Design and Synthesis of N-3-Substituted Quinazolinone Derivatives as Anticancer Agents Targeting EGFR

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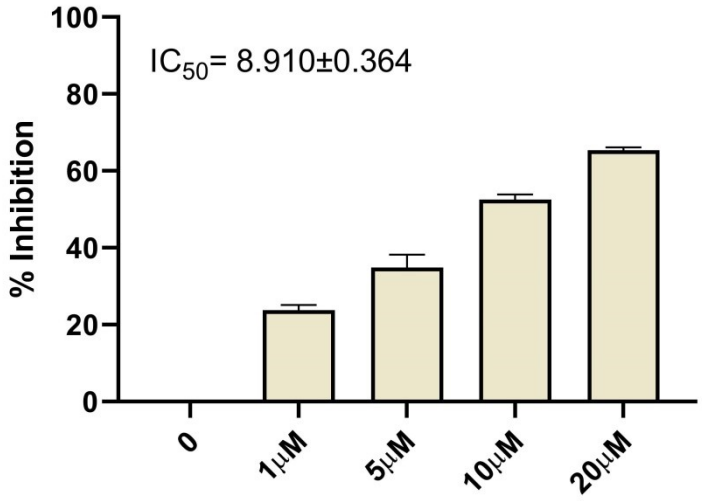


Fig. S1 Antiproliferative activities of 4c at 1, 5, 10, and 20μM conc. for 24h against PC-3 cells. Data are presented as mean± SEM for three independent experiments.

Compound	IC_{50} (μM) ± SEM
4c	>100
4e	>100
4f	67.947±2.745
4h	>100
4i	>100
4j	>100

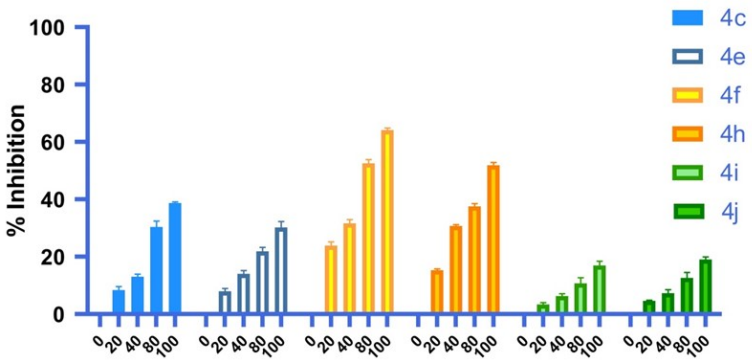


Fig. S2 Antiproliferative activities of 4c, 4e, 4f. 4h, 4i and 4j against HEK-293 cells at 20, 40, 80 and 100 μM concentration. Data are presented as mean± SEM for three independent experiments.

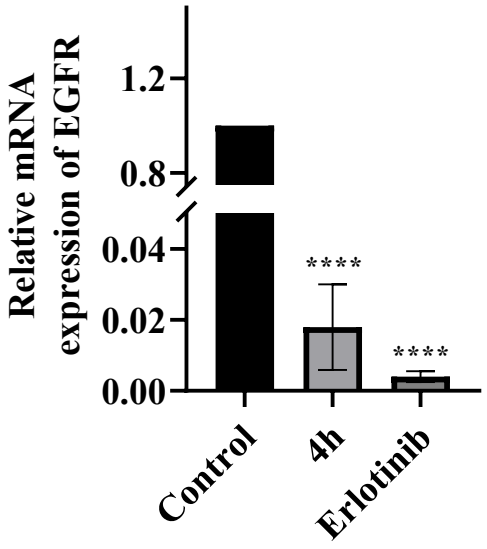


Fig. S3 Effect of 4h on relative mRNA expression of EGFR in MCF-7 cell line. All values are expressed as mean ± SEM. Analysis was done using one-way ANOVA followed by Tukey's Post hoc test. ****$p < 0.0001$ "*" versus control.

Table S1: Selectivity Index of compound 4c. 4e, 4f, 4h, 4i and 4j against MCF-7 compared

MCF-7	
Compound	**Selective index**
4c	>17
4e	>15
4f	10.49
4h	>15
4i	>13
4j	>16

MM-GBSA study:

Results:

The binding free energies of the 4h and 4i were calculated using the MM-GBSA (molecular mechanics generalized born surface area). The binding energy (ΔG Bind), the Coulomb energy contribution (ΔG Coulomb), the van der Waals energy contribution (ΔG van der Waals) and the lipophilic energy contribution (ΔG Lipo) are tabulated in Table 2. Compound 4i showed an improved ΔG Bind, ΔG Coulomb, ΔG vdW, and ΔG Lipo energy compared to 4h.

Table S2: MM-GBSA binding free energy results of compounds 4h and 4i against EGFR

Entry	ΔG Bind	ΔG Bind Coulomb	ΔG Bind vdW	ΔG Bind Lipo
4i	-46.22	-11.21	-44.65	-13.51
4h	-39.76	-7.86	-41.47	-12.21

Methods:

The binding energy of the ligand in the EGFR binding pocket was analyzed by molecular mechanics with generalized Born and surface area solvation (MM-GBSA) study. The docking output file was incorporated into the MMGBSA study in the 'Prime' module of Maestro. The docking complexes were minimized, and binding energies were calculated using the VSGB solvation model.

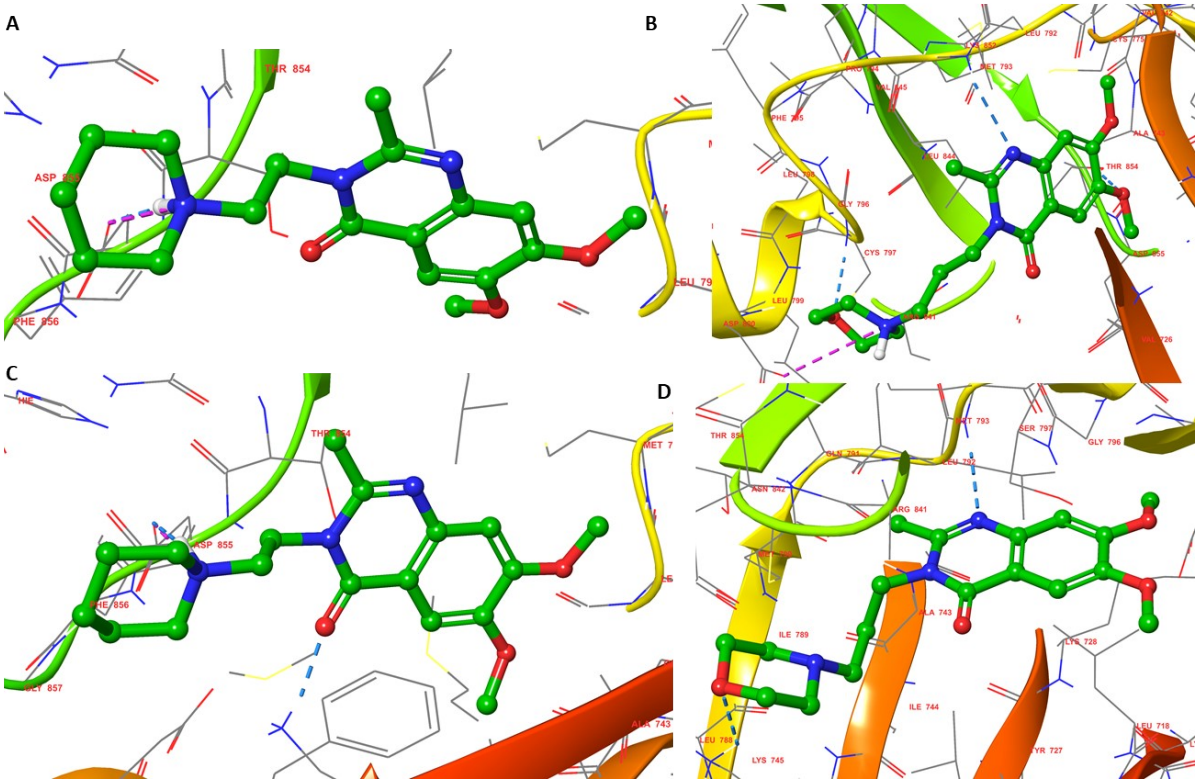


Fig. S4 Binding pose of 4h and 4i at L858R/T790M EGFR (A and B) and L858R/T790M/C797S EGFR (C and D) respectively.

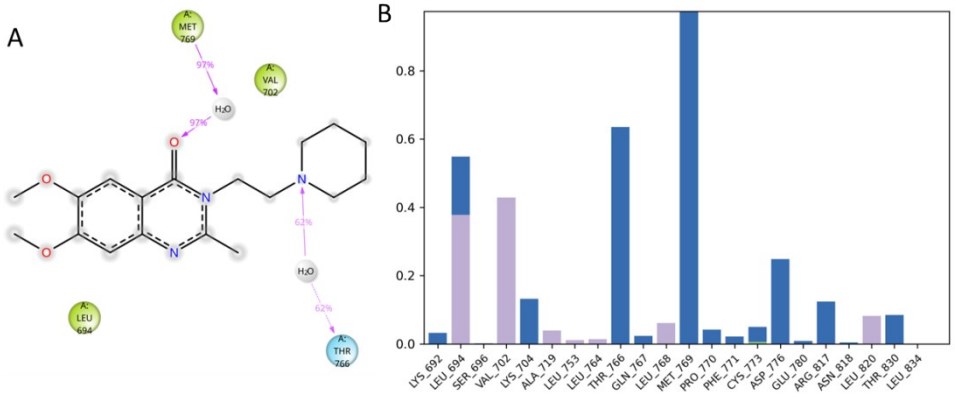


Fig. S5 2D Protein-ligand interaction diagram (A) and Protein-ligand interaction histogram (B) from the MD simulations of **4h** in the EGFR binding site; (H-bonds are shown in green, and lipophilic contacts in grey).

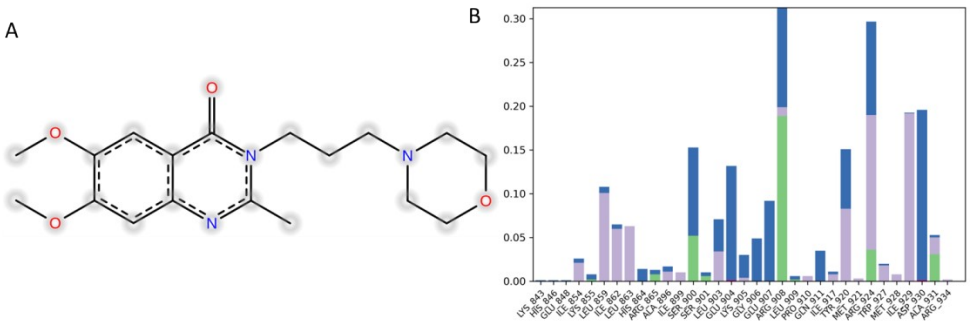


Fig. S6 2D Protein-ligand interaction diagram (A) and Protein-ligand interaction histogram (B) from the MD simulations of **4i** in the EGFR binding site; (H-bonds are shown in green, and lipophilic contacts in grey).

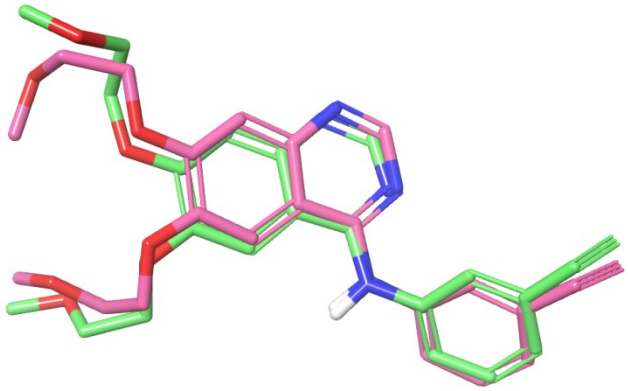
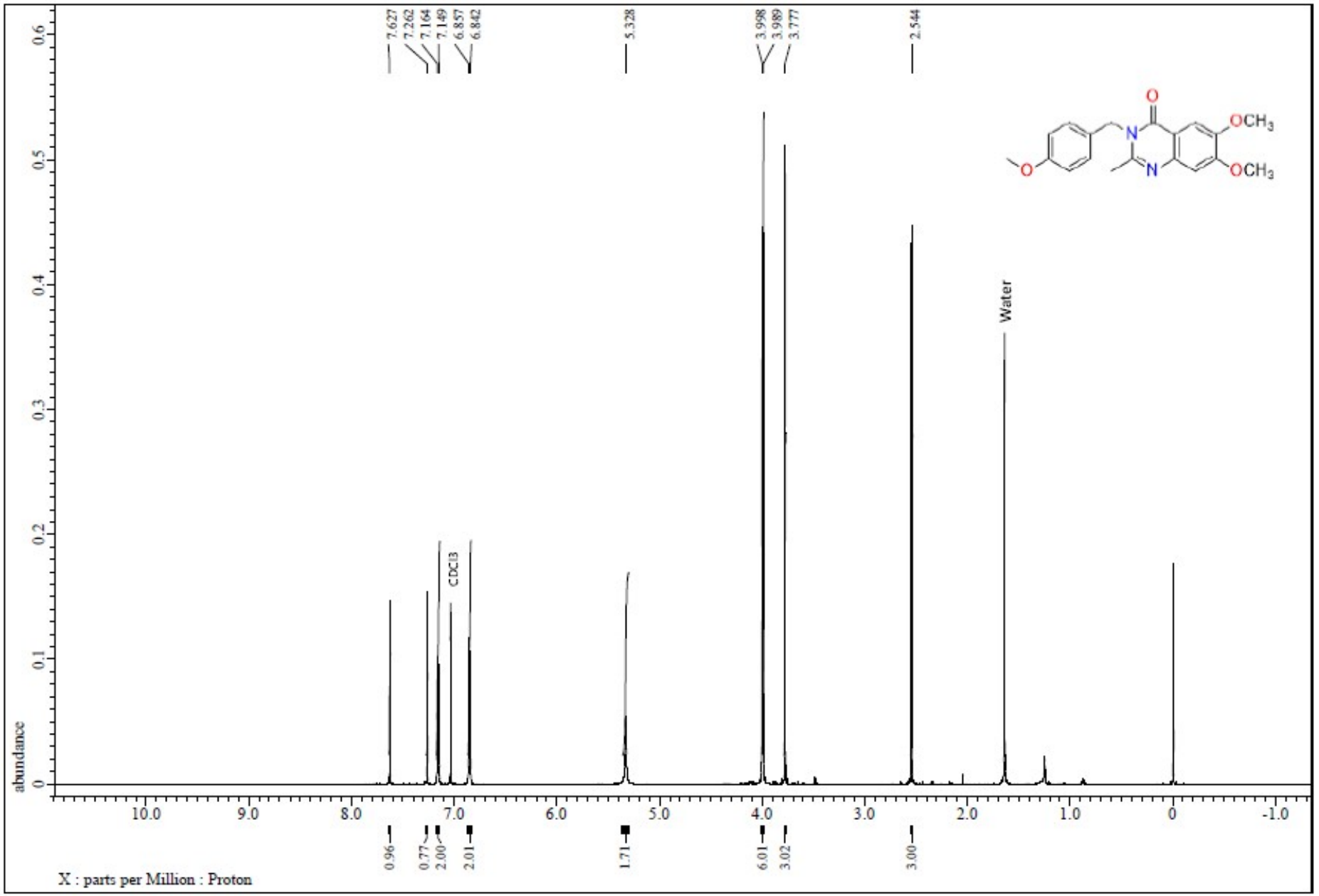


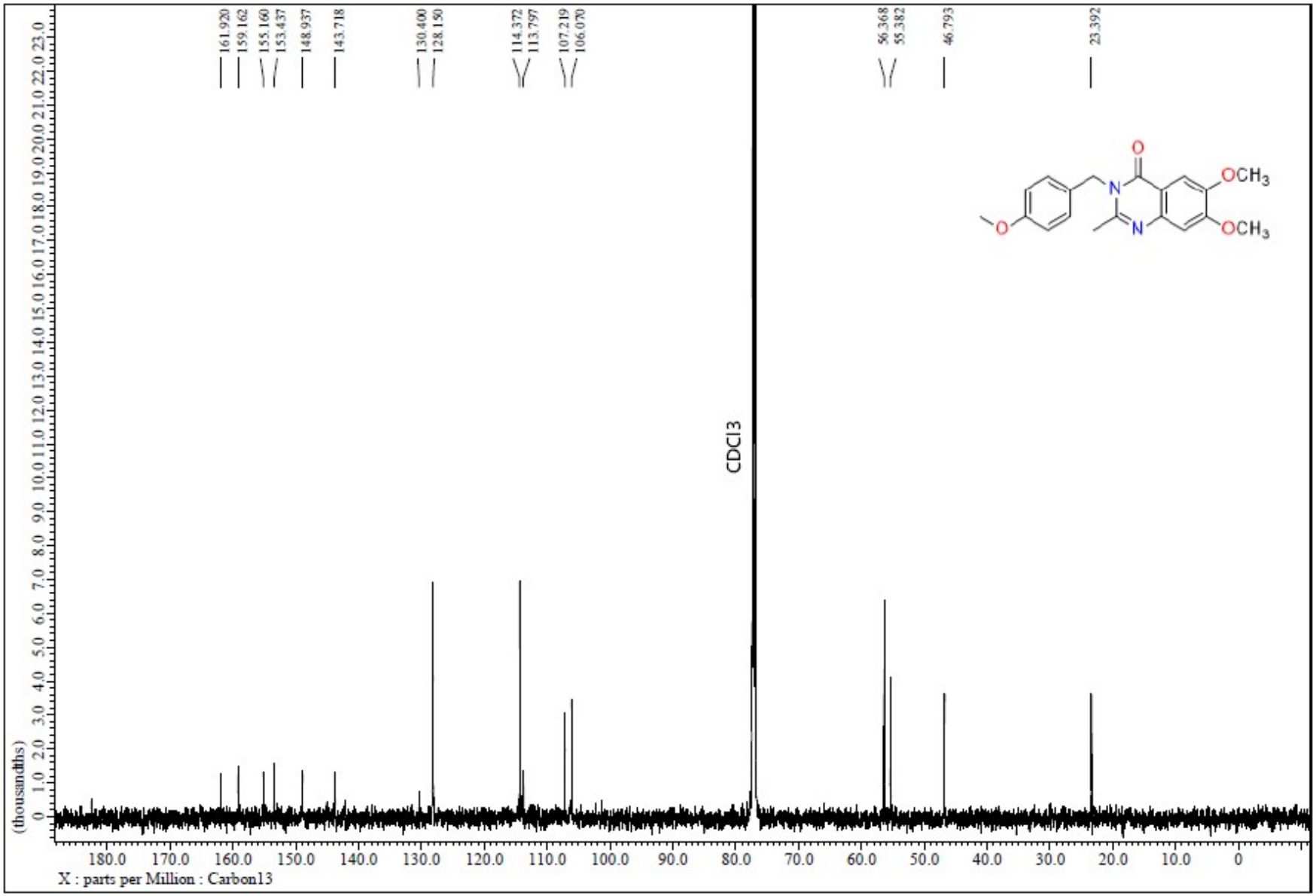
Fig. S7 Superimposed structures (with RMSD 1.01Å) of co-crystallized erlotinib (green) and re-docked erlotinib (pink) at the binding pocket of EGFR.

