Design, synthesis, and biological evaluation of new thiazolo[5,4-d]pyrimidine derivatives as potent antiproliferative agents

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Contents

1. Experiment

1.1 MTT assay: Exponentially growing cells were seeded into 96-well plates at a concentration of 3,000 cells per well. After 24 h of incubation, the culture medium was removed and fresh medium containing various concentrations of the candidate compounds was added to each well. The cells were then incubated for 72 h, thereafter MTT assays were performed and cell viability was assessed at 570 nm by a microplate reader (Biotech, Shanghai, China).

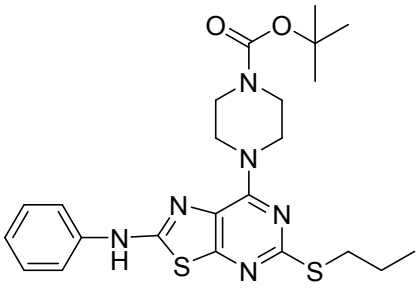
1.2 General procedure for the synthesis of compounds 7a-j

The intermediate derivatives **5a~b** were synthesized following the previously reported procedure.[1] Then the isothiocyanate analogs reacted with **5a~b** under alkaline conditions (cesium carbonate) in acetonitrile to give the key active intermediates **6a~f.**[2] The mixture of **6** (1 eq), appropriate amine (1.1 eq) and TEA (2 eq) in isopropanol was refluxed for 6 h and monitored by TLC (PE/EA = 4:1 ~ 1:1). After the completion of the reaction, the reaction mixture was cooled to room temperature and diluted with ethyl acetate, then washed with water for three times. The organic phase was dried with anhydride sodium sulfate and concentrated under reduced

vacuum. The residue was purified by flash column chromatography (PE/EA = 4:1 ~ 1:1) to give the target products.

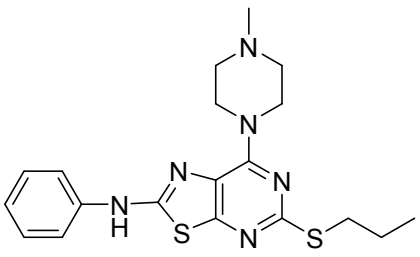
2. Characterization data of compounds

Compound 7a



White solid, yield 61 %, Mp 188~189 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (s, 1H), 7.59-7.61 (m, 2H), 7.35-7.39 (m, 2H), 7.01-7.05 (m, 1H), 4.14 (br, 4H), 3.48-3.50 (t, J = 5.2 Hz, 4H), 3.02-3.05 (t, J = 7.1 Hz, 2H), 1.66-1.71 (m, 2H), 1.44 (s, 9H), 0.97-1.00 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.87, 161.09, 154.51, 153.89, 151.35, 140.19, 129.05, 125.40, 122.21, 117.62, 79.12, 45.12, 32.32, 28.03, 22.47, 13.27. HR-MS (ESI): Calcd. $C_{23}H_{30}N_6O_2S_2$, $[M+H]^+$m/z: 487.1950, found: 487.1945.

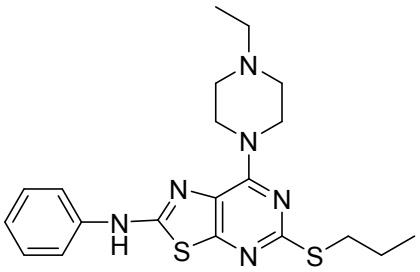
Compound 7b



White solid, yield 52 %, Mp 170~172 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (s, 1H), 7.62-7.64 (m, 2H), 7.29-7.33 (m, 2H), 6.97-7.00 (m, 1H), 4.16 (br, 4H), 2.99-3.03 (t, J = 7.2 Hz, 2H), 2.44-2.46 (t, J = 5.1 Hz, 4H), 2.22 (s, 3H), 1.65-1.70 (m, 2H), 0.96-0.99 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.71, 161.07, 154.32, 151.36, 140.37, 128.85, 125.25, 121.93, 117.52, 54.55, 45.67, 45.23, 32.32,

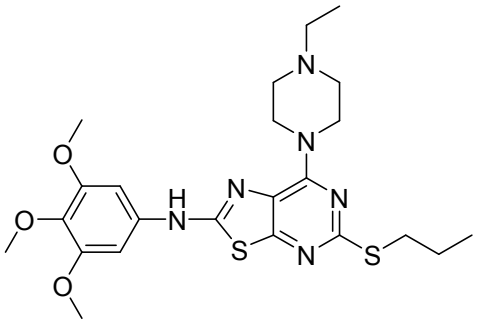
22.54, 13.26. HR-MS (ESI): Calcd. $C_{19}H_{24}N_6S_2$, $[M+H]^+$m/z: 401.1582, found: 401.1581.

Compound 7c



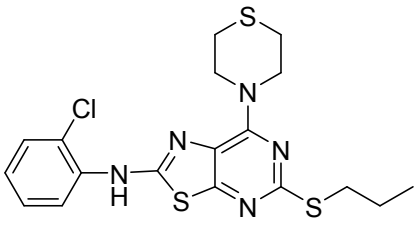
Gray solid, yield 55 %, Mp 179~180 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.51 (s, 1H), 7.59-7.61 (m, 2H), 7.34-7.38 (m, 2H), 7.00-7.04 (m, 1H), 4.16 (br, 4H), 3.01-3.04 (t, J = 7.2, 2H), 2.49-2.51 (t, J = 5.0 Hz, 4H), 2.34-2.39 (m, 2H), 1.63-1.72 (m, 2H), 1.02-1.06 (t, J = 7.1 Hz, 3H), 0.96-1.00 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.85, 160.94, 154.26, 151.35, 140.24, 129.04, 125.23, 122.12, 117.56, 52.34, 51.54, 45.33, 32.30, 22.52, 13.27, 11.88. HR-MS (ESI): Calcd. $C_{20}H_{26}N_6S_2$, $[M+H]^+$m/z: 415.1739, found: 415.1736.

Compound 7d



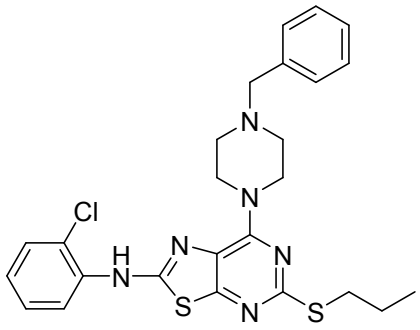
White solid, yield 58 %, Mp 158~160 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.45 (s, 1H), 6.94 (s, 2H), 4.16 (m, 4H), 3.78 (s, 6H), 3.63 (s, 3H), 3.00-3.04 (t, J = 7.2 Hz, 2H), 2.44-2.46 (t, J = 5.0 Hz, 4H), 2.33-2.38 (m, 2H), 1.65-1.70 (m, 2H), 1.01-1.04 (t, J = 7.1 Hz, 3H), 0.96-1.00 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.26, 161.44, 154.69, 153.48, 151.81, 136.82, 133.05, 125.82, 95.65, 60.61, 56.05, 52.72, 52.00, 45.67, 32.79, 23.02, 13.77, 12.28. HR-MS (ESI): Calcd. $C_{23}H_{32}N_6O_3S_2$, $[M+H]^+$m/z: 505.2056, found: 505.2057.

Compound 7e



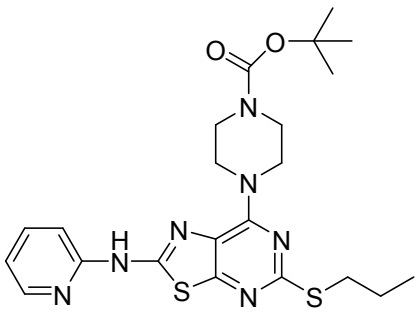
White solid, yield 75 %, Mp 188~190 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 7.97 (m, 1H), 7.33-7.40 (m, 2H), 7.06 (m, 1H), 4.44 (m, 4H), 3.01-3.05 (t, J = 7.2 Hz, 2H), 2.76-2.79 (t, J = 4.9 Hz, 4H), 1.66-1.71 (m, 2H), 0.97-1.00 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.82, 160.94, 154.37, 151.74, 141.96, 133.92, 131.14, 125.75, 122.10, 117.28, 116.51, 48.70, 32.83, 27.00, 23.02, 13.79. HR-MS (ESI): Calcd. $C_{18}H_{20}ClN_5S_3$, $[M+H]^+$m/z: 438.0648, found: 438.0647.

Compound 7f



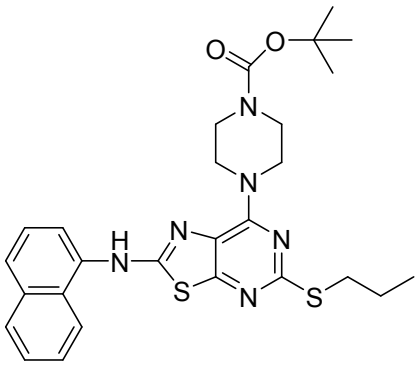
Pink solid, yield 62 %, Mp 180~182 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (s, 1H), 8.03 (m, 1H), 7.31-7.35 (m, 5H), 7.24-7.29 (m, 2H), 7.01-7.04 (m, 1H), 4.15 (m, 4H), 3.52 (s, 2H), 3.34 (s, 2H), 3.01 (t, J = 7.2 Hz, 2H), 2.51-2.53 (m, 4H), 1.64-1.69 (m, 2H), 0.95-0.98 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.80, 161.59, 154.29, 151.96, 141.99, 138.21, 133.87, 131.05, 129.51, 128.63, 127.50, 125.43, 122.00, 117.38, 116.53, 62.42, 53.05, 45.82, 32.80, 22.98, 13.76. HR-MS (ESI): Calcd. $C_{25}H_{27}ClN_6S_2$, $[M+H]^+$m/z: 511.1505, found: 511.1505.

