Supplementary Information

Pillararene-Based Rare-Earth Luminescent Probe for Two-Channel and Simultaneous Detection of ROS and ATP in Living Cells

Shanshan Bao, †[a] Yanan Guo, †[a] Dandan Lv,[a] Congcong Wang,[a] Yao Kou,[a] Yuchen Yang,[a] Nan Song,*[a] and Yu Tang*[a,b]

[a]*State Key Laboratory of Applied Organic Chemistry, Key Laboratory of Nonferrous Metal Chemistry and Resources Utilization of Gansu Province, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou, 730000, P. R. China.*

[b]*State Key Laboratory of Baiyunobo Rare Earth Resource Researches and Comprehensive Utilization, Baotou Research Institute of Rare Earths, Baotou, 014030, P. R. China. Emails: tangyu@lzu.edu.cn; songn@lzu.edu.cn*

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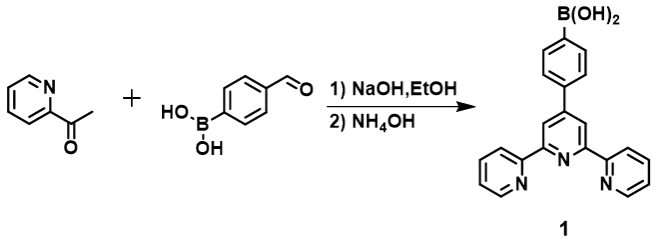
1. Experimental Section

1.1 Materials and Instruments

4-formylphenylboronic acid, 2-acetylpyridine, 2-thiophenoyltrifluoroacetone, europium nitrate hexahydrate were purchased from Heowns Biochem Technologies, LLC, Tianjin, China. Carbazole, 2-dimethylaminochloroethane hydrochloride, sodium hydride, acetyl chloride were purchased from Beijing InnoChem Science & Technology Co., Ltd. Ethyl trifluoroacetate, 1,4-dimethoxybenzene, polyformaldehyde, boron trifluoride ethyl ether, boron Tribromide, potassium iodide, ethyl bromoacetate were obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. Anhydrous aluminium chloride and hydrogen peroxide (30% H_2O_2) were purchased from Damao Chemical Reagent Factory. Iodomethane were purchased from Energy Chemical. 1,10-Phenanthroline were purchased from J&K Scientific. Adenosine 5'-triphosphate disodium salt were purchased from Bide Pharmatech Co., Ltd. All biological reagents were purchased from Beijing Solarbio Science&Technology Co., Ltd. Hela cells and RAW264.7 cells were obtained from Gansu University Of Chinese Medicine. The other reagents used in this work were purchased from the supplier, and the water used in the experiment was ultrapure water.

^{1}H NMR spectra were obtained at 400 MHz using Bruker AVANCE NEO 400 and JEOL JNM-ECS 400M NMR spectrometers, and ^{13}C NMR spectra were recorded at 100 MHz. The mass spectra of compound were recorded on a time of flight mass spectrometer and the complexes were obtained from the Bruker Maxis 4G ultra-high-resolution MS system. Absorption spectra were recorded on a UV-Visible spectrophotometer (Cary 5000, Agilent). Fourier Transform infrared spectra (FTIR) were obtained from an infrared spectrometer (Nicolet iS5, Thermo scientific). Isothermal Titration Calorimetry (ITC) data were recorded on an isothermal titration calorimeter (iTC200, GE). Fluorescence spectra were obtained with a Horiba FL3 fluorescence spectrophotometer. All microstructure were characterized with field emission scanning electron microscope (SEM) and Transmission Electron Microscope (TEM). Zeta-potential were recorded on Brookhaven 90Plus PALS. CCK-8 assay was performed using a microplate reader (Multiskan Sky, Thermo scientific). Confocal imaging was performed on Nikon A1 RMP + Ti2-E high-resolution fluorescence microscope.

1.2 Synthesis



Scheme S1 Synthetic route of TPBA[1]

Synthesis of compound 1 (TPBA)

NaOH (9.6 g, 240 mmol) and ethanol (200 mL) were added to a 500 mL round-bottomed flask and dissolved by ultrasonication, followed by the addition of 4-formylphenylboronic acid (6.0 g, 40 mmol) and 2-acetylpyridine (10.6 g, 88 mmol). The mixture was stirred at 25 ℃ for 24 h. Then, 150 mL ammonia (28%) were added to the flask and reacted at 95 ℃ for 20 h. At the end of the reaction, the solution was cooled down to room temperature, and the precipitate was collected by centrifugation at 10000 rpm, 4 min. Finally, it was washed with isopropanol five times and $CHCl_3$ twice. The resulting precipitate was dried under vacuum at 40 ℃ overnight to obtain a light purple powdery solid (10.61 g, 75.1% yield). ^{1}H NMR (400 MHz, CD_3OD): δ 8.70 (ddd, J = 4.9, 1.7, 0.8 Hz, 2H), 8.67 (s, 2H), 8.65 (dd, J = 8.0, 0.9 Hz, 2H), 8.01 (td, J = 7.8, 1.8 Hz, 2H), 7.75 (q, J = 8.2 Hz, 4H), 7.48 (ddd, J = 7.5, 4.9, 1.2 Hz, 2H). ^{13}C NMR (101 MHz, CD_3OD): δ 157.58 (s), 156.96 (s), 153.23 (s), 150.03 (s), 138.77 (s), 135.32 (d, J = 30.1 Hz), 135.12-134.68 (m), 125.97 (s), 125.30 (s), 123.02 (s), 119.56 (s). ESI-MS calculated MS: M= 353.13, found m/z: $[M+H]^+$= 354.2283.

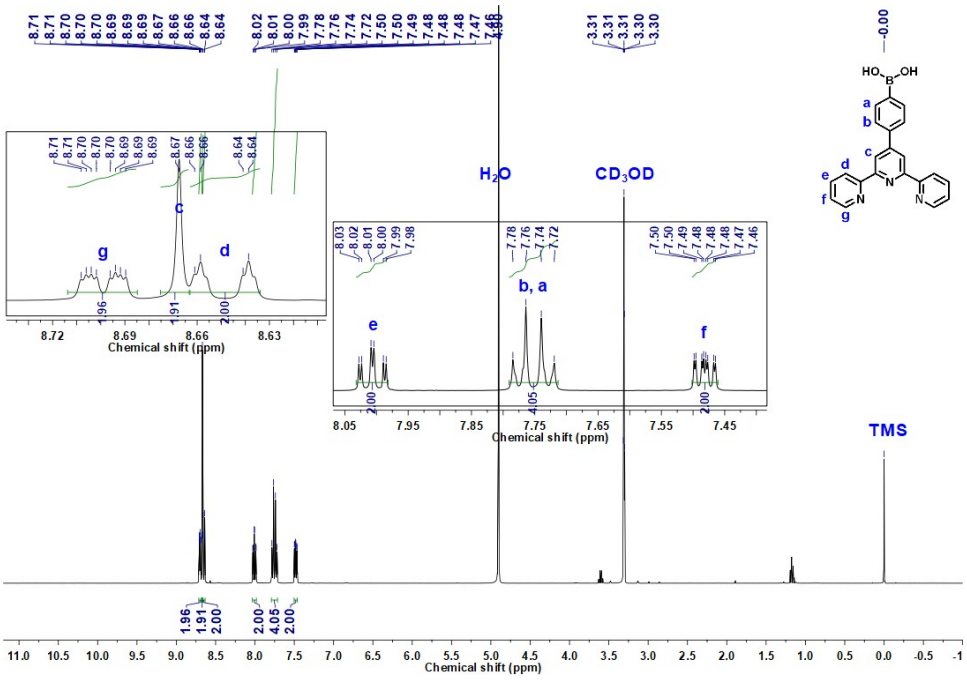


Fig. S1. ^{1}H NMR spectrum of compound 1 (400 MHz, CD_3OD)

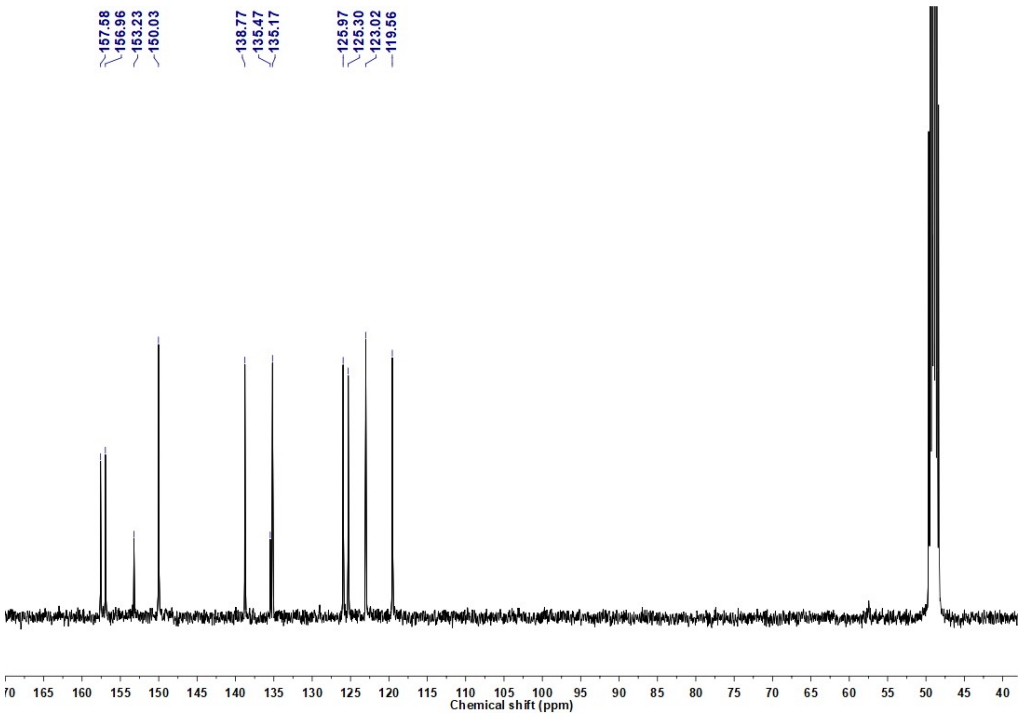


Fig. S2. ^{13}C NMR spectrum of compound 1 (101 MHz, CD_3OD)

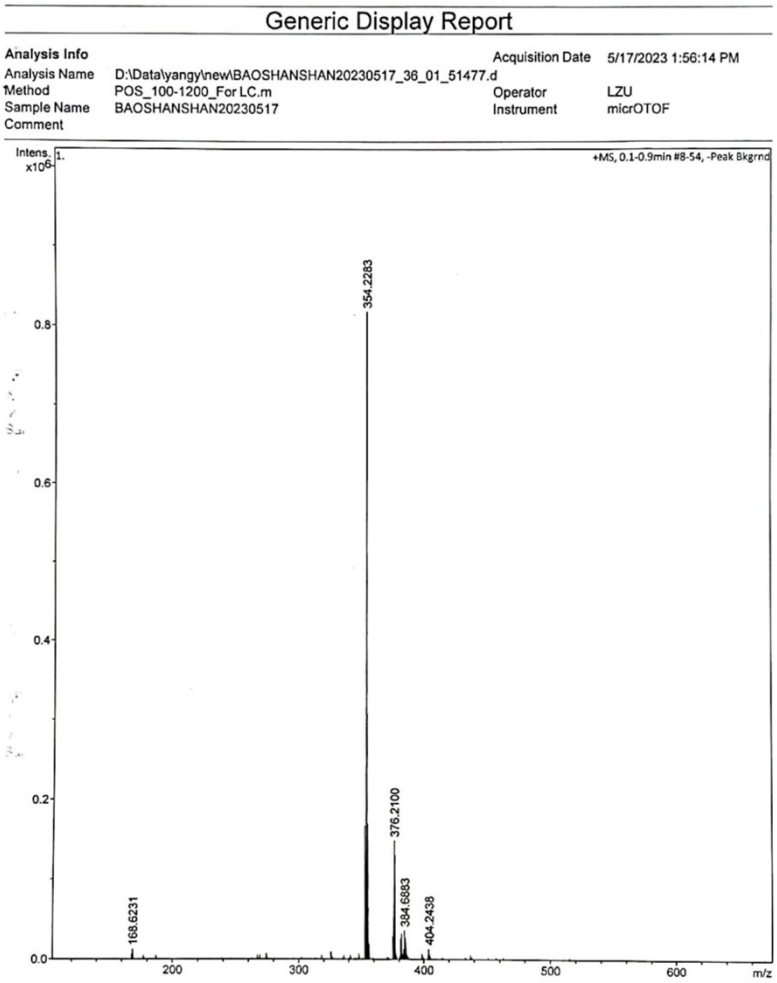
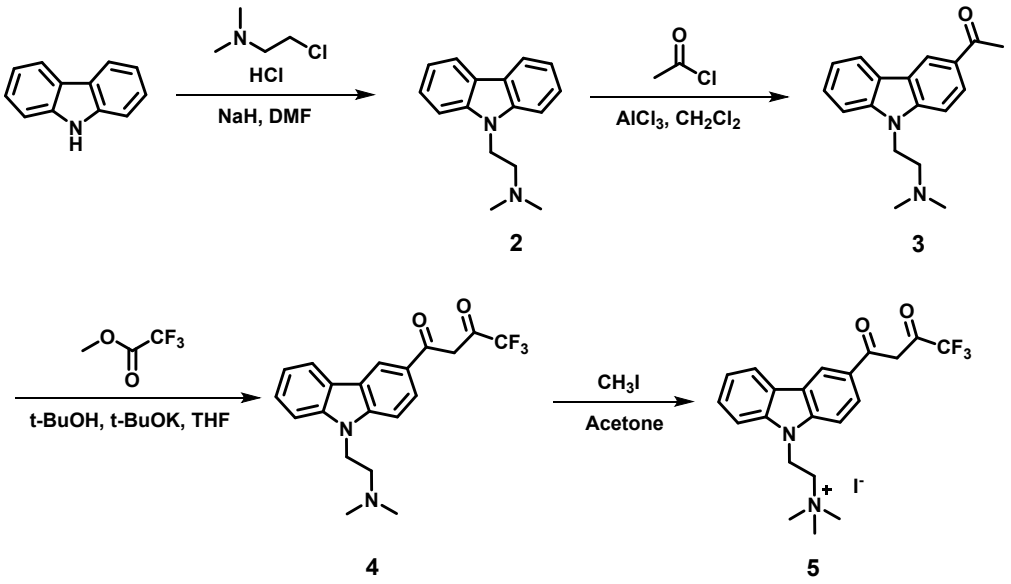


