Supplementary Information

An oxidative cleavage-based ratiometric fluorescent probe for hypochlorous acid detection and imaging

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1. General Information

Materials. All the chemicals were of analytical reagent grade and used as received without further purification unless specified. Di-(2-picolyl)amine (DPA) was purchased from Sigma-Aldrich. Carbon disulfide (CS_2), ammonium hydroxide (25%), absolute ethanol, and dichloromethane were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Potassium superoxide (KO_2) and TBHP were purchased from Aladdin Chemical Reagent Co. Ltd. Ultrapure water (18.2 MΩ·cm) was obtained from a Millipore water purification system and used in the whole experiment.

Instruments. NMR spectra were recorded on Bruker AVANCE AV-400 machines, in $CDCl_3$. Chemical shifts were reported in ppm with respect to residual solvent protons, coupling constants (JX-X') were reported in Hz. ESI-MS spectra were taken on a Thermo Proteome X-LTQ mass spectrometer employing a regular ESI source setup. UV-vis absorption spectra were recorded on a Shimadzu UV-2550 spectrometer in a quartz cell (10 mm × 10 mm) under ambient condition. Fluorescent measurements were performed on a Perkin-Elmer LS-55 Fluorescence spectrometer (Liantriant, U.K.) equipped with a plotter unit at room temperature with excitation and emission slit widths of 10 nm. Fluorescent photos were taken with a Canon 350D digital camera under a UV lamp (365 nm, 8W).

2. Synthesis and characterization of the probe PIPT

PIPT was synthesized according to the following procedure. Typically, 50 μL of DPA and 30 μL of ammonium hydroxide were added into 2.0 mL of solvent mixture (dichloromethane/ethanol, 1:1) in a flask. The mixture was pre-cooled in an ice bath for 30 minutes, followed by the dropwise addition of CS_2 (35 μL) dissolved in the same solvent mixture (1.0 mL). The reaction was then kept under stirring at room temperature for 10 hours and a yellow solution was obtained. The solvent was removed by reduced pressure distillation and the residue was chromatographed on silica gel column with dichloromethane/methanol (200:1) as eluent followed by dried in vacuum to obtain pure product. Yield: 48 mg, 80%. ESI-MS (m/z): 242.07 $(M+H)^+$; ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.58 (d, J=5.2 Hz, 1H), 8.28 (dd, J=7.6 Hz, J=1.2 Hz, 1H), 7.66 (td, J=7.6 Hz, J=1.6 Hz, 1H), 7.45 (d, J=8.0 Hz, 1H), 7.23 (q,

J=4.8 Hz, 1H), 7.18 (s, 1H), 7.11 (d, J=9.2 Hz, 1H), 6.73 (q, J=6.0 Hz, 1H), 6.52 (td, J=7.2 Hz, J=1.2 Hz, 1H), 5.66 (s, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 155.02, 153.77, 149.52, 137.07, 127.50, 125.36, 123.39, 123.15, 122.36, 117.31, 112.19, 106.91, 52.97

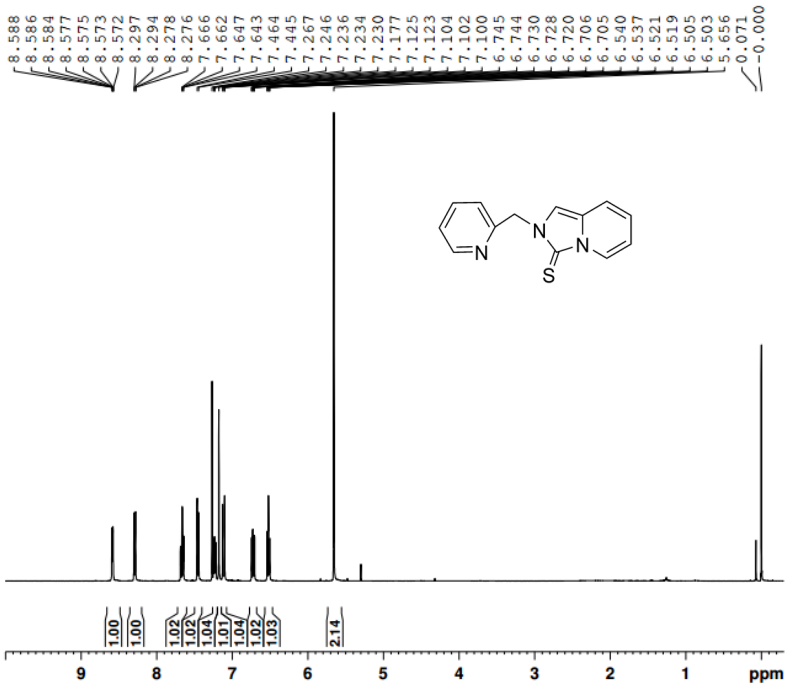

Fig. S1 The ^{1}H NMR spectrum of the probe PIPT obtained in $CDCl_3$ (400 MHz).

