Electronic Supplementary Information for

Diversity-Oriented Synthesis for Novel, Selective and Drug-like Inhibitors for a Virulent Phosphatase from Mycobacterium Tuberculosis

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Kinetic studies were carried out to probe the mechanism of inhibition of **11h**, and Lineweaver-Burk plot indicates that **11h** is a noncompetitive inhibitor against mPTPB with a K_i of 1.5 μM.

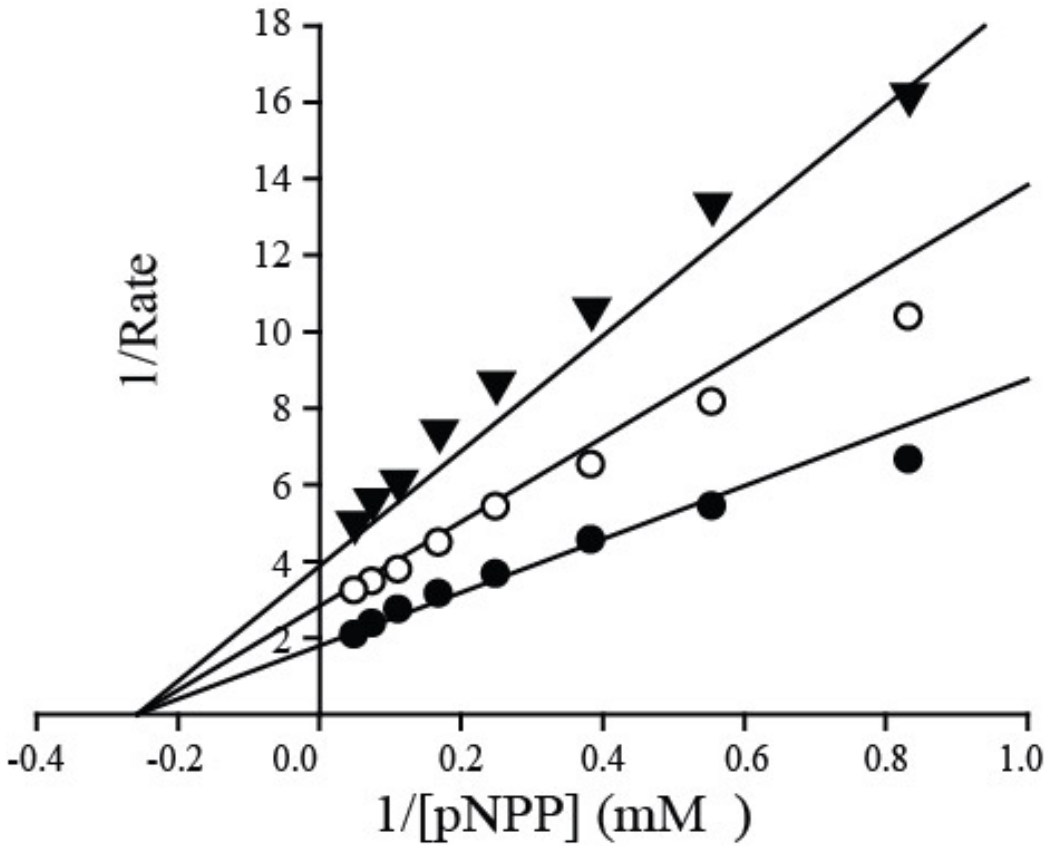


Fig. S1 Lineweaver-Burk plot for **11h** mediated mPTPB inhibition. **11h** concentrations were 0 (●), 1.25 (○), 2.5 (▼) μM, respectively.

To get an insight of **11h**'s good activity and specificity against mPTPB, the binding mode of **11h** with mPTPB was built using AutoDock 4.2.5[1] based on the mPTPB●OMTS complex structure (PDB ID: 2OZ5[2]). As shown in Fig. S2 A, **11h** locates in the deep and broad active site pocket of mPTPB, 5-salicylic acid head group interacts with the catalytic P-loop, while benzthiazole and 3-bromo benzene structure motifs interact with three helices α3A, α7, α8. In detail, the carboxylic acid group of **11h** forms H-bonds with backbone amide of F161, A162, and side chain of R166; the hydroxyl group forms H-bonds with K164 and D165 (Fig. S2 B). These polar interactions provide essential binding affinity between **11h** and mPTPB. In addition, benzothiazole is sandwiched by F161 and F98 and forms π-π interactions with them; and bromobenzene moiety resides in a hydrophobic pocket constituted by F98, L199, I203, M206, I207, F211 and L227. These hydrophobic and Van der Waals force strengthen the binding between **11h** and mPTPB.

