Supporting Information

Novel potential artificial MRSA DNA intercalators: synthesis and biological evaluation of novel berberine-derived thiazolidinediones

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1. Biological assay protocols

1.1 Antibacterial assay

The berberine-derived thiazolidinediones **4**, **6**, **8**, **10**, **12,** and **14** and intermediate **11** were evaluated for their biological activities against five Gram-positive bacteria (Methicillin-resistant *Staphylococcus aureus*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213) and six Gram-negative bacteria (*Klebsiella pneumoniae*, *Escherichia coli*, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa*, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumanii*). The MIC (minimum inhibitory concentration) values were determined by using a protocol according to the Clinical and Laboratory Standard Institute (CLSI) guidelines. The microbial suspension was adjusted with to a concentration of 1×10^5 CFU. Initially, the test compounds were dissolved in DMSO to prepare the stock solutions, then the compounds and reference drugs were prepared in MuellereHinton broth (Guangdong Huaikai Microbial Sci. & Tech Co., Ltd, Guangzhou, Guangdong, China) to obtain the required concentrations. These dilutions were inoculated and incubated at 37 ^{o}C for 24 h.

1.2 Antifungal activity

The newly synthesized compounds **4**, **6**, **8**, **10**, **12,** and **14** and intermediate **11** were evaluated for their antifungal activities against *Candida albicans*, *Candida tropicalis*, *Aspergillus fumigatus*, *Candida albicans* ATCC 90023, *Candida parapsilokis* ATCC 20019. A spore suspension in sterile distilled water was prepared from one day old culture of the fungi growing on Sabouraud Agar (SA) media. The final spore concentration was 1–5 × 10^3 spore mL^{-1}. From the stock solutions of the tested compounds and reference antifungal drug Fluconazole, dilutions in sterile RPM1 1640 medium (Neuronbc Laboraton Technology C1., Ltd, Beijing, China) were made resulting in eleven wanted concentrations of each tested compound. These dilutions were

inoculated and incubated at 35 °C for 24 hours.

Table S1 *In vitro* MIC (μmol/mL) values for intermediate **11** and berberine-derived thiazolidinediones **4**, **6**, **8**, **9**, **10**, **12** and **14** toward fungi[a]

Compds	*C. albicans*	*C. tropicals*	*A. fumigatus*	*C. albicans* 90023	*C. parapsilosis* 22019
4	0.566	0.566	0.566	0.141	0.283
6a	1.065	1.065	1.065	1.065	1.065
6b	1.007	0.503	1.007	0.016	0.008
6c	0.954	0.477	0.477	0.060	0.015
6d	0.907	0.453	0.907	0.907	0.907
6e	0.216	0.432	0.216	0.108	0.216
6f	0.825	0.825	0.825	0.206	0.825
6g	1.031	1.031	1.031	1.031	1.031
6h	1.007	1.007	1.007	1.007	1.007
8	1.039	1.039	1.039	0.065	0.032
10a	0.522	0.522	0.261	0.130	0.016
10b	1.042	0.260	0.521	0.521	1.042
11a	1.250	1.250	1.250	1.250	0.156
11b	0.583	0.583	0.291	0.583	0.146
11c	1.035	1.035	0.518	0.259	0.259
11d	1.066	1.066	1.066	0.133	1.066
12a	0.503	1.007	0.252	0.063	0.031
12b	0.475	0.951	0.951	0.238	0.238
12c	0.057	0.057	0.913	0.457	0.228
12d	0.442	0.883	0.221	0.110	0.221
14	0.054	0.108	0.862	0.862	0.862
Berberine	0.344	0.344	0.689	0.172	0.172
Fluconazole	0.209	0.836	1.672	0.007	0.013

[a] *C. albicans*, *Candida albicans*; *C. albicans* 90023, *Candida albicans* ATCC 90023; *C. tropicalis*, *Candida tropicalis*; *A. fumigatus*, *Aspergillus fumigatus*; *C. parapsilokis* 20019, *Candida parapsilokis* ATCC 20019.

1.3 Cytotoxicity assay

The cytotoxicity of compound **6b** against hepatocyte LO2 cells was evaluated by the standard CCK-8 assay. Cells were seeded in a 96-well plate at a density of about 5×10^4 cells per well and cultured in 100 μL of cell suspension in an incubator at 37 °C under 5% CO_2 atmosphere for 24 h. Samples of different concentrations were then added into different wells and incubated with cells for another 48 h. Afterwards, 10 μL of the CCK stock solution was added to each well. After incubation for another 4 h, the optical density (OD) at 570 nm of each well was measured on a microplate reader (Bio-Rad 680). The absorbance values were normalized to wells in which cells were not treated with compounds **6b**. Data were presented as the average values with standard deviations.

1.4 Resistance study

We selected representative compound **6b** to investigate the developing rate of bacterial resistance according to the reported method. The resistant strain of MRSA was exposed to sub-MIC of compound **6b** for sustained passages and the new values of compound **6b** for each passage of MRSA were determined. The bacteria from sub-MIC concentration of the compound (0.5×MIC) was diluted for the next MIC experiment. And after 12 h incubation period, the bacteria from sub-MIC concentration of the compound (0.5×MIC) was diluted for the next MIC experiment. This process was repeated for 15 passages. The new MIC values of compound **6b** against each

passage of MRSA were determined.

1.5 Bacterial membrane permeabilization

The grown culture of MRSA was harvested (4000 rpm, 6 min), washed, and resuspended in 5 mM glucose and 5 mM HEPES buffer (pH 7.4) in 1:1 ratio. Then an amount of 10 of the tested compound **6b** (12 × M1C) was added to a cuvette containing 2 mL of bacterial suspension and 10 μM propidium iodide (PI). Fluorescence was monitored at excitation wavelength of 535 nm (slit width of 10 nm) and emission wavelength of 617 nm (slit width of 5 nm). As a measure of inner membrane permeabilization, the uptake of PI was monitored by the increase in fluorescence for 10 min.

1.6 Interaction of compound **6b** and sensitive MRSA DNA

1.6.1 Procedures for isolating genomic DNA from drug-resistant MRSA bacteria

(a) An overnight culture (1 mL) was added to a microcentrifuge tube (1.5 mL), and then centrifuged at 13,000−16,000 r/min for 2 minutes to pellet the cells. The supernatant was removed and the cells were resuspended thoroughly in EDTA (480 μL, 50 mM). The lytic enzyme (120 μL) was added appropriately to the resuspended cell pellet and mixed gently. The purpose of this pretreatment is to weaken the cell wall so that efficient cell lysis could take place. After the sample was incubated at 37 °C for 30–60 minutes, it was centrifuged for 2 minutes at 13,000–16,000 r/min and the supernatant was removed. Nuclei Lysis solution (600 μL) was added to the sample, it was gently pipetted until the cells are resuspended. It was incubated at 80 °C for 5 minutes to lyse the cells, and then it was cooled to room temperature. RNase solution (3 μL) was added to the cell lysate, the tube was inverted to mix for 2–5 times. After the sample was incubated at 37 °C for 15–60 minutes, then it was cooled to room temperature.
(b) Protein precipitation solution (200 μL) was added to the RNase-treated cell lysate, and vortex vigorously at high speed for 20 seconds to mix the protein precipitation solution with the cell lysate. The sample was incubated on ice for 5 minutes, and centrifuged at 13,000–16,000 r/min for 3 minutes.
(c) The supernatant containing the DNA was transferred to a clean microcentrifuge tube (1.5 mL) containing room temperature isopropanol (600 μL). It was gently mixed by inversion until the thread-like strands of DNA form a visible mass, and was centrifuged at 13,000–16,000 r/min for 2 minutes. The supernatant was carefully poured off and the tube was drained on a clean absorbent paper. Ethanol (70%, 600 μL) was added at room temperature to the tube and it was gently shake several times to wash the DNA pellet. After it was centrifuged at 13,000–16,000 r/min for 2 minutes, and then the ethanol was carefully aspirated. The tube was drained on a clean absorbent paper to allow the pellet to air-dry for 10–15 minutes.
(d) DNA rehydration solution (100 μL) was added to the tube and incubated at 65 °C for 1 hour. The solution was mixed periodically by gently tapping the tube. Alternatively, the DNA was rehydrated by incubating the solution overnight at 4 °C. The obtained DNA was stored at 2–8 °C.

1.6.2 Absorption spectra of DNA in the presence of compound **6b**

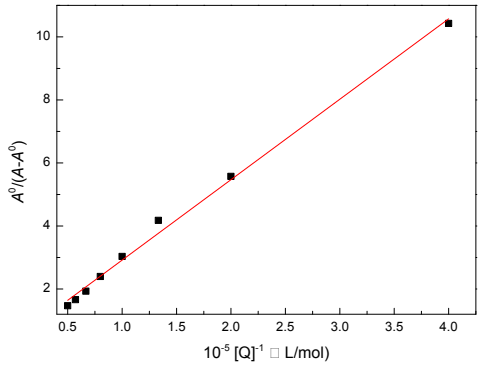


Fig. S1 The plot of $A^0/(A-A^0)$ vs 1/[compound **6b**].

1.6.3 Absorption spectra of NR interactions with DNA

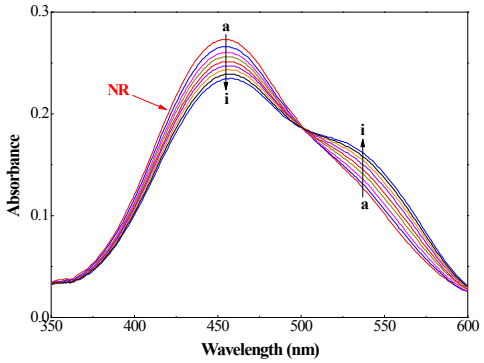


Fig. S2 UV absorption spectra of NR in the presence of MRSA DNA at pH 7.4 and room temperature. c(NR) = 2 × 10^{-5} mol/L, and c(DNA) = 0–3.81 × 10^{-5} mol/L.

1.7 Interaction of compound **6b** with HSA

1.7.1 UV-vis absorption spectral study of HSA

The fluorescence emission spectra of HSA with and without compound **6b** are displayed in Fig. S3. The intrinsic fluorescence of HSA is primarily contributed to Tyr and Trp, which can be quenched upon interaction with small ligands. As shown in Fig. S3, the fluorescence intensity of HSA gradually decreased with increasing concentration of compound **6b**, which suggested that compound **6b** could effectively bind to HSA.

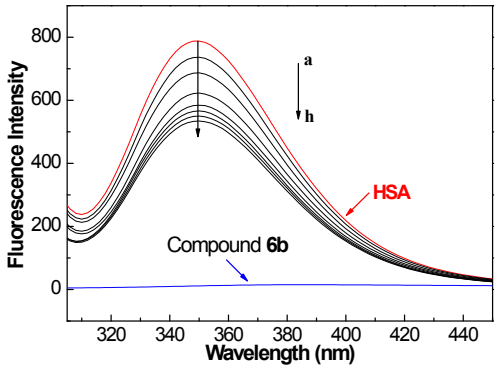


Fig. S3 Emission spectra of HSA with various concentrations of compound **6b**. c(HSA) = 1.0 × 10^{-5} mol/L; c(compound **6b**)/(10^{-5} mol/L), a–h: from 0.0 to 1.22 at increments of 0.174; blue and red lines are the emission spectrum of compound **6b** only and HSA only, respectively; T = 298 K, λex = 295 nm.

1.7.2 Binding mode and thermodynamic parameters

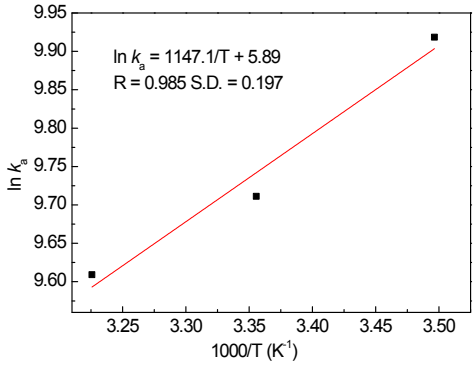


Fig. S4 Van't Hoff plots of the **6b**-HSA system.

