Supporting Information

HEPES is not suitable for fluorescence detection of HClO: a novel probe for HClO in absolute PBS

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Contents

Materials and apparatus

All solvents and chemical reagents were analytical grade and purchased from commercial suppliers. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 500 MHz AVANCE III spectrometer with chemical shifts reported in ppm at room temperature. Mass spectra were obtained with Thermo Fisher LCQ Fleet mass spectrometer (USA) and a LC/Q-Tof MS spectrometry (USA). Absorption spectra were collected by using a Shimadzu 1750 UV-visible spectrometer (Japan). Fluorescence spectra were measured with a Shimadzu RF-5301 fluorescence spectrometer (Japan). The pH of the testing systems was determined by a PHS-3C pH Meter (China). A three-electrode cell with an Electrochemical Workstation (CHI660D, Shanghai Chenhua Device Company, China), comprised the ITO working electrode, a Pt wire counter electrode and an Ag/AgCl reference electrode were used. The mouse imaging was conducted by a vivo imaging system FX Pro (Kodak In-Vivo imaging system FX Pro, USA).

All of the experiments were performed in compliance with the relevant laws and institutional guidelines, and were approved by Northwest A&F University.

Preparation of analyte solution

ROS and RNS were prepared according to literature report.[1,2] The solutions of anions were prepared from their sodium salts. Hydroxyl radical (•OH) was obtained from the Fenton reaction of Fe^{2+} and H_2O_2. Nitric oxide was generated from SNP (sodium nitroferricyanide (III) dihydrate). Superoxide solution (O_2^-) was prepared by adding KO_2 (1.0 mg) to dry dimethylsulfoxide (1.0 mL) and stirring vigorously for 10 min. Single oxygen ($^1O_2$) was generated by mixing H_2O_2 with NaClO sequentially. ROO• was generated from 2,2′-azobis(2-amidinopropane)dihydrochloride. $ONOO^-$ was obtained from the reaction of H_2O_2 and isoamyl nitrite at pH $\geq$ 12.0. The concentration was determined by using an extinction coefficient of 1670 ± 50 cm^{-1} $(mol/L)^{-1}$ at 302 nm.

The calculation of LOD

The detection limit was calculated based on the fluorescence titration according to the literature.[3-4] The fluorescence intensity of ten reagent blank samples containing no HClO was measured, and the mean as well as the standard deviation (SD) was calculated.

$$LOD = 3\sigma/slope$$

Where σ is the standard deviation of the blank solution measured by 10 times; slope comes from the calibration curve.

Synthetic procedures of YDN

21 mg sodium nitrite was dissolved in 1 mL water, then dropwise to a mixture of 174 mg (0.5 mmol) 5-amino fluorescein and 1.5 mL concentrated hydrochloric acid at ice bath, stirring for 20 min. A pre-cooling solution of 65 mg (0.6 mmol) m-phenylenediamine in 5 mL methanol was added to the mixture above over 10 min. Then stirred for another 10 h. Filtered and washed with water to afford **YDN,** in 45% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.14 (s, 2H), 8.14 (s, 1H), 8.06 (d, J = 8.2 Hz, 1H), 7.42 (d, J = 8.8 Hz, 1H), 7.29 (s, 2H), 7.26 (d, J = 8.2 Hz, 1H), 6.73 – 6.64 (m, 4H), 6.61 – 6.54 (m, 2H), 6.14 (s, 2H), 6.06 (d, J = 8.8 Hz, 1H), 5.89 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.19, 159.97, 155.33, 154.61, 152.37, 150.76, 130.20, 129.66, 129.05, 127.92, 125.04, 115.70, 113.11, 110.22, 107.00, 102.73, 96.48, 83.51. HRMS($C_{26}H_{18}N_4O_5$) calc. m/z = 466.1277, found m/z = 467.1347, $[M+H]^+$.

