Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Imaging dynamic changes of intracellular cysteine pool that respond to the stimulation of external oxidative stress

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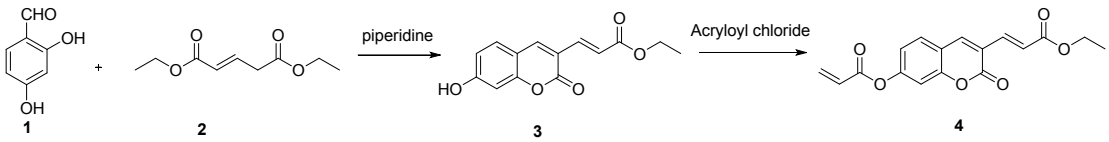
1. Reagents and apparatus

All chemical reagents and solvents were purchased from commercial sources and used without further purification. Cells were purchased from the Committee on type Culture Collection of Chinese Academy of Sciences. Nematode and *Arabidopsis Thaliana* were obtained from Nanjing University. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DRX-600 spectrometer. Mass spectra were carried out by Mass Spectrometry Facility at Nanjing University. All pH measurements were determined by a HI 2221 calibration check pH/ORP meter. Fluorescence measurements were measured on Hitachi Fluorescence Spectrophotometer F-7000. Absorption spectra were recorded on a UV spectrometer. All fluorescent imaging experiments were carried out using a confocal fluorescent microscope at Nanjing University (Leica TCS SP8 MP, or single-photon confocal microscope (Olympus)).

Amino acids were dissolved in doube distilled water for obtaining a series of 10 mM stock solutions. Probe CyP was dissolved in DMSO to obtain a 1 mM stock solution. The system of testing was set: 100 μL 1x PBS buffer(pH 7.4), 2 μL 1mM solution of the probe, 2 μL 10 mM solution of Cys, and added dd-H_2O to get a 200 μL testing system for fluorescent measurements. For all fluorescent measurement, the excitation wavelength was 428 nm, the slit widths were 5 nm, the voltage was 400 V, and then the emission spectrum was scanned from 450 nm to 650 nm at 1200 nm/min by fluorescence spectrophotometer F-7000.

2. Synthesis and characterization of compounds



Synthesis of ethyl (E)-3-(7-hydroxy-2-oxo-2H-chromen-3-yl)acrylate(3)

Piperidine (44.64 μL, 0.57 mmol) and diethyl (E)-pent-2-enedioatee(1.05 g, 5.65 mmol) were added into anhydrous ethanol solution of 2,4-dihydroxybenzaldehyde 740 mg, 5.36 mmol) with stirring at 80 ^{o}C for 12 h. After confirming completion of

the reaction by TLC, and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 10/1, v/v) to afford compound **3** as yellow solid. Yield: 1.98 g (76%). ^{1}H NMR (600 MHz, DMSO-*d*6) δ 8.44 (s, 1H), 7.59-7.47 (m, 2H), 6.90-6.79 (m, 2H), 6.74 (d, *J* = 2.0 Hz, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 1.25 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (150 MHz,DMSO-d_6) δ166.74, 163.69, 159.71, 155.89, 146.02, 139.44, 131.32, 120.05, 116.52, 114.63, 118.7, 102.44, 60.52, 14.65. ESI MS: 283.0 $[M+Na]^+$.

Synthesis of ethyl (E)-3-(7-(acryloyloxy)-2-oxo-2H-chromen-3-yl)acrylate(4)

Acryloyl chloride (415.8 μL, 4.62 mM) and triethylamine (77 mg, 0.77 mM) were dropped to the solution of compound **3** (200 mg, 0.77 mM) in dry dichloromethane under N_2 at 0 ^{o}C. After warming up to room temperature, the reaction was continually stirred for another 12 h. The solvents were concentrated under reduced pressure and the crude product was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 10/1, v/v) to afford compound **4.** Yield: 278 mg, (88.54%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.59 (s, 1H), 7.80 (d, *J* = 8.5 Hz, 1H), 7.56 (d, *J* = 16.0 Hz, 1H), 7.43 (d, *J* = 2.1 Hz, 1H), 7.28 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.96 (d, *J* = 16.0 Hz, 1H), 6.59 (dd, *J* = 17.3, 1.1 Hz, 1H), 6.45 (dd, *J* = 17.3, 10.4 Hz, 1H), 6.22 (dd, *J* = 10.4, 1.1 Hz, 1H), 4.21 (q, *J* = 7.1 Hz, 2H), 1.27 (t, *J* = 7.1 Hz, 3H).^{13}C NMR(150 MHz, DMSO-d_6) δ166.43, 164.04, 159.14, 154.18, 154.00, 144.64, 138.60, 134.97, 130.66, 127.70, 122.29, 121.01, 119.62, 117.4, 110.47, 60.76, 14.62. ESI MS: 337.0 $[M+Na]^+$.

