

Electronic Supplementary Information (ESI)

for

**CO release with ratiometric fluorescence changes: A promising
visible-light-triggered metal-free CO-releasing molecule**

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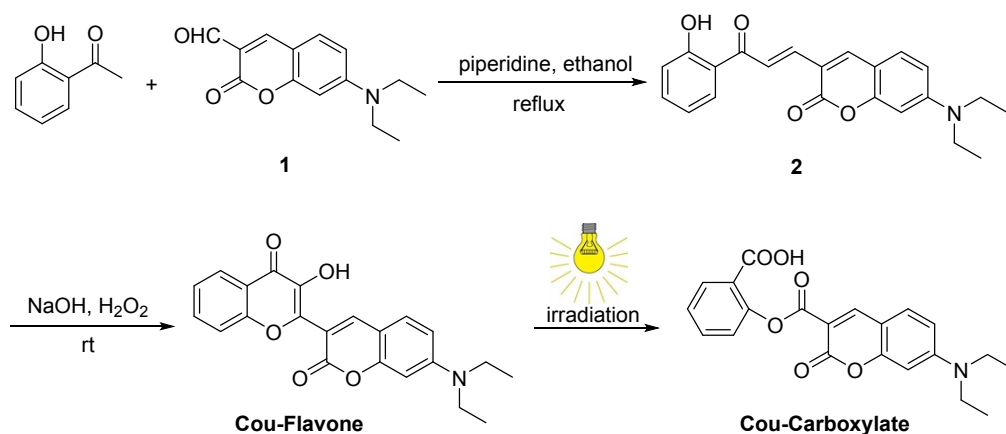
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1. Experimental

1.1. Materials and instruments

Unless otherwise stated, all chemicals and reagents were purchased from commercial suppliers and used as received. Carboxy-hemoglobin (COHb) detection kit was purchased from Leagene biotechnology company (Beijing, China) and was used in accordance with the instructions provided. Reactive oxygen species and reactive nitrogen species (ROS/RNS) were prepared and used freshly according to our published procedures.^[1-3] The wild zebrafish (3 days old) were purchased from China Zebrafish Resource Center, Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). Kunming mice (weight 20 ± 2 g) were purchased from Wuhan Center for Disease Control and Prevention. All animal experiments were proceeded with the permission of Wuhan Animal Ethics Committee and were carried out strictly in accordance with the requirements of the National Act on the use of experimental animals (China). NMR spectra were recorded on a Varian 400 NMR spectrometer. Mass spectra were collected on a Thermo Scientific DSQ II GC/MS spectrometer and a Bruker micro TOF-Q instrument. UV-Vis and fluorescence spectra were recorded on an Agilent Cary-100 UV-Vis spectrophotometer and an Agilent Cary Eclipse fluorescence spectrophotometer, respectively. The spectra of gas chromatography (GC) were recorded on a Fuli 9790IIA-2 GC spectrometer with a flame ionization detector (FID) detector and a Nickel conversion furnace. Cell and zebrafish imaging were performed on TCS SP8 Laser Scanning Confocal Microscopy (Leica, Germany) with 40 \times and 10 \times objective lens, respectively. In vivo imaging experiments were carried out in a Bruker In Vivo-Xtreme imaging system.

1.2. Synthetic procedures for compound 2, *Cou-Flavone* and *Cou-Carboxylate*



Scheme S2. Synthesis route for compound **2**, **Cou-Flavone** and **Cou-Carboxylate**.

Synthesis of compound 2. To a solution of 2-hydroxy acetophenone (272 mg, 2 mmol) and 7-(diethylamino)-2-oxo-2H-chromene-3-carbaldehyde (coumarin **1**^[4], 490 mg, 2 mmol) in ethanol (15 mL) was added 200 μ L of piperidine. The mixture was then heated to reflux for 24 h. After cooling, the red solid product was collected by filtration, and then recrystallized from methanol to afford compound **2** as red crystals (550 mg, 76%). Mp: 203-204 °C. TLC (silica plate): R_f ~0.7 (dichloromethane). ¹H NMR (400 MHz, CDCl₃): δ 8.32 (d, J = 15.1 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H), 7.77 (s, 1H), 7.67 (d, J = 15.0 Hz, 1H), 7.47 (t, J = 7.7 Hz, 1H), 7.32 (d, J = 8.8 Hz, 1H), 6.99 (d, J = 8.3 Hz, 1H), 6.94 (t, J = 7.6 Hz, 1H), 6.59 (d, J = 9.1 Hz, 1H), 6.48 (s, 1H), 5.52 (br s, 1H), 3.43 (q, J = 6.7 Hz, 4H), 1.23 (t, J = 7.1 Hz, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 194.39, 163.51, 160.22, 156.70, 152.18, 147.15, 140.82, 136.15, 130.27, 121.11, 120.29, 118.88, 118.28, 114.44, 109.71, 108.95, 96.83, 45.12, 12.53. EI-MS: m/z found 362.78 (M⁺).

Synthesis of Cou-Flavone. Compound **2** (363 mg, 1 mmol) was dissolved in methanol (10 mL) and followed by addition of 50 mL of NaOH aqueous solution (10%). Then 1 mL of hydrogen peroxide (30%) was added dropwise to the reaction mixture. The resulting mixture was then stirred under room temperature for 24 h. After that, the pH of the reaction solution was adjusted to 6-7 with diluted HCl. Water (10 mL \times 3) was used to wash the resulting solution for three times, and the dichloromethane phase was dried over Na₂SO₄. After filtered and removal of the organic solvent, the crude product was purified by silica column chromatography to afford **Cou-Flavone** as a

yellow solid (168 mg, 45 %). Mp: 216-217 °C. TLC (silica plate): R_f ~0.65 (dichloromethane:methanol, 10:1, v/v). ^1H NMR (400 MHz, CDCl_3): δ 8.24 (d, J = 5.4 Hz, 2H), 7.66 (t, J = 7.8 Hz, 1H), 7.54 (d, J = 8.5 Hz, 1H), 7.39 (q, J = 8.2, 7.4 Hz, 2H), 7.01 (s, 1H), 6.66 (d, J = 8.4 Hz, 1H), 6.52 (s, 1H), 3.46 (q, J = 7.0 Hz, 4H), 1.25 (t, J = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 173.37, 160.02, 157.11, 155.56, 152.25, 145.73, 143.49, 138.95, 133.45, 130.49, 125.57, 124.42, 121.59, 118.30, 110.43, 109.91, 108.21, 96.85, 45.13, 12.48. EI-MS: m/z found 377.1 (M^+). ESI-MS: m/z found 378.27 ($\text{M} + \text{H}^+$). HR-MS: calcd for $\text{C}_{22}\text{H}_{20}\text{NO}_5^+$ ($\text{M} + \text{H}^+$): 378.1336; found 378.1335 and calcd. for $\text{C}_{22}\text{H}_{19}\text{NO}_5\text{Na}^+$ ($\text{M} + \text{Na}^+$): 400.1155; found 400.1158.

Synthesis of Cou-Carboxylate. **Cou-Flavone** (38 mg, 0.1 mmol) was dissolved in 50 mL of 10 mM HEPES buffer (pH 7.4, with 10 % DMSO). Then the mixture was stirred under a portable 460 nm light irradiation (7 w, 12 mW / cm^2) at room temperature for 6 h. After the reaction finished, 50 mL of dichloromethane was added and the solution was washed by water (10 mL \times 3). Finally, the organic phase was dried over Na_2SO_4 . After filtered and removal of the organic solvent, the crude solid product was collected and further purified by silica column chromatography to afford **Cou-Carboxylate** as a light-yellow solid (25 mg, 66 %). Mp: 235-236 °C. TLC (silica plate): R_f ~ 0.35 (dichloromethane:methanol, 10:1, v/v). ^1H NMR (400 MHz, CDCl_3): δ 10.58 (s, 1H), 8.67 (s, 1H), 7.93 (d, J = 8.0 Hz, 1H), 7.52 (t, J = 7.8 Hz, 1H), 7.46 (d, J = 8.9 Hz, 1H), 7.01 (d, J = 8.3 Hz, 1H), 6.93 (t, J = 7.4 Hz, 1H), 6.71 (d, J = 8.9 Hz, 1H), 6.53 (s, 1H), 3.50 (q, J = 7.0 Hz, 4H), 1.27 (t, J = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 174.01, 165.59, 165.05, 162.16, 158.11, 153.87, 150.47, 136.72, 132.09, 130.90, 119.51, 117.74, 111.56, 111.00, 108.61, 105.29, 96.84, 45.40, 12.43. EI-MS: m/z found 381.1 (M^+). ESI-MS: m/z found 382.36 ($\text{M} + \text{H}^+$). HR-MS: calcd. for $\text{C}_{21}\text{H}_{20}\text{NO}_6^+$ ($\text{M} + \text{H}^+$): 382.1285; found 382.1222.

