Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information (SI)

for

An ICT Based Ultraselectivity Fluorescent Probe for Detection of HClO in Living Cells

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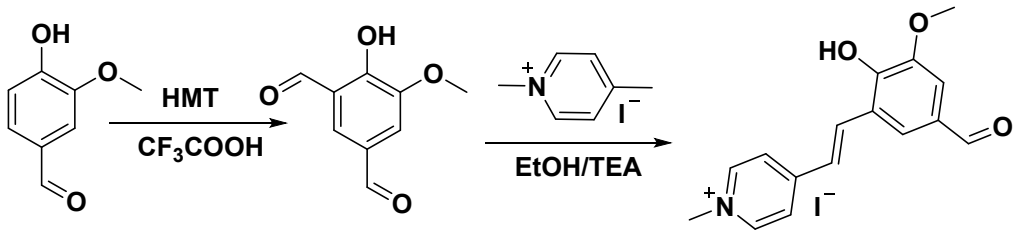
Materials and Chemicals

All chemicals were purchased from commercial suppliers and used without further purification. All solvents were purified prior to use. Distilled water was used after passing through a water ultrapurification system. TLC analysis was performed using precoated silica plates. Hitachi F−7000 fluorescence spectrophotometer was employed to measure fluorescence spectra. Shanhai Huamei Experiment Instrument Plants, China provided a PO-120 quartz cuvette (10 mm).^{1}H NMR and ^{13}C NMR experiments were performed with a Bruker AVANCE-300 MHz and 75 MHz NMR spec-trometer, respectively (Bruker, Billerica, MA). Coupling constants (*J* values) are reported in hertz. ESI determinations were carried out on an LTQ-MS (Thermo) instrument. The cell imaging experiment was measured by laser confocal fluorescence imaging using a Leica TCS SP5 laser scanning microscope. The red single crystal of probe was mounted on a glass fiber for data collection. Cell constants and an orientation matrix for data collection were obtained by least-squares refinement of diffraction data from reflections within 2.556–27.475°, using a Bruker SMART APEX CCD automatic diffractometer. Data were collected at 173 K using Mo Kα radiation (λ = 0.71073 Å) and the ω-scan technique, and corrected for the Lorentz and polarization effects (SADABS).[s1] The structures were solved by direct methods (SHELX97),[s2] and subsequent difference Fourier maps were inspected and then refined in *F*2 using a full-matrix least-squares procedure and anisotropic displacement parameters.



Synthesis of Compound 2. Vanilline (0.304 g, 2 mM) and hexamethylenetetramine (1.2 equiv.) were dissolved in TFA and the solution was heated at 100 °C for 4 h. Approximately 10 mL 3 M HCl was added and the solution was heated at 100 °C for 1 h. After cooling to room temperature, the mixture was extracted with 20mL CH_2Cl_2 for three times. The organic layer was dried over magnesium sulfate and concentrated under reduced pressure to afforded 0.299 g (1.6 mM, 83%) yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) =3.95 (s, 3 H), 7.61 (s, 1 H), 7.88 (s, 1 H), 9.88 (s, 1 H), 10.36 (s, 1 H), 11.34 (s, 1 H); ^{1}C NMR (DMSO-d_6, 75 MHz): 55.1, 112.1, 121.2, 123.6, 126.8, 148.0, 154.8, 189.0, 189.9. ESI-MS m/z: Calcd for $C9H7O4^-$ 179.03; Found 178.83 (Fig. S1)

Synthesis of Probe 1. Compound **2** (0.18 g, 1 mM), trimethylamine (1 equiv.) and 1,4-dimethylpyridin-1-ium iodide (0.235 g, 1 mM) was dissolved in anhydrous ethanol (20 mL). The reaction mixture was then refluxed for 14 h. The solution was then removed under reduced pressure. The crude product was purified on a silica gel column using ethanol and then methanol to afford 0.201 g (0.5 mM, 51%) probe **1** as red solid. ^{1}H NMR (DMSO-d_6, 300 MHz): 3.64 (s, 3H), 4.09 (s, 3H), 6.80 (s, 1H), 7.47 (s, 1H), 7.60 (d, 1H, J = 15.6), 7.87 (d, 1H, J = 6.3), 7.99 (d, 1H, J = 15.9), 8.47 (d, 1H, J = 6.3), 9.35 (s, 1 H); ^{1}C NMR (DMSO-d_6, 75 MHz): 45.4, 54.4,

115.0, 117.5, 120.7, 142.1, 143.2, 153.0, 154.8, 171.6, 186.5. Crystal data for $C_{16}H_{16}NO_3I$: crystal size: 0.14 × 0.11 × 0.08 mm^3, space group Pnma. a = 6.385 (13) Å, b = 16.788 (4) Å, c = 7.974 (16) Å, V = 854.1 (16) $Å^3$, Z = 2, T = 173 K, θ_{max} = 27.48°, 9558 reflections measured, 3768 unique (R_{int} = 0.0317) Final residual for 251 parameters and 3768 reflections with $I > 2\sigma(I)$: R_1= 0.0353, wR_2= 0.0915 and GOF = 1.084 (Fig. S4).

