Supporting Information

Construction of a novel asymmetric imidazole-cored AIE probe for ratiometric imaging of endogenous leucine aminopeptidase

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Contents

Experimental section

Apparatus and reagents. ^{1}H-NMR (400 and 500 MHz) and ^{13}C-NMR spectra (100 and 125 MHz) were recorded on a Bruker Advance spectrometer with tetramethylsilane (TMS) as an internal standard. High-resolution mass spectra (HRMS) were measured on a Thermo Scientific instrument. A Shimadzu UV-2450 UV-visible spectrophotometer was used to record the ultra-violet spectra. Fluorescence measurements were collected on a HITACHI F-2700 PC spectrofluorophotometer (Shimadzu, Japan). The pH measurements were conducted with Rex PHS-3C pH meter. Zestier Nano ZS (Malvern Instruments Ltd., U.K.) was used to determine the particle sizes. Thin-layer chromatography (TLC) analysis was performed on silica gel GF254 plates. Unless specifically noted, all chemical reagents were purchased from Energy Chemical Co., Ltd. (China), all solvents in analytic grade were supplied from Sinopharm Chemical Reagent Co., Ltd. (China). Leucine aminopeptidase (LAP, microsomal from porcine kidney, type IV-S) and bestatin were obtained from Sigma-Aldrich Co., Ltd and Aladdin Company (Shanghai, China), respectively. Ultrapure water prepared by Millipore water purification system was used throughout.

General procedure for LAP detection. The stock solution (1.0 mM) of ASSI-Leu was prepared in DMSO. Typically, optical measurements were performed in aqueous system containing 10% DMSO (v/v) via the following procedures. In a 2 mL test tube, probe solution was diluted with ultrapure water to obtain the desired concentration (5 μM), followed by the addition of appropriate volume of LAP standard solution. After incubation at 37 ℃ for a period time, the fluorescence spectra were collected from 400 to 675 nm with an excitation of 365 nm.

Theoretical methods. The quantum chemical calculations were carried out by the Gaussian 16 program. Density functional theory (DFT) and time-dependent density functional theory (TDDFT) were used to optimize the geometric construction of the compounds. In order to simulate the experimental solvent environment, the calculations were based on the polarizable continuum model (PCM) with DMSO as the solvent to consider the solvation effect. To explore the ESIPT process, the potential energy curves were scanned in the ground state (S_0) and the first excited state (S_1) as a

function of the bond length of O_{31}-H_{58} based on PBE0/def2-SVP level. For investigation of TICT process, the potential energy surface scanning as a function of dihedral angle C_6-C_1-C_{30}-N_{32} in both enol and keto state was carried out through relaxed scan method based on PBE0/def2-SVP level.

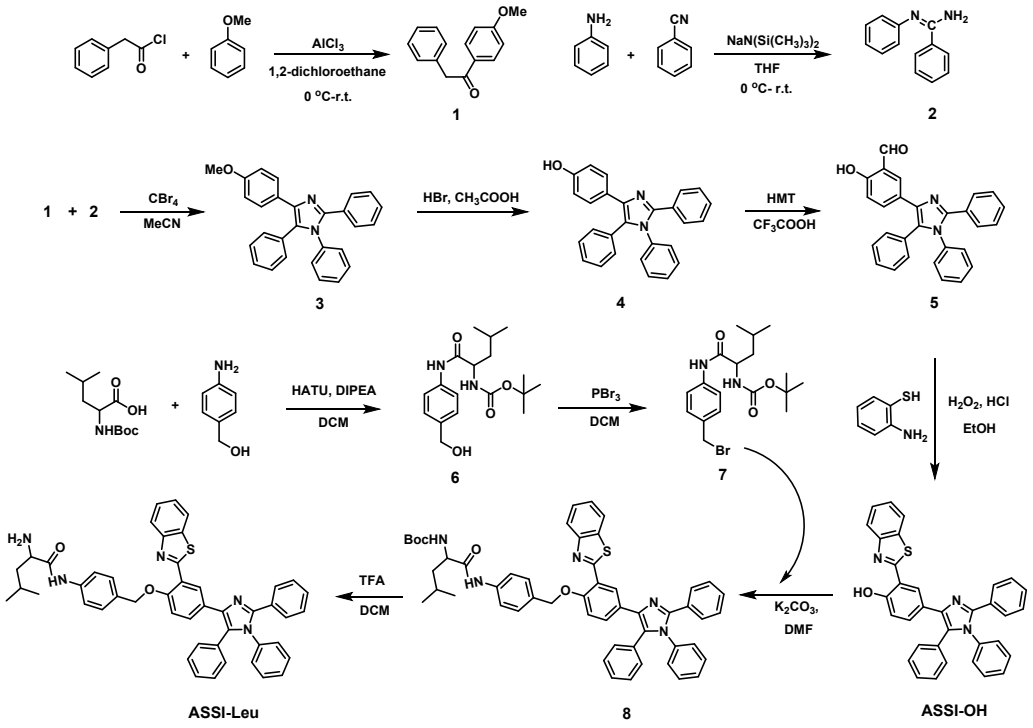
Cell incubation and cytotoxicity assay. HepG2 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS, Invitrogen) and 1% penicillin-streptomycin at 37 ℃ in a humidified incubator containing 5% carbon dioxide. The Cell Counting Kit 8 (CCK8) assay was used to evaluate cytotoxicity. Firstly, the cells were evenly seeded in a 96-well plate with about 5000 to 8000 cells in each well plate, and allowed to grow for 24 h. Next treated the cells with various concentrations of probe and further cultured for 24 h. After removal of the culture medium, CCK8 reagent diluted with DMEM medium (10% FBS) was added to the well plate for 1h incubation. The absorbance at 450 nm was acquired from a microplate reader. The cell viability was calculated with equation: viability (%) = $[(OD - OD_{blank}) / (OD_{control} - OD_{blank})] \times 100\%$.

Confocal fluorescence imaging in the living cells. Prior to imaging, the HepG2 cells were seeded in a 6-well plate and grown to 50% to 70% confluency under the above culture conditions. ASSI-Leu in DMEM was added to the well plate for coincubation, then washed the treated cells with prewarmed Tris-HCl buffer for three times. The fluorescence cell images were achieved by confocal laser scanning microscope. Meanwhile, another section of HepG2 cells was pretreated with bestatin for 1 h before the stain of the probe system. In the colocalization studies, probe in fresh DMEM medium was added to the wells of adherent cells for 1h incubation and then labeled with 100 nM Lyso-Tracker Red for 30 min. Linear ROIs were drawn over the areas by using ImageJ software.

Zebrafish pretreatment and fluorescence imaging. The animal experiment was approved by the Animal Ethics and welfare Committee, at the Third Xiangya Hospital, Central South University (No. 2021-S137). All animal studies were carried out using the Institutional Animal Care and Use Committee (IACUC) approved procedures. Zebrafish embryos were purchased from Nanjing Eze-Rinka company Co., Ltd. Zebrafish embryos were transferred into 12-well plates and cultured in E3 media containing 1-phenyl-2-thiourea (PTU) at a constant temperature of 28 ℃. All zebrafish

experiments were conducted in accordance with international ethics guidelines. 7-day old zebrafish were pretreated with cisplatin for 12 h aimed to induce the overexpression of LAP, and then incubated with probe in E3 media. In the inhibitor experiment, the inhibitor bestatin diluted with E3 media was added before incubation with the probe. Then, the culture medium was removed by washing with E3 media for five times. Immediately, the zebrafishes were transferred to a 35 mm glass bottom dish to obtain fluorescence images under confocal microscope.

Synthesis



Scheme S1. Synthetic routes of **ASSI-OH** and **ASSI-Leu.**

Synthesis of compound 1. To the solution of phenylacetyl chloride (620 mg, 4 mmol) in 1,2-dichloroethane (20 mL) was added aluminum trichloride (802.8 mg, 6 mmol) at 0 ℃. Then the anisole (432 mg, 4 mmol) was added dropwise at a maintained temperature of 0- 5 ℃. The mixture was warmed to room temperature and stirred for 2 h. Then the resulting mixture was poured into a mixture of ice water and 36% hydrochloric acid. The aqueous solution was extracted with CH_2Cl_2 and washed with brine. The organic layer was dried over anhydrous sodium sulfate and concentrated by vacuum-rotary evaporation procedure. Finally, the residue was purified by column chromatography with ethyl

acetate/petroleum ether (1: 60, v/v) as eluent to obtain **1** as a white solid (464 mg, 51.3%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.04 – 8.00 (m, 2H), 7.27 (ddd, *J* = 14.5, 7.9, 4.1 Hz, 4H), 7.22 – 7.19 (m, 1H), 6.98 (d, *J* = 9.0 Hz, 2H), 4.26 (s, 2H), 3.85 (s, 3H). HRMS: m/z calcd for $C_{15}H_{15}O_2$ ($[M + H]^+$) 227.1072, found 227.1064.

Synthesis of compound 2. The aniline (279.4 mg, 3 mmol) solution in dry tetrahydrofuran was added dropwise to sodium hexamethyldisilazide (1.5 mL, 1 M solution in THF) at 0 ℃ under nitrogen protection. After stirring for 20 min, a solution of benzonitrile (309.4 mg, 3 mmol) in THF was slowly added. The reaction was stirred overnight at room temperature, then poured into ice water (400 mL) and extracted with dichloromethane. The collected organic solution was dried over anhydrous magnesium sulfate and concentrated by reduced pressure. The concentrate was recrystallized in a system of dichloromethane and petroleum ether to obtain **2** as a brown solid (298.2 mg, 50.6%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.97 (d, *J* = 7.2 Hz, 2H), 7.49 – 7.42 (m, 3H), 7.31 (t, *J* = 7.7 Hz, 2H), 6.98 (t, *J* = 7.3 Hz, 1H), 6.86 (s, 2H), 6.28 (s, 2H). HRMS: m/z calcd for $C_{13}H_{13}N_2$ ($[M + H]^+$) 197.1079, found 197.1068.

Synthesis of compound 3. To the solution of compound 1 (110.0 mg, 0.4 mmol) in acetonitrile (5 mL) was added the carbon tetrabromide (132.6 mg, 0.4 mmol) dissolved in acetonitrile slowly. Then added compound **2** (156.8 mg, 0.8 mmol) slowly and the reaction mixture was stirred overnight at 70 ℃. The solvent was evaporated under reduced pressure and purified by silica column chromatography with ethyl acetate/petroleum ether (1: 50, v/v) as eluent to afford **3** as a white solid (80.1 mg, 49.8%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.41 (d, *J* = 8.8 Hz, 2H), 7.38 – 7.35 (m, 2H), 7.32 (d, *J* = 2.5 Hz, 2H), 7.31 (s, 1H), 7.29 (s, 1H), 7.29 – 7.26 (m, 5H), 7.24 (d, *J* = 2.2 Hz, 1H), 7.24 – 7.21 (m, 3H), 6.83 (d, *J* = 8.9 Hz, 2H), 3.71 (s, 3H). ^{13}C NMR (125 MHz, DMSO) δ 158.45, 146.23, 137.26, 137.20, 131.62, 130.98, 130.89, 130.70, 129.60, 129.20, 129.15, 128.91, 128.75, 128.70, 128.60, 128.07, 127.34, 114.10, 55.44. HRMS: m/z calcd for $C_{28}H_{23}N_2$ ($[M + H]^+$) 403,1810, found 403.1819.

