SUPPORTING INFORMATION

Novel Tacrine Derivatives as Potential CDK9 Inhibitors with Low Cholinesterase Inhibitory Property: Design, Synthesis, and Biological Evaluation

Wenwu Liu,‡[ab] Yaoguang Huang,‡[b] Limeng Wu,‡[b] Wenjie Liu,[b] Xiaowen Jiang,[b] Zihua Xu[ac] and Qingchun Zhao*[ab]

[a] Teaching Hospital of Shenyang Pharmaceutical University, General Hospital of Northern Theater Command, Shenyang 110840, People's Republic of China

[b] School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, People's Republic of China

[c] School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, People's Republic of China

* Corresponding authors:

Qingchun Zhao: E-mail address: zhaoqingchun1967@163.com (Q.C. Zhao).

‡These authors contributed equally to this work.

Table S1 The L02 cells cytotoxicity of compounds **5a**-**5u**

Comp.	Inhibition % at 10 μM or IC_{50} (μM）
5a	72.9%
5b	84.0%
5c	6.28
5d	4.61
5e	7.04
5f	0.91
5g	94.0%
5h	2.64
5i	2.87
5j	> 10
5k	71.3%
5l	> 10
5m	100%
5n	93.4%
5o	74.8%
5p	38.4%
5q	67.4%
5r (ZLWT-48)	3.83
5s	60.0%
5t	63.7%
5u	72.9%
Tacrine	> 100
ZLWT-37	0.26
Dinaciclib	0.014

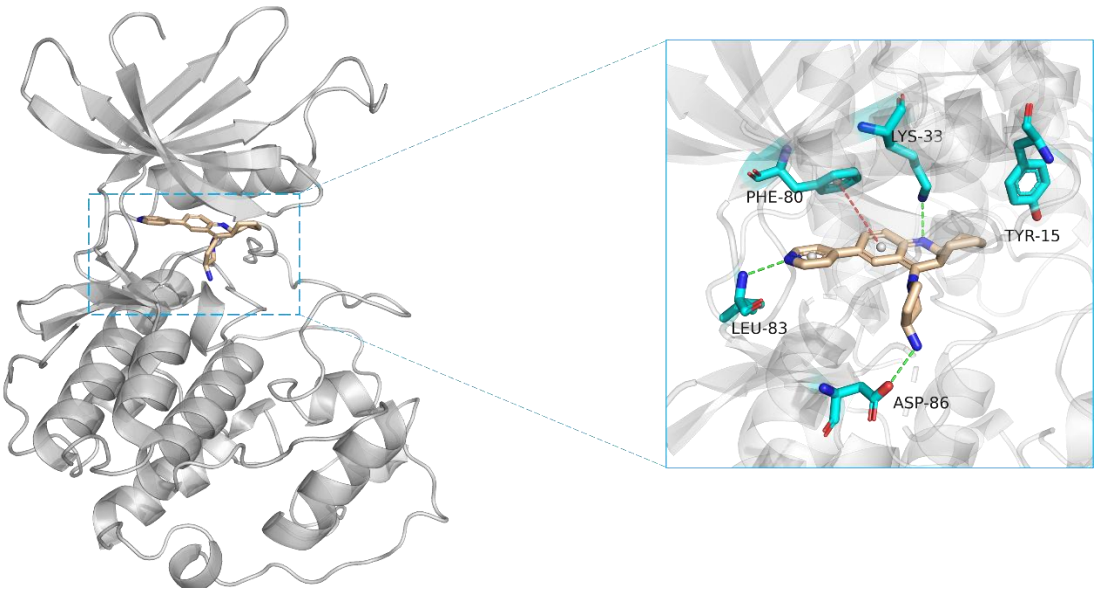
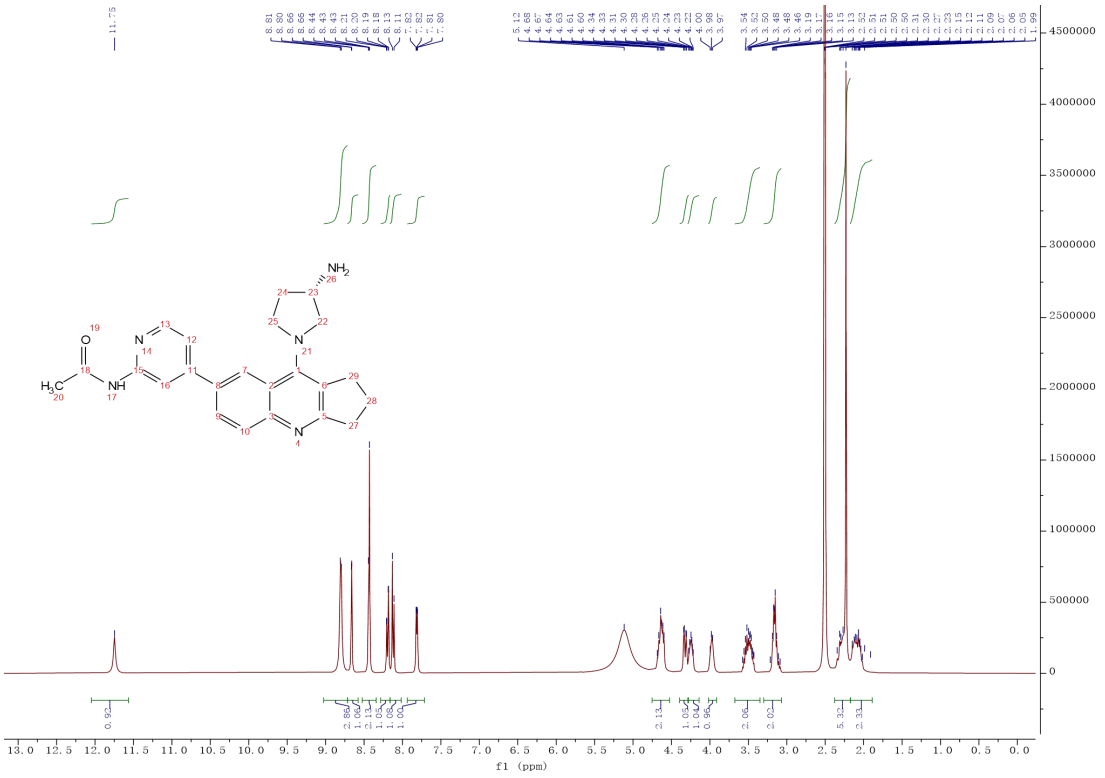
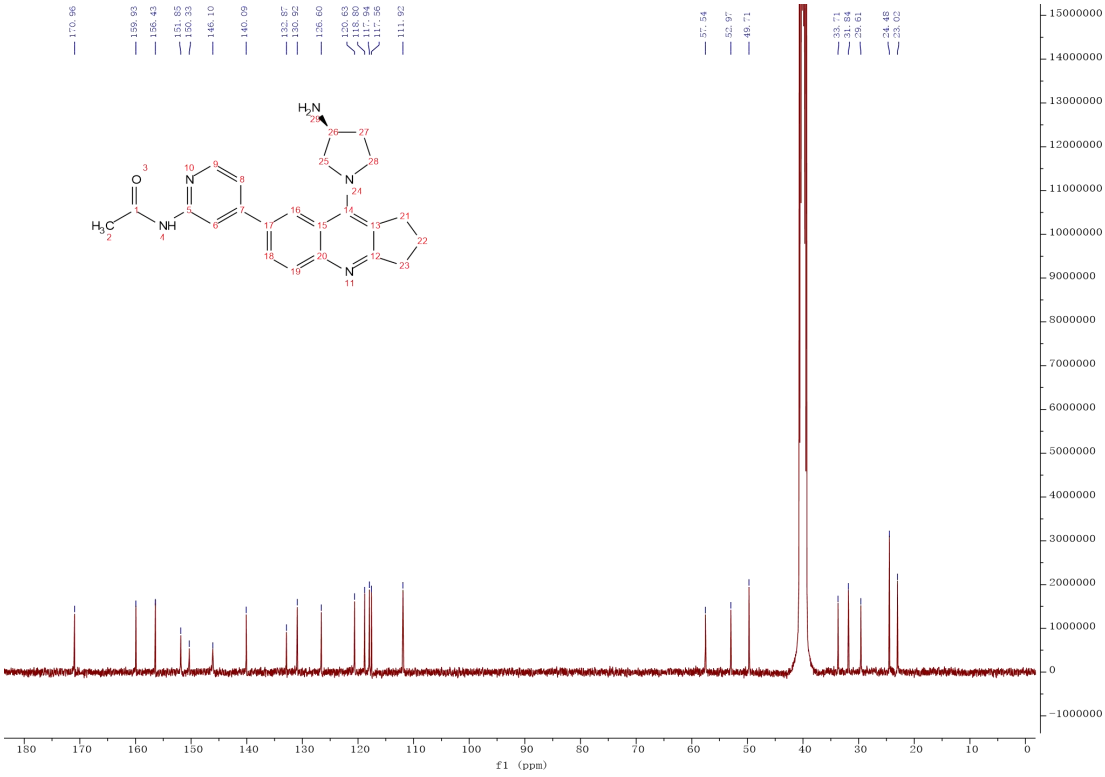


Fig. S1 Docking pose of compound **ZLWT-48** in the binding site of CDK2 (PDB code:3EID). Docking was performed with Glide, and images were generated with Pymol. Green-dashed lines indicate H-bond interactions, red-dashed lines indicate hydrophobic interactions. The compound binding pocket with interacting residues was shown in stick mode (wheat).

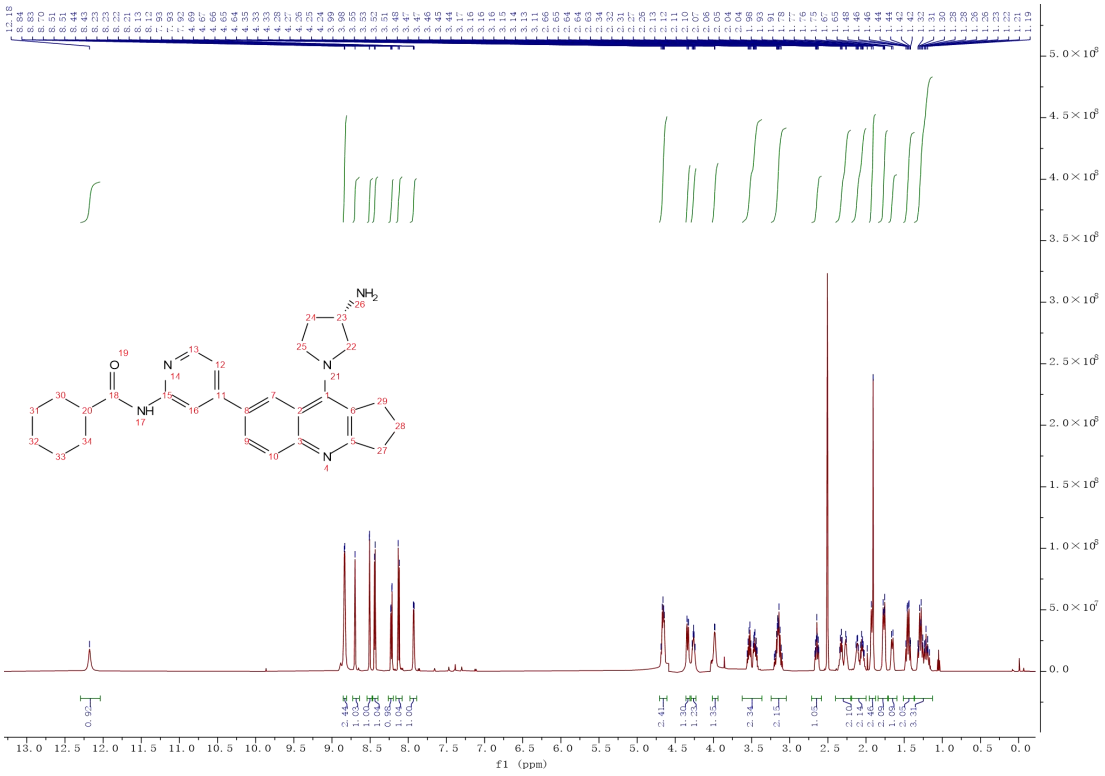
^{1}H NMR Spectra, ^{13}C NMR Spectra, HR-MS, and HPLC traces of Representative Compounds.



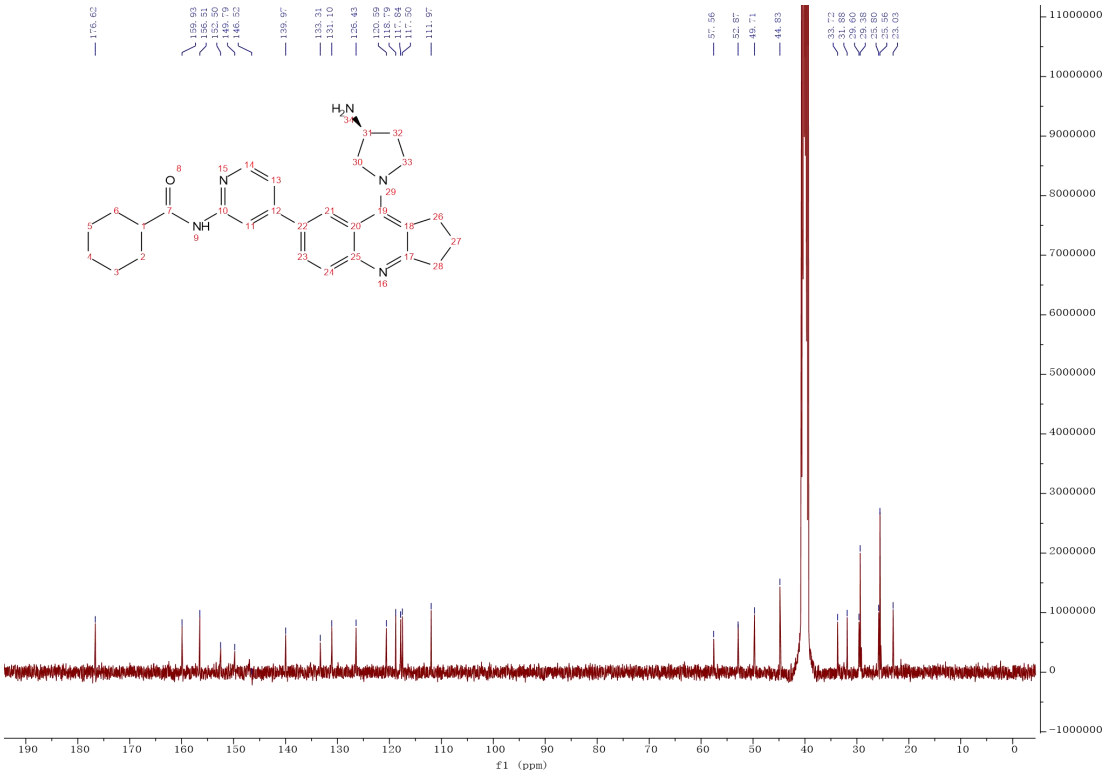
^{1}H NMR spectrum of **5a** in DMSO-d_6.



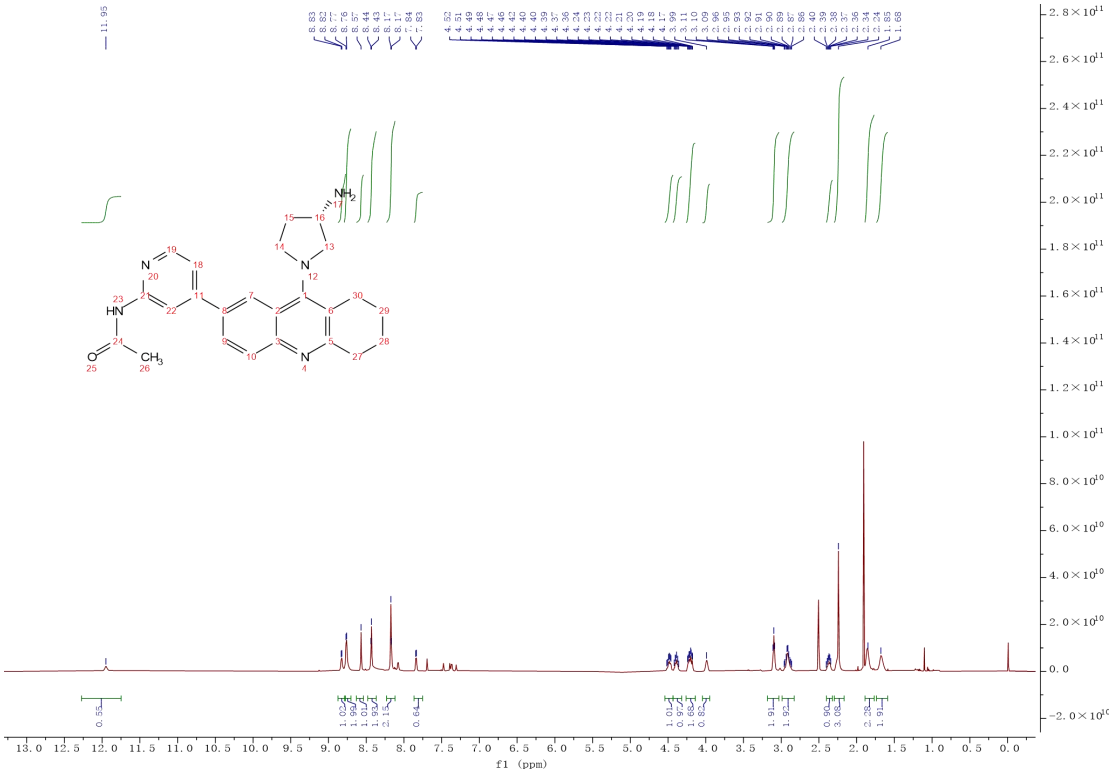
^{13}C NMR spectrum of **5a** in DMSO-d_6.



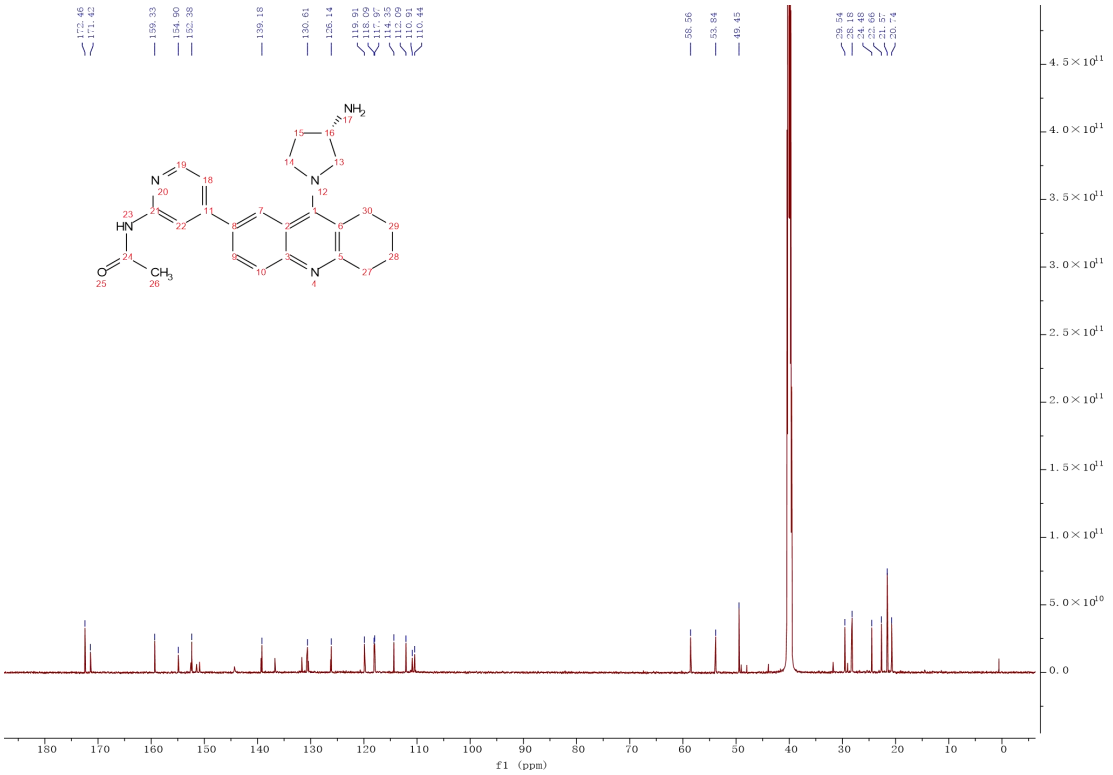
^{1}H NMR spectrum of **5b** in DMSO-d_6.



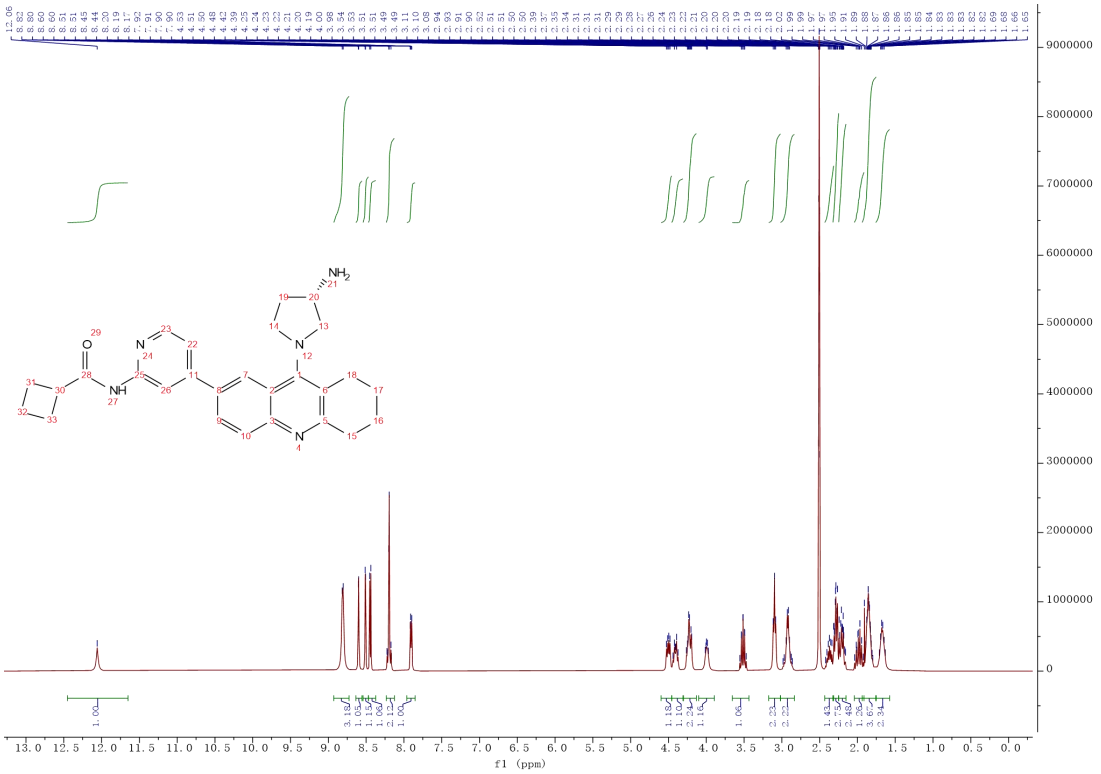
^{13}C NMR spectrum of **5b** in DMSO-d_6.



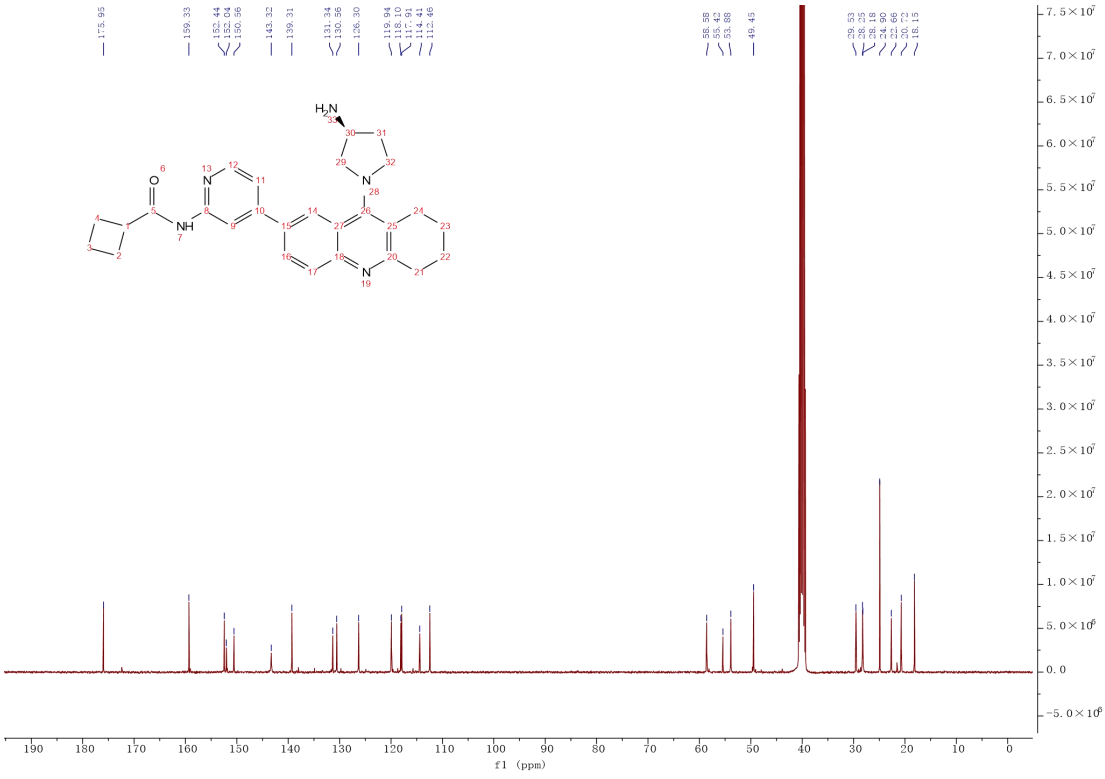
^{1}H NMR spectrum of **5c** in DMSO-d_6.



