Electronic Supplementary Information

A novel dual-site ICT/AIE fluorescent probe for detecting hypochlorite and polarity in living cells

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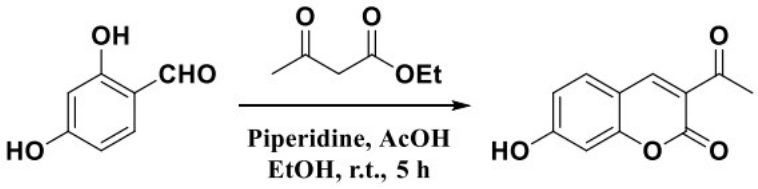
Synthesis

Synthesis of 3-acetyl-7-hydroxycoumarin (compound 1)

The compound **1** was synthesized by 2,4-dihydroxybenzaldehyde and ethyl acetoacetate through a Knoevenagel condensation reaction.[1] In briefly, 2.76 g (20.0 mmol) 2,4-dihydroxybenzaldehyde and 3.12 g (24.0 mmol) ethyl acetoacetate was added into 20 mL ethanol in a 100 mL flask, and then 1.70 g (20.0 mmol) piperidine dissolved in 2 mL ethanol was added dropwise into the mixture, after which 0.1 mL acetic acid was added by drops. The mixture was stirred at room temperature for about 5 h. Crude product was obtained after the solvent was removed by evaporation and the residue was washed by a small amount of ethanol. The crude product was further purified by column chromatography (ethyl acetate) and the pure compound **1** was obtained as a yellow solid (79.1% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.15 (s, 1H), 8.59 (s, 1H), 7.79 (d, J= 8.6 Hz, 1H), 6.85 (dd, J = 8.6 Hz, J = 2.3 Hz, 1H), 6.75 (d, J = 2.2 Hz, 1H), 2.55 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.0, 164.6, 159.5, 157.7, 148.3, 133.1, 119.5, 114.6, 111.2, 102.2, 30.5 (Fig. S9-S10, ESI†).

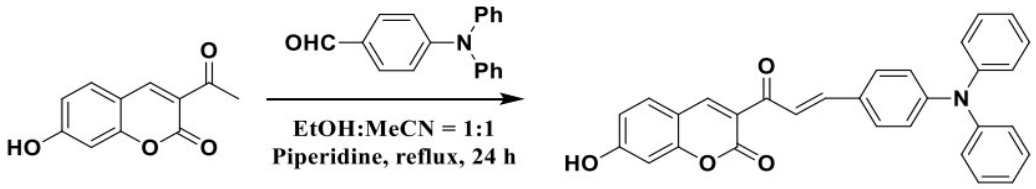


compound 1

Synthesis of (E)-3-(3-(4-(diphenylamino) phenyl)-7-hydroxy coumarin (CTPA-OH)

The **CTPA-OH** was synthesized by compound **1** (1.02 g, 5.0 mmol) and 4-diphenylamine benzaldehyde (1.78 g, 6.5 mmol) with ethanol (10 mL) and acetonitrile (10 mL) as the mixed solvent and piperidine (15 drops) as well as acetic acid (15 drops) as catalysts. The reaction system was heated to 80 ℃ and refluxed for about 24 h in the atmosphere of nitrogen. The solvent is removed by evaporation after the reaction system was cooled to room temperature, and a large amount of dark red solid was obtained by dissolving the oil with dichloromethane and adding petroleum

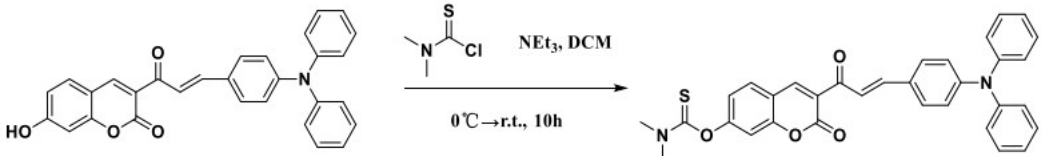
ether. The crude product was further purified by column chromatography (petroleum ether: ethyl acetate =2:1, v/v), and **CTPA-OH** which still contained a small amount of impurity matter was obtained (0.97 g, 42.3% yield). The product was directly applied to the next reaction. HRMS (ESI positive): calc. for $C_{30}H_{21}NO_4^+$, $[M+H]^+$ 460.1543, found 460.1545 (Fig. S11, ESI†).



compound 1 **CTPA-OH**

Synthesis of the probe CTPA

The probe **CTPA** was prepared by **CTPA-OH** (0.46 g, 1.0 mmol) and N,N-dimethylthioacyl chloride (0.73 g, 6.0 mmol) with dichloromethane (10 mL) as the solvent and triethylamine (0.30 g, 3.0 mmol) as the acid binding agent. The N,N-dimethylthioacyl chloride (dissolved in 2 mL dichloromethane) was added at 0 ℃ and the mixture was stirred for 1 h, after which the reaction system was sequentially stirred for 10 h at the room temperature. The crude product was obtained after removing solvent by evaporation, and was further purified by column chromatography (dichloromethane). Finally, the **CTPA** was obtained as an orange solid (0.25 g, 45.6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, *J* = 22.8 Hz, 1H), 7.81 (q, *J* = 15.6 Hz, 2H), 7.67 (dd, *J* = 8.4 Hz, *J* = 3.6 Hz, 1H), 7.52 (d, *J* = 8.6 Hz, 1H), 7.31 (t, *J* = 7.8 Hz, 4H), 7.21 – 7.06 (m, 10H), 7.01 (d, *J* = 8.6 Hz, 1H), 3.47 (s, 3H), 3.39 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.3, 186.0, 159.1, 158.2, 155.8, 150.5, 147.2, 146.7, 145.2, 130.4, 130.3, 129.5, 127.7, 125.6, 124.9, 124.3, 121.3, 121.2, 120.6, 116.5, 111.5, 43.4, 39.1, 29.7. HRMS (ESI positive): calc. for $C_{33}H_{27}N_2O_4S^+$, $[M+H]^+$ 547.1686, found 547.1657 (Fig. S12-S14, ESI†).



