Supporting Information for

A near-infrared emission fluorescent probe with multi-rotatable moieties for high sensitivity detection of mitochondrial viscosity in inflammatory cell model

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Materials and instruments

Unless otherwise stated, ultrapure water was used in all experiments and all reagents were acquired from commercial suppliers without further purification. The pH measurements were performed with a Mettler-Toledo Delta 320 pH meter. UV−vis absorption spectra were measured on a Shimadzu UV-2700 spectrophotometer and fluorescence spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer. Viscosity value were carried out on a NDJ-8 rotational viscometer. MTT was purchased from *J&K Scientific Ltd*. Fluorescence imaging experiments were performed with Nikon A1MP confocal microscopy. TLC analysis carried out on silica gel plates and column chromatography was conducted over silica gel (mesh 200−300), both of them were purchased from the *Qingdao Ocean Chemicals*. ^{1}H and ^{13}C NMR spectra were performed on an AVANCE III 400 MHz digital NMR spectrometer. High resolution mass spectrometric (HRMS) analyses were carried out on an Agilent 1100 HPLC/MSD spectrometer.

General procedure for viscosity determination and spectral measurements

The solvents were acquired by mixing water-glycerol systems in different proportions. Each viscosity value recorded by using NDJ-8 rotational viscometer. The stock solution of **TPE-V** (1 mM) was prepared in DMSO. The test solution contained **TPE-V** (10.0 μM), water-glycerol systems in different proportions. These solutions were sonicated for 5 min to remove air bubbles. After standing at room temperature for 1 h, then measured in a UV spectrophotometer and a fluorescence spectrophotometer.

Fluorescence quantum yields measurements

The relative fluorescence quantum yields were determined with Rhodamine 6G (F = 0.95) in water as a standard and calculated using the following equation:

$$\Phi_x = \Phi_s(F_x/F_s)(A_s/A_x)(\lambda_{exs}/\lambda_{exx})(n_x/n_s)^2$$

Where Φ stands for quantum yield; F represents integrated area under the appropriate emission spectrum; A stands for absorbance at the excitation wavelength; λ_{ex} is the excitation wavelength; n is the refractive index of the solution (because of the low concentrations of the solutions (1-20 μM), the refractive indices of the difference solutions are similar, which can be omitted); and the subscripts x and s refer to test sample and reference substance, respectively.

Fluorescence lifetime measurement

The solutions of **TPE-V** (10 μM) in different viscosity were prepared in solvent mixture (water–glycerol). These solutions were sonicated for 5 min to eliminate air bubbles. After standing for 1 h at room temperature, the solutions were measured in a fluorescence lifetime measuring equipment (Edinburgh Instruments) with the

excitation wavelength at 460 nm and detection at 650nm.

The Forster–Hoffmann equation

The Forster–Hoffmann equation[s1] was utilized to correlate the relationship between the fluorescence emission intensity of **TPE-V** and the value of solvent viscosity.

$$\log I = C + x \log \eta$$

$$\log \tau = C + x \log \eta$$

Where η is the value of viscosity, I is the emission intensity, τ is the fluorescence lifetime, C is a constant, and x represents the sensitivity of the fluorescent probe to viscosity.

Cytotoxicity assays

The cytotoxicity of **TPE-V** to HeLa cells was performed by standard MTT assays. 2 × 10^4 cells/mL HeLa cells were seeded in 96-well plates and then incubated with different concentrations of **TPE-V** (0-50 μM) for 24 h. Subsequently, HeLa cells were incubated with 5 mg/mL MTT (10 mL per well) and treated for 4 h. After that the supernatants were aspirated and 100 μL DMSO per well was added. The absorbance of the solution at 570 nm was recorded using microplate reader. The cell viability (%) = $(OD_{sample}-OD_{blank}) / (OD_{control}-OD_{blank}) \times 100$ %. OD_{sample} denotes cells treated with various concentrations of **TPE-V**; OD_{blank} denotes the plates with DMEM; $OD_{control}$ denotes cells without treated with **TPE-V**. Each concentration was conducted with three parallel samples, and the results were expressed as mean ± standard deviation (SD).

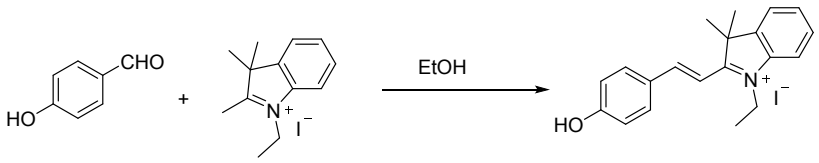
Cell culture and fluorescence imaging

HeLa and NHA cells were cultured in Dulbecco's Modified Eagle Medium media (DMEM, Hyclone) supplemented with 10 % fetal bovine serum (FBS, Sijiqing), penicillin (100 U/ml, Hyclone) and streptomycin sulfate (100 U/ml, Hyclone) under an atmosphere of 5% CO_2 and 95% air at 37 °C.

