Supporting Information

In vitro and *in vivo* activity of (trifluoromethyl)pyridines as anti-*Chlamydia trachomatis* agents

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Contents:

Table S1: LogS of the synthesized compounds

Compound ID	LogS
5	-2.96
6	-3.99
7	-3.54
8	-3.82
9	-3.59
10	-3.71
11	-3.72
12	-5.34
13	-4.85
16	-5.23
17	-5.91
18	-5.43
19	-5.26
20	-5.92
21	-5.92
22	-5.65
23	-6.17

XTT cell viability assay

During the anti-chlamydial investigation, the cell viability was monitored under the microscope for abnormalities and compared with control samples. Besides this, we studied the toxicity of the most active compound **20** towards HEp-2 cells using a Cell Proliferation Kit II (XTT). We incubated HEp-2 cells with two concentrations of the tested compounds, 25 and 50 μg/mL, for 48 hours (**Figure S6**). The tested compound was tolerable at both concentrations.

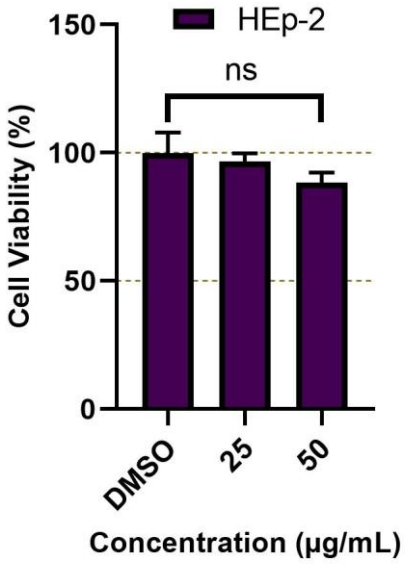


Figure S1: Cytotoxicity analysis of compound **20** at 25 and 50 μg/mL on HEp-2 cells. The absorbance values represent an average of triplicate samples. Data represent two biological replicates. ns = not significant (ANOVA).

Indirect immunofluorescence assay (IFA)

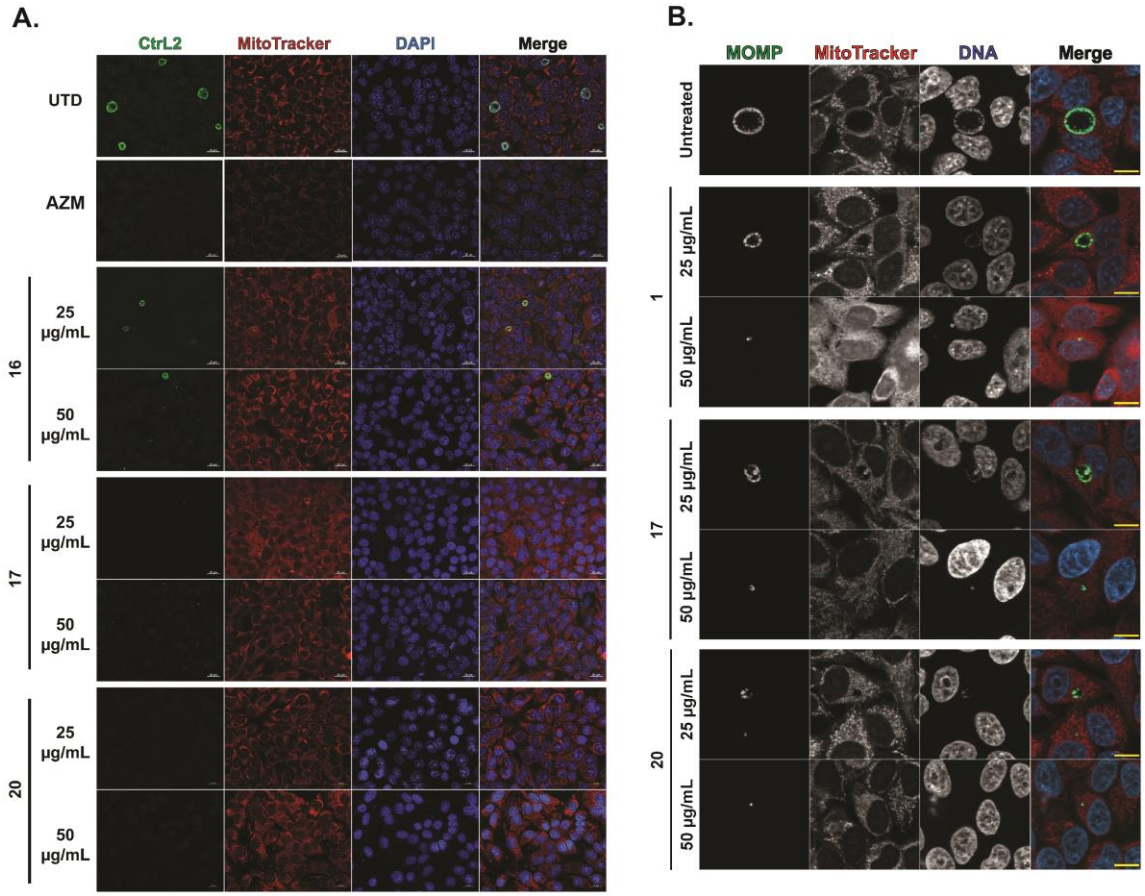


Figure S2. (a) Immunofluorescence analysis of derivatives **16**, **17**, and **20** at 24hpi. Samples were stained for MOMP (Ctr L2; green), mitochondria (MitoTracker; red), and DNA (DAPI; blue). Scale bar = 20 µm. (b) Zoomed images of a single chlamydial inclusion of **17** and **20** treated, *Chlamydia*-infected cells in comparison with **1** and untreated samples at 24hpi. The infected cells were fixed and stained, as indicated above. Scale bar = 10 µm.

***In-vitro* antichlamydial investigations of AZM and Doxy against preformed EBs.**

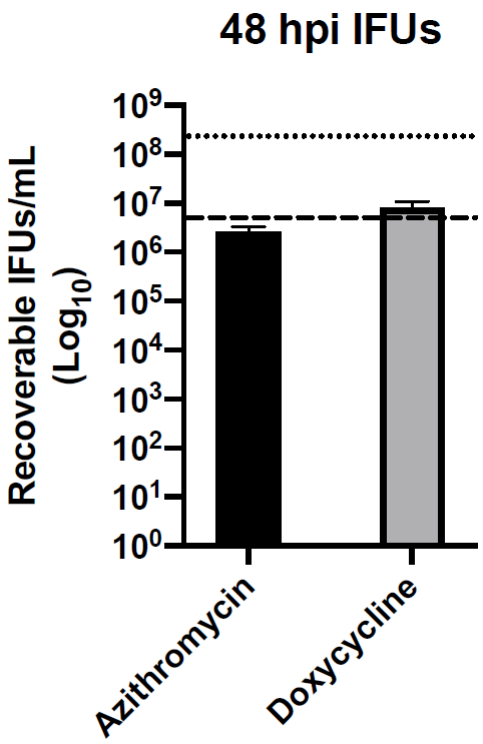


Figure S3. Effect of Azithromycin and Doxycycline (1μg/mL) on preformed EBs. The infected cells were treated starting at 24 hpi, which was maintained throughout the remainder of the experiment. Data represent three biological replicates.

Dose-response effect.

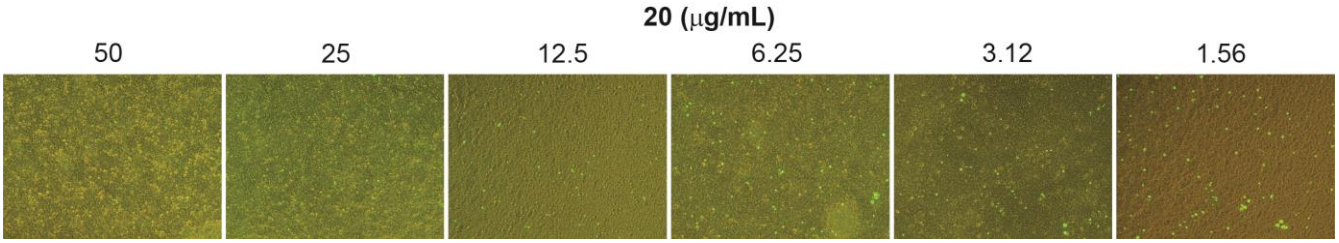


Figure S4. Dose-response analysis of **20** inhibitory effect.

Antimicrobial activity against *E.coli* and *S. aureus*

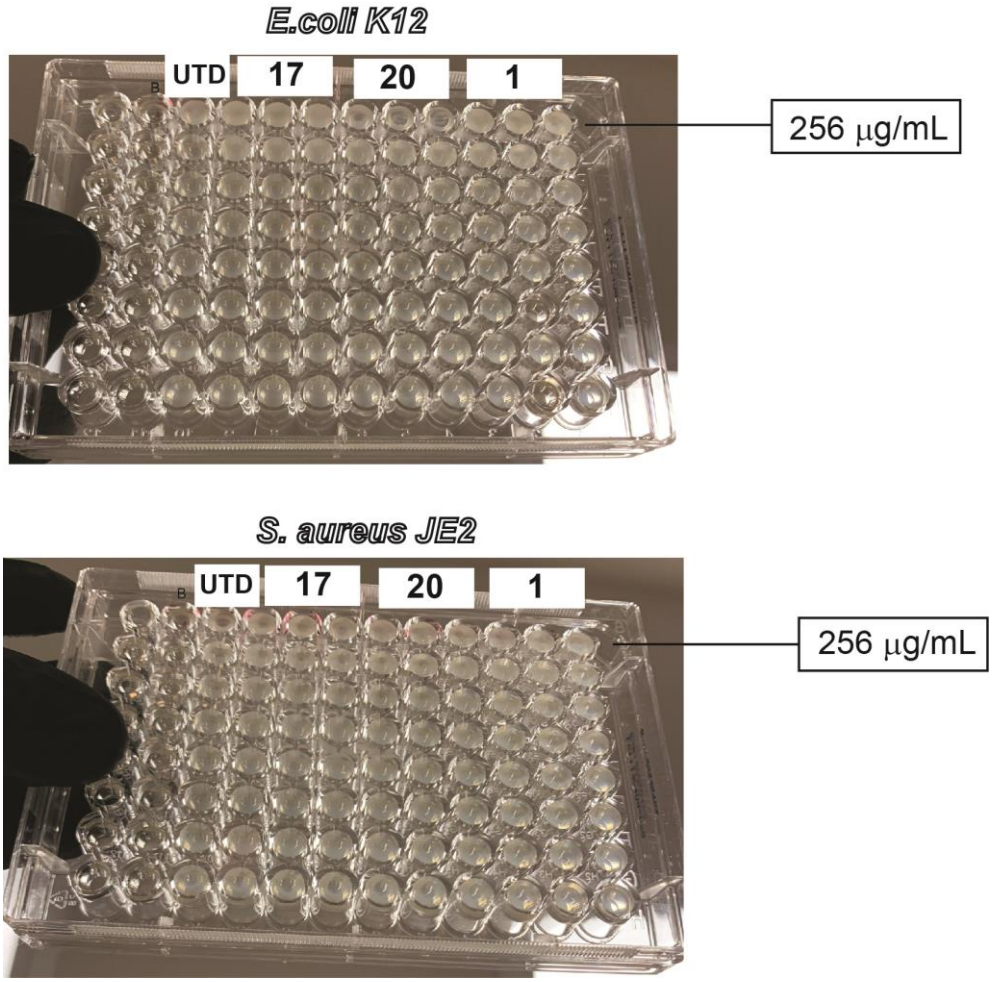


Figure S5. Antibacterial investigation of **1**, **17**, and **20** against *E.coli* K12 and *S.aureus* JE2. Data represent two biological replicates.

Three-dimensional (3D) stratified squamous culture:

To generate our model, human keratinocytes (HaCaT) were cultured on top of mouse fibroblasts (NIH-3T3), which were seeded into a hydrogel scaffold.[1] Here, we used a HaCaT keratinocyte-based 3D culture model[2] to mimic the stratified differentiating state of the lower genital tract and to test the ability of *Chlamydia* to grow and spread into stratified epithelium as a part of our *in vitro* investigations. Initially, HaCaT cells were seeded over a collagen rafts contain NIH-3T3 cells and allowed to proliferate for two days. Once confluent, the media was removed from the raft and only added to the lower well to create an air-surface interface that enabled differentiation and stratification.

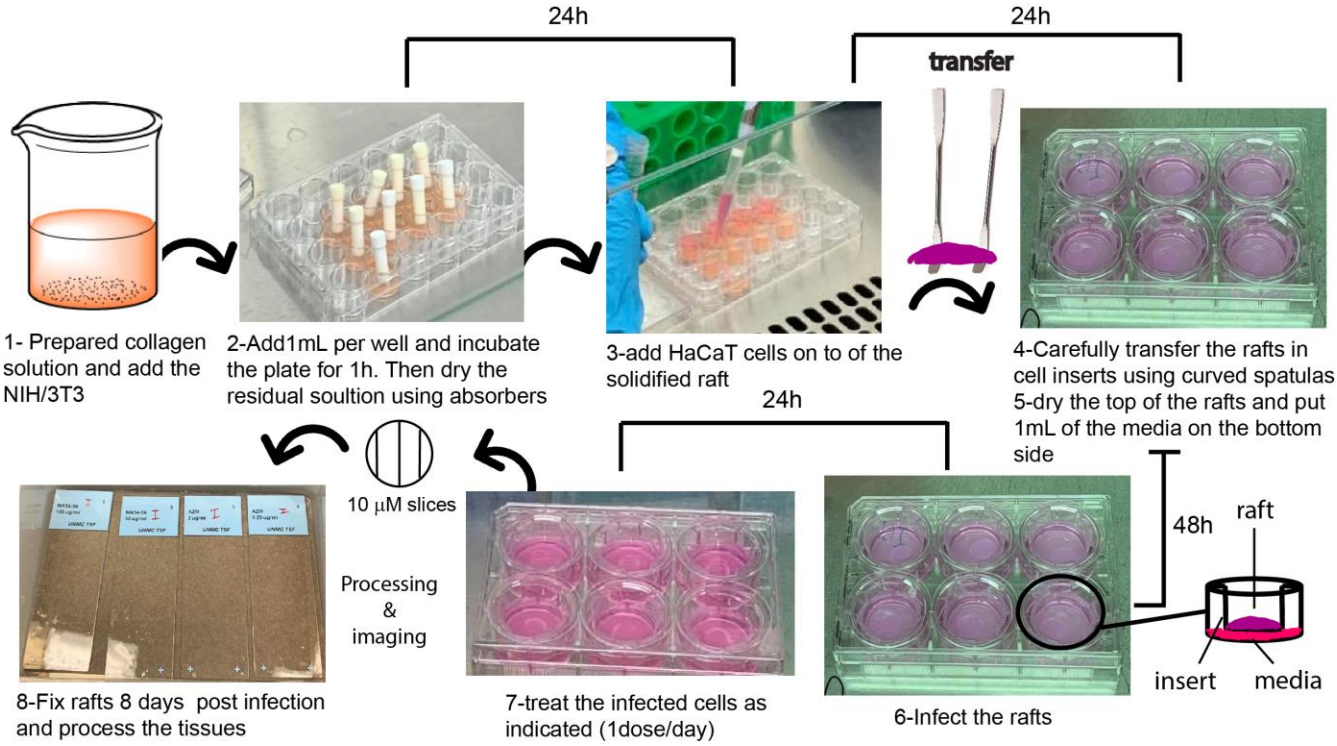


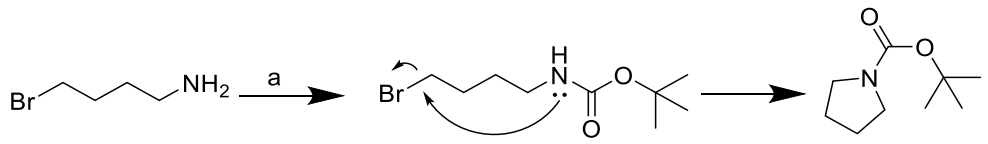
Figure S6. Schematic of the experiment procedures

Chemistry

Synthesis of 4-((2-Chlorophenyl)thio)butan-1-amine (17h):

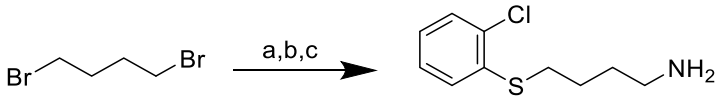
The synthesis of the right side of the molecule needed some protective group manipulation. The first set of conditions we tested led to cyclization of 1-bromobutylamine as detected by ^{1}HNMR.

Scheme S1: Alternative method to prepare **17h**



Reagents and conditions: (a) Boc_2O, Et_3N, DCM, 23 °C, 6h.

Scheme S2: Alternative method to prepare **15h**



15h

Reagents and conditions: (a) Phthalimide, acetone, 40 °C, overnight; (b) o-chlorothiophenol, sodium hydride, DMF, 80 °C, 4h; (c) Hydrazine hydrate, ethanol, 80 °C, overnight.

To a solution of 1,4-dibromobutane (1.00 mL, 5.50 mmol) in acetone (20 mL), was added potassium phthalimide (1.25 g, 6.75 mmol) and anhydrous potassium carbonate (1.10 g, 1 eq). The reaction was stirred at room temperature overnight. After reaction completion (as indicated by TLC), the white precipitate was filtered off. The organic solvent was then evaporated to afford the required compounds as yellowish-white powder, which was used directly in the next step without further purification.[3]

In another flask, 2-(4-bromobutyl)isoindoline-1,3-dione (200 mg, 0.70 mmol) was dissolved in DMF (5 mL), and the reaction was brought to 0 °C before NaH (1.2 eq, 60% in mineral oil) was added portion-wise. After 15 minutes, o-chlorothiophenol (0.08 mL, 1 eq) in DMF (0.5 mL) was added dropwise. The reaction mixture was heated up to 80 °C and stirred for 18 h. After consuming the starting material (TLC monitored), the reaction was quenched with ice water, extracted with ethyl acetate, and dried over Na_2SO_4. The cured product was then purified using flash chromatography to afford the desired product as light-yellow oil (57 %), which was subjected to deprotection using hydrazine hydrate in ethanol to afford the desired free amine which was used directly in the coupling step without further purification.

Replacement of PyBOP with HBTU to avoid tri(pyrrolidin-1-yl)phosphine oxide byproduct

In some derivatives, tri(pyrrolidin-1-yl)phosphine oxide (TPPO) was obtained as a byproduct that was confirmed by ^{1}HNMR (**Figure S7**). Thus, we used HBTU instead in some derivatives, as indicated in **Scheme 2**. The water solubility of tetraethyl urea that formed as a byproduct allowed us to remove it by washing the organic extract with water.

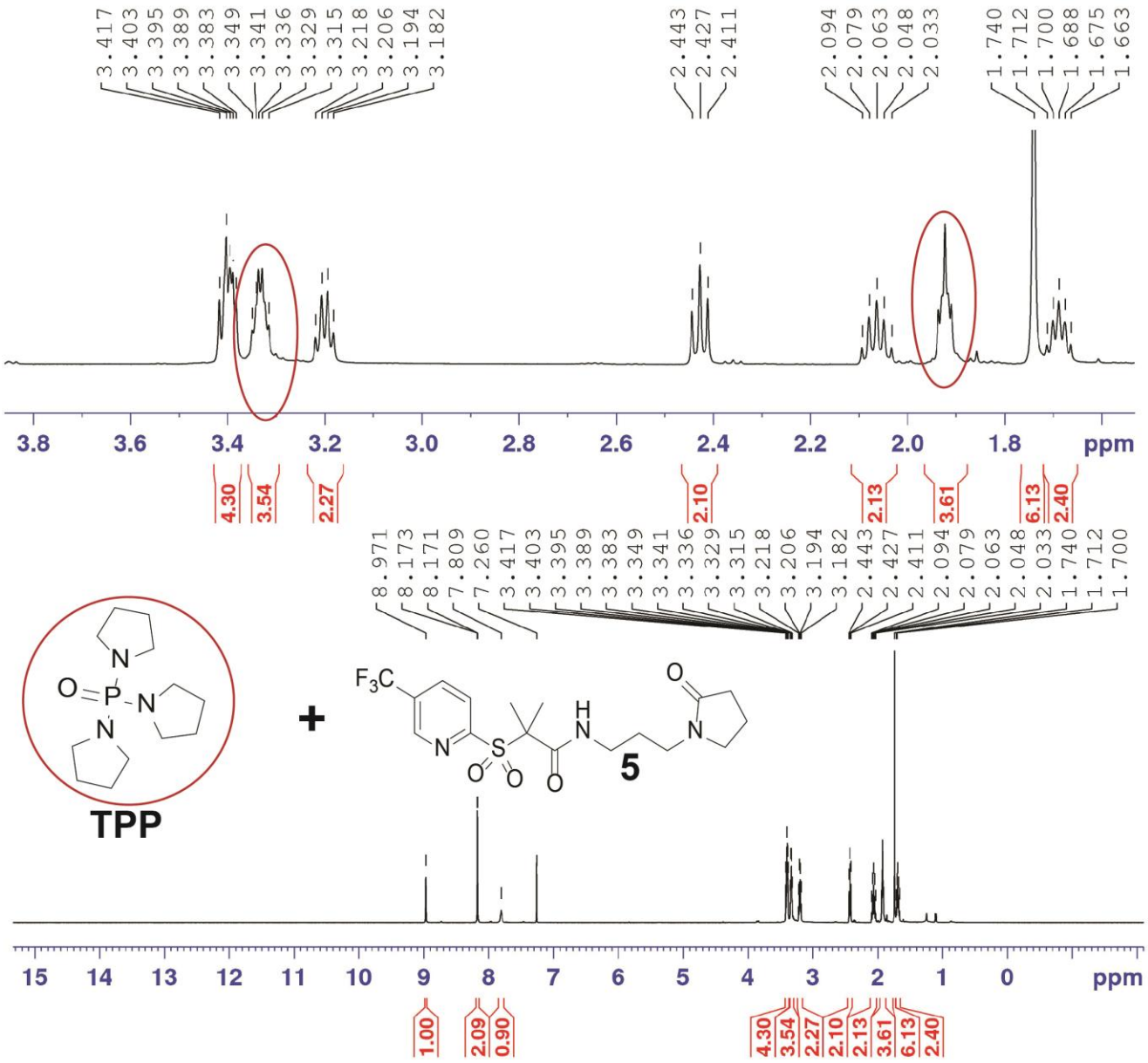
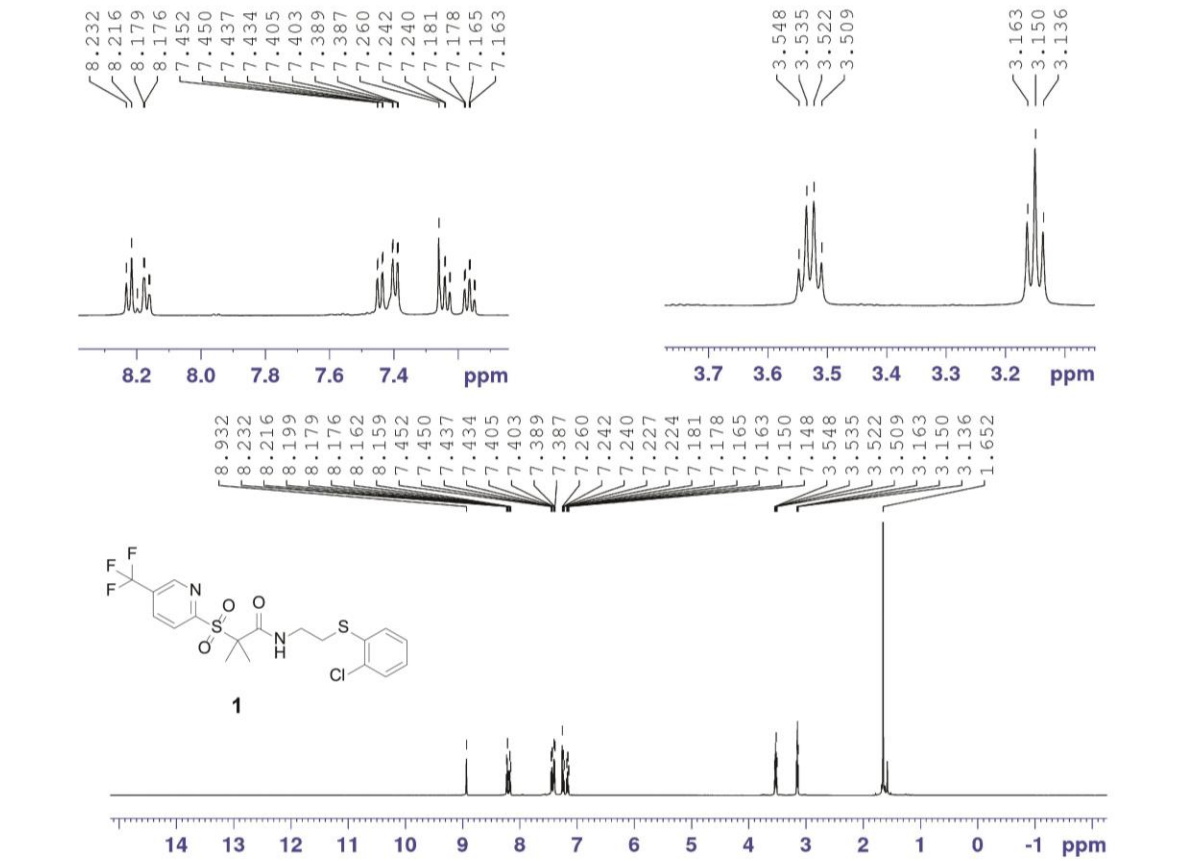


Figure S7: [1]HNMR of compound **5** contaminated with TPP as a side product released after the amine coupling.