CTPA-OH **CTPA**

Preparation of solution of probe CTPA and analytes

Probe stock solutions of **CTPA** (1×10^{-3} M) were prepared in dimethyl sulfoxide (DMSO) or tetrahydrofuran (THF) and stored at 4 ℃. Absorption and emission spectra related to sodium hypochlorite were tested in PBS buffer solution (pH = 7.4) containing 1% THF. Spectral tests of polar response were performed in THF/H_2O (containing 1% DMSO) and DMSO/H_2O. The polarity of mixed solution was adjusted by changing the volume ratio of THF or DMSO to H_2O, and expressed by polar empirical constant E_T (30) according to the literature.[2] pH stability experiment was conducted in H_2O (containing 1% DMSO) by adjusting the above system with 0.1 M hydrochloric acid solution and 0.1 M sodium hydroxide solution. Viscosity study was conducted in glycerol/H_2O system by adjusting the volume ratio of glycerol to H_2O. The test solution (1×10^{-5} M) was obtained by adding 100 μL of probe stock solutions to 10 mL colorimetric tube and diluting the probe solution with the corresponding solvent system to the scale line.

The preparation of sodium hypochlorite solution was completed by diluting the commercial sodium hypochlorite solution, which concentration was calculated according to the absorbance of UV-vis absorption spectral at 292 nm.[3] The solutions (1×10^{-1} M) of various amino acids, cations, anions as well as reactive sulfur species (RSS) were prepared with glutamic acid (Glu), arginine (Arg), glycine (Gly), lysine (Lys), histidine(His), tryptophan (Trp), cysteine (Cys), homocysteine (Hcy), $FeCl_3\cdot6H_2O$, $AlCl_3$, $FeCl_2\cdot4H_2O$, $CdCl_2$, $BaCl_2\cdot2H_2O$, $CuSO_4\cdot5H_2O$, $ZnSO_4\cdot7H_2O$, $CaCl_2$, $MgSO_4$, NaCl, KCl, NH_4Cl, $KF\cdot2H_2O$, NaBr, KI, $Na_2S_2O_3$, $Na_2S_2O_5$, $KHSO_4$, Na_2SO_3, NaHS, Na_2S in deionized water. Other reactive oxygen species and reactive

nitrogen species need be prepared just before the testing moment. Tert-butyl hydroperoxide (TBHP) was delivered from 10% commercial aqueous solution. Nitric oxide (NO) was generated from sodium nitroprusside (SNP, CAS: 13755-38-9) [4]. Other ROS including hydroxyl radical (·OH), tert-butoxy radical (·O^{t}Bu), peroxynitrite ($ONOO^-$) and singlet oxygen ($^1O_2$) were obtained by reported procedures.[3-5] The concentration of $ONOO^-$ was determined according to the absorbance at 302 nm ($\varepsilon_{302\ nm}$ = 1670 $M^{-1}cm^{-1}$). The appropriate aliquot of analyte was added to the testing solution according to need of experiments in the text and was subsequently stored at 37 ℃ before recording the spectra.

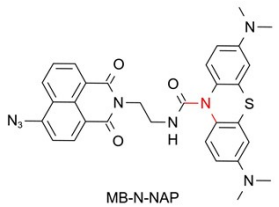
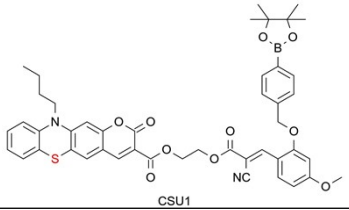
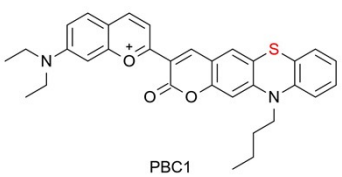
Determination of the detection limit for ClO^-

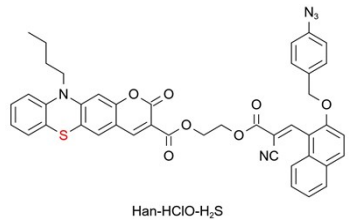
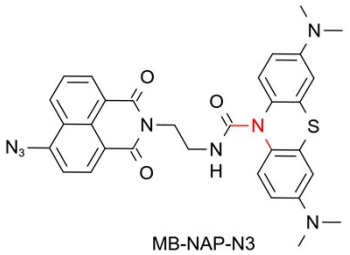
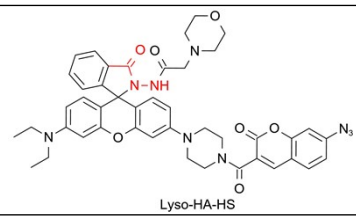
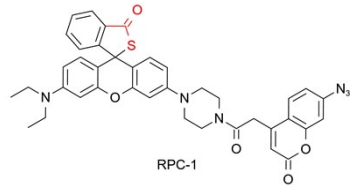
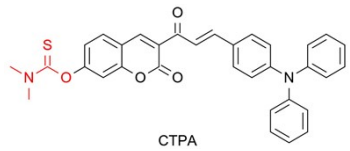
The detection limit of probe **CTPA** for ClO^- was obtained by the following equation:

$$LOD = 3\sigma/k$$

Where σ means the standard deviation and k means the slope of the titration spectra curve within a limited range.

Table S1. Dual-fluorescent probes for the detection of ClO^-.

