

## *Electronic Supplementary Information*

### A novel ratiometric fluorescent probe for selectively determining HClO based on ESIPT mechanism and its application in real samples

Jingrui Li<sup>a,b</sup>, Aijun Gong<sup>a,b\*</sup>, Guoqing Shi<sup>a</sup>, Chengwen Chai<sup>a</sup>

<sup>a</sup>School of chemistry and biological engineering, University of Science and Technology Beijing, Beijing 100083, China. <sup>b</sup>Beijing Key Laboratory for Science and Application of Functional Molecular and Crystalline Materials, University of Science and Technology Beijing, Beijing 100083, China.

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## 1. ROS preparation

The other analytes with oxidizing properties including  $\cdot\text{OH}$ ,  $^1\text{O}_2$ ,  $\text{Fe}^{3+}$ ,  $\text{H}_2\text{O}_2$ ,  $\text{ONOO}^-$ ,  $t\text{-BuOOH}$ ,  $\text{NO}$ , and  $\text{HClO}$  were prepared according to the following methods in literature. The species of  $\cdot\text{OH}$  was generated in the Fenton system consisting of ferrous ammonium sulfate and hydrogen peroxide<sup>1</sup>. The species of  $^1\text{O}_2$  was prepared through adding  $\text{NaOCl}$  into the solution of  $\text{H}_2\text{O}_2$ <sup>2</sup>.  $\text{H}_2\text{O}_2$  solution was prepared through diluting the commercial  $\text{H}_2\text{O}_2$  solution. The exact concentration of  $\text{H}_2\text{O}_2$  was determined based on the molar extinction coefficient of  $\text{H}_2\text{O}_2$  at 240 nm ( $43.6 \text{ M}^{-1} \text{ cm}^{-1}$ ). The species of  $\text{ONOO}^-$  was obtained by using 3-morpholinopyrrolidine as a donor<sup>3</sup>.  $t\text{-BuOOH}$  was obtained commercially from Alfa Aesar.  $\text{NO}$  was generated by using sodium nitroferricyanide(III)dihydrate as a donor. The source of  $\text{Fe}^{3+}$  was obtained from the solution of  $\text{FeCl}_3$ . The stock solution of  $\text{HClO}$  was prepared by diluting a commercial  $\text{NaOCl}$  solution. The concentration of  $\text{HClO}$  was determined based on the molar extinction coefficient of  $\text{HClO}$  at 292 nm ( $350 \text{ M}^{-1} \text{ cm}^{-1}$ ).

## 2. HPLC analysis for detection mechanism

HPLC analysis was performed on a Shimadzu UFLC system (Shimadzu, Kyoto, Japan) consisting of two LC-20AD pumps, an SPD-M20A diode-array detector, a CTO-20A oven, and an SIL-20A auto sampler. The detection wavelengths were 345 nm. The mobile phase was water-methanol (gradient from 5% to 90% in 12 min). The flow rate was 1.0 mL/min. A Dikma Diamonsil  $\text{C}_{18}$  column (250 mm $\times$ 4.6 mm, 5  $\mu\text{m}$ , Dikma Technologies Inc, Beijing, China) was used throughout.

## 3. Optical properties of the probe

Table S1. Linear equation of the probe toward to  $\text{HClO}$

Entry	Equation	$R^2$	Detection limit/nM
The probe	$Y=0.109 X + 0.086$	0.996	14.6

Table S2. Fluorescent properties of the compounds and the probe

Entry	QY %	$\lambda_{\text{em}}$ nm	$\lambda_{\text{ex}}$ nm	$\epsilon$ $10^4 \text{ M}^{-1} \text{ cm}^{-1}$
The probe	0.11	600	340	2.71/1.89
The probe + $\text{HClO}$	0.34	485/600	340	3.52/2.43

