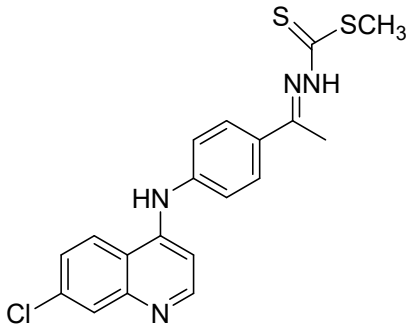
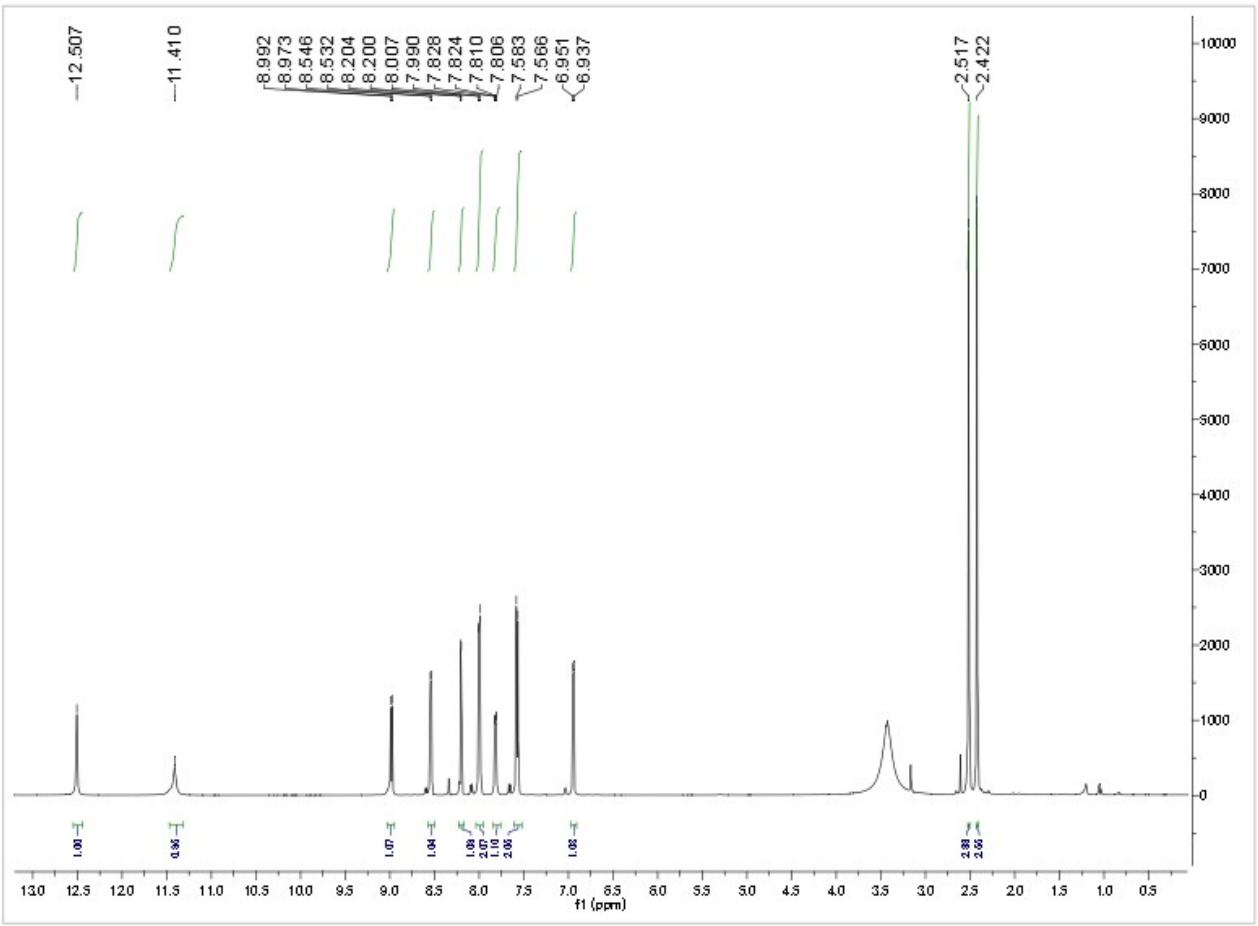
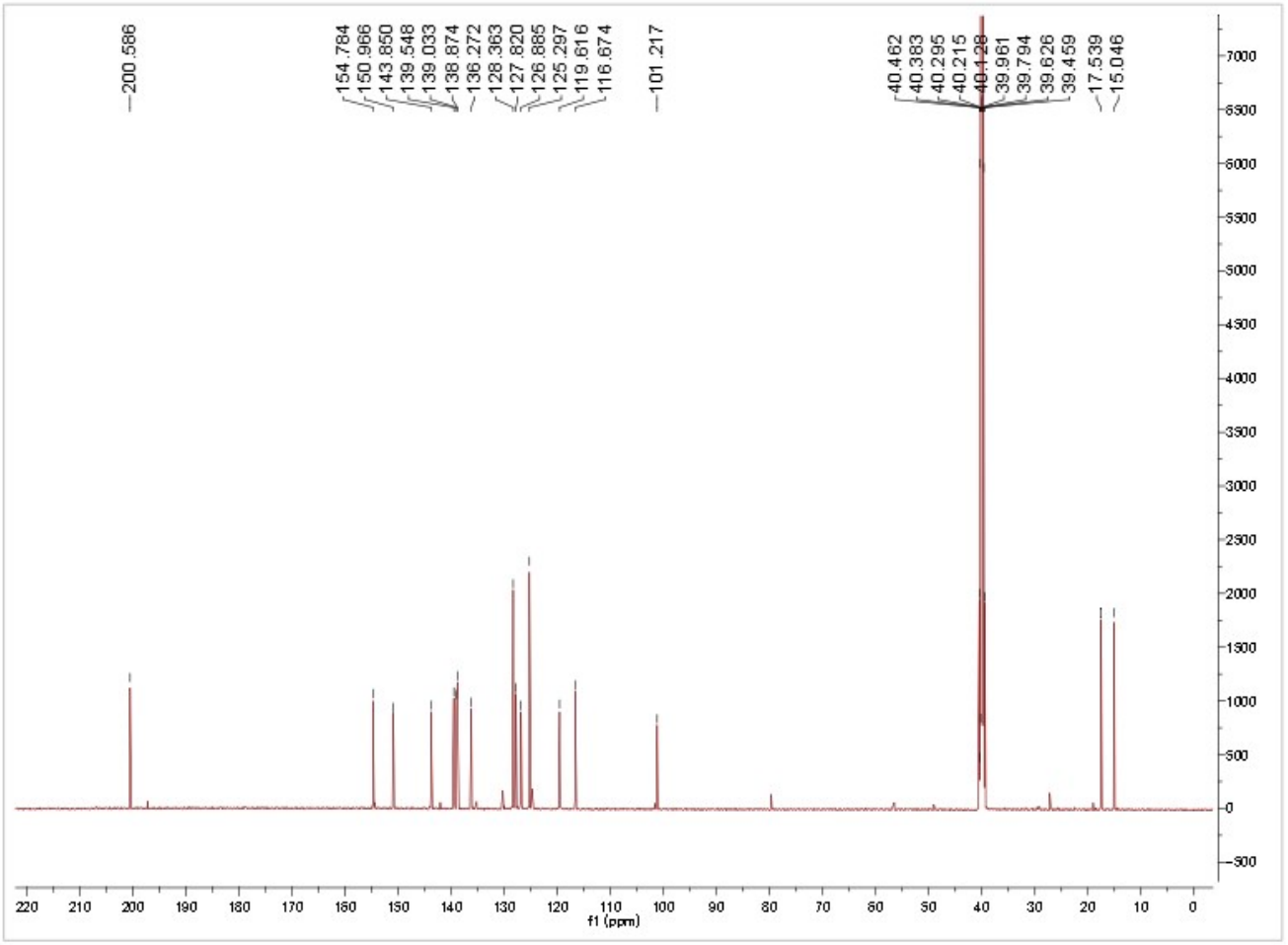
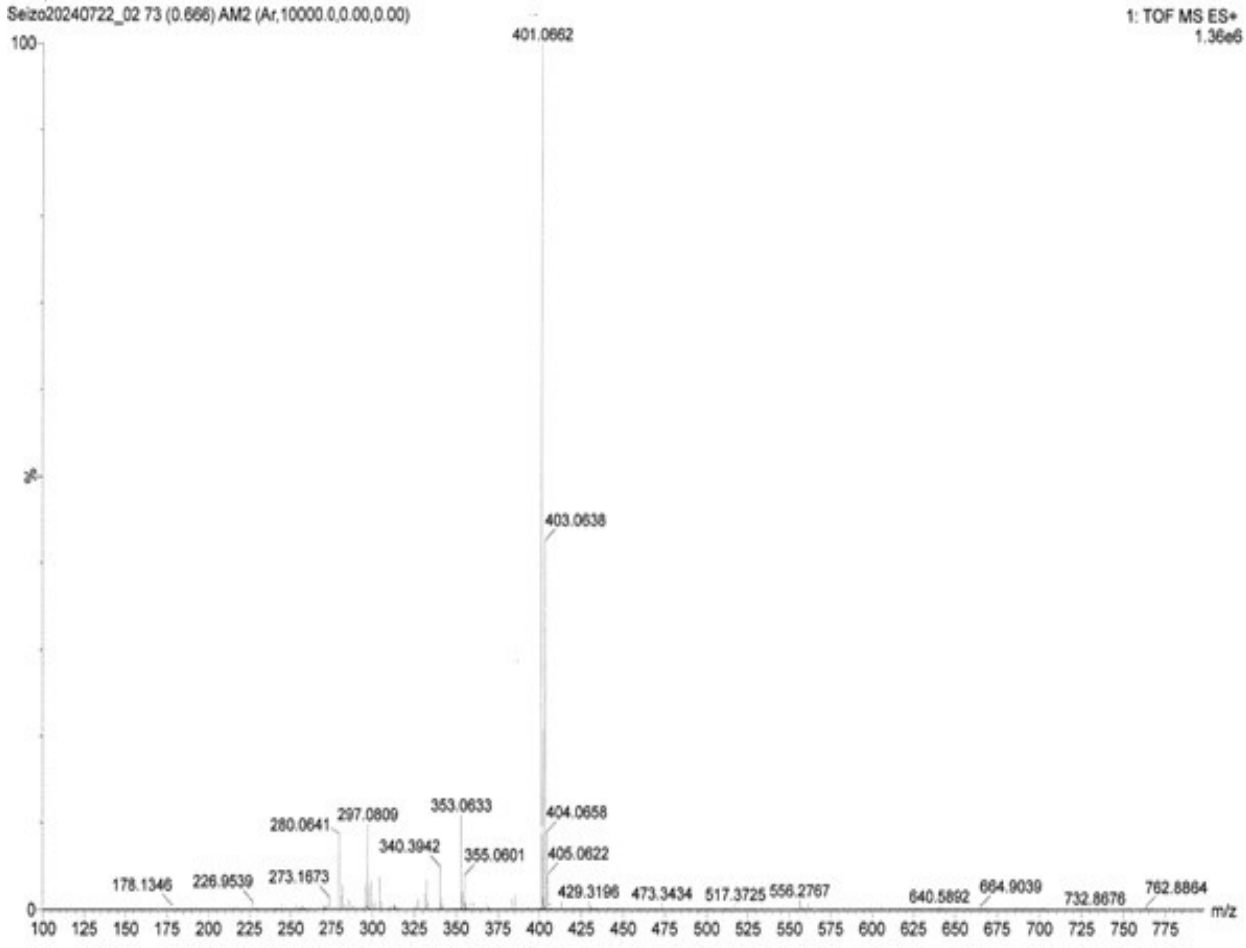
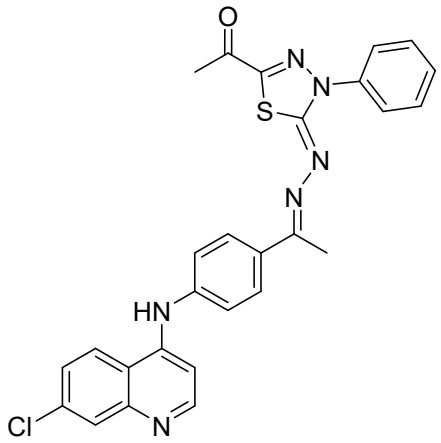


5

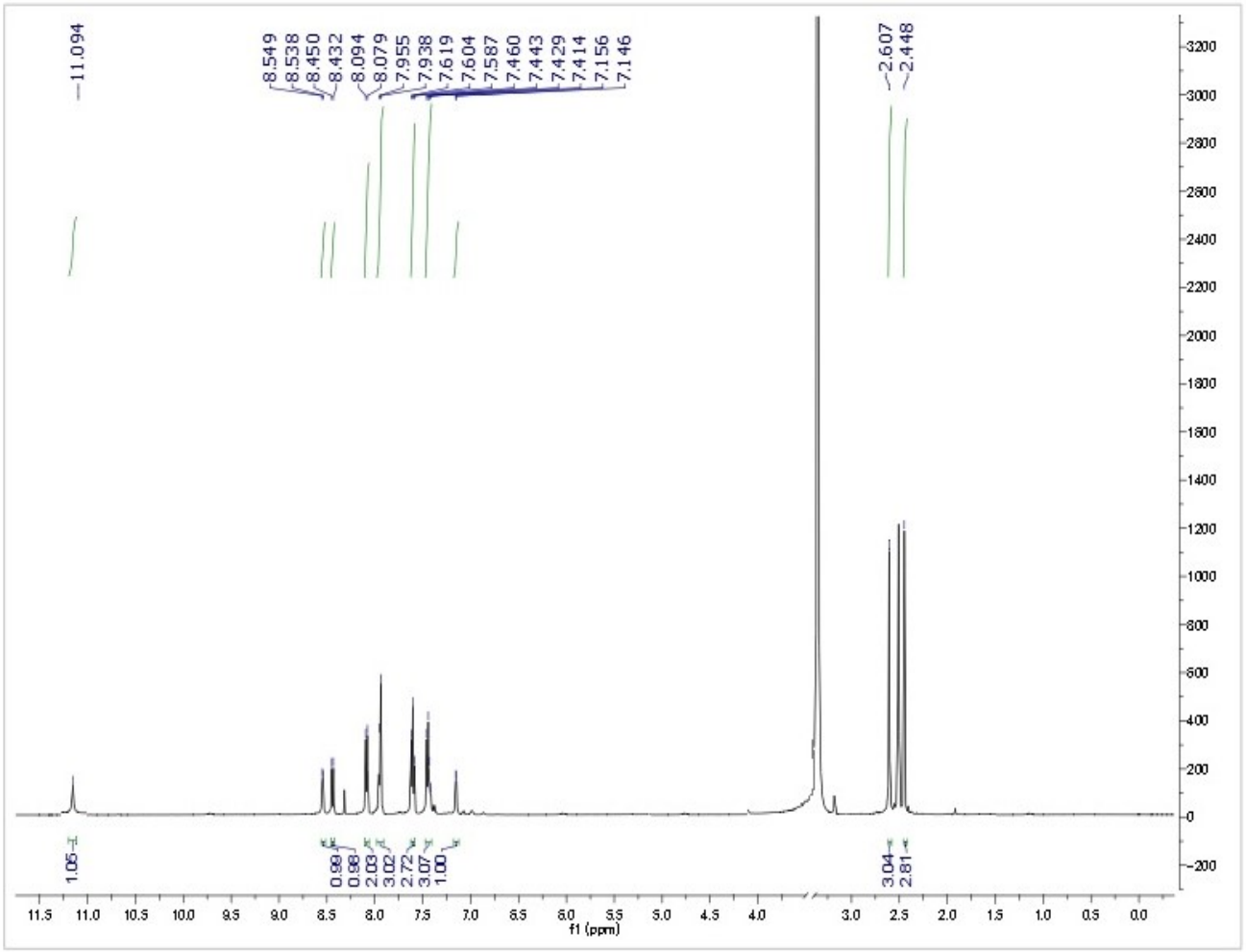


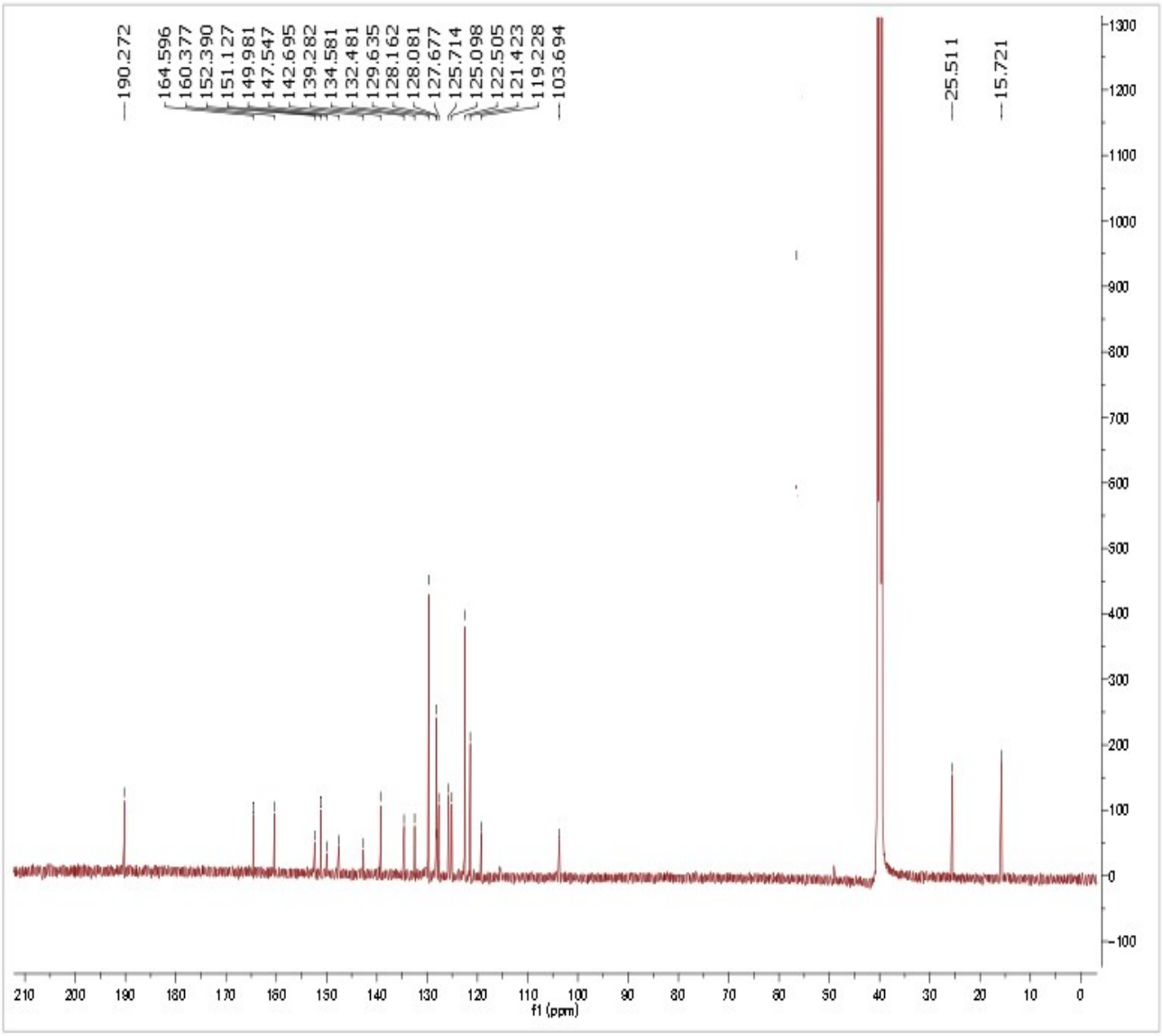

200.586
154.784
150.966
143.850
139.548
139.033
138.874
136.272
128.363
127.820
126.885
125.297
119.616
116.674
101.217
40.462
40.383
40.295
40.215
40.128
39.961
39.794
39.626
39.459
17.539
15.046
f1 (ppm)