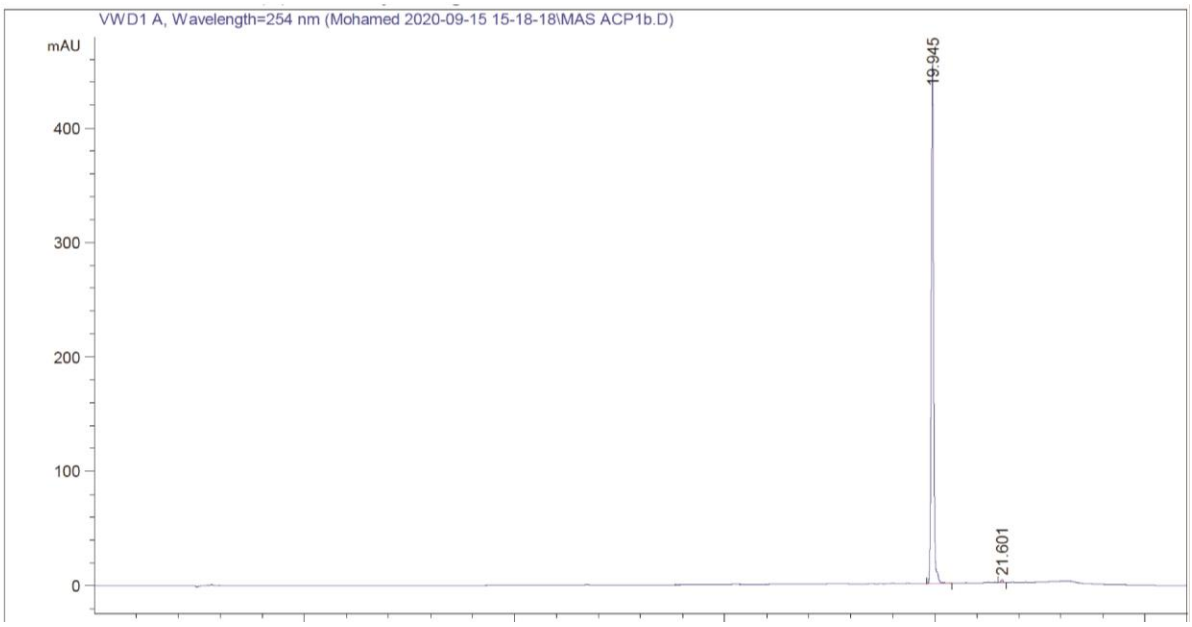
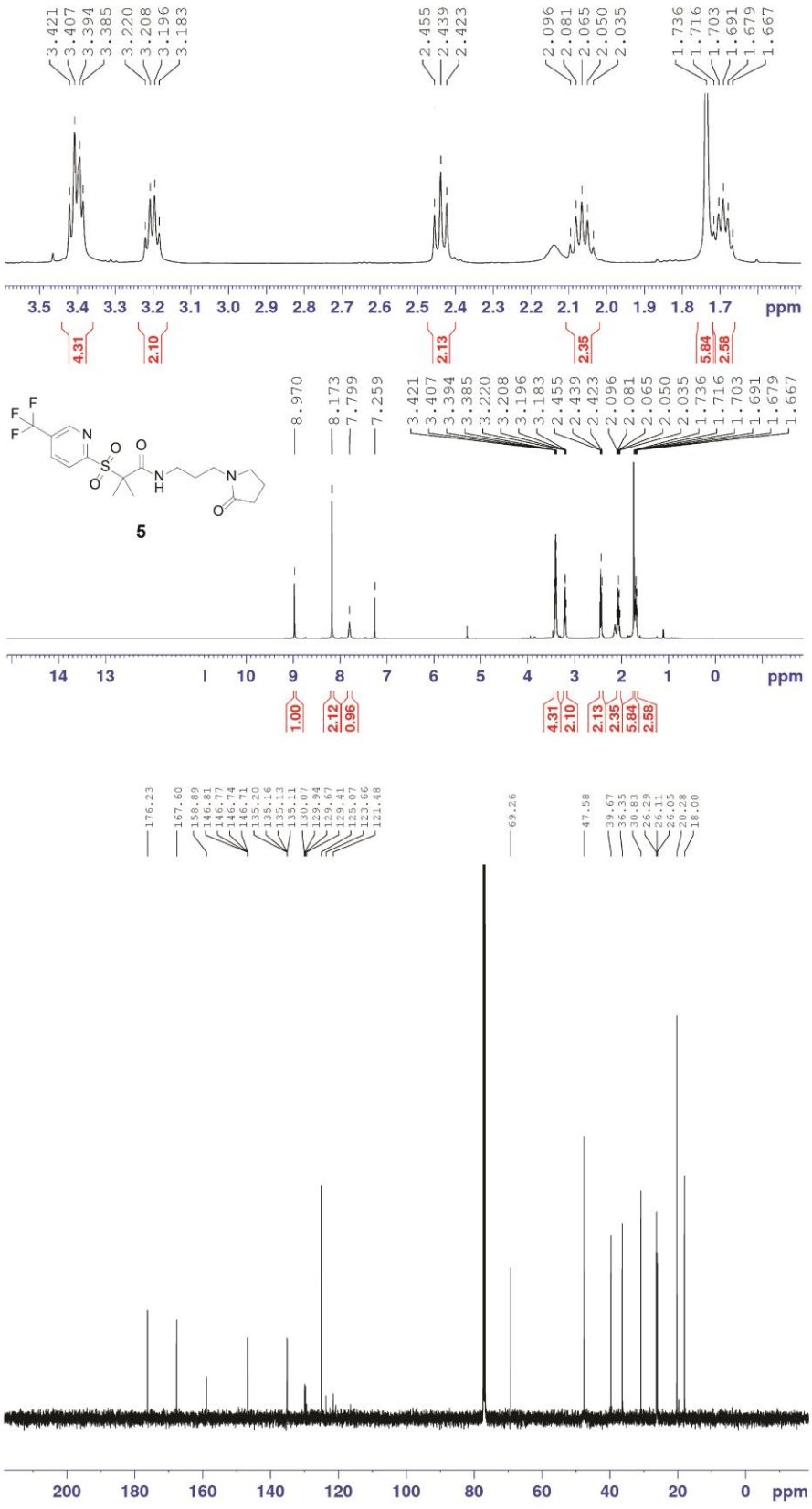


Figure S8. [1]HNMR and HPLC spectra of compound **1**


3.421
3.407
3.394
3.385
3.220
3.208
3.196
3.183
2.455
2.439
2.423
2.096
2.081
2.065
2.050
2.035
1.736
1.716
1.703
1.691
1.679
1.667
ppm
4.31
2.10
2.13
2.35
5.84
2.58
8.970
8.173
7.799
7.259
5
1.00
2.12
0.96
176.23
167.60
158.89
146.81
146.77
146.74
146.71
135.20
135.16
135.13
135.11
130.07
129.94
129.67
129.41
125.07
123.66
121.48
69.26
47.58
39.67
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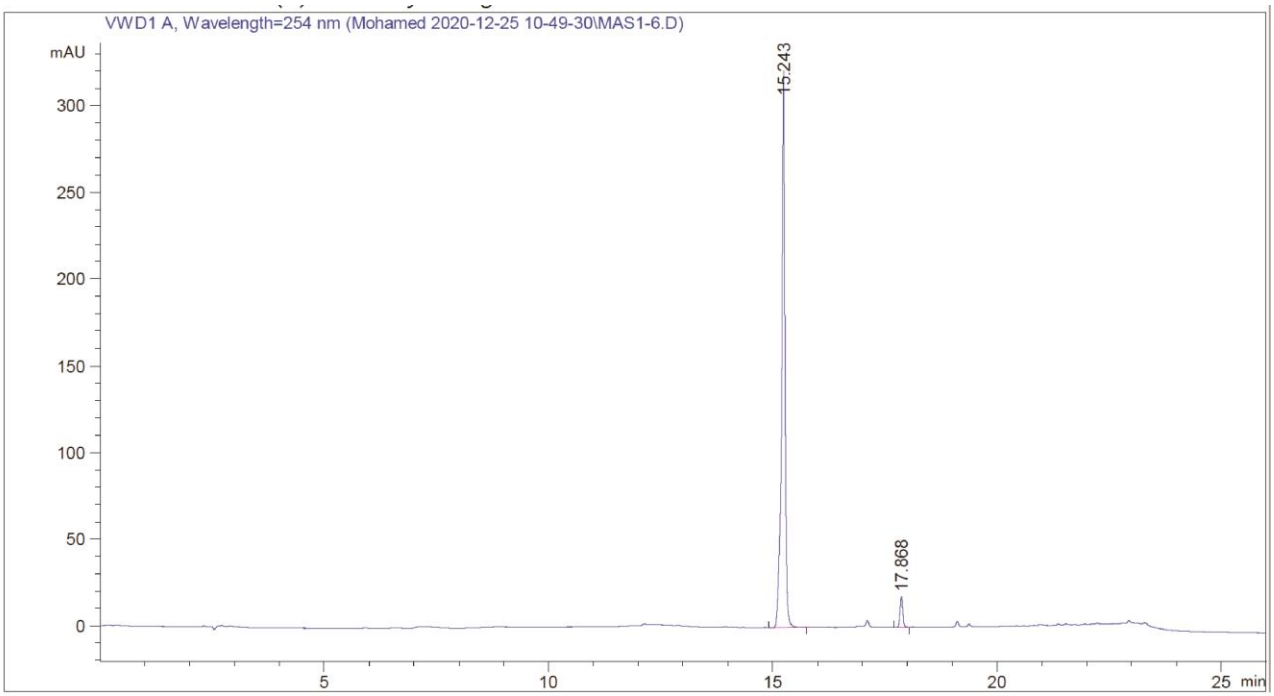
30.83
26.29
26.11
26.05
20.28
18.00



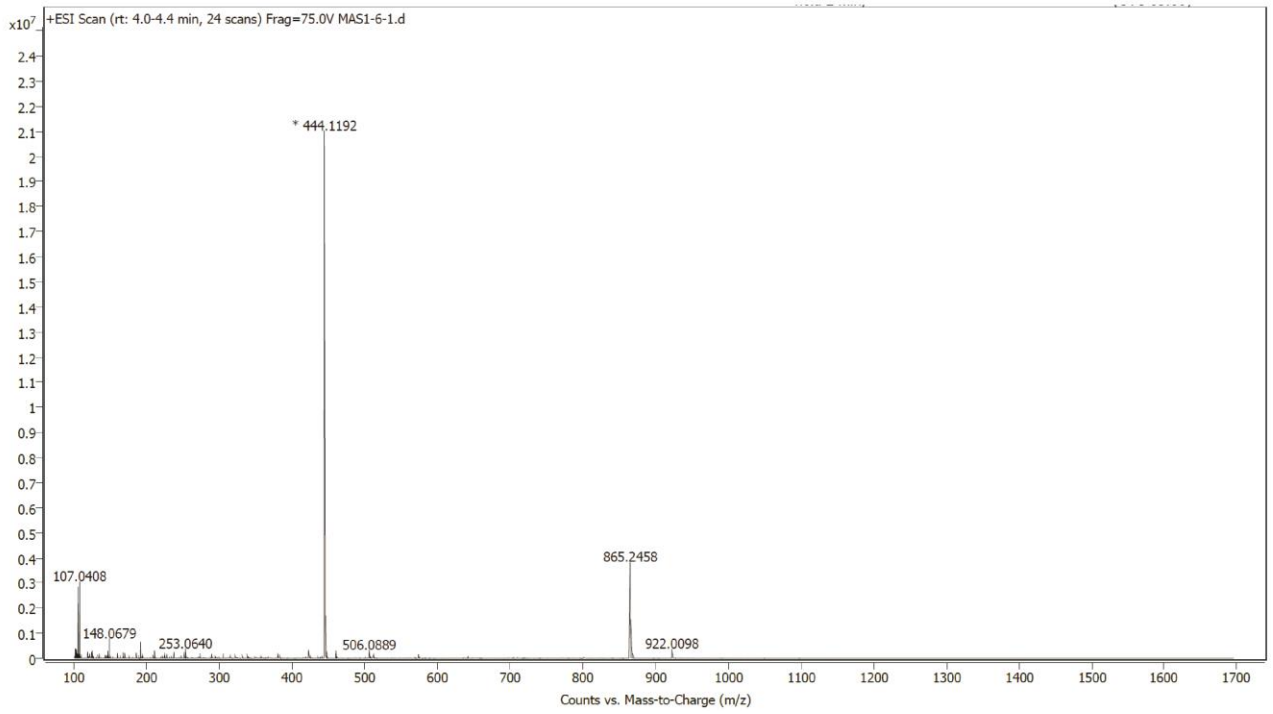
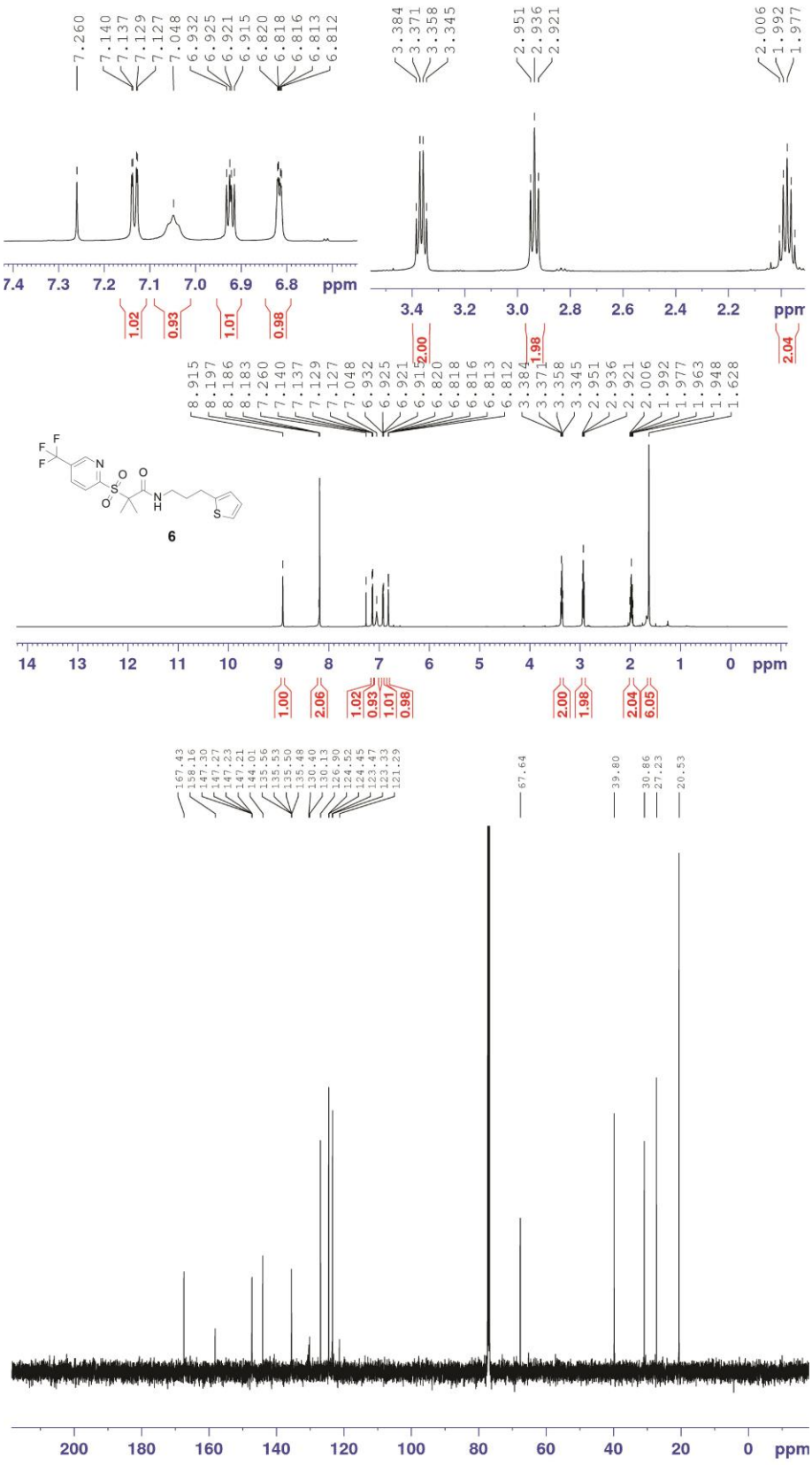


Figure S9. NMR, HPLC, and MS spectra of compound **5**


7.260
7.140
7.137
7.129
7.127
7.048
6.932
6.925
6.921
6.915
6.820
6.818
6.816
6.813
6.812
3.384
3.371
3.358
3.345
2.951
2.936
2.921
2.006
1.992
1.977
7.4 7.3 7.2 7.1 7.0 6.9 6.8 ppm
3.4 3.2 3.0 2.8 2.6 2.4 2.2 ppm
1.02
0.93
1.01
0.98
2.00
1.98
2.04
8.915
8.197
8.186
8.183
1.963
1.948
1.628
6
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
1.00
2.06
1.02
0.93
1.01
0.98
2.00
1.98
2.04
6.05
167.43
158.16
147.30
147.27
147.23
147.21
144.01
135.56
135.53
135.50
135.48
130.40
130.13
126.90
124.52
124.45
123.47
123.33
121.29
67.64
39.80
30.86
27.23
20.53
200 180 160 140 120 100 80 60 40 20 0 ppm

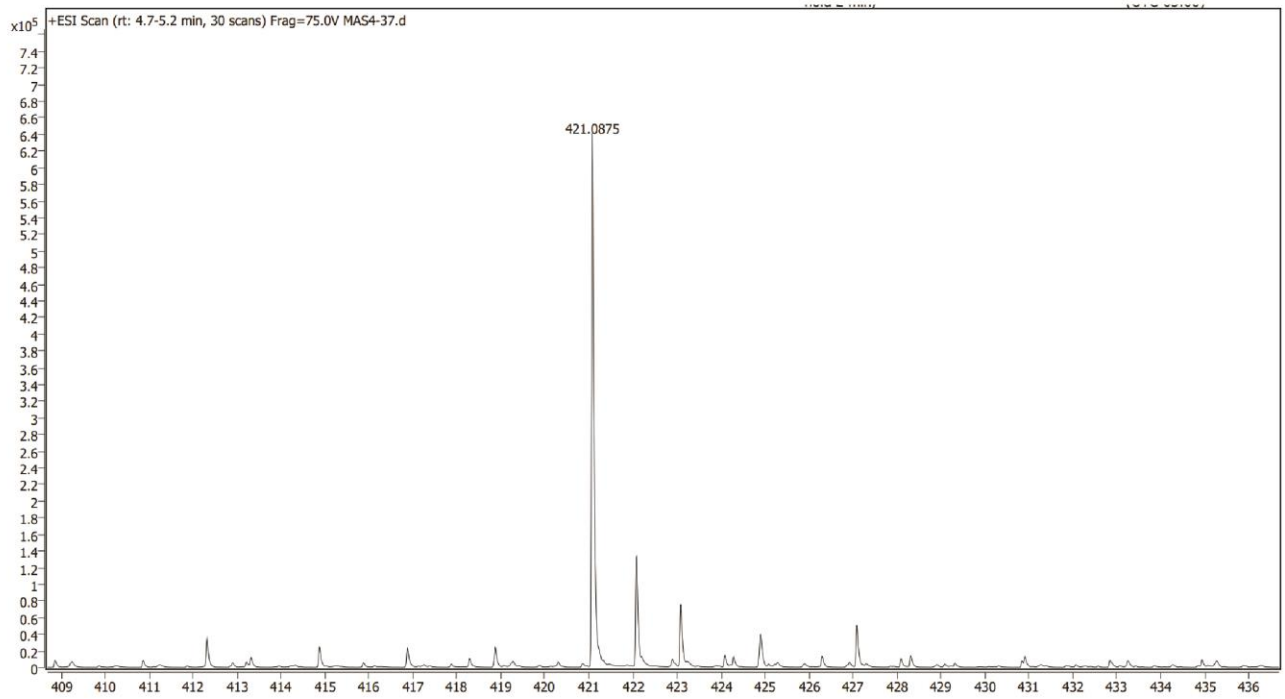
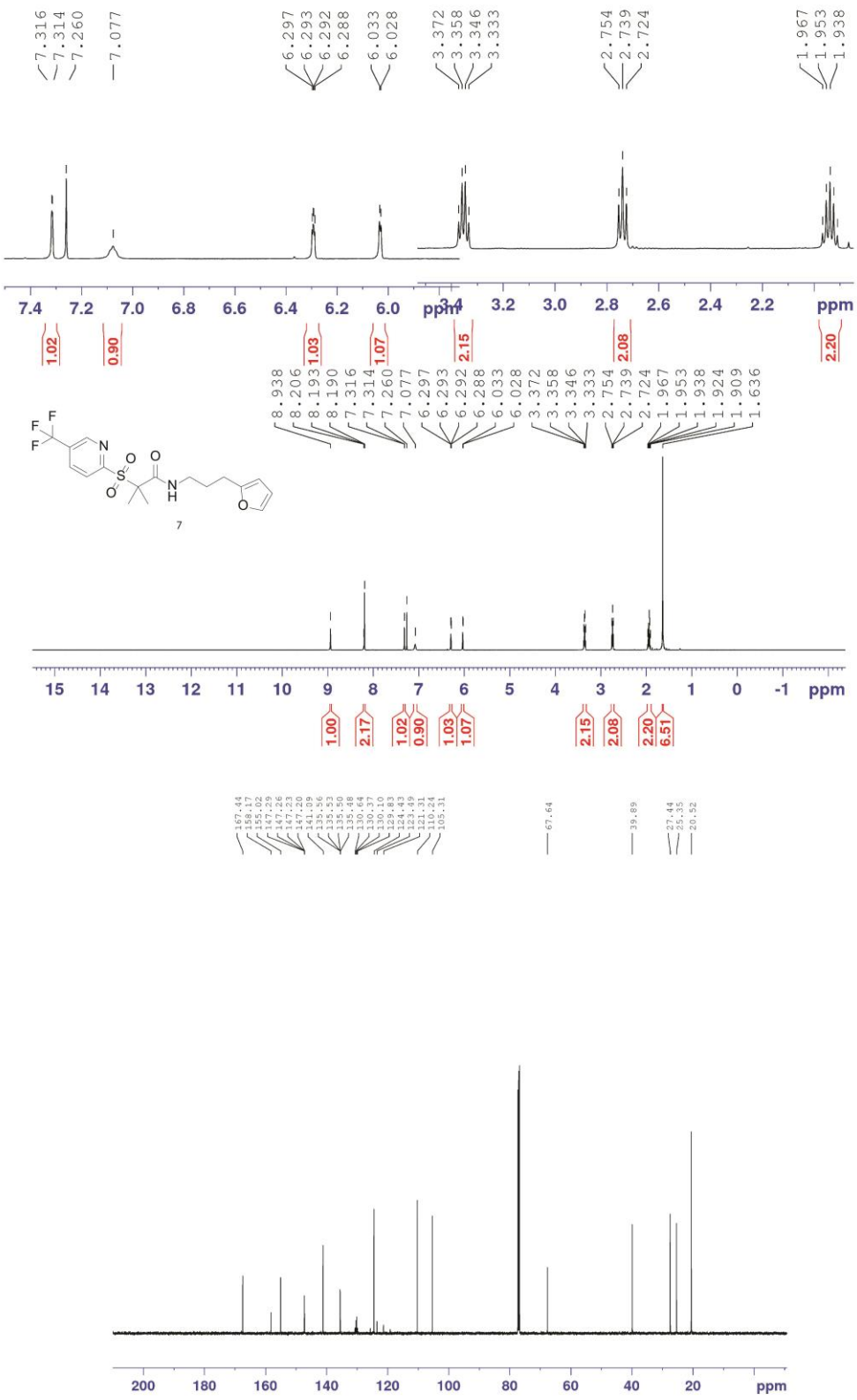
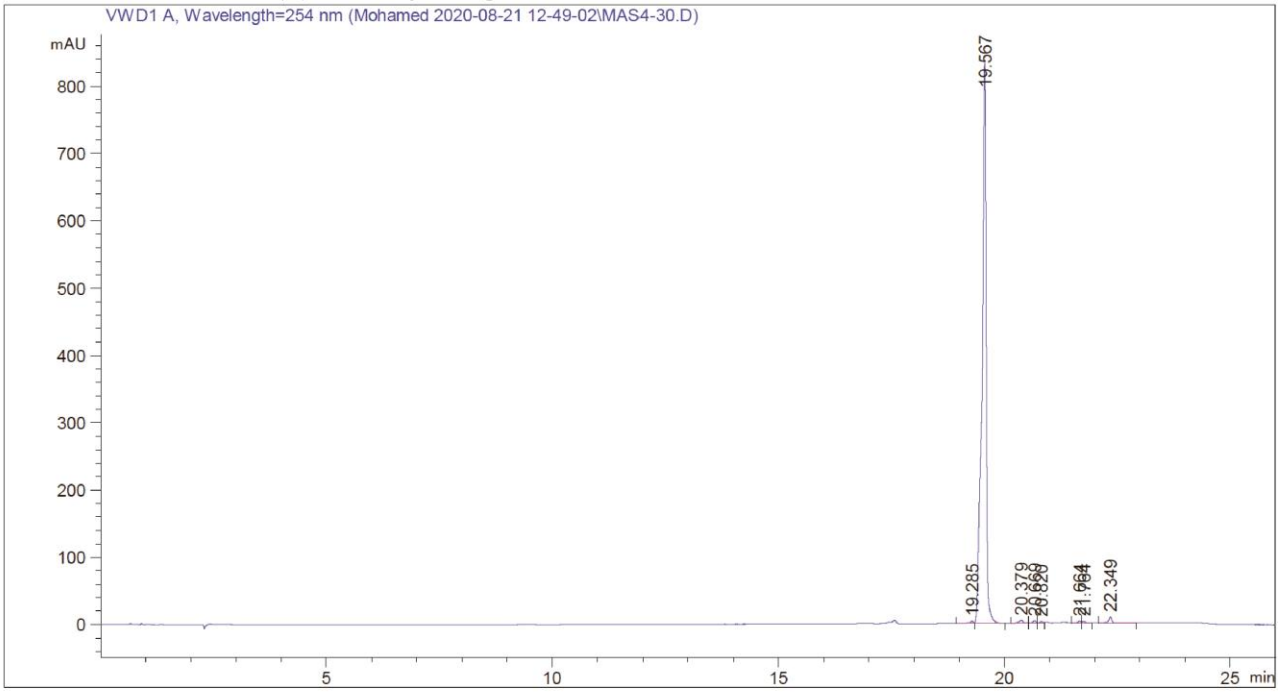


