Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Supporting Information

Robustanoids A and B, two novel pyrrolo[2,3-*b*]indole alkaloids from *Coffea canephora*: isolation and total synthesis

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General experimental details

For isolation:

Optical rotations were measured on an Autopol VI automatic polarimeter (Rudolph Research Analytical) at room temperature. IR spectra were recorded on a Nicolet 6700 (Thermo Fisher Scientific) spectrometer. UV spectra were measured on a Shimadzu UV-2550 UV-visible spectrophotometer. 1D and 2D NMR data were recorded on a Varian 400/600 MHz instrument with TMS as internal standard. HRESIMS data were acquired using a micrOTOF-Q II mass spectrometer (Bruker). Semi-preparative HPLC separations were performed on a Hitachi Chromaster system consisting of an 5110 pump, 5210 autosampler, 5310 column oven, 5430 diode array detector and a Phenomenex Luna C18 column (250 × 10 mm, S-5 μm), all operated using EZChrom Elite software. All solvents were of ACS or HPLC grade, and were obtained from Tansoole (Shanghai, China), Sigma-Aldrich (St. Louis, MO), respectively. Silica gel (300–400 mesh), C18 reverse-phased silica gel (150-200 mesh, Merck), and MCI gel (CHP20P, 75−150 μM, Mitsubishi Chemical Industries Ltd.) were used for column chromatography (CC), and pre-coated silica gel GF254 plates (Qingdao Marine Chemical Plant, Qingdao, People's Republic of China) were used for TLC.

For synthesis:

All reactions were performed under an argon atmosphere in flame-dried glassware with magnetic stirring. Air- and moisture-sensitive liquids were transferred by syringe. Purification of the reaction products was carried out by flash column chromatography using 200−300 mesh silica gel. Visualization on TLC (analytical thin layer chromatography) was achieved by the use of UV light (254 nm) and treatment with aqueous ceric ammonium molybdate staining followed by heating. High-resolution mass spectra (HRMS) were recorded using LTQ Orbitrap XL (Thermo Fisher Scientific). Other general experimental details are the same as those for isolation.

Plant materials

Robusta coffee beans (*Coffee canephora*) were bought from Wanning coffee market, Hainan Province (Hainan, China), and identified by Dr. Jun Wang (Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences). A voucher specimen (RC-201511) is deposited in the Key Laboratory of Plant Resources and Chemistry of Arid Zone, Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences (Xinjiang, China).

Extraction and isolation

Air-dried ground powder of *C. canephora* beans (500.0 g) was extracted with MeOH (4.5 L × 3) by maceration at room temperature (7 days each time) to afford a crude methanol extract (135.2 g). The extract was applied to a column of RP-18 silica gel (MeOH-H_2O, 10:90 to 100:0, v/v) in gradient to yield seven fractions (A–G). Fraction C (6.4 g) was then subjected to silica gel column chromatography (CC) eluted with a $CHCl_3$:MeOH (10:1 to 1:1, v/v) gradient to obtain five fractions (C1–C5). Fraction C3 (3.0 g) was further separated by silica gel CC eluted with a $CHCl_3$: MeOH (10:1 to 1:1 v/v) gradient to yield six sub-fractions (C3a–C3F). Purification of sub-fraction C3b (79.7 mg) by semi-preparative HPLC, eluting with MeOH-H_2O (0-15 min: 45:55 to 47:53; 15-16 min: 47:53 to 100:0; 16-17 min: 100:0; 17-18 min: 100:0 to 45:55; 18-25 min: 45:55; v/v, 3 mL/min), yielded compounds **1** (17.0 mg) and **2** (7.2 mg).

Robustanoid A (1): colorless amorphous powder; $[\alpha]^{20}_{D}$ = - 414.5 (*c* 0.2, MeOH); UV (MeOH) λ_{max}: 207, 294, 336 nm; IR ν_{max} 3423, 2923, 1685, 1655, 1601, 1522, 1460, 1384, 1174, 1022, 765 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; HR-ESI-MS: *m/z* 381.1124 $[M+H]^{+}$ (Calcd. for $C_{20}H_{17}N_2O_6$, 381.1087).

Robustanoid B (2): colorless amorphous powder; $[\alpha]^{20}_{D}$ = - 496.0 (*c* 0.2, MeOH); UV (MeOH) λ_{max}: 203, 293, 339 nm; IR ν_{max} 3422, 2923, 1685, 1654, 1603, 1522, 1459, 1176, 1022 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; HR-ESI-MS: *m/z* 395.2669 $[M+H]^{+}$ (Calcd. for $C_{21}H_{19}N_2O_6$, 395.1243).

Evaluation of inhibition of α-glucosidase enzyme activity

A mixture of 50 μL of different concentrations of the test compounds and 100 μL of 0.1 M phosphate buffer (pH 6.9) containing yeast α-glucosidase (from *Saccharomyces cerevisiae*) solution (1.0 U/mL) was incubated in 96 well plates at 25 °C for 10 min. After pre-incubation, 50 μL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25 °C for 5 min. Before and after incubation, absorbance was recorded at 405 nm by a micro-plate reader (SpectraMax M2, Molecular Devices Corp., operated by SoftmaxPro v.4.6 software, Sunnyvale, CA, USA) and compared to that of the control which had 50 μL buffer solutions instead of the compounds. The α-glucosidase inhibitory activity was expressed as % inhibition and was calculated as follows:

$$\%\,\text{inhibition} = \left(\frac{\Delta\text{Abs}_{\text{control}} - \Delta\text{Abs}_{\text{sample}}}{\Delta\text{Abs}_{\text{control}}} \right) \times 100$$

Calculated ECD data of compound 1

The absolute configuration of **1** was determined by quantum chemical TDDFT calculations of the theoretical ECD spectrum. Firstly, conformational analysis was carried out via Monte Carlo searching using molecular mechanism with MMFF94 force field in the Spartan 08 program.[1] In the relative energy window of 0-2 Kcal/mol, the result showed two lowest energy conformers. The conformers were then reoptimized using DFT at the B3LYP/6-311++G(2d,2p) level in vacuum in the Gaussian 09 program.[2] The B3LYP/6-311++G(2d,2p) harmonic vibrational frequencies were further calculated to confirm their stability. The energies, oscillator strengths, and rotational strengths of the first 60 electronic excitations were calculated using the TDDFT methodology at the B3LYP/6-311++G(2d,2p) level in vacuum. The ECD spectra were simulated by the overlapping Gaussian function (σ = 0.45 eV),[3] in which velocity rotatory strengths of the first 40 exited states were adopted. To get the conformationally averaged ECD spectra, the simulated spectra of the lowest energy conformers were averaged according to the Boltzmann distribution theory and their relative Gibbs free energy (ΔG).

(1) *Spartan 08*; Wavefunction Inc.:Irvine, CA.

(2) *Gaussian 09*, Revision A.1, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.;

Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian, Inc., Wallingford CT, 2009.

(3) Stephens, P. J.; Harada, N. ECD cotton effect approximated by the Gaussian curve and other methods. *Chirality* **2010**, *22*, 229–233.

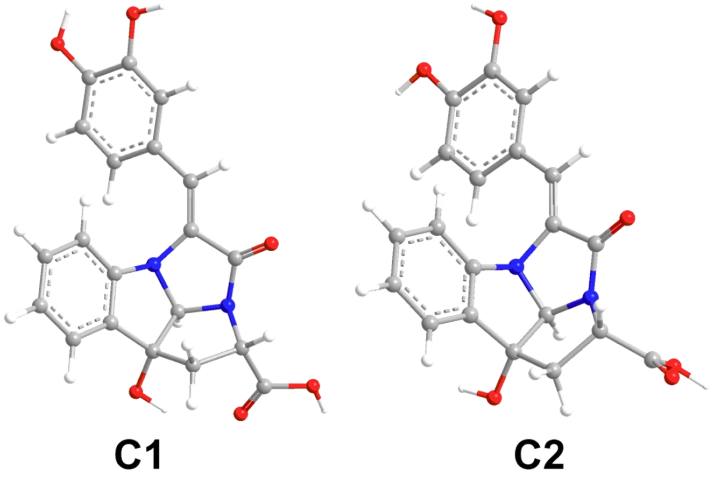


Fig. S1. B3LYP/6-311++G(2d,2p) optimized lowest energy 3D conformers of **1**.

ECD simulation:

ECD spectrum of each conformation is simulated according to the overlapping Gaussian functions expressed as:

$$\Delta\varepsilon(E)=\frac{1}{2.296\times10^{-39}\sqrt{\pi}\sigma}\sum_{i}^{A}\Delta E_i R_i e^{[-(E-\Delta Ei)^2/\sigma^2]}$$

Where σ is half the bandwidth at 1/e peak height and expressed in energy units. The parameters ΔE_i and R_i are the excitation energies and rotational strengths for the transition i, respectively.

The above function is converted to Δε, λ (wavelength) correlations as:

$$\Delta\varepsilon(\lambda)=\frac{1}{2.296\times10^{-39}\sqrt{\pi}\sigma}\sum_{i}^{A}\Delta E_i R_i e^{[-(1240/\lambda-\Delta Ei)^2/\sigma^2]}$$

and then simulation were accomplished by using the Excel 2003 and the Origin 7.0 software.

To get the final spectra, all the simulated spectra of conformations of each compound were averaged according to their energy and the Boltzmann distribution theory expressed as:

$$\frac{N_i^*}{N}=\frac{g_i e^{-\varepsilon_i/k_B T}}{\sum g_i e^{-\varepsilon_i/k_B T}}$$

Energy analysis:

conf.	Gibbs free energy (298.15 K)		
	G (Hartree)	ΔG (Kcal/mol)	Boltzmann Distribution
C1	-1332.745896	0	0.687
C2	-1332.745153	0.46624	0.313

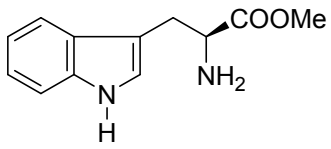
*ECD data for **1**:*

State	**C1**		**C2**	
	Excitation energies(eV)	Rotatory Strengths*	Excitation energies(eV)	Rotatory Strengths*
1	3.3439	-11.6082	3.5955	14.8246
2	3.961	-69.7087	3.9745	-78.3656
3	4.1057	0.8845	4.2747	39.3199
4	4.2862	37.9911	4.3988	-74.5683
5	4.3733	-46.0512	4.5423	-0.8729
6	4.4366	-1.5311	4.6188	-6.0928
7	4.6041	5.522	4.7076	0.726
8	4.6256	0.1517	4.7264	27.8388
9	4.708	-5.4375	4.7659	-7.2183
10	4.7398	12.0613	4.8849	6.016
11	4.8234	4.1442	4.9824	-2.2775
12	4.8398	11.8767	5.002	-4.0265
13	5.0056	-5.658	5.1349	-3.0052
14	5.0297	7.2646	5.1854	3.0156
15	5.0574	-5.2419	5.2342	-17.7617
16	5.1132	3.8971	5.2958	3.9948
17	5.1621	2.4692	5.3245	-8.5935
18	5.2185	-10.8242	5.3348	2.6438
19	5.2422	5.0312	5.3724	-5.1722
20	5.2485	-3.6349	5.4079	5.6897
21	5.3264	-13.4606	5.444	-16.6864
22	5.4147	-7.8615	5.4813	-12.0822
23	5.4379	-2.4937	5.5072	0.8112
24	5.4717	-6.4301	5.5323	-2.6063
25	5.5427	-16.0239	5.5542	6.2016
26	5.5452	-4.2791	5.5882	-7.3174
27	5.5565	-4.6096	5.647	-8.202
28	5.5787	27.6885	5.6884	-15.4958
29	5.5994	1.0663	5.7357	-2.6021
30	5.6507	6.2866	5.7742	2.2182
31	5.7219	8.5271	5.7862	0.6757
32	5.7711	7.5348	5.8039	20.6685
33	5.792	-2.3634	5.826	17.4899

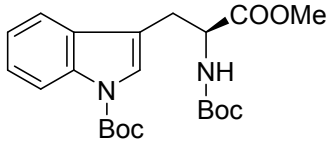
34	5.8129	1.5663	5.8824	-16.5783
35	5.8451	29.8495	5.8844	21.0426
36	5.8834	-0.8516	5.9298	0.5951
37	5.9036	-10.9879	5.9747	-13.1539
38	5.9193	0.6923	6.0027	3.4256
39	5.9289	21.1126	6.0195	13.2002
40	5.9609	-23.5614	6.0237	-0.3827
41	5.9925	-2.5635	6.032	9.1069
42	6.0195	-11.6411	6.0476	-9.9716
43	6.0422	-13.7401	6.0629	8.648
44	6.0524	3.8401	6.0805	44.8102
45	6.0885	-1.4896	6.1193	-20.8543
46	6.0933	11.2961	6.1448	10.7569
47	6.1064	0.0761	6.1628	-0.1353
48	6.122	0.7214	6.1833	-1.7842
49	6.182	-27.2753	6.1976	-14.5276
50	6.1944	-16.9452	6.2064	-5.9745
51	6.2167	6.3985	6.2205	2.0892
52	6.2216	18.0563	6.224	-14.8182
53	6.2428	-1.7105	6.2692	16.0205
54	6.2652	17.2132	6.3053	1.6664
55	6.292	2.5385	6.324	-2.5468
56	6.3259	45.4311	6.3299	-6.4455
57	6.3352	-72.7018	6.3426	23.4022
58	6.3445	28.1814	6.3523	-21.3668
59	6.3601	20.3435	6.3723	10.4022
60	6.3778	-1.0413	6.3972	14.3663

* R(velocity) 10**-40 erg-esu-cm

Procedure for the total synthesis and analytical data of related compounds

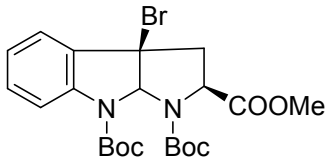


L-Tryptophan methyl ester: Thionyl chloride (7.15 mL, 98 mmol) was added dropwise to a cold (0 °C) solution of anhydrous methanol (220 mL) under magnetic stirring. The solution was stirred at 0 °C for 30 min, and then L/-Trp (8.00 g, 39.2 mmol) was added and the resulting solution was heated at 60 °C for 18 h. After evaporation of the solvent, a white residue of hydrochloride salt was obtained, which was neutralized by a saturated Na_2CO_3 solution (25 mL) and the ester was extracted with equal volume of ethyl acetate three times. The organic layer was dried over anhydous. Na_2SO_4 and evaporated under reduced pressure yielding pale yellow oil, which solidified upon standing to a pale yellow crystalline solid. (8.48 g, 99%) of L-Trp methyl ester. Physical properties and spectroscopic data in accordance with the literature [1].

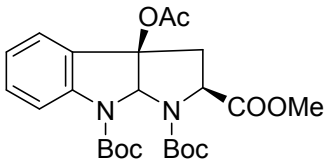


Compound 3: Powdered sodium hydroxide (7.4 g, 185 mmol) was added to a solution of L-tryptophan methyl ester (8.48 g, 37 mmol) and tetrabutylammonium hydrogen sulfate (1.25 g, 3.7

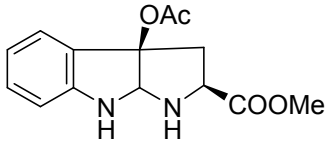
mmol) in CH_2Cl_2 (300 mL) and the mixture stirred for two and half hours at room temperature. Bis(*tert*-butyloxy)carbonic anhydride (24 g, 111mmol) was then added and the mixture allowed to stir for 20 h then filtered through a pad of celite and evaporated in vacuo. The residue was purified by flash chromatography (silica gel, petroleum / ethyl acetate = 5:1) to afford the title compound (15.0 g, 93%) as a white waxy solid. Physical properties and spectroscopic data in accordance with the literature[2].



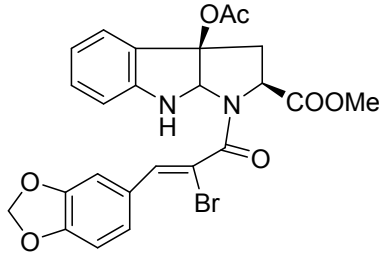
Compound 4: To a solution of the **3** (15.0 g, 36 mmol) in CH_2Cl_2 (300 mL) under argon was added *N*-bromosuccinimide (6.4 g, 36 mmol) and pyridinium *p*-toluenesulfonate (9.0 g, 36 mmol). The resulting yellow solution was stirred at 25 ºC for 25 min. The mixture was diluted with brine, extracted with AcOEt, dried over Na_2SO_4, The residue was purified by flash chromatography (silica gel, petroleum / ethyl acetate = 5:1) to afford (16.0 g, 91%) of the title compound as a white foam. Physical properties and spectroscopic data in accordance with the literature[3].



