ELECTRONIC SUPPLEMENTARY INFORMATION

Ratiometric Fluorescence Probe for Selective and Sensitive Detection of Leucine Aminopeptidase in Lysosome

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Contents:

1. Materials and general methods

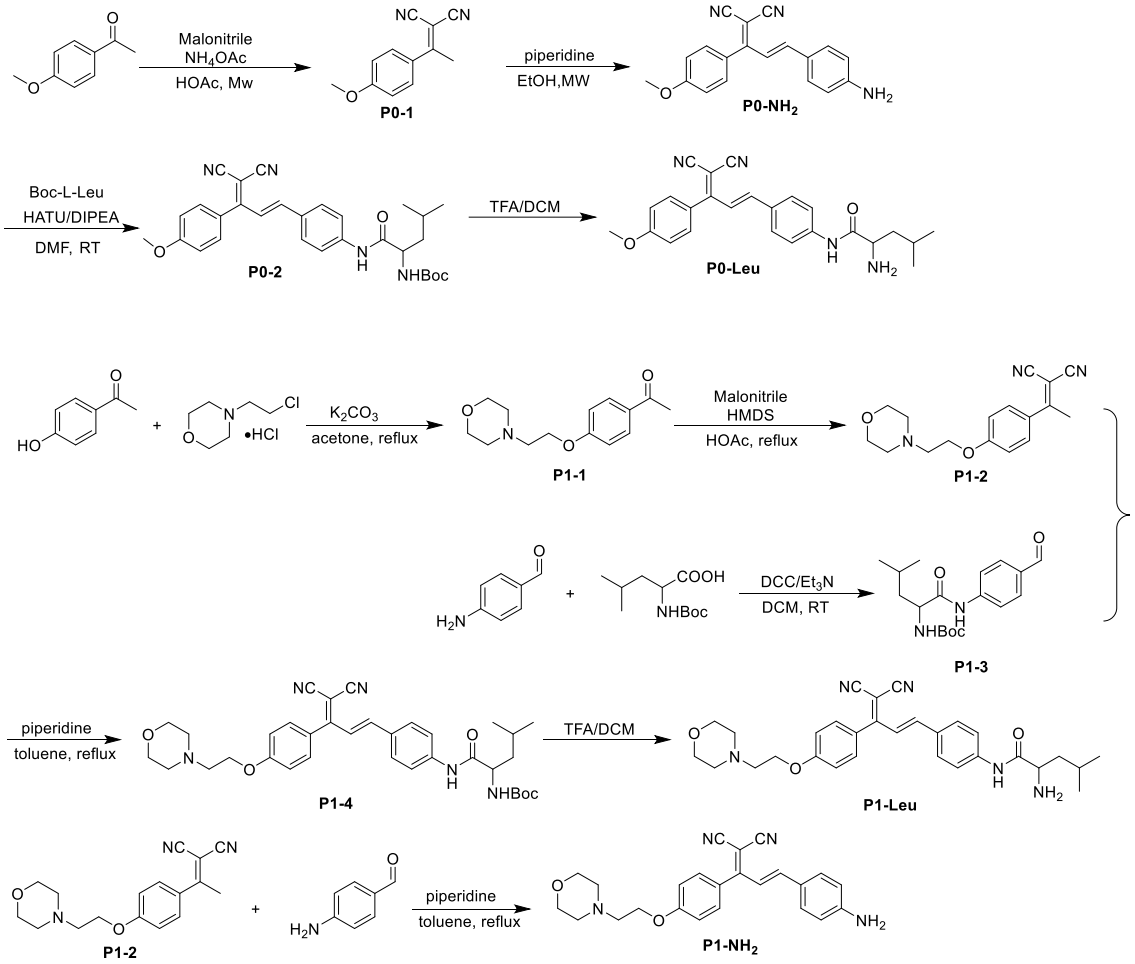
1.1. Chemicals

Unless otherwise stated, all reagents for synthesis were obtained commercially and used without further purification. Dicyclohexylcarbodiimide (DCC) and N-(2-chloroethyl) morpholine hydrochloride were purchased from Aladdin, malonitrile was purchased from TCI. Boc-*L*-Leucine and trifluoroacetic acid (TFA) were purchased from Innochem. 4-aminobenzaldehyde and 4-methoxyacetophenone were purchased from Bidepharm, 4-hydroxyacetophenone was purchased from Macklin, HATU was purchased from oka, N,N-Diisopropylethylamine (DIPEA) was purchased from csbio. LysoBlue was obtained from KeyGEN BioTECH Co., Ltd, Nanjing, China. Dulbecco's modified Eagle's media (DMEM) was purchased from gibco. Other chemical reagents were purchased from Beijing Chemical Plant or Beijing Reagent.

1.2. Instrumentation

^{1}H NMR and ^{13}C NMR spectrum were measured with a Bruker Avance 400 MHz spectrometer. The fluorescence spectrum was measured by the Hitachi F-4600 fluorescence spectrophotometer. Mass spectrometry was obtained by the liquid chromatography-mass spectrometer (Xevo G2 Qtof). UV absorption was obtained by UV-3600 ultraviolet visible near infrared spectrophotometer. The cell image was taken using a laser scanning confocal microscope (Leica SP8 and Zeiss LSM880). Calculation of Pearson correlation coefficient and fluorescence intensity of cells were done by Image Pro. Molecular docking calculations were done by Discovery Studio.

1.3. Synthesis



Scheme S1. Synthetic route for probe probe **P1-Leu** and **P0-Leu**.

2-(1-(4-methoxyphenyl)ethylidene)malononitrile (P0-1)

P0-1 was synthesized on the basis of previous work [refer. A, Didier Villemin, et al. "Solventless convenient synthesis of new cyano-2-aminopyridine derivatives from enaminonitriles." *Tetrahedron Letters* 54. 13(2013):1664-1668.]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 9.0 Hz, 2H), 7.02 (d, *J* = 9.0 Hz, 2H), 3.90 (s, 3H), 2.64 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.09, 163.14, 129.87, 127.90, 114.49, 113.73, 113.45, 81.87, 55.64, 23.84. HR-MS (ESI): calculated for $C_{12}H_9N_2O$ ([M]$^-$) 197.0715, found 197.0709.

(E)-2-(3-(4-aminophenyl)-1-(4-methoxyphenyl)allylidene)malononitrile(P0-NH_2)

To a stirred solution of **P0-1** (198 mg, 1 mmol, 1 equiv.) and 4-aminobenzaldehyde (121 mg, 1 mmol, 1 equiv.) in EtOH (5 mL), was added piperidine (85 mg, 1 mmol, 1 equiv.). The mixture was heated to 85 °C in microwave synthesizer within 5 min and held for 30 min. After the reaction was completed, the mixture was allowed to cool down to RT and red solids were precipitated. The mixture was filtered and the resulting precipitate was recrystallized with ethanol to obtain the purplish red product **P0-NH_2** (210 mg, yield 70%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.30 (m, 5H), 7.07 – 6.96 (m, 2H), 6.83 (d, *J* = 15.3 Hz, 1H), 6.66 (d, *J* = 8.2 Hz, 2H), 3.89 (s, 3H). HR-MS (ESI): calculated for $C_{19}H_{15}N_3O$ ([M]$^-$) 300.1137, found 300.1010.

tert-butyl (1-((4-formylphenyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (P0-2).

To a stirred solution of HATU (184 mg, 0.48 mmol, 3 equiv), Boc-*L*-Leucine (56 mg, 0.24 mmol, 1.5 equiv.) and DIPEA (62 mg, 0.48 mmol, 3 equiv) in dry DMF (2 mL), was added **P0-NH$_2$** (48 mg, 0.16 mmol, 1 equiv) in dry DMF (2 mL) drop by drop at 0 °C. The mixture was stirred at room temperature overnight. After the reaction was completed, monitored by thin-layer chromatography (TLC), the mixture was quenched by water (100 mL). The yellow precipitation was centrifuged, then dissolved with ethyl acetate and washed with saturated salt water. After drying with anhydrous sodium sulfate, the solvent was evaporated to obtain the crude product **P0-2**, which was directly used for the next step without further purification.

(E)-2-amino-N-(4-(4,4-dicyano-3-(4-methoxyphenyl)buta-1,3-dien-1-yl)phenyl)-4-methylpentanamide (P0-Leu)

A solution of **P0-2** (51 mg, 0.1 mmol, 1 equiv.) in dichloromethane (DCM)/TFA = 1:1 (2 mL) was stirred at RT for 4 hours. Then the reaction mixture was neutralized with $NaHCO_3$ until pH = 7. The solution was extracted by EtOAc (10 mL*3), and the organic phase was dried over by anhydrous Na_2SO_4. After removing the solvent in vacuo, **P0-Leu** was purified by column chromatography using DCM/methanol (V:V = 20:1) as eluent to get the product (37 mg, yield 90%).^{1}H NMR (400 MHz, DMSO) δ 7.74 (t, *J* = 12.5 Hz, 2H), 7.68 (d, *J* = 8.8 Hz, 2H), 7.48 (dd, *J* = 17.2, 12.1 Hz, 3H), 7.16 (d, *J* = 8.7 Hz, 2H), 6.94 (d, *J* = 15.5 Hz, 1H), 3.88 (s, 3H), 1.75 (dq, *J* = 13.1, 6.7 Hz, 1H), 1.47 (ddd, *J* = 13.6, 8.3, 5.5 Hz, 1H), 1.33 (ddd, *J* = 14.8, 10.3, 5.9 Hz, 1H), 0.94 – 0.83 (m, 6H). ^{13}C NMR (101 MHz, DMSO) δ 175.25, 170.85, 161.69, 148.49, 142.15, 131.28, 130.17, 129.05, 125.09, 122.68, 119.29, 114.58, 114.37, 113.70, 78.70, 55.46, 54.05, 43.78. HR-MS (ESI): calculated for $C_{25}H_{26}N_4O_2$ ([M]$^-$) 413.1978, found 413.1960.

