Electronic Supplementary Information

Highly Selective and Sensitive Chemiluminescent Probe for Leucine Aminopeptidase Detection in *Vitro*, in *Vivo* and in human Liver Cancer Tissue

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1. General Materials and Methods

General Materials

All reagents and solvents were of analytical grade available from commercial sources, used as received or purified by standard techniques. Thin-layer chromatography (TLC) plates were employed for monitoring the reaction. ^{1}H nuclear magnetic resonance (NMR) was obtained on 400 MHz (Hertz) spectrometer (Bruker Co., Ltd., Swiss). All chemical shifts (δ) recorded in ppm were related to internal standard tetramethyl silane (δ = 0.0 ppm) or signals of residual solvent $CDCl_3$ (7.26 ppm for ^{1}H, 77.16 ppm for ^{13}C), and the coupling constants were given in Hz. Mass spectra were recorded on liquid chromatography/mass spectrometry (Orbitrap Fusion™ Tribrid™, Thermo Scienfific). UV-vis absorption spectra were recorded on UV spectrometer (U-3010, Hitachi, Japan). The fluorescence spectra were recorded on fluorescence spectrometer (Hitachi FluoroMax-4/PLUS). The chemiluminescence signals were captured by Spectramax M5 (MDC). The chemiluminescence images of cells and mice were measured by using Xenogen IVIS® Spectrum imaging system (Caliper Life Science, USA). Leucine aminopeptidase, cathepsin B and ubenimex were purchased from Sigma-Aldrich. Boc-L-Leucine and other reagent were obtained from Macklin.

General procedure for the detection of LAP with probe 1 and 2.

The work solution of probe 1 and 2 (20 μM) was prepared by diluting appropriate volume of stock solution (20 mM) of probe 1 and 2 into enzyme reaction buffer (1×PBS buffer, pH 7.4, 10% DMSO). probe 1 and 2 (20 μM, 100 μL, in enzyme reaction buffer) were mixed with enzyme assay buffer containing LAP (100 μL, in PBS) in white or black 96-well plants, and the mixed reaction solution was then incubated at 37 ℃. The LAP-triggered reaction of probe 1 was analyzed by LC-MS and UV-vis absorption, chemiluminescence, and fluorescence spectroscopy. The LC-MS were recorded on liquid chromatography/mass spectrometry (Orbitrap Fusion™ Tribrid™, Thermo Scienfific). The UV-vis absorption spectra were obtained from 200 to 700 nm. The chemiluminescence spectrum was recorded (white 96-well plants) on Spectramax M5 (MDC), the integration time is 1 s. Chemiluminescence imaging of the solution was carried out using IVIS Lumina XR III system (black 96-well plants). The fluorescence spectra were recorded from 450 nm to 700 nm with an excitation at 400 nm on the fluorescence spectrophotometer.

Chemiluminescence kinetic profile.

Chemiluminescence response of probe 1 and 2 (10 μM, 200 μL) in the absence or presence of LAP (100 U/L) was investigated. The reaction solutions were incubated at 37 ℃, and the real-time

chemiluminescence was recorded (white 96-well plants) on Spectramax M5 (MDC) every 80 Second, last for 6000 Second. The integration time is 1 s.

Determination of the sensitivity of probe 1 and Leu-AMC toward LAP in vitro.

To evaluate the sensitivity of probe 1 toward LAP, probe 1 (10 μM, 200 μL) on a black 96-well plate was incubated with varying levels of LAP (0, 10, 20, 40, and 80 U/ L) at 37 ℃ for 10 min. The resulting chemiluminescence images were acquired using the IVIS Lumia XR III system. The chemiluminescence intensities in each well were quantified using ROI, and plot to the levels of LAP added. After linear fitting, the slope (k) from each plot was abtained, and the limit of detection (LOD) was calculated from 3δ/k, where δ represents the standard deviation of 11 blank measurements.

To study the sensitivity of Leu-AMC toward LAP, Leu-AMC (10 μM, 200 μL) on a black 96-well plate was incubated with varying levels of LAP (0, 6.25, 12.5, 25, 50, and 100 U/ L) at 37 ℃ for 30 min. The fluorescence Intensity were recorded at 460 nm with an excitation at 355 nm on the Spectramax M5 (MDC). After linear fitting, the slope (k) from each plot was abtained, and the limit of detection (LOD) was calculated from 3δ/k, where δ represents the standard deviation of 11 blank measurements.

Determination of the specificity toward LAP.

To examine the selectivity of probe 1, the chemiluminescence intensity of probe 1 incubated with different analytes was investigated. Probe 1 (10 μM) in LAP assay buffer (200 μL) on a white 96-well palte was incubated with Hcy (100 μM), L-Cys (100 μM), Glu (100 μM), KCl (100 μM), Na_2S (100 μM), $Al_2(SO_4)_3$ (100 μM), $CuSO_4$ (100 μM), $ZnCl_2$ (100 μM), $FeSO_4$ (100 μM), $MgSO_4$ (100 μM), $CaCl_2$ (100 μM), β-galactosidase (β-gal, 100 U/L), cathepsin B (100 U/L), alkaline phosphatase (ALP, 100 U/L), pyroglutamyl aminopeptidase I (100 U/L), LAP (100 U/L), or LAP (100 U/L) together with it inhibitor Ubenimex (Ube, 40 μM) at 37 ℃ for 10 min. The chemiluminscence spectra in each well was recorded on the microplate reader uner chemiluminescence mode, with an integration time of 1 s.

Determination of the hydrolysis effect of LAP at different temperatures.

Temperature is an important factor that affects enzymatic hydrolysis ability. Therefore, the hydrolysis effect of LAP on probe 1 (10 μM) at different temperatures was investigated. Probe 1 (10 μM) in LAP assay buffer (200 μL) on a white 96-well palte was incubated with LAP (100 U/L), the temperature increased from 25 ℃ to 37 °C for 10 min. The chemiluminscence spectra in each well was recorded on the microplate reader uner chemiluminescence mode, with an integration time of 1 s.

Cell culture.

HepG2 cells and LO2 normal cells were grown in high glucose Dulbecco's modified Eagle's medium (DMEM) (Gibco) containing 10% fetal bovine serum (FBS) (Gibco), and 1% penicillin/streptomycin (Gibco). All cells were cultured in a 5% CO_2 humidified incubator at 37 ℃.

Cytotoxicity studies.

HepG2 or LO2 cells were seeded on flat-bottomed 96-well plates (5000 cells/well) and incubated at 37 ℃ for 24 h. Varying concentrations of probe 1 (0, 5, 10, 20, 40 μM) in the medium were then added. After being incubation for 24 h, MTT solution (50 μM, 1× in PBS) was added into each well. The cells were kept at 37 ℃ for another 4 h, and the medium containing unreacted MTT in each well was then removed carefully. The resulting purple crystals in the wells were dissolved by addition of 150 μL DMSO. The absorbance (OD) of formazan at 490 nm in each well was recorded on a microplate reader (Tcan). The absorbance of cells without any treatment ($OD_{control}$) were used as the control, and the percentage of cell viability in each treatment was calculated by dividing OD to $OD_{control}$. Every experiment was repeated three times.

Chemiluminescence determination of LAP activity in live cells.

Liver cancer cells (HepG2) cells (4×10^4/well) were seeded into white 96-well plates and allowed to grow a day. HepG2 cells were pretreated with Ube (40 μM) in the medium for 1 h. After the removal of the medium, probe 1 (10 μM) in LAP assay buffer (200 μL) was added into each well. The chemiluminescence intensity of LAP activity in live cells was recorded at 37 ℃ in real time by using SparkTM 10M multimode microplate reader with integration time of 1 s.

HepG2, LO2, cells (0, 2.5×10^3, 5×10^3, 1×10^4, 2×10^4, 4×10^4, and 4×10^4 pretreated with Ube [40 μM]) in each well were seeded into black 96-well plates and allowed to grow a day. After the removal of the medium, probe 1 (10 μM) in LAP assay buffer (200 μL) incubated with probe 1 (10 μM) at 37 °C for 30 min. The chemiluminescence image of the cells solution was acquired by using the IVIS Lumina XR III system, with acquirement time of 60 s.

Fluorescence imaging and colocalization studies.

HepG2 or LO2 cells (5×10^4) were seeded on a glass-bottom cell culture dish, and kept growing overnight. To inhibit LAP activity, HepG2 cells were pretreated with Ube (40 μM) for 1 h. Probe 1 (10 μM) in FBS free DMEM medium were added into cells and incubated at 37 ℃ for 1 h. After being washed with cold PBS (1×) for three times, fluorescence images of the cells were then acquired form 500-650 nm on a LSM880 With Airyscan confocal laser scanning microscope, using excitation of 405 nm.

For co-localization studies, HepG2 cells were incubated with probe 1 or product 6-1 (10 μM) for 1 h. After being washed with cold PBS for three times, the cells were then incubated with 10 μg/mL Lyso Tracker Green for 20 min. The fluorescence images of the cells were acquired on a LSM880 With Airyscan confocal laser scanning microscope after three times of washes with PBS (1×, pH 7.4). The fluorescence of probe 1 or product 6-1 was acquired from 500 nm to 550 nm with an excitation at 405 nm. The fluorescence of LysoTracker Green was acquired from 550 nm to 600 nm with an excitation at 488 nm.

Chemiluminescence imaging of LAP activity in vivo.

All animal studies were conducted according to institutional animal care and use regulations approved by the Animal Research Center of Southern Medical University (Permit number SCKK 2016-0041). Nine female, athymic, 4–5-week-old BALB/c nude mice were injected subcutaneously with 1×10^7 HepG2 cells to establish xenograft HepG2 tumors in the armpit. When the tumors grew to a mean volume of ~150 mm^3, the mice were randomly grouped into three (n = 3). PBS (100 μL) was used for intratumoral injection. Probe 1 (100 μM in 100 μL PBS) was used for intratumoral injection. LAP activity in the tumor was inhibited by directly injecting Ube (200 μM in 100 μL PBS) into tumors at 1 h before the intratumoral injection of probe 1 (100 μM in 100 μL PBS). The whole body chemiluminescence images of mice were recorded by the IVIS Lumia XR III system (open filter) at 0, 5, 10, 15, 20, 25, 30, and 35 min. The acquirement time was 60 s.

Sensing LAP activities in HepG2-tumor-bearing nude mice tissues.

The Urine, serum, were acquied from the HepG2-tumor-bearing nude mice. The heart, liver, spleen, lung, kidney, brain, and tumor tissues were acquied from the HepG2-tumor-bearing nude mice, and then 10% tissue homogenates in saline were prepared. After centrifugation at 10,000 rpm for 20 min, the supernatant was collected. Approximately 10 μL of supernatant or Urine or serum was 10-fold diluted in saline, and then mixed with probe 1 (10 μM). The chemiluminescence image was captured using the IVIS Lumia XR III system at 10 min. The acquirement time was 60 s. The supernatant or Urine or serum (diluted 1:10 with saline) was incubated with probe 1 (10 μM, incubated 10 minutes) or Leu-AMC (10 μM, incubated 30 minutes) at 37°C, respectively. Correspondingly, fluorescence signal was captured using SparkTM 10M multimode microplate reader.

Sensing LAP activities in human tissue samples.

The Human tissue samples were provided by Guangdong Provincial People's Hospital. The 10% tissue homogenates in saline were prepared. After centrifugation at 10,000 rpm for 20 min, the

supernatant was collected. Approximately 10 μL of supernatant was 10, 20, 40, 80-fold diluted in saline, and then mixed with probe 1 (10 μM). The chemiluminescence image was captured using the IVIS Lumia XR III system at 10 min. The acquirement time was 60 s. The 10% tissue homogenate supernatant (diluted 1:10 with saline) was incubated with probe 1 (10 μM, incubated 10 minutes) or Leu-AMC (10 μM, incubated 30 minutes) at 37 ℃, respectively. Correspondingly, chemiluminescence and fluorescence signal was captured using SparkTM 10M multimode microplate reader.

2. Abbreciations

PE- petroleum ethe, **EA**- Ethyl acetate, **DCM**- Dichloromethane, **THF**- tetrahydrofuran, **ACN**- acetonitrile, **DMF**- N, N-Dimethylformamide.

3. Synthesis and characterization of probe 1

Synthesis of compound OH-LAP

Boc-L-Leucine (1.16 g, 5 mmol) was dissolved in DCM (100 mL), followed by addition of HATU (2.28 g, 6 mmol) and DIPEA (1.65 mL, 10 mmol). After further stirring over 30 min, (4-aminophenyl) methanol (492 mg, 4 mmol) was added, and then the mixture reacted for 12 h at room temperature. The resultant was washed with water and brine. The organic layer was separated and dried with anhydrous Na_2SO_4. The crude product was purified by silica-gel column chromatography using (PE: EA=2:1) to afford compound OH-LAP (618 mg, 68 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.28 (s, 1H), 7.36 (d, *J* = 8.0, 2H), 7.07 (d, *J* = 8.0, 2H), 5.67 (d, *J* = 8.0, 1H), 4.50 (s, 2H), 4.36 (s, 1H), 1.73 – 1.70 (m, 1H), 1.62 – 1.59 (m, 2H), 1.37 (s, 9H), 0.92 – 0.88 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.18, 150.56, 137.22, 136.43, 127.36, 119.86, 80.08, 64.34, 53.92, 41.42, 28.32, 24.73, 23.06, 21.60.

Synthesis of compound 5

To a solution of compound OH-LAP (840 mg, 2.5 mmol) in THF (50 mL) at 0 ℃, CBr_4 (1.66 g, 5.0 mmol) and Triphenylphosphine (1.3 g, 5.0 mmol) was added. The resulting reaction mixture was warmed up to room temperature and stirred for 5 h. Then, the mixture was diluted with EA (20 mL), and washed with water and brine. The organic layer was separated and dried with anhydrous Na_2SO_4. The crude product was purified by silica-gel column chromatography using PE: EA (5:1) to afford compound 5 (696.7 mg, 70 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.29 (s, 1H), 7.43 (d, *J* = 8.0, 2H), 7.19 (d, *J* = 8.0, 2H), 5.55 (d, *J* = 8.0, 1H), 4.45 (s, 3H), 1.80 – 1.75 (m, 1H), 1.69 – 1.66 (m, 2H), 1.44 (s, 9H), 0.99 – 0.96 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.75, 156.51, 138.15, 133.06, 129.52, 119.82, 80.37, 53.92, 41.04, 33.57, 28.31, 24.73, 22.99, 21.66.

Synthesis of compound 7

Compound 6[1] (50 mg, 0.13 mmol), KI (216 mg, 1.3mmol) and K_2CO_3 (35 mg, 0.26 mmol) were dissolved in DMF (1 mL) and stirred was stirred for 10 min before compound 5 (52 mg, 0.13 mmol) was added to the mixture. The reaction mixture was stirred for 6 h at room temperature with the reaction progress being monitored by TLC analysis (PE: EA= 5:1). Upon completion, the reaction mixture diluted with EtOAc (50 mL) and was washed with saturated solution of brine, the solvent was concentrated under reduced pressure and the product was purified bysilica gel columnchromatography (PE: EA= 6:1). The product 7 was obtained as solid (76 mg, yield: 83 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.01 (s, 1H), 7.93 (s, 1H), 7.54 (d, *J* = 8.1 Hz, 2H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.37 (s, 2H), 7.06 (d, *J* = 8.0 Hz, 1H), 6.45 (d, *J* = 16.2 Hz, 1H), 5.39 (s, 1H), 4.93 (s, 2H), 4.35 (d, *J* = 30.3 Hz, 1H), 3.79 (s, 3H), 3.29 (d, *J* = 13.3 Hz, 4H), 2.06 (s, 1H), 1.93 (d, *J* = 15.2 Hz, 5H), 1.86 – 1.61 (m, 10H), 1.45 (s, 9H), 0.97 (t, *J* = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.39, 167.00, 156.35, 153.64, 139.34, 138.80, 138.30, 138.02, 132.16, 131.48, 129.49, 127.61, 125.03, 119.82, 119.60, 80.33, 75.70, 57.09, 51.69, 40.98, 39.11, 38.95, 38.52, 36.98, 32.84, 29.61, 28.27, 24.73, 22.94, 21.79.

