Supporting information for

A novel ER-targeted two-photon fluorescent probe for monitoring abnormal concentration of HClO in diabetic mice

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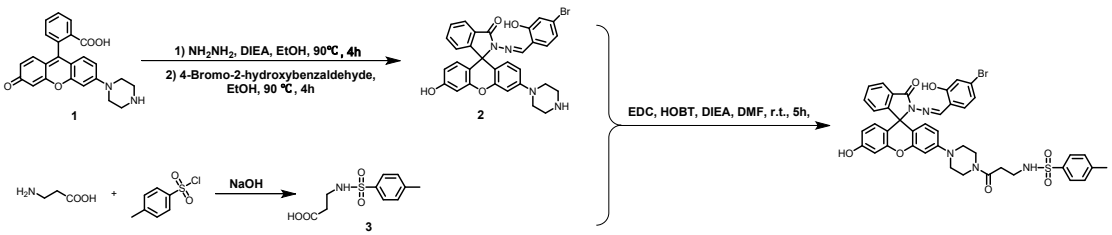
Contents

1 Materials and instruments

The reagents used in this experiment were commercially available and had not been subjected to any further treatment. The UV-Vis spectra were obtained by a UV-2700 spectrophotometer (Shimadzu, Japan). In addition, all fluorescence spectrum was tested at the room temperature using an F-4600 fluorescence spectrophotometer (Japan Hitachi). High resolution mass spectra was acquired on Aglient 7250& JEOL-JMS-T100LP AccuTOF (Bruker Daltonics, Billerica, MA, USA). And liquid chromatography-mass spectrometry (LC-MS) data were measured with an Agilent 6510 Q-TOF LC / MS (Agilent, USA). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE III 400 MHz digital nuclear magnetic resonance spectrometer (Bruker Biospin, Billerica, MA, USA) with an internal standard of tetramethylsilane (TMS).

2 The synthesis route of HClO-ER



Scheme S1. The synthetic route for **HClO-ER** .

Compound 1 was synthesized from existing literature[1]. Compound 1 (145 mg, 0.36 mmol), hydrazine hydrate (275 mg, 1.5 mmol) and DIEA (130 mg, 1.0 mmol) was dissolved in 50 mL of ethyl alcohol for 4 hours at 90℃. Then the 4-Bromo-2-hydroxybenzaldehyde (72.29 mg, 0.36 mmol) was added the reaction systerm and continue to strring for 4 hours at the same condition. After the reaction completed, ethyl alcohol was removed and the crude was purified by column chromatography (PE : EA=6:1, v/v) to get the compound 2 (130 mg, 60.5%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.80 (s, 2H), 8.51 (s, 3H), 8.39 (s, 1H), 7.96 (s, 2H), 7.92 (d, J = 9.3 Hz, 1H), 7.48 (d, J = 8.3 Hz, 2H), 6.80 (s, 2H), 6.62 (d, J = 2.3 Hz, 2H), 6.22 (s, 1H), 3.17 (s, 2H), 1.29 - 1.19 (m, 6H), 0.86 (t, J = 6.9 Hz, 1H).

β-aminopropionic acid (600 mg, 6.7 mmol) was dissolved in 4 mL ultrapure water, and 3.4 mL NaOH solution (2 M) and *p*-toluenesulfonyl chloride (1.83 g, 9.6 mmol) were added. And the mixture was stirred at 35℃ for 4 h. NaOH was added to keep the

pH of the solution at 9, and the reaction was continued for 1 hour after the base was completely dissolved. Then the residual *p*-toluenesulfonyl chloride was filtered to removed and pH was adjusted to 2 with HCl at 0℃ with a large number of precipitated white solids. Next, the solids were filtered and washed with distilled water for two times. Then the compound was dried at 35℃ and get the white aciculate crystallization 3. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.27 (s, 1H), 7.68 (d, J = 8.2 Hz, 2H), 7.59 (t, J = 5.7 Hz, 1H), 7.41 (d, J = 8.1 Hz, 2H), 2.91 (t, J = 9.9 Hz, 2H), 2.39 (s, 3H), 2.35 (t, J = 7.1 Hz, 2H).

