Electronic supplementary information for

Phenothiazine and semi-cyanine based colorimetric and fluorescent probe for the rapid detection of hypochlorous acid

Zhong-Chao Lin and Xue-Fei Wang*

Department of Biological Science and Biotechnology, School of Chemical Engineering and Life Sciences, Wuhan University of Technology, Wuhan, 430070, China.

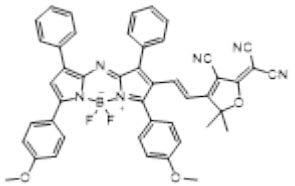
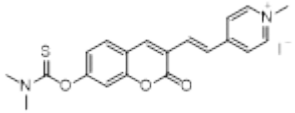
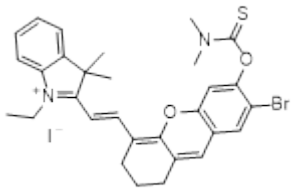
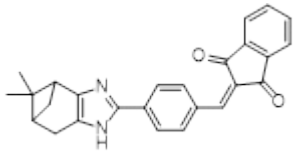
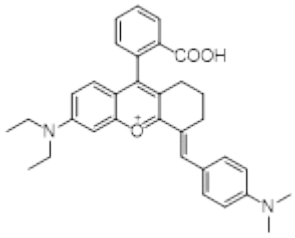
*Corresponding author: Xue-Fei Wang.

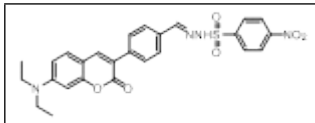
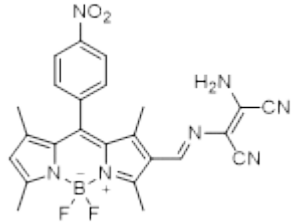
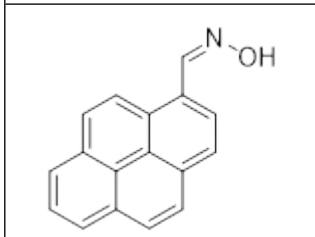
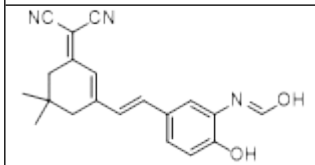
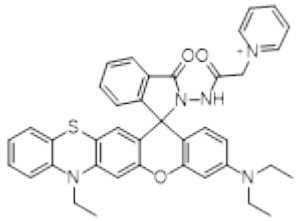
E-mail address: xuefei@whut.edu.cn.

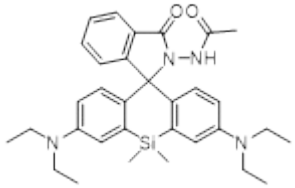
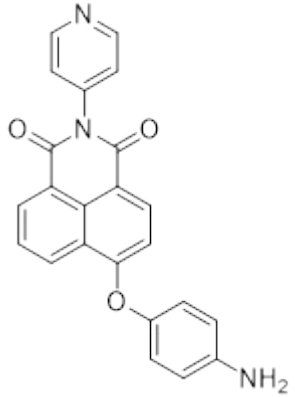
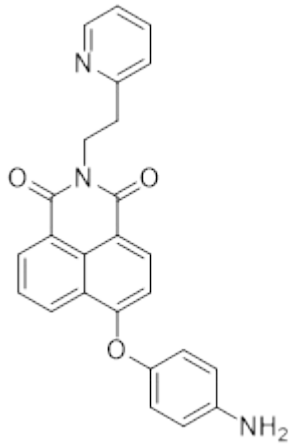
Contents

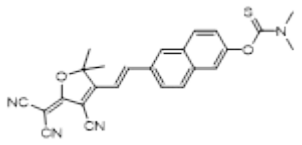
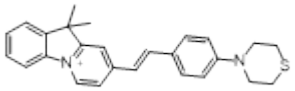
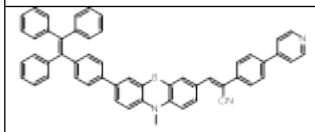
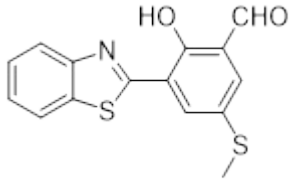
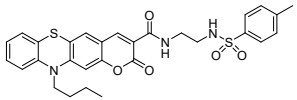
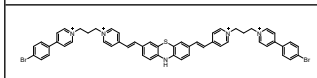
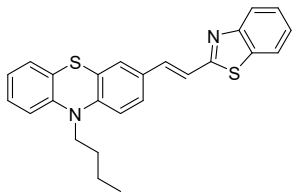
1. Properties of reported probes

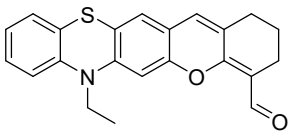
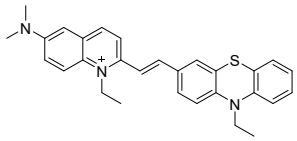
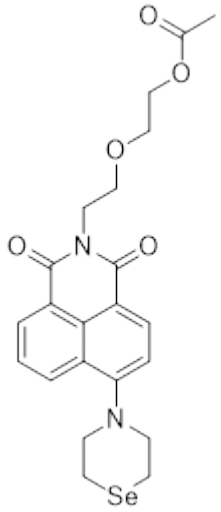
Table S1. Summary of the relevant properties of reported probes

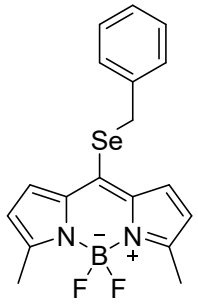
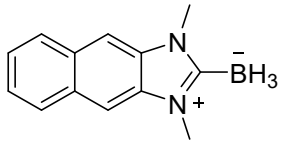
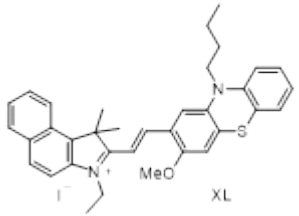
Probe	Solvent	Sensing mode	LOD	Time（equiv.）	Application	Ref.
	PBS/CH_3CN (1/4, v/v)	OFF-ON	0.36 μM	2 min (30 equiv.)	RAW264.7 cells	14
	PBS buffer (pH 7.4)	OFF-ON	2.8 nM	<5 s (12 equiv.)	HeLa cells and Zebrafish	15
	EtOH/PBS buffer (pH 7.4, 1:9, v/v)	OFF-ON	89.7 nM	within 5 s (1 equiv.)	MCF-7 cells	16
	THF/PBS buffer (v/v = 5/5, pH = 7.4)	Ratiometric	13.2 nM	within 0.5 min (2 equiv.)	Test strips, real water samples and RAW264.7 cells	17
	PBS buffer (1% DMSO, pH = 7.4)	ON-OFF	28.3 nM	<60 s (8 equiv.)	RAW264.7 cells, tissues of rats, arthritis rat	18

