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# Supplementary information

## Near-infrared Chemiluminescent Probes for Monitoring Leucine Aminopeptidase Activity

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## 1.General Materials and Methods

### General Materials

All reagents and solvents in analytical grade were purchased from commercial sources and used without further purification. The $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer, using TMS as an internal standard. High resolution mass spectrometry data were obtained with a Waters LCT Premier XE spectrometer. UV-vis absorption spectra were recorded on Varian Cary 500 UV spectrometer. The fluorescence spectra were recorded on Varian Cary Eclipse fluorescence spectrometer. Chemiluminescence signal acquisition and chemiluminescence kinetic profile were recorded on Tecan Spark microplate reader. Chemiluminescence signals in 96-well plates were recorded on ImageQuant LAS4000 system (GE Healthcare). The intensity of each well was quantified by the ImageQuant TL software (GE Healthcare). Cell fluorescence images were taken on a Leica TCS SP8 (63×oil lens). The chemiluminescence images of cells were measured with IVIS Spectrum Imaging System.

### Preparation of test solutions

Three chemiluminescent probes were dissolved in DMSO and prepared as test stock solutions at concentrations of $1\times10^{-3}$ M; Bovine serum albumin (BSA) was dissolved in total water and prepared as a test stock solution at a concentration of 40 mg/mL; Trimethyl-β-cyclodextrin was dissolved in water and prepared as a test stock solution of $2\times10^{-2}$ M; (n-Hexadecyl)tri-n-butyl phosphonium bromide (TBHP) was dissolved in water and prepared as a test stock solution of $2\times10^{-2}$ M; Bengal rose red (RB) was dissolved in water and prepared as a test stock solution at a concentration of $1\times10^{-3}$ M. The solution was stored at 4 °C in the dark.

### Optical tests of LAP response

Probes 1 and 2 were dissolved in DMSO to make a $1\times10^{-3}$ M test stock solution, and 20 μL of the stock solution was pipetted into PBS to make a 2 mL test solution with a dye concentration of 10 μM. LAP solution (500 U/L) was then added and placed in a cuvette to test the UV and fluorescence spectra of the probe's response to LAP over time.

### Fluorescence quantification test

Pipette 20 μL of stock solution and add it to PBS solution to prepare 2 mL of test solution and place it in a cuvette, place the cuvette in a fluorescence spectrometer, add different concentrations of LAP solution, and collect its fluorescence intensity at 625 nm to test the linear relationship between fluorescence intensity and LAP concentration.

### Chemiluminescence quantification test

2 μL of dye mother liquor, 2 μL of enhancer were added to a black 96-well plate, incubated with different concentration of LAP. The DMSO/PBS mixture was then added to prepare a total volume of 200 μL of test solution (10 μM dye concentration). After 1-second white light irradiation (96 $mW/cm^2$), immediately collect CL signals with IVIS Spectrum Imaging System, the acquisition time is 2 min.

### Chemiluminescence kinetic profile

Chemiluminescence kinetic profile of probe 1 and 2 (10 μM, 200 μL) in the absence or presence of

LAP (500 U/L) was investigated. The test solutions were incubated at 37 ℃, and the real-time chemiluminescence was recorded (black 96-well plates) on microplate reader every 60 seconds, last for 24 h. The integration time is 1 s.

**Selective tests of DCM-LAP**

2 μL of dye mother liquor, 2 μL of enhancer were added to a black 96-well plate, incubated with other substances. The DMSO/PBS mixture was then added to prepare a total volume of 200 μL of test solution (10 μM dye concentration). After 1-second white light irradiation (96 mW/cm$^2$), immediately collect CL signals with IVIS Spectrum Imaging System, the acquisition time is 2 min.

**Stability tests**

Dissolve the DCM-LAP and DCM-LAP-C in methanol, the probes were exposed to 10 mW/cm$^2$ of white LED light and its degradation were monitored by HPLC at 10 min intervals.

**Cell Experiment**

**Cell culture**

The HepG2 cells were purchased from the Institute of Cell Biology (Shanghai, China). Cells were all propagated in T-25 flasks cultured at 37 ℃ under a humidified 5% $CO_2$ atmosphere in DEME medium (GIBCO/Invitrogen, Camarillo, CA, USA), which were supplemented with 10% fetal bovine serum (FBS, Biological Industry, Kibbutz Beit HaEmek, Israel) and 1% penicillin-streptomycin (10000 U/mL penicillin and 10 mg/mL streptomycin, Solarbio life science, Beijing, China).

**Cell cytotoxicity Assay**

The cell cytotoxicity of Probe 1 and probe 2 to HepG2 cells were measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were plated in 96-well plated in 0.1 mL volume of DMEM medium with 10% FBS, at a density of $1\times10^4$ cells/well and added with desired concentrations of probes (0,5,10,20,40 μM). After incubation for 24 h, absorbance was measured at 500 nm with a Tecan GENios Pro multifunction reader (Tecan Group Ltd, Maennedorf, Switzerland). Each concentration was measured in three times. The relative cell viability was calculated by the equation: cell viability (%) = $(OD_{treated}/OD_{control})\times100\%$.

**In Vitro Cellular CL Imaging in 96-Well Plates**

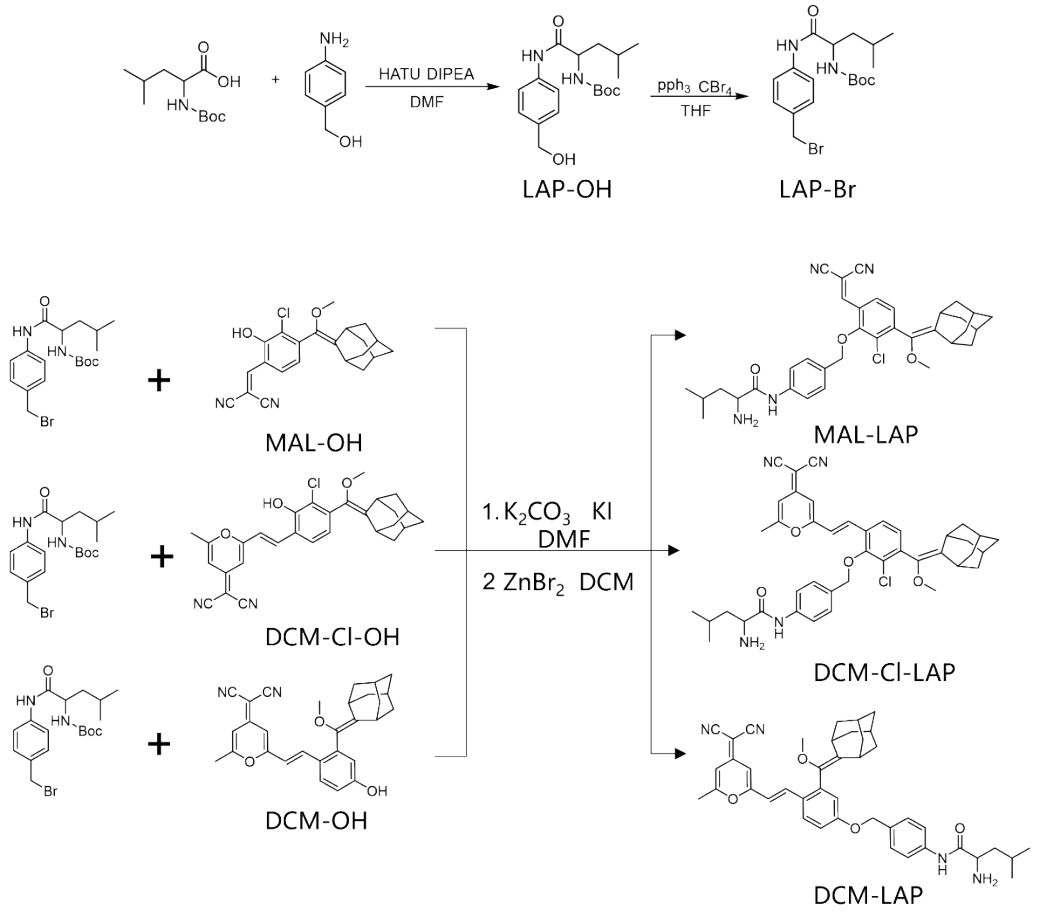
The HepG2 cells at $2.5\times10^4$ cells/well were placed into the wells of a black 96-well plate with complete medium (200 μL) for 12 h. The cells pro-incubated with 20 μM probes and RB for 2 h, followed by white light irradiation 3 min (96 mW/cm$^2$). After the irradiation was completed, immediately collect CL signals with IVIS Spectrum Imaging System (acquisition time of 5 min).