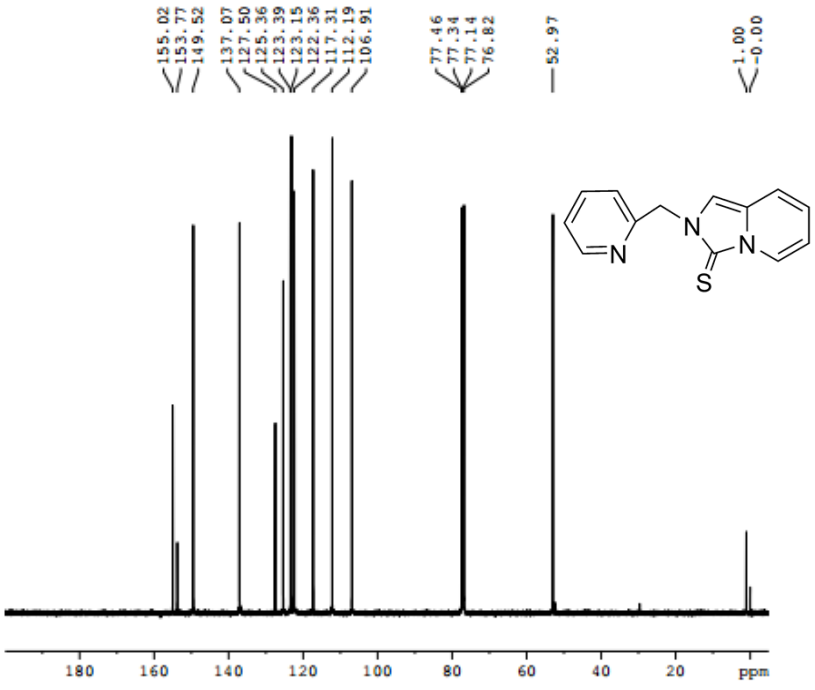

Fig. S2 The ^{13}C NMR spectrum of the probe PIPT obtained in $CDCl_3$ (400 MHz).

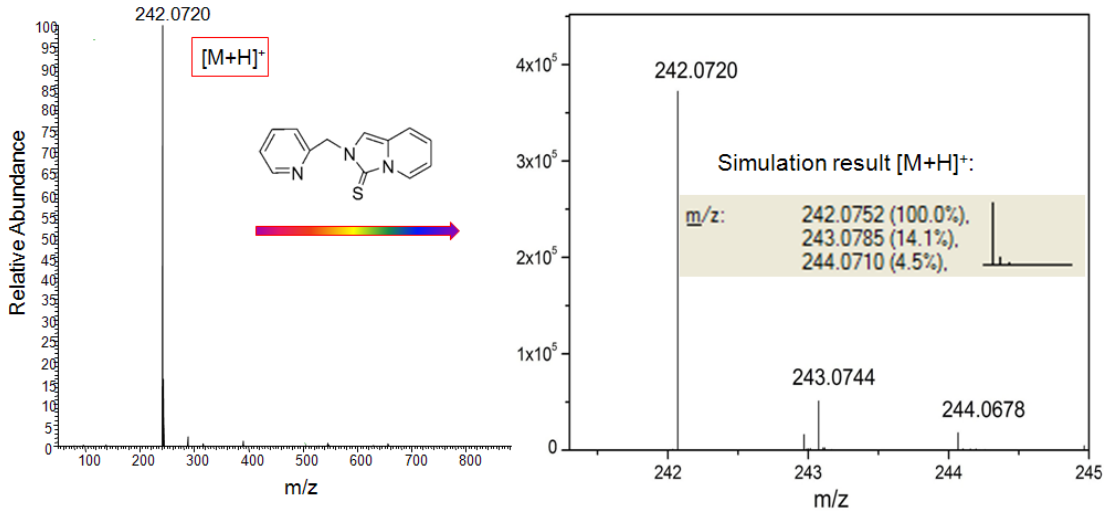

Fig. S3 Left: The mass spectrum of probe PIPT in positive mode. Right: The high resolution mass spectrum of PIPT shows the isotope distribution and the simulated isotope distribution of the probe.