3. Cell culture and confocal microscopy imaging

Fluorescent imaging of exogenous Cys in living cells

Hela cells were cultured in DMEM medium supplemented with 10 % fetal bovine serum, 1 % penicillin and streptomycin at 37 ^{o}C in a humidified atmosphere containing 5% CO_2, and then cells were transferred into 6-well plates. After culturing overnight, Hela cells were washed with supplemented medium three times, and then incubated with 2 mM N-ethylmaleimide (NEM) to remove intracellular thiols for 30 min at 37 ^{o}C. Then, cells were pretreated with 20 μM, 40 μM, 60 μM, 80 μM and

100 μM Cys after washing with PBS three times, cells were further co-incubated with 10 μM probe for another 30 min before imaging. All imaging experiments were performed on a confocal fluorescent microscope after washing with PBS buffer three times.

4. Fluorescent imaging of endogenous Cys in living cells

Hela cells were cultured in DMEM medium supplemented with 10 % fetal bovine serum, 1 % penicillin and streptomycin at 37 ^{o}C in a humidified atmosphere containing 5% CO_2 overnight. Cells were washed with PBS and then pretreated with 500 μM H_2O_2 or 2 mM NAC for 1 h. After washed with PBS three times, cells were incubated with 10 μM probe for another 30 min. Confocal images were performed on a single-photon confocal fluorescent microscope (Olympus, Nanjing University), and the excitation wavelength was 428 nm and the emission wavelength was 450-550 nm.

5. Fluorescent imaging in live *Arabidopsis Thaliana*

The root tissue of 5-days *Arabidopsis Thaliana* were chosen for fluorescent imaging. The root tip of *Arabidopsis Thaliana* was pretreated with 500 μM H_2O_2 or 2 mM NAC for 1 h, washed, and 10 μM probe was added and incubated for another 30 min. Then, confocal images were carried out on a two-photon confocal fluorescent microscope (Leica TCS SP8 MP). The excitation wavelength was 428 nm, and the emission wavelength was 450-550 nm.

6. Fluorescent imaging in live nematode

The nematode for imaging was hermaphrodite and adult. The adult nematodes were pre-incubated with 500 μM H_2O_2 or 2 mM NAC for 1 h, and then 10 μM probe was added and incubated for another 30 min. Subsequently, live nematodes were imaged by using a confocal microscope. The excitation wavelength was 428 nm, and the emission wavelength was 450-550 nm.

7. Fluorescent imaging in live Arabidopsis Thaliana under cadmium-induced oxidative stress

The root tissues of 5 -days *Arabidopsis Thaliana* were chosen for fluorescent imaging. The root tip of *Arabidopsis Thaliana* was pretreated with 0, 50 μM, 100 μM, 150 μM and 200 μM Cd^{2+} at 25 ^{o}C for 8h, washed, and then 10 μM probe was added and incubated for another 30 min. confocal images of *Arabidopsis Thaliana* were carried out on a confocal fluorescent microscope. The excitation wavelength was 428 nm, and the emission wavelength was 450-550 nm.

8. The limit of detection (LOD) of CyP

The emission spectrum of free CyP in PBS buffer (10 mM, 1% DMSO, pH 7.4) was collected for 20 times to confirm the background noise σ. The linear regression curve was then fitted according to the data in the range of cysteine from 1 to 10 μM and obtained the slope. The detection limit (3σ/slope) was then determined to be 0.93 μM.

9. Quantum yield of CyP

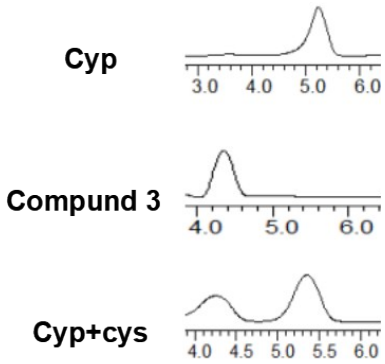
The fluorescence quantum yield Φu was estimated through participation ratio method, where using the ethanol solution of 5 μM rhodamine B (Φ = 0.69, λ_{ex} = 365 nm) for the sample and reference. Through testing the absorption and fluorescence spectra of CyP (5 μM), the fluorescence quantum yield was calculated using equation as follows:

$\Phi_u=[(A_sF_un^2)/(A_uF_sn_0^2)]\Phi_s$.

