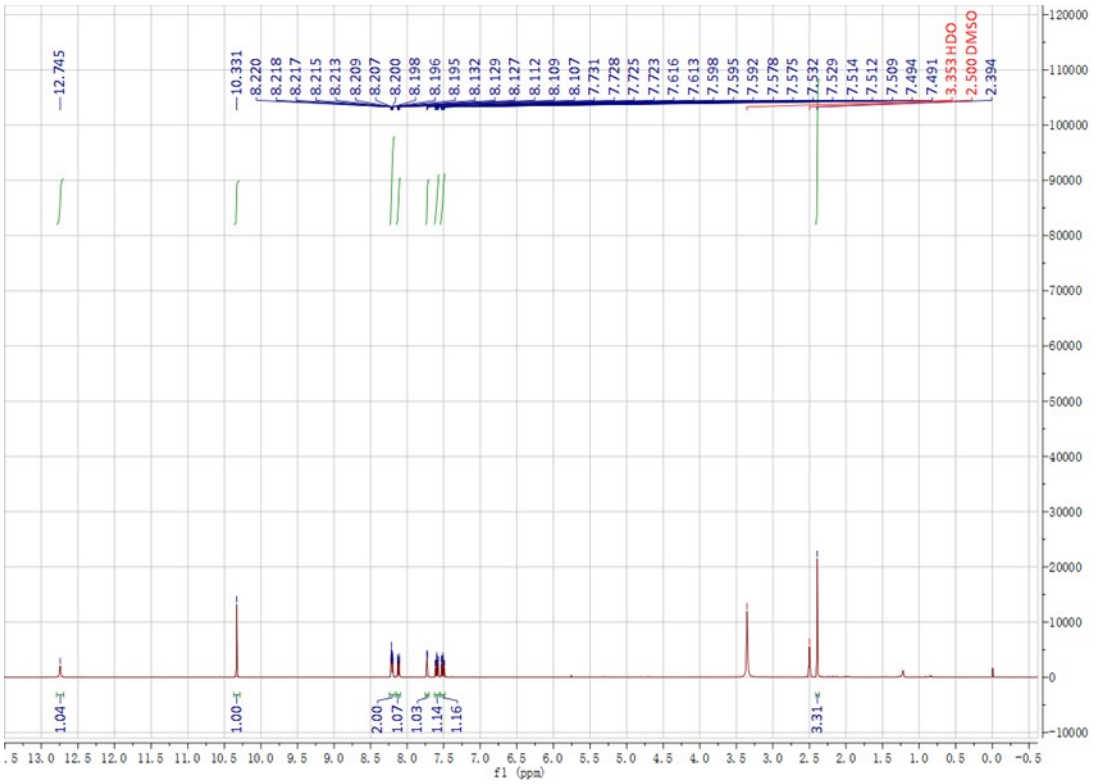

Figure S3. [1]H NMR (400 MHz) spectra of HBTOF in DMSO-d_6.

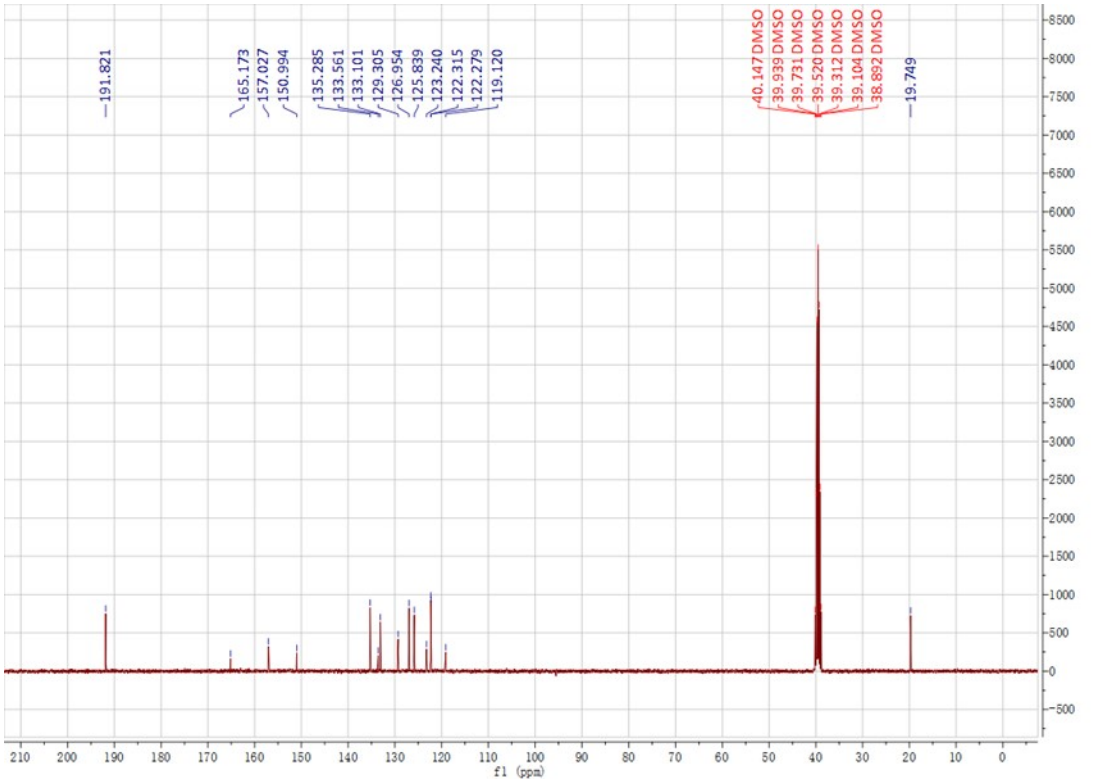

Figure S4. [13]C NMR (100 MHz) spectra of HBTOF in DMSO-d_6.