1.3. Optical studies

The optical studies of **Cou-Flavone** (10 μM) were performed in HEPES buffer (10 mM, pH 7.4, with 10% DMSO, v/v) and in a quartz cuvette with 10 mm of lightpath. A portable UV-light (460 nm, 7 w, 12 mW / cm^2) was used as the light source for

irradiation of **Cou-Flavone** to release CO. The UV-Vis or fluorescent spectra of the **Cou-Flavone** solution were recorded after irradiation at 460 nm or addition of an analyte at room temperature. $\lambda_{\text{ex}} = 420$ nm and slit width: $d_{\text{ex}} = d_{\text{em}} = 5$ nm were set for fluorescence measurements. The fluorescence quantum yield was reported with fluorescein as the reference.

1.4. Cytotoxicity and anti-proliferation experiments

All of the cytotoxicity and anti-proliferation experiments were measured by standard MTT assays (MTT: 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyltetrazolium bromide). The cytotoxicity of **Cou-Flavone** and its product **Cou-Carboxylate** were measured using living MCF-7 cells and HeLa cells. The cytotoxicity of 460 nm irradiation (7w, 12 mW/cm²) were measured using living MCF-7 cells. The anti-proliferation experiments were measured using living MCF-7 cells.

For cytotoxicity experiments, the cells were cultured in Dulbecco Modified Eagle Medium (DMEM) with high glucose medium, supplemented with 10% fetal bovine serum (FBS), 100 µg/mL penicillin and 100 µg/mL streptomycin in a 5% CO₂, water saturated incubator at 37 °C. After the cells were treated with various concentrations of **Cou-Flavone**, **Cou-Carboxylate**, and different times of irradiation (460 nm, 7w, 12 mW/cm²), then with MTT, the absorbance of the resulting solution dissolved with DMSO was measured at 490 nm with a microplate reader. The cell viability was expressed as the optical density ratio of the treatment to control.

For anti-proliferation experiments, the cells were cultured in Dulbecco Modified Eagle Medium (DMEM) with high glucose medium, supplemented with 10% fetal bovine serum (FBS), 100 µg/mL penicillin and 100 µg/mL streptomycin in a 5% CO₂, water saturated incubator at 37 °C. After 12 h, the blank group of cells were treated with MTT, the absorbance of the resulting solution dissolved with DMSO was measured at 490 nm with a microplate reader. The experimental groups were continue cultured in the absence and in the presence of different concentrations of **Cou-Flavone** (0, 10, 20, 30, 40, and 50 µM) with 15 min irradiation (460 nm, 7w, 12 mW/cm²) for another 24 h, then with MTT and measured by the absorbance. The relative numbers of cells was expressed as the optical density ratio of the experimental group to blank

group.

*1.5. CO-releasing and imaging with **Cou-Flavone** in living cells, zebrafish and mice*

CO-releasing and Imaging with Cou-Flavone in Living Cells. MCF-7 cells were seeded in a 24-well culture plate for one night before cell imaging experiments. In the experiment of cell imaging, living cells were incubated with **Cou-Flavone** (10 μM) at 37 °C for 15 min as the control. For imaging of CO-releasing, cells were incubated with **Cou-Flavone** (10 μM) for 15 min followed with an illumination (460 nm, 7w, 12 mW/cm²) for 5, 10, or 15 min, respectively, and then imaged after incubation with our recently published NIR fluorescent CO probe system^[5] (10 μM **Probe 1** + 10 μM PdCl₂) for another 15 min at 37 °C. All images were taken after washing the cells with PBS buffer for three times.

CO-releasing and Imaging with Cou-Flavone in Living Zebrafish. The wild type zebrafish (3 dpf) were cultivated in a 6-well culture plate and costained with **Cou-Flavone** (10 μM) for 15 min. After washed with PBS for three times, the wild type zebrafish were given an illumination (460 nm, 7 w, 12 mW/cm²) by using a portable corn lamp for 0, 5, and 15 min, respectively. The imaging of CO-releasing was then carried out after incubation with our NIR fluorescent CO probe system (10 μM **Probe 1** + 10 μM Pd²⁺) for another 15 min. The images were obtained with excitation at 405, 458 and 670 nm, and emissions were collected at 420–510 nm, 520–620 nm, and 670–750 nm for blue channel, yellow channel, and red channel, respectively.

CO-releasing and Imaging with Cou-Flavone in Living Mice. For in vivo imaging of CO-releasing, Kunming mice were used as model. Before the mice experiments, all of the mice were anesthetized by intraperitoneal (ip) injection of 1% pelltobarbitalumnatricum solution (0.01 mL/g). The mice were then divided into three groups. The first group was only given an ip injection of 100 μL of saline for 5 min as the blank group. The second group was given an ip injection of 100 μL of our **probe 1** system (10 μM **Probe 1** + 10 μM Pd²⁺) for 5 min and imaged as another control. The third group was first given an ip injection of 100 μL of **Cou-Flavone** (20 μM) for 15 min. Then the injection area of the abdominal cavity was given an illumination (460 nm, 7w, 12 mW/cm²) with a portable corn lamp for 0, 5, and 20 min, respectively.

Finally, 100 μL of the **probe 1** system (10 μM **Probe 1** + 10 μM Pd^{2+}) was ip injected to the same area and the mouse was then imaged after 5 min. Imaging was carried out in a Bruker In Vivo-Xtreme imaging system with three different channels for fluorescence imaging. The blue channel has an excitation filter of 420 nm and an emission filter of 470 nm, which was used for detection of **Cou-Carboxylate** (the photolysis product of **Cou-Flavone**). The yellow channel has an excitation filter of 460 nm and an emission filter of 550 nm, which was used for detection of **Cou-Flavone**. The red channel has an excitation filter of 670 nm and an emission filter of 710 nm, which was used for detection of the released CO with the **probe 1** system.

Note: For living zebrafish studies, the illumination was given to the whole body of zebrafish due to their small sizes. For illumination in living mice, we covered the mouse with an opaque black cloth with a square hole ($\sim 3 \times 3$ cm) which was exposed to the injection area of abdomen for illumination.

*1.6. Detection of CO released from **Cou-Flavone** by gas chromatography*

The CO released from **Cou-Flavone** was also recorded by gas chromatography with a FID detector. In this experiment, 1.5 mL of transparent glass bottles with sealing ring were prepared at first, followed with addition of 1 mL of **Cou-Flavone** (200 μM) solution (dissolved in 10 mM HEPES buffer, pH 7.4, with 10% DMSO, v/v). The bottles were capped immediately and the air inside was removed by vacuum. Then the bottles were given different times (1, 5, 10, and 15 min) of irradiation with a portable light (460 nm, 7 w, 12 mW / cm^2), respectively. After that, the bottles were placed in a 70 $^{\circ}\text{C}$ water bath for 10 min to release CO into the vial headspace. Analysis of the released CO was completed by injecting 500 μL from the headspace of each bottle into the GC system after the bottle content was cooled to room temperature.

*1.7. Detection of CO released from **Cou-Flavone** in vivo by colorimetry*

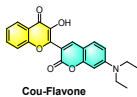
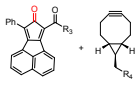
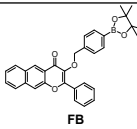
For further confirm the CO release with **Cou-Flavone** in living systems, we measured the COHb production in vivo by colorimetric method. Kunming mice were used as model. Before the experiments, all of the mice were anesthetized by intraperitoneal (ip) injection of 1% pelltobarbitalumnatricum solution (0.01 mL/g). The mice were then divided into two groups. The first group was only given an ip injection of 100 μL of

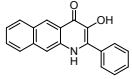
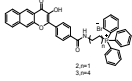
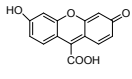
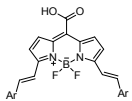
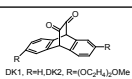
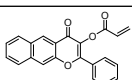
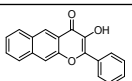
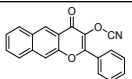
saline as the blank group. The second group was given an ip injection of 100 μL of **Cou-Flavone** (200 μM), then the injection area of the abdominal cavity was illuminated by a portable corn lamp (460 nm, 7w, 12 mW/cm^2) for 0, 5, 10 and 20 min, respectively. After 1 h, the fresh blood was collected from the heart of mice. 0.1 mL of blood was then quickly added to an Alkaline Buffer (2 mL) followed by addition of $\text{Na}_2\text{S}_2\text{O}_4$ (20 mg). The Alkaline Buffer and $\text{Na}_2\text{S}_2\text{O}_4$ were used from the purchased COHb detection kit. After mixing well, 0.2 mL of the resulting solution was added to a 96-well plate. The absorbance at 538 nm and 578 nm was then measured immediately with a microplate reader. The amount of COHb in the total amount of Hb (ω in %) was calculated based on the method of $\text{COHb} (\%) = \{2.44 \cdot (A_{538}/A_{578}) - 2.68\} \cdot 100\%$ in accordance with the instructions of the detection kit provided.