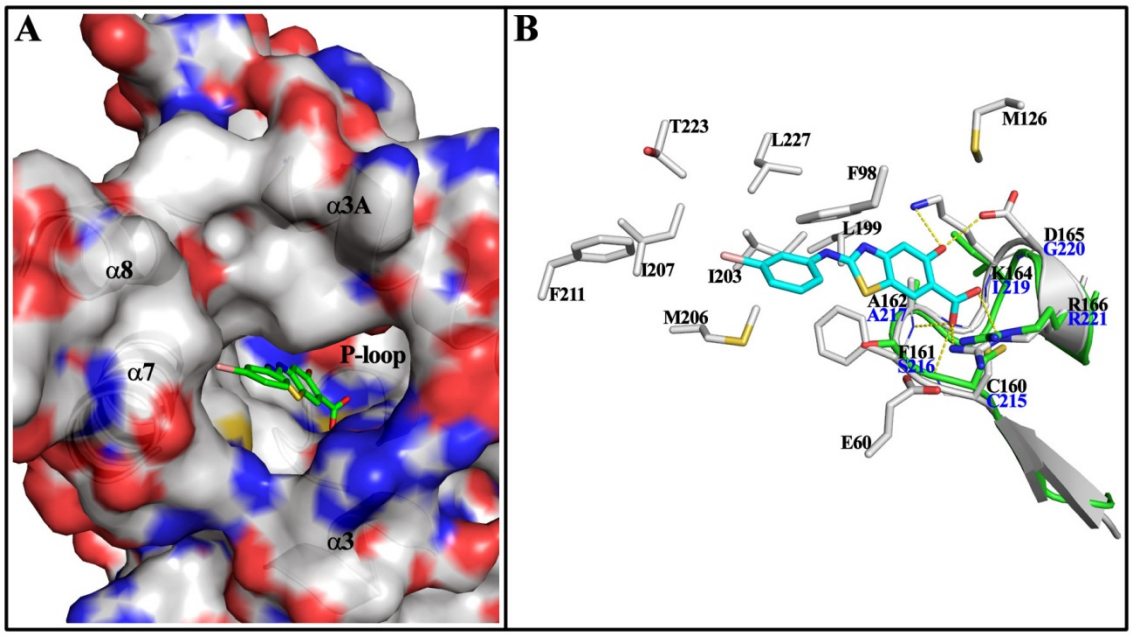


Fig. S2 Proposed binding mode of **11h** with mPTPB. (**A**) A global view of **11h** binding into the deep and broad active site of mPTPB. (**B**) A detailed view of **11h**●mPTPB complex. **11h** (in cyan) and protein residues (in gray) within 5Å distance are shown in stick, polar interactions are highlighted in dotted yellow line. P-loop of PTP1B (PDBID: 2HNQ)[3] (in green, side chain residues written in blue) was superimposed into **11h**●mPTPB complex, which shows that **11h** has weaker interactions with P-loop of PTP1B than that of mPTPB.

11h has at least 20-fold selectivity against a panel of mammalian PTPs. To understand the molecular basis of its excellent specificity, we carried out sequence alignment and structure comparison analysis. As shown in Fig. S3 A, three of the seven P-loop residues (CFAGKDR) (three residues highlighted in blue) that interact with compound **11h** are unique to mPTPB when compared with over 50 human PTPs. These three residues significantly contribute to the binding affinity in our binding mode (Fig S1 B), specifically, K164 and D165 make strong polar interactions with the hydroxyl group of **11h**, and F161 interacts with the benzothiazole ring through π-π interaction. In contrast, the cognate residues in human PTPs can only provide limited Van der Waals interaction with **11h**. The structure superimposition indicates that three helices of α3A, α7 and α8 are also unique to mPTPB (Fig. S3 B and C), which provides hydrophobic interactions with the 3-bromobenzene group, and these interactions would be absent in PTP1B and VHR. Collectively, these unique features of mPTPB likely contribute to the observed specificity of compound **11h** against mPTPB.