Fig. S3. MS spectrum of compound 1



Scheme S2 Synthetic route of THB[2]

Synthesis of compound 2

9-H-carbazole (8.36 g, 50 mmol), 2-dimethylaminoethyl chloride hydrochloride (8.64 g, 60 mmol), NaH (5.0 g, 125 mmol) were dissolved in 40 mL DMF and reacted at 120 °C for 24 h. After the mixture cooled to room temperature, 50 mL deionized water was added to the mixture with stirring and extracted with CH_2Cl_2 (30 mL×3), the organic phase was collected and dried with anhydrous sodium sulfate. The solvent was removed by spin evaporation, and the crude product obtained was purified by silica gel column chromatography (eluent dichloromethane: methanol = 100:1, v/v), and then dried under vacuum to obtain compound 2 (8.20 g, 68.8% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, J = 7.8 Hz, 2H), 7.42 – 7.35 (m, 2H), 7.30 (d, J = 8.2 Hz, 2H), 7.16 (dd, J = 11.3, 4.3 Hz, 2H), 4.27 – 4.20 (m, 2H), 2.59 – 2.52 (m, 2H), 2.23 (s, 6H). ESI-MS calculated MS: M= 238.15, found m/z: $[M+H]^+$= 239.1547.

Synthesis of compound 3

Compound 2 (4.76 g, 20 mmol) and 40 mL dichloromethane were added to a 500 mL aubergine flask under an ice-water bath and stirred until completely dissolved, then anhydrous $AlCl_3$ (6.0 g, 45 mmol) was slowly added to the flask. Acetyl chloride (0.785 g, 10 mmol) dispersed in 20 mL of dichloromethane was added to the above reaction system slowly by dropping with a constant pressure dropping funnel, and the reaction was carried out at room temperature for 3 h. After that, 50 mL deionized water and 1 M NaOH solution was slowly added to the mixture with stirring and the pH was adjusted to about 7. Then it was extracted with CH_2Cl_2 three times. The organic phase was collected and dried with anhydrous sodium sulfate. The solvent was removed by rotary evaporation after standing for a period of time. The residue was purified by column chromatograph using dichloromethane and methanol as eluent to give the

purified title compound (2.17 g, 59.7% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (s, 1H), 7.99 (t, *J* = 7.7 Hz, 2H), 7.42 (t, *J* = 7.7 Hz, 1H), 7.31 (d, *J* = 8.2 Hz, 1H), 7.22 (dd, *J* = 12.9, 4.6 Hz, 2H), 4.19 (t, *J* = 7.5 Hz, 2H), 2.62 – 2.55 (m, 5H), 2.26 (s, 6H). ESI-MS calculated MS: M= 280.16, found m/z: $[M+H]^+$= 281.1654.

Synthesis of compound 4

Trifluoroacetate (7.96 g, 56 mmol) and a solution of tert-butanol (15 mL) and anhydrous THF (15 mL) were added to a 250 mL round-bottomed flask under an ice-water bath. Then potassium tert-butanol (3.77 g, 33.6 mmol) was slowly added to the flask. After stirring for 30 min, a solution of compound 3 (1.57 g, 5.6 mmol) dispersed in 15 mL dichloromethane was slowly added dropwise over 5 minutes with a constant pressure dropping funnel to the mixture. The reaction was carried out at room temperature for 24 h. At the end of the reaction, 50 mL of deionized water was slowly added to the mixture with stirring, and the pH was adjusted to about 7 with dilute hydrochloric acid. Then the mixture was extracted with CH_2Cl_2, and the organic layer was collected and dried with anhydrous Na_2SO_4. The organic solvent was removed by rotary evaporation. The residue was purified by chromatography on a silica gel with an eluent of dichloromethane : methanol=40:1~20:1, dichloromethane : methanol = 10:1 unfolding. (0.56 g, 26.5% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (d, *J* = 1.3 Hz, 1H), 8.23 (d, *J* = 7.7 Hz, 1H), 7.96 (dd, *J* = 8.6, 1.5 Hz, 1H), 7.59 (dd, *J* = 17.0, 8.4 Hz, 2H), 7.49 – 7.44 (m, 1H), 7.22 (t, *J* = 7.4 Hz, 1H), 6.09 (s, 1H), 4.48 (t, *J* = 6.8 Hz, 2H), 3.35 (s, 6H), 2.62 (t, *J* = 6.8 Hz, 2H), 2.21 (s, 6H). ESI-MS calculated MS: M= 376.14, found m/z: $[M+H]^+$= 377.1467.

Synthesis of compound 5 (THB)

Compound 4 (0.559 g, 1.5 mmol) and 20 mL of acetone were added to a 100 mL round-bottomed flask and stirred at 55 °C until complete dissolution, then CH_3I (1 mL, 15 mmol) was added slowly and dropwise to the flask. Continue heating and refluxing for 24 h. At the end of the reaction, a yellow precipitate was formed, filtered, washed with acetone, and finally dried under vacuum to obtain the pure product 4 (0.626 g, 80.5% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.19 (s, 1H), 8.41 (d, *J* = 7.7 Hz, 1H), 8.34 – 8.29 (m, 1H), 7.91 (d, *J* = 8.8 Hz, 1H), 7.82 (d, *J* = 8.3 Hz, 1H), 7.64 (t, *J* = 7.3 Hz, 1H), 7.41 (t, *J* = 7.4 Hz, 1H), 7.25 (s, 1H), 5.05 – 4.97 (m, 2H), 3.79 – 3.73 (m, 2H), 3.28 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 187.45, 172.92(q), 143.27, 140.21, 127.23, 126.02, 123.83, 122.96(q), 122.62, 121.31, 121.08, 119.00, 116.19, 110.30, 110.24, 92.59, 61.22, 52.78, 36.46. ESI-MS calculated MS: M= 518.07, $[M-I]^+$= 391.16, found m/z: $[M-I]^+$= 391.1761.

Synthesis of europium complex Eu(TPBA)THB(TTA)$_2$ (G_1).

TPBA (35 mg, 0.1 mmol), THB (51.8 mg, 0.1 mmol), 2-thiophenoyltrifluoroacetone (TTA) (44.4 mg, 0.2 mmol) and 15 mL methanol were added into a 100 mL round-bottomed flask and dissolved by ultrasonication. Then 1 M

NaOH solution (4 mg, 0.1 mmol) was slowly added to the mixture with stirring and the pH was adjusted to 7~8. At this time the solution was yellow, clear and transparent. $Eu(NO_3)_3 \cdot 6H_2O$ (44.6 mg, 0.1 mmol) was dissolved in 100 μL water, and slowly added into the above mixture while stirring. A yellow precipitate was generated immediately, and the reaction was heated and stirred at 60 ℃ for 12 hours. After that, the solution was cooled to room temperature, and centrifuged to enrich the precipitate. The precipitate was washed three times with a small amount of methanol and water and dried under vacuum at 40 ℃ for several hours to obtain a light yellow solid (105 mg, 75.9%).

The remaining complexes were synthesized as above.

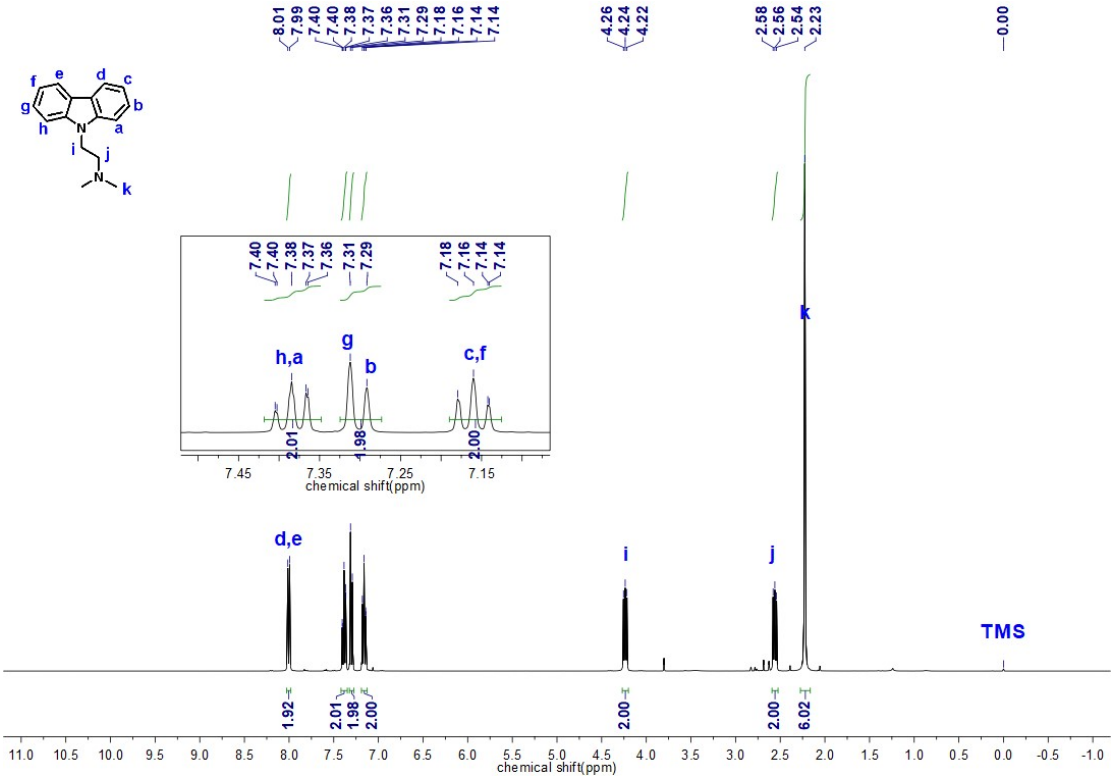


Fig. S4. ^{1}H NMR spectrum of compound 2 (400 MHz, $CDCl_3$)

