Supporting information

Sensing carboxylesterase 1 in living systems by a practical and isoform-specific fluorescent probe

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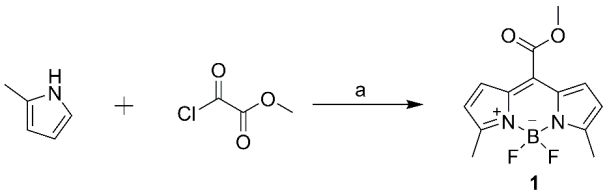
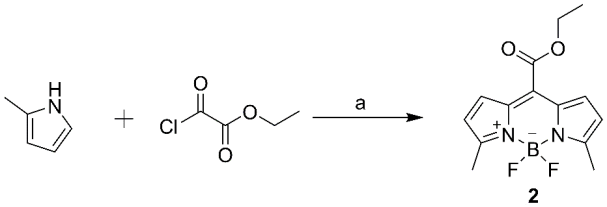
Experiment section



Scheme S1. Synthetic scheme for probe **1**. (a) i) CH2Cl2, -78 °C, 5 h; ii) Et3N, BF3·OEt2, -78 °C to RT, 6 h, 7%.

Synthesis of Probe 1. Probe **1** was synthesized according to known literature [1]. To a dry dichloromethane (CH_2Cl_2) solution (150 mL) of 2-methyl-1H-pyrrole (810.0 mg, 10.0 mmol) at -78 °C under an argon atmosphere, a solution of methyl chlorooxoacetate (428.8 mg, 3.5 mmol) in dry CH_2Cl_2 (50 mL) was added dropwise over 1 hour, and the mixture was stirred at this temperature for 5 hours, before the sequential addition of Et_3N (2.1 mL, 15.0 mmol) and $BF_3{\cdot}OEt_2$ (3.1 mL, 24.1 mmol). The mixture was slowly warmed to room temperature (RT), and further stirred at RT for 6 hours. After removing the solvent, the residues were further purified by a silica gel column chromatograph using petroleum ether (PE)/CH_2Cl_2 (v/v: 3/2) as the mobile phase to afford probe **1** as a red solid (68 mg, yield: 7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (d, *J* = 4.1 Hz, 2H), 6.31 (d, *J* = 4.2 Hz, 2H), 3.99 (s, 3H), 2.63 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.31, 160.73, 133.54, 130.87, 127.87, 120.70, 53.08, 15.24.



Scheme S2. Synthetic scheme for probe **2**. (a) i) CH2Cl2, -78 °C, 5 h; ii) Et3N, BF3·OEt2, -78 °C to RT, 6 h, 4%.

Synthesis of Probe 2. Probe **2** was synthesized according to the same procedure as that used for probe **1**, with 2-methyl-1H-pyrrole (810.0 mg, 10.0 mmol) and ethyl chlorooxoacetate (477.9 mg, 3.5 mmol) as the raw materials. The crude compound was purified over a silica column with PE/CH_2Cl_2 (v/v: 3/2) to afford probe **2** as a red powder (41 mg, yield: 4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, J = 4.1 Hz, 2H), 6.30 (d, *J* = 4.2 Hz, 2H), 4.45 (q, *J* = 7.1 Hz, 2H), 2.62 (s, 6H), 1.43 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.81, 160.57, 133.49, 130.75, 128.44, 120.62, 62.54, 15.22, 14.22. HRMS (ESI positive) calcd for $[M+H]^+$ 293.1267, found 293.1261.

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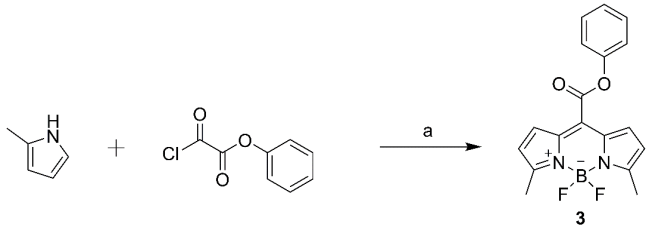
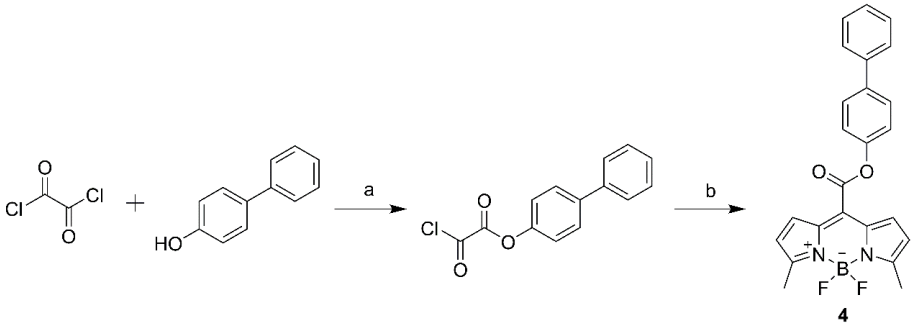


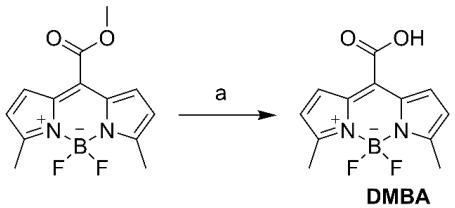
Scheme S3. Synthetic scheme for probe **3**. (a) i) CH2Cl2, -78 ˚C, 5 h; ii) Et3N, BF3·OEt2, -78 ˚C to RT, 6 h, 10%.

Synthesis of Probe 3. Similar to the synthetic procedures for probe **1**, probe **3** was synthesized using 2-methyl-1H-pyrrole (810.0 mg, 10.0 mmol) and phenyl chlorooxoacetate (646.1 mg, 3.5 mmol) as the raw materials. The crude compound was purified over a silica column with PE/CH_2Cl_2 (v/v: 2/1) to afford probe **3** as a violet powder (120 mg, yield: 10%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (t, *J* = 7.6 Hz, 2H), 7.38 (d, *J* = 2.9 Hz, 2H), 7.34 (t, *J* = 7.4 Hz, 1H), 7.25 (d, *J* = 8.5 Hz, 2H), 6.36 (d, *J* = 3.6 Hz, 2H), 2.67 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.44, 161.23, 150.13, 133.75, 130.95, 129.84, 126.98, 126.75, 121.24, 121.06, 15.33. HRMS (ESI positive) calcd for $[M+H]^+$ 341.1267, found 341.1273.



Scheme S4. Synthetic scheme for probe **4**. (a) CH2Cl2, 0 ˚C, 2 h. (b) i) CH2Cl2, -78 ˚C, 8 h; ii) Et3N, BF3·OEt2, -78 ˚C to RT, 12 h, 5%.

Synthesis of Probe 4. To a stirred dichloromethane (CH_2Cl_2) solution (50 mL) of oxalyl chloride (6.35 g, 50.0 mmol) at 0 ˚C, a solution of 4-phenylphenol (4.25 g, 25.0 mmol) in CH_2Cl_2 (20 mL) was added dropwise over 20 min, and the resulting mixture was stirred at room temperature for 2 hours. After the reaction was completed, the solvent was removed under reduced pressure to give crude biphenyl chlorooxoacetate. To a dry dichloromethane (CH_2Cl_2) solution (150 mL) of 2-methyl-1H-pyrrole (810.0 mg, 10.0 mmol) at -78 ˚C under an argon atmosphere, a solution of crude biphenyl chlorooxoacetate (1.0 g) in dry CH_2Cl_2 (50 mL) was added dropwise over 1 hour, and the mixture was stirred at this temperature for 8 hours, before the sequential addition of Et_3N (2.1 mL, 15.0 mmol) and BF_3·OEt_2 (3.1 mL, 24.1 mmol). The mixture was slowly warmed to room temperature, and further stirred at RT for 12 hours. After removing the solvent, the residues were further purified by a silica gel column chromatograph using PE/CH_2Cl_2 (3/2 v/v) as the mobile phase to afford probe **4** as a violet solid (72 mg, yield: 5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 8.6 Hz, 2H), 7.59 (d, *J* = 7.5 Hz, 2H), 7.47 (t, *J* = 7.5 Hz, 2H), 7.38 (dd, *J* = 9.5, 5.6 Hz, 3H), 7.33 (d, *J* = 8.5 Hz, 2H), 6.37 (d, *J* = 4.3 Hz, 2H), 2.67 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.50, 161.27, 149.48, 140.05, 140.00, 133.77, 130.96, 128.91, 128.52, 127.65, 127.18, 126.87, 121.51, 121.10, 15.34. HRMS (ESI positive) calcd for $[M+H]^+$ 417.1580, found 417.1569.



Scheme S5. Synthetic scheme for metabolite **DMBA**. (a) HLMs, PBS, 37 ˚C, overnight，80%.

