Supporting information

A near-infrared fluorescent probe for monitoring Leucine aminopeptidase in living cells

Yun Chai,[a, #] Yuting Gao,[b, #] Huiwen Xiong,[a] Wanqian Lv,[a] Guichun Yang,[a] Cuifen Lu,[a] Junqi Nie,[a] Chao Ma,[a] Zuxing Chen, *, [a] Jun Ren,[a] Feiyi Wang, *, [a]

[a] Ministry of Education Key Laboratory for the Synthesis and Application of Organic Functional Molecules & Hubei Collaborative Innovation Center for Advanced Organic chemical Materials, Hubei University, Wuhan 430062, P. R. China.

[b] National Engineering Research Center for Nanomedicine, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, P. R. China.

[#] These authors contributed equally to this work.

E-mail: wangfyi@hubu.edu.cn; chzux@hubu.edu.cn

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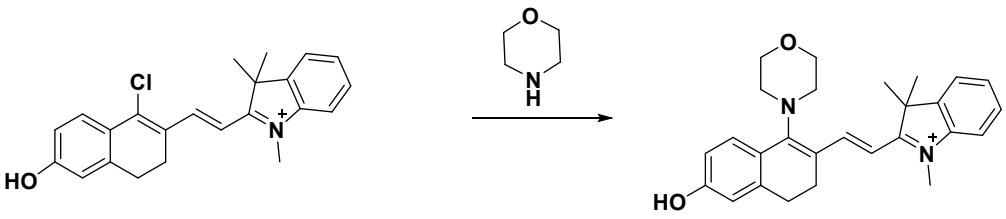
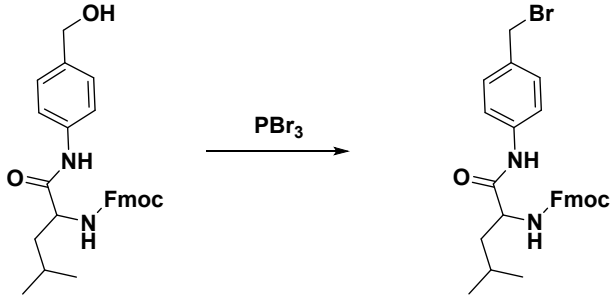
1. Synthesis of CHMC-M-Leu.



CHMC1 CHMC-M

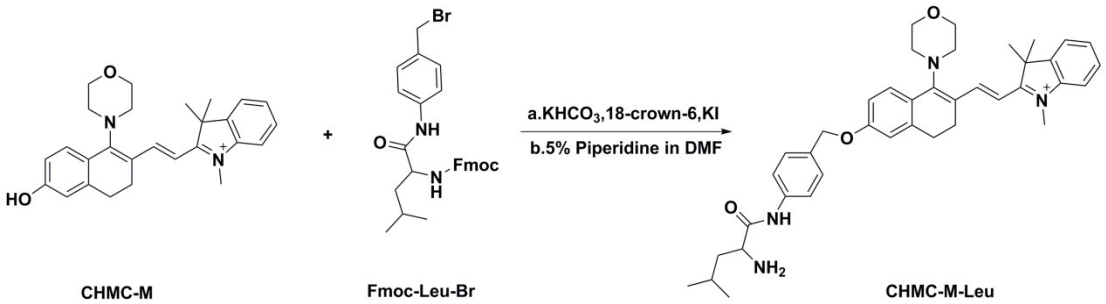
Synthesis of compound CHMC-M. CHMC1 (364 mg, 1.00 mmol) was added to an appropriate amount of CH_3CN and stirred for 10 min, followed by the addition of Morpholine (0.26 mL, 3.00 mmol), and the mixture reacted at room temperature for 30 min. It is then extracted with CH_2Cl_2 and purified by Silica gel column chromatography to give product CHMC-M (336 mg, 0.81 mmol), yield was 81%. ^{1}H NMR (400 MHz, CD_3OD): δ 7.97-7.93 (m, 1H), δ 7.65-7.63 (d, 1H), δ 7.51-7.49 (d, 1H), δ 7.44-7.40 (m, 1H), δ 7.29-7.24 (d, 2H), δ 6.76-6.71 (m, 3H), δ 6.20-6.16 (d, 1H), δ 4.00 (m, 8H), δ 3.62 (s, 3H), δ 2.18 (m, 3H), δ 1.71 (s, 6H); HRMS (ESI) calcd for $C_{27}H_{31}N_2O_2^+$: 415.23800, Found: 415.23845 $[M]^+$.



Fmoc-Leu-OH Fmoc-Leu-Br

Synthesis of compound Fmoc-Leu-Br. Compound Fmoc-Leu-OH (458 mg, 1.00 mmol) was dissolved in 10.0 mL of dry THF, followed by the addition of PBr_3 (142 uL, 1.50 mmol) in an ice bath, and the mixture was stirred at 0 °C for 2 h. The mixture was added to 10 mL of a saturated $NaHCO_3$ solution and then diluted with 100 mL of ice water. It is then extracted with EA and purified by Silica gel column chromatography to give product Fmoc-Leu-Br (396 mg, 0.76 mmol), yield was 76%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.07 (s, 1H), δ 7.77-7.75 (d, 2H), δ 7.57-7.37 (d, 6H), δ 7.28-7.20 (m, 4H), δ 6.02-6.00 (d, 1H), δ 4.51-4.46 (m, 1H), δ 4.43 (s, 2H), δ 4.39-

4.27 (m, 2H), δ 4.18-4.11 (m, 1H), δ 1.78-1.70 (m, 2H), δ 1.32-1.28 (m, 1H), δ 0.96-0.92 (d, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.34, 156.94, 143.67, 143.46, 141.25, 137.92, 133.55, 129.72, 127.79, 127.14, 124.99, 120.02, 67.39, 54.46, 46.97, 41.08, 33.42, 24.79, 22.94, 21.99; HRMS (ESI) calcd for $C_{28}H_{29}BrN_2O_3$: 520.13616, Found: 521.13772 $[M+H]^+$.