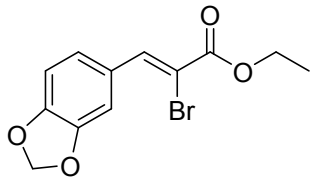
Compound 5: To a solution of **4** (8.1 g, 16.3 mmol) in AcOH (150 mL) was added silver acetate (3.22 g, 19.6 mmol), and the reaction mixture was heated at reflux for 20 min. After the mixture was cooled to ambient temperature, Et_2O (100 mL) was added. The mixture was filtered, and the filter cake was washed with Et_2O (100 mL). The solvents were removed under reduced pressure, and the residue was purified by flash chromatography (silica gel, petroleum / ethyl acetate = 5:1) to afford (7.1 g, 91%) as a white foam. Physical properties and spectroscopic data in accordance with the literature[4].



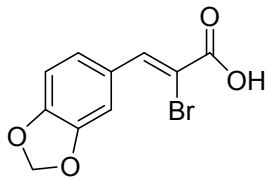
Compound 6: To a solution of **5** (2.4 g, 5 mmol) in CH_3CN (30 mL) was added TMSI (1.7 mL, 2.5 mmol) and the reaction mixture was at 0 °C stirred for 20 min and then ambient temperature 2 h. The reaction mixture was quenched by $NaHCO_3$ solution, diluted with brine, extracted with AcOEt, dried over Na_2SO_4. The residue was purified by flash chromatography (silica gel, dichloromethane / methanol=10:1) to afford (1.3 g, 93%) of the title compound as a brown foam. Physical properties and spectroscopic data in accordance with the literature[4].



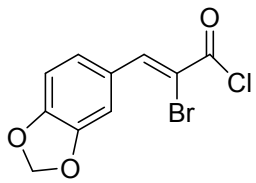
Amide x1: To a solution of DMAP (1.0 g, 6.5 mmol, 1.2 equiv) and acid chloride **7** (1.98 g, 6.5 mmol, 1.2 equiv) in CH_2Cl_2 at 0 °C. And a solution of **6** (1.5 g, 5.4 mmol, 1.0 equiv) in CH_2Cl_2 was added to the reaction mixture at 0 °C. The mixture was stirred at 0 °C for 2 h and then diluted with brine, extracted with AcOEt, dried over Na_2SO_4. The residue was purified by flash chromatography (silica gel, petroleum / ethyl acetate = 3:1) to afford (2.1 g, 73%) of the title compound as a white foam. Analytical data for amide: HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{24}H_{21}BrNaN_2O_7$ 551.0424; found 551.0429.



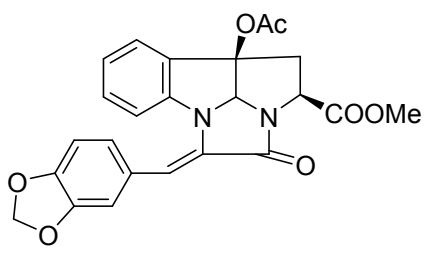
Compound x2: To a solution of piperonyl aldehyde (1.5 g, 10 mmol, 1.0 equiv) and ethyl bromoacetate (1.83 g, 11 mmol, 1.1equiv) in DCM (25 mL) under a nitrogen atmosphere was added $TiCl_4$ (12 mmol, 1.0 M soln in DCM, 1.2 equiv) in drops over a period of 10 minutes. The mixture was then stirred at room temperature for 30 min. To this mixture was added Et_3N (2.02 g, 20 mol, 2.0 equiv) in drops over a period of 10 minutes while maintaining the reaction temperature below 30 °C. The resulting brown mixture was then stirred at room temperature for 5 h. The mixture was diluted with DCM (20 mL) and washed with 1.0 N aqueous HCl (15 mL), water and brine. The organic phase was dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was passed through a plug of silica (silica gel, petroleum / ethyl acetate = 9:1) to afford (2.78 g, 93%) of the title compound as a yellow powder. Analytical data for **x2**: m.p. 69.5–70.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (s, H), 7.64 (s, 1H), 7.30 (dd, J = 0.8 Hz, 8.0 Hz, 1H), 6.86 (d, J = 8.4, 1H), 6.03 (s, 1H), 4.34 (q, J = 7.2 Hz, 1H), 1.38 (t, J =7.2 Hz, 3H) ; ^{13}C NMR (100 Hz, $CDCl_3$) δ 163.7, 149.5, 147.8, 140.3, 127.9, 127.1, 110.9, 109.6, 108.5, 101.8, 62.8, 14.4 ; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{12}H_{11}BrNaO_4$ 320.9733; found 320.9729.



Compound x3: To a solution of **x2** (1.8 g, 6 mmol, 1.0 equiv) in CH_2Cl_2/CH_3OH (9:1) (50 mL), and the solution was cooled to 5 °C in ice-water bath. NaOH (480 mg, 12 mmol, 2.0 equiv) was added, and the mixture was stirred for 12 h at room temperature. The solvents were then removed under vacuum, the residue was diluted with water, and the aqueous solution was extracted with diethyl ether to remove the remaining ester. The aqueous phase was then cooled, acidified to pH 2-3 with dilute HCl, and extracted with Et_2O. The combined organic layer was dried over Na_2SO_4, and the solvent was removed to afford (1.5 g, 92.5%) of the title compound as a yellow powder. Analytical data for **x3**: m.p. 186.9–188.4 °C; $[\alpha]_D^{25}$ +12.0 (*c* 0.1, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.49 (s, H), 8.15 (s, 1H), 7.63 (d, J = 1.2 Hz, 1H), 7.46 (dd, J = 1.2 Hz, 8.4 Hz, 1H), 7.03 (d, J = 8 Hz, 1H), 6.11 (s, 1H) ; ^{13}C NMR (100 Hz, DMSO-d_6) δ 164.2, 149.1, 147.3, 139.6, 127.2, 126.5, 111.7, 109.2, 108.5, 101.8 ; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{10}H_7BrO_4$ 292.9420; found 292.9426.

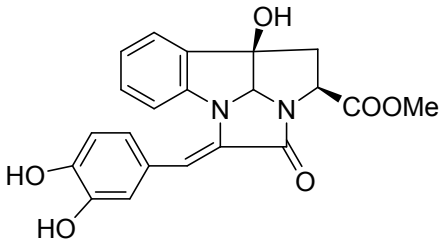


Compound 7: To a solution of **x3** (2.7 g, 9.3 mmol) in toluene (20 mL) at 0 °C was added oxalyl chloride (1.41 g, 11.1 mmol) and dimethylformamide (0.1 mL) in 0 °C. The mixture was stirred for 2 h. The solvents were then removed under vacuum. The crude product was used for next step without purification.

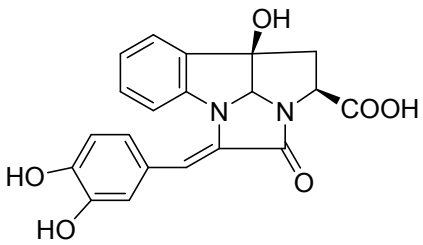


Compound 8: To a solution of amide **x1** (1.6 g, 3 mmol, 1.0 equiv) , $Pd(OAc)_4$ (5 mol%), P*t*Bu_3·HBF_4 (6 mol%), and K_2CO_3 (828 mg, 6 mmol, 2.0 equiv). The flask was flushed with argon for 5 minutes, after which toluene (20 mL) was added and the vial sealed. The vial was stirred at room temperature for 5 minutes and then placed in a pre-

heated oil bath at 100 °C. After stirring for 14 hours, the vial was removed from the oil bath and allowed to cool to room temperature, then filtered through a pad of celite and evaporated in vacuo. The residue was purified by flash chromatography (silica gel, petroleum / ethyl acetate = 3:1) to afford (1.12 g , 83%) of the title compound as a white foam. Analytical data for **8**: m.p. 112.2–114.1 ℃; $[\alpha]_D^{25}$ –471 (*c* 0.1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (d, *J* = 1.6 Hz, 1H), 7.55 (dd, *J* = 7.6 Hz, 0.8 Hz, 1H), 7.36 (dd, *J* = 1.6 Hz, 8.4 Hz, 1H), 7.14 –7.18 (m, 1H), 7.02 – 7.06 (m, 1H), 6.91 (s, 1H), 6.86 (d, *J* = 8.0 Hz, 1H), 6.65 (d, *J* = 8.0 Hz, 1H), 6.01 (dd, *J* =1.2 Hz, 4.4 Hz, 1H), 5.77 (s, 1H), 4.67 (q, *J* = 9.6 Hz, 1H), 3.82 (s, 3H), 3.32 (dd, *J* = 5.6 Hz, 14.4 Hz, 1H), 3.13 (dd, *J* =4.4 Hz, 14.8 Hz, 1H), 2.10 (s, 3H) ; ^{13}C NMR (100 Hz, $CDCl_3$) δ 171.4, 170.2, 167.3, 152.1, 148.8, 148.4, 137.3, 131.7, 131.2, 128.0, 126.8, 126.4, 124.4, 122.5, 116.9, 109.7, 108.8, 101.6, 90.7, 88.1, 57.3, 53.1, 45.3, 21.5; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{24}H_{20}NaN_2O_7$ 471.1163; found 471.1168.

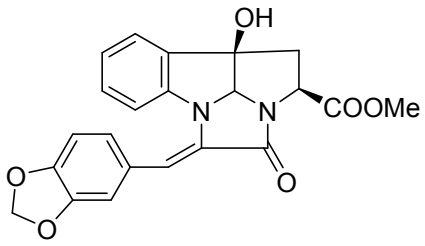


Compound 2: To a solution of **8** (450 mg, 1 mmol, 1.0 equiv) in toluene (15 mL) at –30 ℃ was added trifluoromethanesulfonic acid (excess, 2 mL). The resulting solution was stirred at 0 °C for 5 min and then at room temperature for 5 minutes, then was diluted with $NaHCO_3$ solution (10 mL) and extracted with ethyl acetate (2 × 15 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4), concentrated, and evaporated in vacuo. The residue was purified by flash chromatography (silica gel, petroleum / ethyl acetate = 1:2) to afford (268 mg, 68%) of the title compound as a white foam. Analytical data for **2**: $[\alpha]_D^{25}$ –546 (*c* 0.2, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.49 (s, 1H), 9.16 (s, 1H), 7.62 (d, *J* = 1.6 Hz, 1H),7.44 (d, *J* = 8.0 Hz, 1H), 7.25 (dd, *J* = 1.6 Hz, 8.4 Hz, 1H), 7.15 –7.19 (m , 1H), 7.05 –7.09 (m , 1H), 6.81 (d, *J* =8.0 Hz, 1H), 6.72 (s, 1H), 6.56 (dd, *J* = 8.0 Hz, 1H), 6.33 (s, 1H), 5.30 (s, 1H), 4.49 (dd, *J* = 9.6 Hz, 5.6 Hz, 1H), 3.74 (s, 3H), 3.08 (dd, 13.6 Hz, 9.6Hz, 1H) , 2.73 (dd, *J* = 5.6 Hz, 14.0 Hz, 1H) ; ^{13}C NMR (100 Hz, DMSO-d_6) δ 171.4, 167.0, 151.1, 147.3, 145.5, 136.8, 135.3, 130.2, 125.3, 124.8, 123.9, 123.5, 121.6, 116.8, 115.9, 115.7, 89.2, 84.7, 56.7, 52.5, 46.6; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{21}H_{18}NaN_2O_6$ 417.1057; found 417.1063.



Compound 1: To a solution of **2** (14 mg, 0.035 mmol, 1.0 equiv) in CH_3OH/H_2O (1:1, v/v, 2 mL) at 0 ℃ was added lithium hydroxide (2 mg, 0.7 mmol. 2.0 equiv) and the resulting solution was stirred at 0 °C for 30 min and then was diluted with water. The solution was extracted with diethyl ether to remove the remaining ester. The aqueous phase was acidified to pH 2-3 with 1N HCl, and extracted with Et_2O. The combined organic layer was dried over Na_2SO_4, and the solvent was removed to afford (12.5 mg, 92%) of the title compound as a brown oil. Analytical data for **1**: $[\alpha]_D^{25}$–328 (c 0.1, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.10 (s, 1H), 9.48 (s, 1H), 9.15 (s, 1H), 7.62 (d, *J* = 1.6 Hz, 1H), 7.44 (d, *J* = 7.6 Hz, 1H), 7.25 (dd, *J* = 1.6 Hz, 8.4 Hz, 1H), 7.15–7.19 (m , 1H), 7.07 (t, *J* = 7.2 Hz, 1H), 6.81 (dd, *J* = 8.4 Hz, 1H), 6.71 (s, 1H), 6.55 (d, *J* = 8.0 Hz, 1H), 6.32 (s, 1H) , 5.28 (s, 1H), 4.35 (dd, J = 9.6 Hz, 5.6 Hz,1H), 3.05 (dd, 13.6 Hz, 9.6Hz, 1H), 2.71 (q, 13.6

Hz, 1H) ; 13C NMR (100 Hz, DMSO-d_6) δ 172.4, 166.9, 151.1, 147.2, 145.5, 137.0, 135.5, 130.1, 125.3, 124.8, 123.8, 123.5, 121.3, 116.7, 115.9, 115.8, 89.3, 84.7, 56.8, 46.9; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{20}H_{16}NaN_2O_6$ 403.0901; found 403.0903.



Compound 9: To a solution of **8** (45 mg, 0.1 mmol, 1.0 equiv) in CH_3OH (2 mL) at room temperature was added NaOMe (11 mg, 0.2 mmol, 2.0 equiv) and the resulting solution was stirred at room temperature for 2 h. The aqueous phase was acidified to pH 2–3 with dilute HCl, and extracted with EtOAc. The combined organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (silica gel, petroleum / ethyl acetate = 1:1) to afford (39 mg, 96%) of the title compound as a white foam. Analytical data for **10**: m.p. 130.1−131.8 ℃; $[\alpha]_D^{25}$ – 437 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (s, 1H), 7.39 (d, *J* = 7.6 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.15 (t, *J* = 8.0 Hz, 1H), 7.06 (t, *J* = 7.6 Hz, 1H), 6.87 (s, 2H), 6.62 (d, *J* = 8.0 Hz, 1H), 6.01 (d, *J* = 4.8 Hz, 1H), 5.52 (s, 2H), 4.65 (q, *J* =4.8 Hz, 1H), 3.83 (s, 3H), 2.78–2.98 (m, 3H); ^{13}C NMR (100 Hz, $CDCl_3$) δ 171.9, 167.9, 151.1, 148.7, 148.4, 137.1, 134.2, 131.4, 128.1, 126.3, 124.6, 124.5, 121.9, 117.2, 109.6, 108.8, 101.6, 90.2, 86.6, 57.7, 53.1, 47.4; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{22}H_{18}NaN_2O_6$ 429.1052; found 429.1056.

