Supporting information for

Turn-On Fluorescent Probe Based on Dicyanisophorone for Rapid Detecting of Peroxynitrite in Aqueous Medium and Bioimaging

Jianwei Wu, Jia Li, Yaqiao Shi, Liting Jiang, Chenming Chan, Ru Feng*, Yue Wang and Zhaoli Xue*

School of Chemistry and Chemical Engineering, Jiangsu University, Zhenjiang 212013, PR China

2.1 Materials

All related chemicals and solvents obtained from J&K Scientific Ltd were analytical grades without further purification. For the determination of UV-vis spectra and fluorescence spectra, spectral grade solvents (HPLC containing isopentane stabilizer) were purchased from J&K Scientific Ltd. (4,5-dimethylthiazol)-2,5-diphenyltetrazolium-bromide (MTT), dimethyl sulphoxide (DMSO), Dulbecco's modified Eagles medium (DMEM) cell culture medium, fetal bovine serum (FBS), phosphate buffer solutions (PBS) and Pancreatic enzyme were purchased from Jiangsu Biooer Scientific Instrument Co. Ltd. $ONOO^-$ stock solution was prepared by a published method: HCl (0.6 M, 10 mL) was added to a vigorously stirred solution of $NaNO_2$ (0.6 M, 10 mL) and H_2O_2 (0.7 M, 10 mL) in deionized H_2O at 0 °C, immediately followed by the rapid addition of NaOH (1.5 M, 20 mL).The aqueous solution of ClO^- was prepared from the NaClO solution contain 6-14 % reactive chlorine, and the original concentration of the ClO^- was determined by titration with $S_2O_3^{2-}$. H_2O_2 solution was diluted from the stock H_2O_2 solution purchased from Sigma-Aldrich Chemical Co. ROO• was generated from 3-Chloroperbenzoic acid. •OH was obtained by Fenton reaction. The $O_2•^-$ solution was prepared by dissolving KO_2 in DMSO. The aqueous solution of Cu^{2+}, Fe^{3+}, Ag^+ and Hg^{2+} were prepared from $CuSO_4$, $FeCl_3•6H_2O$, $AgNO_3$ and $Hg(CF_3SO_3)_2$. The deionized water was used for preparing the metal ion solutions. Thin-layer chromatography (TLC), flush column chromatography and gravity column chromatography were performed on Art. 5554 (Merck KGaA), Silica Gel 60 (Merck KGaA), and Silica Gel 60N (Kanto Chemical Co.) respectively.

2.2. Equipment

UV–vis spectroscopy test was performed by UV-2450 spectrometer. And fluorescence spectroscopy test was performed by CaryEclipse spectrometer with 5 nm excitation and emission slit widths. ^1H NMR and ^{13}C NMR experiments were performed on a Bruker Avance 400 MHz. Chemical shifts are reported in units of ppm relative to the solvent residue peaks (DMSO-d_6, δ = 2.50 ppm for ^1H, 39.52 ppm for ^{13}C). MALDI-TOF mass spectra were recorded on a Bruker Daltonics autoflexII MALDI-TOF MS spectrometer. The pH measurements were conducted with a Sartorius PB-10 (Sartorius scientific instruments, Beijing, China) containing a Composite glass electrode. The date of MTT was measured by Spectramax microwell plate reader (SpectraMax

190). Cell imaging experiments were performed by using Two-photon laser confocal microscopy (Leica DMi8, Germany).

2.3. Calculations of detection limit (LOD)

The LOD results were based on fluorescence titration experiments. The calculation based on a plot of absorption intensity and concentration of $ONOO^-$.

$$DL = 3\sigma/k$$

where σ was the standard deviation of blank sample, k is the slope of the calibration curve[29, 30].

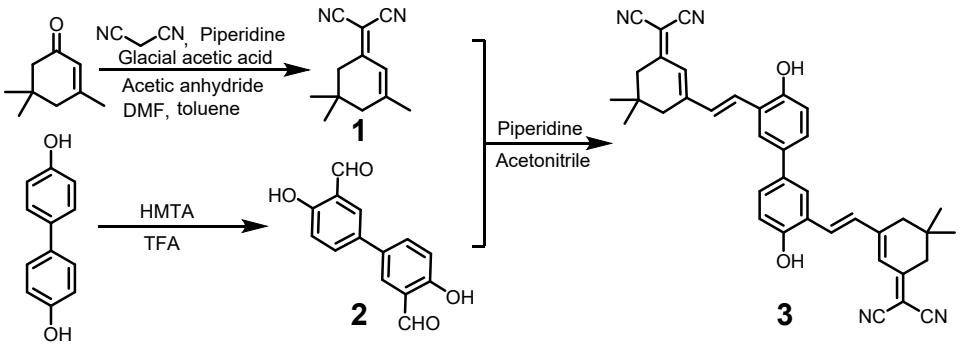
2.4 Cytotoxicity experiments

Living MCF-7 cells were provided by the college of pharmacy of Jiangsu University. The cells with the concentration of 5×10^{-3} / mL were seeded into a 96-well cell-culture plate in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FBS (fetal calf serum) at 37 °C under an atmosphere of 5 % CO_2 for 24 h. After dilution with DMEM medium, various concentrations (10 μM, 15 μM and 20 μM) of chemosensor **3** were added into the cell culture plate after the cells were washed with phosphate buffered saline (PBS) for three times. The cells were incubated at 37 °C under an atmosphere of 5 % CO_2 overnight. After the incubation time, the original medium was wiped off and washed with PBS for three times followed by adding 100 μL MTT (1 mg/mL). 4 h later, the medium was taken off and 100 μL DMSO was added into each well. The absorbance at 490 nm was measured by Spectramax microwell plate reader, the cell viability towards MCF-7 cell line was measured using the given equation:

Cell viability (%) = Mean absorbance (Treated cell) / Mean absorbance (Control cell)

2.5 Cell fluorescence imaging

The cells were divided into four groups and imaged after different treatments. All groups of cells were incubated with 10 μM probe for 0.5 h. As a control group, the first group was not treated with $ONOO^-$. The next three groups were incubated with 10 μM, 50 μM and 100 μM $ONOO^-$, respectively, for another 0.5 h. All the cells were rinsed with PBS buffer for three times before imaging. The fluorescence images were collected on a confocalmicroscope (Leica DMi8, Germany).



Scheme 1: Synthesis of chemosensor **3**.