Preparation of Solutions of Probe 1 and Analytes. Stock solution of probe **1** (2 mM) was prepared in DMSO. Stock solutions of ClO^- (2 mM), ClO_2^-, IO_4^-, CN^-, HS^- and HSO_3^- (200mM) were prepared by direct dissolution of proper amounts of sodium salts in deionized water. All chemicals used were of analytical grade. Superoxide solution ($O_2^{\bullet -}$) was prepared by adding KO_2 (1.0 mg) to dry dimethylsulfoxide (1.0 mL) and stirring vigorously for 10 min. Hydroxyl radical (•OH) was obtained from the Fenton reaction of Ferrous perchlorate and hydrogen peroxide. Single oxygen ($^1O_2$) was generated by mixing H_2O_2 with NaOCl sequentially. ROO• was generated from 2,2'-azobis(2-amidinopropane)dihydrochloride. Nitric oxide was generated from SNP (Sodium Nitroferricyanide (III) Dihydrate). SNP in deionizer water was added then stirred for 30 min at 25°C. [s4−s6]

General fluorescence spectra measurements. All the detection experiments were measured in DMSO−Hepes buffer (3:1, v/v, pH 7.4). The procedure was as follows: into a DMSO−Hepes buffer (3:1, v/v, pH 7.4) solution, containing 50 μM probe **1**, a HClO sample was gradually titrated. The process was monitored by fluorescence spectrometer (λ_{ex}= 373 nm, λ_{em}= 468 nm, slit: 5 nm/5 nm).

Cell Culture and Imaging. The HepG2 cells were cultured in 1× SPP medium (1% proteose peptone, 0.2% glucose, 0.1% yeast extract, 0.003% EDTA ferricsodium salt) at 30 ˚C. Some of the HepG2 were treated with 30 μM of probe **1** (DMSO stock solution) in culture media for 30 min at 37 ˚C and washed 3 times with PBS. Meanwhile, another portion of HepG2 cells were incubated with probe **1** (30 μM DMSO stock solution) for 30 min at 37 ˚C with 10 μM, 20 μM, 30 μM of HClO added for the final 30 min. Cell imaging was then carried out after washing cells with PBS buffer.

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s2 G.M. Sheldrick, Program for the Refinement of Crystal Structure, University of Goettingen, Germany, 1997.Sheldrick, G.M. Program for the Refinement of Crystal Structure, University of Goettingen, Germany, **1997**.

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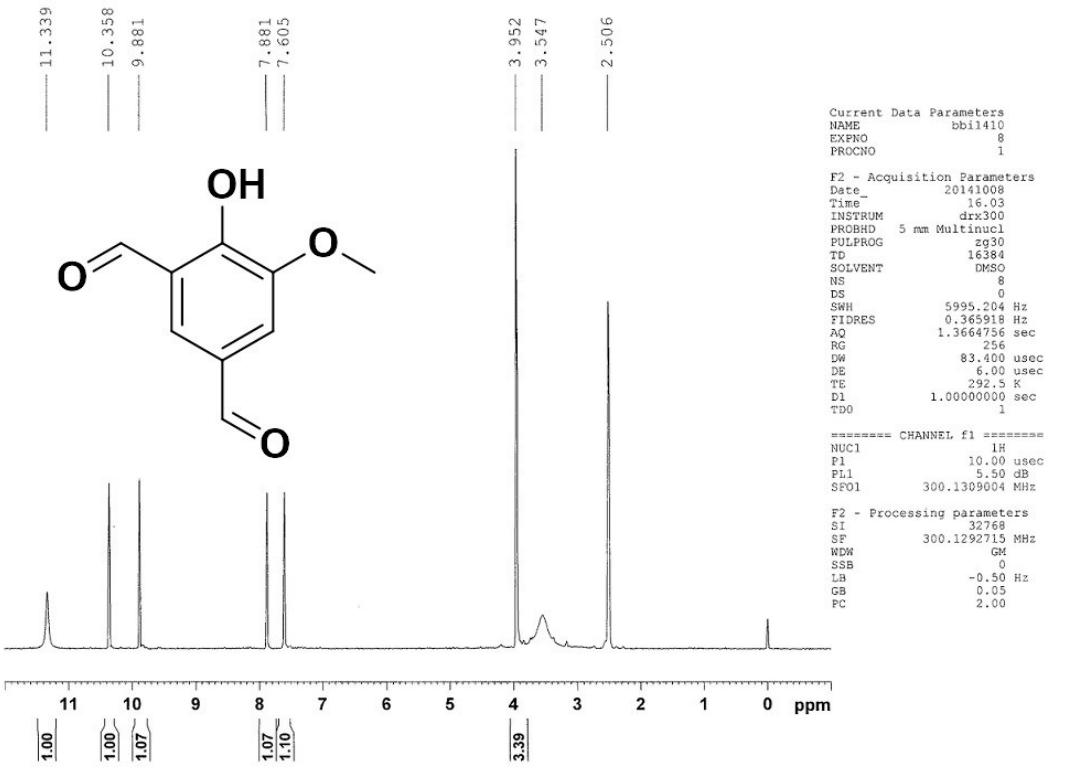
s4 X.H. Li, G.X. Zhang, H.M. Ma, D.Q. Zhang, J. Li and D.B. Zhu, *J. Am. Chem. Soc.*, 2004, **126**, 11543.

