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Supporting Information

Long wavelength emission two-photon fluorescent probe for highly selective detection of cysteine in living cells and inflamed mouse model

Dongjie Liu[a], Yun Lv[b], Mei Chen[a,*], Dan Cheng[b,*], Zhiling Song[c], Lin Yuan[b], Xiaobing Zhang[b]

[a]*College of Materials Science and Engineering, Hunan University, Changsha 410082, PR China*

[b]*State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, 410082, PR China*

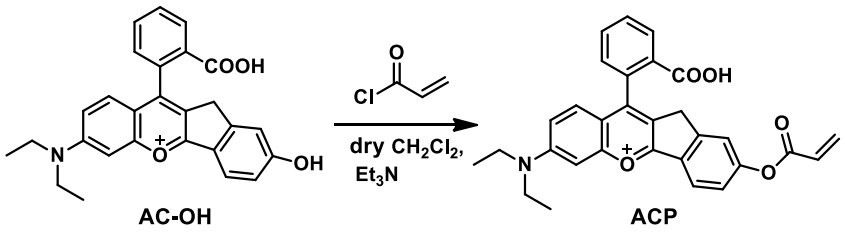
[c]*Key Laboratory of Optic-electric Sensing and Analytical Chemistry for Life Science, MOE, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, PR China.*

**Email - chenmei@hnu.edu.cn*

1. Materials and General Experimental Methods

All chemicals were purchased from commercial suppliers and used without further purification. Distilled water was used for all experimental procedures. Mass spectra were acquired using a Thermo Finnigan LCQ advantage ion trap mass spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker-400 spectrometer, which was referenced to tetramethylsilane (TMS) as an internal standard. Fluorescence spectra were obtained with a HITACHI F4600 fluorescence spectrophotometer. The fluorescence images were taken by an Olympus FV1000 confocal laser scanning microscope equipped with a CCD camera and Nikon confocal microscope. Thin-layer chromatography (TLC) analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200-300). Both gels were purchased from the Yantai Jiangyou Silica Gel Development Company Limited.

2. Synthesis and Characterization



Scheme S1. The synthetic route of probe **ACP**.

3. Spectrometric Studies

For photophysical characterization, the compound **ACP** was dissolved in CH_3CN to make the stock solutions (500 μM), which were diluted to 10 μM as the testing solutions with PBS buffer solution (25 mM, 20% CH_3CN, pH 7.4). Absorption and fluorescence spectroscopic studies were performed on a Shimadzu UV-1800 power spectrometer; a Hitachi F-4600 fluorescence spectrophotometer.

4. Determination of the fluorescence quantum yield:

Fluorescence quantum yield for **ACP** was determined by using Rhodamine B (Φ_f = 0.65 in EtOH) as a fluorescence standard.[1] The quantum yield was calculated using the following equation[2, 3]:

$$\Phi_{F(X)} = \Phi_{F(S)} (A_S F_X / A_X F_S) (n_X / n_S)$$

Where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts S and X refer to the standard and to the unknown, respectively.

5. Fluorescence Microscopic Studies

Cell culture. HepG2/HeLa cells were cultured in high glucose Dulbecco's Modified Eagle Medium (DMEM, Hyclone) containing 10% fetal bovine serum and 1% antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin) in 5% CO_2 at 37 °C. Cells were then carefully harvested

and split when they reached 80% confluence for maintaining exponential growth.