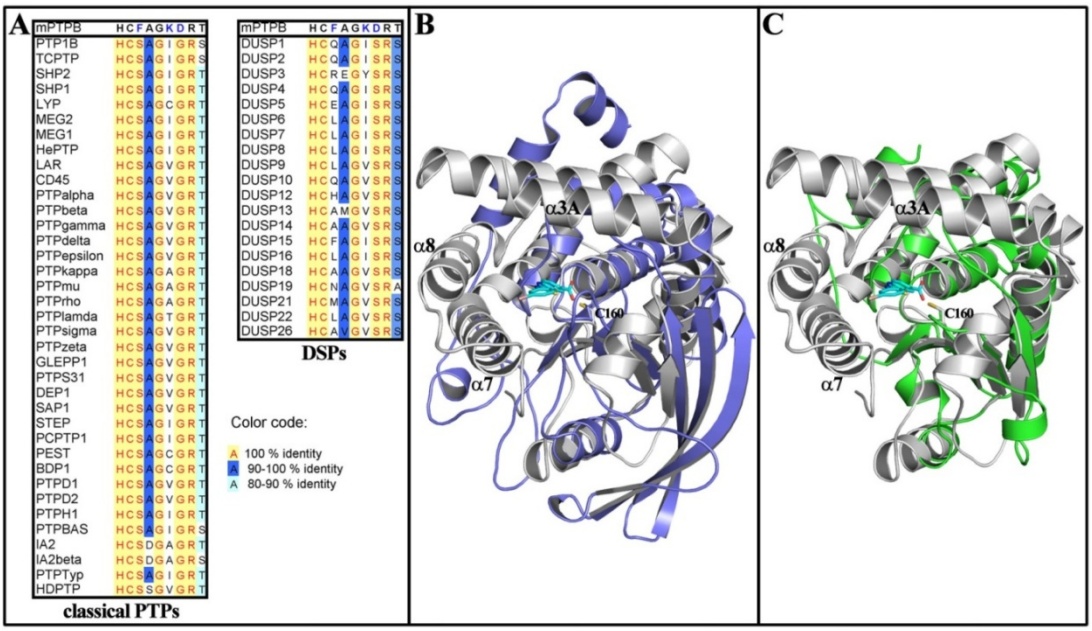


Fig. S3 Sequence alignment and structure comparison analysis between mPTPB and mammalian PTPs. (**A**) Sequence alignment of the catalytic P-loop of mPTPB with classical PTPs and DSPs. (**B**) Structure superimposition of mPTPB (PDB ID: 2OZ5, represented in gray ribbon) and PTP1B (PDBID: 2HNQ[3], represented in blue ribbon). (**C**) Structure superimposition of mPTPB (PDB ID: 2OZ5, represented in gray ribbon) with VHR (PDB ID: 1VHR[4], represented in green ribbon). The structures were superimposed based on the nine residues used for alignment in panel **A**.

mPTPB secreted by *Mtb* down-regulates Erk1/2 activation in macrophage cells to block IL-6 production, while up-regulates Akt to promote survival, and mPTPB inhibitors can rescue these processes.[5] In our studies, **11h** was able to increase Erk1/2 phosphorylation and decrease Akt phosphorylation induced by IFN-γ in mPTPB transfected Raw264.7 cells at 5 μM, 10 μM, in a dose dependent manner. In contrast, compound **11a** with an IC_{50} of 25 μM has no such effects at 10 μM, indicating **11h**'s excellent cellular activity in targeting mPTPB.

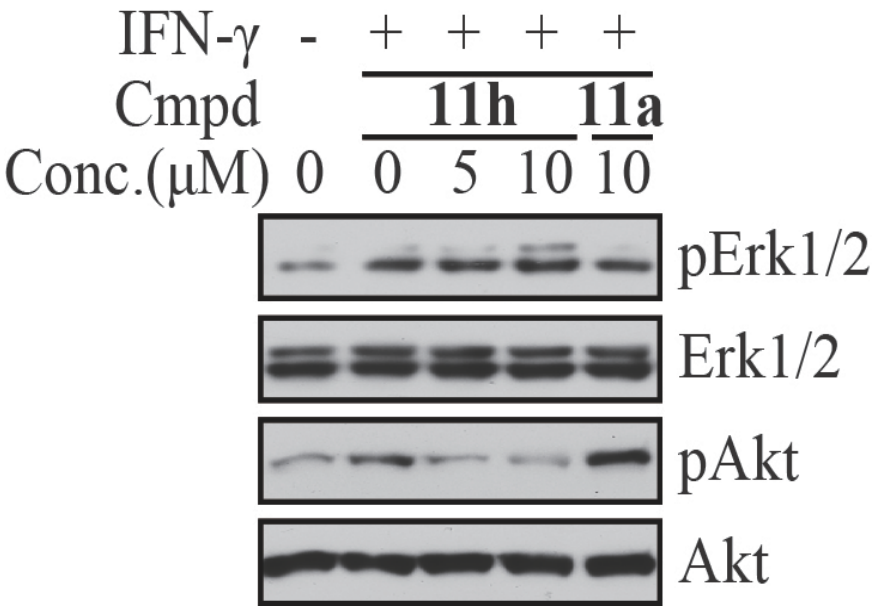
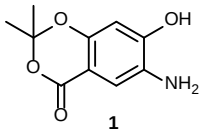


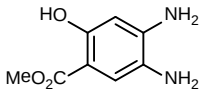
Fig. S4 Cellular studies of compound **11h** in mPTPB transfected Raw264.7 cells

Characterizations of compounds **1-11**



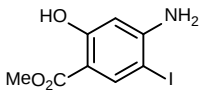
1

1. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.12 (s, 1H), 6.38 (s, 1H), 1.66 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.2, 155.1, 151.8, 126.3, 116.8, 105.9, 103.9, 102.5, 25.2. ESI-MS Cacld. for $C_{10}H_{12}NO_4$ ($M+H^+$): m/z 210.0761; found 210.0760.



