# Supplementary Information

Design, synthesis and biological evaluation of novel Schiff base-bridged tetrahydroprotoberberine triazoles as new type of potential antimicrobial agents

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# 1 Experimental Protocols

## 1.1 General Methods

TLC analysis was done using pre-coated silica gel plates. FT-IR spectra were carried out on Bruker RFS100/S spectrophotometer (Bio-Rad, Cambridge, MA, USA) using KBr pellets in the 400–4000 $cm^{-1}$ range. $^{1}H$ NMR, $^{13}C$ NMR, $^{1}H$-$^{1}H$ COSY and $^{13}C$-$^{1}H$ COSY spectra were recorded on a Bruker AV 300 spectrometer using TMS as an internal standard. The high-resolution mass spectra (HRMS) were recorded on an IonSpec FT–CR mass spectrometer with ESI resource. UV spectra were recorded at room temperature on a TU-2450 spectrophotometer (Puxi Analytic Instrument Ltd. of Beijing, China) equipped with 1.0 cm quartz cells.

The stock solution of compound **7a** was prepared in DMF. Calf thymus DNA (Sigma Chemical Co., St. Louis, MO) was used without further purification, and its stock solution was prepared by dissolving an appropriate amount of DNA in doubly distilled water. The solution was allowed to stand overnight and store at 4 °C in the dark for about a week. The concentration of DNA in stock solution was determined by UV absorption at 260 nm using a molar absorption coefficient $\xi_{260}$ = 6600 L $mol^{-1}$ $cm^{-1}$ (expressed as molarity of phosphate groups) by Bouguer-Lambert-Beer law. The purity of DNA was checked by monitoring the ratio of the absorbance at 260 nm to that at 280 nm. The solution gave a ratio of > 1.8 at A260/A280, which indicated that DNA was sufficiently free from protein. NR stock solution was prepared by dissolving its solid (Sigma Chemical Co.) in doubly distilled water and was kept in a cool and dark place. All the solutions were adjusted with Tris-HCl buffer solution (pH = 7.4), which was prepared by mixing and diluting Tris solution with HCl solution. All chemicals were of analytical reagent grade, and doubly distilled water was used throughout. HSA was dissolved in Tris-HCl buffer solution (0.05 M Tris, 0.15 M NaCl, pH = 7.4). Sample masses were weighed on a microbalance with a resolution of 0.1 mg. All other chemicals and solvents were commercially available, and were used without further purification.

The pH of the solution was determined by using a Sartorius PB-10 pH meter (Sartorius Scientific Instrument (Beijing) Co., Ltd., P.R. China). DNA cleavage was analysed by gel electrophoresis on a DYY-12 electrophoresis meter (Beijing Liuyi Biotechnology Co., Ltd., P.R. China) and gel image analysing system (Vilber Lourmat BIO-1D, France). Plasmid pUC19 DNA, 50×TAE, 6×loading buffer, gold view dye, and agarose were purchased from Beijing Changsheng Biotechnology Co., Ltd. Trishydroxymethylaminomethane (Tris), HCl, NaCl, and NaOH were analytical grade products and used as supplied. All other chemicals were purchased from Chongqing chemical Co. and, unless otherwise indicated, were of analytical grade. The water used for experiments was doubly distilled.

## 1.2 Biological Assay Procedures

Minimal inhibitory concentration (MIC, μg/mL) is defined as the lowest concentration of target compounds that completely inhibited the growth of bacteria, by means of standard two-fold serial dilution method in 96-well microtest plates according to the National Committee for Clinical Laboratory Standards (NCCLS). The tested microorganism strains were provided by the School of Pharmaceutical Sciences, Southwest University and the College of Pharmacy, Third Military Medical University. Berberine, Chloromycin, Norfloxacin and Fluconazole, were used as control drugs. DMSO with inoculation bacterial not medicine was used as positive control to ensure that the solvent had no effect on bacteria growth. All the bacteria and fungi growth was monitored visually and spectrophotometrically, and the experiments were performed in triplicate.

### 1.2.1 Antibacterial Assays

The prepared compounds were evaluated for their antibacterial activities against Gram-positive bacteria (*Staphylococcus aureus* ATCC25923, Methicillin-resistant *Staphylococcus aureus* N315 (MRSA), *Bacillus subtilis* ATCC6633) and *Micrococcus luteus* ATCC4698), Gram-negative bacteria (*Escherichia coli* JM109, *Escherichia coli* DH52, *Shigella dysenteriae*, *Pseudomonas aeruginosa* ATCC27853, *Bacillus proteus* ATCC13315 and *Bacillus typhi*). The bacterial suspension was adjusted with sterile saline to a concentration of $1 \times 10^5$ CFU. Initially the compounds were dissolved in DMSO to prepare the stock solutions, then the tested compounds and reference drugs were prepared in Mueller–Hinton broth (Guangdong huaikai microbial sci. & tech co., Ltd, Guangzhou, Guangdong, China) to obtain the required concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5 μg/mL. These dilutions were inoculated and incubated at 37 ºC for 24 h.

### 1.2.2 Antifungal Assays

The newly synthesized compounds were evaluated for their antifungal activities against *Candida albicans* ATCC10231, *Candida mycoderma* ATCC9888, *Candida utilis* ATCC9950, *Aspergillus flavus* ATCC204304 and *Beer yeast*. 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 $1-5 \times 10^3$ spore $mL^{-1}$. From the stock solutions of the tested compounds and reference antifungal drug Fluconazole, dilutions in sterile RPMI 1640 medium (Neuronbc Laboraton Technology CO, Ltd, Beijing, China) were made resulting in eleven wanted concentrations (0.5 to 512 μg/mL) of each tested compound. These dilutions were inoculated and incubated at 35 ºC for 24 hours.

**Table S1** Antifungal data as MIC (μg/mL) for compounds **4–7**[a].

| Compds | Fungi | | | | |
|---|---|---|---|---|---|
| | *C. albicans* | *C. mycoderma* | *C. utilis* | *A. flavus* | *B. yeast* |
| **4** | 128 | 128 | 512 | 128 | 1 |
| **5** | 512 | 512 | 512 | 512 | 256 |
| **6a** | 4 | 128 | 256 | 512 | 512 |
| **6b** | 32 | 8 | 16 | 8 | 32 |
| **6c** | 2 | 8 | 8 | 8 | 32 |
| **6d** | 512 | 8 | 8 | 64 | 16 |
| **6e** | 256 | 64 | 16 | 256 | 64 |
| **6f** | 512 | 256 | 512 | 256 | 256 |
| **7a** | 4 | 64 | 32 | 16 | 0.5 |
| **7b** | 0.5 | 256 | >512 | 512 | 256 |
| **7c** | 16 | 256 | 32 | 16 | 16 |
| **7d** | 256 | 16 | 8 | 64 | 64 |
| **7e** | 256 | 8 | 512 | 128 | 512 |
| **7f** | 8 | 8 | 0.5 | 4 | 32 |
| **7g** | 8 | 64 | 8 | 32 | 256 |
| **7h** | 256 | 256 | 256 | 8 | 512 |
| **Fluconazole** | 1 | 4 | 8 | 256 | 16 |

[a]*C. albicans*, *Candida albicans* (ATCC76615); *C. mycoderma*, *Candida mycoderma*; *C. utilis*, *Candida utilis*; *S. cerevisia*, *Saccharomyces cerevisia*; *A. flavus*, *Aspergillus flavus*.

## 1. 3 Development of Aqueous Solubility in Phosphate Buffer (pH 7.4)

A standard solution was prepared by precisely weighing amount (generally 1 mg) of sample and dissolving in 10 mL $CH_3OH$. The standard solution was scanned by UV to determine the wavelength of maximum absorbance, diluting as necessary. A saturated solution was prepared by stirring magnetically a small volume of pH 7.4 phosphate buffer solution in the presence of excess sample for 3 h, then the excess solid compound was filtered. Afterwards, the saturated solution was scanned by UV at the wavelength of the absorption maximum. Total solubility was dedermined by the relationship C' = A'C/A. Where C = concentration of standard solution in mg/mL, A = absorbance of the standard solution (correcting for any dilutions), A' = absorbance of the saturated solution (correcting for any dilutions), and C' = concentration of saturated solution in mg/mL.

**Table S2** ClogP values of compounds **6a**, **6d-e**, **7b**, **7d**, **7e** and **7g**[b].

| Compds | ClogP | Compds | ClogP |
|---|---|---|---|
| **6a** | 1.59 | **7d** | 3.54 |
| **6d** | 4.76 | **7e** | 3.54 |
| **6e** | 5.82 | **7g** | 4.25 |
| **7b** | 2.97 | | |

[b]ClogP values were calculated by ChemDraw Ultra 14.0.

## 1. 4 Time−Kill Kinetics Assay

The bactericidal activity evaluated by performing time kill kinetics assay. This gives the imformation about the rate at which the compounds are acting on bacteria. Briefly, MRSA bacterium was grown in suitable growth medium at 37 °C for 6 h and diluted. Compound **7a** was added to the bacterial solution (MRSA of approximately $1.8 \times 10^5$ CFU/mL) at concentrations of 4 × MIC in a 96-well plate and the mixture was incubated at 37 °C. At different time intervals (0, 1, 2, 3, 4, 5, 6, 7h), aliquots (20 μL) from the solution were taken out and serially diluted 10-fold in 0.9% sodium chloride. Then 20 μL of the dilutions was plated on yeast−dextrose agar plates and incubated at 37 °C for 24 h. The bacterial colonies were counted, and results are represented in logarithmic scale: $\log_{10}$(CFU/mL) vs time (in hours).

## 1. 5 Development of Resistance to Compound 7a

Considering the high-level resistance of Norfloxacin to MRSA strains, we selected the representative compound **7a** to investigate the developing rate of bacterial resistance according to the paper "J. Hoque; M. M. Konai; S. Gonuguntla; G. B. Manjunath; S. Samaddar; V. Yarlagadda and J. Halar. *J. Med. Chem.*, 2015, **58**, 5486". We exposed a standard strain of MRSA towards increasing concentrations of compound **7a** from original MIC for sustained passages and determined the MIC values of compound **7a** for each passage of MRSA.

## 1.6 Interactions with Calf Thymus DNA

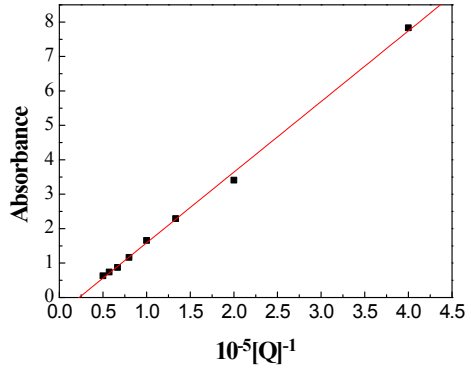



**Fig. S1** The plot of $A^0/(A-A^0)$ versus 1/[compound **7a**].

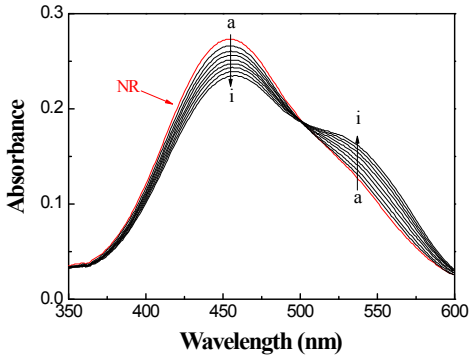



**Fig. S2** UV absorption spectra of NR in the presence of DNA at pH 7.4 and room temperature. *c*(NR) = $2 \times 10^{-5}$ mol/L, and *c*(DNA) = 0–$3.81 \times 10^{-5}$ mol/L for curves a–i respectively at increment $0.48 \times 10^{-5}$ mol/L.

## 1.7 DNA Cleavage Eexperiments

The plasmid pUC19 DNA was treated with different compounds in the buffer(5 mM Tris-HCl/50 mM NaCl, pH = 7.0) to a total volume of 20 μL. After incubation at 37 °C for a certain time, the solution was quenched by the addition of 6×loading buffer (2 μL). Then, the resulting solutions were loaded on a 0.8% agarose gel containing gold view dye. Gel electrophoresis was carried out at 100 V for 0.5 h in 1 × TAE buffer, and then the plasmid bands were visualized by a transilluminator and photographed.

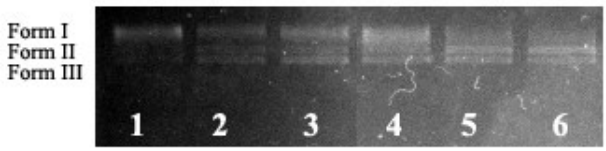



**Fig. S3** Agarose gel electrophoresis patterns for the cleavage of pUC19 plasmid DNA ($6.25 \times 10^{-3}$ μg/μL) by **7a**–$Zn^{2+}$ and berberine–$Zn^{2+}$ complexes in buffer (50 mM Tris-HCl/50 mM NaCl, pH = 7.0) at 37°C after 16 h of incubation. Lane 1: DNA control; lane 2: DNA + berberine (0.0125 mM); lane 3: DNA + **7a** (0.0125 mM); lane 4: DNA + $Zn^{2+}$ (0.0125 mM); lane 5: DNA + berberine (0.0125 mM) + $Zn^{2+}$ complex (0.0125 mM); lane 6: DNA + **7a** (0.0125 mM) + $Zn^{2+}$

(0.0125 mM).