1-(4-(2-morpholinoethoxy)phenyl)ethan-1-one (P1-1)

To a stirred solution of 4-hydroxyacetophenone (136 mg, 1 mmol, 1 equiv.) in acetone (2 mL), was added K_2CO_3 (346 g, 2.5 mmol, 2.5 equiv.) and N-(2-chloroethyl)morpholine hydrochloride (186 mg, 1 mmol, 1 equiv.). The mixture was refluxed at 60^{o}C for 2 hours. Then the reaction was allowed to cool down to RT, the reaction mixture was concentrated in vacuo, the solid was treated with 20 mL water, the aqueous phase was extracted by EtOAc (20 mL*3), and **P1-1** was purified by column chromatography using ethyl acetate: triethylamine = 40:1 (V/V) as eluent to get the product (187 mg, yield 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.9 Hz, 2H), 6.96 (d, *J* = 8.9 Hz, 2H), 4.20 (t, *J* = 5.7 Hz, 2H), 3.87 – 3.66 (m, 4H), 2.85 (t, *J* = 5.7 Hz, 2H), 2.64 – 2.58 (m, 4H), 2.57 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 196.65, 162.58, 130.55, 130.42, 114.22, 66.86, 66.04, 57.41, 54.08, 26.32. HR-MS (ESI): calculated for $C_{14}H_{20}NO_3$ ([M]$^+$) 250.1443, found 250.1467.

2-(1-(4-(2-morpholinoethoxy)phenyl)ethylidene)malononitrile (P1-2)

To a stirred solution of **P1-1** (249 mg, 1 mmol, 1 equiv.) and malonitrile (132 mg, 2 mmol, 2 equiv.) in HOAc (1 mL), was added hexamethyldisilazane (HMDS, 194 mg, 1.2 mmol, 1.2 equiv.). The mixture was refluxed at 60℃ for 8 hours. Then the reaction was allowed to cool down to RT, and the reaction mixture was neutralized with Na_2CO_3 until there was a white solid precipitated. Then the solution was extracted by EtOAc (10 mL*3), and dried over anhydrous Na$_2$SO4, and the organic solvent was concentrated in vacuo to get the yellow oily product, which was directly used for the next step without further purification.

tert-butyl (1-((4-formylphenyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (P1-3)

To a stirred solution of 4-aminobenzaldehyde (121 mg, 1 mmol, 1 equiv.) and Boc-L-Leucine (231 mg, 1 mmol, 1 equiv.) in DCM (2 mL), was added DCC (206 mg, 1 mmol, 1 equiv.). The mixture was stirred at room temperature for 14 hours. The filtrate was concentrated by vacuum and dissolved with ethyl acetate. The solution was washed successively with aqueous $NaHCO_3$, NH_4Cl, and saturated NaCl. The organic phase

was dried with Na_2SO_4, then purified by column chromatography using dichloromethane/methanol (V:V=30:1) to get the crude product, which was used directly for the next step.

(E)-2-amino-N-(4-(4,4-dicyano-3-(4-(2-morpholinoethoxy)phenyl)buta-1,3-dien-1-yl)phenyl)-4-methylpentanamide (P1-Leu)

To a stirred solution of **P1-2** (89 mg, 0.3 mmol, 1 equiv.) and **P1-3** (100 mg, 0.3 mmol, 1 equiv.) in toluene (1 mL), was added piperidine (26 mg, 0.3 mmol, 1 equiv.). The mixture was refluxed at 115 °C for 4 hours. After the reaction was completed as indicated by TLC, the mixture was allowed to cool down to RT, and purified by preparative TLC, using DCM/methanol (20/1) as eluent to obtain the crude product **P1-4**, which was directly used for the next step without further purification. A solution of **P1-4** (60 mg, 0.1 mmol, 1 equiv.) in DCM/TFA = 1:1 (2 mL) was stirred at RT for 4 hours. Then the reaction mixture was neutralized with $NaHCO_3$ until pH=7. The solution was extracted by EtOAc (10 mL*3), and the organic phase was dried over by anhydrous Na_2SO_4. After removing the solvent in vacuo, **P1-Leu** was purified by column chromatography using DCM/methanol (V:V =10:1) as eluent to get the product (46 mg, yield 89%).^{1}H NMR (400 MHz, DMSO) δ 7.76 (d, *J* = 8.8 Hz, 2H), 7.68 (d, *J* = 8.8 Hz, 2H), 7.47 (dd, *J* = 12.1, 10.8 Hz, 3H), 7.17 (d, *J* = 8.8 Hz, 2H), 6.96 (s, 1H), 4.20 (t, 2H), 3.63 – 3.56 (m, 4H), 2.74 (t, *J* = 5.6 Hz, 2H), 1.75 (dq, *J* = 19.7, 6.5 Hz, 1H), 1.48 (ddd, *J* = 13.6, 8.2, 5.5 Hz, 1H), 1.40 – 1.30 (m, 1H), 0.90 (dd, *J* = 9.4, 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.13, 171.09, 161.29, 148.62, 141.16, 131.11, 130.12, 129.98, 125.53, 123.44, 119.55, 115.07, 114.26, 113.53, 80.20, 77.48, 77.36, 77.16, 76.84, 66.96, 66.12, 57.56, 54.18, 54.04, 43.87, 25.11, 23.51, 21.40. HR-MS (ESI): calculated for $C_{30}H_{35}N_5O_3$ ($[M]^-$) 512.2622, found 512.2635.

(E)-2-(3-(4-aminophenyl)-1-(4-(2-morpholinoethoxy)phenyl)allylidene)malononitrile (P1-NH_2)

To a stirred solution of **P1-2** (48 mg, 0.16 mmol, 1 equiv.) and 4-aminobenzaldehyde (20 mg, 0.16 mmol, 1 equiv.) in toluene (1 mL), was added piperidine (14mg, 0.16mmol, 1 equiv.). The mixture was refluxed at 115 °C for 4 hours. After the reaction was completed as indicated by TLC, the mixture was allowed to cool down to RT, and purified by preparative TLC, using DCM/methanol (V/V= 20:1) as eluent to obtain the purplish red product **P1-NH_2** (40 mg, yield 63%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.30 (m, 5H), ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.28 (m, 5H), 7.02 (d, *J* = 8.7 Hz, 2H), 6.82 (d, *J* = 15.3 Hz, 1H), 6.63 (d, *J* = 8.5 Hz, 2H), 4.18 (t, *J* = 5.6 Hz, 4H), 3.77 – 3.72 (m, 4H), 2.85 (t, *J* = 5.6 Hz, 2H), 2.69 – 2.51 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.40, 160.89, 150.43, 149.73, 131.29, 130.97, 125.92, 124.61, 120.34, 114.84, 114.73, 113.99, 66.81, 65.92, 57.48, 54.06. HR-MS (ESI): calculated for $C_{24}H_{23}N_4O_2$ ($[M]^-$) 399.1821, found 300.1798.

2. General Procedure for Spectral Measurements.

Unless otherwise noted, all the spectral measurements were performed in 20 mM phosphate buffer saline (pH 7.4, PBS) according to the following procedure. To a test tube, a certain amount of 20 mM phosphate buffer was added, followed by addition of 10 μL **P1-Leu** stock solution in DMSO (2 mM) and appropriate volume of LAP solution, and the final volume was 1 mL with 1% DMSO (v/v). After incubation on shaker at 37 °C in thermostat for 2 h, the absorption and fluorescence spectra were measured.

3. Colocalization Fluorescence Imaging of HepG2 cells.

The DMEM was added 10% fetal bovine serum, 1% penicillin, 1% streptomycin as medium, and cells were cultured in an environment at 37 °C with 5% CO_2 and 95% air.
Co-localization experiments were performed according to literature method. In brief, cells were seeded in glass bottom dishes and allowed to adhere for 24 h, then LysoBlue (10 μM) and **P1-Leu** (5 μM) were added in serum free media to incubate for 1 h at 37 °C. In the fluorescence imaging experiments, LysoBlue was excited at 405 nm, and the emissions were collected at 410-450 nm; **P1-Leu** was excited at 405 nm and emissions were collected at 490-540 nm for green channel and 555-605 nm for red channel. **P1-NH_2** was excited at 405 nm and emissions were collected at 555-605 nm.

4. Cytotoxicity Assay.

HepG2 cells and L02 cells were placed in 96-well plates and incubated in cell culture tanks for 24 h. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was used to determine cytotoxicity. Cells in 96-well plates were added with different concentrations of **P1-Leu** and incubated in an incubator for 12 h. Cells were then treated with 20 μL MTT solution (5 mg/ml) for 4 h. Finally, the supernatant was removed, 100 μL DMSO was added to each well, and the UV absorption intensity of 490 nm was measured after 15 minutes.

