Supporting Information

Design of a near-infrared fluoro-photoacoustic probe for rapid imaging of carboxylesterase in liver injury

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1.Materials and instruments

All chemicals were purchased from commercial suppliers and used without further purification. All enzymes and analytes mentioned were purchased from Sigma-Aldrich. Water was purified and doubly distilled by a Milli-Q system (Millipore, USA). Mass spectra were performed using a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (UltrafleXtreme). High resolution mass spectrometry was performed using Bruker Daltonics micr-OTOF-Q Ⅱ spectrometer. NMR spectra were recorded on a Bruker DRX-400 spectrometer using TMS as an internal standard. The absorbance was recorded by UV-vis absorption spectrometry (UV-3600, Shimadzu) or microplate reader (SpectraMax iD3). Photoluminescence spectra were recorded on an Edinburgh Instruments F-S5 fluorescence spectrometer with a 1 cm standard quartz cell. Fluorescence images of cells were obtained from Olympus FV1000-MPE laser scanning confocal microscope (Japan). In vivo imaging was carried out on an IVIS Lumina XR (IS1241N6071) imaging system. Photoacoustic images of mice were obtained from MSOT inVision 256-TF (iThera Medical GmbH, Munich, Germany). The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter. TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300) columns, obtained from the Yantai Jiangyou silica gel Development Company Limited. H&E staining images was acquired from Wuhan Servicebio Technology CO., LTD.

For all cell imaging, Olympus FV1000-MPE laser scanning confocal microscope (Japan) was used：Emission wavelength: 700-790 nm；Excitation wavelength: 640 nm； Software: NIS-Elements Viewer.

For animal fluorescence imaging, IVIS spectral imaging system was used: Imaging parameters: Fluorescence; Emission Filter: Cy5.5 channel (690-770 nm); Excitation Filter:

675 nm; Exposure time: 1s in Figure 4A, 4B and S9; Software: Living Image 4.0 software. For photoacoustic imaging, a commercial small animal photoacoustic imaging system was used: Model: MSOT inVision 256-TF (iThera Medical GmbH, Munich, Germany); Optical parametric oscillator (OPO): Nd:YAG laser; Excitation pulses: 11 ns; Wavelengths range: 680 nm to 750 nm; Repetition rate: 10 Hz; Wavelength tuning speed:10 ms; Peak pulse energy: 100 mJ; Center frequency of ultrasound transducers: 5 MHz (60% bandwidth). All mice were imaged using isoflurane as an anesthetic, and were killed by cervical dislocation before dissection.

2. Experiment Detail

2.1 Spectral measurements.

The stock solution of QHD-CE (10 mM) was prepared in DMSO (Dimethyl sulfoxide). The various testing analytes stock solution was prepared in pure water and stored in -80℃ refrigerator (Stock solution concentration: L-lysine, L-methionine, L-serine, L-glutamic acid, Cu^{2+}, Ca^{2+}, L-alanine, glucose, sarcosine, glycine, nitroreductase, L-histidine, hydrogen peroxide, 5` -adenosine triphosphate, Fe^{3+}, L-leucine, L-tyrosine, L-arginine, cys, L-ascorbic acid sodium, proline were 100 mM; HClO 10 mM; bovine serum albumin 5 %, GGT 1000U/ L, MAO-B 1mg/mL, LAP 3000 U/L, ARS 300 U/mL, BSA 10%, GSH 500 μM, acetylcholinesterase, butyrylcholinesterase 10 U/mL, CE 200 U/mL). In selectivity experiment, 1 is blank, the order of the remaining analytes is the same as that of the stock solution mentioned. Concentrations of acetylcholinesterase, butyrylcholinesterase and CE is 1, 1, 10 U/mL, concentrations of rest analytes is diluted 100 times from the stock solution. For all the spectra measurements, the excitation wavelength was 719 nm, the excitation slit widths and emission slit widths were 10 nm.

2.2 Cytotoxicity assays.

The cytotoxicity of QHD-CE to HepG2 cells was performed by MTT. HepG2 cells were seeded in 96-well plates for 24 h. Then cells were incubated with various concentrations of QHD-CE (0-8 μM) for 24 h. After that, solutions in each well were replaced with 100 μL MTT (0.5 mg/mL). After incubated for 4 h, the supernatants were aspirated and 100 μL DMSO was added. The absorbance of the solution at 560 nm was

recorded using microplate reader. The cell viability (%) = (OD_{sample} - OD_{blank})/($OD_{control}$ - OD_{blank}) × 100%.

OD_{sample} denotes the cells cultured with different concentrations of QHD-CE, $OD_{control}$ denotes the cells incubated with culture medium, OD_{blank} denotes only the culture medium.

2.3 Cell Culture

HepG2 and HEK293 cells were cultured in high glucose Dulbecco's Modified Eagle Medium (DMEM, Hyclone) supplemented with 10% fetal bovine serum (FBS, BI), and 1% antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin, Hyclone) at 37℃ and 5% CO_2. L02 cells were cultured in Medium RPMI 1640 supplemented with 10% fetal bovine serum (FBS, BI), and 1% antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin, Hyclone) at 37℃ and 5% CO_2. Cells were carefully harvested and split when they reached 80% confluence to maintain exponential growth.

