Supporting Information

Highly Selective and Sensitive Photoinduced Electron Transfer (PET) Based HOCl Fluorescent Probe in Water and Its Endogenous Imaging in Living Cells

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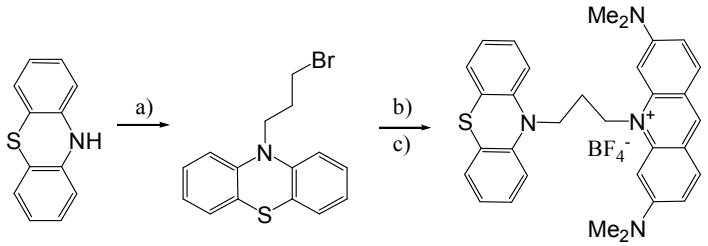
Experimental section

General considerations

All cations in the form of nitrate salts, all anions in the form of sodium salts were purchased from Sigma-Aldrich Chemical Company and used without further purification. All other chemicals used were local products of analytical grade. All solvents (analytical grade and spectroscopic grade) were obtained commercially and used as received unless otherwise mentioned. NMR spectra were recorded on a Bruker spectrometer at 400 (^{1}H NMR) MHz and 100 (^{13}C NMR) MHz, or 300 (^{1}H NMR) MHz and 75 (^{13}C NMR) MHz. Chemical shifts (δ values) were reported in ppm down field from internal Me4Si (^{1}H and ^{13}C NMR). High-resolution mass spectra (HRMS) were acquired on an Agilent 6510 Q-TOF LC/MS instrument (Agilent Technologies, Palo Alto, CA) equipped with an electrospray ionization (ESI) source. UV absorption spectra were recorded on a UV-2550 UV-VIS spectrophotometer (Shimadzu, Japan). Fluorescence measurements were performed using an F-4600 fluorescence spectrophotometer (Hitachi, Japan) and a quartz cell (1 cm × 1 cm). Melting point was recorded on a Boethius Block apparatus and was uncorrected. Live cell fluorescent images were taken under a FV1000 Confocal Microscope (Olympus), For all the experiments, the 'Laser 1 Wavelength' for all experiments are 488 nm, and the 'Emission Wavelength' are 520 nm.



Ptz **1** **Ptz-AO**

Scheme 1 Synthesis of the probe Ptz-AO. Reagents and conditions: a) NaH, 1,3-dibromopropane, DMF, r. t., 4 h; b) acridine orange, KI, toluene, reflux, 24 h; c) KBF_4, CH_2Cl_2/EtOH (1:1, v/v), reflux, 3 h.

Synthesis of Compound 1

N-(4-bromopropyl)phenothiazine (1).

Phenothiazine (1.21 g, 6.0 mmol) was stirred in a slurry of sodium hydride (60% dispersion in mineral oil, 3.0 equivalents) in dry DMF (15 mL) at room temperature for 4 h. 1,3-Dibromopropane (3.0 equivalents) was added dropwise and the suspension was stirred at 0 ^{o}C for 3 h. The reaction mixture was poured into water (150 mL). The crude product was extracted with CH_2Cl_2 (3 × 40 mL), and dried over Na_2SO_4. The crude product was then purified by column chromatography (SiO_2, petroleum ether 100%) to give **1** (1.25 g, 65% yield) as a colorless stick oil; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 7.18-7.16 (m, 4H), 6.97-6.91 (m, 4H), 4.09 (t, J = 6.4 Hz, 2H), 3.53 (t, J = 6.2 Hz, 2H), 2.32 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): 145.0, 127.6, 127.3, 125.7, 122.7, 115.6, 45.1, 30.9, 29.7.

Synthesis of Ptz-AO.

Commercial available acridine orange base (3.0 g) was washed with concentrated ammonia (10 mL) three times to remove the coordinated zinc salt. The insoluble solid materials were collected by filtration, and then dried in vacuum. Then, a mixture of acridine orange (490 mg, 1.84 mmol) and *N*-(4-bromopropyl)phenothiazine (**1**) (578 mg, 1.8 mmol) in 25 mL toluene was refluxed for 24 h. After cooling to room temperature, the solid product was filtered and washed with toluene (3 × 10 mL) and ether (3 × 5 mL), respectively. The solid product was dissolved in dichloromethane (10 mL) and ethanol (10 mL), and then KBF_4 (1.0 g) was added in one portion. The suspension was refluxed for 3 h in dark for anion exchange. The insoluble inorganic salts were filtered out. The filtrate was condensed to dryness. The crude product was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 8:1, v/v) to give Ptz-AO as red powder (618 mg, 58% yield); m.p. > 300 ^{o}C; HRMS: 505.2425 $(M\text{-}BF_4^-)^+$; calcd: 505.2426; ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 8.77 (s, 1H), 7.90 (d, J = 8.7 Hz, 2H), 7.22-7.15 (m, 8H), 6.99 (t, J = 7.2 Hz, 2H), 6.56 (s, 2H), 4.83 (t, J = 7.2 Hz, 2H), 4.31 (t, J = 5.6 Hz, 2H), 3.17 (s, 12H), 2.30 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6, ppm): 155.4, 144.5, 142.9, 142.2, 133.0, 127.5, 127.3, 123.9, 122.7, 116.5, 115.6, 114.3, 92.0, 44.9, 43.4, 22.4.