	PBS buffer (pH 7.4, containing 5% EtOH)	OFF-ON	2.89 μmol/L	20 s (10.0 equiv.)	HeLa cells, RAW264.7 cells, tissues and Zebrafish	19
	EtOH–PBS solution (v/v = 6:4, pH = 7.4)	OFF-ON	0.27 μM	5 min (12 equiv.)	Tap water and HeLa cells	20
	PBS buffer (pH = 7.4)	ON-OFF	667 pM	--[a]	Zebrafish	21
	EtOH/PBS (6:4, v/v, pH = 7.4)	OFF-ON	11.51 nM	≤2 s (200 equiv.)	HeLa cells and mice	22
	10 mM PBS containing 33% acetonitrile	OFF-ON	1.3 nM	100 s (1.6 equiv.)	BV-2 cells and mice	23

	aqueous solution	OFF-ON	0.23 μM	35 s (5 equiv.)	HeLa cells, RAW264.7 cells and mice	24
	PBS solution (pH = 5.0)	OFF-ON	16 nM	Rapid response (1 equiv.)	cells	25
	EtOH/PBS solution (v/v, 1/9, pH 7.4)	OFF-ON	147 nM	1 min (10 equiv.)	HepG2 and liver tissues	26

	ethanol-PBS buffer (1/99, V/V, pH 7.4)	OFF-ON	24 nM	< 1 min (--)	HeLa cells, real water samples, cellular organization	27
	Tris-HCl buffer solution (pH 7.4, containing 1% DMSO)	OFF-ON	35.2 nM	40 s (20 equiv.)	HeLa cells, Zebrafish	28
	--	Ratiometric	--	3 min (4 equiv.)	HeLa cell, tissue	29
	PBS buffer (pH 7.4, 2% DMSO)	Ratiometric	0.14 μM	Within 10 s (1.4 equiv.)	HeLa cell, paper	30
	CH3CN/PBS (10 mM, pH = 7.4, v/v, 3:7)	Ratiometric	11.8 nM	5 min (40 equiv.)	MCF-7 cells	31
	--[a]	OFF-ON	147 nmol/L	few seconds (5 equiv.)	RAW 264.7 cells; nude mice	32
	PBS (pH 7.6)	ON-OFF	5.2 nM	10 s (1 equiv.)	RAW 264.7 cells	33

	pH = 7.0, PBS/EtOH = 7:3, v/v	OFF-ON	6 nM	<10 s (1 equiv.)	HeLa cells	34
	PBS buffer (PBS/ DMF, 9:1, v/v, 10 mM, pH 7.4, 37 ℃)	OFF-ON	0.32 μM	2 s (4 equiv.)	H460 cells; mice	35
	PBS buffer (10 mM, pH 7.4)	OFF-ON	13.3 nM	within 8 s (--)	HeLa cells, Zebrafish	36

	PBS buffer (pH = 7.4, 20 mM, 0.1% CH_3CN)	OFF-ON	0.8 nM	5 s (1 equiv.)	RAW 264.7 cells	37
	PBS/CH_3CN (v/v, 9/1, 10 mM PBS, pH 7.4)	Ratiometric	3.6 μM	within 1 min (30 equiv.)	Raw 264.7 cells, rat hippocampal slices	38
	PBS/CH_3CN (v/v, 9/1, 10 mM PBS, pH 7.4)	OFF-ON	28 nM	within 10 s (2 equiv.)	Test strips, real water samples HeLa cells, Zebrafish	This work

[a] Not mentioned (--)

2. Preparation of various other analysts

The various ROS/RNS and other analytes were prepared as follows:

(a) ClO^- (NaClO), H_2O_2 and TBHP (tert-butyl hydroperoxide) were purchased from the company, and then diluted with deionized water to use.

(b) $O_2^{\bullet-}$: KO_2 was dissolved in dry DMSO to make the 1 mM stock solution;

(c) •OH: $FeSO_4$ solution (1.0 mM, 0.1 mL) was added to a solution of H_2O_2 (1.0 mM, 1.0 mL) in PBS (10 mM, pH 7.4) to give a 0.1 mM stock solution at room temperature;

(d) $ONOO^-$: A stirred solution of $NaNO_2$ (0.6 M, 10 mL) and H_2O_2 (0.7 M, 10 mL) in deionized water was added to HCl (0.6 M, 10 mL) at 0 °C, followed immediately by a rapid addition of NaOH (1.5 M, 20 mL). Excess hydrogen peroxide was removed by MnO_2. The concentration of $ONOO^-$ was determined by UV analysis with an extinction coefficient of 302 nm (ε= 1670 M^{-1} cm^{-1}), and the solution was stored at −20 ℃ for use;

(e) $^1O_2$: $NaMoO_4$ solution (10 mM) and H_2O_2 solution (10 mM) were prepared with PBS (10 mM, pH=7.4), and an aliquot of these two solutions was mixed to obtain a 5 mM of $^1O_2$ stock solution;

(f) NO: H_2SO_4 solution (3.6 M) was added dropwise to a stirred $NaNO_2$ solution (7.3 M). The resulting gas was passed through a solution of NaOH (2 M) and then deionized water to give a 2.0 mM stock solution.

Other analysts were purchased from commercial suppliers and used directly.