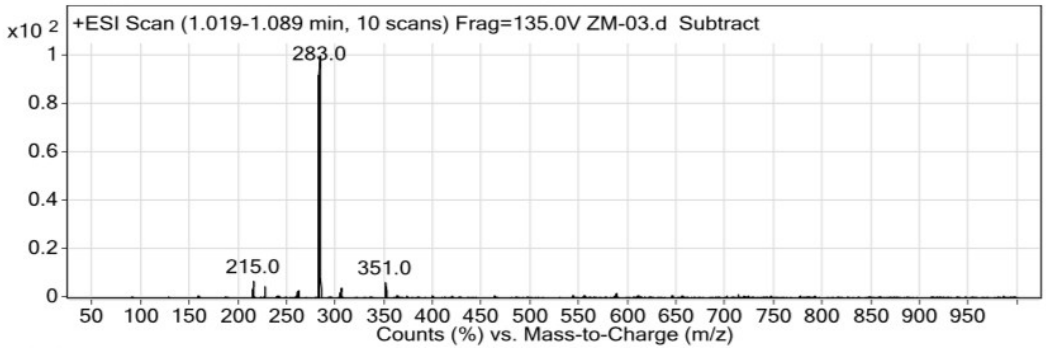
Φ_s is the quantum yields of the reference substance, A_s and A_u represents the absorbance of the reference and testing sample at the excitation wavelength, F_s and F_u refer to the integrated emission band areas under the same conditions, n and n_0 are the solvent refractive indexes of determined and reference, respectively. In the process of detection should control the absorbance to be lower than 0.05.

Quantum yield: Φ = 0.488.

10. Supplementary Figures



Compound 3



Probe CyP

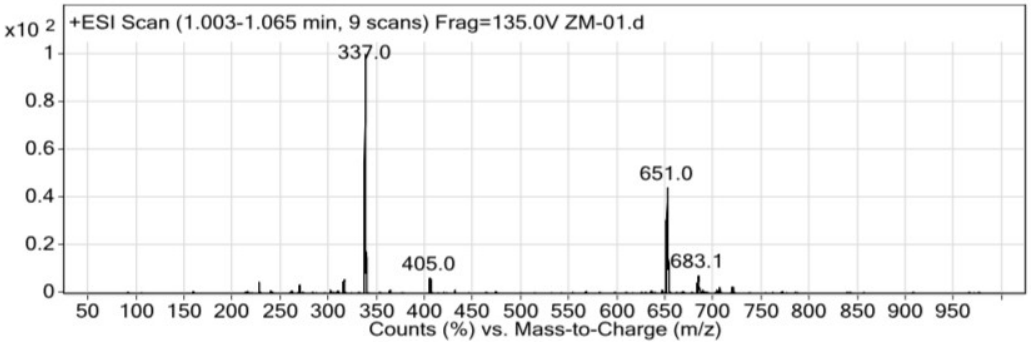


Fig. S1. LC-MS analysis of CyP solution after incubation with cysteine, a new m/z peak (m/z = 283.0) identified as the released fluorophore (compound 3)**.**

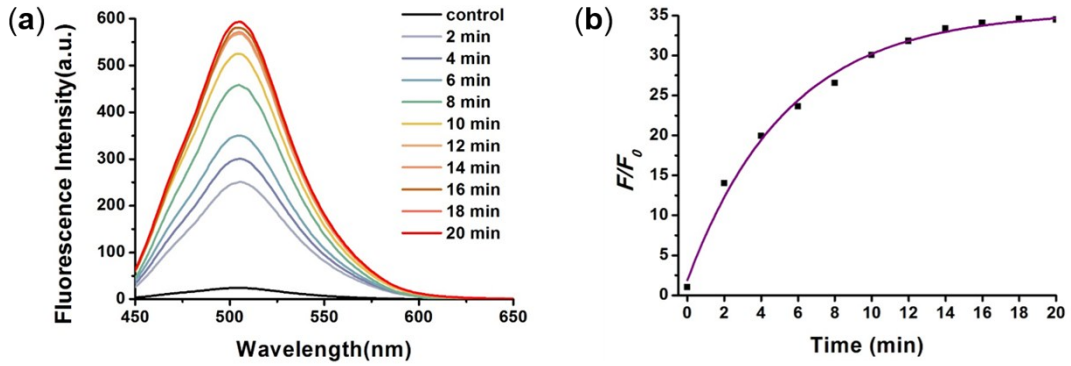


Fig. S2. The fluorescent spectra of CyP (10 μM) incubated with Cys (100 μM) in PBS buffer (10 mM, 1% DMSO, pH 7.4) at 37 °C for different reaction time (0-20 min). (b) Time-dependent fluorescence intensity of CyP at 505 nm *versus* increasing incubation time in PBS buffer (10 mM, 1% DMSO, pH 7.4) at 37 °C. Excitation: 428 nm, emission: 450-650 nm. The data represent the average of three independent experiments.