2.4 Animal Experiment

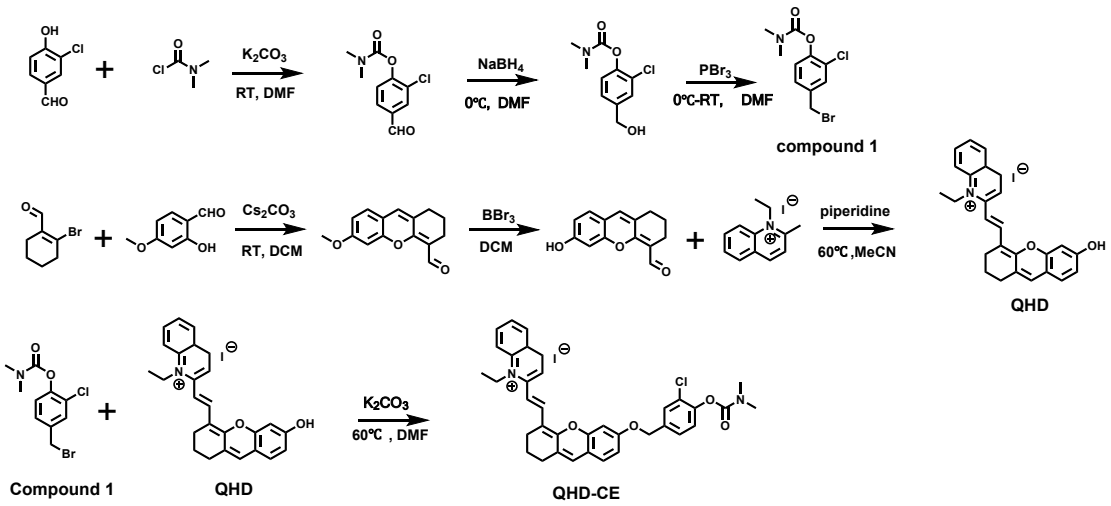
All animal experiments were in compliance with relevant laws and approved by the Institutional Animal Care and Use Committee of Hunan University (SYXK 2018-0006). Nude female mice (4-5 weeks) were obtained from m Hunan Anshengmei Drug Research Institute Co. Ltd (Changsha, China). Mice were intraperitoneal injected with 400 mg/kg acetaminophen (APAP) solution for 12 h as liver injury group and normal mice were intraperitoneally injected with saline solution as normal group. Prior to imaging, mice were fasted for 12 h and then were injected with 120 μL QHD-CE (200 μM in PBS, 20% DMSO) via the tail vein followed by being imaged.

Kunming male mice (4-5 weeks) were obtained from m Hunan Anshengmei Drug Research Institute Co. Ltd (Changsha, China). Experiment mice were intraperitoneal injected with streptozotocin (STZ) dissolved freshly in 0.01 M citrated buffer (pH 4.5) at a dose of 180 mg/kg body weight (BW). Normal mice were intraperitoneally injected with saline solution as normal group. After 4 days, the blood glucose levels of mice were higher than 16.7, indicating the diabetic mice model was successfully established. Prior

to imaging, mice were fasted for 12 h and then were injected with 120 μL QHD-CE (200 μM in PBS, 20% DMSO) via the tail vein followed by being imaged.

2.5 Synthesis of Probe QHD-CE

The starting materials compound 1 and QHD were prepared according to reported procedures.[1,2] As shown in Scheme S1, QHD (30.0 mg, 0.059 mmol) and potassium carbonate (16.1 mg, 0.118 mmol) were dissolved in 5 mL DMF (N,N-Dimethylformamide) and then compound 1 (26.0 mg, 0.088 mmol) were added the above stirred solution. The mixture solution was stirred for 3 h under N_2 atmosphere at 60℃. After TLC (thin-layer chromatography) showed that the starting materials were consumed completely and a new main spot was formed, the solvent was removed with reduced pressure and the residue was purified with chromatography (TLC, MeOH/Dichloromethane = 1:40) to give QHD-CE purple product (30.0 mg, 70% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.72 (d, J = 9.1 Hz, 1H), 8.60 (d, J = 12.2, 2.9 Hz, 2H), 8.38 (d, J = 9.0 Hz, 1H), 8.25 (d, J = 8.0 Hz, 1H), 8.05 (t, J = 8.0 Hz, 1H), 7.80 (t, J = 7.5 Hz, 1H), 7.69 (d, J = 2.0 Hz, 1H), 7.48 (d, J = 8.2, 2.0 Hz, 1H), 7.36 (s, 1H), 7.34 (s, 1H), 7.32 (d, J = 2.3 Hz, 1H), 7.07 (s, 1H), 6.92 (dd, J = 8.6, 2.4 Hz, 1H), 6.75 (m, 1H), 5.23 (s, 2H), 4.94 (d, J = 7.5 Hz, 2H), 3.10 (s, 3H), 2.94 (s, 3H), 2.65 (m, 5.9 Hz, 4H), 1.54 (d, J = 7.0 Hz, 3H), 1.26 (d, J = 3.9 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.6, 155.7, 154.5, 153.5, 152.9, 146.9, 141.9, 138.2, 135.9, 133.9, 130.1, 129.1, 128.0, 127.8, 127.5, 126.9, 126.26, 124.6, 120.5, 118.2, 115.3, 112.2, 111.9, 111.3, 101.9, 69.8, 68.5, 62.1, 56.0, 48.6, 45.0, 37.1, 36.2, 29.0, 28.7, 13.4. HRMS m/z: $[C_{36}H_{34}ClN_2O_4]^+$ calcd for 593.2202, found 593.2200.



