Supplementary material

Design, Synthesis, and Biological Evaluation of Quinoline Derivatives as Dual-target Inhibitors of Chitin Synthase and Glucosamine-6-Phosphate Synthase

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1. Antibacterial activity

1.1 Results and discussion

The compounds **8a-i, 9,** and **14a-o** were evaluated for their *in vitro* antibacterial activities against three Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*) and three Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *methicillin-resistant Staphylococcus aureus*), with levofloxacin and chloramphenicol serving as control drugs in this study. The antibacterial efficacy of the compounds was assessed by determining their minimum inhibitory concentrations (MIC values). Blank controls and negative controls were included, with all experiments performed in triplicate. As shown in Table 1, the MIC values of all tested compounds were ≥ 256 μg/mL, which were significantly higher than those of the control drugs. These results indicate that compounds **8a-i, 9,** and **14a-o** exhibited extremely poor antibacterial activity and demonstrated no apparent inhibitory effects against the tested bacterial strains.

Table 1. The MIC value (μg/mL) of compounds **8a-i, 9,** and **14a-o** against antibacterial *in vitro*.

Compounds	Gram-Negative bacteria			Gram-positive organisms		
	Escherichia coli	*Pseudomonas aeruginosa*	*Bacillus proteus*	*Staphylococcus aureus*	*Bacillus subtilis*	*methicillin-resistant Staphylococcus aureus*
	(JM 109)	(ATCC 9027)	(ATCC 8427)	（ATCC 25923）	(ATCC 6633)	(N 315)
8a	256	＞512	512	512	512	512
8b	256	256	256	256	512	512
8c	256	＞512	256	256	256	＞512
8d	512	＞512	256	256	512	512
8e	256	＞512	512	256	512	512
8f	＞512	＞512	512	512	512	256
8g	512	256	256	256	256	256
8h	256	256	512	512	＞512	512
8i	512	＞512	512	256	256	256

9	512	＞512	256	256	512	256
14a	256	256	512	＞512	256	＞512
14b	256	256	512	256	＞512	512
14c	256	＞512	＞512	256	512	512
14d	＞512	＞512	512	512	512	256
14e	256	＞512	256	256	256	＞512
14f	512	256	256	256	512	512
14g	512	＞512	256	256	256	256
14h	256	256	＞512	512	＞512	512
14i	512	＞512	＞512	512	256	256
14j	256	＞512	256	256	512	256
14k	256	512	256	512	＞512	＞512
14l	512	256	＞512	256	256	256
14m	256	＞512	＞512	＞512	256	512
14n	256	256	256	512	512	512
14o	＞512	256	512	256	＞512	＞512
Chloramphenicol	8	4	8	4	2	4
Norfloxacin	2	4	2	4	2	2
Blank control	+ᵃ	+	+	+	+	+
Negetive control	+	+	+	+	+	+

ᵃ Colonies grew normally in the blank and negative controls (containing 0.5% DMSO solution) and the solution was turbid.

1.2 Antibacterial assay

In this experiment, three Gram-positive organisms including *Methicillin-resistant staphylococcus aureus* (N 315), *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), and three Gram-negative organisms including *Escherichia coli* (JM 109), *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus proteus* (ATCC 8427) was selected as the experimental strains. Chloramphenicol and Norfloxacin were used as positive

controls. The test compounds (10.24 mg) and control drugs (chloramphenicol and norfloxacin, 10.24 mg each) were accurately weighed and transferred to sterile 15 mL centrifuge tubes. A stock solution of 1024 µg/mL was prepared by adding dimethyl sulfoxide (DMSO, 1 mL) followed by sterile water (9 mL). Serial two-fold dilutions were performed in 96-well plates to obtain final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, and 1 µg/mL. The blank control group was prepared by adding 200 µL of sterile water containing 0.5% DMSO to column 11 of the 96-well plate, while the negative control group consisted of 100 µL sterile water containing 0.5% DMSO mixed with 100 µL bacterial suspension in column 12. All experimental groups, including three parallel replicates, were incubated at 37°C for 24 h in a biochemical incubator. Minimum inhibitory concentration (MIC) values were determined following the incubation period.

2. ^{1}H NMR and HRMS spectra of compound 6

^{1}H NMR spectrum of compound 6

Meas. m/z	#	Ion Formula	m/z	Adduct	err [ppm]	mSigma	# mSigma	Score	rdb	e¡¥	Conf	N-Rule
247.1075	1	C13H15N2O3	247.1077	M+H	1.0	18.0	1	100.00	8.0	even		ok
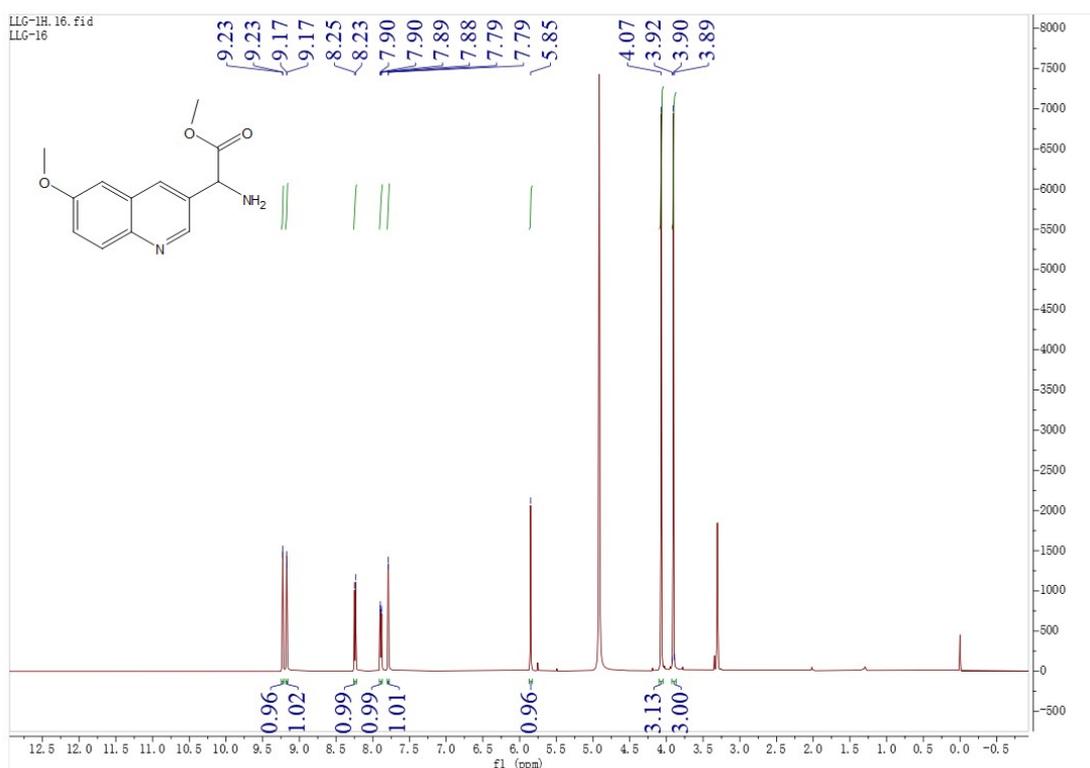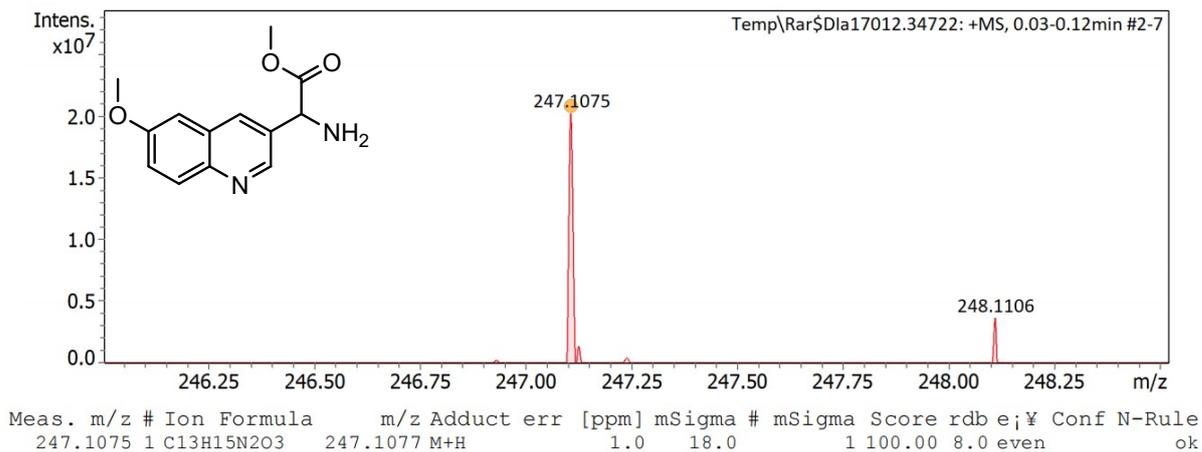
HRMS spectrum of compound **6**

3.¹H NMR, ¹³C NMR and HRMS spectra of the target compounds
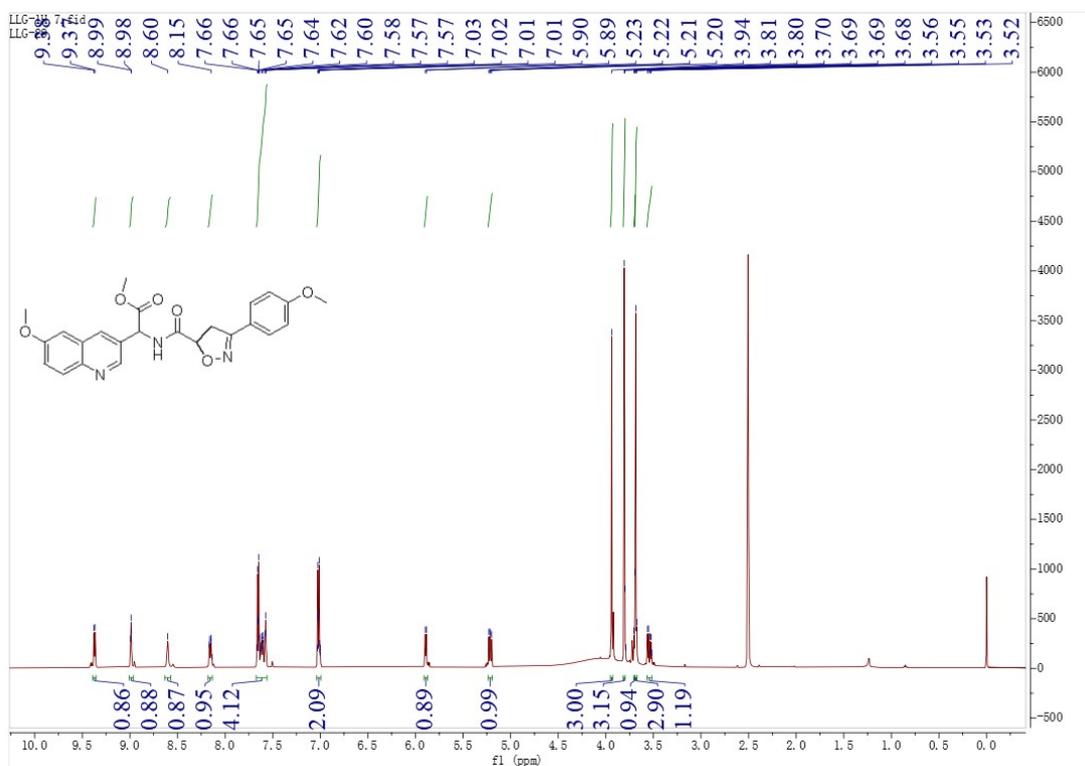
¹H NMR spectrum of compound **8a**

