Green and Facile Assembly of Diverse Fused N-Heterocycles using Gold-Catalyzed Cascade Reactions in Water

Supplementary Materials

Xiuwen Jia[1], Pinyi Li[1], Xiaoyan Liu[1], Jiafu Lin[1*], Yiwen Chu[1], Jinhai Yu[2], Jiang Wang[3,4], Hong Liu[3,4*] and Fei Zhao[1*]

[1] Antibiotics Research and Re-evaluation Key Laboratory of Sichuan Province, Sichuan Industrial Institute of Antibiotics, Chengdu University, Chengdu 610052, China; jiaxiuwen2018@126.com (X.J.); pinyiLi19950206@126.com (P.L.); 19940826097@163.com (X.L.); siiakyb@139.com (Y.C.)

[2] School of Biological Science and Technology, University of Jinan, Jinan 250022, China; bio_yujh@ujn.edu.cn

[3] State Key Laboratory of Drug Research and CAS Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China; jwang@simm.ac.cn

[4] University of Chinese Academy of Sciences, Beijing 100049, China.

***** Correspondence: linjiafu@cdu.edu.cn (J.L.); hliu@simm.ac.cn (H.L.); zhaofei@cdu.edu.cn (F.Z.); Tel.: +86-17360061902 (J.L.); Tel.: +86-021-5080-7042 (H.L.); Tel.: +86-18780255276 (F.Z.)

Survey of the solvents on the yield of product SF1a……...……..2

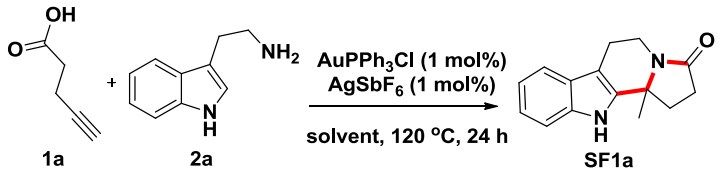
NMR and ESI(+)MS spectrum of SF5a, $[D]_n$-SF5a, SF5b, $[D]_n$-SF5b, SF1a, $[D]_n$-SF1a……...…3

Antibacterial bioassay……..…...10

Antibacterial results and discussion……..…...11

Copies of ^{1}H and ^{13}C NMR spectra of new compounds……..…….....…17

Table S1. Survey of the solvents on the yield of product SF1a [a].



Entry	Solvent	Yield (%) [b]
1	H_2O	91
2	Toluene	86
3	Xylene	88
4	DCE	90
5	THF	75
6	CH_3CN	63
7	DMSO	41
8	MeOH	35

[a] Reaction conditions: 4-pentynoic acid **1a** (0.6 mmol), tryptamine **2a** (0.5 mmol), $AuPPh_3Cl/AgSbF_6$ (0.005 mmol), solvent (4.0 ml), 120 °C, 24 h. [b] Yield refers to isolated yield.

NMR and ESI(+)MS spectrum of SF5a, $[D]_n$-SF5a, SF5b, $[D]_n$-SF5b, SF1a, $[D]_n$-SF1a

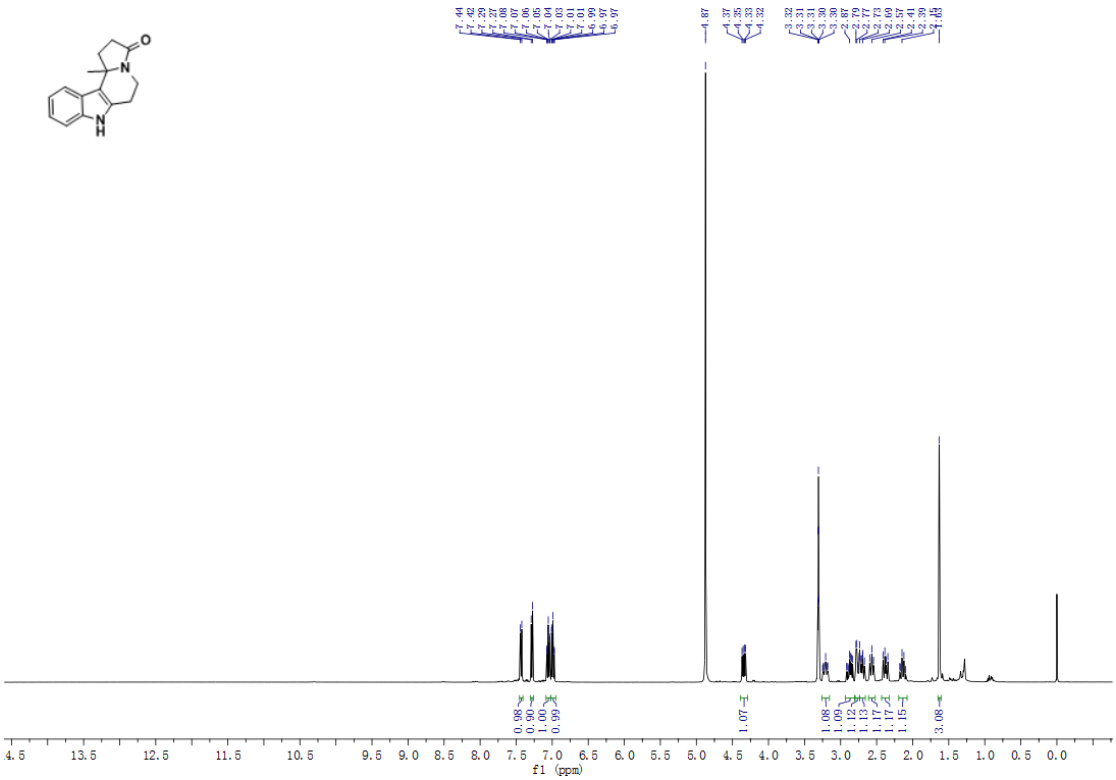


Figure S1 ^{1}H NMR spectrum of **SF5a** in methanol-d_4.

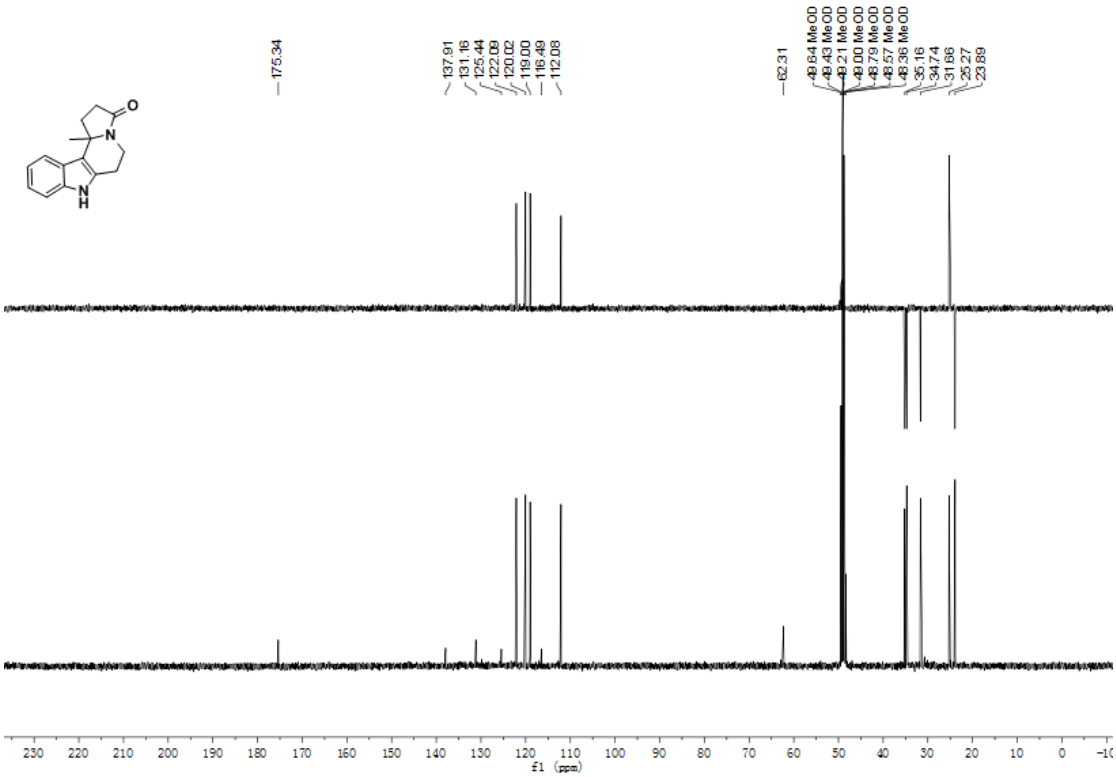


Figure S2 ^{13}C NMR spectrum of **SF5a** in methanol-d_4.

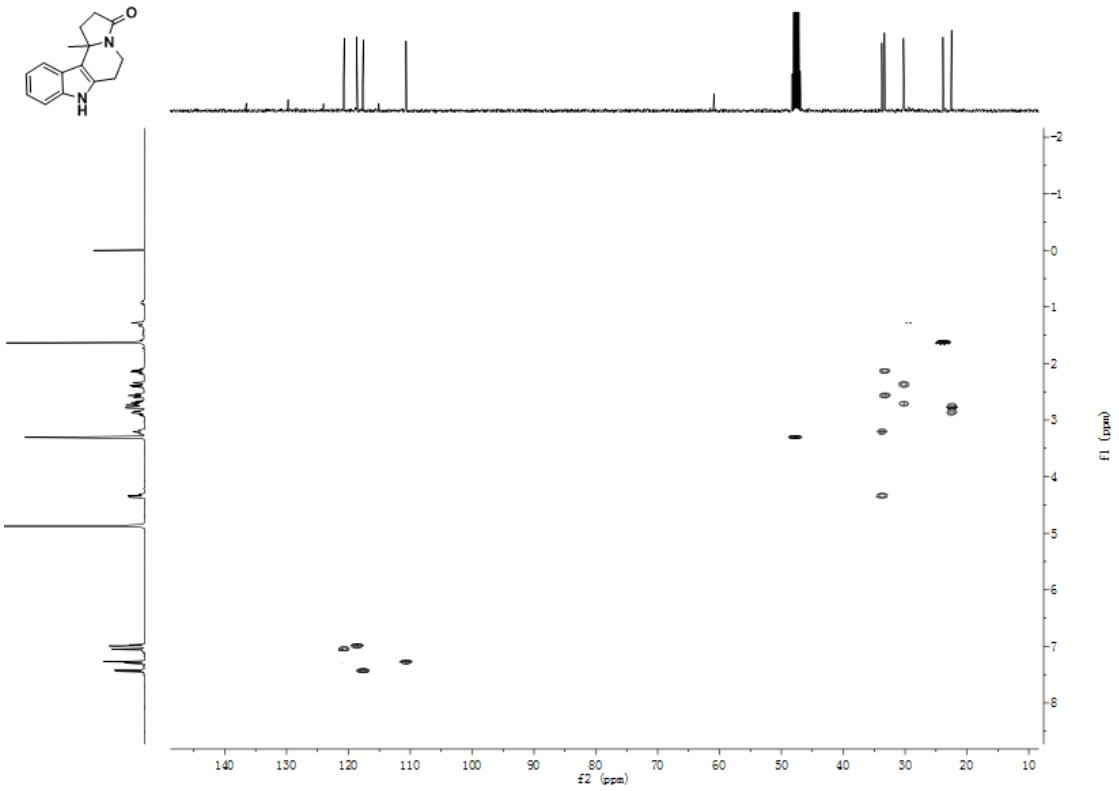


Figure S3 HSQC spectrum of **SF5a** in methanol-d_4.

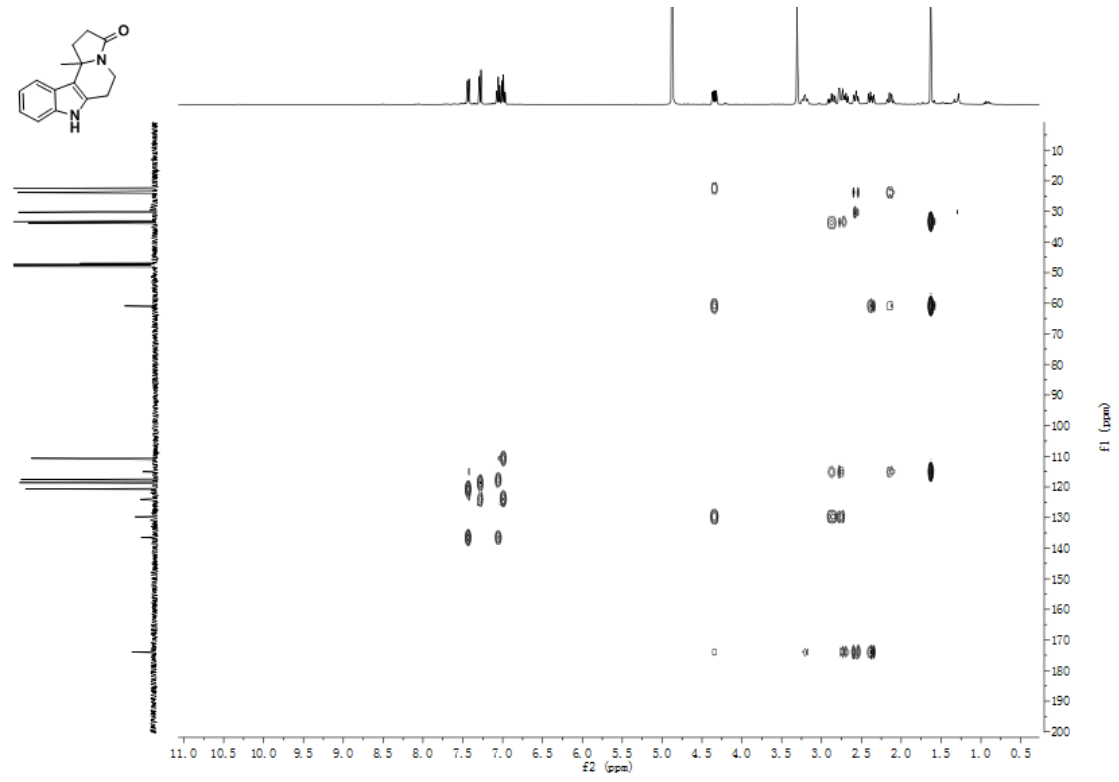


Figure S4 HMBC spectrum of **SF5a** in methanol-d_4.