Synthesis of compound 4. The compound **3** (215.2 mg, 0.8 mmol) was dissolved in a mixture of acetic acid (2.5 mL) and 48% aqueous hydrobromic acid (10 mL), then heated to reflux for 11 h. The resulting mixture was poured into ice water followed by adjusting pH value to neutral with sodium hydroxide.

Then the solution was extracted with ethyl acetate, dried over dried over anhydrous magnesium sulfate and concentrated by vacuum-rotary evaporation procedure. The crude product was purified by silica column chromatography with ethyl acetate/petroleum ether (1: 2, v/v) as eluent to obtain **4** as a white solid (183.7 mg, 58.0%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.37 (s, 1H), 7.35 (dd, J = 6.7, 3.2 Hz, 2H), 7.32 (d, J = 2.9 Hz, 2H), 7.30 (d, J = 4.1 Hz, 2H), 7.28 (d, J = 2.2 Hz, 5H), 7.27 (s, 2H), 7.23 (d, J = 2.5 Hz, 1H), 7.22 (d, J = 3.5 Hz, 2H), 7.20 – 7.19 (m, 1H), 6.64 (d, J = 8.7 Hz, 2H). ^{13}C NMR (125 MHz, DMSO) δ 156.63, 146.08, 137.70, 137.27, 131.61, 131.09, 130.98, 130.27, 129.59, 129.21, 128.83, 128.67, 128.61, 128.58, 128.21, 115.43.

Synthesis of compound 5. Take hexamethylenetetramine (323.9 mg, 2.3 mmol), 10 mL of trifluoroacetic acid in a dried round-bottomed flask, equipped with magnetic stirring bar. To the mixture was added compound **4** (183.7 mg, 0.5 mmol) and stirred at 90 ℃ with reflux for 7 h. The reaction mixture was cooled to room temperature, quenched with H_2O and the pH value was adjusted to neutral with sodium hydroxide. Then the solution was extracted with ethyl acetate, dried over anhydrous magnesium sulfate, concentrated by rotary evaporation. Further purification was conducted on silica gel chromatography with ethyl acetate/petroleum ether (1: 25, v/v) as eluent to obtain **5** as a canary yellow solid (79.0 mg, 41.1 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.70 (s, 1H), 10.21 (s, 1H), 7.89 (d, J = 1.9 Hz, 1H), 7.51 (dd, J = 8.7, 2.1 Hz, 1H), 7.39 – 7.36 (m, 2H), 7.34 (s, 1H), 7.32 (d, J = 3.4 Hz, 2H), 7.31 - 7.28 (m, 5H), 7.28 – 7.27 (m, 1H), 7.25 (d, J = 1.5 Hz, 1H), 7.24 (d, J = 2.1 Hz, 2H), 7.23 (s, 1H), 6.87 (d, J = 8.7 Hz, 1H). HRMS: m/z calcd for $C_{28}H_{21}N_2O_2$ ($[M + H]^+$) 417.1603, found 417.1589.

Synthesis of ASSI-OH. Compound **5** (79.0 mg, 0.18 mmol) was dissolved in 8 mL absolute alcohol and then 2-aminothiophenol (46.0 mg, 0.36 mmol) was added under stirring. To the system was added a few drops of hydrogen peroxide (30%) and hydrochloric acid, and the reaction was performed at room temperature for 2 h. Quenched reaction with H_2O and extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over anhydrous magnesium sulfate and evaporated. The crude product was purified by silica column chromatography using ethyl acetate/petroleum ether (1: 45, v/v) as eluent to afford **ASSI-OH** as a yellow solid (42.2 mg, 43.7%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.60 (s, 1H), 8.42 (s, 1H), 8.12 (d, J = 7.9 Hz, 1H), 8.04 (d, J = 8.1 Hz, 1H), 7.54 (t, J

= 7.6 Hz, 1H), 7.45 (t, *J* = 7.6 Hz, 1H), 7.41 (d, *J* = 6.8 Hz, 3H), 7.34 (s, 6H), 7.32 – 7.28 (m, 6H), 7.28 – 7.26 (m, 1H), 6.95 (d, *J* = 8.6 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-*d6*) δ 165.89, 155.54, 151.89, 146.45, 137.15, 136.63, 134.46, 131.60, 131.08, 130.86, 129.62, 129.21, 129.05, 128.90, 128.78, 128.64, 126.99, 126.86, 126.70, 125.64, 122.58, 122.47, 118.43, 117.30. HRMS: m/z calcd for $C_{34}H_{24}N_3OS$ ($[M + H]^+$) 522.1640, found 522.1620.

Synthesis of compound 6. To a solution of Boc-L-Leucine (0.925g, 4 mmol) in 30 mL anhydrous CH_2Cl_2 was added HATU (1.9 g, 5 mmol) and DIPEA (1.034 g, 8 mmol) under nitrogen atmosphere. After stirring for 30 min, added (4- aminophenyl) methanol (0.984 g, 8 mmol) was added dropwise, and further stirring for 24 h at room temperature. After dilution with water, the mixture was extracted with CH_2Cl_2, dried over anhydrous magnesium sulfate, and evaporated to afford a crude oil. Then the crude product was purified by flash chromatography using dichloromethane/methanol (100:1, v/v) as eluent to obtain **6** as a colorless oil (840.2 mg, 62.5%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.91 (s, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.24 (d, *J* = 8.3 Hz, 2H), 6.99 (d, *J* = 8.0 Hz, 1H), 5.13 (s, 1H), 4.44 (d, *J* = 5.4 Hz, 2H), 4.14 (dd, *J* = 13.6, 8.8 Hz, 1H), 1.67 – 1.49 (m, 2H), 1.44 (dd, *J* = 8.2, 5.3 Hz, 1H), 1.38 (s, 9H), 0.89 (d, *J* = 4.0 Hz, 6H). HRMS: m/z calcd for $C_{18}H_{28}N_2NaO_4$ ($[M + Na]^+$) 359.1947, found 359.1969.

Synthesis of compound 7. Cooling the round-bottom flask of compound **6** (247.3 mg, 0.74 mmol) in 15 mL anhydrous dichloromethane to -10 ℃. Tribromophosphine (398.0 mg, 1.47 mmol) was added dropwise over 15 min. After stirring for 30 min at constant -10 ℃, the reaction mixture was poured into ice-water carefully and extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated by rotary evaporation under reduced pressure. The obtained product **7** was used directly in the following reaction without further purification.

Synthesis of compound 8. To a solution of compound **ASSI-OH** (41.4 mg, 0.08 mmol) in acetone (6 mL) was added K_2CO_3 (22.1 mg, 0.16 mmol), compound **7** (63.9 mg, 0.16 mmol), 18-crown-6 (42.3 mg, 0.16 mmol), KI (132.8 mg, 0.8 mmol). The mixture was stirred at 60 ℃ under nitrogen for 8 h. Subsequently, the mixture was extracted with DCM, and the organic phase was dried over anhydrous Na_2SO_4. After removing the solvent under a reduced pressure, the residue was purified by silica gel

chromatography (ethyl acetate/petroleum ether = 1: 5, v/v) to afford a white solid **8** (48.1 mg, yield 71.6%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm 10.05 (s, 1H), 8.91 (s, 1H), 8.08 (d, J = 7.9 Hz, 1H), 8.03 (d, J = 8.1 Hz, 1H), 7.66 (d, J = 8.0 Hz, 2H), 7.52 (s, 2H), 7.51 (s, 1H), 7.42 – 7.39 (m, 3H), 7.37 (d, J = 9.0 Hz, 2H), 7.33 (s, 6H), 7.32 (s, 2H), 7.31 (s, 2H), 7.28 (s, 2H), 7.24 (d, J = 8.9 Hz, 1H), 7.06 (d, J = 7.9 Hz, 1H), 5.32 (s, 2H), 4.17 – 4.08 (m, 1H), 1.66 – 1.52 (m, 2H), 1.46 – 1.41 (m, 1H), 1.38 (s, 9H), 0.89 (s, 6H). ^{13}C-NMR (125 MHZ, DMSO-d_6) δ 172.38, 162.50, 155.93, 155.14, 151.97, 146.54, 139.48, 137.07, 136.43, 135.91, 131.64, 131.42, 131.23, 130.77, 129.98, 129.61, 129.49, 129.20, 129.07, 128.87, 128.67, 127.61, 126.69, 125.34, 122.87, 122.29, 121.91, 119.64, 113.98, 78.49, 70.77, 55.37, 41.10, 28.67, 24.82, 23.43, 22.02. HRMS: m/z calcd for $C_{52}H_{50}N_5O_4S$ ([M + H]$^+$) 840.3584, found 840.3517.

Synthesis of ASSI-Leu. To a stirring solution of compound **8** (46.1 mg, 0.05 mmol) in dry CH_2Cl_2 (2 mL) at - 5 ℃ was added anisole (0.5 mL, 4.5 mmol) and CF_3COOH (0.5 mL, 6.5 mmol) dropwise, then the mixture was stirred at room temperature overnight. After evaporation of solvent, the crude product was purified by a flash column chromatography using MeOH/ CH_2Cl_2 (1: 100, v/v) as eluent to obtain a white solid ASSI-Leu (16.0 mg, yield 39.5%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm 8.90 (s, 1H), 8.06 (d, J = 7.8 Hz, 1H), 8.03 (d, J = 8.1 Hz, 1H), 7.68 (d, J = 8.0 Hz, 2H), 7.53 (s, 1H), 7.51 (s, 2H), 7.40 (s, 4H), 7.39 (s, 1H), 7.36 (s, 1H), 7.33 (s, 6H), 7.32 (s, 2H), 7.31 (s, 2H), 7.28 (s, 2H), 7.24 (d, J = 9.3 Hz, 1H), 5.33 (s, 2H), 3.53 (s, 1H), 1.77 – 1.71 (m, 1H), 1.51 (d, J = 6.0 Hz, 2H), 0.90 (dd, J = 11.1, 6.5 Hz, 6H). ^{13}C-NMR (125 MHz, DMSO- d_6) δ 174.19, 162.41, 156.04, 155.00, 151.99, 146.49, 140.69, 136.98, 136.15, 135.87, 132.41, 131.63, 131.45, 130.66, 130.00, 129.61, 129.17, 129.07, 128.89, 128.82, 128.67, 127.70, 127.08, 126.72, 125.39, 122.91, 122.25, 121.96, 116.02, 114.02, 70.39, 55.32, 41.12, 24.56, 23.55, 22.56, 21.92. HRMS: m/z calcd for $C_{47}H_{42}N_5O_2S$ ([M + H]$^+$) 740.3059, found 740.3071.

Optical properties and biological studies

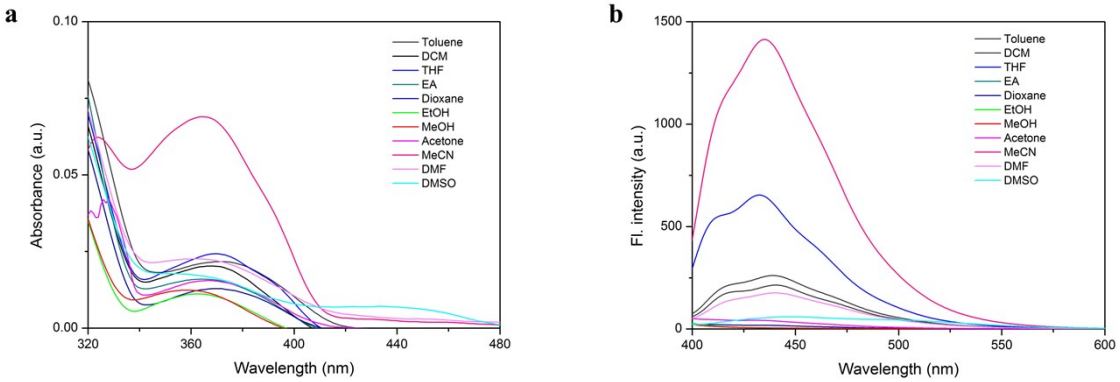


Figure S1. a) Absorption spectra and b) Fluorescence spectra of **ASSI-OH** (5 μM) in solvents with different polarities. λ_{ex} = 365 nm.