3. UV/Vis absorption and fluorescence spectra of the XL and XLO

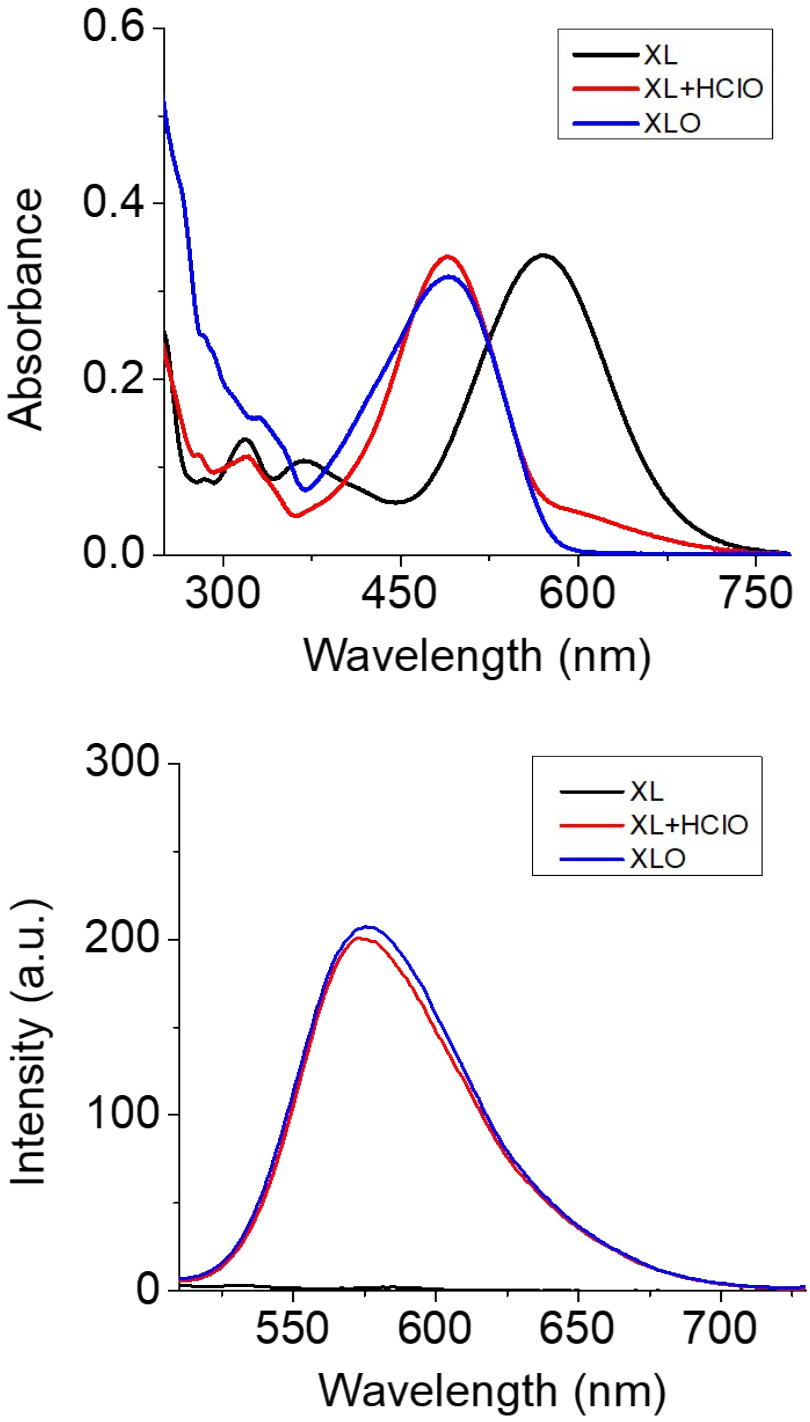


Figure S1. UV/vis absorption (upper) and fluorescence spectra (bottom) of XL, XLO, and 10 μM XL with 15 μM HClO in PBS (10% CH_3CN containing, pH=7.4), excitation at 488 nm.

4. Measurement of the detection limit

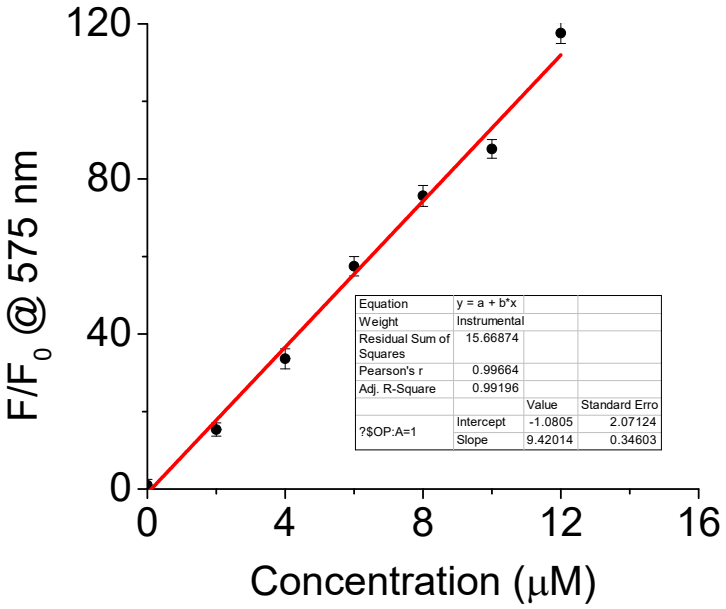


Figure S2. Linear correlation between the fluorescence increment (F/F_0) of XL at 575 nm and the concentration of HClO.

The detection limit was calculated based on the fluorescence titration:

The fluorescence intensity at 575 nm was fitted linearly with the increasing concentrations of HClO over a range of 0-20 μM. From the plot, the slope (k) was obtained to be 9.4 μM^{-1}, shown in Figure S2. The detection limit of XL to HClO was calculated to be 28 nM in term of the formula ($3\sigma/k$), where σ is the standard deviation of blank measurement.