Compound 2 (298.05 mg, 0.5 mmol), compound 3 (121.53 mg, 0.5 mmol), EDC (191.70 mg, 1 mmol), HOBT (33.78 mg, 0.25 mmol) and 100 μL DIEA were dissolved in 1.5 mL DMF, and the mixture was stirred at room temperature for 5 h under nitrogen atmosphere. After the reaction completed, the crude was purified by column chromatography (PE : EA=5:1, v/v) to get the **HClO-ER** (196 mg, 47.8%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.11 (s, 2H), 8.39 (s, 1H), 8.14 (d, J = 10.7 Hz, 1H), 8.03 (d, J = 14.3 Hz, 3H), 7.91 (d, J = 2.1 Hz, 2H), 7.68 (dd, J = 8.6, 3.8 Hz, 4H), 7.50 (dd, J = 5.9, 2.7 Hz, 2H), 7.39 (d, J = 8.0 Hz, 3H), 7.19-7.12 (m, 2H), 4.38 (s, 1H), 3.23-3.19 (m, 1H), 3.17-3.13 (m, 1H), 2.88 (t, J = 7.3 Hz, 4H), 2.38 (s, 5H), 2.24 (t, J = 7.2 Hz, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.57, 167.17, 166.02, 162.02, 161.37, 160.47, 159.51, 144.54, 143.33, 143.11, 137.79, 130.10, 127.92, 127.03, 123.85, 123.24, 118.63, 112.57, 111.60, 110.18, 102.86, 44.89, 35.43, 21.42, 18.75. $[M]^+$ 821.1519 ; $[M]^+$ found 821.1530; $[M+NH_4]^+$ found 841.1825.

3 General procedures for spectral test

The probe **HClO-ER** was prepared with dimethyl sulfoxide (DMSO) into reserve liquid with a concentration of 10 mM. The final test concentration of probe is 5 μM in the 4 mL PBS buffer containing 5% ethanol. Before testing the spectrum, a certain amount of NaClO solution is added to the probe solution and mixed. All test parameters were fired at 500 nm.

4 Two-photon active cross-section (δΦ)

The two-photon absorption cross-section (δΦ) of the probe was measured with fluorescein as reference by two-photon fluorescence induction method.

$$\delta_2 = \delta_1 \times \frac{F_2}{F_1} \times \frac{\phi_1}{\phi_2} \times \frac{c_1}{c_2}$$

F_1 and F_2 are the two-photon induced fluorescence intensity of the standard sample and the sample to be measured; φ1 and φ2 respectively represent the fluorescence quantum yield of the standard sample and the sample to be measured; c1 and c2 represent the solution concentration of the standard sample and the sample to be tested; δ_1 and δ_2 are the two-photon absorption cross-section of the standard sample and the sample to be tested.

5 Cells culture and imaging

HeLa cells were cultured in DMEM (Dulbecco's Modified Eagle's medium) containing 10% FBS and 1% double antibody at 37℃ in air containing 5% CO_2. After incubation for 24 h, the original medium was removed and new medium was added. Then the cell was incubated using 5 μM for 30 minutes. Later, the cell was washed with sterile PBS (pH 7.4) buffer for 3 times and then treated with 100 μM and 200 μM NaClO for 30 min respectively for imaging.

Raw 264.7 cells were cultured in RPIM1640 medium containing 10% FBS and 1% double antibody in air containing 5% CO_2 at 37 ℃. After 24 hours of culture, the original medium in the culture dish was washed, and then the first group were incubated with 10 μM DTT (dithiothreitol) for 1 h. The second group was incubated with 20 μM β-ME (β-mercaptoethanol) for 2 h. The third group was incubated with 4 mmol glucose for 2.5 h. Later, the cell was washed three times with sterile PBS buffer (pH 7.4) and incubated with 5 μM **HClO-ER** for 30 min for imaging.

6 Zebrafish and tissue culture and imaging

The zebrafish used in the experiment were purchased from Nanjing Yishu Lihua Biological Technology Co., Ltd. The zebrafish was placed on a clean glass-bottom petri dish. Then zebrafish was incubated using 5 μM **HClO-ER** for 60 min. Later, the zebrafish was treated with 100 μM and 200 μM NaClO for 60 min respectively for imaging. In addition, zebrafish were stimulated with drugs and fluorescence imaging was performed. The first group is that Zebrafish was pretreated with 5 μM **HClO-ER** for 60 min were then incubated with 10 μM DTT for 1.5 h. The second group was that zebrafish was pretreated with 5 μM **HClO-ER** for 60 min were then incubated with 20 μM β-ME for 2.5 h. The last group was that zebrafish was pretreated with 5 μM **HClO-ER** for 30 min were then incubated with 4 mmol glucose for 3 h.

The animals were purchased from School of Pharmaceutical Sciences, Guangxi Medical University, and the studies were approved by the Animal Ethical Experimentation Committee of Guangxi Medical University. All animals were kept during experiment according to the requirements of the National Act on the use of experimental animals (China).The establishment of diabetic mouse model was carried out according to literature[2]. After the model was established, the tissue was sectioned and two-photon imaging was performed.