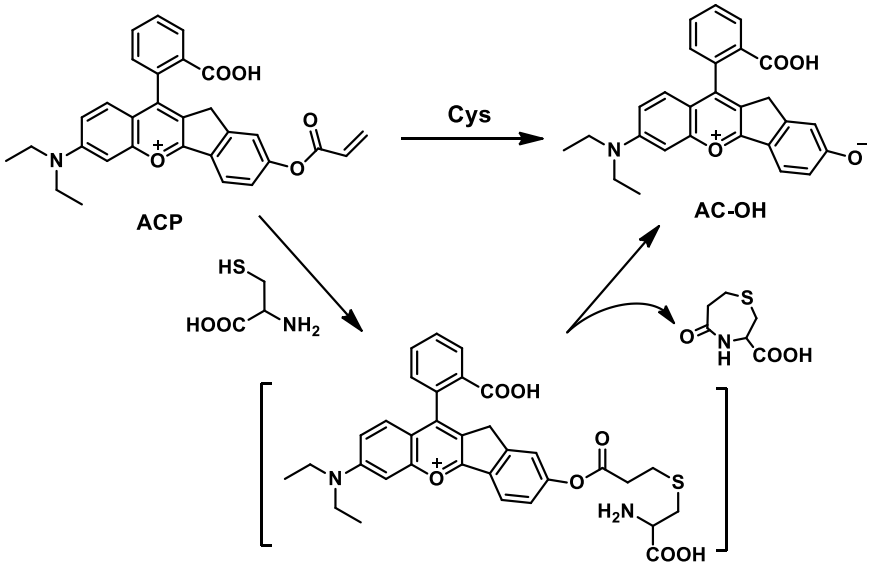
Cell cytotoxicity. Cell cytotoxicity was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Typically, HepG2 cells were plated at 1×10^5 cells per well into a 96-well flat bottom plateand incubated overnight at 37 °C prior to exposure to **ACP**. Then the MTT (0.5 mg/mL) solutionwas added into each well. After incubation for 4 h, Dimethyl sulfoxide (DMSO) (100 μL/well) was added and incubated with cells to dissolve the precipitated formazan crystals. The absorbance at 490 nm was recorded by a multi-detection microplate reader.

6. Two-photon Imaging Studies

The two-photon cell experiment can be divided into two groups. The first group is that the HepG2 cells were incubated with 10 μM **ACP** for 30 min. Then the cells were washed by PBS buffer before imaging. The second group, HepG2 cells were pre-incubated with 400 μM N-ethylmaleimide (NEM) for 30 min, and then treated with 10 μM **ACP** for 30 min. Then the cells were washed by PBS prior to imaging. The two-photon microscopic imaging uses OLYMPUS FV1000 (TY1318) confocal microscope with an excitation filter of 810 nm and the collection wavelength range is from 550-650 nm.

Experiments on animals were well designed and approved by the Animal Ethics Committee of College of Biology (Hunan University). All the related procedures are following closely the Guidelines for Care and Use of Laboratory Animals of Hunan University.

7. Supplemental Figures



Scheme S2. The proposed reaction mechanism of probe **ACP** and Cys.

Table S1. Properties of respresentive fluorescent probes for Cys

Probes	λ_{ex}/nm	λ_{em}/nm	detection limit/μM	Sensitivity	Two photon property measurement	Biomaging application
CCy[4]	445	550	0.411	--	Yes	Cell and tissues
Co-Cys[5]	400	496	0.1080	29.88-fold	Yes	Cell, tissues and zebrafish
Cou-SBD-Cl[6]	450	580/481	1.4	56.1-fold	NO	Cell and zebrafish
ANBI[7]	460	590	2.3 / 0.35	--	Yes	Cell and tissues
CP-1[8]	463	556	5.0	12-fold	NO	Cell
CAC[9]	408	490	0.19	--	NO	Cell and zebrafish
Ly-1[10]	430	498	1.2	90-fold	NO	Cell-zebrafish
CyA[11]	670	697	0.13 / 0.16	--	NO	Cell
Probe-2[12]	450 / 332	472/540	0.084	62.4-fold	NO	Cell
TP-NIR[13]	557	702	0.2	35-fold	Yes	Cell
CP-NIR[14]	600	760	0.048	40-fold	NO	Cell and in vivo
TP-Ratio-Cys[15]	375	460 / 580	--	36-fold	Yes	Cell and tissues
ACP (this-work)	550	611	0.0241	51-fold	Yes	Cell and tissues