References

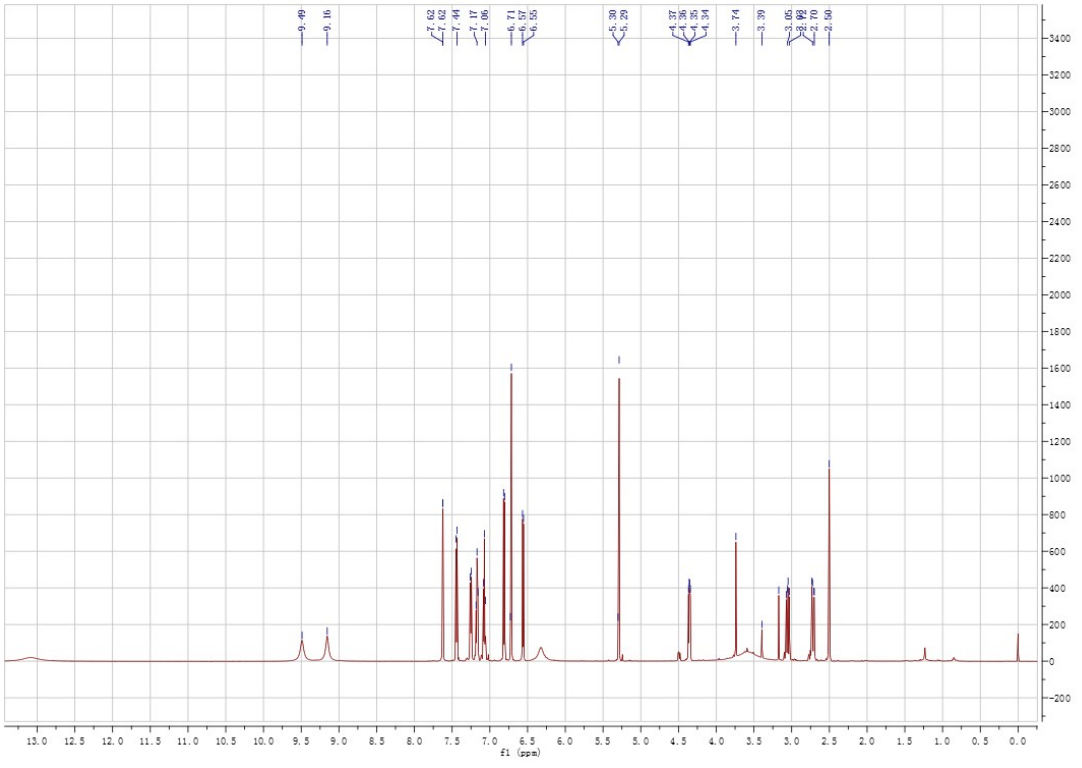
(1) Alqahtani, N.; Porwal, S. K.; James, E. D.; Bis, D. M.; Karty, J. A.; Lane, A. L.; Viswanathan, R. *Org. Biomol. Chem*. **2015**, *13*, 9323.

(2) Depew, K. M.; Marsden, S. P.; Zatorska, D.; Zatorski, A.; Bornmann, W. G.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 11953.

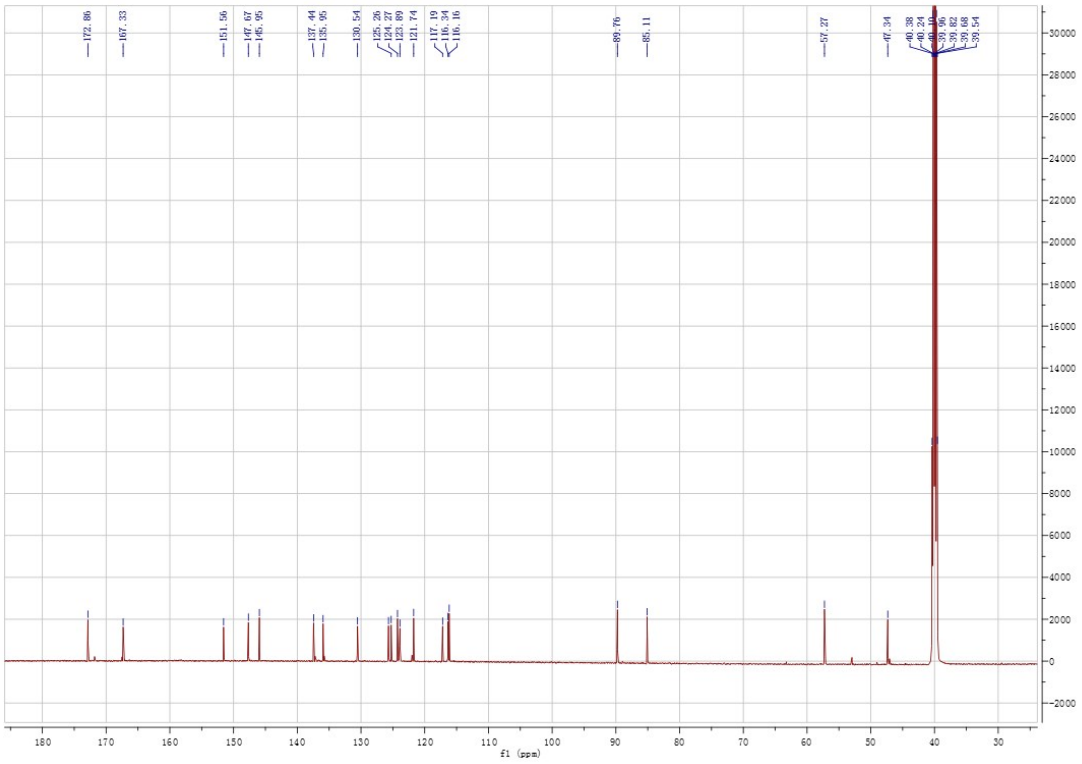
(3) Sun, Y.; Li, R.; Zhang, W.; Li, A. *Angew. Chem. Int. Ed.* **2013**, *52*, 9201.

(4) Lorenzo, P.; Álvarez, R.; de Lera, Á. R. *Eur. J. Org. Chem.* **2014**, *2014*, 2557.

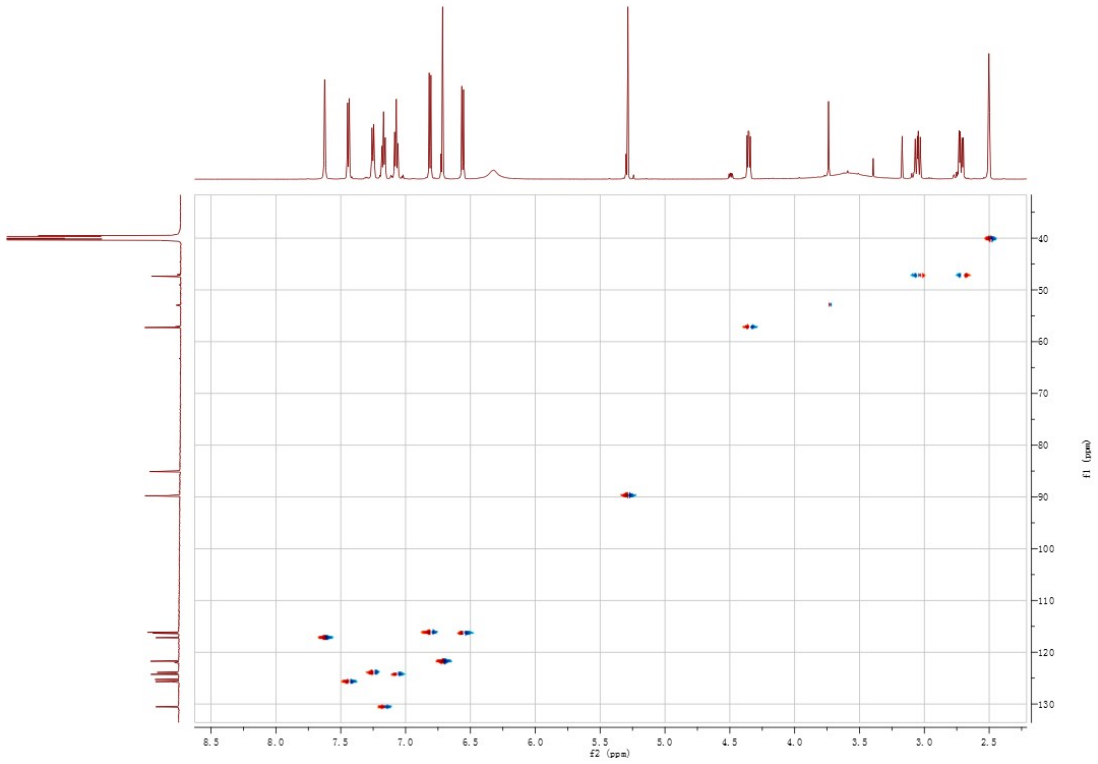
NMR, HRMS, and IR spectra for compounds 1 and 2



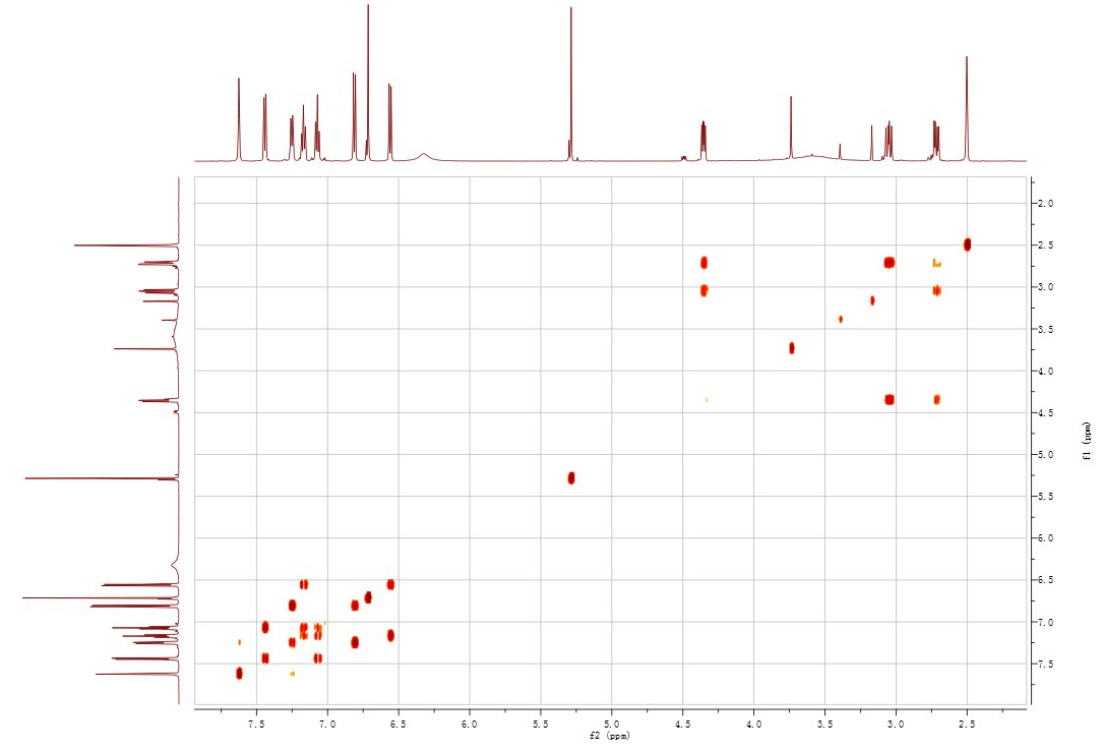
^{1}H NMR spectrum (DMSO-d_6, 600 MHz) of **1**



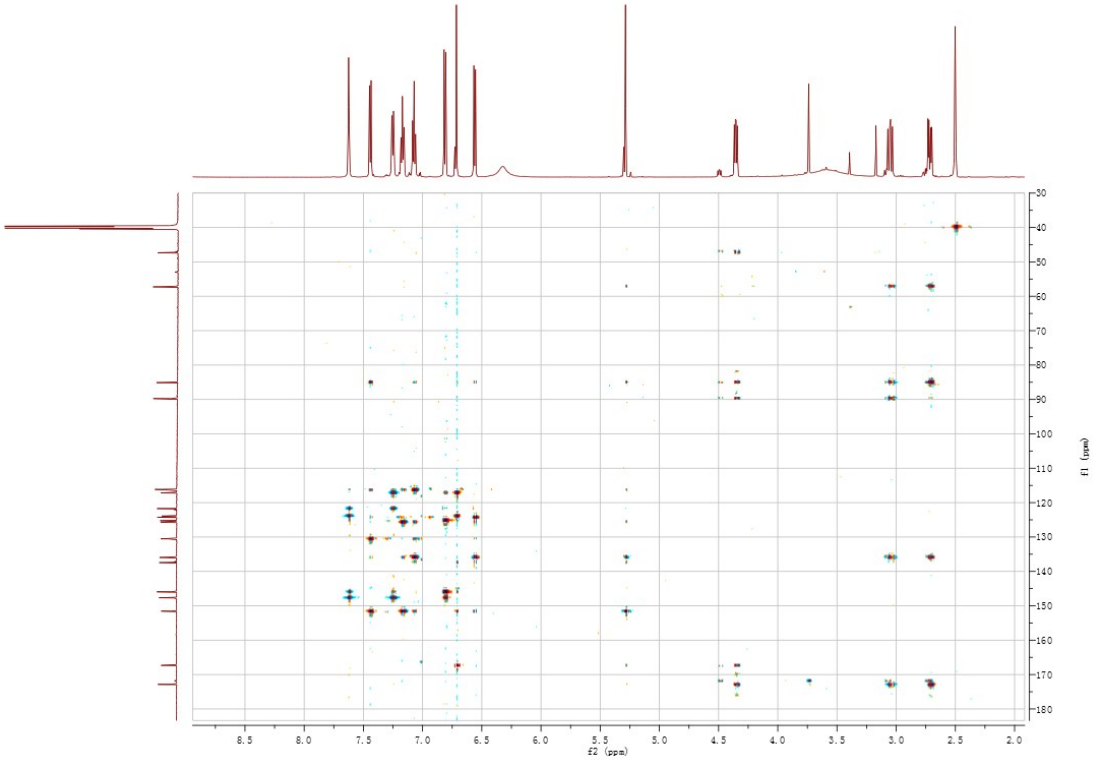
^{13}C NMR spectrum (DMSO-d_6, 150 MHz) of **1**



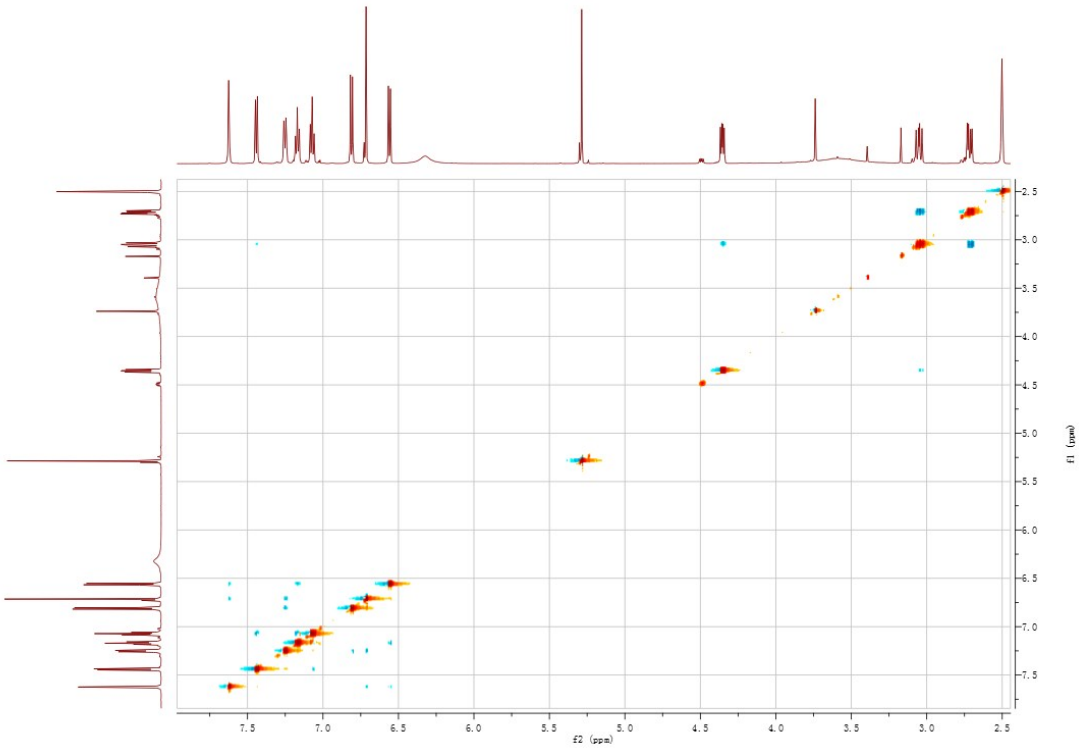
HSQC spectrum (DMSO-d_6) of **1**



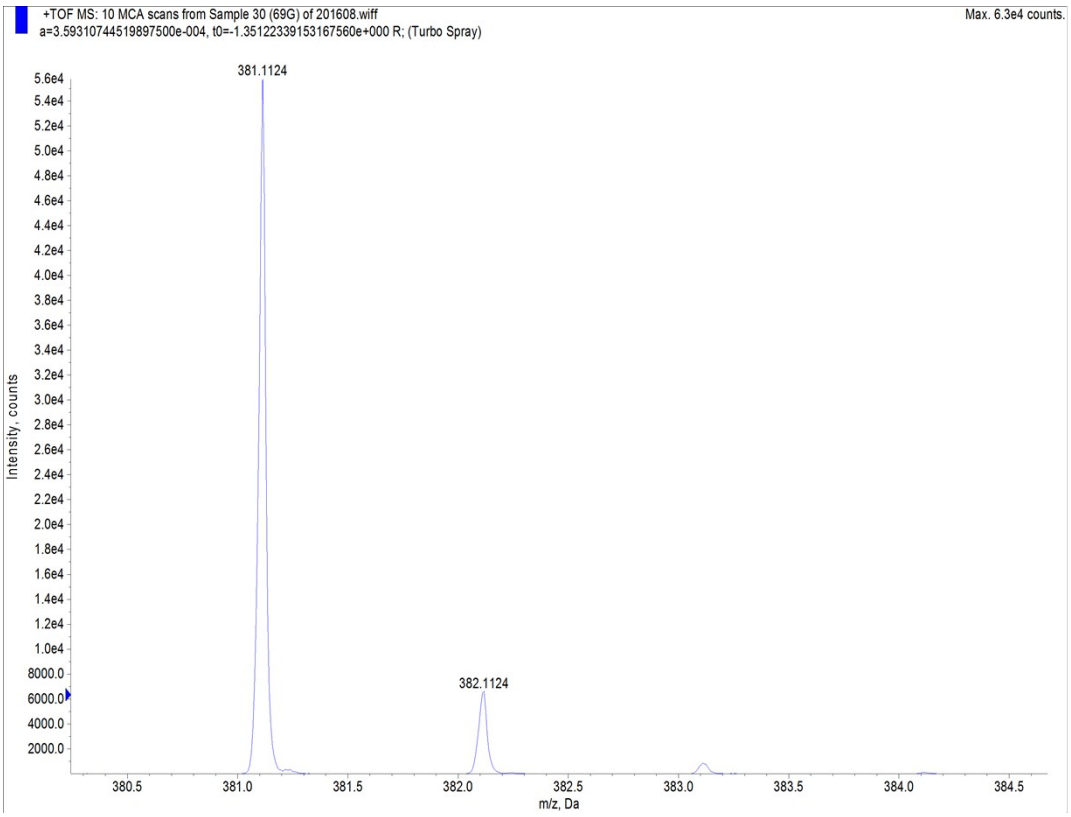
^{1}H-^{1}H COSY spectrum (DMSO-d_6) of **1**



