Supporting Information

Migratory fluorescent probe for monitoring dynamic changes in lysosomal pH during apoptosis

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Experimental

1. Experimental instruments and reagents

The reagents used in this experiment were commercially available and had not been subjected to any further purification. 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). The UV-Vis spectra were obtained by a UV-2700 spectrophotometer (Shimadzu, Japan). In addition, all fluorescent spectra were tested at the room temperature using an F-4600 fluorescent spectrophotometer (Japan Hitachi). HepG2(human hepatocellular carcinoma cells) were purchased from Beyotime(Gene expression databases: ArrayExpress). High resolution mass spectra were acquired on Aglient 7250& JEOL-JMS-T100LP AccuTOF (Bruker Daltonics, Billerica, MA, USA). ^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance III HD 600 MHz NMR spectrometer (United States of America). The fluorescent imaging of the cells was performed with a Leica TCS SP8 CARS confocal microscope.

2. Optical studies and analysis

A stock solution (5 mM) of probe **KY-1** was obtained by weighing 5.03 mg of **KY-1** probe solid powder and dissolving it in 2 mL dimethyl sulfoxide (DMSO) analytical solution. All spectrometric probes were used at a concentration of 10 μM.

A stock solution (5 mM) of probe **KY-OH** was obtained by weighing 5.34 mg of **KY-OH** probe solid powder and dissolving it in 2 mL N,N-Dimethylformamide (DMF) analytical solution. All spectrometric probes were used at a concentration of 10 μM.

The stock solutions (10 mM) of DL-Homocysteine, L-Cysteine, L-Alanine, L-Methionine, L-Glutamic Acid, L-Arginine, L-Ascorbic Acid, L-Lysine, L-Tyrosine, L-Proline, L-Tryptophan, L-Serine, L-Aspartic acid, L-Histidine, Dextrose, Phenylalanine, Glycine, Glutamic acid, Glutathione, $S_2O_8^{2-}$, HSO_5^-, Leucine, S^{2-}, HS^-, Fe^{2+}, K^+, Na^+, Cu^+, SO_4^{2-}, Fe^{3+}, CO_3^{2-}, F^-, $S_2O_3^{2-}$, SO_3^{2-}, HSO_3^-, HSO_4^-, CH_3COO^- were freshly prepared in ultrapure water, respectively.

3. The fluorescence quantum yield of probes

Calculation formula of fluorescence quantum yield:

$$\Phi_{sample} = \Phi_{standard}\left(\frac{A_{standard}F_{sample}}{A_{sample}F_{standard}}\right)$$

In the formula, Φ_{sample} represents the fluorescence quantum yield of the sample under test; $\Phi_{standard}$ is the fluorescence quantum yield of the standard sample; $A_{standrad}$ denotes the absorbance of the standard sample at the excitation wavelength; A_{sample} is the absorbance of the sample under test at the excitation wavelength; F_{sample} is the integral area of the fluorescence emission of the sample under test; $F_{standrad}$ represents the integral area of the fluorescence emission of the standard sample.

The fluorescence quantum yield of probe **KY-1** was obtained using Rhodamine B as the standard sample and water as the solvent. The fluorescence quantum yield of probe **KY-1** was measured in different solvents. The fluorescence quantum yield of **KY-OH** was obtained using a standard sample of quinine sulfate and a solvent of 0.1 M H_2SO_4 aqueous solution. The fluorescence quantum yield of probe **KY-OH** in different solvents was measured.

4. Cytotoxicity assay

The cytotoxicity of the probe **KY-1** to HepG2 cells was studied by standard MTT assays. About 2×10^4 cells/mL HepG2 cells were incubated with various concentrations of **KY-1** (1, 2, 3, 5, 10, 20 and 50 μM) in 96-well plates for 24 h. Subsequent work was that 10 μL MTT (5 mg/mL) was added to each well and continued to incubate for another 4 h. Then, violet formazan crystals were dissolved with DMSO (100 μL). The plate was shaken for about 10 min, and each well was analyzed by the microplate reader and detected at the absorbance of 490 nm.

$$\text{Cell viability} = \frac{OD_{sample} - OD_{blank}}{OD_{control} - OD_{blank}} \times 100\%$$

OD_{sample} was the results obtained by cell incubated with different probe concentrations; OD_{blank} was the result obtained after adding DMSO; $OD_{control}$ was the results obtained by cell incubated DMEM culture solution only.

5. Culture and preparation of HepG2 cells

HepG2 cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 °C. Before the experiments, the HepG2 cells in 35-mm glass-bottomed dishes were cultured to a density of 2×10^5 cells per dish. Cells were incubated for 24 h. Cells will attach to the glass surface during this time. When the cell density reached 80% – 90%, digested with trypsin solution, then the cells were inoculated into 20 mm

confocal imaging dishes with a density of 1×10^5 / mL and incubated for 24 h to make them wall-adherent. When the cells reached a density of about 4×10^5 /mL, the original medium in the dish were washed and new medium were replaced, imaging experiments were performed.

6. Cell fluorescent imaging

5.1. Bioimaging experiment of stimulated by Rapamycin (RAPA).

HepG2 cells were cultured as above. The cultured HepG2 cells were seeded into glass culture dishes and cultured to 4×10^5 cells / mL for fluorescent imaging. HepG2 cells were treated with fresh medium containing of Rapamycin (5 μM) for 2 h. After the Rapamycin pretreatment was completed, the medium containing Rapamycin was removed, and the cells were washed three times with PBS. Fresh medium was then added, followed by the addition of 10 μM **KY-OH** to the cell culture, which was incubated for 30 min. The cell images were obtained using the upright fluorescence microscope.

5.2. Bioimaging experiment of stimulated by Chloroquine (CQ).

HepG2 cells were cultured as above. The cultured HepG2 cells were seeded into glass culture dishes and cultured to 4×10^5 cells / mL for fluorescent imaging. HepG2 cells were treated with fresh medium containing of Chloroquine (20 μM) for 3 h. After the Chloroquine pretreatment was completed, the medium containing Chloroquine was removed, and the cells were washed three times with PBS. Fresh medium was then added, followed by the addition of 10 μM **KY-OH** to the cell culture, which was incubated for 30 min. The cell images were obtained using the upright fluorescence microscope.

5.3. Bioimaging experiment of stimulated by NH_4Cl.

HepG2 cells were cultured as above. The cultured HepG2 cells were seeded into glass culture dishes and cultured to 4×10^5 cells / mL for fluorescent imaging. HepG2 cells were treated with fresh medium containing of NH_4Cl (4 mM) for 12 h. After the NH_4Cl pretreatment was completed, the medium containing NH_4Cl was removed, and the cells were washed three times with PBS. Fresh medium was then added, followed by the addition of 10 μM **KY-OH** to the cell culture, which was incubated for 30 min. The cell images were obtained using the upright fluorescence microscope.

5.4. Bioimaging experiment of stimulated by LPS.

HepG2 cells were cultured as above. The cultured HepG2 cells were seeded into glass culture dishes and cultured to 4×10^5 cells / mL for fluorescent imaging. HepG2

cells were treated with fresh medium containing of LPS (1 mg / mL) for 1 h. After the LPS pretreatment was completed, the medium containing LPS was removed, and the cells were washed three times with PBS. Fresh medium was then added, followed by the addition of 10 μM **KY-OH** to the cell culture, which was incubated for 30 min. The cell images were obtained using the upright fluorescence microscope.

5.5. Bioimaging experiment of stimulated by LPS+ Dexamethasone.

HepG2 cells were cultured as above. The cultured HepG2 cells were seeded into glass culture dishes and cultured to 4×10^5 cells/mL for fluorescent imaging. HepG2 cells were treated with fresh medium containing of LPS (1 mg/mL) for 1 h, After the LPS pretreatment was completed, the medium containing LPS was removed, and the cells were washed three times with PBS. Fresh medium was then added, followed by the addition of 20 μM Dexamethasone for 0.5 h, After the Dexamethasone pretreatment was completed, the medium containing Dexamethasone was removed, and the cells were washed three times with PBS. Fresh medium was then added, followed by the addition of 10 μM **KY-OH** to the cell culture, which was incubated for 30 min. The cell images were obtained using the upright fluorescence microscope.

5.6. Bioimaging Experiments of H_2O_2-Induced Apoptosis

HepG2 cells were cultured as above. The cultured HepG2 cells were seeded into glass culture dishes and cultured to 4×10^5 cells/mL for fluorescent imaging. HepG2 cells were treated with fresh medium containing of H_2O_2 (15 μM) for 0.5 h, After the H_2O_2 pretreatment was completed, the medium containing H_2O_2 was removed, and the cells were washed three times with PBS. Fresh medium was then added, followed by the addition of 10 μM **KY-OH** to the cell culture, which was incubated for 30 min. The cell images were obtained using the upright fluorescence microscope.

5.7. Bioimaging Experiments of Colchicine-Induced Apoptosis

HepG2 cells were cultured as above. The cultured HepG2 cells were seeded into glass culture dishes and cultured to 4×10^5 cells/mL for fluorescent imaging. HepG2 cells were treated with fresh medium containing of Colchicine (10 μM) for 24 h, After the Colchicine pretreatment was completed, the medium containing Colchicine was removed, and the cells were washed three times with PBS. Fresh medium was then added, followed by the addition of 10 μM **KY-OH** to the cell culture, which was

incubated for 30 min. The cell images were obtained using the upright fluorescence microscope.

5.8. **Bioimaging Experiments of Rotenone-Induced Apoptosis**

HepG2 cells were cultured as above. The cultured HepG2 cells were seeded into glass culture dishes and cultured to 4×10^5 cells/mL for fluorescent imaging. HepG2 cells were treated with fresh medium containing of Rotenone (10 μM) for 24 h, After the Rotenone pretreatment was completed, the medium containing Rotenone was removed, and the cells were washed three times with PBS. Fresh medium was then added, followed by the addition of 10 μM **KY-OH** to the cell culture, which was incubated for 30 min. The cell images were obtained using the upright fluorescence microscope.