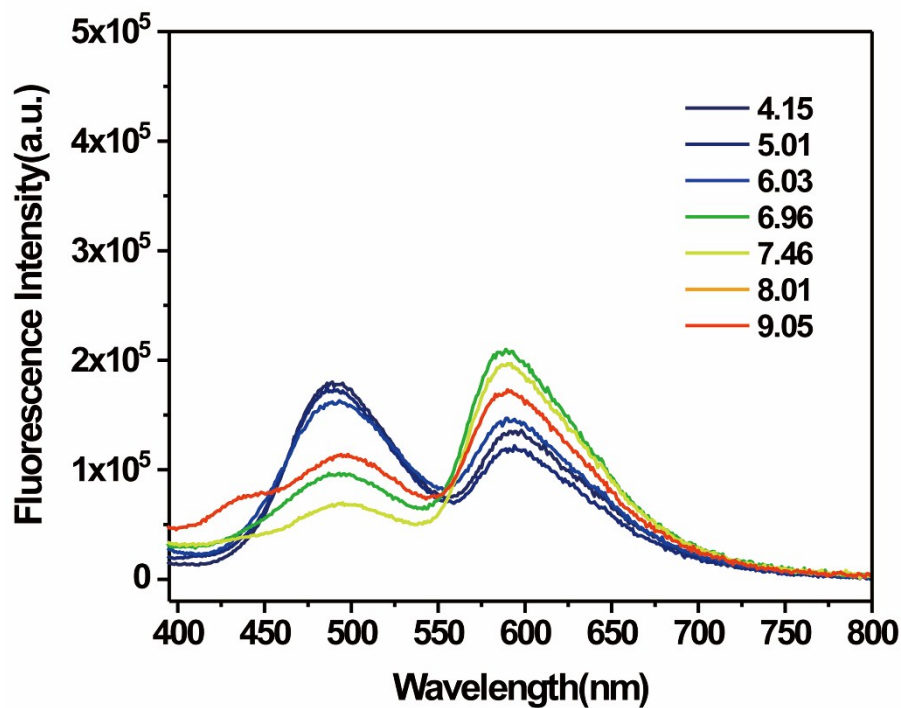


Figure S1. Effects of pH on the fluorescence of the probe (20  $\mu\text{M}$ ) reacting with HClO (10 $\mu\text{M}$ ) for 30 s in PBS/MeOH(v/v, 4:1, pH=7.4, 10mM).  $\lambda_{\text{ex}}$ =340, slit width: 2/8 nm.

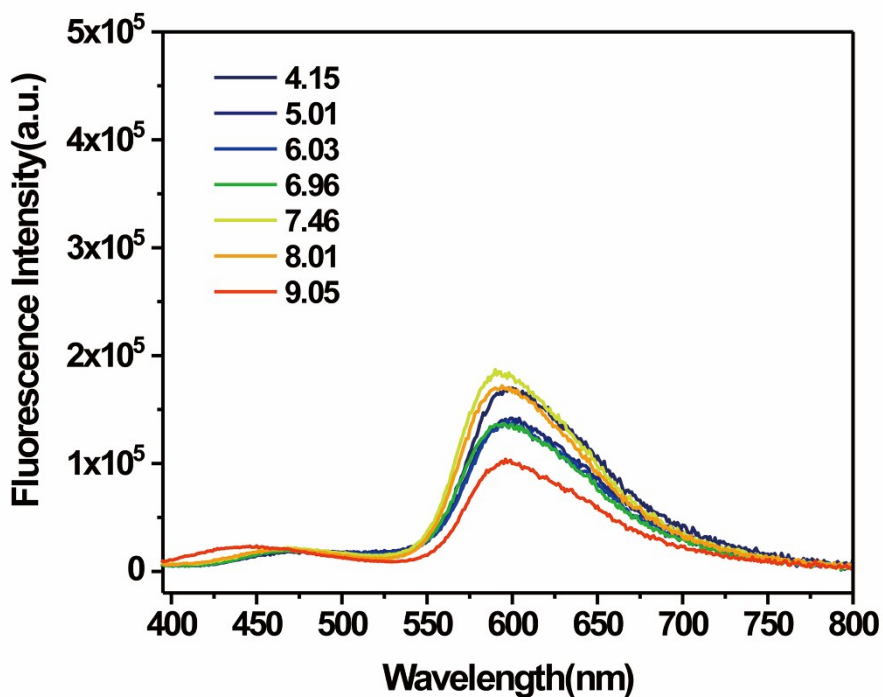


Figure S2. Fluorescent spectrum of the probe (20  $\mu\text{M}$ ) in PBS/MeOH(v/v, 4:1, pH=7.4, 10mM) with different pH value.  $\lambda_{\text{ex}}$ =340, slit width: 2/8 nm.