3. Spectral properties of PIPT

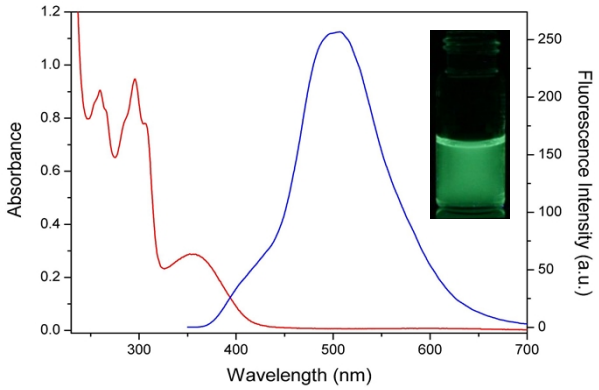

Fig. S4 The UV-vis absorption and fluorescence emission spectra of PIPT in PBS/EtOH (3:1) at pH 7.4. PIPT exhibits an emission maximum at 505 nm. The inset is the fluorescence image of PIPT solution (1.0 μM) under irradiation of a UV lamp (365 nm).

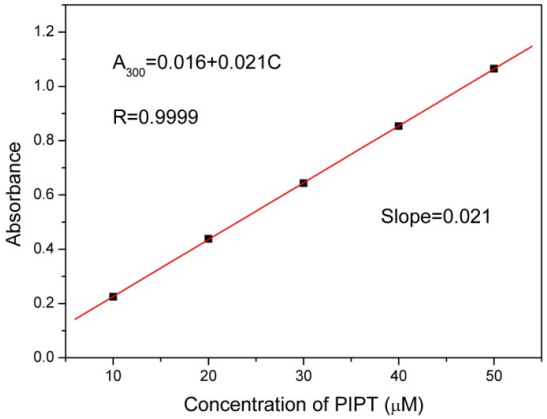

Fig. S5 A Beer's Law plot (concentration versus absorbance) of PIPT at various concentrations. The molar absorption coefficient (ε) equals the slope of the graph and is calculated to be 2100 $M^{-1}cm^{-1}$.

4. Fluorescence Quantum Yield Measurement

Fluorescence quantum yield of PIPT was calculated according to the following equation:[1]

$$\Phi_{F\ sample} = \Phi_{F\ standard} \cdot \frac{Abs_{standard}}{Abs_{sample}} \cdot \frac{\Sigma F_{sample}}{\Sigma F_{standard}} \cdot \frac{\eta_{sample}^{2}}{\eta_{standard}^{2}}$$

where Φ_F stands for fluorescence quantum yields; Abs and ΣF denote the absorbance at the excitation wavelength and the measured integrated fluorescence intensity, and η is the refractive index of the solvent used. Fluorescein in 0.1 M NaOH (Φ_F = 0.95) was selected as a standard for the measurement.

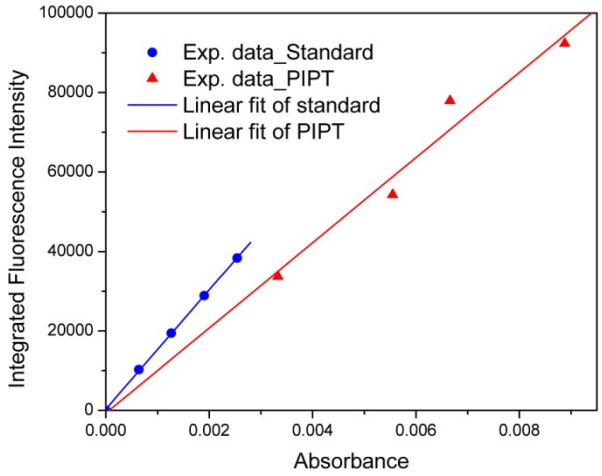

Fig. S6 Determination of the fluorescence quantum yield of PIPT based on fluorescein in 0.1 M NaOH as standard.

5. Stability study

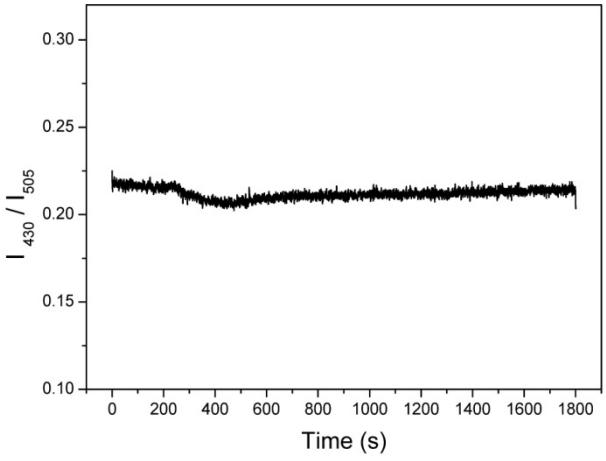

Fig. S7 The fluorescence photostability of PIPT solution (1.0 μM, intensity ratio of I_{430}/I_{505}) against irradiation time in PBS/EtOH (3:1) at pH 7.4 recorded by consecutive irradiation at 300 nm for 30 minutes.