5.9. Bioimaging experiment of stimulated by CCCP (Carbonyl Cyanide3-ChloroPhenylhydrazone).

HepG2 cells were cultured as above. The cultured HepG2 cells were seeded into glass culture dishes and cultured to 4×10^5 cells / mL for fluorescent imaging. HepG2 cells were treated with fresh medium containing of CCCP (20 μM) for 0.5 h. After the CCCP pretreatment was completed, the medium containing CCCP was removed, and the cells were washed three times with PBS. Fresh medium was then added, followed by the addition of 10 μM **KY-1** to the cell culture, which was incubated for 30 min. The cell images were obtained using the upright fluorescence microscope.

5.10. Co-location experimental imaging

HepG2 cells were cultured as above. The cultured HepG2 cells were seeded into glass culture dishes and cultured to 4×10^5 cells/mL to fluorescent imaging. HepG2 cells were incubated with **KY-1** (10 μM) and Mito-Tracker Green (10 μM, a commercial dye targeting Mitochondria) for 30 min, cells were washed three times with PBS prior to imaging. The confocal fluorescent imaging was manipulated with red channel (λ_{ex} = 510 nm) for **KY-1**, green channel (λ_{ex} = 490nm) for Mito-Tracker Green. The Mitochondria localization ability of **KY-1** was analyzed by the Pearson coefficient.

HepG2 cells were cultured as above. The cultured HepG2 cells were seeded into glass culture dishes and cultured to 4×10^5 cells/mL to fluorescent imaging. HepG2 cells were incubated with **KY-OH** (10 μM) and Lyso-Tracker Green (10 μM, a commercial dye targeting Lysosomes) for 30 min, cells were washed three times with PBS prior to

imaging. The confocal fluorescent imaging was manipulated with red channel (λ_{ex} = 470 nm) for **KY-OH**, green channel (λ_{ex} = 504 nm) for Lyso-Tracker Green. The Lysosomes localization ability of **KY-OH** was analyzed by the Pearson coefficient.

5.11. Model and parameters of inverted microscope upright fluorescence microscope

Cellular imaging was performed using a Revolve FL upright fluorescence microscope (Echo Laboratories) and an RVL-100-G microscope equipped with a 40× objective lens (Discover-Echo, USA).

Channel Name	Excitation Wavelength	Emission Wavelength
DAPI	385/30	450/50
FITC	470/40	525/50
TxRed	560/40	635/60
Cy5	640/30	690/50

5.12. performing ^{1}H NMR titration experiments on the probe under different pH conditions

we conducted ^{1}H NMR experiments on the probe under different pH conditions. We used deuterated chloroform, deuterated methanol, deuterated trifluoroacetic acid, and deuterated sodium hydroxide to prepare three different pH ^{1}H NMR reagents:

From the experiment, we can see that the probe exhibits different ^{1}H NMR characteristic peaks under acidic and alkaline conditions, which are consistent with the ^{1}H NMR results predicted by our designed **KY-OH** structure under acidic and alkaline conditions, indicating that the probe exhibits different structures under acidic and alkaline conditions.

7. Synthesis of probes

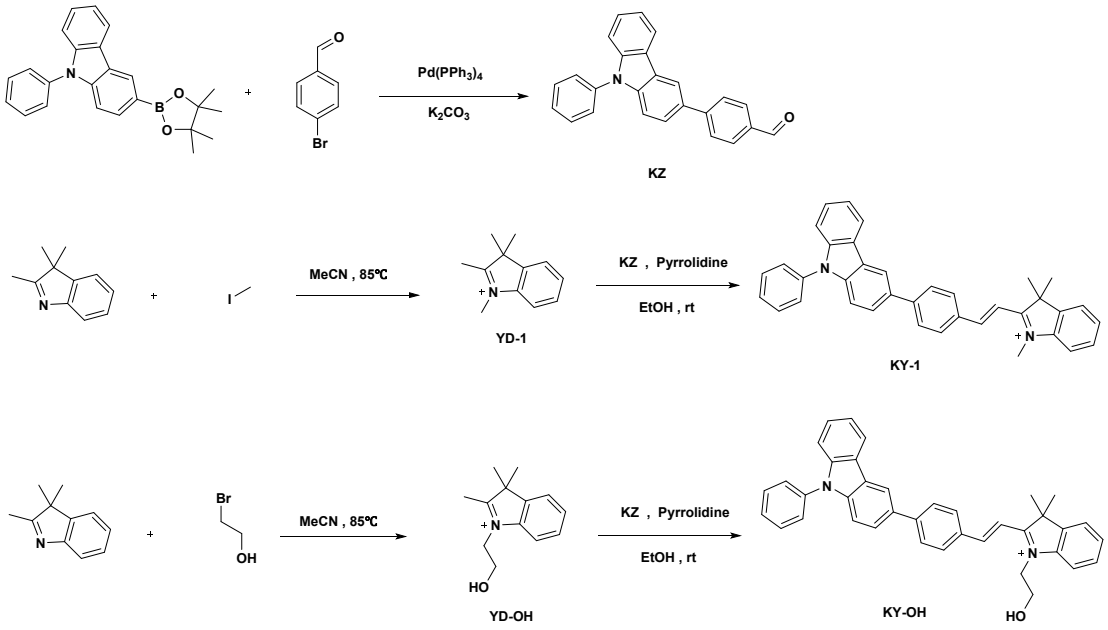

Fig. S1. Steps in the synthesis of the compound **KY-1** and **KY- OH.**

Synthesis of **YD-1**:

Dissolve the starting material 2,3,3-trimethylindolenine (1 g, 6.28 mmol) and iodomethane (1.07 g, 7.54 mmol) in acetonitrile. The mixture was refluxed and stirred at 85°C for 12 h. After completion of the reaction, the solvent was removed under reduced pressure. Diethyl ether was added, and the mixture was sonicated. The residue was filtered under vacuum and washed with diethyl ether to afford the pink solid product **YD-1** (1.8 g, 95% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.95 – 7.90 (m, 1H), 7.87 – 7.81 (m, 1H), 7.63 (tt, J = 7.4, 6.0 Hz, 2H), 3.98 (d, J = 1.4 Hz, 3H), 2.78 (d, J = 1.4 Hz, 3H), 1.54 (s, 6H).

Synthesis of **YD-OH**:

Dissolve the starting materials 2,3,3-trimethylindolenine (1 g, 6.28 mmol) and 2-bromoethanol (1 g, 7.54 mmol) in acetonitrile. The mixture was refluxed and stirred at 85°C for 12 h. After completion of the reaction, the solvent was removed under reduced pressure. Diethyl ether was added, and the mixture was sonicated. The solid was filtered under vacuum and washed with diethyl ether to afford the pink solid product **YD-OH** (1.7 g, 95% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.01 – 7.96 (m, 1H), 7.89 – 7.84

(m, 1H), 7.66 – 7.60 (m, 2H), 4.61 (t, J = 5.1 Hz, 2H), 3.89 (dd, J = 5.8, 4.5 Hz, 2H), 2.84 (s, 3H), 1.56 (s, 6H).

Synthesis of **KZ**:

React the starting material 9-Phenyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9H-carbazole (2 g, 5.38 mmol), 4-Bromobenzaldehyde (1 g, 5.38 mmol), $Pd(PPh_3)_4$ (0.062 g, 0.054 mmol), and K_2CO_3 (1.2 g, 8.08 mmol) in a solvent prepared by mixing tetrahydrofuran: deionized water = 3:1. After nitrogen displacement, the mixture was refluxed and stirred at 70°C for 12 h. Upon completion, the solvent was removed under reduced pressure. The crude product was purified by column chromatography (DCM:PE = 2:1), yielding 1.8 g of the white solid product **KZ** in 95% yield. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 10.10 (s, 1H), 8.45 (dd, *J* = 1.9, 0.7 Hz, 1H), 8.24 (dt, *J* = 7.8, 1.0 Hz, 1H), 8.03 – 8.00 (m, 2H), 7.93 – 7.90 (m, 2H), 7.73 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.66 (tt, *J* = 7.7, 1.9 Hz, 2H), 7.63 – 7.61 (m, 2H), 7.55 – 7.50 (m, 2H), 7.50 – 7.45 (m, 2H), 7.36 (ddd, *J* = 7.9, 6.3, 1.7 Hz, 1H).

8. Supplementary Figure and Table

Table S1 Fluorescence quantum yield of KY-1 in different solvents.

Solvent	$A_{standrad}$	A_{sample}	$F_{standrad}$	F_{sample}	$\Phi_{standard}$	Φ_{sample}
DMF	0.049	0.05	428152.2	10111.92	0.31	0.007175
DMSO	0.049	0.05	42800.58	11802.29	0.31	0.083773
H_2O	0.048	0.049	428468.8	9358.371	0.31	0.006633
EtOH	0.05	0.049	426562	10741.99	0.31	0.007966
1.4-Dioxane	0.049	0.049	426143	11090.48	0.31	0.008068
MeOH	0.049	0.049	425315.3	12468.2	0.31	0.009088
MePh	0.05	0.05	422466	9480.078	0.31	0.006956
THF	0.049	0.05	420211.1	9230.746	0.31	0.006674
Glycerol	0.049	0.047	417857.7	50200.24	0.31	0.038827

Table S2 Fluorescence quantum yield of KY-OH in different solvents.