Before the imaging experiments, HeLa and NHA cells were seeded in 35 mm glass-bottom culture dishes (Nest). After 24 h, the cells were pretreated with Monensin (10 μM) or nystatin (10 μM) or LPS (20 μM) for 40 min, then incubated with **TPE-V** (10 μM) for 30 min at 37 °C respectively. Finally, the cells were washed twice with PBS and the fluorescence imaging was performed with Nikon A1R confocal microscope with a 40× objective lens. The fluorescence emission was collected at TRITC channel (570-620nm) upon excitation at 488 nm.

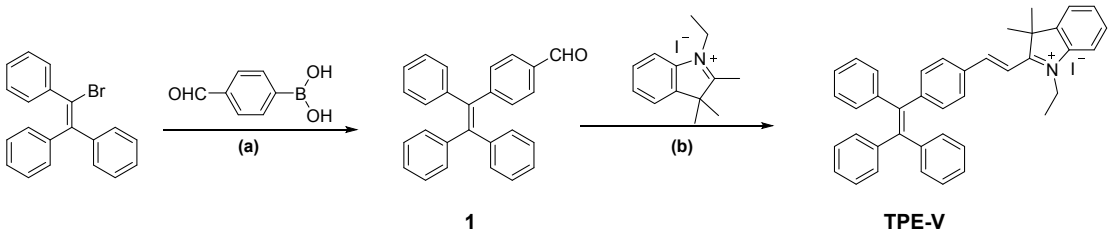
Synthesis

Synthesis of compound In-OH.



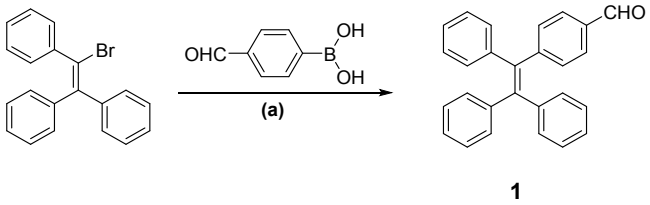
4-Hydroxy benzaldehyde (122 mg, 1 mmol) and 1,2,3,3-Tetramethyl-3H-indolium (315 mg, 1 mmol) were dissolved in ethanol (20 mL). The reaction mixture was refluxed for 6 hours under nitrogen. After cooled to room temperature, and evaporation of the solvent, the residue was purified by column chromatography (dichloromethane: methanol = 30:1, v/v) to give red solid.

Synthesis route of the fluorescent probe TPE-V.



Scheme S1. Synthesis route of the fluorescent probe **TPE-V**: (a) THF, 2M K_2CO_3 aqueous solution, TBAB, Pd $(PPh_3)_4$, reflux, overnight; (b) ethanol, reflux, overnight.

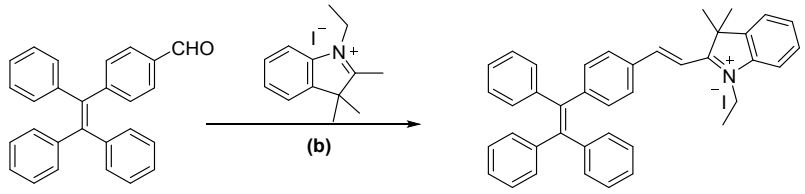
Synthesis of compound 1.



Bromotriphenylethylene (335 mg, 1 mmol), 4-Formylphenylboronic acid (181 mg, 1.2 mmol), tetrakis(triphenylphosphine)palladium(0) (62 mg, 0.05 mmol), potassiuim carbonate (166 mg, 1.2 mmol) and tetrabutylammonium bromide (TBAB, 2 mg) were added in a Schlenk tube. After thorough deoxygenation, 5 mL distilled

THF and 600 μL deionized water were added into the sealed tube. The solution was kept stirring at 80 °C overnight. After cooling to room temperature and evaporation of the solvent, the residue was dissolved in chloroform and extracted with water three times followed by the usual work. After drying by anhydrous sodium sulfate, the crude product was purified by column chromatography (ethyl acetate: petroleum ether = 1:10, v/v) to afford compound **1** (pale yellow solid, yield 80%). [S2] ^{1}H NMR (400 MHz, DMSO) δ = 9.89 (s, 1H), 7.68 (d, J=8.0, 2H), 7.17 (m, 11H), 7.00 (m, 6H).

Synthesis of compound TPE-V.