1.8 Drug combination study

The drug combination studies of compound **6b** and clinical drugs norfloxacin and fluconazole against microbia were performed by 2-fold dilution checkerboard assay with concentration values from 1/32 to 4 times the MIC value of each molecule. The combination effects were determined by the fractional inhibitory concentration (FIC) index. The FIC value can be calculated as FIC = MIC of compound A in mixture/MIC of compound A alone + MIC of compound B in mixture/MIC of compound B alone, where, FIC ≤ 0.5, FIC > 0.5 and ≤ 1.0, FIC > 1 and ≤ 2, and FIC > 2 represent synergism, additivism, indifferent effect, antagonism, respectively.

1.9 Molecular docking

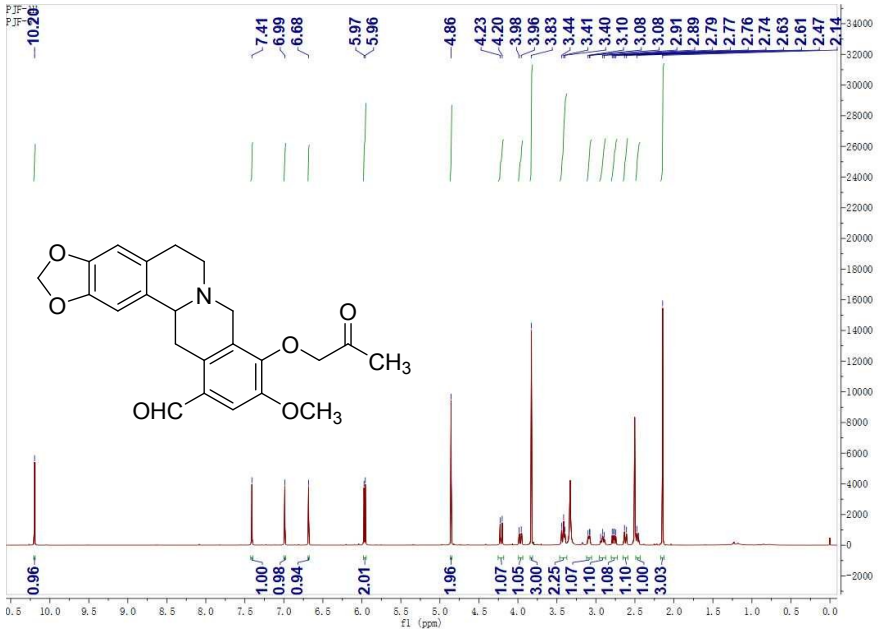
SYBYL-X 2.0 was used to perform the docking study. The Lamarkian Genetic Algorithm (LGA) was applied for the conformational search.

2. Experimental protocols

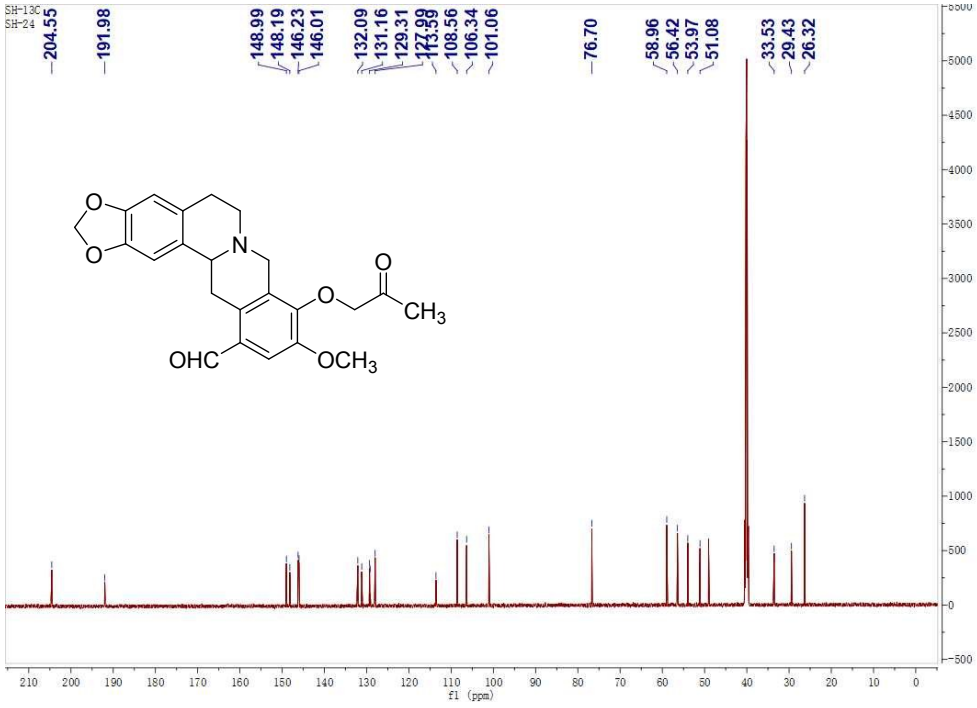
2.1 Characterizations of some representative compounds