2.6 ***Synthesis of 2-(3,5,5-trimethylcyclohex-2-en-1-ylidene) alononitrile 1***

Isophorone (3.3 mL, 21.7 mmol) and malononitrile (1.2 mL, 19.7mmol) were injected into the 250 mL Shrek tube and then 10 mL DMF and 10 mL toluene were added under nitrogen environment. Follow by glacial acetic acid (0.2 mL, 3.5 mmol), acetic anhydride (0.2 mL, 2.1 mmol) and piperidine (0.4 mL, 4 mmol) were added dropwise. The mixture was stirred at room temperature for 1.0 h and then refluxed at 120 ℃ for 4.0 h. After cooling down to room temperature, the solvent was removed under reduced pressure and then extracted by using deionized water and CH_2Cl_2. The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Then the crude product was purified by silica-gel flash column chromatography (silica gel, CH_2Cl_2: Petroleum ether = 1 : 2) to obtain fine brown needles powder. Yield: 2.43 g, 60%. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K): δ = 6.54 (s, 1H, alkyl), 2.51 (s, 2H, vinyl), 2.20 (s, 2H, vinyl), 2.03 (s, 3H, –CH_3), 0.93 (s, 6H, –CH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6, 298 K) δ = 171.8, 162.9, 119.9, 113.9, 113.1, 76.5, 45.3, 42.4, 32.4, 27.7, 25.4 ppm. UV-vis (in CH_2Cl_2) λ [nm] (ε [$M^{-1}cm^{-1}$]): 305 (60,800).

2.7 ***Synthesis of 4,4'-dihydroxy-[1,1'-biphenyl]-3,3'-dicarbaldehyde 2***

4, 4'-Dihydroxybiphenyl (2.12 g, 10.74 mmol) and methenamine (3.02 g, 21.48 mmol) were added into a 250 mL round-bottom flask. Afterwards, TFA (75.3 mL) was injected into the flask and the mixture was stirred for 3.0 h at 70 ℃ in N_2 atmosphere. The resulted solution was added into a certain amount of cold distilled water and then stirred for 3.0 h at room temperature. The organic phase was extracted from the mixture which had been filtered by acetate (2 × 30 mL). Dry the combined organic solution over Na_2SO_4 and evaporate dryness. Collect the crude product and purify by column chromatography (Silica gel, CH_2Cl_2: Petroleum ether = 3:1) to obtain the yellow

solid. Yield: 0.98 g, 38 %. ^{1}H NMR (400 MHz, DMSO-d_6, 298 K) δ = 10.80 (s, 2H, aldehyde), 10.30 (s, 2H, hydroxy), 7.88 (s, 2H, phenyl), 7.80 (d, J = 8.6 Hz, 2H, phenyl), 7.07 (d, J = 8.6 Hz, 2H, phenyl) ppm; ^{13}C NMR (100 MHz, DMSO-d_6, 298 K) δ = 192.4, 160.4, 130.8, 126.9, 122.8, 118.6 ppm; UV-vis (in CH_2Cl_2) λ [nm] (ε [$M^{-1}cm^{-1}$]): 247 (30,000); Calcd. for $C_{14}H_{10}O_4$ [$M–H^+$]$^-$: 241.0501, Found: 241.0504.