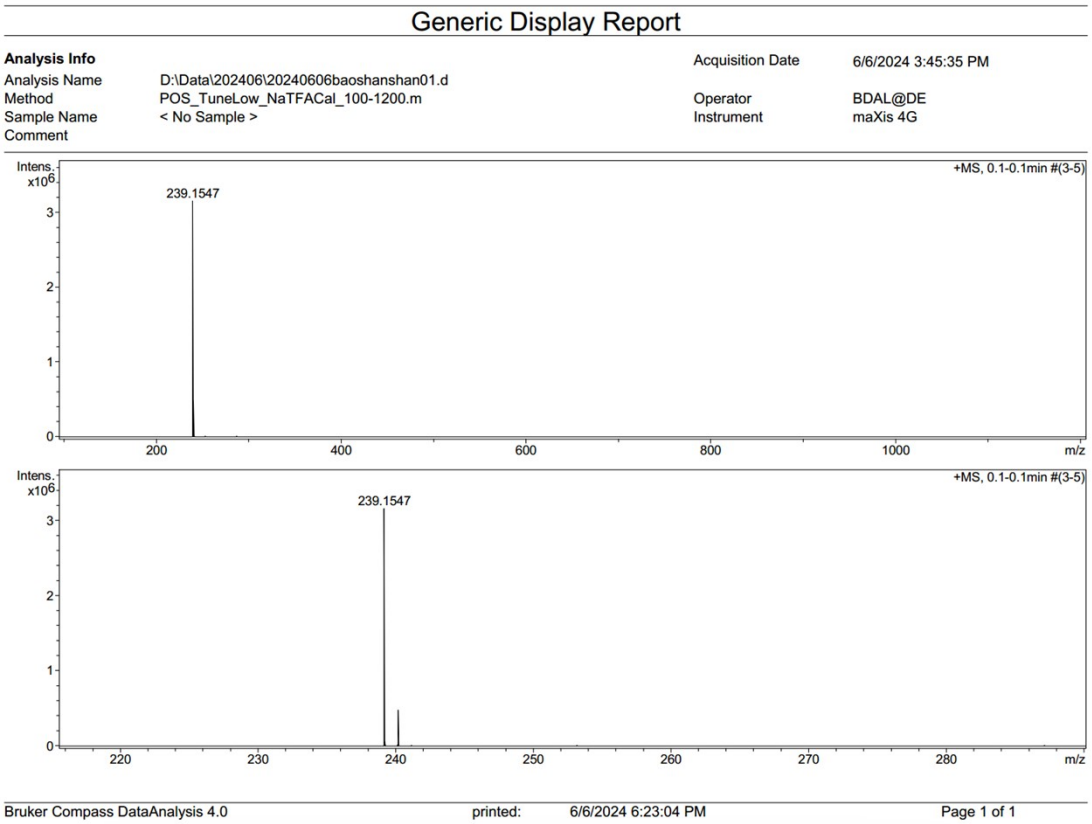


Fig. S5. ESI-MS of compound 2

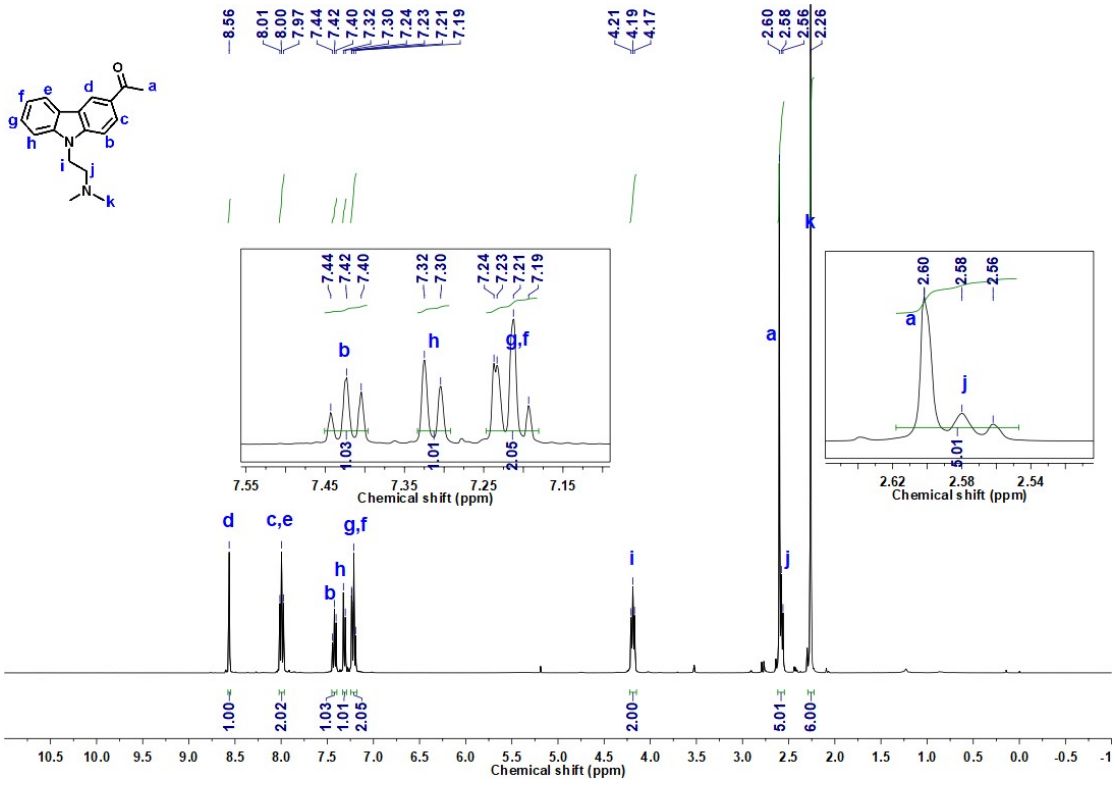


Fig. S6. ^{1}H NMR spectrum of compound 3 (400 MHz, $CDCl_3$)

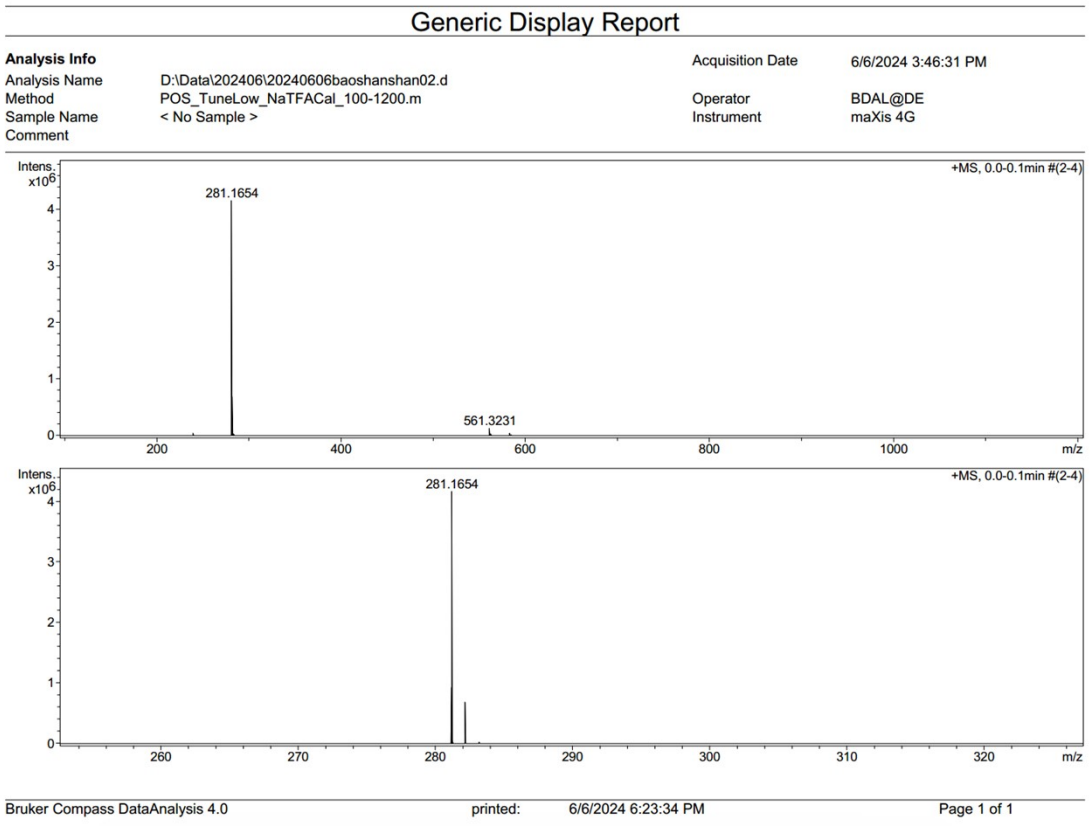


Fig. S7. ESI-MS of compound 3

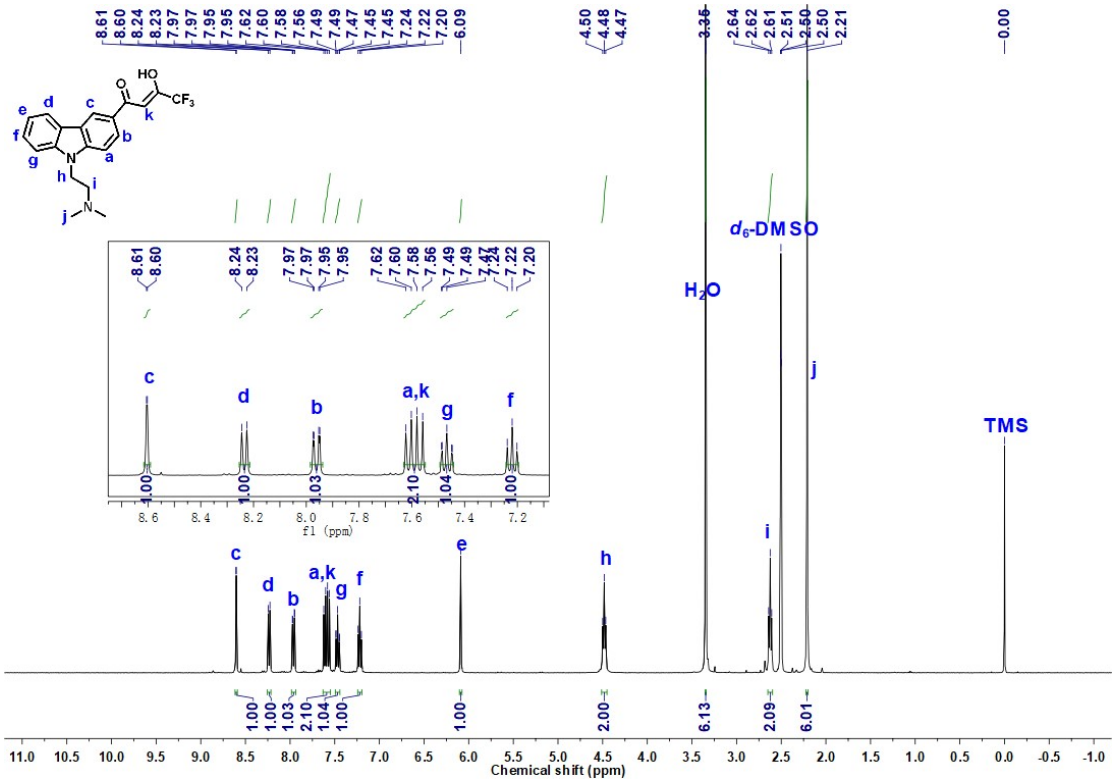


Fig. S8. ^{1}H NMR spectrum of compound 4 (400 MHz, DMSO-d_6)

