

Design, synthesis and biological evaluation of novel potent STAT3 inhibitors based
on related heterocycle-fused naphthoquinones for cancer therapy

Yuanguang Chen,^{a†} Huashen Xu,^{a†}, Lu Chen,^a Maoying Zhang,^a Dexiao, Yang^c Minghui
Tong,^c Xuan Shi,^c Zhongbo Liu^{b*} * Guoliang Chen^{a*}

^aKey Laboratory of Structure-Based Drug Design & Discovery of Ministry of Education,
School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016,
China

^bSchool of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China

^c3D BioOptima Co., Ltd., Suzhou 215104, PR China

[†]These authors contributed equally to this work

Figure S1. Antiproliferative Activities of **A1-A21** against HepG2, K562 cell and L-02
cell lines2
Figure S2. ¹H NMR, ¹³C NMR, HRMS and HPLC Spectrum of
Compounds.....3

Figure S1. Antiproliferative Activities of A1-A21 against HepG2 (A), K562 (B) cell lines and L-02 (c) cell lines

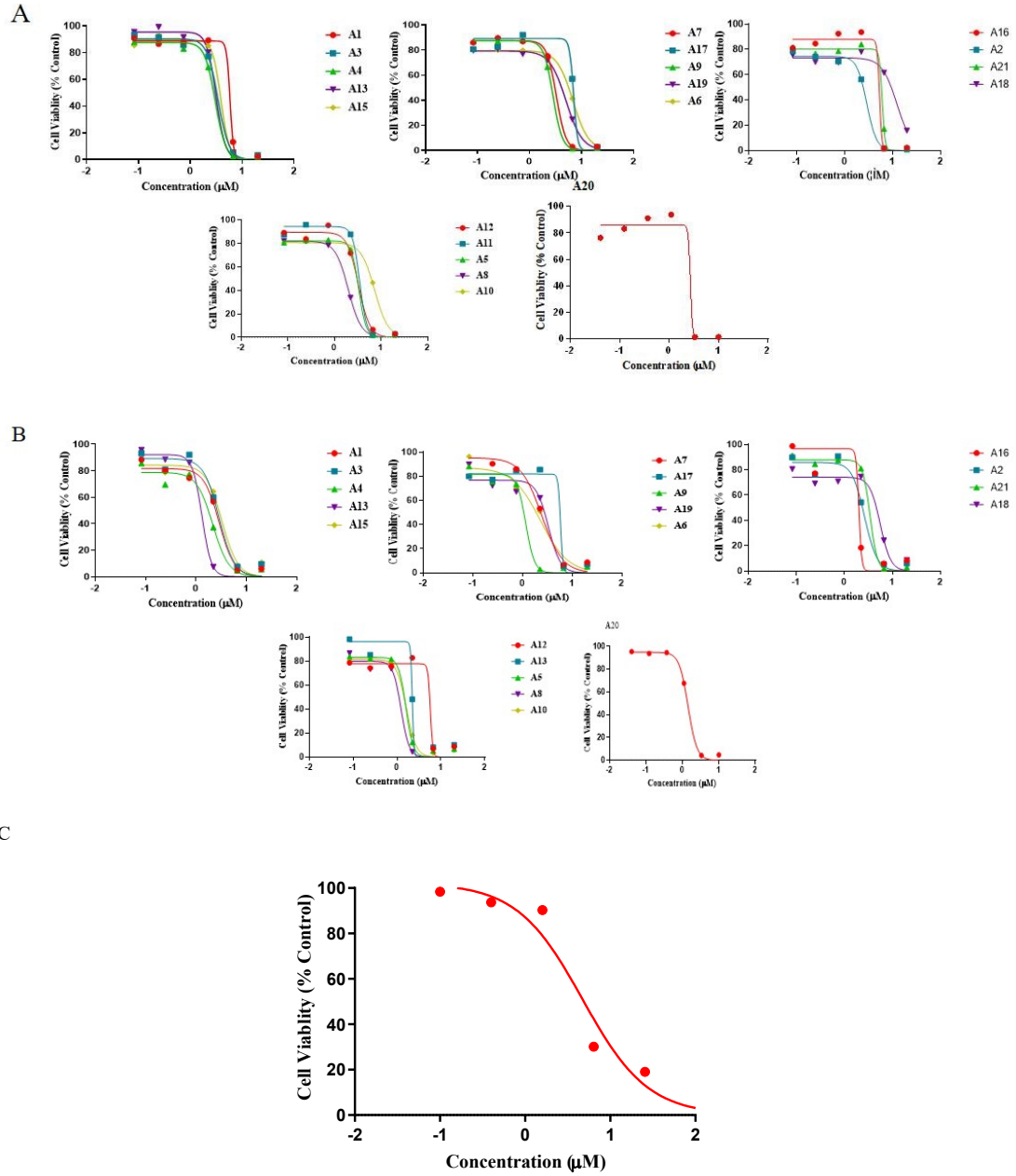
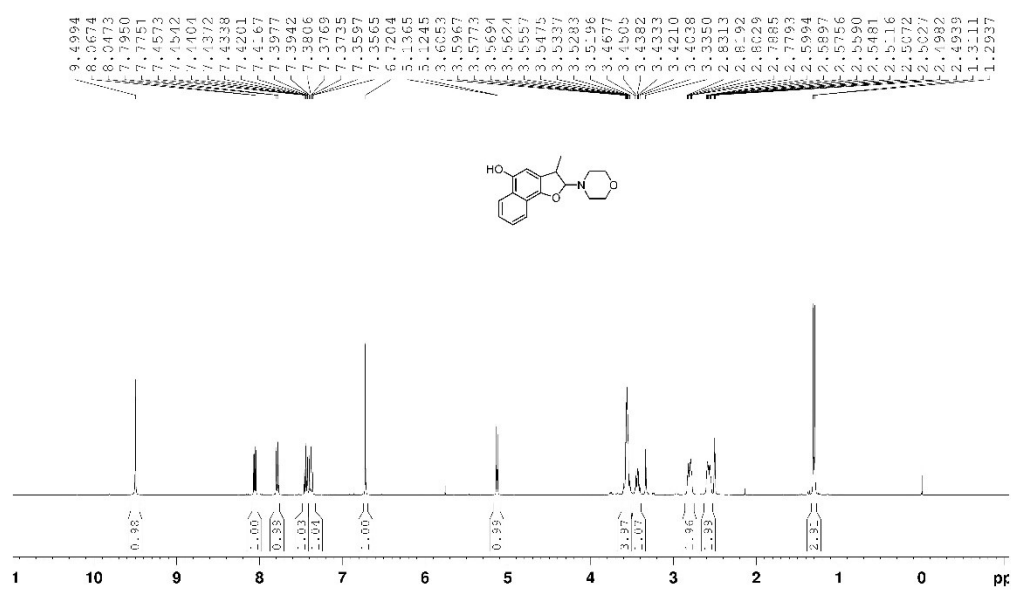
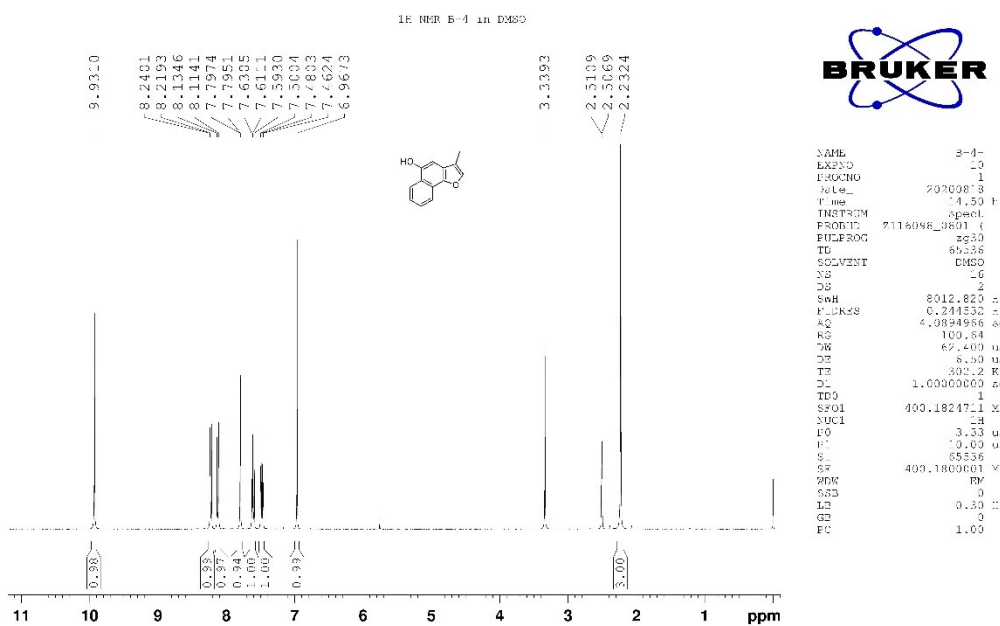


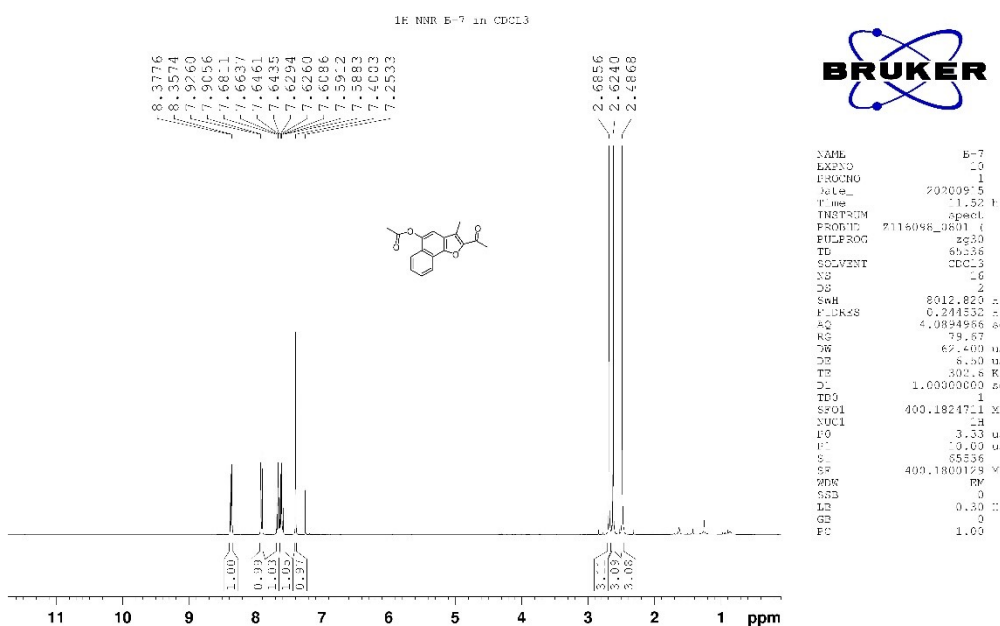
Figure S2. ^1H NMR, ^{13}C NMR, HRMS and HPLC Spectrum of Compounds