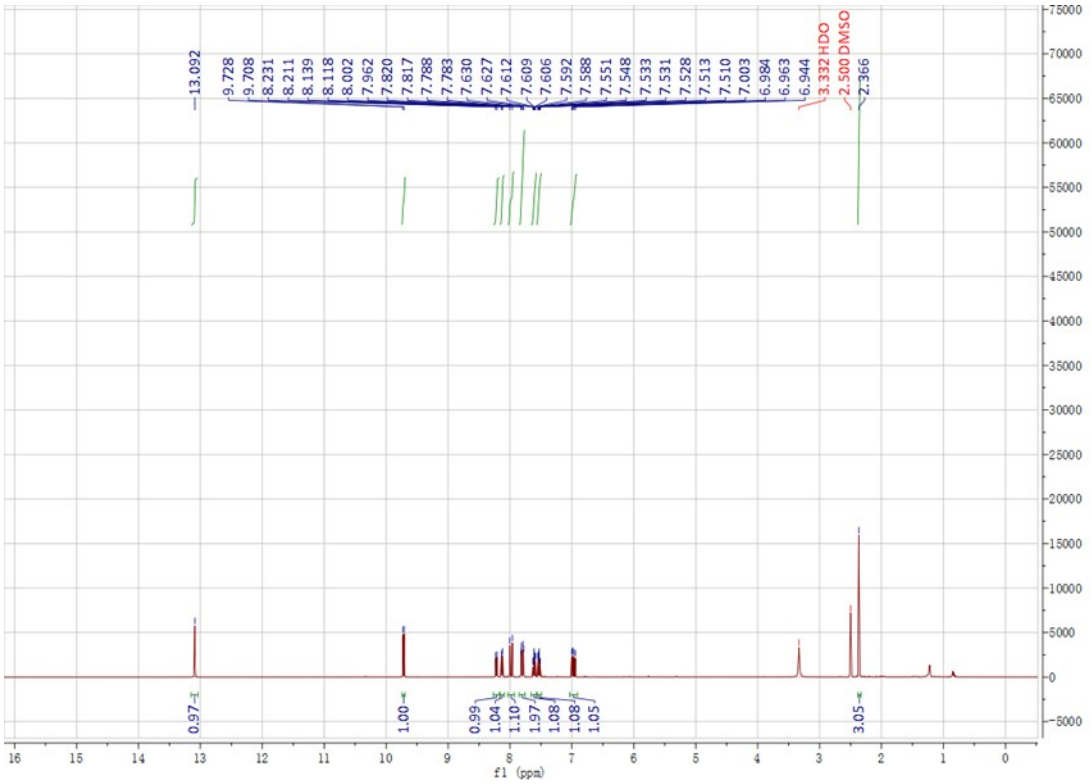

Figure S5. ^{1}H NMR (400 MHz) spectra of HBTDF in DMSO-d_6.

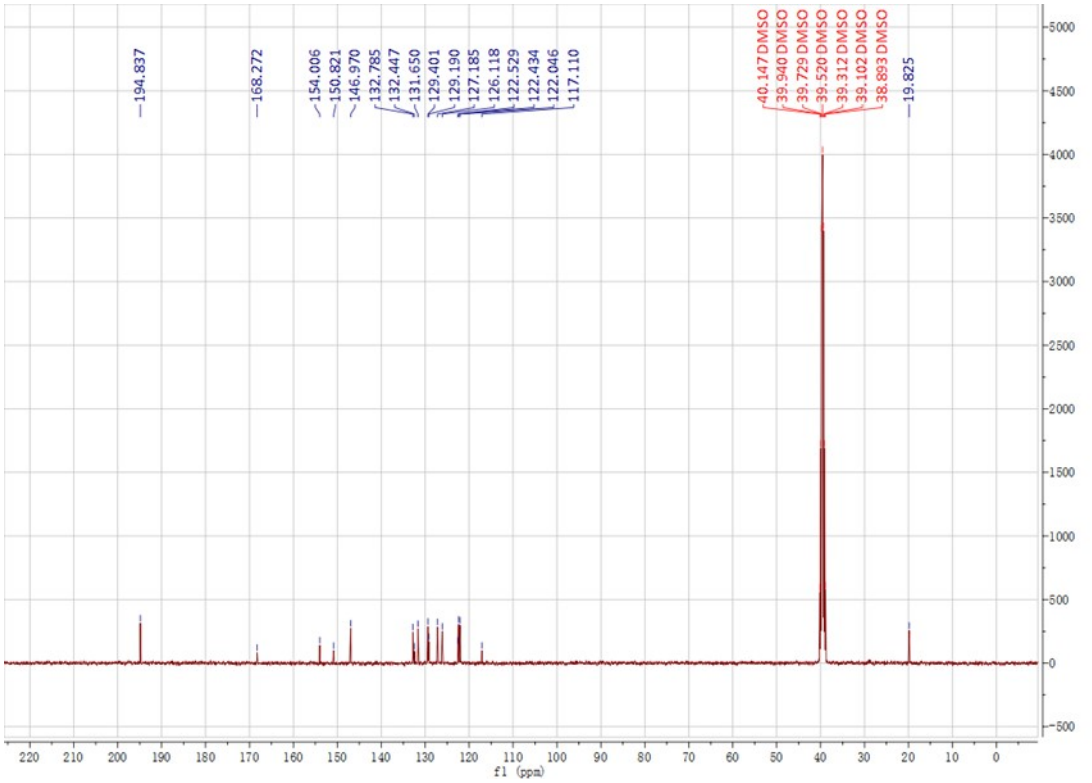

Figure S6. ^{13}C NMR (100 MHz) spectra of HBTDF in DMSO-d_6.