## 1.8 Interactions of Compound 7a with HSA

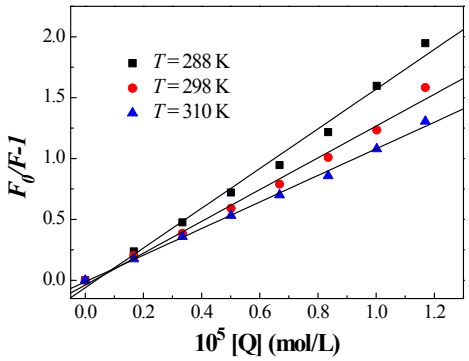



**Fig. S4** Stern–Volmer plots of **7a**–HSA system at different temperatures.

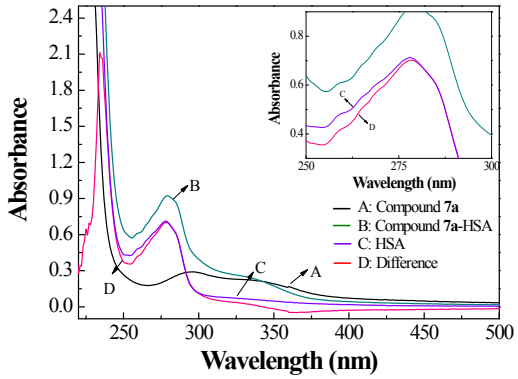



**Fig. S5** UV–vis spectra of HSA in the presence of compound **7a**: A, absorption spectrum of compound **7a** only; B, absorption spectrum of compound **7a**/HSA 1:1 complex; C, absorption spectrum of HSA only; D, difference between absorption spectrum of compound **7a**/HSA 1:1 complex and compound **7a**, *c*(HSA) = *c*(compound **7a**) = $1.0 \times 10^{-5}$ mol/L. The curves C and D for the wavelength ranging from 250–300 nm were depicted in the inset.

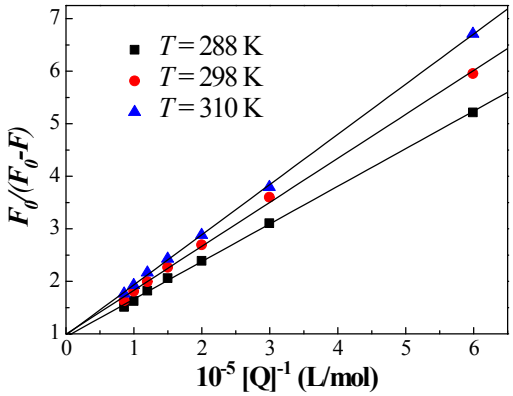



**Fig. S6** Modified Stern–Volmer plots of **7a**–HSA system at different temperatures.

**Table S3** Stern–Volmer quenching constants for the interaction of compound **7a** with HSA at various temperatures.

| pH | *T* (K) | $K_{SV}$ (L/mol) | $K_q$ (L/mol $s^{-1}$) | R[a] | S.D.[b] |
|---|---|---|---|---|---|
| | 288 | $1.63 \times 10^5$ | $2.55 \times 10^{13}$ | 0.995 | 0.0717 |
| 7.4 | 298 | $1.31 \times 10^5$ | $2.05 \times 10^{13}$ | 0.996 | 0.0532 |
| | 310 | $1.09 \times 10^5$ | $1.70 \times 10^{13}$ | 0.999 | 0.0256 |

R[a] is the correlation coefficient. S.D.[b] is standard deviation.

**Table S4** Binding constants and sites of **7a**–HSA system at pH = 7.4

| *T*(K) | Modified Stern–Volmer Method | | | Scatchard equation | | | |
|---|---|---|---|---|---|---|---|
| | $10^{-5}K_a$ (L/mol) | R | S.D | $10^{-5}K_b$ (L/mol) | R | S.D | *n* |
| 288 | 1.32 | 0.999 | 0.0345 | 3.35 | 0.998 | 0.022 | 1.07 |
| 298 | 1.19 | 0.999 | 0.0639 | 2.02 | 0.998 | 0.021 | 1.04 |
| 310 | 1.02 | 0.999 | 0.0324 | 1.26 | 0.999 | 0.012 | 1.01 |

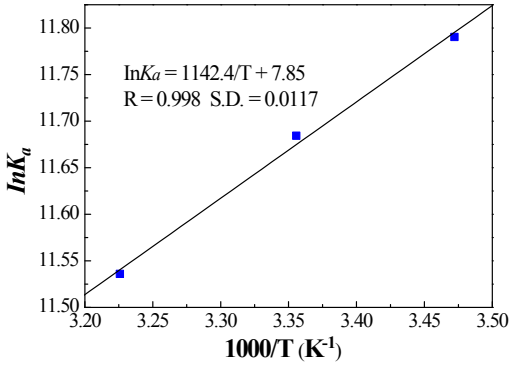



**Fig. S7.** Van't Hoff plots of the compound **7a**–HSA system.

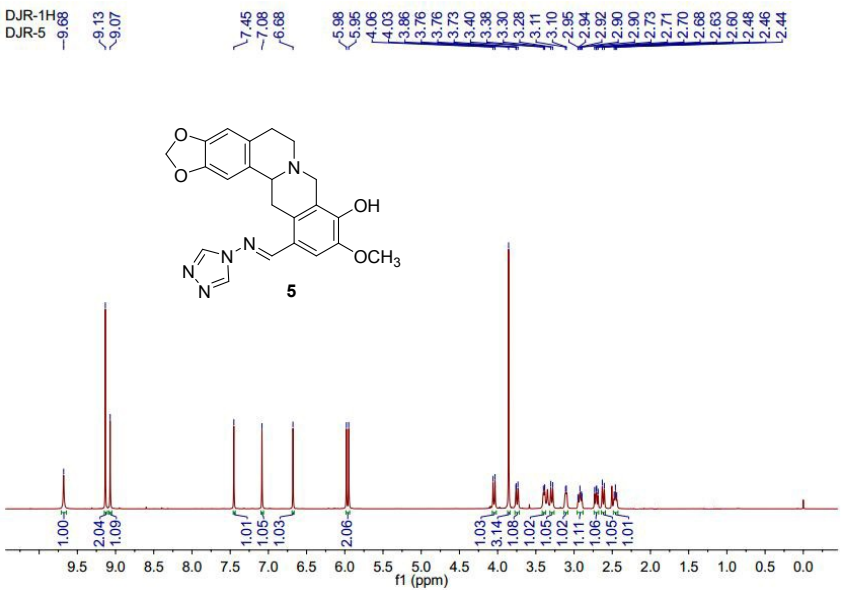
**Table S5** Thermodynamic parameters of **7a**–HSA system at different temperatures.

| *T* (K) | Δ*H* (kJ/mol) | Δ*G* (kJ/mol) | Δ*S* (J/mol·K) |
|---|---|---|---|
| 288 | | –28.23 | |
| 298 | –9.498 | –28.96 | 65.27 |
| 310 | | –29.72 | |

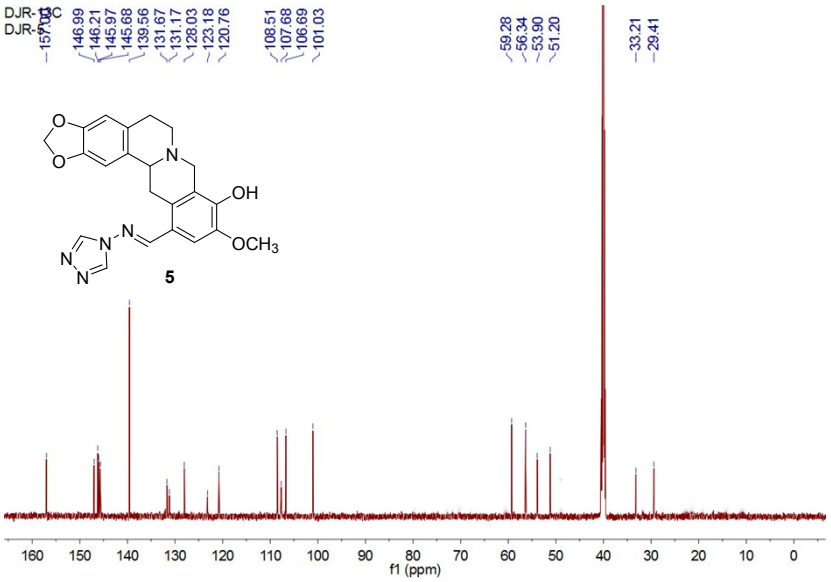
# 2 Some Representative Spectra

## 2.1 Spectra of Compound 5

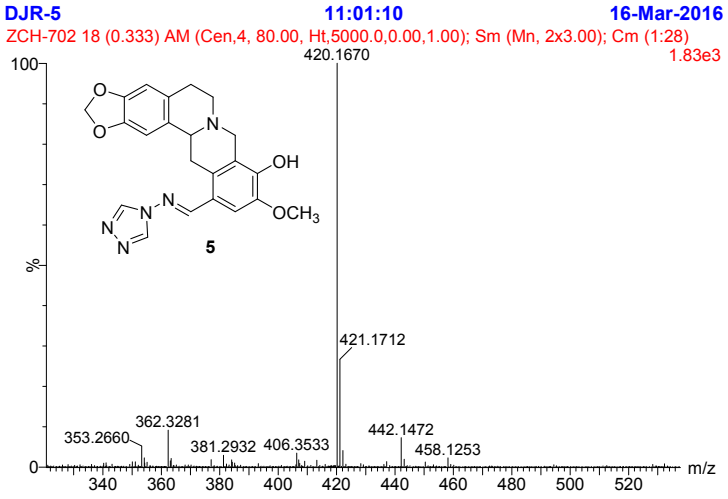
### $^1$H NMR Spectrum



**$^{13}$C NMR Spectrum**

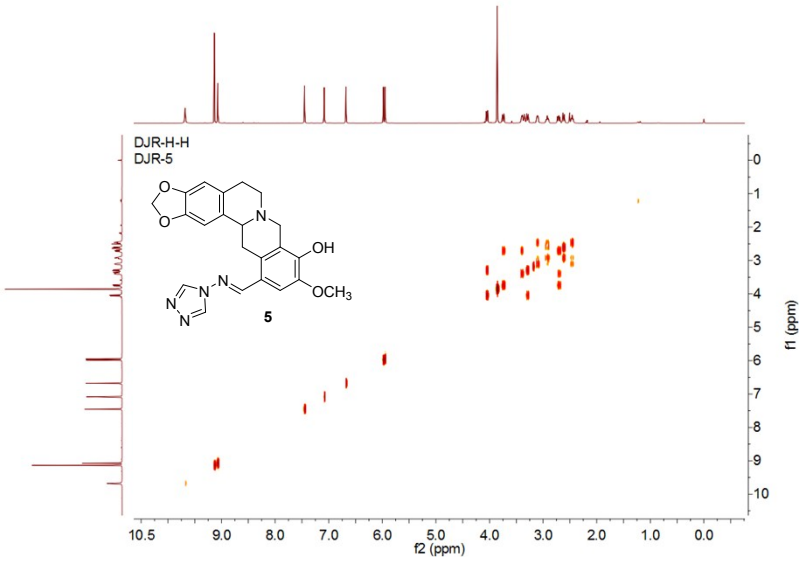



**HRMS Spectrum**

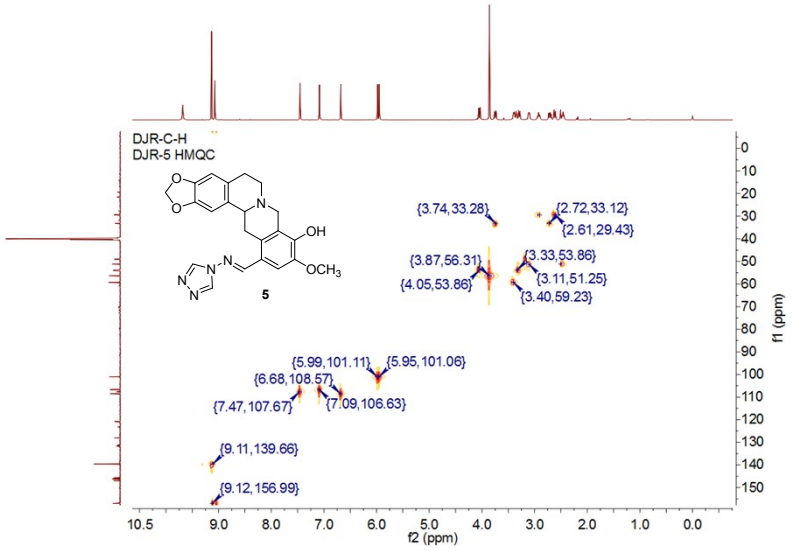



HR-MS (TOF) calcd for $C_{22}H_{21}N_5O_4$: $[M+H]^+$, 420.1672; found, 420.1670.