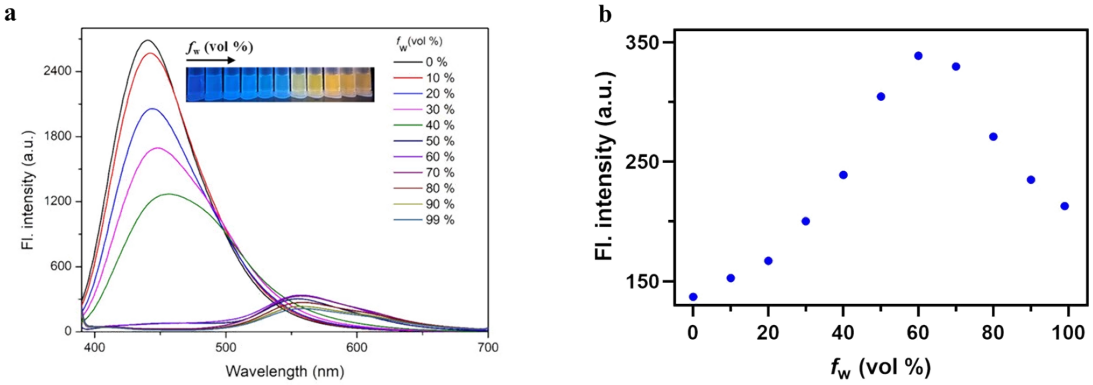


Figure S2. a) Fluorescence spectra of **ASSI-OH** (10 μM) in mixtures of DMSO/water with different water fraction (f_w). Inset: the fluorescence changes from blue to yellow of **ASSI-OH** with the increasing water fraction under a hand-held UV lamp. b) Fluorescence emission at 554 nm of **ASSI-OH** (10 μM) in mixtures of DMSO/water. λ_{ex} = 365 nm.

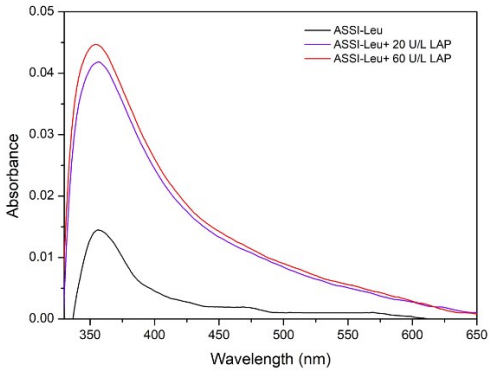


Figure S3. Absorption spectra of **ASSI-Leu** (5 μM) after incubation with LAP (20 U/L or 60 U/L) in aqueous solution (with 10% of DMSO, v/v) at 37 °C.

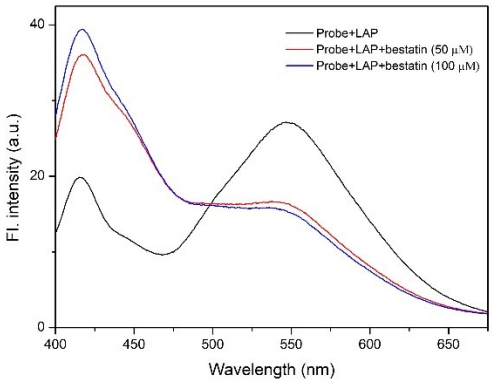


Figure S4. Fluorescence spectra of **ASSI-Leu** (5 μM) after incubation with LAP in the presence of 0, 50, 100 μM bestatin in aqueous solution (10% DMSO, v/v) at 37 °C. λ_{ex} = 365 nm.

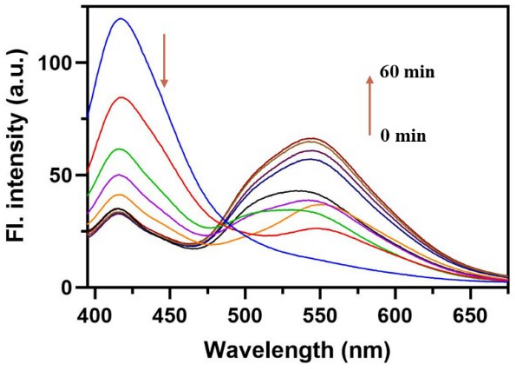


Figure S5. Time-dependent fluorescence spectra of **ASSI-Leu** (5 μM) with 0.06 U mL^{-1} LAP in

aqueous solution (10% DMSO, v/v) at 37 ℃.

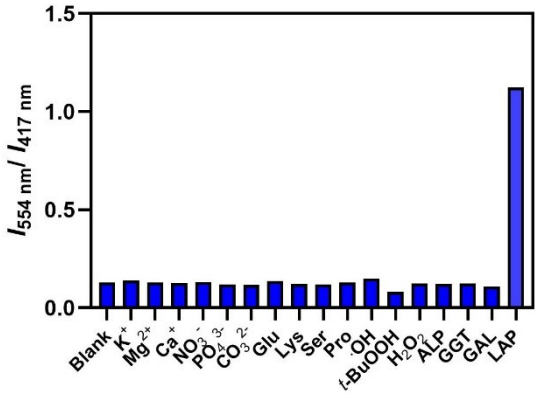


Figure S6. Fluorescence ratio ($I_{554\ nm}/I_{417\ nm}$) of **ASSI-Leu** (5 μM) in presence of various analytes (0.05 U mL^{-1} LAP or other interferents) in aqueous system (containing 10% DMSO, 37 ℃). The concentration of the ions, amino acids and reactive oxygen species is 100 μM and that of other proteases is 0.1 U mL^{-1}, λ_{ex} = 365 nm.

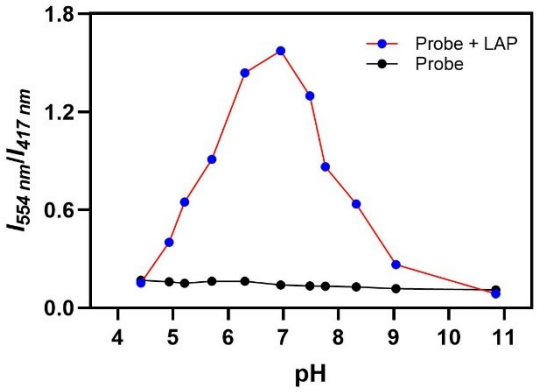


Figure S7. Effects of the system pH on the fluorescence ratio of **ASSI-Leu** (5 μM) in the absence or presence of LAP (0.06 U mL^{-1}) at 37 °C. λ_{ex} = 365 nm.

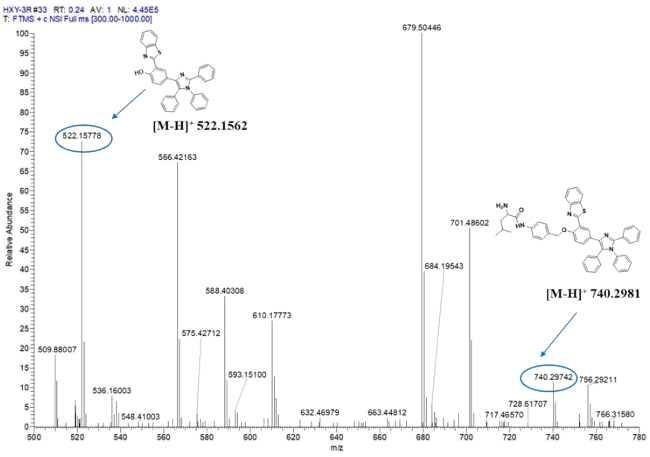


Figure S8. ESI spectrum of the reaction mixture of **ASSI-Leu** with LAP.

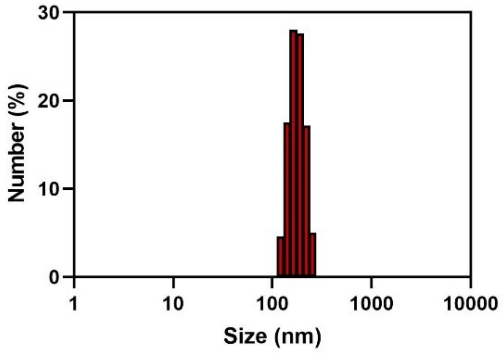


Figure S9. Hydrodynamic diameter distribution of **ASSI-Leu** after incubation with LAP obtained from dynamic light scattering (DLS).

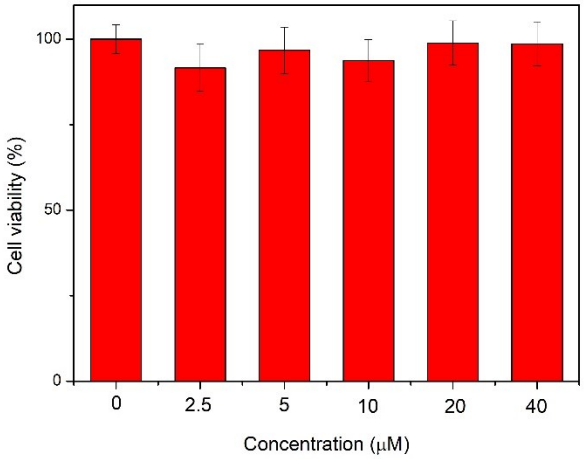


Figure S10. Relative cell viability of HepG2 cells after incubation with **ASSI-Leu** at various concentrations for 24 hrs.

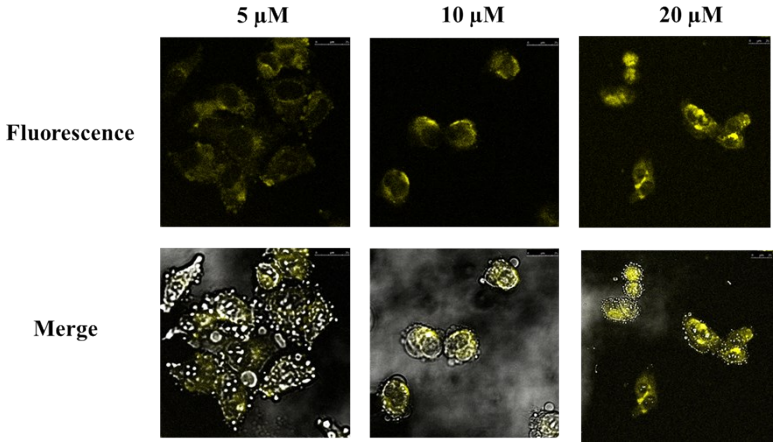


Figure S11. Confocal fluorescence images of HepG2 cells incubated with different concentrations of probe (5 μM, 10 μM, 20 μM) for 30 min respectively at 37 ℃. The fluorescence signal is collected at 490–560 nm, λ_{ex}= 405 nm. Scale bar: 25 μm.