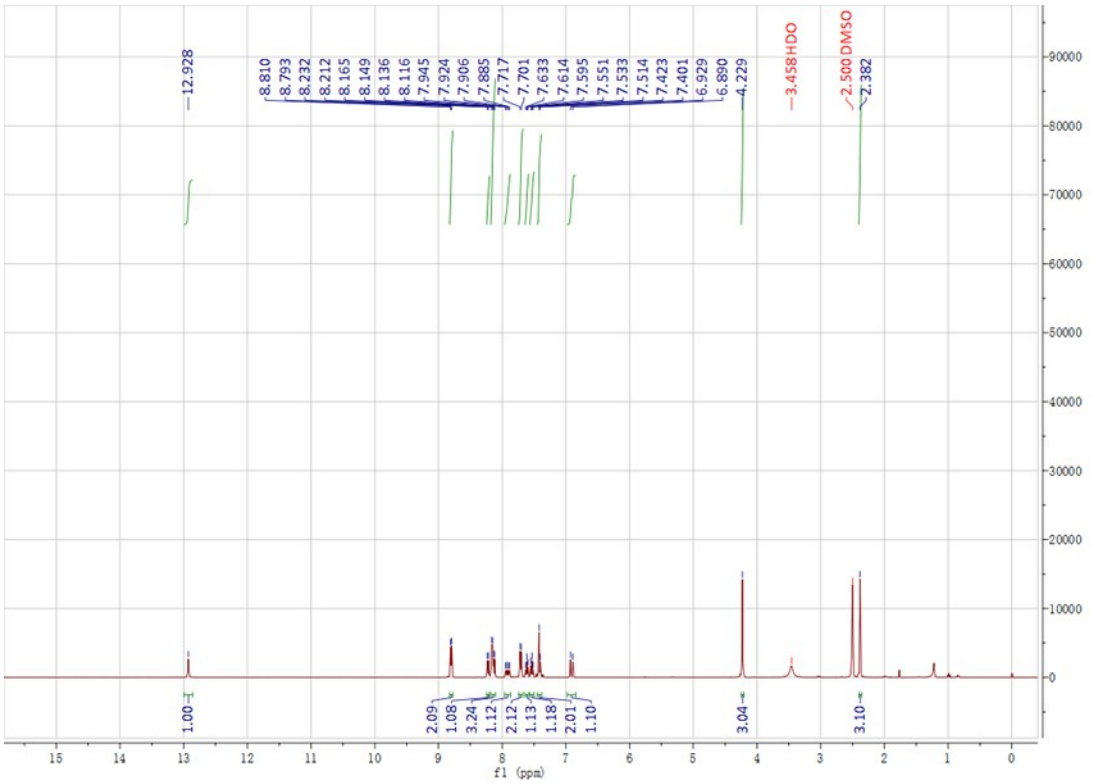

Figure S7. ^{1}H NMR (400 MHz) spectra of probe HBTN in DMSO-d_6.

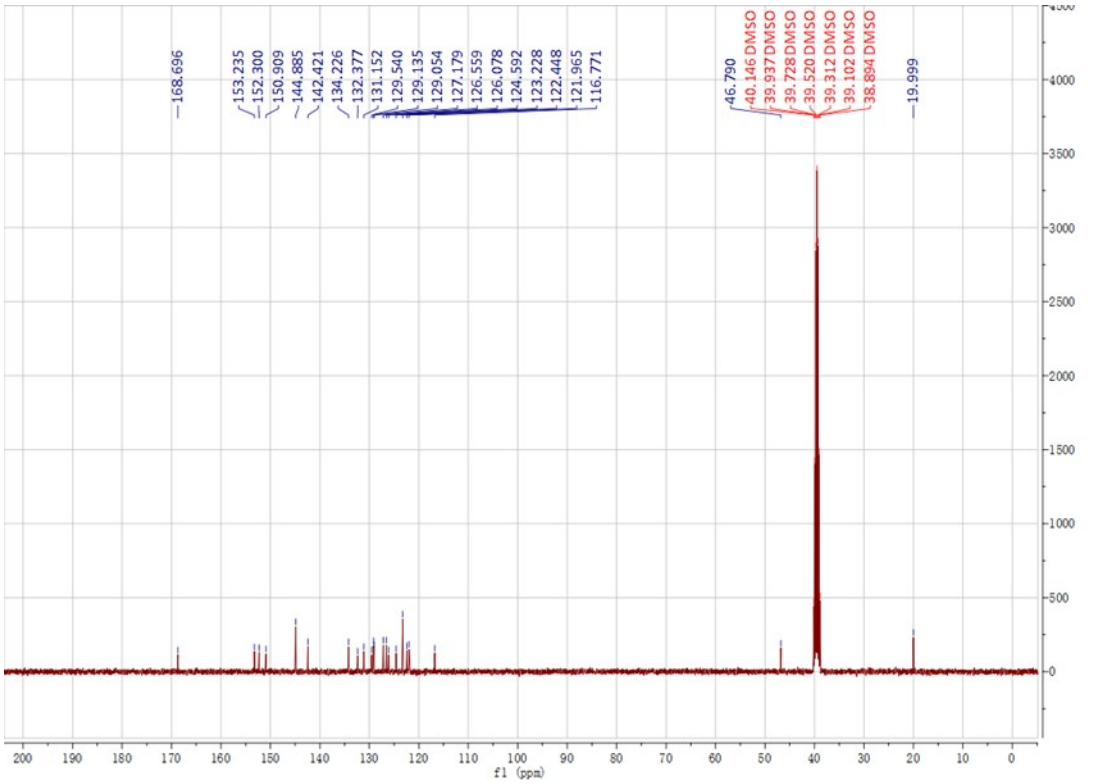

Figure S8. ^{13}C NMR (100 MHz) spectra of probe HBTN in DMSO-d_6.

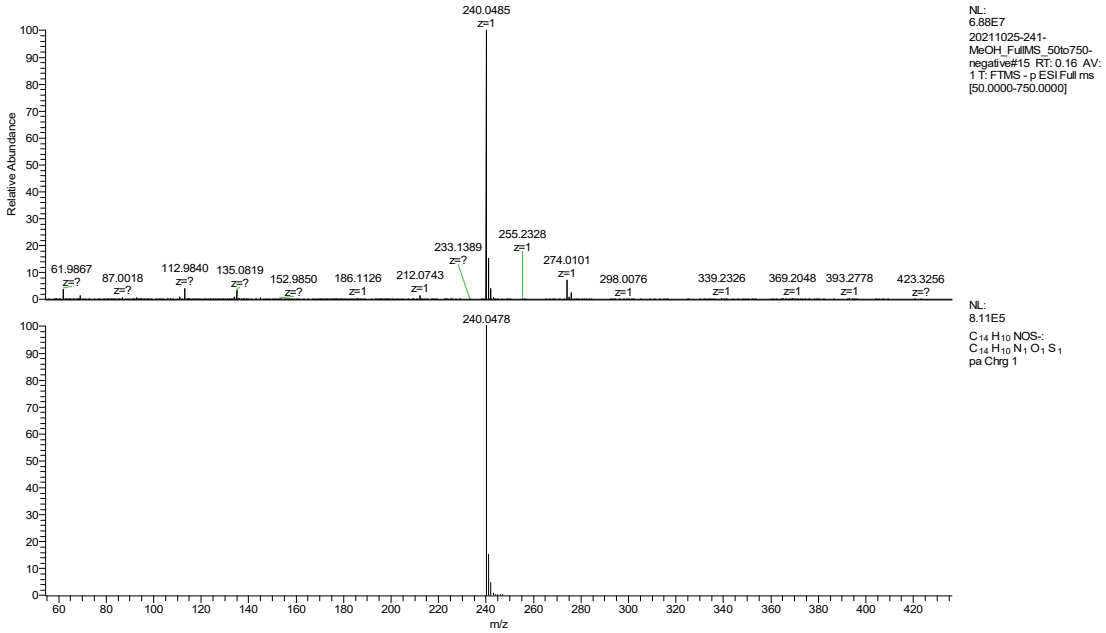

Figure S9. HRMS spectra of HBTOH.