1 **TPE-V**

Compound **1** (360 mg, 1 mmol) and 1,2,3,3-Tetramethyl-3H-indolium (315 g, 1 mmol) were dissolved in ethanol (20 mL). The reaction mixture was refluxed for 6 hours under nitrogen. After cooled to room temperature, and evaporation of the solvent, the residue was purified by column chromatography (dichloromethane: methanol = 30:1, v/v) to give red solid. (0.54 g, 70 % yield). ^{1}H NMR (400 MHz, DMSO) δ 9.01 (d, J=16.4, 1H), 8.48 (m, 3H), 8.36 (m, 1H), 8.28 (d, J=16.4, 1H), 8.148 (m, 2H), 7.58 (m, 17H), 5.37 (q, J=7.2, 2H), 2.38 (s, 6H), 2.06 (t, J=7.2, 3H). ^{13}C NMR (101 MHz, DMSO) δ 206.75, 154.46, 144.68, 143.72, 143.63, 143.44, 133.12, 132.52, 131.55, 131.41, 130.71, 130.30, 130.29, 129.89, 128.48, 128.22, 127.61, 127.38, 123.58, 115.70, 112.55, 53.24, 43.44, 25.98, 13.90. HRMS (m/z): [M + H]$^+$ calcd for $C_{40}H_{36}N^+$, 530.2842; found, 530.2846.

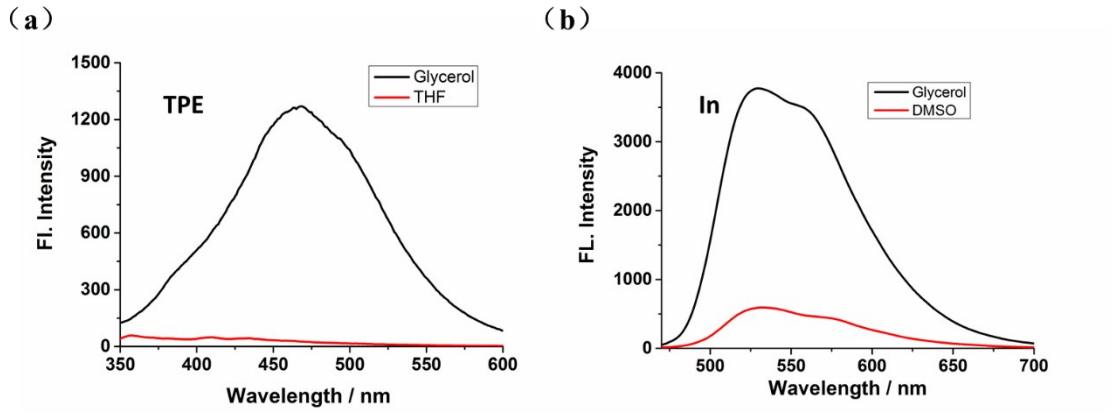


Fig. S1. (a) Fluorescence spectra of 10 μM **TPE** in THF and glycerol solutions. λ_{ex} = 320 nm. (b) Fluorescence spectral of **In-OH** in DMSO and glycerol solutions. λ_{ex} = 450 nm.

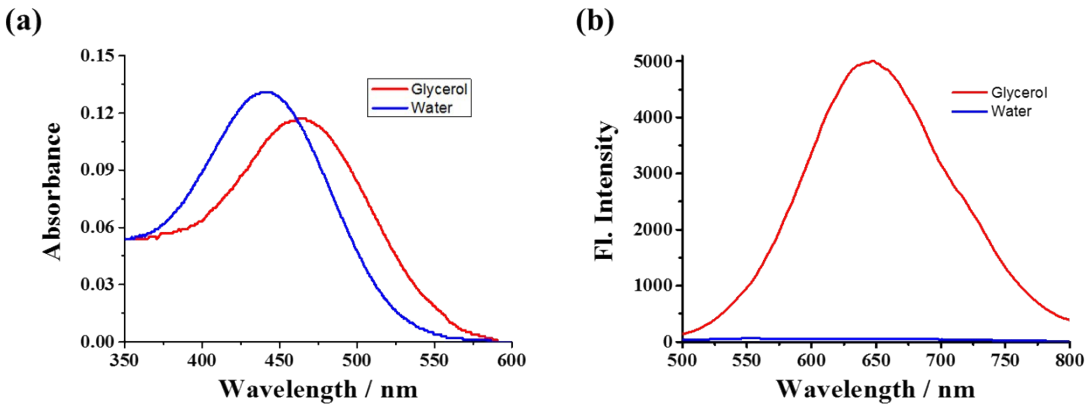


Fig. S2. Absorption (a) and fluorescence spectral of the probe **TPE-V** in water and glycerol solutions. λ_{ex} = 460 nm.

Table S1. Properties of the representative fluorescent probes developed for detection viscosity.

