Electronic Supportng Information (ESI) for

A novel mitochondrial-targeted fluorescent probe with two rotors based on 1,4-dihydropyridine,visualize and monitor the viscosity of live cells and mice in vivo

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Theoretical calculation method

The calculations are performed through DFT (density functional theory) and TDDFT (time shift DFT) on the B3LYP/6-31G (d, p) level of the Gaussian 09 program. The hole electron distribution analysis was performed using the Multiwfn program. PCM solvation model was used with water as solvent in all the calculations.

Cytotoxicity experiments and fluorescence imaging

We used standard CCK8 to evaluate the cytotoxicity of probe PTI. The specific experimental steps are as follows: After the Hela cells are resuscitated, place an appropriate amount of the cell line in the culture medium and culture it in a constant temperature incubator at 37°C and 5% CO_2. The medium is made up of 10% fetal bovine serum, 1% penicillin streptomycin double antibody DMEM high glucose medium. Take the Hela cells that survive the logarithmic growth phase in a centrifuge tube,

centrifuge, remove the supernatant, add 2 ml of medium, and blow evenly. Then take 10 ml of cell suspension and dilute to ten times. Pipette 1 μL of cell suspension and place it in the counting chamber of the cell counting plate. Count directly under the microscope to calculate the average value n, which is calculated by the formula: cell number/mL=n×10×10^4/ml. Take an appropriate amount of cell suspension by calculation and place it in a 96-well plate to ensure that the constant volume is 100 μL per well, and each well contains approximately 1×10^4 cells. Then put it in a 37 ℃, 5% CO_2 incubator for 24 h. After the cells adhere to the wall, remove the culture medium, rinse 3 times with PBS buffer solution, add probes and culture medium, probe concentrations are 0, 5, 10, 15, 20, 50, 100, 200 mM, 5 wells in each group . Then place it in a 37 ℃, 5% CO_2 incubator and continue to incubate for 24 h. After taking it out, add 10 μL of LCCK8 solution to each well, incubate it in an incubator for 4 h, and measure the absorbance at 450 nm with a microplate reader. Then carry out three parallel tests and take the average value. The cell survival rate is calculated by the following formula:

Cell survival rate (%)=[A(adding medicine)-A(blank)]/[A(0 adding medicine)-A(blank)]×100%

A (addition): absorbance value of experimental group; A (addition of 0): absorbance value of control group; A (blank): absorbance value of blank group

Cell fluorescence imaging test: The test is divided into three groups: A Hela cells + probe N (10.0 μM); B Hela cells + probe PTI (10.0 μM) + Mon; C Hela cells + probe PTI (10.0 μM) + Nys . Take the cells in the logarithmic growth phase and dilute to a cell suspension of 2.0×10^4 cells, place them in a petri dish, and culture them in a 37°C, 5% CO_2 incubator for 24 h. After the cells adhere to the wall, remove the medium, and rinse three times with buffer PBS. Then add the probe solution (10.0 μM) prepared by the culture medium to group A, group B and group C, and place them in a 37 ℃, 5% CO_2 incubator for 30 min, then add PBS buffer solution to group A, group B Add the same amount of PBS buffer prepared by Mon, and add the same amount of PBS buffer prepared by Nys to group C, then continue to incubate for 30 min, and observe the cell imaging with a laser confocal microscope.