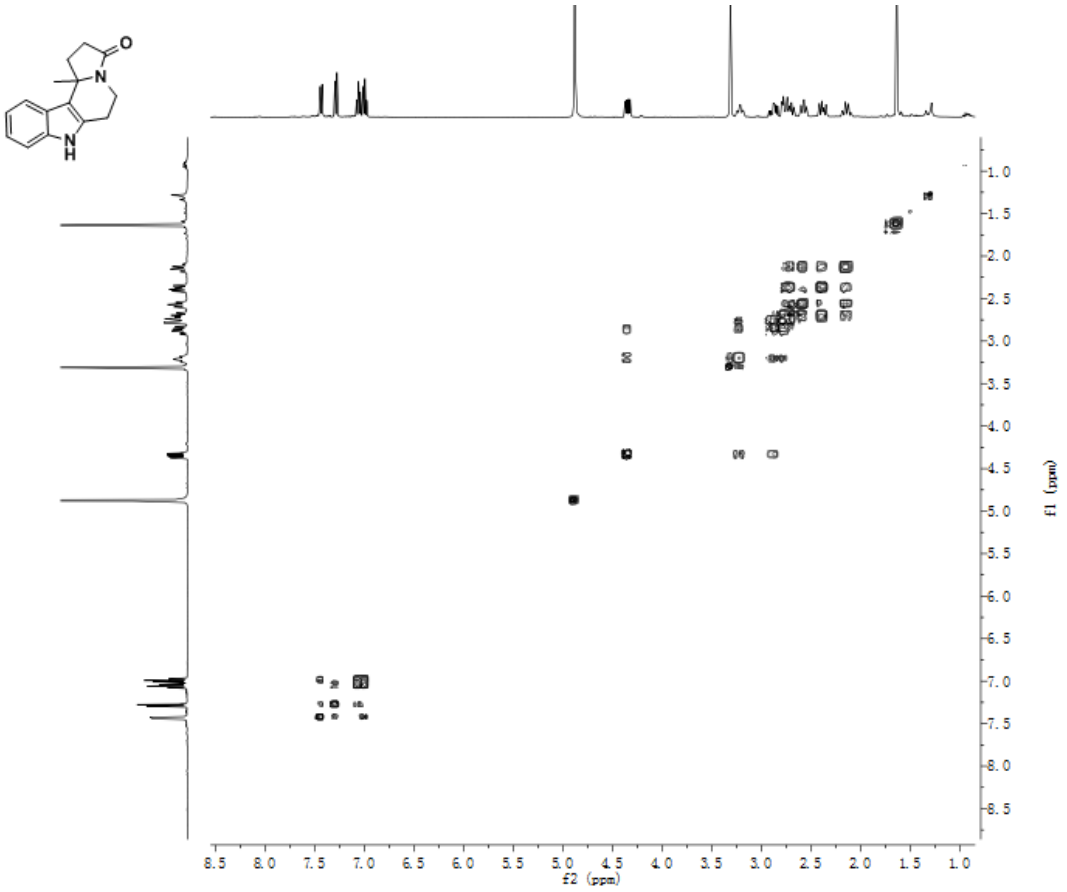


Figure S5 ^{1}H-^{1}H COSY spectrum of **SF5a** in methanol-d_4.

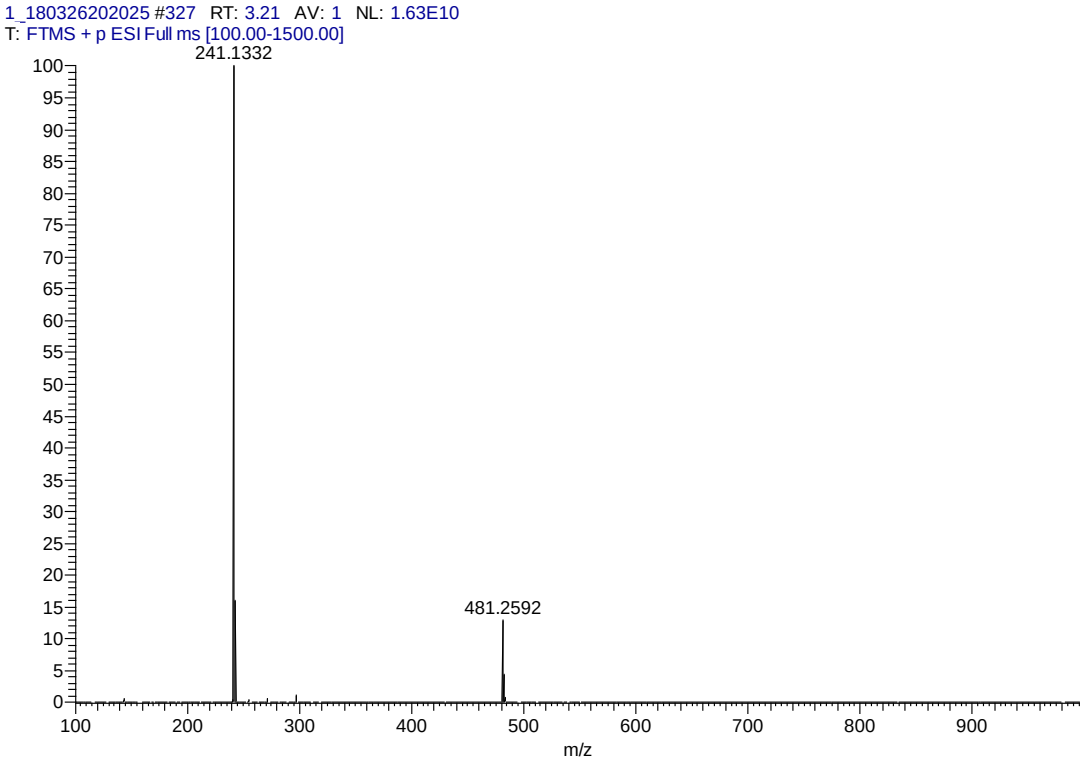


Figure S6 ESI(+)MS spectrum of **SF5a**.

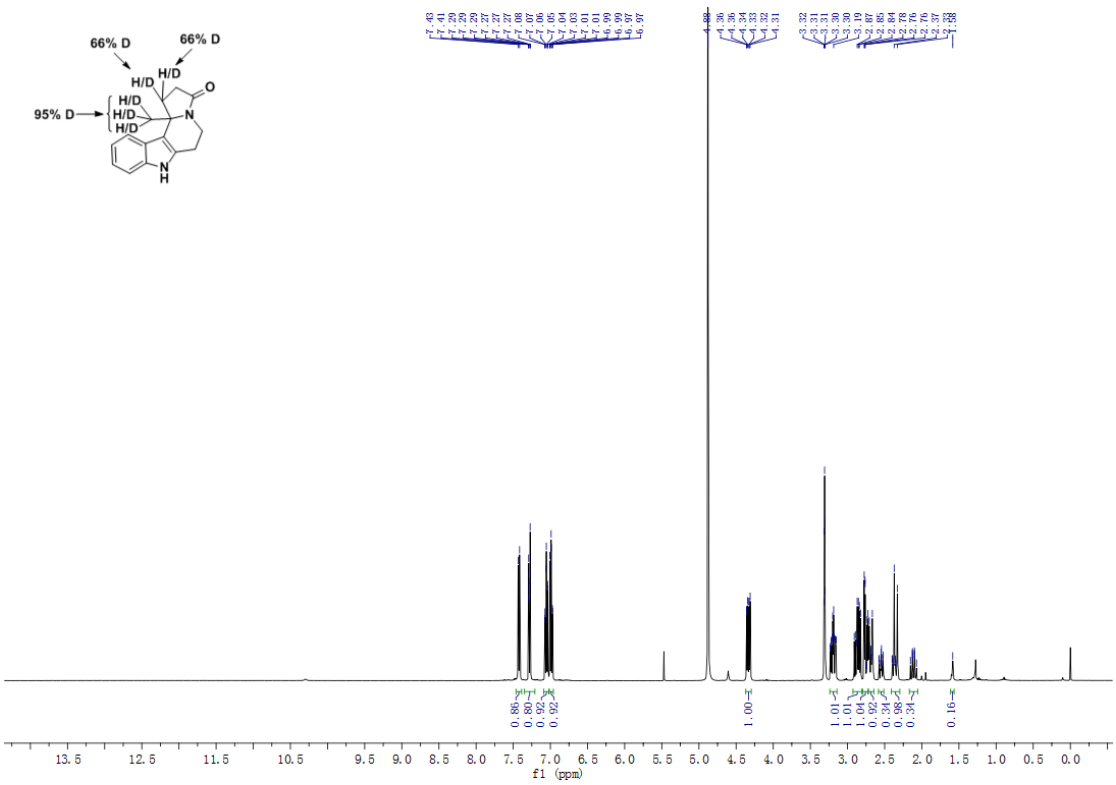


Figure S7 ^{1}H NMR spectrum of $[D]_n$-**SF5a** in methanol-d_4.

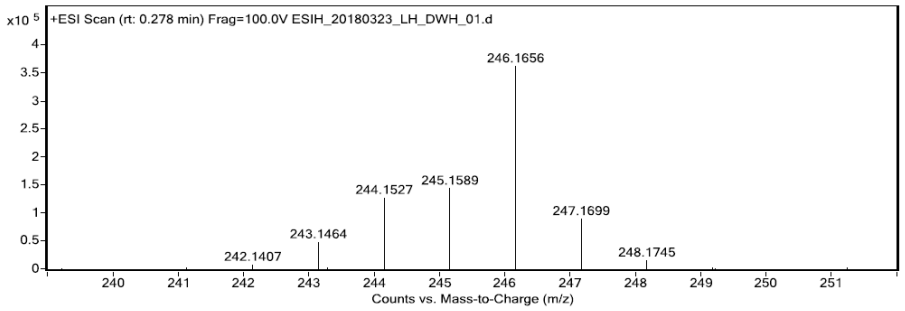
Qualitative Analysis Report

Data Filename	ESIH_20180323_LH_DWH_01.d	**Sample Name**	B6-ZF-0321
Sample Type	Sample	**Position**	P1-B1
Instrument Name	Agilent G6520 Q-TOF	**User Name**	
Acq Method	20160322_MS_ESIH_POS_1min.m	**Acquired Time**	3/23/2018 12:03:23 PM
IRM Calibration Status	Success	**DA Method**	small molecular data analysis method.m
Comment	ESIH by ZZY		

Sample Group **Info.**
Acquisition SW Version 6200 series TOF/6500 series Q-TOF B.05.01 (B5125.3)

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
100	0	ESI



Formula Calculator Element Limits

Element	Min	Max
C	3	17
H	0	100

Qualitative Analysis Report

O	0	1
N	0	2
S	0	0
D	0	10

Formula Calculator Results

m/z	Calc m/z	Diff (mDa)	Diff (ppm)	Ion Formula	Score
242.1407	242.1398	-0.86	-3.57	C15 H16 D N2 O	44.58
242.1407	242.1416	0.88	3.64	C16 H4 D8 N O	44.48
243.1464	243.1461	-0.35	-1.46	C15 H15 D2 N2 O	47.09
243.1464	243.1478	1.38	5.71	C16 H3 D9 N O	40.2
243.1464	243.1492	2.74	11.32	C15 H19 N2 O	24.48
244.1527	244.1524	-0.36	-1.49	C15 H14 D3 N2 O	47.07
244.1527	244.1541	1.38	5.66	C16 H2 D10 N O	40.3
244.1527	244.1555	2.73	11.24	C15 H18 D N2 O	24.63
245.1589	245.1586	-0.22	-0.9	C15 H13 D4 N2 O	47.42
245.1589	245.1617	2.88	11.78	C15 H17 D2 N2 O	23.02
245.1589	245.1556	-3.32	-13.59	C15 H9 D6 N2 O	18.1
246.1656	246.1649	-0.99	-4.06	C15 H12 D5 N2 O	82.39
246.1656	246.168	2.1	8.58	C15 H16 D3 N2 O	71.18

--- End Of Report ---

Figure S8 ESI(+)MS spectrum of $[D]_n$-**SF5a**.

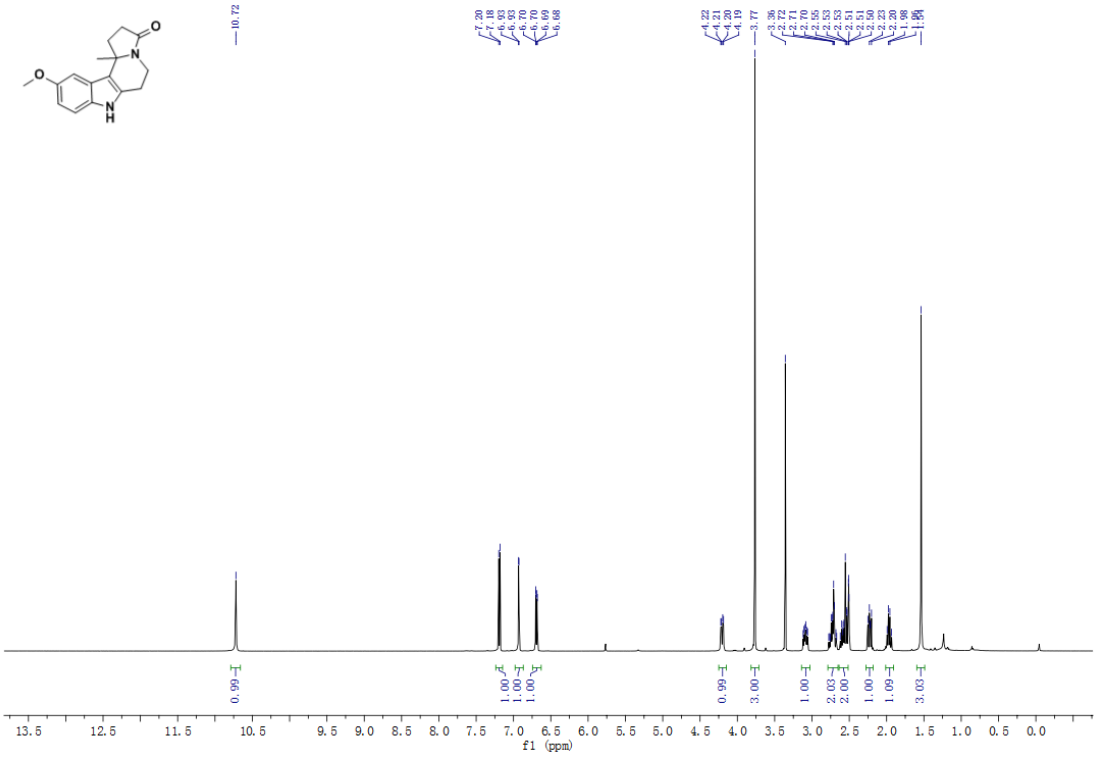


Figure S9 ^{1}H NMR spectrum of **SF5b** in dimethyl sulfoxide-d_6.