Scheme S1. Synthesis route of QHD-CE

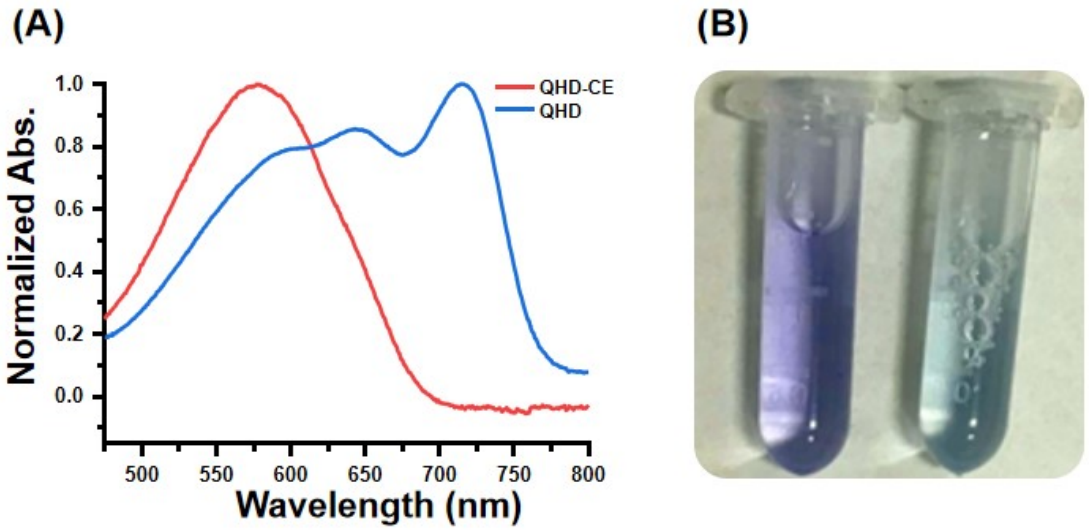


Figure S1. (A) Normalized absorption spectra of 3 μM QHD-CE (red curve) and 3 μM QHD (blue curve) in PBS/DMSO solution (10 mM, pH 7.4, v/v, 7/3). (B) 10 μM QHD-CE (left) and 10 μM QHD-CE reacted with CE (right) in PBS/DMSO solution (10 mM, pH 7.4, v/v, 7/3).

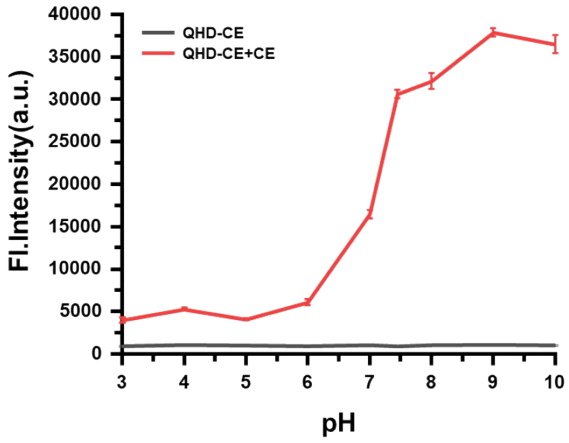


Figure S2. Effect of pH on the fluorescence of QHD-CE (3 μM) before and after reaction with CE (10 U/mL). Error bar represents the standard deviation (± SD) (n = 3).

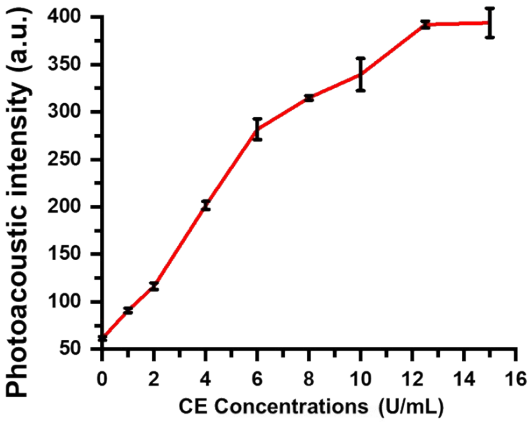


Figure S3. The PA_{720} quantitative analysis of Figure 2A.