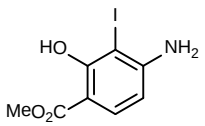
2

2. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (s, 1H), 6.20 (s, 1H), 3.84 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 158.5, 145.6, 124.8, 118.4, 102.1, 101.6, 51.6. ESI-MS Cacld. for $C_8H_{11}N_2O_3$ ($M+H^+$): m/z 183.0764; found 183.0766.



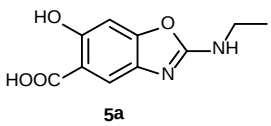
3

3. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.77 (s, 1H), 8.07 (s, 1H), 6.25 (s, 1H), 4.53 (br, 2H), 3.84 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.2, 163.1, 152.6, 140.5, 105.4, 100.3, 71.6, 51.9. ESI-MS Cacld. for $C_8H_9INO_3$ ($M+H^+$): m/z 293.9622; found 293.9622.



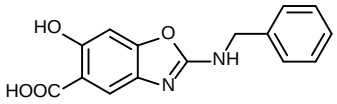
4

4. ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.00 (s, 1H), 7.60 (d, J = 8.6 Hz, 1H), 6.26 (d, J = 8.6 Hz, 1H), 4.72 (brs, 2H), 3.89 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.1, 162.2, 153.3, 130.8, 105.7, 102.5, 72.4, 52.1. ESI-MS Cacld. for $C_8H_9INO_3$ ($M+H^+$): m/z 293.9622; found 293.9627.



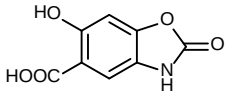
5a

5a. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.47 (s, 1H), 7.95 (t, J = 5.5 Hz, 1H), 7.54 (s, 1H), 6.96 (s, 1H), 3.32-3.27 (m, 2H), 1.17 (t, J = 7.2 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.6, 162.3, 157.7, 153.2, 136.0, 114.8, 107.9, 97.6, 37.2, 14.6. ESI-MS Cacld. for $C_{10}H_{11}N_2O_4$ ($M+H^+$): m/z 223.0713; found 223.0711.



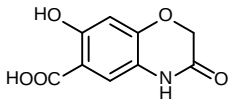
5b

5b. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.50 (s, 1H), 8.56 (t, J = 6.0 Hz, 1H), 7.55 (s, 1H), 7.55-7.32 (m, 5H), 7.00 (s, 1H), 4.50 (d, J = 5.9 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.48, 162.4, 157.7, 153.2, 138.7, 135.6, 128.4, 127.2, 115.0, 108.0, 99.5, 97.6, 45.7. ESI-MS Cacld. for $C_{15}H_{13}N_2O_4$ (M+H$^+$): m/z 285.0870; found 285.0878.



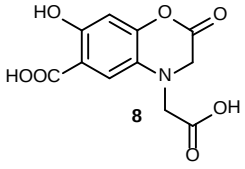
6

6. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.63 (s, 1H), 7.37 (s, 1H), 6.91 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.1, 158.8, 154.5, 149.0, 123.2, 109.7, 108.0, 99.0. ESI-MS Cacld. for $C_8H_4NO_5$ (M-H$^+$): m/z 194.0095; found 194.0087.



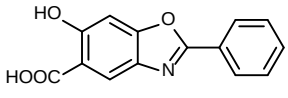
7

7. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.16 (s, 1H), 10.67 (s, 1H), 7.31 (s, 1H), 6.52 (s, 1H), 4.63 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.35, 163.4, 158.2, 149.4, 119.7, 116.0, 106.3, 104.0, 66.6. ESI-MS Cacld. for $C_9H_6NO_5$ (M-H$^+$): m/z 208.0251; found 208.0243.



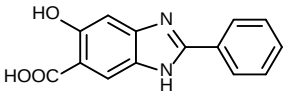
8

8. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.29 (s, 1H), 6.62 (s, 1H), 4.78 (s, 2H), 4.63 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.2, 163.1, 158.6, 150.7, 121.3, 115.7, 106.8, 104.6, 99.5, 66.8, 42.4. ESI-MS Cacld. for $C_{11}H_8NO_7$ (M-H$^+$): m/z 266.0306; found 266.0306.



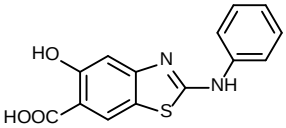
9

9. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.16-8.12 (m, 3H), 7.63-7.58 (m, 3H), 7.32 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.1, 162.5, 160.2, 154.7, 134.2, 132.0, 129.3, 127.1, 126.0, 121.0, 110.7, 98.7. ESI-MS Cacld. for $C_{14}H_8NO_4$ (M-H$^+$): m/z 254.0459; found 254.0457.