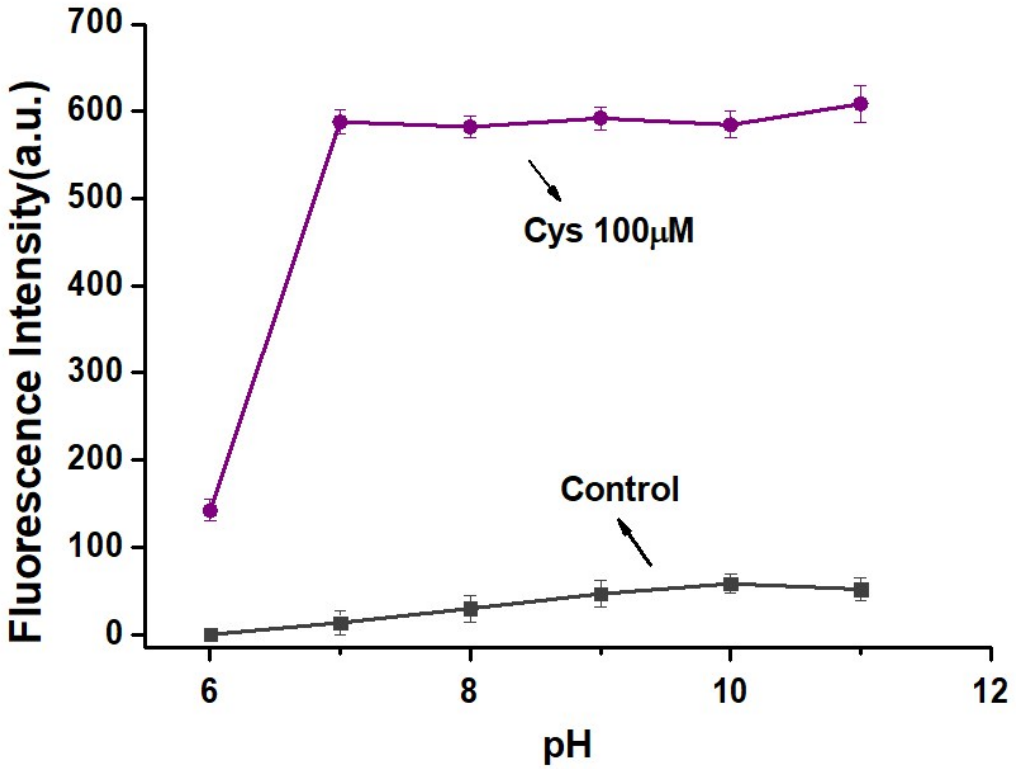


Fig. S3. The fluorescent intensity of 10 μM probe and 100 μM Cys in different pH PBS buffer (10 mM PBS, 1% DMSO) at 25 ^{o}C for 20 min. Excitation:428 nm,emission:505 nm, slid width:5 nm. The data represents the average of three independent experiments.

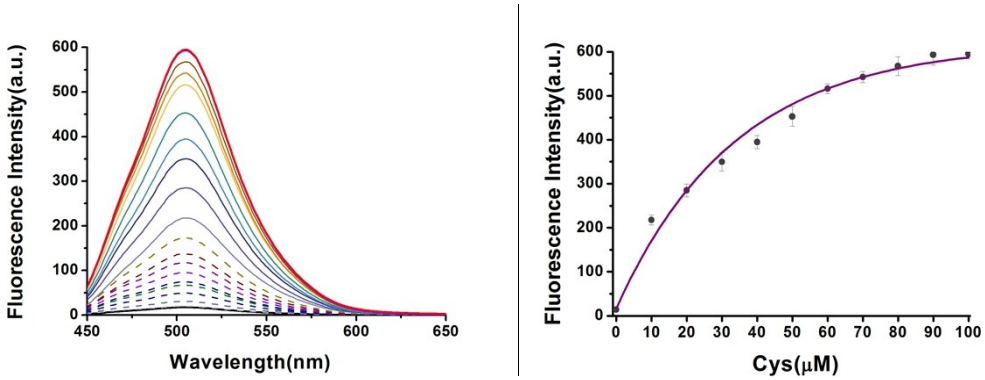


Fig. S4. (a) The fluorescent spectra of CyP (10 μM) upon addition of different concentration of Cys (0-100 μM) in PBS buffer (10 mM, 1% DMSO, pH 7.4) at 37 °C for 20 min. (b) The plot of fluorescent intensity at 505 nm versus different concentration of Cys (0-100 μM). Excitation: 428 nm, emission: 450-650 nm. The data represent the average of three independent experiments.