Fluorescence imaging in living mice

Select Kunming mice for the experiment. The handling of mice complies with the requirements of the Animal Ethics Committee of Jilin University. LPS, Mon, Nys and normal saline were injected into the abdominal cavity of mice. After 12 h, 100 μL of the probe sample (2 mM) was injected into the mice by tail vein injection. One hour later, the mice were anesthetized by injecting 100 μL of 1% sodium pentobarbital. After general anesthesia, the abdomen was depilated with depilatory cream and photographed with a small animal in vivo imager.
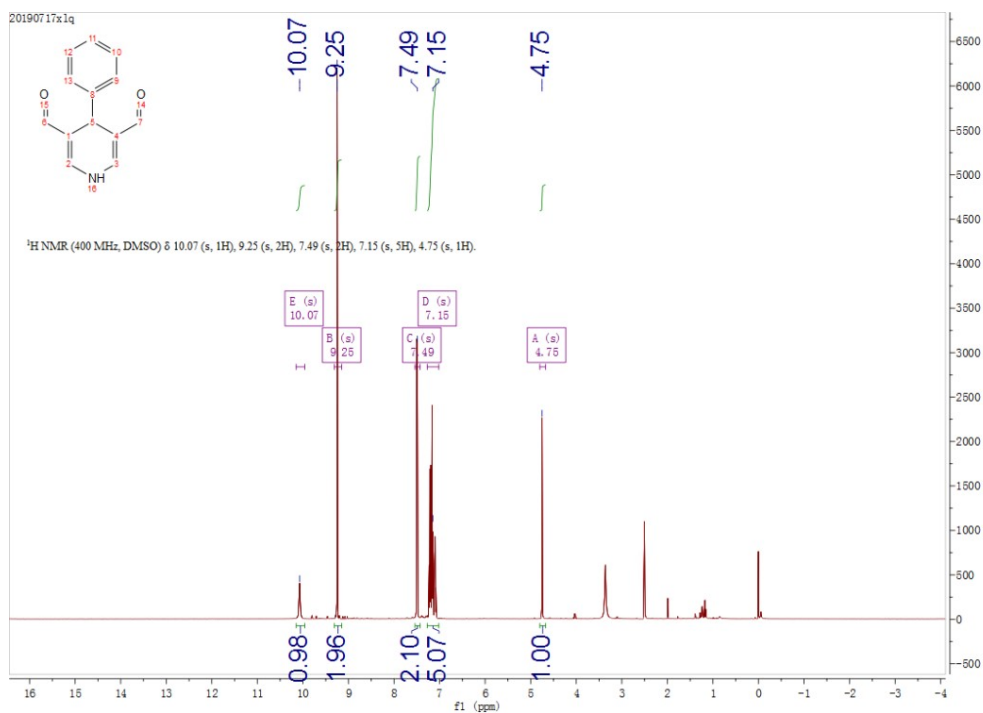
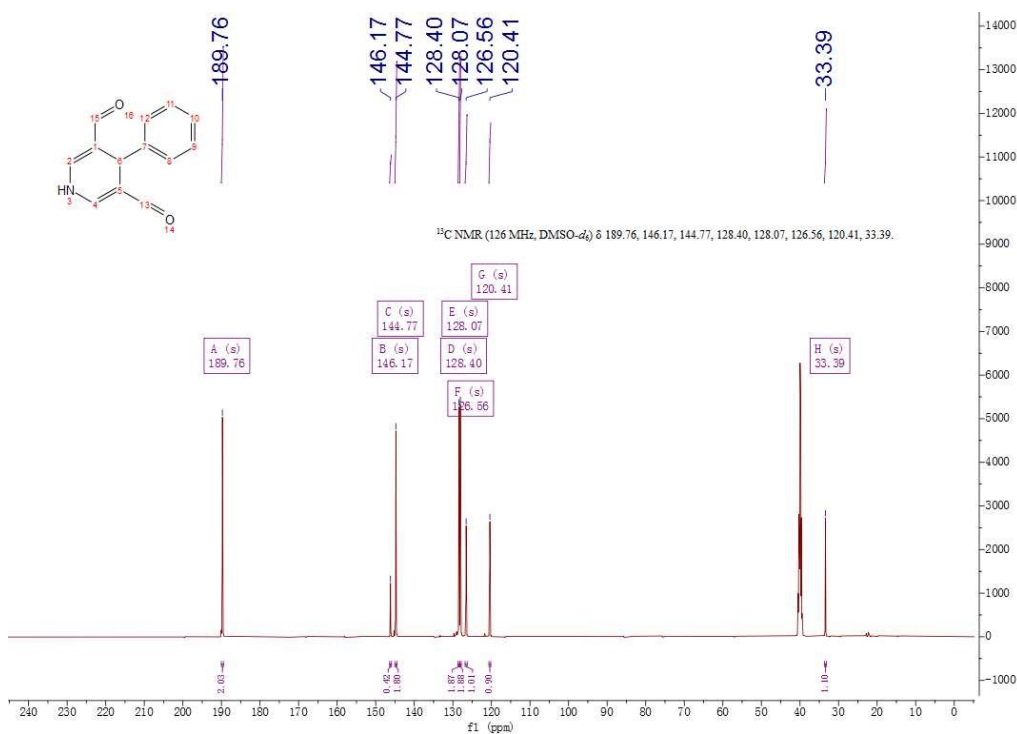Figure S1 ^{1}H NMR spectrum of Compound 1 in DMSO-d6.

^{1}H NMR (400 MHz, DMSO) δ 10.07 (s, 1H), 9.25 (s, 2H), 7.49 (s, 2H), 7.15 (s, 5H), 4.75 (s, 1H).

