Electronic Supporting Information

Construction of cationic *meso*-thiazolium-BODIPY AIE fluorescent probes for viscosity imaging in dual organelles

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Contents

oxidizing agents. (B) The corresponding intensity changes at 553 nm of (A). All the spectra were compared with that in glycerol.

Fig. S5 (A) Fluorescence spectra of **3** in PBS solutions (0.1 M PBS, pH 7.4) before and after the addition of biologically relevant amino acid, metal ions, anions and oxidizing agents. (B) The corresponding intensity changes at 560 nm of (A). All the spectra were compared with that in glycerol.

Fig. S6 (A) MTT and (B) CCK-8 assay of HeLa cells with different concentration of **3** for 24 h.

Fig. S7 (A) MTT and (B) CCK-8 assay of HeLa cells with different concentration of **2** for 24 h.

Fig. S8 Confocal fluorescence images of HeLa cells co-incubated with **2**, and Mito-tracker red or Lyso-tracker red at 37 ℃ for 30 min, respectively.

Fig. S9 Confocal fluorescence images of HeLa cells co-incubated with **3**, and Hoechst or Nile red at 37 ℃ for 45 min.

Fig. S10 Confocal fluorescence images of HeLa cells co-incubated with **2**, and Hoechst or Nile red at 37 ℃ for 45 min.

Fig. S11 (A) Confocal fluorescence images of **2** in HeLa cells, which were pre-incubated with none, LPS or monensin for 40 min, and then incubated with **2** for further 30 min; (B) The intensity of **2** with none, LPS or monensin.

Fig. S12. ^{1}H and ^{13}C NMR spectrum of **2** in DMSO-d_6.

Fig. S13. ^{1}H and ^{13}C NMR spectrum of **3** in DMSO-d_6.

Fig. S14 High-resolution ESI mass spectrum of **2**.

Fig. S15 High-resolution ESI mass spectrum of **3**.

Experimental section

1. General

All solvents and reagents with analytical or reagent grade were used with received for the measurements and reactions. All the reactions were performed under nitrogen protection. The spectroscopic measurements were performed in deionized water and analytical solvents. All the chromatographic purifications were through silica gel (Qingdao Haiyang, 300-400 mesh). ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were recorded with a Bruker AVANCE III 500 spectrometer (^{1}H, 500; ^{13}C, 126 MHz) in DMSO-d_6, which were referenced internally using the residual solvent (^{1}H: δ 2.50 ppm for DMSO-d_6) or solvent (^{13}C: δ 39.6 ppm for DMSO-d_6) resonances relative to $SiMe_4$. Electrospray ionization (ESI) mass data was recorded on an AB SCIEX TripleTOF 4600 mass spectrometer. The electronic absorption and fluorescence spectra were performed on a UV-1780 spectrophotometer (Shimadzu, Japan) and a F-4600 spectrofluorometer (Hitachi, Japan), respectively. The corresponding fluorescence quantum yields (Φ_F) were calculated using the equation [S1]: $\Phi_{F(sample)} = (F_{sample}/F_{ref}) \times (A_{ref}/A_{sample}) \times (n_{sample}^2/n_{ref}^2) \times \Phi_{F(ref)}$, where F, A and n stand for the integrated area, the absorbance at the excitation wavelength and the corresponding refractive index of solvents. The subscripts sample and ref stand for unknown and the standard samples, respectively. The subscripts of sample and ref represent unknown samples and reference samples, respectively. Rhodamine B in ethanol was used as the reference [$\varphi_{F(ref)} = 0.49$] [S2]. The Förster-Hoffmann equation ($\log I = C + x \log \eta$) was used to calculate the linear relationship between the intensity of probe and solvent viscosity,

in which η, I, C and x represent the viscosity, the emission intensity, a constant and the sensitivity of a fluorescent probe towards the viscosity, respectively [S3].

2. Synthesis

2.1. Synthesis of meso-thiazolium *BODIPY **2***

A mixture of BODIPY **1** (30.0 mg, 90.6 μmol) in of N, N-dimethylformamide (1.0 mL) and iodomethane (**4**) (0.5 mL) was heated at 80 ℃ for 1 day, and the reaction process was monitored using thin-layer chromatography. After cooling to room temperature, water was added to remove DMF under reduced pressure to give crude products. Further purification was carried out on column chromatography using dichloromethane/methanol (20:1, v/v) as the eluent to yield a deep pink solid (8.8 mg, 28%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.75 (d, J = 3.6 Hz, 1 H, thiazole-H), 8.73 (d, J = 3.6 Hz, 1 H, thiazole-H), 6.41 (s, 2 H, pyrrole-H), 4.11 (s, 3 H, NCH_3), 2.52 (s, 6 H, CH_3), 1.48 (s, 6 H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6): δ 161.6, 159.9, 142.3, 139.9, 130.3, 129.6, 123.4, 119.4, 45.8, 34.5, 18.9, 14.7, 12.6, 8.8. HRMS (ESI): m/z calcd for $C_{17}H_{19}BF_2N_3S$ $[M]^+$: 346.1355, found: 346.1366.

2.2. Synthesis of meso-thiazolium *BODIPY **3***

A mixture of BODIPY **1** (30.0 mg, 90.6 μmol) and benzyl bromide (**5**) (0.25 mL) was heated at 80 ℃ for 1 day, and the reaction process was monitored by thin-layer chromatography. After cooling, the crude product was purified by column chromatography using dichloromethane/methanol (20:1, v/v) as the eluent to give a

deep pink solid (5.0 mg, 13%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.17 (d, J = 4.0 Hz, 1 H, thiazole-H), 8.79 (d, J = 4.0 Hz, 1 H, thiazole-H), 7.26 (m, 5 H, ArH), 6.29 (s, 2 H, pyrrole-H), 5.67 (s, 2 H, NCH_2), 2.54 (s, 6 H, CH_3), 1.18 (s, 6 H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6): δ 161.7, 159.9, 142.3, 139.2, 131.1, 130.4, 130.3, 129.8, 128.9, 123.4, 119.2, 57.1, 14.8, 12.8. HRMS (ESI): m/z calcd for $C_{23}H_{23}BF_2N_3S$ $[M]^+$: 422.1668, found: 422.1678.

3. Preparation of stock solutions for spectroscopic measurements

BODIPYs **2** and **3** (1.5 mM) in N, N-dimethylformamide were prepared as the stock solutions for all the spectroscopic experiments. All the interfering agents, including the amino acids, metal ions, anions and oxidants were prepared with concentration of 2 mM in aqueous solutions. Solutions of probe (2 or 10 μM) in different solvents or with different concentration of interfering species (100 μM) in phosphate buffer saline (abbreviated as PBS, 0.1 M PBS, pH 7.4) were prepared for use and stored in refrigerator for one night.

For preparing different fractions of glycerol and water mixtures (20 mL), we firstly calculated the corresponding weight of glycerol and water in each mixture, and then prepared them on a balance. Finally, the probes in stock solution were added to the each mixture (3 mL) and shook up the mixtures. At 20 °C, water has a viscosity (η) of 1.00 cP and glycerol has a viscosity of 950.17 cP. Viscosity for the mixtures was calculated using the following equation [S4]:

$$\ln (\eta_{mix}) = \Sigma\, w_i \times \ln (\eta_i)$$

where η_{mix} is the viscosity of the mixture, η_i is the viscosity of each component, and w_i is the weighting factor ($0<w<1$) of each component.