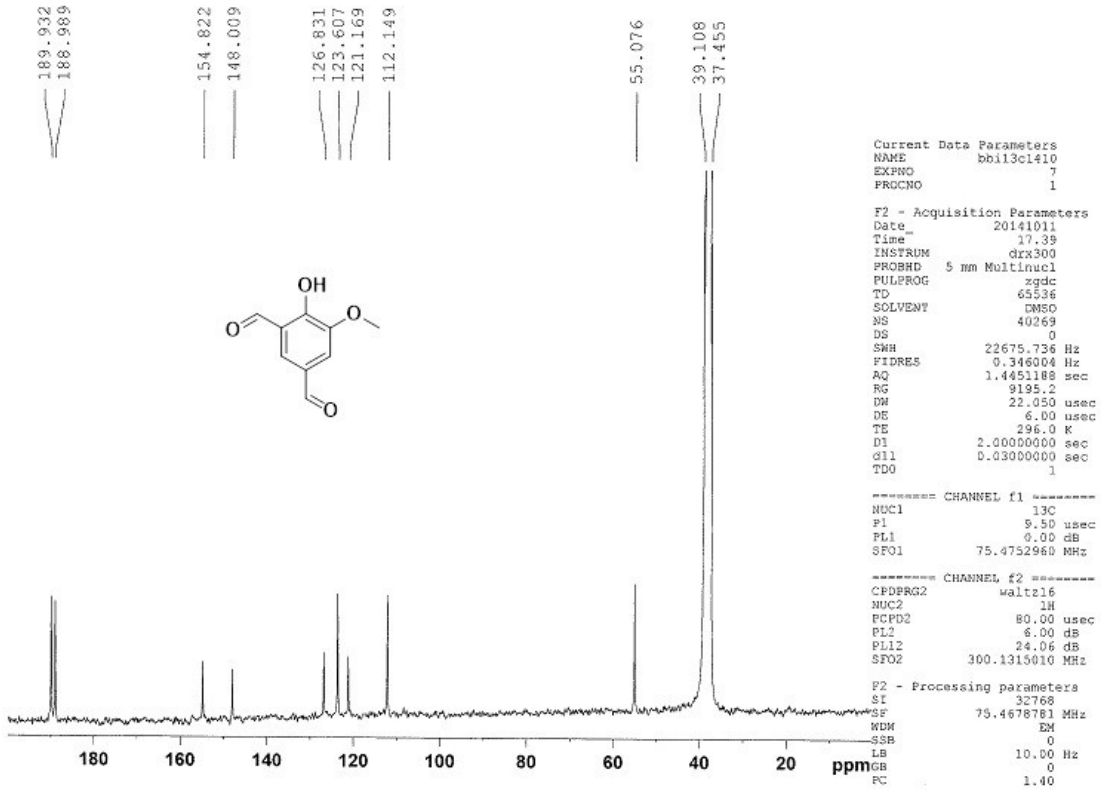
s5 H.T. Zhao, S. Kalivendi, H. Zhang, J. Joseph, K. Nithipatikom, J. Vasquez-Vivar and B. Kalyanaraman, *Free Radical Biology & Medicine* 2003, **34**, 1359.

s6 Z.N. Sun, F.Q. Liu, Y. Chen, P.K. H. Tam and D. Yang, *Org. Lett.*, 2008, **10**, 2171.

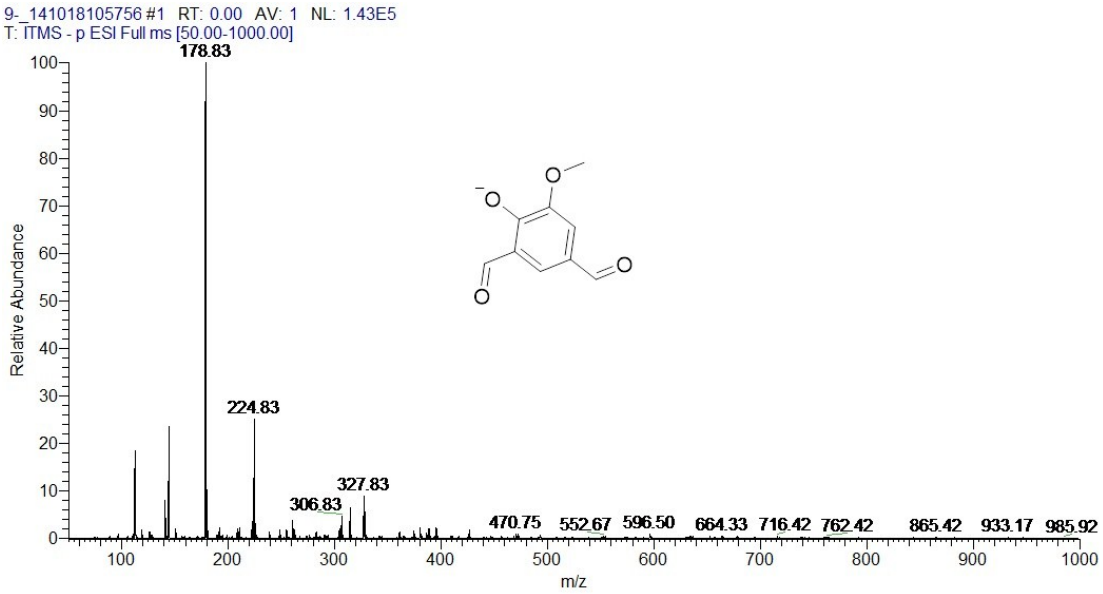
Figure S1: Structure characterization of compound **2**