5. Selectivity of probe XL

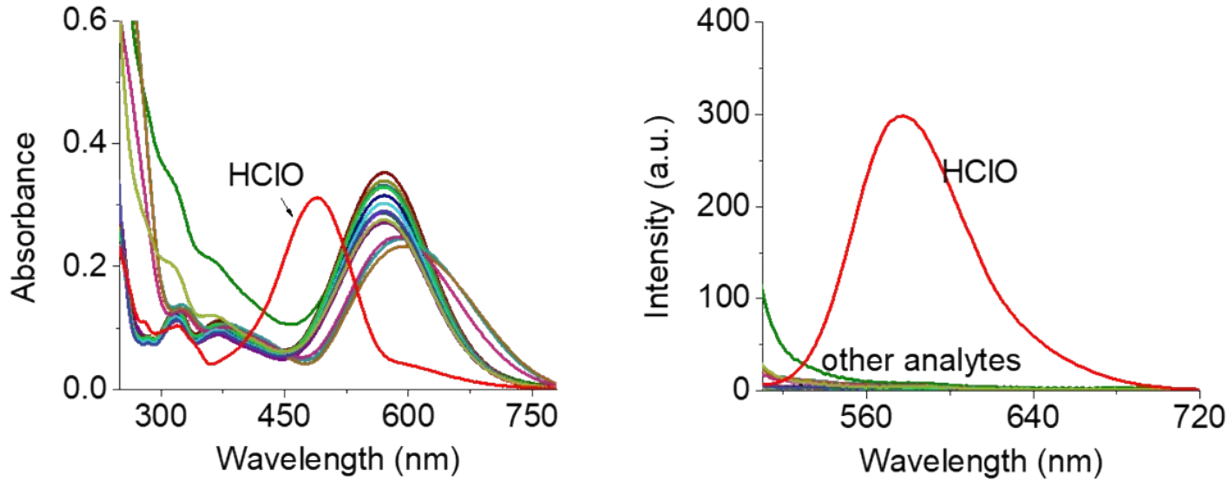


Figure S3. UV/vis absorption (left) and fluorescence (right) spectra of probes with various analytes. (Analytes: 0, blank; 1, Na^+; 2, K^+; 3, Cu^{2+}; 4, Zn^{2+}; 5, Cl^-; 6, I^-; 7, SO_4^{2-}; 8, Cys; 9, Hcy; 10, GSH; 11, H_2O_2; 12, TBHP; 13, $O_2^{\bullet-}$; 14, •OH; 15, $ONOO^-$; 16, $^1O_2$; 17, NO; 18, ClO^-)

6. High-resolution mass spectra for the reaction mixture of XL with HClO

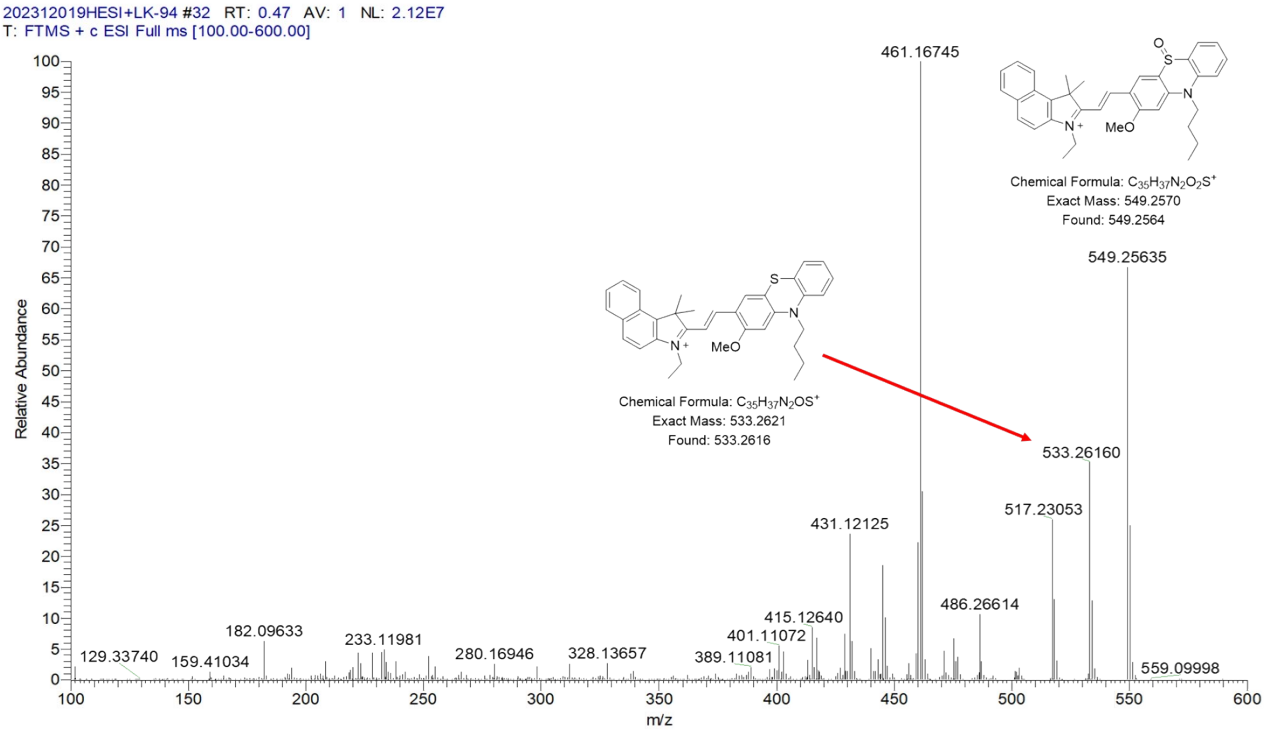


Figure S4. High-resolution mass spectrum for the mixture of XL with HClO.