7 Supplementary Figure

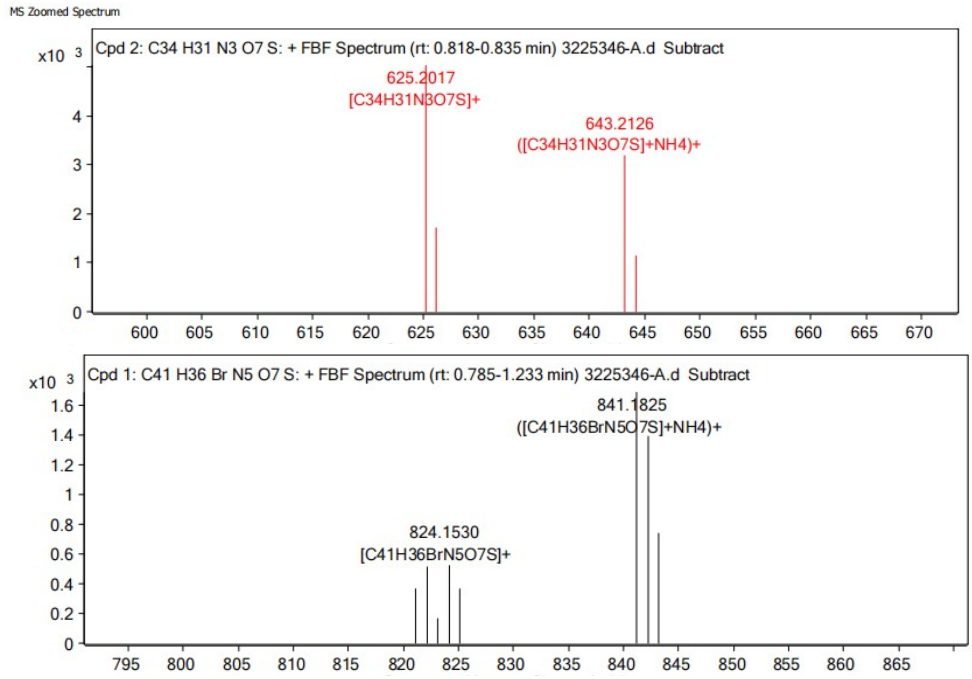


Fig. S1. HRMS data of the probe **HClO-ER** in presence of NaCl**O** in PBS.

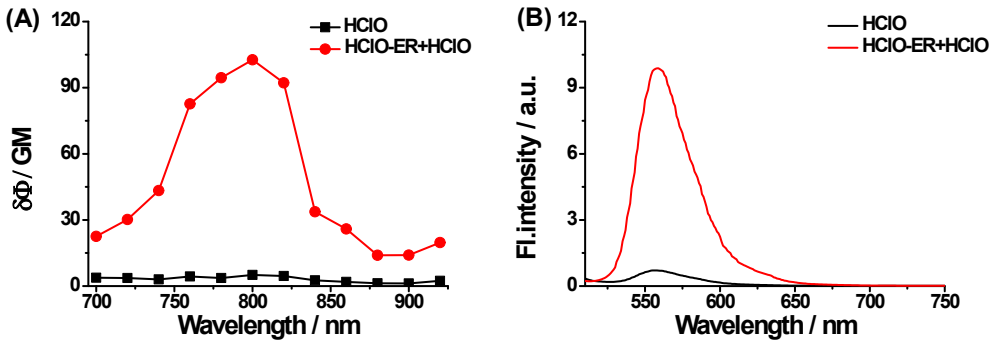


Fig. S2. Two-photon absorption cross-section (δΦ) (A) and fluorescence spectra (B) of HClO (5 μM) with or without HClO (200 μM) in PBS. (pH = 7.4, 0.1 M), λ_{ex}= 800 nm.