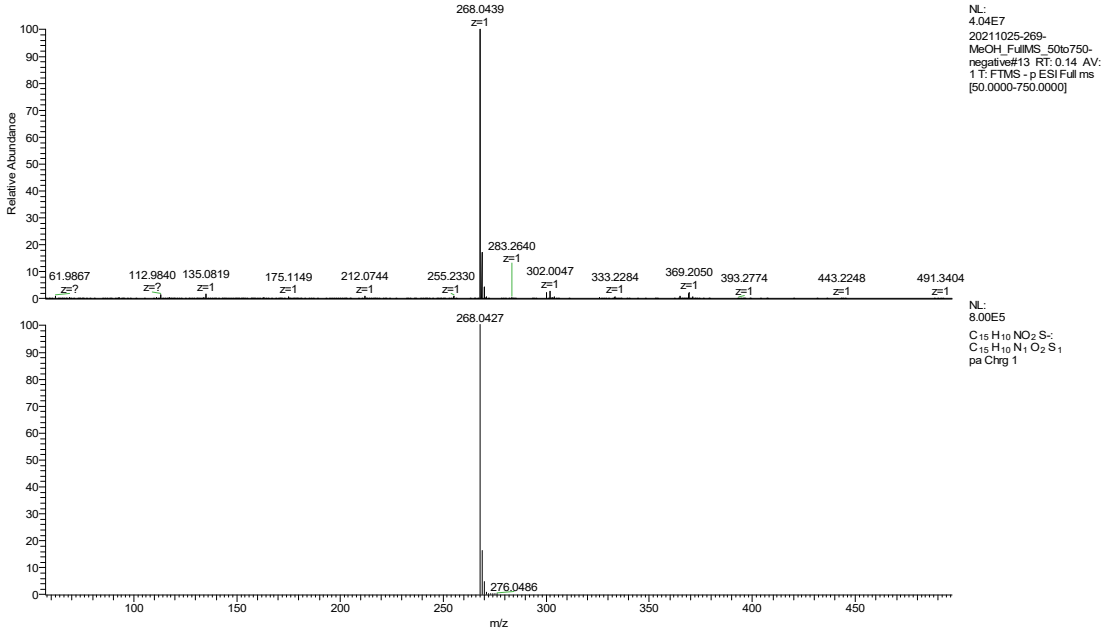

Figure S10. HRMS spectra of HBTOF.

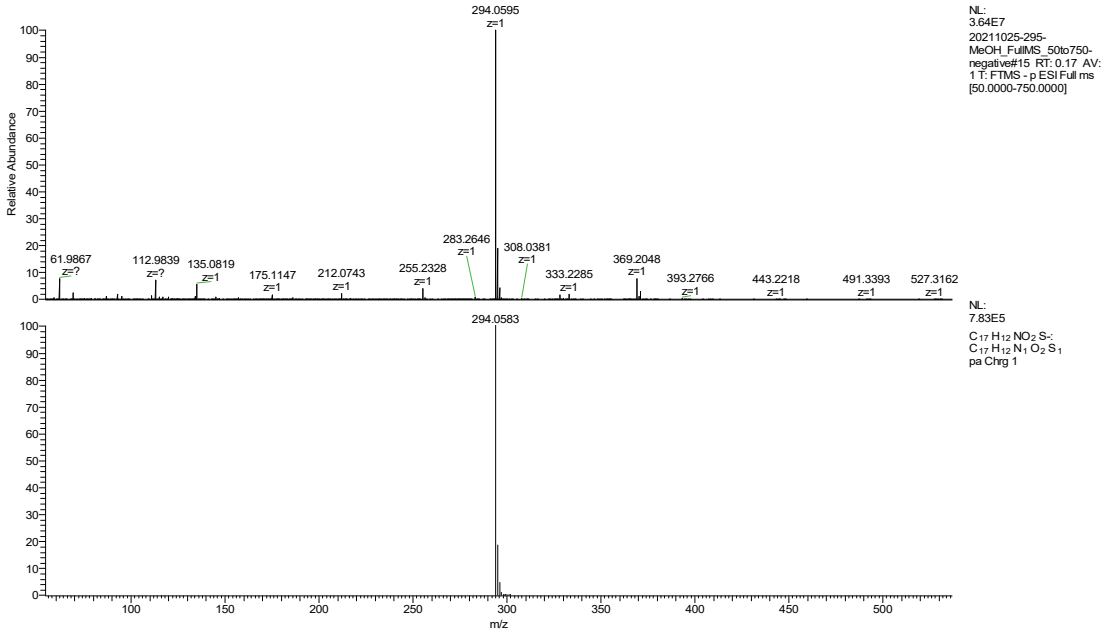

Figure S11. HRMS spectra of HBTDF.

