Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary material

A novel fluorescent probe based on ESIPT and AIE processes for detection of hydrogen peroxide and glucose and its application in nasopharyngeal carcinoma imaging

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1. Synthesis and characterization of the compounds HPQ and HPQB

***General Information*.** Unless otherwise stated**,** other reactants were purchased commercially, and used as received without further purification. Double distilled water from a Millipore Milli-Q plus system was used throughout the work. ^{1}HNMR and ^{13}CNMR spectra were recorded on Brucker spectrometers. Chemical shifts were reported in ppm relative to a tetramethyslilane (TMS) standard in $CDCl_3$. High Resolution Mass spectra (HRMS) were obtained on LCMS Orbitrap Velos Pro ETD. UV-Vis absorption spectra were measured with a Shimadzu UV-2450 UV-visible spectrophotometer. Fluorescence spectra were recorded on Shimadzu RF-5301 PC spectrofluorometer. All solvents were purified and dried following standard procedures unless special statements. Compound **2** was prepared according to reported procedures.[S1] The nasopharyngeal carcinoma (C666-1) cells are from cancer research center of Central South University. All cell experiments were performed in compliance with the Chinese laws and institutional guidelines, and our protocol was reviewed and approved by the Ethic Committee of the Xiangya Hospital of Central South University.

HPQB stocks were made at a concentration of 1mM in THF. H_2O_2, tert-butyl hydroperoxide (t-BuOOH) and hypochlorite (ClO^-) were delivered from 30, 70, and 5% aqueous solutions, respectively. Hydroxyl radical (·OH) was generated by reaction of H_2O_2 (5 mM) with Co^{2+} (50 mM), and single oxygen ($^1O2$) was obtained by addition NaOCl (50 mM) to H2O2 (5 mM). Peroxynitrite ($ONOO^-$) and superoxide were delivered using 3-morpholinosydnonimine (SIN-1) and xanthine/xanthine oxidase (9mm/90 mU), respectively.

***Synthesis of compound 1*.** To a 100 mL round bottom flask fitted with a magnetic stirrer was added ptolylboronic acid (680.0 mg, 5.0 mmol), anhydrous pinacol (649.0 mg, 5.5 mmol), and diethyl ether (50 mL). After stirring for 5 minutes $MgSO_4$ (360.0 mg, 3.0 mmol) was added and the flask sealed with a rubber septum. The reaction was stirred vigorously overnight at room-temperature, the solids were then removed by filtration, and washed with diethyl ether (3 × 15 mL). The crude filtrate was then recrystallized to obtain white crystalline solid (1.01 g, 92% yield) which was used without further purification.

Synthesis of compound 2. To a 100 mL round bottom flask fitted with a magnetic stirrer was added compound **1** (872.0 mg, 4.0 mmol), N-bromosuccinimide (783.2 mg, 4.4 mmol), and acetonitrile (30mL). The solution was stirred for 5 minutes prior to the addition of AIBN (131.4 mg, 0.8 mmol). A condensor was then fitted to the flask and the solution refluxed at 90 °C for 4 hours under an air atmosphere. After cooling to room temperature the solution was concentrated on a rotary evaporator remove the remaining acetonitrile. The crude material was then separated and purified by silica gel column chromatography to obtain white powder (1.01 g, 92% yield).