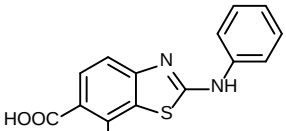
10

10. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.16 (d, J = 7.3 Hz, 2H), 8.07 (s, 1H), 7.59-7.54 (m, 3H), 7.02 (s, 1H). ESI-MS Cacld. for $C_{14}H_{11}N_2O_3$ ($M+H^+$): m/z 255.0764; found 255.0760.



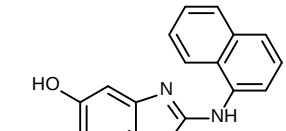
11a

11a. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.40 (s, 1H), 10.76 (s, 1H), 8.23 (s, 1H), 7.76 (d, J = 7.9 Hz, 2H), 7.38 (t, J = 7.8 Hz, 2H), 7.08-7.05 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.9, 166.1, 160.4, 158.3, 140.0, 129.1, 122.9, 122.9, 121.1, 118.5, 107.8, 105.8. ESI-MS Cacld. for $C_{14}H_9N_2O_3S$ ($M-H^+$): m/z 285.0339; found 285.0337.



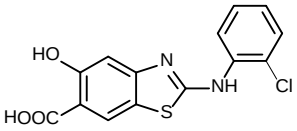
11b

11b. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.95 (s, 1H), 10.82 (s, 1H), 7.78-7.74 (m, 3H), 7.39 (t, J = 7.7 Hz, 2H), 7.18 (d, J = 8.5 Hz, 1H), 7.07 (t, J = 7.3 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.1, 164.8, 158.2, 156.0, 140.1, 129.1, 128.0, 122.8, 118.2, 115.8, 111.1, 106.0. ESI-MS Cacld. for $C_{14}H_{11}N_2O_3S$ ($M+H^+$): m/z 287.0485; found 287.0491.



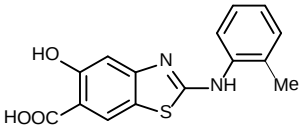
11c

11c. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.39 (s, 1H), 8.17-8.16 (m, 2H), 8.00-7.98 (m, 2H), 7.83 (d, J = 8.2 Hz, 1H), 7.59-7.56 (m, 3H), 6.94 (s, 1H). ESI-MS Cacld. for $C_{18}H_{13}N_2O_3S$ ($M+H^+$): m/z 337.0641; found 337.0644.



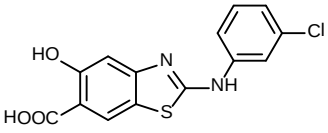
11d

11d. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.20 (s, 1H), 8.05 (d, J = 7.5 Hz, 1H), 7.54 (dd, J = 1.4, 8.0 Hz, 1H), 7.41 (m, 1H), 7.21 (m, 1H), 6.95 (s, 1H). ESI-MS Cacld. for $C_{14}H_{10}ClN_2O_3S$ ($M+H^+$): m/z 321.0095; found 321.0086.



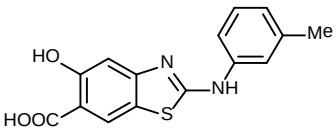
11e

11e. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.15 (s, 1H), 7.68 (d, J = 6.4 Hz, 1H), 7.28-7.24 (m, 2H), 7.17-7.14 (m, 1H), 6.90 (s, 1H), 2.27 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.8, 169.2, 160.4, 158.3, 138.2, 131.7, 130.8, 126.6, 125.7, 124.3, 122.8, 121.3, 107.2, 105.0, 17.8, ESI-MS Cacld. for $C_{15}H_{13}N_2O_3S$ ($M+H^+$): m/z 301.0641; found 301.0638.



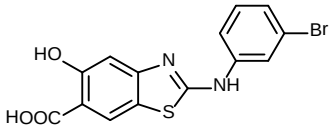
11f

11f. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.46 (s, 1H), 10.93 (s, 1H), 8.27 (s, 1H), 7.99 (s, 1H), 7.62 (d, J = 8.4 Hz, 1H), 7.40 (t, J = 8.1 Hz, 1H), 7.12-7.10 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.8, 165.7, 160.4, 157.9, 141.4, 133.4, 130.7, 123.1, 122.1, 121.0, 117.6, 116.7, 106.2, 99.5. ESI-MS Cacld. for $C_{14}H_{10}ClN_2O_3S$ ($M+H^+$): m/z 321.0095; found 321.0102.