Probes[a]	LOD	Linear interval	Response time	Another analyte	Application	Ref.
 MB-N-NAP	2.74 μM	0-20 μM	＜5 s	H_2S	H9C2 cells and HepG2 cells	35
 CSU1	13 nM	100.0-275.0 μM	----	H_2O_2	MCF-7 cells	36
 PBC1	13 nM	40-200 μM	within 20 s	SO_2	HeLa cells and zebrafish	37

 Han-HClO-H_2S	17 nM	50-300 mM	----	H_2S	MCF-7 cells	38
 MB-NAP-N3	32 nM	0-90 μM	40 s	H_2S	HeLa cells and zebrafish	39
 Lyso-HA-HS	7.3 nM	0-50 μM	within seconds	H_2S	HeLa cells	40
 RPC-1	19.8 nM	0-100 μM	within seconds	H_2S	RAW264.7 cells and mice	41
 CTPA	11.9 nM	0-19 μM	＜30 s	Polarity	Hela cell and NIH-3T3 cell	this work

a. The red part in the structure is the recognition site for ClO^-.

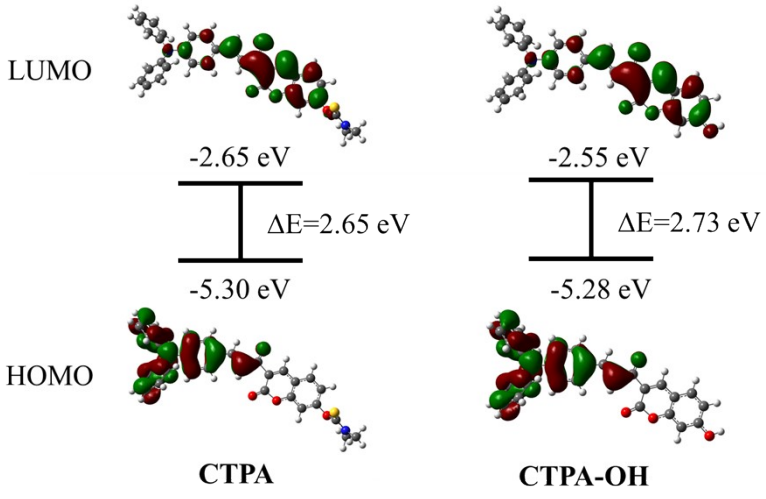


Fig. S1. Molecular orbital amplitude plots of HOMO/LUMO energy levels of **CTPA** and **CTPA-OH**

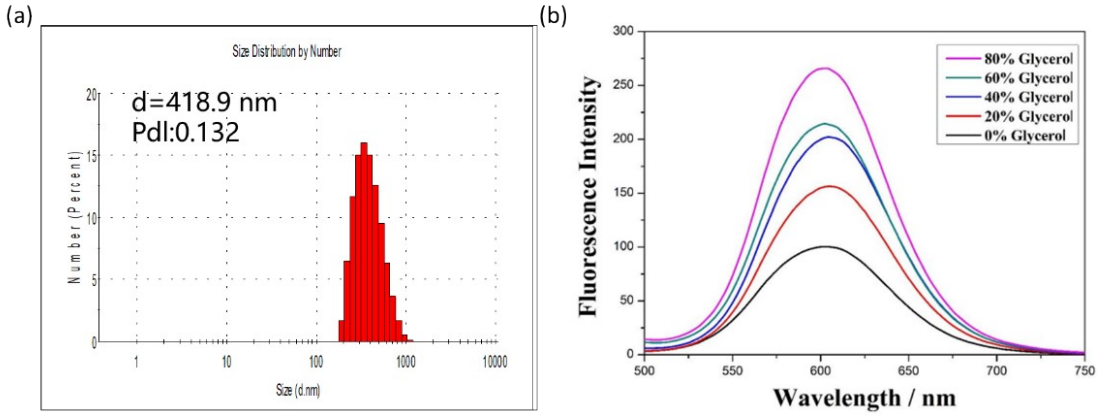


Fig. S2. (a). DLS data of **CTPA** (10 μM) (b). Fluorescence spectra of probe **CTPA** (10 μM) in the mixture of H_2O and glycerol (glycerol from 0% to 80%)

