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Assembly of indole fluorophore in situ for hydrogen sulfide signaling through substrate triggered intramolecular reduction/cyclization cascade: A sensitive and selective probe in aqueous solution

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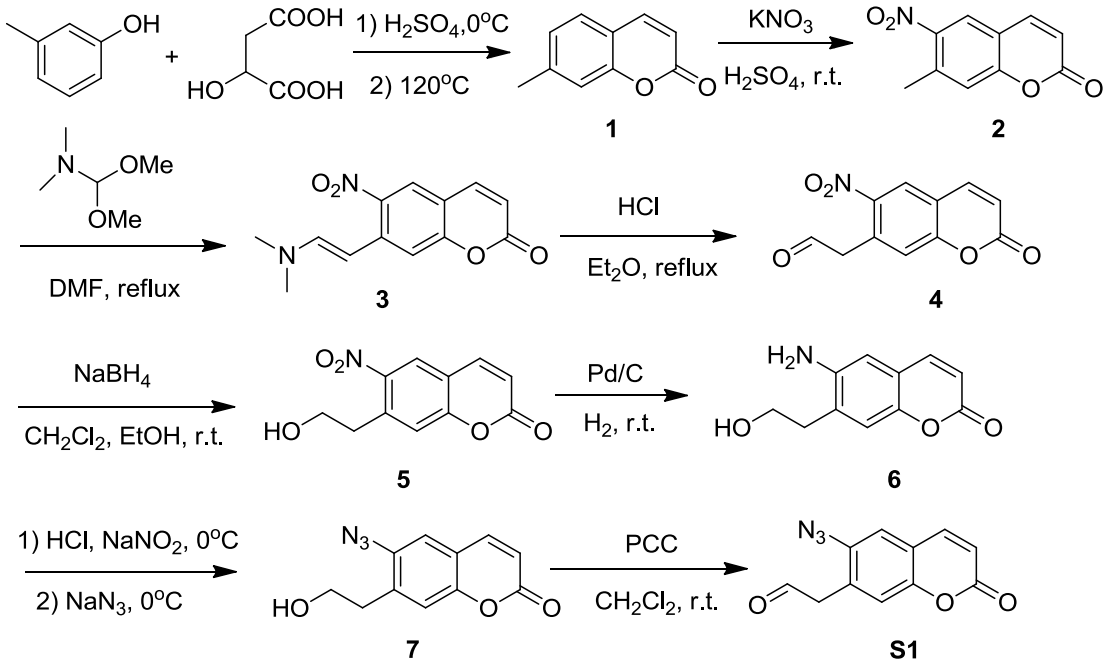
1. Materials and instruments

Materials: All chemical reagents were purchased from Aladdin Corporation and J&K Corporation without further purification. All the organic solvents were of analytical grade. DMF was distilled using CaH_2 to remove the water before used. Thin-layer chromatography was performed on silica gel plates. Silica gel P60 (Qingdao, 300-400 mesh) was used for column chromatography. Water was purified by a Milli-Q system.

Instruments: Fluorescence spectra were collected by a Varian Cary Eclipse Fluorescence Spectrometer. Absorption spectra were recorded by a Varian Cary 100 UV-Vis spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were taken in $CDCl_3$ and DMSO-d_6 at 25 ^{o}C on a Bruker AV-400 spectrometer in NMR Facility of East China University of Science and Technology (ECUST). The chemical shifts were reported in ppm (TMS as internal standard). Mass spectra were performed in the Analysis Center of East China University of Science and Technology (ECUST).

2. Synthesis



Scheme S1 The synthesis of probe **S1**

Synthesis of 7-methylcoumarin (compound 1)[1]

To a solution of 2-hydroxysuccinic acid (25.40 g, 0.19 mol) in m-cresol (20 mL, 0.19 mol) at 0 ^{o}C was added concentrated sulfuric acid (50 mL) dropwise. The reaction mixture was stirred at room temperature for 0.5 h, then heated up to 120 ^{o}C for 3 h until there was no bubble out. After cooling down to room temperature, the mixture was poured onto ice. The product was

extracted with EtOAc, washed with brine and dried over Na_2SO_4. After the solvent was removed in vacuo, the residue was recrystallized with 95% ethanol to give the title compound (12 g, 40%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (d, J = 9.6 Hz, 1H), 7.38 (d, J = 7.6 Hz, 1H), 7.16 (s, 1H), 7.11 (d, J = 7.6 Hz, 1H), 6.37 (d, J = 9.6 Hz, 1H), 2.47 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.08, 154.12, 143.46, 143.09, 127.57, 125.62, 116.96, 116.45, 115.35, 21.75.

Synthesis of 7-methyl-6-nitrocoumarin (compound 2)[2]

To a solution of compound 1 (9.31 g, 58.2 mmol) in concentrated sulfuric acid (60 mL) at 0 ^{o}C was added KNO_3 (7.08 g, 69.8 mmol) portionwise. The reaction mixture was stirred at 0 ^{o}C for 2 h, and poured onto ice. The mixture was filtered and the filter cake was washed with water (3×900 mL). The filter cake was dried off to give the title compound (8.4 g, 70%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.54 (s, 1H), 8.16 (d, J = 9.6 Hz, 1H), 7.56 (s, 1H), 6.63 (d, J = 9.6 Hz, 1H), 2.63 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.58, 156.02, 145.24, 143.53, 138.09, 125.84, 120.52, 117.88, 117.72, 20.66.

Synthesis of 7-formylmethyl-6-nitrocoumarin (compound 4)[2]

Compound 2 **(**6 g, 28.8 mmol**)** and N, N-Dimethylformamidedimethyl acetal (7.2 mL, 51.84 mmol) were refluxed under Ar in 60 mL of anhydrous DMF for 2 h. The reaction mixture was concentrated in vacuo to get the crude product 3, 60 mL of Et2O and 60 mL of 3 N aq. HCl were added and the mixture was refluxed for 2 h. 400 mL of CH_2Cl_2 and 400 mL of water were then added. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel flash chromatography with 1:3 PE / CH_2Cl_2 to give the title compound (2.1 g, 30%).^{1}H NMR (400 MHz, DMSO-d_6): δ 9.75 (s, 1H), 8.64 (s, 1H), 8.20 (d, J = 9.6 Hz, 1H), 7.57 (s, 1H), 6.67 (d, J = 9.6 Hz, 1H), 4.35 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 198.7, 159.4, 156.2, 145.1, 143.4, 133.6, 126.1, 121.6, 118.7, 118.5, 48.2.

Synthesis of 7-hydroxyethyl-6-nitrocoumarin (compound 5)[2]

To a solution of compound 4 (1.5 g, 6.4 mmol) in anhydrous CH_2Cl_2 (50 mL) at 0 ^{o}C was added $NaBH_4$ (242 mg, 6.4 mmol) suspension in EtOH (45 mL) dropwise over 30 min. The reaction was completed in 5 min, and quenched with 1N HCl. The solution was concentrated in vacuo. Then CH_2Cl_2 and water were added. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel flash chromatography with 1:20 EtOAc/ CH_2Cl_2 to give the title compound (902 mg, 60%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.43 (s, 1H), 8.13 (d, J = 9.6 Hz, 1H), 7.52 (s, 1H), 6.62 (d, J = 9.6 Hz, 1H), 4.82 (s, 1H), 3.68 (t, J = 6.4 Hz, 2H), 3.10 (t, J = 6.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.55, 155.62, 146.05, 143.39, 138.99, 125.50, 120.38, 118.01, 117.81,

61.07, 35.95.

Synthesis of 7-hydroxyethyl-6-aminecoumarin (compound 6)[2]

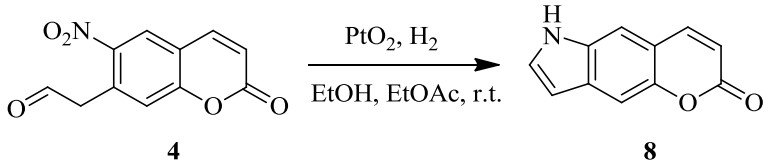
Compound 5 (900 mg, 3.8 mmol) was dissolved in 50 mL of MeOH / EtOAc (1:1). The mixture was hydrogenated at 1 atm of H_2 using Pd/C (45 mg) as the catalyst. After completion of the reaction as evidence by TLC, the solution was filtered on diatomite and the diatomite cake was washed with methanol. Then the filtrate was concentrated in vacuo to give the title compound (624 mg, 80%) without any further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.87 (d, J = 9.2 Hz, 1H), 7.04 (s, 1H), 6.81 (s, 1H), 6.31 (d, J = 9.2 Hz, 1H), 5.06 (s, 2H), 3.66 (br, 2H), 2.70 (t, J = 6.8 Hz, 2H).

Synthesis of 7-hydroxyethyl-6-azidecoumarin (compound 7)[3]

To a 0 ^{o}C solution of compound 6 (400 mg, 1.95 mmol) in EtOAc (1 mL) and water (1 mL) was added concentrated hydrochloric acid (2 mL). The mixture was stirred for 15 min. To this solution was added a solution of sodium nitrite (245 mg, 3.51 mmol) in water (0.74 mL) over 5 min. After completion of the addition, the reaction was stirred for an additional 1.5 h. A solution of sodium azide (230 mg, 3.51 mmol) in water (0.7 mL) was added over 5 min. After 1 h, the reaction mixture was diluted with water (100 mL), extracted with ethyl acetate (2×50 mL). The combined organic layer was washed with dilute sodium hydroxide solution, then with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel flash chromatography with 1:30 EtOAc/ CH_2Cl_2 to give the title compound (225 mg, 50%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.07 (d, J = 9.6 Hz, 1H), 7.67 (s, 1H), 7.32 (s, 1H), 6.50 (d, J = 9.6 Hz, 1H), 4.73 (t, J = 5.2 Hz, 1H), 3.61 (q, J = 6.4 Hz, 2H), 2.77 (t, J = 6.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.32, 150.89, 143.75, 135.91, 134.90, 118.99, 118.40, 117.77, 116.78, 60.70, 34.81. HRMS (EI): Calcd for $C_{11}H_9N_3O_3$ 231.0644; Found, 231.0646.