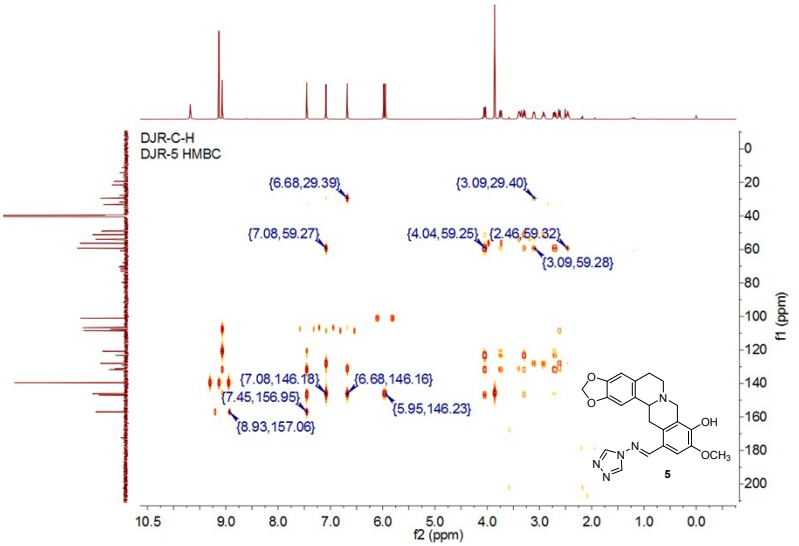
**$^1$H–$^1$H COSY Spectrum**



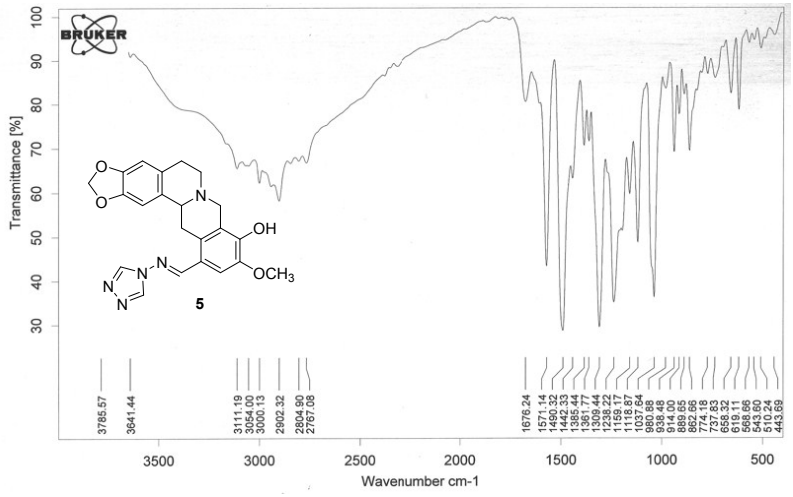
**HMQC Spectrum**




**HMBC Spectrum**

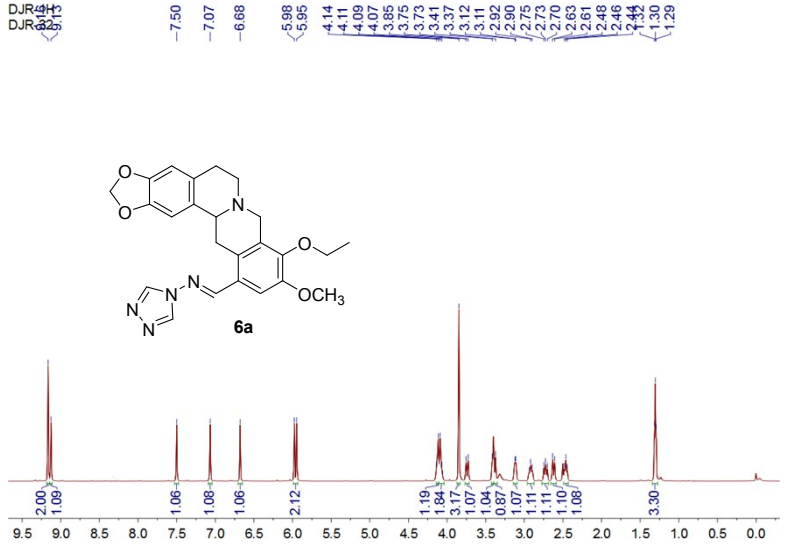



**IR Spectrum**

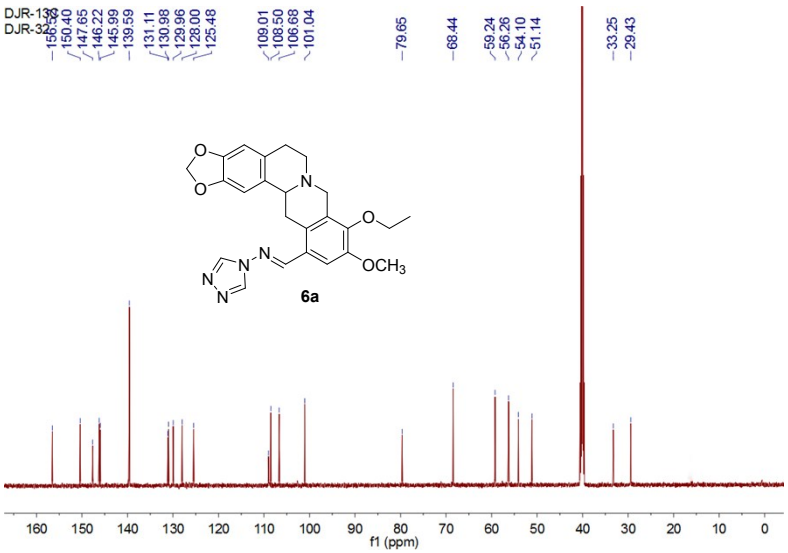


## 2.2 Spectra of Compound 6a

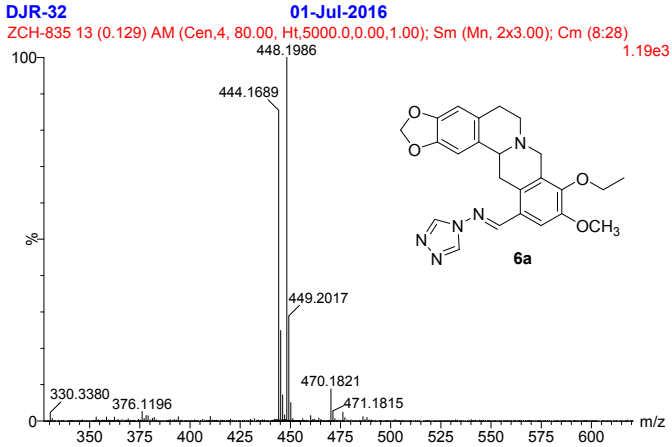
### $^{1}$H NMR Spectrum




### $^{13}$C NMR Spectrum

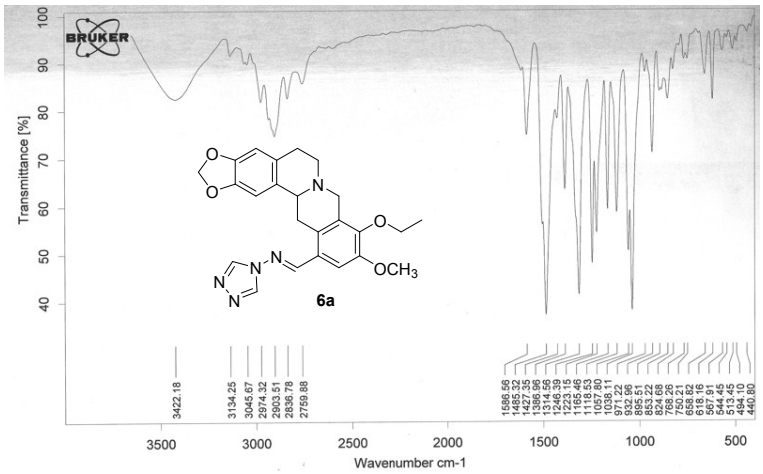



### HRMS Spectrum



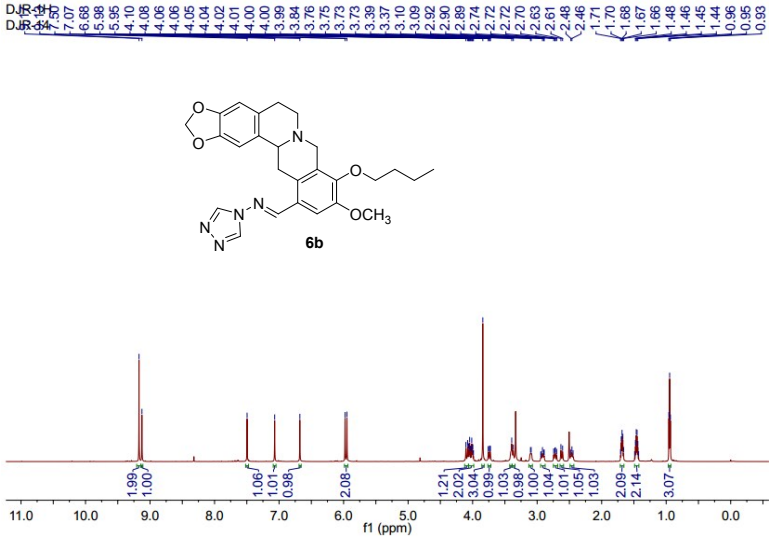

HR-MS (TOF) calcd for $C_{24}H_{25}N_5O_4$: $[M+H]^+$, 448.1985; found, 448.1986.

