Supporting Information

Visualization of carboxylesterase 2 with a near-infrared two-photon fluorescent probe and its potential evaluation of anticancer drug effects in orthotopic colon carcinoma mice model

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Contents:

Materials. All reactions were performed under argon protection at dark environment and monitored by thin-layer chromatography (TLC) (Hailang, Yantai). Flash chromatography was carried out using silica gel (200 - 300 mesh). For imaging experiments, **DCM-CES2** with a purity of above 95% was used. All organic solvents were purchased from Energy Chemical, Shanghai, China. The fetal bovine serum (FBS), Dulbecco's Modified Eagle Medium (DMEM), were purchased from Gibco, American. All chemicals used in synthesis were analytical reagent grade and were used as received. Ultrapure water was used throughout all the experiments. Phosphate buffer saline (PBS) and all inorganic reagents were obtained from Aladdin and CCK-8 (Cell Counting Kit-8) was purchased from Dojindo, Shanghai. Different cell lines including HT-29 cells and HepG2 cells were purchased from the Committee on Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). All chemicals were commercial products of analytical grade. Paraoxonases including PON1 and PON2 were obtained from bio-world, acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and human serum albumin (HSA) were all obtained from Sigma. The recombinant CESs isoforms CES1 and CE2 were purchased from BD company.

Instruments. Thin-layer chromatography (TLC) was performed on silica gel plates. Silica gel P60 (SiliCycle) was used for column chromatography (Hailang, Yantai) 200 - 300 mesh. Absorption spectra were obtained on UV-Vis spectrophotometer (Hitachi, U-2910). Fluorescence spectra were obtained with a Hitachi F4600 Fluorescence Spectrophotometer. ^{1}H NMR and ^{13}C NMR were obtained from Bruker AM 400 MHz and 100 MHz spectrometers, respectively. High-resolution mass spectra were carried on Agilent 1290 infinity 6540 UHD accurate mass Q-TOF MS (Agilent, USA). The fluorescence images of cells were acquired using Zeiss LSM 880 NLO two-photon laser scanning confocal microscope from Carl Zeiss AG company (set at wavelength 830 nm) through 60× oil and zoom objective. Flow cytometry data were collected by BD Biosciences FACSAria and Sysmex CyFlow Cube6. Ultrathin sections were cut by Leica EM UC7. T. Mice fluorescence images were collected by PerkinElmer IVIS® Lumina XR Series Ⅲ System. Mice pathological splices were imaged by Nikon Model Eclipse Ci-L microscope.

Spectral experiments. Both absorption and fluorescence spectra were measured in 10 mM PBS (pH 7.4, 37 ^{o}C) solution. PBS was treated with N_2 for 5 min before using. The pH gradient of PBS buffer solution from 4.0 to 9.0 was achieved by adding different volumes of HCl or NaOH solution. Ultrapure water was used to prepare all aqueous solutions. Absorption spectra were obtained with 1.0 - cm glass cells. Fluorescence emission spectra were obtained with a Xenon lamp and 1.0-cm quartz cells. The fluorescence intensity was measured at $\lambda_{ex}/_{em}$= 560/650 - 750 nm. The detection limit of the probe for CE2 was calculated based on the method of 3σ/k, where σ was the standard deviation of blank measurement, and k referred to the slope between the fluorescence enhancement versus the CE2

concentration. The mixtures were equilibrated requested time at 37 °C before measurement.

Enzymatic kinetics assays. Probe (1 - 100 μM) were evaluated in triplicate with CE2 at a final concentration of 10 μg/mL in 10 mM PBS (pH 7.4). The amount of enzyme was chosen to allow an accurate measurement of the initial rate. Probe and enzyme solutions were incubated separately at 37 °C for 10 min. Prior to the addition of the enzyme to the probe, the reaction was monitored by measuring fluorescence change at 685 nm by **DCM-CES2** (excited at 560 nm) at 37 °C. The initial velocity was calculated from the slope of each progress curve. The parameters such as K_m and K_{cat} with CE2 for probe **DCM-CES2** were determined by Lineweaver-Burk plot: $V = V_{max}\cdot[\text{probe}]/(K_m+[\text{probe}])$, where V was the reaction rate, [probe] referred to the concentration of the probe, and K_m was the Michaelis constant.

Measurement of Two-Photon Cross-Section. The two-photon cross-section of **DCM-CES2** and **DCM** (50 μM) were measured in PBS buffer (pH = 7.4) as described in literature [1]. The cross-sections of **DCM-CES2** and **DCM** were calculated by using the following formula: $\delta = \delta r \times (Fs \times \phi r \times nr)/ (Fr \times \phi s \times ns)$, where δ, F, ϕ and n stood for TP cross-section, spectral integral area, quantum yield and concentration, respectively; s and r stood for sample and reference, respectively. Fluorescein (aqueous, 1 μM, pH = 11) was used as a reference probe. From 700 nm to 900 nm, **DCM** was calculated to have a two-photon absorption cross-section of δ_{max} = 26 GM (1 GM = $10^{-50}\cdot cm^4\cdot s\cdot photon^{-1}\cdot molecule^{-1}$) at 830 nm (Fig. S2). The results demonstrated that **DCM-CES2** could be used for CE2 imaging by two-photon microscopy in cells.

Cell culture and confocal imaging. HT-29 cells HepG2 cells were incubated in DMEM supplemented with 10% fetal bovine serum (FBS). The cultures were maintained at 37 ℃ in a 95% humidified atmosphere with 5% CO_2. Living cells were firstly inoculated to confocal plate following by adding 1 mL fresh complete culture media and cultured for 24 h. Then, the cells were incubated with 10 μM probe **DCM-CES2** solution before performing imaging experiment. Confocal Images of cells were obtained using Zeiss LSM 880 NLO two-photon laser scanning confocal microscope (set at wavelength 830 nm) with an oil objective lens 60 × and zoom, respectively. For red channel: the excitation wavelength was selected 830 nm and the collected wavelengths were selected 650 - 750 nm. For green channel: the excitation wavelength was selected 490 nm, and the collected wavelengths were selected 515 nm. For blue channel: the excitation wavelength was selected 405 nm, and the collected wavelengths were selected 420 - 480 nm.

Flow cytometry analysis of CES2 by DCM-CES2. Flow Cytometry assay was performed for the detection of CE2 with probe **DCM-CES2**. The cells were cultured at 2.0×10^5 cells/well in 6-well plates, and then treated with **DCM-CES2** for 10 min. After harvest, cells were washed, digested by Trypsin-EDTA Solution, and resuspended in PBS and analyzed by flow cytometry. (λ_{ex} = 560 nm, λ_{em}

= 650 - 750 nm).

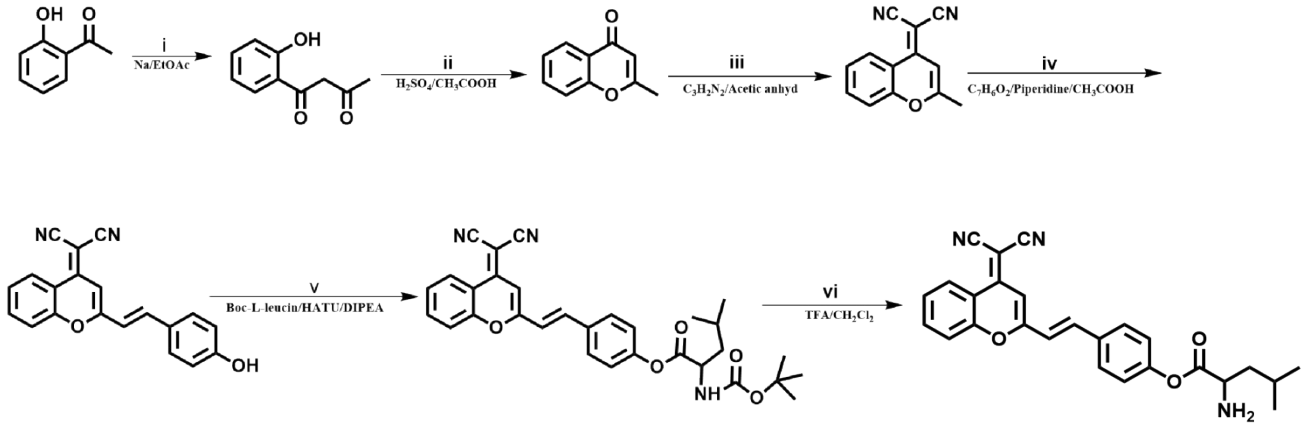
Flow cytometry assays for apoptosis and necrosis. The five group of cells were inoculated in six-well plates of ~ 2.0×10^5 cells per well and then treated with irinotecan and capecitabine as described in the paper. Afterwards, the cells were washed and resuspended with PBS buffer solution. Annexin V-FITC/PI (Propidium Iodide) staining was carried out using an Apoptosis and Necrosis Assay Kit according to the manufacturer's instructions. The tested cells (2.0×10^5 cells/well) were resuspended by staining buffer, and then added 5 μL of Annexin V-FITC and 10 μL PI staining solution. After mixing, the samples were incubated in ice bath for 30 min and then analyzed by flow cytometry.

