Supporting Information for

Lipid droplet-specific fluorescent probe for acute liver injury and tumor diagnosis via aberrant polarity changes

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Experimental

1. Materials and Instruments

Chemical reagents or materials were purchased from commercial suppliers without further purification except as otherwise showed. The UV-Vis spectra were acquired by a UV-2700 spectrophotometer (Shimadzu, Japan), and fluorescence spectra were measured on a HITACHI F4700 fluorescence spectrophotometer (Japan). High-resolution mass spectra were obtained on Agilent 7250& JEOL-JMS-T100LP AccuTOF (Bruker Daltonics, Billerica, MA, USA). The fluorescence imaging of cells was performed with Leica TCS SP8 CARS confocal microscope (Germany). The fluorescence imaging of the mice was performed with Small Animal In Vivo Imaging System (IVIS Lumina Series III). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE III 600 MHz digital nuclear magnetic resonance spectrometer (Bruker Biospin, Billerica, MA, USA) with an internal standard of tetramethyl silane (TMS). TLC analysis was carried out on silica gel plates and column chromatography was conducted over silica gel (mesh 200-300), both of which were purchased from the Qingdao Ocean Chemicals. All aqueous solutions were prepared with ultrapure water obtained from a Milli-Q water purification system (18.2 MΩ·cm).

2. Synthesis and characterization

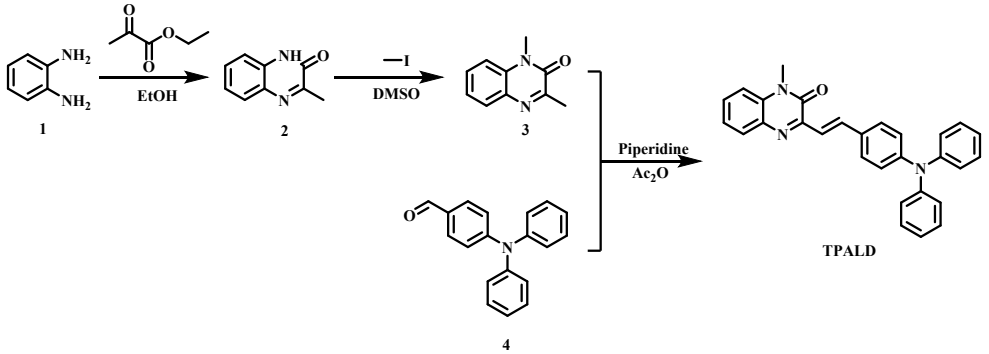
2.1 Synthesis of compound 2

In a clean dry flask, o-Phenylenediamine (3.24 g, 30 mmol) was dissolved in

absolute ethanol (40 mL). Then, ethyl pyruvate (5.60 g, 48 mmol) dissolved in ethanol (20 mL) was added dropwise. After completion, the reaction was stirred at room temperature for 6 h. After completion of the reaction, the product was filtered off and wash with EtOH, then dry in a vacuum oven to obtain white crystalline compound **2** (4.08 g, yield: 84 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.29 (s, 1H), 7.69 (dd, J = 8.1, 1.4 Hz, 1H), 7.52 – 7.36 (m, 1H), 7.33 – 7.17 (m, 2H), 2.40 (s, 3H).

2.2 Synthesis of compound 3

Compound **2** (3.17 g, 20 mmol), K_2CO_3 (8.27 g, 60 mmol), 35 mL DMSO and CH_3I (3.69 g, 26 mmol) were added into a 150 mL round bottom flask, and reacted at 55 ℃ for 6 h. At the end of the reaction (monitored by TLC), the mixture was poured into H_2O, and then extract with ethyl acetate twice (each 30 mL). The obtained organic layer was dried over anhydrous Na_2SO_4. The solvent ethyl acetate was removed by rotary evaporator and then purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 20:1, v/v), obtaining light-yellow powder compound **3** (2.85 g, yield: 83 %). ^{1}H NMR (600 MHz, Chloroform-d) δ 7.82 (dd, J = 8.0, 1.5 Hz, 1H), 7.54 (ddd, J = 8.6, 7.3, 1.5 Hz, 1H), 7.42 – 7.29 (m, 2H), 3.72 (s, 3H), 2.62 (s, 3H).



Scheme S1. Synthesis route of **TPALD**

3. Spectral experiments

3.1 Optical studies and analysis

A stock solution (1 mM) of the probe **TPALD** was initially prepared in dimethyl sulfoxide (DMSO). All spectrometric probes were used at a concentration of 10 μM. The adjunction of 20 μL of stock solution was added to 2.0 mL of different solvent systems to obtain the probe **TPALD** diluent. The solutions of various interfering substances (cations, anions, amino acids and active small molecules) were prepared with twice-distilled water. The providing solutions were mixed well before texting the spectra. Polarity response of **TPALD** (10 μM) was measured in 1,4-dioxane/water mixture. Unless otherwise specified, the required fluorescence spectral measurement is generally an excitation wavelength of 460 nm, an excitation slit width of 5.0 nm, and an emission slit width of 5.0 nm.

4. Cell fluorescence imaging

4.1 Cell culture

HepG2 cells were cultured in DMEM (Dulbecco's modified Eagle's medium), supplemented with 10% FBS, 1% streptomycin sulfate, 1% penicillin in a humidified 5% CO_2/ 95% air incubator at 37 ℃. Replace the growth medium every two days. When the cells had grown to 80%, they were digested with trypsin and then sub-cultured before experiments.

4.2 Colocalization experiments

HepG2 cells were first treated with **TPALD** (10 μM) and incubated for 30 min. Then, BODIPY 493/503 (500 nM), the commercial tracker staining dye for LDs, was added to continue to incubate for 15 min, the confocal fluorescence imaging was manipulated with green channel (λ_{ex} = 493 nm, λ_{em} = 500 - 540 nm) for BODIPY 493/503, Red channel (λ_{ex} = 488 nm, λ_{em} = 550 - 650 nm) for **TPALD**.

4.3 Cell imaging

HepG2 cells were cultured using the methods above and divided into several different experiment groups. (1) HepG2 cells were first incubated with different volume (0 μL, 5 μL, 10 μL, 20 μL) of 100 μM oleic acid for 30 min and then 10 μL of **TPALD** (10 μM) was added into each group with continuous incubation for 30 min. **TPALD** was excited at 488 nm, and emission range at 550 - 650 nm.