¹³C NMR spectrum of compound **8a**

Meas. m/z	#	Ion Formula	m/z	Adduct	err [ppm]	mSigma	# mSigma	Score	rdb	e¯	Conf	N-Rule
450.1659	1	C24H24N3O6	450.1660	M+H	0.2	12.8	1	100.00	15.0	even		ok
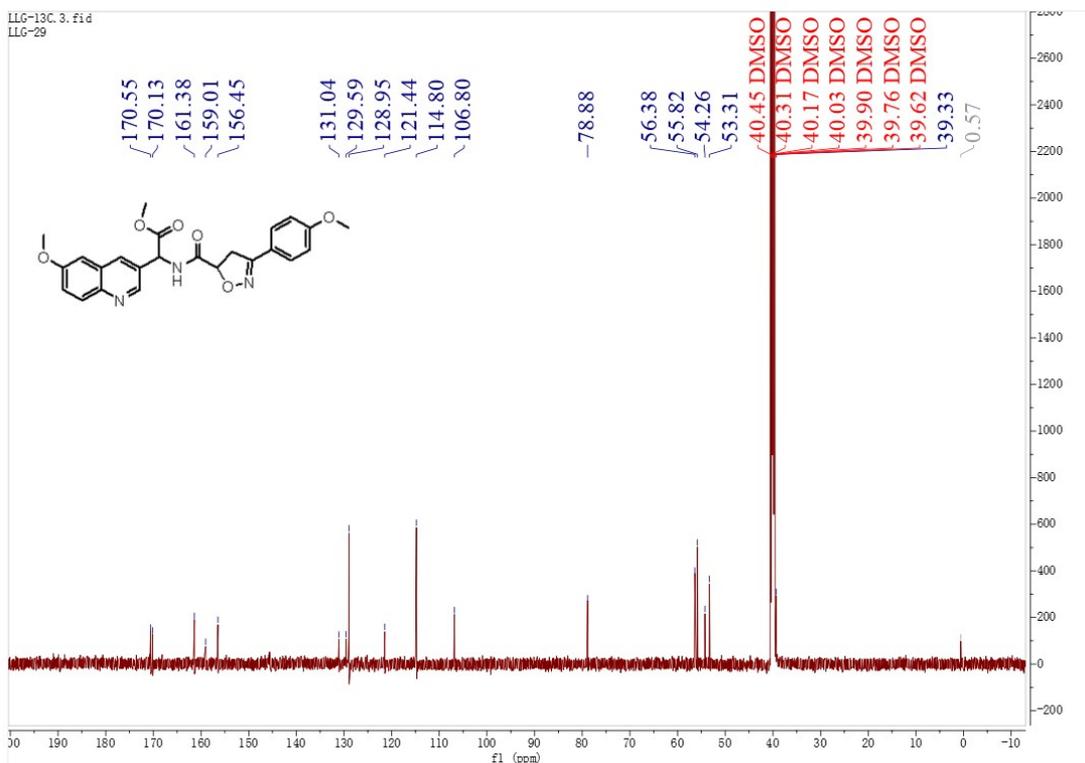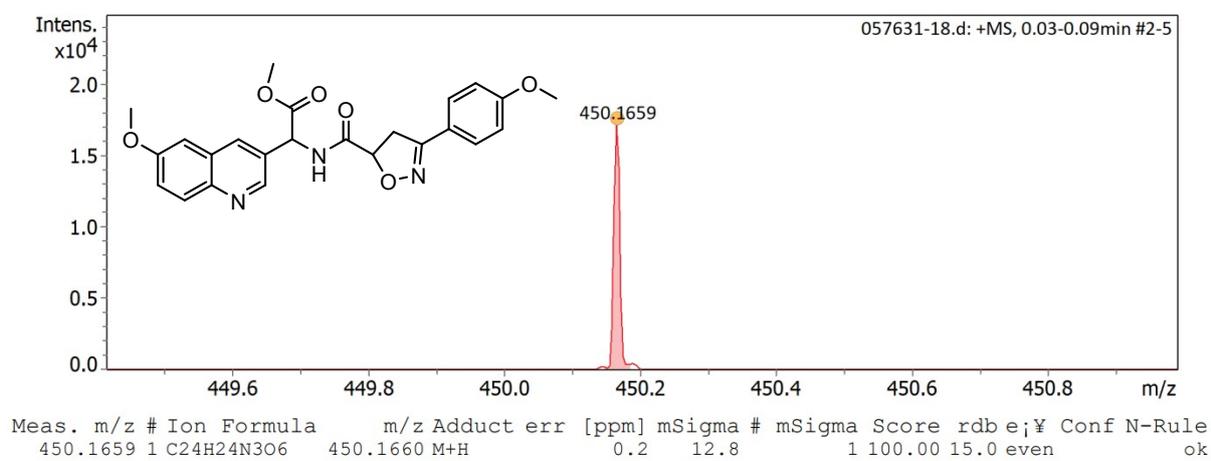
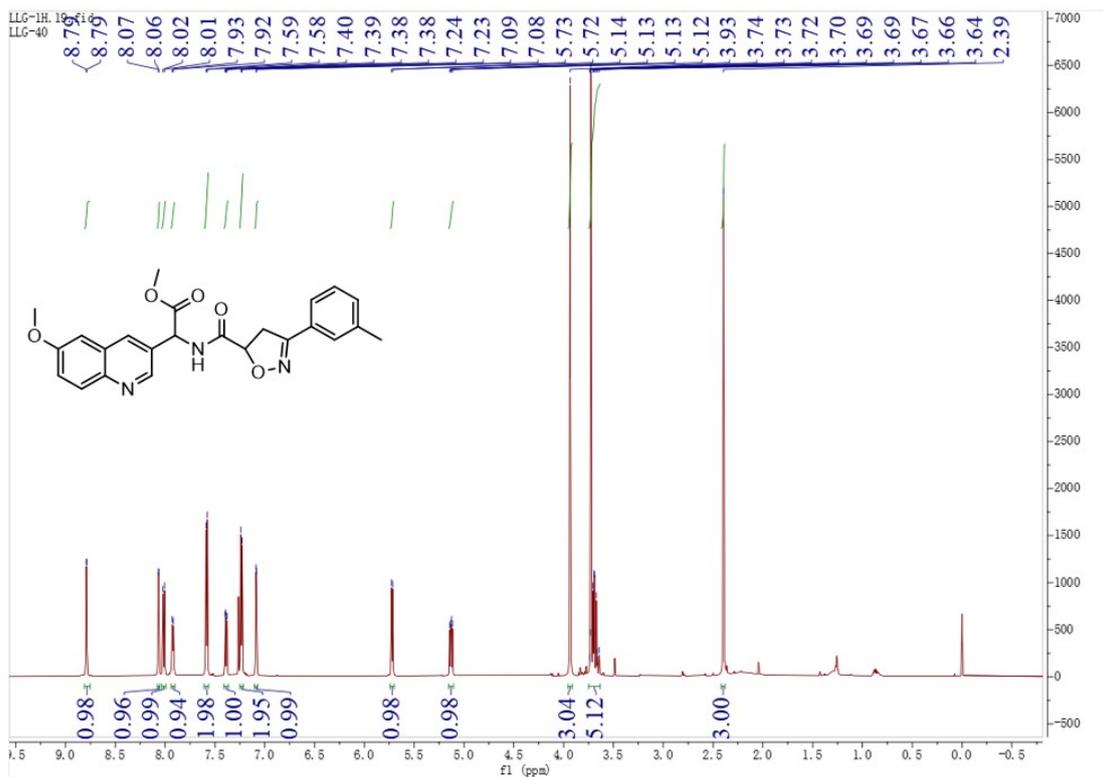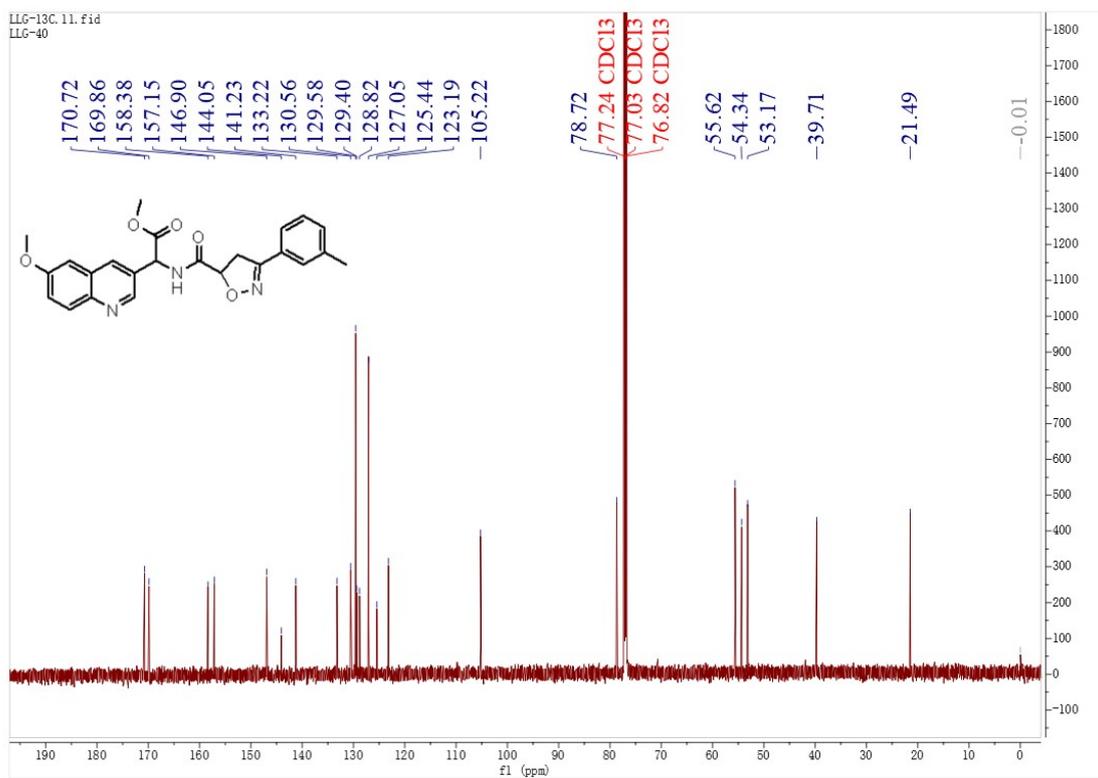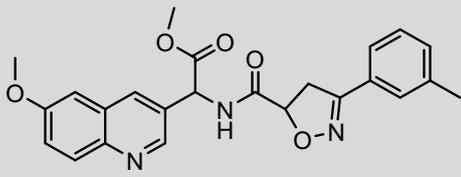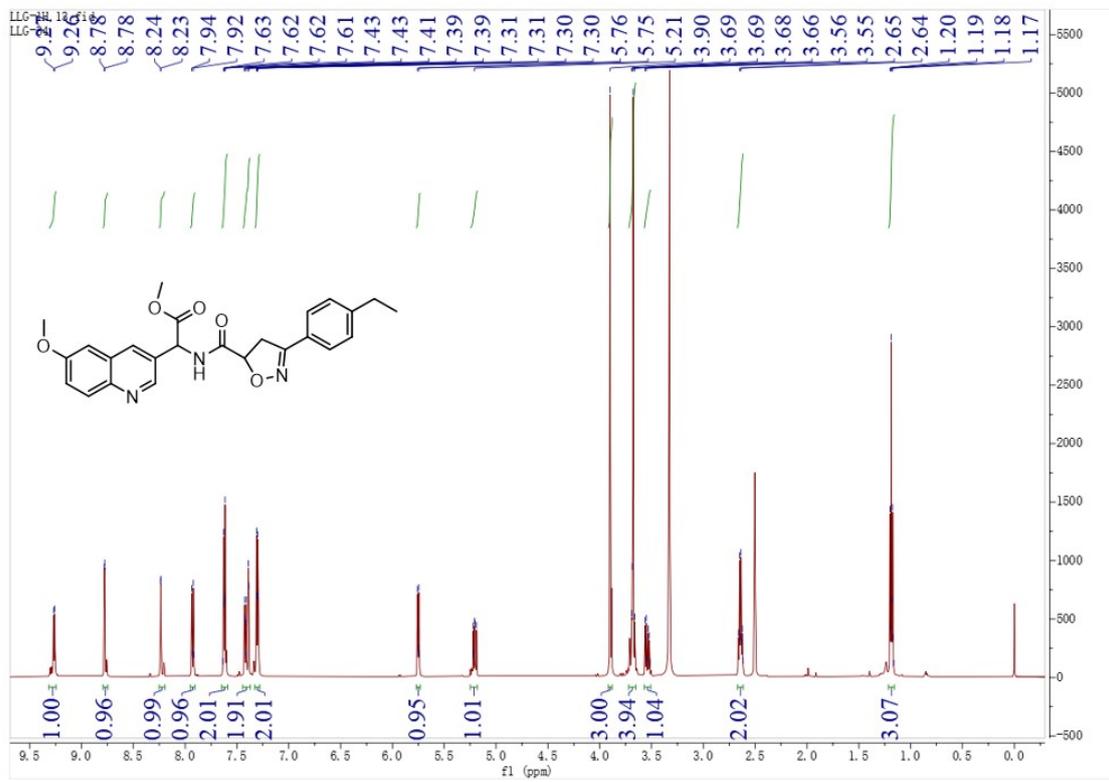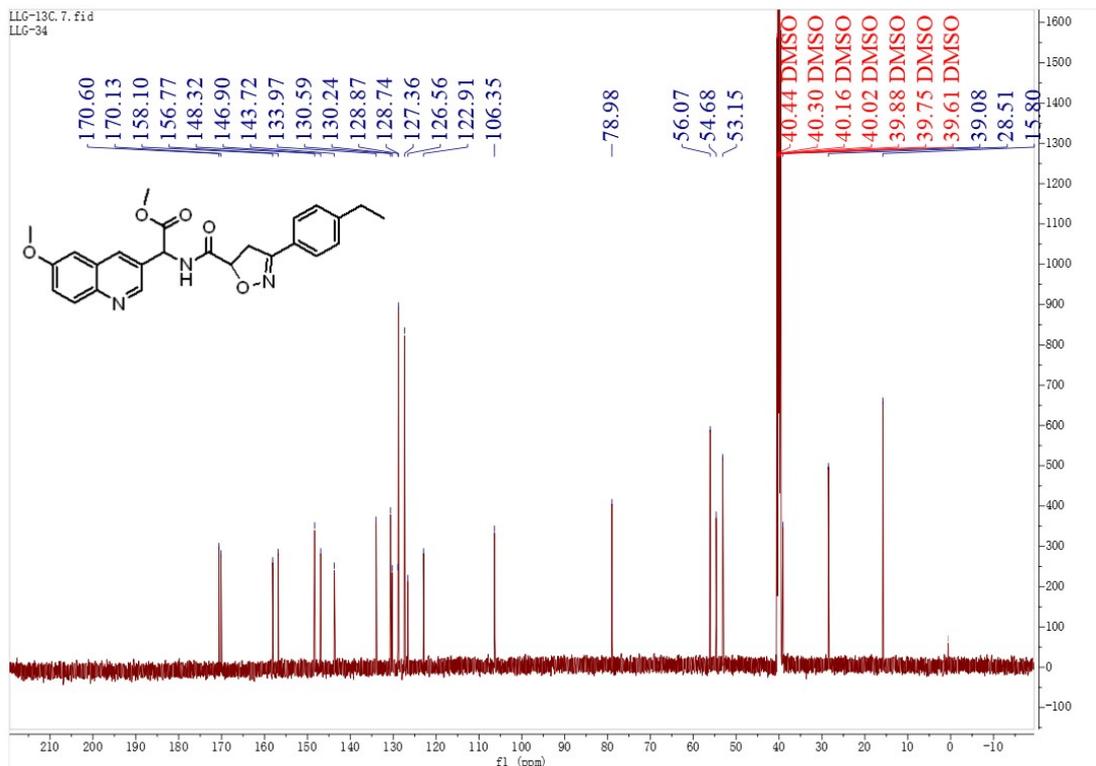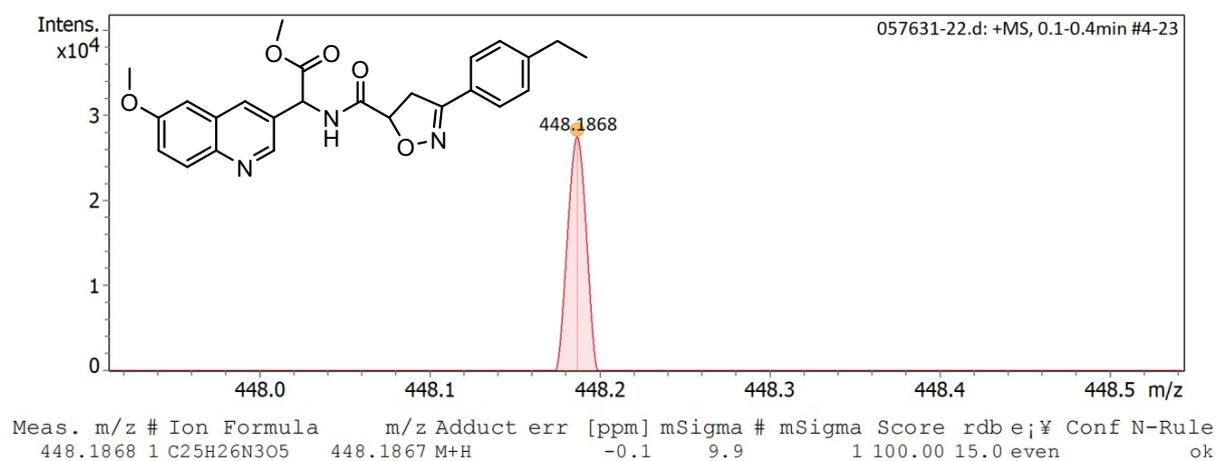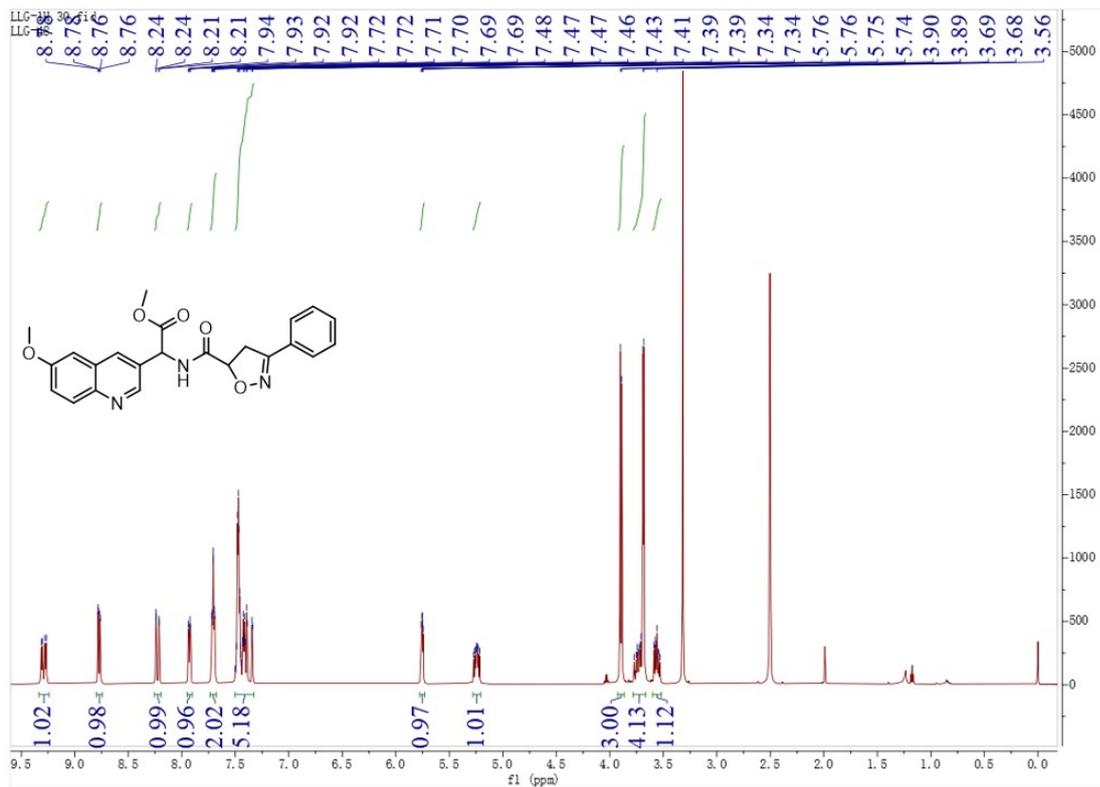HRMS spectrum of compound **8a**
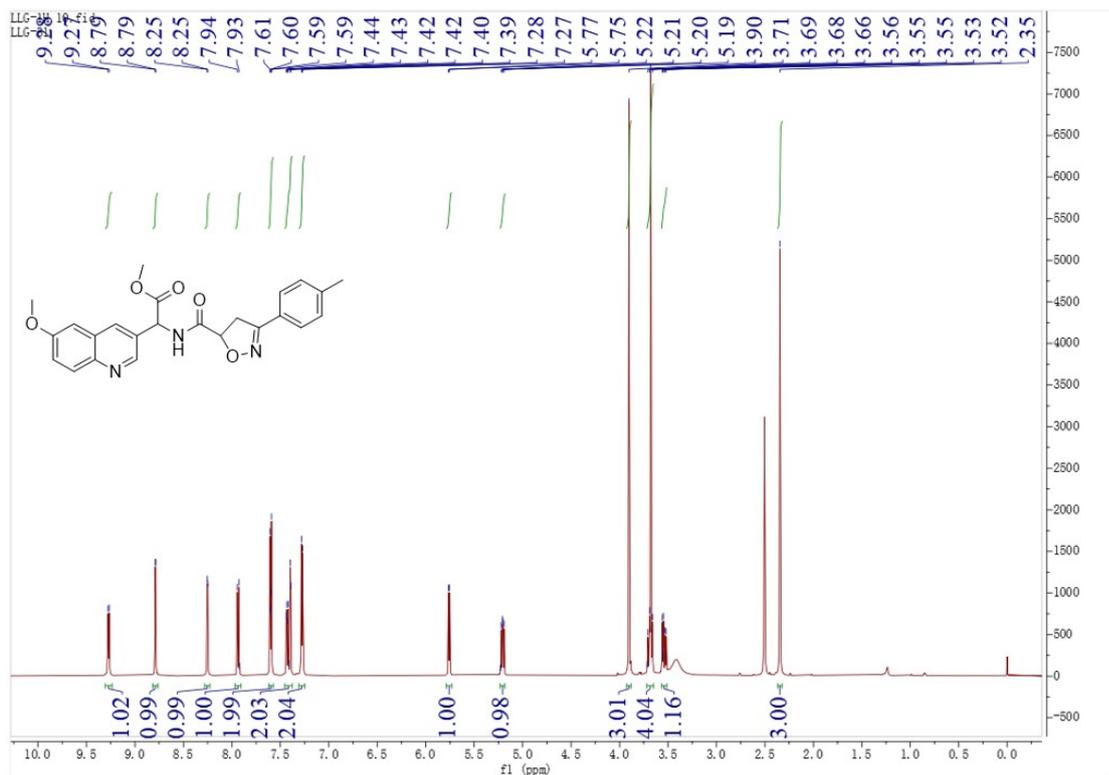
¹H NMR spectrum of compound **8b**
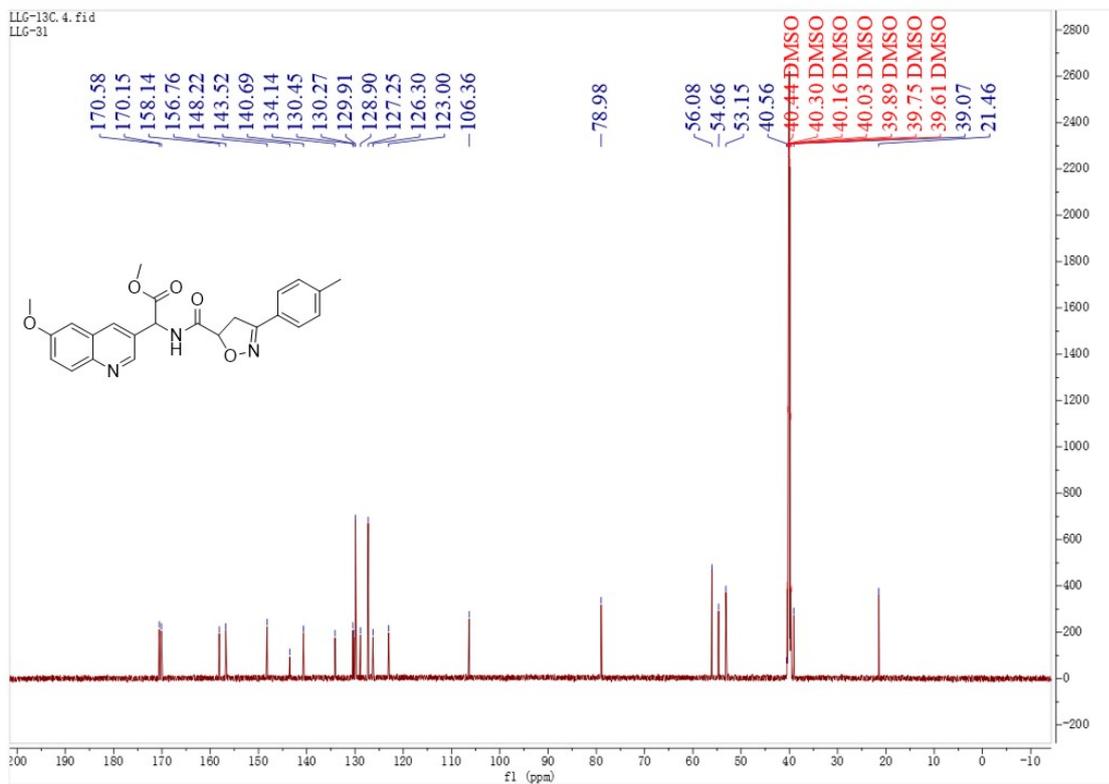
¹³C NMR spectrum of compound **8b**

HRMS spectrum of compound **8b**

Meas. m/z	# Ion Formula	m/z Adduct	err [ppm]	mSigma	# mSigma Score	rdb	e¡¥ Conf	N-Rule
434.1708	1 C24H24N3O5	434.1710 M+H	0.5	23.2	1 100.00	15.0	even	ok
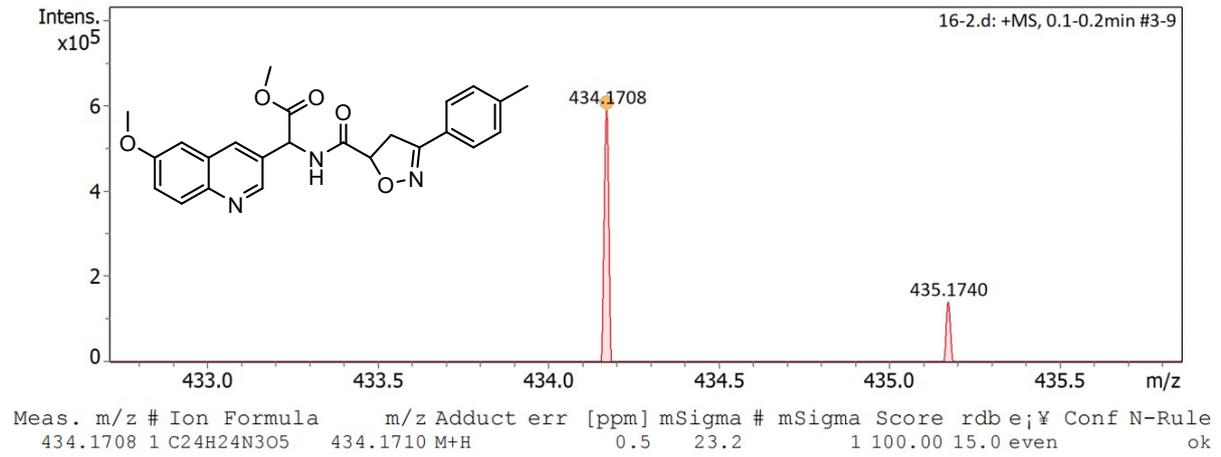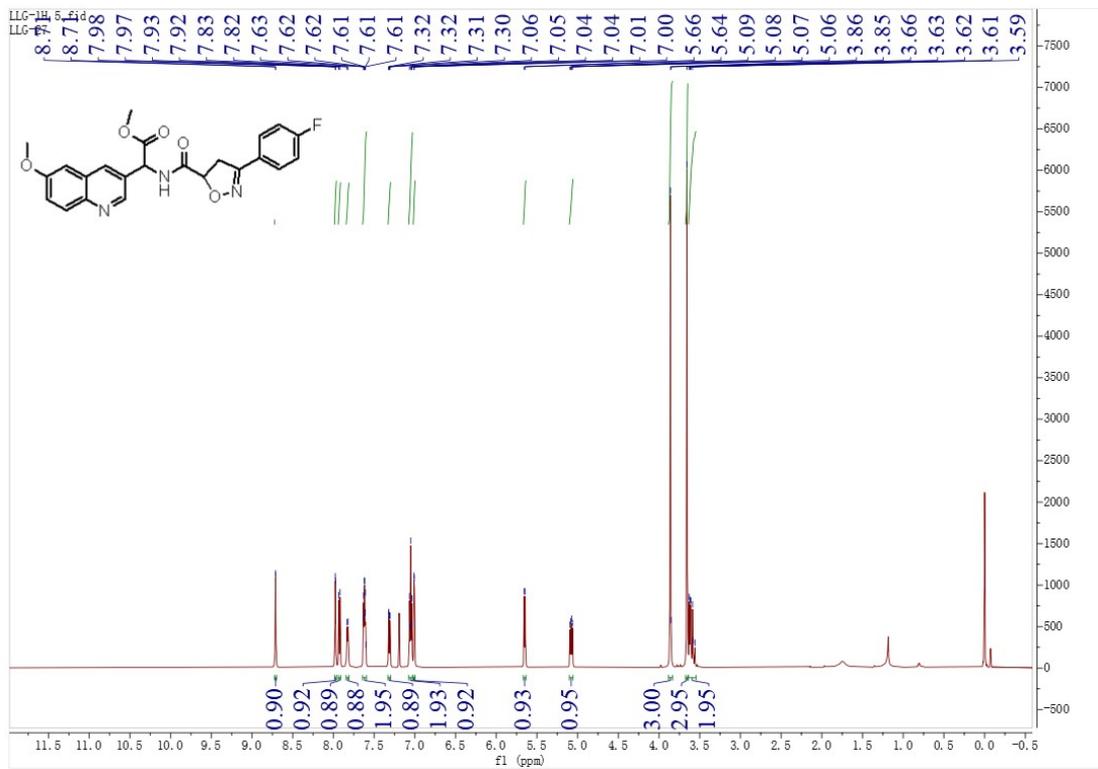
^{1}H NMR spectrum of compound **8c**