Cytotoxicity of DCM-CES2. The cytotoxicity of probe **DCM-CES2** was assessed by the CCK-8 assay. The cells were seeded into a 96-well cell culture plate at a final density of 5×10^3 cells/well. After the cells were incubated for 24 h at 37 °C under 5% CO_2, different concentrations of **DCM-CES2** (0 - 100 μM) were added to the wells. Subsequently, the cells were incubated for 24 h at 37 °C under 5% CO_2. Then, 10 μL CCK-8 solution was added to each well of the plate and incubated for 4 h at 37 °C under 5% CO_2.

***In vivo* imaging in living mice models.** Kunming female mice (KM) with 6 - 8 weeks old and weighing 18 - 22 g, were from Hainan Medical University. All animals were quarantined as required. During the quarantine period, the animal's activities, diet and other performances were observed. The animals were inspected before the test, and the quarantined animals were used for the test. Tumor-bearing mice: after 5 days of adaptive feeding of KM mice, each mouse was inoculated with 0.1 mL of MC38 colon cancer cell suspension under the midline of the back. We could notice the tumor when time extended to 7 - 10 days. As for *in situ* tumor: 1.5% pentobarbital sodium was injected to anesthetize KM mice after feeding for 5 days. MC38 cell suspension (0.1 mL) was added to mice which were opened in the abdominal cavity and 1 mL syringe needle was lightly swept the mouse colon. At last the abdominal cavity was sutured and we put mice back in the cage and continued to raise them normally. Mice would form tumors in about 28 days. Images were taken by Perkinelmer IVIS Lumina XRMS Series III In Vivo Imaging System, with an excitation filter of 560 nm and emission windows from 650 nm to 750 nm after *in situ* injection probe **DCM-CES2** to the tumor.

The Ethics Committee of Hainan Medical University approved this study. All surgical procedures and experimental protocols were approved by the Institutional Animal Care and Use Committee in Hainan Medical University, Haikou, China.

2. Synthetic routes of the probe DCE-CES2.



Scheme S1 Synthesis route for probe **DCM-CES2**.

Ⅰ): 1-(2-hydroxyphenyl) ethanone (5.0 g, 36.75 mmol) was dissolved in 30 mL ethyl acetate, and then sodium (4.00 g, 0.17 mmol) was added into the solution. Adding 5 mL of methanol along the wall of the round bottom flask to remove the remaining sodium from the reaction after violently stirring for 18 h at ambient temperature. Then we added 50 mL of 0.5 M HCl dropwise until the solution turned black and all the remaining HCl solution was added. After that we extracted with ethyl acetate to give a black organic phase. The organic phase was dried over Na_2SO_4, filtered, and concentrated to yield the final crude product as a yellow solid (3.6 g, 55%) which was directly used in the next reaction without further purification.

Ⅱ): Concentrated hydrochloric acid (1 mL) was slowly added to a solution of 1-(2-hydroxyphenyl) butane-1,3-dione (3.6 g, 20.2mmol) in methanol (30 mL). After that, the solution mixture was heated to reflux for 4 h, and the solvent was evaporated under reduced pressure, and then extracted with 50 mL of ethyl acetate and 50 mL of saturated saline solution. The organic phase was concentrated on a rotary evaporator, and the residues were purified by silica chromatography (200-300 mush) eluted with Petroleum ether: EAC = 10:1 - 5:1 (v / v) to give a yellow solid (2.4 g, 75.0 %). ^{1}H NMR (400 MHz, CD_3OD) δ(ppm): 8.16 - 8.19 (m, 1H), 7.62-7.66 (m, 1H), 7.37-7.42 (m, 2H), 6.17 (s, 1H), 2.38 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ(ppm): 178.27, 166.23, 156.48, 133.44, 125.62, 124.92, 123.54, 117.66, 110.44.

Ⅲ): 2-methyl-4H-chromen-4-one (2.4 g, 15.6 mmol) and malononitrile (1.20 g, 18.1 mmol) were dissolved in 15 mL acetic anhydride. The solution was refluxed for 14 h and then the solvent was evaporated in vacuo. Deionized water (80 mL) was added to the residue and the mixture was refluxed for another 0.5 h, followed by extraction with methylene dichloride. The organic layers were dried over Na_2SO_4, filtered, and concentrated. The obtained crude product was purified by silica column chromatography to yield an orange solid (1.3 g, 40 %). ^{1}H NMR (400 MHz, CD_3OD) δ(ppm): 8.89-8.91 (m, 1H), 7.7-7.74 (m, 1H), 7.45-7.48 (m, 2H), 6.71 (s, 2H), 2.44 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 161.74, 153.24, 152.91, 134.61, 126.04, 125.79, 118.67, 116.58, 104.47, 62.26, 20.49.

Ⅳ): 2-(2-methyl-4H-chromen-4-ylidene) malononitrile (126 mg, 0.60 mmol), and N-(4-formyl-phenyl) acetamide (90.0 mg, 0.56 mmol) were dissolved in toluene (15 mL) with piperidine (0.20 mL) and acetic acid (0.20 mL) under argon protection at room temperature. Then the mixture was refluxed for 3 h to give an orange precipitate. After filtration, the orange solid was refluxed in a solution of conc. HCl and ethanol (2:1, 50 mL) for another 2 h before the pH of the solution was adjusted to neutral. The aqueous solution was extracted with ethyl acetate and then the organic layers were dried over Na_2SO_4, filtered, and concentrated to obtain the crude product which was purified by silica column chromatography to yield a crimson solid (84.24 mg, 45%). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.73 (d, 1H), 7.91 (t, 1H), 7.79 (d, 1H), 7.70 (d, 1H), 7.61 (dd, 3H), 7.27 (d, 1H), 6.95 (s, 1H), 6.85 (d, 2H). ^{13}C NMR (100 MHz, DMSO-d6) δ 160.1, 158.8, 152.7, 152.0, 139.2, 135.2, 130.3, 126.0, 124.6, 118.9, 117.4, 117.1 116.0, 115.8, 105.6, 59.0.

Ⅴ)**:** Boc-L-leucine (440 mg, 1.6 mmol), HATU (760 mg, 2 mmol) and DIPEA (260 μL, 1.2 mmol) were dissolved in dry CH_2Cl_2 (40 mL) with stirring at 0 °C for 30 min. Then DCM (164 mg, 0.4 mmol) in CH_2Cl_2 (5 mL) was introduced, and the reaction mixture was further stirred at room temperature for 4 h. The precipitate was filtered, and the filtrate was evaporated under reduced pressure, and produced compound Boc-DCM-CES2 as dark-blue solid.