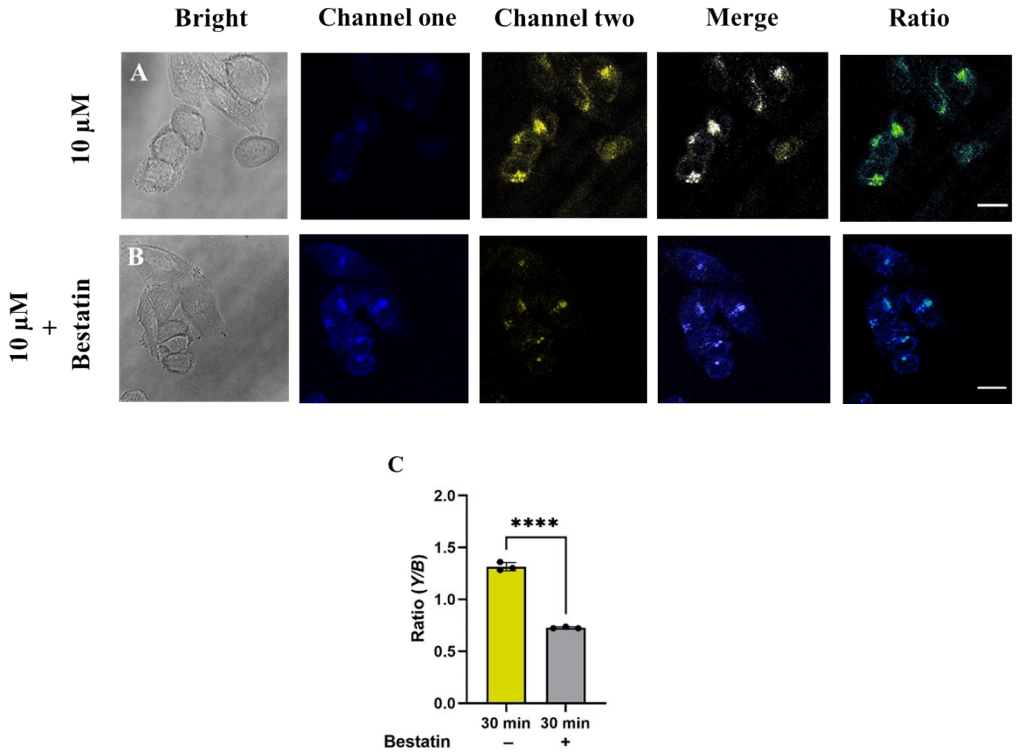


Figure S12. Confocal fluorescence images of HepG2 cells (A) incubated with probe **ASSI-Leu** (10 μM) for 30 min and (B) pretreated with 100 μM bestatin for 1 h and then incubated with probe **ASSI-**

Leu (10 μM) for 30 min respectively at 37 ℃. Channel one was collected from 410 to 470 nm (Blue); Channel two was collected from 490 to 560 nm (Yellow), λ_{ex} = 405 nm. Scale bar: 10 μm. (C) The relative fluorescence intensity ratio from channel one to channel two (Y/B). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (****p < 0.0001).

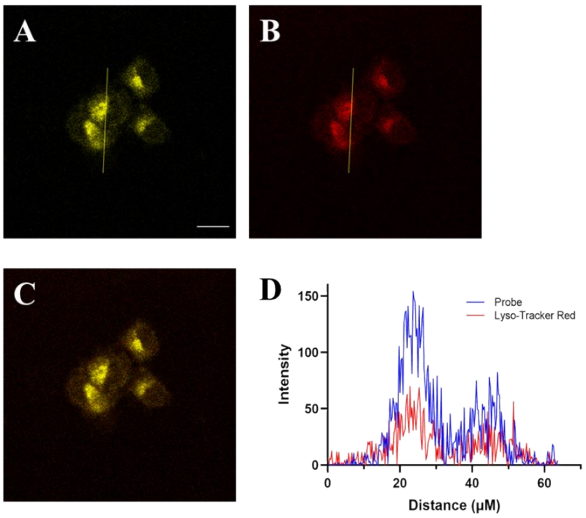


Figure S13. The colocalization of **ASSI-Leu** in HepG2 cells. (A) Channel two: **ASSI-Leu** (10 μM) stain (λ_{ex}= 405 nm, λ_{em}= 490–560 nm); (B)Red channel: LysoTraker red (0.1 μM) stain (λ_{ex} = 570 nm, λ_{em} = 580–650 nm); (C) Merged images of (A, B); (D) FL intensity profile of ROI across the line from (A, B). Scale bar is 20 μm.

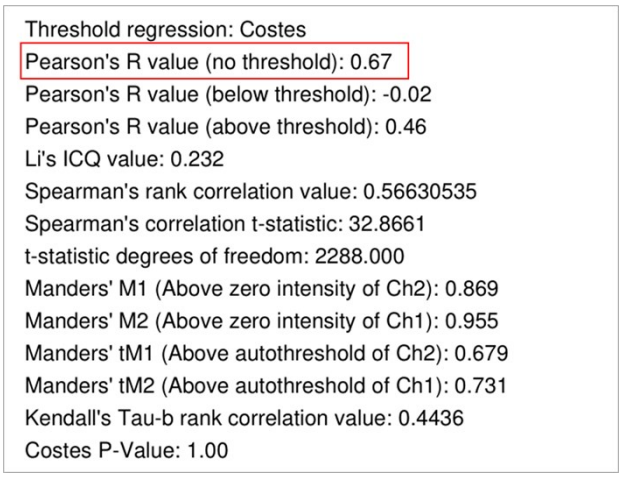


Figure S14. The colocalization of **ASSI-Leu** in HepG2 cells (Pearson's correlation coefficient (r) = 0.67).

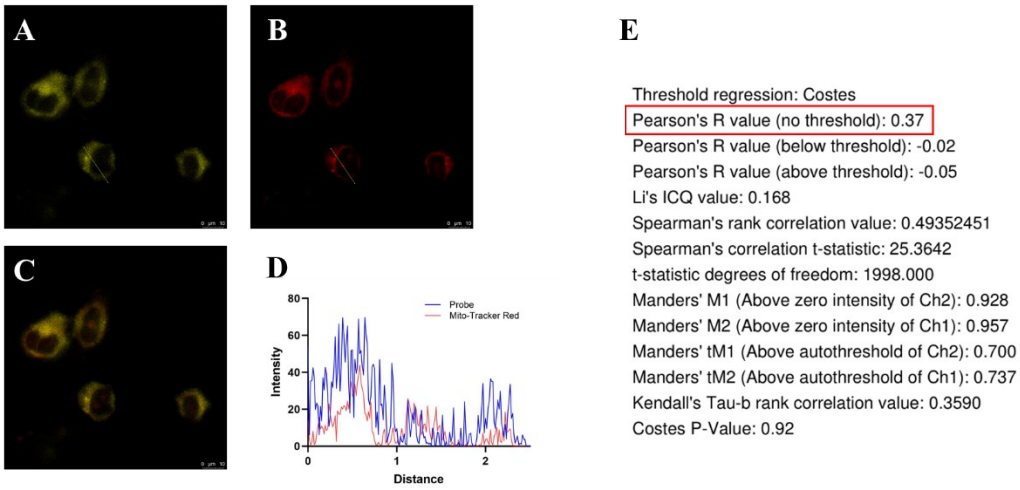


Figure S15. The colocalization of **ASSI-Leu** in HepG2 cells. (A) Channel two: ASSI-Leu (10 μM) stain (λ_{ex}= 405 nm, λ_{em}= 490–560 nm); (B)Red channel: Mito-Tracker red (0.1 μM) stain (λ_{ex} = 570 nm, λ_{em} = 590–630 nm); (C) Merged images of (A, B); (D) FL intensity profile of ROI across the line from (A, B). Scale bar is 10 μm. (F) The colocalization of **ASSI-Leu** in HepG2 cells (Pearson's correlation coefficient (r) = 0.37).

Characterization of compounds

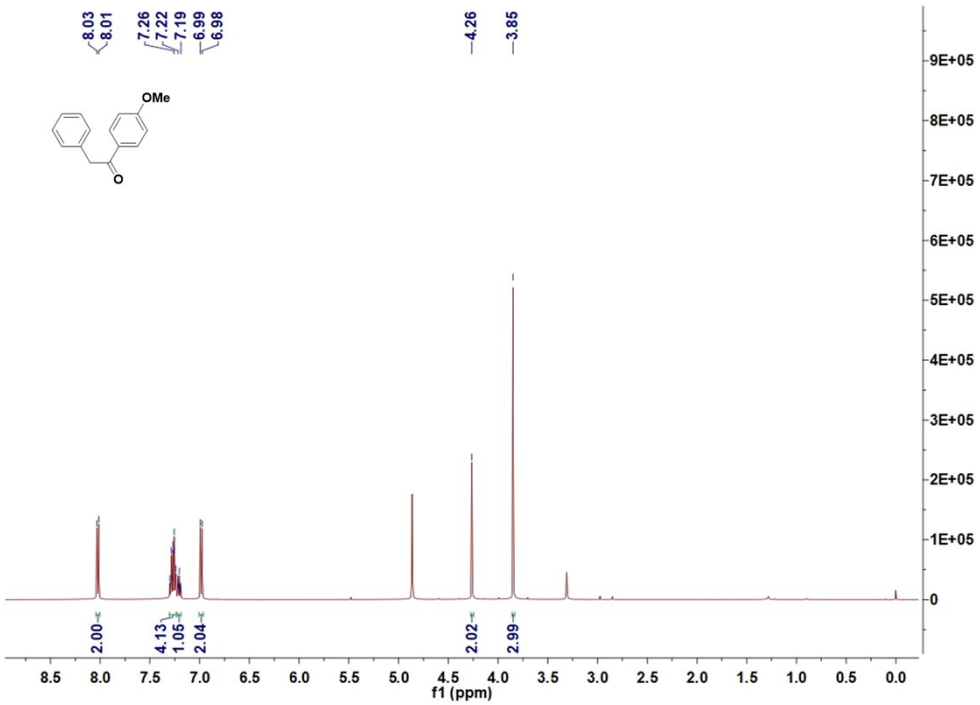


Figure S16. ^{1}H NMR spectra (CD_3OD) of compound **1**.

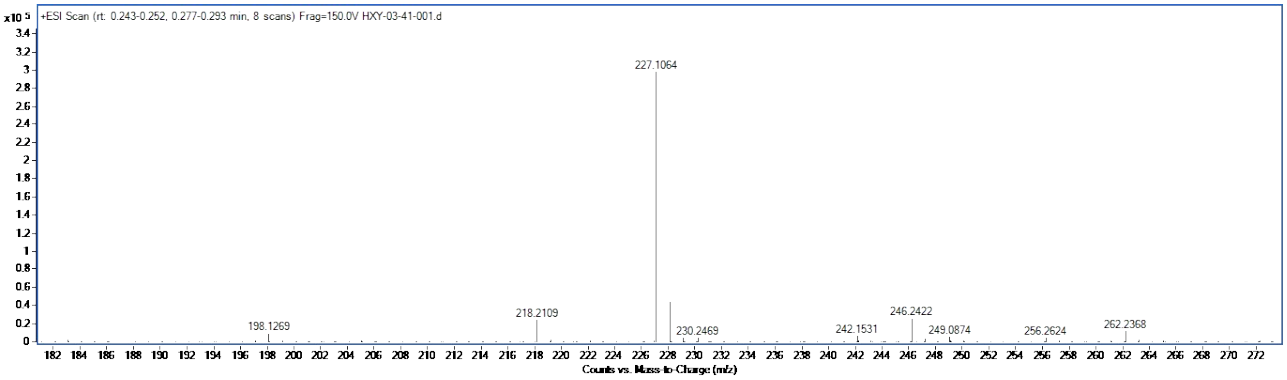


Figure S17. HRMS spectrum of compound **1**.