Compound 7g



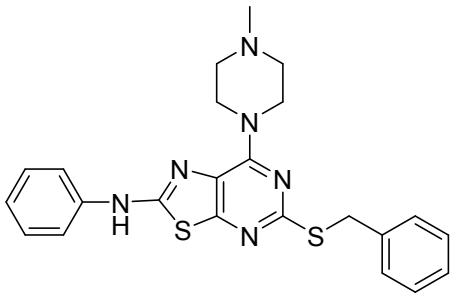
Pale yellow solid, yield 74 %, Mp 176~177 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.48 (s, 1H), 8.32-8.34 (m, 1H), 7.74-7.78 (m, 1H), 7.11-7.14 (m, 1H), 6.99-7.02 (m, 1H), 4.19 (m, 4H), 3.46-3.49 (t, J = 5.3 Hz, 4H), 3.04-3.07 (t, J = 7.0 Hz, 2H), 1.67-1.72 (m, 2H), 0.97-1.01 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.88, 161.79, 153.88, 151.93, 151.69, 151.15, 146.36, 138.37, 123.54, 116.91, 111.05, 79.13, 44.96, 32.32, 28.02, 22.56, 13.28. HR-MS (ESI): Calcd. $C_{22}H_{29}N_7O_2S_2$, $[M+H]^+$m/z: 488.1902, found: 488.1905.

Compound 7h



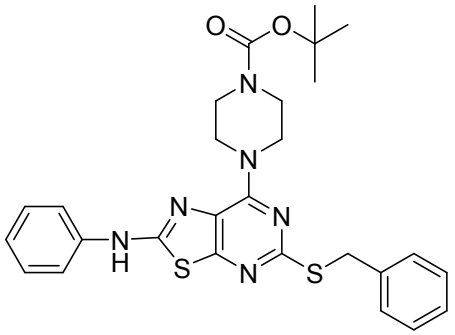
Pale yellow solid, yield 82 %, Mp 175~177 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.43 (s, 1H), 8.22-8.25 (m, 1H), 8.05-8.08 (m, 1H), 7.96-7.99 (m, 1H), 7.74-7.76 (m, 1H), 7.52-7.59 (m, 3H), 4.09 (m, 4H), 3.41-3.44 (t, J = 5.2 Hz, 4H), 3.02-3.05 (t, J = 7.0 Hz, 2H), 1.65-1.71 (m, 2H), 1.43 (s, 9H), 0.96-1.00 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.76, 161.34, 156.89, 153.86, 151.32, 135.51, 133.94, 128.30, 126.40, 126.21, 125.87, 125.82, 125.24, 124.37, 122.22, 117.93, 79.11, 54.86, 45.06, 32.31, 28.03, 22.49, 13.26. HR-MS (ESI): Calcd. $C_{27}H_{32}N_6O_2S_2$, $[M+H]^+$m/z: 537.2106, found: 537.2105.

Compound 7i



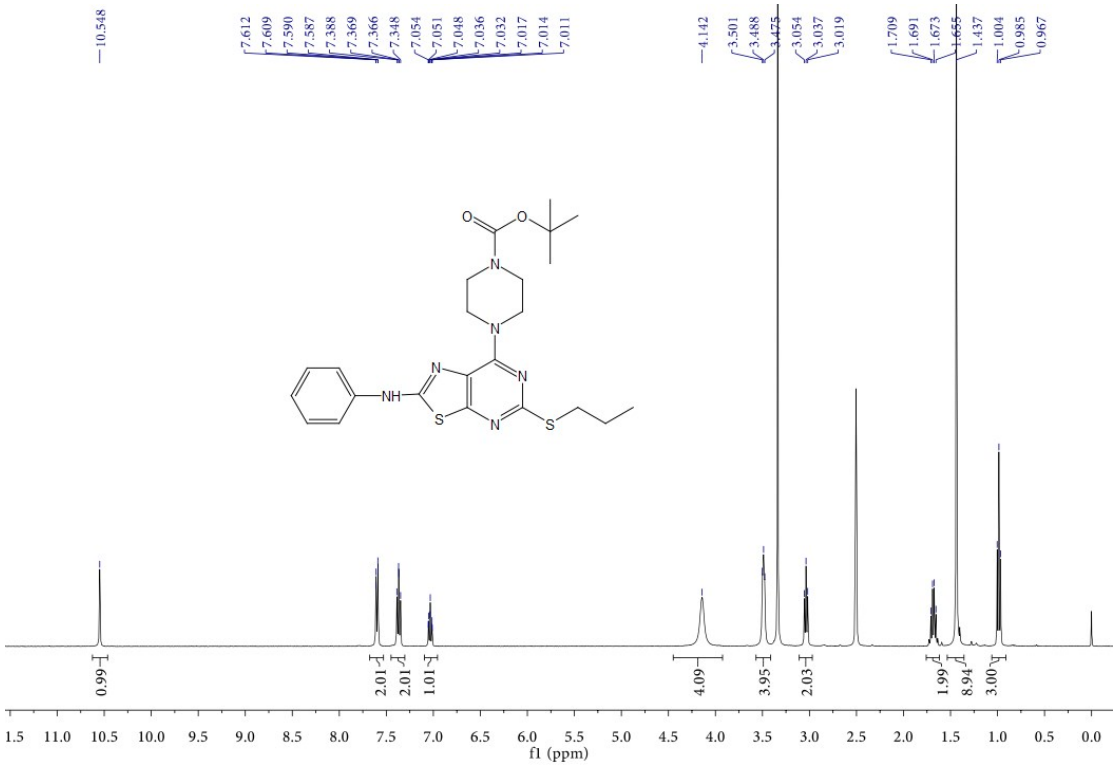
Pink solid, yield 59 %, Mp 175~177 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.59 (s, 1H), 7.59-7.61 (m, 2H), 7.41-7.43 (m, 2H), 7.29-7.37 (m, 4H), 7.22-7.25 (m, 1H), 7.00-7.04 (m, 1H), 4.36 (s, 2H), 4.16 (m, 4H), 2.42-2.45 (t, J = 5.0 Hz, 4H), 2.22 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.79, 161.32, 154.97, 151.83, 140.69, 138.73, 129.55, 129.19, 128.82, 127.36, 125.89, 122.70, 118.10, 54.99, 46.07, 45.72, 35.03. HR-MS (ESI): Calcd. $C_{23}H_{24}N_6S_2$, $[M+H]^+$m/z: 449.1582, found: 449.1580.