Ⅵ)**:** Compound Boc-DCM-CES2 was stirred in CH_2Cl_2 (5 mL) containing trifluoroacetic acid (1 mL) at room temperature for 3 h. The solvent was removed by evaporation under reduced pressure, and the

crude product was purified by silica gel chromatography eluted with CH_2Cl_2/CH_3OH (v/v, 10:1), affording probe **DCM-CES2** as orange solid (136 mg, yield 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 8.69 - 8.71 (m, 1H), 7.87 - 7.93 (m, 2H), 7.74-7.76 (m, 1H), 7.55 - 7.67 (m, 4H), 7.2 - 7.24 (d, 1H), 6.84 - 6.91 (t, 3H), 4.01 - 4.04 (m, 1H), 2.5 - 2.51 (s, 1H), 1.91 - 2.33 (m, 3H), 1.43 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d6) δ 177.45, 174.88, 172.60, 160.52, 159.33, 153.27, 152.47,139.74, 135.69, 130.81, 126.57, 126.49, 125.05, 119.45, 117.83, 117.57, 116.50, 116.35, 106.14, 81.35, 59.61, 55.86, 55.21, 29.39, 29.07, 29.05.

3. Effect of pH on DCM-CES2 and its metabolite DCM.

Fluorescence spectra were obtained with a Hitachi F4600 Fluorescence Spectrophotometer and fluorescence spectra were measured in 10 mM PBS (pH 7.4, 37 °C) solution. The pH gradient was achieved in PBS buffer solution from 4.0 to 9.0. As shown in Fig. S2, during the pH range from 4.0 to 9.0, there was negligible fluorescence change at the maximum emission (685 nm) for **DCM-CES2** with excitation at 560 nm, revealing the high stability of the sensing probe. Upon the addition of CE2, a substantial increase of fluorescence intensity at 685 nm was observed in the pH of 6 to 7.4 and then reached a platform, demonstrating that **DCM-CES2** could be used to monitor CE2 over extensive pH range.

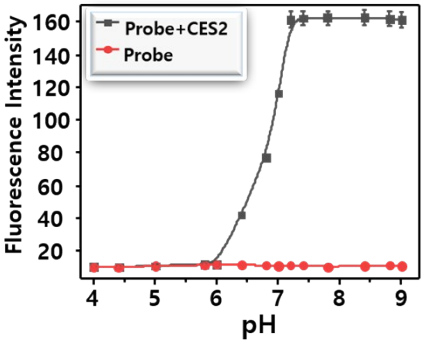


Fig. S1 Effect of pH values on the fluorescence intensity of **DCM-CES2** and its metabolite DCM (10 μM) in different PBS buffer (10 mM) at 37 °C, respectively. pH values: 4.0, 4.4, 5.0, 5.8, 6.0, 6.8, 7.0, 7.2, 7.4, 7.6, 7.8, 8.0, 8.4, 8.8, 9.0 (λ_{ex} = 560 nm, λ_{em} = 650 - 750 nm). The experiments were repeated for three times and the data were shown as mean (±S.D.).

4. Measurement of Two-Photon Cross-Section.

The two-photon cross-section of **DCM-CES2** (50 μM) and **DCM** (50 μM) were measured in PBS buffer (pH = 7.4) as described in literature.[1] The cross-sections of **DCM-CES2** and **DCM** were calculated by using the following formula: δ = δr × (Fs × ϕr × nr) / (Fr ×ϕs × ns), where δ, F, ϕ and n stood for TP cross-section, spectral integral area, quantum yield and concentration, respectively; s and r stood for sample and reference, respectively. Fluorescein (aqueous, 1 μM, pH = 11) was used as a

reference probe. From 700 nm to 900 nm, **DCM** was calculated to have a two-photon absorption cross-section of δ_{max} = 26 GM (1 GM = 10^{-50}·cm^4·s·$photon^{-1}$·$molecule^{-1}$) at 830 nm (Fig. S4). The results demonstrated that **DCM-CES2** could be used for CE2 imaging by two-photon microscopy in cells.

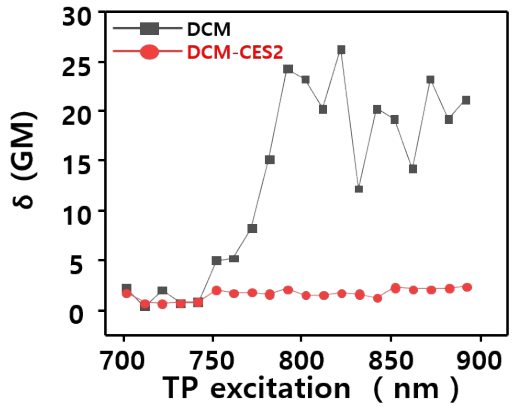


Fig. S2 The cross-sections of **DCM-CES2** (50 μM) and **DCM** (50 μM) were calculated by using the following formula: δ = δr × (Fs × ϕr × nr)/(Fr ×ϕs × ns), where δ, F, ϕ and n stood for TP cross-section, spectral integral area, quantum yield and concentration, respectively; s and r stood for sample and reference, respectively.

5. Selectivity of DCM-CES2.

The ability for **DCM-CES2** to specifically react with CE2 over various potential interfering species such as amino acids, metal ions and several redox active species under physiological conditions was crucial to achieve accurate detection. We tested three types of metal ions, six kinds of amino acids, vitamin, several active species and leucine aminopeptidase, which were commonly existed in living systems. The results indicated that the probe **DCM-CES2** did not respond to all the potential candidates other than CE2 (Fig. S3). These results provided strong evidence that **DCM-CES2** could serve as an effective chemical tool to detect CE2 with high selectivity in complicated physiological systems.

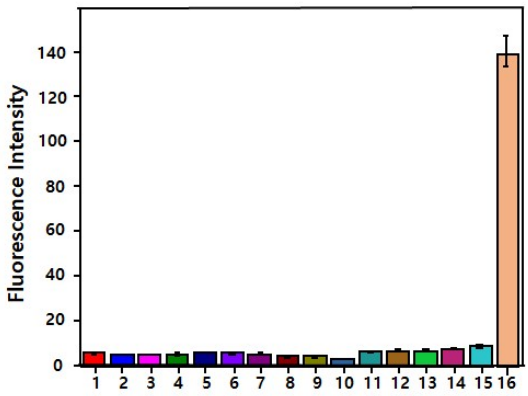


Fig. S3 Fluorescent responses of **DCM-CES2** (10 μM) towards various interfering substances compared with CE2 in cells at 37 °C for 10 min in PBS. 1, Cysteine (1 mM); 2, Alanine (1 mM); 3,

Glutamine (1 mM); 4, Glutamic acid (1 mM); 5, Arginine (1 mM); 6, Serine (1 mM); 7, Vitamin (1 mM); 8, Ca^{2+} (10 μM); 9, Zn^{2+} (10 μM); 10, Cu^{2+} (10 μM); 11, •OH (50 μM); 12, HOCl (50 μM); 13, H_2O_2 (50 μM); 14, H_2S_2 (50 μM); 15, Leucine aminopeptidase (0.6 U) ;16,CE2. (λ_{ex} = 560 nm, λ_{em} = 650 - 750 nm). The experiments were repeated three times and the data were shown as mean (± S.D.).

6. Cell Counting Kit-8 assay for DCM-CES2.

The cytotoxicity of the probe was evaluated by Cell Counting Kit-8 assay (CCK-8, Fig. S5) with human hepatocellular liver carcinoma cell line (HepG2) and human colorectal cancer cell line HT-29, as described previously.[2] The viability rates of the selected model cell lines were more than 85% when treated with various concentrations of DCM-CES2 ranging from 0 to 100 μM for 24 h at 37 ℃, which indicated the relatively low cytotoxicity and satisfactory biocompatibility of DCM-CES2.