Synthesis of 7-formylmethyl-6-azidecoumarin (compound S1)[4]

To a solution of compound 7 (231 mg, 1 mmol) in anhydrous CH_2Cl_2 (30 mL) was added PCC (323 mg, 1.5 mmol). Then the mixture was stirred at room temperature for 4 h and filtered on diatomite. Then the diatomite cake was washed with CH_2Cl_2. To the filtrate was added water (100 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel flash chromatography with 1:2 PE/ CH_2Cl_2 to give the title compound (45 mg, 20%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.78 (s, 1H), 7.70 (d, J = 9.6 Hz, 1H), 7.27 (s, 1H), 7.20 (s, 1H), 6.50 (d, J = 9.6 Hz, 1H), 3.81 (br, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 196.90, 160.04, 150.96, 141.93, 135.55, 128.71, 120.09, 119.06, 117.86, 116.19, 46.11. HRMS (EI): Calcd for $C_{11}H_7N_3O_3$ 229.0487; Found, 229.0486.



4 **8**

Scheme S2 The synthesis of compound 8

Synthesis of compound 8[2]

To a solution of compound 4 (300 mg, 1.3 mmol) in 20 mL of EtOH / EtOAc (1:1) was added PtO_2 (20 mg, 0.09 mmol) as the catalyst. The mixture was hydrogenated at 1 atm of H_2 for 4h. The reaction mixture was concentrated in vacuo. The residue was purified by silica gel flash chromatography with 1:5 PE/ CH_2Cl_2 to give the title compound (168 mg, 69%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.53 (s, 1H), 8.15 (d, J = 9.6 Hz, 1H), 7.75 (s, 1H), 7.65 (s, 1H), 7.53 (s, 1H), 6.56 (s, 1H), 6.32 (d, J = 9.6 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.45, 147.91, 146.05, 133.55, 131.13, 130.98, 114.23, 113.30, 110.72, 105.79, 101.91.

3. Methods and data

(1) The pH titration

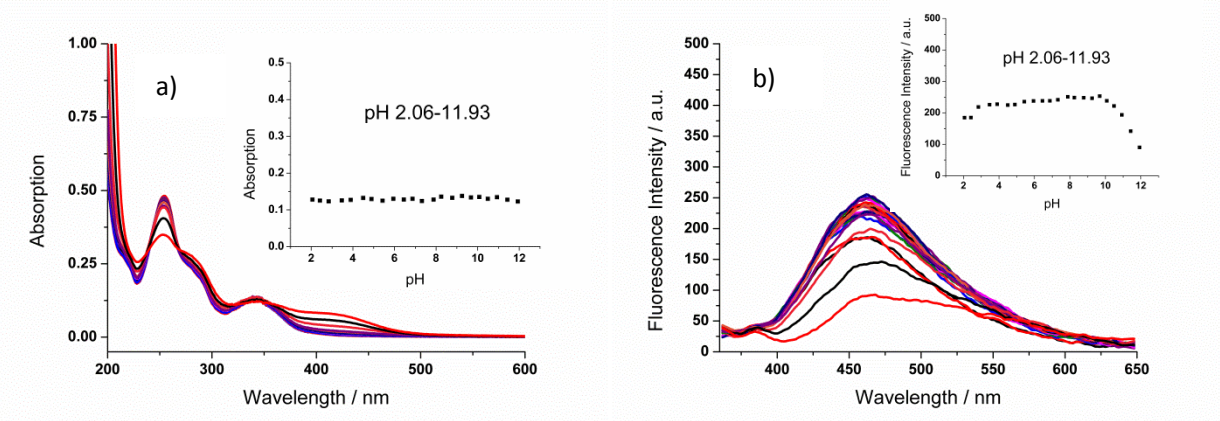


Fig.S1 (a) Absorption response of **S1** (30 μM) to various pH in water with 5% CH_3CN as the co-solvent. The insert figure was about the absorption responses of the probe **S1** to different pH. (b) Fluorescence response of **S1** (30 μM) to various pH in water with 5% CH_3CN as the co-solvent. The insert figure was about the fluorescence responses of the probe **S1** to different pH. The excitation wavelength was 340 nm. pH 2.06-11.93, slit:10 nm, 10 nm.

(2) The absorption and emission spectrum of the reaction product compound **8**

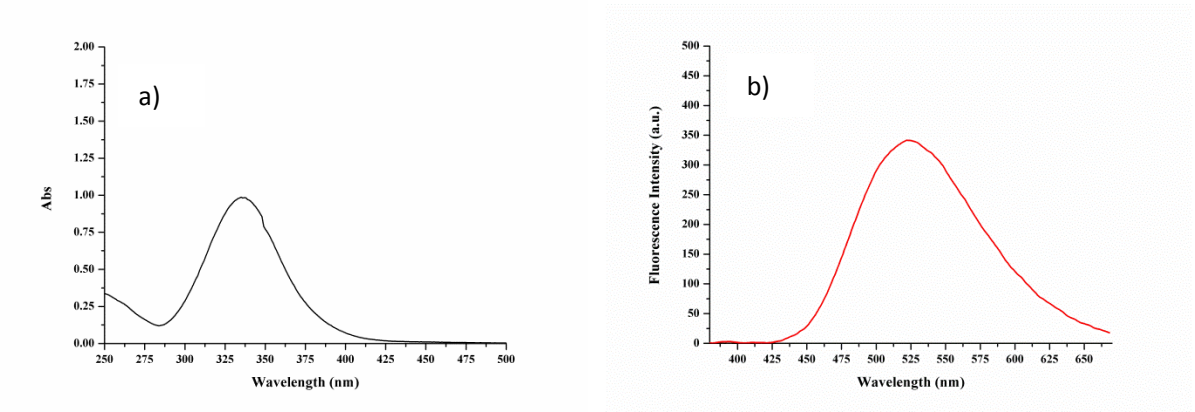


Fig.S2 (a) The absorption spectrum of the reaction product compound **8**. (b) The emission spectrum of compound **8** (60 μM) in the water. The excitation wavelength was 340 nm. Slit: 5 nm, 10 nm.

(3) Fluorescence changes of S1 to different concentrations of NaSH in the water (with 5% CH_3CN).

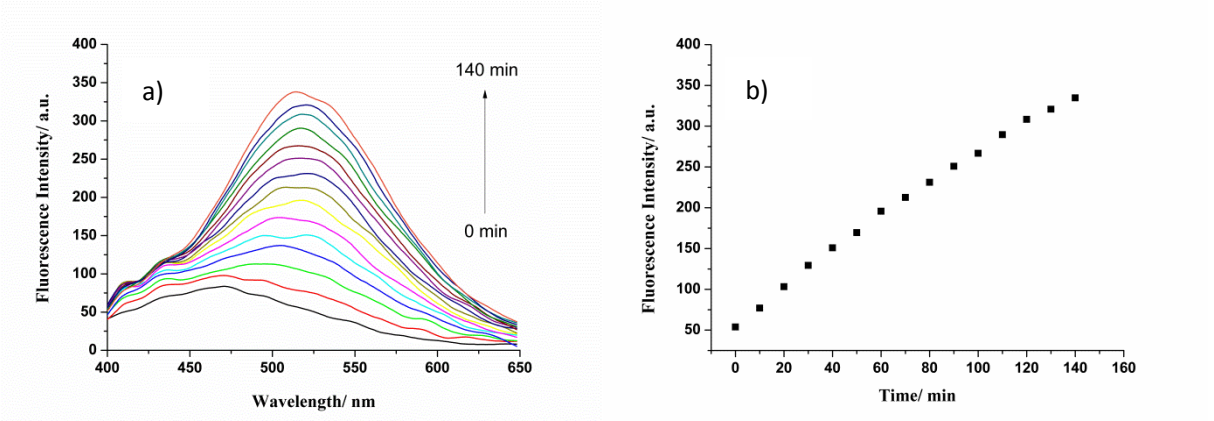
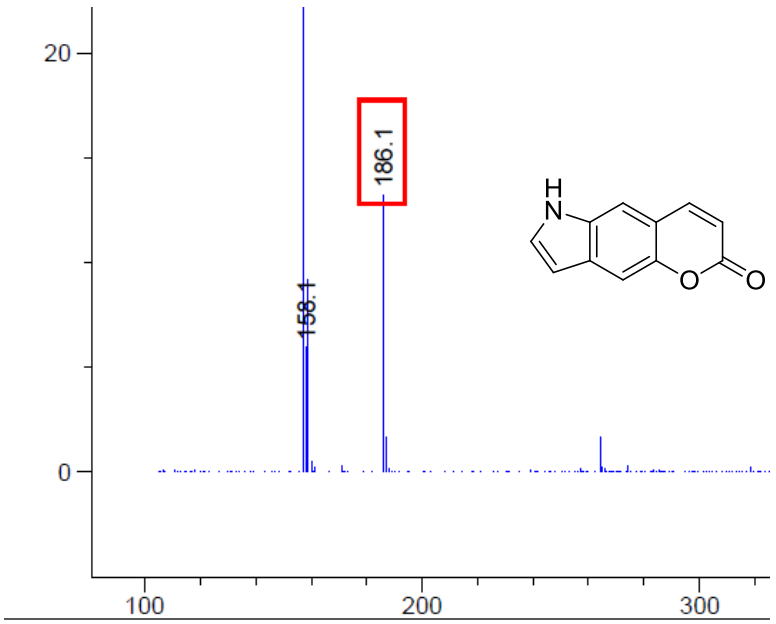