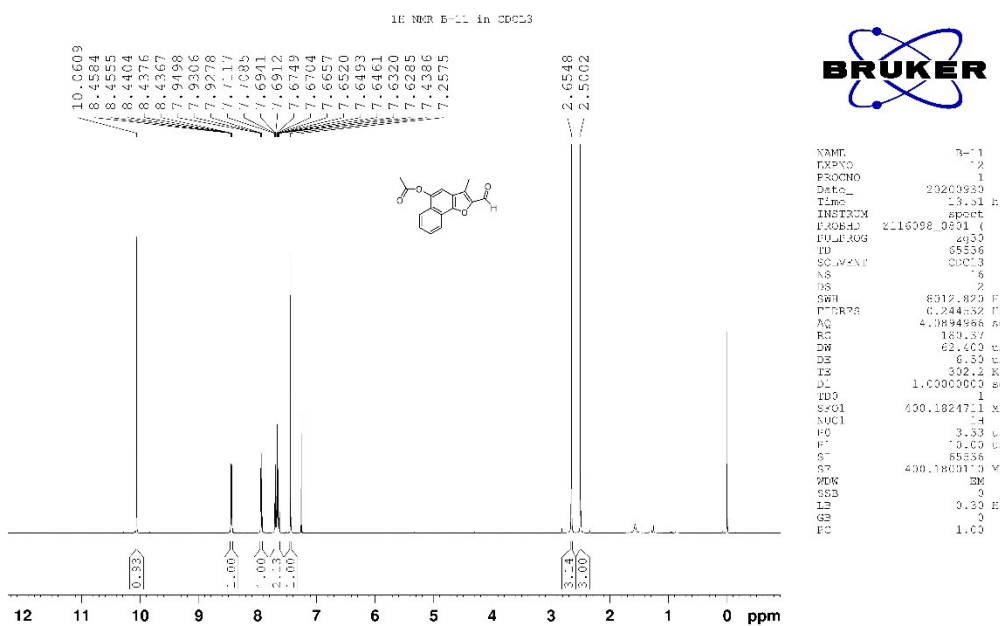
^1H NMR spectrum of 2



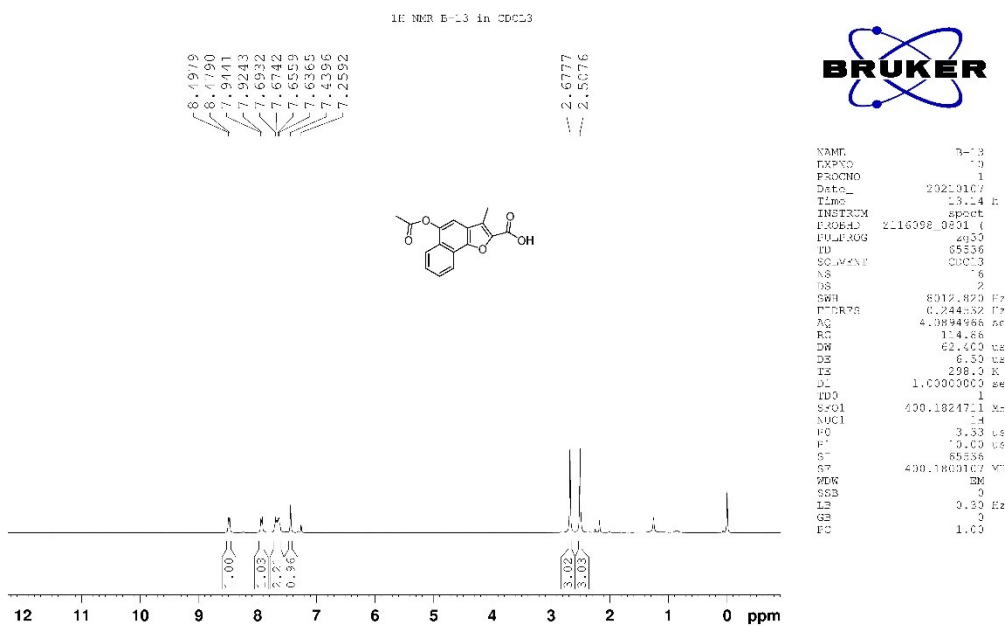
¹H NMR spectrum of 3



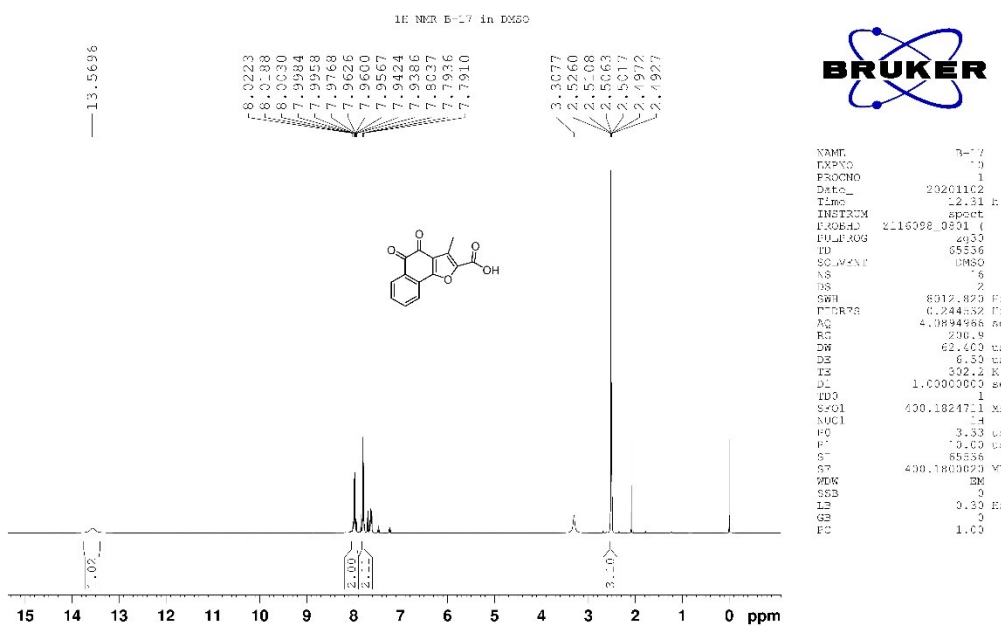
¹H NMR spectrum of 4



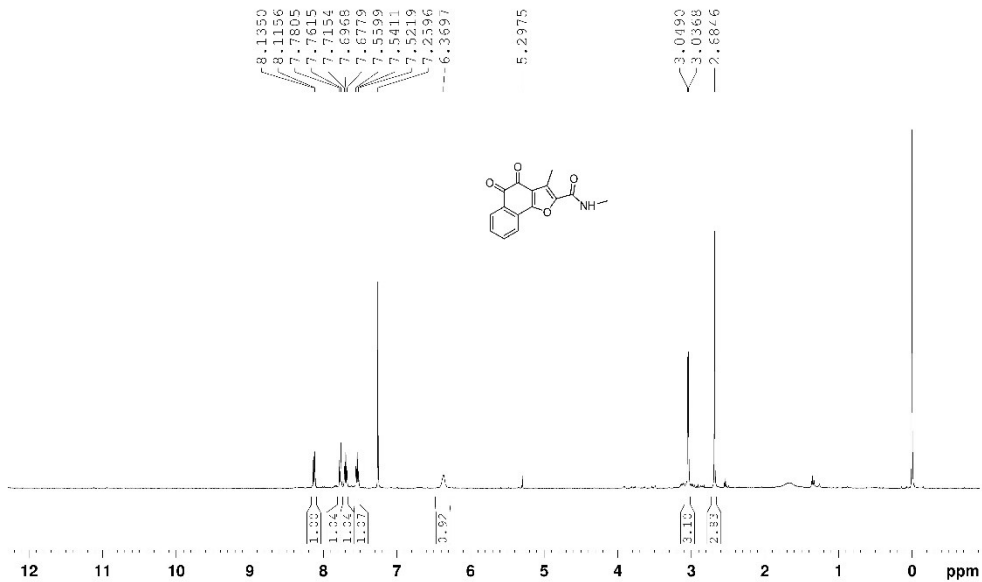
¹H NMR spectrum of 5



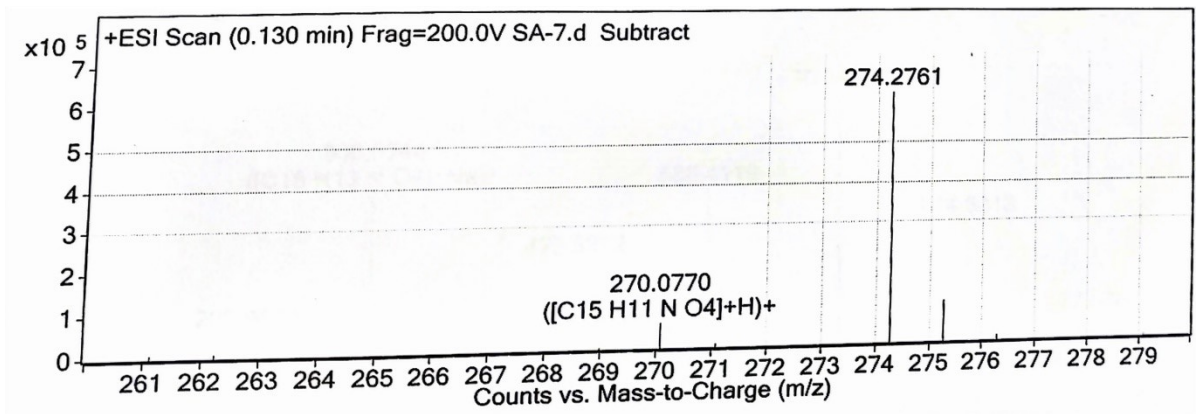
¹H NMR spectrum of 6



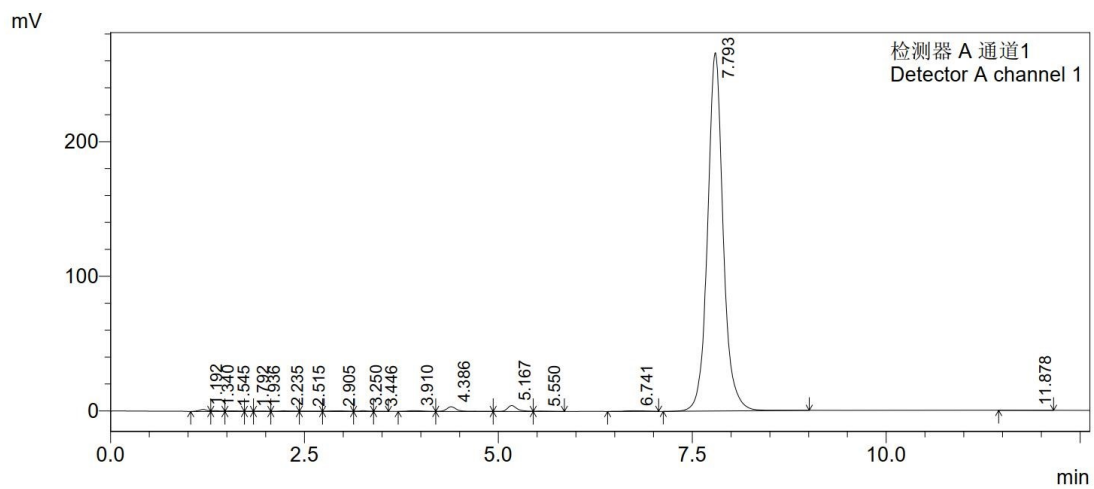
¹H NMR spectrum of 1



¹H NMR spectrum of A1

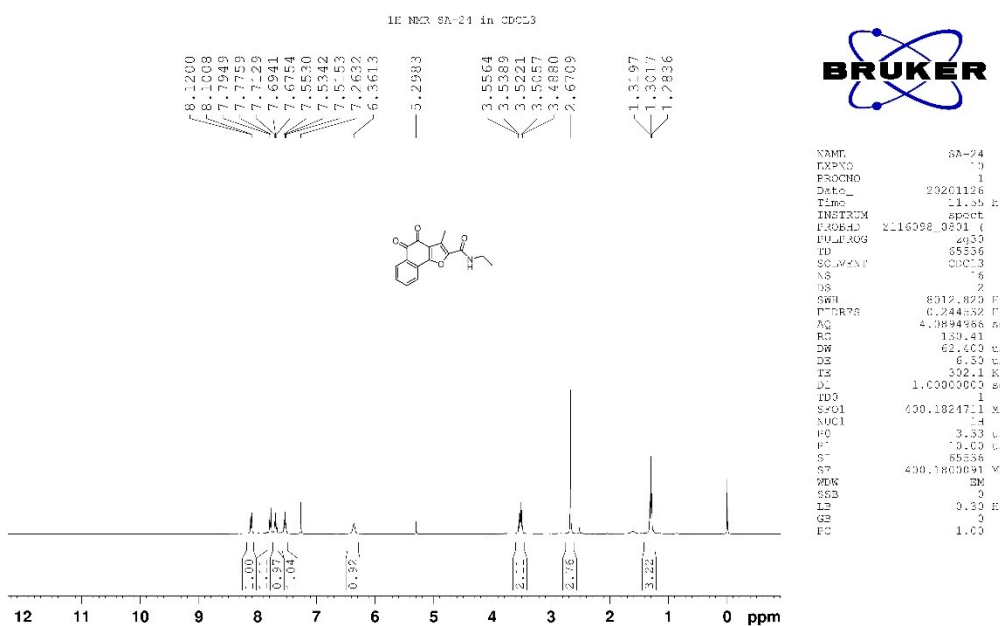


HRMS analysis of A1

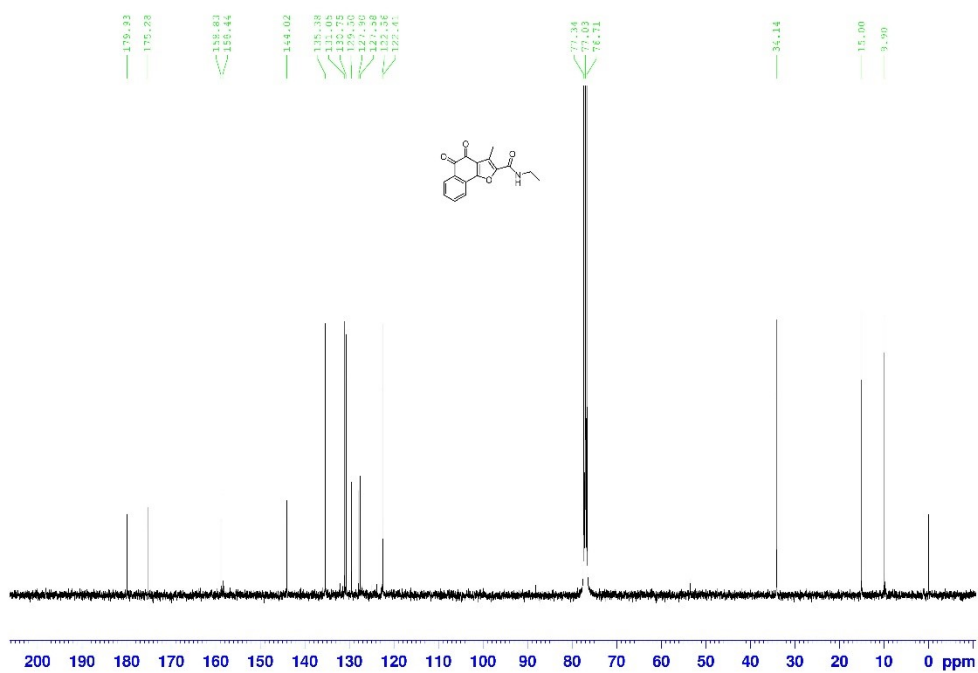


peak	time (min)	Area	height	Aera %	height %
1	1.192	11272	1495	0.292	0.532
2	1.340	5024	596	0.130	0.212
3	1.545	5512	464	0.143	0.165
4	1.792	1836	267	0.048	0.095
5	1.936	3717	310	0.096	0.110
6	2.235	6611	485	0.171	0.172
7	2.515	3890	289	0.101	0.103
8	2.905	7043	405	0.183	0.144
9	3.250	4988	565	0.129	0.201
10	3.446	1626	170	0.042	0.060
11	3.910	8403	734	0.218	0.261
12	4.386	32577	3544	0.844	1.261
13	5.167	38162	4378	0.989	1.558
14	5.550	3391	256	0.088	0.091
15	6.741	7838	602	0.203	0.214
16	7.793	3712358	266243	96.235	94.755
17	11.878	3368	178	0.087	0.063
total		3857614	280981	100.000	100.000

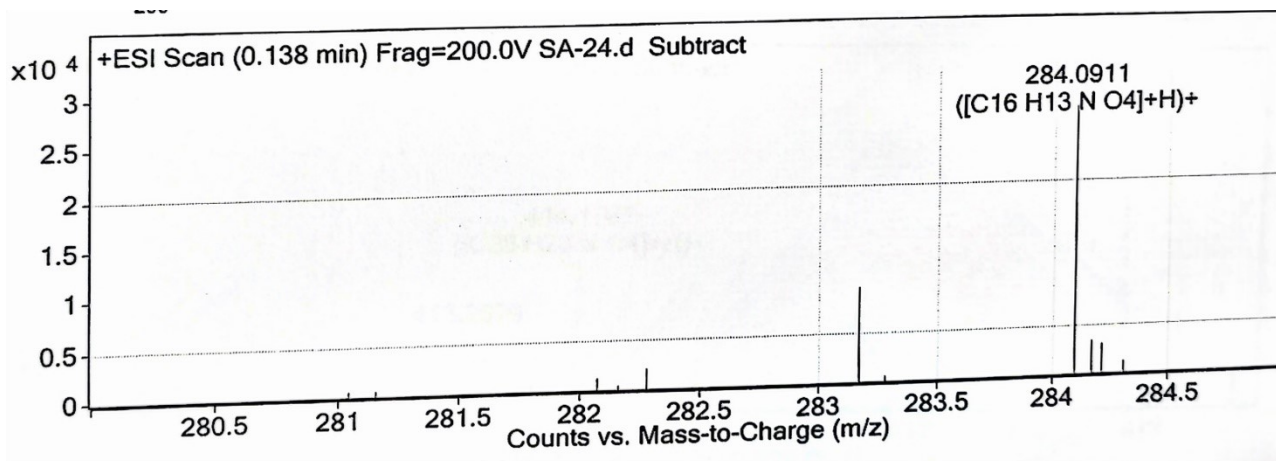
HPLC analysis of A1



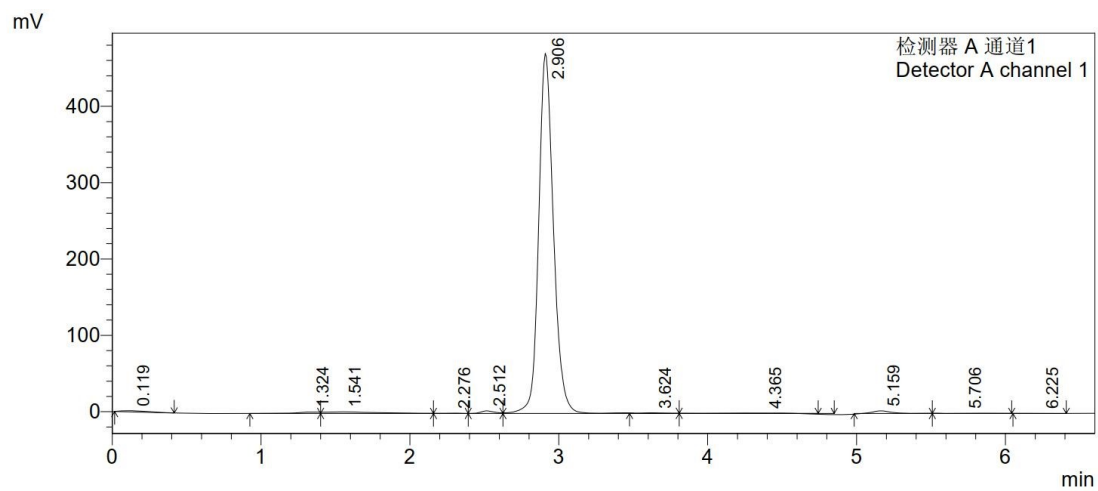
¹H NMR spectrum of A2



^{13}C NMR spectrum of A2

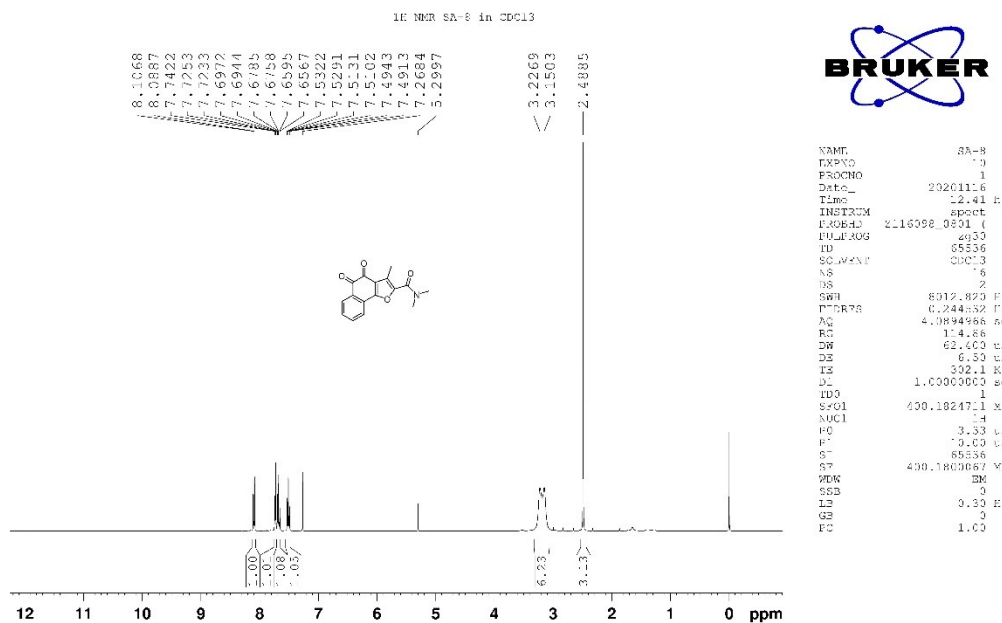


HRMS analysis of A2

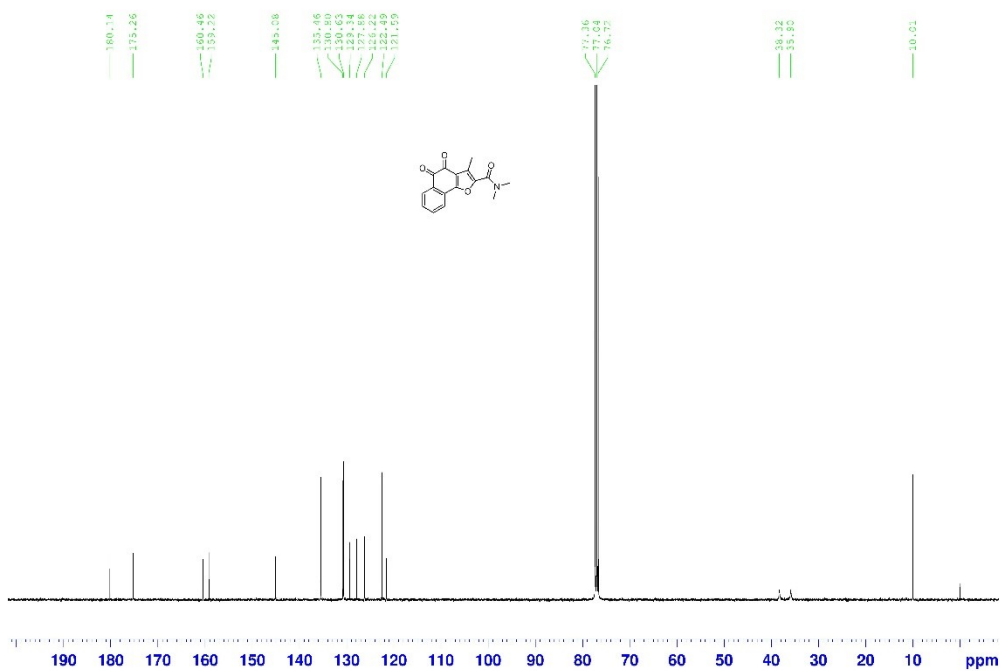


peak	time (min)	Area	height	Area %	height %
1	0.110	1588	128	0.542	0.309
2	1.302	2026	189	0.692	0.456
3	2.512	1695	275	0.579	0.662
4	2.906	286227	40837	97.782	98.301
5	3.590	1183	113	0.404	0.273
total		292719	41543	100.000	100.000

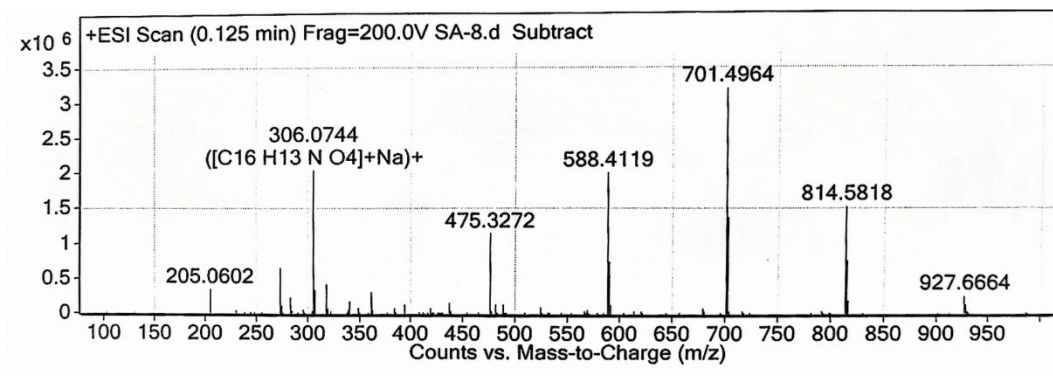
HPLC analysis of A2



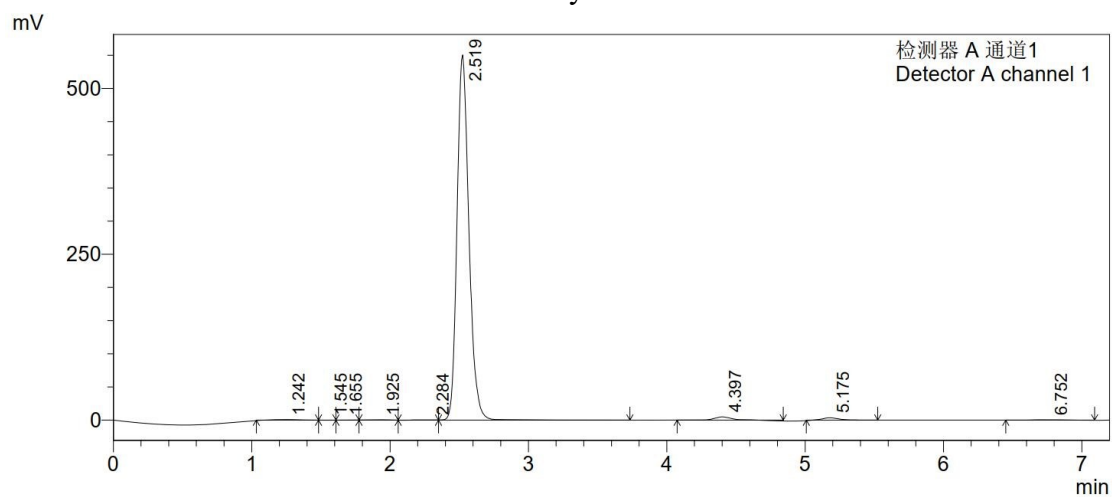
¹H NMR spectrum of A3



^{13}C NMR spectrum of A3

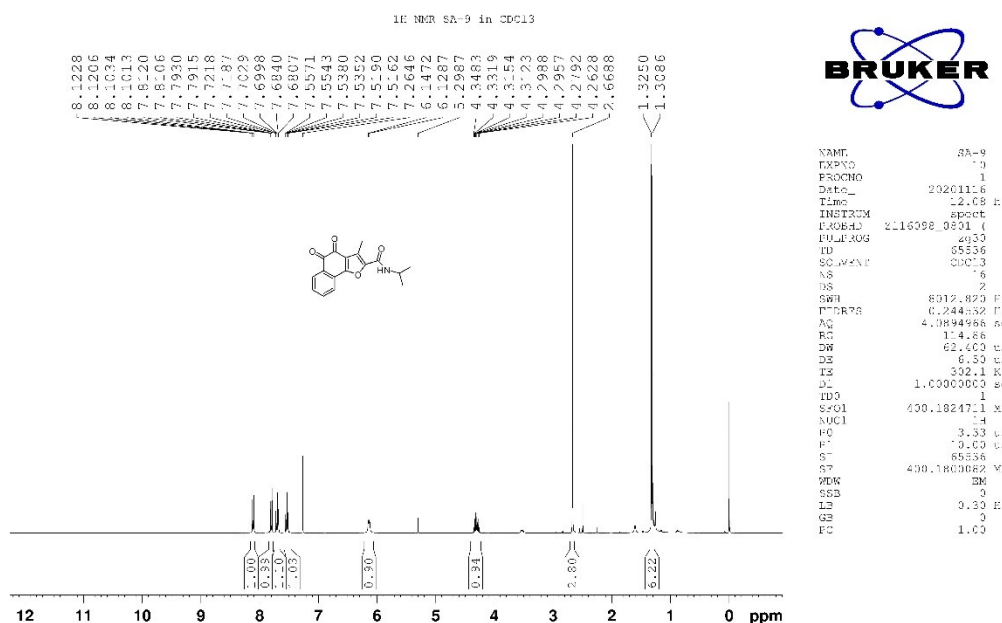


HRMS analysis of A3

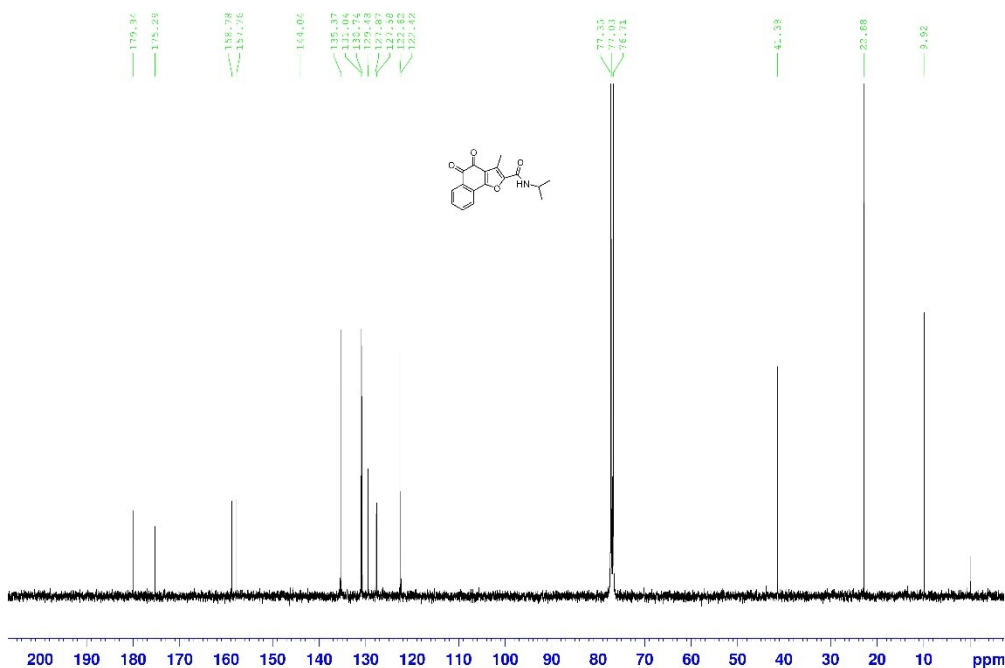


peak	time (min)	Area	height	Area %	height %
1	1.242	10993	890	0.326	0.159
2	1.545	1525	248	0.045	0.044
3	1.655	1707	219	0.051	0.039
4	1.925	4441	555	0.132	0.099
5	2.284	3231	295	0.096	0.052
6	2.519	3279781	550658	97.111	98.056
7	4.397	41396	4788	1.226	0.853
8	5.175	27910	3425	0.826	0.610
9	6.752	6366	496	0.188	0.088
total		3377350	561574	100.000	100.000