^{13}C NMR spectrum of compound **8c**

Meas. m/z # Ion Formula	m/z Adduct	err [ppm]	mSigma # mSigma	Score	rdb e¡¥ Conf N-Rule
438.1459 1 C23H21FN3O5	438.1460 M+H	0.2	11.5	1 100.00	15.0 even ok
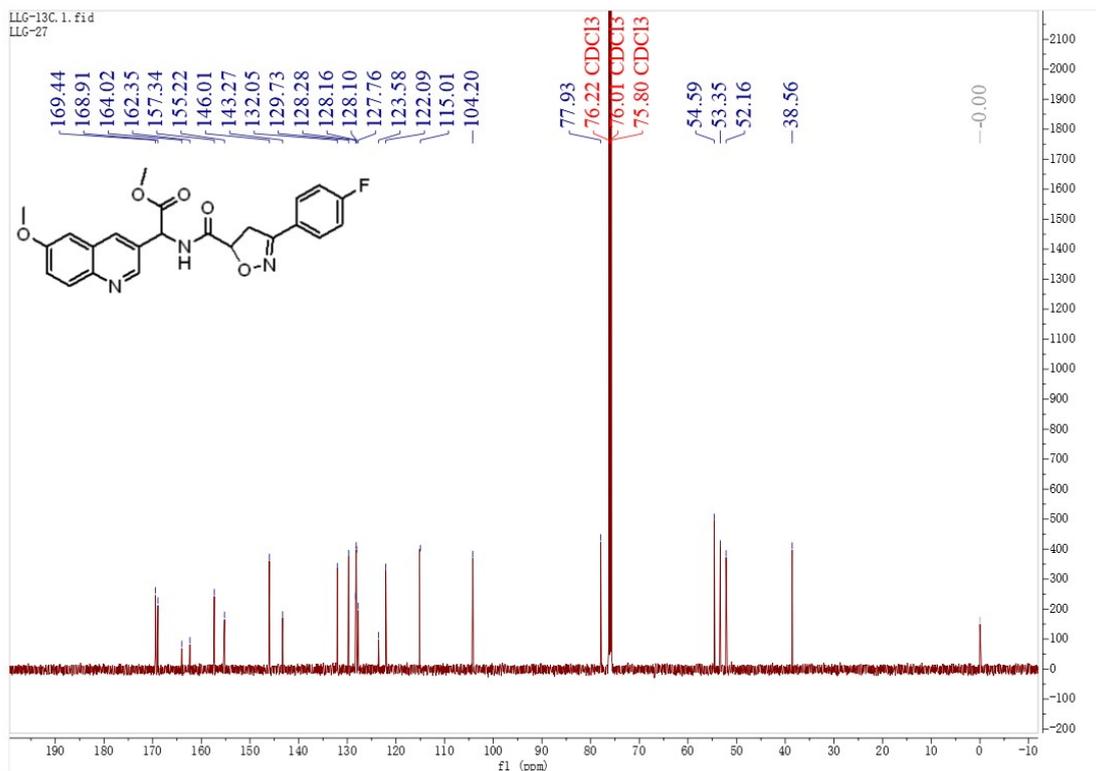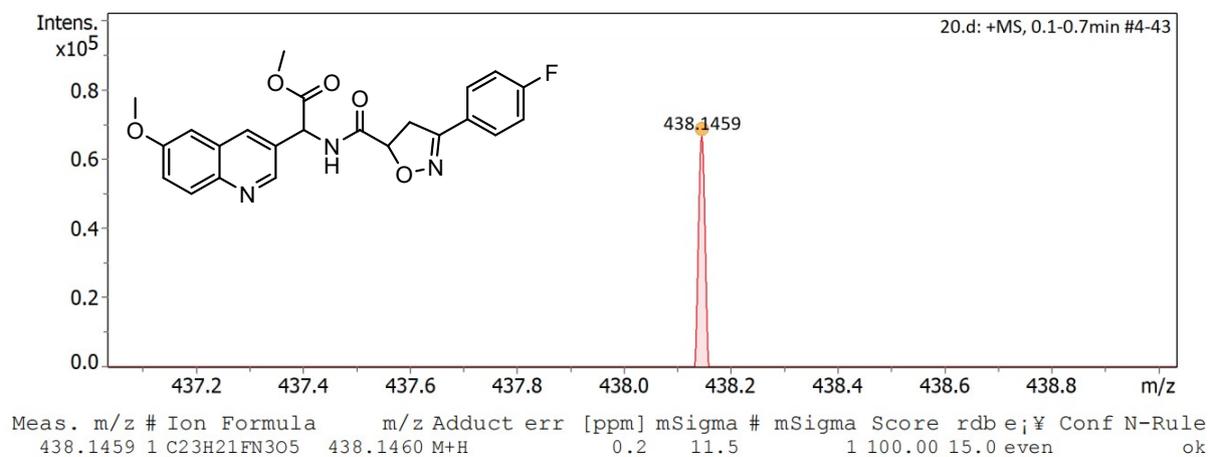
HRMS spectrum of compound **8c**
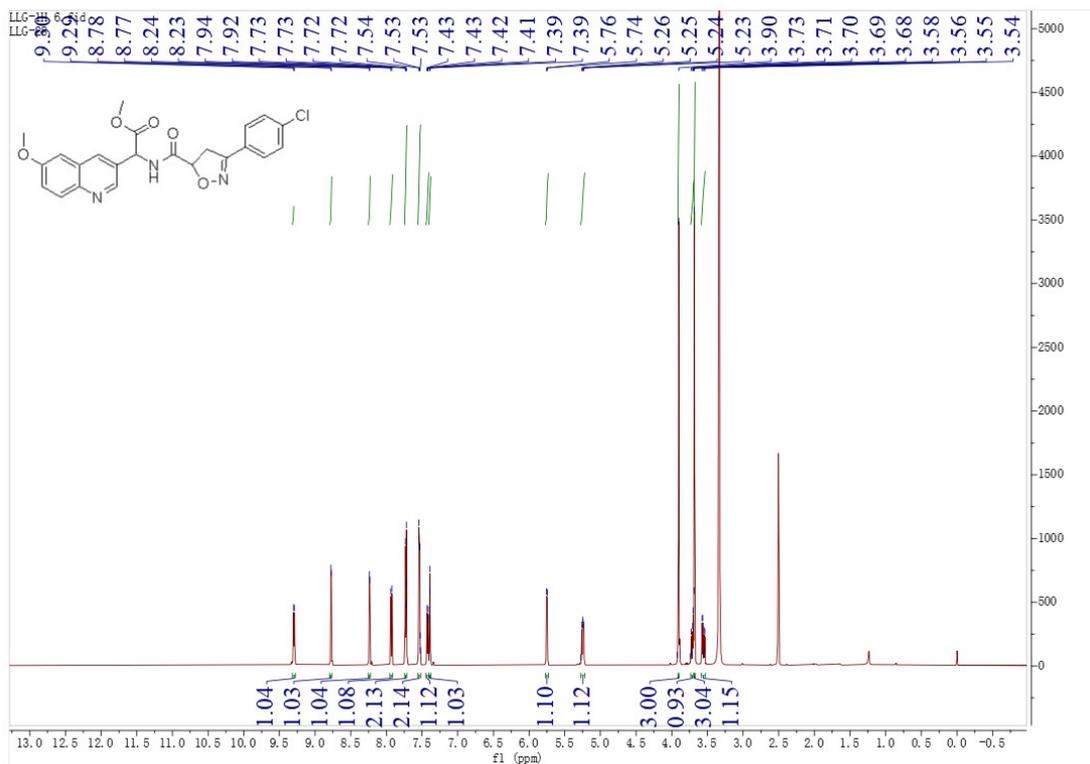
^{1}H NMR spectrum of compound **8d**
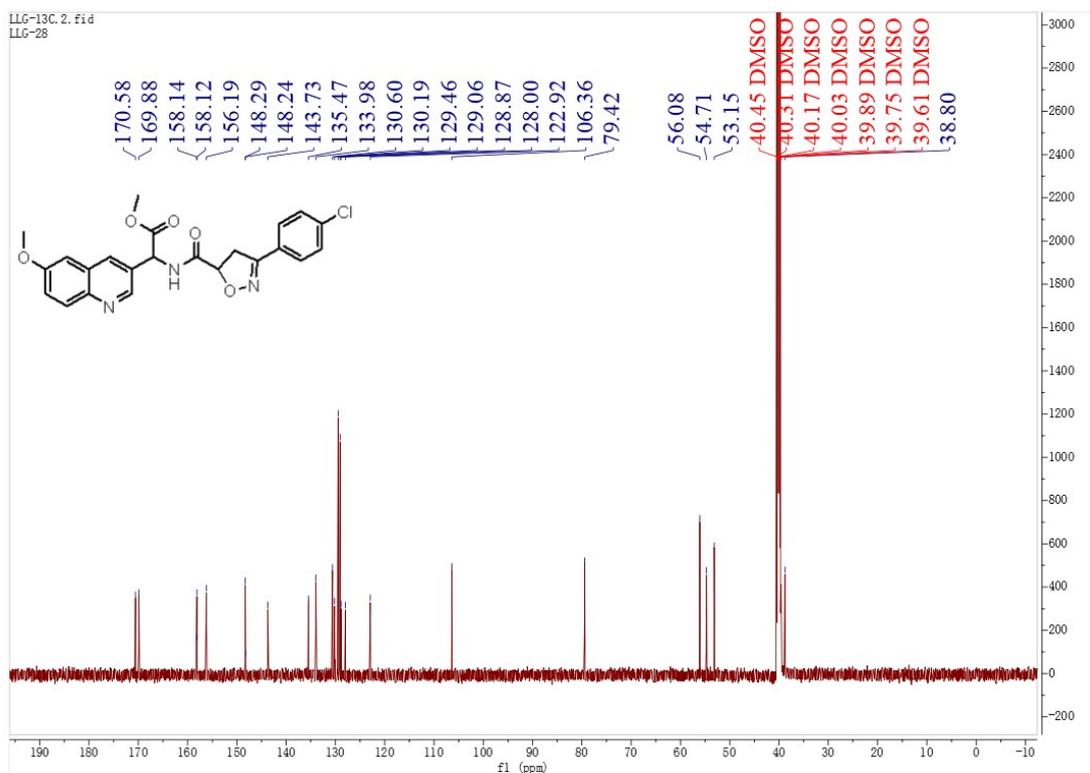
^{13}C NMR spectrum of compound **8d**

HRMS spectrum of compound **8d**

Meas. m/z	#	Ion Formula	m/z	Adduct	err [ppm]	mSigma #	mSigma Score	rdb	e¡¥ Conf	N-Rule
454.1165	1	C23H21ClN3O5	454.1164	M+H	-0.1	23.7	1 100.00	18.0	even	ok

¹H NMR spectrum of compound **8e**

170.62
170.39
158.12
157.82
155.94
148.29
143.72
133.95
132.28
130.62
130.32
130.18
129.45
128.84
122.92
121.12
117.95
112.81
106.27
78.82
56.07
54.70
53.13
41.65
40.45 DMSO
40.31 DMSO
40.17 DMSO
40.03 DMSO
39.89 DMSO
39.75 DMSO
39.62 DMSO
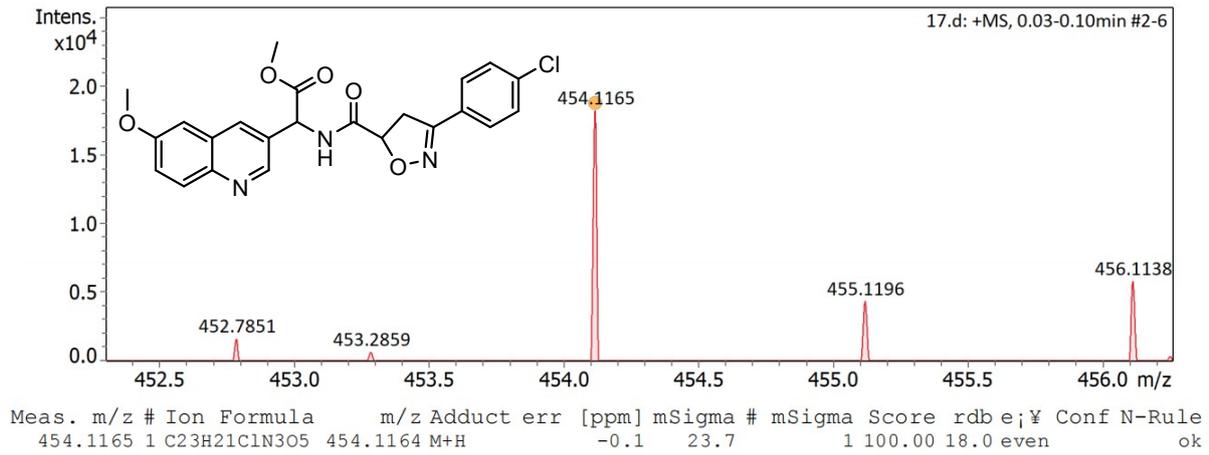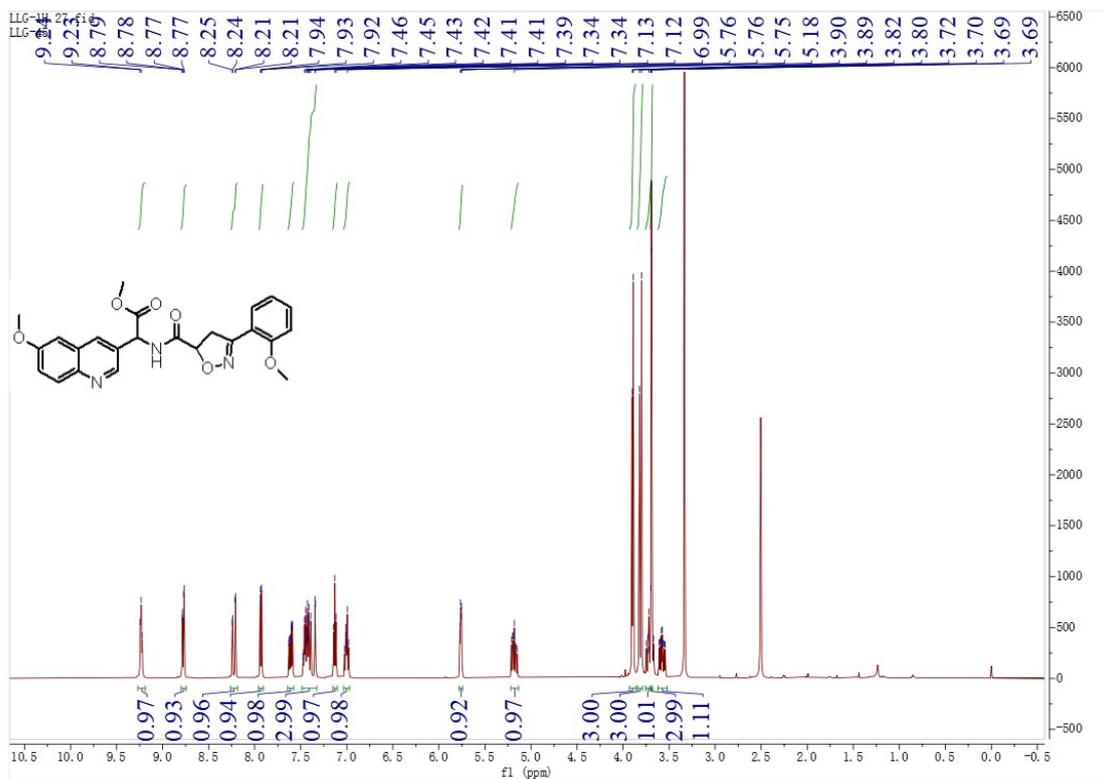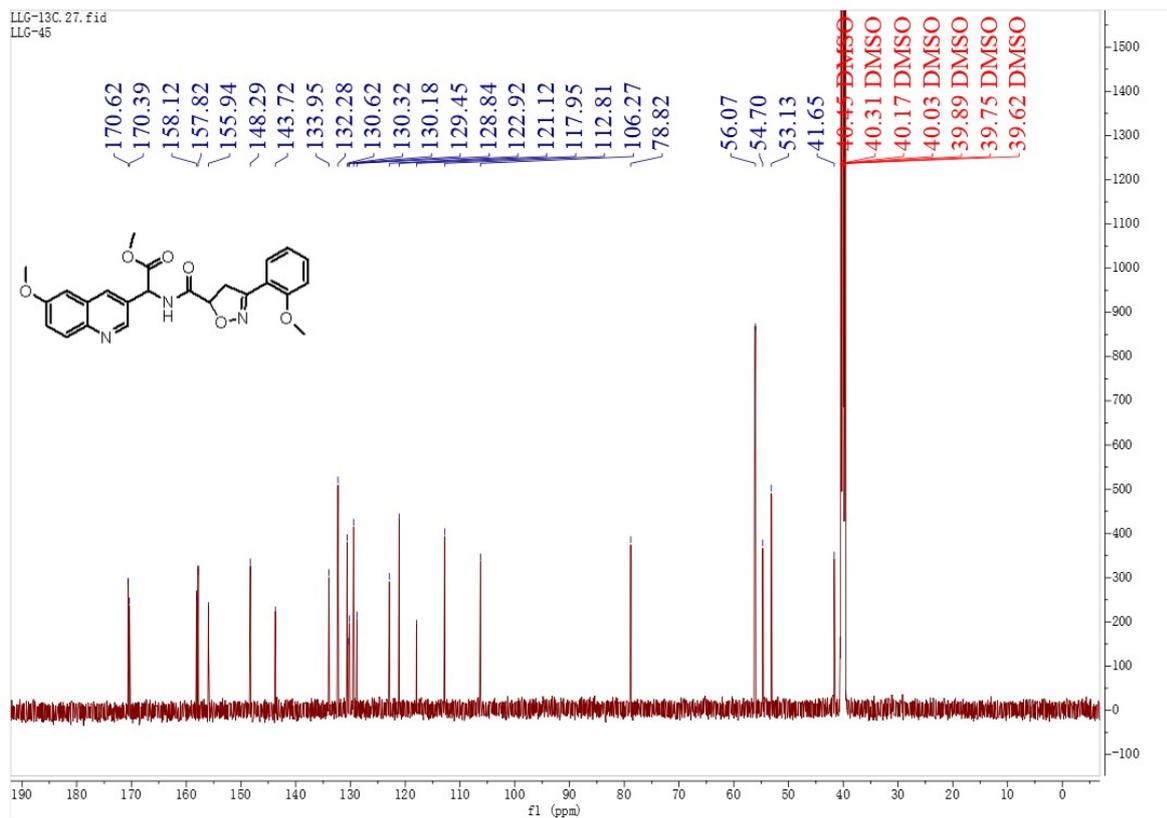
^{13}C NMR spectrum of compound **8e**
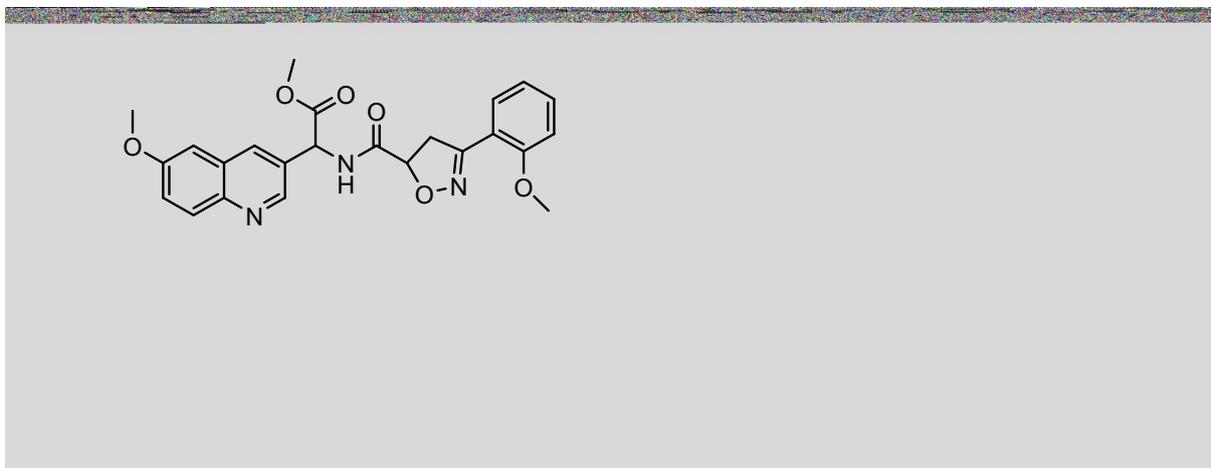
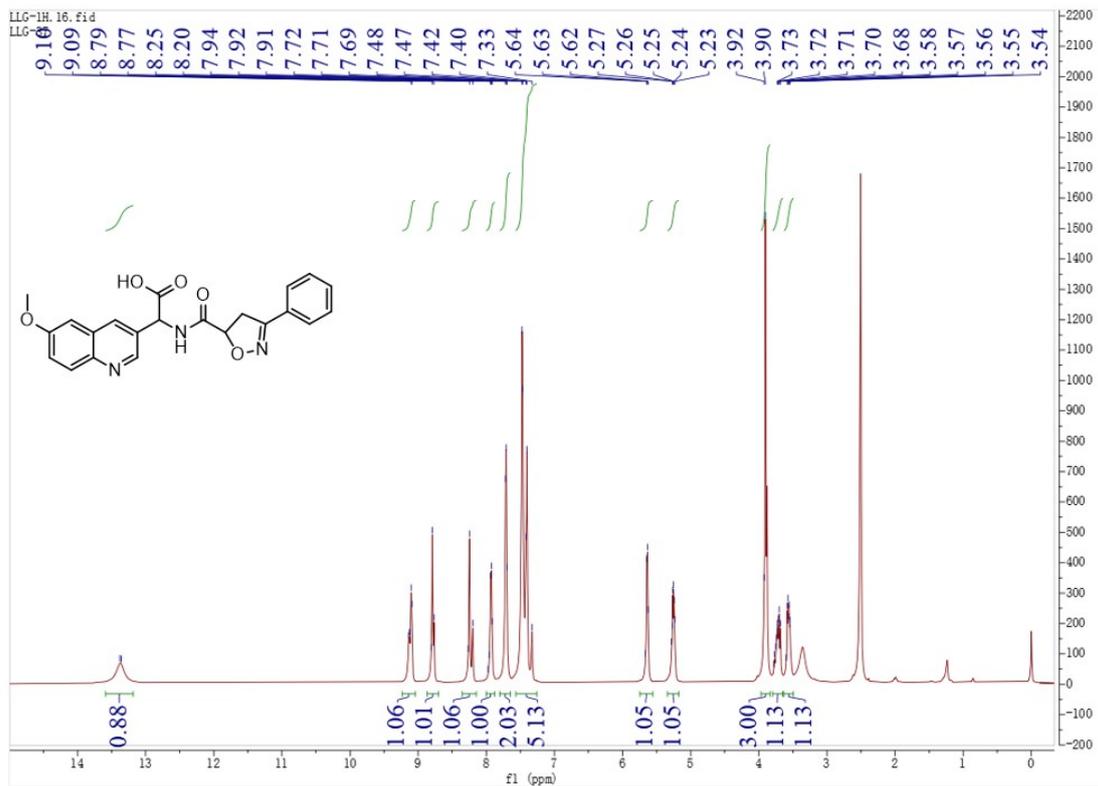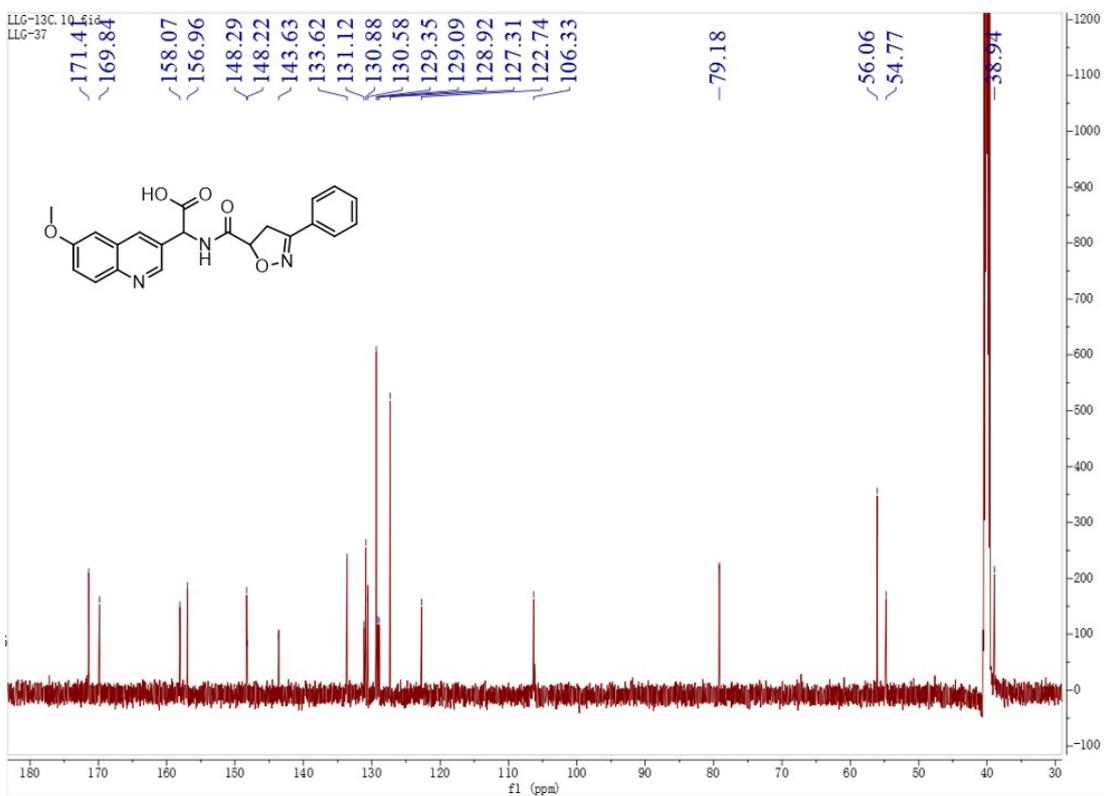HRMS spectrum of compound **8e**

^{1}H NMR spectrum of compound **8f**

^{13}C NMR spectrum of compound **8f**

HRMS spectrum of compound **8f**

^{1}H NMR spectrum of compound **8g**

^{13}C NMR spectrum of compound **8g**

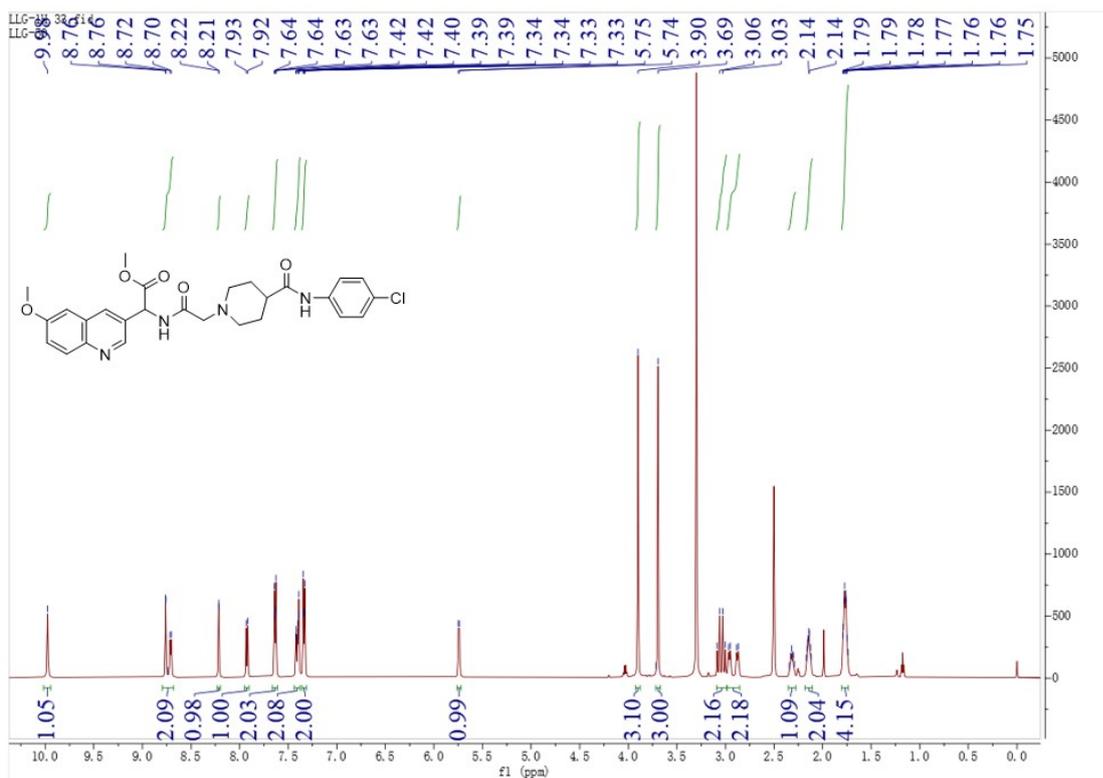| Meas. m/z	#	Ion Formula	m/z	Adduct	err [ppm]	mSigma	# mSigma	Score	rdb	e¡¥ Conf	N-Rule
448.1868	1	C25H26N3O5	448.1867	M+H	-0.1	9.9	1	100.00	15.0	even	ok