HepG2 cells were seeded at a density of 5×10^4 cells/mL in a 96-well micro-assay culture plate and growth for 24 h. The cell DMEM (Dulbecco's modified Eagle's medium) culture medium of each well was then replaced with the fresh medium containing increasing concentrations of **TPALD**, 0, 2, 5, 10, 20 and 50 μM. The wells with the culture medium only were marked as the blank. After incubation under a 5 % CO_2/95 % air circumstance for 24 h, cell culture medium was removed and then were washed with PBS three times. Subsequent work was that 10 μL MTT (5 mg/mL) was added to each well and continued to incubate for another 4 h. After 4 h, the MTT solution was then discarded from each well, and the formed formazan crystals were

dissolved in 100 μL of DMSO. The optical density of each well was measured at a wavelength of 490 nm using a microplate reader (Bio-Tek, USA). The results from the five experiments were averaged respectively. The following formula was used to calculate the viability of cell growth: Viability = (mean of absorbance value of treatment group–blank) / (mean absorbance value of control – blank) × 100%. All of the measurements were performed five times.

5. Animal experiments

Seven-week-old Kunming mice were purchased from Guangxi Medical University. The farming system of animals was under standard laboratory conditions. All animal experiments were reviewed and approved by the Animal Care and Experiment Committee of Guangxi University (protocal number: Gxu-2021-115).

Acetaminophen (APAP) inducing method was used to create the ALI model mice. APAP was dissolved in normal saline solution at a concentration of 20 mg/mL. The mice were given a intraperitoneal injection at a dosage of 2 mL/100 g. The mice's kidney, liver, spleen, lung, and heart were removed, and animal optical imaging system was used to accomplish the fluorescence imaging.

Herein, the tumor model mice were constructed by subcutaneous injection of 4T1 cells into the right leg of Kunming mice for about 7 days.

6. Supplementary Figures and tables

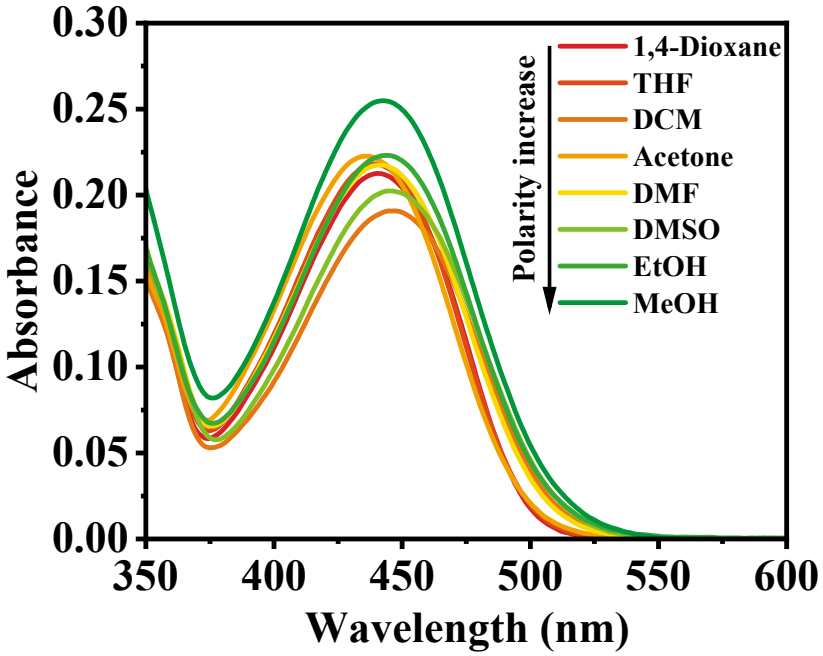


Fig. S1. Absorption spectra of **TPALD** (10 μM) in different solvents.

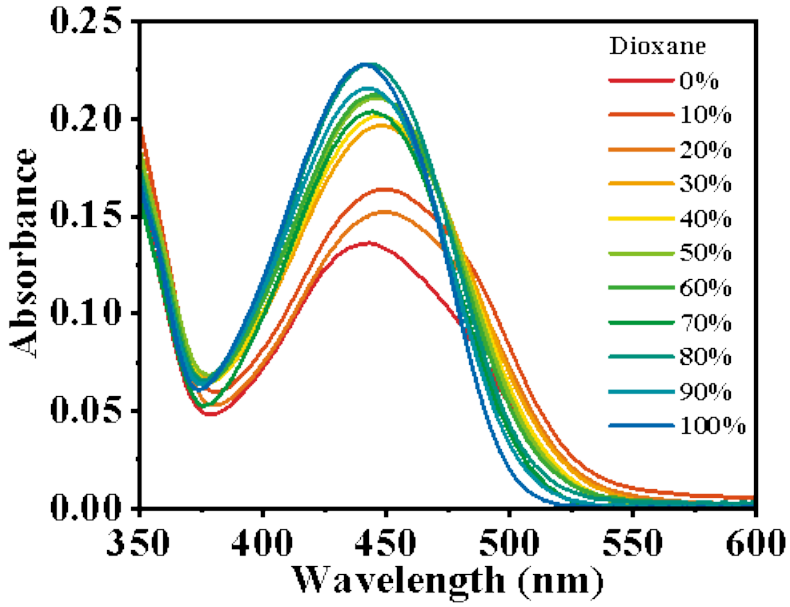


Fig. S2. Absorption spectra of **TPALD** (10 μM) in a 1,4-dioxane-H_2O system with the fraction of dioxane from 0 to 100 %.

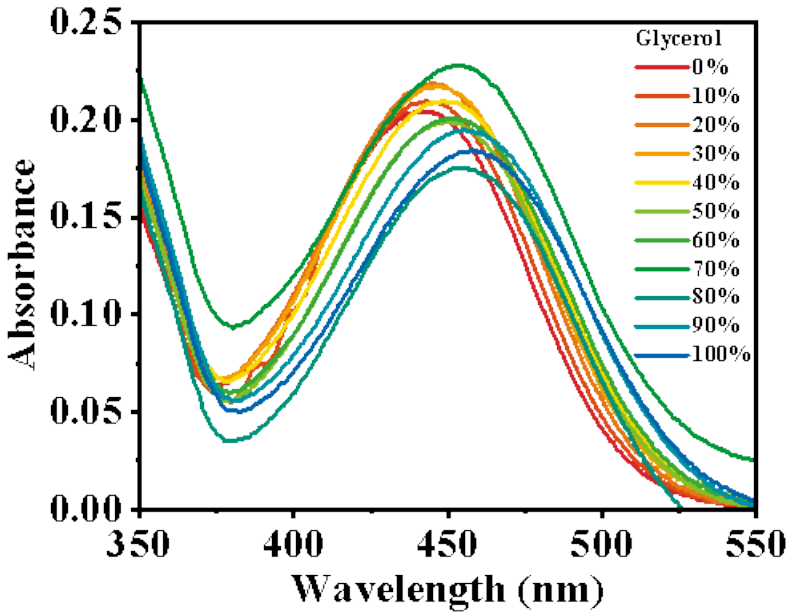


Fig. S3. Absorption spectra of **TPALD** (10 μM) in a glycerol-MeOH system with the fraction of glycerol from 0 to 100 %.