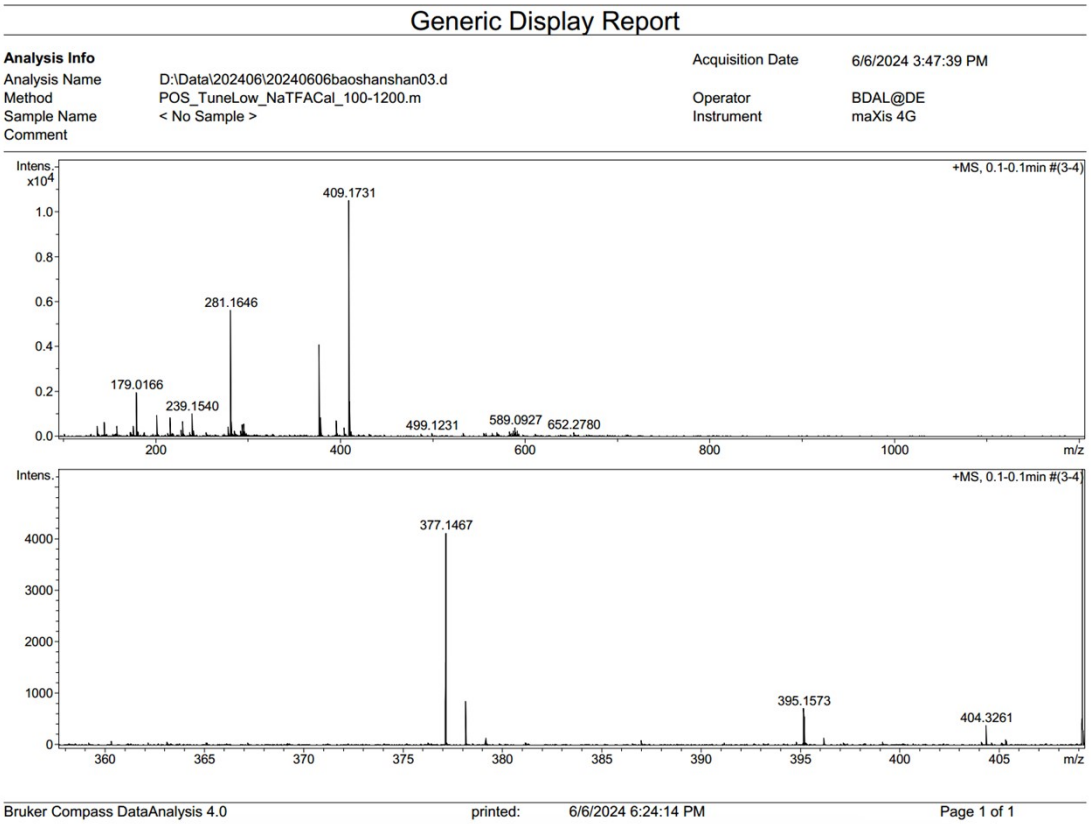


Fig. S9. ESI-MS of compound 4

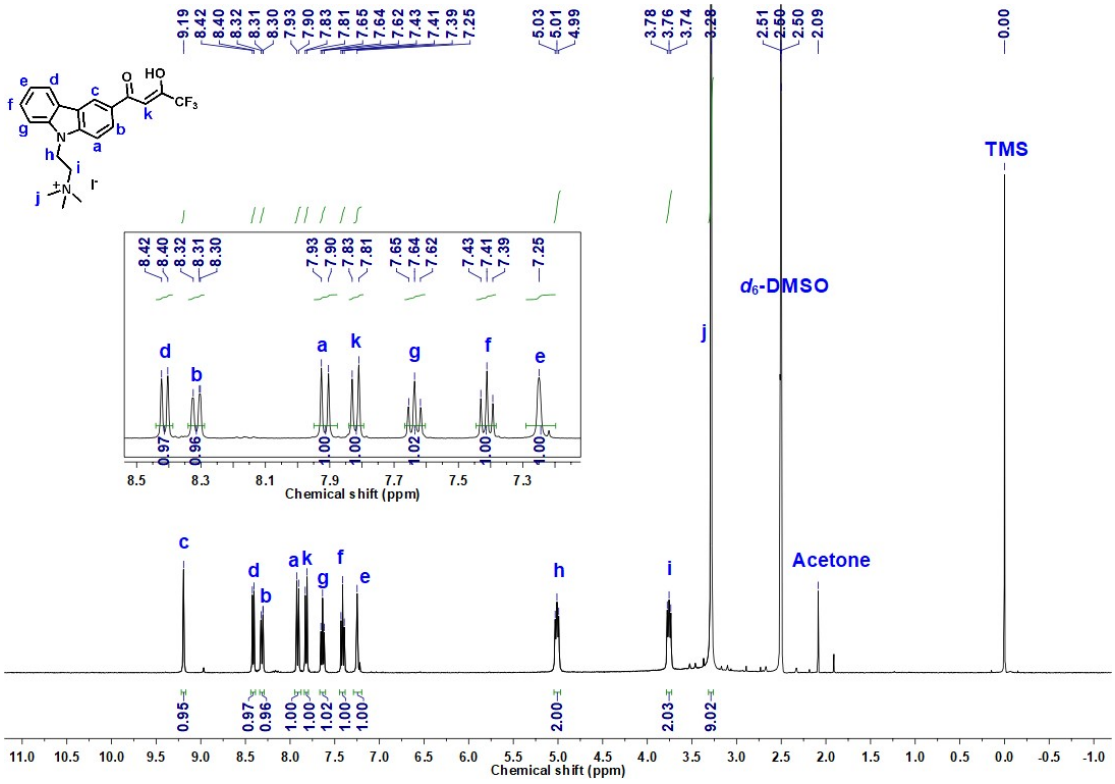


Fig. S10. ^{1}H NMR spectrum of compound 5 (400 MHz, DMSO-d_6)

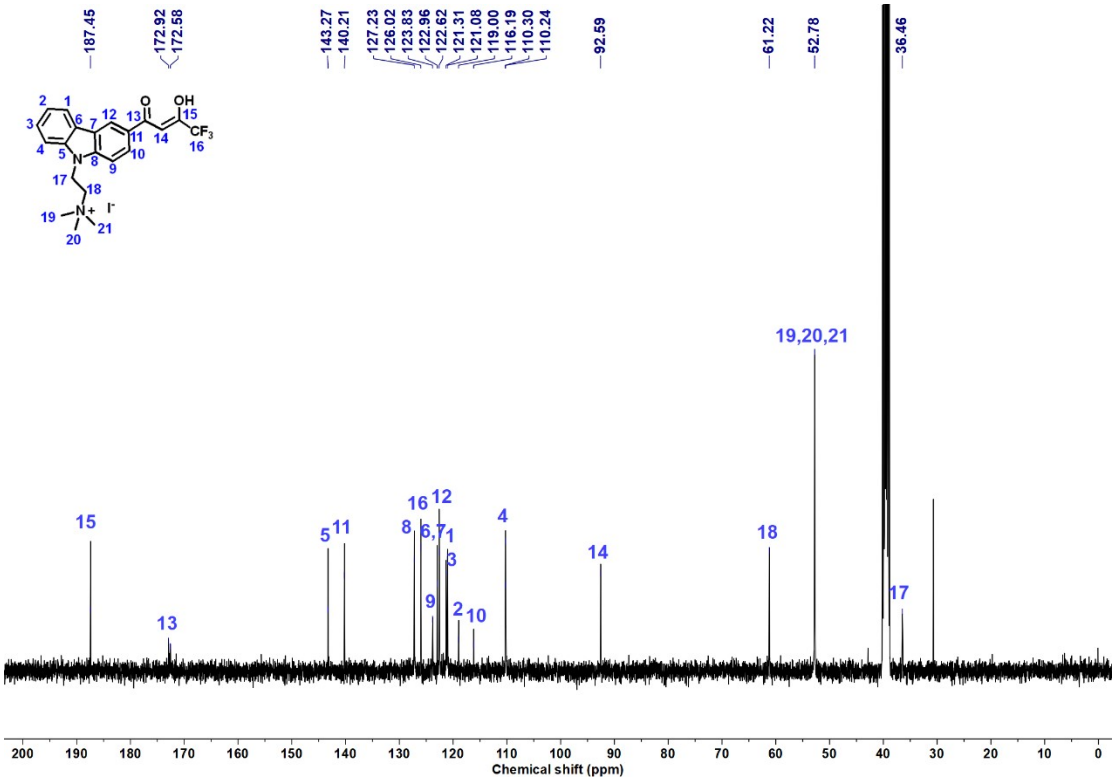


Fig. S11. ^{13}C NMR spectrum of compound 5 (101 MHz, DMSO-d_6)