**In Vitro Cellular Fluorescence Imaging of Probes**

The HepG2 cells at $2.5\times10^4$ cells/well were seeded onto glass-bottom petri dishes with complete medium (1.5 mL) for 12 h. Probe 1 and Probe 2 (10 μM) in FBS free DMEM medium were added into cells and incubated at 37 ℃ for 4 h. After being washed with cold PBS(1×) for three times, fluorescence images of the cells were photographed by using a Confocal laser scanning microscope Leica TCS SP8 (63×oil lens) with 500 nm as the excitation wavelength and 600-700 nm as the

emission wavelength.

**2.Synthesis of Probes**




Scheme S1. Synthesis route of DCM-Cl-LAP, MAL-LAP and DCM-LAP

The specific synthesis steps of DCM-Cl-OH and the Chemiluminescent aldehyde precursor of DCM-OH refer to our group's previous studies[1-3].

**Synthesis of DCM-OH**

Chemiluminescent aldehyde precursor (500 mg, 1.67 mmol) and pyranonitrile (432 mg, 2.51 mmol) were added to a round-bottom flask, dissolved with 20 mL anhydrous acetonitrile, and then 0.1 mL pyrrolidine was added to the flask, stirred under argon atmosphere at 85 ℃ for 10 hours. After the reaction completed, the solvent was removed by evaporation, and the column chromatography (PE : EA = 10 : 1) was used to obtain 312 mg of orange-yellow solid with a yield of 41.3%. $^{1}$H NMR (400 MHz, $CDCl_3$, ppm): $\delta$ 7.75-7.71 (d, $J$ = 16 Hz, 1H, alkene-H), 7.63-7.61 (d, $J$ = 8.8 Hz, 1H, phenyl-H), 6.90-6.87 (dd, $J_1$ = 8.8 Hz, $J_2$ = 2.8 Hz, 1H, phenyl-H), 6.78-6.77 (d, $J$ = 2.4 Hz, 1H, phenyl-H), 6.61 (d, $J$ = 1.6 Hz, 1H, phenyl-H), 6.60-6.56 (d, $J$ = 16 Hz, 1H, alkene-H), 6.52 (s, 1H, phenyl-H), 5.75 (s, 1H, -OH), 3.33 (s, 3H, -O-$CH_3$), 3.26 (s, 1H, adamantane-H), 2.34 (s, 3H, -$CH_3$), 2.11 (s, 1H, adamantane-H), 1.97-1.68 (m, 12H, adamantane-H). $^{13}$C NMR (100 MHz, $CDCl_3$, ppm): $\delta$ 161.86, 159.78, 156.53, 139.69, 138.55, 136.32, 132.24, 127.49, 126.81, 117.78, 116.10,

115.94, 115.18, 106.72, 106.40, 58.66, 56.95, 36.95, 32.63, 29.77, 28.26, 19.85. Mass spectrometry (ESI-MS, m/z): $[M - H]^-$ calcd for $C_{29}H_{27}O_3N_2$, 451.2016; found 451.2007.

**Synthesis of LAP-OH**

Boc-L-leucine (4694 mg, 20.31 mmol), HATU (9266 mg, 24.37 mmol) and DIPEA (5203 mg, 40.62 mmol) were added to a round-bottom flask, dissolved with 100 mL ultra-dry DCM, stirred for 10 min, and then 4-aminobenzyl alcohol (2000 mg, 16.25 mmol) was added to the flask, stirred under argon atmosphere for 12 h. After the reaction completed, the reaction solution was washed with saturated $NH_4Cl$ solution (100 mL×3), the organic phase was dried with anhydrous $Na_2SO_4$, and the solvent was removed by evaporation, and the column chromatography (PE : EA = 3 : 1) was used to obtain 3206 mg of light yellow solid with a yield of 58.57%. $^1H$ NMR (400 MHz, $CDCl_3$, ppm): $\delta$ 8.93 (s, 1H, CONH), 7.43-7.41 (d, $J$ = 8.4 Hz, 2H, phenyl-H), 7.17-7.15 (d, $J$ = 7.2 Hz, 2H, phenyl-H), 5.30-5.28 (d, $J$ = 8.0 Hz, 1H, NH-Boc), 4.57 (s, 2H, $CH_2$-Br), 4.35 (s, 1H, -CH-), 1.78-1.67 (m, 4H, -$CH_2$-), 1.64-1.59 (m, 1H, -CH-), 1.44 (s, 9H, -Boc), 0.98-0.94 (m, 6H, -$CH_3$). $^{13}C$ NMR (100 MHz, $CDCl_3$, ppm): $\delta$ 171.65, 156.55, 137.36, 136.43, 127.80, 119.65, 80.46, 64.79, 53.77, 38.74, 28.31, 24.84, 22.95, 21.88. Mass spectrometry (ESI-MS, m/z): $[M + Na]^+$ calcd for $C_{18}H_{28}O_4N_2Na^+$, 359.1941, found 359.1941.

**Synthesis of LAP-Br**

LAP-OH (3000 mg, 8.92 mmol) and $CBr_4$ (5849 mg, 17.85 mmol) were added to a round-bottom flask, dissolved with 30 mL ultra-dry THF. Triphenylphosphine (4681 mg, 17.85 mmol) was dissolved in 30 mL ultra-dry THF, placed in a constant pressure dropping funnel, slowly dropped into the reaction flask under an ice bath, and then transferred to room temperature and stirred under an argon atmosphere for 4 h. After the reaction completed, 100 mL ethyl acetate was added to the reaction mixture, washed with saturated $NH_4Cl$ solution (150 mL×2), the organic phase was dried with anhydrous $Na_2SO_4$, and the solvent was removed by evaporation, then column chromatography (PE : EA = 5 : 1) was used to obtain 2200 mg of light yellow solid with a yield of 61.9%. $^1H$ NMR (400 MHz, $CDCl_3$, ppm): $\delta$ 8.61 (s, 1H, CONH), 7.48-7.46 (d, $J$ = 8.4 Hz, 2H, phenyl-H), 7.30-7.28 (d, $J$ = 8.4 Hz, 2H, phenyl-H), 5.01 (d, $J$ = 8.0 Hz, 1H, NH-Boc), 4.46 (s, 2H, $CH_2$-Br), 4.27 (s, 1H, -CH-), 1.79-1.74 (m, 4H, -$CH_2$-), 1.59-1.55 (m, 1H, -CH-), 1.45 (s, 9H, -Boc), 0.98-0.94 (m, 6H, -$CH_3$). $^{13}C$ NMR (100 MHz, $CDCl_3$, ppm): $\delta$ 171.33, 156.50, 138.11, 133.26, 129.68, 119.86, 80.62, 53.89, 40.74, 33.55, 28.36, 24.79, 23.02, 21.80. Mass spectrometry (ESI-MS, m/z): $[M + Na]^+$ calcd for $C_{18}H_{27}O_3N_2BrNa^+$, 421.1097, found 421.1080.