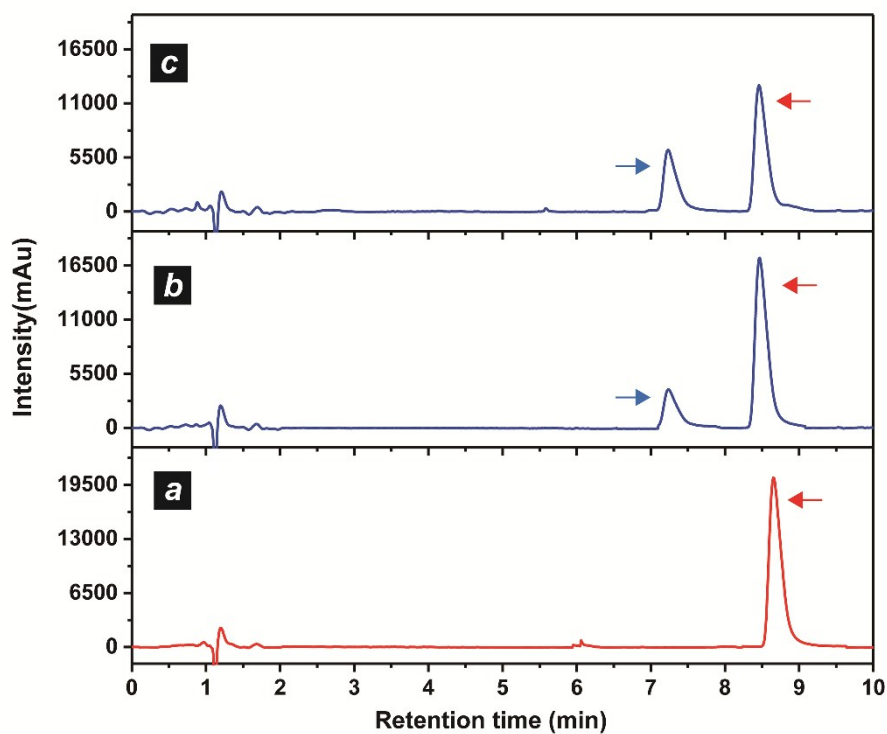


Figure S3. The HPLC chromatography of the probe (a, 30 $\mu$ M, marked with red arrow) treated with HClO (b, 15 $\mu$ M; c, 45 $\mu$ M; new product was marked with blue arrow)