Figure S2 ^{13}C NMR spectrum of Compound 1 in DMSO-d6.

^{13}C NMR (126 MHz, DMSO-d_6) δ 189.76, 146.17, 144.77, 128.40, 128.07, 126.56, 120.41, 33.39.
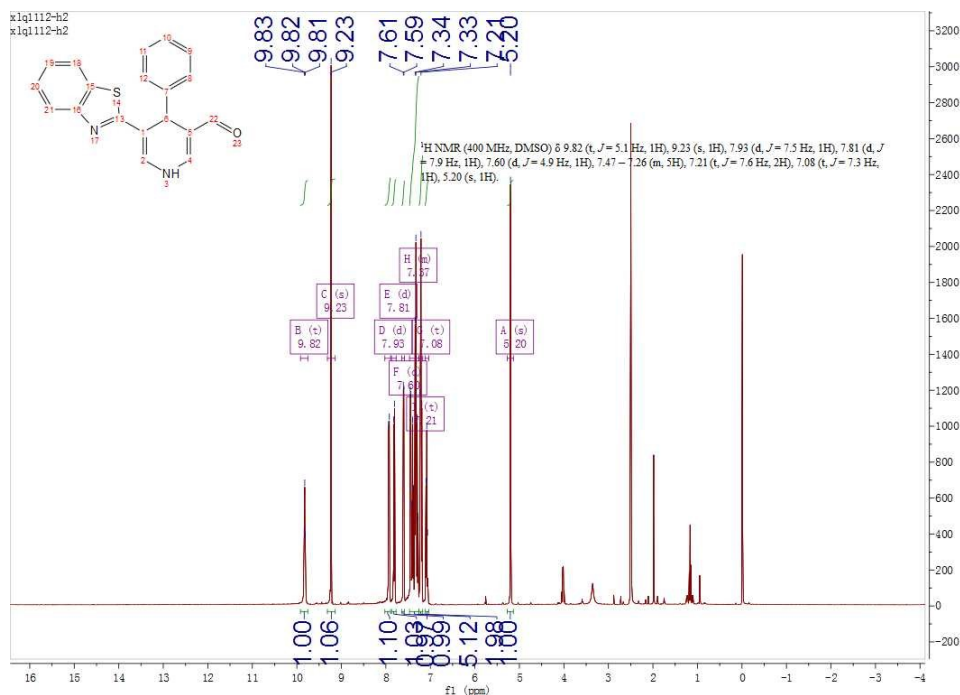
Figure S3 ^{1}H NMR spectrum of Compound 2 in DMSO-d6.

^{1}H NMR (400 MHz, DMSO) δ 9.82 (t, J = 5.1 Hz, 1H), 9.23 (s, 1H), 7.93 (d, J = 7.5 Hz, 1H), 7.81 (d, J = 7.9 Hz, 1H), 7.60 (d, J = 4.9 Hz, 1H), 7.47 – 7.26 (m, 5H), 7.21 (t, J = 7.6 Hz, 2H), 7.08 (t, J = 7.3 Hz, 1H), 5.20 (s, 1H).
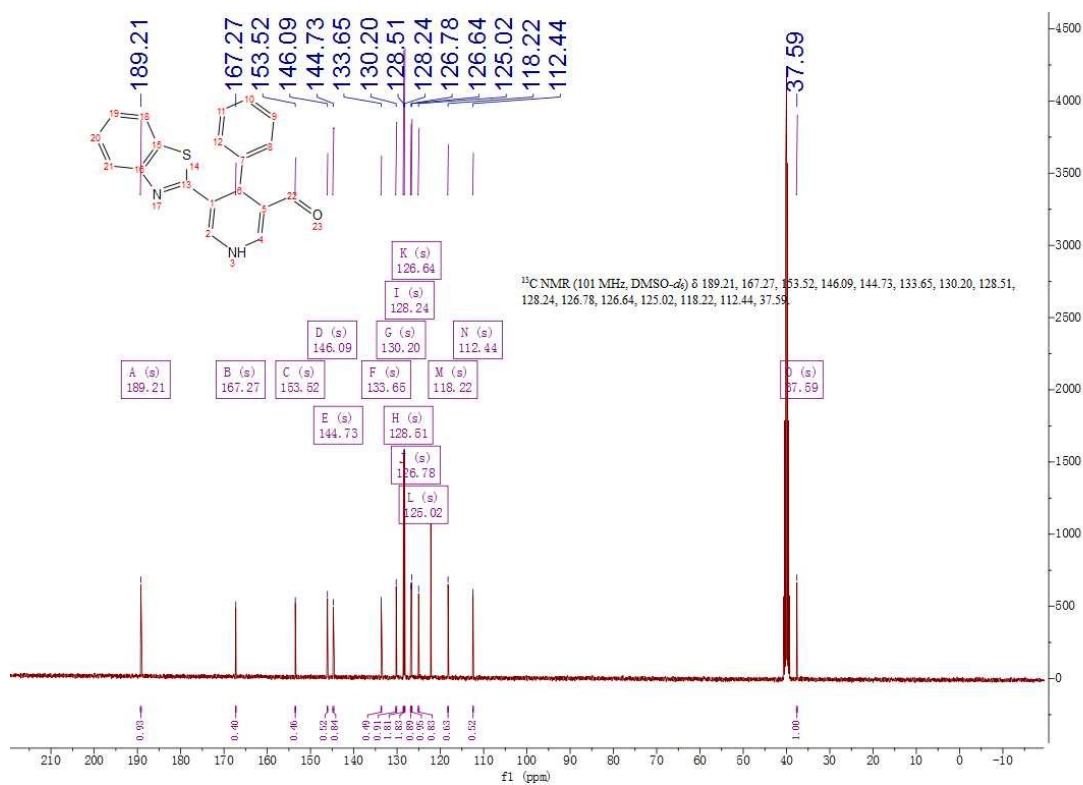
Figure S4 ^{13}C NMR spectrum of Compound 2 in DMSO-d6.