The effect of pH for YDN

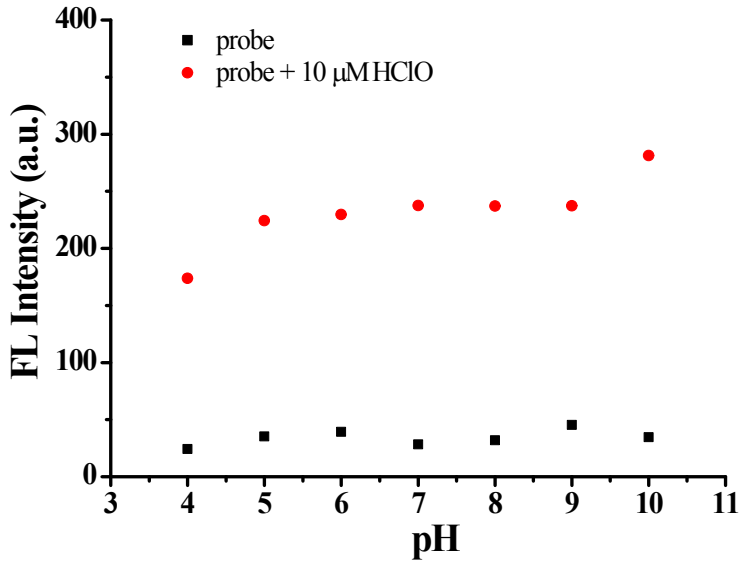

Fig.S1 The effect of pH value on the fluorescence intensity of **YDN** (5μM).

The effect of solution on YDN

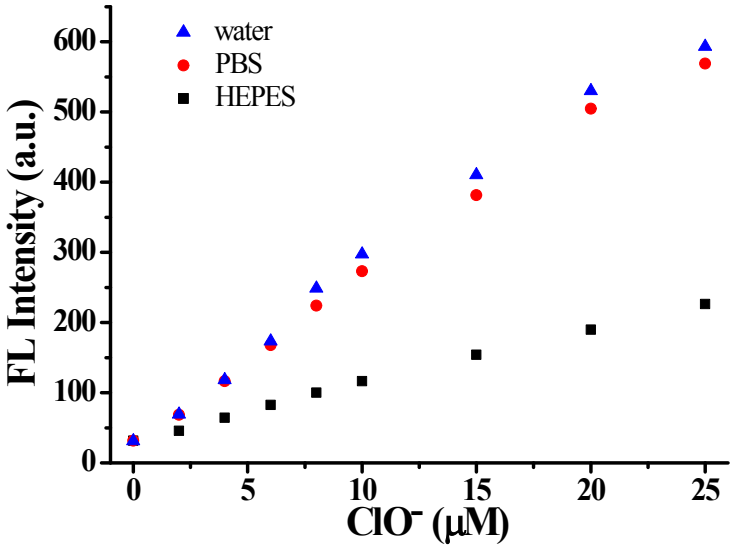

Fig.S2 Fluoresce intensity of **YDN** (5 μM) in 10 mM HEPES (black, pH = 7.4), 10 mM PBS (red, pH = 7.4) and water (blue, pH = 7.4) with the addition of HClO (0-25 μM) at room temperature. λ_{ex} = 485 nm, λ_{em} = 516 nm.