4. General procedures of cell culture and MTT assay

HeLa cells were used with Dulbecco's modified Eagle's medium (DMEM) with high glucose, as the culture medium under the atmosphere of 5% CO_2 at 37 °C for 48 h. To evaluate the cytotoxicity of these two probes, MTT assay were used. HeLa cells were seeded onto a 96-well cell-culture plate for 24 h. Then, various concentrations of probe (0, 3.125, 6.25, 12.5, 25 and 50 μM) in DMEM were added to the wells. After another 48 h, MTT (10 μL, 5 mg/mL) was added to each well and incubated at 37 °C for another 4 h. The MTT solutions were removed and purple precipitates (formazan) observed in plates, which were further dissolved in dimethyl sulfoxide (DMSO, 100 μL). Microplate reader was used to measure the absorbance for each well.

CCK-8 assay was also used. HeLa cells were seeded onto a 96-well cell-culture plate for 24 h. Then, various concentrations of probe (0, 3.125, 6.25, 12.5, 25 and 50 μM) in DMEM were added to the wells. After another 24 h, 10 μL CCK-8 solution was added to each well and incubated at 37 °C for another 2 h. Orange yellow water-soluble Formazan was observed in the tablet, and the absorbance of each well at a wavelength of 450 nm was measured using Microplate reader.

5. Cell imaging

The confocal imaging experiments were performed using a 20-mm confocal dish and HeLa cells. The cells were firstly passed and adhered for two days. The cells were firstly cultured with lipopolysaccharide (LPS) (250 ug/mL) or monensin (20 μM) for 40 min, and then PBS was used to wash the cells for three times. Then, the freshly prepared stock solutions of **2** or **3** (5 μM in DMEM) were added to the cells, which were pretreated with LPS, monensin or none-pretreated ones. The cells were further cultured for 30 min at 37 °C. A Leica TCS SP8 confocal microscope (Germany) was used to perform Confocal imaging with a 63 × oil-immersion objective lens. The samples were excited at 488 nm with an argon ion laser and emissions were collected at 506-600 nm.

For the subcellular cell imaging, HeLa cells were chosen and cultured for for 48 h. And then, probes (8 μM) with Mito-tracker red or Lyso-tracker red (1 μM) in DMEM were used to culture the cells for 30 min. After washing the cells with PBS for three times, confocal imaging was performed for Mito-Tracker or Lyso-Tracker red, with excitation wavelength of 561 nm and emission wavelength of 580-700 nm. Meanwhile, probes (8 μM) with Hoechst or Nile red (2 μM) in DMEM were used to culture the cells for 45 min. After washing the cells with PBS for three times, confocal imaging was performed with excitation wavelength of 405 nm and emission wavelength of 420-500 nm for Hoechst, and with excitation wavelength of 514 nm and emission wavelength of 580-700 nm for Nile red. Two fluorescence probes were excited at 488 nm and emission spectra were collected at 508-560 nm.

References

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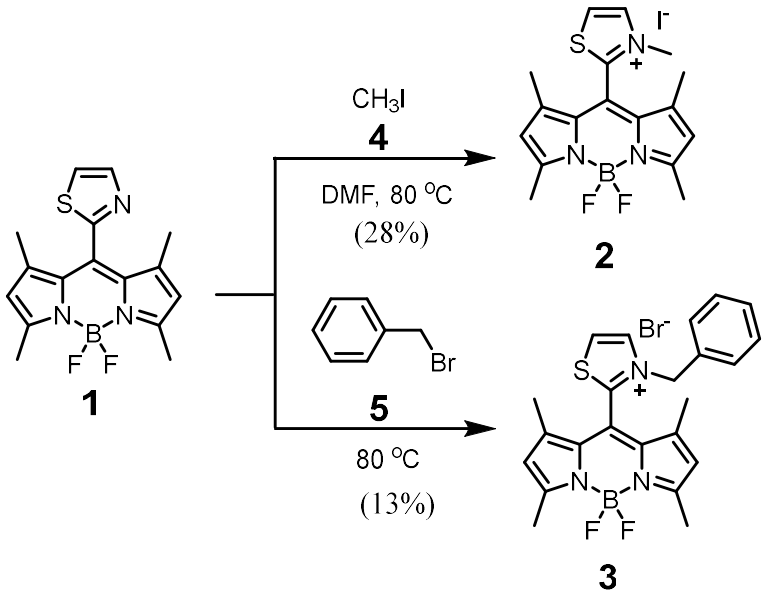
[S2] K. G. Casey and E. L. Quitevis, *J. Phys. Chem*. 1988, **92***,* 6590-6594.

[S3] T. Förster and G. Z. Hoffmann, *Z. Phys. Chem*. 1971, **75**, 63-76.

[S4] A. Dragan, A. E. Graham and C. D. Geddes, *J. Fluoresc.* 2014, **24**, 397.



Scheme S1 Synthetic route of fluorescent probes **2** and **3**.

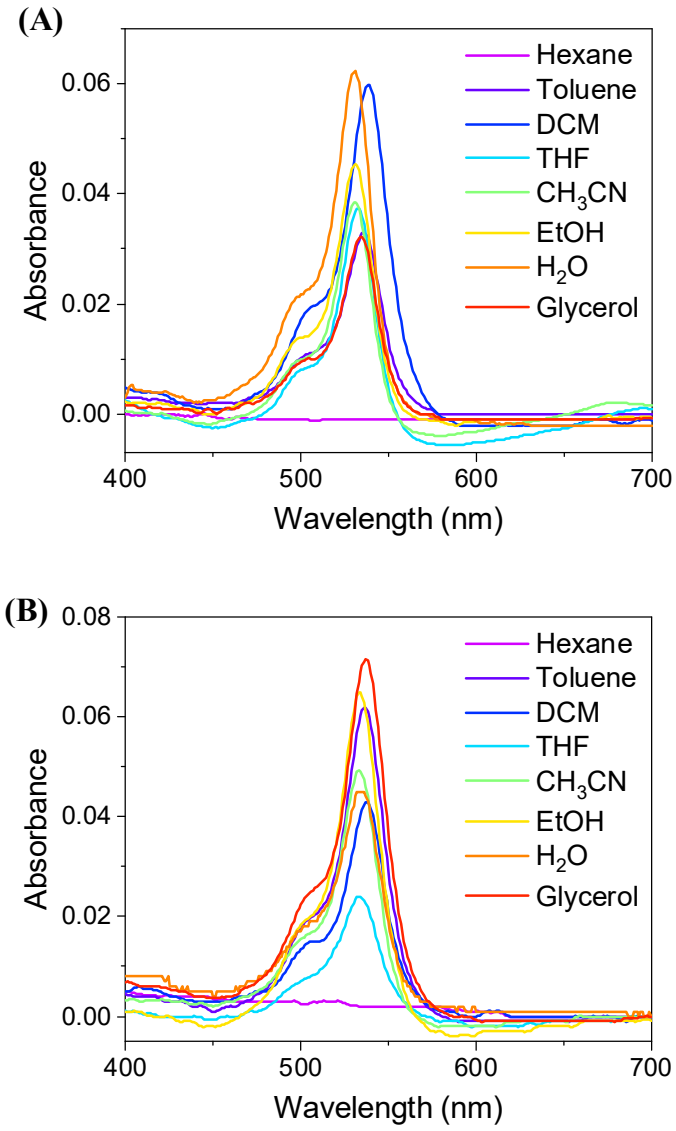


Fig. S1 UV-Vis absorption spectra of (A) **2** and (B) **3** (2 μM) in different solvents.