Synthesis of CHMC-M-Leu. CHMC-M (32.0 mg, 0.077 mmol), Fmoc-Leu-Br (160.2 mg, 0.308 mmol), $KHCO_3$ (20.6 mg, 0.15 mmol), 18-crown-6 (40.4 mg, 0.15 mmol) and KI (85.2 mg, 0.385 mmol) were mixed in 10.0 mL acetone, and the mixture was stirred at 40 ℃ under nitrogen for 10 h. After evaporation of the solvent, the residue was added to 5% Piperidine in DMF and stirred at room temperature for another 5 h. It is then extracted with CH_2Cl_2 and purified by Silica gel column chromatography to obtain the product: CHMC-M-Leu (6.3 mg, 0.010 mmol), yield was 13%. ^{1}H NMR (400 MHz, CD_3OD): δ 8.04-8.01 (m, 1H), δ 7.77-7.75 (d, 1H), δ 7.44-7.43 (d, 1H), δ 7.39-7.35 (m, 1H), δ 7.18-7.15 (d, 2H), δ 6.87-6.84 (d, 1H), δ 6.81 (s, 1H), δ 6.60-5.97 (d, 1H), δ 3.95-3.91 (m, 3H), δ 3.53 (s, 3H), δ 2.89-2.86 (m, 3H), δ 2.78-2.74 (m, 3H), δ 1.95-1.88 (m, 3H), δ 1.68 (s, 6H), δ 1.42-1.31 (m, 9H), δ 0.99-0.94 (m, 5H); ^{13}C NMR (100 MHz, CD_3OD): δ 170.96, 166.91, 163.09, 146.25, 143.32, 142.69, 140.04, 128.67, 128.13, 123.24, 121.65, 120.27, 114.86, 114.45, 109.04, 50.26, 38.15, 29.36, 29.03, 28.64, 27.47, 24.22, 21.93, 12.88; HRMS (ESI) m/z =633.4168 $[M]^+$.

2. Stability of CHMC-M-Leu and effects of the system temperature and pH.

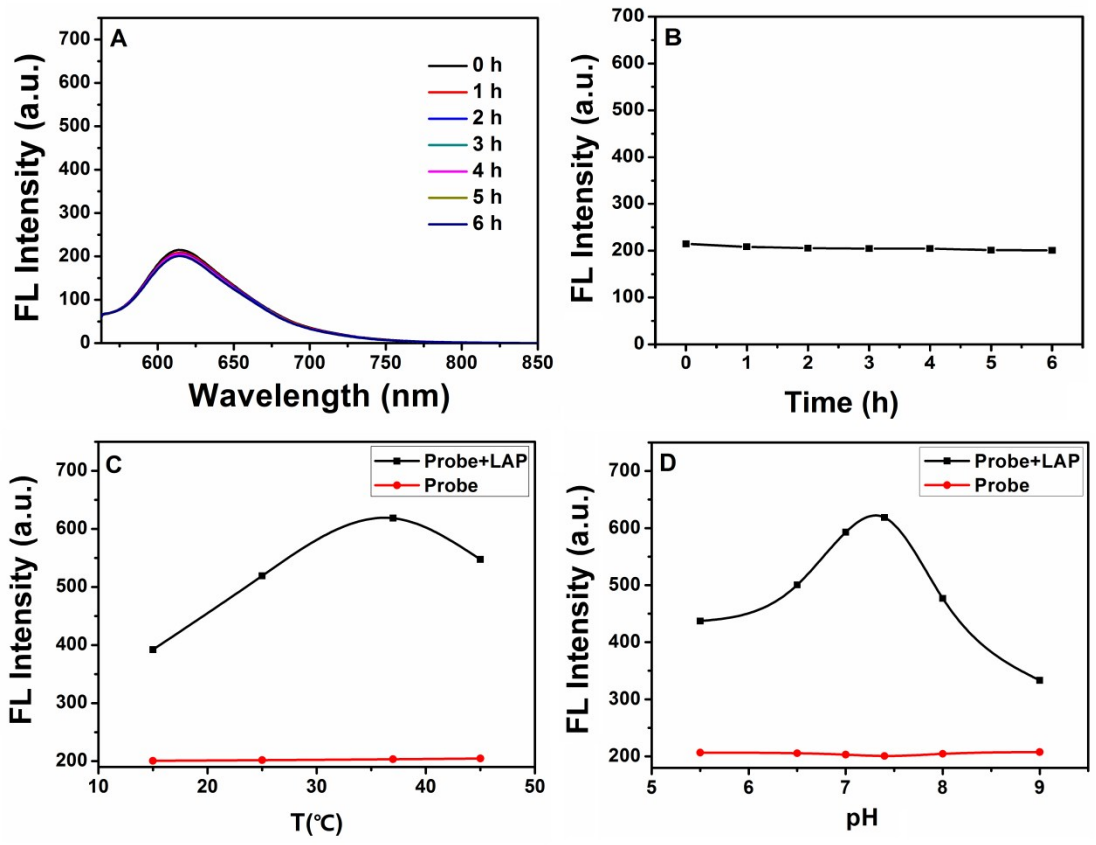


Figure S1. Stability of CHMC-M-Leu in PBS buffer solution (A and B, pH = 7.4, 37 ◦C). Effects of reaction temperature (C) and pH (D) on the fluorescence intensity at 625 nm of CHMC-M-Leu (10 μM) in the absence and presence of 0.15 U·mL^{-1} LAP for 30 min, λ_{ex} = 530 nm.

