Electronic Supplementary Information (ESI†)

A new red fluorescent probe based on the rosamine-phenothiazine for highly selective and rapid detection of hypochlorite and its bioimaging in live cells

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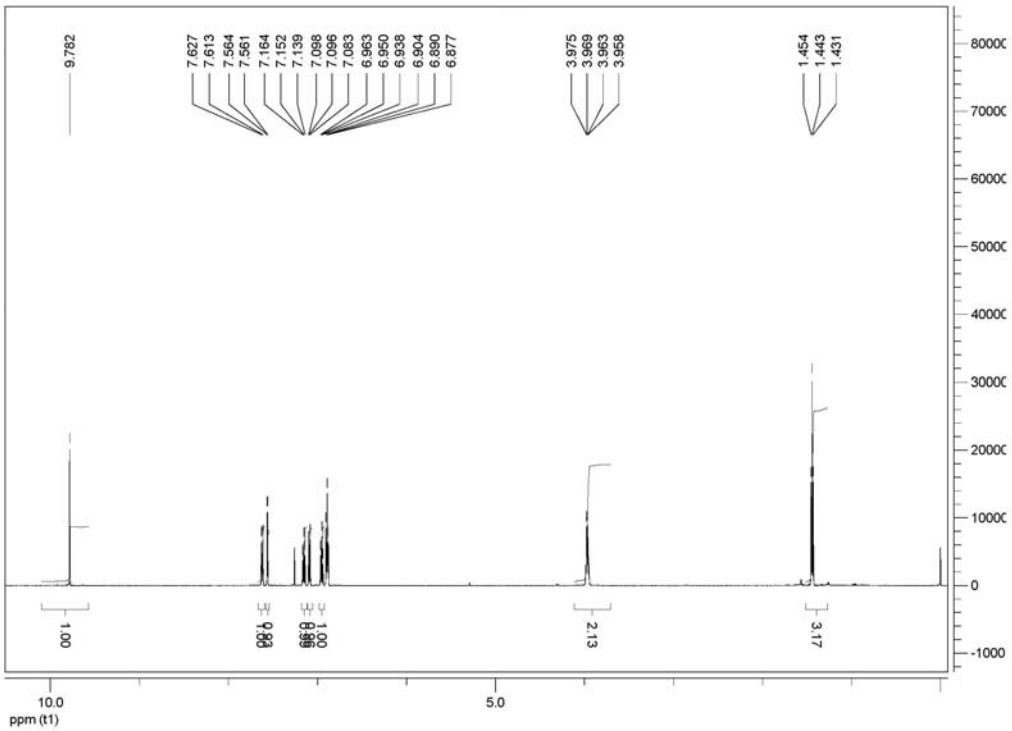


Fig. S1 ^{1}H NMR chart of compound **M1** ($CDCl_3$, 600 MHz).