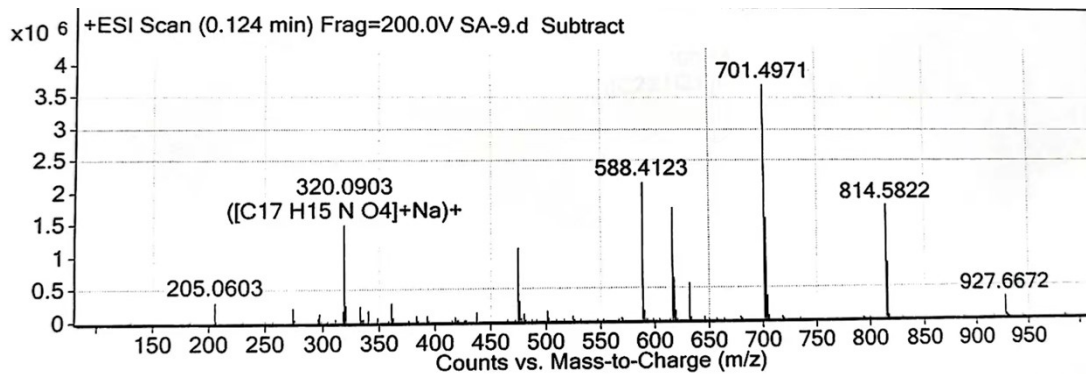
HPLC analysis of A3



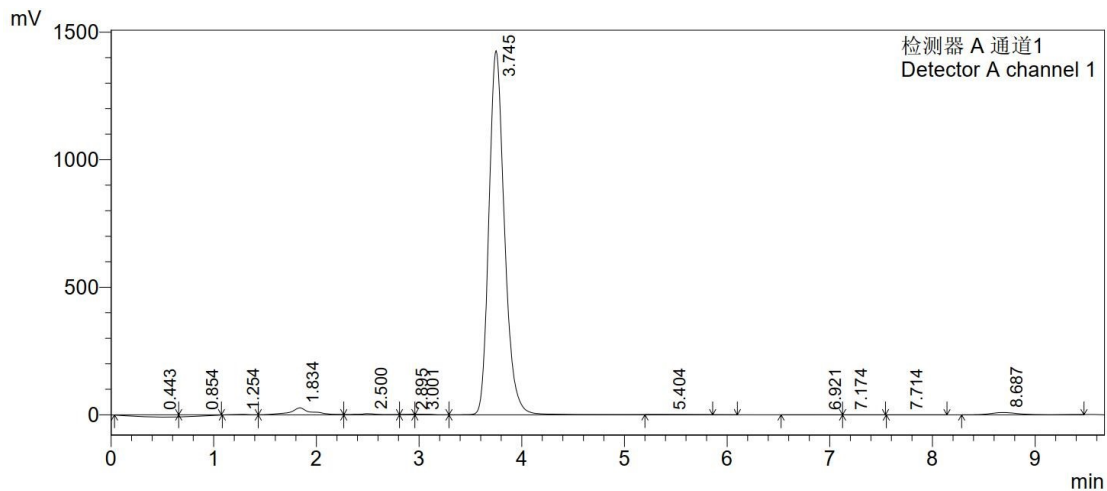
¹H NMR spectrum of A4



¹³C NMR spectrum of A4

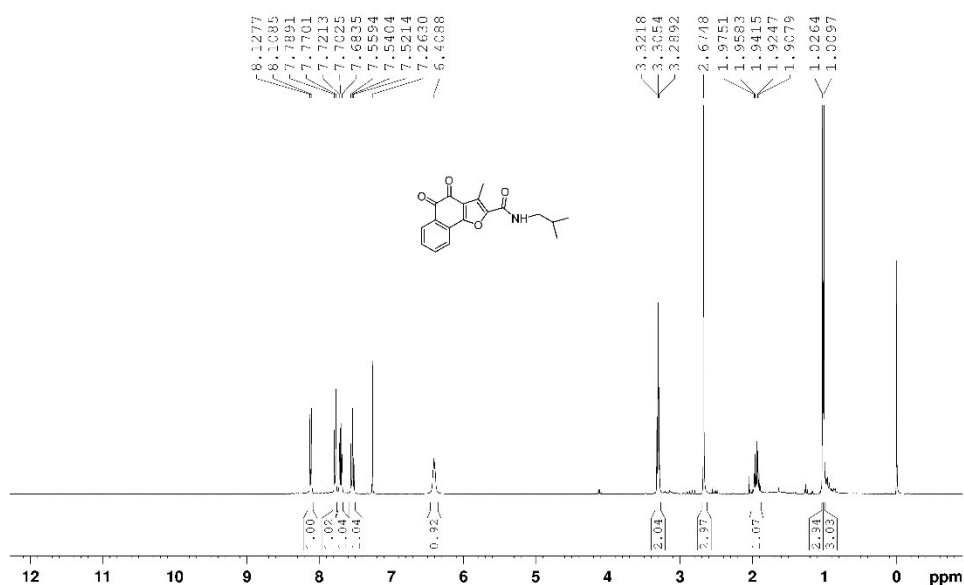


HRMS analysis of A4

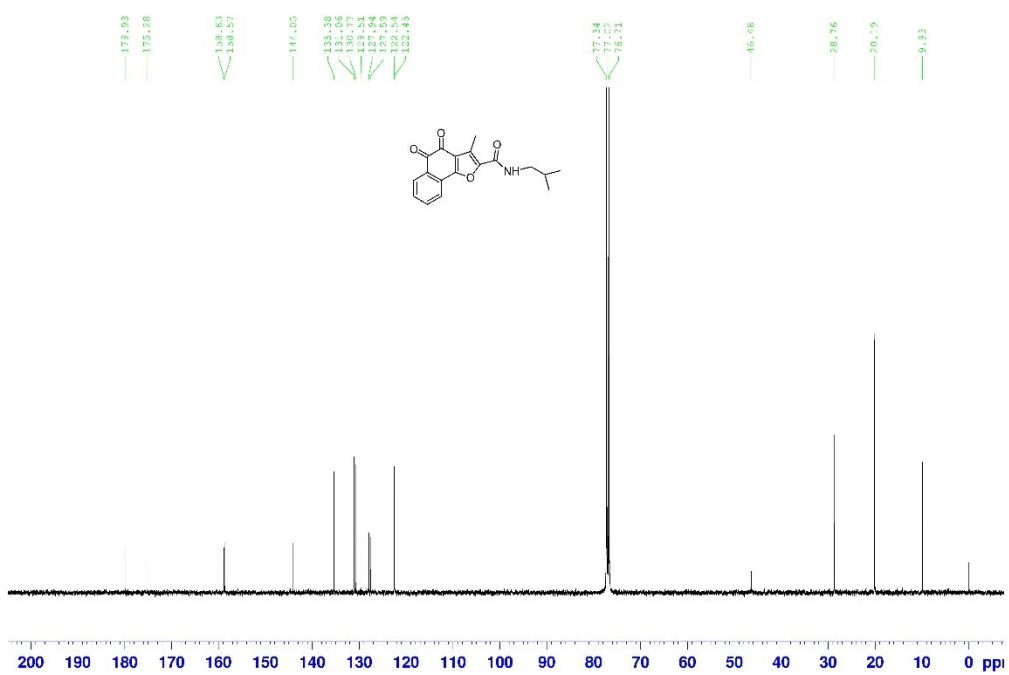


peak	time (min)	Area	height	Area %	height %
1	0.443	2381	120	0.015	0.008
2	0.854	1890	128	0.012	0.009
3	1.254	15590	1839	0.099	0.125
4	1.834	391990	27514	2.498	1.867
5	2.500	59903	4494	0.382	0.305
6	2.895	5603	709	0.036	0.048
7	3.001	6804	664	0.043	0.045
8	3.745	15015884	1427866	95.672	96.871
9	5.404	13008	807	0.083	0.055
10	6.921	8206	419	0.052	0.028
11	7.174	3631	245	0.023	0.017
12	7.714	1684	116	0.011	0.008
13	8.687	168651	9057	1.075	0.614
total		15695223	1473980	100.000	100.000

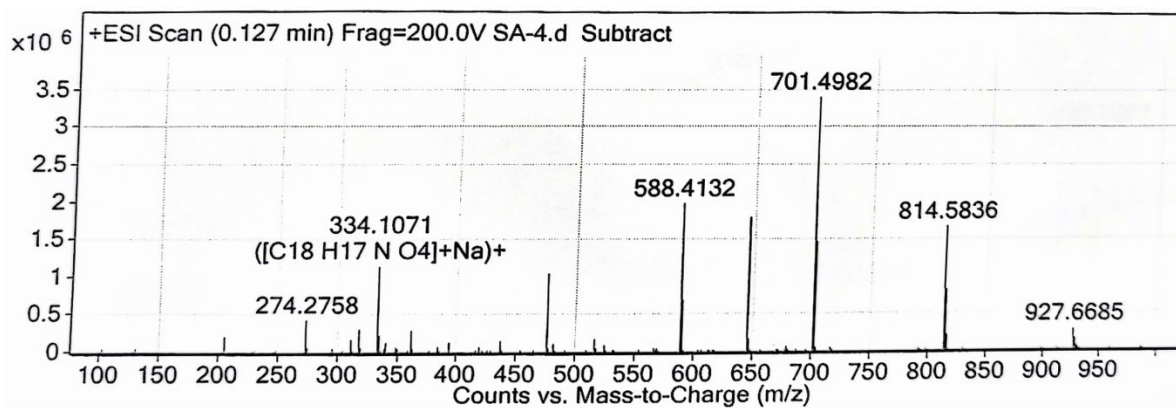
HPLC analysis of A4



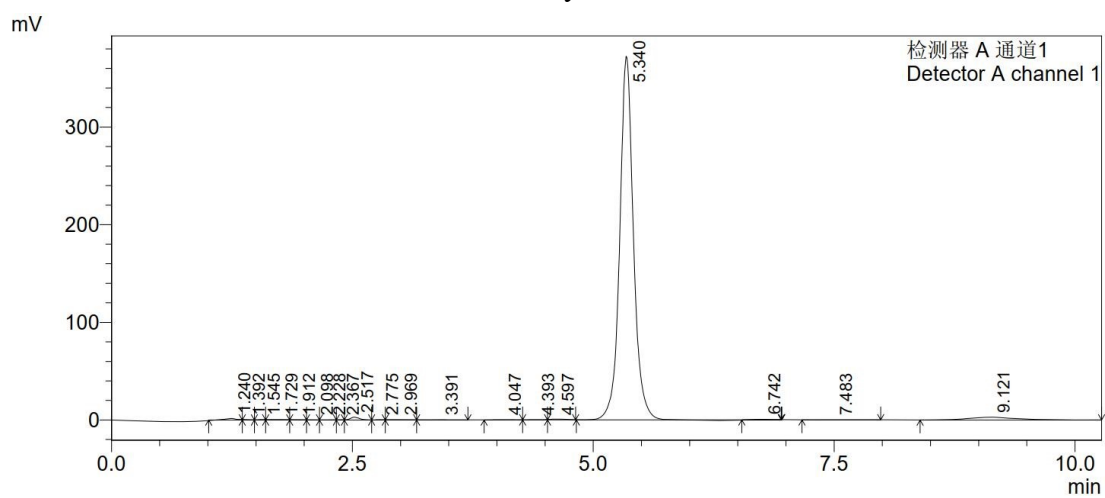
¹H NMR spectrum of A5

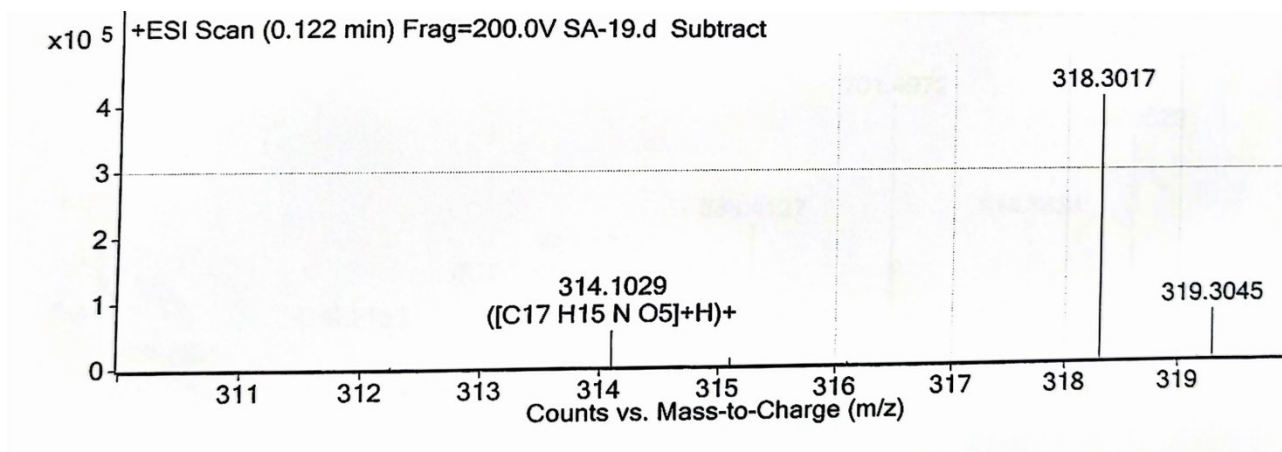


¹³C NMR spectrum of A5

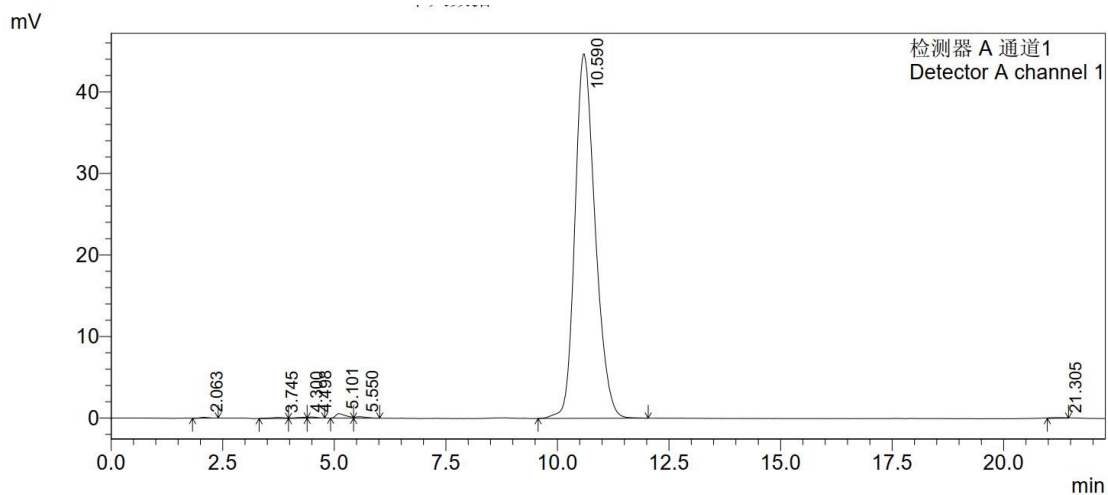


HRMS analysis of A5



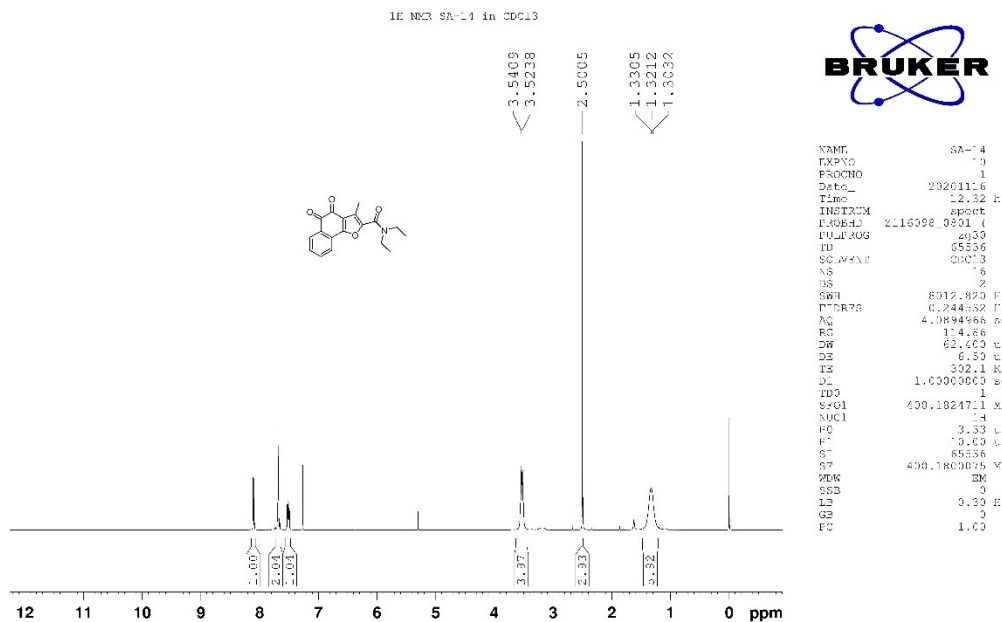


HRMS analysis of A6

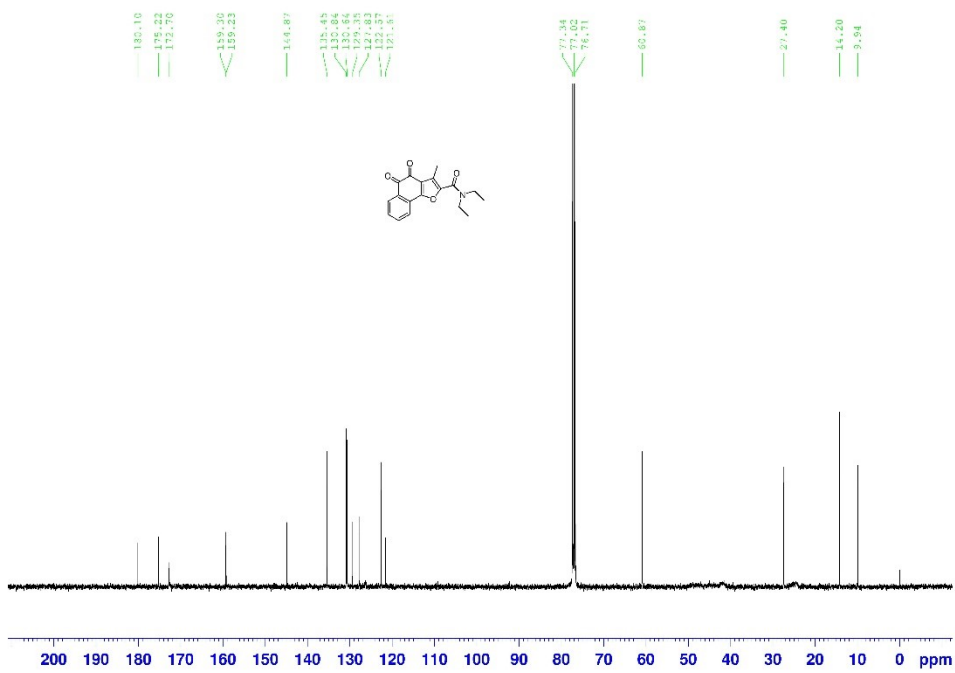


peak	time (min)	Area	height	Area %	height %
1	2.063	1597	110	0.111	0.240
2	3.745	3058	131	0.213	0.285
3	4.300	2143	114	0.150	0.249
4	4.498	1896	152	0.132	0.331
5	5.101	8465	545	0.591	1.184
6	5.550	3079	171	0.215	0.372
7	10.590	1410719	44714	98.442	97.165
8	21.305	2086	80	0.146	0.174
total		1433043	46019	100.000	100.000