^{13}C NMR (101 MHz, DMSO-d_6) δ 189.21, 167.27, 153.52, 146.09, 144.73, 133.65, 130.20, 128.51, 128.24, 126.78, 126.64, 125.02, 118.22, 112.44, 37.59.

Synthesis of compound 3：Compound 1 (0.213 g, 1.0 mmol) and 1,2,3,3-tetramethyl-3H-indolium iodide (0.30 g, 1.0 mmol) were added to a 50 ml single-necked flask. Add 20 ml of ethanol, add two drops of piperidine, and under the protection of nitrogen, the reaction was stirred at 85°C under reflux overnight. The solvent was removed, and the obtained crude product was separated by

dichloromethane/methanol (100:1, v/v) column chromatography, and finally 0.36 g of a red solid was obtained with a yield of 72%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.76 (s, 1H), 9.30 (s, 1H), 8.17 (d, J = 15.4 Hz, 1H), 8.00 (s, 1H), 7.68 (dd, J = 19.5, 7.7 Hz, 2H), 7.60 – 7.38 (m, 5H), 7.29 (t, J = 7.6 Hz, 2H), 7.15 (t, J = 7.3 Hz, 1H), 6.44 (d, J = 15.4 Hz, 1H), 5.10 (s, 1H), 3.76 (s, 3H), 1.60 (d, J = 8.8 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d6) δ 189.94, 179.72, 154.92, 145.16, 144.81, 142.76, 142.36, 129.11, 128.81, 128.57, 128.09, 127.24, 123.03, 122.46, 119.21, 114.16, 105.55, 51.23, 35.68, 33.33, 26.54, 26.16. MS, m/z calcd. for $C_{25}H_{25}N_2O^+$:369.1961; found[M]$^+$: 369.1951.
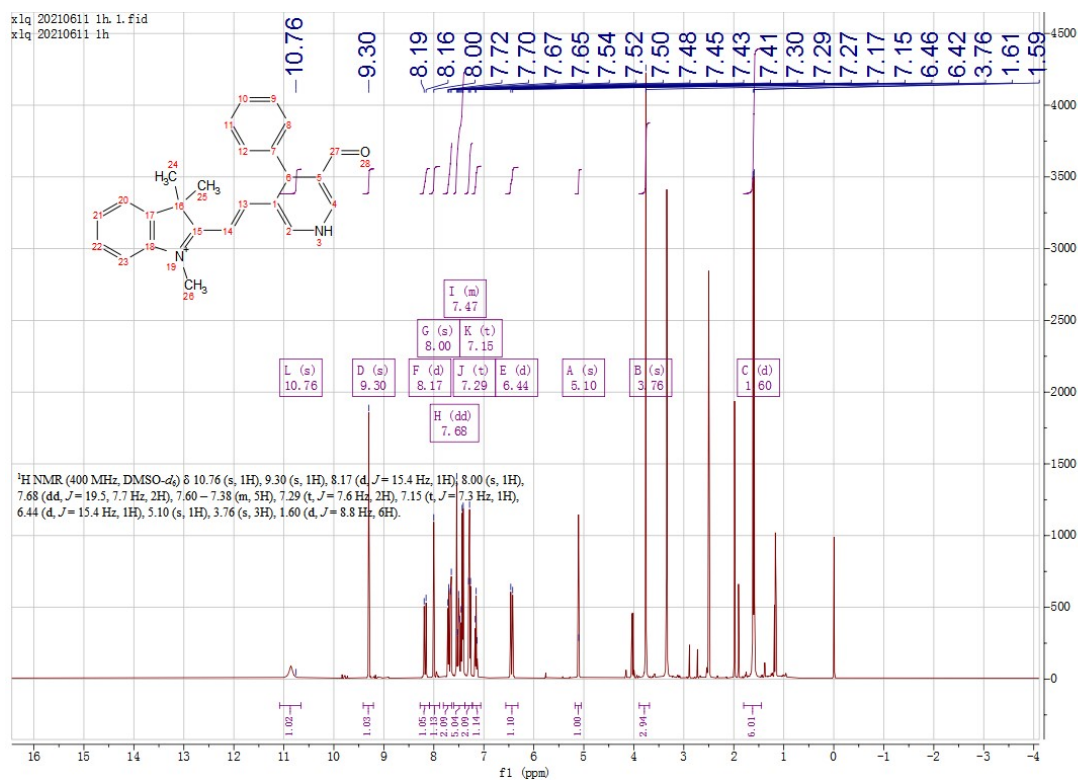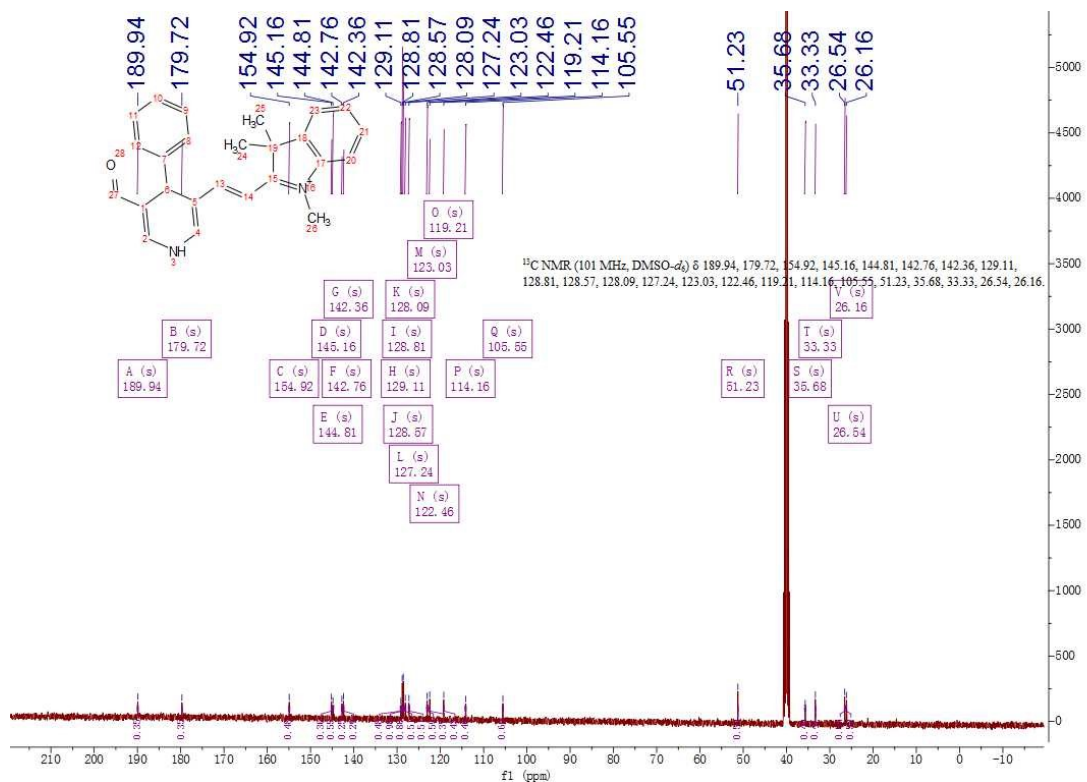