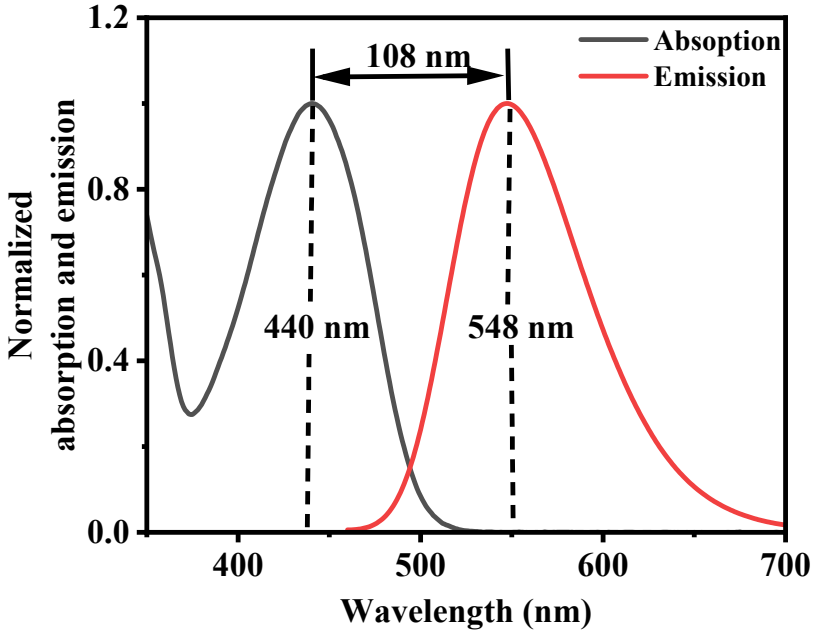


Fig. S4. The Stokes Shift of **TPALD** in 1,4-dioxane solution.

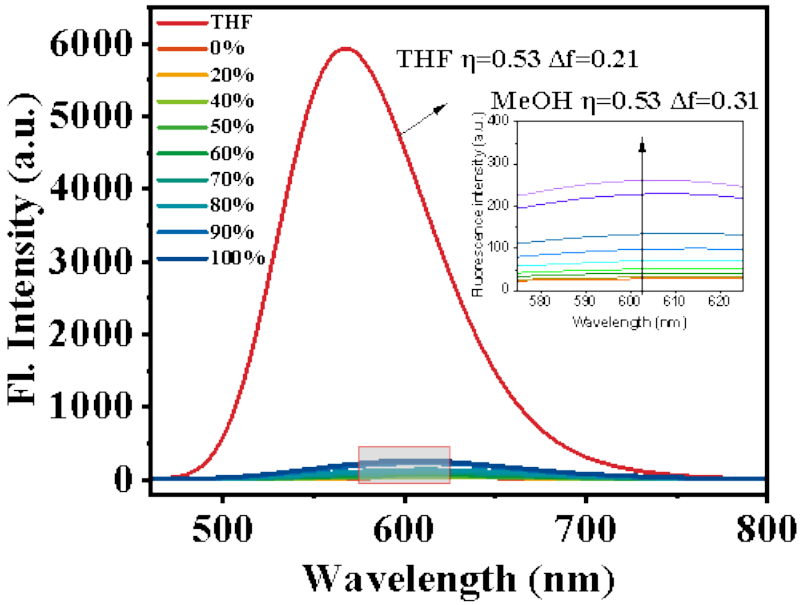


Fig. S5. Fluorescence spectra of **TPALD** (10 μM) in a glycerol-MeOH system and THF.

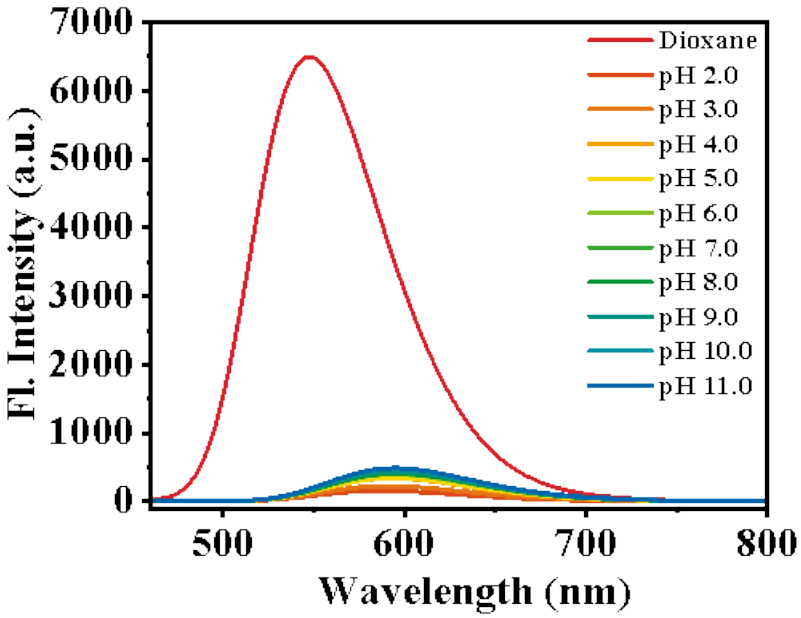


Fig. S6. Fluorescence spectra of **TPALD** (10 μM) under 100% water with different pH from 2.0 to 11.0.

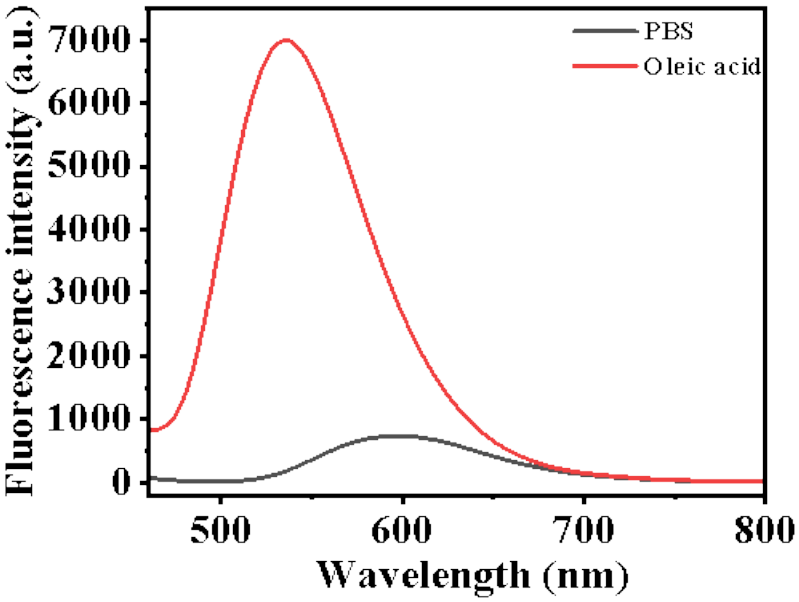


Fig. S7. Fluorescence spectra of **TPALD** (10 μM) in PBS and oleic acid.