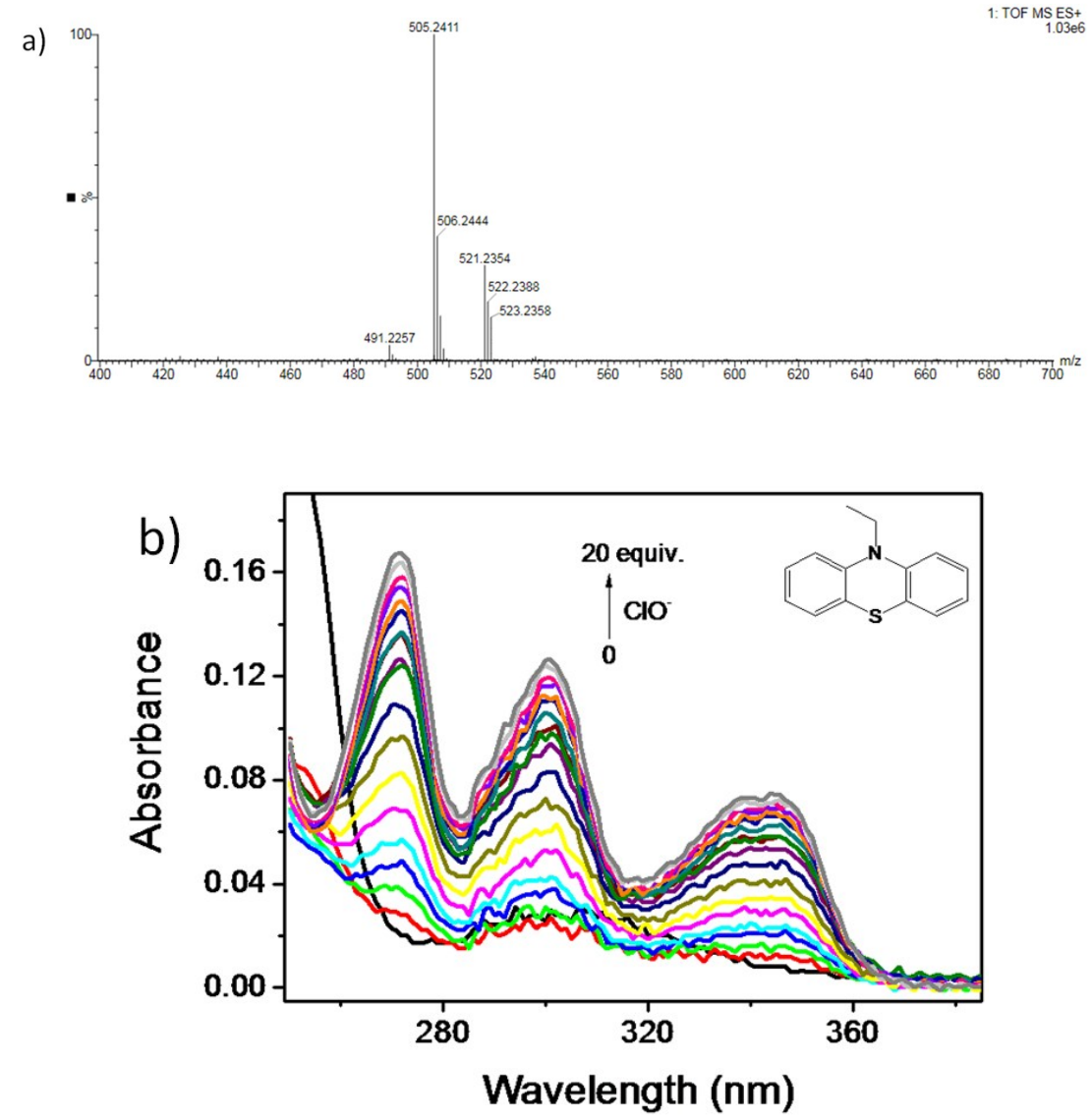


Fig. S1 a) HRMS spectra of Ptz-AO upon addition of ClO^- (2.0 equivalents). The peak (m/z) at 505.2431 corresponds to Ptz-AO (Calcd: 505.2426), and the peak (m/z) at 521.2379 corresponds to OPtz-AO (Calcd: 521.2370). b) UV-Vis spectra of the model compound N-ethylphenothiazine in the presence of different amounts of NaOCl (0 - 20 equivalents); c) HRMS spectra of the model compound N-ethylphenothiazine in the presence of NaOCl (10 equivalents). The peak (m/z) at 244.0796 corresponds to the N-ethylphenothiazine sulfoxide (Calcd: 244.0791), and the peak (m/z) at 227.0775 corresponds to the N-ethylphenothiazinium cation (Calcd: 227.0769).