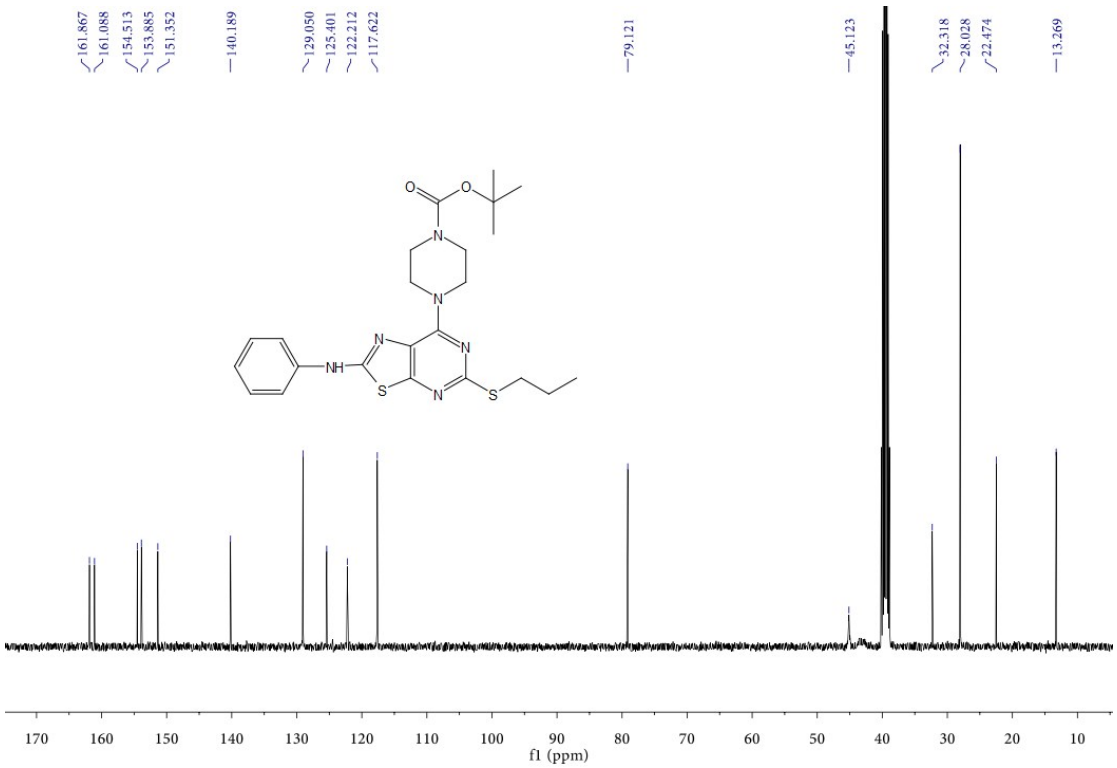
Compound 7j

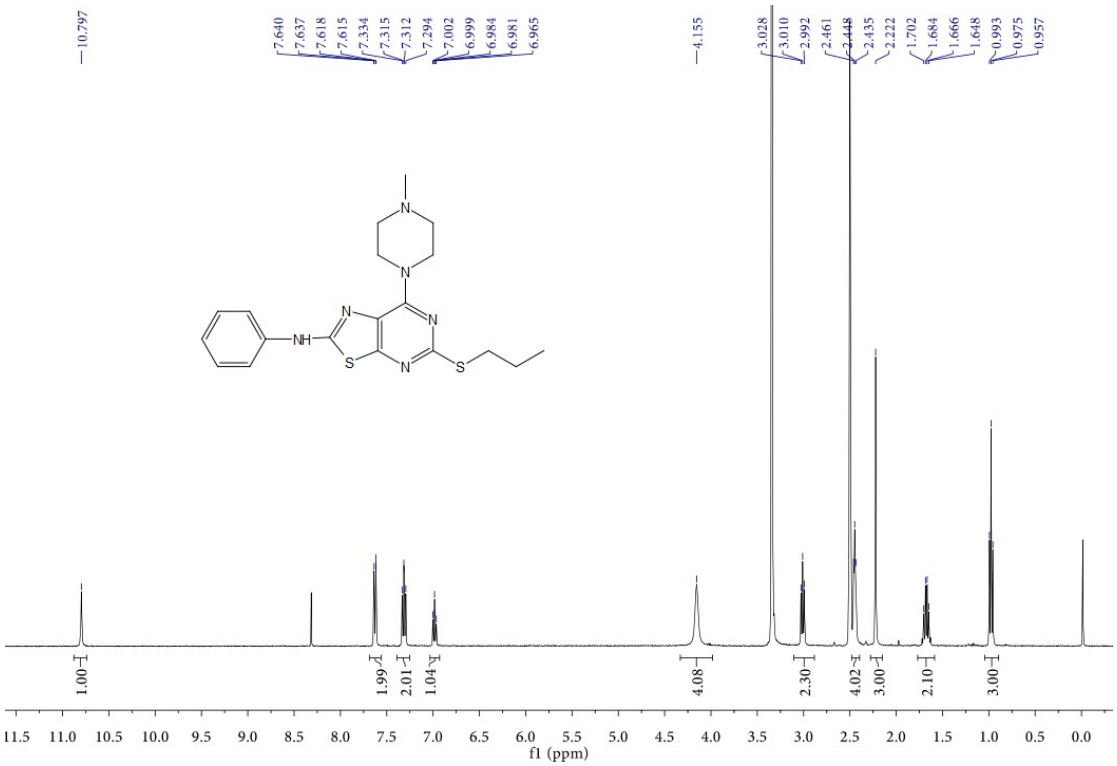


Pale yellow solid, yield 68 %, Mp 130~133 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 7.59-7.61 (m, 2H), 7.41-7.43 (m, 2H), 7.35-7.39 (m, 2H), 7.30-7.33 (m, 2H), 7.22-7.26 (m, 1H), 7.02-7.06 (m, 1H), 4.37 (s, 2H), 4.14 (m, 4H), 3.45-3.47 (t, J = 5.2 Hz, 4H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.36, 160.95, 154.69, 153.88, 151.34, 140.14, 138.18, 129.10, 128.70, 128.34, 126.89, 125.55, 122.32, 117.68, 79.13, 45.19, 34.57, 28.03. HR-MS (ESI): Calcd. $C_{27}H_{30}N_6O_2S_2$, $[M+H]^+$m/z: 535.1950, found: 535.1953.