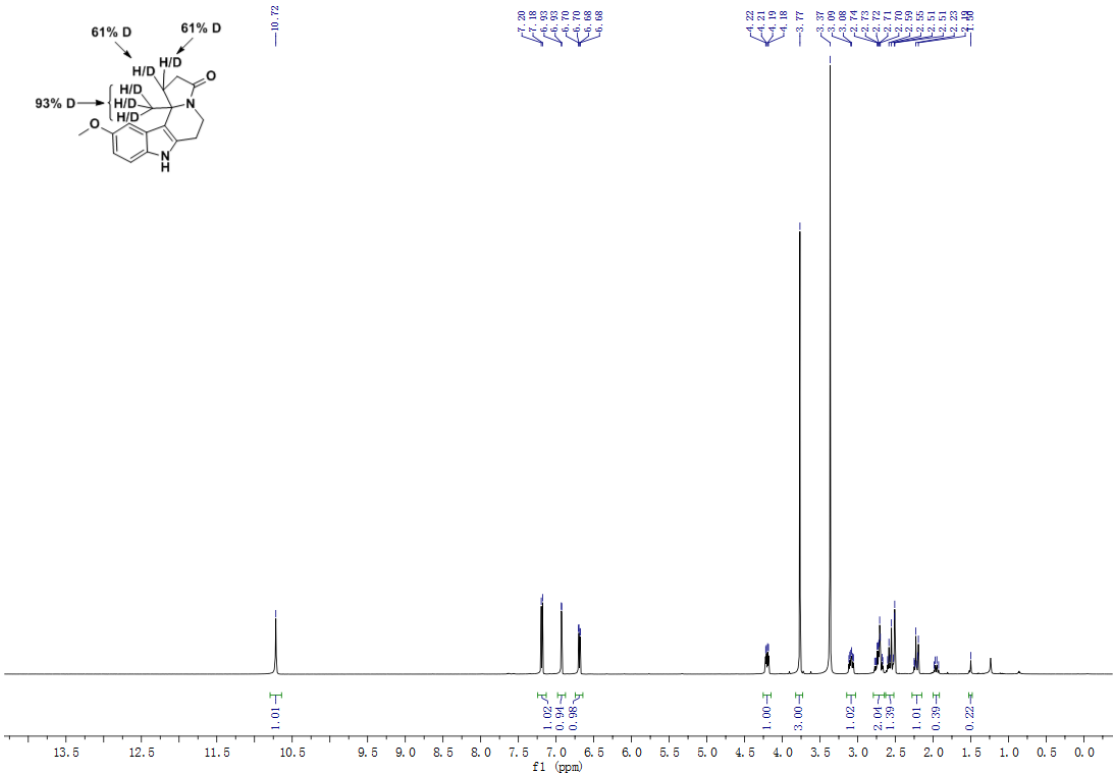


Figure S10 ^{1}H NMR spectrum of $[D]_n$-**SF5b** in dimethyl sulfoxide-d_6.

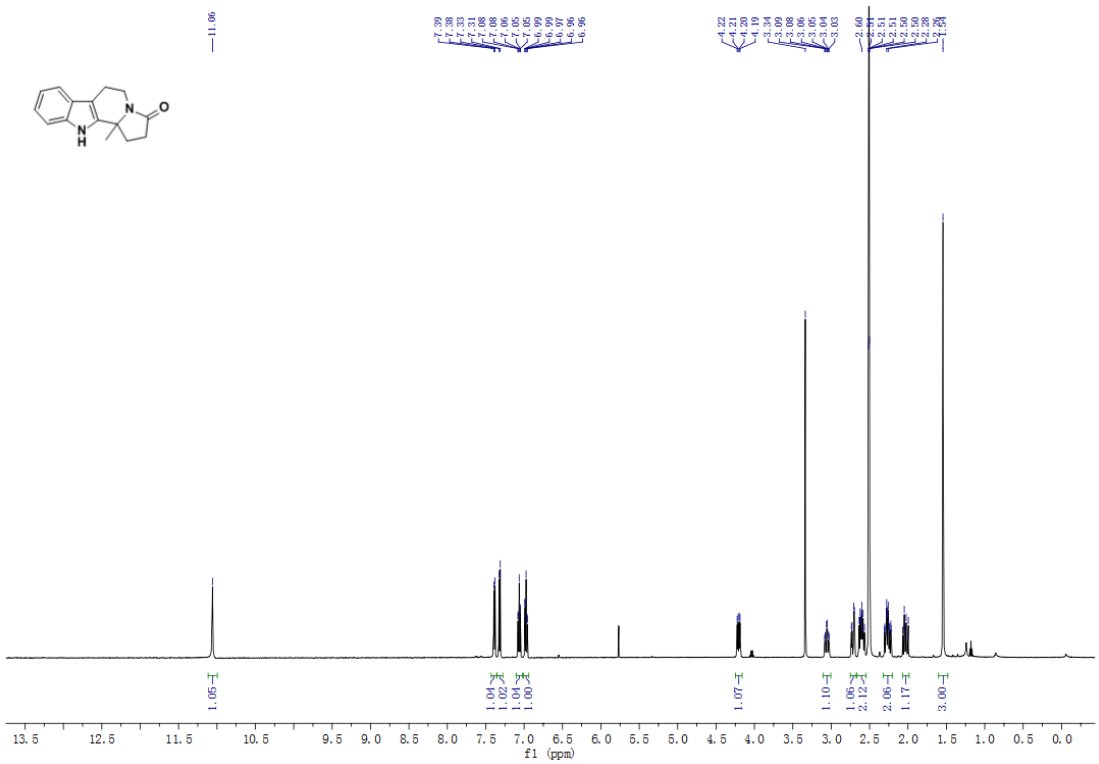


Figure S11 ^{1}H NMR spectrum of **SF1a** in dimethyl sulfoxide-d_6.

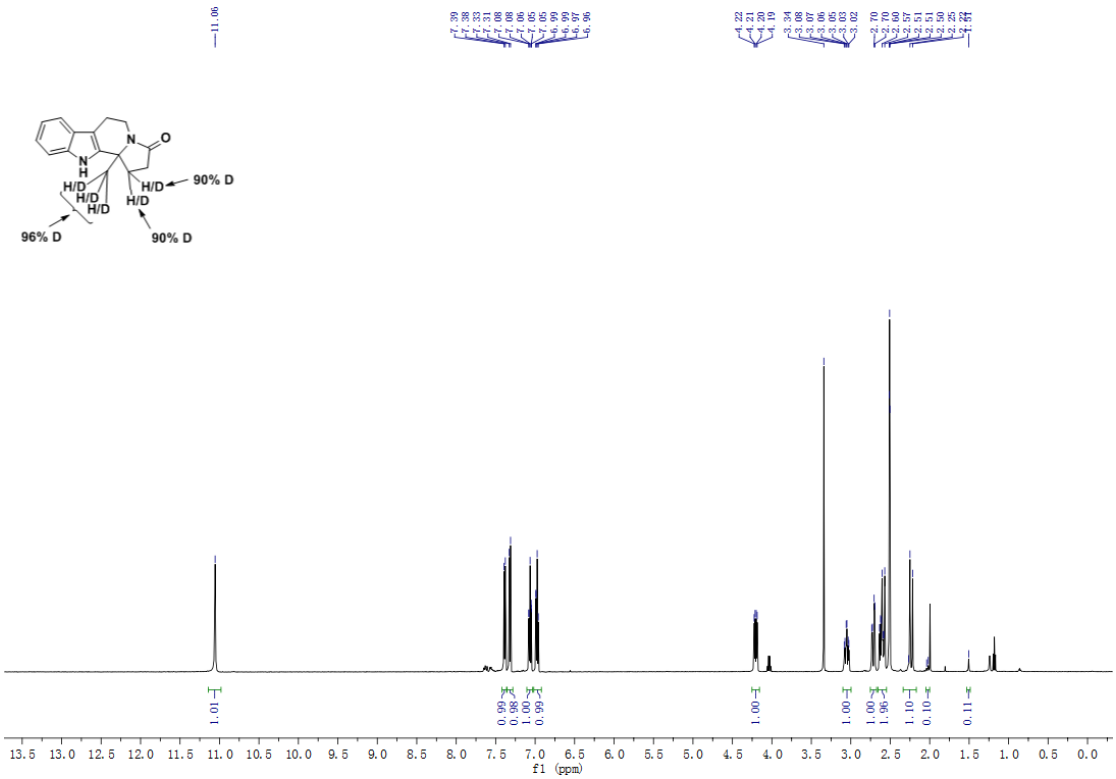


Figure S12 ^{1}H NMR spectrum of $[D]_n$-**SF1a** in dimethyl sulfoxide-d_6.

Antibacterial bioassay

Bacterial strains, culture and growth conditions, and sample preparation

Staphylococcus aureus (S. aureus) was used in this study and cultured at 37 ^{o}C in Mueller-Hinton broth (MH broth). 5 mg compounds were dissolved in 100 μl DMSO, and the resulting solution was used as the sample stock. All the experiments were repeated at least three times.

Preliminary screening of antibacterial activities

The preliminary antibacterial activities against *S. aureus* strain were investigated in 96-well plates, and DMSO was used as the blank control. Briefly, *S. aureus* strain was seeded into 200 μl MH broth per well to make a density of 1×10^5 CFU/ml. Subsequently, an aliquot of the sample stock was added to make a final compound concentration of 100 μg/ml. After that, the optical density (OD) of the mixture in each well at 600 nm wavelength was immediately measured by a spectrometer, and recorded as OD_0. Then the plate was incubated at 37 ^{o}C for 24 h, after that, the OD of the mixture in each well was immediately measured again and recorded as OD_{24}. ΔOD ($\Delta OD = OD_{24} - OD_0$) was calculated and used to evaluate the antibacterial potency of the compounds. Finally, compounds with ΔOD lower than 0.1 were selected out for further study.

Minimal inhibitory concentration (MIC) study

The determination of minimal inhibitory concentration (MIC) of tested compounds was carried out in 96-well plates with DMSO as the blank control. Briefly, *S. aureus* strain was seeded into 200 μl MH broth per well to make a density of 1×10^5 CFU/ml. Subsequently, an aliquot of the sample stock was added to make 5 final compound concentrations (5 μg/ml, 10 μg/ml, 25 μg/ml, 50 μg/ml, 100 μg/ml and 200 μg/ml). After that, the OD of the mixture in each well at 600 nm wavelength was immediately measured by a spectrometer, and recorded as OD_0. Then the plates were incubated at 37 ^{o}C for 24 h, after that, the OD of the mixture in each well was immediately measured again and recorded as OD_{24}. ΔOD ($\Delta OD = OD_{24} - OD_0$) was calculated and used to determine the MIC_{90}. MIC_{90} was determined as the lowest concentration that inhibited 90% bacteria growth as compared with DMSO control group.

Time-Kill Assays

Time-kill assays were performed in 96-well plates, and DMSO was used as the blank control. Briefly, *S. aureus* strain was seeded into 200 μl MH broth per well to make a density of 1×10^5 CFU/ml. Subsequently, an aliquot of the sample stock was added to make 5 final compound concentrations (5 μg/ml, 10 μg/ml, 25 μg/ml, 50 μg/ml, 100 μg/ml and 200 μg/ml). After that, the OD of the mixture in each well at 600 nm wavelength was instantly measured by a spectrometer, and recorded as OD_0. Then the plate was incubated at 37 ^{o}C for 2 h, 4 h, 6 h, 8 h, 10 h, 12 h and 24 h, after that, the OD of the mixture in each well was immediately measured again and recorded as OD_t. ΔOD ($\Delta OD = OD_t - OD_0$) was calculated and used to draw the time-kill curves.

Colony-forming units (CFU) study

At the end of the time-kill assays, the plates were used for CFU study. Concisely, parallel wells were randomly selected and diluted by 10^5 times with MH broth. Then 100 μl diluent was taken and spread on Mueller-Hinton agar. The agar plates were incubated at 37 ^{o}C for 24 h. After that, the agar plates were recorded.

Statistical analysis: Statistical calculations were processed with Origin Pro 7.5 and Excel 2016.

Antibacterial results and discussion

Preliminary screening results

Preliminary screening disclosed that 21 compounds from the library showed antibacterial activities against the growth of *S. aureus* strain at the concentration of 100 μg/ml (Figure S13), and five of them (compounds **SF9d**, **SF29b**, **SF33**, **SF36** and **SF41**) showed good antibacterial activities, which were selected for further study.

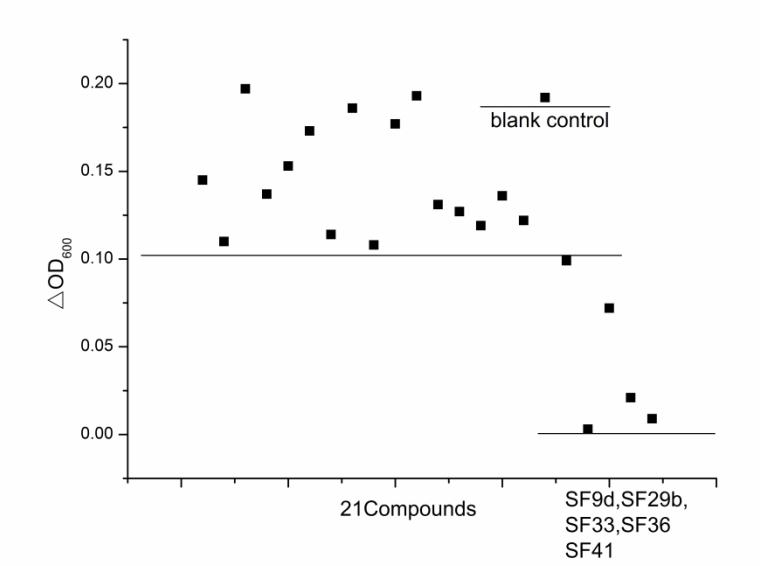


Figure S13 Preliminary screening of antibacterial activities of compounds at 100 μg/ml.