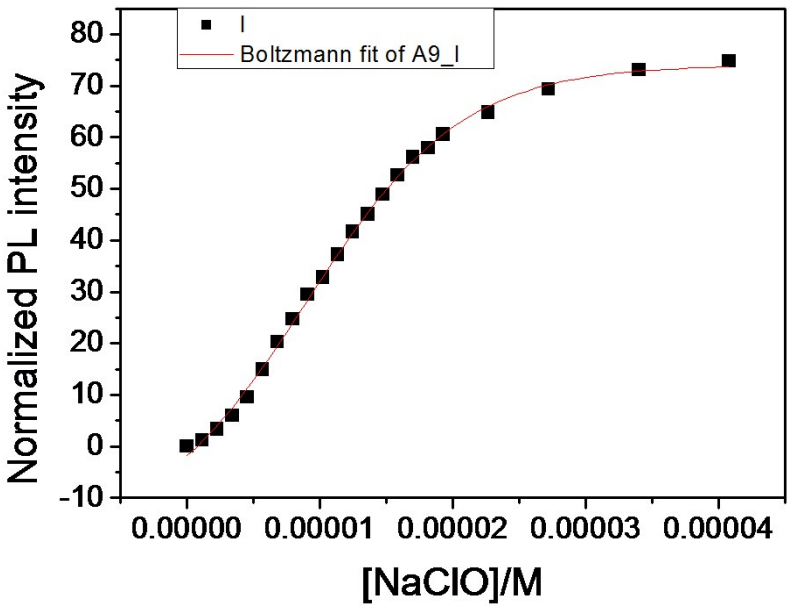


Fig. S2 Measurement of the fluorescence turn-on constant ($K_{turn\text{-}on}$) of Ptz-AO.[S1]

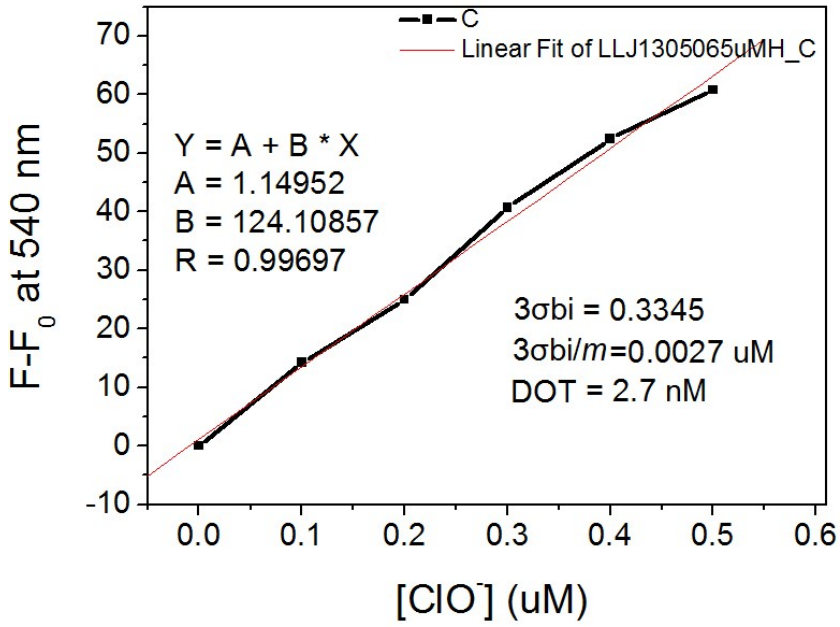


Fig. S3 Emission (at 540 nm) of Ptz-AO at different concentrations of ClO^- added. A linear relationship between the fluorescence intensity and the ClO^- concentration could be obtained in the 0-0.5 μM concentration range (R = 0.997). The detection limit was then calculated with the equation: detection limit = 3σbi/m, where σbi is the standard deviation of blank measurements (3σbi = 0.3345, derived from ten measurements), m is the slope between intensity *versus* sample concentration. The detection limit was measured to be 2.7×10^{-9} M.

[S1] P. Du and S. J. Lippard, *Inorg. Chem*., 2010, **49**, 10753.