^{1}H, ^{13}C NMR and HRMS of all synthesized final compounds (4a-4j)

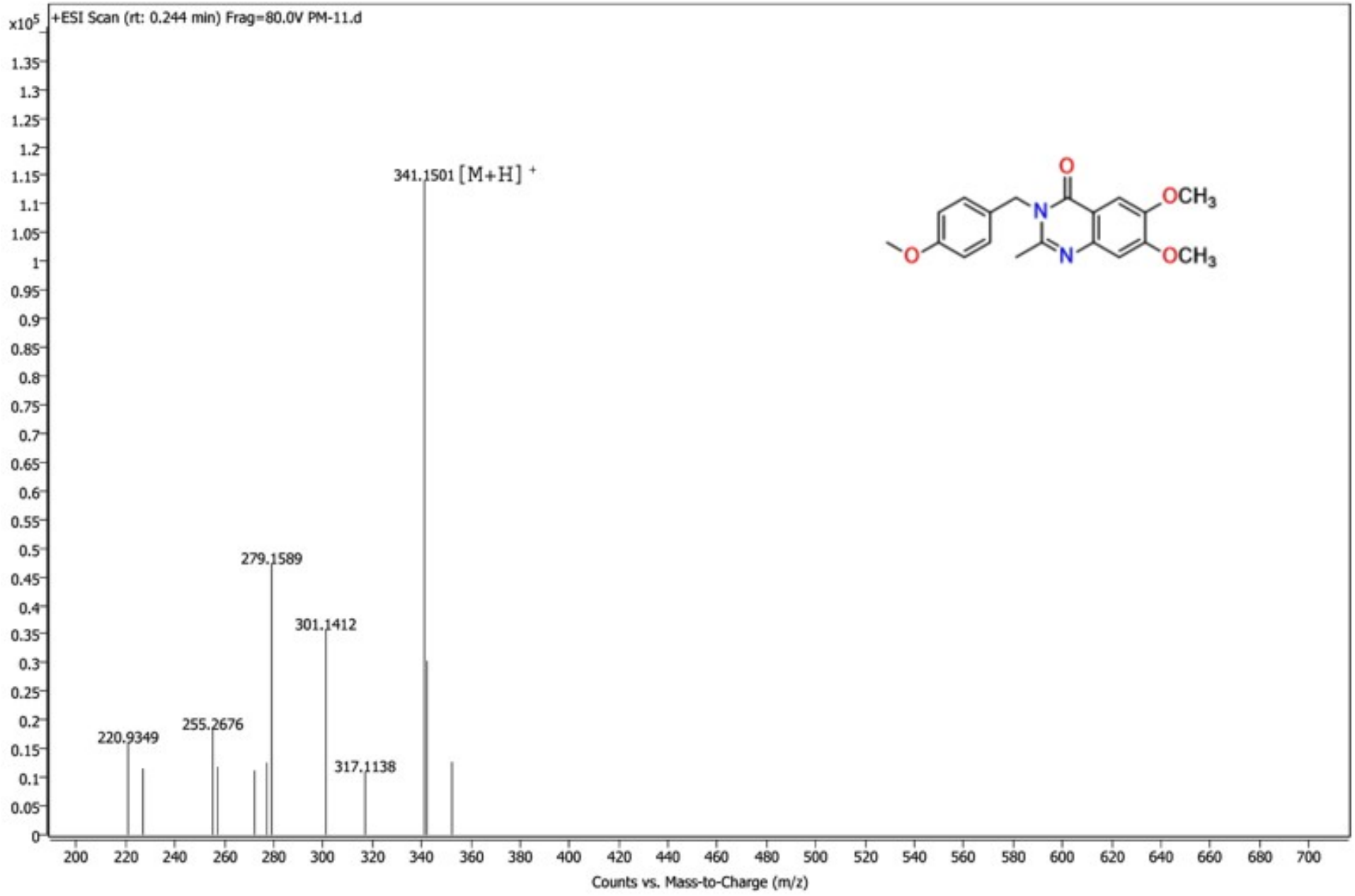
^{1}H NMR of **4a**



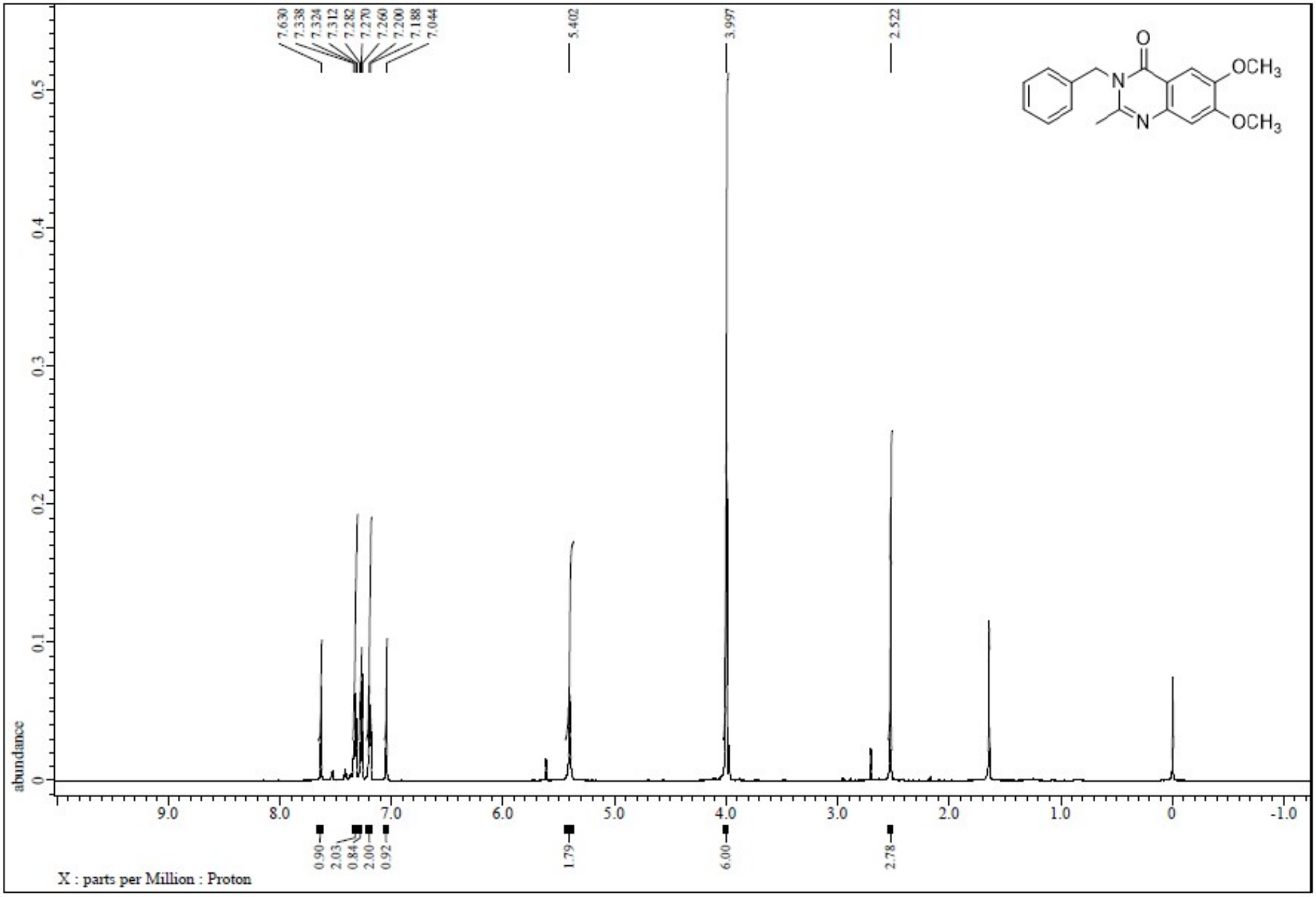
^{13}C NMR of **4a**


161.920
159.162
155.160
153.437
148.937
143.718
130.400
128.150
114.372
113.797
107.219
106.070
56.368
55.382
46.793
23.392
CDCl3
OCH3
OCH3
O
N
N
O
(thousandths)
X : parts per Million : Carbon13

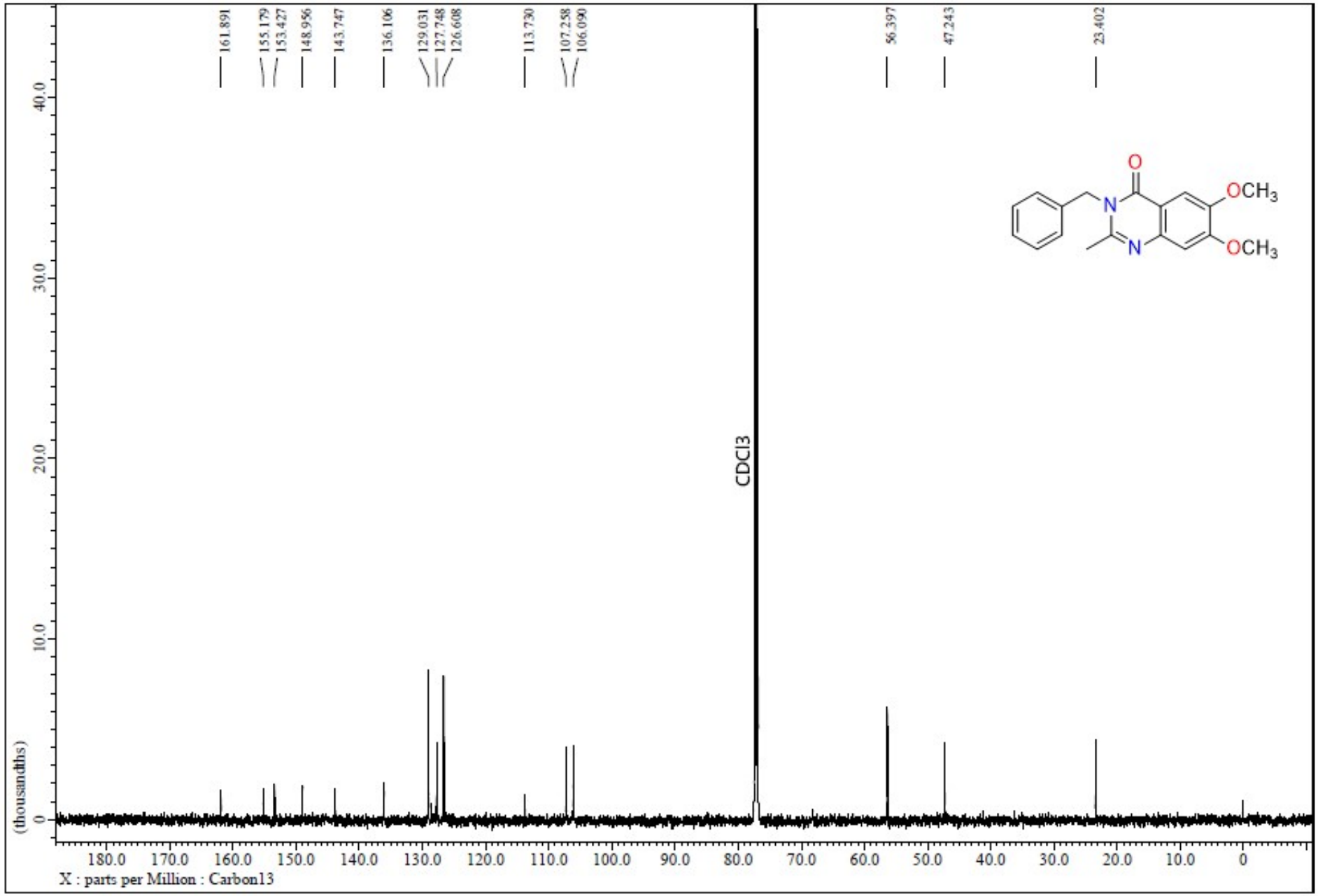
HRMS of 4a


+ESI Scan (rt: 0.244 min) Frag=80.0V PM-11.d
x10^5
341.1501 [M+H]+
279.1589
301.1412
255.2676
220.9349
317.1138
OCH3
OCH3
O
N
N
O
Counts vs. Mass-to-Charge (m/z)

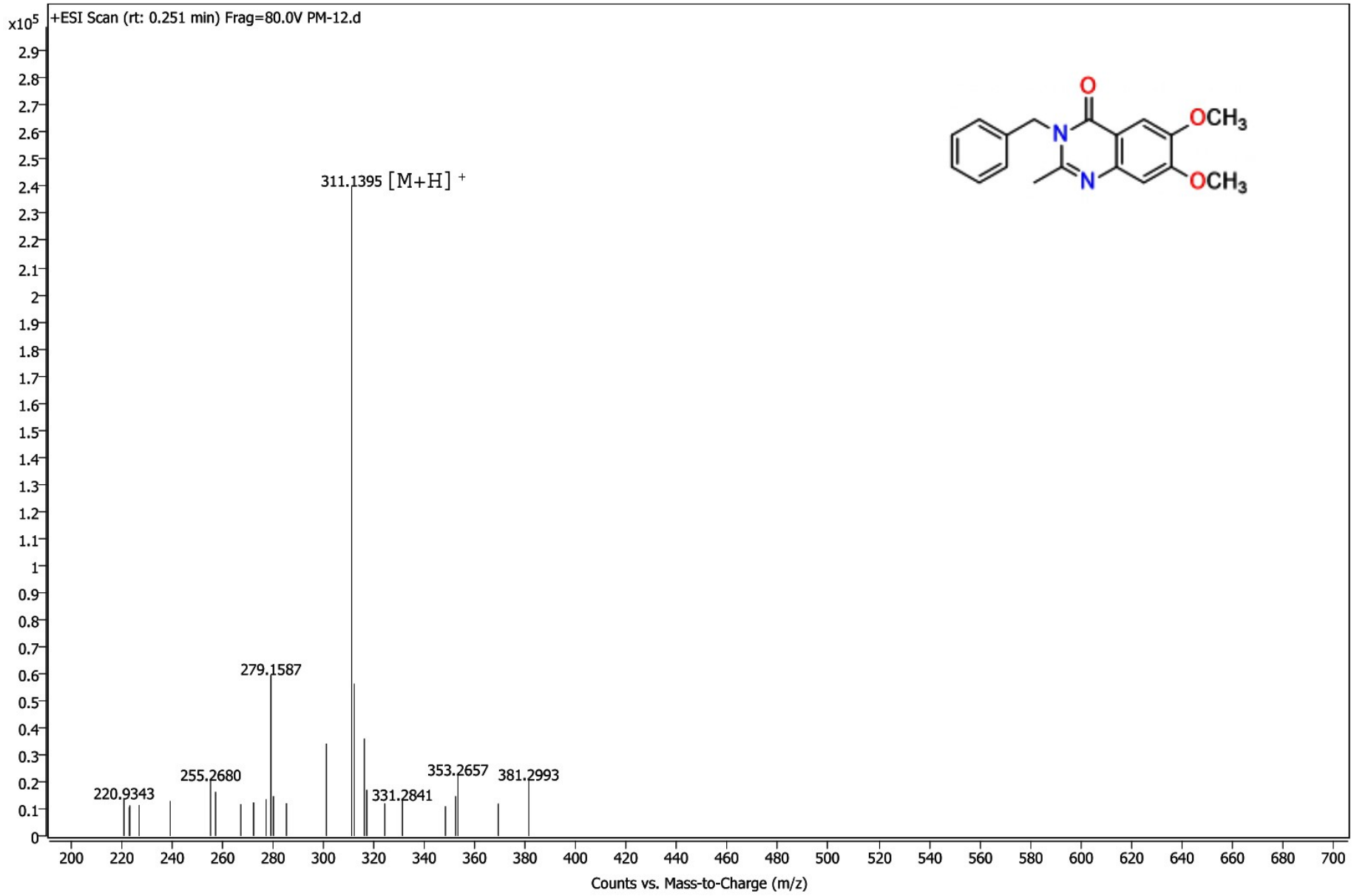
^{1}H NMR of **4b**


7.630
7.338
7.324
7.312
7.282
7.270
7.260
7.200
7.188
7.044
5.402
3.997
2.522
O
N
N
OCH3
OCH3
abundance
0
0.1
0.2
0.3
0.4
0.5
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
-1.0
0.90
2.03
0.84
2.00
0.92
1.79
6.00
2.78
X : parts per Million : Proton

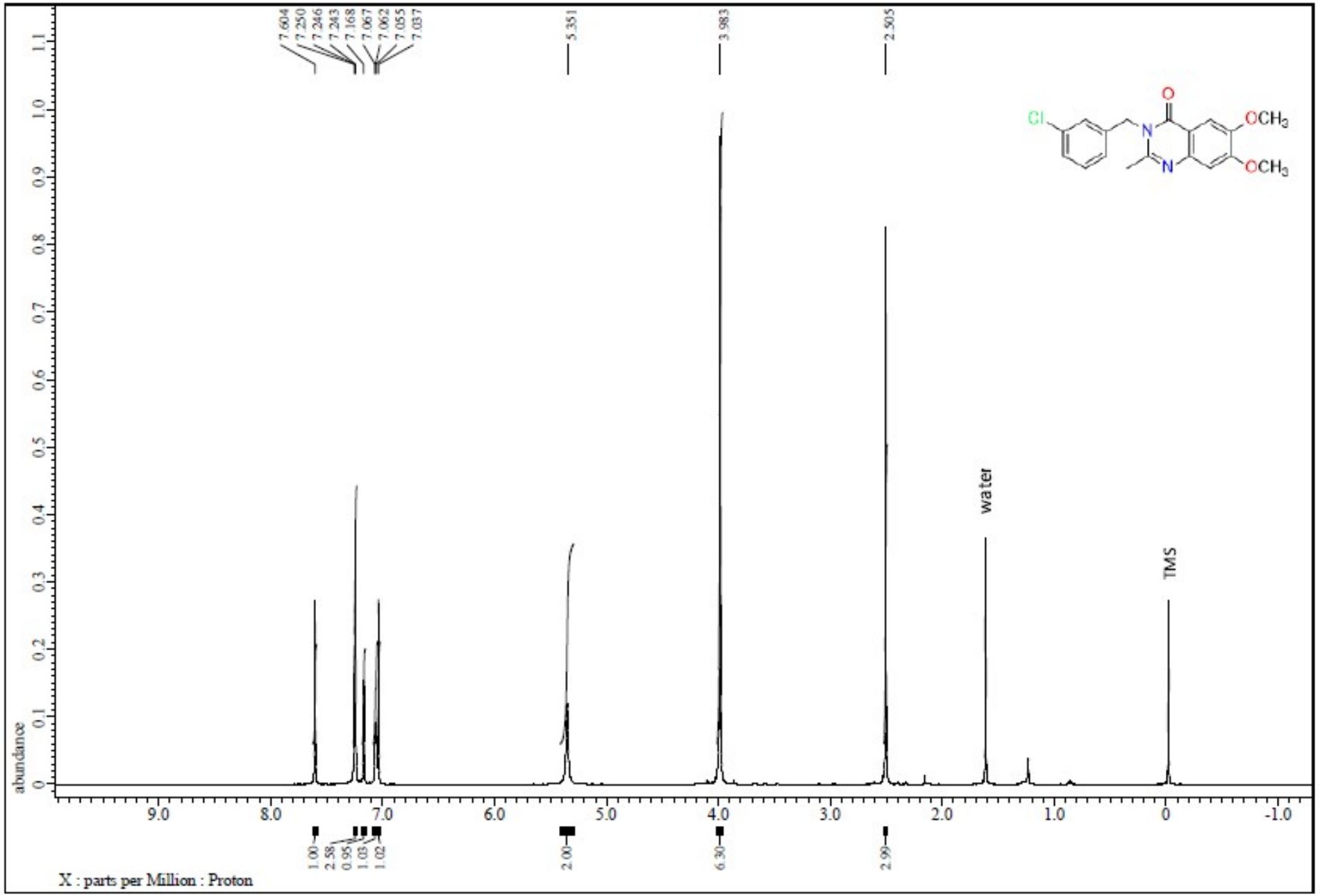
^{13}C NMR of **4b**


161.891
155.179
153.427
148.956
143.747
136.106
129.031
127.748
126.608
113.730
107.258
106.090
56.397
47.243
23.402
CDCl3
O
N
N
OCH3
OCH3
(thousandths)
40.0
30.0
20.0
10.0
0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
X : parts per Million : Carbon13