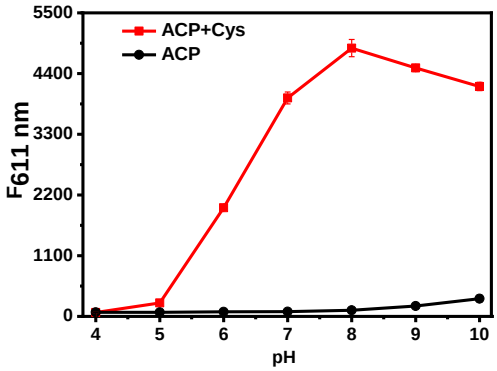


Figure S1. Fluorescence intensity of probe **ACP** (10 μM) with or without Cys (40 μM) at different pH conditions (25 mM PBS, containing 20% CH_3CN).

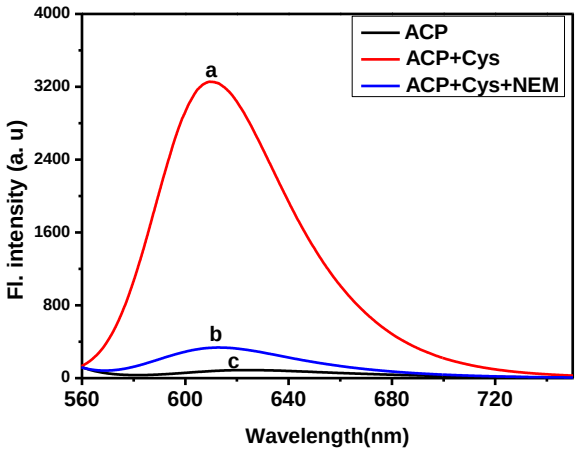


Figure S2. Fluorescence emission spectra of different reaction systems. (a): system (c) + Cys (25 μM); (b): system (a) + NEM (200 μM); (c): **ACP** (10 μM). $\lambda_{ex/em}$= 550/611 nm.