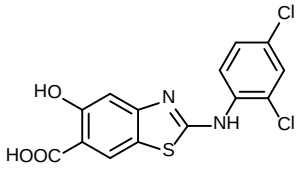
11g

11g. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.22 (s, 1H), 7.60 (d, J = 9.0 Hz, 1H), 7.51 (s, 1H), 7.26 (t, J = 7.8 Hz, 1H), 7.03 (s, 1H), 6.89 (d, J = 7.5 Hz, 1H), 2.30 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.8, 166.1, 160.4, 158.3, 140.0, 138.3, 128.9, 123.7, 122.9, 121.1, 119.0, 115.8, 107.7, 105.7, 21.2. ESI-MS Cacld. for $C_{15}H_{13}N_2O_3S$ ($M+H^+$): m/z 301.0641; found 301.0639.



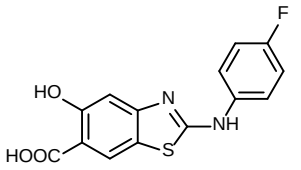
11h

11h. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.45 (br, 1H), 10.91 (br, 1H), 8.27 (s, 1H), 8.10 (s, 1H), 7.68 (d, J = 7.8 Hz, 1H), 7.34 (t, J = 8.0 Hz, 1H), 7.24 (d, J = 7.8 Hz, 1H), 7.09 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.7, 165.6, 160.4, 157.9, 141.5, 131.0, 125.2, 123.1, 121.8, 121.0, 120.5, 117.1, 108.3, 106.1. ESI-MS Cacld. for $C_{14}H_{10}BrN_2O_3S$ ($M+H^+$): m/z 364.9590; found 364.9594.



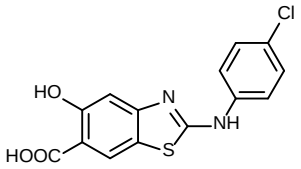
11i

11i. ^{1}H NMR (500 MHz, DMSO-d_6) δ ^{1}H NMR (500 MHz, DMSO-d_6) 9.42 (s, 1H), 8.69 (s, 1H), 8.14 (s, 1H), 8.02 (d, J = 9.0 Hz, 1H), 7.65 (d, J = 2.5 Hz, 1H), 7.56 (s, 1H), 7.42-7.40 (m, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.1, 166.4, 161.3, 157.9, 151.9, 145.4, 140.3, 134.6, 128.7, 127.6, 124.3, 124.2, 109.5, 99.5. ESI-MS Cacld. for $C_{14}H_7Cl_2N_2O_3S$ ($M-H^+$): m/z 352.9560; found 352.9562.



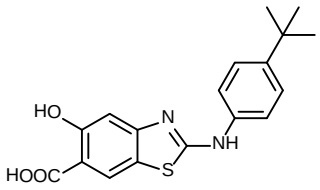
11j

11j. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.78 (s, 1H), 8.21 (s, 1H), 7.77-7.75 (m, 2H), 7.22 (t, J = 8.9 Hz, 2H), 7.02 (s, 1H). ESI-MS Cacld. for $C_{14}H_{10}FN_2O_3S$ ($M+H^+$): m/z 305.0391; found 305.0396.



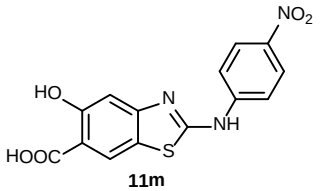
11k

11k. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.25 (s, 1H), 7.80 (d, J = 8.9 Hz, 2H), 7.43 (d, J = 8.9 Hz, 2H), 7.06 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.8, 165.7, 160.4, 158.1, 139.0, 128.9, 126.2, 123.0, 121.0, 119.8, 108.1, 106.0. ESI-MS Cacld. for $C_{14}H_{10}ClN_2O_3S$ ($M+H^+$): m/z 321.0095; found 321.0095.



11l

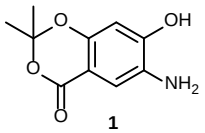
11l. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.40 (s, 1H), 10.66 (s, 1H), 8.21 (s, 1H), 7.64 (d, J = 8.6 Hz, 2H), 7.40 (d, J = 8.6 Hz, 2H), 7.00 (s, 1H), 1.28 (s, 9H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.85, 166.29, 160.4, 158.5, 145.4, 137.5, 125.7, 122.8, 121.1, 118.5, 107.6, 105.6, 34.0, 31.2. ESI-MS Cacld. for $C_{18}H_{19}N_2O_3S$ ($M+H^+$): m/z 343.1111; found 343.1114.