2.1.1 Spectra of compound **11a**.

^{1}H NMR spectrum

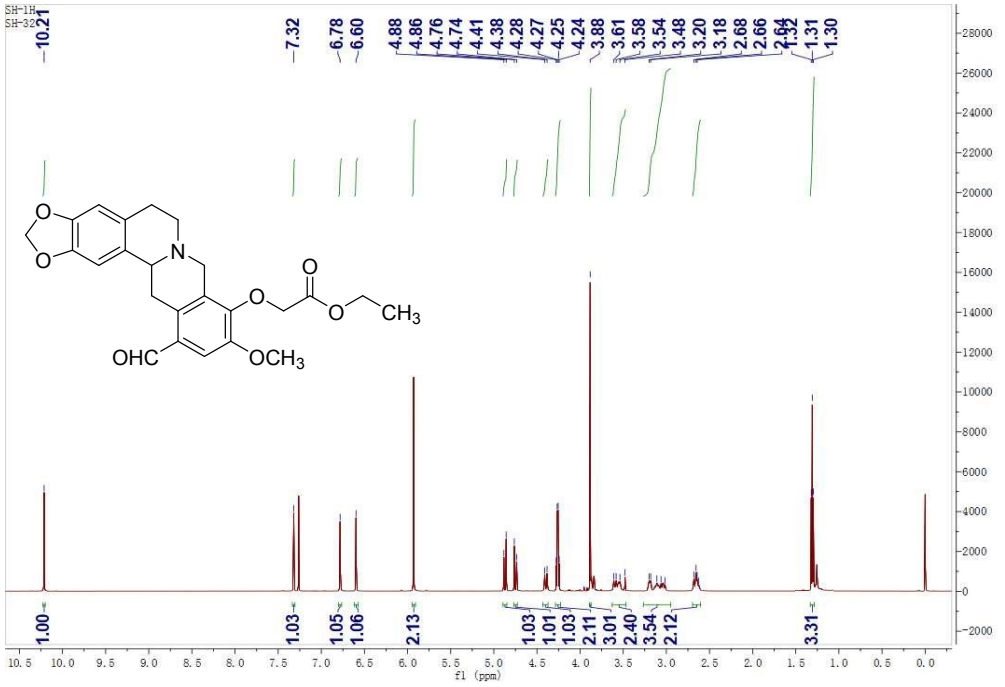


^{13}C NMR spectrum

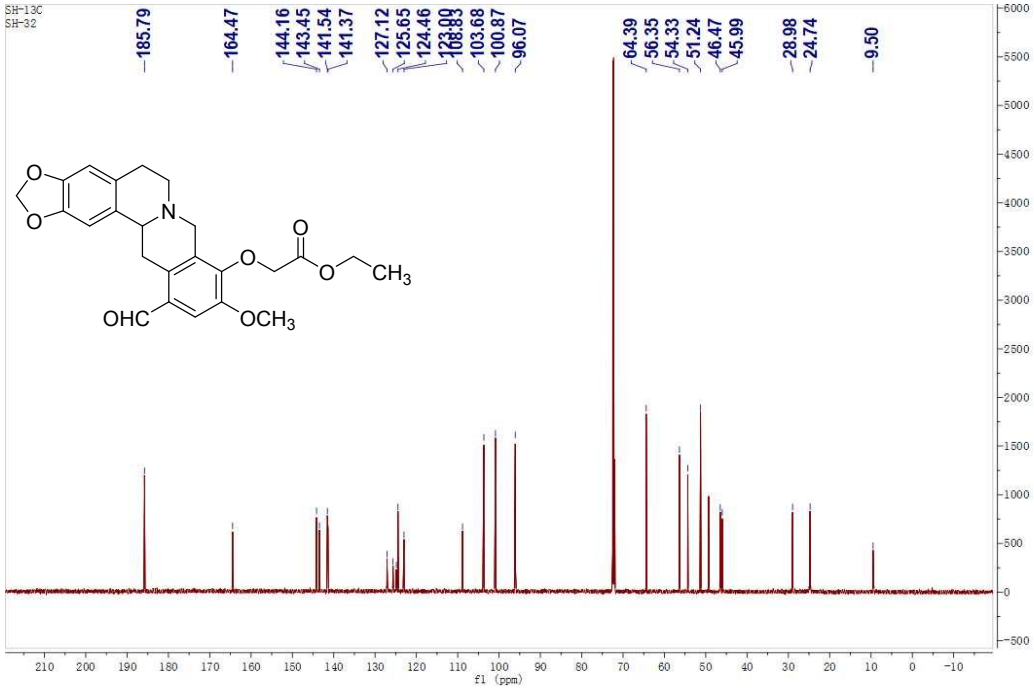


2.1.2 Spectra of compound **11b**

^{1}H NMR spectrum

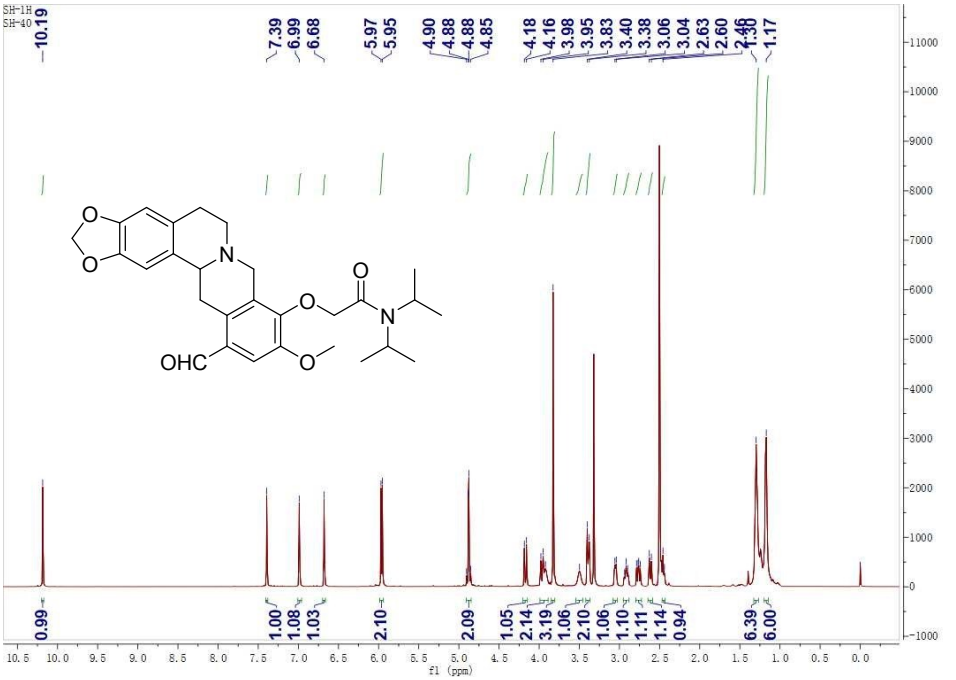


^{13}C NMR spectrum



2.1.3 Spectra of compound **11c**

^{1}H NMR spectrum



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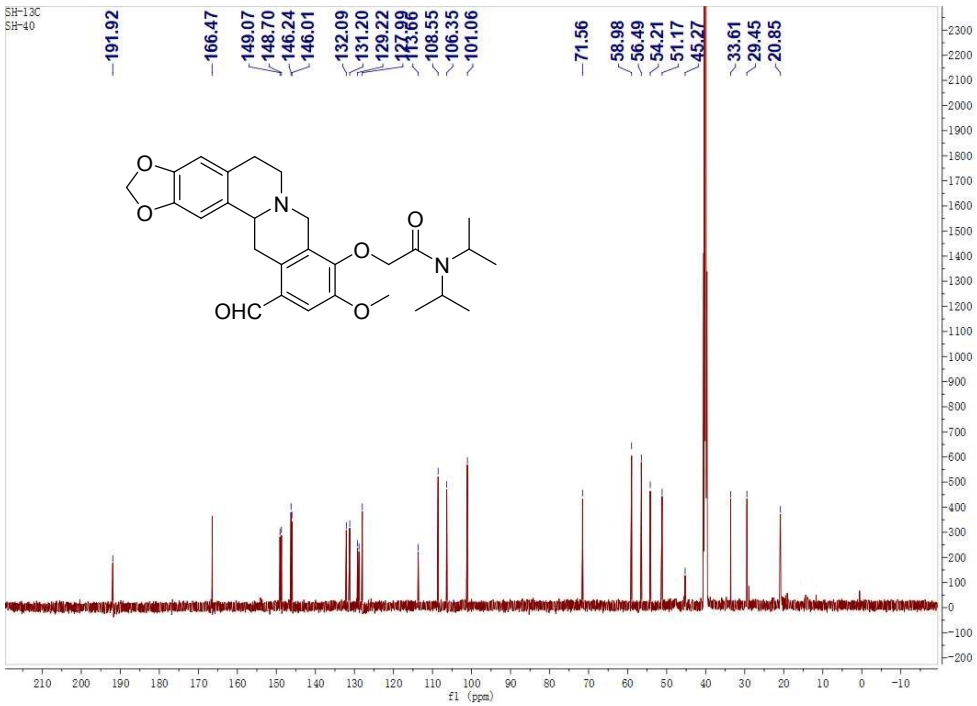
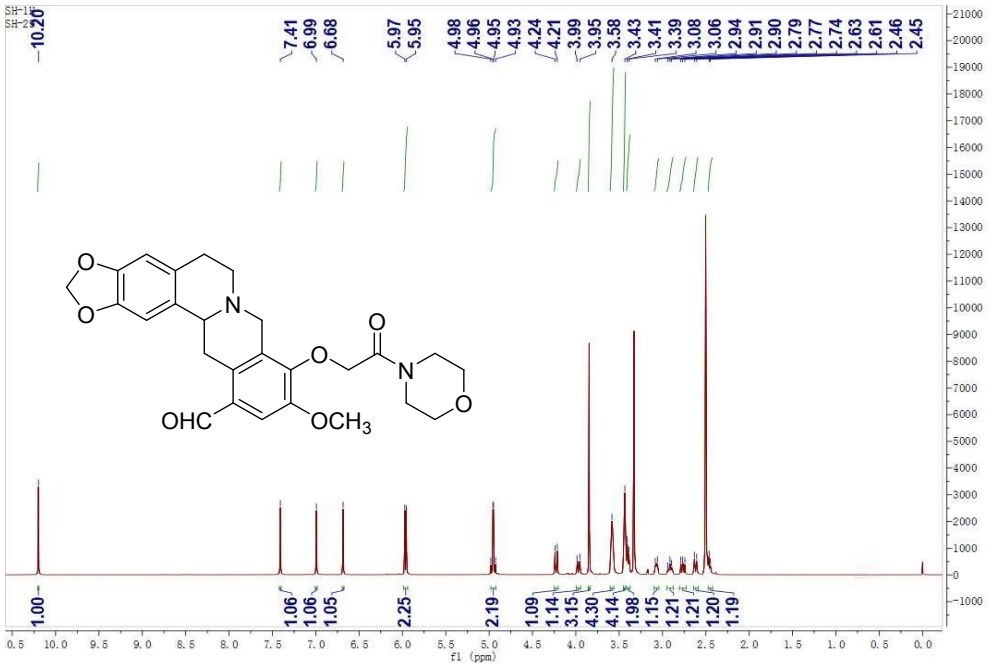
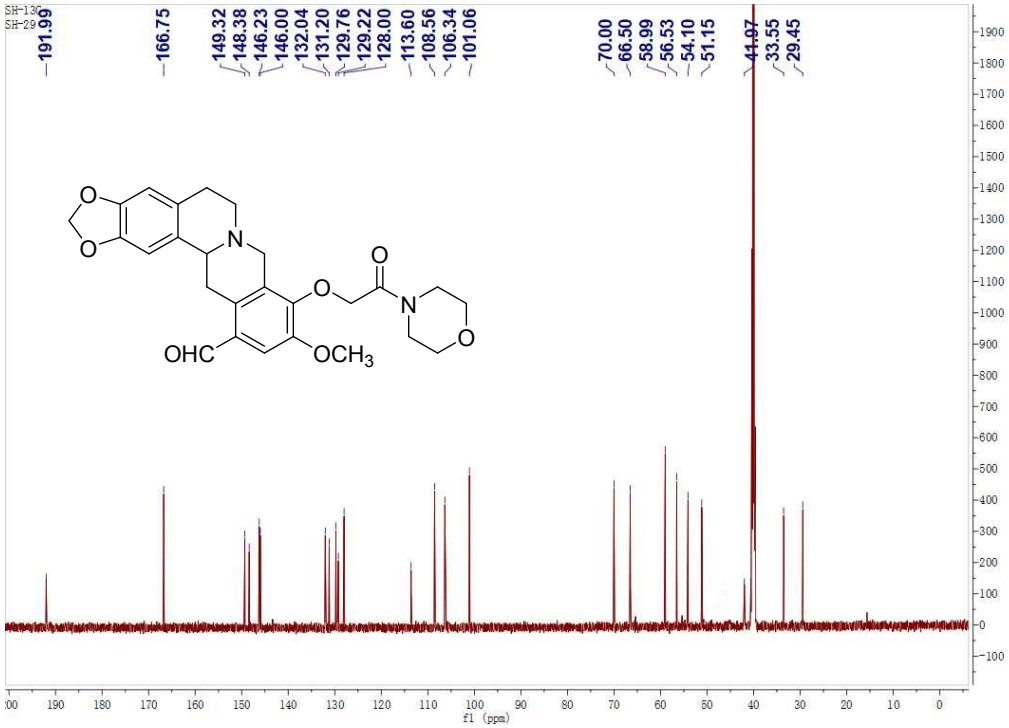