^{1}H-NMR spectrum of compound **2** in DMSO-d_6

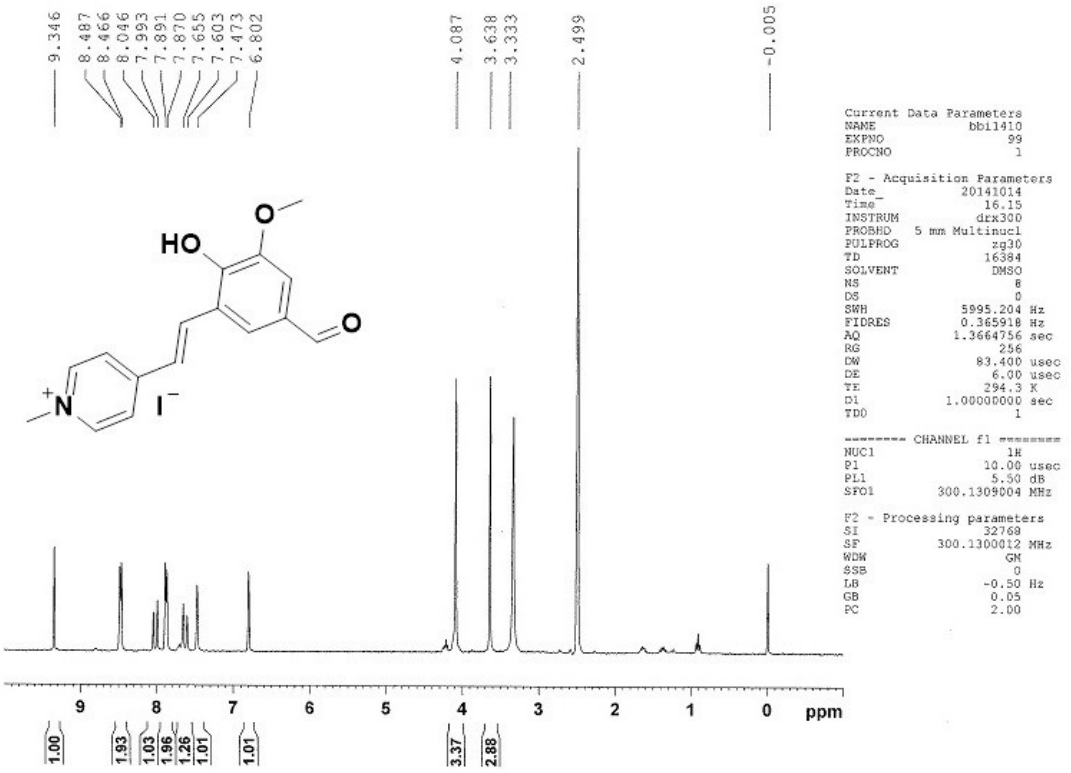


^{13}C-NMR spectrum of compound **2** in DMSO-d_6

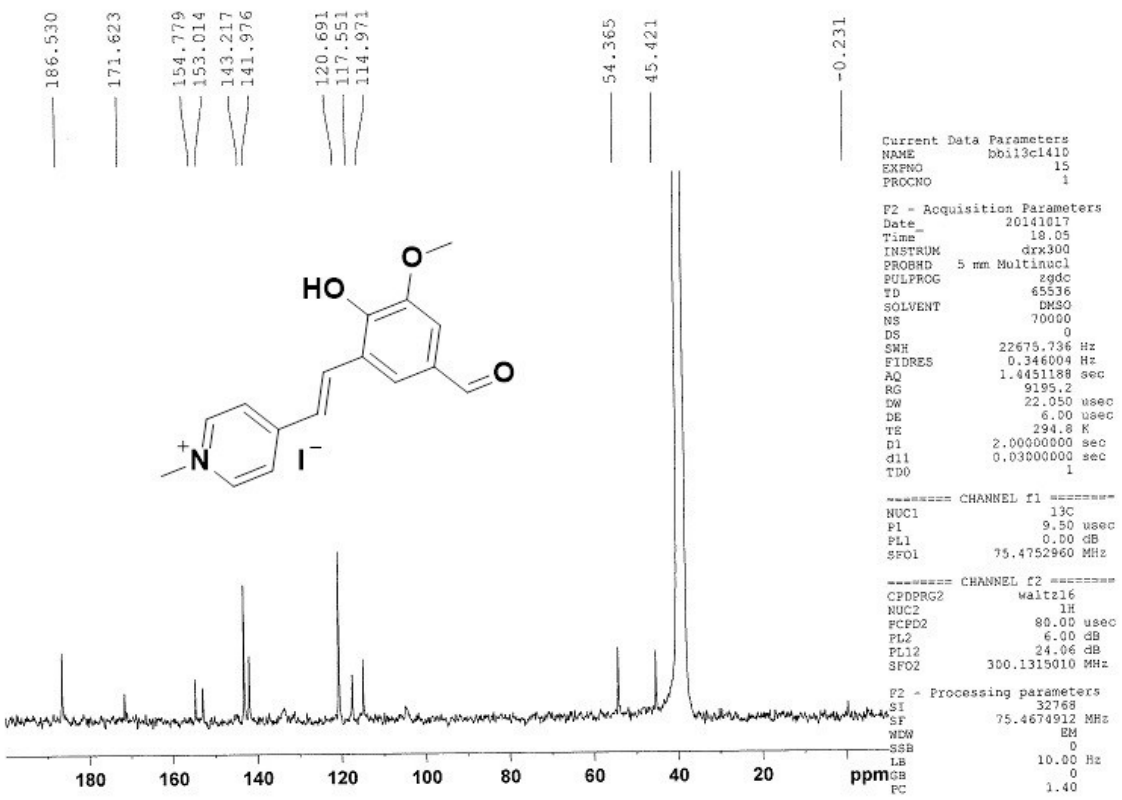


MS (ESI) spectrum of compound **2**

Figure S2: Structure characterization of probe **1**

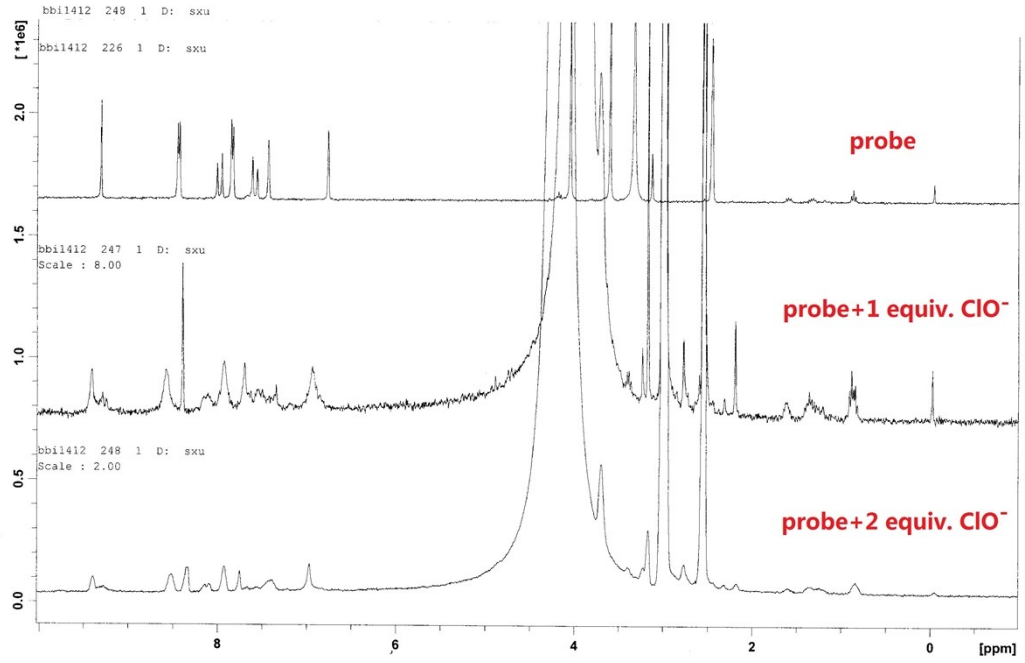


