A new C_{19}-diterpenoid alkaloid in *Aconitum georgei* Comber

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Abstract: Nine diterpenoid alkaloids were isolated from *Aconitum georgei* Comber belonging to the genus *Aconitum* in Ranunculaceae family. Their structures were determinated by using HR-ESI-MS and 1D/2D NMR spectra as geordine (**1**), yunaconitine (**2**), chasmanine (**3**), crassicauline A (**4**), forestine (**5**), pseudaconine (**6**), 14-acetylalatisamine (**7**), austroconitine B (**8**), and talatisamine (**9**). Among them, compound **1** is a previously undescribed aconitine-type C_{19}-diterpenoid alkaloid, and compounds **3**, and **5**-**9** have not previously been isolated from this species. The results of *in vitro* experiments indicated that new compound **1** possesses mild anti-inflammatory activity, which inhibited the production of NO in LPS-activated RAW 264.7 cells with an inhibition ratio of 29.75% at 50 μM.

Keywords: *Aconitum georgei* Comber; Ranunculaceae; diterpenoid alkaloid; geordine; anti-inflammatory

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1 Activity Test of *Geordine (1)*

1.1 NO inhibition assay

NO inhibition activity was evaluated according to a referenced method. RAW264.7 cells were inoculated into 96-well plates, stimulated with 1 μg/mL LPS, and treated with the compound under test (final concentration 50 μM). The control group was the nondrug group and the L-NMMA-positive drug group. After overnight cell culture, NO production was detected in the medium, and light absorption was measured at 570 nm. MTS was added to the remaining medium for the cell viability test to exclude the toxic effects of the compounds on the cells.

NO Generate the inhibition rate (%) = (nondrug treatment group $OD_{570\ nm}$- Sample group $OD_{570\ nm}$) / nondrug treatment group $OD_{570\ nm}$ × 100%)

1.2 Anti-proliferation assay

The following human tumor cell lines were used: the HL-60 human myeloid leukemia cell line, the SMMC-7721 human hepatocarcinoma cell line, the A549 lung cancer cell line, the MDA-MB-231 breast cancer cell line, and the SW-480 human pancreatic carcinoma. Ten percent fetal bovine serum was used as culture medium (DMEM or RMPI1640). Cells were seeded in 96-well plates at a density of 3000~15000 cells per well in 100 μL of complete culture medium. After cell attachment for 12~24 hours. The compounds were dissolved in DMSO and screened at a concentration of 40 μM. The final volume of each hole was 200 μL. After incubation at 37 °C for 48 hours, 20 μL MTS solution and 100 μL culture medium were added. After incubating for 2 ~ 4

hours, the light absorption value (λ = 492 nm) was read with a multifunctional enzyme marker (kan multifc). Two positive compounds, cisplatin (DDP) and Taxol (Taxol), were selected in each experiment [cisplatin (0.06, 0.32, 1.6, 8.0, and 40.0 μM) and Taxol (0.008, 0.04, 0.2, 1.0 and 5.0 μM)]. Three duplicate wells were used for each concentration, and all the tests were repeated three times. The cell growth curve was drawn with the concentration as the horizontal coordinate and the cell survival rate as the vertical coordinate, two-point method (Reed and Muench method) was used to calculate the IC_{50} value of the compound.

1.3 antimicrobial activity test

The main purpose of this experiment is to detect the *Candida albicans* (*C.albicans*), *Bacillus subtilis* (*B.subtilis*), *Escherichia coli* (*E.coli*) and *Staphylococcus aureus* (*S.aureus*). In the experiment, PDA, PDB and LB media were used to activate strains, fungi and bacteria respectively. The results were expressed as minimum inhibitory concentration (MIC). After making PDA and PDB media, different fungi or bacteria mother liquor were inoculated into PDB media or LB media respectively according to their own characteristics. The fungi were cultured at 28℃ for 18 hours, the bacteria were incubated at 37℃ for 3 days. The substance in question was dissolved in DMSO and prepared into 10.24 mg/mL sample solution. Nystatin was used as the control substance for fungus and kanamycins as the control substance for bacteria, 2.56 mg/mL solution was prepared with DMSO solution, and 200 μL solution was put into corresponding culture medium and diluted to 1 mL. The 5μL sample and the

control were added to the two holes in the first row of the 96-well plate, and then diluted to 90μg with the corresponding medium solution, the concentration of sample solution in each hole was 512, 256, 128, 64, 32, 8, 4, 2, 1 μg/mL, and then 10μL diluted solution was added to each hole to make the volume of each hole 100μL. Then the orifice plate was placed in an incubator to observe the growth of the strain and record the minimal inhibitory concentration (MIC) of the strain.

1.4 detection of anti-acetylcholine activity

Acetycholinesterase (AChE) converts Acetylcholine iodide (ATCI) into thiocholine. However, DTNB can react specifically with thiocholine to form yellow products. Using this characteristic, the antiacetylcholine activity of the substance can be determined by observing the color of the substance. The experiment measured the absorbance of the yellow substance at 405 nm, calculations based on absorbance determine the degree to which the compound suppresses AChE. The concrete steps of the experiment are: the phosphate buffer solution with pH = 8.0 and concentration of 0.1 mol/L was prepared. The compound was diluted to 1mM with 2% DMSO-water as solvent, and the final concentration of the compound was 50μM after the reaction. The positive control in the experiment was Tacrine, 0.333μM was prepared with 2% DMSO-water solution, while the negative control was DMSO-PB solution with 2% . The positive control group changed the 10μL phosphate buffer to 10μL Tacrine, while the negative control group changed the 10μL phosphate buffer to 10μL 2% DMSO, the blank group changed the 150μL phosphate buffer and 10μL DMSO 2% .

The negative control group changed the 10μL phosphate buffer to 10μL 2% DMSO, the blank group changed the 150μL phosphate buffer and 10μL DMSO 2% . The absorbance of the substances in each group was determined by enzyme-labeled instrument, and the background value was measured twice at 405 nm.

The inhibition rate T% = $[(A_E-A_B)- (A_S-A_B)] (A_E-A_B) \times 100\%$ A_E was the negative control group, A_B was the background value and A_S was the sample group.

The results showed that the MIC values of compound **1** were higher than 512, which indicated that compound **1** had no antibacterial activity. The anti-acetylcholine activity (6.50%) is less than 10% and has no inhibitory effect, and its activity is much lower than Tacrine (48.02%).