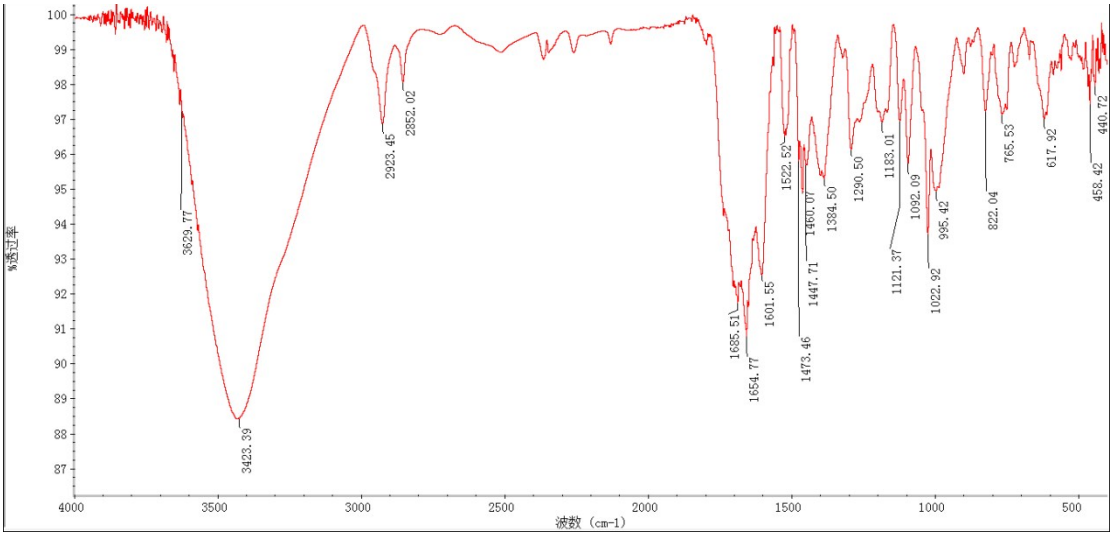
HMBC spectrum (DMSO-d_6) of **1**



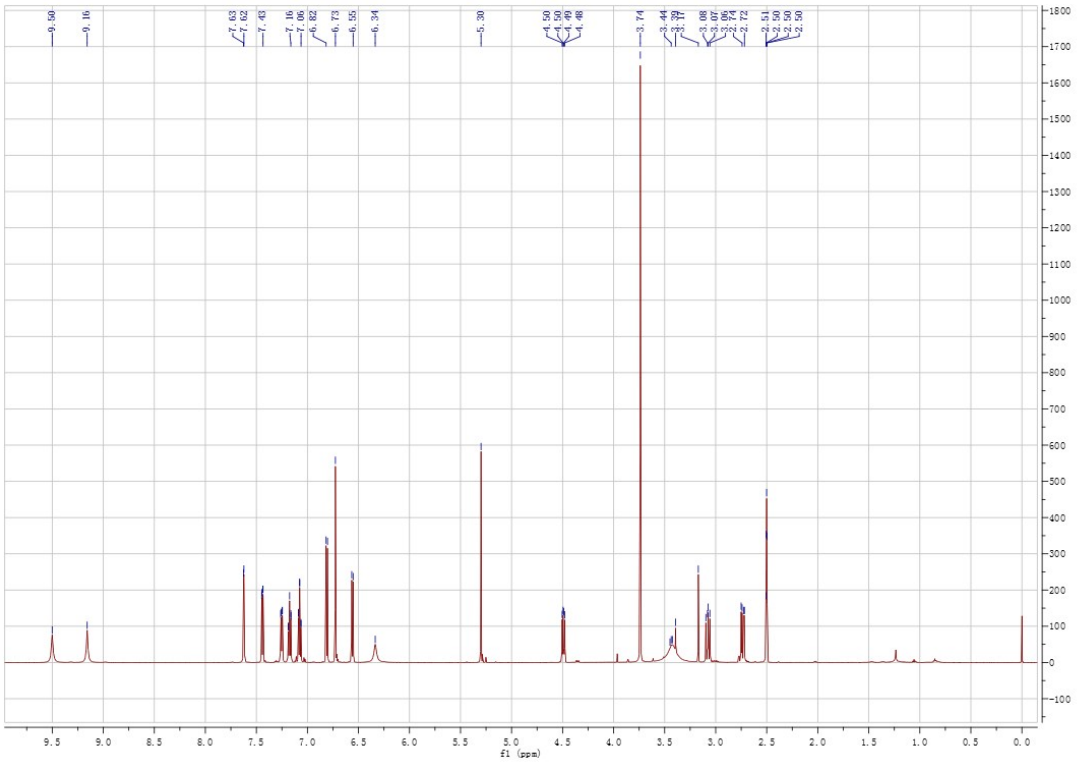
NOESY spectrum (DMSO-d_6) of **1**



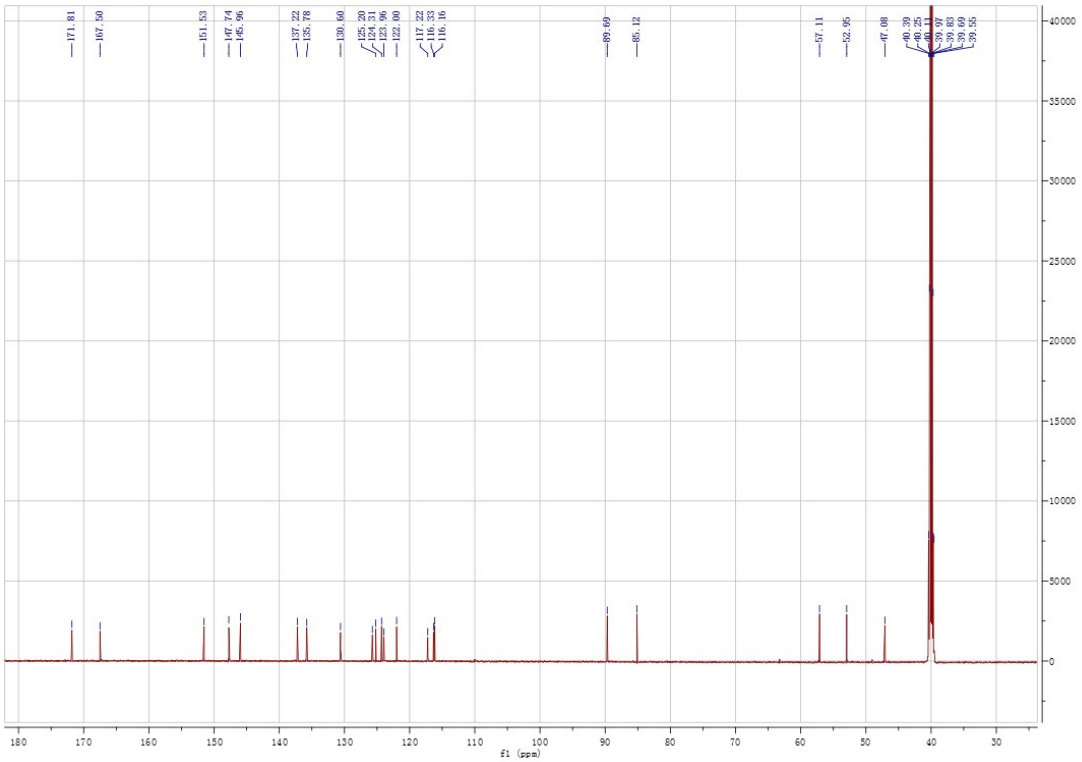
HRESIMS (+) spectrum of **1**



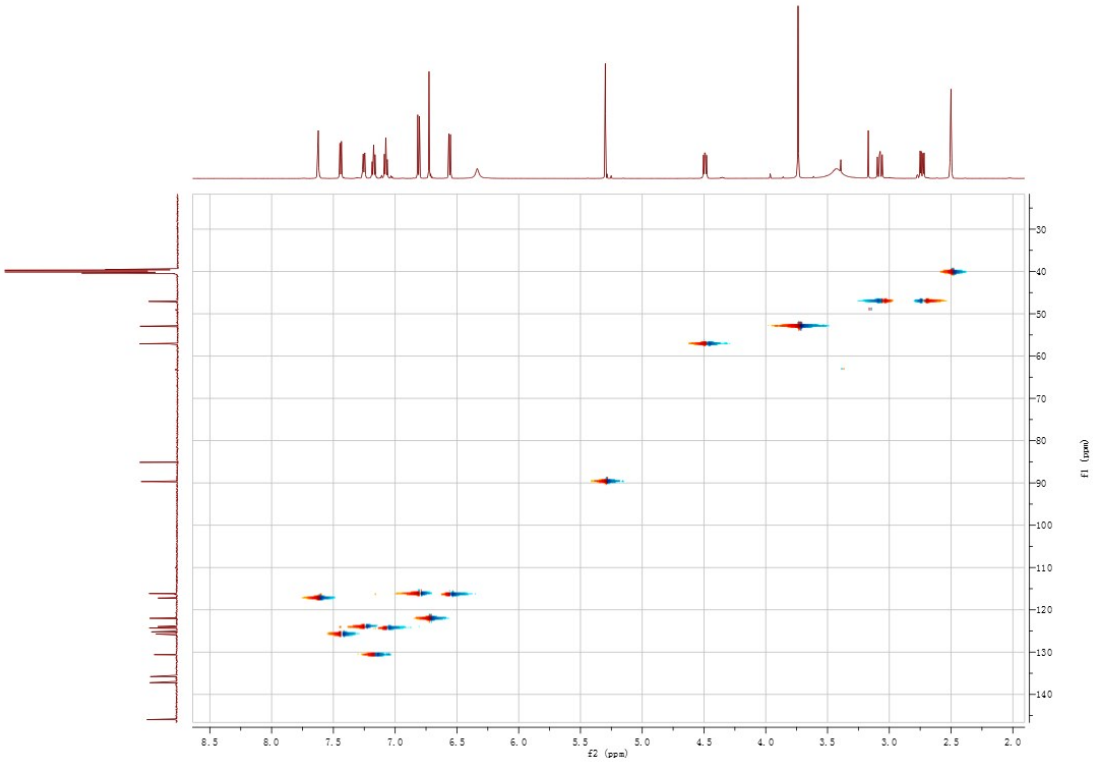
IR spectrum of **1**



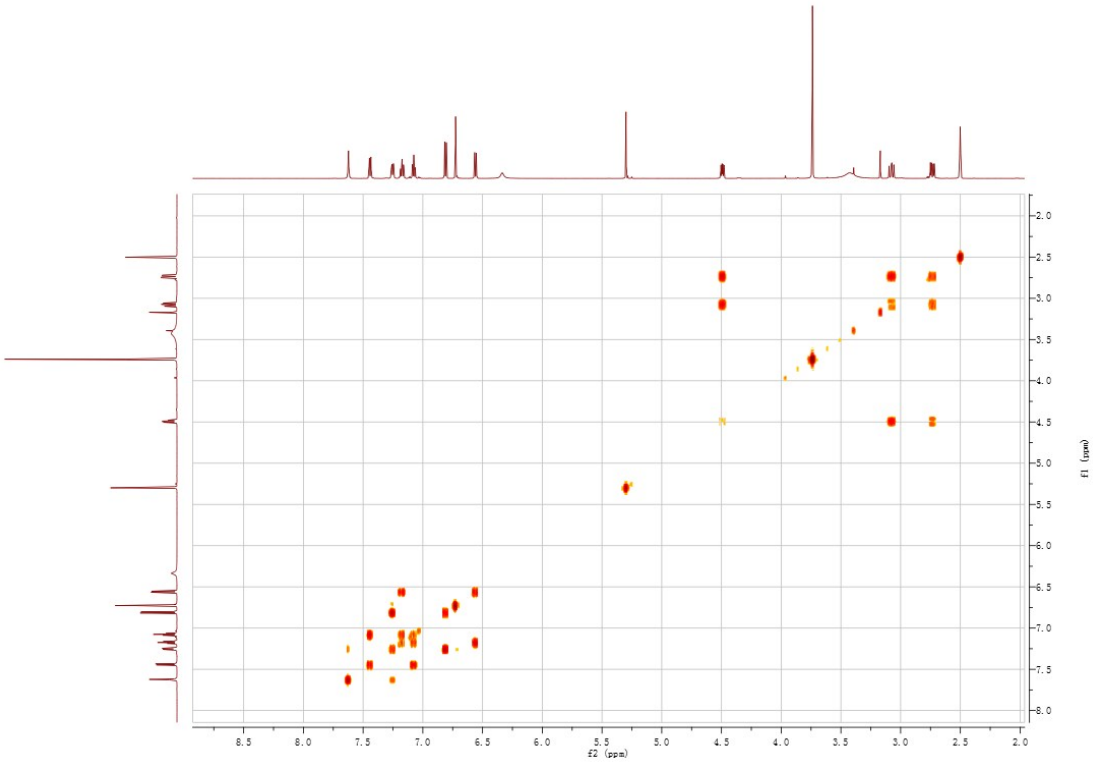
^{1}H NMR spectrum (DMSO-d_6, 600 MHz) of **2**