Biosynthesis of the hydrolytic metabolite DMBA. The metabolite **DMBA** was biosynthesized using human liver microsomes, followed by isolation, purification, structural elucidation and quantitative analysis. Briefly, Probe **1** (10.0 mg, 0.036 mmol) was dissolve in DMSO solution with a concentration of 10 mM. Then, 20 μM probe **1** was incubated with 2 μg protein/mL liver microsomes, in the presence of 100 mM PBS (pH 7.4) overnight at 37 ˚C. The reaction was terminated by the addition of acetonitrile. In parallel, an SPE cartridge (C18 and anion exchange resin, 1000 mg; Dalian Sipore, Dalian, China) was activated by sequential washing using 6 mL of methanol and 6 mL of Millipore water. After removal of the protein from the biosynthesis reaction by centrifugation at 20,000 × g for 20 min at 4°C, the combined supernatants were subjected to the SPE cartridge, which was then subsequently eluted with 12 mL of Millipore water, 12 mL of methanol and 12 mL of 3% formic acid methanol. The

entire process was monitored by HPLC, and the metabolite-containing fractions were collected and evaporated to dryness in vacuo, yielding 7.6 mg of metabolite **DMBA**. ^{1}H NMR (400 MHz, MeOD) δ 7.30 (d, *J* = 4.1 Hz, 2H), 6.39 (d, *J* = 4.2 Hz, 2H), 2.57 (s, 6H). ^{13}C NMR (100 MHz, MeOD) δ 165.49, 159.84, 132.93, 130.84, 130.36, 119.96, 119.93, 13.65. HRMS (ESI negative) calcd for $[M-H]^-$ 263.0809, found 263.0813.

LC-UV-FD analysis. Probe **1** and its metabolite **DMBA** in human CES1 or PBS were identified by LC-UV-FD. As mentioned above, probe **1** (10 μM) was incubated in presence or absence of CES1 at 37 °C for 60 min with a total volume of 0.2 mL, then, equal volume of cold acetonitrile was added to terminate the reaction, and centrifuged at 20,000 g for 20 minutes. The supernatants were analyzed by a Shimadzu UFLC system. A Shim-Pack VP-ODS (75 mm × 2.0 mm, 2 μm) analytical column kept at a temperature of 40 °C was applied for analysis. The mobile phase consisted of CH_3CN (A) and water containing 0.2% formic acid (B) with the following gradient: 0.01–3.00 min, 68-10% B; 3.00–3.31 min, 10% B; 3.31–4.50 min, 10–68% B; 4.51–6.50 min, 68% B; and the flow rate at 0.4 mL/min. probe **1** and corresponding metabolite **DMBA** can be quantified by this LC-UV method monitored at 505 nm and LC-FD method monitored at 560 nm with 505nm as excitation wavelength.

Reaction phenotyping assays. To determinate the specificity of probe **1** toward CES1, the hydrolytic abilities of a number of hydrolases distributed in the human body towards probe **1** were tested under physiological conditions (pH 7.4, 37 °C) for 60 min. The tested enzymes and the final concentrations as follows, carboxylesterases (CES1 and CES2, 5 μg/mL), α-chymotrypsin (α-CT, 5 μg/mL), trypsin (5 μg/mL), carbonic anhydrase I (CA, 5 μg/mL), human serum albumin (HSA, 50 μg/mL), acetyl-cholinesterase (AChE, 5 μg/mL), butyryl-cholinesterase (BChE, 20 U/L), paraoxonase (PON1 and PON2, 5 μg/mL), dipeptidyl peptidase 4 (DPP4, 5 μg/mL) and proteinase K (PK, 5 μg/mL). The fluorescence intensities were recorded in PBS-acetonitrile (v: v = 1: 1, pH 7.4, $\lambda_{ex/em}$ =505/560 nm).

Chemical inhibition assays. To ensure the change of fluorescence intensities was CES1 dependent, the inhibitory effects of several selective enzyme inhibitors toward esterase may involve in probe **1** hydrolysis were investigated with recombinant human CES1 and pool human liver microsomes as enzyme source. Several potent inhibitors of esterases including BNPP (inhibitors of CES, 100 μM), LPA (inhibitor of CES2, 100 μM), UKA (inhibitor of CES1, 100 μM), GA (inhibitor of cholinesterase, 100 μM) and EDTA (inhibitor of PONs, 100 μM) were pre-incubated with the two enzyme sources at 37 °C for 10 min, respectively. Then, probe **1** were added to start the reaction and incubated at 37 °C for 60 min, the fluorescence intensities were measured in PBS-acetonitrile (v: v = 1: 1, pH = 7.4, $\lambda_{ex/em}$ =505/560 nm).

General procedures for sensing human CES1 activities. All the measurements for sensing human CES1 activities were carried out in following incubation system. In brief, the total volume of incubation system is 200 μL which contain PBS (100 mM, pH 7.4) and human tissue microsomes (2 μg/mL, final concentration). After mixed gently and pre-incubate at 37 °C for 3 min, probe **1** was added to active the reactions (the final concentration of DMSO was 2%). After incubation at 37 °C for 30 min, equal volume of ice-cold acetonitrile was added to terminate this reactions. Then, the fluorescent intensities of supernatant were analyzed by microplate reader. To ensure the formation of the hydrolytic metabolite was enzyme dependent, control experiment (without enzyme sources) were execute. All assays were performed in duplicates.

Enzyme kinetic analyses. A mount of HLM or CES1 was incubated with probe **1** (0.1-20 μM) in 200 μL PBS (100 mM, pH 7.4), respectively. Before kinetic analysis, protein concentrations and incubation time were optimized to sure probe **1** hydrolysis within a linear range response. Then, enzyme reaction was started by addition of probe **1** (a series of substrates concentrations) to enzyme mixture which have been pre-incubated at 37 °C for 3 min. After incubated for 30 min, the reaction was terminated by addition of equal volume ice-cold acetonitrile. The formation rates of corresponding metabolite was determined by measuring the change of fluorescence intensities at 560 nm (excitation wavelength were set as 505 nm). Kinetic parameters (K_m and V_{max}) were calculated by nonlinear regression analysis using the Michaelis-Menten equation (Eq. S1).

$$v = \frac{V_{max} \times [S]}{K_m + [S]} \qquad \text{(S1)}$$

Quantification and correlation studies of CES1 in human liver microsomes. According to the procedure mentioned above, probe **1** (10 μM) was incubated with a group of 12 individual human liver microsomes (HLMs) at 37 °C, respectively. Then, the reactions were terminated with equal volume ice-cold acetonitrile and the hydrolytic rates of probe **1** were measured by synergy H^1 Hybrid Multi-Mode Micro-plate Reader. Clopidogrel, a reported substrate for CES1 was used to assay CES1 activities of the 12 individual HLMs as described before.[2] The hydrolytic rates of clopidogrel and probe **1** in 12 individual HLMs were compared, to verify the reliability of probe **1** in monitoring CES1 activities in HLMs. The correlation between clopidogrel and probe **1** hydrolysis were displayed by the linear regression coefficient ($P<0.005$ was considered statistically significant).

Preparation of cell lysate. Cells were washed twice with cold PBS before harvest. Cellular proteins were extracted by sonicating 30 s (200 W) for three times with 1 min on ice between two pluses. Then the cell lysate was centrifuged for 20 minutes at 9,000 g and the supernatant was collected for inhibition assays.

Cytotoxicity assays. The cytotoxicity effects of probe **1** on the viability of cells were carried out through CCK-8 method. A549 Cells (2×10^4/mL, 200 μL) were seeded in 96-well plate in DMEM/F12 culture medium containing 10% fetal bovine serum (FBS) and maintained in humidified incubator containing 5% CO_2 at 37 ˚C for 24 h. Then, the cells were incubated with varied concentrations of probe **1** (Prepared in FBS-free culture medium, 200 μL) for another 2 h. subsequently, CCK8 solution were added to incubated with cells at humidified incubator for another 4 h, and the absorbance at 450 nm was measured. Cell viability was calculated by A/A0×100% (A and A0 are the absorbance of experimental group and control group, respectively).

Confocal fluorescence imaging. A549 cells were cultured in DMEM/F12 culture medium (10% FBS) with a density of 10000 cells per dish (Φ 20 mm), after incubated overnight in a humidified incubator (37 °C, 5% CO_2). The adherent cells were washed twice with DMEM/F12 culture medium (FBS free) to remove suspending cells and FBS, then, adherent cells were pre-incubated with 100 μM BNPP (preparing in FBS free DMEM/F12 culture medium) for 30 min in humidified incubator. Then, the cells were incubated with probe **1** (final concentration 2 μM) and Hoechst 33342 (diluted according to instruction), which were diluted into DMEM/F12 culture medium (FBS free), in humidified incubator for another 30 min. Followed by rinsing with PBS (pH 7.4) for three times to remove the extracellular probe. For biological imaging of Ces in zebrafish, the junior zebrafish were maintained in zebrafish embryo culture solution, following grow for 7 days, they were pre-treated with 100 uM BNPP for 30 min. After that, the zebrafish were incubated with probe **1** (2 μM) for another 30 min at room temperature. Then, washed twice with zebrafish embryo culture solution to remove the extracellular probe. The fluorescence imagining were conducted by confocal microscope (Leica SP8, Germany and FV1000, Olympus, Japan).

Preparation of fresh mouse liver slices for fluorescence imaging. The fresh mouse livers were cut to slices with a thickness of 100 μm by a microtome and put into confocal dish. Then, the fresh liver slices were incubated with probe **1** (2 μM) in PBS at 37 ˚C for 30 min, after wash twice with PBS to remove extracellular probe, the fluorescence signals were recorded by confocal microscopy (Leica SP8, Germany).

Living-cells based inhibitors screening. HepG-2 cells were cultured in MEM culture medium (10% FBS) with a density of 8000 cells per well in 96 microplate and incubated overnight in humidified incubator (37 °C, 5% CO_2). After washed twice with MEM culture medium (FBS free) to remove suspending cells and FBS, the cells were pre-incubated with inhibitors (BNPP and UKA) at various concentrations (preparing in FBS free MEM culture medium) for 30 min in humidified incubator containing 5% CO_2 at 37 °C. After then, FBS free culture medium contain probe **1** (2 μM) and inhibitors at various concentrations were used to incubate with HepG-2 cells for another 30 min. Finally, the fluorescence intensities of the hydrolytic metabolite were detected by fluorescence microplate reader (BioTek, Winooski, Vermont, USA).