2.1.4 Spectra of compound **11d**

^{1}H NMR spectrum

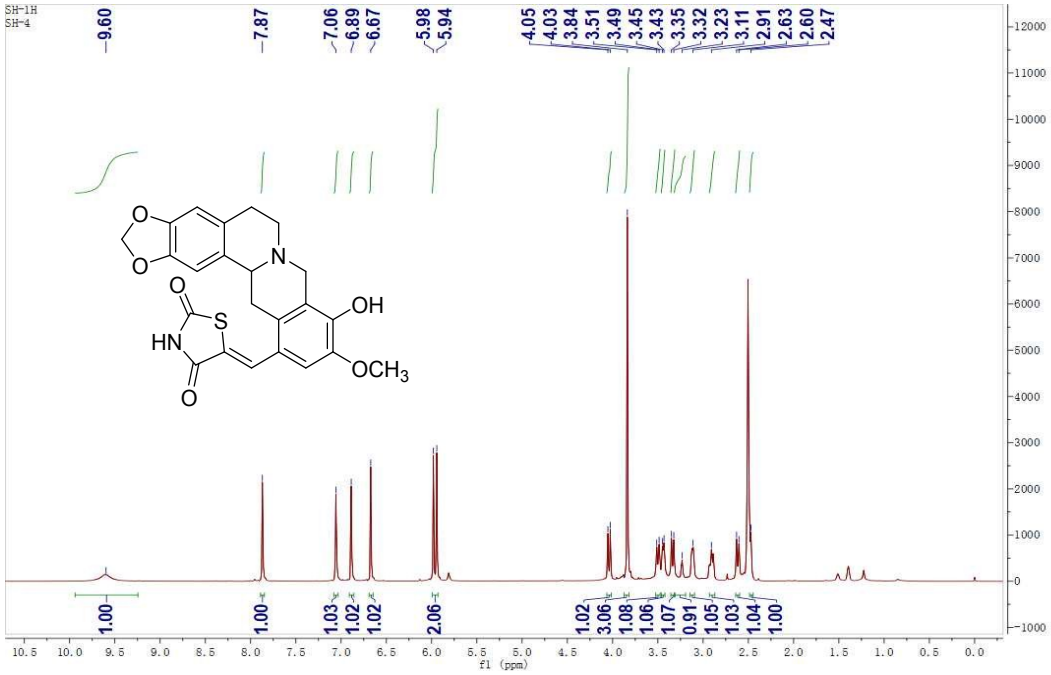


^{13}C NMR spectrum

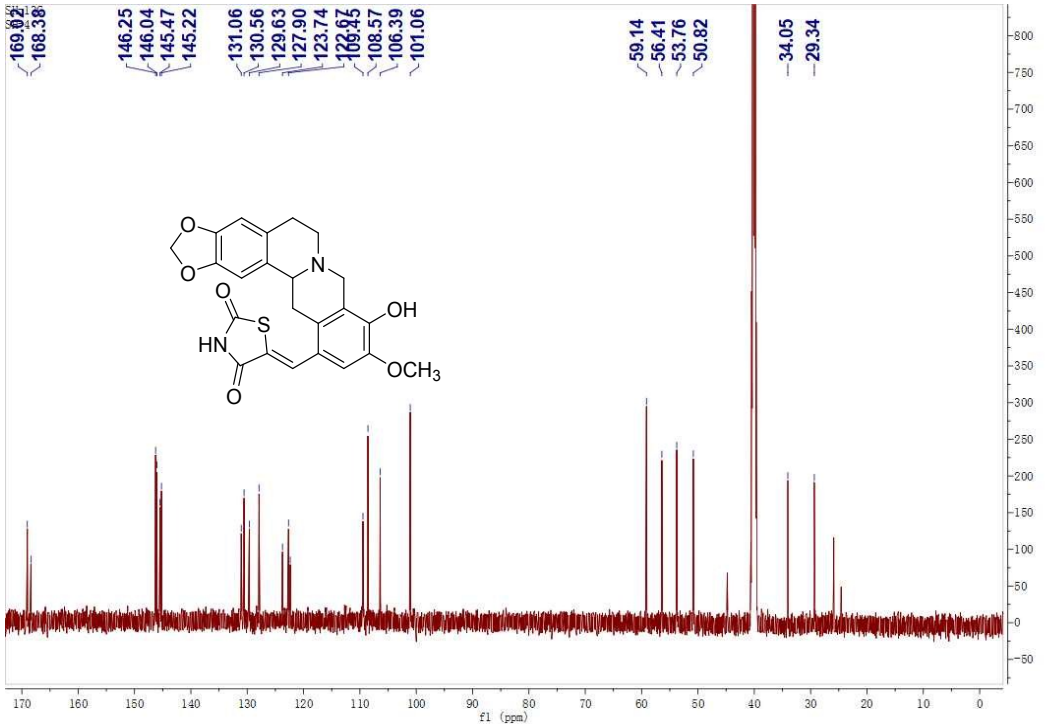


2.1.5 Spectra of compound **4**

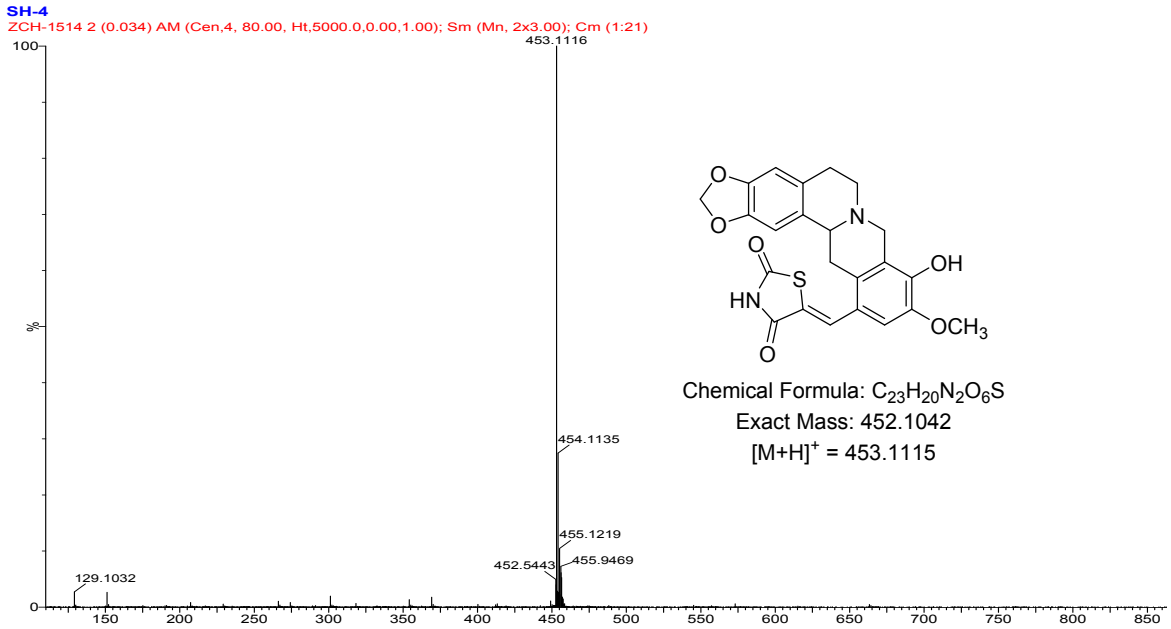
^{1}H NMR spectrum



^{13}C NMR spectrum

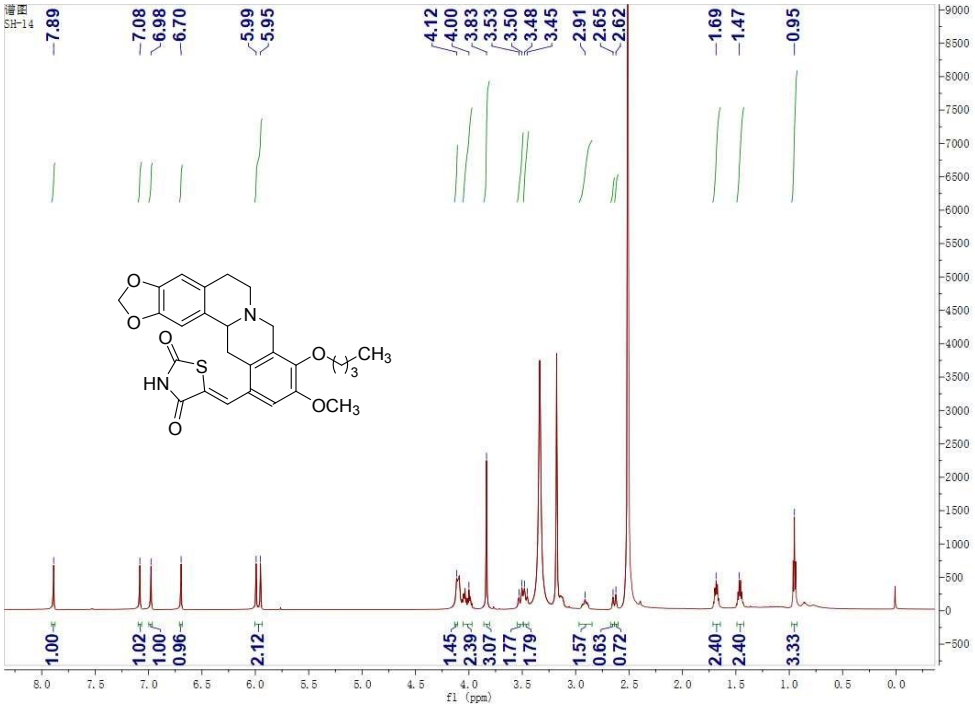


HRMS spectrum



2.1.6 Spectra of compound **6b**

^{1}H NMR spectrum



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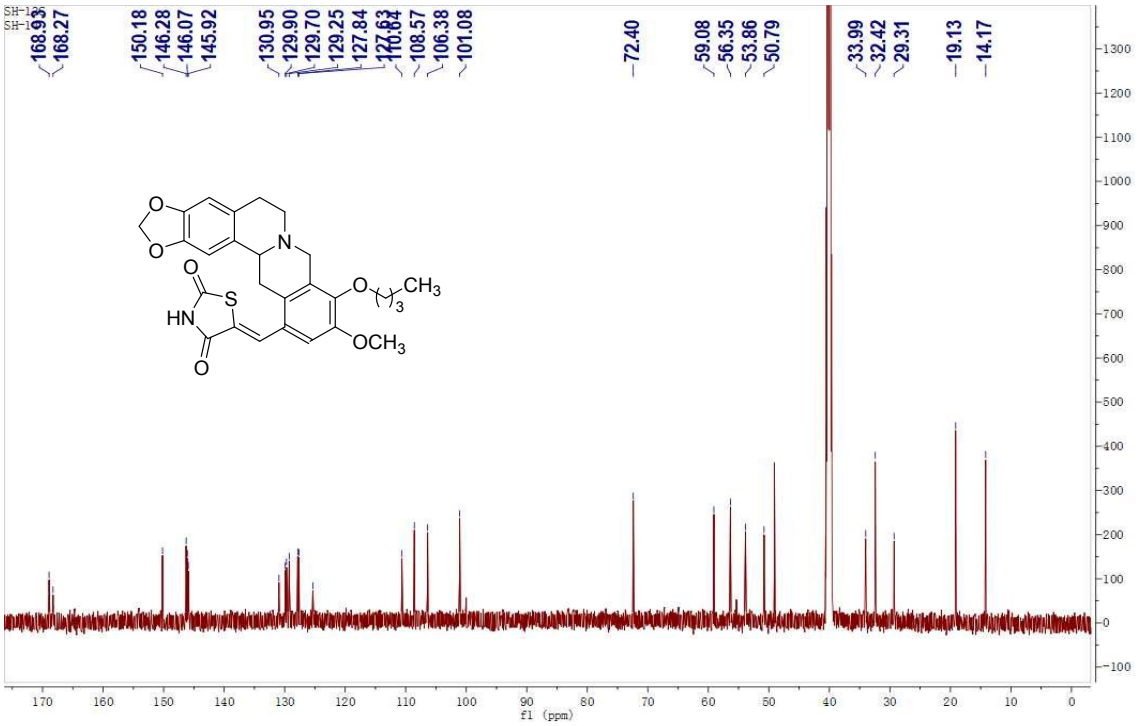
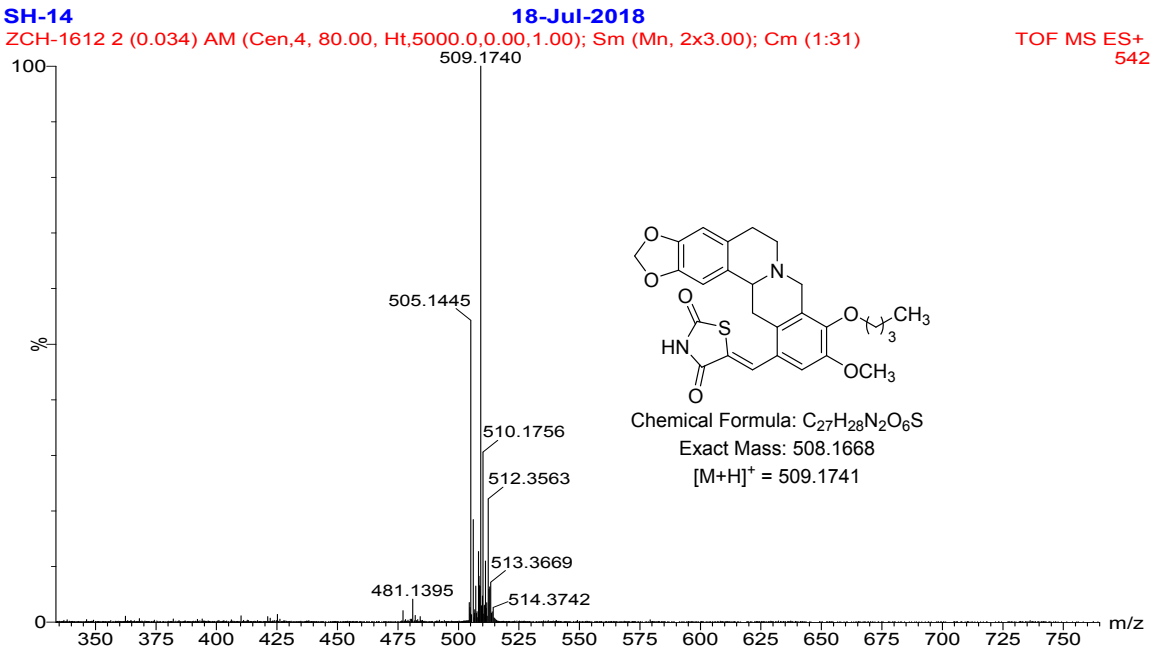
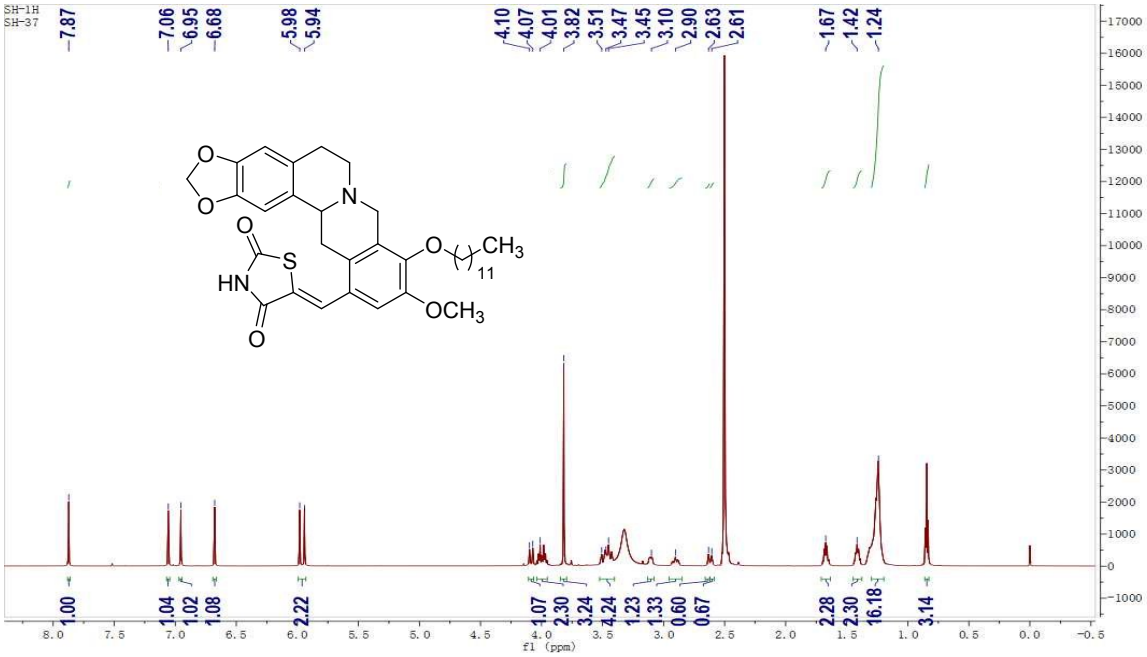