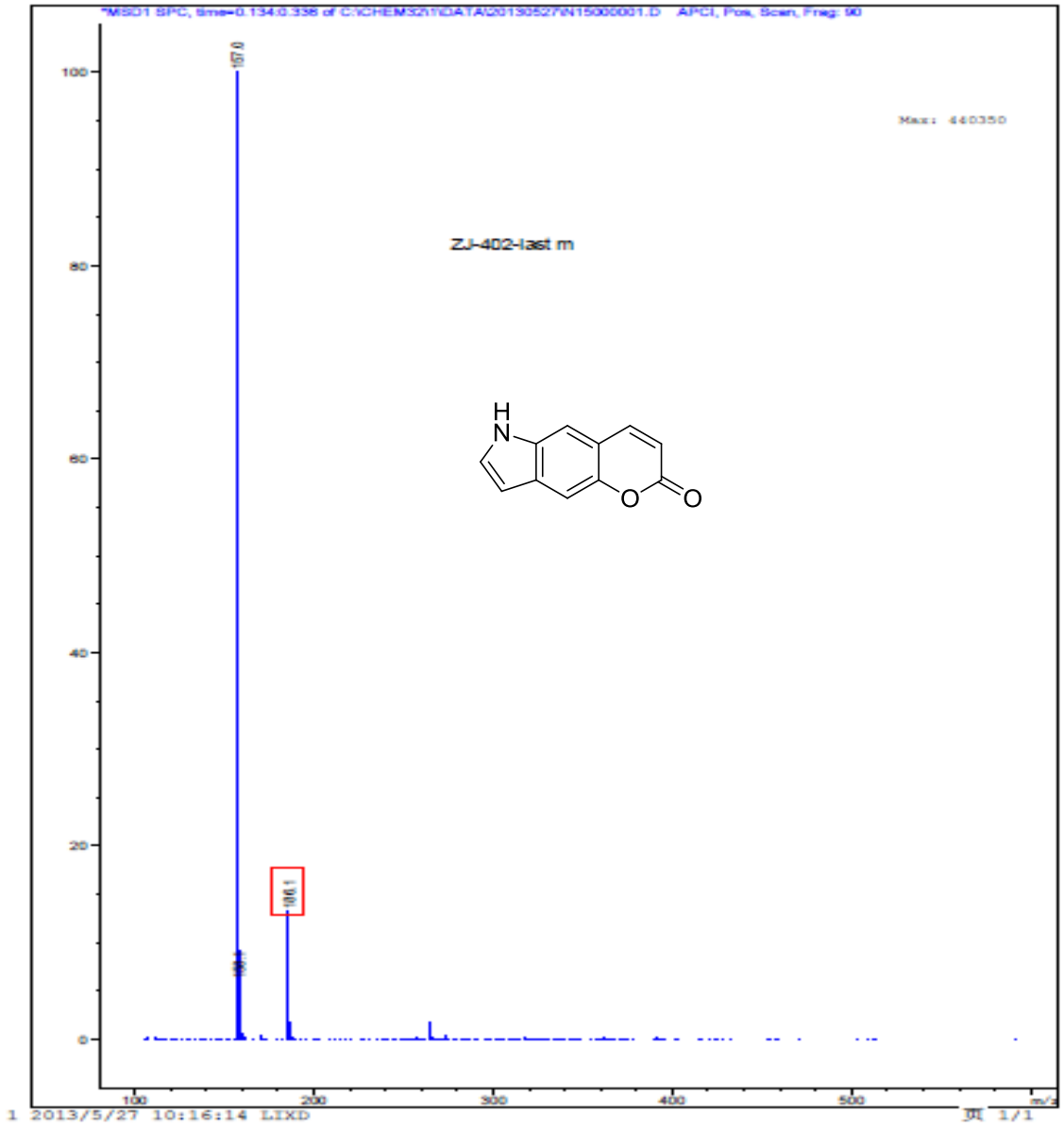
Fig.S3 (a) The fluorescence responses of **S1** (15 μM) with H_2S (50 μM) in the water with 5% CH_3CN as a co-solvent at 25°C. The data were recorded every 10 min. (b) Fluorescence responses ($I_{520\ nm}$) of **S1** (15 μM) to H_2S (50 μM). Excitation wavelength was 340 nm. Slit: 10 nm, 10 nm.

(4) The detection limit

The concentration of H_2S ranges from nano- to millimolar levels in the blood. The detection limit was calculated according to the previous literature[5]. The fluorescence intensity of S1 was measured by ten times and the standard deviation was calculated. The fluorescence intensity at 520 nm was plotted as a concentration of H_2S. By using detection limit $3\sigma/k$, the detection limit was calculated as 0.22 μM. σ is the standard deviation of the fluorescence intensity of S1, k is the slope between the fluorescence intensity at 520 nm versus the H_2S concentration.

(5) MS (ESI) of the product resulting from the reaction between the probe **S1** and H_2S.


20
0
186.1
158.1
100
200
300
H
N
O
O


ZJ-402-last m
100
80
60
40
20
0
157.0
186.1
100
200
300
400
500
m/z
1 2013/5/27 10:16:14 LIXD
1/1
H
N
O
O

The reaction was performed in CH_3OH and water.

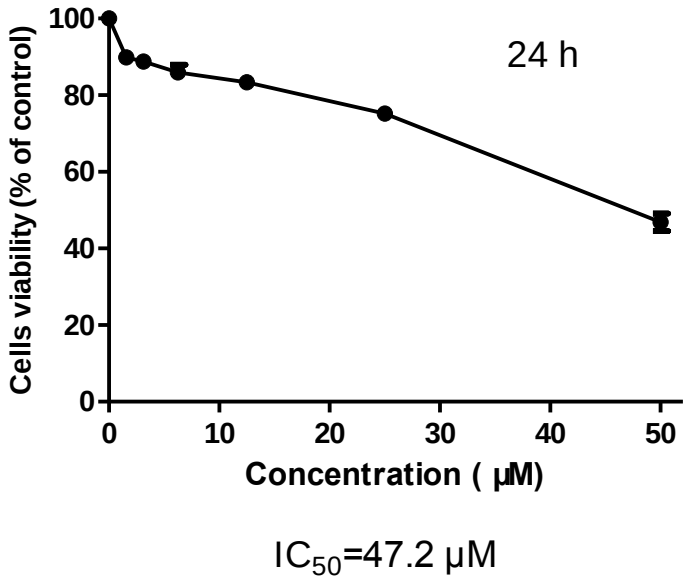
(6) Cell Culture and Imaging

HeLa cells were obtained from American Type Culture collection, and grown in Dulbecco's modification of Eagle's medium Dulbecco (DMEM/high: with 4500 mg/L Glucose, 4.0 mM L-Glutamine, and 110 mg/L Sodium Pyruvate) supplemented with 10% foetal bovine serum (FBS). Cells were incubated in a 5% CO_2 humidified incubator at 37 ^oC and typically passaged with sub-cultivation ratio of 1:4 every two days.

For fluorescence microscopy, HeLa cells were seeded in 24-well culture plate for one night. The cells were first incubated with **S1** (15 μM) for 20 min at 37 ^oC and washed with phosphate buffer (pH 7.4). Then the cells were treated with or without NaHS (250 μM) for another 40 min at 37 ^oC and washed with phosphate buffer (pH 7.4) for three times. Fluorescence imaging was performed with Nikon Ti-S with Xenon lamp. Exposure time is 20 s for green emission.