3. Time dependent fluorescence changes of probes with LAP and inhibitor .

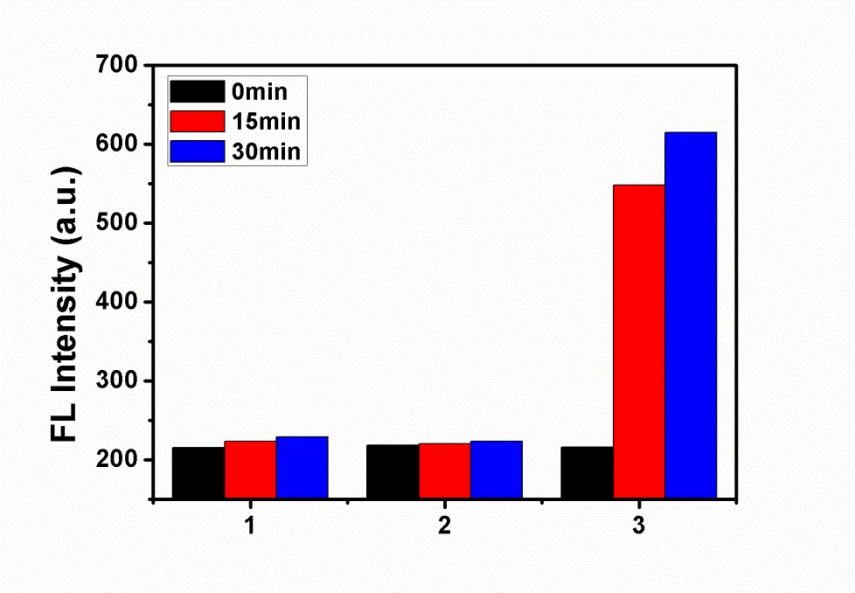


Figure S2. Fluorescence emission intensity at 625 nm (λ_{ex} = 530 nm) in different reaction system. 1) probe (10 μM) + 0.15 U·mL^{-1} LAP + 1.0 mM inobestin; 2) probe (10 μM) + 1.0 mM inobestin; 3) probe (10 μM) + 0.15 U·mL^{-1} LAP. The reaction was performed in PBS buffer solution (pH=7.4 , 37 ℃) .

4. HPLC and HRMS spectra for monitoring the reaction system.

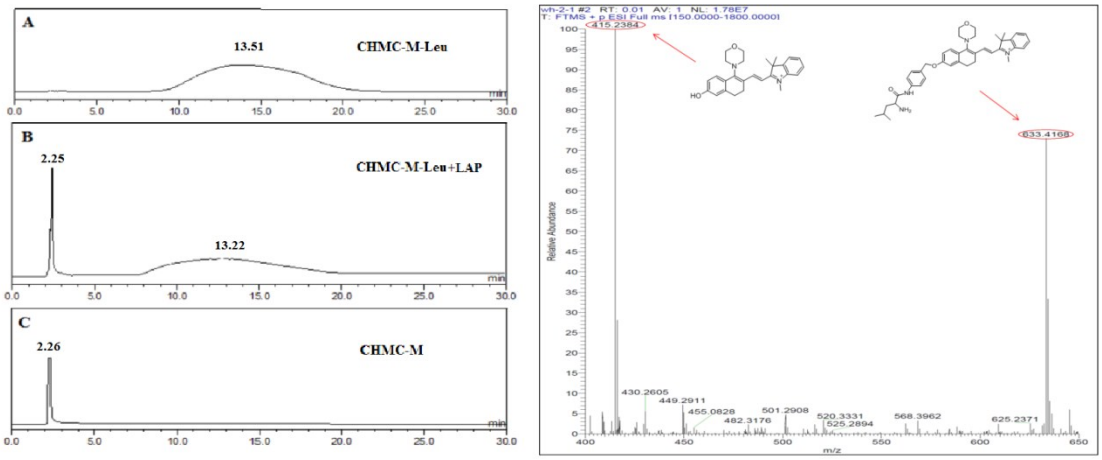


Figure S3. (A, B, C) HPLC analysis and HRMS spectra for monitoring the reaction between CHMC-M-Leu and LAP.

5. The titration experiments of probes with LAP.

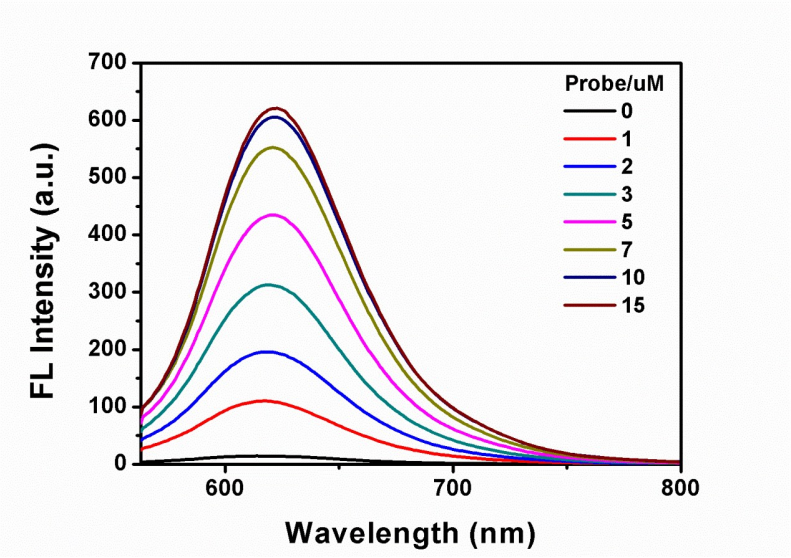


Figure S4. The emission spectra of different concentrations of CHMC-M-Leu (0, 1, 2, 3, 5, 7, 10, 15 μM, respectively) in the presence of LAP (0.15 $U \cdot mL^{-1}$). Data are recorded 30 min after the addition of analytes, λ_{ex} = 530 nm.