Table S1 The Fl. Intensity of **TPALD** in different fractions of glycerol in the Gly-MeOH system

Fraction (%)	Fl. Intensity (a.u.)	Fraction (%)	Fl. Intensity (a.u.)
0	28.31	60	71.68
10	30.97	70	99.16
20	32.68	80	135.3
30	35.47	90	230
40	42.2	100	261.7
50	52.39	THF	5937

Table S2 The Fl. Intensity of **TPALD** in different pH buffer solution

pH	Fl. Intensity (a.u.)	pH	Fl. Intensity (a.u.)
2	78.58	7	178.2
3	104.3	8	162
4	142.3	9	174
5	141.3	10	192
6	160.1	11	193.4
		1,4-Dioxane	6494

Table S3 The Fl. Intensity of **TPALD** in 30 % 1,4-dioxane with different interfering analytes

Analytes	Fl. Intensity (a.u.)	Analytes	Fl. Intensity (a.u.)
30 % 1,4-Dioxane	74.24	PO_4^{3-}	85.86
SO_4^{2-}	89.96	SO_3^{2-}	85.52
SCN^-	66.43	$S_2O_3^{2-}$	82.13
AcO^-	93.81	S^{2-}	64.91
NO_2^-	77.28	CO_3^{2-}	94.99
HSO_3^-	79.24	Na^+	73.12
F^-	97.99	Mn^{2+}	70.6
Br^-	91.04	Mg^{2+}	77.81
I^-	76.57	K^+ (10 μM)	89.71
Cu^{2+}	56.21	L-Gly	59.33
K^+ (100 μM)	80.79	L-Hcy	77.15
Cu^+	85.65	L-His	58.92

Ca^{2+}	88.32	L-Lys	70.51
GSH	53.93	L-Phe	80.79
L-Ala	70.13	L-Pro	67.41
L-Arg	65.58	L-Val	68.15
L-Cys	58.53	TBHP	54.29

Table S4 The fluorescence quantum yield of the probe **TPALD** in mixed solutions of 1,4-dioxane and H_2O at different ratios

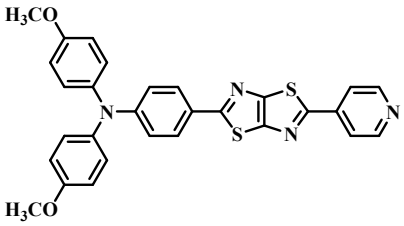
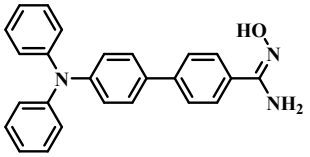
1,4-Dioxane (v %)	H_2O (v %)	Polarity/ Δf	Integral area	Fluorescence quantum yield
100%	0%	0.1222	2373	0.2083
98%	2%	0.1634	2329	0.2037
97%	3%	0.2246	2270	0.1982
95%	5%	0.2862	2183	0.1898
93%	7%	0.2958	1798	0.1558
92%	8%	0.3132	1637	0.1416
90%	10%	0.3291	1460	0.1258
85%	15%	0.3491	1034	0.0882
80%	20%	0.3609	709	0.0600
70%	30%	0.3747	235.7	0.0192
60%	40%	0.3828	227.0	0.0185
50%	50%	0.3885	123.7	0.0100
40%	60%	0.3929	70.14	0.0055
30%	70%	0.3966	46.07	0.0035

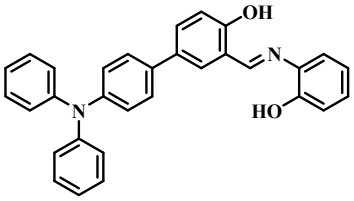
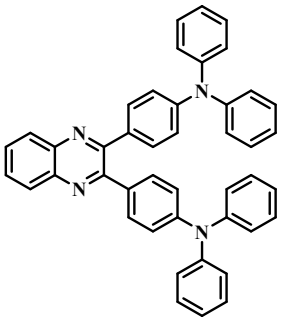
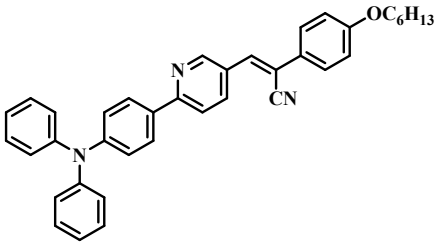
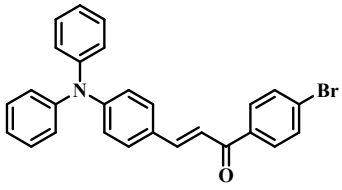
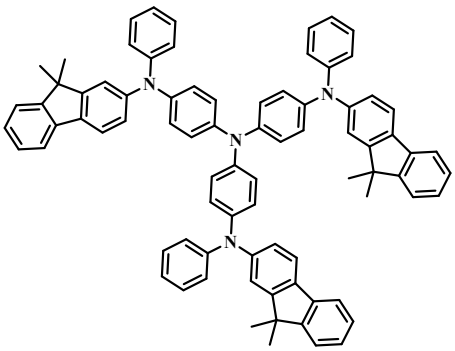
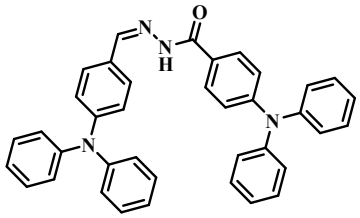
Rhodamine B (In ethanol)	/	11508	0.89

Table S5 The fluorescence quantum yield of the probe **TPALD** in different solvents with different polarity

Solvent	E_T (30)/ kcal·mol^{-1}	Integral area	Fluorescence quantum yield
Dioxane	36	2664	0.2294
THF	37.4	2276	0.1960
DCM	41.1	2155	0.1856
Acetone	42.2	1856	0.1598
DMF	43.8	1747	0.1504
DMSO	45	1380	0.1188
EtOH	51.9	200.1	0.0172
MeOH	55.5	79.85	0.0069
Rhodamine B	/	11615	0.89

Table S6 The Stokes Shift of the probe **TPALD** compared with probes in some relevant works

Molecular structure	Excitation wavelength (nm)	Emission wavelength (nm)	Stokes shift (nm)	Reference
	460	518	58	*Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2025, **330**, 125694
	300	393	93	*Dyes and Pigments*, 2025, **232**,

				112436
	450	525	75	*Polyhedron*, 2024, **248**, 116759
	420	482	62	*Asian Journal of Organic Chemistry*, 2024, **13**, 202400282
	410	487	77	*Journal of Molecular Structure*, 2025, **1327**, 141241
	410	500	90	*Journal of Molecular Structure*, 2025, **1327**, 142106
	354	383	29	*Journal of Photochemistry and Photobiology A: Chemistry*, 2025, **459**, 116078
	368	430	62	*Optical Materials*, 2024, **149**, 114992