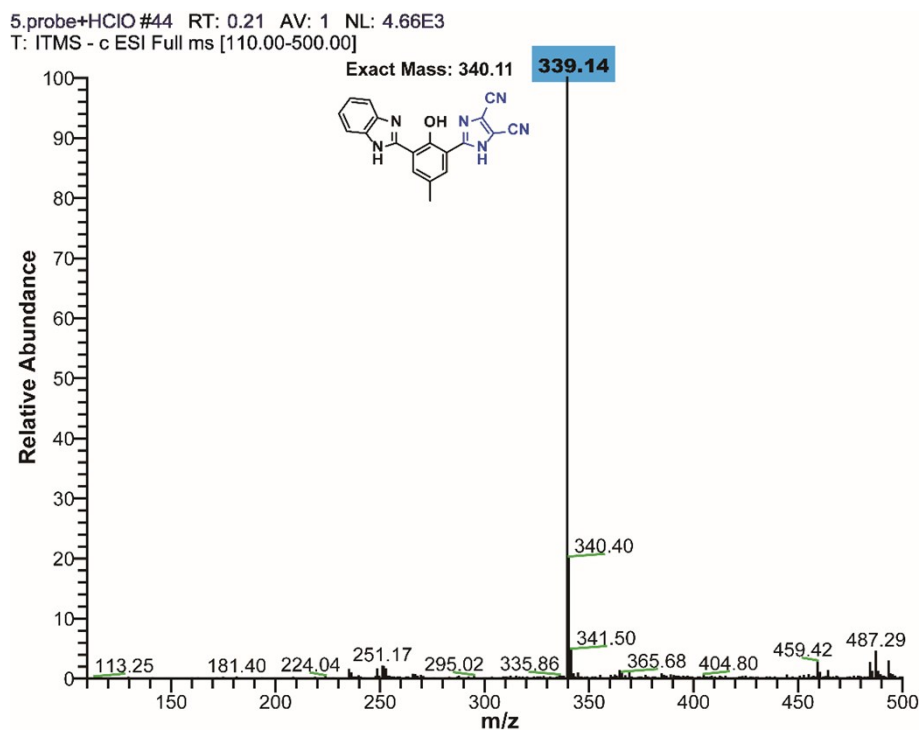


Figure S4. The mass spectrum of the new species at retention time 7.25 min

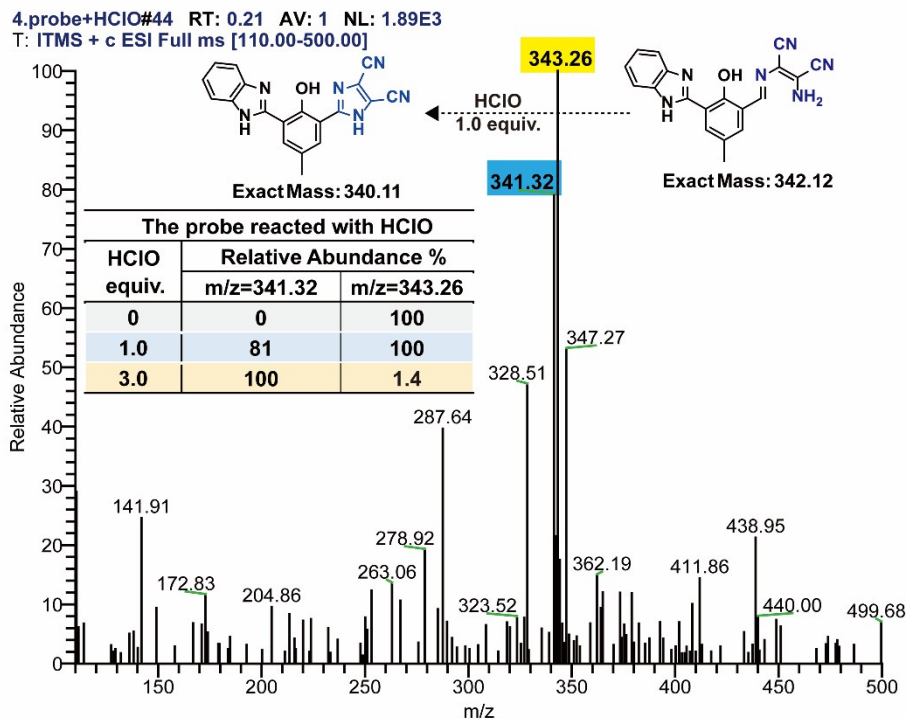
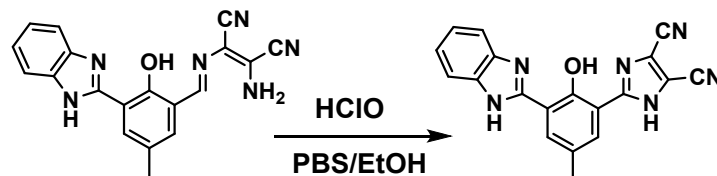


Figure S5. The mass spectrum of the probe (10 $\mu$ M) treated with HClO (10 $\mu$ M)

#### 4. Purification of the product generated from the probe



Scheme S1. Synthesis of the product generated from the probe

To a solution of the probe (50 mg) in 20 mL of PBS buffer (10mM, pH7.4, containing 10% ethanol) was added the solution of HClO. TLC monitored the reaction process. The mixture was stirred at room temperature for 10 min and poured into 20 mL of water. Then, 10 mL of CH<sub>2</sub>Cl<sub>2</sub> was added to extract the final products. Repeat the above procedure twice to collect the organic phase. The obtained organic phase of CH<sub>2</sub>Cl<sub>2</sub> was isolated and concentrated to 1mL. The residue was purified by preparative SiO<sub>2</sub> plates using petroleum ether-ethyl acetate (4:1, v/v) as an eluent to give a yellow solid (5.2 mg). The obtained products were characterized by <sup>1</sup>H NMR.