^{1}H-NMR spectrum of probe **1** in DMSO-d_6

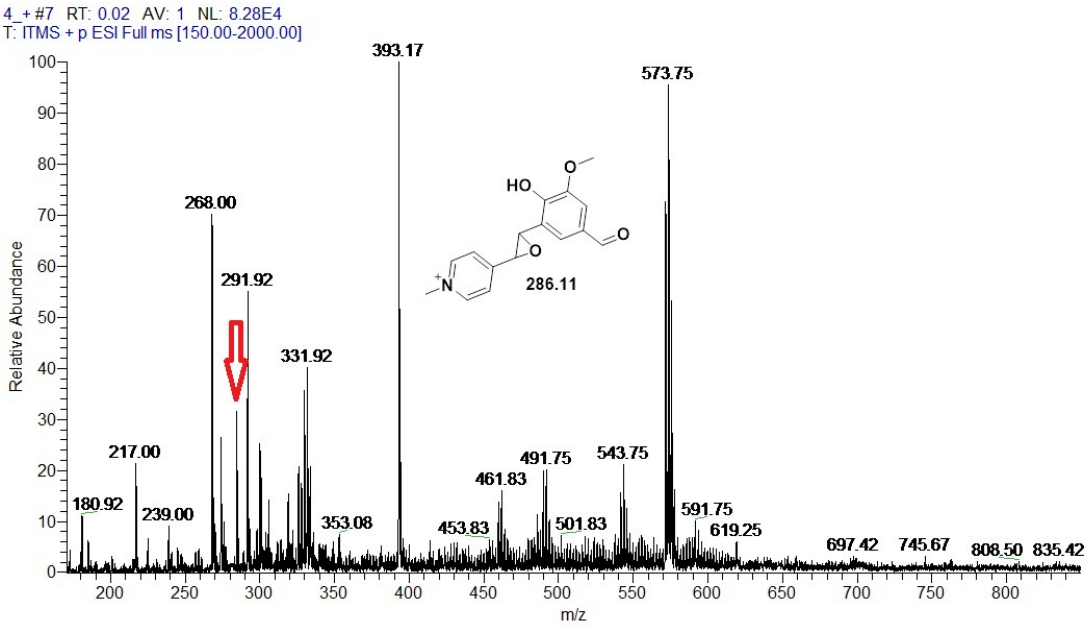


^{13}C-NMR spectrum of probe **1** in DMSO-d_6

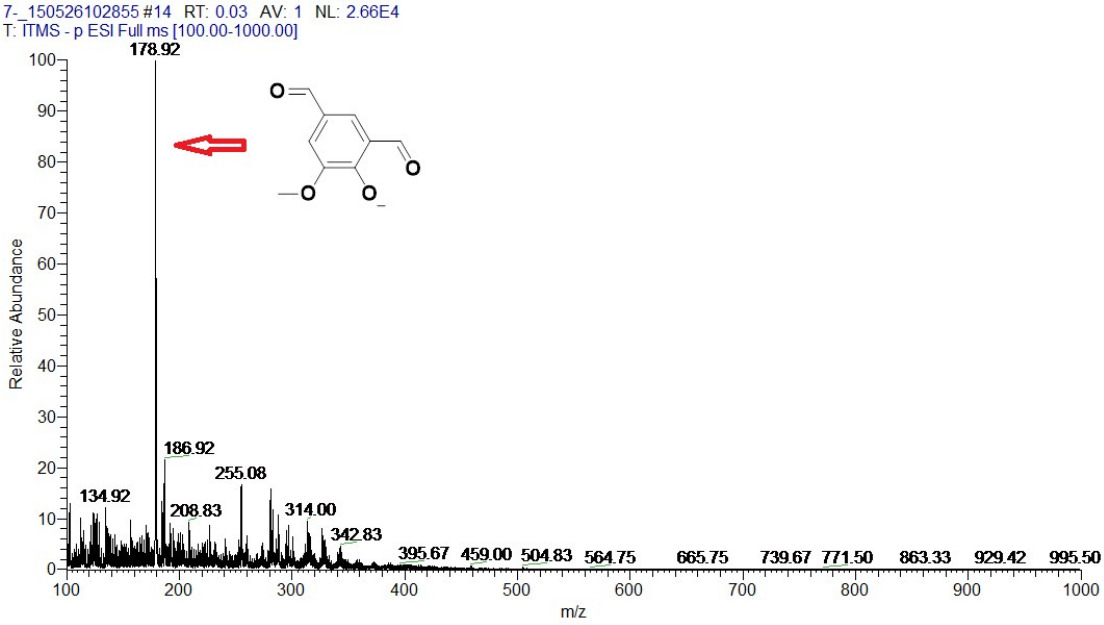
Figure S3: Structure characterization of compound **3**



^{1}H-NMR titration experiments of probe **1** upon addition of HClO in DMSO-d_6