HRMS spectrum of compound **8g**

^{1}H NMR spectrum of compound **8h**

^{13}C NMR spectrum of compound **8h**
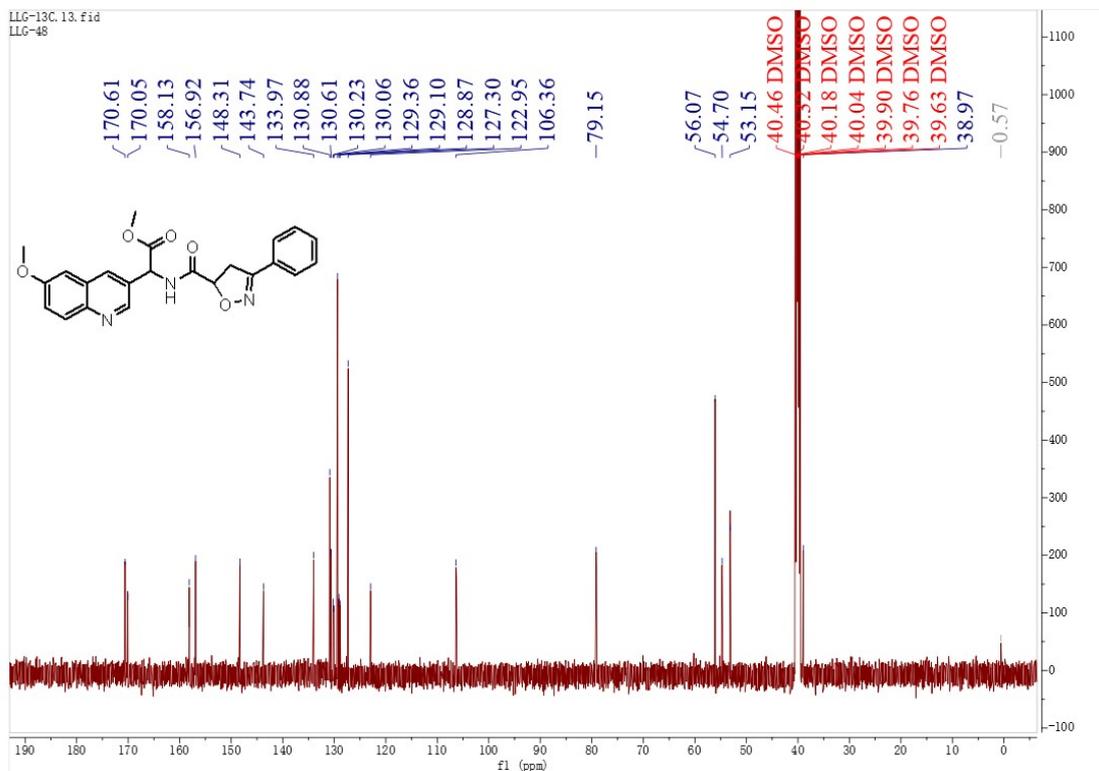
HRMS spectrum of compound **8h**
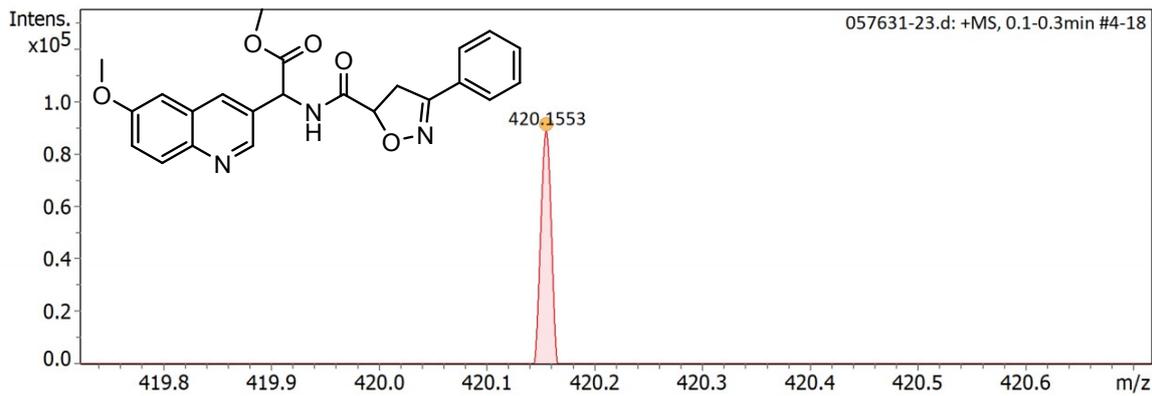
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420.1553	1	C23H22N3O5	420.1554	M+H	0.2	30.3	1	100.00	15.0	even		ok
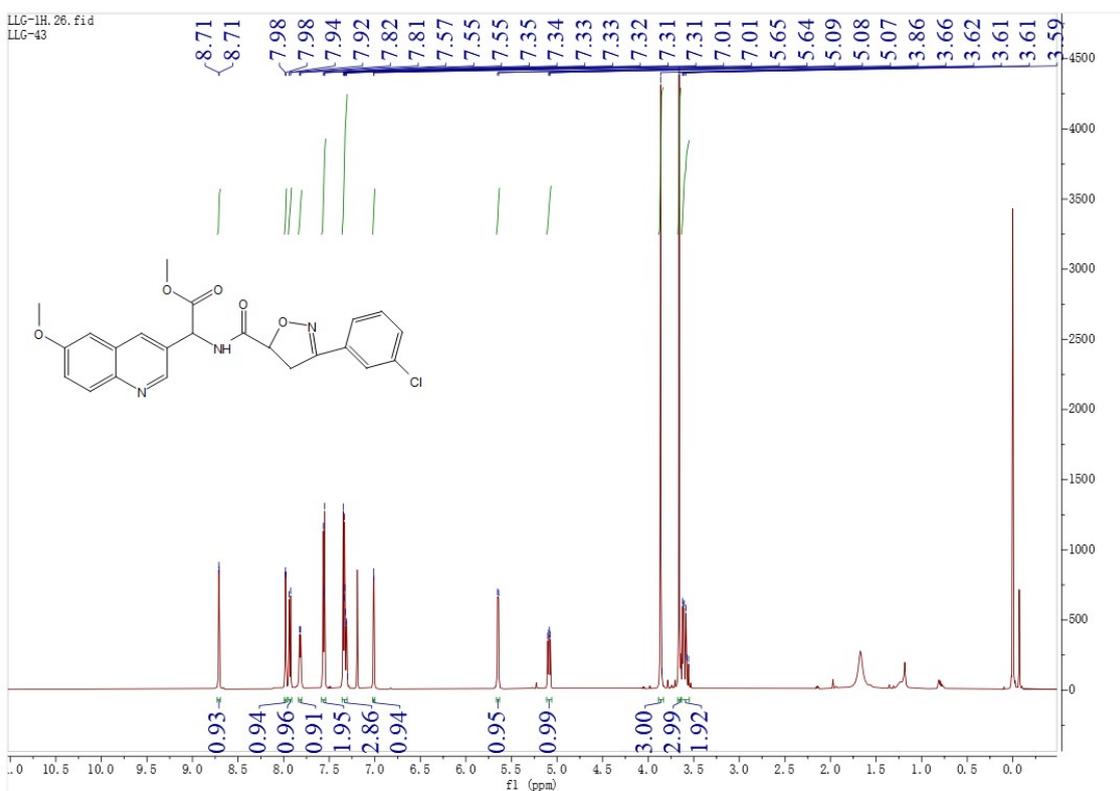
^{1}H NMR spectrum of compound **8i**
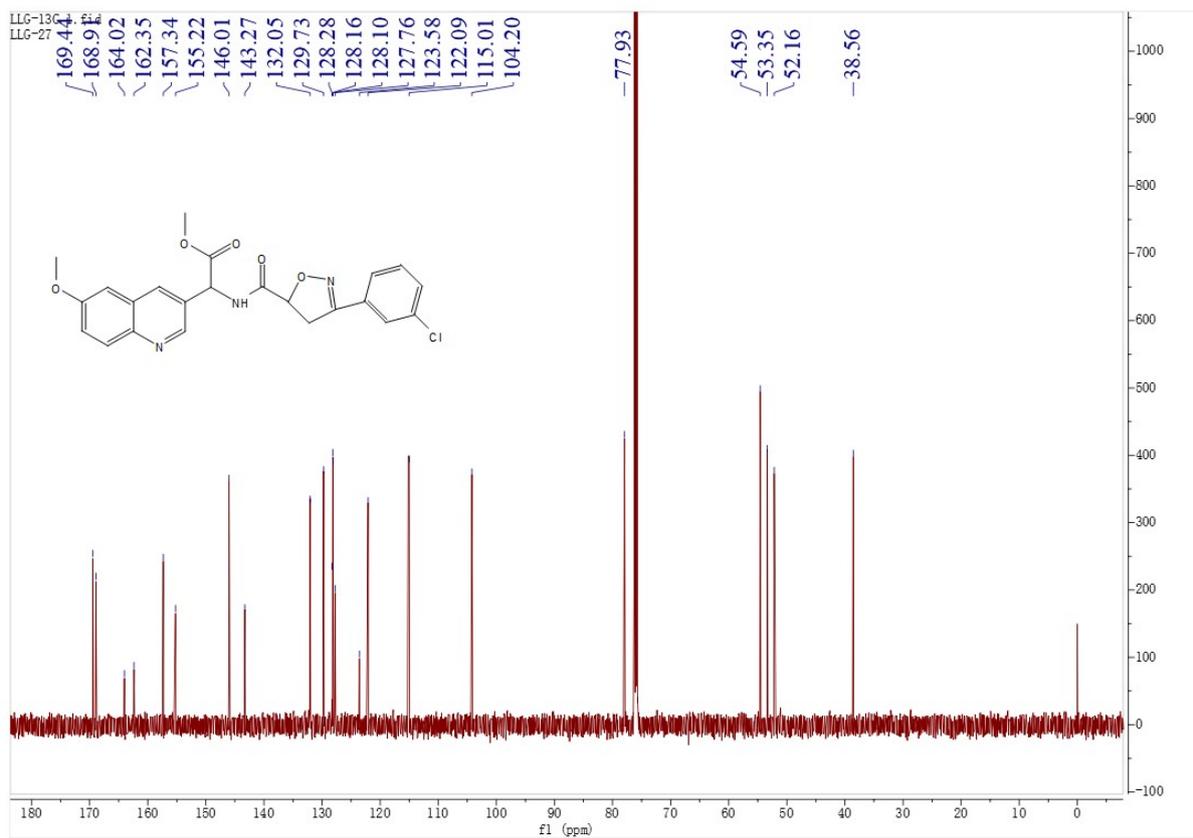
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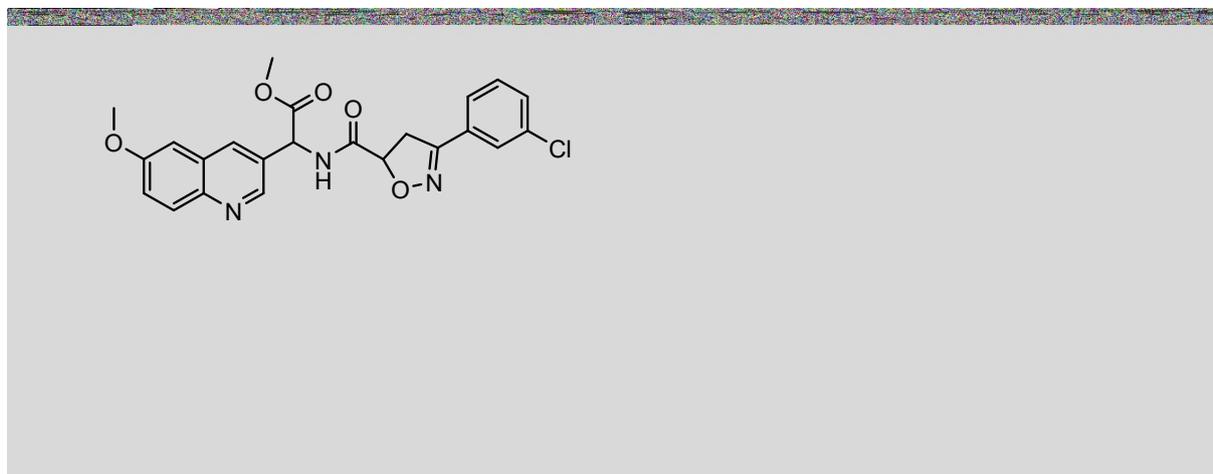
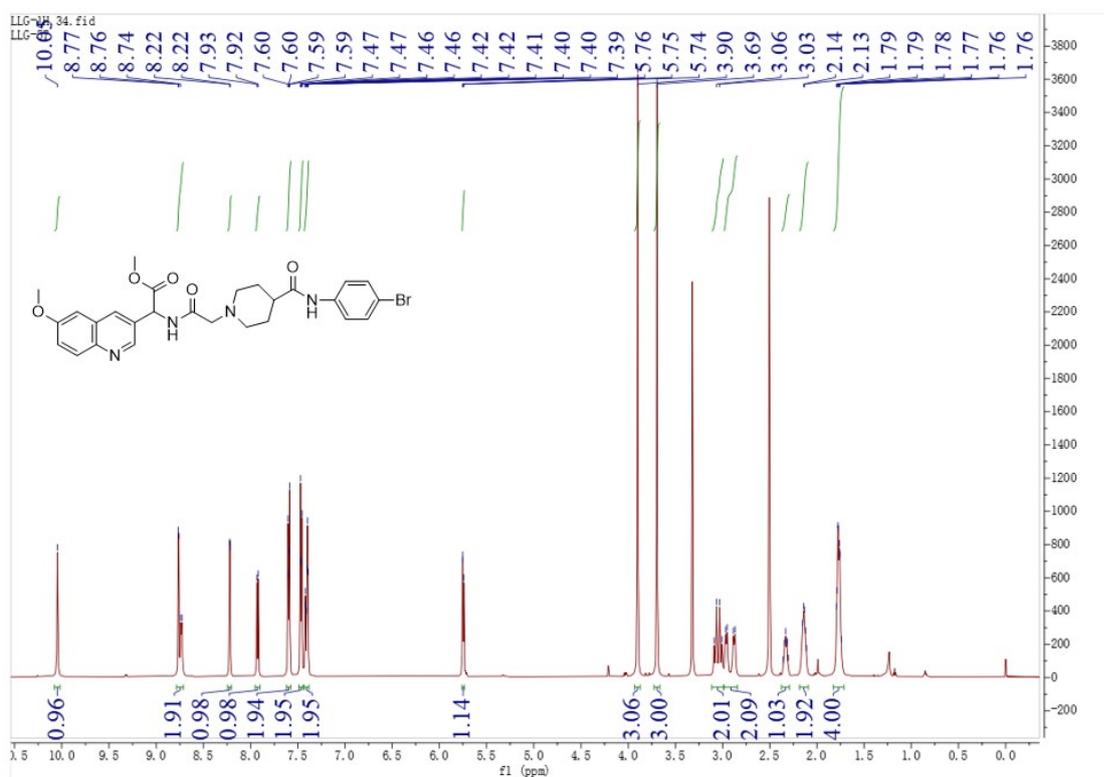HRMS spectrum of compound **8i**

^{1}H NMR spectrum of compound **9**

^{13}C NMR spectrum of compound **9**

Meas. m/z # Ion Formula   m/z Adduct err [ppm] mSigma # mSigma Score rdb e¡¥ Conf N-Rule
406.1398 1 C22H20N3O5   406.1397 M+H   -0.0   3.6   1 100.00 15.0 even   ok
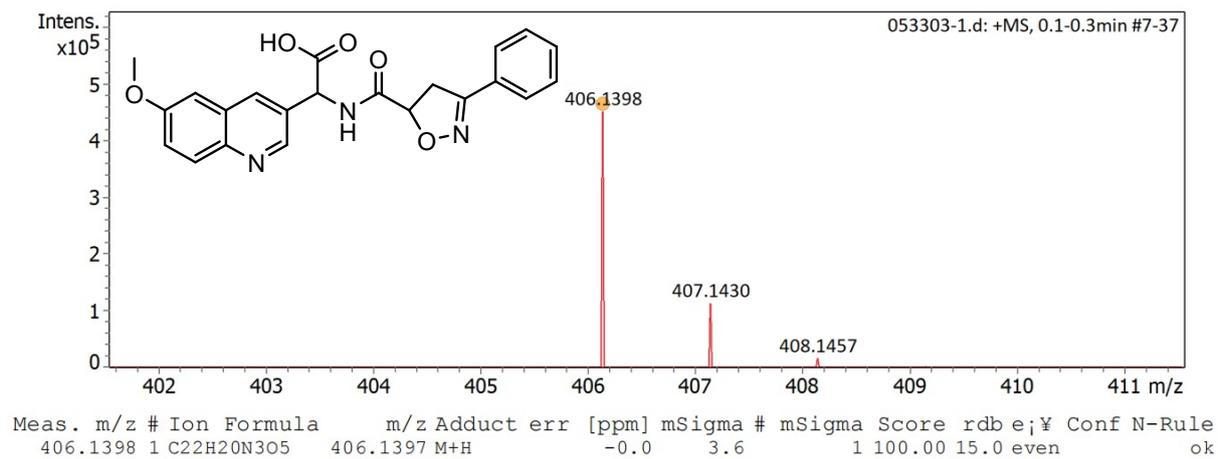
HRMS spectrum of compound **9**
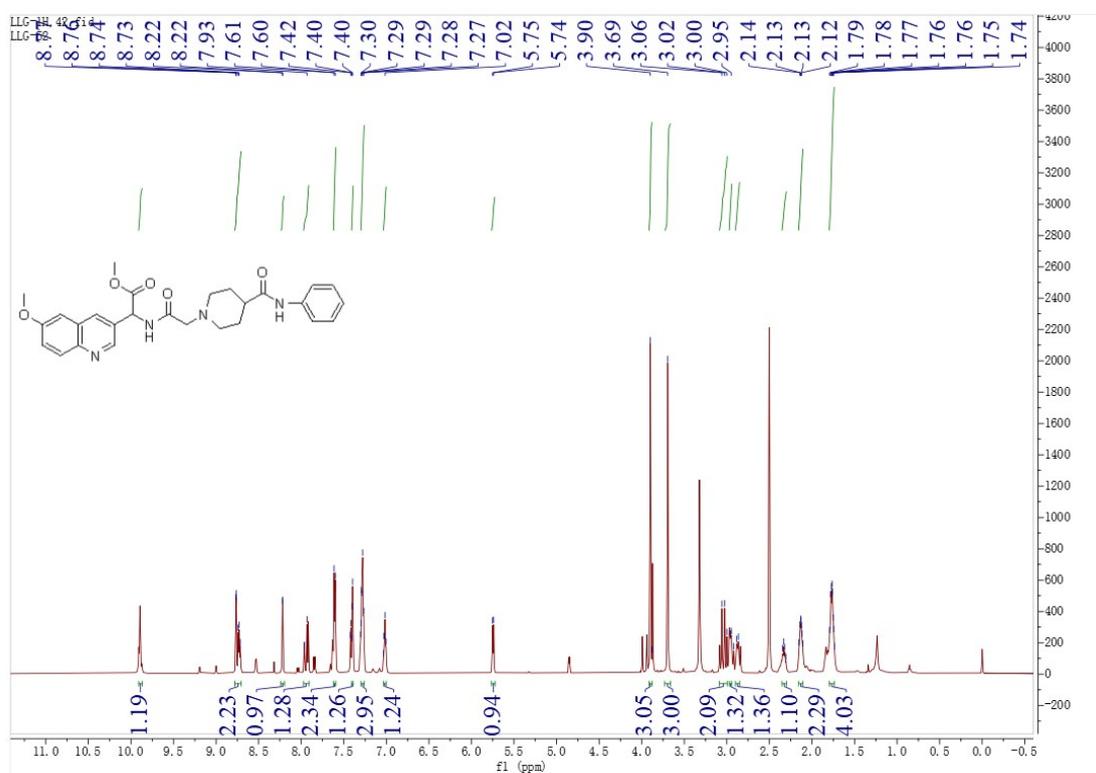
^{1}H NMR spectrum of compound **14a**