Figure S10. NMR, HPLC, and MS spectra of compound **6**


7.316
7.314
7.260
7.077
6.297
6.293
6.292
6.288
6.033
6.028
3.372
3.358
3.346
3.333
2.754
2.739
2.724
1.967
1.953
1.938
ppm
1.02
0.90
1.03
1.07
2.15
2.08
2.20
8.938
8.206
8.193
8.190
7.316
7.314
7.260
7.077
6.297
6.293
6.292
6.288
6.033
6.028
3.372
3.358
3.346
3.333
2.754
2.739
2.724
1.967
1.953
1.938
1.924
1.909
1.636
7
1.00
2.17
1.02
0.90
1.03
1.07
2.15
2.08
2.20
6.51
167.44
158.17
155.02
147.29
147.26
147.23
147.20
141.09
135.56
135.53
135.50
135.48
130.64
130.37
130.10
129.83
124.43
123.49
121.31
110.24
105.31
67.64
39.89
27.44
25.35
20.52
ppm



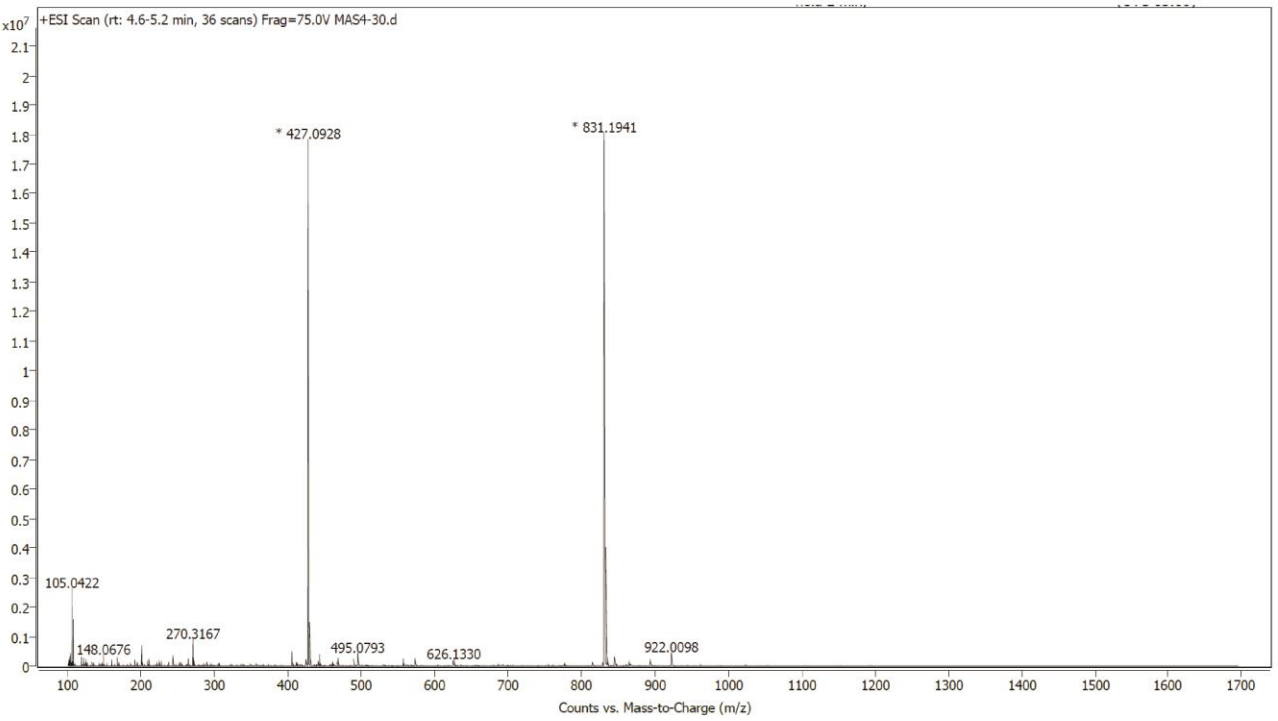
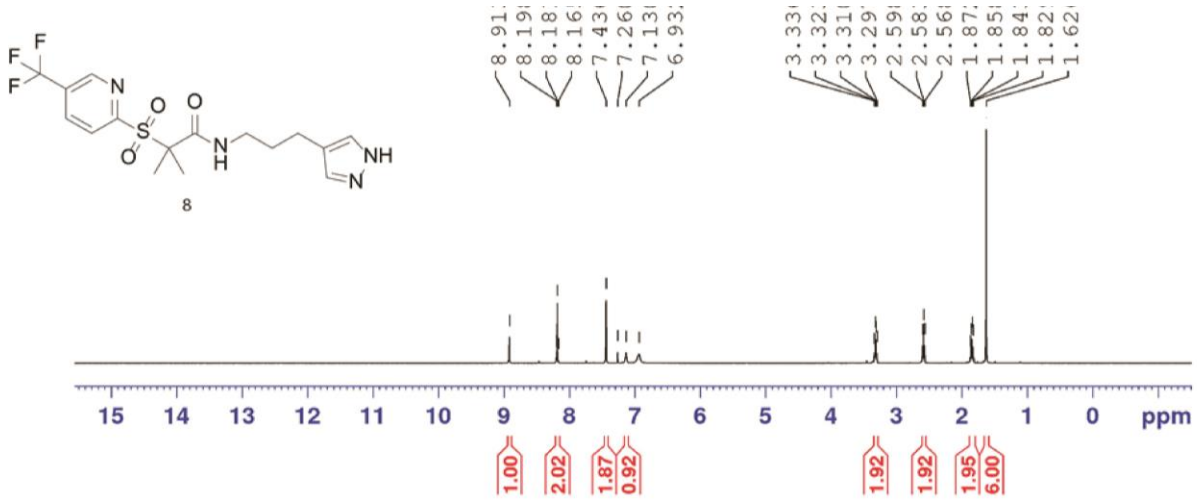
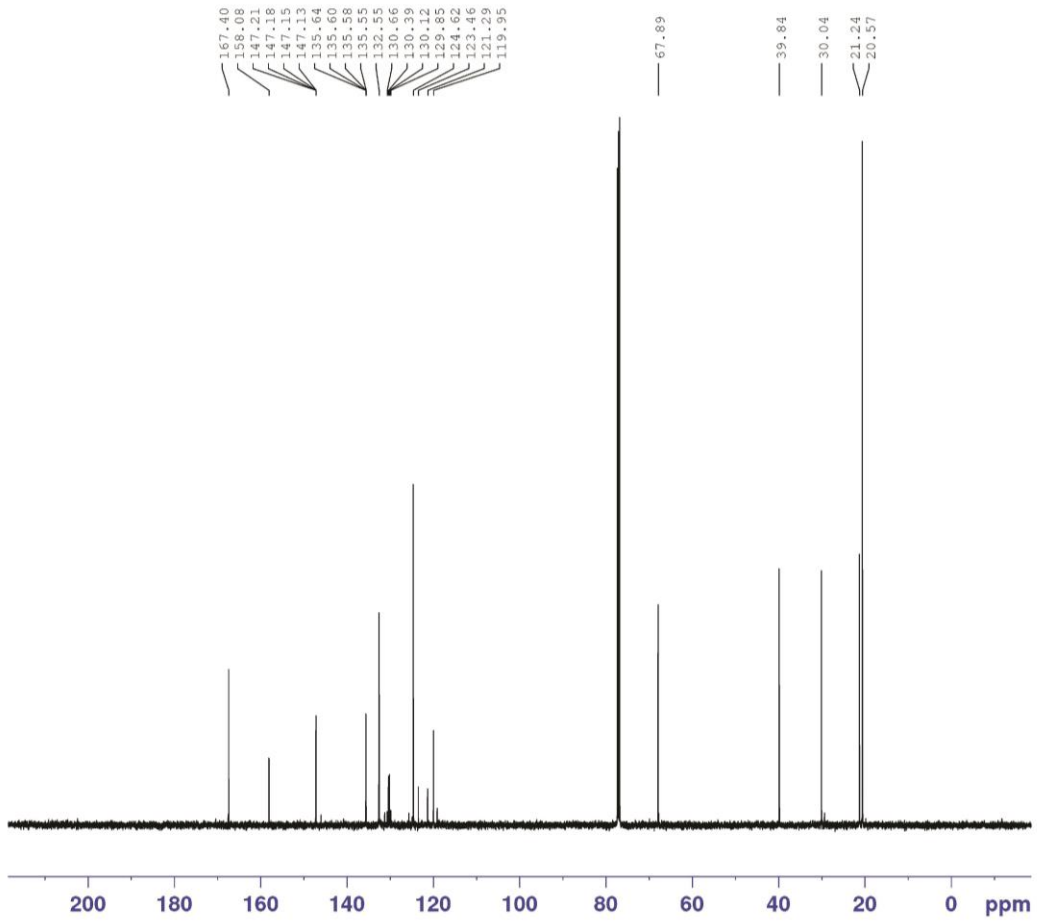
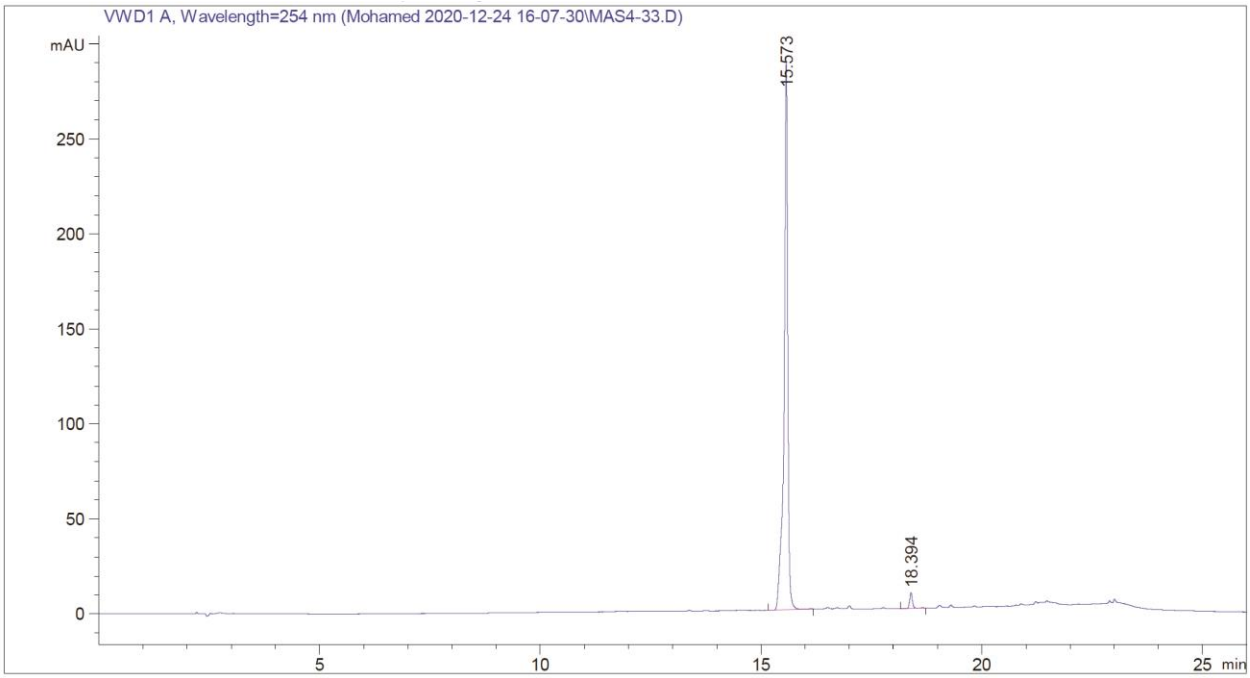


Figure S11. NMR, HPLC, and MS spectra of compound **7**


8.91
8.19
8.18
8.16
7.43
7.26
7.13
6.93
3.33
3.32
3.31
3.29
2.59
2.58
2.56
1.87
1.85
1.84
1.82
1.62
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
1.00
2.02
1.87
0.92
1.92
1.92
1.95
6.00


167.40
158.08
147.21
147.18
147.15
147.13
135.64
135.60
135.58
135.55
132.55
130.66
130.39
130.12
129.85
124.62
123.46
121.29
119.95
67.89
39.84
30.04
21.24
20.57
200 180 160 140 120 100 80 60 40 20 0 ppm



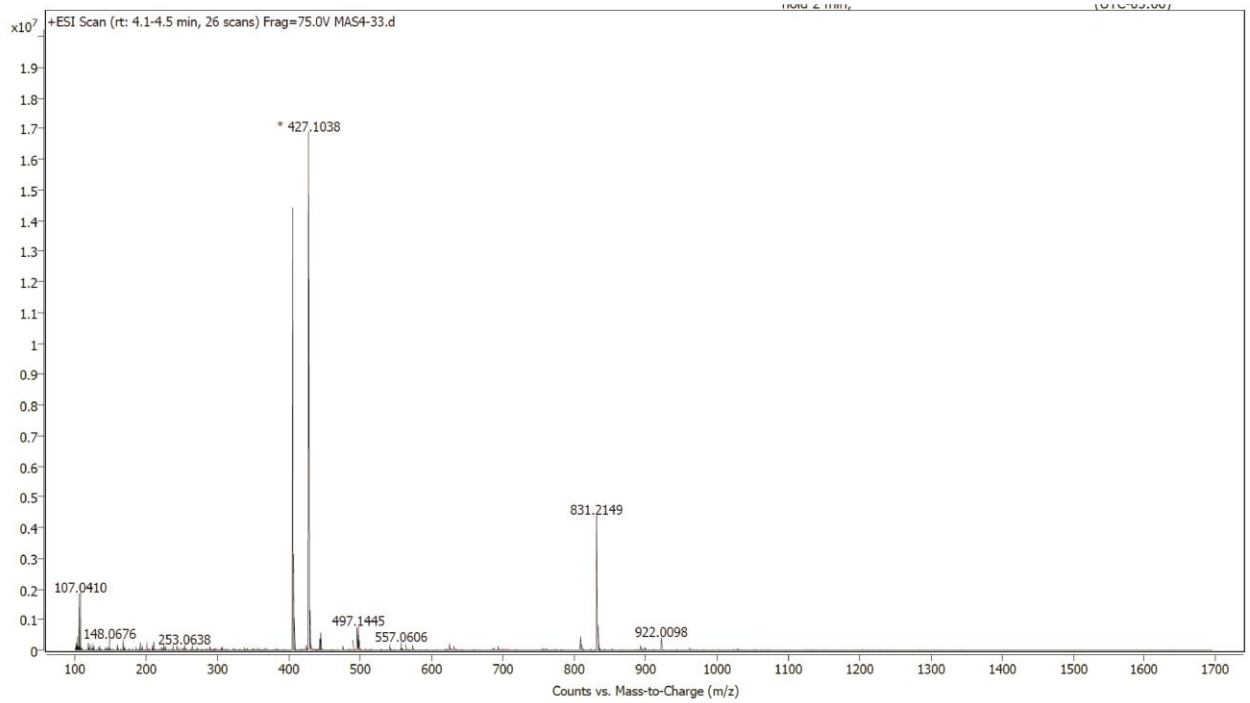
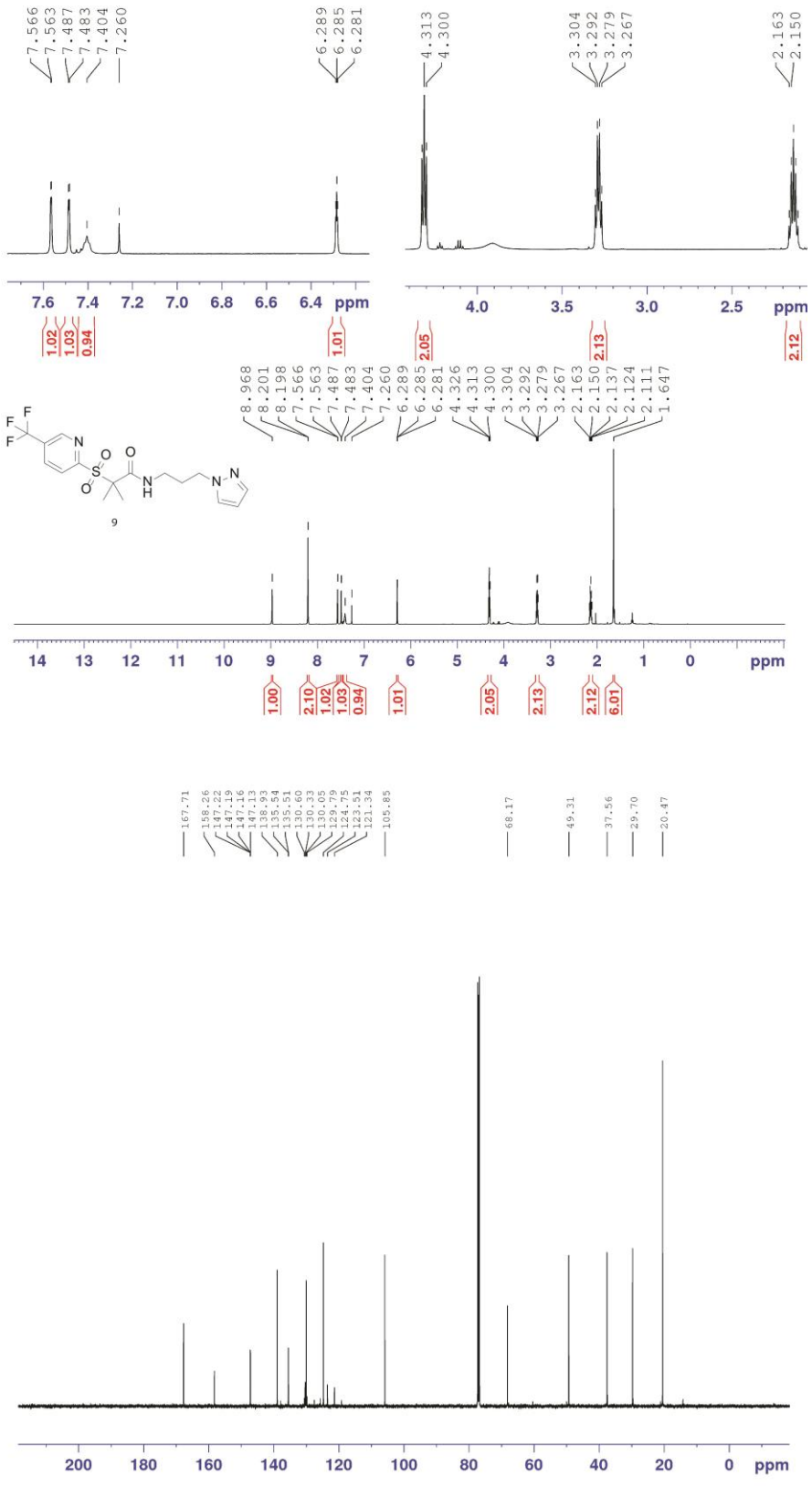


Figure S12. NMR, HPLC, and MS spectra of compound **8**


7.566
7.563
7.487
7.483
7.404
7.260
6.289
6.285
6.281
4.313
4.300
3.304
3.292
3.279
3.267
2.163
2.150
ppm
1.02
1.03
0.94
1.01
2.05
2.13
2.12
8.968
8.201
8.198
7.566
7.563
7.487
7.483
7.404
7.260
6.289
6.285
6.281
4.326
4.313
4.300
3.304
3.292
3.279
3.267
2.163
2.150
2.137
2.124
2.111
1.647
9
1.00
2.10
1.02
1.03
0.94
1.01
2.05
2.13
2.12
6.01
167.71
158.26
147.22
147.19
147.16
147.13
138.93
135.54
135.51
130.60
130.33
130.05
129.79
124.75
123.51
121.34
105.85
68.17
49.31
37.56
29.70
20.47