References

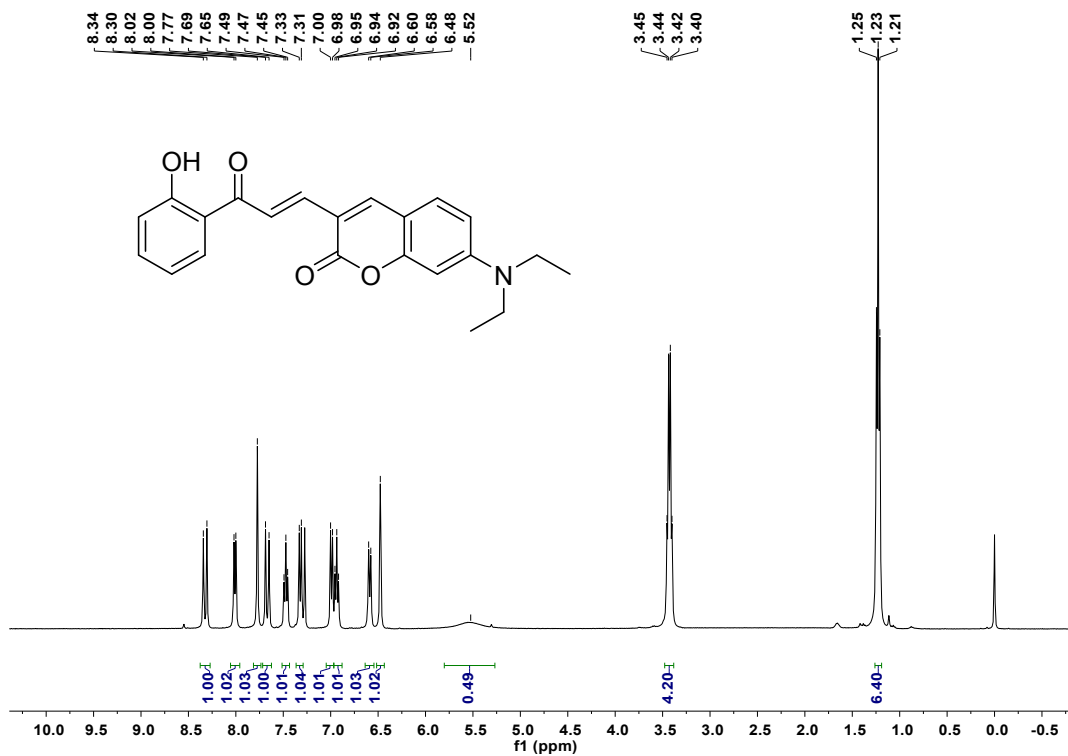
- [1] Yu, D.; Huang, F.; Ding, S.; Feng, G. *Anal. Chem.* **2014**, *86*, 8835–8841.
- [2] Liu, D.; Feng, S.; Feng, G. *Sens. Actuators B* **2018**, *269*, 15–21.
- [3] Feng, W.; Liu, D.; Feng, S.; Feng, G. *Anal. Chem.* **2016**, *88*, 10648–10653.
- [4] Zhang, Q.; Zhang, Y.; Ding, S.; Zhang, H.; Feng, G. *Sens. Actuators B* **2015**, *211*, 377–384.
- [5] Feng, W.; Feng, G. *Sens. Actuators B* **2018**, *255*, 2314–2320.

2. Table S1. Comparison of metal-free fluorescent CORMs

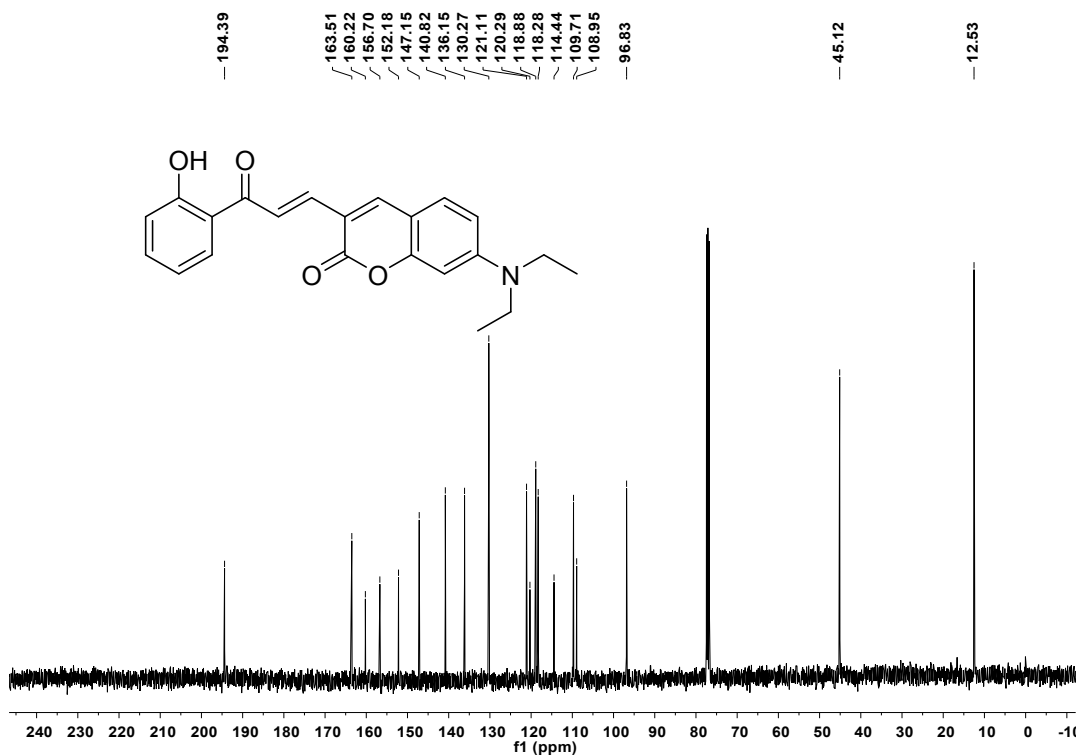
Reference	Structure	Conditions	Ex/Em (nm)	Ratiometric Fluorescence changes
This work	 Cou-Flavone	HEPES buffer (10 mM, 10% DMSO, pH 7.4).	Ex 420 Em 475/555	Yes
Nat. Chem. 2018 , <i>10</i> , 787–794	 Click and release	20% of DMSO/PBS	370/461	No
Angew. Chem., Int. Ed. 2018 , <i>57</i> , 12415–12419.	 FB	PBS buffer solution (50 mM, pH 7.4) with 10% CH_3CN	405/585	No

J. Am. Chem. Soc. 2018 , 140, 9721–9729.		PBS buffer (10 mM, 0.5% DMSO, pH 7.4).	428/600	No
ACS Chem. Biol. 2018 , 13, 2220–2228		CH ₃ CN:DMSO (10:1)	410/600	No
Org. Lett. 2013 , 15, 4552–4555		Aqueous phosphate buffer (pH = 7.4, I = 0.1 M)	488/532	No
J. Am. Chem. Soc. 2016 , 138, 126–133.		Aqueous phosphate buffer solutions (pH = 7.4).	652/750	No
Org. Biomol. Chem. 2013 , 11, 6671–6674.		1% DMSO–water mixture	490/520	No
J. Am. Chem. Soc. 2017 , 139, 9435–9438		CH ₃ CN:PBS (1:1 v/v, with 3% DMSO, 10 mM, pH = 7.4)	410/575	No
ChemistryOpen 2015 , 4, 590– 594.		1:1 aqueous DMSO	409/582	No
ChemPlusChem 2017 , 82, 1408–1412.		PBS buffer (pH 7.4, 10 mM phosphate, 3.0 mM CTAB)	410/460(58 2)	No