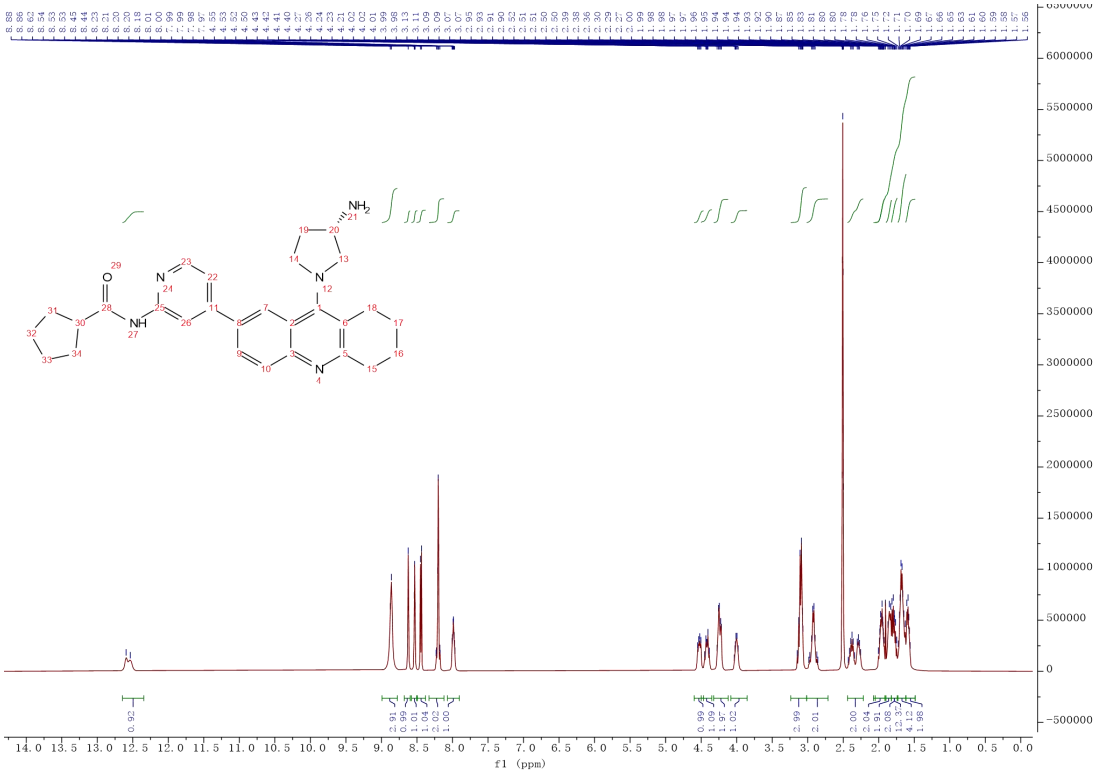
^{13}C NMR spectrum of **5c** in DMSO-d_6.



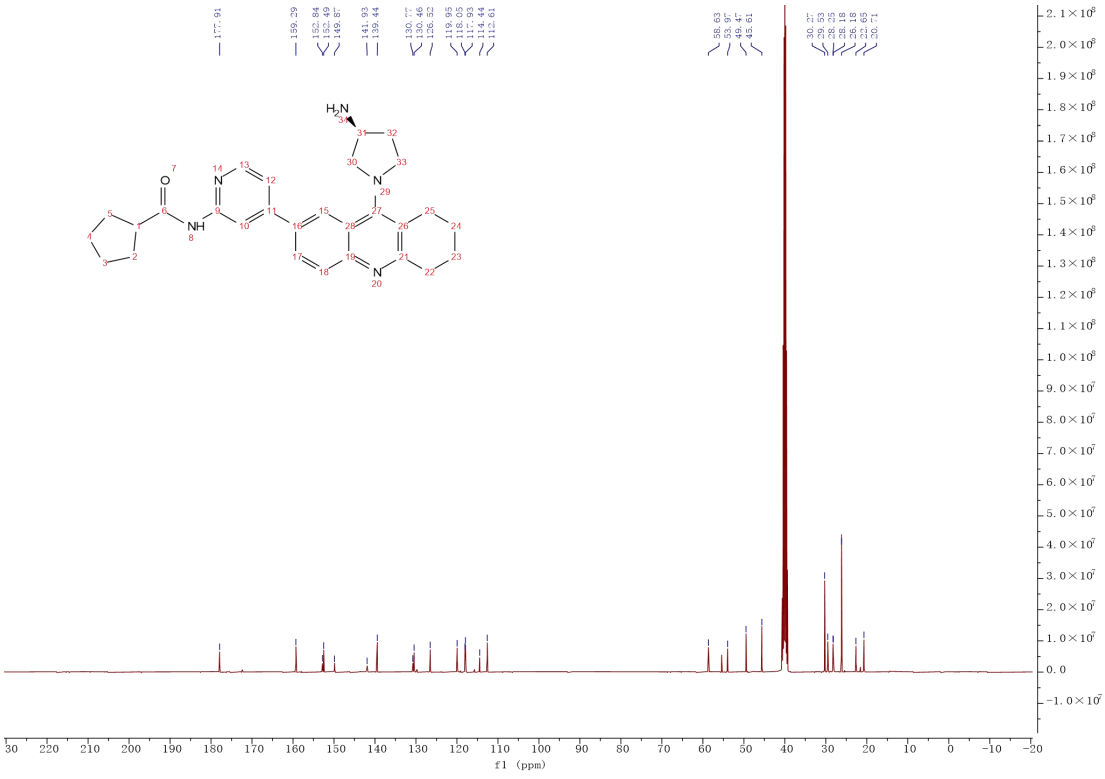
^{1}H NMR spectrum of **5d** in DMSO-d_6.



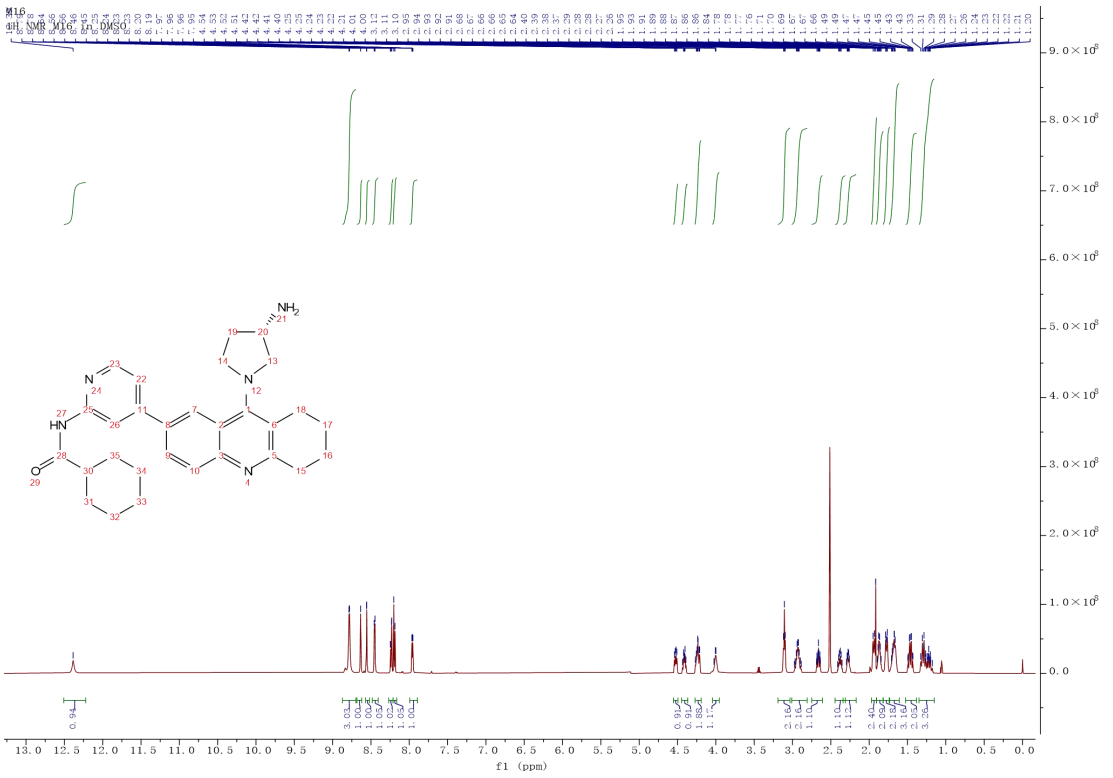
^{13}C NMR spectrum of **5d** in DMSO-d_6.



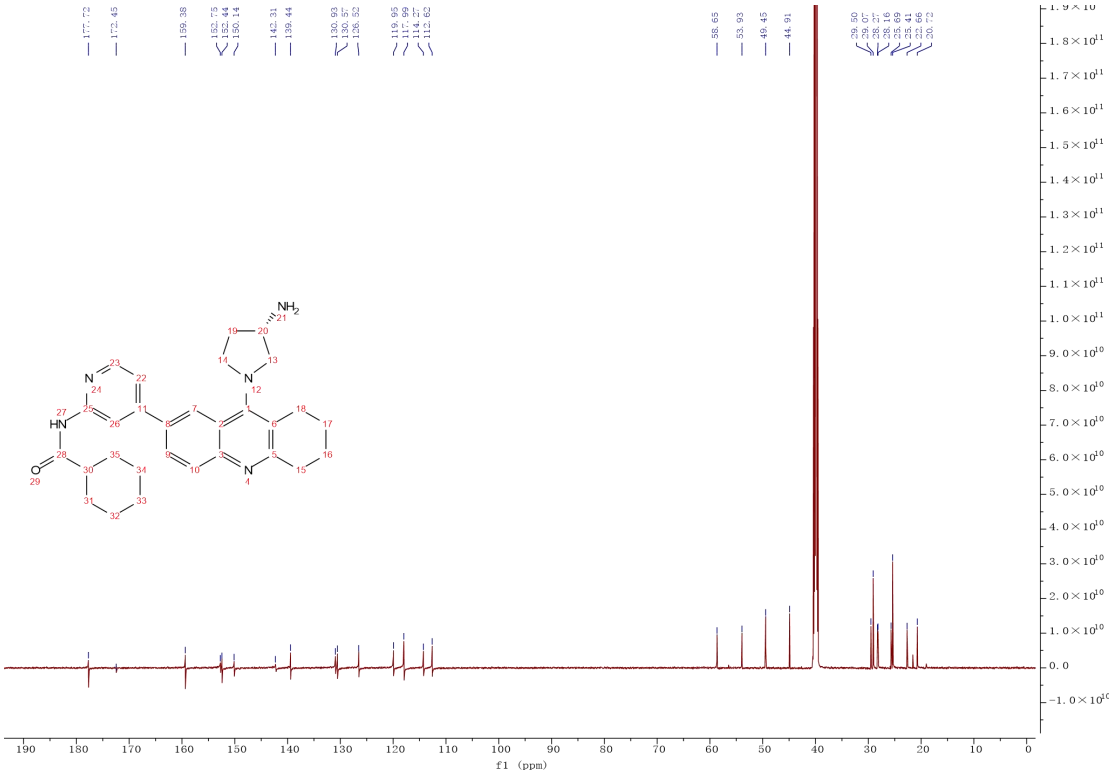
^{1}H NMR spectrum of **5e** in DMSO-d_6.



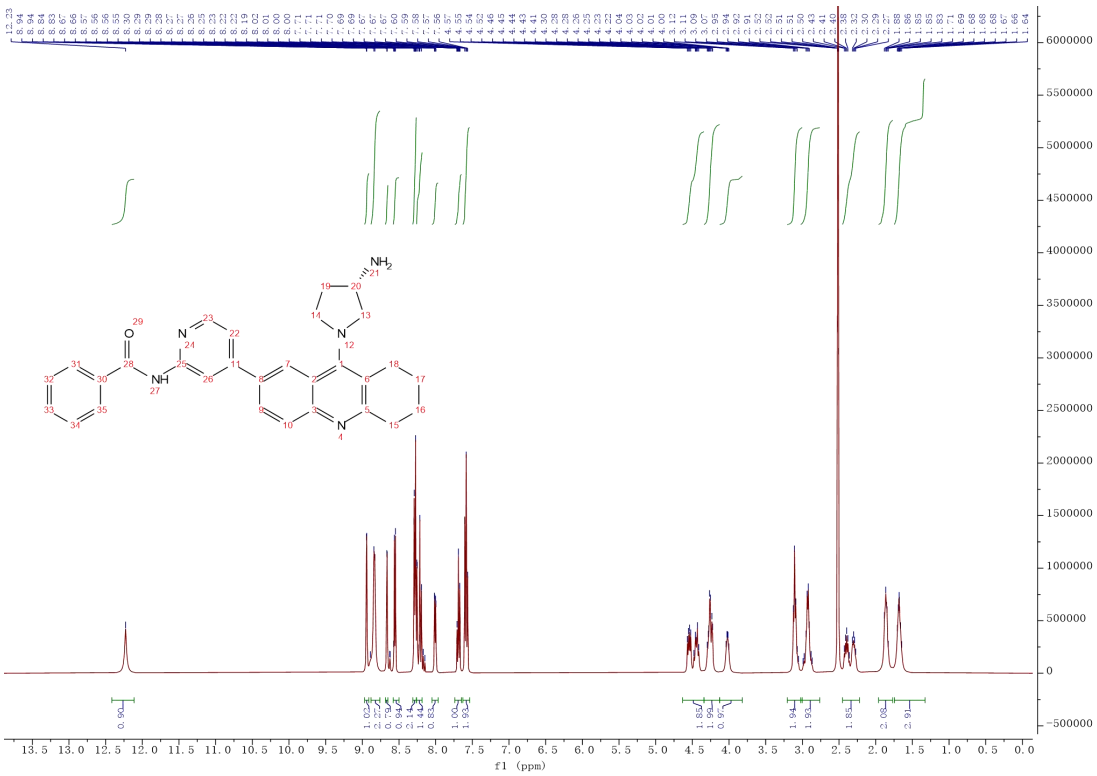
^{13}C NMR spectrum of **5e** in DMSO-d_6.



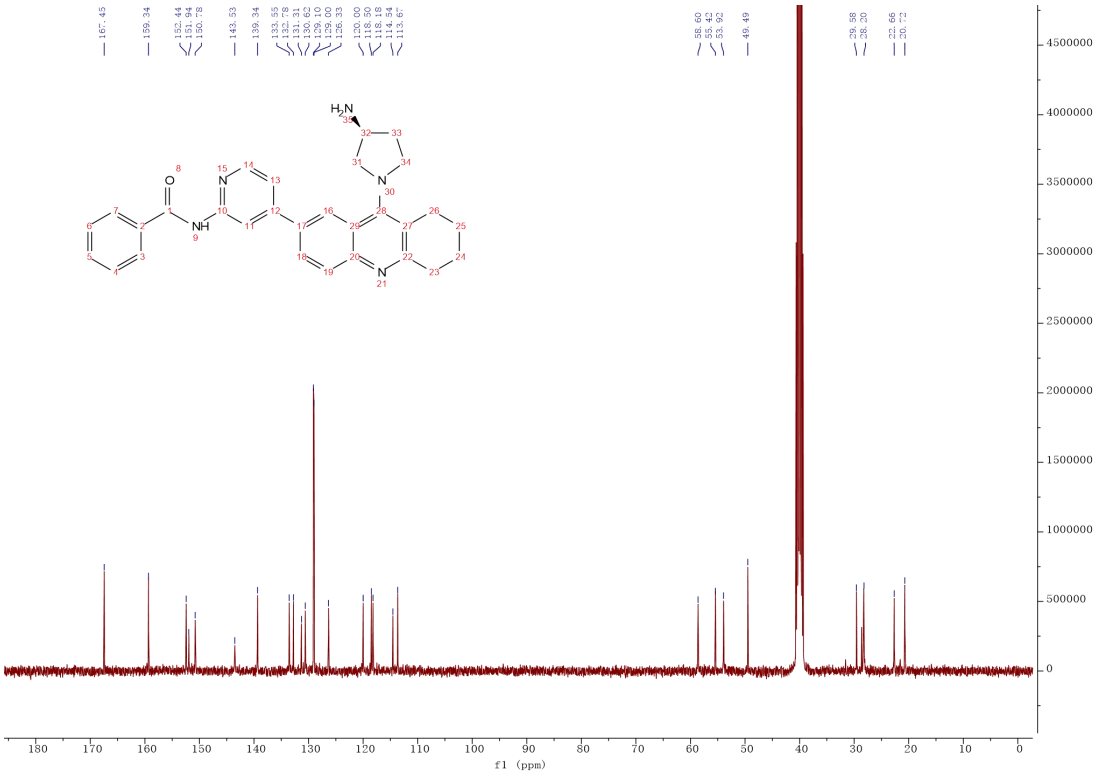
^{1}H NMR spectrum of **5f** in DMSO-d_6.



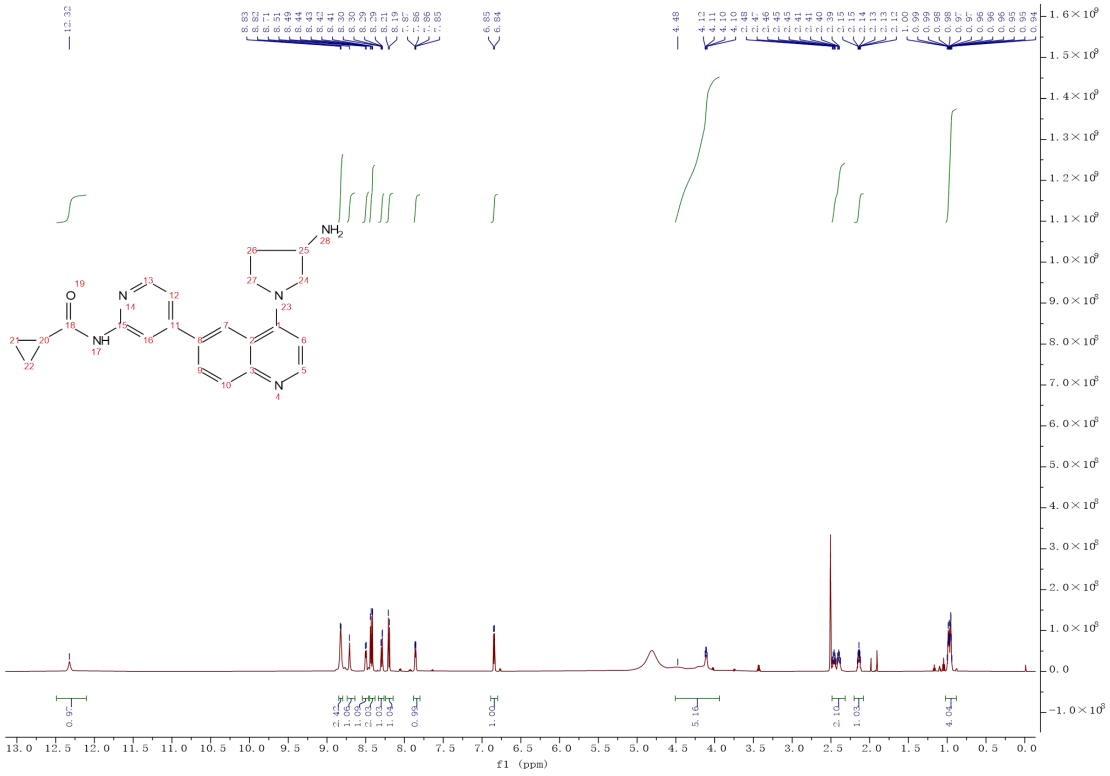
^{13}C NMR spectrum of **5f** in DMSO-d_6.



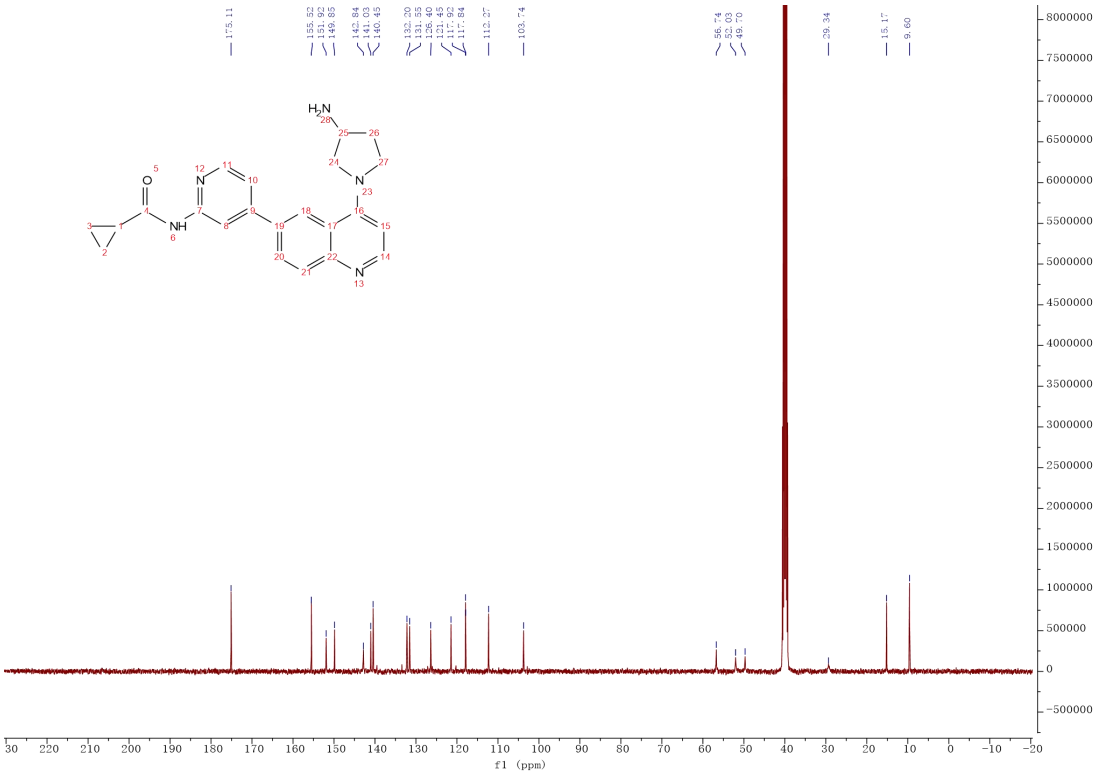
^{1}H NMR spectrum of **5g** in DMSO-d_6.



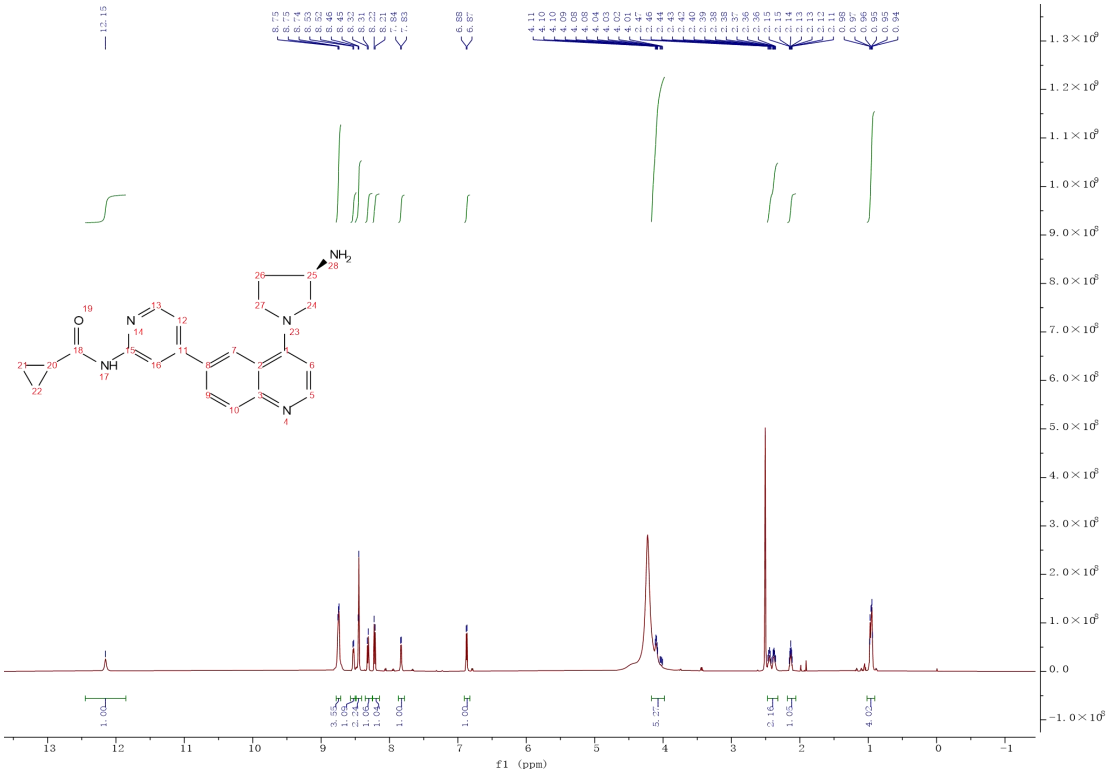
^{13}C NMR spectrum of **5g** in DMSO-d_6.



