A mitochondria-targeted fluorescent probe for hypochlorite sensing and its application in bioimaging

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List of contents

1. Chemicals and apparatus

4-(diethylamino)-2-hydroxybenzaldehyde, ethyl 3-oxobutanoate, phosphorus oxychloride, piperidine and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Adamas-beta® (Shanghai, China). Ethanol and dimethylformamide were analytical pure and distilled before used. Some other testing species such as Co^{2+}, Cu^{2+}, Fe^{2+}, Pb^{2+}, Zn^{2+}, GSH, Cys, Hcy, HSO_3^-, HSO_4^-,$H_2PO_4^-$,OH^-,NO_2^-, NO_3^-, H_2O_2 were prepared in solutions according to the reported methods. Double-distilled water was used throughout all the experimental solutions. All samples were prepared at room temperature and were shaken for 1 min before each experiment.

Ultraviolet-Visible (UV-vis) absorption was recorded on Lambad 650s spectrophotometer (PerkinElmer, USA) and fluorescence spectra on F-7100 fluorescence spectrophotometer (Hitachi, Japan). 1H NMR and 13C NMR experiments were performed in d6-DMSO on an AVANCE III HD AN-400 MHz spectrometer (Bruker, Germany). Mass spectroscopy was recorded on a LCMS-2020 spectrometer (Shimadzu, Japan). High resolution mass spectroscopy data was obtained on a high resolution Orbitrap Fusion Lumos (Thermo Fisher Scientific, USA). Fluorescence imaging experiments were measured by using A1 confocal laser scanning microscope (Nikon, Japan).