Synthesis of compound 8

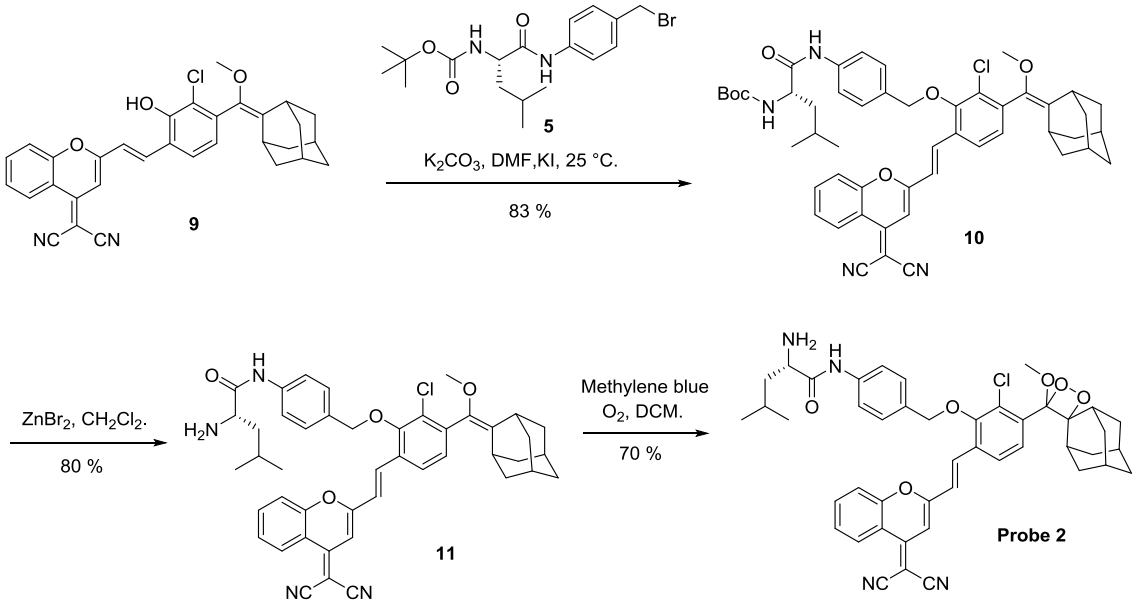
Compound 7 (110 mg 0.156 mmol) was dissolved in DCM (10 mL) and stirred for 10 min before $ZnBr_2$ (146 mg, 0.65 mmol) was added to the mixture. The reaction mixture was stirred for 6 h at room temperature with the reaction progress being monitored by TLC analysis. Upon completion, the reaction mixture diluted with DCM (50 mL) and was washed with saturated solution of brine, the solvent was concentrated under reduced pressure and the product was purified by silica gel columnchromatography (DCM: MeOH= 50:1). The product 8 was obtained as solid (85 mg, yield: 90 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.60 (s, 1H), 7.90 (d, *J* = 16.2 Hz, 1H), 7.61 (d, *J* = 8.3 Hz, 2H), 7.41 (dd, *J* = 8.1, 4.1 Hz, 3H), 7.06 (d, *J* = 8.0 Hz, 1H), 6.42 (d, *J* = 16.2 Hz, 1H), 4.97 (d, *J* = 4.5 Hz, 2H), 3.79 (s, 3H), 3.52 (s, 1H), 3.31 (s, 3H), 3.27 (s, 1H), 2.06 (s, 1H), 1.93 (d, *J* = 14.6 Hz, 5H), 1.87 – 1.76 (m, 8H), 1.73 (s, 2H), 1.03 – 0.88 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.71, 153.56, 138.82, 132.19, 131.24, 129.73, 129.65, 127.64, 125.00, 119.75, 119.13, 75.77, 57.12, 53.87, 51.70, 43.79, 39.10, 38.95, 38.53, 36.98, 32.85, 29.62, 28.13, 24.90, 23.34, 21.29.

Synthesis of probe 1

The mixture of compound 8 (91 mg, 0.15 mmol) and catalytic amountof methylene blue (2 mg) was dissolved in mixture solution of DCM (20 mL). Oxygen was bubbled through the solution while irradiating with white light (LED 150W) for 0.5 h. Upon completion, the reaction mixture diluted with

DCM (50 mL) and was washed with H_2O, the solvent was concentrated under reduced pressure and the product was purified by silica gel columnchromatography (DCM: MeOH=50:1). The probe 1 was obtained as solid (67 mg, yield: 70 %). MS (ESI+): m/z calculated for $C_{35}H_{44}O_7N_2Cl$: 639.2831; found: 639.2832. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.61 (s, 1H), 7.96 – 7.81 (m, 2H), 7.63 (d, *J* = 8.2 Hz, 2H), 7.56 (d, *J* = 8.3 Hz, 1H), 7.40 (d, *J* = 8.2 Hz, 2H), 6.47 (d, *J* = 16.2 Hz, 1H), 4.91 (s, 2H), 3.81 (s, 3H), 3.52 (d, *J* = 10.4 Hz, 1H), 3.22 (s, 3H), 3.02 (s, 1H), 2.01 (s, 1H), 1.76 (d, *J* = 24.0 Hz, 9H), 1.63 (d, *J* = 15.6 Hz, 3H), 1.48 (d, *J* = 10.4 Hz, 2H), 1.33 (s, 2H), 1.03 – 0.93 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.72, 154.06, 138.27, 131.59, 129.82, 128.79, 125.19, 120.97, 119.16, 96.27, 75.97, 53.88, 51.79, 49.63, 43.81, 36.51, 33.81, 33.51, 32.53, 32.15, 31.44, 26.10, 25.74, 24.93, 23.36, 21.28.

4. Synthesis and characterization of probe 2



Scheme S1. Synthetic route of **probe 2**

Synthesis of compound 10

Compound 9[2] (68 mg, 0.13 mmol), KI (216 mg, 1.3mmol) and K_2CO_3 (35 mg, 0.26 mmol) were dissolved in DMF (1 mL) and stirred was stirred for 10 min before compound 5 (52 mg, 0.13 mmol) was added to the mixture. The reaction mixture was stirred for 6 h at room temperature with the reaction progress being monitored by TLC analysis (PE: EA= 5:1). Upon completion, the reaction mixture diluted with EtOAc (50 mL) and was washed with saturated solution of brine, the solvent was concentrated under reduced pressure and the product was purified by silica gel columnchromatography (PE: EA= 6:1). The product 10 was obtained as orange solid (90.7 mg, yield: 83 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (d, *J* = 8.3 Hz, 1H), 8.62 (s, 1H), 7.80 (dd, *J* = 15.2, 6.2 Hz, 2H), 7.58 (d, *J* = 7.9

Hz, 2H), 7.47 (dt, *J* = 16.9, 9.8 Hz, 5H), 7.15 (d, *J* = 8.0 Hz, 1H), 6.84 (d, *J* = 16.0 Hz, 1H), 6.77 (s, 1H), 5.03 (s, 3H), 4.27 (s, 1H), 3.38 (s, 3H), 3.32 (s, 1H), 2.14 (s, 1H), 1.98 (d, *J* = 14.0 Hz, 5H), 1.89 – 1.73 (m, 10H), 1.47 (s, 9H), 1.00 (t, J = 6.4 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.91, 157.06, 153.94, 152.18, 139.34, 138.33, 134.97, 132.95, 132.78, 131.76, 129.61, 129.53, 127.91, 125.98, 125.65, 124.88, 120.44, 119.91, 118.69, 116.60, 107.31, 75.90, 63.02, 57.32, 38.56, 36.99, 32.96, 29.71, 28.26, 24.75, 22.98, 21.79.

Synthesis of compound 11

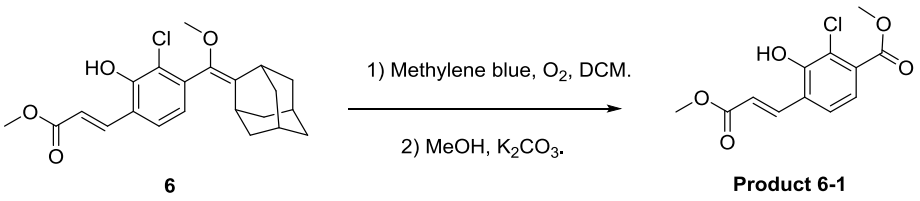
Compound 10 (110 mg, 0.13 mmol) was dissolved in DCM (10 mL) and stirred for 10 min before $ZnBr_2$ 146 mg, 0.65 mol) was added to the mixture. The reaction mixture was stirred for 6 h at room temperature with the reaction progress being monitored by TLC analysis. Upon completion, the reaction mixture diluted with DCM (50 mL) and was washed with saturated solution of brine, the solvent was concentrated under reduced pressure and the product was purified by silica gel columnchromatography (DCM: MeOH= 50:1). The product 11 was obtained as orange solid (77.5 mg, yield: 80 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.61 (s, 1H), 8.91 (d, *J* = 8.2 Hz, 1H), 7.80 (t, *J* = 7.6 Hz, 1H), 7.73 (d, *J* = 16.1 Hz, 1H), 7.63 (d, *J* = 8.2 Hz, 2H), 7.53 – 7.45 (m, 3H), 7.41 (d, *J* = 8.3 Hz, 2H), 7.15 (d, *J* = 8.0 Hz, 1H), 6.77 (d, *J* = 15.6 Hz, 2H), 5.06 (d, *J* = 6.5 Hz, 2H), 3.54 (s, 1H), 3.38 (s, 3H), 3.32 (s, 1H), 2.14 (s, 1H), 2.04 – 1.77 (m, 17H), 1.07 – 0.93 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.81, 152.18, 139.36, 138.31, 134.92, 133.11, 132.73, 131.36, 129.94, 129.85, 127.91, 125.96, 125.64, 124.69, 120.20, 119.31, 118.70, 116.66, 107.16, 76.13, 57.31, 53.80, 43.63, 39.17, 38.65, 37.00, 32.97, 29.71, 28.30, 28.16, 24.94, 23.35, 21.29.

Synthesis of probe 2

The mixture of compound 12 (111 mg, 0.15 mmol) and catalytic amount of methylene blue (2 mg) was dissolved in mixture solution of DCM (20 mL). Oxygen was bubbled through the solution while irradiating with white light (LED 150W) for 0.5 h at 0 ℃. Upon completion, the reaction mixture diluted with DCM (50 mL) and was washed with H_2O, the solvent was concentrated under reduced pressure and the product was purified by silica gel columnchromatography (DCM: MeOH= 50:1). The product probe 2 was obtained as orange solid (77.7 mg, yield: 70 %). MS (ESI+): m/z calculated for $C_{45}H_{45}O_6N_4Cl$: 773.31; found: 773.37. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.64 (s, 1H), 8.92 (d, *J* = 8.2 Hz, 1H), 7.99 (d, *J* = 8.2 Hz, 1H), 7.81 (s, 1H), 7.69 (d, *J* = 16.1 Hz, 1H), 7.62 (d, *J* = 8.2 Hz, 2H), 7.58 – 7.43 (m, 3H), 7.38 (d, *J* = 7.9 Hz, 2H), 6.83 (s, 1H), 6.79 (s, 1H), 4.99 (s, 2H), 3.58 (d, *J* = 8.6 Hz, 1H), 3.29 (s, 3H), 3.07 (s, 1H), 2.06 (s, 1H), 1.91 – 1.74 (m, 11H), 1.69 (s, 1H), 1.65 (s, 1H), 1.51 (d, *J* = 13.0 Hz, 1H), 1.45 (s, 1H), 1.39 (s, 2H), 1.01 (dd, *J* = 10.8, 5.2 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.67, 154.16, 134.97, 132.44, 130.89, 129.96, 129.04, 127.86, 126.05, 125.70, 124.85, 121.33, 119.24,

118.65, 115.48, 111.69, 107.48, 96.34, 76.95, 76.27, 53.80, 49.72, 43.76, 36.52, 33.87, 33.58, 32.61, 32.17, 31.49, 26.85, 26.10, 25.79, 24.96, 23.39, 21.21.

5. Synthesis and characterization of product 6-1

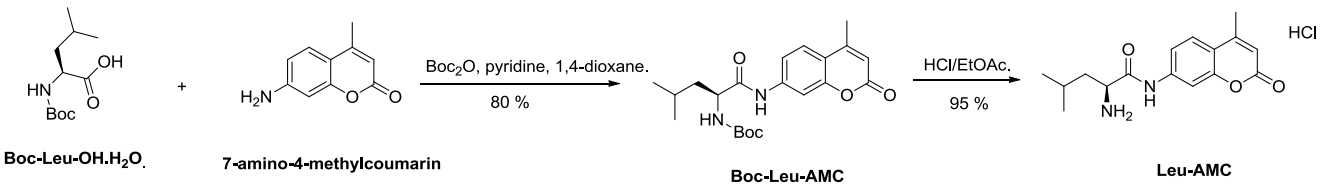


Scheme S2. Synthetic route of **product 6-1**

Synthesis of product 6-1

The mixture of compound 6 (0.1 mmol, 40 mg) and catalytic amount of methylene blue (1 mg) was dissolved in mixture solution of DCM (10 mL). Oxygen was bubbled through the solution while irradiating with white light (LED 150W) for 0.5 h at 0 ℃. Upon completion, the reaction mixture diluted with DCM (10 mL) and was washed with H_2O, the crude orange residue and K_2CO_3 (26 mg, 0.20 mmol) were dissolved in 5 mL of MeOH. The reaction mixture was stirred for 2 h and monitored by TLC. Upon completion, the mixture was diluted with DCM (50 mL) and washed with brine (10 mL). The organic layer was separated, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (PE: EA= 95:5) affording product 6-1 as a pale-yellow solid (10 mg, 37 % yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J* = 16.0 Hz, 1H), 7.51 – 7.46 (m, 2H), 6.66 (d, *J* = 16.0 Hz, 1H), 6.48 (s, 1H), 3.97 (s, 3H), 3.85 (s, 1H).

6. Synthesis and characterization of Leu-AMC



Scheme S3. Synthetic route of **Leu-AMC**

Synthesis of compound Boc-Leu-AMC

Boc-Leu-OH.H_2O (0.87 g, 3.5 mmol, 1 eq) and Boc_2O (0.938 g, 4.3 mmol, 1.25 eq) were dissolved in 1,4-dioxane (14 mL) at room temperature. Pyridine (0.27 mL, 3.4 mmol, 0.96 eq) and 7-amino-4-methylcoumarin (0.76 g, 4.3 mmol, 1.25 eq) were added and the reaction was stirred for 24 hours, after

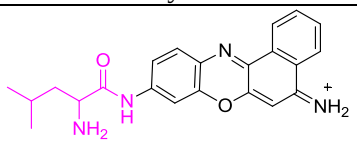
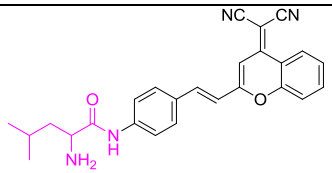
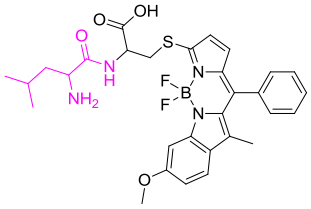
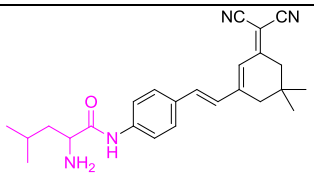
which it was diluted with EtOAc, washed with 10 % $KHSO_4$ (aq), water, saturated aqueous sodium bicarbonate, water and saturated aqueous sodium chloride. The organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (PE: EA= 4:1) affording Boc-Leu-AMC as a pale-yellow solid (1.33 g, 80 % yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.45 (s, 1H), 7.75 (s, 1H), 7.37 (d, *J* = 7.9 Hz, 1H), 7.02 (d, *J* = 8.6 Hz, 1H), 6.08 (s, 1H), 5.28 (s, 1H), 4.41 (s, 1H), 2.36 (s, 3H), 1.80 (d, *J* = 13.3 Hz, 1H), 1.67 (d, *J* = 14.2 Hz, 2H), 1.50 (s, 9H), 1.06 – 0.88 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.95, 160.98, 153.75, 152.38, 141.29, 124.66, 115.42, 115.16, 112.96, 107.01, 80.85, 40.53, 28.37, 24.71, 23.09, 21.30, 18.35.

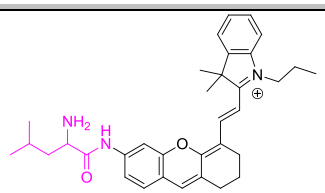
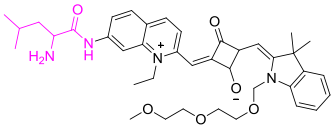
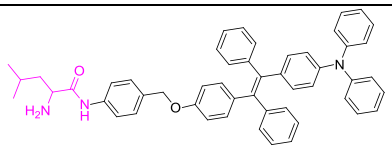
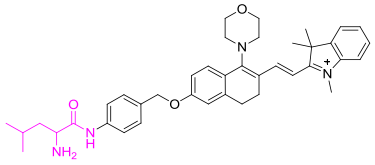
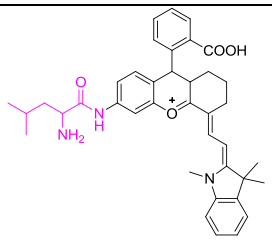
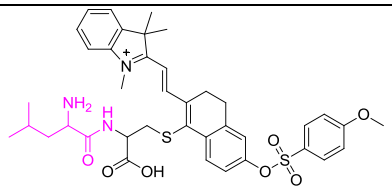
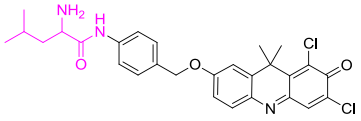
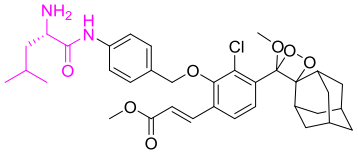
Synthesis of compound Leu-AMC

The Boc-Leu-AMC (100 mg, 0.26 mmol) was dissolved in 4 M HCl/EtOAc (2 mL) at room temperature. The reaction mixture was stirred for 4 h, upon which it was deemed complete by TLC (PE: EA= 4:1), filtering to gave Leu-AMC (80 mg, 95 %) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (s, 1H), 8.53 (s, 3H), 7.85 (s, 1H), 7.74 (d, *J* = 8.2 Hz, 1H), 7.62 (d, *J* = 8.0 Hz, 1H), 6.28 (s, 1H), 4.16 (s, 1H), 2.40 (s, 3H), 1.72 (s, 3H), 0.93 (s, 6H).^{13}C NMR (101 MHz, DMSO-d_6) δ 169.11, 160.28, 153.88, 153.42, 141.93 , 126.43, 115.92, 113.05, 106.50, 52.21, 24.14, 23.09, 22.58, 18.41.

