Supporting Information

Solvent-free and efficient synthesis of bicyclic 2-pyridone derivatives for endoplasmic reticulum imaging

Xiaoyun Ran,[‡a] Qian Zhou,[‡a] Jin Zhang,[a] Shanqiang Wang,[c] Gui Wang,[c] Hui Yang,[a] Xiaochun Liu,[a] Zhouyu Wang*[a] and Xiaoqi Yu*[a, b]

[a.] *Department of Chemistry, Xihua University, Chengdu, 610039, P. R. China.*

E-mail: zhouyuwang@ mail.xhu.edu.cn, xqyu@scu.edu.cn.

[b.] *Key Laboratory of Green Chemistry and Technology, Ministry of Education, College of Chemistry, Sichuan University, Chengdu, 610064, P. R. China.*

[c.] *Department of Pharmaceutics Engineering, Xihua University, Chengdu, 610039, P. R. China.*

[‡] *These authors contributed equally to this work and should be considered as co-first authors.*

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Experimental procedures

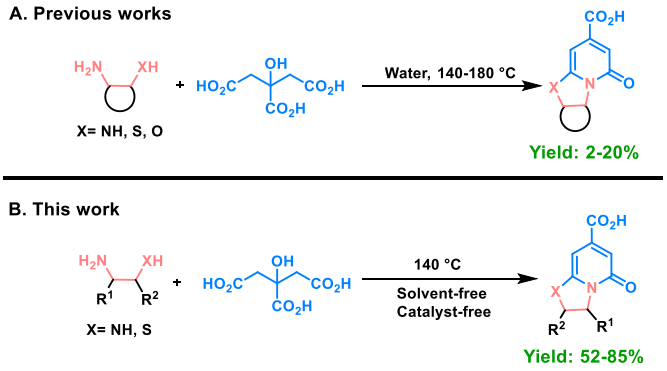
1. Materials and instruments

Unless otherwise noted, all chemical reagents were purchased from commercial suppliers and used without further purification. Citric acid, (1S,2S)-1,2-Diphenyl-1,2-ethanediamine, Methyl iodide were purchased from Adamas. All solvents were dried according to the standard methods prior to use. In the optical spectroscopic studies, all of the solvents were either HPLC or spectroscopic grade.

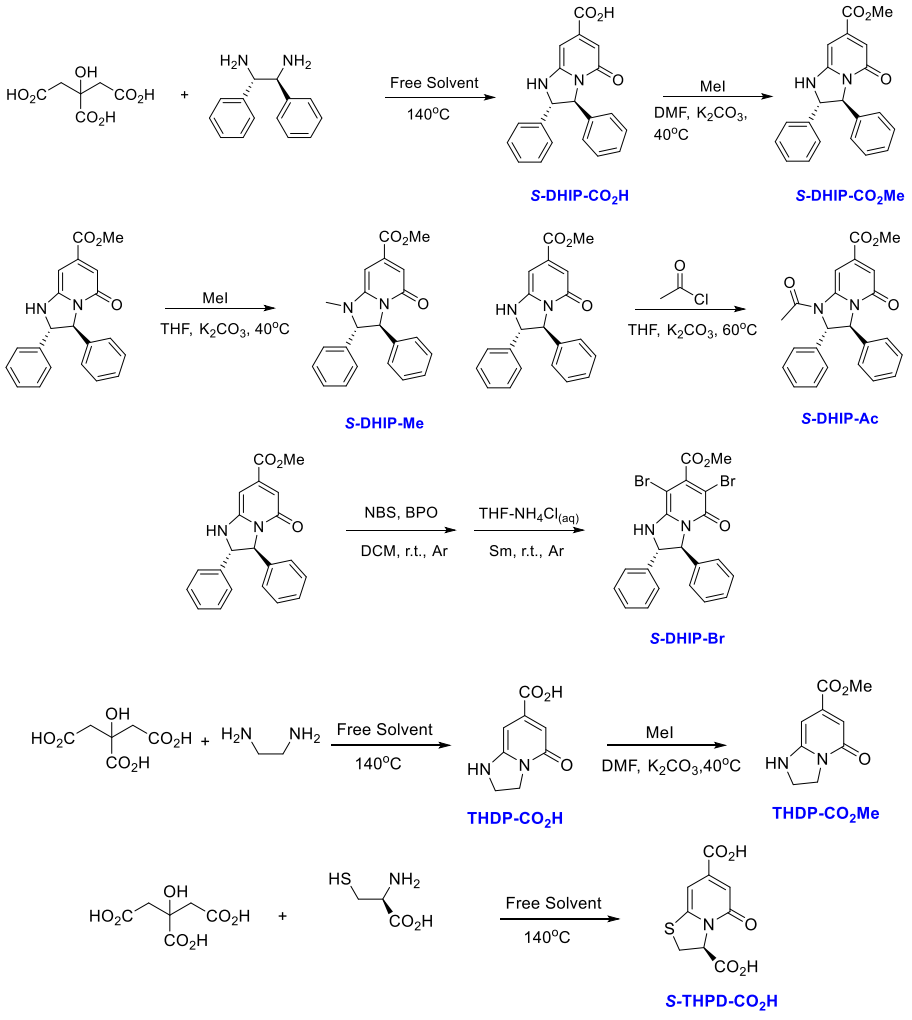
Thin layer chromatography (TLC) was performed on silica gel plates, and spots were visualized under UV light. Column chromatography was carried out using 200-300 mesh silica gel (Qingdao Ocean Chemicals). ^{1}H NMR (300 or 400 MHz) and ^{13}C NMR (75 or 100 MHz, respectively) spectra were recorded on a Bruker 400 MHz NMR spectrometer in $CDCl_3$ or DMSO. ^{1}H NMR chemical shifts are reported in ppm (δ) relative to tetramethylsilane (TMS) with the solvent resonance employed as the internal standard ($CDCl_3$, δ 7.26 ppm, DMSO-d_6 at 2.50 ppm). Proton chemical shifts of NMR spectra were given in ppm relative to internals reference TMS (1H, 0.00 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, brs = broad singlet, d = doublet, t = triplet, td = triplet of doublets, q = quartet, m = multiplet), coupling constants (Hz) and integration. ^{13}C NMR chemical shifts are reported in ppm from tetramethylsilane (TMS) with the solvent resonance as the internal standard ($CDCl_3$, δ 77.0 ppm, DMSO – d_6 at 39.51 ppm). HRMS data were obtained on X500R QTOFB. UV absorption spectra were recorded on a shimadzu UV-2700 UV-visible spectrophotometer. Fluorescence spectra were measured on a Hitachi F-4600 fluorescence spectrophotometer. The absolute fluorescence quantum yield was determined by Hamamatsu quantum yield spectrometer C11347 Quantaurus_QY. Confocal microscopy fluorescence images were acquired on Leica TCS SP8. Single crystal were grown from chloroform/n-heptane via slow evaporation method. Single crystal X-ray diffraction intensity data were collected on Agilent Technologies (Gemini). The ground-state geometries were optimized using the density function theory (DFT) method with B3LYP hybrid functional at the basis set level of 6-31G (d, p). All the calculations were performed using Gaussian 09 package. MTS method was used for testing the cell viability and described in the experimental section. HeLa cells were obtained from Shanghai Institute of Biochemistry and Cell Biochemistry and Cell Biology, Chinese Academy of Science. Confocal lasing scanning microscopic (CLSM) images of single-photo were obtained using LSM 780 (Zeiss).

2. Procedures and characterizations data of compounds.

Generanal chemical method of synthesis the compounds of S-DHIP-CO_2H, S-THDP-CO_2H, S-THDP-CO_2H



Scheme S1 The previous and this methods to synthesis the bicyclic 2-pyridone framework..



Scheme S2 Synthetic routes of terminal compounds.

Ethanediamine derivative (10 mmol) and citric acid (1.92 g, 10 mmol) were added to a hydrothermal synthesis kettle. Then the mixture was reacted at 140 °C for 4 h and was detected by TLC. The crude product was purified by recrystallization with EtOH/H_2O.

(2S,3S)-5-oxo-2,3-diphenyl-1,2,3,5-tetrahydroimidazo[1,2-a]pyridine-7-carboxylic acid (*S*-DHIP-CO_2H): reddish-brown solid, yield: 85%. ^{1}H NMR (400 MHz, DMSO – d_6) δ 13.29 (s, 1H), 8.42 (s, 1H), 7.46 – 7.31 (m, 8H), 7.26 – 7.21 (m, 2H), 6.01 (d, *J* = 1.6 Hz, 1H), 5.91 (d, *J* = 1.6 Hz, 1H), 5.32 (d, *J* = 2.8 Hz, 1H), 4.85 (d, *J* = 2.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO – d_6, Fig.S11) δ 175.0, 171.8, 167.2, 160.1, 154.3, 145.2, 142.1, 139.9, 129.5, 129.3, 128.8, 128.5, 126.2, 126.1, 105.8, 80.8, 72.9, 68.1, 66.6, 43.2. HRMS (ESI) Calcd for [C20H16N2O3 + H] + 332.1234; Found: 333.1229. M.p: 149.5-150.1°C.

(2R,3R)-5-oxo-2,3-diphenyl-1,2,3,5-tetrahydroimidazo[1,2-a]pyridine-7-carboxylic acid (*R*-DHIP-CO_2H): reddish-brown solid, yield: 78%. ^{1}H NMR (400 MHz, DMSO) δ 13.33 (s, 1H), 8.44 (s, 1H), 7.46 – 7.31 (m, 8H), 7.27 – 7.21 (m, 2H), 6.03 (d, *J* = 1.52 Hz, 1H), 5.93 (d, *J* = 1.52 Hz, 1H), 5.33 (d, *J* = 2.6 Hz, 1H), 4.86 (d, *J* = 2.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO – d_6) δ 167.2, 160.1, 154.3, 145.2, 142.1, 139.9, 129.5, 129.3, 128.8, 128.5, 126.2, 126.1, 105.8, 80.8, 68.1, 66.6. HRMS (ESI) Calcd for [C20H16N2O3 -H] + 331.1088; Found: 331.1088. M.p: 135.8-136.0 °C.

5-oxo-1,2,3,5-tetrahydroimidazo[1,2-a]pyridine-7-carboxylic acid[1,2] **(THDP-CO_2H): brown solid, yield: 52%.** ^{1}H NMR (400 MHz, DMSO – d_6) δ 7.49 (s, 1H), 5.94 (d, *J* = 1.6 Hz, 1H), 5.67 (d, *J* = 1.6 Hz, 1H), 4.02 (t, *J* = 8.8 Hz, 2H), 3.64 (t, *J* = 8.8 Hz, 2H). HRMS (ESI, Fig. S14) Calcd for [C8H8N2O3 + H] + 181.0608; Found: 181.0604. M.p: 139.8-139.9°C.

(S)-5-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyridine-3,7-dicarboxylic acid[1,2] **(*S*-THPD-CO_2H): chartreuse solid, yield: 73%.**^{1}H NMR (400 MHz, DMSO – d_6) δ 6.60 (d, *J* = 1.4 Hz, 1H), 6.56 (d, *J* = 1.6 Hz, 1H), 5.47 (dd, *J* = 8.8, 1.2 Hz, 1H), 3.91 (dd, *J* = 12.0, 8.8 Hz, 1H), 3.61 (dd, *J* = 12.0, 1.2 Hz, 1H). M.p: 181.1-182.3°C

Synthesis of compound S-DHIP-CO_2Me

The carboxylic acid **(S-DHIP-CO_2H**, 2 mmol) and K_2CO_3 (3 mmol, 414 mg) was dissolved in DMF (10 mL) and stirred at 40 °C for 30 minutes. Then the MeI (300 mg, 2.1 mmol) was added to the mixture and stirred at 40 °C for 3 h, until full conversion was detected by TLC. The reaction mixture was put into the water and extracted by ethyl acetate (EtOAc) (3 × 30 mL). The combined organic layer was concentrated under reduced pressure. The crude products were purified by silica-gel chromatography with ethyl acetate/petroleum ether to give the pure product.

Methyl (2S,3S)-5-oxo-2,3-diphenyl-1,2,3,5-tetrahydroimidazo [1,2-a]pyridine -7-carboxylate (*S*-DHIP-CO_2Me): light yellow solid，yield: 86%. ^{1}H NMR (400 MHz, DMSO – d_6) δ 8.52 (s, 1H), 7.46 –

7.35 (m, 6H), 7.35 – 7.32 (m, 2H), 7.26 – 7.21 (m, 2H), 6.03 (d, *J* = 1.6 Hz, 1H), 5.93 (d, *J* = 1.6 Hz, 1H), 5.34 (d, *J* = 2.8 Hz, 1H), 4.88 (d, *J* = 2.8 Hz, 1H), 3.84 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.1, 159.9, 154.4, 143.8, 142.0, 139.8, 129.5, 129.3, 128.8, 128.5, 126.2, 126.1, 105.7, 80.5, 68.1, 66.6, 53.1. HRMS (ESI) Calcd for [C21H18N2O3 + H] + 347.1390; Found: 347.1385. M.p: 108.7-109.8°C.

Synthesis of compound *S*-DHIP-Me

The carboxylic acid (***S*-DHIP-CO$_2$H**, 2 mmol) and K_2CO_3 (3 mmol, 414 mg) were dissolved in THF (10 mL) and stirred at 40 °C for 30 minutes. Then the MeI (600 mg, 4.2 mmol) was added to the mixture and stirred at 40 °C for 3h, until full conversion was detected by TLC. The reaction mixture was put into the water and extracted by ethyl acetate (EtOAc) (3 × 30 mL). The combined organic layer was concentrated under reduced pressure. The crude products were purified by silica-gel chromatography with ethyl acetate/petroleum ether to give the pure product.

Methyl (2S,3S)-1-methyl-5-oxo-2,3-diphenyl-1,2,3,5-tetrahydroimidazo [1,2-a] pyridine -7-carboxylate (*S*-DHIP-Me): orange solid, yield: 56%. ^{1}H NMR (400 MHz, DMSO – d_6) δ 7.46 – 7.33 (m, 6H), 7.25 – 7.17 (m, 4H), 6.09 (d, *J* = 1.6 Hz, 1H), 5.87 (d, *J* = 1.6 Hz, 1H), 5.31 (d, *J* = 3.6 Hz, 1H), 4.79 (d, *J* = 3.6 Hz, 1H), 3.86 (s, 3H), 2.79 (s, 3H).^{13}C NMR (100 MHz, DMSO – d_6) δ 166.2, 160.3, 153.8, 144.1, 139.2, 138.5, 129.8, 129.4, 128.7, 126.7, 126.2, 106.2, 79.8, 72.8, 67.1, 53.3, 31.1. HRMS (ESI) Calcd for [C22H20N2O3 + H] + 361.1540; Found: 361.1547. M.p:117.1-118.9°C.

Synthesis of compound *S*-DHIP-Ac

The compound ***S*-DHIP-CO$_2$Me** (692 mg, 2mmol) and K_2CO_3 (3 mmol, 414 mg) were dissolved in THF (10 mL) and stirred at 60 °C for 30 minutes. Then the acetylchloride (236 mg, 3 mmol) was added to the mixture and stirred at 60 °C for 3h, until full conversion was detected by TLC. The reaction mixture was put into the water and extracted by ethyl acetate (EtOAc) (3 × 30 mL). The combined organic layer was concentrated under reduced pressure. The crude products were purified by silica-gel chromatography with ethyl acetate/petroleum ether give the pure product.