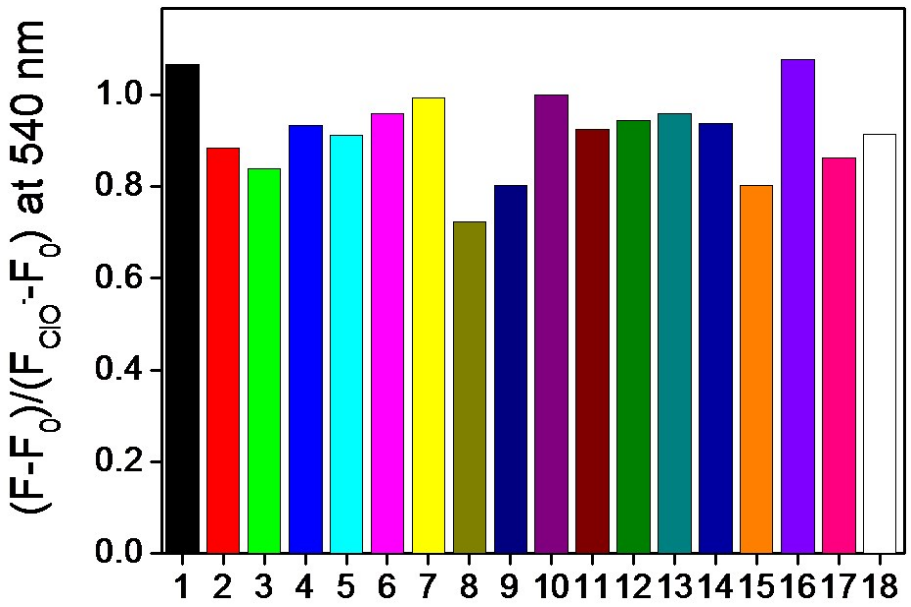


Fig. S4 Change ratio (F - F_0)/ (F_{ClO^-} -F_0) of fluorescence intensity of Ptz-AO upon the addition of 2 equiv. ClO^- in the presence of 2 equiv. other ions and ROS in H_2O. 1: O_2^1; 2: AcO^-; 3: Al^{3+}; 4: Ca^{2+}; 5: Cl^-; 6: Cu^{2+}; 7: Fe^{2+}; 8: Fe^{3+}; 9: H_2O_2; 10: ClO^-; 11: Hg^{2+}; 12: K^+; 13: Na^+; 14: NO_2^-; 15: ·OH; 16: $ONOO^-$; 17: TBHP; 18: Zn^{2+}.

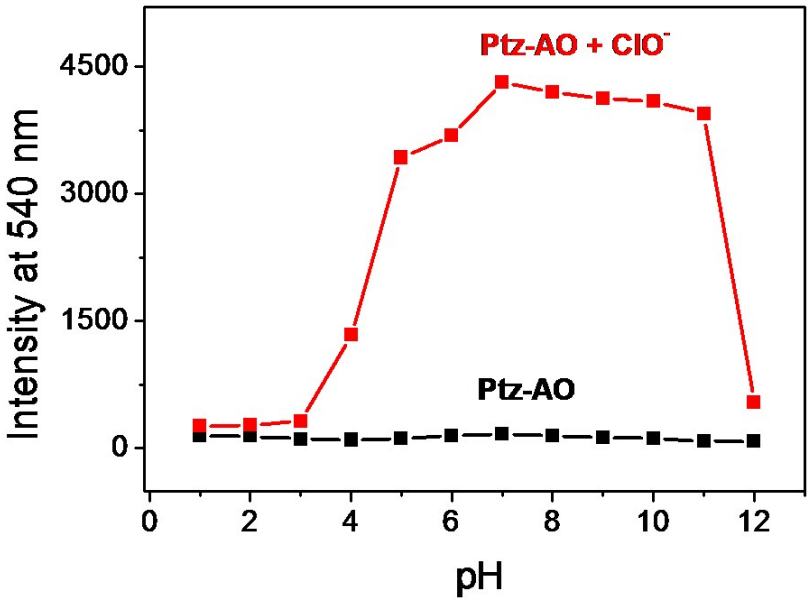


Fig. S5 Fluorescence intensity of Ptz-AO (10 μM) in the absence and presence of NaClO (4.0 equiv.) at various pH values (from 1.0 to 12.0) in H_2O at 540 nm. λ_{ex} = 475 nm, slit: 5 nm; 5 nm.

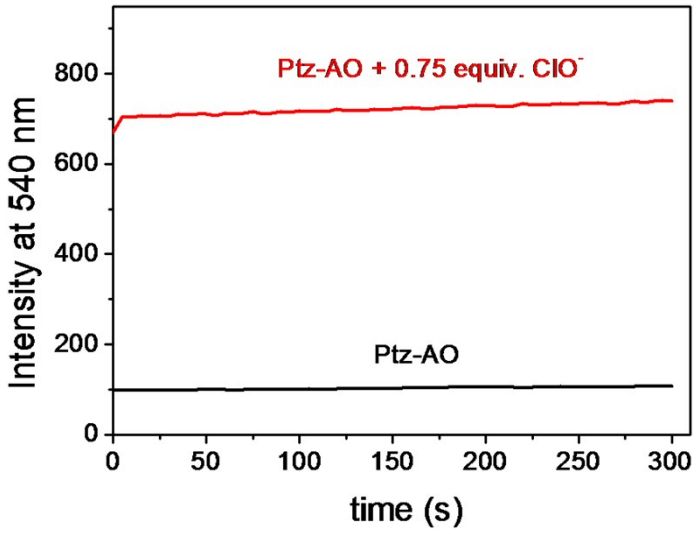


Fig. S6 Time-dependent fluorescence intensity of Ptz-AO (10 μM) upon the addition of 0.75 equiv. ClO^- in H_2O. λ_{ex} = 475 nm, λ_{ex} = 540 nm, Slit = 5 nm.