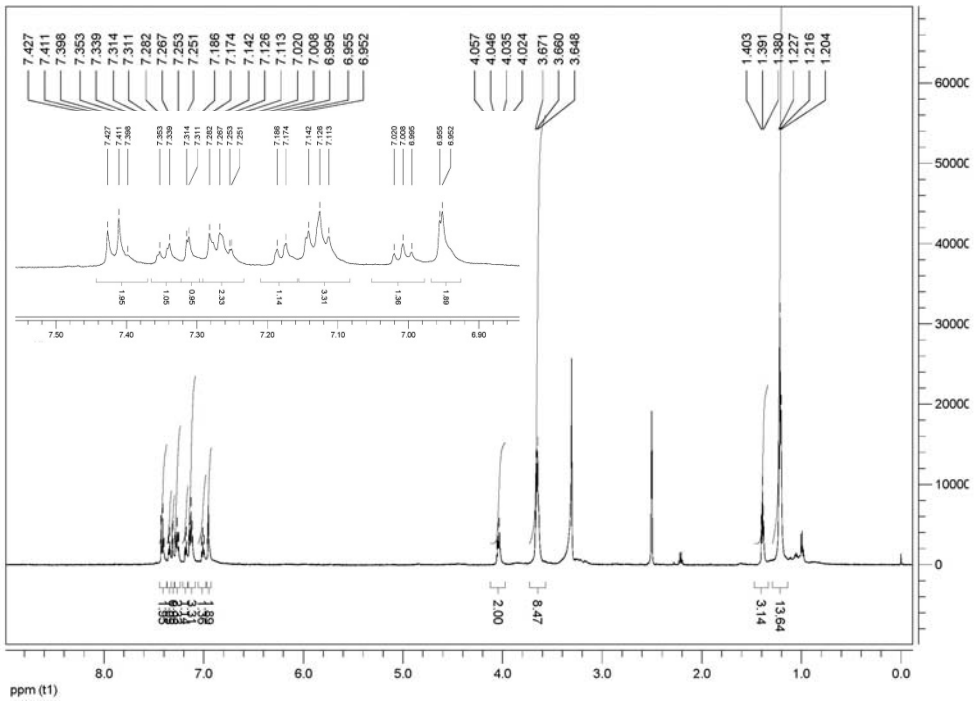


Fig. S2 ^{1}H NMR chart of compound **RClO** ($CDCl_3$, 600 MHz).

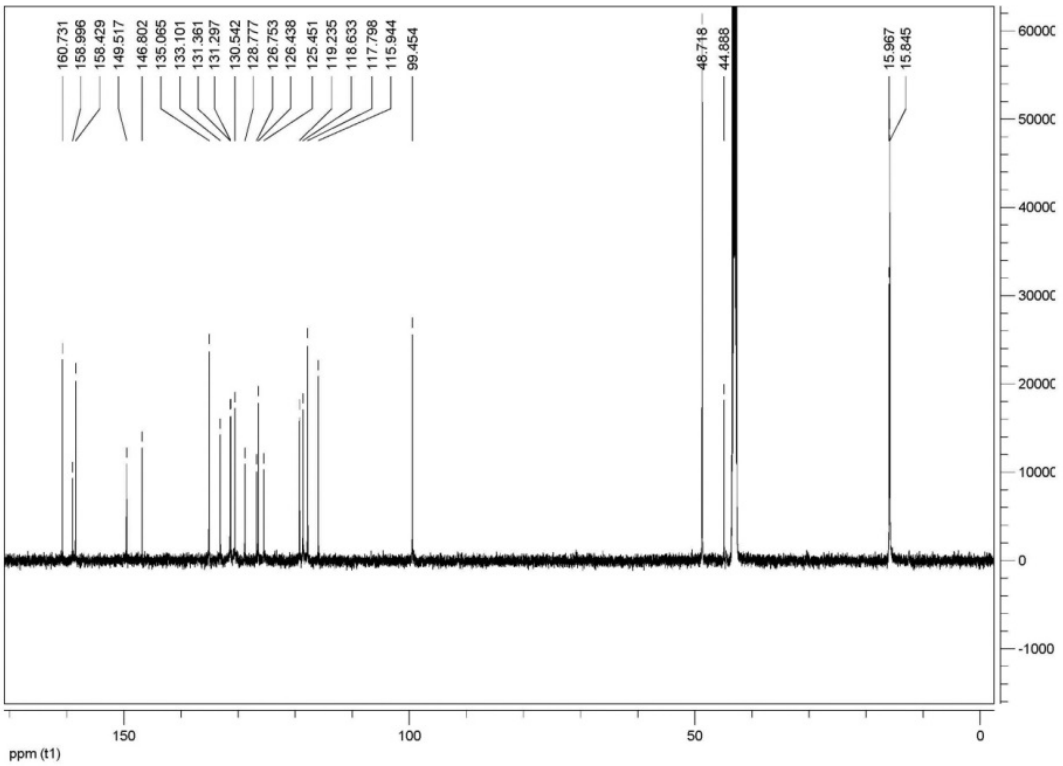


Fig. S3 ^{13}C NMR chart of compound **RClO** ($CDCl_3$, 150 MHz).