3. Structure characterizations.

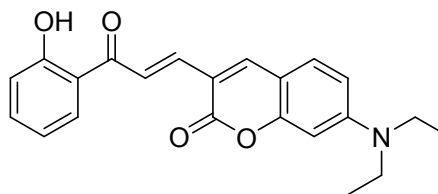
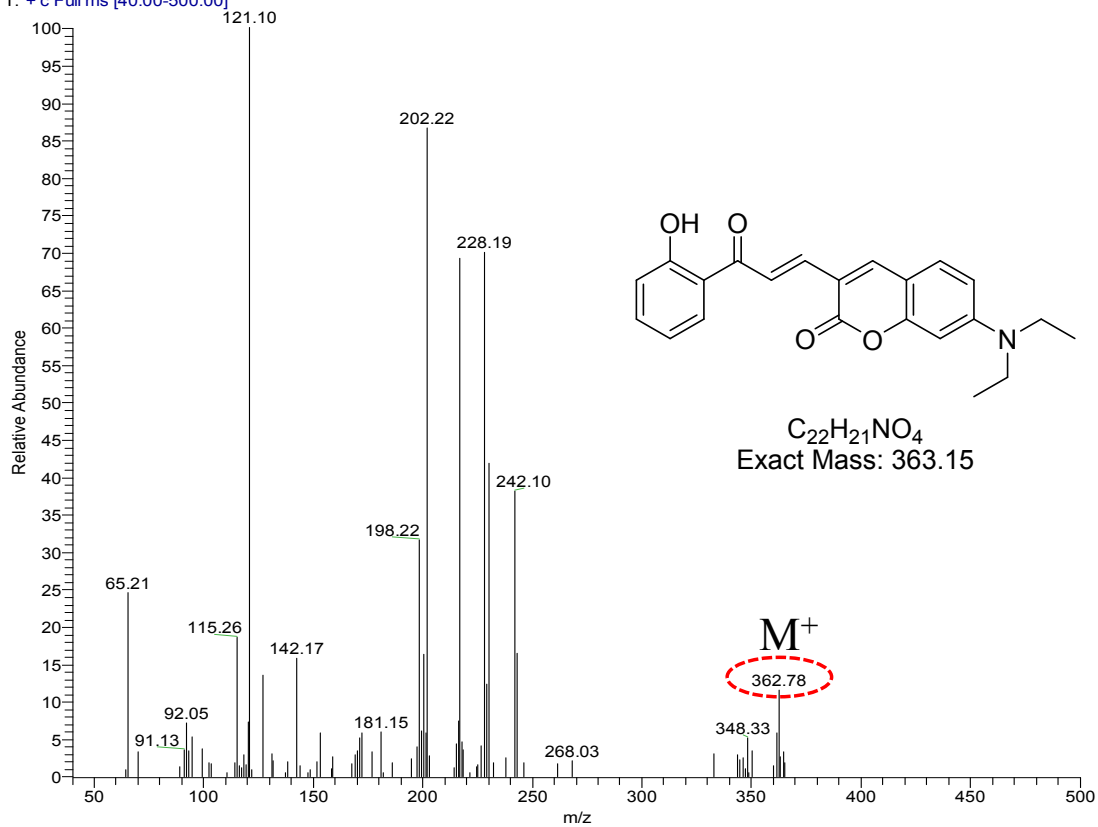


¹H NMR spectrum of compound 2 in CDCl₃.



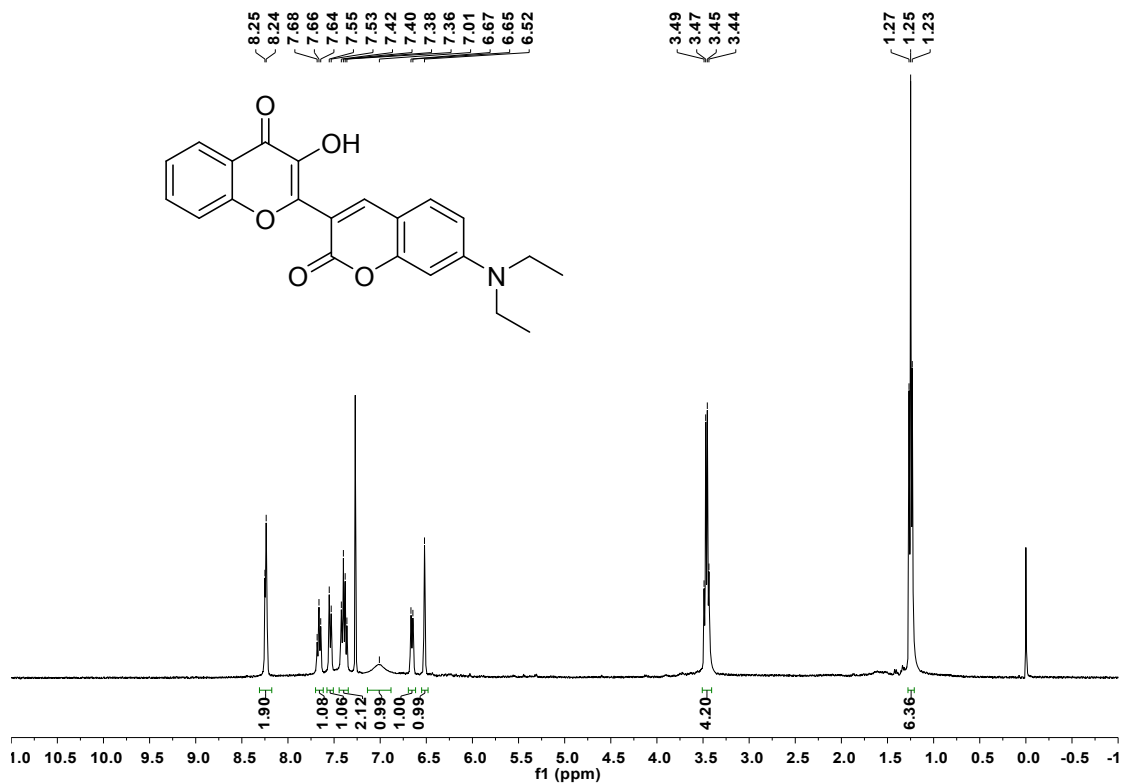
¹³C NMR spectrum of compound 2 in CDCl₃

FWY-273 #163 RT: 2.59 AV: 1 SB: 85 1.52-2.54 , 2.77-3.06 NL: 3.14E3
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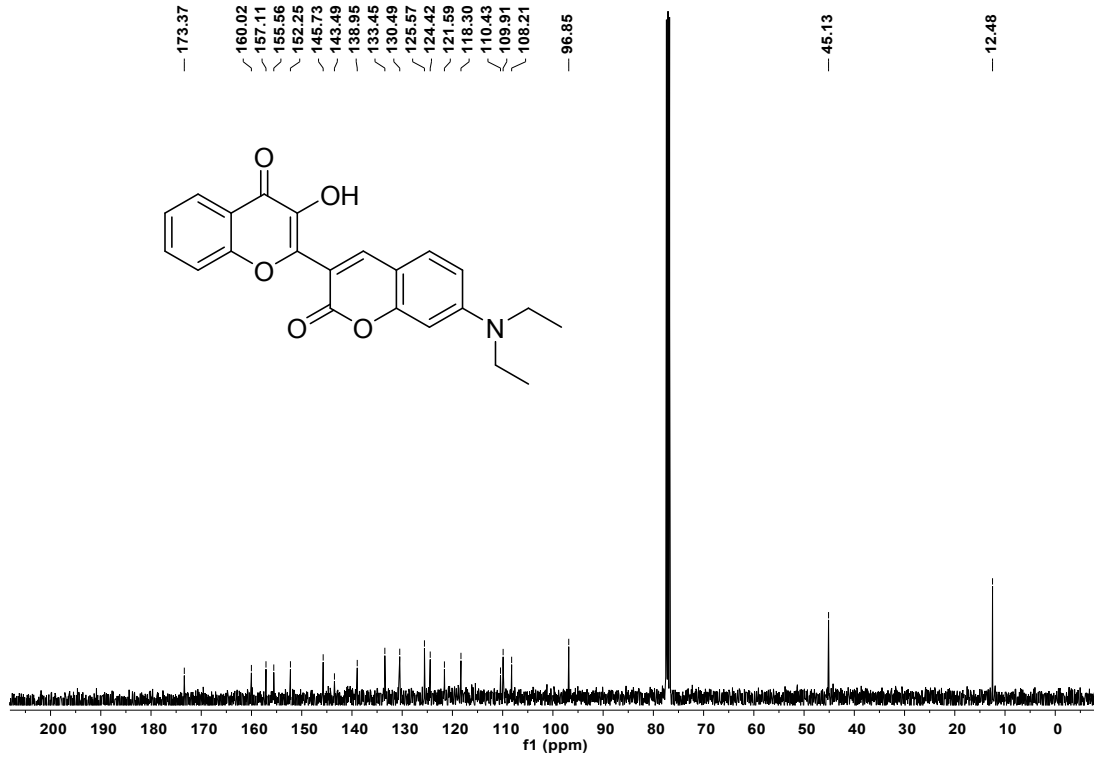