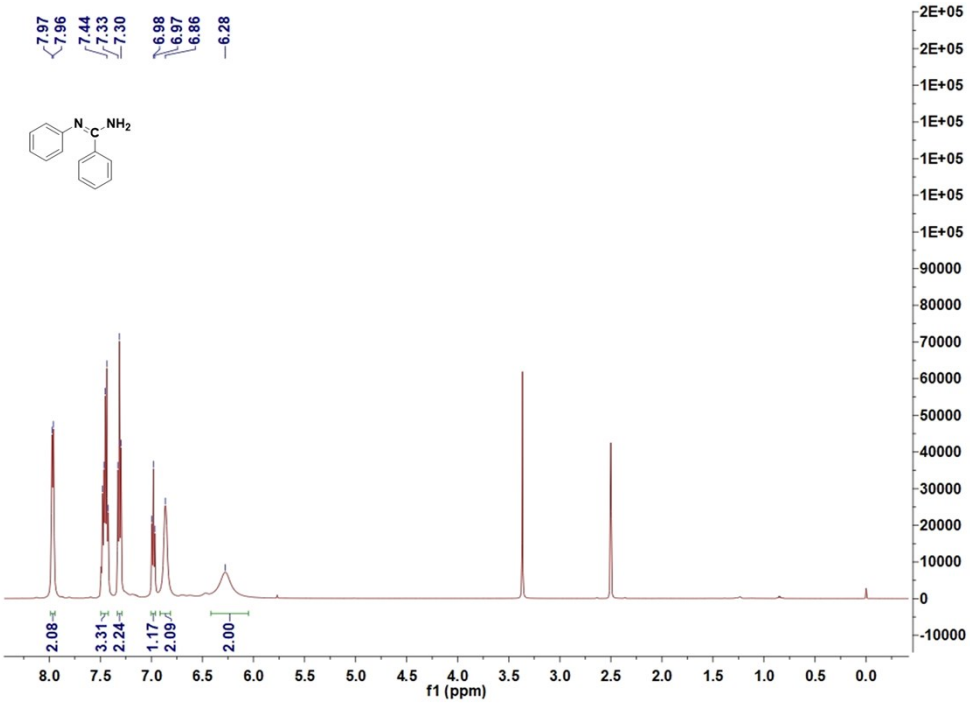


Figure S18. ^{1}H NMR spectra (DMSO-d_6) of compound **2**.

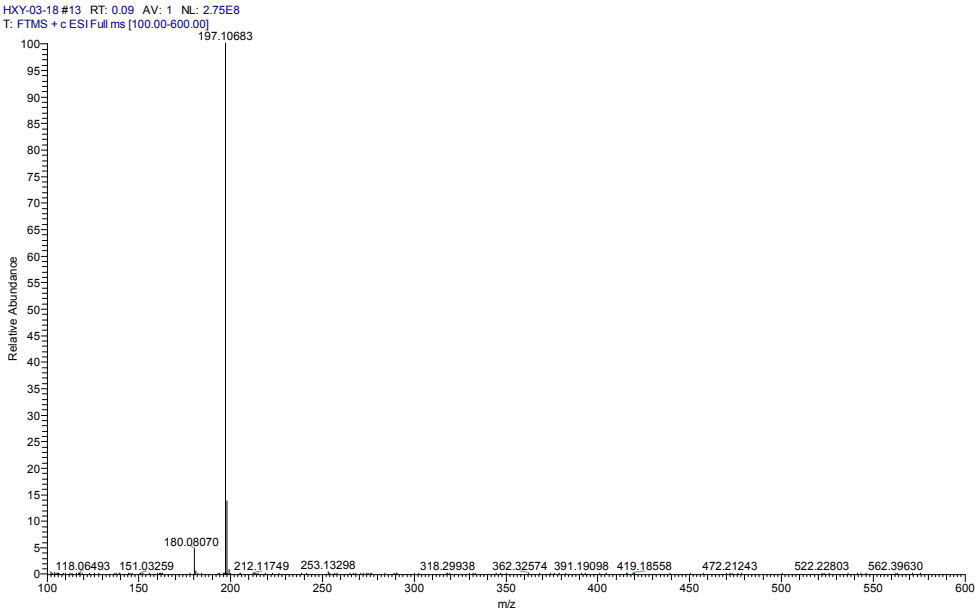


Figure S19. HRMS spectrum of compound **2**.

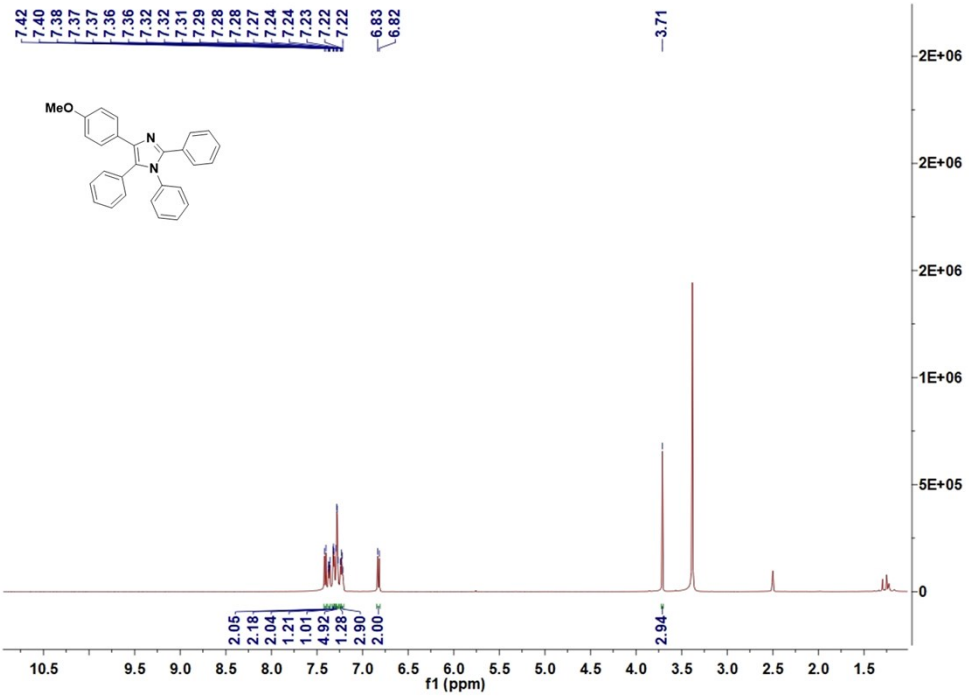


Figure S20. ^{1}H NMR spectra (DMSO-d_6) of compound **3**.

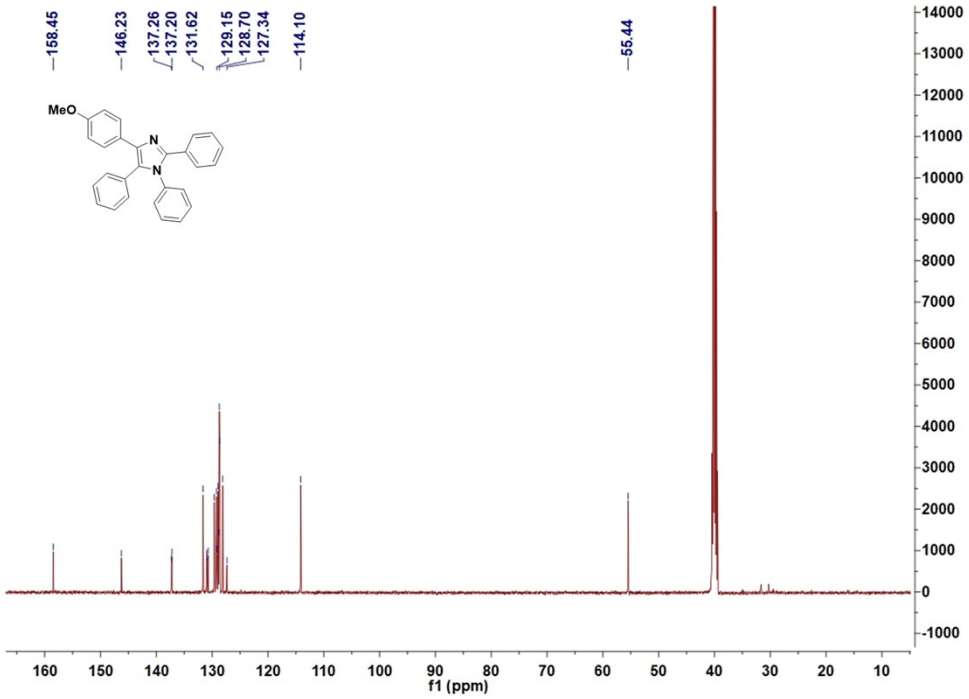


Figure S21. ^{13}C NMR spectra (DMSO-d_6) of compound **3**.

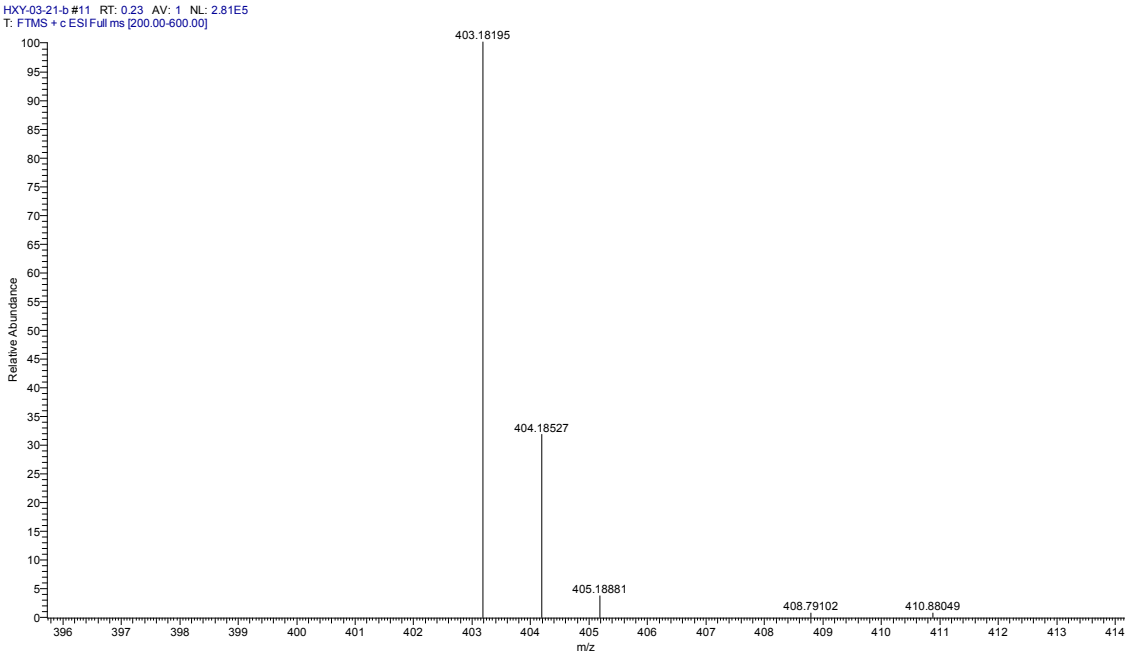


Figure S22. HRMS spectrum of compound **3**.