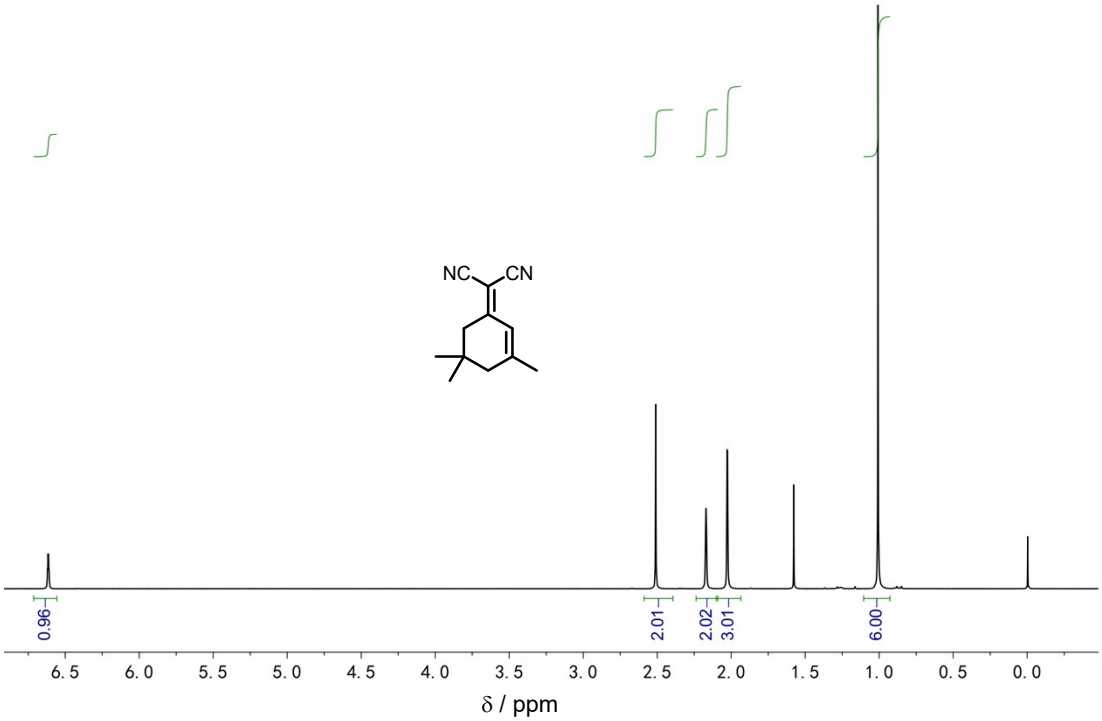


Figure S1. ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **1.**

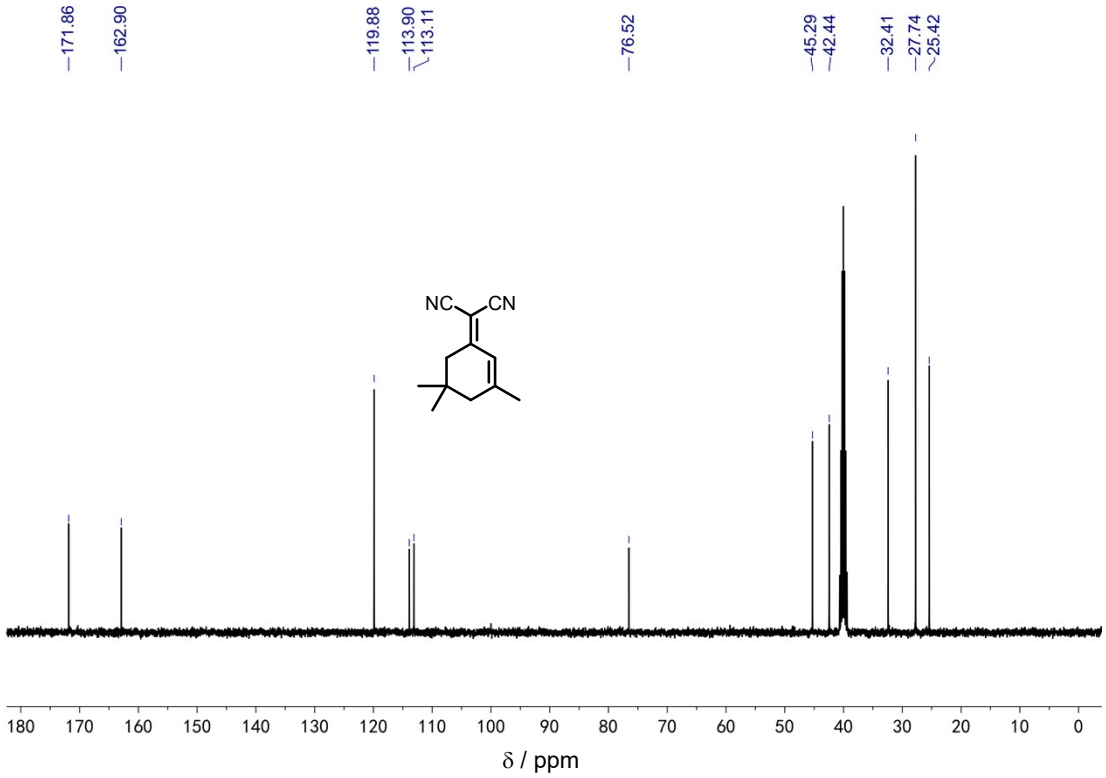


Figure S2. ^{13}C NMR (400 MHz, DMSO-d_6) spectrum of compound **1.**

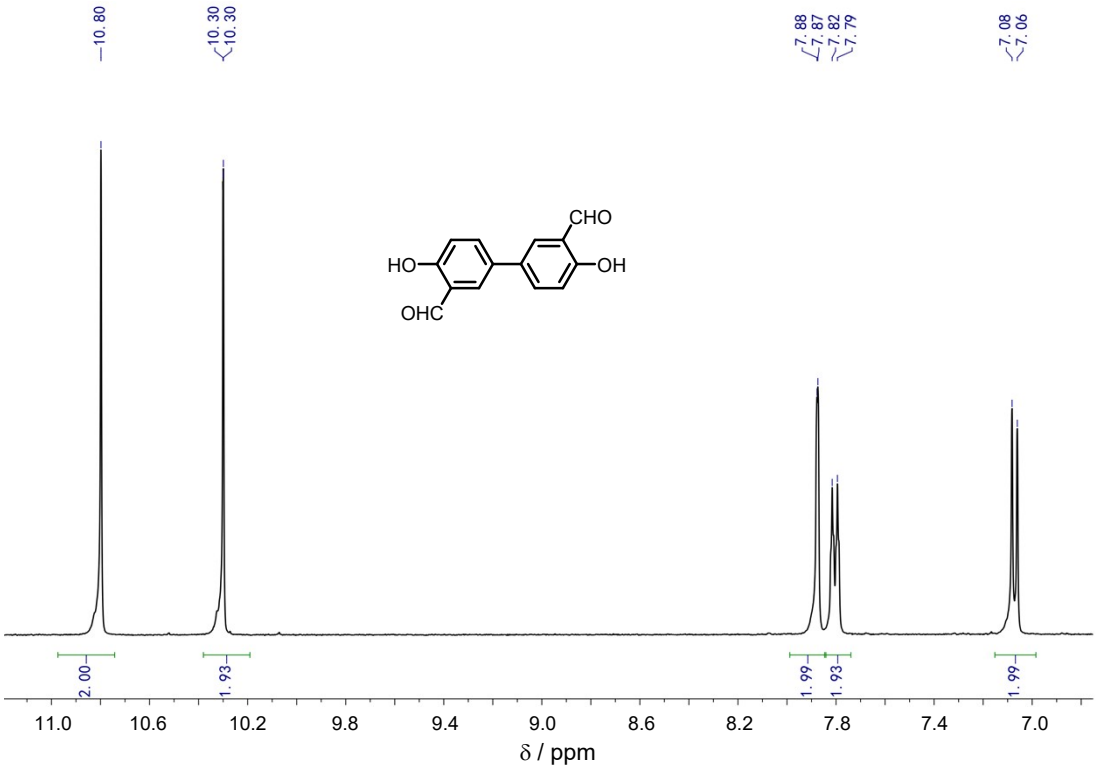


Figure S3. ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound **2.**