**IR Spectrum**

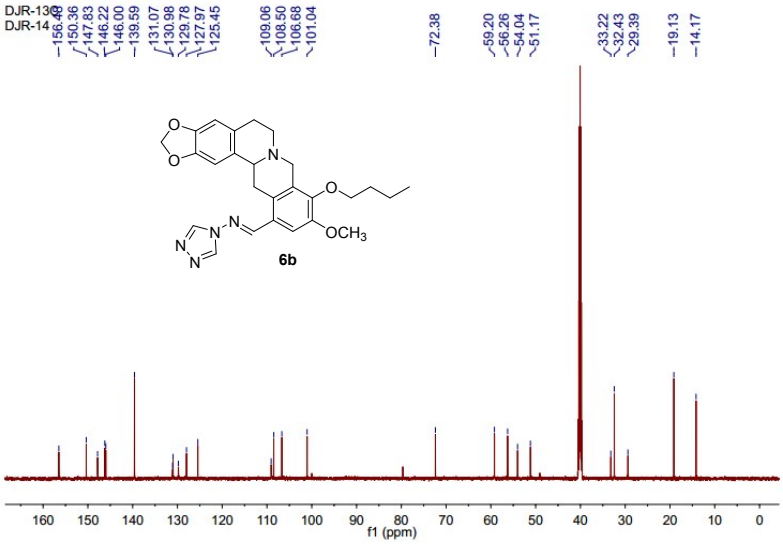



## 2.3 Spectra of Compound 6b

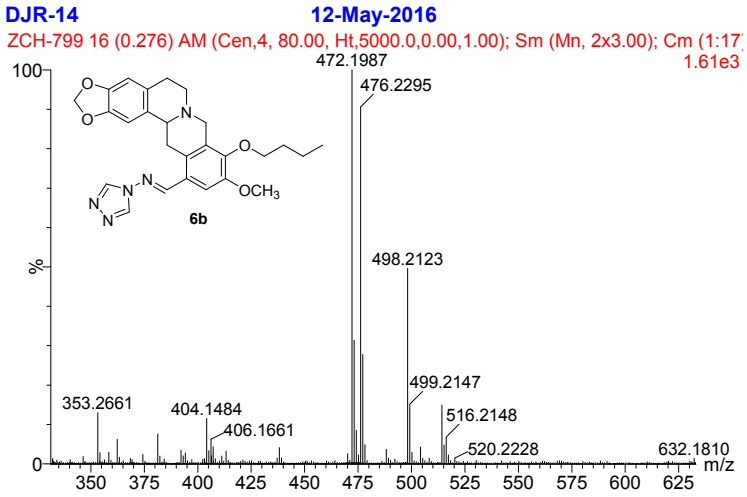
**$^{1}$H NMR Spectrum**




**$^{13}$C NMR Spectrum**

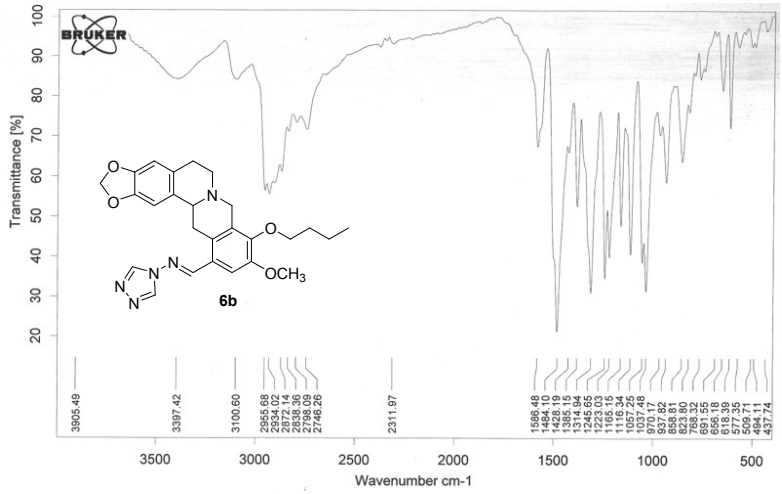


**HRMS Spectrum**



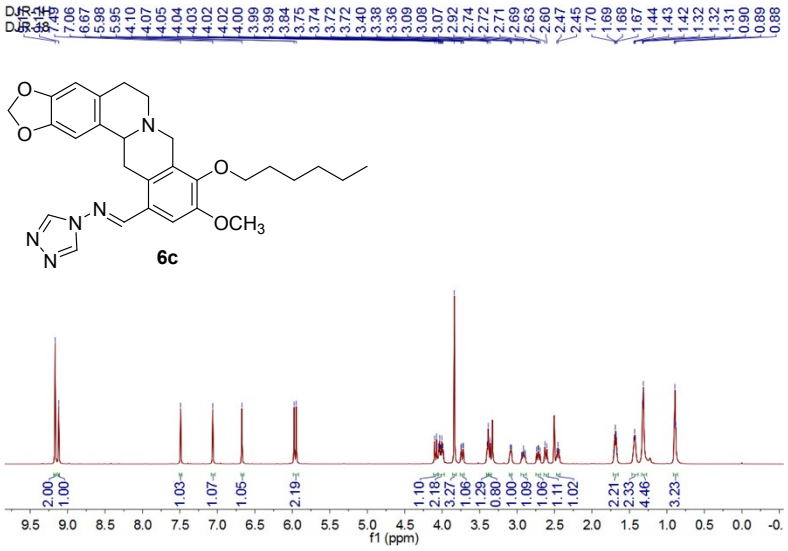

HR-MS (TOF) calcd for $C_{26}H_{29}N_5O_4$: $[M+H]^+$, 476.2298; found, 476.2295.

**IR Spectrum**

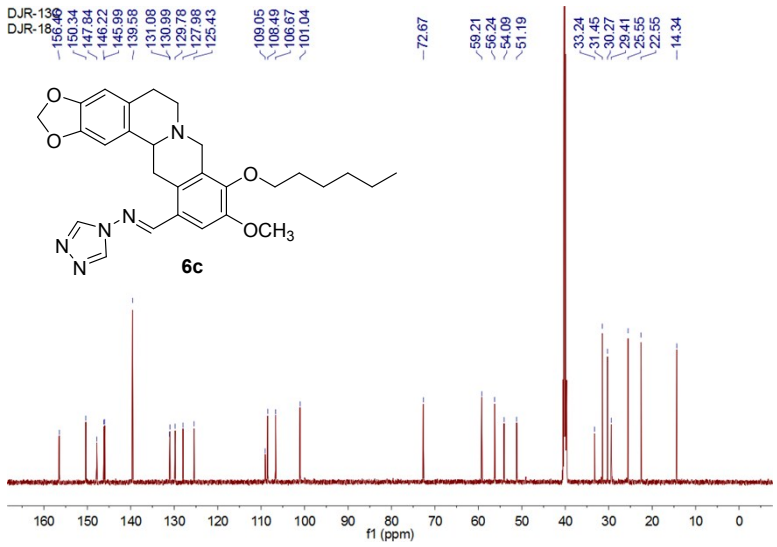



## 2.4 Spectra of Compound 6c

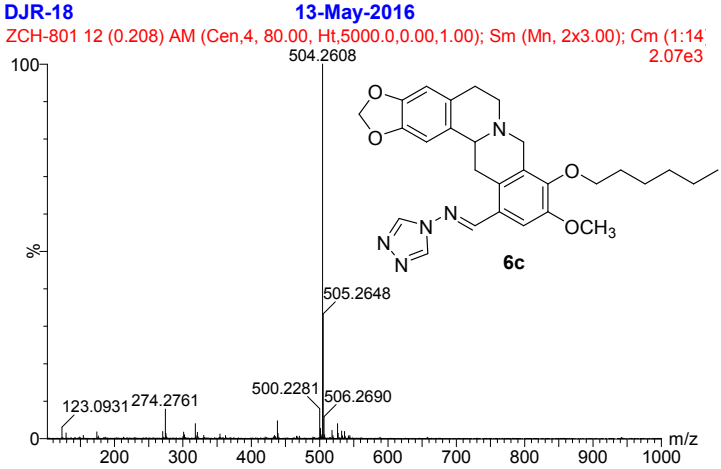
**$^1$H NMR Spectrum**



**$^{13}$C NMR Spectrum**

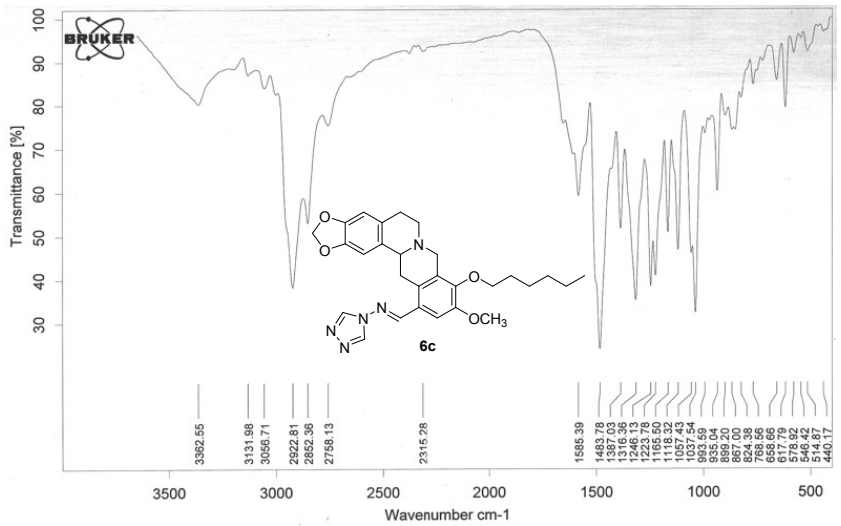



**HRMS Spectrum**



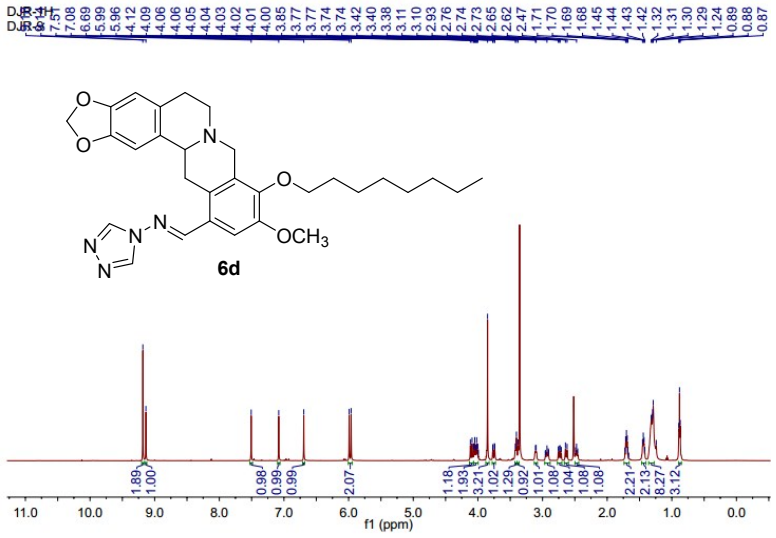

HR-MS (TOF) calcd for $C_{28}H_{33}N_5O_4$: $[M+H]^+$, 504.2611; found, 504.2608.

**IR Spectrum**

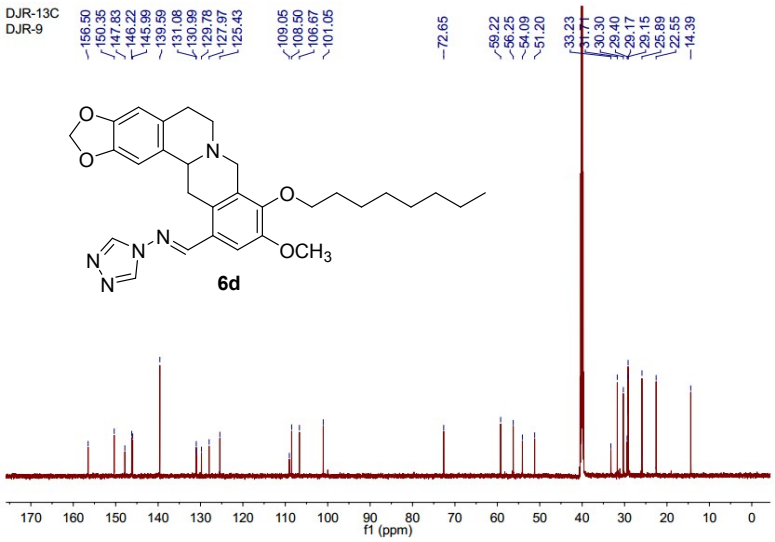



**2.5 Spectra of Compound 6d**

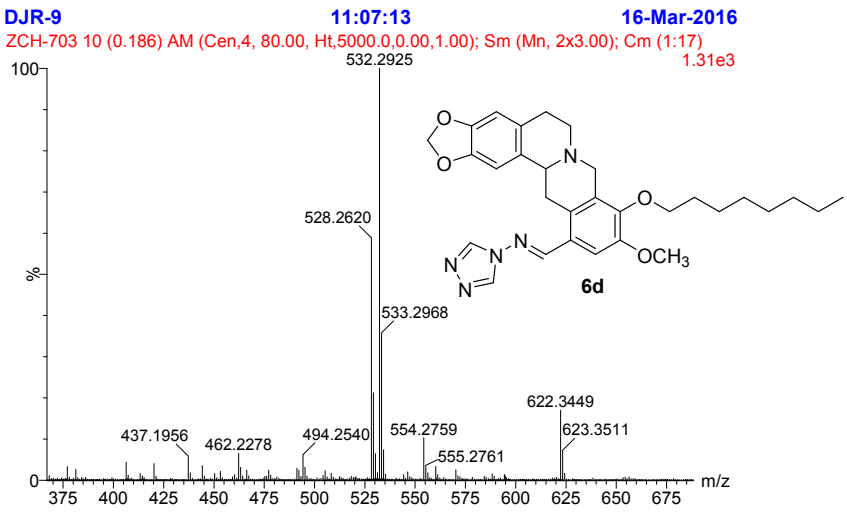
**$^{1}$H NMR Spectrum**




**$^{13}$C NMR Spectrum**

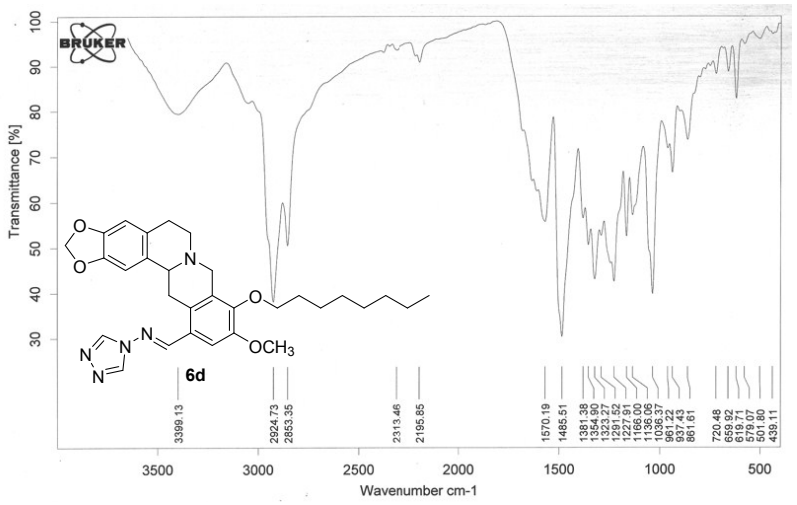



**HRMS Spectrum**



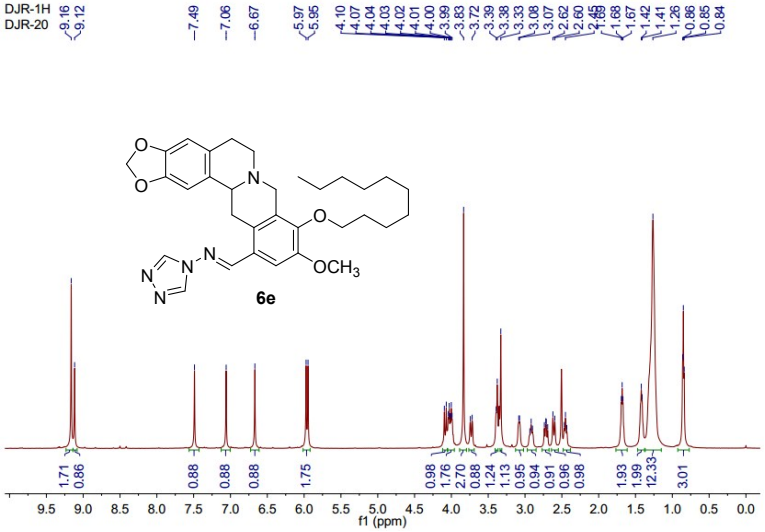

HR-MS (TOF) calcd for $C_{30}H_{37}N_5O_4$: $[M+H]^+$, 532.2924; found, 532.2925.