Methyl (2S,3S)-1-acetyl-5-oxo-2,3-diphenyl-1,2,3,5-tetrahydroimidazo [1,2-a] pyridine-7-carboxylate (*S*-DHIP-Ac): light yellow solid, yield: 85%. ^{1}H NMR (400 MHz, DMSO – d_6) δ 7.51 – 7.37 (m, 7H), 7.32 (d, *J* = 7.2 Hz, 2H), 7.28 – 7.23 (m, 2H), 6.62 (d, *J* = 1.6 Hz, 1H), 5.77 (d, *J* = 3.6 Hz, 1H), 5.44 (s, 1H), 3.89 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (100 MHz, DMSO – d_6) δ 165.3, 159.0, 145.70, 143.6, 139.7, 138.4, 130.1, 129.6, 129.3, 129.1, 126.3, 125.6, 115.5, 89.8, 67.8, 53.5, 24.2. HRMS (ESI) Calcd for [C23H20N2O4 + H] + 389.1496; Found: 389.1487. M.p: 181.0-183.7℃.

Synthesis of compound *S*-DHIP-Br

The compound ***S*-DHIP-CO_2Me** (692 mg, 2 mmol), NBS (10 mmol, 1.78 g) and BPO (0.2 mmol, 46.4mg) were dissolved in DCM (20 mL) and stirred at r.t. for 12 h, until full conversion was detected by TLC. The reaction mixture was put into the water and extracted by DCM (3 × 30 mL). Then the combined organic layer was dried over anhydrous Na_2SO_4. The solvents were removed under reduced pressure. Residue was dissolved in THF-$NH_4Cl_{(aq)}$ (v/v=1:1), then the samarium powder was added to the mixture and stirred at r.t. under Argon for 12 h, detected by TLC. Then the solids were filtered out, and the mixure was extrcated by EtOAc (3 × 30 mL), and the combined organic layer was dried over anhydrous Na_2SO_4. The solvents were removed under reduced pressure, residue was purified by silica-gel chromatography with ethyl acetate/petroleum ether to give the pure product.

Methyl 6,8-dibromo-5-oxo-2,3-diphenyl-1,2,3,5-tetrahydroimidazo[1,2-a] pyridine-7-carboxylate (*S*-DHIP-Br): brown solid, yield: 32%. ^{1}H NMR (400 MHz, DMSO – d_6) δ 9.03 (s, 1H), 7.47 – 7.38 (m, 6H), 7.36 – 7.32 (m, 2H), 7.28 – 7.24 (m, 2H), 5.46 (d, *J* = 4.0 Hz, 1H), 4.93 (d, *J* = 3.6 Hz, 1H), 3.92 (s, 3H). ^{13}C NMR (100 MHz, DMSO – d_6) δ 165.5, 154.8, 151.3, 148.6, 141.0, 139.1, 129.6, 129.5, 129.1, 128.9, 126.5, 126.4, 93.98 (s), 70.75 (s), 67.9, 67.0, 53.7. HRMS (ESI) Calcd for [C21H16Br2N2O3 + H] + 502.9600; Found: 502.9606. M.p: 70.3-71.8^{o}C.

3. Cell culture

Hela cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum and 1% Antibiotic-antimycotic at 37^{o}C in a 5% CO_2/95% air incubator. For fluorescence imaging, cells (4×10^3 /well) were passed on a 96-well plate and incubated for 24 h.

4. Cytotoxicity assay

HeLa cells were incubated in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1‰ antibiotics (penicillin-streptomycin, 10,000 U mL^{-1}) at 37 ^{o}C in a humidified atmosphere containing 5% CO_2. Toxicities of all compounds toward HeLa cells were determined by using MTS (3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium, inner salt) reduction assay following literature procedures. About 1.0×10^4 cells/well was seeded into 96-well plates. After 24 h, various concentrations of all compounds (1.25, 2.5, 5,10, 20 μM) were added to the cells. After another 24 h, 20 μL MTS and 80 μL PBS were added to each well and the plates were incubated at 37^{o}C for another 1h. Then the absorbance of each sample was measured using an ELISA plate reader (model imark 680, BioRad) at a wavelength of 490 nm. The cell viability (%) was obtained according to the

manufacturer' s instruction.

5. Co-localization imaging of cells

Hela cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1‰ antibiotics (penicillin-streptomycin, 10,000 U mL^{-1}) at 37 °C in a humidified atmosphere containing 5% CO_2. The cells were incubated for 2 days before dye loading on an uncoated 35 mm diameter glass-bottomed dish. Then, commercially available endoplasmic reticulum specific staining dyes (ER-Tracker Red) was used for co-localization study. Cells were co-incubated with 10 μM DHIP series probs and 10 μM ER-Tracker at 37 °C for 15 min and mounted on the microscope stage. Fluorescence images were captured using a ZEISS LSM 780 laser-scanning confocal microscope using proper excitation and emission filters for each dye: for probes, λ_{ex}= 405 nm, λ_{em}= 412-497 nm.

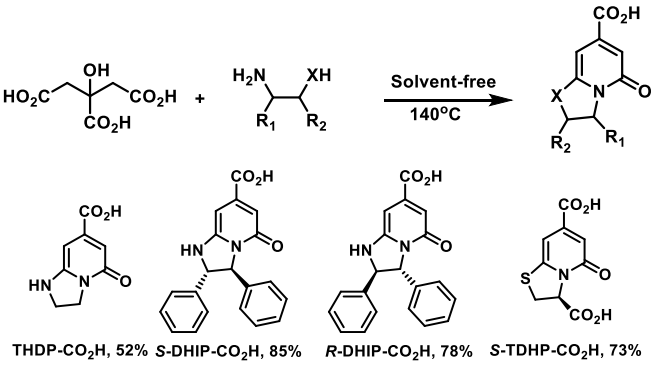
6. Bacteriostatic testing

All compounds against Grame-positive bacteria (*S. aureus* (ATCC-29923)) and Grame-gative bacteria (*E. coli* (ATCC-29922) were investigated using no dosing as the reference based on the National Committee for Clinical Laboratory Standards (NCCLS). The test compounds were dissolved in DMSO to make the concentration of stock solutions be 12800 μg/mL. Then 1 μL of the stock solution was added 99 μL sterile water and diluted to 128 μg/mL as the initial test concentration. Each well of the 96-well plates was added 100 μL of Mueller-Hinton Broth (MHB) and the initial solution was added to the first column to make the concentration of the test compounds be 64 μg/mL. Then pipetting 100 μL of the solution in the first column into the second column and employing the same 2-fold dilution method to adjust the concentrations of the test compounds in column 128 to 64 to 32, 16, 8, 4, 2, 1, 0.5, 0.25 and 0.125 mg/mL. Column 11 was just added only MHB solution while column 12 was only filled with bacterial suspension. The test bacteria were cultured in MHB overnight and cell concentrations of those bacteria were diluted to $1.5*10^5$ CFU/mL. Bacterial suspension with a mount of 100 μL was added to each well of the 96-well plates containing 100 μL of a serial of diluted compounds or drug and the 96-well plates were incubated at 37 °C for 20 h. The bacteriostatic testing values of the test compounds and drug were determined by the OD values which were compared to that of no dosing (blank control). Each concentration of the test compounds inhibiting each bacteria contained three parallels.

Supplementary figures and data

1. Substrate scope of ethylenediamine derivatives.

Table S1 Substrate scope of ethylenediamine derivatives.[a]



[a]Pyridone derivatives (10 mmol) and CA (10 mmol) were stirred at 140 °C for 4 h, isolated yield.

2. Solvent effect of all compounds

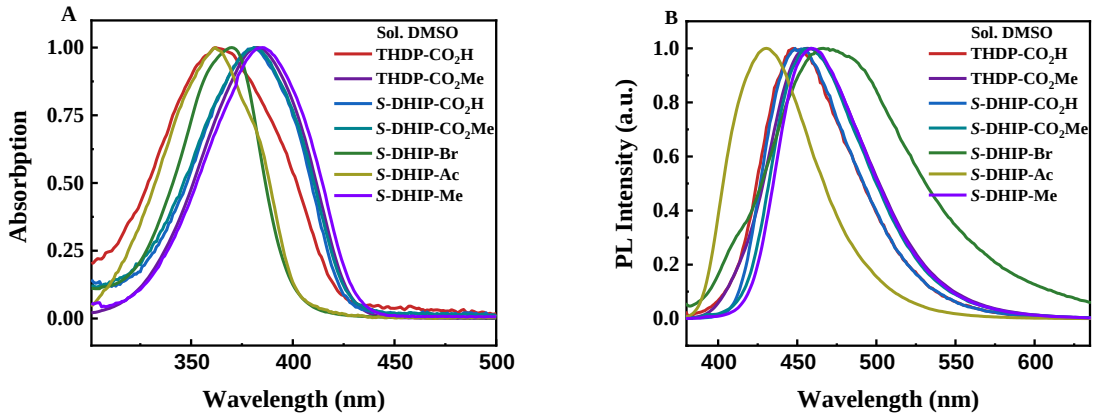
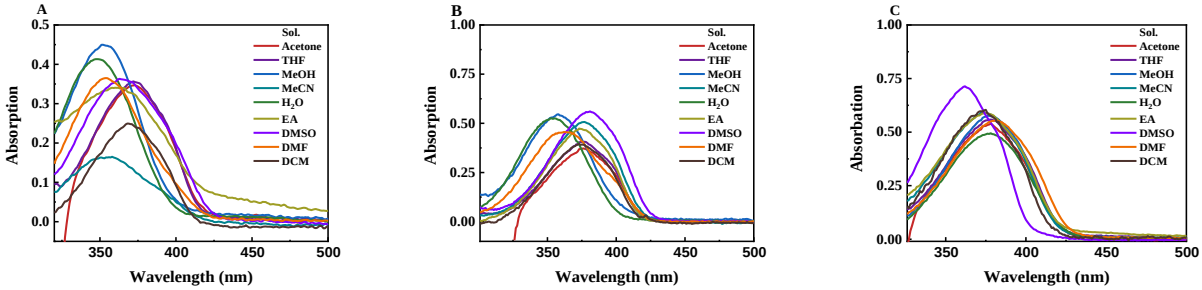


Fig. S1 Uv-vis absorbation and emission spectra of all compounds in DMSO A) c = 100 μM, B) c = 10 μM, $c_{DHIP\text{-}Br}$ = 100 μM, Slite = 2.5nm).



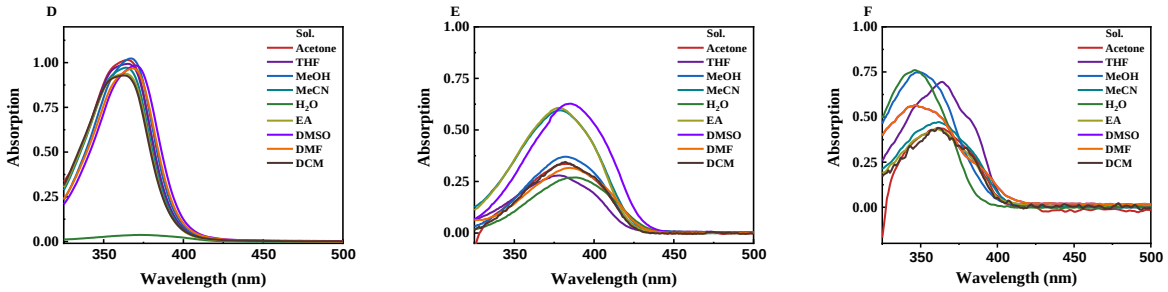


Fig. S2 Uv-vis absorption spectra of all compounds in different solvent A) THDP-CO_2H, B) *S*-DHIP-CO_2H, C) *S*-DHIP-CO_2Me, D) *S*-DHIP-Br, E) *S*-DHIP-Me; F) *S*-TDHP-CO_2H. (Solvent = Acetone, THF, MeOH, MeCN, H_2O, EA, DMSO, DMF, DCM, *c* = 100 μM, temp.= 25 °C).

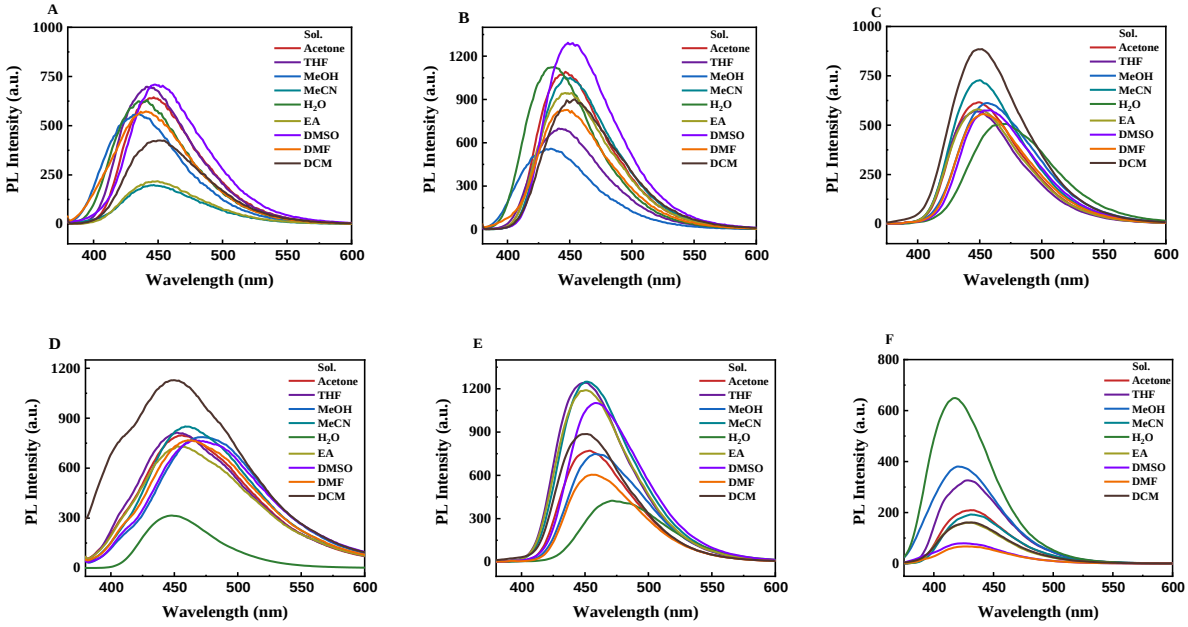


Fig. S3 Fluorescence spectra of all compounds in different solvent A) THDP-CO_2H, B) *S*-DHIP-CO_2H, C) *S*-DHIP-CO_2Me, D) *S*-DHIP-Br, E) *S*-DHIP-Me; F) *S*-TDHP-CO_2H. (Solvent = Acetone, THF, MeOH, MeCN, H_2O, EA, DMSO, DMF, DCM, *c* = 10 μM, $c_{DHIP\text{-}Br}$ = 100 μM, Slite = 2.5 nm, temp.= 25 °C).