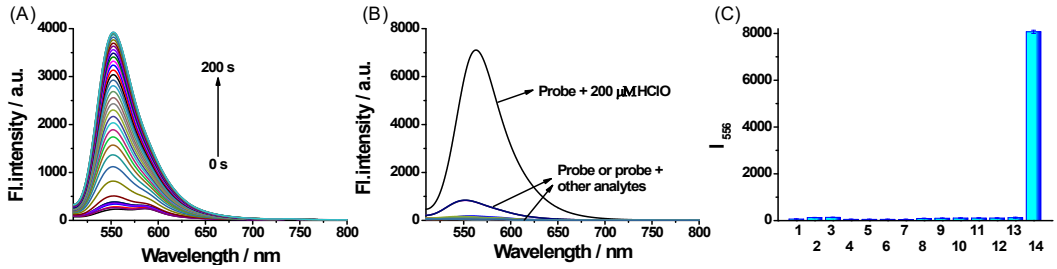


Fig. S3. (A) Time-dependent fluorescence spectra of 5 μM **HClO-ER** in the presence of 300 μM NaClO (10 mM, 5% EtOH, pH = 7.4) in PBS. (B) Comparison of probe with HClO or other analytes. (C) Fluorescence spectra intensity at 556 nm of 5 μM HClO-ER toward various species in PBS buffer (10 mM, 5% EtOH, pH = 7.4) under excitation at 500 nm. The anions and molecules are as follows: Blank; Na^+ (10 mM); Ca^{2+} (70 μM); Mg^{2+} (500 μM); K^+ (180 mM); Zn^{2+} (16 μM); Fe^{2+} (100 μM); Cu^{2+} (1 mM); H_2O_2 (500 μM); SCN^- (120 mM); glucose (5 mM); Cys (360 μM); Hcy (50 μM); GSH (7 mM); OCl^- (400 μM).

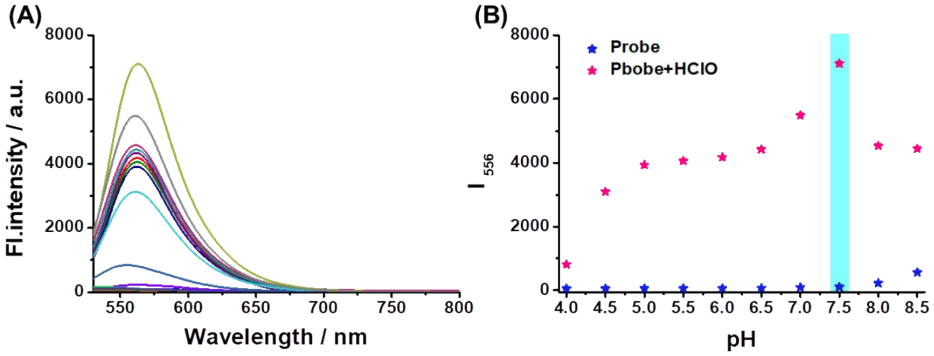


Fig. S4. Fluorescence intensities at 556 nm of 5 μM **HClO-ER** in the absence and presence of 200 μM NaClO at various pH.

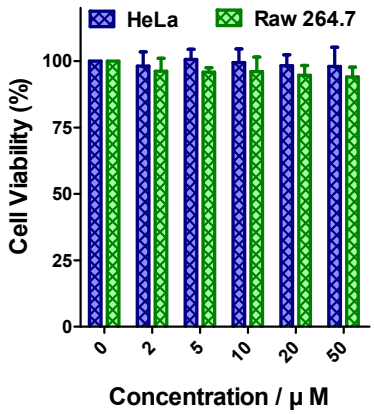


Fig. S5. MTT assay of HeLa and Raw264.7 cells in the presence of various concentrations of **HClO-ER**.