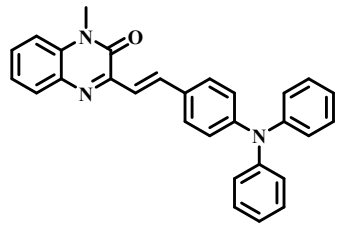
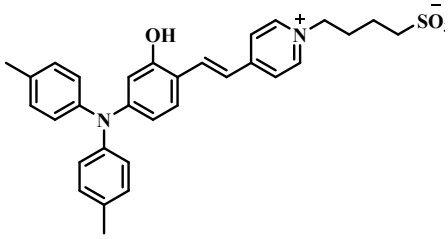
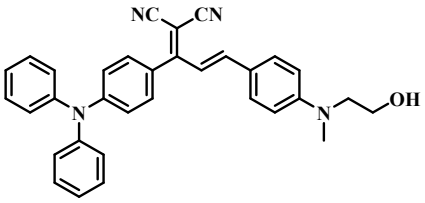
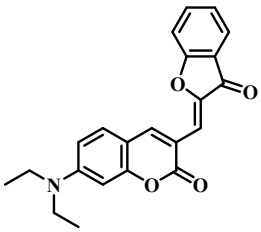
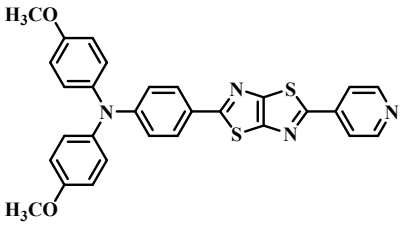
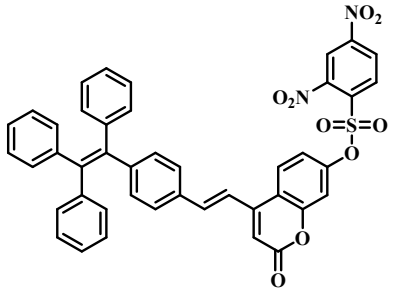
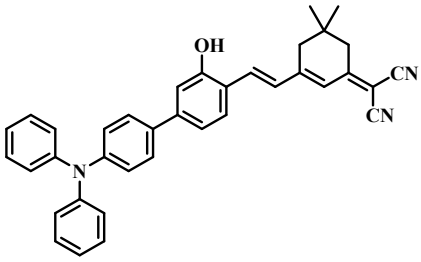
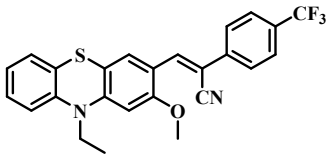
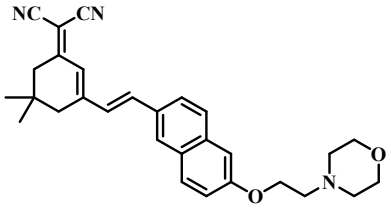
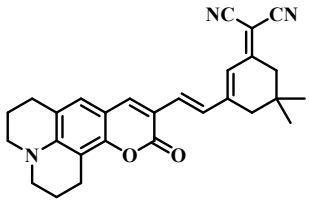
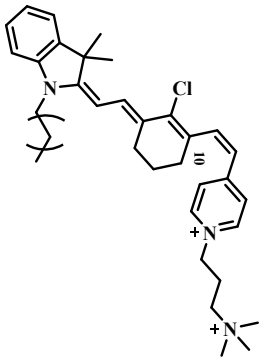
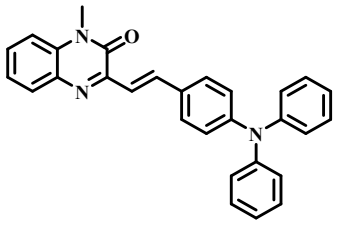
	440	548	108	**This work**

Table S7 Comparison of fluorescence quantum yield between the probe **TPALD** and other polarity-sensitive probes

Molecular structure	Biological applications	Φ_u (%)	Reference
	Viscosity/Polarity detection	1.3 (in THF)	*Sensors and Actuators B: Chemical*, 2025, **435**, 137644.
	Polarity detection	10.8 (in THF)	*Analytica Chimica Acta*, 2025, **1343**, 34370
	Polarity detection	13 (in 1,4-dioxane)	*Sensors and Actuators B: Chemical*, 2025, **426**, 137141
	Polarity detection	8.4 (in toluene)	*Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2025, **330**, 125694

	H_2S/Polarity/Viscosity detection	3.8 (in THF)	*Analytica Chimica Acta*, 2025, **1334**, 343425
	Polarity/pH/HClO detection	15 (in THF)	*Journal of Molecular Liquids*, 2025, **422**, 126953.
	Polarity detection	2.5 (in 1,4-dioxane)	*Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2025, **332**, 125854
	Polarity detection	11 (in water)	*Talanta*, 2024, **280**, 126787
	Polarity detection	6 (in 1,4-dioxane)	*Talanta*, 2024, **275**, 126141
	Polarity detection	2.1 (in 1,4-dioxane)	*Sensors and Actuators B: Chemical*, 2024, **405**, 135331

	Polarity detection	22.9 (in 1,4-dioxane)	**This work**

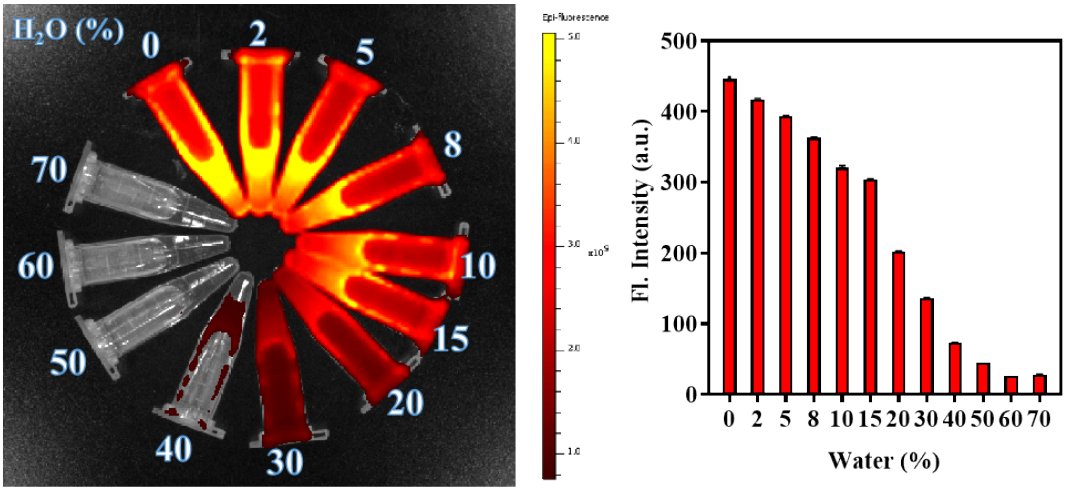


Fig. S8. The infrared imaging photo of the probe **TPALD** under different fractions of dioxane from 0 to 70 %.