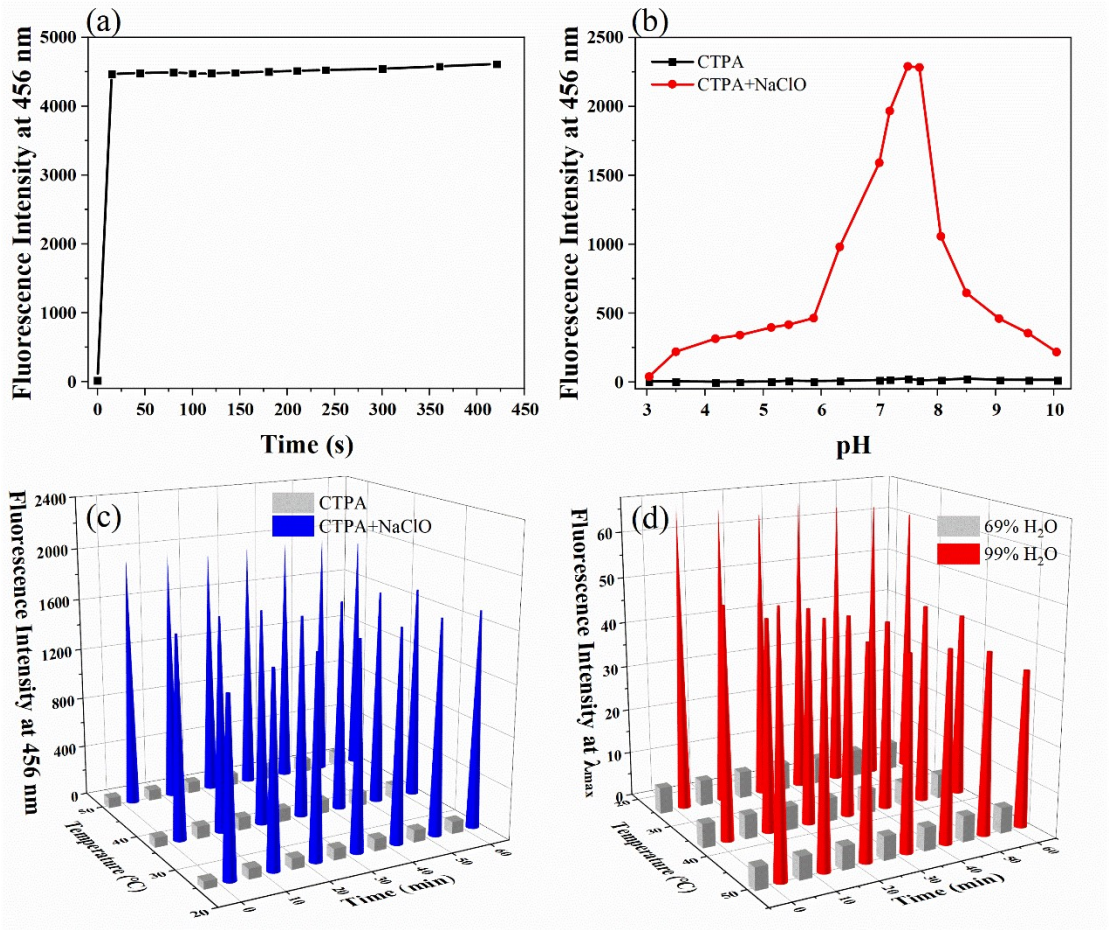


Fig. S3. The fluorescence intensity of **CTPA** (10 μM) (a) within 7 minutes after adding 60 μM NaClO; (b) before and after adding 60 μM NaClO at different pH; at 25°C, 37°C and 50°C (c) before and after adding 100 μM NaClO, λ_{ex} = 410 nm and (d) in THF/H_2O with 69%/99% water content, λ_{ex} = 460 nm.

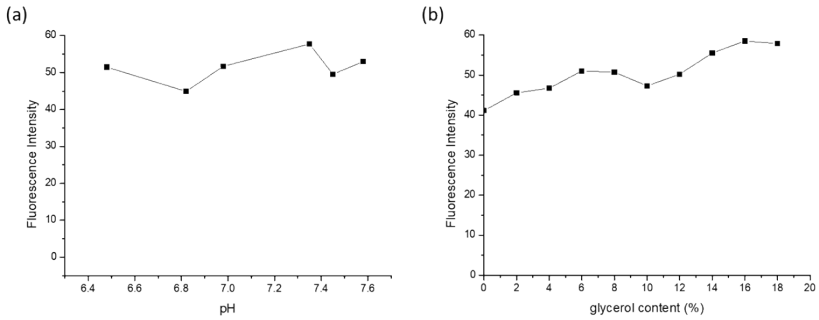


Fig. S4. (a). The pH dependence of fluorescence intensity at the maximum emission of **CTPA** (10 μM) (b). The viscosity dependence of fluorescence intensity at the maximum emission of **CTPA** (10 μM) in the mixture of H_2O and glycerol (glycerol from 0% to 20%), λ_{ex} = 460 nm

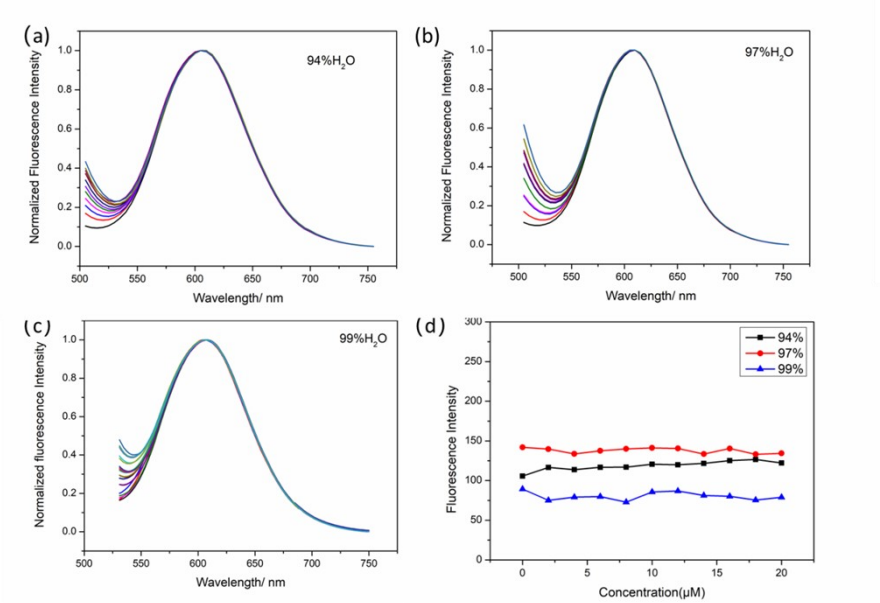


Fig. S5. Normalized fluorescence spectrums of **CTPA** (10 μM) with various concentrations NaClO (0-20 μM) in solvents of (a). 94% (b). 97% (c). 99% water content and (d). fluorescence intensity variety at corresponding maximum emission, λ_{ex} = 460 nm