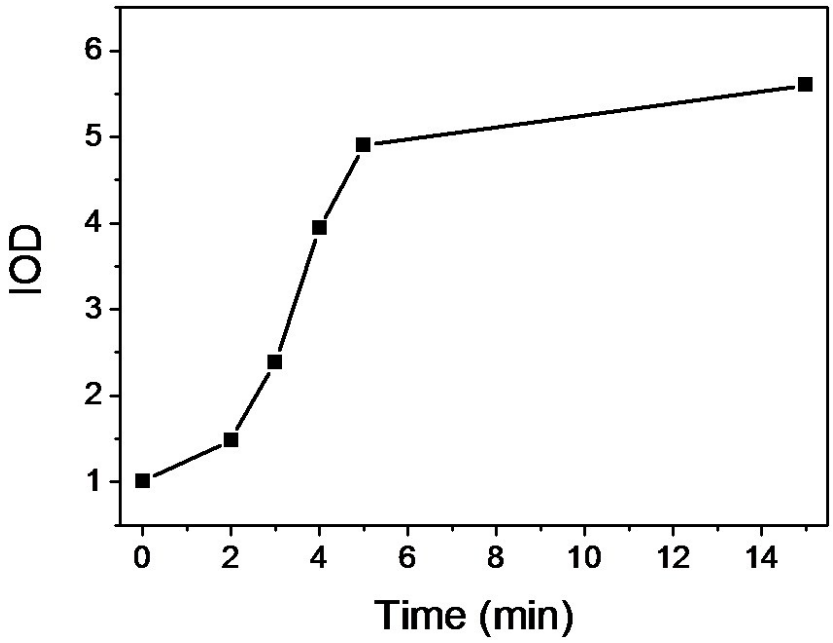


Fig. S7 Dot representing the integrated optical density (IOD) of the probe from the fluorescence images (from Fig. 4) at 0, 2, 3, 4, 5, 15 min, respectively.

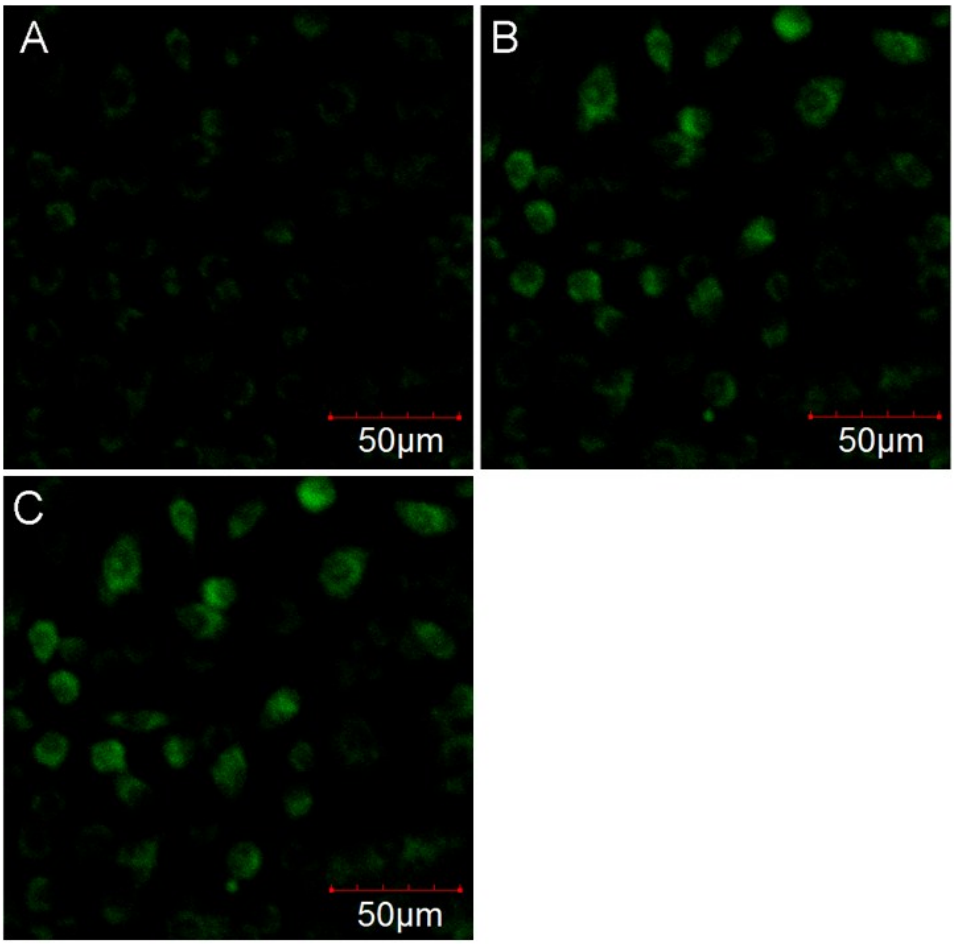


Fig. S8 Fluorescence image of RAW264.7 cells incubated with the dye Ptz-AO (1 μM). A: fluorescence image after staining for 20 min; B and C: fluorescence image after incubated with ClO^- (2 μM) for 2 and 3 min, respectively.