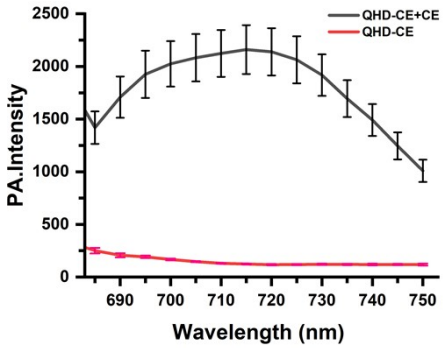


Figure S4. PA spectra of QHD-CE and QHD-CE after reaction with CE. Black line: 10 μM QHD-CE incubated with 10 U/mL CE; Red line: 10 μM QHD-CE incubated in 37 ℃ for 2h. Experiments were performed in PBS/DMSO solution (10 mM, pH 7.4, v/v, 7/3). Error bar represents the standard deviation (±SD) (n = 3)

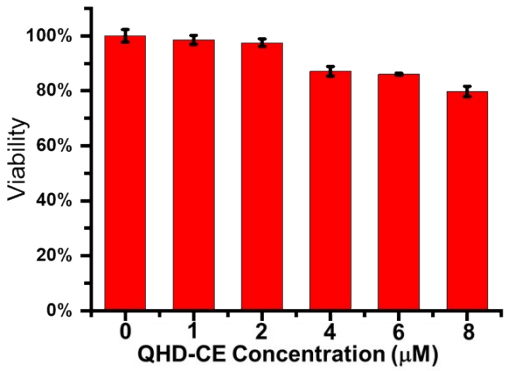


Figure S5. MTT assay for estimating cell viability (%) of HepG2 cells treated with various concentrations of QHD-CE (0-8 μM) after 24 h incubation. Error bar represents the standard deviation (± SD) (n = 3).

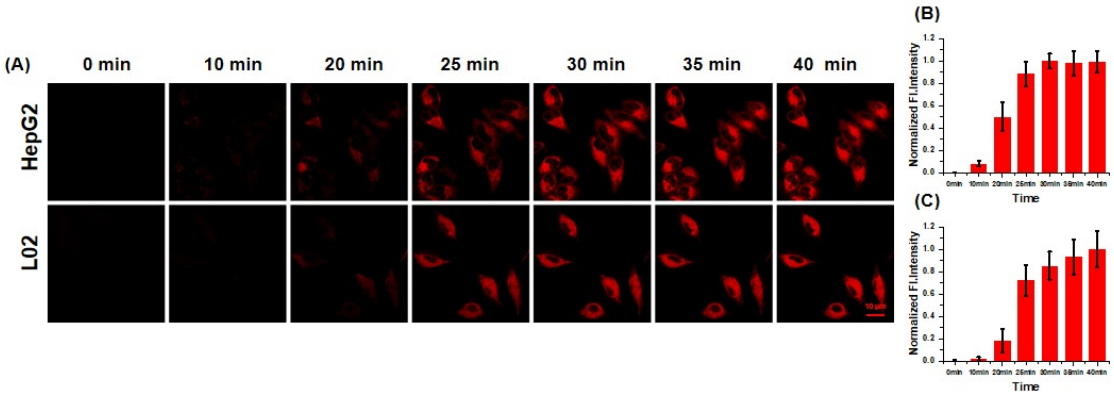


Figure S6. (A) Fluorescence images of HepG2 cells and L02 cells treated with QHD-CE (5 μM) for different time. (B) Corresponding mean intensity of the cells imaged. λ_{ex} = 640 nm, λ_{em} = 700-790 nm. Scar bar: 10 μm. Error bar represents the standard deviation (± SD) (n = 3).

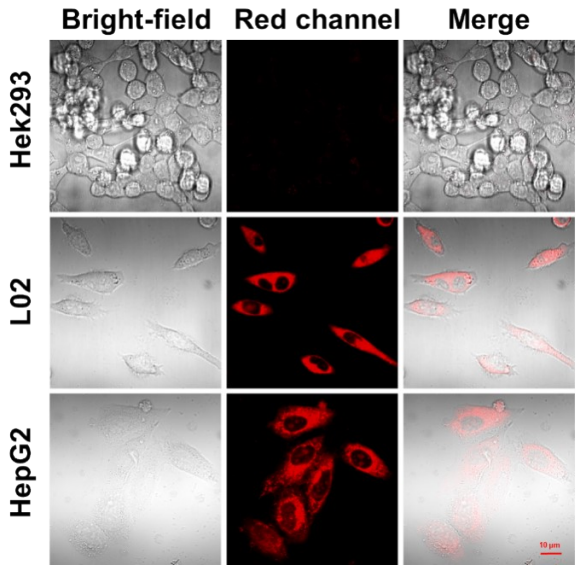


Figure S7. Fluorescence images of Hek293, L02 and HepG2 cells incubated with QHD-CE (3 μM) for 40 min.