## 5. Comparison the $^1\text{H}$ NMR spectra of the product and the probe

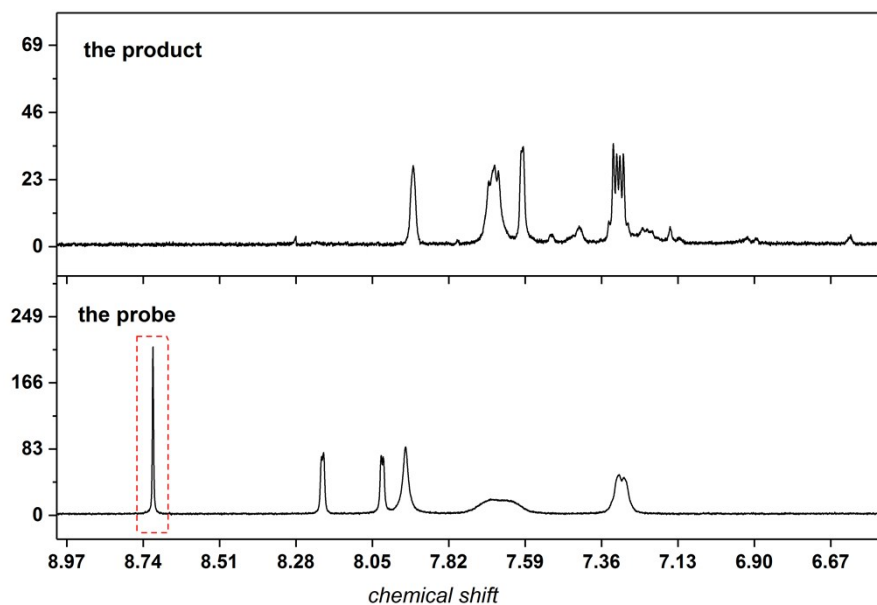


Figure S6 the  $^1\text{H}$ NMR spectra of the product (top, in  $\text{DMSO-}d_6$ , 300 MHz) and the probe (down, in  $\text{CDCl}_3$ , 300 MHz)

## 6. Characterization of the compounds

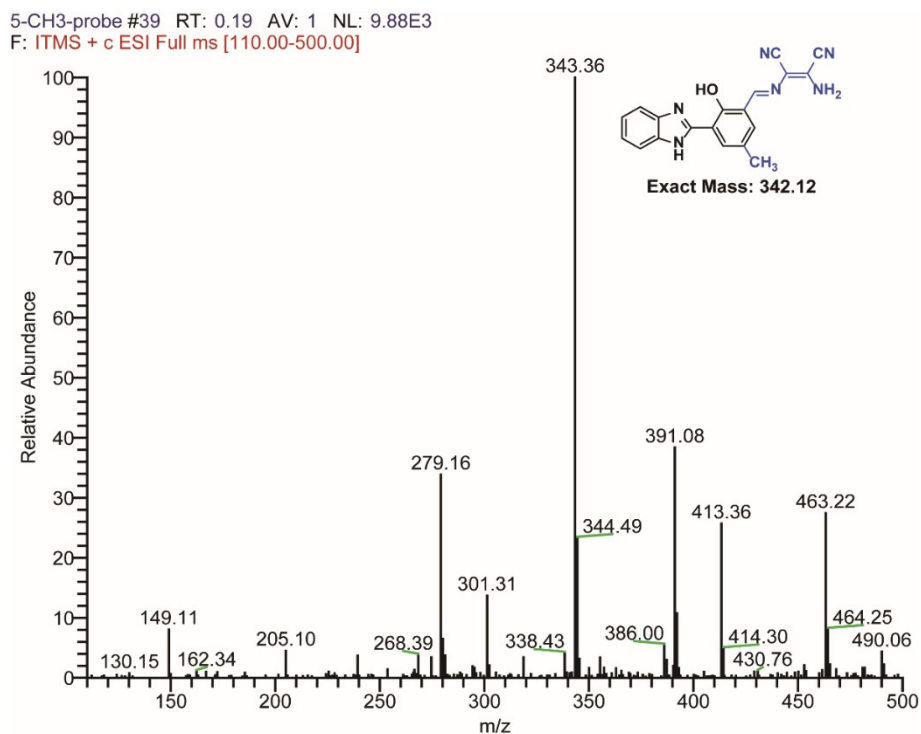


Figure S7. MS spectra of the probe 1

5-CH3 #39 RT: 0.19 AV: 1 NL: 4.84E4  
F: ITMS + c ESI Full ms [110.00-500.00]