Figure S5 ^{1}H NMR spectrum of Compound 3 in DMSO-d6.
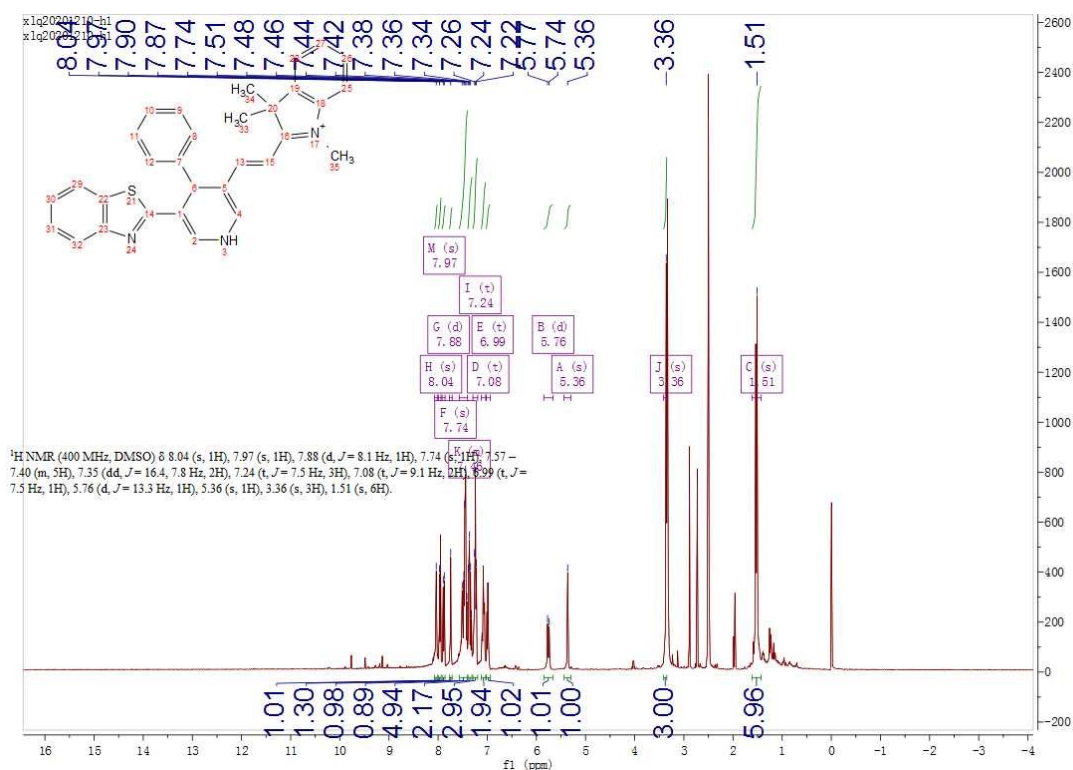
Figure S6 ^{13}C NMR spectrum of Compound 3 in DMSO-d6.

^{13}C NMR (101 MHz, DMSO-d_6) δ 189.94, 179.72, 154.92, 145.16, 144.81, 142.76, 142.36, 129.11, 128.81, 128.57, 128.09, 127.24, 123.03, 122.46, 119.21, 114.16, 105.55, 51.23, 35.68, 33.33, 26.54, 26.16.

Figure S7 ^{1}H NMR spectrum of Compound PTI in DMSO-d6.

^{1}H NMR (400 MHz, DMSO) δ 8.04 (s, 1H), 7.97 (s, 1H), 7.88 (d, J = 8.1 Hz, 1H), 7.74 (s, 1H), 7.57 – 7.40 (m, 5H), 7.35 (dd, J = 16.4, 7.8 Hz, 2H), 7.24 (t, J = 7.5 Hz, 3H), 7.08 (t, J = 9.1 Hz, 2H), 6.99 (t, J = 7.5 Hz, 1H), 5.76 (d, J = 13.3 Hz, 1H), 5.36 (s, 1H), 3.36 (s, 3H), 1.51 (s, 6H).