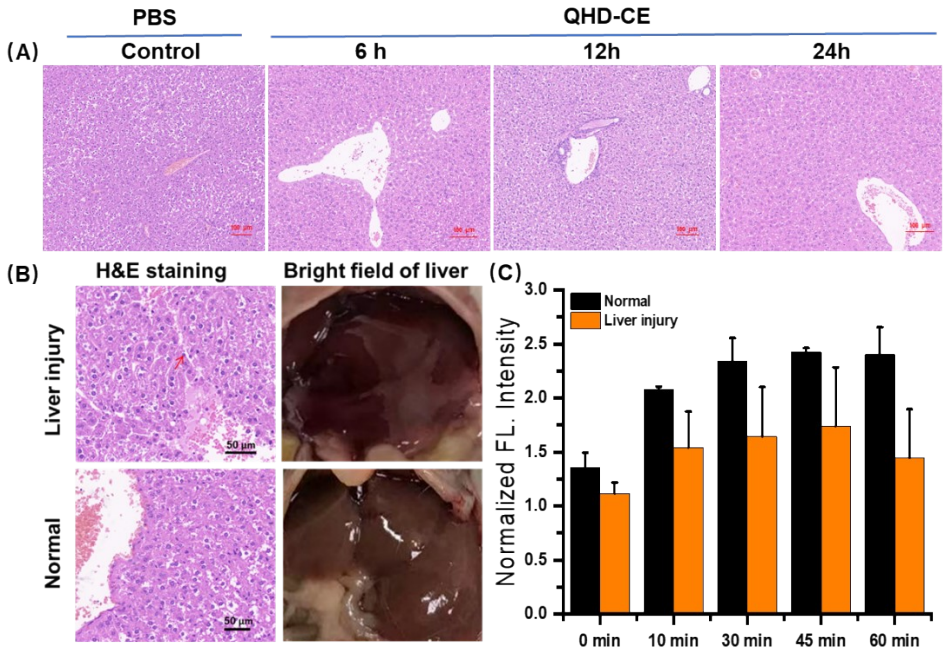


Figure S8. (A) H&E staining of liver tissues after mice were injected with PBS，or QHD-CE at 6 h, 12 h and 24 h, respectively. (B) H&E staining and bright field of livers of normal mice and liver injury mice. Bleeding was observed around the central vein and necrosis of liver cells was observed around the vein in H&E staining of liver injury group; Obvious hepatic congestion was observed in the liver of mice with liver injury. (C) Fluorescence quantitative analysis of Figure 4A. Error bar represents the standard deviation (+SD) (n = 3).

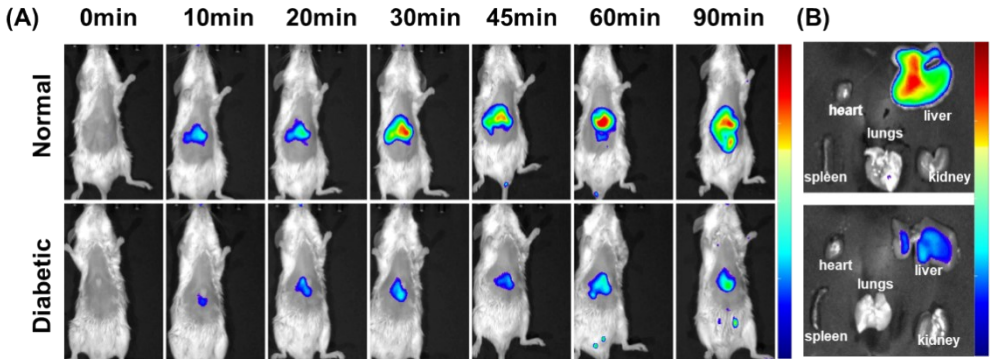


Figure S9. (A) Normal: normal KM mice were injected with QHD-CE via the tail vein followed by being imaged. Diabetic: diabetic KM mice were injected with QHD-CE via the tail vein followed by being imaged. (B) $NIRF_{740}$ images of liver, heart, spleen, lungs, kidney of corresponding mice in 90 min after injection of QHD-CE.