Molecular docking simulations. The protein structure of human CES1 was downloaded from PDB (ID: 1MX5) while the structure modelling of human CES2 was downloaded from SWISS-MODEL repository. This original structure, residue range 30 to 544, was created based on template 5fv4 (PDB ID) on May 3rd, 2017, with QMEAN of -2.86. Discovery Studio (BIOVIA Discovery Studio 2016, Dassault Systèmes, San Diego, USA) were used to performed molecular docking process. The CHARMM 40.1 force field was used to represent the protein and ligand structures. A standard LibDock protocols were conducted to perform docking simulations while protein features were referred to as hotspots. The top scoring ligand poses were obtained by energy-minimization step (allowing the ligand poses to be flexible), and the rigid poses were placed into active site of human CES1 and CES2 while hotspots were matched as triplets. For docking processes, probe **1** was inputted, docking preferences were set as "High Quality" and the poses docked into the active site of human CES1 and CES2 were 46 and 38, respectively. The protein-ligand complexes with the highest LibDock score was taken from the docking results and depicted.

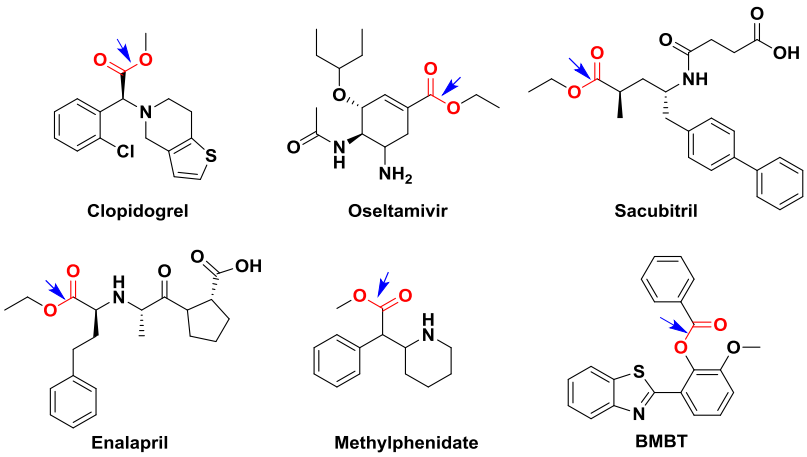


Fig. S1. The chemical structure of the representative substrates of CES1.

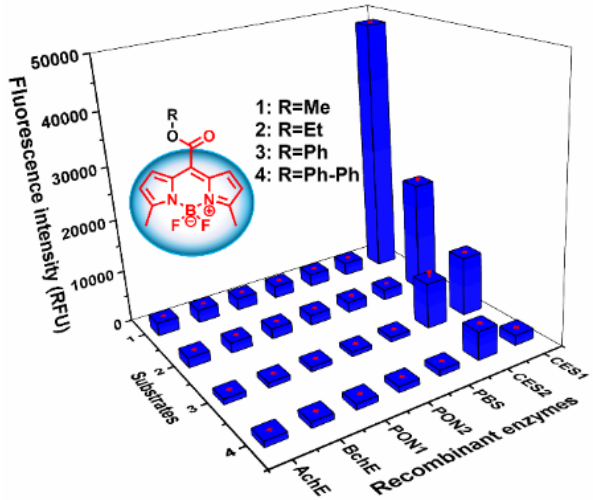


Fig. S2. Fluorescent responds of DMBA derivatives (5 μM) incubated with several hydrolyases at 37 °C for 60 min ($\lambda_{ex}/\lambda_{em}$ = 505/560 nm).

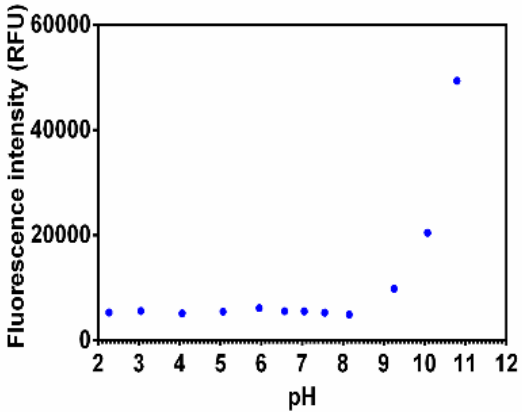


Fig. S3. The effects of pH values on the fluorescence intensity of probe **1**, fluorescence substrates (5 μM) were incubated at 37 ˚C for 30 min before measuring in Synergy H[1] Hybrid Multi-Mode Microplate Reader. (λ_{ex} =505 nm, λ_{em} =560nm).

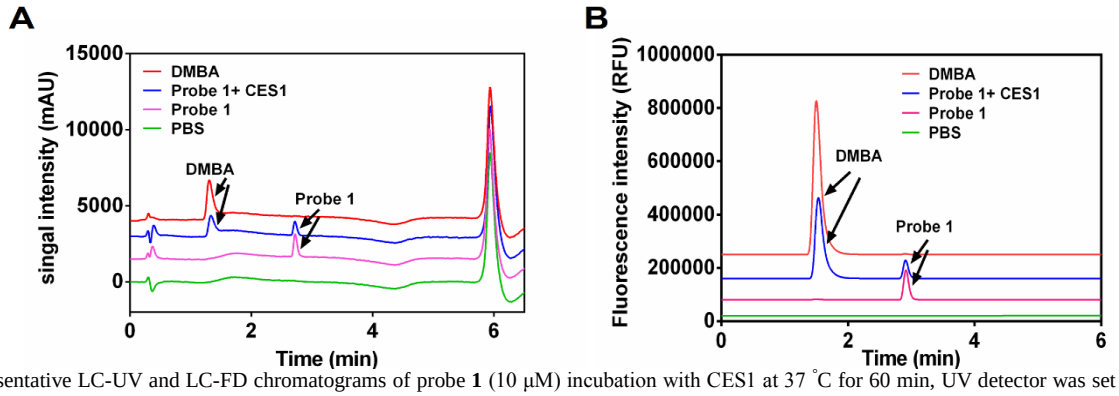


Fig. S4. Representative LC-UV and LC-FD chromatograms of probe **1** (10 μM) incubation with CES1 at 37 ˚C for 60 min, UV detector was set at 505 nm and fluorescence detector was set at 560 nm while excitation wavelength was 505 nm.

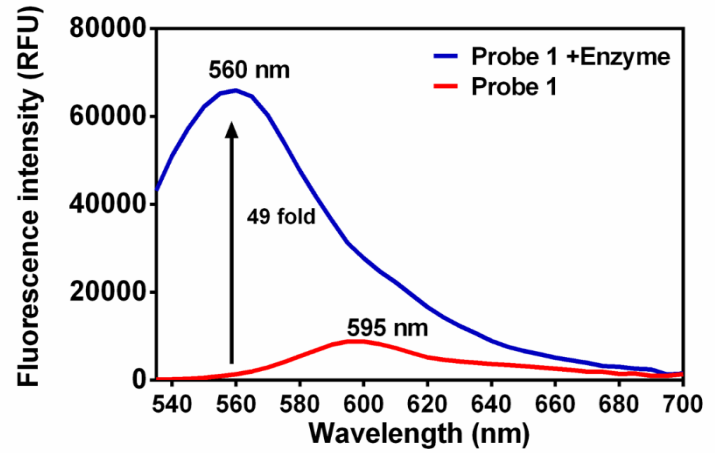


Fig. S5. The change of fluorescence emission of probe **1** (10 μM) upon addition of CES1-containing HLMs as enzyme source.

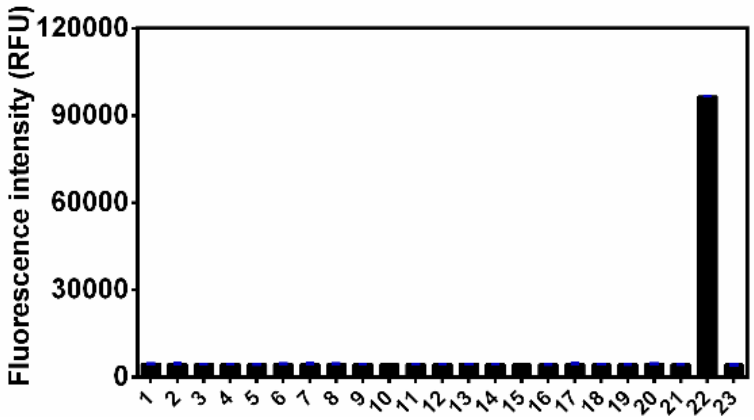


Fig. S6. Fluorescence response of probe **1** (A, 10 μM) to various endogenous compounds (50 μM) in aqueous solution (acetonitrile: PBS = 1:1).1: Mn^{2+},2: Zn^{2+},3: K^{+},4: Ca^{2+},5: Al^{3+},6: Mg^{2+},7: Arg,8: Try,9: Glu,10: Ser,11: Gln,12: Lys,13: Glucose,14: Fe^{2+},15: Gly,16: Co^{2+},17: Myristic acid,18: Cys,19: GSH,20: Vitamin C,21: Fe^{3+},22: CES1,23: PBS.