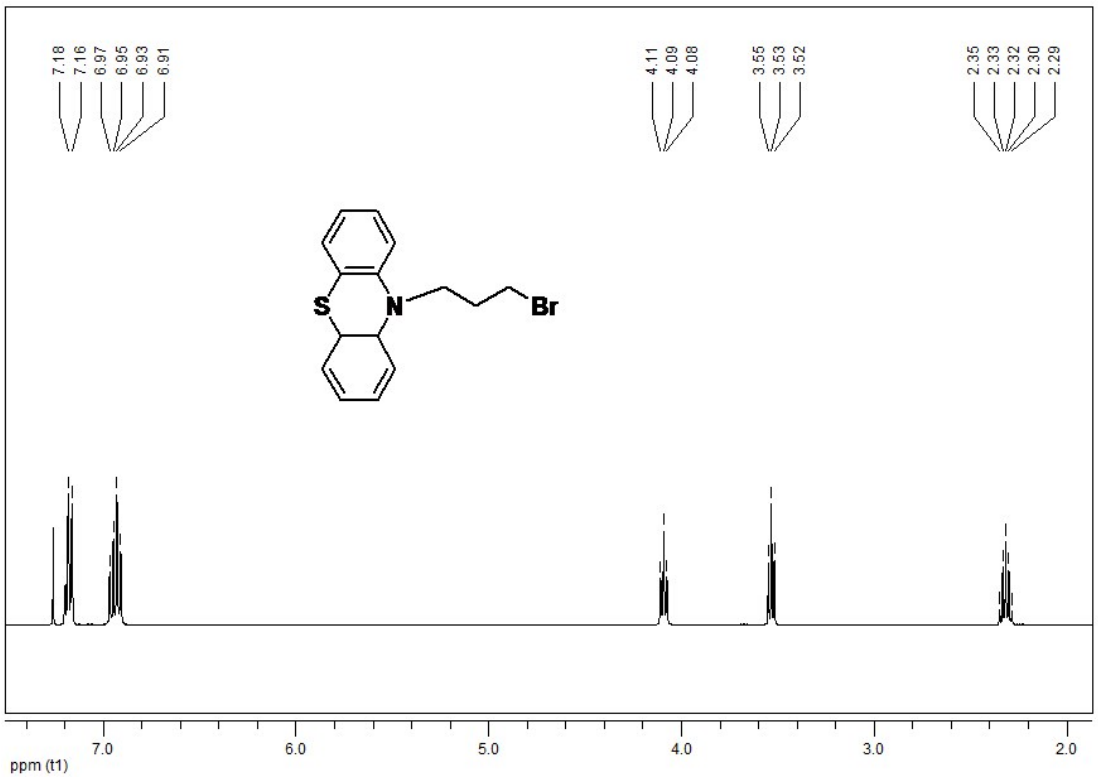


Fig. S9 ^{1}H NMR of compound **1** (400 MHz, $CDCl_3$).