3. Views of the molecular stacking structures in single crystals of THDP-CO_2H, *S*-DHIP-CO_2H, *S*-DHIP-CO_2Me, *S*-DHIP-Br

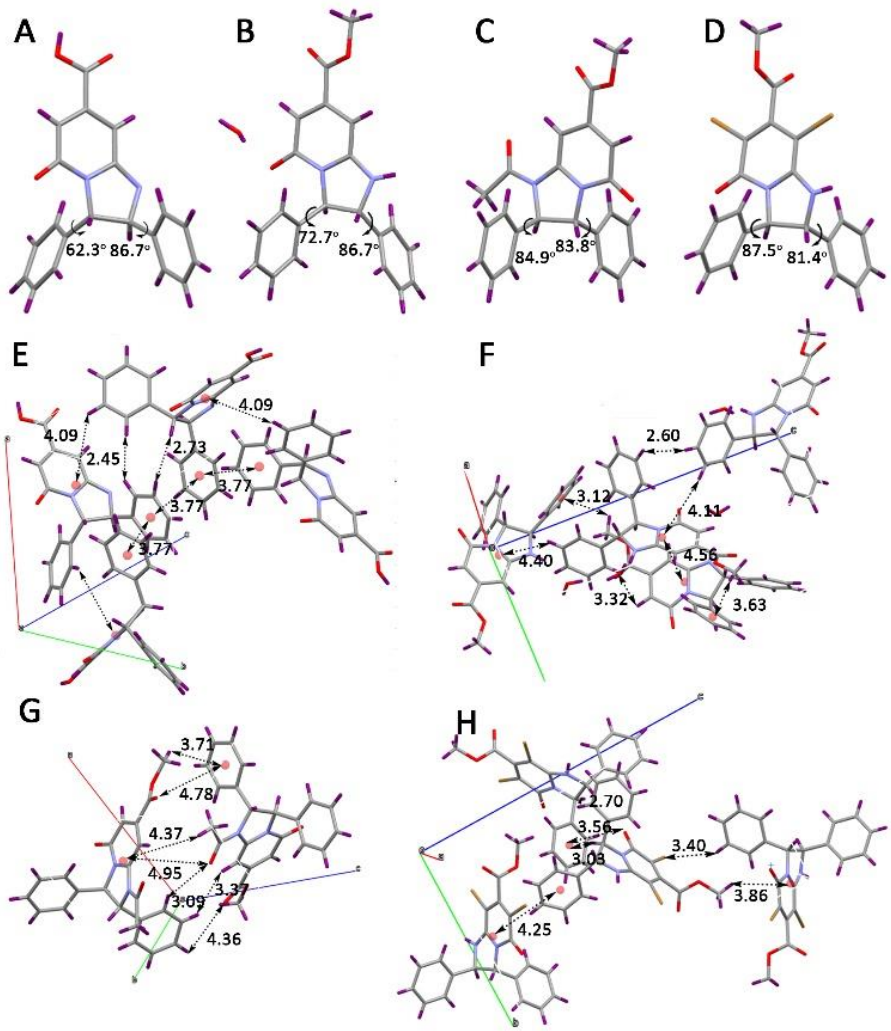


Fig. S4 Single-crystal structures of A) *S*-DHIP-CO_2H, B) S-DHIP-CO_2Me, C) *S*-DHIP-Ac, D) *S*-DHIP-Br and the molecular packing of E) *S*-DHIP-CO_2H, F) *S*-DHIP-CO_2Me, G) *S*-DHIP-Ac, H) *S*-DHIP-Br. carbon, hydrogen, nitrogen and oxygen atoms are shown in grey, mauve, blue, and red, respectively.

4. Theoretical calculation of all compounds

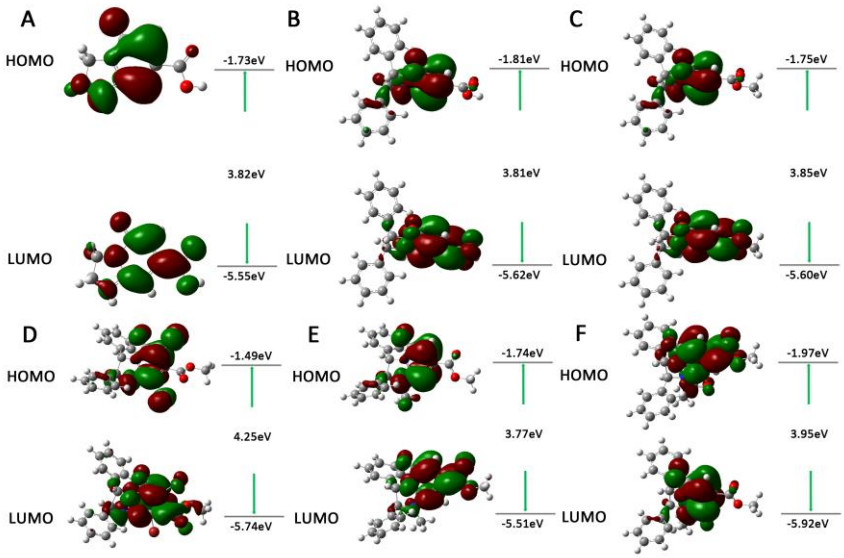


Fig. S5 Molecular orbital amplitude plots of HOMO and LUMO levels of A) THDP-CO_2H, B) *S*-DHIP-CO_2H, C) *S*-DHIP-CO_2Me, D) *S*-DHIP-Br, E) *S*-DHIP-Me, and F) *S*-DHIP-Ac calculated at the B3LYP/6-31G (d, p) level of theory.

Table S2 Optical properties of all compounds

Compounds	λ_{abs}[a] (nm)	ε[a] (M^{-1} cm^{-1})	λem[b](nm)	Φ_F[b] (%)	$\Delta E_{LUMO-HOMO}$[c]（ev）
THDP- CO_2H	367	3.3×10^3	448	80.12	3.82
S-DHIP-CO_2H	380	5.6×10^3	448	65.24	3.81
S-DHIP-CO_2Me	360	6.3×10^3	489	85.84	3.85
S-DHIP-Ac	380	6.9×10^3	430	46.62	4.25
S-DHIP-Br	380	9.8×10^3	467	0	3.77
S-DHIP-Me	390	12.5×10^3	460	92.24	3.95

[a] Absorption maximum in DMSO. [b] Emission maximum of the solution state in DMSO, [c] Energy level of each molecule calculated at the B3LYP/6-31G (d, p) level of theory based on the single crystal (S-DHIP-Me has been optimized based on the B3LYP/6-31G (d, p) level of theory)

Table.S3 Data of Single-structure[a]

Compounds	Crystal system[a]	Space group[b]	$Ø_{I-P1}$[c]	$Ø_{I-P2}$[d]	D_{I-P}[e]	μ[f]
S-DHIP-CO_2H	tetragonal	$P4_1$	62.3	86.7	2.45	9.2662
S-DHIP-CO_2Me	orthorhombic	$P2_12_12_1$	72.7	86.7	4.11	9.5072
S-DHIP-Ac	monoclinic	$P2_1$	83.8	84.9	----	8.2144
S-DHIP-Br	orthorhombic	$P2_12_12_1$	87.5	81.4	4.25	7.7335
S-DHIP-Me	----	----	----	----	----	10.0678

[a] The Crystal system of all compounds, [b]Space group of all compounds, [c, d] dihedral angle of phenyl (P1, P2) and imidazo ring-fused 2-pyridone, [e] The distance of adjacent molecule's imidazo ring-fused 2-pyridone (I) and phenyl (P), [f] Dipole moment of each molecule calculated at the B3LYP/6-31G (d, p) level of theory based on the single crystal (*S*-DHIP-Me has been optimized based on the B3LYP/6-31G (d, p) level of theory).

5. Crystallographic data of *S*-DHIP-CO_2H, *S*-DHIP-CO_2Me, *S*-DHIP-Ac, *S*-DHIP-Br

Crystal data and structure refinements of *S*-DHIP-CO_2H (CCDC Number: 2043200):

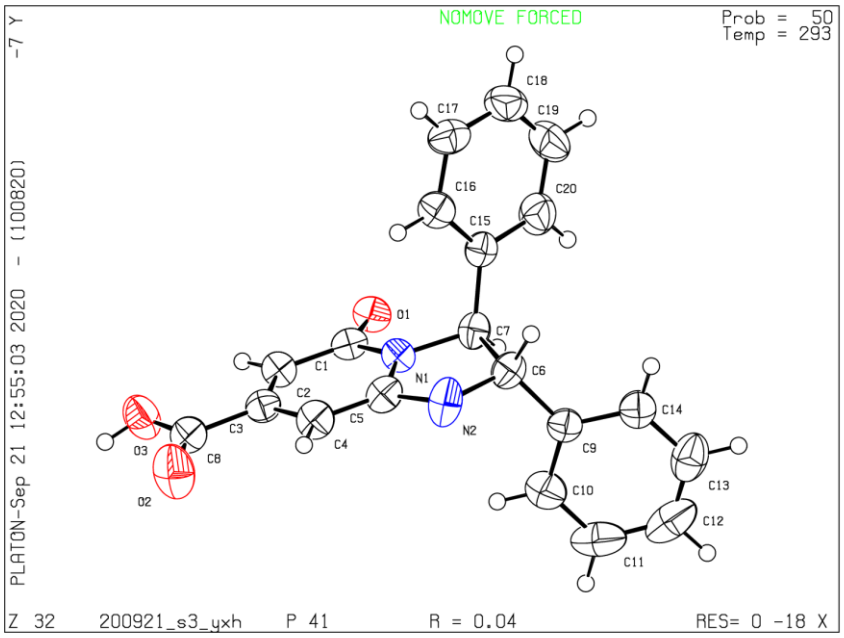


Fig. S6 Ellipsoid plot of ***S*-DHIP-CO_2H**

Identification code	200921_s3_yxh
Empirical formula	C20H15N2O3
Formula weight	331.34
Temperature/K	293.15
Crystal system	tetragonal
Space group	P41
a/Å	10.6876(20)
b/Å	10.6876(20)
c/Å	14.146(4)
α/°	90
β/°	90
γ/°	90
Volume/Å3	1615.8(8)
Z	4
ρcalcg/cm3	1.362
μ/mm-1	0.093
F(000)	692.0
Crystal size/mm3	0.35 × 0.08 × 0.05
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	6.112 to 52.744
Index ranges	$-10 \le h \le 13$, $-10 \le k \le 9$, $-8 \le l \le 17$
Reflections collected	3536
Independent reflections	2393 [Rint = 0.0265, Rsigma = 0.0652]
Data/restraints/parameters	2393/1/227

Goodness-of-fit on F2 0.909
Final R indexes [I>=2σ (I)] R1 = 0.0417, wR2 = 0.0748
Final R indexes [all data] R1 = 0.0730, wR2 = 0.0849
Largest diff. peak/hole / e Å-3 0.29/-0.16
Flack parameter-1.2(10)

Crystal data and structure refinements of *S*-DHIP-CO_2Me (CCDC Number: 2051588):

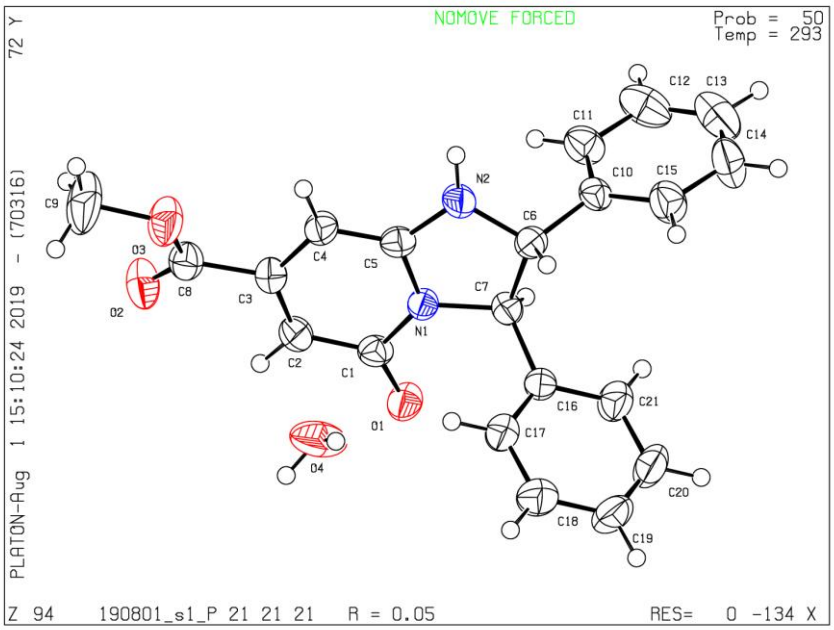


Fig. S7 Ellipsoid plot of ***S*-DHIP-CO_2Me**

Identification code 190801_s1_wzy
Empirical formula C21H20N2O4
Formula weight 364.39
Temperature/K 293.15
Crystal system orthorhombic
Space group P212121
a/Å 9.0116(12)
b/Å 10.7617(18)
c/Å 19.105(3)
α/° 90
β/° 90
γ/° 90
Volume/Å3 1852.8(5)
Z 4
ρcalcg/cm3 1.306
μ/mm-1 0.091
F(000) 768.0
Crystal size/mm3 0.35 × 0.3 × 0.25
Radiation MoKα (λ = 0.71073)
2Θ range for data collection/° 6.216 to 52.74

Index ranges -11 ≤ h ≤ 9, -11 ≤ k ≤ 13, -16 ≤ l ≤ 23
Reflections collected 5708
Independent reflections 3448 [Rint = 0.0173, Rsigma = 0.0456]
Data/restraints/parameters 3448/0/248
Goodness-of-fit on F2 1.043
Final R indexes [I>=2σ (I)] R1 = 0.0474, wR2 = 0.0981
Final R indexes [all data] R1 = 0.0690, wR2 = 0.1101
Largest diff. peak/hole / e Å-3 0.20/-0.19
Flack parameter -0.3(9)

Crystal data and structure refinements of *S*-DHIP-Ac (CCDC Number: 2043201):

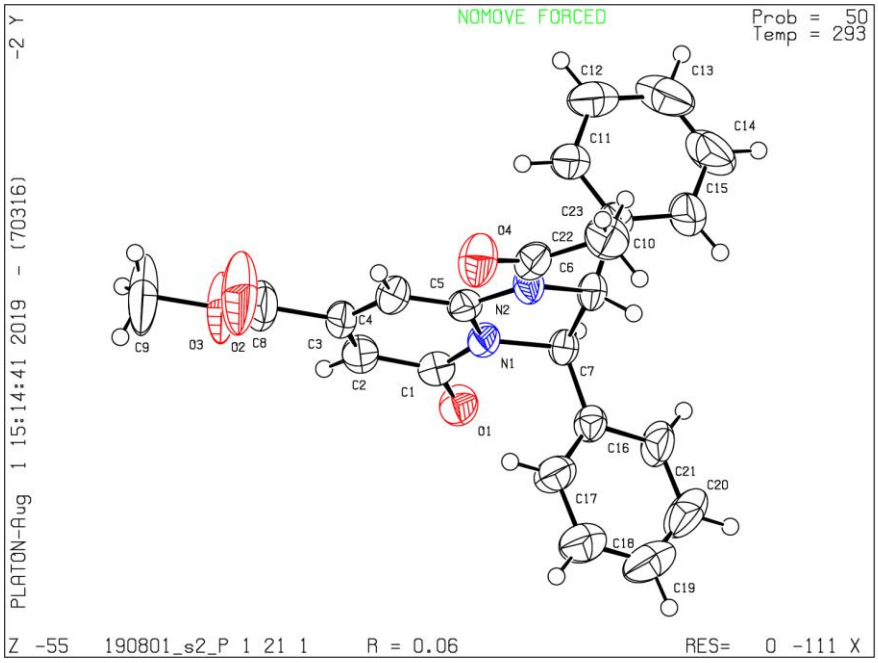


Fig. S8 Ellipsoid plot of ***S*-DHIP-Ac**

Identification code 190801_s2_wzy_2
Empirical formula C23H20N2O4
Formula weight 388.41
Temperature/K 293.15
Crystal system monoclinic
Space group P21
a/Å 10.1065(14)
b/Å 10.7913(11)
c/Å 10.2229(13)
α/° 90
β/° 111.825(16)
γ/° 90
Volume/Å3 1035.0(2)
Z 2
ρcalcg/cm3 1.246