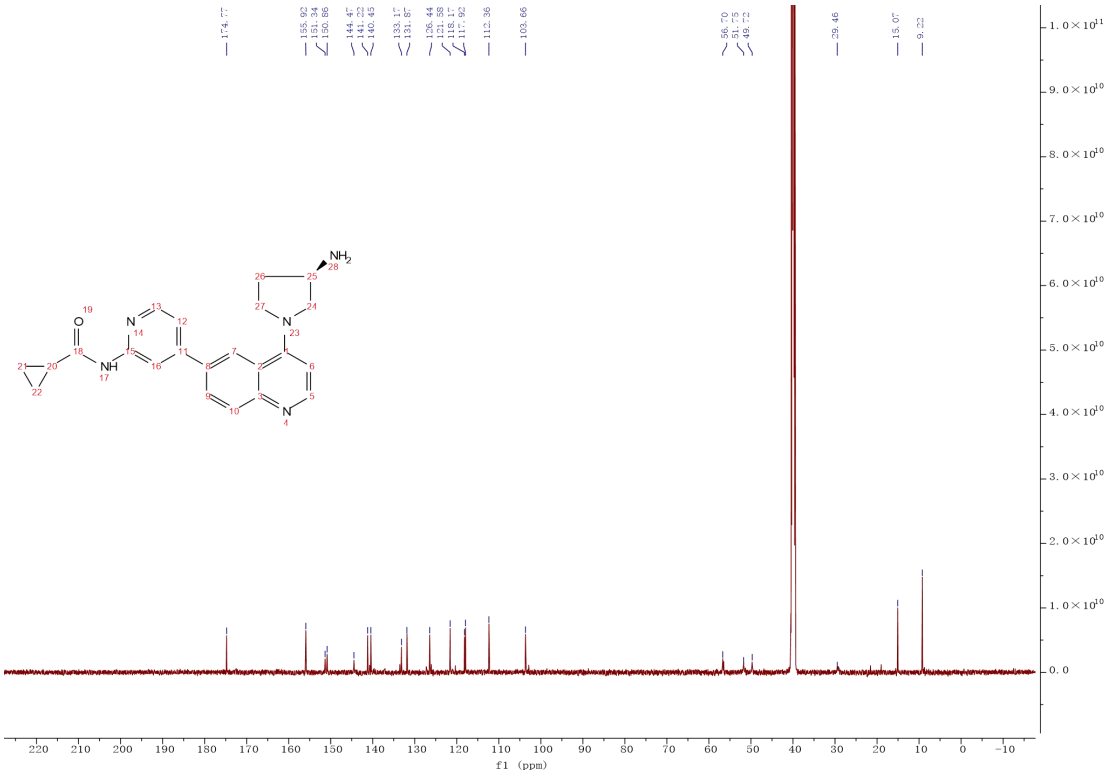
^{1}H NMR spectrum of **5h** in DMSO-d_6.



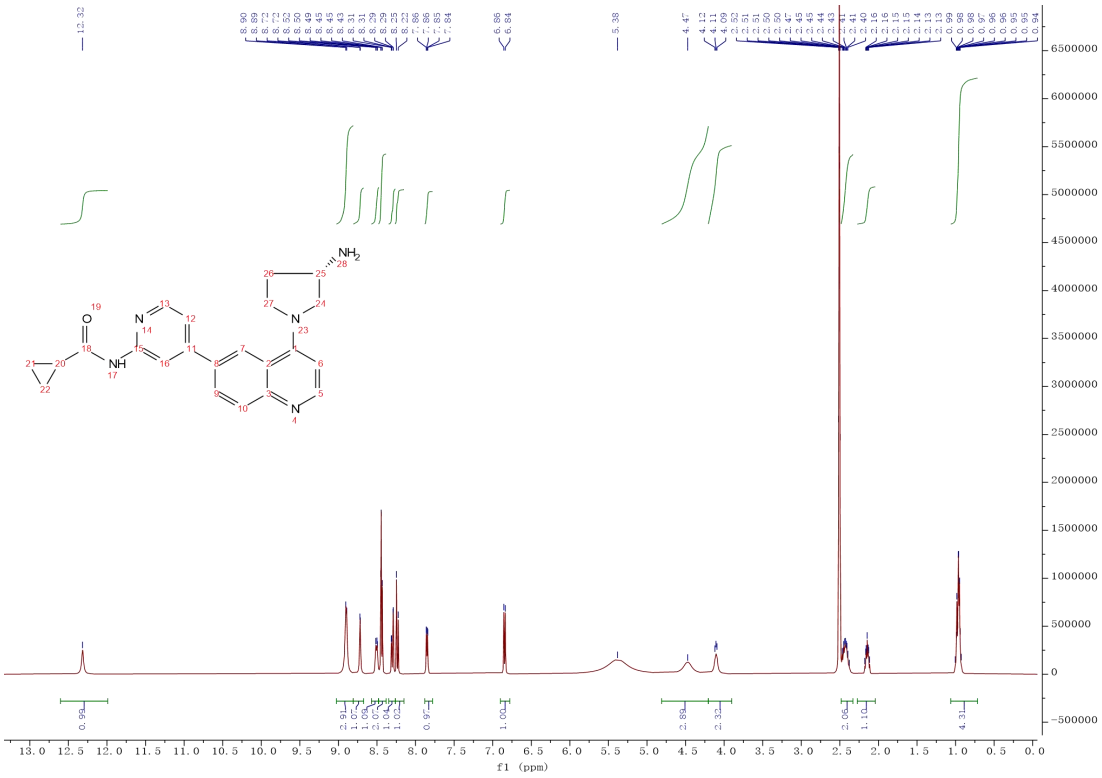
^{13}C NMR spectrum of **5h** in DMSO-d_6.



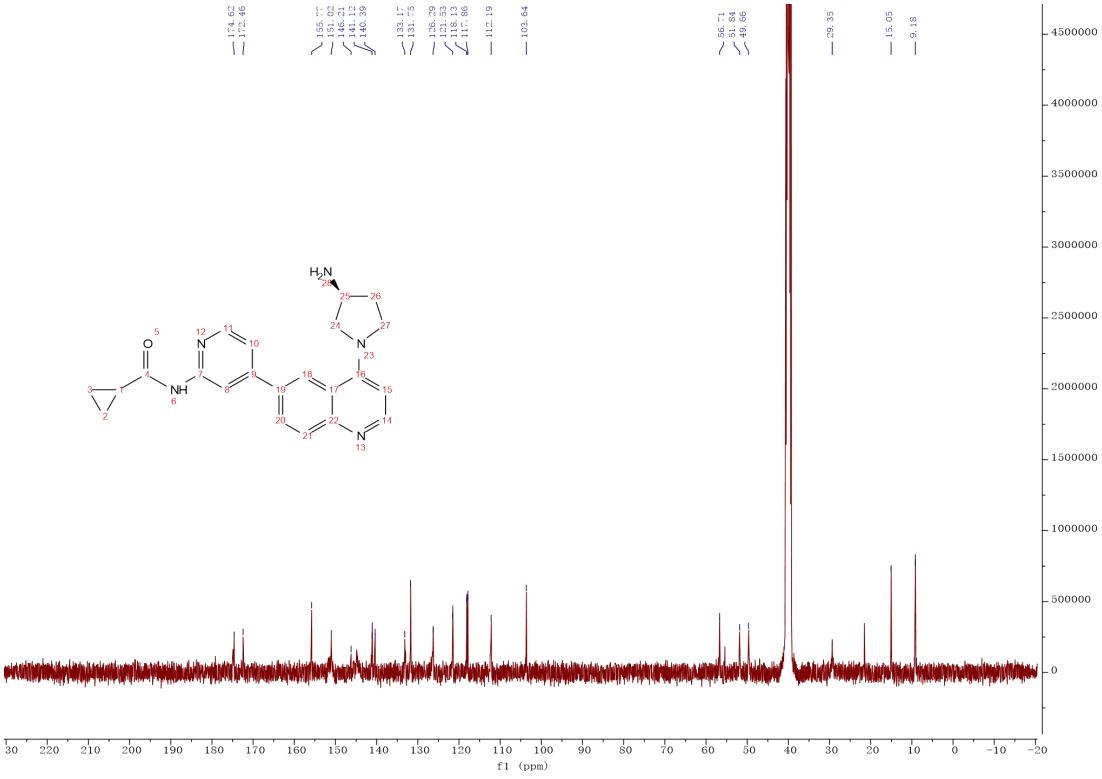
^{1}H NMR spectrum of **5i** in DMSO-d_6.



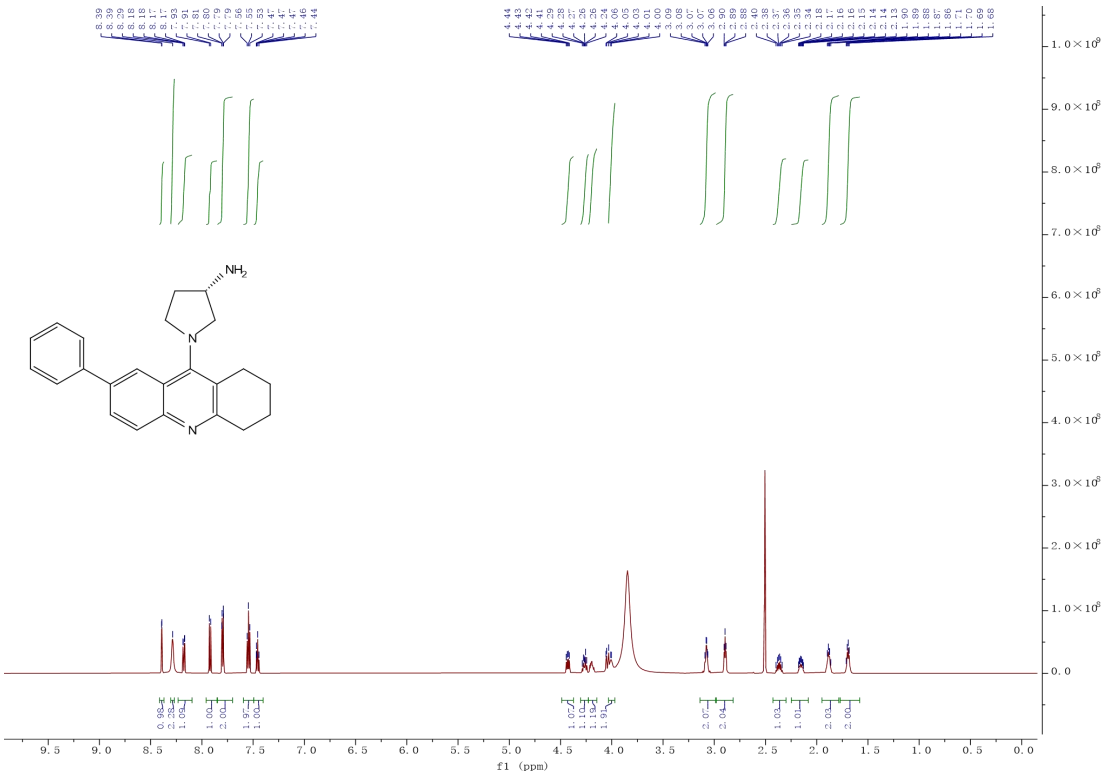
^{13}C NMR spectrum of **5i** in DMSO-d_6.



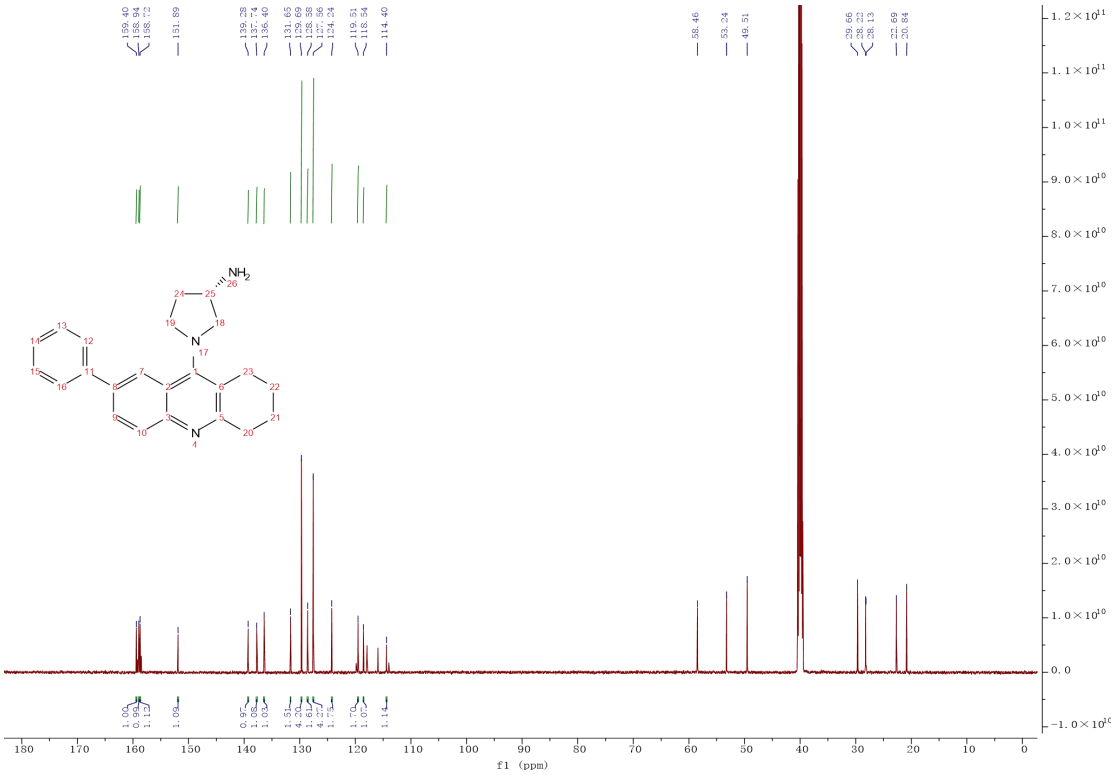
^{1}H NMR spectrum of **5j** in DMSO-d_6.



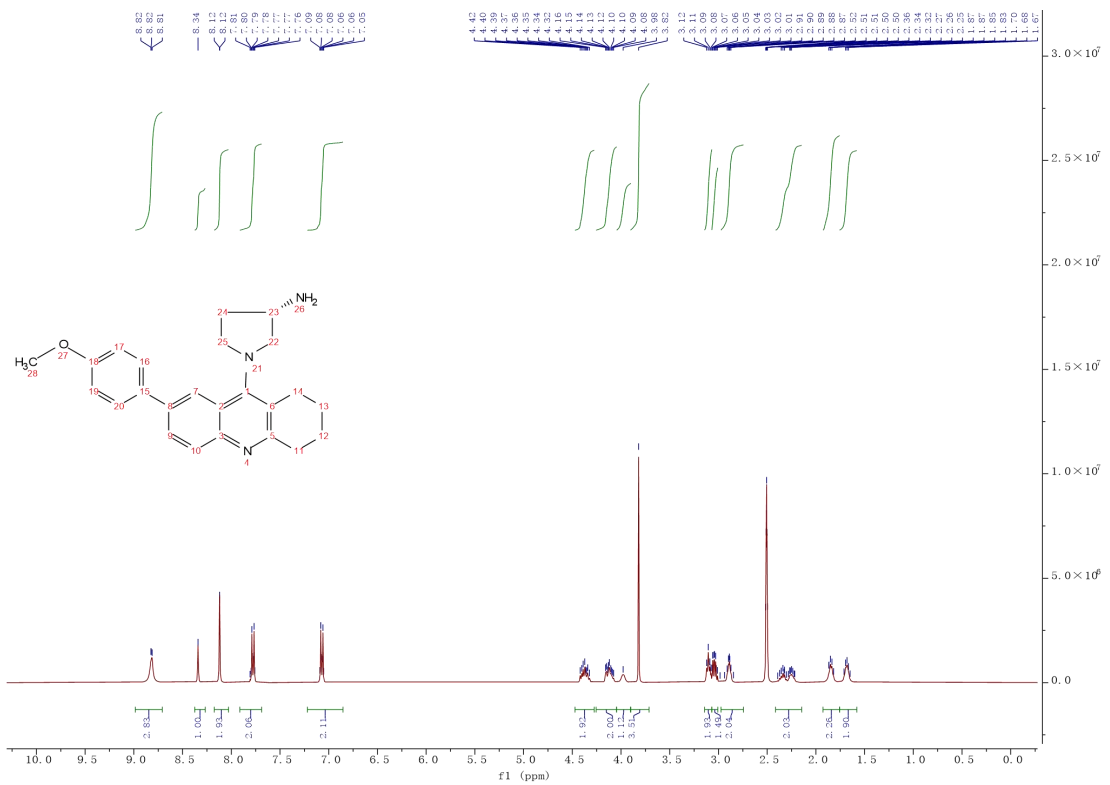
^{13}C NMR spectrum of **5j** in DMSO-d_6.



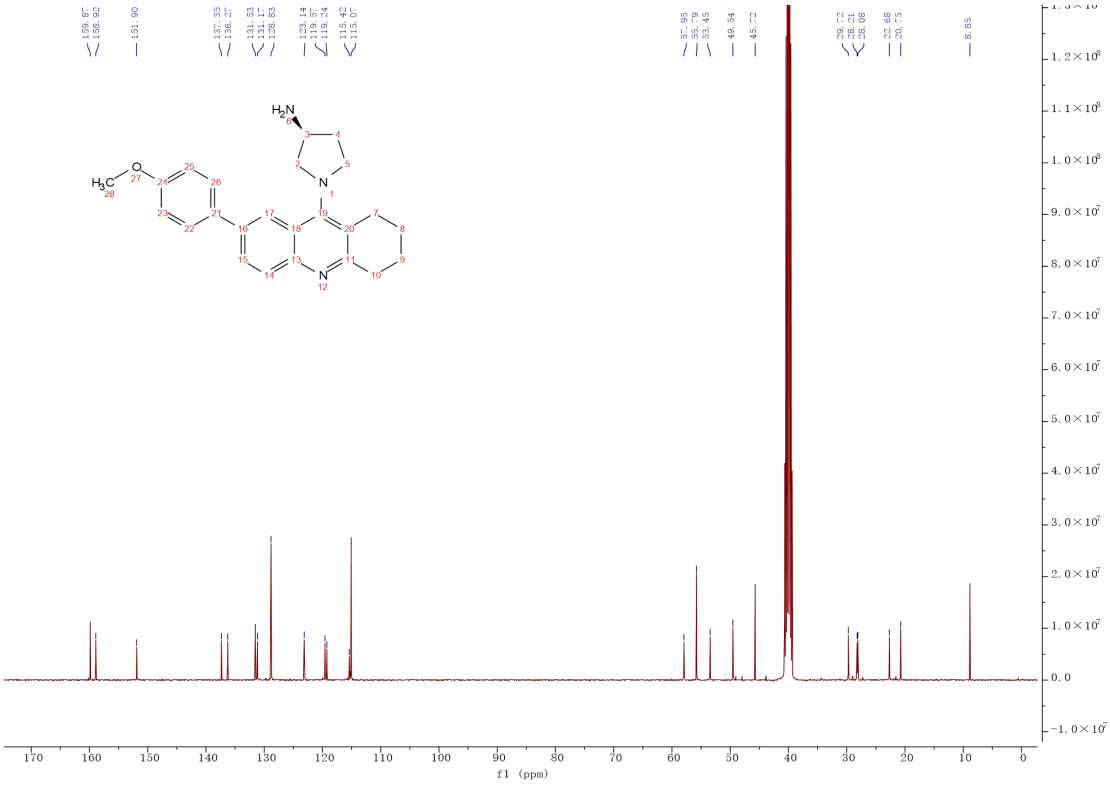
^{1}H NMR spectrum of **5k** in DMSO-d_6.



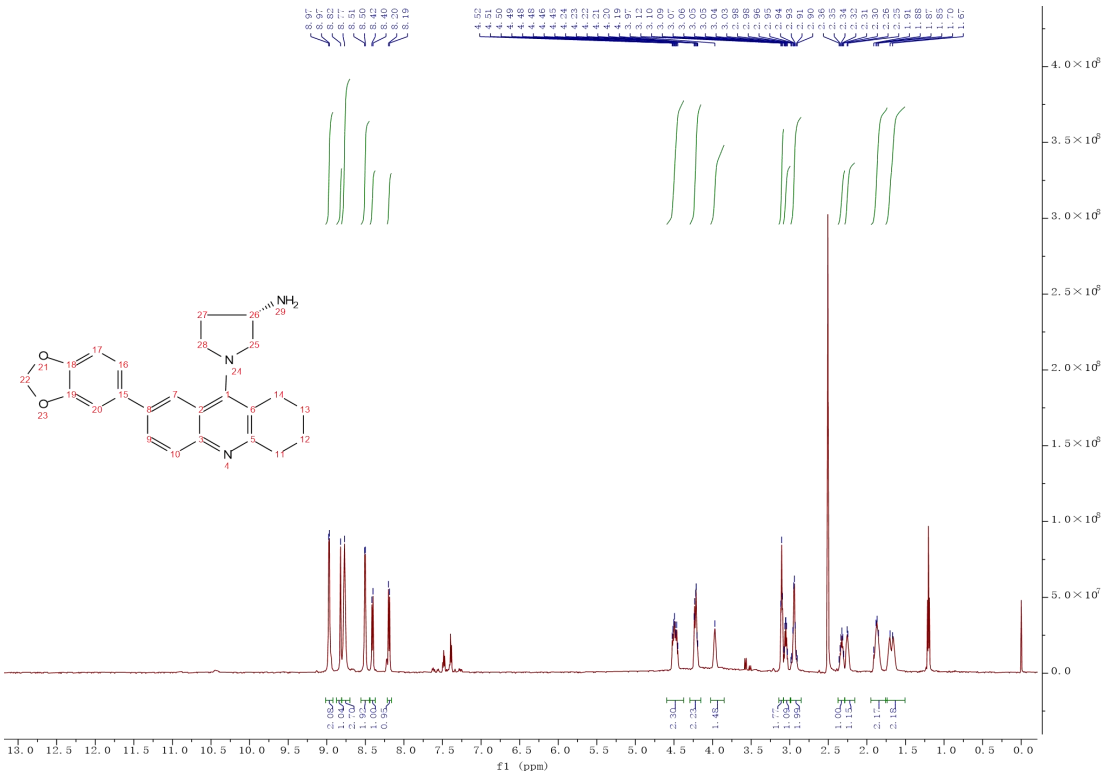
^{13}C NMR spectrum of **5k** in DMSO-d_6.



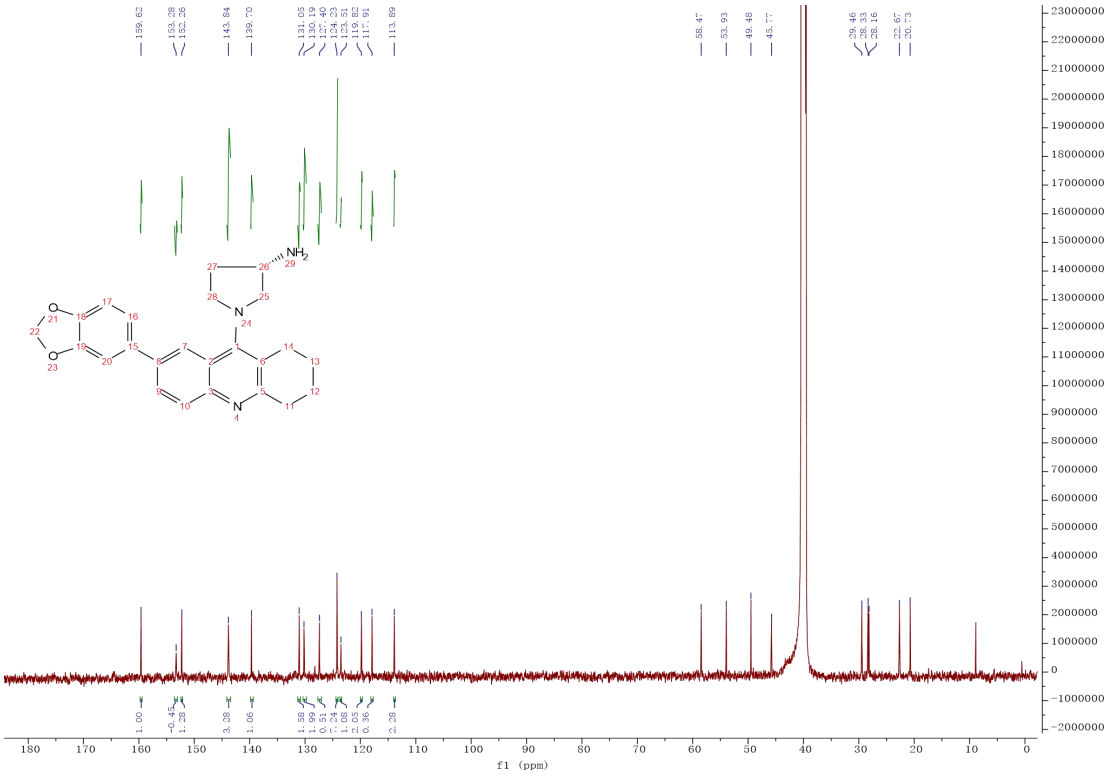
^{1}H NMR spectrum of **5l** in DMSO-d_6.