6. The detection limit of CHMC-M-Leu.

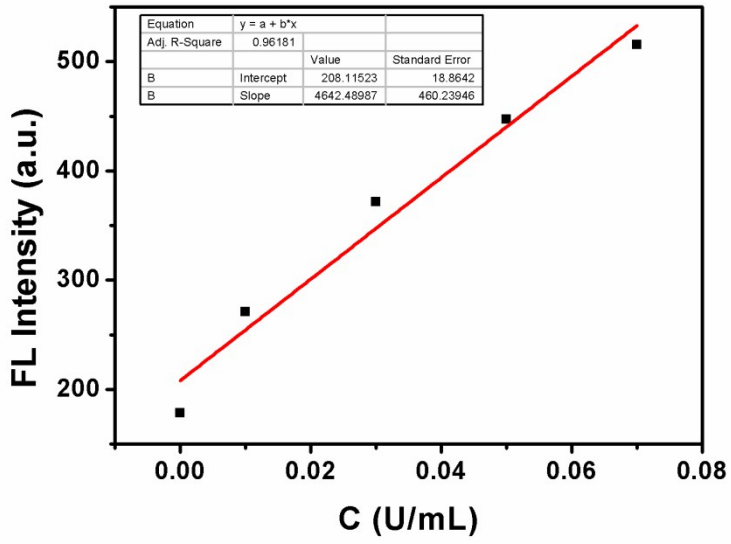


Figure S5. Fluorescence intensity at 625 nm changes with the concentration of LAP. Note: the detection limit was calculated to be 50.0 $ng \cdot mL^{-1}$ (3σ/slope , LAP: 18 $U \cdot mg^{-1}$) for LAP, λ_{ex} = 530 nm.

7. IC_{50} values for CHMC-M-Leu and L-Leucine-p-nitroanilide.

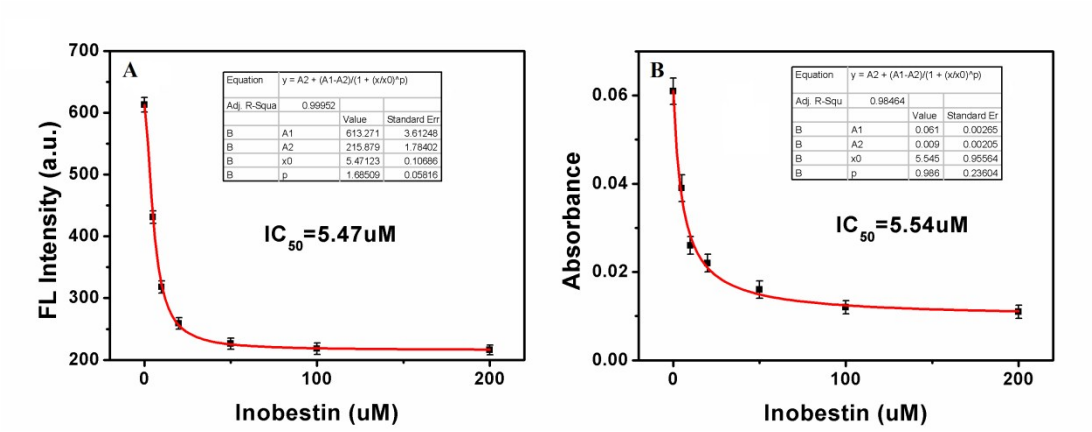


Figure S6. Plots of inhibitory kinetics of inobestin with CHMC-M-Leu (A) and L-Leucine-*p*-nitroanilide (B) as the substrate. The results are the mean ± S. D. (n=3).

8. Detection of LAP in the urine and plasma of normal subjects.

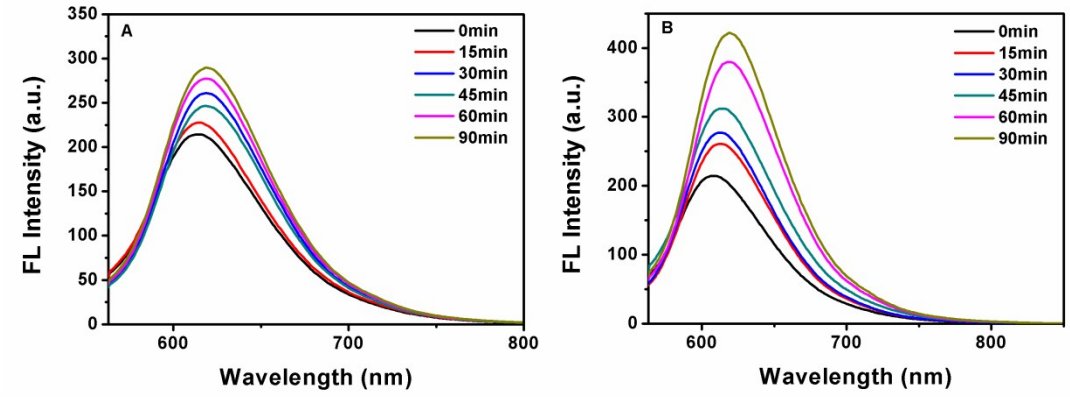


Figure S7. Time-dependent fluorescence and ratiometric intensity changes of CHMC-M-Leu (10 μM) in the presence of urine (20%, v/v , A) and human plasma (1%, v/v , B) in PBS buffer solution at 37 ℃ (pH = 7.4, λ_{ex}= 530 nm).