7. DFT calculations for ICT process

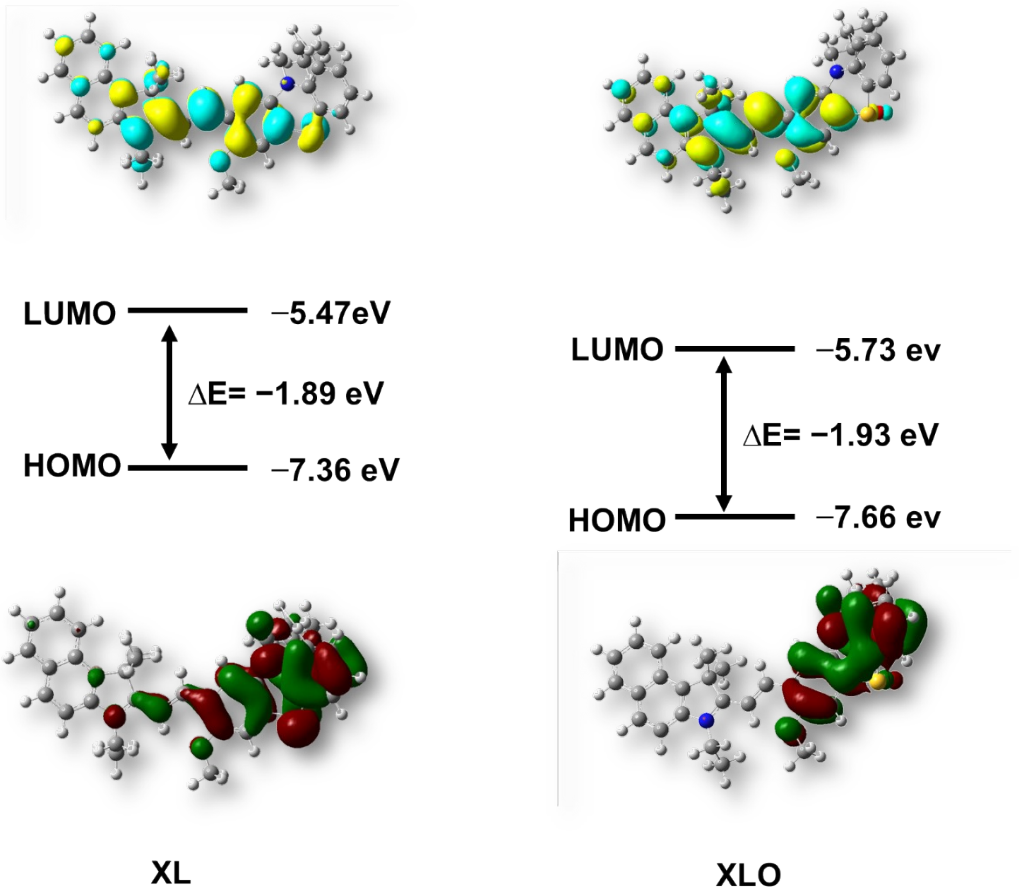


Figure S5. HOMO-LUMO energy level of the probe XL and product XLO.

8. Test strips for the detection of HClO

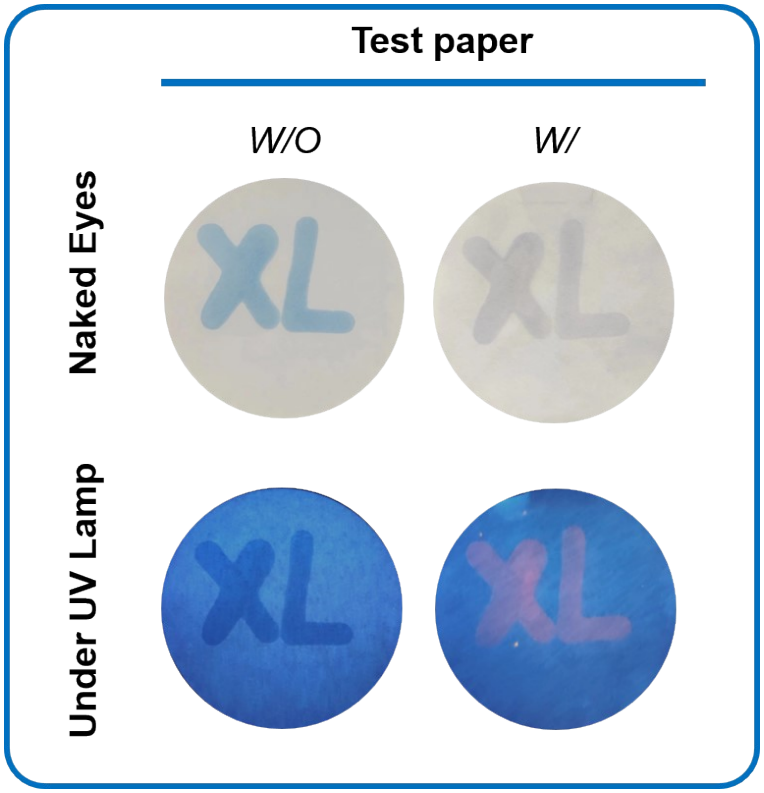


Figure S6. Photographs of test strips containing probe XL before (W/O) and after (W/) the treatment with HClO under naked eye observation (upper) or portable UV lamp irradiation at 365 nm (bottom).

9. pH effect

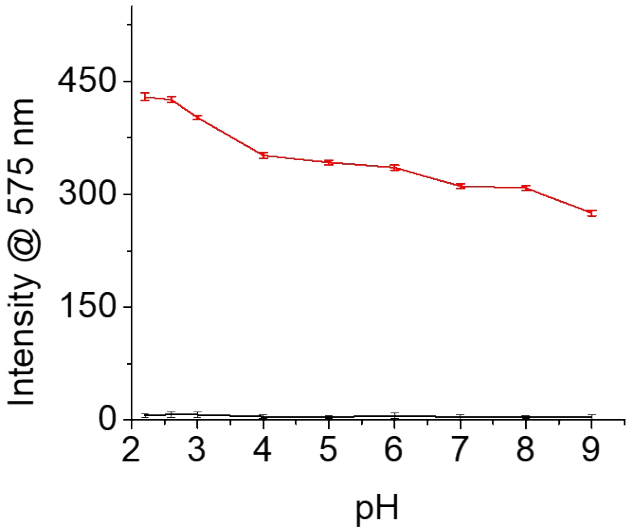


Figure S7. Fluorescence intensity at 575 nm of probe XL before (black) and after (red) the reaction with HClO at different pH.