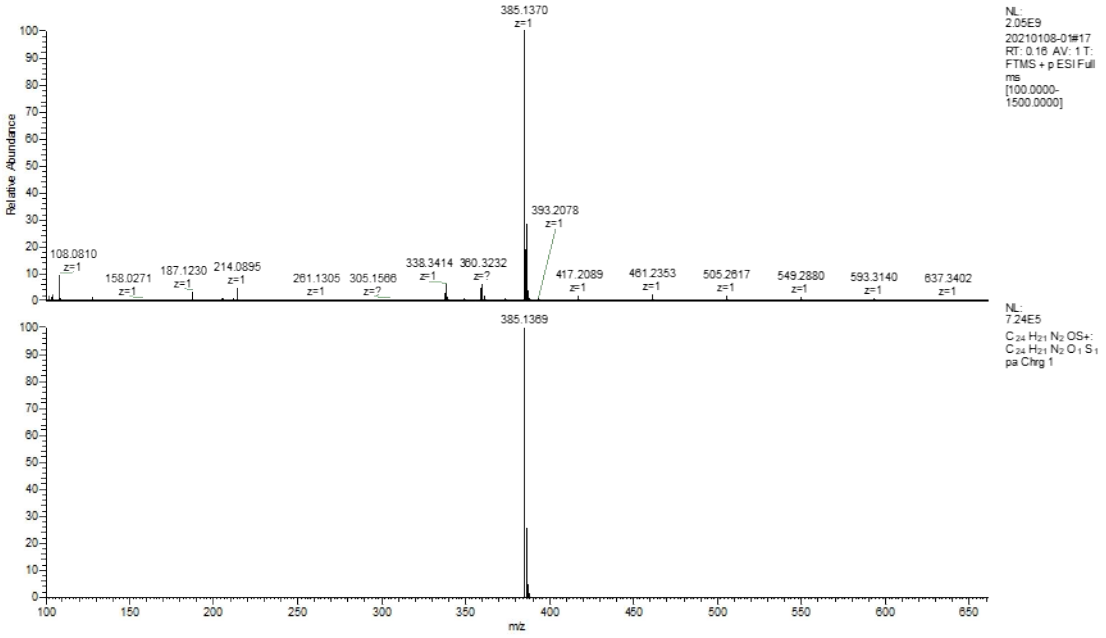

Figure S12. HRMS spectra of probe HBTN.

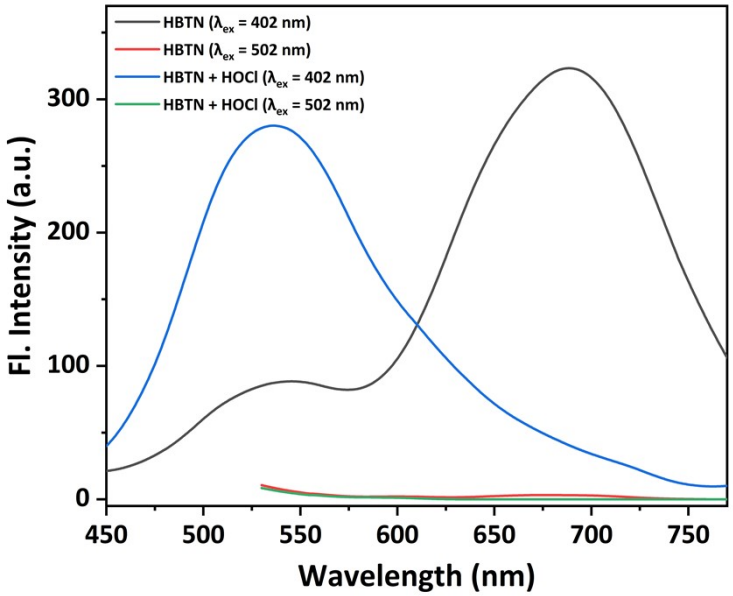

Figure S13. Fluorescence spectra of HBTN and HBTN + HOCl with excitation wavelengths of 402 nm and 502 nm, respectively.

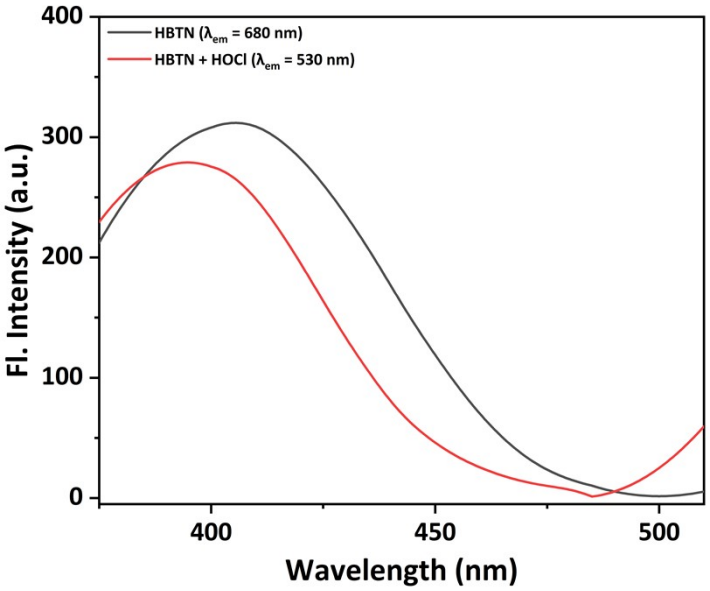

Figure S14. Excitation spectra of HBTN and HBTN + HOCl.

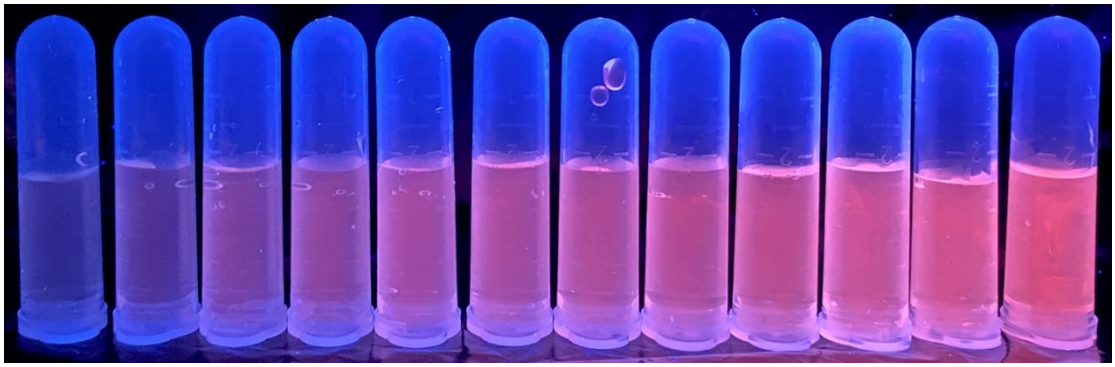

Figure S15. Photograph of the probe HBTN (10 μM) under 365 nm UV light in different viscosity system (water-glycerol mixed solvents: from left to right, $f_{glycerol}$ = 0, 20, 40, 50, 60, 70, 75, 80, 85, 90, 95, 100).