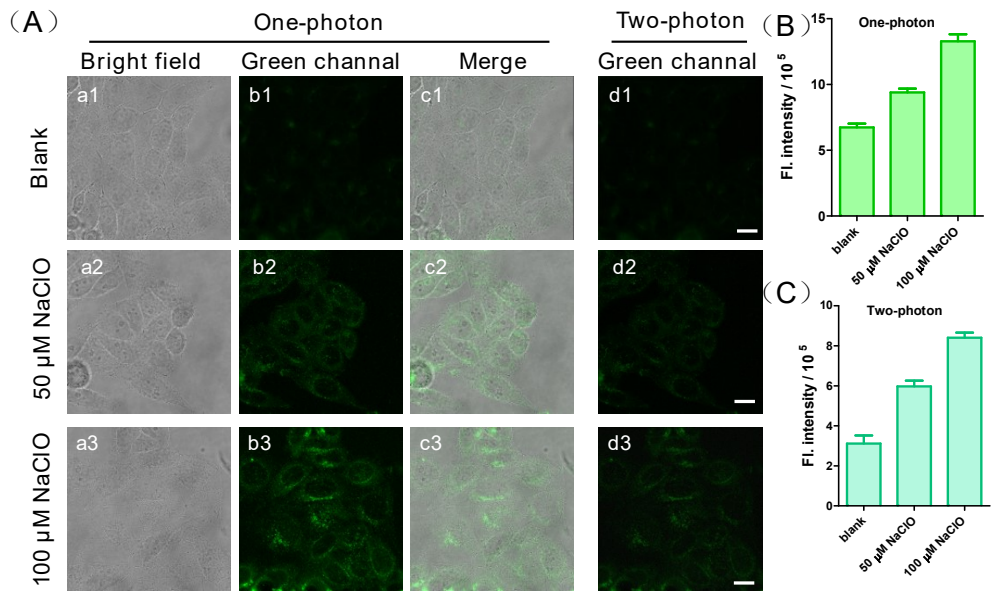


Fig. S6. (A) Confocal fluorescence images of **HClO-ER** in HeLa cells incubated with different concentrations of NaClO. a1-d1: Cells treated with 5 μM **HClO-ER** for 30 min. a2-d2: Cells included with 5 μM **HClO-ER** for 30 min were then incubated with 50 μM NaClO for 30 min. a3-d3: Cells pretreated with 5 μM **HClO-ER** for 30 min were then incubated with 100 μM NaClO for 30 min. One-photon channel: λ_{em} = 510- 610 nm, λ_{ex} =500 nm. Two-photon channel: λ_{em} = 510-610 nm, λ_{ex} =800 nm. Scale bar: 10 μm. (B-C) Quantitative Analysis of one-photon and two-photon fluorescence intensity in (A) using ImageJ software. The error bars represent the standard deviation (±S.D.), n = 5.