**IR Spectrum**

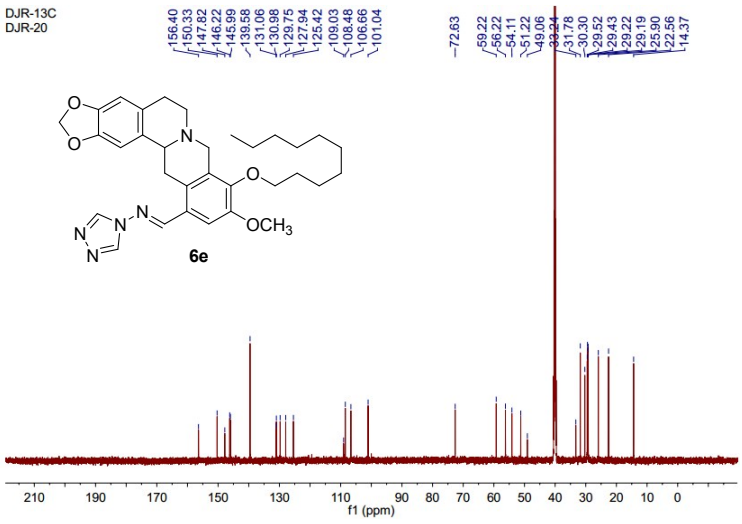



## 2.6 Spectra of Compound 6e

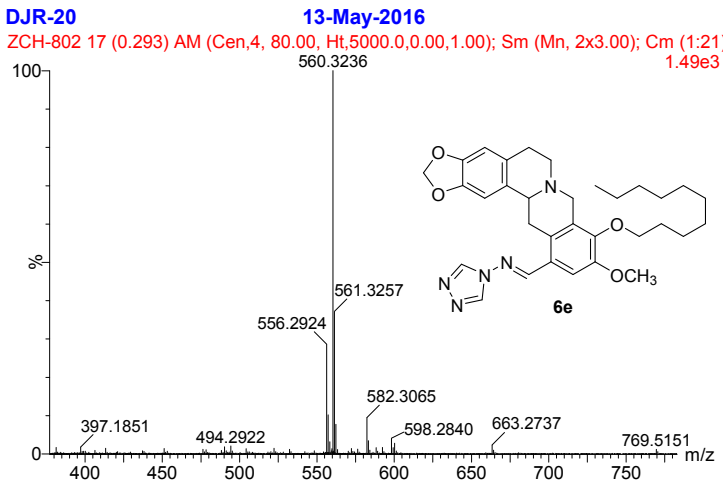
**$^{1}$H NMR Spectrum**




**$^{13}$C NMR Spectrum**

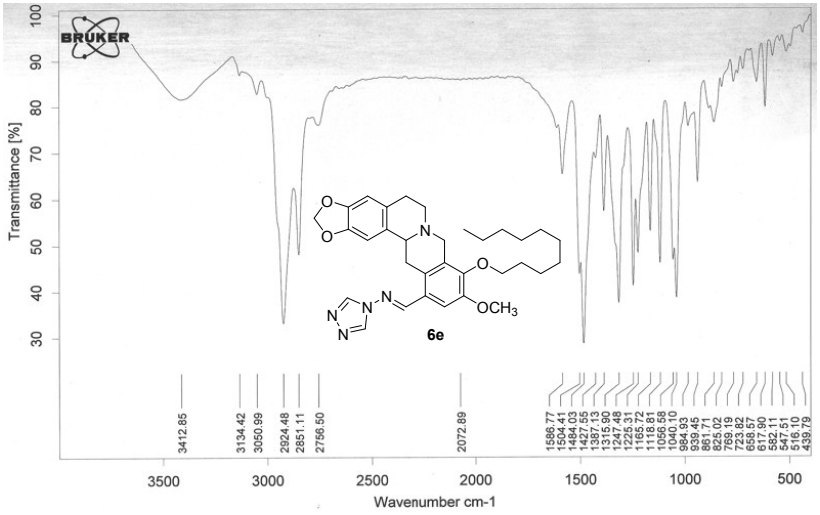


**HRMS Spectrum**



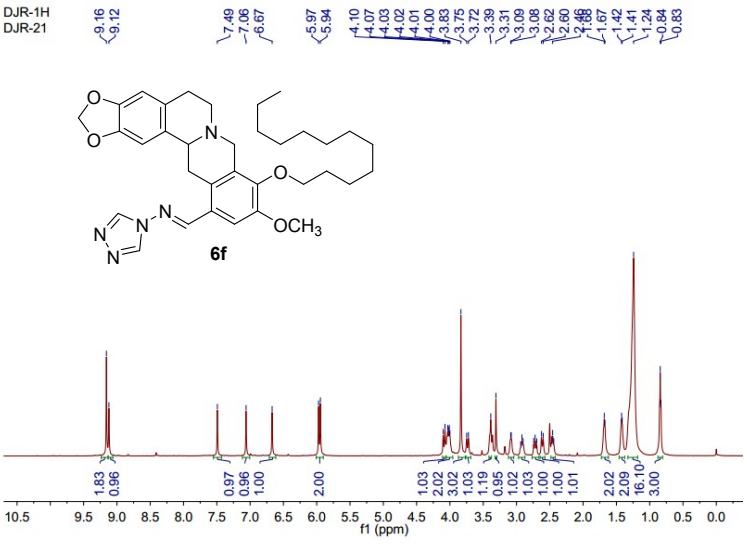

HR-MS (TOF) calcd for $C_{32}H_{41}N_5O_4$: $[M+H]^+$, 560.3237; found, 560.3236.

**IR Spectrum**

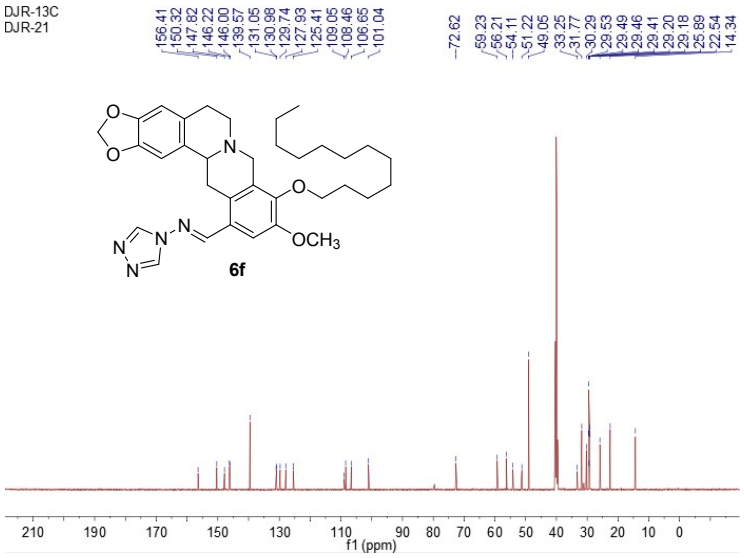



## 2.7 Spectra of Compound 6f

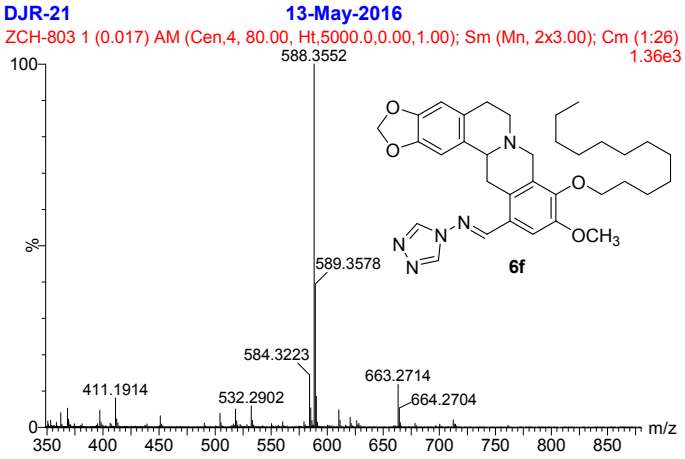
**$^1$H NMR Spectrum**



**$^{13}$C NMR Spectrum**

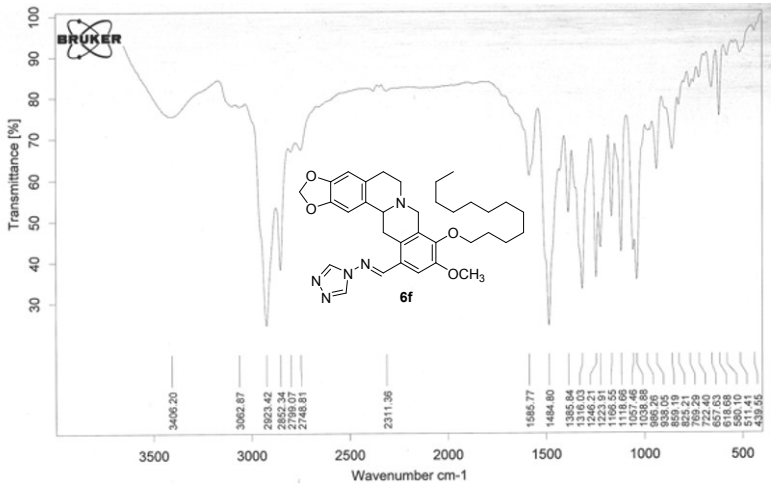



**HRMS Spectrum**



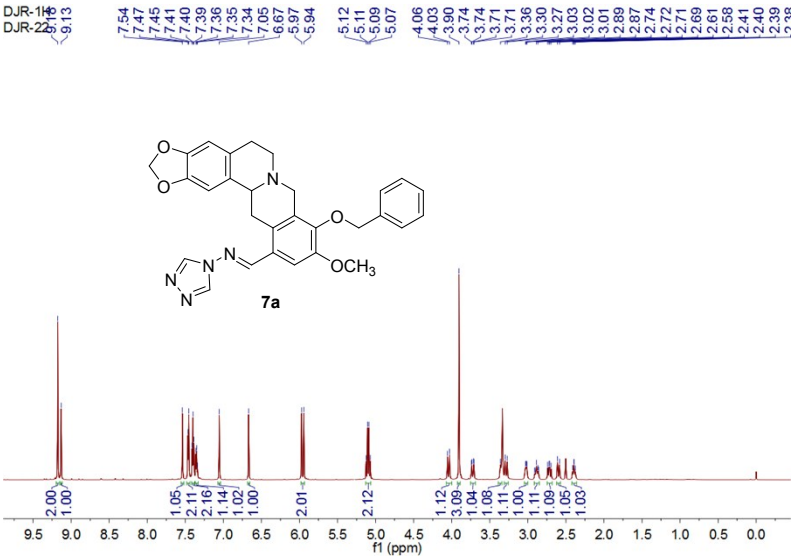

HR-MS (TOF) calcd for $C_{34}H_{45}N_5O_4$: $[M+H]^+$, 588.3550; found, 588.3552.