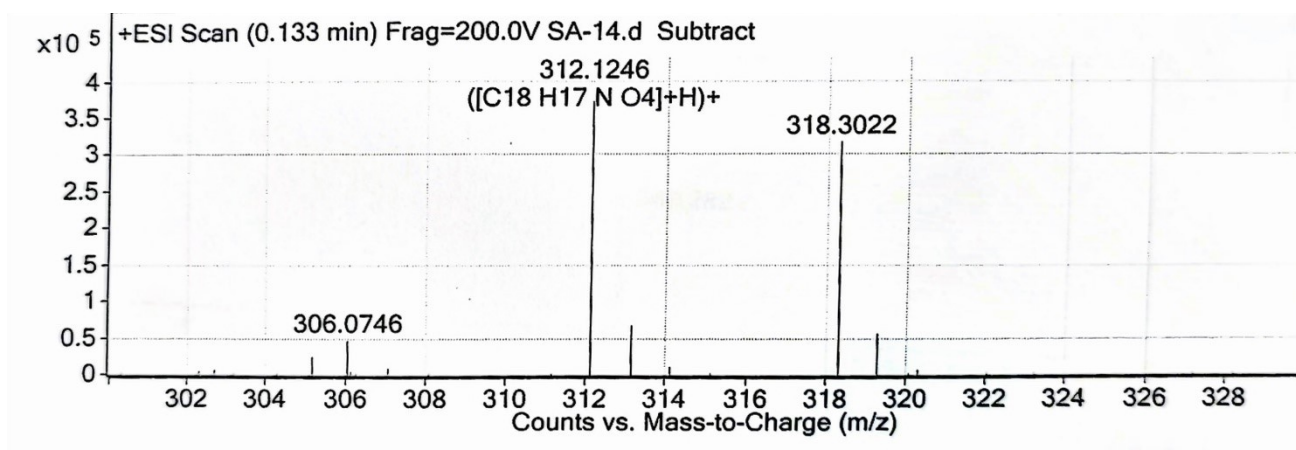
HPLC analysis of A6



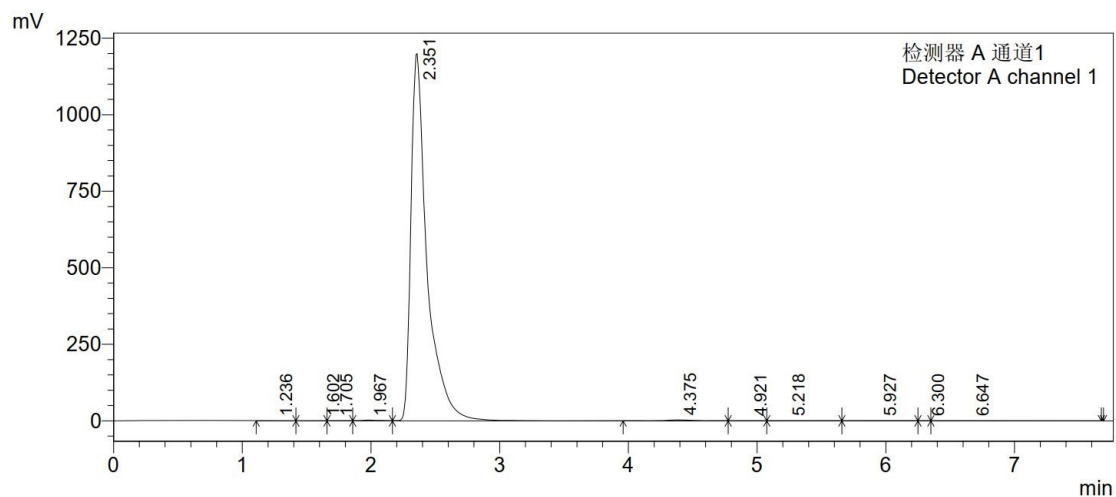
¹H NMR spectrum of A7



^{13}C NMR spectrum of A7

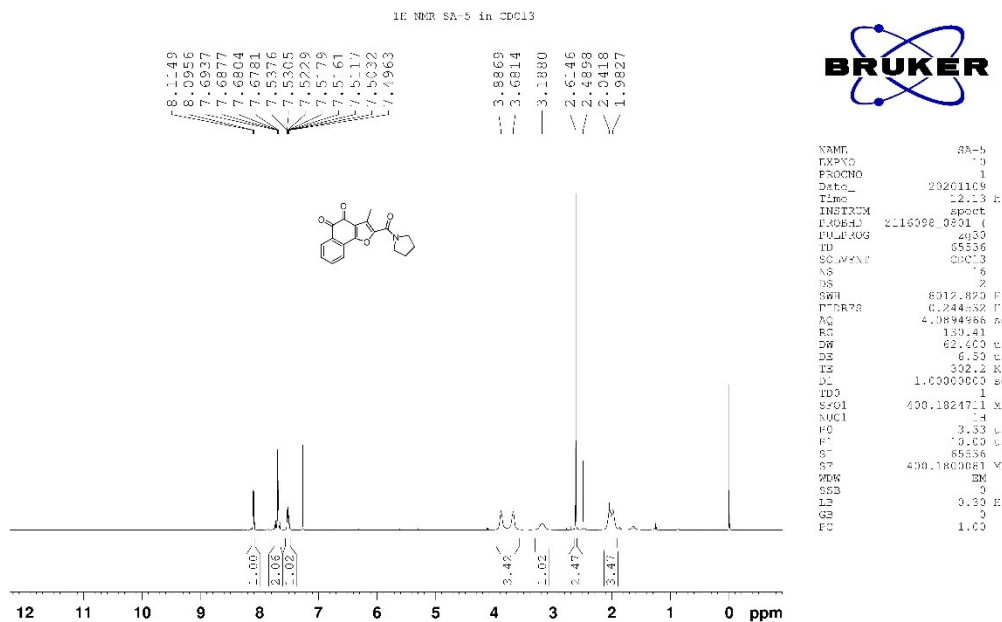


HRMS analysis of A7

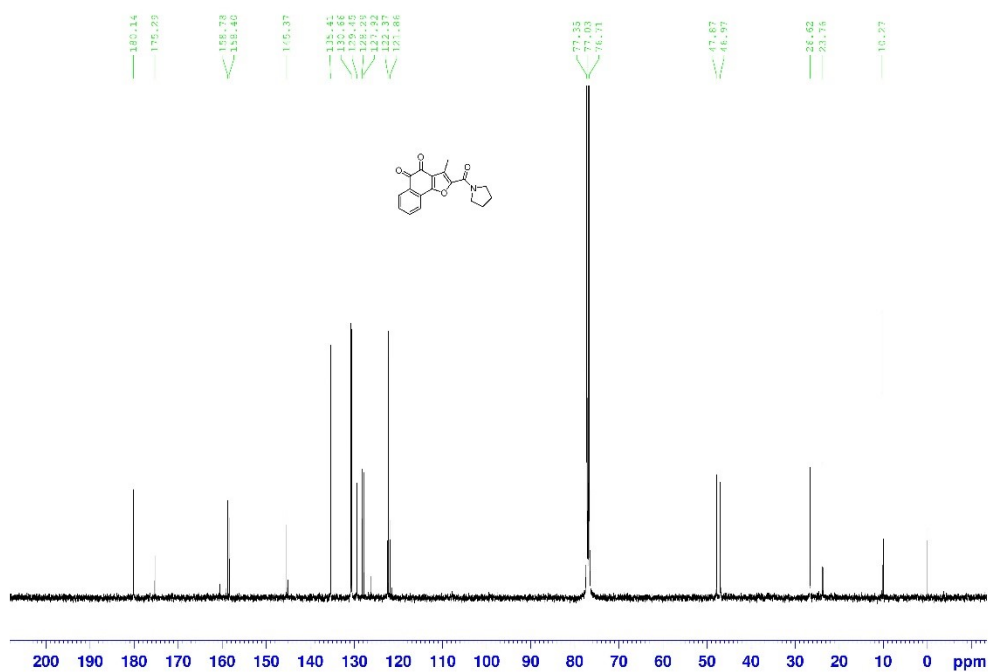


peak	time (min)	Area	height	Area %	height %
1	1.236	11546	1517	0.109	0.125
2	1.602	9827	1037	0.092	0.086
3	1.705	9572	982	0.090	0.081
4	1.967	25494	2120	0.240	0.175
5	2.351	10489402	1199552	98.635	99.068
6	4.375	42120	3099	0.396	0.256
7	4.921	5475	404	0.051	0.033
8	5.218	9698	702	0.091	0.058
9	5.927	13150	637	0.124	0.053
10	6.300	2016	340	0.019	0.028
11	6.647	16287	450	0.153	0.037
total		10634588	1210839	100.000	100.000

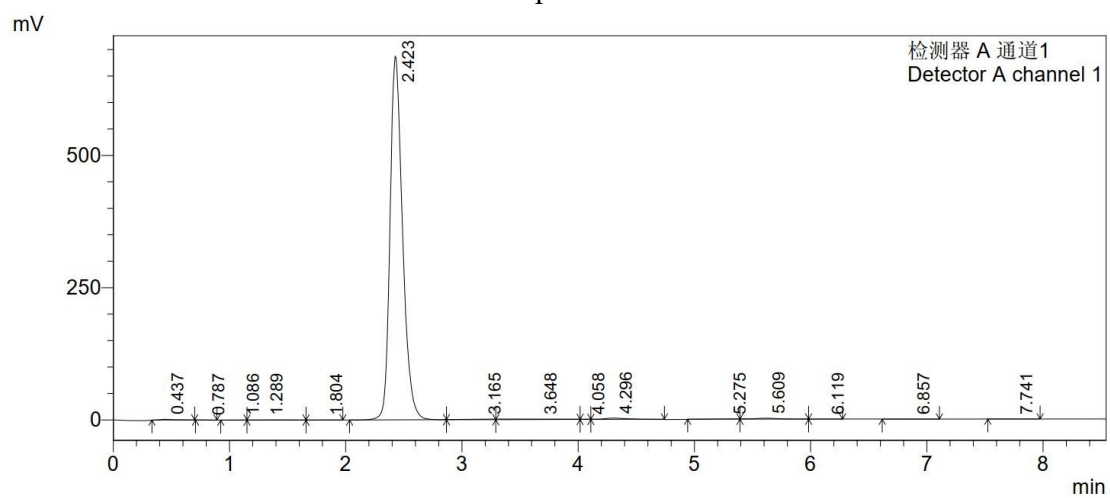
HPLC analysis of A7



¹H NMR spectrum of A8

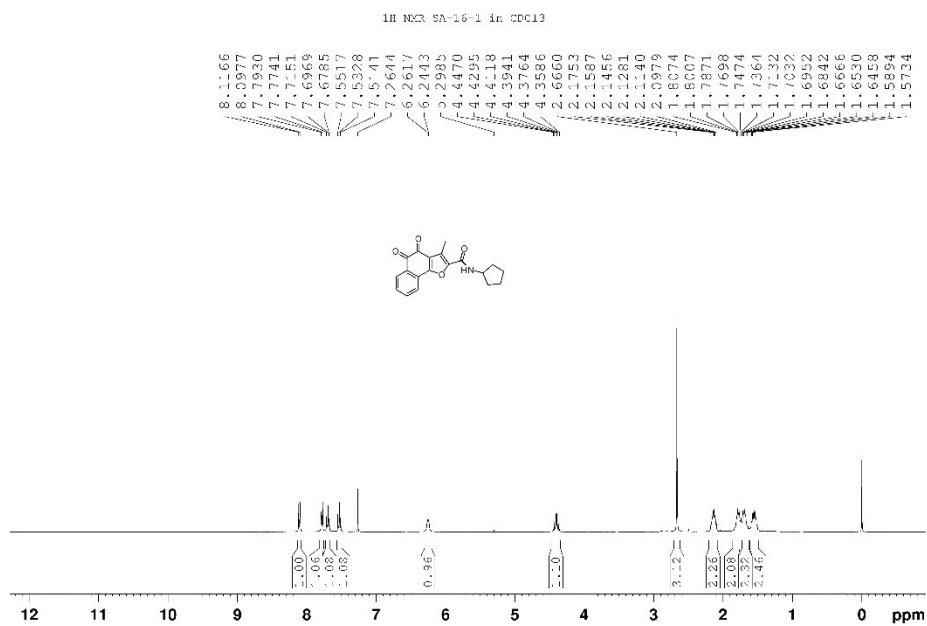


¹³C NMR spectrum of A8

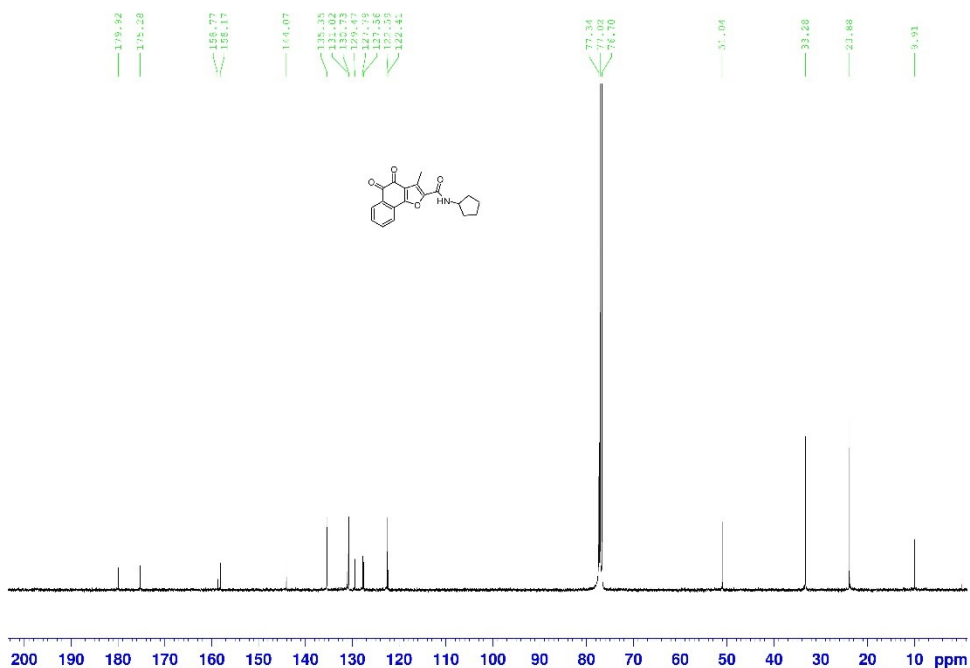


peak	time (min)	Area	height	Area %	height %
1	0.437	8592	1323	0.162	0.190
2	0.787	1075	232	0.020	0.033
3	1.086	1546	233	0.029	0.033
4	1.289	8722	556	0.165	0.080
5	1.804	1864	263	0.035	0.038
6	2.423	5158074	687768	97.437	98.568
7	3.165	17026	829	0.322	0.119
8	3.648	30599	1352	0.578	0.194
9	4.058	1666	307	0.031	0.044
10	4.296	29094	2354	0.550	0.337
11	5.275	4004	291	0.076	0.042
12	5.609	25212	1766	0.476	0.253
13	6.119	1425	112	0.027	0.016
14	6.857	3685	287	0.070	0.041
15	7.741	1184	83	0.022	0.012
total		5293767	697757	100.000	100.000

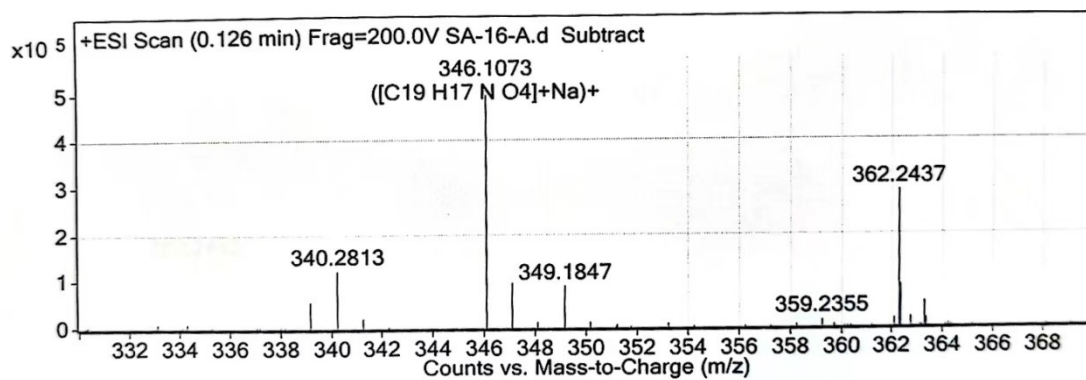
HPLC analysis of A8



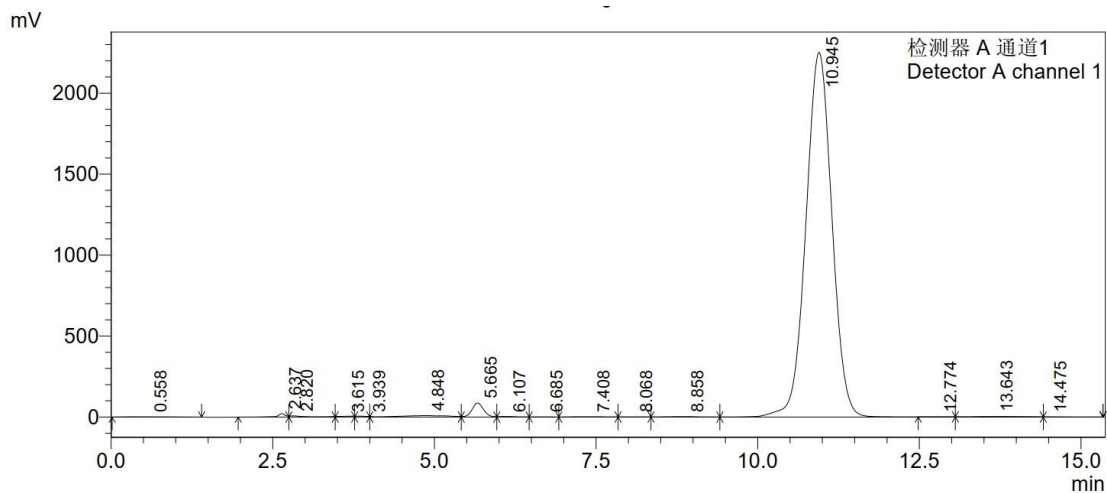
¹H NMR spectrum of A9



¹³C NMR spectrum of A9

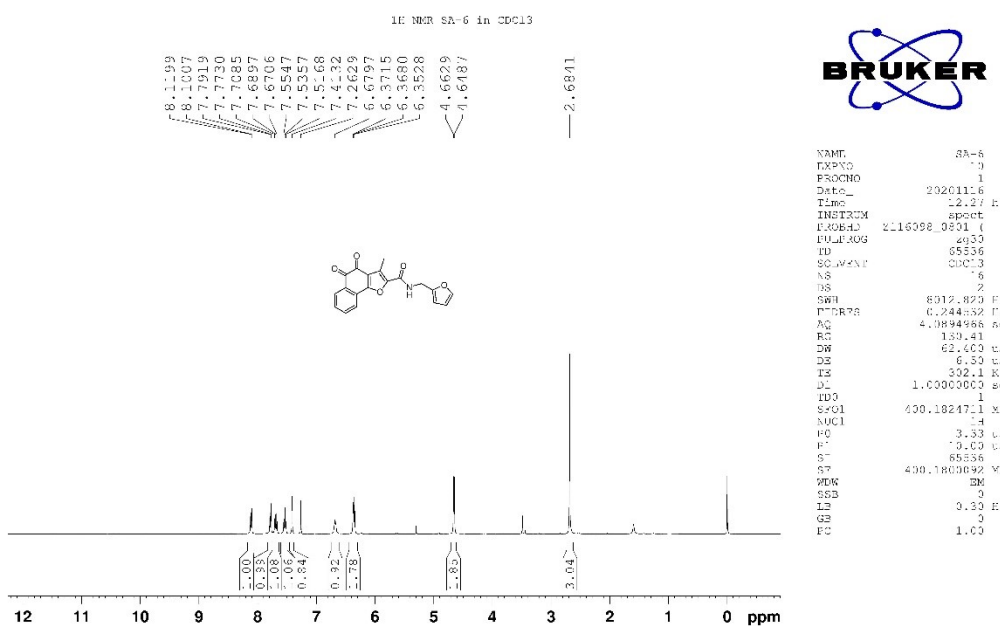


HRMS analysis of A9



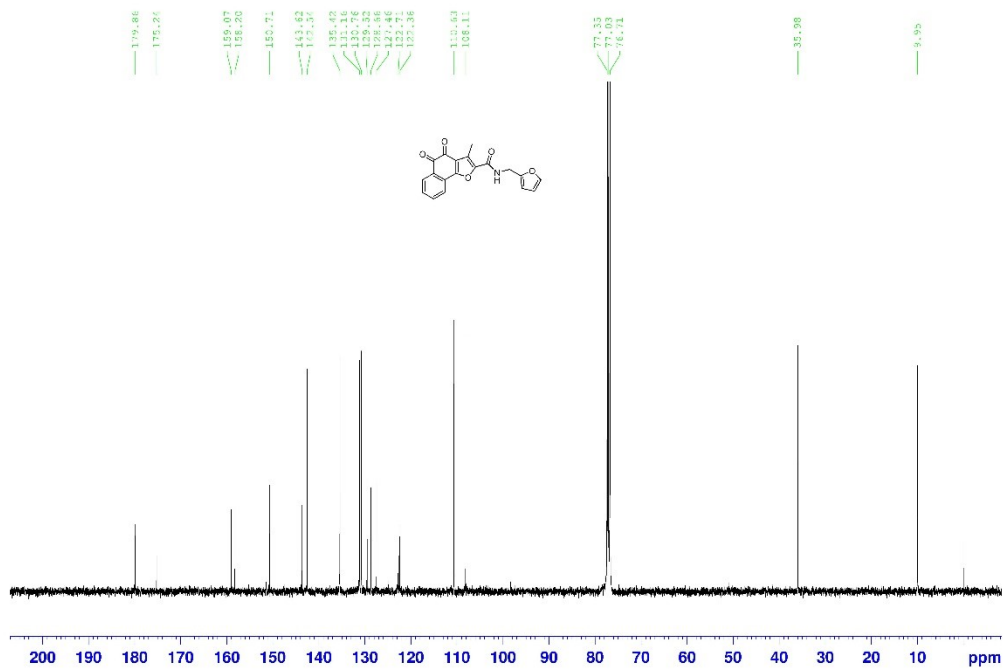
peak	time (min)	Area	height	Area %	height %
1	0.558	39522	939	0.061	0.039
2	2.637	174327	21229	0.267	0.887
3	2.820	123008	7213	0.189	0.301
4	3.615	20017	1285	0.031	0.054
5	3.939	14906	1141	0.023	0.048
6	4.848	466808	8536	0.716	0.357
7	5.665	1079400	86862	1.655	3.628
8	6.107	64432	3855	0.099	0.161
9	6.685	16860	849	0.026	0.035
10	7.408	70791	1879	0.109	0.078
11	8.068	28911	1255	0.044	0.052
12	8.858	89678	2804	0.137	0.117
13	10.945	62926151	2252389	96.459	94.080
14	12.774	2916	134	0.004	0.006
15	13.643	109779	3463	0.168	0.145
16	14.475	8340	289	0.013	0.012
total		65235846	2394120	100.000	100.000