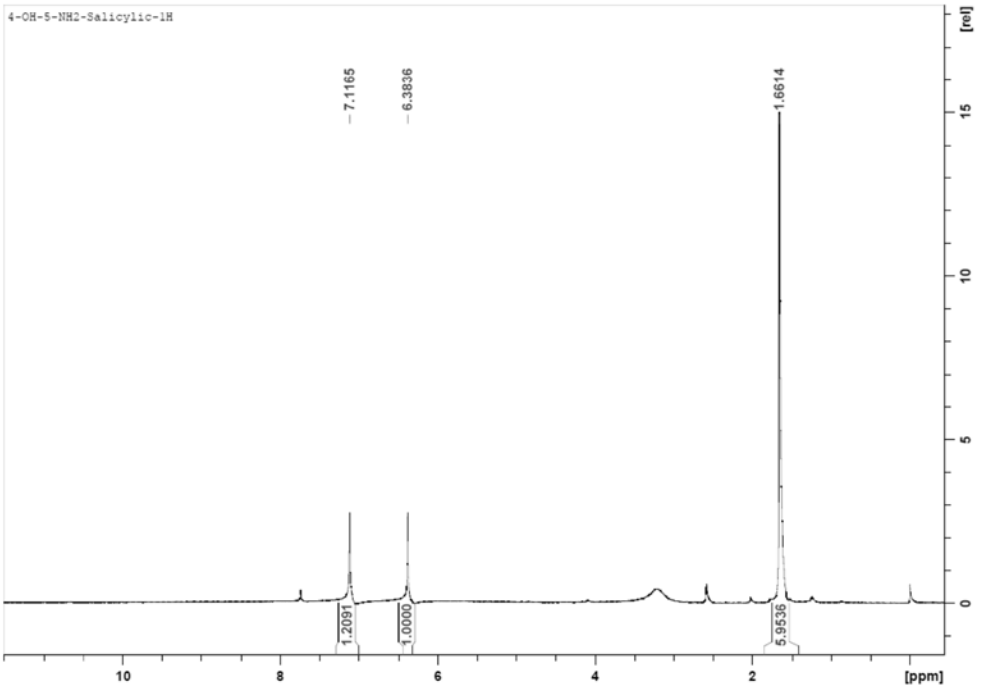
11m

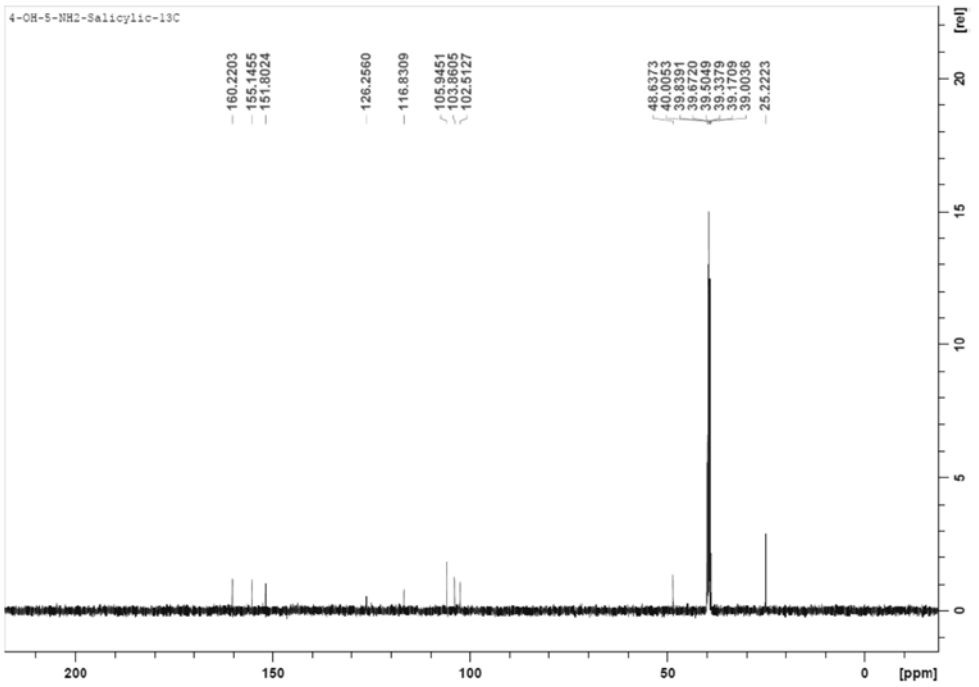
11m. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.40 (s, 1H), 8.30 (s, 1H), 8.25 (d, J = 9.3 Hz, 2H), 7.97 (d, J = 9.3 Hz, 2H), 7.13 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.8, 165.3, 160.4, 157.5, 145.9, 141.5, 125.4, 123.5, 121.4, 117.8, 109.1, 106.7. ESI-MS Cacld. for $C_{14}H_{10}N_3O_5S$ ($M+H^+$): m/z 332.0336; found 332.0337.