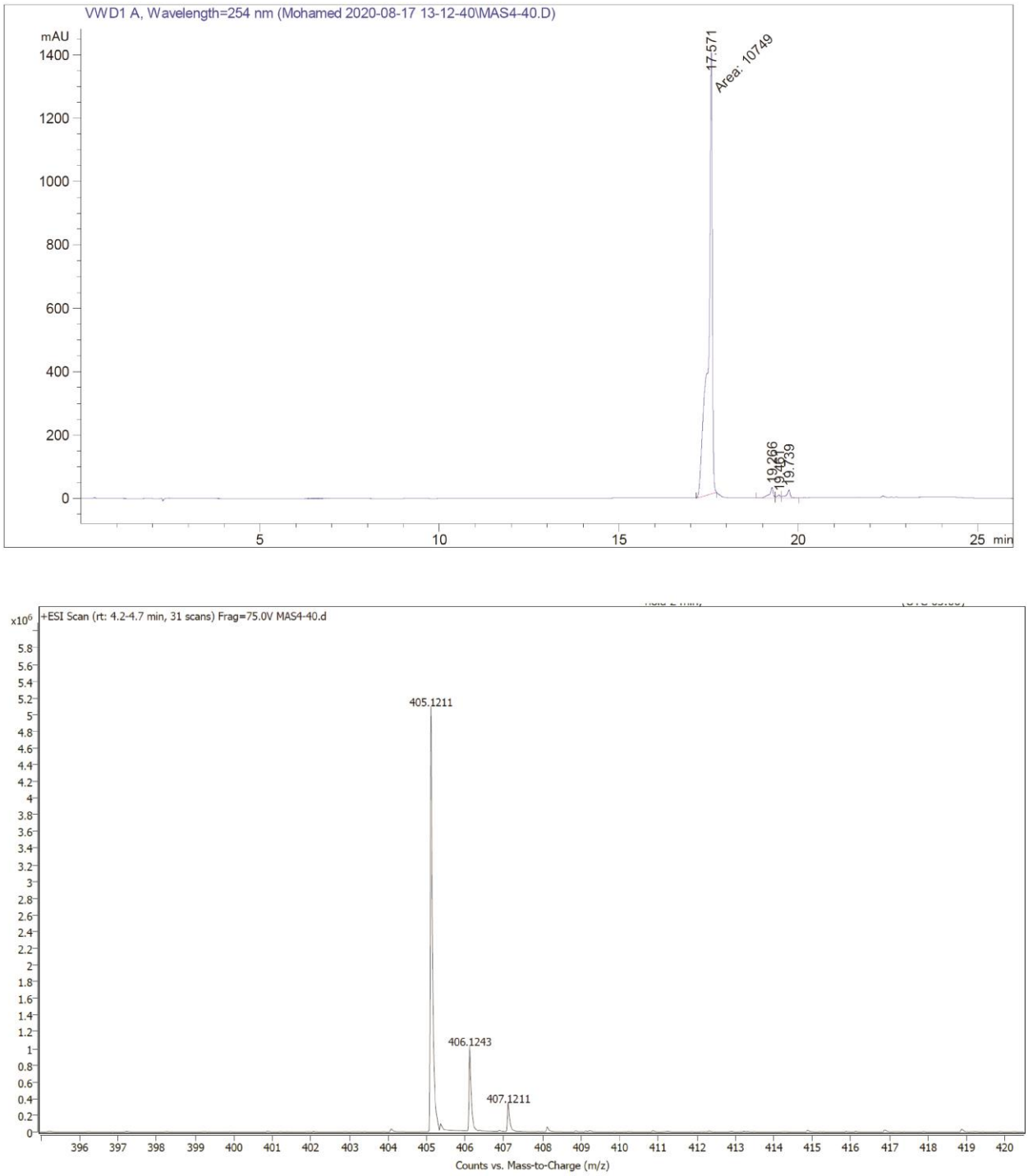
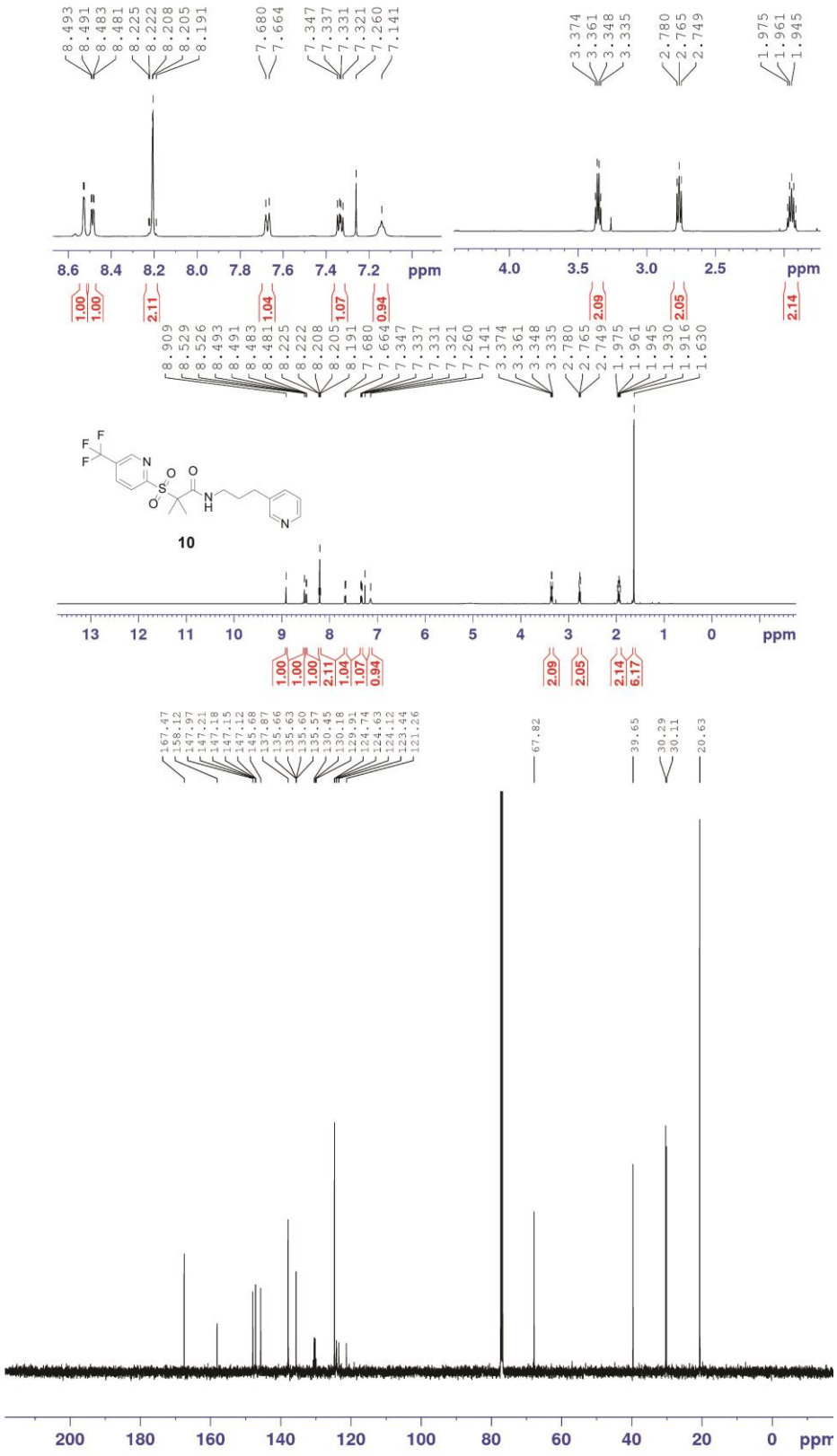


Figure S13. NMR, HPLC, and MS spectra of compound **9**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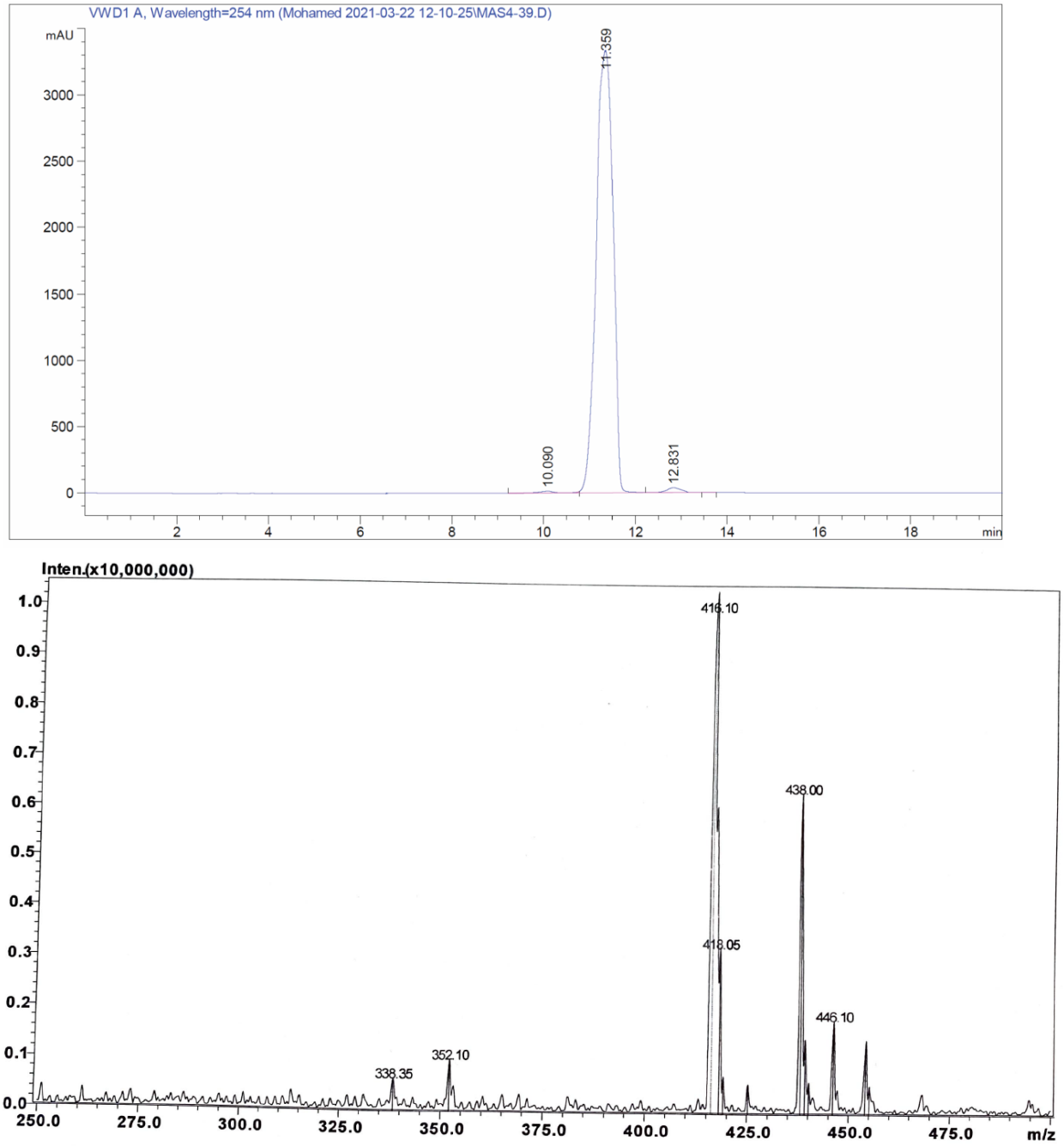
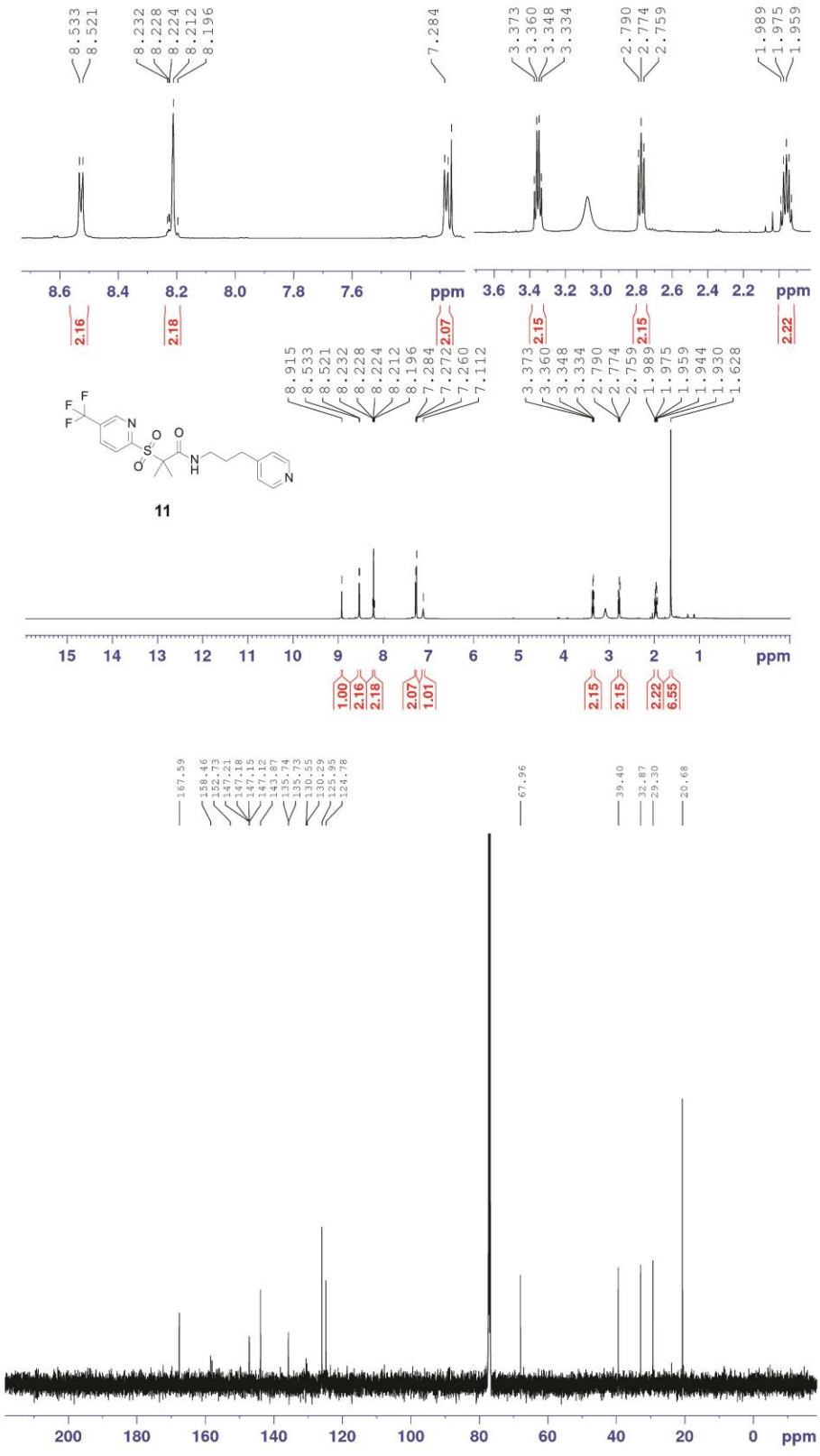
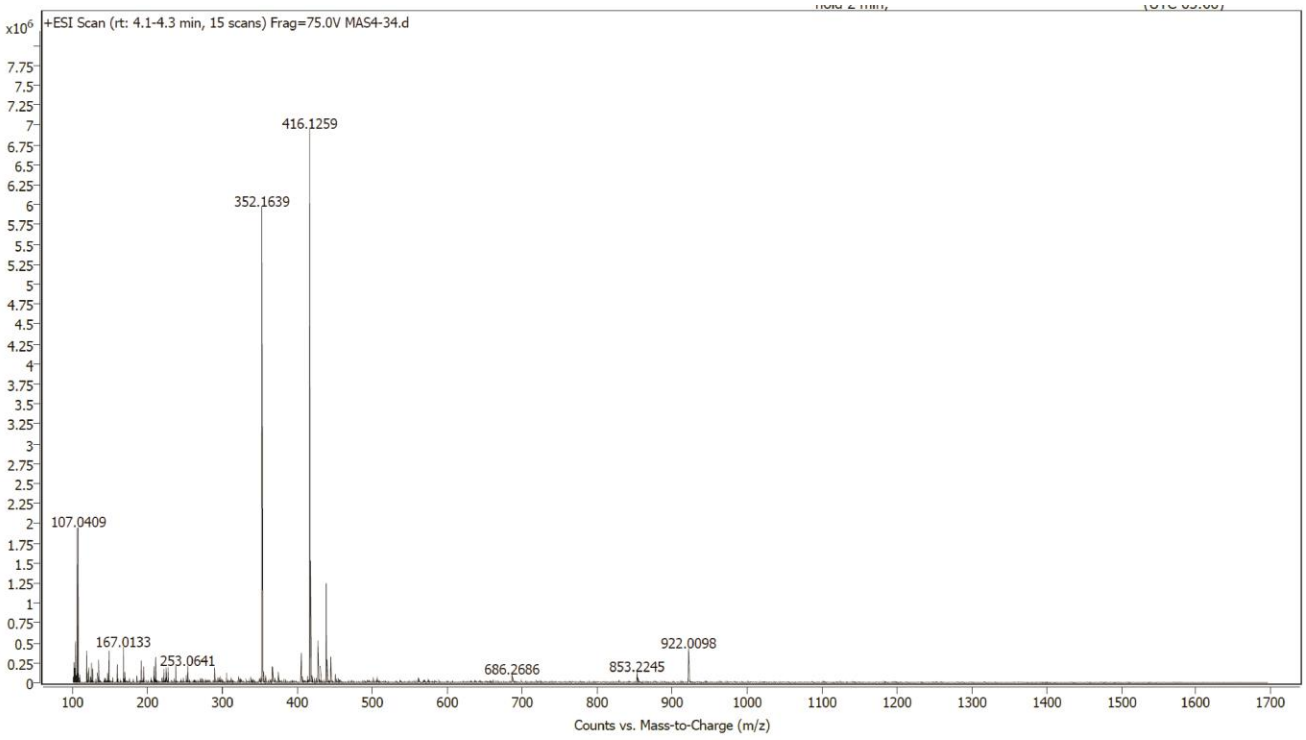


Figure S14. NMR, HPLC, and MS spectra of compound **10**


8.533
8.521
8.232
8.228
8.224
8.212
8.196
7.284
3.373
3.360
3.348
3.334
2.790
2.774
2.759
1.989
1.975
1.959
ppm
2.16
2.18
2.07
2.15
2.15
2.22
8.915
8.533
8.521
8.232
8.228
8.224
8.212
8.196
7.284
7.272
7.260
7.112
3.373
3.360
3.348
3.334
2.790
2.774
2.759
1.989
1.975
1.959
1.944
1.930
1.628
11
1.00
2.16
2.18
2.07
1.01
2.15
2.15
2.22
6.55
167.59
158.46
152.73
147.21
147.18
147.15
147.12
143.87
143.74
135.73
130.55
130.29
125.95
124.78
67.96
39.40
32.87
29.30
20.68



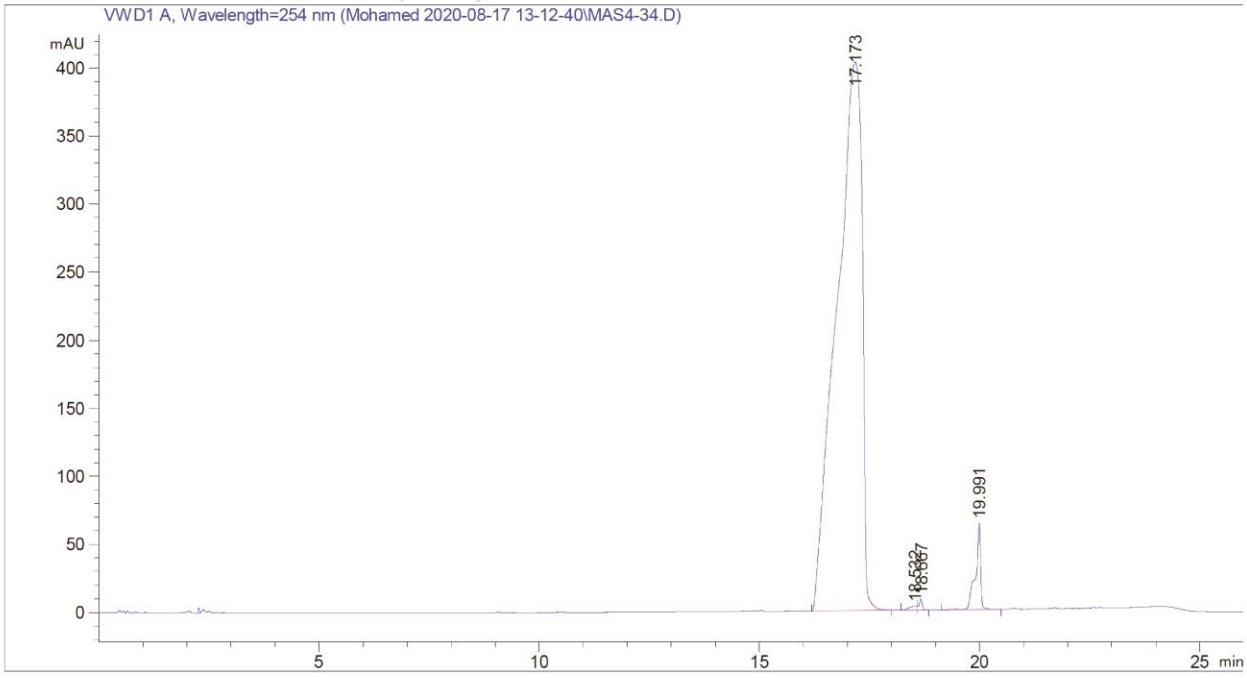
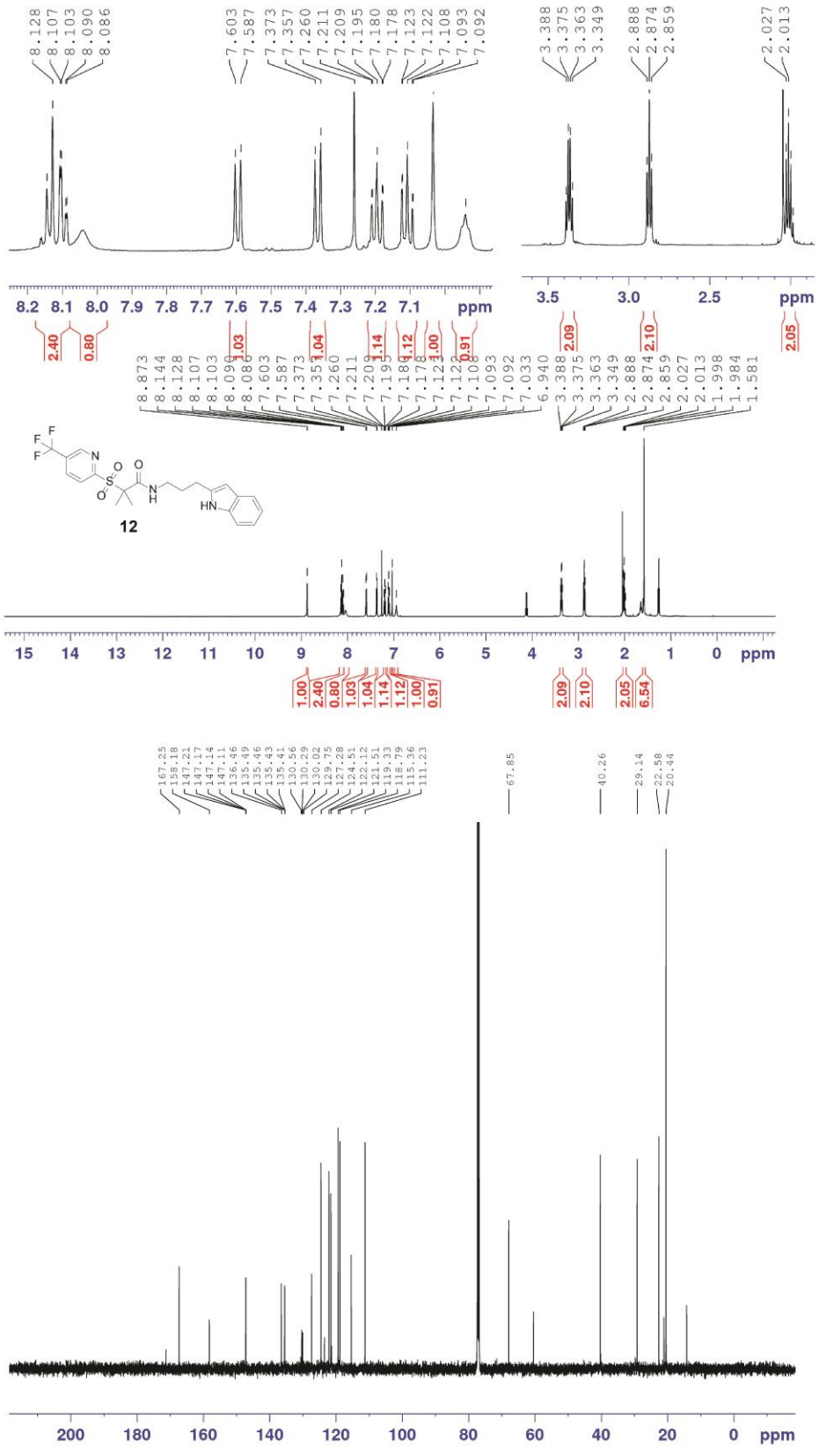
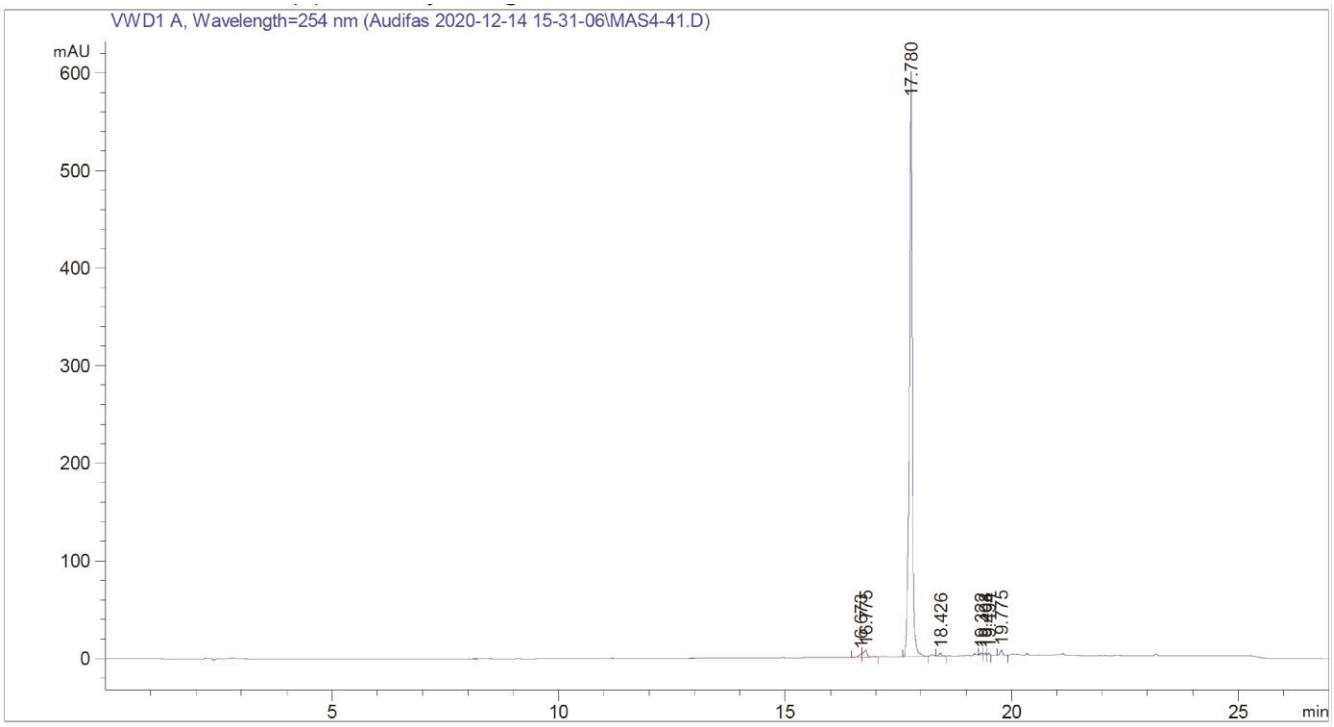