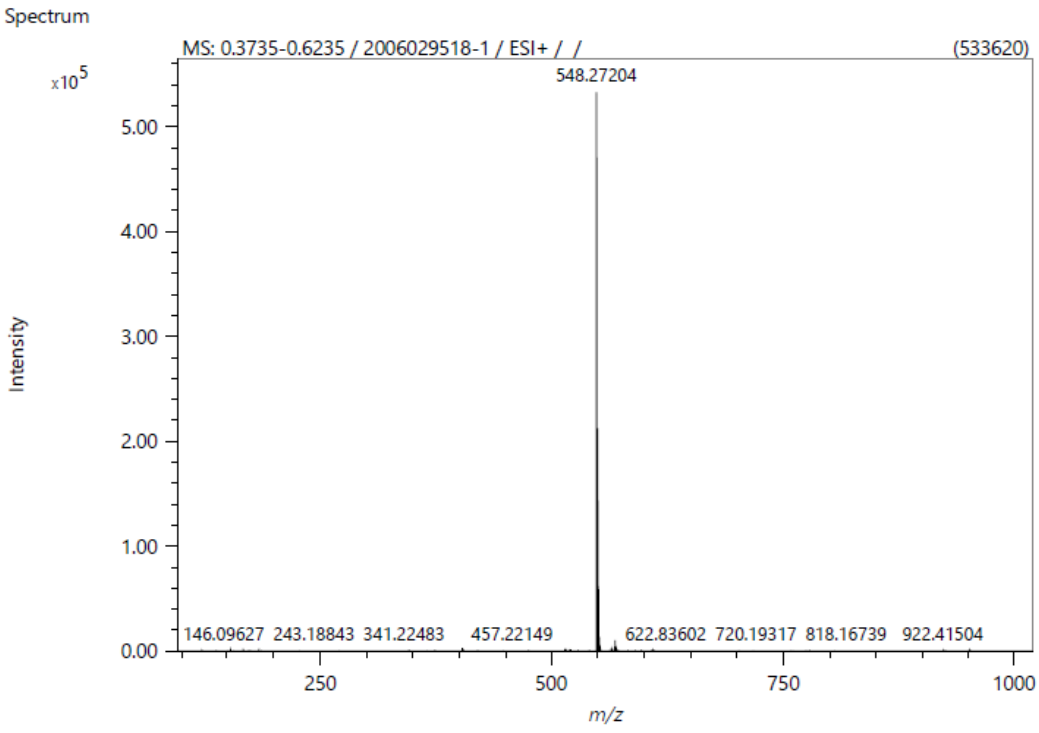


Fig. S4 HRMS chart of compound **RClO**.

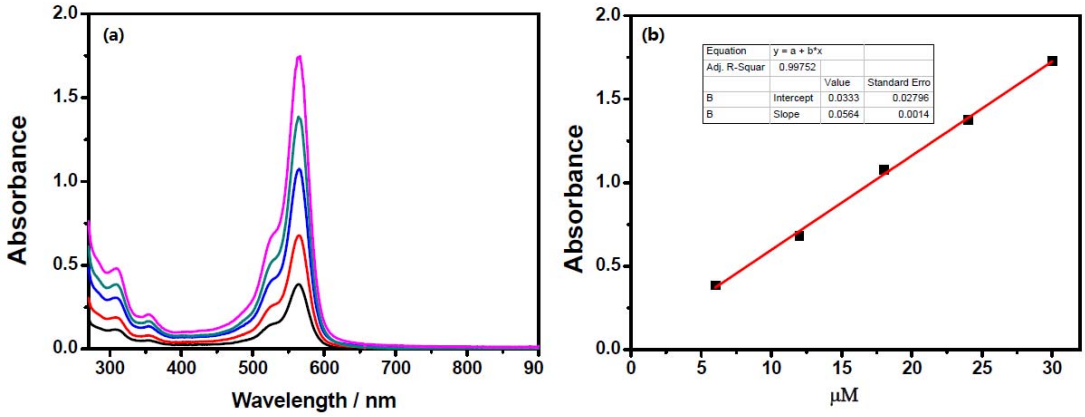


Fig. S5 The solubility of probe **RClO**. (a) Absorption spectra of the probe **RClO** in PB buffer solution (pH 7.4, containing 20 % DMF as a co-solvent) and (b) plot of Absorbance against probe **RClO** concentration 6-36 μM in PBS buffer solution.