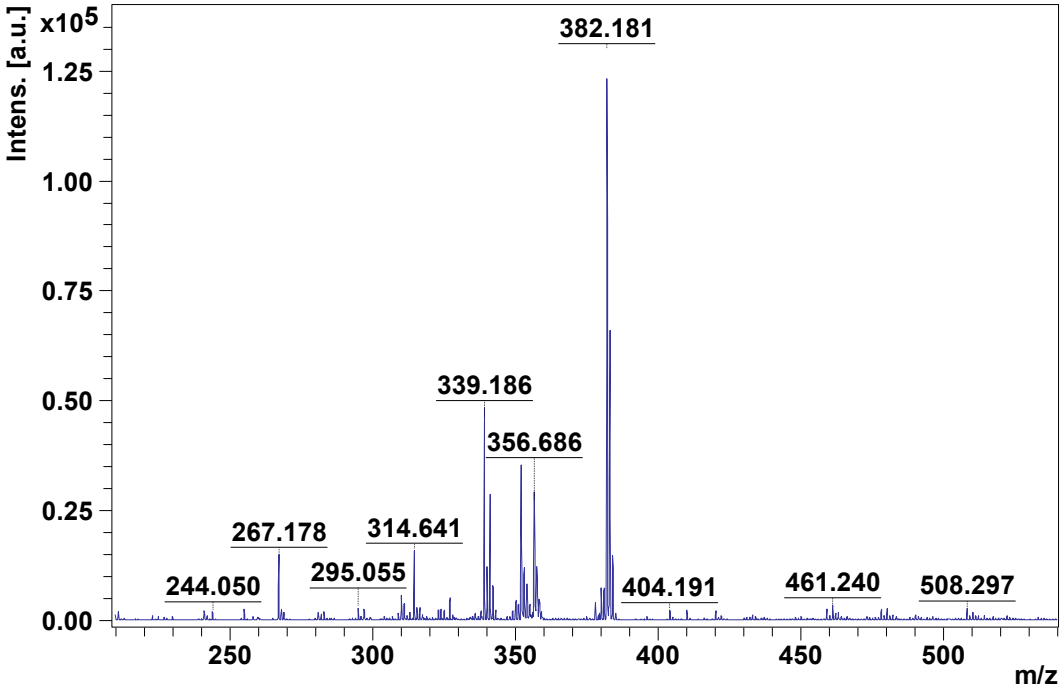


Figure S10. Maldi-Tof/Tof-MS spectrum of QHD.

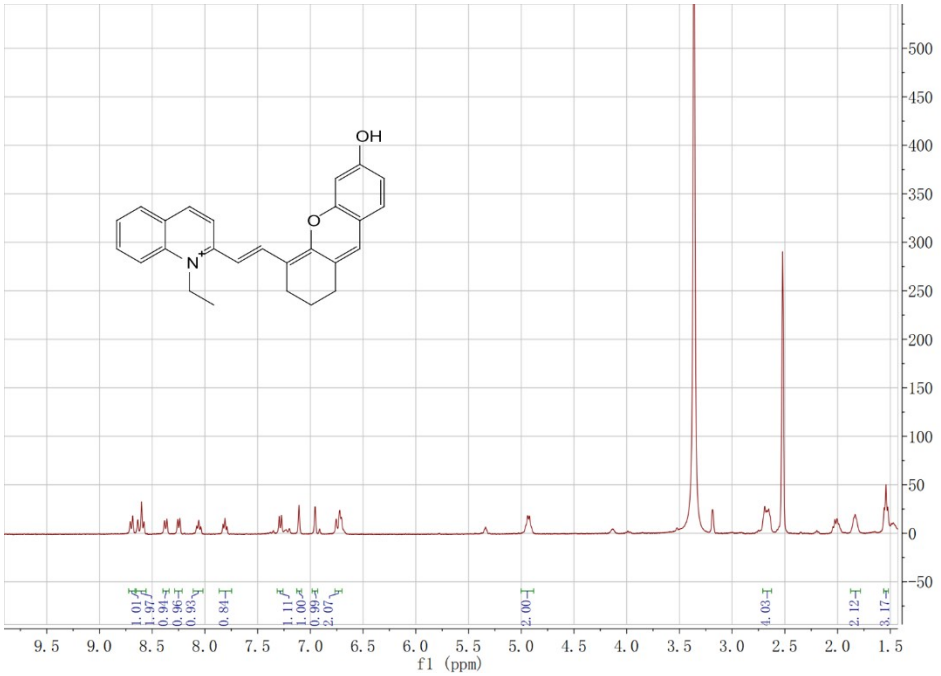


Figure S11. ^{1}H NMR spectrum of Compound QHD in DMSO-d_6.

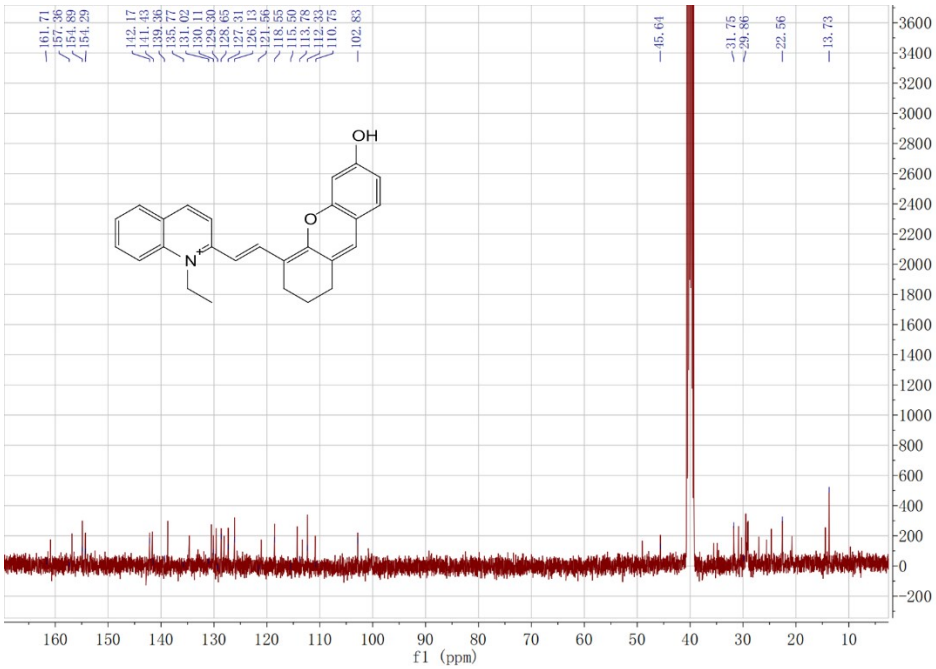


Figure S12. ^{13}C NMR spectrum of Compound QHD in DMSO-d_6.