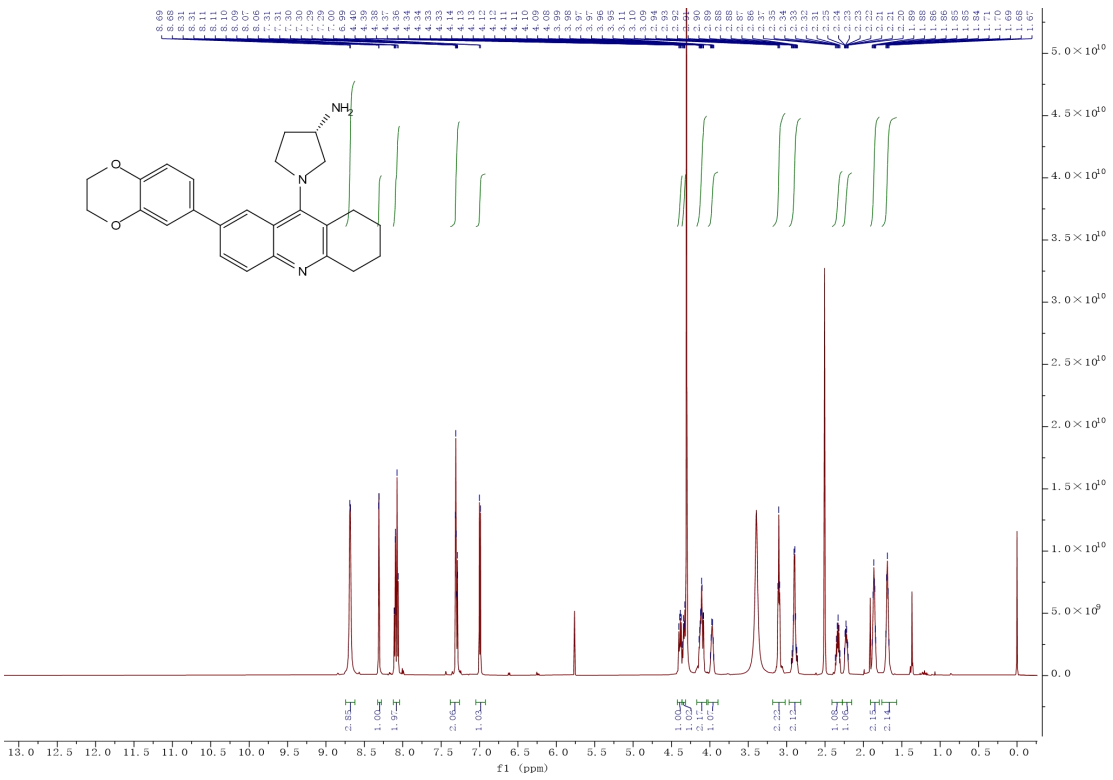
^{13}C NMR spectrum of **5l** in DMSO-d_6.



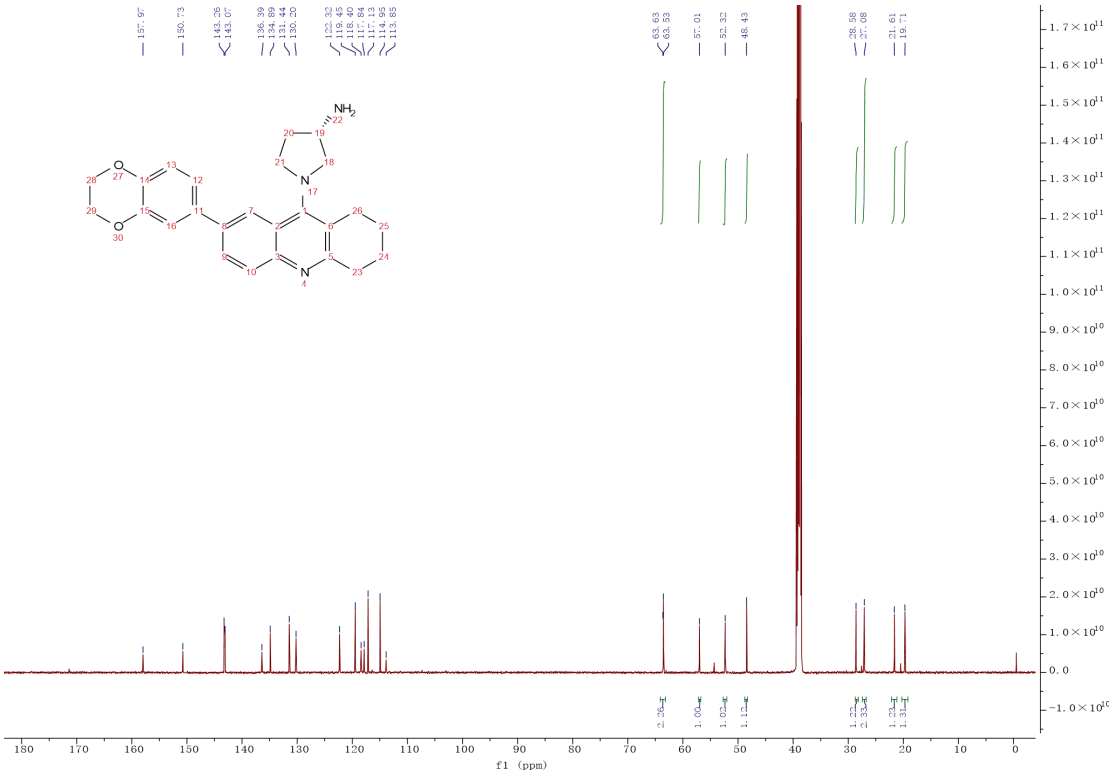
^{1}H NMR spectrum of **5m** in DMSO-d_6.



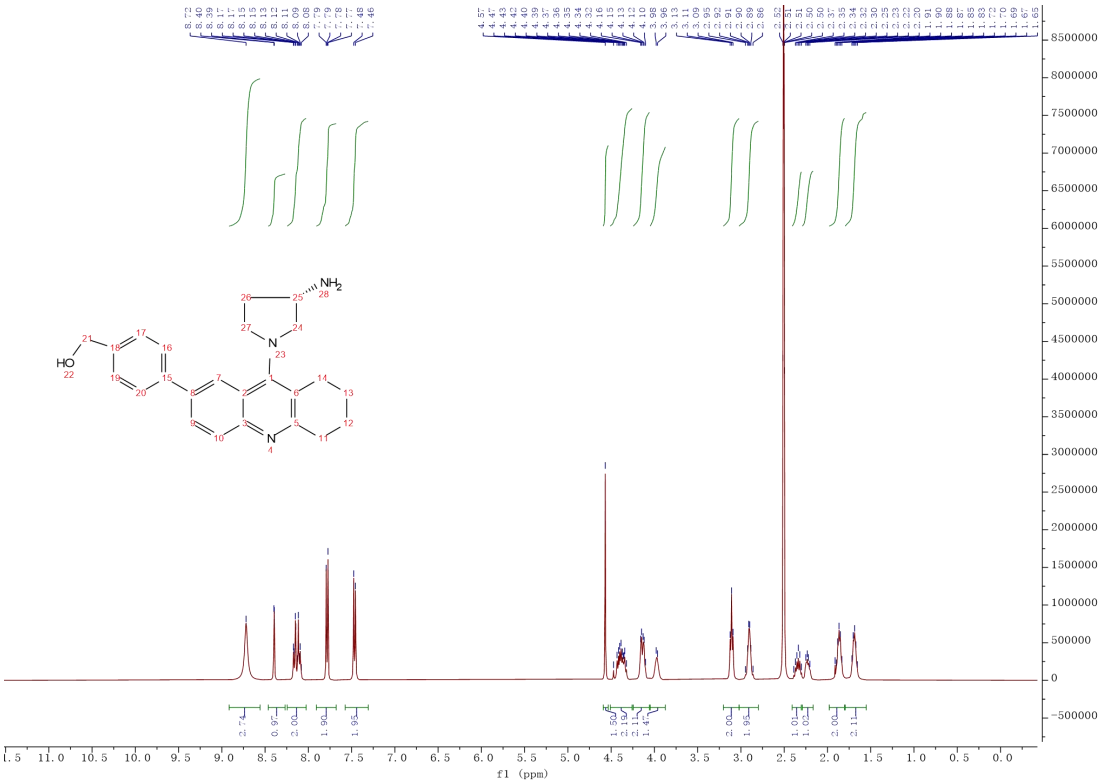
^{1}H NMR spectrum of **5m** in DMSO-d_6.



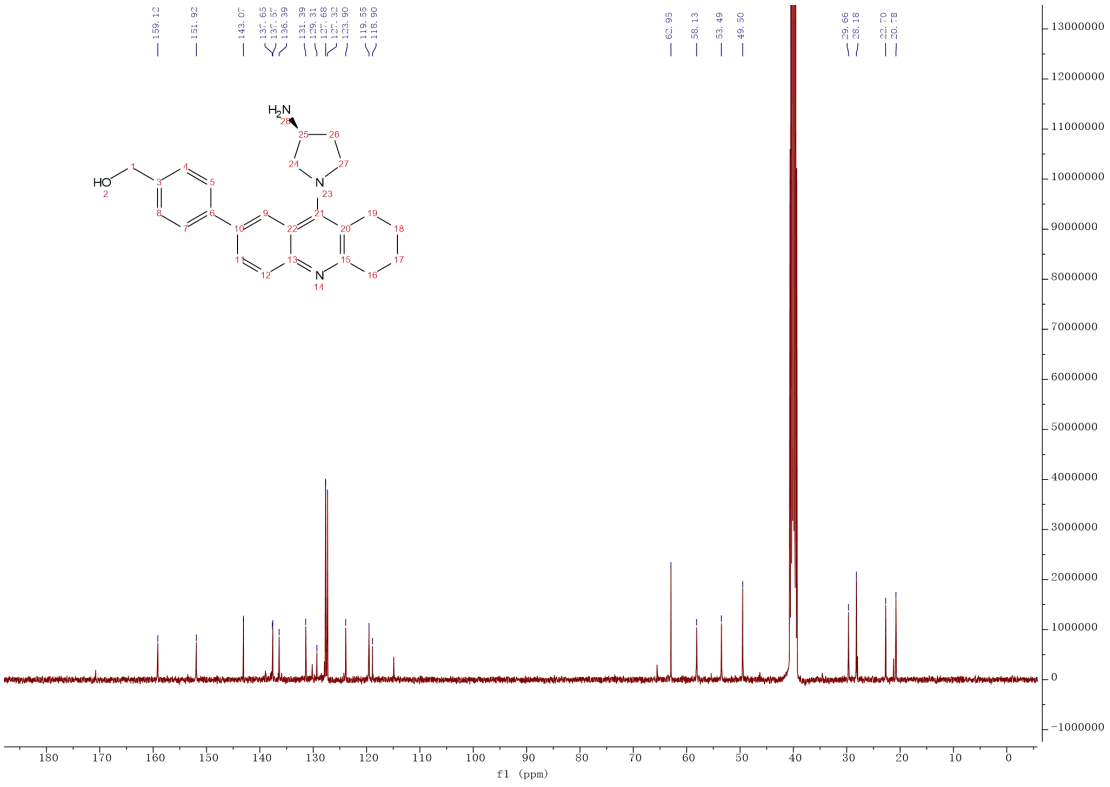
^{1}H NMR spectrum of **5n** in DMSO-d_6.



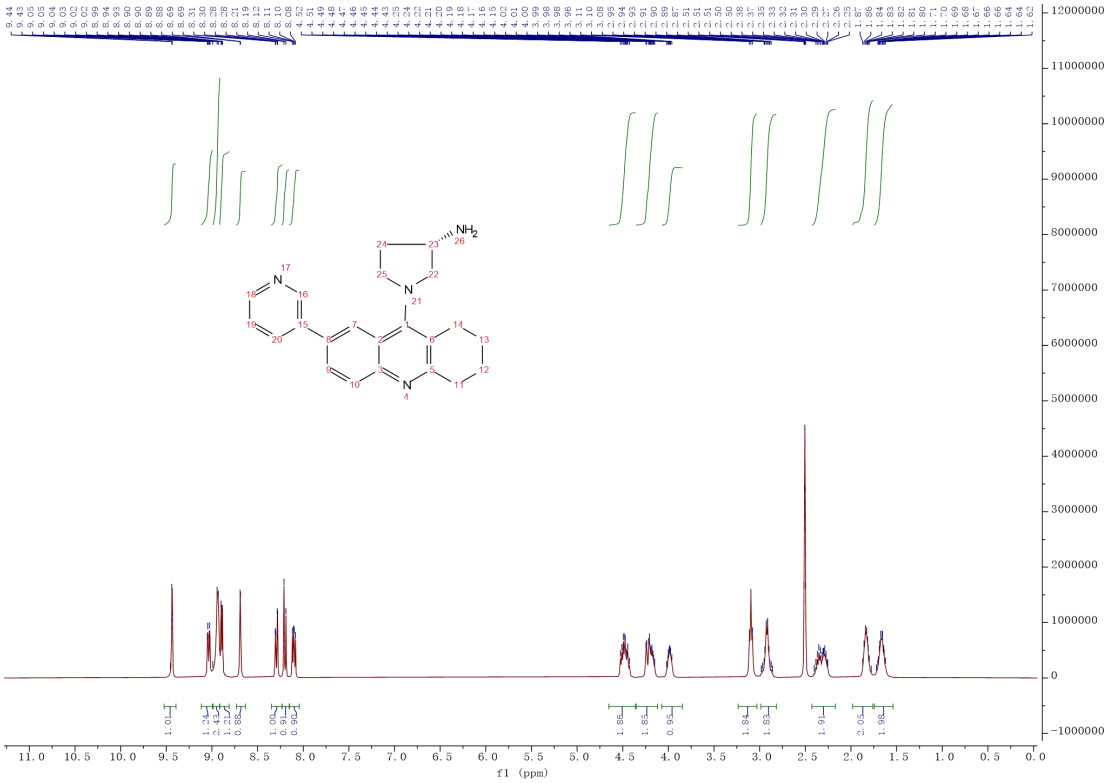
^{13}C NMR spectrum of **5n** in DMSO-d_6.



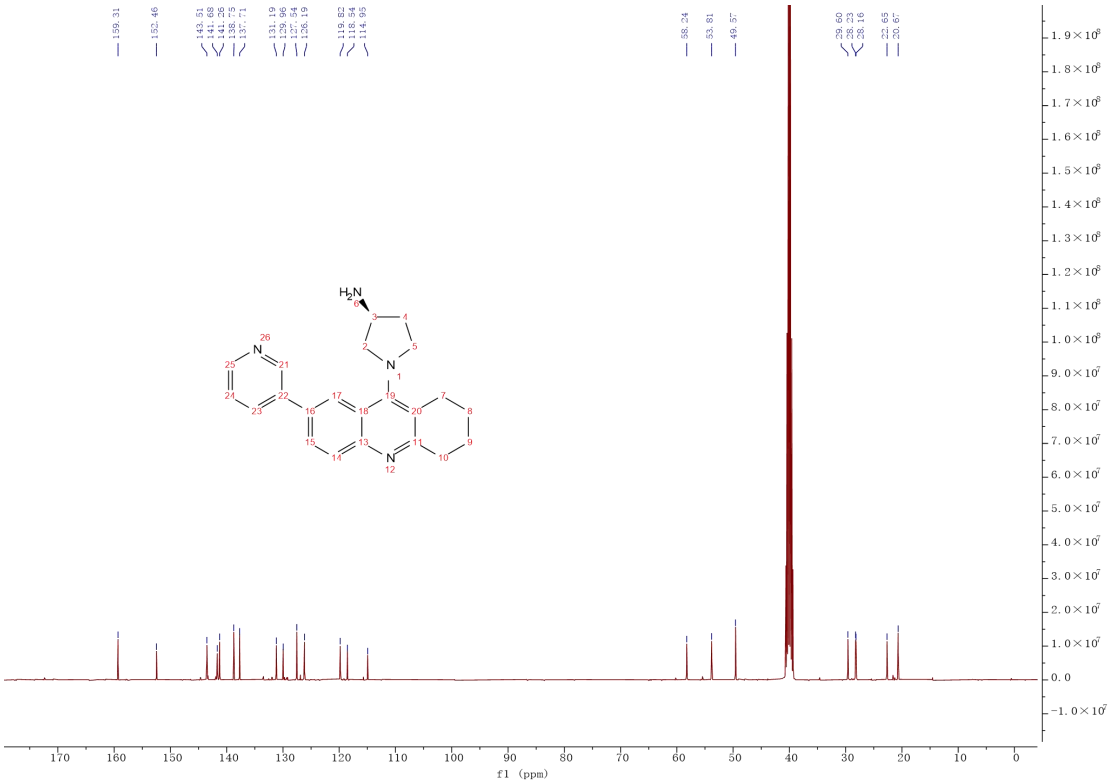
^{1}H NMR spectrum of **5o** in DMSO-d_6.



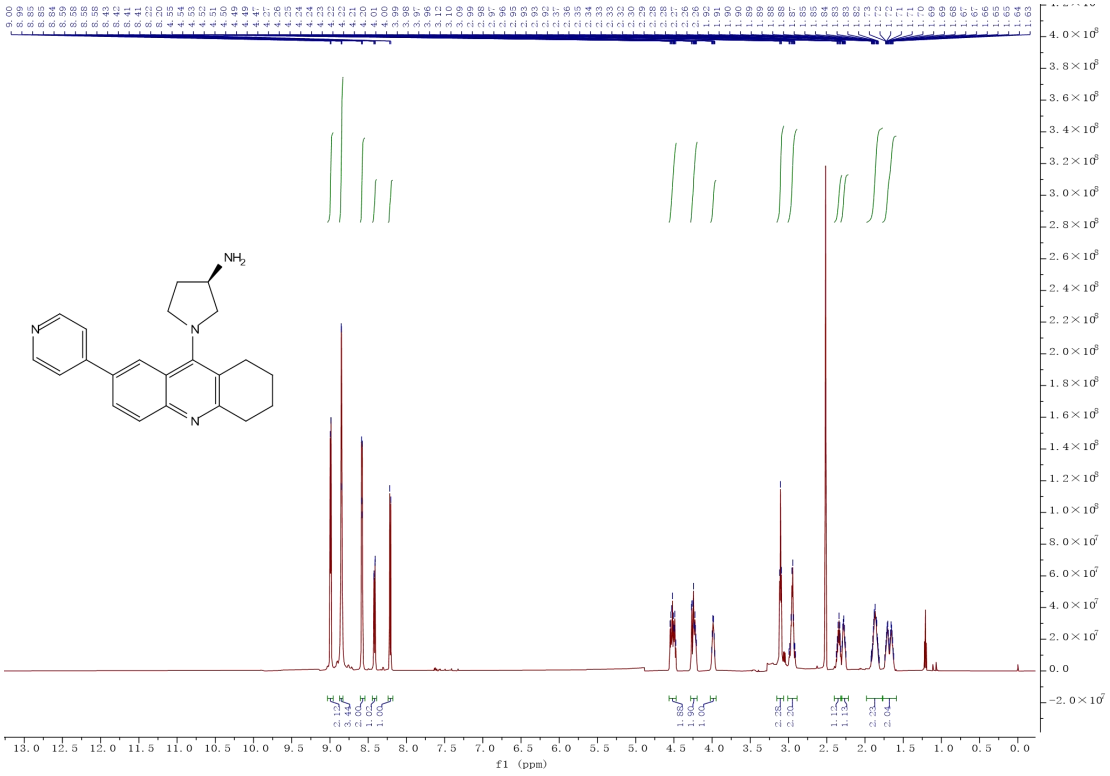
^{1}H NMR spectrum of **5o** in DMSO-d_6.



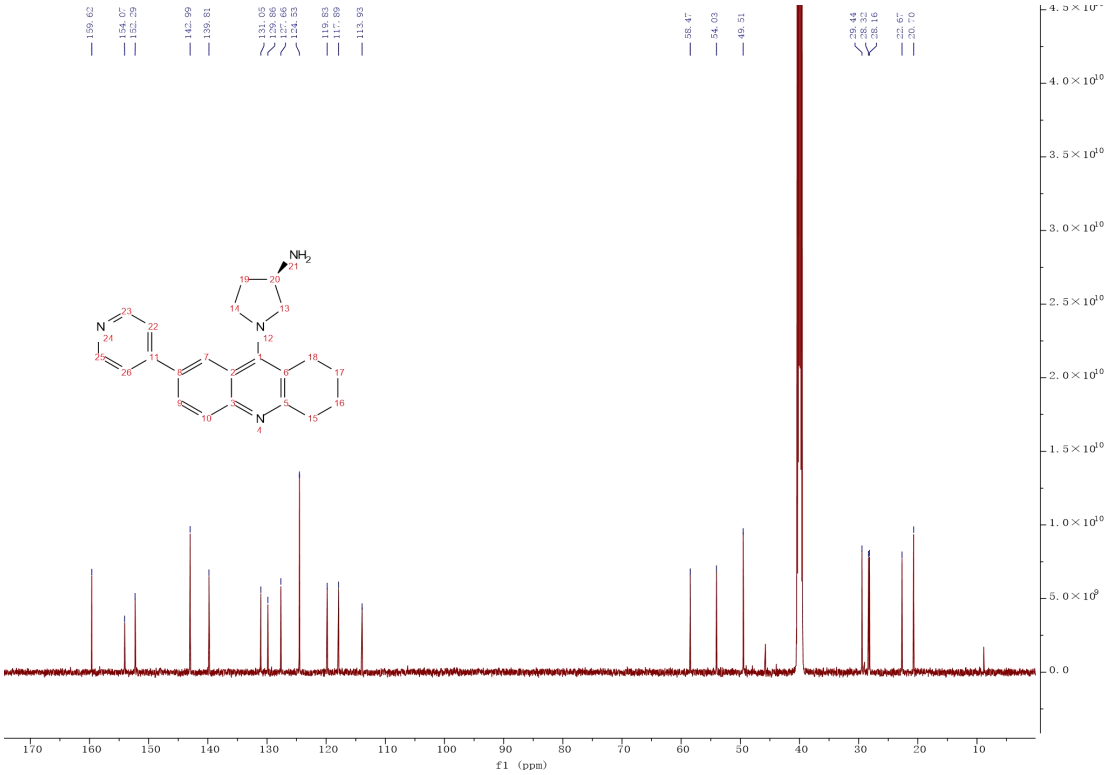
^{1}H NMR spectrum of **5p** in DMSO-d_6.



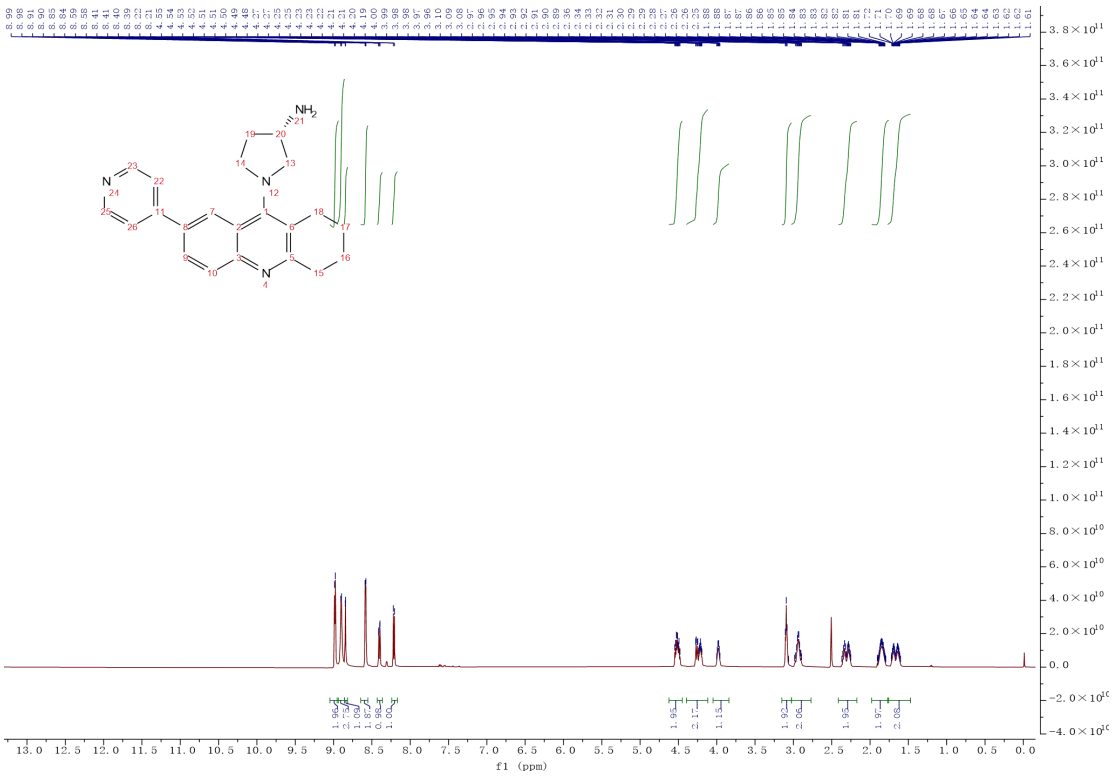
^{13}C NMR spectrum of **5p** in DMSO-d_6.