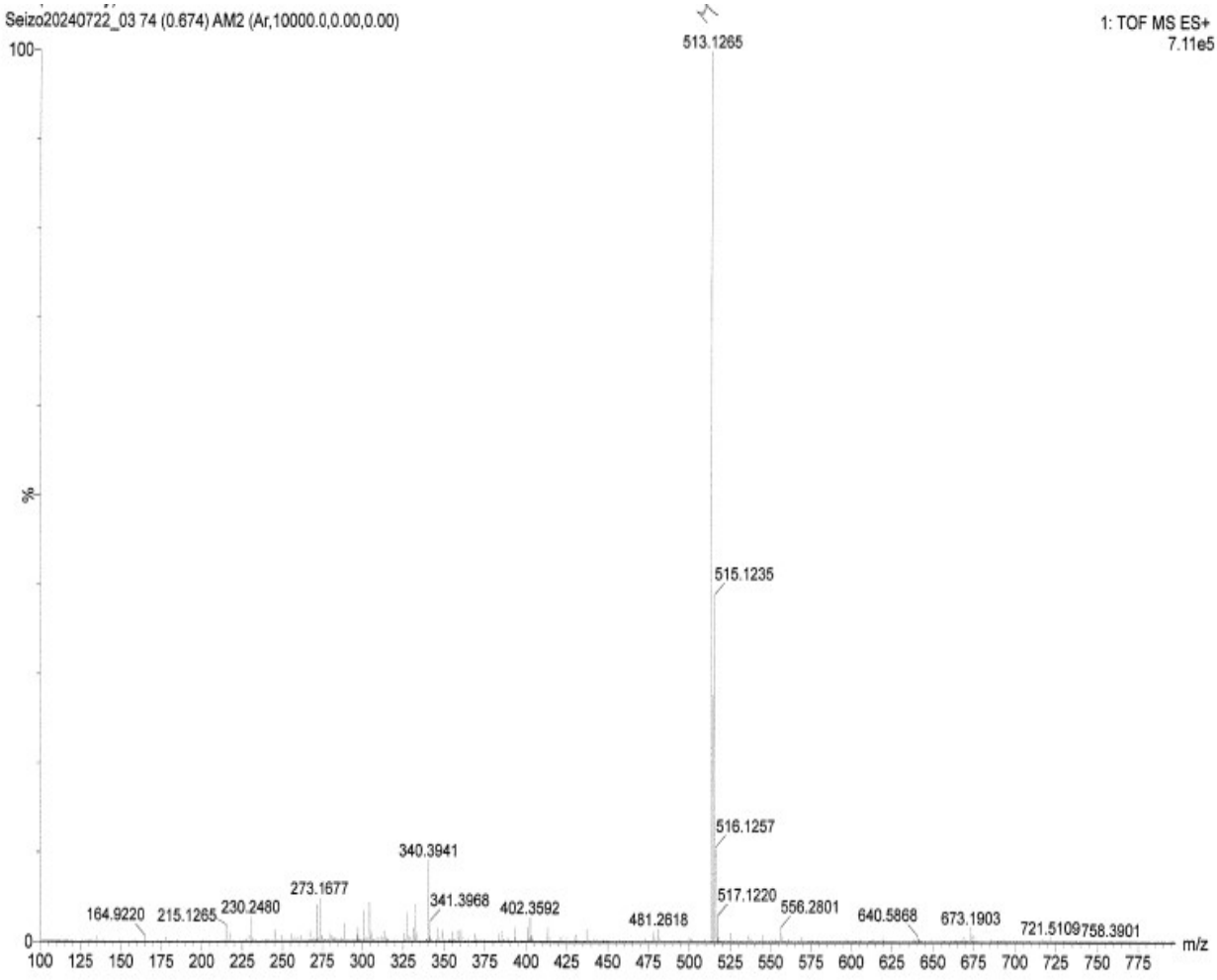

Selzo20240722_02 73 (0.666) AM2 (Ar,10000.0,0.00,0.00)
1: TOF MS ES+
1.36e6
100
%
0
401.0662
403.0638
280.0641
297.0809
353.0633
404.0658
340.3942
355.0601
405.0622
178.1346
226.9539
273.1673
429.3196
473.3434
517.3725
556.2767
640.5892
664.9039
732.8676
762.8864
m/z
100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500 525 550 575 600 625 650 675 700 725 750 775

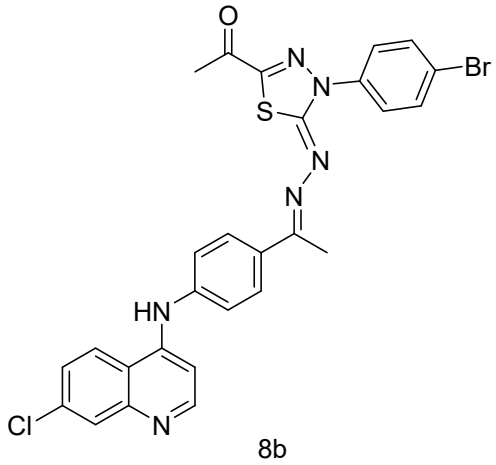

O
N
N
S
N
N
HN
Cl
N

8a

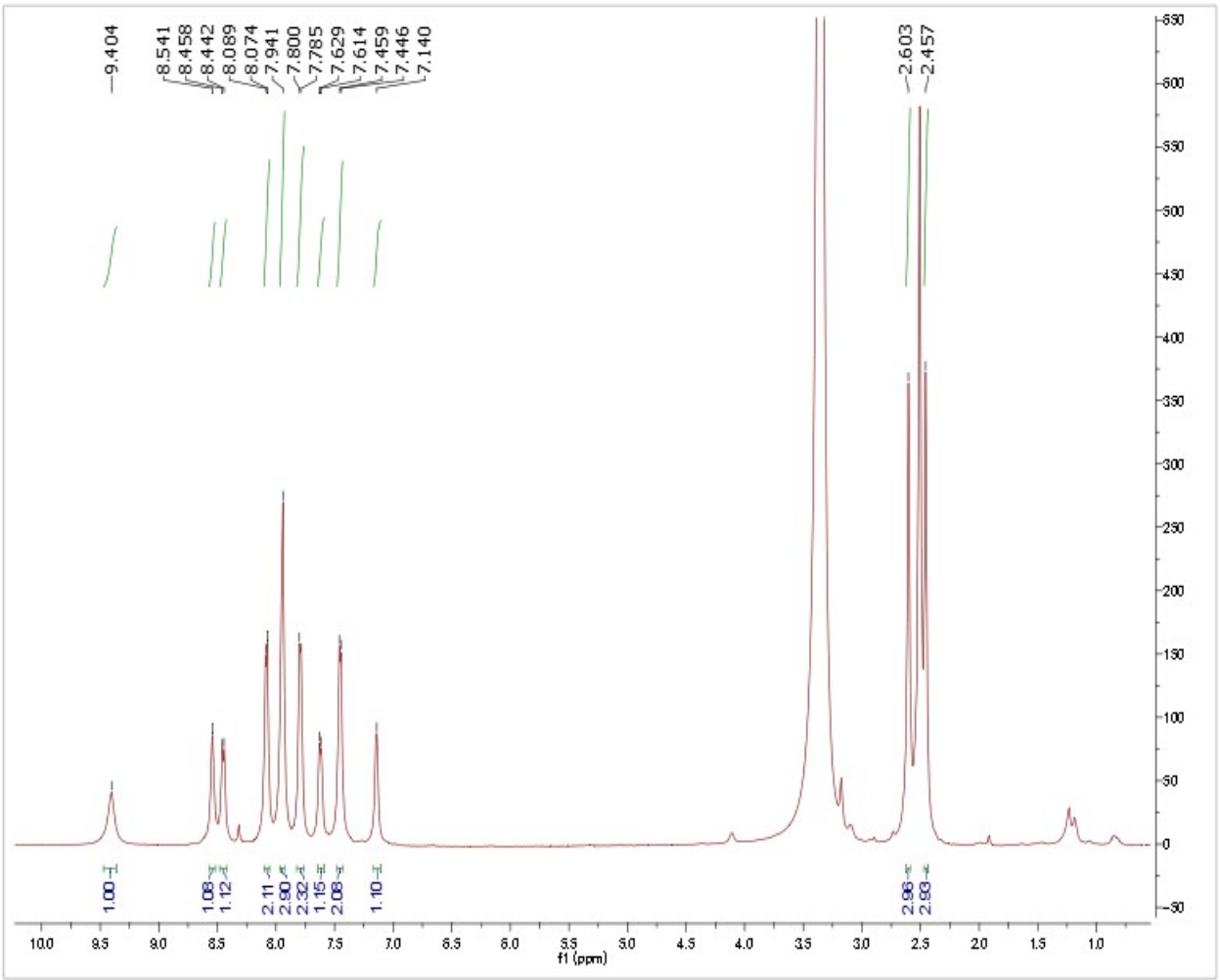

11.094
8.549
8.538
8.450
8.432
8.094
8.079
7.955
7.938
7.619
7.604
7.587
7.460
7.443
7.429
7.414
7.156
7.146
2.607
2.448
1.05
0.99
0.98
2.03
3.02
2.72
3.07
1.00
3.04
2.81
f1 (ppm)

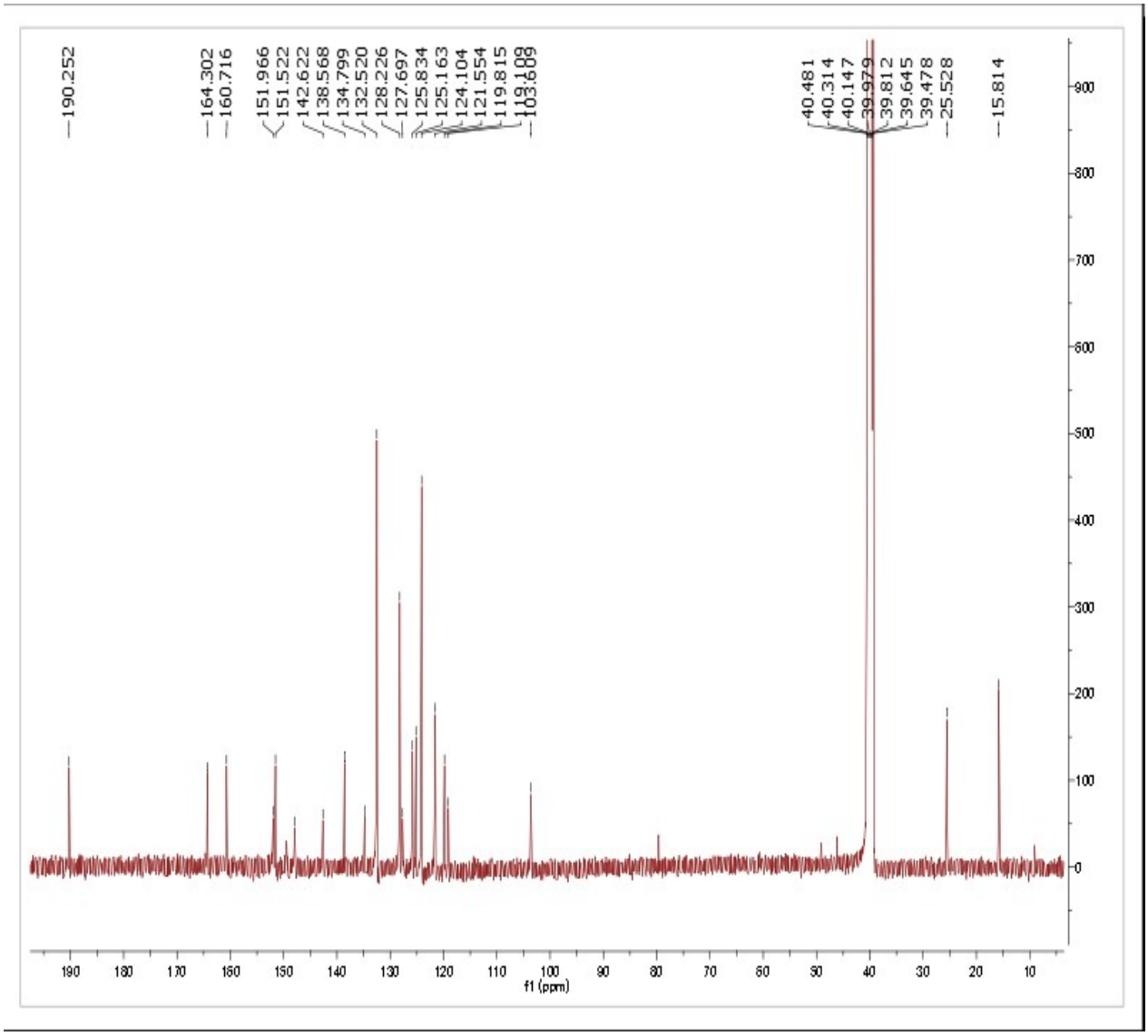

190.272
164.596
160.377
152.390
151.127
149.981
147.547
142.695
139.282
134.581
132.481
129.635
128.162
128.081
127.677
125.714
125.098
122.505
121.423
119.228
103.694
25.511
15.721
f1 (ppm)

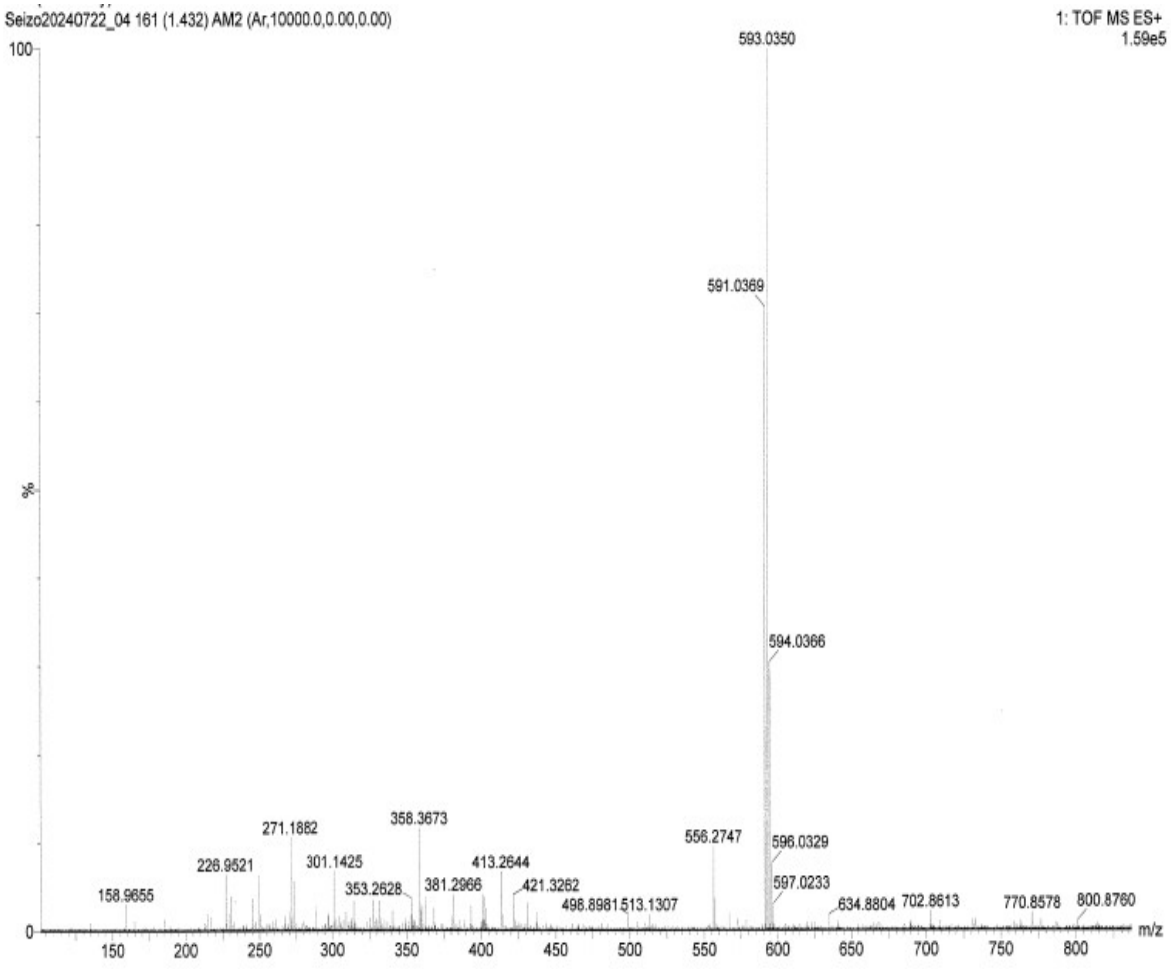

Seizo20240722_03 74 (0.674) AM2 (Ar,10000.0,0.00,0.00)
1: TOF MS ES+
7.11e5
100
%
0
513.1265
515.1235
516.1257
517.1220
340.3941
273.1677
341.3968
164.9220
215.1265
230.2480
402.3592
481.2618
556.2801
640.5868
673.1903
721.5109
758.3901
m/z
100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500 525 550 575 600 625 650 675 700 725 750 775