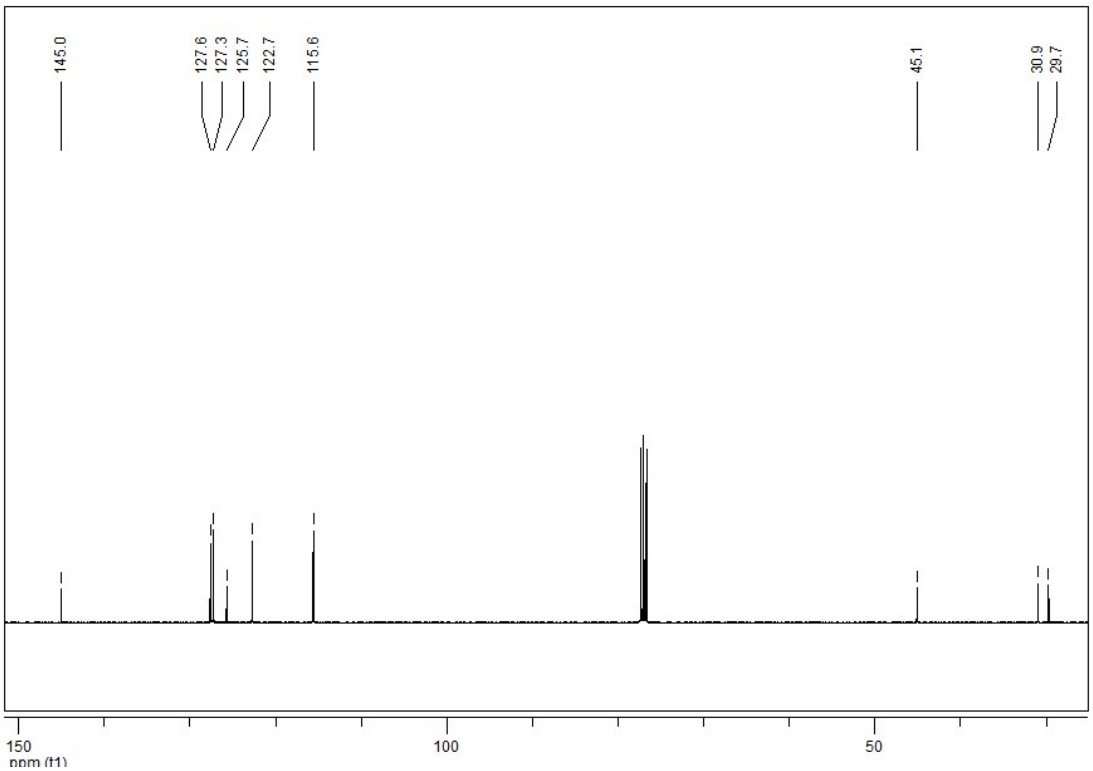


Fig. S10 ^{13}C NMR of compound **1** (100 MHz, $CDCl_3$).

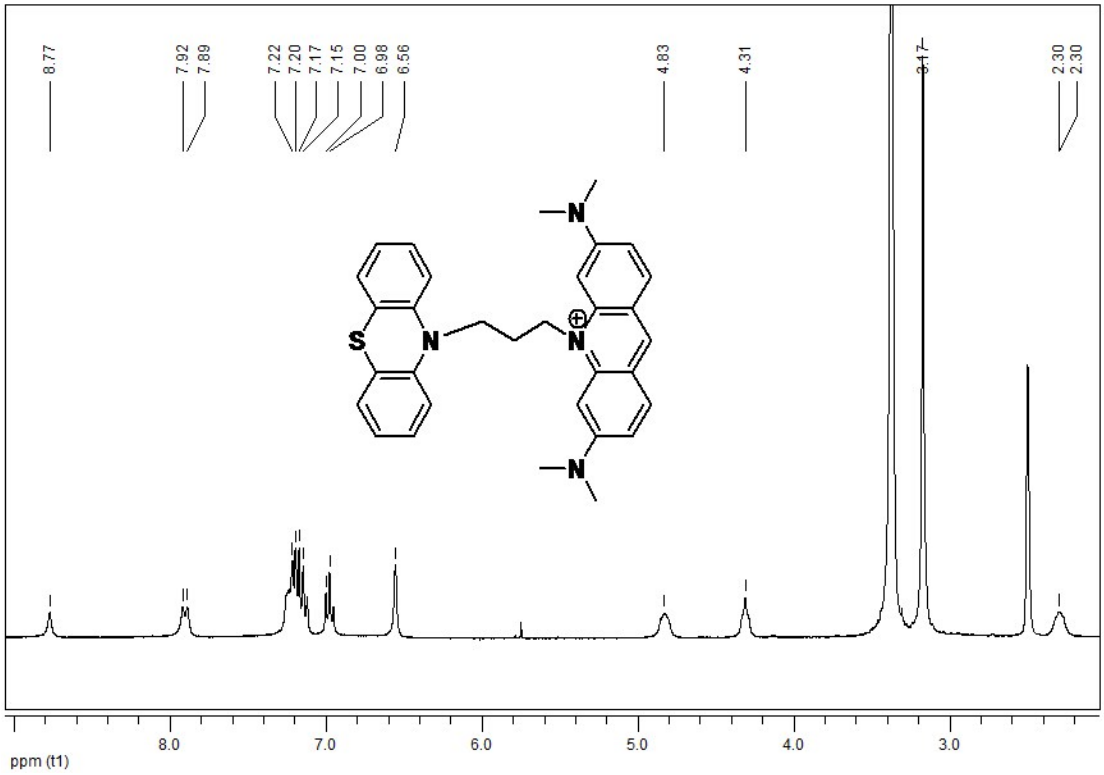


Fig. S11 ^{1}H NMR of Ptz-AO (300 MHz, DMSO-d_6).