HPLC analysis of A9

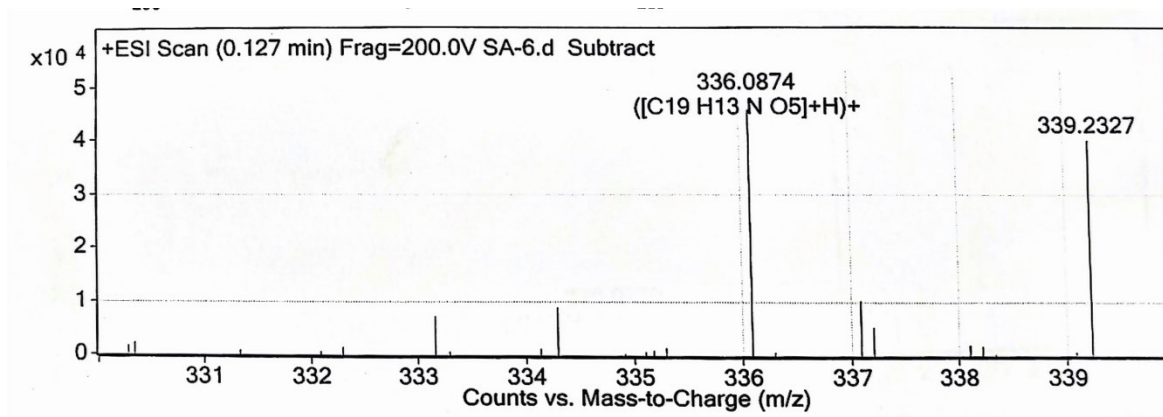


¹H NMR spectrum of A10

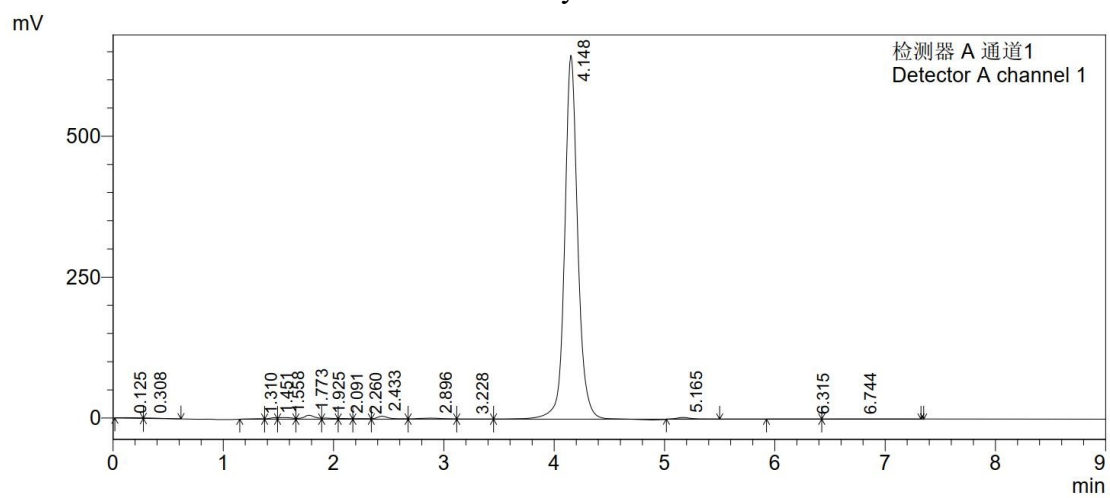
^{13}C NMR SA-6 in CDCl_3



^{13}C NMR spectrum of A10

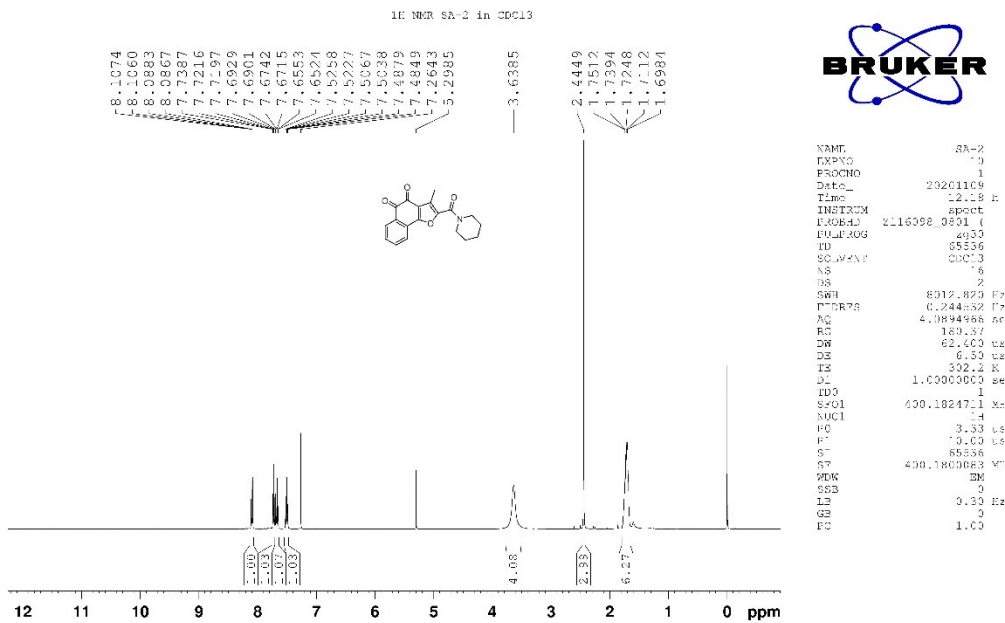


HRMS analysis of A10

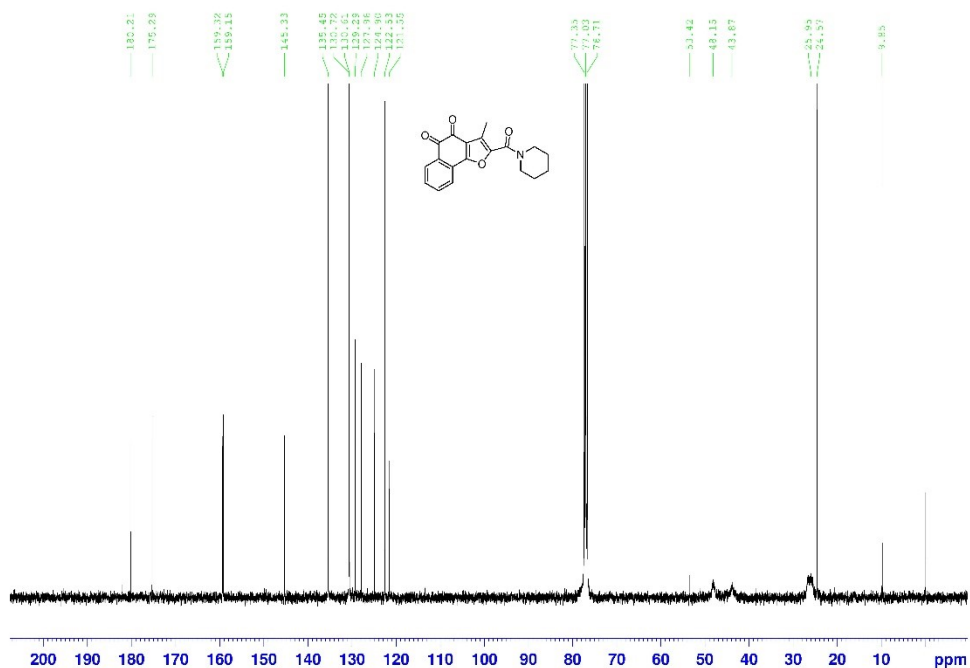


peak	time (min)	Area	height	Area %	height %
1	0.125	6803	438	0.122	0.065
2	0.308	9051	811	0.162	0.120
3	1.310	6961	881	0.125	0.130
4	1.451	12314	2384	0.221	0.353
5	1.558	23544	3038	0.422	0.449
6	1.773	50545	7001	0.905	1.036
7	1.925	13469	1942	0.241	0.287
8	2.091	8338	1239	0.149	0.183
9	2.260	9658	1100	0.173	0.163
10	2.433	41175	5340	0.738	0.790
11	2.896	28228	2025	0.506	0.299
12	3.228	9012	650	0.161	0.096
13	4.148	5329067	645631	95.462	95.501
14	5.165	24432	2967	0.438	0.439
15	6.315	1404	89	0.025	0.013
16	6.744	8398	512	0.150	0.076
total		5582398	676047	100.000	100.000

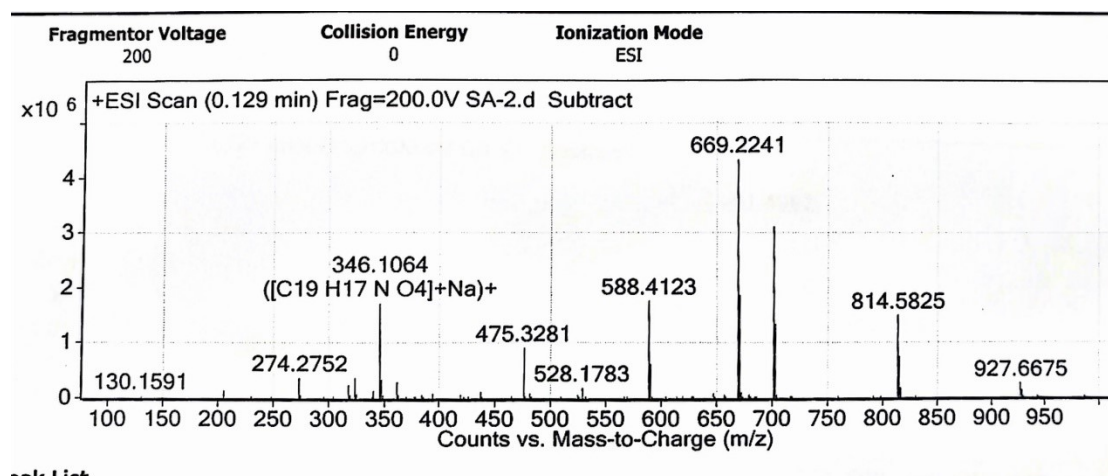
HPLC analysis of A10



¹H NMR spectrum of A11

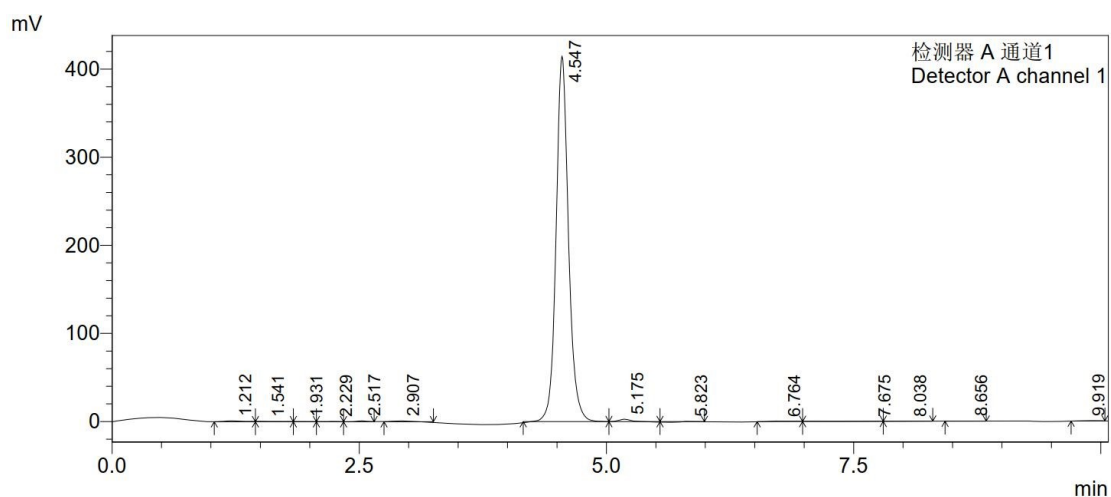


¹³C NMR spectrum of A11



Peak List

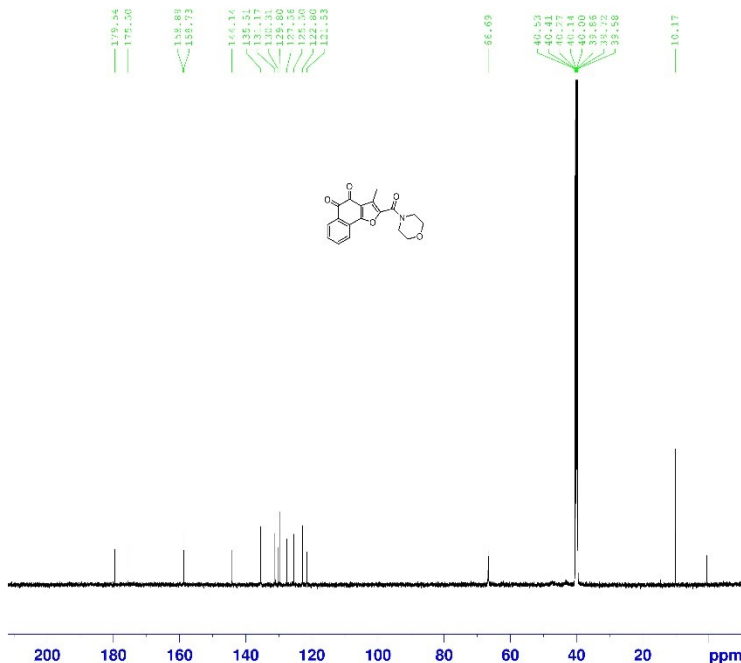
HRMS analysis of A11



peak	time(min)	Area	height	Area %	height %
1	1.212	11017	969	0.297	0.229
2	1.541	5270	405	0.142	0.096
3	1.931	2761	301	0.075	0.071
4	2.229	2990	274	0.081	0.065
5	2.517	6710	881	0.181	0.208
6	2.907	9934	834	0.268	0.197
7	4.547	3614482	415068	97.564	98.106
8	5.175	23543	2755	0.635	0.651
9	5.823	1408	142	0.038	0.034
10	6.764	6247	499	0.169	0.118
11	7.675	8919	225	0.241	0.053
12	8.038	5256	203	0.142	0.048
13	8.656	2046	124	0.055	0.029
14	9.919	4129	399	0.111	0.094
total		3704713	423081	100.000	100.000

HPLC analysis of A11

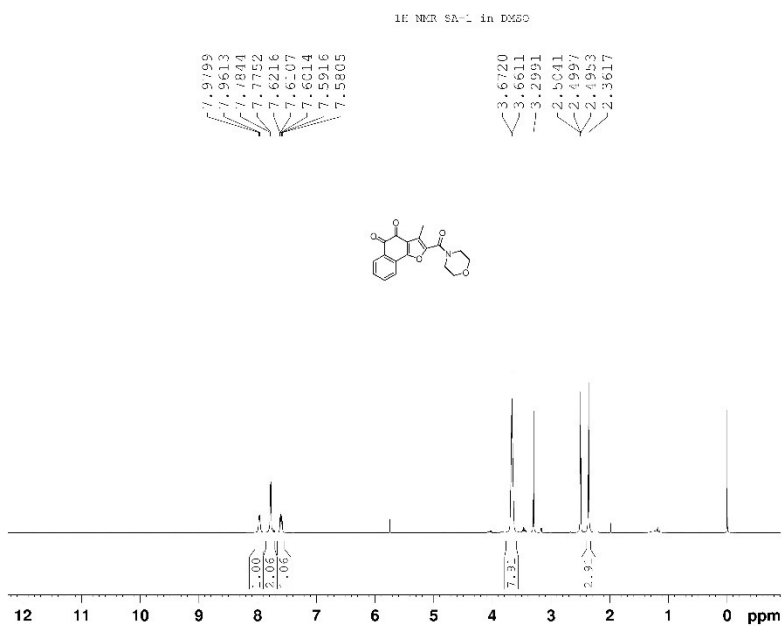
¹³C NMR SA-1 in DMSO



```

NAME SA-1
EXPNO 30
PROCNO 1
Date_ 20201107
Time 9:35 h
INSTRUM Avance Neo
PROBHD Z157363-0004 (
PULPROG zgpg30
TE 300.2K
SOLVENT DMSO
NS 97
DS 2
SWH 37037.035 Hz
FIDRES 1.130285 Hz
AQ 0.8847860 sec
RG 171.5 Hz
DN 13.500 usec
DE 18.00 usec
RF 285.0 K
DI 2.0000000 sec
D11 0.0300000 sec
TEO 1
SFO1 150.9186593 MHz
NUC1 13C
PQ 3.33 usec
PL 10.00 usec
SI 32768
SF 150.9028085 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
  
```

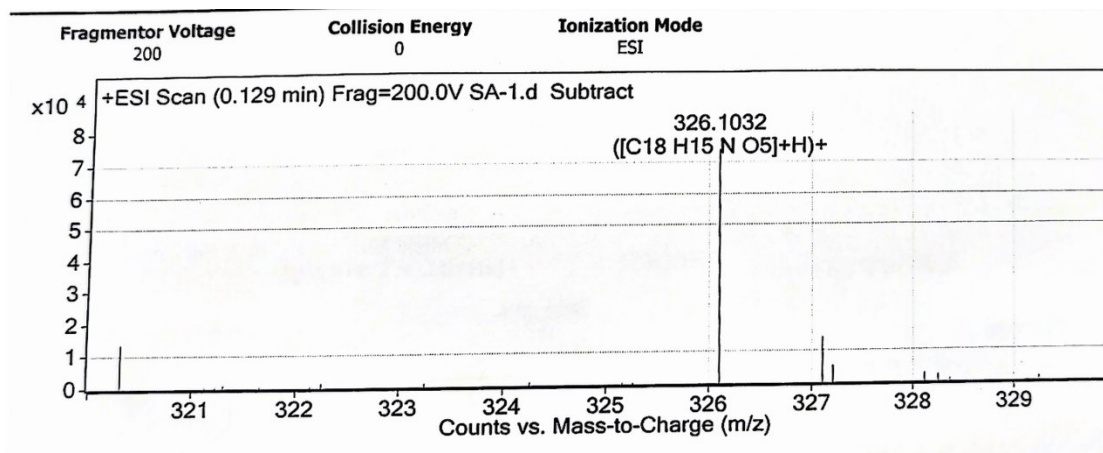
¹H NMR spectrum of A12



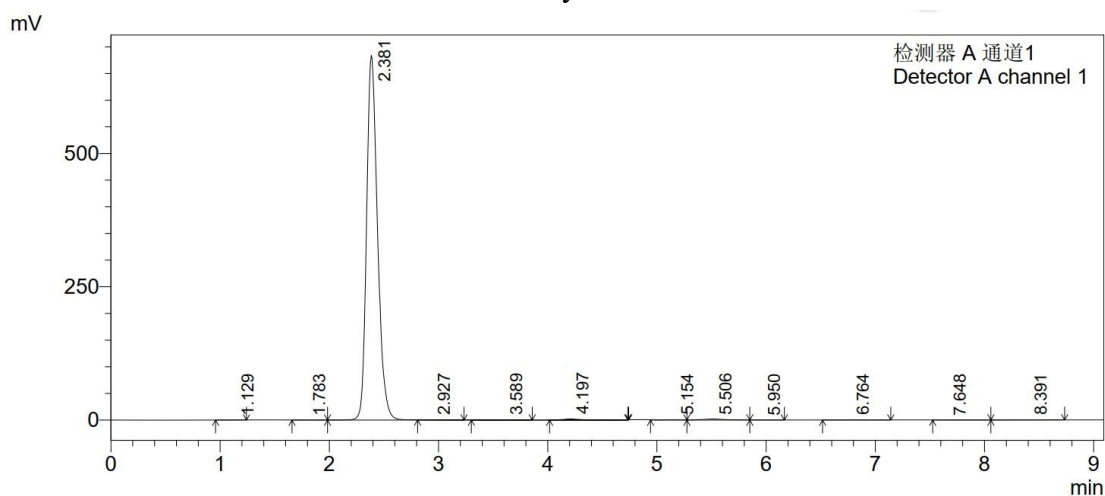
```

NAME SA-1
EXPNO 1
PROCNO 1
Date_ 20201107
Time 12:13 h
INSTRUM spect
PROBHD Z116098-0601 (
PULPROG zgpg30
TE 300.2K
SOLVENT DMSO
NS 97
DS 2
SWH 6012.823 Hz
FIDRES 0.244532 Hz
AQ 4.0894966 sec
RG 236.9 Hz
DN 62.400 usec
DE 6.30 usec
RF 302.1 K
DI 1.0000000 sec
TEO 1
SFO1 400.1824711 MHz
NUC1 1H
PQ 3.33 usec
PL 0.00 usec
SI 65536
SF 400.1863628 MHz
WDW EM
SSB 0
LB 3.33 Hz
GB 0
PC 1.00
  
```

¹³C NMR spectrum of A12

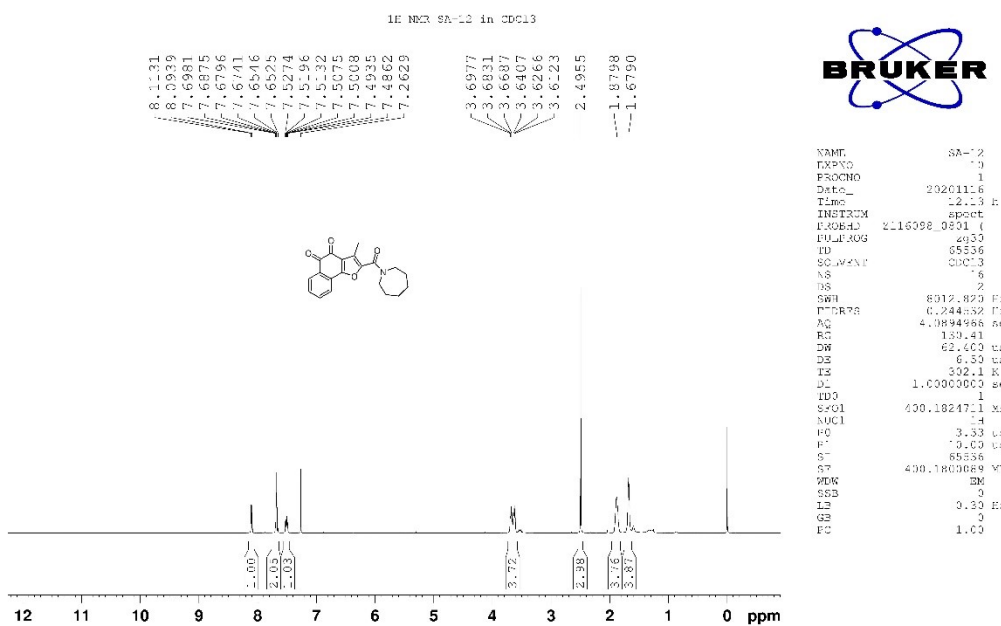


HRMS analysis of A12

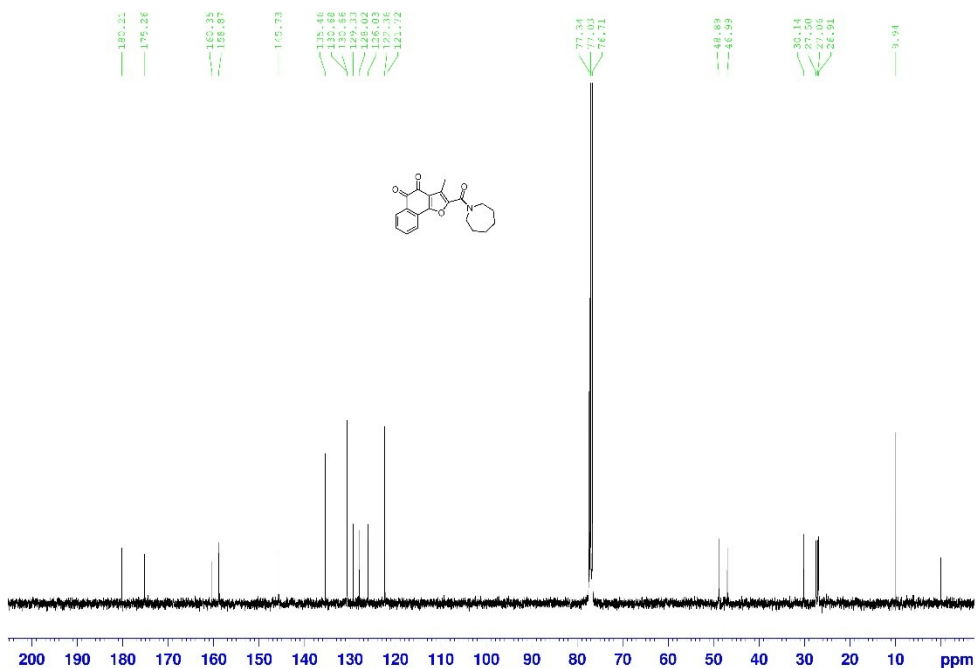


peak	Time (min)	Area	height	Area %	height %
1	1.129	1905	273	0.039	0.039
2	1.783	2000	288	0.041	0.042
3	2.381	4753339	683915	98.358	98.954
4	2.927	4335	489	0.090	0.071
5	3.589	7890	814	0.163	0.118
6	4.197	22426	2213	0.464	0.320
7	5.154	3825	376	0.079	0.054
8	5.506	24682	1866	0.511	0.270
9	5.950	1565	131	0.032	0.019
10	6.764	3831	298	0.079	0.043
11	7.648	1590	101	0.033	0.015
12	8.391	5328	384	0.110	0.056
total		4832716	691148	100.000	100.000