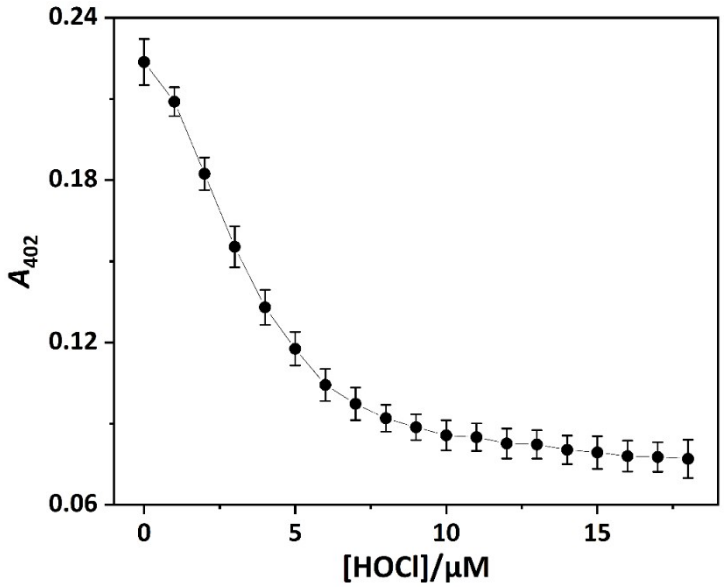

Figure S16. Absorbance (A_{402}) of HBTN (10 μM) upon different amounts of HOCl (0-18 μM).

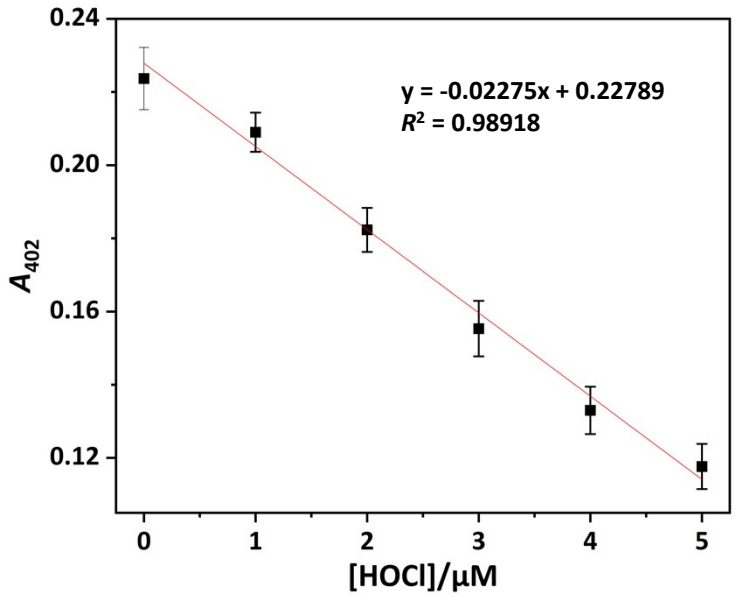

Figure S17. Linear relationship of absorption intensity at 402 nm and HOCl concentration (0-5 μM).

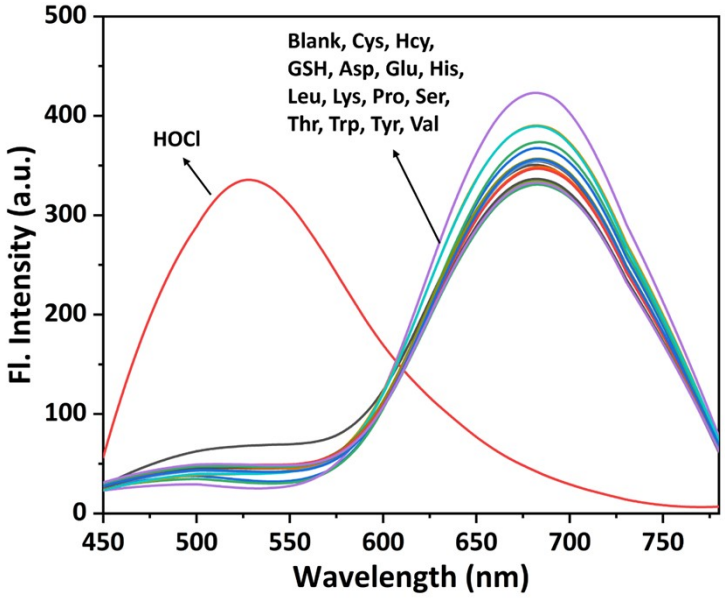

Figure S18. Fluorescence spectra of HBTN (10 μM) in the presence of HOCl (20 μM) or amino acid (500 μM).

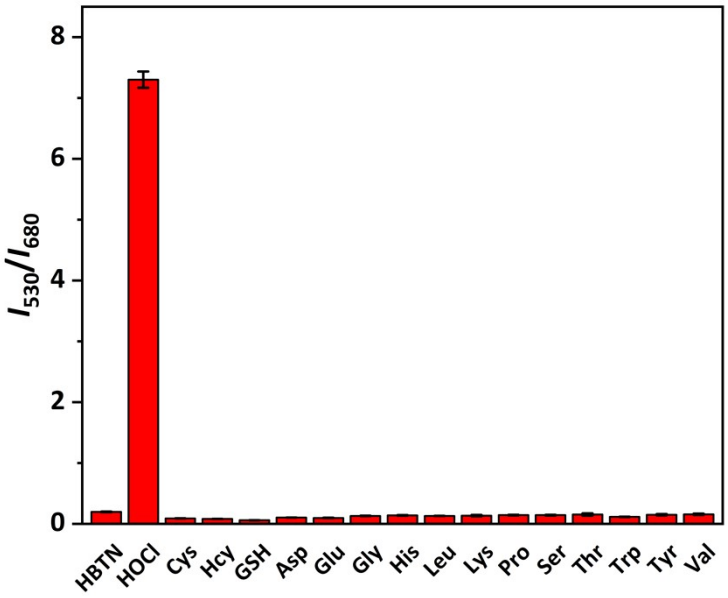

Figure S19. Ratio of fluorescence intensities (I_{530}/I_{680}) of HBTN (10 μM) in the presence of HOCl (20 μM) or amino acid (500 μM).