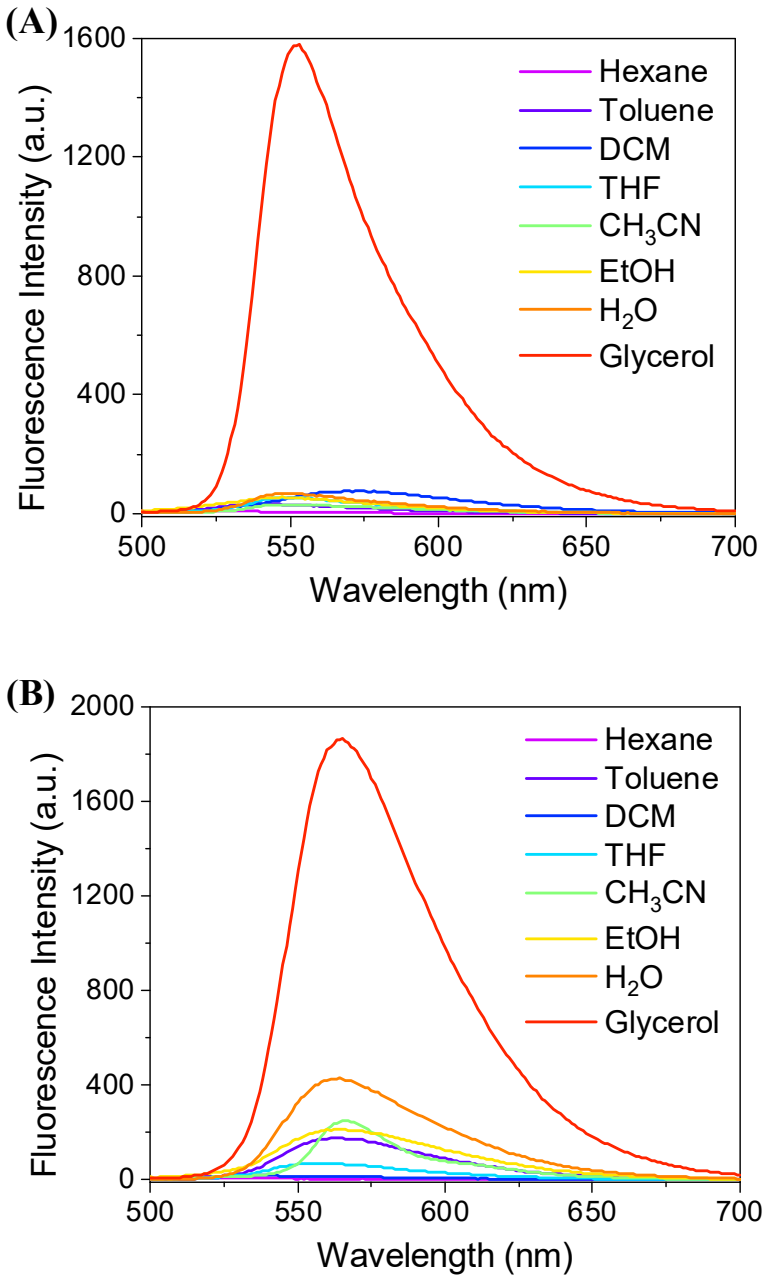


Fig. S2 Fluorescence spectra of (A) **2** and (B) **3** (2 μM) in different solvents. (λ_{ex} = 490 nm)

Table S1 The spectroscopic properties of **2** in different solvents.

Solvents	λ_{max} (nm) (log ε)	λ_{em} (nm)	Φ_F [a]
Hexane	/	/	/
Toluene	537 (4.22)	540	0.031
DCM	538 (4.48)	568	0.059
THF	533 (4.27)	554	0.065
CH_3CN	530 (4.28)	565	0.025
EtOH	531 (4.35)	546	0.031
H_2O	531 (4.49)	550	0.031
Glycerol	531 (4.83)	553	0.349

[a] All was excited at 490 nm and using Rhodamine B in EtOH as the reference (Φ_F = 0.49).

Table S2 The spectroscopic properties of **3** in different solvents.

Solvents	λ_{max} (nm) (log ε)	λ_{em} (nm)	Φ_F [a]
Hexane	/	/	/
Toluene	537 (4.49)	563	0.111
DCM	538 (4.48)	568	0.016
THF	533 (4.08)	556	0.079
CH_3CN	534 (4.39)	566	0.079
EtOH	533 (4.51)	562	0.114
H_2O	535 (4.35)	565	0.159
Glycerol	537 (4.56)	560	0.466

[a] All was excited at 490 nm and using Rhodamine B in EtOH as the reference (Φ_F = 0.49).