3. ^{1}H NMR and ^{13}C NMR spectra



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7.312
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7.002
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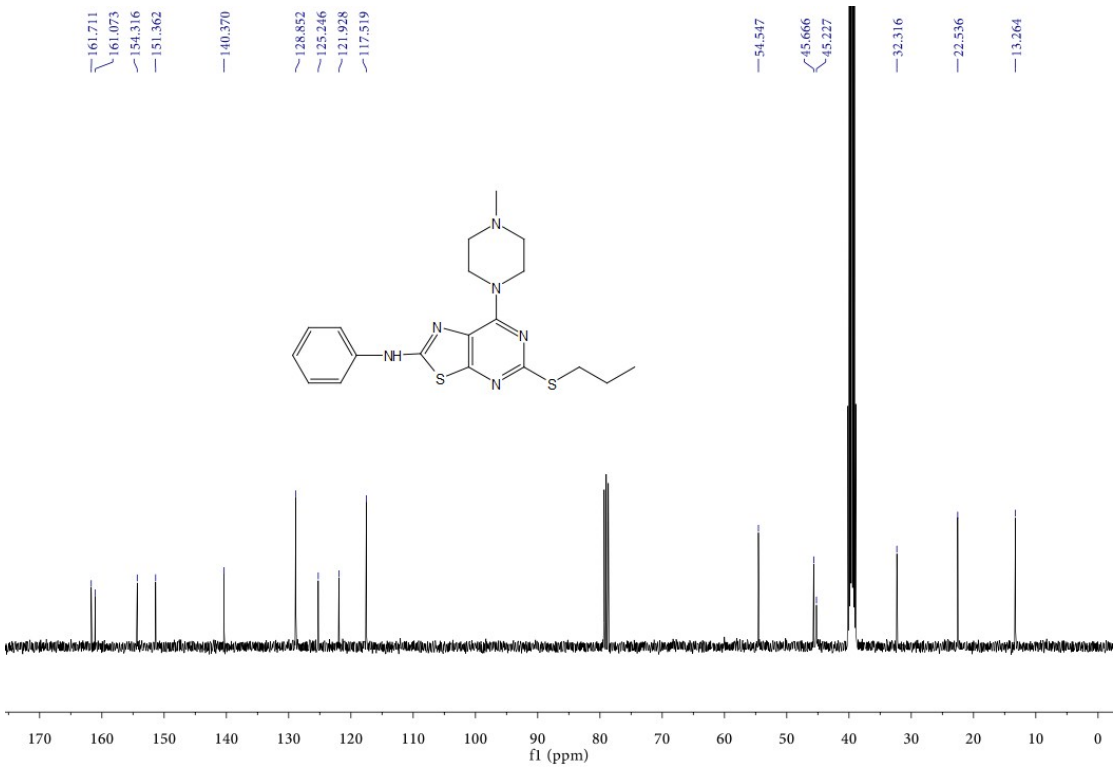
6.981
6.965
4.155
3.028
3.010
2.992
2.461
2.448
2.435
2.222
1.702
1.684
1.666
1.648
0.993
0.975
0.957
1.00
1.99
2.01
1.04
4.08
2.30
4.02
3.00
2.10
3.00
f1 (ppm)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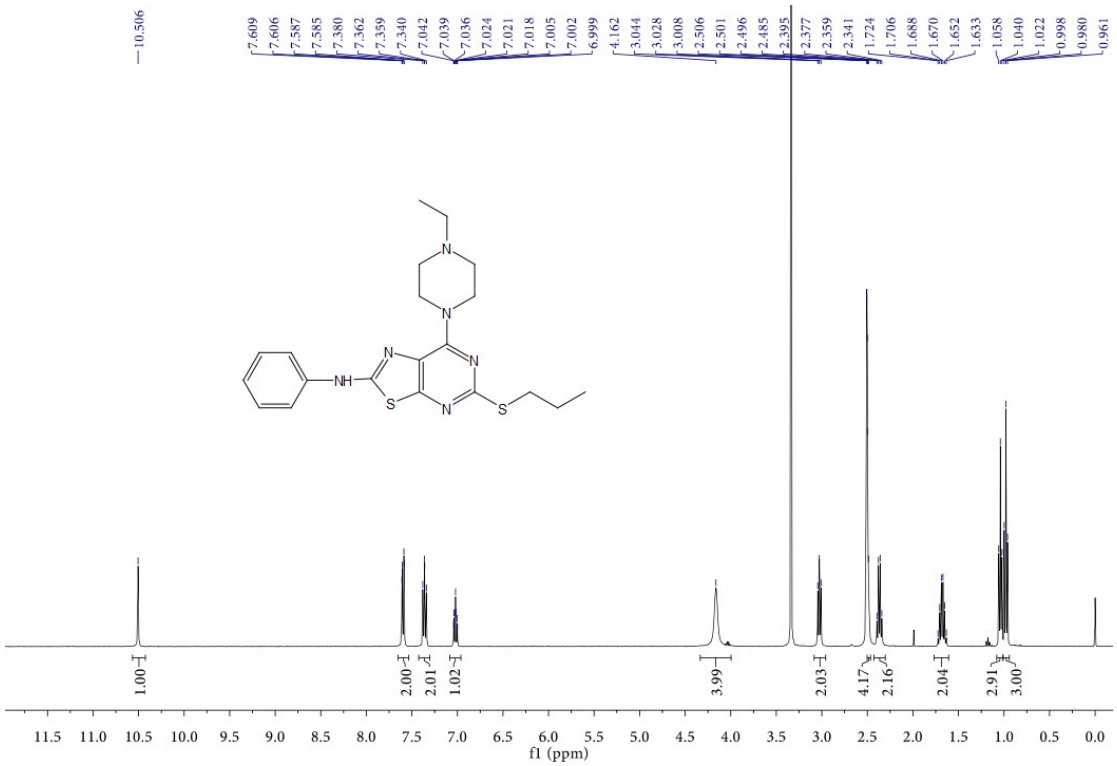
22.536
13.264
f1 (ppm)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7.002
6.999
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2.359
2.341
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1.706
1.688
1.670
1.652
1.633