**Synthesis of DCM-LAP-boc**

LAP-Br (211 mg, 0.53 mmol), $K_2CO_3$ (121 mg, 0.88 mmol), and KI (730 mg, 4.40 mmol) were added to a round-bottom flask, dissolved with 20 mL ultra-dry DMF and stirred for 10 minutes. Then add DCM-OH (200 mg, 0.44 mmol) and stir the mixture at room temperature under an argon atmosphere for 6 hours. After the reaction completed, 100 mL ethyl acetate was added to the reaction mixture and washed with saturated $NH_4Cl$ solution (100 mL×3). Separate the organic phase, dry it over anhydrous $Na_2SO_4$, and the solvent was removed by evaporation. Finally, purify the residue by column chromatography (PE : EA = 5 : 1) to obtain 110 mg yellow solid with a yield of 31.8%. $^1H$ NMR (400 MHz, $CDCl_3$, ppm): $\delta$ 8.34 (s, 1H, -CONH), 7.45-7.41 (d, $J$ = 16 Hz, 1H, alkene-H), 7.66-7.64 (d, $J$ = 8.8 Hz, 1H, phenyl-H), 7.57-7.55 (d, $J$ = 8.4 Hz, 2H, phenyl-

H), 7.40-7.38 (d, $J$ = 8.4 Hz, 2H, phenyl-H), 7.33-7.31 (d, $J$ = 8.4 Hz, 1H, phenyl-H), 7.01-6.98 (dd, $J_1$ = 2.4 Hz, $J_2$ = 8.4 Hz, 1H, phenyl-H), 6.88-6.87 (d, $J$ = 2.4 Hz, 1H, phenyl-H), 6.61-6.57 (d, $J$ = 16 Hz, 1H, alkene-H), 6.52-6.51 (d, $J$ = 1.2Hz, 1H, phenyl-H), 5.06 (s, 2H, -O-$CH_2$), 4.88 (s, 1H, NH-Boc), 4.21 (s, 1H, -CH-), 3.32 (s, 1H, adamantane-H), 3.24 (s, 3H, -O-$CH_3$), 2.34 (s, 3H, -$CH_3$), 2.08 (s, 1H, adamantane-H), 1.85-1.66 (m, 12H, adamantane-H), 1.46 (s, 9H, -$C(CH_3)_3$), 1.25 (s, 2H, -$CH_2$-), 0.99-0.96 (m, 6H, -$CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$, ppm): $\delta$ 171.89, 162.14, 162.10, 160.13, 160.09, 159.90, 158.63, 156.73, 156.66, 139.92, 139.83, 138.49, 138.31, 136.74, 136.39, 132.20, 131.86, 132.20, 131.86, 128.29, 127.45, 127.30, 126.93, 126.02, 120.03, 117.93, 116.94, 116.26, 116.07, 115.38, 106.74, 106.51, 106.35, 80.55, 69.84, 58.22, 57.94, 56.95, 56.91, 41.09, 38.95, 36.95, 32.61, 29.75, 28.35, 24.80, 23.04, 21.78, 19.88, 19.84.

**Synthesis of DCM-LAP**

DCM-LAP-boc (100 mg, 0.13 mmol) was added to a round-bottom flask, dissolved with 15 mL ultra-dry DCM. After stirring for 5 minutes, $ZnBr_2$ (144 mg, 0.65 mmol) was added to the reaction mixture, and stir the mixture under an argon atmosphere for 6 hours at room temperature. After the reaction completed, 50 mL DCM was added to the reaction mixture and washed with saturated $NH_4Cl$ solution (50 mL×3). Separate the organic phase, dry it over anhydrous $Na_2SO_4$, and the solvent was removed by evaporation. Finally, purify the residue by column chromatography (DCM : MeOH = 49 : 1) to obtain 27 mg yellow solid with a yield of 31.0%. $^1$H NMR (400 MHz, $CDCl_3$, ppm): $\delta$ 9.59 (s, 1H, CONH), 7.75-7.71 (d, $J$ = 16.0 Hz, 1H, alkene-H), 7.66-7.64 (d, $J$ = 8.8 Hz, 2H, phenyl-H), 7.65-7.63 (d, $J$ = 8.4 Hz, 1H, phenyl-H), 7.41-7.39 (d, $J$ = 8.4 Hz, 2H, phenyl-H), 7.01-6.98 (dd, $J_1$ = 2.4 Hz, $J_2$ = 8.4 Hz, 1H, phenyl-H), 6.88-6.87 (d, $J$ = 2.4 Hz, 1H, phenyl-H), 6.62-6.61 (d, $J$ = 2.0 Hz, 1H, -CH-), 6.61-6.57 (d, $J$ = 16.4 Hz, 1H, alkene-H), 6.52-6.51 (d, $J$ = 1.2Hz, 1H, phenyl-H), 5.07 (s, 2H, -O-$CH_2$), 3.53-3.50 (dd, $J_1$ = 3.2 Hz, $J_2$ = 8.4 Hz, 1H, -CH-), 3.36 (s, 3H, -O-$CH_3$), 3.30 (s, 1H, adamantane-H), 2.38 (s, 3H, -$CH_3$), 2.36 (s, 1H, -CH-) 2.10 (s, 1H, adamantane-H), 1.98-1.77 (m, 12H, adamantane-H), 1.33 (s, 1H, -CH-), 1.25 (s, 2H, -$NH_2$), 1.02-0.98 (m, 6H, -$CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$, ppm): $\delta$ 171.60, 162.01, 160.08, 159.84, 158.49, 156.59, 139.85, 138.31, 136.36, 132.17, 131.89, 128.33, 126.94, 126.12, 120.00, 117.92, 116.96, 116.06, 115.44, 106.74, 69.84, 56.94, 53.91, 40.92, 38.96, 36.96, 32.63, 31.93, 29.71, 24.80, 22.70, 19.88, 19.83, 14.15. Mass spectrometry (ESI-MS, m/z): $[M + H]^+$ calcd for $C_{42}H_{47}N_4O_4^+$, 671.3592, found 671.3597.