^{13}C NMR spectrum of compound **14a**

Meas. m/z	#	Ion Formula	m/z	Adduct	err [ppm]	mSigma	# mSigma	Score	rdb	e¡¥ Conf	N-Rule
491.2285	1	C27H31N4O5	491.2289	M+H	0.9	10.8	1	100.00	15.0	even	ok
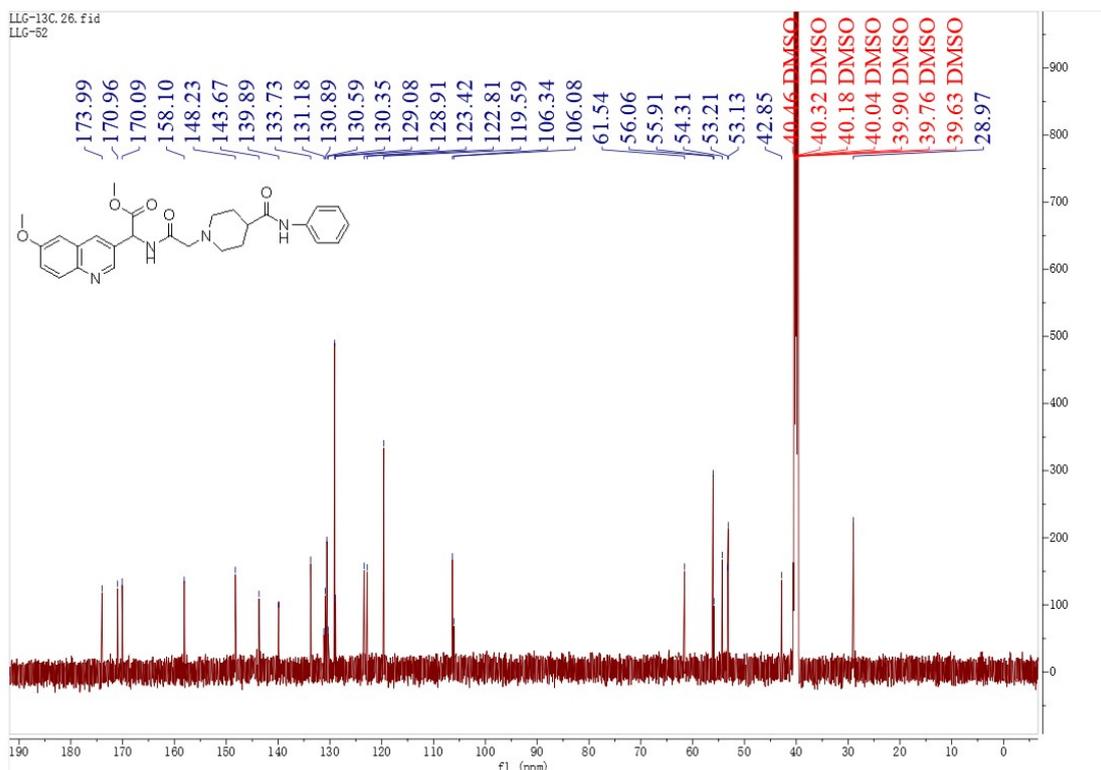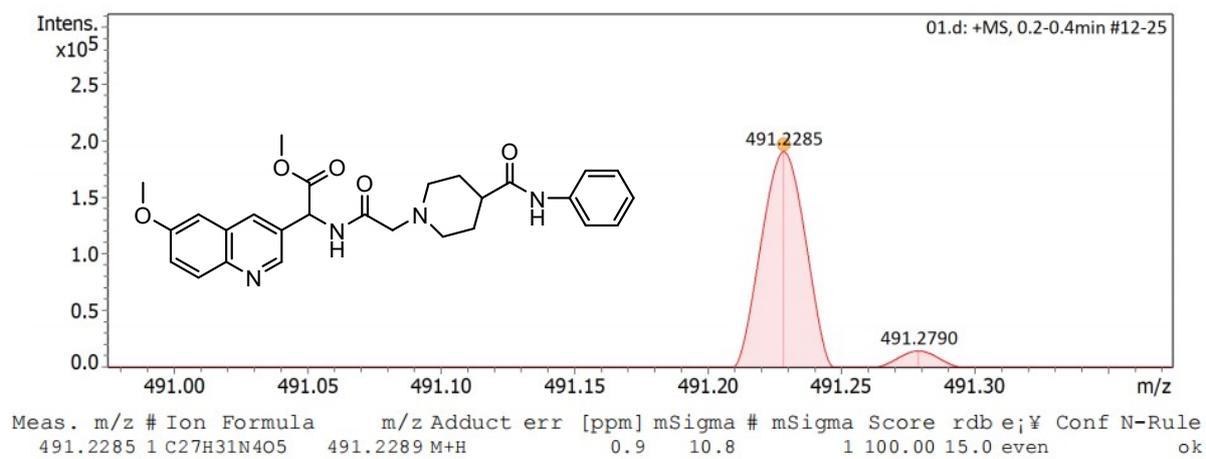
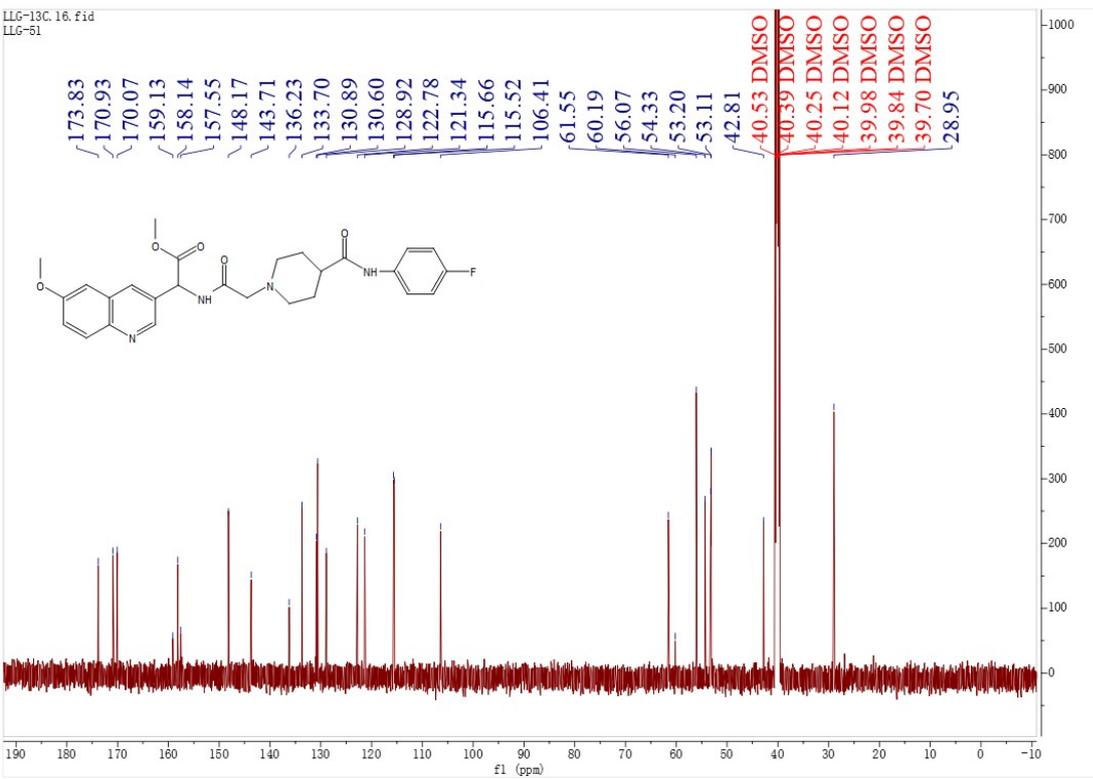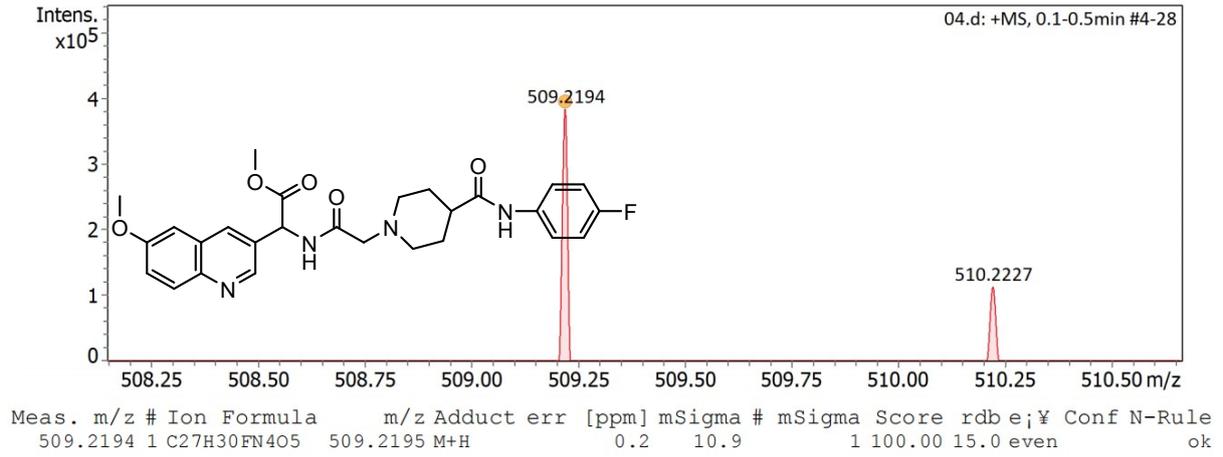HRMS spectrum of compound **14a**
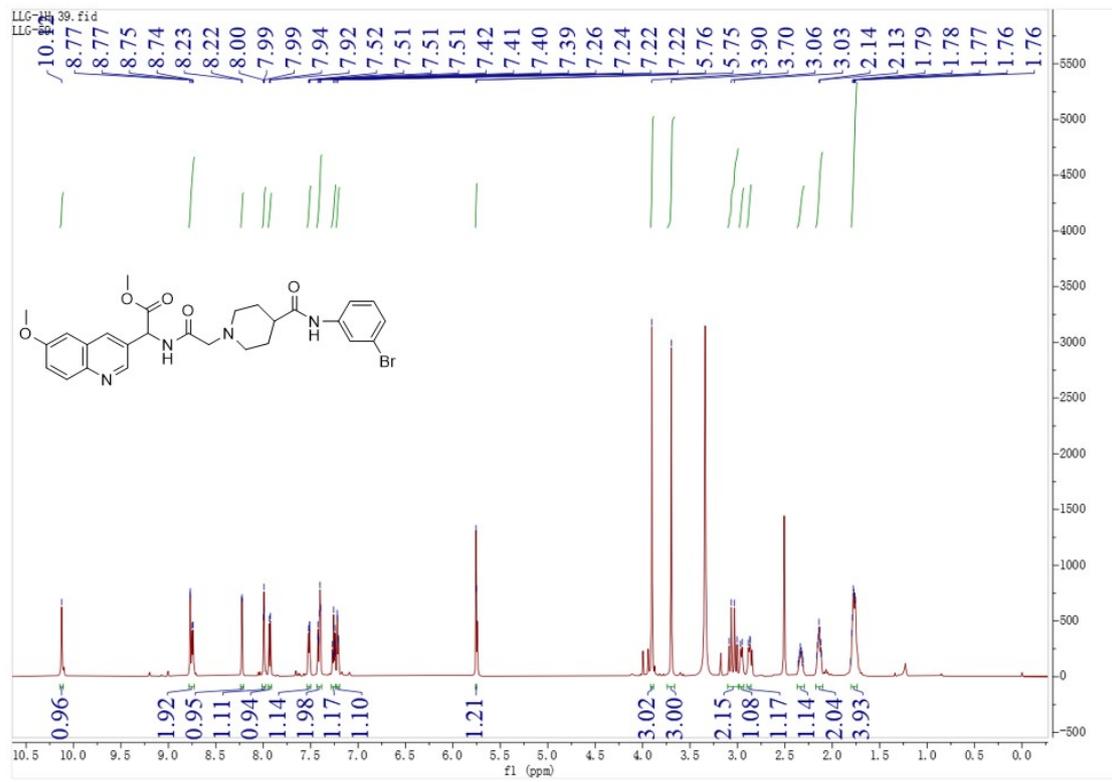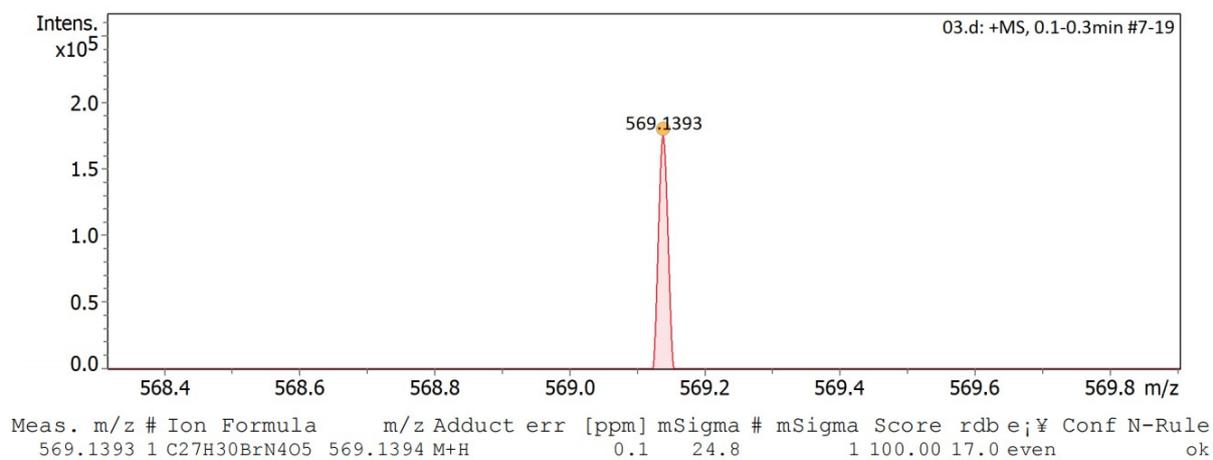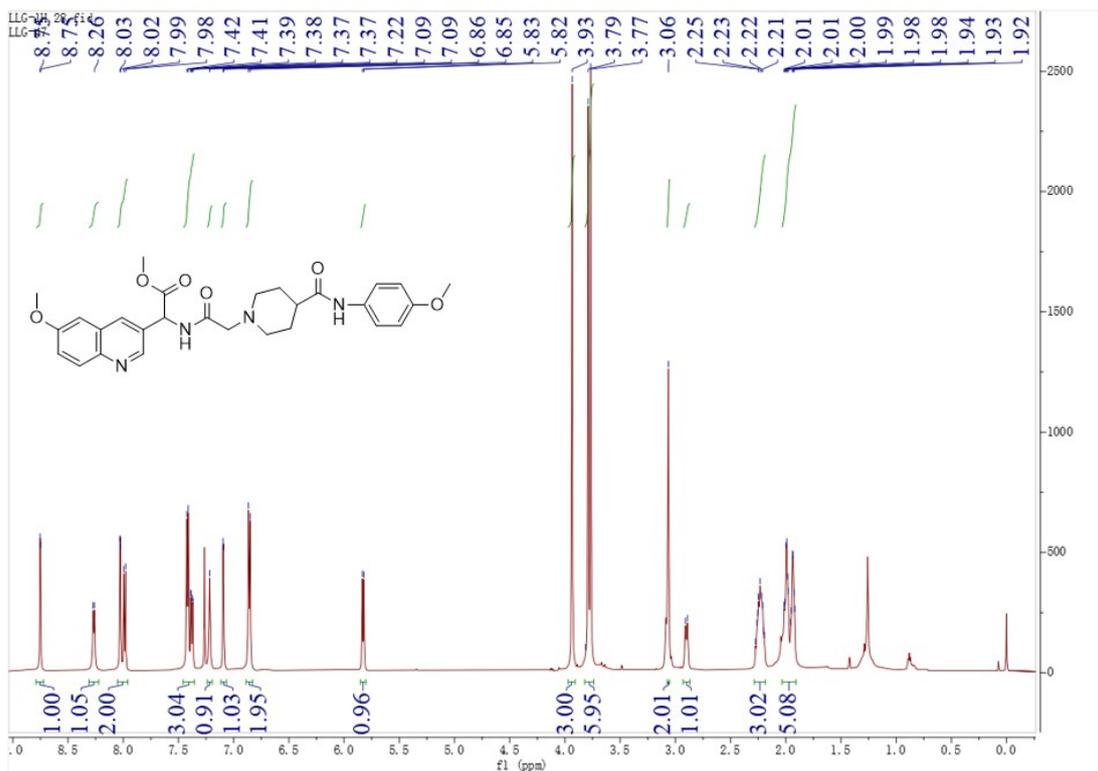
¹H NMR spectrum of compound **14b**

¹³C NMR spectrum of compound **14b**
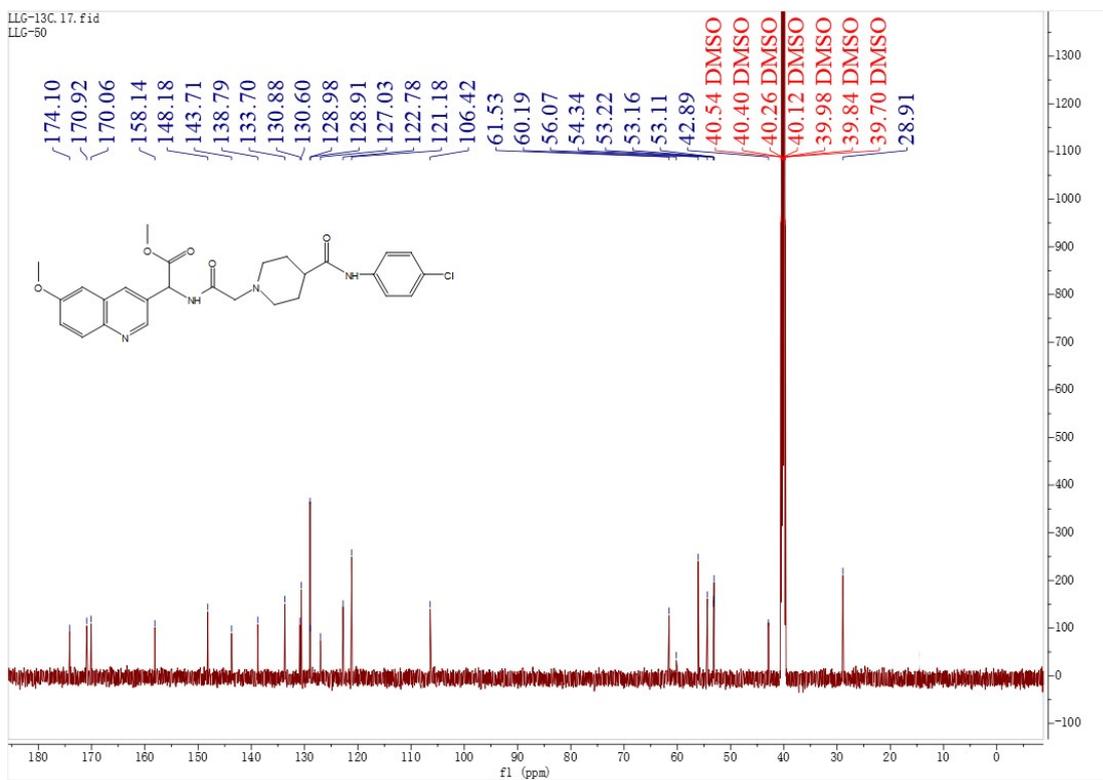
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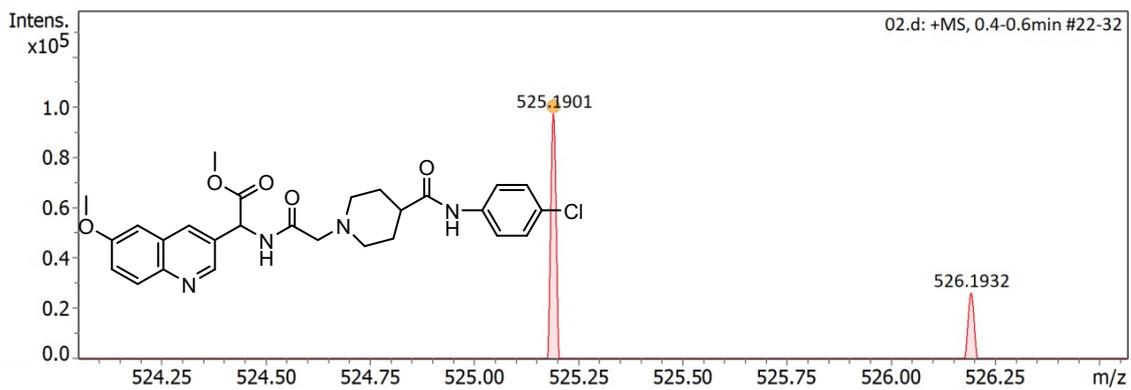
Meas. m/z	#	Ion Formula	m/z	Adduct	err [ppm]	mSigma	# mSigma	Score	rdb	e¡¥ Conf	N-Rule
525.1901	1	C27H30ClN4O5	525.1899	M+H	-0.3	46.9	1	100.00	18.0	even	ok

^{1}H NMR spectrum of compound **14c**

^{13}C NMR spectrum of compound **14c**

Meas. m/z	#	Ion Formula	m/z	Adduct	err [ppm]	mSigma	# mSigma	Score	rdb	e¡¥ Conf	N-Rule
569.1393	1	C27H30BrN4O5	569.1394	M+H	0.2	24.8	1	100.00	17.0	even	ok
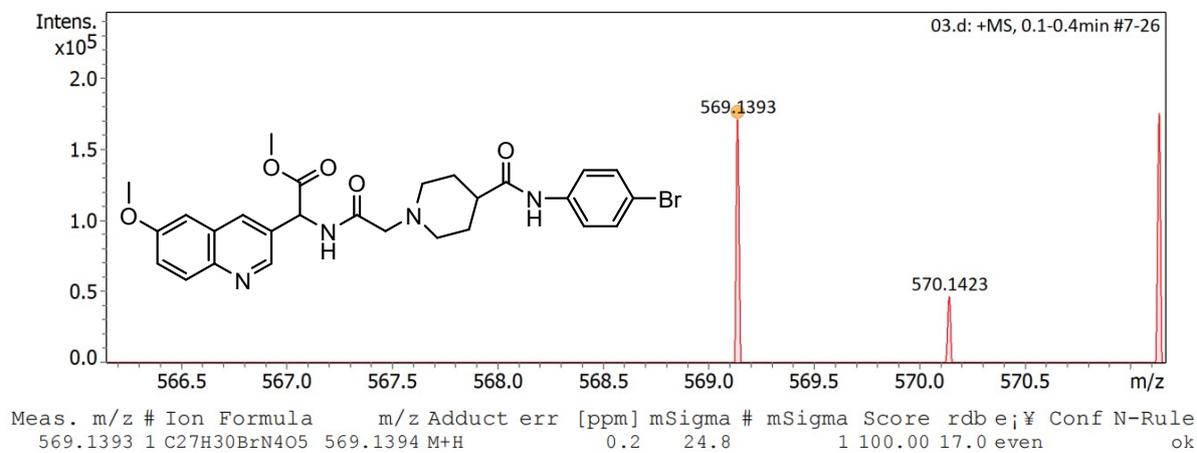
HRMS spectrum of compound **14c**
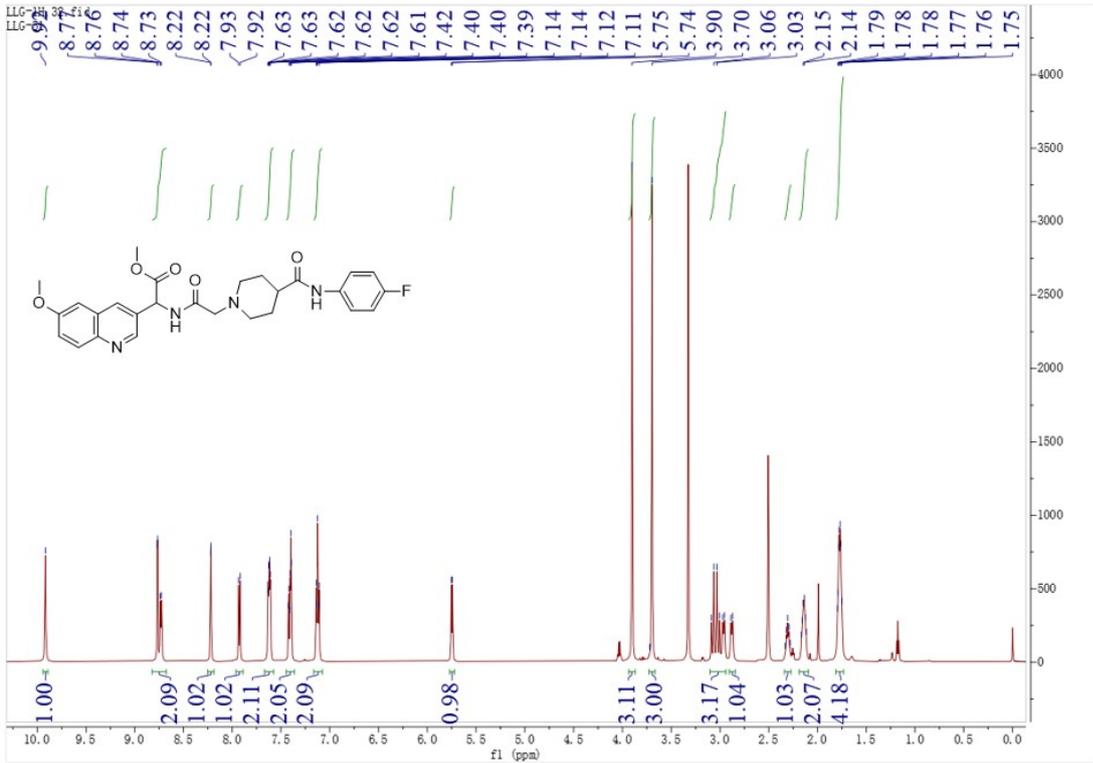
^{1}H NMR spectrum of compound **14d**