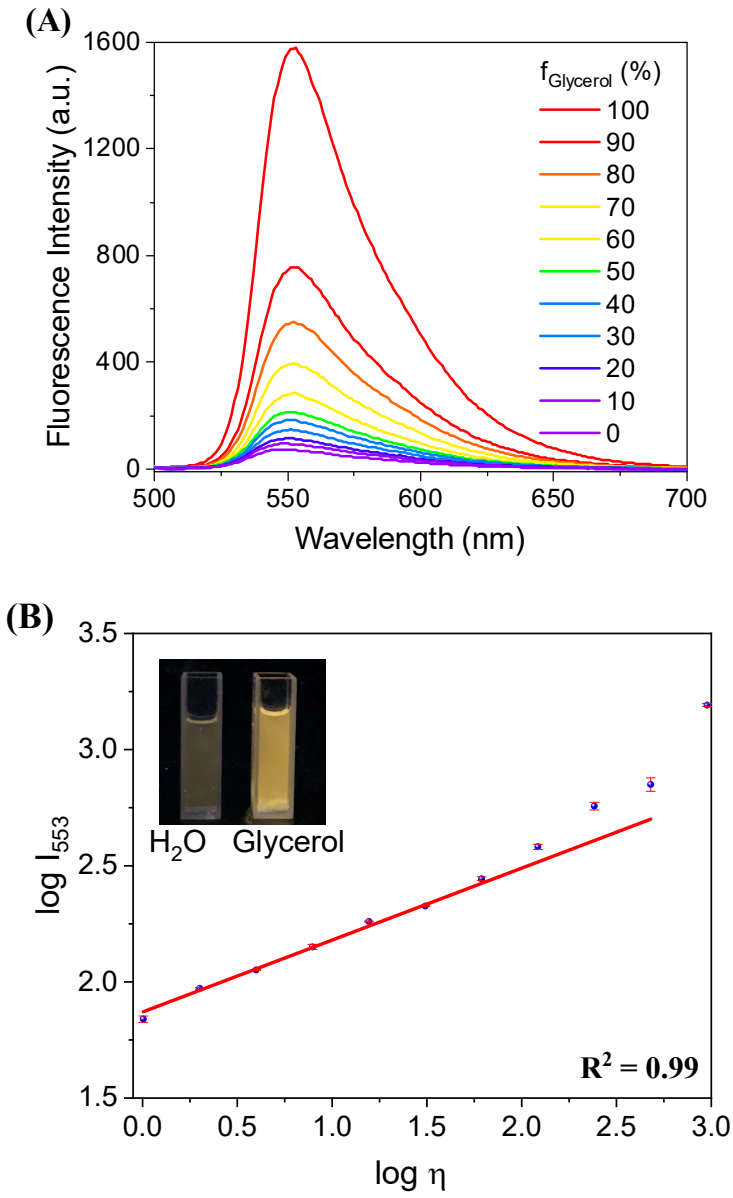


Fig. S3 (A) Fluorescence spectra of **2** (2 μM) in glycerol-H_2O mixtures with increasing viscosity from 1.0 to 950.2 cp. (B) The linear relationship of **2** between log I_{553} (I_{553}: fluorescence intensity at 553 nm) and log η (η: viscosity). The inset shows the corresponding fluorescence observed by naked eyes in water and glycerol under the irradiation at 365 nm UV light. (λ_{ex} = 490 nm)

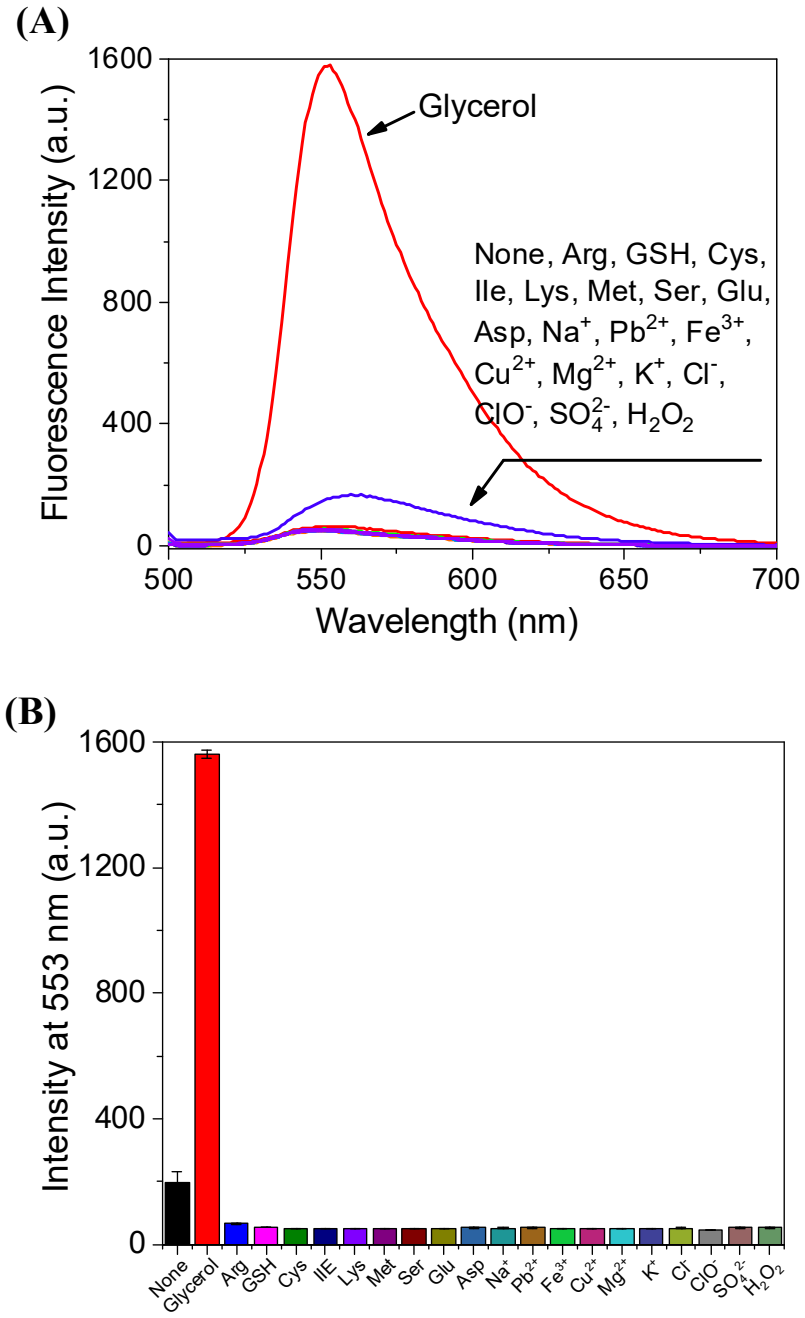


Fig. S4 (A) Fluorescence spectra of **2** (2 μM) in PBS solutions (0.1 M PBS, pH 7.4) before and after the addition of biologically relevant amino acid, metal ions, anions and oxidizing agents. (B) The corresponding intensity changes at 553 nm of (A). All the spectra were compared with that in glycerol. (λ_{ex} = 490 nm)