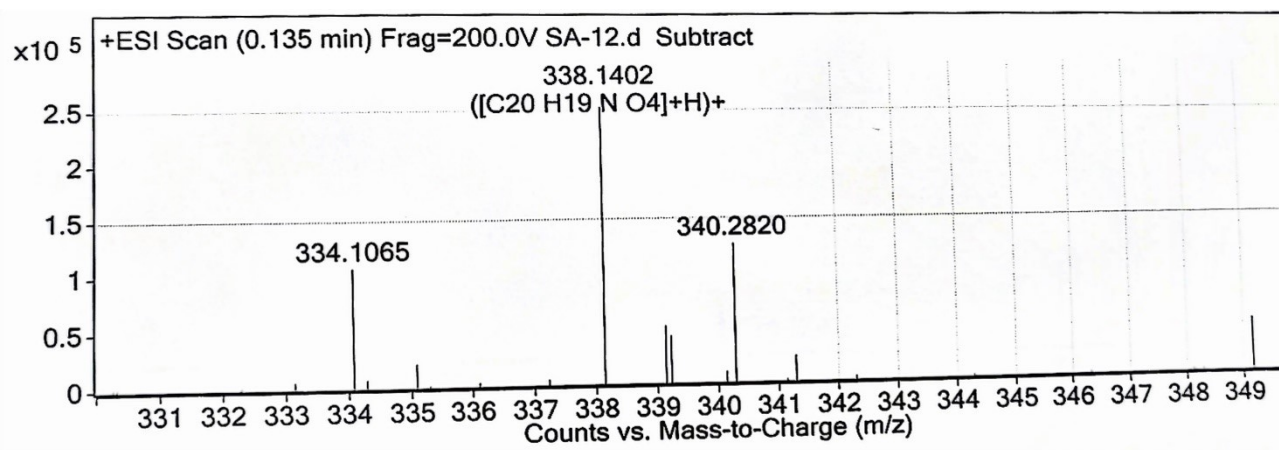
HPLC analysis of A12



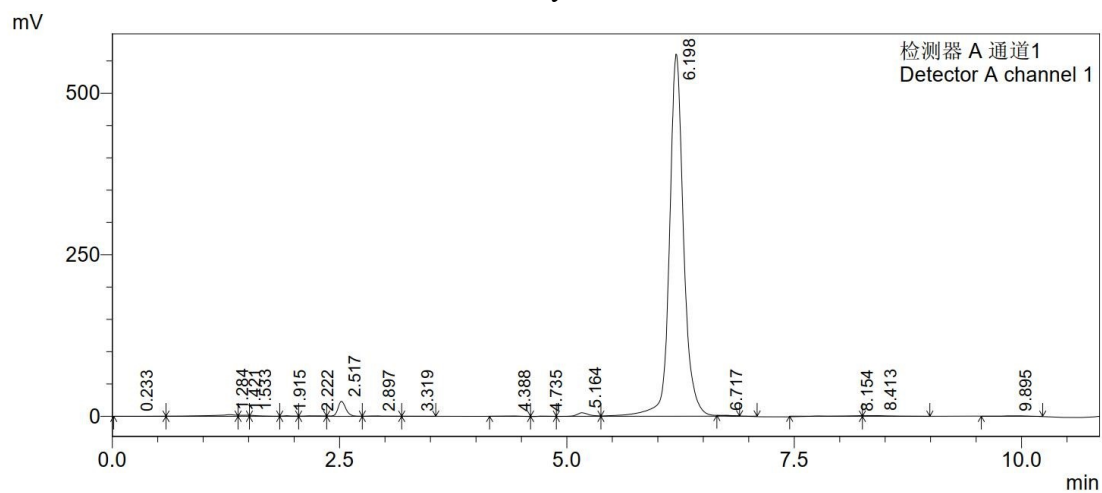
¹H NMR spectrum of A13



¹³C NMR spectrum of A13

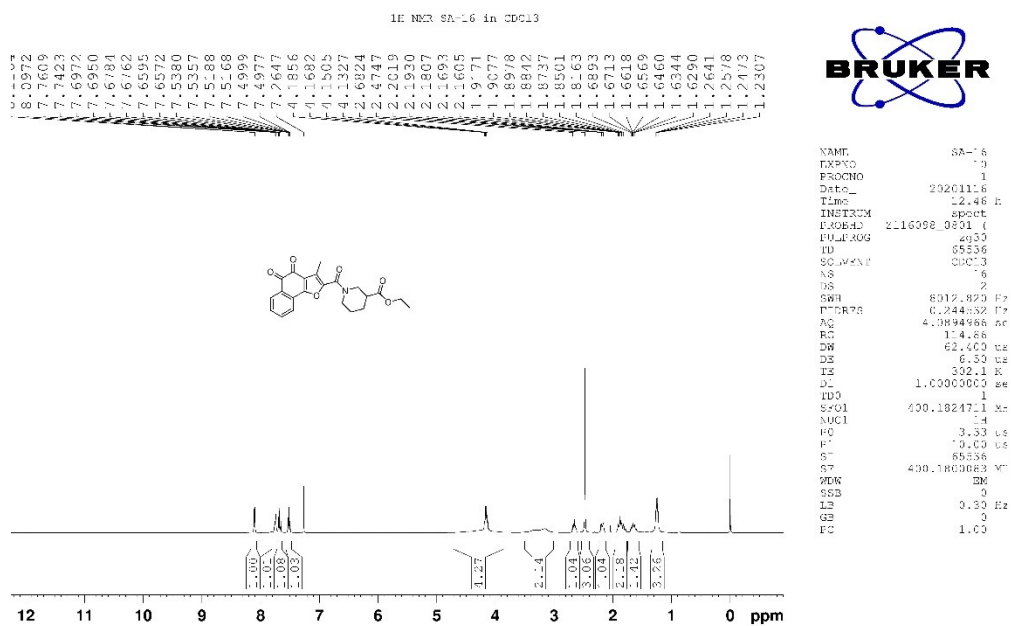


HRMS analysis of A13

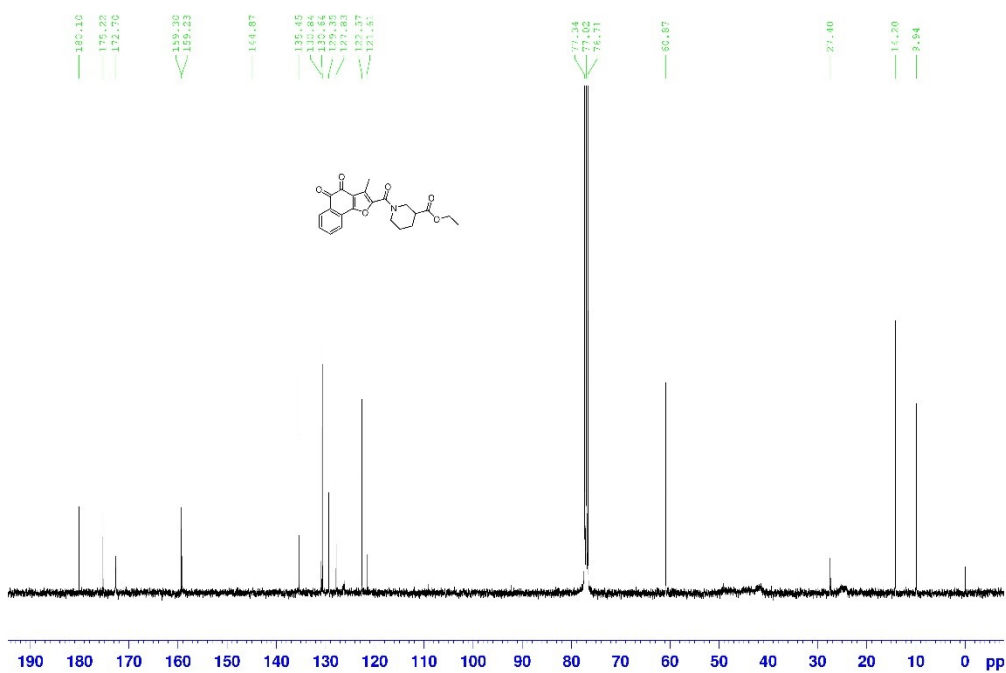


peak	time (min)	Area	height	Area %	height %
1	0.169	1112	77	0.212	0.159
2	1.312	2223	274	0.425	0.562
3	2.517	11529	1856	2.202	3.806
4	6.198	500506	45981	95.606	94.297
5	8.098	1231	113	0.235	0.232
6	8.415	5888	396	1.125	0.813
7	9.897	1019	64	0.195	0.132
total		523507	48762	100.000	100.000

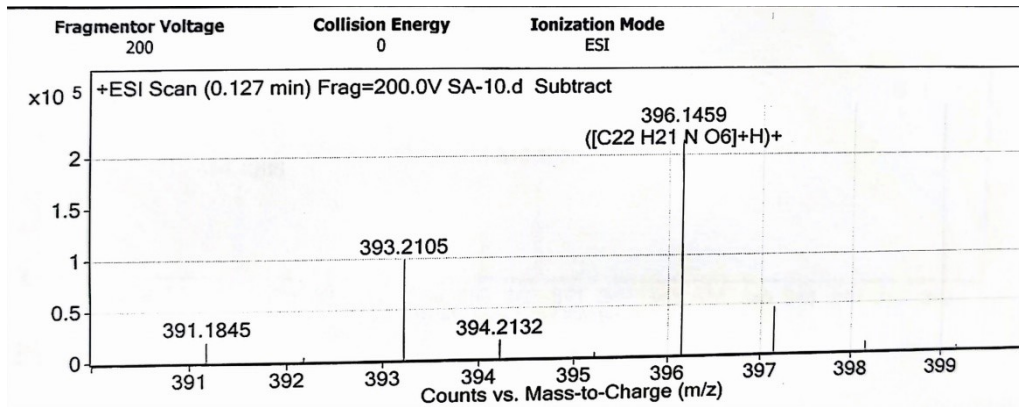
HPLC analysis of A13



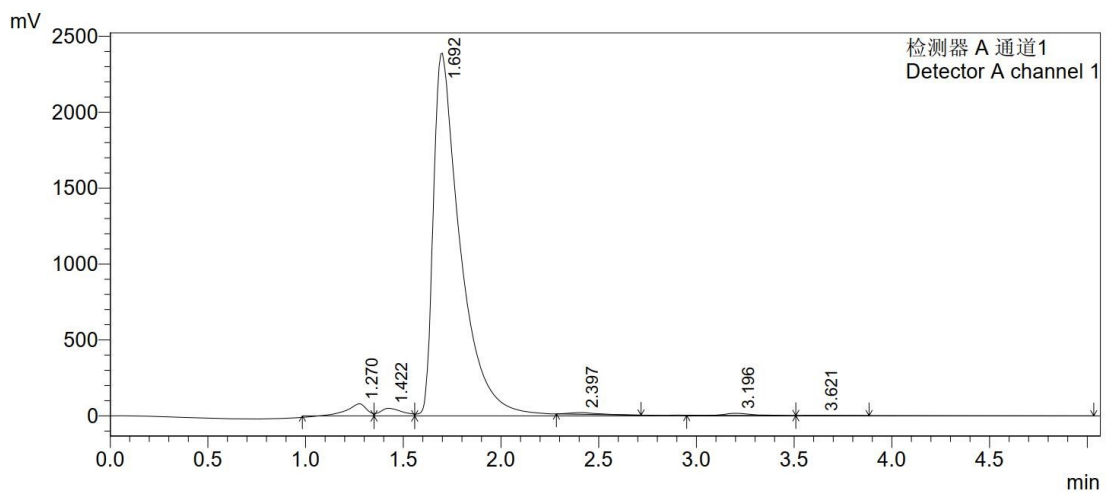
¹H NMR spectrum of A14

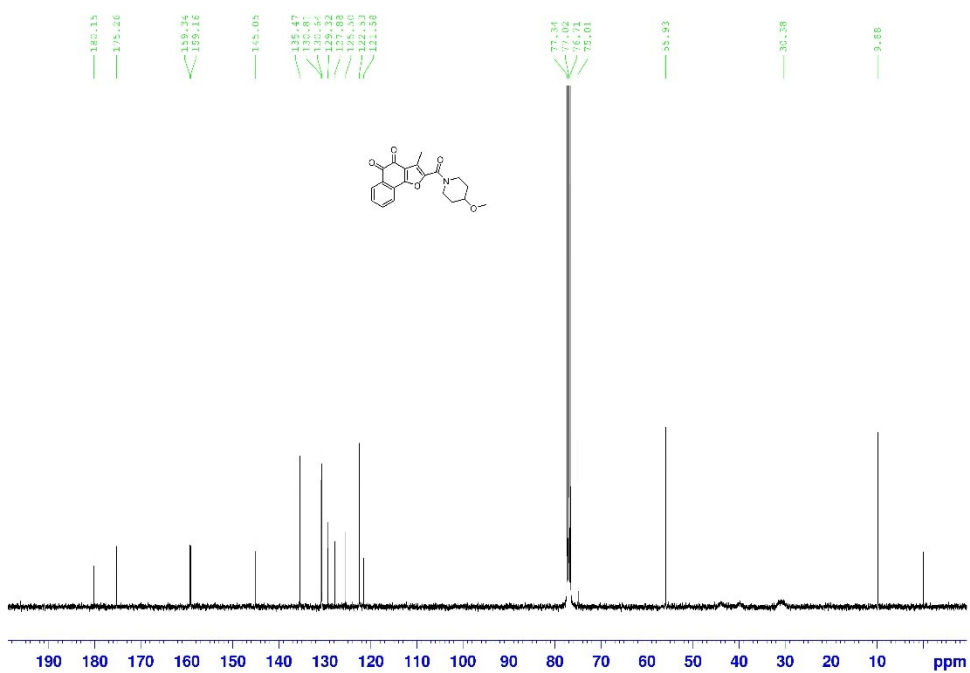


^{13}C NMR spectrum of A14

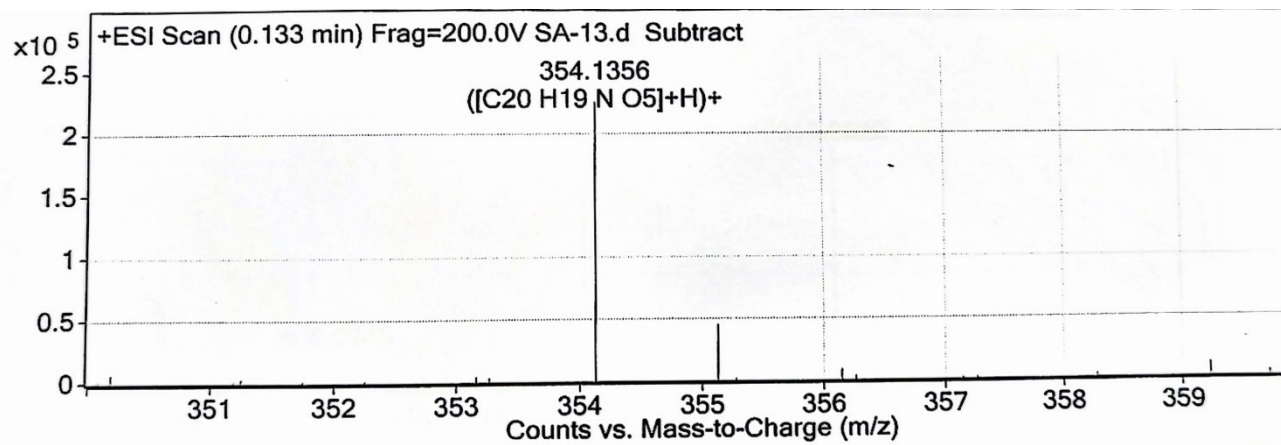


HRMS analysis of A14

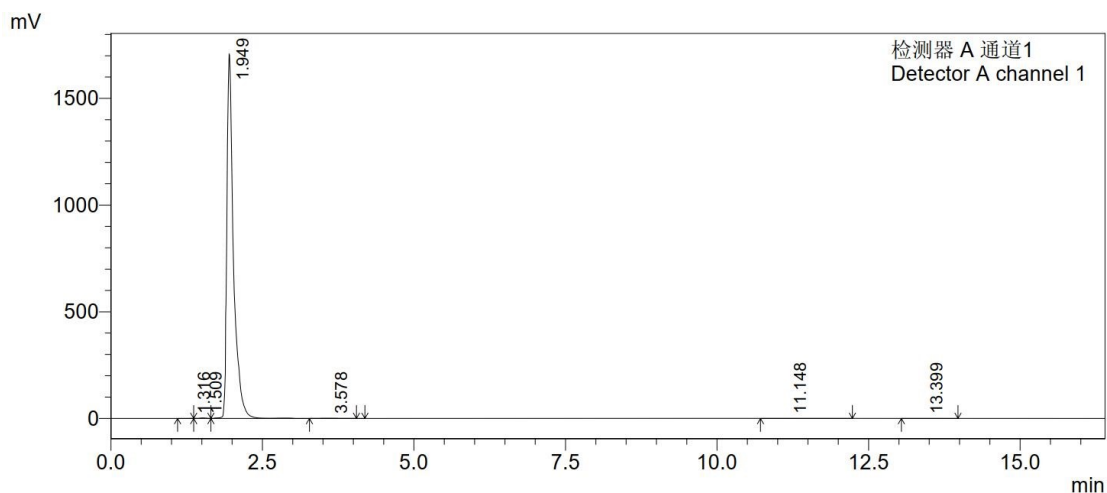




¹³C NMR spectrum of A15

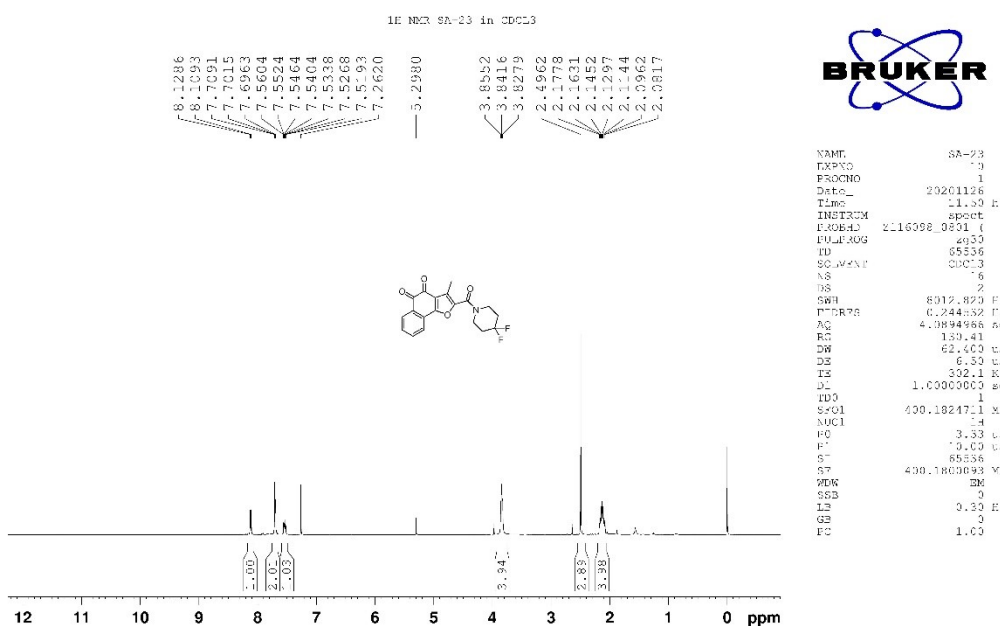


HRMS analysis of A15

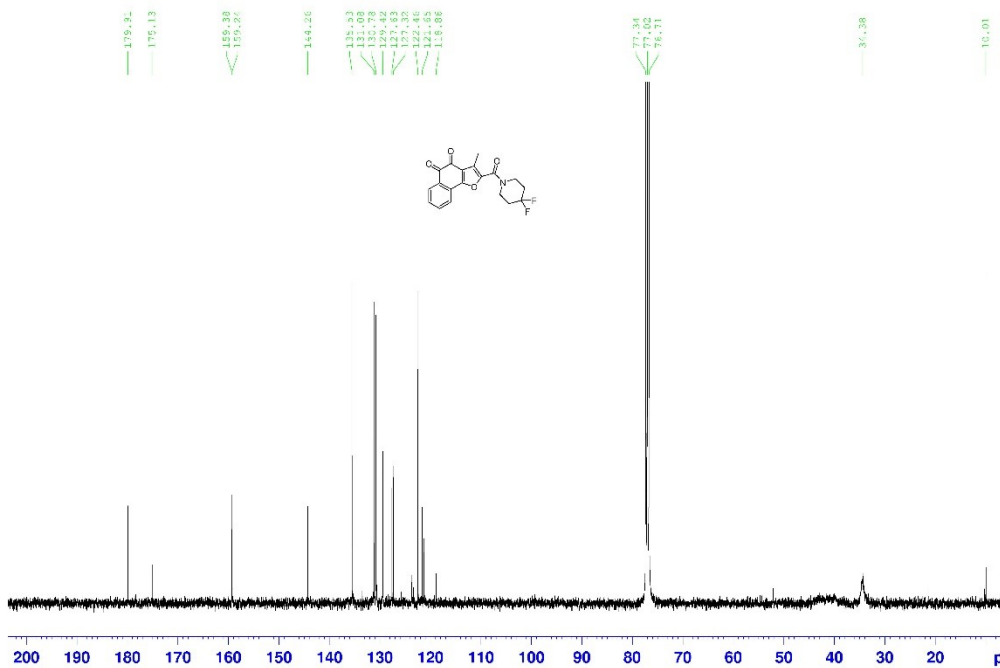


peak	time (min)	Area	height	Area %	height %
1	1.316	3232	458	0.025	0.027
2	1.509	21014	2639	0.161	0.154
3	1.949	13002170	1709035	99.484	99.705
4	3.578	8981	753	0.069	0.044
5	11.148	26573	899	0.203	0.052
6	13.399	7651	300	0.059	0.018
total		13069621	1714084	100.000	100.000