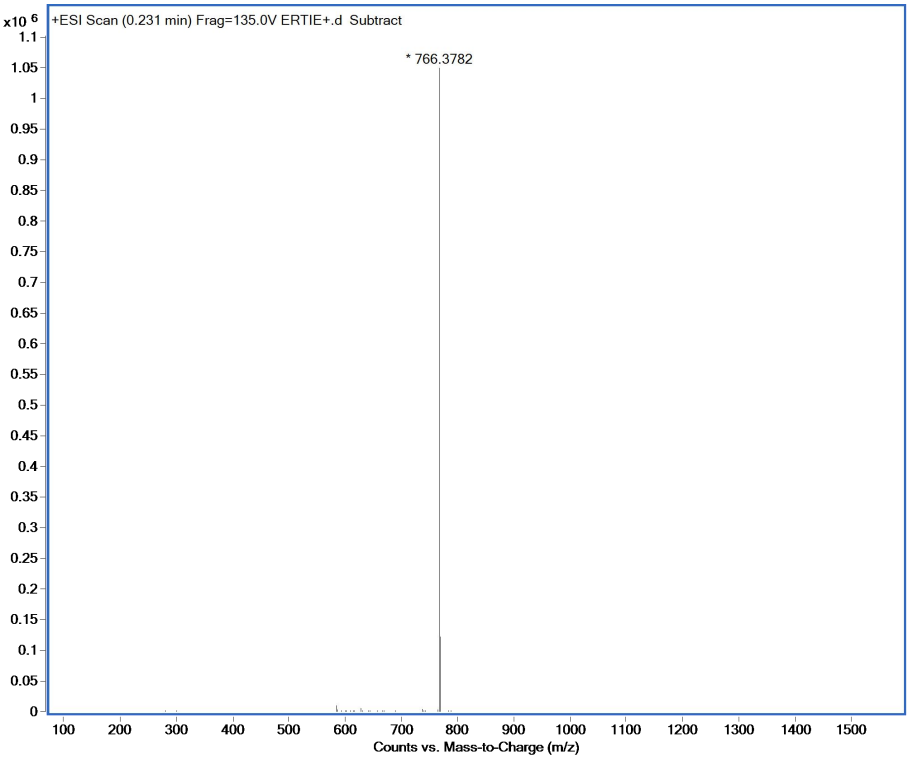

Figure S1 MS spectrum of *Geordine (1)*

Table S1 Rudolph Vplus automatic polarimeter measure condition

鲁道夫 Autopol Vplus 型旋光仪				
Set Temp	Text Temp	T Corr	WLG	Resp
22.0℃	22.1℃	OFF	589nm	2 Sec
Delay	Stable	Cell	Conc./Density	Measure
10 Sec	±0.3℃	50.00mm	0.270g/100ml	Multiple

Table S2 Rudolph Vplus automatic polarimeter measure result

n	Average	Std.Dev	Max	Min				
3.000	50.403	0.2645	50.590	50.029				
S.NO	Time	Result	Scale	OR Arc	WLG.nm	Lg.mm	Conc.g/100ml	Temp
1	8:04:26	50.029	SR	0.068	589	50.00	0.270	22.2
2	8:04:33	50.590	SR	0.068	589	50.00	0.270	22.2
3	8:04:40	50.590	SR	0.068	589	50.00	0.270	22.2
n	Average	Std.Dev	Max	Min				
3.000	50.597	0.4617	51.163	50.032				
S.NO	Time	Result	Scale	OR Arc	WLG.nm	Lg.mm	Conc.g/100ml	Temp
1	8:05:08	50.032	SR	0.068	589	50.00	0.270	22.1
2	8:05:15	50.597	SR	0.068	589	50.00	0.270	22.1
3	8:05:22	51.163	SR	0.068	589	50.00	0.270	22.1