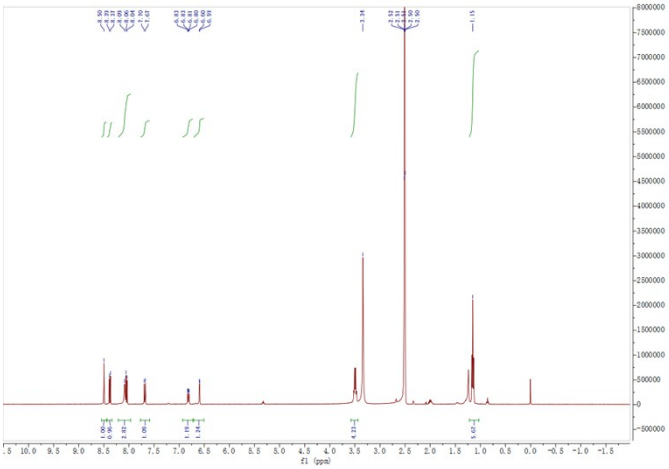

Fig. S1 ^{1}H NMR spectroscopy of C-DAN

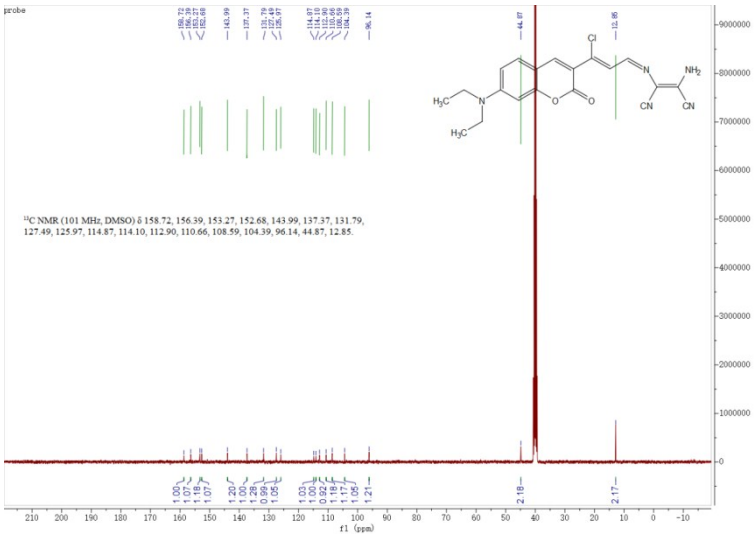

Fig. S2 ^{13}C NMR spectroscopy of C-DAN

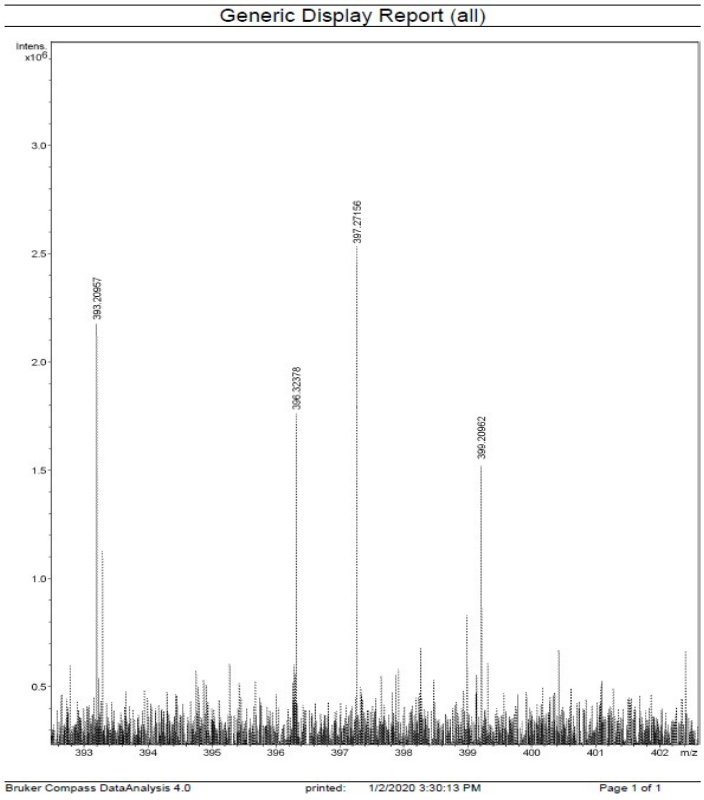

Fig. S3 MS spectroscopy of C-DAN

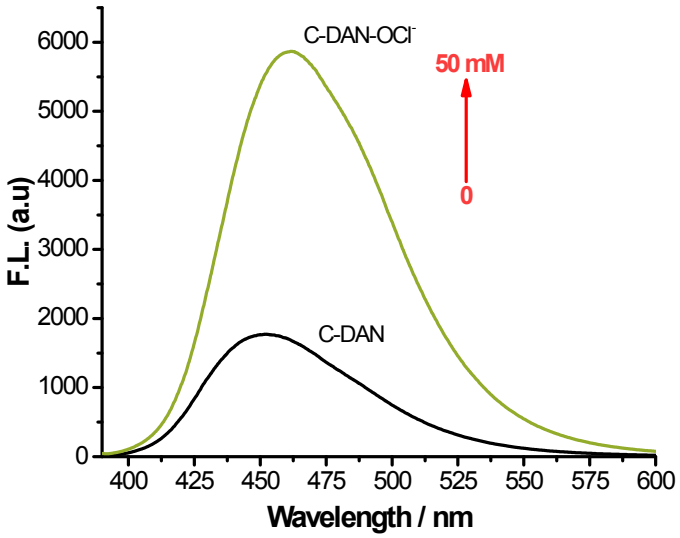

Fig. S4 Fluorescence spectra of C-DAN and C-DAN-OCl^-

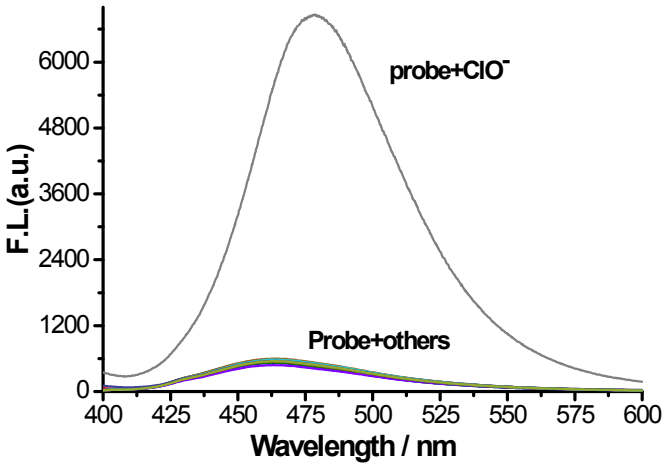

Fig. S5 Fluorescence spectra response of Probe C-DAN in the presence of various anions(Co^{2+}, Cu^{2+}, Fe^{2+}, Pb^{2+}, Zn^{2+}, GSH, Cys, Hcy, HSO_3^-, HSO_4^-, $H_2PO_4^-$, OH^-, NO_2^-, NO_3^-, and H_2O_2) in response to ClO^-.