7. Experimental Graphs of probe 1 and 2

Table. S1. Comparision of LAP dection result between report probers and **probe 1** in this work.

Entry	λ em (nm)	LOD	Type	Ref
	625	0.42 ng/mL	FL [a]	3
	660	46 ng/mL	FL [a]	4
	578	41.9 ng/mL	FL [a]	5
	658	0.38 ng/mL	FL [a]	6

	705	10.5 ng/mL	FL [a]	7
	710	0.61 ng/mL	FL [a]	8
	505	8.87 ng/mL	FL [a]	9
	607	50 ng/mL	FL [a]	10
	703	0.08 U/L	FL [a]	11
	620	42.2 ng/mL	FL [a]	12
	660	0.2 U/L	FL [a]	13
 Probe 1	550	0.008 U/L or 0.4 ng/mL	CL [b]	**This work**

[a].Fluorescence. [b].Chemiluminescence.

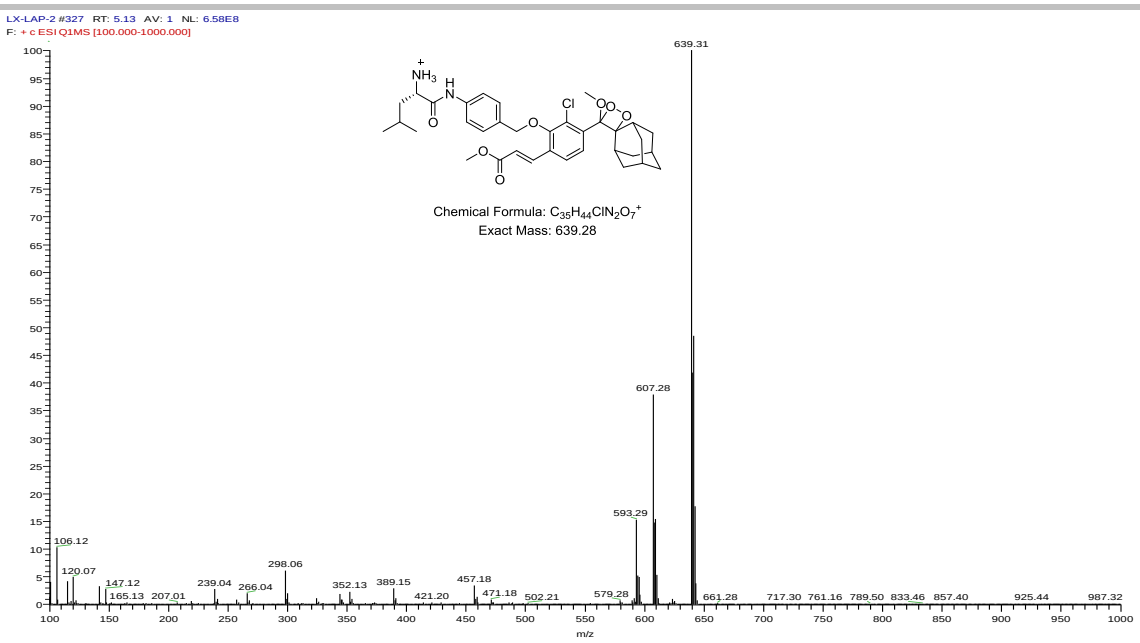


Figure. S1. LC-MS analysis of **probe 1** (10 μM) before (TR = 5.13 min) incubated with LAP (100 U/L) at 37 ^{o}C for 1 h.

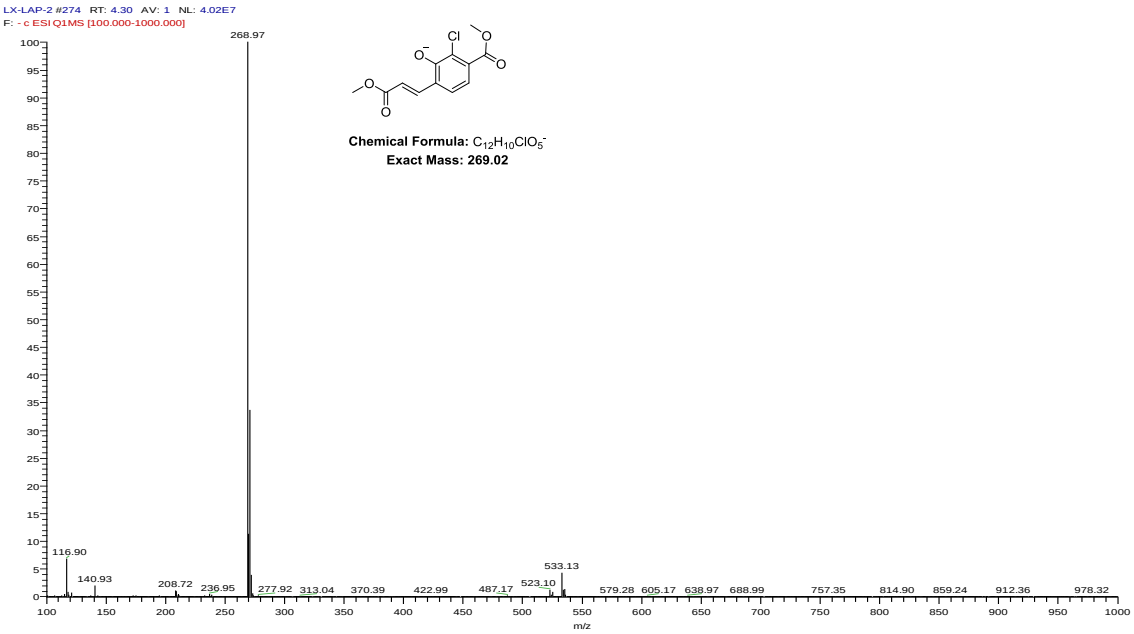


Figure. S2. LC-MS analysis of **probe 1** (10 μM) after (TR = 4.30 min) incubated with LAP (100 U/L) at 37 ^{o}C for 1 h.

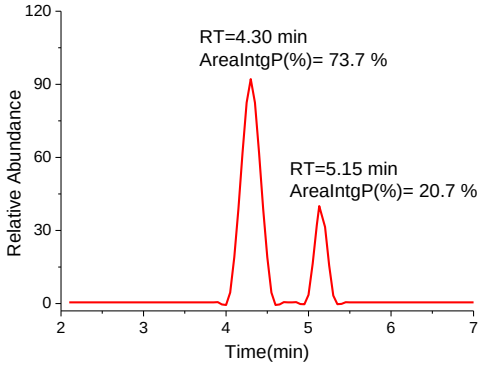


Figure. S3. The HPLC analysis of **probe 1** (10 μM) after incubated with LAP (100 U/L) at 37 °C for 1 h (TR = 4.30 min, 73.7%; TR = 5.15 min, 20.7 %).

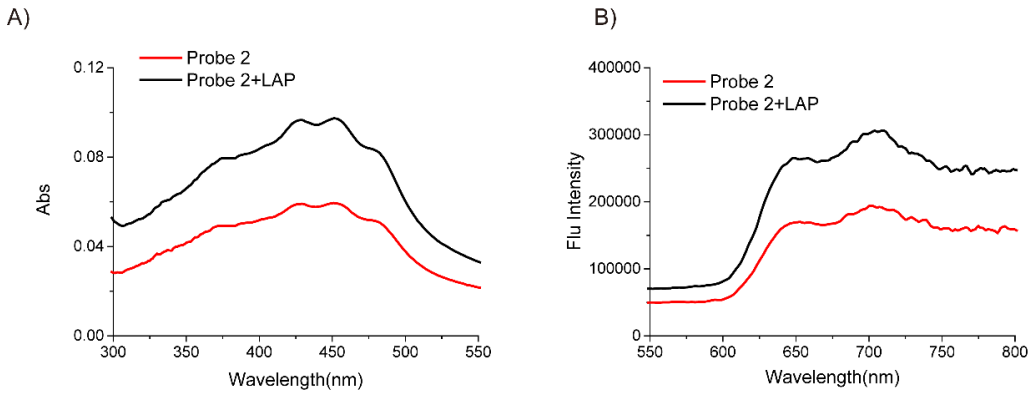


Figure. S4. The absorption (A) and fluorescence (B) emission spectra of **probe 2**. The **probe 2** (10 μM) incubated with or without LAP (100 U/L) in enzyme assay buffer at 37 °C for 6 h.

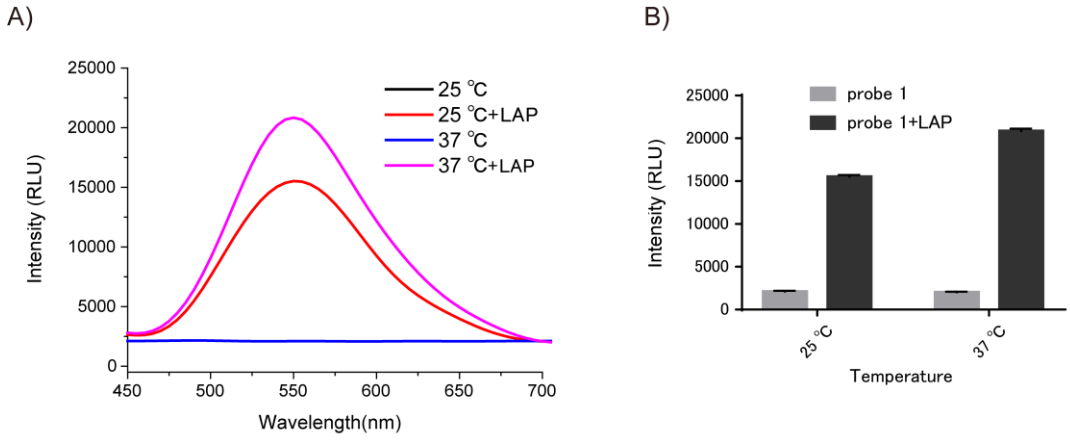


Figure. S5. (A) The chemiluminescence spectra of **probe 1** (10 μM) incubated with or without LAP (100 U/L) at 25 and 37 °C for 10 min. (B) Chemiluminescence quantification of **probe 1** (10 μM) incubated with LAP (100 U/L) at 25 and 37 °C. Data presented are mean ± SD (n=3).

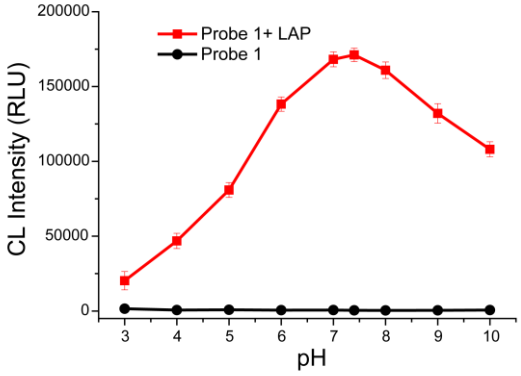


Figure. S6. The chemiluminescence intensity changes of **probe 1** upon incubation with or without LAP (100 U/L) for 10 min in enzyme reaction buffer under different pH values. Data presented are mean ± SD (n=3).

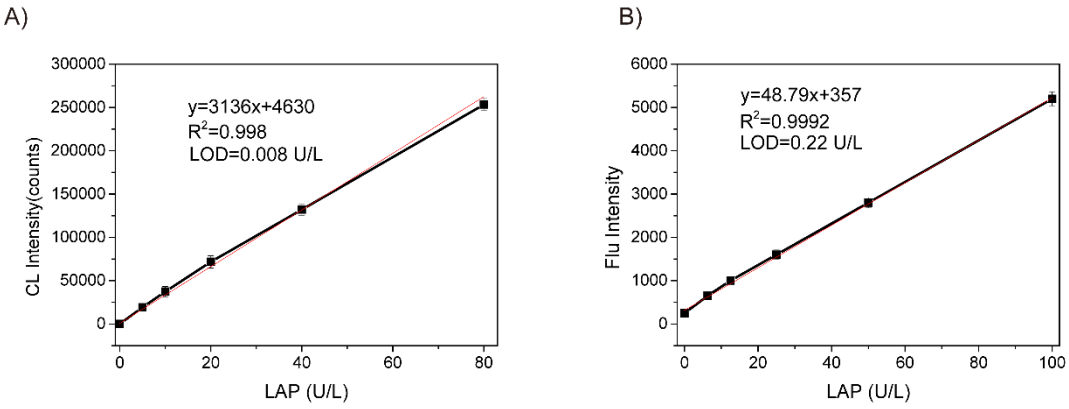


Figure. S7. (A) Linear fitting curve of mean chemiluminescence (CL) intensity from (Figure 4B) versus the LAP concentration in the range of 0 to 80 U/L. (B) Linear fitting curve of mean fluorescence (Flu) intensity from **Leu-AMC** versus the LAP concentration in the range of 0 to 100 U/L. Data presented are mean ± SD (n=3).

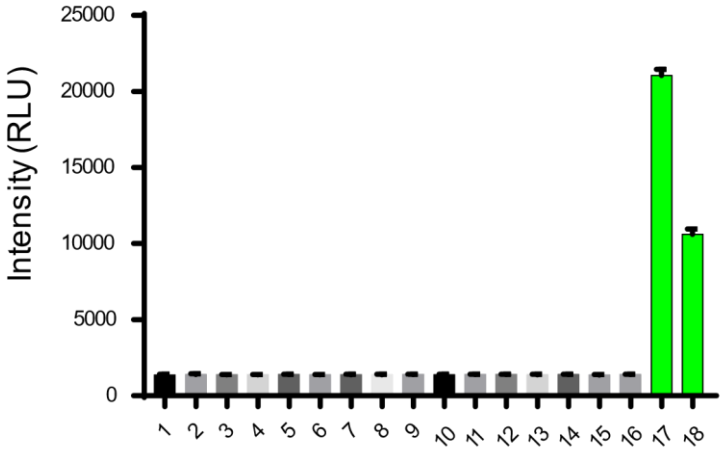


Figure. S8. The chemiluminescence intensity of **probe 1** (10 μM) incubated with various potential interfering substances: (1) control (only probe 1, 10 μM); (2) Hcy (100 μM); (3) L-Cys (100 μM); (4) Glu (100 μM); (5) KCl (100 μM); (6) Na_2S (100 μM); (7) $Al_2(SO_4)_3$ (100 μM); (8) $CuSO_4$ (100 μM); (9) $ZnCl_2$ (100 μM); (10) $FeSO_4$ (100 μM); (11) $MgSO_4$ (100 μM); (12) $CaCl_2$ (100 μM); (13) *β*-galactosidase (100 U/L); (14) cathepsin B (100 U/L); (15) alkaline phosphatase (100 U/L); (16) pyroglutamyl aminopeptidase I (100 U/L); (17) LAP(100 U/L); and (18) LAP together with Ube (40 μM) in enzyme assay buffer at 37 °C for 10 min. The chemiluminescence intensity were acquired on the microplate reader. Data presented are mean ± SD (n=3).

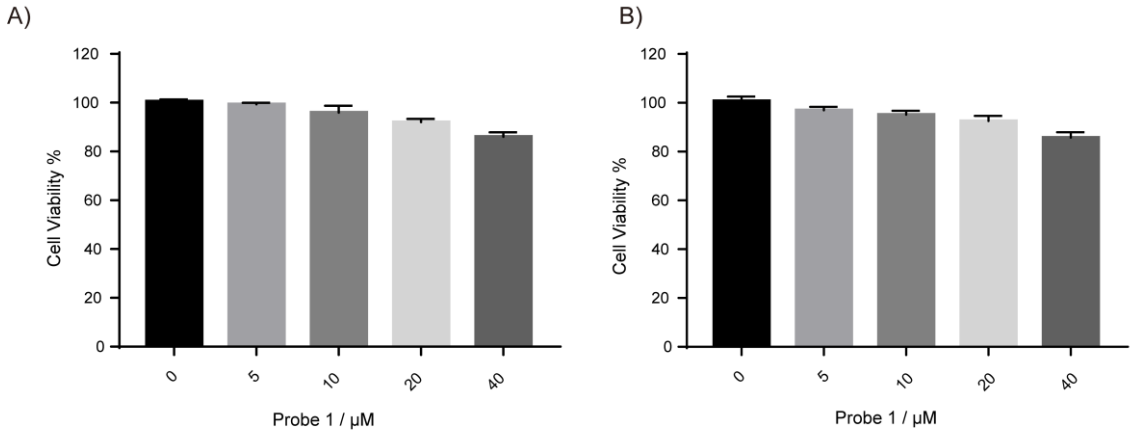


Figure. S9. Cell viabilities of LO2 (A) and HepG2 (B) cells treated with varying concentrations (0, 5, 10, 20, 40 μM) of **probe 1** for 24 h. Data presented are mean ± SD (n=3).