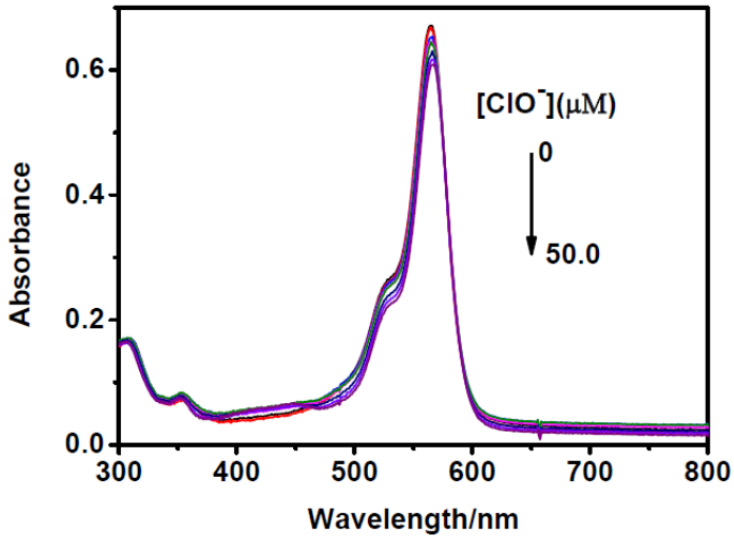


Fig. S6 Absorption respones of the probe **RClO** (12 μM) in the presence of ClO^- (0−50.0μM) in PB buffer (pH 7.4, 20 mM, containing 20% DMF).

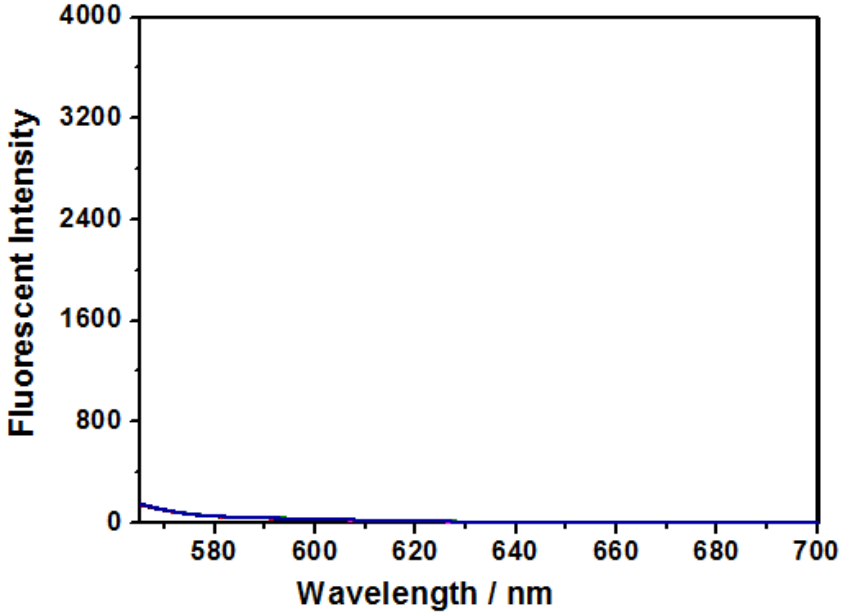


Fig. S7. Time-dependent fluorescence spectra of probe **RClO** (12 μM) excited at 560 nm in PB buffer (pH 7.4, 20 mM, containing 20% DMF). Time scale: 0–30 min. Ex/Em Slit: 5.0 nm, MT Voltage: 600 V