6. Procedures for HOCl detection

The fluorescence responses of the probe to HOCl were examined according to the following procedure. 3.0 mL of PIPT solution with a concentration of 1.0 μM in PBS/EtOH (3:1) was first prepared and the fluorescence spectrum was recorded. HOCl solutions were then added into the above probe solution to get a series of final concentration of 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 μM. The fluorescence spectra were recorded 3 minutes after the addition of HOCl. All fluorescence measurements were operated under ambient conditions.

7. Characterization of the product PIPI

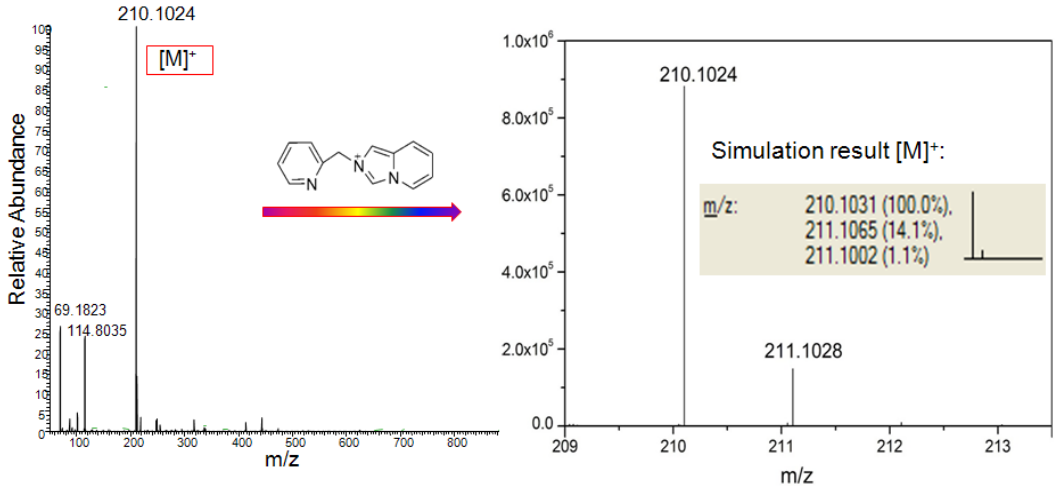

Fig. S8 Left: The mass spectrum (positive mode) of the ylide product PIPI. Right: The high resolution mass spectrum of PIPI and the simulated isotope distribution of the ylide molecule.

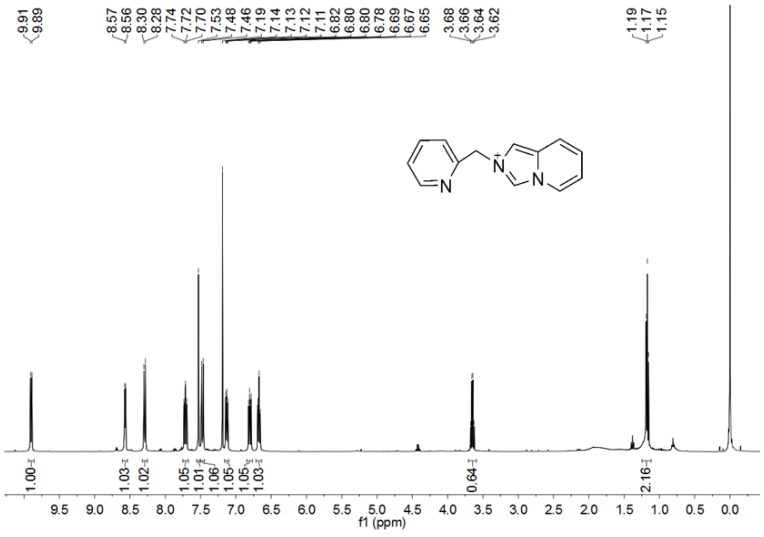

Fig. S9 The ^{1}H NMR spectrum of the ylide product PIPI obtained in $CDCl_3$ (400 MHz). δ 9.90 (d, J = 7.3 Hz, 1H), 8.57 (d, J = 4.9 Hz, 1H), 8.29 (d, J = 8.1 Hz, 1H), 7.74-7.67 (m, 1H), 7.53 (s, 1H), 7.47 (d, J = 9.0 Hz, 1H), 7.13 (dd, J = 7.5 Hz, J = 4.9 Hz, 1H), 6.80 (dd, J = 9.1 Hz, J = 6.4 Hz, 1H), 6.67 (t, J = 6.9 Hz, 1H), 3.65 (q, J = 7.0 Hz, 1H), 1.17 (t, J = 7.0 Hz, 2H).