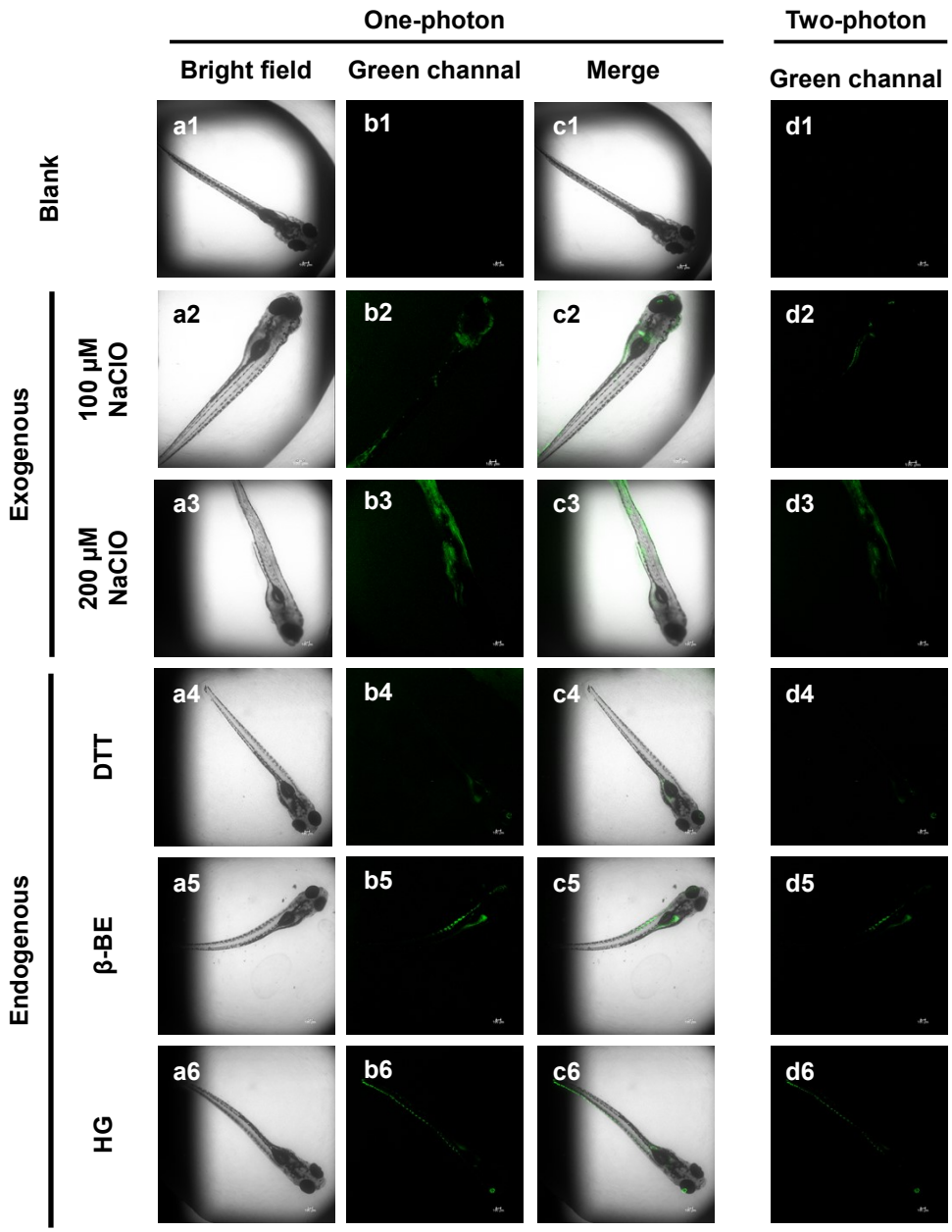


Fig. S7. Confocal fluorescence images of **HClO-ER** in zebrafish incubated with different concentrations of HClO or different irritant. a1-d1: zebrafish treated with 5 μM **HClO-ER** for 60 min. a2-d2: Zebrafish included with 5 μM for 60 min were then incubated with 100 μM NaClO for 60 min. a3-d3: Zebrafish pretreated with 5 μM for 60 min were then incubated with 200 μM NaClO for 60 min. a4-d4: Zebrafish included with 5 μM **HClO-ER** for 60 min were then incubated with 10 μM DTT for 1.5 h. a5-d5: Zebrafish pretreated with 5 μM **HClO-ER** for 60 min were then incubated with 20 μM β-ME for 2.5 h. a6-d6: Zebrafish pretreated with 5 μM **HClO-ER** for 30 min were then incubated with 4 mmol glucose for 3 h. One-photon channel: λ_{em} = 510-610 nm, λ_{ex} = 500 nm. Two-photon channel: λ_{em} = 510-610 nm, λ_{ex} = 800 nm. Scale bar: 100 μm.

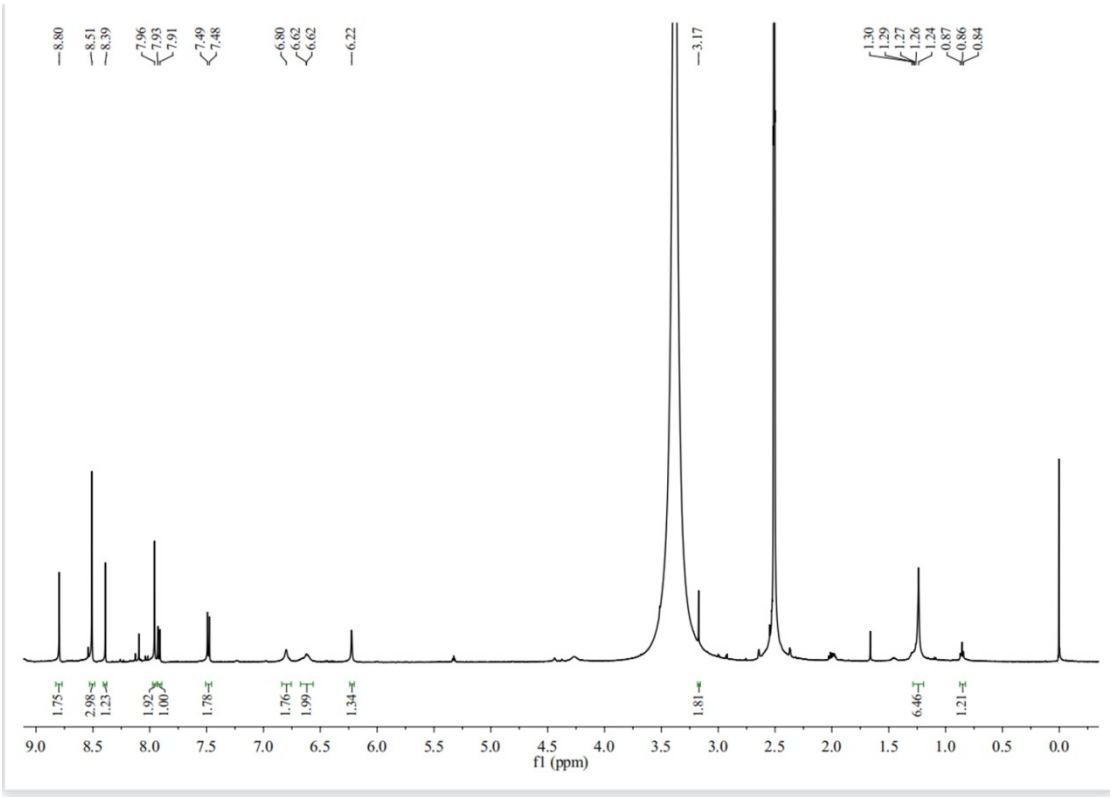


Fig. S8. ^{1}H NMR data of compound 2(DMSO-d_6).