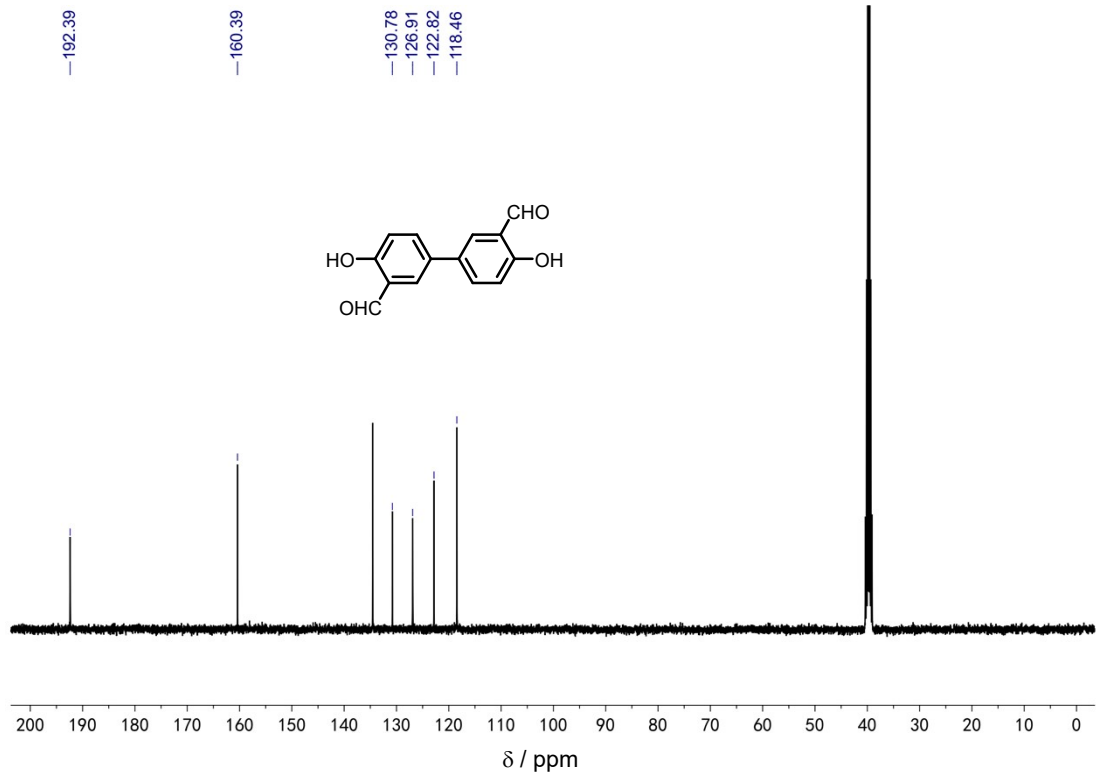


Figure S4. ^{13}C NMR (400 MHz, DMSO-d_6) spectrum of compound **2.**

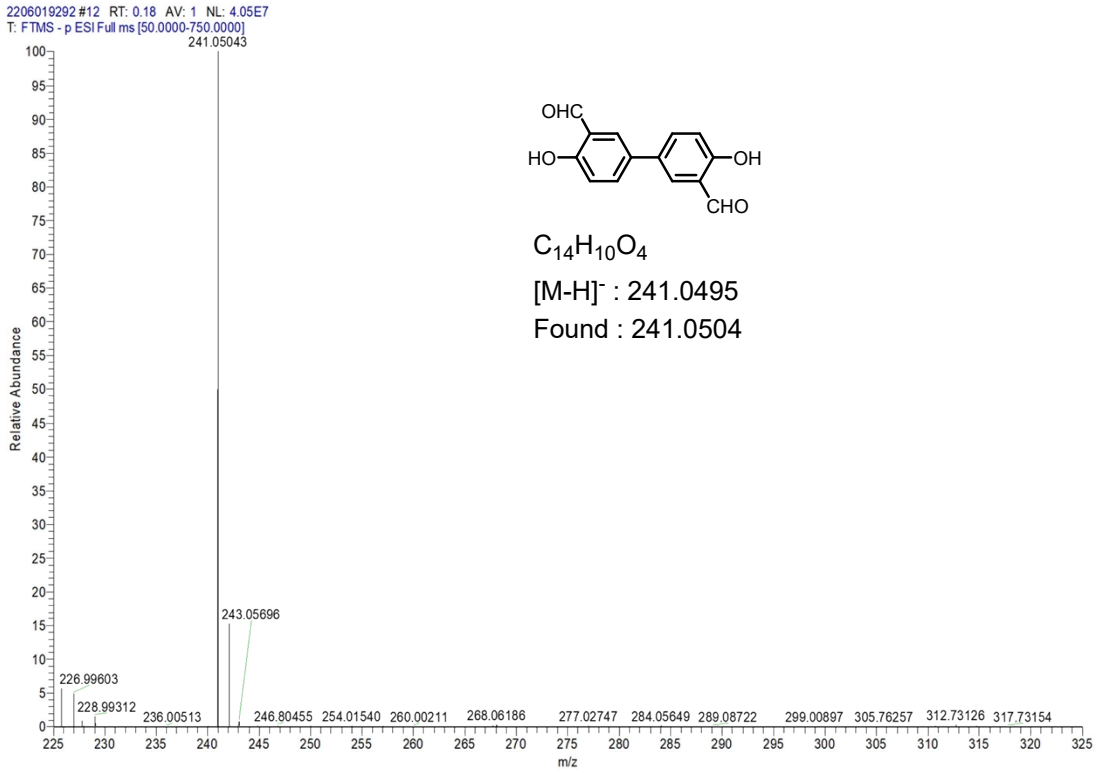


Figure S5. FTMS of compound **2.**

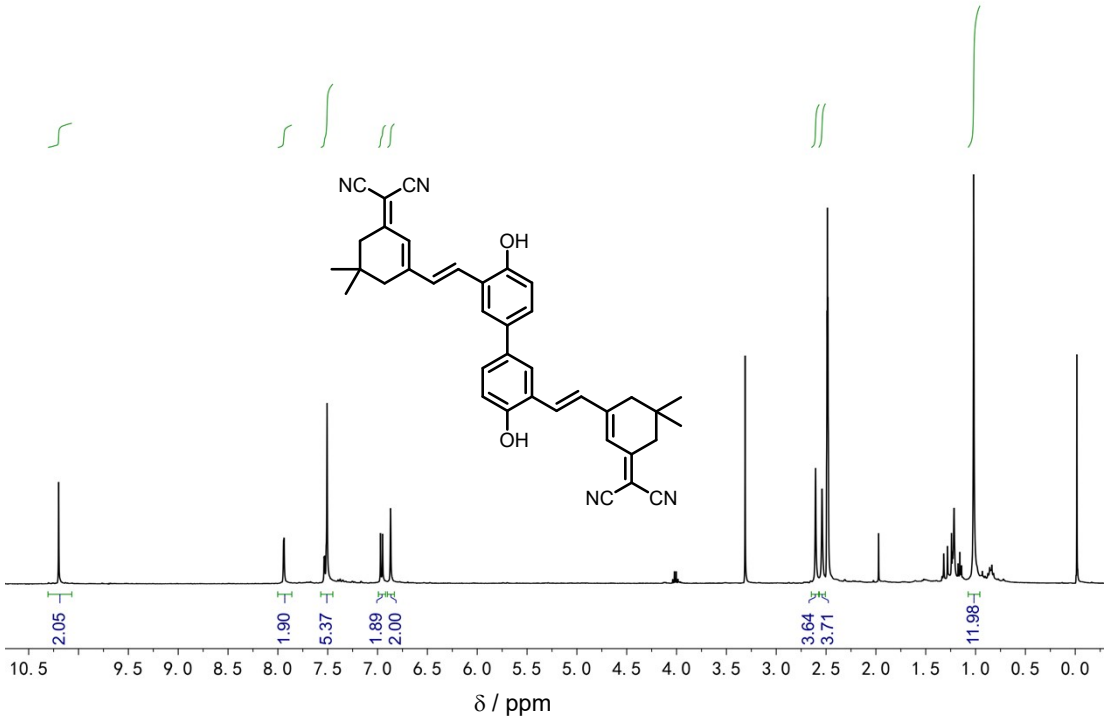


Figure S6. ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of probe **3.**