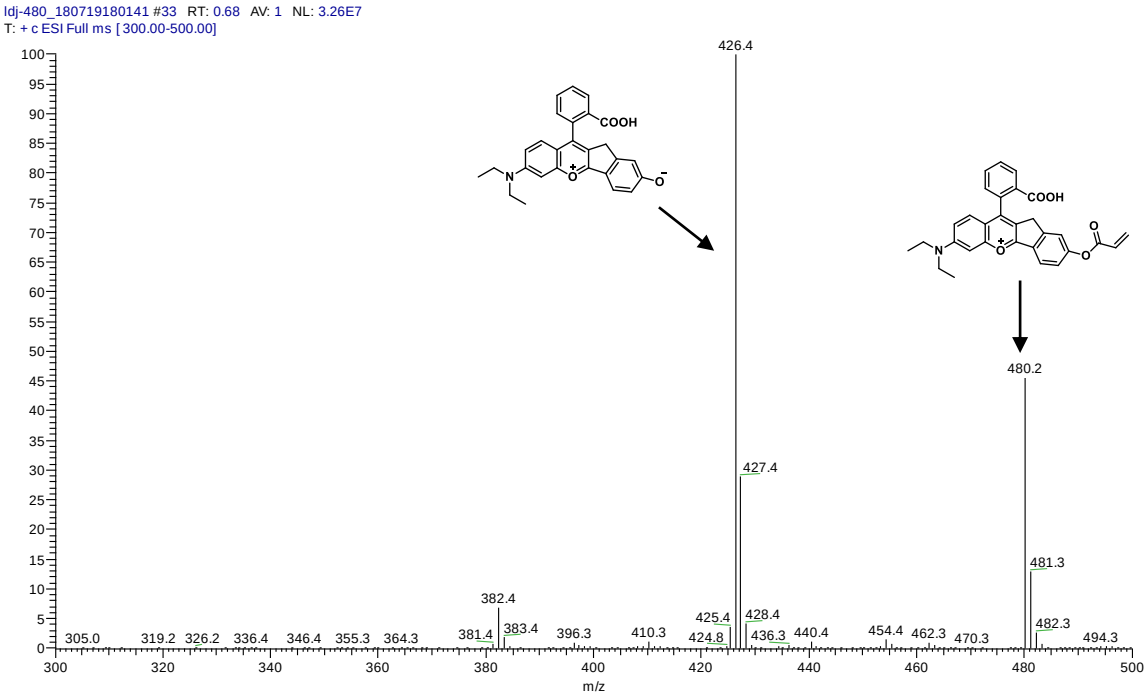


Figure S3. The ESI mass spectrum of probe **ACP** in the presence of Cys.

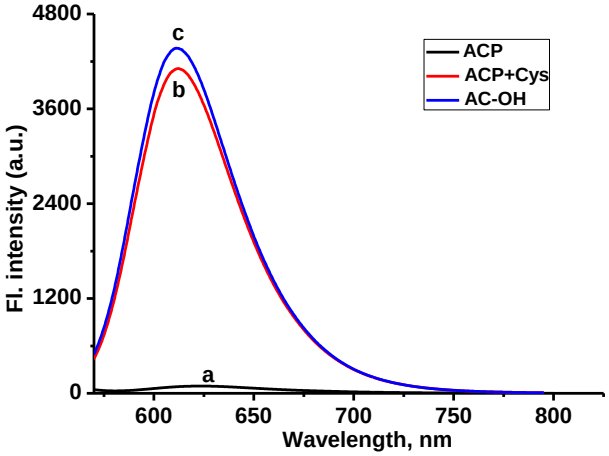


Figure S4. Fluorescence emission spectra of different reaction systems. (a): **ACP**; (b): system (a) + Cys; (c): **AC-OH**. λ_{ex} = 550 nm.

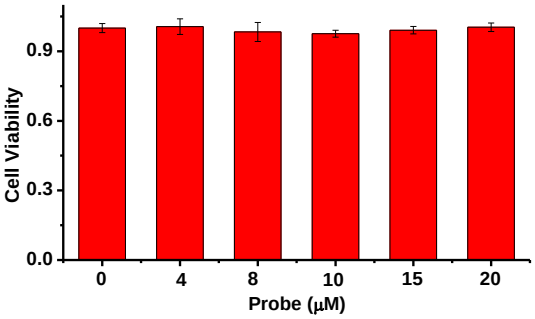


Figure S5. Cytotoxicity of **ACP** in living HepG2 cells. Cells were incubated with the probe at different concentrations for 24 h. Cell viability was measured by MTT assay and the results are reported as percentage relative to untreated cells (mean ± SD).