10. MTT assay of HeLa cells in the presence of XL

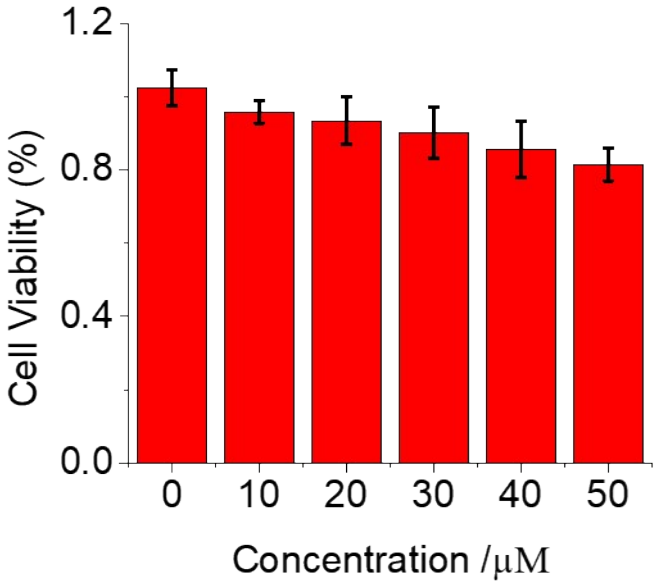


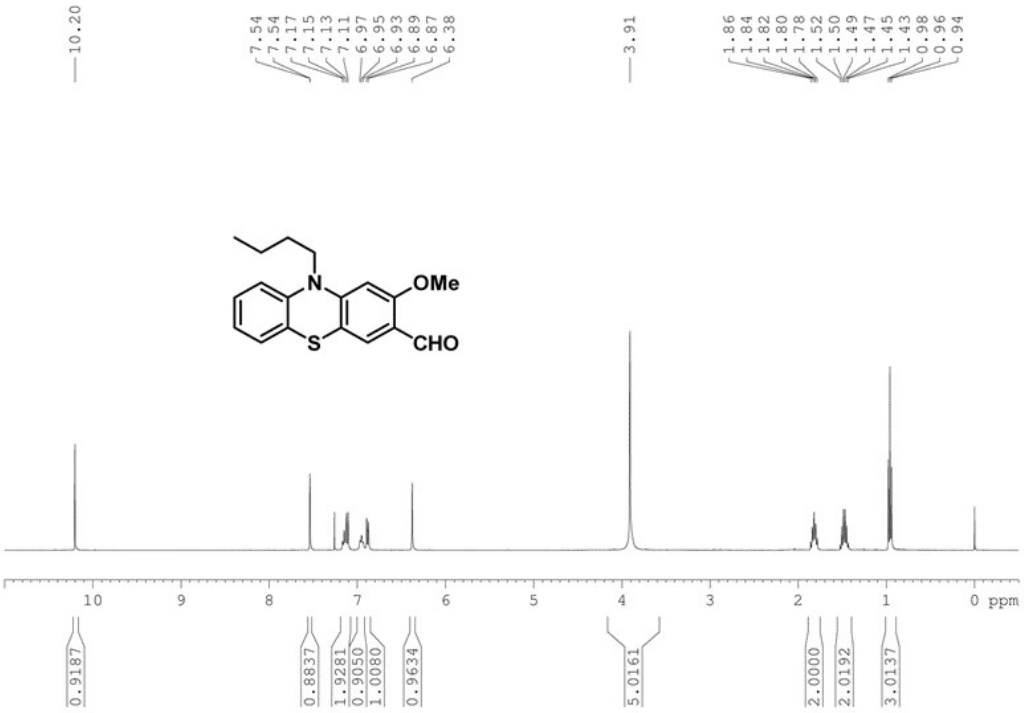
Figure S8. Cytotoxicity assay of probe XL for HeLa cells at different concentrations for 24 h.

11. Zebrafish imaging layer sweep images

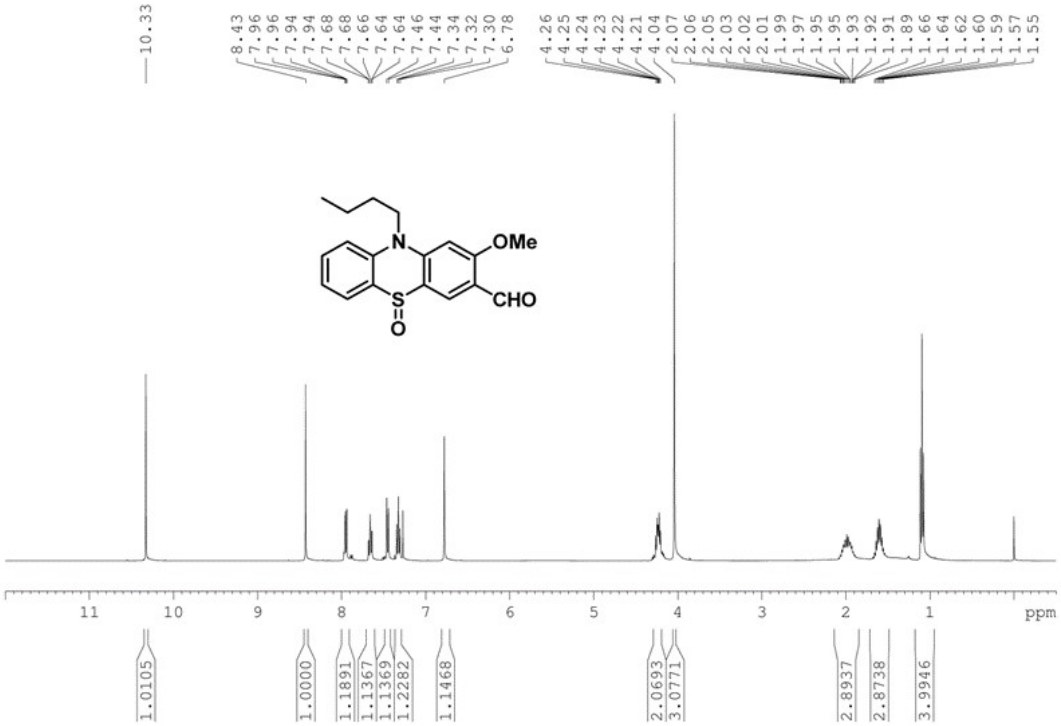
Figure S9. The *z*-axis swept images at different depths.

12. NMR spectra of related compounds

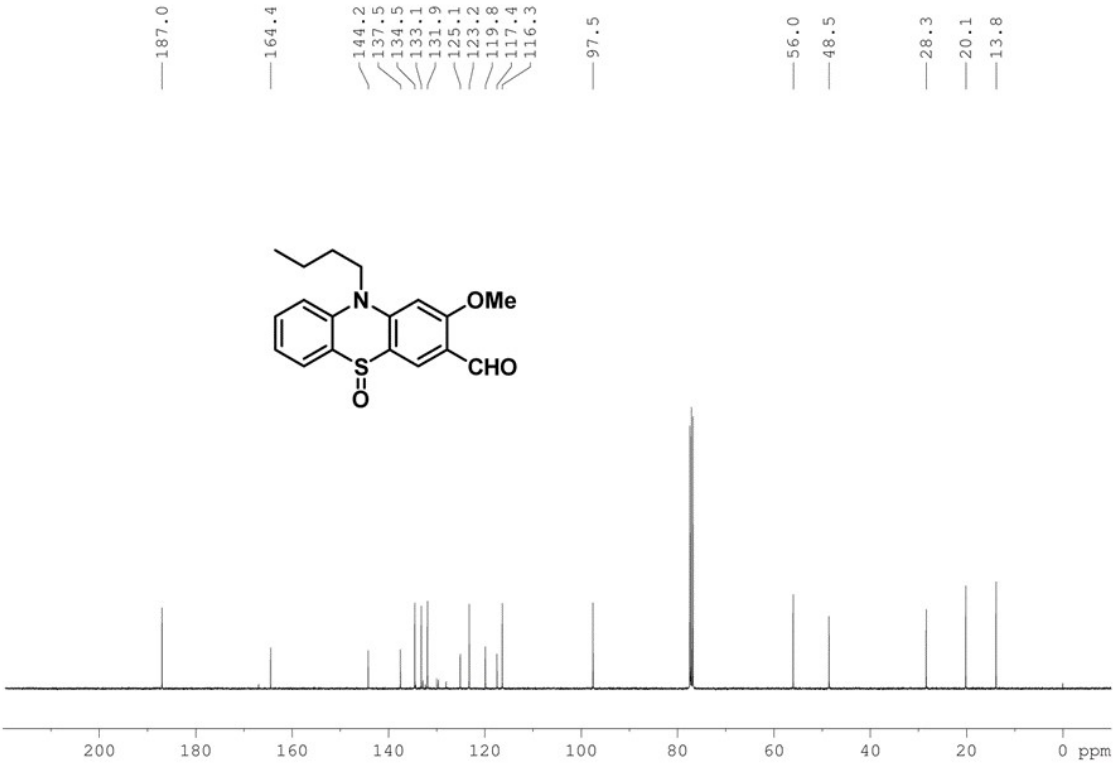
^{1}H NMR of compound **3** in $CDCl_3$, 400 MHz.