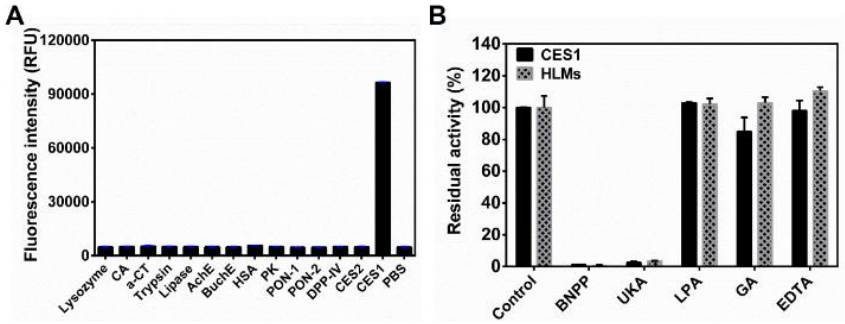


Fig. S7. (A) Fluorescence responses of probe **1** (10 μM) towards various hydrolases in the human body and (B) the inhibitory effects of specific inhibitors of human esterases on probe **1** (10 μM) hydrolysis in both recombinant human CES1 and HLMs ($\lambda_{ex}/\lambda_{em}$ = 505/560 nm).

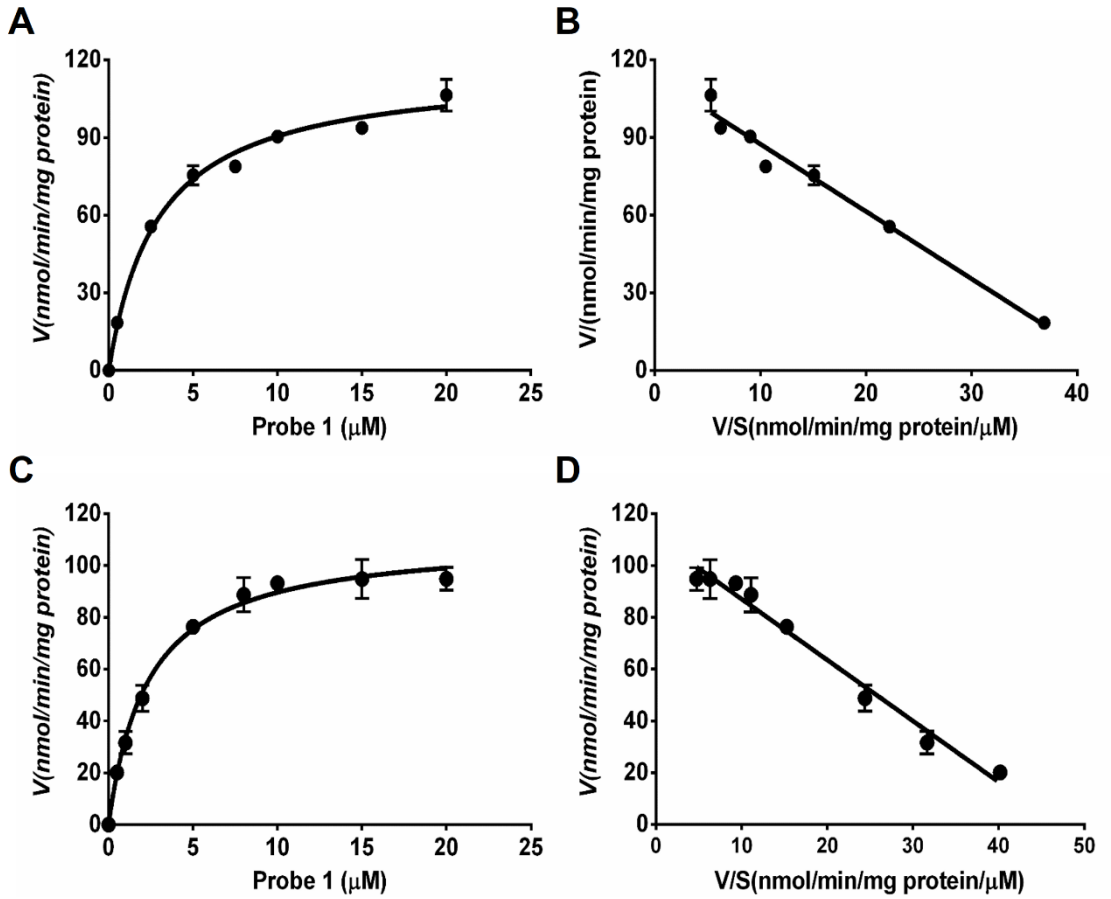


Fig. S8. Michaelis−Menten kinetic plots of probe **1** in recombinant human CES1 (A) and HLMs (C), the corresponding Eadie−Hofstee plots were shown on the right (B and D).

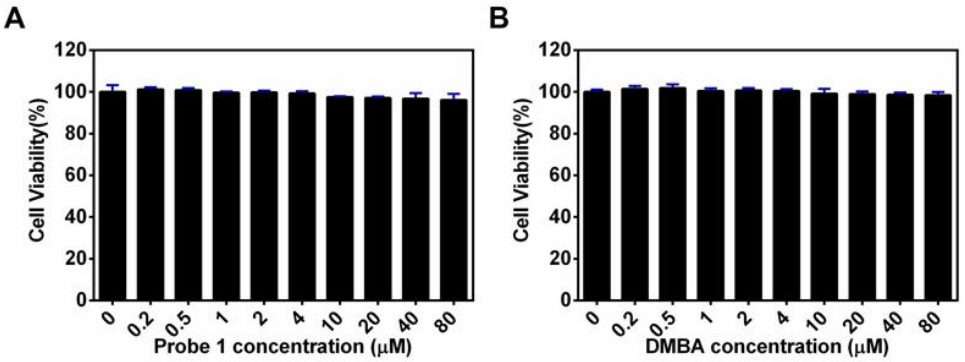


Fig. S9. The cytotoxicity of probe **1** (A) and DMBA (B) toward A549 cells.

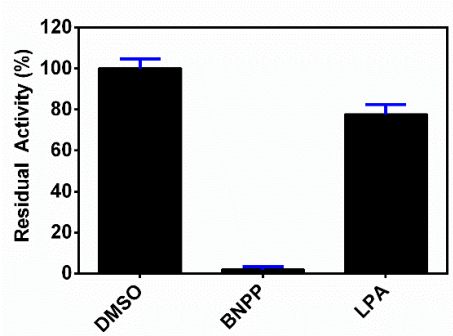


Fig. S10. The inhibitory effects of BNPP (100 μM) and LPA (100 μM) on the hydrolysis of probe **1** (10 μM) in A549 cell lysate (A549 S9).

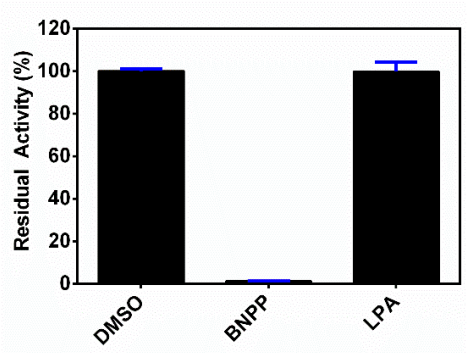


Fig. S11. The inhibitory effects of BNPP (100 μM) and LPA (100 μM) on the hydrolysis of probe **1** (10 μM) in mouse liver tissues preparation (mouse liver microsomes).

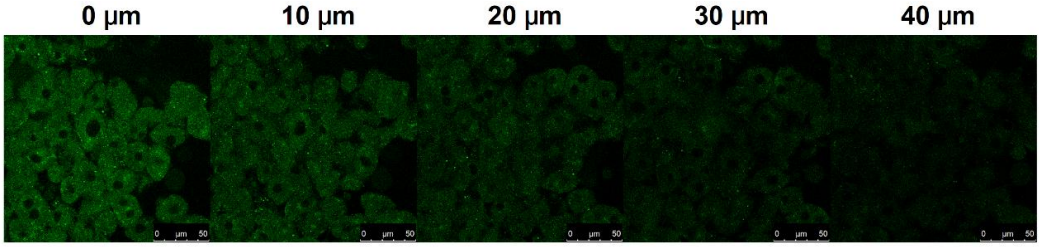


Fig. S12. Confocal imaging (63 × magnification) of CES1 in liver slices. Probe **1** (2 μM) was incubated with liver slices at 37 ˚C for 30 min (excitation at 488 nm and fluorescence emission is 540-580 nm).

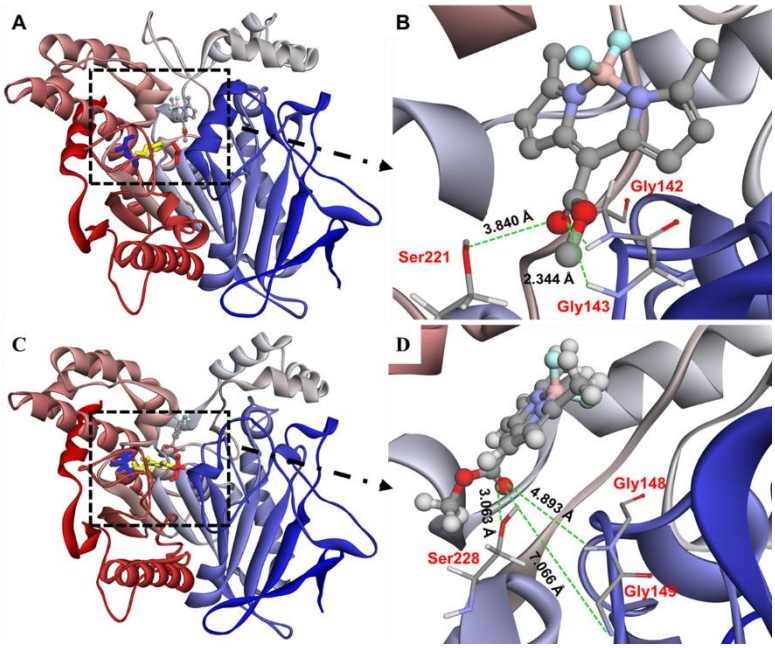


Fig. S13. Molecular docking of probe 1 on human CES1 and CES2. (A) A stereo view of the crystal structure of human CES1 and the stereo-diagram of probe 1 aligned in its active site. (B) A detail view of the binding showed that the Ser221 and the oxyanion hole (Gly142 and Gly143) of human CES1 is near to the carbonyl carbon of probe 1. (C) A stereo view of the crystal structure of modelling of human CE2 and the stereo-diagram of probe **1** aligned in its active site. (D) A detail view of the binding site showed that the hydroxyl group of Ser-228 resides is close to the carbonyl carbon of probe **1** but the orientation of probe 1 hinder the process of hCE2 mediated catalytic cycles and make hCE2 hardly catalyzed probe **1** to DMBA.