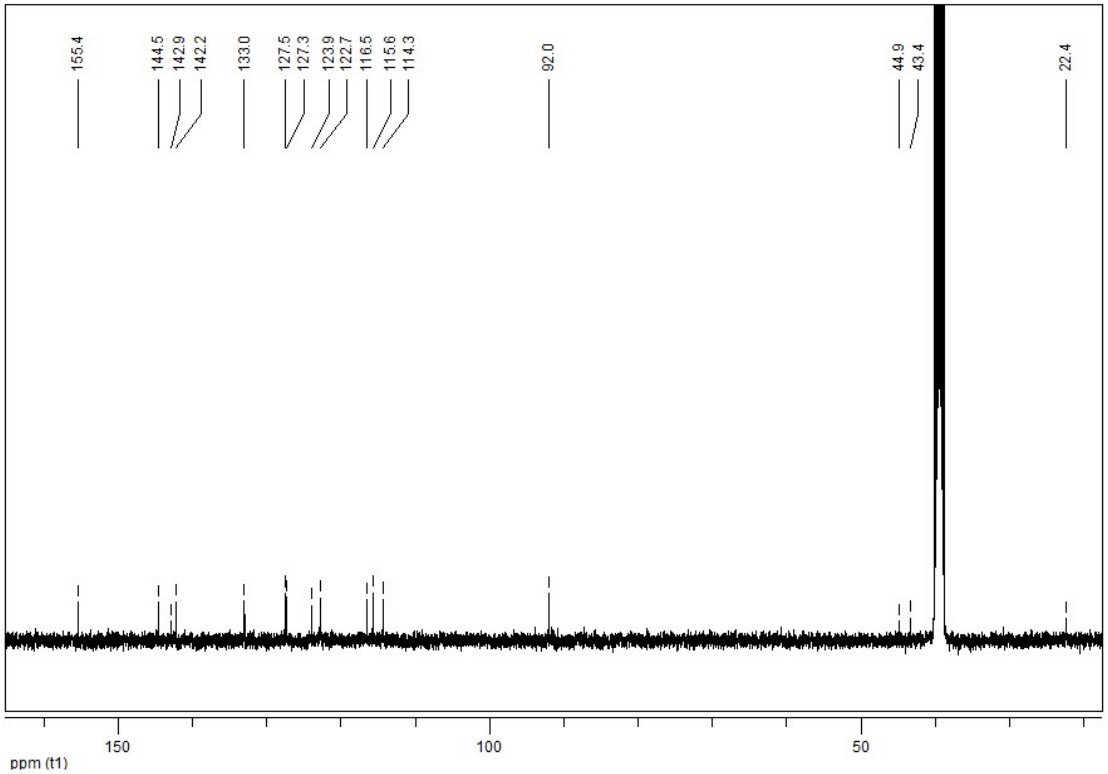


Fig. S12 ^{13}C NMR of Ptz-AO (75 MHz, DMSO-d_6).

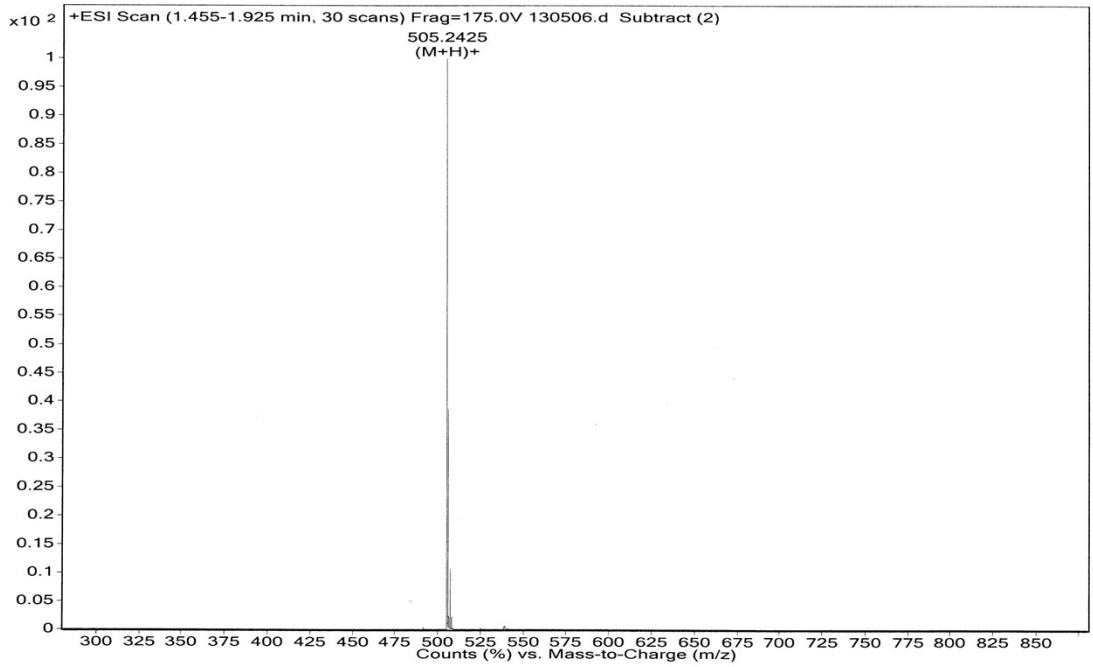


Fig. S13 HRMS spectra of Ptz-AO. The peak (m/z) at 505.2425 corresponds to Ptz-AO (Calcd: 505.2426).