Time-kill assays and colony-forming unit (CFU) studies of compounds SF9d, SF29b, SF33, SF36 and SF41

As shown in Figure S14-S24, time-kill assays and colony-forming units (CFU) studies were also conducted with compounds **SF9d**, **SF29b**, **SF33, SF36** and **SF41**. Among them, compound **SF36** displayed the most potent antibacterial activity against *S. aureus* strain. Time-kill assay showed that **SF36** was bactericidal within 2-24 h at the concentration of 25 μg/ml, preventing bacterial growth of *S. aureus* strain completely (Figure 17). Colony-forming units (CFU) study of **SF36** was also carried out (Figure 23). The results showed that the number of clones on the agar plate decreased significantly in a dose-dependent manner, and only few clone was observed at the concentration of 50 μg/ml, indicating the antibacterial potency of this compound intuitively.

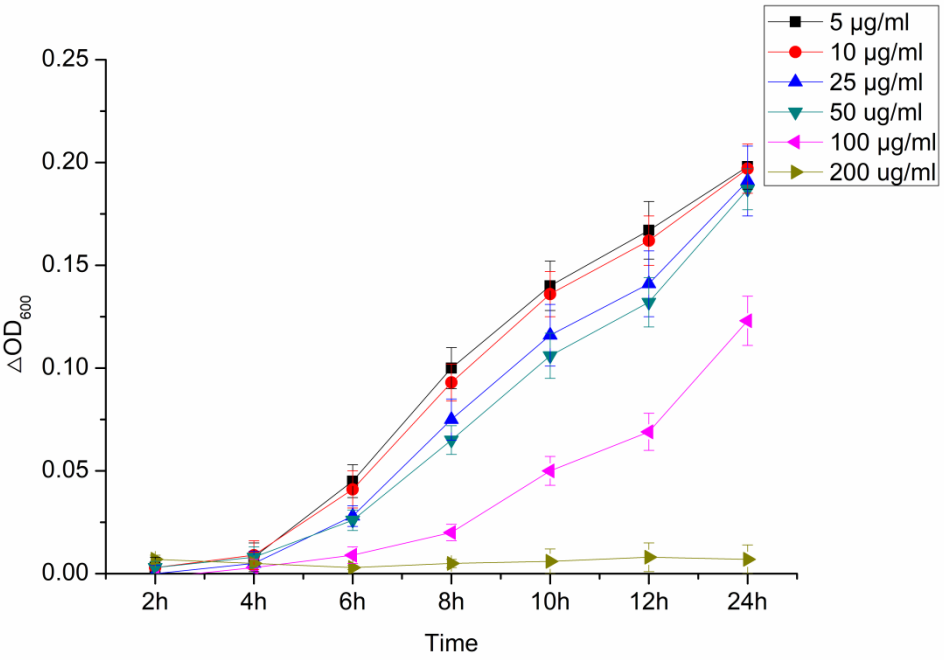


Figure S14 Time-kill results of compound **SF9d** against *S. aureus* strain.

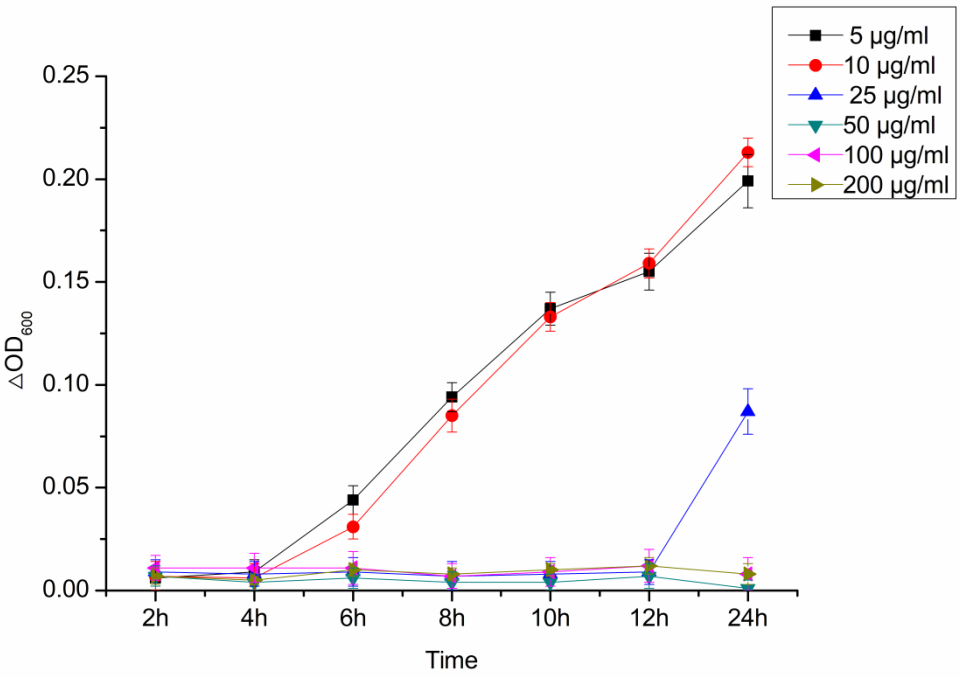


Figure S15 Time-kill results of compound **SF29b** against *S. aureus* strain.

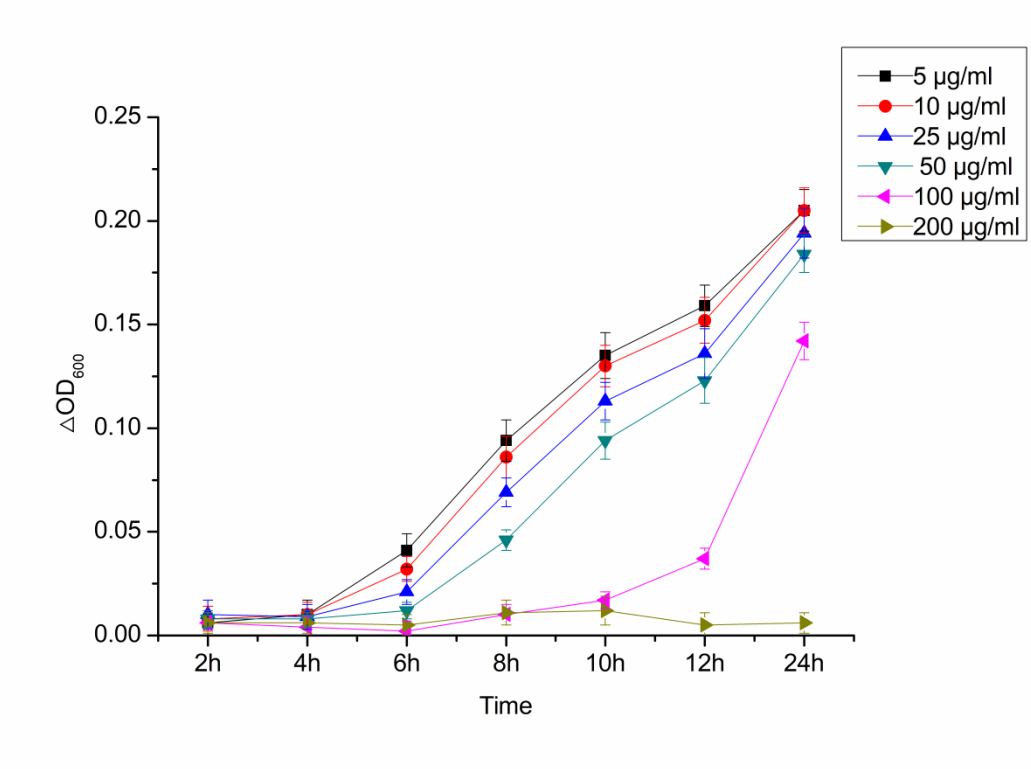


Figure S16 Time-kill results of compound **SF33** against *S. aureus* strain.

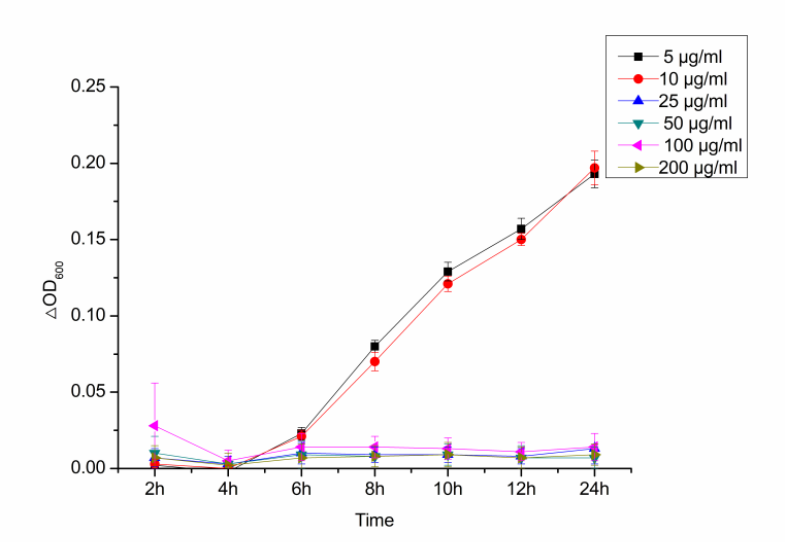


Figure S17 Time-kill results of compound **SF36** against *S. aureus* strain.

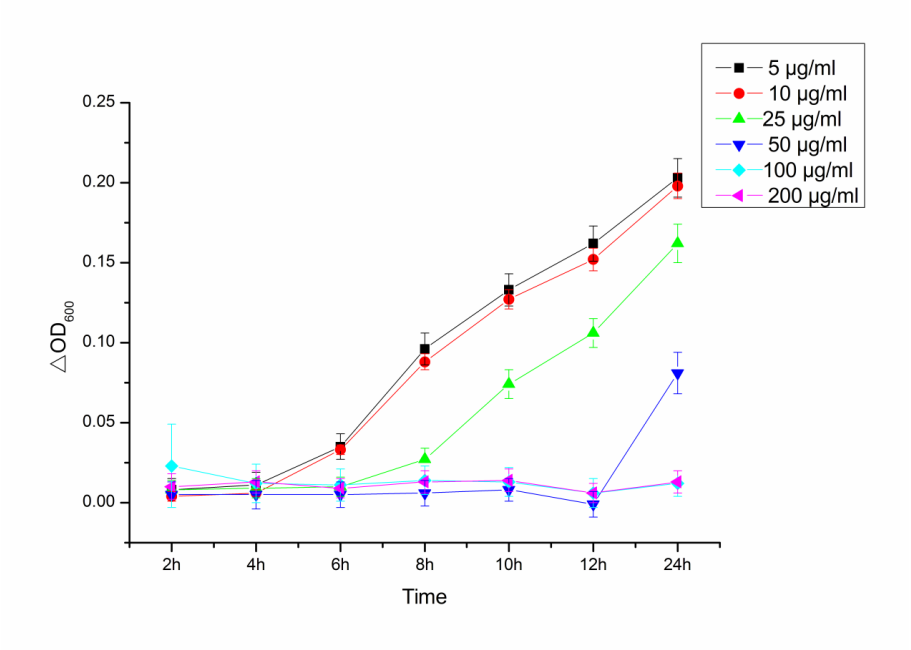


Figure S18 Time-kill results of compound **SF41** against *S. aureus* strain.

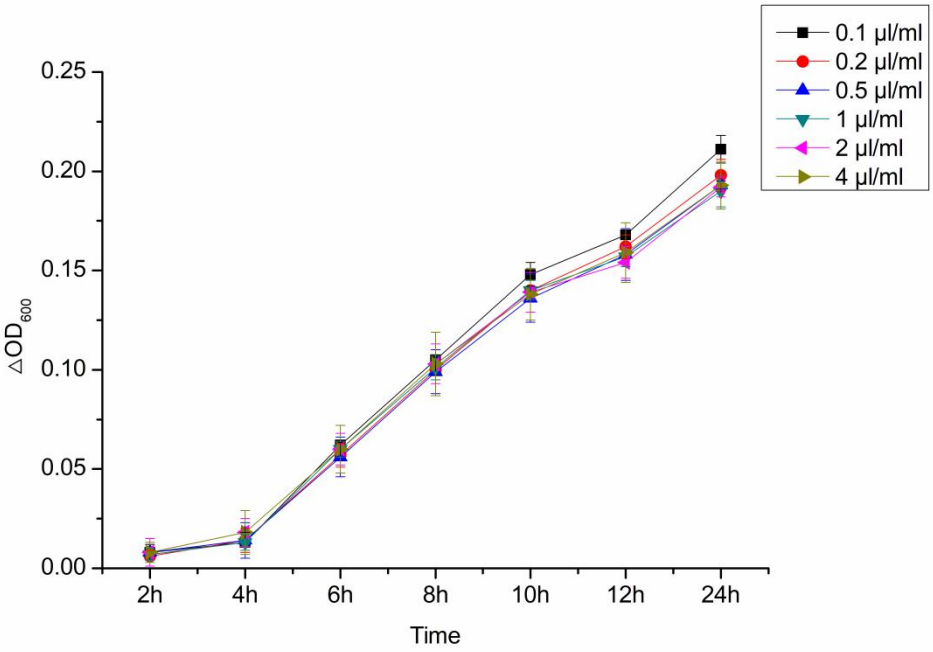


Figure S19 Time-kill results of DMSO against *S. aureus* strain.

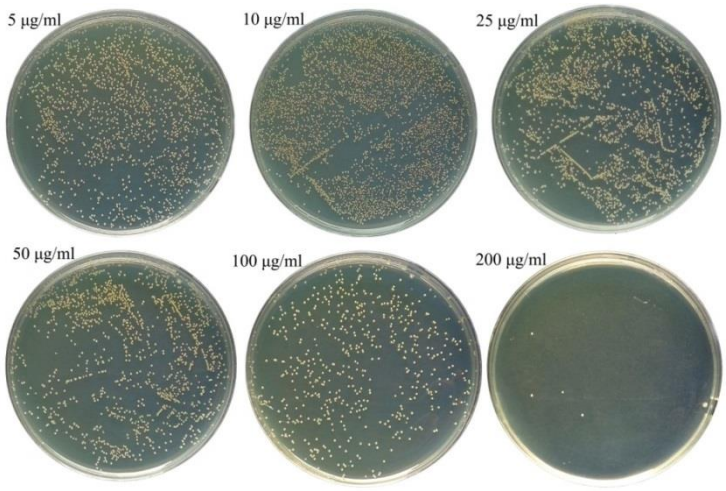


Figure S20 CFU results of compound **SF9d**.