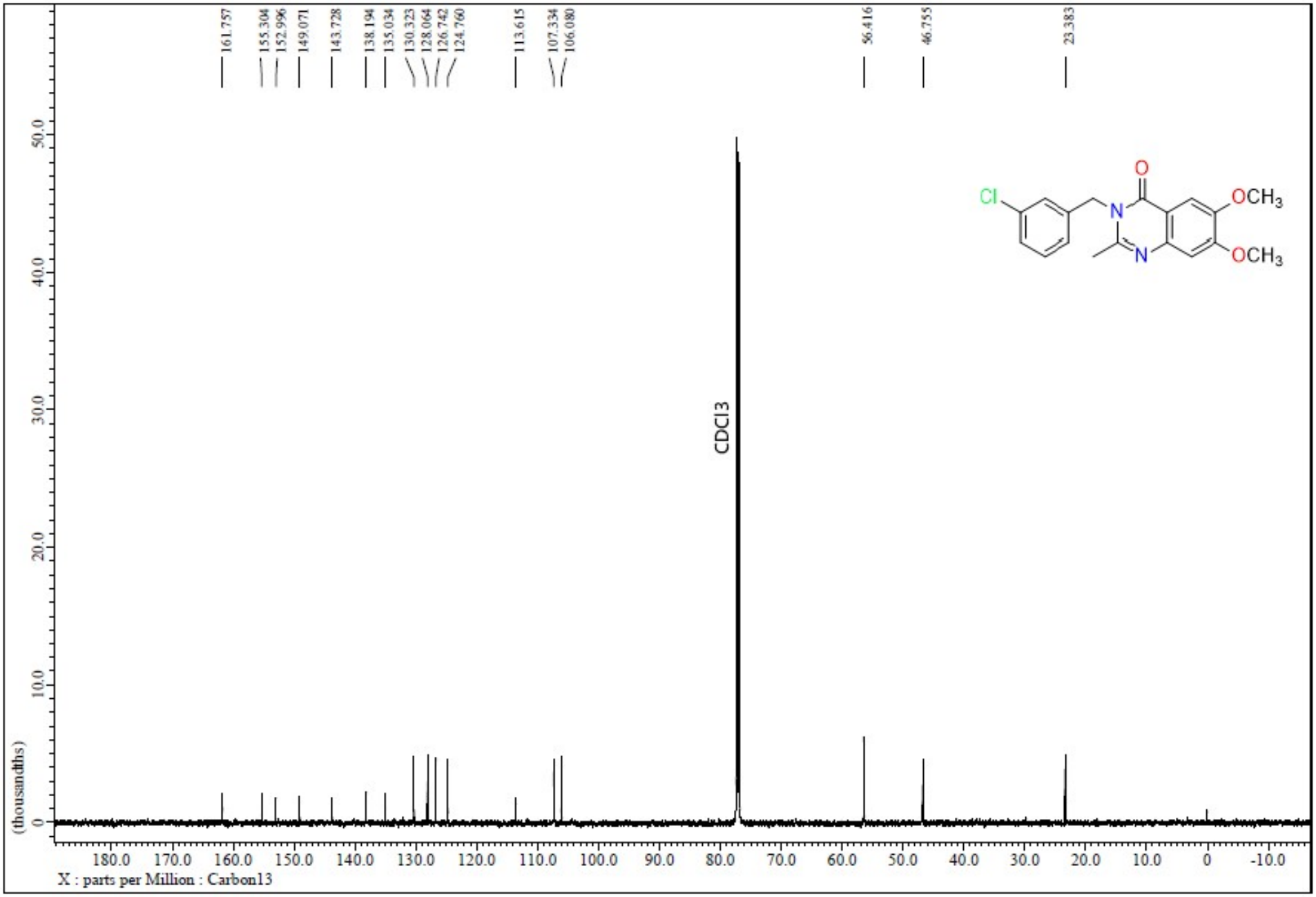
HRMS of 4b


x10^5
+ESI Scan (rt: 0.251 min) Frag=80.0V PM-12.d
311.1395 [M+H]+
279.1587
255.2680
220.9343
331.2841
353.2657
381.2993
O
N
N
OCH3
OCH3
Counts vs. Mass-to-Charge (m/z)

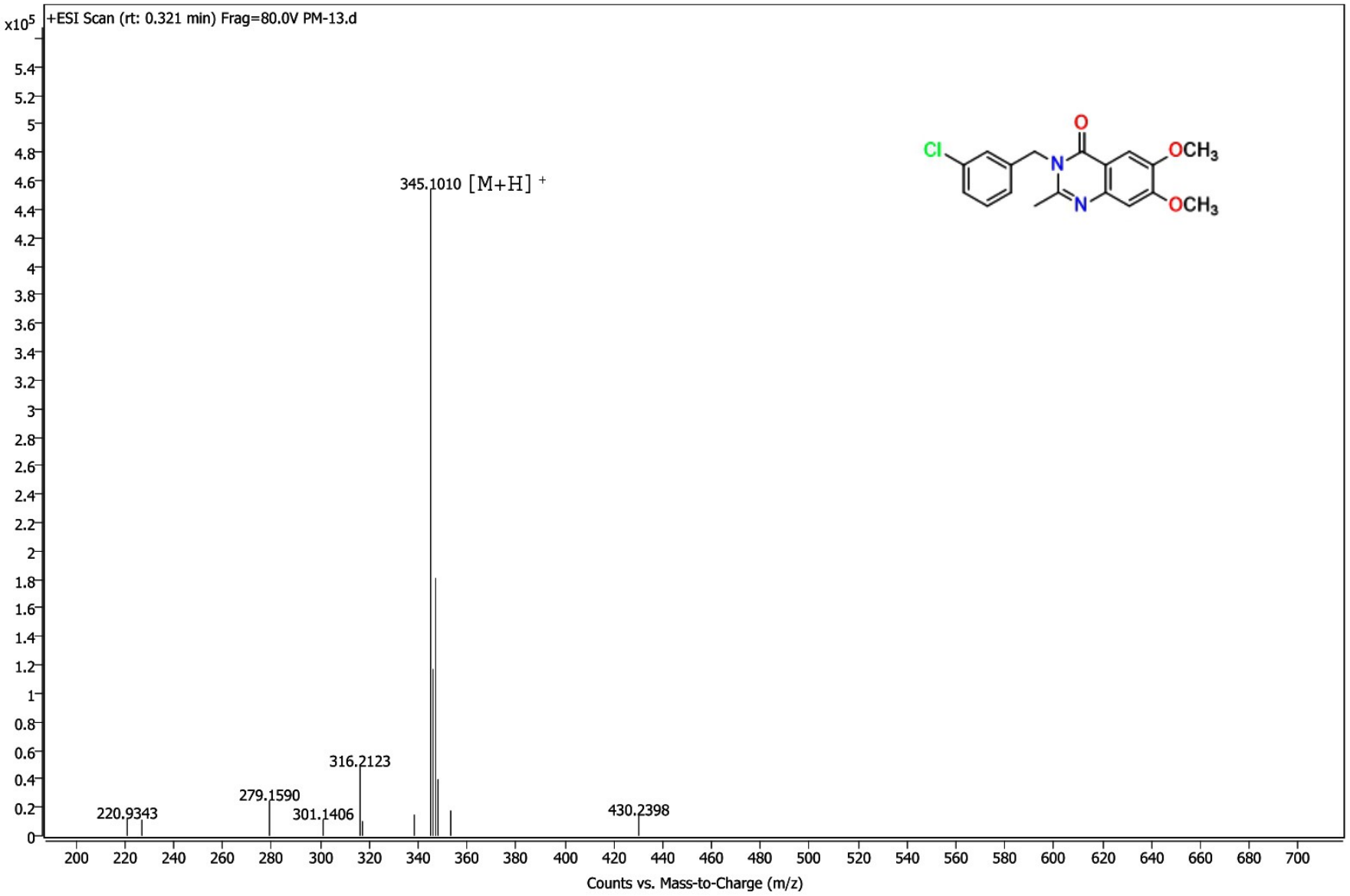
^{1}H NMR of **4c**


7.604
7.250
7.246
7.243
7.168
7.067
7.062
7.055
7.037
5.351
3.983
2.505
water
TMS
abundance
X : parts per Million : Proton
1.00
2.58
0.95
1.03
1.02
2.00
6.30
2.99
Cl
O
N
N
OCH3
OCH3

^{13}C NMR of **4c**


161.757
155.304
152.996
149.071
143.728
138.194
135.034
130.323
128.064
126.742
124.760
113.615
107.334
106.080
56.416
46.755
23.383
CDCl3
Cl
O
N
N
OCH3
OCH3
(thousandths)
50.0
40.0
30.0
20.0
10.0
0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
-10.0
X : parts per Million : Carbon13

HRMS of 4c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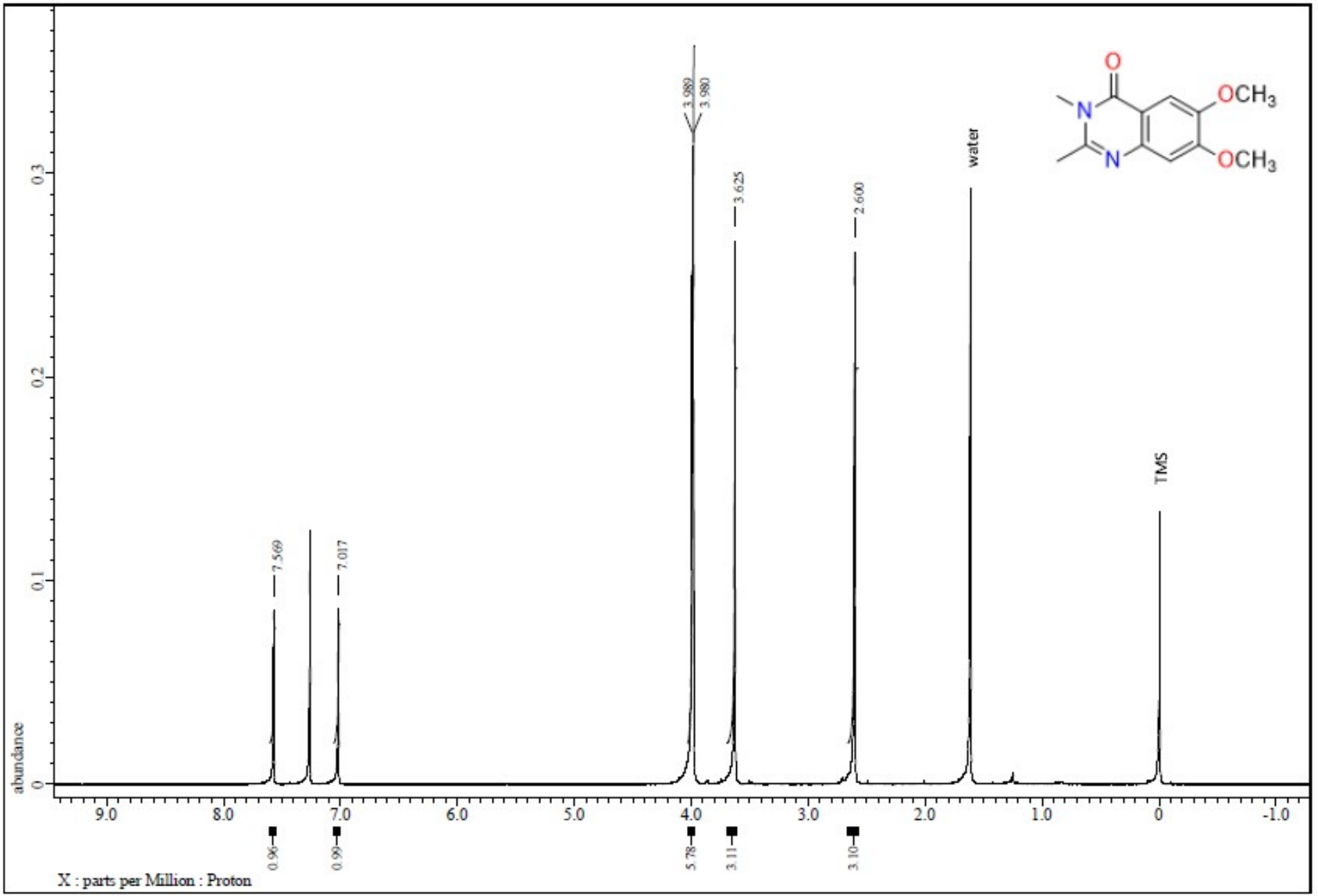
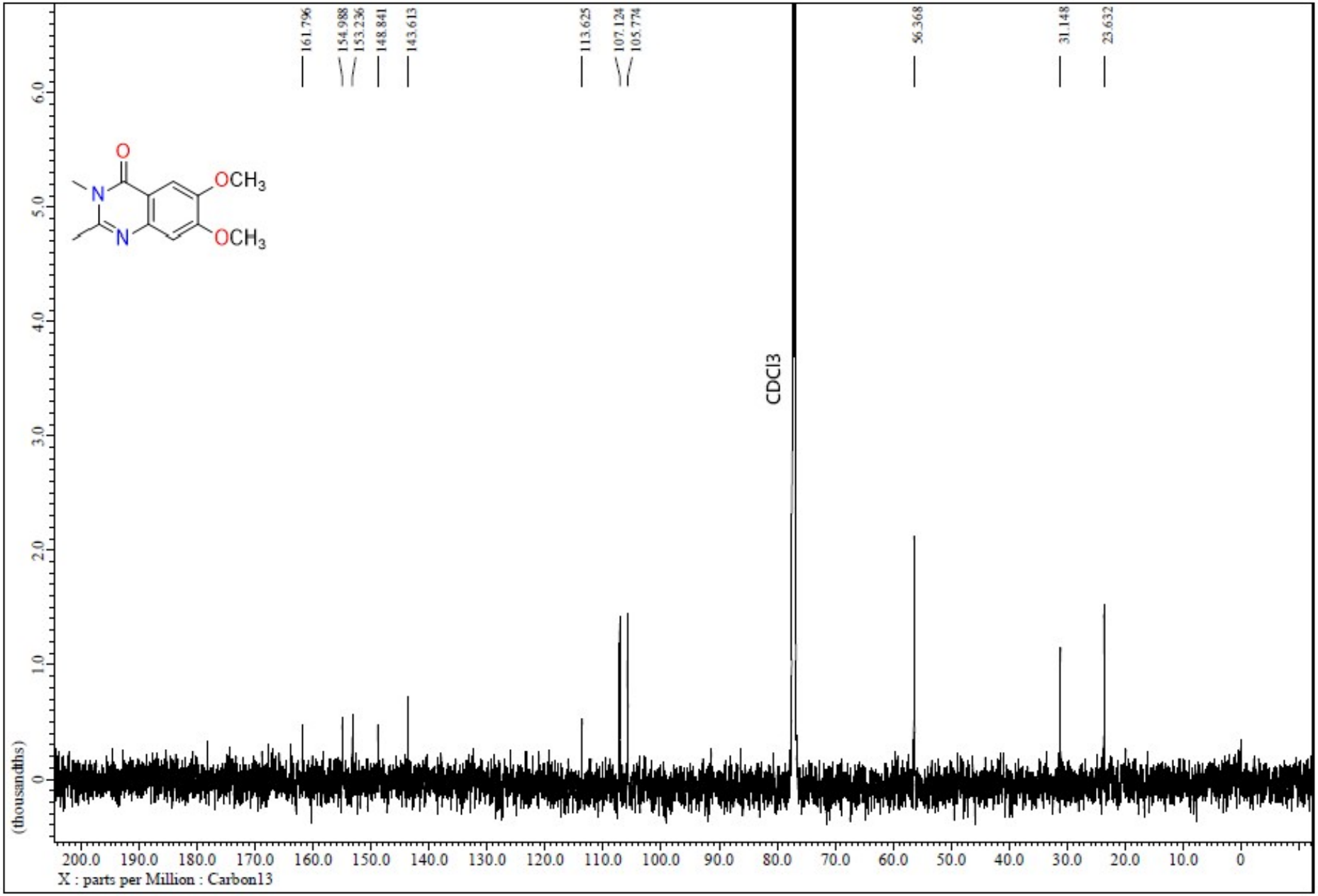
x10^5
345.1010 [M+H]+
316.2123
279.1590
301.1406
220.9343
430.2398
Cl
O
N
N
OCH3
OCH3
Counts vs. Mass-to-Charge (m/z)

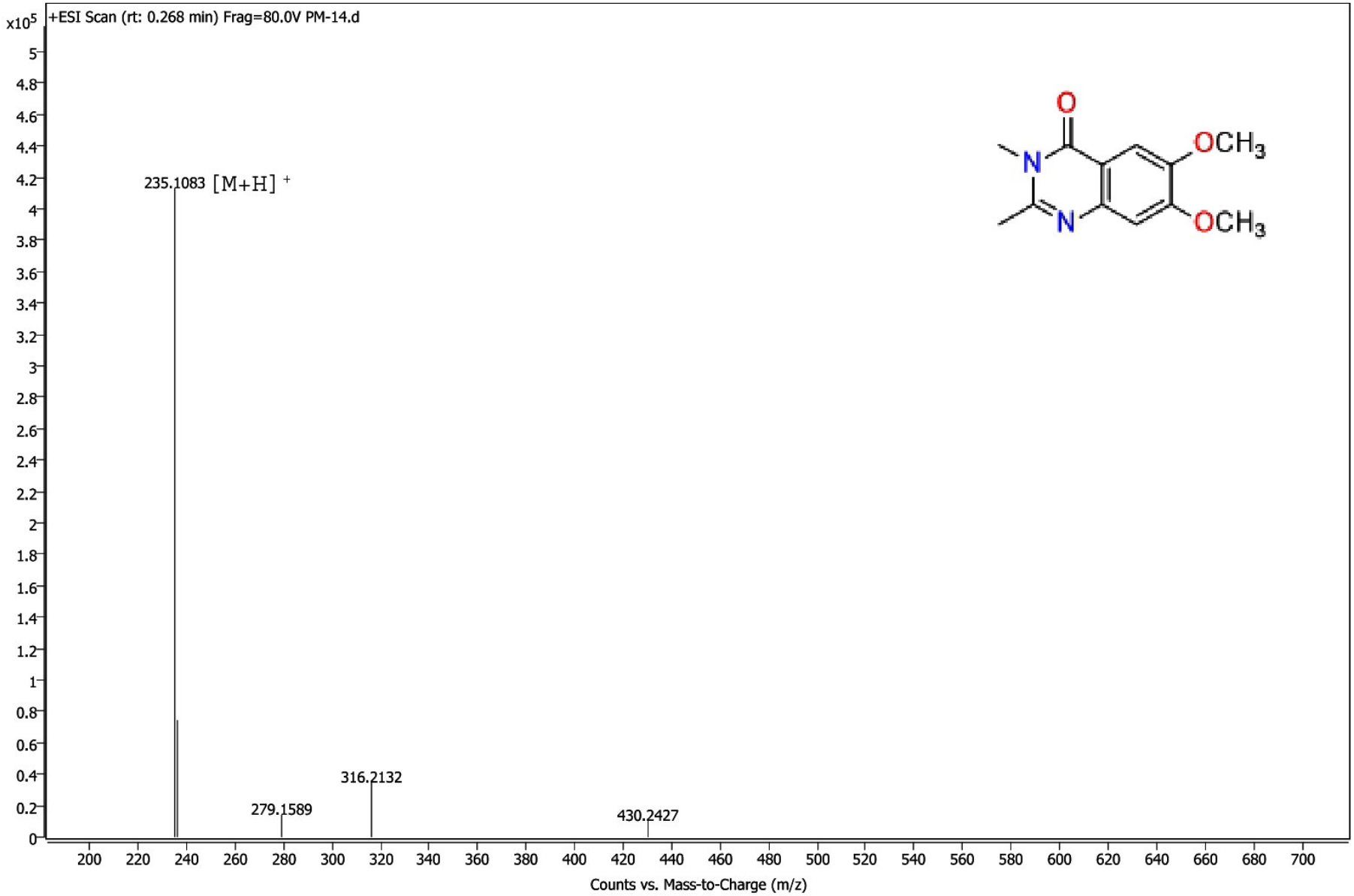
^{1}H NMR of **4d**


7.569
7.017
3.989
3.980
3.625
2.600
water
TMS
abundance
X : parts per Million : Proton
0.96
0.99
5.78
3.11
3.10
O
N
N
OCH3
OCH3