Solvent	$A_{standrad}$	A_{sample}	$F_{standrad}$	F_{sample}	$\Phi_{standard}$	Φ_{sample}
DMF	0.05	0.049	34421.12	25209.59	0.58	0.433454
DMSO	0.049	0.05	34890.59	26541.36	0.58	0.432383
H_2O	0.05	0.048	35316.15	10713.88	0.58	0.183286
EtOH	0.05	0.05	35802.58	22077.25	0.58	0.35765
1.4-Dioxane	0.049	0.048	36229.22	22830.41	0.58	0.373111
MeOH	0.05	0.05	36638.88	20176.26	0.58	0.319394
MePh	0.05	0.05	36890.43	11668.96	0.58	0.183462
THF	0.049	0.05	37115.81	21005.74	0.58	0.321687
Glycerol	0.05	0.05	37365.54	9864.32	0.58	0.153117

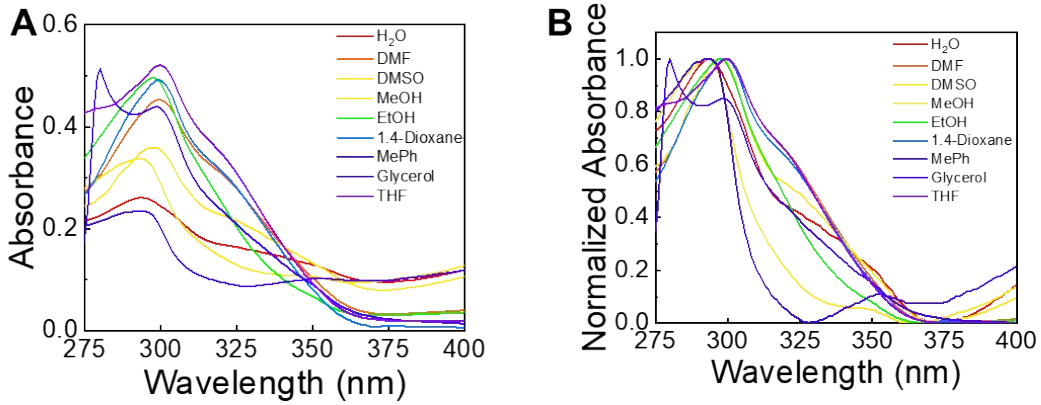

Fig. S2. (A) Ultraviolet absorption spectra of probe **KY-1** in different solvents; (B) normalized ultraviolet absorption spectra.

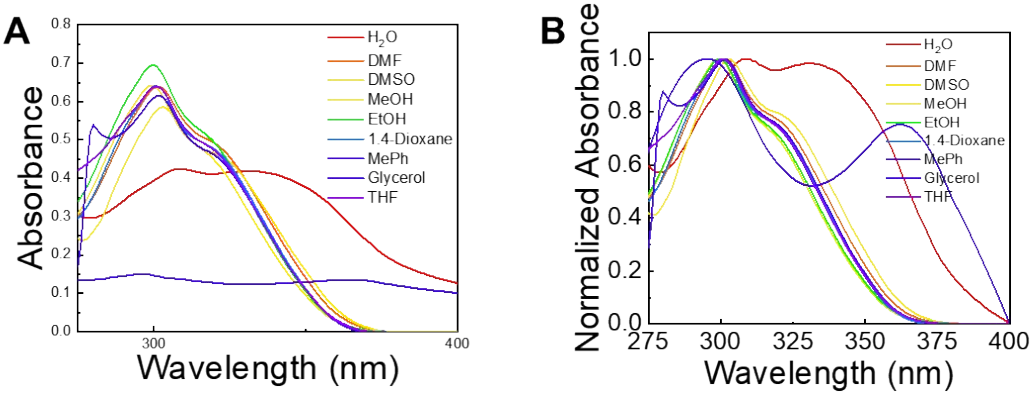

Fig. S3. (A) Ultraviolet absorption spectra of probe **KY-OH** in different solvents; (B). normalized ultraviolet absorption spectra.

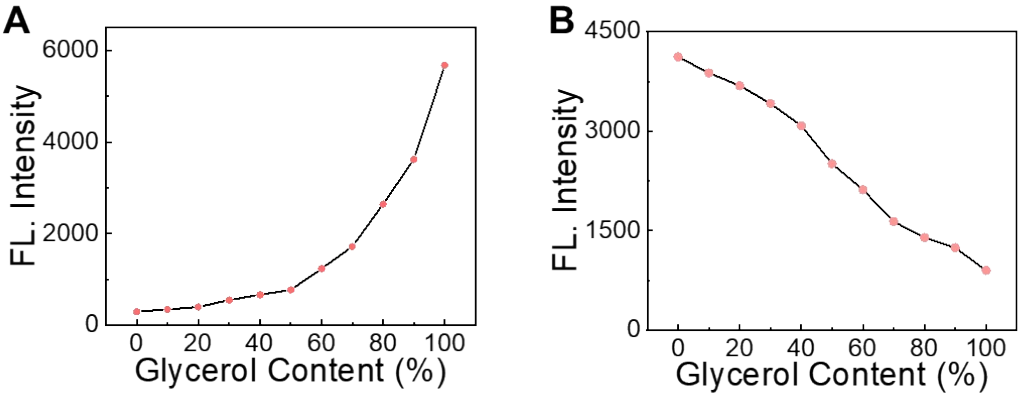

Fig. S4. (A) Fluorescence emission intensity of probe **KY-1** at λ= 580 nm under different viscosity conditions; (B) Fluorescence emission intensity of probe **KY-OH** at λ= 398 nm under different viscosity conditions.

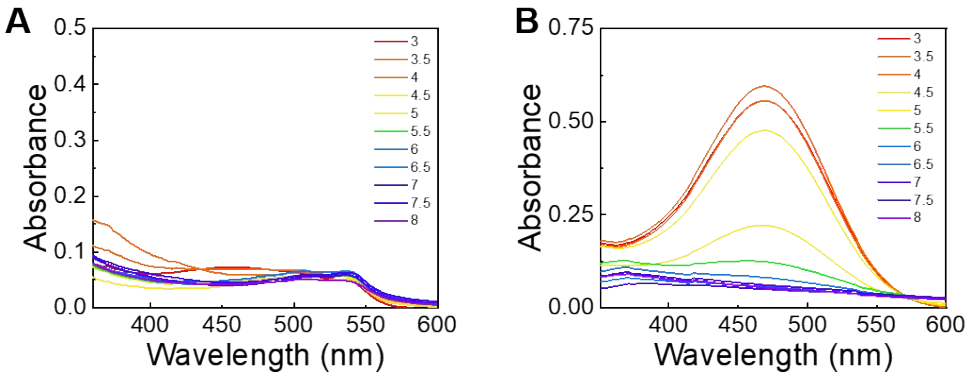

Fig. S5. (A) Ultraviolet absorption spectra of probe **KY-1** in different pH buffers; (B) Ultraviolet absorption spectra of probe **KY-OH** in different pH buffers.

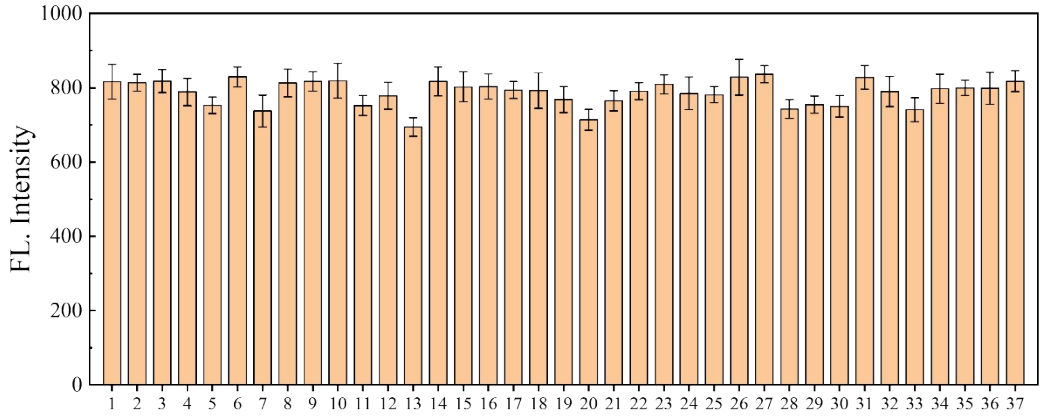

Fig. S6. Fluorescence intensity of **KY-1** (10 μM) at 564 nm under the influence of different substances (10 μM); 1. DL-Homocysteine, 2. L-Cysteine, 3. L-Alanine, 4. L-Methionine, 5. L-Glutamic Acid, 6. L-Arginine, 7. L-Ascorbic Acid, 8. L-Lysine, 9. L-Tyrosine, 10. L-Proline, 11. L-Tryptophan, 12. L-Serine, 13. L-Aspartic acid, 14. L-Histidine, 15. Dextrose, 16. Phenylalanine, 17. Glycine, 18. Glutamic acid, 19. Glutathione, 20. $S_2O_8^{2-}$, 21. HSO_5^-, 22. Leucine, 23. S^{2-}, 24. HS^-, 25. Fe^{2+} 26. K^+, 27. Na^+, 28. Cu^+, 29. Fe^{2+}, 30. Fe^{3+}, 31. CO_3^{2-}, 32. F^-, 33. $S_2O_3^{2-}$, 34. SO_3^{2-}, 35. HSO_3^-, 36. HSO_4^- ,37. CH_3COO^-.

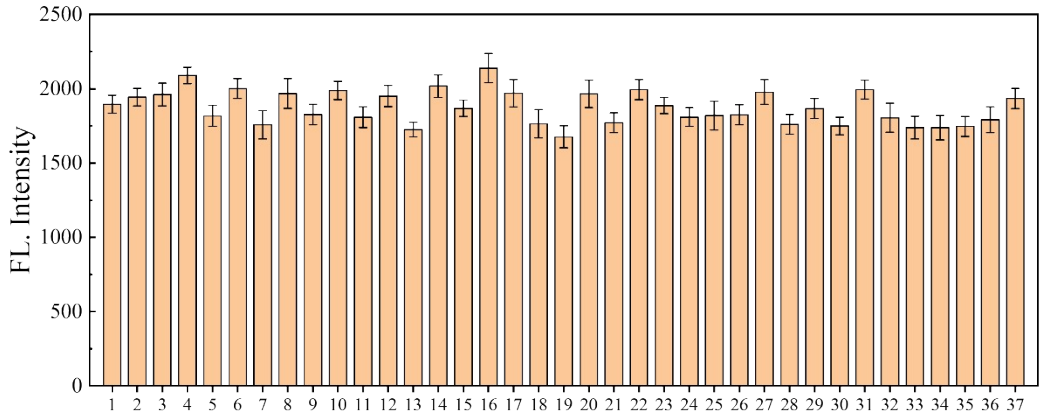

Fig. S7. Fluorescence intensity of **KY-OH** (10 μM) at 412 nm under the influence of different substances (10 μM); 1. DL-Homocysteine, 2. L-Cysteine, 3. L-Alanine, 4. L-Methionine, 5. L-Glutamic Acid, 6. L-Arginine, 7. L-Ascorbic Acid, 8. L-Lysine, 9. L-Tyrosine, 10. L-Proline, 11. L-Tryptophan, 12. L-Serine, 13. L-Aspartic acid, 14. L-Histidine, 15. Dextrose, 16. Phenylalanine, 17. Glycine, 18. Glutamic acid, 19. Glutathione, 20. $S_2O_8^{2-}$, 21. HSO_5^-, 22. Leucine, 23. S^{2-}, 24. HS^-, 25. Fe^{2+} 26. K^+, 27. Na^+, 28. Cu^+, 29. Fe^{2+}, 30. Fe^{3+}, 31. CO_3^{2-}, 32. F^-, 33. $S_2O_3^{2-}$, 34. SO_3^{2-}, 35. HSO_3^-, 36. HSO_4^- ,37. CH_3COO^-.