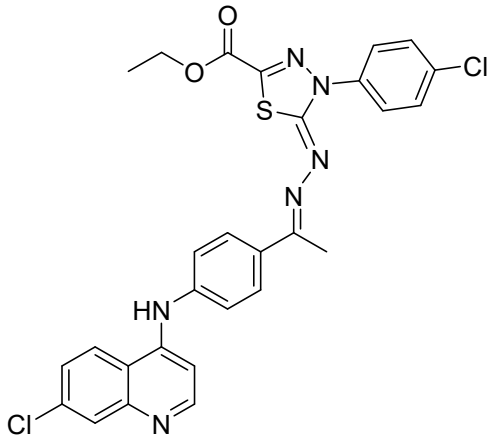

O
N
N
S
Br
N
N
HN
Cl
N

8b

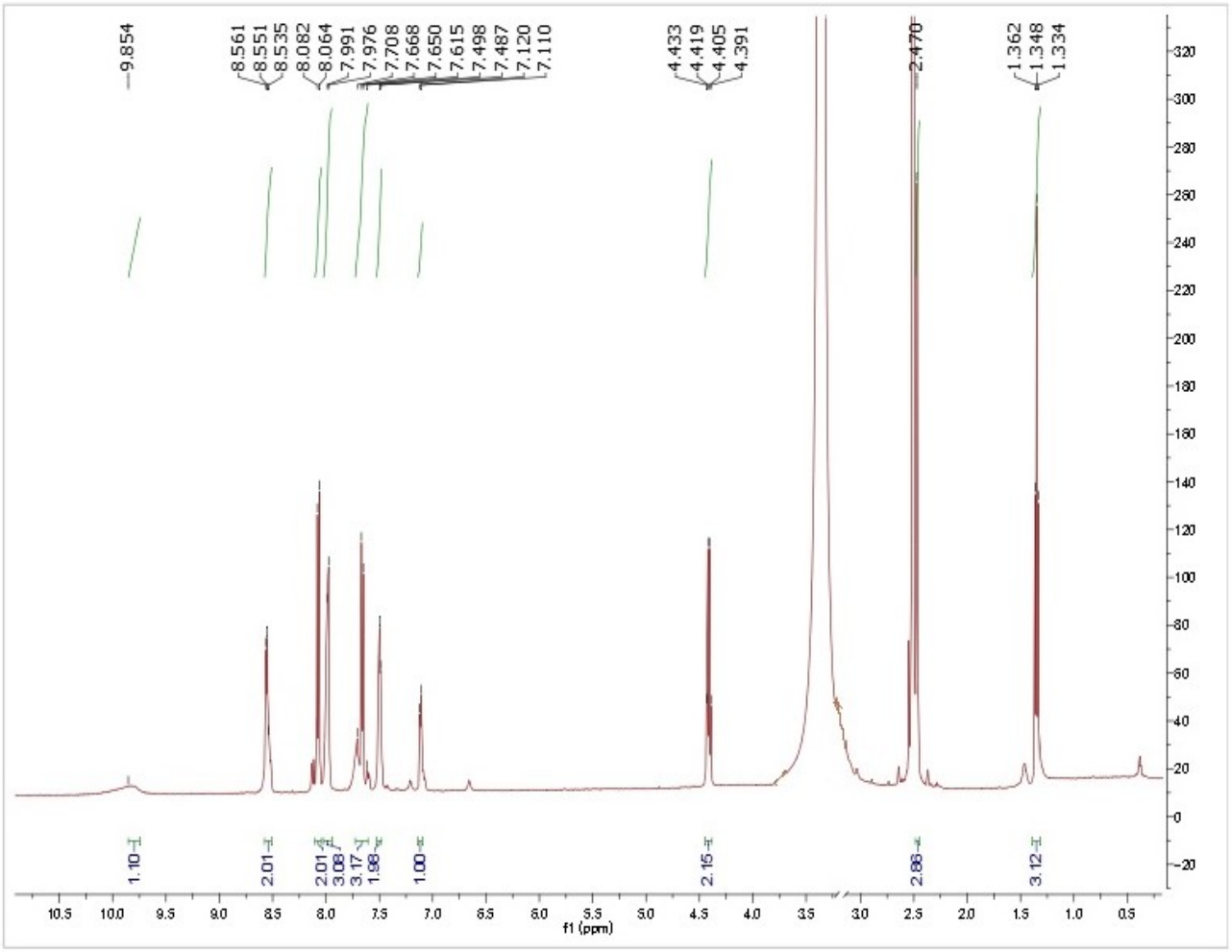

9.404
8.541
8.458
8.442
8.089
8.074
7.941
7.800
7.785
7.629
7.614
7.459
7.446
7.140
2.603
2.457
1.00
1.08
1.12
2.11
2.90
2.32
1.15
2.08
1.10
2.96
2.93
f1 (ppm)

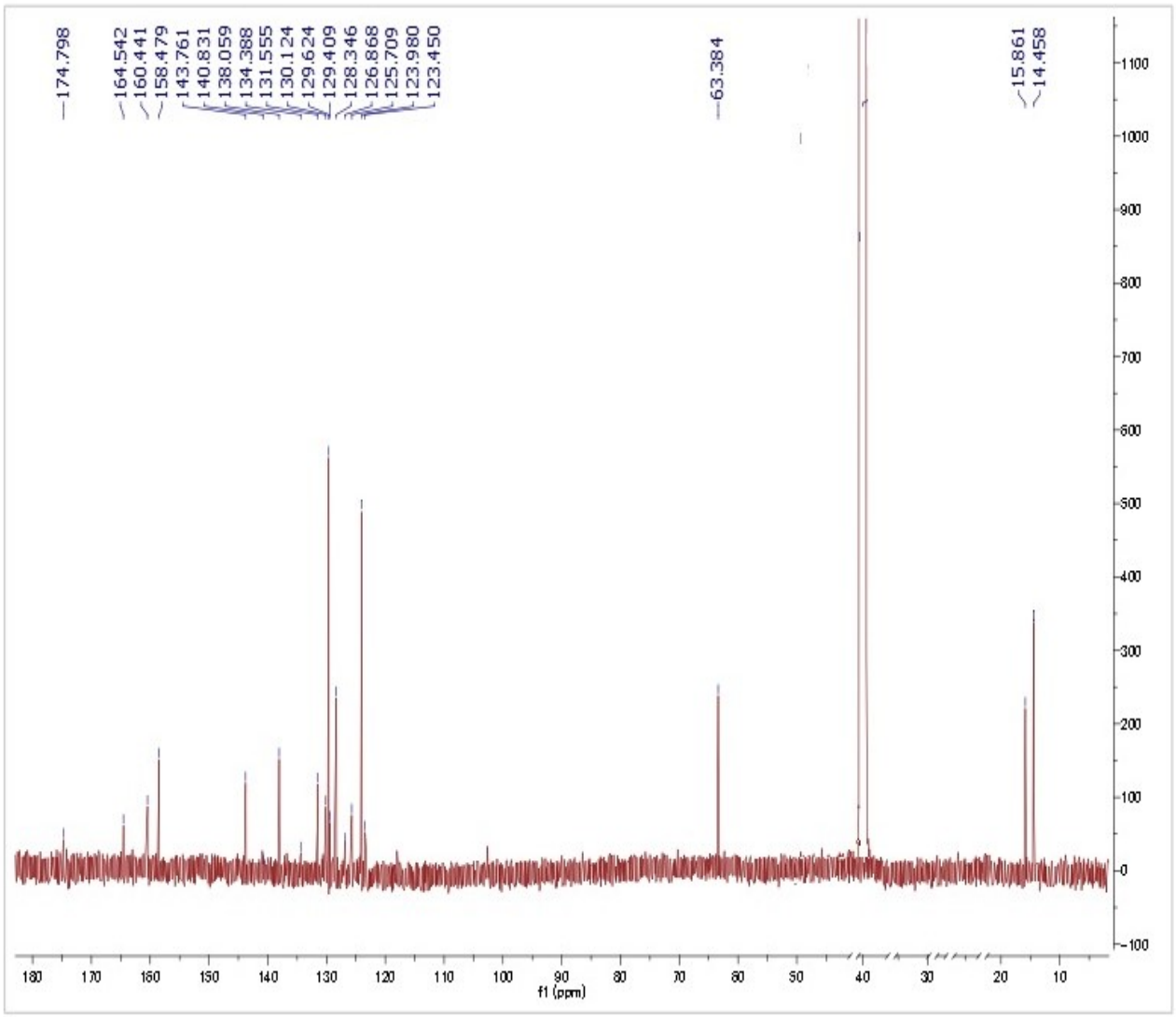

190.252
164.302
160.716
151.966
151.522
142.622
138.568
134.799
132.520
128.226
127.697
125.834
125.163
124.104
121.554
119.815
119.109
103.609
40.481
40.314
40.147
39.979
39.812
39.645
39.478
25.528
15.814
f1 (ppm)

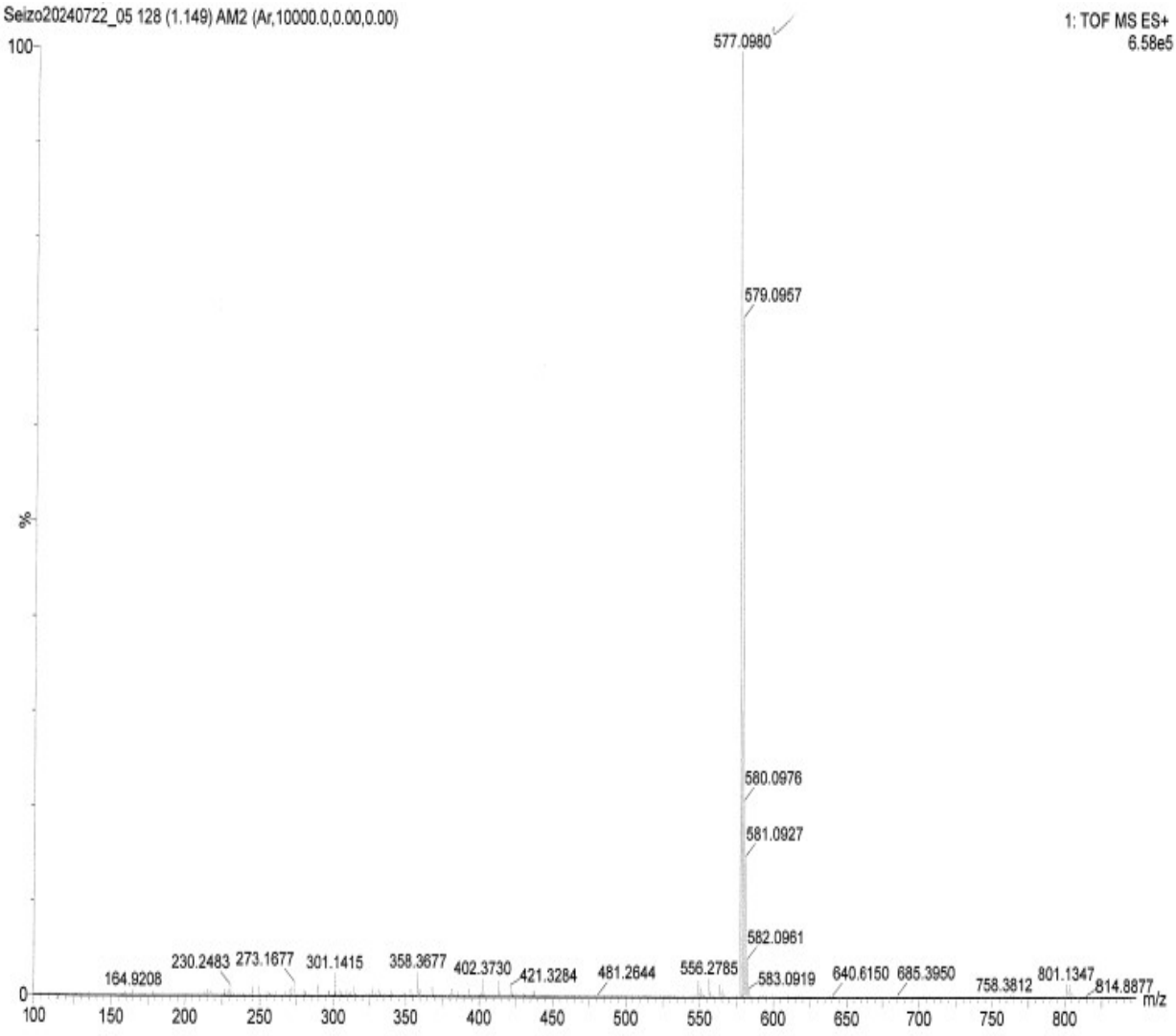

Seizo20240722_04 161 (1.432) AM2 (Ar,10000.0,0.00,0.00)
1: TOF MS ES+
1.59e5
100
%
0
593.0350
591.0369
594.0366
596.0329
597.0233
556.2747
358.3673
271.1882
301.1425
226.9521
158.9655
353.2628
381.2966
413.2644
421.3262
498.8981
513.1307
634.8804
702.8613
770.8578
800.8760
150
200
250
300
350
400
450
500
550
600
650
700
750
800
m/z

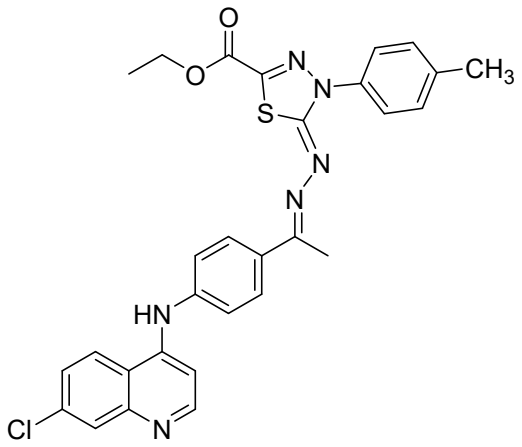

O
O
N
N
Cl
S
N
N
HN
Cl
N

8c

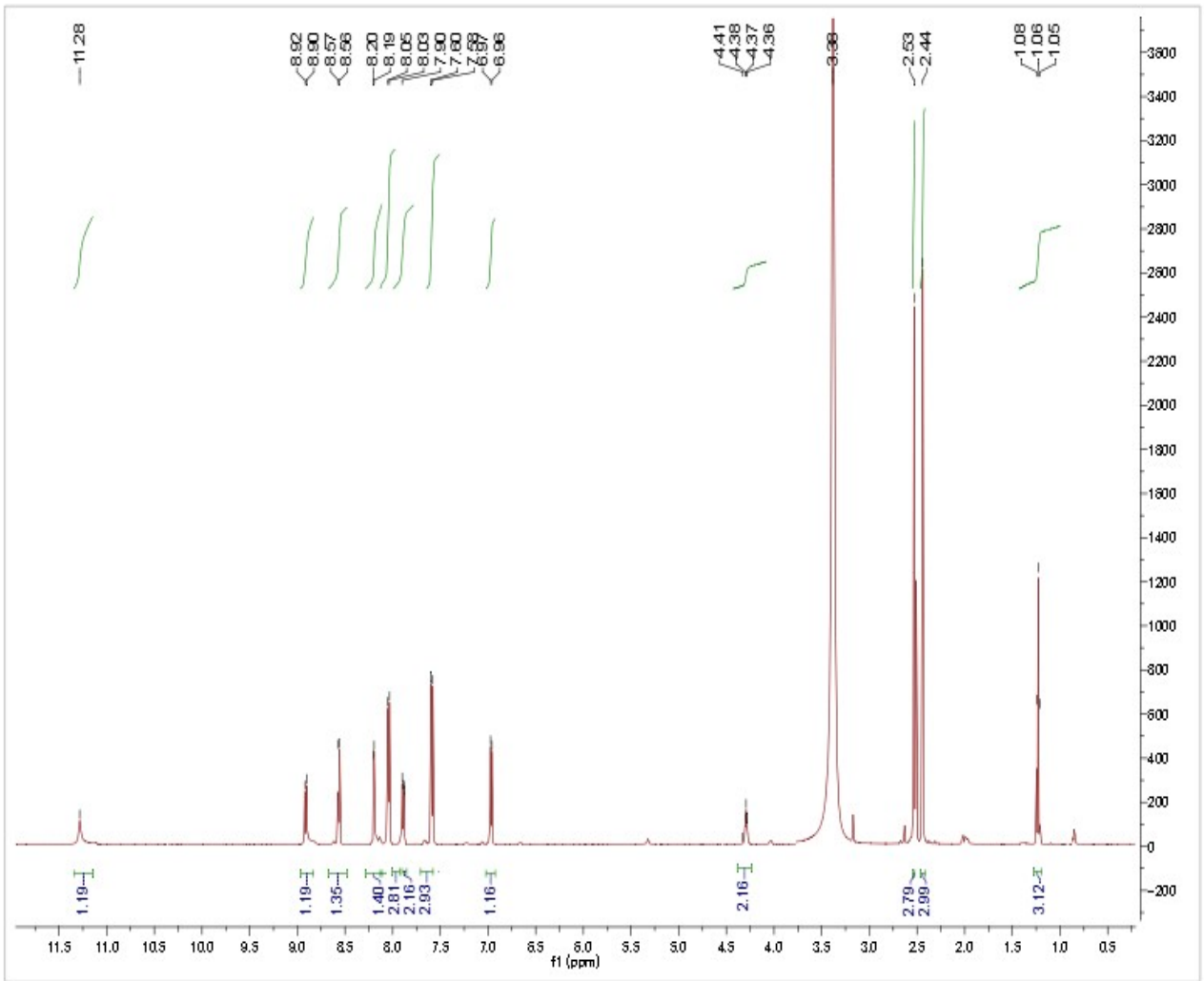

9.854
8.561
8.551
8.535
8.082
8.064
7.991
7.976
7.708
7.668
7.650
7.615
7.498
7.487
7.120
7.110
4.433
4.419
4.405
4.391
2.470
1.362
1.348
1.334
1.10
2.01
2.01
3.08
3.17
1.98
1.00
2.15
2.88
3.12
f1 (ppm)