The linear relationship in water

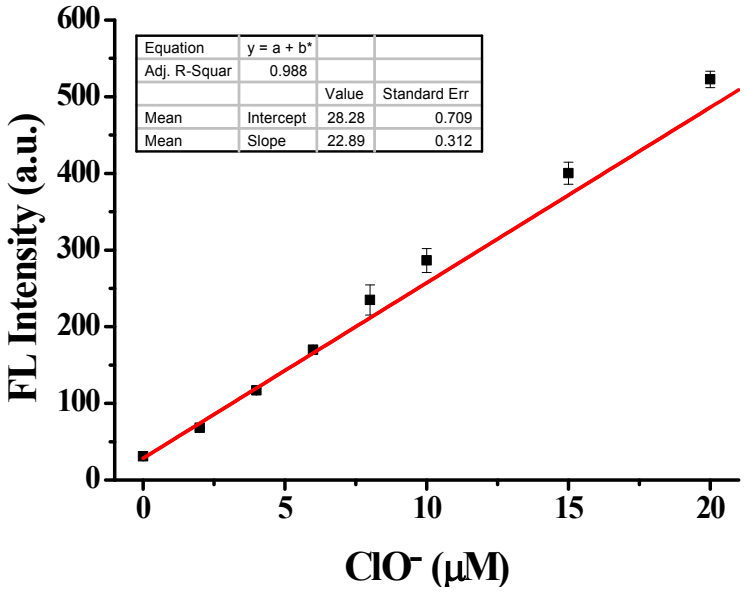

Fig.S3 The linear relationship between the fluorescent intensity of **YDN** (5 μM) and HClO concentration in water. All data were collected at 2 min after the addition of HClO. λ_{ex} = 485 nm, λ_{em} = 516 nm. Error bars standed for the mean value of three experiments.

DPV detection of PBS, HEPES and YDN

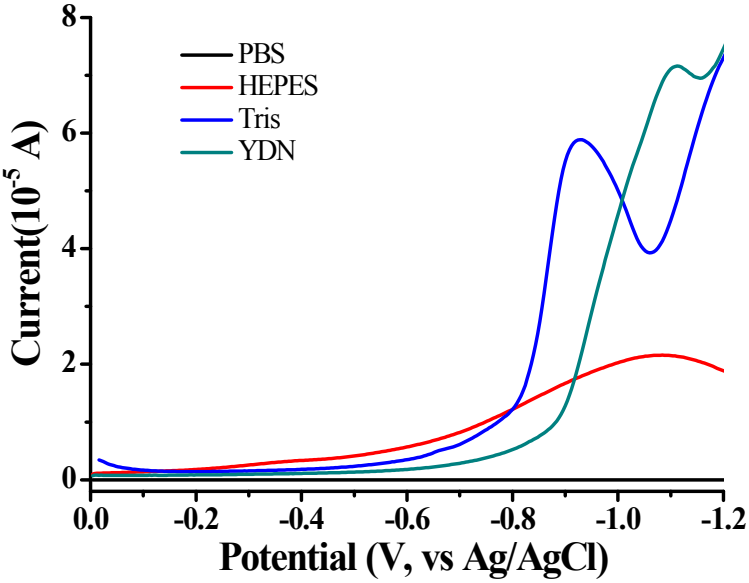

Fig.S4 Differential pulse voltammograms (DPV) of 10 mM PBS (black), 10 mM HEPES (red), 10 mM Tris (blue), and **YDN** (green, in 10 mM PBS). DPV was measured on a CHI 660D instrument using a three-electrode system consisting of a Ag/AgCl as reference electrode, a platinum wire as counter electrode, an ITO plate as working electrode, Scan rate: v = 25 mV s^{-1}.