^{13}C NMR of **4d**


161.796
154.988
153.236
148.841
143.613
113.625
107.124
105.774
56.368
31.148
23.632
CDCl3
O
N
N
OCH3
OCH3
(thousandths)
6.0
5.0
4.0
3.0
2.0
1.0
0
200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0
X : parts per Million : Carbon13

HRMS of 4d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x10^5
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316.2132
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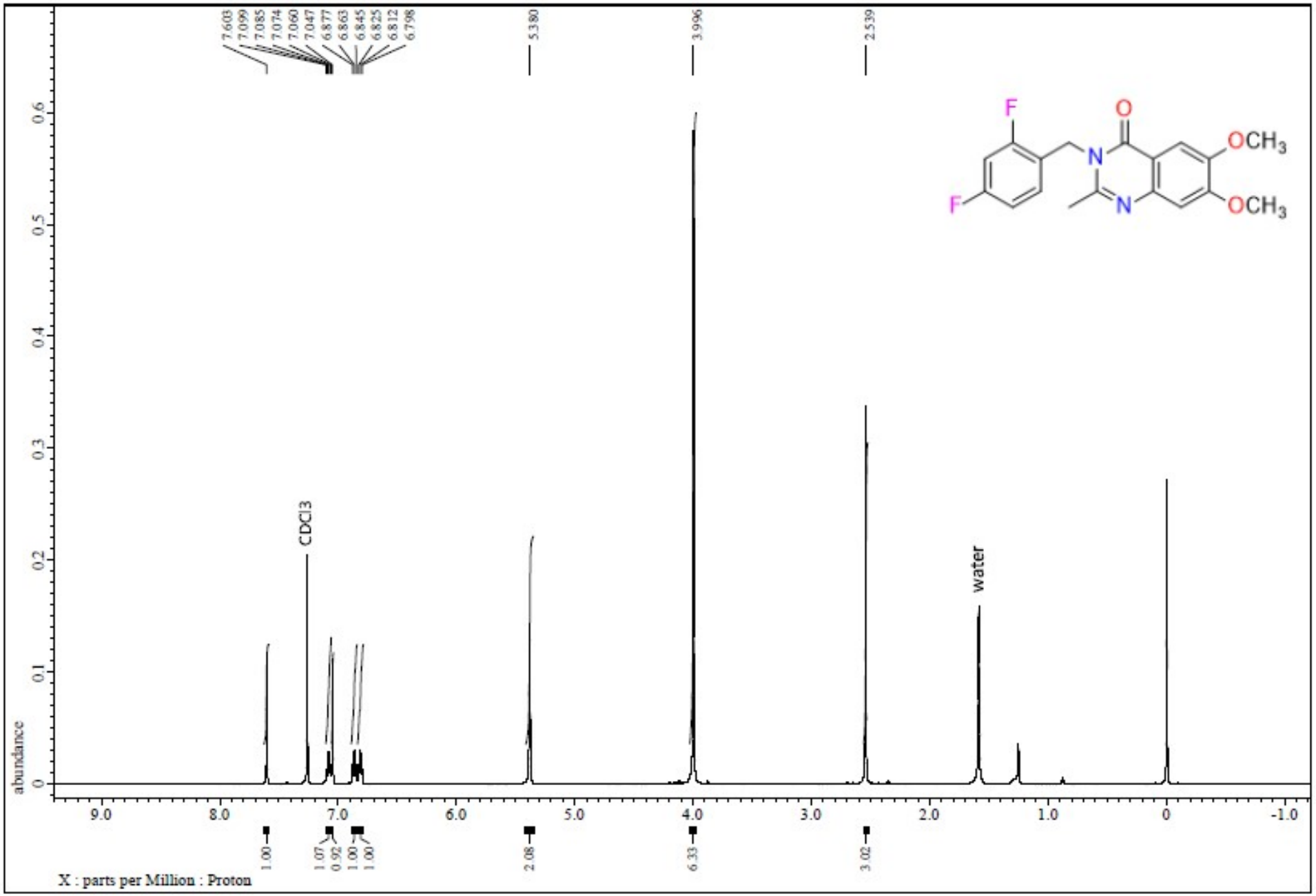
O
N
N
OCH3
OCH3
Counts vs. Mass-to-Charge (m/z)

^{1}H NMR of **4e**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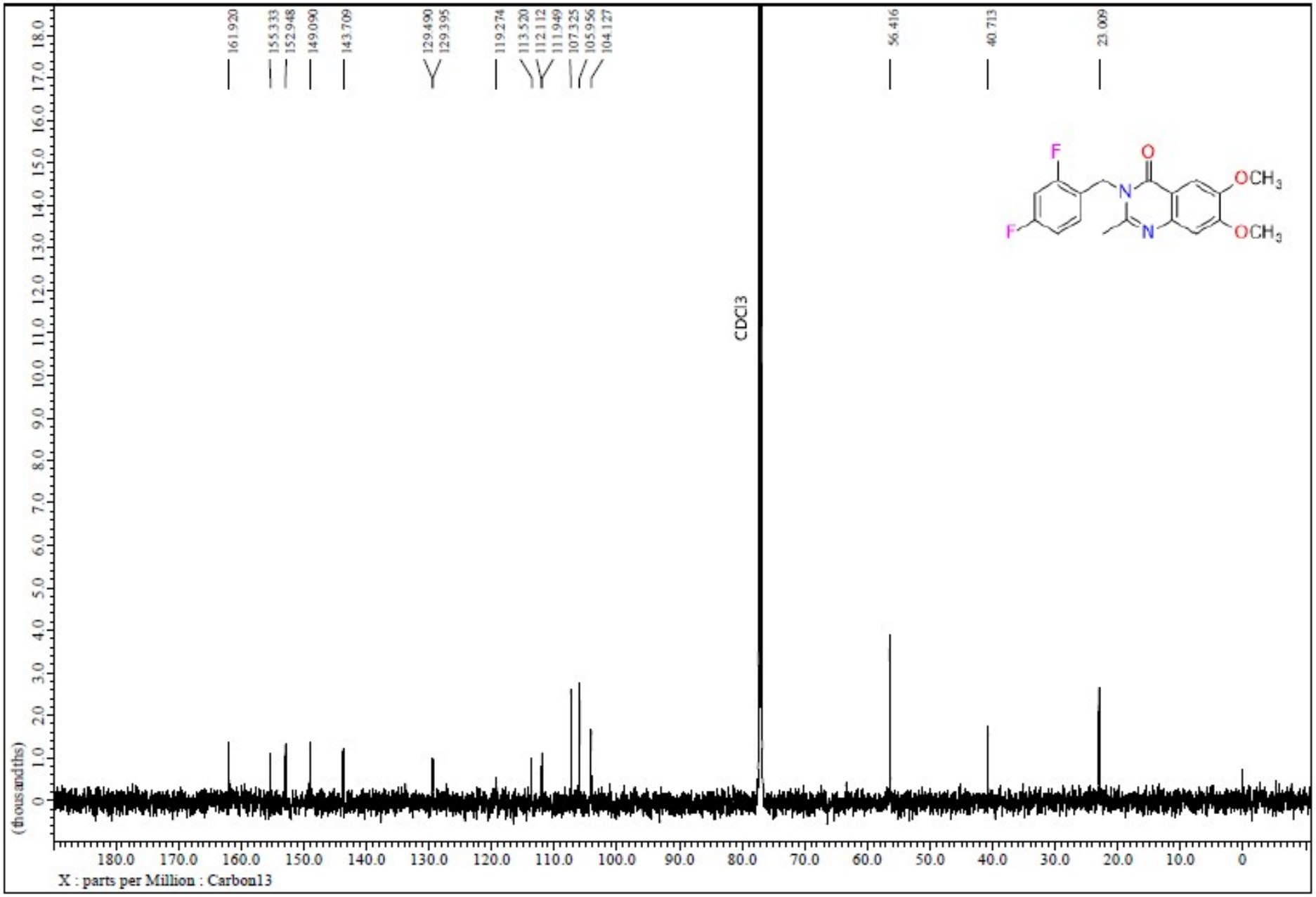
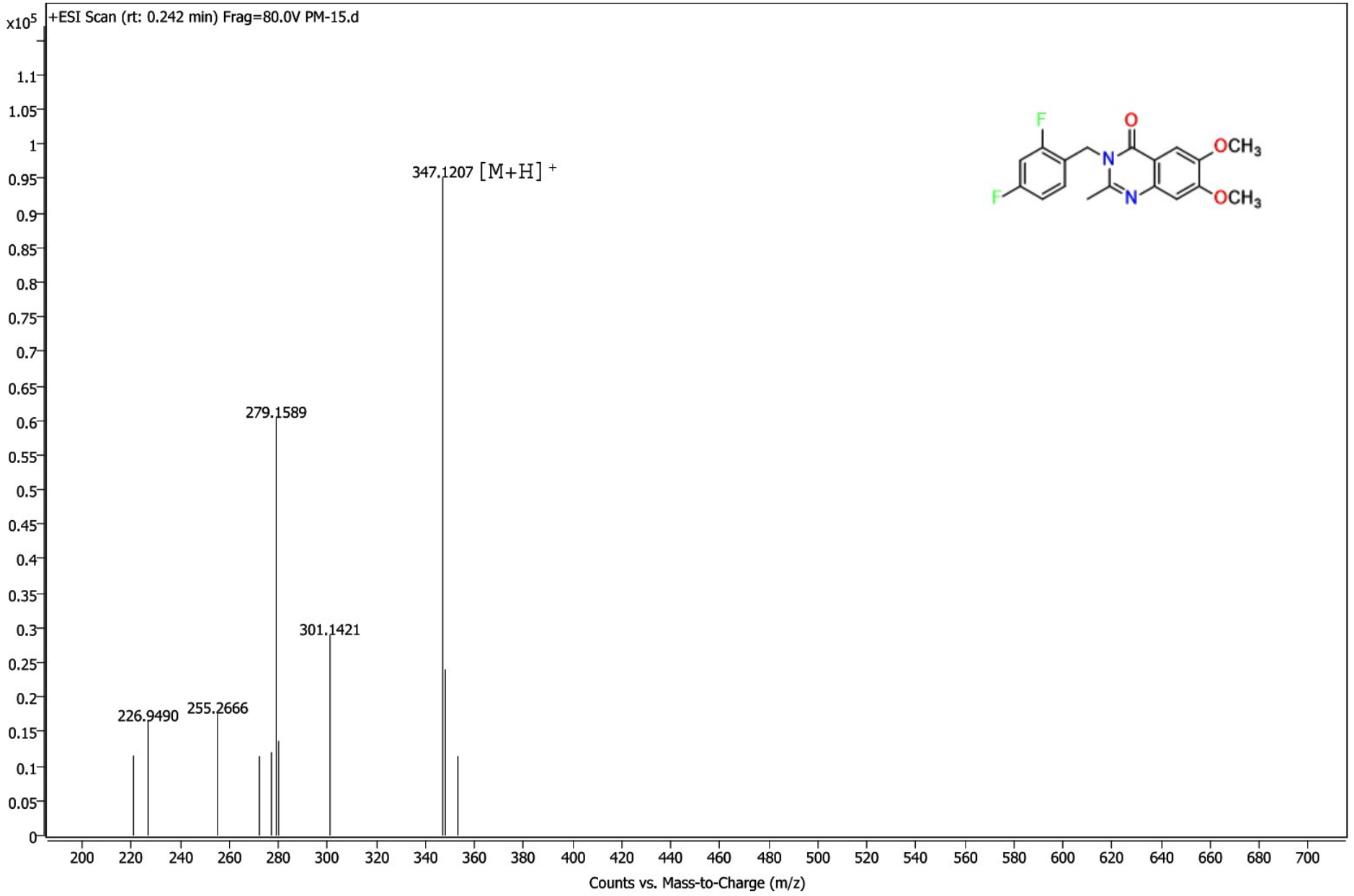
7.099
7.085
7.074
7.060
7.047
6.877
6.863
6.845
6.825
6.812
6.798
5.380
3.996
2.539
CDCl3
water
abundance
X : parts per Million : Proton
1.00
1.07
0.92
1.00
1.00
2.08
6.33
3.02
F
O
N
N
F
OCH3
OCH3

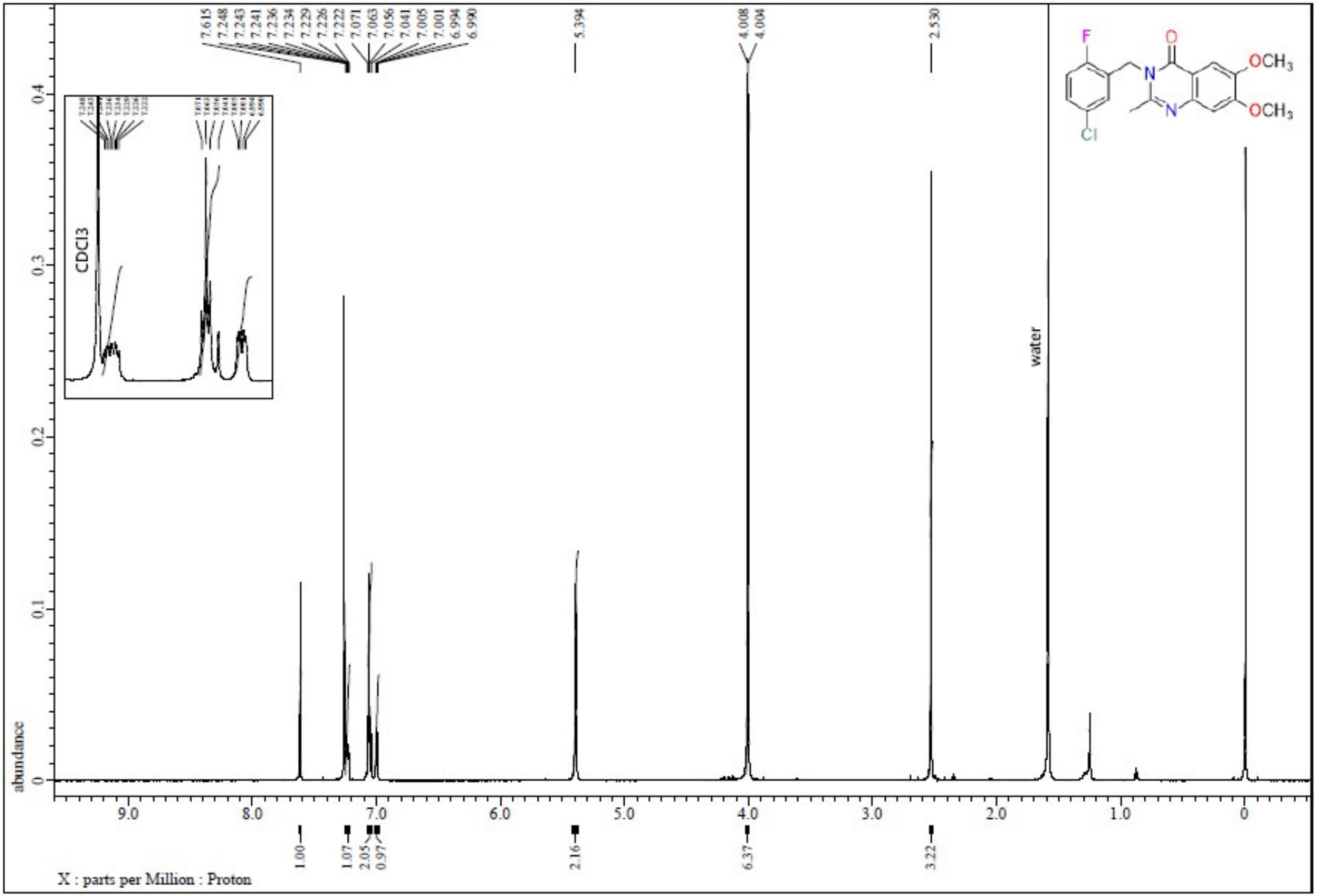
^{13}C NMR of **4e**


161.920
155.333
152.948
149.090
143.709
129.490
129.395
119.274
113.520
112.112
111.949
107.325
105.956
104.127
56.416
40.713
23.009
CDCl3
F
O
N
N
F
OCH3
OCH3
(thousandths)
X : parts per Million : Carbon13