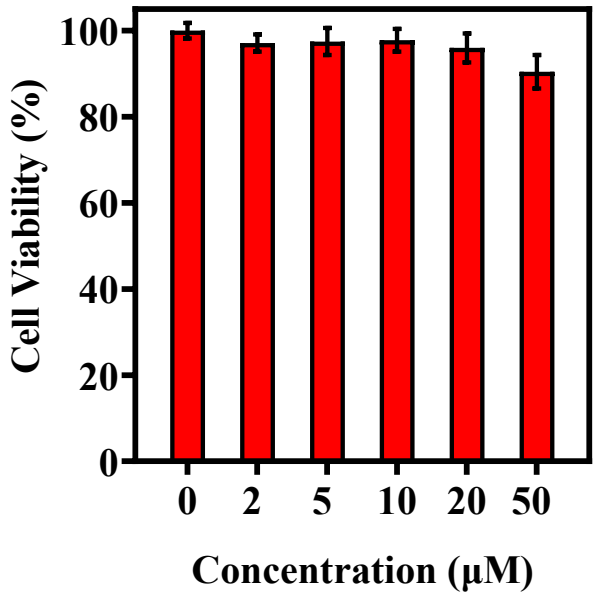


Fig. S9. Cytotoxicity assays of the probe **TPALD** at different concentrations for HepG2 cells.

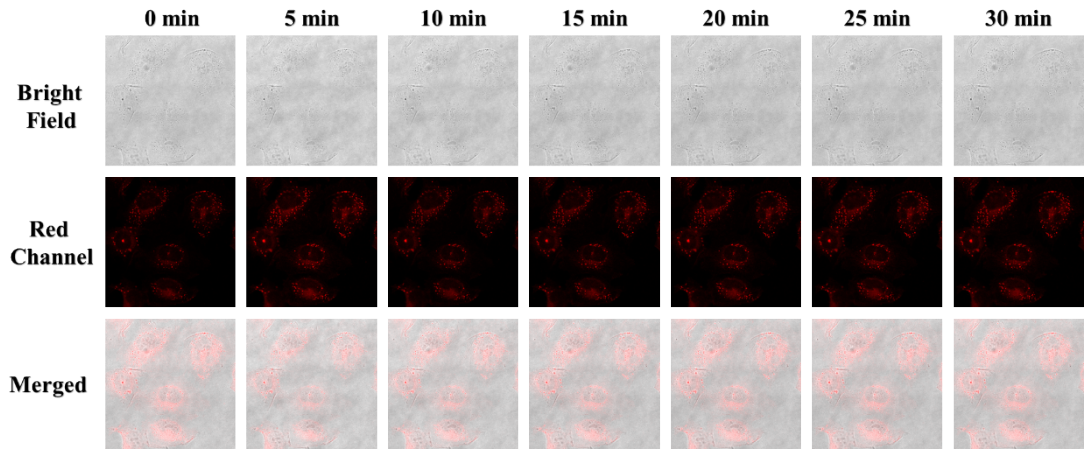


Fig. S10. Photostability of the probe **TPALD** in living HepG2 cells. HepG2 cells were incubated with 10 μM **TPALD** for 30 min, and then the culture medium was removed and the cells were rinsed three times with PBS (10 mM, pH 7.4) for confocal imaging. λex = 488 nm, λem = 560-660 nm.

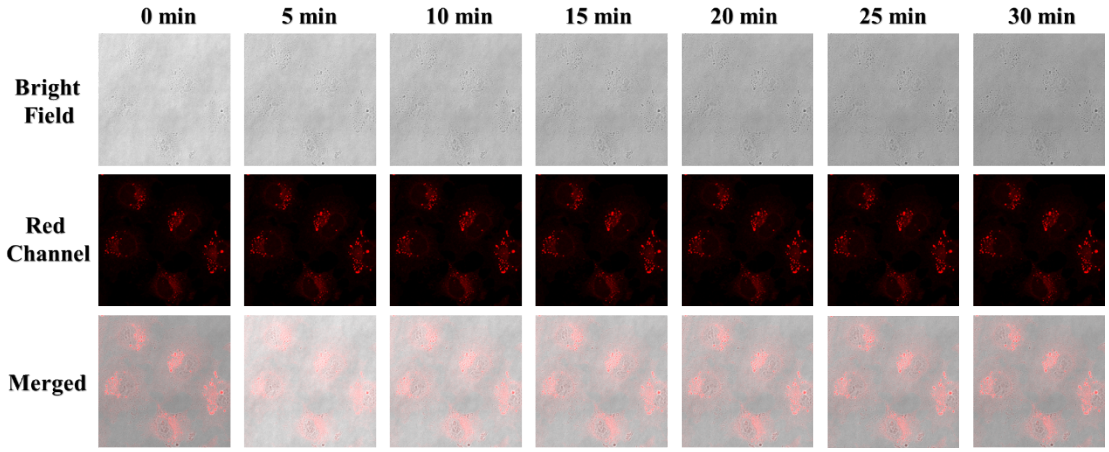


Fig. S11. Photostability of **TPALD** with OA-induced (5 μM) HepG2 cells. HepG2 cells were first incubated with 5 μM OA for 30 min incubated and then incubated with 10 μM **TPALD** for 30 min, then washed three times with PBS (10 mM, pH 7.4) for confocal imaging.

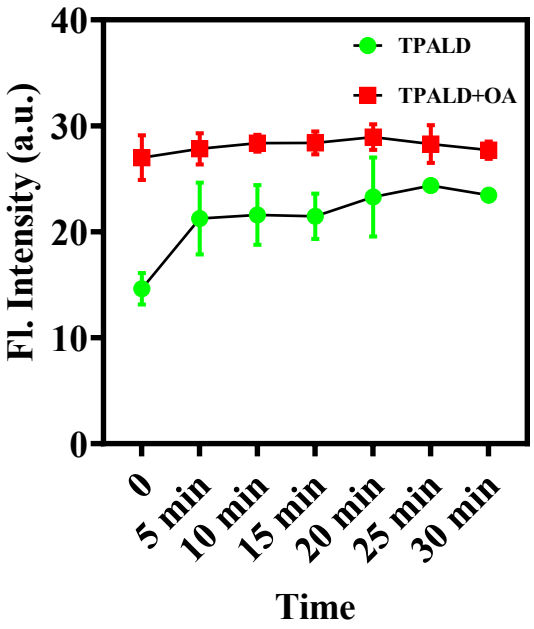


Fig. S12. The mean fluorescence intensity at different times of S9&S10.