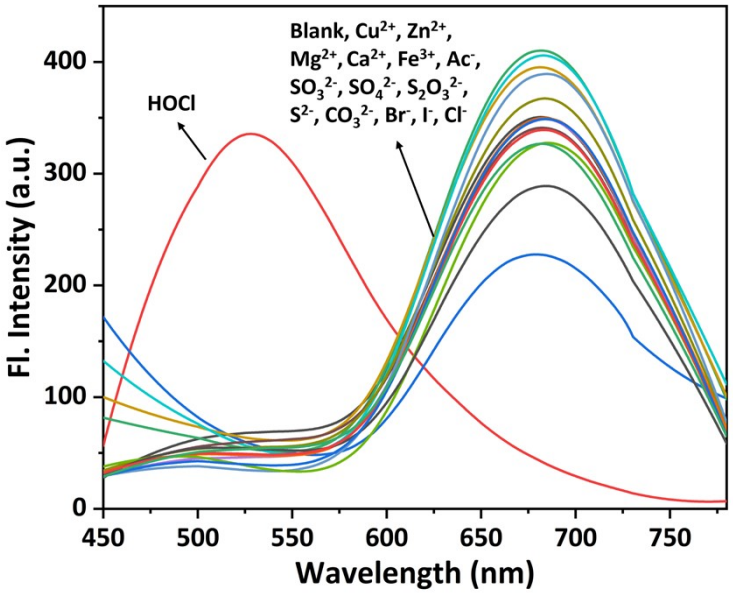

Figure S20. Fluorescence spectra of HBTN (10 μM) in the presence of HOCl (20 μM), metal ion (200 μM) or acid anion (200 μM).

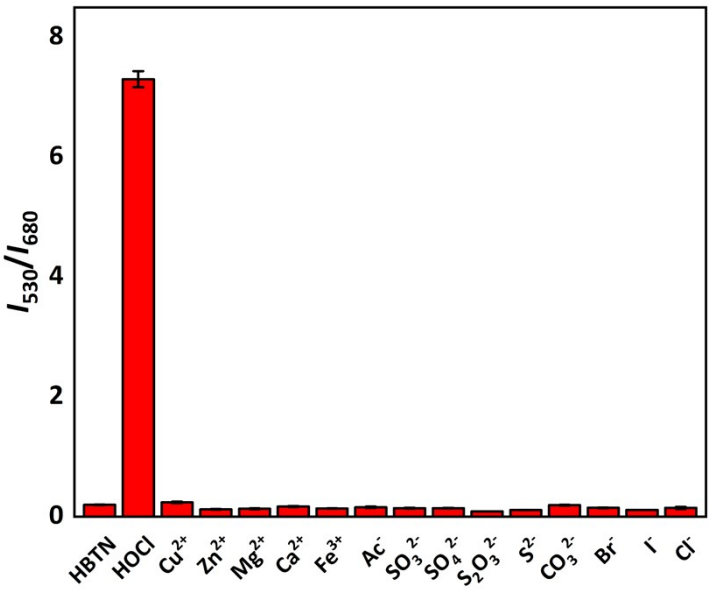

Figure S21. Ratio of fluorescence intensities (I_{530}/I_{680}) of HBTN (10 μM) in the presence of HOCl (20 μM), metal ion (200 μM) or acid anion (200 μM).

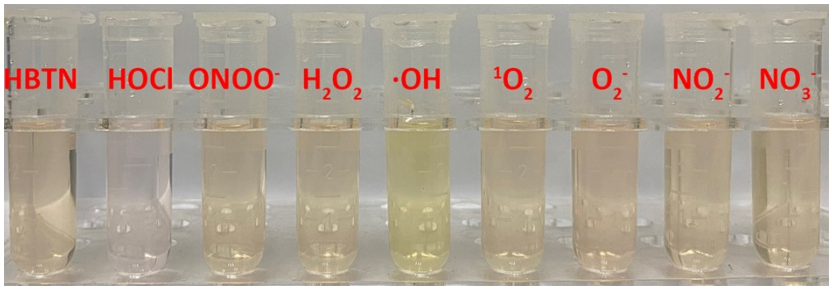

Figure S22. Photograph under visible light of HBTN (10 μM) in the presence of HOCl (20 μM)

or other ROS (200 μM).

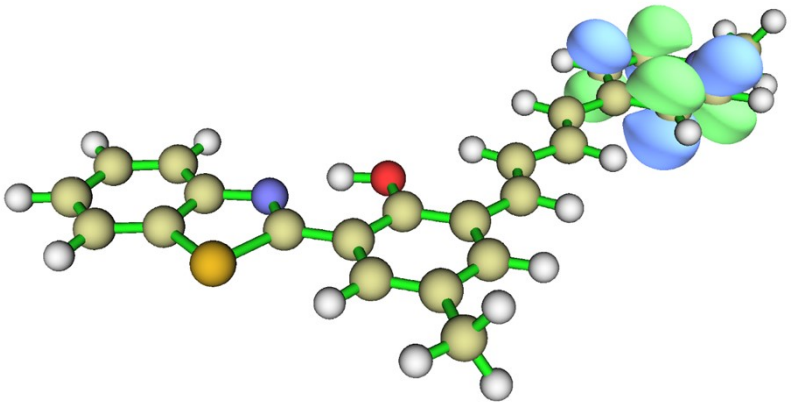

Figure S23. LUMO+1 of HBTN.