Synthesis of compound HPQ. A mixture of 2-aminobenzamide (272 mg, 2 mmol),

salicylaldehyde (244 mg, 2 mmol), and iodine (508 mg, 2 mmol) in 15 mL of ethanol was stirred at reflux for 6 h. After the reaction completed, the excessive iodine was removed by adding $Na_2S_2O_3$ aqueous solution (5 wt %). The generated precipitation was filtered and further washed by water (10 mL × 2) and ethanol (10 mL × 2), the resulting white solid was then dried under vacuum to afford HPQ as a white solid (380 mg, 80% yield). ^{1}H NMR (500 MHz, DMSO-d_6, TMS): δ 13.80 (s, 1H), 12.49 (s, 1H), 8.24 (d, J=8.19 Hz, 1H), 8.16 (d, J=7.80 Hz, 1H), 7.86 (m, J=1H), 7.77(d, J=8.22 Hz, 1H), 7.55 (t, J=7.58 Hz, 1H), 7.46 (t, J=7.94 Hz, 1H), 7.02 (d, J=8.43 Hz, 1H), 6.97 (t, J=7.58 Hz, 1H); ^{13}C (125 MHz, DMSO -d_6): 161.36, 160.01, 153.69, 146.13, 135.02, 133.70,127.69, 126.95, 126.03, 120.73, 118.81, 117.87, 113.74.

Synthesis of compound HPQB. A mixture of **HPQ** (238 mg, 1 mmol), compound 2 (445.5 mg, 1.5 mmo) and potassium carbonate (207 mg, 1.5 mmol) was dissolved in 10 ml dimethylformamide and then refluxed at 120 ^{o}C for 24 hrs. After evaporation of solvent under reduced pressure, chromatography of the crude product to afford product **HPQB**(190.5 mg，42% yield). ^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ 11.03 (s, 1H), 8.56 (dd, J=2.04 Hz, J=1.73 Hz, 1H), 8.32 (d, J=8.37 Hz, 1H), 7.88 (d, J=8.19 Hz, 2H), 7.78-7.83(m, 2H), 7.47-7.51(m, 4H), 7.20 (m, 1H), 7.10 (d, J=8.09 Hz, 1H), 5.40 (s, 2H), 1.37 (s, 12H); ^{13}C (100 MHz, $CDCl_3$-d_6):166.05, 161.26, 148.78, 138.88, 135.08, 134.27, 133.01, 129.44, 127.33, 126.80, 126.07, 123.79, 118.66, 117.73, 114.94, 83.86, 68.81, 24.82; HRMS-EI ($C_{27}H_{27}BN_2O4$) m/z: calculated for $[M+H]^+$: 455.2064, found $[M+H]^+$: 455.2143.

2. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The fluorescence emission spectrum of probe **HPQB** was measured by ten times and the standard deviation of blank measurement was achieved. To gain the slop, the standard deviation of blank measurement was achieved. To gain the slop, the fluorescence intensity (500 nm) was plotted as a concentration of H_2O_2. So the detection limit was calculated with the following equation:

Detection limit = 3σ/k

Where σ is the standard deviation of blank measurement, k is the slop between the emission intensity (500 nm) of versus H_2O_2 concentration.

3. Cell culture

Nasopharyngeal carcinoma (C666-1) cells were maintained following the protocols. Cells were first grown in a circular petri dish (60 mm) using RPMI 1640 medium with 10% fetal bovine serum (FBS), $NaHCO_3$ (2 g L^{-1}), and 1% antibiotics (penicillin/streptomycin,100 U/mL), and were maintained in a humidified incubator at 37 ^{o}C, in 5% CO_2/95% air. One day before imaging, cells were passed and plated on 18 mm glass coverslips in culture dish.