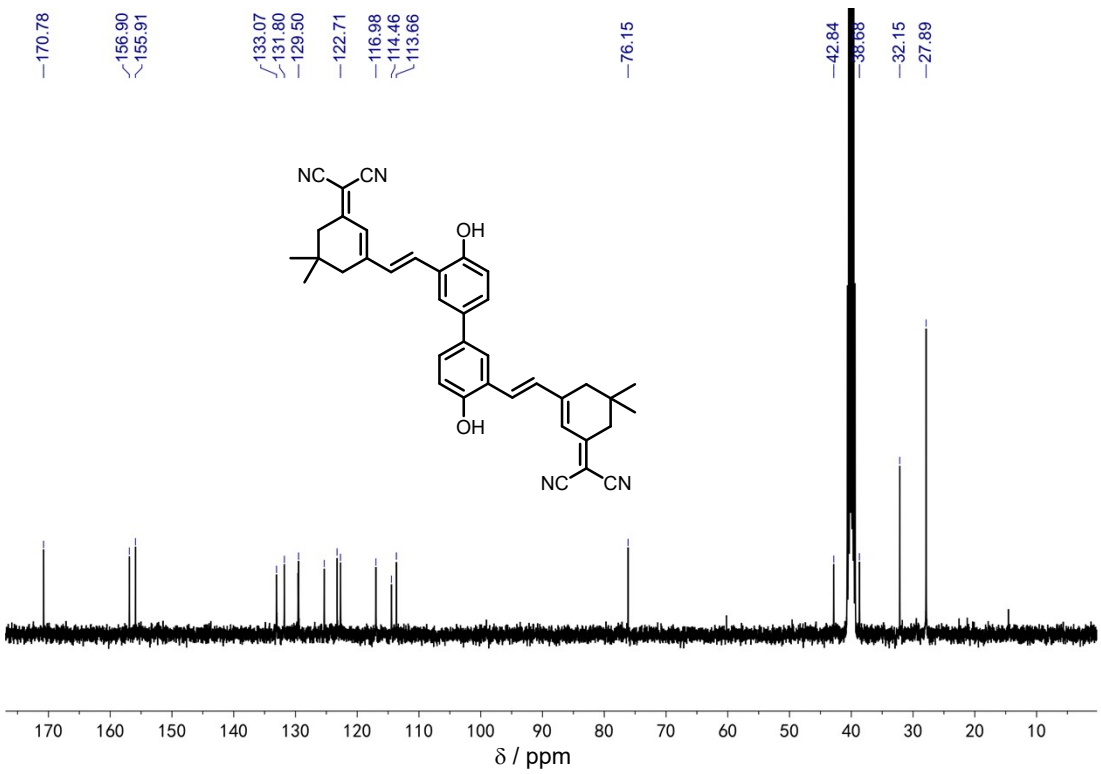


Figure S7. ^{13}C NMR (400 MHz, DMSO-d_6) spectrum of probe **3.**

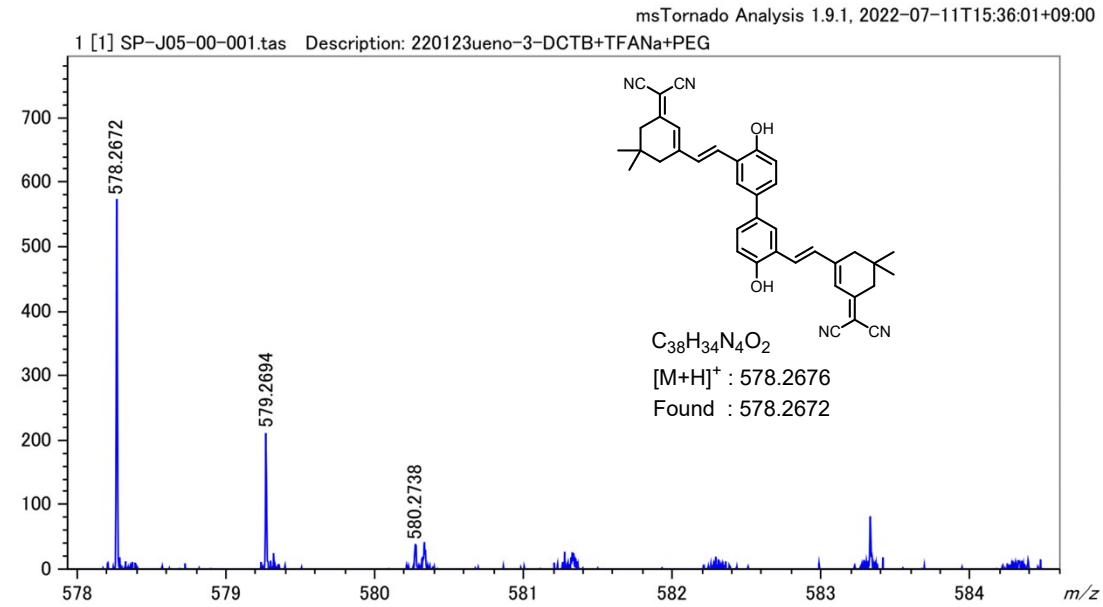


Figure S8. MALDI-TOF MS of probe **3.**

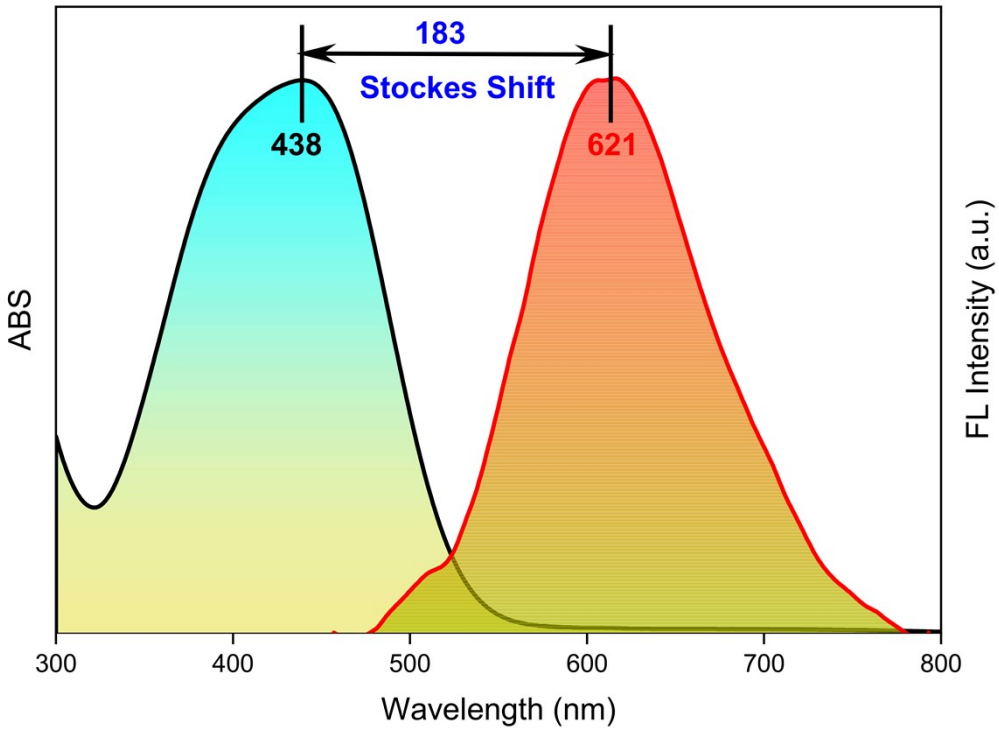


Figure S9. Absorption and emission spectra of probe **3** in DMSO solution excited at 438 nm.