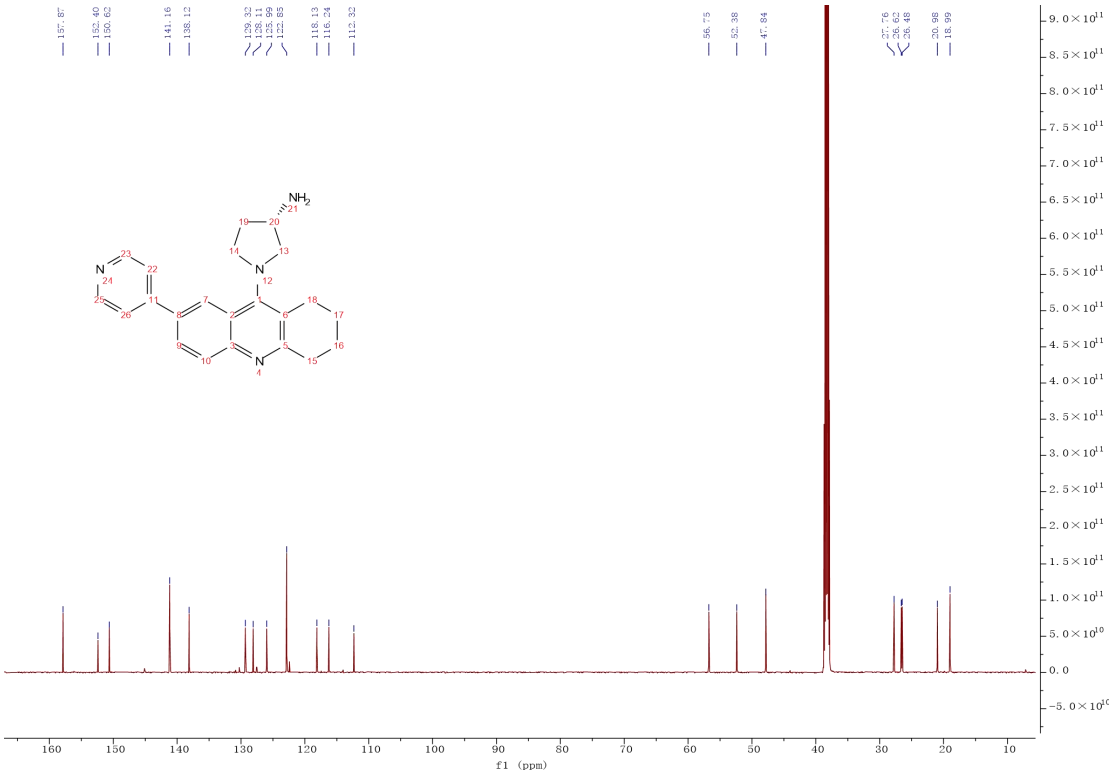
^{1}H NMR spectrum of **5q** in DMSO-d_6.



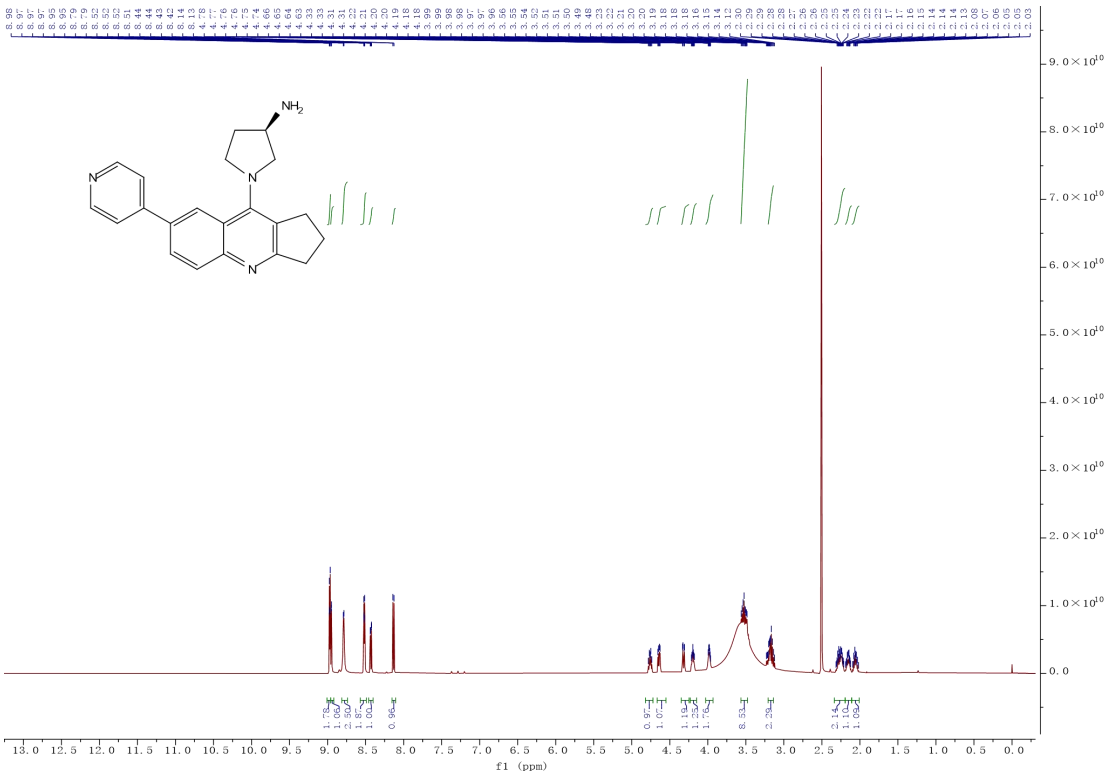
^{13}C NMR spectrum of **5q** in DMSO-d_6.



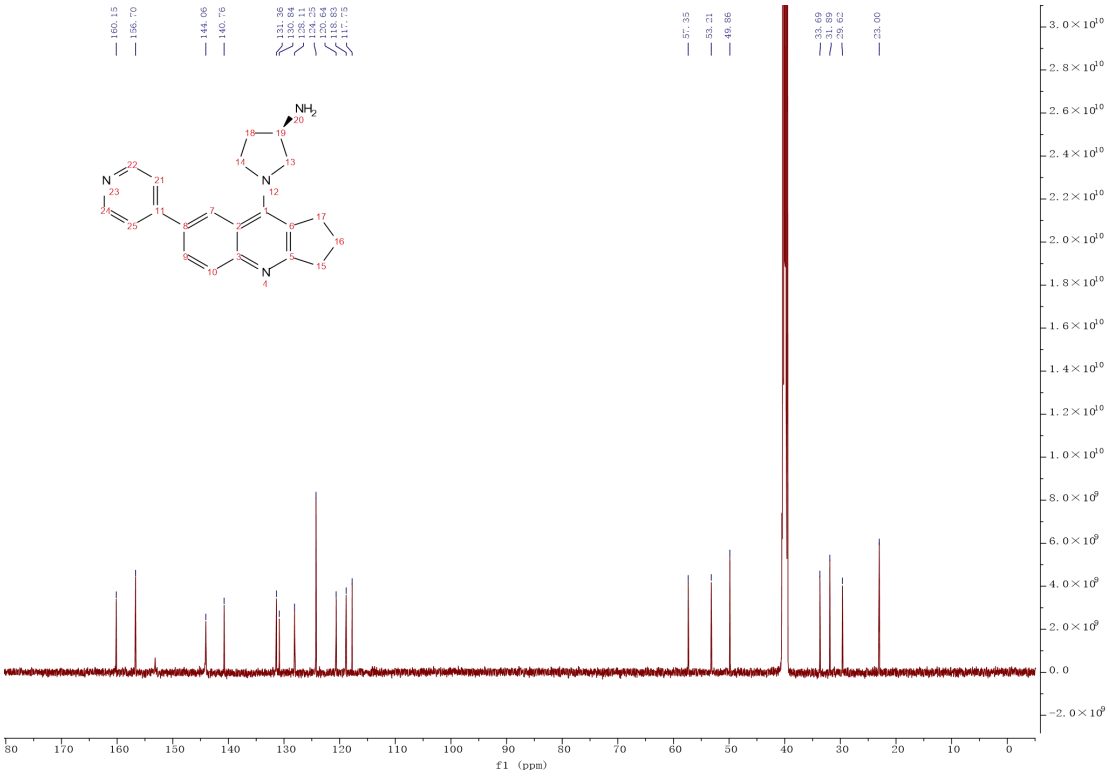
^{1}H NMR spectrum of **5r** in DMSO-d_6.



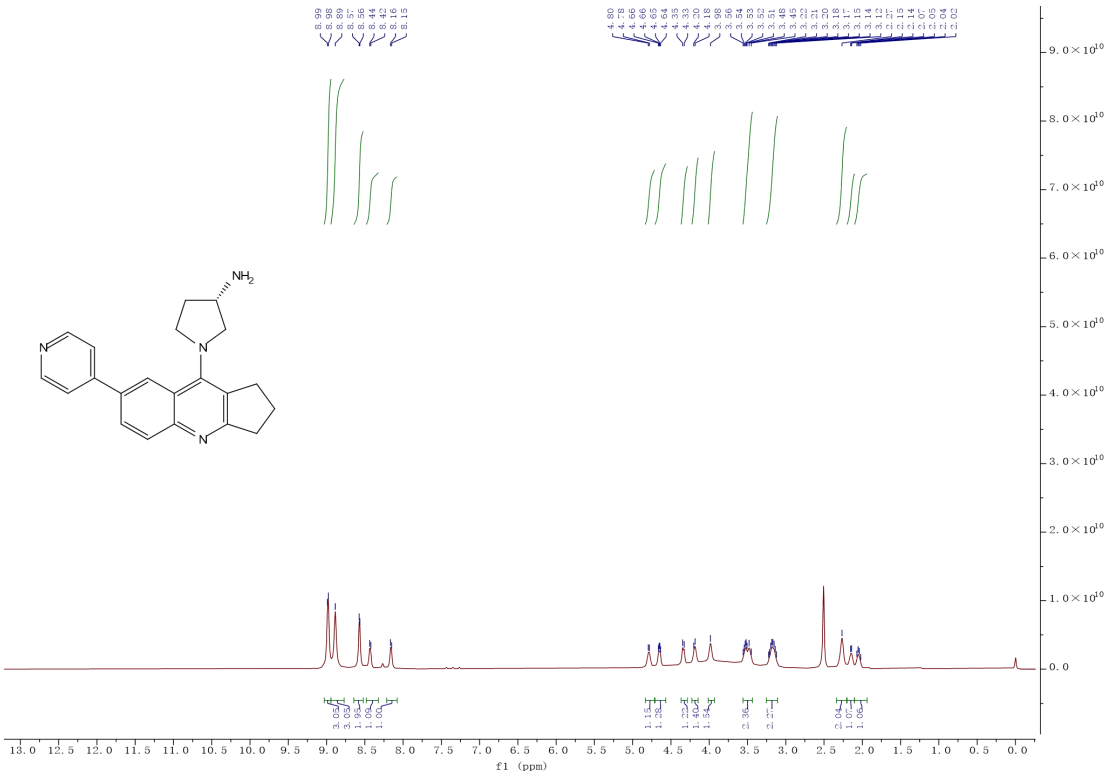
^{13}C NMR spectrum of **5r** in DMSO-d_6.



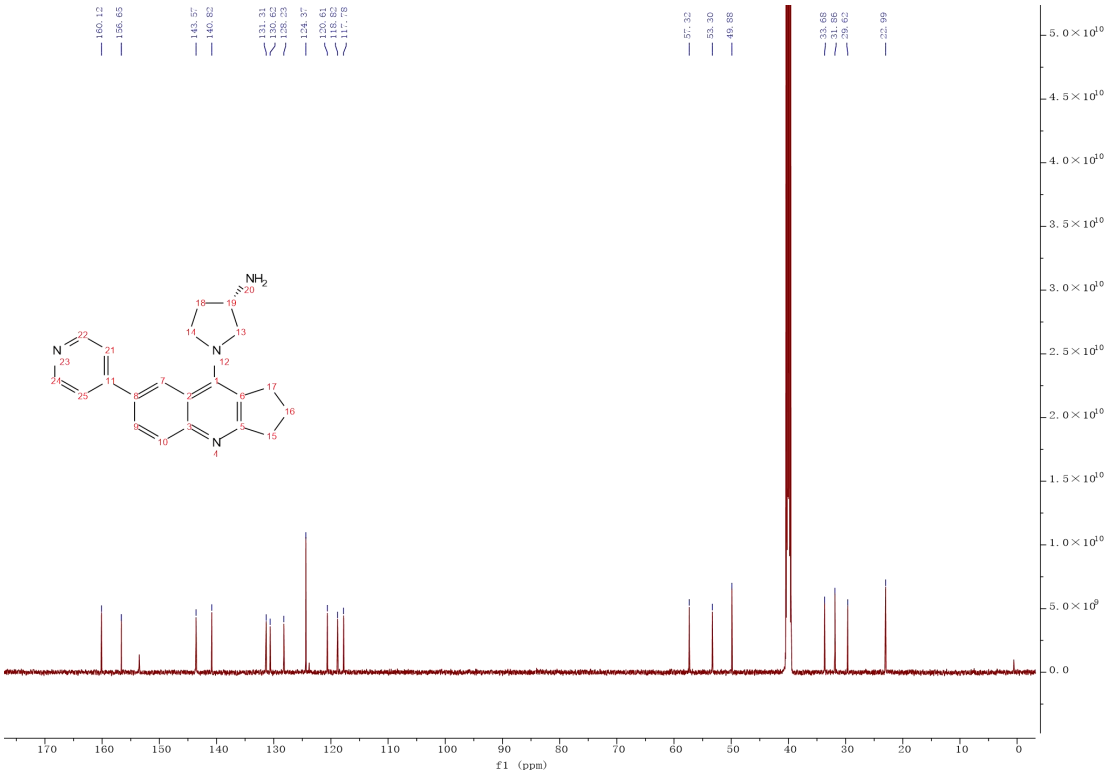
^{1}H NMR spectrum of **5s** in DMSO-d_6.



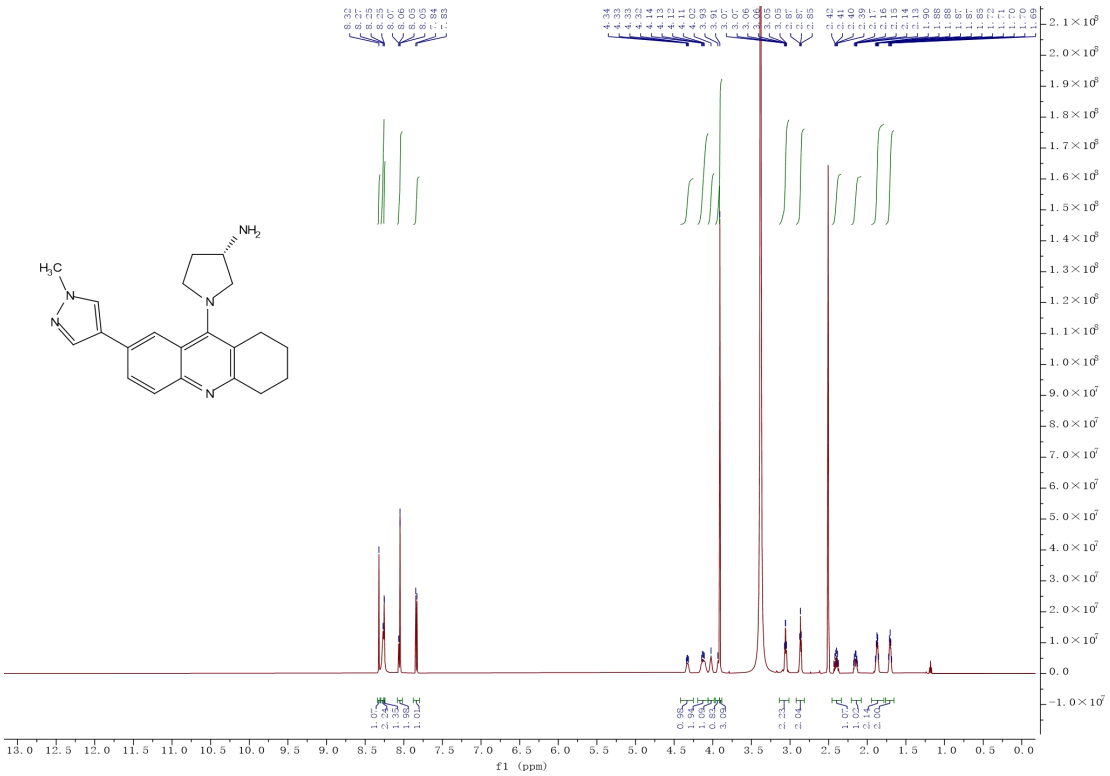
^{13}C NMR spectrum of **5s** in DMSO-d_6.



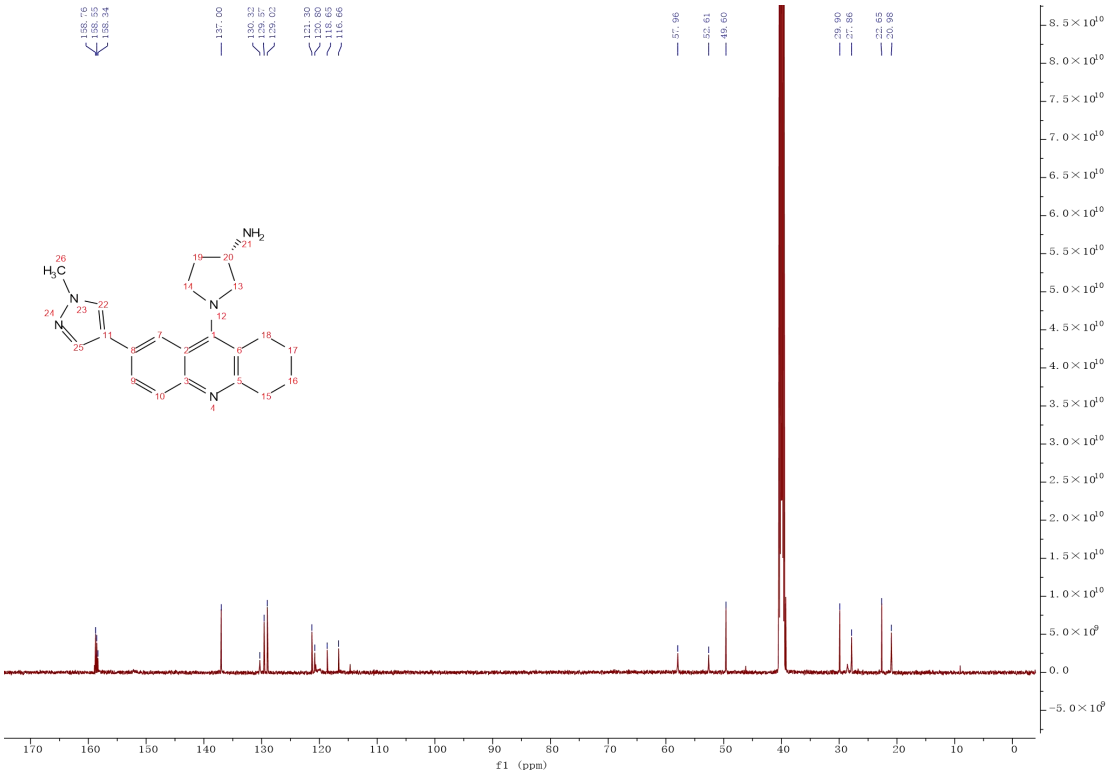
^{1}H NMR spectrum of **5t** in DMSO-d_6.



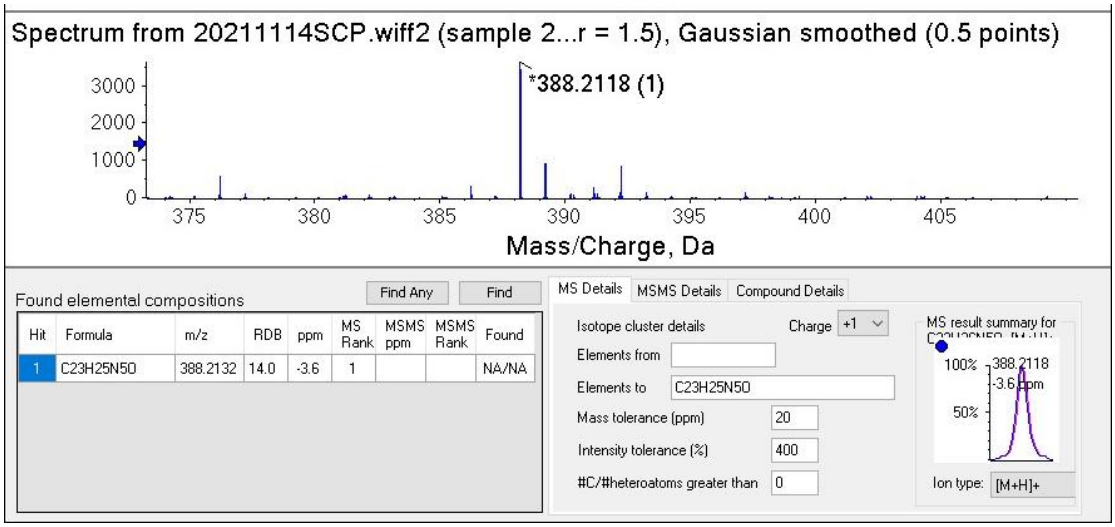
^{13}C NMR spectrum of **5t** in DMSO-d_6.



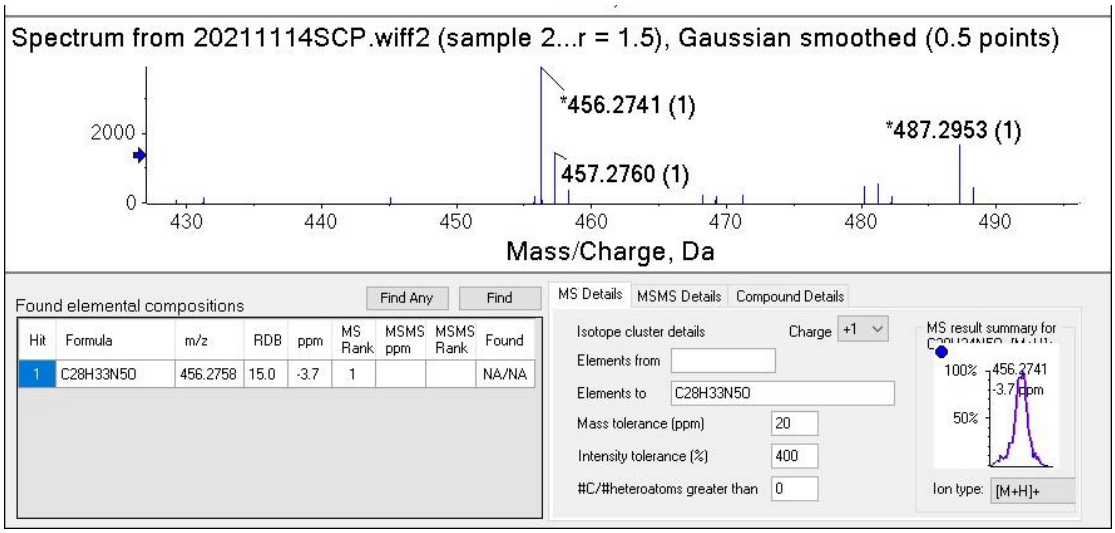
^{1}H NMR spectrum of **5u** in DMSO-d_6.



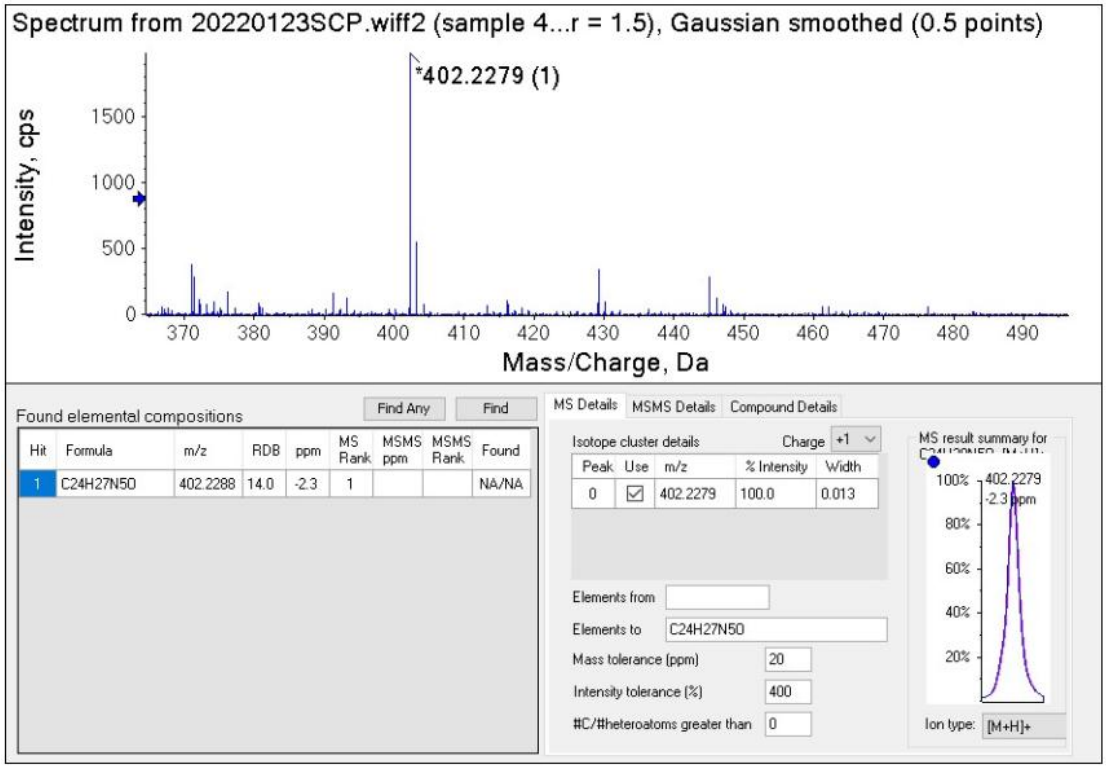
^{13}C NMR spectrum of **5u** in DMSO-d_6.