Probes	Emission wavelength	Response fold	Stoke's shift	Imaging application	Ref.
TPE-V	650	~100	~190	inflammatory cell model	This work
Vis-A	517	—	~16	SMMC7721 cells	*J. Mater. Chem. B, 2017, 5,*

					360—368.
MCN	470	~90	~105	HeLa cells	*J. Mater. Chem. B, 2017, 5, 2743-2749.*
Lyso-NA	610	~50	~60	RAW.264.7 cells	*J. Mater. Chem. B, 2018, 6, 580-585.*
TDHC	600	~25	~75	HeLa cells and neurons	*Biosens. Bioelectron, 2016, 86, 885-891.*
BODIPY - 2	510	~39	~25	plasma membranes of SK-OV-3 cells in	*Chem. Commun., 2014, 50, 5282--5284*

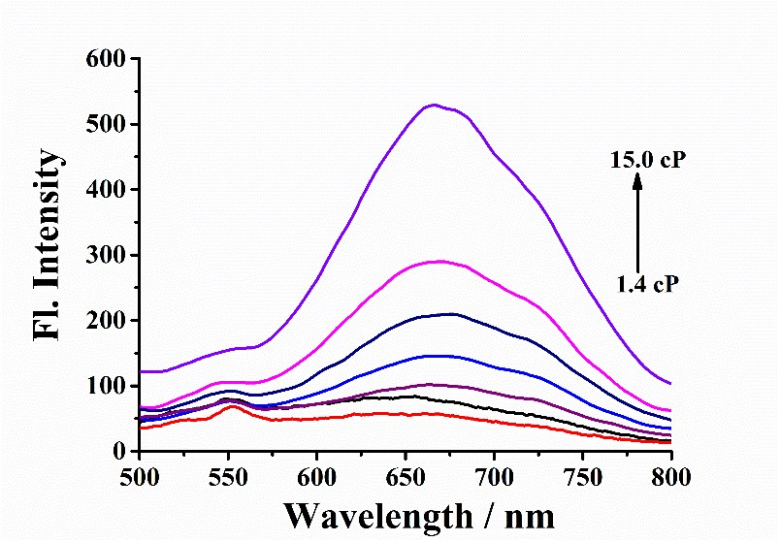


Fig. S3. Fluorescence spectra of 10 μM **TPE-V** in variation ratios of water-glycerol system, the viscosities being 1.4−15 cP, respectively. λ_{ex} = 460 nm.

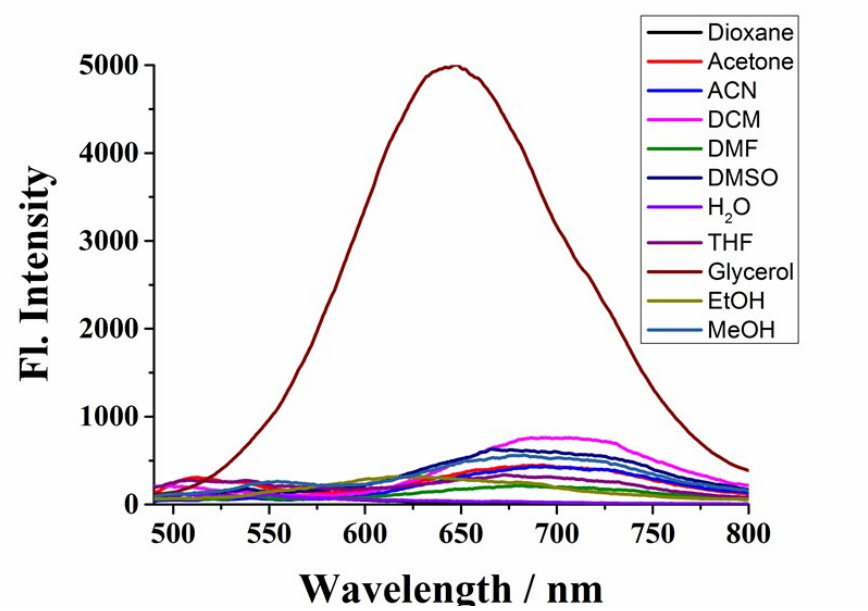


Fig. S4. Fluorescence spectra of 10 μM **TPE-V** in variation solvents. λ_{ex} = 460 nm.

Table S2 the photo-physical data of probe **TPE-V** in different solvent systems

Solvents	λ_{ab}(nm)	λ_{em}(nm)	Fluorescence Quantum $\Phi^{[a]}$ (%)
H_2O	436	557	0.014
DMF	440	560	0. 054
DMSO	443	670	0. 024
MeOH	453	683	0.025
EtOH	457	674	0. 021
THF	457	540	0. 025
ACN	444	690	0. 023
Acetone	447	680	0. 025
Dioxane	463	539	0. 022
DCM	492	690	0. 043
Glycerol	460	650	0. 286

[a] Rhodamine 6G was used as a standard reference with a quantum yield of 0.95 in water. [s3]