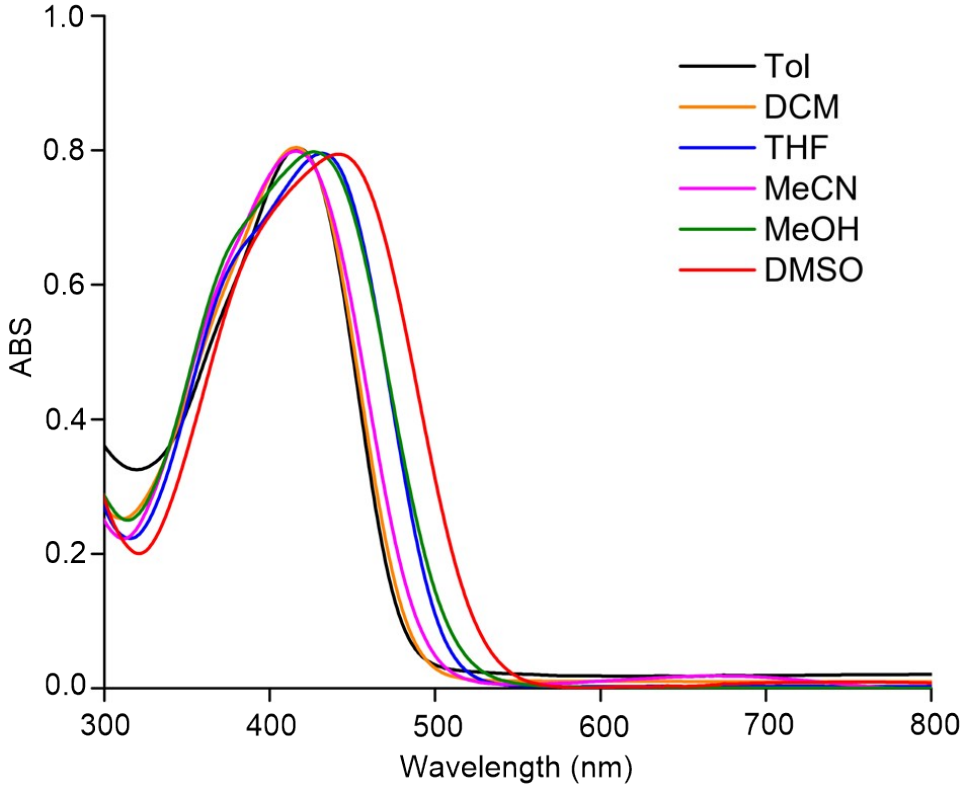


Figure S10. The absorption spectra of probe **3** in different solvent.

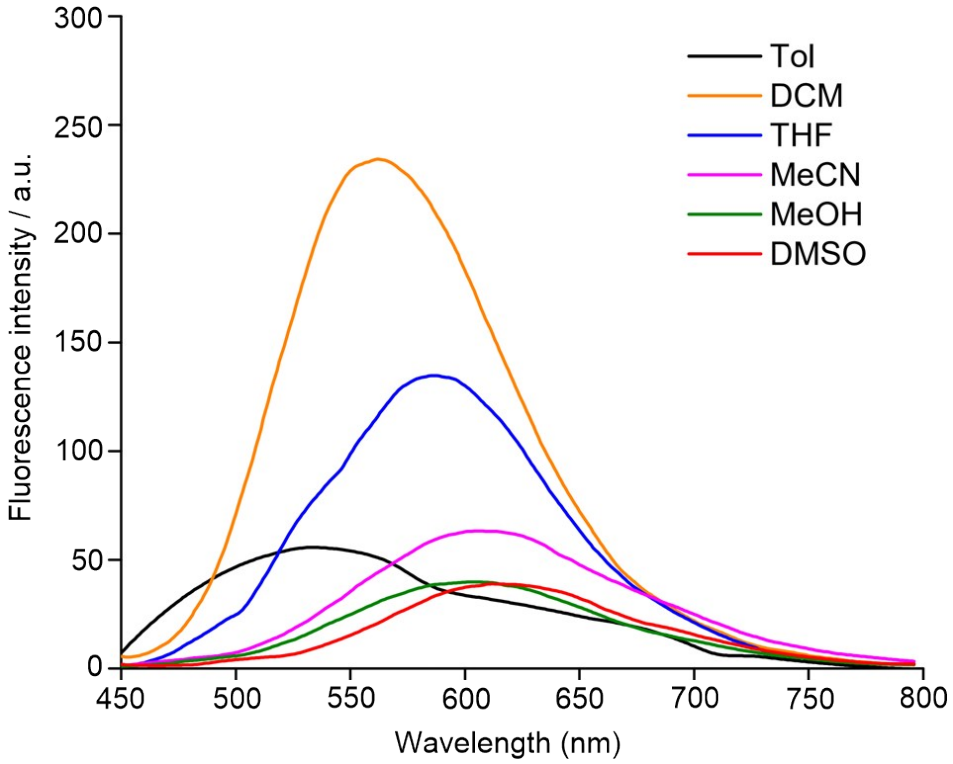


Figure S11. Fluorescence intensity spectra of probe **3** in different solvent (Ex.: 438 nm).