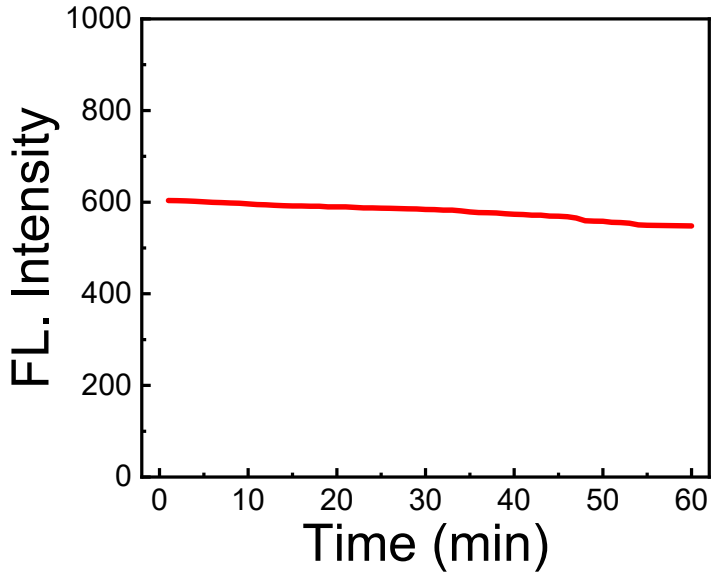

Fig. S8. Photostability test of probe **KY-OH** (10 μM) involved continuous kinetic scanning to measure changes in fluorescence intensity at 410 nm (λ_{ex}=340 nm) in aqueous solution.

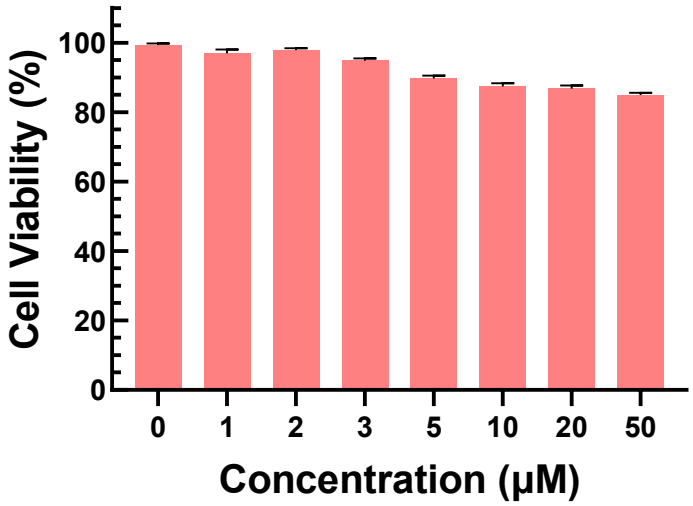

Fig. S9. Cytotoxicity assays conducted via the MTT method demonstrated that HepG2 cells treated with **KY-1** at various concentrations (1 – 50 μM) for 24 h.

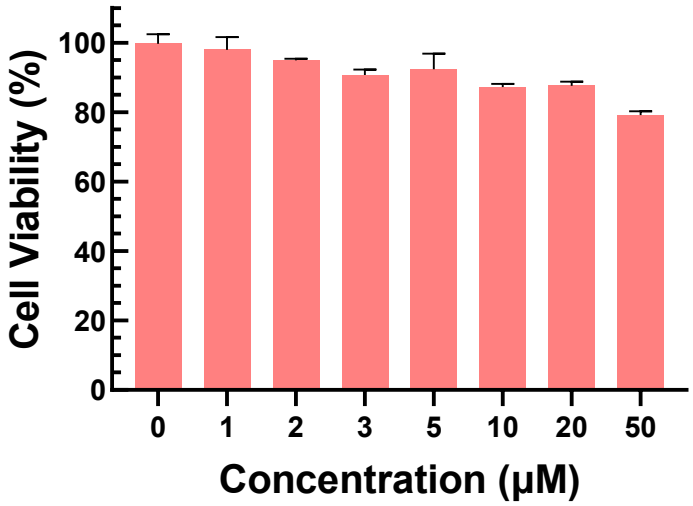

Fig. S10. Cytotoxicity assays conducted via the MTT method demonstrated that HepG2 cells treated with **KY-OH** at various concentrations (1 – 50 μM) for 24 h.

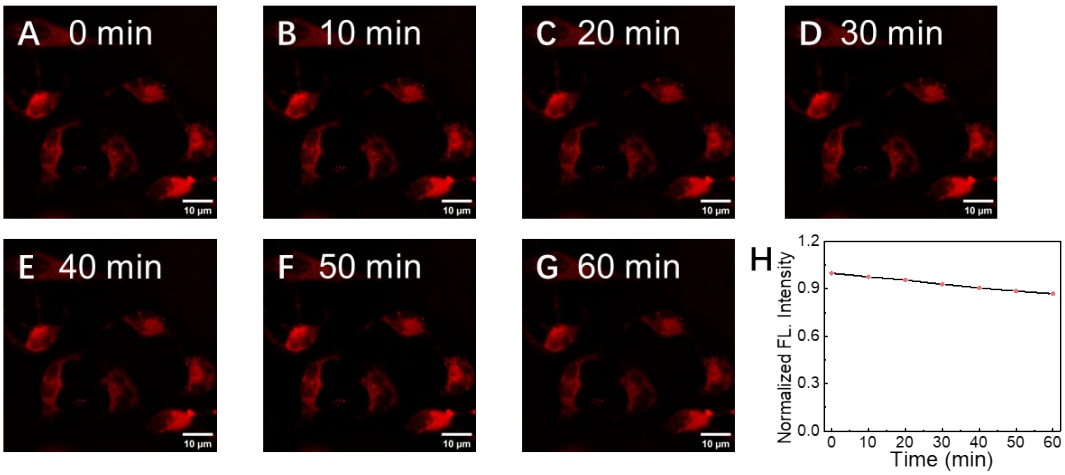

Fig. S11. (A-G) Time-lapse imaging of **KY-OH** in cells; (H). Fluorescence intensity analysis curve in cell imaging images. Scale bar: 10 μm..

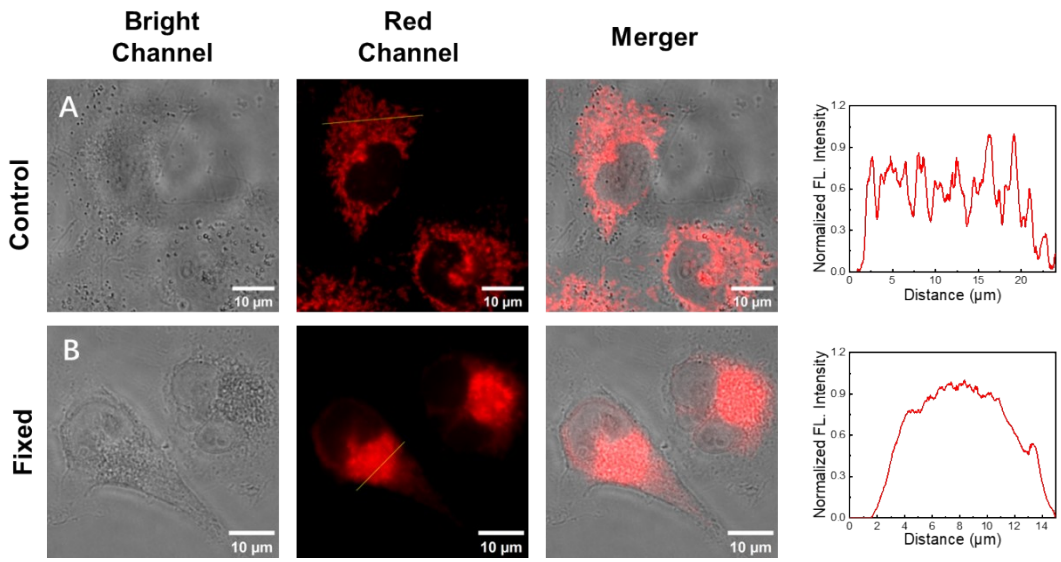

Fig. S12. (A)HepG2 cells stained with 10 μM **KY-1** for 30 min and imaged using an upright fluorescence microscope; (B) HepG2 cells stained with 10 μM **KY-1** for 30 min, fixed with 4% paraformaldehyde, and imaged using an upright fluorescence microscope. Scale bar: 10 μm.