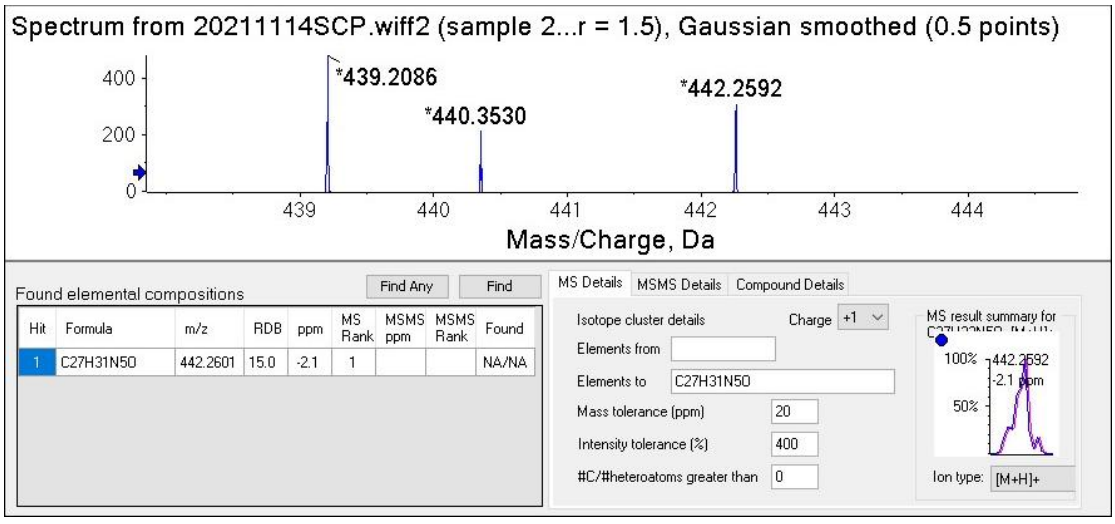
HR-ESI-MS of compound **5a**: 388.2118 $[M+H]^+$, (calcd for $C_{23}H_{25}N_5O$, 388.2132).



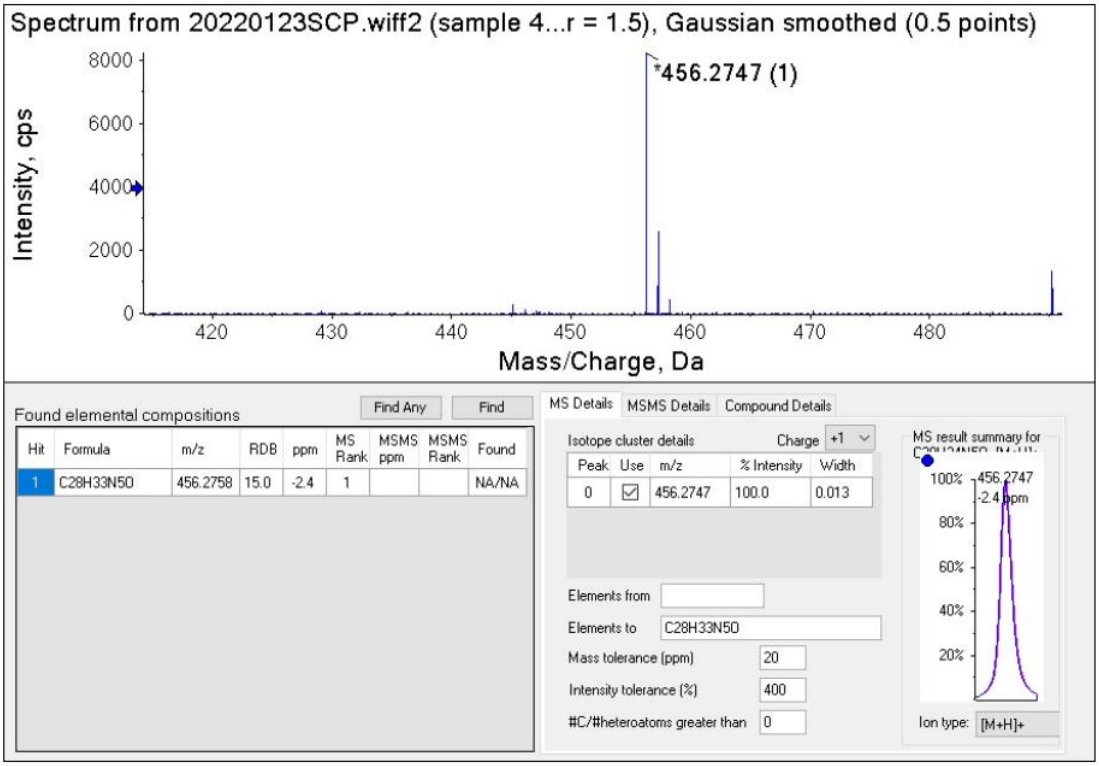
HR-ESI-MS of compound **5b**: 456.2741 $[M+H]^+$, (calcd for $C_{28}H_{33}N_5O$, 456.2758).



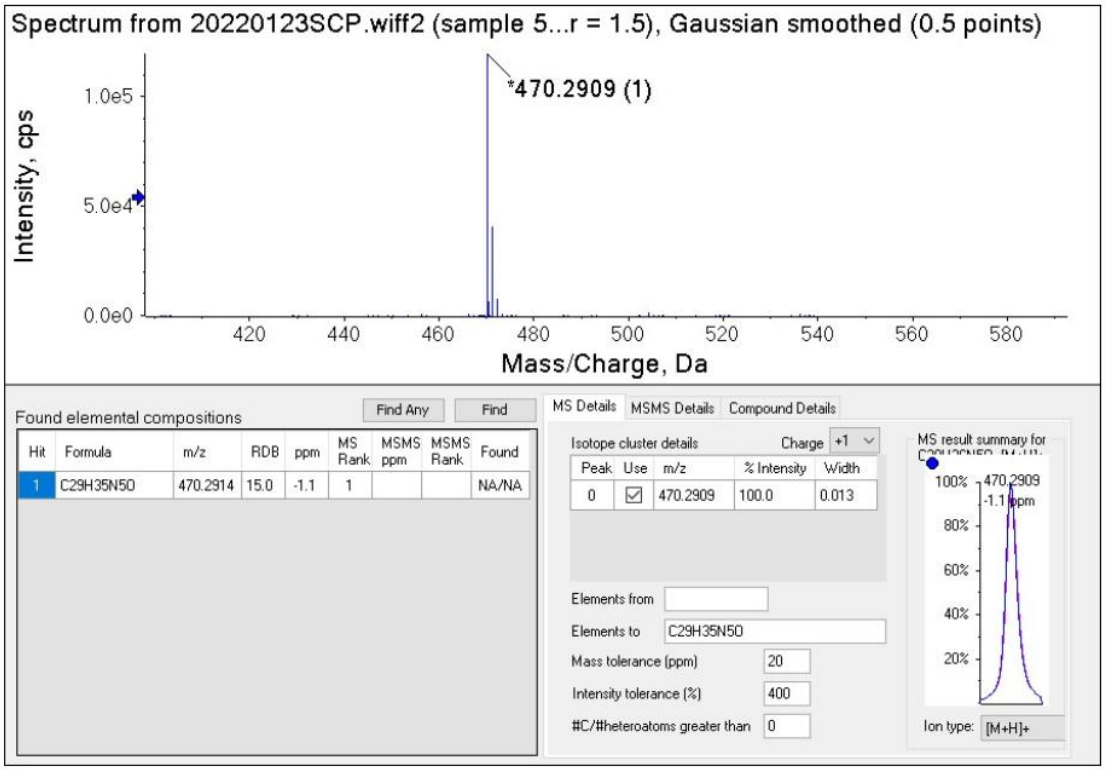
HR-ESI-MS of compound **5c**: 402.2279 $[M+H]^+$, (calcd for $C_{24}H_{27}N_5O$, 402.2288).



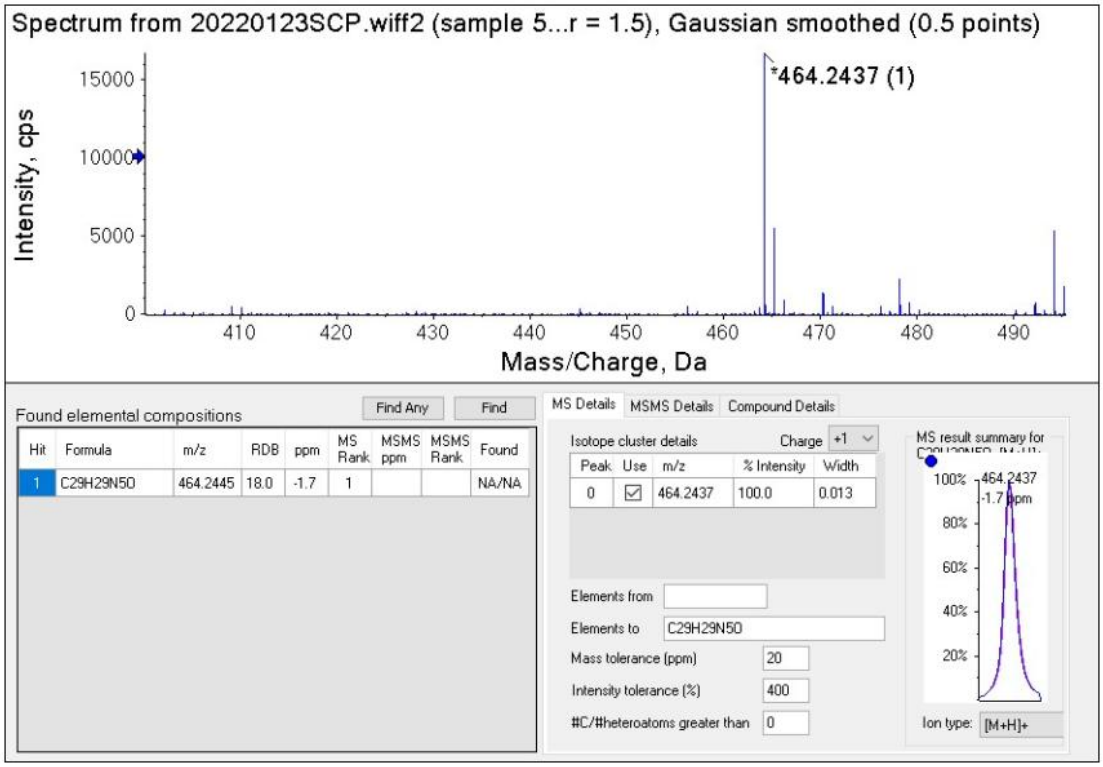
HR-ESI-MS of compound **5d**: 442.2592 $[M+H]^+$, (calcd for $C_{27}H_{31}N_5O$, 442.2601).



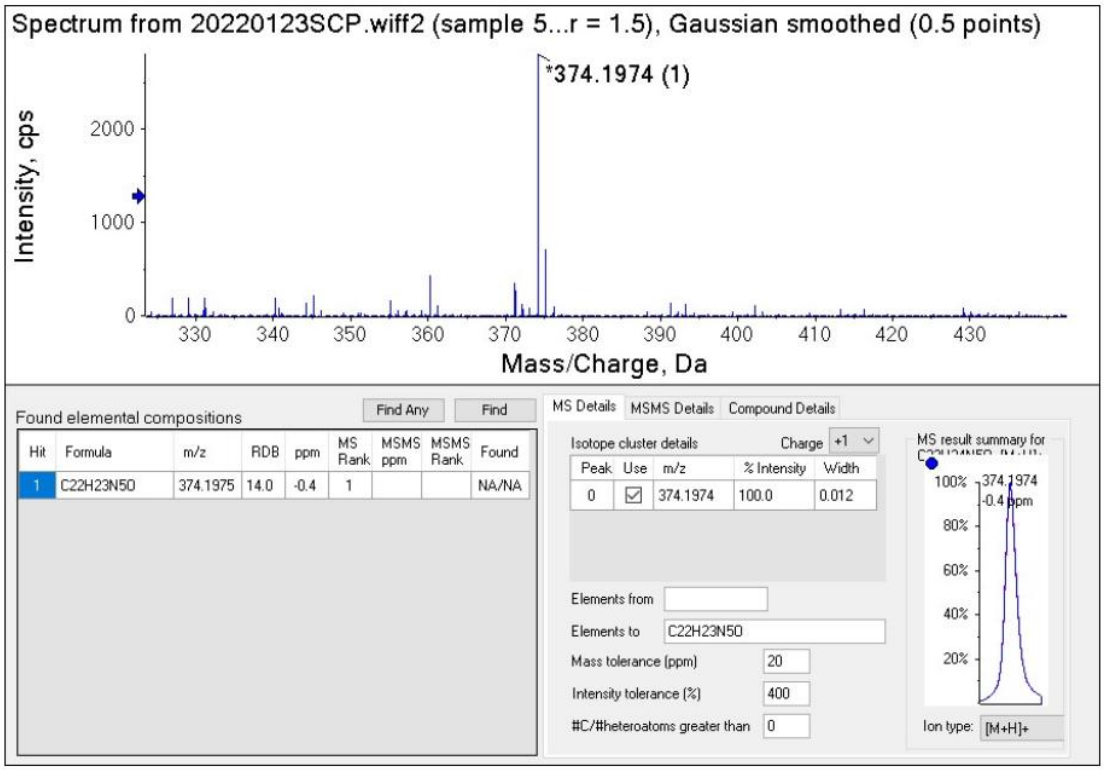
HR-ESI-MS of compound **5e**: 456.2747 $[M+H]^+$, (calcd for $C_{28}H_{33}N_5O$, 456.2758).



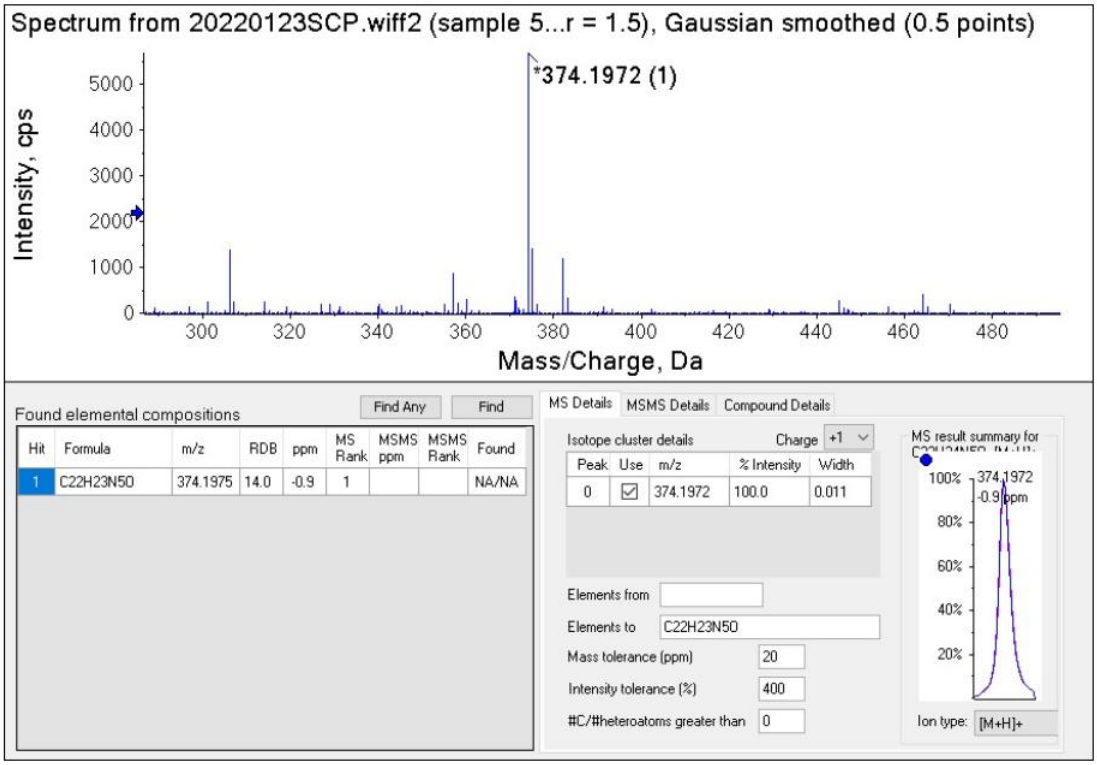
HR-ESI-MS of compound **5f**: 470.2909 $[M+H]^+$, (calcd for $C_{29}H_{35}N_5O$, 470.2914).



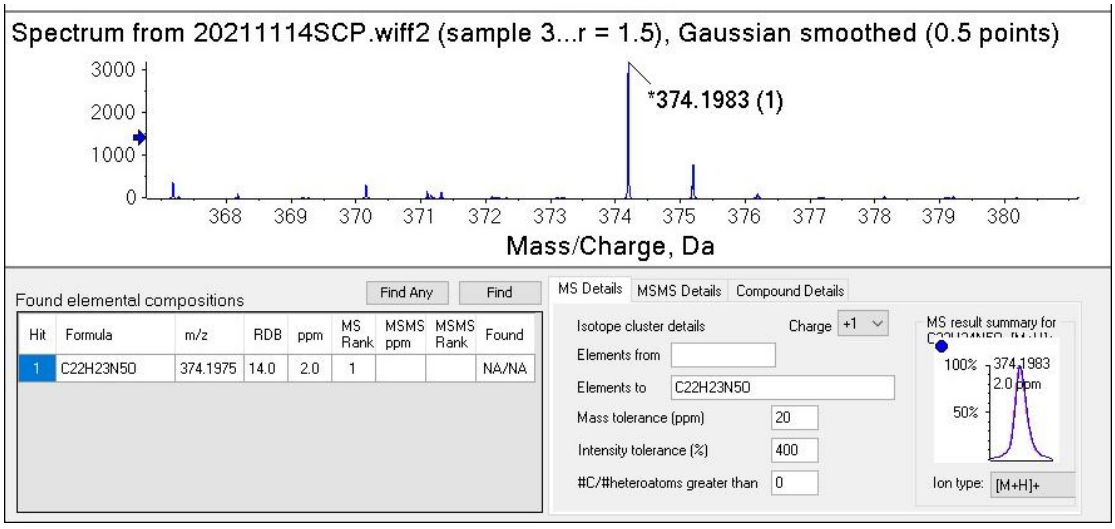
HR-ESI-MS of compound **5g**: 464.2437 $[M+H]^+$, (calcd for $C_{29}H_{29}N_5O$, 464.2445).



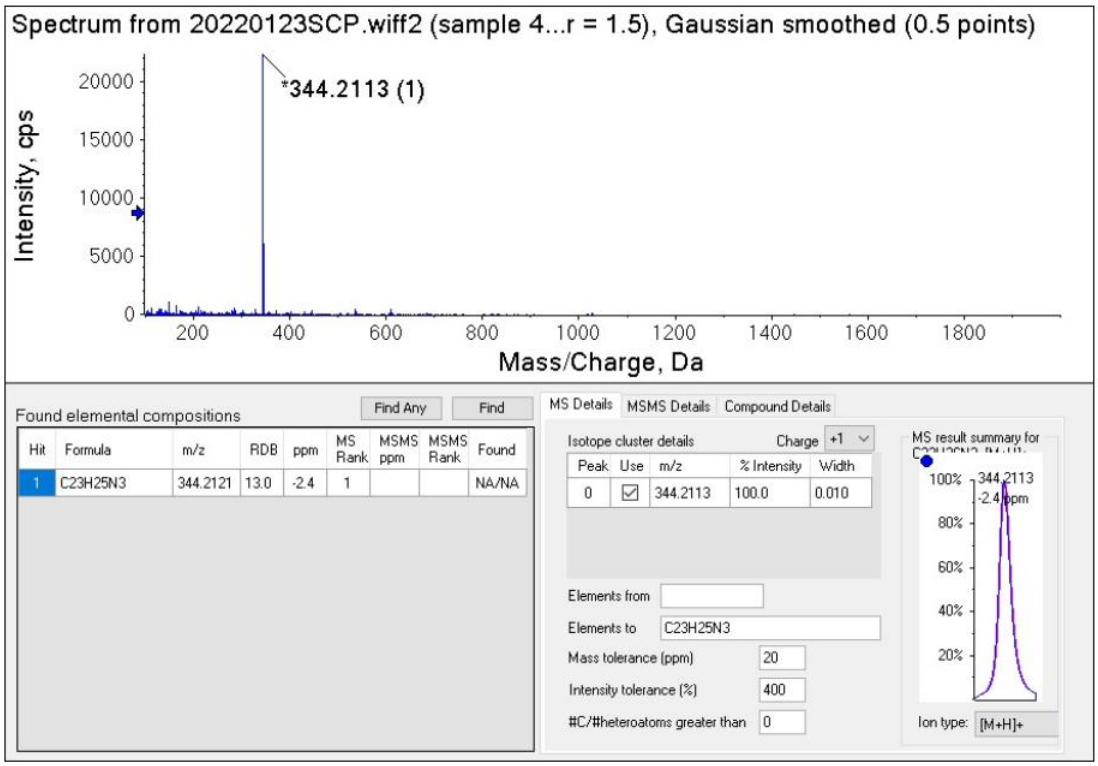
HR-ESI-MS of compound **5h**: 374.1974 $[M+H]^+$, (calcd for $C_{22}H_{23}N_5O$, 374.1975).



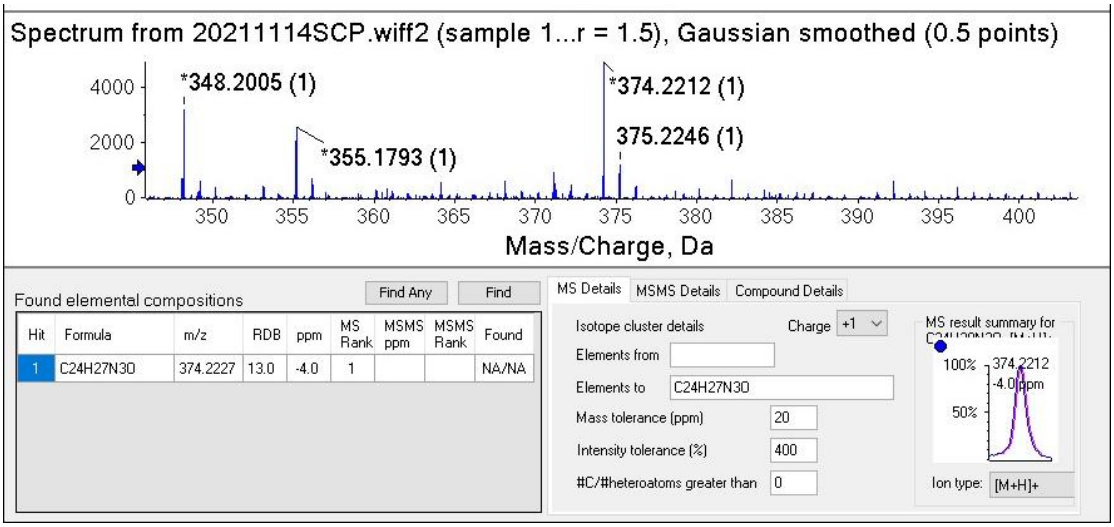
HR-ESI-MS of compound **5i**: 374.1972 $[M+H]^+$, (calcd for $C_{22}H_{23}N_5O$, 374.1975).



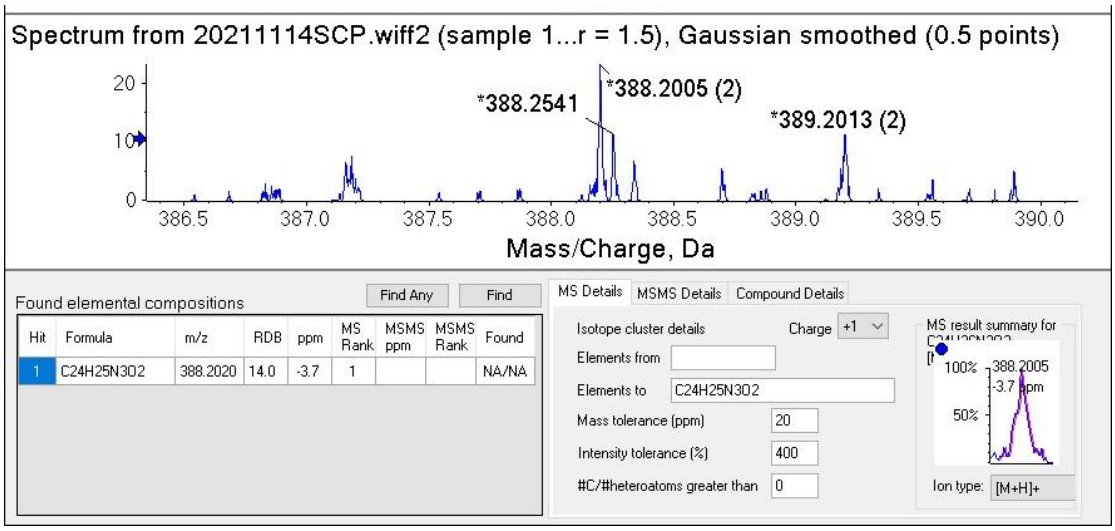
HR-ESI-MS of compound **5j**: 374.1983 $[M+H]^+$, (calcd for $C_{22}H_{23}N_5O$, 374.1975).