μ/mm-1 0.086
F(000) 408.0
Crystal size/mm3 0.35 × 0.15 × 0.15
Radiation MoKα (λ = 0.71073)
2Θ range for data collection/° 5.716 to 52.74
Index ranges -12 ≤ h ≤ 9, -13 ≤ k ≤ 8, -8 ≤ l ≤ 12
Reflections collected 4390
Independent reflections 3191 [Rint = 0.0556, Rsigma = 0.1143]
Data/restraints/parameters 3191/1/264
Goodness-of-fit on F2 0.833
Final R indexes [I>=2σ (I)] R1 = 0.0571, wR2 = 0.1202
Final R indexes [all data] R1 = 0.1030, wR2 = 0.1324
Largest diff. peak/hole / e Å-3 0.21/-0.26
Flack parameter -1.8(10)

Crystal data and structure refinements of *S*-DHIP-Br (CCDC Number: 2043204):

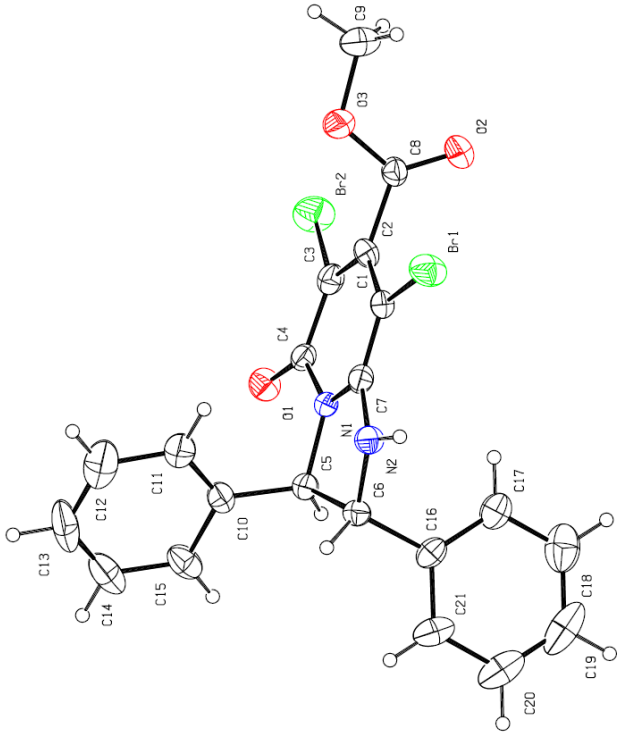


Fig. S9 Ellipsoid plot of ***S*-DHIP-Br**

Identification code 191122_s2_wzy_rxy
Empirical formula C21H16Br2N2O3
Formula weight 504.18
Temperature/K 293.15
Crystal system orthorhombic
Space group P212121
a/Å 9.0188(9)
b/Å 11.8068(9)
c/Å 19.0898(18)

α/° 90
β/° 90
γ/° 90
Volume/Å3 2032.7(3)
Z 4
ρcalcg/cm3 1.647
μ/mm-1 4.012
F(000) 1000.0
Crystal size/mm3 0.35 × 0.3 × 0.25
Radiation MoKα (λ = 0.71073)
2Θ range for data collection/° 6.072 to 52.742
Index ranges -11 ≤ h ≤ 10, -14 ≤ k ≤ 14, -23 ≤ l ≤ 23
Reflections collected 15533
Independent reflections 4116 [Rint = 0.0907, Rsigma = 0.0978]
Data/restraints/parameters 4116/0/258
Goodness-of-fit on F2 0.904
Final R indexes [I>=2σ (I)] R1 = 0.0591, wR2 = 0.1409
Final R indexes [all data] R1 = 0.1083, wR2 = 0.1783
Largest diff. peak/hole / e Å-3 0.69/-0.60
Flack parameter 0.008(11)

6. Cytotoxicity of all probes on Hela cells evaluated by MTS assay

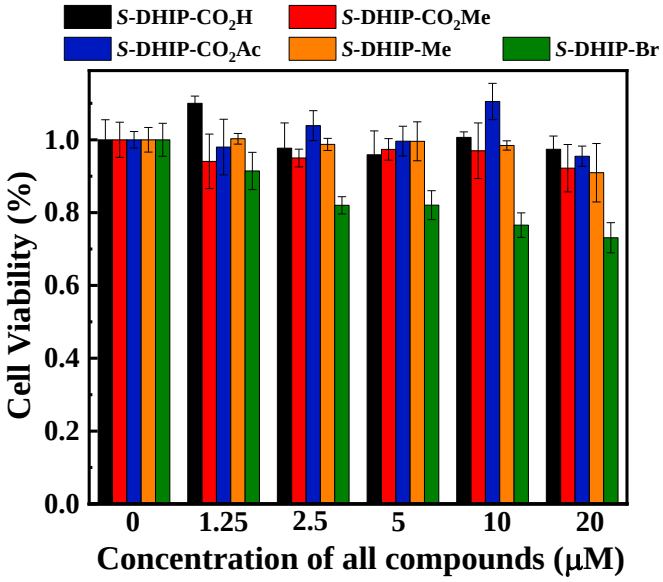


Fig. S10 Cytotoxicity of *S*-DHIP-CO_2H, *S*-DHIP-Ac, *S*-DHIP-Me, *S*-DHIP-Br, *S*-DHIP-CO_2Me. Hela cells were incubated with compounds (0-20 μM) for 24 h. Results are mean ± SD.

7. Confocal fluorescence images

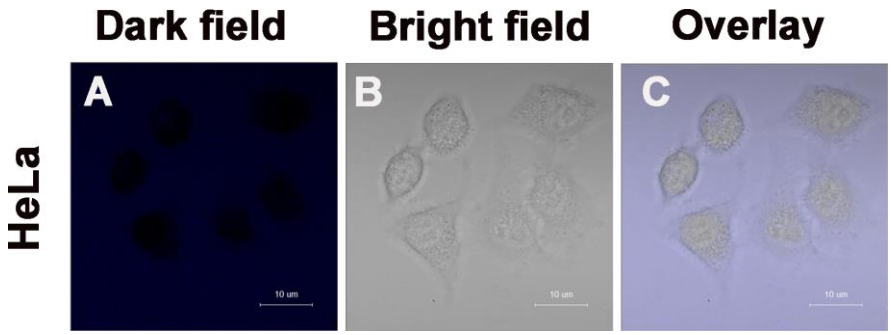


Fig. S11 Confocal fluorescence images of Hela cell co-stained with 10 μM ***S*-DHIP-Ac** probe (blue channel: λ_{ex} = 405 nm, λ_{em} = 412-497 nm) for 15 min. Scale bar: 10 μm.

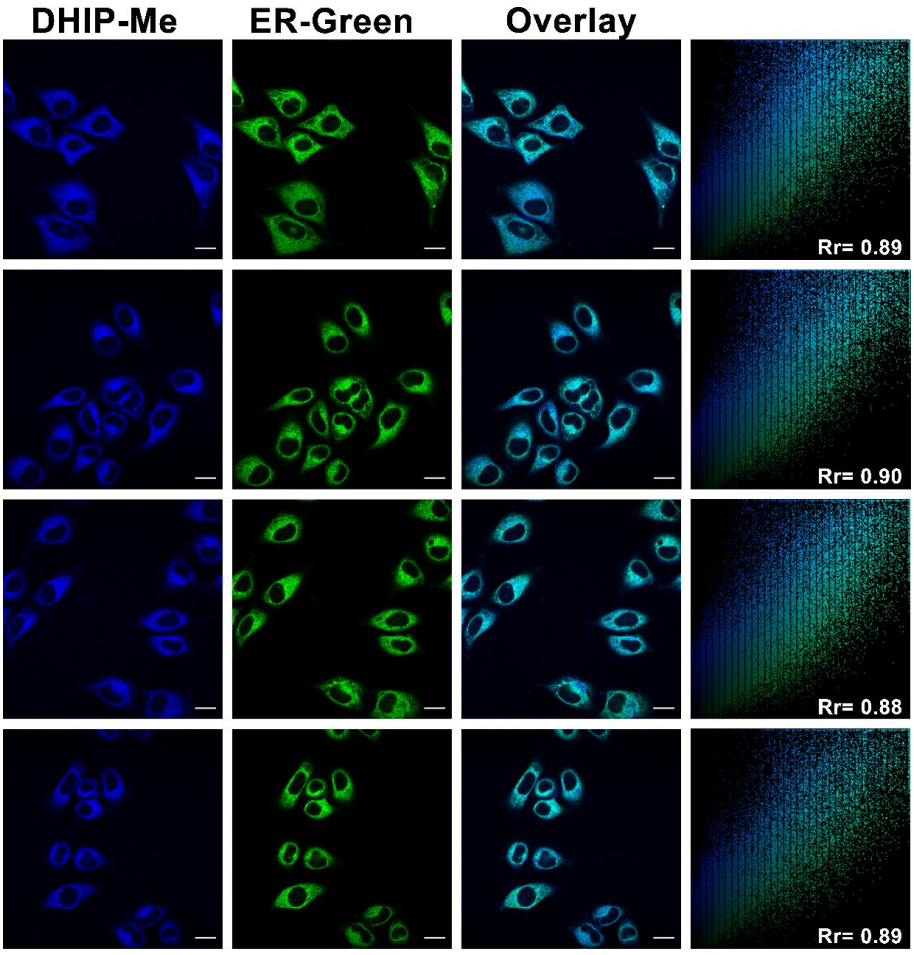


Fig. S12 Confocal fluorescence images of Hela cell co-stained with 10 μM **S-DHIP-Me** (blue channel: λ_{ex} = 405 nm, λ_{em} = 412-497 nm) and ER-Tracker Green at 37 °C for 15 min. Scale bar: 20 μm.

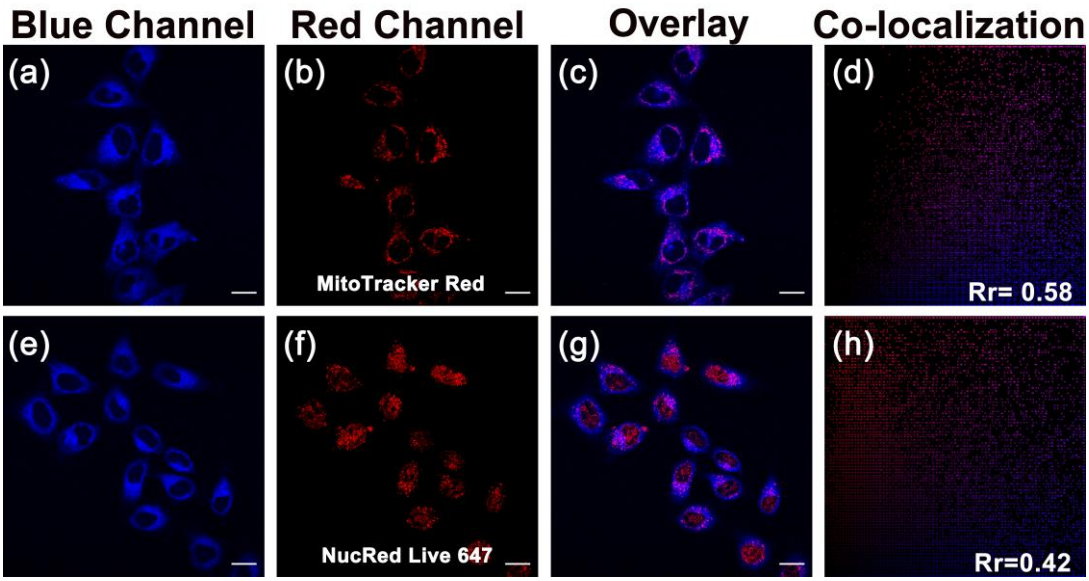


Fig. S13 Confocal fluorescence images of Hela cell co-stained with 10 μM **S-DHIP-Me** (blue channel: λ_{ex} = 405 nm, λ_{em} = 412-497 nm) and MtioTracker Red or NucRed Live 647 at 37 °C for 15 min. Scale bar: 20 μm.

8. Bacteriostatic Activity

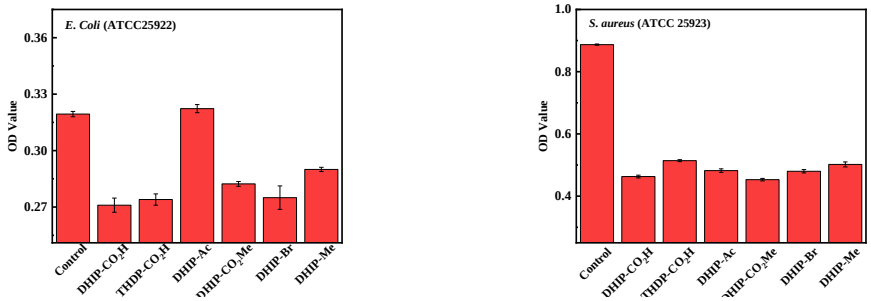


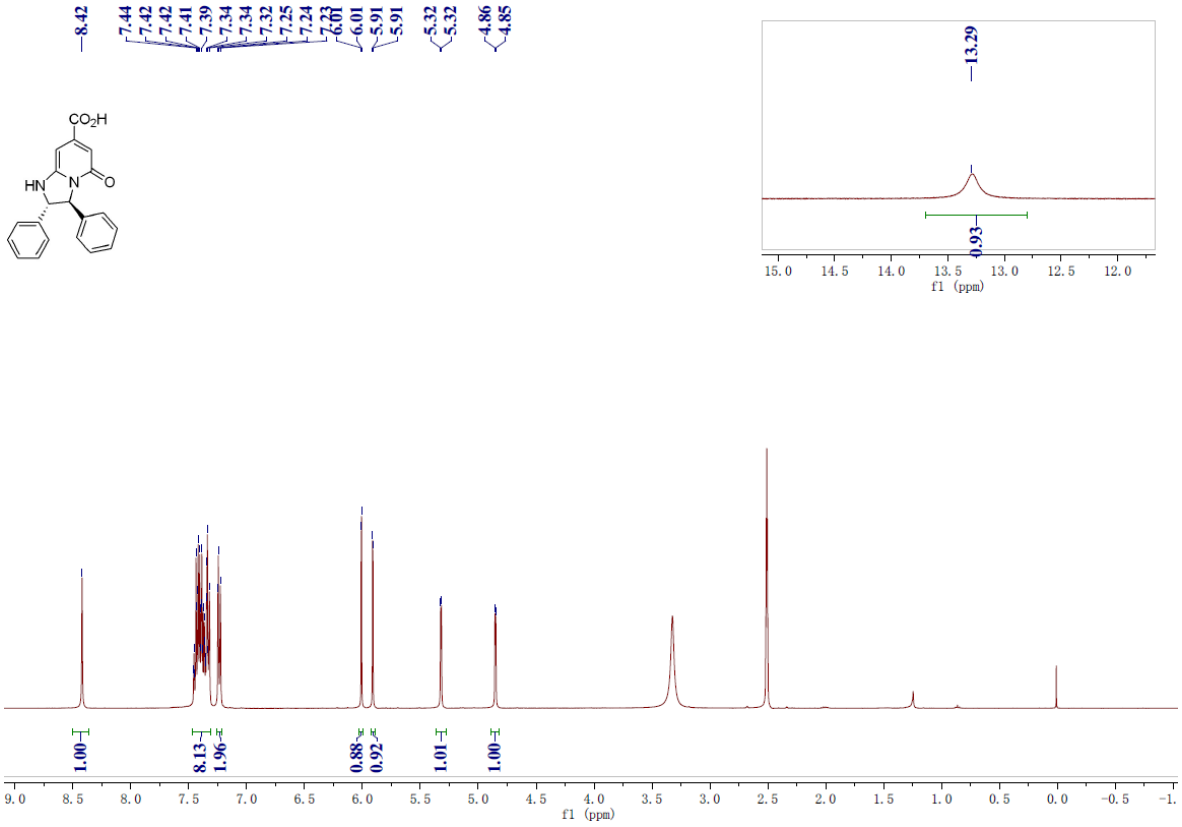
Fig. S12 *E. Coli.* and *Saureus.* were incubated with compounds (0.125 μg/ml) for 24 h. Results are mean ± SD.