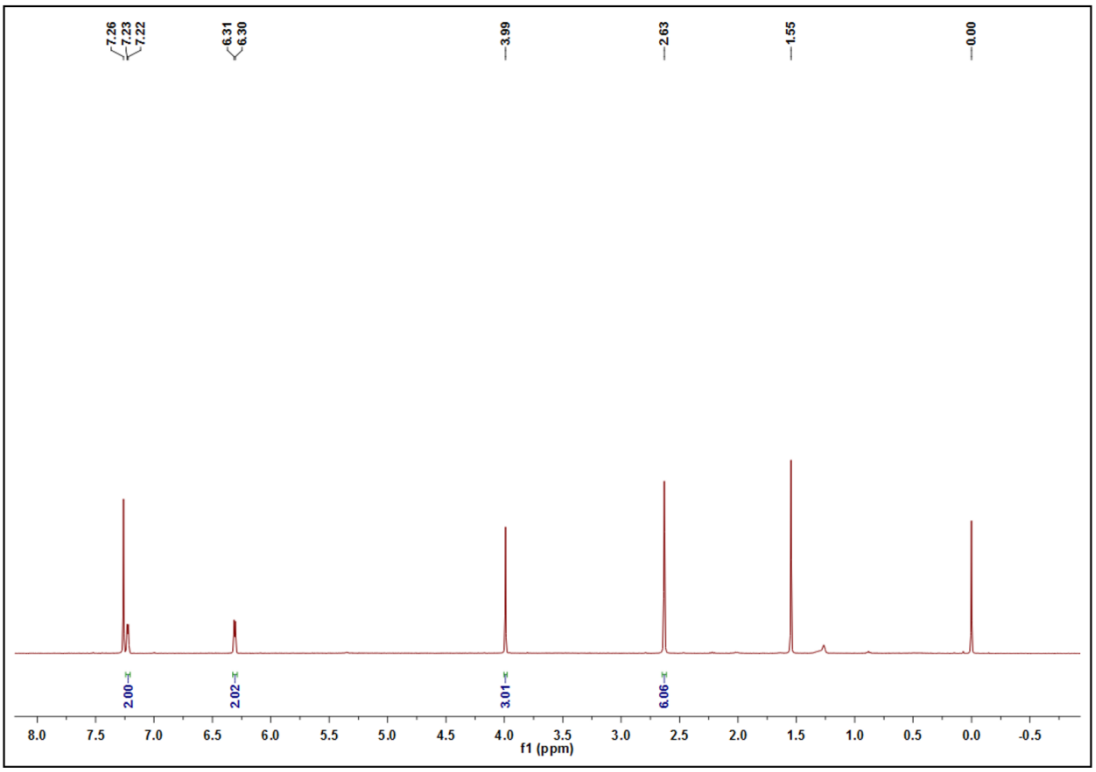


Fig. S14. ^{1}H NMR spectrum of probe **1** in $CDCl_3$.

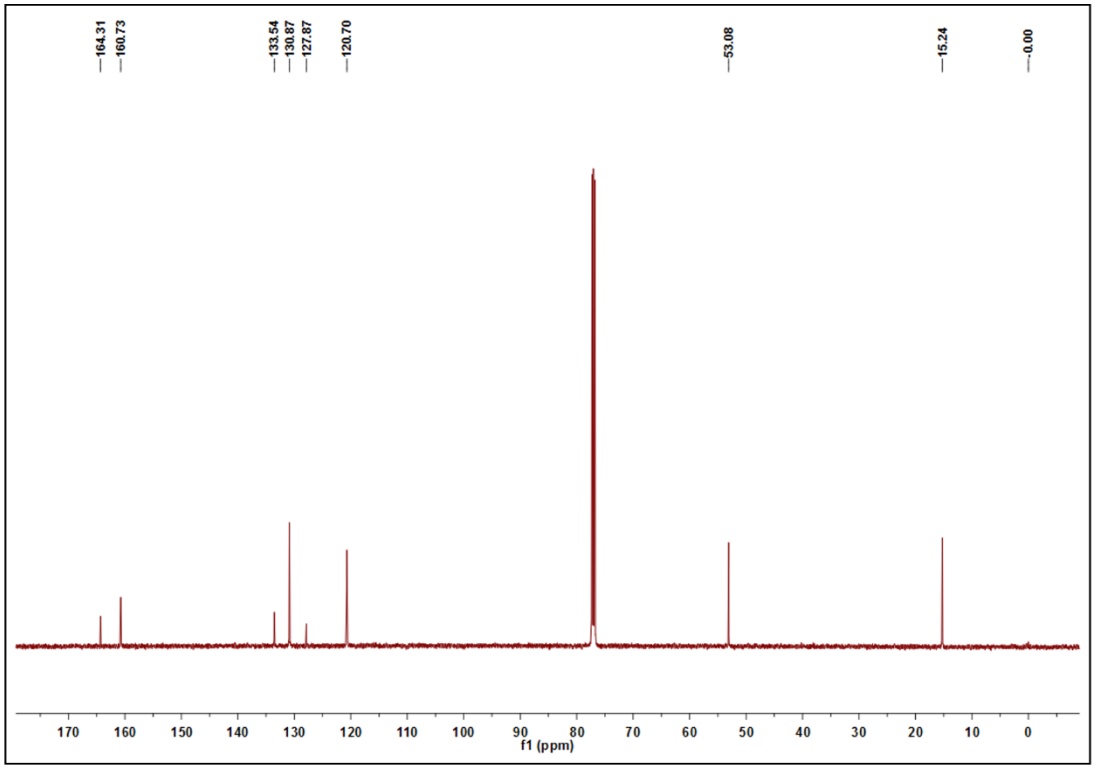


Fig. S15. ^{13}C NMR spectrum of probe **1** in $CDCl_3$.

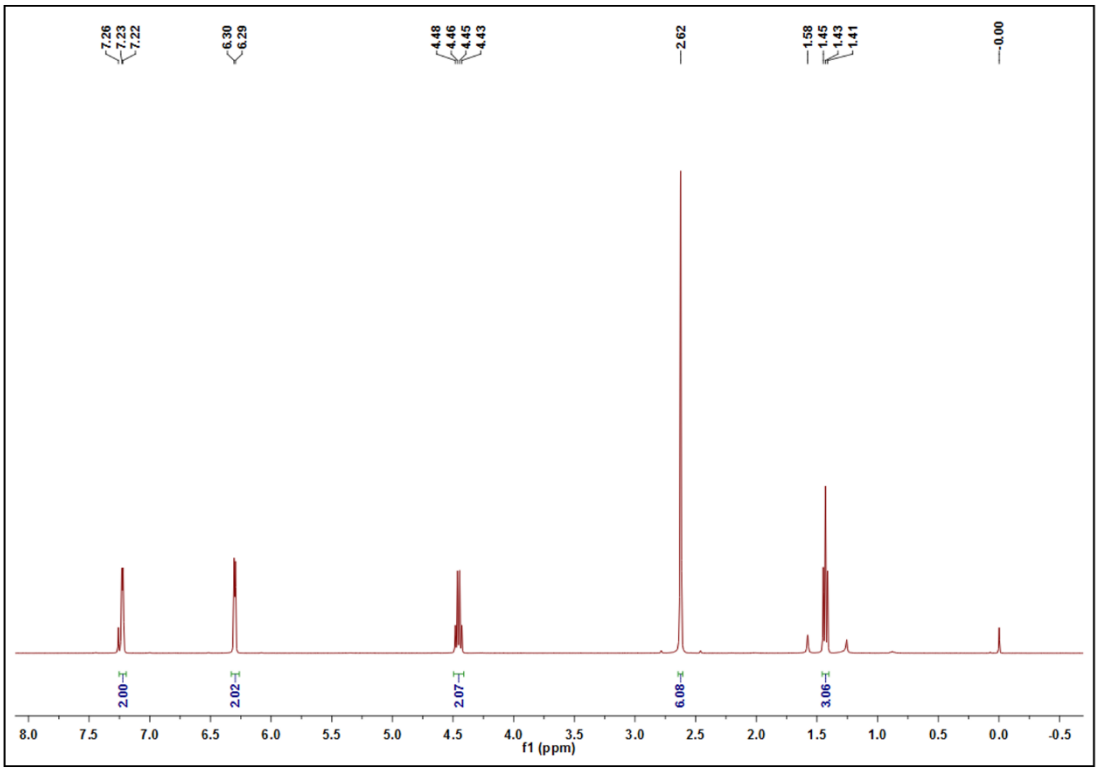


Fig. S16. ^{1}H NMR spectrum of probe **2** in $CDCl_3$.