9. The MTT assays.

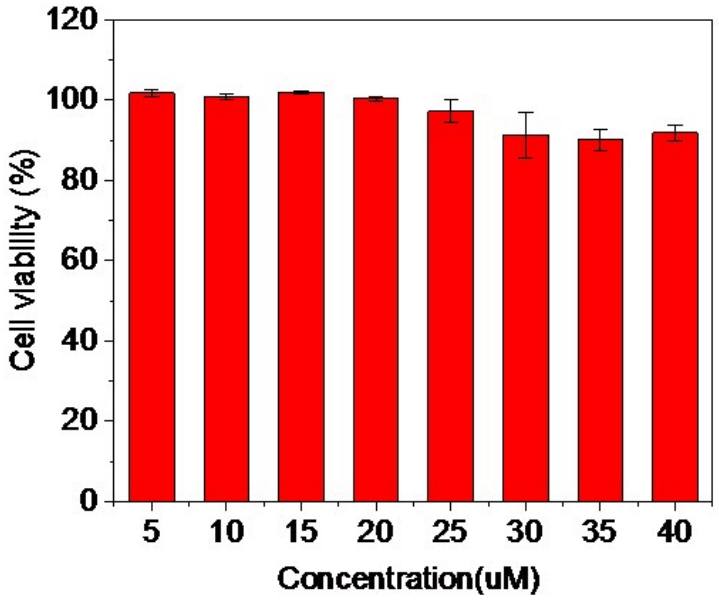


Figure S8. Viability of HeLa cells upon incubation with various concentrations of CHMC-M-Leu (5 – 40 μM) for 24 h. The results are the mean ± standard deviation of five separate measurements.

10. Cell imaging.

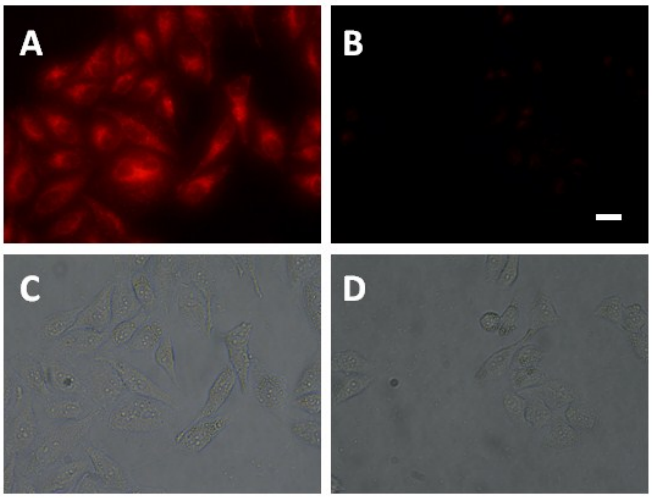
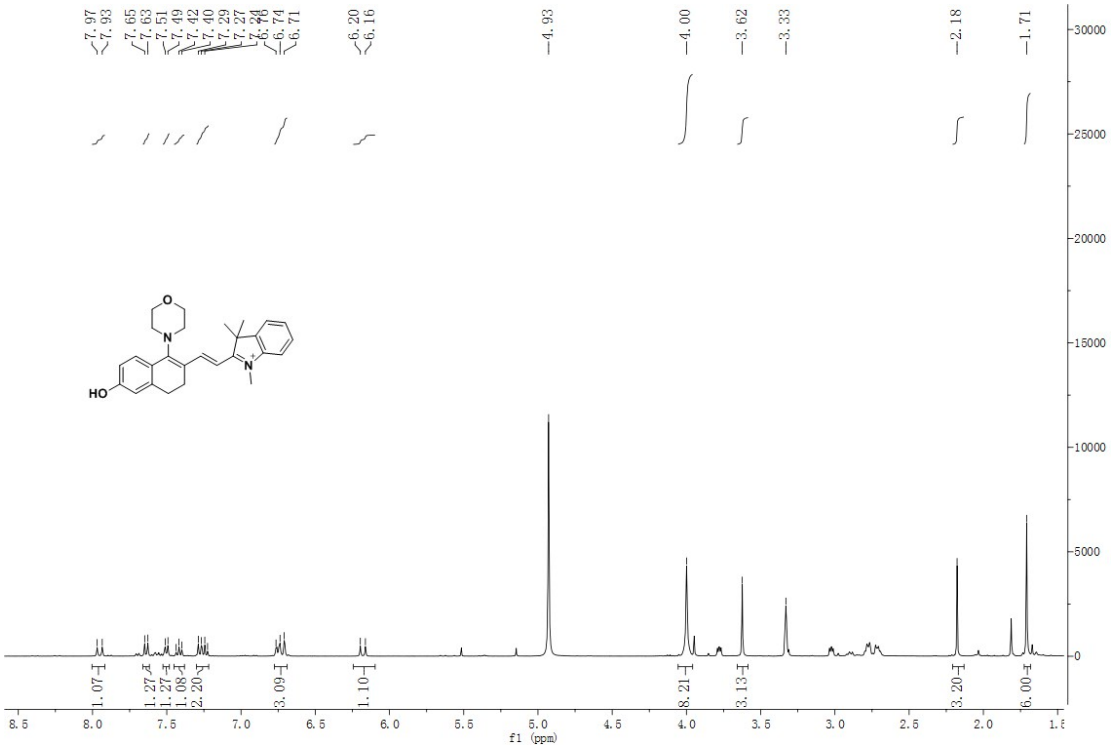


Figure S9. HeLa cells were pretreated with 2.0 $mg \cdot L^{-1}$ cisplatin for 6 h, and then treated with (B, D) or without (A, C) inobestin (100 μM) for 30 min. After that, CHMC-M-Leu (10 μM) was added and incubated for another 1 h.