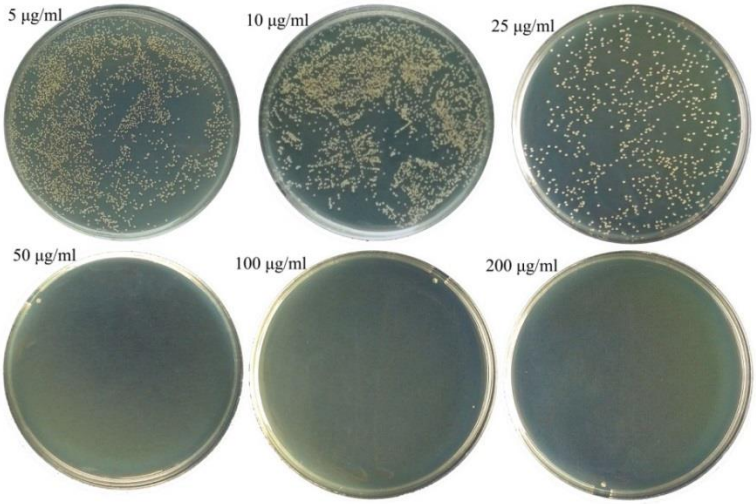


Figure S21 CFU results of compound **SF29b**.

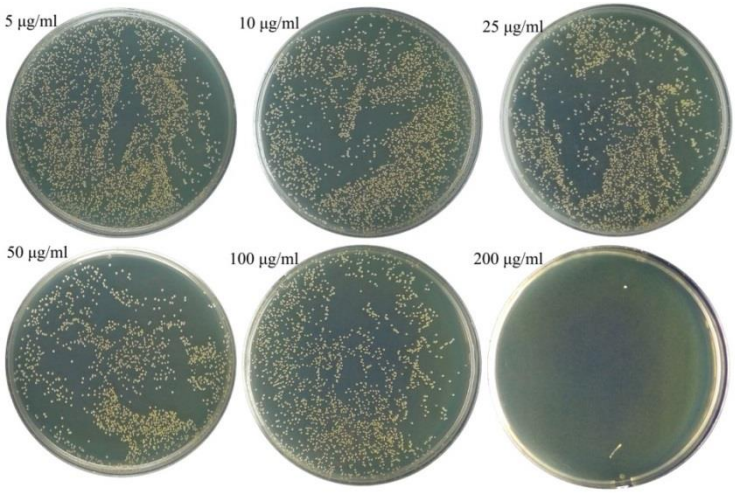


Figure S22 CFU results of compound **SF33**.

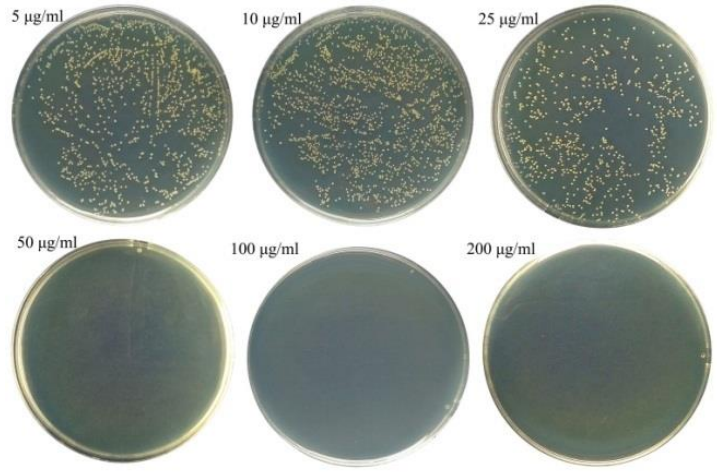


Figure S23 CFU results of compound **SF36**.

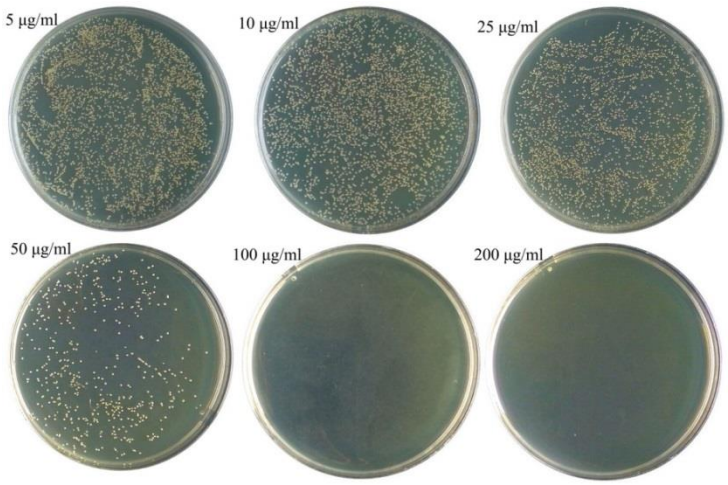
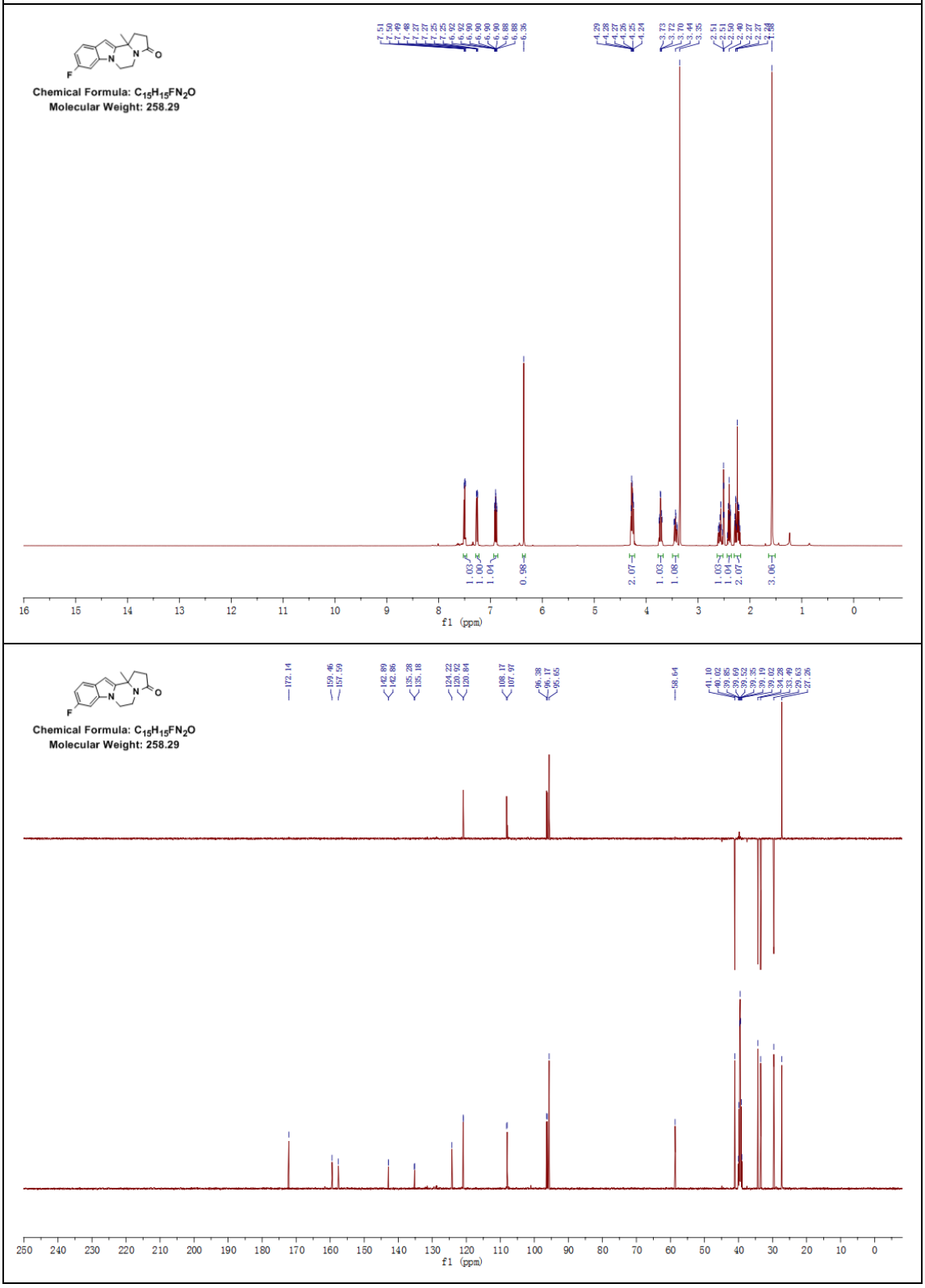


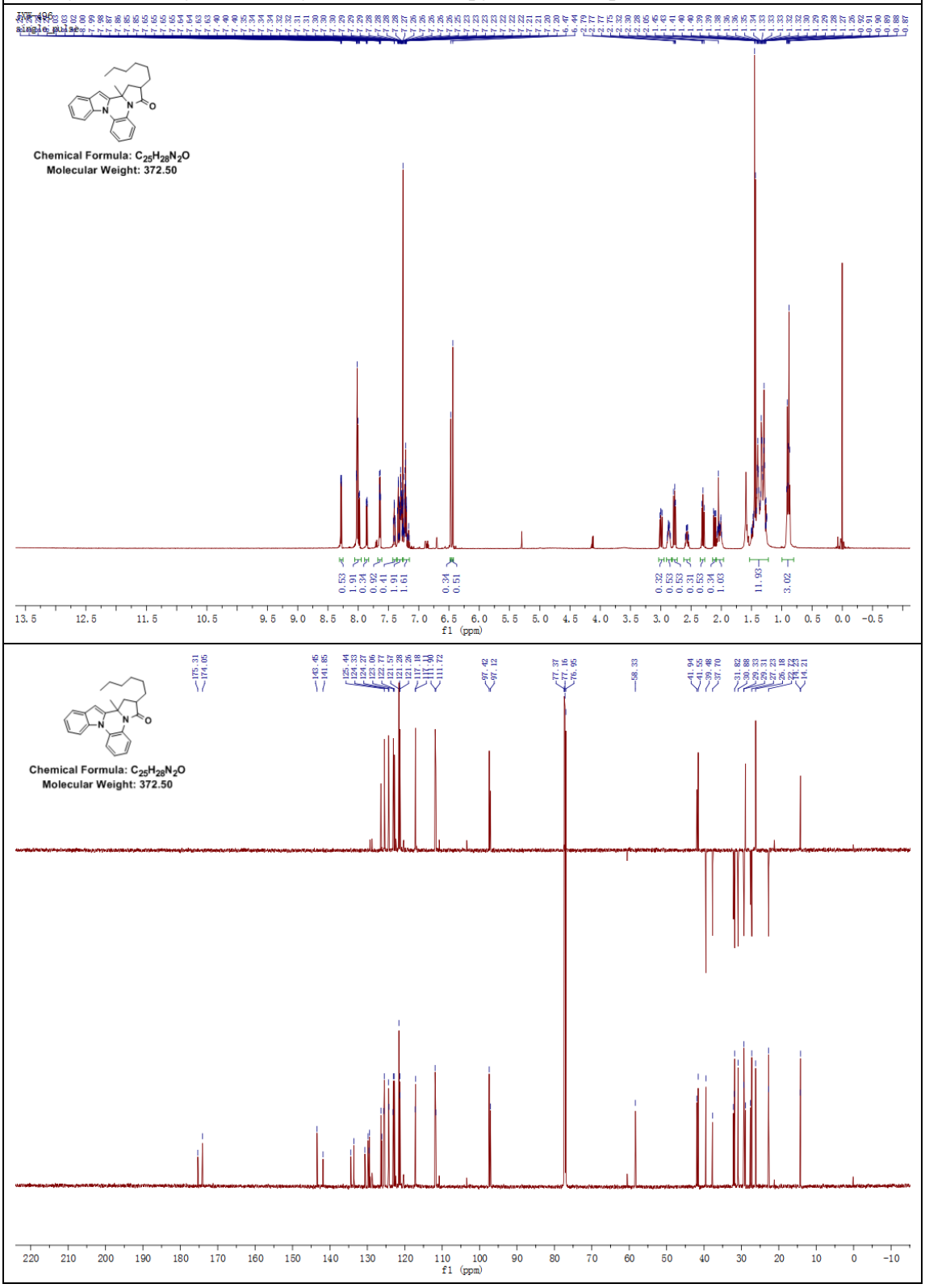
Figure S24 CFU results of compound **SF41**.