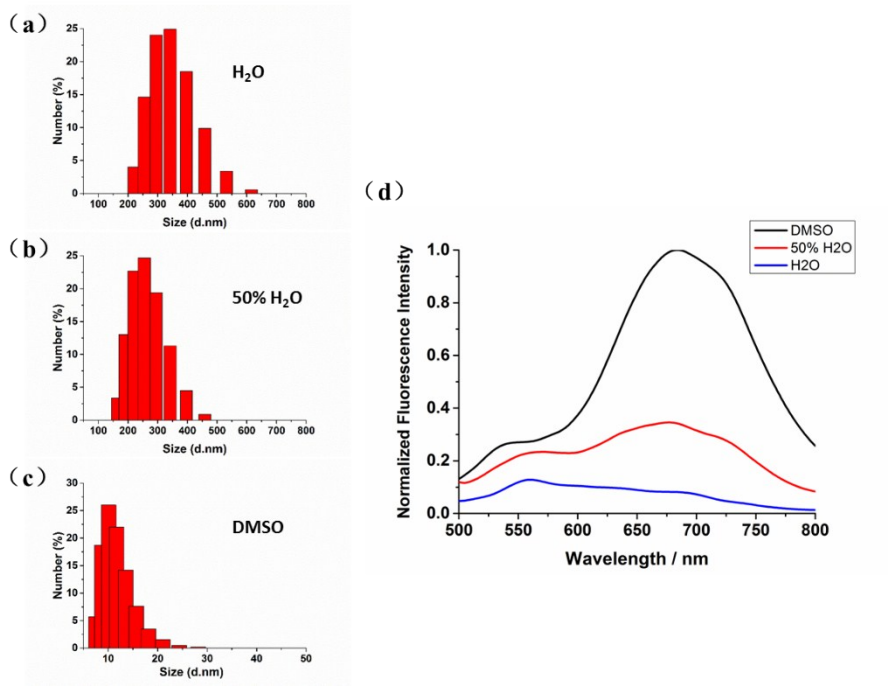


Fig. S5. DLS measurements of **TPE-V** in DMSO (a), 20% H_2O (b) and H_2O solutions. (d) Fluorescence spectra of 10 μM **TPE-V**.

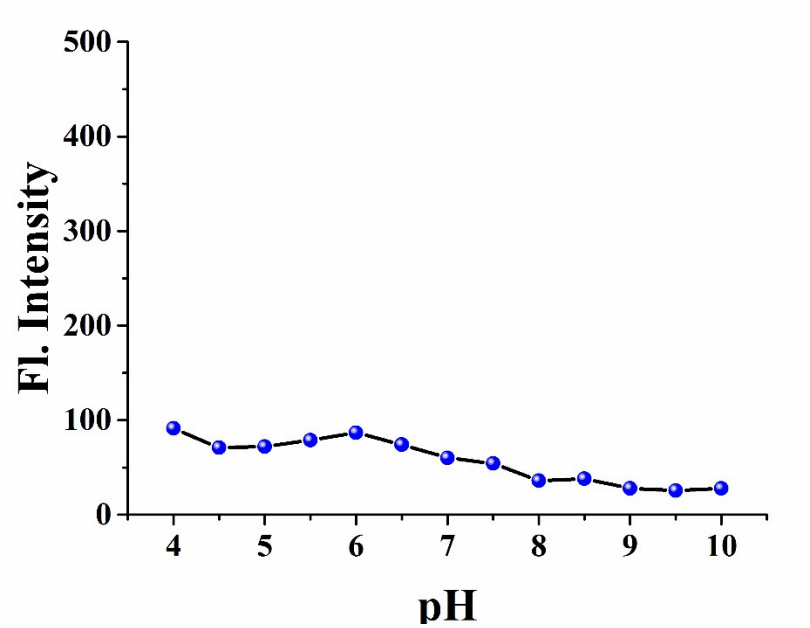


Fig. S6. Fluorescence intensity of 10 μM **TPE-V** at 650 in variation pH values. λ_{ex} = 460 nm.

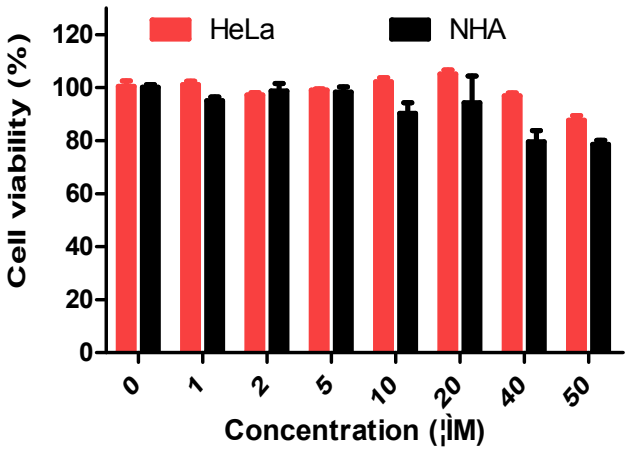


Fig. S7. Viability of HeLa and NHA cells treated with different concentrations (0 - 50 μM) of **TPE-V**.