9. NMR and HRMS spectra.



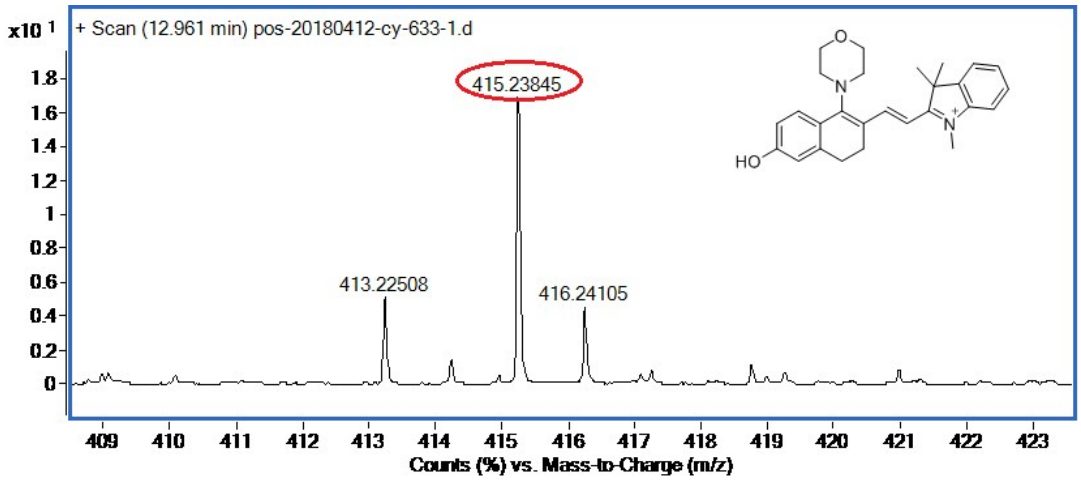
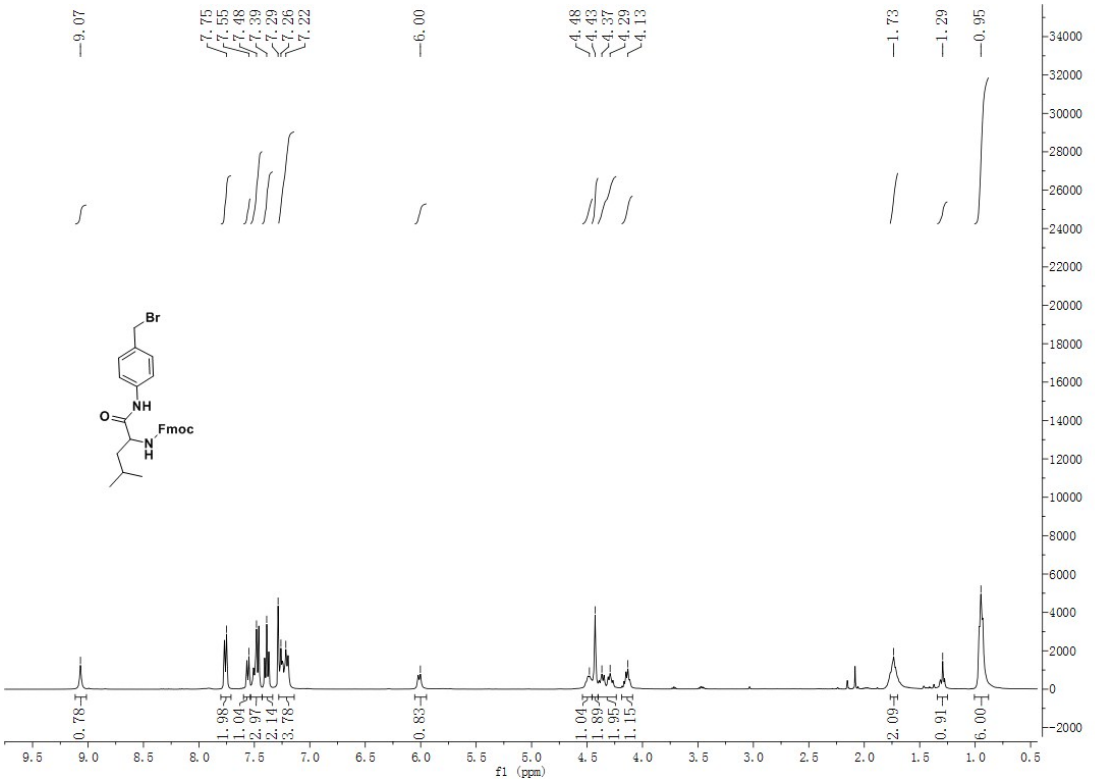


Figure S10. NMR and HRMS spectra of CHMC-M.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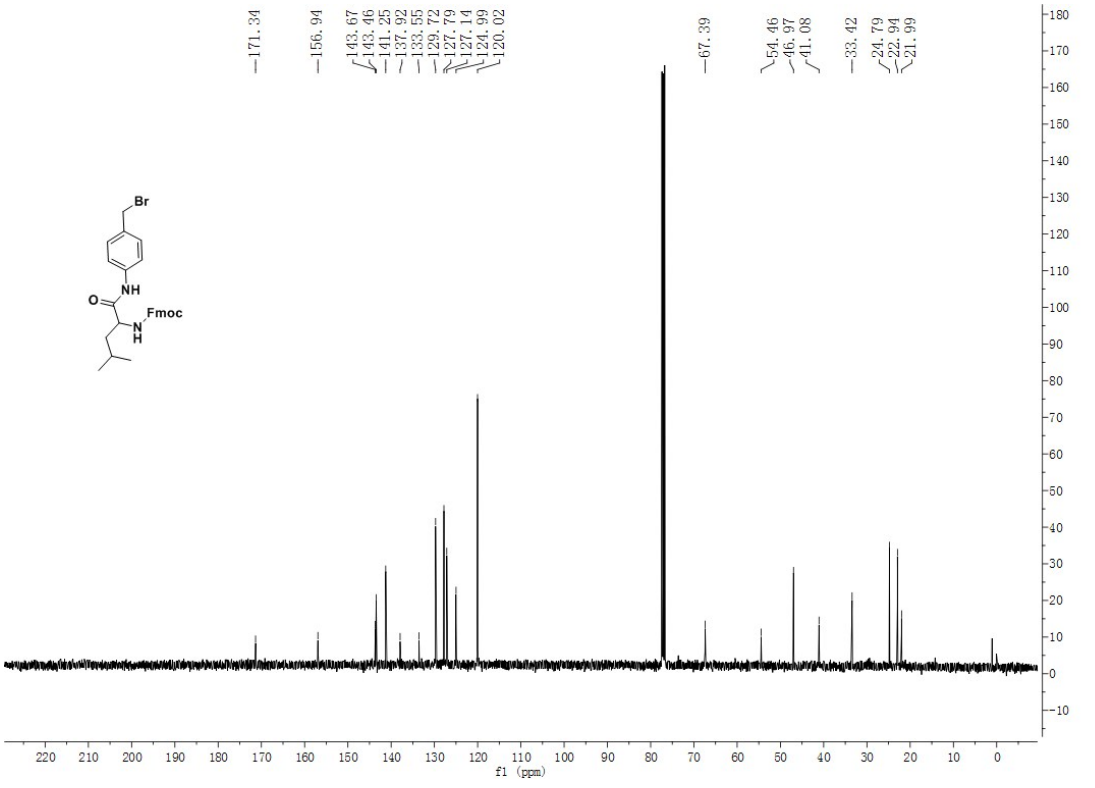
7.48
7.39
7.29
7.26
7.22
6.00
4.48
4.43
4.37
4.29
4.13
1.73
1.29
0.95
Br
NH
O
Fmoc
N
H
0.78
1.98
1.04
2.97
2.14
3.78
0.83
1.04
1.89
1.95
1.15
2.09
0.91
6.00
f1 (ppm)