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1.040
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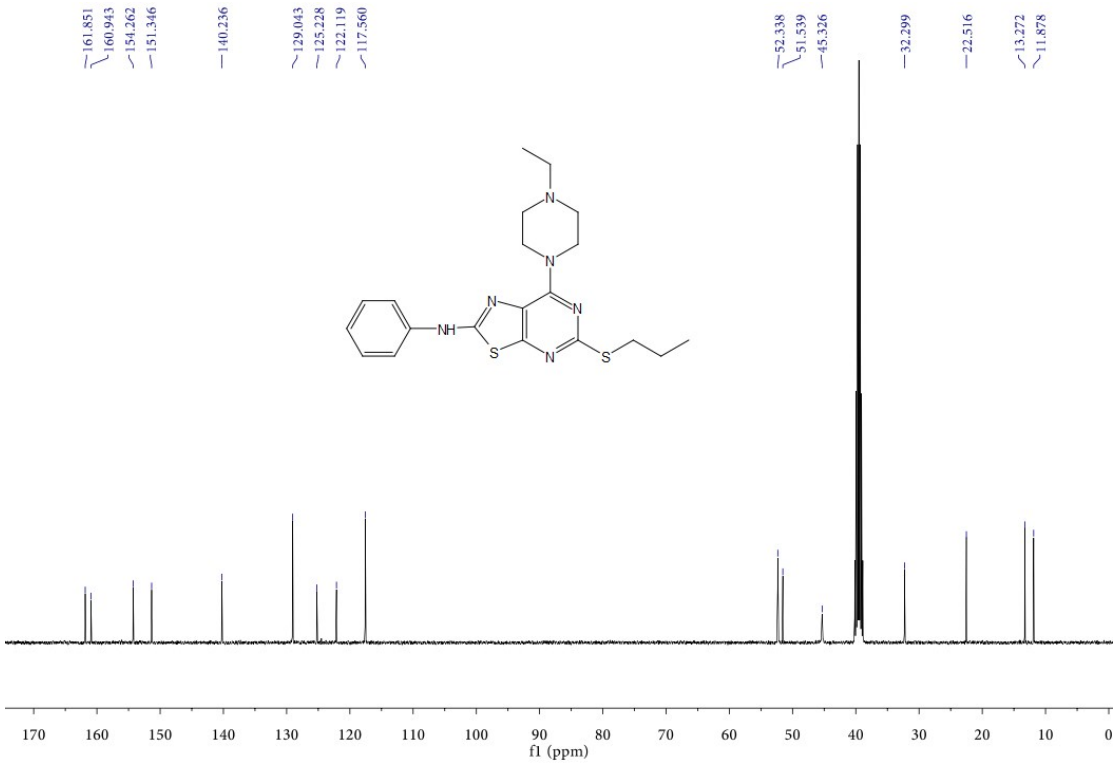
0.980
0.961
1.00
2.00
2.01
1.02
3.99
2.03
4.17
2.16
2.04
2.91
3.00
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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122.119
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52.338
51.539
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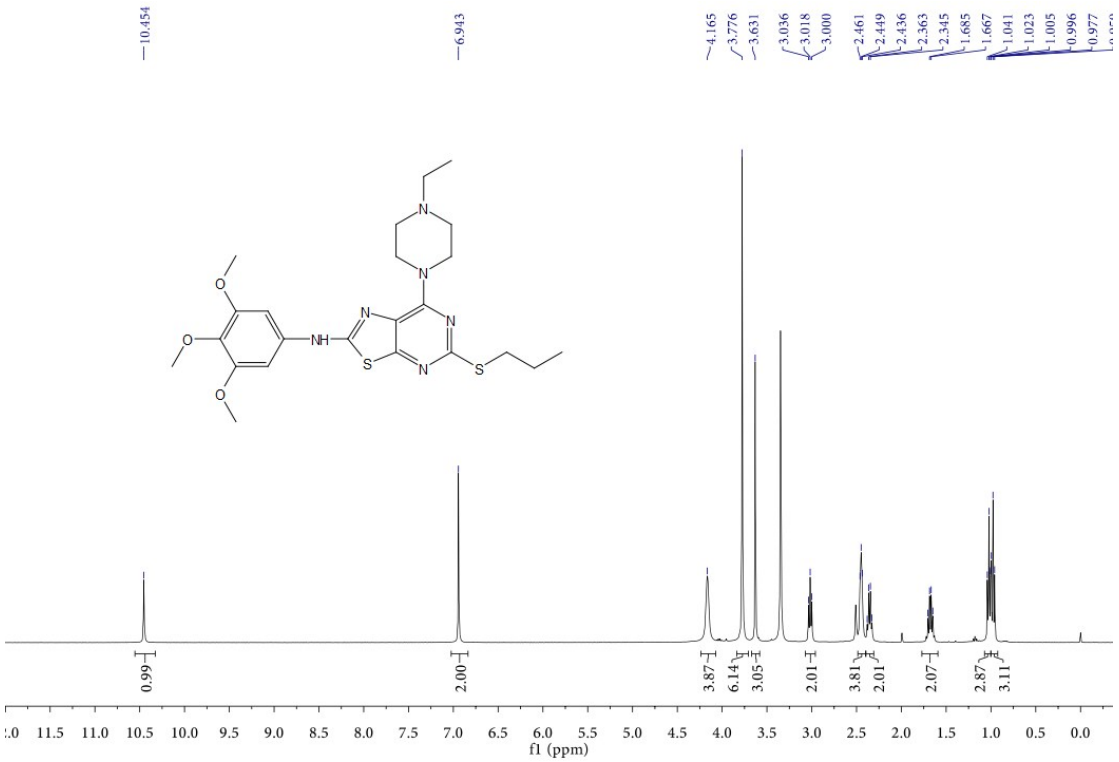
32.299
22.516
13.272
11.878
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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1.685
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0.996
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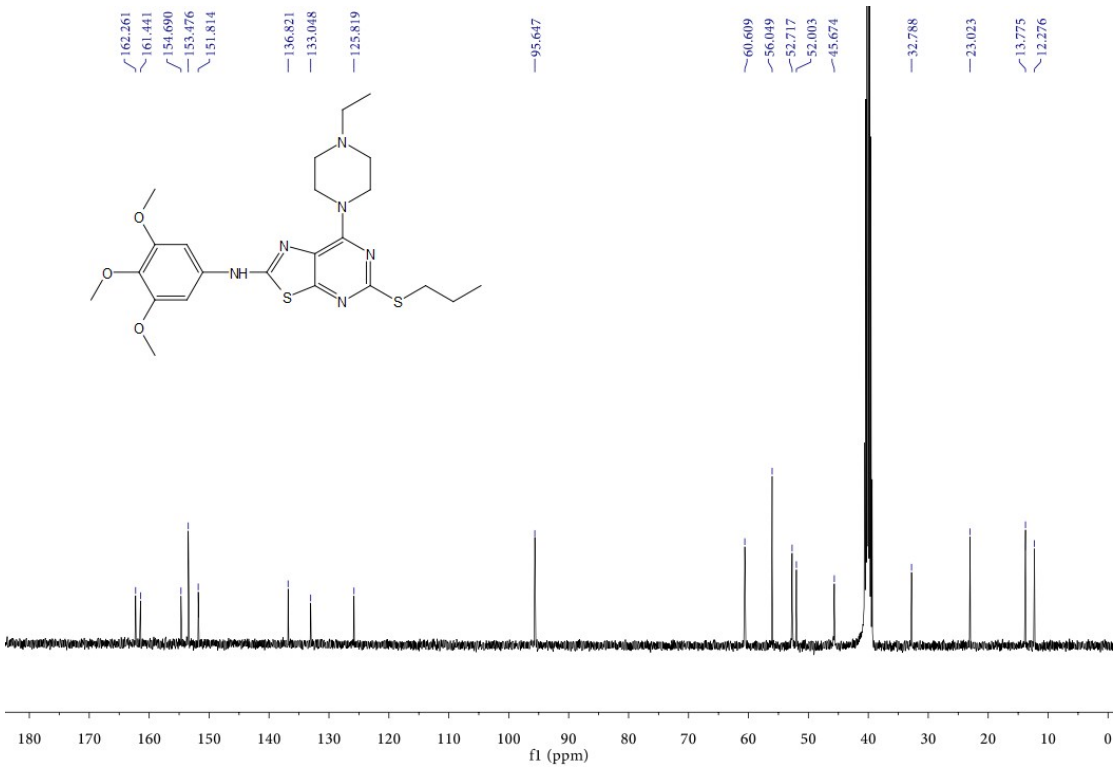