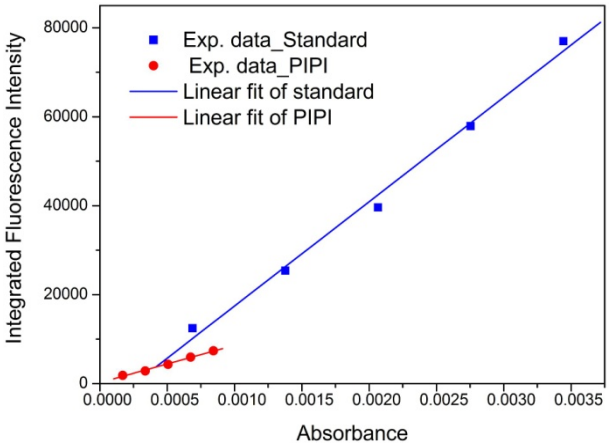

Fig. S10 Determination of the fluorescence quantum yield of the fluorescent product PIPI using quinine sulfate in 0.1 M H_2SO_4 as standard.

8. Effect of pH

The effects of pH values on the fluorescence spectra of the probe were examined by recording the fluorescence spectra of the probe at different pH values in the absence and presence of HOCl.

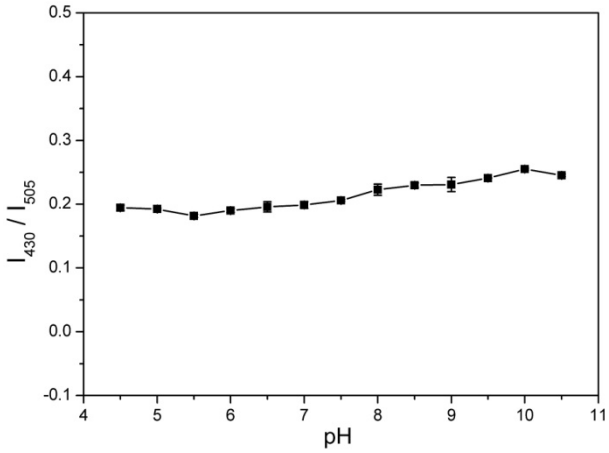

Fig. S11 Effect of the pH value on the fluorescence intensity ratio of the probe PIPT (1.0 μM) in PBS/EtOH (3:1).

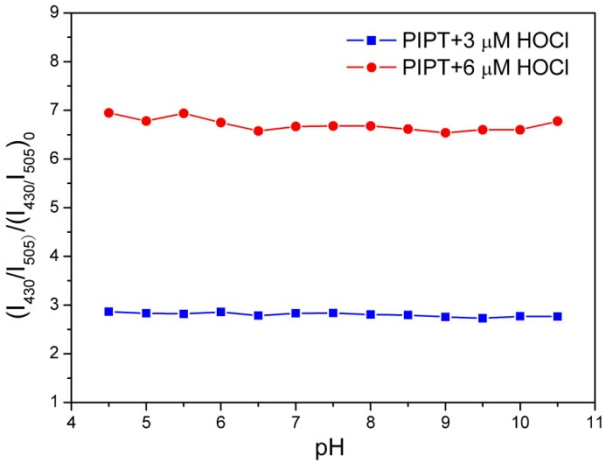

Fig. S12 The dependence of fluorescence intensity of the probe (1.0 μM) on different pH values in the presence of 3.0 μM and 6.0 μM HOCl in PBS/EtOH (3:1). The pH values were altered in intervals of 0.5. All the experiments were performed at ambient conditions.

9. Selectivity study

Generally, 3.0 μL of the PIPT stock solution (1.0 mM) in ethanol was diluted in 3.0 mL of PBS/ethanol (3:1) to get a final concentration of 1.0 μM. Hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2), *tert*-butylhydroperoxide (TBHP), superoxide (O_2^-), nitrite (NO_2^-) and sulfide (S_2^-) were freshly prepared with a stock solution of 3.0 mM. Peroxynitrite ($ONOO^-$) was prepared from the oxidation of nitrous acid by H_2O_2 under acid catalyzation.[2] Hydroxyl radical (·OH) was generated in situ using Fenton reaction by adding ferrous sulfate in the presence of 10 equivalents of H_2O_2. The concentration of OH was equal to the Fe(II) concentration (10 mM). The fluorescence spectra were recorded before and after the addition of ROS. PBS buffer was used in the control experiments.