(7) Cell viability assay

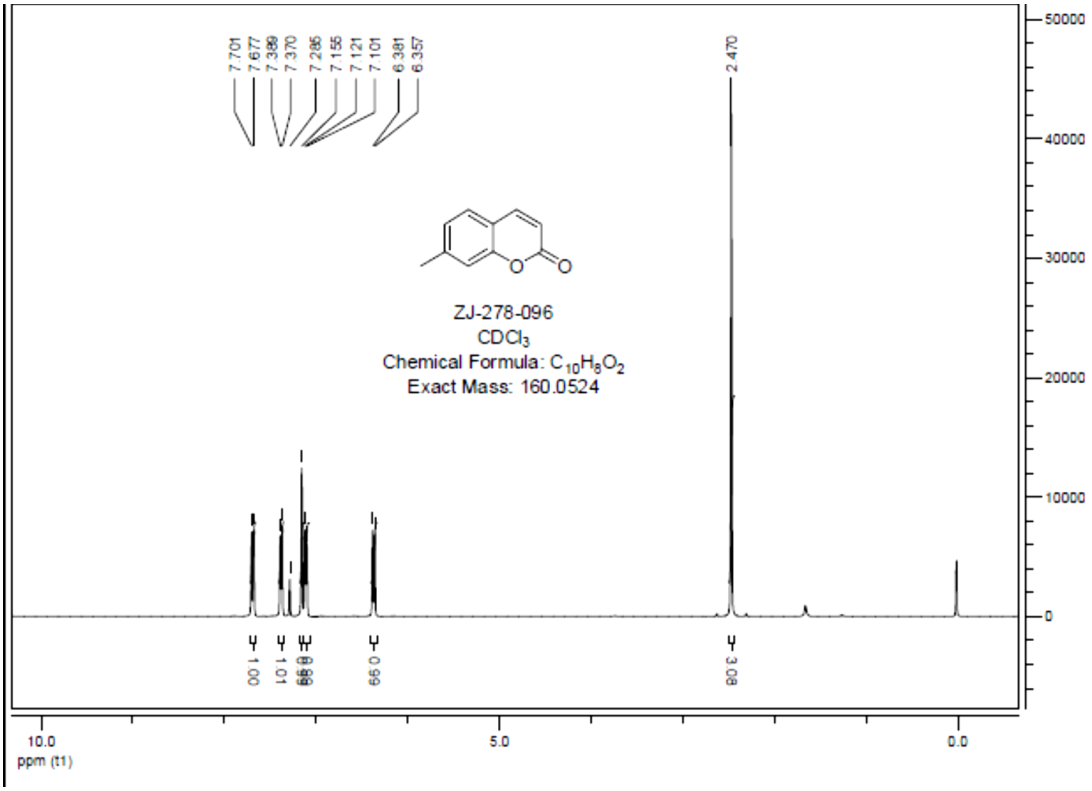
MCF-7 cells (1×10^4) were seeded into a 96-well plate and treated with various concentrations of **S1** for 24 h. Cells were irradiated under light emitted from a 100-watt quartz-halogen lamp.The fluence rate was 240 mW/cm^2 measured by a photo radiometer (Delta Ohm, Padua, Italy). Cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The IC_{50} value was 47.2 μM.