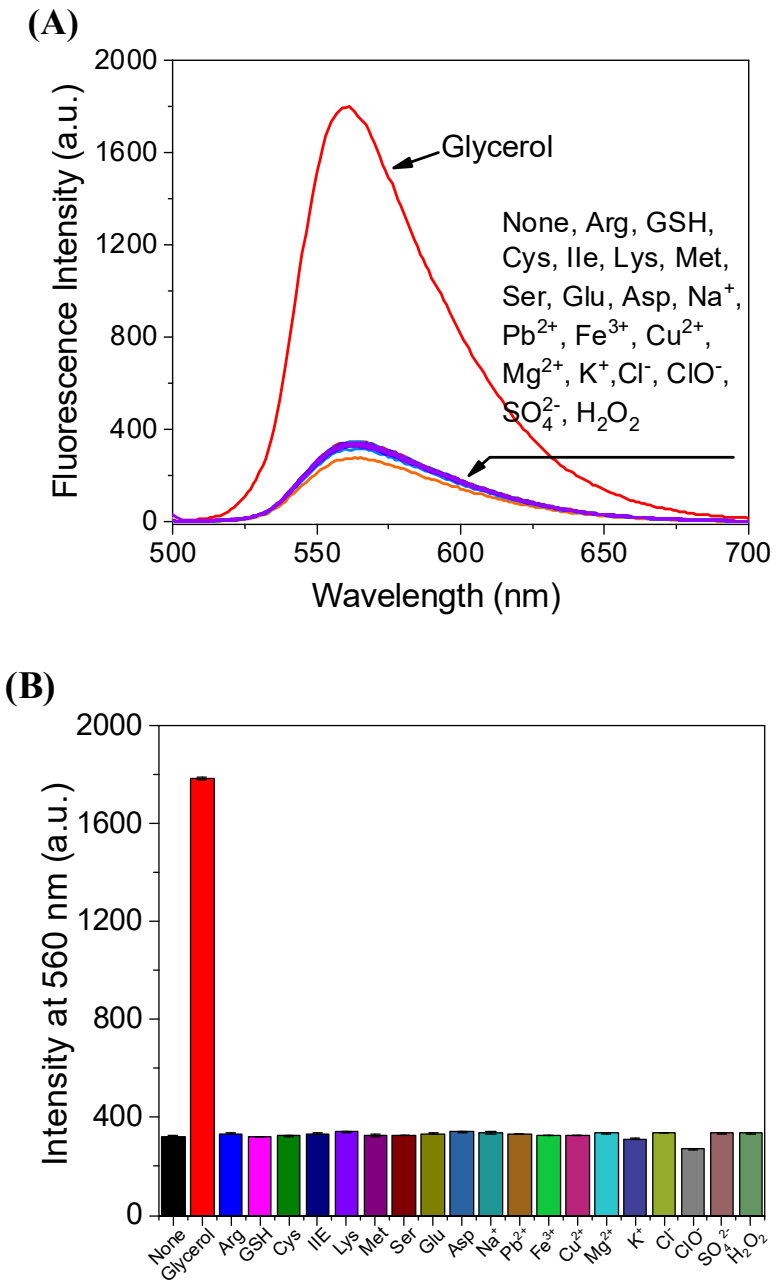


Fig. S5 (A) Fluorescence spectra of **3** (2 μM) in PBS solutions (0.1 M PBS, pH 7.4) before and after the addition of biologically relevant amino acid, metal ions, anions and oxidizing agents. (B) The corresponding intensity changes at 560 nm of (A). All the spectra were compared with that in glycerol. (λ_{ex} = 490 nm)

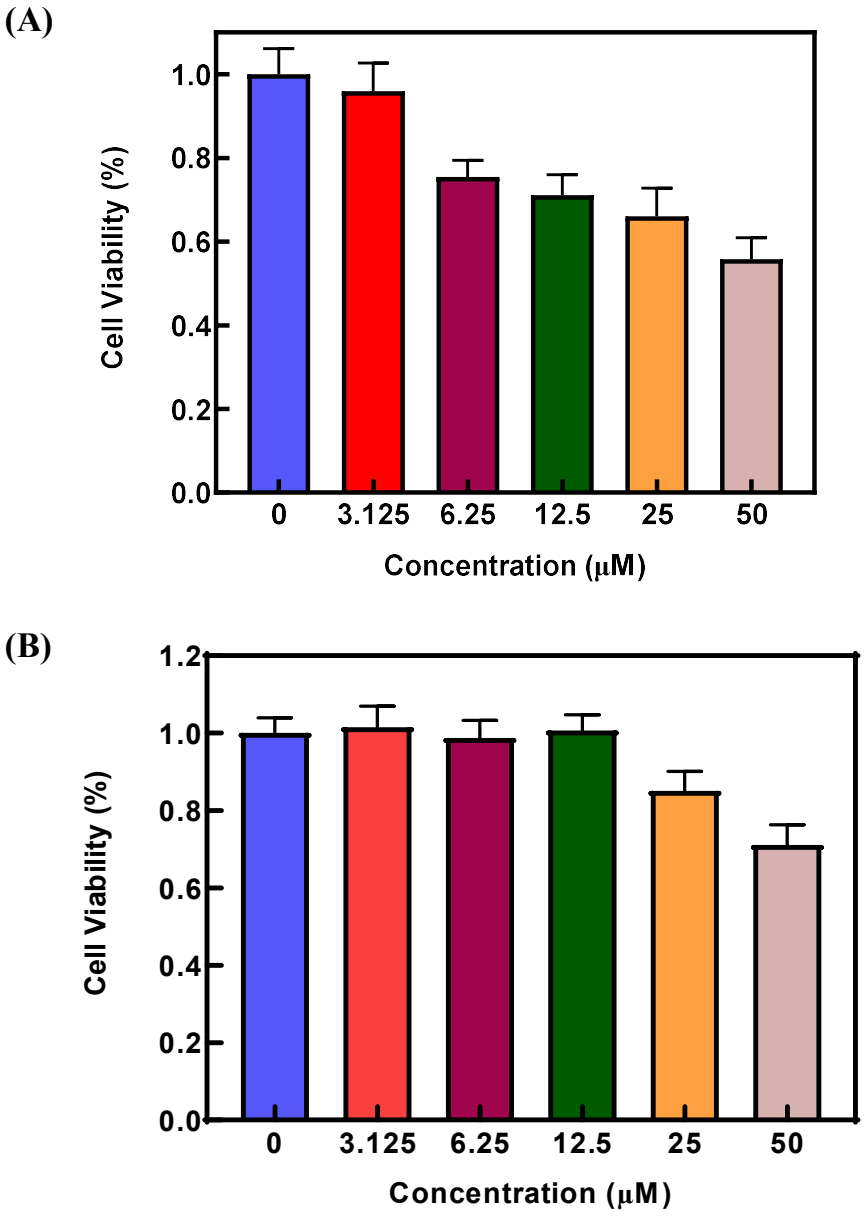


Fig. S6 (A) MTT and (B) CCK-8 assay of HeLa cells with different concentration of **2** for 24 h.