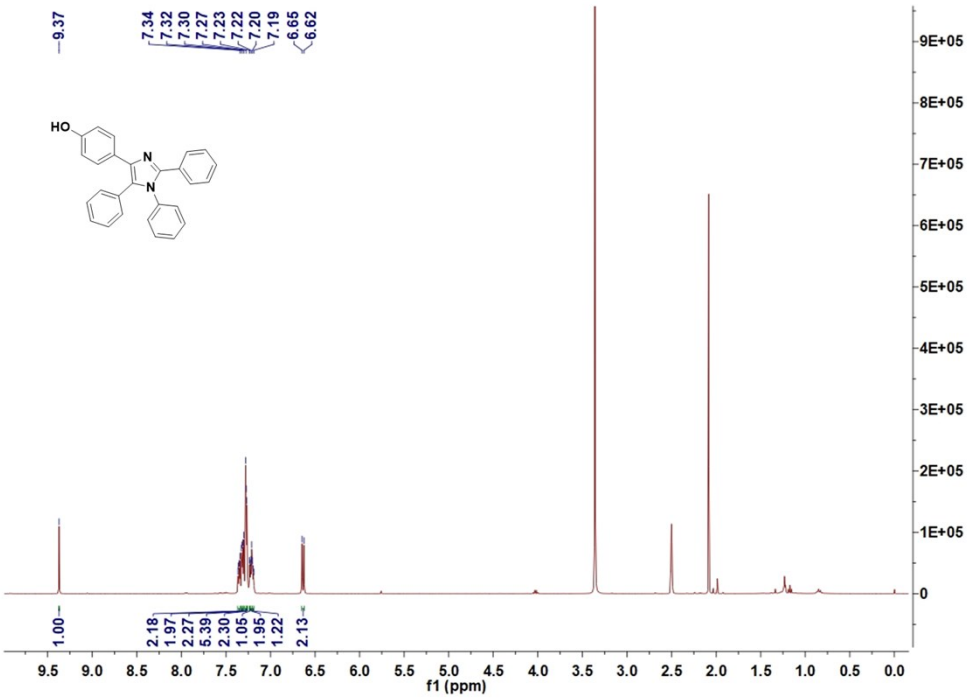


Figure S23. ^{1}H NMR spectra (DMSO-d_6) of compound **4**.

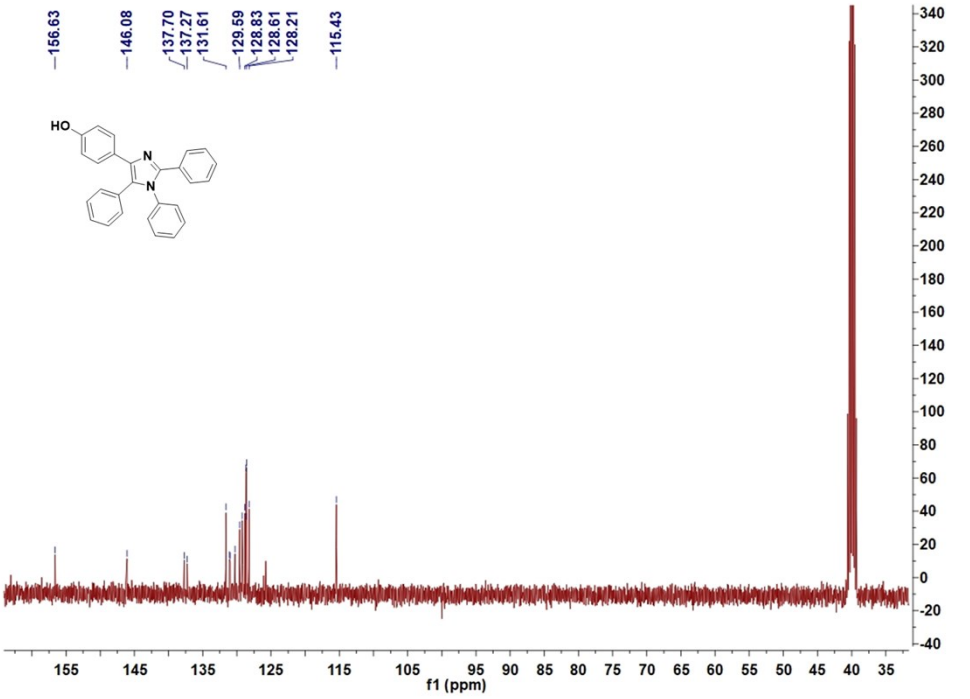


Figure S24. ^{13}C NMR spectra (DMSO-d_6) of compound **4**.

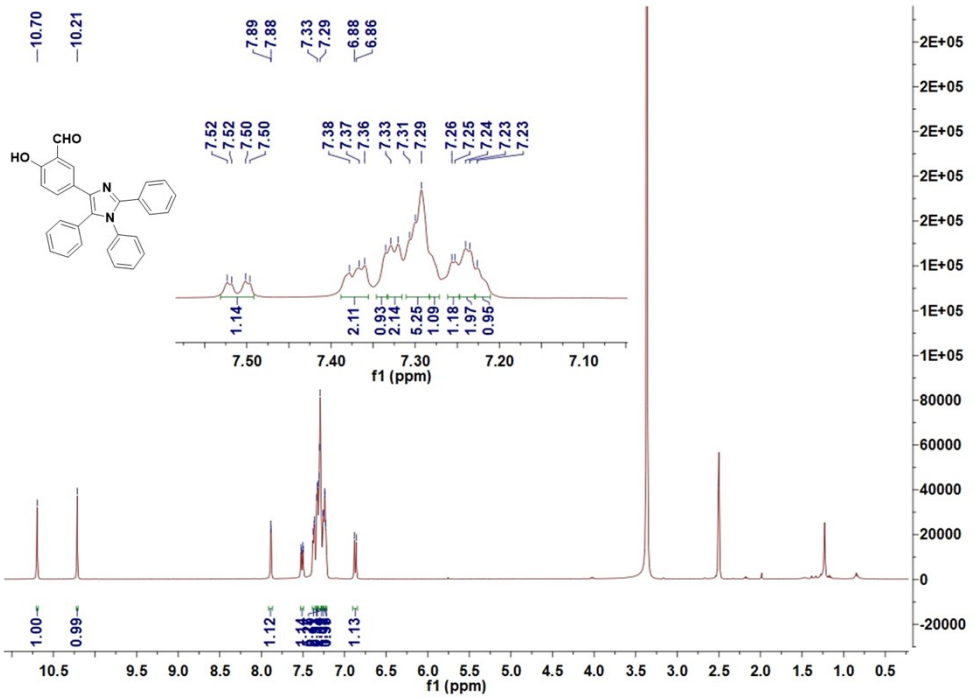


Figure S25. ^{1}H NMR spectra (DMSO-d_6) of compound **5**.

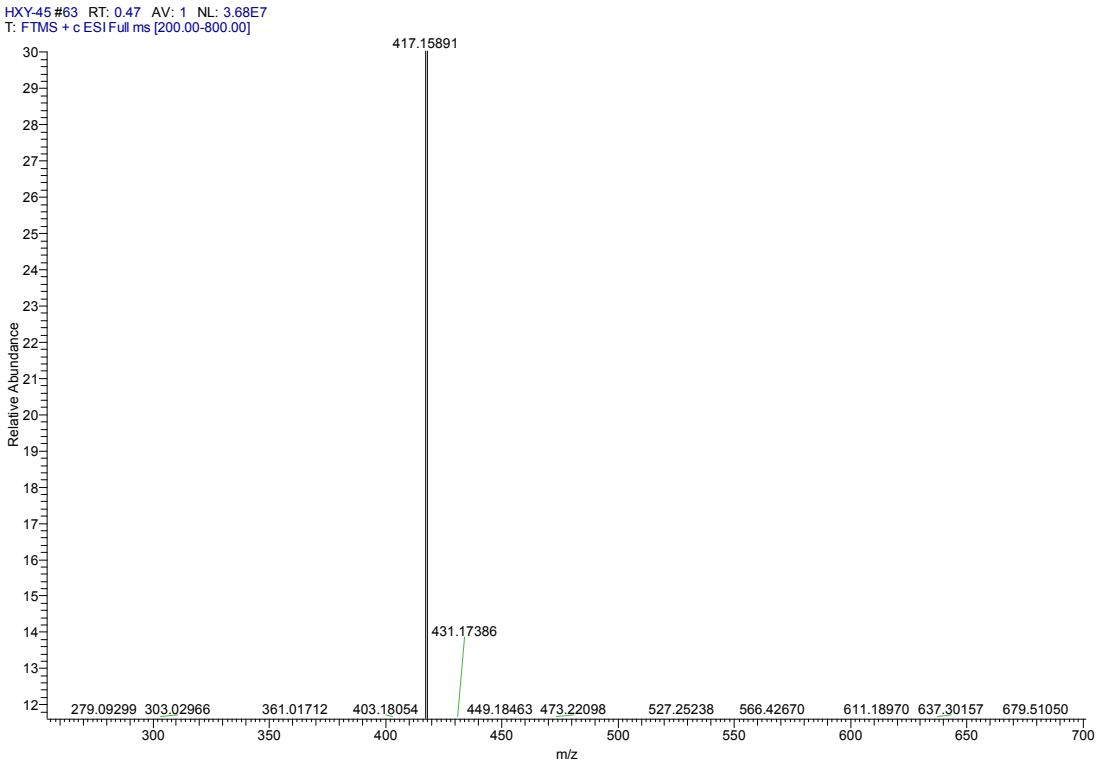


Figure S26. HRMS spectrum of compound **5**.

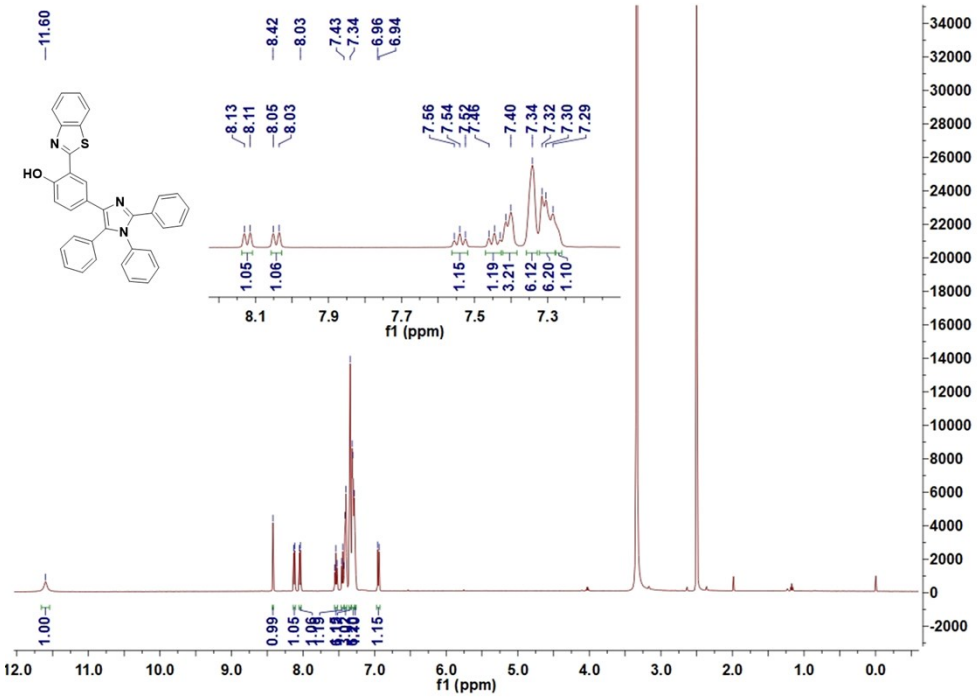


Figure S27. ^{1}H NMR spectra (DMSO-d_6) of **ASSI-OH**.