Table S4. The MIC（ug/mL）of compounds

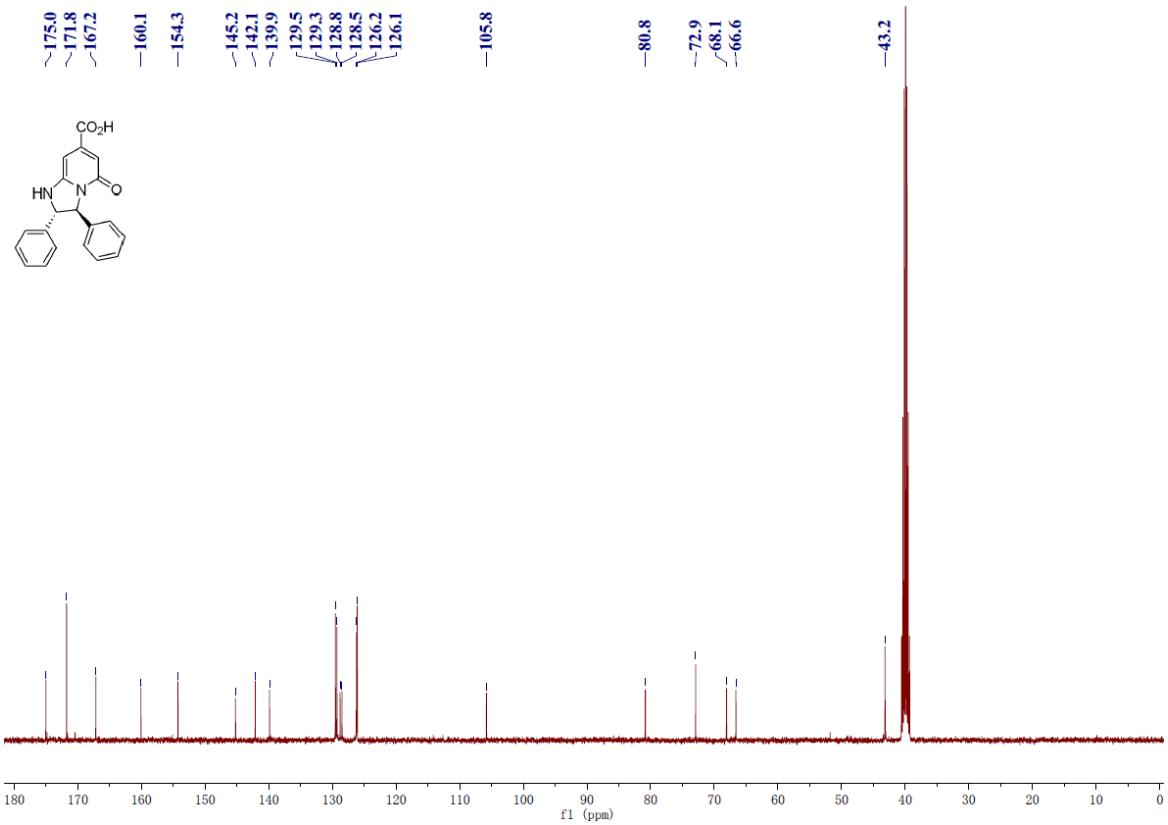
Compounds	*E. coli* ATCC 25922	*S. aureus* ATCC25923
Blank control	＞128	＞128
***S*-DHIP-CO_2H**	0.5	1
THDP-CO_2H	8	4
***S*-DHIP-Ac**	64	64
***S*-DHIP-CO_2Me**	64	64
***S*-DHIP-Br**	2	8
***S*-DHIP-Me**	32	16

Compound ***S*-DHIP-CO$_2$H**, **THDP-CO$_2$H**, ***S*-DHIP-Ac**, ***S*-DHIP-CO$_2$Me**, ***S*-DHIP-Br**, ***S*-DHIP-Me** exhibited antibacterial activities against tested gram-positive strains and gram-negative strains, especially for S. aureus ATCC 25923 with the MIC 1–64 μg/mL, which was over 2-128 folds compared with blank control, and their activity against E. coli ATCC 25922 was 0.5-64 μg/mL at the same time. ***S*-DHIP-CO$_2$H** displayed potent bactericidal effect as the MIC =1 μg/mL against E. coli ATCC 25922, while the MIC =0.5 μg/mL against S. aureus ATCC 25923.

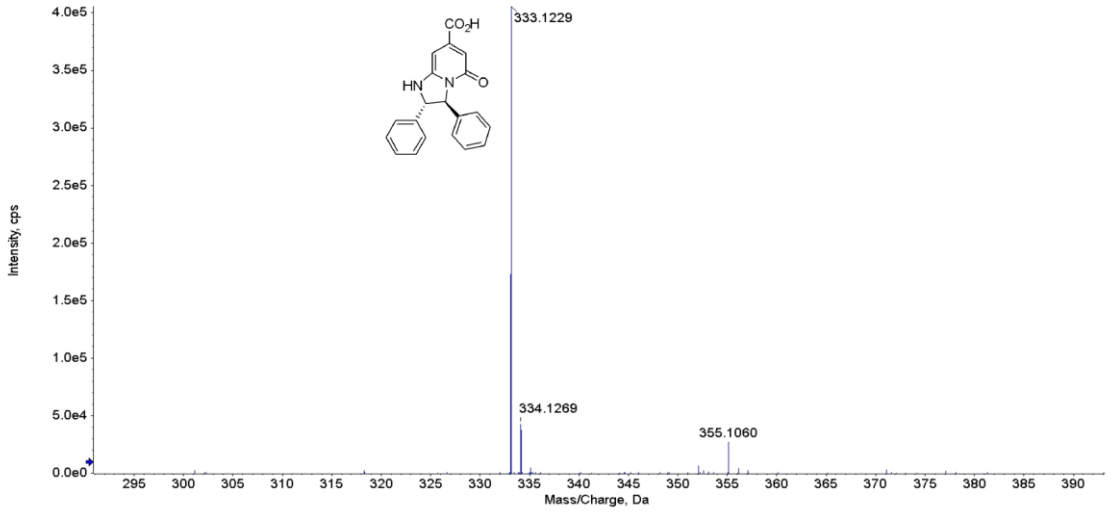
^{1}H NMR, ^{13}C NMR and ESI-MS spectra of synthesized compounds



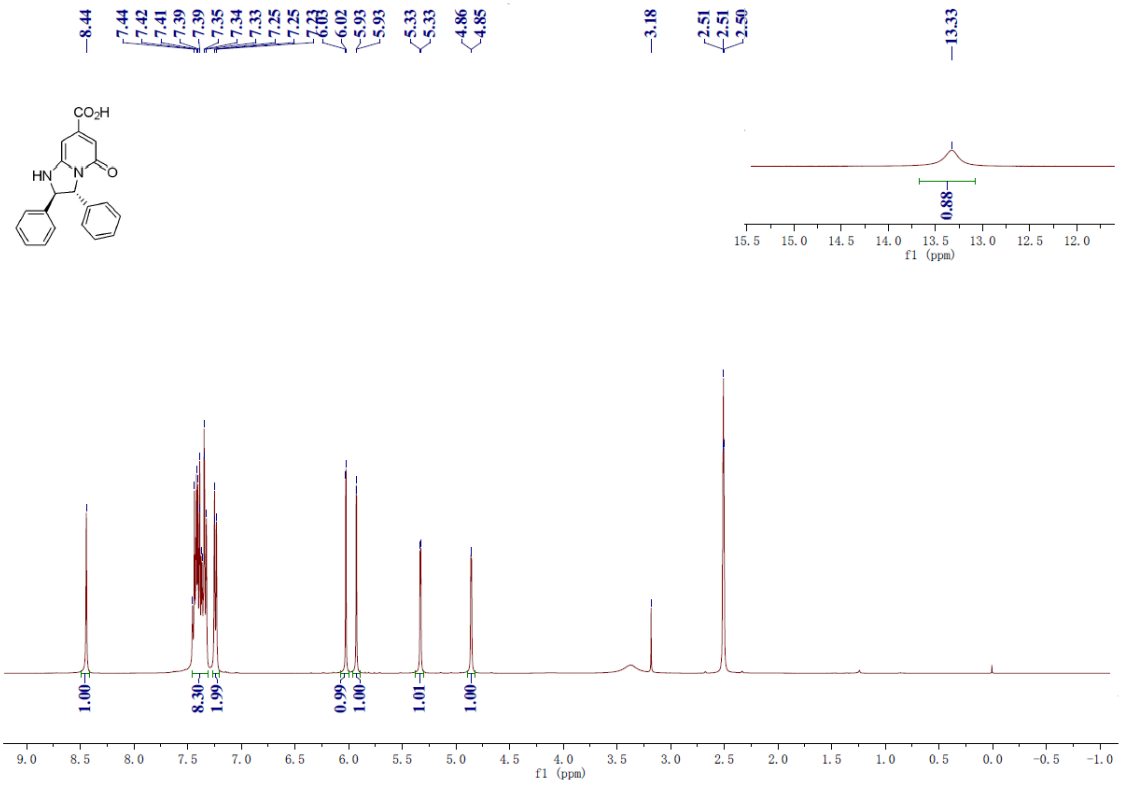
The ^{1}H NMR spectrum of compound ***S*-DHIP-CO$_2$H** in DMSO-d$_6$.



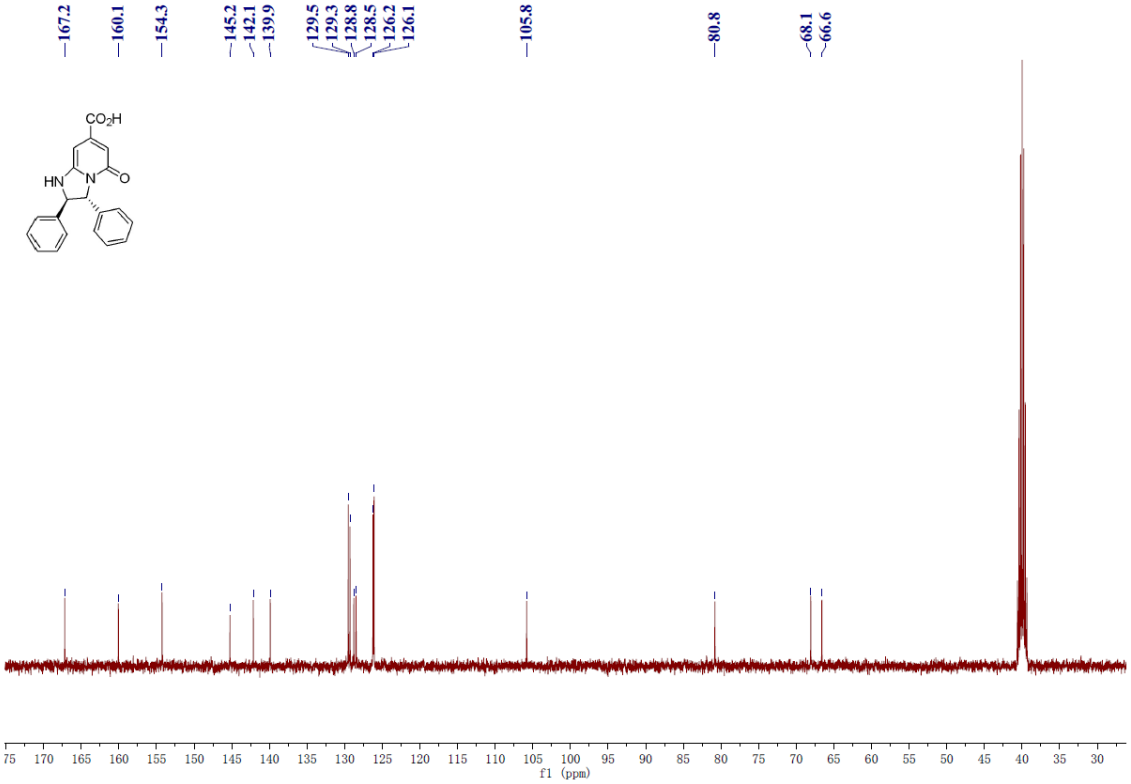
The ^{13}C NMR spectrum of compound ***S*-DHIP-CO$_2$H** in DMSO-d$_6$.



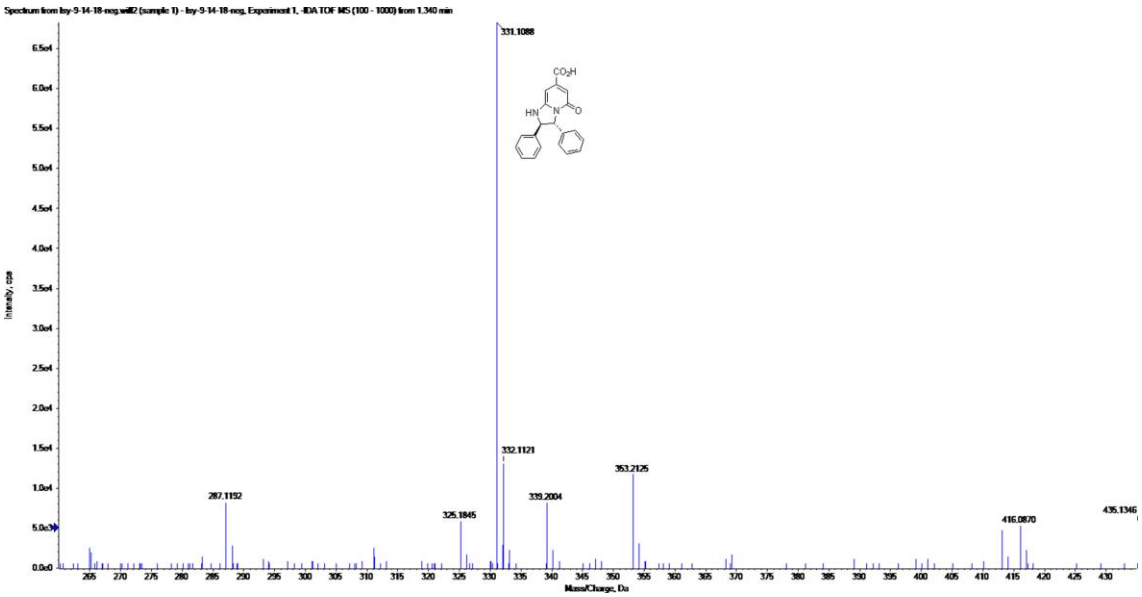
The ESI-MS spectrum of compound ***S*-DHIP-CO$_2$H**.