C₂₂H₂₁NO₄
 Exact Mass: 363.15

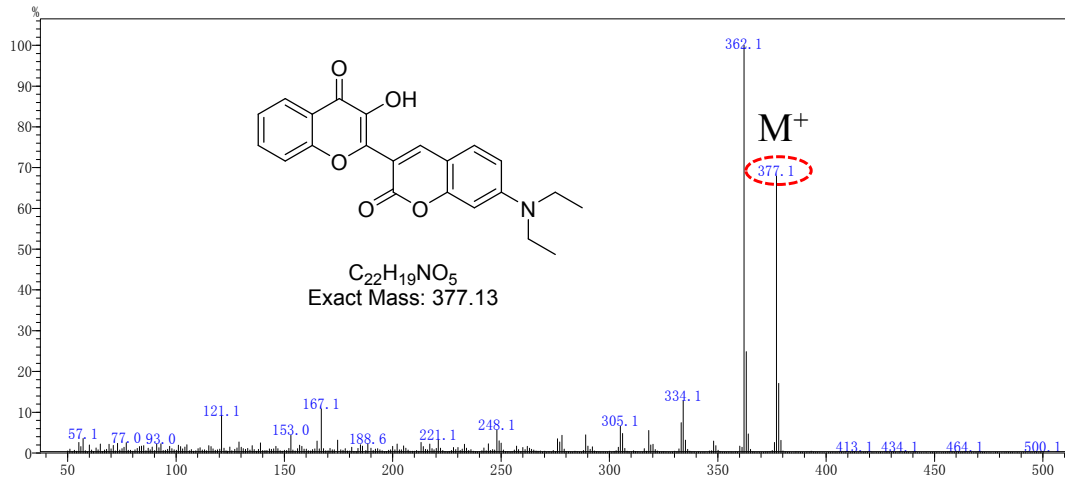
EI-MS spectrum of compound 2



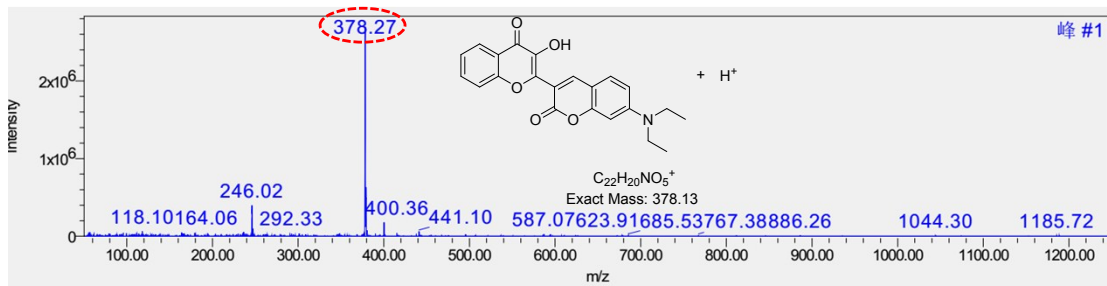
¹H NMR spectrum of Cou-Flavone in CDCl₃.



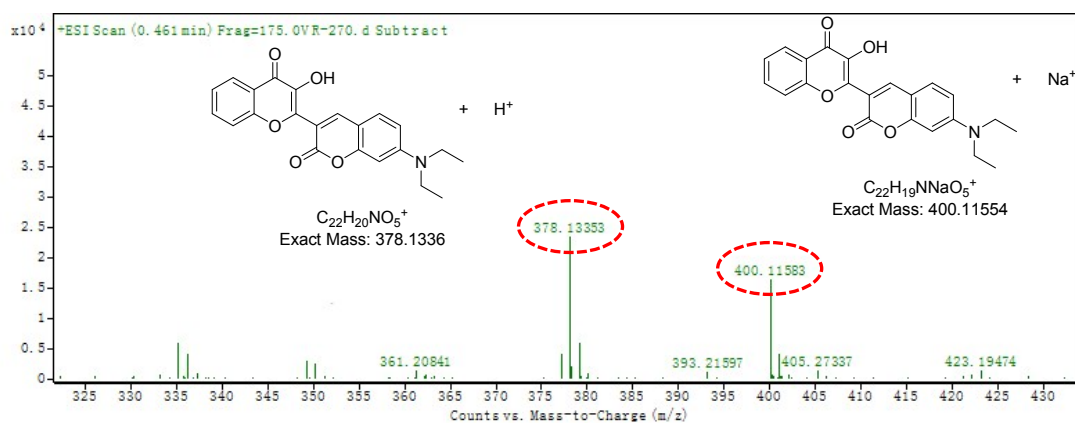
^{13}C NMR spectrum of Cou-Flavone in CDCl_3 .