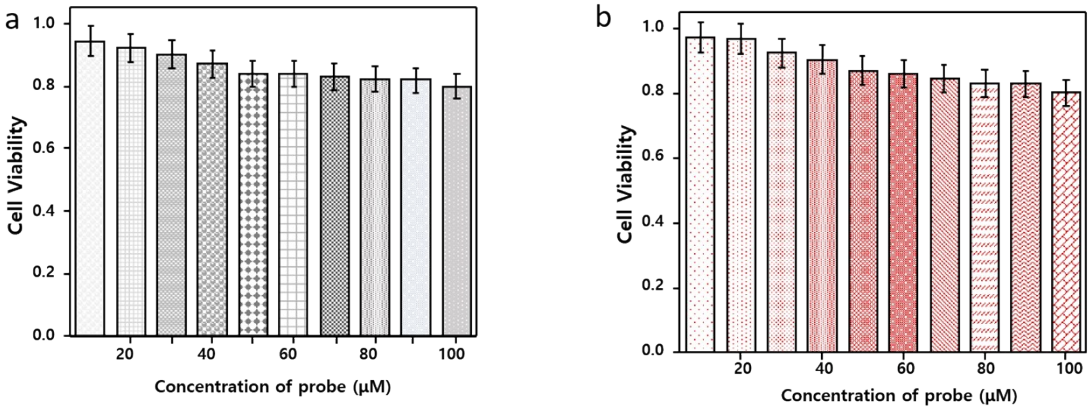


Fig. S4 (a) Cell viability of HepG2 cells and (b) HT-29 cells in different concentrations of **DCM-CES2** (0 - 100 μM) were determined by CCK-8 assay. The experiments were repeated three times and the data were shown as mean (± S.D.).

7. Exploration of incubation time for probe in HepG2 cells.

The cells were incubated with 10 μM probe **DCM-CES2** solution before performing imaging experiment. Confocal Images of cells were obtained using Zeiss LSM 880 NLO two-photon laser scanning confocal microscope (set at wavelength 830 nm) with an oil objective lens 60 ×. As descripted in Fig. S6, HepG2 cells exhibited a negligible intracellular background fluorescence while there initially appeared red emission signal after pretreatment with **DCM-CES2** for 5 min and the fluorescence intensity gradually increased as time extended. Further incubation led to equilibrium and the fluorescence intensity was no longer enhanced about at 30 min, which had been fixed as the optimum incubation time.

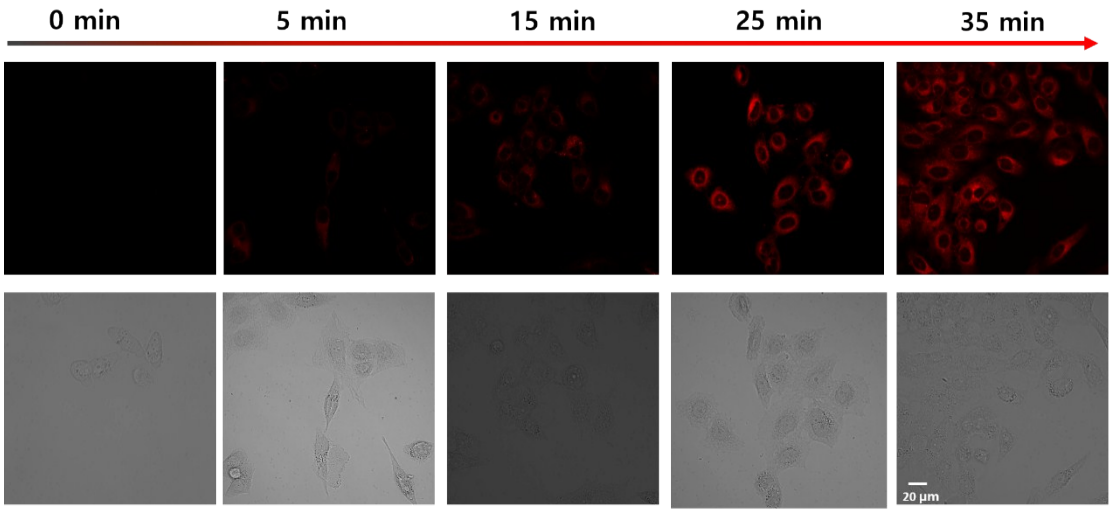


Fig. S5 Cell images obtained using two-photon confocal fluorescence imaging excitation wavelength of 830 nm and a band-path (650 - 750 nm) emission filter with an objective lens (60 x). Fluorescent imaging of CES2 in HepG2 cells at time points: 0, 5, 15, 25, and 35 min. scale bar: 20 μm.

8. Two-photon confocal fluorescence imaging and flow cytometry assay for detection of CE2

To elucidate the localization of the probe in cells, HepG2 cells and HT-29 cells were both incubated with **DCM-CES2** for 30 min, cytoplasmic dye Calcein-AM for 15 min, and the nuclear staining dye Hoechst 33258 for 30 min, respectively. As shown in Fig. S6a – S6f, the selected cells exhibited green and blue fluorescence, respectively in the Calcein-AM and Hoechst 33258 channel when separately excited at 490 nm and 405 nm. A red fluorescence was obtained from the probe **DCM-CES2** with TP mode at 830 nm. The merged images further demonstrated that **DCM-CES2** worked in cytoplasm without entering nucleus.