Copies of ^{1}H and ^{13}C NMR spectra of new compounds

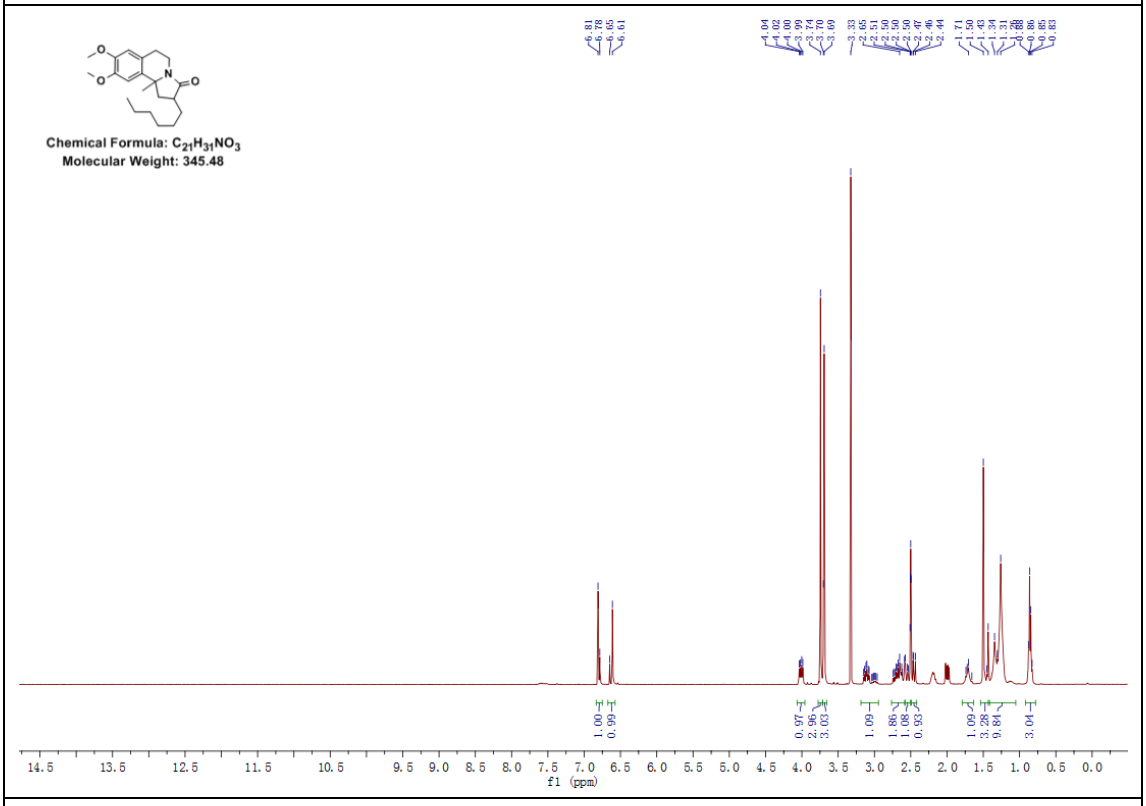
9-fluoro-12b-methyl-1,5,6,12b-tetrahydropyrrolo[2',1':3,4]pyrazino[1,2-*a*]indol-3(2*H*)-one (SF9d)

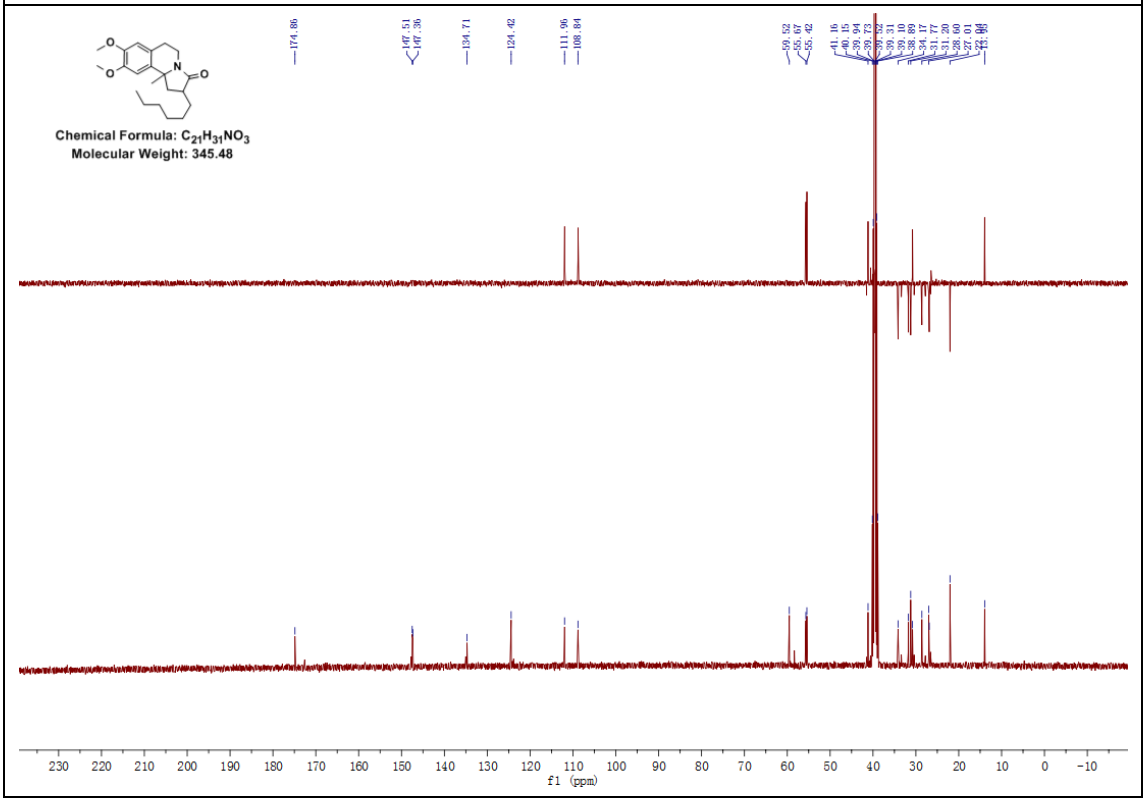


2-hexyl-14b-methyl-1,14b-dihydroindolo[1,2-*a*]pyrrolo[2,1-*c*]quinoxalin-3(2*H*)-one (SF14b)

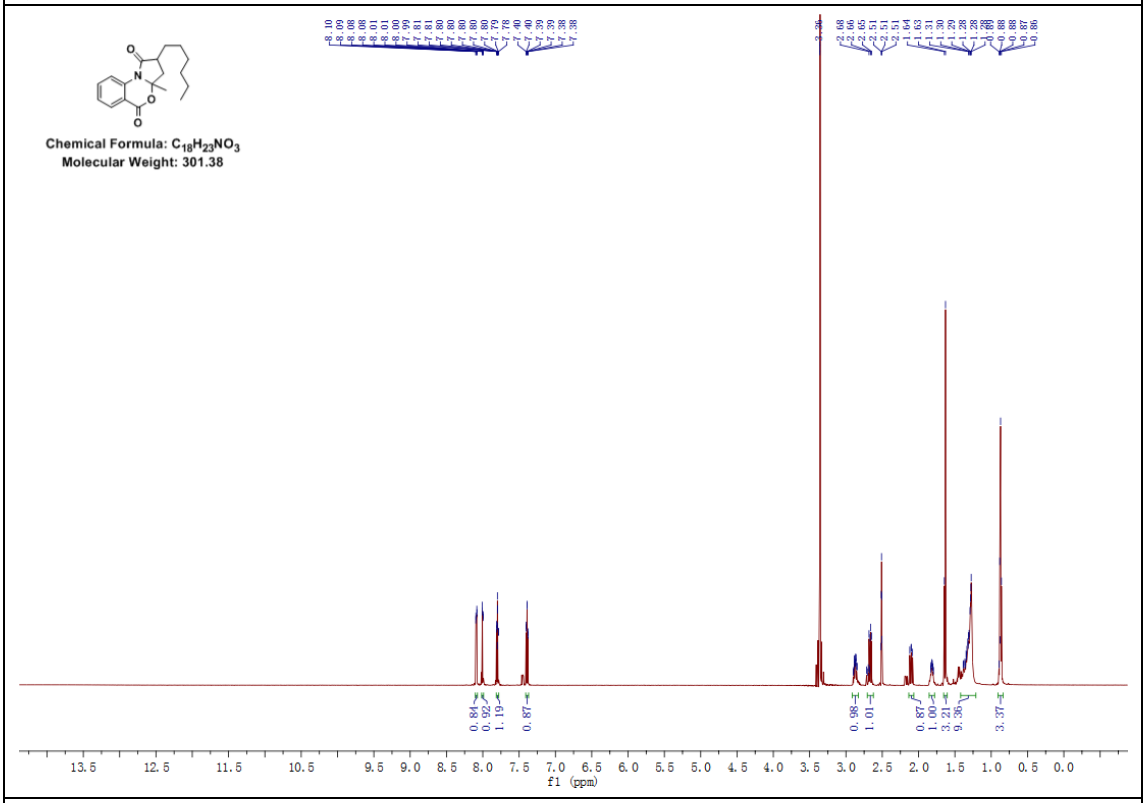


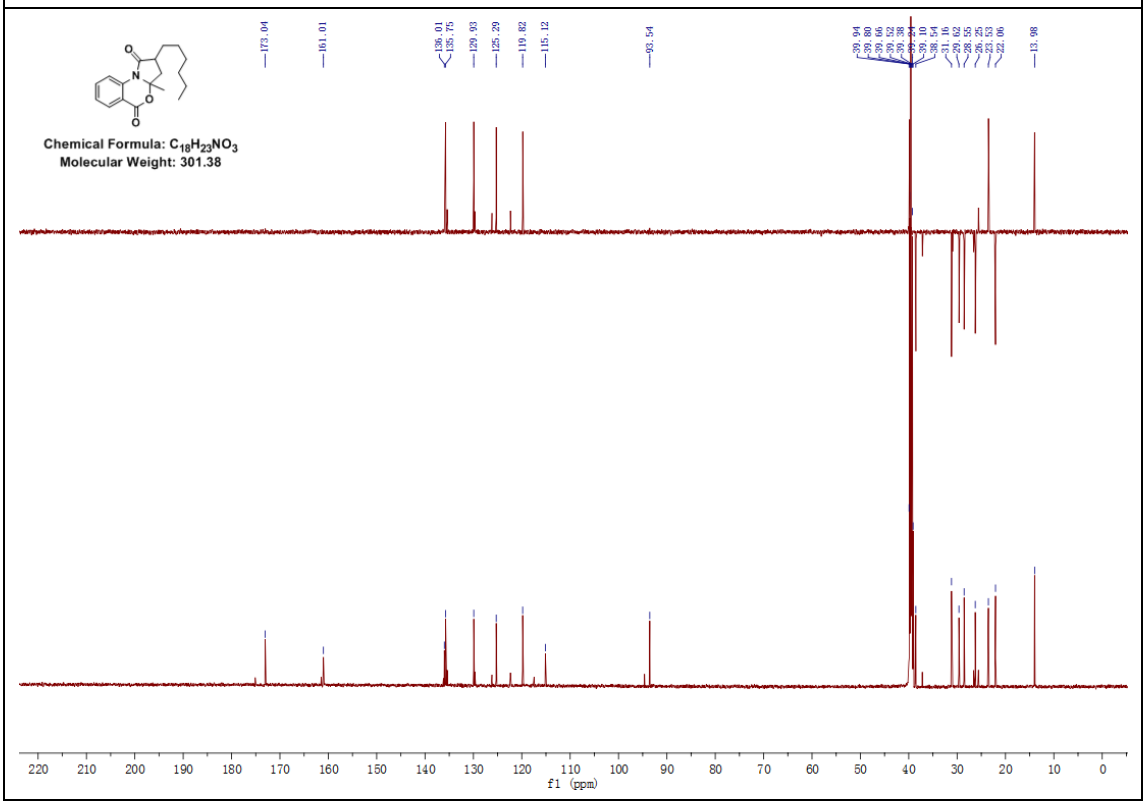
2-hexyl-8,9-dimethoxy-10b-methyl-1,5,6,10b-tetrahydropyrrolo[2,1-*a*]isoquinolin-3(2*H*)-one (SF24b)