5. Imaging of Endogenous LAP Levels in HepG2 cells.

HepG2 cells were seeded in glass-bottom dishes and adhered, followed by incubating with 5 μM **P1-Leu** in serum free media for 0.5 h at 37 °C. Then fluorescence imaging is measured as above. For inhibiting assays, HepG2 or L02 cells were pretreated with bestatin (100 μM) for 1 h before incubation with **P1-Leu**.

6. Linear range and detection limit of this work and other references

Table S1. Linear range and Detection limit of this D-A-D probe and other D-π-A probes

Analytes	Linear range	Limit of detection	References
γ-Glutamyltranspeptidase	0-20 U/L	0.16 U/L	*Chem. Commun*., **2016**, 52, 10400-10402
Monoamine oxidase A	0.5-1.5 and 0.5-2.5 μg/mL	1.1 and 10 ng/mL	*Anal. Chem*. **2016**, 88, 1440−1446
Alkaline phosphatase	0.5-5 U/L	0.38 U/L	*J. Mater. Chem. B*. **2015**, 3, 1042-1048
Alkaline phosphatase	0-150 U/L	0.15 U/L	*ACS Appl. Mater. Interfaces.* **2014**, 6, 19, 17245–17254
LAP	0-0.6 U/mL	46 ng/mL	*ACS Appl. Mater. Interfaces.* **2016**, 8, 40, 26622–26629
LAP	0-0.2 U/mL	41.9 ng/mL	*Anal. Chem*. **2017**, 89, 21, 11576–11582
β-Galactosidase	0-12 U/mL	0.17 U/L	*J. Am. Chem. Soc.* **2016**, 138, 16, 5334–5340
β-Galactosidase	0-8 μg/mL		*Sensor Actuat. B: Chem*. **2018**, 262, 508–515
Tyrosinase	0-160 U/mL	0.2 U/mL	*Analyst*. **2018**, 143, 4476-4483
γ-Glutamyltranspeptidase	1-20 U/L	0.3 U/L	*New J. Chem.* **2018**, 42, 5403-5407
LAP	0-10 U/L	0.016 U/L (2.2 ng/mL)	This work

Table S2. Linear range and detection limit of this LAP probe and other references

Linear range	Limit of detection	References
0-100 U/L	50.35 ng/mL	*Dyes Pigm*. **2021**, *187*, 109145.
0-70 U/L	50 ng/mL	*Analyst.* **2019**, *144*, 463−467.
0.1-0.6 U	46 ng/mL	*ACS Appl. Mater. Inter*. **2016**, *8*, 26622-26629.
0-250 U/L	42.2 ng/mL	*Sensor Actuat. B: Chem*. **2020**, *321*, 128631.
0-100 U/L	41.9 ng/mL	*Anal. Chem.* **2017**, *89*, 11576−11582.
0-5 U/L	8.9 ng/mL	*Anal. Chim. Acta.* **2018**, 1031, 169−177.
0-40 U/L	0.61 ng/mL	*Anal. Chem.* **2018**, *90*, 9359-9365.
0-26 ng/mL	0.42 ng/mL	*Chem. Sci.* **2016**, *7*,788−792.
0.4-14 ng/mL	0.38 ng/mL	*Anal. Chem*. **2017**, *89*, 12319−12326.
0-75 U/L	6.7 U/L	*Chem. Sci.* **2021**, *12*, 14855–14862
0-50 U/L	3.0 U/L	*Chem. Commun*. **2021**, *57*, 6608–6611
0-140 U/L	0.37 U/L	*Acta, Part A.* **2021**, *249*, 119328.
0-50 U/L	0.2 U/L	*Anal. Chim. Acta.* **2021**, *1168*, 338621
1-50 U/L	0.19 U/L	*Chem. Sci*. **2017**, *8*,3479−3483
0.1-1 U/L	0.08 U/L	*J. Am. Chem. Soc.* **2019**, 141, 6352−6361
0-70 U/L	0.049 U/L	*Tetrahedron.* **2021**, *99*, 132449
0-2 U/L	0.047 U/L	*Anal. Chem.* **2019**, *91*, 8085−8092
2-12 U/L	0.011 U/L	*J. Mater. Chem. B.* **2021**, *9*, 8842–8850
0-10 U/L	0.016 U/L (2.2 ng/mL)	This work

7. Fluorescence analysis, HR-MS, molecular docking calculations and cell experiments.

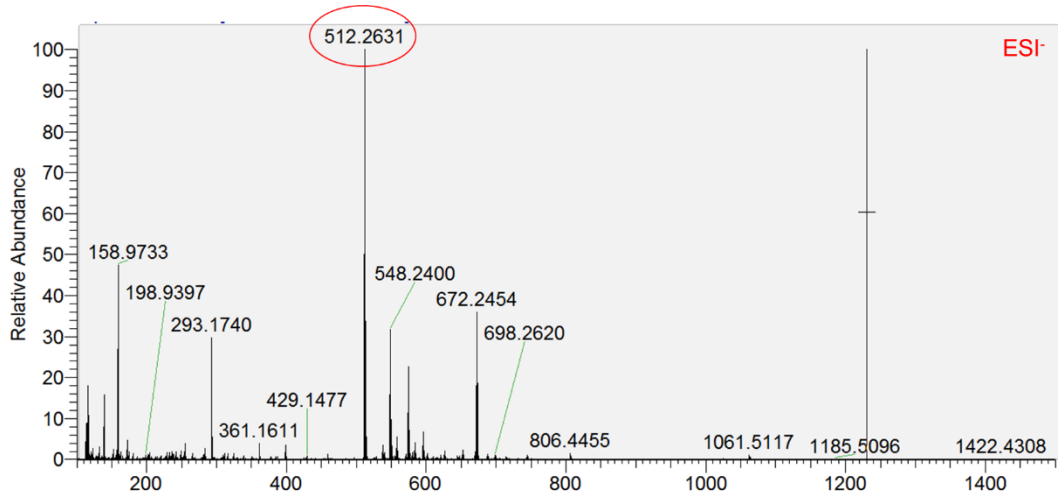


Figure S1. Electronspray ionization mass spectral of **P1-Leu**.

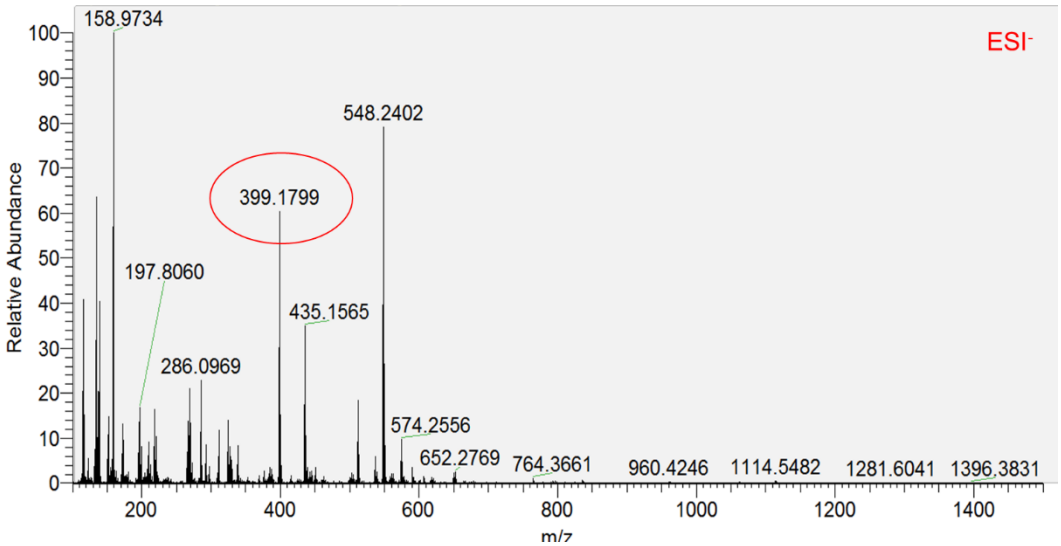


Figure S2. Electronspray ionization mass spectral of **P1-Leu** after the response with LAP.