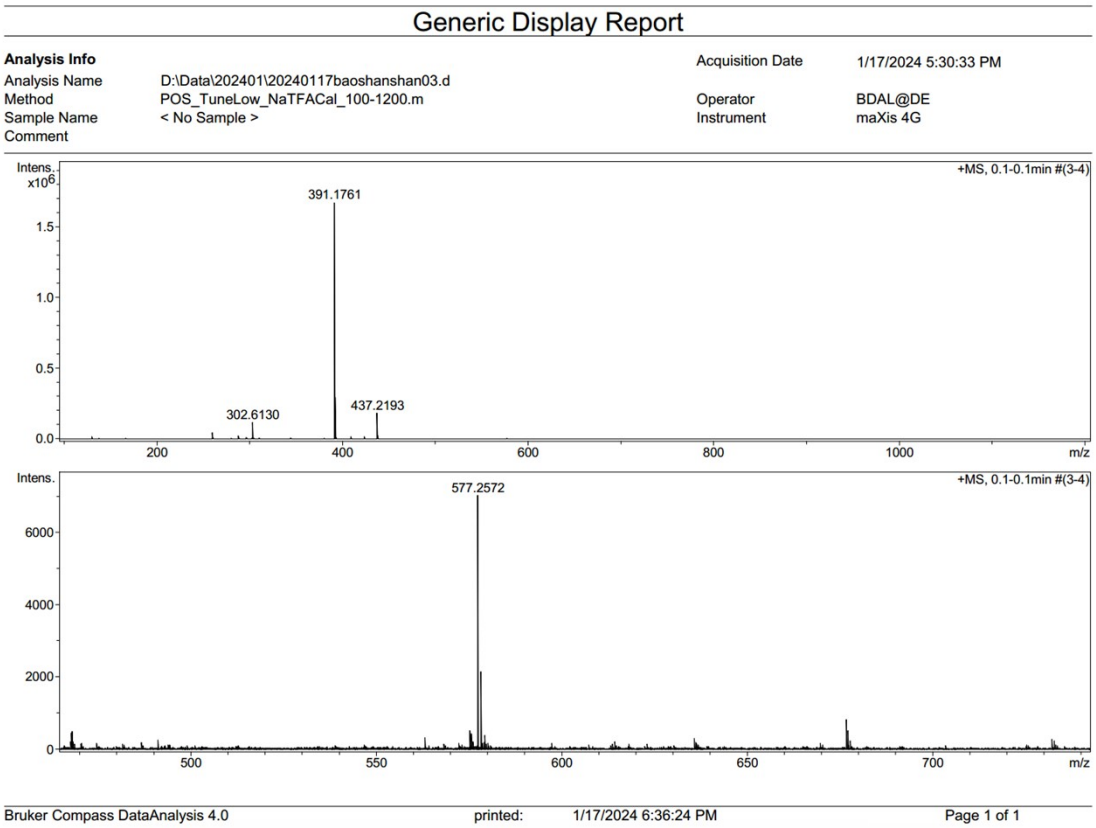


Fig. S12. HRMS spectrum of compound 5

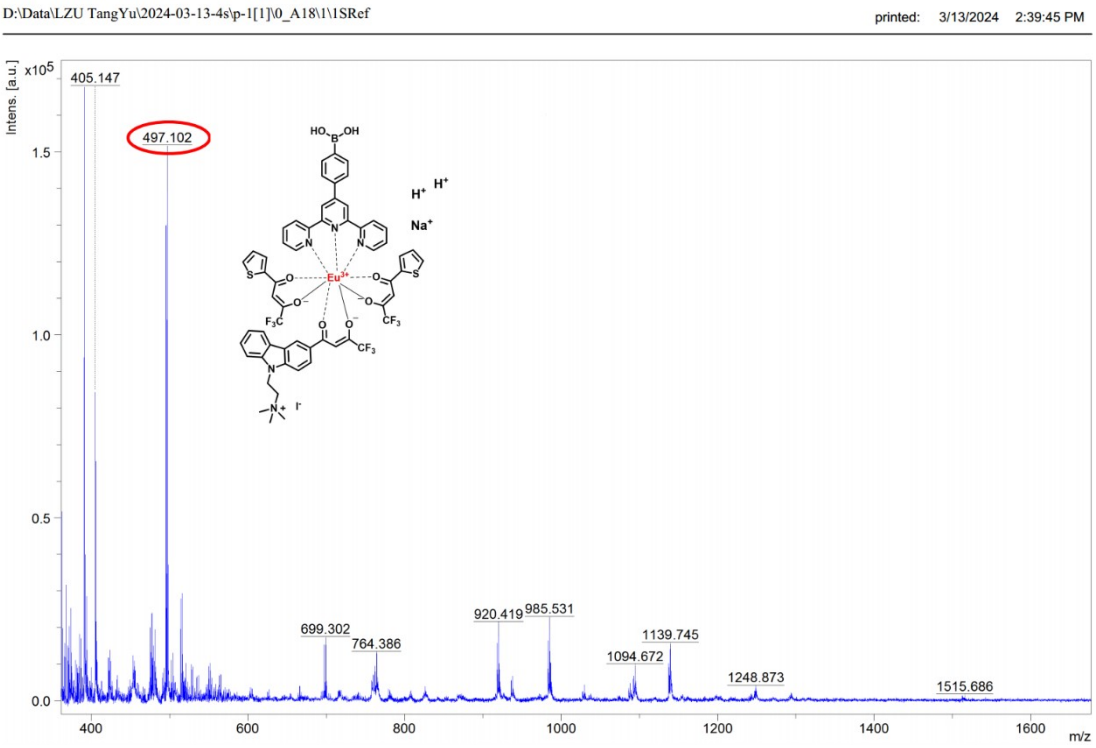


Fig. S13. MALDI-TOF MS spectrum of Eu(TPBA)THB(TTA)$_2$

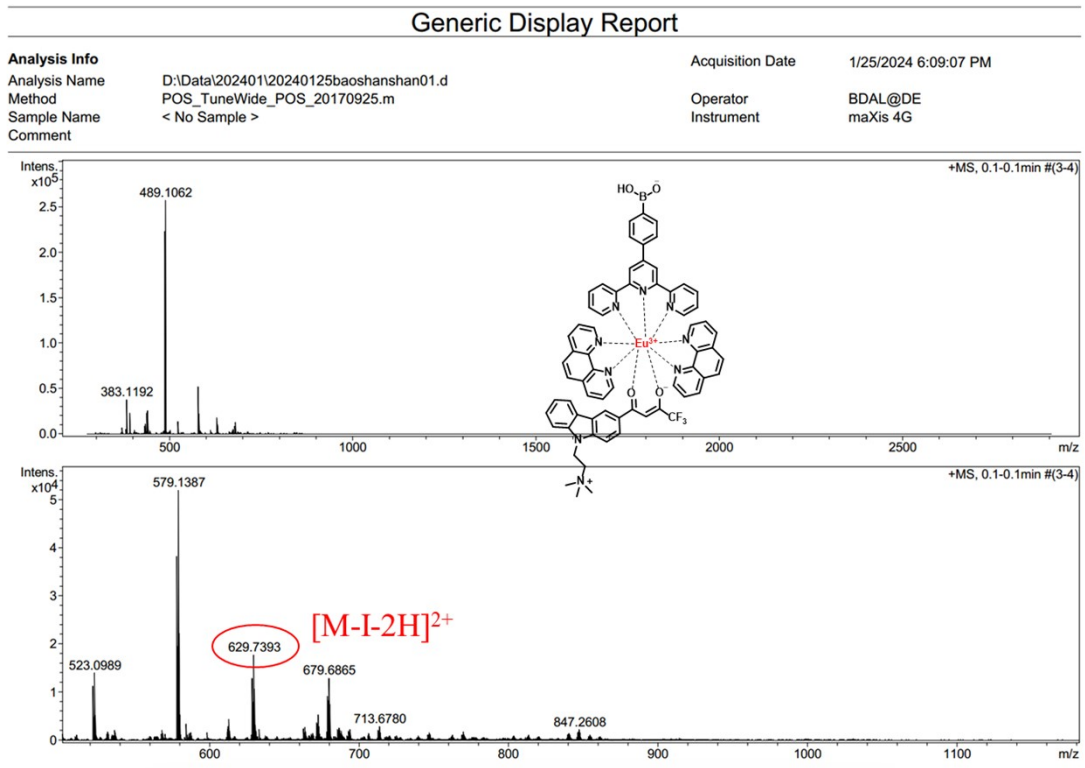


Fig. S14. HRMS spectrum of Eu(TPBA)THB(Phen)$_2$

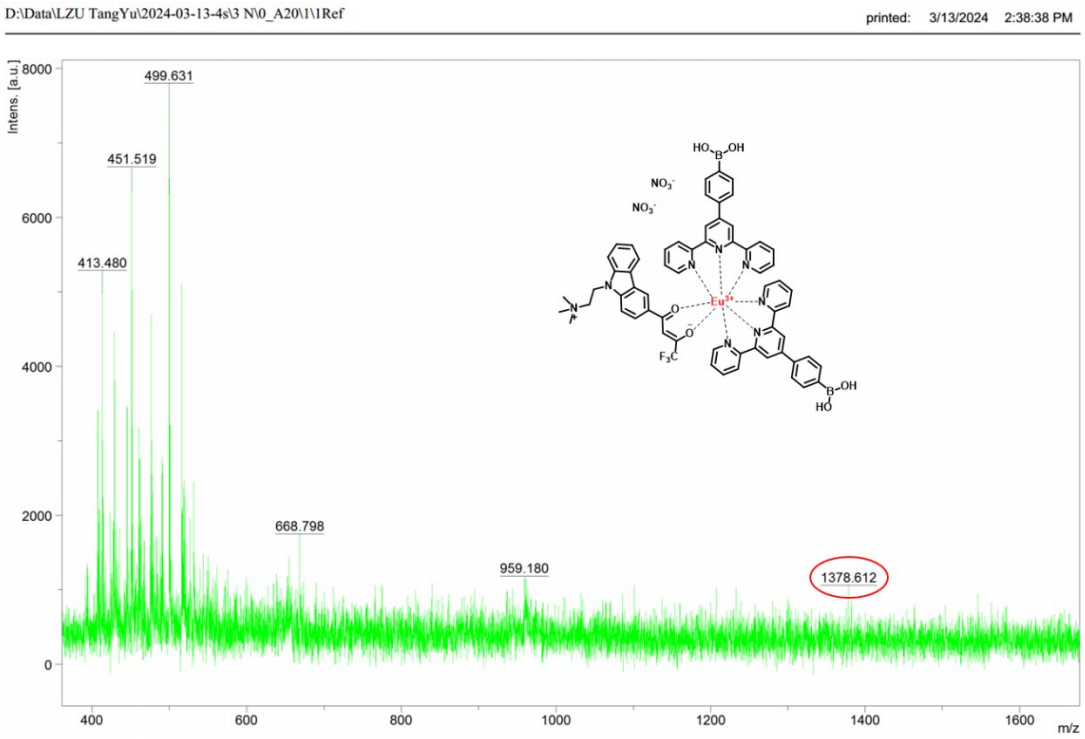
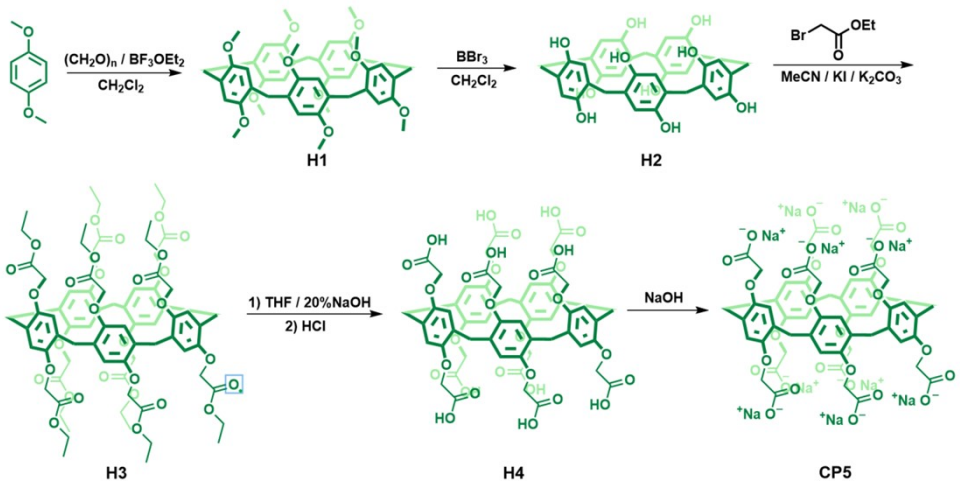


Fig. S15 MALDI-TOF MS spectrum of Eu(TPBA)$_2$THB

Table S1. The elemental analysis of four europium complexes G_1~G_4.

complex	w_i/%		
	C	H	N
G_1	49.195 49.321	3.390 3.417	4.288 4.240
G_2	46.194 46.336	3.743 3.743	8.214 8.131
G_3	49.701 50.179	3.889 3.951	8.031 8.226
G_4	72.572 72.601	4.414 4.387	11.768 11.789



Scheme S3. Synthetic route of CP5[3]

Synthesis of compound H4

The synthetic method of H1-H4 was according to the reported literature. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.94 (s, 10H), 7.11 (s, 10H), 4.70 (d, J = 15.9 Hz, 11H), 4.41 (d, J = 15.8 Hz, 11H), 3.74 (s, 10H).

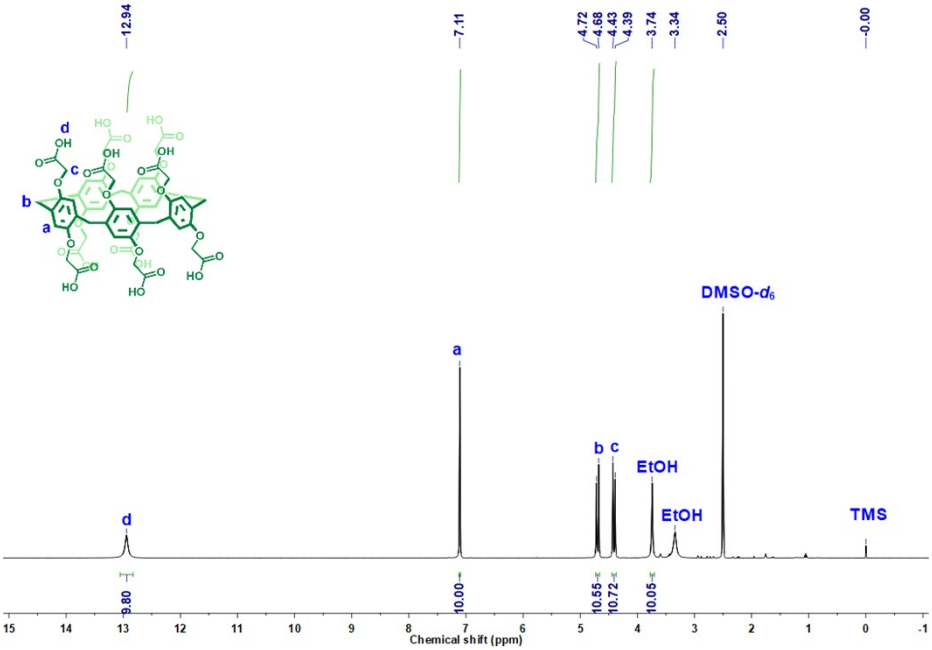


Fig. S16. ^{1}H NMR spectrum of compound H4 (400 MHz, DMSO-d_6)

Synthesis of CP5.

10 eq. NaOH was added to an aqueous solution of 1 eq. of H4 under sonication conditions to obtain CP5. In this work, water-soluble CP5 was used as the host molecule.

Synthesis of Eu(TPBA)$_2$THB⊂CP5 (G$_3$⊂CP5).

Eu(TPBA)$_2$THB (0.01 mmol) and 5 mL DMF were added into a 25 mL glass vial and dissolved by ultrasonication to obtain yellow transparent solution. CP5 (0.01 mmol) and 300 μL high-purity water were added into another centrifugal tube,

dissolving by ultrasonication. Then the aqueous solution of CP5 was slowly added dropwise to the glass vial under stirring. Afterwards, the mixture was stirred for 6 h at room temperature to get milky white turbid solution. Finally, the solution was enriched by centrifugation and dried under vacuum for 10 hours to obtain a white solid.

The compounds (Eu(TPBA)THB$(TTA)_2$⊂CP5 **(G_1⊂CP5)** and Eu(TPBA)THB$(Phen)_2$⊂CP5 **(G_2⊂CP5)**) were synthesized as above.

1.3 Characterization of Photophysical Properties

Fluorescence spectra of Europium complex toward H_2O_2.

Firstly 50 mL, 10 μM ethanol solution of europium complex was prepared as stock solution. According to the literature[4], the concentration of 30% H_2O_2 stock solution was 9.0 M as measured by the UV-Vis absorbance at 240 nm (ε = 43.6 $M^{-1}\cdot cm^{-1}$), and the 30% H_2O_2 was diluted into a series of aqueous H_2O_2 solutions with concentration gradients before each test. 10 μL H_2O_2 diluted solution was added into 3 mL the probe stock solution, ultrasonicating for 15 min to make the reaction complete, and then immediately used for fluorescence testing.

Fluorescence spectra of Europium complex-CP5 compounds toward H_2O_2 and ATP.

To demonstrate that our complex probe is a dual-detection probe responsive to H_2O_2 and ATP, the optical properties of the probe were investigated under simulated physiological conditions. Subsequently, the concentration-dependent fluorescence spectra of the probes were measured in PBS buffer solution (0.01 mM, pH 7.4) with the addition of different concentrations of H_2O_2 or ATP.

The details are as follows: 530 μL (1/10) was pipetted from the resulting europium complex-CP5 complex solution and fixed to 100 mL with PBS buffer (0.01 mM, pH 7.4) to make 10 μM stock solution. H_2O_2 test solution: 30% H_2O_2 is taken before each test and diluted into a series of aqueous H_2O_2 solutions with a concentration gradient; ATP test solution: 1 M ATP masterbatch (0.5514 g, 1 mmol, dissolved in 1 mL water) was prepared, then diluted with water to obtain a series of 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2, 1.4, 1.6, 1.8, and 2.0 mM ATP for the test solution. For testing, 10 μL H_2O_2 or 10 μL ATP diluent was added into 3 mL stock solution, and the solution was sonicated for 15 min to complete the reaction, after which it was used immediately for fluorescence testing.

In addition, to investigate whether G_3⊂CP5 has a specific fluorescence response to H_2O_2 among many ROS. We measured the fluorescence intensity of probe G_3⊂CP5 (100 μM) to various common ROS (Blank, 2 mM H_2O_2, 2 mM $^1O_2$, 2 mM ClO^-, 2 mM •OH) at 614 nm in PBS buffer solution (λ_{ex} = 368 nm, slit 2 nm).