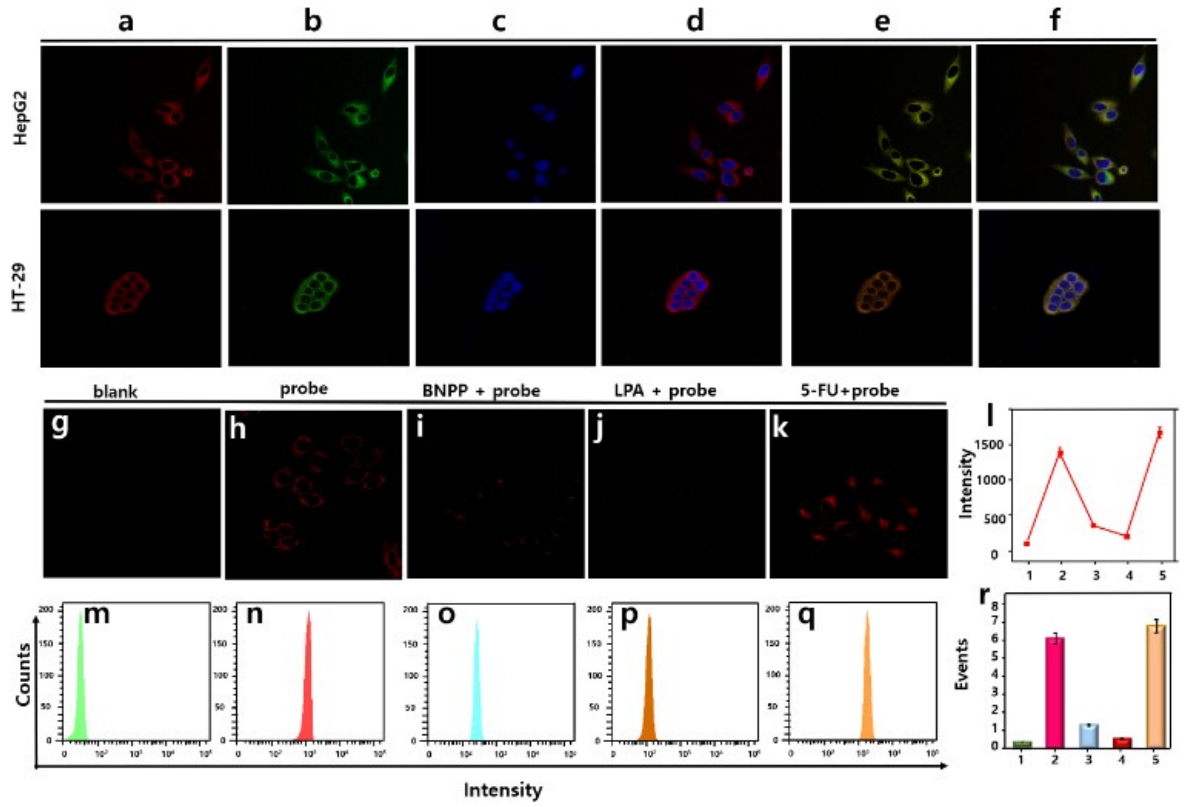


Fig. S6 Two-photon confocal fluorescence imaging and flow cytometry assay for detection of CE2 in HepG2 cells (human hepatocellular liver carcinoma cell line) and HT-29 cells (human colorectal cancer cell line). a) Cells pretreated with **DCM-CES2** (10 μM) for 30 min. b) Cells pretreated with cytoplasmic dye Calcein-AM (10 μM) for 15 min. c) Cells pretreated with nucleus staining dye Hoechst (100 ng/mL) for 30 min. Image was merged by d) Hoechst and probe, e) Calcein-AM and probe, f) Hoechst, Calcein-AM and probe, respectively; Red fluorescence image of **DCM-CES2** (λ_{ex} = 830 nm, λ_{em} = 650 - 750 nm), Green fluorescence image of Calcein-AM (λ_{ex} = 490 nm, λ_{em} = 515 nm); Blue fluorescence image of Hoechst (λ_{ex} = 405 nm, λ_{em} = 420 - 480 nm). g) only HepG2 cells. h) HepG2 cells incubated with 10 μM **DCM-CES2**. i) BNNP (300 μM) and j) LAP (300 μM) were added into HepG2 cells for 60 min, and then incubated with 10 μM **DCM-CES2**. k) HepG2 cells were pretreated with 70 μM 5-FU for 48 h, and then incubated with 10 μM **DCM-CES2**. l) Mean fluorescence intensities of images in g - k. m – q) Flow cytometry assay corresponding to g – k, (λ_{ex} = 560 nm, λ_{em} = 650 - 750 nm) respectively. r). Mean values of m - q. The data were shown as mean (± s.d.).

9. Optimization of imaging time in mice.

As for *in situ* tumor: 1.5% pentobarbital sodium was injected to anesthetize KM mice after feeding for 5 days. MC38 cell suspension (0.1 mL) was added to mice which were opened in the abdominal cavity and 1 mL syringe needle was lightly swept the mouse colon. At last the abdominal cavity was sutured and we put mice back in the cage and continued to raise them normally. Mice would form tumors in about 28 days. Images were taken by Perkinelmer IVIS Lumina XRMS Series III In Vivo Imaging System, with an excitation filter of 560 nm and emission windows from 650 nm to 750 nm after *in situ* injection probe **DCM-CES2** (200 μM, 30 μL in PBS) to the tumor. As displayed in Fig. S7, the control group was injected with PBS. As expected, the injection of **DCM-CES2** resulted in gradually fluorescence increase indicating its rapid activation by CE2 in vivo. The intensity reached maximum at 150 min after injection and remained stable up to 180 min.

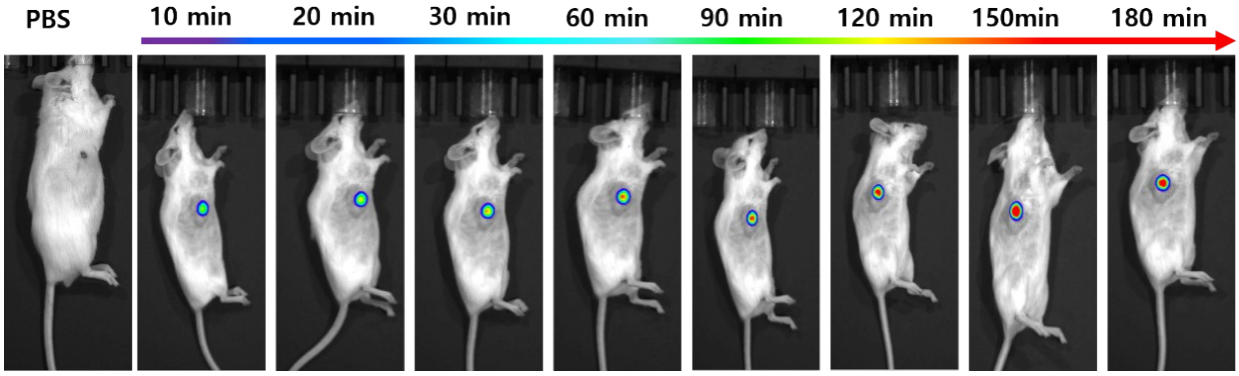


Fig. S7 *In vivo* imaging of CE2 with DCM-CES2 (intratumoral injection, 100 μM, 30 μL in PBS) in tumor-bearing nude mice. (λ_{ex} = 560 nm, λ_{em} = 650 - 750 nm).

10. Bright-field imaging of Fig. S6 and Fig. 2.

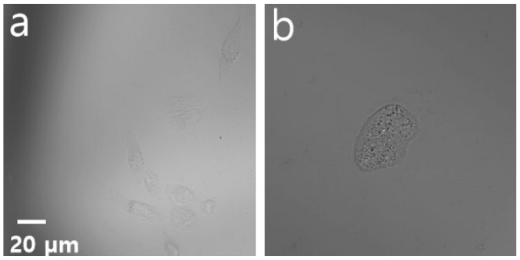


Fig. S8 (a) Bright field of HepG2 cells and (b) of HT-29 cells in **Fig. S6.**

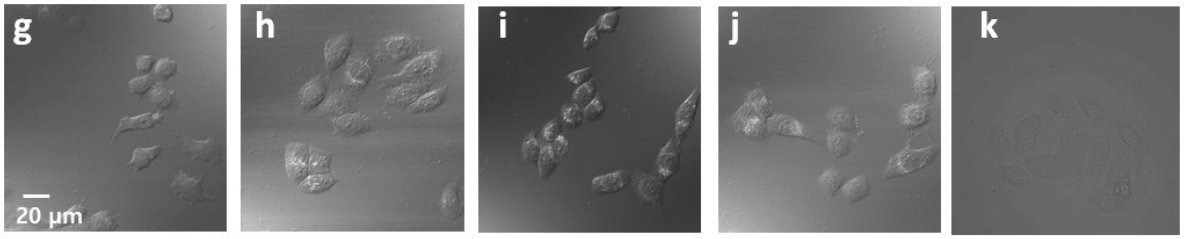


Fig. S9 Bright field of HepG2 cells in **Fig. S6g - 6k**.

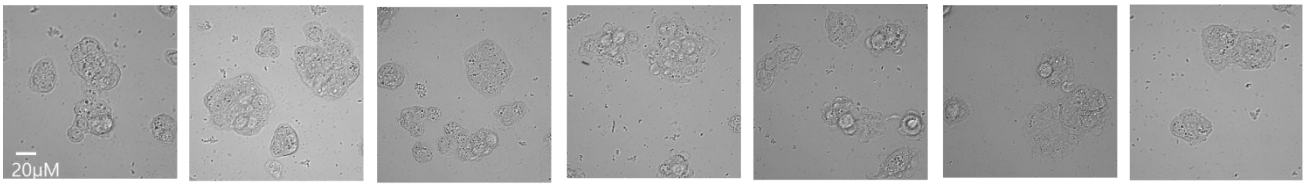


Fig. S10 Bright field of HT-29 cells treated with Irinotecan in **Fig. 2a**.

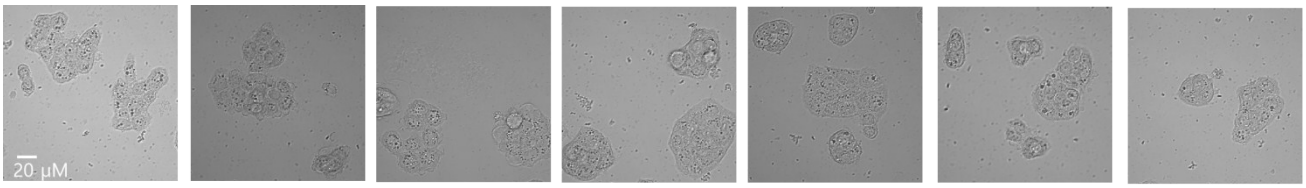


Fig. S11 Bright field of HT-29 cells treated with Capecitabine in **Fig. 2b**.