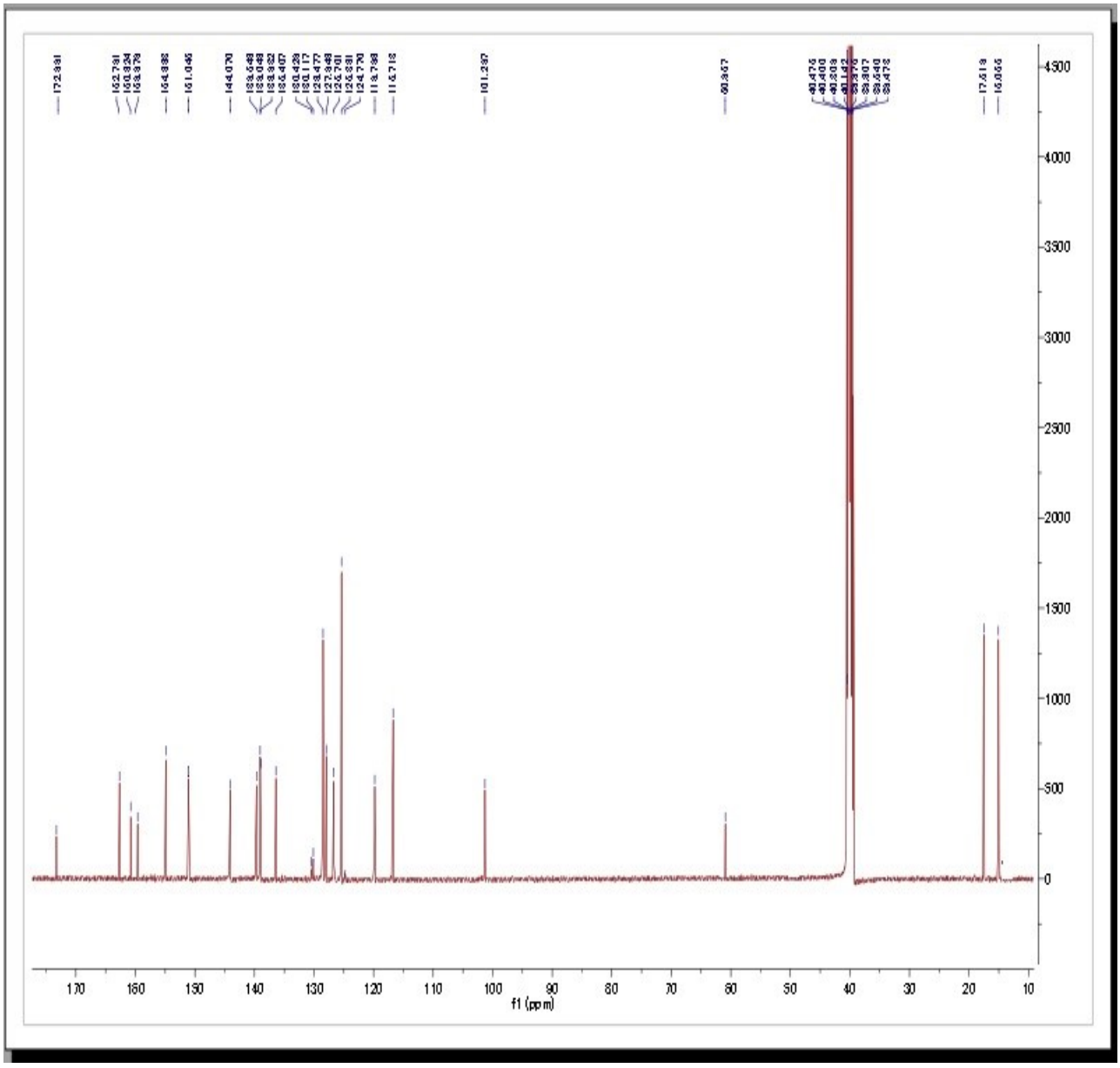

174.798
164.542
160.441
158.479
143.761
140.831
138.059
134.388
131.555
130.124
129.624
129.409
128.346
126.868
125.709
123.980
123.450
63.384
15.861
14.458
f1 (ppm)

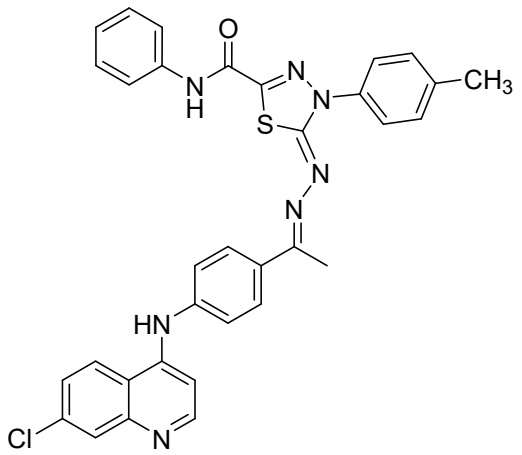

Seizo20240722_05 128 (1.149) AM2 (Ar,10000.0,0.00,0.00)
1: TOF MS ES+
6.58e5
100
%
0
577.0980
579.0957
580.0976
581.0927
582.0961
583.0919
164.9208
230.2483
273.1677
301.1415
358.3677
402.3730
421.3284
481.2644
556.2785
640.6150
685.3950
758.3812
801.1347
814.8877
m/z
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800


O
O
N
N
S
N
N
HN
Cl
N
CH3

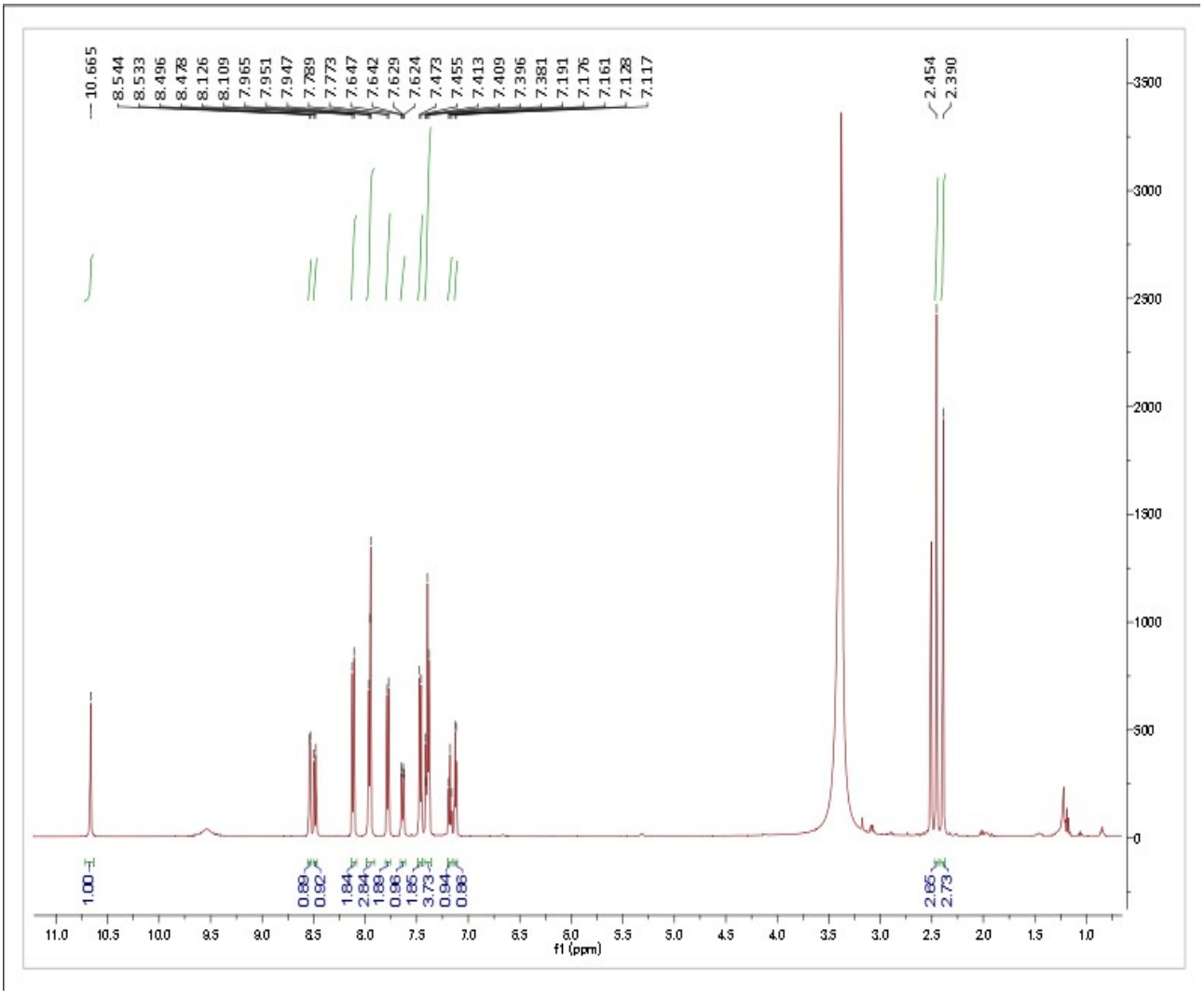
8d

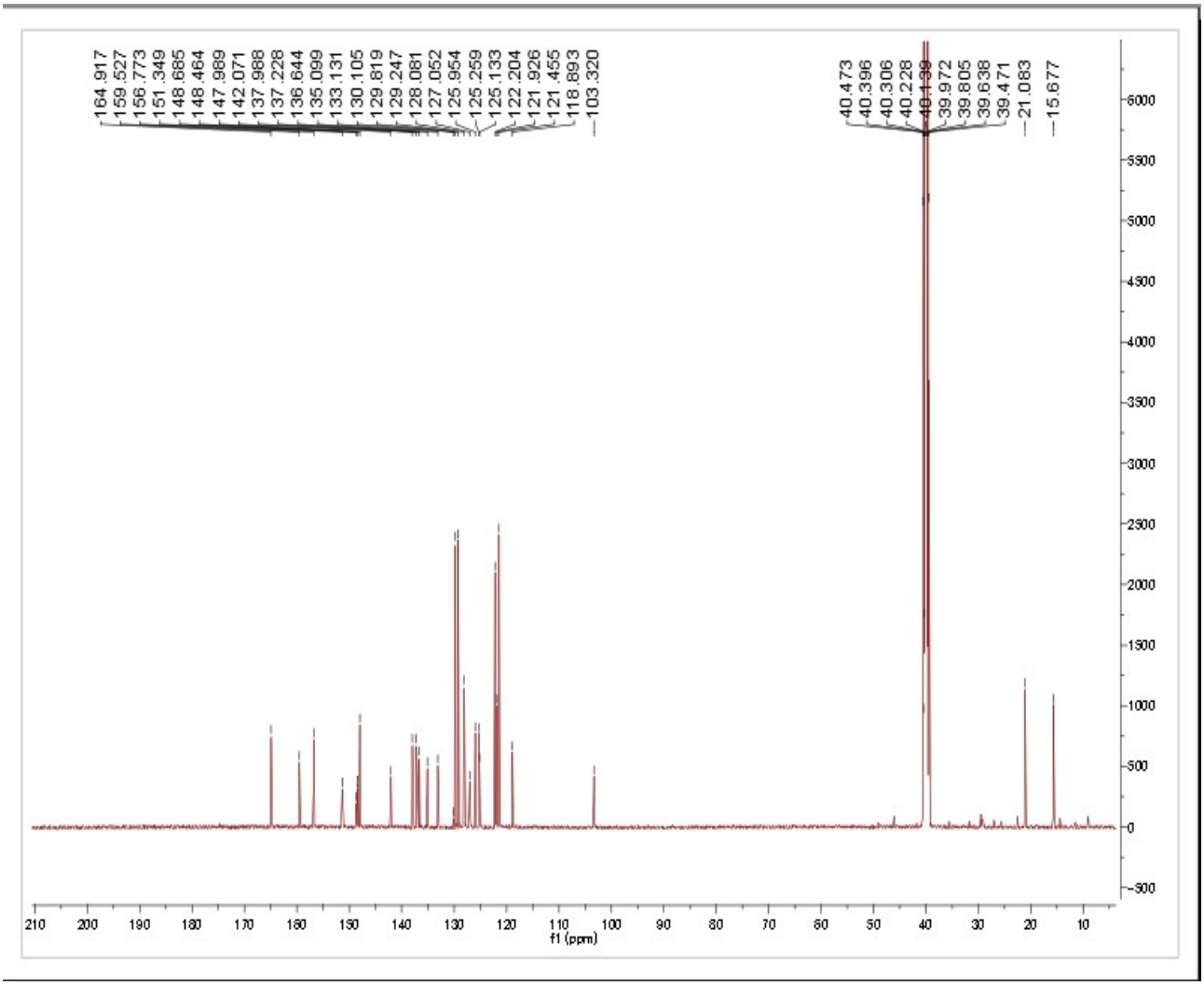

11.28
8.92
8.90
8.57
8.56
8.20
8.19
8.05
8.03
7.90
7.80
6.96
4.41
4.38
4.37
4.36
3.38
2.53
2.44
1.08
1.06
1.05
1.19
1.19
1.35
1.40
2.81
2.16
2.93
1.16
2.16
2.79
2.99
3.12
f1 (ppm)

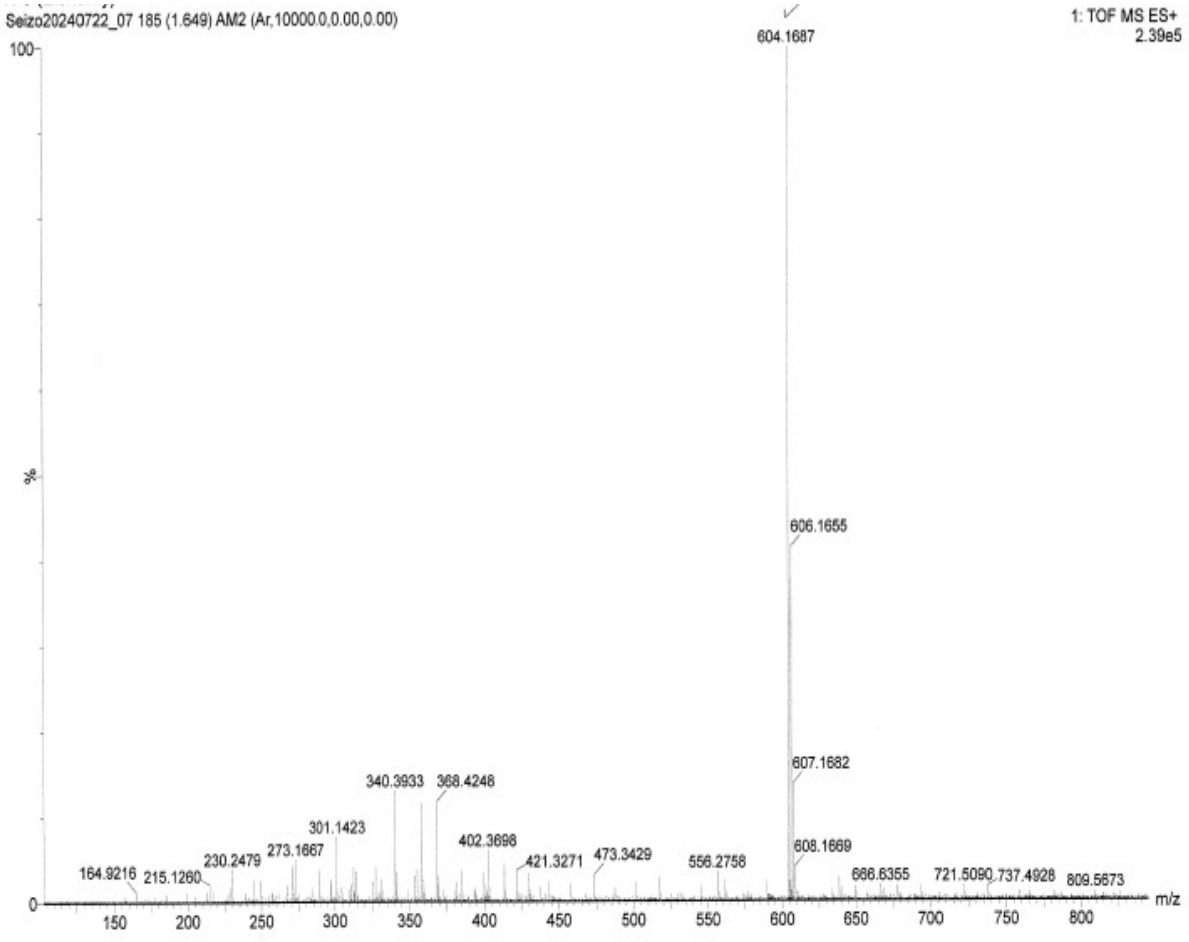

172.331
162.731
160.324
158.378
154.388
151.045
144.070
133.648
133.048
132.922
130.407
130.429
129.117
124.477
123.949
122.701
122.331
124.770
113.788
111.718
101.287
59.307
40.475
40.400
40.203
39.992
39.807
39.640
39.473
17.519
16.066
4500
4000
3500
3000
2500
2000
1500
1000
500
0
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