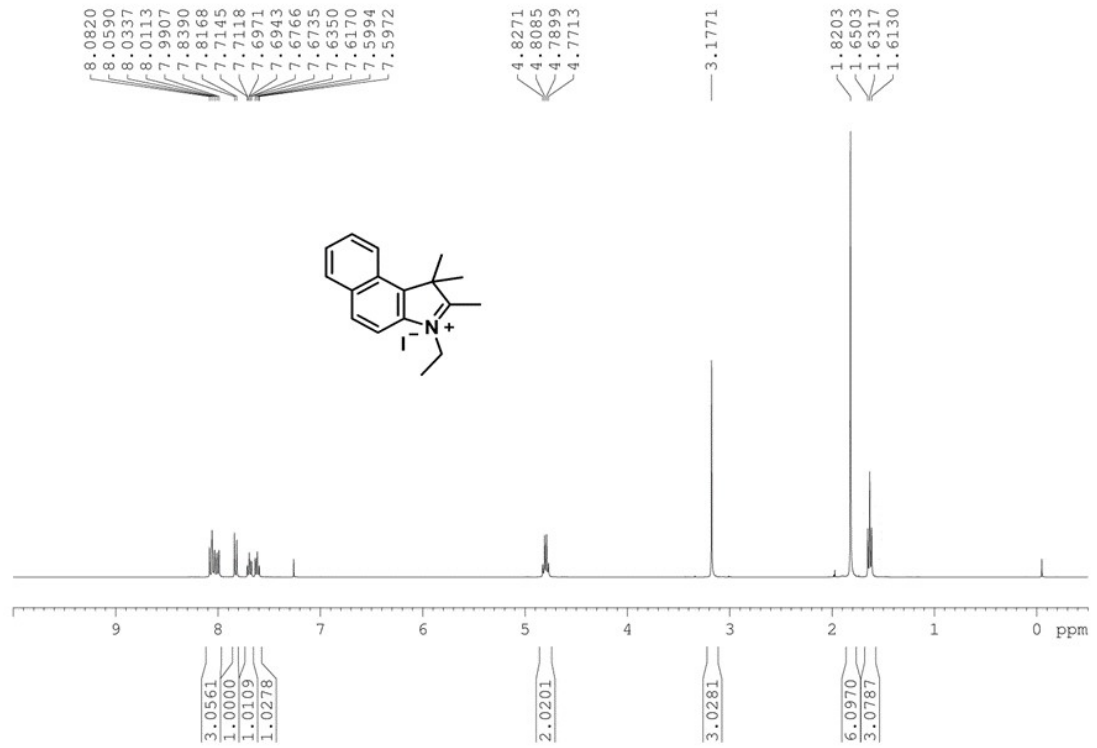
^{1}H NMR of compound **4** in $CDCl_3$, 400 MHz.



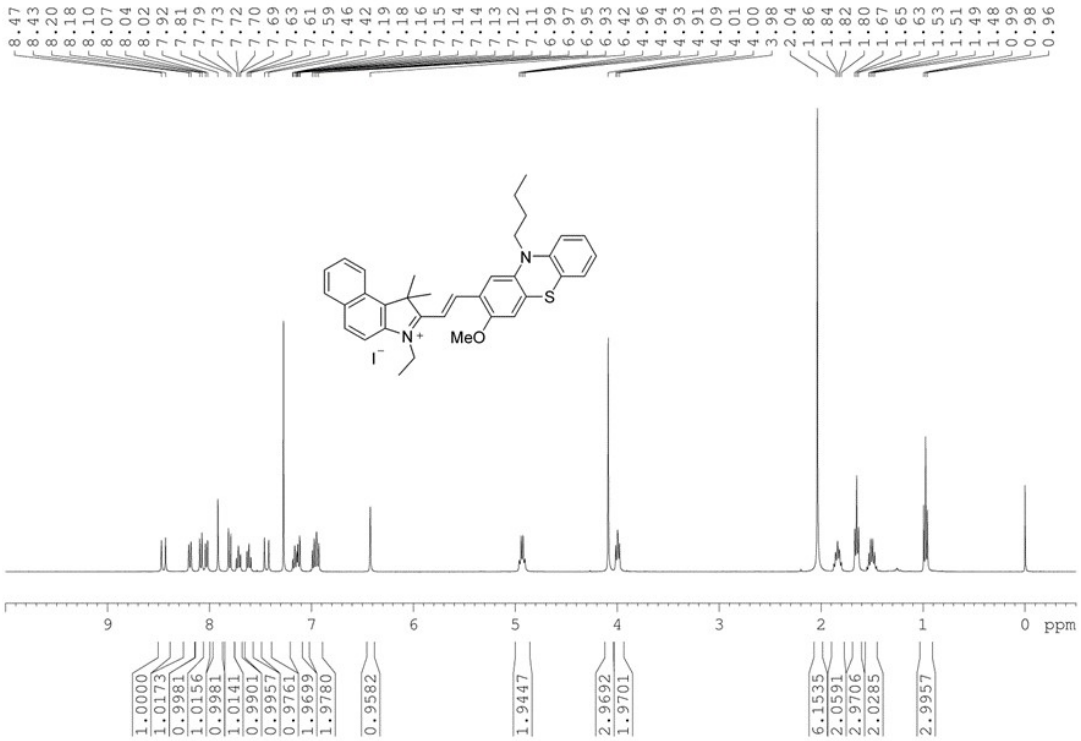
^{13}C NMR of compound **4** in $CDCl_3$, 400 MHz.