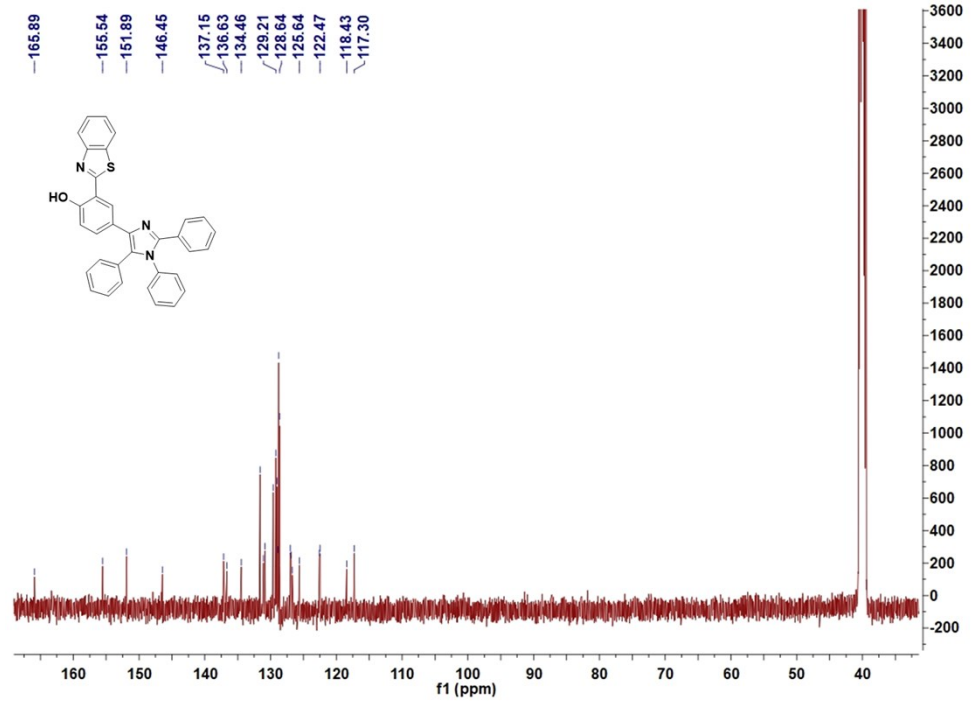


Figure S28. ^{13}C NMR spectra (DMSO-d_6) of **ASSI-OH**.

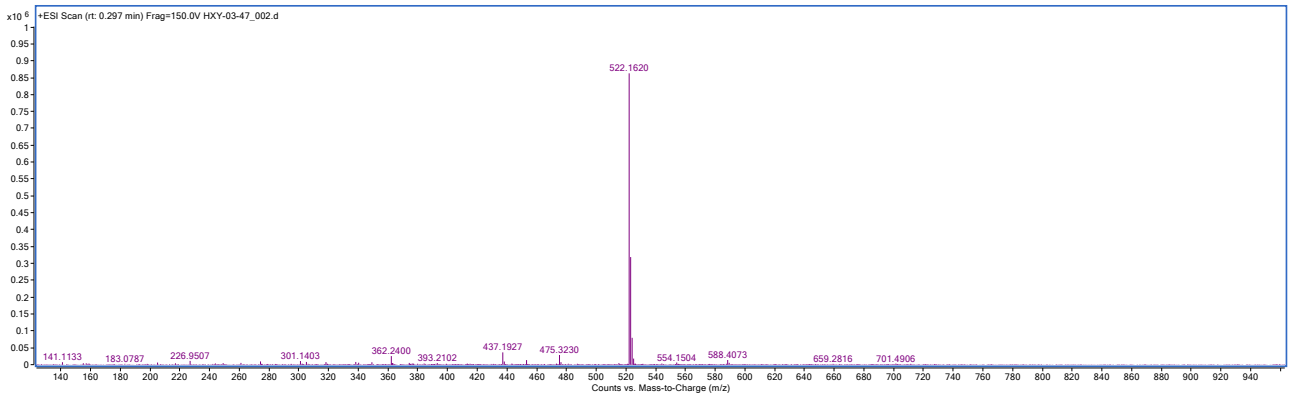


Figure S29. HRMS spectrum of **ASSI-OH**.

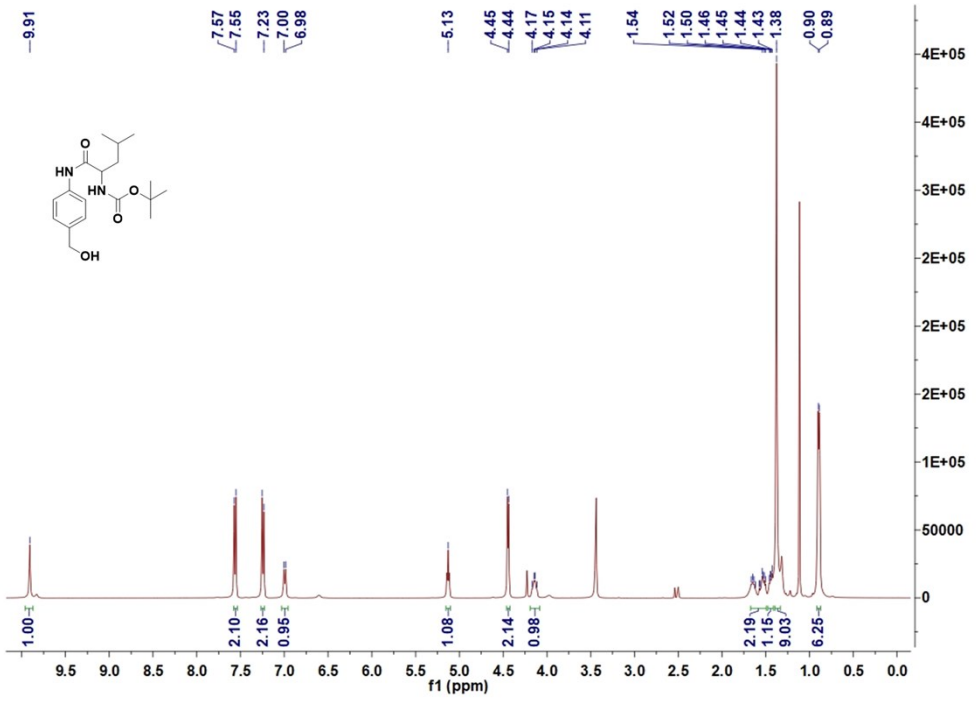


Figure S30. ^{1}H NMR spectra (DMSO-d_6) of compound **6**.

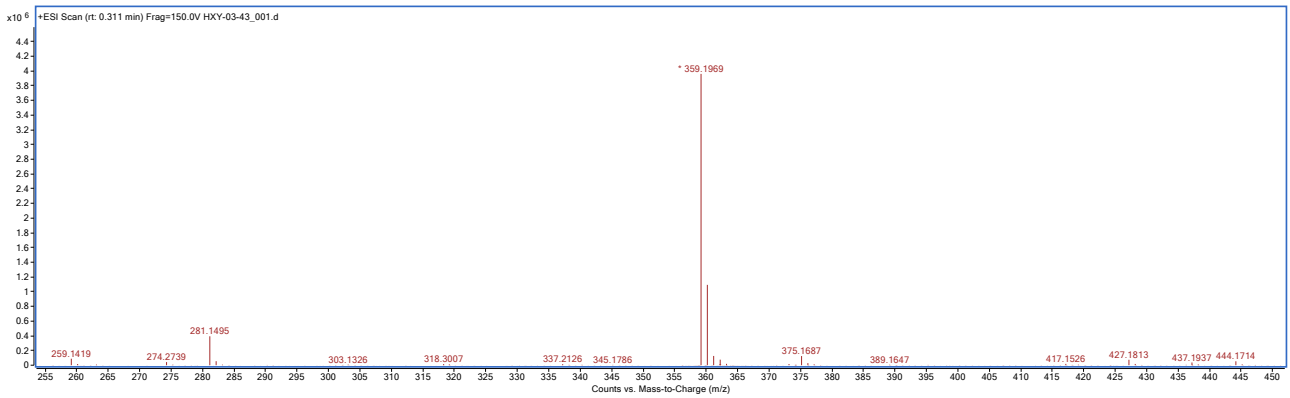


Figure S31. HRMS spectrum of compound **6**.