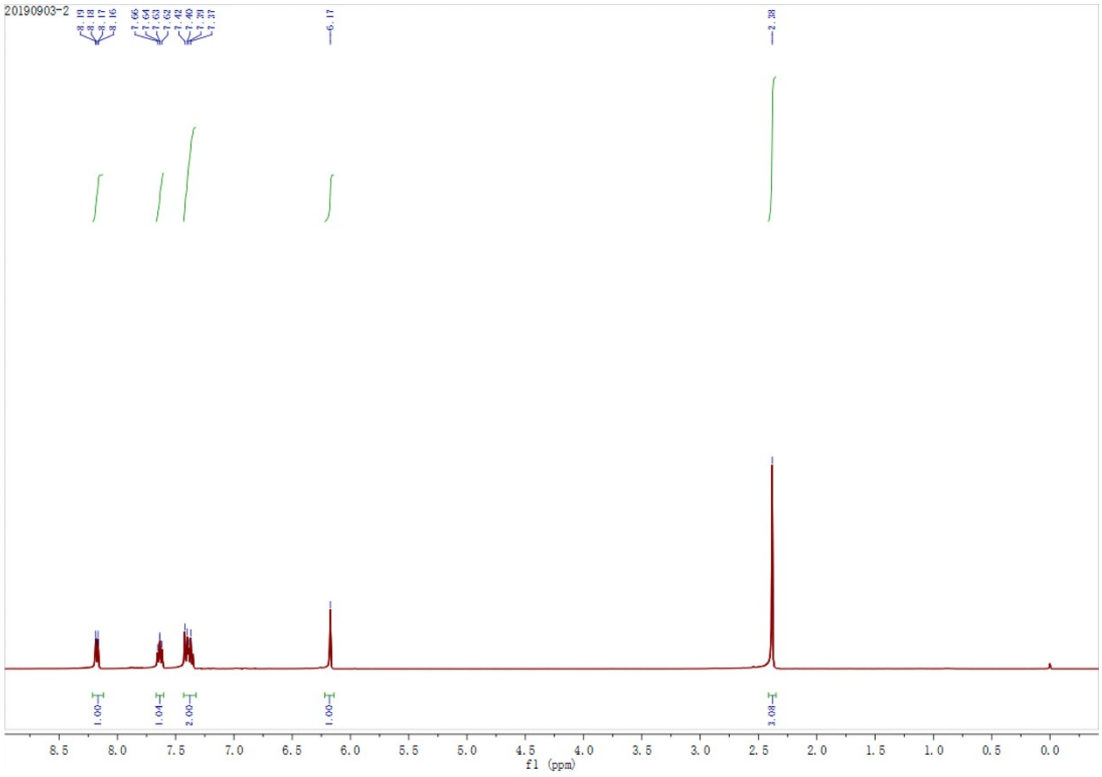
11. References.

1. J. Wang, B. Li, W. Zhao, X. Zhang, X. Luo, M. E. Corkins, S. L. Cole, C. Wang, Y. Xiao, X. Bi, Y. Pang, C. A. McElroy, A. J. Bird and Y. Dong, *ACS Sens.*, 2016, **1**, 882-887.

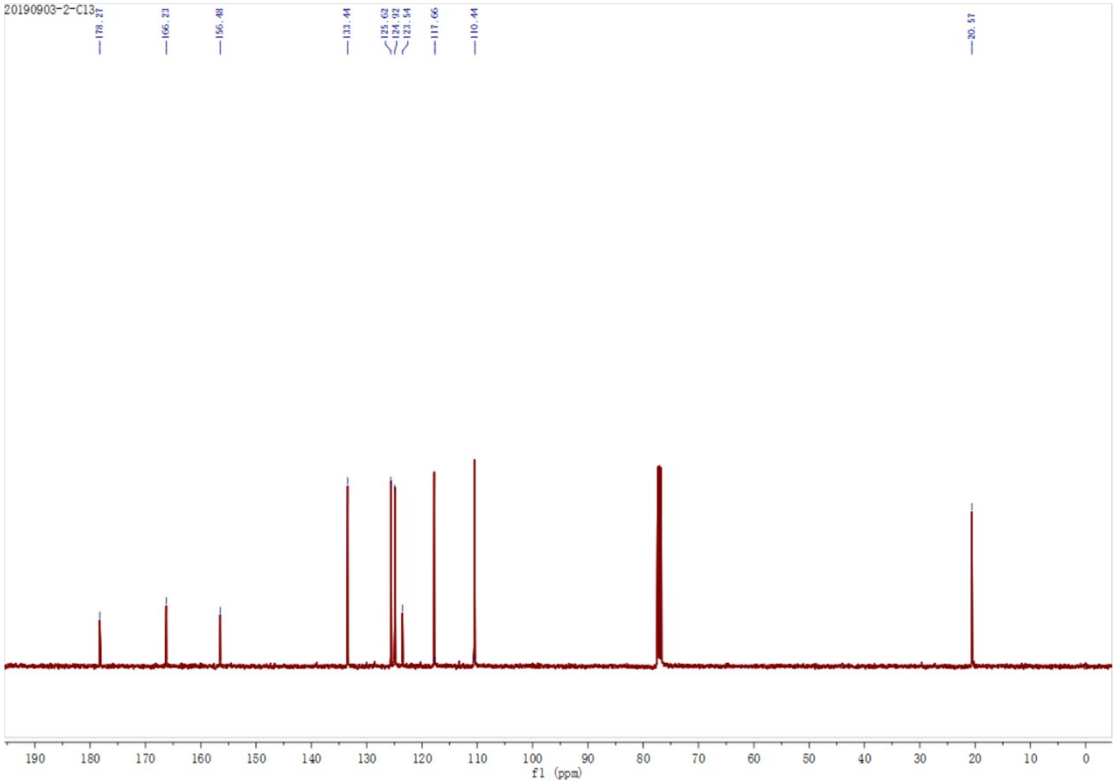
2. K. Gu, Y. Xu, H. Li, Z. Guo, S. Zhu, S. Zhu, P. Shi, T. D. James, H. Tian and W.-H. Zhu, J. *Am. Chem. Soc*., 2016, **138**, 5334-5340.

12. ^{1}H NMR, ^{13}C NMR and HR-MS of DCM-CES2

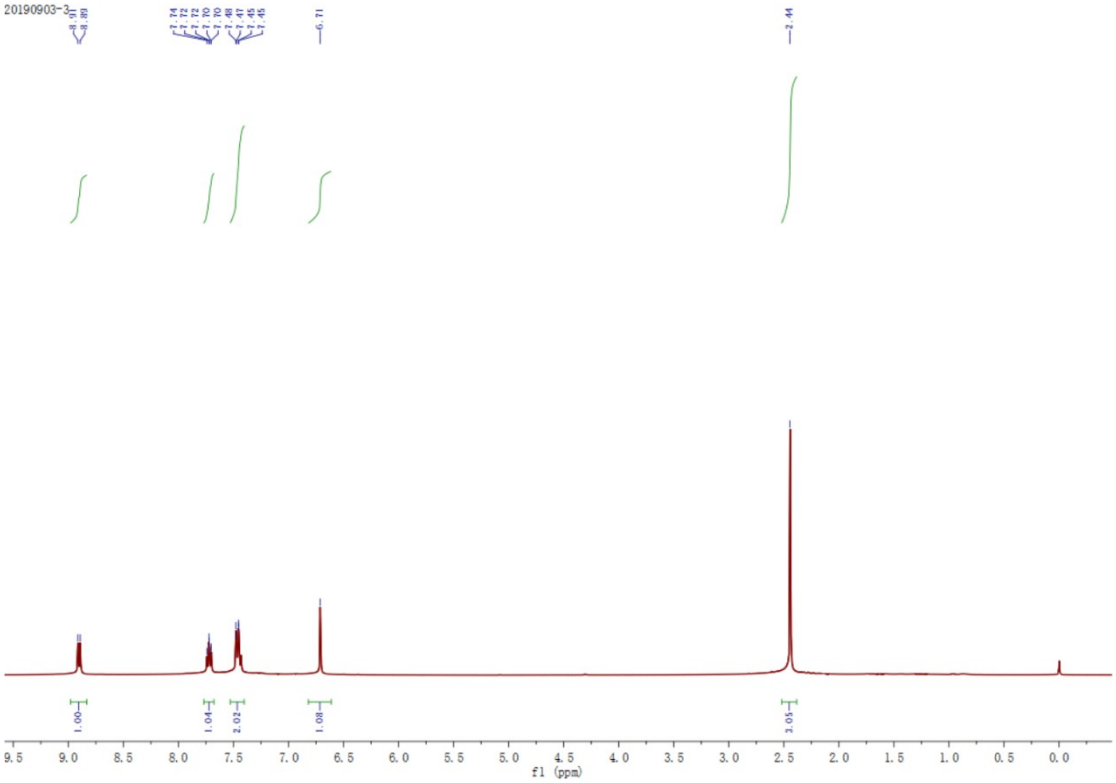
^{1}H-NMR of 1-(2-hydroxyphenyl) butane-1,3-dione