HRMS spectrum of **6b**



2.1.7 Spectra of compound **6f**

^{1}H NMR spectrum



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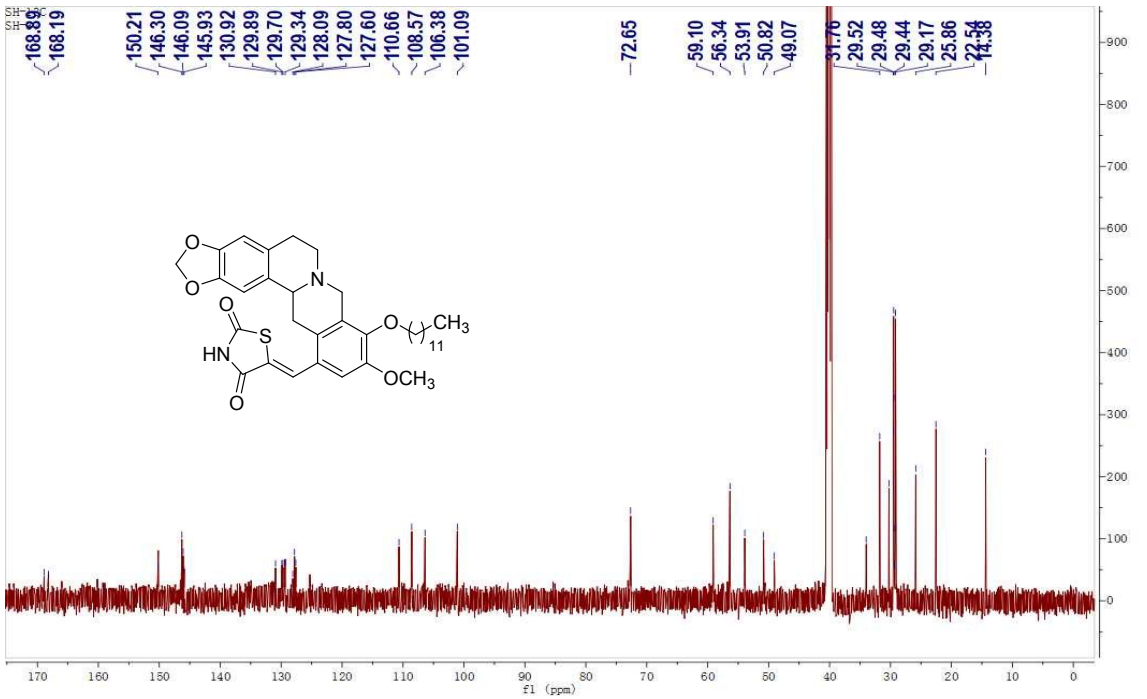

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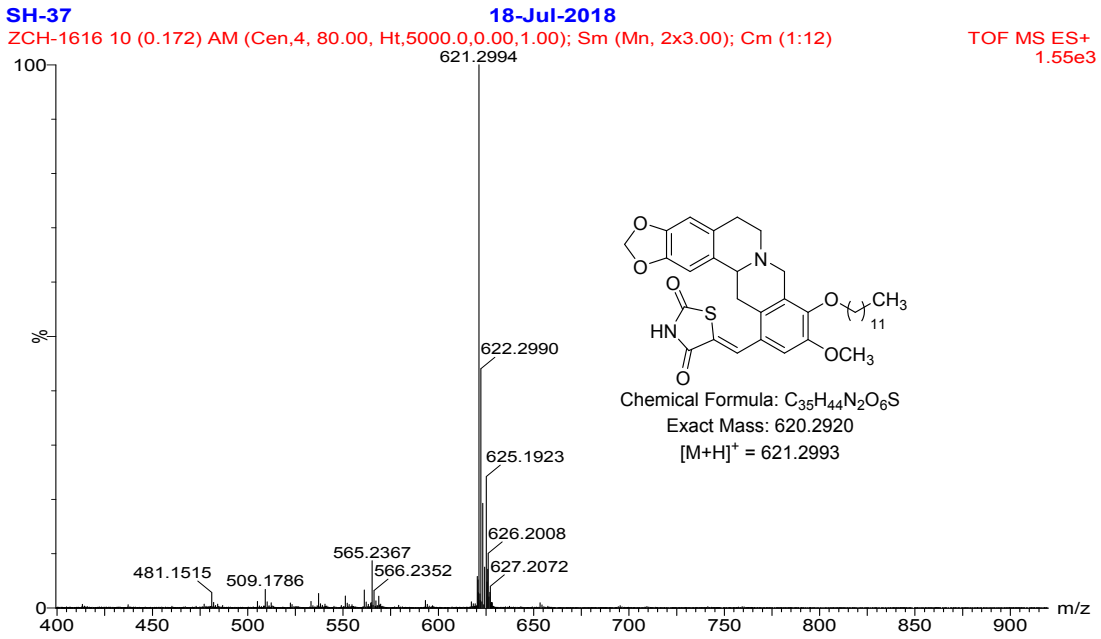
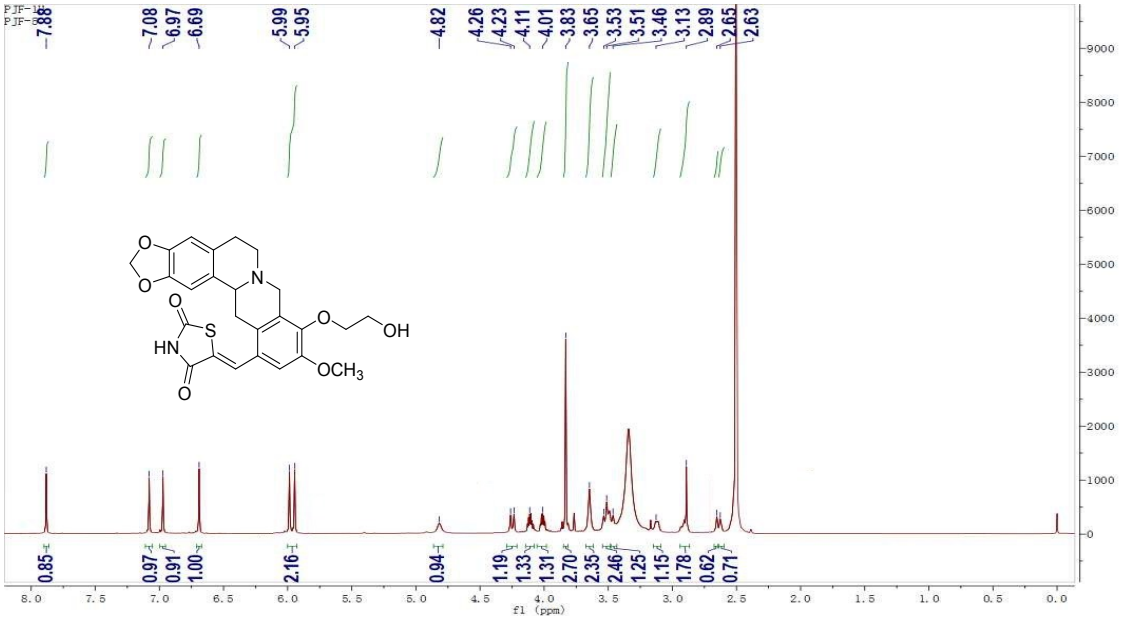
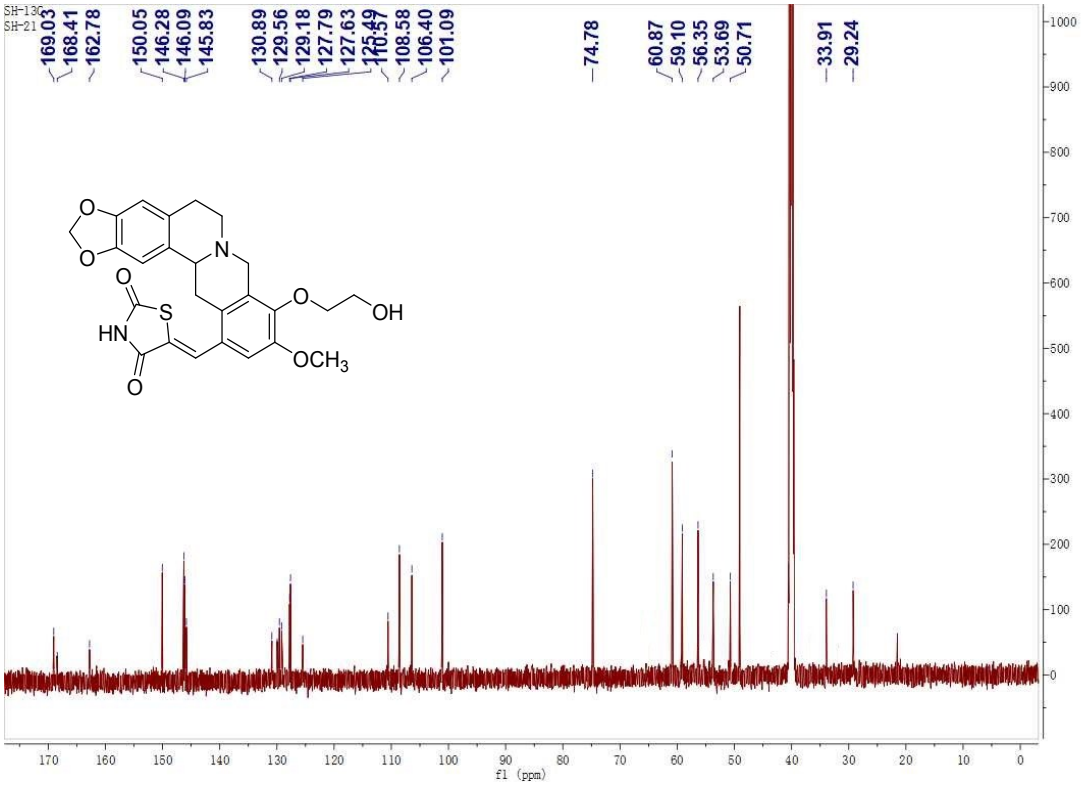