**Synthesis of DCM-Cl-LAP-boc**

LAP-Br (98.3 mg, 0.25 mmol), $K_2CO_3$ (56.8 mg, 0.41 mmol), and KI (341.7 mg, 2.06 mmol) were added to a round-bottom flask, dissolved with 5 mL ultra-dry DMF and stirred for 10 minutes. Then add DCM-OH (100 mg, 0.21 mmol) and stir the mixture at room temperature under an argon atmosphere for 6 hours. After the reaction completed, 100 mL ethyl acetate was added to the reaction mixture and washed with saturated $NH_4Cl$ solution (50 mL×3). Separate the organic phase, dry it over anhydrous $Na_2SO_4$, and the solvent was removed by evaporation. Finally, purify the residue by column chromatography (PE : EA = 6 : 1) to obtain 51 mg yellow solid with a yield of 30.8%. $^1$H NMR (400 MHz, $CDCl_3$, ppm): $\delta$ 8..65 (s, 1H, CONH), 7.56-7.54 (d, $J$ = 8.8 Hz, 2H, phenyl-H), 7.53-7.49 (d, $J$ = 15.6 Hz, 1H, alkene-H), 7.43-7.41 (d, $J$ = 8.0 Hz, 1H, phenyl-H), 7.37-7.35 (d, $J$ = 8.0 Hz, 2H, phenyl-H), 7.26 (s, 1H, phenyl-H), 7.12-7.12 (d, $J$ = 8.0 Hz, 1H, phenyl-H), 6.74-6.70 (d, $J$ = 16.0 Hz), 0.96 (s, 1H, -NH-boc), 5.07-4.98 (dd, 2H, -O-$CH_2$), 4.28 (s,

1H, -CH), 3.35 (s, 3H, -O-$CH_3$), 3.29 (s, 1H, adamantane-H), 2.35 (s, 3H, -$CH_3$), 2.10 (s, 1H, adamantane-H), 1.97-1.77 (m, 12H, adamantane-H), 1.61-1.59 (d, $J$ = 9.2 Hz, -$CH_2$), 1.46 (s, 9H, -$(CH_3)_3$), 1.25 (s, 1H, -CH-), 0.98-0.96 (m, 6H, -$CH_3$). Mass spectrometry (ESI-MS, m/z): $[M + H]^+$ calcd for $C_{47}H_{54}ClN_4O_4^+$, 805.3726, found 805.3737.

**Synthesis of DCM-Cl-LAP**

DCM-Cl-LAP-boc (100 mg, 0.12 mmol) was added to a round-bottom flask, dissolved with 10 mL ultra-dry DCM. After stirring for 5 minutes, $ZnBr_2$ (137 mg, 0.62 mmol) was added to the reaction mixture, and stir the mixture under an argon atmosphere for 6 hours at room temperature. After the reaction completed, 50 mL DCM was added to the reaction mixture and washed with saturated $NH_4Cl$ solution (50 mL×3). Separate the organic phase, dry it over anhydrous $Na_2SO_4$, and the solvent was removed by evaporation. Finally, purify the residue by column chromatography (DCM : MeOH = 50 : 1) to obtain 36 mg yellow solid with a yield of 42.6%. $^1$H NMR (400 MHz, $CDCl_3$, ppm): $\delta$ 9.63 (s, 1H, CONH), 7.61-7.59 (d, $J$ = 8.8 Hz, 2H, phenyl-H), 7.50-7.46 (d, $J$ = 16.4 Hz, 1H, alkene-H), 7.40-7.38 (d, $J$ = 8.0 Hz, 1H, phenyl-H), 7.34-7.32 (d, $J$ = 8.4 Hz, 2H, phenyl-H), 7.12-7.10 (d, $J$ = 8.0 Hz, 1H, phenyl-H), 6.63-6.59 (d, $J$ = 16.4 Hz, 1H, alkene-H), 6.53 (s, 1H, phenyl-H), 6.52 (s, 1H, phenyl-H), 5.03-5.01 (d, 2H, -O-$CH_2$), 3.54-3.51 (dd, $J_1$ = 3.2 Hz, $J_2$ = 8.4 Hz, 1H, -CH-), 3.33 (s, 1H, adamantane-H), 3.24 (s, 3H, -O-$CH_3$), 2.34 (s, 3H, -$CH_3$), 2.08 (s, 1H, adamantane-H), 1.96-1.67 (m, 12H, adamantane-H), 1.33 (s, 1H, -CH-), 1.28 (s, 2H, -$NH_2$), 1.00-0.96 (m, 6H, -$CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$, ppm): $\delta$ 162.29, 158.84, 139.40, 138.50, 132.81, 131.38, 129.91, 127.94, 119.76, 119.31, 114.98, 107.64, 106.52, 59.26, 57.37, 43.60, 38.61, 29.76, 28.31, 24.99, 23.43. Mass spectrometry (ESI-MS, m/z): $[M + H]^+$ calcd for $C_{42}H_{46}ClN_4O_4^+$, 705.3192, found 705.3205.

Specific steps of the synthetic route of MAL-LAP are the same as those of DCM-LAP and DCM-Cl-LAP. Its NMR spectra are detailed in Part IV of the Supporting Information.

**3. Experiments**

**Table S1**. The fluorescence quantum yields four compounds.

| | DCM-OH | DCM-Cl-OH | DCM-LAP | DCM-Cl-LAP |
|---|---|---|---|---|
| $\Phi_f$[a] | 0.013 | 0.012 | 0.009 | 0.009 |

[a]The fluorescence quantum yields $\Phi_f$ value was determined using fluorescein (0.92, 0.1 M NaOH solution) as an internal reference.

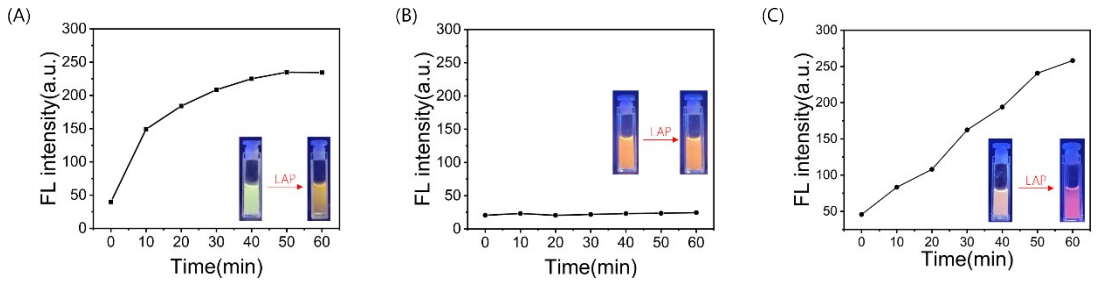



Fig. S1. (A) Time-dependent fluorescence intensity of MAL-LAP at 525 nm. Insert, its fluorescence color changes before and after LAP response. (B) Time-dependent fluorescence intensity of DCM-Cl-LAP at 625 nm. Insert, its fluorescence color changes before and after response. (C) Time-dependent fluorescence intensity of DCM-LAP at 625 nm. Insert, its fluorescence color changes before and after response.

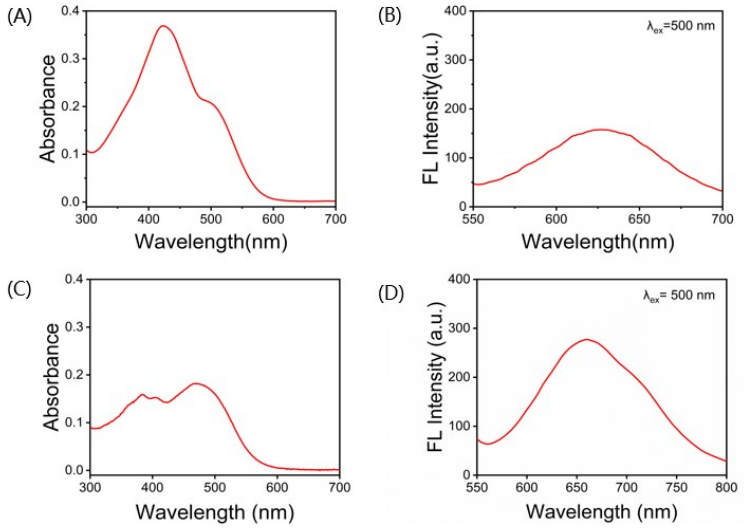



Fig. S2. Spectral property testing：Absorption spectra(A) and emission spectra(B) of DCM-OH in aqueous solution (10 μM, PBS: DMSO=7:3, v: v). Absorption spectra(C) and emission spectra(D) of DCM-Cl-OH in aqueous solution (10 μM, PBS: DMSO=7:3, v: v).