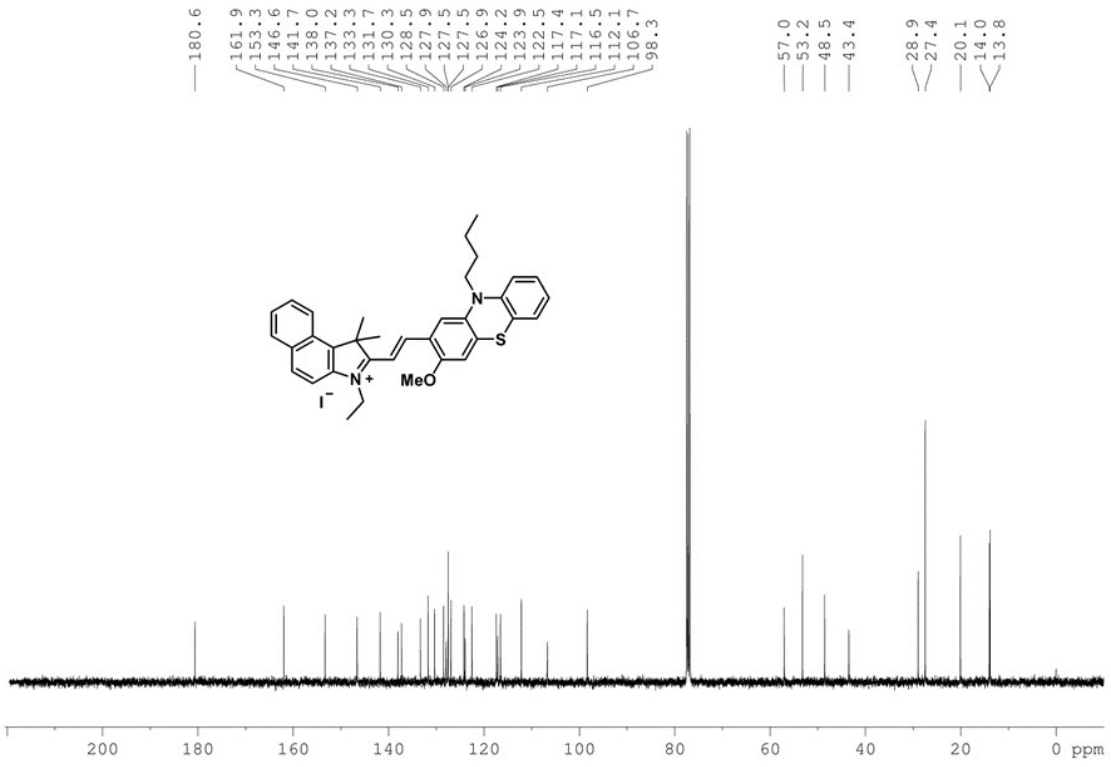
^{1}H NMR of Compound **5** in $CDCl_3$, 400 MHz.



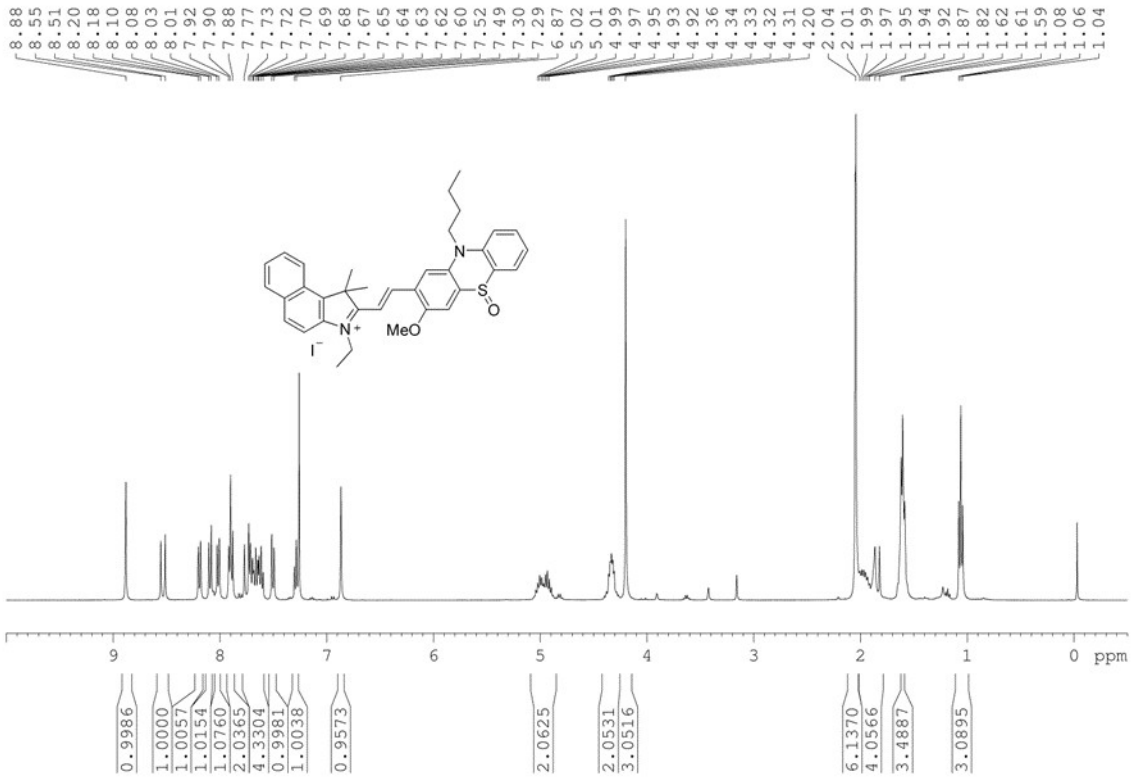
^{1}H NMR of XL in $CDCl_3$, 400 MHz.



^{13}C NMR of XL in $CDCl_3$, 100 MHz.



^{1}H NMR of XLO in $CDCl_3$, 400 MHz.



^{13}C NMR of XLO in $CDCl_3$, 100 MHz.