^{13}C NMR spectrum of compound **14d**

HRMS spectrum of compound **14d**

Meas. m/z	#	Ion Formula	m/z	Adduct	err [ppm]	mSigma	#	mSigma Score	rdb	e¡¥	Conf	N-Rule
509.2194	1	C27H30FN4O5	509.2195	M+H	0.2	10.9	1	100.00	15.0	even		ok

^{1}H NMR spectrum of compound **14e**

^{13}C NMR spectrum of compound **14e**

Meas. m/z	#	Ion Formula	m/z	Adduct	err [ppm]	mSigma	# mSigma	Score	rdb	e¡¥ Conf	N-Rule
569.1393	1	C27H30BrN4O5	569.1394	M+H	0.1	24.8	1	100.00	17.0	even	ok
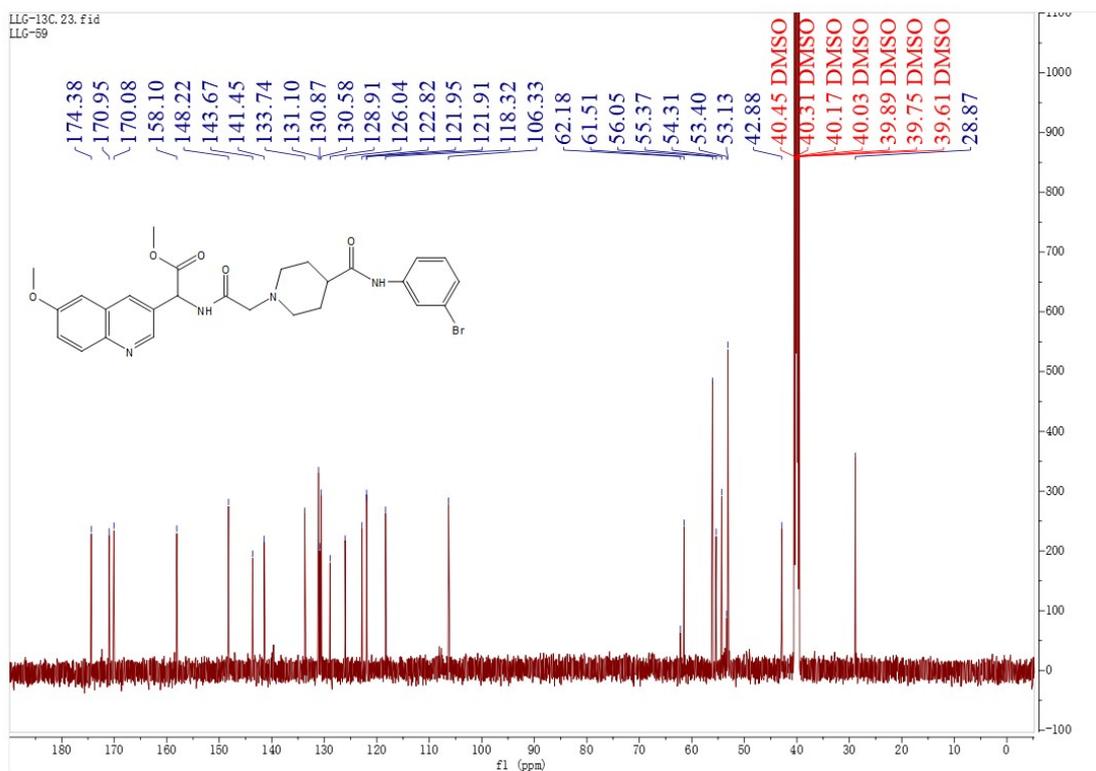
HRMS spectrum of compound **14e**

^{1}H NMR spectrum of compound **14f**
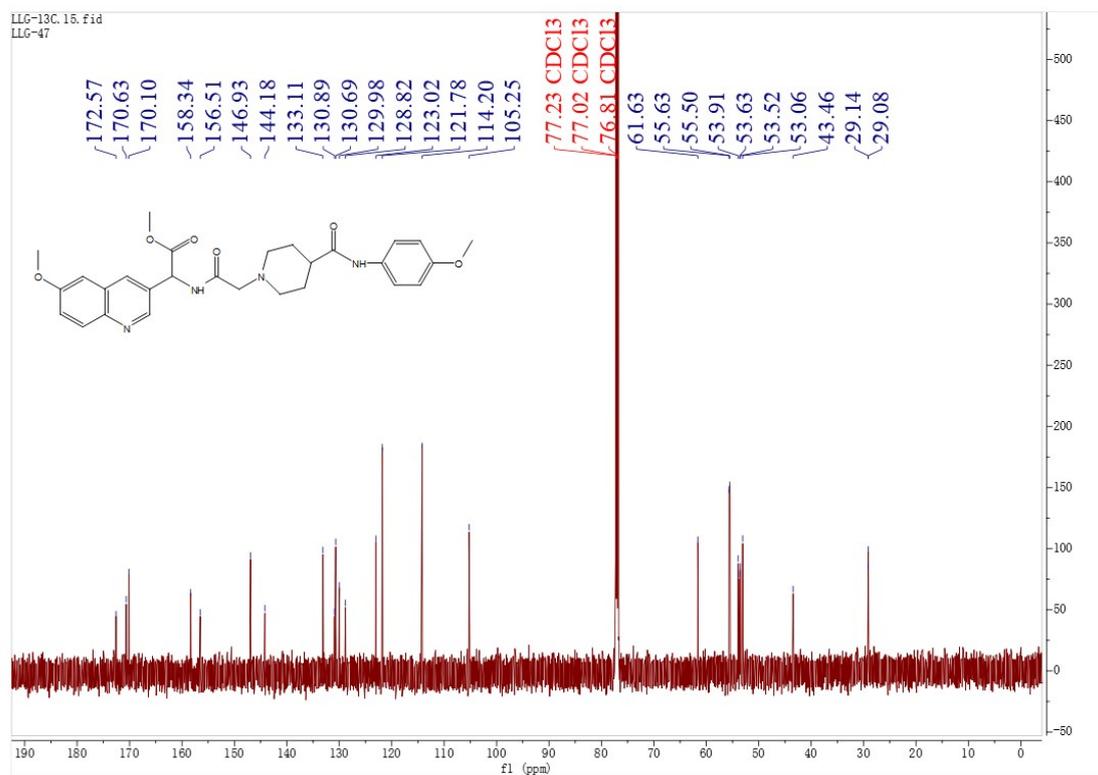
^{13}C NMR spectrum of compound **14f**
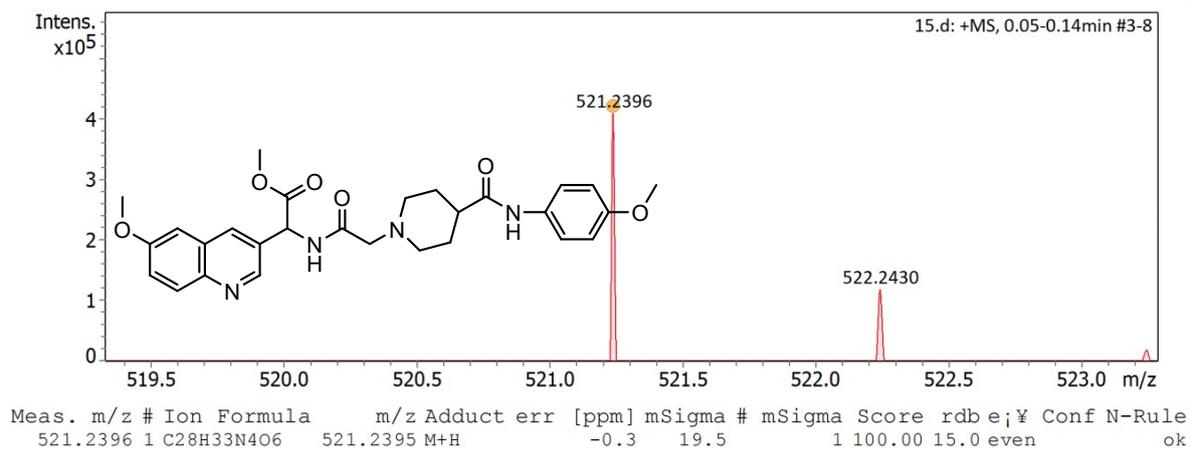
HRMS spectrum of compound **14f**

Meas. m/z	#	Ion Formula	m/z	Adduct	err [ppm]	mSigma	# mSigma	Score	rdb	e¡¥	Conf	N-Rule
521.2396	1	C28H33N4O6	521.2395	M+H	-0.3	19.5	1	100.00	15.0	even		ok

¹H NMR spectrum of compound **14g**

174.25
170.95
170.08
158.10
154.17
152.57
148.21
143.67
137.12
133.73
130.86
130.58
128.90
122.81
120.93
119.82
119.40
117.19
106.33
79.66
61.50
56.04
54.32
53.38
53.12
42.82
40.44 DMSO
40.31 DMSO
40.17 DMSO
40.03 DMSO
39.89 DMSO
39.75 DMSO
39.61 DMSO
28.86

^{13}C NMR spectrum of compound **14g**

Meas. m/z	#	Ion Formula	m/z	Adduct	err [ppm]	mSigma	# mSigma	Score	rdb	e¡¥ Conf	N-Rule
543.1805	1	C27H29ClFN4O5	543.1805	M+H	0.0	27.3	1	100.00	18.0	even	ok

HRMS spectrum of compound **14g**
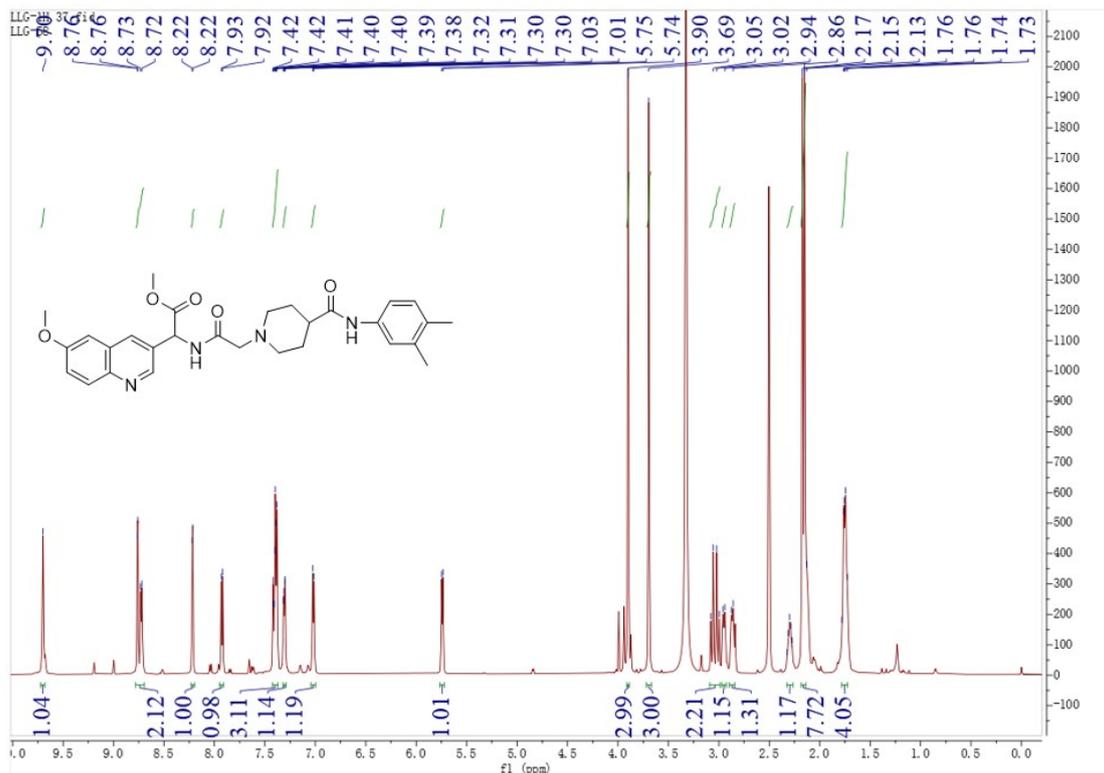
^{1}H NMR spectrum of compound **14h**

^{13}C NMR spectrum of compound **14h**
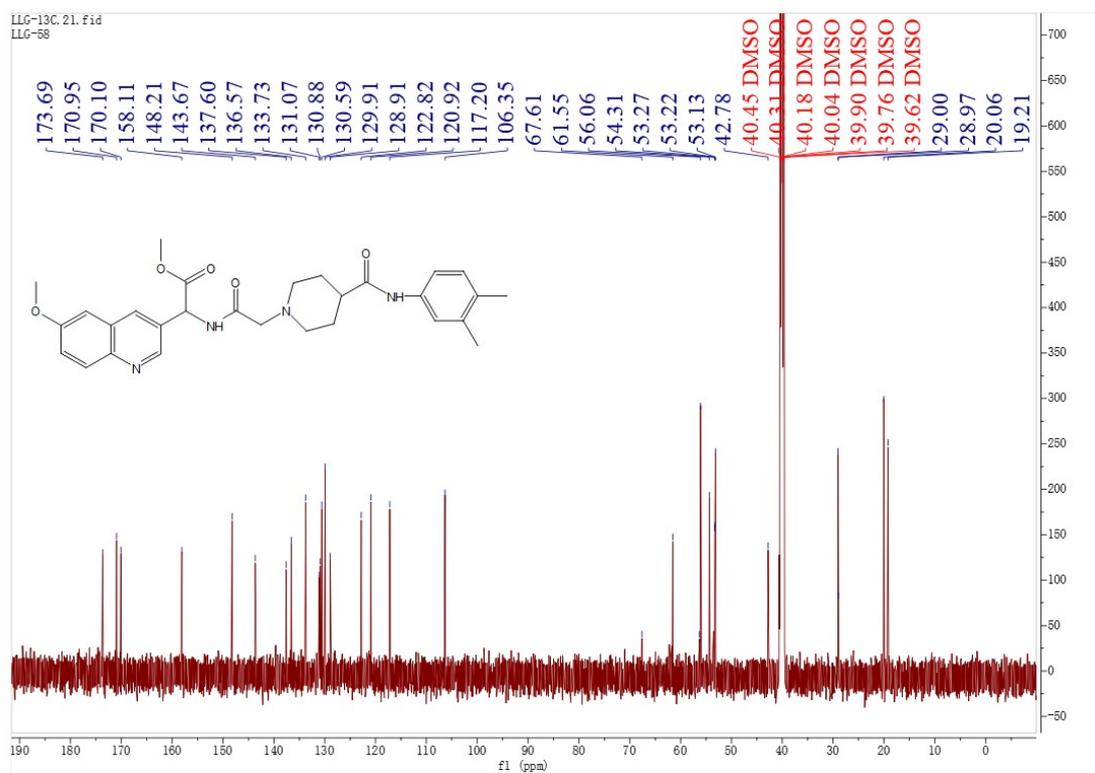
HRMS spectrum of compound **14h**
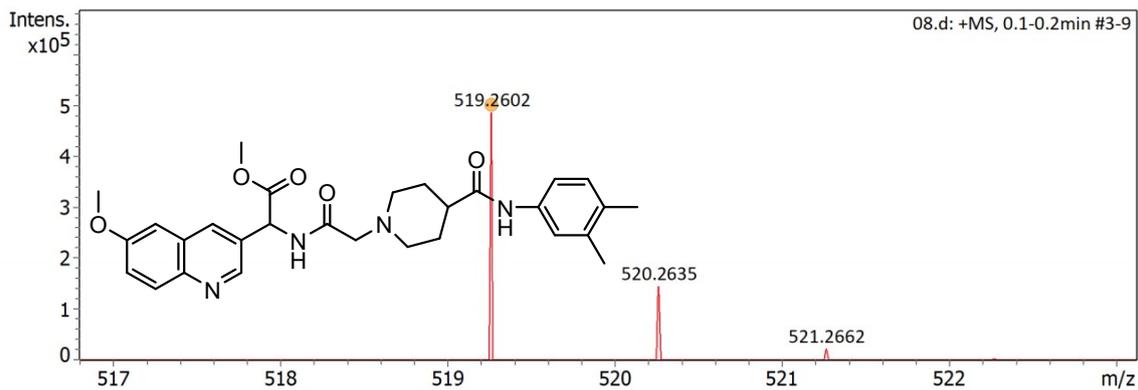
Meas. m/z	# Ion Formula	m/z	Adduct	err [ppm]	mSigma	# mSigma Score	rdb	e¡¥	Conf	N-Rule
519.2602	1 C29H35N4O5	519.2602	M+H	-0.0	20.3	1 100.00	15.0	even		ok
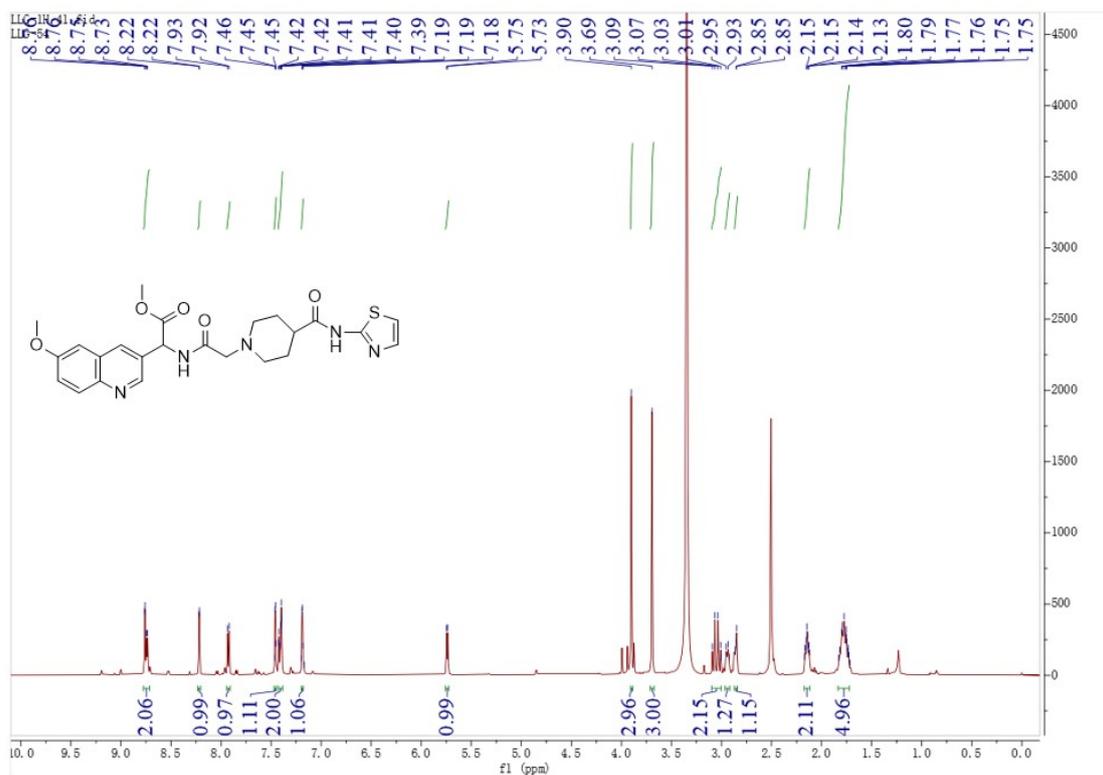
¹H NMR spectrum of compound **14i**