1.4 Fluorescence Imaging of G_3⊂CP5 in Living Cells

We have already demonstrated the excellent detection capabilities of the probe for H_2O_2 and ATP in vitro, Subsequently, we investigated the feasibility of using the probe for simultaneous imaging and dynamic monitoring of endogenous H_2O_2 and ATP in living cells. Here, we selected two cell models: mouse monocyte macrophage RAW264.7 cells and Hela cells. The cells were cultured at 37 °C in a constant temperature incubator containing 5% carbon dioxide, and the medium was DMEM complete medium containing 15% fetal bovine serum. The concentration of the host-guest complex used in the cell imaging experiments was 100 μg·mL^{-1}.

Cytotoxicity assays.

Collected Hela and RAW264.7 cells in the logarithmic phase, seeding them into a 96-well microtiter plate at a density of 1×10^5 cells per well. After 24 hours of incubation, various concentrations of $G_3\subset$CP5 (0, 10, 20, 40, 80, 100 μg·mL^{-1}) were added with six replicates per group. Cells without probe treatment were served as controls, and a zero-point adjustment group was established. Then after 24 hours of incubation in the incubator the medium was aspirated and washed with PBS. Afterwards, 10 μL CCK-8 solution was added to each well. After 4 hours of incubation, the absorbance of each well at 490 nm was measured using a microplate reader.

Cell survival rate = $(OD_{\text{treated group}} - OD_{\text{zero wells}})/(OD_{\text{control wells}} - OD_{\text{zero wells}}) \times 100\%$, The cell survival rate for each group was calculated.

Cellular uptake assay.

Hela and RAW264.7 cells in logarithmic growth phase were taken and inoculated at a density of 1×10^5 cells into 20 mm glass-bottomed cell culture dishes. After incubation for 24 hours, added Eu(TPBA)$_2$THB$\subset$CP5 diluted with DMEM and incubated for an additional 4 hours. Afterwards, the cells were gently rinsed three times with PBS. After fixation by adding paraformaldehyde, the cells were rinsed three more times with PBS and stained using a nuclear staining kit (DAPI, blue fluorescence). Finally, the staining solution was removed and the cells were rinsed with PBS. The petri dishes were placed under a laser confocal microscope for observation and photographs.

Co-localization fluorescence imaging in mitochondria.

Hela and RAW264.7 cells were taken, counted and inoculated into 20 mm glass-bottomed cell culture dishes at a density of 1×10^5 cells. After incubation for 24 hours, the cells were incubated with the material Eu(TPBA)$_2$THB$\subset$CP5 for 2 hours, and then washed three times with PBS, and then incubated for 30 minutes by adding the commercial fluorescent dye Mito Tracker Green FM (excitation wavelength 488 nm, emission wavelength 510 nm). A laser confocal and super-resolution imaging system was used for observation, and the bright field and fluorescence channels were recorded.

Fluorescence imaging of H_2O_2 and ATP under external H_2O_2 and ATP stimulation.

Hela and RAW264.7 cells were taken, counted and inoculated into 20 mm glass-

bottomed cell culture dishes at a density of 1×10^5 cells. After the cells were attached, the experiment was divided into four groups: blank group (PBS), PBS+H_2O_2, PBS+ATP, $G_3\subset$CP5, $G_3\subset$CP5+H_2O_2, $G_3\subset$CP5+ATP, added to the corresponding group and incubated for 4 hours, washed three times with PBS. After 30 minutes of fixation with paraformaldehyde, the cells were rinsed three times with PBS, and finally the petri dishes were placed under a laser confocal microscope for observation and photographs.

2. Photophysical Properties

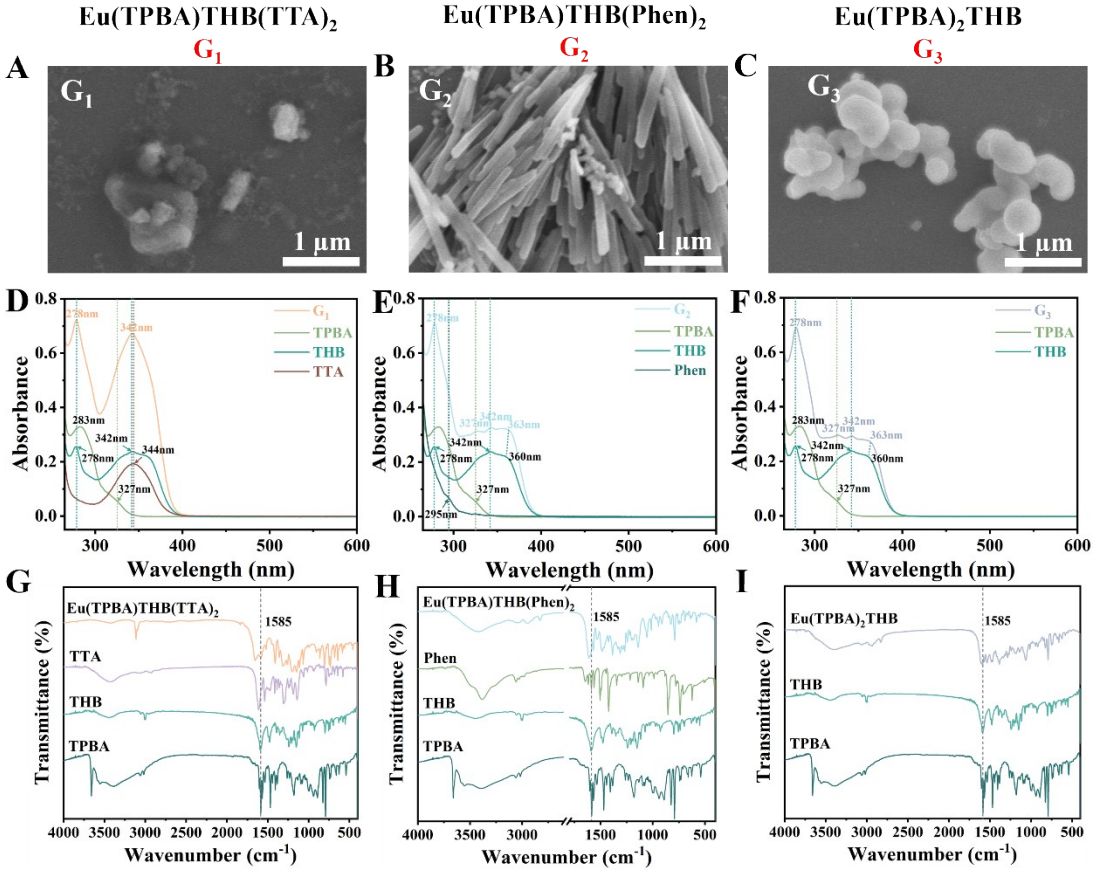


Fig. S17. Characterization of three europium complexes. (A-C) SEM of G_1, G_2, G_3. (D-F) UV-vis absorbance spectra of G_1, G_2, G_3 and their corresponding ligands (10 μM in DMF solution). (G-I) FTIR spectra of G_1, G_2, G_3 and their corresponding ligands.

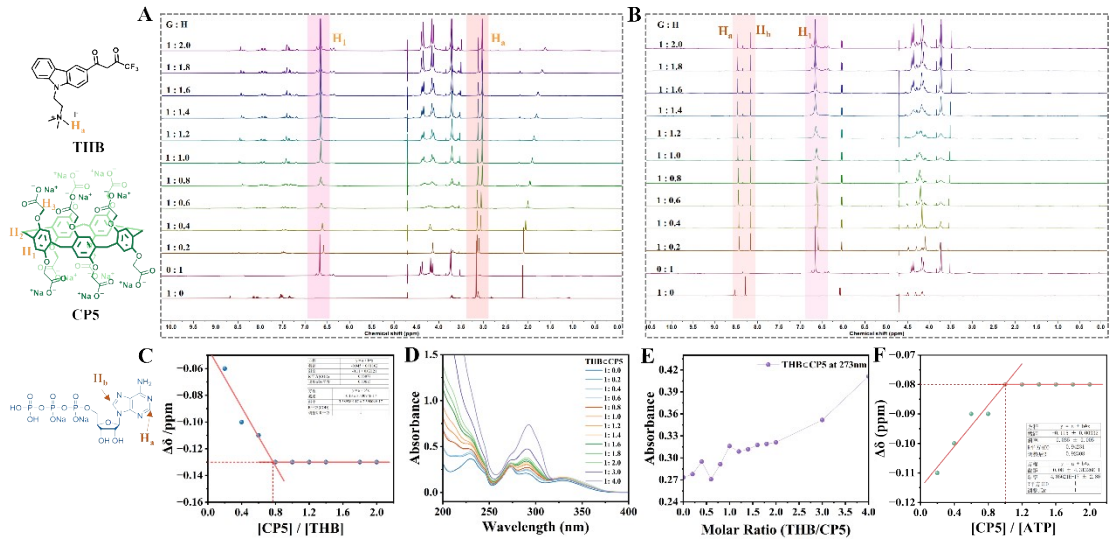


Fig. S18. (A) ^{1}H NMR spectra (400 MHz, D_2O), THB (G, 1 mM) and CP5 (H, 0 ~ 2 mM). (B) ^{1}H NMR spectra (400 MHz, D_2O), ATP (G, 1 mM) and CP5 (H, 0 ~ 2 mM). (C) Job-plot curves of the chemical shift of H_a in the host-guest complex relative to the pure THB shift. (D)(E) Ultraviolet titration spectrum of THB (0.5 mM) and CP5 (0 ~ 1 mM) in deionized water. (F) Job-plot curves of the chemical shift of H_a in the host-guest complex relative to the pure ATP shift.

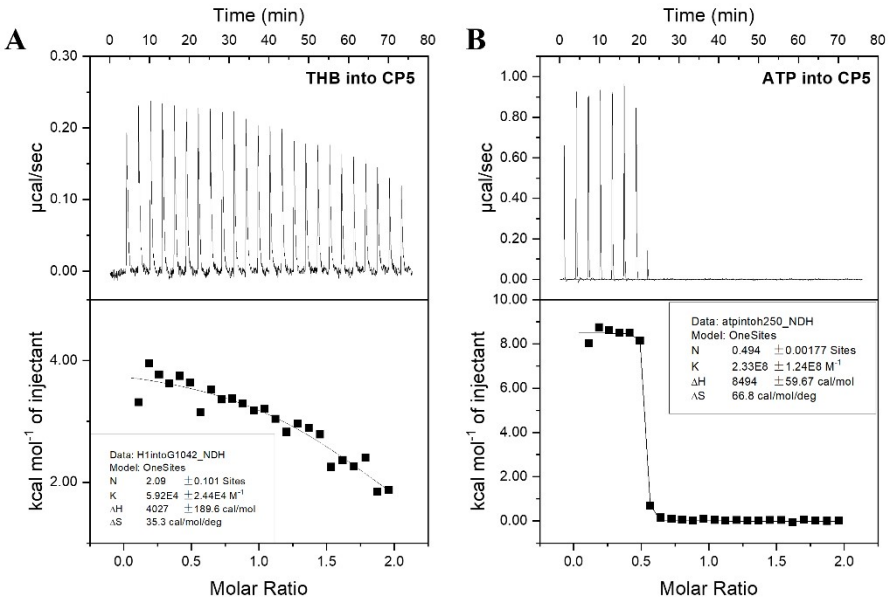


Fig. S19. Raw ITC data at 25 ℃ for 25 sequential injections (0.8 µL per injection initially, 1.5 µL per injection thereafter) of the guest solution into CP5 solution and net reaction heat obtained by subtracting the dilution heat from the reaction heat, fitted by "one set of binding sites" model. (A) CP5 (0.1 mM) with THB (1 mM) in deionized water. (B) CP5 (0.1 mM) with ATP (1 mM) in deionized water.