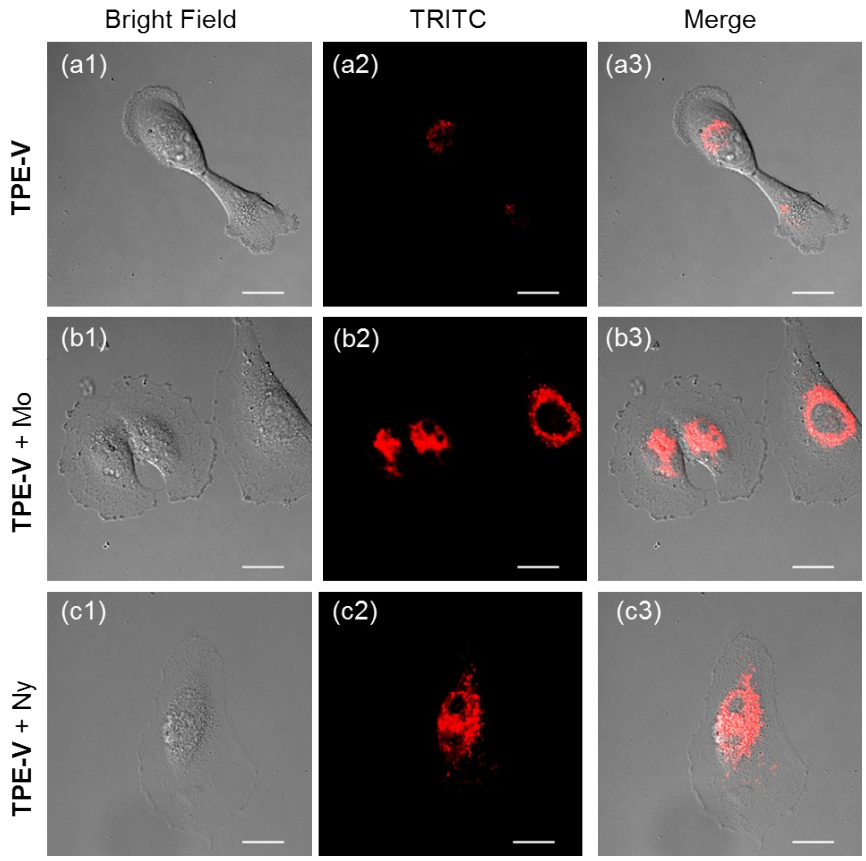


Fig. S8. The fluorescence imaging of NHA cells. (a1-a3) images of NHA cells stained with 10 μM **TPE-V** for 30 min. (b1-b3) NHA cells exposed to 10 μM monensin for 40 min, and then incubated with 10 μM **TPE-V** for another 30 min. (c1-c3) cells pretreated with 10 μM nystatin for 40 min, and then treated with 10 μM **TPE-V** for another 30 min. (a1-c1) Bright-field images of NHA cells. (a2-c2) Fluorescence images of NHA cells in red channel. (a3-c3) the overlay of bright-field and red channel. λ_{ex} = 488 nm, λ_{em} = 570−620 nm. Scale bar: 20 μm.

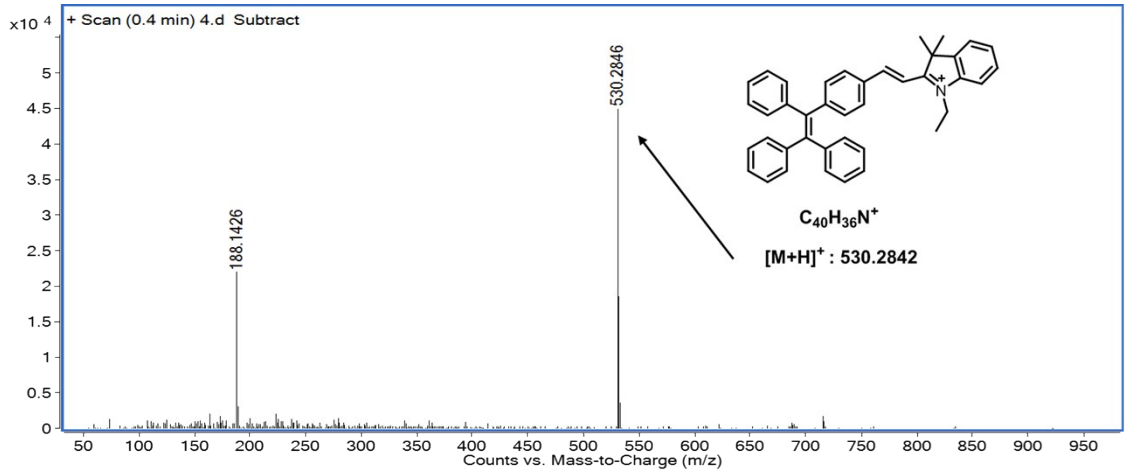


Fig. S9. HR-MS spectrum of **TPE-V**.