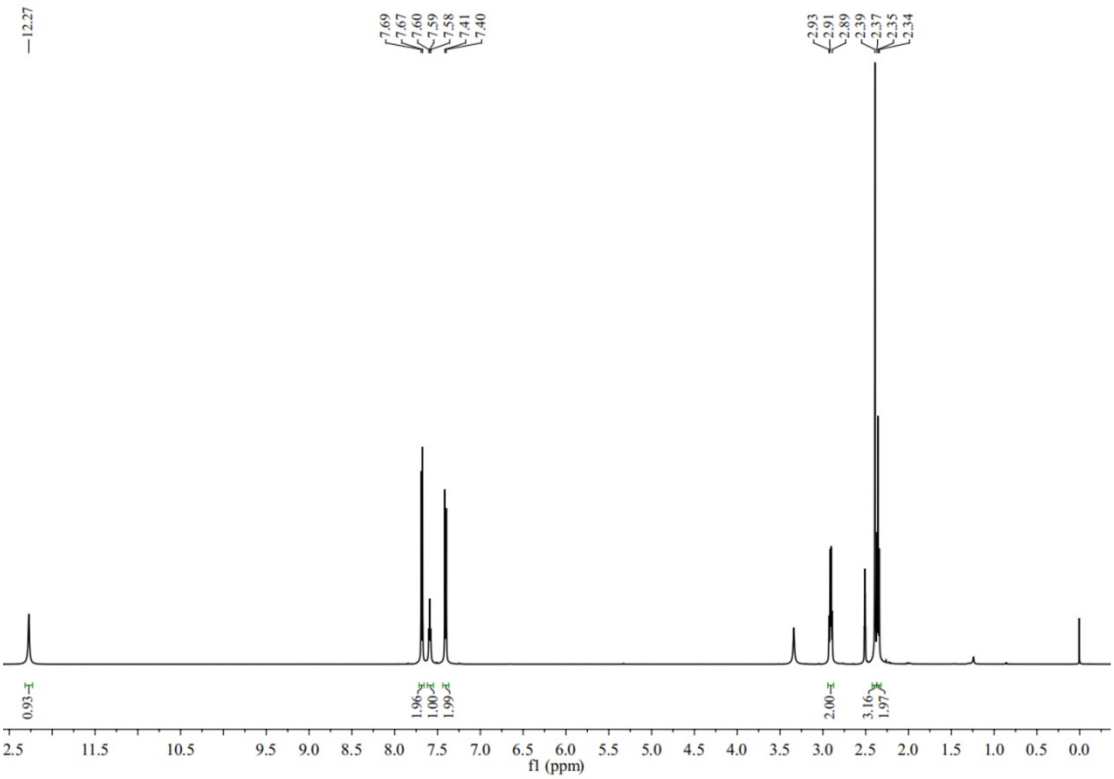


Fig. S9. ^{1}H NMR data of compound 3(DMSO-d_6).