MS (ESI) spectrum of probe **1** upon addition of 1 equiv. HClO in MeOH



MS (ESI) spectrum of probe **1** upon addition of 2 equiv. HClO in MeOH

Figure S4: Crystal data of probe 1

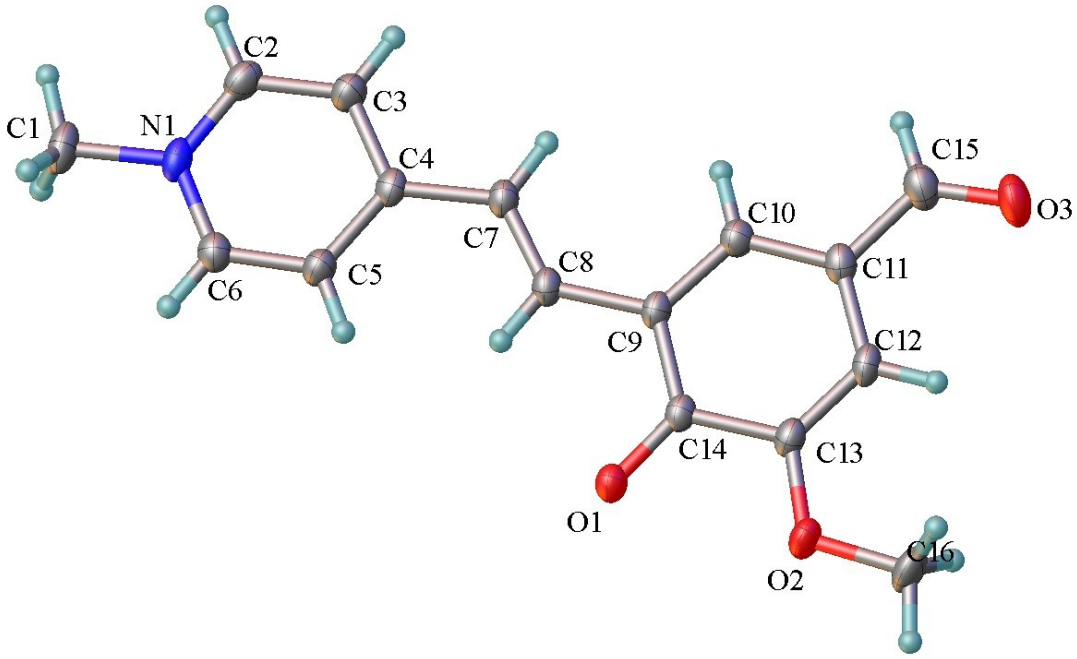


Table 1. Crystal data and structure refinement for mx3426.