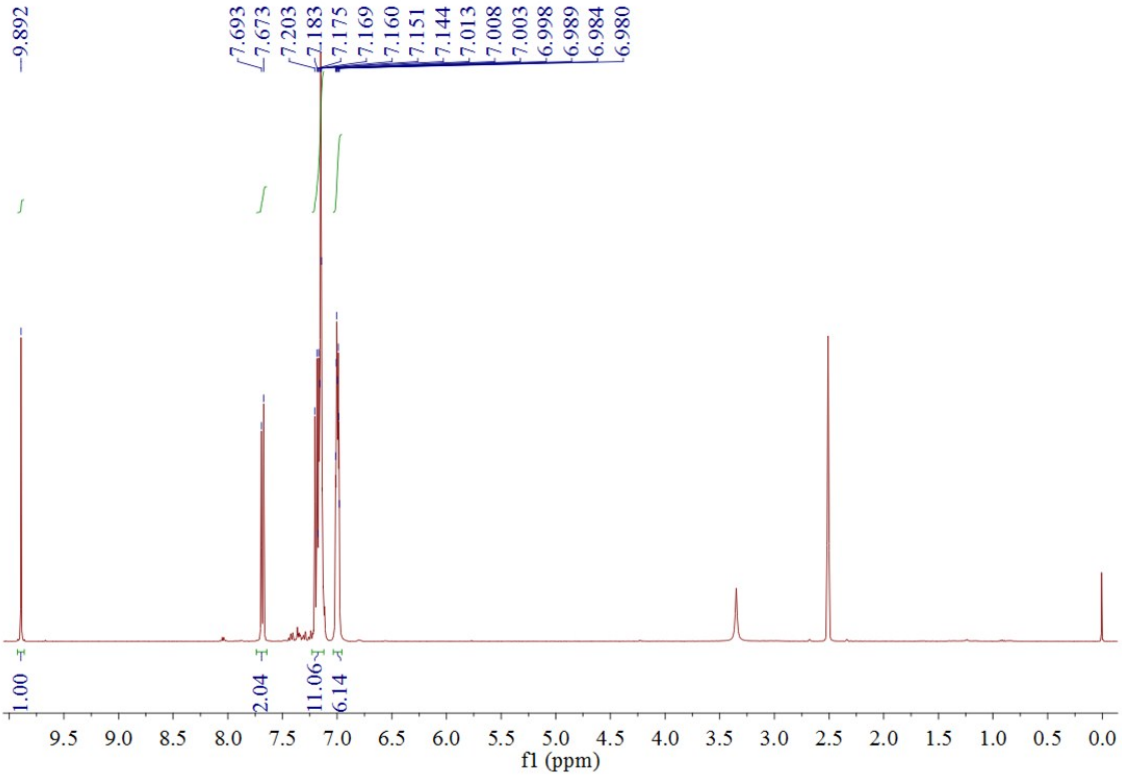


Fig. S10. ^{1}H NMR spectrum of Compound **1** in d_6-DMSO.