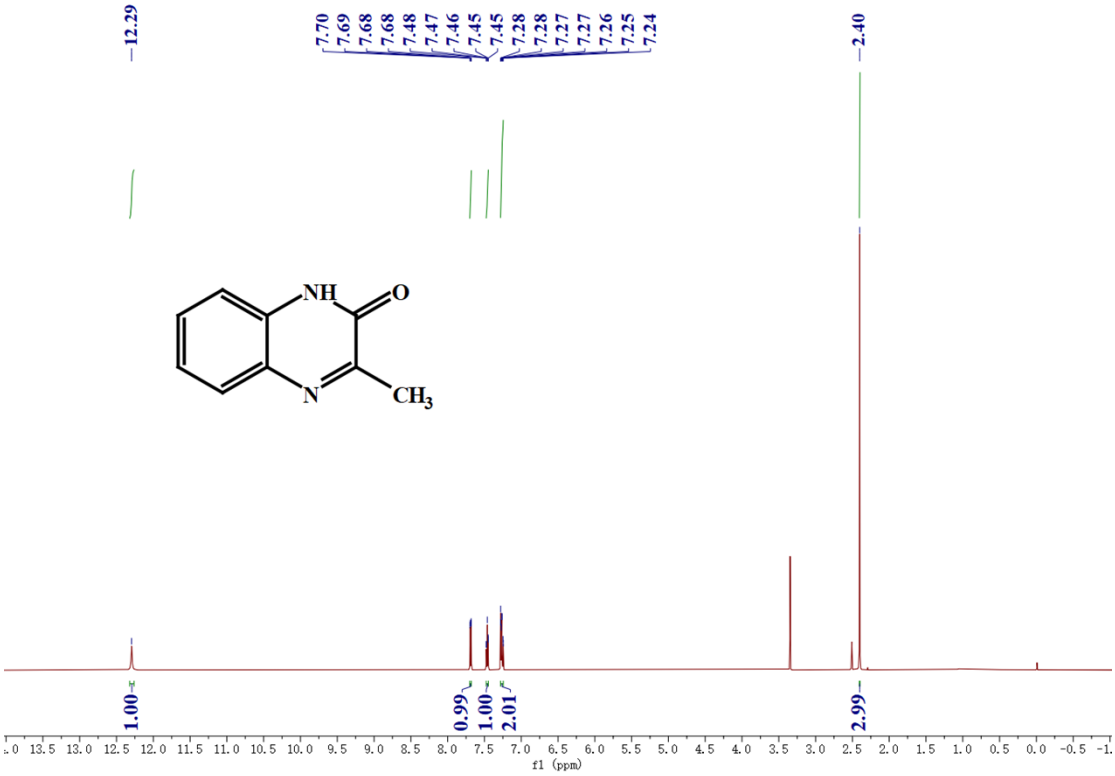


Fig. S13. ^{1}H NMR spectrum of compound **2**.

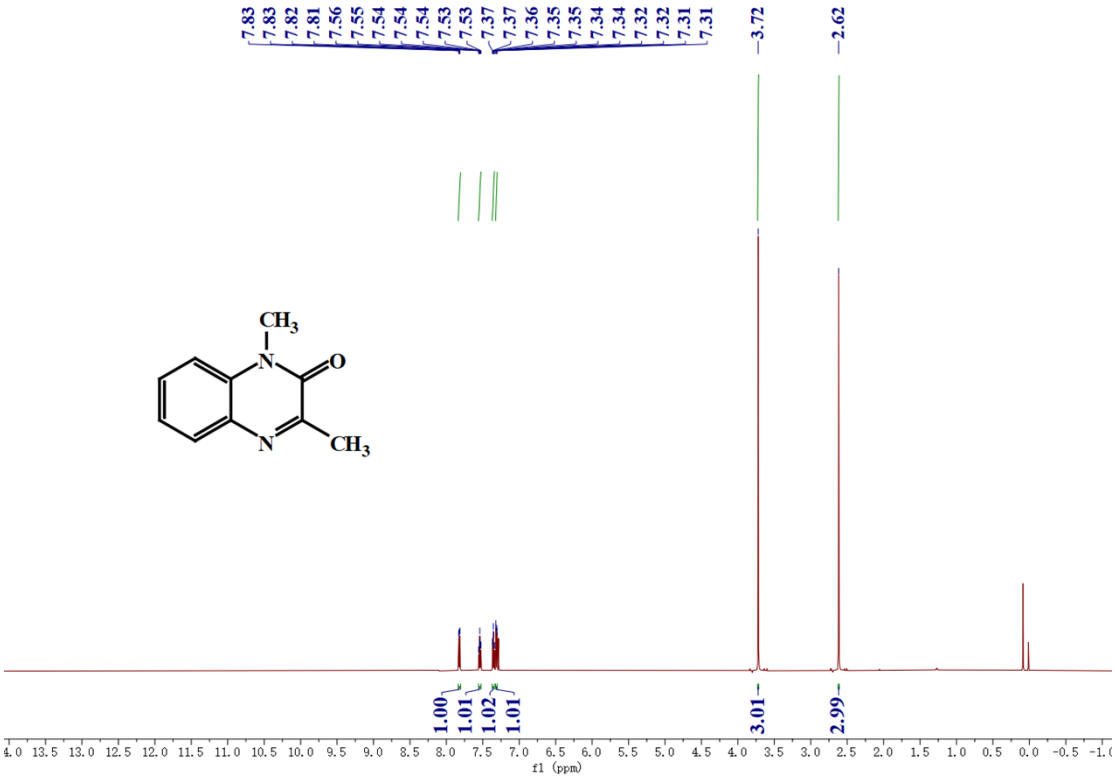


Fig. S14. [1]H NMR spectrum of compound **3**.