Figure S15. NMR, HPLC, and MS spectra of compound **11**


12



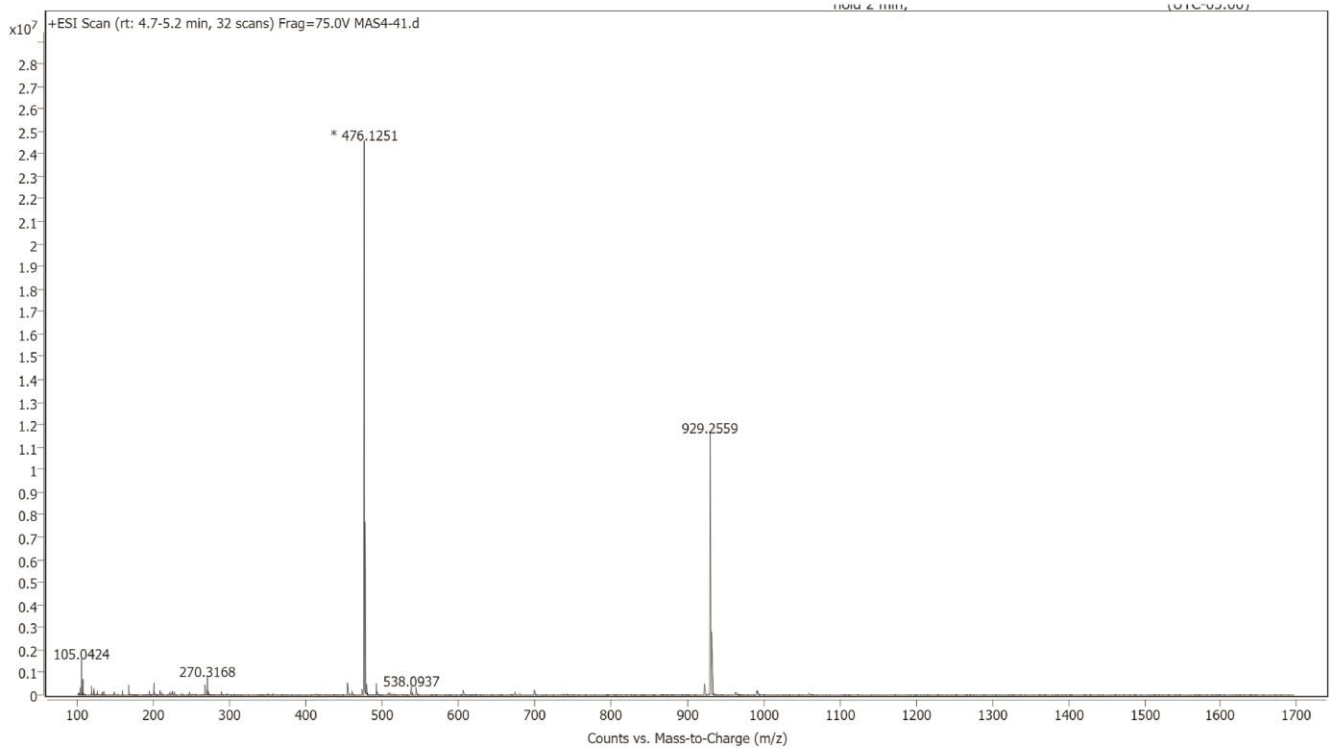
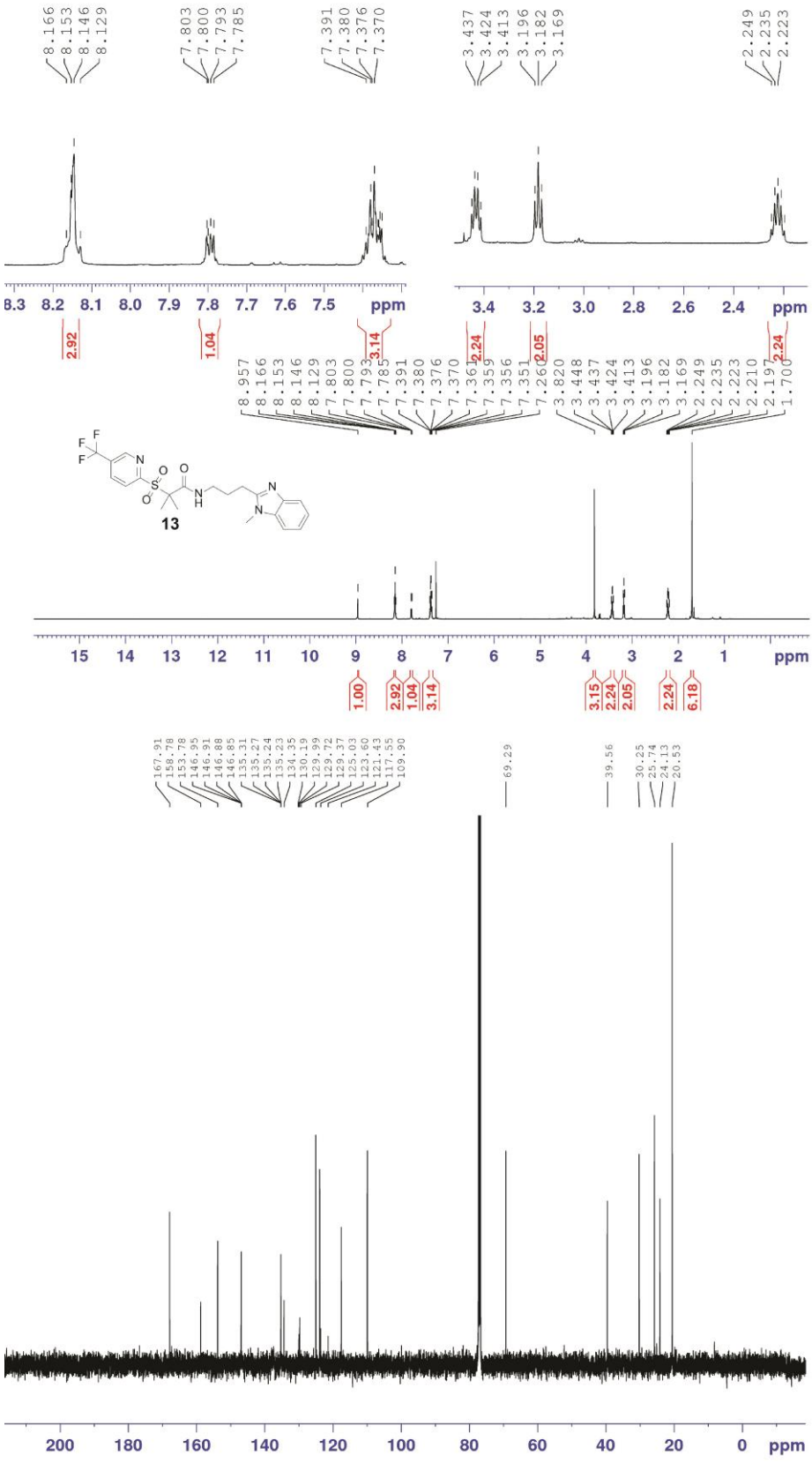
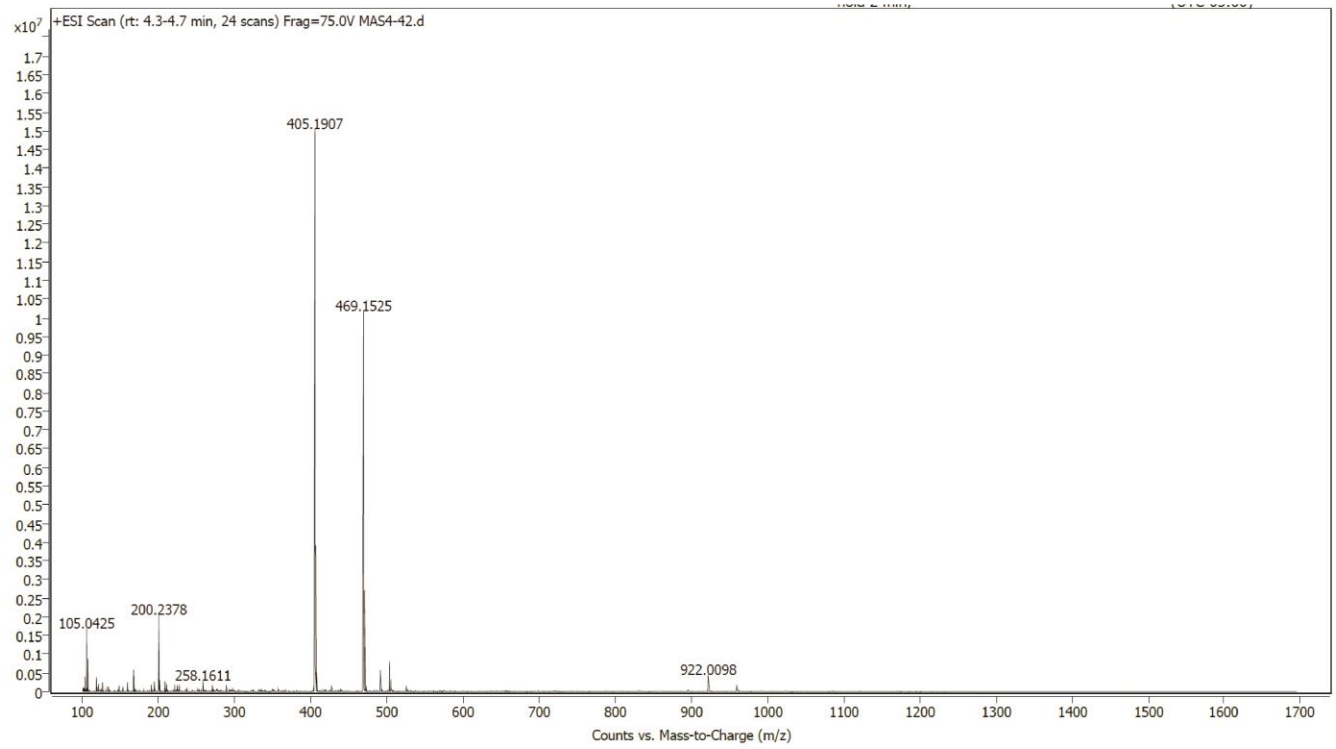


Figure S16. NMR, HPLC, and MS spectra of compound **12**


8.166
8.153
8.146
8.129
7.803
7.800
7.793
7.785
7.391
7.380
7.376
7.370
3.437
3.424
3.413
3.196
3.182
3.169
2.249
2.235
2.223
ppm
2.92
1.04
3.14
2.24
2.05
2.24
13
1.00
2.92
1.04
3.14
3.15
2.24
2.05
2.24
6.18
167.91
158.78
153.78
146.95
146.91
146.88
146.85
135.31
135.27
135.24
135.23
134.35
130.19
129.99
129.72
129.37
125.03
123.60
121.43
117.55
109.90
69.29
39.56
30.25
25.74
24.13
20.53



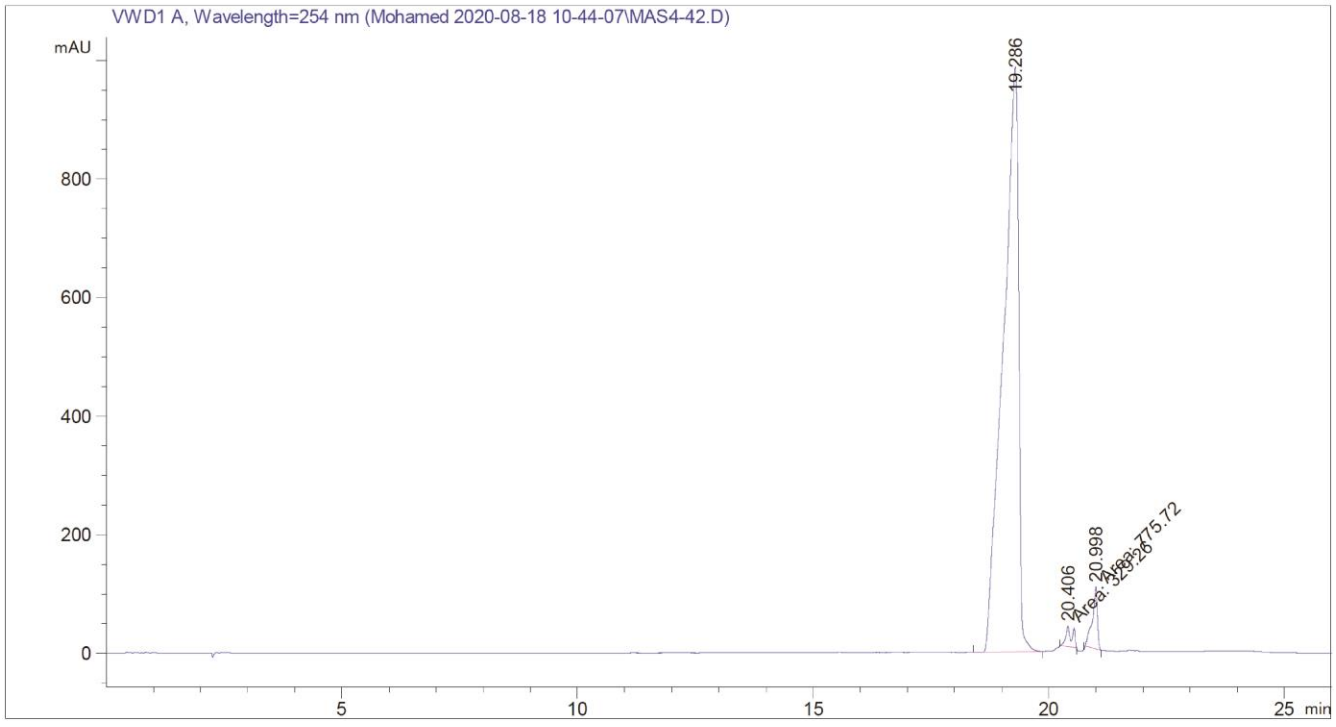
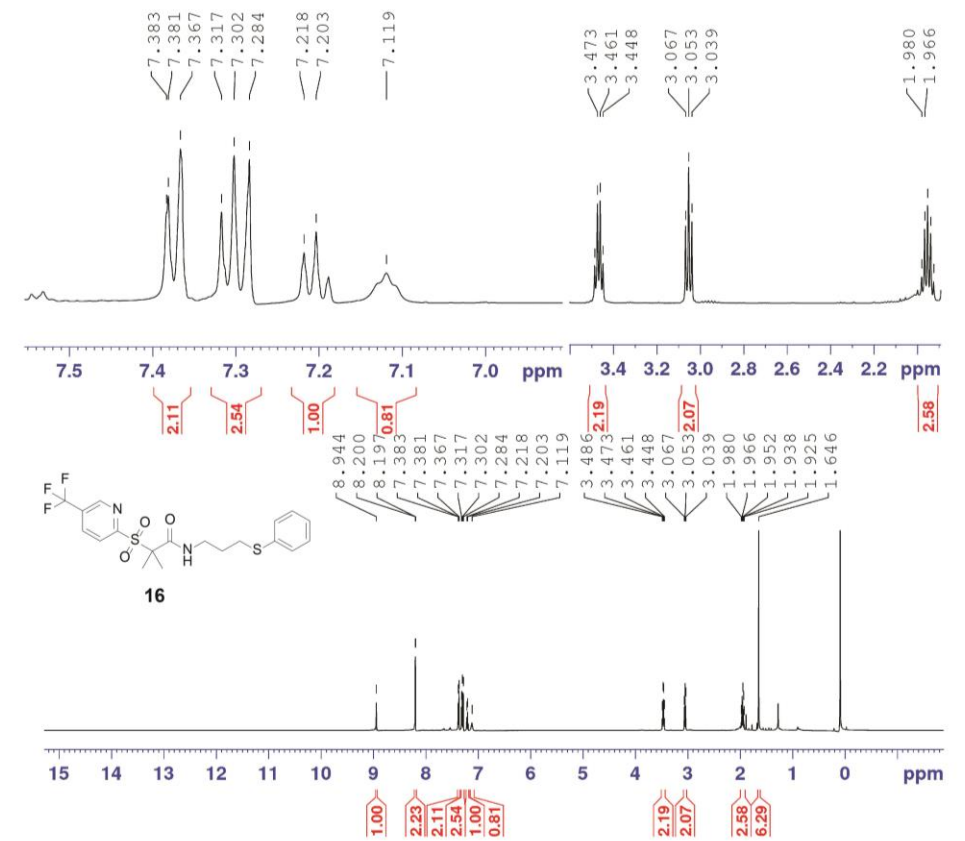
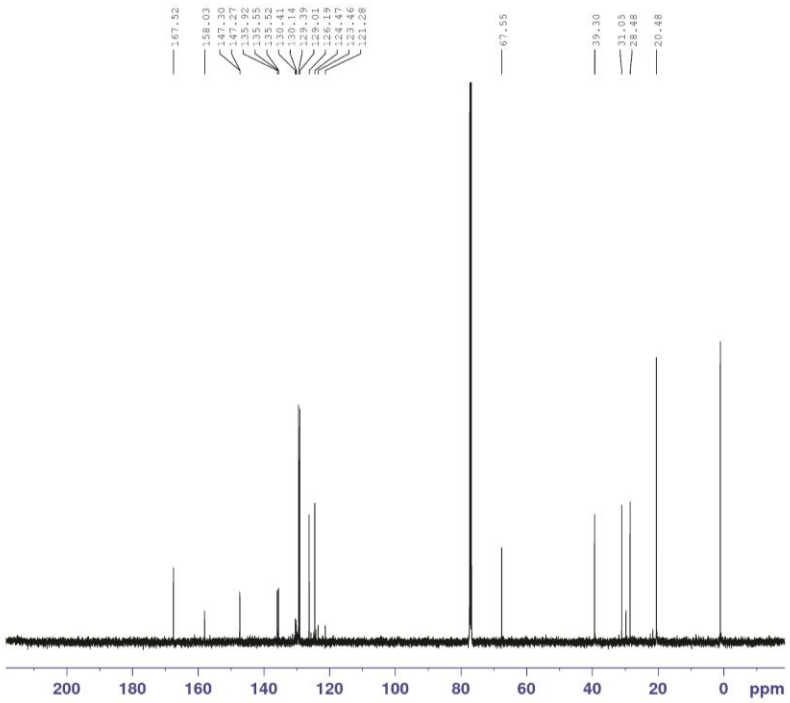
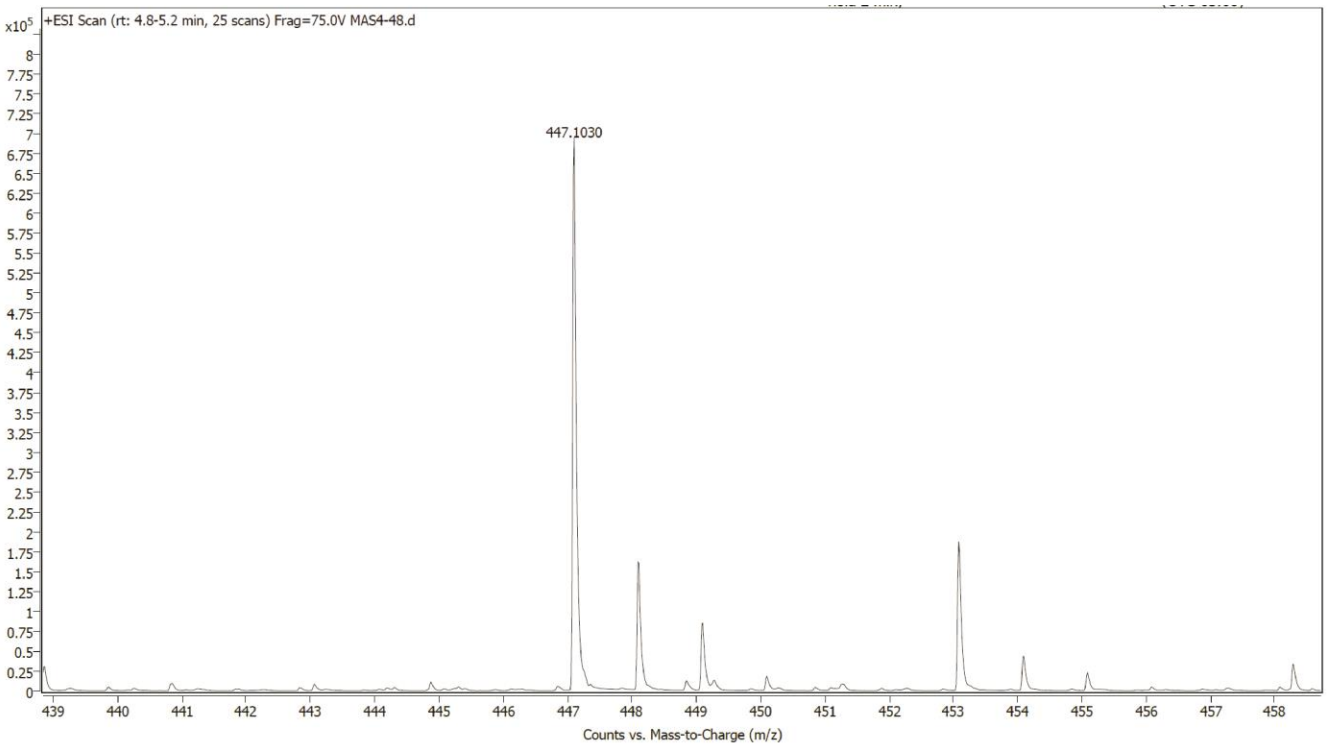