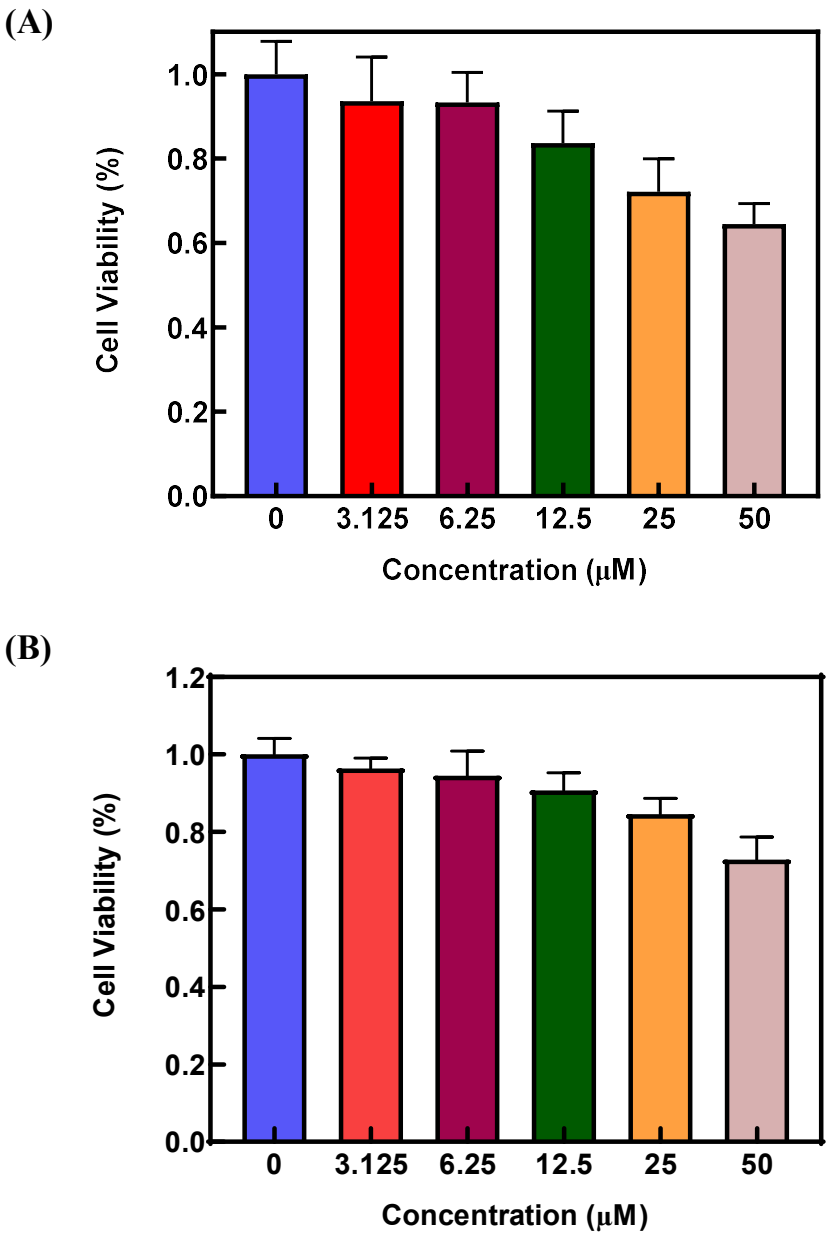


Fig. S7 (A) MTT and (B) CCK-8 assay of HeLa cells with different concentration of **3** for 24 h.

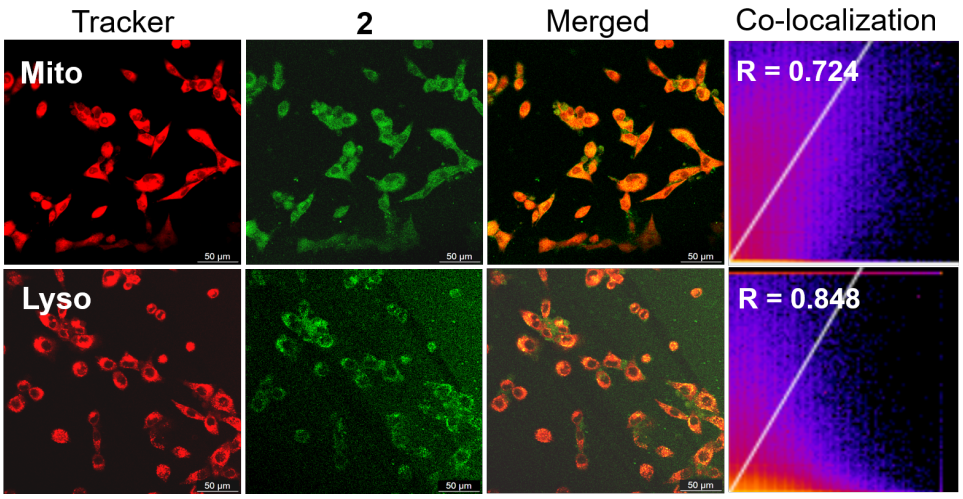


Fig. S8 Confocal fluorescence images of HeLa cells co-incubated with **2** (8 μM), and Mito-tracker red or Lyso-tracker red (1 μM) at 37 ℃ for 30 min, respectively. For Mito-tracker red or Lyso-tracker red, λ_{ex} = 561 nm, λ_{em} = 580-700 nm; For **2**, λ_{ex} = 488 nm, λ_{em} = 508-560 nm.

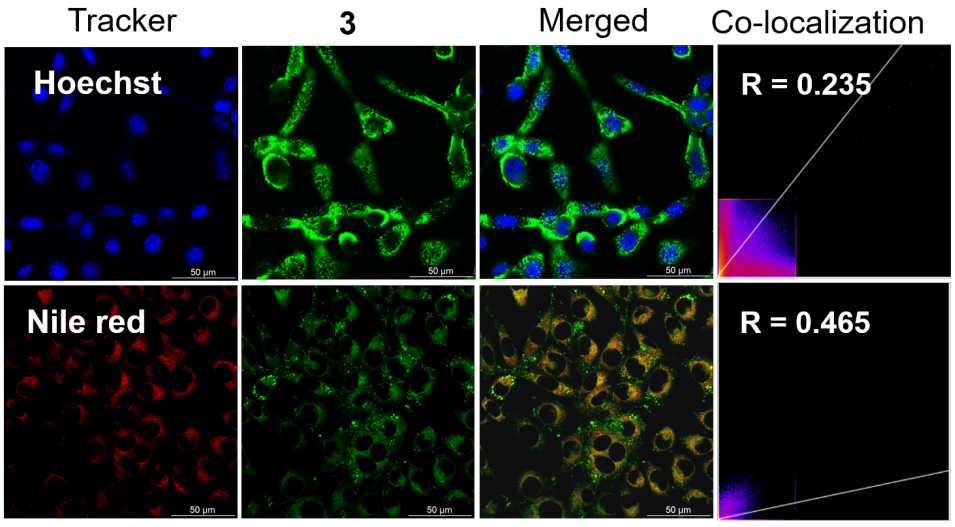


Fig. S9 Confocal fluorescence images of HeLa cells co-incubated with **3** (8 μM), and Hoechst (2 μM) or Nile red (2 μM) at 37 ℃ for 45 min. For Hoechst, λ_{ex} = 405 nm, λ_{em} = 420-500 nm; For Nile red, λ_{ex} = 514 nm, λ_{em} = 580-700 nm; For **3**, λ_{ex} = 488 nm, λ_{em} = 508-600 nm.

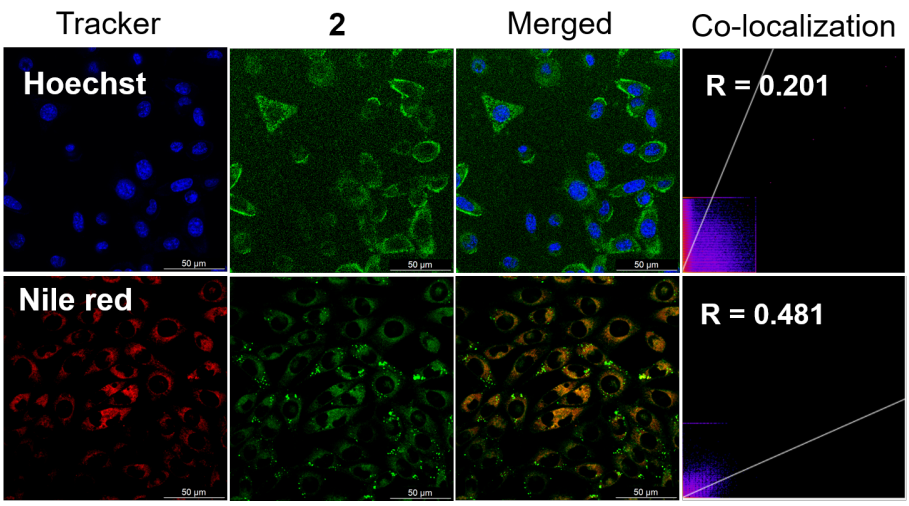


Fig. S10 Confocal fluorescence images of HeLa cells co-incubated with **2** (8 μM), and Hoechst (2 μM) or Nile red (2 μM) at 37 °C for 45 min. For Hoechst, λ_{ex} = 405 nm, λ_{em} = 420-500 nm; For Nile red, λ_{ex} = 514 nm, λ_{em} = 580-700 nm; For **2**, λ_{ex} = 488 nm, λ_{em} = 508-600 nm.