¹³C NMR (101 MHz, DMSO) δ 167.24 (s), 163.05 – 162.65 (m), 153.66 (s), 144.08 (s), 142.52 (s), 140.45 (s), 139.74 (s), 133.81 (s), 129.11 – 128.21 (m), 126.90 (s), 126.67 (s), 125.44 (s), 122.53 (s), 122.26 (d, *J* = 12.9 Hz), 108.89 (s), 93.46 (s), 47.24 (s), 36.23 (s), 28.15 (s).
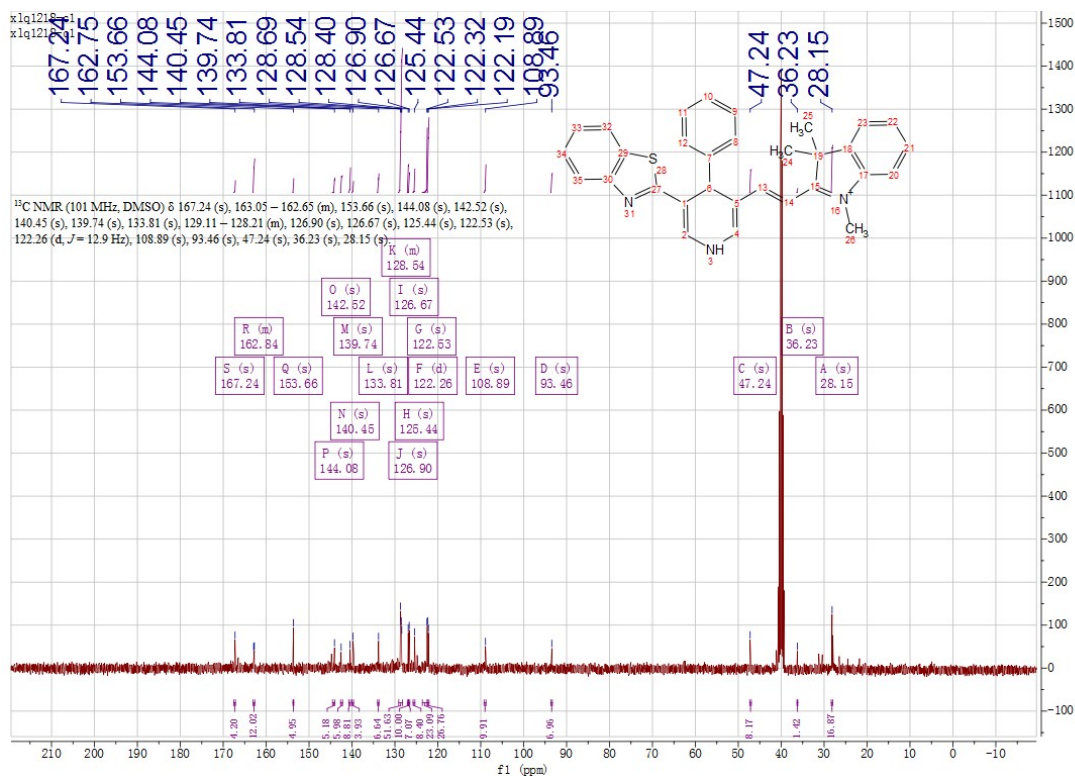
Figure S8 ¹³C NMR spectrum of Compound PTI in DMSO-d6.

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	G:\ÖÊÆ×²âÊÔ\É«È©Ó«¹åÍÀ¸ß·Ö±æÖÊÆ×	Acquisition Date	2019/9/27 11:19:19
Method	lc-ms-hr-low.m	Operator	zlwei
Sample Name	XLQ-7	Instrument / Ser#	micrOTOF-Q II 10351
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	5.0 l/min
Scan End	1200 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste

C$_{13}$H$_{11}$NO$_2$: 213.0790

+MS, 0.5min #30

136.0411
214.0870
241.0480
347.5873

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e¡¥ Conf	mSigma	Std I	Std I Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
214.0870	1	C 13 H 12 N O 2	214.0863	-3.4	-3.3	8.5	ok	even	10.52	0.0192	0.0007	0.0081	0.0003	0.8427

Figure S9 High-resolution mass spectra of Compound 1.

Figure S10 High-resolution mass spectra of Compound 2.

Figure S11 High-resolution mass spectra of Compound 3.