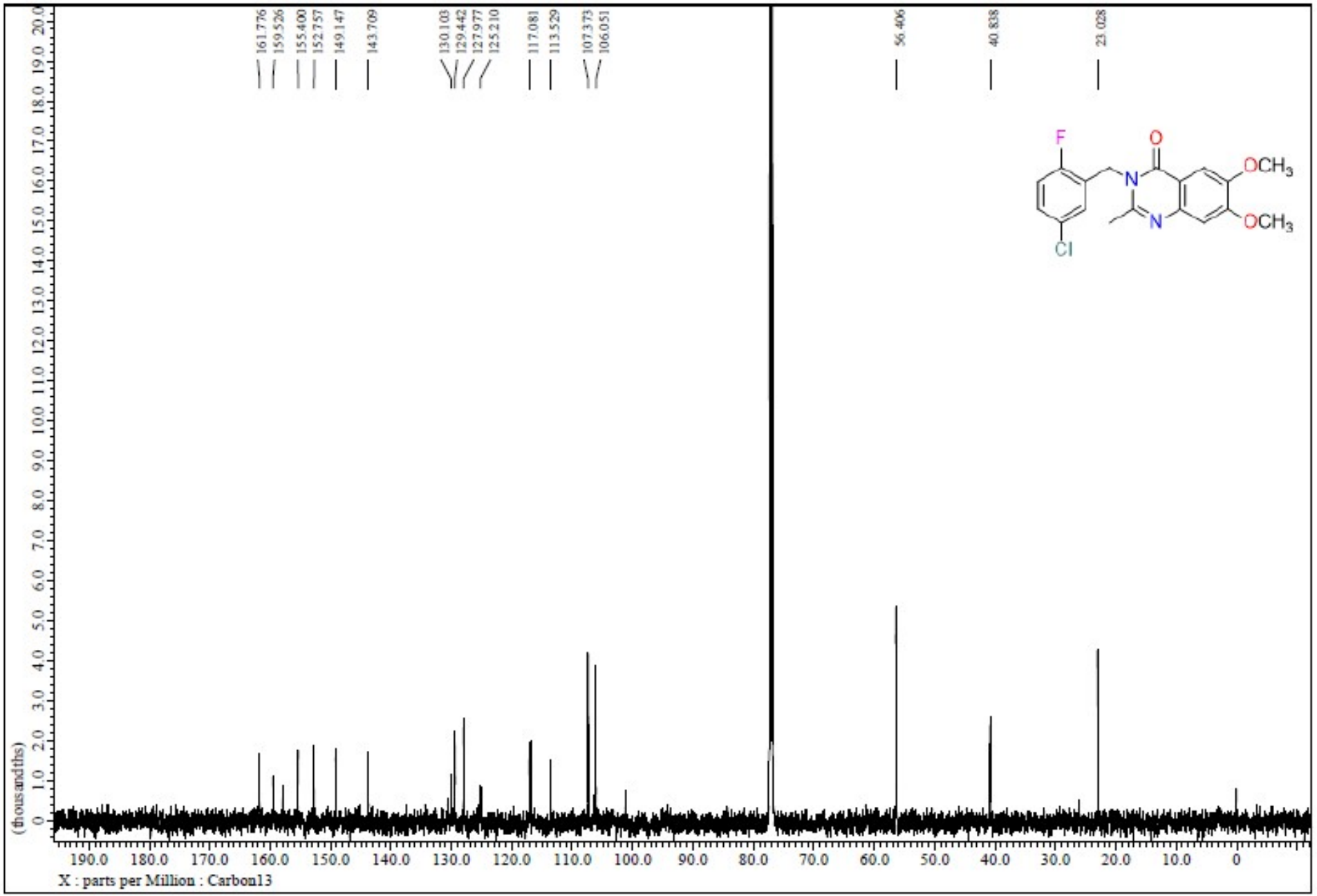
HRMS of 4e


x10^5
+ESI Scan (rt: 0.242 min) Frag=80.0V PM-15.d
347.1207 [M+H]+
279.1589
301.1421
226.9490
255.2666
F
O
N
N
F
OCH3
OCH3
Counts vs. Mass-to-Charge (m/z)

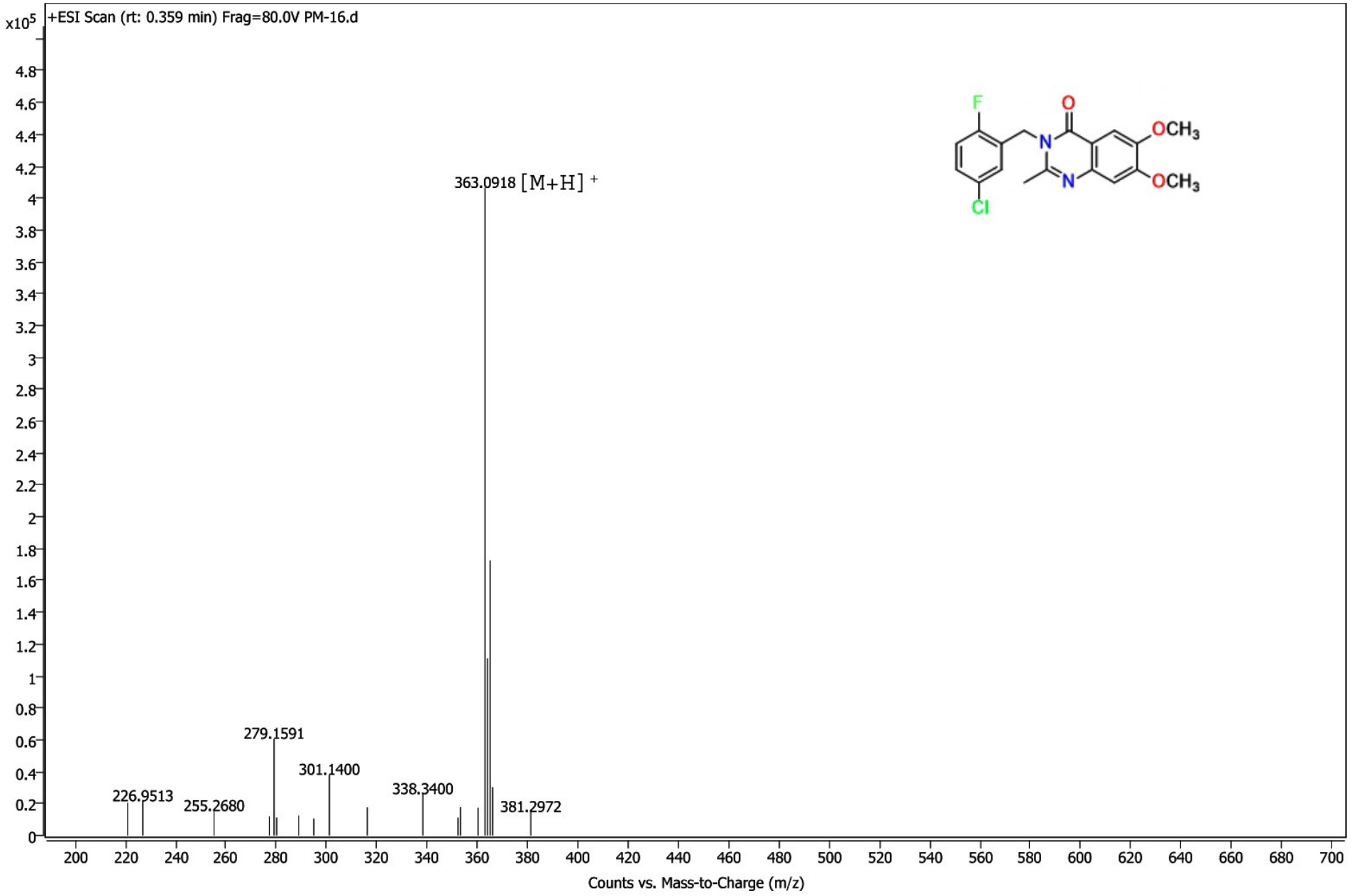
^{1}H NMR of **4f**


7.615
7.248
7.243
7.241
7.236
7.234
7.229
7.226
7.222
7.071
7.063
7.056
7.041
7.005
7.001
6.994
6.990
5.394
4.008
4.004
2.530
CDCl3
water
abundance
X : parts per Million : Proton
1.00
1.07
2.05
0.97
2.16
6.37
3.22
F
O
N
N
OCH3
OCH3
Cl

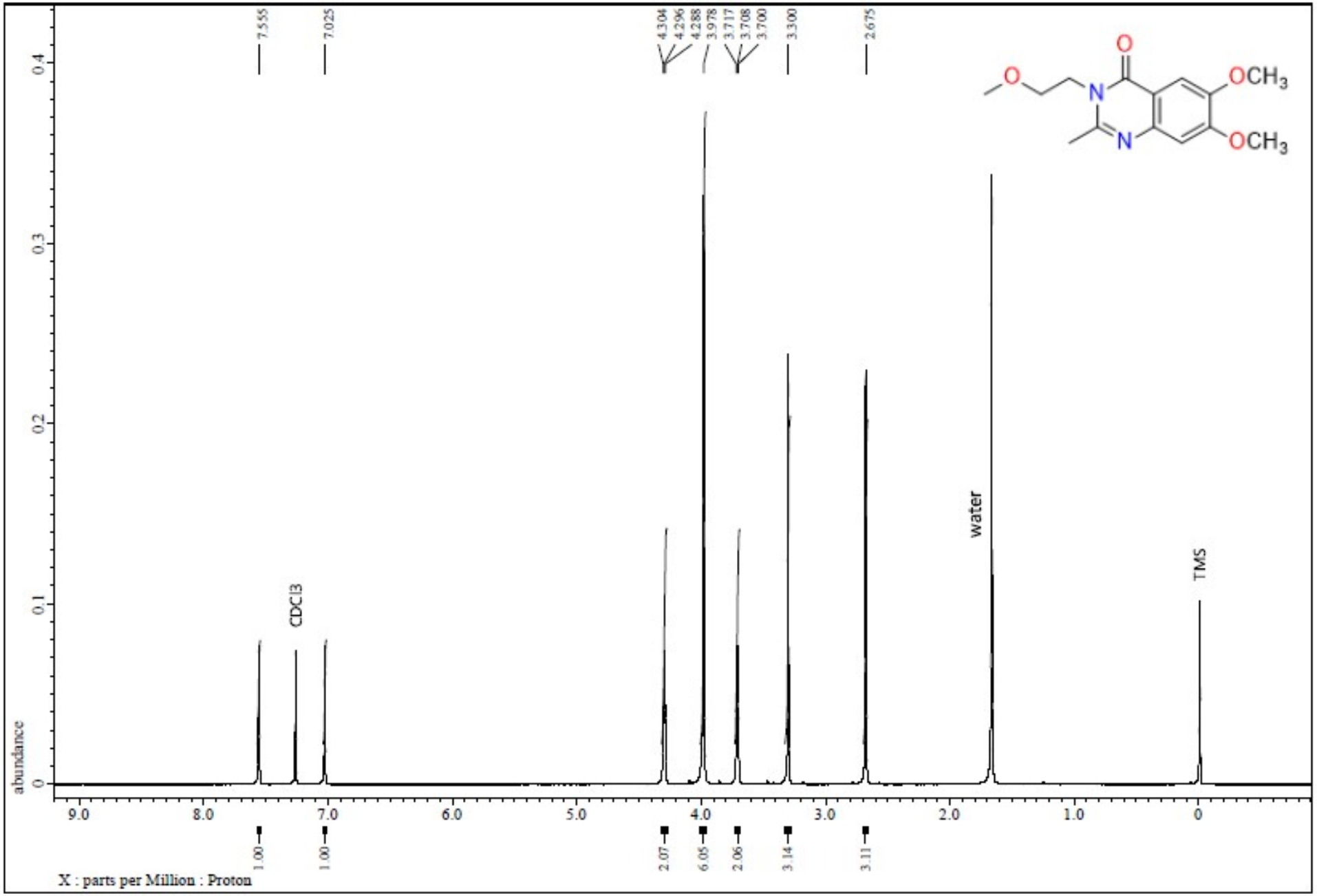
^{13}C NMR of **4f**


161.776
159.526
155.400
152.757
149.147
143.709
130.103
129.442
127.977
125.210
117.081
113.529
107.373
106.051
56.406
40.838
21.028
F
O
N
N
OCH3
OCH3
Cl
(thousandths)
X : parts per Million : Carbon13

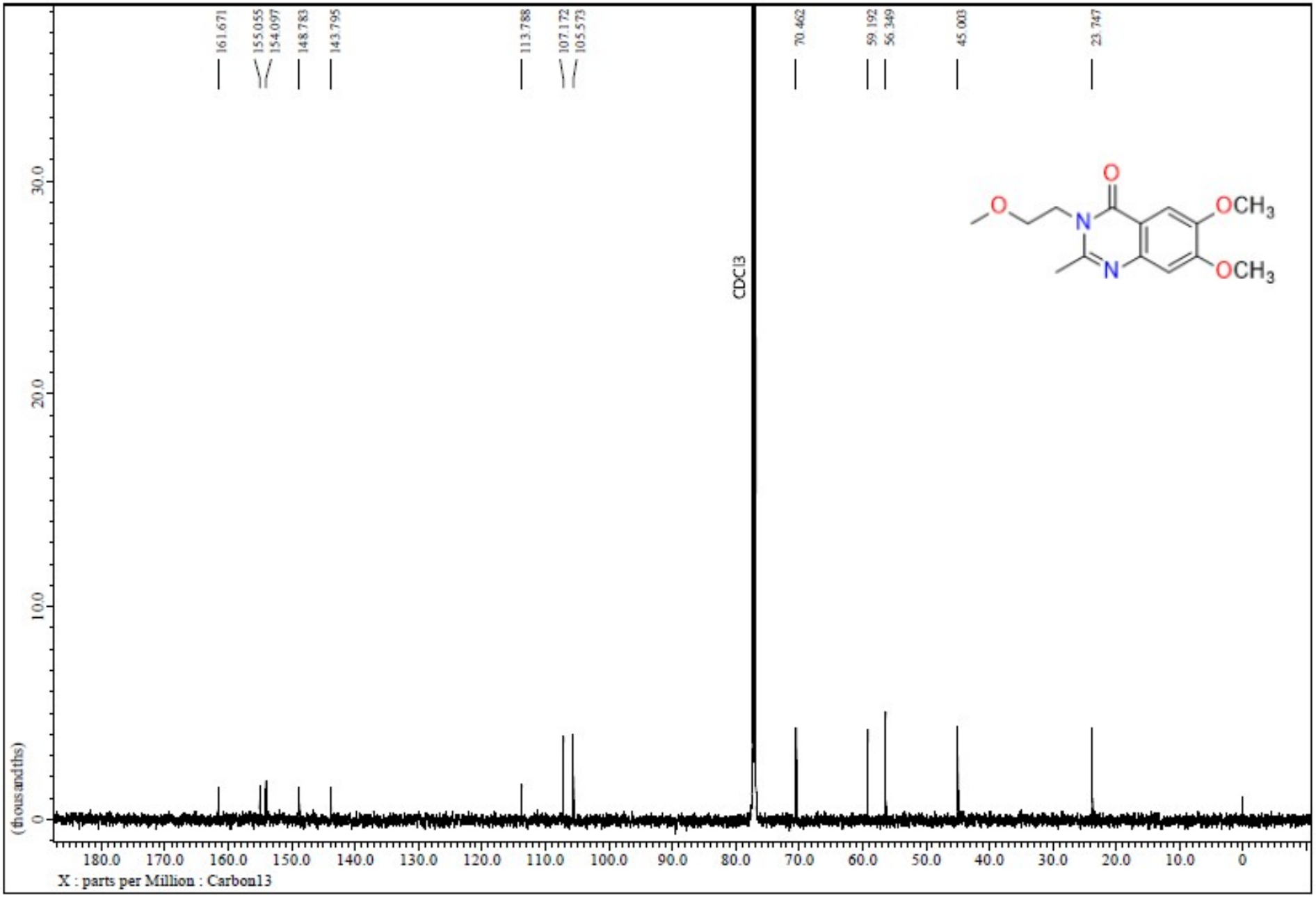
HRMS of 4f


x10^5
+ESI Scan (rt: 0.359 min) Frag=80.0V PM-16.d
363.0918 [M+H]+
279.1591
301.1400
226.9513
255.2680
338.3400
381.2972
F
O
N
N
OCH3
OCH3
Cl
Counts vs. Mass-to-Charge (m/z)

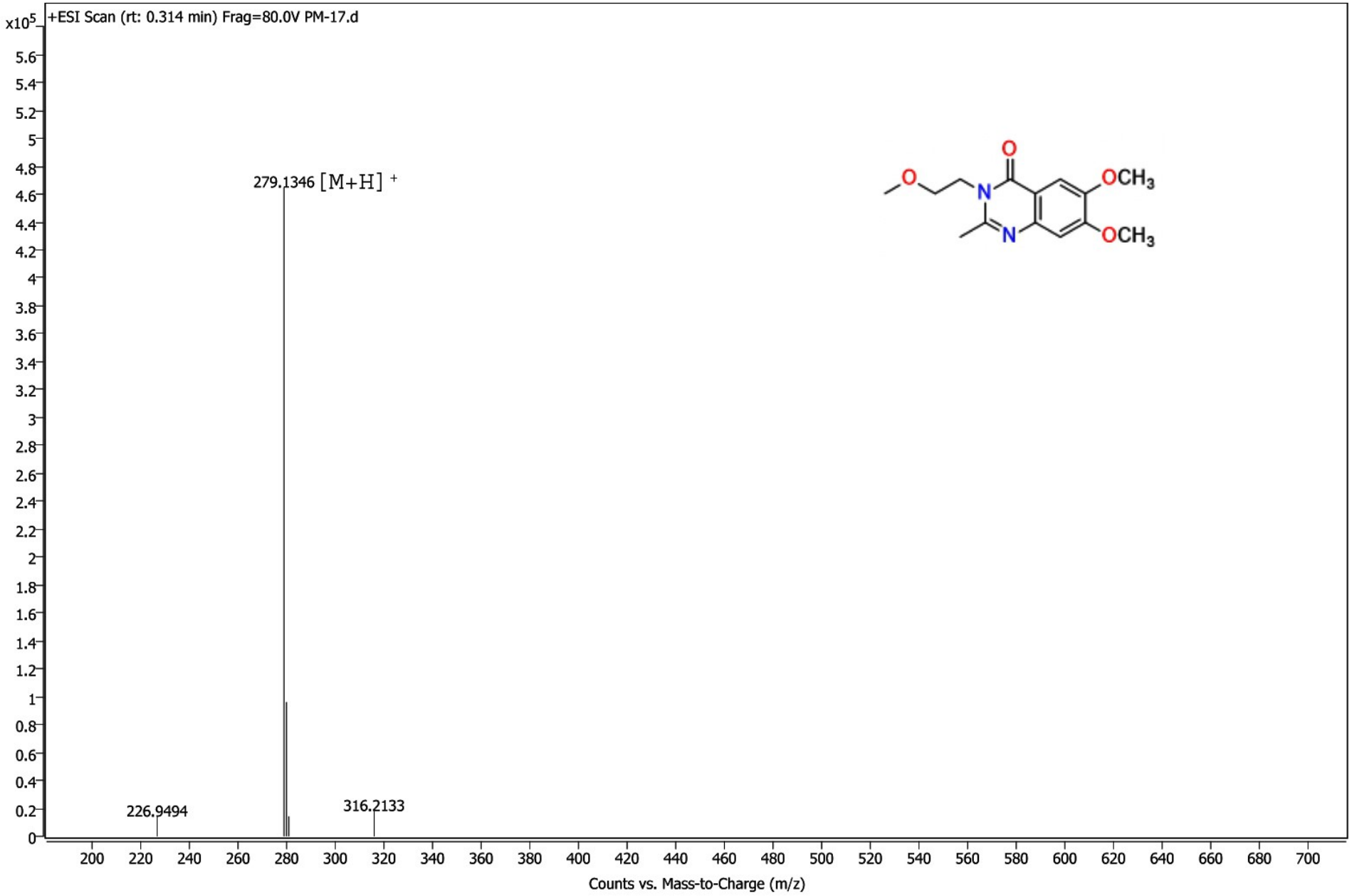
^{1}H NMR of **4g**


7.555
7.025
4.304
4.296
4.288
3.978
3.717
3.708
3.700
3.300
2.675
water
TMS
CDCl3
abundance
X : parts per Million : Proton
1.00
1.00
2.07
6.05
2.06
3.14
3.11
O
N
N
O
OCH3
OCH3