Identification code	mx3426	
Empirical formula	C16 H23 N O7	
Formula weight	341.35	
Temperature	173.1500 K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 1 21 1	
Unit cell dimensions	a = 6.3846(13) Å	α= 90°.
	b = 16.788(4) Å	β= 92.052(4)°.
	c = 7.9738(16) Å	γ = 90°.
Volume	854.1(3) $Å^3$	
Z	2	
Density (calculated)	1.327 Mg/m^3	
Absorption coefficient	0.104 mm^{-1}	
F(000)	364	
Crystal size	0.14 x 0.11 x 0.08 mm^3	
Theta range for data collection	2.556 to 27.475°.	
Index ranges	-8<=h<=8, -21<=k<=21, -10<=l<=10	

Reflections collected	9558
Independent reflections	3768 [R(int) = 0.0317]
Completeness to theta = 26.000°	99.5 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	1.0000 and 0.7965
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3768 / 1 / 251
Goodness-of-fit on F^2	1.084
Final R indices [I>2sigma(I)]	R1 = 0.0353, wR2 = 0.0915
R indices (all data)	R1 = 0.0363, wR2 = 0.0926
Absolute structure parameter	-0.4(4)
Extinction coefficient	n/a
Largest diff. peak and hole	0.210 and -0.145 e.$Å^{-3}$

Figure S5: UV-vis spectra of probe **1** towards HClO

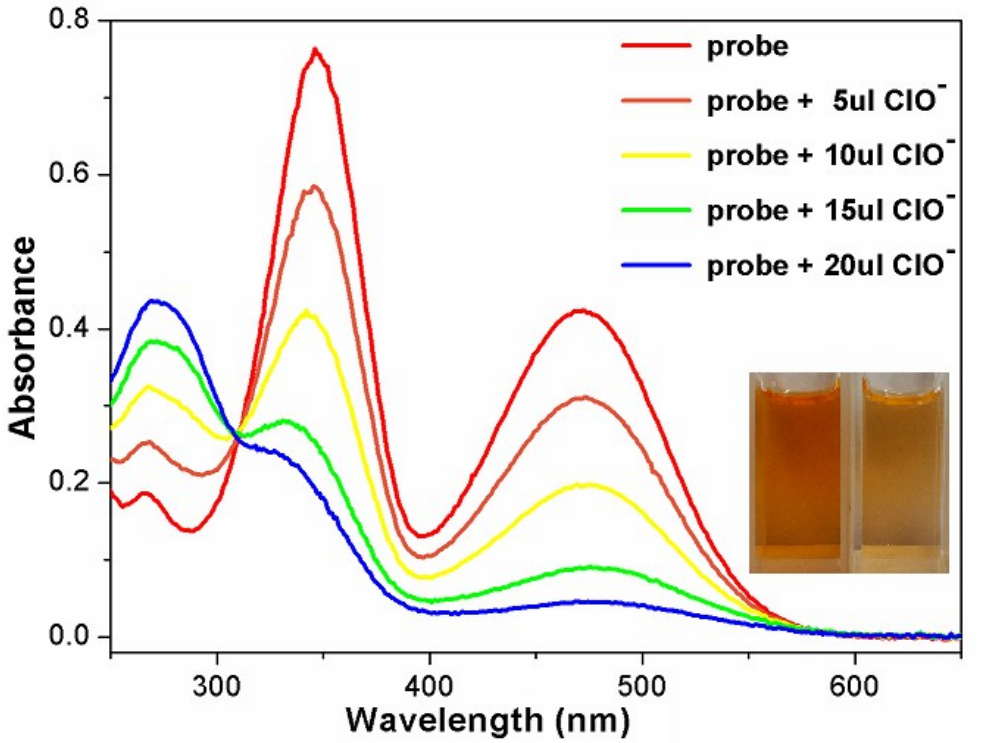


Figure S5a: UV-vis spectra of **1** (25 μM) upon addition of HClO (20 μM) in DMSO−Hepes buffer (3:1, v/v, pH 7.4) at 25 ˚C. Each spectrum was collected 30 s after adding of HClO.

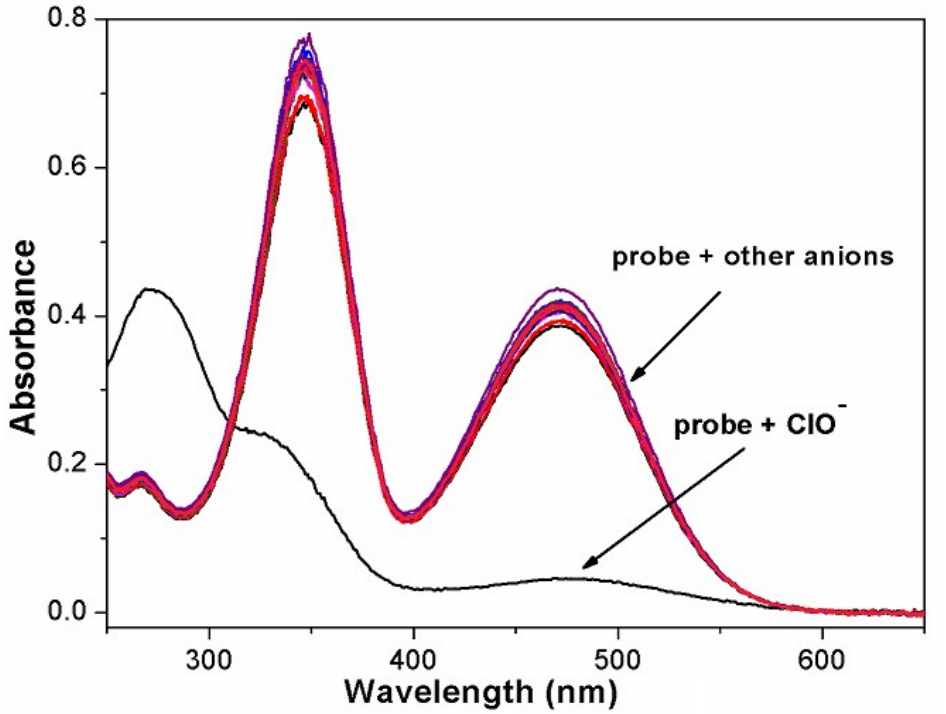


Figure S5b: UV-vis interference spectrum of probe **1** towards various analytes including ClO^-, H_2O_2, $^1O_2$, NO, $O_2^{\bullet-}$, HO•, ClO_2^-, IO_4^-, $ONOO^-$, ROO•, CN^-, HS^-, HSO_3^- and Cys (20 μM of **1** towards 1 equiv. of HClO and 100 equiv. of other anions in DMSO−Hepes buffer (3:1, v/v, pH 7.4)).