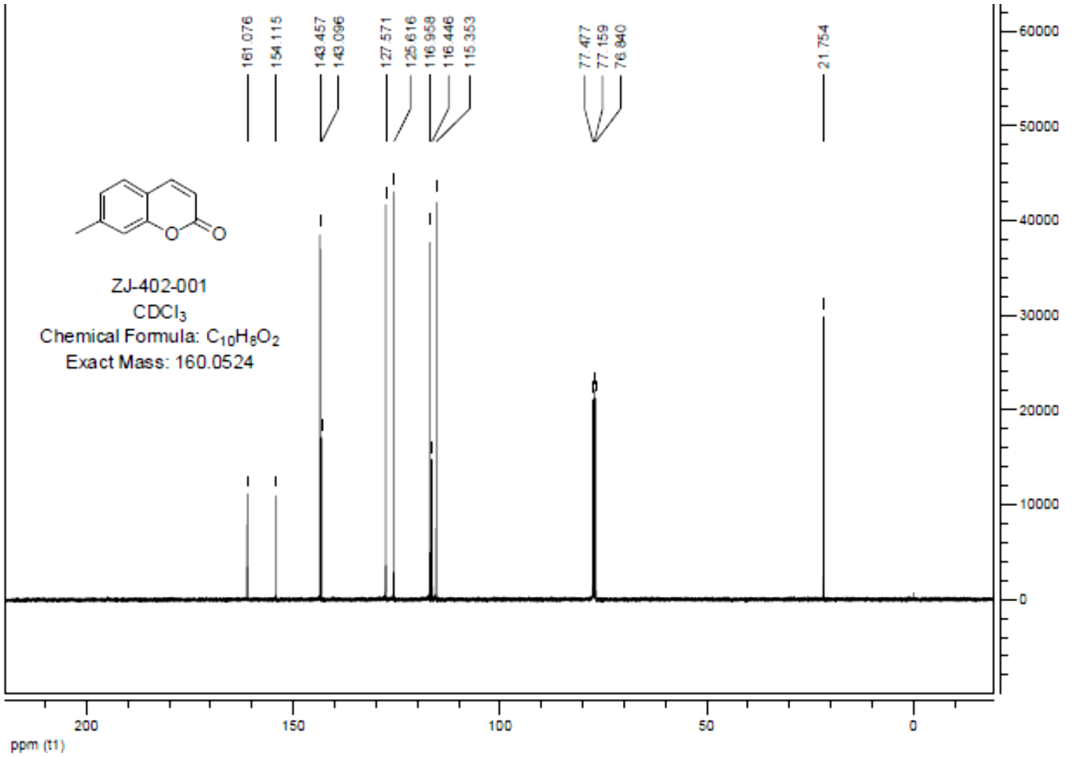
IC_{50}=47.2 μM

4. **NMR and MS spectra**

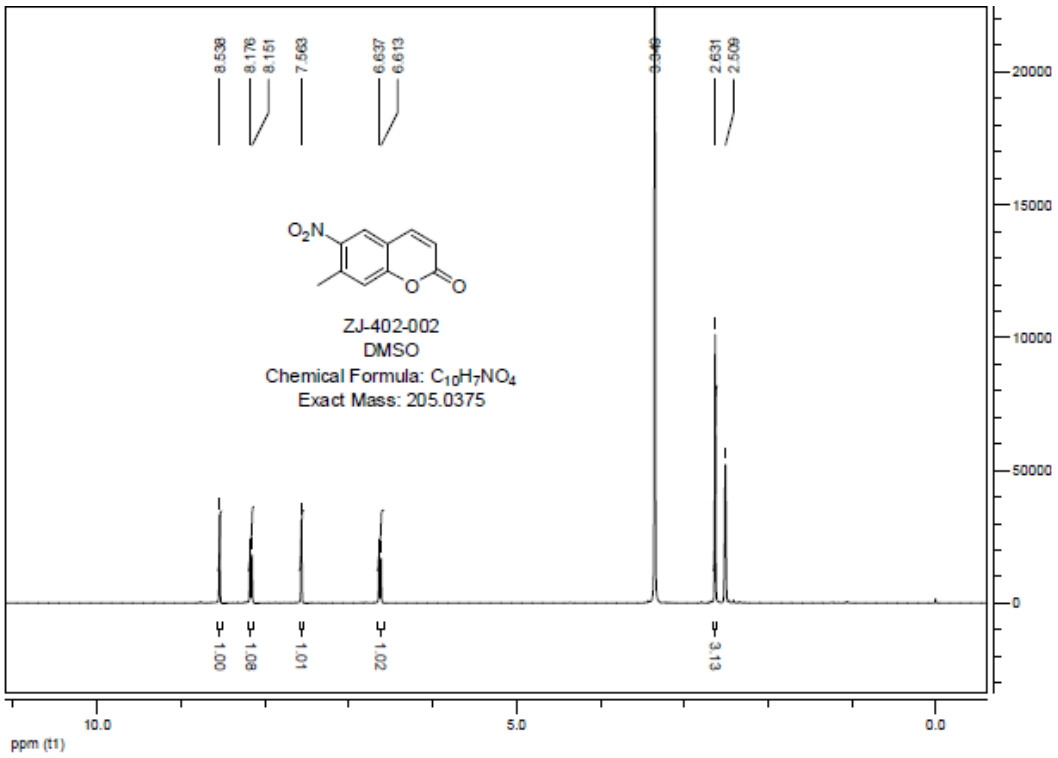
Compound 1-^{1}H NMR



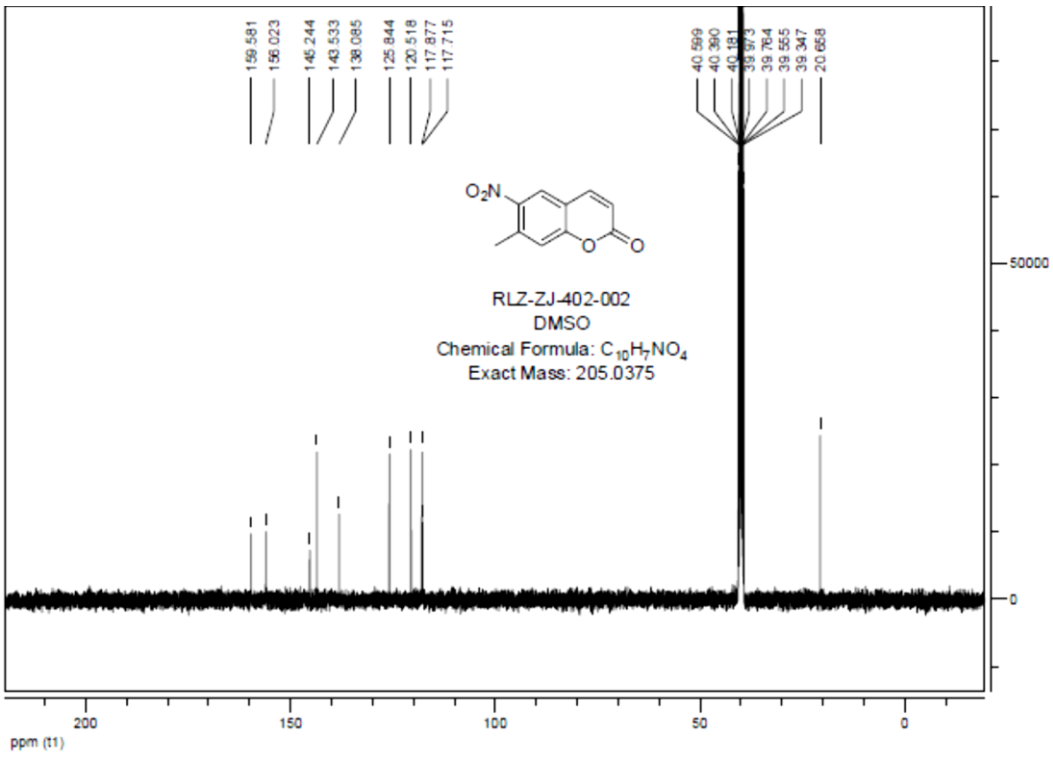
Compound 1-^{13}C NMR



Compound 2-^{1}H NMR



Compound 2-^{13}C NMR



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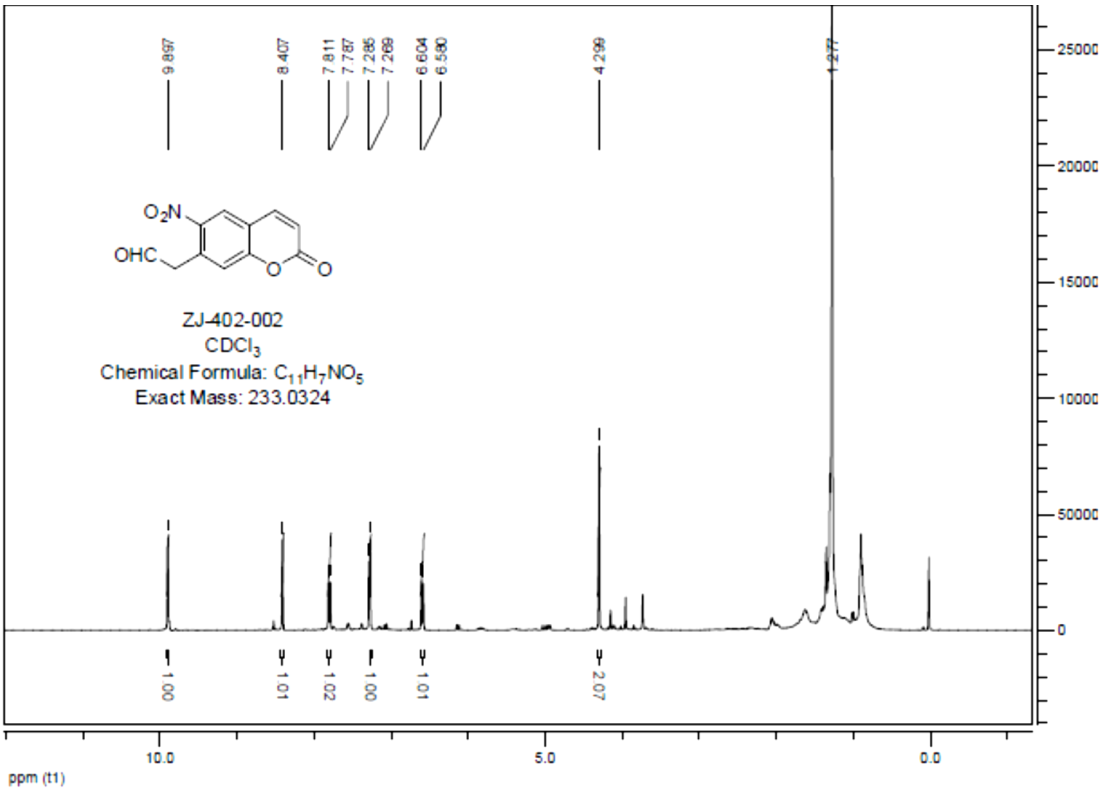
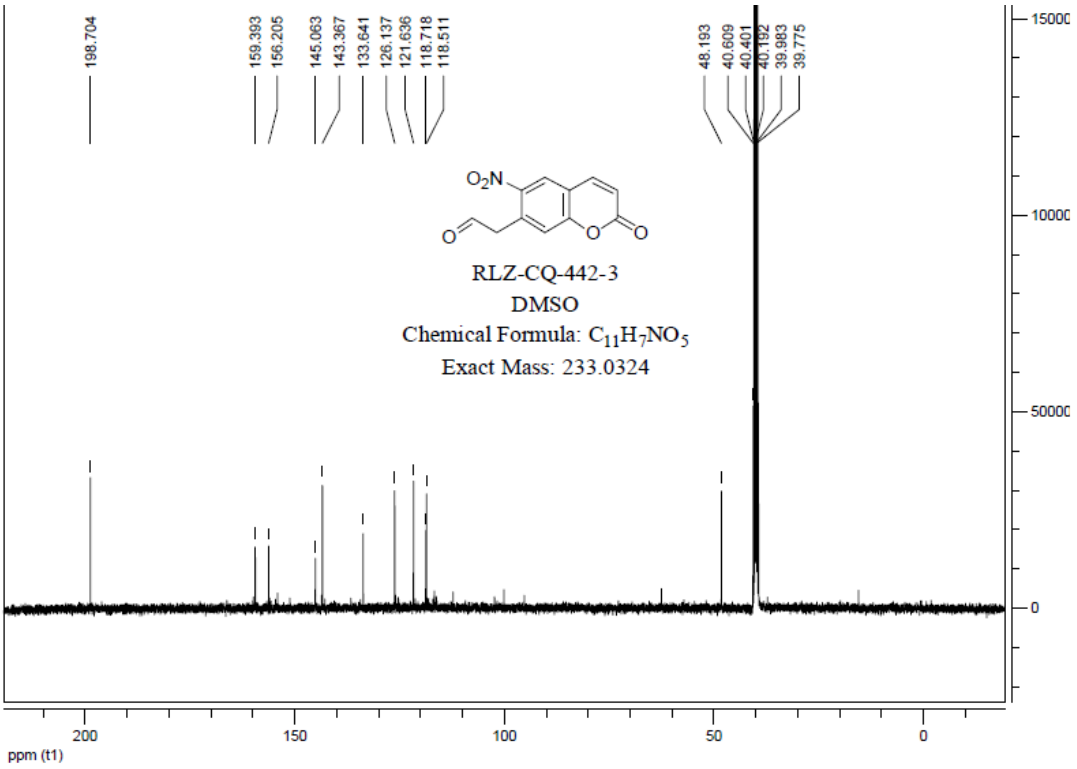
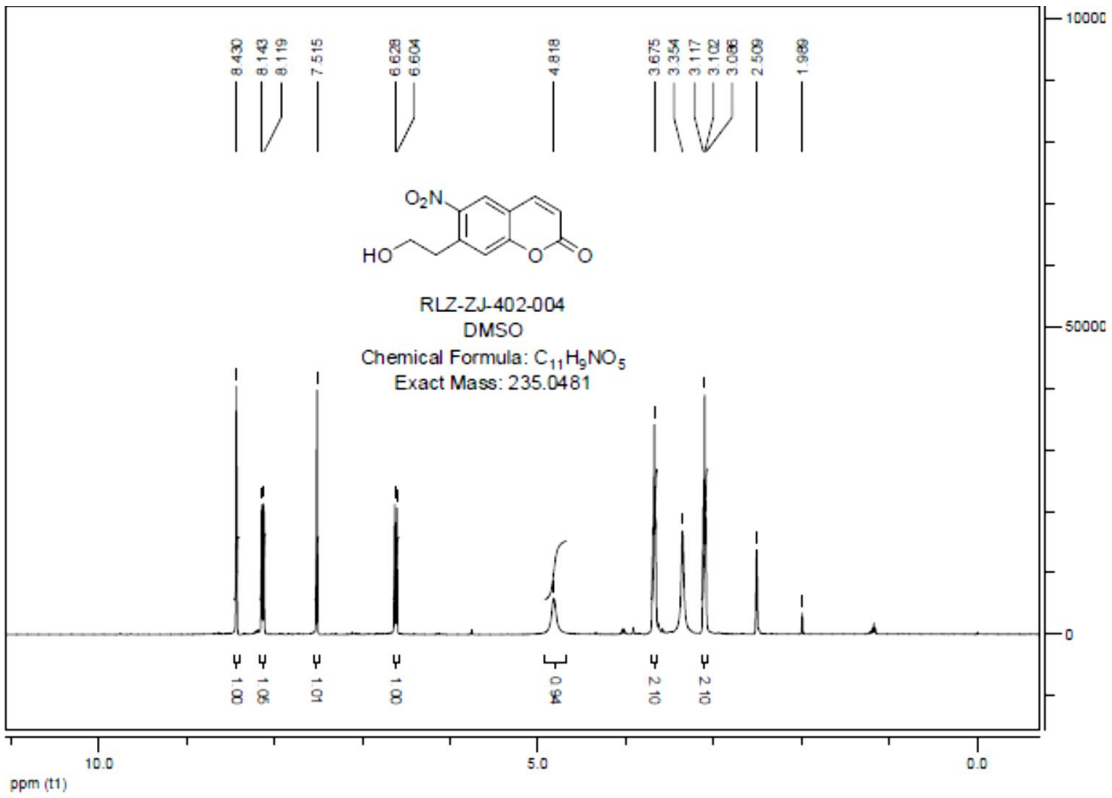