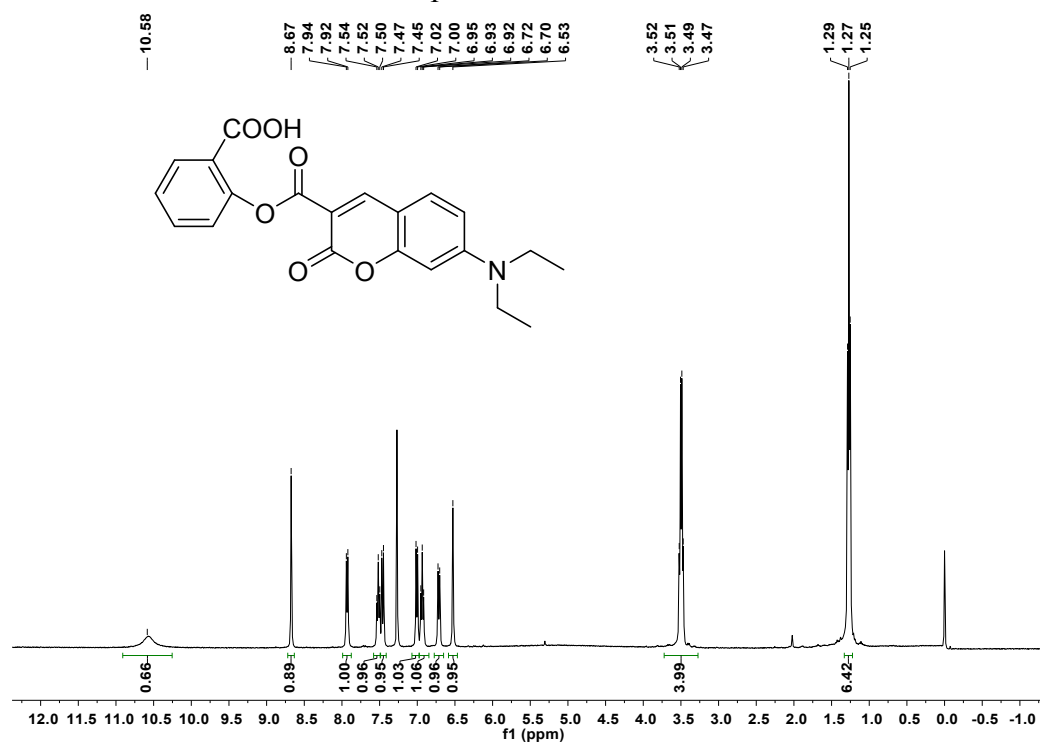
EI-MS spectrum of Cou-Flavone

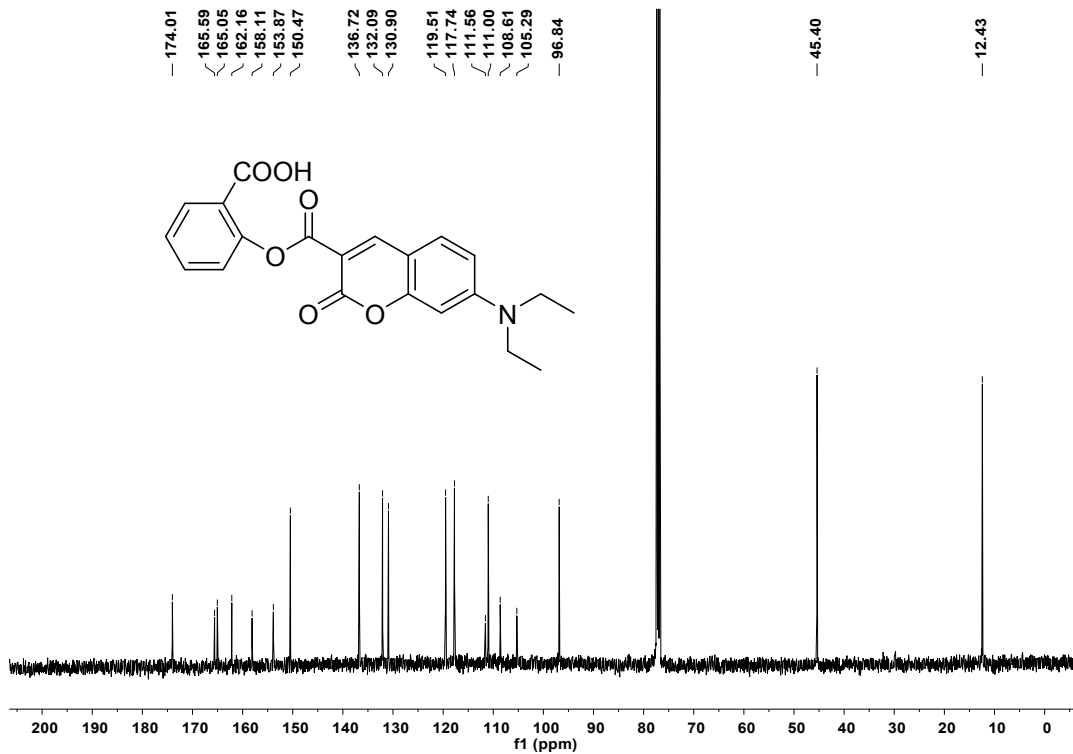


ESI-MS spectrum of Cou-Flavone

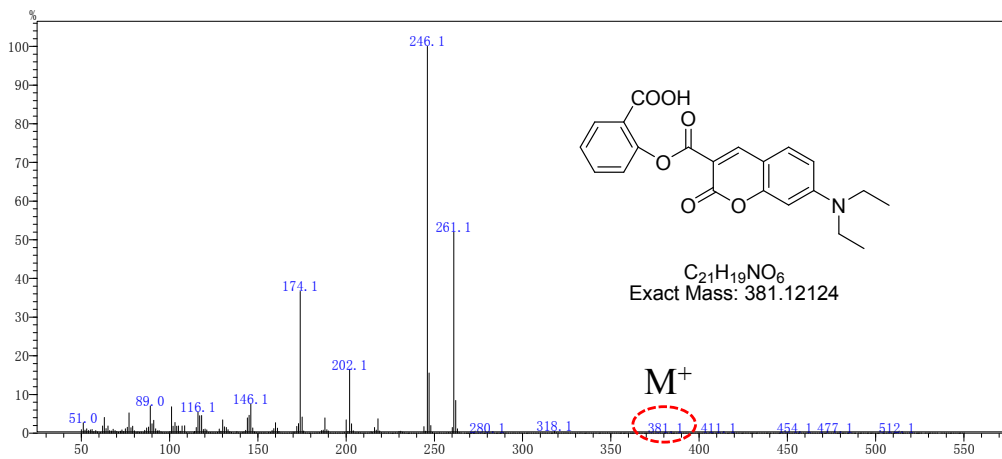


HR-MS spectrum of Cou-Flavone

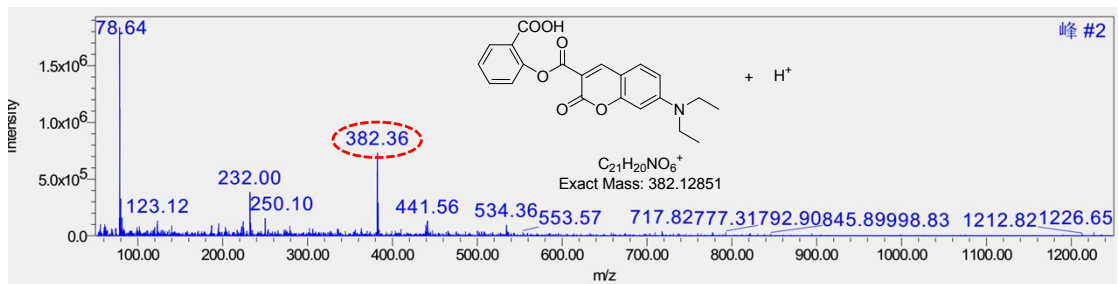




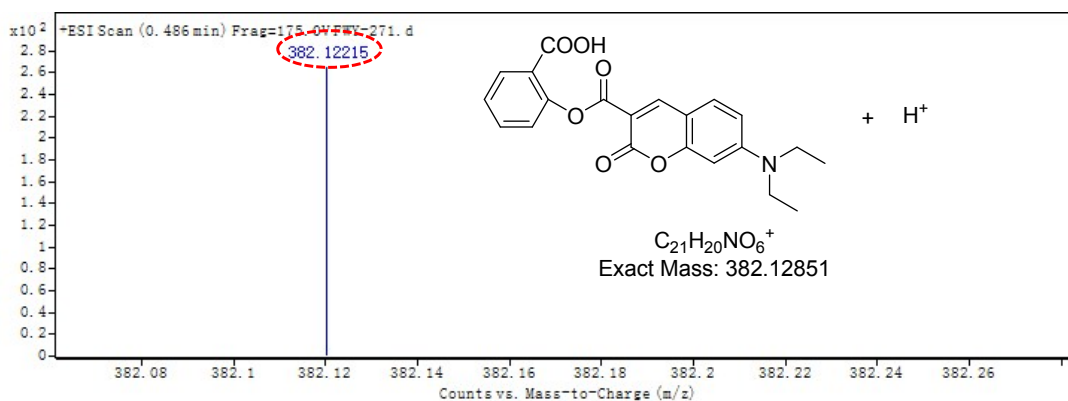
^{13}C NMR spectrum of **Cou-Carboxylate** in CDCl_3 .



EI-MS spectrum of **Cou-Carboxylate**



LC-MS spectrum of **Cou-Carboxylate**



HR-MS spectrum of Cou-Carboxylate

4. Additional Data.

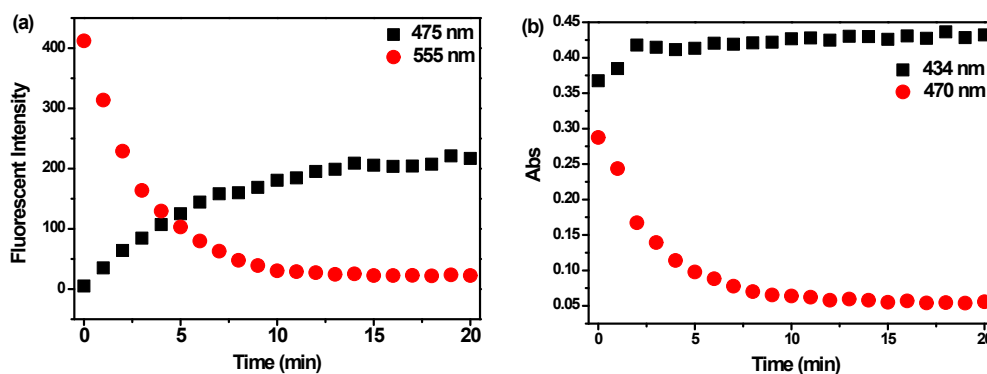


Figure S1. (a) The fluorescent intensity changes at 555 and 475 nm of the **Cou-Flavone** (10 μ M) solution with 460 nm light irradiation. (b) Abs changes at 470 and 434 nm of the **Cou-Flavone** (10 μ M) solution with 460 nm light irradiation. All data were collected in HEPES buffer (10 mM, pH 7.4, 10% DMSO, v/v) at 25 $^{\circ}$ C and each data was collected after 1 min irradiation (460 nm, 7 w, 12 mW/cm²). For fluorescent measurements, λ_{ex} = 420 nm, slit width: d_{ex} = d_{em} = 5 nm.

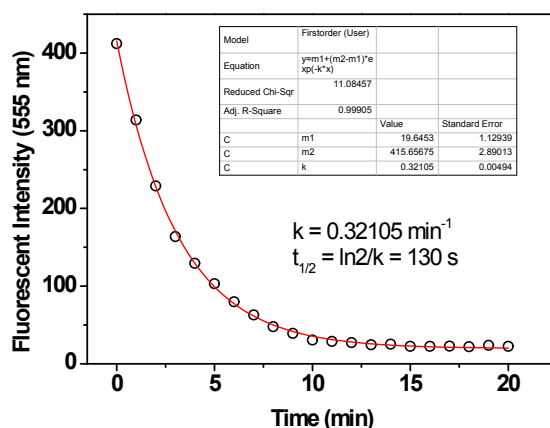


Figure S2. The fluorescent intensity changes of the **Cou-Flavone** (10 μM) solution at 555 nm during light irradiation (460 nm, 7w, 12 mW/cm^2) in HEPES buffer (10 mM, with 10% DMSO, v/v) at 25 $^\circ\text{C}$. $\lambda_{\text{ex}} = 420 \text{ nm}$, slit width: $d_{\text{ex}} = d_{\text{em}} = 5 \text{ nm}$. The data was fitted (red line) by a first order reaction scheme.

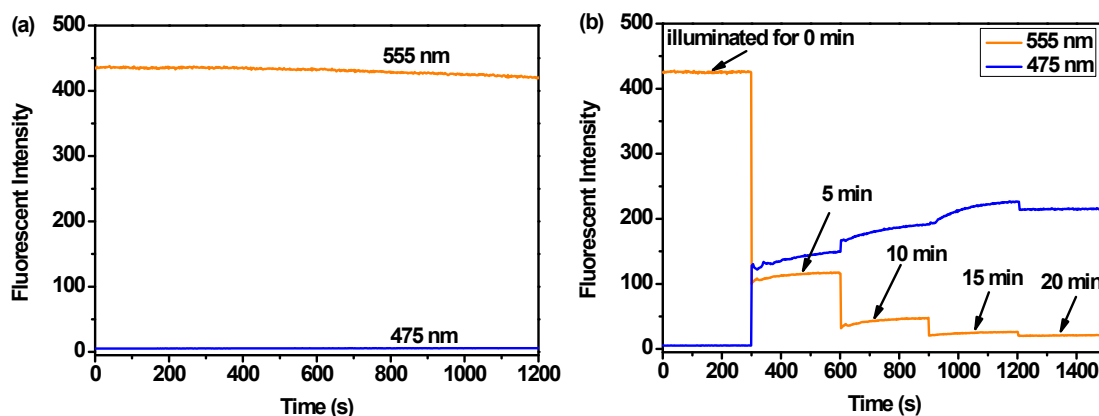


Figure S3. (a) The fluorescent intensity changes of the **Cou-Flavone** (10 μM) solution at 555 and 475 nm in the absence of irradiation. (b) Time-dependent of fluorescent intensity changes of the **Cou-Flavone** (10 μM) solution at 555 and 475 nm after the periodic irradiation every 5 min (460 nm, 7 w, 12 mW/cm^2). All data were collected in HEPES buffer (10 mM, pH 7.4, 10% DMSO, v/v) at 25 $^\circ\text{C}$. $\lambda_{\text{ex}} = 420 \text{ nm}$, slit width: $d_{\text{ex}} = d_{\text{em}} = 5 \text{ nm}$.