171.34
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143.46
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67.39
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f1 (ppm)

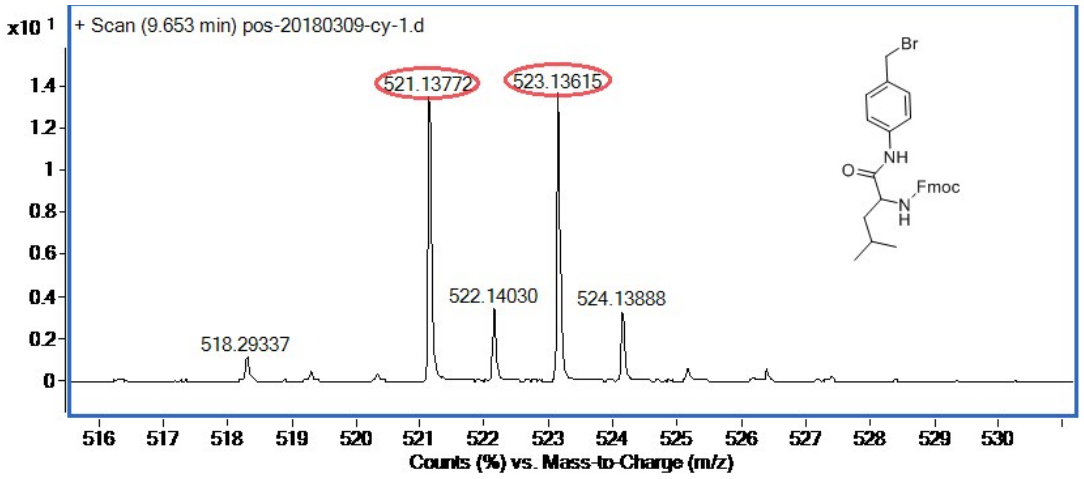
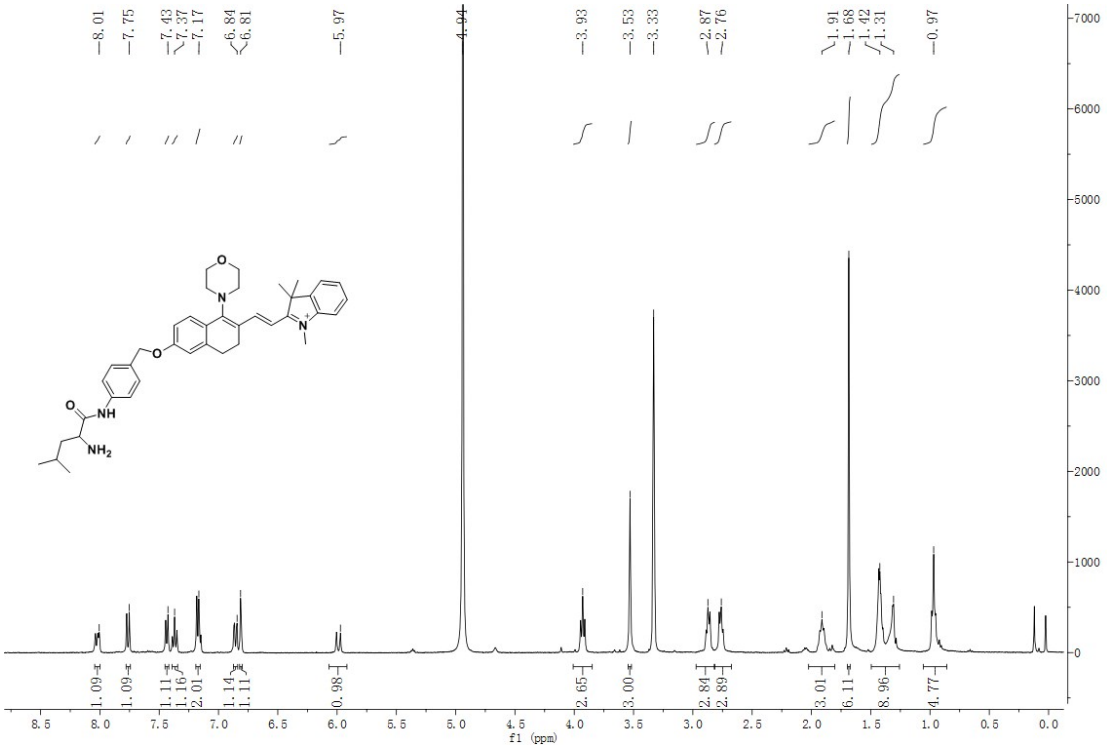
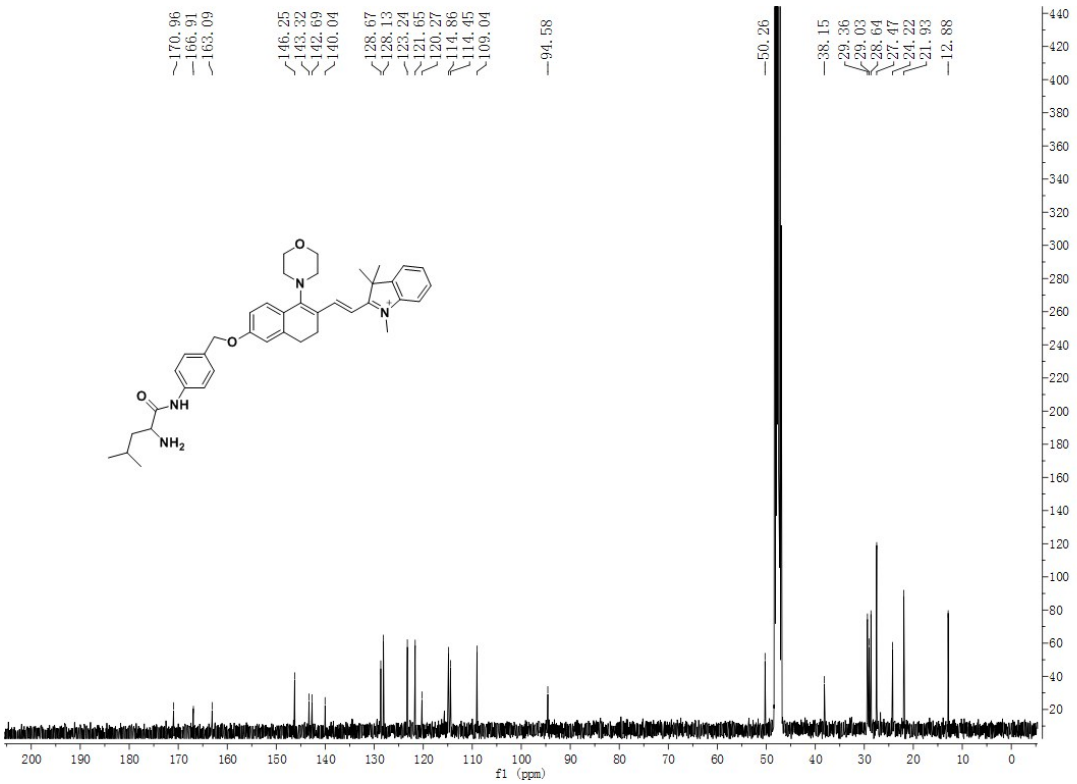