4. Time-dependent fluorescence of 1 in the presence of different amounts of hydrogen peroxide

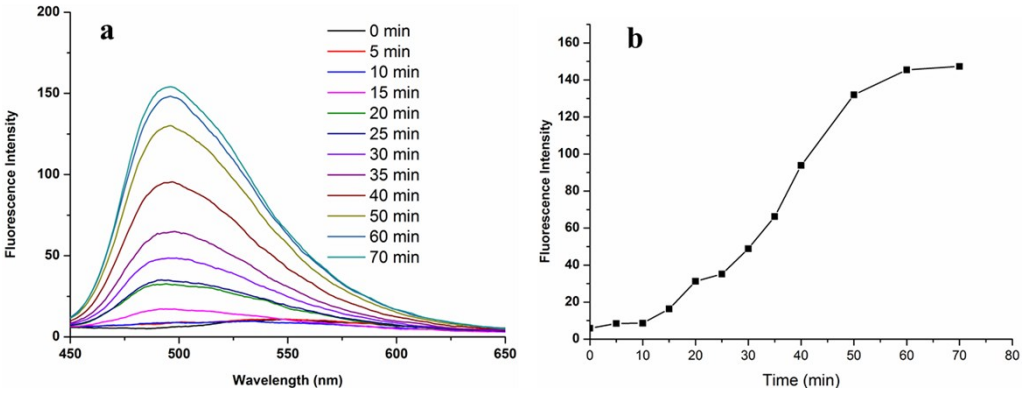

Figure S1. (a)Time-dependent PL spectra of **HPQB** in THF/ buffer mixture solution (10 mM HEPES, pH = 7.4) in the presence of H_2O_2 (140 μM) at 37 °C; (b) Plot of PL intensity of **HPQB** in the presence and absence of H2O2 versus incubation time.

5. Evidences for the formation of compound HPQ after incubation of compound HPQB with H_2O_2

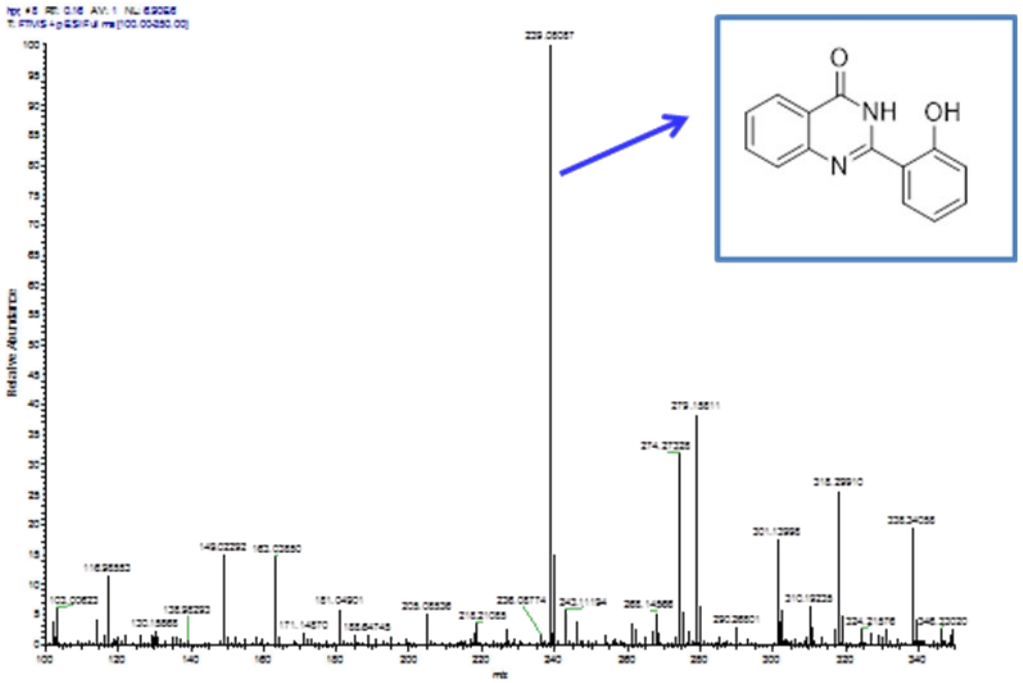

Figure S2. HRMS spectrum of reaction residue after (in positive ion mode); a signal at m/z = 239.0809 [M+H$^+$] corresponding to compound **HPQ [**calcd. for (m/z): 238.0742**]**.