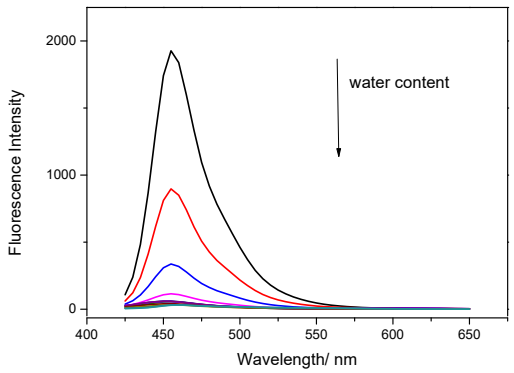


Fig. S6. Fluorescence spectrums of **CTPA** (10 μM) adding 10 μM NaClO in the mixture of H_2O and THF (f_w = 77% to 99%), λ_{ex} = 410 nm

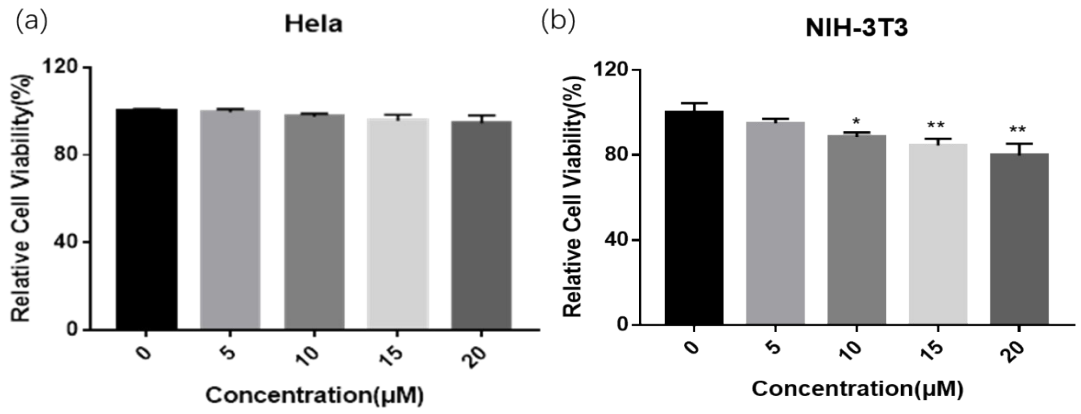


Fig. S7. Effects of the probe **CTPA** with various concentrations (0-20 μM) on the viability of the (a). Hela cells and (b). NIH-3T3 cells.

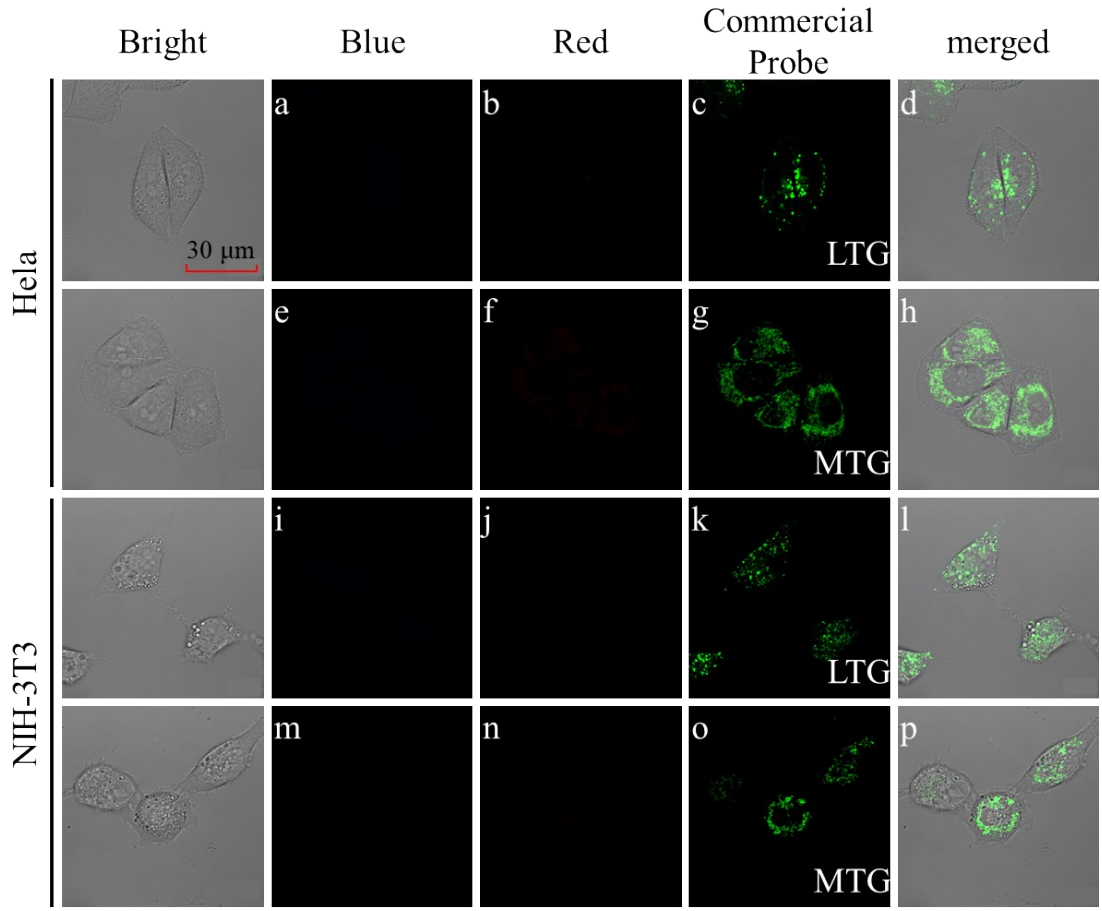


Fig. S8. Co-localization experiment of **CTPA** and (a-d, i-l) LTG and (e-h, m-p) MTG in Hela and NIH-3T3 cells respectively.