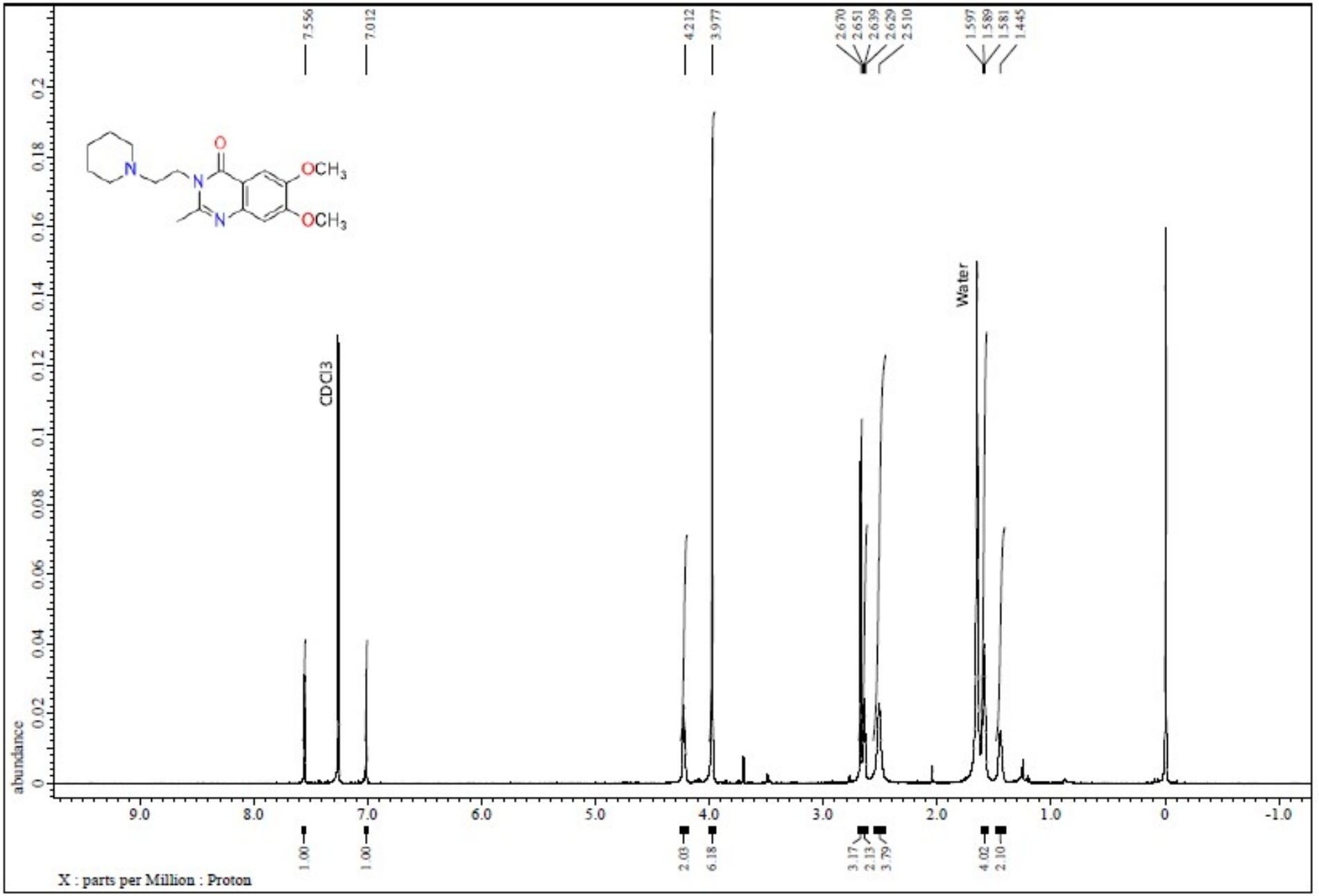
^{13}C NMR of **4g**


161.671
155.055
154.097
148.783
143.795
113.788
107.172
105.573
70.462
59.192
56.349
45.003
23.747
CDCl3
O
O
N
N
OCH3
OCH3
30.0
20.0
10.0
0
(thousandths)
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
X : parts per Million : Carbon13

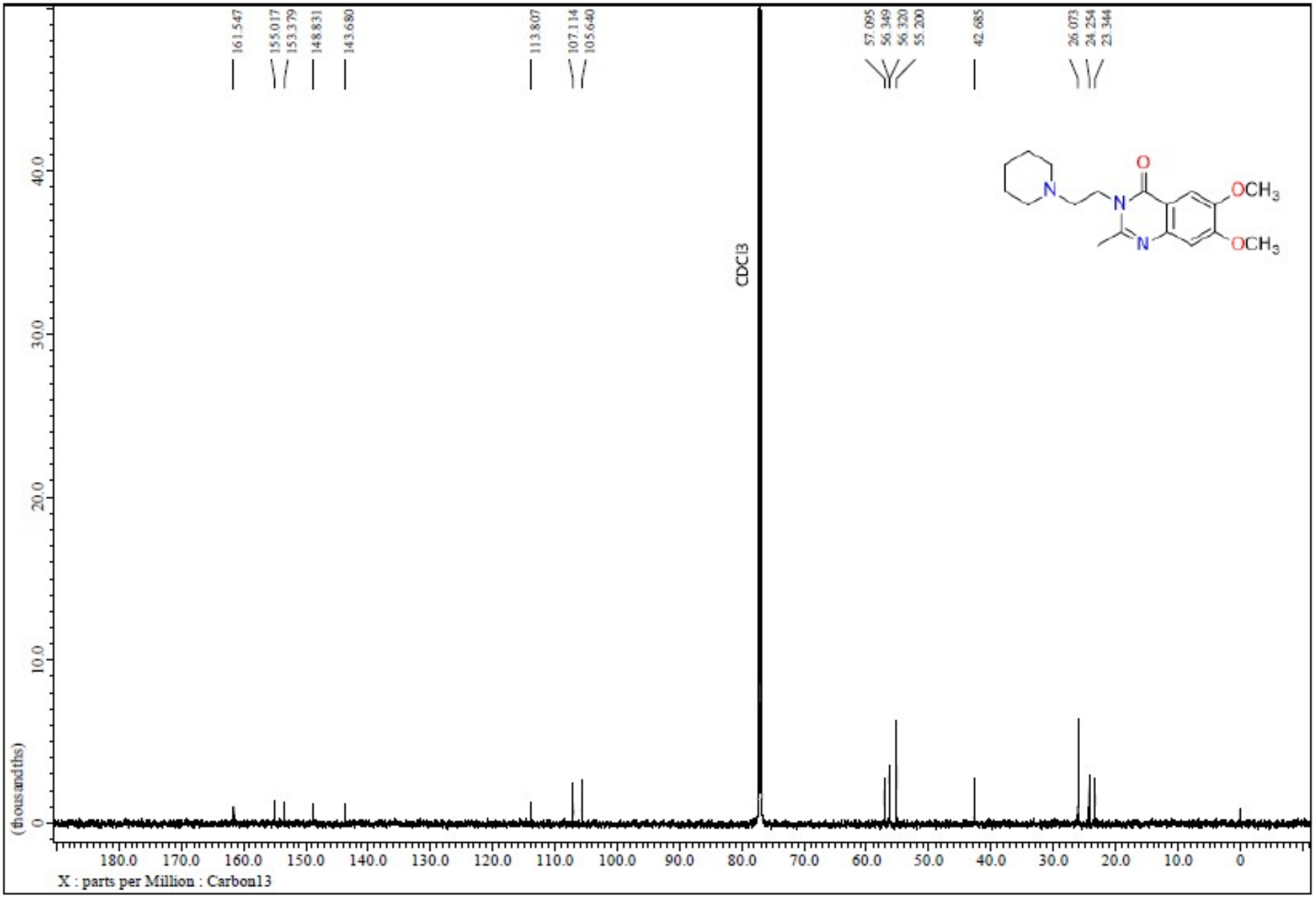
HRMS of 4g


+ESI Scan (rt: 0.314 min) Frag=80.0V PM-17.d
x10^5
279.1346 [M+H]+
226.9494
316.2133
Counts vs. Mass-to-Charge (m/z)

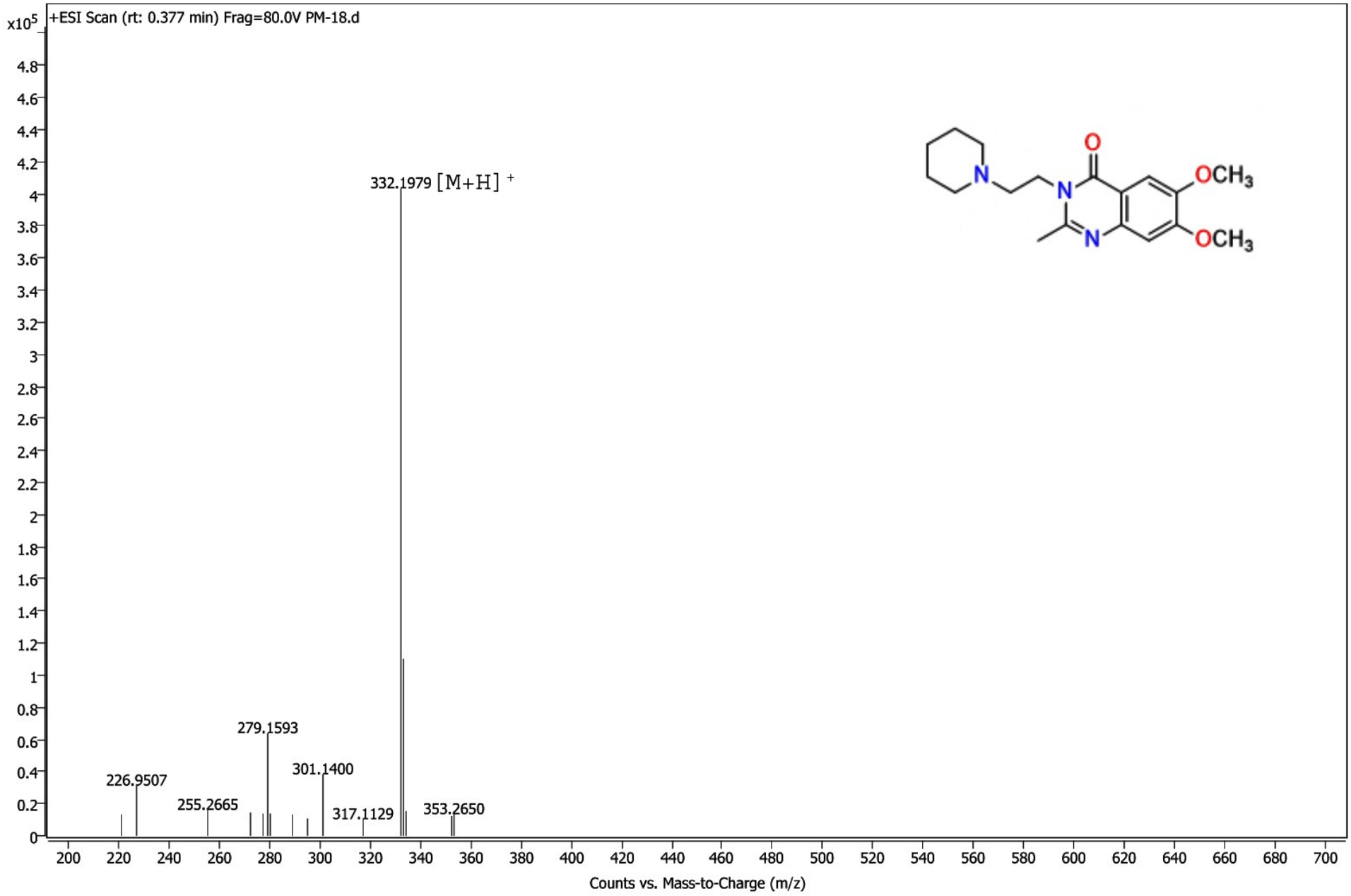
^{1}H NMR of **4h**


7.556
7.012
4.212
3.977
2.670
2.651
2.639
2.629
2.510
1.597
1.589
1.581
1.445
CDCl3
Water
OCH3
OCH3
abundance
X : parts per Million : Proton
1.00
1.00
2.03
6.18
3.17
2.13
3.79
4.02
2.10

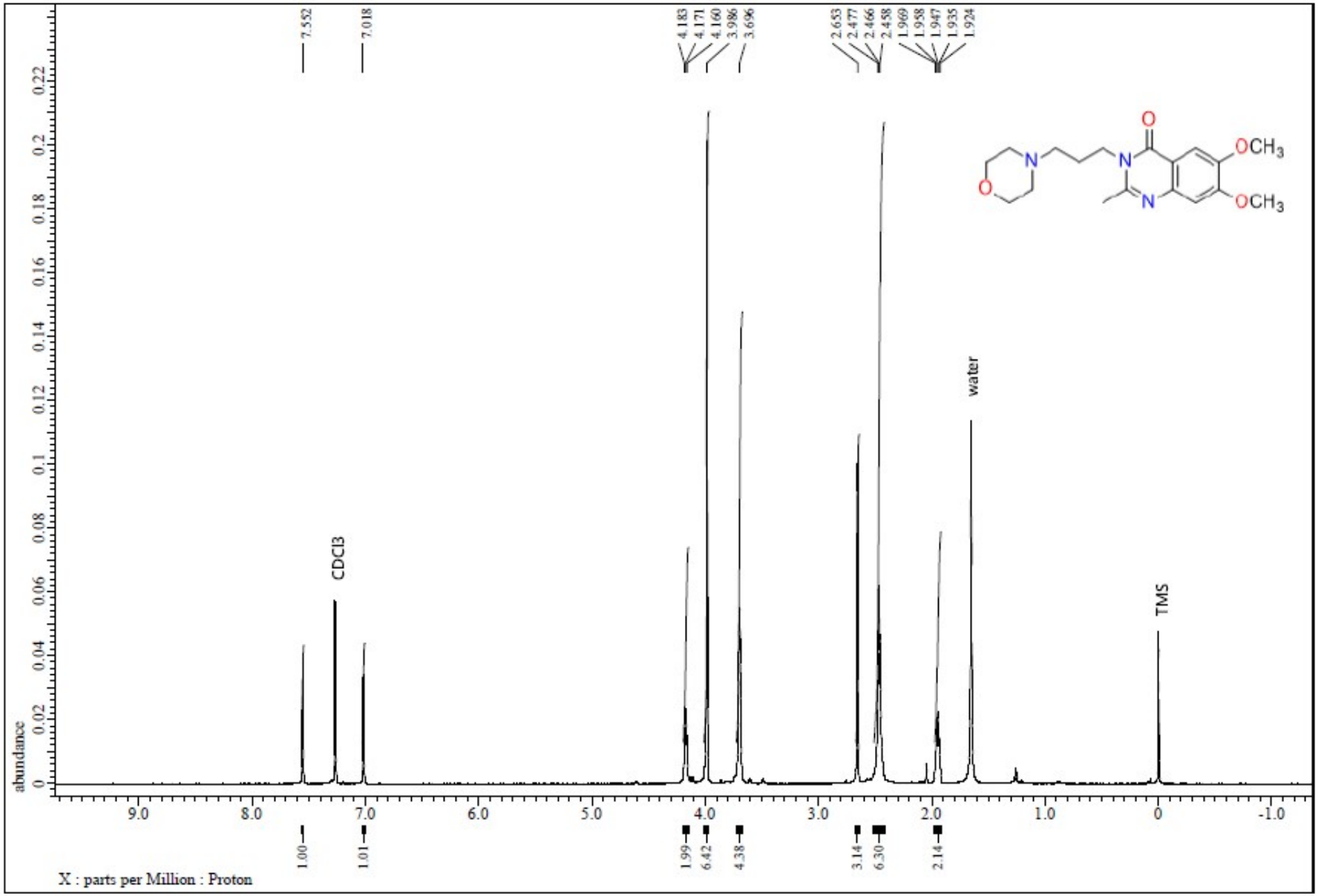
^{13}C NMR of **4h**


161.547
155.017
153.379
148.831
143.680
113.807
107.114
105.640
57.095
56.349
56.320
55.200
42.685
26.073
24.254
23.344
CDCl3
OCH3
OCH3
(thousandths)
X : parts per Million : Carbon13

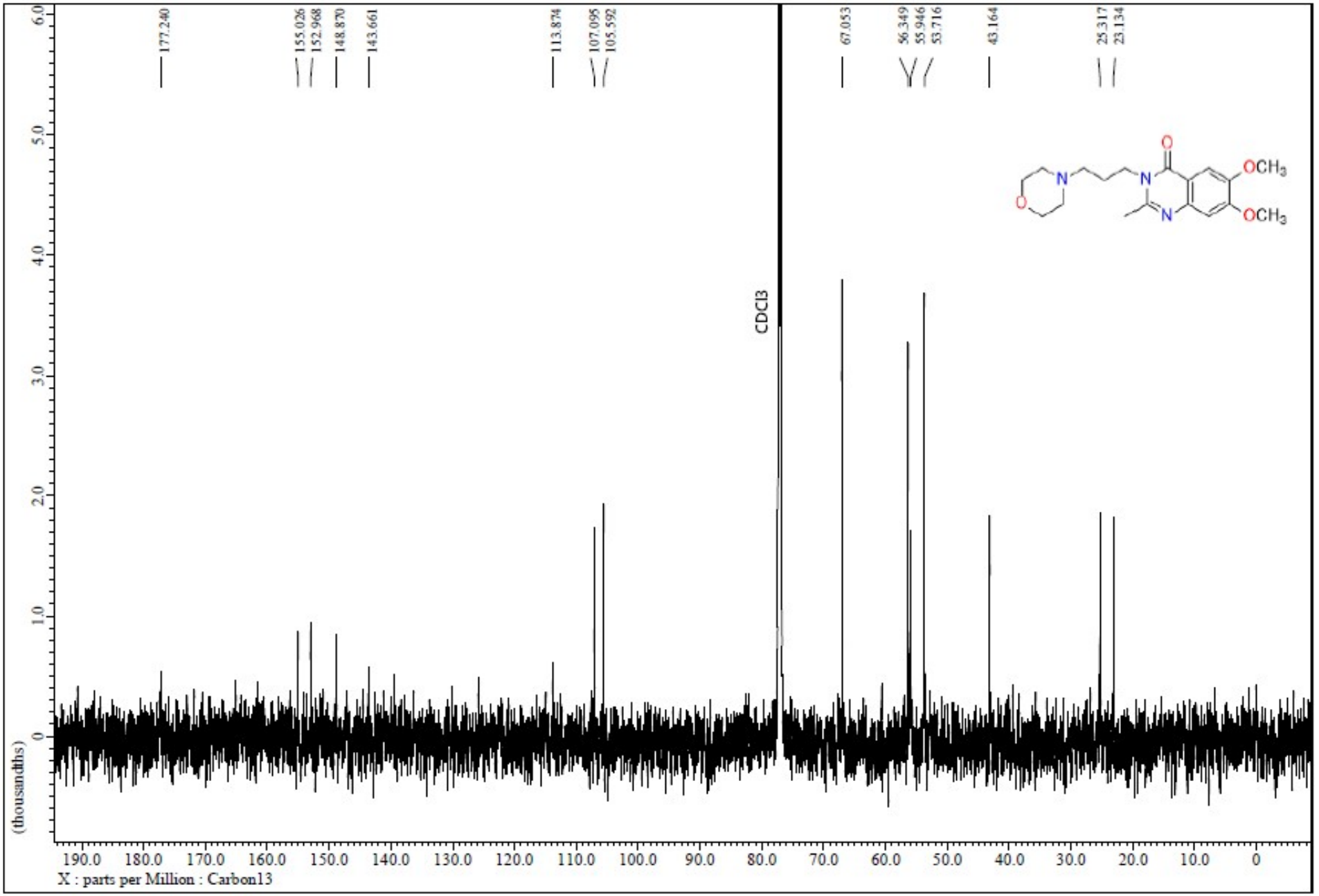
HRMS of 4h


+ESI Scan (rt: 0.377 min) Frag=80.0V PM-18.d
x10^5
332.1979 [M+H]+
279.1593
301.1400
226.9507
255.2665
317.1129
353.2650
Counts vs. Mass-to-Charge (m/z)