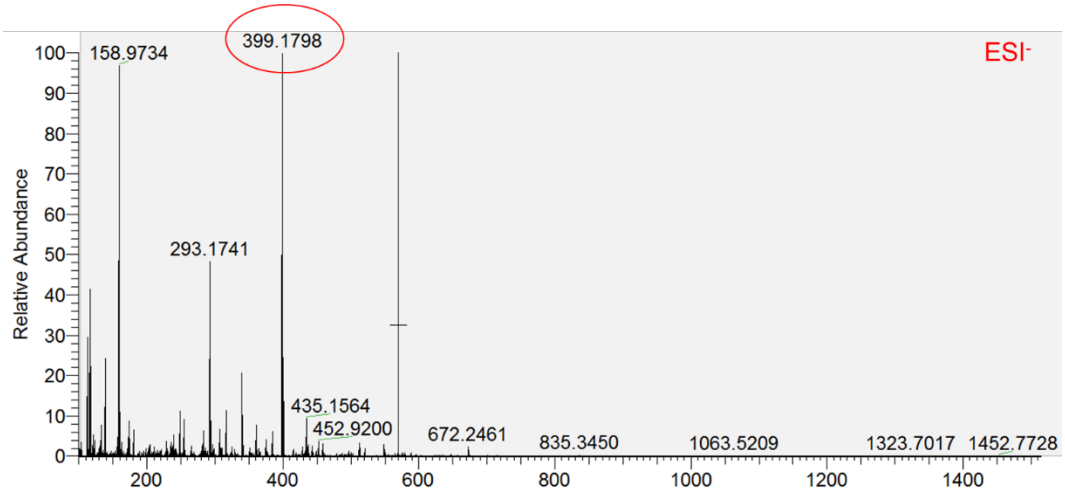


Figure S3. Electronspray ionization mass spectral of **P1-NH$_2$.**

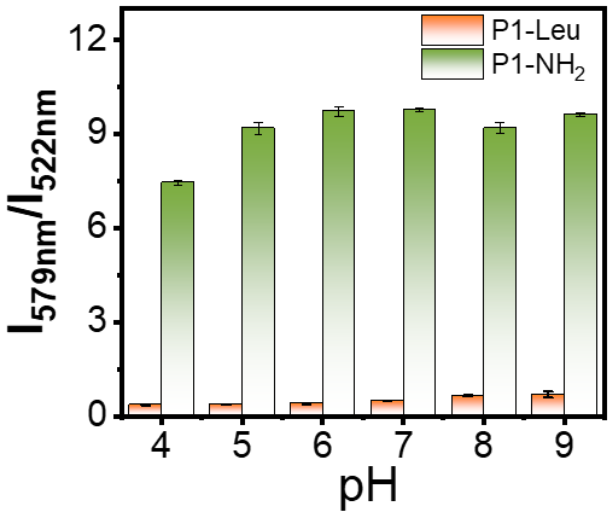


Figure S4. Fluorescence ratio $I_{579\ nm}/I_{522\ nm}$ of **P1-NH$_2$** and **P1-Leu** under different pH conditions.

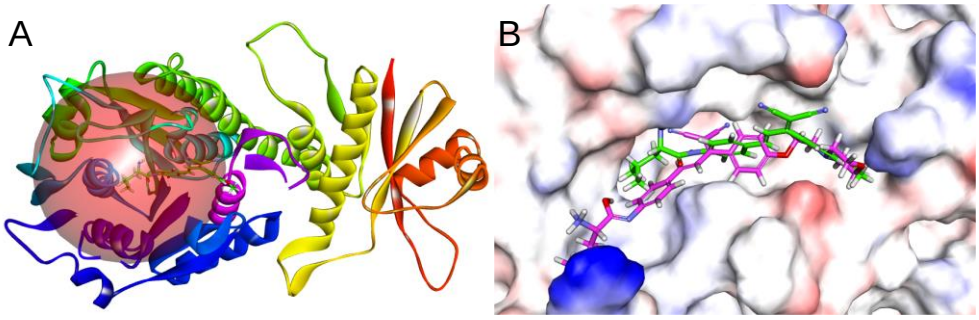


Figure S5. Docking of **P1-Leu** and **P0-Leu** on LAP based on Discovery Studio platform. (A) Structure of LAP. The binding site is indicated by the red transparent sphere. (B) Partial enlargement of (A).

Molecular docking experiment was done by Discovery Studio to simulate the binding between the probes and LAP. The crystal structure was extracted from LAP (PDB ID: 1LAN), downloaded from Protein Data Bank database. Detailed docking process and MD simulation was conducted in a similar manner as describe[1]. As demonstrated in Figure S5-S7, **P0-Leu** tends to bind with LAP mainly through hydrogen bonds and hydrophobic interactions on these critical residues, including Ala333, Ala451, Leu360, Thr359, Gly362 and Asp332 (Figure S6). **P1-Leu** binds with LAP mainly on Ile421, Arg425, Gly362, Gly452, Thr359 and Thr455 through hydrogen bonds and hydrophobic interactions (Figure S7), which demonstrate a strong affinity between **P1-Leu** and LAP.

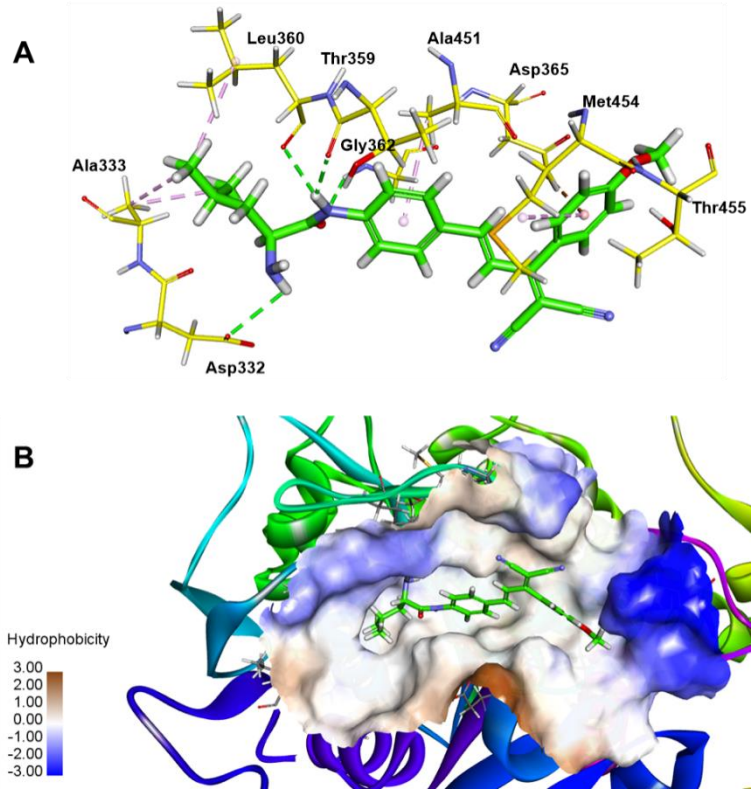


Figure S6. (A) Interactions between **P0-Leu** and LAP including hydrogen bond (green) and hydrophobic interaction (magenta) and π-sulfur bond (yellow). (B) Hydrophobic interactions between **P0-Leu** and LAP.

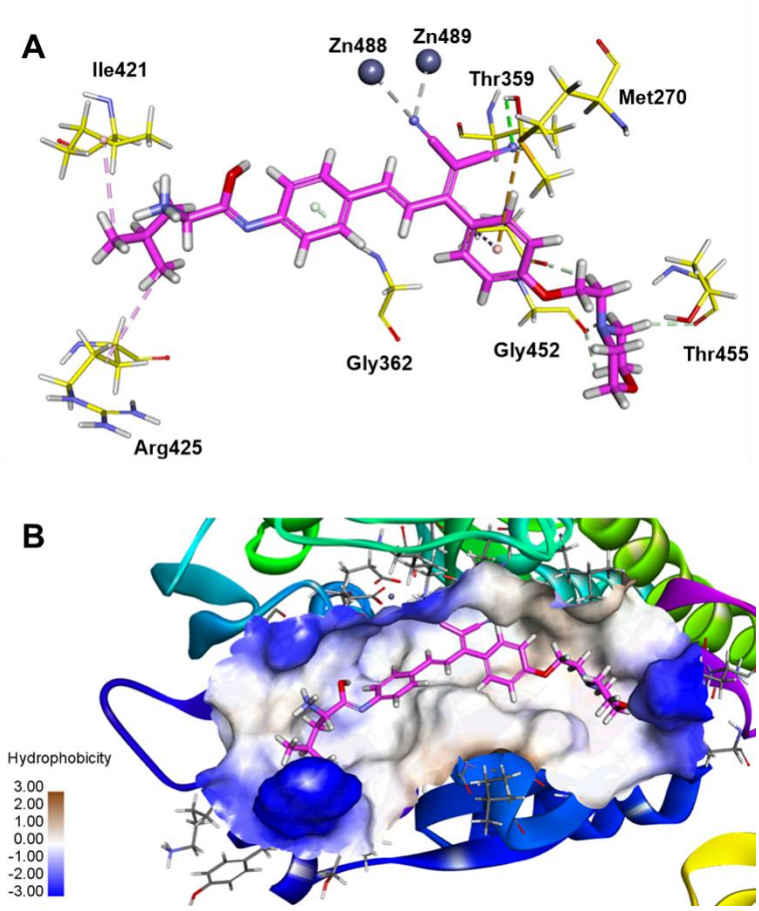


Figure S7. (A) Interactions between **P1-Leu** and LAP including hydrogen bond (green) and hydrophobic interaction (magenta), π-sulfur bond (yellow), π-donor hydrogen bond (pale green) and Zn-N coordination bond (gray). (B) Hydrophobic interactions between **P1-Leu** and LAP.

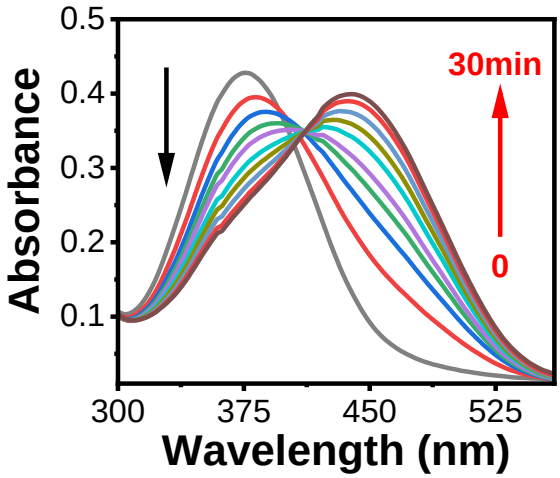


Figure S8. Time-dependent absorption spectra of **P1-Leu** (20 μM) incubated with 500 U/L LAP.