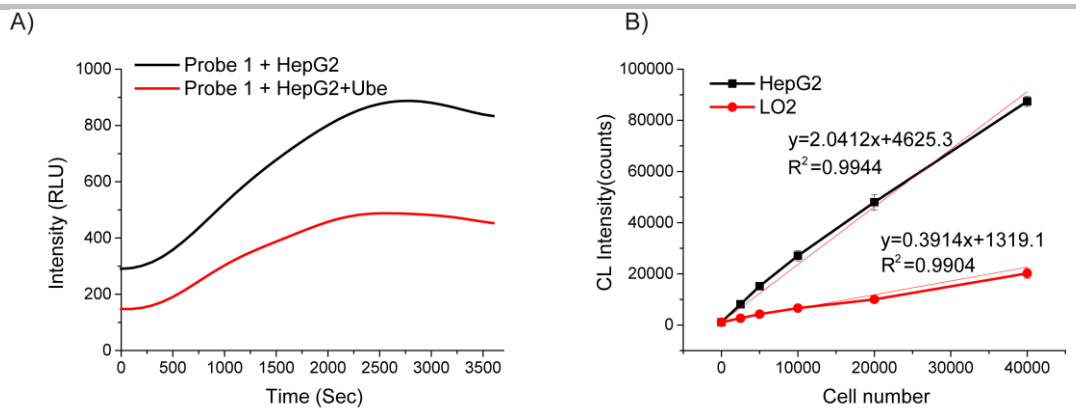


Figure. S10. (A) Real-time measurement of chemiluminescence intensity in indicated live cells (~4×10^4/well) upon incubated with **probe 1** (10 μM). HepG2 Values are expressed as means ± SD (n= 3). The chemiluminescence intensities were acquired using a microplate reader. (B) Linear fitting curve of mean chemiluminescence intensity versus HepG2 and LO2 cell numbers as indicated in (Figure 5A). Data presented are mean ± SD (n=3).

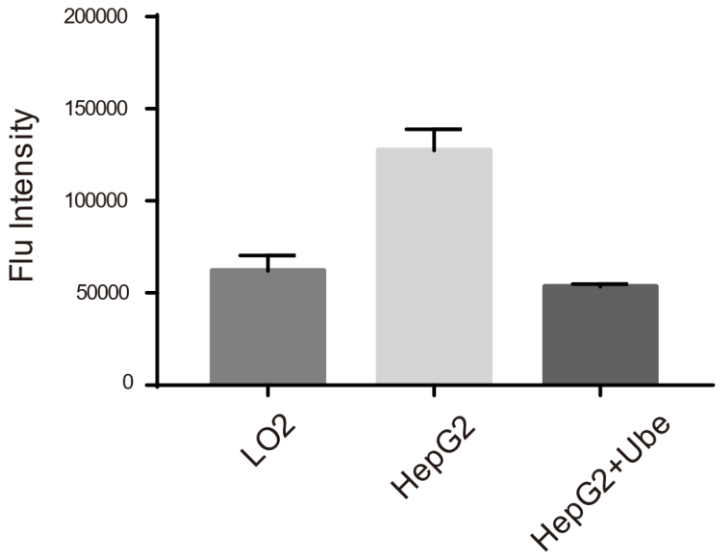


Figure. S11. Quantification of part (Figure 6). Data presented are mean ± SD (n=3).

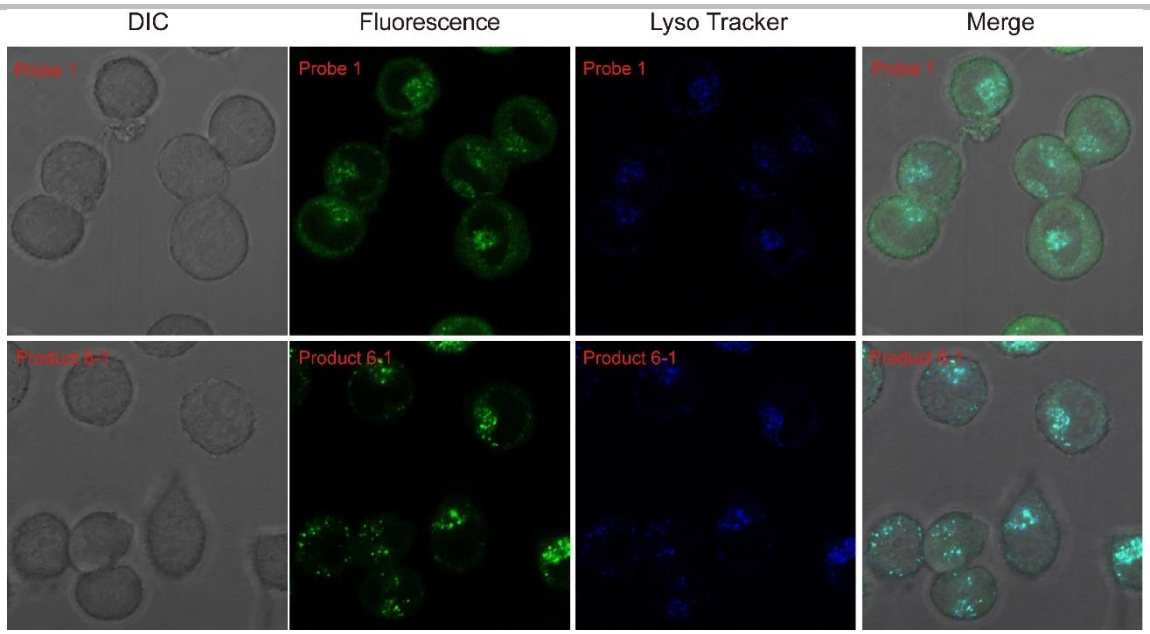


Figure. S12. Fluorescence images of HepG2 cells treated with **probe 1** and **product 6-1** following staining with Lyso Tracker Green. Cells were incubated with probe 1 (10 μM) for 1 h and then costained with 10 μg/mL Lyso Tracker Green for 20 min. Scale bar: 63 μm.

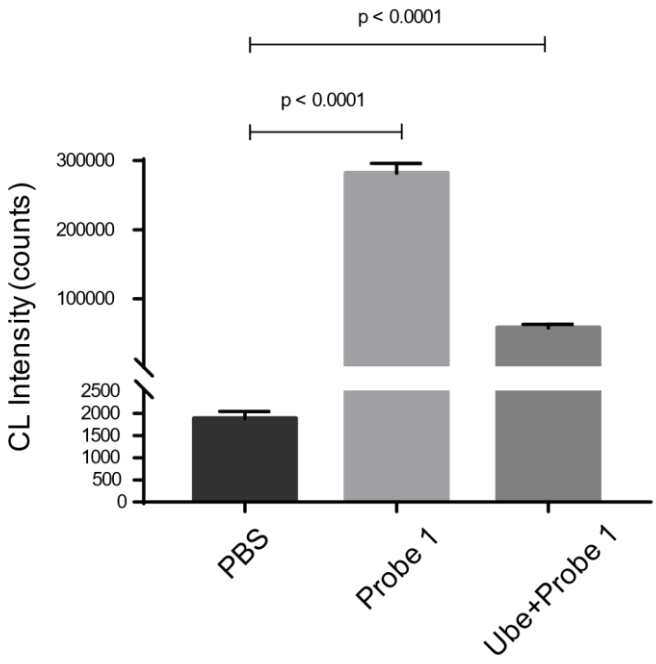


Figure. S13. Quantification of part (Figure. 5C). Data presented are mean ± SD (n=3, $p < 0.0001$).