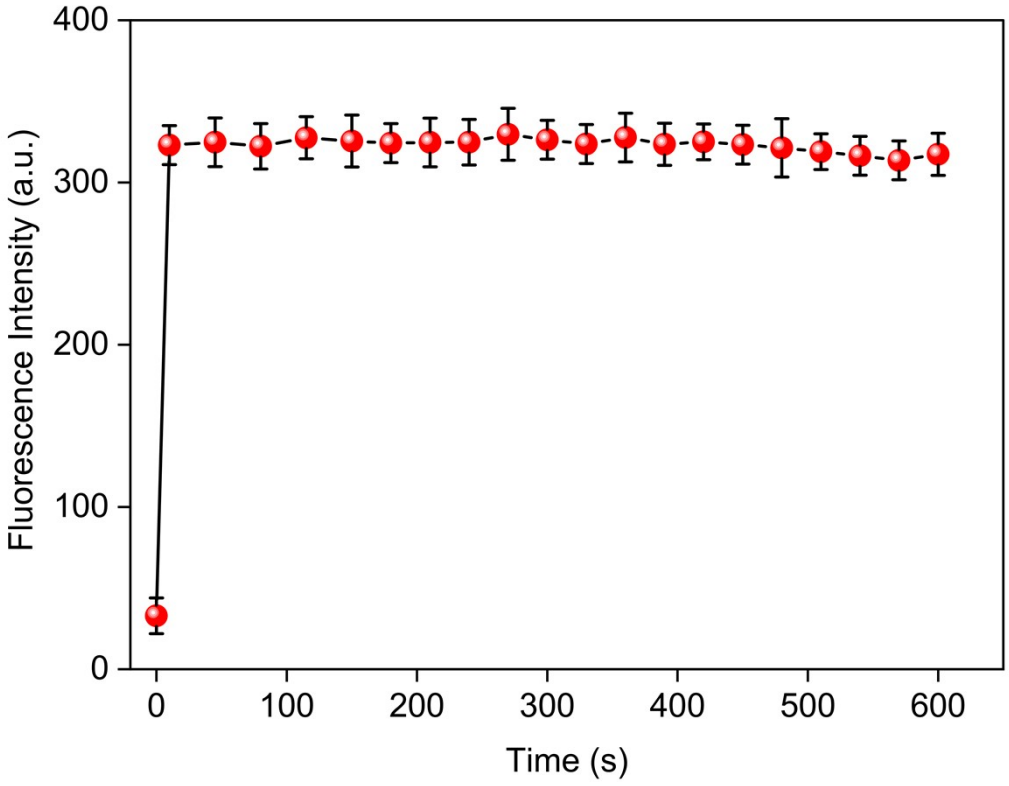


Figure S12. Time-dependent reaction profiles of probe **3** with **ONOO**$^-$ solution (E.m.: 621 nm).

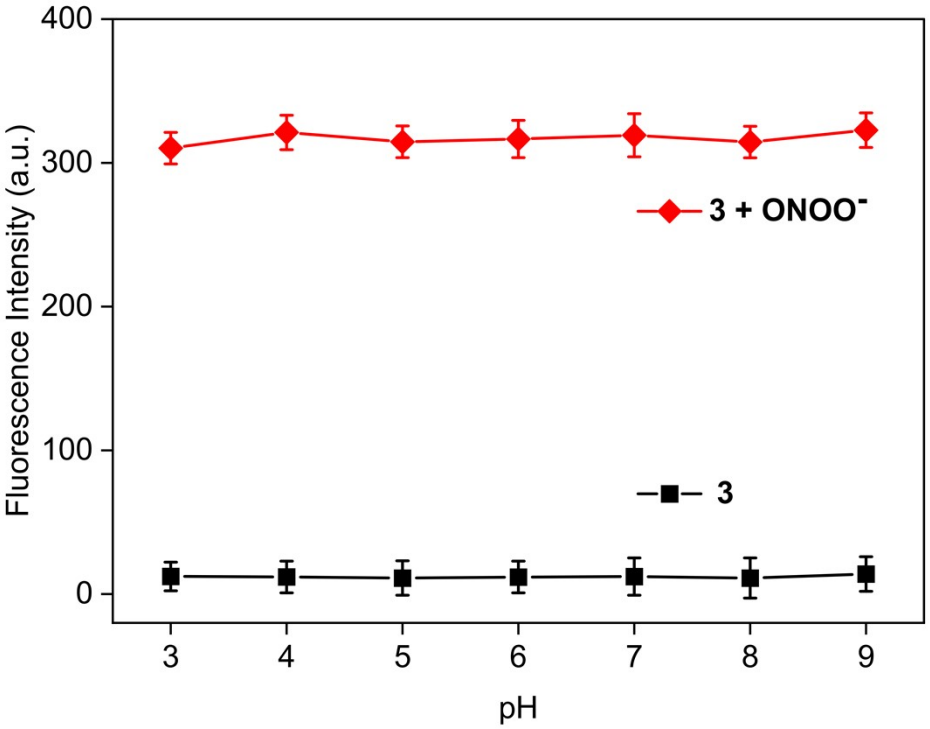


Figure S13. pH range of probe **3** in DMSO and water (V_{DMSO} : V_{Water} = 9 : 1)

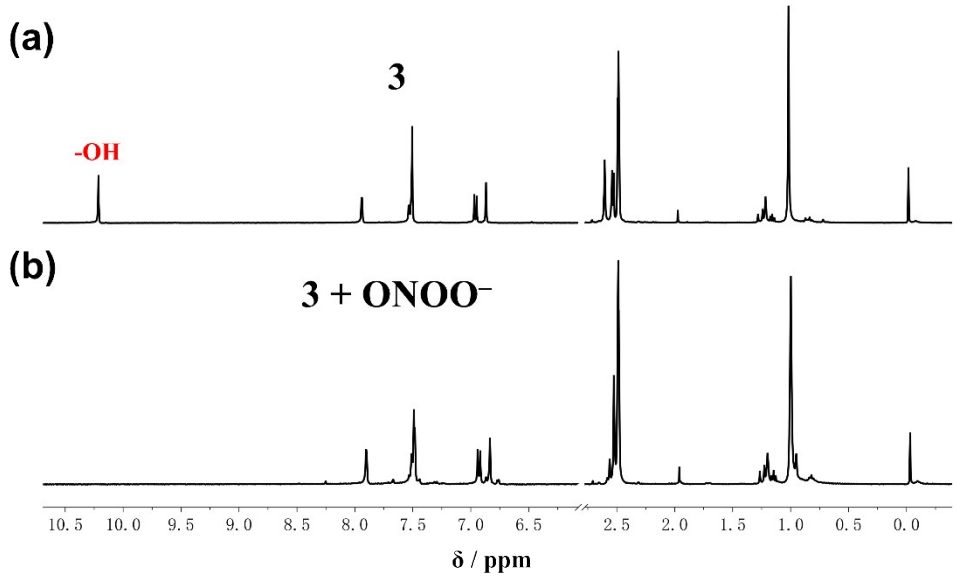


Figure S14. ^{1}H NMR titration of probe **3** before and after react with **ONOO**$^-$ in d_6-DMSO.