Dynamics of YDN to HClO

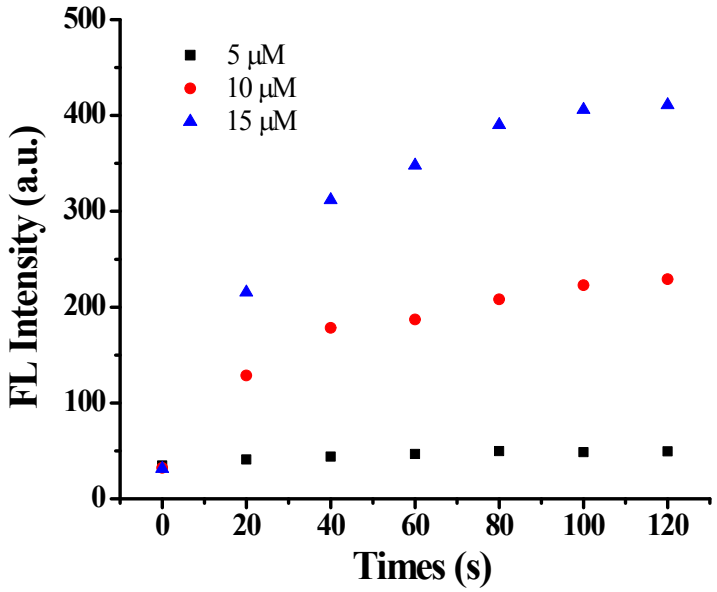

Fig.S5 Time-dependent fluorescence intensity changes of **YDN** (5 μM) at 516 nm upon addition of varied concentrations of HClO. All data were recorded in PBS buffer (10 mM, pH 7.4) at room temperature. λ_{ex} = 485 nm.

Absorbance response to HClO

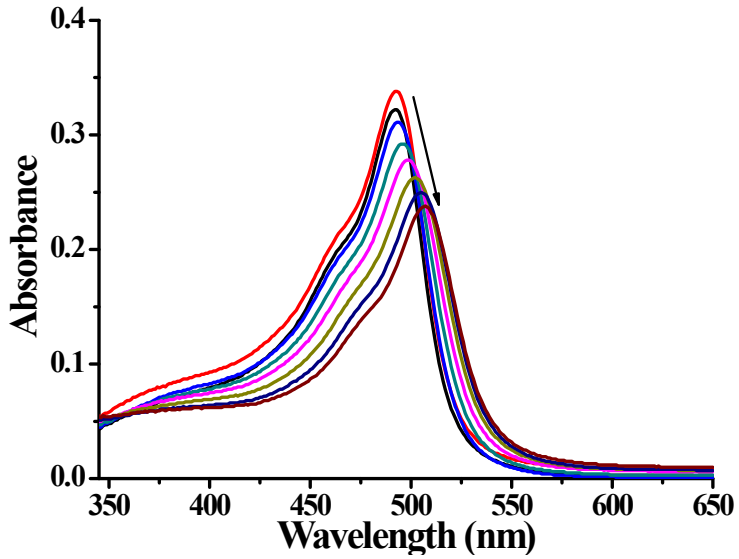

Fig.S6 Concentration-dependent absorption spectra of **YDN** (5 μM) in the presence of 0-25μM HClO in PBS buffer (pH = 7.4) at room temperature.

Comparison about the detection limits for HClO

Table S1 Comparison table about the detection limits for HClO

Probe	Linear range	Solvent	LOD (M)
Ref. 14	0−10 nM	PBS-EtOH, 9:1	5.6×10^{-10}
Ref. 16	0–80 μM	PBS-0.05% DMSO	1.79×10^{-8}
Ref. 19a	2–20 μM	PBS	3.0×10^{-7}
Ref. 19b	0–0.5 μM	PBS-CH_3CN, 1:9	9.3×10^{-8}
Ref. 19c	0–100 μM	CH_3CN-Water, 4:1	2.8×10^{-8}
Ref. 19d	0–10 μM	PBS-0.5% DMSO	4.3×10^{-7}
This work	0–23 μM	PBS	8.7×10^{-9}