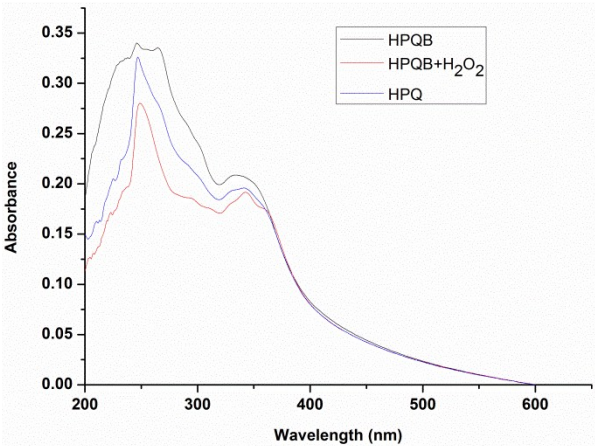

Figure S3. Normalized UV−vis absorption spectra in THF/ buffer mixture solution, **HPQB**, **HPQ** and the reaction product of **HPQB** with H_2O_2; [**HPQB**] = [**HPQ**] = 10 μM.) **HPQB** (0.1 mM) + H_2O_2 (200 μM) after incubation for 60 min.

6. Fluorescence response of HPQB in the presence of *D*-glucose and glucose oxidase

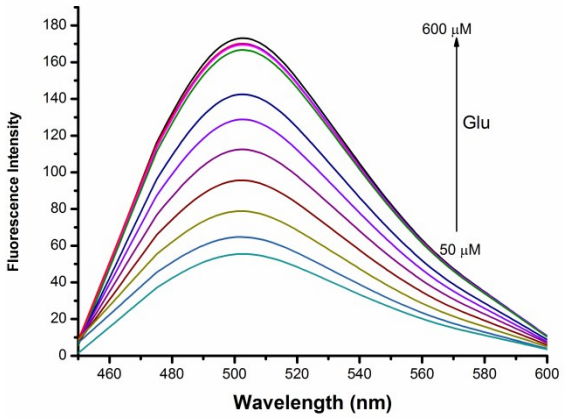

Figure S4. Fluorescence intensity at 500 nm of **HPQB** (10 μM) after incubation with glucose oxidase (2 U/mL) and different concentrations of D-glucose; the reaction was performed at 37 °C in 10 mM HEPES buffer at pH 7.4. Fluorescence intensity at 500 nm was measured with excitation at 330 nm.

7. The plot of fluorescence intensity at 500 nm *vs.* the concentration of *D*-glucose

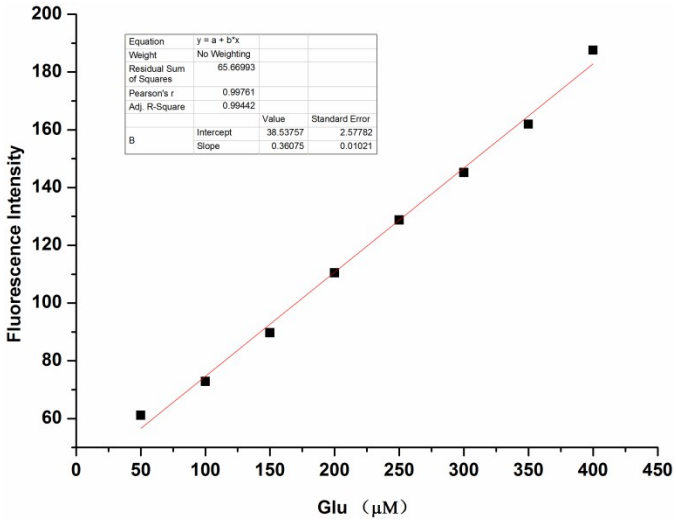

Figure S5 The plot of fluorescence intensity at 500 nm *vs.* the concentration of *D*-glucose; inset shows the linear relation for concentration of *D*-glucose in the range of 50-400 μM.