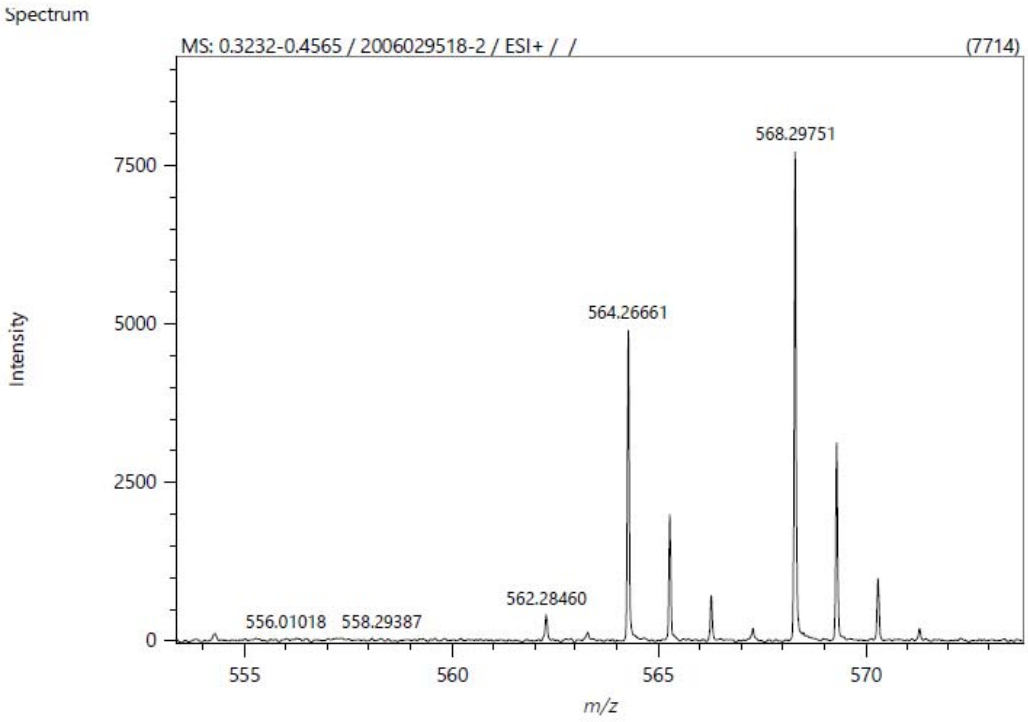


Fig. S8 HRMS charts of probe **RClO** upon treatment with ClO^-.

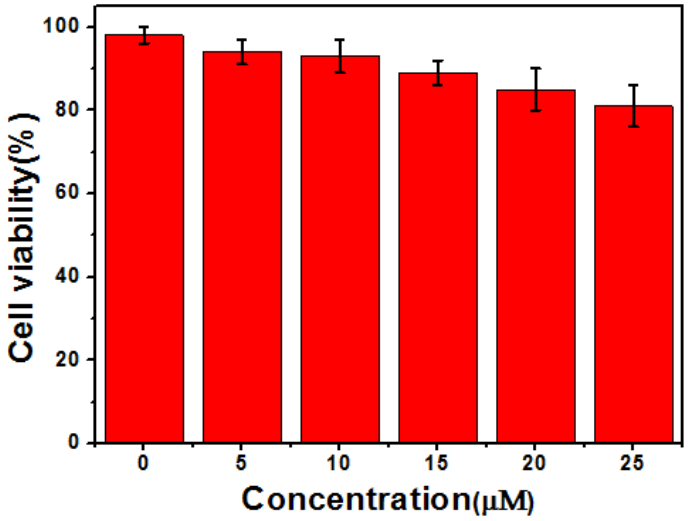


Fig. S9 Cell viability of HepG2 cells after treated with different concentrations of probe **RClO** for 12 h. Cell viability was assayed by the MTT method (values: mean ± standard deviation).