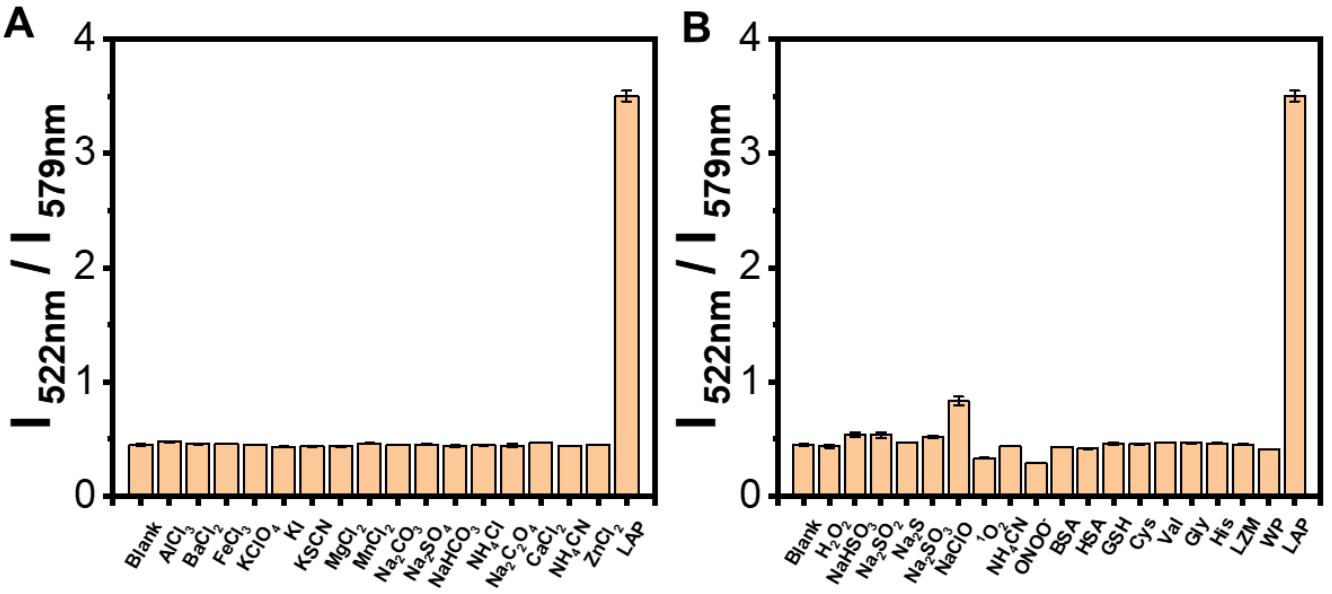


Figure S9. Fluorescence ratio ($I_{579\ nm}/I_{522\ nm}$) of **P1-Leu** (20 μM) after incubation with LAP (10 U/L) and other different analytes ($FeCl_3$: 100 μM, $ZnCl_2$: 100 μM, $NaHSO_3$:100 μM, Na_2S: 100 μM, CN^-: 100 μM, $ONOO^-$: 100 μM, Na_2SO_3: 20 μM, H_2O_2: 20 μM, NaClO: 20 μM, $CaCl_2$: 2.5 mM, whey protein (WP): 0.5 mg/mL, lysozyme: 0.2 U/mL, other analytes: 1 mM).

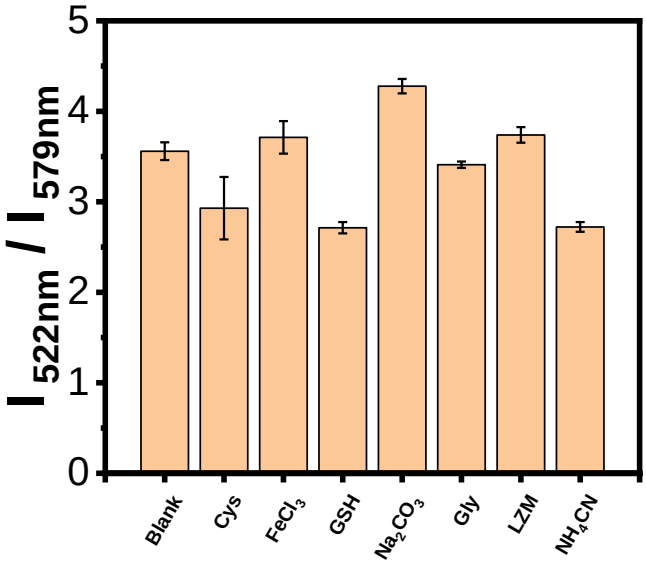


Figure S10. Competitive experiments of **P1-Leu** (20 μM) co-incubated with 500 U/L LAP and competing species (Cys: 100 μM, Gly: 100μM, LZM: 200 U/L, $FeCl_3$: 100 μM, Na_2CO_3: 100 μM, NH_4CN: 100 μM).

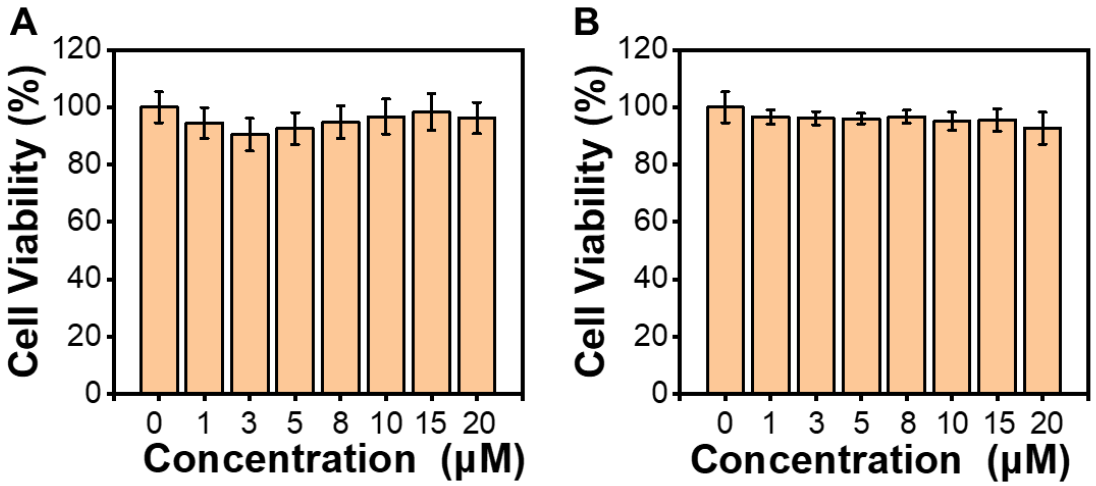


Figure S11. The cytotoxicity assay of different concentrations of **P1-Leu** to cells after incubation with HepG2 cells (A) and L02 cells (B) for 12 h.

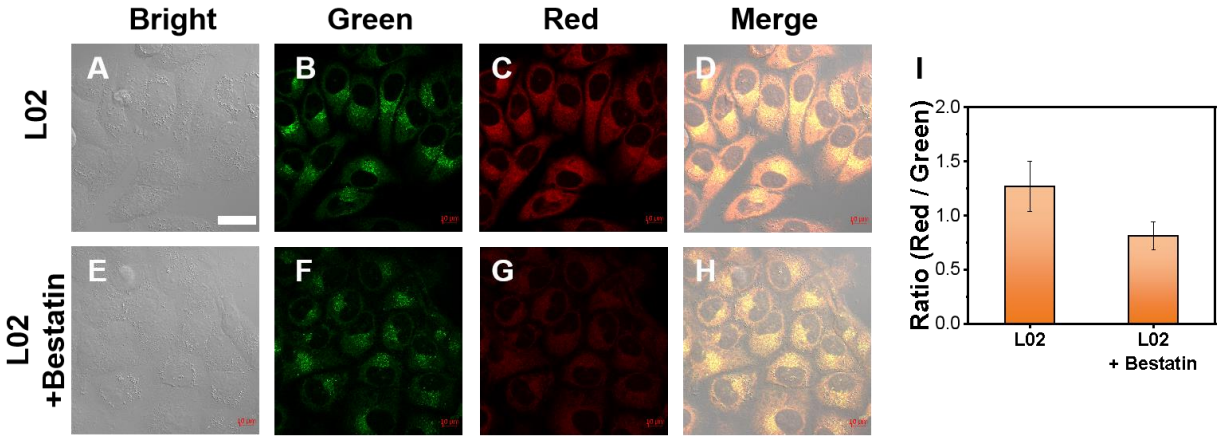


Figure S12. Confocal fluorescence images of the L02 cells with P1-Leu (5 μM). (A-D) L02 cells incubated with the **P1-Leu** for 30 min. (E-H) L02 cells pretreated with bestatin (100 μM) for 1 h, and then treated with P1-Leu for 0.5 h. (I) Changes in F_{red}/F_{green} ratio in cells. Green channel: 490-540 nm, λ_{ex} = 405 nm; Red channel: 555-605 nm, λ_{ex} = 488 nm. Scale bar: 25 μm.