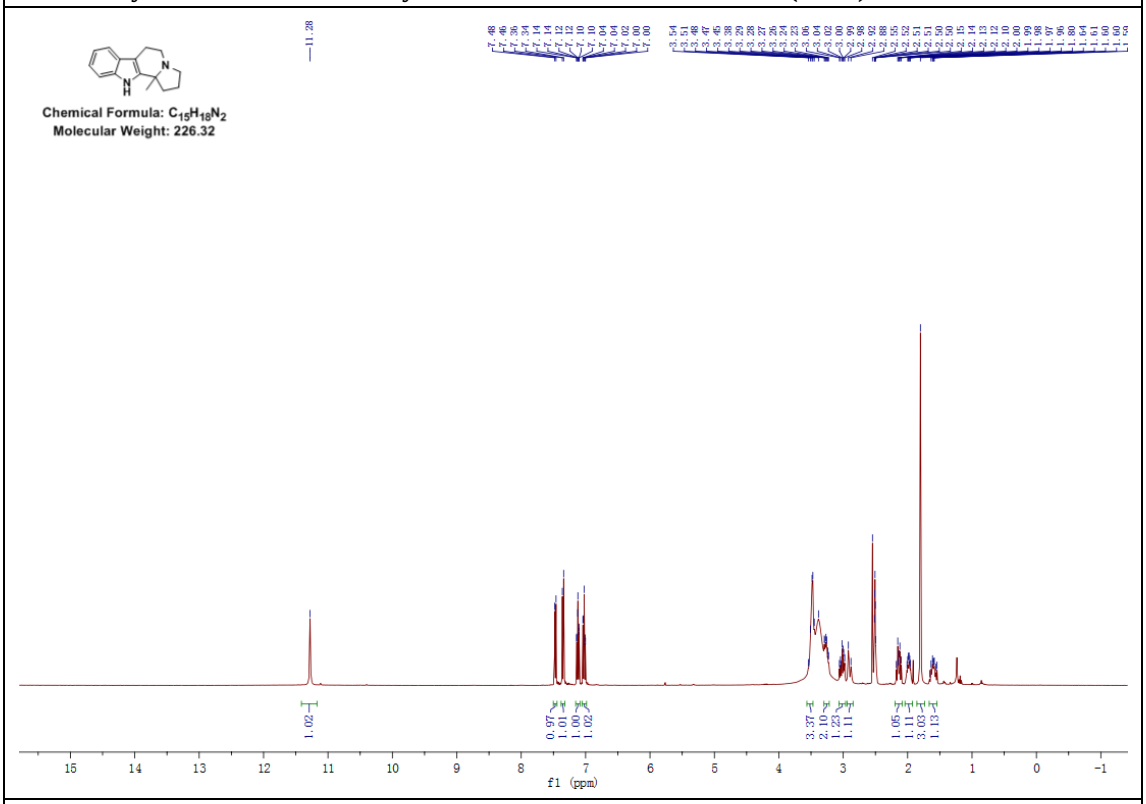


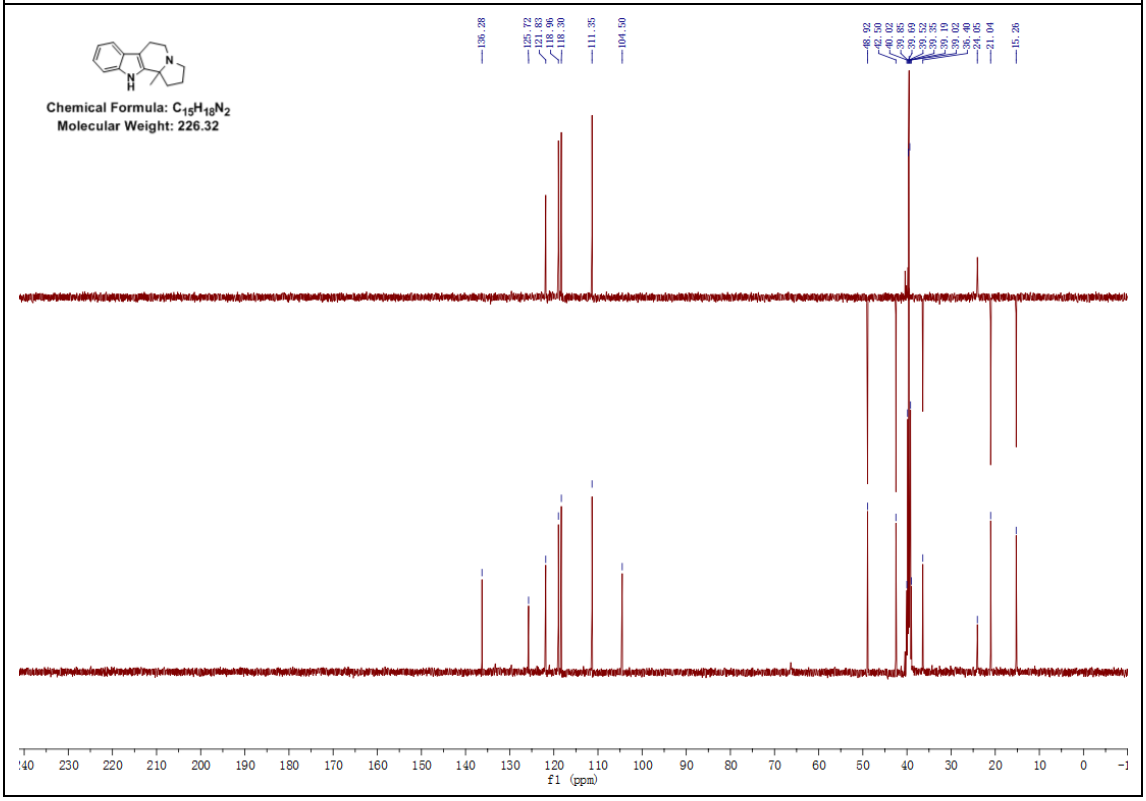
2-hexyl-3a-methyl-3,3a-dihydro-1*H*-benzo[*d*]pyrrolo[2,1-*b*][1,3]oxazine-1,5(2*H*)-dione (SF35b)



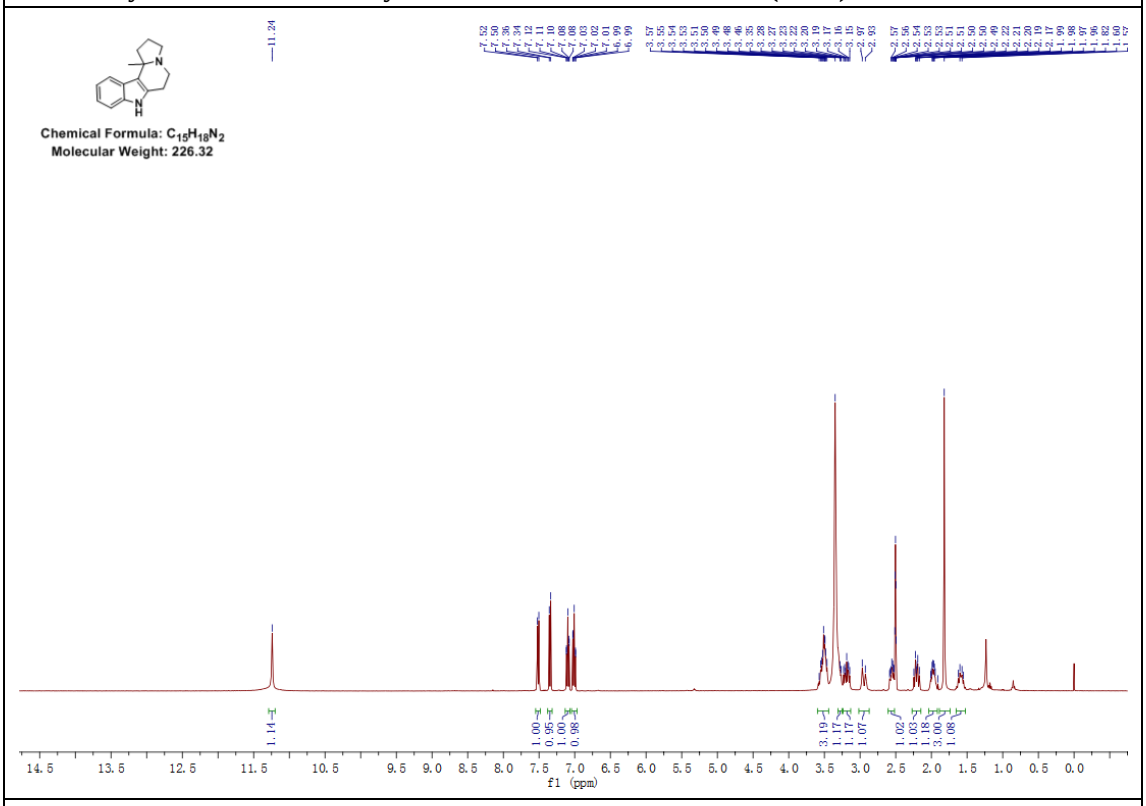


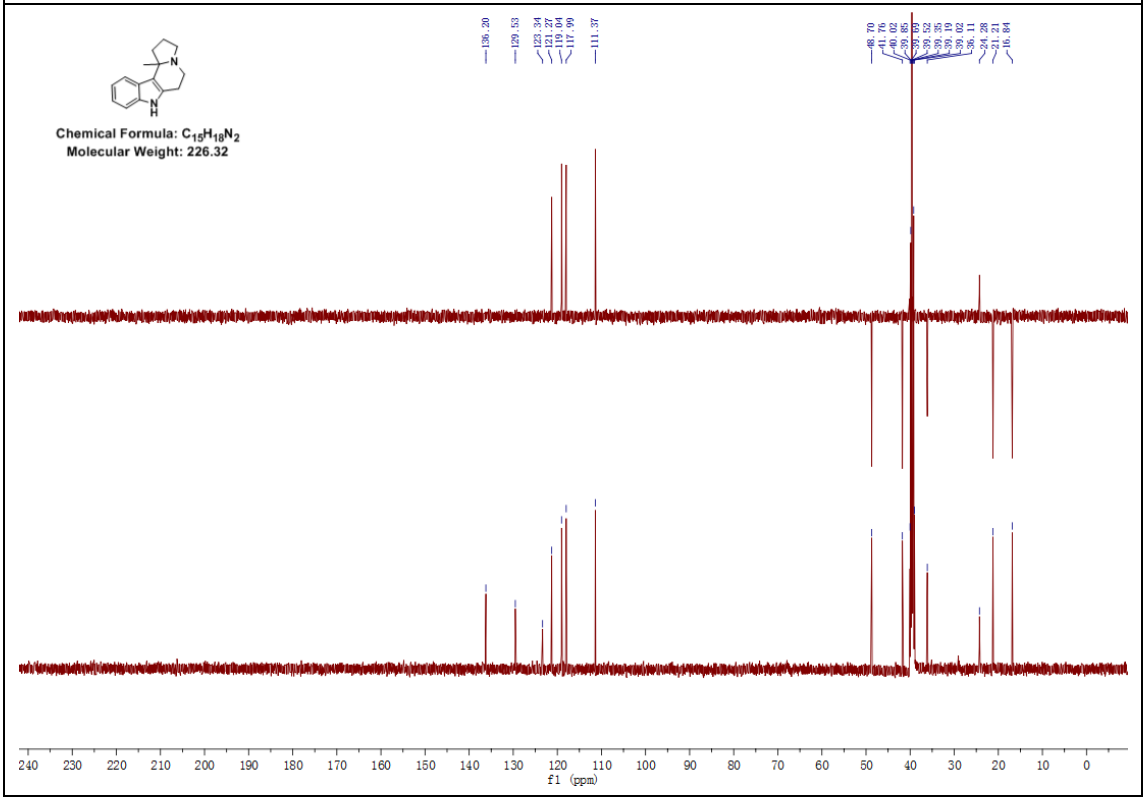
11b-methyl-2,3,5,6,11,11b-hexahydro-1*H*-indolizino[8,7-*b*]indole (SF47)



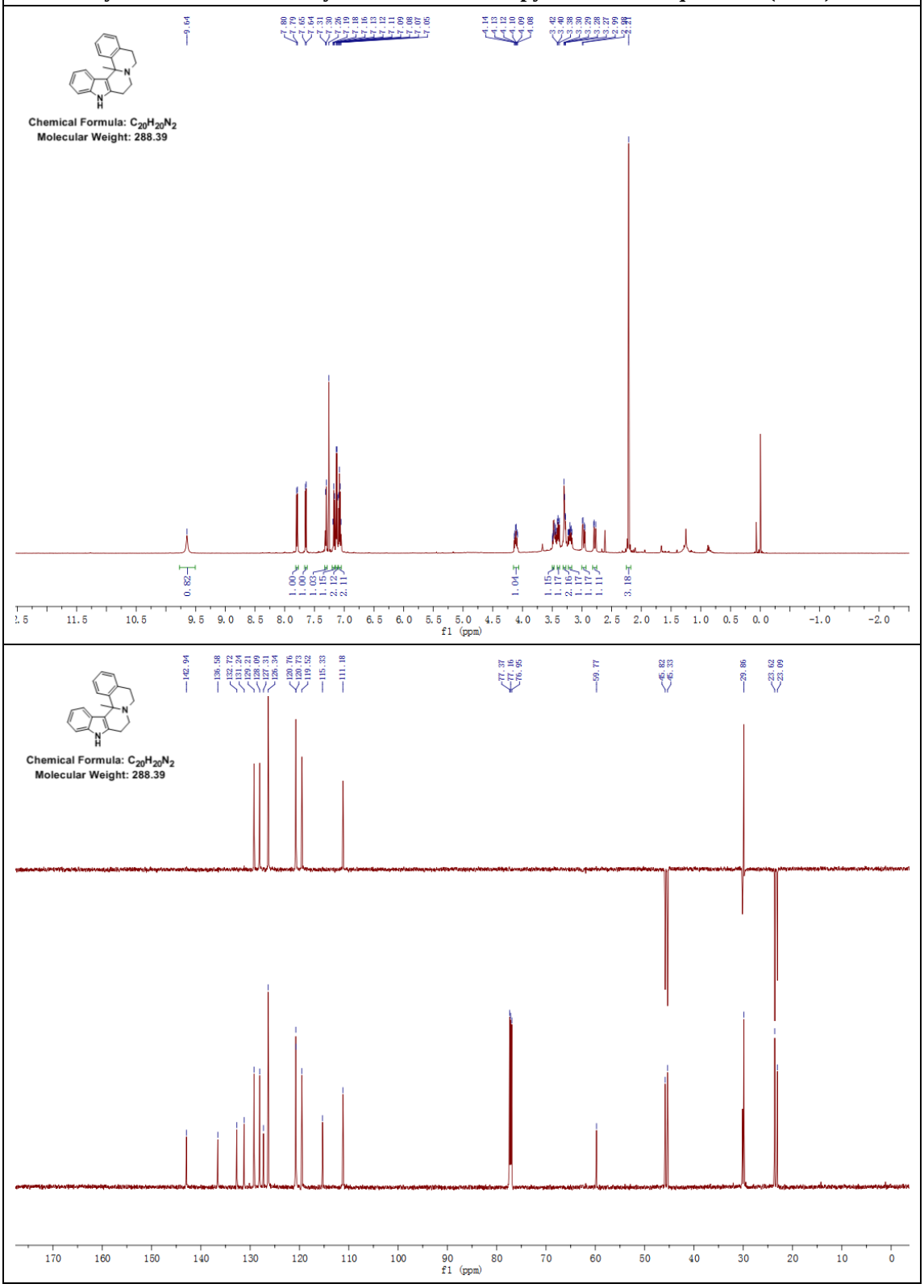


11c-methyl-2,3,5,6,7,11c-hexahydro-1*H*-indolizino[7,8-*b*]indole (SF50)