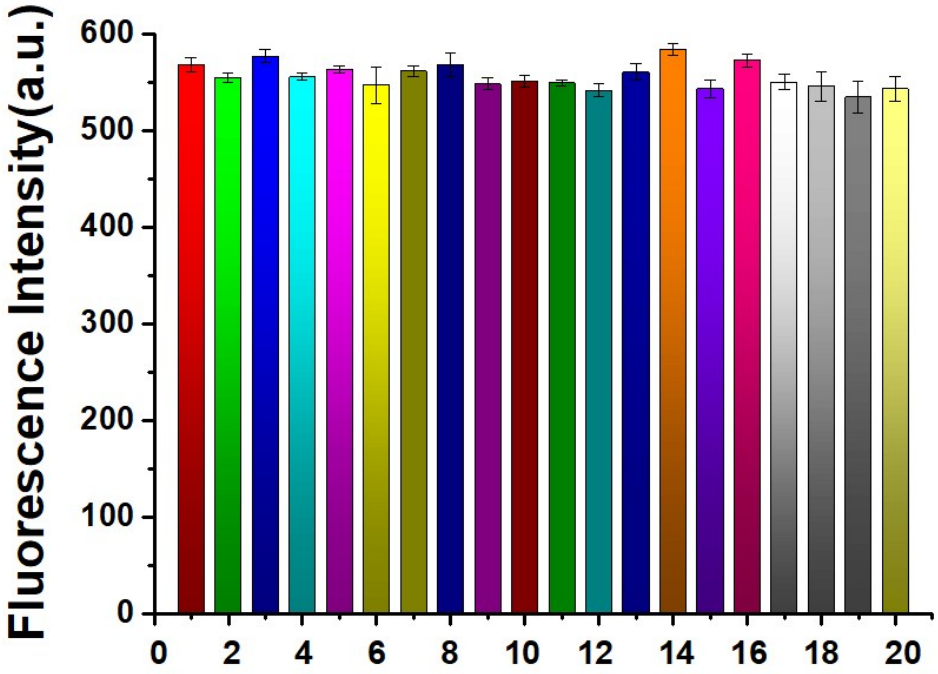


Fig. S5. Changes of fluorescence intensity of Cys (100 μM) and other analytes (100 μM) co-existed in the buffer (10 mM PBS, pH 7.4, 1% DMSO) with CyP (10 μM) at 25 ^{o}C for 20 min. Sample: 1)Cys, 2)Val, 3)Lys, 4)Ala, 5)Alg, 6)Leu, 7)Ile, 8)Pro, 9) Phe, 10)Tyr, 11)Trp, 12)Thr, 13)Ser, 14)Met, 15)Asn, 16)Gln, 17)Asp, 18)Asn, 19)His, 20)Gly. Excitation:428 nm, emission:505 nm, slid width:5 nm. The data represents the average of three independent experiments.

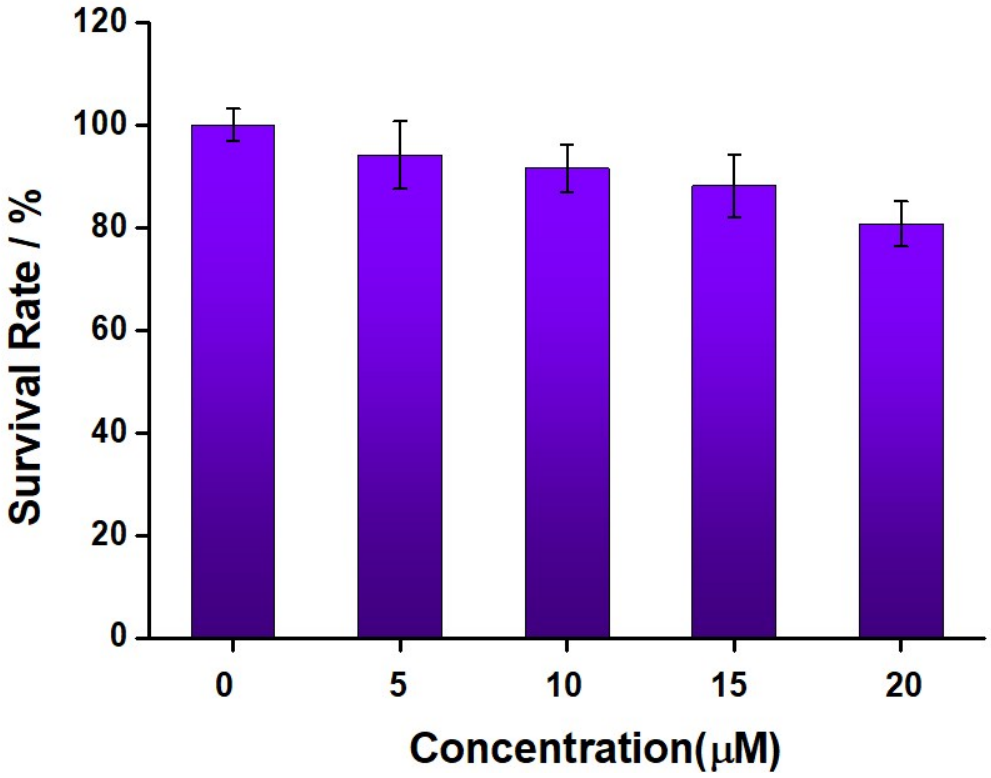


Fig. S6. The MTT assay was performed to test the cytotoxicity of **CyP**. Hela cells were incubated in DMEM with 5 % fetal calf serum at 37 ^{o}C in 96-well plates, then different concentration **CyP** (0, 5, 10, 15, 20μM) were added into 96-well plates and further incubated at 37 ^{o}C for 12 h, the MTT assay was analysis by Microplate Reader. The survival rate of cells was over 80% after added 20μM **CyP**, demonstrating its low cytotoxicity.