Colour changes under natural light and hand-held UV lamp

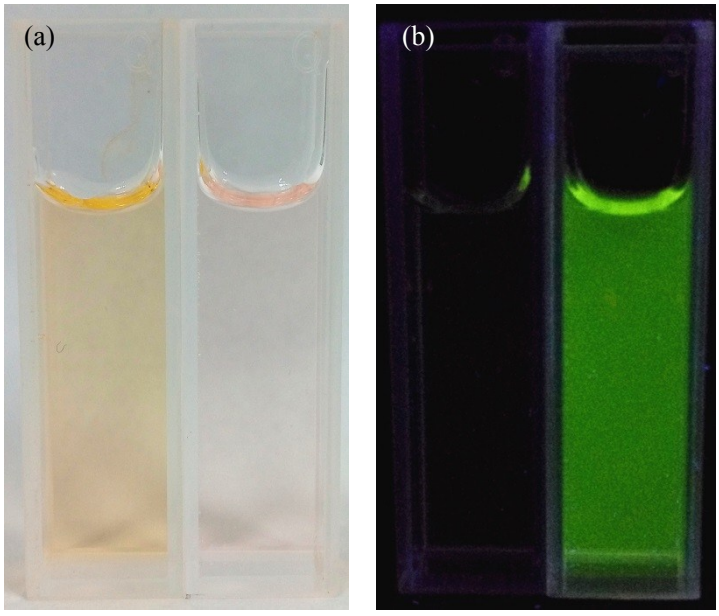

Fig.S7 (a) The colour changes under natural light of **YDN** (5 μM) in the absence and presence of HClO (25 μM, from left to right); (b) Fluorescence images of **YDN** (5 μM) in the absence and presence of HClO (25 μM, from left to right) upon excitation under a hand-held UV lamp (365 nm).

Fluorescence images of YDN to HClO and other ROS/RNS

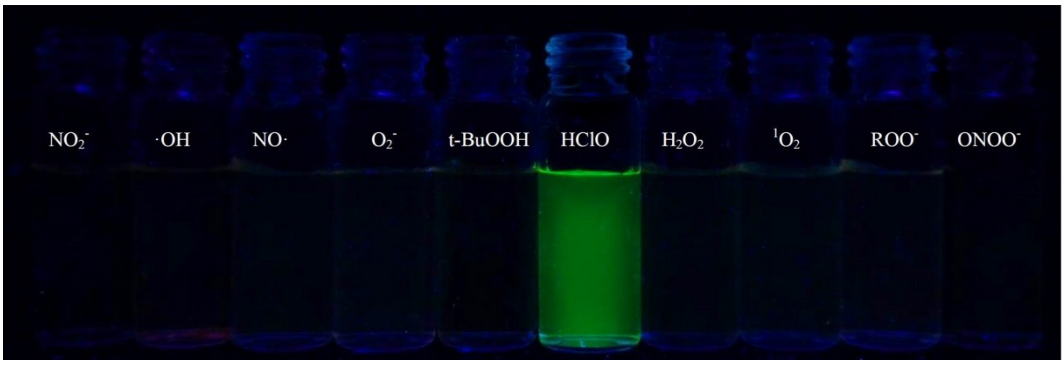

Fig.S8 Fluorescence images of **YDN** (5 μM) towards HClO (15 μM) and other ROS/RNS (200 μM for each) in PBS buffer (10 mM, pH 7.4) upon excitation under a hand-held UV lamp (365 nm).

Fluorescence response to HClO and other analytes

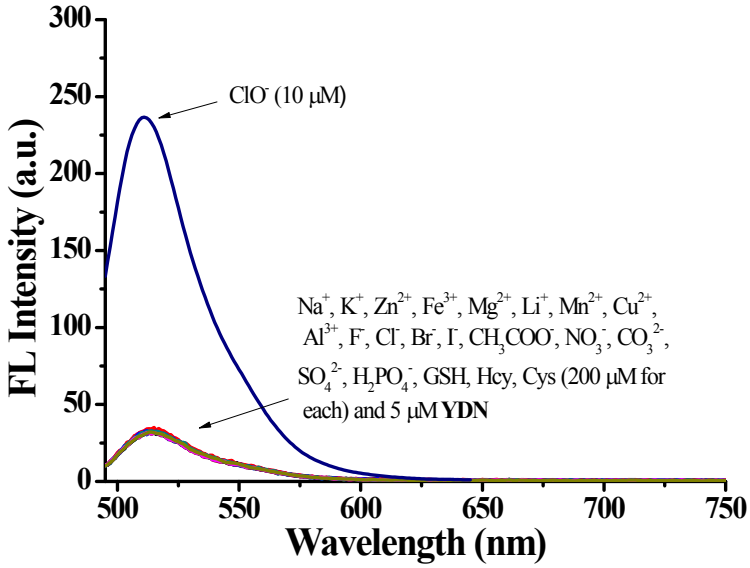

Fig.S9 Fluorescence spectra of **YDN** (5 μM) with the addition of 10 μM HClO or other analytes (200 μM for each) in PBS buffer (10 mM, pH 7.4) at room temperature. All data were collected 2 min after the addition of HClO. λ_{ex} = 485 nm, λ_{em} = 516 nm.