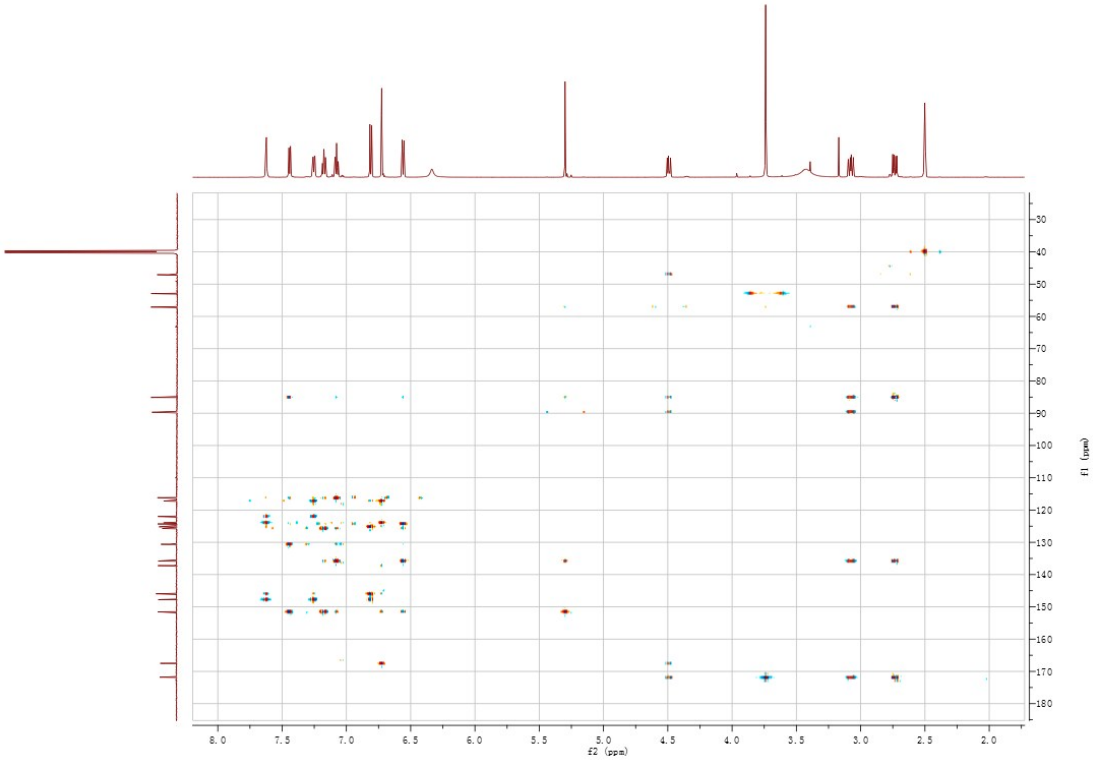
^{13}C NMR spectrum (DMSO-d_6, 150 MHz) of **2**



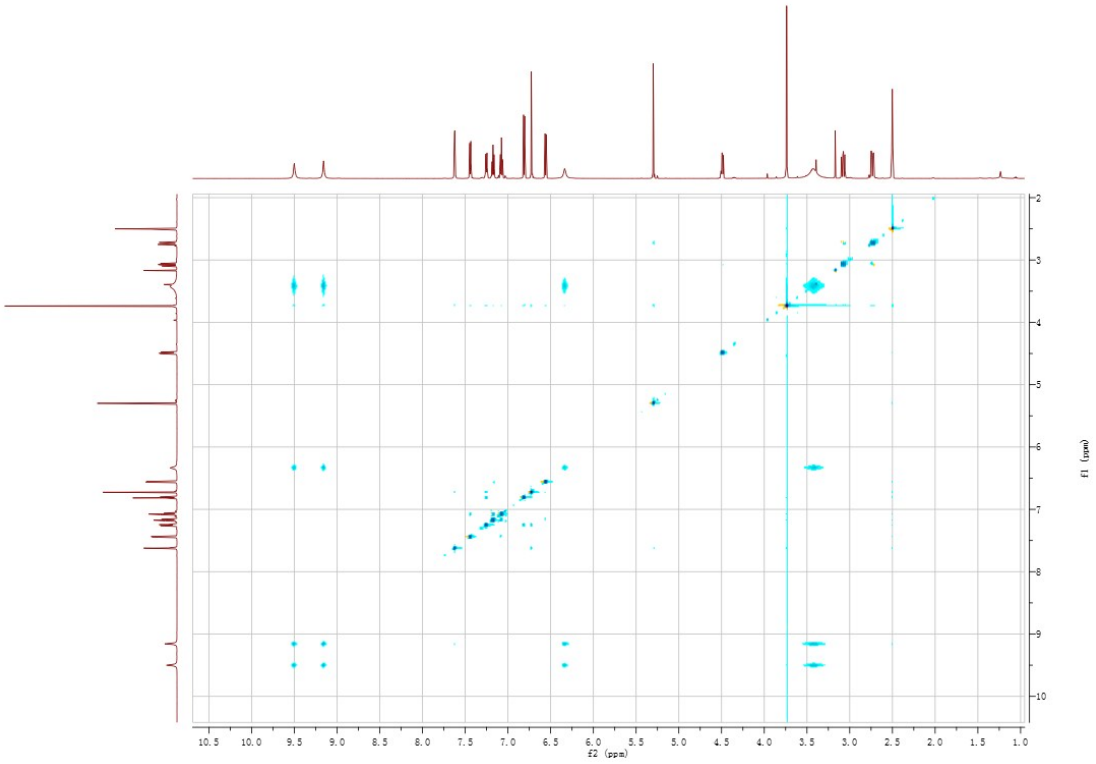
HSQC spectrum (DMSO-d_6) of **2**



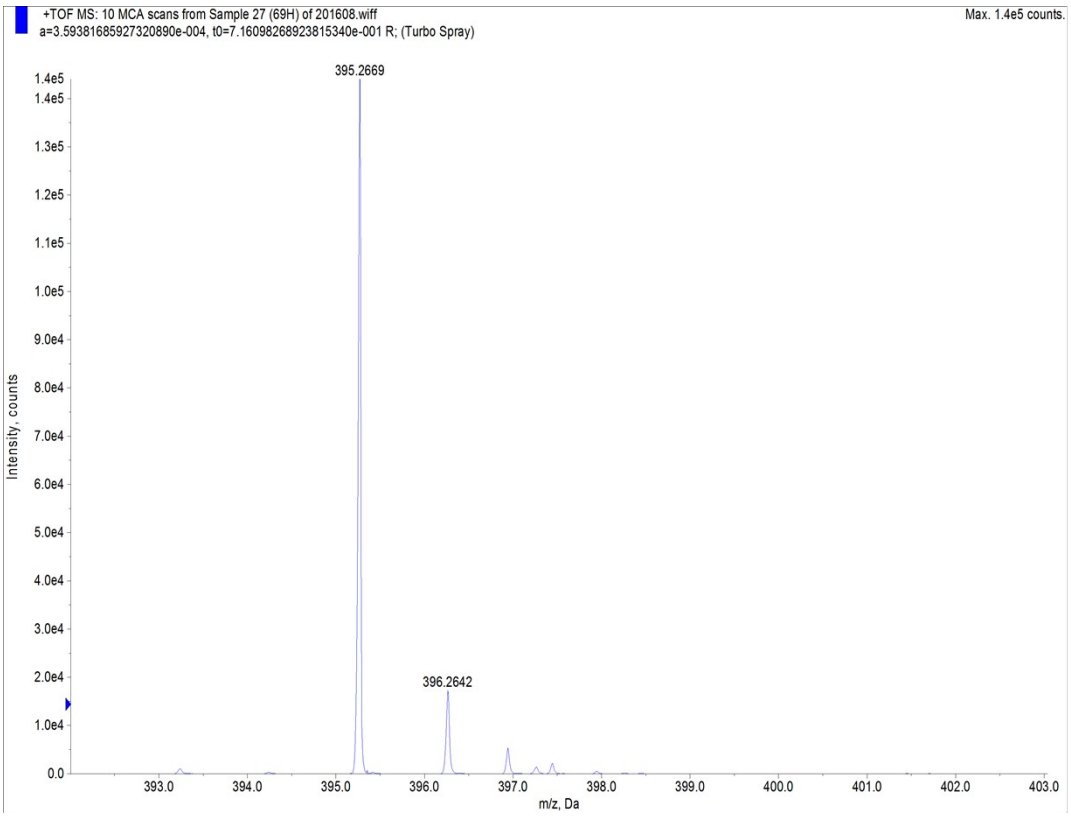
^{1}H-^{1}H COSY spectrum (DMSO-d_6) of **2**



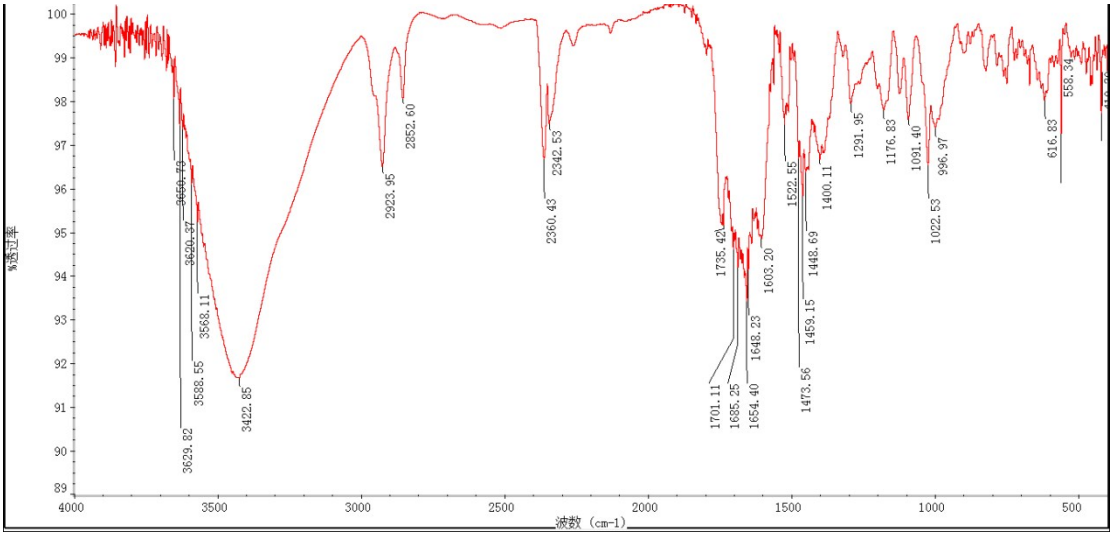
HMBC spectrum (DMSO-d_6) of **2**



NOESY spectrum (DMSO-d_6) of **2**

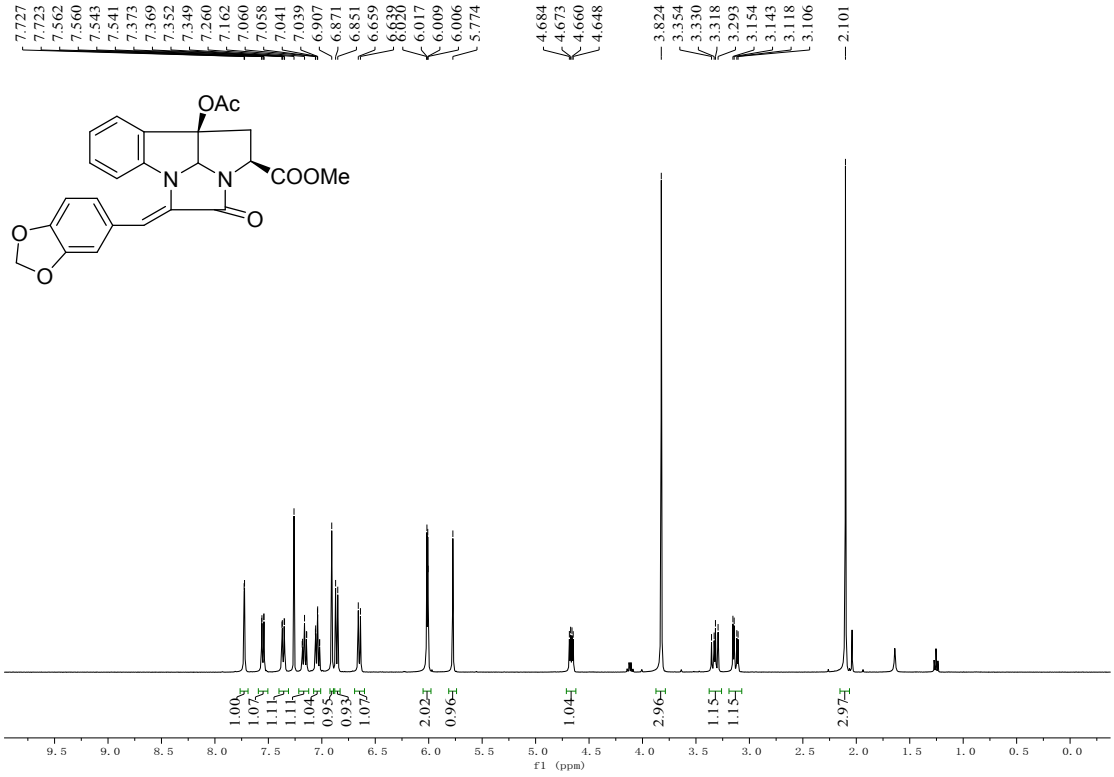


HRESIMS (+) spectrum of **2**

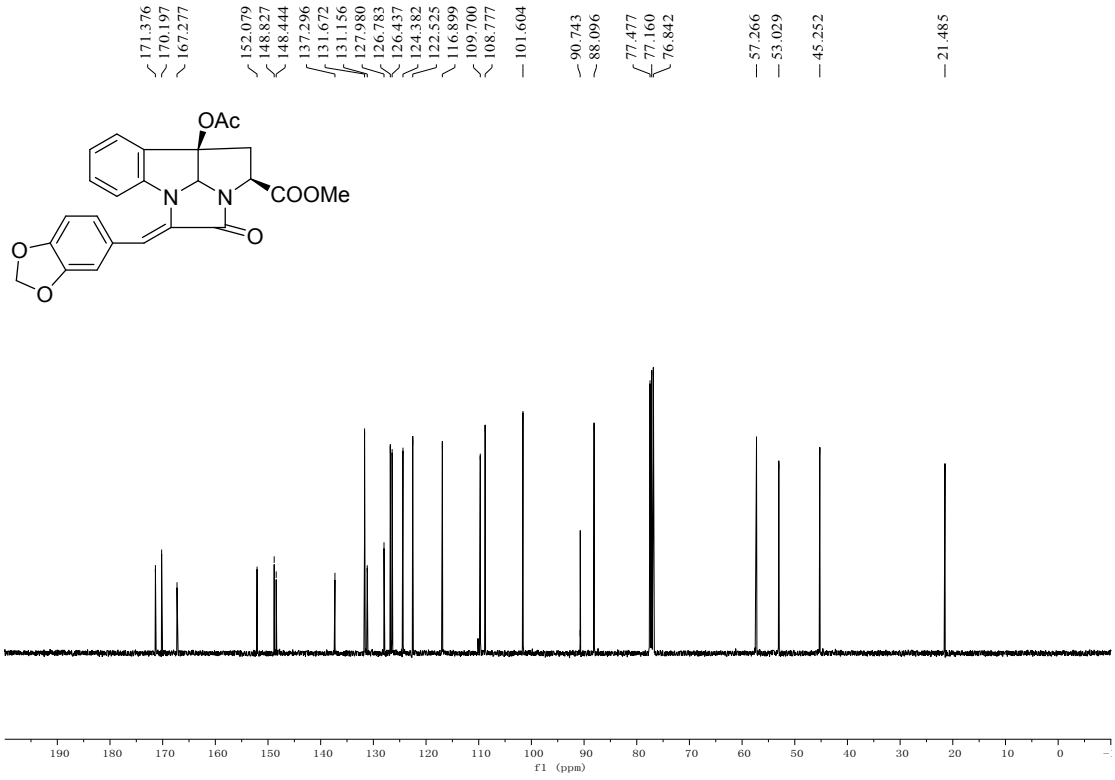


IR spectrum of **2**

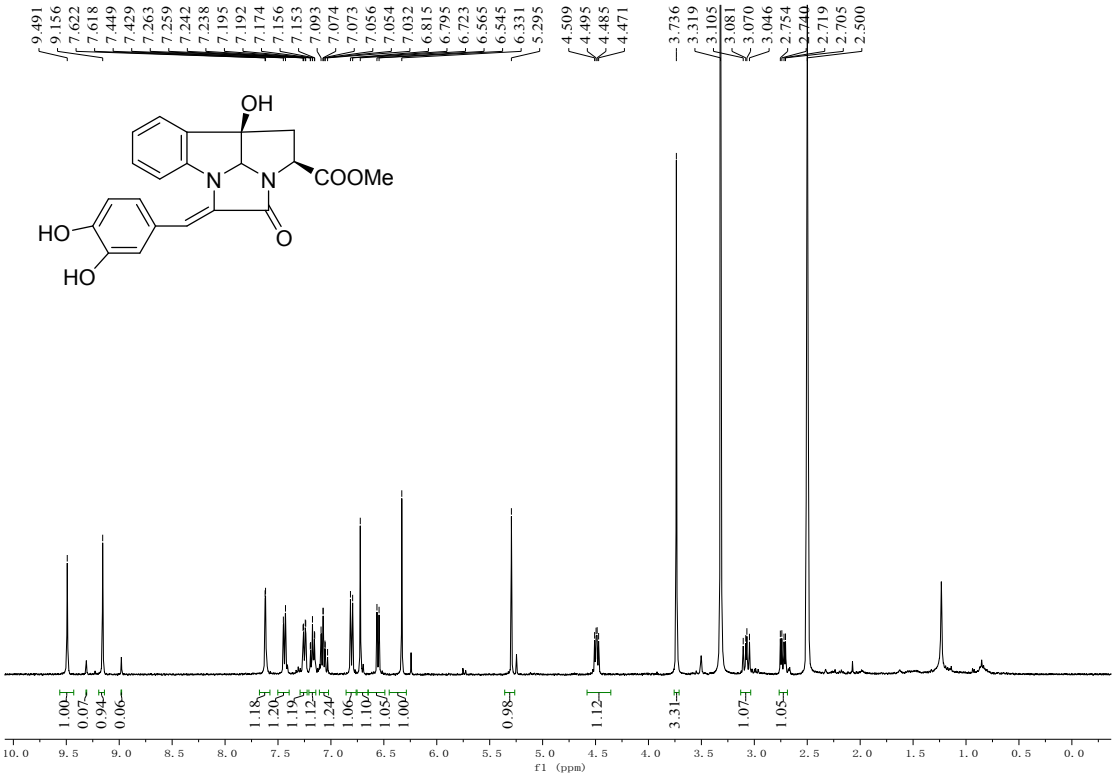
^{1}H NMR and ^{13}C NMR spectra for all synthetic new compounds