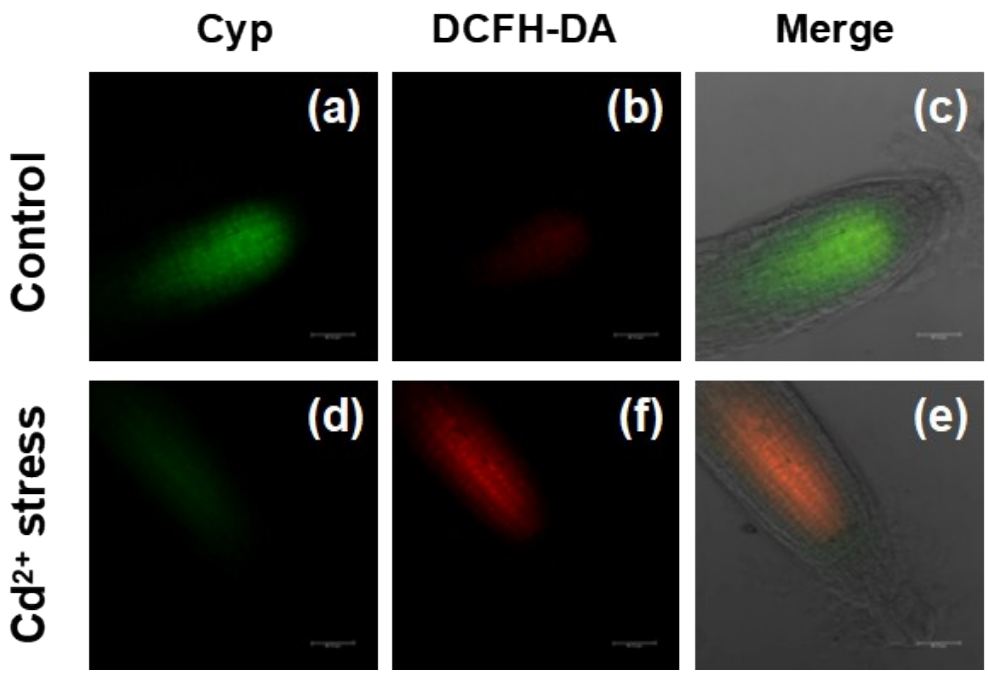
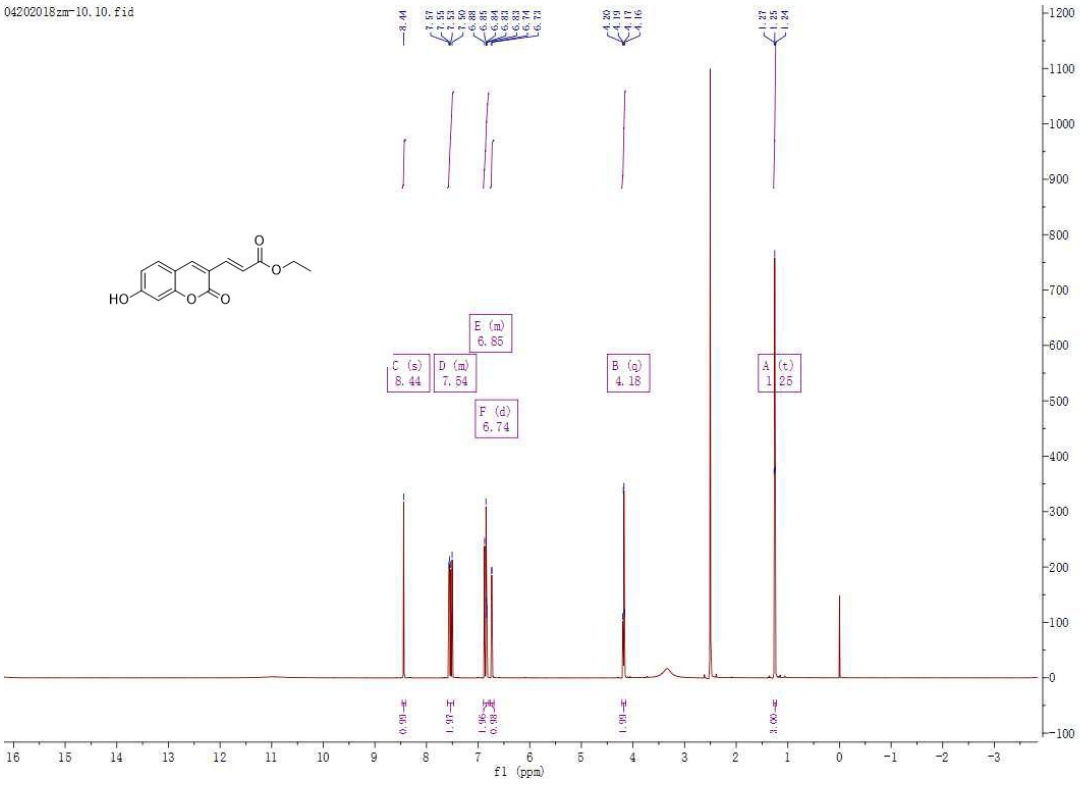