**IR Spectrum**

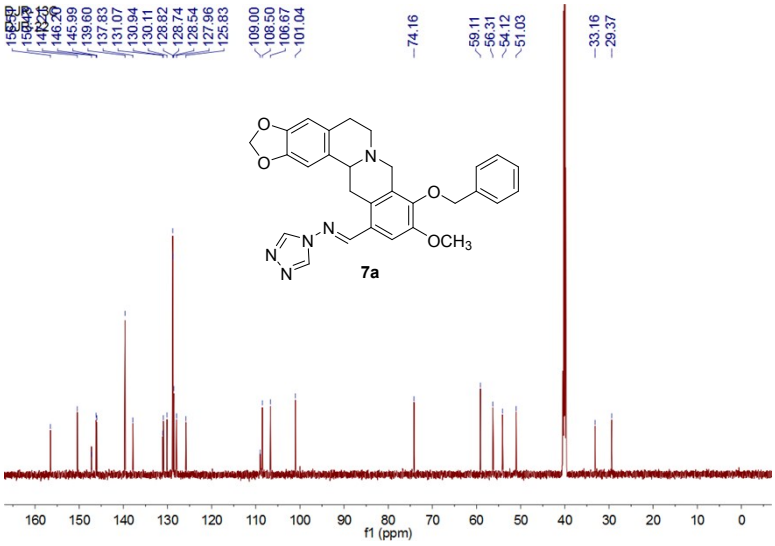



## 2.8 Spectra of Compound 7a

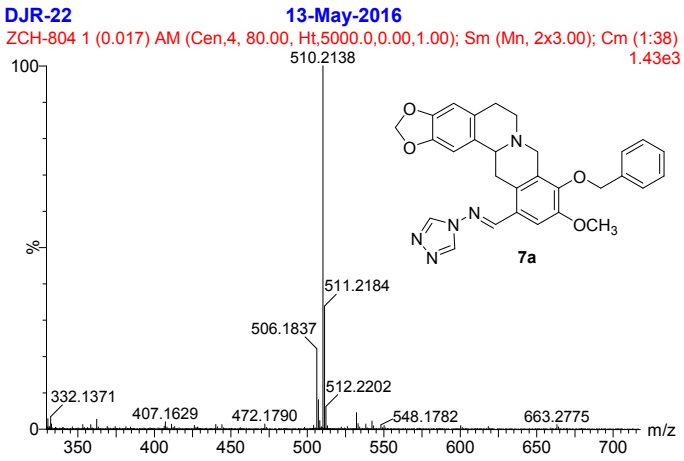
**$^{1}$H NMR Spectrum**




**$^{13}$C NMR Spectrum**

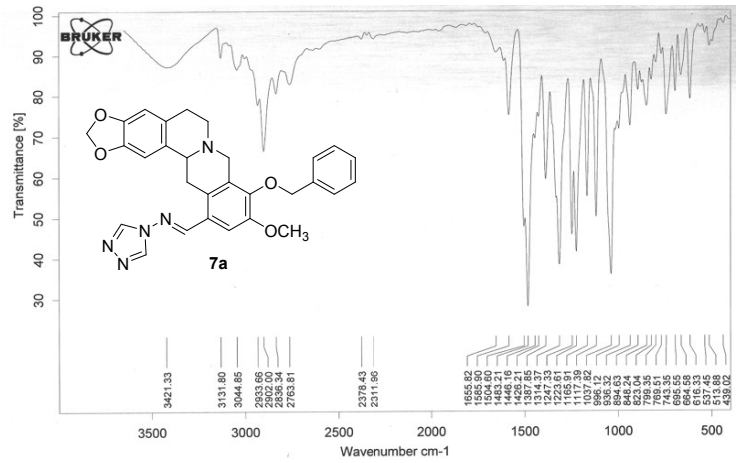



**HRMS Spectrum**



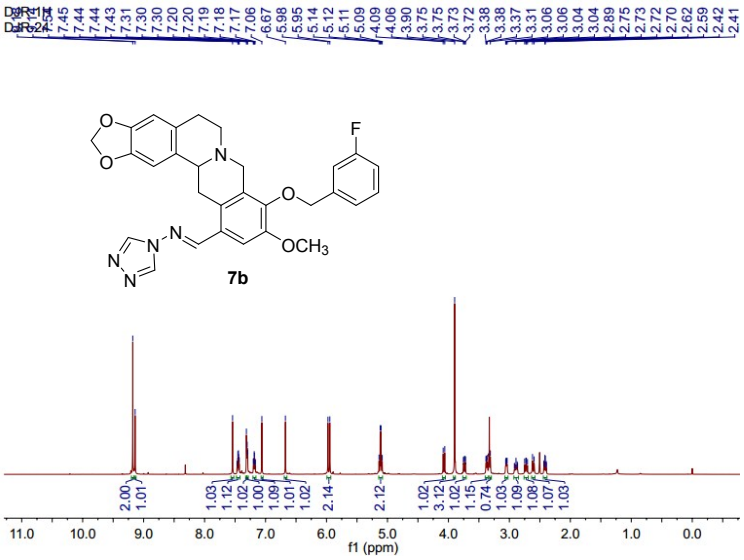

HR-MS (TOF) calcd for $C_{29}H_{27}N_5O_4$: $[M+H]^+$, 510.2138; found, 510.2141.

**IR Spectrum**

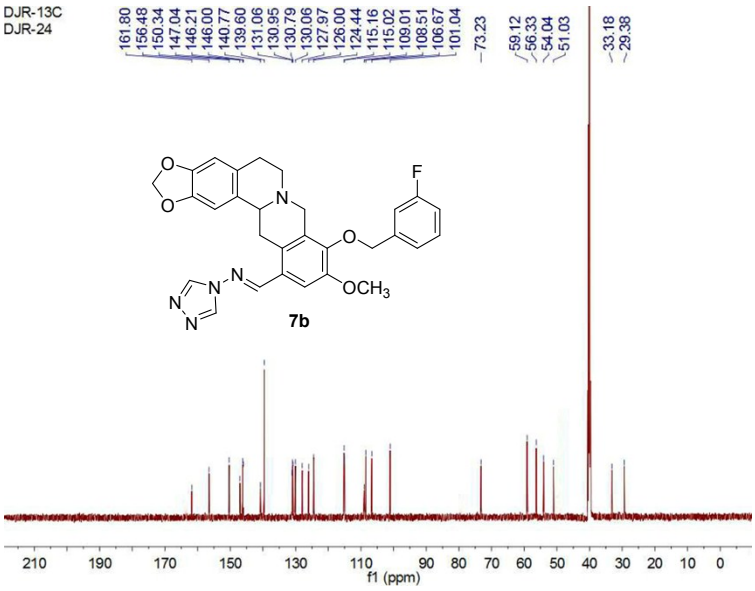



## 2.9 Spectra of Compound 7b

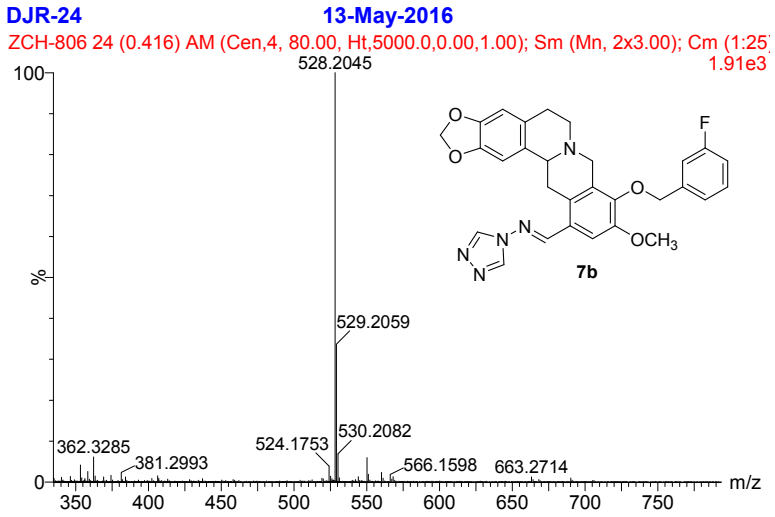
### $^{1}$H NMR Spectrum




### $^{13}$C NMR Spectrum

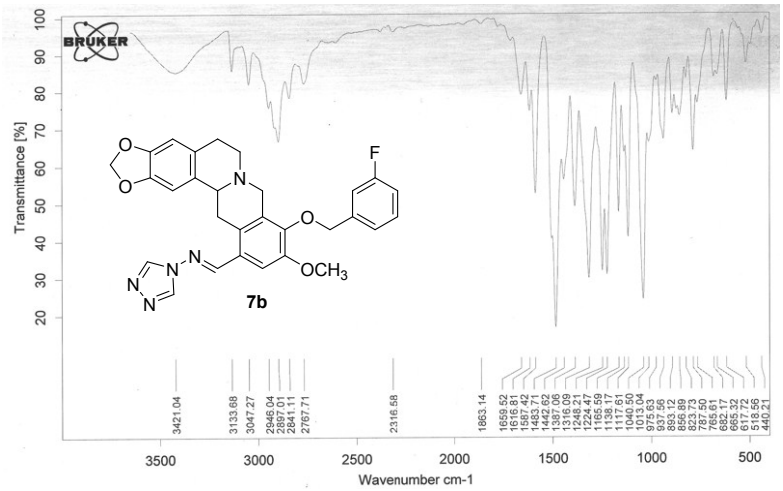


**HRMS Spectrum**



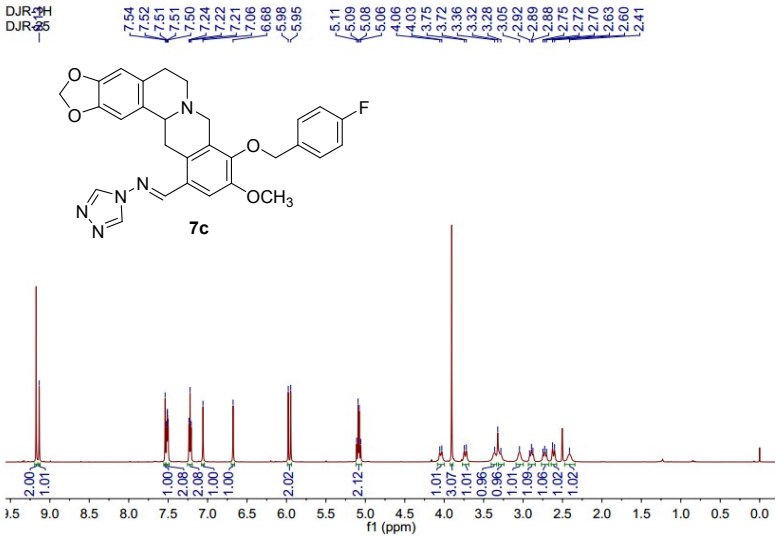

HR-MS (TOF) calcd for $C_{29}H_{26}FN_5O_4$: $[M+H]^+$, 528.2047; found, 528.2045.

**IR Spectrum**

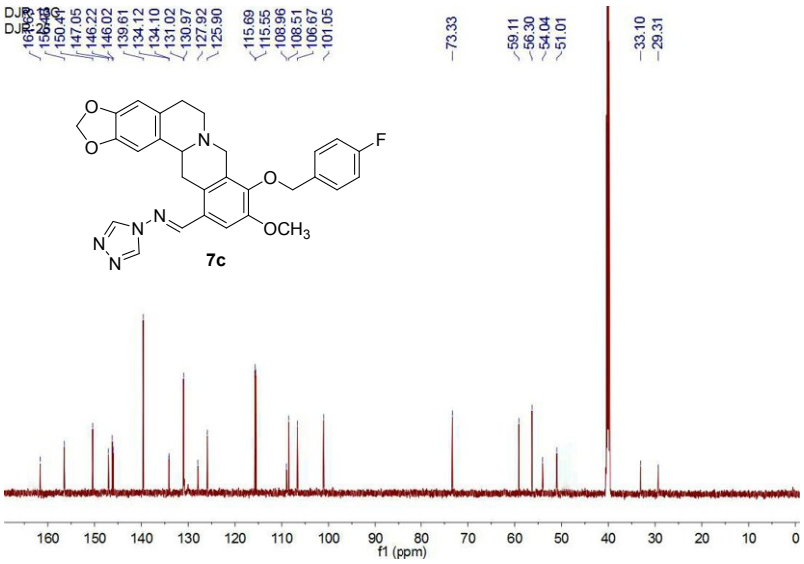



## 2.10 Spectra of Compound 7c

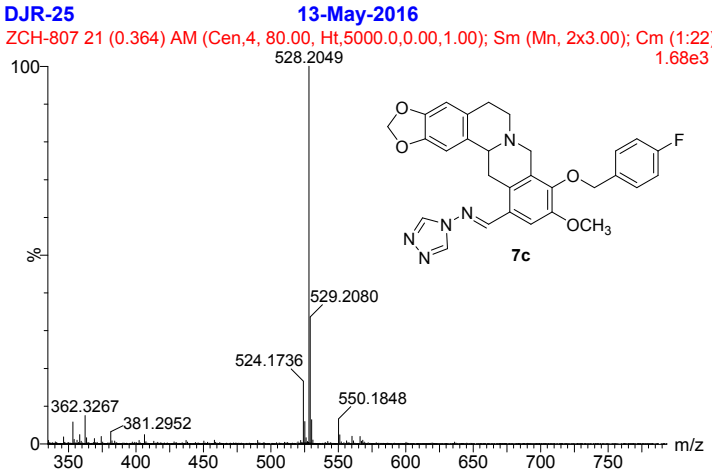
**$^1$H NMR Spectrum**



**$^{13}$C NMR Spectrum**

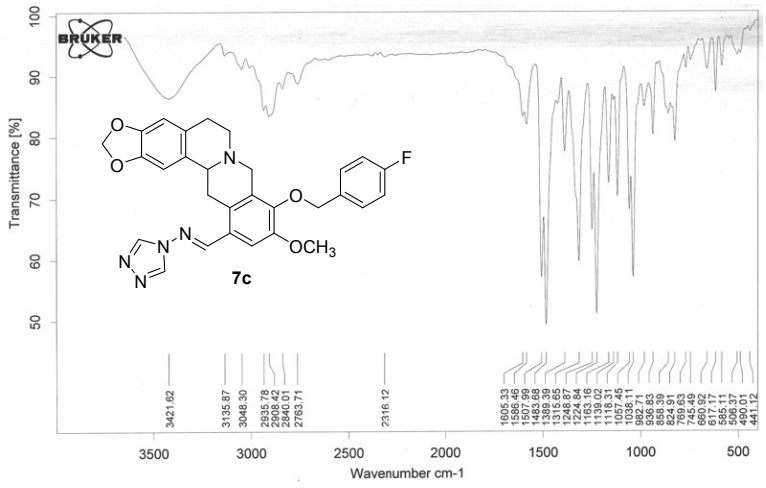



**HRMS Spectrum**



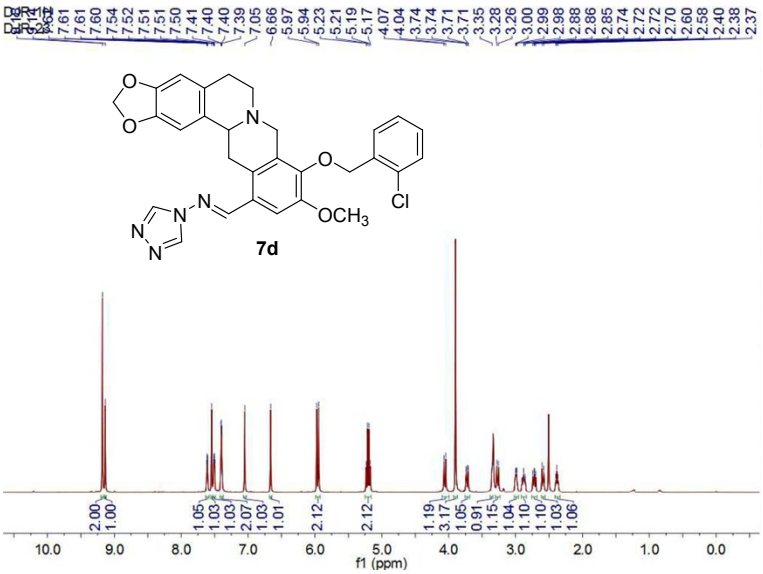

HR-MS (TOF) calcd for $C_{29}H_{26}FN_5O_4$: $[M+H]^+$, 528.2047; found, 528.2049.