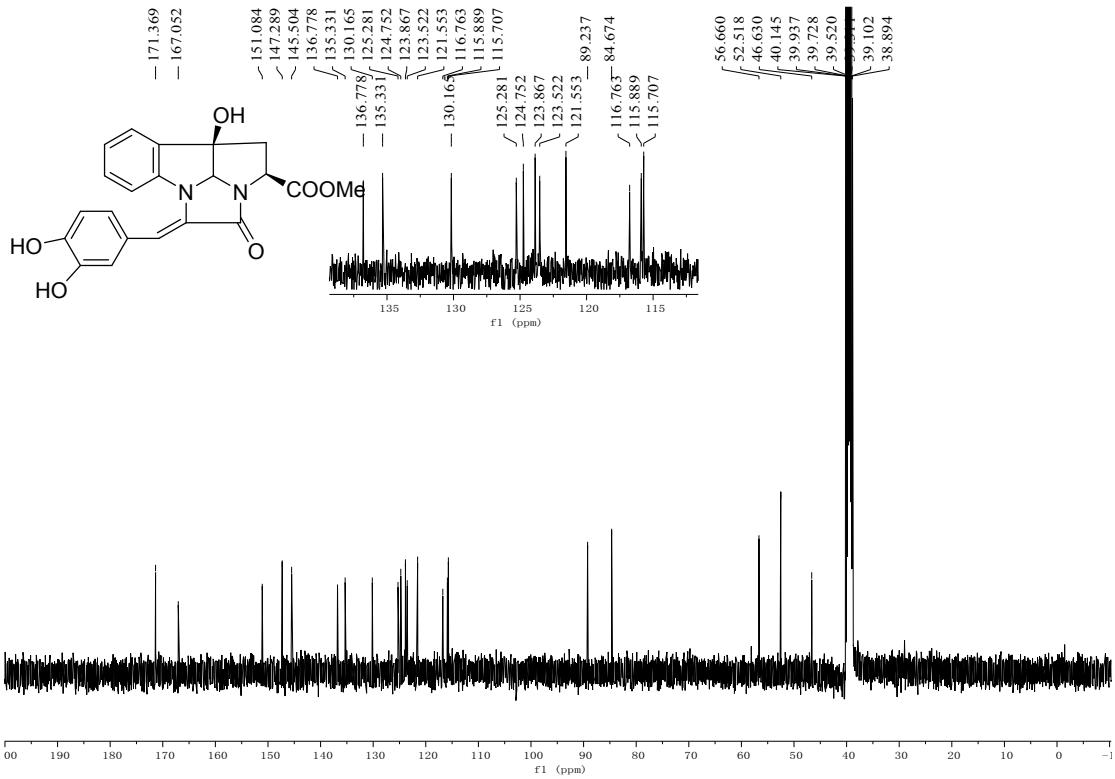
^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of **8**



^{13}C NMR spectrum ($CDCl_3$, 100 MHz) of **8**



^{1}H NMR spectrum (DMSO-d_6, 400 MHz) of **2**



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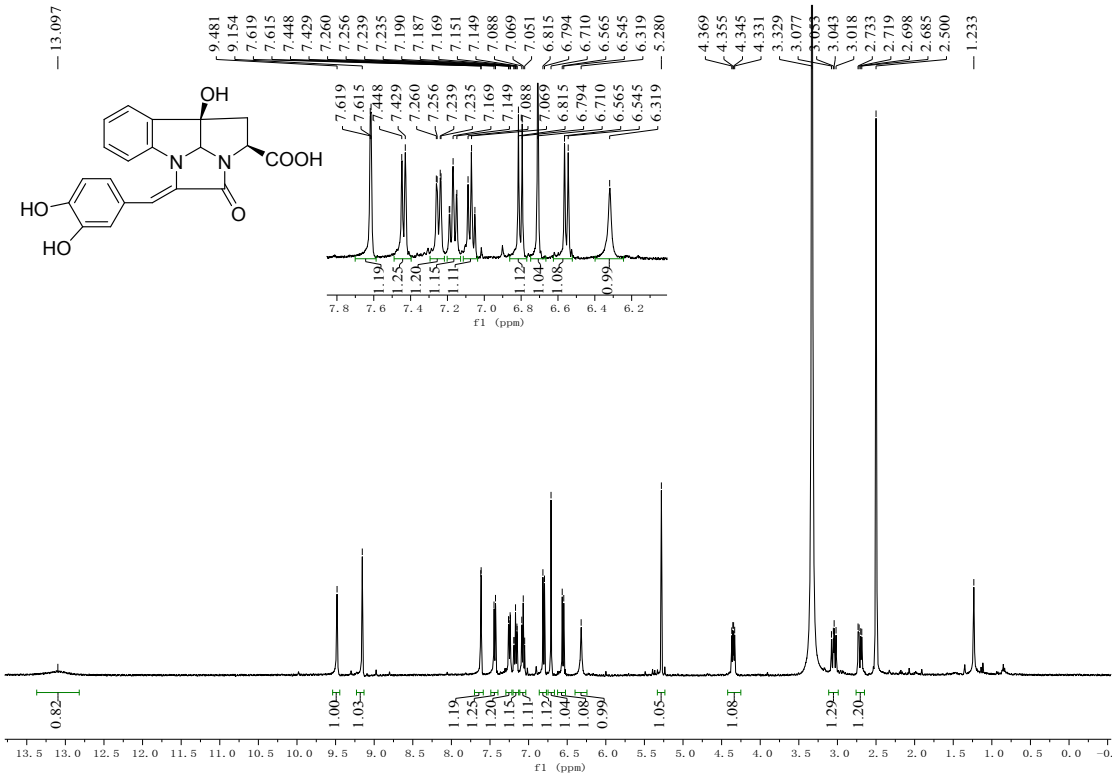
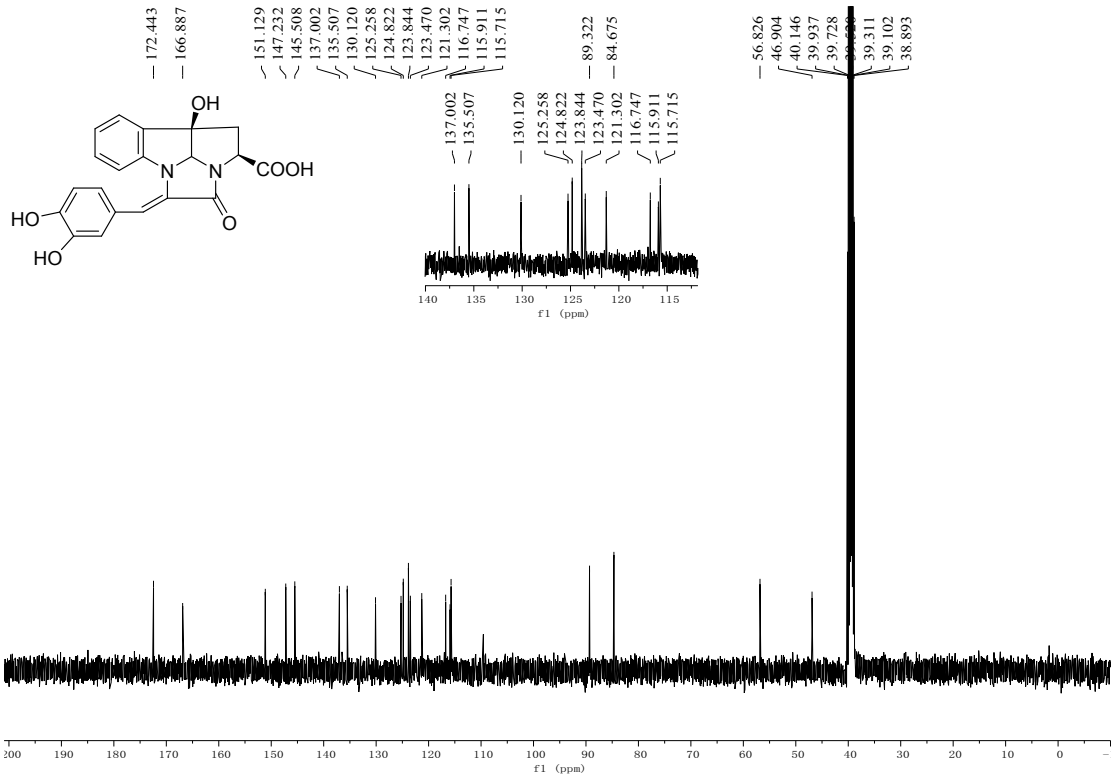
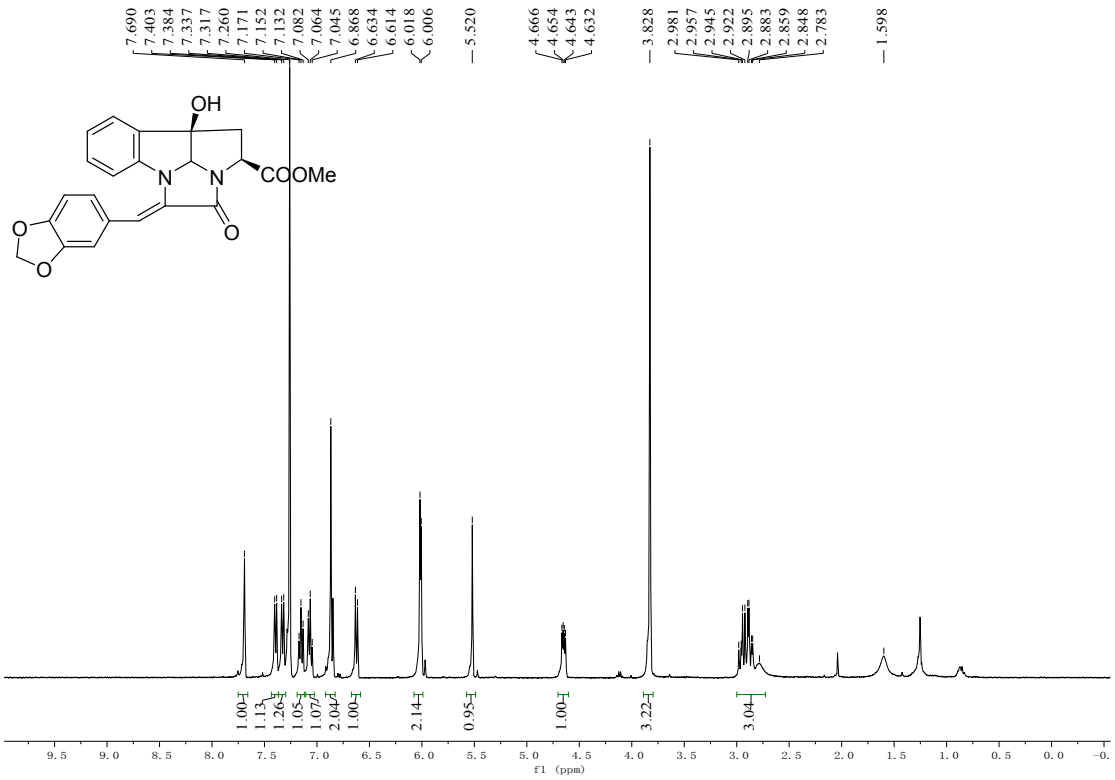


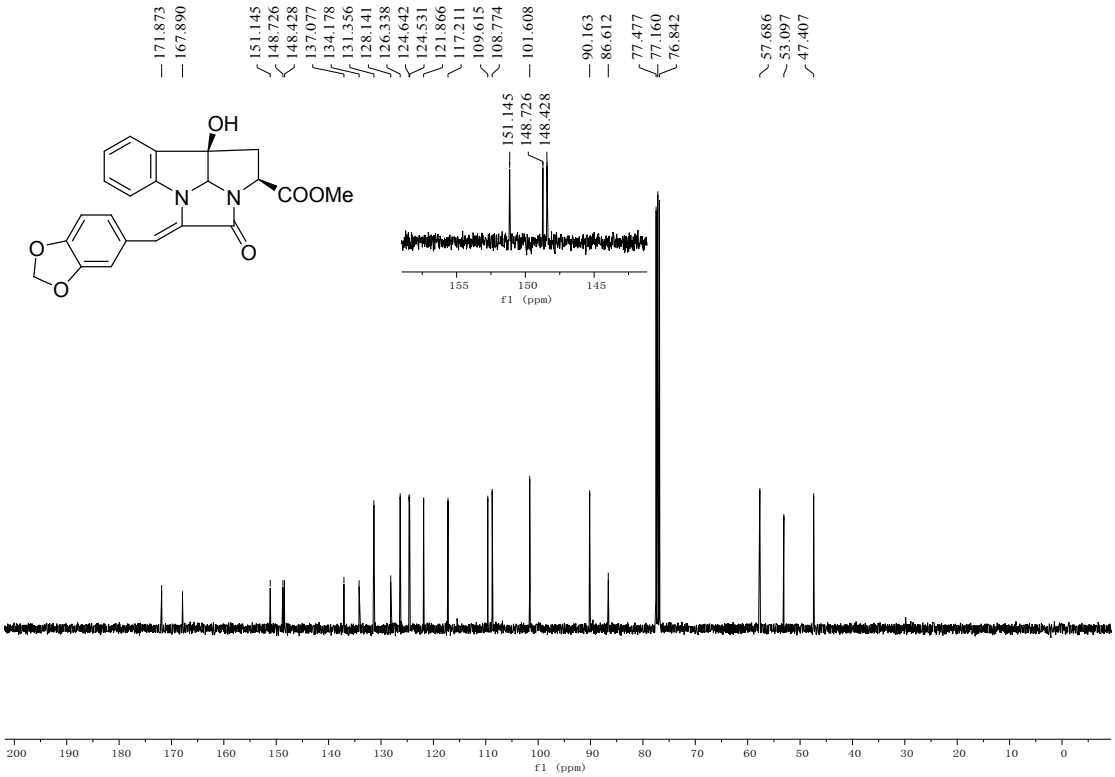
^{1}H NMR spectrum (DMSO-d_6, 400 MHz) of **1**