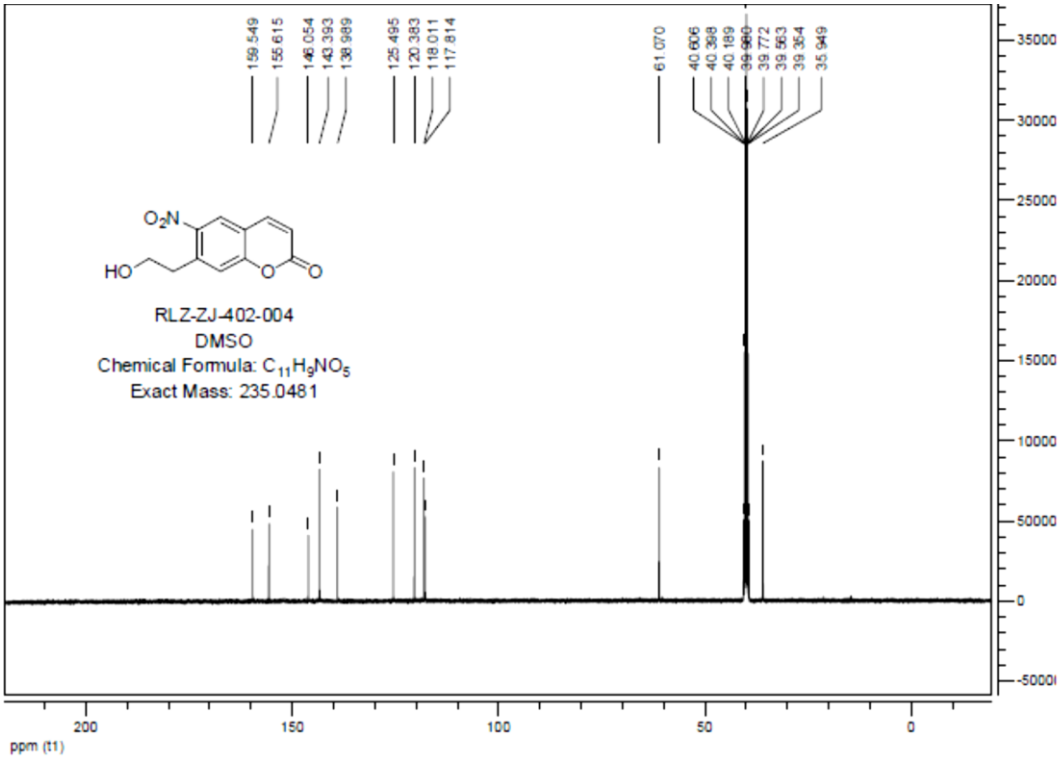
Compound 4-^{13}C NMR



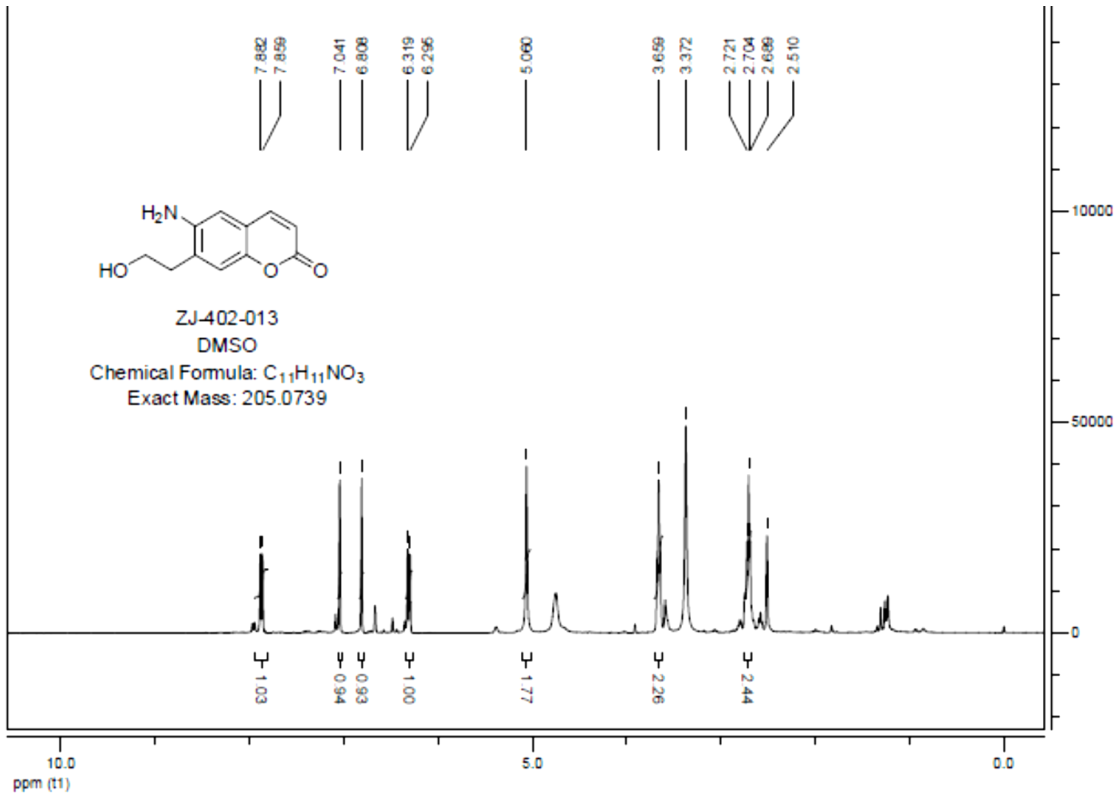
Compound 5-^{1}H NMR



Compound 5-^{13}C NMR



Compound 6-^{1}H NMR



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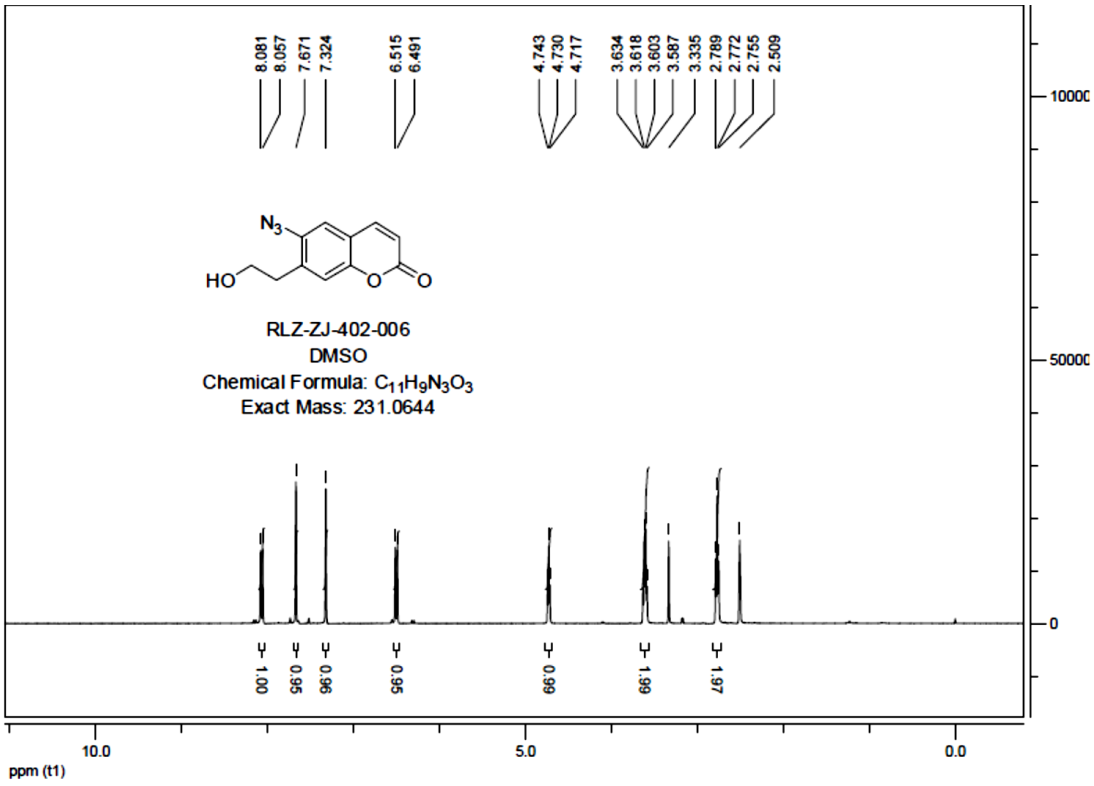