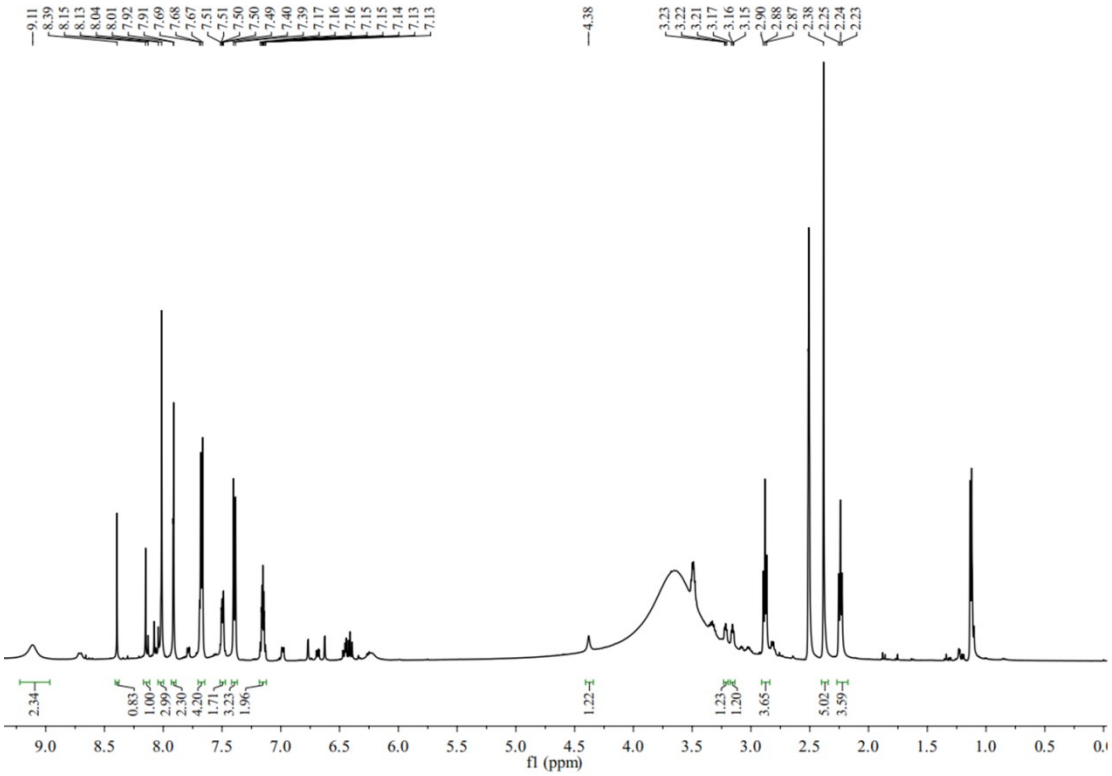


Fig. S10. ^{1}H NMR data of **HClO-ER** (DMSO-d_6).

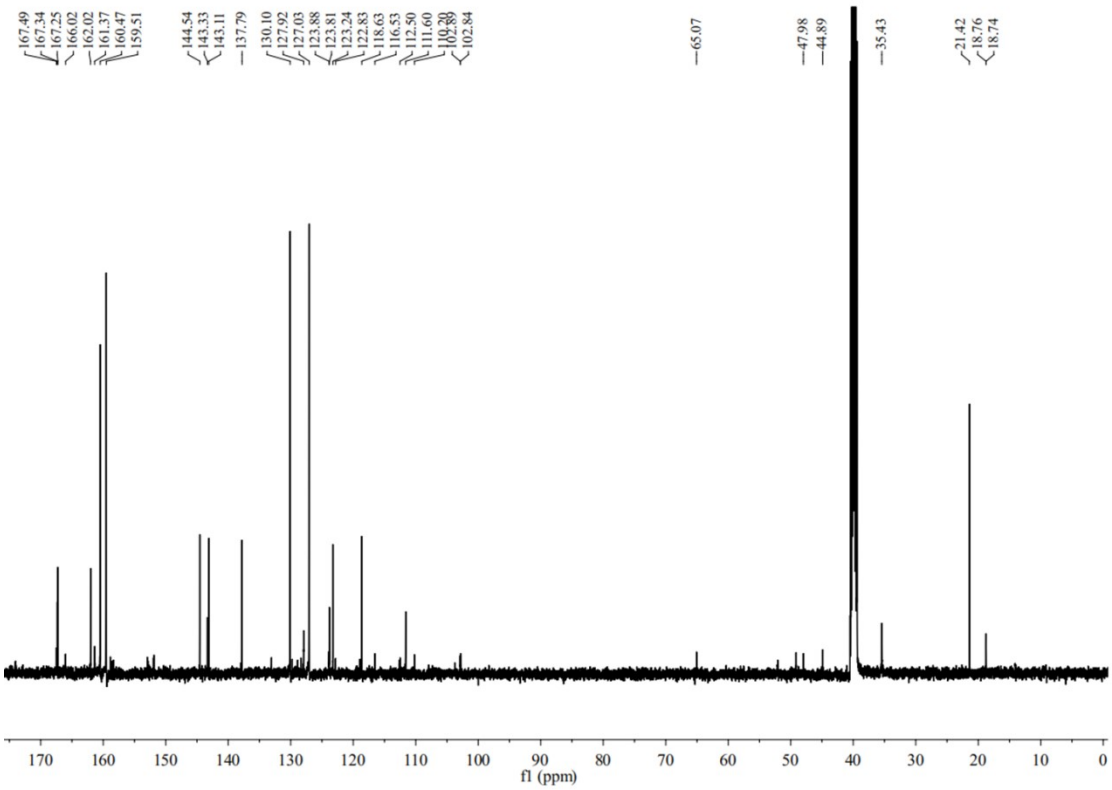


Fig. S11. ^{13}C NMR data of **HClO-ER** (DMSO-d_6).

References

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2. W. Song, B. Dong, Y. Lu, Z. Li, W. Zhang and W. Lin, *Sens Actuators B Chem.*, 2021, **332**, 129537.