Figure S17. NMR, HPLC, and MS spectra of compound **13**


7.383
7.381
7.367
7.317
7.302
7.284
7.218
7.203
7.119
3.473
3.461
3.448
3.067
3.053
3.039
1.980
1.966
7.5 7.4 7.3 7.2 7.1 7.0 ppm
3.4 3.2 3.0 2.8 2.6 2.4 2.2 ppm
2.11 2.54 1.00 0.81 2.19 2.07 2.58
8.944
8.200
8.197
7.383
7.381
7.367
7.317
7.302
7.284
7.218
7.203
7.119
3.486
3.473
3.461
3.448
3.067
3.053
3.039
1.980
1.966
1.952
1.938
1.925
1.646
16
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
1.00 2.23 2.11 2.54 1.00 0.81 2.19 2.07 2.58 6.29


167.52
158.03
147.30
147.27
135.92
135.55
135.52
130.41
130.14
129.39
129.01
126.19
124.47
123.46
121.28
67.55
39.30
31.05
28.48
20.48
200 180 160 140 120 100 80 60 40 20 0 ppm



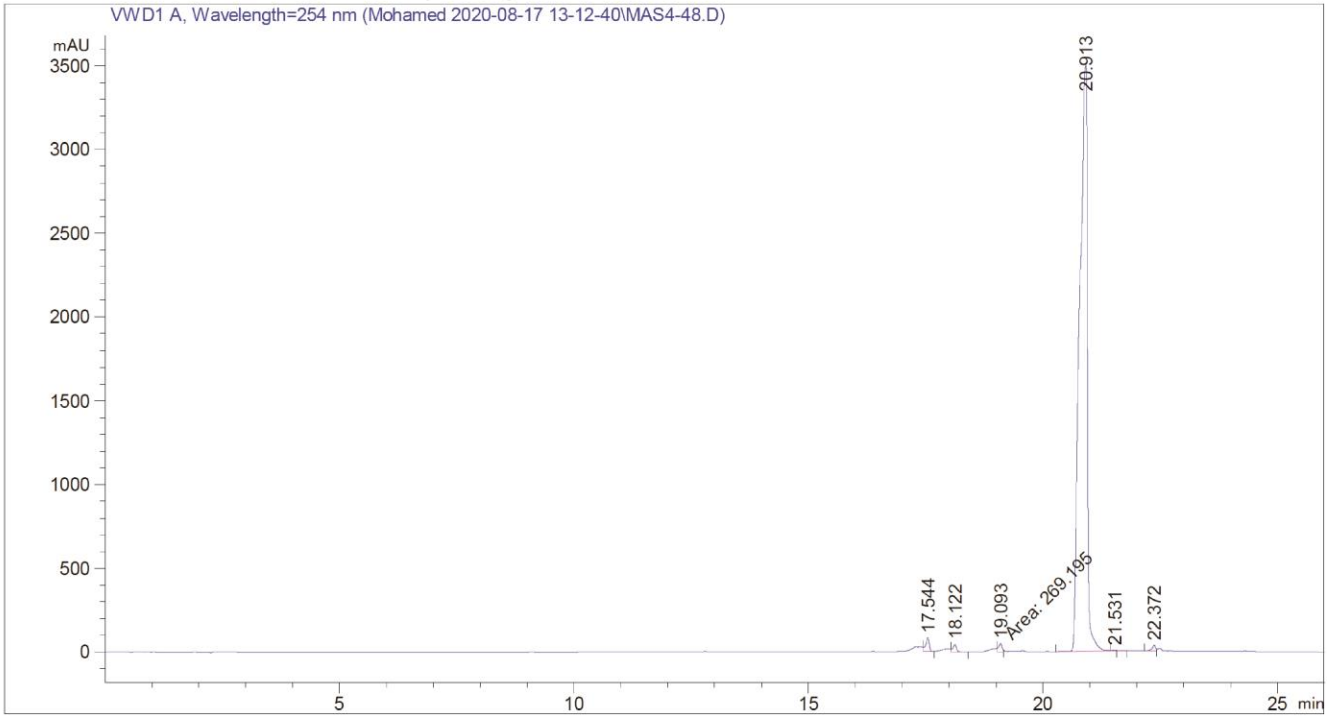
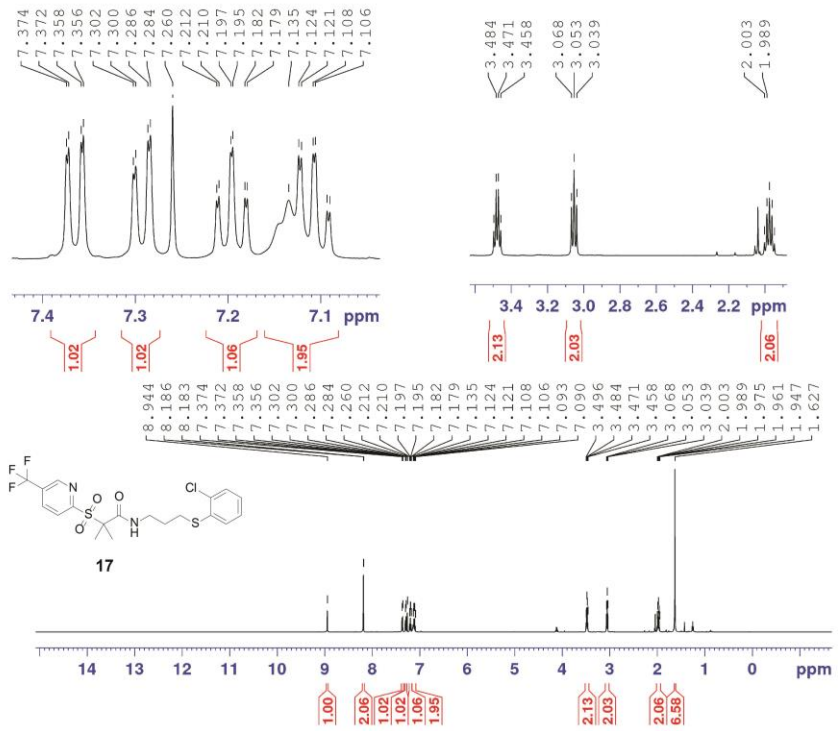
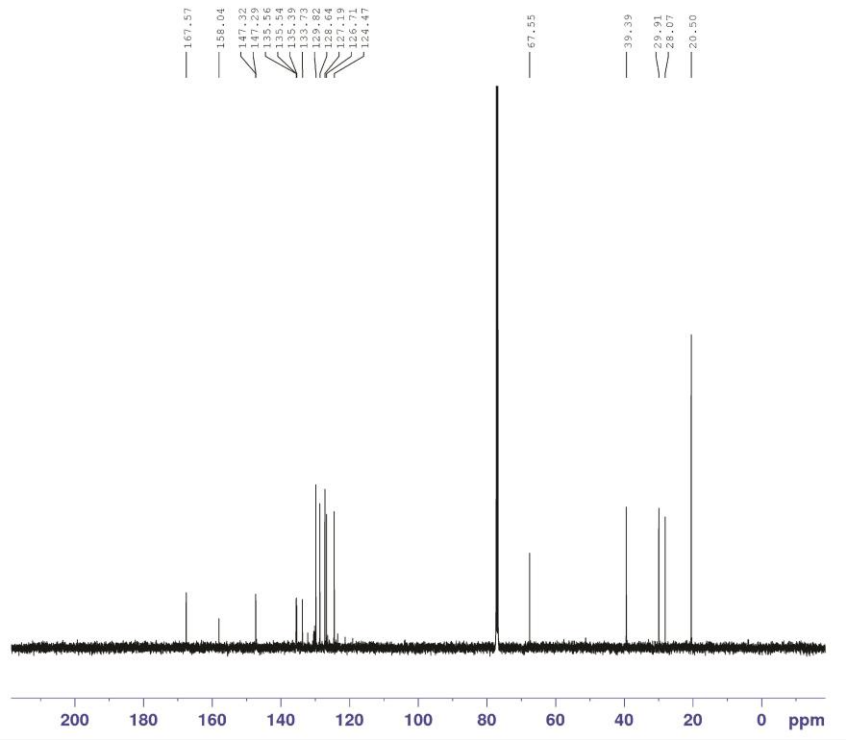
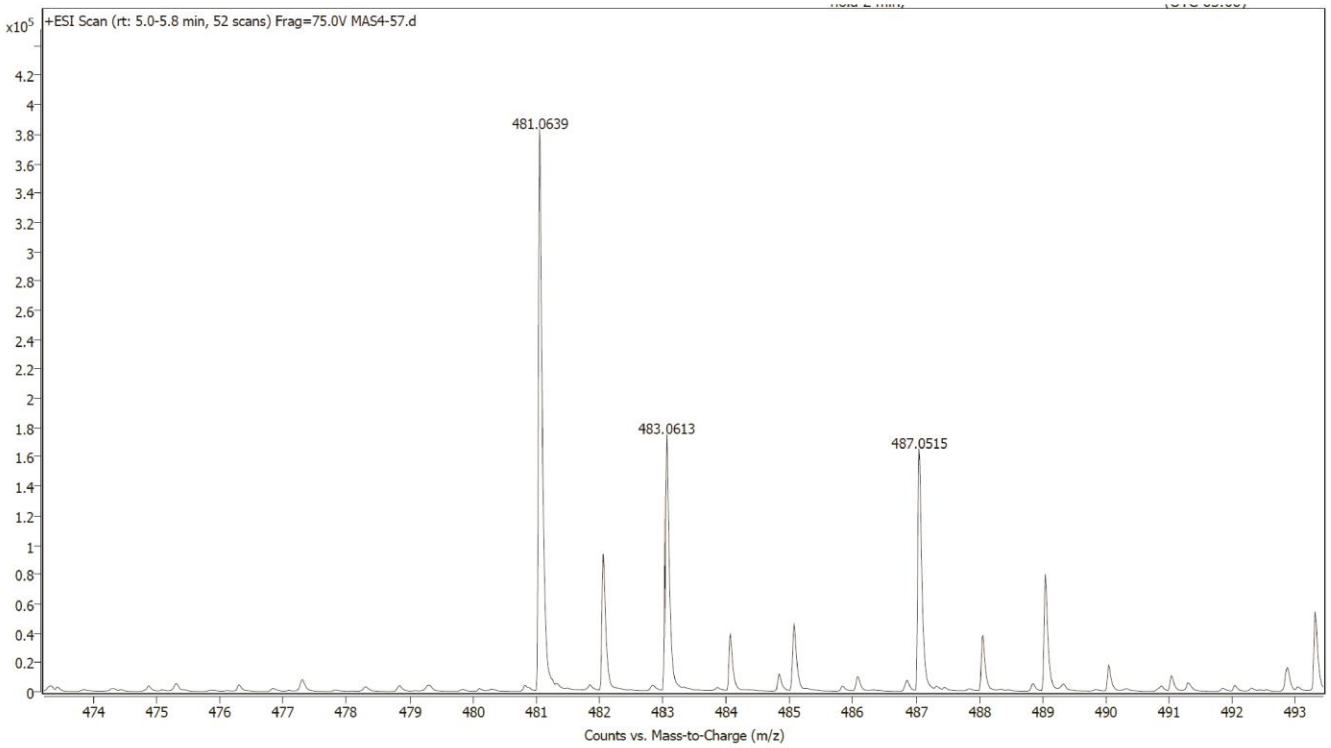


Figure S18. NMR, HPLC, and MS spectra of compound **16**


17





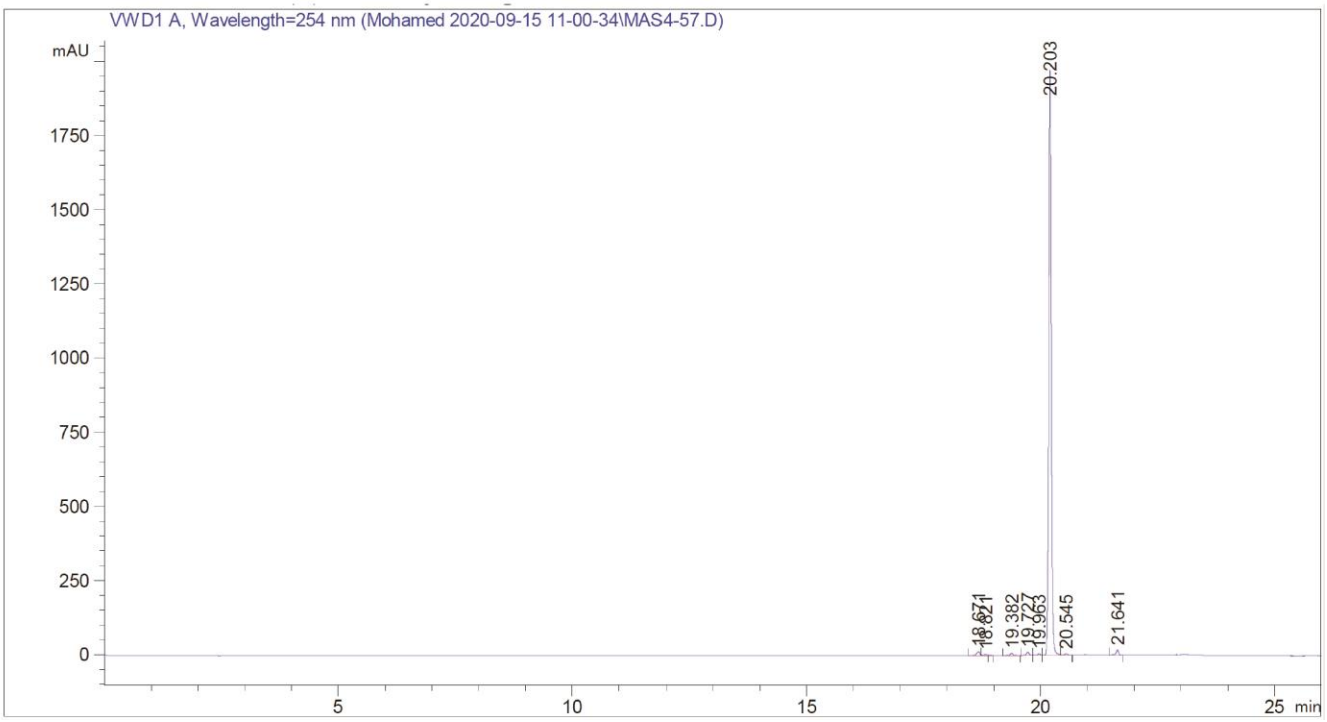
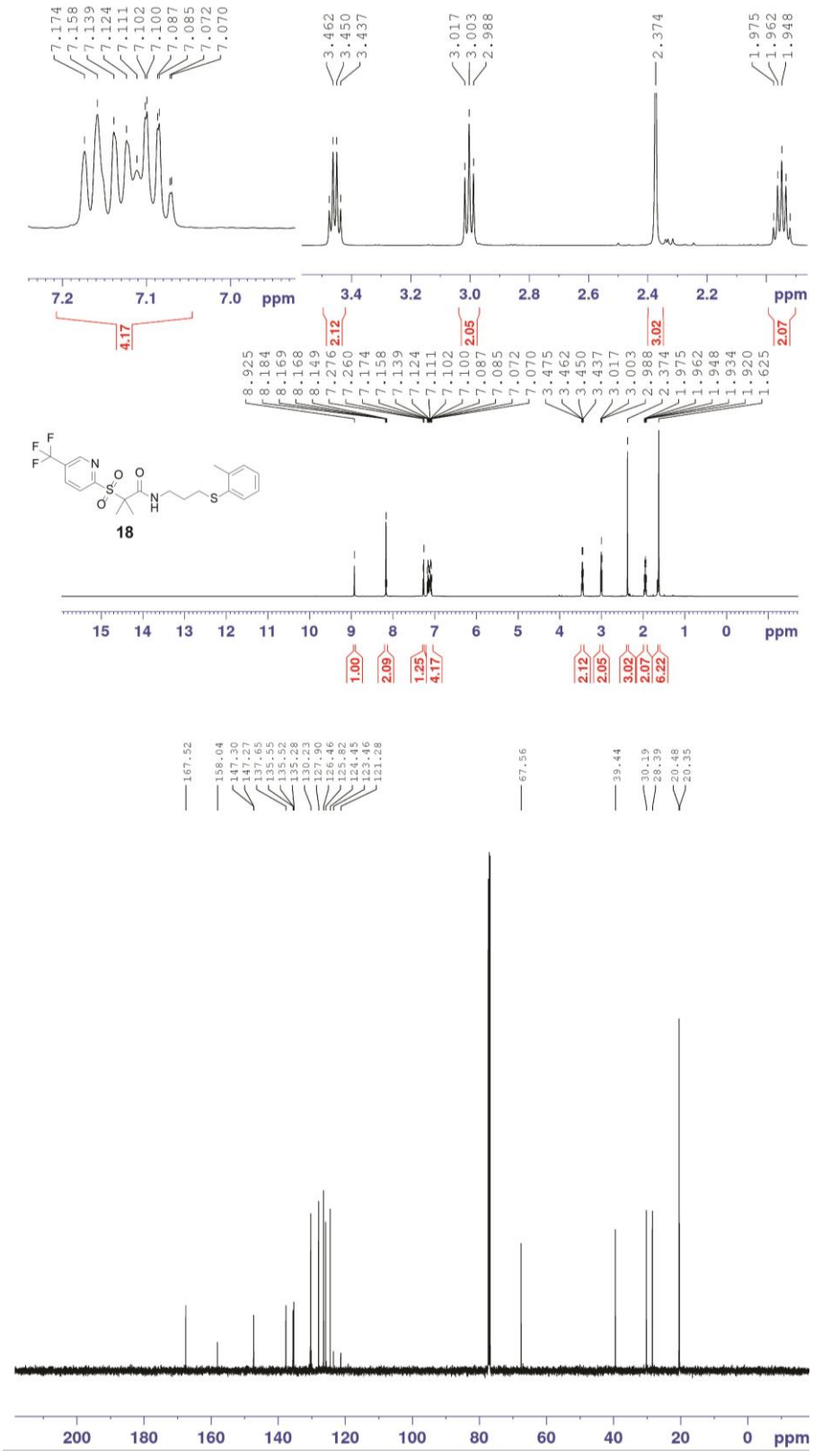
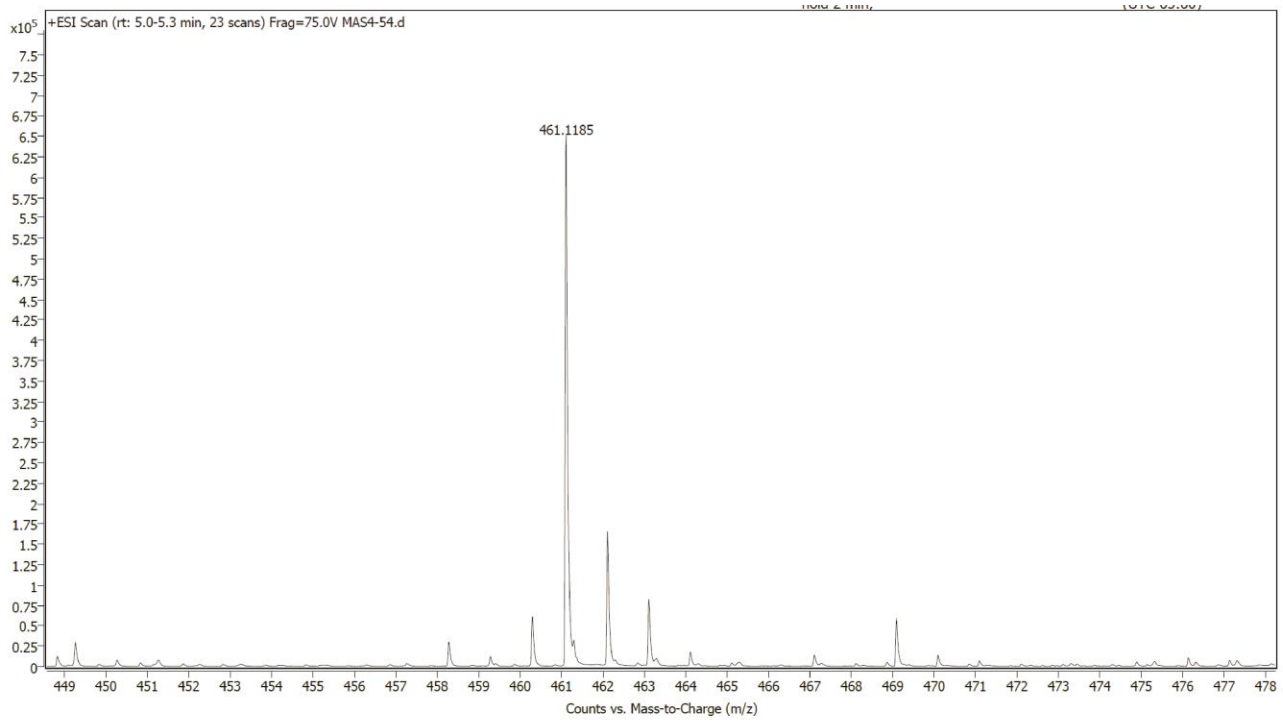