Cell imaging of YDN

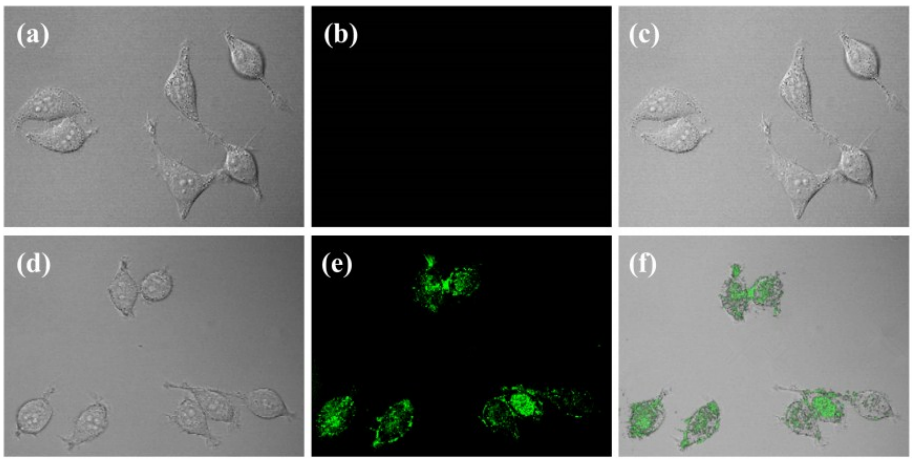

Fig. S10 Confocal fluorescence images of HClO in Hela cells. (a) Bright-field image of cells incubated with probe **YDN** (5 μM) for 30 min; (d) Bright-field image of cells after treatment with 20 μM HClO for 15 min and subsequent treatment with 5 μM **YDN** for 30 min; (b) and (e) Fluorescence-field images of the Hela cells in green emission (515–555 nm); (c) and (f) Overlap of bright-field and fluorescence. λ_{ex} = 488 nm.

MTT essay of YDN

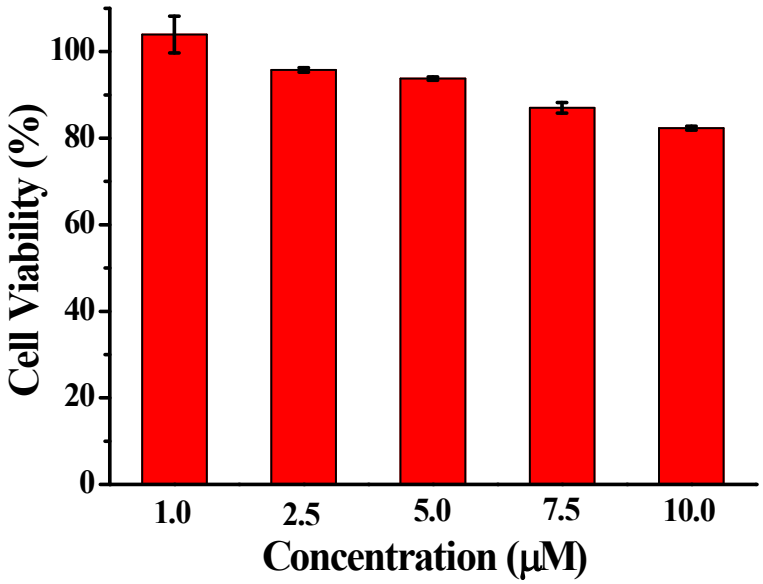

Fig.S11 Cell viability of Hela cells in the presence of different concentrations of **YDN** after 24 h of incubation determined by the MTT assay. Error bars standed for the mean value of five experiments.