8. ^{1}H NMR and ^{13}C NMR spectra of compounds HPQ and HPQB

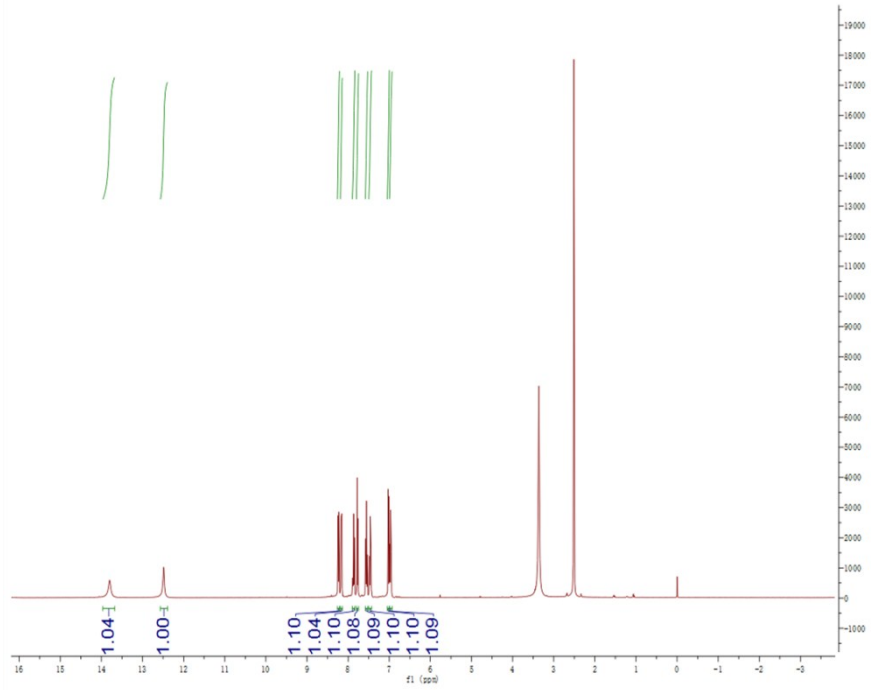

Figure S6. ^{1}H NMR (500 MHz, DMSO-d_6) of **HPQ**.

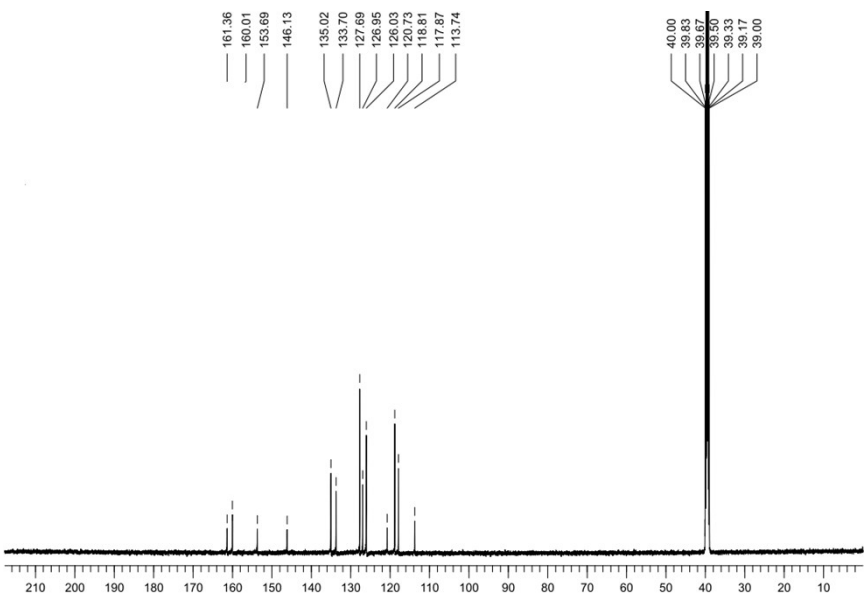

Figure S7. ^{13}C NMR (125 MHz, DMSO-d_6) of **HPQ**.