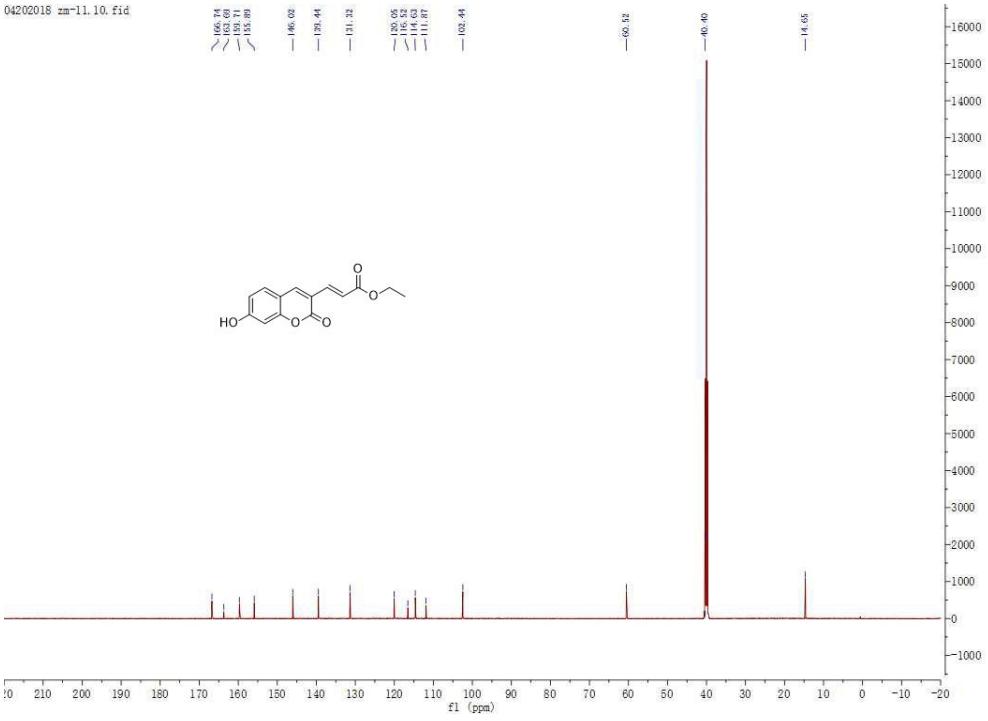