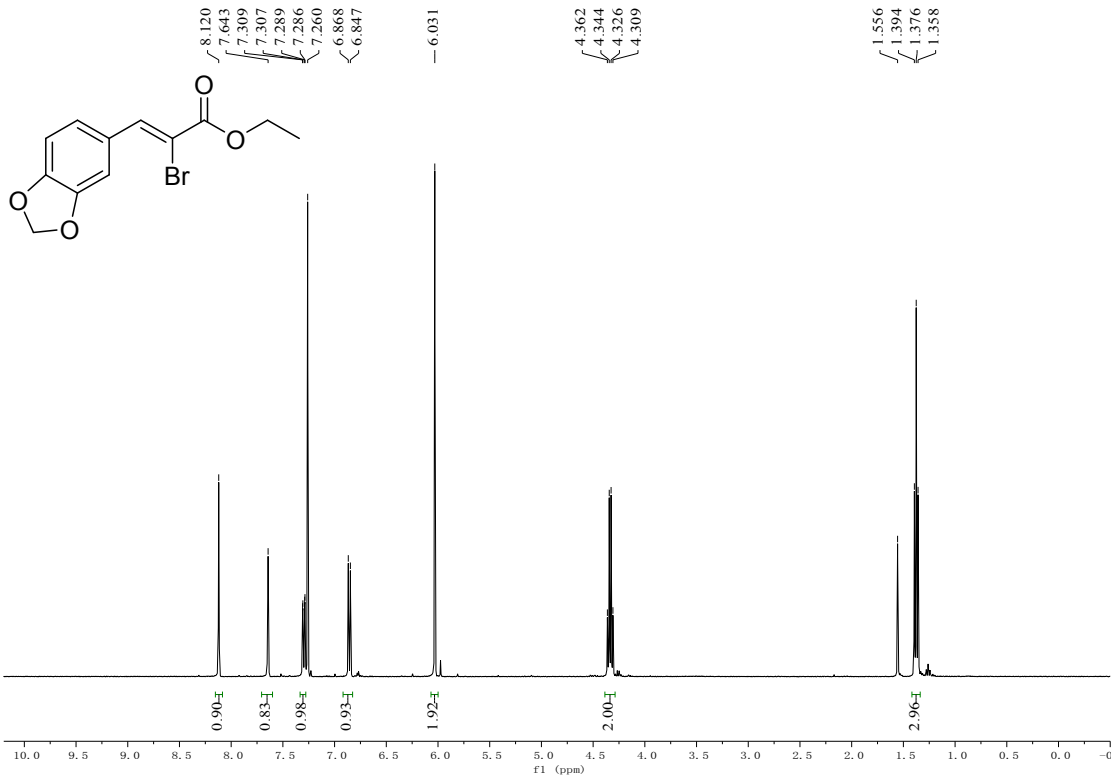
^{13}C NMR spectrum (DMSO-d_6, 100 MHz) of **1**



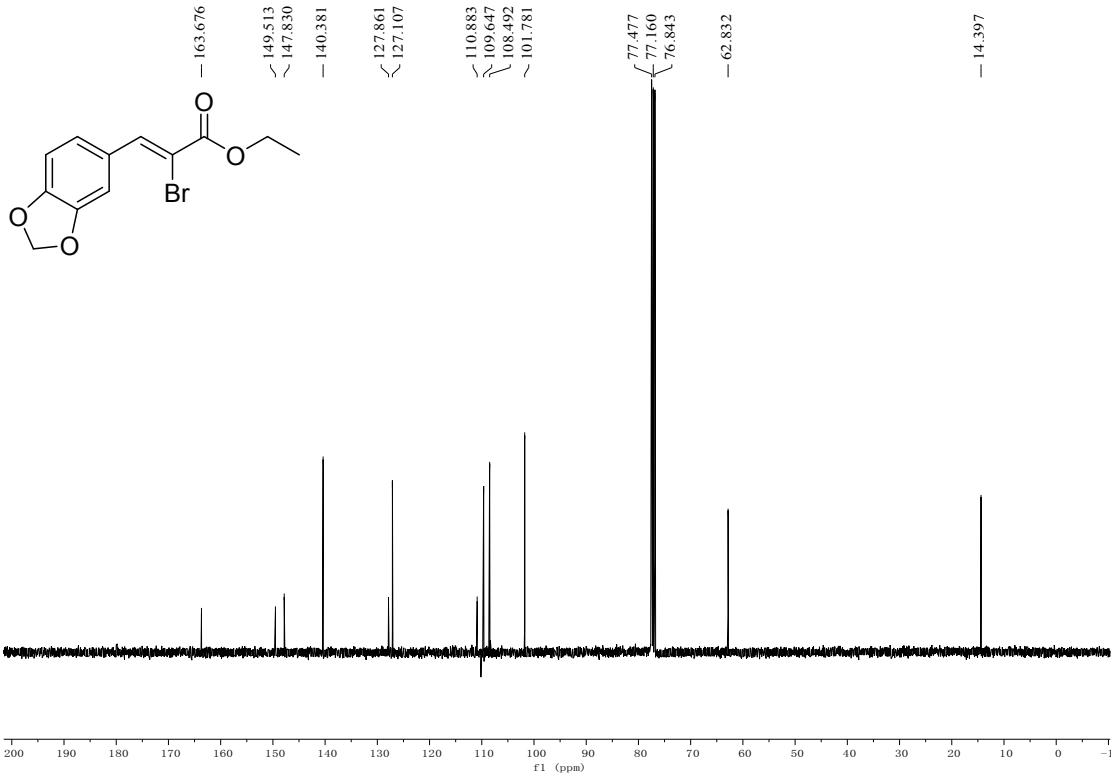
^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of **9**



^{13}C NMR spectrum ($CDCl_3$, 100 MHz) of **9**



^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of **x2**



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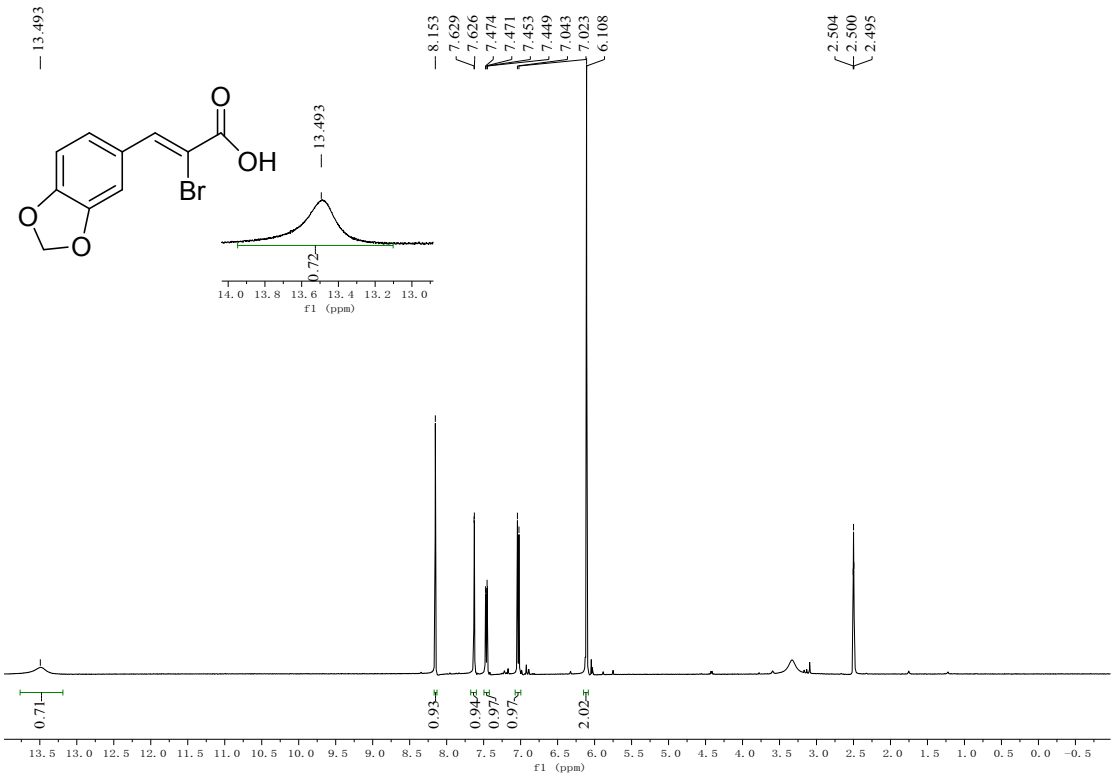
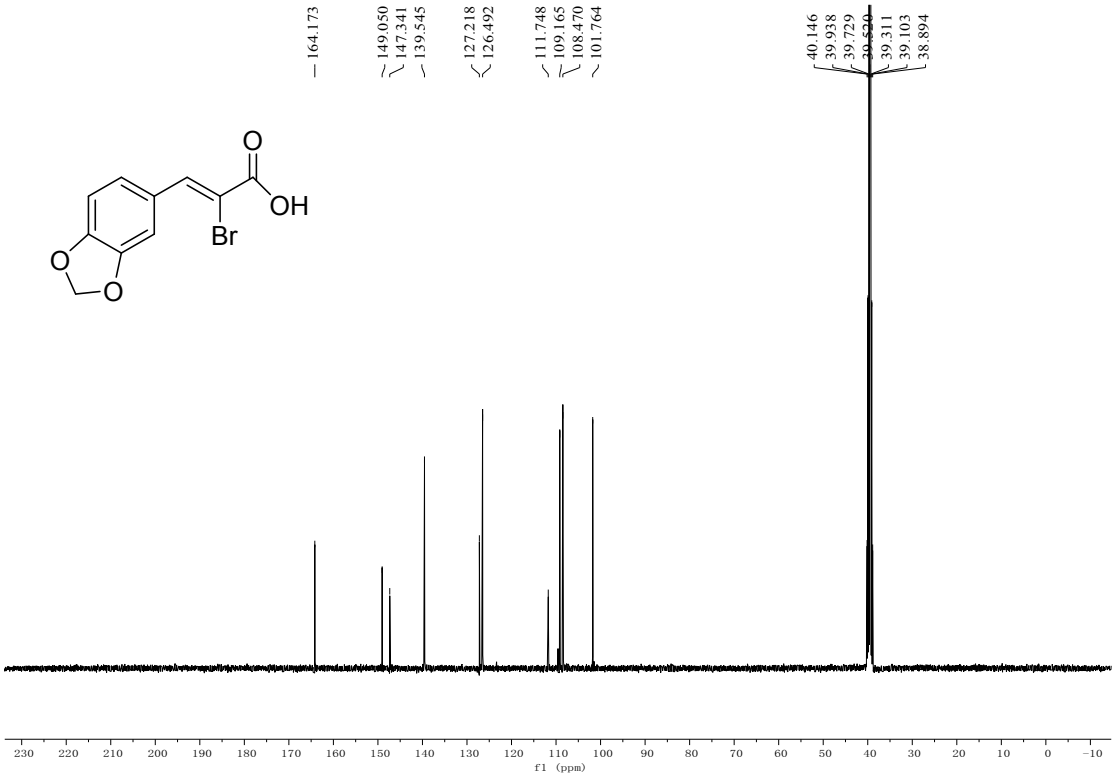


^{1}H NMR spectrum (DMSO-d_6, 400 MHz) of **x3**



^{13}C NMR spectrum (DMSO-d_6, 100 MHz) of **x3**

X-Ray crystal structure (ORTEP) of compound **9** with the thermal ellipsoids shown at a 50% probability level (The crystals of compound **9** suitable for X-ray diffraction were grown by slow evaporation of its CH_2Cl_2/MeOH solution)

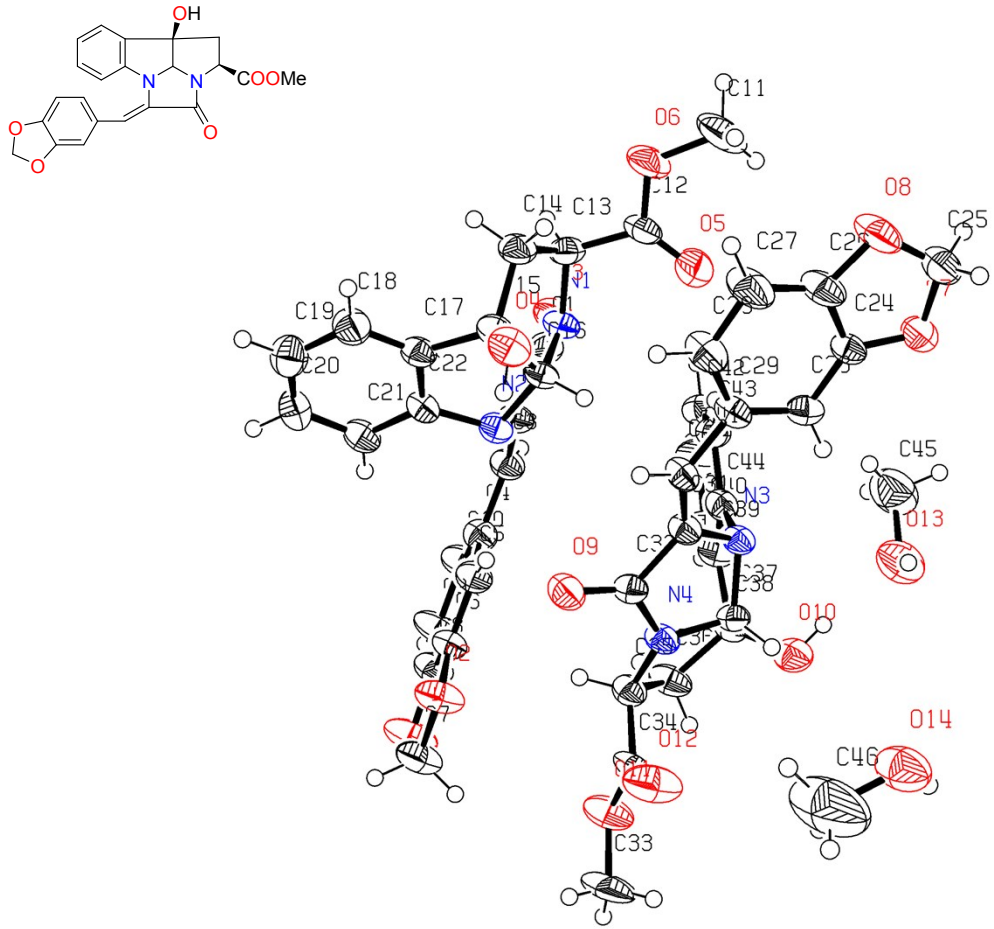


Table S1. Bond Angles for compound 9

Identification code	**9**
Empirical formula	$C_{23}H_{22}N_2O_7$
Formula weight	438.43
Temperature/K	293(2)
Crystal system	triclinic
Space group	P1
a/Å	9.297(7)
b/Å	11.515(9)
c/Å	11.957(9)
α/°	61.974(7)
β/°	80.333(11)
γ/°	67.602(10)
Volume/Å^3	1044.6(14)
Z	2
ρ_{calc}g/cm^3	1.394
μ/mm^{-1}	0.104
F(000)	460.0
Crystal size/mm^3	0.210 × 0.200 × 0.190
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	4.74 to 50.018
Index ranges	$-11 \le h \le 9$, $-13 \le k \le 7$, $-14 \le l \le 14$
Reflections collected	5277
Independent reflections	4450 [R_{int} = 0.0400, R_{sigma} = 0.0910]
Data/restraints/parameters	4450/3/586
Goodness-of-fit on F^2	1.028
Final R indexes [I>=2σ (I)]	R_1 = 0.0699, wR_2 = 0.1773
Final R indexes [all data]	R_1 = 0.0863, wR_2 = 0.2000
Largest diff. peak/hole / e Å^{-3}	0.40/-0.28
Flack parameter	-0.3(10)

Table S2. Bond Lengths for compound 9

Atom	Atom	Length/Å	Atom	Atom	Length/Å
O1	C8	1.369(5)	C4	C5	1.395(7)
O1	C7	1.410(8)	C5	C6	1.376(5)
O2	C6	1.357(6)	C6	C8	1.373(7)
O2	C7	1.431(6)	C8	C9	1.346(8)