8. Structure characterizations

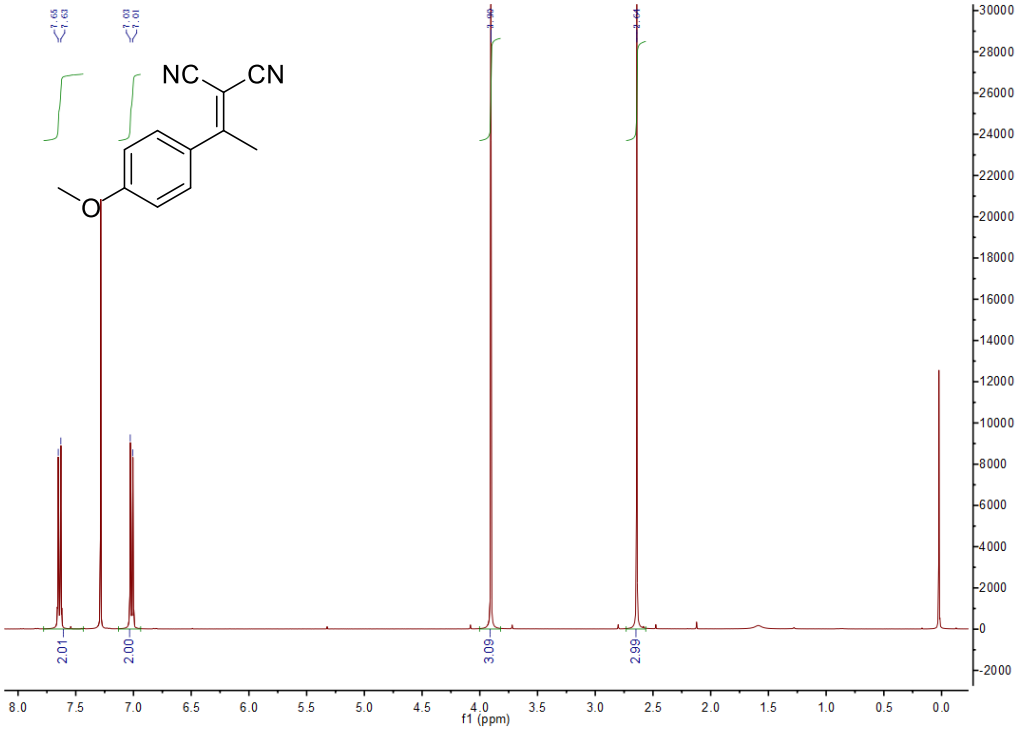


Figure S13. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **P0-1**.

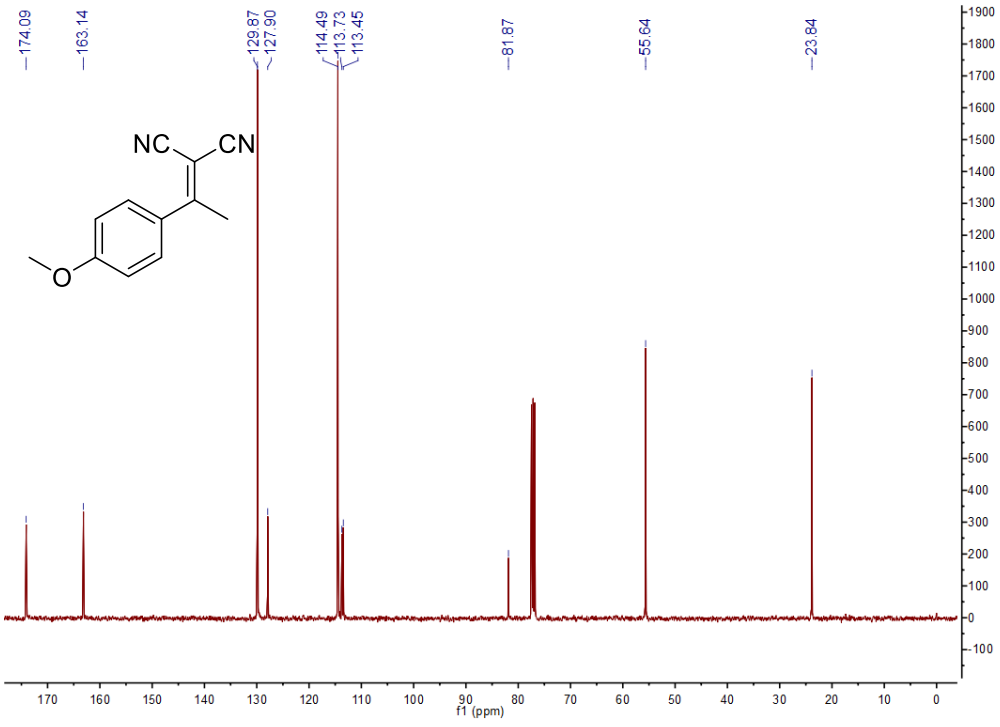


Figure S14. ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of **P0-1**.

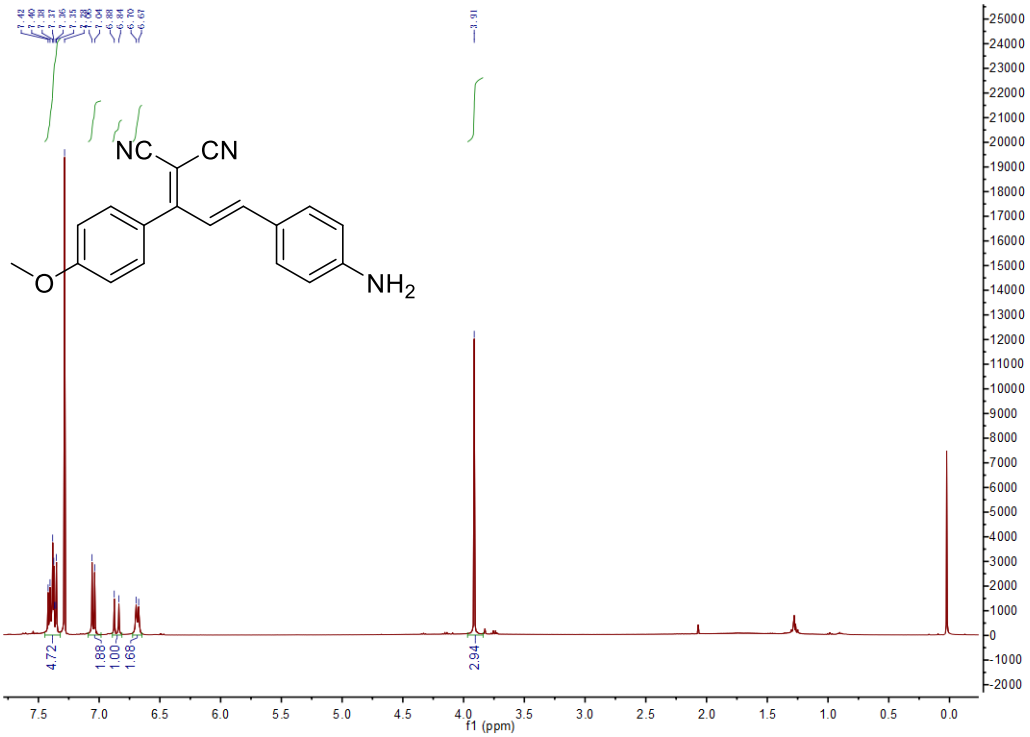


Figure S15. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **P0-NH$_2$**.

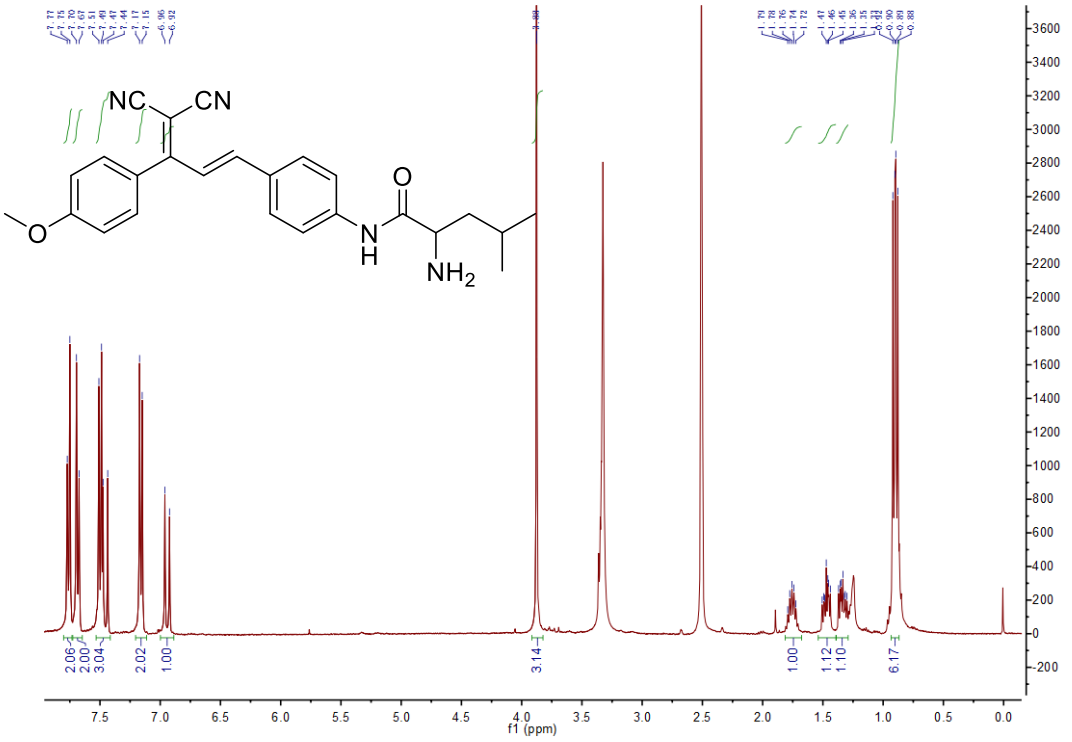


Figure S16. ^{1}H NMR (400 MHz, DMSO) spectrum of **P0-Leu**.