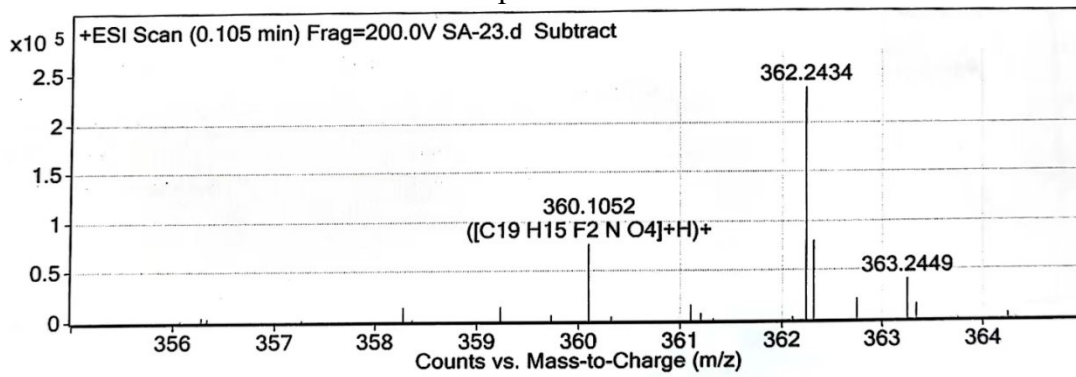
HPLC analysis of A15



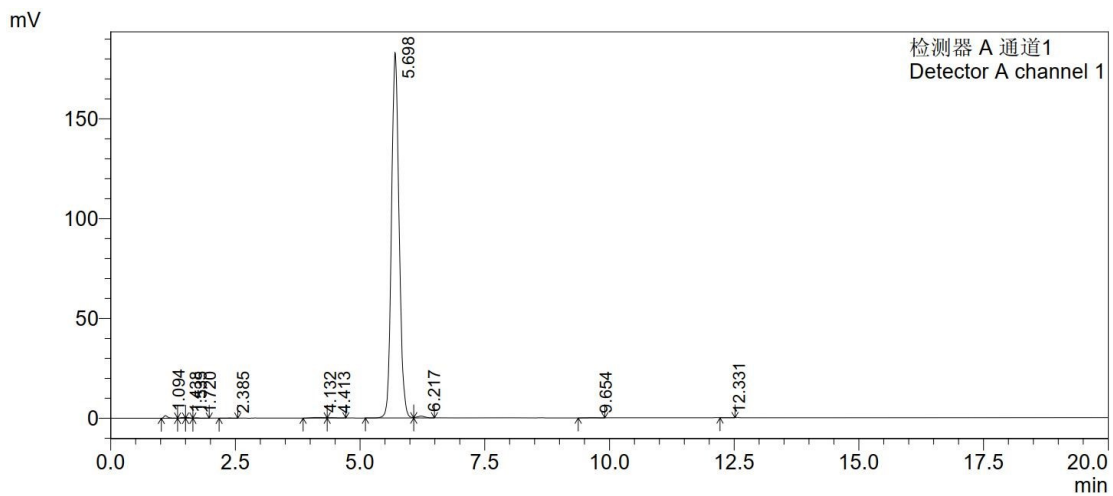
¹H NMR spectrum of A16



¹³C NMR spectrum of A16

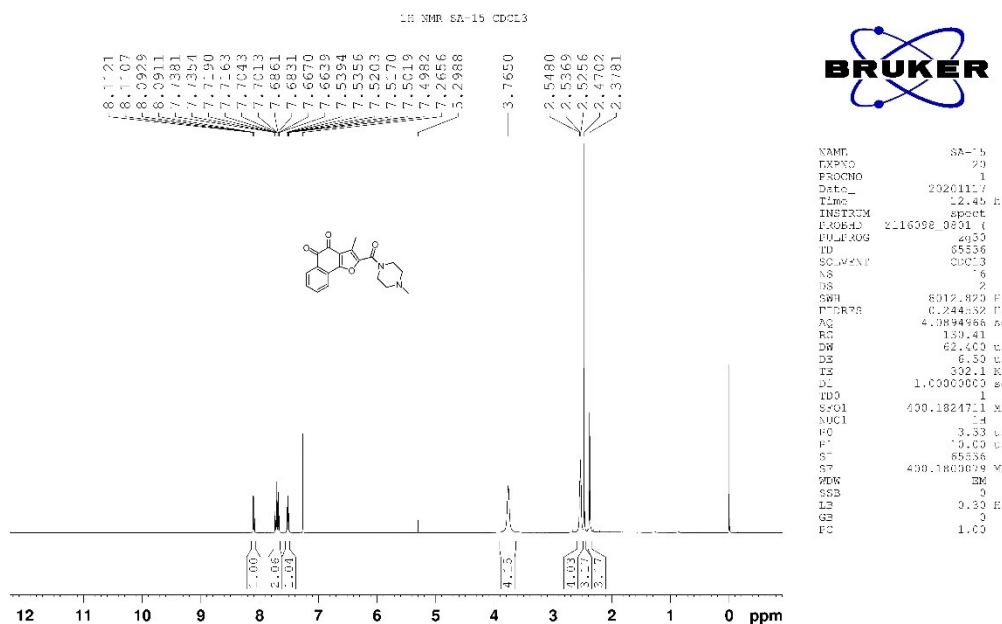


HRMS analysis of A16

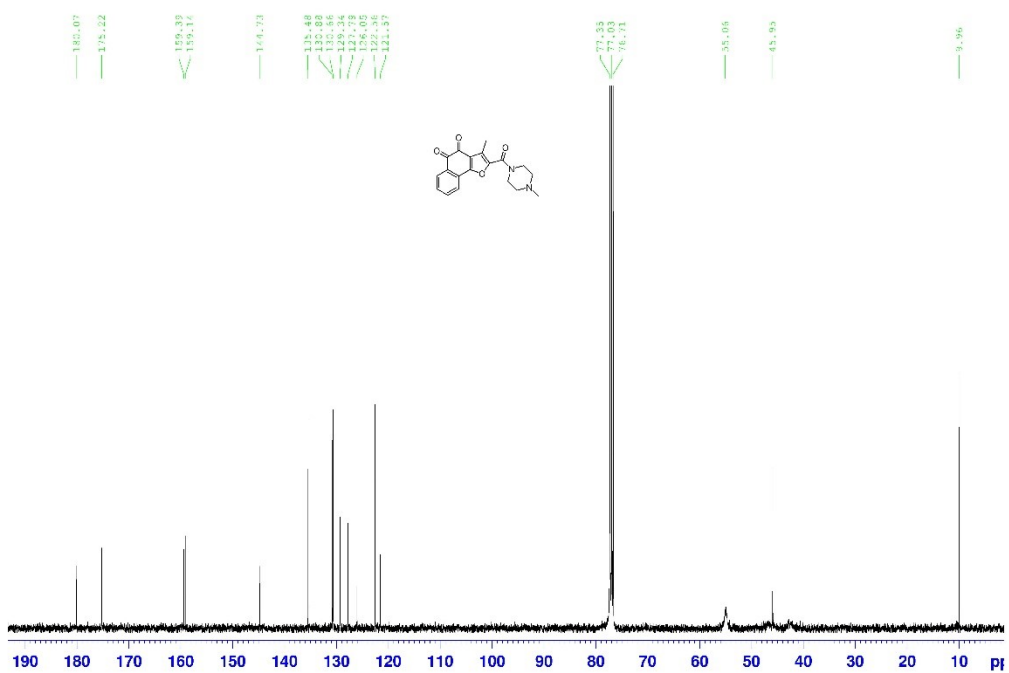


peak	time (min)	Area	height	Area %	height %
1	1.094	7104	1294	0.359	0.691
2	1.438	2363	360	0.119	0.192
3	1.535	2031	336	0.103	0.180
4	1.720	1907	197	0.096	0.105
5	2.385	1283	94	0.065	0.050
6	4.132	5467	304	0.276	0.162
7	4.413	2244	208	0.113	0.111
8	5.698	1942698	183252	98.203	97.901
9	6.217	9761	914	0.493	0.488
10	9.654	1929	128	0.098	0.068
11	12.331	1459	94	0.074	0.050
total		1978245	187181	100.000	100.000

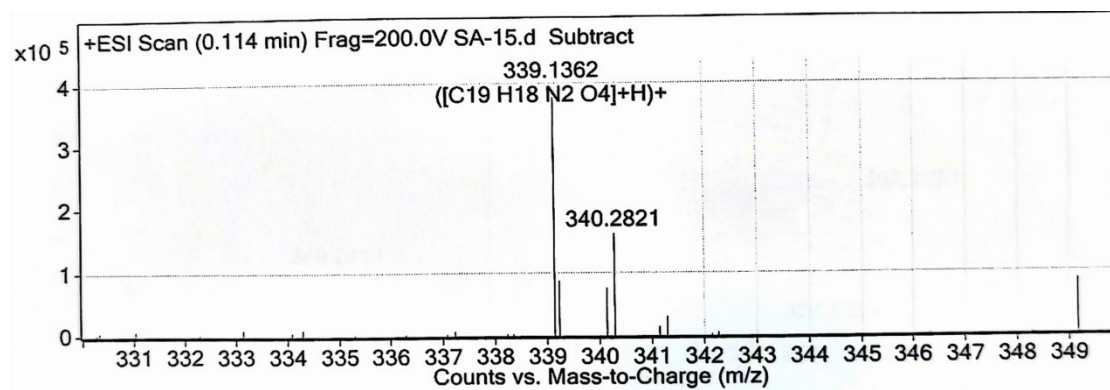
HPLC analysis of A16



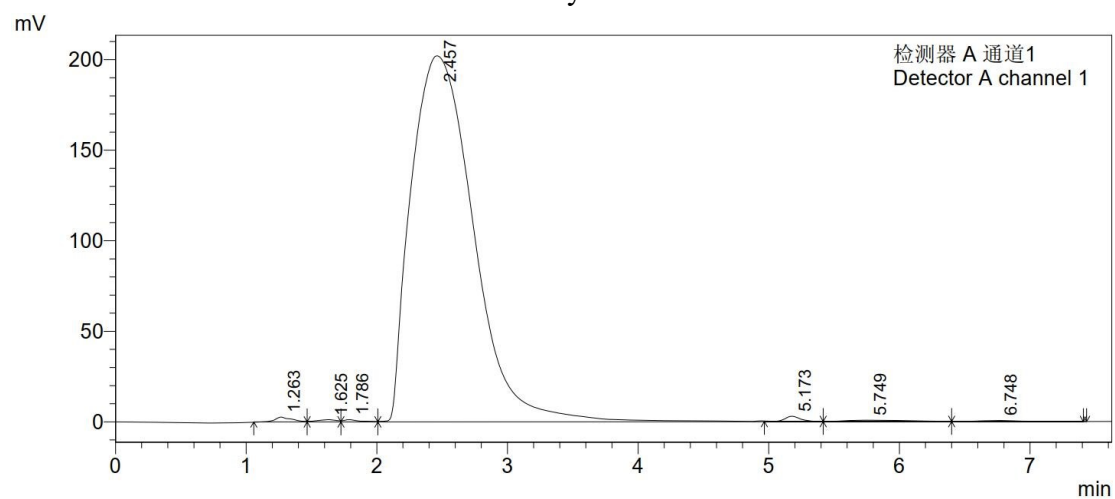
¹H NMR spectrum of A17



^{13}C NMR spectrum of A17

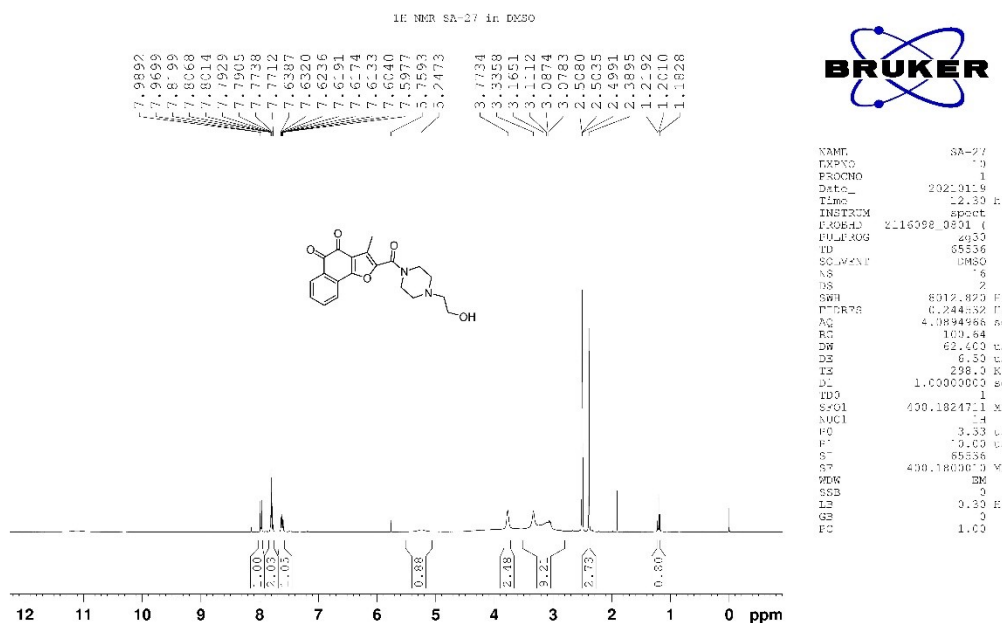


HRMS analysis of A17

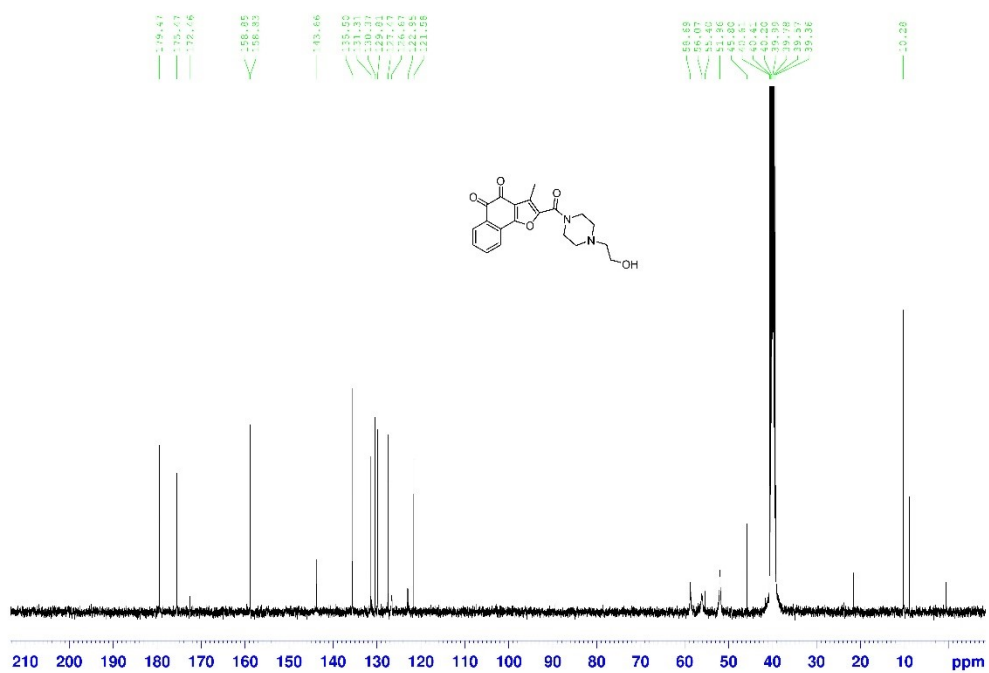


peak	time (min)	Area	height	Area %	height %
1	1.173	51582	6101	0.159	0.183
2	1.333	86664	4087	0.267	0.122
3	2.055	4240	456	0.013	0.014
4	2.336	5057	380	0.016	0.011
5	2.959	89662	2151	0.276	0.064
6	3.960	2763	281	0.009	0.008
7	4.959	31990687	3307616	98.428	99.120
8	6.274	20930	1314	0.064	0.039
9	6.566	20491	1166	0.063	0.035
10	7.176	220865	12957	0.680	0.388
11	8.302	3775	189	0.012	0.006
12	9.019	4833	295	0.015	0.009
total		32501549	3336994	100.000	100.000

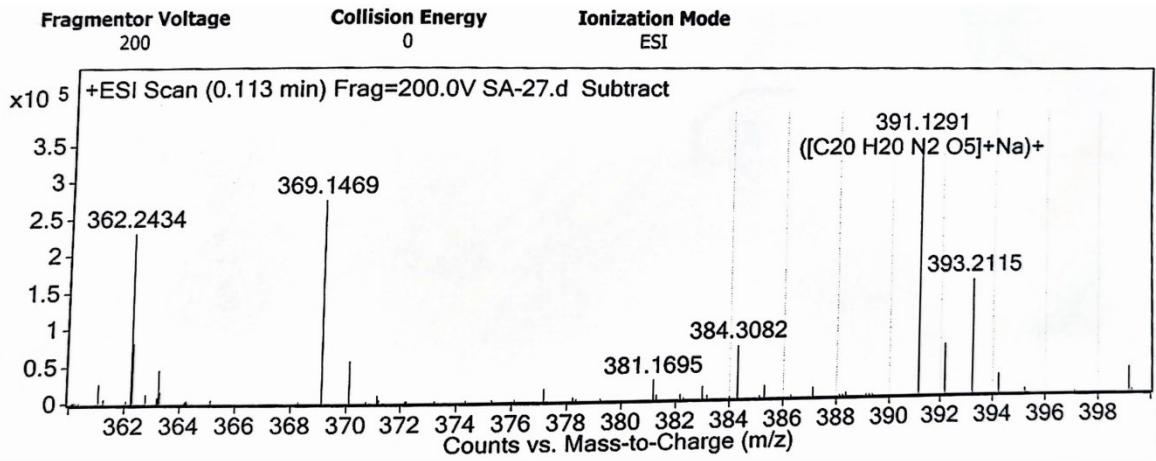
HPLC analysis of A17



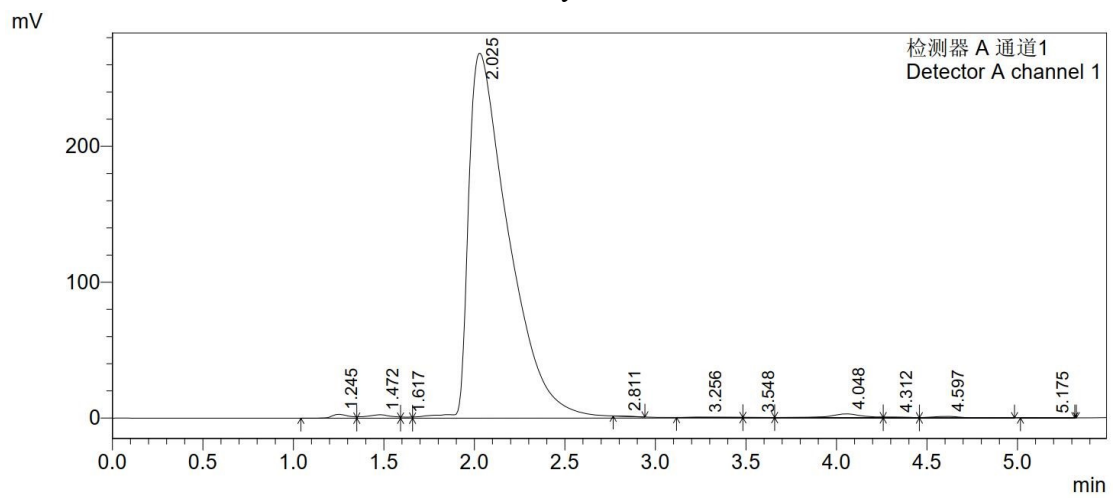
¹H NMR spectrum of A18



¹³C NMR spectrum of A18

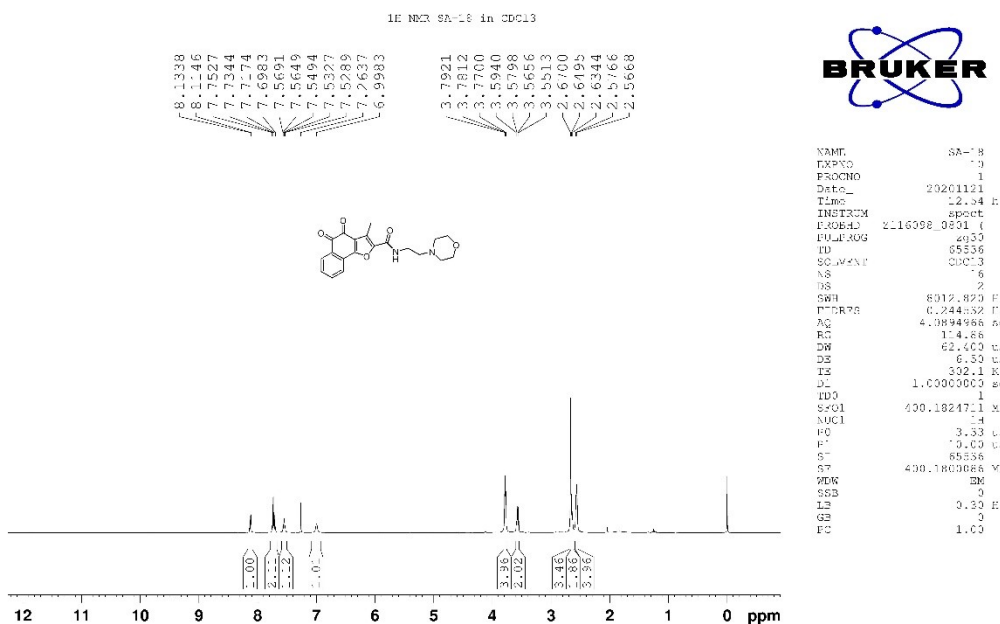


HRMS analysis of A18

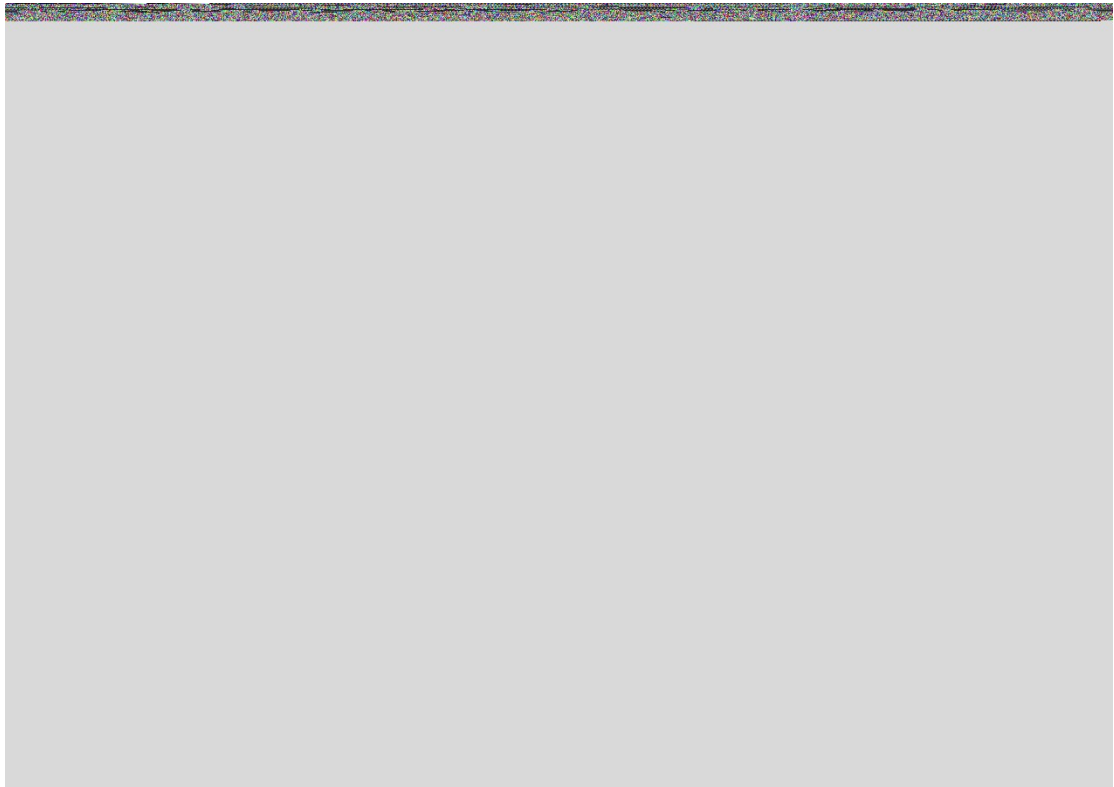


peak	time (min)	Area	height	Area %	height %
1	1.245	18581	2827	0.435	1.008
2	1.472	23588	2566	0.552	0.915
3	1.617	3715	945	0.087	0.337
4	2.025	4161844	268490	97.455	95.723
5	2.811	1625	321	0.038	0.114
6	3.256	5151	444	0.121	0.158
7	3.548	1772	195	0.041	0.070
8	4.048	35509	2835	0.831	1.011
9	4.312	5712	581	0.134	0.207
10	4.597	11738	1112	0.275	0.396
11	5.175	1313	172	0.031	0.061
total		4270547	280488	100.000	100.000