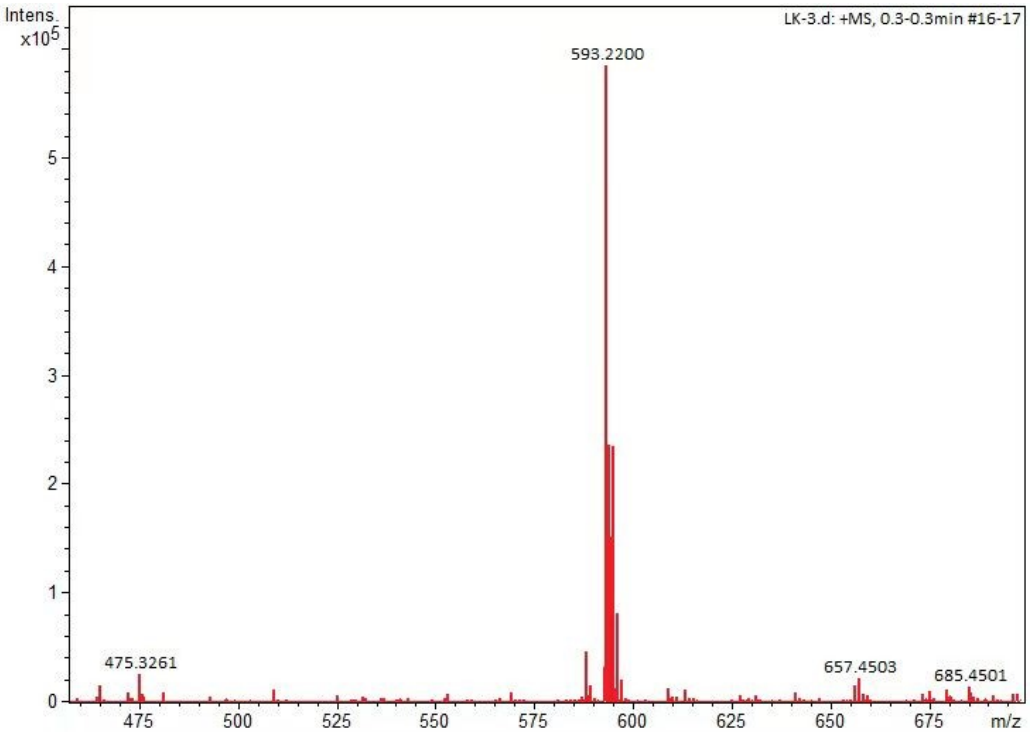


Figure S13. HR-MS spectrum of QHD-CE.

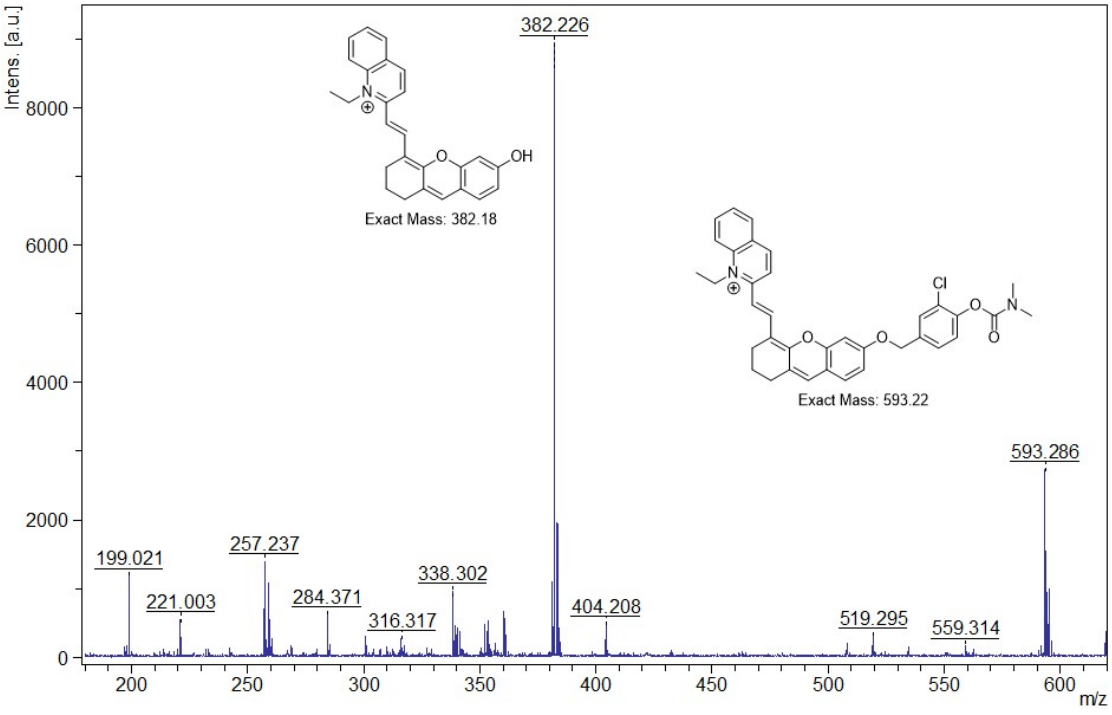


Figure S14. Maldi-Tof/Tof-MS spectrum of QHD-CE reacted with CE.