¹³C NMR spectrum of compound **14i**

Meas. m/z # Ion Formula	m/z Adduct	err [ppm]	mSigma # mSigma	Score	rdb e¡¥ Conf N-Rule
498.1805 1 C24H28N5O5S	498.1806 M+H	0.2	27.0	1 100.00	16.0 even ok
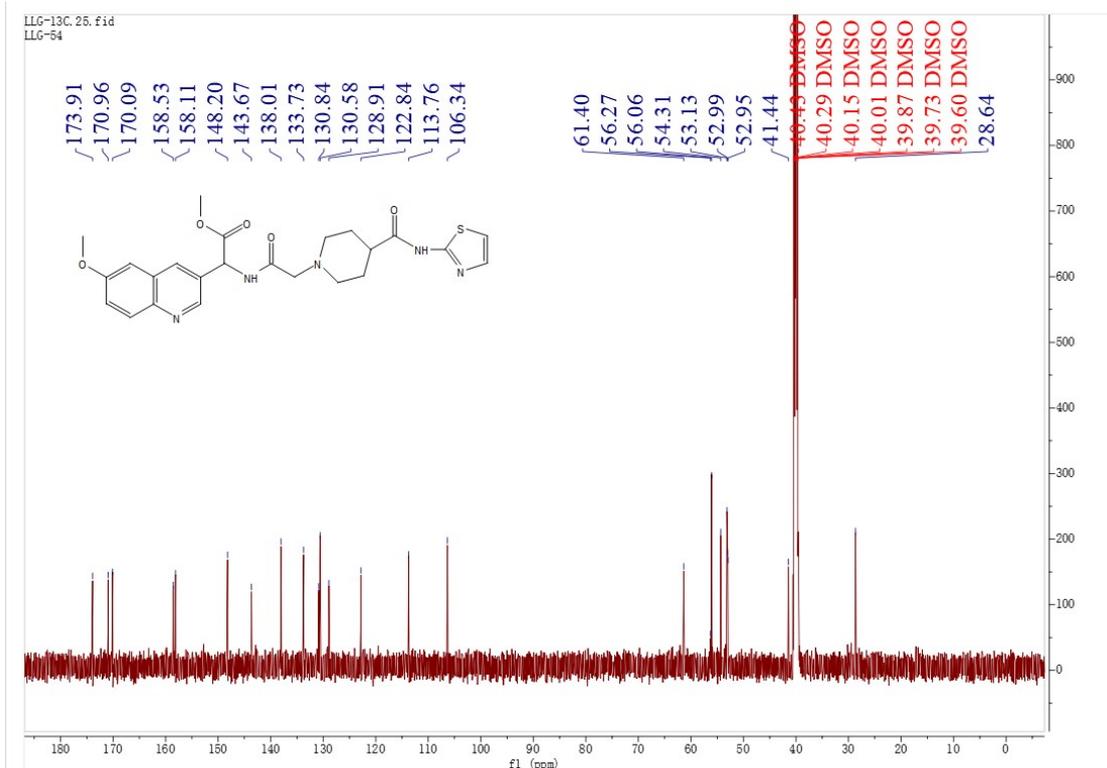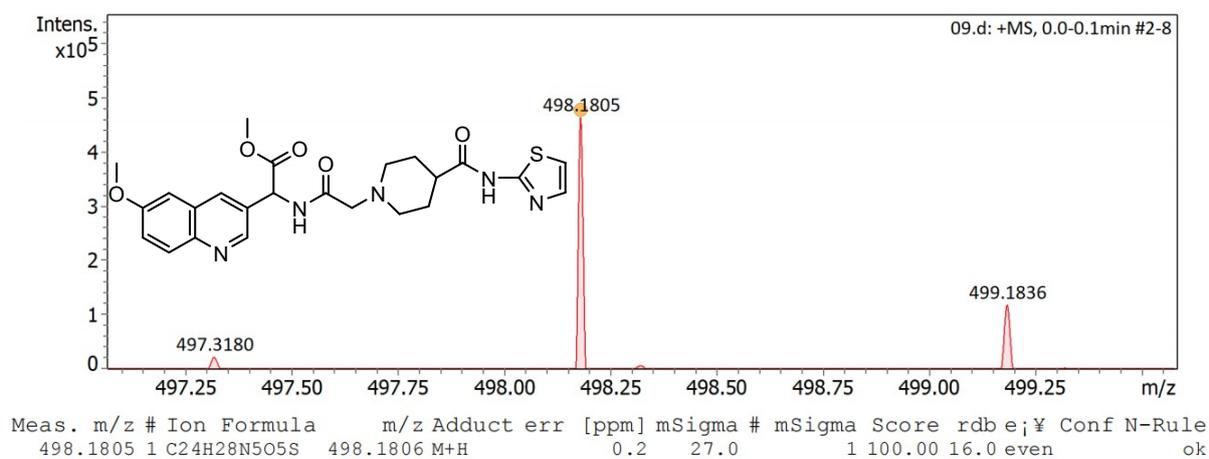
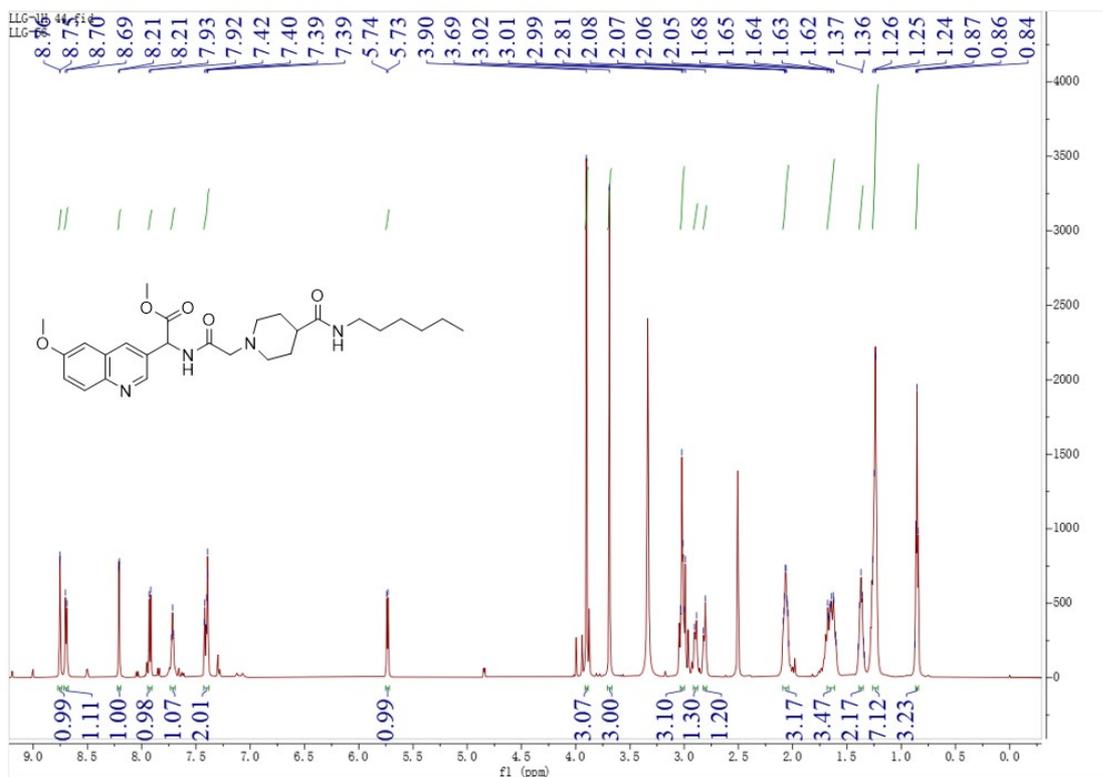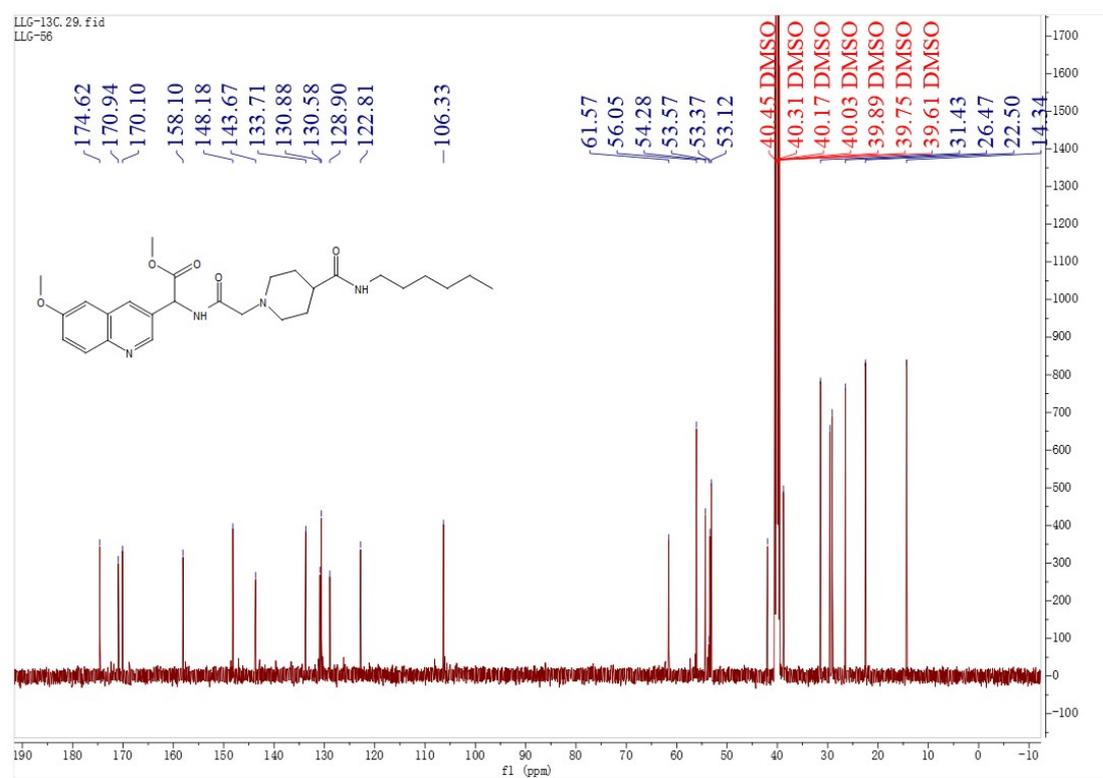HRMS spectrum of compound **14i**
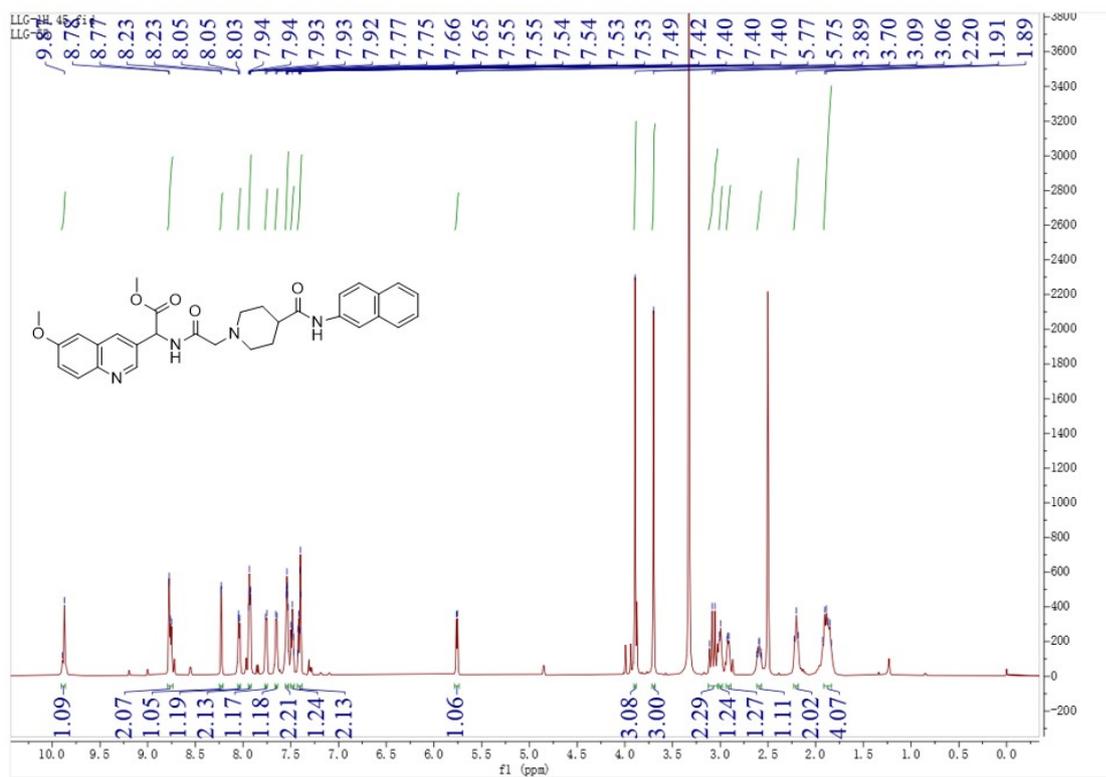
^{1}H NMR spectrum of compound **14j**

^{13}C NMR spectrum of compound **14j**
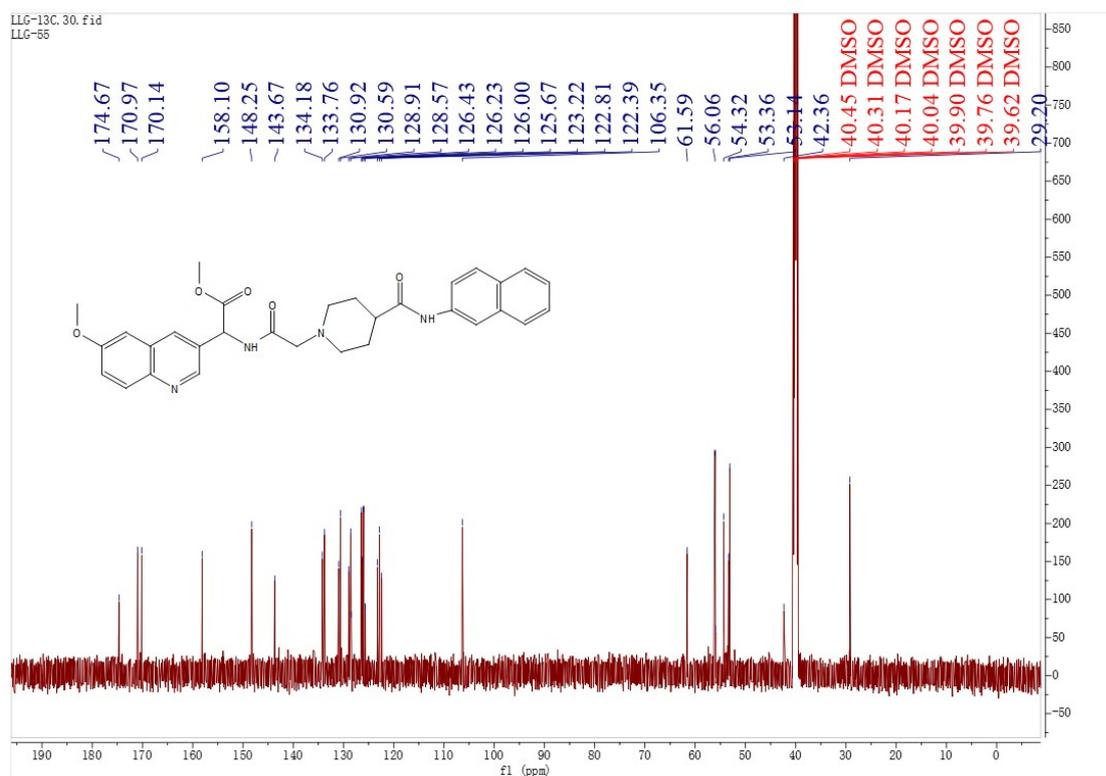
HRMS spectrum of compound **14j**
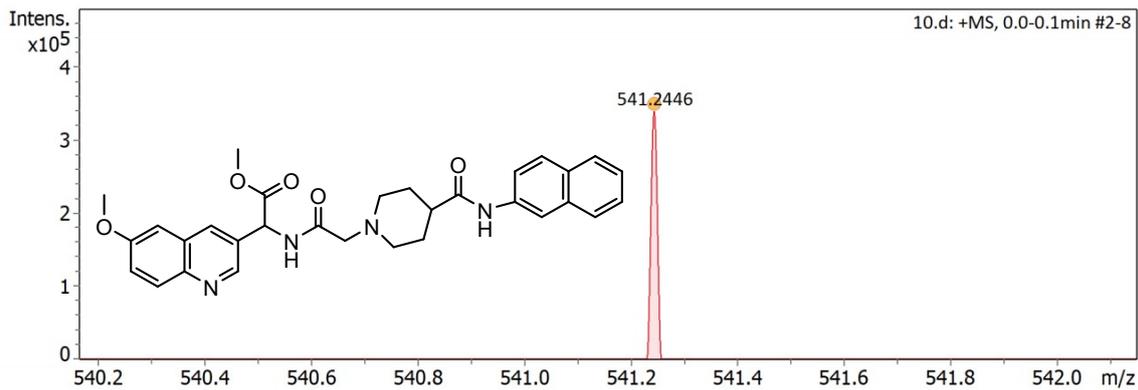
Meas. m/z	#	Ion Formula	m/z	Adduct	err [ppm]	mSigma	#	mSigma	Score	rdb	e¡¥	Conf	N-Rule
541.2446	1	C31H33N4O5	541.2445	M+H	-0.0	24.9		1	100.00	18.0	even		ok

^{1}H NMR spectrum of compound **14k**

^{13}C NMR spectrum of compound **14k**
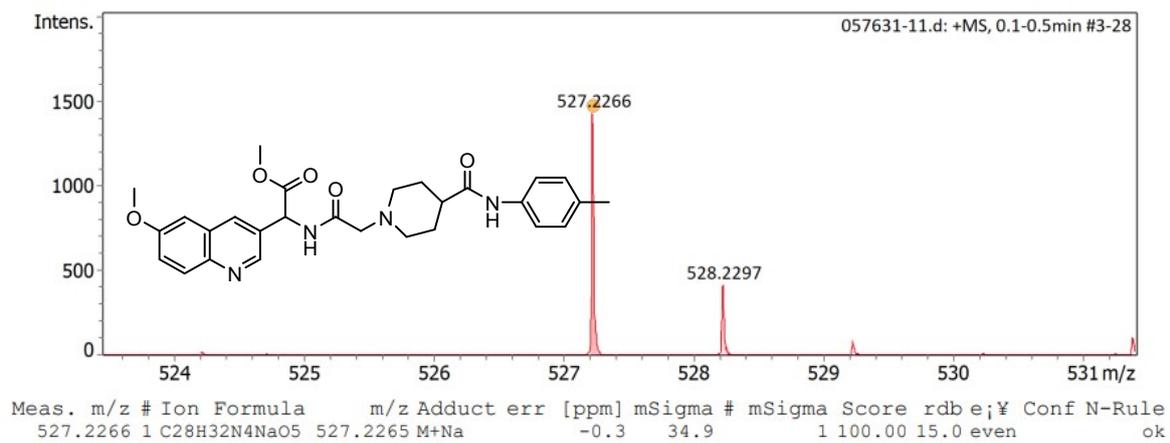
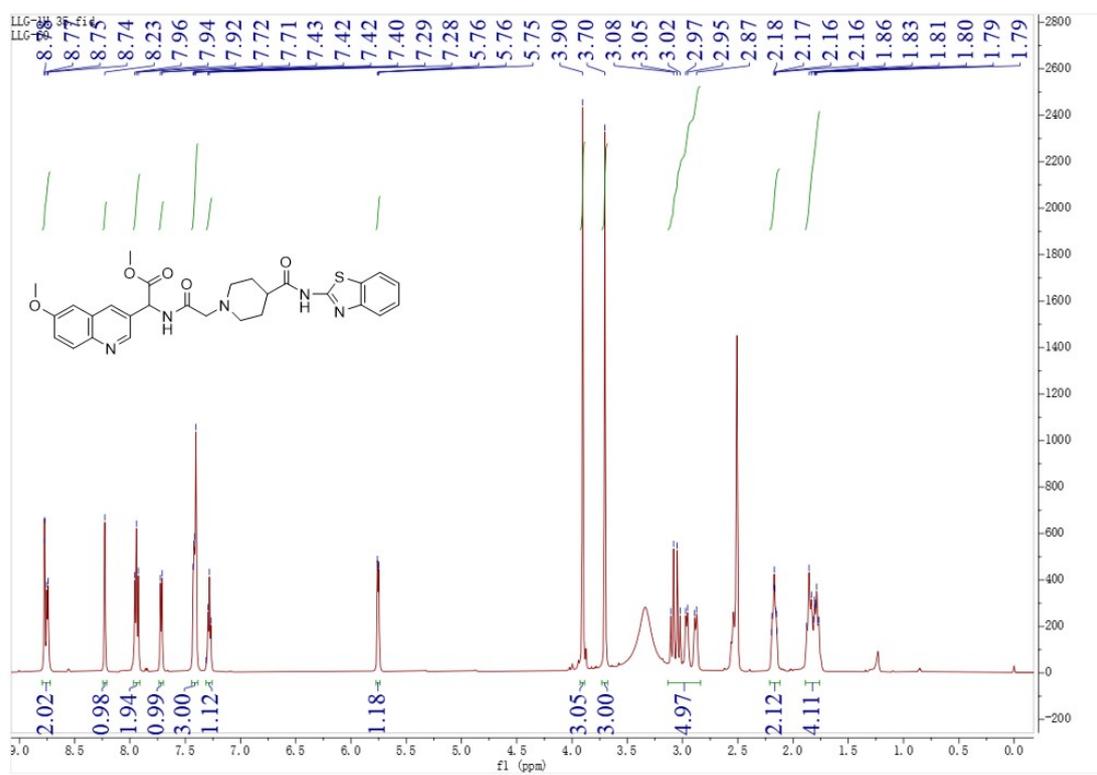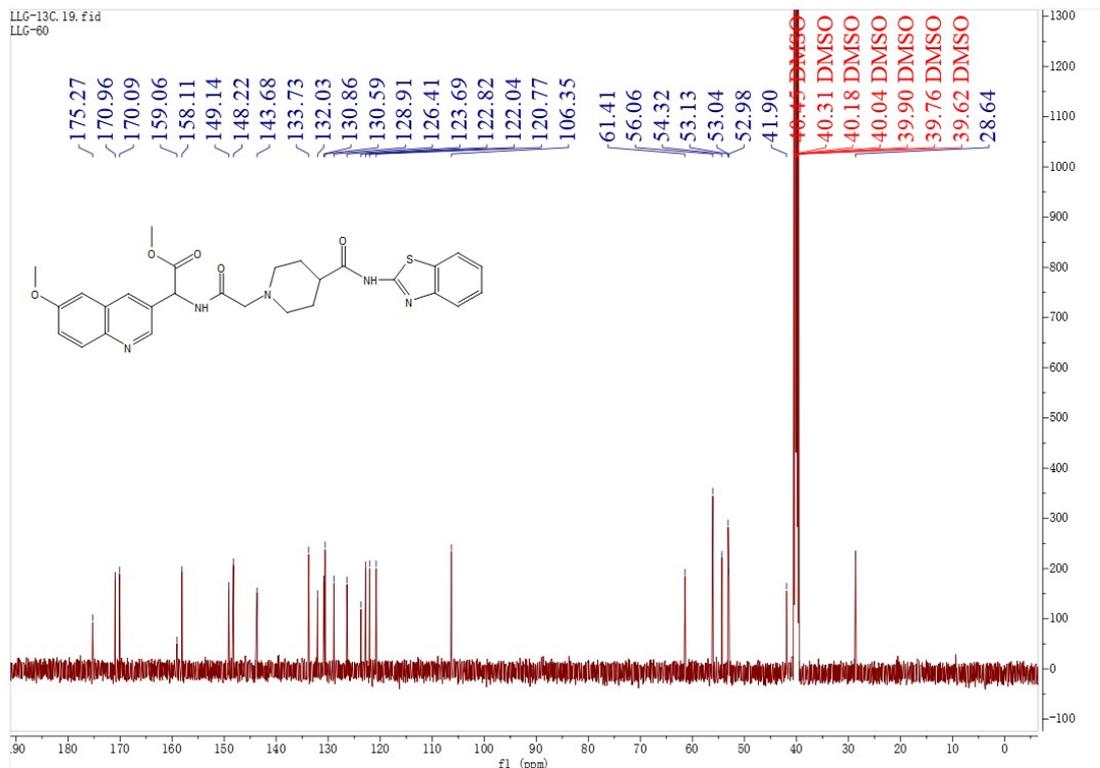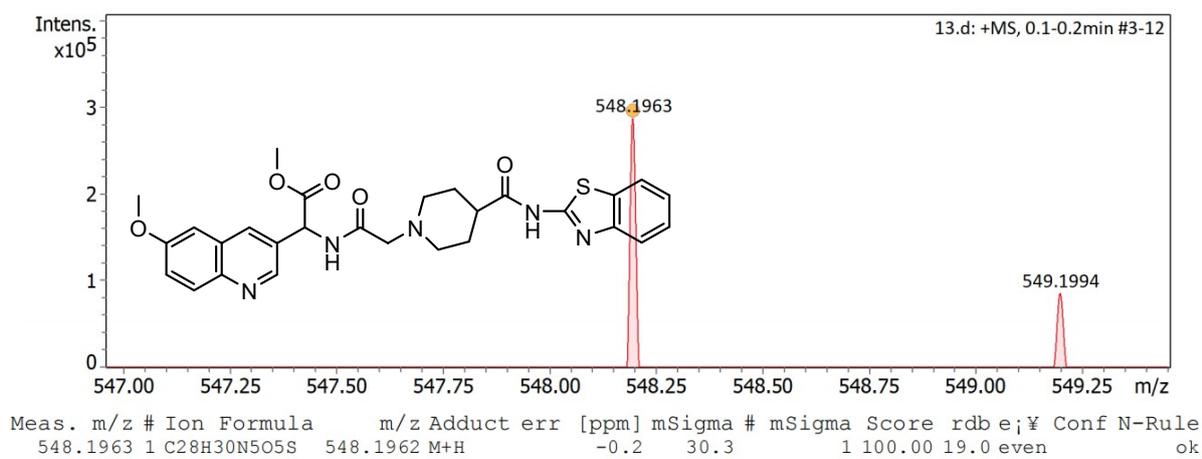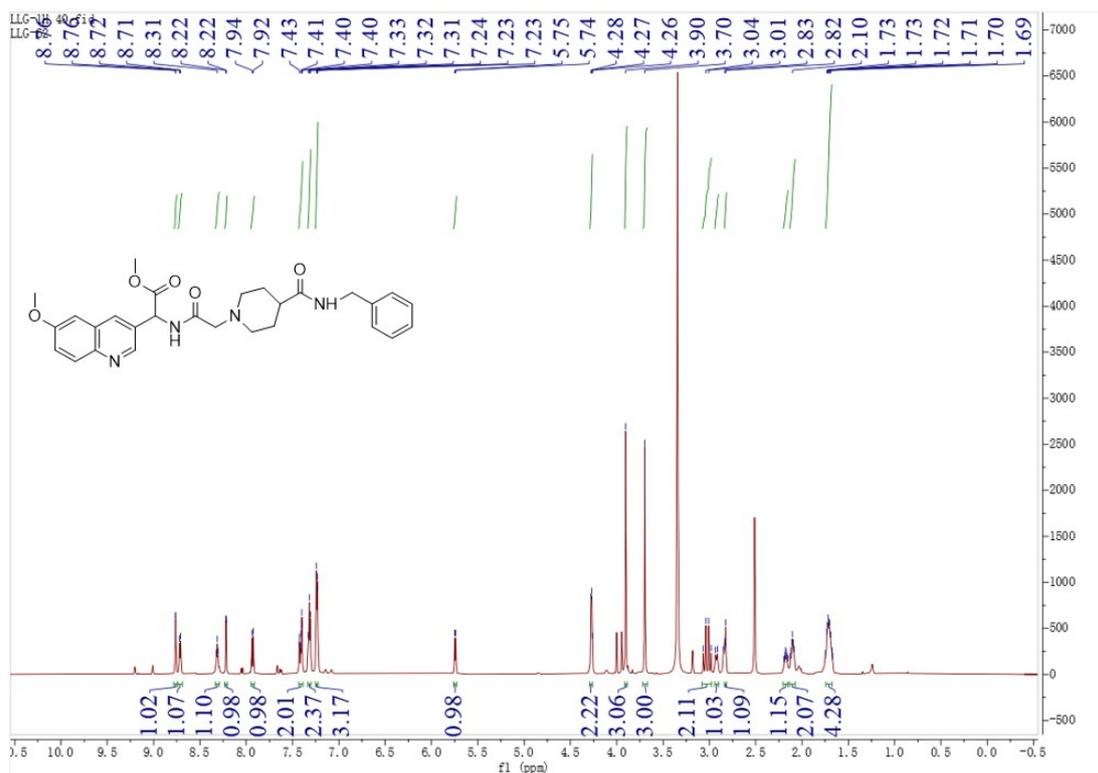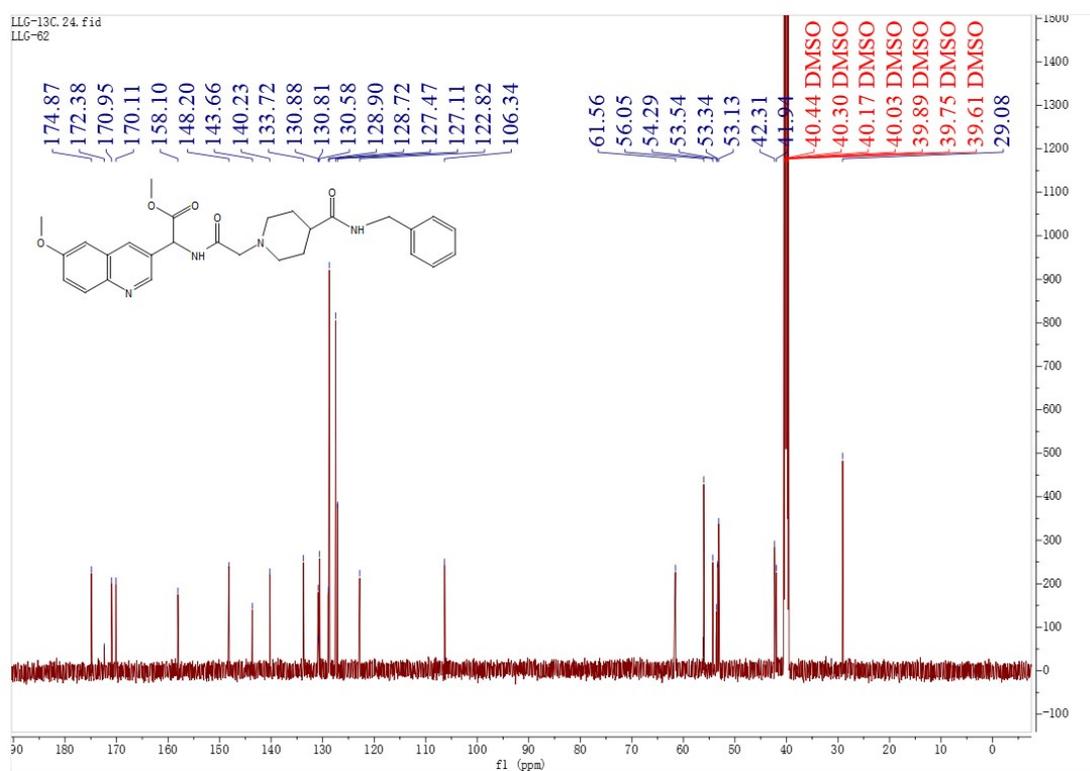HRMS spectrum of compound **14k**