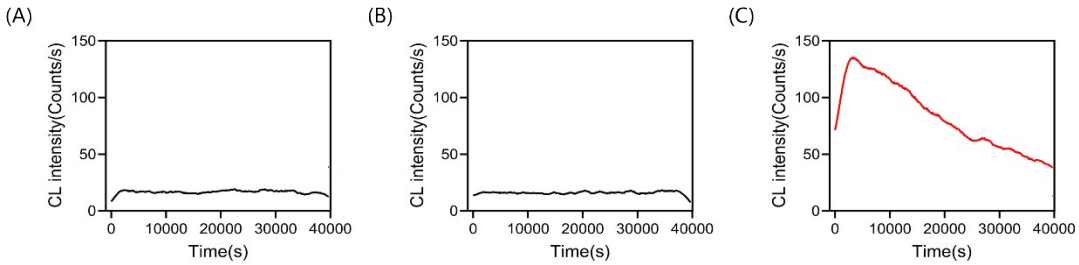



Fig. S3. (A) Photoactivated chemiluminescence kinetic curves of probe MAL-LAP (10 μM) with incubation of LAP, under light exposure (96 mW/cm$^2$ white LED for 1s). (B) Photoactivated chemiluminescence kinetic curves of probe DCM-LAP (10 μM) with incubation of LAP, under light exposure (96 mW/cm$^2$ white LED for 1s). (C) Photoactivated chemiluminescence kinetic curves of probe DCM-Cl-LAP (10 μM) with incubation of LAP, under light exposure (96 mW/cm$^2$ white LED for 1s).

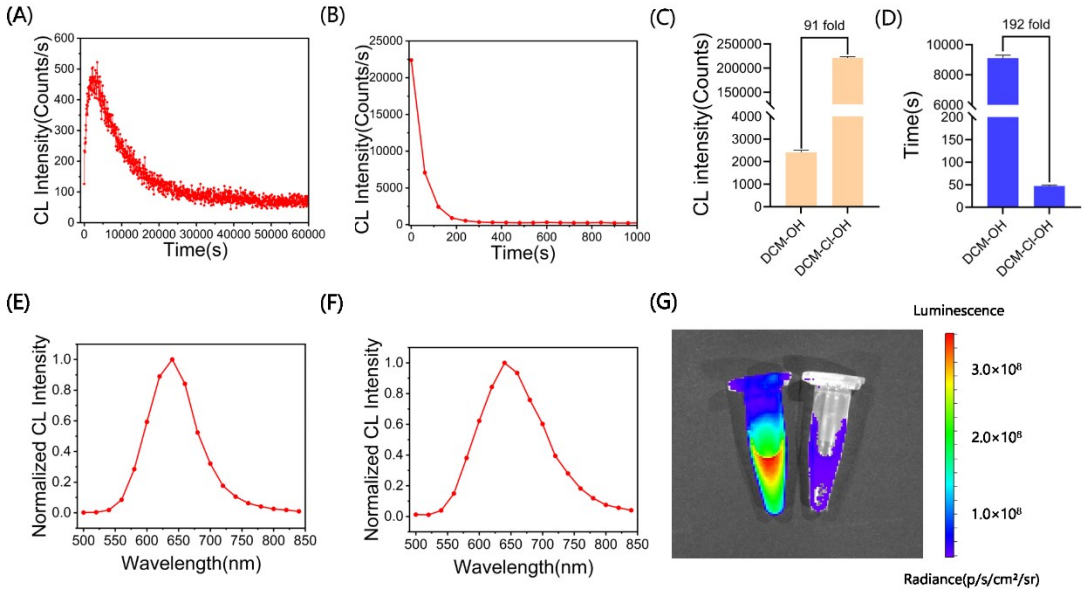



Fig. S4. (A) Chemiluminescence kinetic curves of DCM-OH(10 μM) in Tris-HCl buffer solution(pH=8.8), containing 20eq trimethyl-β-cyclodextrin, under light exposure(96 mW/cm$^2$ white LED for 1s). (B) Chemiluminescence kinetic curves of DCM-Cl-OH(10 μM) in PBS buffer solution(pH=7.4), containing 10% 40 mg/mL BSA, under light exposure(96 mW/cm$^2$ white LED for 1s). (C) and (D) Comparison of DCM-OH and DCM-Cl-OH in chemiluminescence intensity and half-life. (E) Normalized chemiluminescence spectra of DCM-OH. (F) Normalized chemiluminescence spectra of DCM-Cl-OH. (G) Photoactivated chemiluminescence intensities of DCM-OH(10 μM) and DCM-Cl-OH(10 μM) captured by IVIS, under light exposure(96 mW/cm$^2$ white LED for 1s).

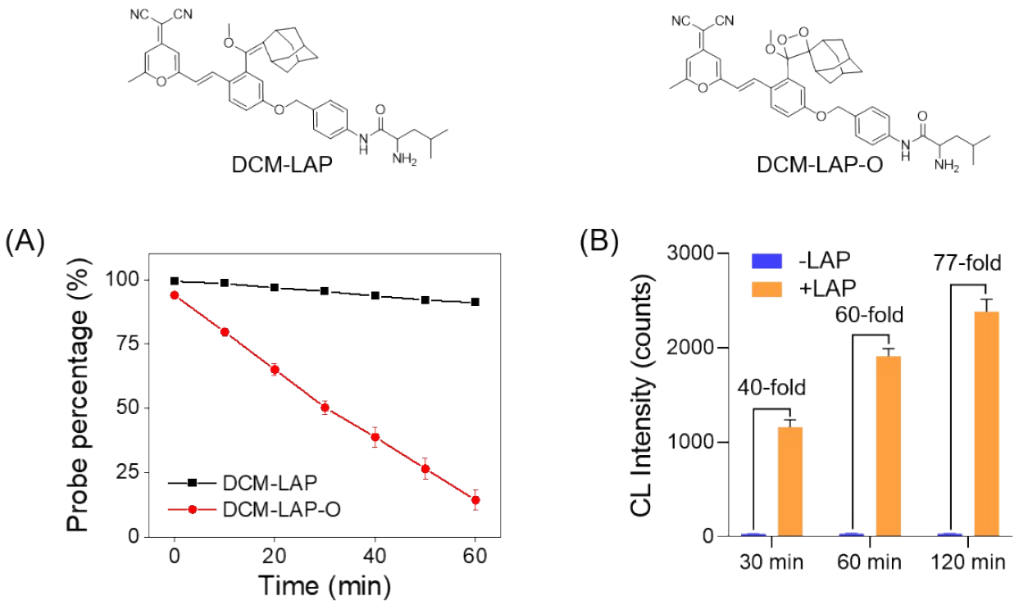



Fig. S5. (A) Stability test of DCM-LAP and DCM-LAP-O under sustained illumination (10 $mW \cdot cm^{-2}$). (B) The photoactivated chemiluminescence intensity of DCM-LAP upon incubation with LAP (100 U/L) for different times.