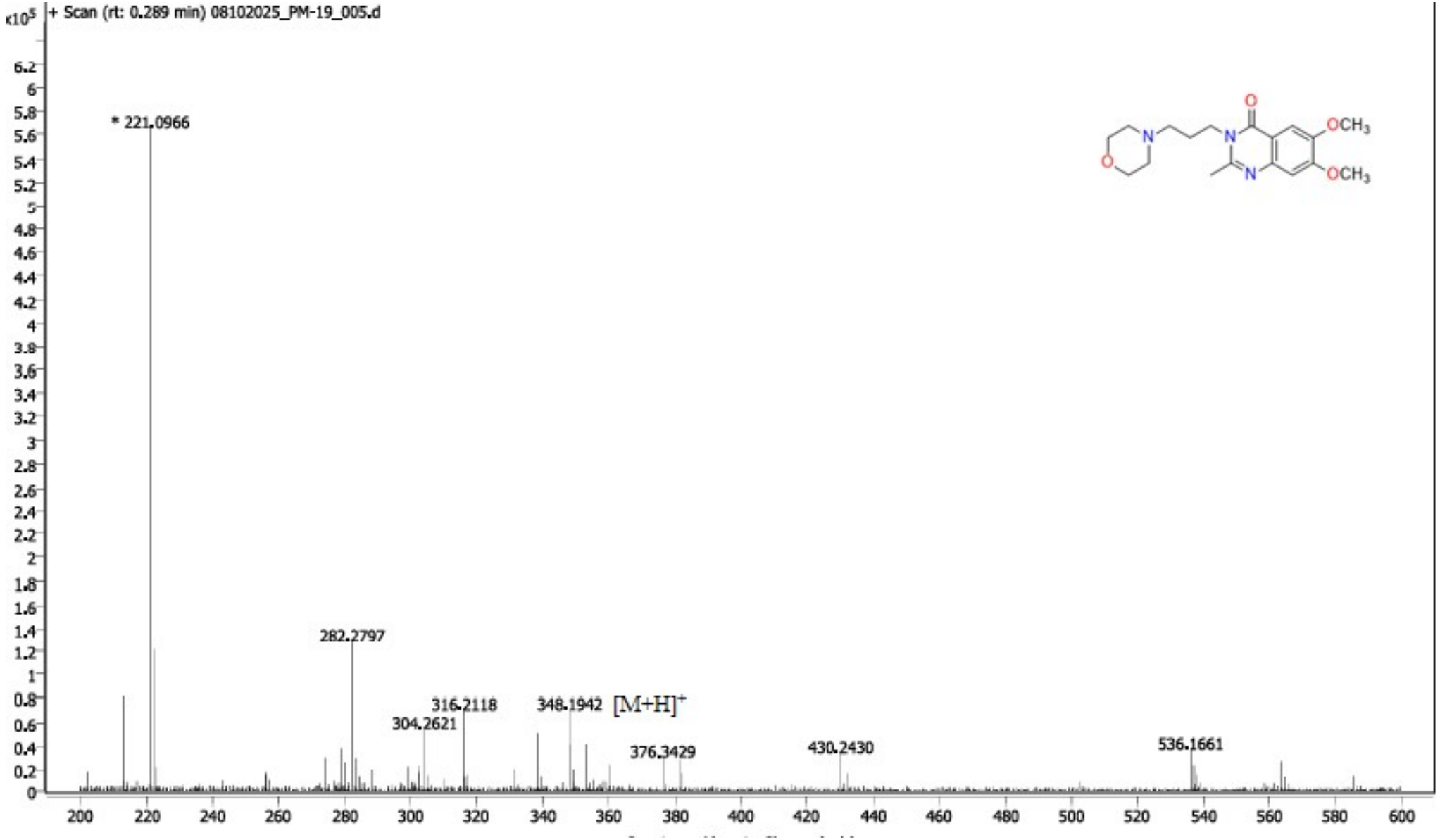
^{1}H NMR of **4i**


7.552
7.018
4.183
4.171
4.160
3.986
3.696
2.653
2.477
2.466
2.458
1.969
1.958
1.947
1.935
1.924
CDCl3
water
TMS
abundance
X : parts per Million : Proton
1.00
1.01
1.99
6.42
4.38
3.14
6.30
2.14
OCH3
OCH3

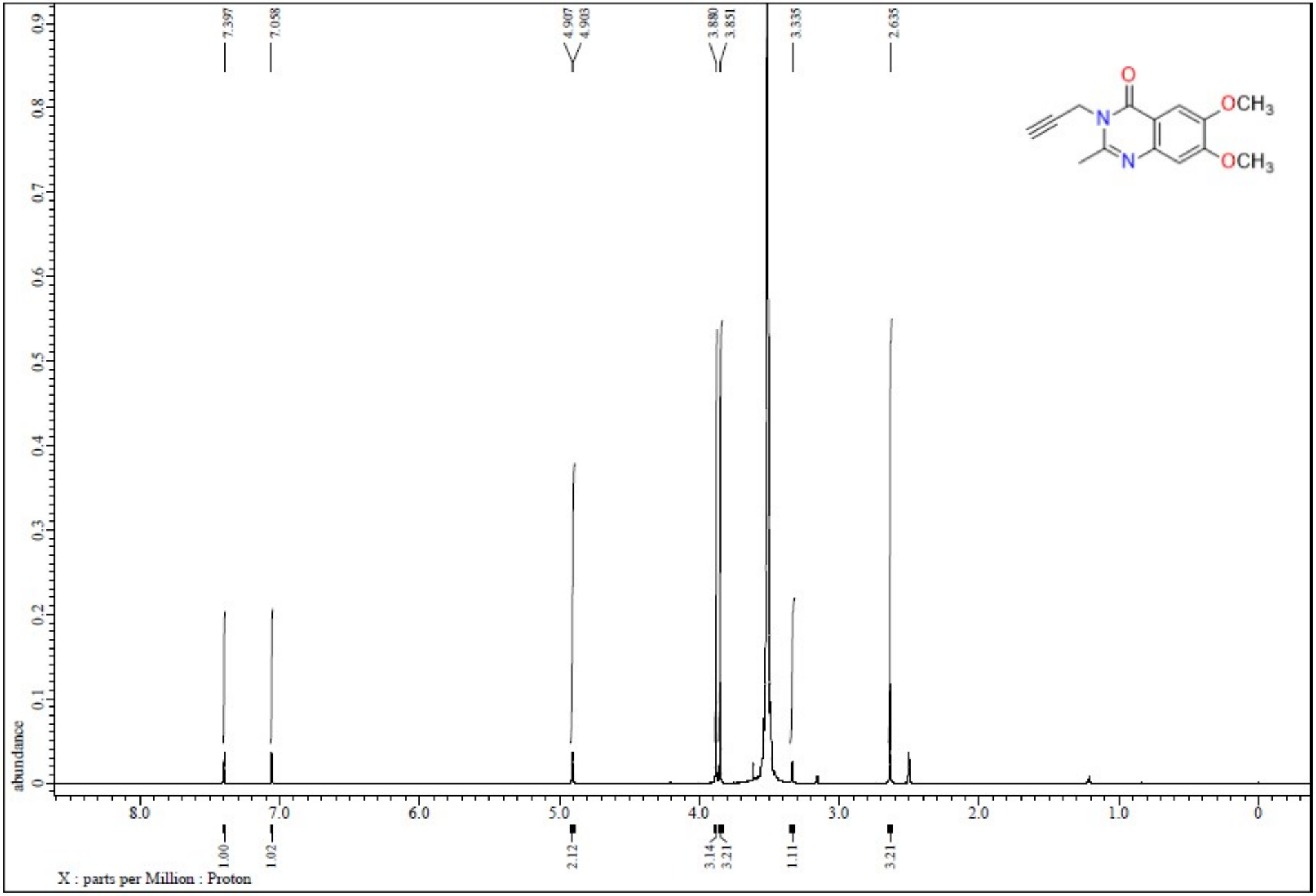
^{13}C NMR of **4i**


177.240
155.026
152.968
148.870
143.661
113.874
107.095
105.592
CDCl3
67.053
56.349
55.946
53.716
43.164
25.317
23.134
OCH3
OCH3
(thousandths)
X : parts per Million : Carbon13

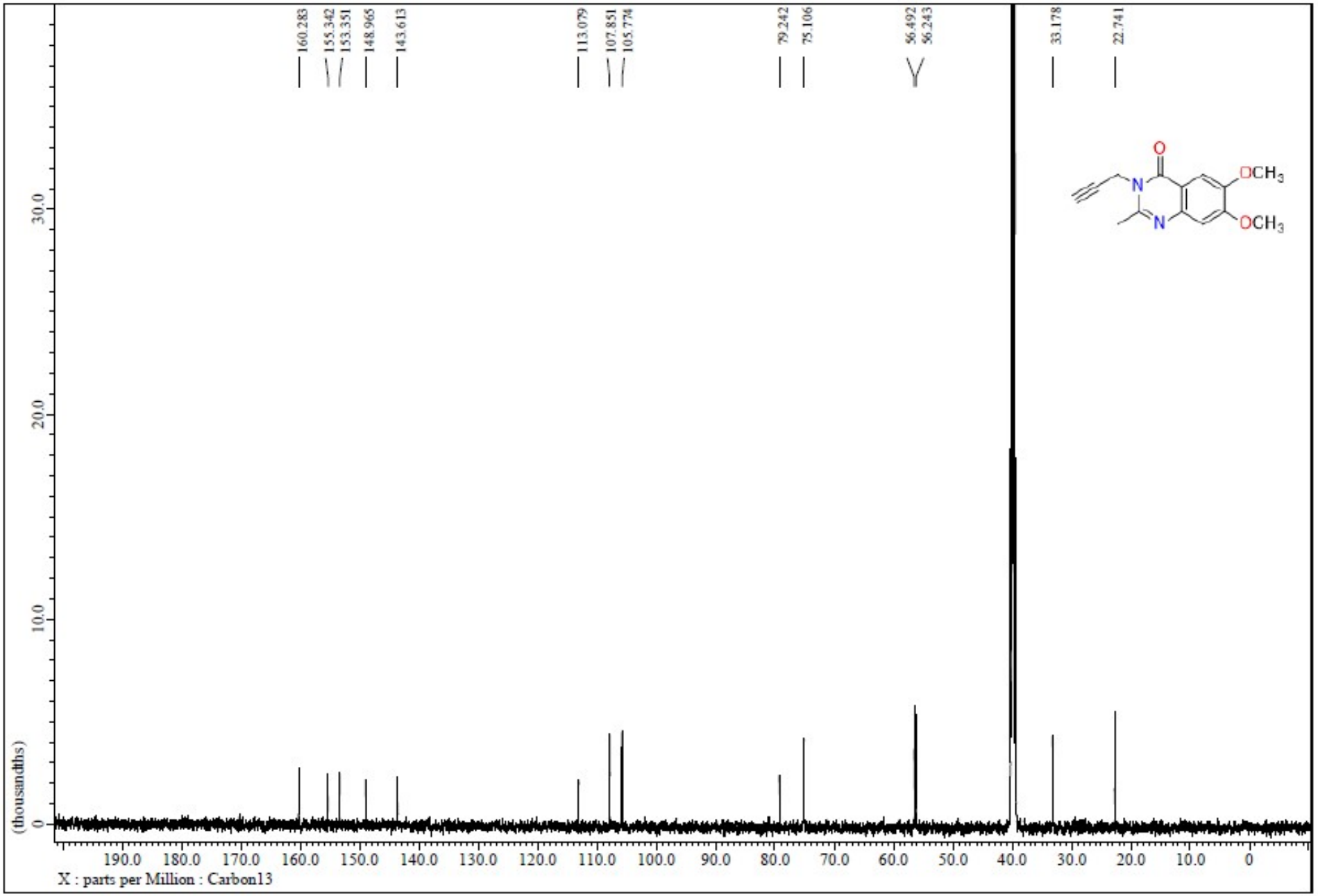
HRMS of **4i**


+ Scan (rt: 0.289 min) 08102025_PM-19_005.d
x10^5
* 221.0966
282.2797
304.2621
316.2118
348.1942
[M+H]+
376.3429
430.2430
536.1661
O
N
N
N
O
OCH3
OCH3

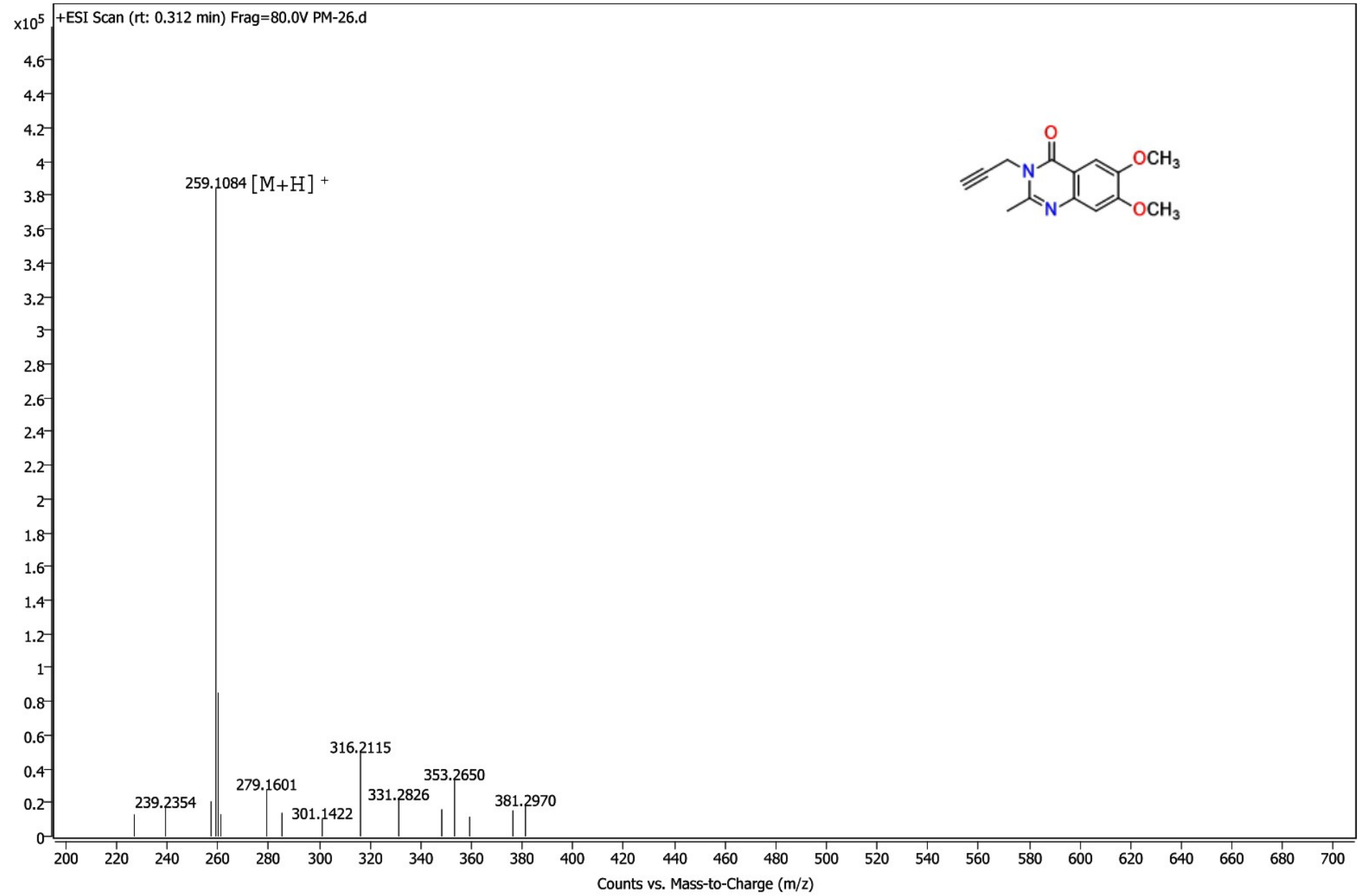
^{1}H NMR of **4j**


7.397
7.058
4.907
4.903
3.880
3.851
3.335
2.635
O
N
N
OCH3
OCH3
abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
1.00
1.02
2.12
3.14
3.21
1.11
3.21
X : parts per Million : Proton

^{13}C NMR of **4j**


160.283
155.342
153.351
148.965
143.613
113.079
107.851
105.774
79.242
75.106
56.492
56.243
33.178
22.741
O
N
N
OCH3
OCH3
30.0
20.0
10.0
0
(thousandths)
190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0
X : parts per Million : Carbon13

HRMS of 4j

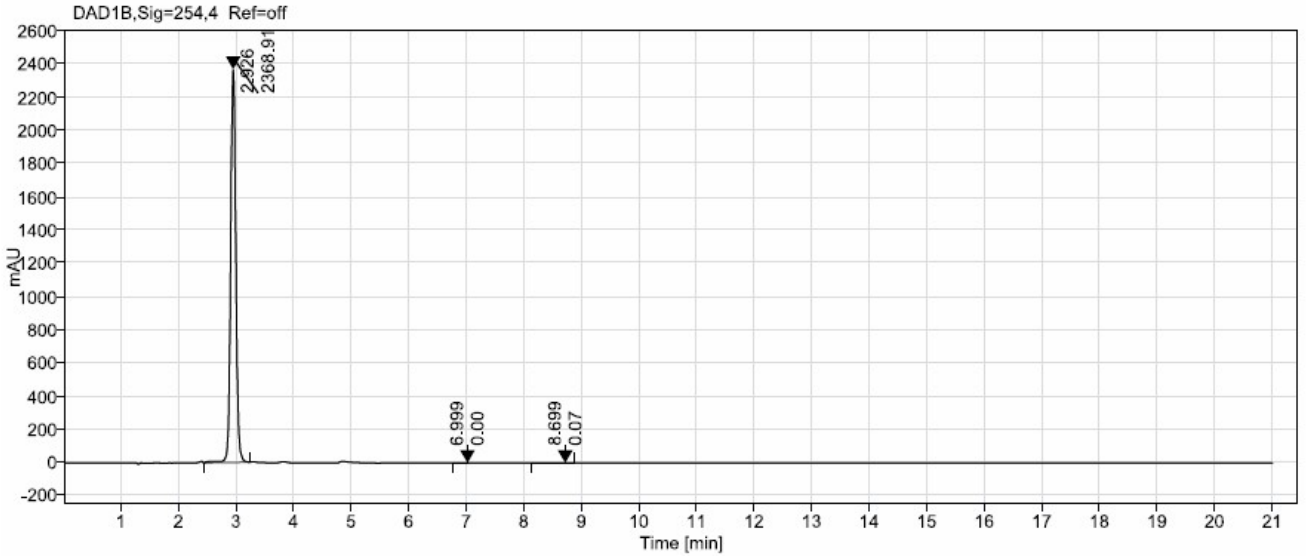

+ESI Scan (rt: 0.312 min) Frag=80.0V PM-26.d
x10^5
259.1084 [M+H]+
239.2354
279.1601
301.1422
316.2115
331.2826
353.2650
381.2970
Counts vs. Mass-to-Charge (m/z)

HPLC method and % purity of all synthesized final compounds (4a-4j)

HPLC method

An Agilent 1260 Infinity II system was used for HPLC analysis. The UV-Vis detector DAD 1260WAR, autosampler G7129A, and a Hypersil C-18 column (4.6 × 250 mm) were used in the system. The study was performed at 25–28°C using a mobile phase consisting of water (A) and acetonitrile (B) with a flow rate of 1 mL/min. The gradient program started with 40% water and 60% acetonitrile at 0.00 min followed by 30% water and 70% acetonitrile for 3.00 min, 10% water and 90% acetonitrile at 7.00 min, 0% water and 100% acetonitrile for 10.00 min. The total run time was 20 minutes. The maximum pressure limit was set to 400 bar throughout the run.