2.1.8 Spectra of compound **6g**

^{1}H NMR spectrum



^{13}C NMR spectrum



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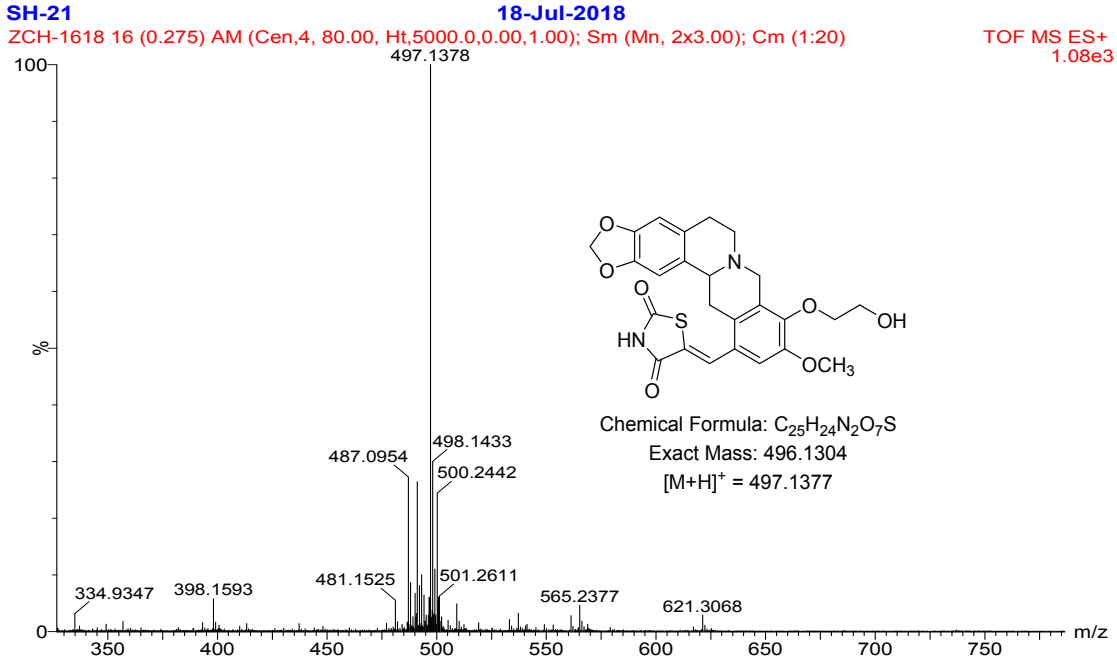
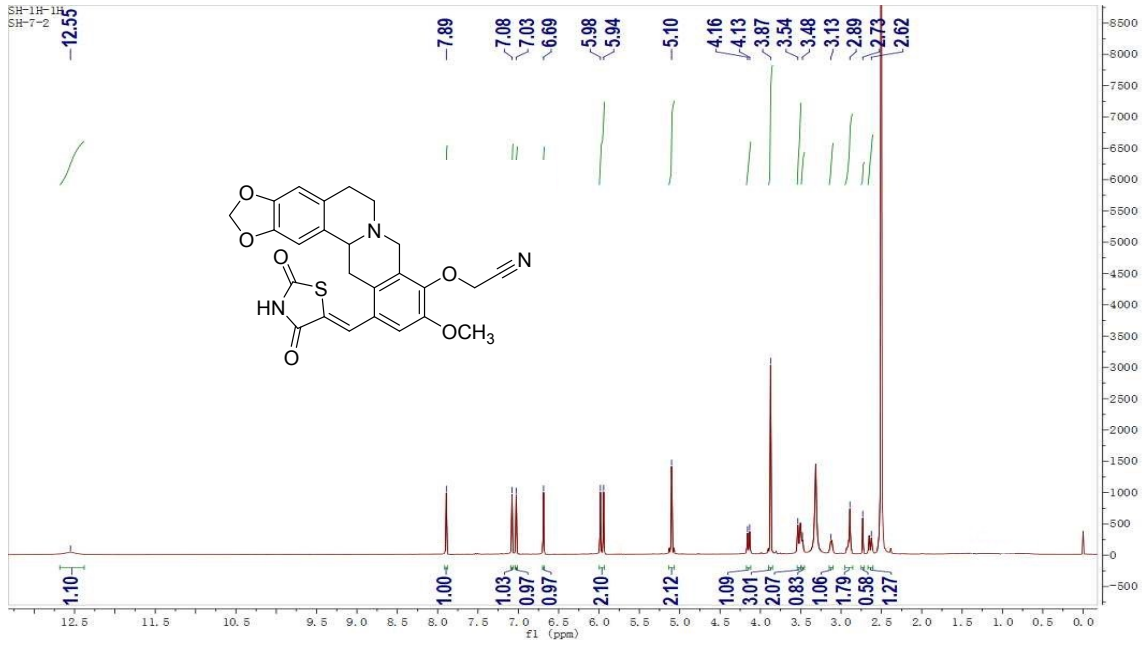
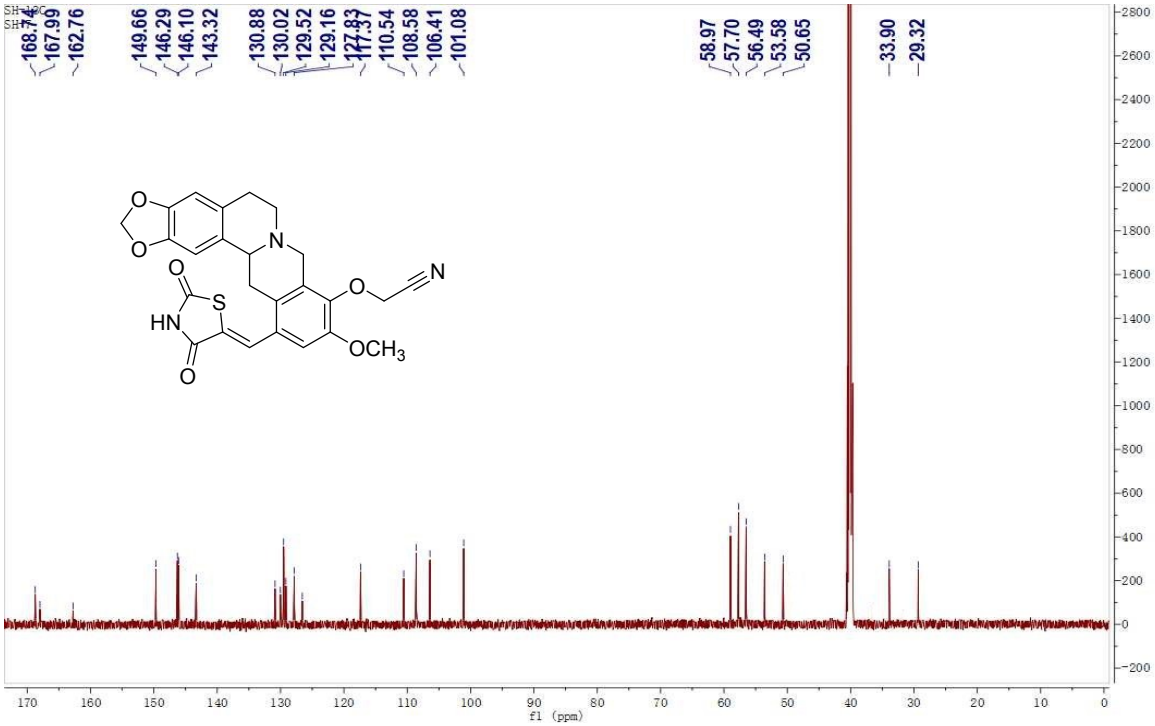


2.1.9 Spectra of compound **10b**

^{1}H spectrum



^{13}C NMR spectrum



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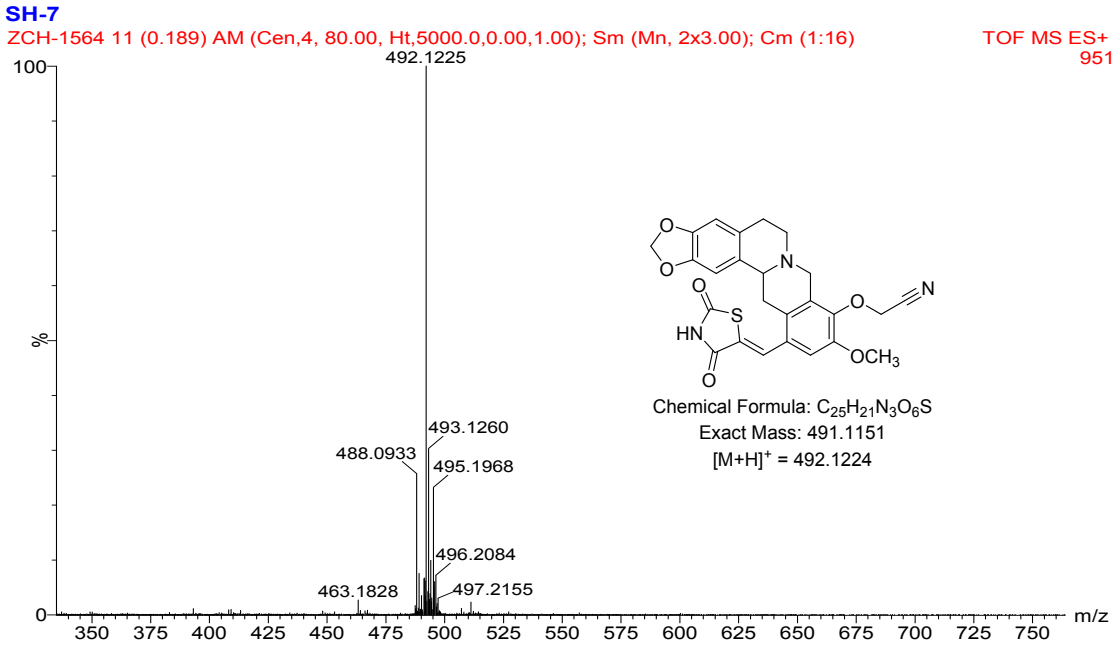
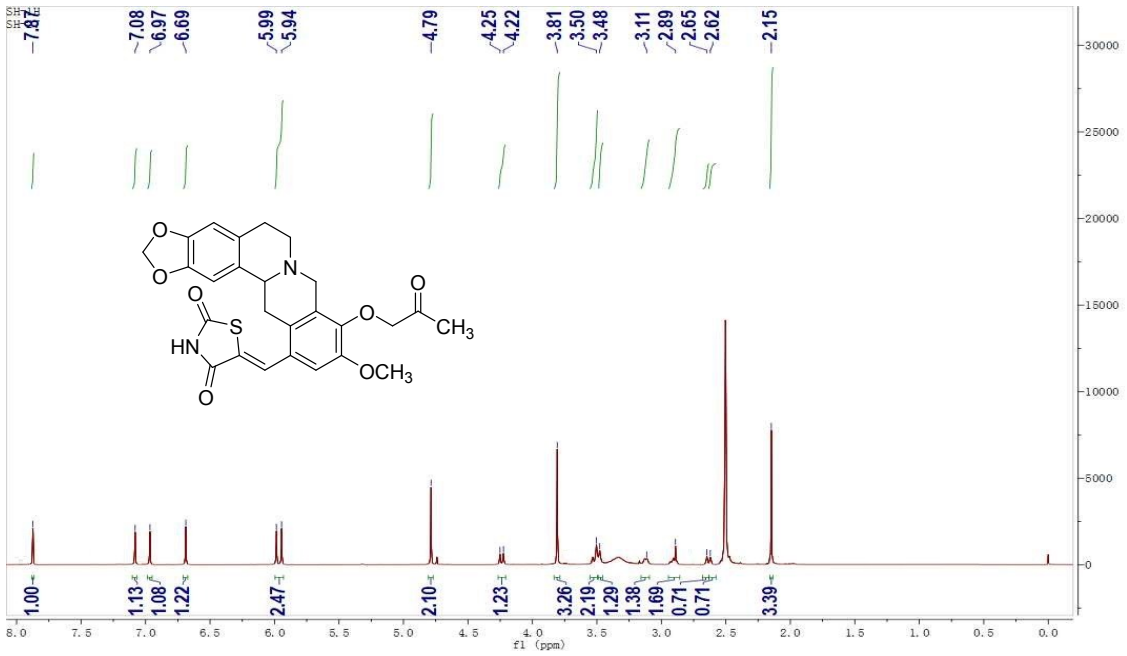
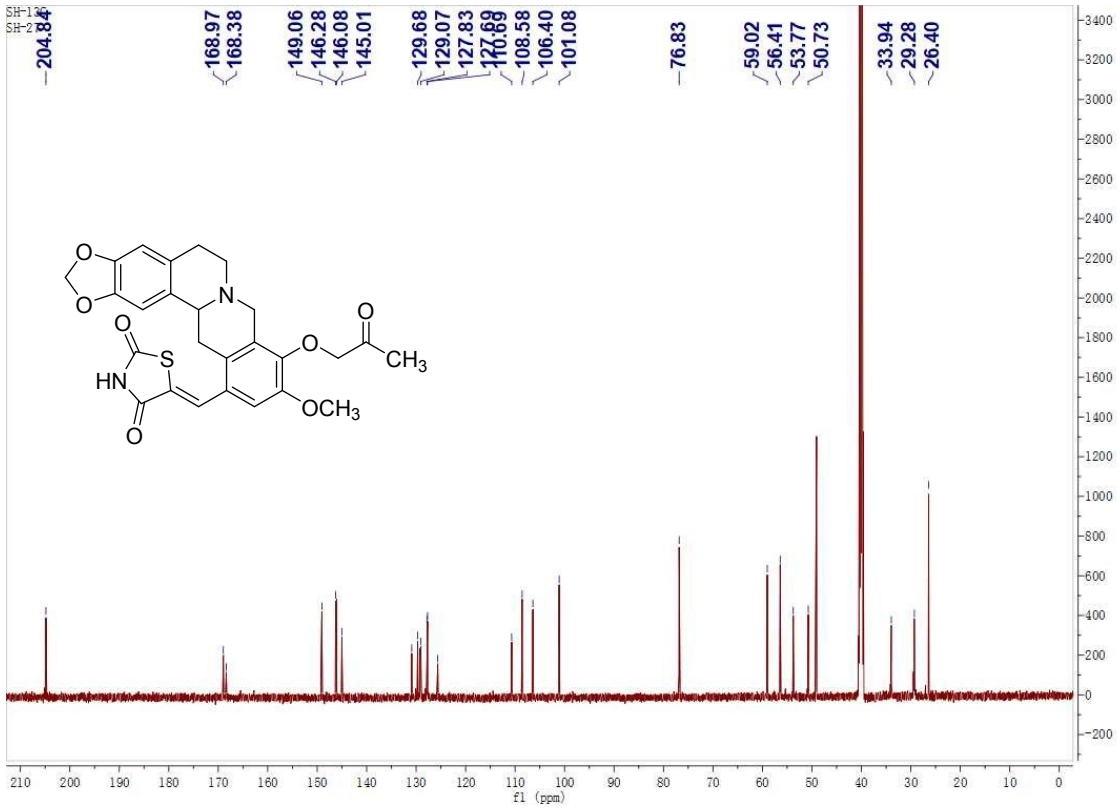