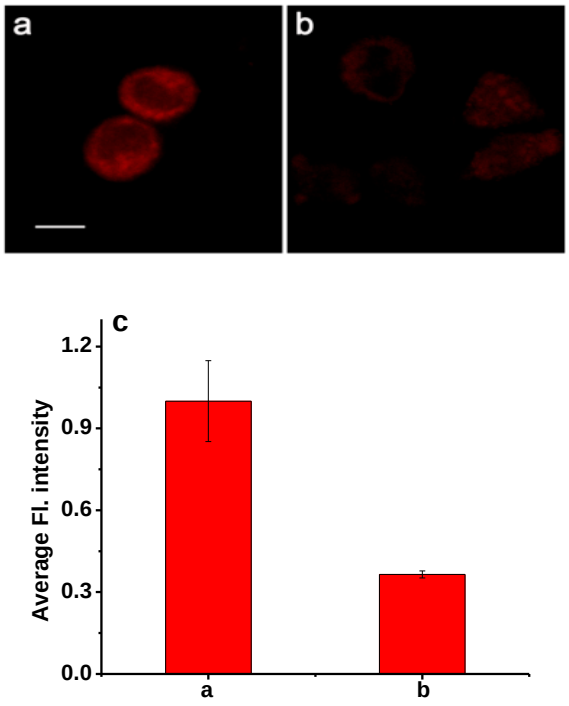


Figure S6. Fluorescence images of probe **ACP** responding to Cys in living HeLa cells by confocal fluorescence imaging. (a) Cells were incubated with probe **ACP** (10 μM, 30 min), and then imaged. (b) Cells were pretreated with NEM (400 μM, 30 min), then treated with probe **ACP** (10 μM, 30 min), and then imaged. The fluorescence images were captured from the red channel of 550-650 nm with excitation at 559 nm. (c) Average fluorescence intensity in panels a-b. Data are expressed as mean ± SD of three parallel experiments. Scale bar = 10 μm.

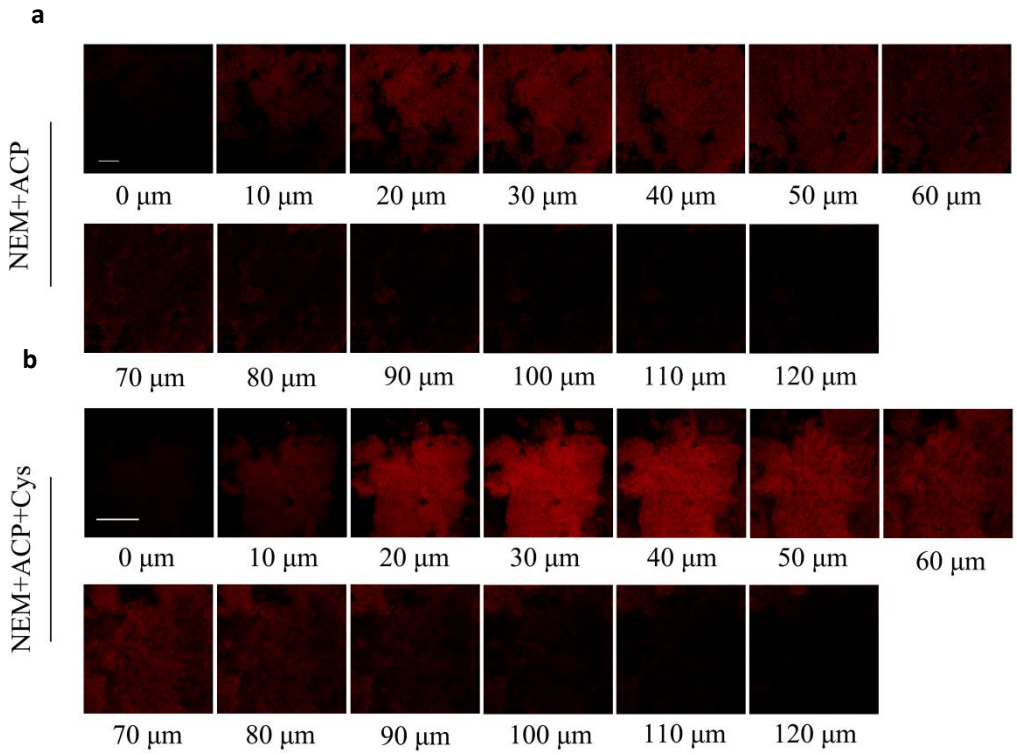


Figure S7. Two-photon fluorescence images of fresh mouse liver slice pretreated with 1000 μM NEM for 30 min and then incubated with **ACP** (10 μM**)** for 30 min (a), and pretreated with Cys (50 μM) for 30 min after incubated with NEM (1000 μM) for 30 min, subsequently incubated with probe **ACP** (10 μM) for 30 min (b) at the depths of approximately 0~120 μm, respectively. Excitation at 810 nm. Scale bar = 200 μm.