8. NMR spectra

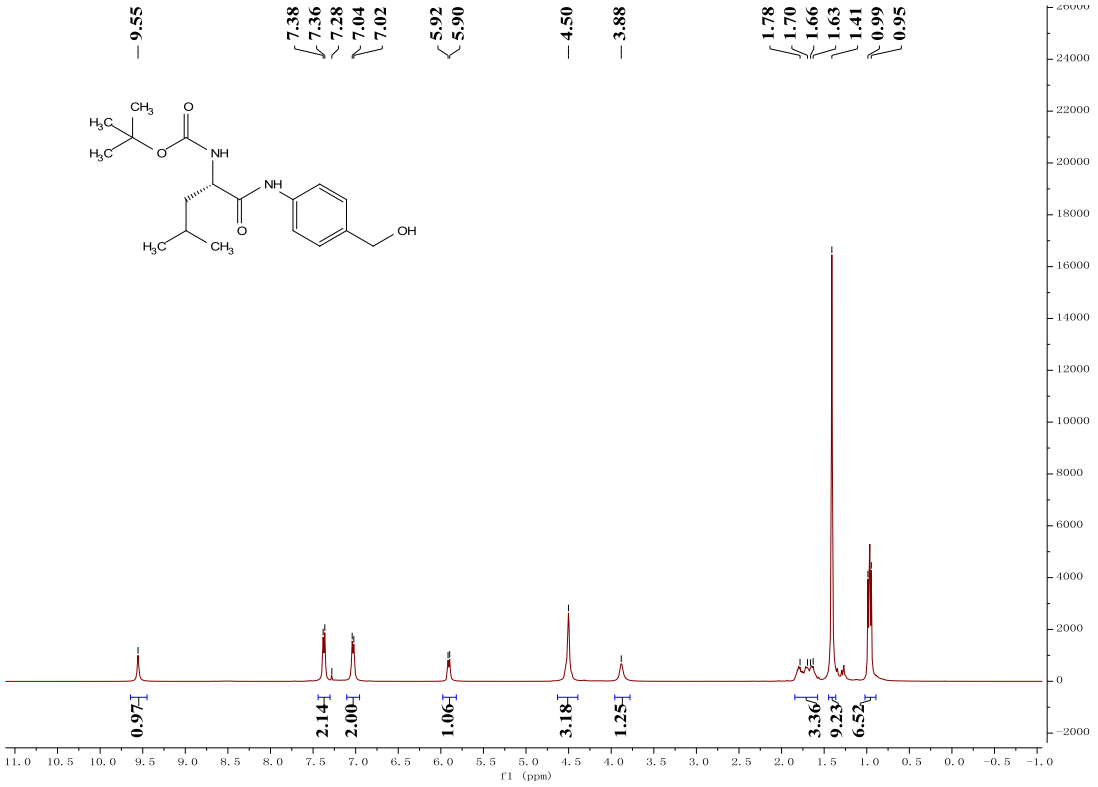


Figure. S14. ^{1}H NMR (400 MHz) spectrum of **OH-LAP** in $CDCl_3$

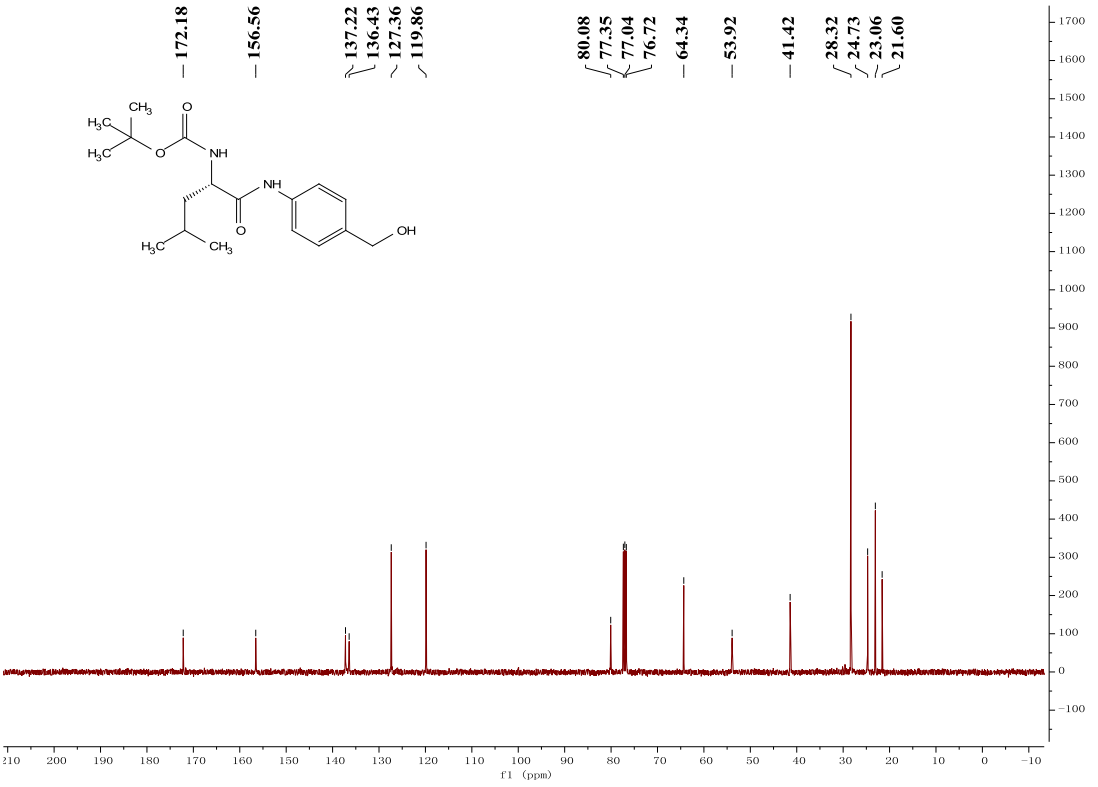


Figure. S15. ^{13}C NMR (101MHz) spectrum of **OH-LAP** in $CDCl_3$

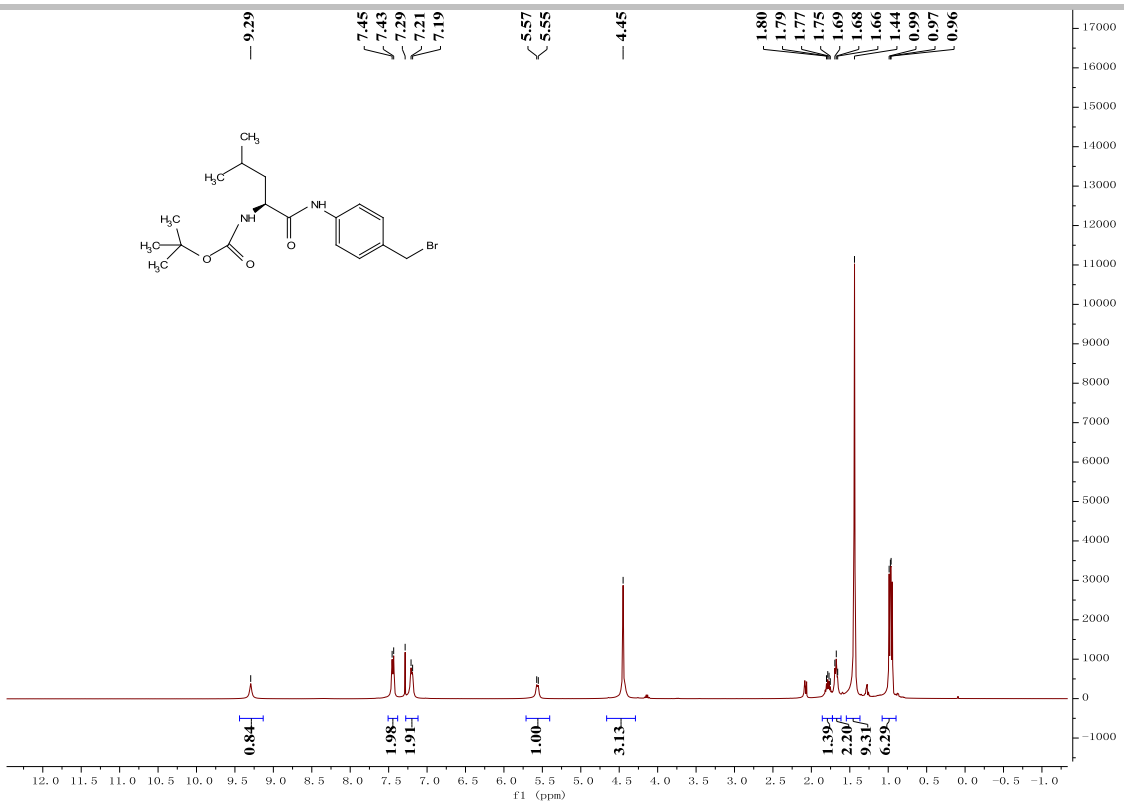


Figure. S16. ^{1}H NMR (400 MHz) spectrum of **compound 5** in $CDCl_3$

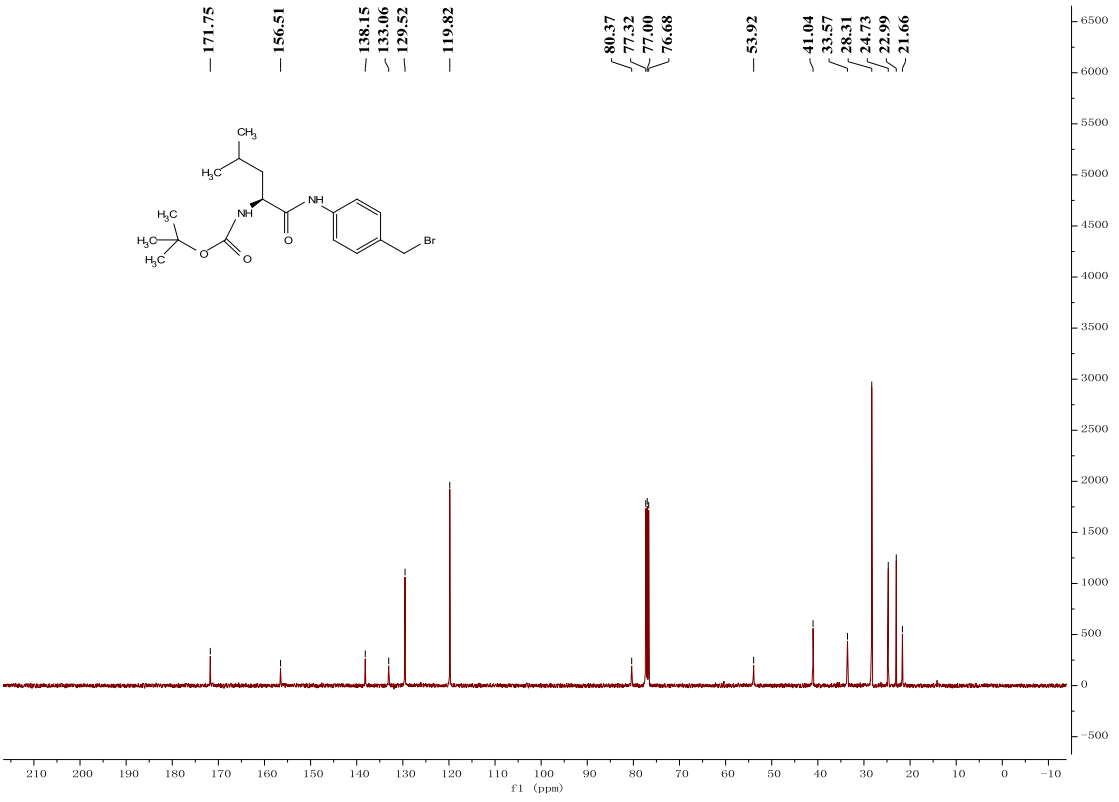


Figure. S17. ^{13}C NMR (101MHz) spectrum of **compound 5** in $CDCl_3$

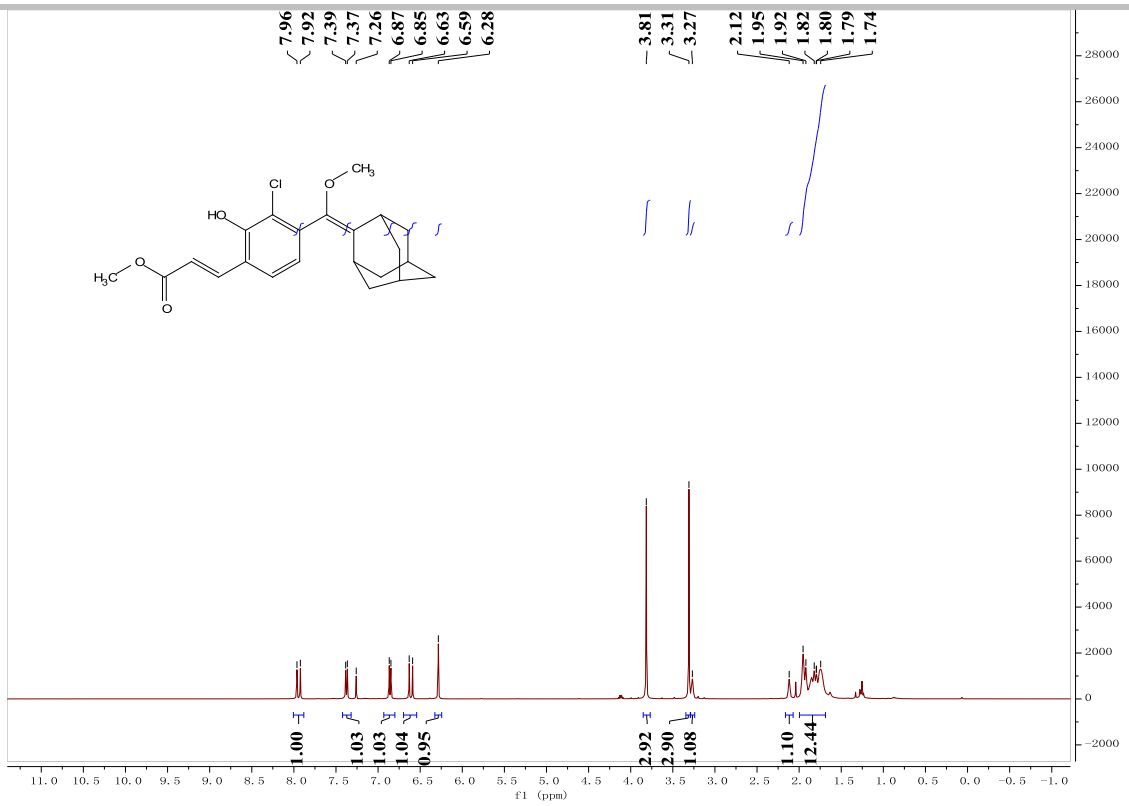


Figure. S18. ^{1}H NMR (400 MHz) spectrum of **compound 6** in $CDCl_3$

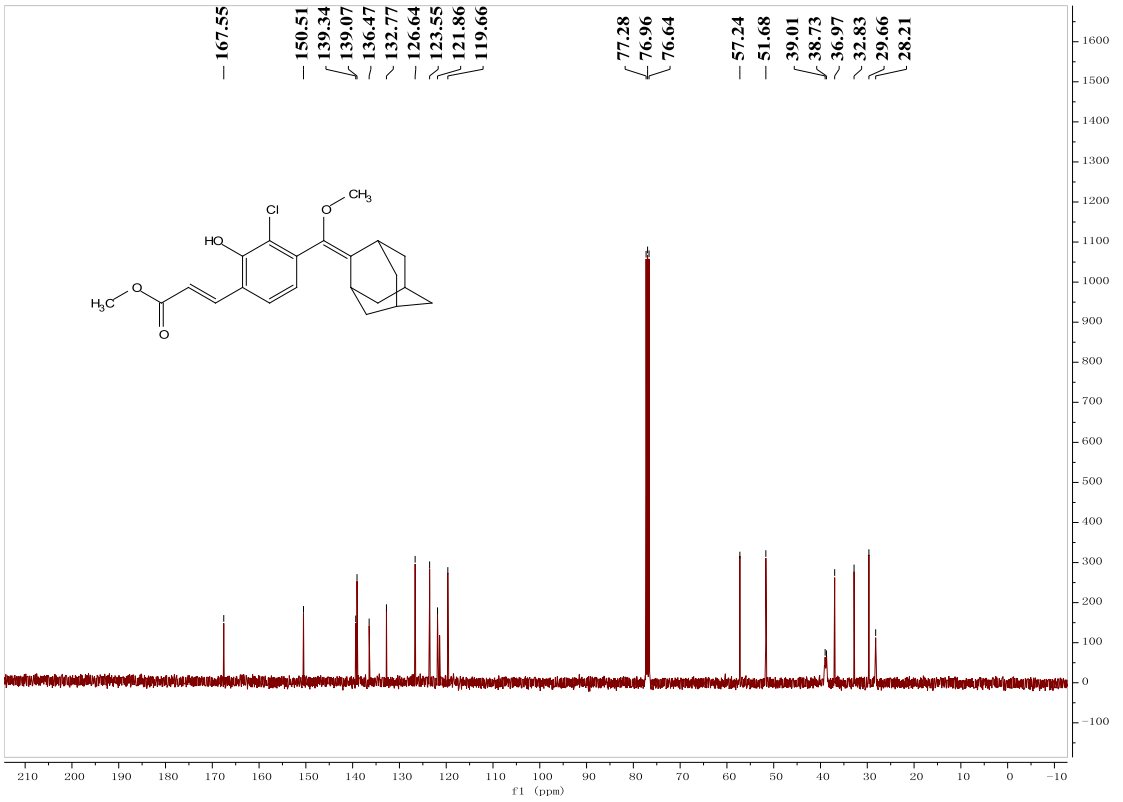


Figure. S19. ^{13}C NMR (101MHz) spectrum of **compound 6** in $CDCl_3$

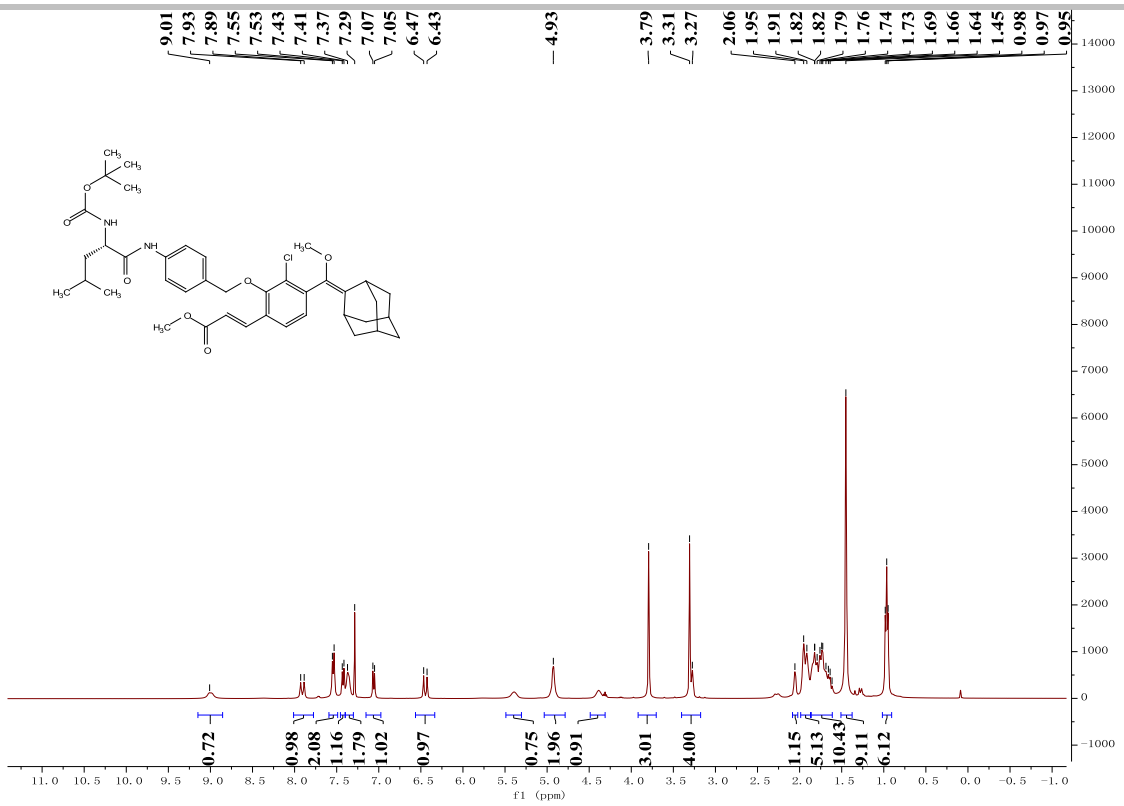


Figure. S20. ^{1}H NMR (400 MHz) spectrum of **compound 7** in $CDCl_3$