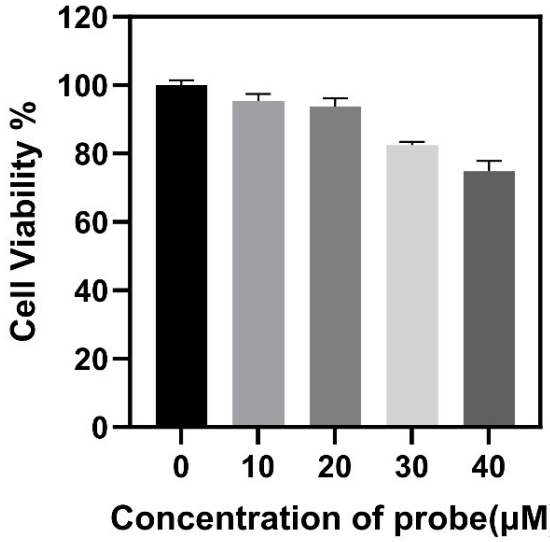
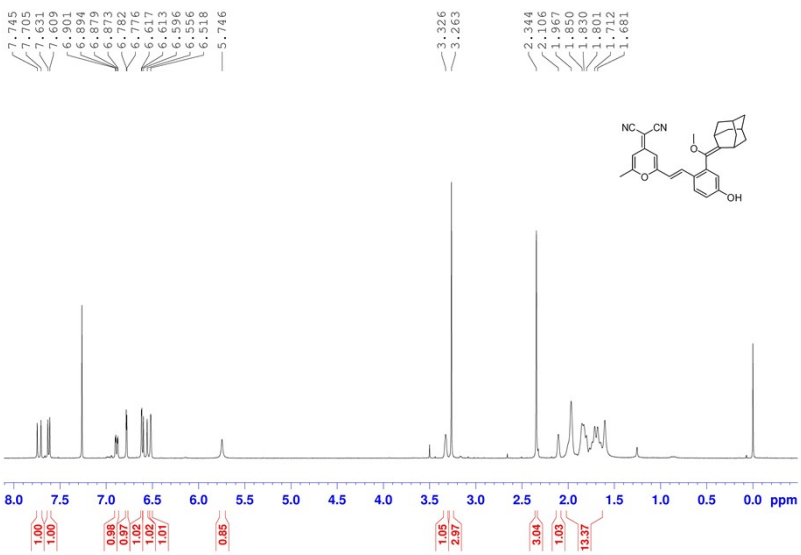
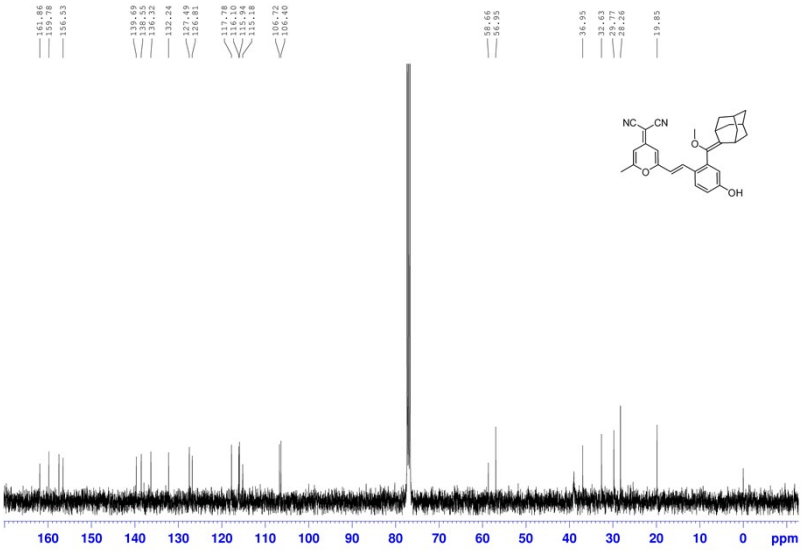



Fig.S6. Relative viability of HepG2 cells in vitro after incubation with DCM-LAP at various concentrations of LAP.

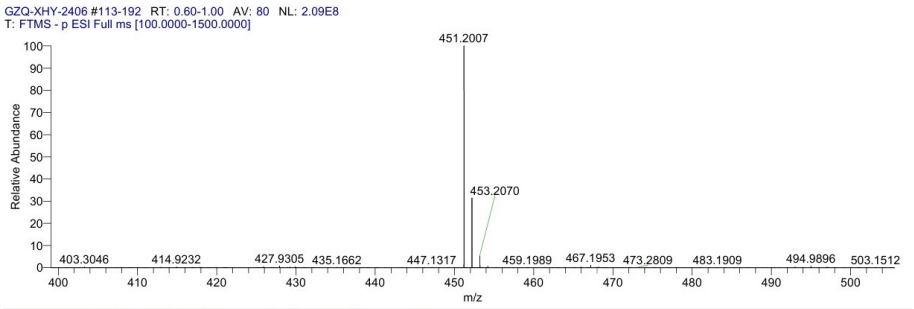
## 4. $^{1}$H NMR Spectra, $^{13}$C NMR Spectra, and HRMS Spectra of Compounds




1.$^{1}$H NMR spectrum of DCM-OH in $CDCl_3$



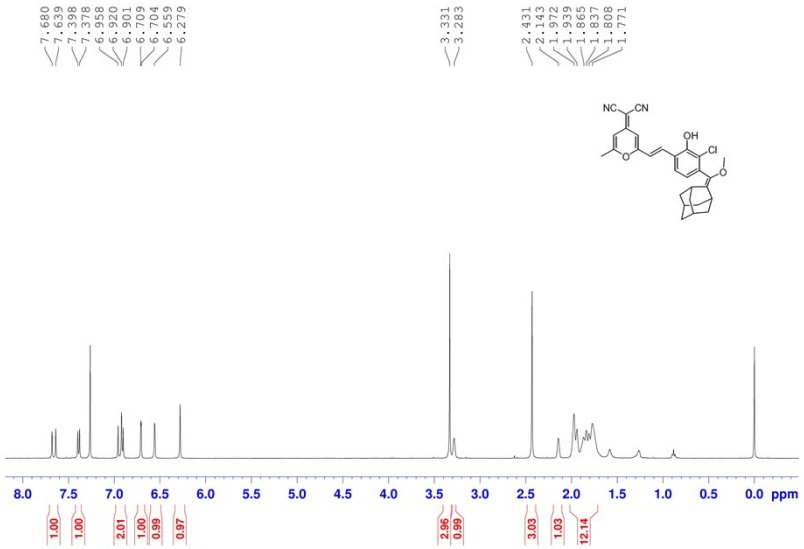

2.$^{13}$C NMR spectrum of DCM-OH in $CDCl_3$



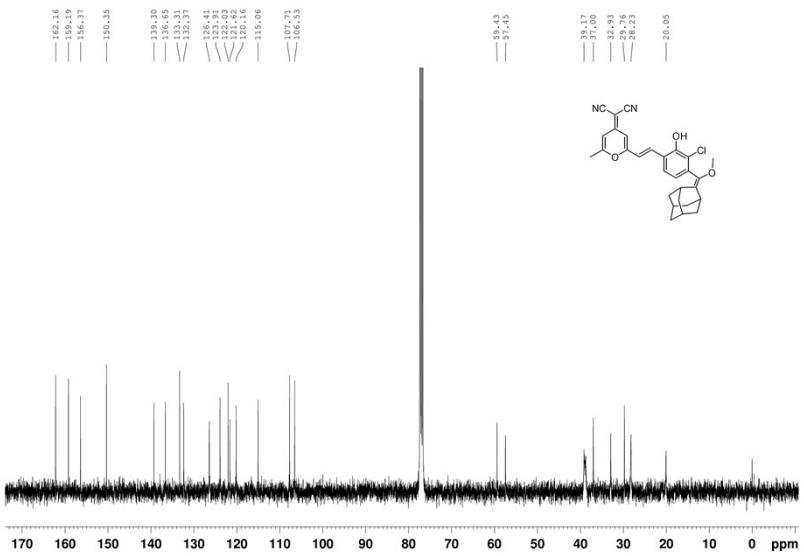

GZQ-XHY-2406#112-192 RT: 0.59-1.00 AV: 81
T: FTMS - p ESI Full ms [100.0000-1500.0000]
m/z= 450.71-451.74

| m/z | Intensity | Relative | Theo. Mass | Delta (ppm) | Composition |
|---|---|---|---|---|---|
| 451.2007 | 207626336.0 | 100.00 | 451.2016 | -0.89 | $C_{29}H_{27}O_3N_2$ |