Figure S6: Fluorescent kinetic analysis

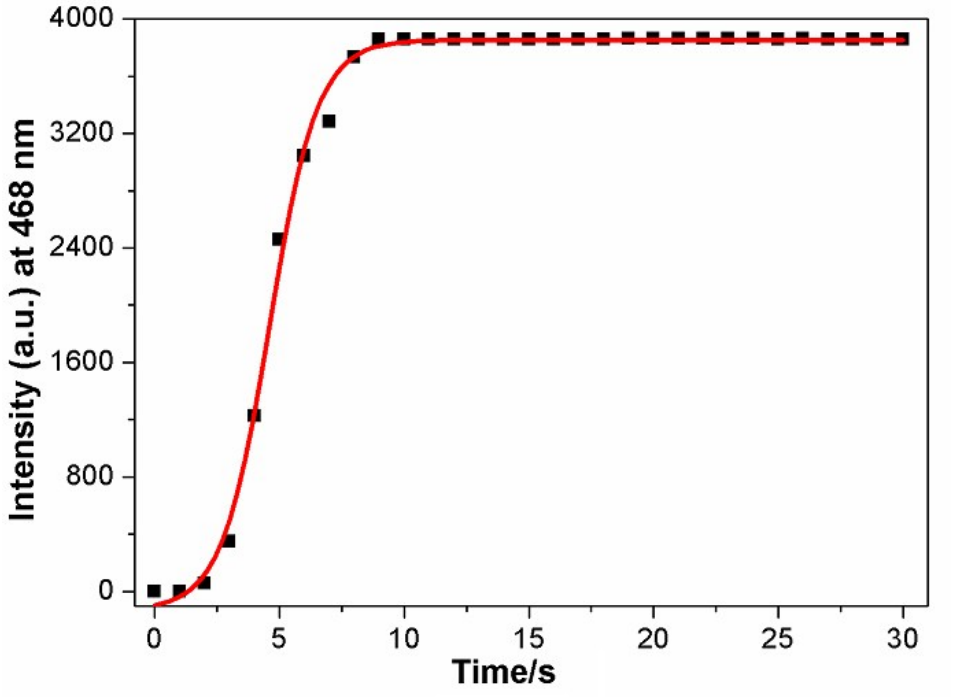


Figure S6: Kinetic analysis of probe **1** towards HClO (50 μM **1** with 10 equiv. of HClO).

Figure S7: The viability test of probe **1** in HepG2 cells

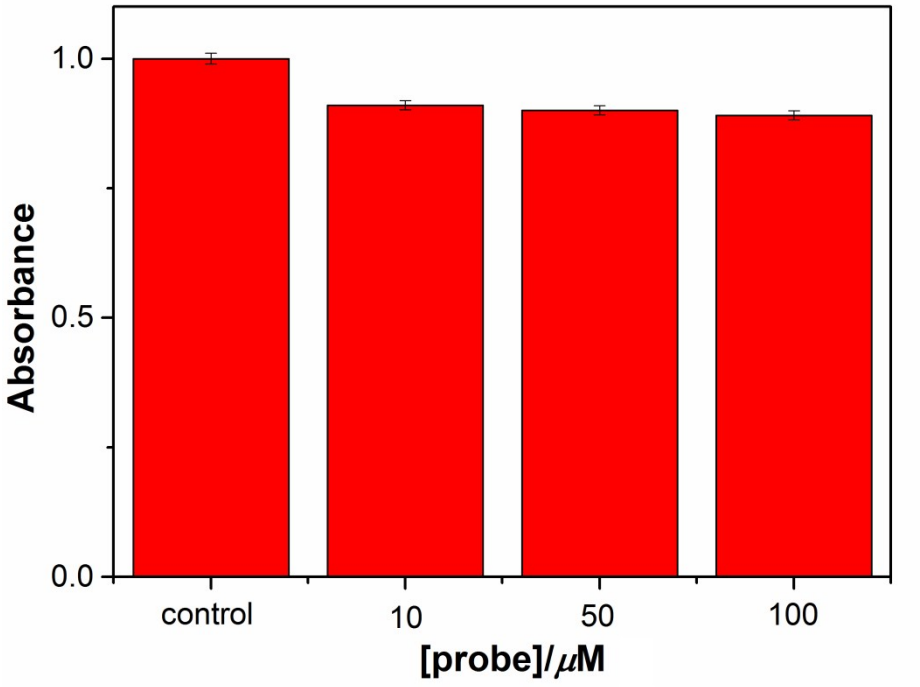


Figure S7: Viability of HepG2 cells in the presence of probe **1** (0-100 μM) as measured by CCK-8 kit. The cells were incubated with probe **1** for 1 h.