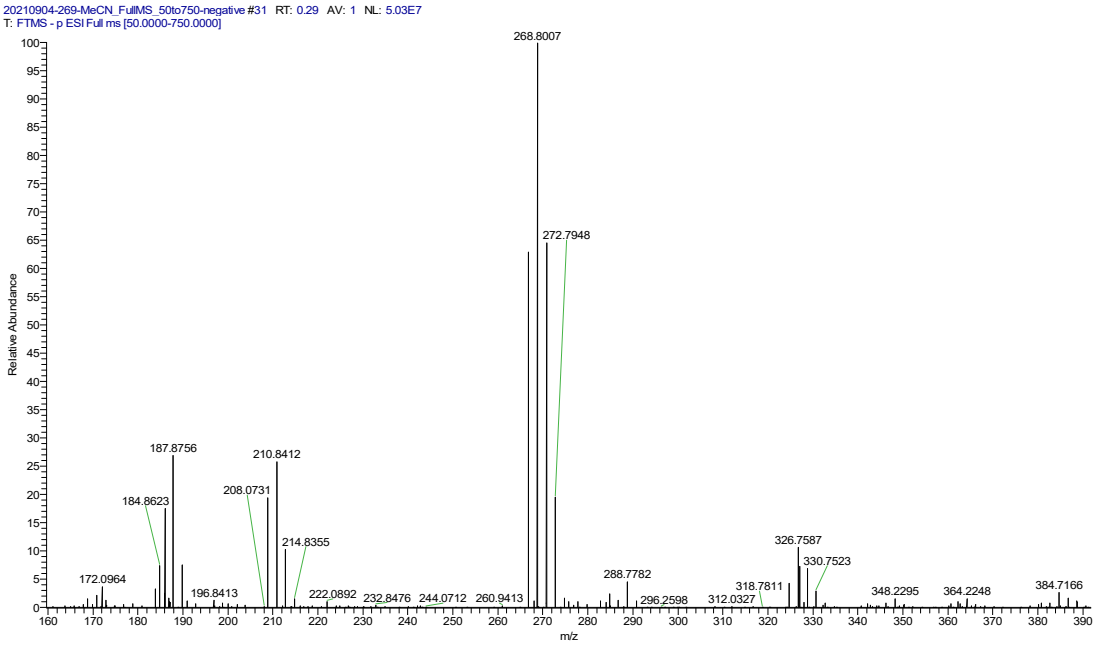

Figure S24. HRMS spectra of HBTN in the presence of HOCl.

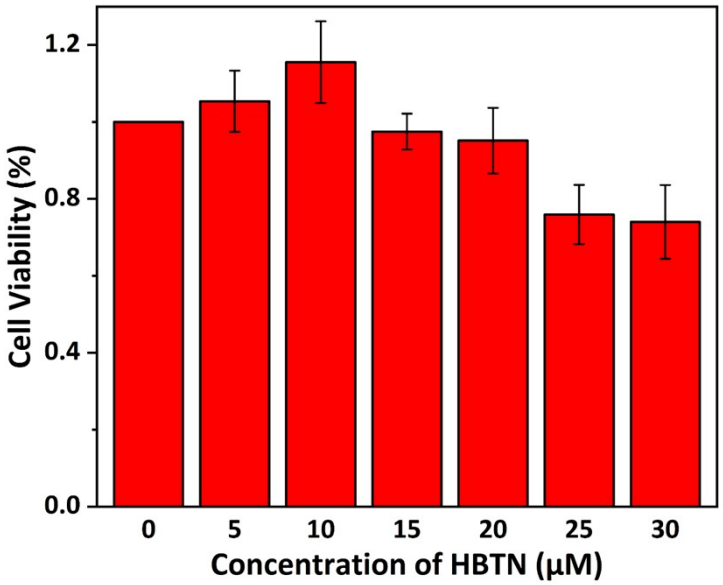

Figure S25. Cell viability of MCF-7 cells treated with different concentrations of HBTN (0, 5, 10, 15, 20, 25 and 30 μM) for 12 h, The cell viability was observed via MTT assay.

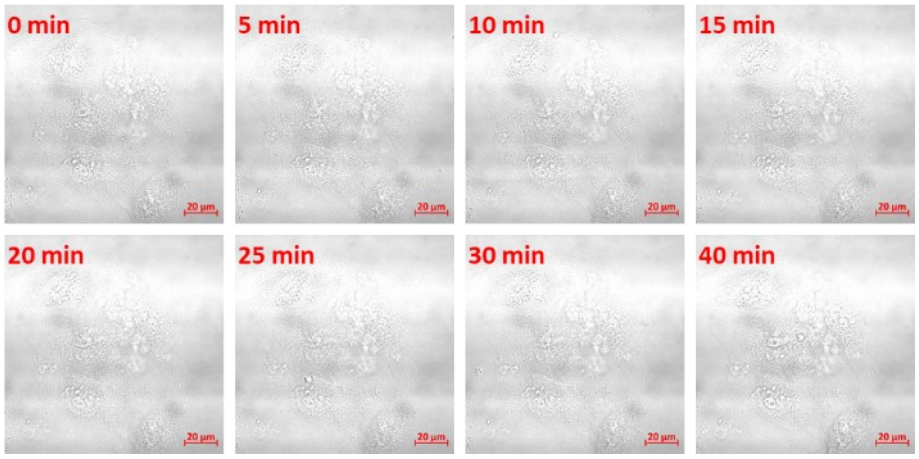

Figure S26. Brightfield images of MCF-7 cells irradiated with 405 nm laser light for different durations.

Table S1. The photophysical properties of HBTN, HBTN + glycerol and HBTN + HOCl.

Samples	λmax abs(nm)	ε($M^{-1}\cdot cm^{-1}$)	λmax em(nm)	$\Delta\lambda$(nm)	Φ_F	B($M^{-1}\cdot cm^{-1}$)	τ(ns)
HBTN	402	22525	680	278	0.0460	1036	5.465
HBTN + glycerol (100%)	419	24400	680	261	0.1943	4741	8.839

HBTN + HOCl	288	14915	530	242	0.0324	483	4.421

λmax abs: Maximum absorbance wavelength. ε: Molar extinction coefficient. λmax em: Maximum emission wavelength. $\Delta\lambda$: Stokes shift. Φ_F: Fluorescence quantum yield. B: Brightness. The brightness was calculated using $B = \varepsilon \times \Phi_F$. τ: Fluorescence lifetime. Fluorescence quantum yield and fluorescence lifetime were tested by Edinburgh FLS980 steady-state transient fluorescence spectrometer.

Table S2. Comparison of fluorescent probe for viscosity and HOCl.

Probes	Emission peak	Stokes shift	Subcellular localization	Bioimaging	Reference
HBTN	680 nm/530 nm	278 nm	Mitochondrial targeting	MCF-7 cells and zebrafish	This work
Lyso-VH	580 nm/500 nm	144 nm	Lysosomal targeting	A549 cells and mouse	*Anal. Chem.*, 2022, **94**, 12144-12151
TPP-AN	414 nm/600 nm	230 nm	Mitochondrial targeting	HeLa cells	*Talanta*, 2022, **241**, 123235
CBRV	571 nm/710 nm	225 nm	No	HeLa cells	*Spectrochim. Acta Part A*, 2021, **246**, 119059
PTZ-TPP/ PTZ-MP	610 nm/580 nm/500 nm	125 nm	No	INS-1 β-islet cells	*J. Mol. Struct.*, 2021, **1227**, 129523
BDP-CY	600 nm/510 nm	48 nm	No	MCF-7 cells	*Dyes Pigments*, 2016, **125**, 89-94