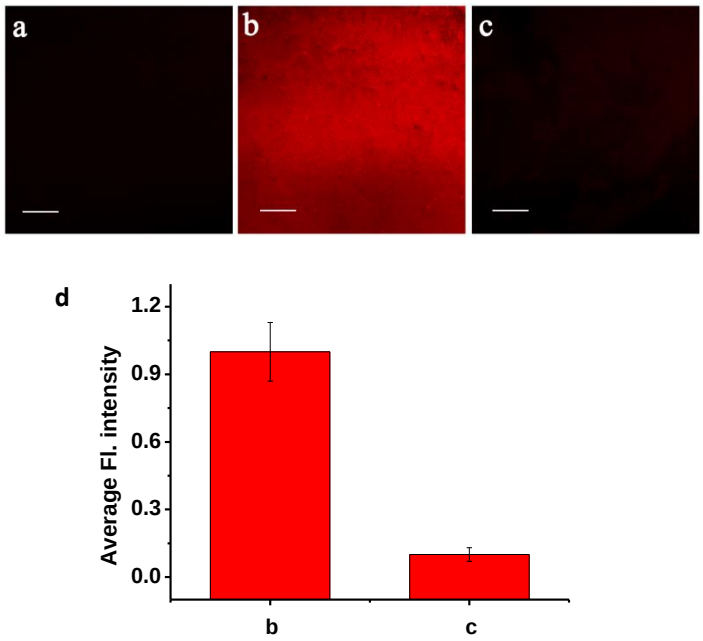


Figure S8. One-photon fluorescence images of fresh mouse liver slice with a magnification of 10×. First column, intact tissues (a); Second column, tissues were treated only **ACP** (10 μM) for 30 min (b); Third column, tissues were pretreated with 1000 μM NEM for 30 min and then with probe **ACP** (10 μM) incubated for 30 min (c). (d) Average fluorescence intensity in (b-c). Excitation at 559 nm and emission band at 550-650 nm. Scale bar: 200 μm.

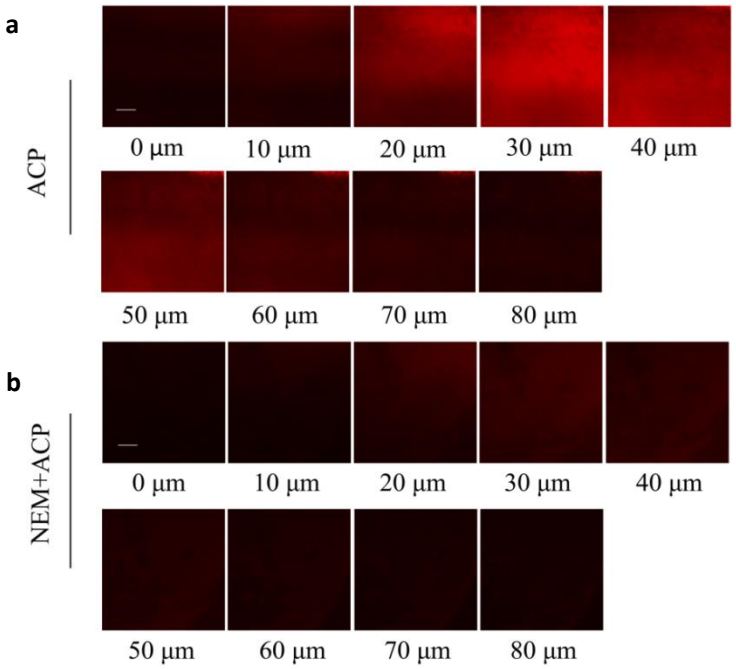


Figure S9. One-photon fluorescence images of fresh mouse liver slice incubated with only **ACP** (10 μM**)** for 30 min (a) and pretreated with 1000 μM NEM for 30 min and then incubated with **ACP** (10 μM**)** for 30 min (b) at the depths of approximately 0~80 μm. Excitation at 559 nm. Scale bar = 200 μm.