Figure S11. NMR and HRMS spectra of Fmoc-Leu-Br.





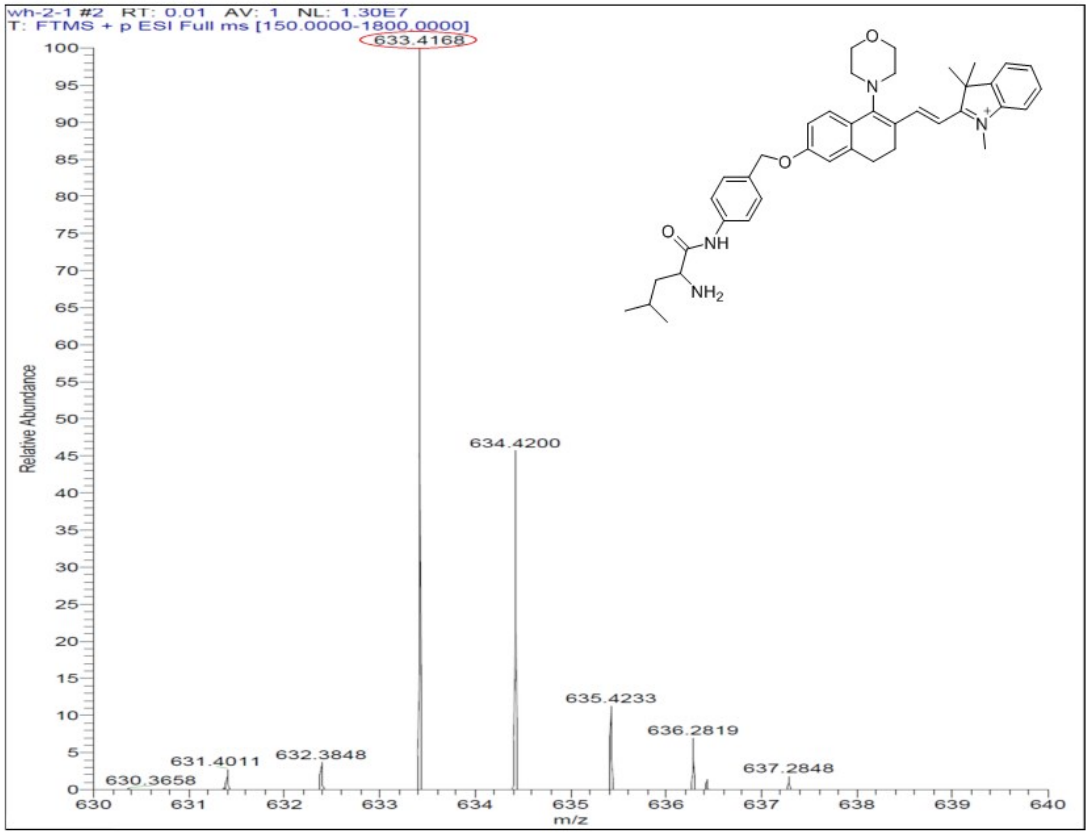


Figure S12. NMR and HRMS spectra of CHMC-M-Leu.