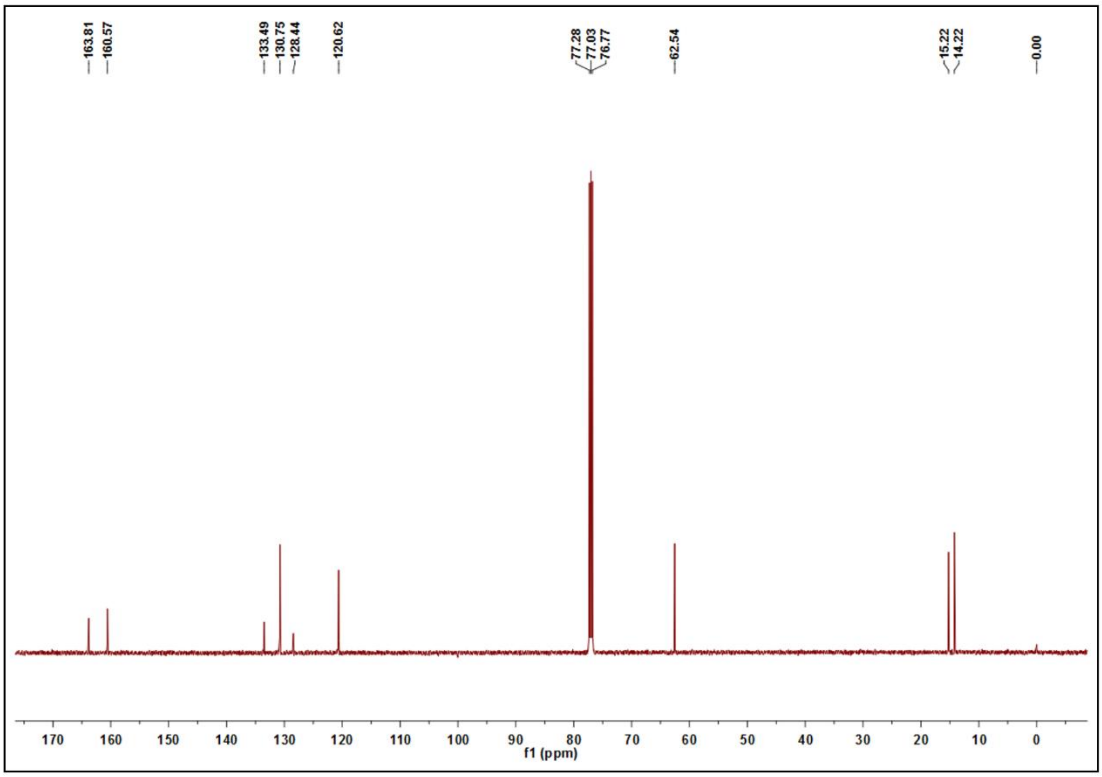


Fig. S17. ^{13}C NMR spectrum of probe **2** in $CDCl_3$.

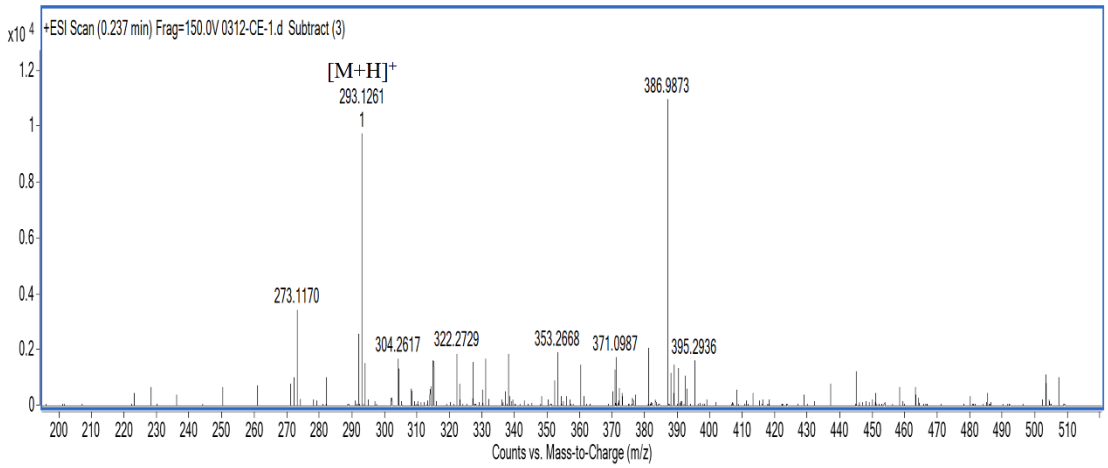


Fig. S18. HRMS spectrum of probe **2**.

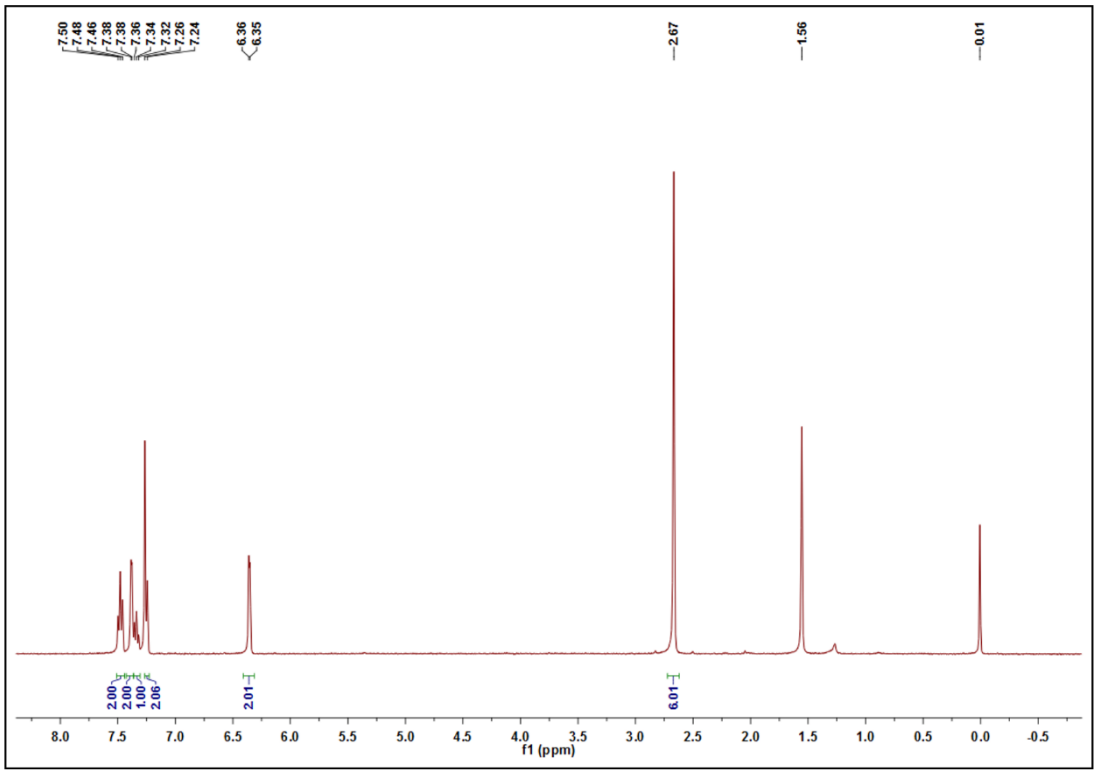


Fig. S19. ^{1}H NMR spectrum of probe **3** in $CDCl_3$.

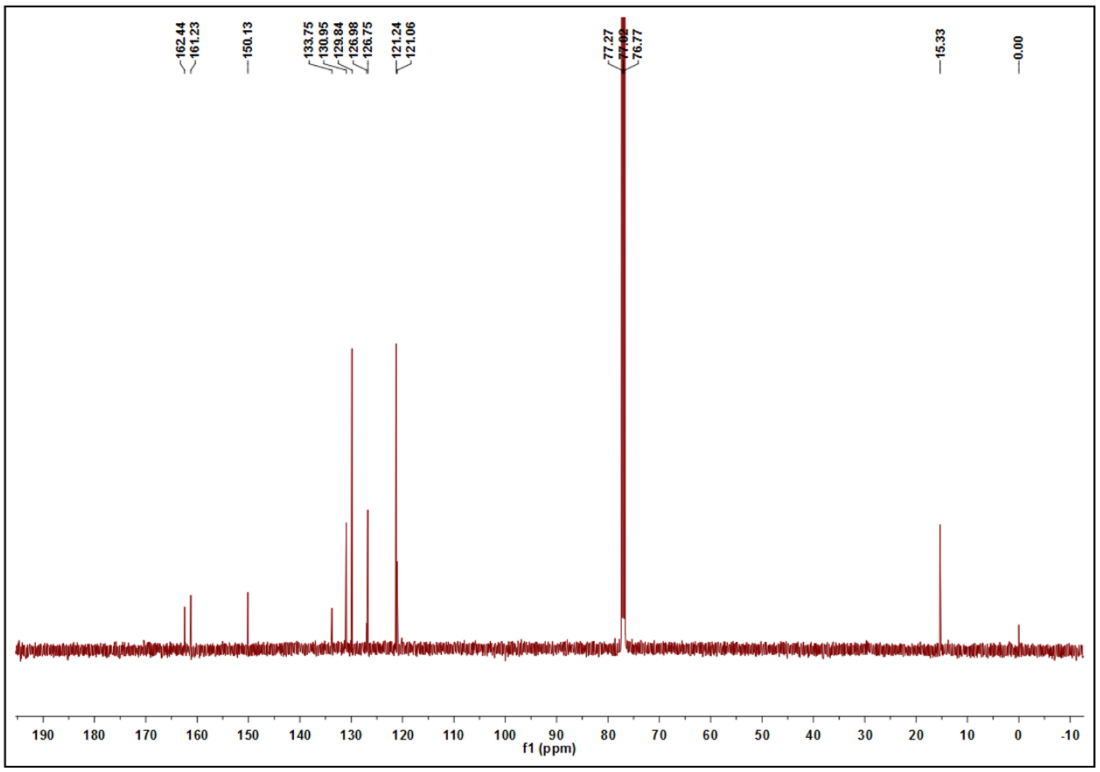


Fig. S20. ^{13}C NMR spectrum of probe **3** in $CDCl_3$.