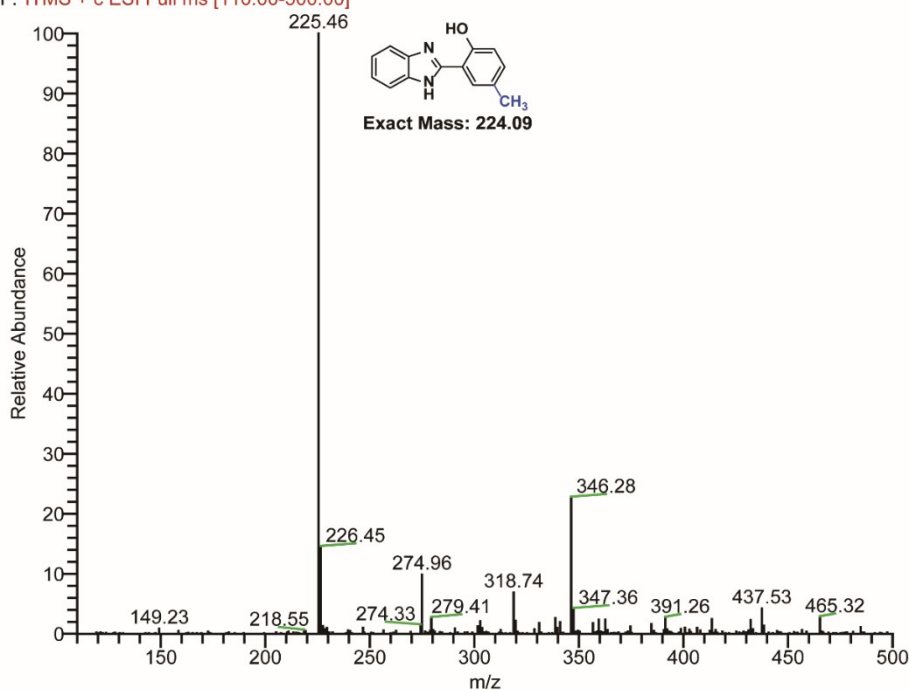


Figure S8. MS spectra of the 5-CH<sub>3</sub>-HBI

5-CH3-CHO #35 RT: 0.17 AV: 1 NL: 3.36E4  
F: ITMS + c ESI Full ms [110.00-500.00]