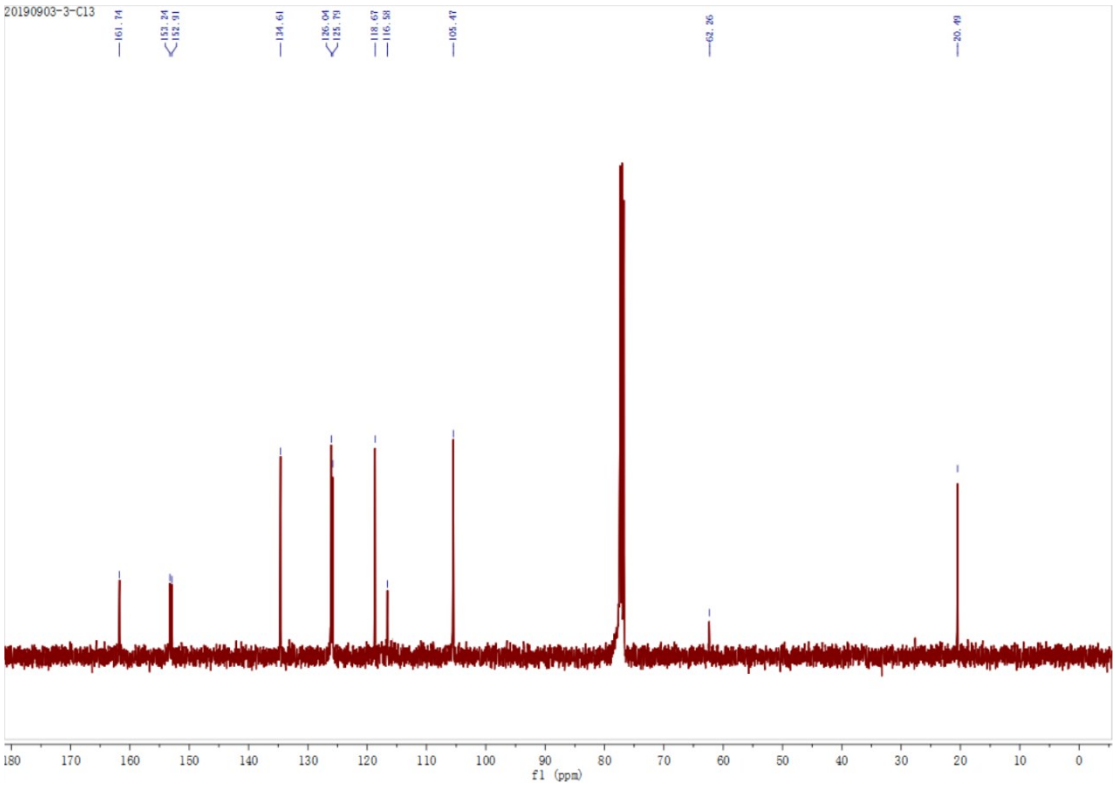
^{13}C-NMR of 1-(2-hydroxyphenyl) butane-1,3-dione