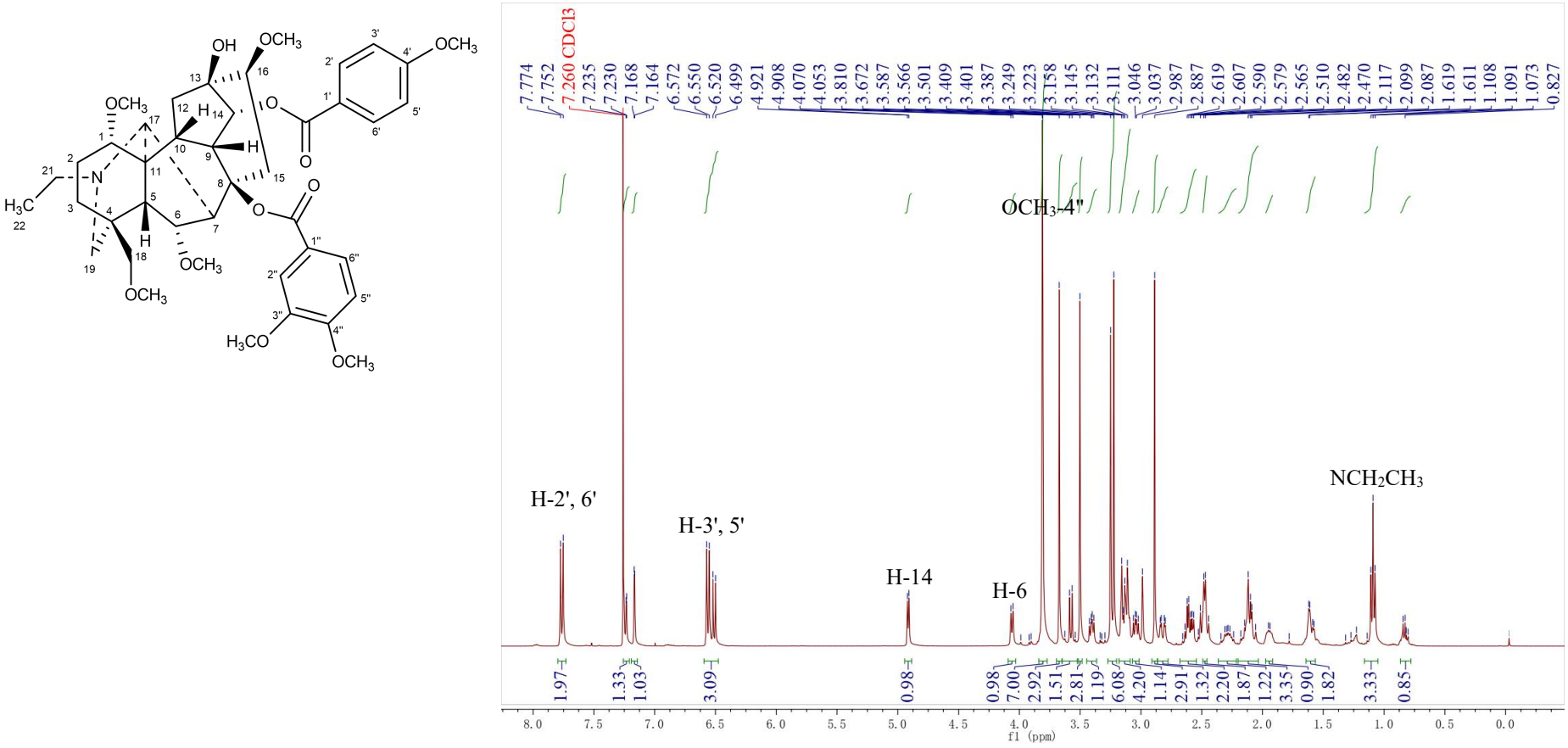

Figure S2 ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of *Geordine (1)*

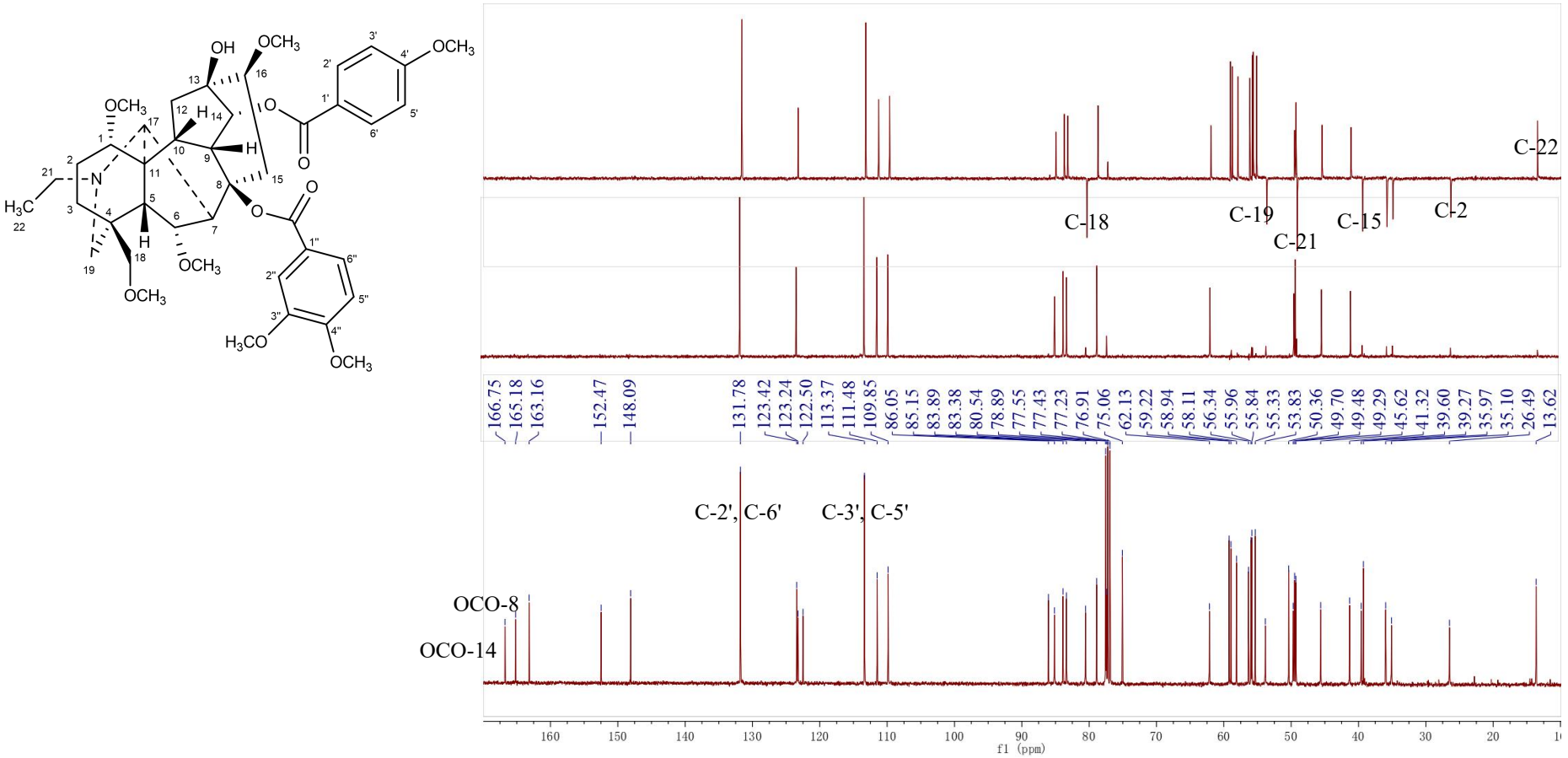

Figure S3 ^{13}C NMR spectrum ($CDCl_3$, 400 MHz) of *Geordine (1)*