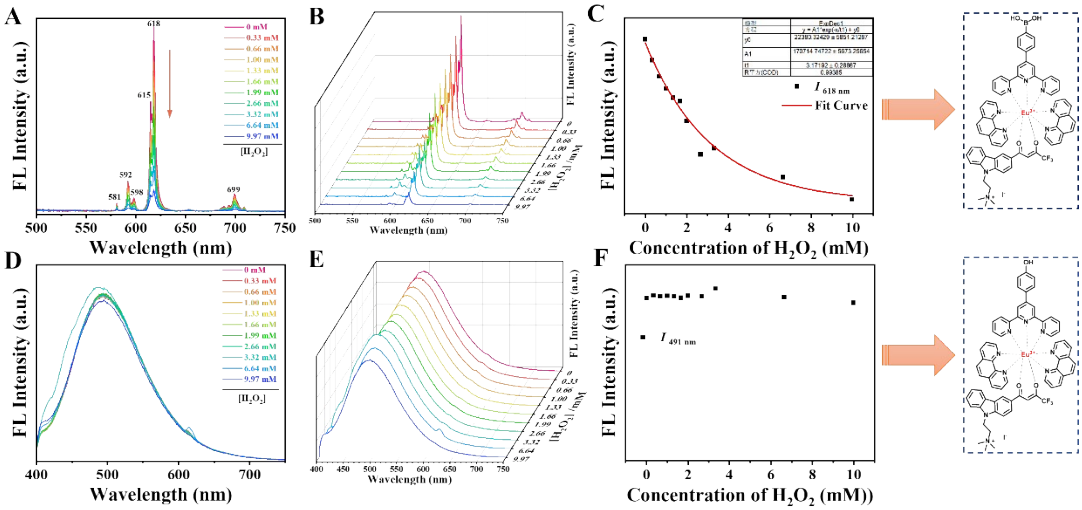


Fig. S20. Fluorescence emission spectra of G_2 and control group G_4. (A)(B)(D)(E) Fluorescence emission spectra after addition of different concentrations of H_2O_2 to the ethanol solutions of the complexes G_2 and G_4. (C) Linear fit of the fluorescence intensity of G_2 at 618 nm (λ_{ex} = 366 nm, λ_{em} = 618 nm, slit 5 nm). (F) Fluorescence intensity of G_4 at 491 nm (λ_{ex} = 333 nm, λ_{em} = 491 nm, slit 5 nm).

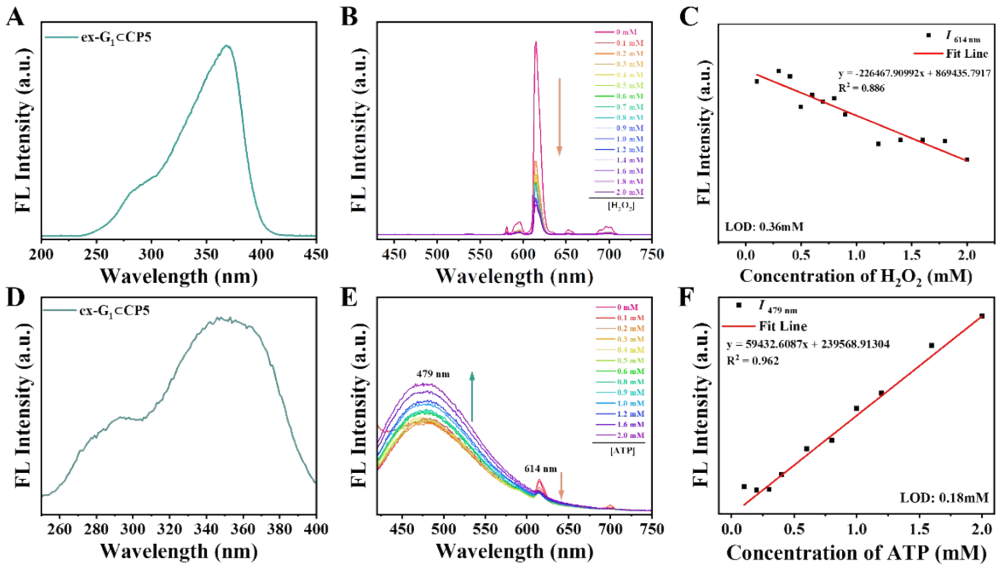


Fig. S21. H_2O_2 detection of G_1⊂CP5: (A) Fluorescence excitation spectra (λ_{em} = 614 nm, slit 1 nm). (B) Fluorescence titration spectra (C) Linear fit between the fluorescence intensity of the emission peak at 614 nm and the concentration of H_2O_2 in ethanol solution (λ_{ex} = 369 nm, slit 1 nm). ATP detection of G_1⊂CP5: (D) Fluorescence excitation spectra (λ_{em} = 615 nm, slit 5 nm). (E) Fluorescence titration spectra. (F) Linear fit between the fluorescence intensity of the emission peak at 479 nm and the concentration of H_2O_2 in ethanol solution (λ_{ex} = 363 nm, slit 5 nm).

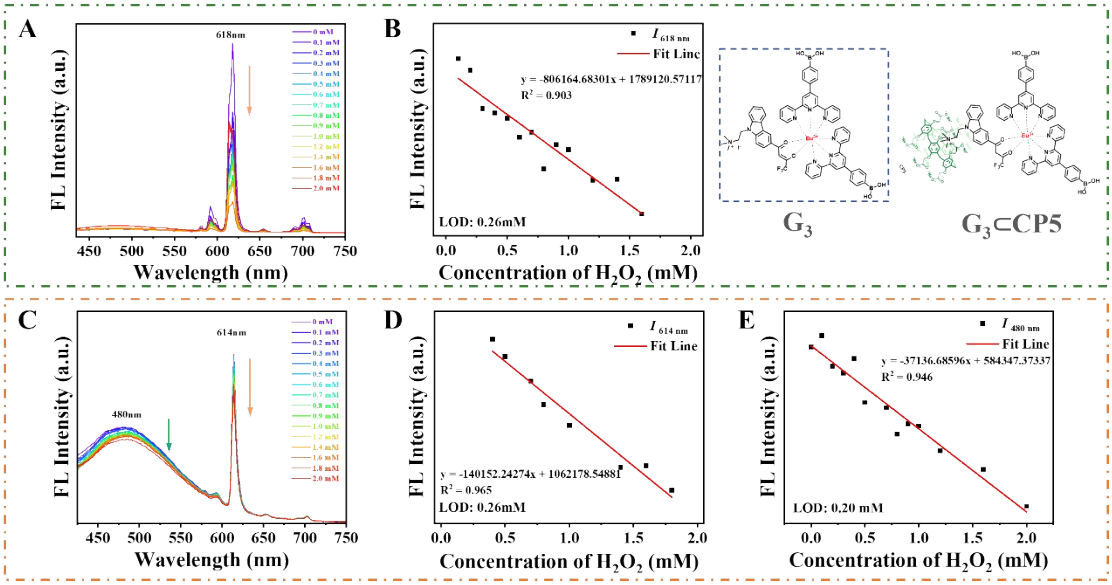


Fig. S22. Comparison of H_2O_2 detection capability between G_3 and G_3⊂CP5 in PBS buffer solution: (A) Fluorescence emission spectra of G_3 (λ_{ex} = 370 nm, slit 3 nm). (B) Linear fit between the fluorescence intensity of the emission peak at 618 nm and the concentration of H_2O_2. (C) Fluorescence emission spectra of G_3⊂CP5 (λ_{ex} = 331 nm, slit 3 nm). (D) Linear fit between the fluorescence intensity of the emission peak at 614 nm and the concentration of H_2O_2.

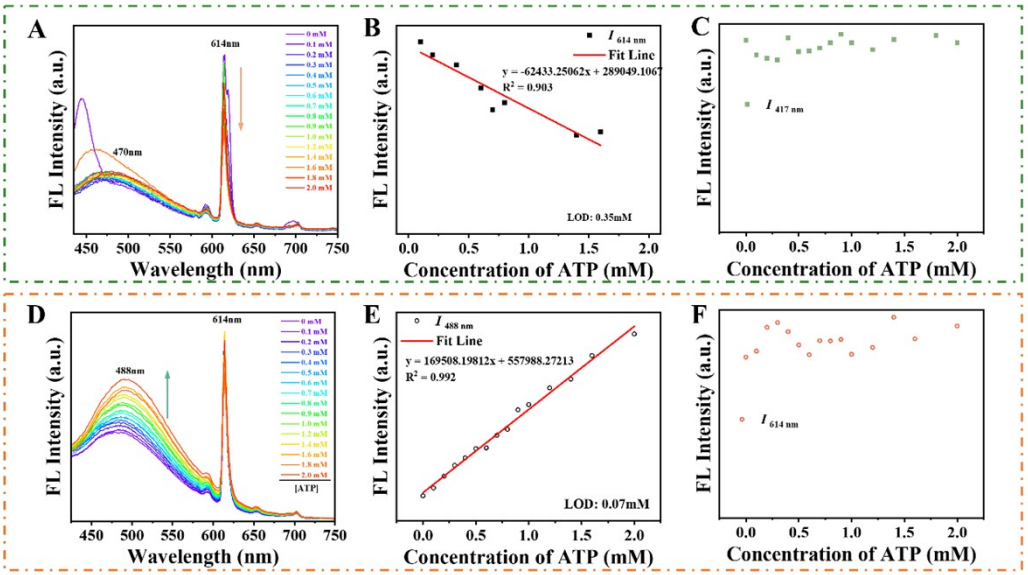


Fig. S23. Comparison of ATP detection capability between G_3 and G_3⊂CP5 in PBS buffer solution: (A) Fluorescence emission spectra of G_3 (λ_{ex} = 370 nm, slit 3 nm). (B) Linear fit between the fluorescence intensity of the emission peak at 614 nm and the concentration of ATP. (C) Fluorescence intensity of G_3 at 471 nm. (D) Fluorescence emission spectra of G_3⊂CP5 (λ_{ex} = 331 nm, slit 3 nm). (E) Linear fit between the fluorescence intensity of the emission peak at 488 nm and the concentration of ATP. (F) Fluorescence intensity of G_3⊂CP5 at 614 nm.

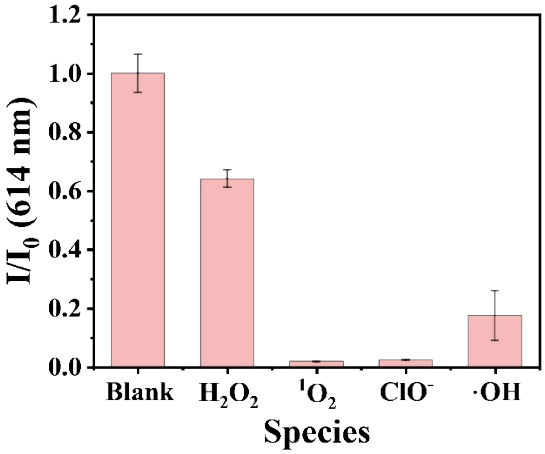


Fig. S24. Selectivity of G_3⊂CP5 (100 μM) to common ROS interfering substances (Blank, 2 mM H_2O_2, 2 mM $^1O_2$, 2 mM ClO^-, 2 mM •OH) in H_2O_2 channel (λ_{ex} = 368 nm, slit 2 nm).

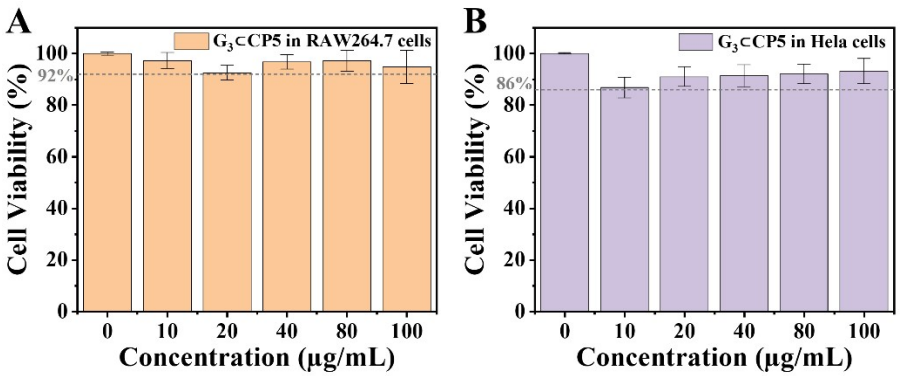


Fig. S25. Cell viability of (A) RAW264.7 and (B) Hela cells after 24 hours of incubation with different concentrations (0~100 μg·mL^{-1}) of G_3⊂CP5.