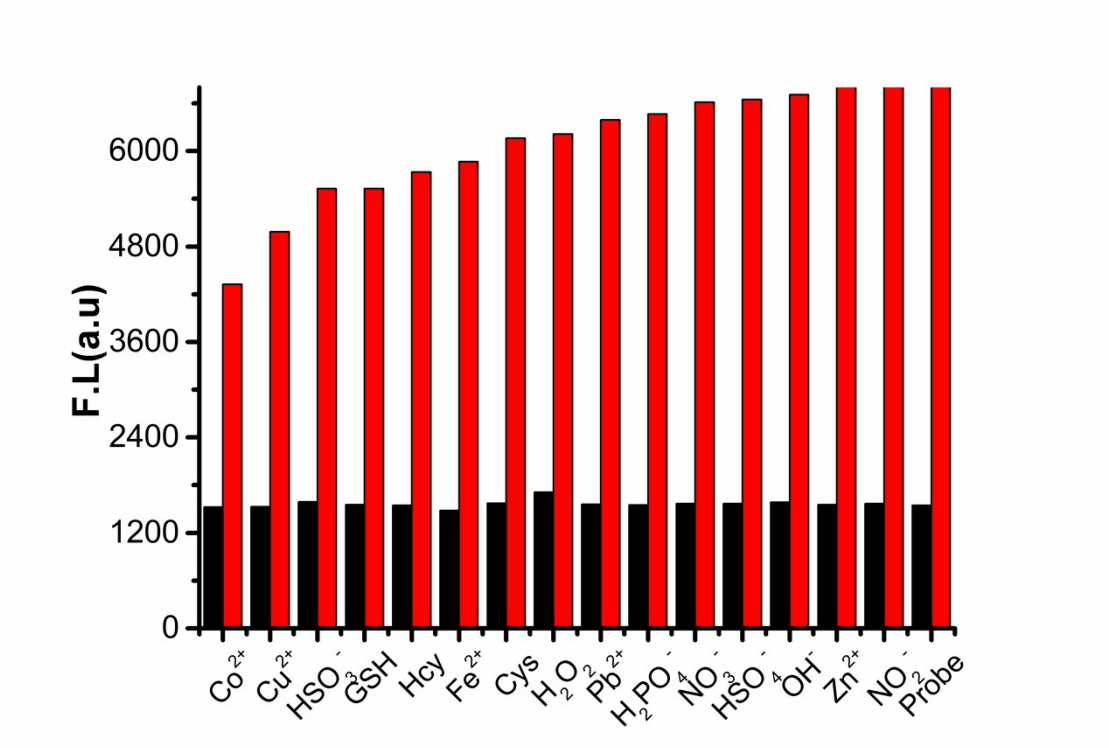

Fig. S6 The fluorescence intensity when the probe C-DAN (10 mM) was added to the solutions in PBS (pH = 7.4). The black bars indicate the fluorescence response of C-DAN to the interfering substance.

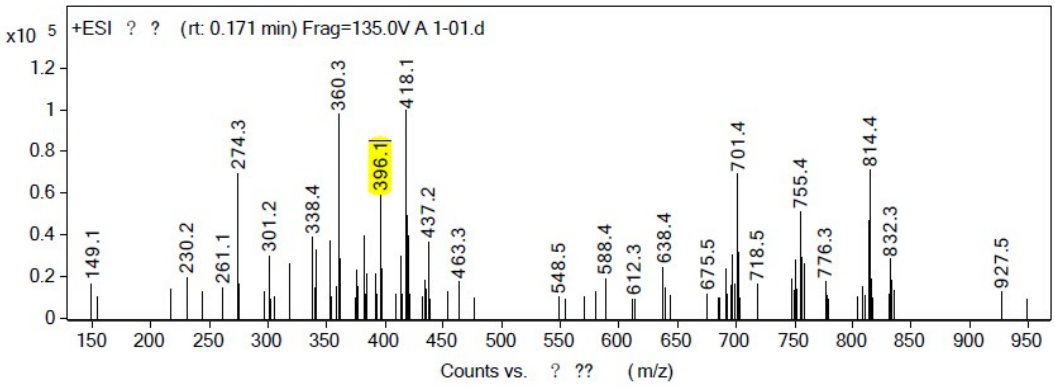

Fig. S7 The MS spectroscopy of C-DAN /ClO^-.

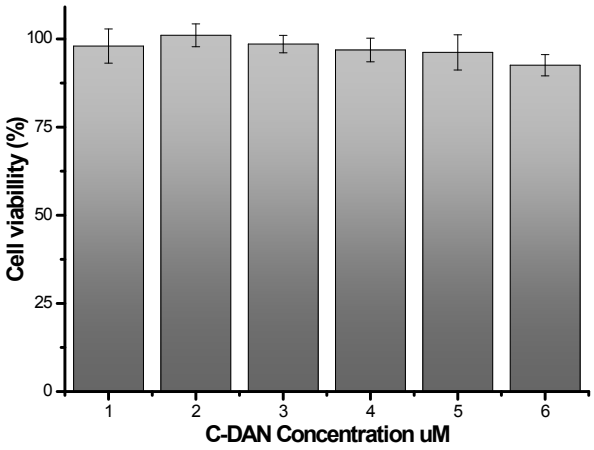

Fig. S8 Cytotoxicity of C-DAN by a MTT assay (n = 3).

Table S1 The testing results of ClO− concentration in tap water and river water samples (n=3)

Samples	Addition (mM)	Detection (mM)	Recovery (%)	RSD (%)
Tap water	10	9.86	98.6	1.85
	15	14.75	98.3	1.92
	20	19.88	99.4	1.88
River water	10	9.78	97.8	1.86
	15	15.02	102	1.98
	20	19.65	98.3	1.93

RSD value reported is for n = 3.