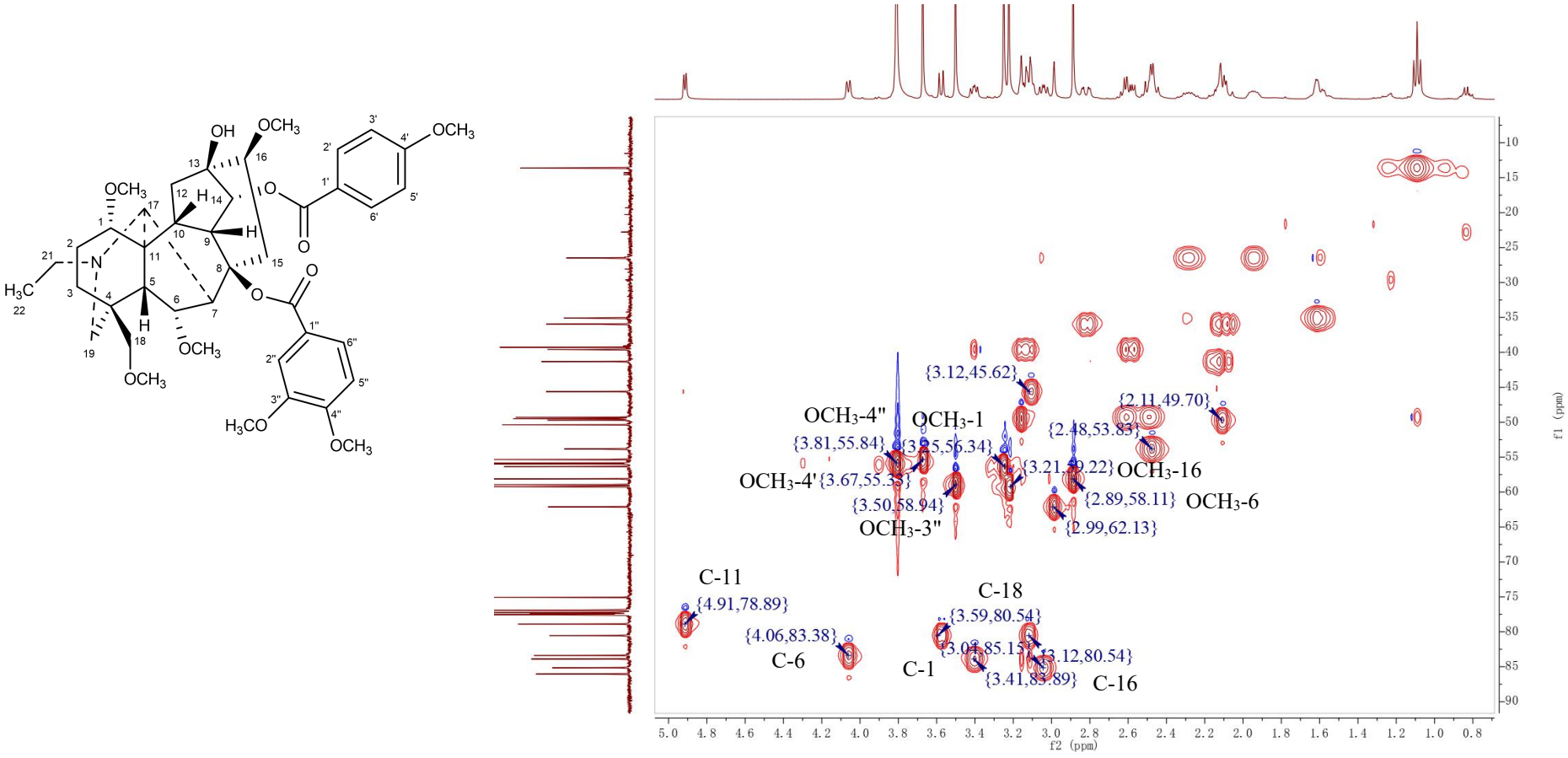

Figure S4 HSQC spectrum ($CDCl_3$, 400 MHz) of *Geordine (1)*

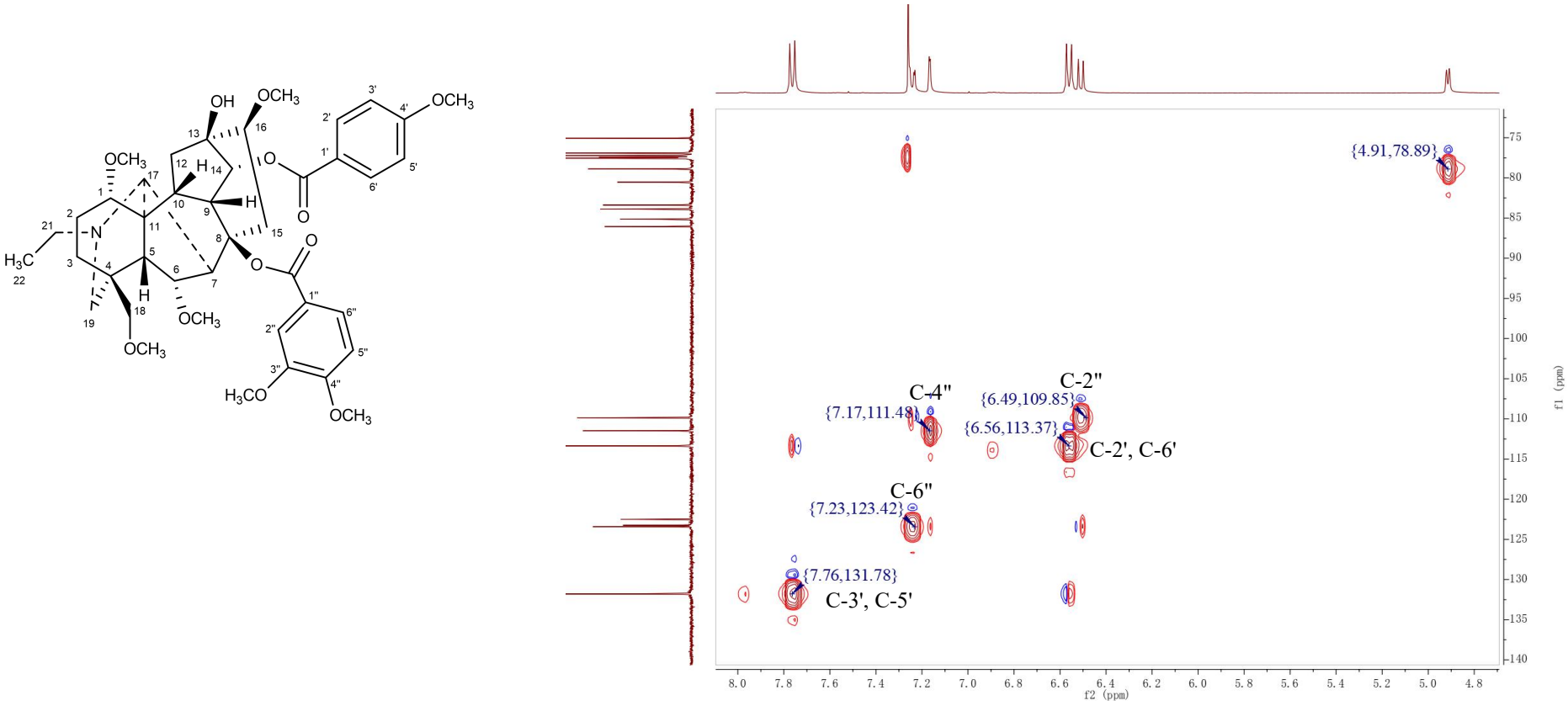

Figure S5 HSQC spectrum ($CDCl_3$, 400 MHz) of *Geordine (1)*

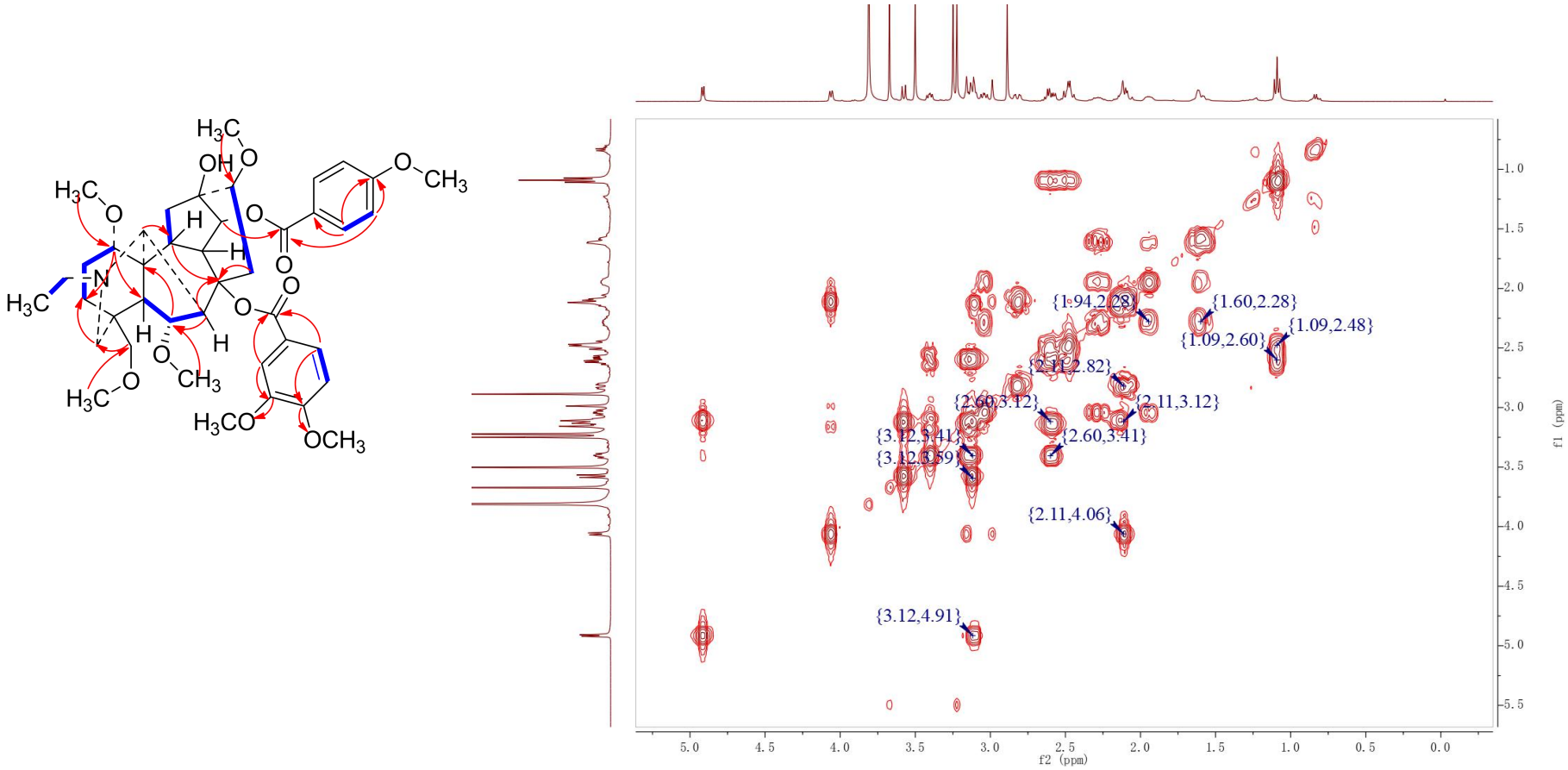