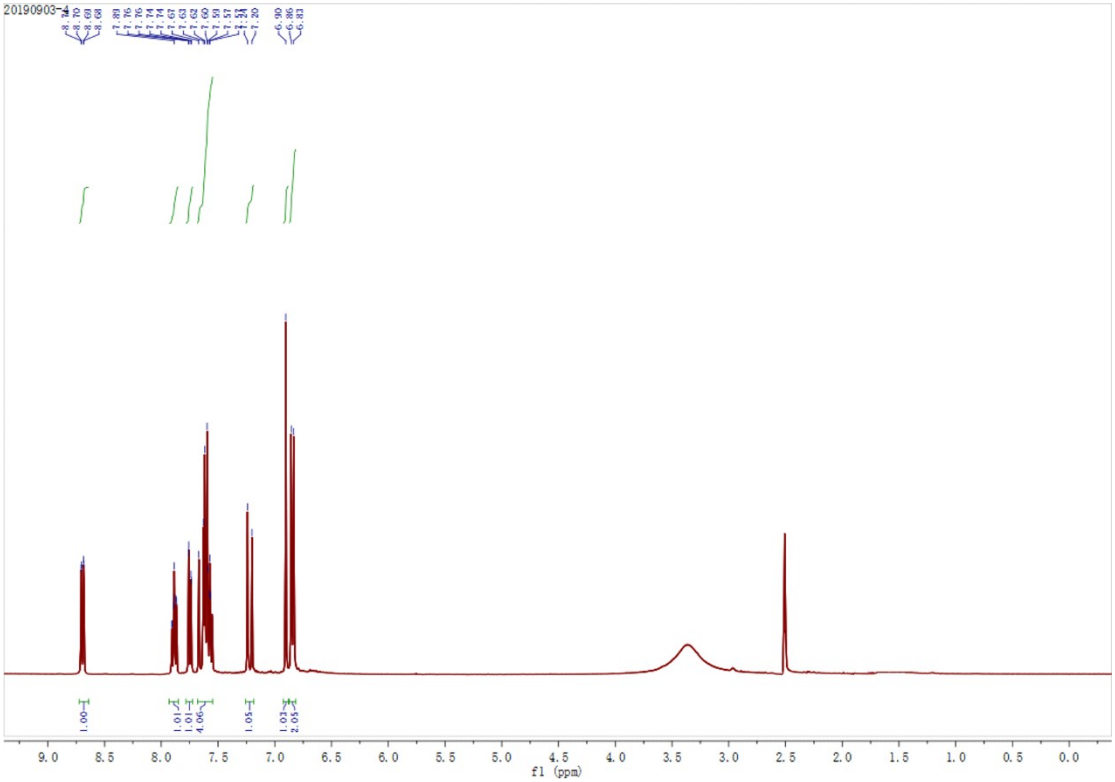
^{1}H-NMR of 2-methyl-4H-chromen-4-one



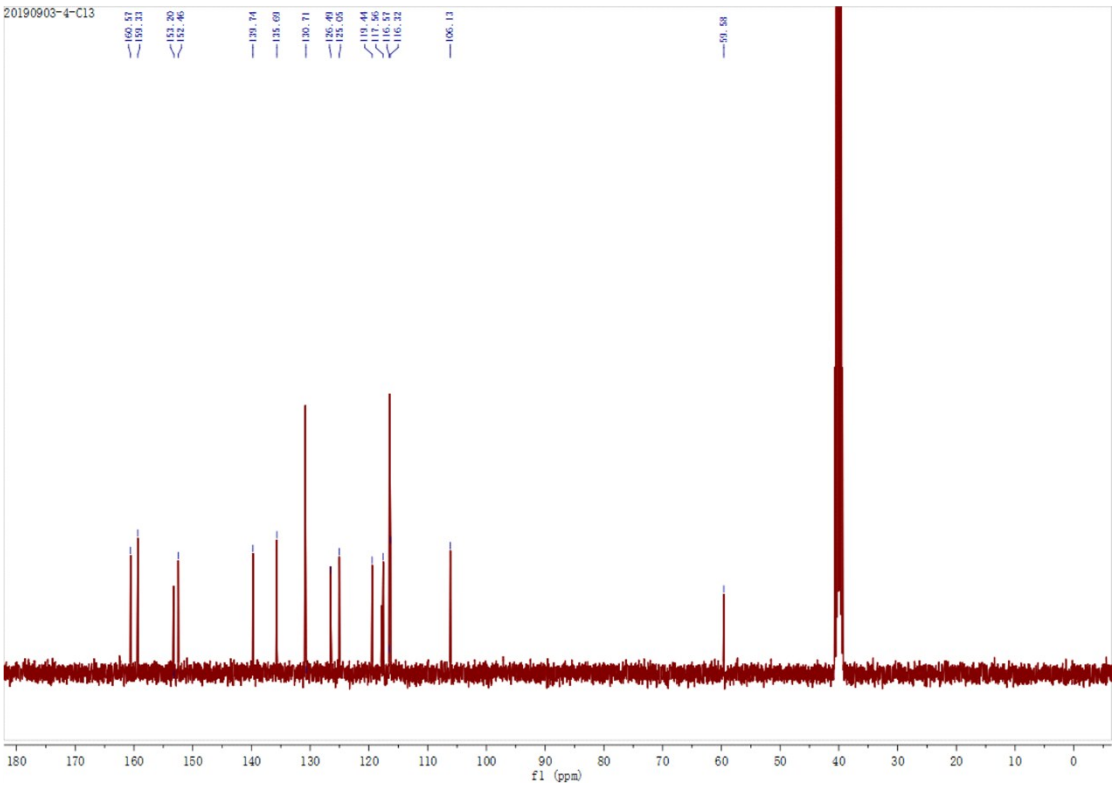
^{13}C-NMR of 2-methyl-4H-chromen-4-one



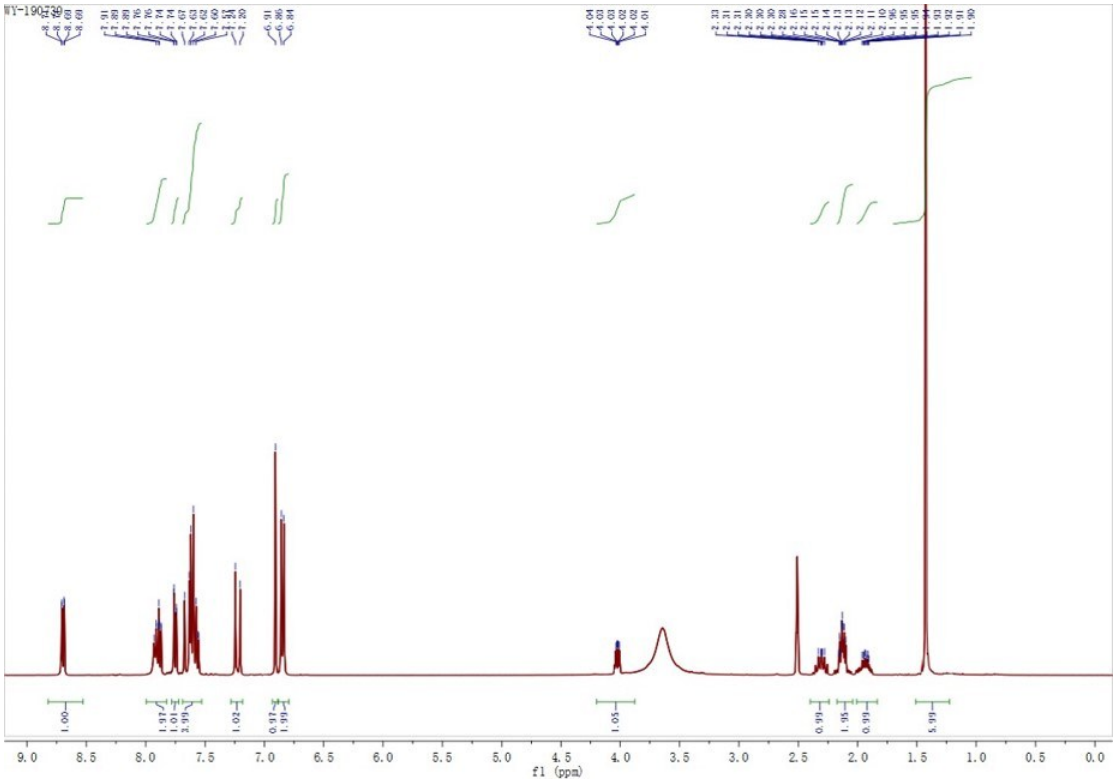
^{1}H-NMR of DCM



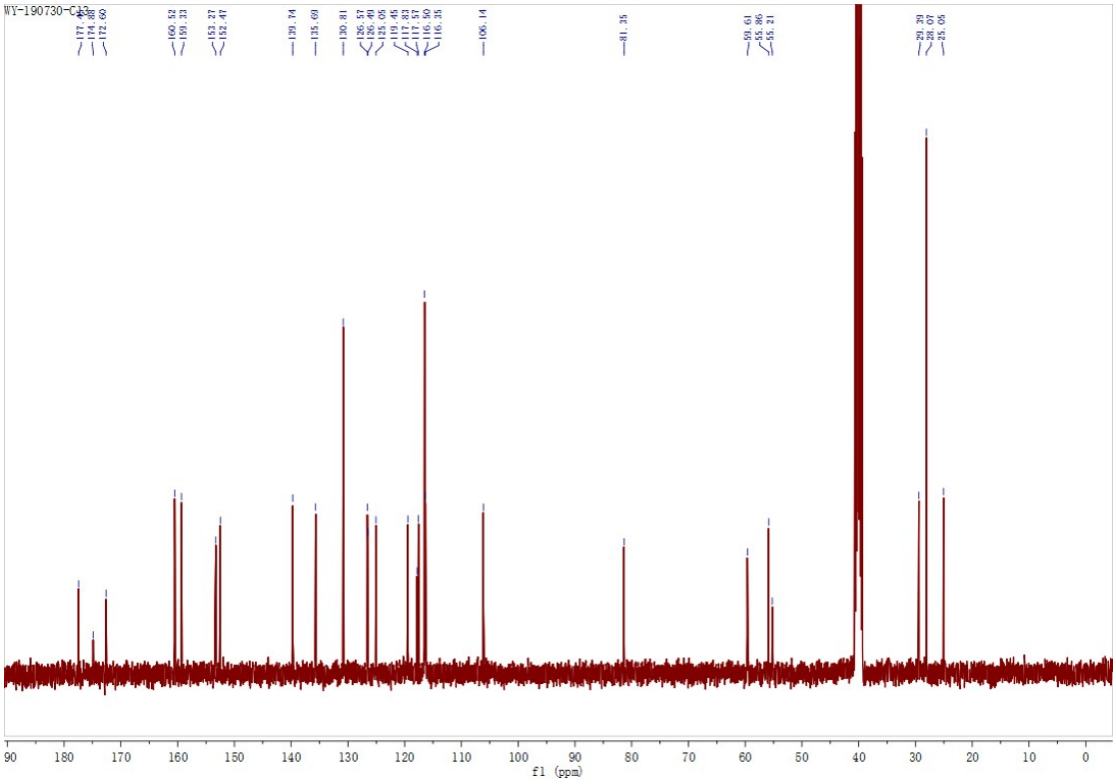
^{13}C-NMR of DCM



^{1}H-NMR of DCM-CES2



^{13}C-NMR of DCM-CES2



HR-MS of DCM-CES2