Characterization

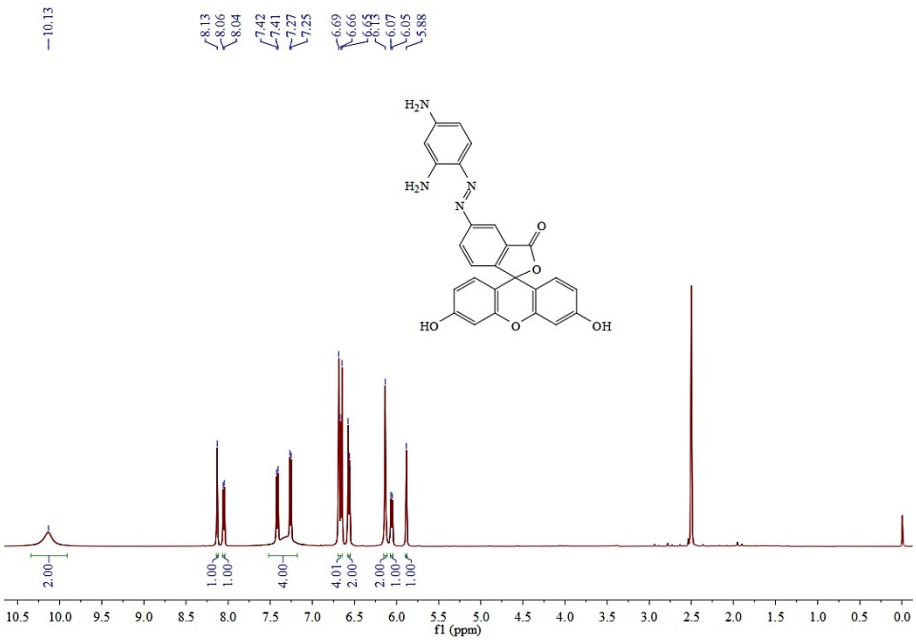

Fig.S12 ^{1}H NMR of **YDN** in DMSO-d_6.

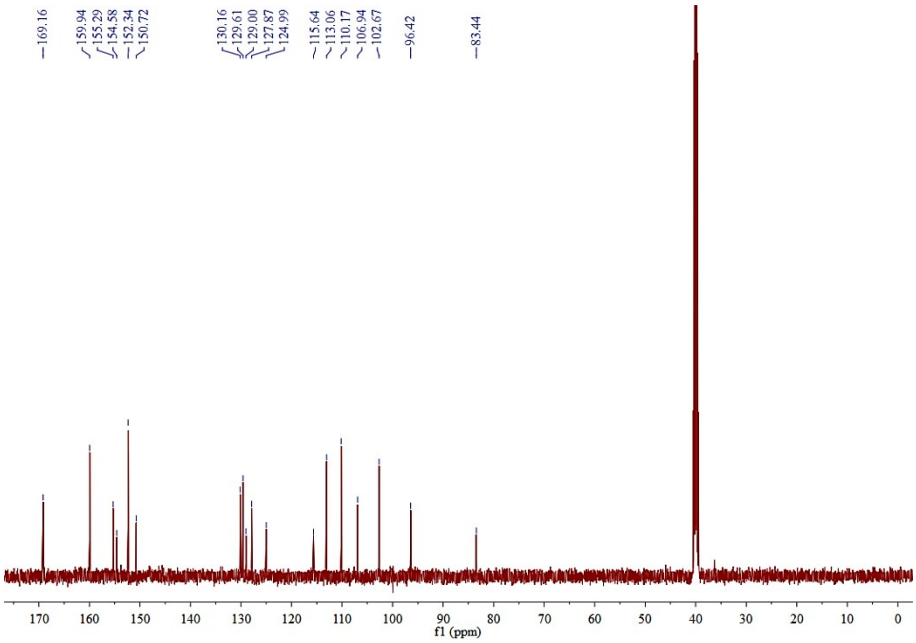

Fig.S13 ^{13}C NMR of **YDN** in DMSO-d_6.