Figure S6 ^{1}H-^{1}H COSY spectrum ($CDCl_3$, 400 MHz) of *Geordine (1)*

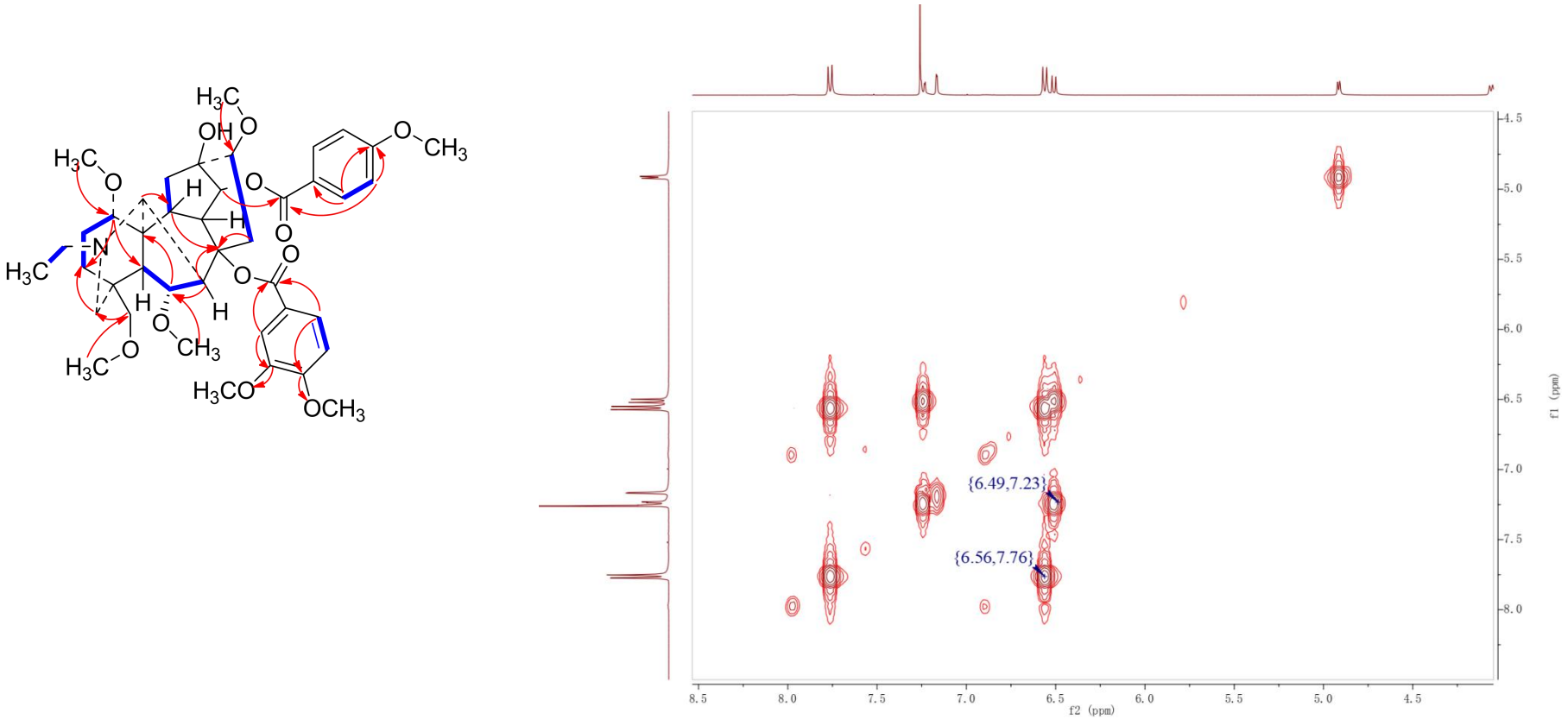

Figure S7 ^{1}H-^{1}H COSY spectrum ($CDCl_3$, 400 MHz) of *Geordine (1)*

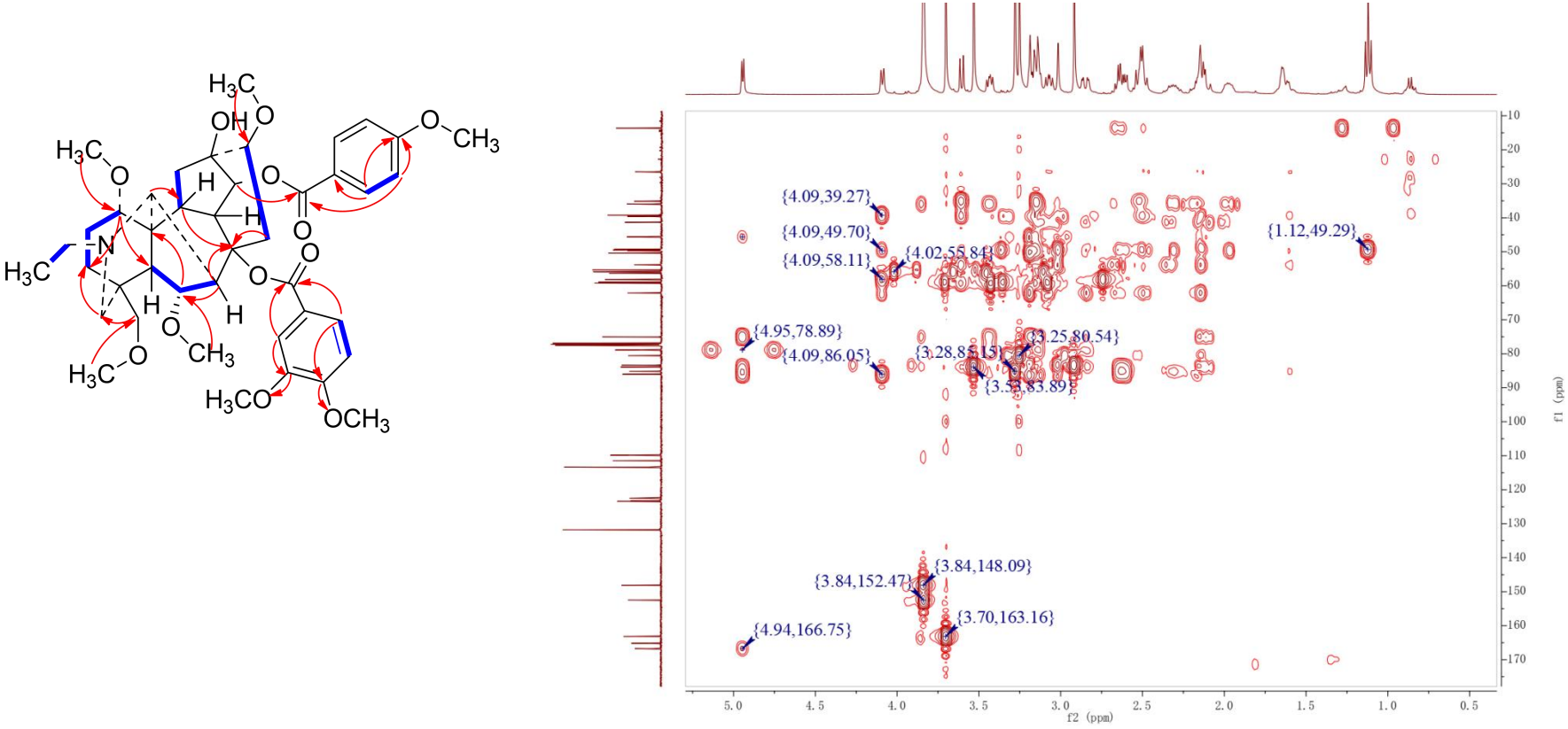