**IR Spectrum**

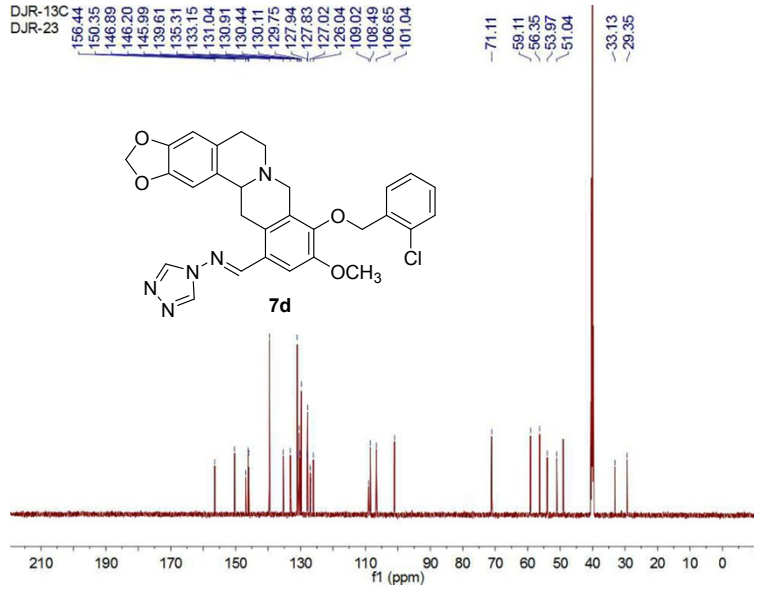



**2.11 Spectra of Compound 7d**

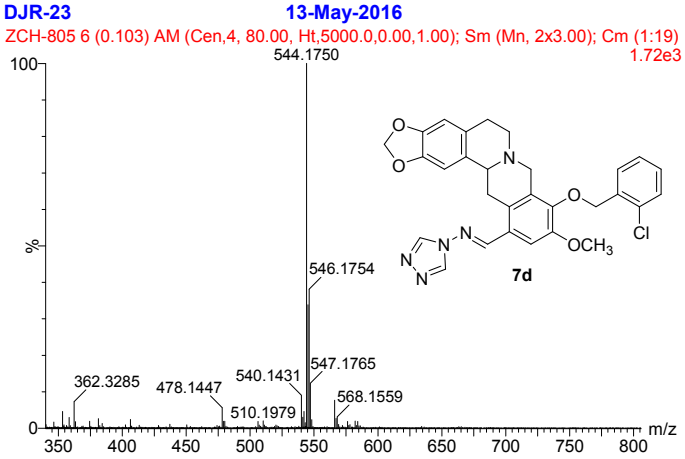
**$^{1}$H NMR Spectrum**




**$^{13}$C NMR Spectrum**

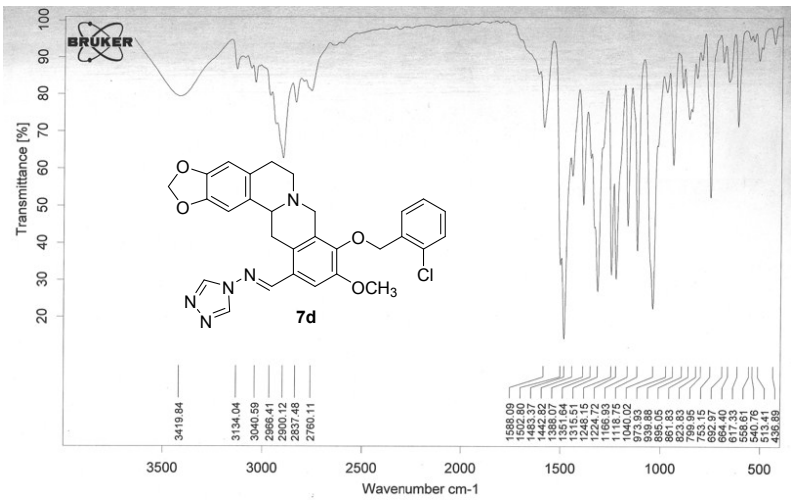



**HRMS Spectrum**



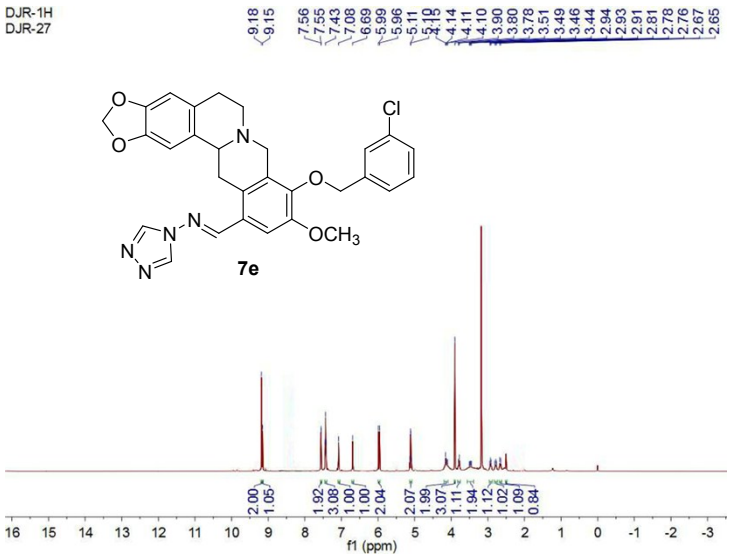

HR-MS (TOF) calcd for $C_{29}H_{26}ClN_5O_4$: $[M+H]^+$, 544.1752; found, 544.1750.

**IR Spectrum**

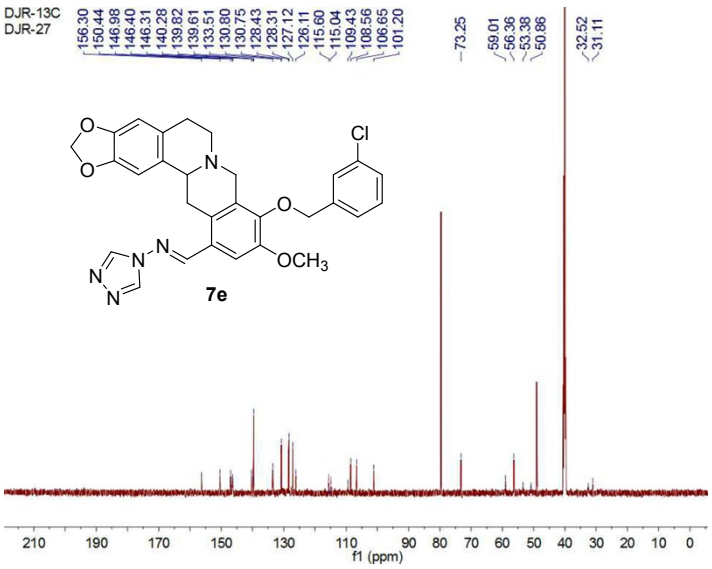



## 2.12 Spectra of Compound 7e

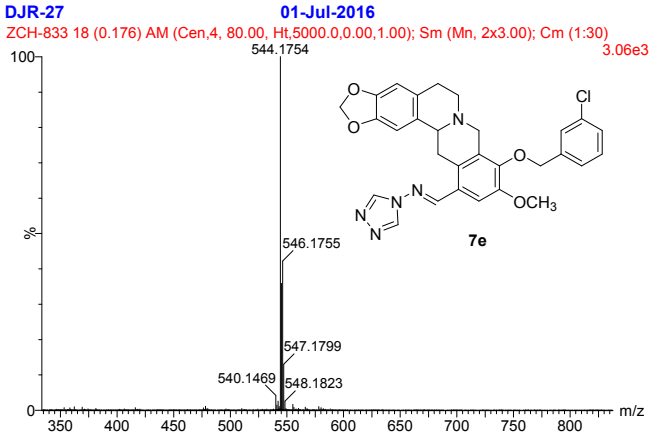
**$^{1}$H NMR Spectrum**




**$^{13}$C NMR Spectrum**

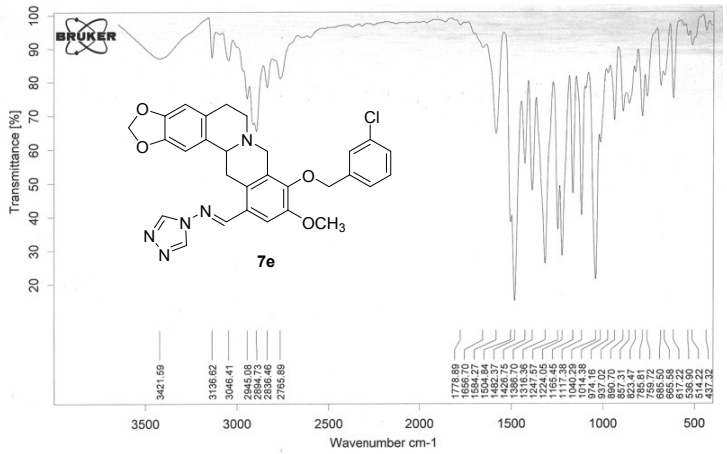


**HRMS Spectrum**



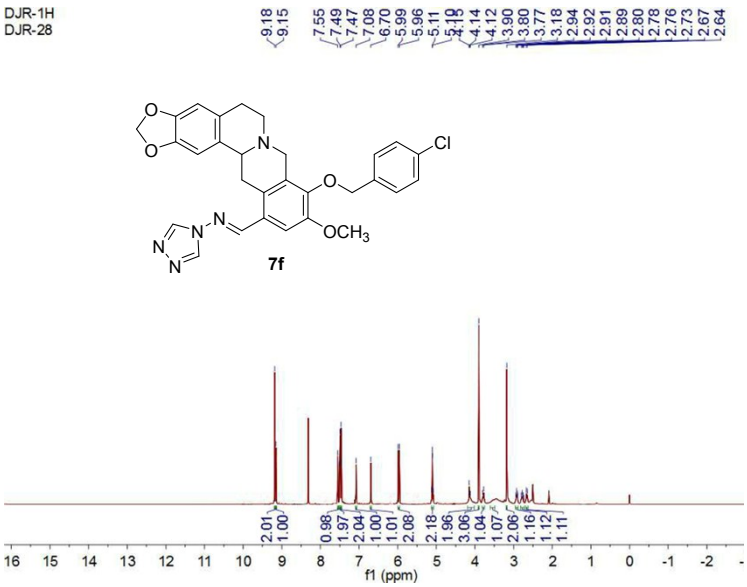

HR-MS (TOF) calcd for $C_{29}H_{26}ClN_5O_4$: $[M+H]^+$, 544.1752; found, 544.1754.