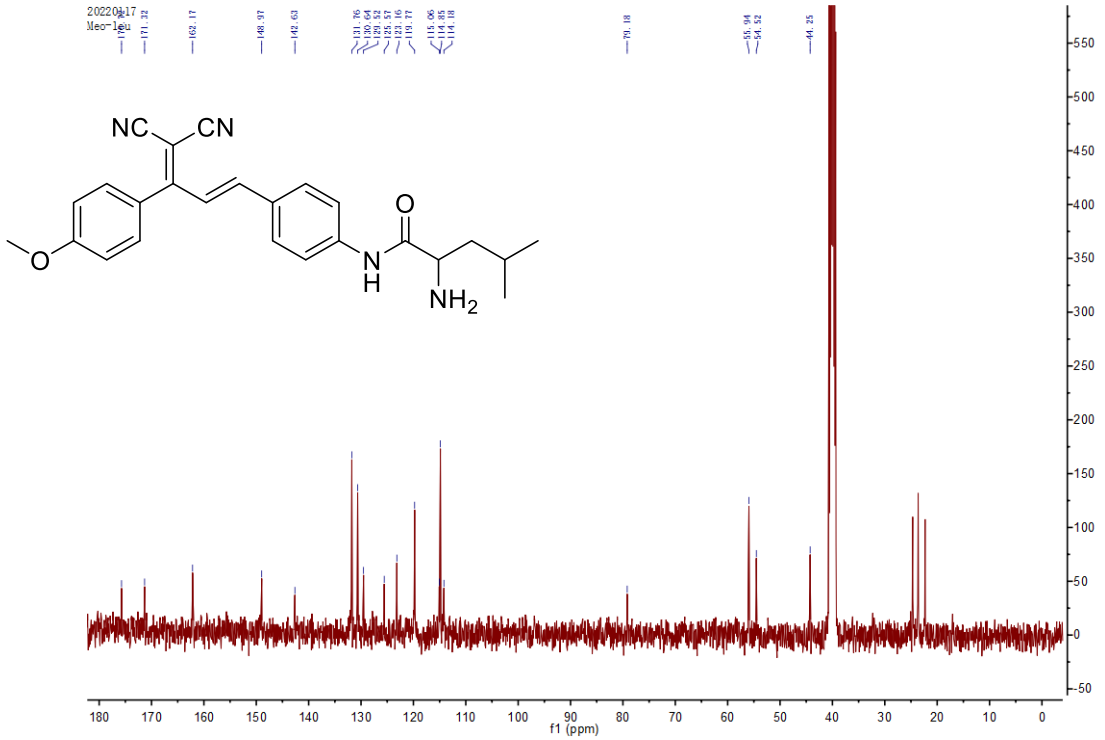


Figure S17. ^{13}C NMR (101 MHz, DMSO) spectrum of **P0-Leu.**

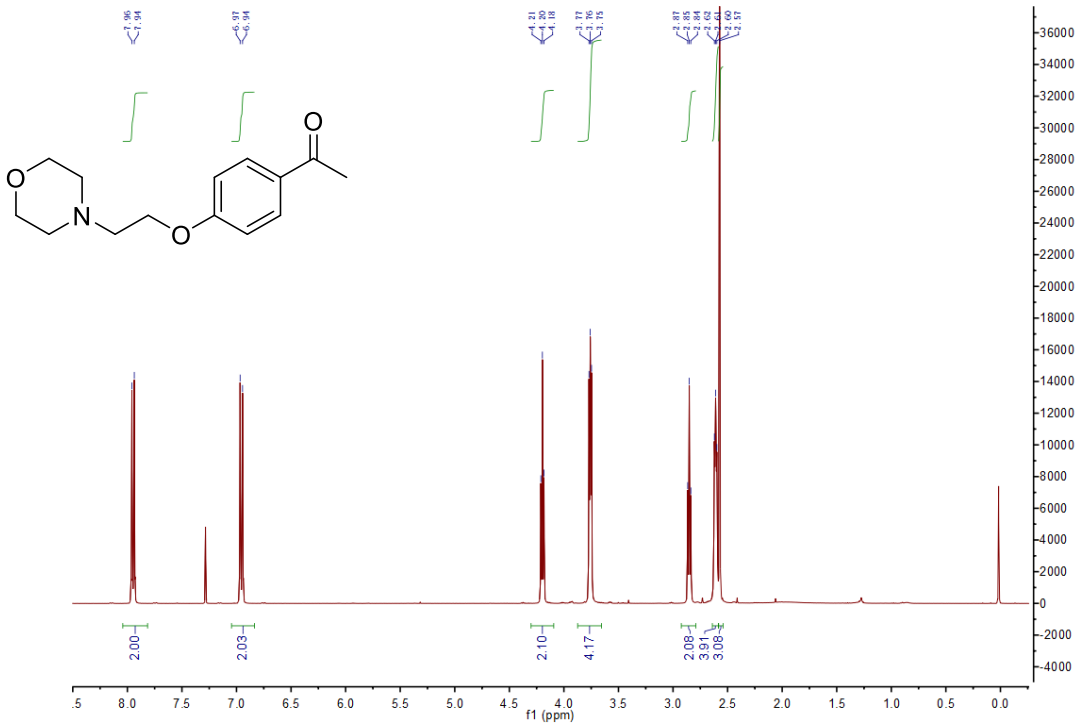


Figure S18. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **P1-1**.

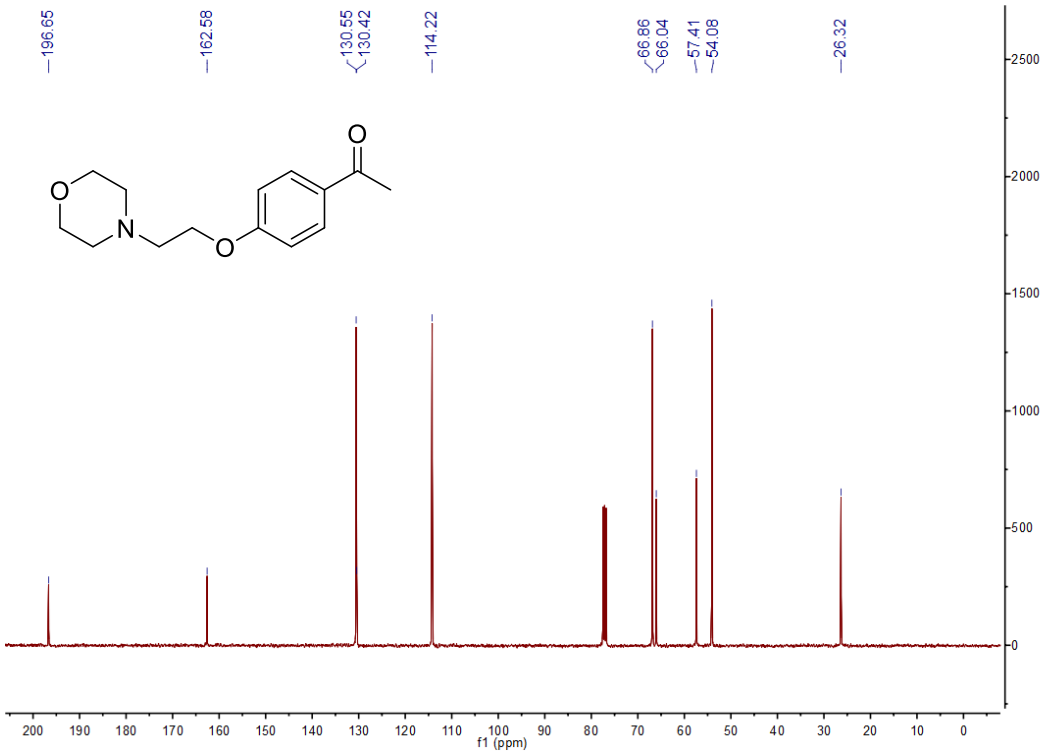


Figure S19. ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of **P1-1**.