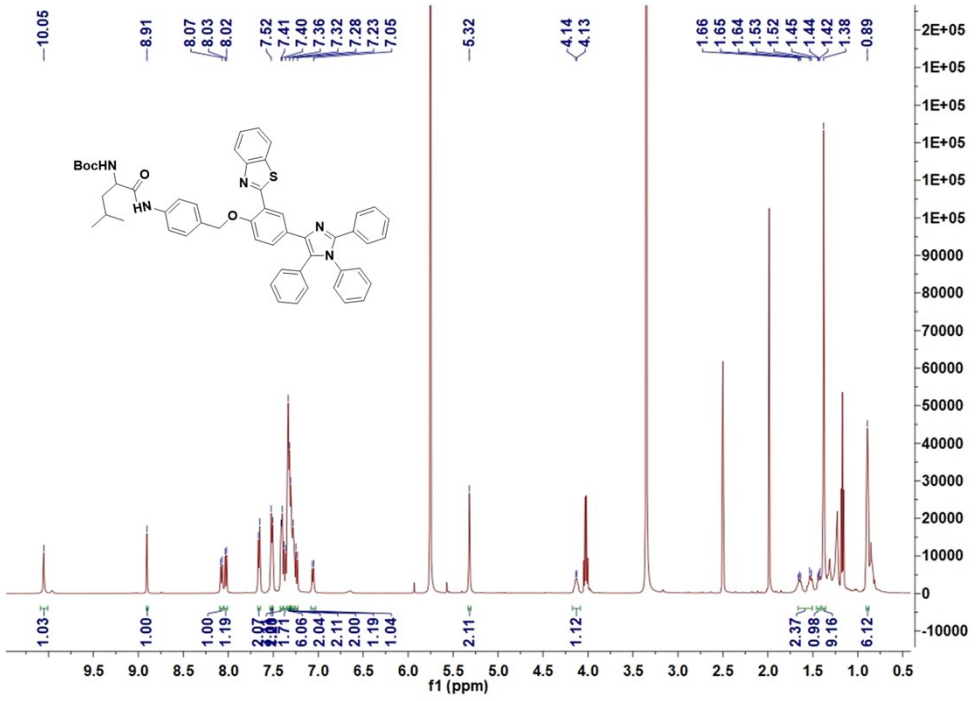


Figure S32. ^{1}H NMR spectra (DMSO-d_6) of compound **8.**

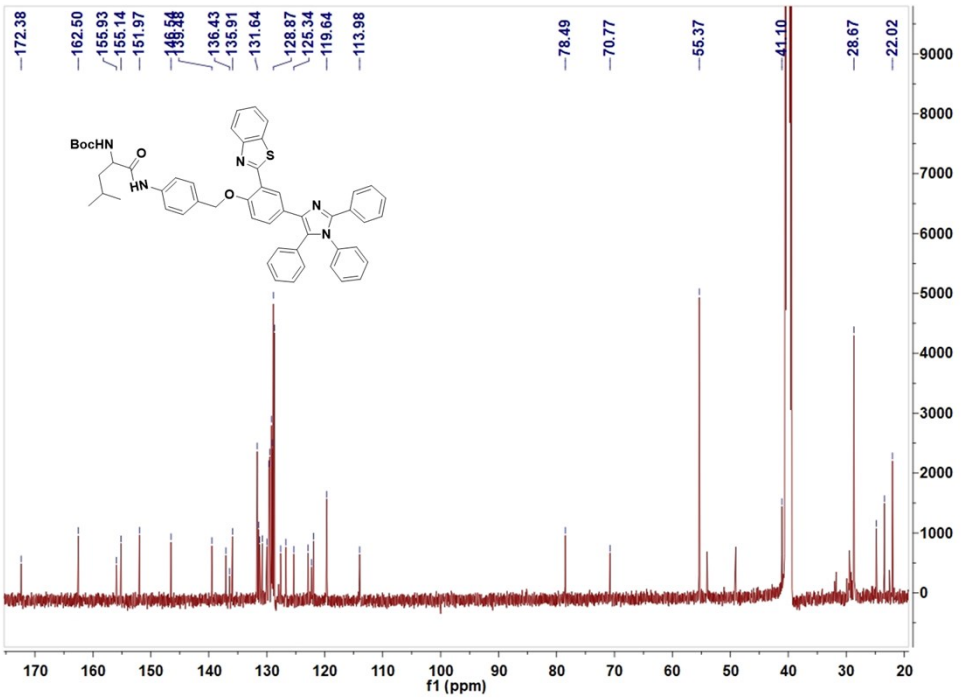


Figure S33. ^{13}C NMR spectra (DMSO-d_6) of compound **8**.

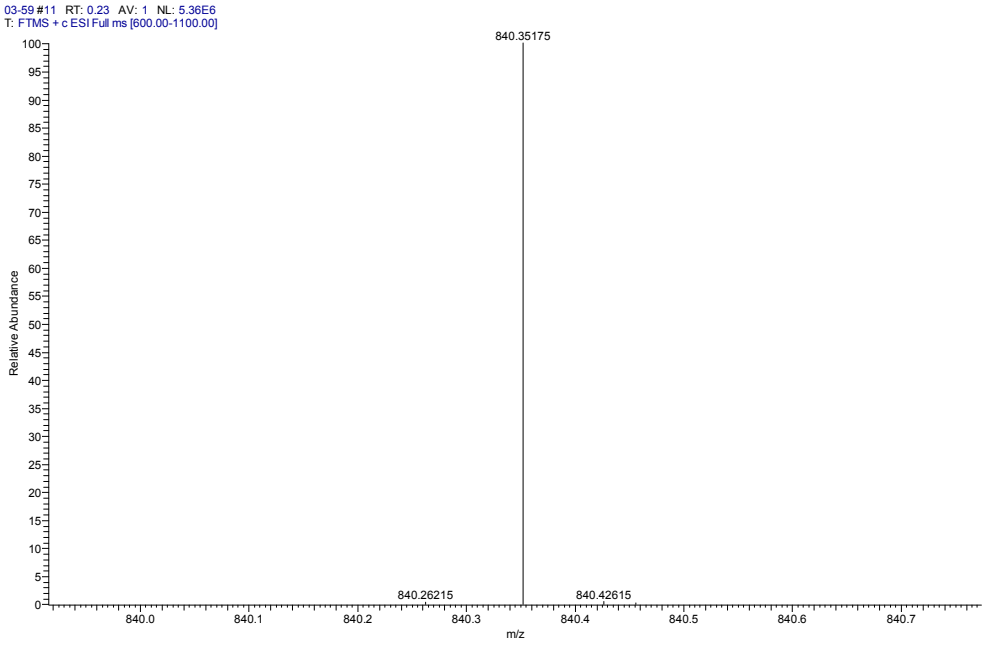


Figure S34. HRMS spectrum of compound **8**.

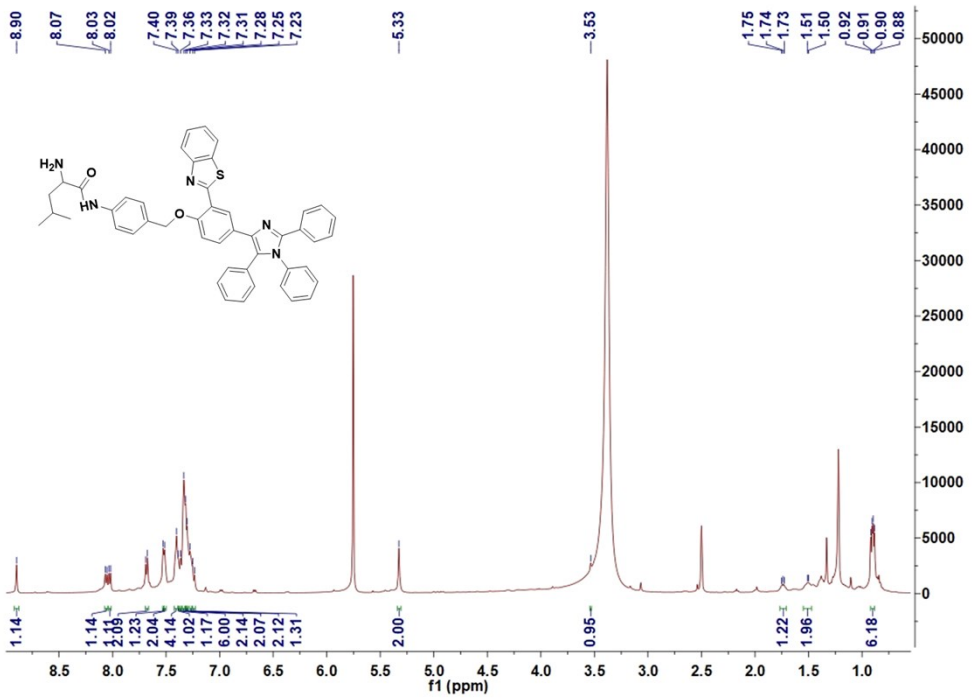


Figure S35. ^{1}H NMR spectra (DMSO-d_6) of **ASSI-Leu.**

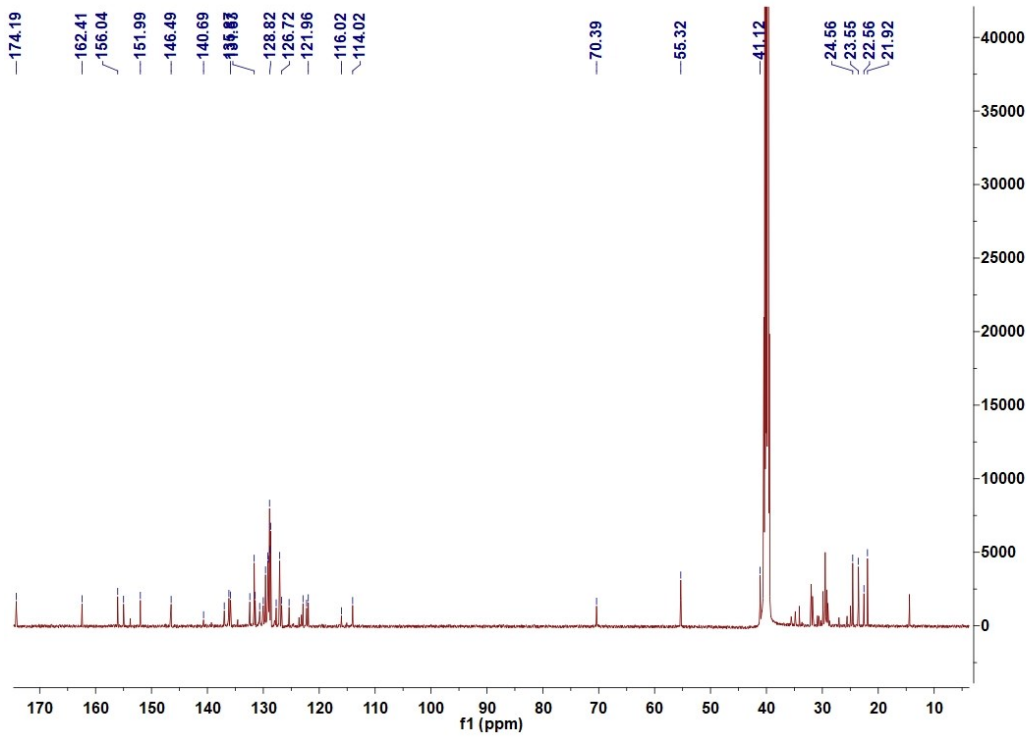


Figure S36. ^{13}C NMR spectra (DMSO-d_6) of **ASSI-Leu**.

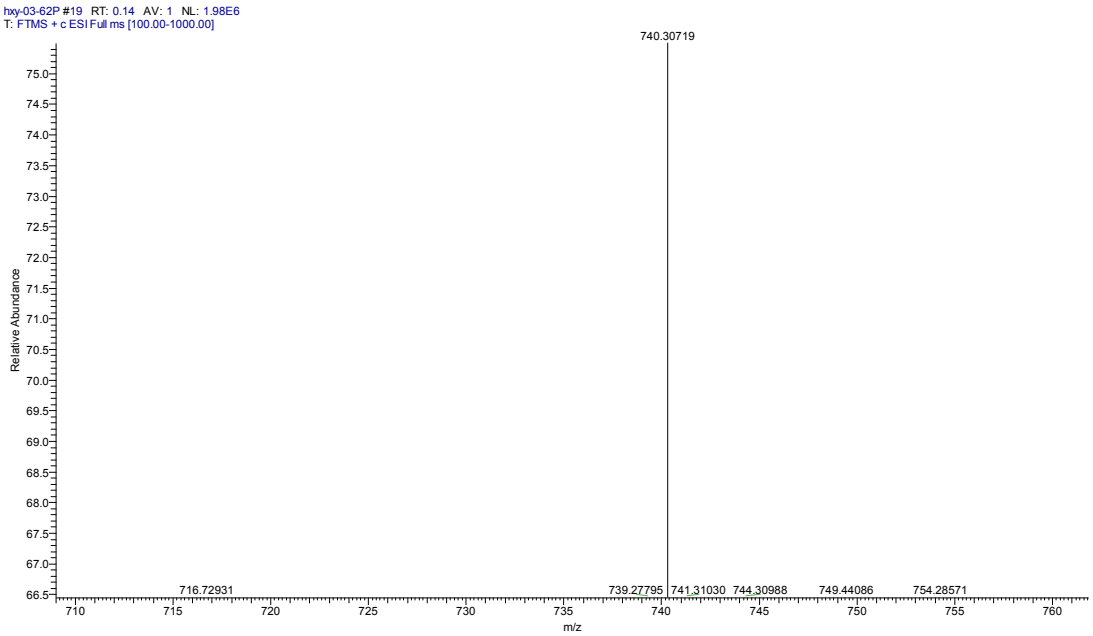


Figure S37. HRMS spectrum of **ASSI-Leu**.