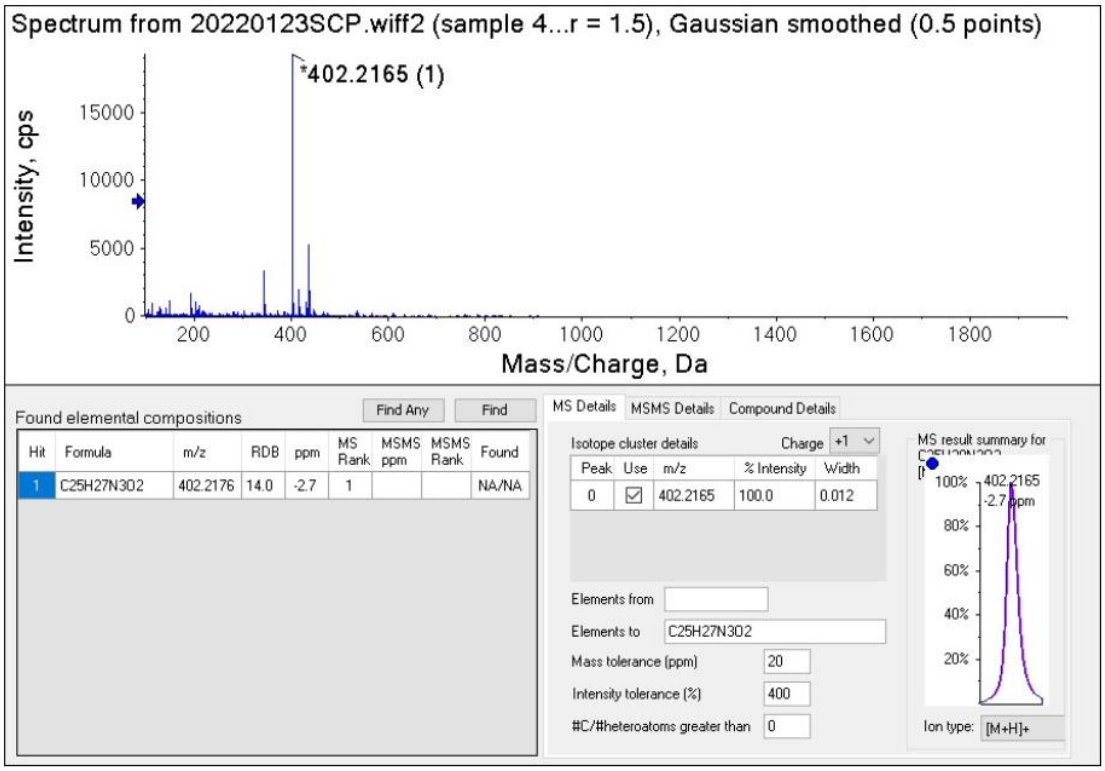
HR-ESI-MS of compound **5k**: 344.2113 $[M+H]^+$, (calcd for $C_{23}H_{25}N_3$, 344.2121).



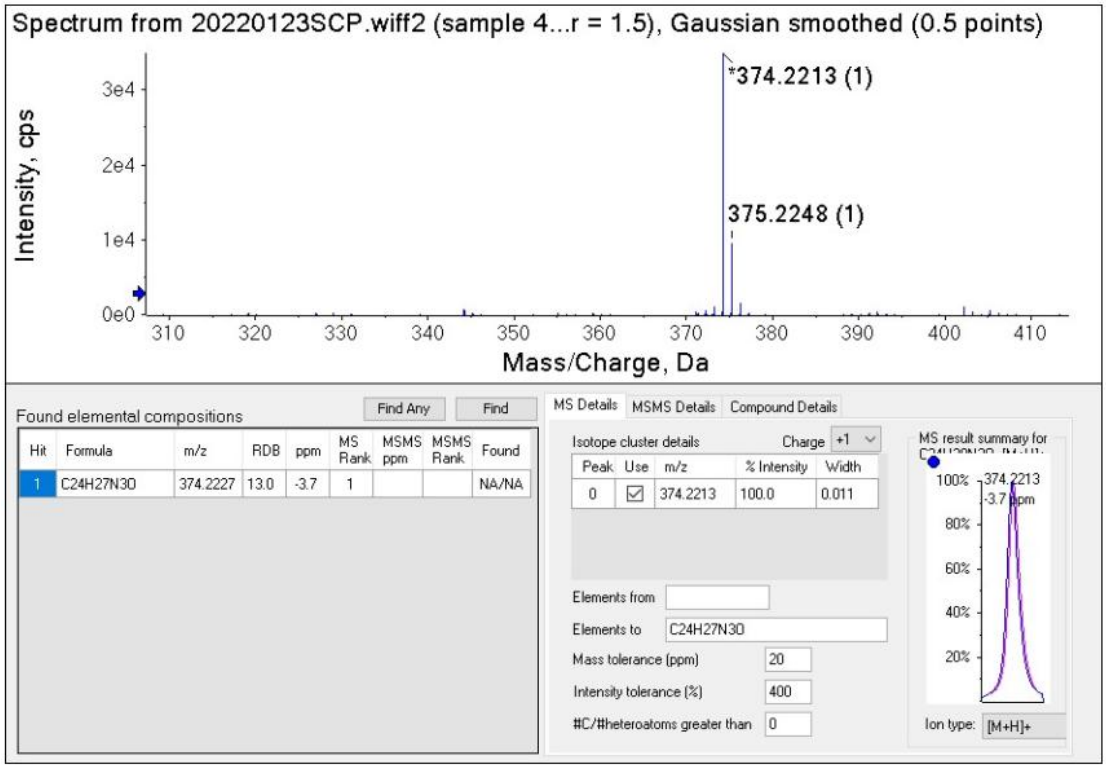
HR-ESI-MS of compound **5l**: 374.2212 $[M+H]^+$, (calcd for $C_{24}H_{27}N_3O$, 374.2227).



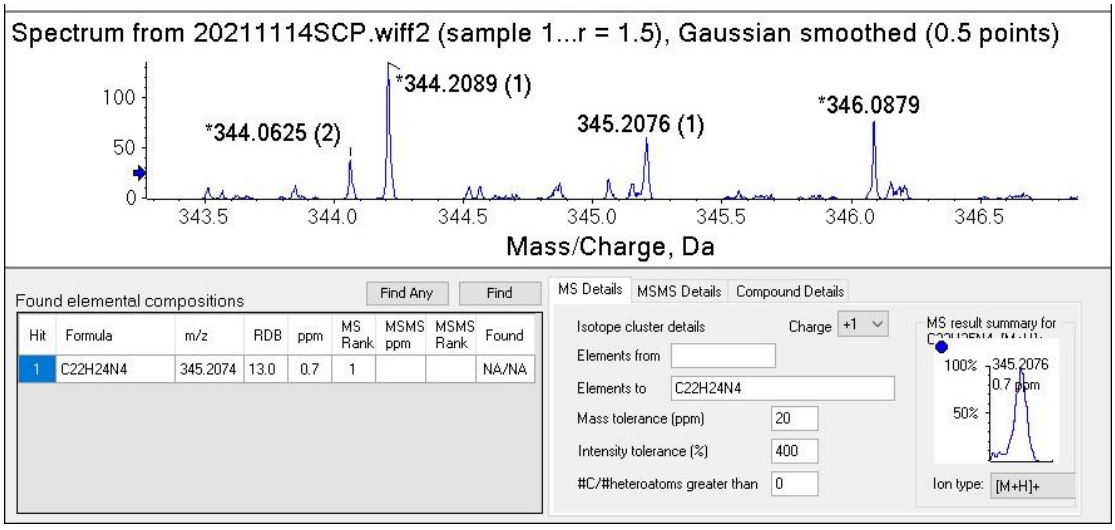
HR-ESI-MS of compound **5m**: 388.2005 $[M+H]^+$, (calcd for $C_{24}H_{25}N_3O_2$, 388.2020).



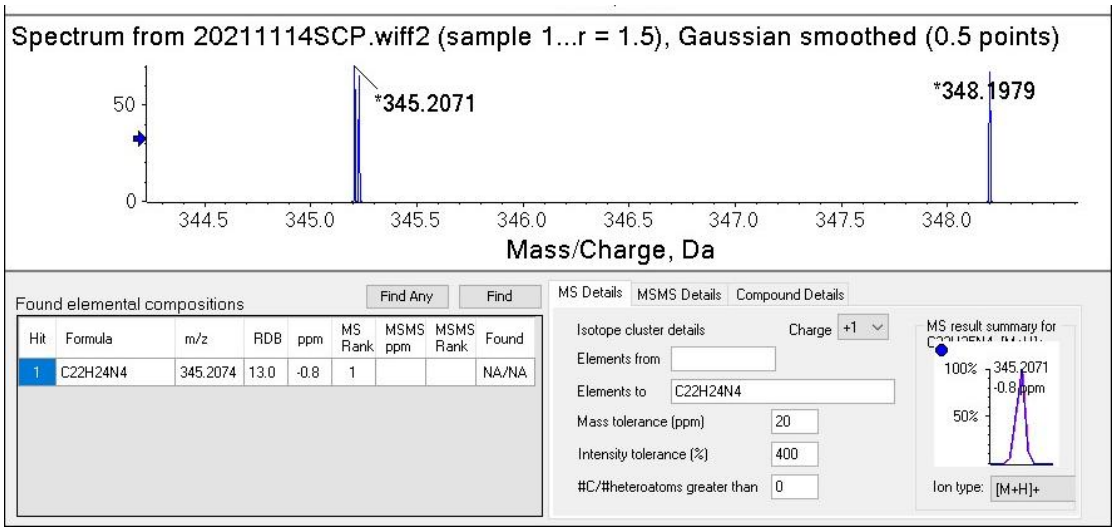
HR-ESI-MS of compound **5n**: 402.2165 $[M+H]^+$, (calcd for $C_{25}H_{27}N_3O_2$, 402.2176).



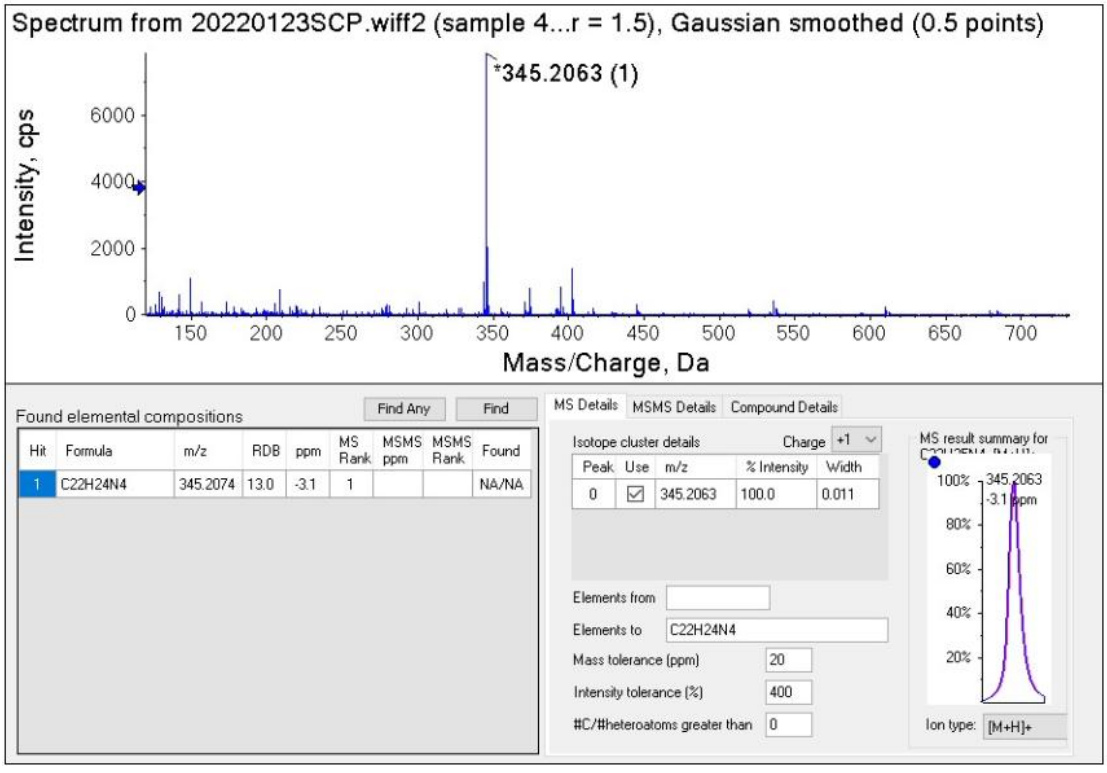
HR-ESI-MS of compound **5o**: 374.2213 $[M+H]^+$, (calcd for $C_{24}H_{27}N_3O$, 374.2227).



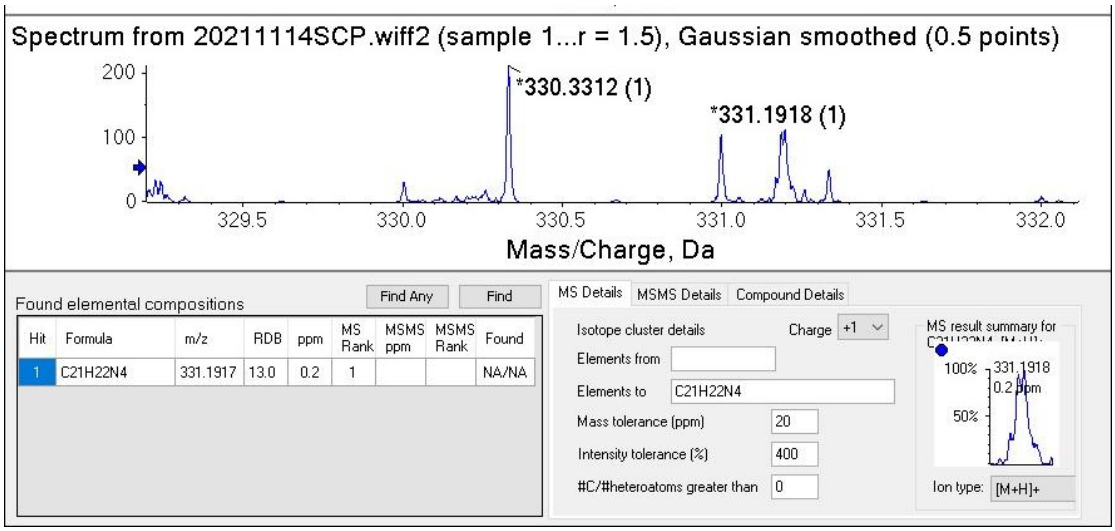
HR-ESI-MS of compound **5p**: 345.2076 $[M+H]^+$, (calcd for $C_{22}H_{24}N_4$, 345.2074).



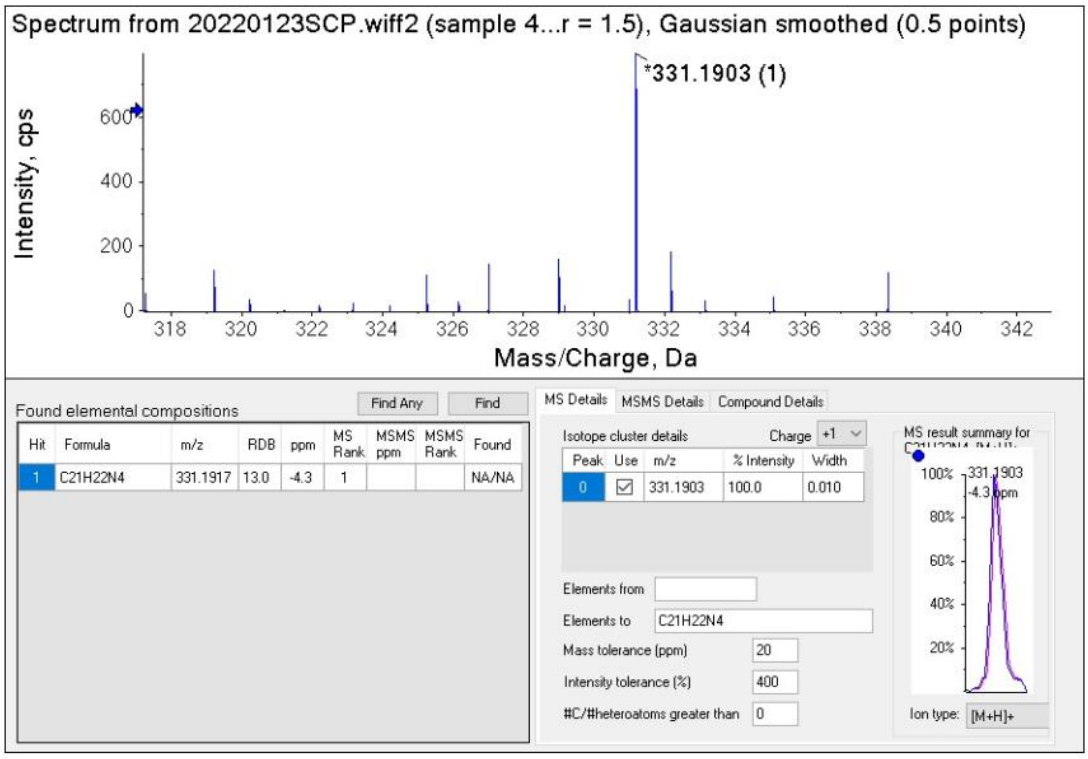
HR-ESI-MS of compound **5q**: 345.2071 $[M+H]^+$, (calcd for $C_{22}H_{24}N_4$, 345.2074).



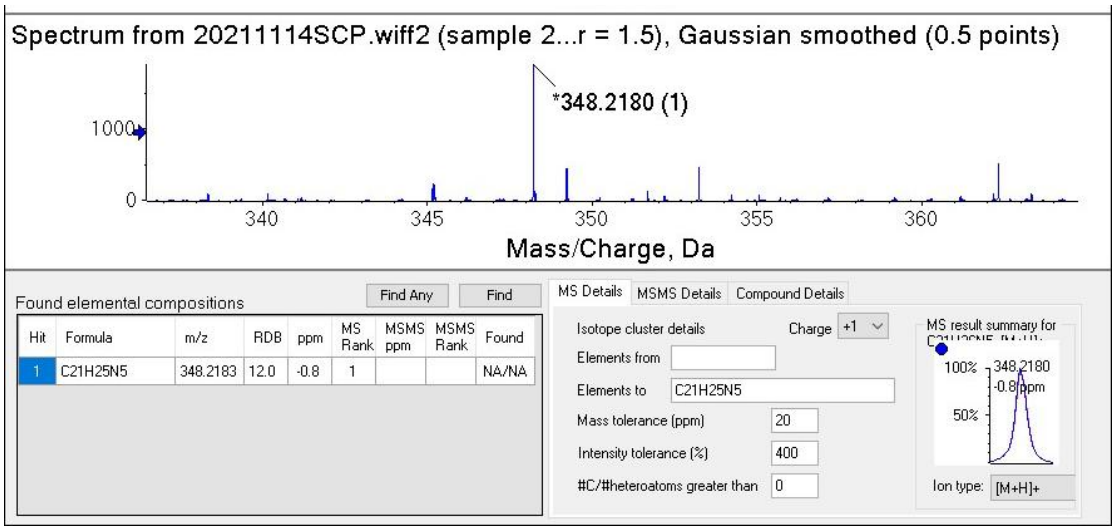
HR-ESI-MS of compound **5r**: 345.2063 $[M+H]^+$, (calcd for $C_{22}H_{24}N_4$, 345.2074).



HR-ESI-MS of compound **5s**: 331.1918 $[M+H]^+$, (calcd for $C_{21}H_{22}N_4$, 331.1917).



HR-ESI-MS of compound **5t**: 331.1903 $[M+H]^+$, (calcd for $C_{21}H_{22}N_4$, 331.1917).

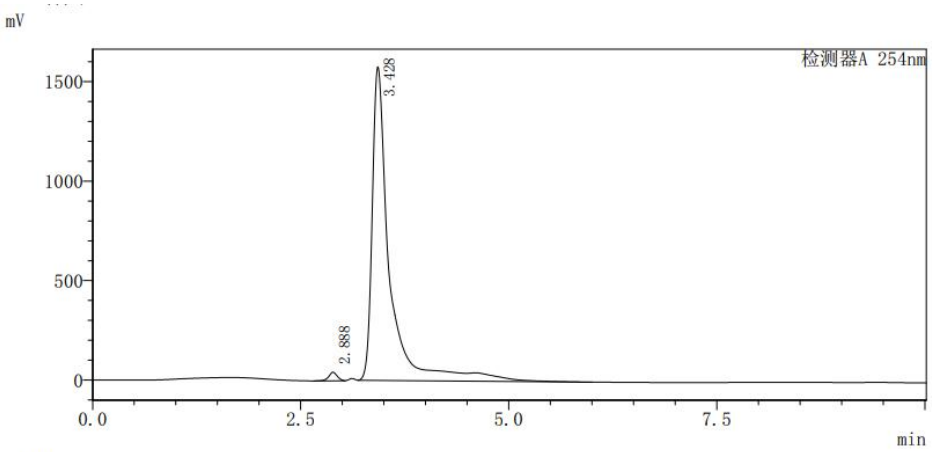


HR-ESI-MS of compound **5u**: 348.2180 $[M+H]^+$, (calcd for $C_{21}H_{25}N_5$, 348.2183).

The corresponding data of purity was estimated by HPLC (Essentia SIL-16, UV detection at λ =254 nm) using Agilent ZORBAX Extend C18 (4.6 × 250 mm, 5 μM) column. And the elution method used for target compounds was shown below. Mobile phase A (aqueous phase) was 1000 mL purified water. Mobile phase B (organic phase) was chromatographic grade methanol. Gradient elution was set as 30% B for 0-5 min, 50% B for 5-10 min, 70% B for 10-15 min, 95% B for 15-18 min, 30% for 18-21 min. The flow rate and injection volume were set as 1.0 mL/min, and 10 μ*L*, respectively.

For compounds **5a**, **5d**, **5e**, **5g**, **9j, 5l**, **5o**, and **5p**: Mobile phase A (aqueous phase) was 1000 mL purified water. Mobile phase B (organic phase) was chromatographic grade methanol. Gradient elution was set as 50% B for 0-10 min. The flow rate and injection volume were set as 1.0 mL/min, and 10 μ*L*, respectively.

The HPLC spectra of representative hybrids were as follows.