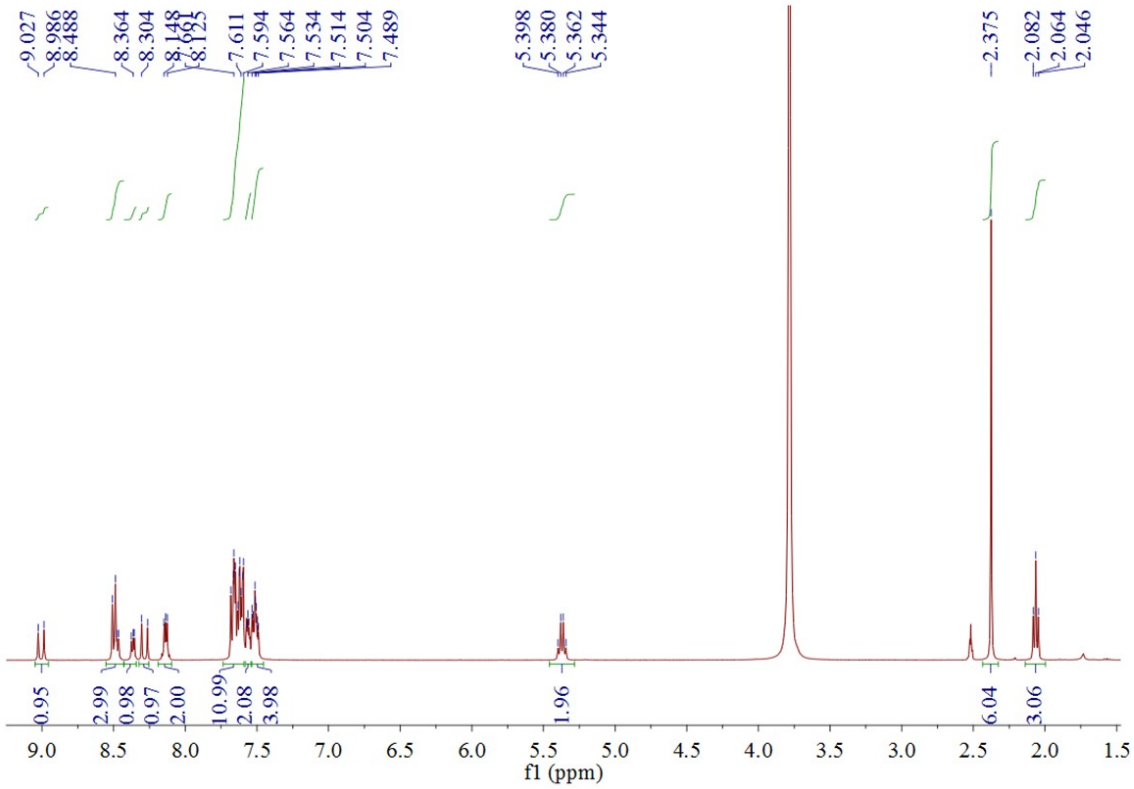


Fig. S11. ^{1}H NMR spectrum of **TPE-V** in d_6-DMSO.

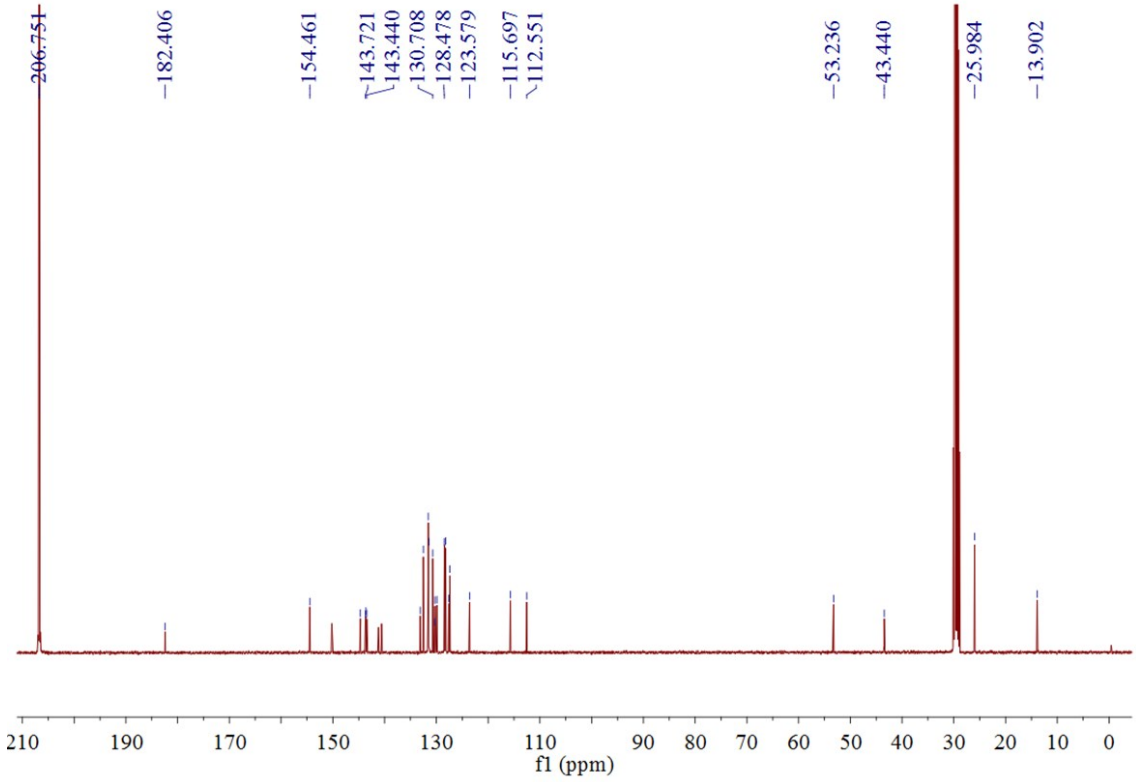


Fig. S12. ^{13}C NMR spectrum of **TPE-V** in d_6-DMSO.

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(s2) Z. Ma, Z. Wang, X. Meng, Z.Ma, Z.Xu, Y. Ma and X. Jia. *Angew. Chem. Int. Ed*. 2016, 55, 519-.522.
(s3) D. Magde, G.E. Rojas, and P. Seybold. *Photochem. Photobiol.* 1999, 70, 737-744.