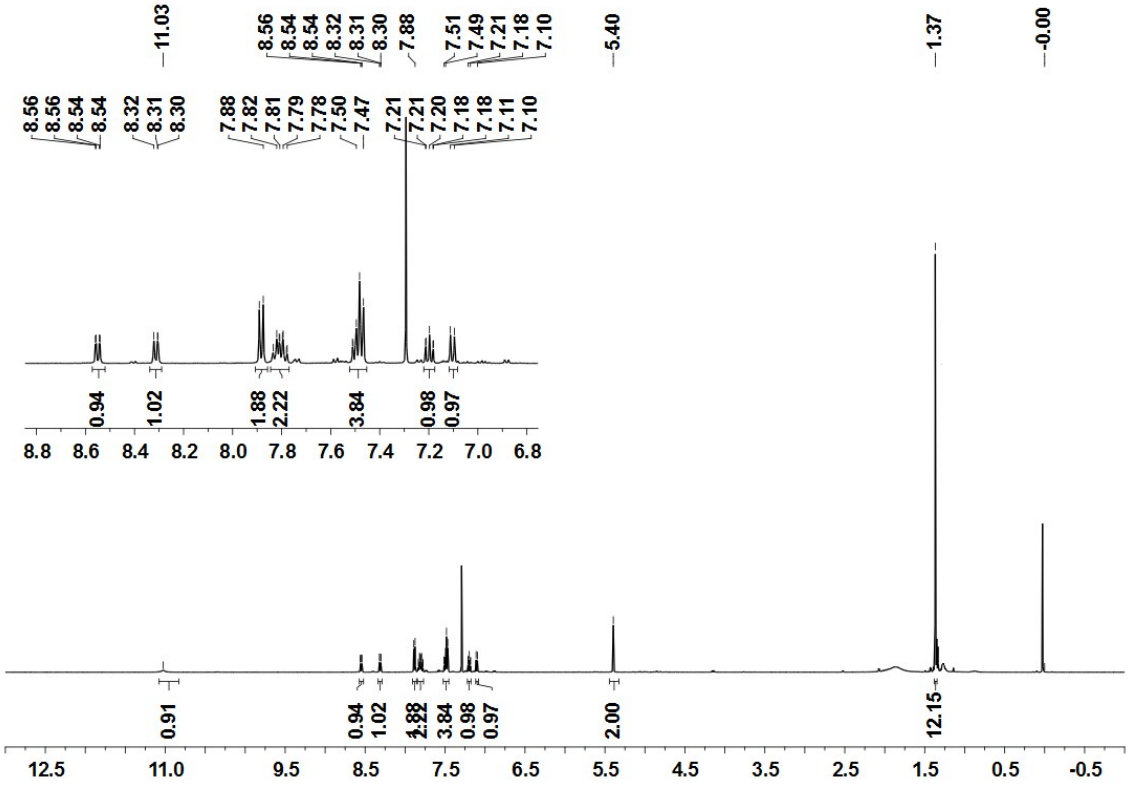

Figure S8. ^{1}H NMR (500 MHz, $CDCl_3$) of **HPQB**.