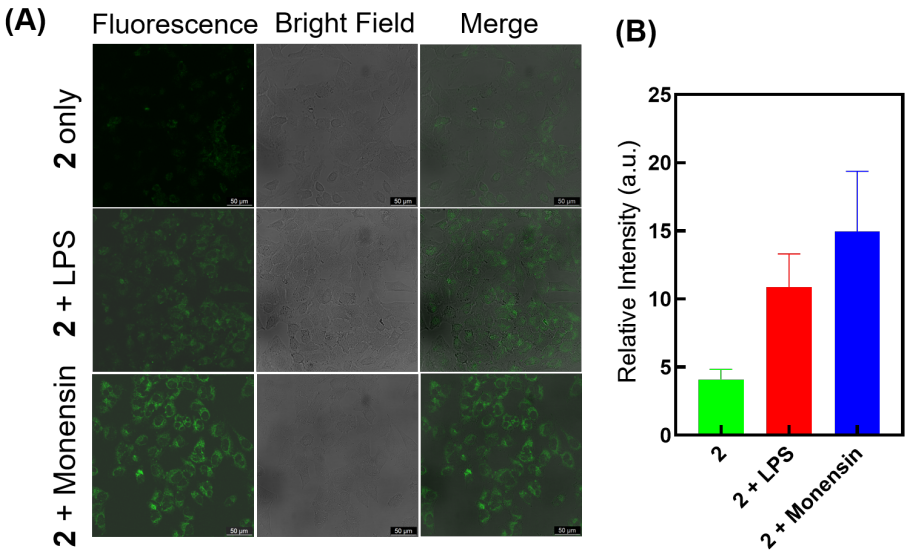


Fig. S11 (A) Confocal fluorescence images of **2** in HeLa cells, which were pre-incubated with none, LPS (250 μg/mL) or monensin (20 μM) for 40 min, and then incubated with **2** (5 μM) for further 30 min; (B) The intensity of **2** with none, LPS or monensin. (λ_{ex} = 488 nm, λ_{em} = 506-650 nm)

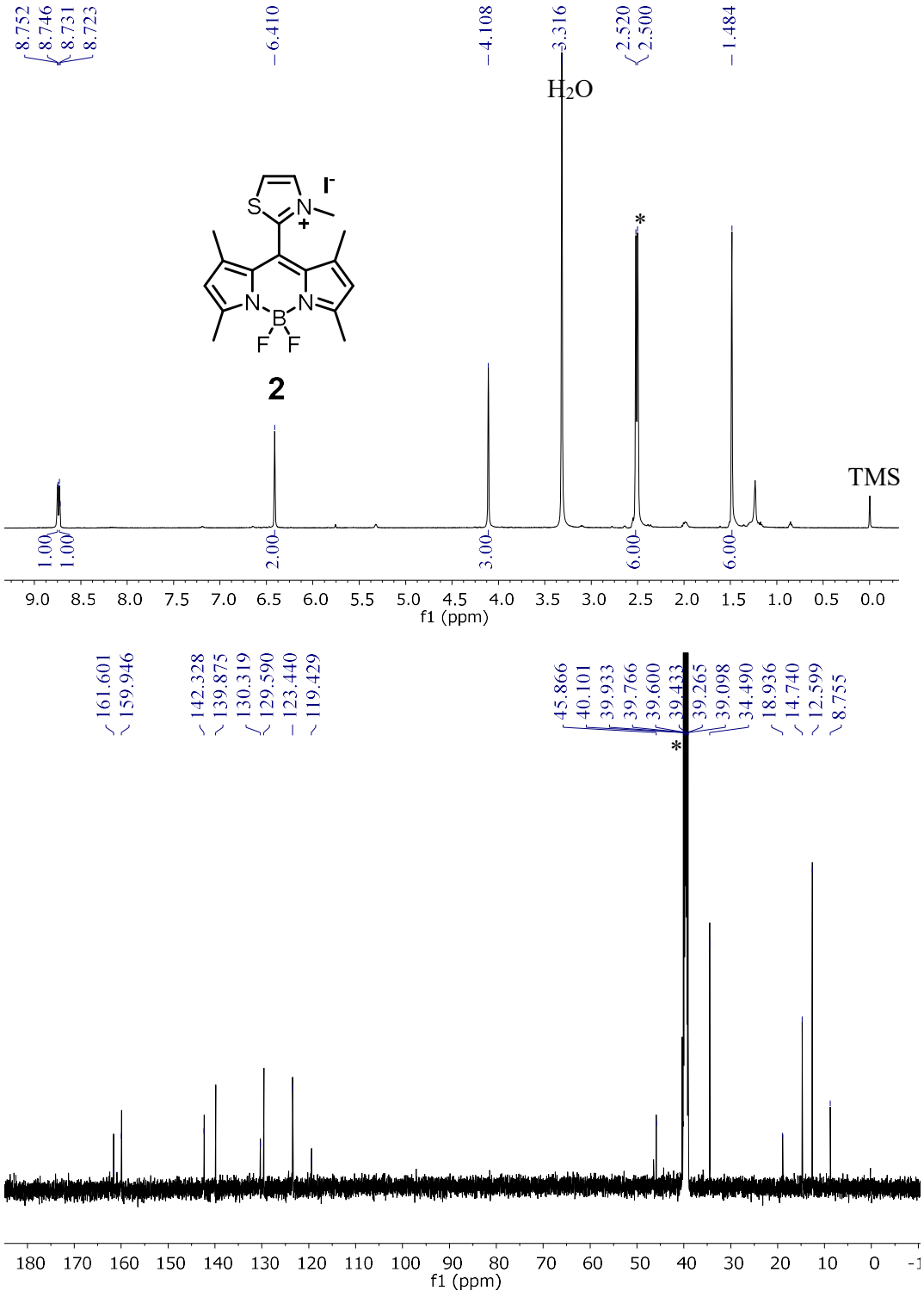


Fig. S12 ^{1}H and ^{13}C NMR spectrum of **2** in DMSO-d_6.