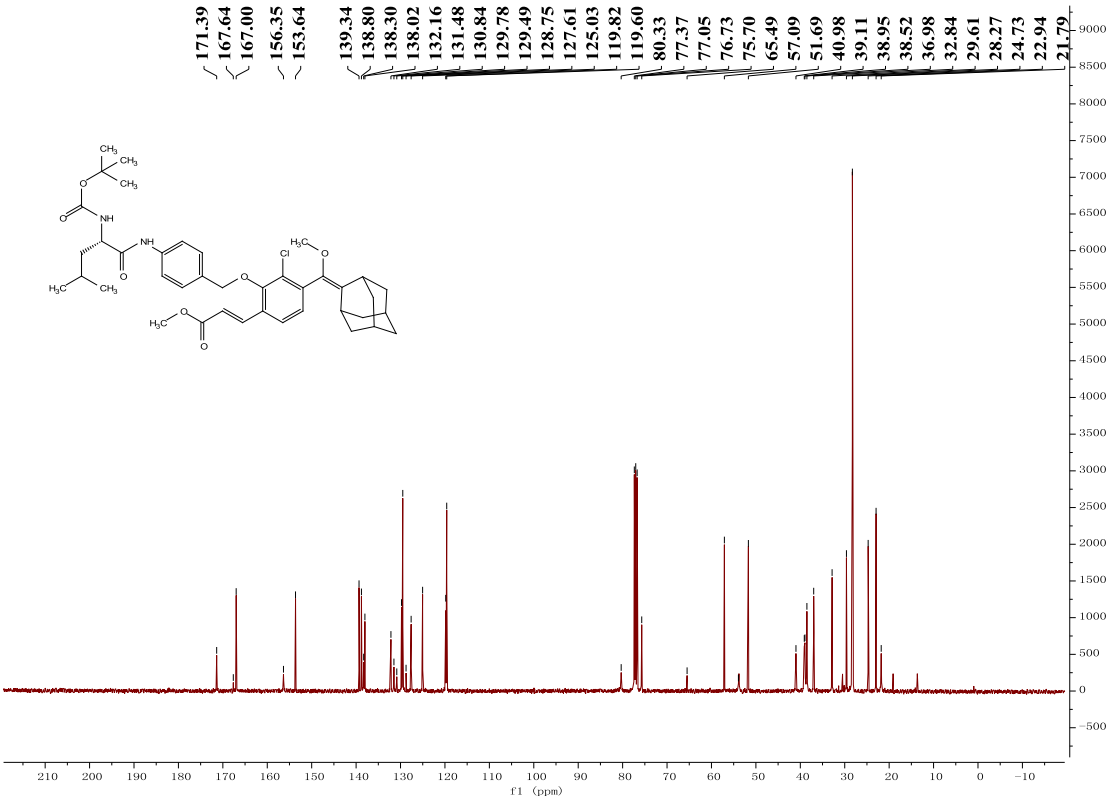


Figure. S21. ^{13}C NMR (101 MHz) spectrum of **compound 7** in $CDCl_3$

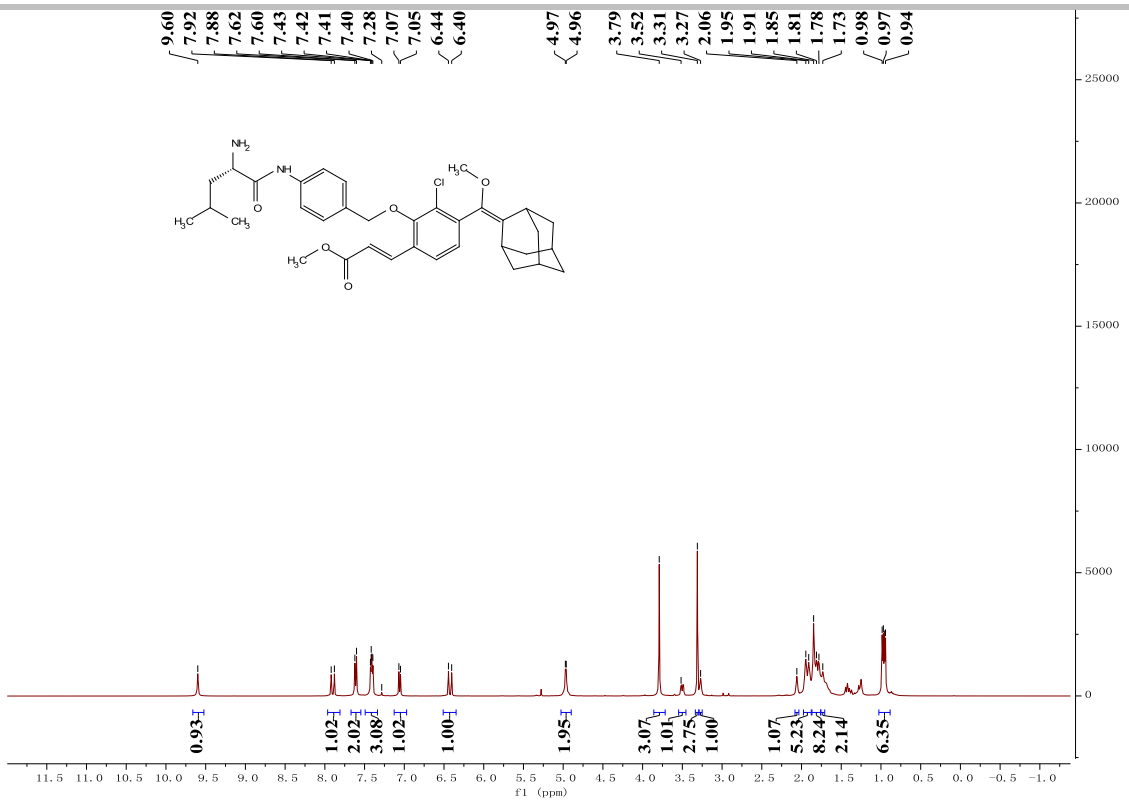


Figure. S22. ^{1}H NMR (400 MHz) spectrum of **compound 8** in $CDCl_3$

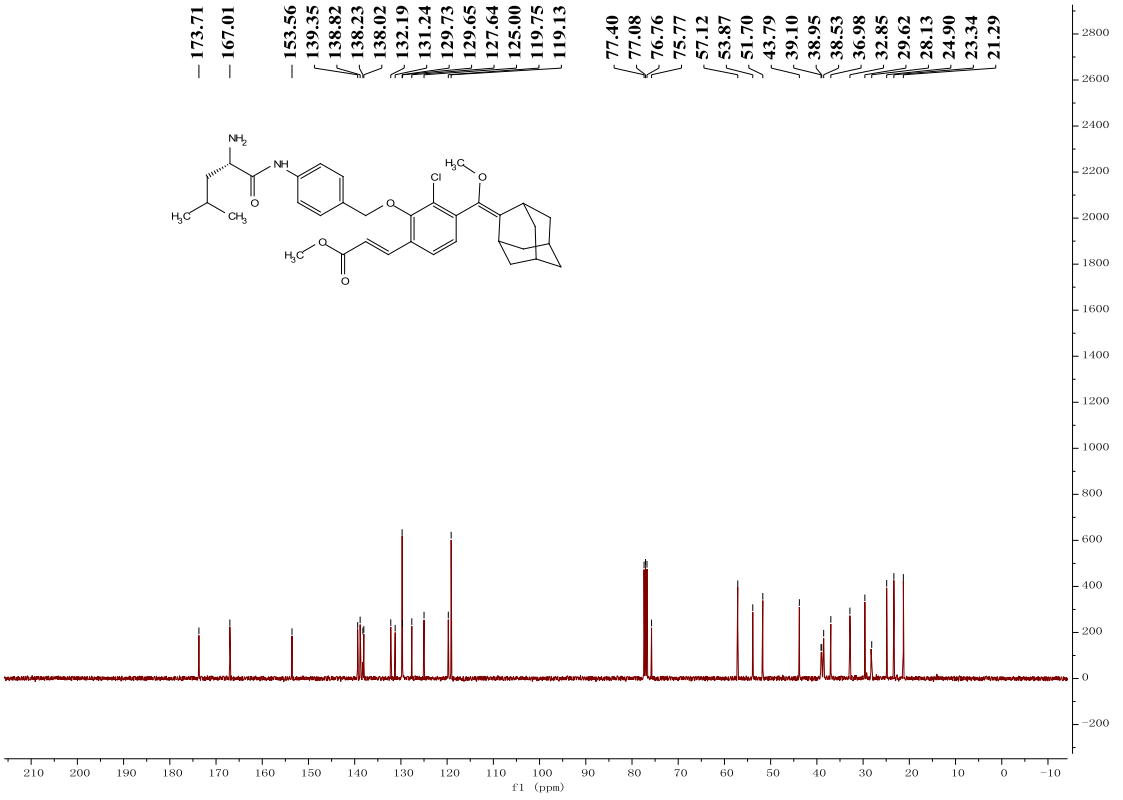


Figure. S23. ^{13}C NMR (101 MHz) spectrum of **compound 8** in $CDCl_3$

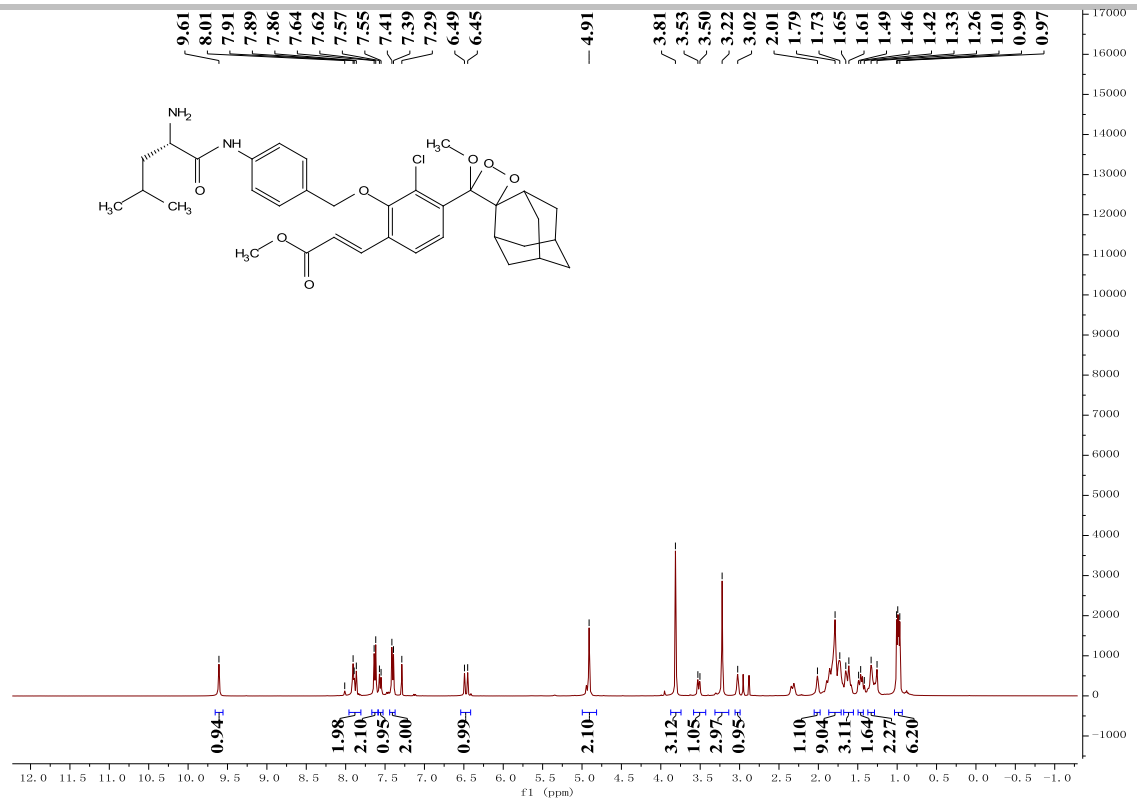


Figure. S24. ^{1}H NMR (400 MHz) spectrum of **probe 1** in $CDCl_3$

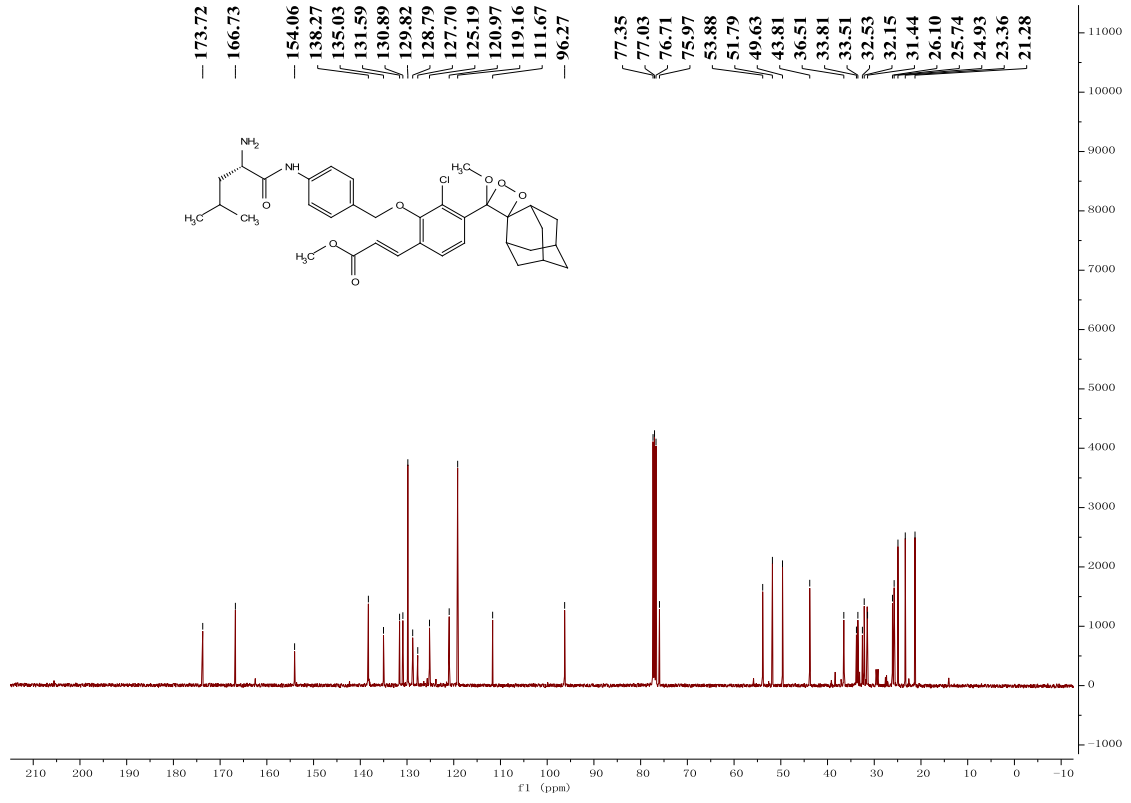


Figure. S25. ^{13}C NMR (101 MHz) spectrum of **probe 1** in $CDCl_3$

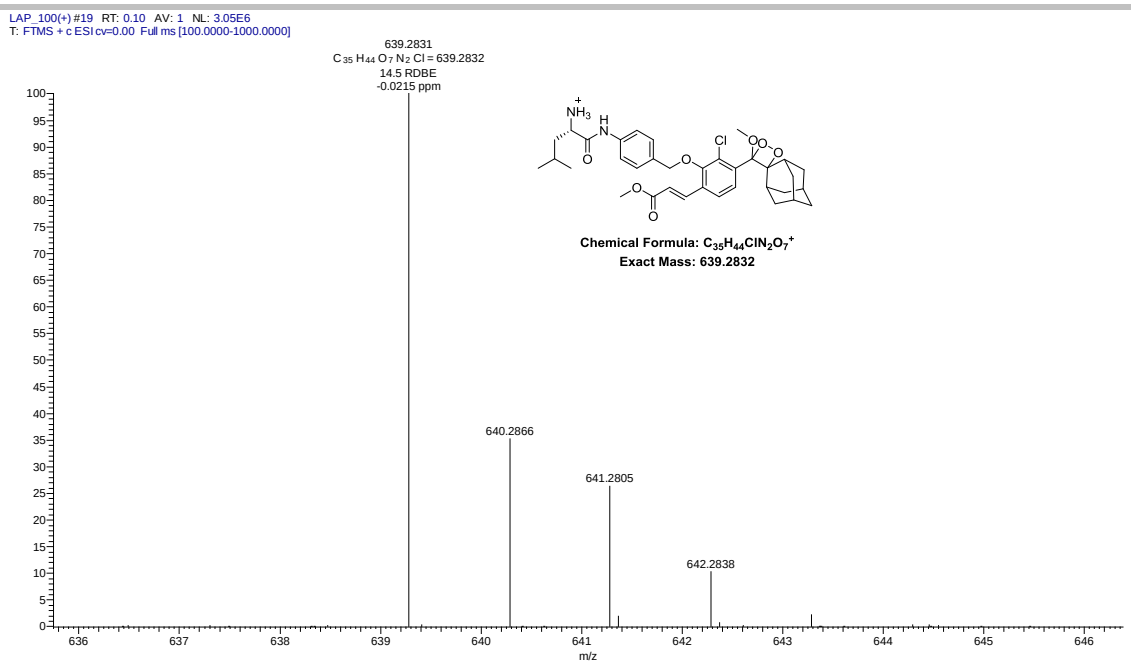


Figure. S26. HRMS spectrum of **probe 1** in MeOH

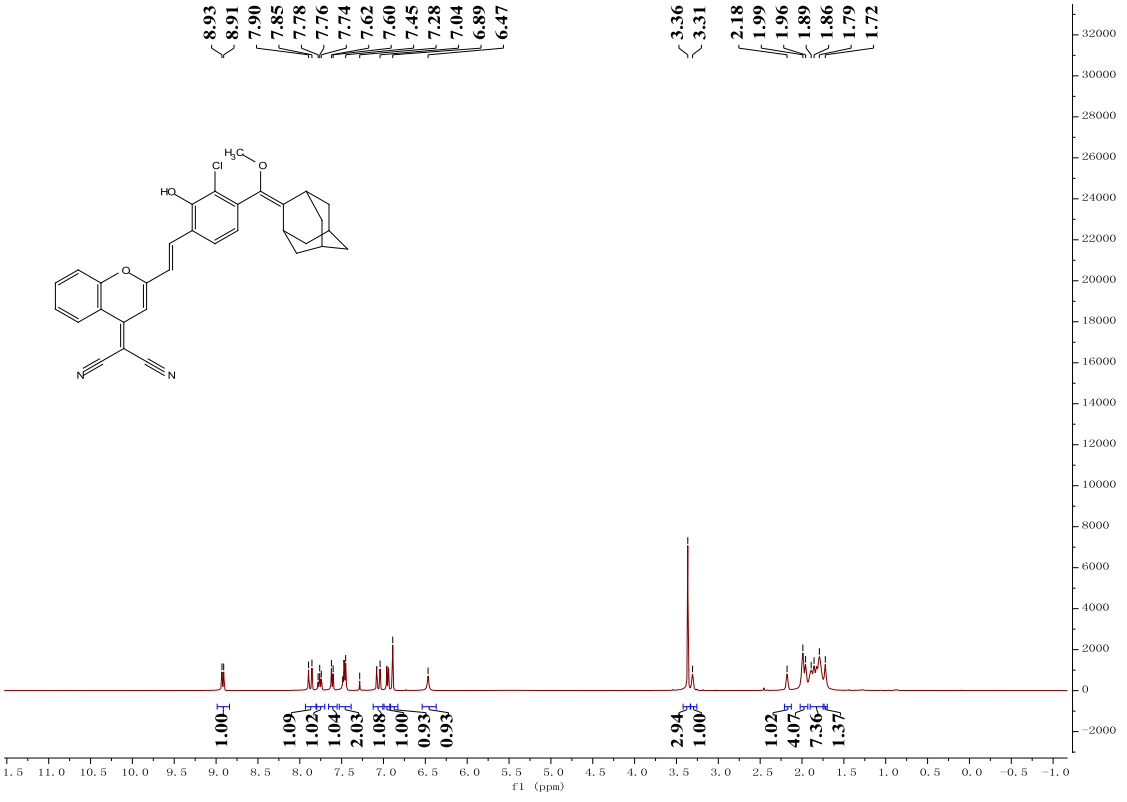


Figure. S27. ^{1}H NMR (400 MHz) spectrum of **compound 9** in $CDCl_3$