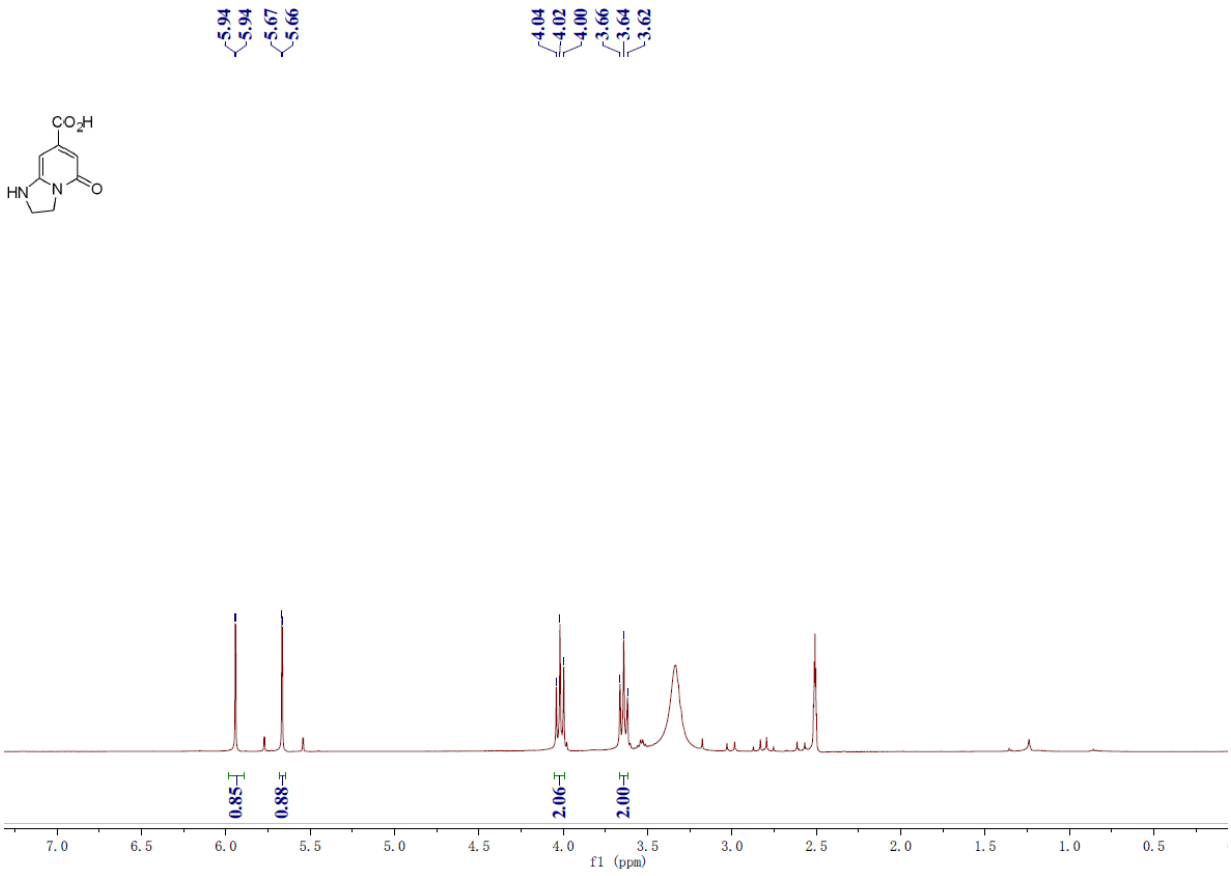
The ^{1}H NMR spectrum of compound ***R*-DHIP-CO$_2$H** in DMSO-d$_6$.



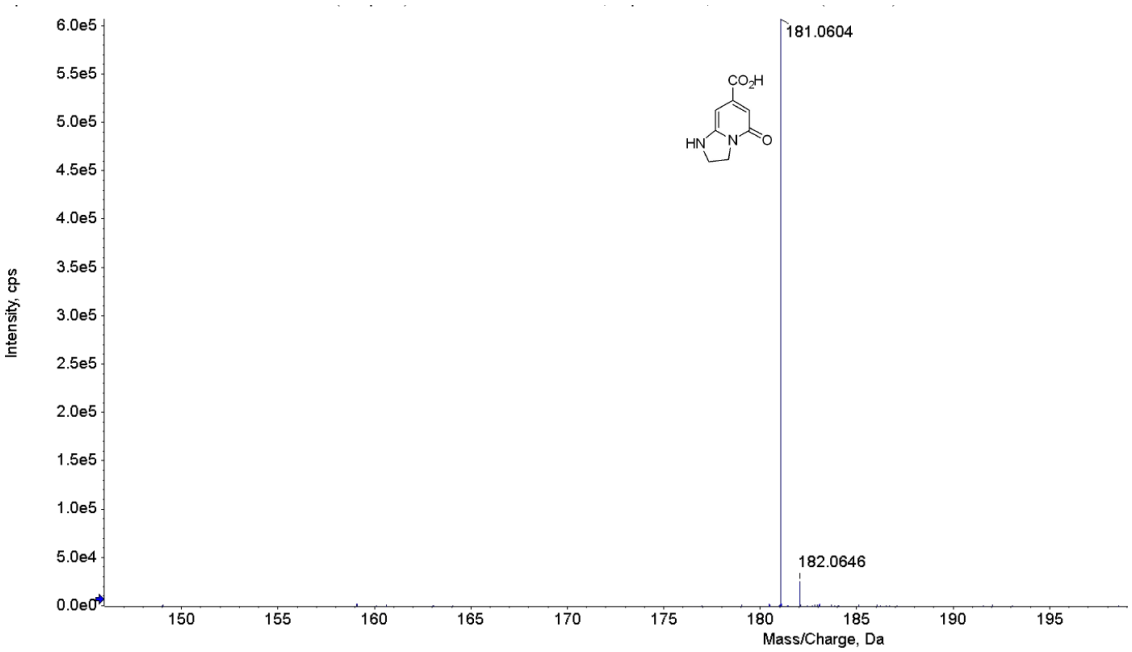
The ^{13}C NMR spectrum of compound ***R*-DHIP-CO$_2$H** in DMSO-d$_6$.



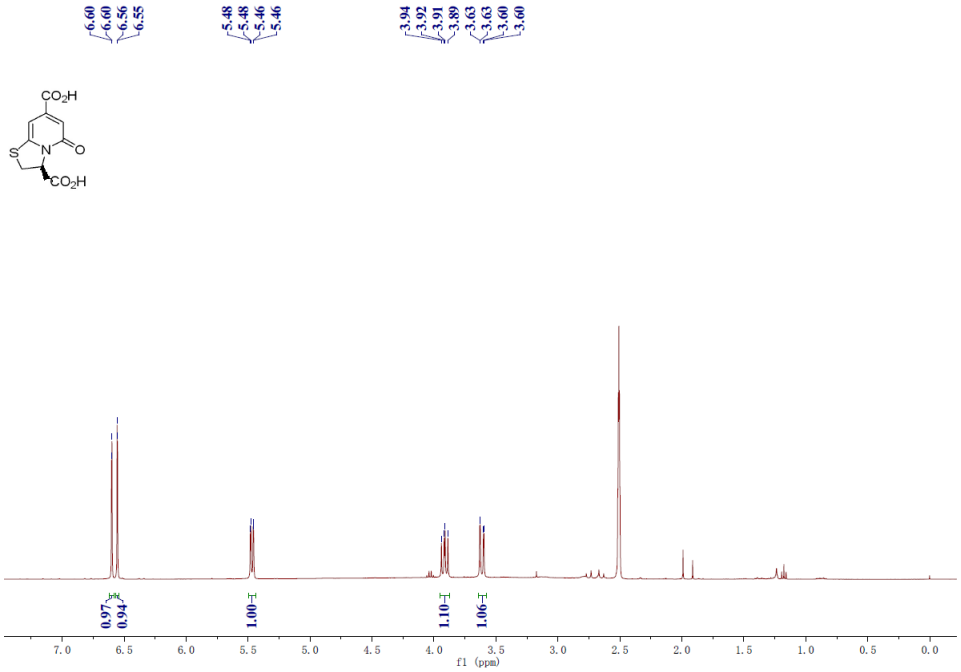
The ESI-MS spectrum of compound ***R*-DHIP-CO_2H.**



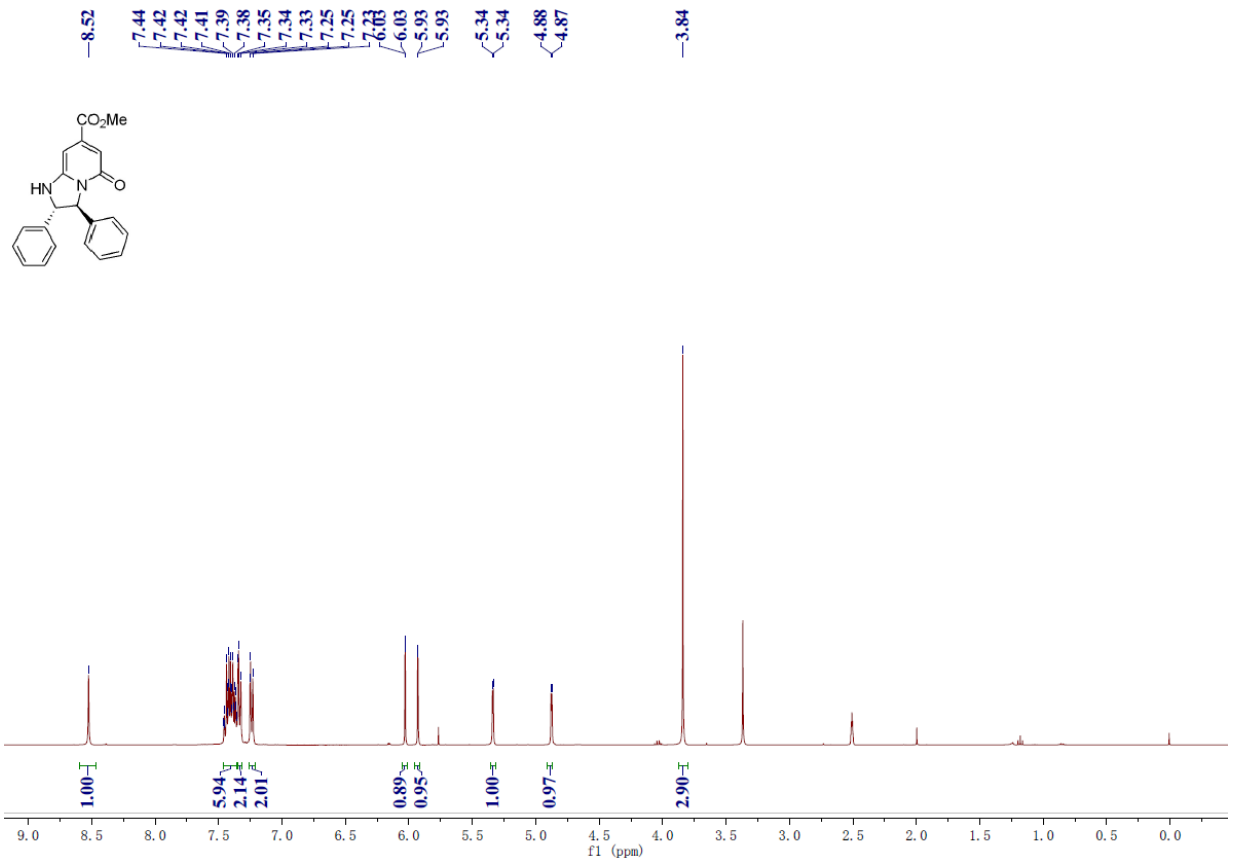
The ^{1}H NMR spectrum of compound **THDP-CO_2H** in DMSO-d_6.



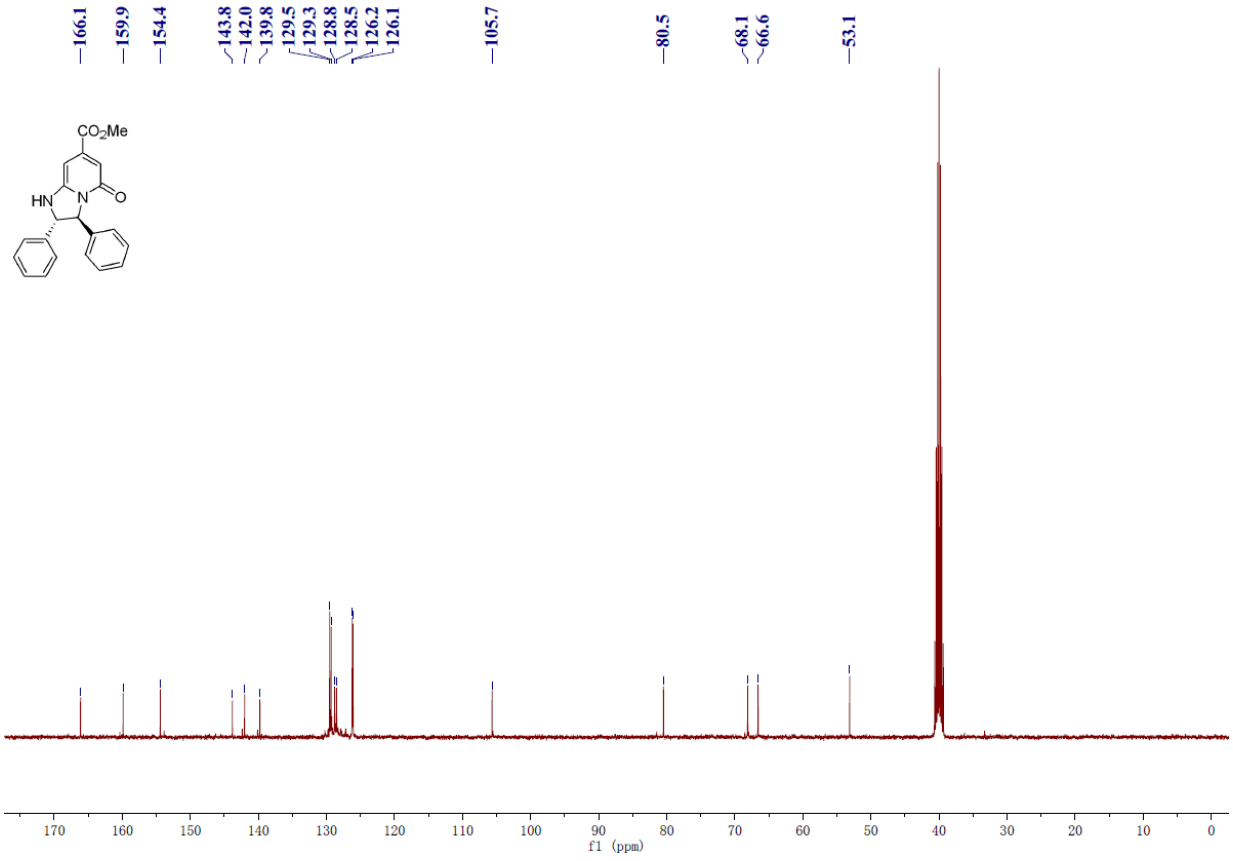
The ESI-MS spectrum of compound **THDP-CO_2H**.