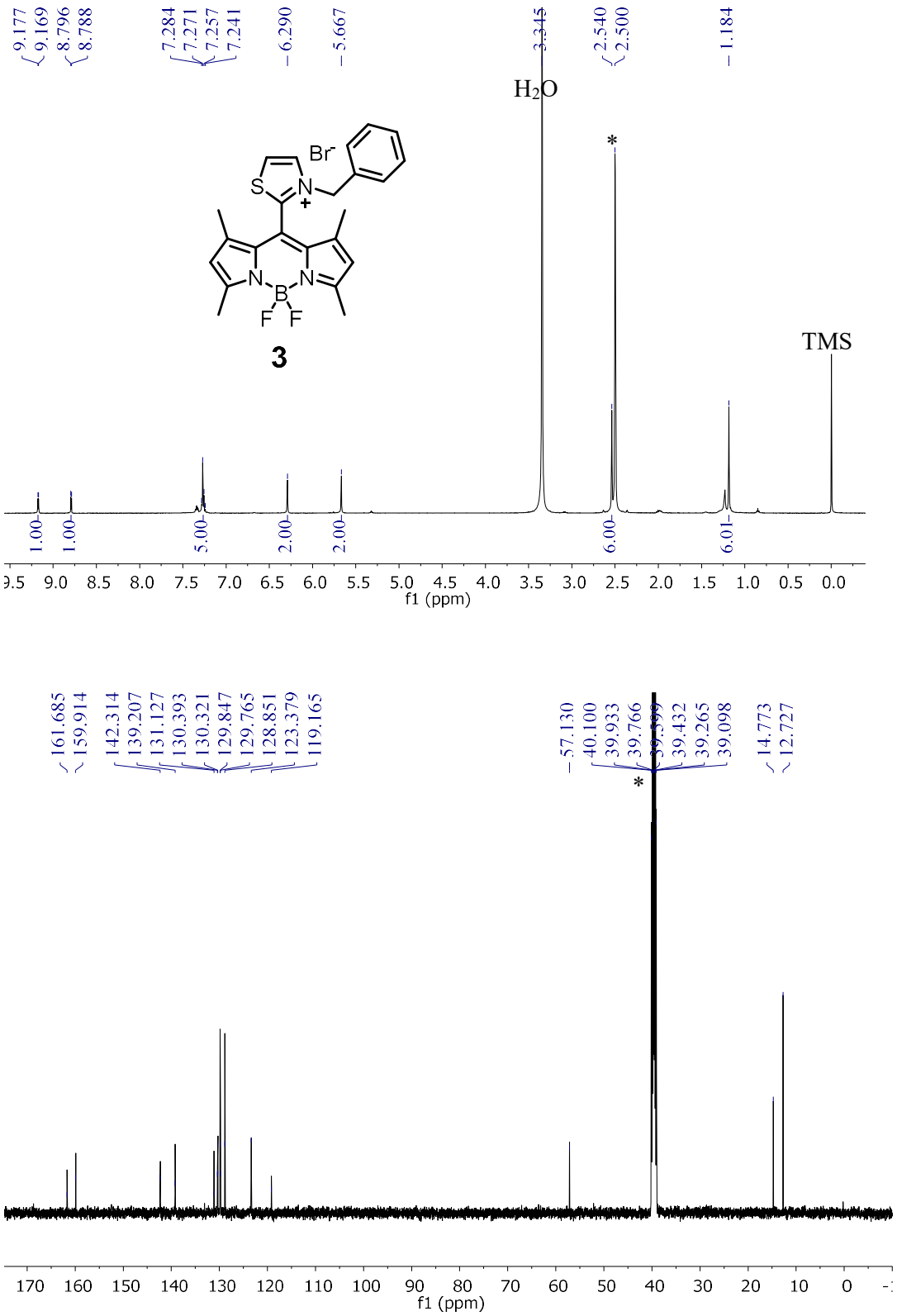


Fig. S13 ^{1}H and ^{13}C NMR spectrum of **3** in DMSO-d_6.

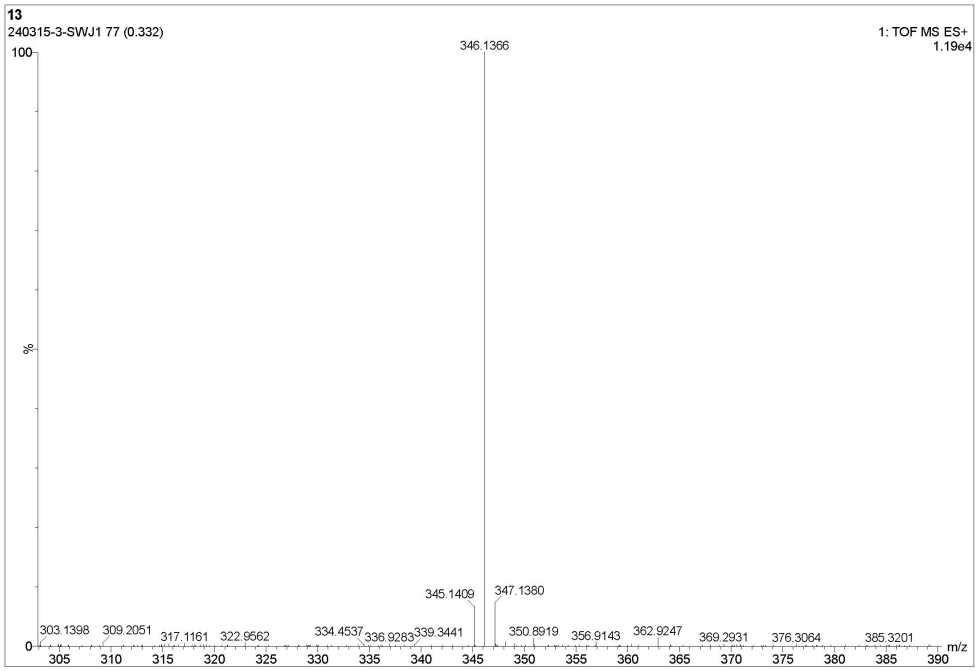


Fig. S14 High-resolution ESI mass spectrum of **2**.

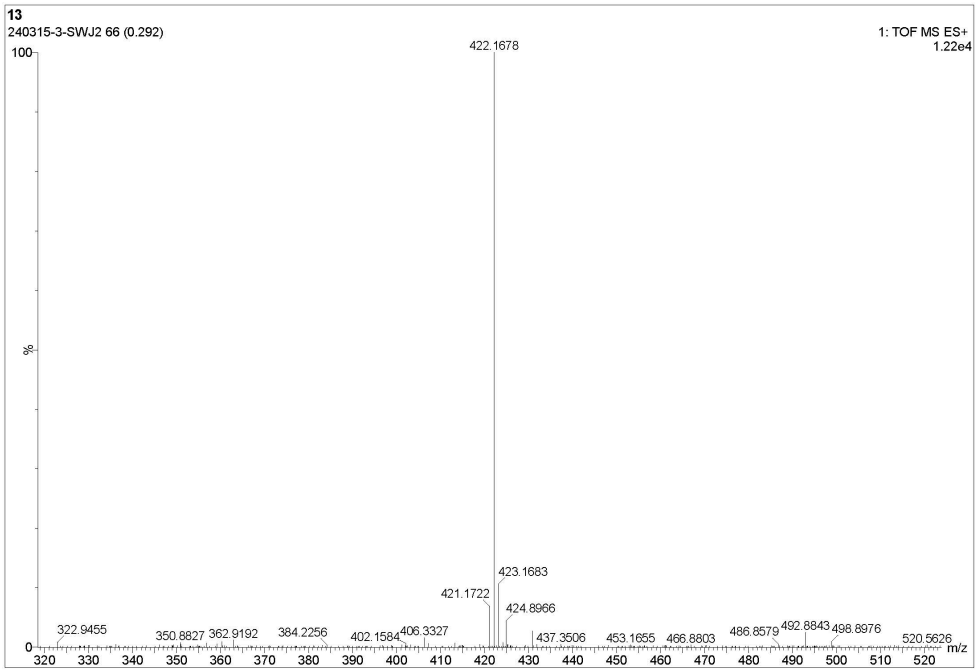


Fig. S15 High-resolution ESI mass spectrum of **3**.