Figure S8 HMBC spectrum ($CDCl_3$, 400 MHz) of *Geordine (1)*

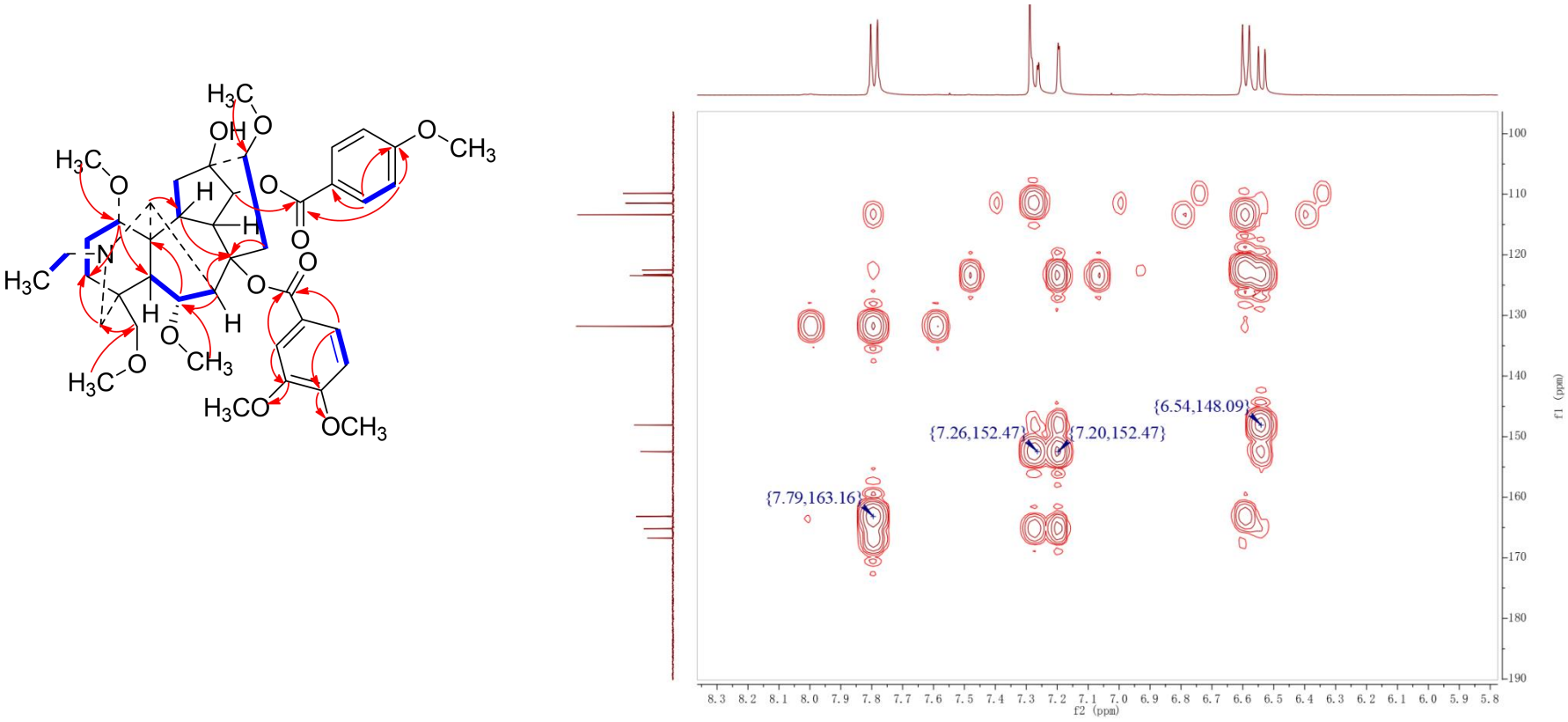

Figure S9 HMBC spectrum ($CDCl_3$, 400 MHz) of *Geordine (1)*

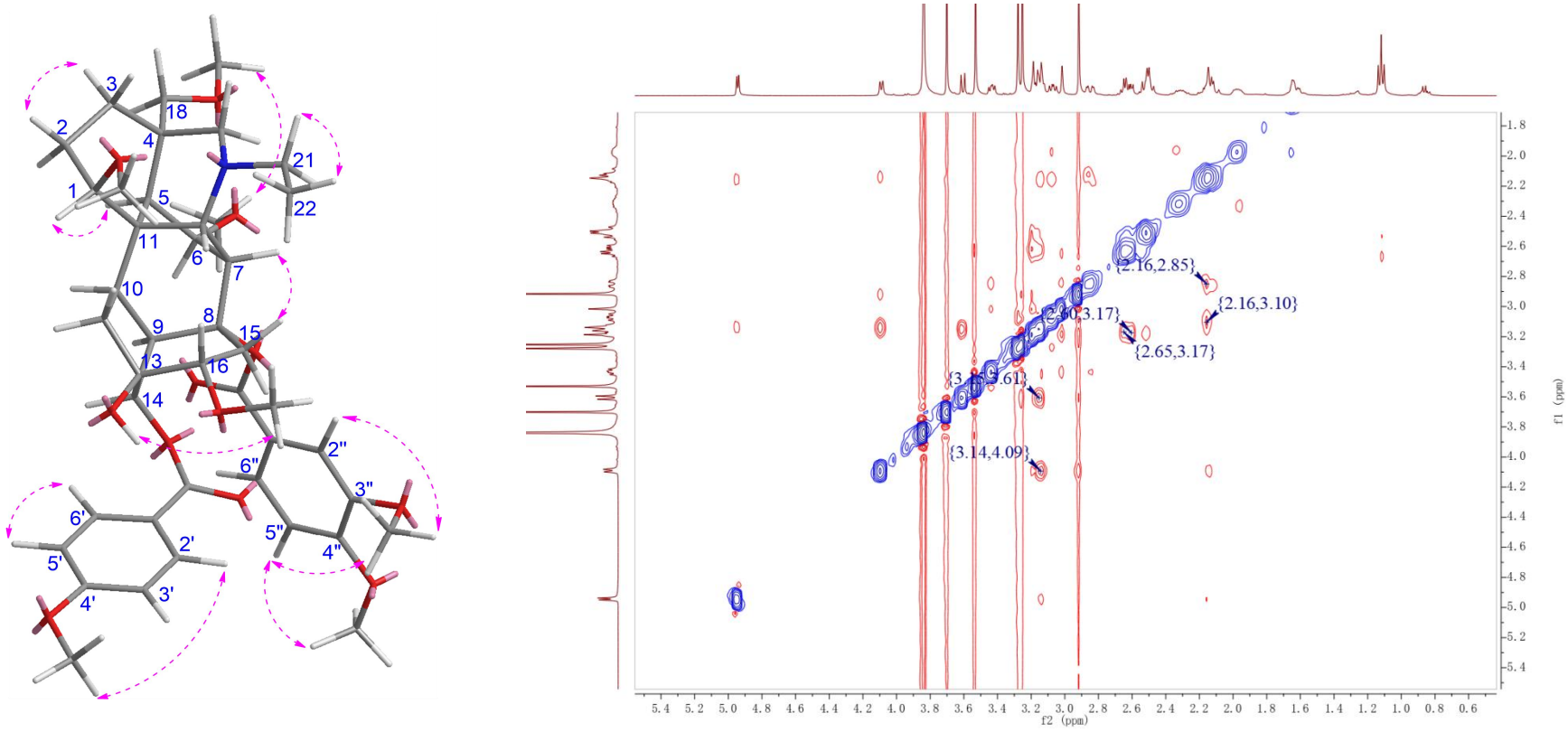