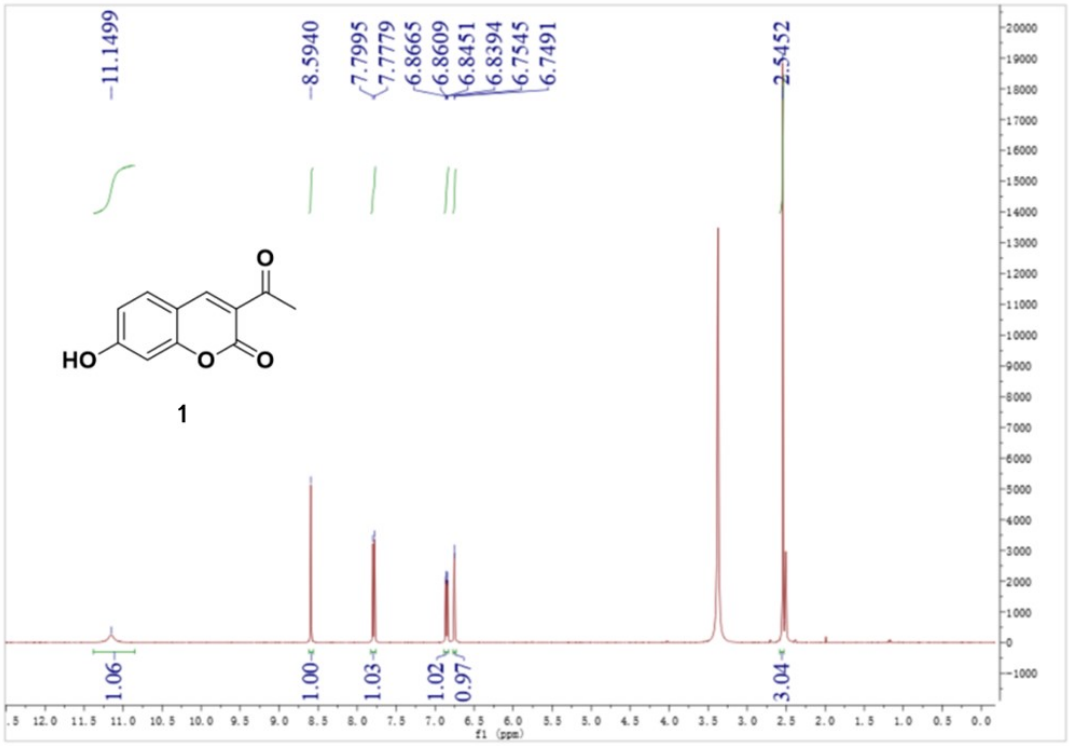


Fig. S9. ^{1}H NMR spectrum of compound **1** in DMSO-d_6.

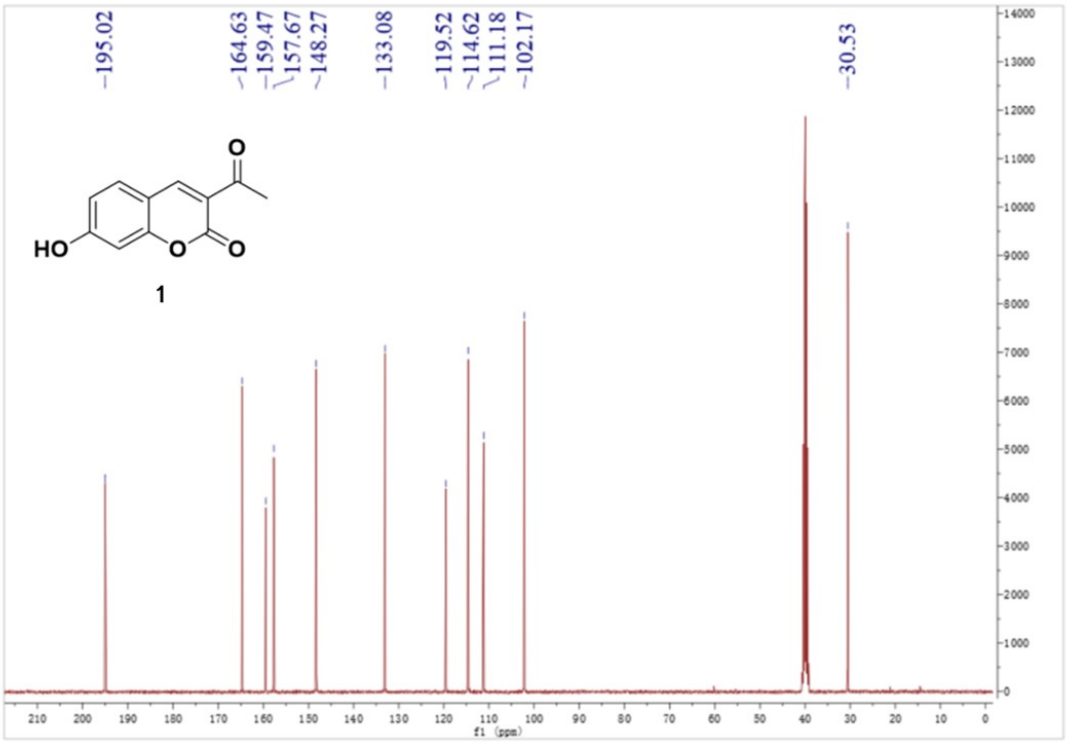


Fig. S10. ^{13}C NMR spectrum of compound **1** in DMSO-d_6.