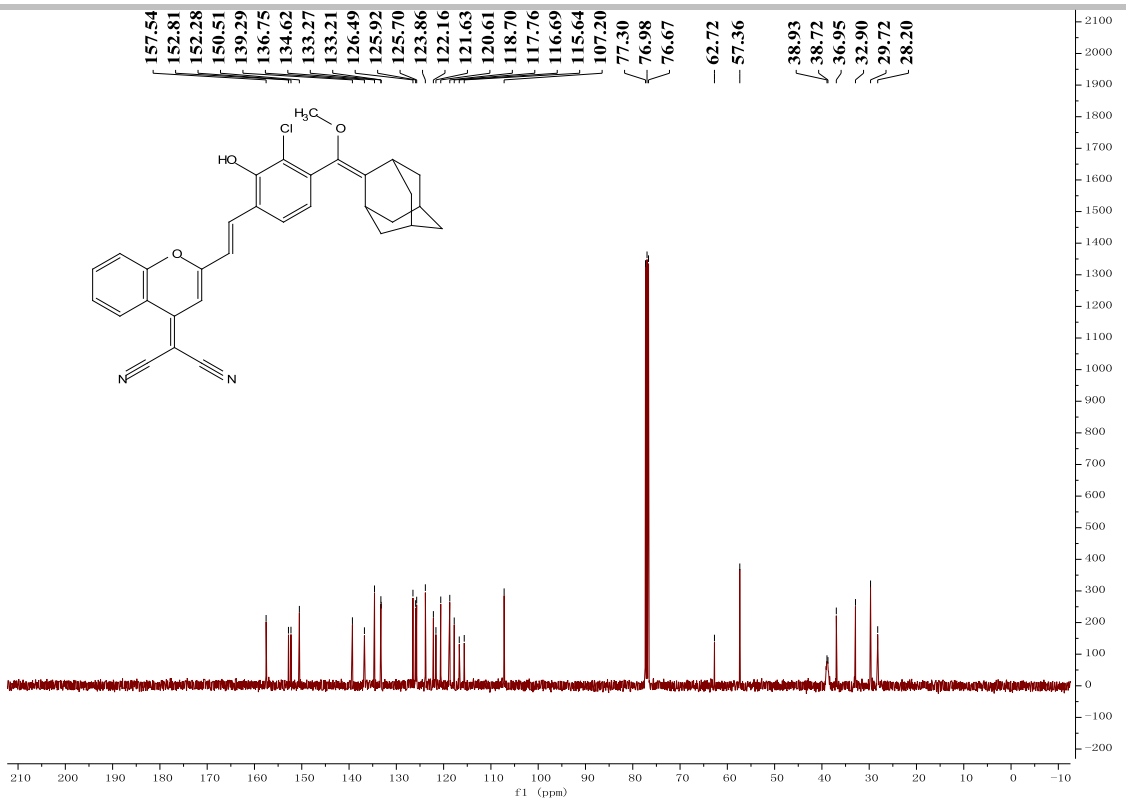


Figure. S28. ^{13}C NMR (101 MHz) spectrum of **compound 9** in $CDCl_3$

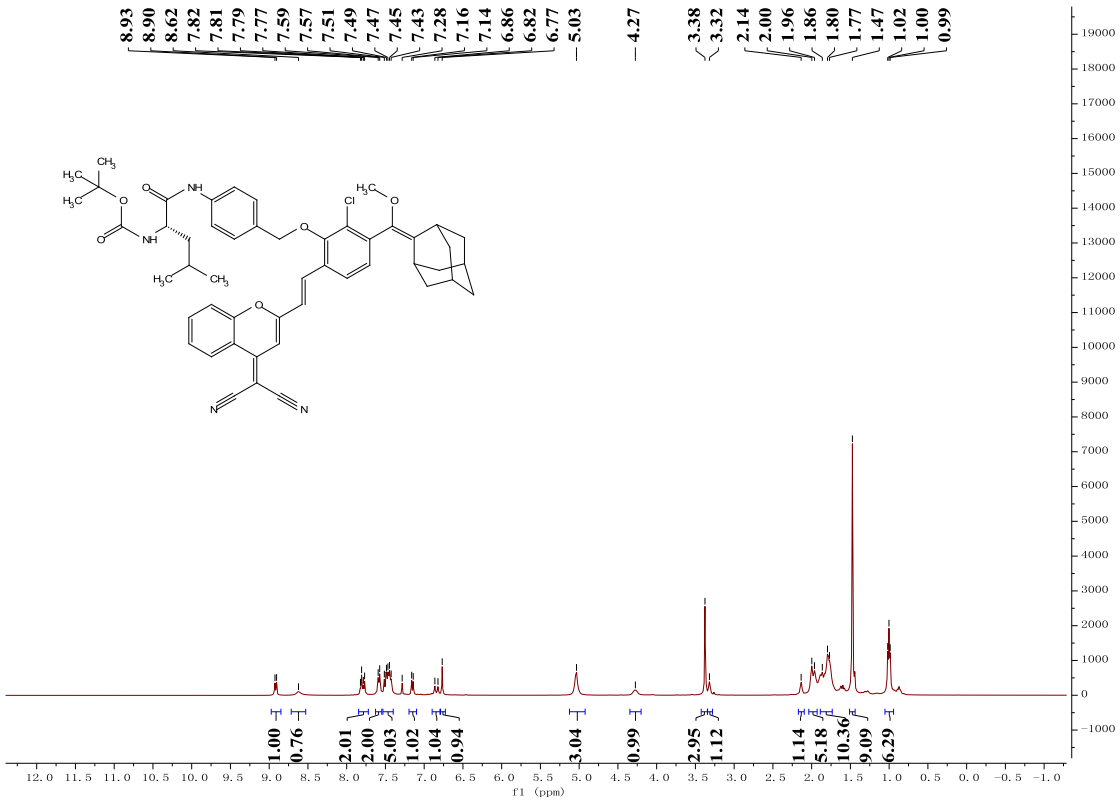


Figure. S29. ^{1}H NMR (400 MHz) spectrum of **compound 10** in $CDCl_3$

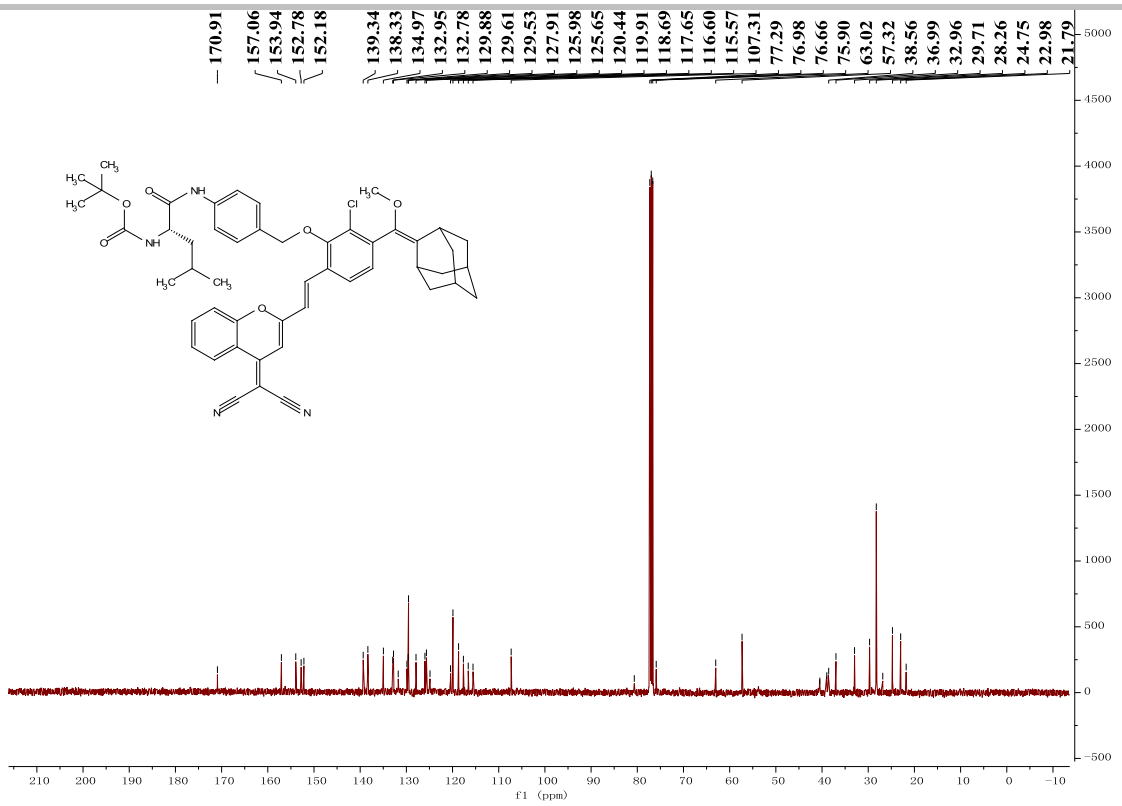


Figure. S30. ^{13}C NMR (101 MHz) spectrum of **compound 10** in $CDCl_3$

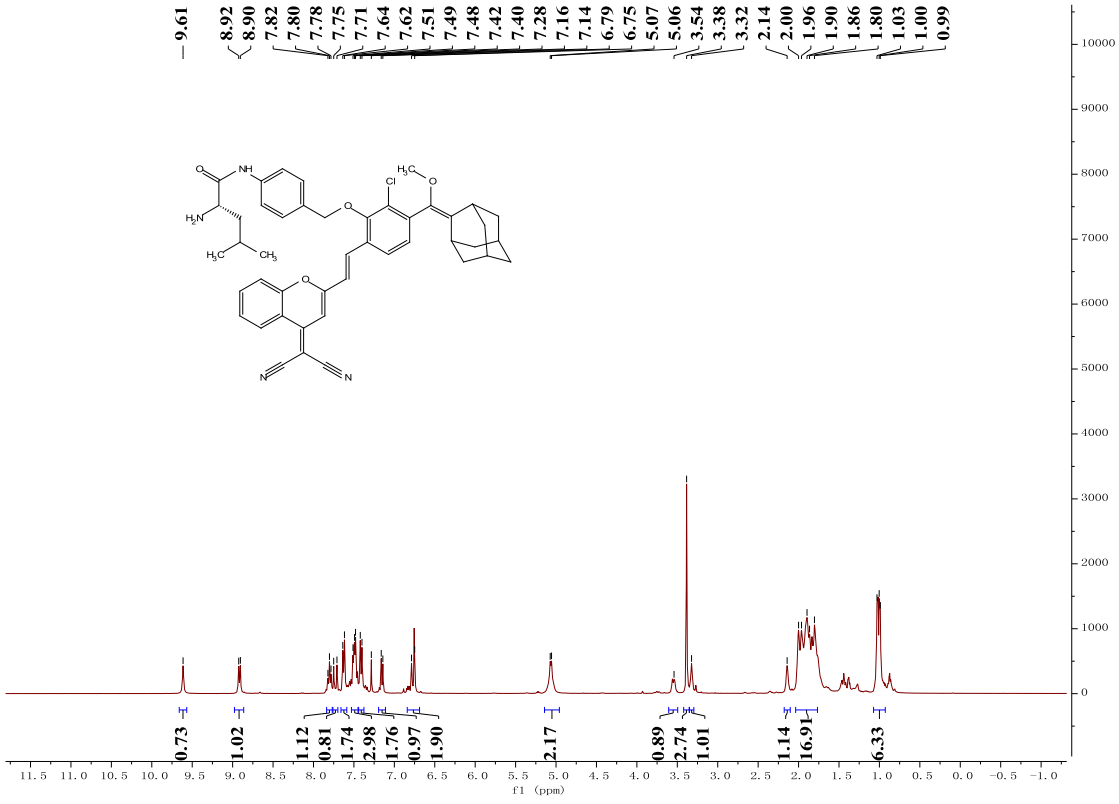


Figure. S31. ^{1}H NMR (400 MHz) spectrum of **compound 11** in $CDCl_3$

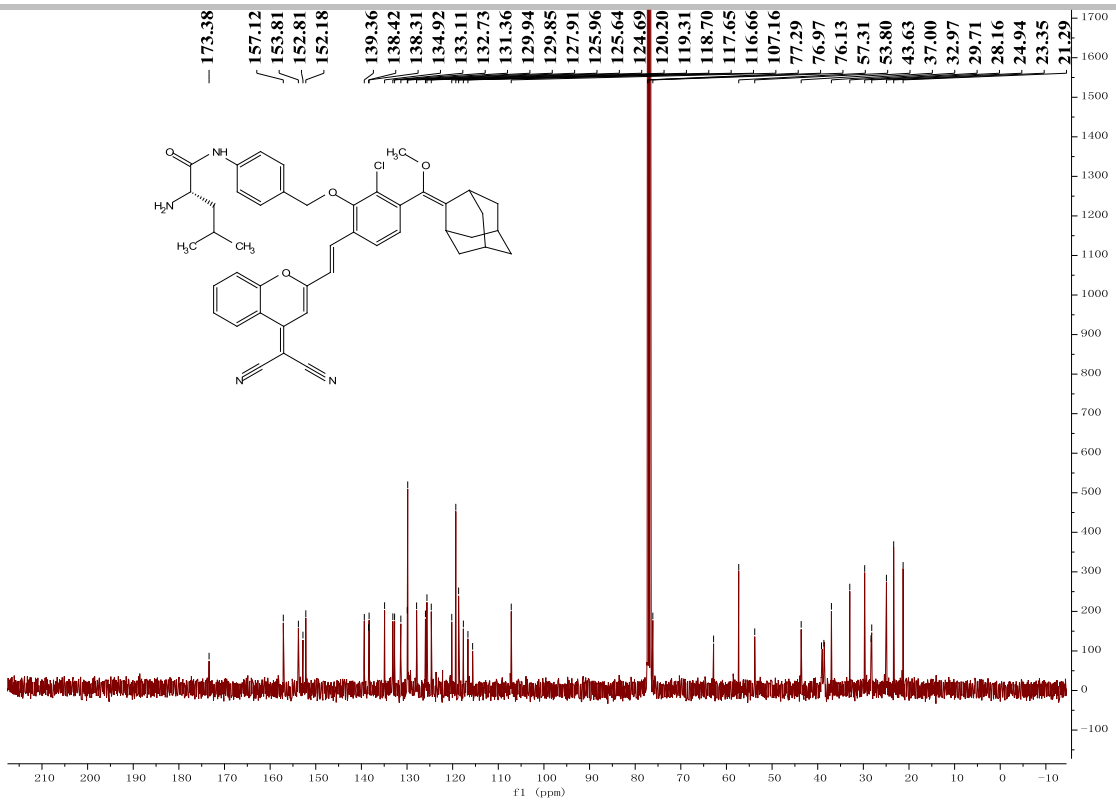


Figure. S32. ^{13}C NMR (101 MHz) spectrum of **compound 11** in $CDCl_3$

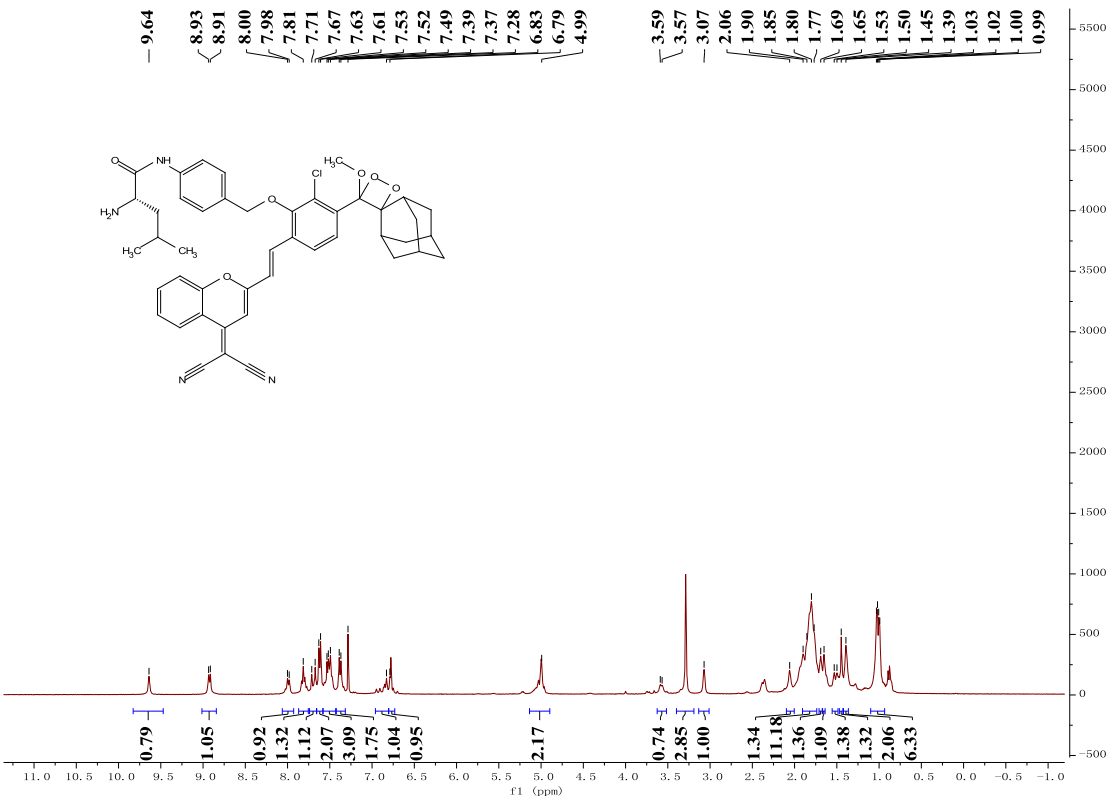


Figure. S33. ^{1}H NMR (400 MHz) spectrum of **probe 2** in $CDCl_3$

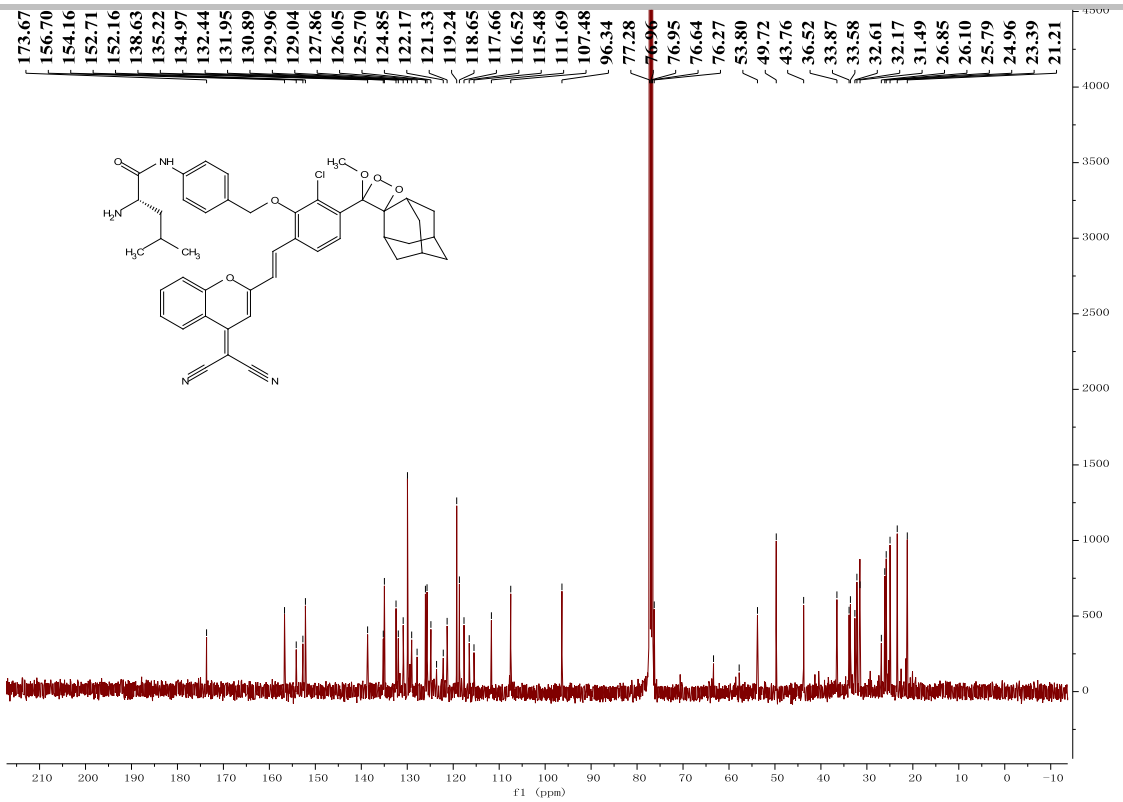