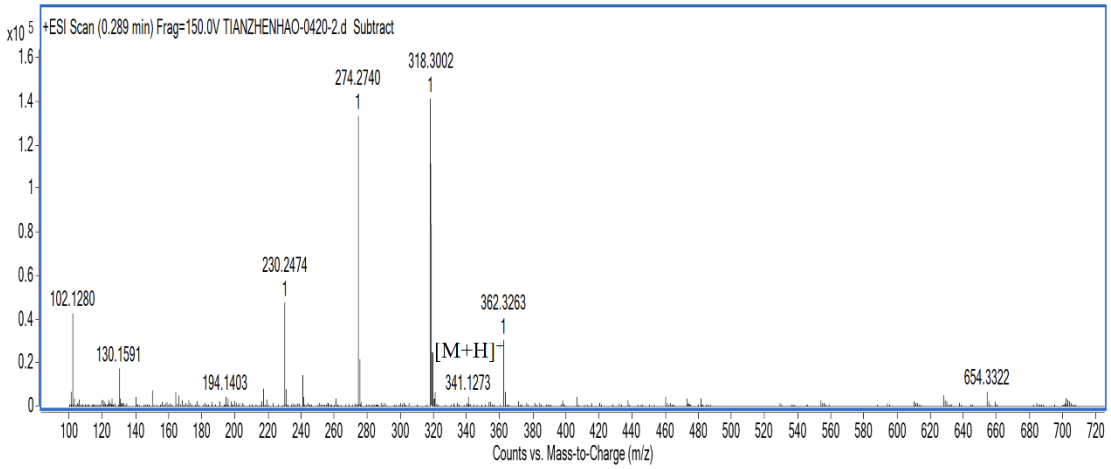


Fig. S21. HRMS spectrum of probe **3**.

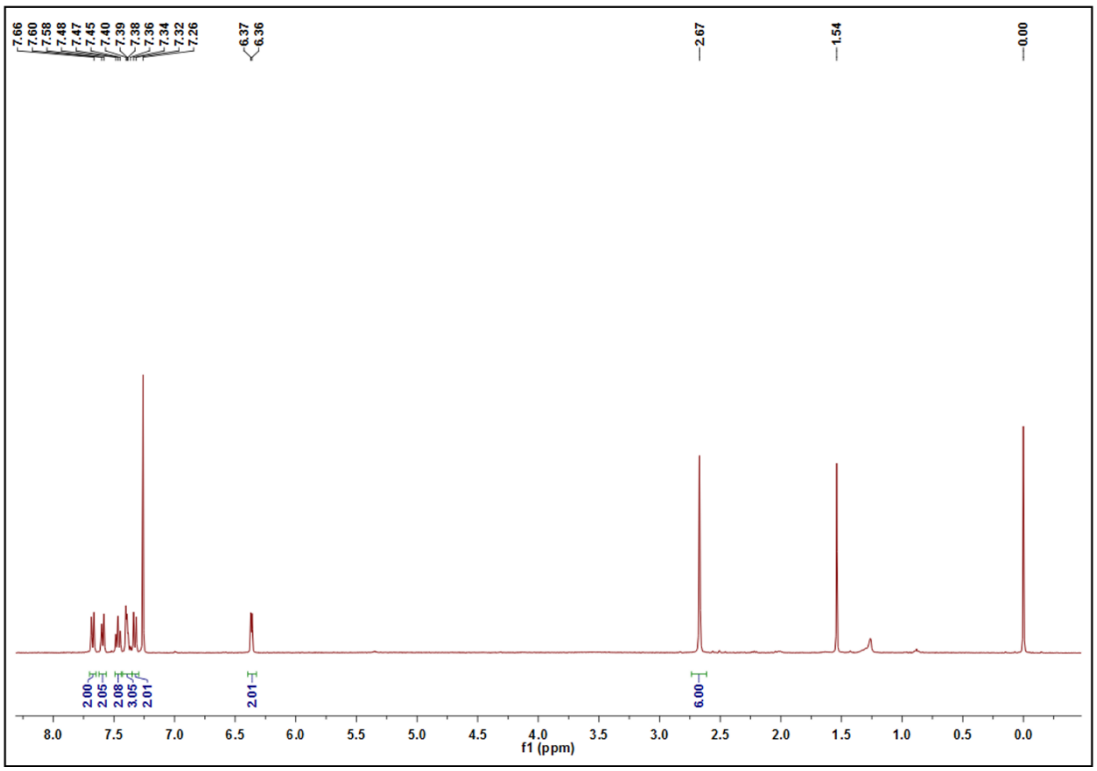


Fig. S22. ^{1}H NMR spectrum of probe **4** in $CDCl_3$.

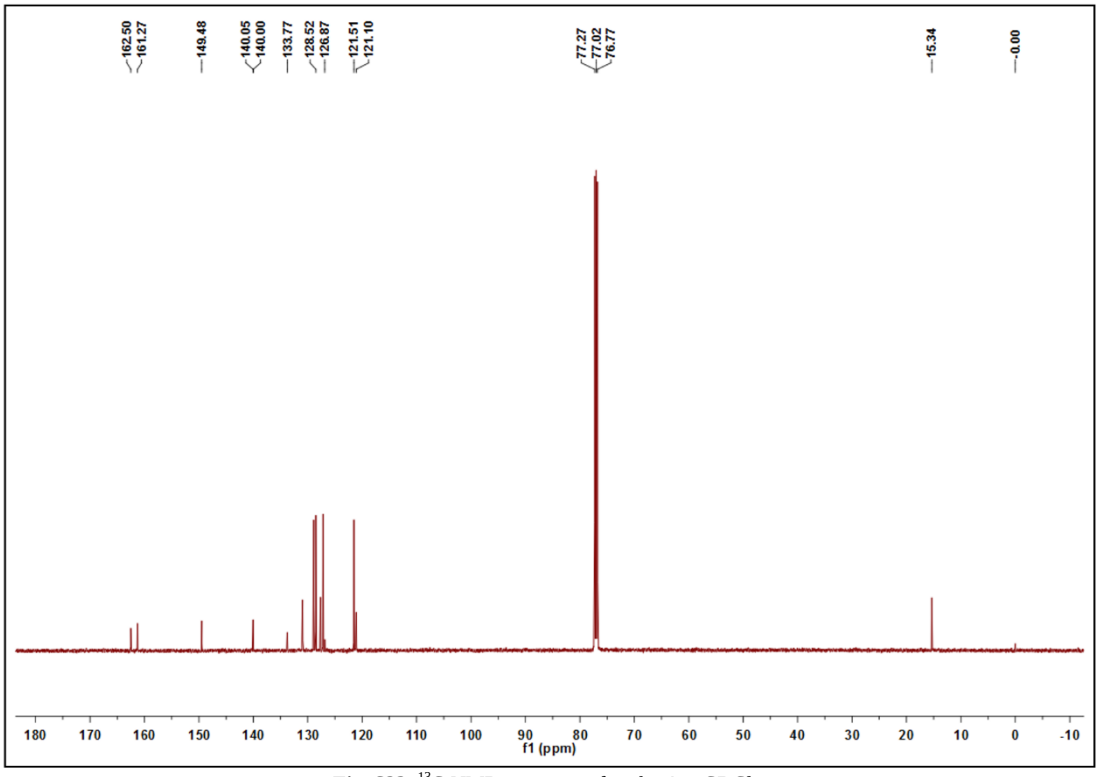


Fig. S23. ^{13}C NMR spectrum of probe **4** in $CDCl_3$.

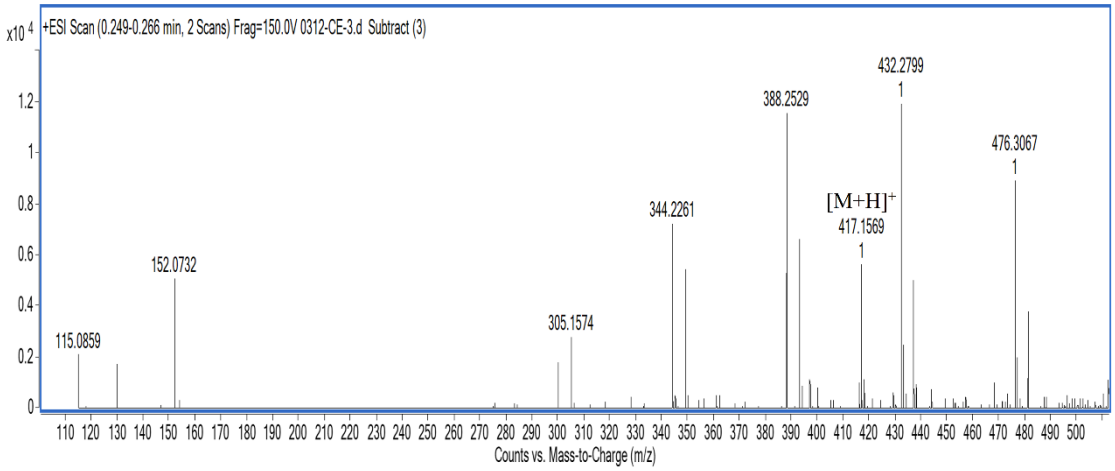


Fig. S24. HRMS spectrum of probe **4**.