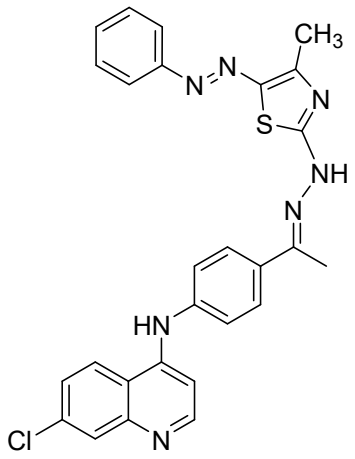

O
N
H
N
N
S
CH3
N
N
HN
Cl
N

8e

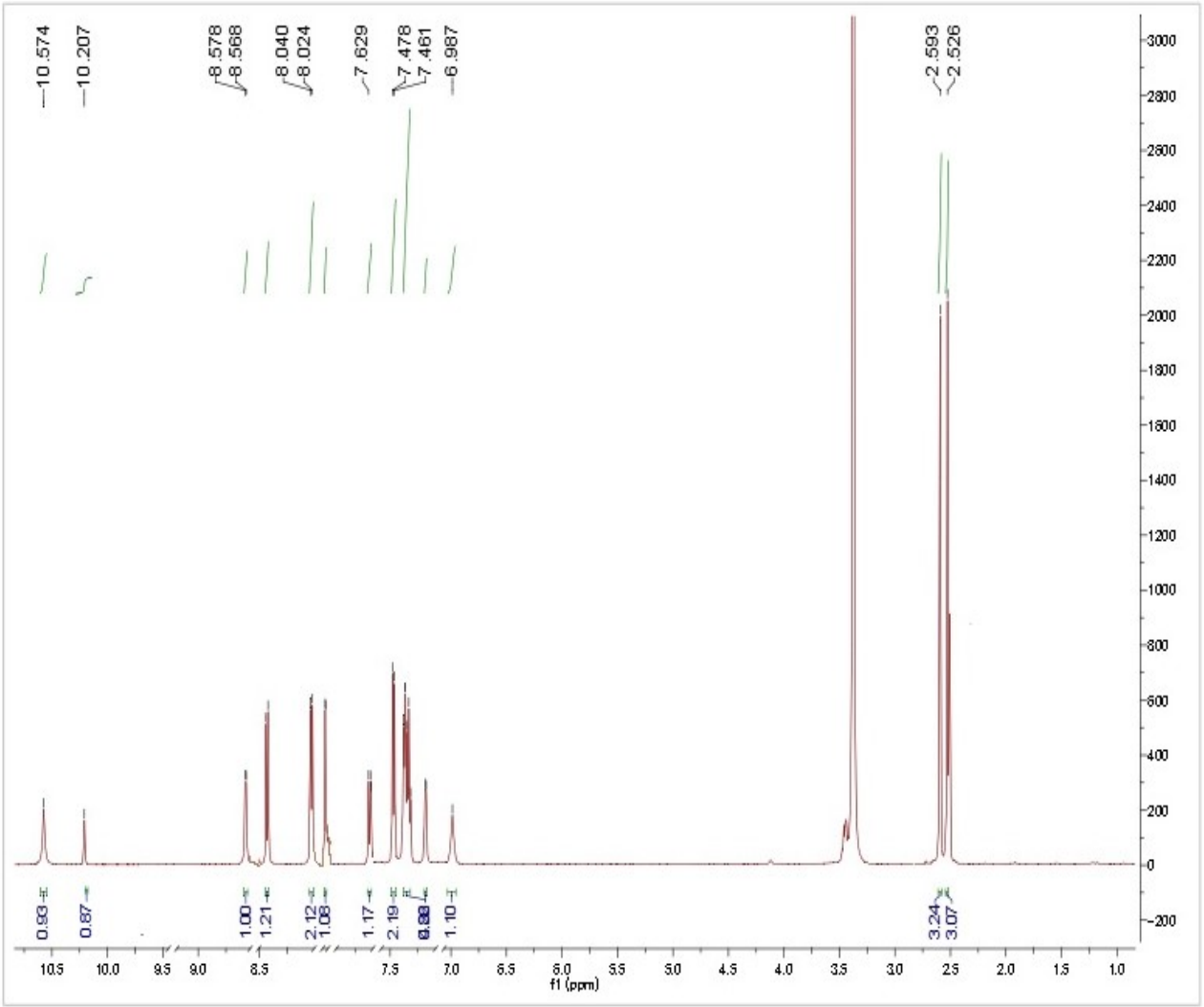

10.665
8.544
8.533
8.496
8.478
8.126
8.109
7.965
7.951
7.947
7.789
7.773
7.647
7.642
7.629
7.624
7.473
7.455
7.413
7.409
7.396
7.381
7.191
7.176
7.161
7.128
7.117
2.454
2.390
1.00
0.89
0.92
1.84
2.84
1.88
0.96
1.85
3.73
0.94
0.86
2.85
2.73
f1 (ppm)

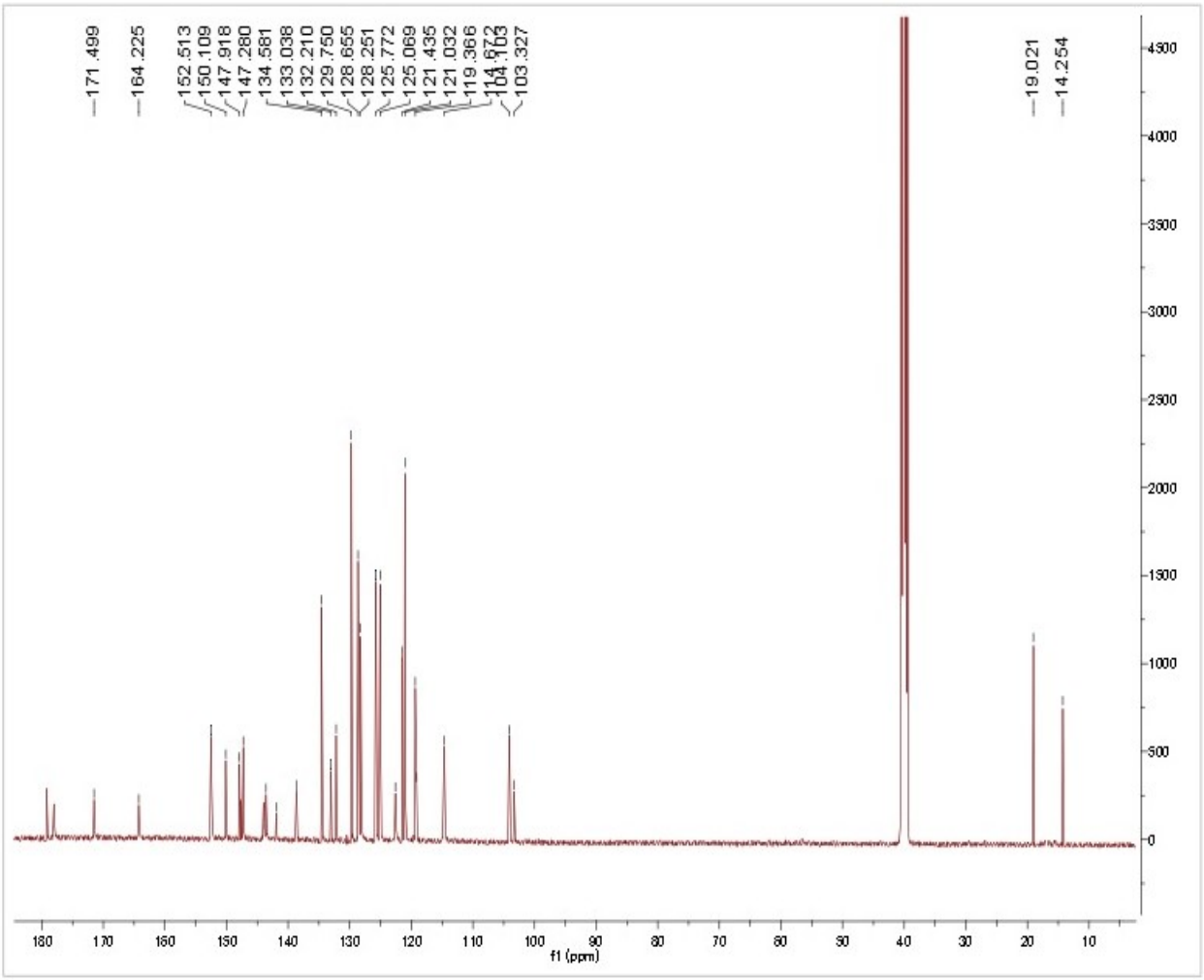

164.917
159.527
156.773
151.349
148.685
148.464
147.989
142.071
137.988
137.228
136.644
135.099
133.131
130.105
129.819
129.247
128.081
127.052
125.954
125.259
125.133
122.204
121.926
121.455
118.893
103.320
40.473
40.396
40.306
40.228
40.139
39.972
39.805
39.638
39.471
21.083
15.677
f1 (ppm)

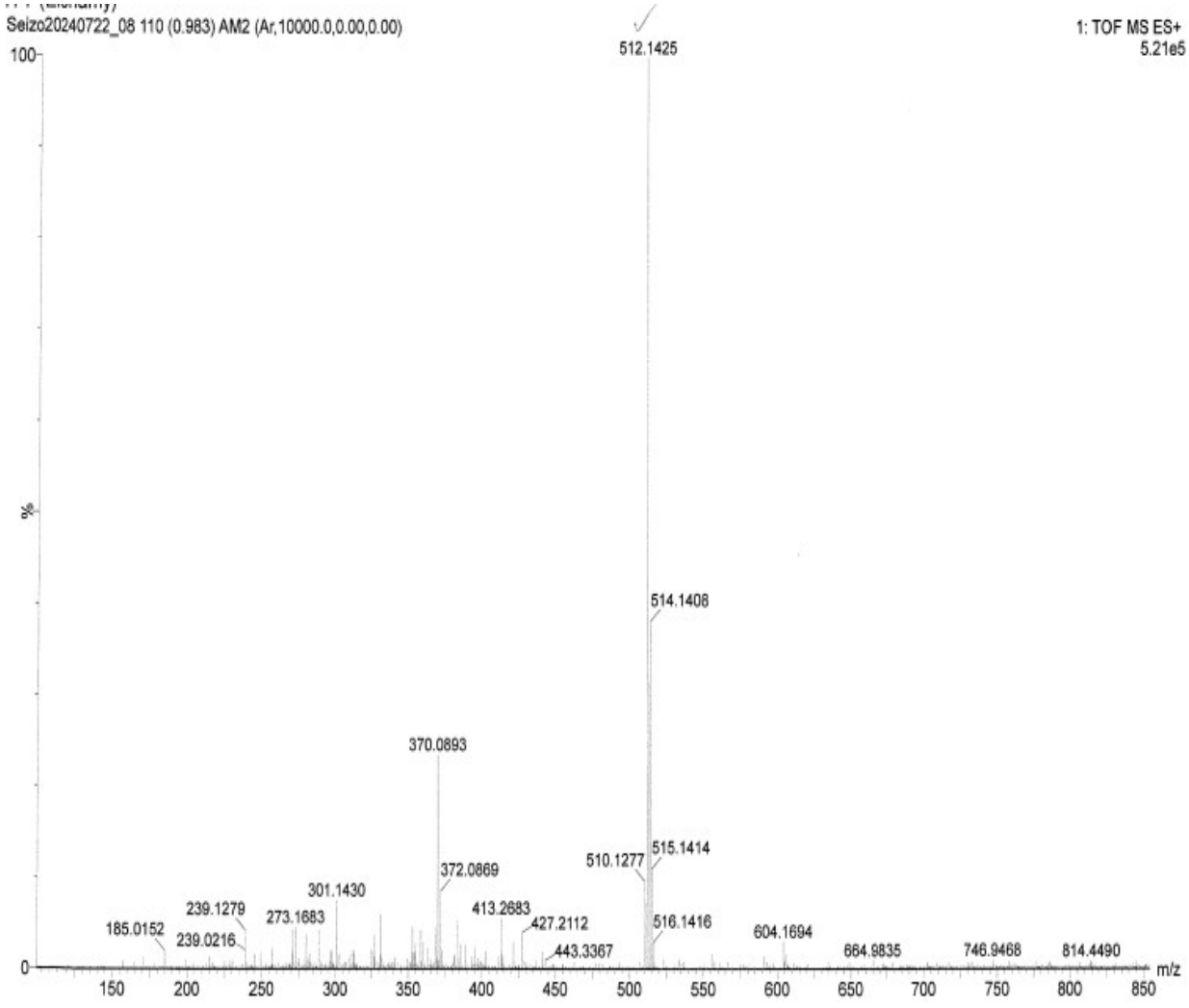

Seizo20240722_07 185 (1.649) AM2 (Ar,10000.0,0.00,0.00)
1: TOF MS ES+
2.39e5
100
%
0
604.1687
606.1655
607.1682
608.1669
340.3933
368.4248
301.1423
402.3698
273.1667
230.2479
164.9216
215.1260
421.3271
473.3429
556.2758
686.6355
721.5090
737.4928
809.5673
m/z
150
200
250
300
350
400
450
500
550
600
650
700
750
800

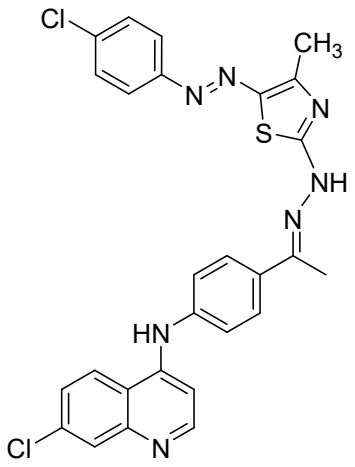

CH3
N
N
S
N
NH
N
HN
Cl
N

12a

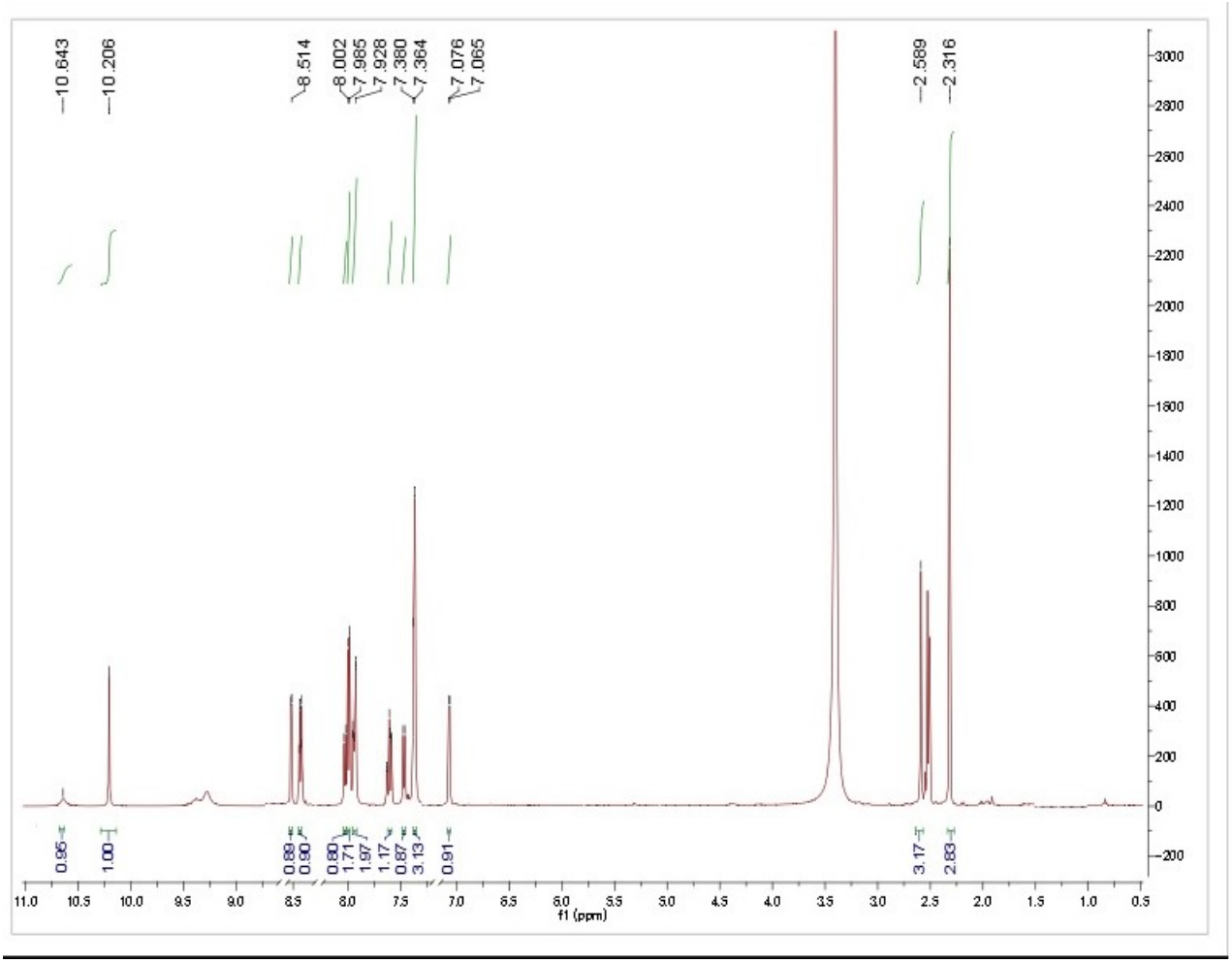

10.574
10.207
8.578
8.568
8.040
8.024
7.629
7.478
7.461
6.987
2.593
2.526
0.93
0.87
1.00
1.21
2.12
1.08
1.17
2.19
1.10
3.24
3.07
f1 (ppm)