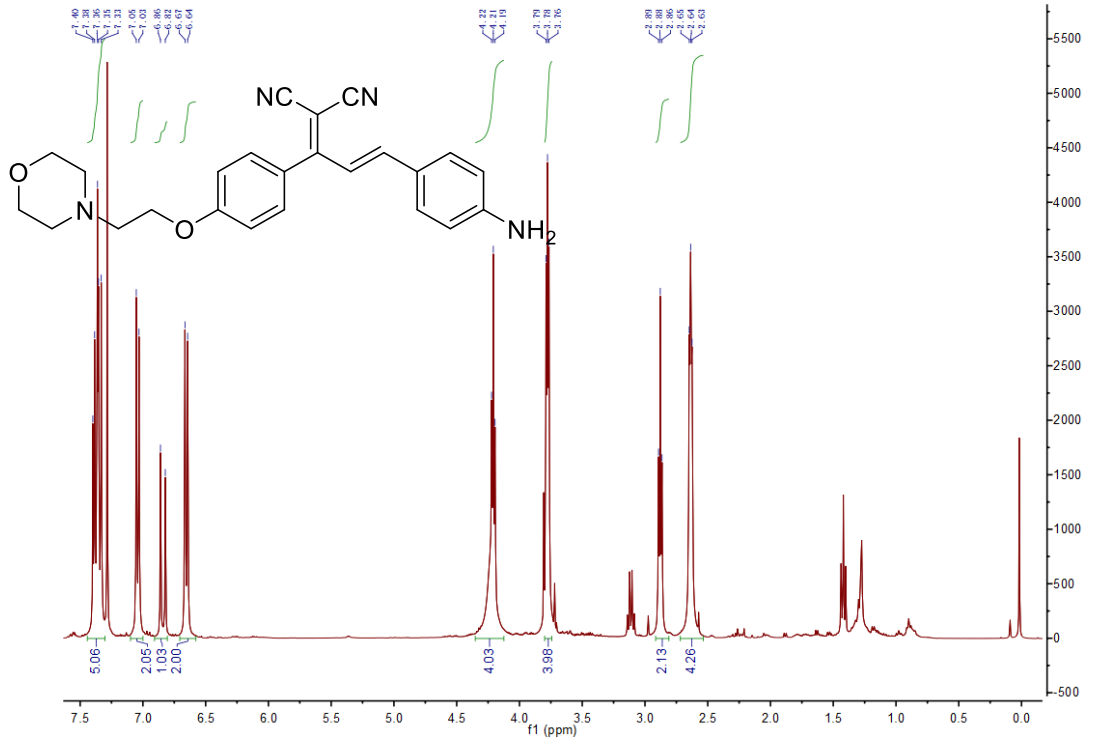


Figure S20. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **P1-NH$_2$**.

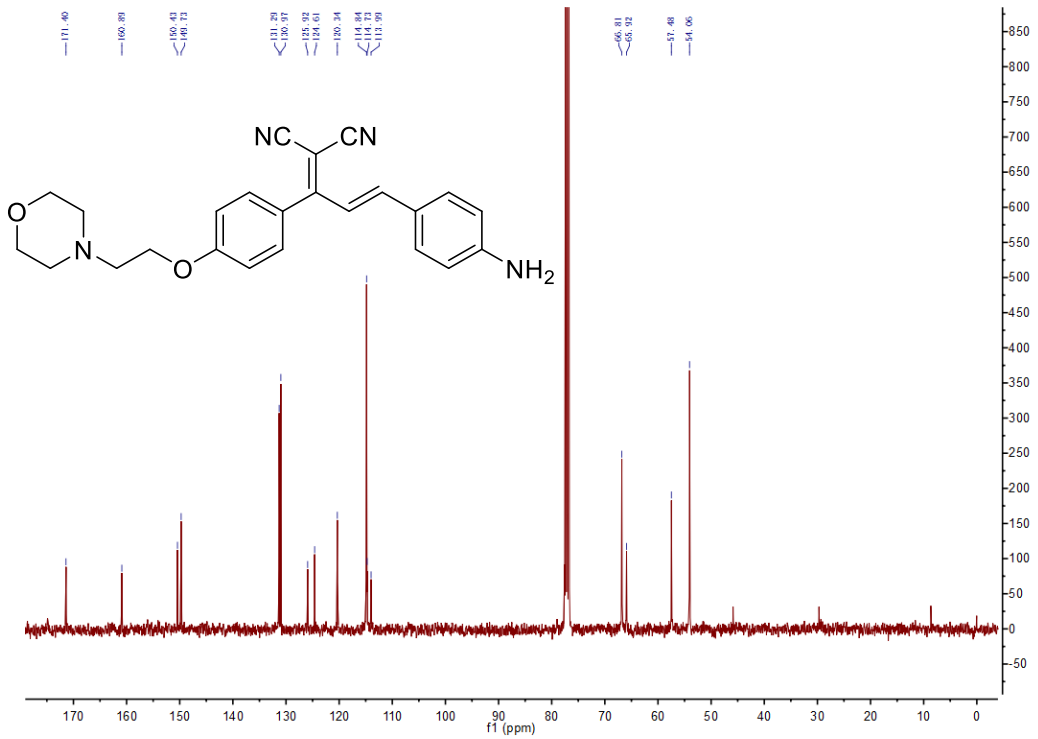


Figure S21. ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of **P1-NH$_2$**.

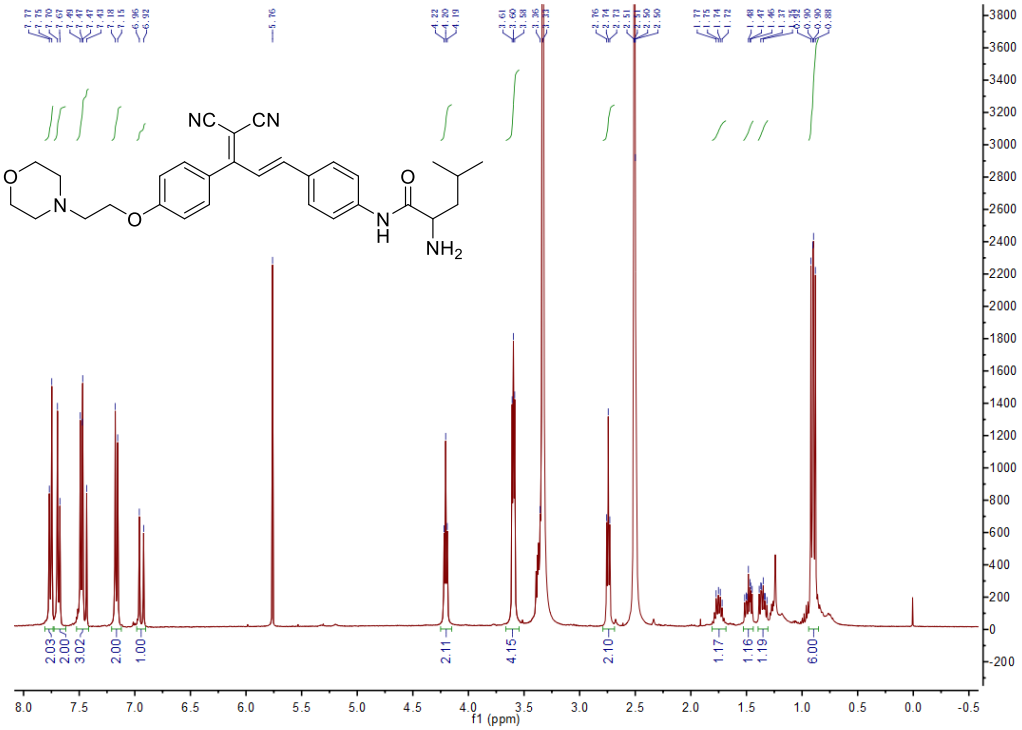


Figure S22. ^{1}H NMR (400 MHz, DMSO) spectrum of **P1-Leu**.

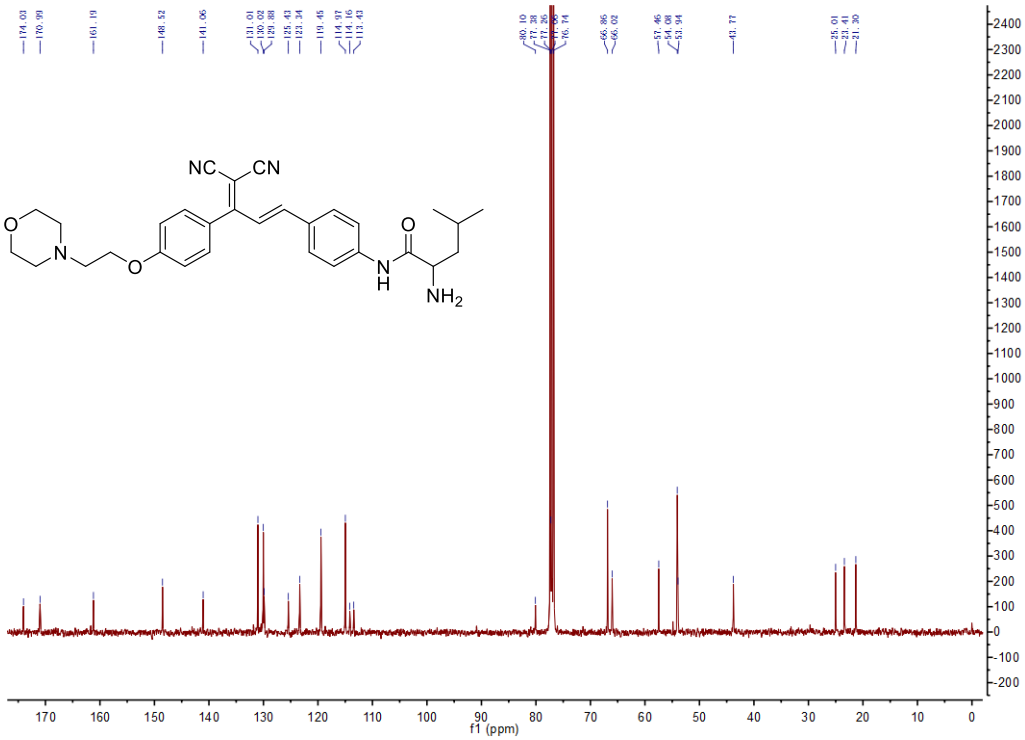


Figure S23. ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of **P1-Leu.**

9. References

[1] J. Ye, X. Yang and C. Ma, *Int. J. Mol. Sci*., 2022, 23, 4085.