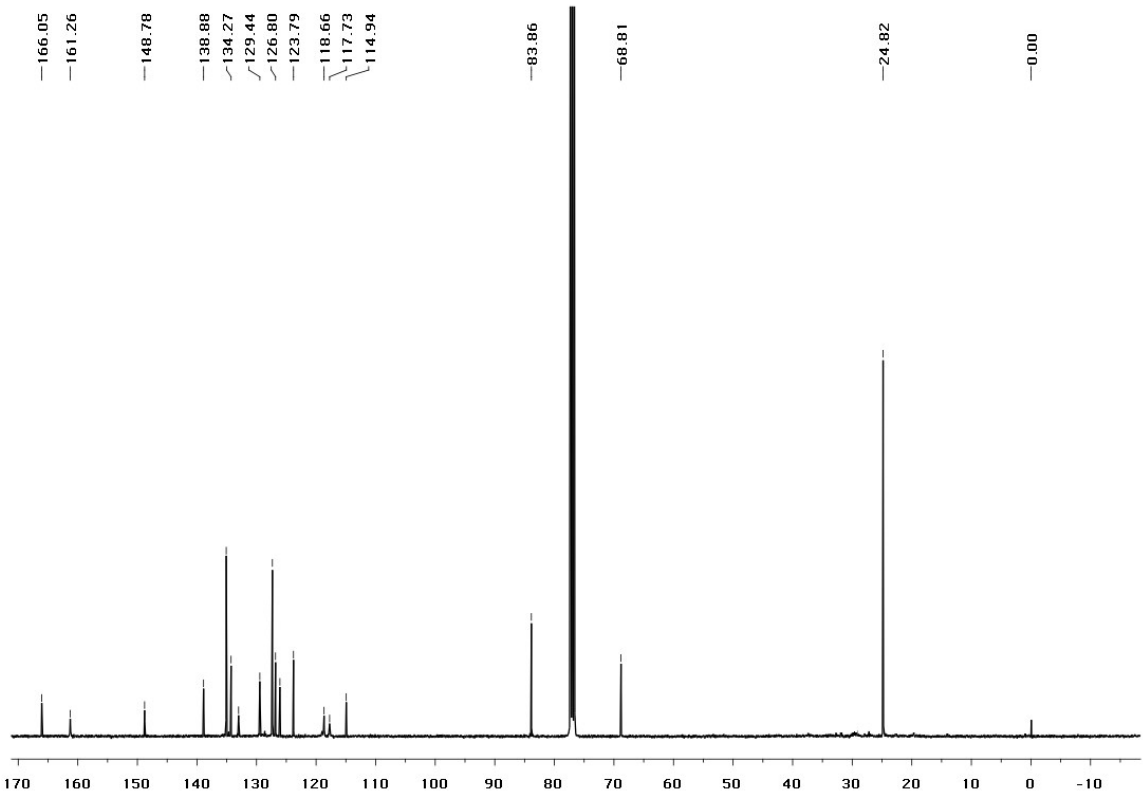

Figure S9. ^{13}C NMR (100 MHz, $CDCl_3$) of **HPQB**.

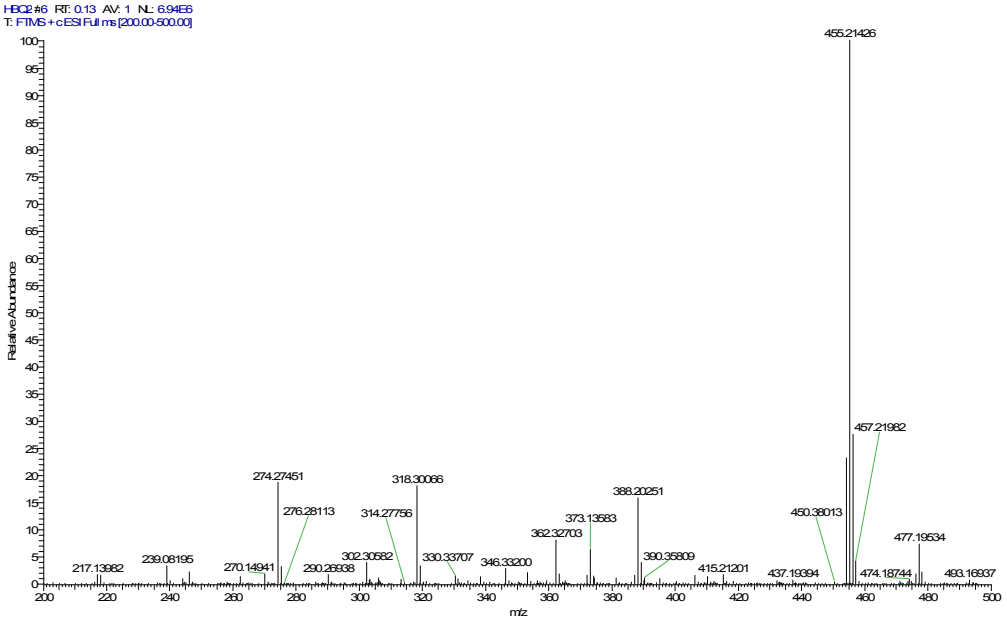

Figure S10. HRMS spectra of **HPQB**.