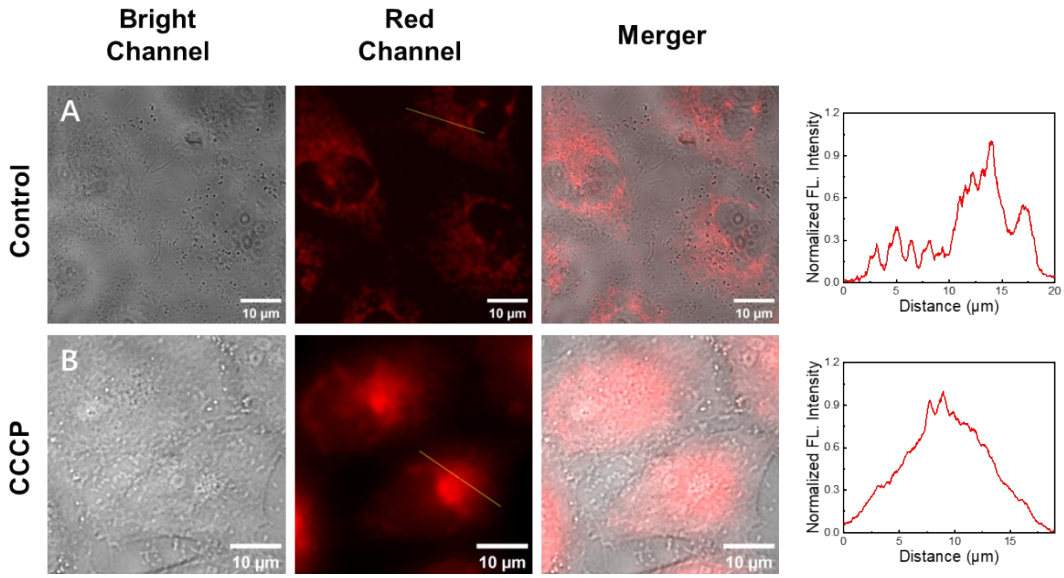

Fig. S13. (A) HepG2 cells were stained with 10 μM **KY-1** for 30 minutes and imaged using an upright fluorescence microscope; (B) HepG2 cells treated with 20 μM CCCP (Carbonyl Cyanide 3-ChloroPhenylhydrazone) for 30 min, followed by staining with 10 μM **KY-1** for 30 min, were imaged using an upright fluorescence microscope. Scale bar: 10 μm.

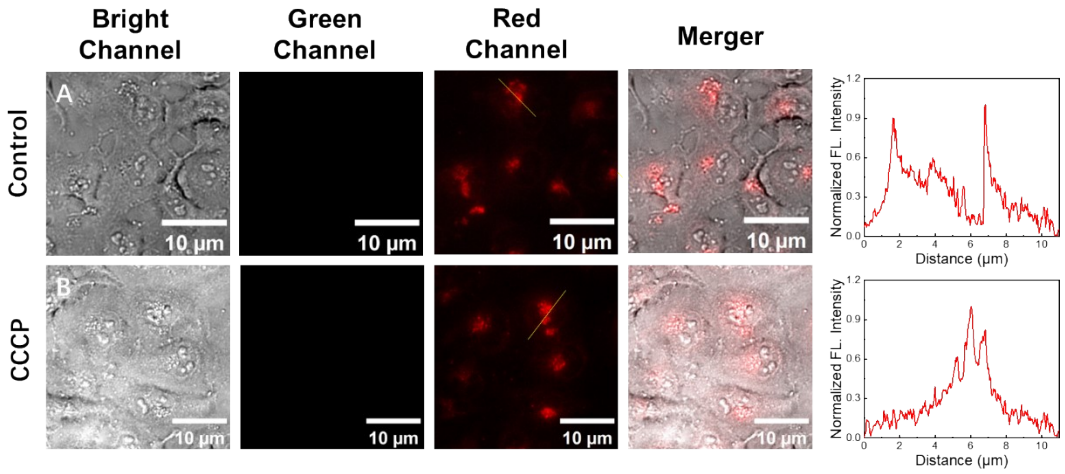

Fig. S14. (A) Imaging images and fluorescence intensity analysis curves of HepG2 cells after incubation with 10 μM acidified **KY-OH** for 30 min. (B) Imaging images and fluorescence intensity analysis curves of HepG2 cells after pre-incubation with CCCP for 30 min, followed by incubation with 10 μM acidified **KY-OH** for 30 min. Scale bar: 20 μm.

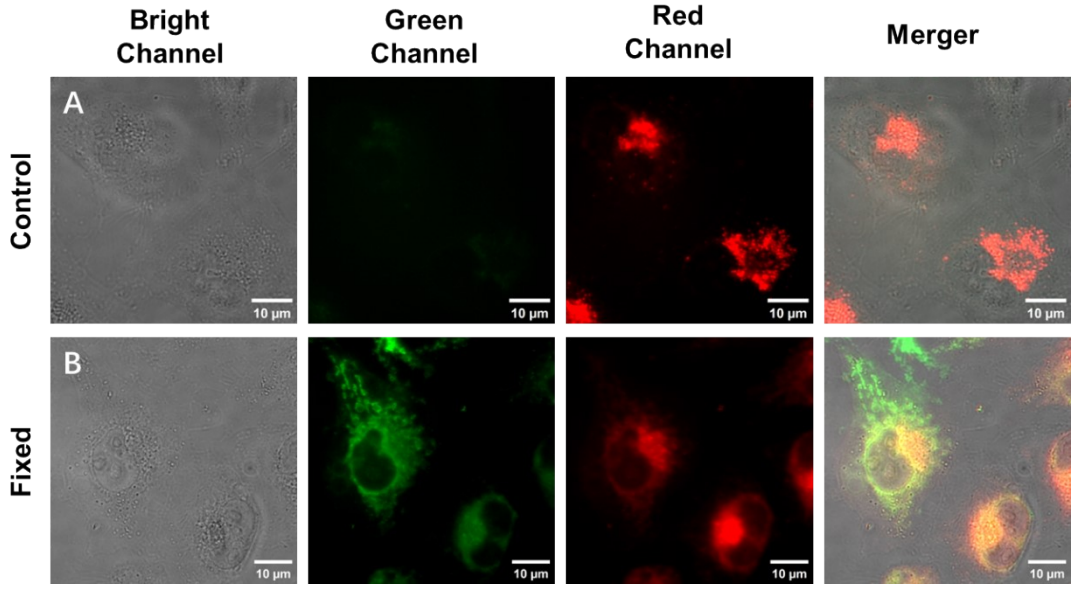

Fig. S15. (A) HepG2 cells stained with 10 μM **KY-OH** for 30 min and imaged using an upright fluorescence microscope; (B) HepG2 cells stained with 10 μM **KY-OH** for 30 min, fixed with 4% paraformaldehyde, and imaged using an upright fluorescence microscope. Scale bar: 10 μm.

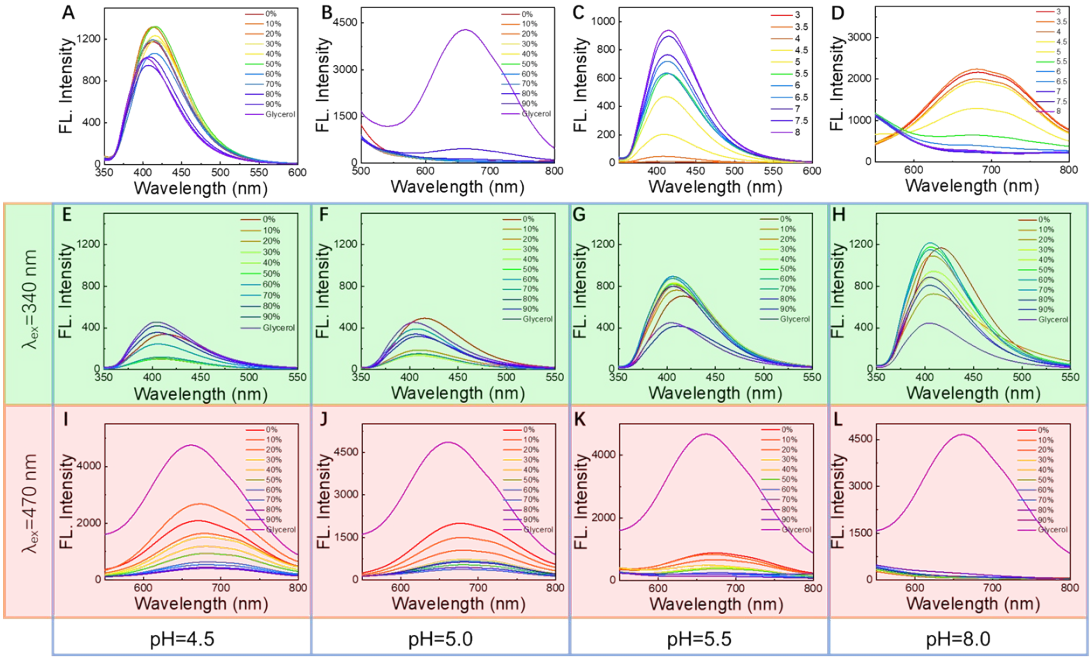

Fig. S16. (A-B) Fluorescence spectra of 10 μM **KY-OH** at different viscosities (PBS: Glycerol) at λ_{ex} = 340 nm and λ_{ex} = 470 nm, respectively; (C-D) Fluorescence spectra of 10 μM **KY-OH** at different pH conditions with λ_{ex} = 340 nm and λ_{ex} = 470 nm, respectively; (E-H) Fluorescence spectra of 10 μM **KY-OH** at λ_{ex} = 340 nm under different pH and viscosity conditions; (I-L) Fluorescence spectra of 10 μM **KY-OH** at λ_{ex} = 470 nm under different pH and viscosity conditions.

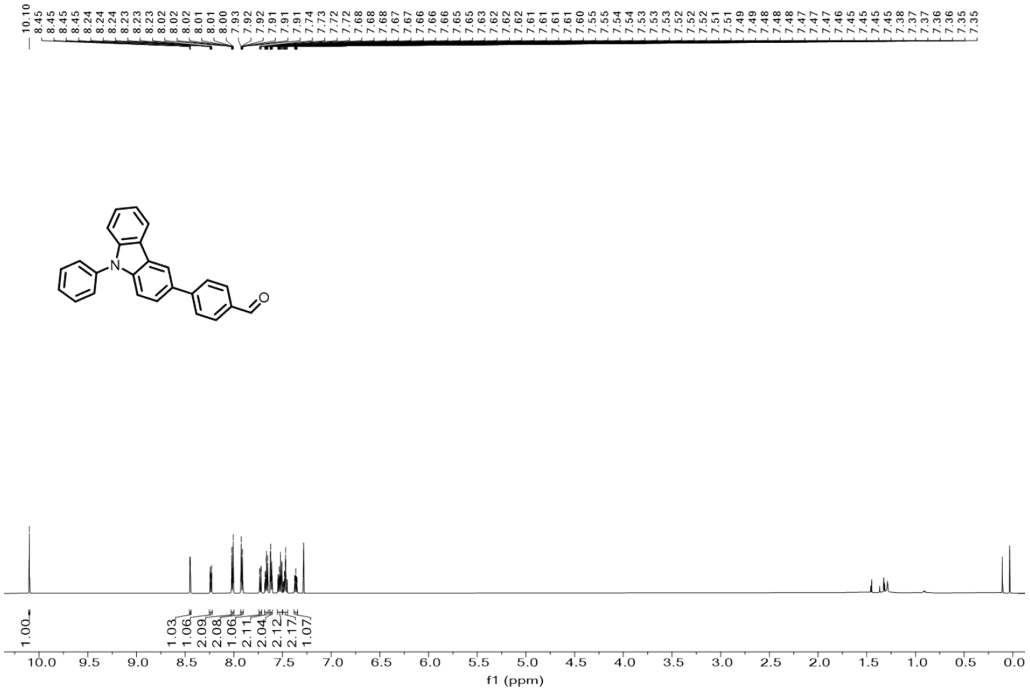

Fig. S17. The ^{1}H NMR spectrum of compound **KZ** in Chloroform-*d*.