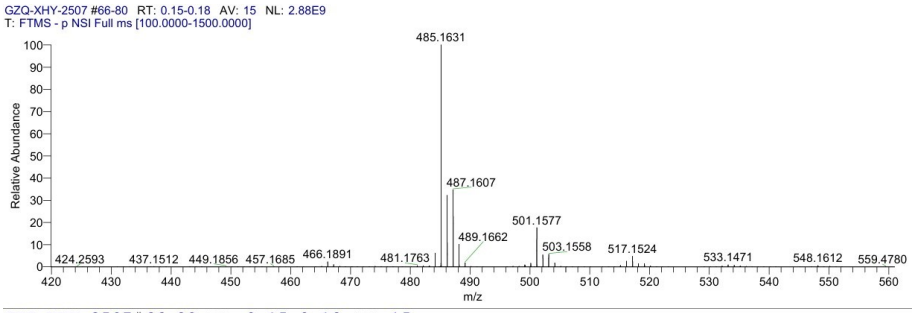
3.HRMS spectrum of DCM-OH




4.$^1$H NMR spectrum of DCM-Cl-OH in $CDCl_3$



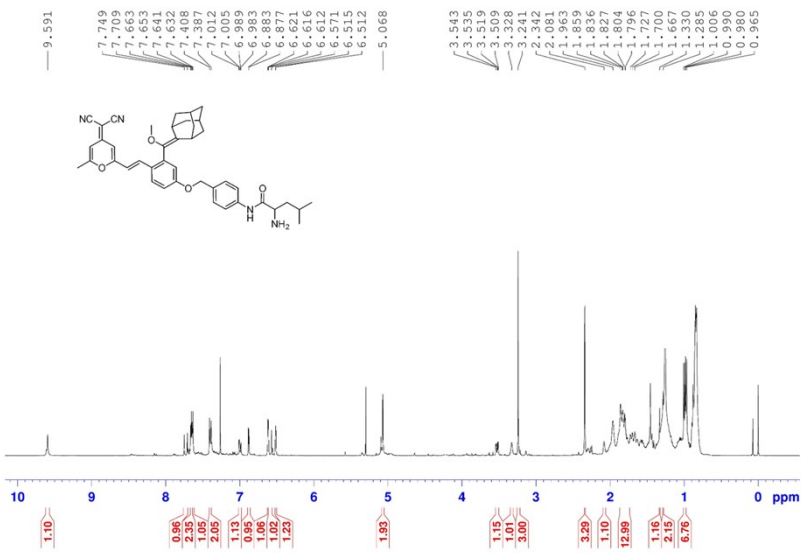

5.$^{13}$C NMR spectrum of DCM-Cl-OH in $CDCl_3$



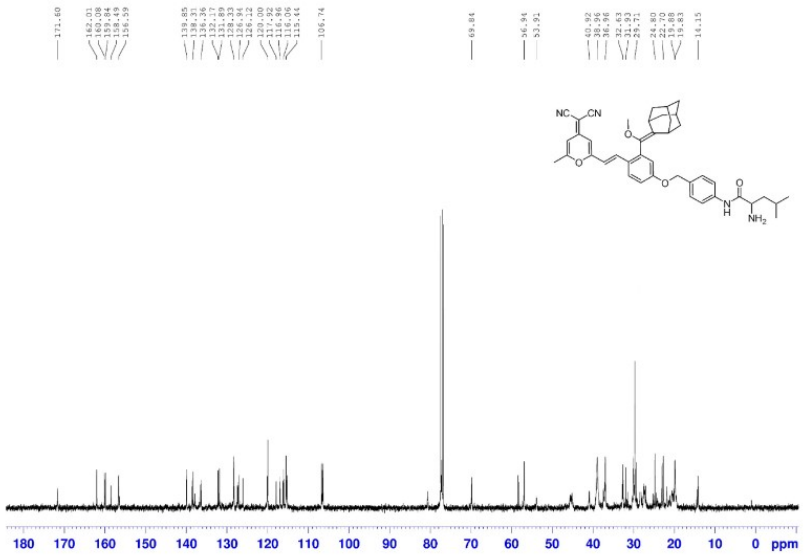

GZQ-XHY-2507#66-80 RT: 0.15-0.18 AV: 15
T: FTMS - p NSI Full ms [100.0000-1500.0000]
m/z= 484.68-485.67

| m/z | Intensity | Relative | Theo. Mass | Delta (ppm) | Composition |
|---|---|---|---|---|---|
| 485.1631 | 2939332352.0 | 100.00 | 485.1626 | 0.47 | $C_{29}H_{26}O_3N_2Cl$ |