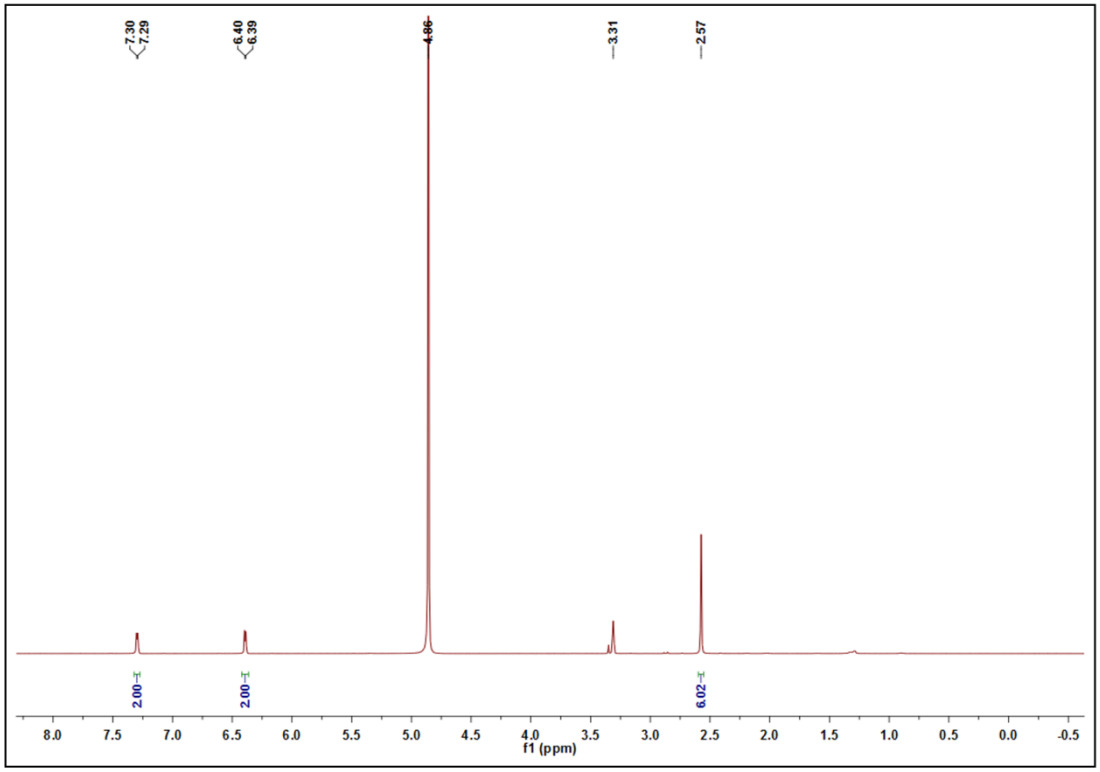


Fig. S25. ^{1}H NMR spectrum of metabolite **DMBA** in MeOD.

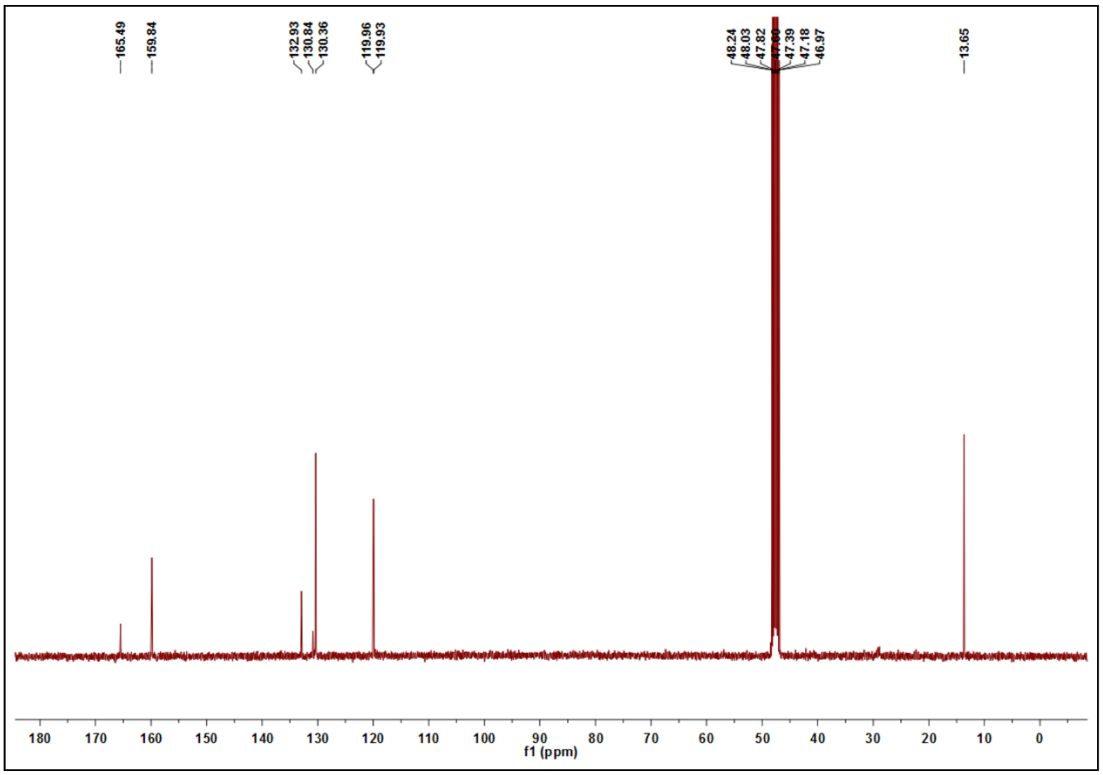


Fig. S26. ^{13}C NMR spectrum of metabolite **DMBA** in MeOD.

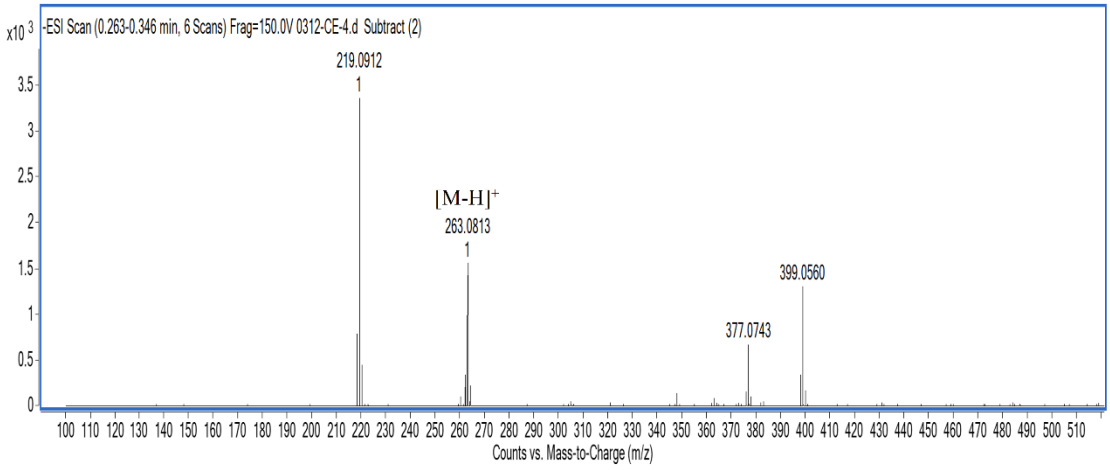


Fig. S27. HRMS spectrum of metabolite **DMBA**.

Table S1

Kinetic parameters for probe **1** hydrolysis in different enzyme sources.

Enzyme source	k_{cat}	k_m (μmol/L)	k_{cat}/k_m
Human CES1	116.8 ± 3.5	2.9 ± 0.3	40.3
HLMs	110.6 ± 2.8	2.3 ± 0.2	48.1

k_{cat} values were in nmol min^{-1} mg^{-1} protein for liver microsomes or in nmol min^{-1} mg^{-1} for recombinant human CES1, and k_{cat}/k_m values were in mL min^{-1} mg^{-1} protein for liver microsomes or in nmol min^{-1} mg^{-1} for CES1.

Table S2

The IC_{50} values of two CES1 inhibitors against human CES1 in different enzyme sources.

Inhibitors	IC_{50} values (nmol/L)		
	CES1	HLMs	Living cells
BNPP	74 ± 1.87	109 ± 3.27	854 ± 6.55
UKA	122 ± 2.09	172 ± 4.47	18052 ± 9.06

References

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2. Wang, D. D.; Jin, Q.; Hou, J.; Feng, L.; Li, N.; Li, S. Y.; Zhou, Q.; Zou, L. W.; Ge, G. B.; Wang, J. G.; Yang, L., Highly sensitive and selective detection of human carboxylesterase 1 activity by liquid chromatography with fluorescence detection. *J. Chromatogr. B* **2016,** *1008*, 212-218.