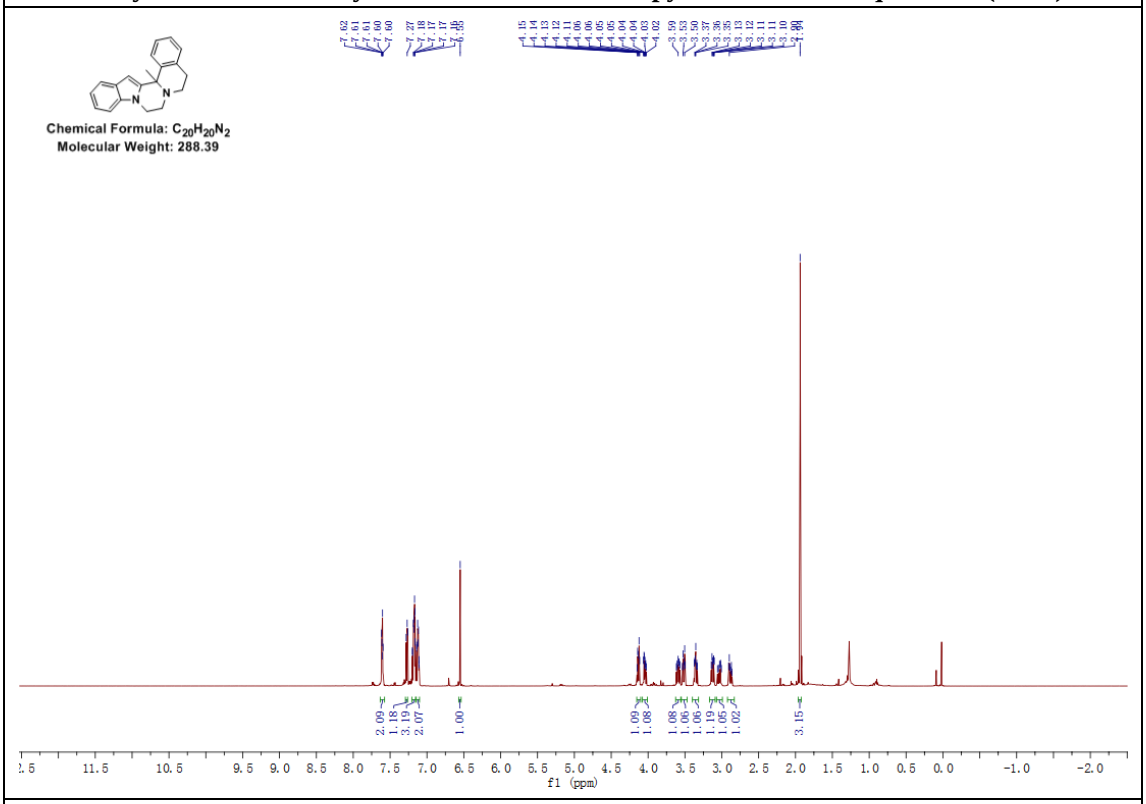


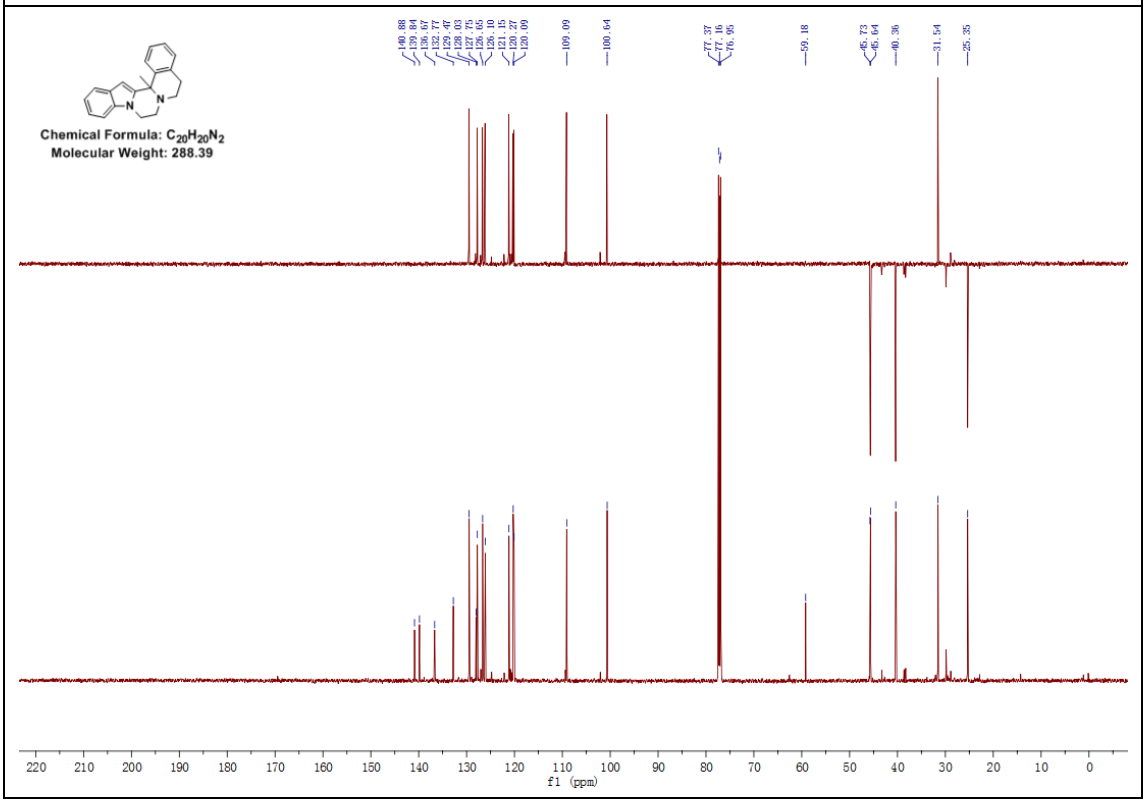


14c-methyl-5,6,8,9,10,14c-hexahydroindolo[3',2':3,4]pyrido[2,1-*a*]isoquinoline (SF51)

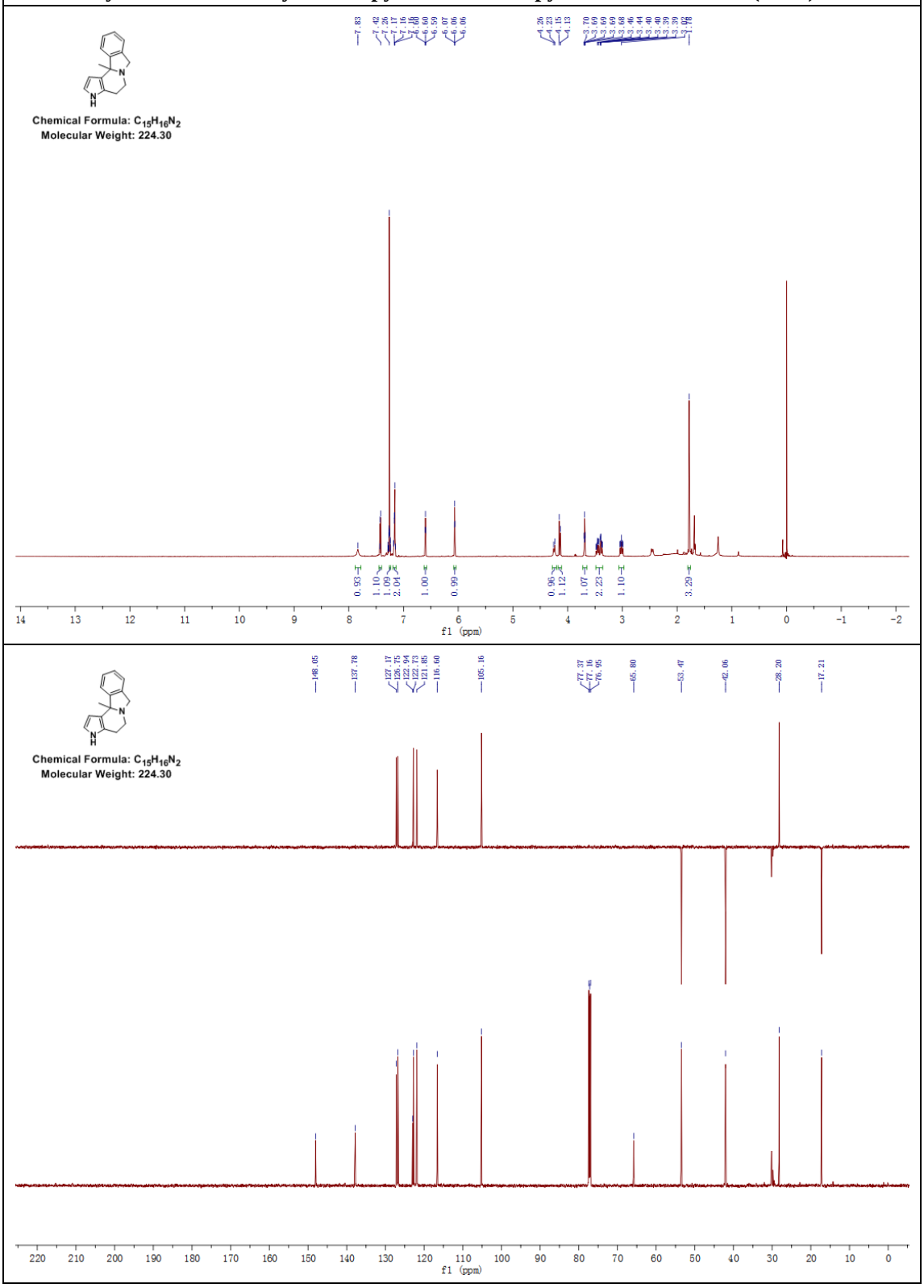


15b-methyl-6,8,9,15b-tetrahydro-5*H*-indolo[2',1':3,4]pyrazino[2,1-*a*]isoquinoline (SF52)

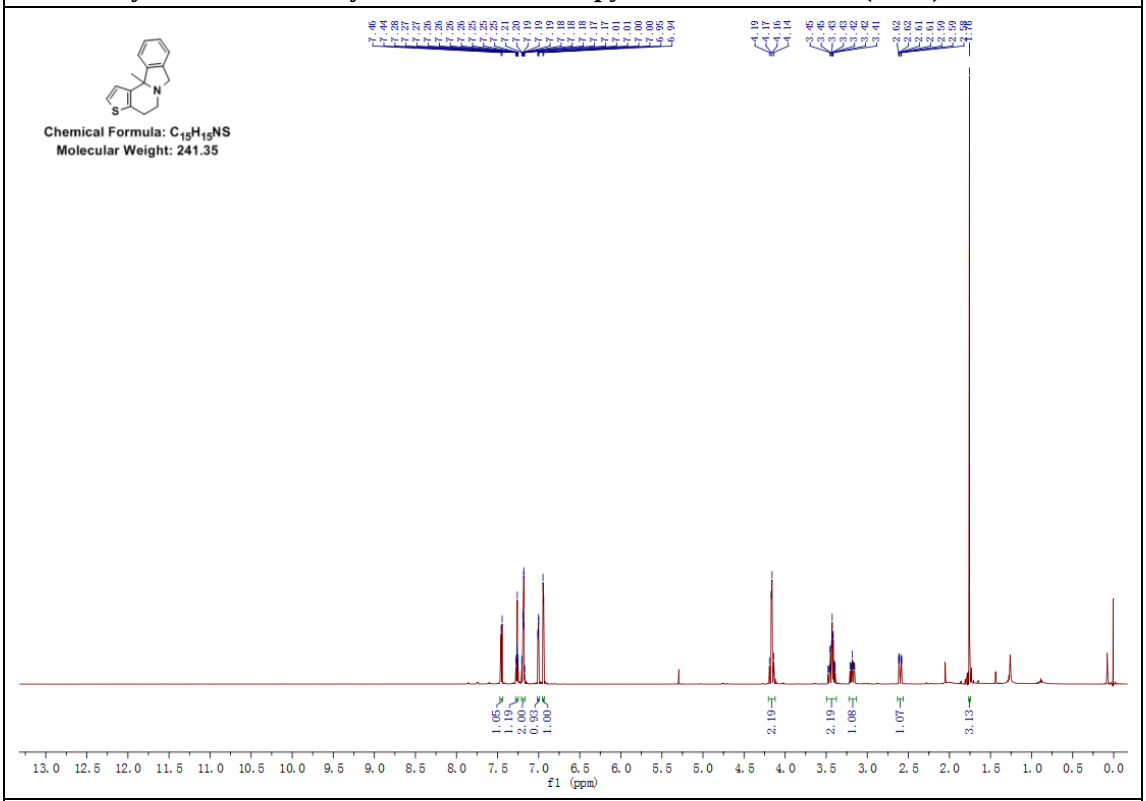


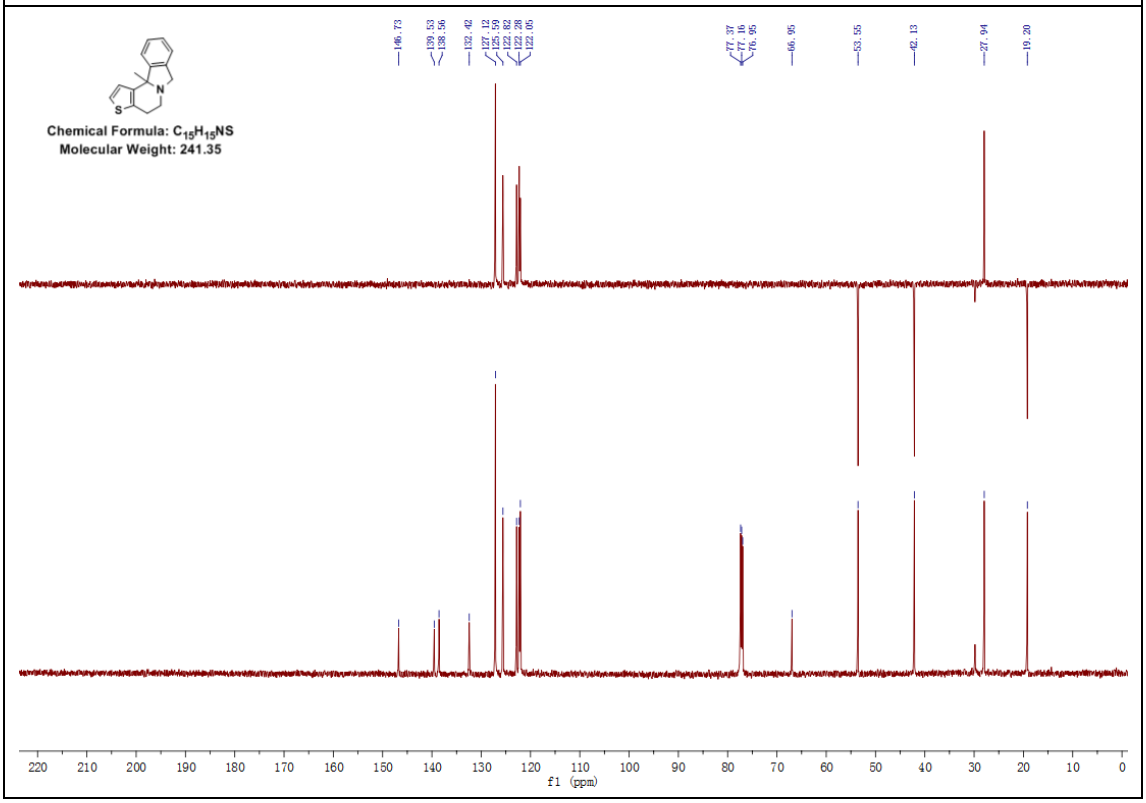


11b-methyl-4,5,7,11b-tetrahydro-3*H*-pyrrolo[3',2':3,4]pyrido[2,1-*a*]isoindole (SF53)



11b-methyl-4,5,7,11b-tetrahydrothieno[3',2':3,4]pyrido[2,1-*a*]isoindole (SF54)





11b-methyl-4,5,7,11b-tetrahydrothieno[2',3':3,4]pyrido[2,1-*a*]isoindole (SF55)