3.11
f1 (ppm)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136.821
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125.819
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52.003
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23.023
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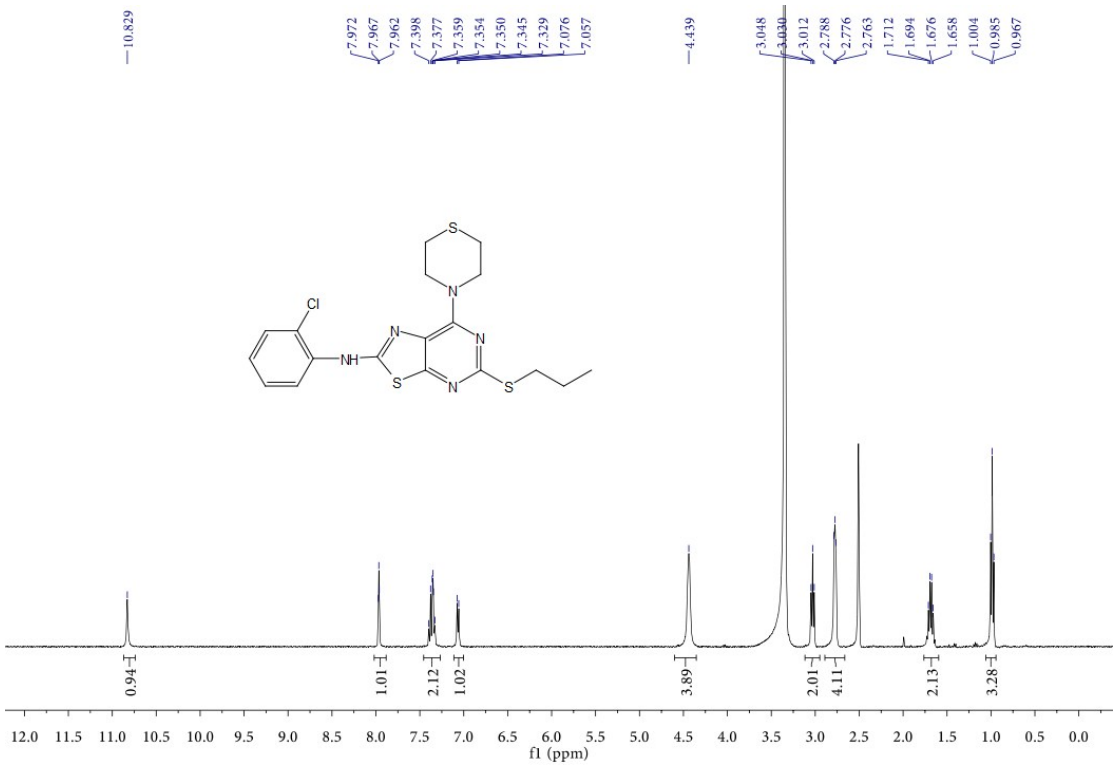
12.276
f1 (ppm)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7.962
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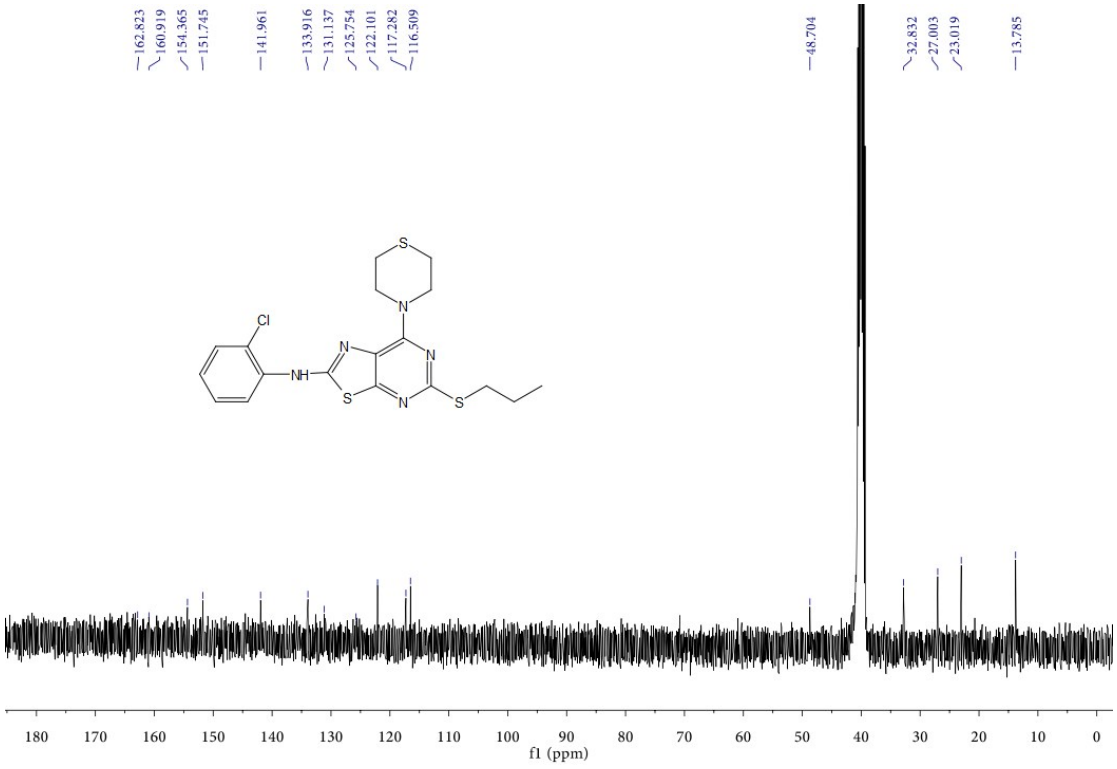
7.377
7.359
7.354
7.350
7.345
7.329
7.076
7.057
4.439
3.048
3.030
3.012
2.788
2.776
2.763
1.712
1.694
1.676
1.658
1.004
0.985
0.967
0.94
1.01
2.12
1.02
3.89
2.01
4.11
2.13
3.28
f1 (ppm)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27.003