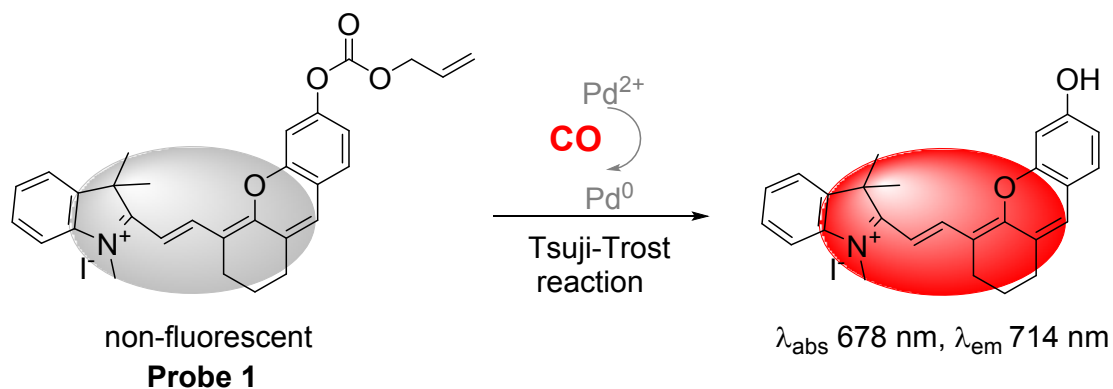


Figure S4. The structure of **Probe 1** for NIR fluorescent turn-on detection of CO (see Feng, W.; Feng, G. *Sens. Actuators B*. **2018**, 255, 2314–2320.).

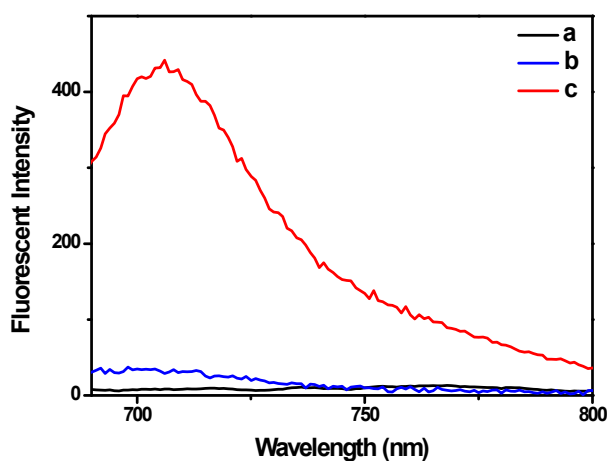


Figure S5. The release of CO from **Cou-Flavone** was proved by **probe 1**. (a) The NIR fluorescence spectrum of **Cou-Flavone** (10 μM) after irradiated (460 nm, 7w, 12 mW/cm^2) for 15 min as blank. (b) The fluorescence spectrum of 5 μM **Probe 1** and 5 μM Pd^{2+} as control. (c) The fluorescence spectrum of **Cou-Flavone** (10 μM) after irradiated (460 nm, 7w, 12 mW/cm^2) for 15 min and then indicated with 5 μM **Probe 1** and 5 μM Pd^{2+} . All spectra were measured in HEPES buffer (10 mM, with 10% DMSO, v/v) at 25 $^\circ\text{C}$ with $\lambda_{\text{ex}} = 670$ nm, slit width: $d_{\text{ex}} = 5$ nm; $d_{\text{em}} = 10$ nm.

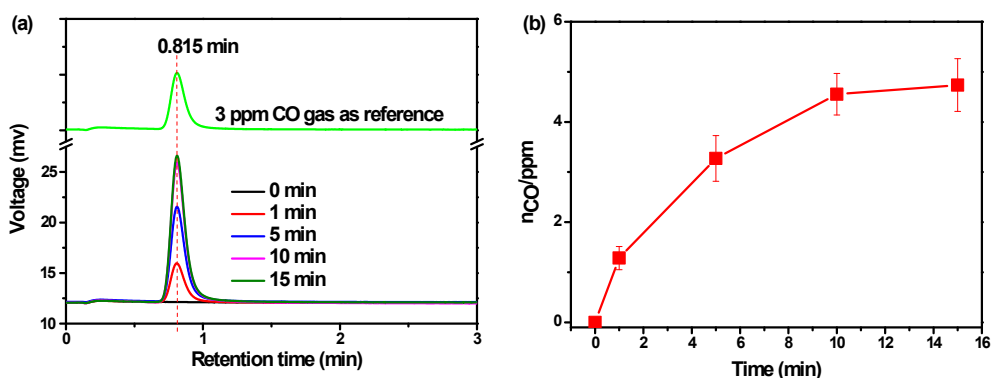


Figure S6. (a) Gas chromatographic peak of CO and (b) amount of CO (ppm) released from **Cou-Flavone** (200 μ M) with an irradiation (460 nm, 7w, 12 mW/cm²) in HEPES buffer (10 mM, pH 7.4, 10% DMSO, v/v) at 25 °C for different times (0, 1, 5, 10, and 15 min). CO gas (3 ppm) was used as reference.

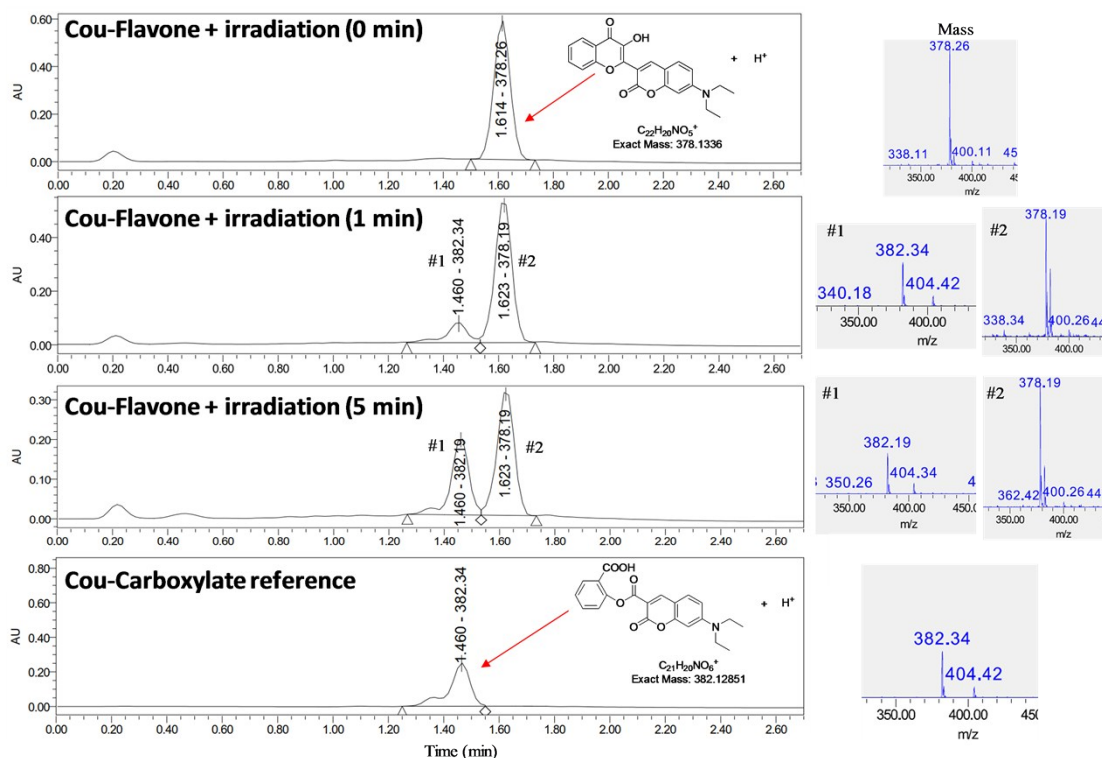


Figure S7. LC-MS analysis of the reaction mixture of **Cou-Flavone** (50 μ M) after irradiation (460 nm, 7w, 12 mW/cm²) for different times (0, 1, and 5 min) in HEPES buffer (10 mM, pH 7.4, with 10 % DMSO, v/v) at room temperature. With **Cou-Carboxylate** as reference, this data showed that **Cou-Carboxylate** was generated and

its amount was increased over the irradiation time. The right part shows the mass spectra. The detection wavelength was 254 nm for LC analysis.