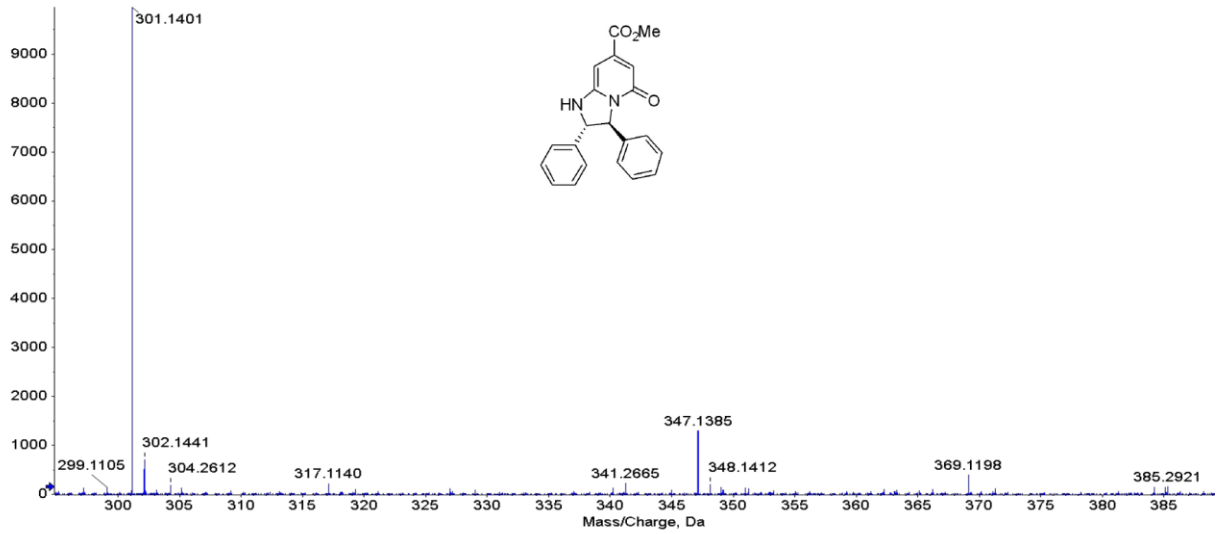
The ^{1}H NMR spectrum of compound **THPD-CO_2H**.



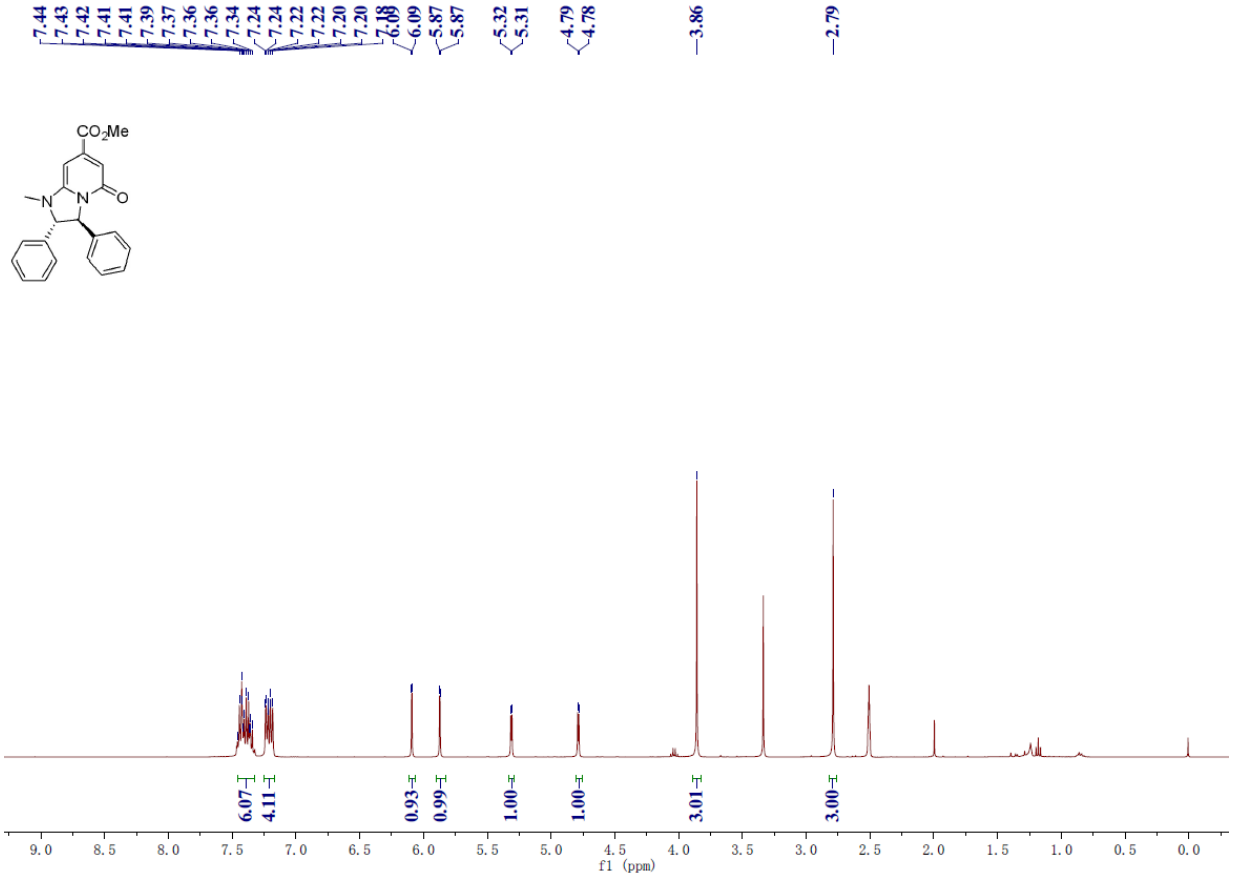
The ^{1}H NMR spectrum of compound ***S*-DHIP-CO$_2$Me** in DMSO-d$_6$.



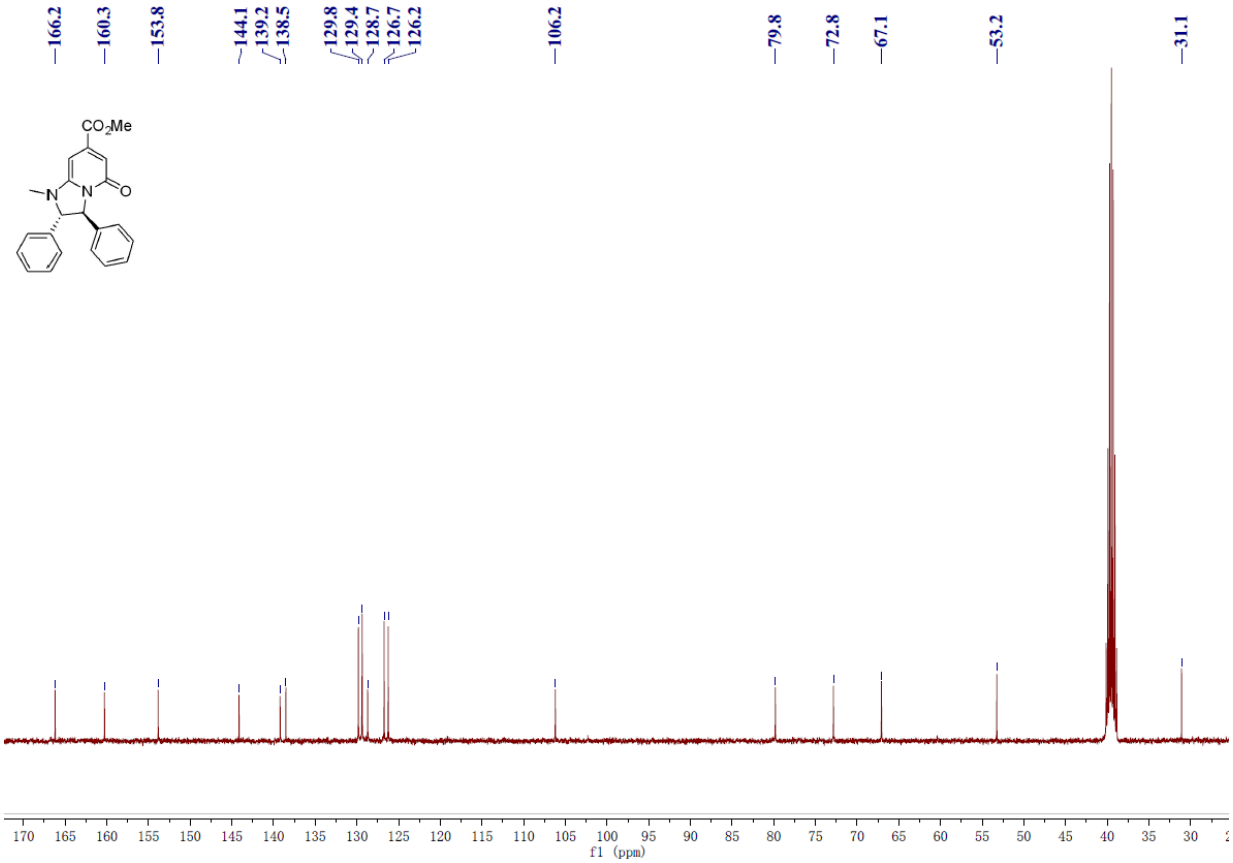
The ^{13}C NMR spectrum of compound ***S*-DHIP-CO$_2$Me** in DMSO-d$_6$.



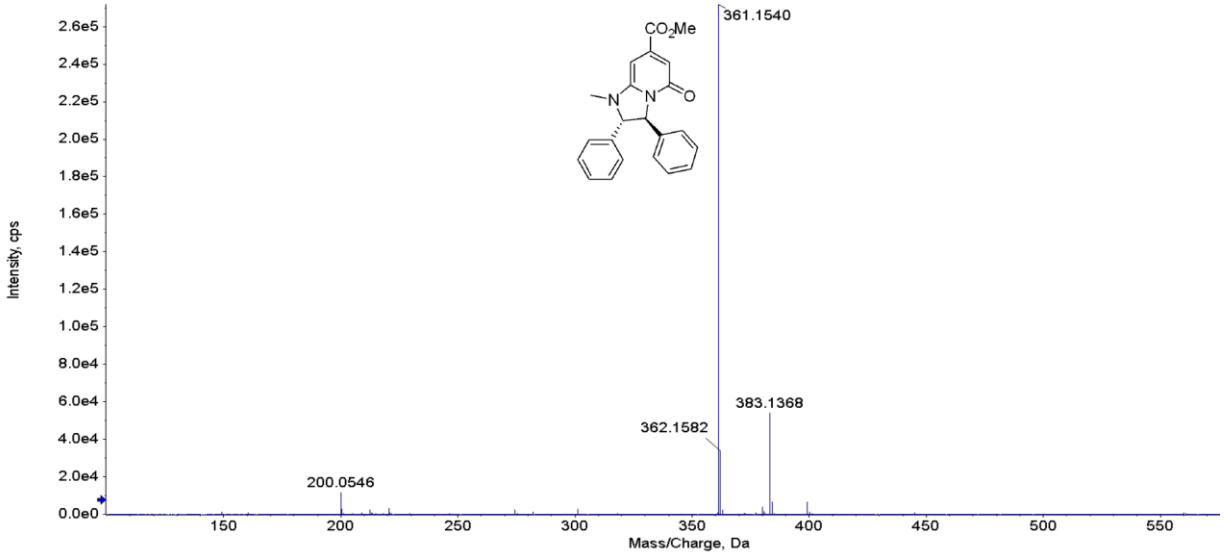
The ESI-MS spectrum of compound ***S*-DHIP-CO_2Me**.



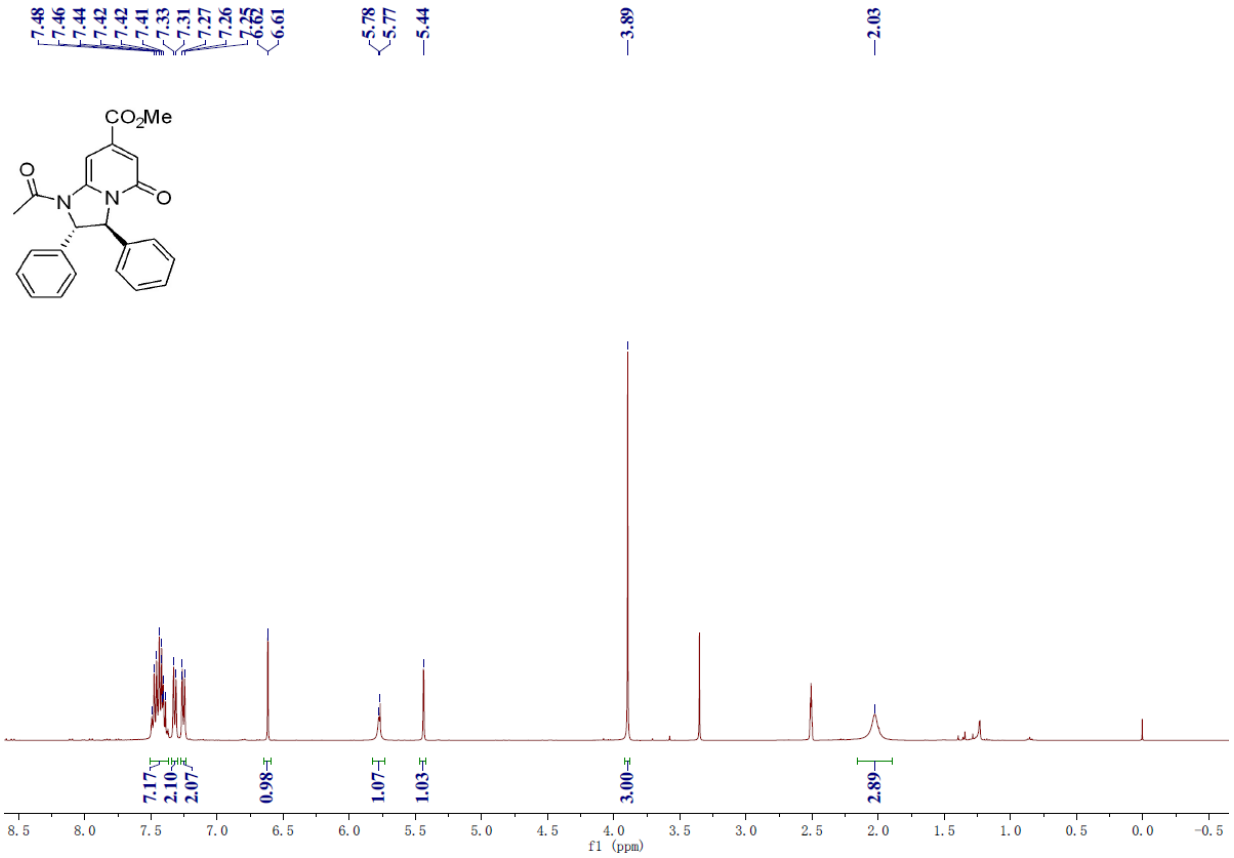
The ^{1}H NMR spectrum of compound ***S*-DHIP-Me** in DMSO-d_6.