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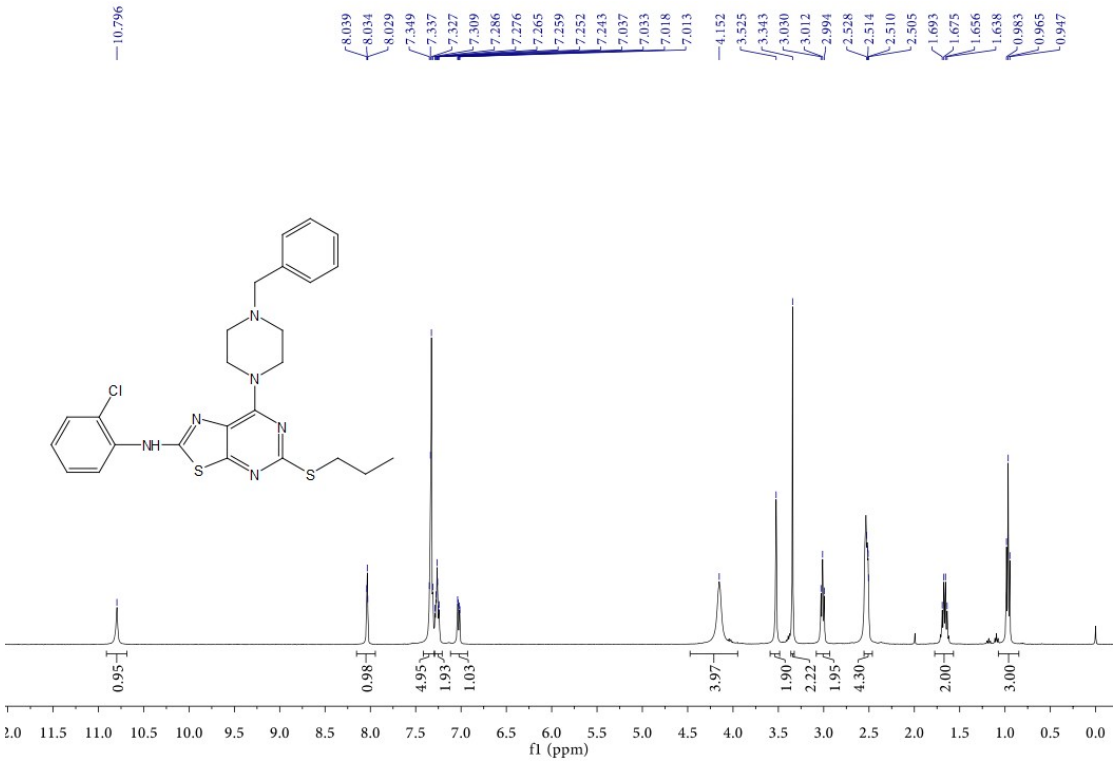
13.785
f1 (ppm)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7.037
7.033
7.018
7.013
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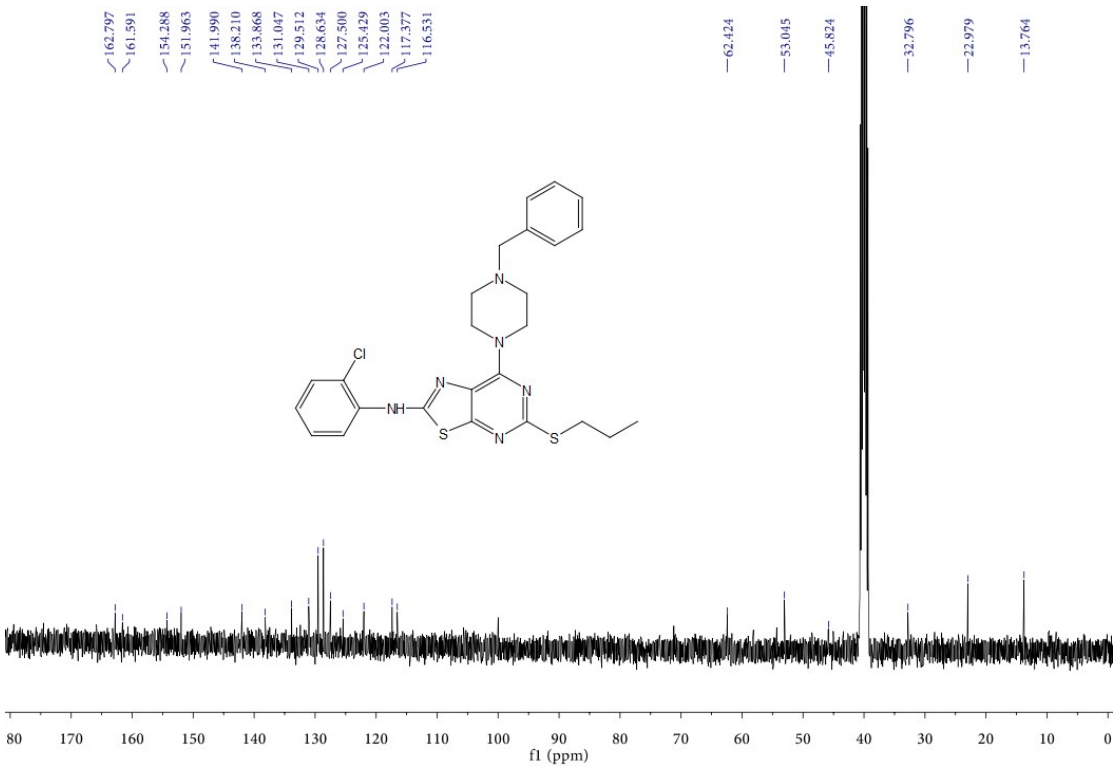
3.343
3.030
3.012
2.994
2.528
2.514
2.510
2.505
1.693
1.675
1.656
1.638
0.983
0.965
0.947
Cl
NH
N
S
0.95
0.98
4.95
1.93
1.03
3.97
1.90
2.22
1.95
4.30
2.00
3.00
f1 (ppm)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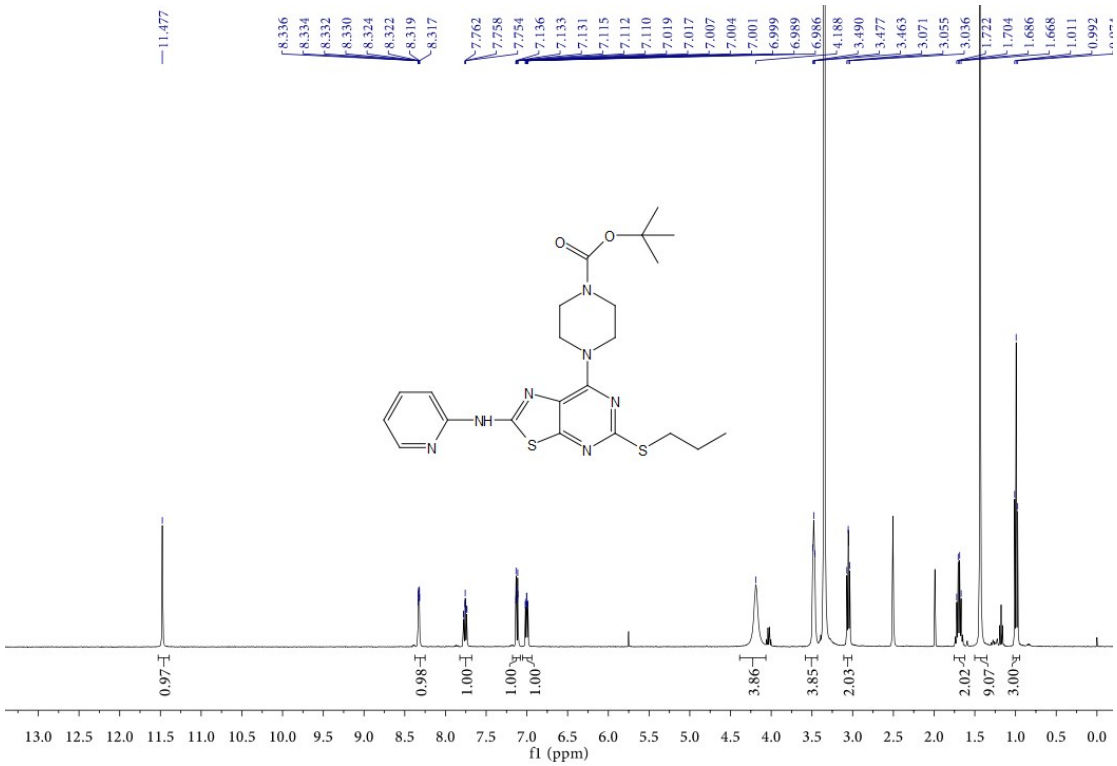
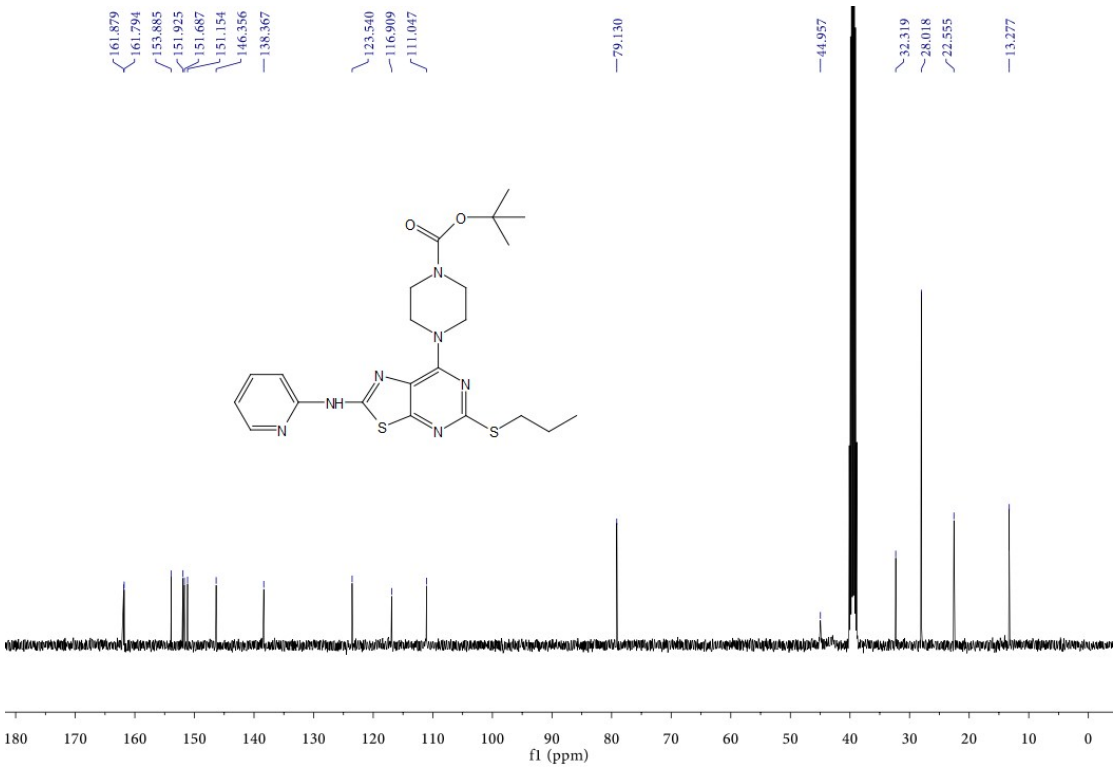
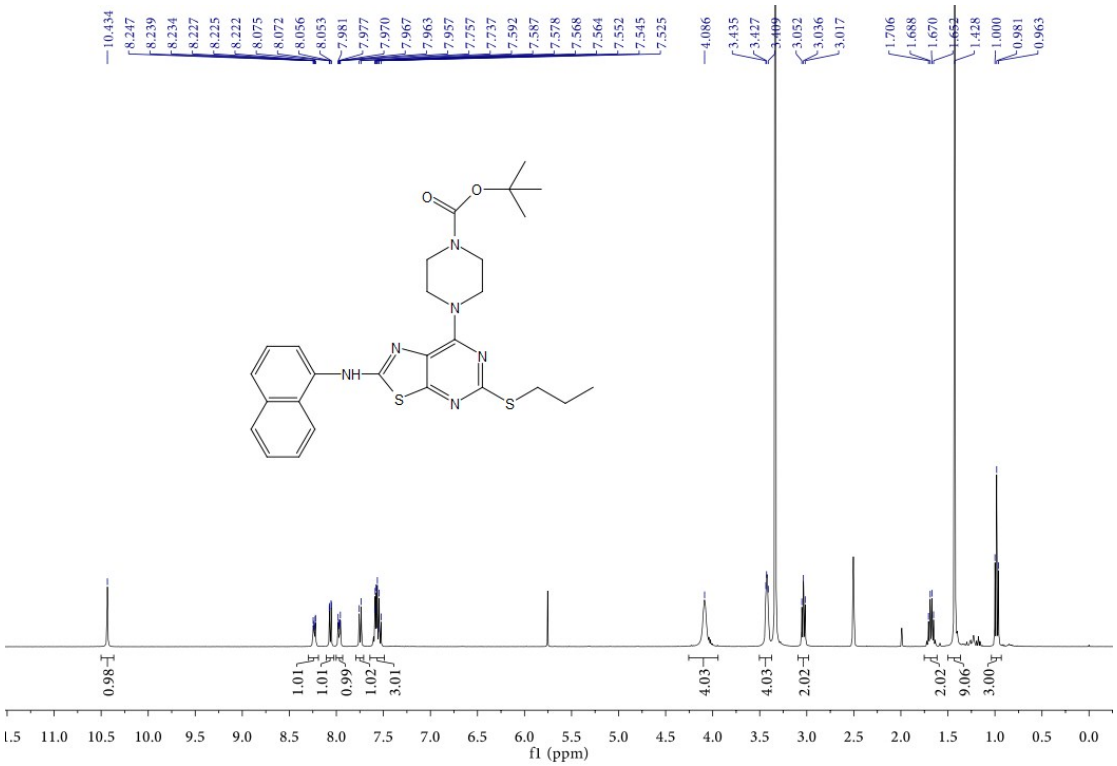
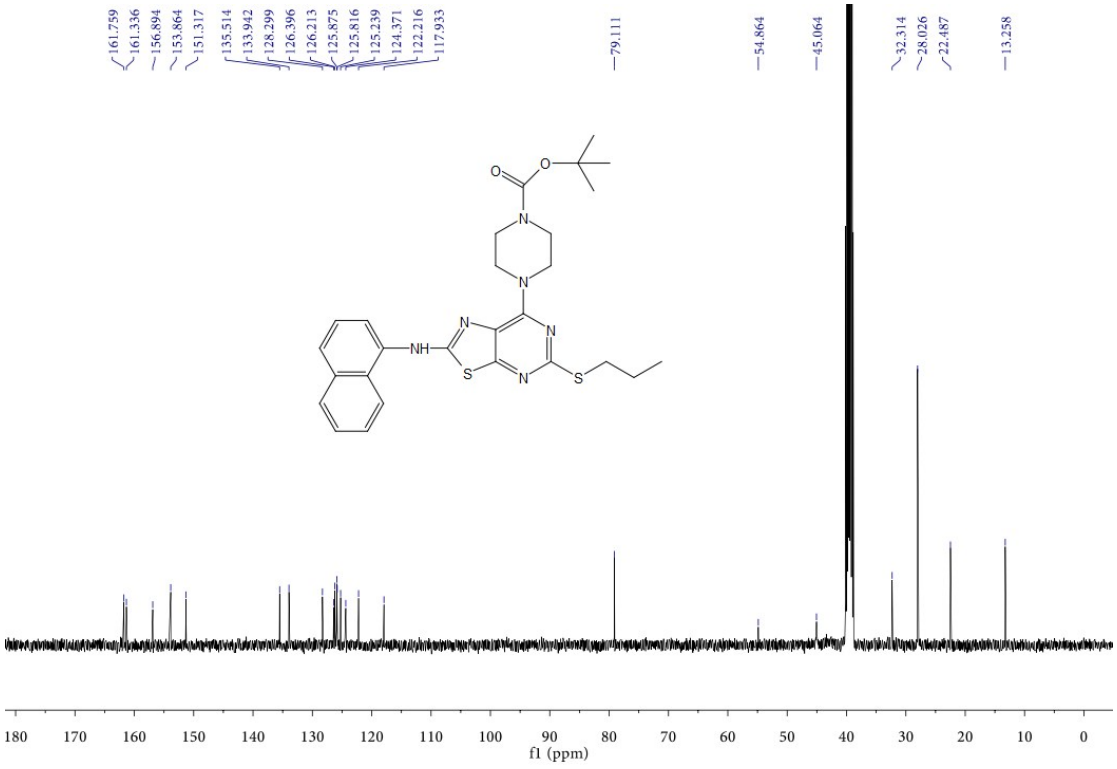
117.377
116.531
62.424
53.045
45.824
32.796
22.979
13.764
Cl
NH
N
S
f1 (ppm)