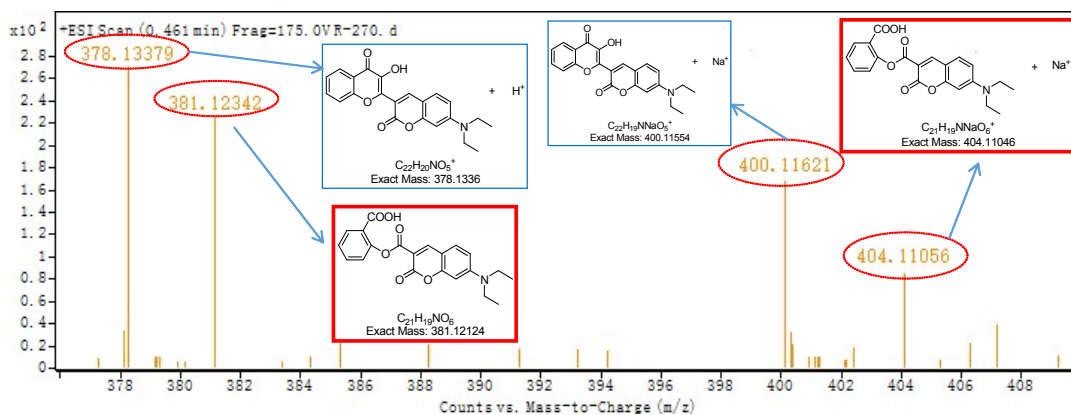


Figure S8. HR-MS analysis of the reaction mixture of **Cou-Flavone** (10 μ M) with irradiation (460 nm, 7w, 12 mW/cm²) in HEPES buffer (10 mM, pH 7.4, with 10 % DMSO, v/v) for 5 min at room temperature. This result showed that **Cou-Carboxylate** was generated.

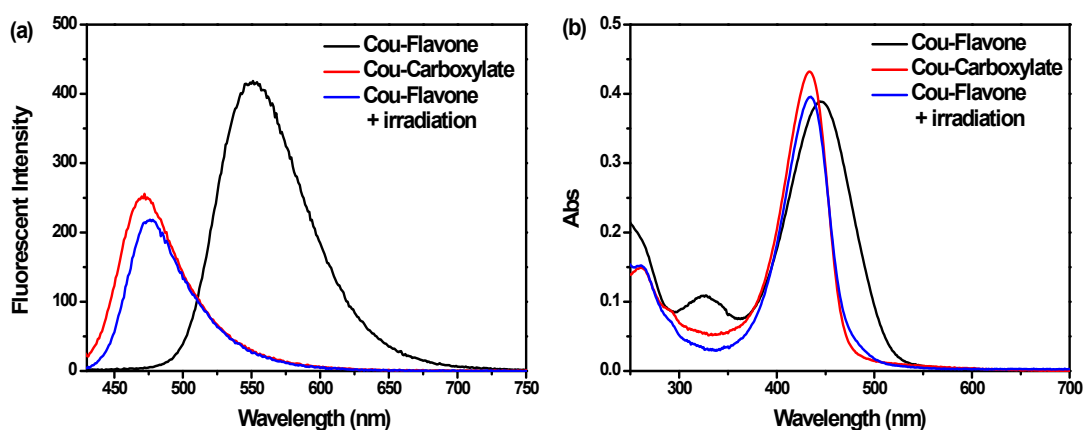


Figure S9. The (a) fluorescence and (b) UV-vis spectra of **Cou-Flavone**, **Cou-Flavone** after irradiation (460 nm, 7w, 12 mW/cm²), and **Cou-Carboxylate** in HEPES buffer (10 mM, pH 7.4, 10 % DMSO, v/v) at 25 °C. For fluorescent measurements, λ_{ex} = 420 nm, slit width: d_{ex} = 5 nm, d_{em} = 5 nm.

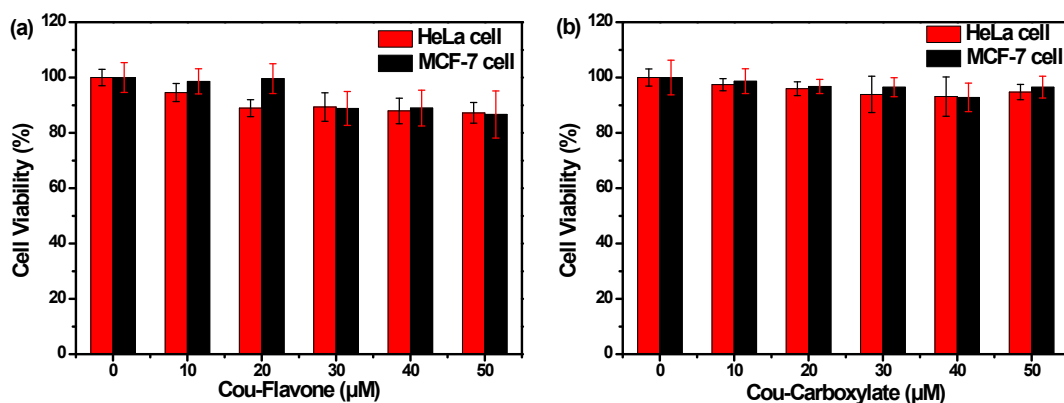


Figure S10. Percentage of viable HeLa and MCF-7 cells after treated with different concentrations of the (a) **Cou-Flavone** and (b) **Cou-Carboxylate** for 24 hours, respectively. The cell viability was obtained via MTT assay.

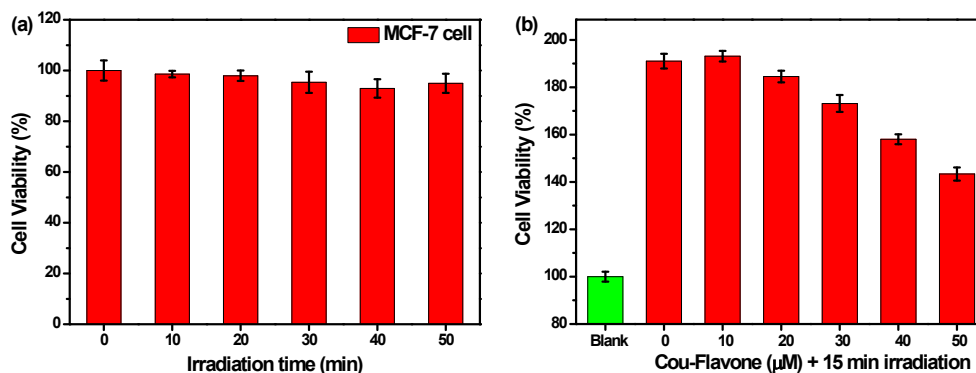


Figure S11. (a) Percentage of viable MCF-7 cells after irradiation (460 nm, 7w, 12 mW/cm²) for different times. (b) The result of anti-proliferation experiments (the green column means the blank control cells without treatment of **Cou-Flavone**. The red columns mean the cell viability after treatment of different concentrations of **Cou-Flavone** with 15 min irradiation (460 nm, 7w, 12 mW/cm²). The cell viability of MCF-7 cells were obtained via MTT assay.

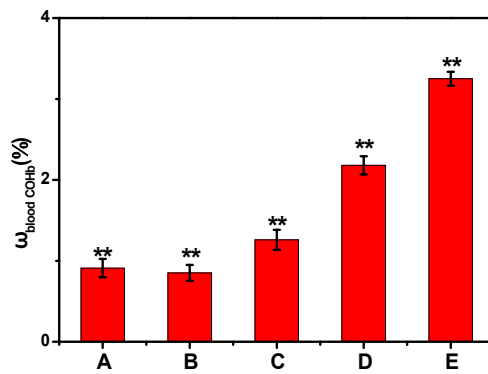


Figure S12. The COHb production in vitro. For groups A-E: (A) normal mouse as control group. (B) **Cou-Flavone** (100 μ L, 200 μ M in saline with 10% DMSO) treated mouse without irradiation as blank group. (C) **Cou-Flavone** (100 μ L, 200 μ M in saline with 10% DMSO) treated group with irradiation for 5 min (460 nm, 7w, 12 mW/cm²). (D) **Cou-Flavone** (100 μ L, 200 μ M in saline with 10% DMSO) treated group with irradiation for 10 min (460 nm, 7w, 12 mW/cm²). (E) **Cou-Flavone** (100 μ L, 200 μ M in saline with 10% DMSO) treated group with irradiation for 20 min (460 nm, 7w, 12 mW/cm²). Results are presented as mean \pm s.d. n = 5 per group. **P < 0.01, versus group A.