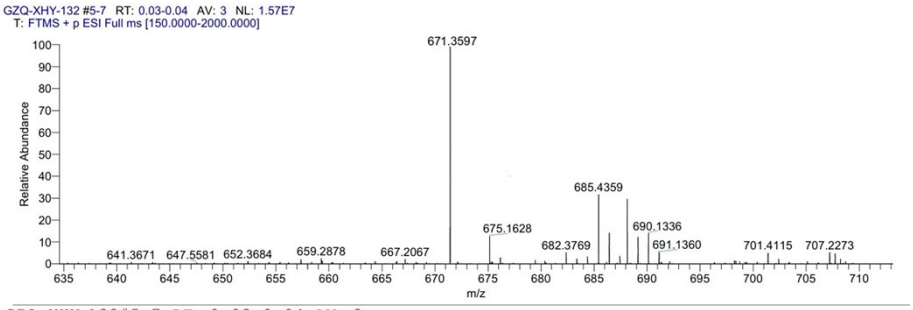
6.HRMS spectrum of DCM-Cl-OH




7. $^{1}H$ NMR spectrum of DCM-LAP in $CDCl_3$



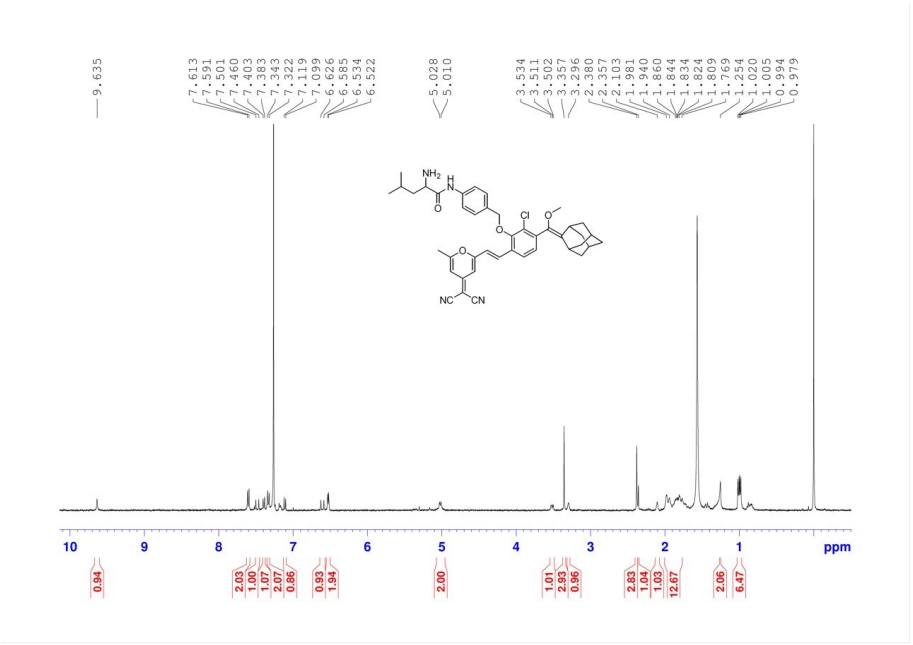

8. $^{13}C$ NMR spectrum of DCM-LAP in $CDCl_3$



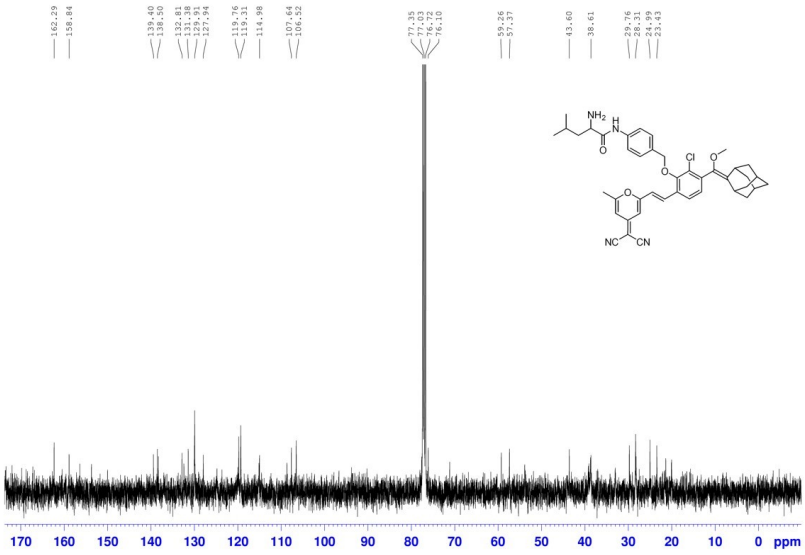

GZQ-XHY-132#5-7 RT: 0.03-0.04 AV: 3
T: FTMS + p ESI Full ms [150.0000-2000.0000]
m/z= 670.58-672.56

| m/z | Intensity | Relative | Theo. Mass | Delta (ppm) | Composition |
|---|---|---|---|---|---|
| 671.3597 | 15686281.0 | 100.00 | 671.3592 | 0.25 | $C_{42}H_{47}O_4N_4$ |

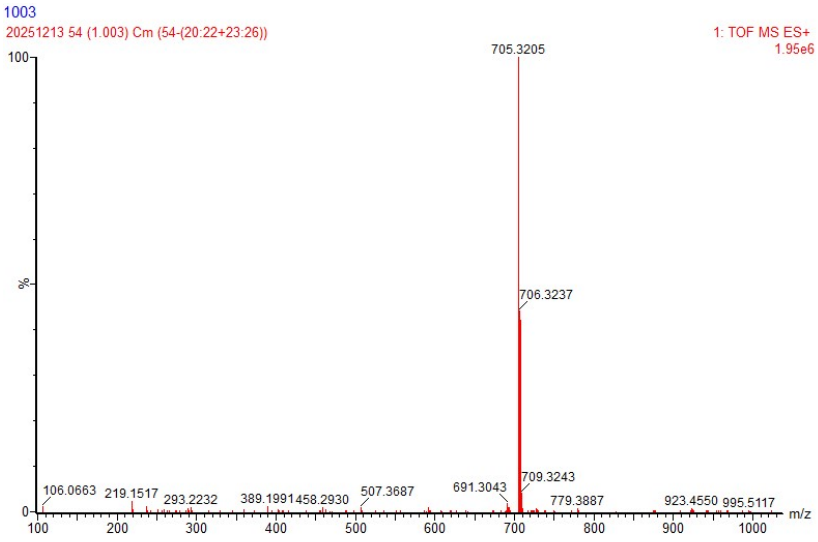
9.HRMS spectrum of DCM-LAP



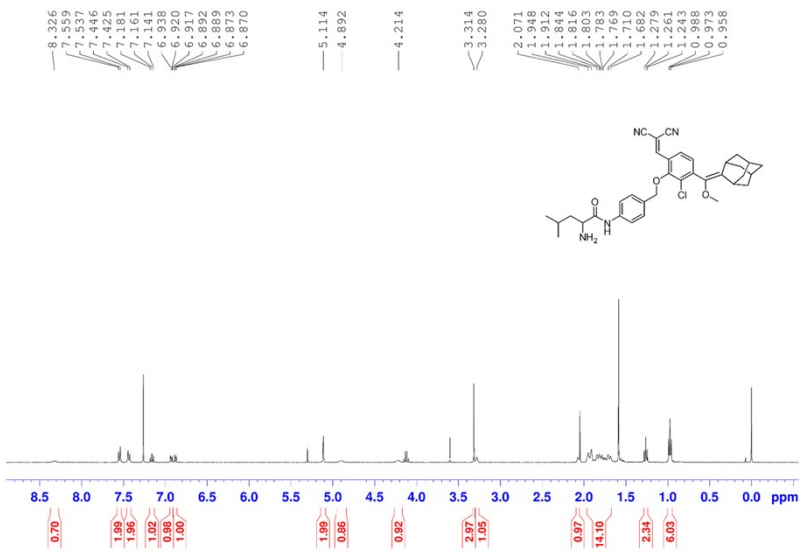

10.$^1$H NMR spectrum of DCM-Cl-LAP in $CDCl_3$



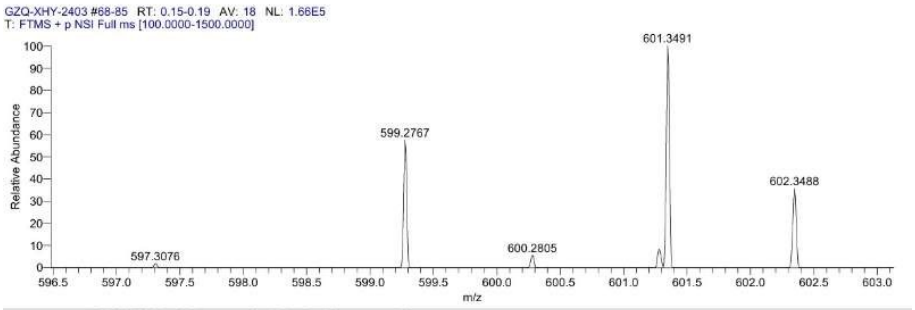

11. $^{13}C$ NMR spectrum of DCM-Cl-LAP in $CDCl_3$




12.HRMS spectrum of DCM-Cl-LAP




13. $^{1}H$ NMR spectrum of MAL-LAP in $CDCl_3$




GZQ-XHY-2403#67-85 RT: 0.15-0.19 AV: 19
T: FTMS + p NSI Full ms [100.0000-1500.0000]
m/z= 599.21-599.32

| m/z | Intensity | Relative | Theo. Mass | Delta (ppm) | Composition |
|---|---|---|---|---|---|
| 599.2767 | 91222.5 | 100.00 | 599.2783 | -1.63 | $C_{35}H_{40}O_3N_4Cl$ |

14.HRMS spectrum of MAL-LAP

**Reference**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