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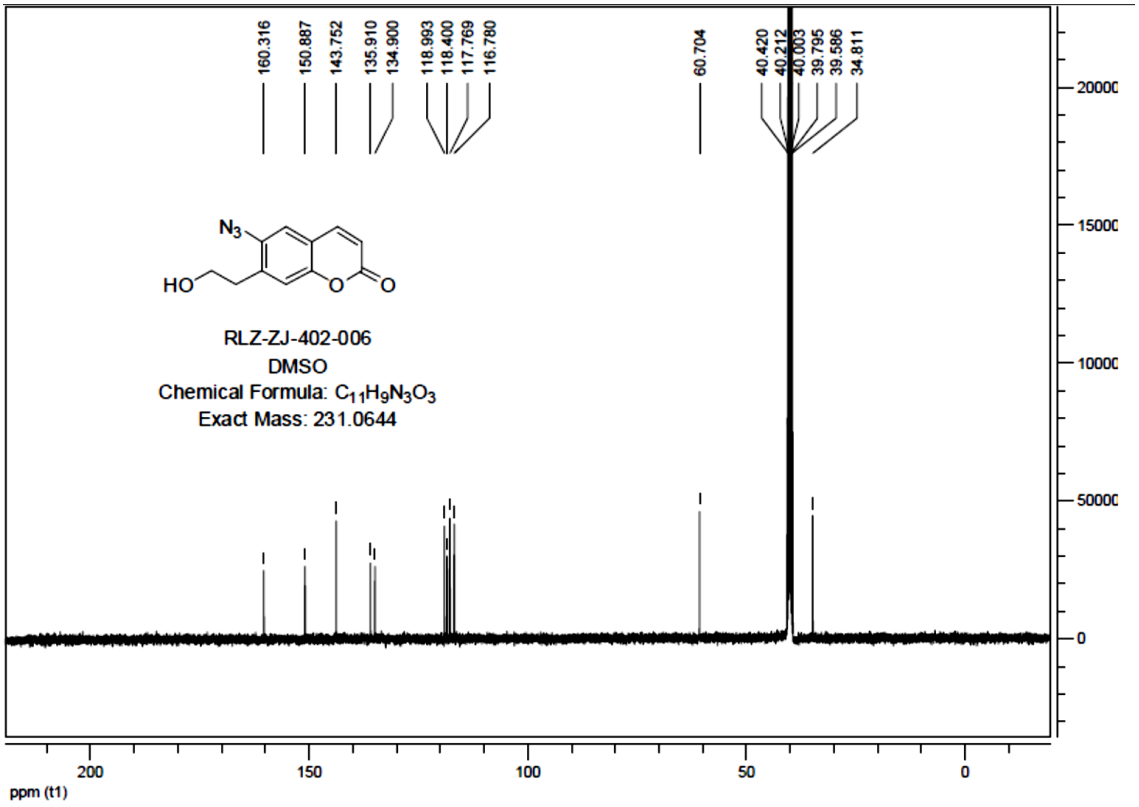
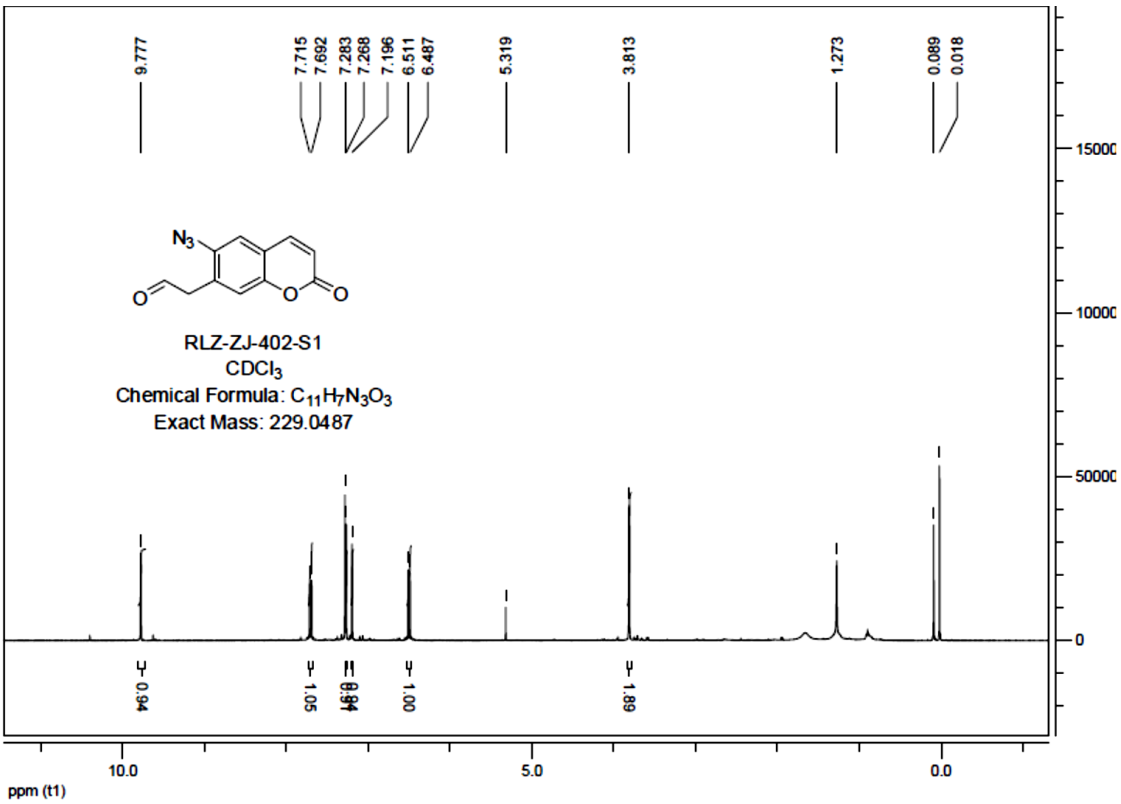
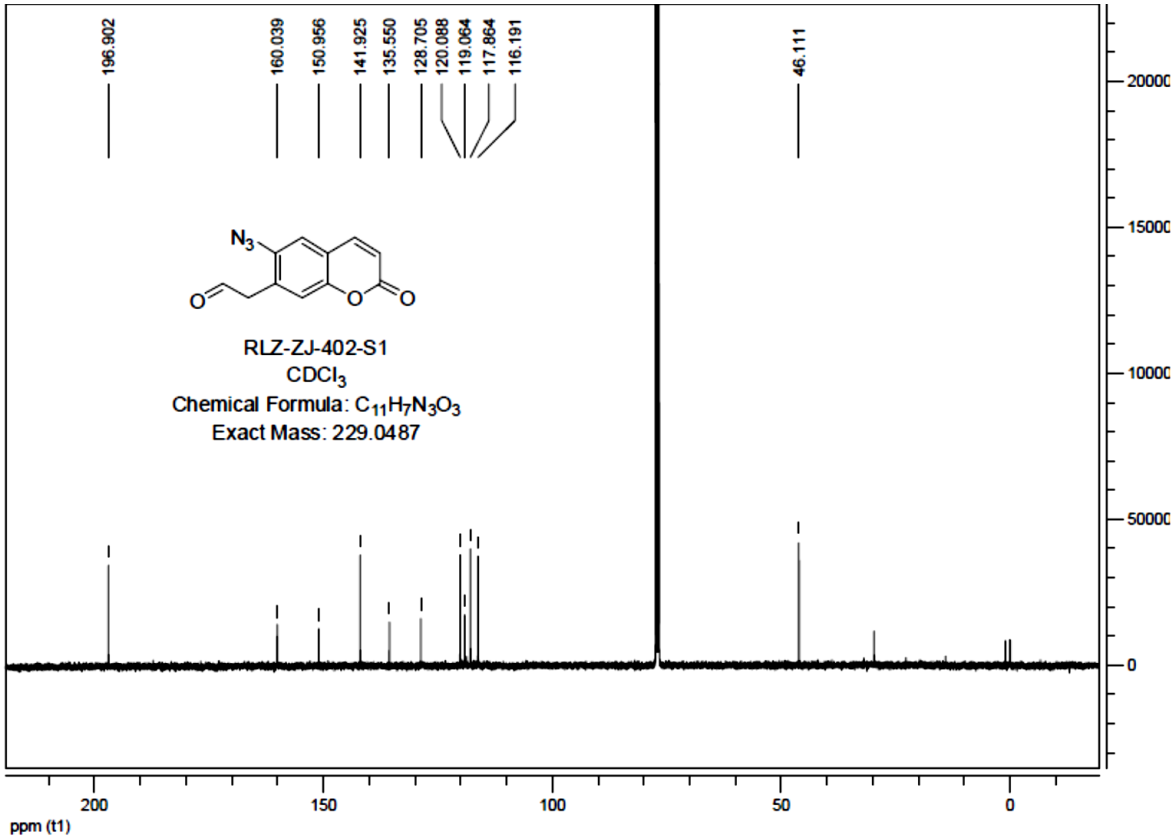