**IR Spectrum**

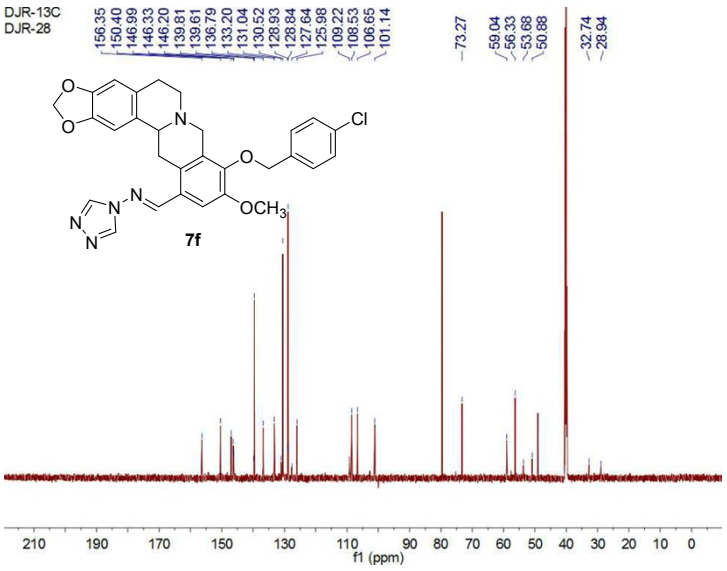



## 2.13 Spectra of Compound 7f

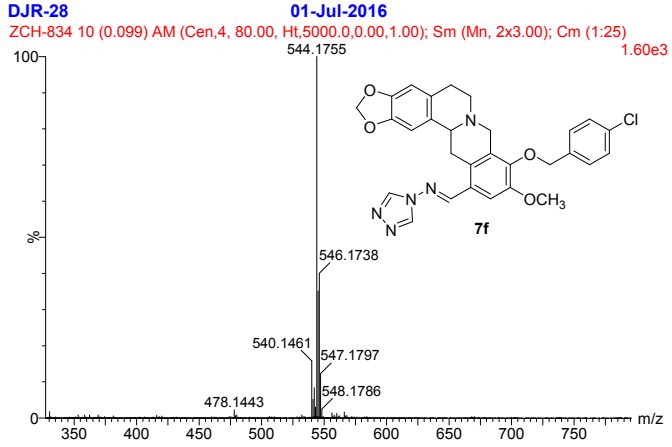
**$^1$H NMR Spectrum**




**$^{13}$C NMR Spectrum**

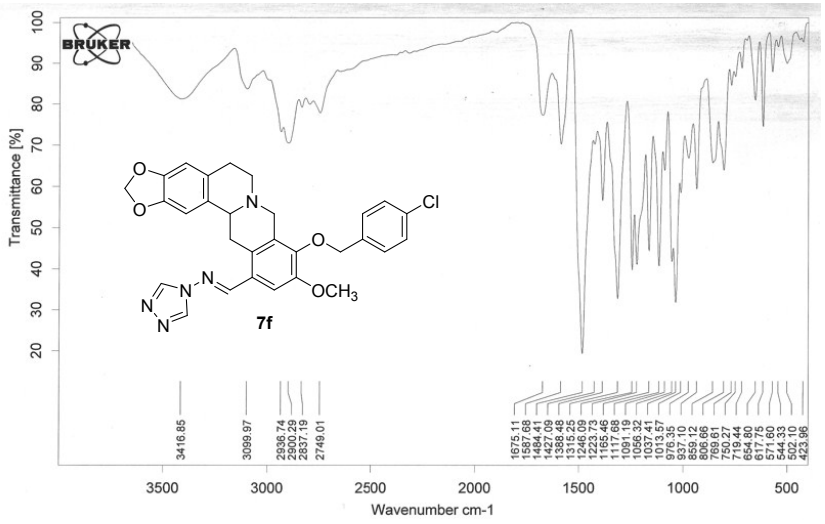



**HRMS Spectrum**



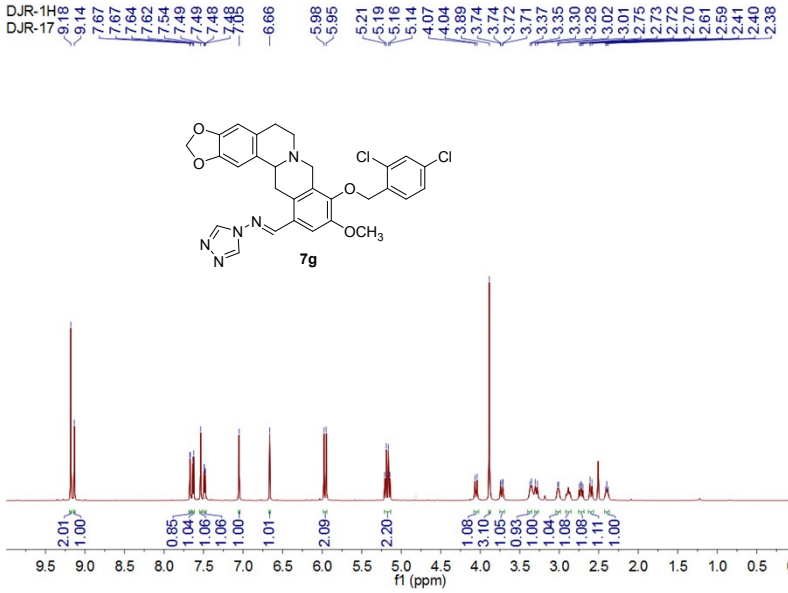

HR-MS (TOF) calcd for $C_{29}H_{26}ClN_5O_4$: $[M+H]^+$, 544.1752; found, 544.1755.

**IR Spectrum**

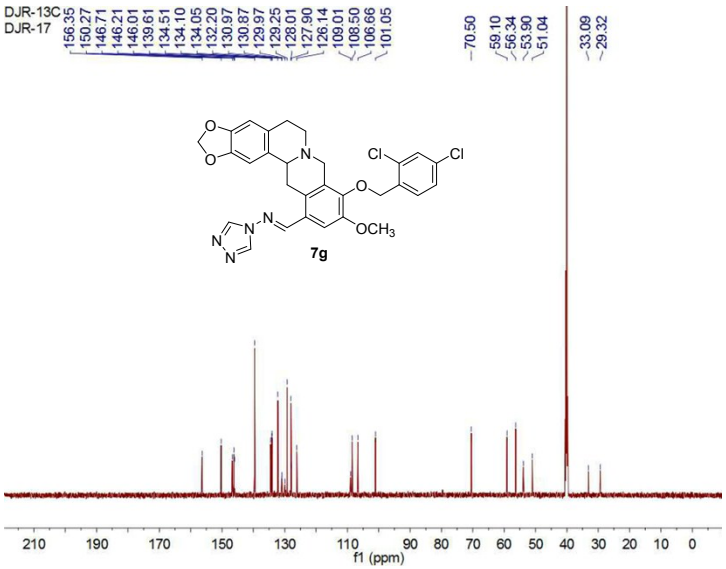



## 2.14 Spectra of Compound 7g

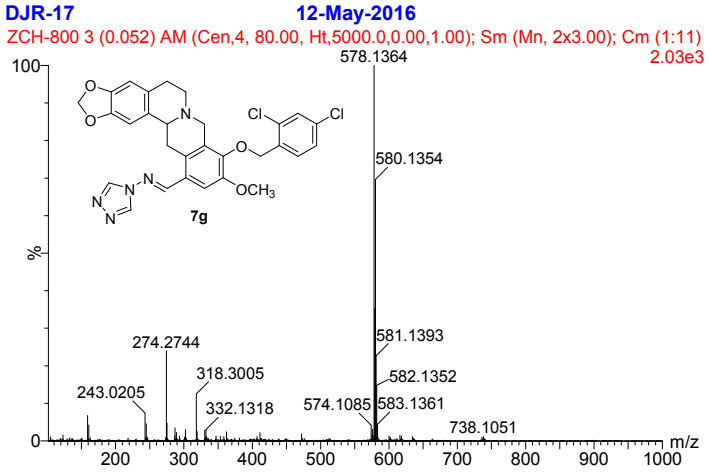
**$^1$H NMR Spectrum**




## $^{13}$C NMR Spectrum

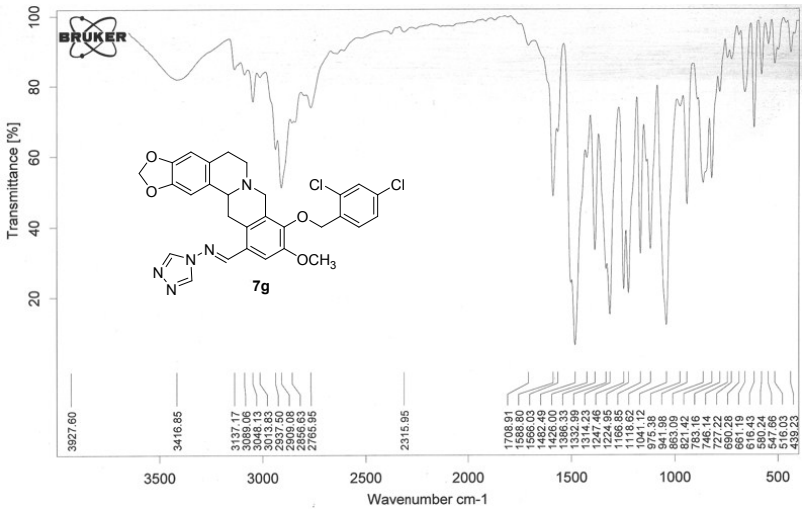



## HRMS Spectrum



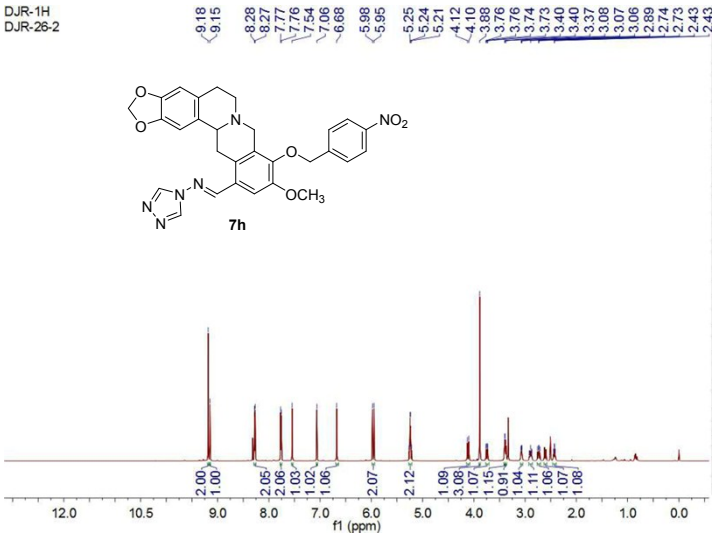
HR-MS (TOF) calcd for $C_{29}H_{25}Cl_2N_5O_4$: $[M+H]^+$, 578.1362; found, 578.1364.

**IR Spectrum**

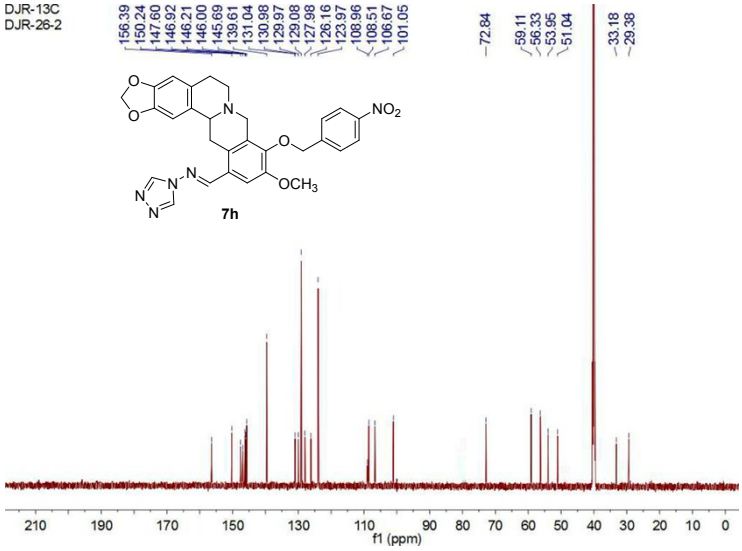



## 2.15 Spectra of Compound 7h

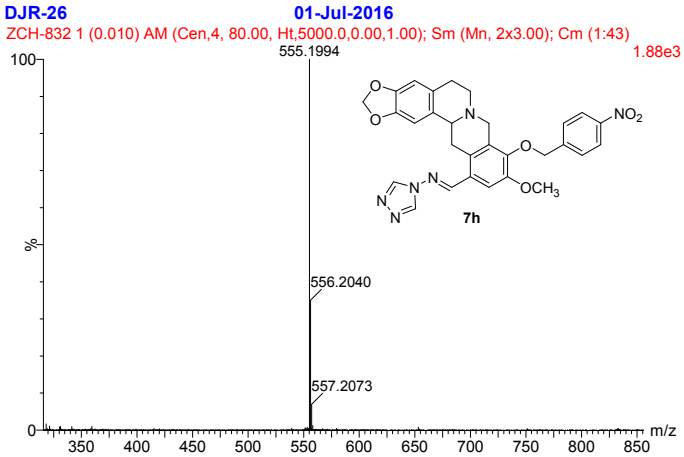
**$^1$H NMR Spectrum**




**$^{13}$C NMR Spectrum**




## HRMS Spectrum




## IR Spectrum