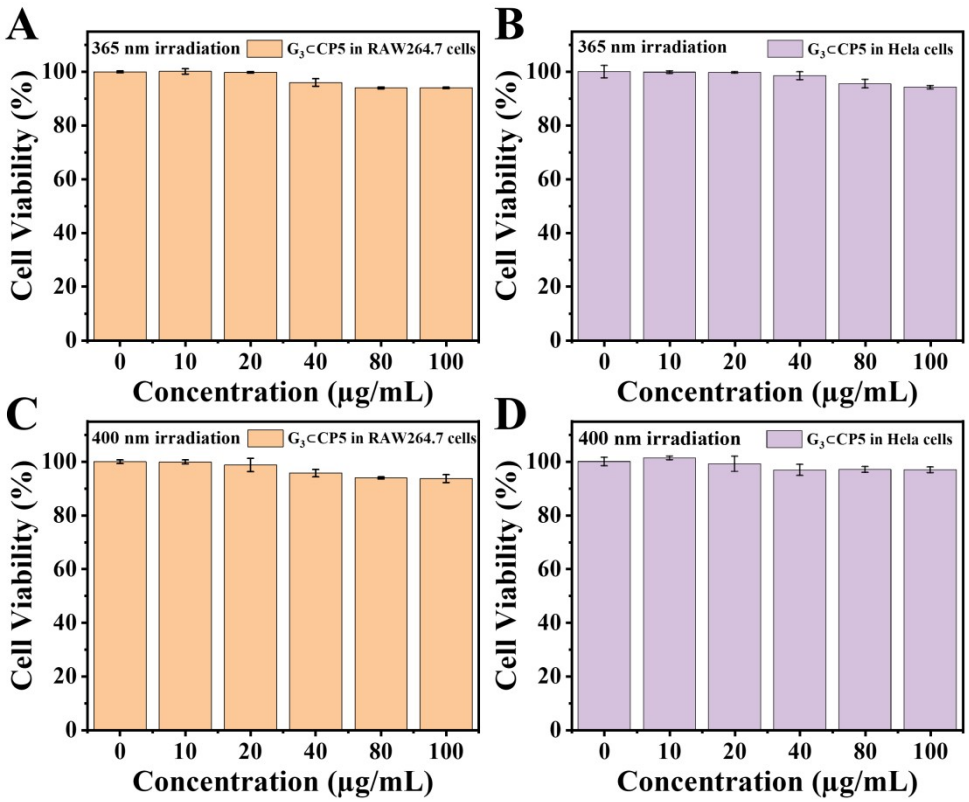


Fig. S26. Cell viability of (A) RAW264.7 and (B) Hela cells after 24 hours of incubation with of G_3⊂CP5 (0~100 μg·mL^{-1}) under the irradiation of 365 nm (pulsed irradiation for 3 minutes every half hour, last for 3 hours in total); Cell viability of (C) RAW264.7 and (D) Hela cells after 24 hours of incubation with of G_3⊂CP5 (0~100 μg·mL^{-1}) under the UV irradiation of 400 nm (pulsed irradiation for 3 minutes every half hour, last for 3 hours in total).

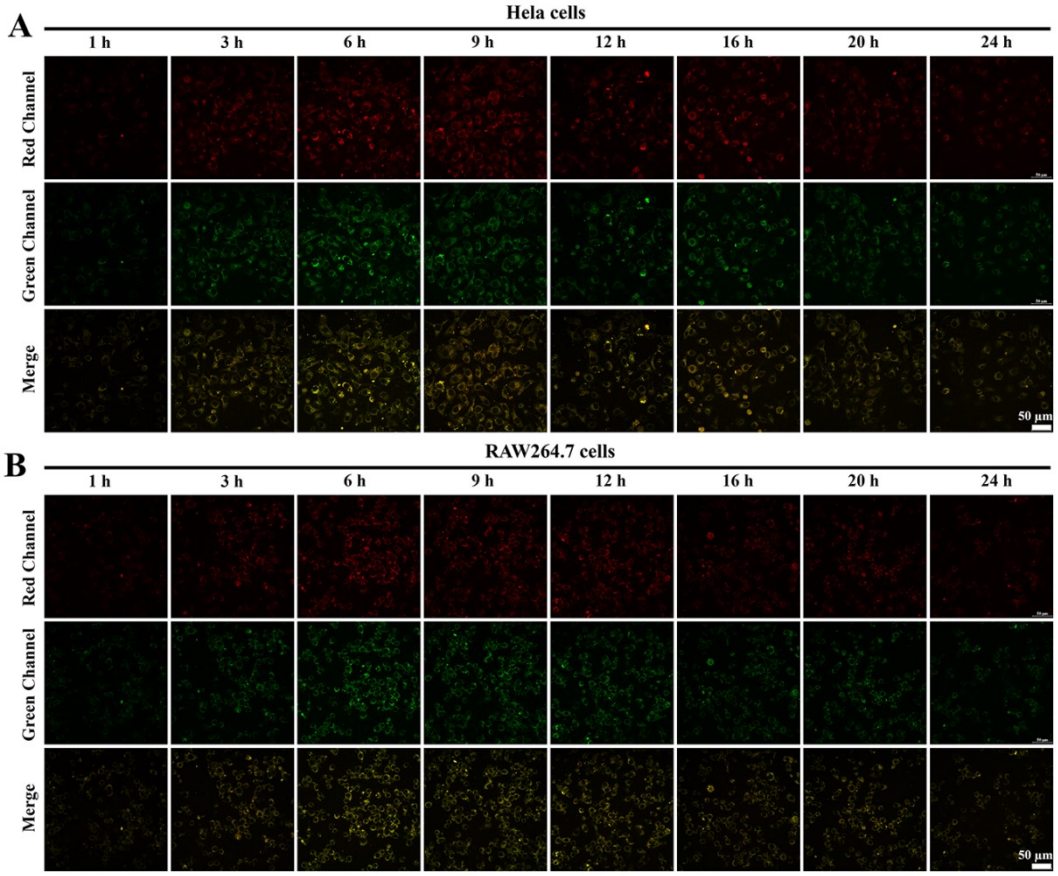


Fig. S27. Cellular uptake assay of G_3⊂CP5 in Hela cells and RAW264.7 cells.

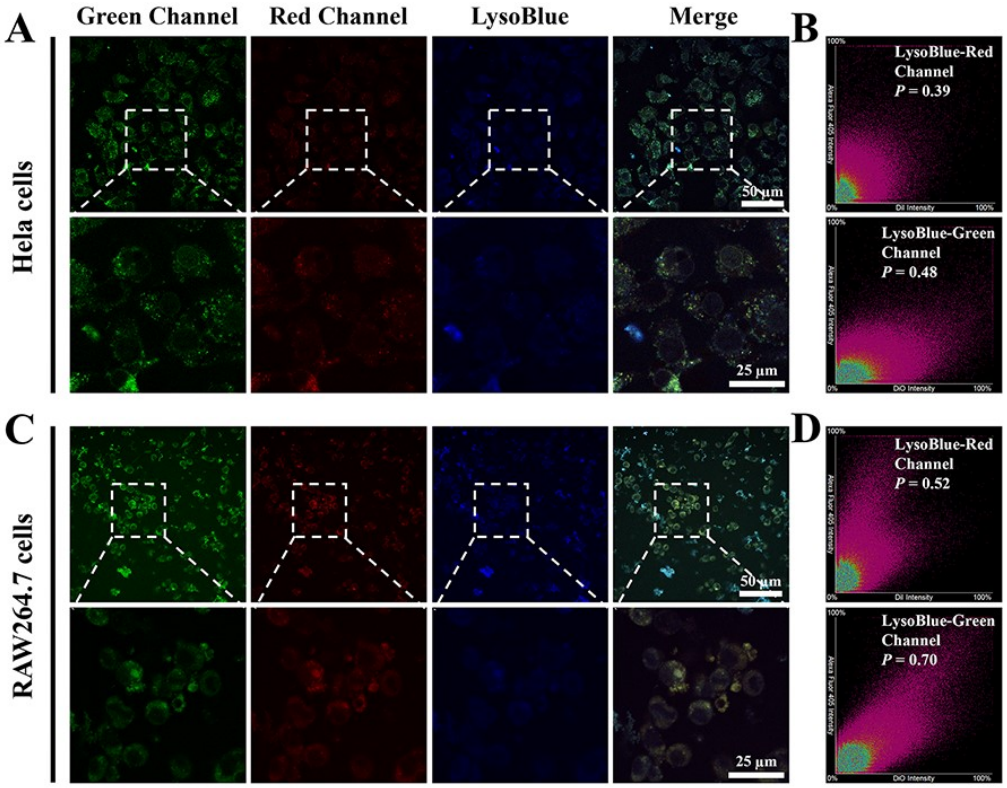


Fig. S28. (A) and (C) CLSM images of co-localization imaging in lysosomes. (C) and (D) The Pearson's correlation of lysosome targeting.

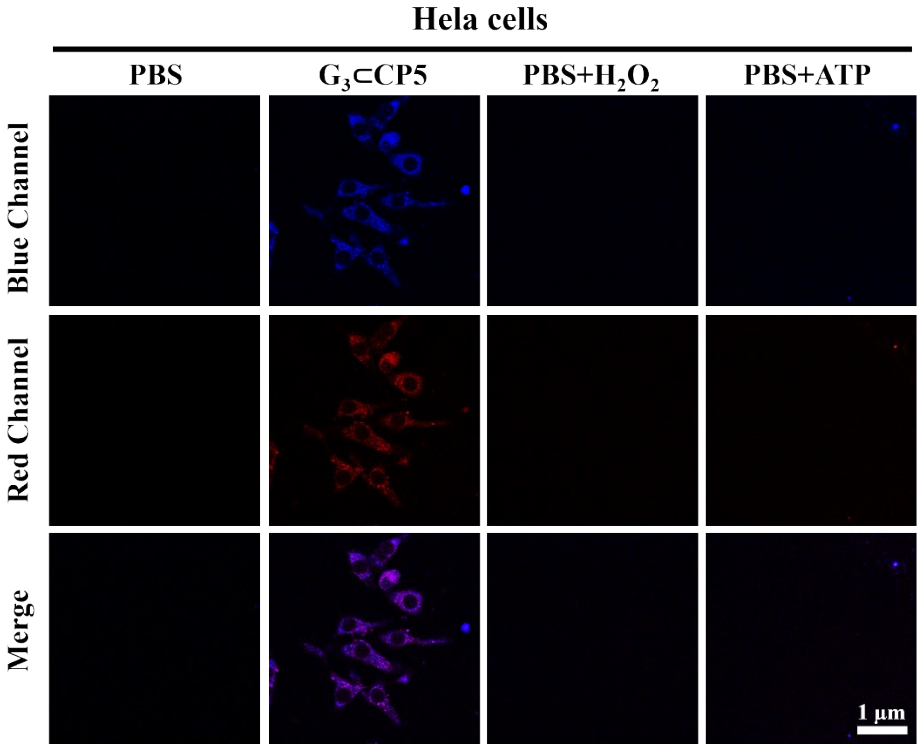


Fig. S29. Fluorescence imaging of PBS, G_3⊂CP5, PBS+H_2O_2, PBS+ATP in blue channel, red channel and merge respectively in Hela cells.

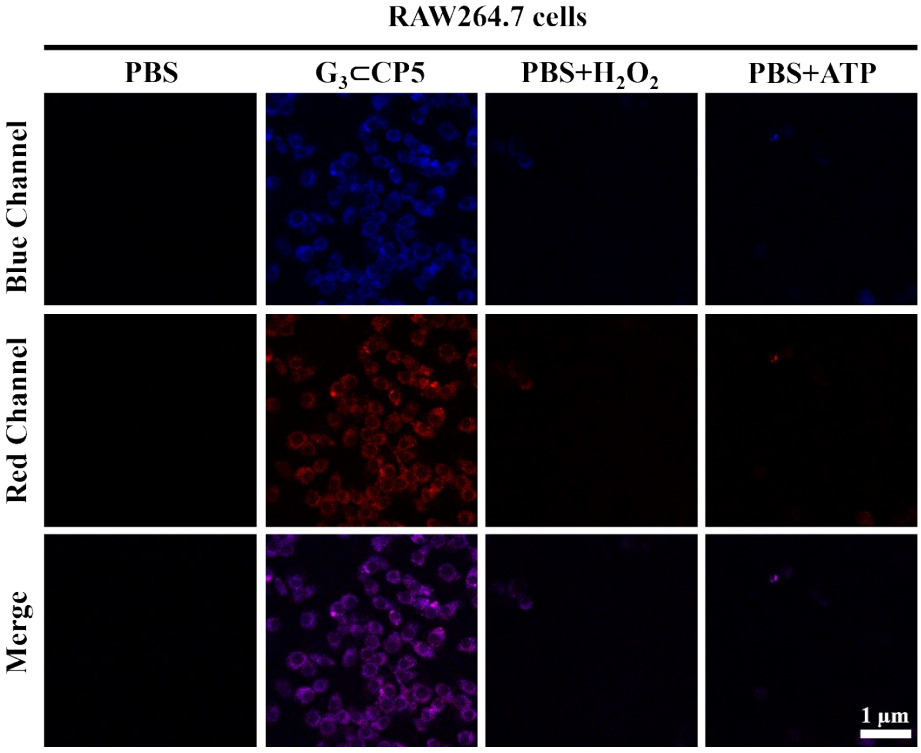


Fig. S30. Fluorescence imaging of PBS, G_3⊂CP5, PBS+H_2O_2, PBS+ATP in blue channel, red channel and merge respectively in RAW264.7 cells.

3. References

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