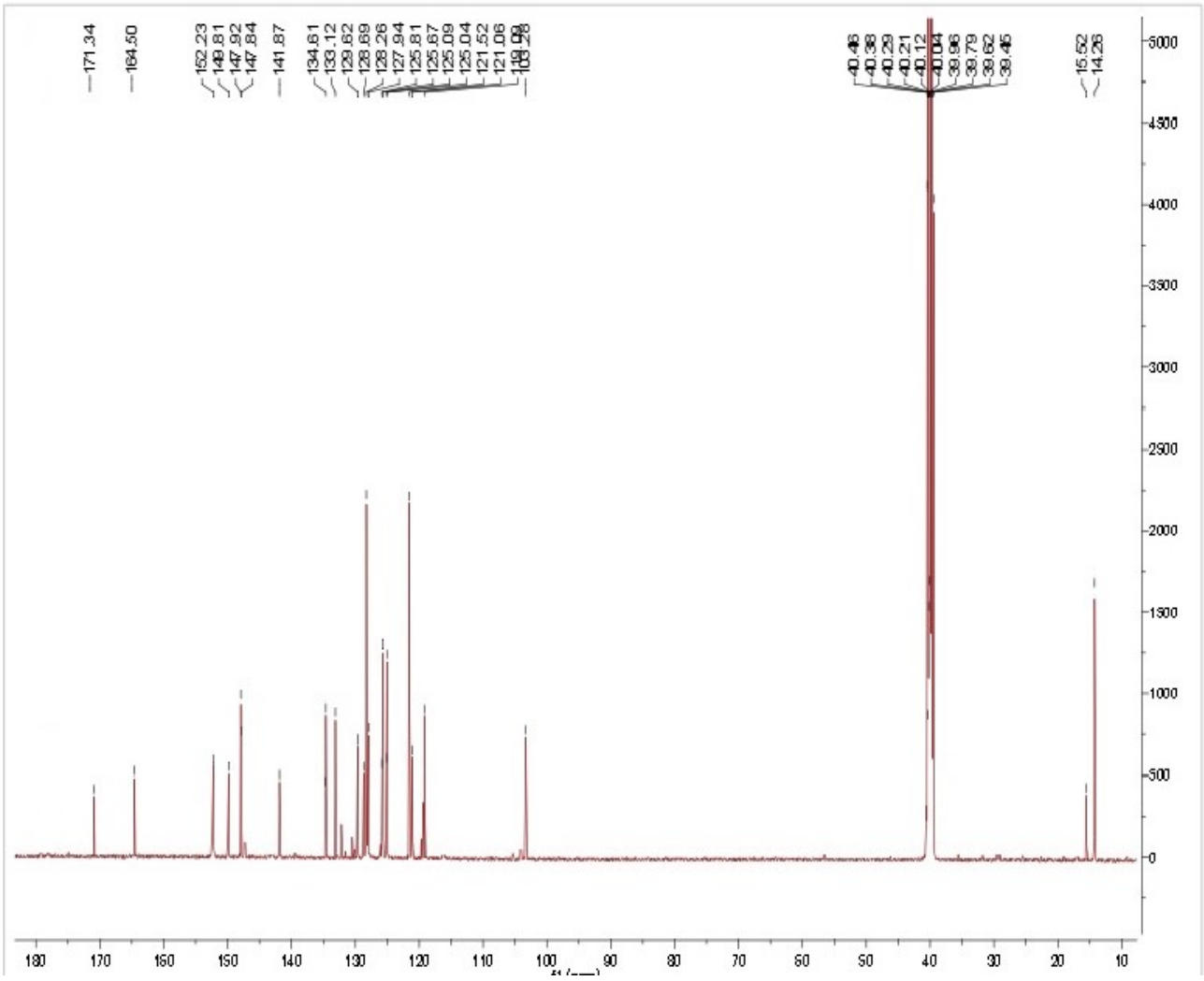

171.499
164.225
152.513
150.109
147.918
147.280
134.581
133.038
132.210
129.750
128.655
128.251
125.772
125.069
121.435
121.032
119.366
114.673
104.103
103.327
19.021
14.254
f1 (ppm)

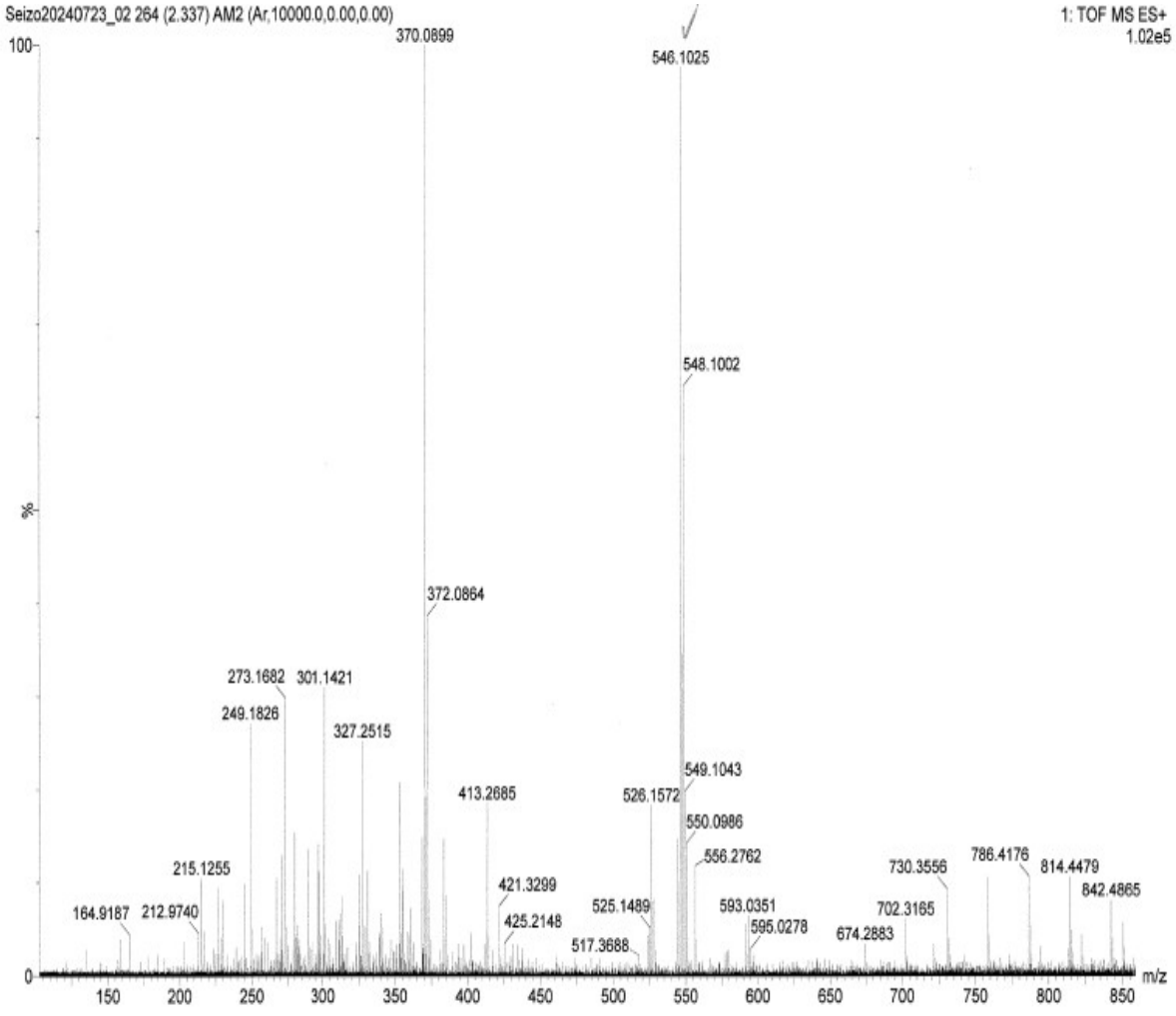

Seizo20240722_08 110 (0.983) AM2 (Ar,10000.0,0.00,0.00)
1: TOF MS ES+
5.21e5
512.1425
514.1408
370.0893
372.0869
510.1277
515.1414
301.1430
239.1279
273.1683
413.2683
427.2112
516.1416
185.0152
239.0216
443.3367
604.1694
664.9835
746.9468
814.4490
100
%
0
m/z
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850


Cl
CH3
N
N
S
N
NH
N
HN
Cl
N

12b


10.643
10.206
8.514
8.002
7.985
7.928
7.380
7.364
7.076
7.065
2.589
2.316
0.95
1.00
0.89
0.90
0.80
1.71
1.97
1.17
0.87
3.13
0.91
3.17
2.83
f1 (ppm)


171.34
164.50
152.23
149.81
147.92
147.84
141.87
134.61
133.12
129.62
128.69
128.26
127.94
125.81
125.67
125.09
125.04
121.52
121.06
40.46
40.38
40.29
40.21
40.12
40.04
39.96
39.79
39.62
39.45
15.52
14.26
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10


Seizo20240723_02 264 (2.337) AM2 (Ar,10000.0,0.00,0.00)
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100
%
0
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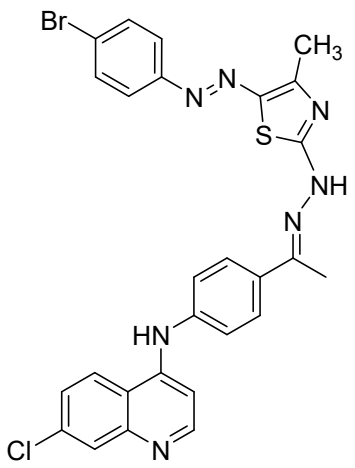
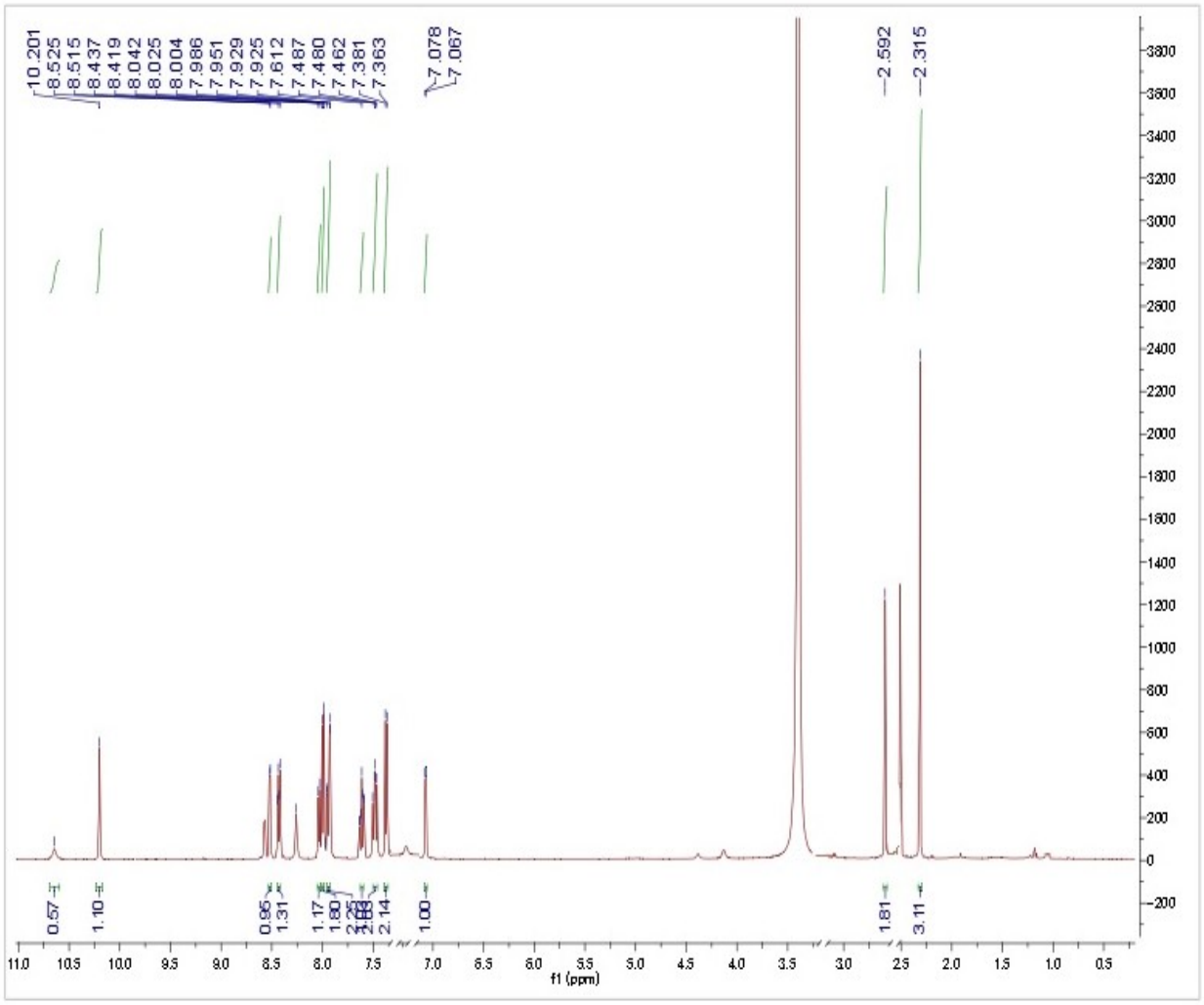
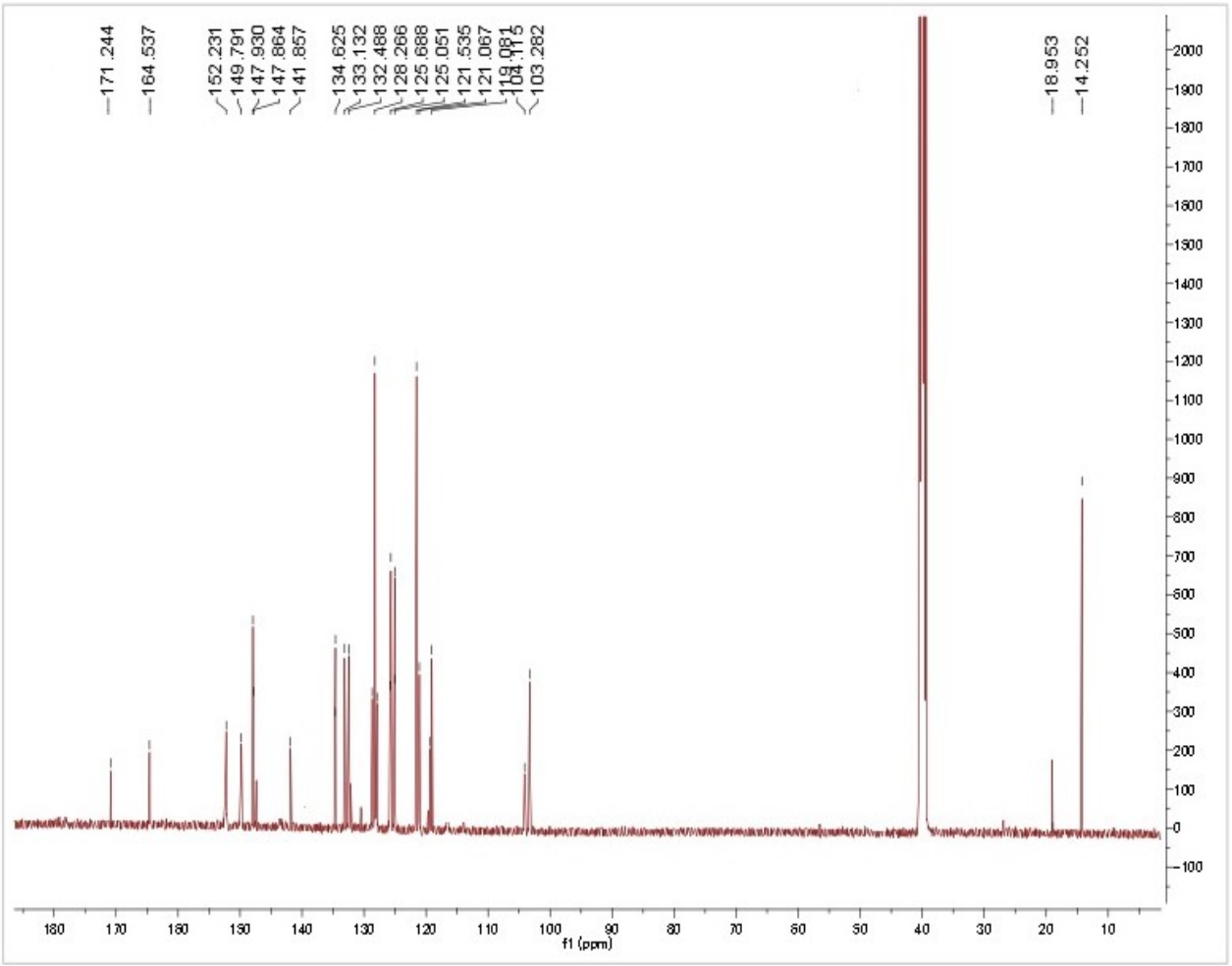
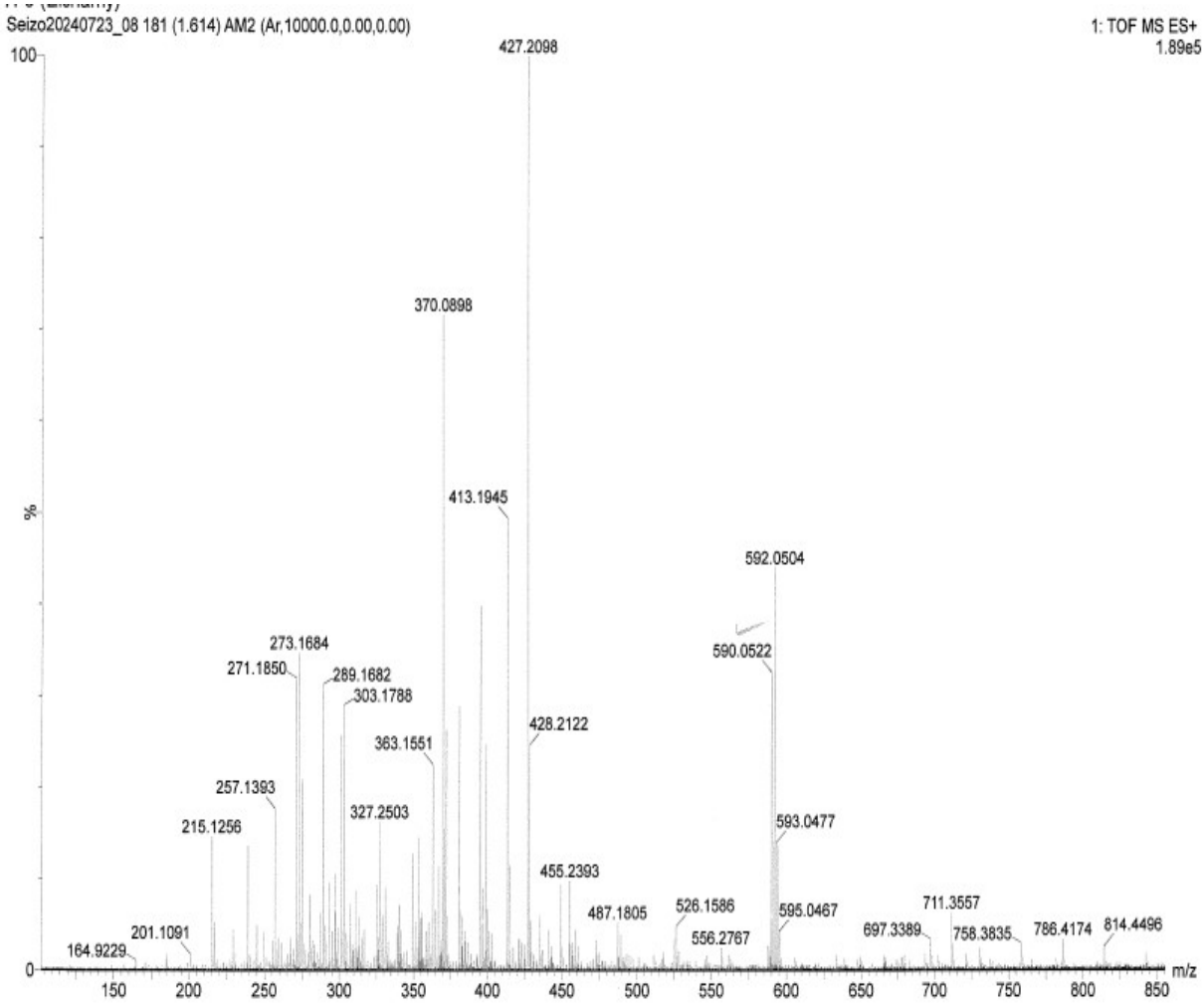
546.1025
548.1002
372.0864
273.1682
301.1421
249.1826
327.2515
549.1043
413.2685
526.1572
550.0986
556.2762
215.1255
421.3299
730.3556
786.4176
814.4479
842.4865
164.9187
212.9740
525.1489
593.0351
702.3165
425.2148
595.0278
674.2883
517.3688
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
m/z