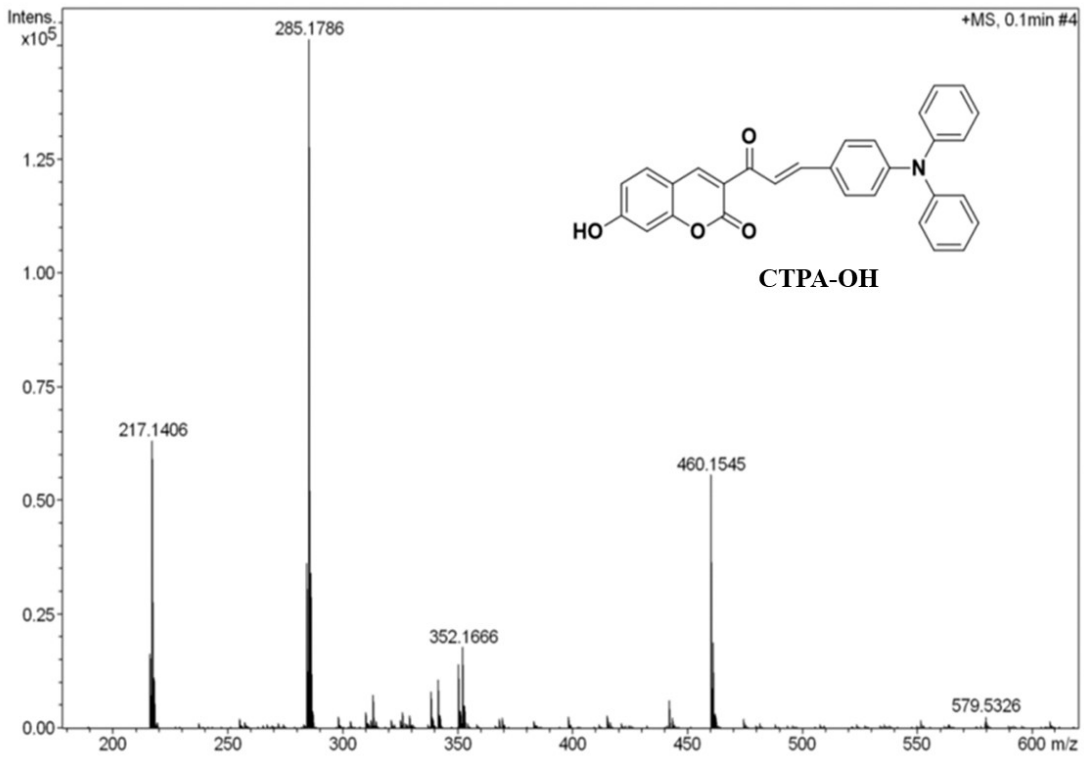


Fig. S11. HRMS spectrum of **CTPA-OH**

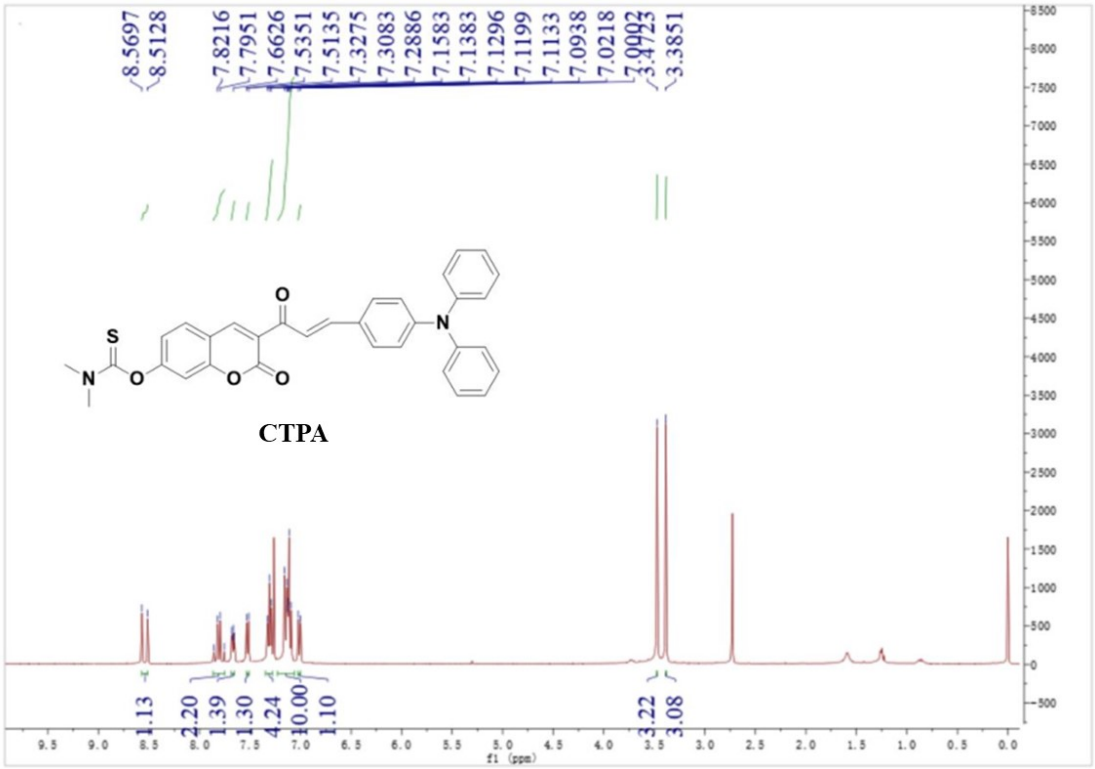


Fig. S12. ^{1}H NMR spectrum of **CTPA** in $CDCl_3$

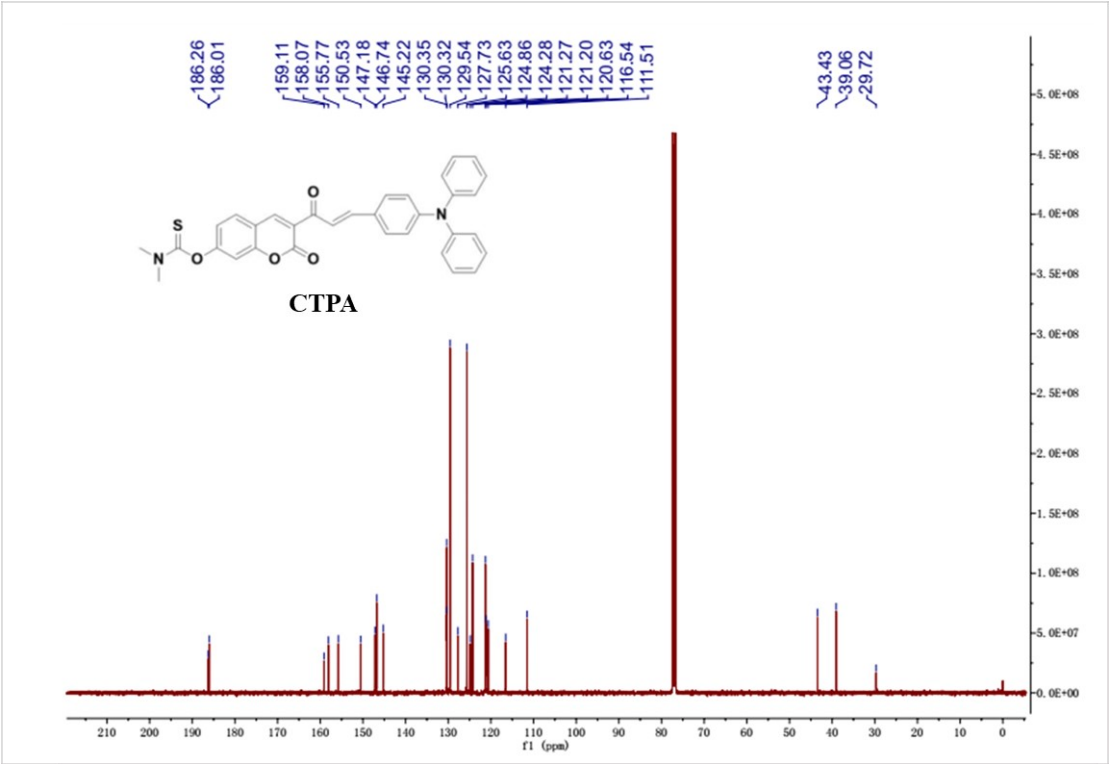


Fig. S13. ^{13}C NMR spectrum of **CTPA** in $CDCl_3$

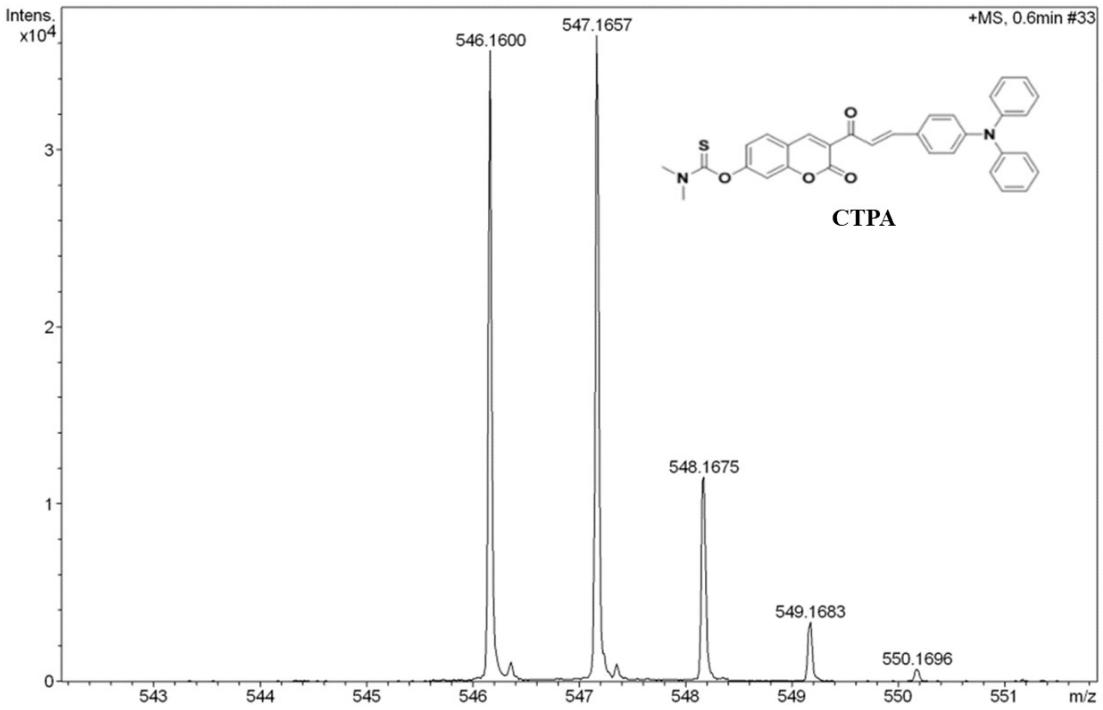


Fig. S14. HRMS spectrum of **CTPA**

References:

[1] Dai X, Wu QH, Wang PC, Tian J, Xu Y, Wang SQ, Miao JY and Zhao BX, *Biosensors and Bioelectronics*, 2014, **59**, 35-39.

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[3] Mao ZQ, Jiang H, Li Z, Zhong C, Zhang W and Liu ZH, *Chemical Science*, 2017, **8**(6), 4533-4538.

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