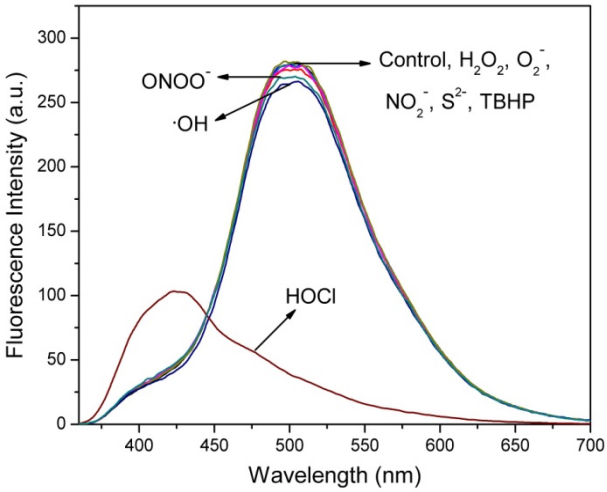

Fig. S13 The fluorescence response of PIPT (1.0 μM) in the presence of 7.0 μM of various selected reactive oxygen species PBS/EtOH (3:1) at pH 7.4. PBS was added with the same volume as the analytes solution for control experiment.

10. Density Functional Theory (DFT) Calculations

The Gaussian 09 program has been used for the DFT calculations of PIPT before and after reaction with HOCl to figure out the difference in spectral and chemical properties between them. The intermolecular interaction energies of the compounds were calculated by the DFT method using the Parr's correlation functional B3LYP/6-31+G (d). The HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) of the molecules were visualized with Gauss View 5.0.

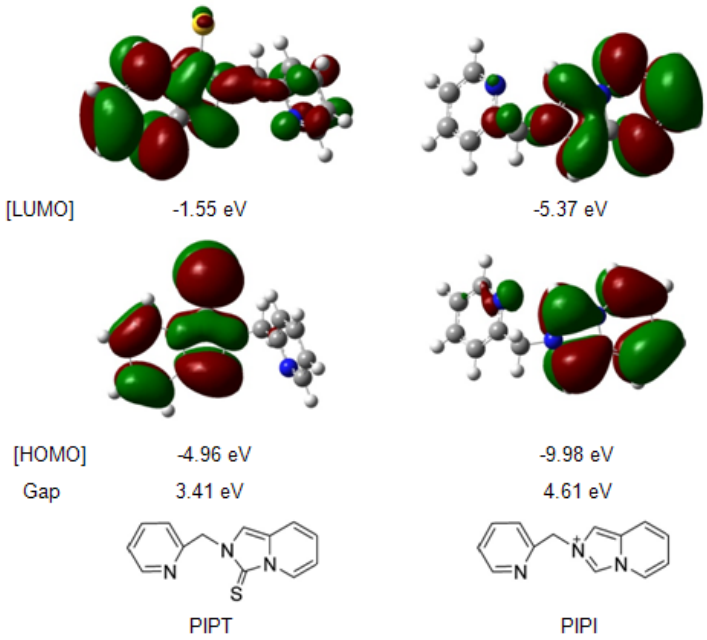

Fig. S14 The ground state geometry optimization and electron density distribution of compound PIPT and PIPI calculated with DFT at the B3LYP/6-31G (d) level.

11. UV-vis titration study

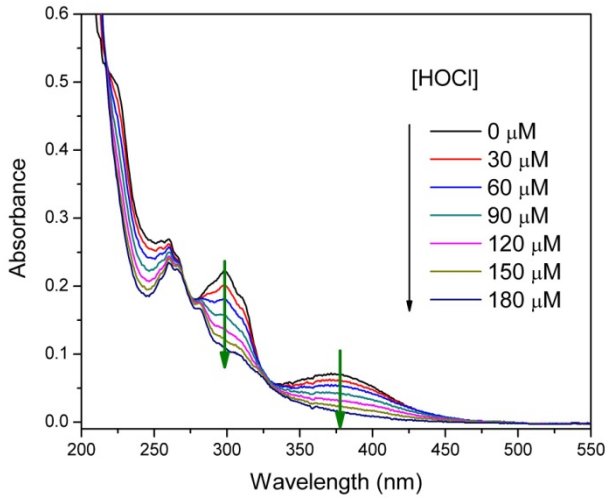

Fig. S15 The UV-vis absorption spectra of the probe (30 μM) upon addition of different concentration of HOCl in PBS/EtOH (3:1) at pH 7.4.