11.477
8.336
8.334
8.332
8.330
8.324
8.322
8.319
8.317
7.762
7.758
7.754
7.136
7.133
7.131
7.115
7.112
7.110
7.019
7.017
7.007
7.004
7.001
6.999
6.989
6.986
4.188
3.490
3.477
3.463
3.071
3.055
3.036
1.722
1.704
1.686
1.668
1.011
0.992
0.97
0.98
1.00
1.00
1.00
3.86
3.85
2.03
2.02
9.07
3.00
13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


161.879
161.794
153.885
151.925
151.687
151.154
146.356
138.367
123.540
116.909
111.047
79.130
44.957
32.319
28.018
22.555
13.277
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


10.434
8.247
8.239
8.234
8.227
8.225
8.222
8.075
8.072
8.056
8.053
7.981
7.977
7.970
7.967
7.963
7.957
7.757
7.737
7.592
7.587
7.578
7.568
7.564
7.552
7.545
7.525
4.086
3.435
3.427
3.409
3.052
3.036
3.017
1.706
1.688
1.670
1.652
1.428
1.000
0.981
0.963
0.98
1.01
1.01
0.99
1.02
3.01
4.03
4.03
2.02
2.02
9.06
3.00
f1 (ppm)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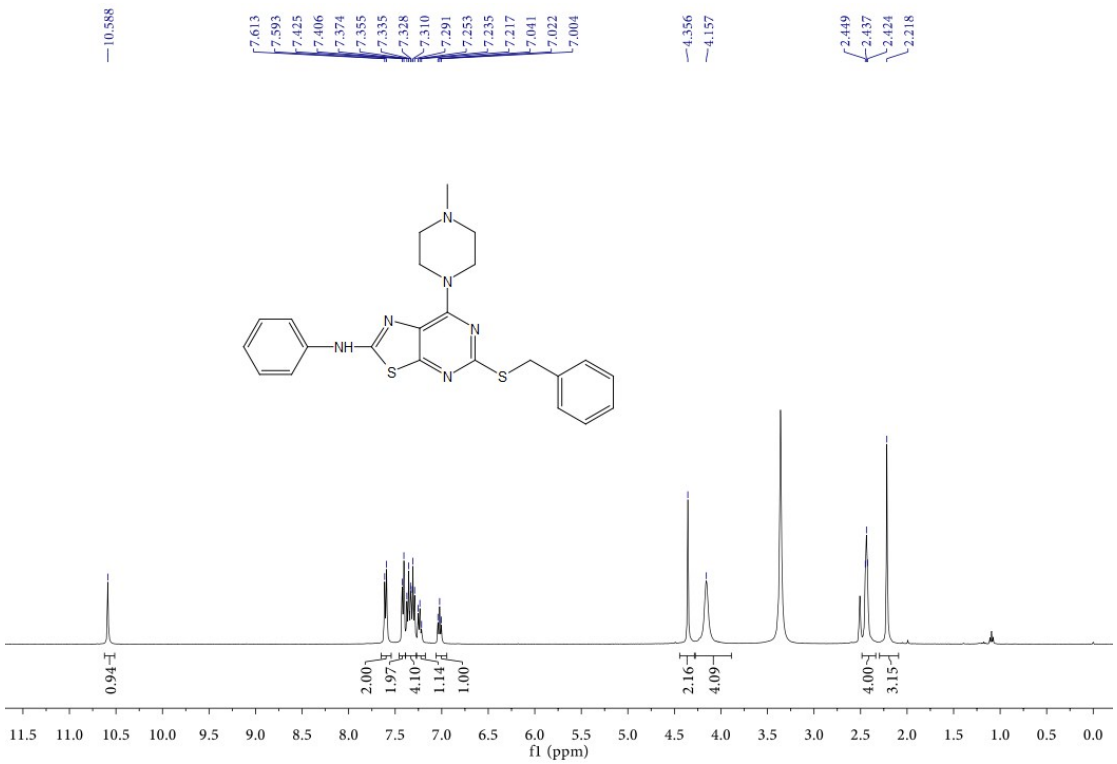
161.336
156.894
153.864
151.317
135.514
133.942
128.299
126.396
126.213
125.875
125.816
125.239
124.371
122.216
117.933
79.111
54.864
45.064
32.314
28.026
22.487
13.258
f1 (ppm)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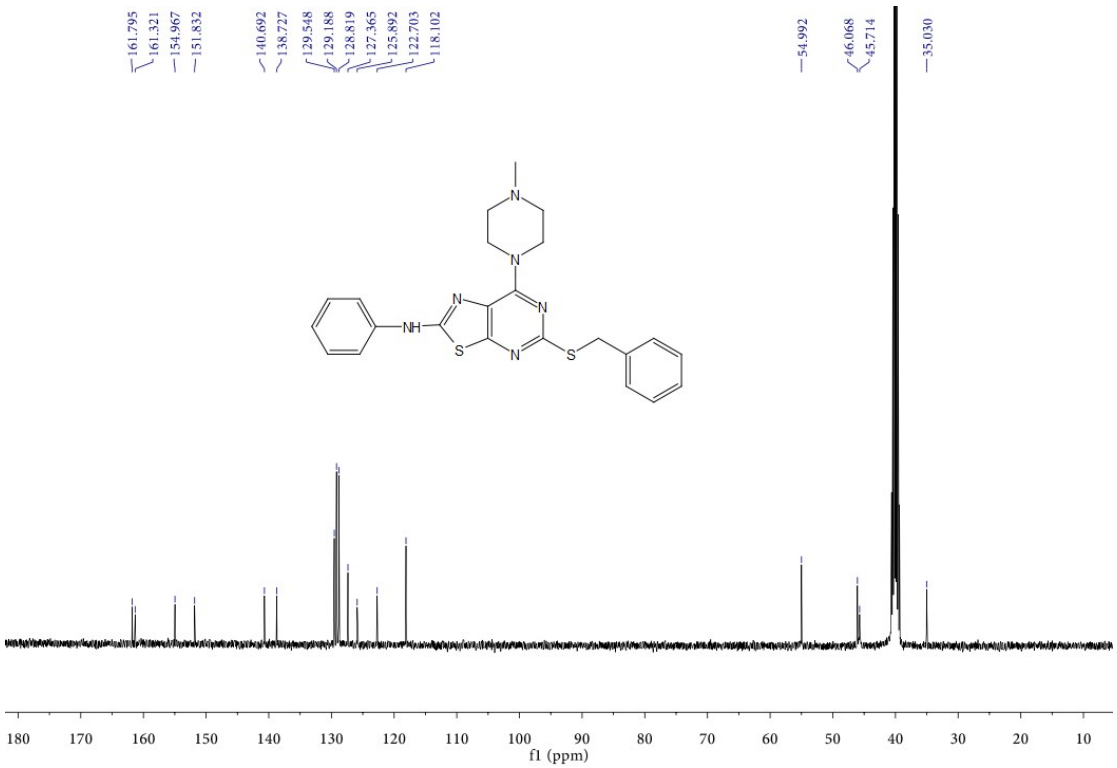
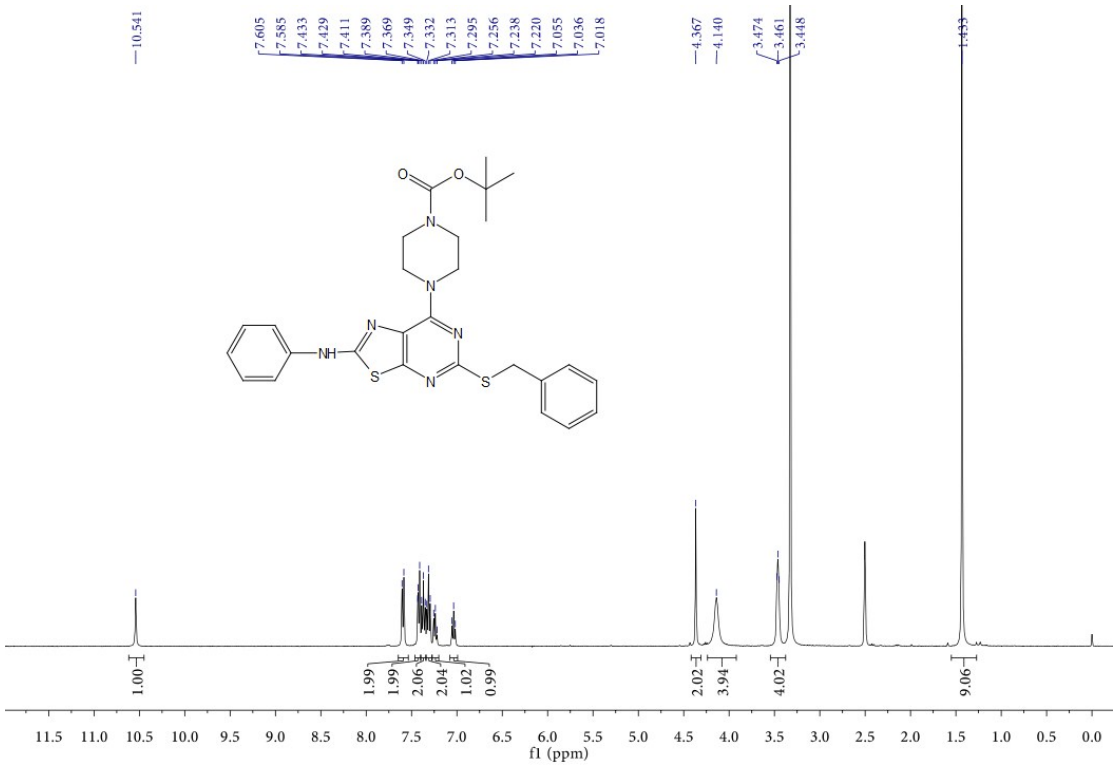
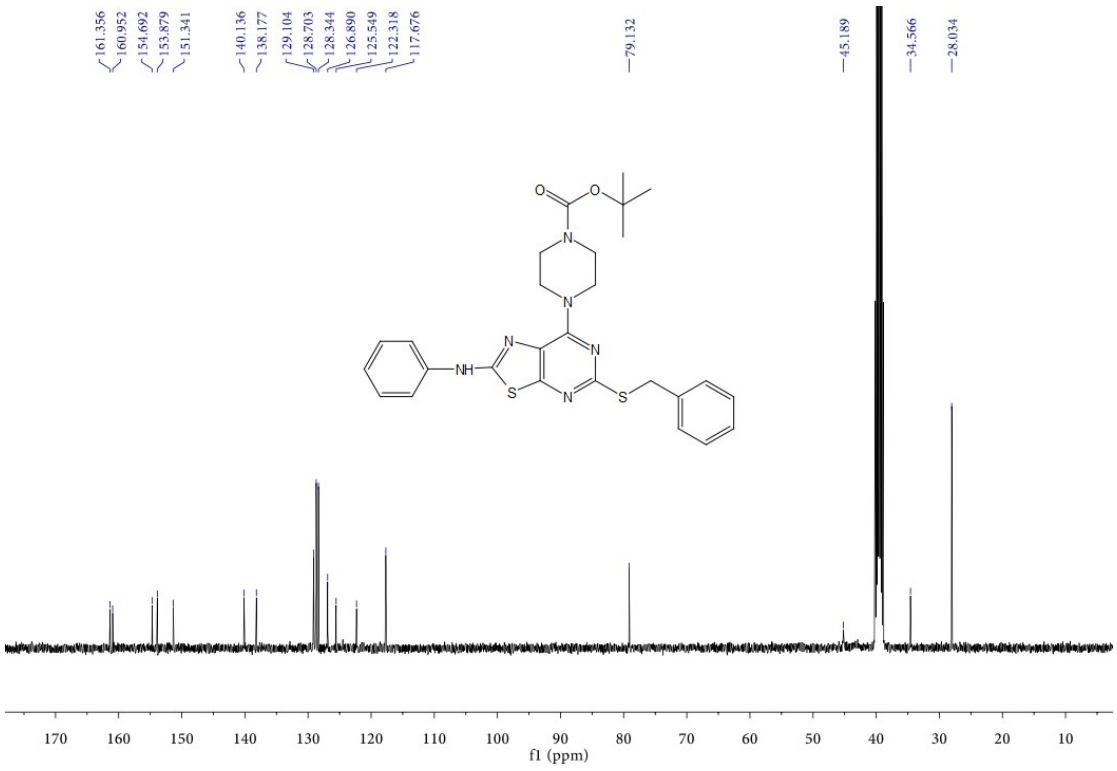
7.613
7.593
7.425
7.406
7.374
7.355
7.335
7.328
7.310
7.291
7.253
7.235
7.217
7.041
7.022
7.004
4.356
4.157
2.449
2.437
2.424
2.218
0.94
2.00
1.97
4.10
1.14
1.00
2.16
4.09
4.00
3.15
f1 (ppm)


161.795
161.321
154.967
151.832
140.692
138.727
129.548
129.188
128.819
127.365
125.892
122.703
118.102
54.992
46.068
45.714
35.030
f1 (ppm)


10.541
7.605
7.585
7.433
7.429
7.411
7.389
7.369
7.349
7.332
7.313
7.295
7.256
7.238
7.220
7.055
7.036
7.018
4.367
4.140
3.474
3.461
3.448
1.433
1.00
1.99
1.99
2.06
2.04
1.02
0.99
2.02
3.94
4.02
9.06
f1 (ppm)


161.356
160.952
154.692
153.879
151.341
140.136
138.177
129.104
128.703
128.344
126.890
125.549
122.318
117.676
79.132
45.189
34.566
28.034
f1 (ppm)

References

1. Li ZH, Yang DX, Geng PF, et al. Design, synthesis and biological evaluation of [1,2,3]triazolo[4,5-d]pyrimidine derivatives possessing a hydrazone moiety as antiproliferative agents. *European journal of medicinal chemistry.* 2016;124: 967-980.

2. Liu J, Patch RJ, Schubert C, Player MR. Single-step syntheses of 2-amino-7-chlorothiazolo[5,4-d]pyrimidines: intermediates for bivalent thiazolopyrimidines. *J Org Chem.* 2005;70(24): 10194-10197.