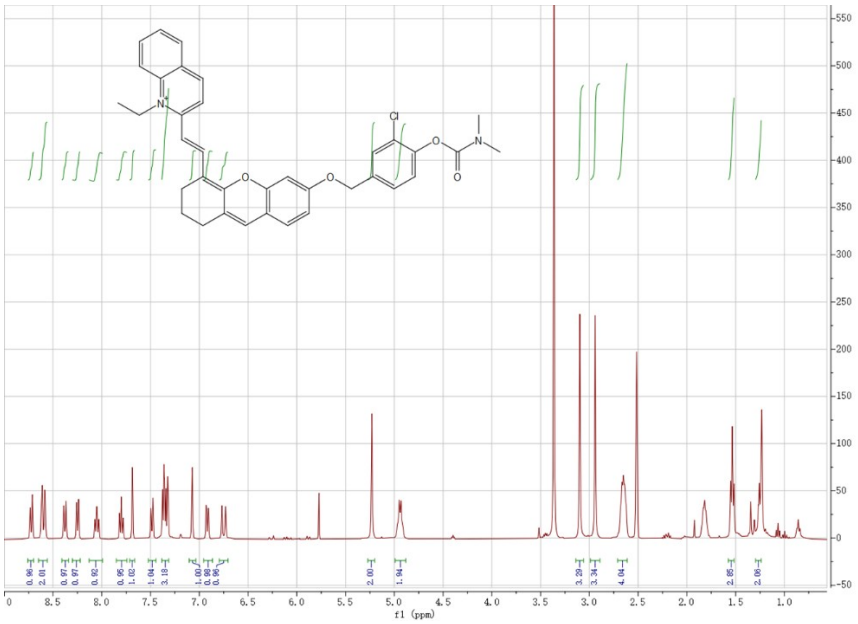


Figure S15. ^{1}H NMR spectrum of Compound QHD-CE in DMSO-d_6.

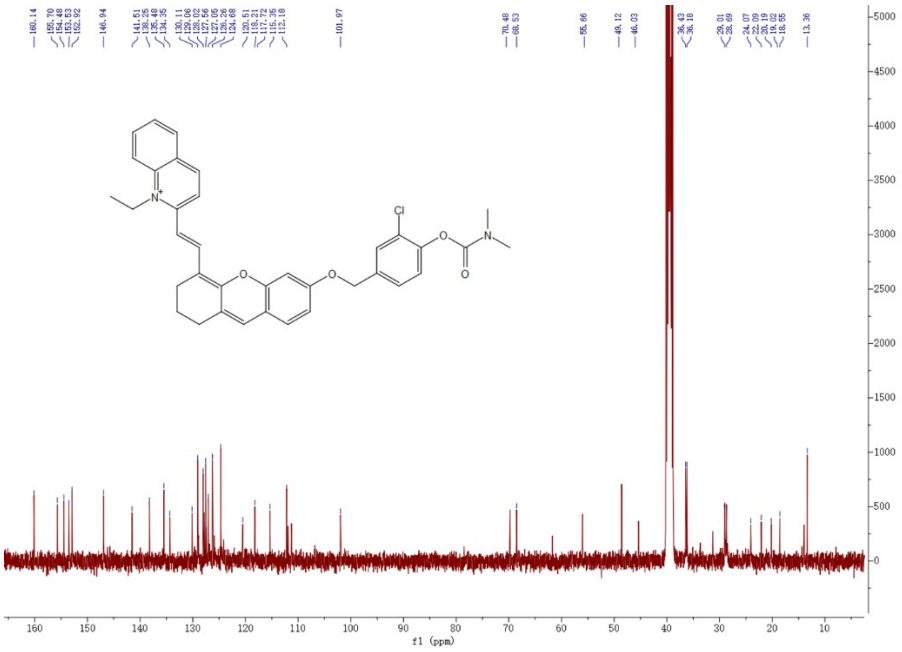


Figure S16. ^{13}C NMR spectrum of Compound QHD-CE in DMSO-d_6.

References

1. W. Li, R. Li, R. Chen, S. Ai, H. Zhu, L. Huang and W. Lin, *Anal Chem*, 2022, **94**, 7996-8004.
2. X. Wu, R. Wang, S. Qi, N. Kwon, J. Han, H. Kim, H. Li, F. Yu and J. Yoon, *Angew Chem Int Ed Engl*, 2021, **60**, 15418-15425.