2.1.10 Spectra of compound **12a**

^{1}H spectrum



^{13}C NMR spectrum



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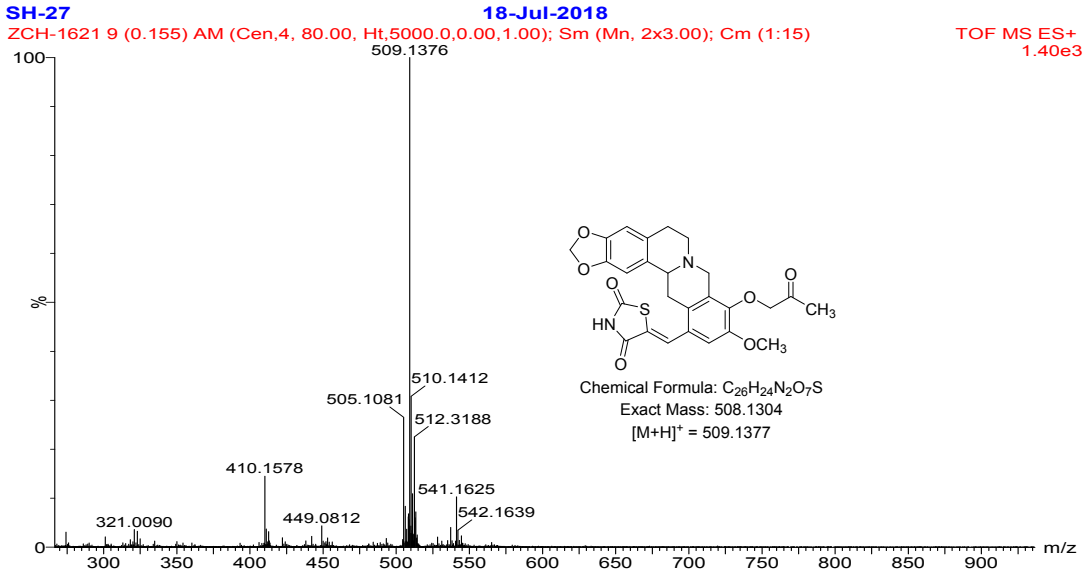
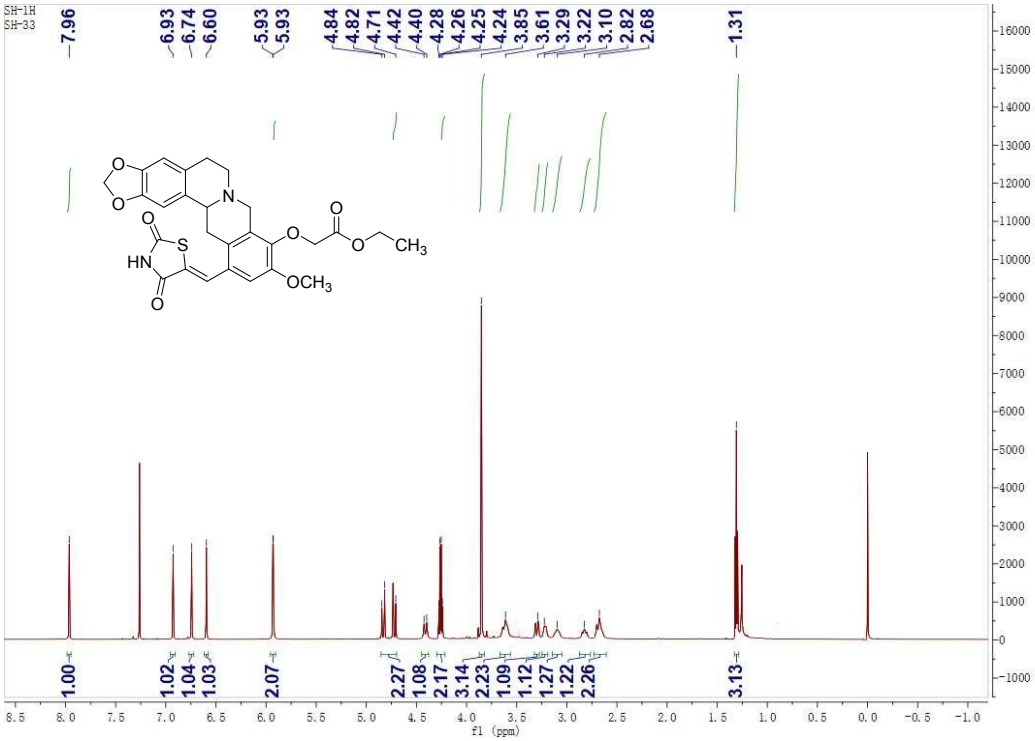
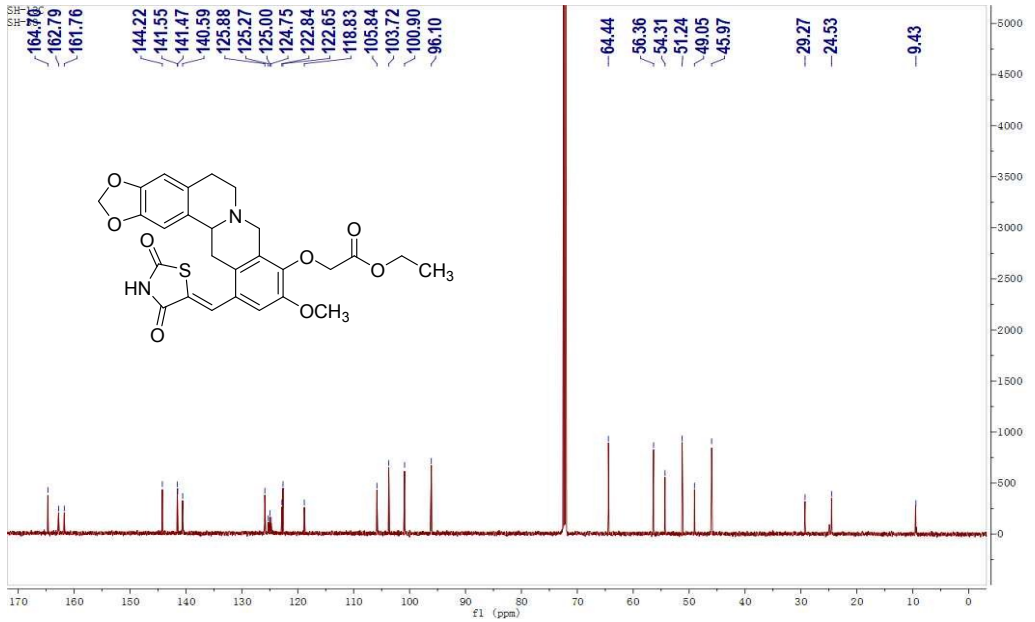