Br
CH3
N
N
S
N
NH
N
HN
Cl
N

12c


10.201
8.525
8.515
8.437
8.419
8.042
8.025
8.004
7.986
7.951
7.929
7.925
7.612
7.487
7.480
7.462
7.381
7.363
7.078
7.067
2.592
2.315
0.57
1.10
0.96
1.31
1.17
1.80
2.14
1.00
1.81
3.11
f1 (ppm)


171.244
164.537
152.231
149.791
147.930
147.864
141.857
134.625
133.132
132.488
128.266
125.688
125.051
121.535
121.067
119.081
104.915
103.282
18.953
14.252
f1 (ppm)


Seizo20240723_08 181 (1.614) AM2 (Ar,10000.0,0.00,0.00)
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100
%
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413.1945
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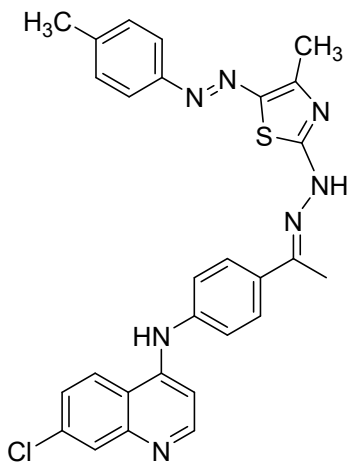
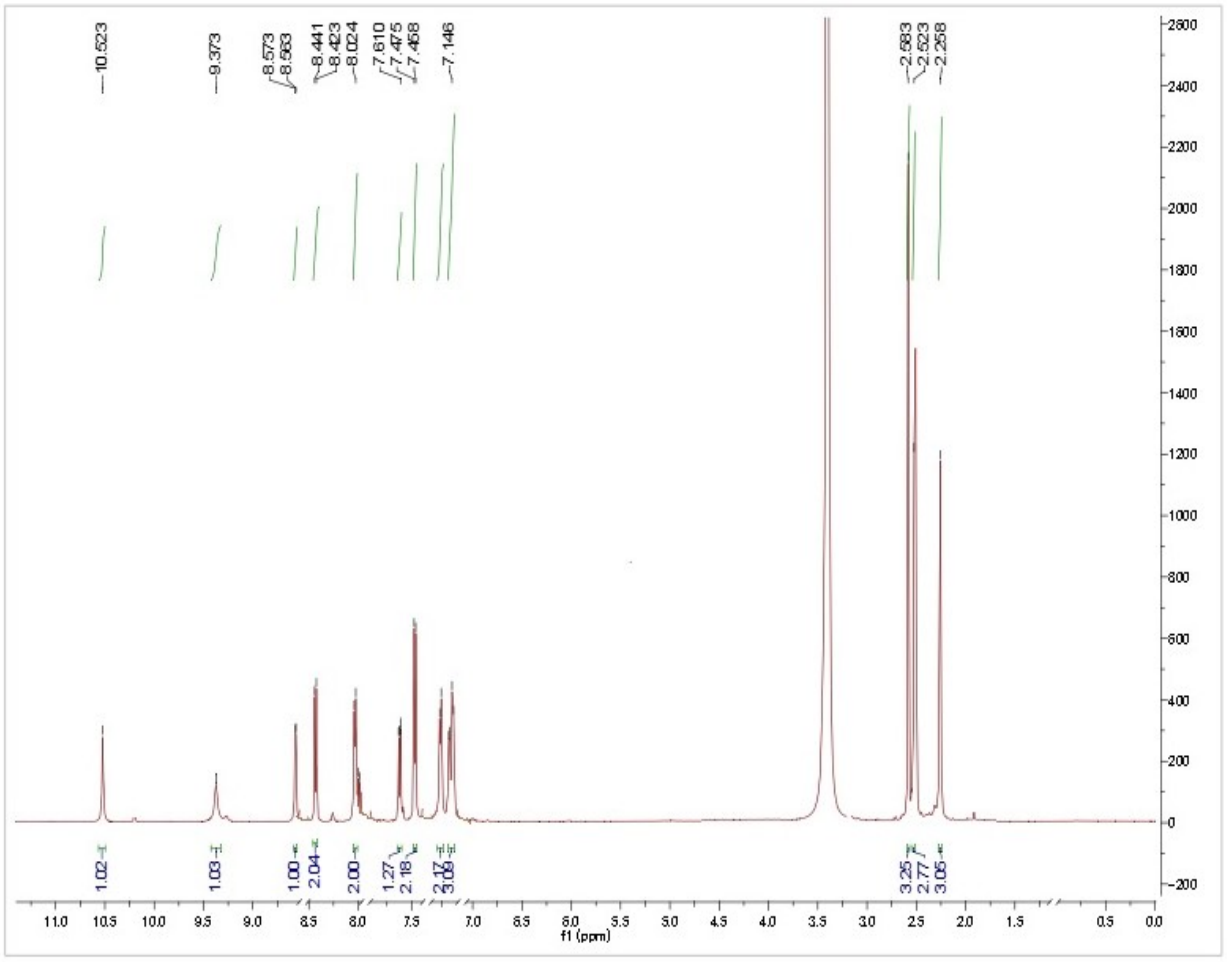
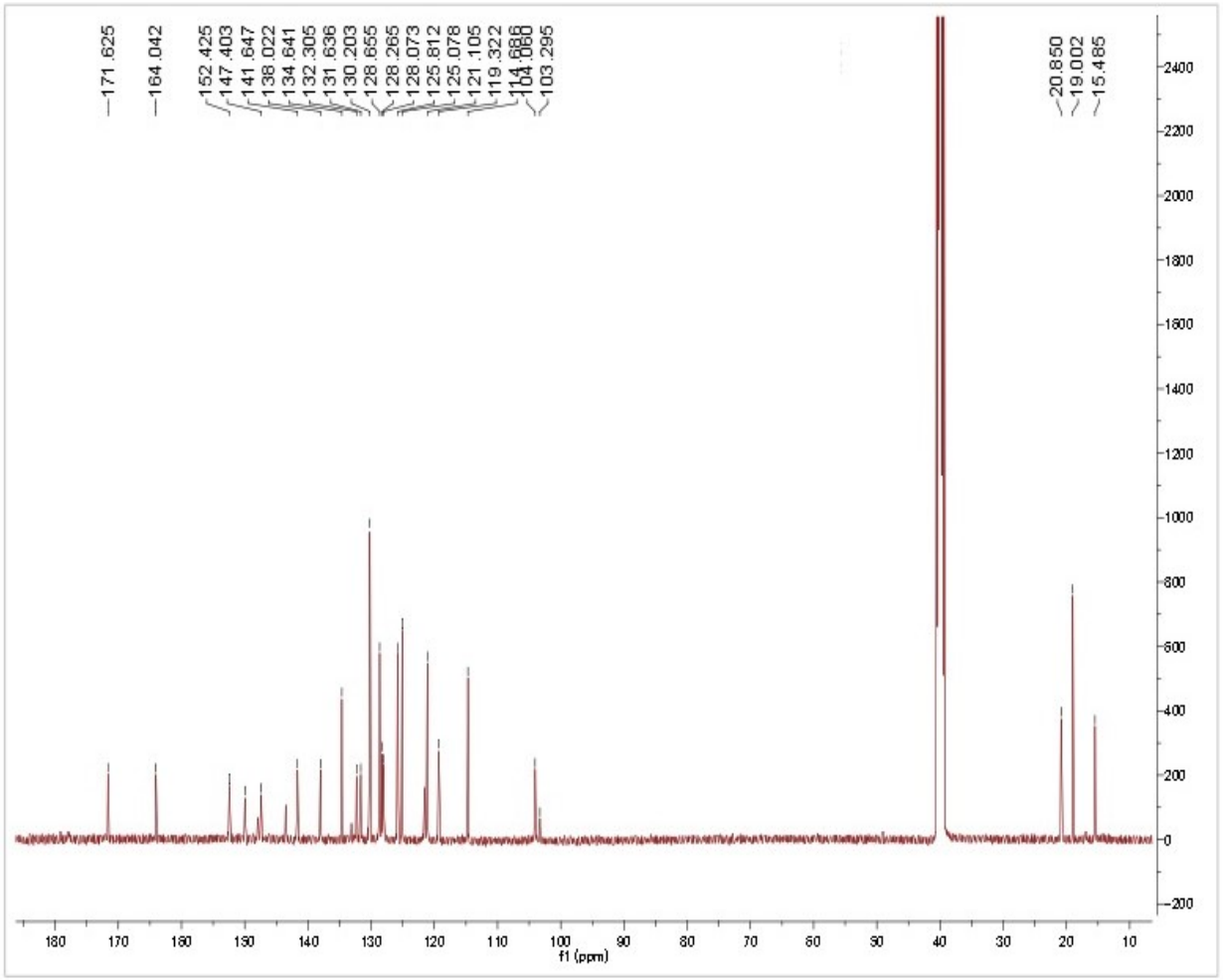
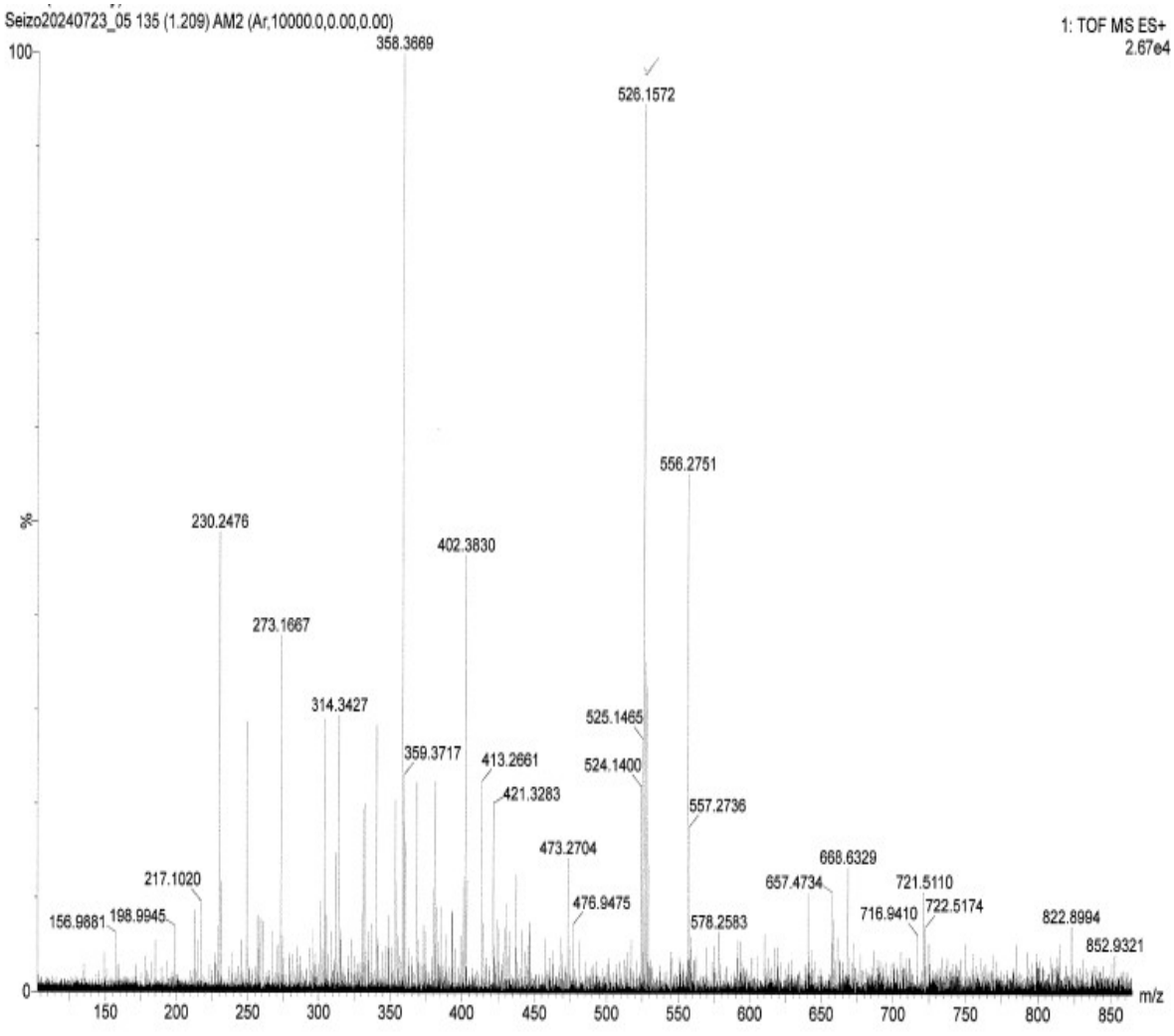
590.0522
273.1684
271.1850
289.1682
303.1788
428.2122
363.1551
257.1393
327.2503
215.1256
593.0477
455.2393
487.1805
526.1586
595.0467
711.3557
697.3389
758.3835
786.4174
814.4496
556.2767
201.1091
164.9229
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
m/z



12d


10.523
9.373
8.573
8.563
8.441
8.423
8.024
7.610
7.475
7.458
7.146
2.583
2.523
2.258
1.02
1.03
1.00
2.04
2.00
1.27
2.18
2.17
3.09
3.25
2.77
3.05
f1 (ppm)


171.625
164.042
152.425
147.403
141.647
138.022
134.641
132.305
131.636
130.203
128.655
128.265
128.073
125.812
125.078
121.105
119.322
114.686
114.060
103.295
20.850
19.002
15.485
f1 (ppm)


Seizo20240723_05 135 (1.209) AM2 (Ar,10000.0,0.00,0.00)
1: TOF MS ES+
2.67e4
100
%
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358.3669
526.1572
556.2751
230.2476
402.3830
273.1667
314.3427
525.1465
359.3717
413.2661
524.1400
421.3283
557.2736
473.2704
668.6329
217.1020
657.4734
721.5110
476.9475
716.9410
722.5174
156.9881
198.9945
578.2583
822.8994
852.9321
150
200
250
300
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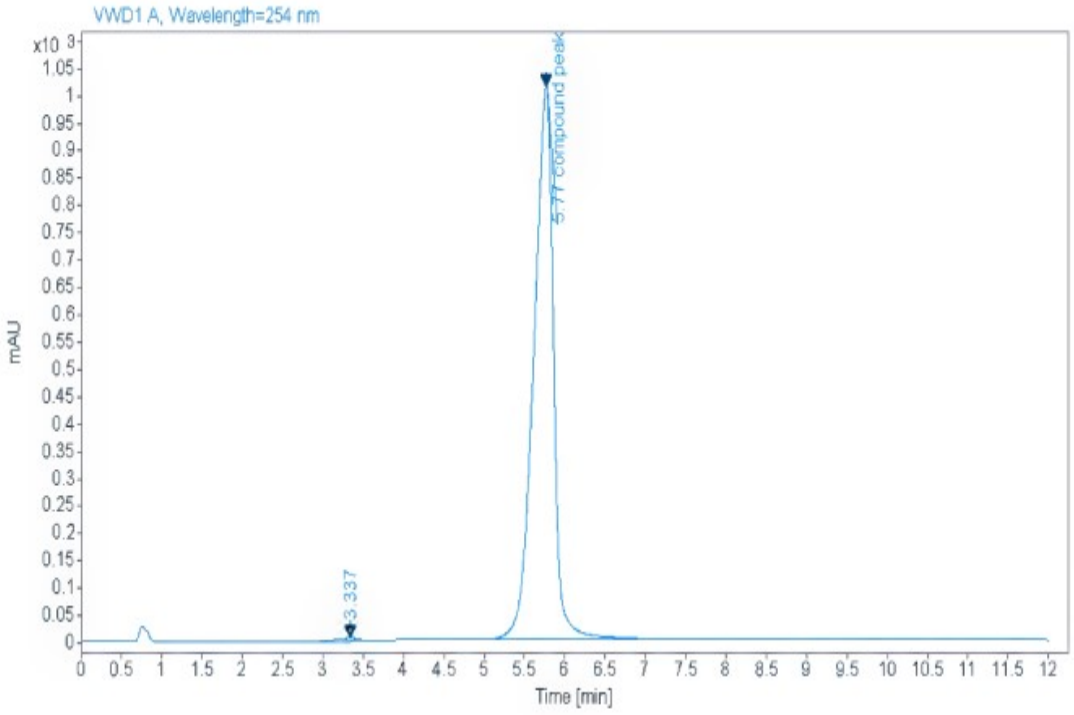
400
450
500
550
600
650
700
750
800
850
m/z