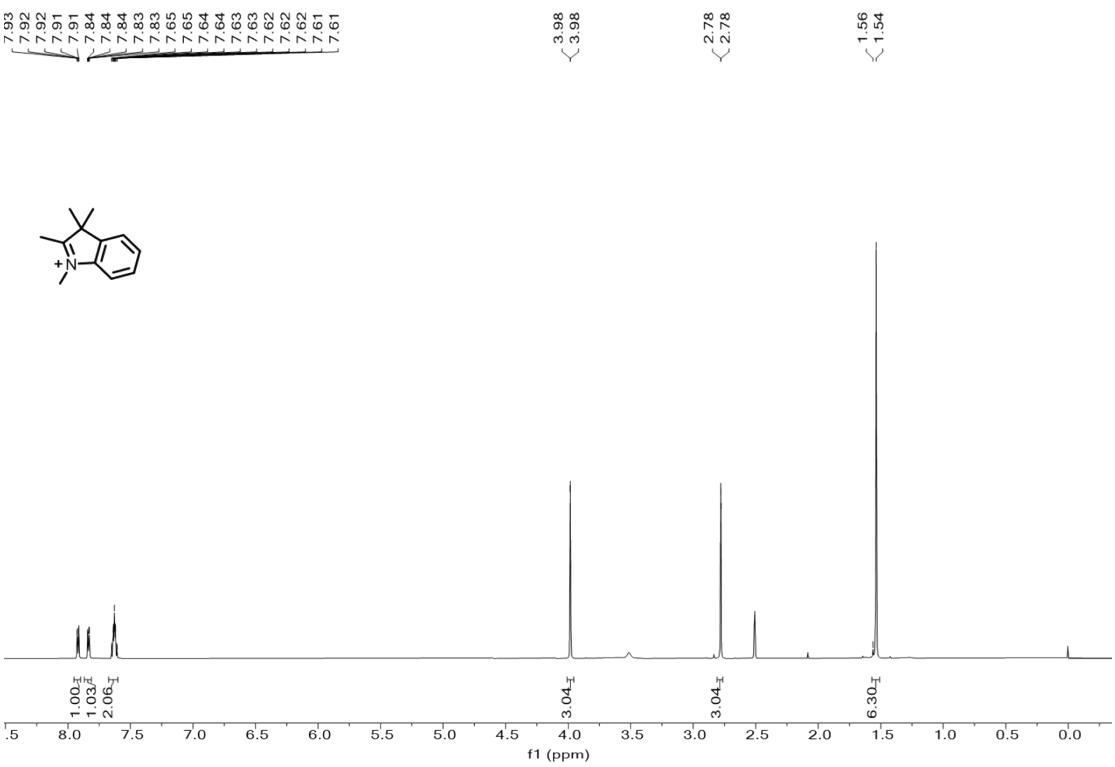

Fig. S18. The ^{1}H NMR spectrum of compound **YD-1** in DMSO-*d6*

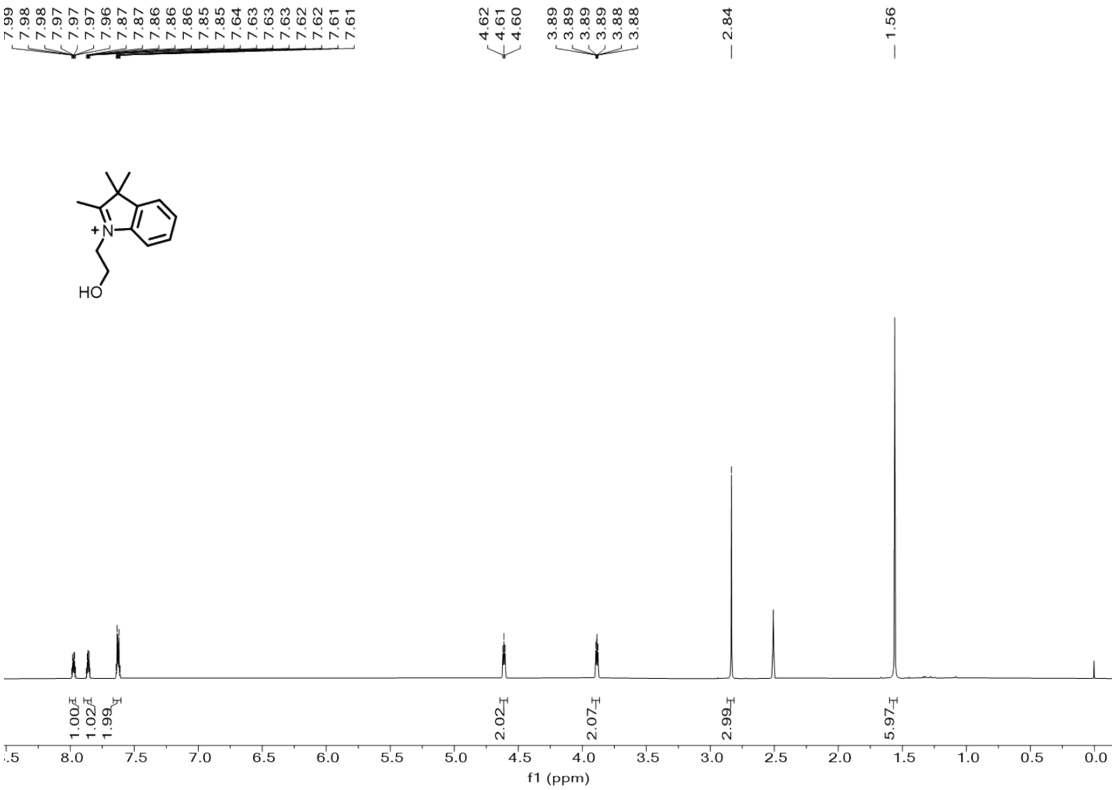

Fig. S19. The ^{1}H NMR spectrum of compound **YD-OH** in DMSO-*d6*

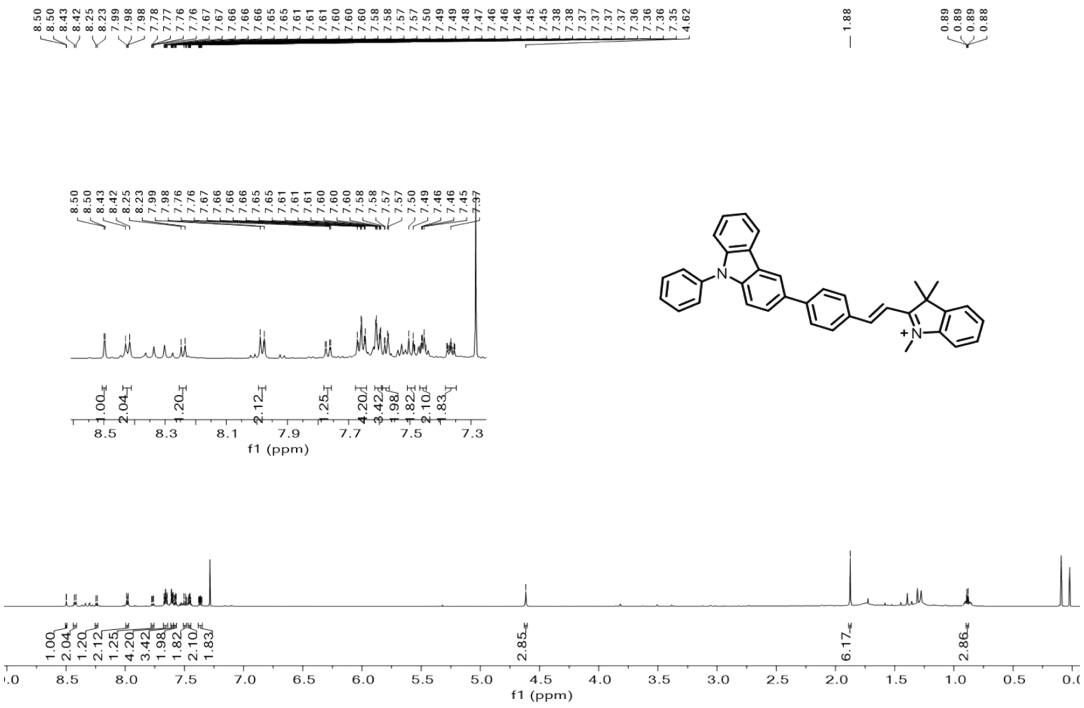

Fig. S20. The ^{1}H NMR spectrum of compound **KY-1** in Chloroform-*d*.