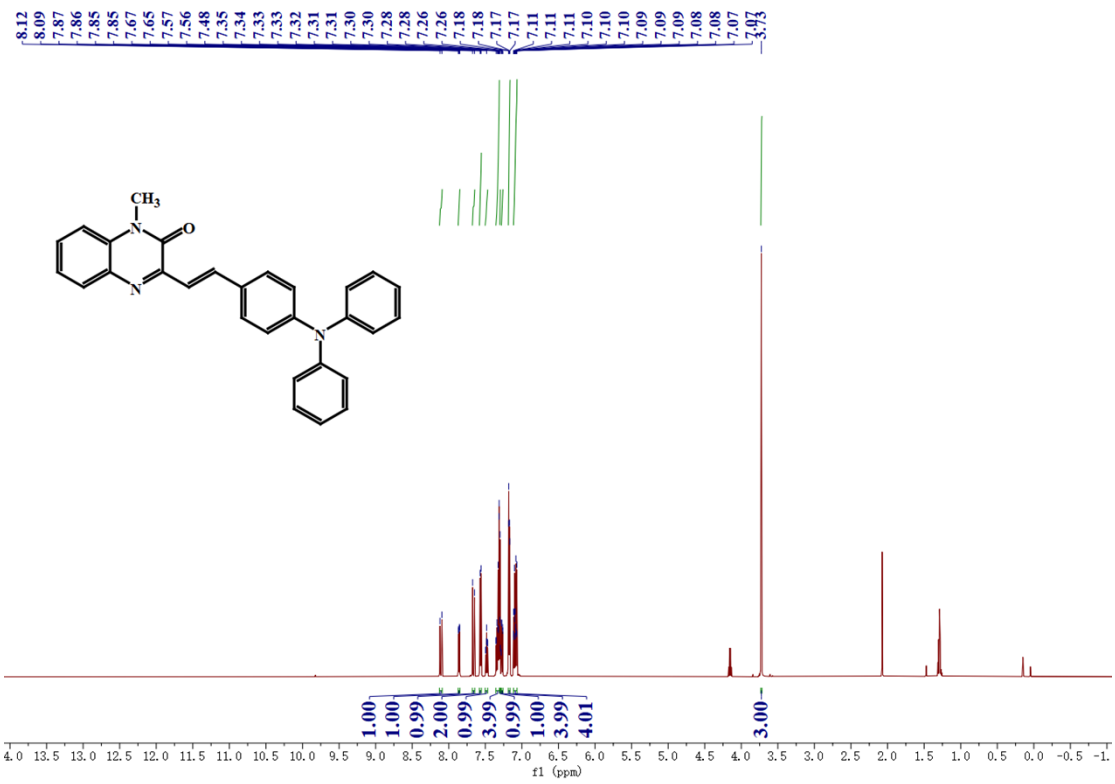


Fig. S15. [1]H NMR spectrum of compound **TPALD**.

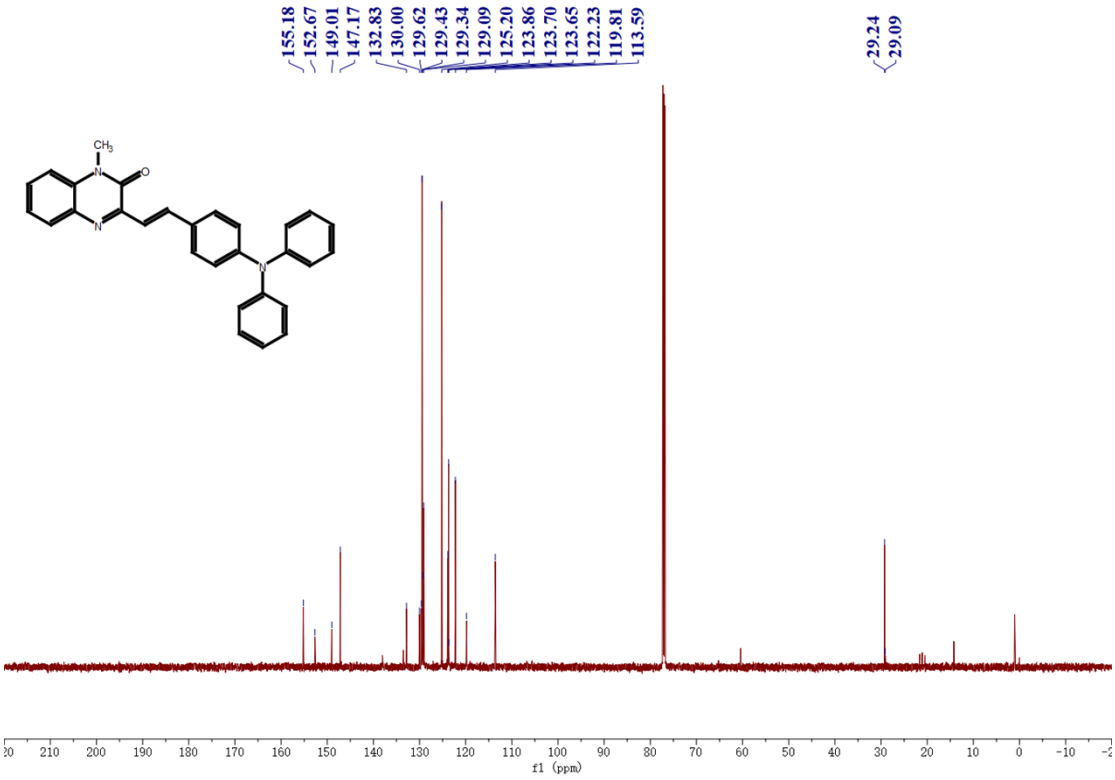


Fig. S16. ^{13}C NMR spectrum of compound **TPALD**.

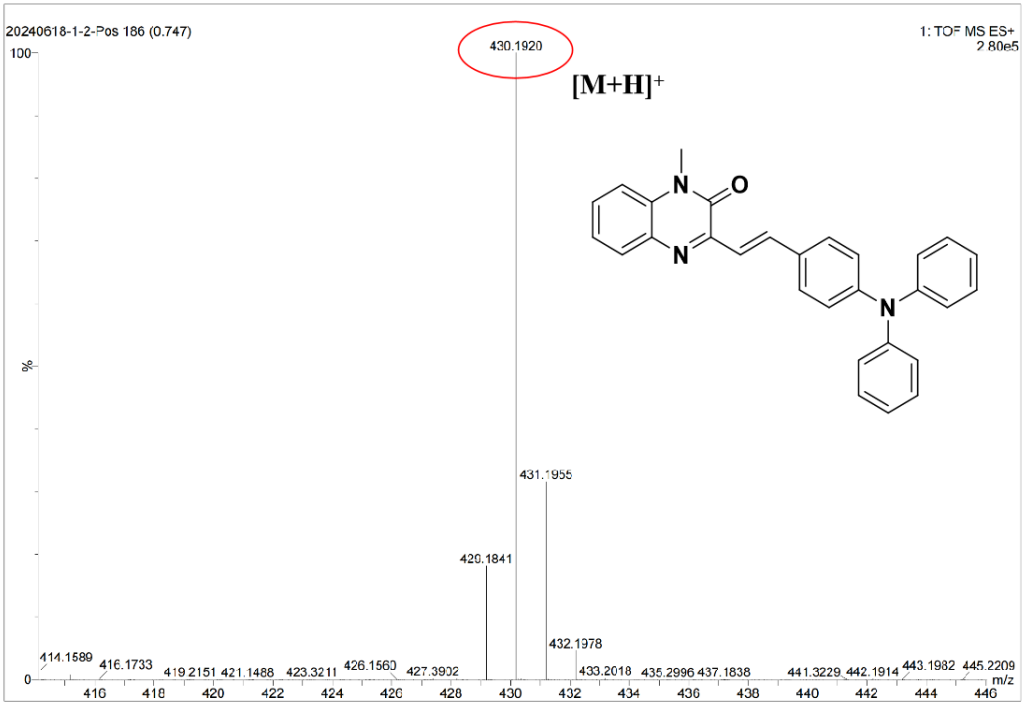


Fig. S17. HRMS spectrum of compound **TPALD**.