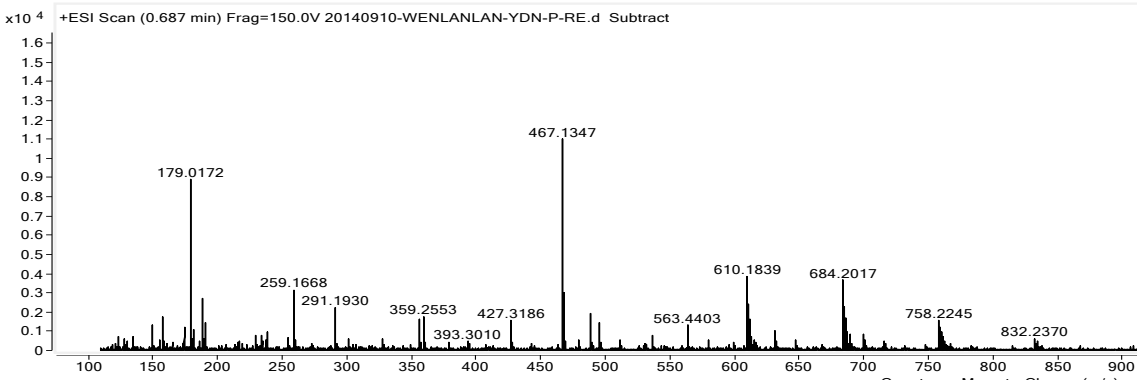

Fig.S14 HRMS of **YDN**.

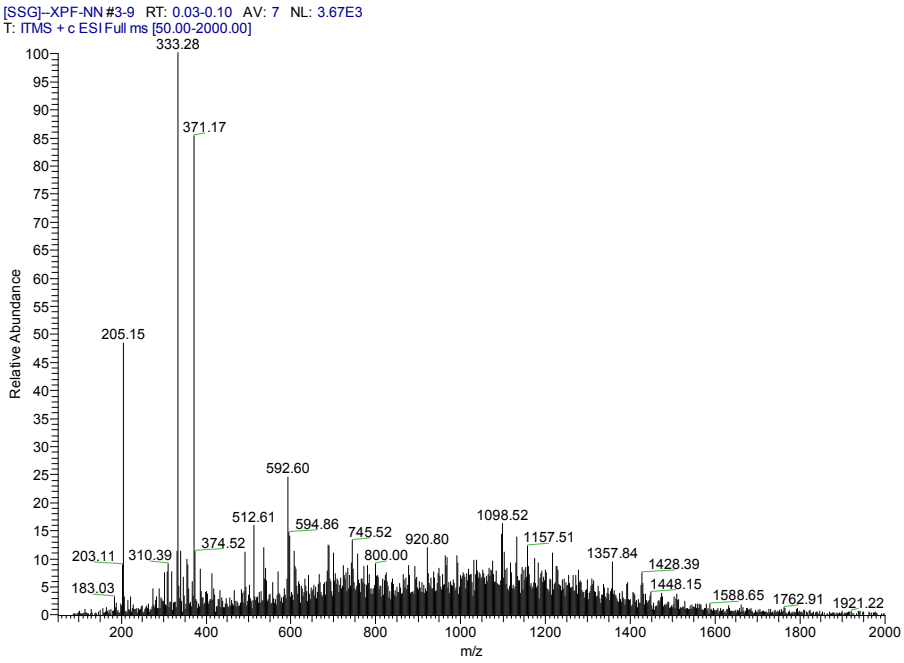

Fig.S15 MS spectra of **YDN** + HClO.

References

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