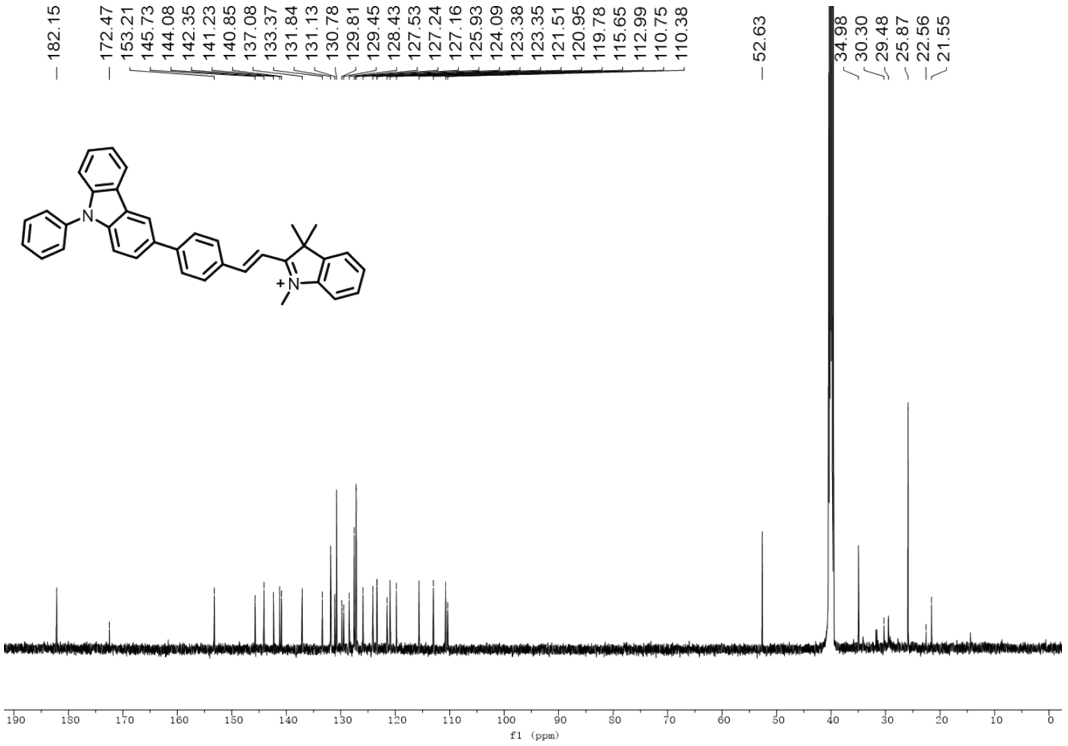

Fig. S21. The ^{13}C NMR spectrum of compound **KY-1** in Chloroform-*d*.

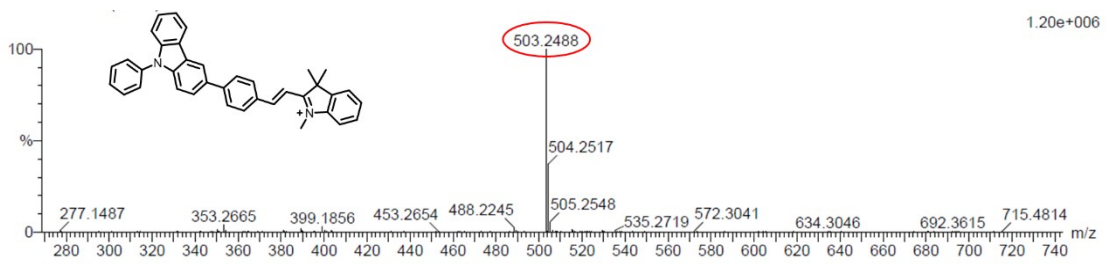

Fig. S22. HRMS spectrum of compound **KY-1**.

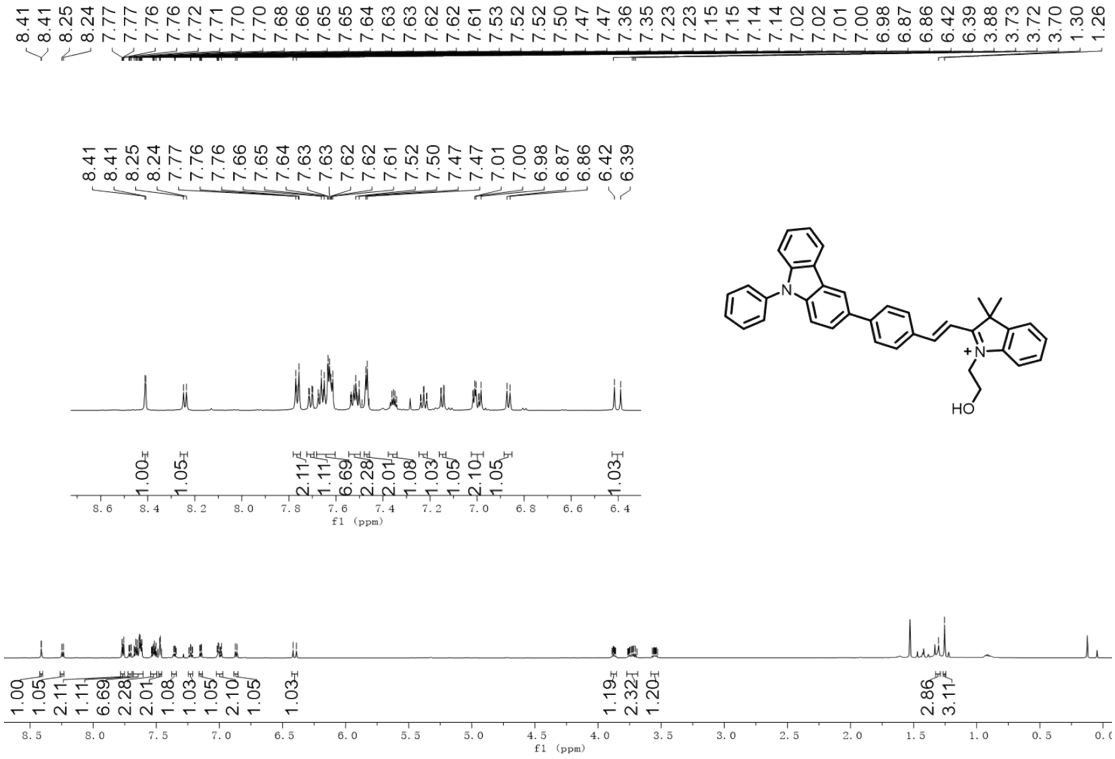

Fig. S23. The ^{1}H NMR spectrum of compound **KY-OH** in Chloroform-*d*.

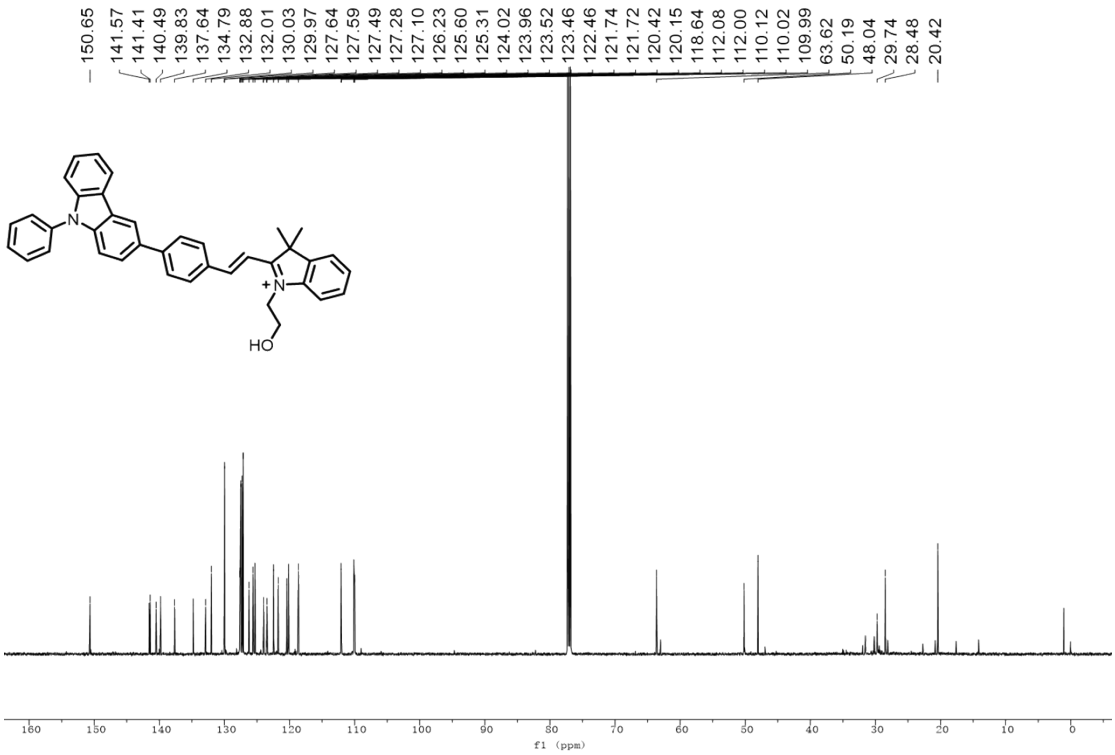

Fig. S24. The ^{13}C NMR spectrum of compound **KY-OH** in Chloroform-d.

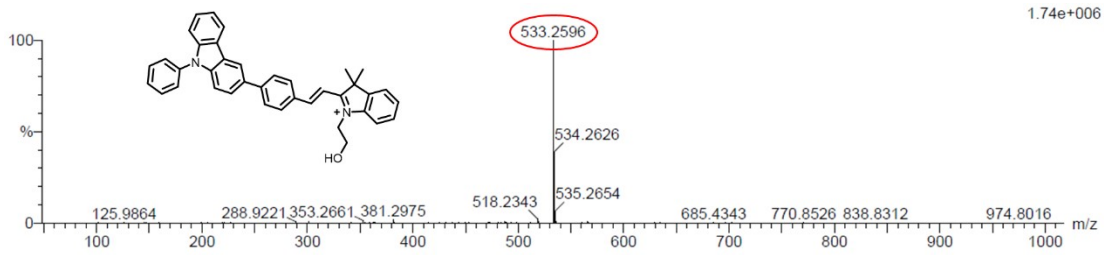

Fig. S25. HRMS spectrum of compound **KY-OH**.