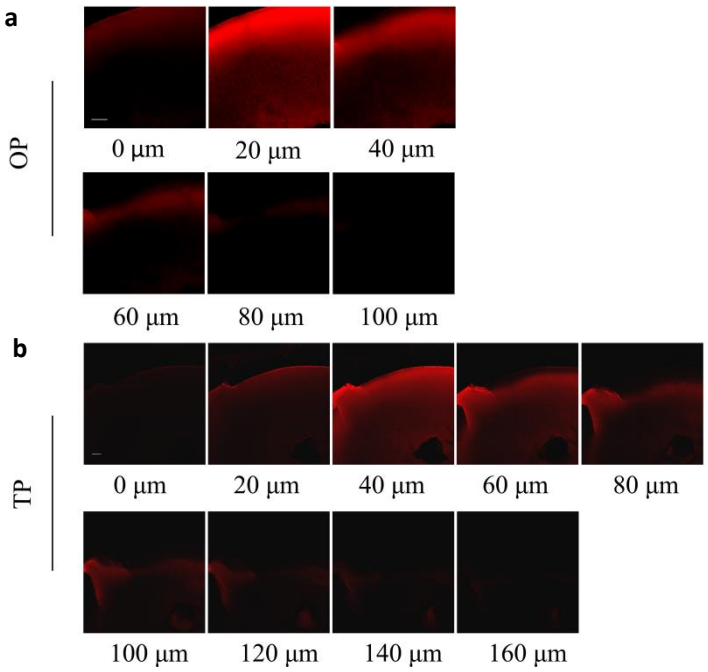


Figure S10. One-photon (a) and two-photon (b) fluorescence images of fresh mouse liver slice incubated with **AC-OH** (10 μM**)** for 30 min at the depths of approximately 0~100 μm and 0~160 μm. Scale bar = 100 μm.