Figure S19. NMR, HPLC, and MS spectra of compound **17**


18



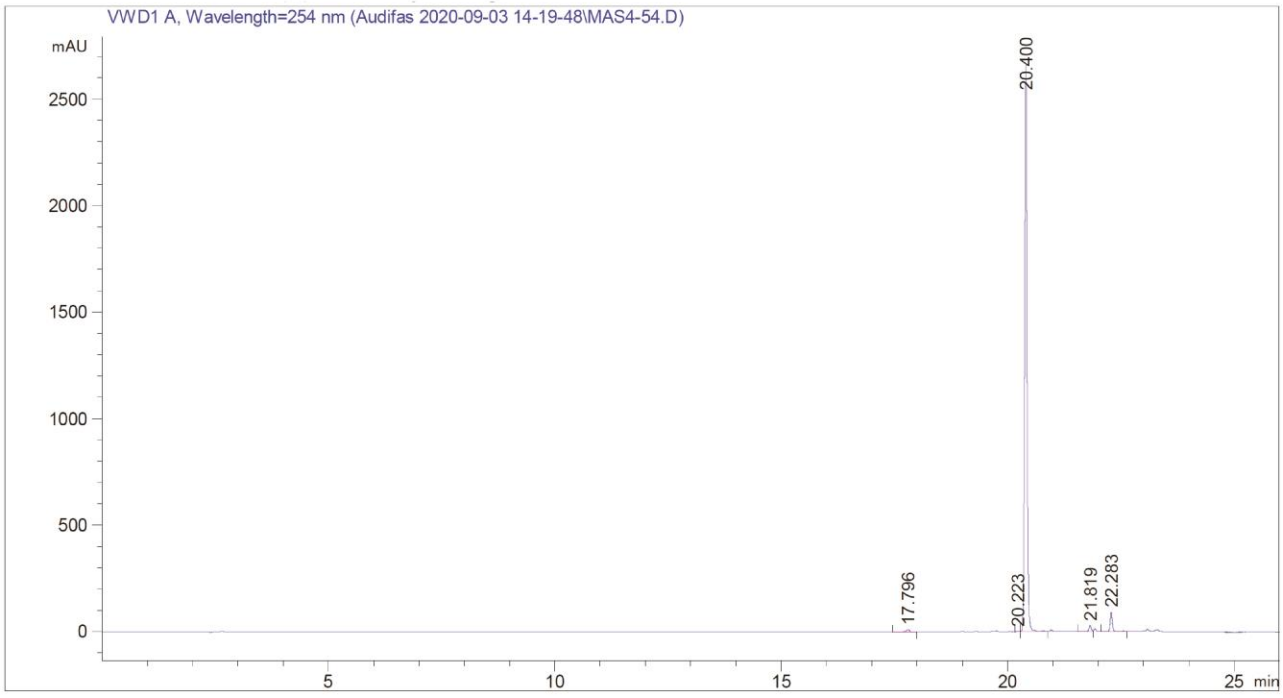
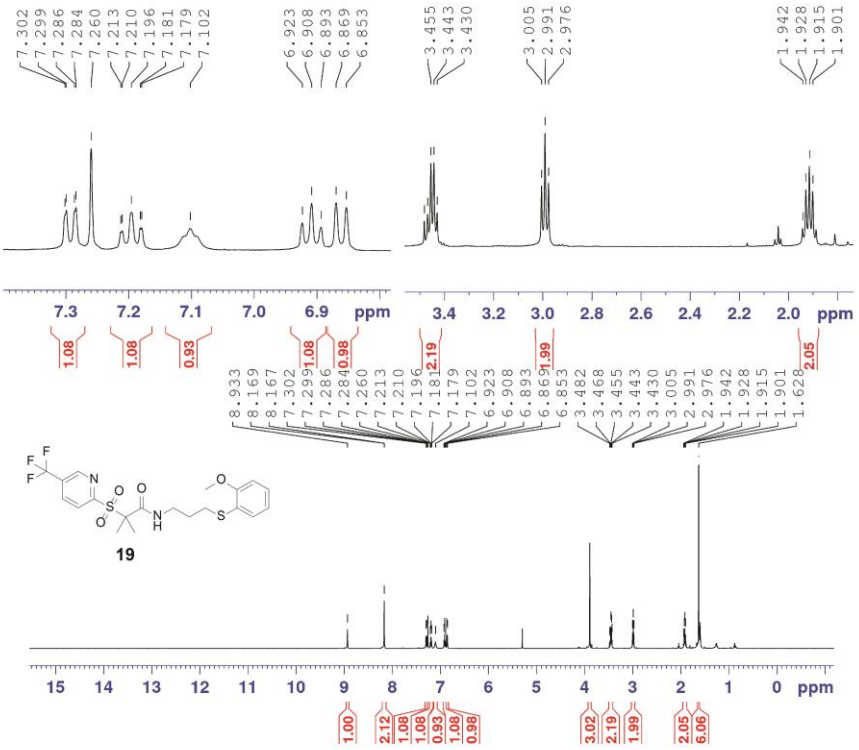
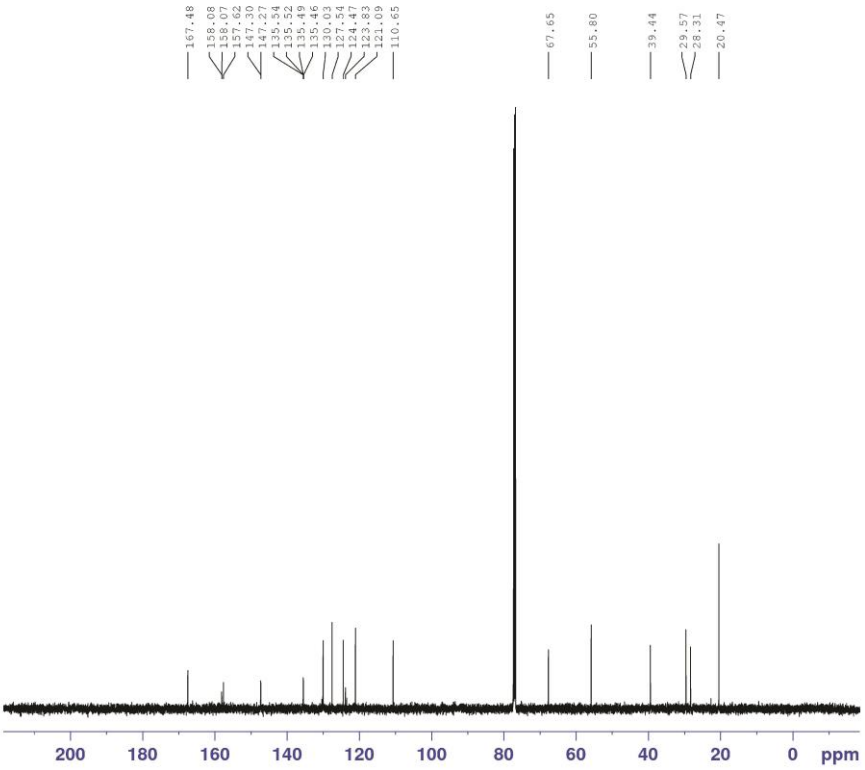


Figure S20. NMR, HPLC, and MS spectra of compound


19



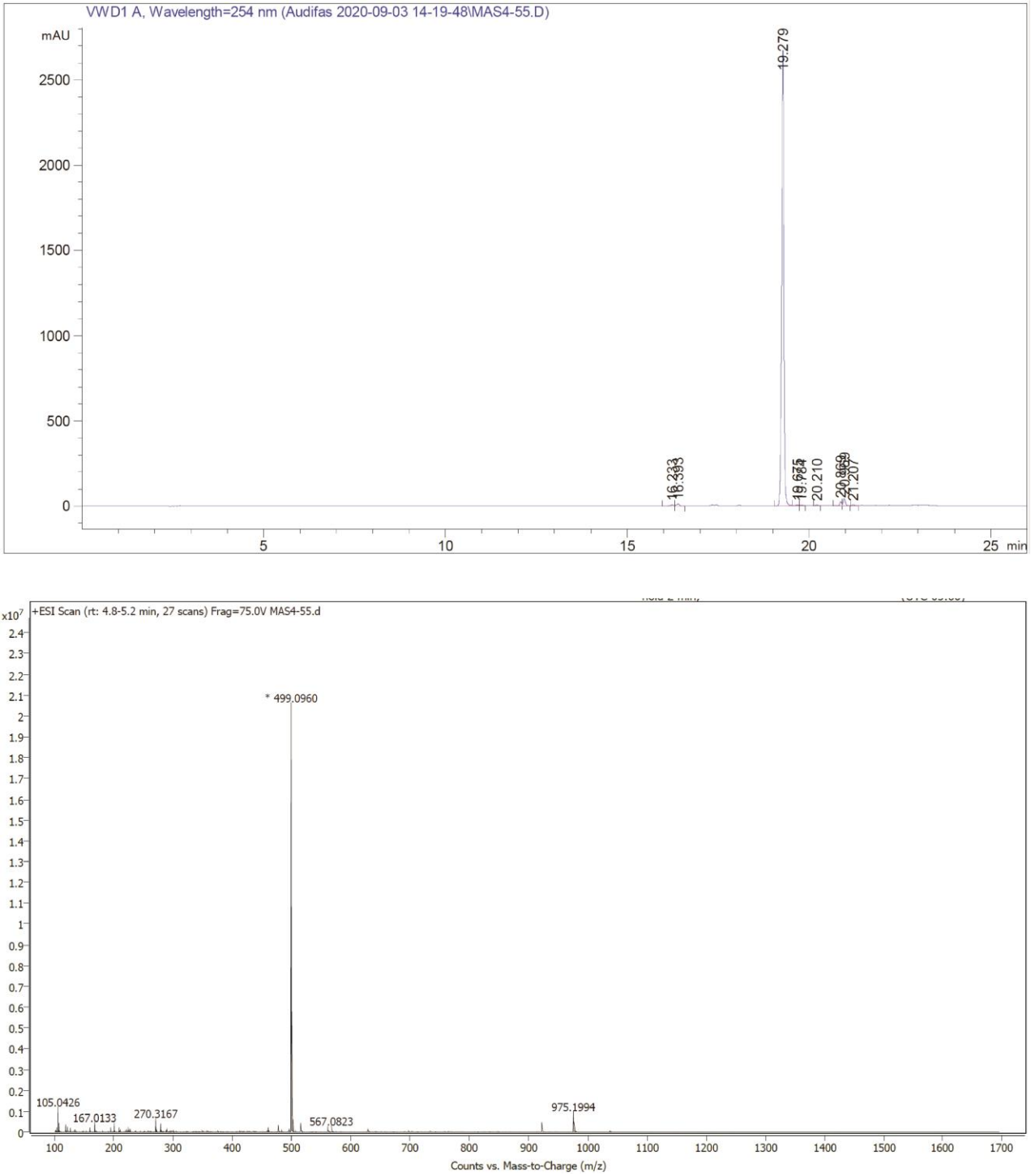
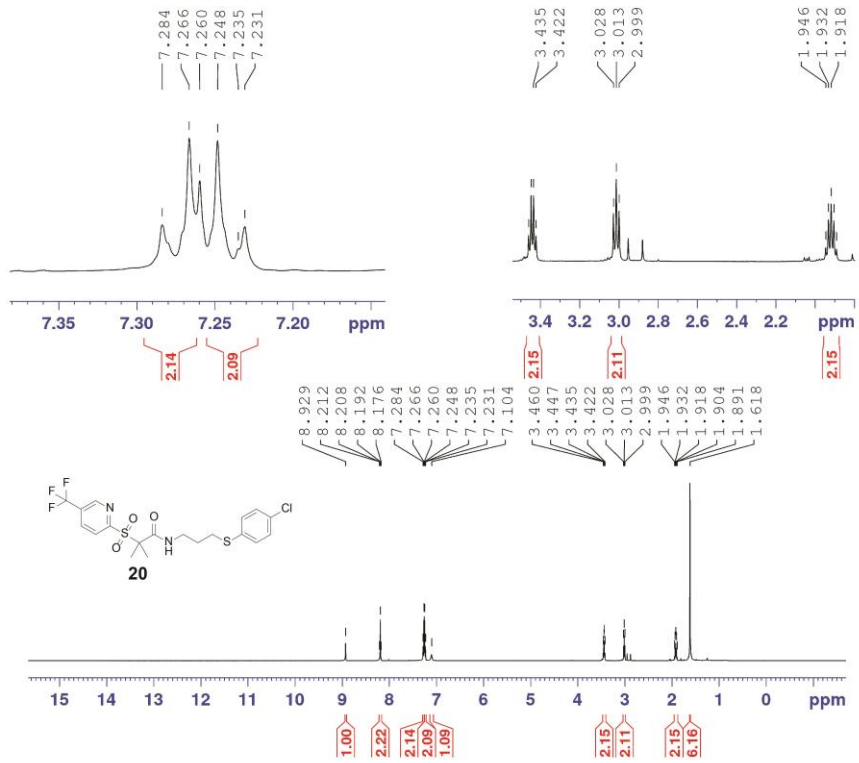
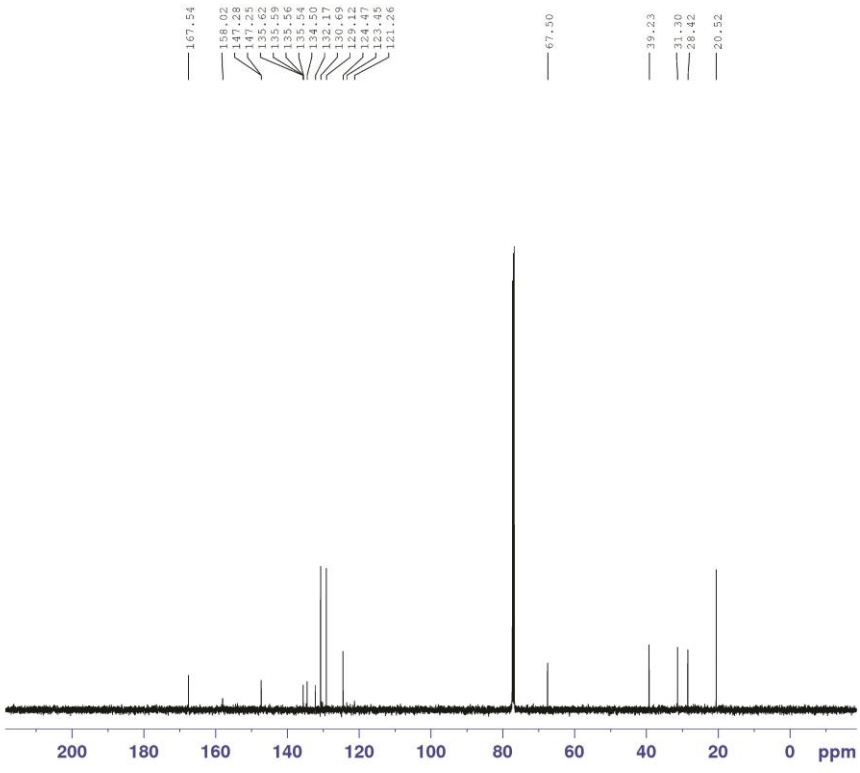


Figure S21. NMR, HPLC, and MS spectra of compound **19**


7.284
7.266
7.260
7.248
7.235
7.231
3.435
3.422
3.028
3.013
2.999
1.946
1.932
1.918
7.35
7.30
7.25
7.20
ppm
2.14
2.09
3.4
3.2
3.0
2.8
2.6
2.4
2.2
ppm
2.15
2.11
2.15
8.929
8.212
8.208
8.192
8.176
7.284
7.266
7.260
7.248
7.235
7.231
7.104
3.460
3.447
3.435
3.422
3.028
3.013
2.999
1.946
1.932
1.918
1.904
1.891
1.618
F
F
F
N
O
O
S
O
O
N
H
S
Cl
20
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
2.22
2.14
2.09
1.09
2.15
2.11
2.15
6.16


167.54
158.02
147.28
147.25
135.62
135.59
135.56
135.54
134.50
134.17
130.69
129.12
124.47
123.45
121.26
67.50
39.23
31.30
28.42
20.52
200
180
160
140
120
100
80
60
40
20
0
ppm

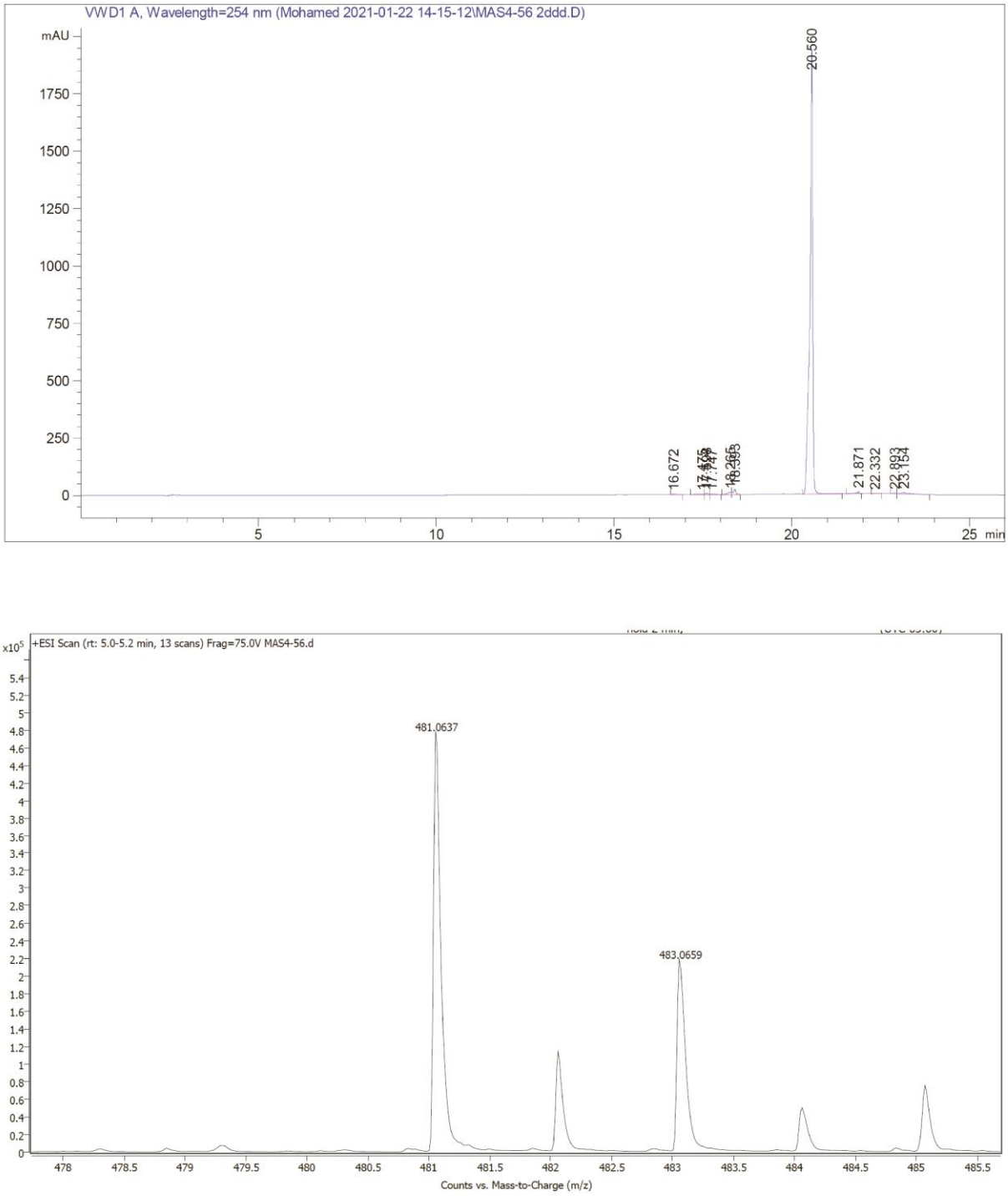
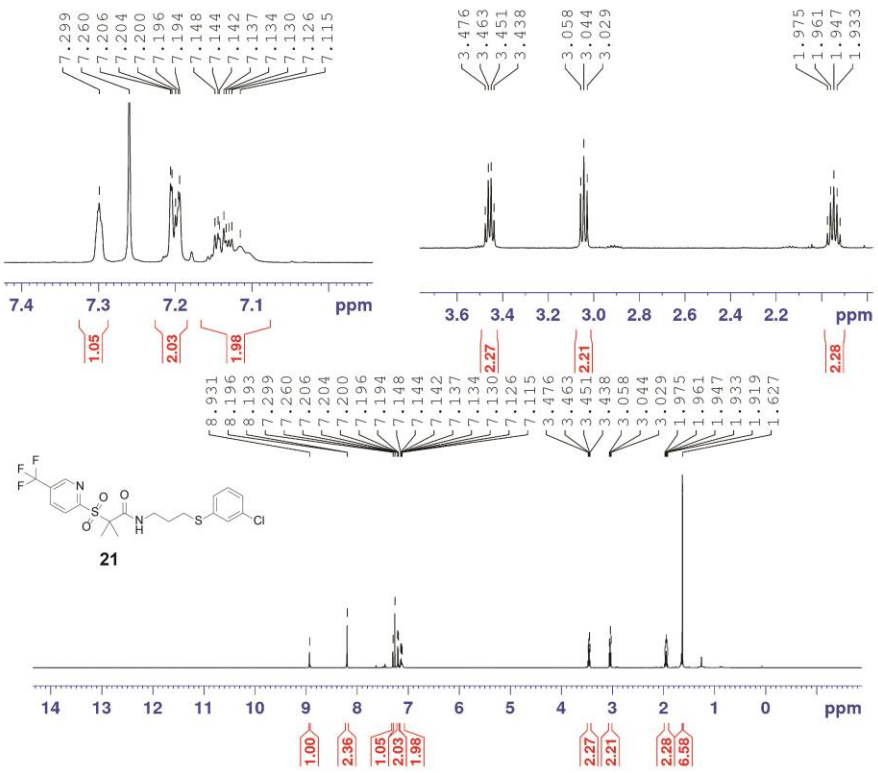
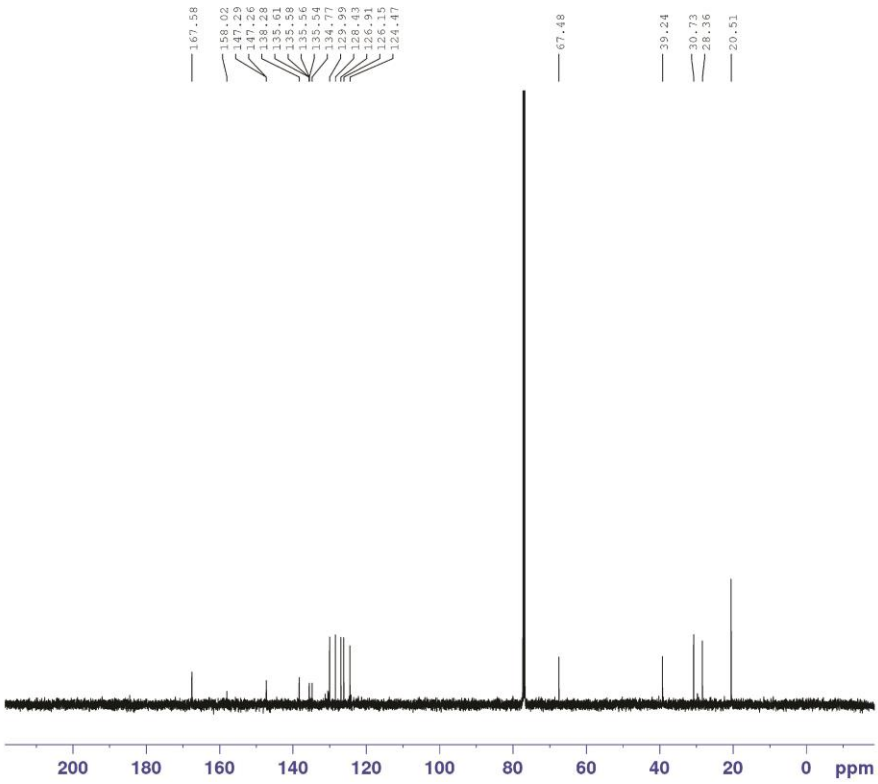


Figure S22. NMR, HPLC, and MS spectra of compound **20**


7.299
7.260
7.206
7.204
7.200
7.196
7.194
7.148
7.144
7.142
7.137
7.134
7.130
7.126
7.115
3.476
3.463
3.451
3.438
3.058
3.044
3.029
1.975
1.961
1.947
1.933
7.4
7.3
7.2
7.1
ppm
1.05
2.03
1.98
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
ppm
2.27
2.21
2.28
8.931
8.196
8.193
7.299
7.260
7.206
7.204
7.200
7.196
7.194
7.148
7.144
7.142
7.137
7.134
7.130
7.126
7.115
3.476
3.463
3.451
3.438
3.058
3.044
3.029
1.975
1.961
1.947
1.933
1.919
1.627
21
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
2.36
1.05
2.03
1.98
2.27
2.21
2.28
6.58