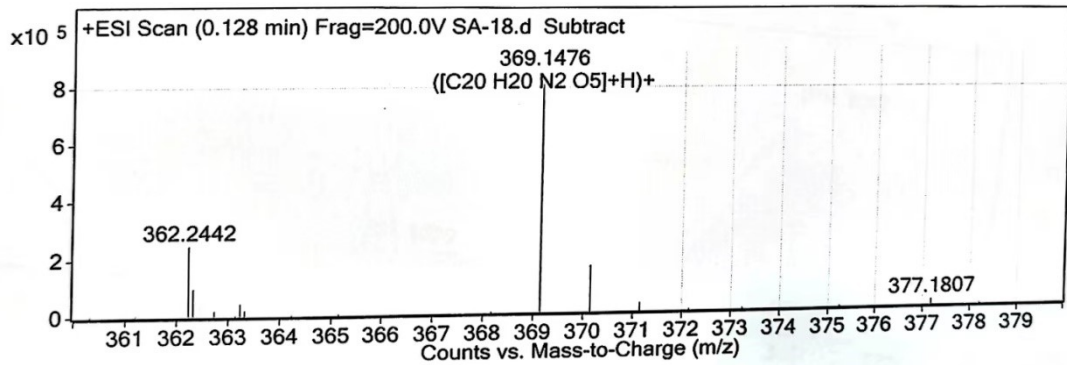
HPLC analysis of A18



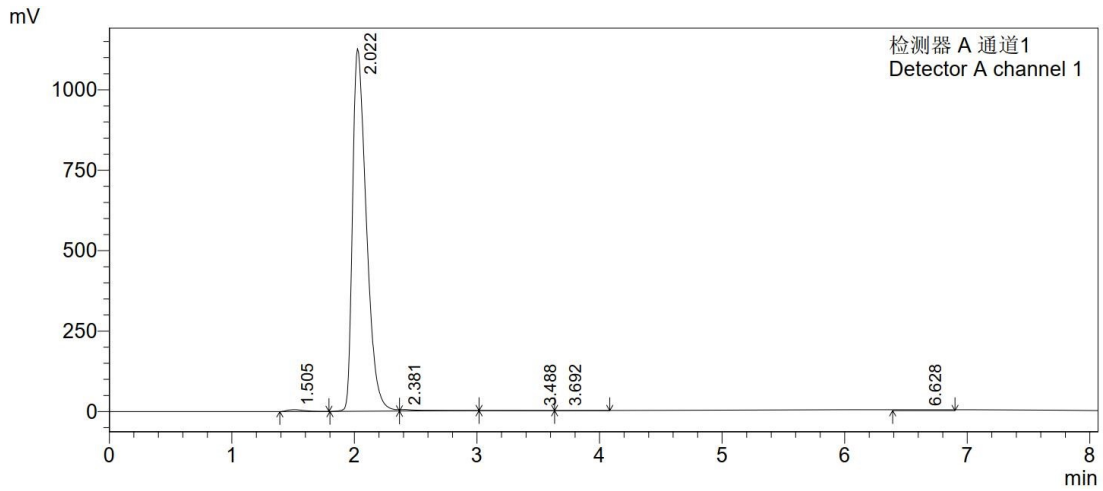
¹H NMR spectrum of A19



^{13}C NMR spectrum of A19

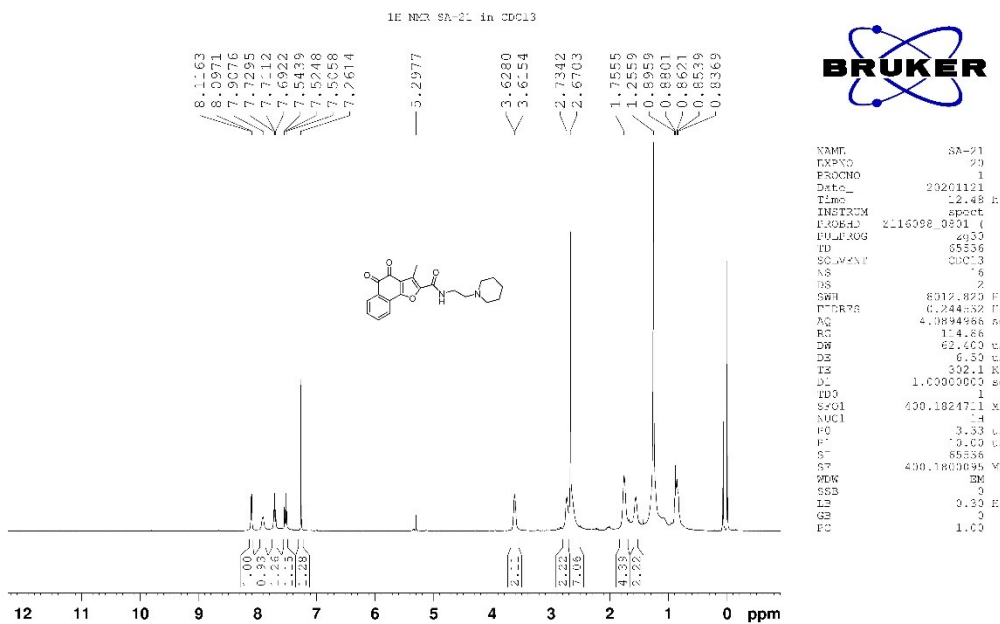


HRMS analysis of A19

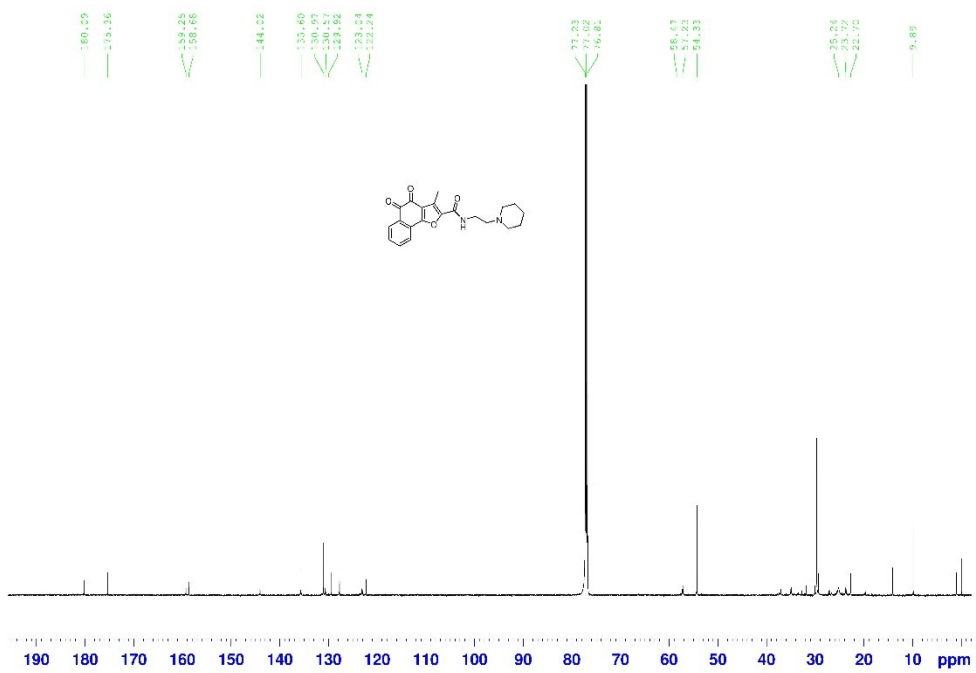


peak	time (min)	Area	height	Area %	height %
1	1.505	53265	5533	0.596	0.486
2	2.022	8809859	1127821	98.646	99.043
3	2.381	56503	4851	0.633	0.426
4	3.488	5950	236	0.067	0.021
5	3.692	4134	210	0.046	0.018
6	6.628	1059	67	0.012	0.006
total		8930769	1138720	100.000	100.000

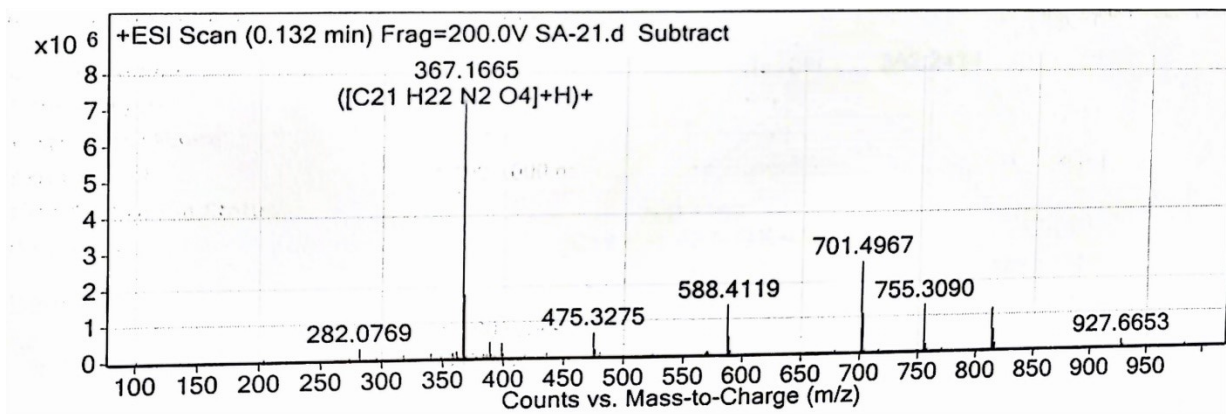
HPLC analysis of A19



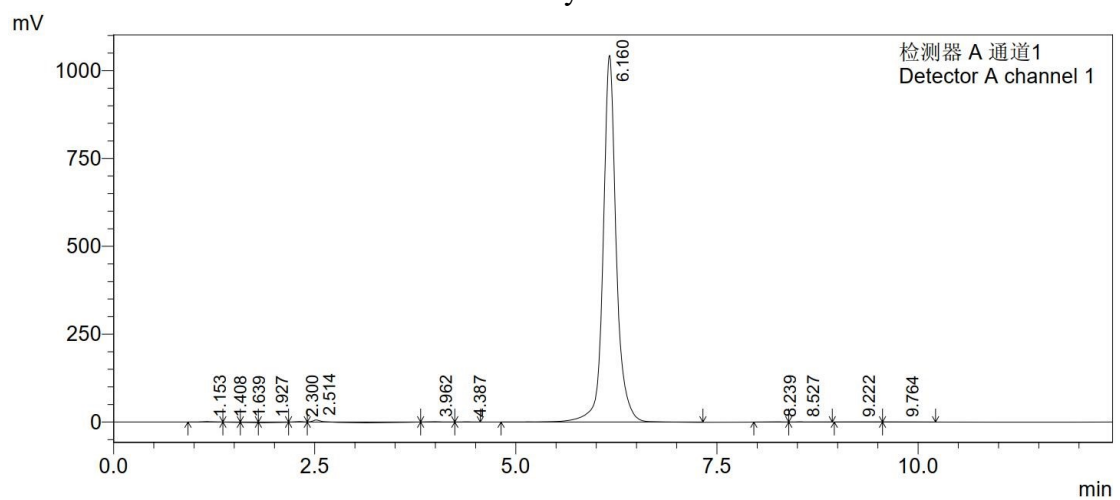
¹H NMR spectrum of A20



¹³C NMR spectrum of A20

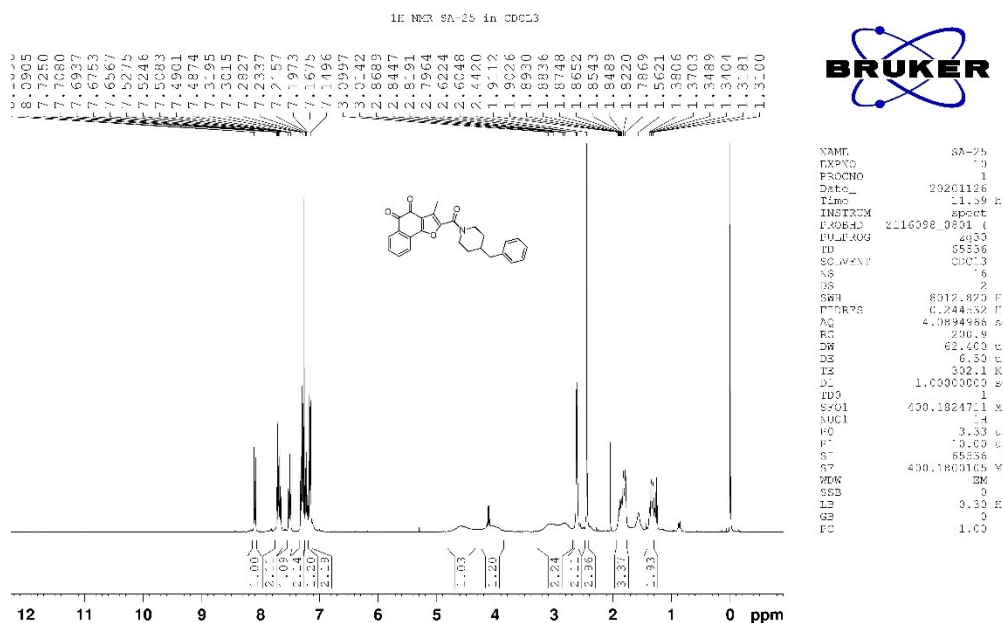


HRMS analysis of A20

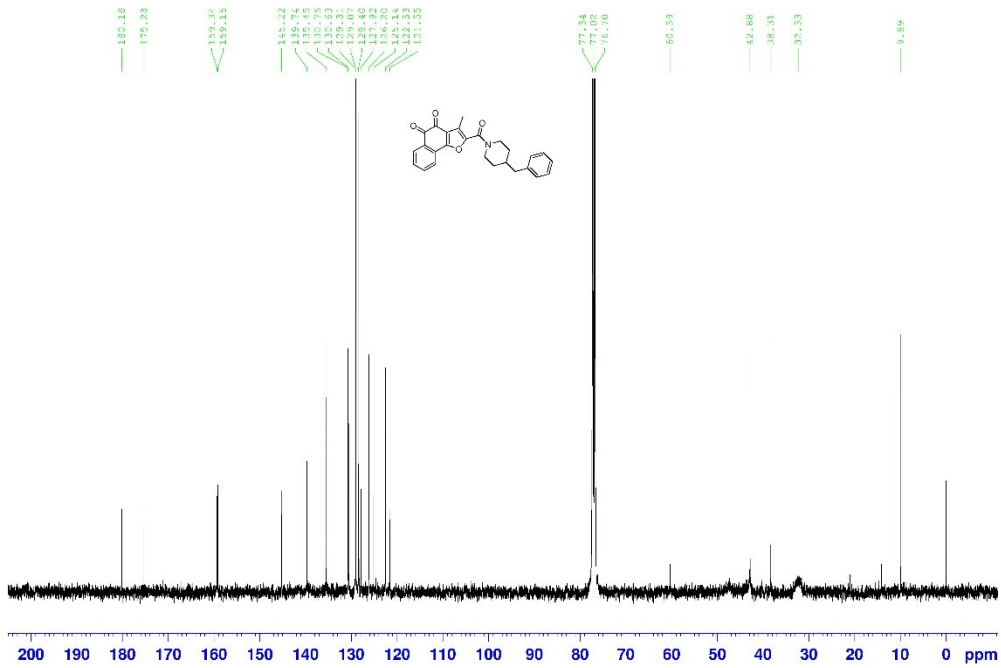


peak	time (min)	Area	height	Area %	height %
1	1.153	11107	1111	0.093	0.105
2	1.408	2100	191	0.018	0.018
3	1.639	1977	201	0.017	0.019
4	1.927	3052	220	0.026	0.021
5	2.300	7953	1191	0.067	0.113
6	2.514	42410	5790	0.357	0.549
7	3.962	7895	890	0.066	0.084
8	4.387	2664	295	0.022	0.028
9	6.160	11787924	1043499	99.203	98.968
10	8.239	3231	249	0.027	0.024
11	8.527	3594	234	0.030	0.022
12	9.222	5677	369	0.048	0.035
13	9.764	2995	137	0.025	0.013
total		11882579	1054377	100.000	100.000

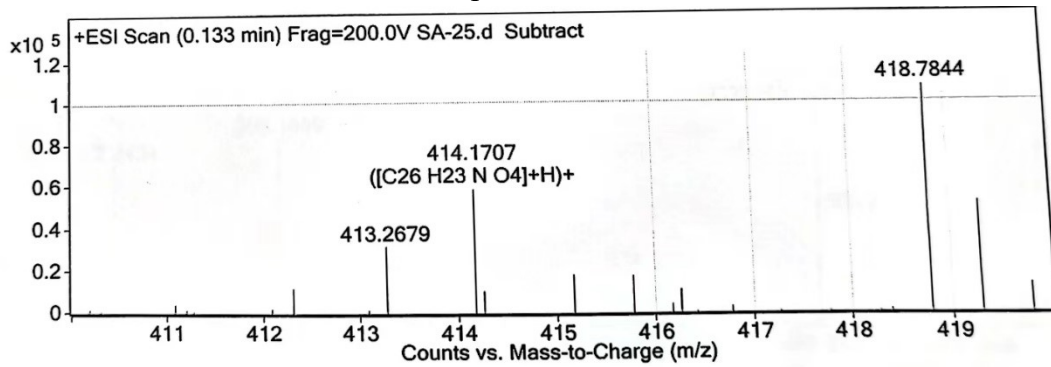
HPLC analysis of A20



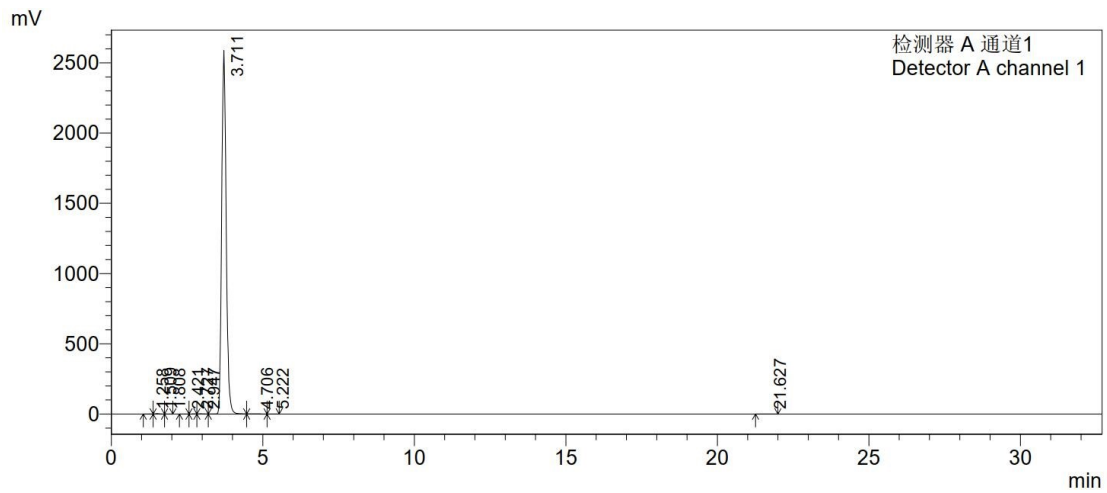
¹H NMR spectrum of A21



¹³C NMR spectrum of A21



HRMS analysis of A21



peak	time (min)	Area	height	Area %	height %
1	1.258	4681	525	0.017	0.020
2	1.509	25721	3427	0.095	0.132
3	1.808	1582	145	0.006	0.006
4	2.421	1214	98	0.004	0.004
5	2.727	8561	984	0.032	0.038
6	2.947	19992	1890	0.074	0.073
7	3.711	26900826	2588822	99.616	99.659
8	4.706	32911	1251	0.122	0.048
9	5.222	3620	217	0.013	0.008
10	21.627	5365	321	0.020	0.012
total		27004473	2597679	100.000	100.000

HPLC analysis of A21