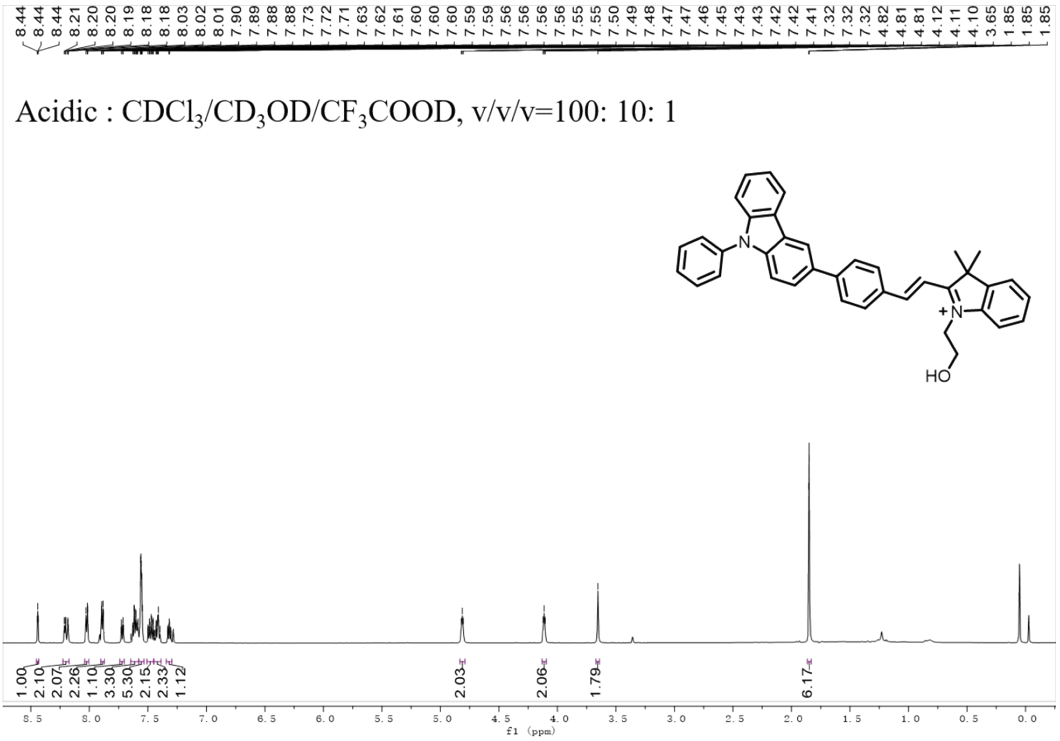

Fig. S26. The ^{1}H NMR spectrum of compound **KY-OH**. ($CDCl_3/CD_3OD/CF_3COOD$, v/v/v=100: 10: 1)

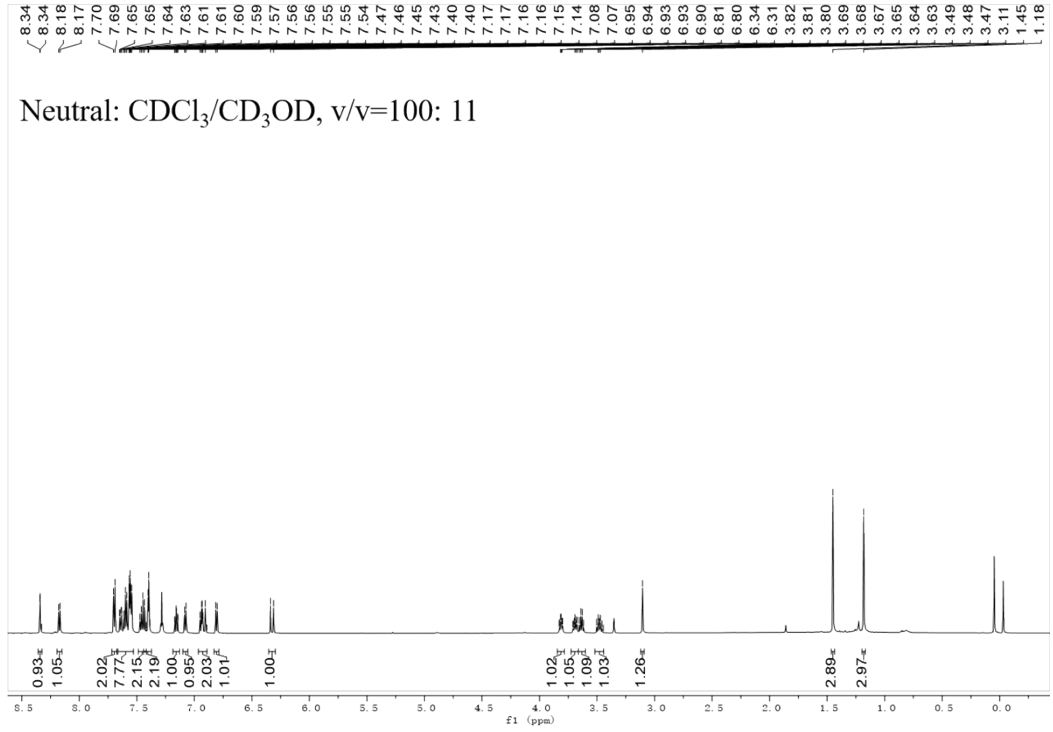

Fig. S27. The ^{1}H NMR spectrum of compound **KY-OH**. ($CDCl_3/CD_3OD$, v/v=100: 11)

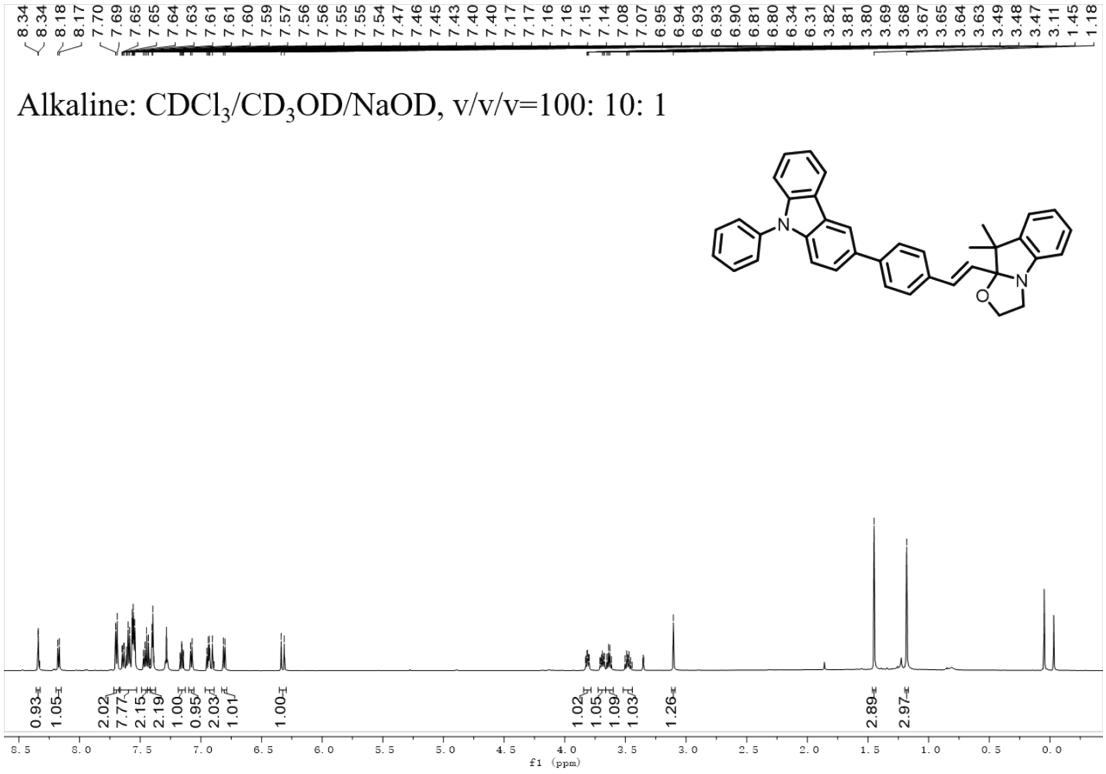

Fig. S28. The ^{1}H NMR spectrum of compound **KY-OH**. ($CDCl_3/CD_3OD/NaOD$, v/v/v=100: 10: 1)

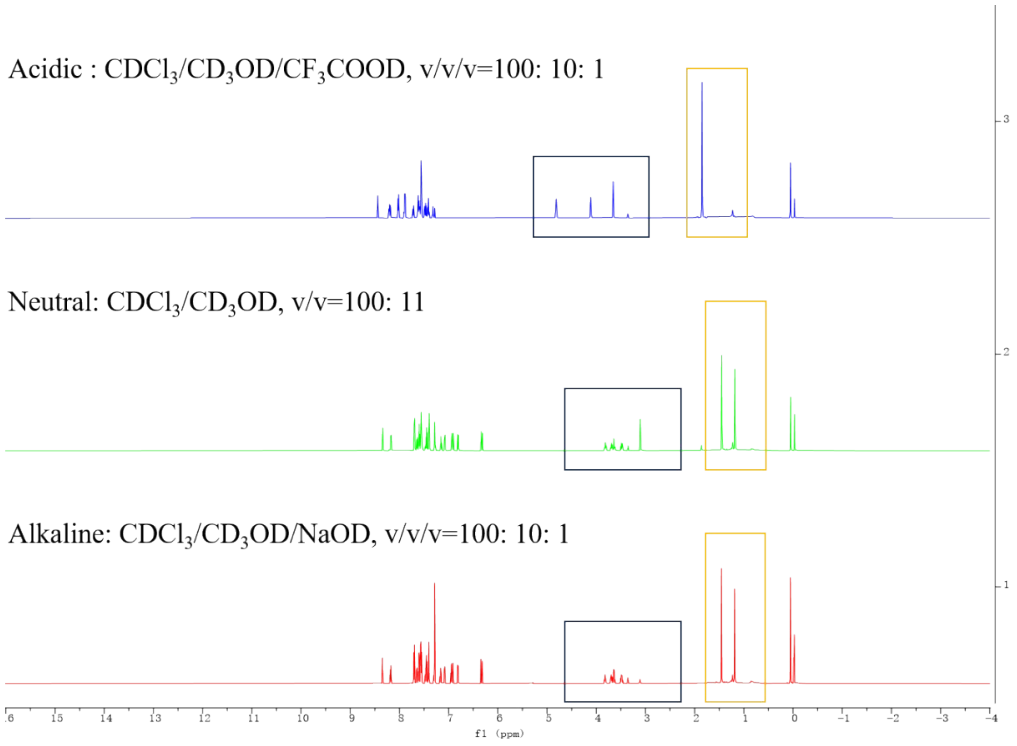

Fig. S29. Comparison of ^{1}H NMR spectra of compound KY-OH under different pH conditions.