Figure S10 NOESY spectrum ($CDCl_3$, 400 MHz) of *Geordine (1)*

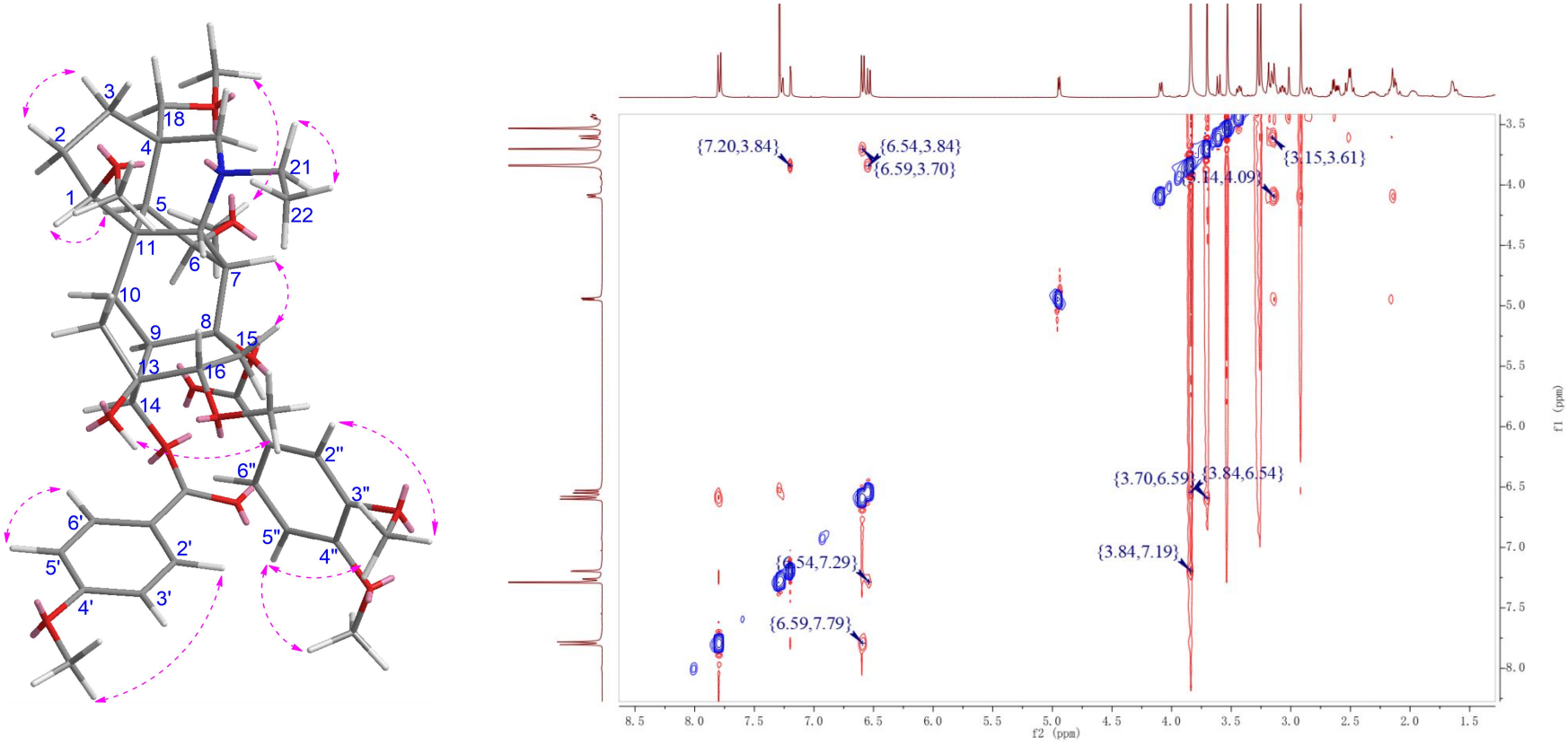

Figure S11 NOESY spectrum ($CDCl_3$, 400 MHz) of *Geordine (1)*

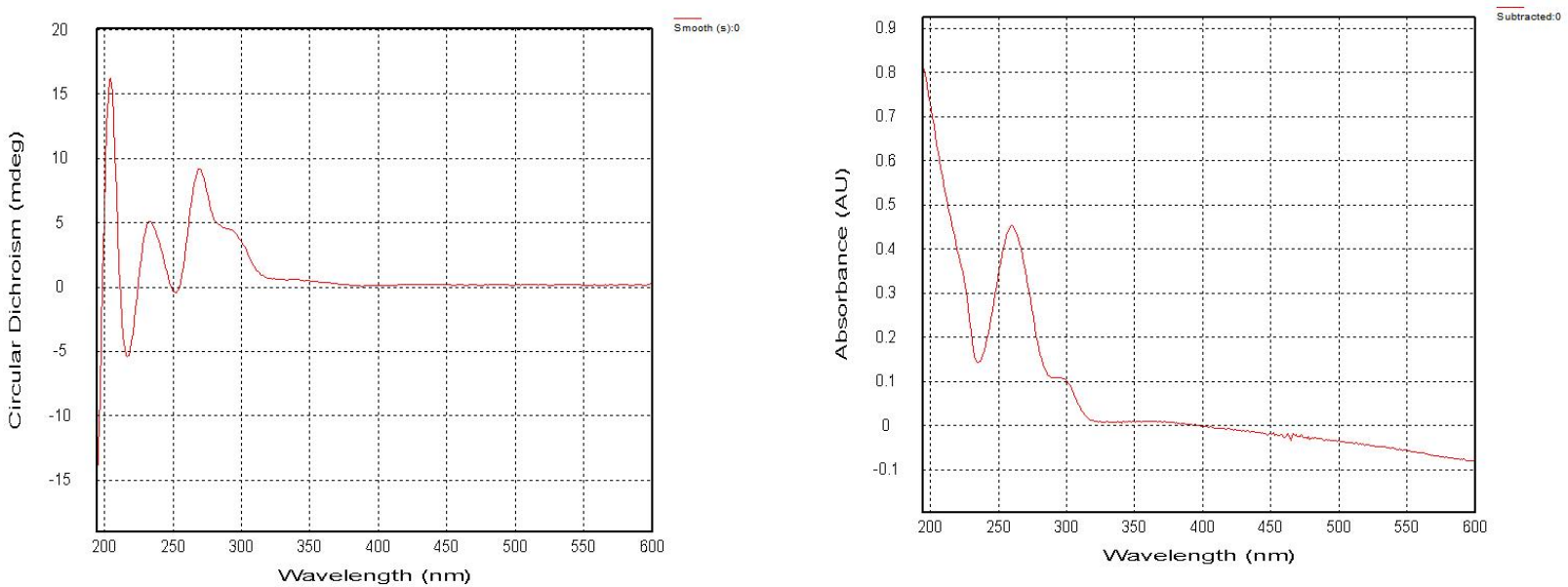

Figure S12 ECD and UV date of *Geordine (1)*