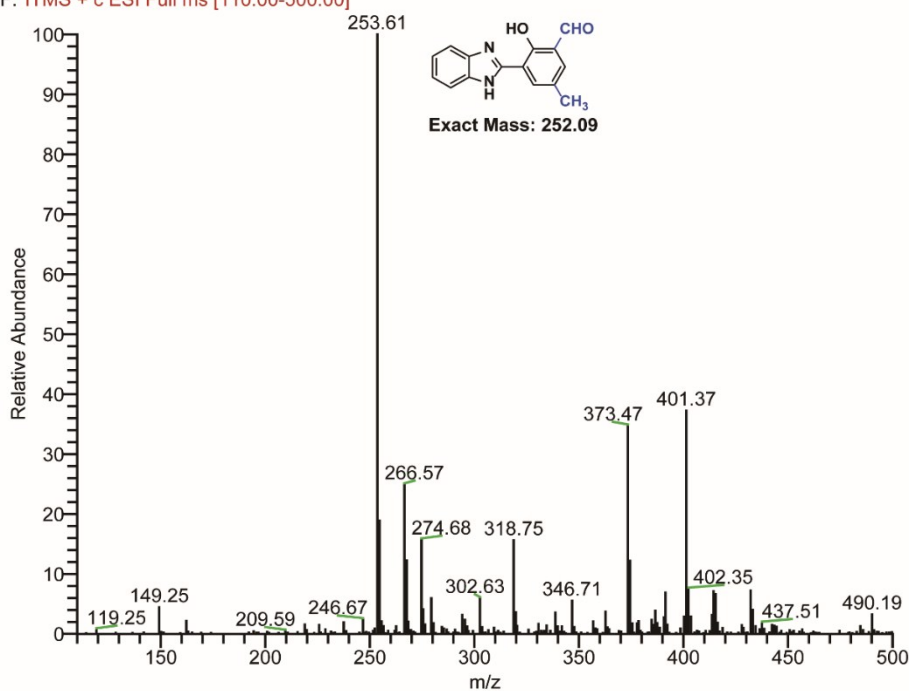


Figure S9. MS spectra of the 5-CH<sub>3</sub>-HBI-CHO

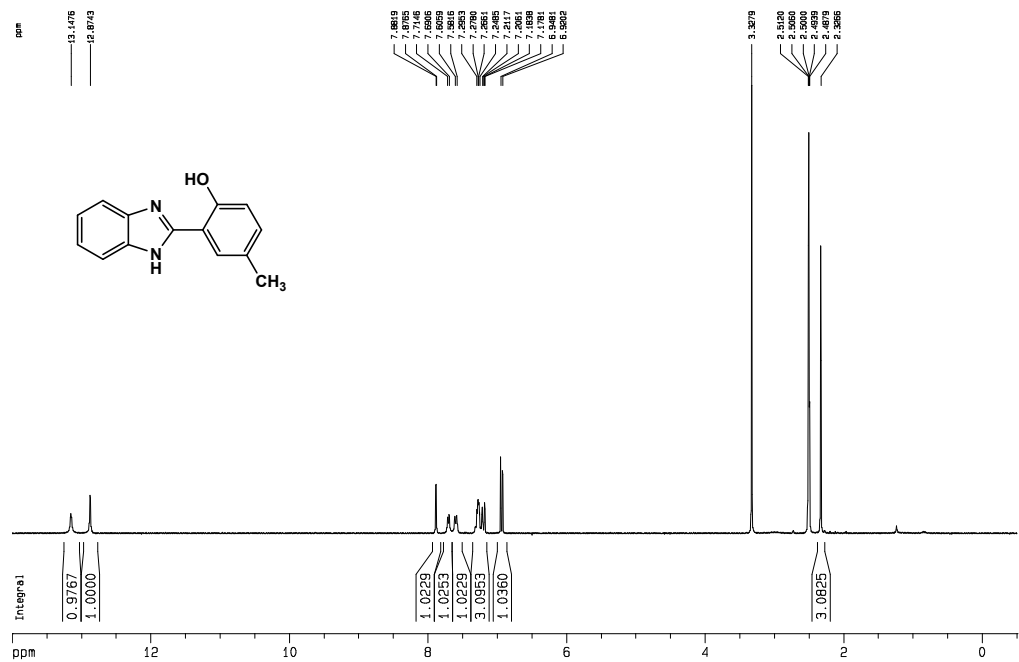


Figure S10. <sup>1</sup>H NMR spectra of the 5-CH<sub>3</sub>-HBI

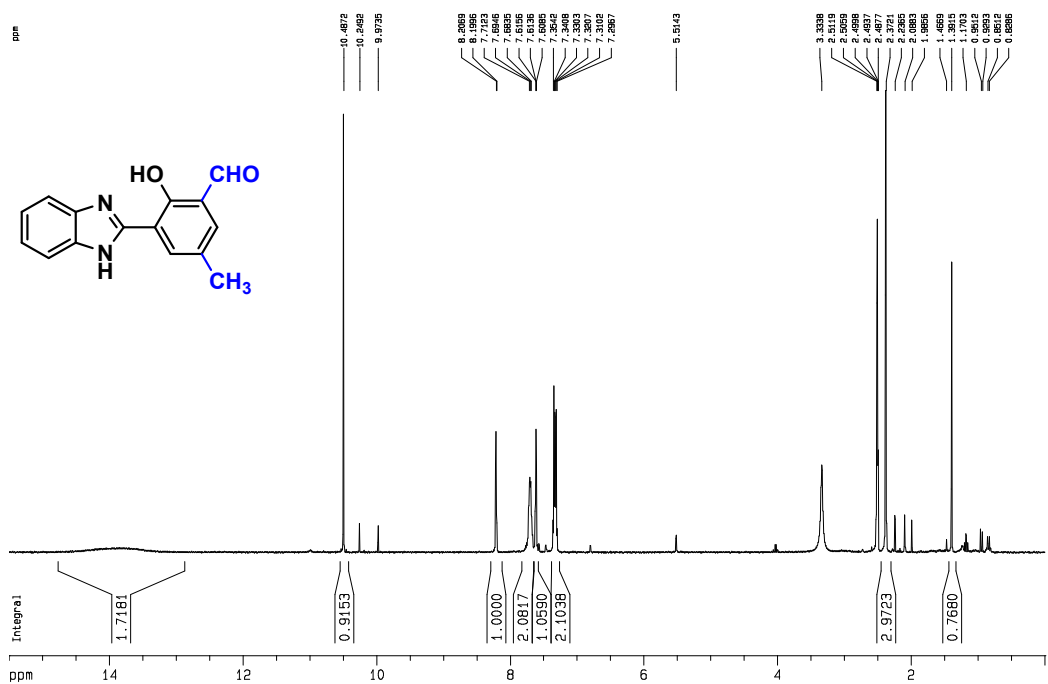


Figure S11. <sup>1</sup>H NMR spectra of the 5-CH<sub>3</sub>-HBI-CHO



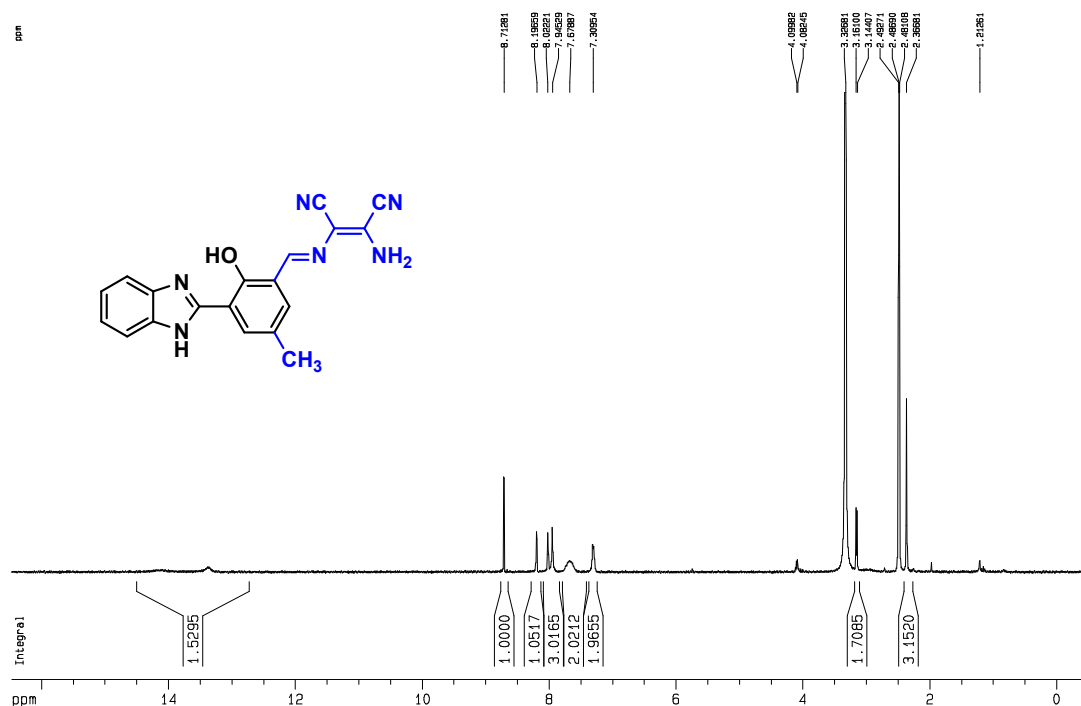


Figure S12.  $^1\text{H}$ NMR spectra of the 5- $\text{CH}_3$ -HBI probe

## 7. Reference

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- 2 X. Li, G. Zhang, H. Ma, D. Zhang, J. Li, D. Zhu, 4,5-Dimethylthio-4'-[2-(9-anthryloxy)ethylthio]tetrathiafulvalene, a highly selective and sensitive chemiluminescence probe for singlet oxygen. *J. Am. Chem. Soc.*, 2004, 126 (37) 11543-11548.
- 3 C. Song, Z. Ye, G. Wang, J. Yuan, Y. Guan, A lanthanide-complex-based ratiometric luminescent probe specific for peroxyxynitrite. *Chem.-Eur. J.* 2010, 16(22) 6464-6472.