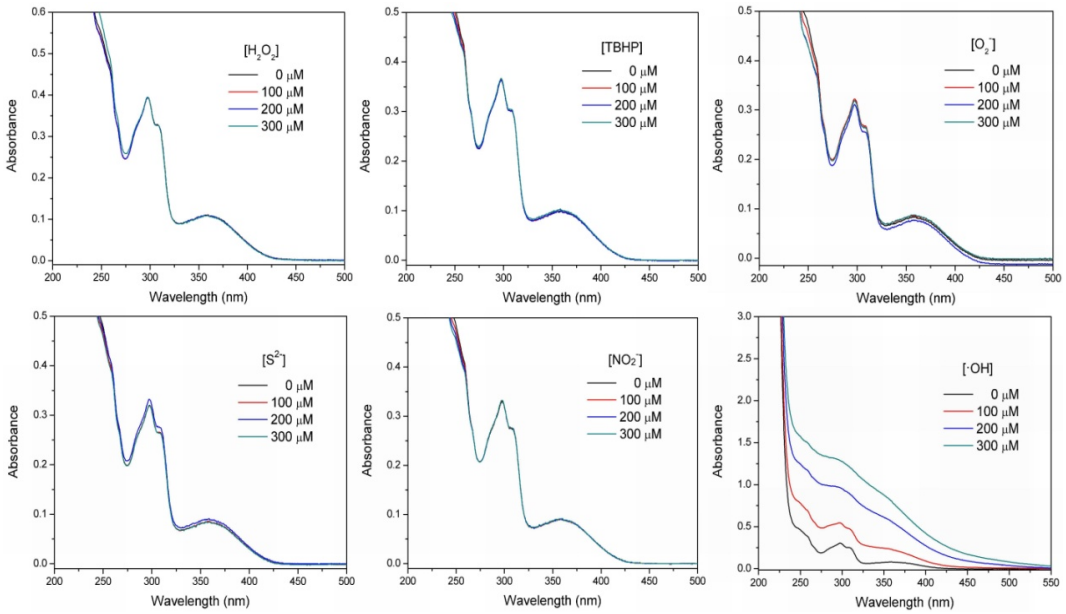

Fig. S16 Absorbance spectral changes of PIPT in the presence of other ROS including hydrogen peroxide (H_2O_2), *tert*-butyl hydroperoxide (TBHP), superoxide (O_2^-), sulfide (S^{2-}), nitrite (NO_2^-) and hydroxyl radical (·OH).

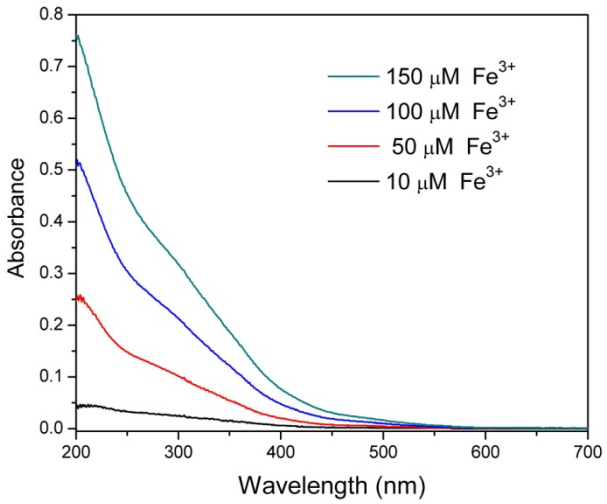

Fig. S17 The UV-vis absorption spectra of Fe^{3+} at different concentrations of 10, 50, 100 and 150 μM, respectively.