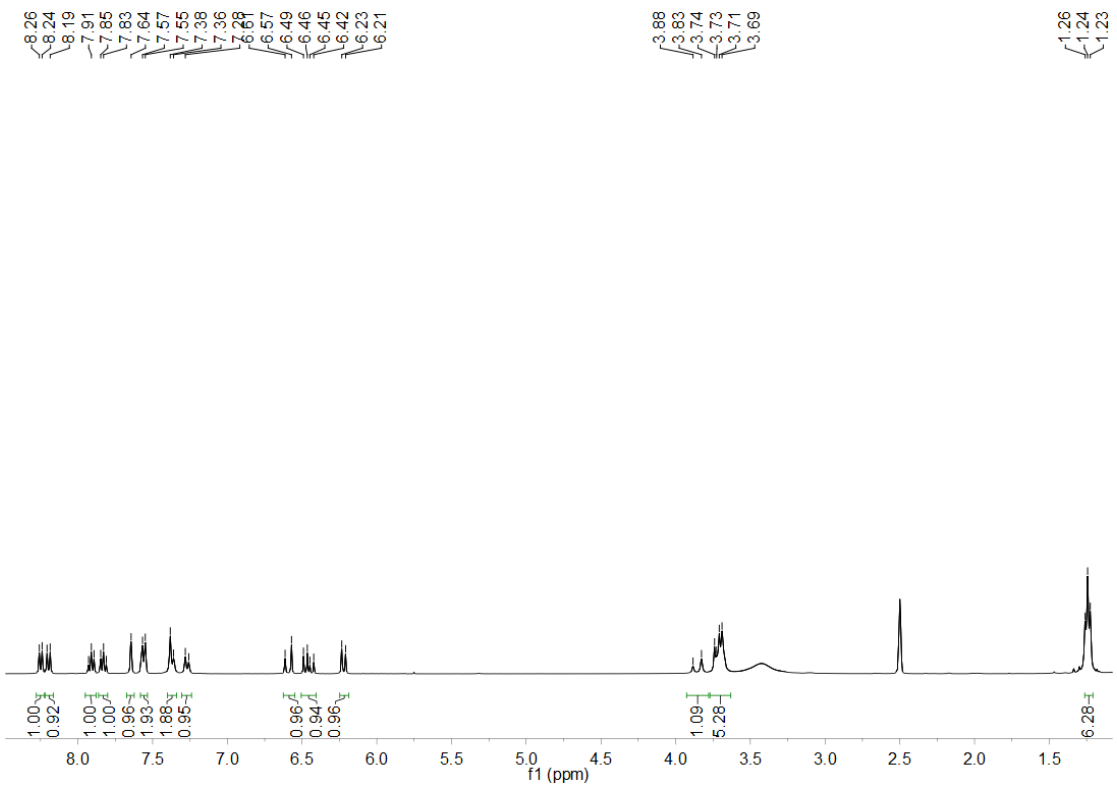


Figure S11. The [1]HNMR spectrum of compound **ACP** (DMSO-d_6)

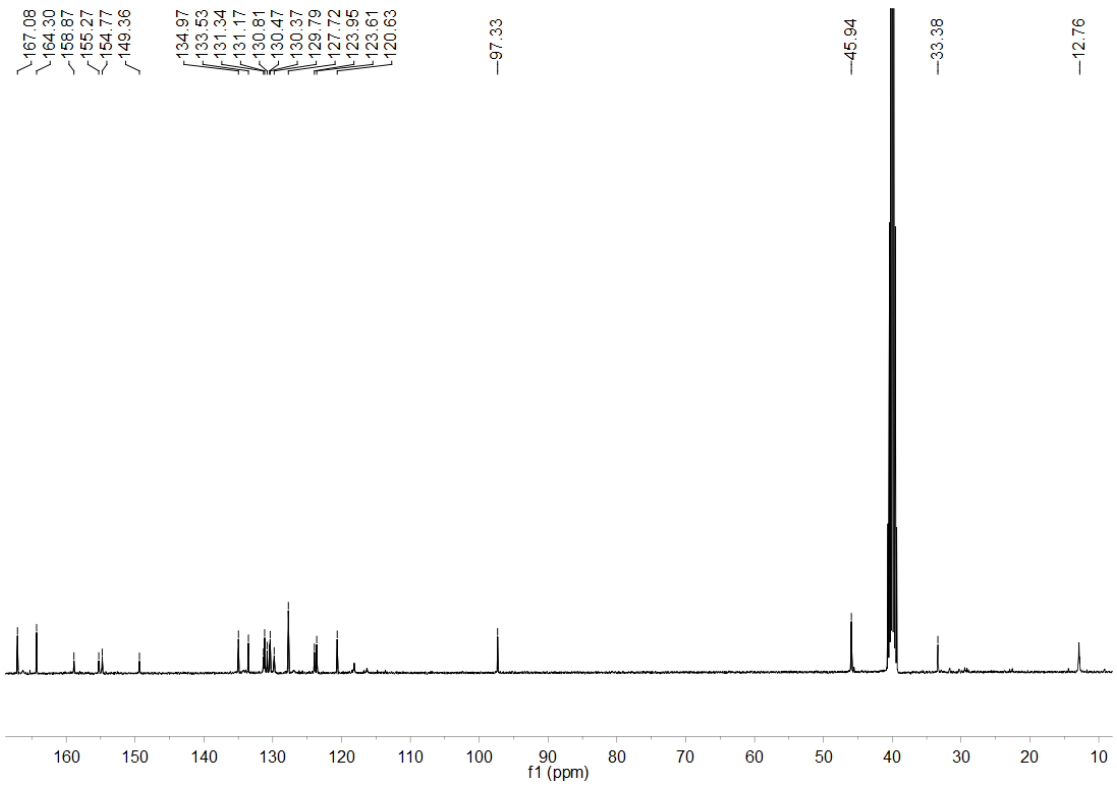


Figure S12. The [13]CNMR spectrum of compound **ACP** (DMSO-d_6)

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