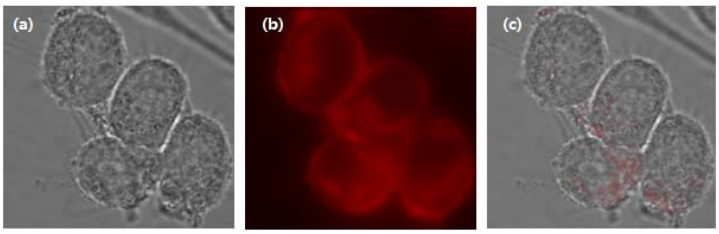
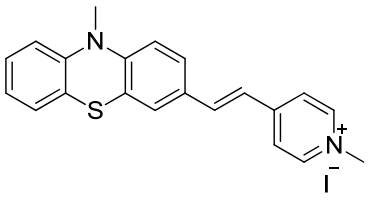
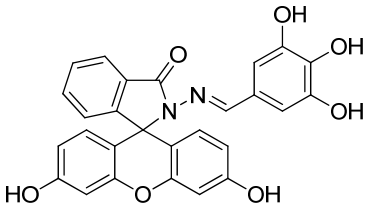
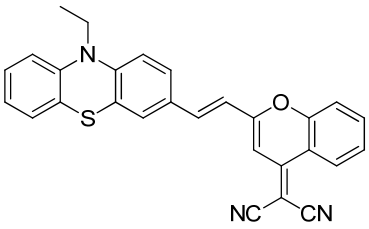
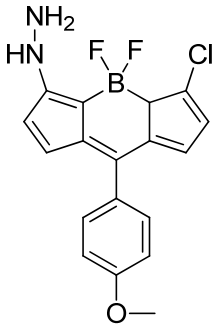


Fig. S10 Fluorescence images were obtained in a EVOS FL Auto imaging system. (a) Bright field image of the HepG2 cells; (b) HepG2 cells were pretreated with probe **RClO** (12μM) for 30 min and subsequently treated with ClO^- (25μM) for another 30 min ; (c) overlay of (a) and (b) in bright field image;

Table S1. Comparison of probe **RClO** for the detection of ClO^-

Reference	**Response time**	**Detection limit**	**Probe structure**
Chem. Commun., 2015[1]	5 min	17.9nM	

Sens. Actuators B: Chem.，2016 [2]	30min	500nM	
Sensors and Actuators B, 2018[3]	-----	720nM	
Dyes Pigments, 2016[4]	1min	93nM	
This work	20s	17.8 nM	PBS buffer (pH 7.4, 20 mM, 20% DMF

[1] Xiao H, Xin K, Dou H, Yin G, Quan Y, Wang R. A fast-responsive mitochondria-targeted fluorescent probe detecting endogenous hypochlorite in living RAW 264.7 cells and nude mouse. Chemical Communications 2015;51:1442-1445.

[2] Jin X, Jia Y, Chen W, Chui P, Yang Z, A reaction-based fluorescent probe for rapid detection of hypochlorite in tap water, serum, and living cells, Sens. Actuators B: Chem. 232 (2016) 300-305.

[3] Deng B B, Ren M G, Kong X Q, Zhou K, Lin W Y. Development of an enhanced turn-on fluorescent HOCl probe with a large Stokes shift and its use for imaging

HOCl in cells and zebrafish. Sensors and Actuators B-Chemical 2018;255:963-969.

[4] Cheng T, Zhao J, Wang Z, An J, Xu Y, Qian X, Liu G, A highly sensitive and selective hypochlorite fluorescent probe based on oxidation of hydrazine via free radical mechanism, Dyes Pigments 126 (2016) 218-223

Table S2. Repeatability and reproducibility of probe **RClO** sensing ClO^- in 20 mM potassium phosphate buffer / DMF (1:1 v/v, pH 7.4) at room temperature.

Sample	ClO^- spiked (μM)	S_R	RSD_R (%)
S1	10	23.5	2.86
S2	20	25.5	1.39
S3	30	52.8	1.89
S4	40	28.9	0.79
S5	50	57.8	1.48

Number of independent measurements per sample: n=3; S_R: reproducibility standard deviation; RSD_R (%): reproducibility relative standard deviation.

Determination of relative standard deviation (RSD): The RSD were calculated based on the following equations.

$$\bar{x} = \frac{x_1 + x_2 + \cdots + x_n}{n}$$

$$s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$$

$$\mathrm{RSD} = \frac{s}{\bar{x}} \times 100\%$$

Where x_1, ..., x_n are measured values of the sample items; $\bar{x}$ is the mean value of these measurements; n is the total number of measurements; *i* is the number of measurement; s is the standard deviation.