2.1.11 Spectra of compound **12b**

^{1}H spectrum



^{13}C NMR spectrum



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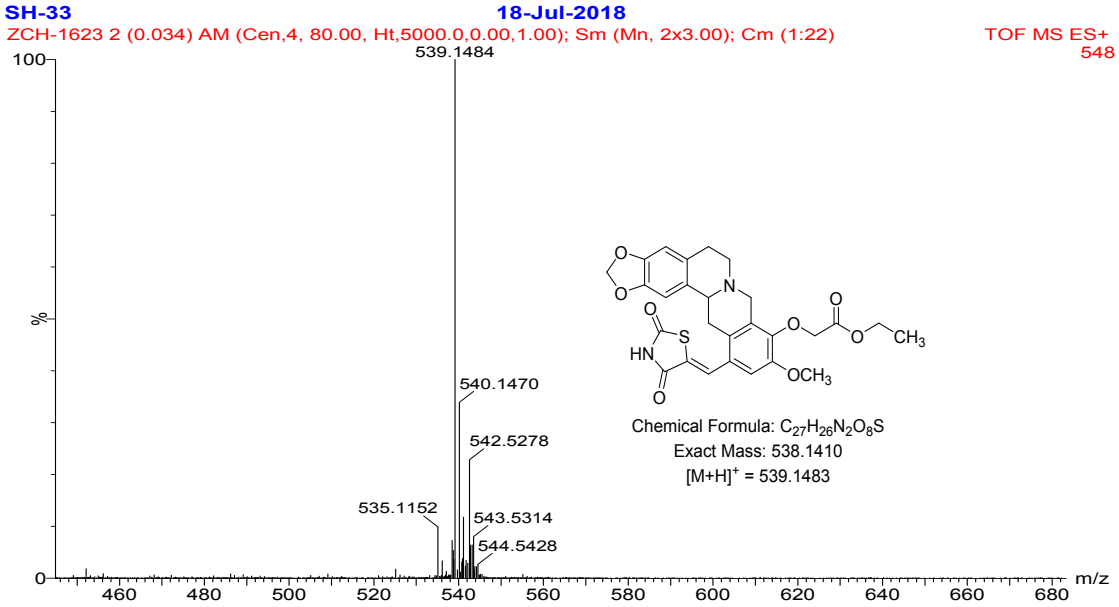
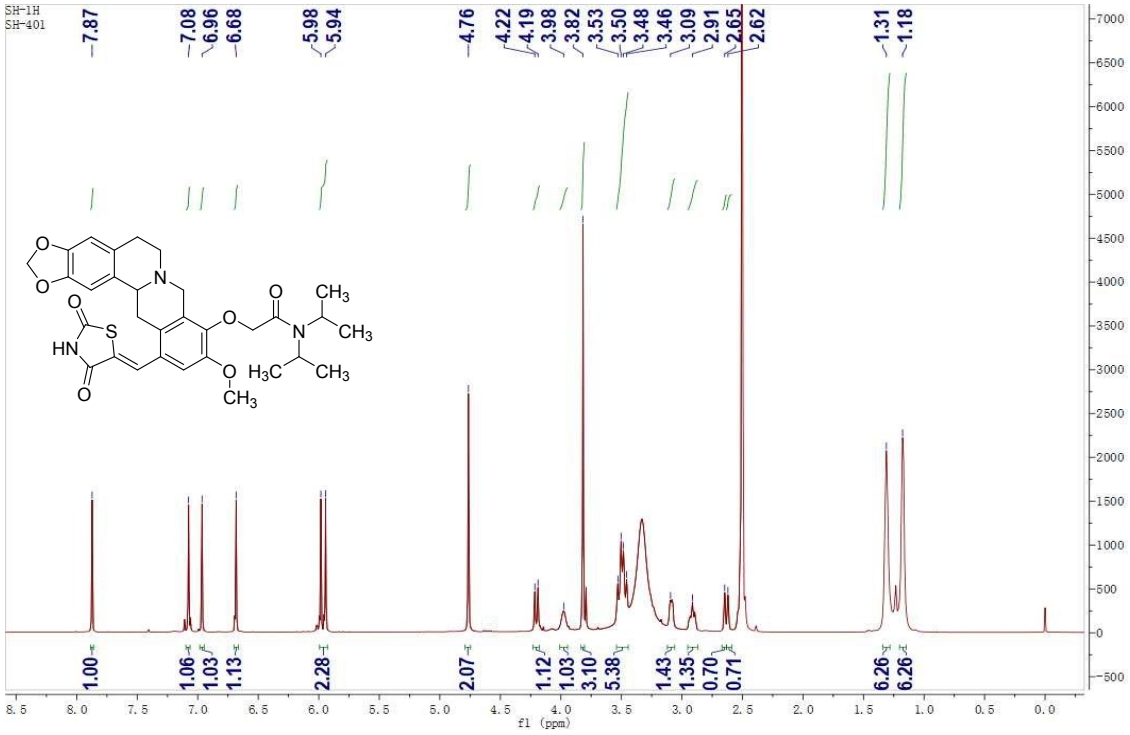
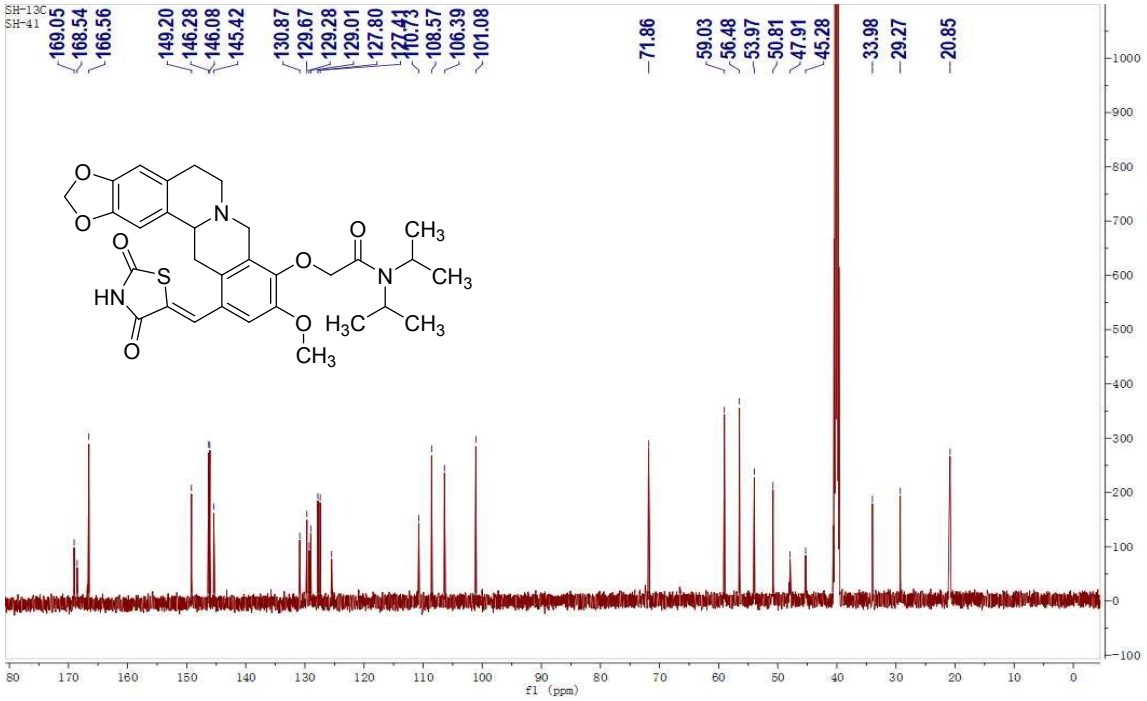


2.1.12 Spectra of compound **12c**

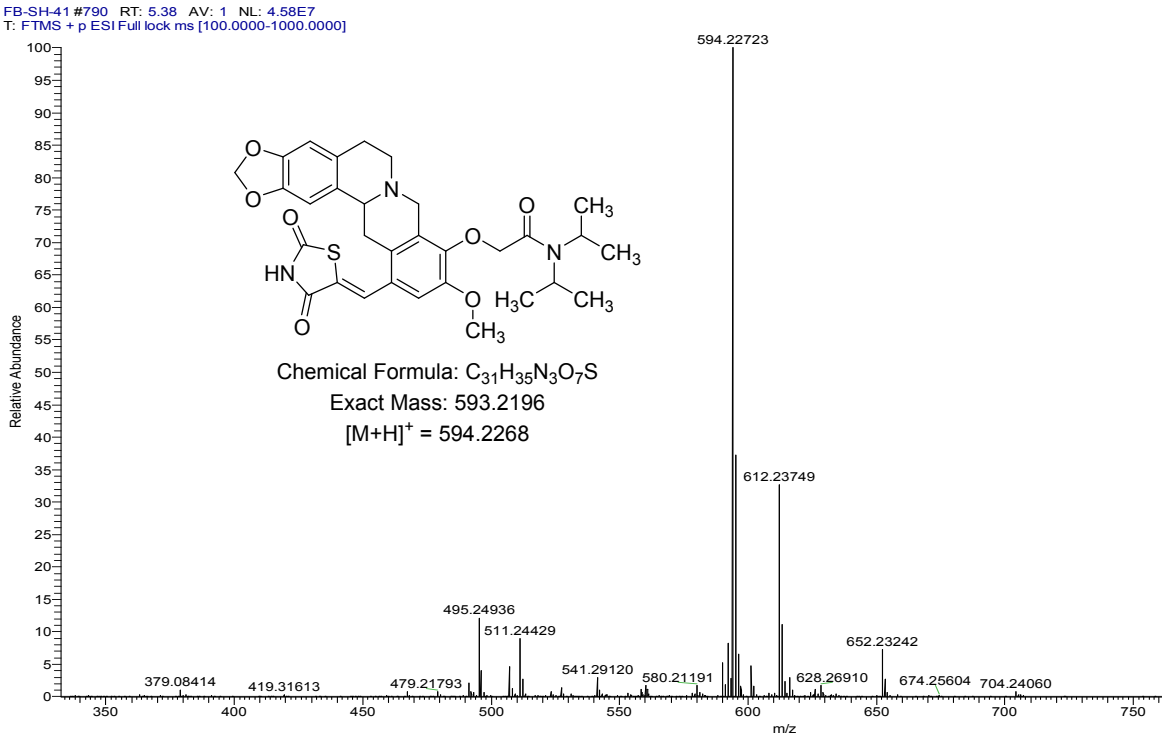
^{1}H spectrum



^{13}C spectrum

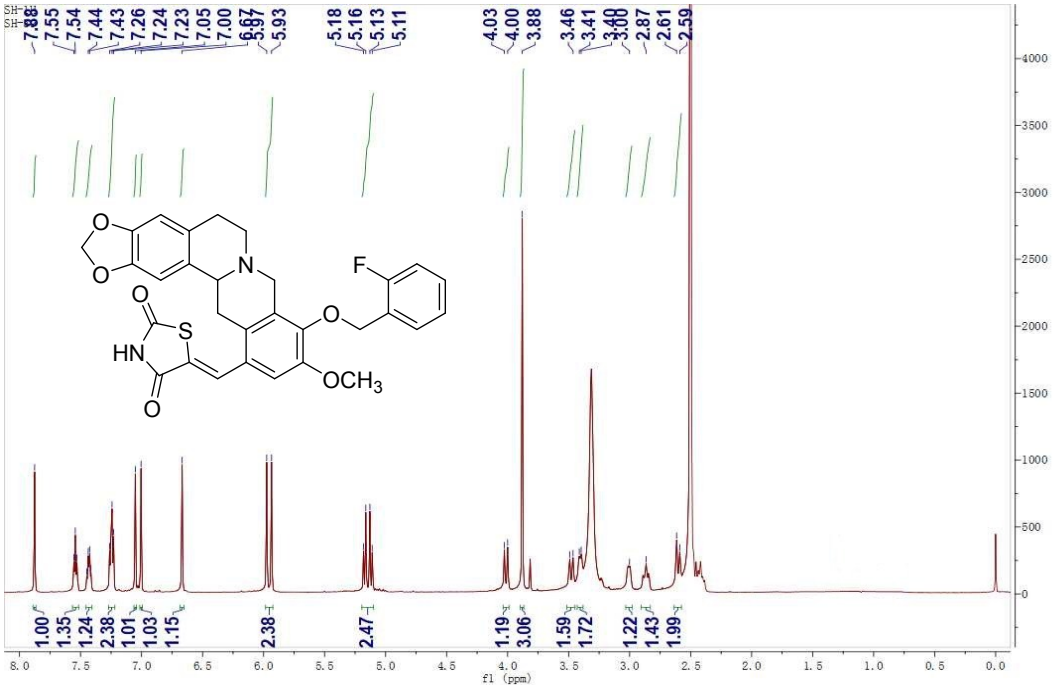


HRMS spectrum

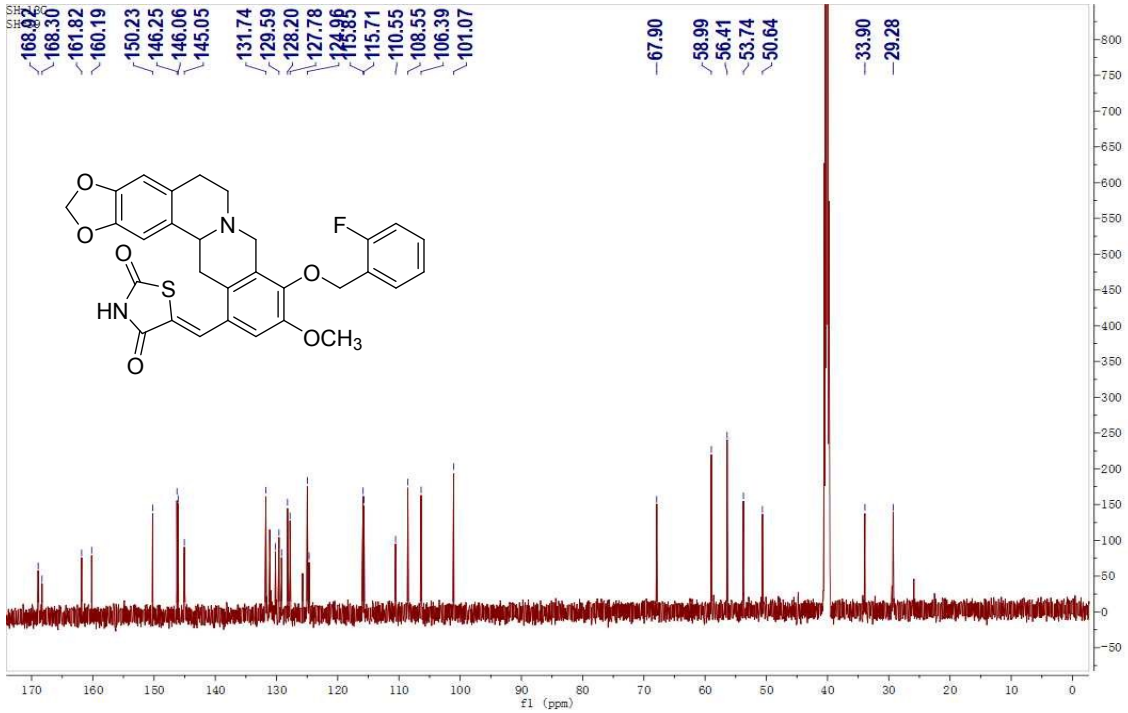


2.1.13 Spectra of compound **14**

^{1}H NMR spectrum



^{13}C NMR spectrum



HRMS spectrum