12. Reaction mechanism between PIPT and HOCl using mass spectrometry

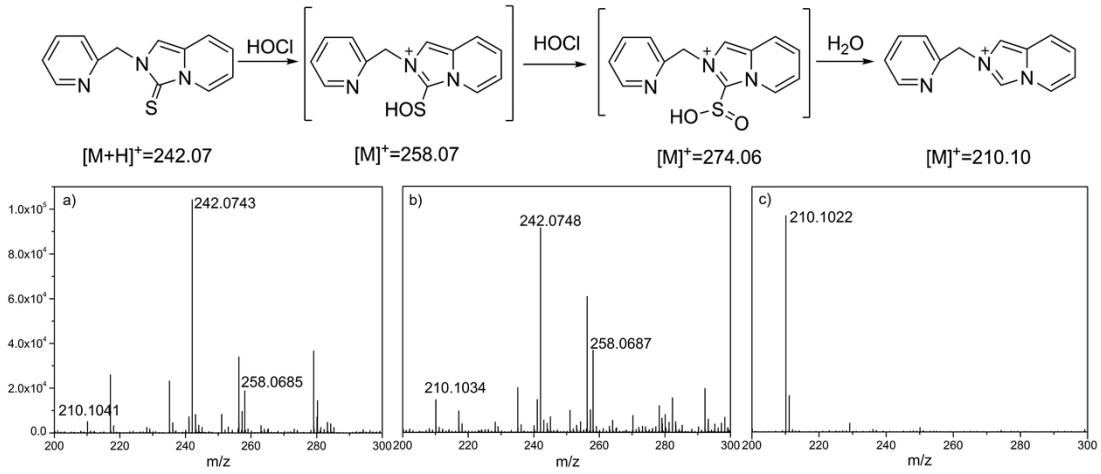

Fig. S18. The probable reaction mechanism of PIPT with HOCl, which could be supported by the mass spectra results of PIPT reacted with HOCl at different molar ratio: (a) 1:1, (b) 1:2, and (c) 1:7.

13. Visual detection of gaseous HOCl using fluorescent indicating paper

We fabricated a portable fluorescent indicating paper following our previous method.[3] Briefly, a symbol of "PIPT" was printed on a piece of cellulose acetate paper using PIPT ethanol solution (5.0 μM) as ink. For the visual detection of gaseous HOCl, the test paper was first put into a glass vial covered with parafilm. The gaseous HOCl was prepared by bubbling a concentrated HOCl solution with strong flow of nitrogen gas. The nitrogen flow carried the HOCl gas into the above indicating vial containing the test paper. The fluorescence colors of the indicating paper before and after exposure to gaseous HOCl were recorded using a digital camera under illumination of a UV lamp (365 nm). The test paper initially shows a bright green symbol "PIPT" before exposed to HOCl under a UV lamp. After exposure to the HOCl gas (the final concentration of total HOCl is estimated to be 10 ppm), the bright green symbol "PIPT" changes to blue, which can be easily observed with the naked eyes (the left panel). This visual method is potential in some practical applications where requiring convenient and rapid determination of gaseous HOCl.

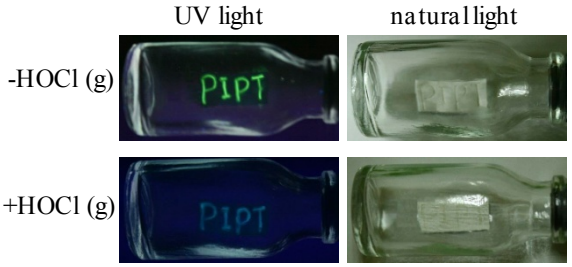

Fig. S19 The visual identification of HOCl in gaseous phase based on the test paper. The images on the left were taken under a 365 nm UV lamp. The corresponding images under natural light were shown on the right as a comparison.

14. Cell Culture and Fluorescence Imaging of HOCl

The human lung adenocarcinoma A549 cells from the hospital of Hefei Institute of Physical Science were cultured in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% fetal bovine serum in an atmosphere of 5% CO2 and 95% air at 37 ºC. The cell suspension was first seeded on glass bottomed plates (Φ = 35 mm) in a 37 ºC humidified incubator for two days. For fluorescence imaging, the cells were incubated in new culture medium with 7.5 μM of PIPT overnight. The cells were then washed three times with phosphate buffered saline (PBS) buffer (pH = 7.4) to remove excessive PIPT. For detection of HOCl, the cells preloaded with PIPT were treated with 100 μM of HOCl and incubated for 2 hours. Control experiment was performed at incubation without HOCl. Confocal fluorescence imaging was performed with Carl Zeiss microscopy (LSM710) equipped with a cooled CCD camera using 400 nm-excitation.

References

1. Q. S. Mei, K. Zhang, G. J. Guan, B. H. Liu, S. H. Wang and Z. P. Zhang, *Chem. Commun.*, 2010, **46**, 7319.

2. J. S. Beckman, T. W. Beckman, J. Chen, P. A. Marshall and B. A. Freeman, *P. Natl. Acad. Sci. USA*, 1990, **87**, 1620.

3. C. Yuan, K. Zhang, Z. P. Zhang and S. H. Wang, *Anal. Chem.*, 2012, **84**, 9792.