Figure. S34. ^{13}C NMR (101 MHz) spectrum of **probe 2** in $CDCl_3$

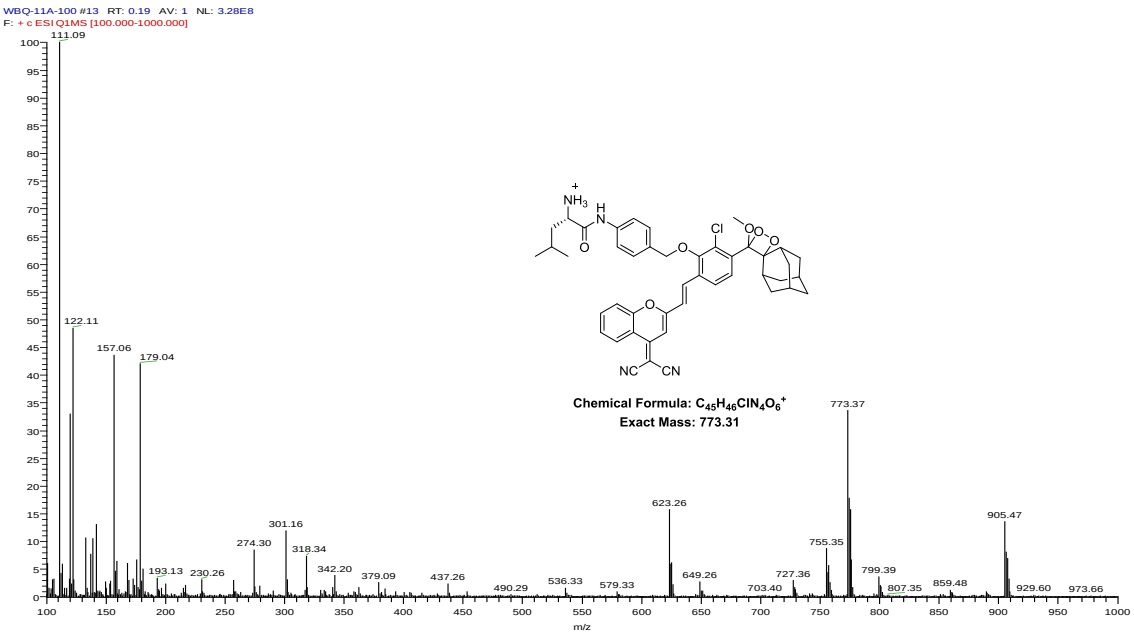


Figure. S35. MS spectrum of **probe 2** in MeOH

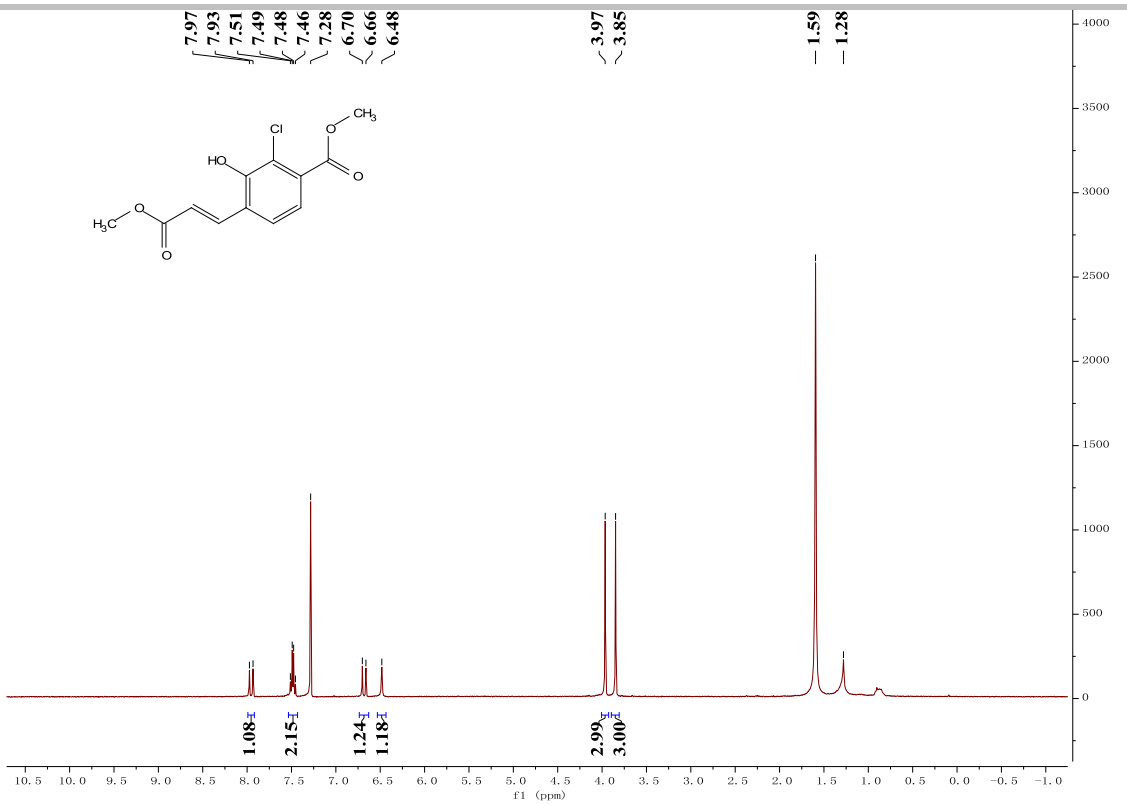


Figure. S36. ^{1}H NMR (400 MHz) spectrum of **product 6-1** in $CDCl_3$

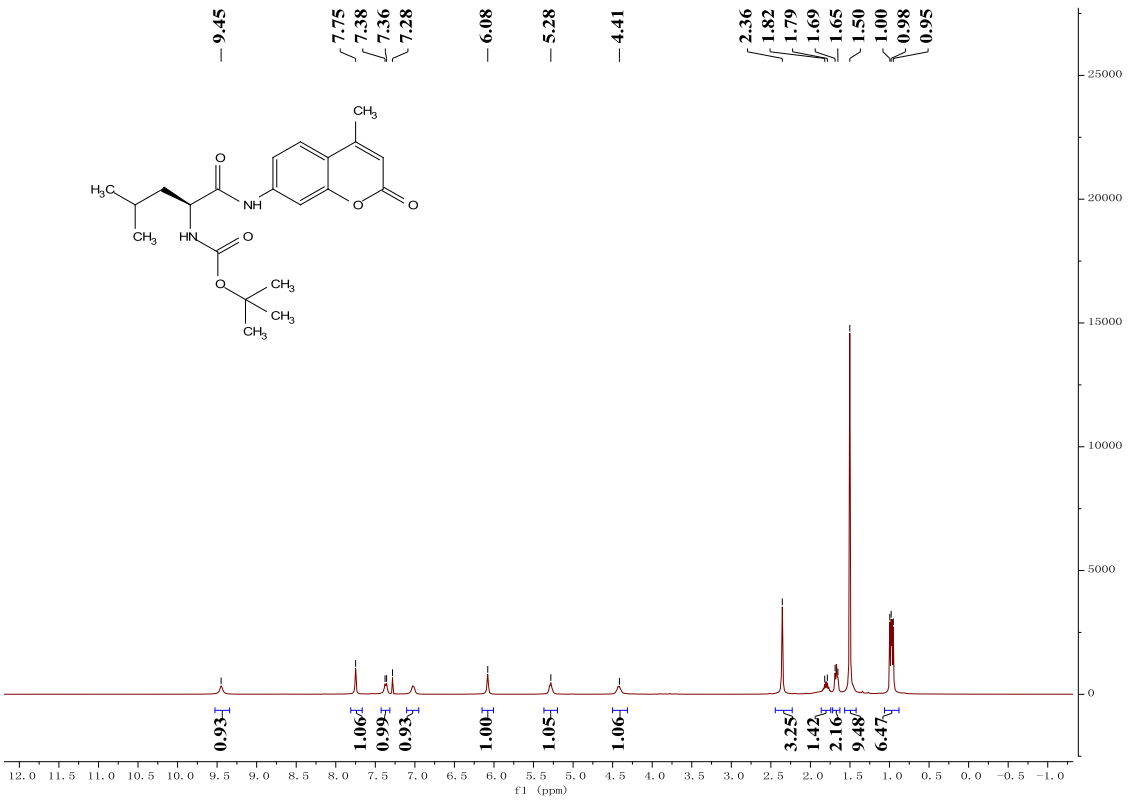


Figure. S37. ^{1}H NMR (400 MHz) spectrum of **Boc-Leu-AMC** in $CDCl_3$

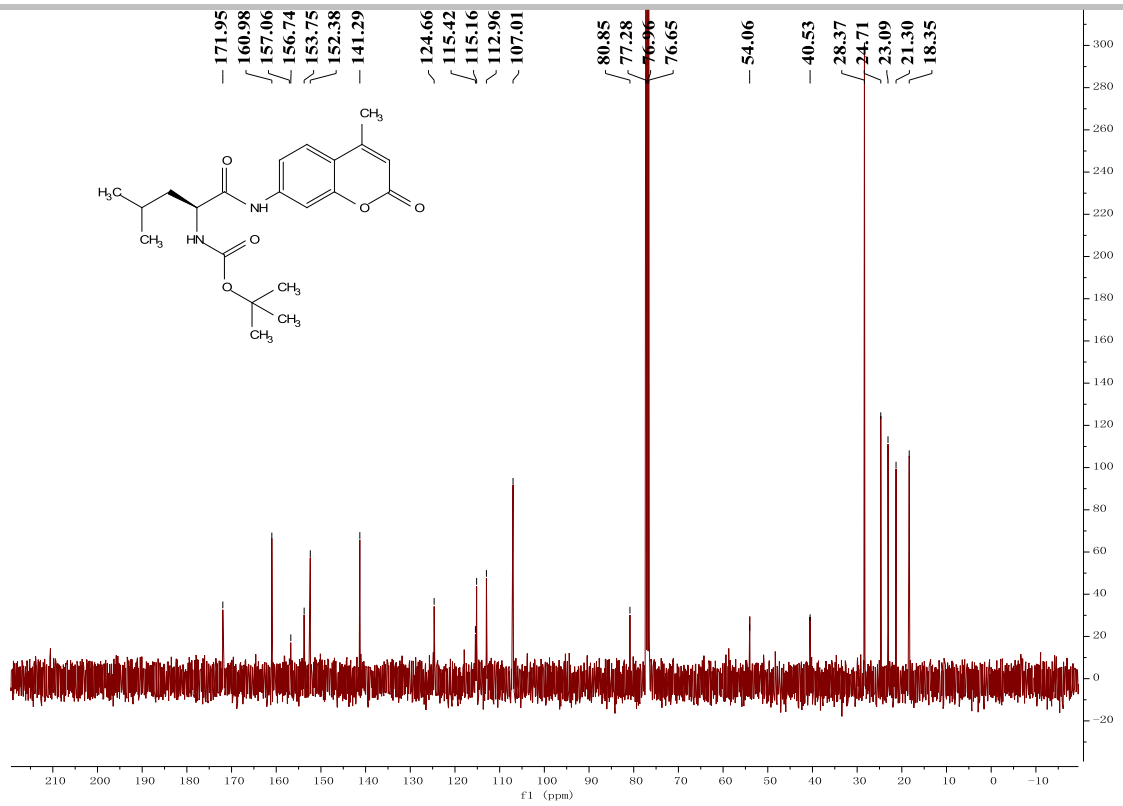


Figure. S38. ^{13}H NMR (101 MHz) spectrum of **Boc-Leu-AMC** in $CDCl_3$

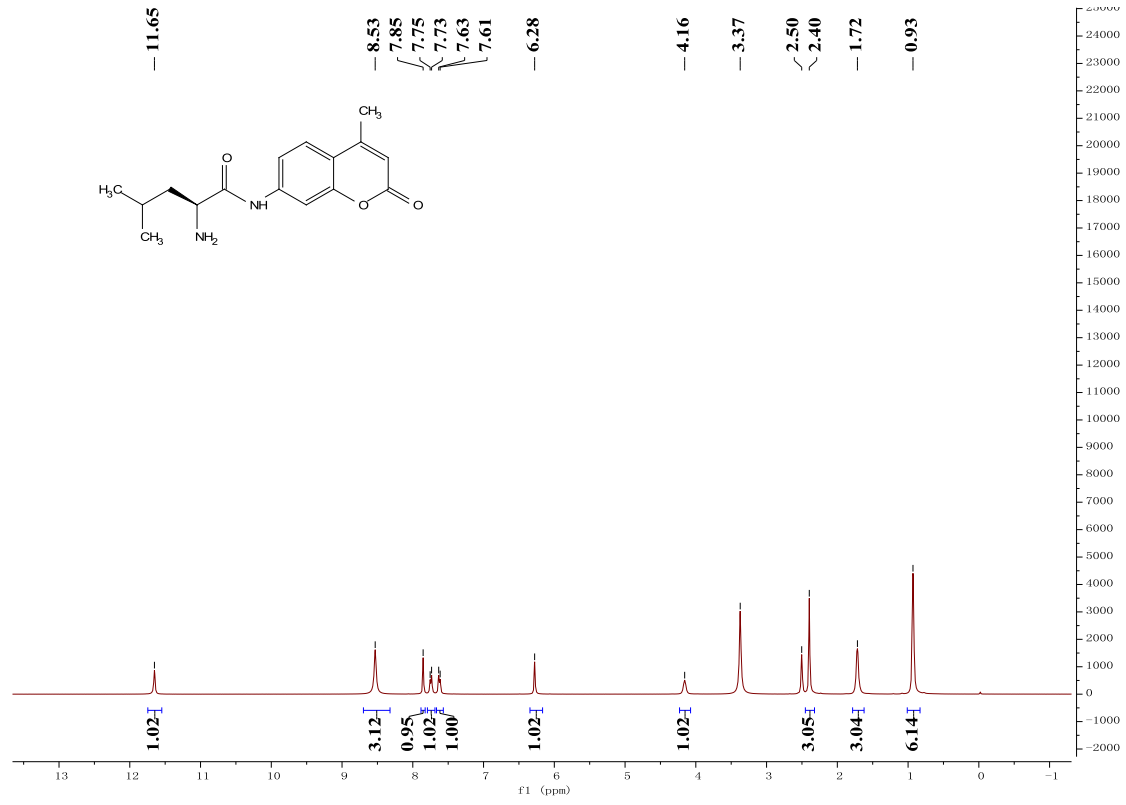


Figure. S39. ^{1}H NMR (400 MHz) spectrum of **Leu-AMC** in DMSO-d_6

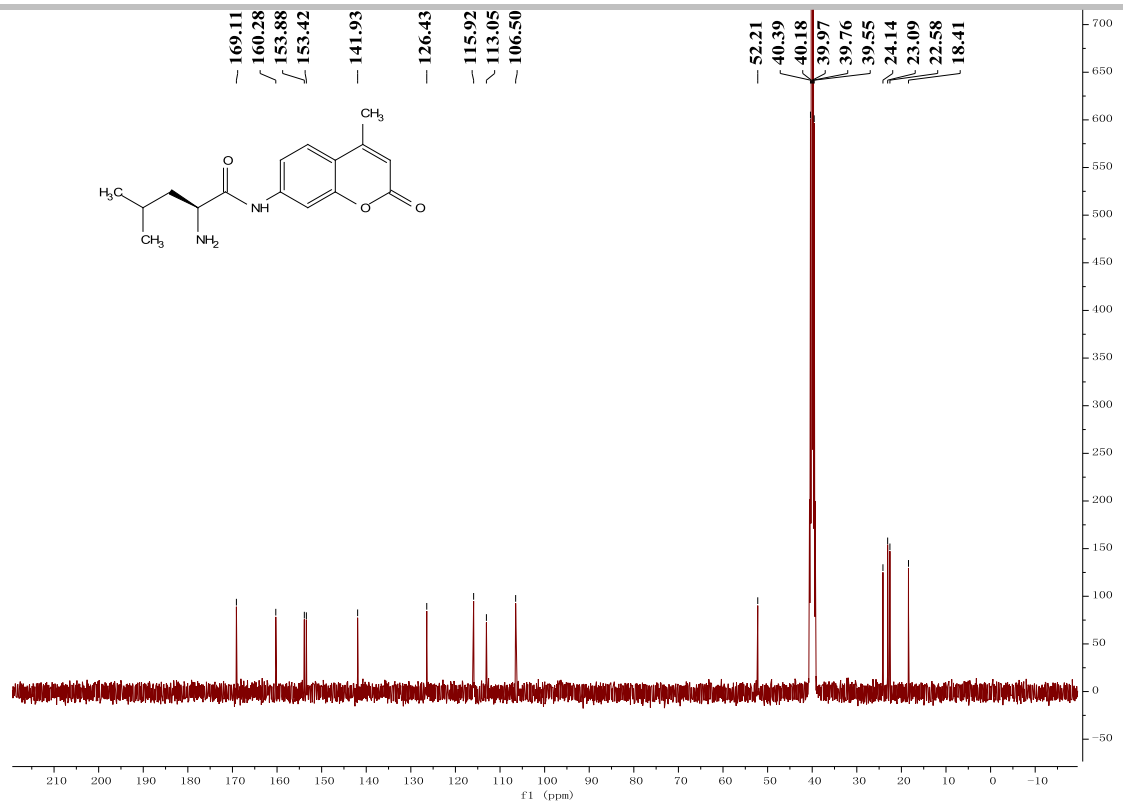


Figure. S40. ^{13}H NMR (101MHz) spectrum of **Leu-AMC** in DMSO-d_6

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