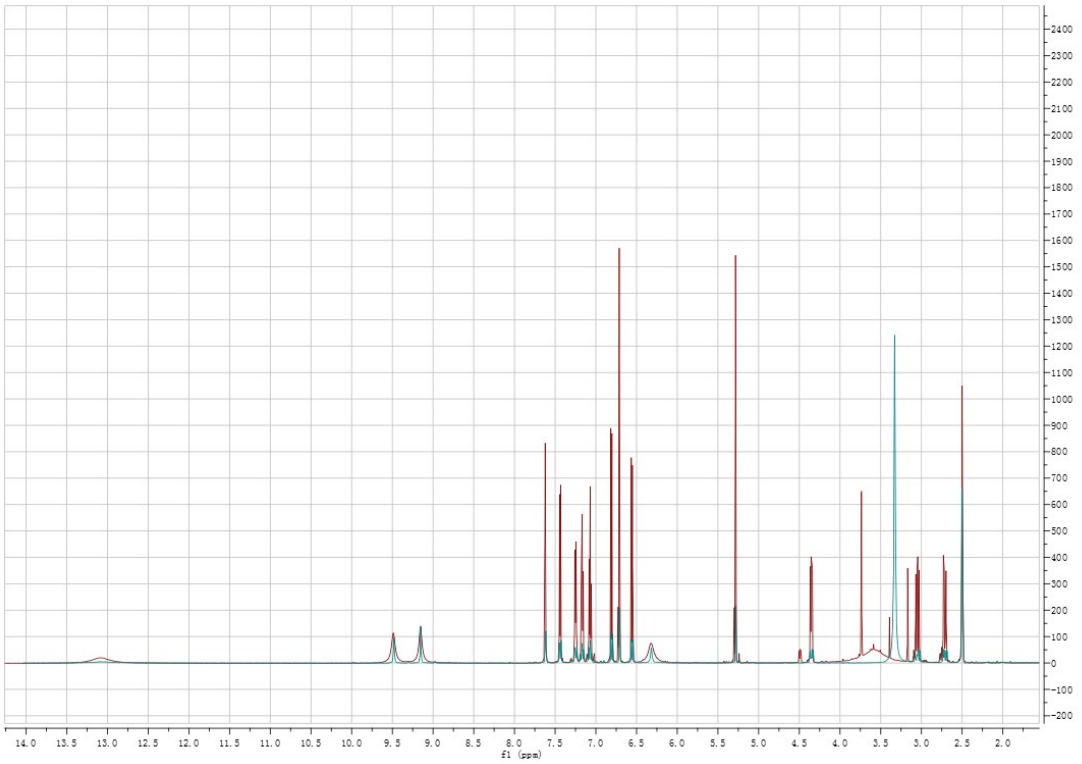
O3	C1	1.222(6)	C9	C10	1.406(6)
O4	C15	1.397(6)	C12	C13	1.486(6)
O5	C12	1.198(6)	C13	C14	1.568(7)
O6	C12	1.314(5)	C14	C15	1.558(5)
O6	C11	1.455(7)	C15	C17	1.519(6)
O7	C24	1.362(5)	C15	C16	1.540(6)
O7	C25	1.436(6)	C17	C18	1.357(6)
O8	C26	1.367(5)	C17	C22	1.406(6)
O8	C25	1.371(6)	C18	C19	1.371(7)
O9	C32	1.243(5)	C19	C20	1.371(7)
O10	C38	1.428(5)	C20	C21	1.398(6)
O11	C34	1.316(5)	C21	C22	1.376(6)
O11	C33	1.437(7)	C23	C24	1.370(5)
O12	C34	1.203(8)	C23	C29	1.385(6)
O13	C45	1.414(8)	C24	C26	1.375(6)
O14	C46	1.368(10)	C26	C27	1.384(8)
N1	C1	1.358(6)	C27	C28	1.389(6)
N1	C13	1.443(5)	C28	C29	1.379(6)
N1	C16	1.444(6)	C29	C30	1.461(5)
N2	C22	1.439(5)	C30	C31	1.330(5)
N2	C2	1.449(6)	C31	C32	1.478(5)
N2	C16	1.484(5)	C34	C35	1.508(6)
N3	C44	1.429(6)	C35	C36	1.538(7)
N3	C31	1.447(5)	C36	C38	1.551(6)
N3	C37	1.479(4)	C37	C38	1.526(7)
N4	C32	1.340(5)	C38	C39	1.507(6)
N4	C37	1.442(5)	C39	C44	1.373(6)
N4	C35	1.442(5)	C39	C40	1.398(8)
C1	C2	1.474(5)	C40	C41	1.394(6)
C2	C3	1.332(6)	C41	C42	1.369(7)
C3	C4	1.458(5)	C42	C43	1.397(8)
C4	C10	1.391(7)	C43	C44	1.389(5)

Table S3. Bond Angles for compound 9

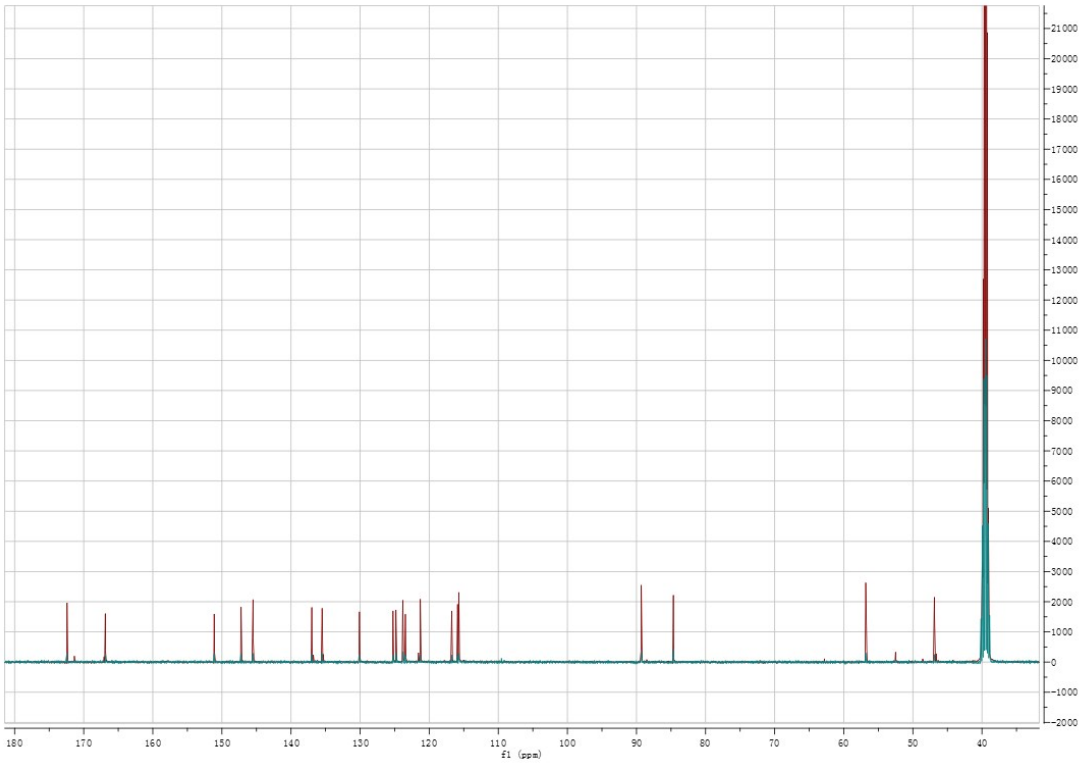
Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C8	O1	C7	106.0(4)	C18	C17	C15	129.6(4)
C6	O2	C7	105.6(4)	C22	C17	C15	110.3(4)
C12	O6	C11	117.6(4)	C17	C18	C19	120.2(4)
C24	O7	C25	105.5(3)	C20	C19	C18	119.9(5)

C26	O8	C25	106.1(3)	C19	C20	C21	121.7(4)
C34	O11	C33	115.3(5)	C22	C21	C20	117.4(4)
C1	N1	C13	128.6(4)	C21	C22	C17	120.7(4)
C1	N1	C16	113.5(3)	C21	C22	N2	127.0(4)
C13	N1	C16	112.9(4)	C17	C22	N2	112.3(4)
C22	N2	C2	114.9(4)	C24	C23	C29	117.8(4)
C22	N2	C16	104.8(3)	O7	C24	C23	129.3(4)
C2	N2	C16	104.3(3)	O7	C24	C26	108.5(3)
C44	N3	C31	114.4(3)	C23	C24	C26	122.1(4)
C44	N3	C37	104.1(3)	O8	C25	O7	108.2(4)
C31	N3	C37	104.1(3)	O8	C26	C24	110.0(4)
C32	N4	C37	112.6(3)	O8	C26	C27	129.0(4)
C32	N4	C35	129.6(4)	C24	C26	C27	121.0(4)
C37	N4	C35	112.9(3)	C26	C27	C28	116.6(4)
O3	C1	N1	126.0(3)	C29	C28	C27	122.4(5)
O3	C1	C2	129.0(4)	C28	C29	C23	120.0(4)
N1	C1	C2	104.9(4)	C28	C29	C30	117.0(4)
C3	C2	N2	126.7(3)	C23	C29	C30	123.0(4)
C3	C2	C1	125.2(4)	C31	C30	C29	131.4(4)
N2	C2	C1	108.1(4)	C30	C31	N3	127.6(3)
C2	C3	C4	131.4(5)	C30	C31	C32	125.3(4)
C10	C4	C5	120.2(4)	N3	C31	C32	107.1(3)
C10	C4	C3	117.6(5)	O9	C32	N4	125.5(3)
C5	C4	C3	122.2(4)	O9	C32	C31	128.3(3)
C6	C5	C4	116.7(4)	N4	C32	C31	106.2(3)
O2	C6	C8	110.2(3)	O12	C34	O11	123.3(4)
O2	C6	C5	127.3(5)	O12	C34	C35	125.4(4)
C8	C6	C5	122.4(5)	O11	C34	C35	111.3(5)
O1	C7	O2	108.1(4)	N4	C35	C34	111.0(4)
C9	C8	O1	128.1(5)	N4	C35	C36	104.1(3)
C9	C8	C6	122.3(4)	C34	C35	C36	114.1(4)
O1	C8	C6	109.5(5)	C35	C36	C38	106.8(4)
C8	C9	C10	116.7(5)	N4	C37	N3	104.4(3)
C4	C10	C9	121.6(5)	N4	C37	C38	104.1(3)
O5	C12	O6	122.6(4)	N3	C37	C38	110.3(4)
O5	C12	C13	125.1(4)	O10	C38	C39	113.3(4)
O6	C12	C13	112.3(4)	O10	C38	C37	113.6(4)
N1	C13	C12	112.0(3)	C39	C38	C37	100.0(3)
N1	C13	C14	103.3(3)	O10	C38	C36	108.2(3)
C12	C13	C14	112.9(4)	C39	C38	C36	116.5(4)

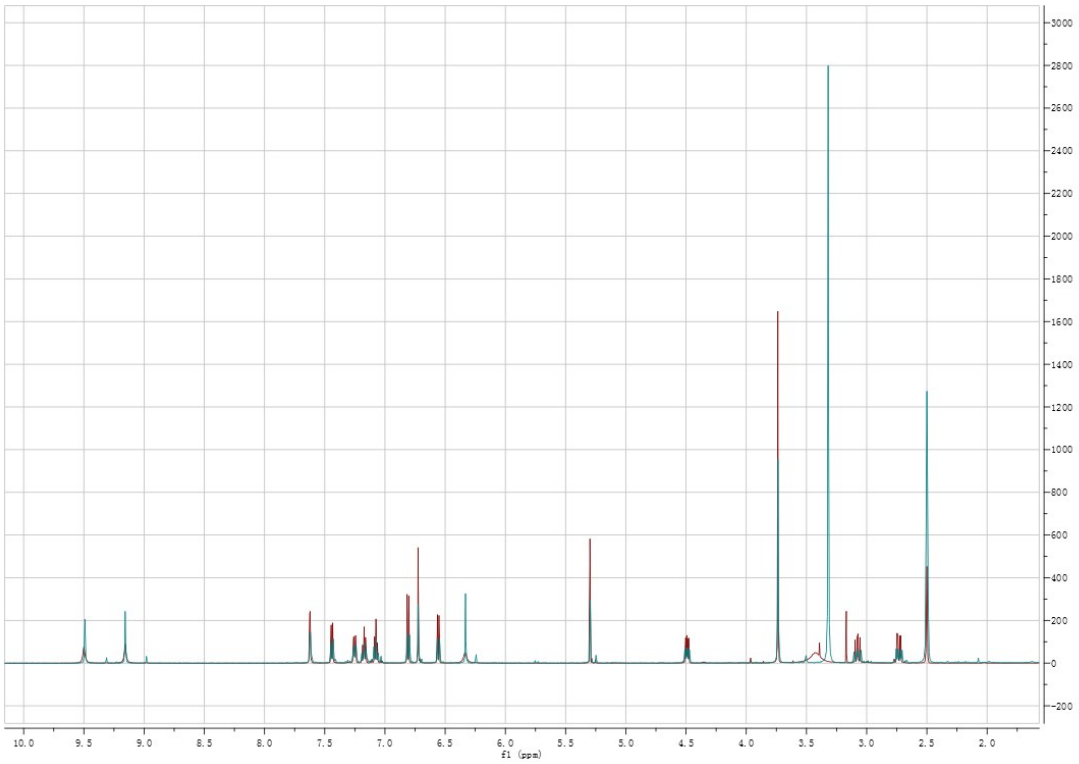
C15	C14	C13	107.1(3)	C37	C38	C36	104.9(4)
O4	C15	C17	113.0(4)	C44	C39	C40	120.2(4)
O4	C15	C16	113.8(4)	C44	C39	C38	111.5(4)
C17	C15	C16	101.0(3)	C40	C39	C38	128.1(4)
O4	C15	C14	108.7(4)	C41	C40	C39	117.5(5)
C17	C15	C14	115.7(4)	C42	C41	C40	121.9(6)
C16	C15	C14	104.2(3)	C41	C42	C43	120.6(4)
N1	C16	N2	103.8(4)	C44	C43	C42	117.4(4)
N1	C16	C15	105.2(3)	C39	C44	C43	122.3(5)
N2	C16	C15	110.1(3)	C39	C44	N3	112.2(3)
C18	C17	C22	120.1(4)	C43	C44	N3	125.5(4)



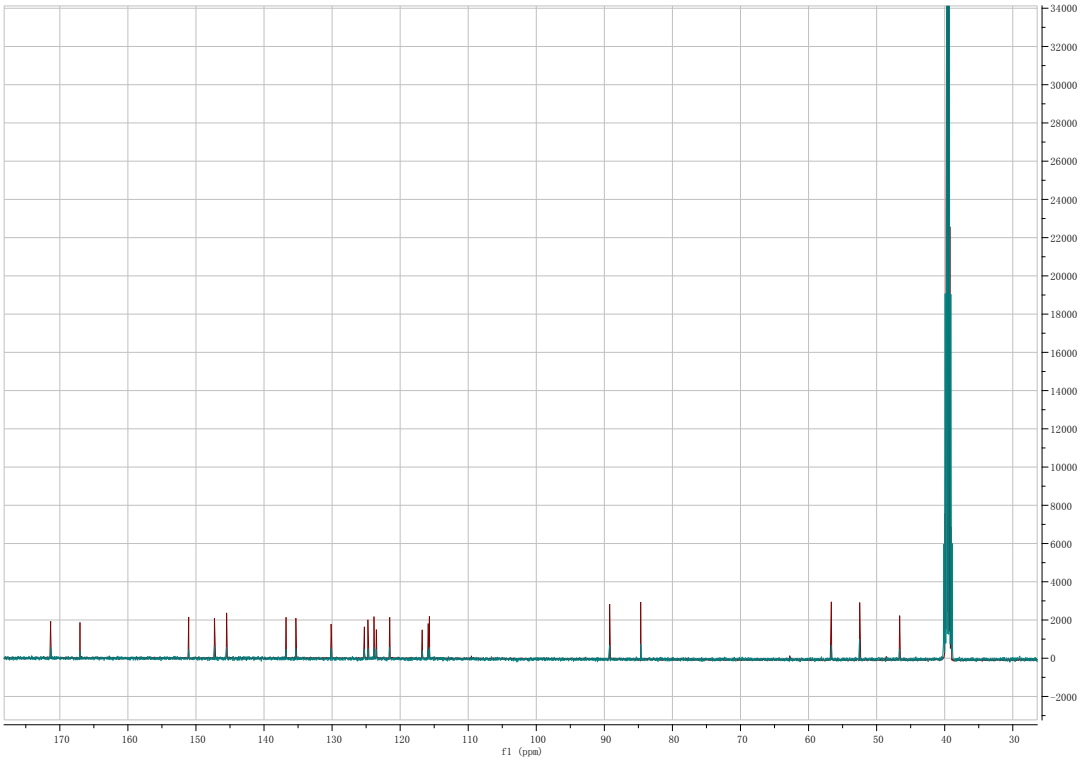
^{1}H NMR spectra of **1** (natural and synthetic products).



^{13}C NMR spectra of **1** (natural and synthetic products).



^{1}H NMR spectra of **2** (natural and synthetic products).



^{13}C NMR spectra of **2** (natural and synthetic products).