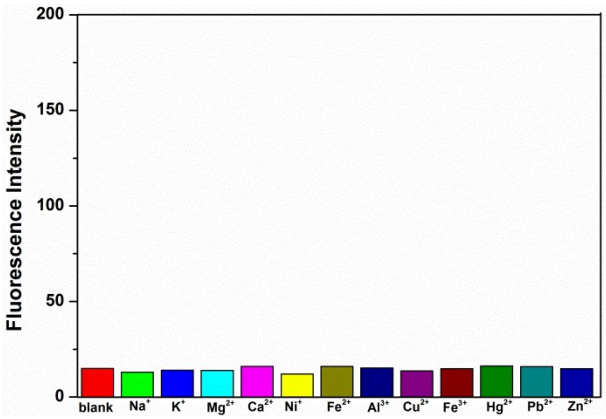

Figure S11. Fluorescence responses of Probe HPQB (10 μM) to different metal ions (100 μM), λex= 333 nm.

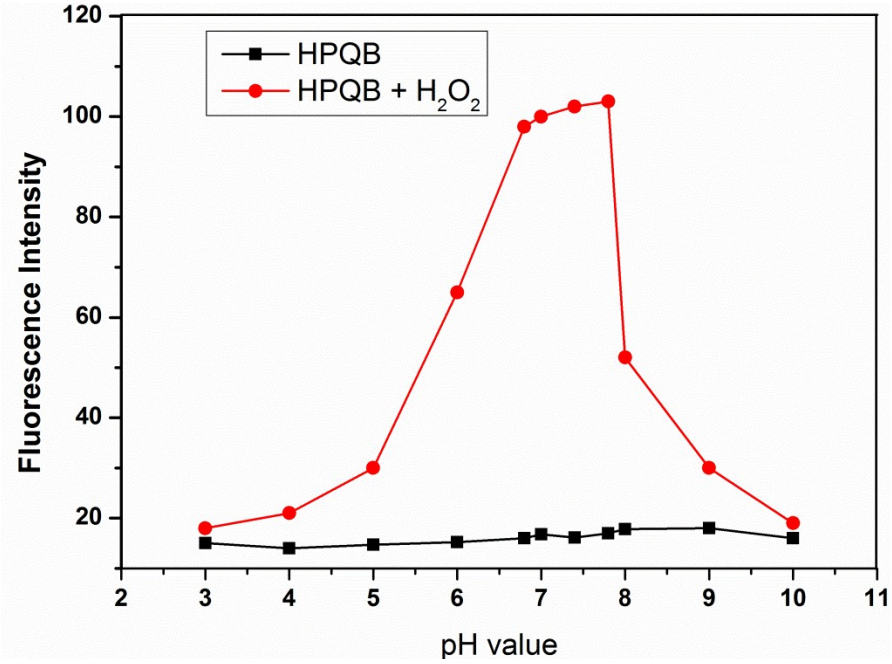

Figure S12. The fluorescence intensity ratio of probe HPQB (10 μM) as a function of pH in PBS (10 mM) solutions (■). The fluorescence intensity of probe HPQB (10 μM) in the presence of H2O2 (100 μM) as a function of pH in PBS (10 mM) solutions (●)

Reference

S1 Zhao, W.; Li, Y.; Yang, S.; Chen, Y.; Zheng, J.; Liu, C.; Qing, Z.; Li, J.; Yang, R. *Anal.Chem* **2016**, *88*, 4833.