167.58
158.02
147.29
147.26
138.28
135.61
135.58
135.56
135.54
134.77
129.99
128.43
126.91
126.15
124.47
67.48
39.24
30.73
28.36
20.51
200
180
160
140
120
100
80
60
40
20
0
ppm

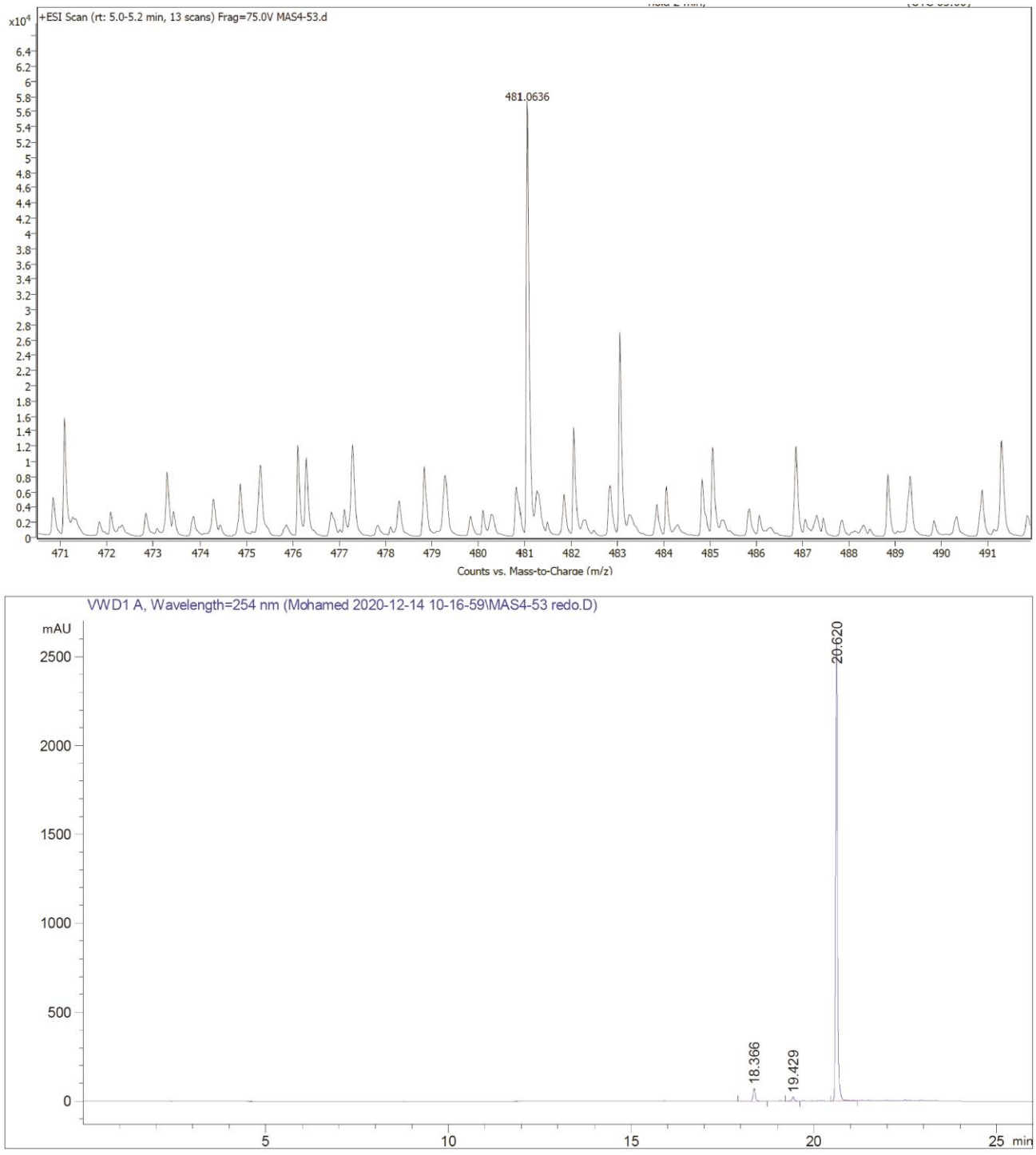
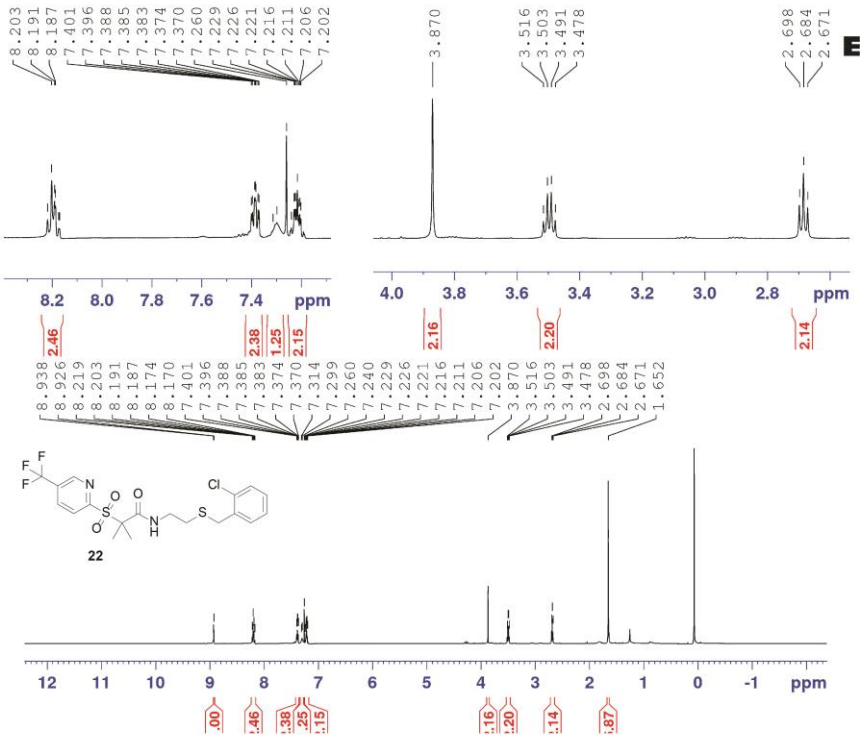
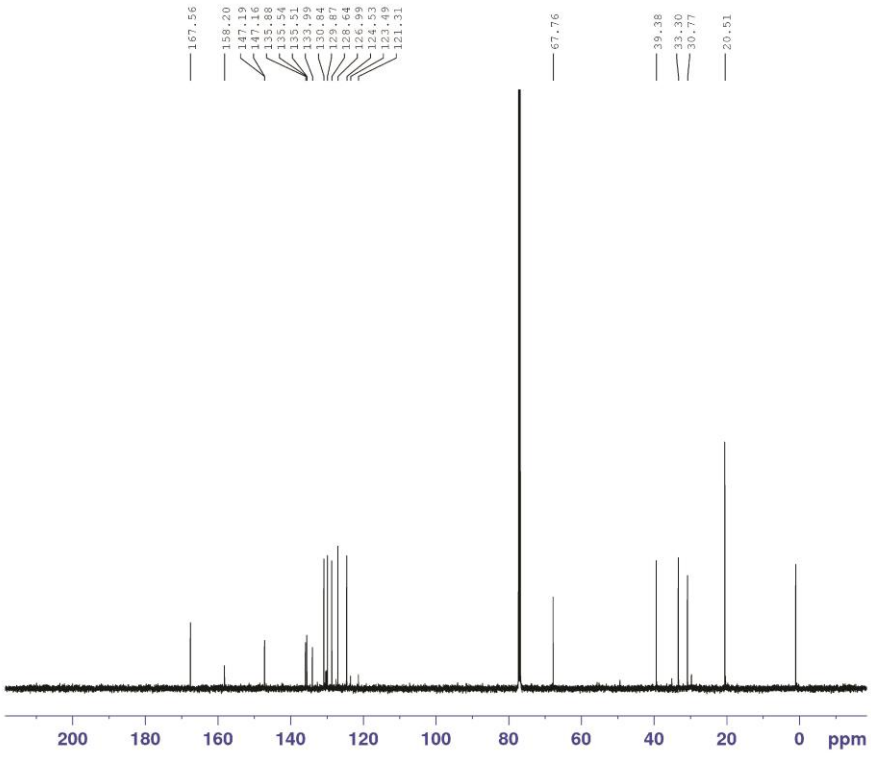
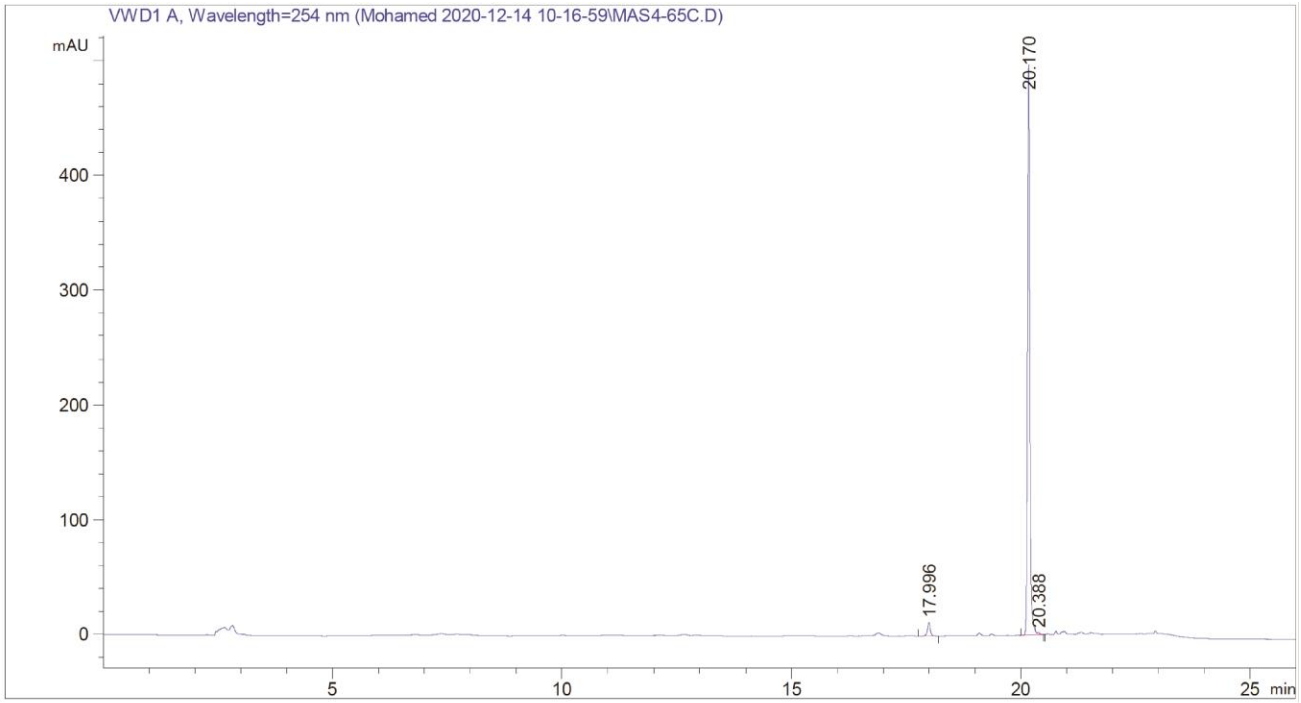


Figure S23. NMR, HPLC, and MS spectra of compound **21**


8.203
8.191
8.187
7.401
7.396
7.388
7.385
7.383
7.374
7.370
7.260
7.229
7.226
7.221
7.216
7.211
7.206
7.202
3.870
3.516
3.503
3.491
3.478
2.698
2.684
2.671
E
8.2 8.0 7.8 7.6 7.4 ppm
4.0 3.8 3.6 3.4 3.2 3.0 2.8 ppm
2.46
2.38
1.25
2.15
2.16
2.20
2.14
8.938
8.926
8.219
8.203
8.191
8.187
8.174
8.170
7.401
7.396
7.388
7.385
7.383
7.374
7.370
7.314
7.299
7.260
7.240
7.229
7.226
7.221
7.216
7.211
7.206
7.202
3.870
3.516
3.503
3.491
3.478
2.698
2.684
2.671
1.652
F
F
F
N
O
O
S
O
O
N
H
S
Cl
22
12 11 10 9 8 7 6 5 4 3 2 1 0 -1 ppm
.00
.46
.38
.25
.15
.16
.20
.14
.87


167.56
158.20
147.19
147.16
135.88
135.54
135.51
133.99
130.84
129.87
128.64
126.99
124.53
123.49
121.31
67.76
39.38
33.30
30.77
20.51
200 180 160 140 120 100 80 60 40 20 0 ppm



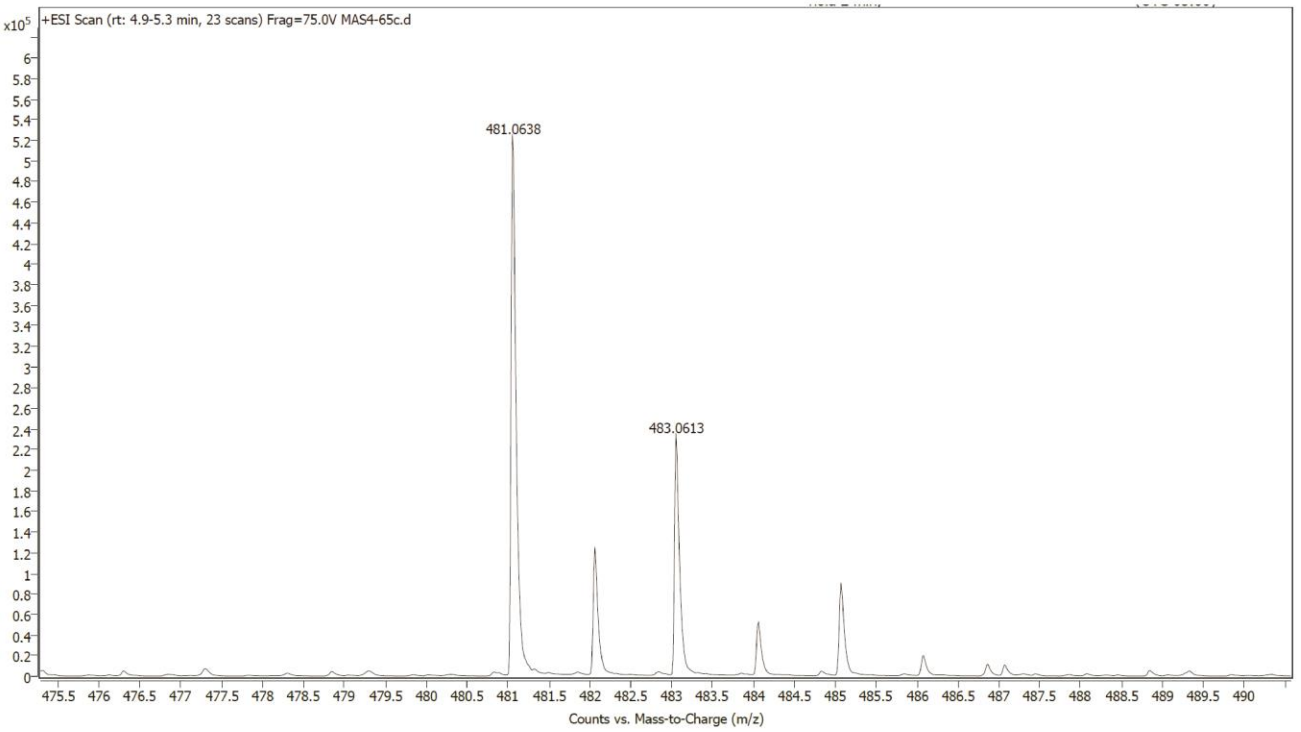
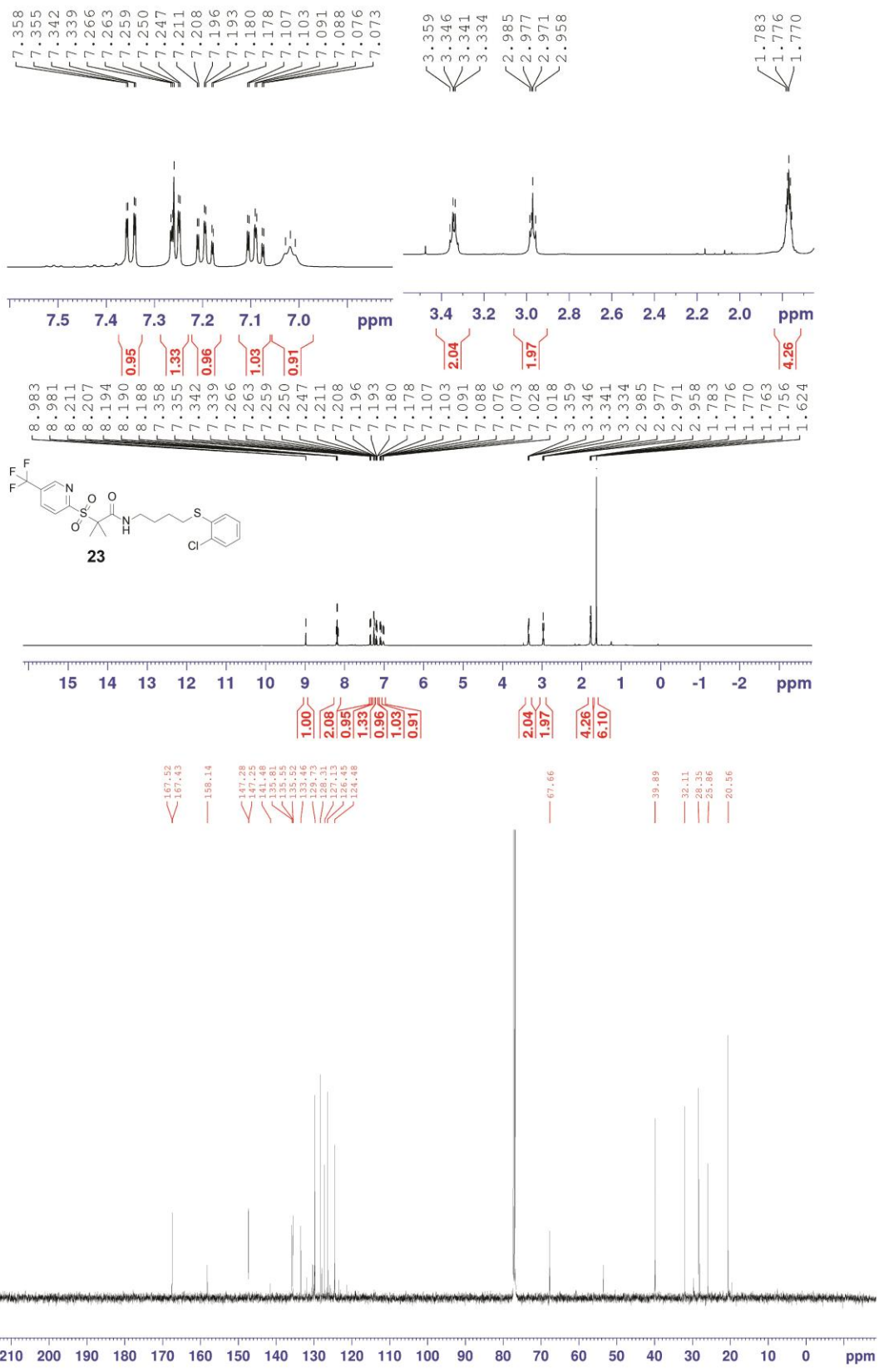


Figure S24. NMR, HPLC, and MS spectra of compound **22**


23

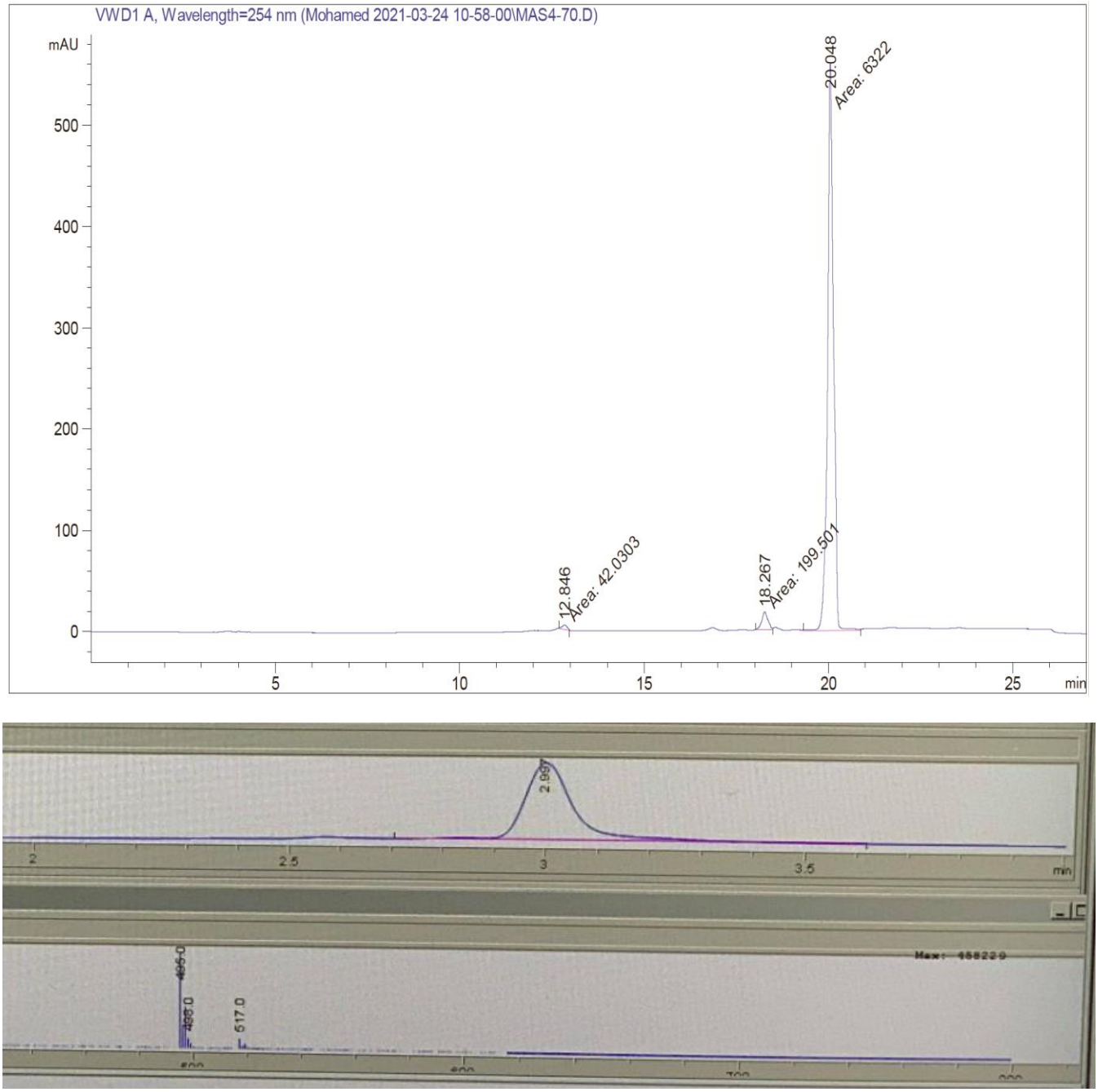


Figure S25. NMR, HPLC, and MS spectra of compound **23**

References

1. Lee, S.; Jin, S.-P.; Kim, Y. K.; Sung, G. Y.; Chung, J. H.; Sung, J. H. Construction of 3D multicellular microfluidic chip for an in vitro skin model. *Biomed. Microdevices* **2017,** 19, 22.
2. Nogueira, A. T.; Braun, K. M.; Carabeo, R. A. Characterization of the Growth of Chlamydia trachomatis in In vitro-generated Stratified Epithelium. *Front. Cell. Infect. Microbiol.* **2017,** 7, 438.
3. Yongqing, K.; Shengyong, Z.; Linlin, W. Synthesis of N-(4-bromobutyl) phthalimide. *Chemical Reagents* **2001,** 23, 359-359.