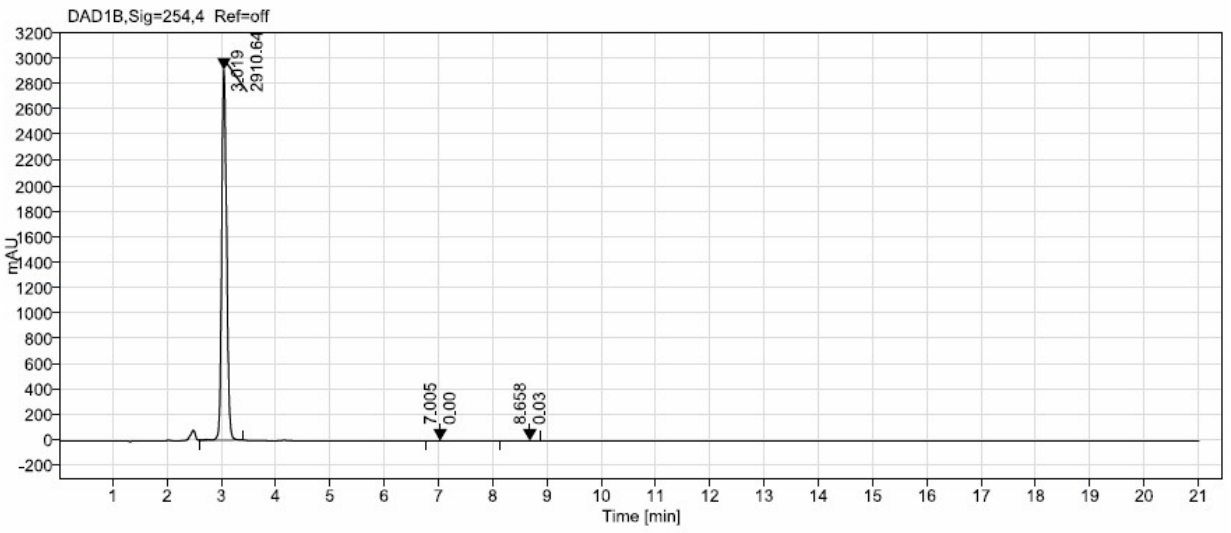
HPLC of **4a**



Signal: DAD1B,Sig=254,4 Ref=off

Name	RT [min]	Area	Area %	Height	Height %
	2.926	14698.418	100.00	2368.907	100.00
	6.999	0.156	0.00	0.000	0.00
	8.699	0.343	0.00	0.074	0.00

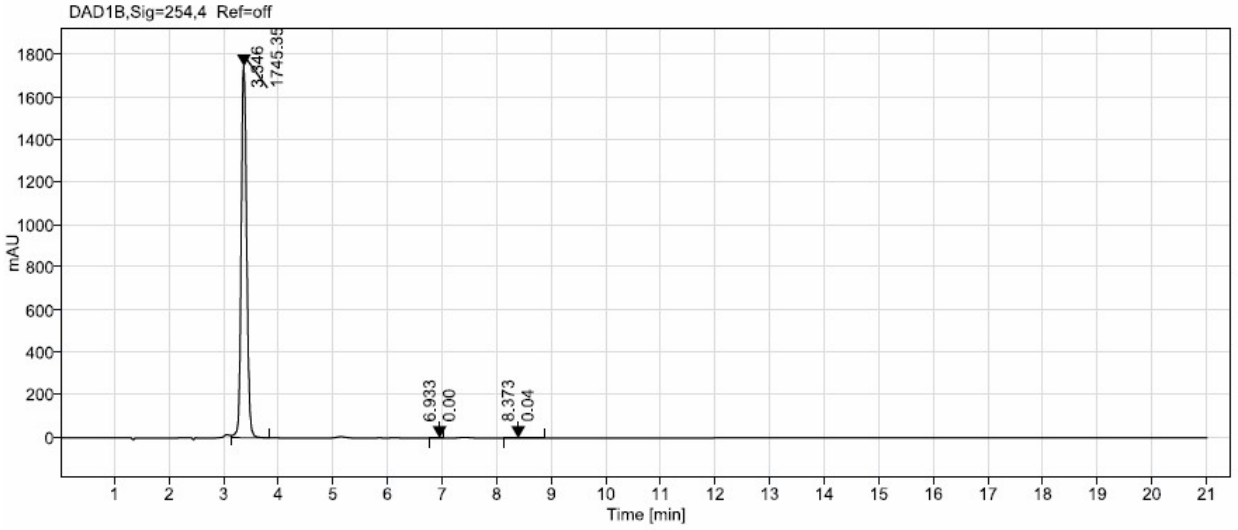
HPLC of 4b



Signal: DAD1B,Sig=254,4 Ref=off

Name	RT [min]	Area	Area %	Height	Height %
	3.019	18444.005	99.99	2910.635	100.00
	7.005	0.281	0.00	0.002	0.00
	8.658	1.060	0.01	0.026	0.00

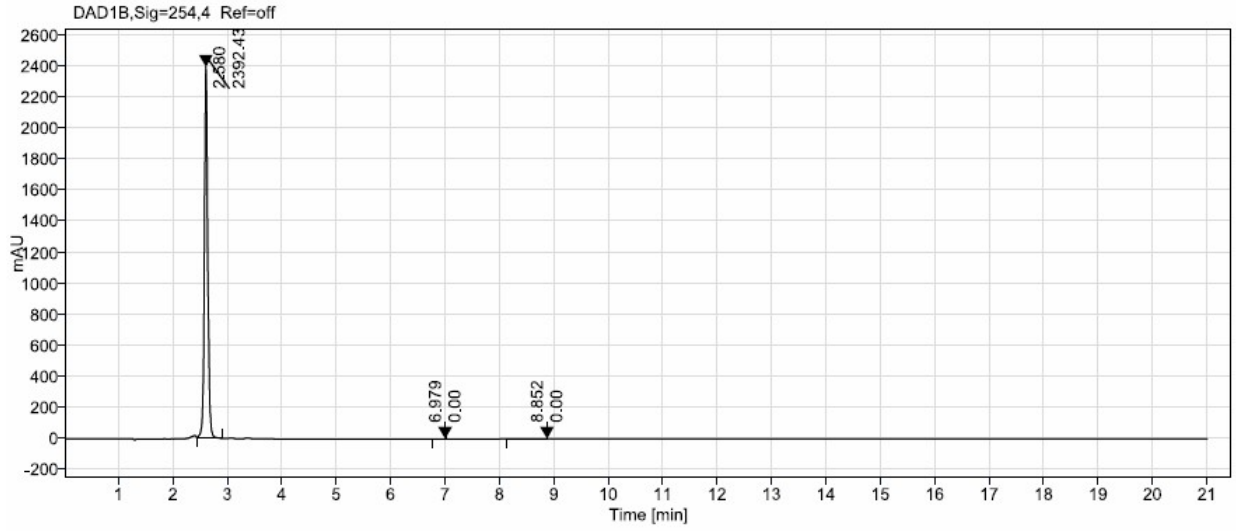
HPLC of 4c



Signal: DAD1B,Sig=254,4 Ref=off

Name	RT [min]	Area	Area %	Height	Height %
	3.346	11962.702	99.99	1745.351	100.00
	6.933	0.025	0.00	0.004	0.00
	8.373	1.006	0.01	0.045	0.00

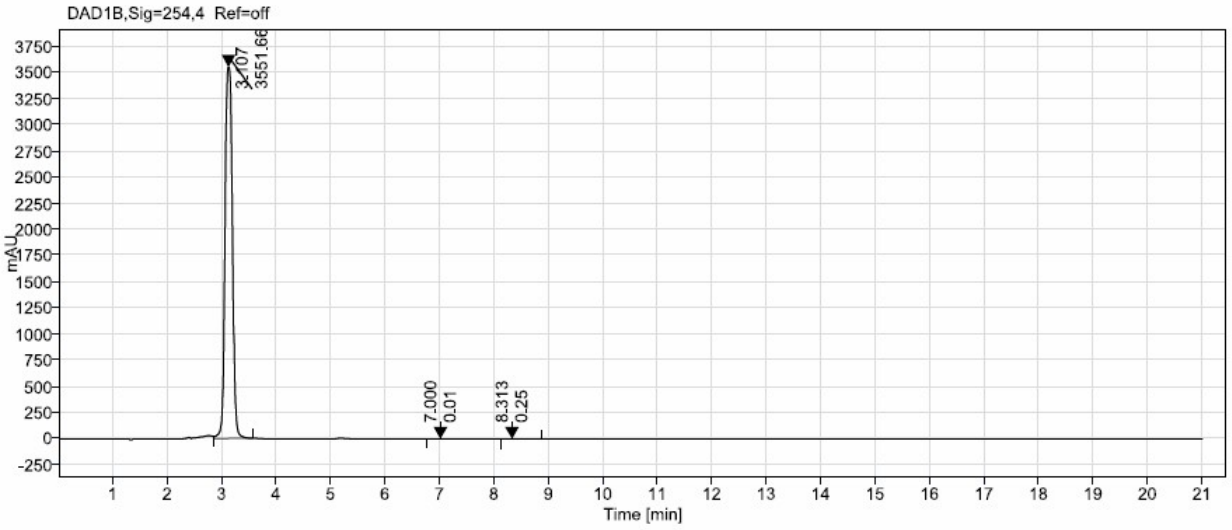
HPLC of 4d



Signal: DAD1B,Sig=254,4 Ref=off

Name	RT [min]	Area	Area %	Height	Height %
	2.580	10408.209	99.99	2392.427	100.00
	6.979	0.019	0.00	0.003	0.00
	8.852	0.528	0.01	0.000	0.00

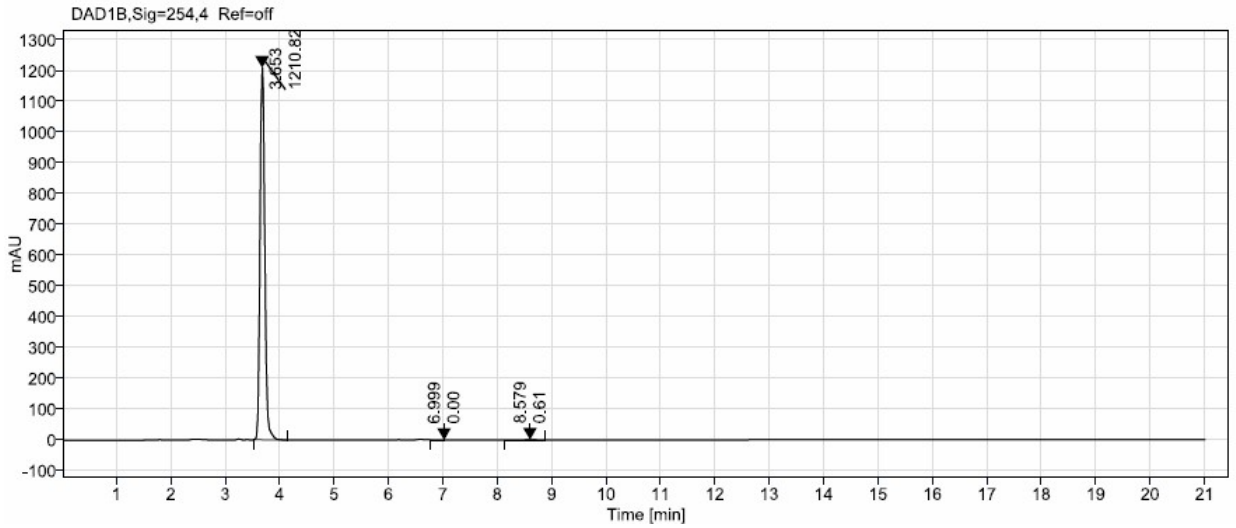
HPLC of 4e



Signal: DAD1B,Sig=254,4 Ref=off

Name	RT [min]	Area	Area %	Height	Height %
	3.107	33000.670	99.98	3551.657	99.99
	7.000	1.468	0.00	0.008	0.00
	8.313	4.443	0.01	0.251	0.01

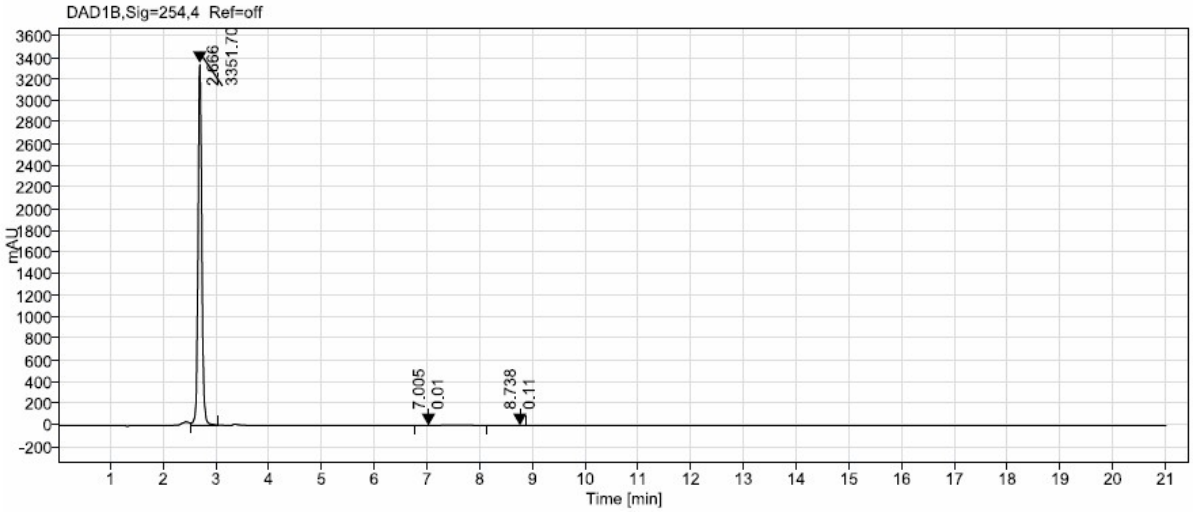
HPLC of 4f



Signal: DAD1B,Sig=254,4 Ref=off

Name	RT [min]	Area	Area %	Height	Height %
	3,653	7314,850	99,91	1210,824	99,95
	6,999	0,539	0,01	0,000	0,00
	8,579	5,938	0,08	0,606	0,05

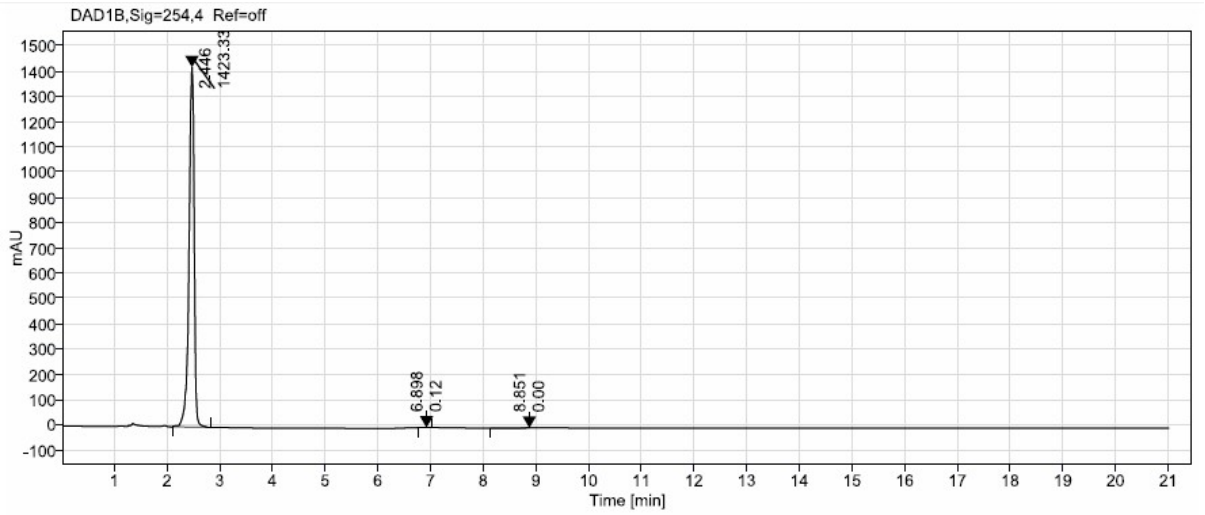
HPLC of 4g



Signal: DAD1B,Sig=254,4 Ref=off

Name	RT [min]	Area	Area %	Height	Height %
	2,666	17057,110	99,96	3351,697	100,00
	7,005	0,439	0,00	0,012	0,00
	8,738	6,617	0,04	0,109	0,00

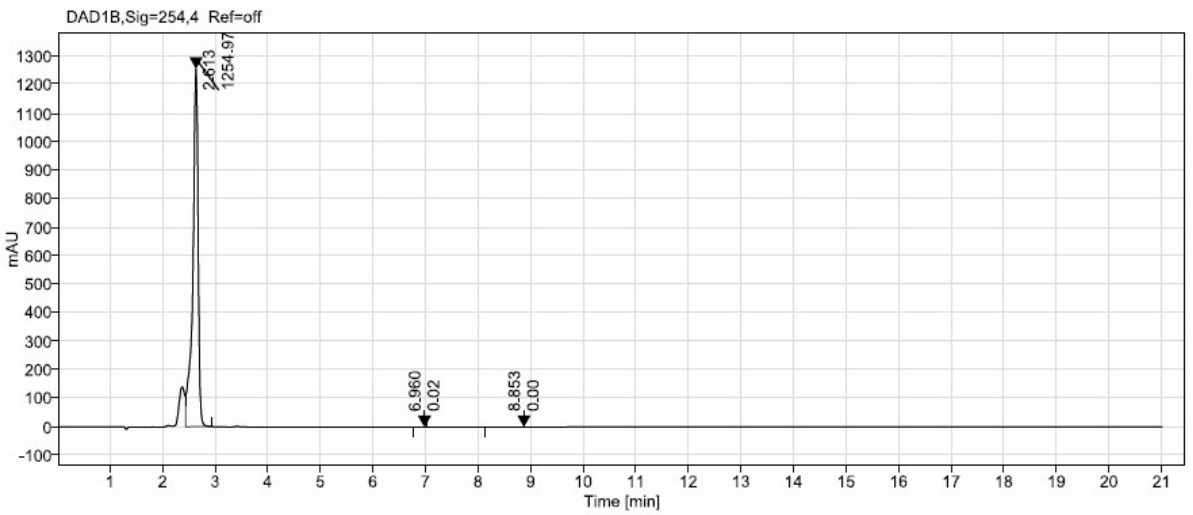
HPLC of 4i



Signal: DAD1B,Sig=254,4 Ref=off

Name	RT [min]	Area	Area %	Height	Height %
	2.446	9603.727	99.98	1423.329	99.99
	6.898	1.278	0.01	0.118	0.01
	8.851	1.057	0.01	0.000	0.00

HPLC of 4j



Signal: DAD1B,Sig=254,4 Ref=off

Name	RT [min]	Area	Area %	Height	Height %
	2.613	8722.115	99.99	1254.972	100.00
	6.960	0.288	0.00	0.021	0.00
	8.853	0.349	0.00	0.000	0.00