Fig. S7. Confocal imaging of hydrogen peroxide level of *Arabidopsis Thaliana* which exposed by Cd^{2+}. The root tissues of *Arabidopsis Thaliana* were pretreated with Cd^{2+}(0 or 200 μM) for 8 h at 25 ^{o}C, and then *Arabidopsis Thaliana* were incubated with CyP (10 μM) at 25 ^{o}C for another 30min. After washed with PBS three times, tissues were further incubated with DCFH-DA (200 μM) for 30 min at 25 ^{o}C (a and d) **CyP**, green channel, the excitation wavelength was 405 nm, and the emission wavelength was 450-550 nm. (b and f) DCFH-DA, red channel, the excitation wavelength was 488 nm, and the emission wavelength was 520-580 nm. (c and e) Overly channel of green, red and bright field imaging.

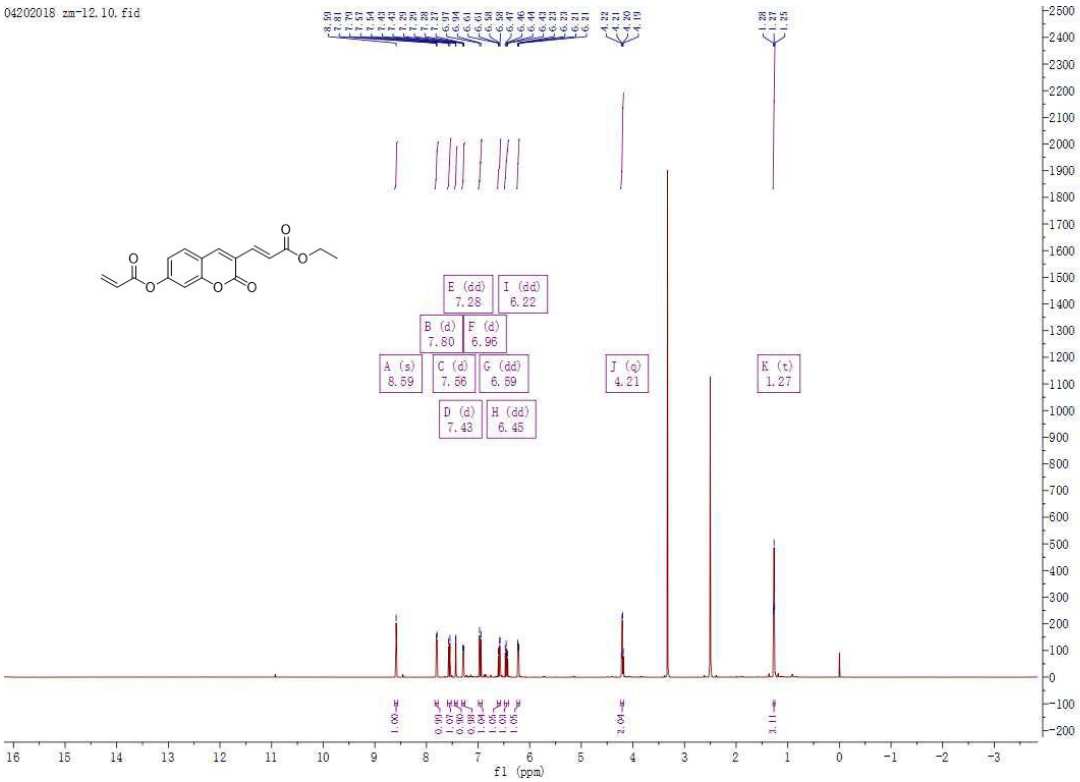
11. NMR spectrum of compounds



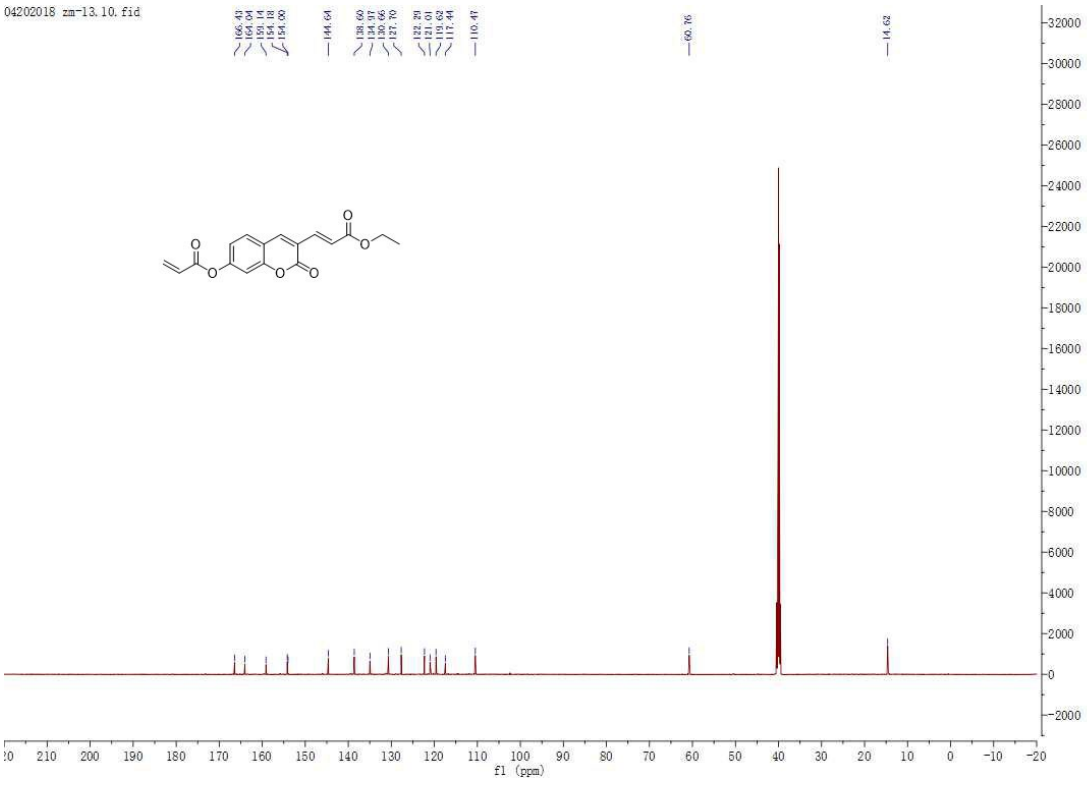
^{1}H NMR of compound 3



^{13}C NMR of compound 3



^{1}H NMR of compound 4



^{13}C NMR of compound 4