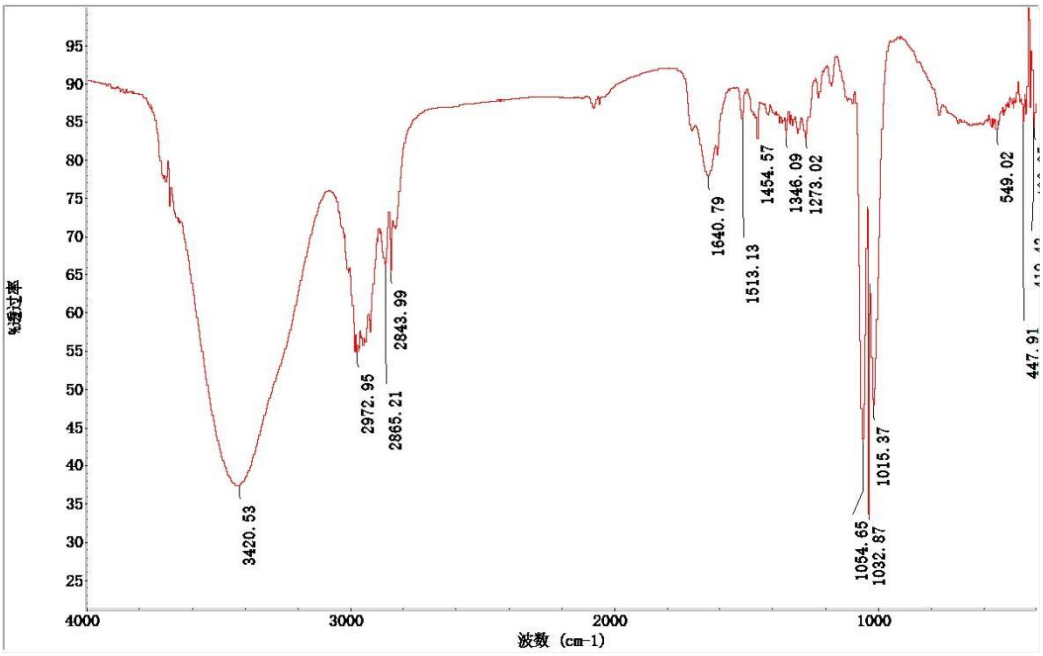

Figure S13 An IR map of *Geordine (1)*

Table S3　Nitric oxide (NO) generation inhibitor screening result table of *Geordine (1)*

样品	浓度（μM）	NO 生成抑制率（%）
L-NMMA	50	57.42±1.41
YD403	50	29.75±1.95