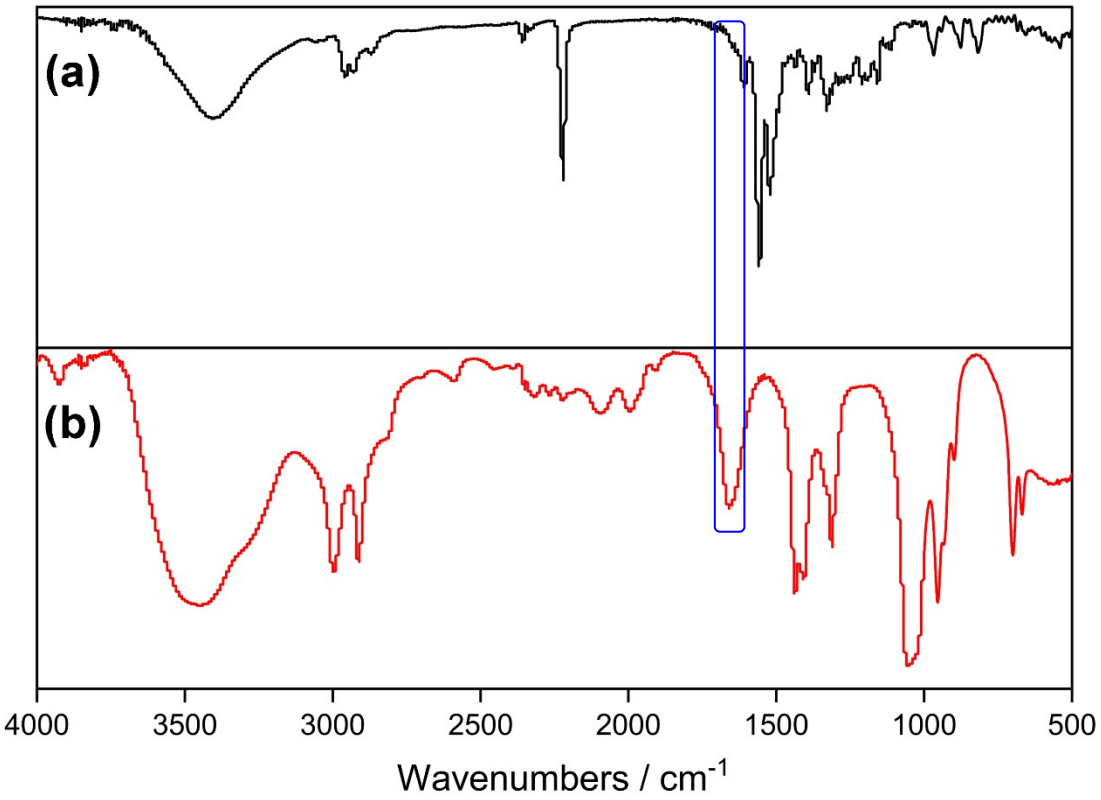


Figure S15. The FTIR spectra for probe **3** (**a**) and after react with **ONOO**$^-$ (**b**).

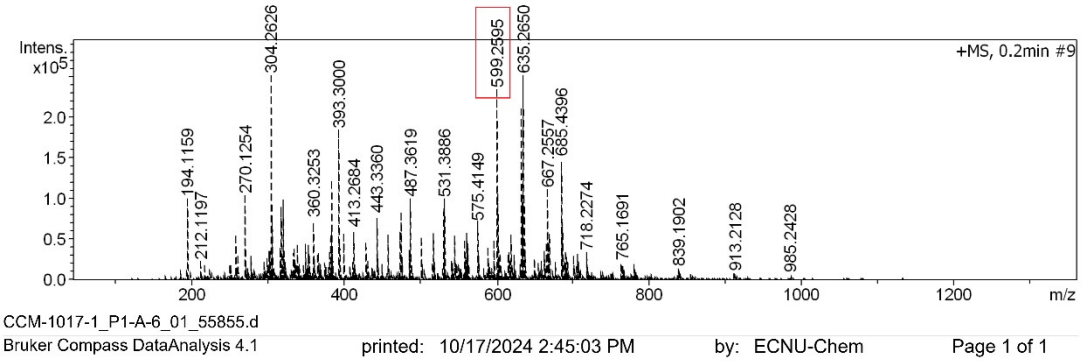


Figure S16. The ESI-MS of probe **3** after react with **ONOO**$^-$.

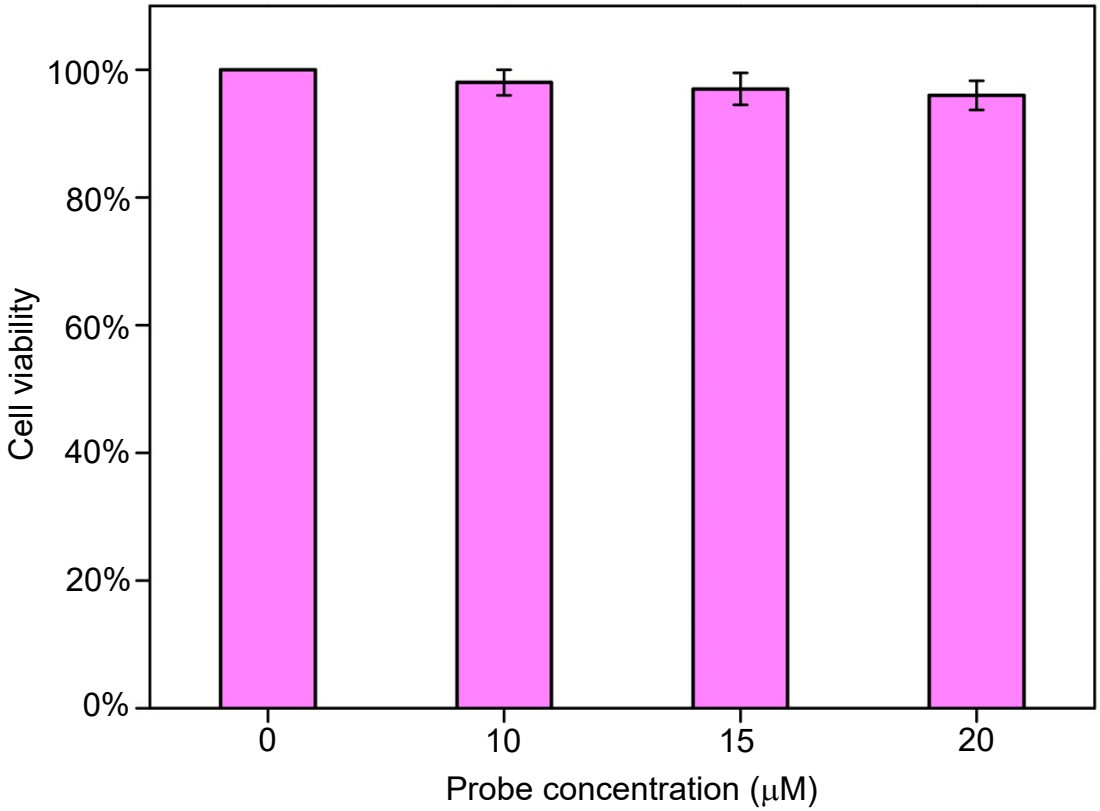


Figure S17. Viability of MCF-7 cells incubated with different concentrations of probe **3**.

Reference

[S1] C. Lee, W. Yang, R.G. Parr, Phys. Rev. B 37 (1988) 785.

[S2] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery, Jr., J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, O. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, Gaussian 09, Revision A.02, Gaussian Inc, Wallingford CT (2009)