¹H NMR spectrum of compound **14l**

¹³C NMR spectrum of compound **14l**

HRMS spectrum of compound **14l**

Meas. m/z	#	Ion Formula	m/z	Adduct	err [ppm]	mSigma	# mSigma	Score	rdb	e¡¥	Conf	N-Rule
499.2918	1	C27H39N4O5	499.2915	M+H	-0.5	20.0	1	100.00	11.0	even		ok
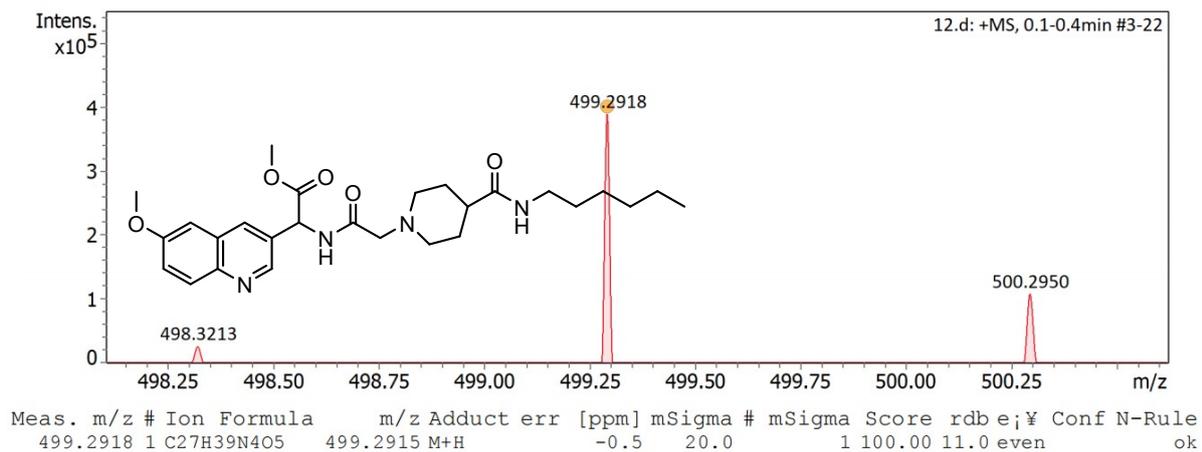
^{1}H NMR spectrum of compound **14m**

¹³C NMR spectrum of compound **14m**

Meas. m/z	#	Ion Formula	m/z Adduct	err [ppm]	mSigma	# mSigma Score	rdb	e¯ Conf N-Rule
548.1963	1	C28H30N5O5S	548.1962 M+H	-0.2	30.3	1 100.00	19.0	even ok

HRMS spectrum of compound **14m**

^{1}H NMR spectrum of compound **14n**

^{13}C NMR spectrum of compound **14n**
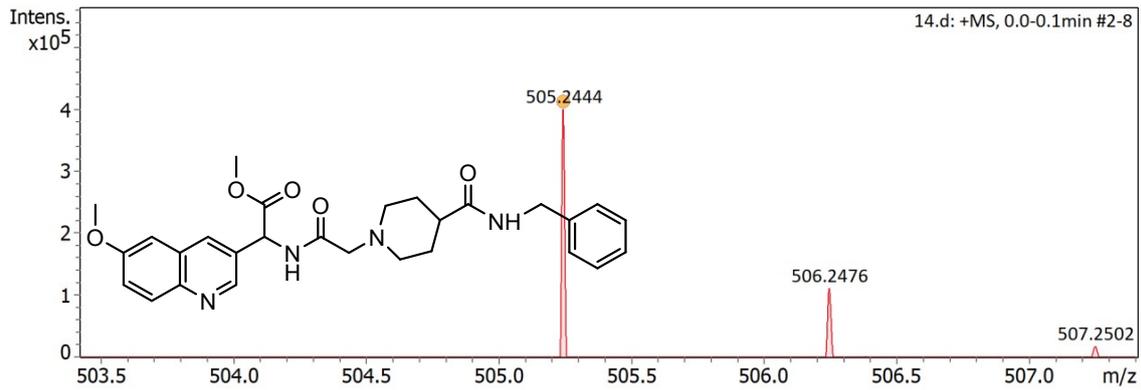
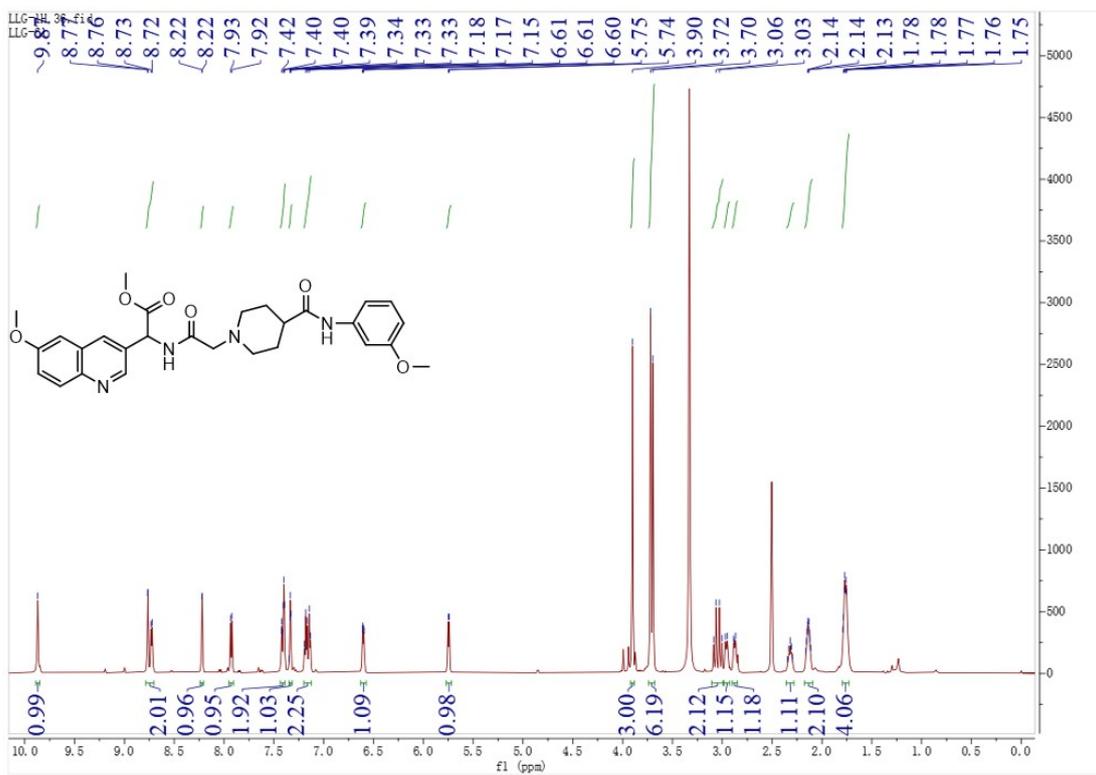HRMS spectrum of compound **14n**

¹H NMR spectrum of compound **14o**
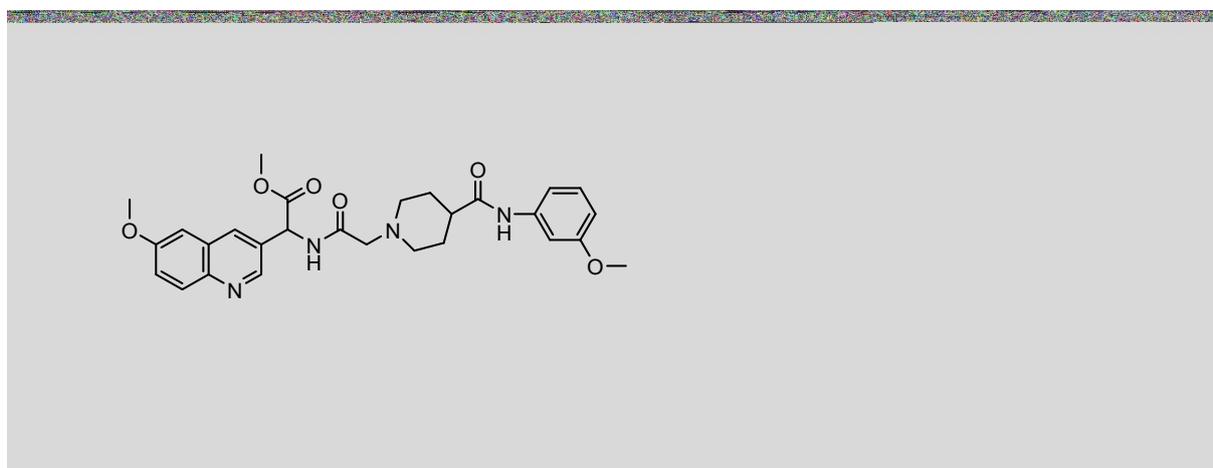
174.05
170.95
170.09
159.96
158.11
148.20
143.67
141.05
133.73
130.88
130.58
129.85
128.91
122.82
111.89
108.96
106.34
105.38
61.52
56.06
55.41
54.31
53.24
53.19
53.13
42.92
40.45 DMSO
40.31 DMSO
40.18 DMSO
40.04 DMSO
39.90 DMSO
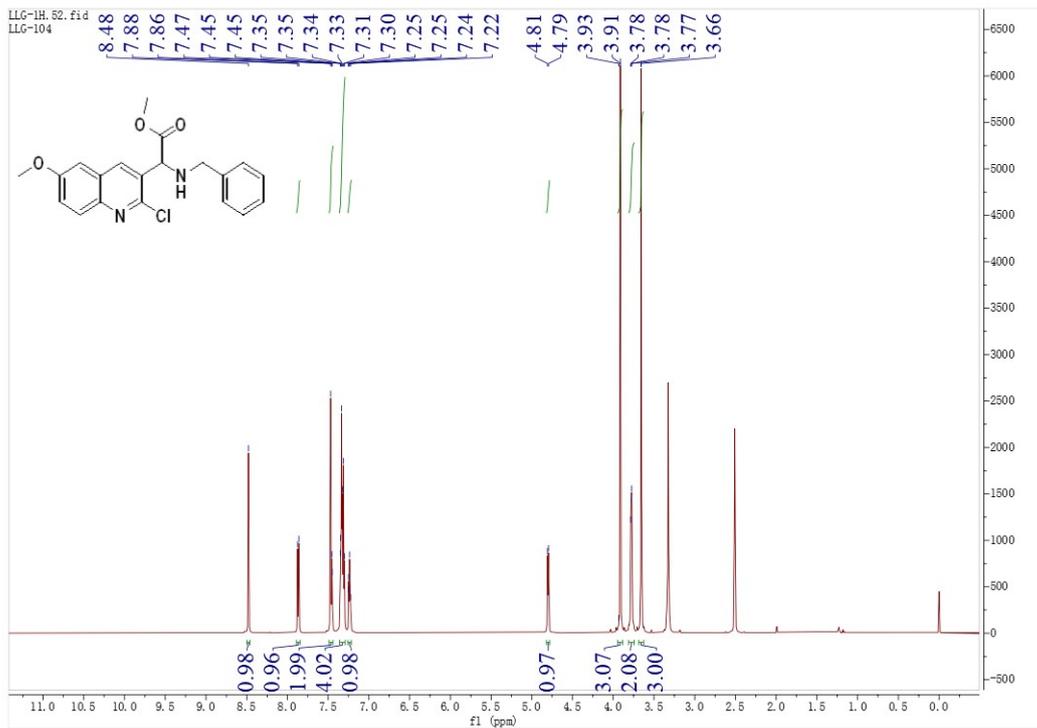39.76 DMSO
39.62 DMSO
28.96

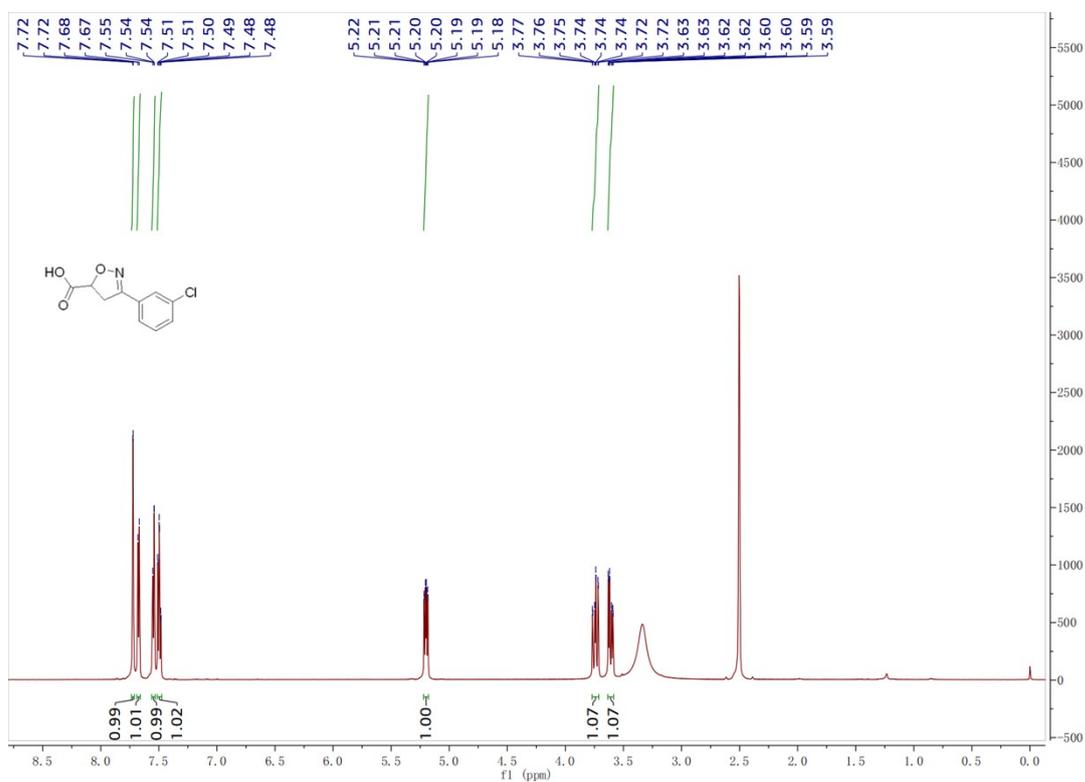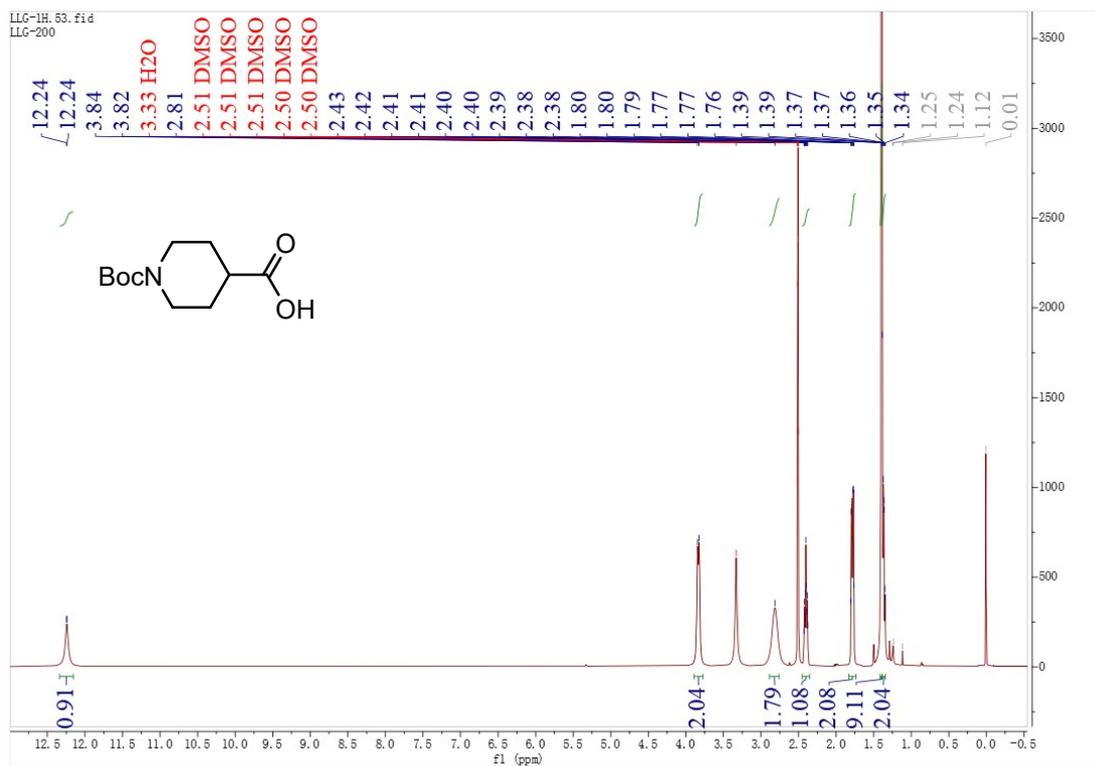f1 (ppm)
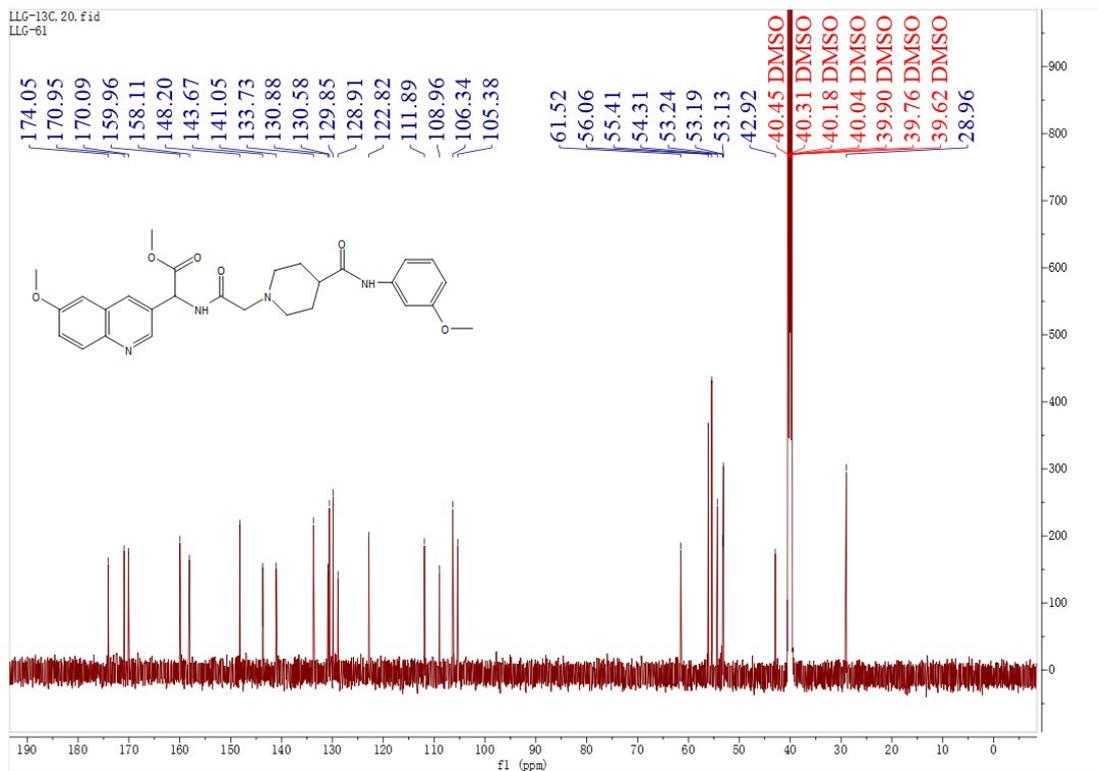
^{13}C NMR spectrum of compound **14o**

HRMS spectrum of compound **14o**

4. ^{1}H NMR spectra of compounds **5, 7i, 11,** and **13**

^{1}H NMR spectrum of compound **5**

^{1}H NMR spectrum of compound **7i**

¹H NMR spectrum of compound **11**

¹H NMR spectrum of compound **13**