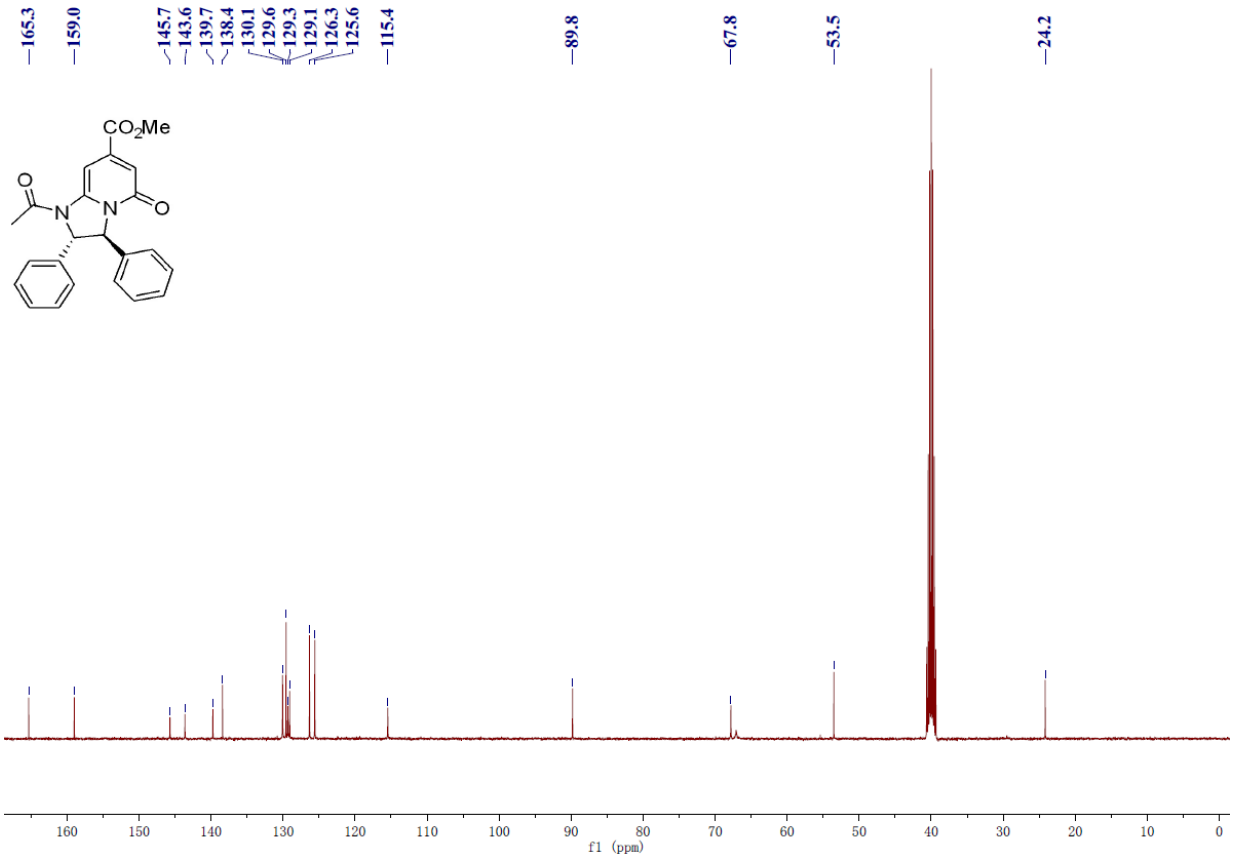
The ^{13}C NMR spectrum of compound ***S*-DHIP-Me** in DMSO-d_6.



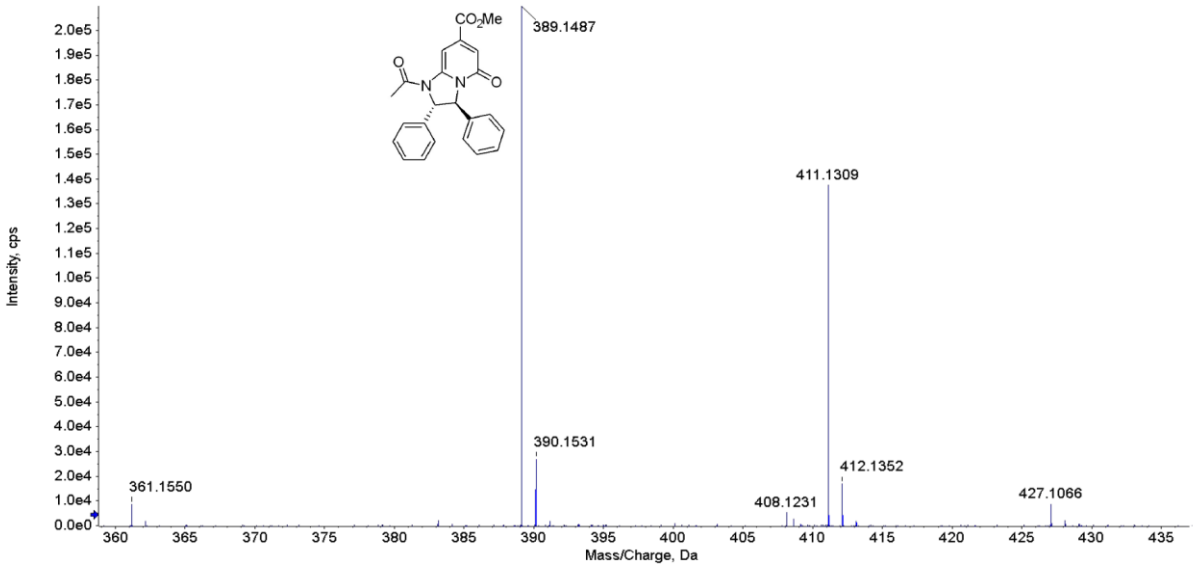
The ESI-MS spectrum of compound ***S*-DHIP-Me**.



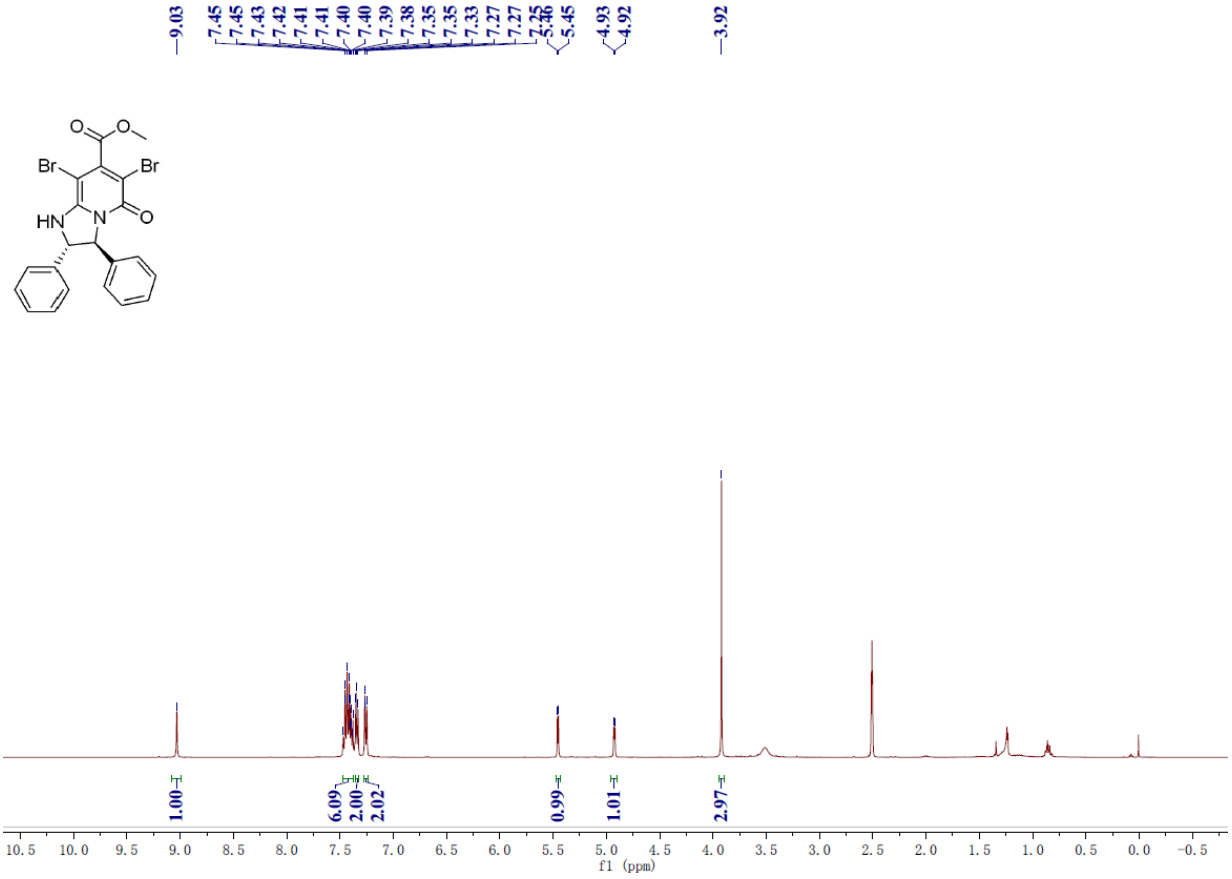
The ^{1}H NMR spectrum of compound ***S*-DHIP-Ac** in DMSO-d$_6$.



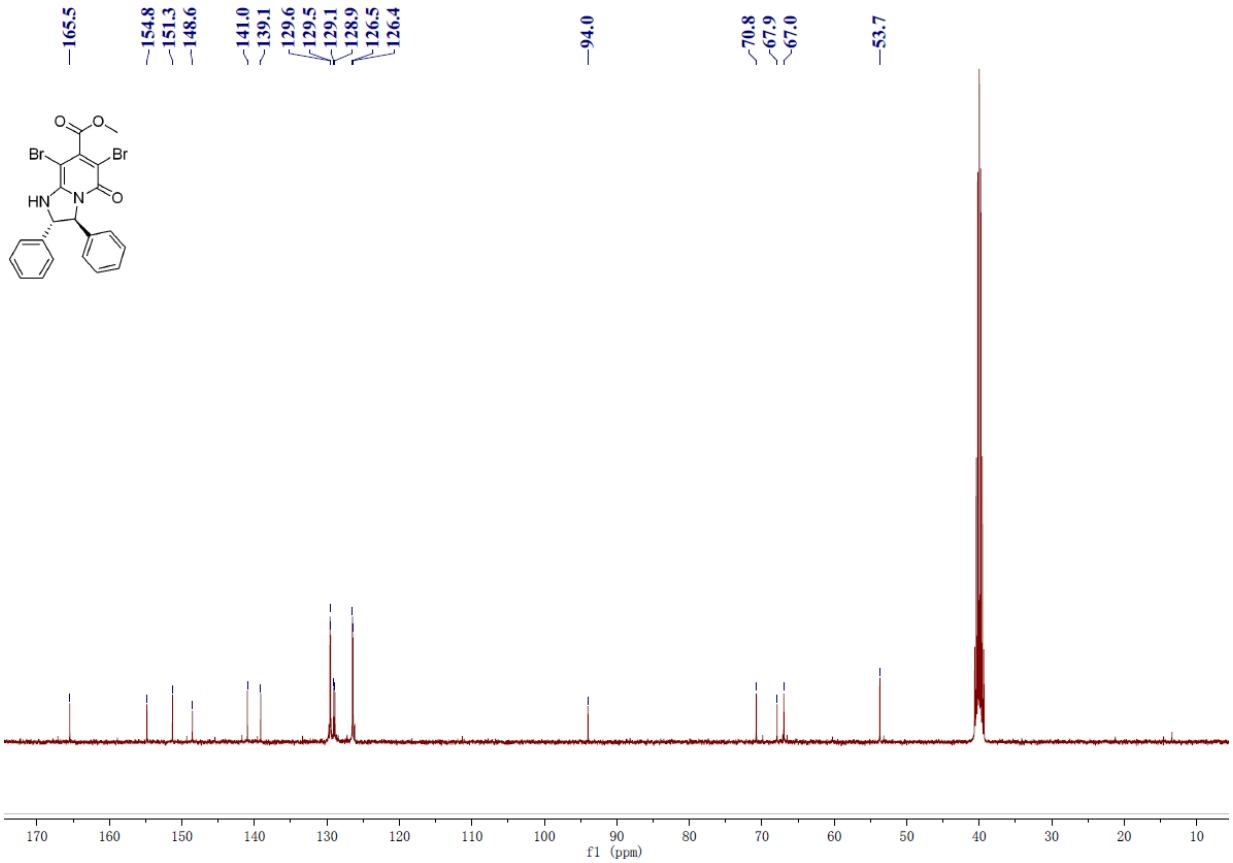
The ^{13}C NMR spectrum of compound ***S*-DHIP-Ac** in DMSO-d$_6$.



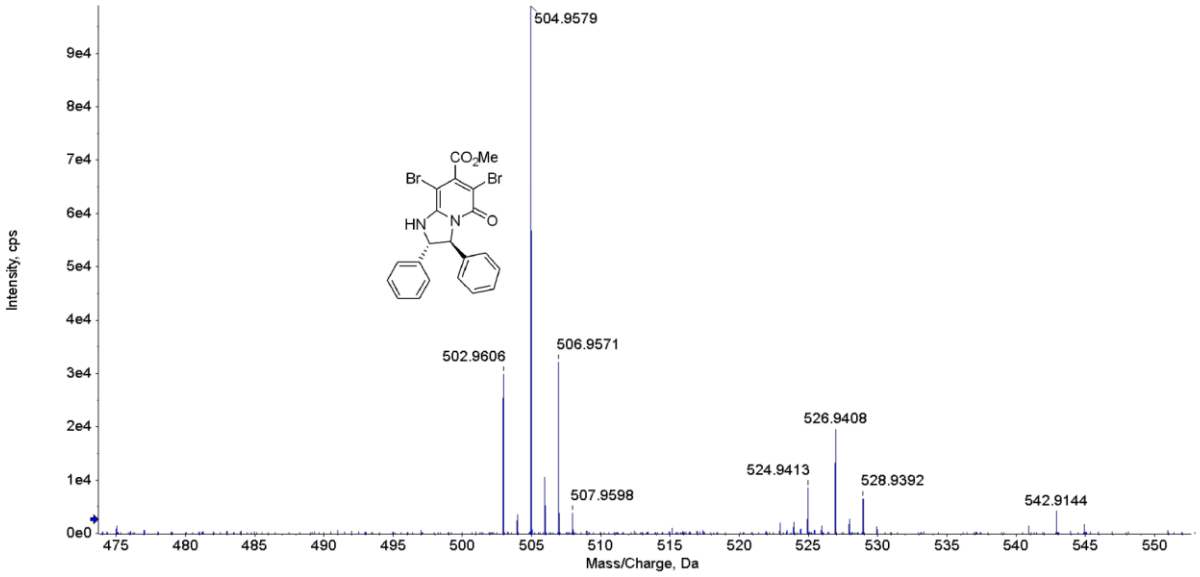
The ESI-MS spectrum of compound ***S*-DHIP-Ac**.



The ^{1}H NMR spectrum of compound ***S*-DHIP-Br** in DMSO-d_6.



The ^{13}C NMR spectrum of compound ***S*-DHIP-Br** in DMSO-d_6.



The ESI-MS spectrum of compound ***S*-DHIP-Ac**.

Reference

1. Y. Song, S. Zhu, S. Zhang, Y. Fu, L. Wang, X. Zhao and B. Yang, Investigation from chemical structure to photoluminescent mechanism: a type of carbon dots from the pyrolysis of citric acid and an amine, *J. Mater. Chem. C*, 2015, **3**, 5976-5984.
2. J. A. D. Good, M. Kulén, J. Silver, K. S. Krishnan, W. Bahnan, C. Núñez-Otero, I. Nilsson, E. Wede, E. de Groot, Å. Gylfe, S. Bergström and F. Almqvist, Thiazolino 2-Pyridone Amide Isosteres As Inhibitors of *Chlamydia trachomatis* Infectivity, *J. Med. Chem.*, 2017, **60**, 9393-9399.