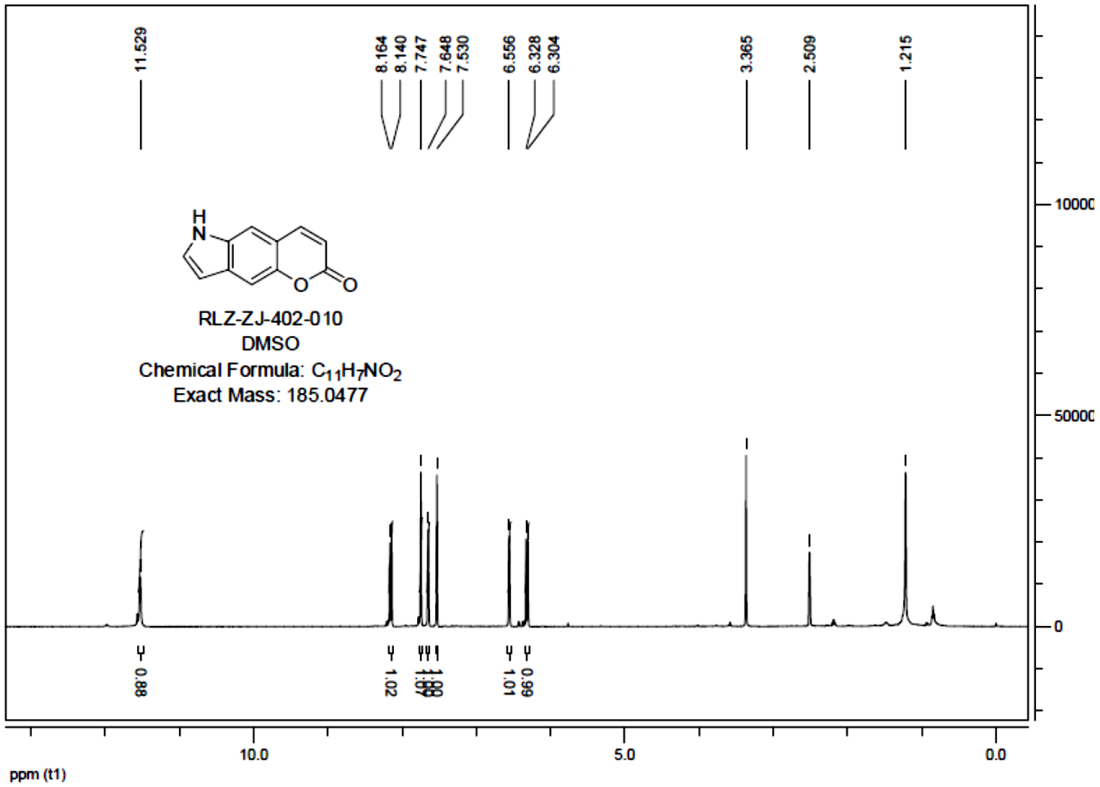
Compound S1-^{1}H NMR



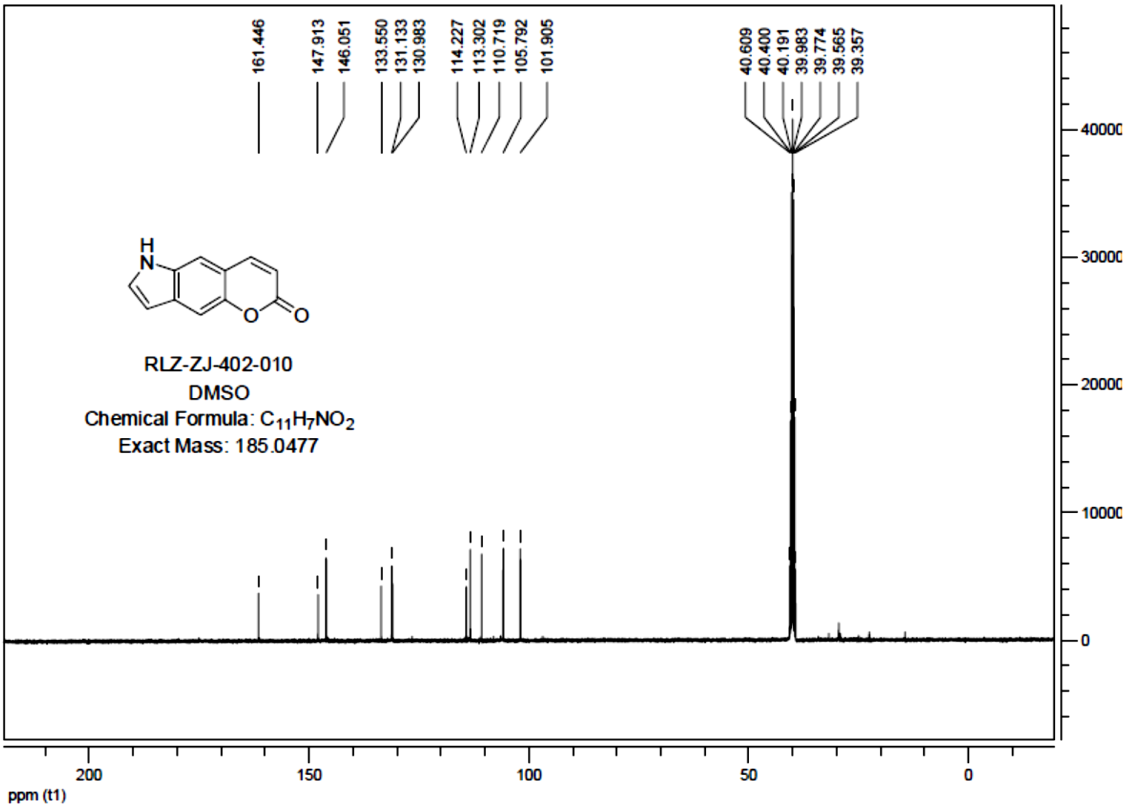
Compound S1-^{13}C NMR



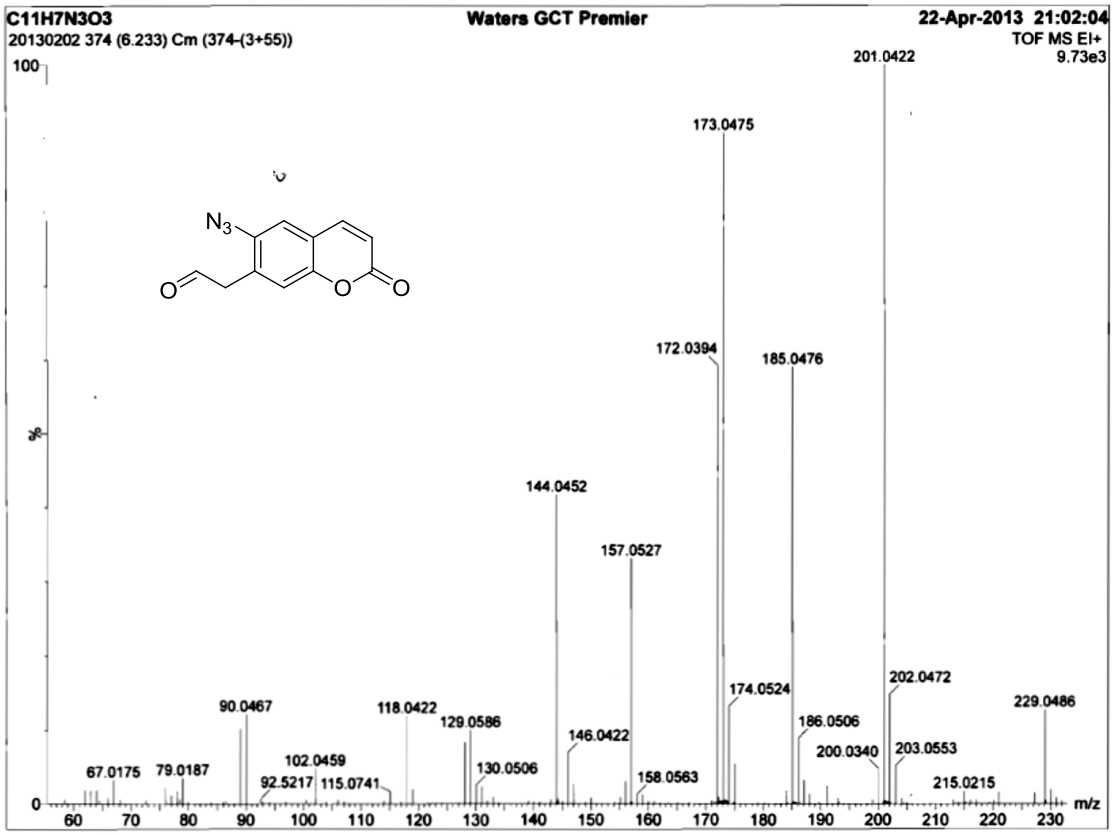
Compound 8-^{1}H NMR



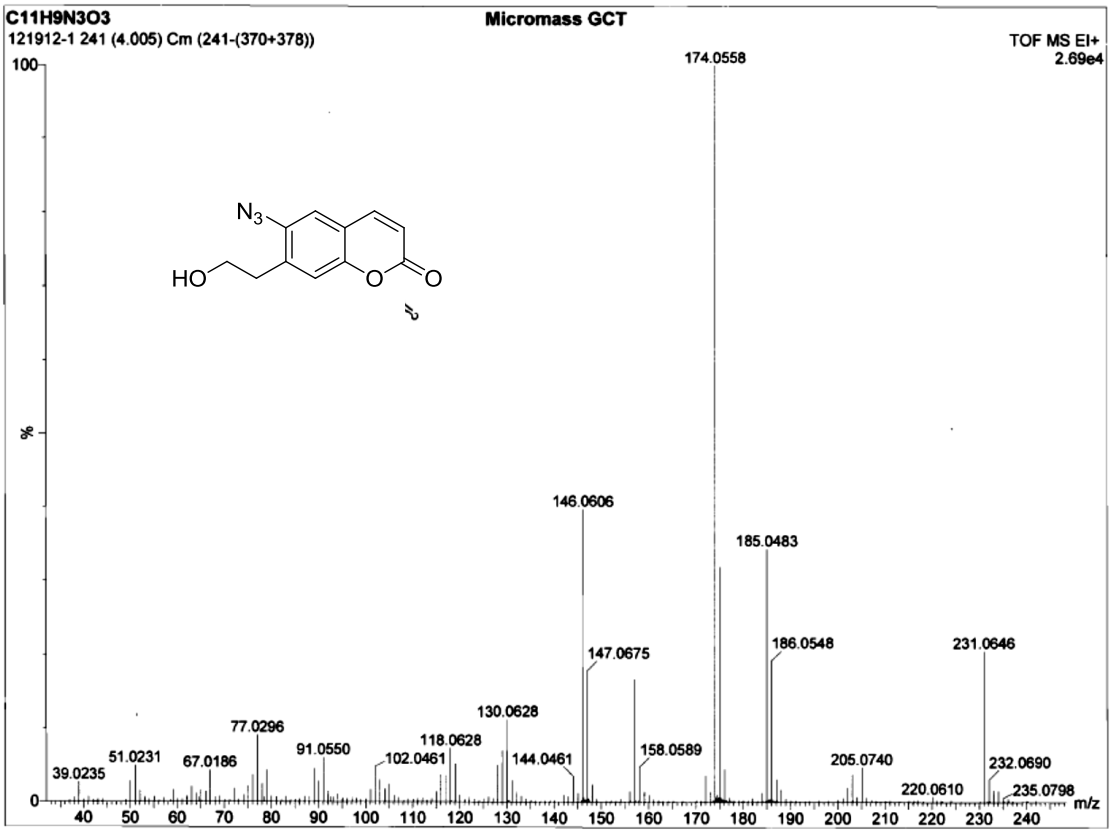
Compound 8-^{13}C NMR



Compound S1-HRMS (EI)



Compound 7-HRMS (EI)



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