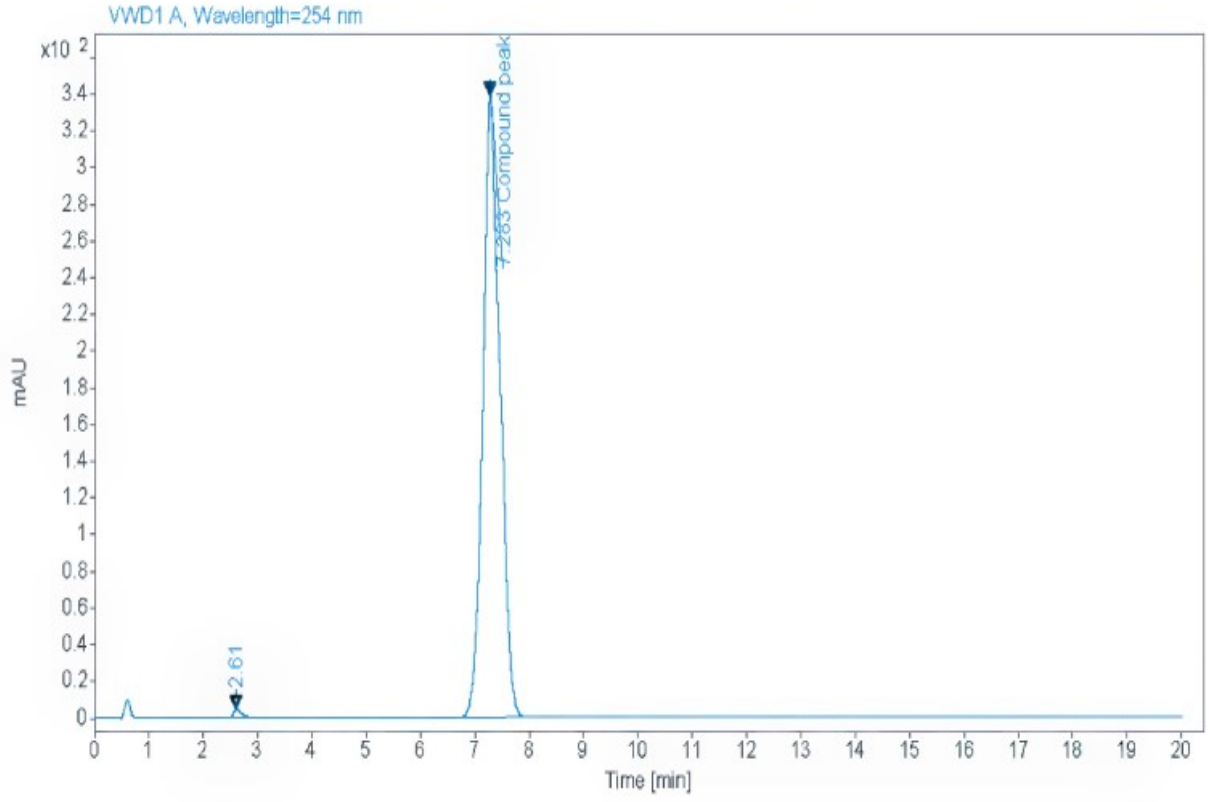
Charts of HPLC



Signal: VWD1 A, Wavelength=254 nm

RT [min]	Type	Width [min]	Area	Height	Area%	Name
3.337	BV	0.2564	101.3273	5.3740	0.5929	
5.770	BB	0.2646	16991.2751	1011.5413	99.4071	compound peak
		Sum	17092.6024			

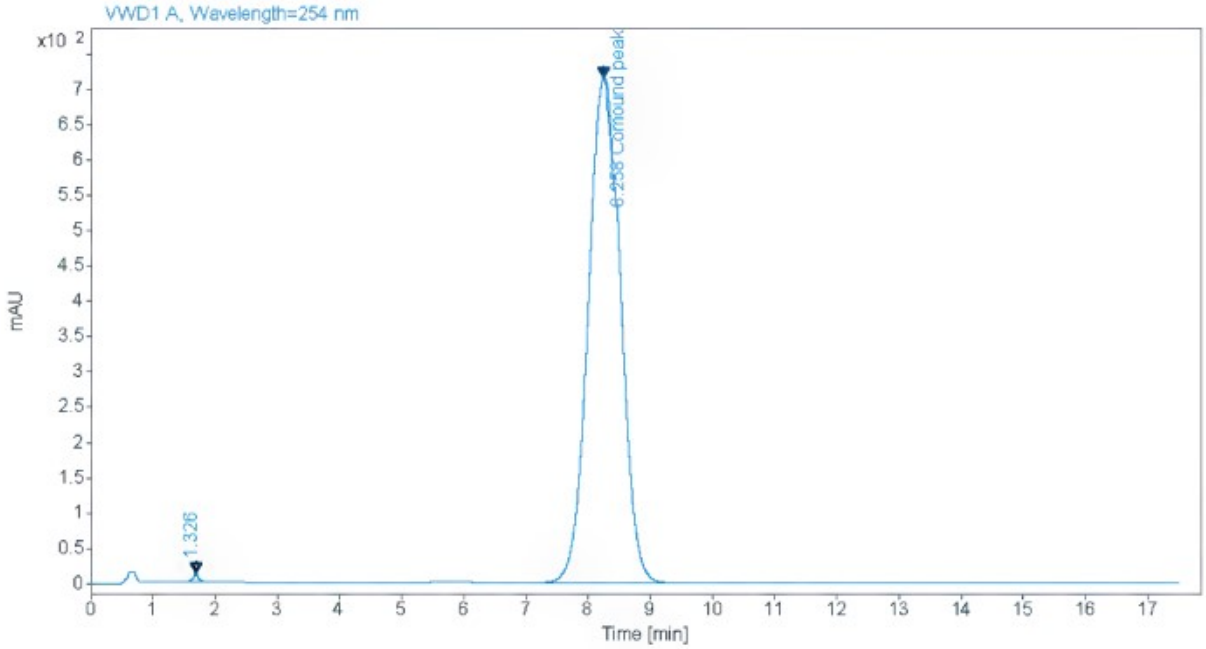
HPLC for compound 5



Signal: VWD1 A, Wavelength=254 nm

RT [min]	Type	Width [min]	Area	Height	Area%	Name
2.610	VV	0.1804	50.1489	4.5290	0.6038	
7.283	BV	0.3404	8255.9166	339.0104	99.3962	Compound peak
		Sum	8306.0655			

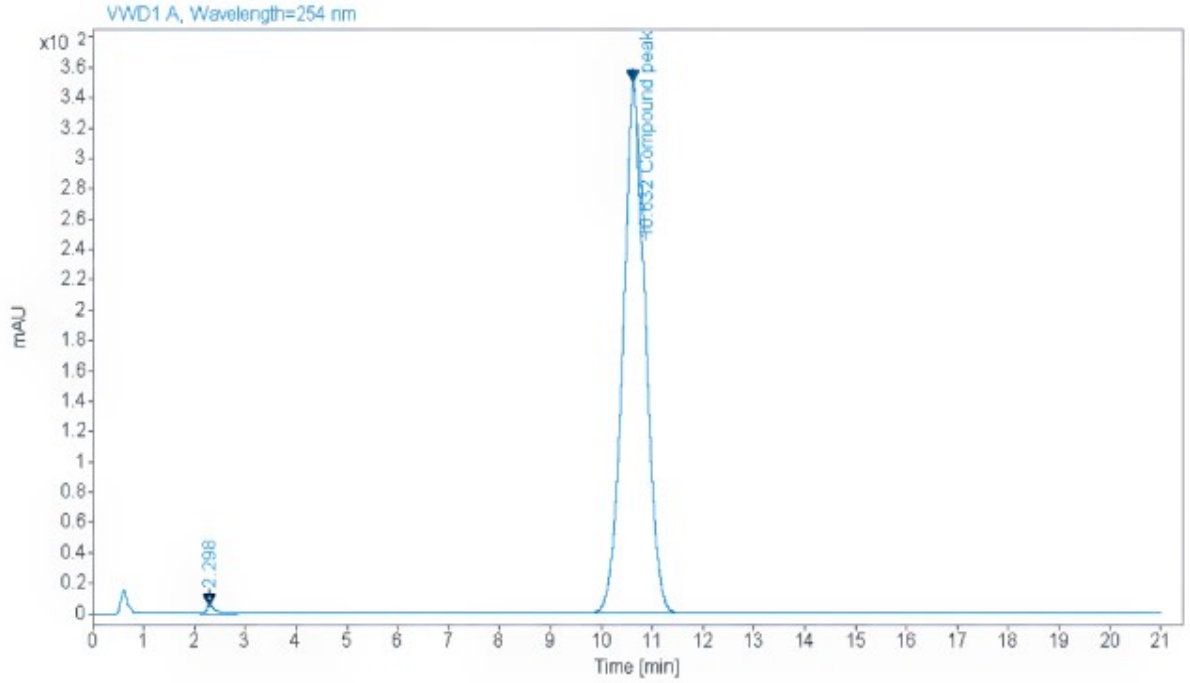
HPLC for compound 8b



Signal: VWD1 A, Wavelength=254 nm

RT [min]	Type	Width [min]	Area	Height	Area%	Name
1.326	VB	0.0651	47.2621	11.1678	0.1562	
8.258	VB	0.6022	30221.1952	715.9852	99.8438	Comound peak
		Sum	30268.4573			

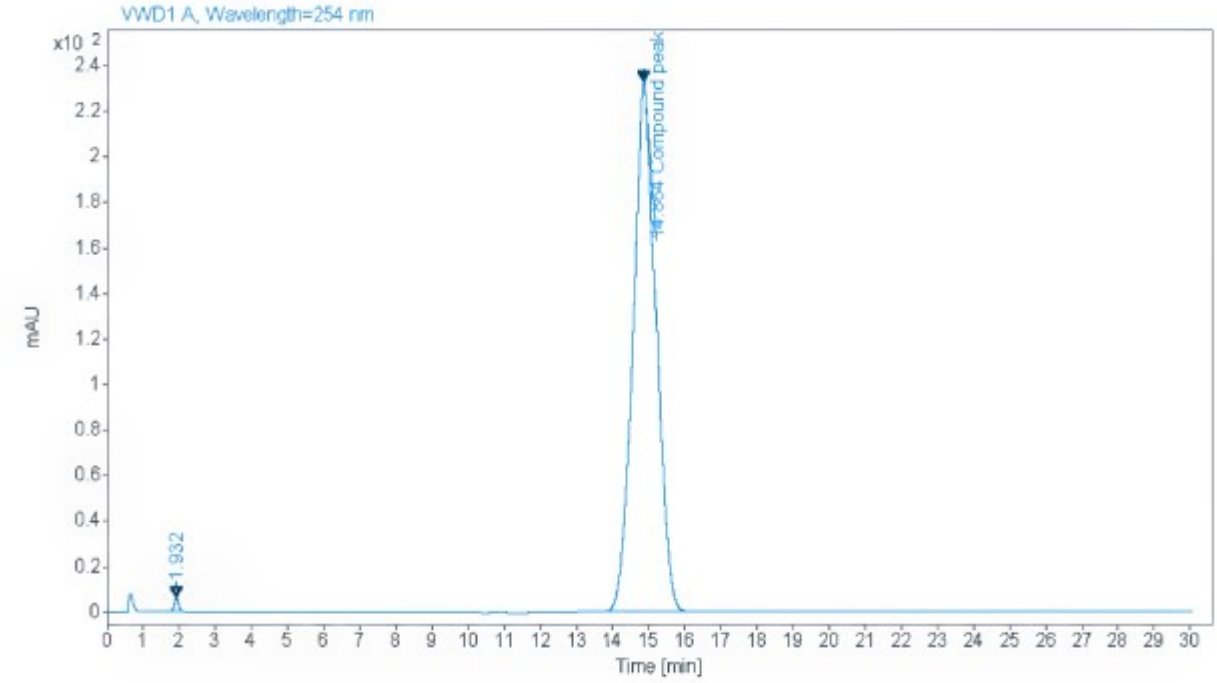
HPLC for compound 8d



Signal: VWD1 A, Wavelength=254 nm

RT [min]	Type	Width [min]	Area	Height	Area%	Name
2.298	VV	0.1846	60.9981	5.2683	0.4273	
10.632	BV	0.5386	14214.2833	350.2096	99.5727	Compound peak
		Sum	14275.2814			

HPLC for compound 12a



Signal: VWD1 A, Wavelength=254 nm

RT [min]	Type	Width [min]	Area	Height	Area%	Name
1.932	BB	0.5998	261.5290	5.3801	1.8811	
14.864	BB	0.7763	13641.7191	230.5098	98.1189	Compound peak
		Sum	13903.2481			

HPLC for compound 12c

Biology part

Culture conditions

L. cultured in tissue flasks containing RPMI 1640 medium supplemented with 10% HIFCS and 100 IU penicillin and 100 µgml-1 streptomycin solution at 26°C (Tariku et al., 2010; Habtemariam, 2003; Seifert et al., 2010). Stock solution and working concentration preparation All the compounds tested were dissolved in DMSO to a final concentration of 1 mg/ml Both test and standard solutions were serially diluted to appropriate concentrations using complete media. The test compounds were prepared by three-fold serial dilutions from 10 µgml-1 to 0.04 µg ml-1 Amphotericin B deoxycholate and miltefosine which were used as a positive control for comparison of the antileishmanial activities of the test compounds, were also made in three-fold serial dilutions (Foroumadi et al., 2005)

Tariku Y, Hymete A, Hailu A, Rohloff J (2010). Constituents, Antileishmanial Activity and Toxicity Profile of Volatile Oil from Berries of Croton macrostachyus. Nat. Prod. Commun. 5:975-980.

Habtemariam S (2003). In vitro antileishaminial effects of antibacterial diterpenes from two Ethiopian premna species: P.schimperi and P. oligotricha. BMC pharmacol 3:1-6.

Seifert K, Escobar P, Croft SL (2010). In vitro activity of anti-leishmanial drugs against Leishmania donovani is host cell dependent. J. Antimicrob. Chemother. 3:508-11

Foroumadi A, Pournourmohammadi S, Soltani F, Asgharian-Rezaee M, Dabiri S, Kharazmi A, Shafiee A (2005). Synthesis and in vitro leishmanicidal activity of 2-(5-nitro-2-furyl) and 2-(5-nitro-2-thienyl)-5- substituted-1, 3, 4-thiadiazoles. Bioorg. Med. Chem. Lett. 15:1983- 1985.

Docking part

Table S1. RMSD values between the best-scoring binding mode obtained with CmDock and the best-scoring binding mode obtained with AutoDock Vina.

AutoDock Vina Compound 5 affinity to Lm-PTR1 (kcal/mol)	CmDock Compound 5 affinity to Lm-PTR1 (kcal/mol)	RMSD (Å)
-8.4	-20.73	1.69

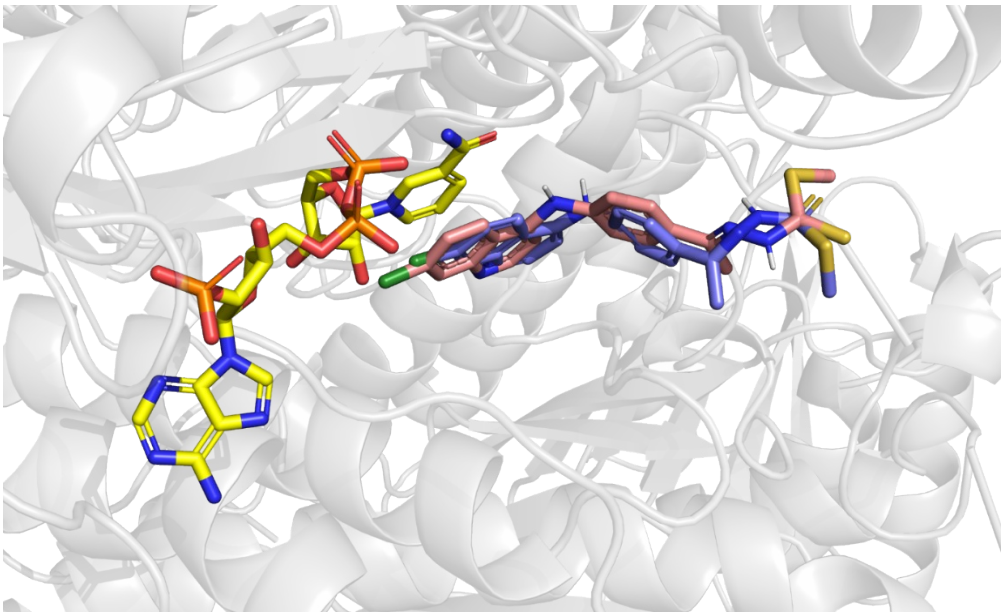


Figure S1. The binding mode of compound 5 obtained with CmDock (pink) in comparison with the binding mode of compound 5 obtained with AutoDock Vina (purple). The cofactor NADPH is presented in yellow.

The calculated RMSD value between the best-scoring docked poses, obtained with CmDock and AutoDock Vina equals 1.69 Å, which indicates that the binding modes obtained with both docking programs are indeed very simular. This additionally confirms the validity of the applied molecular docking protocol in CmDock.

To demonstrate the stability of the observed intermolecular interactions during 100 ns MD simulation, the occupancies of the observed intermolecular interactions in 25 ns intervals are presented in **Table S2**.

Table S2. Occupancy of intermolecular interactions throughout 100 ns MD simulation of compound 5-Lm-PTR1 complex.

Residue	Interaction type	Occupancy
0-25 ns		
TYR194	H-bond	100.00%
PHE113	Hydrophobic	97.21%
HIS241	H-bond	88.45%
LEU229	Hydrophobic	76.10%
LEU226	Hydrophobic	70.60%
LEU188	Hydrophobic	62.67%
GLY225	Waterbridge	56.29%
PHE113	Pi-stack	50.77%
26-50 ns		
PHE113	Hydrophobic	99%
TYR194	H-bond	97%
LEU229	Hydrophobic	77%
HIS241	H-bond	74%
LEU226	Hydrophobic	65%
LEU188	Hydrophobic	63%
GLY225	H-bond	52%
PHE113	Pi-stack	50.9%
51-75 ns		
PHE113	Hydrophobic	98%
TYR194	H-bond	98%
LEU229	Hydrophobic	81%
HIS241	H-bond	78%
LEU226	Hydrophobic	68%
LEU188	Hydrophobic	65%
GLY225	H-bond	52%
PHE113	Pi-stack	50%
76-100 ns		
PHE113	Hydrophobic	99%
HIS241	H-bond	98%
TYR194	H-bond	97%
LEU229	Hydrophobic	78%
LEU188	Hydrophobic	67%
LEU226	Hydrophobic	66%
GLY225	H-bond	57%
PHE113	Pi-stack	54%