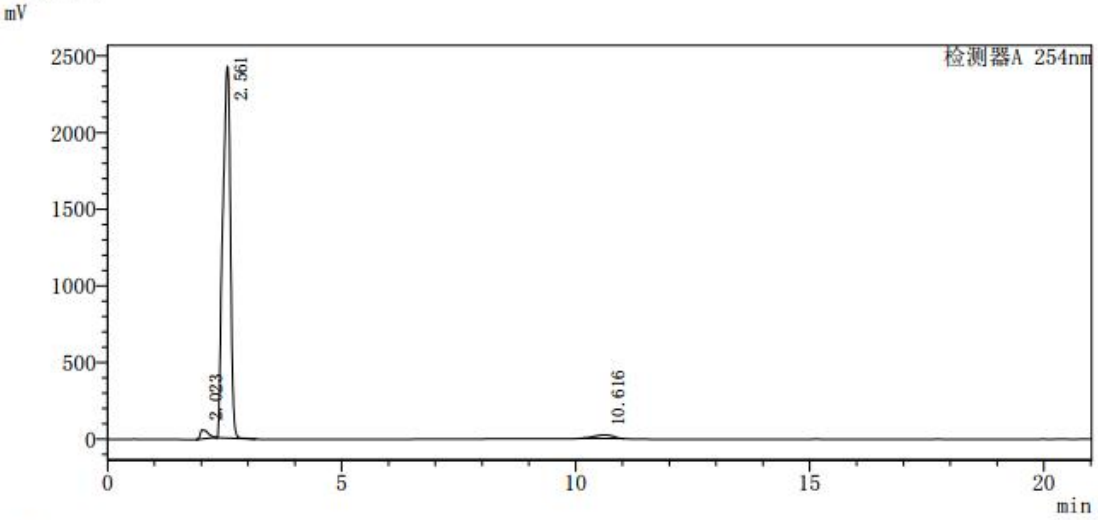
〈峰表〉

检测器A 254nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	2.888	298743	42695	1.279			
2	3.428	23051057	1576057	98.721		S	
总计		23349800	1618752				

The HPLC trace of **5a**

<色谱图>

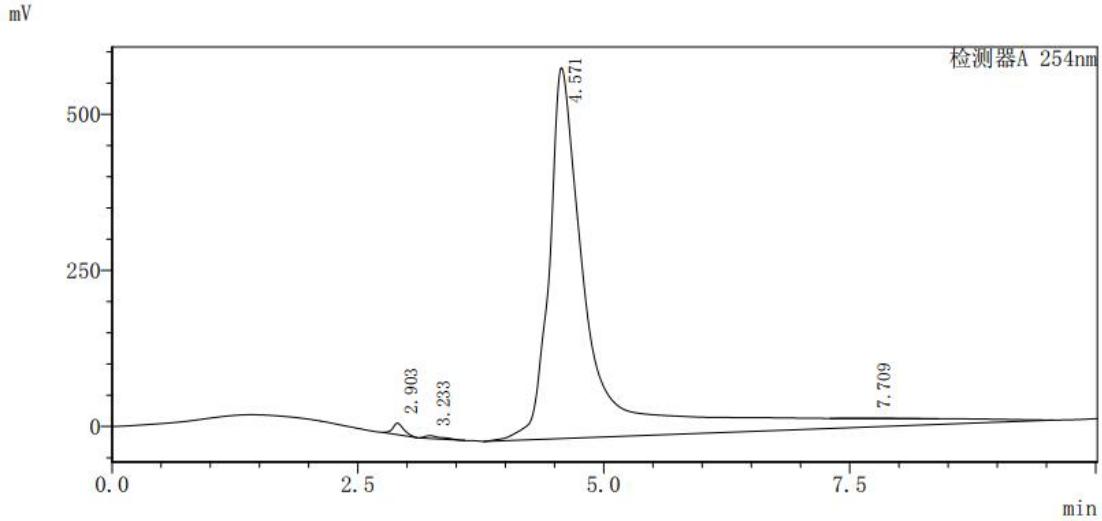


<峰表>

检测器A 254nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	2.023	662233	60029	2.306		M	
2	2.561	27440024	2425572	95.534		S	
3	10.616	620636	23332	2.161		M	
总计		28722892	2508932				

The HPLC trace of **5b**

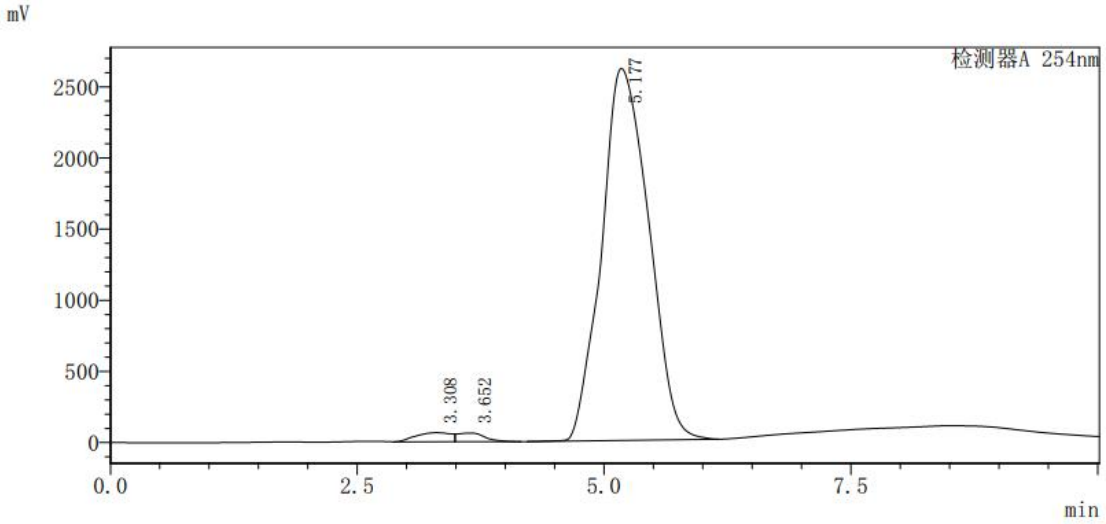


<峰表>

检测器A 254nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	2.903	143812	18379	0.799			
2	3.233	57716	4643	0.321			
3	4.571	17791100	593590	98.797		S	
4	7.709	15026	402	0.083		T	
总计		18007655	617014				

The HPLC trace of **5d**

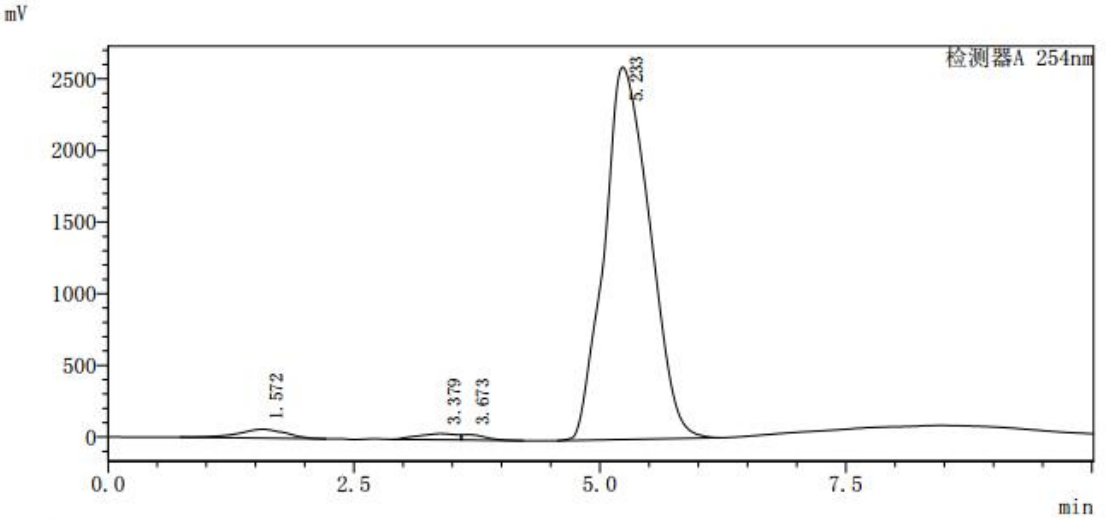


〈峰表〉

检测器A 254nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	3.308	1596466	63604	1.786			
2	3.652	1147242	60445	1.283		V	
3	5.177	86662698	2614147	96.931			
总计		89406407	2738196				

The HPLC trace of **5e**



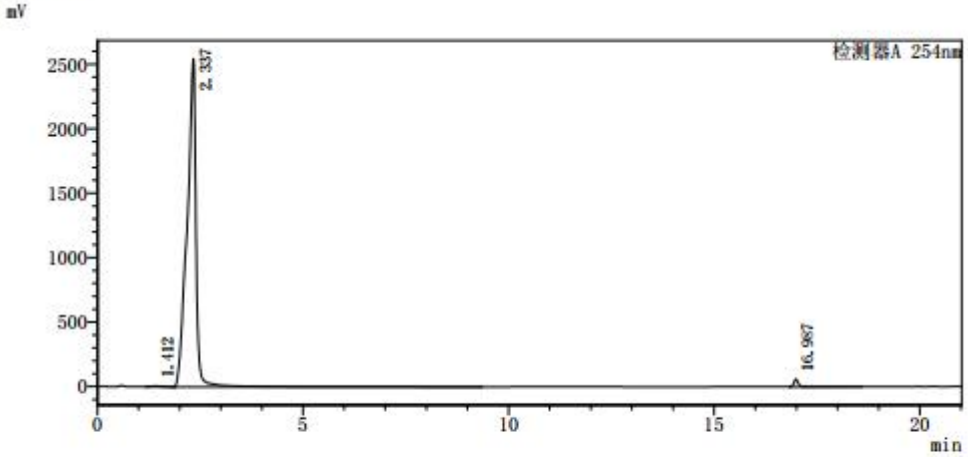
〈峰表〉

检测器A 254nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	1.572	2045322	62236	2.331			
2	3.379	1181049	42894	1.346			
3	3.673	605209	37898	0.690		V	
4	5.233	83900037	2599461	95.633			
总计		87731617	2742489				

The HPLC trace of **5g**

<色谱图>



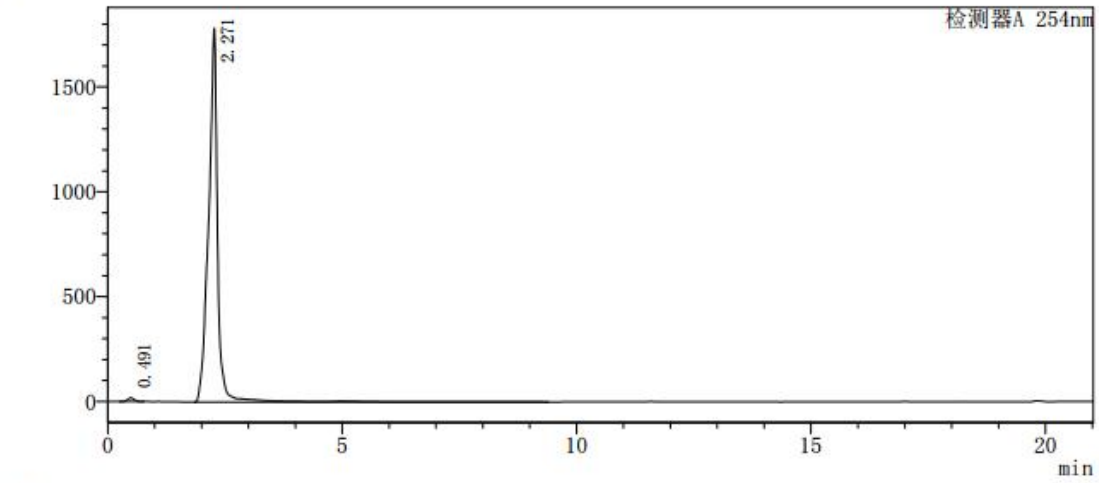
<峰表>

检测器A 254nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	1.412	101601	6551	0.261		V	
2	2.337	38398408	2544712	98.616			
3	16.987	437403	58732	1.123		SV	
总计		38937411	2609994				

The HPLC trace of **5h**

<色谱图>

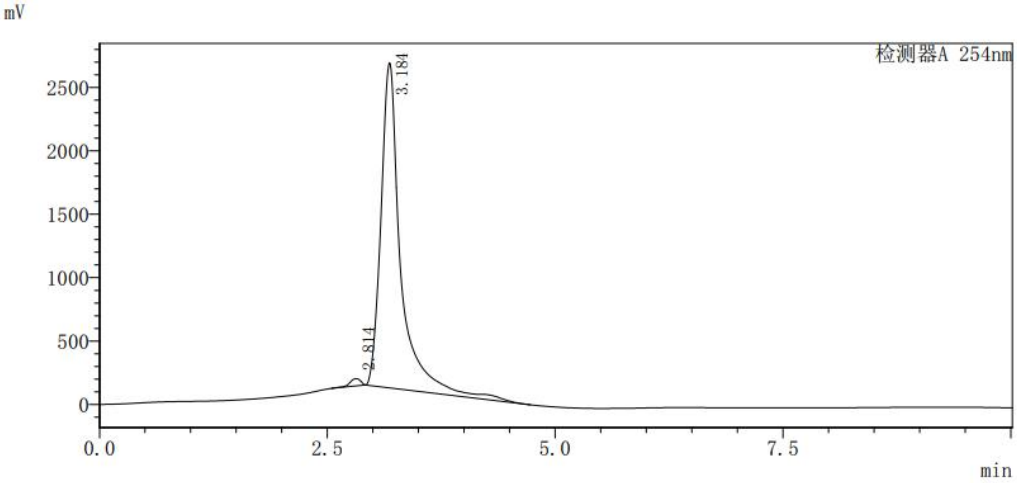


<峰表>

检测器A 254nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	0.491	167969	17710	0.697			
2	2.271	23931541	1781828	99.303		S	
总计		24099510	1799538				

The HPLC trace of **5i**



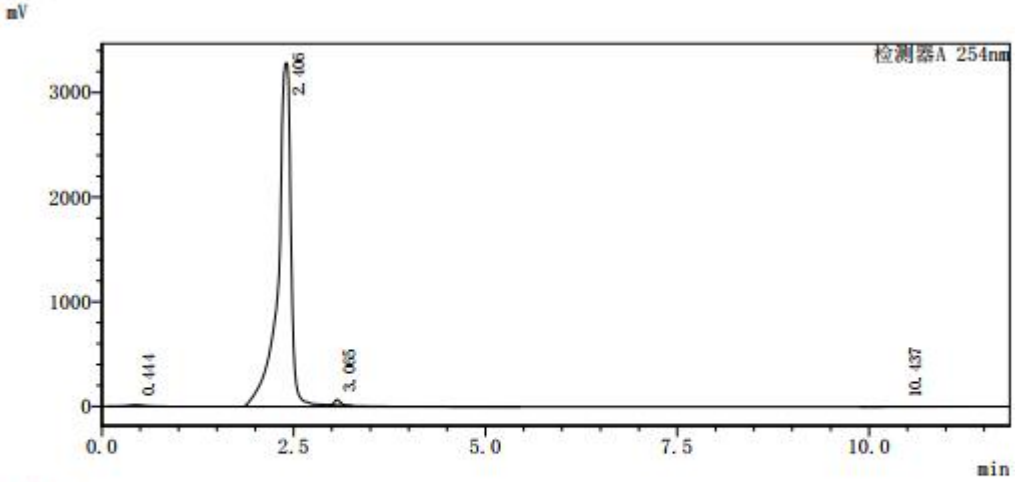
〈峰表〉

检测器A 254nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	2.814	472212	57717	1.205		M	
2	3.184	38706970	2565155	98.795			
总计		39179182	2622872				

The HPLC trace of **5j**

〈色谱图〉

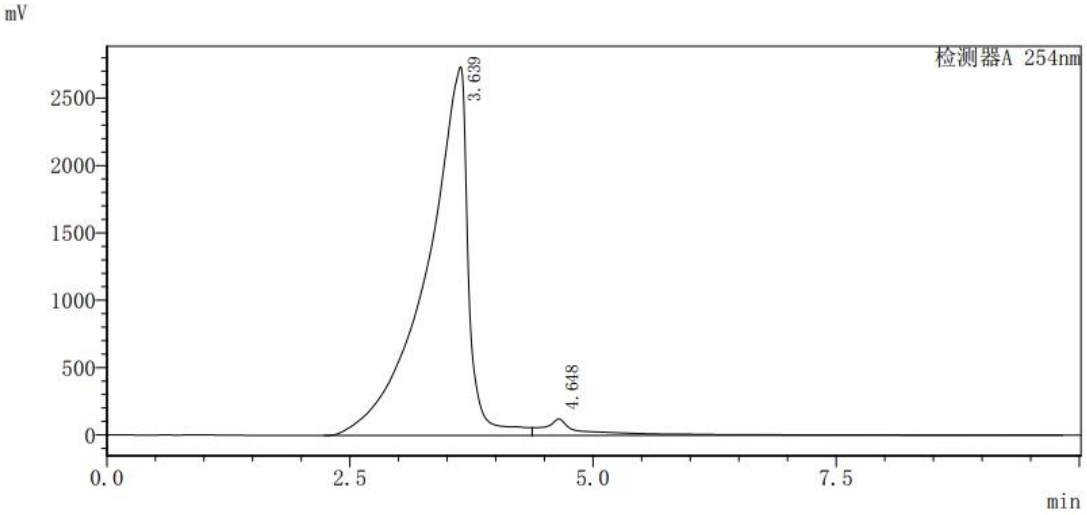


〈峰表〉

检测器A 254nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	0.444	247496	14985	0.623			
2	2.406	39164025	3286447	98.525		SV	
3	3.065	296812	53575	0.747		T	
4	10.437	42057	1570	0.106			
总计		39750390	3356577				

The HPLC trace of **5k**

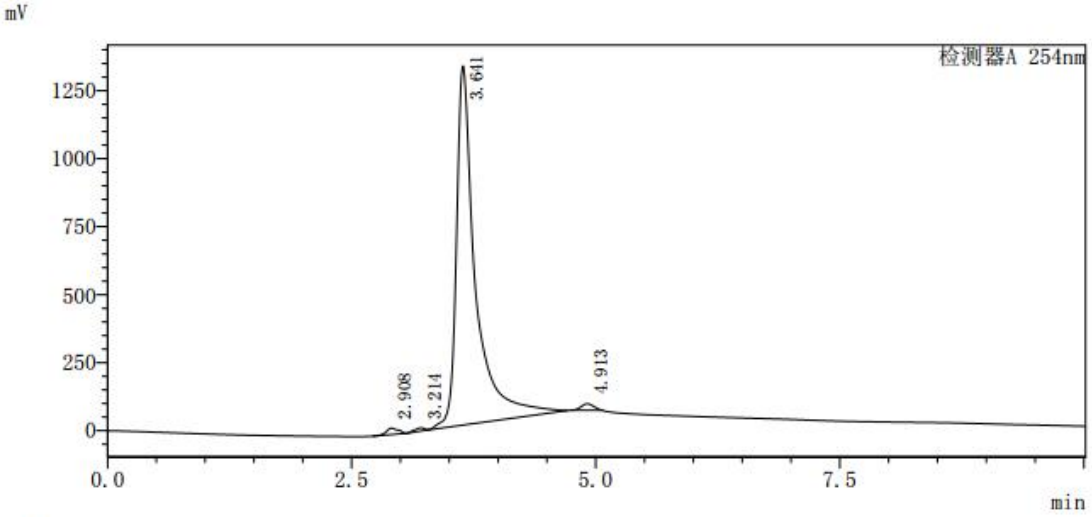


〈峰表〉

检测器A 254nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	3.639	81239852	2735635	95.604			
2	4.648	3735714	121808	4.396		SV	
总计		84975566	2857442				

The HPLC trace of **5l**

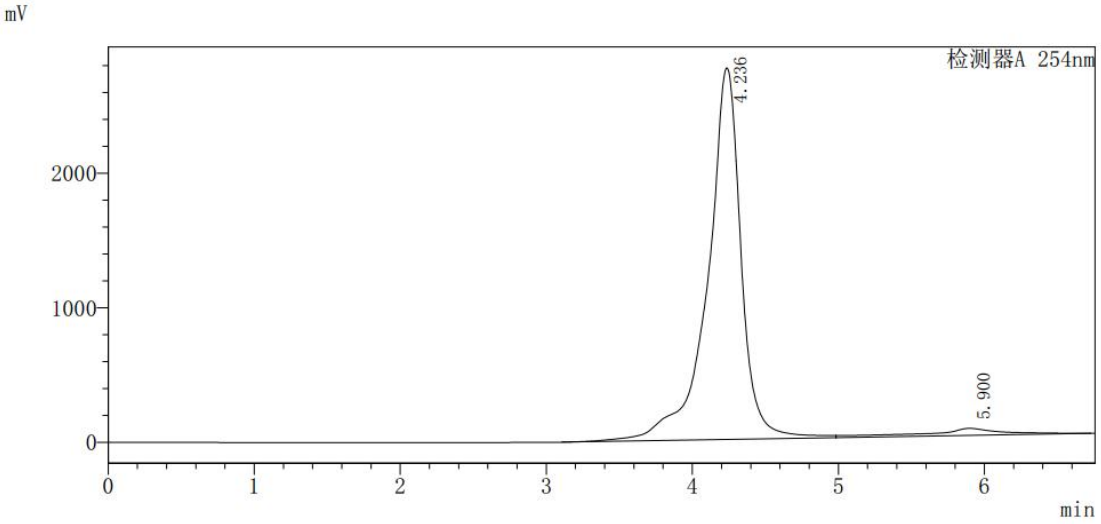


〈峰表〉

检测器A 254nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	2.908	202016	23446	1.093			
2	3.214	84805	11033	0.459			
3	3.641	17975869	1319931	97.264		M	
4	4.913	218820	24060	1.184		M	
总计		18481510	1378469				

The HPLC trace of **5o**

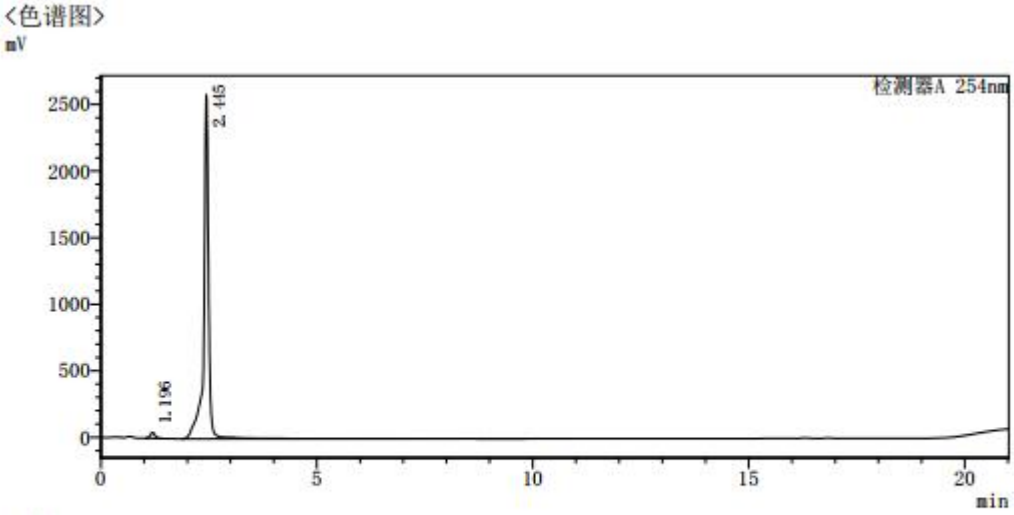


〈峰表〉

检测器A 254nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	4.236	44184130	2759688	95.831			
2	5.900	1922104	52401	4.169		V	
总计		46106234	2812089				

The HPLC trace of **5p**



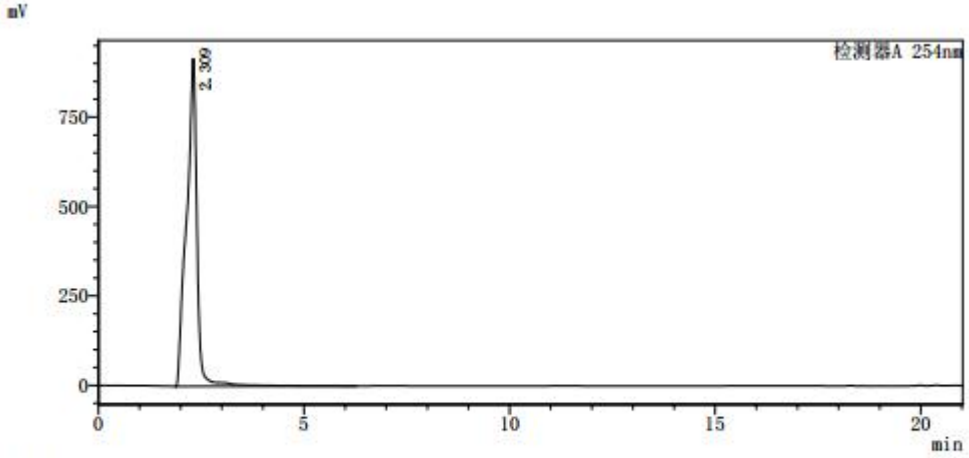
〈峰表〉

检测器A 254nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	1.196	325459	43938	1.603		V	
2	2.445	19974429	2583315	98.397		S	
总计		20299888	2627254				

The HPLC trace of **5r**

<色谱图>



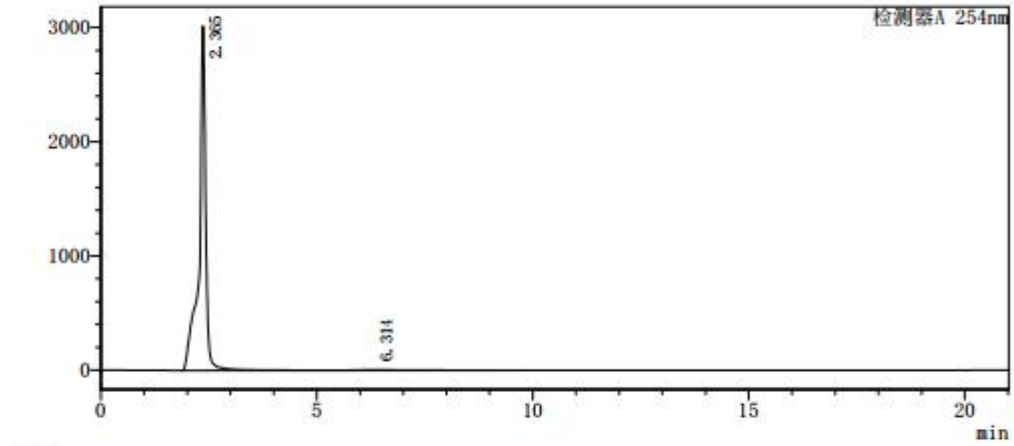
<峰表>

检测器A 254nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	2.309	16213124	915667	100.000		S	
总计		16213124	915667				

The HPLC trace of **5s**

<色谱图>



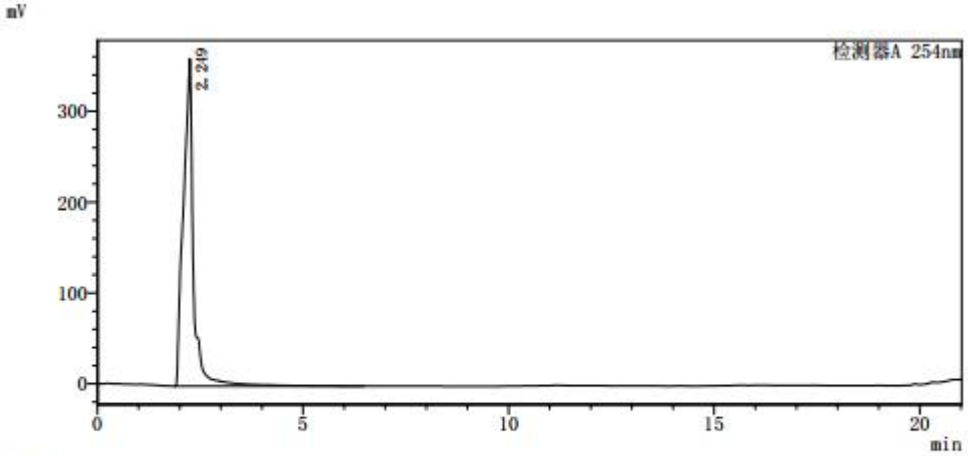
<峰表>

检测器A 254nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	2.365	32315679	3015990	98.573		S	
2	6.314	467680	4652	1.427		T	
总计		32783360	3020642				

The HPLC trace of **5t**

<色谱图>



<峰表>

检测器A 254nm

峰号	保留时间	面积	高度	浓度	浓度单位	标记	化合物名
1	2.249	6154694	360519	100.000			
总计		6154694	360519				

The HPLC trace of **5u**