+MS, 2.7min #163

C 31 H 28 N 3 S
474.1995

$C_{31}H_{28}N_3S^+$: 474.1998

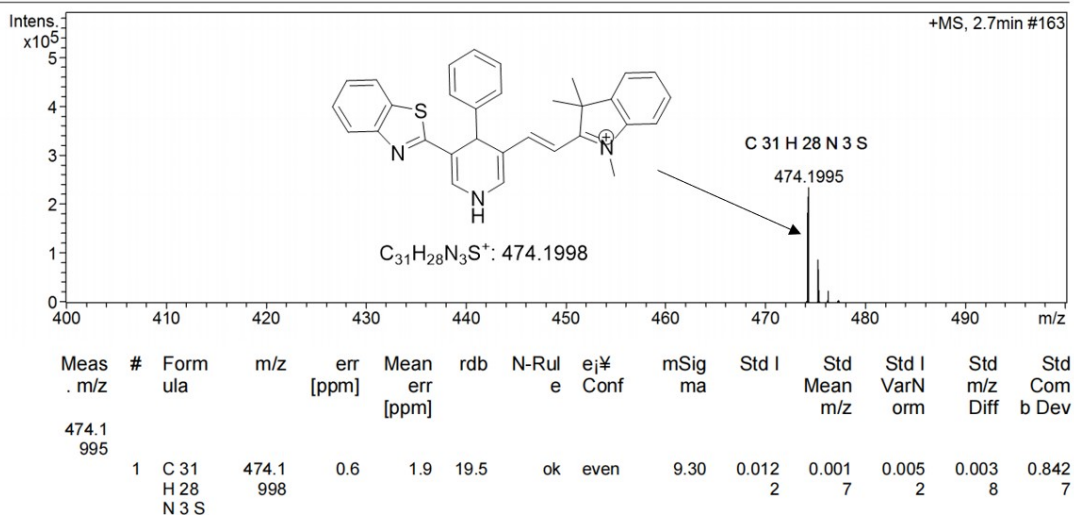| Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e¡¥ Conf	mSigma	Std I	Std Mean m/z	Std I VarN orm	Std m/z Diff	Std Comb Dev
474.1995														
	1	C 31 H 28 N 3 S	474.1998	0.6	1.9	19.5	ok	even	9.30	0.0122	0.0017	0.0052	0.0038	0.8427

Figure S12 High-resolution mass spectra of Compound PTI.

Table 1:Viscosity value corresponding to different glycerol volume fraction

Gly/V%	0	10	20	30	40	50	60	70	80	90	99
Viscous/cp	1.5	2.5	6.0	9.8	18.0	36.5	74.0	140.0	270.0	665.0	1100.0

Table 1:Viscosity value corresponding to different glycerol volume fraction

Table 2:The corresponding viscosity of the probe solution at different temperatures

temperature/°C	20	25	30	35	40	45	50
Viscosity/cp	635	425	300	210	150	115	90

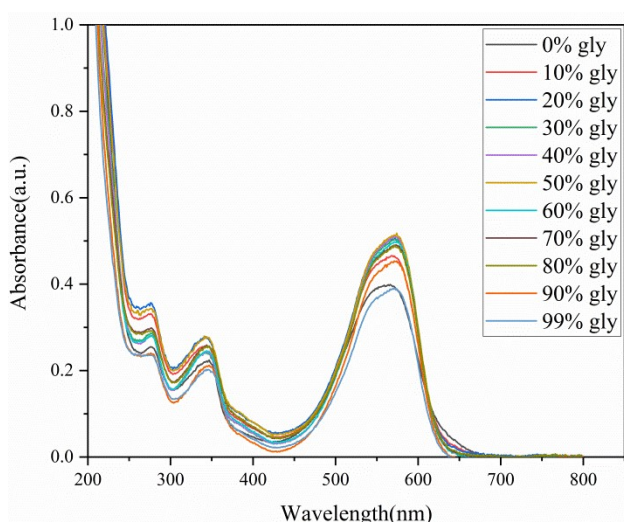Table 2: The corresponding viscosity of the probe solution at different temperatures

Figure S13 absorbe spectrum of probe PTI (10 μM) with increasing viscosity.

Table 3:Comparison table of different fluorophore viscosity probes

Fluorophore	Viscosity solvent system	Mitochondrial targeting	Emission wavelength	References
diethylamino coumarin	methanol and glycerol	No	634nm	[1]
benzothiazole-based	methanol and glycerol	Yes	583nm	[2]
"distorted-BODIPY"-based	water and glycerol	No	538nm	[3]
4-aminobenzaldehyde	water and glycerol	Yes	583nm	[4]
6-substituted quinoline	water and glycerol	No	490nm	[5]
carbazole	ethanol and glycerol	Yes	545nm	[6]
1,4-dihydropyridine	ethanol and glycerol	Yes	625nm	This work

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6.   K. Zhou, M. Ren, B. Deng and W. Lin, *New Journal of Chemistry*, 2017, **41**, 11507-11511.