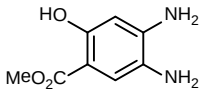
Copies of ^{1}H and ^{13}C NMR Spectra of compounds **1-11**



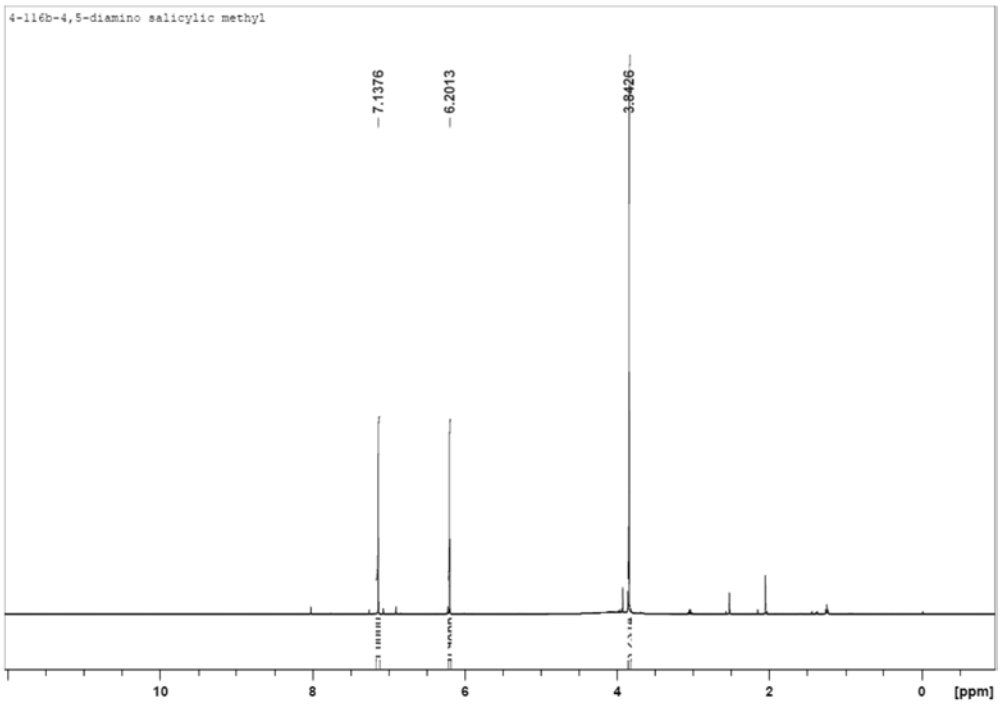
1






HO
NH2
MeO2C
NH2

2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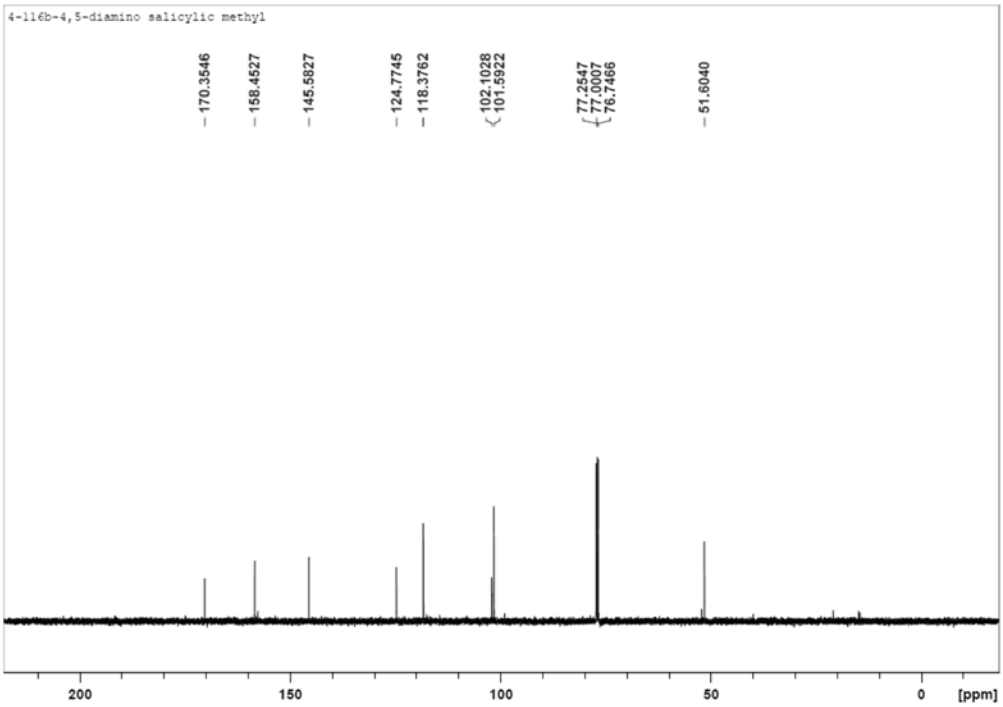
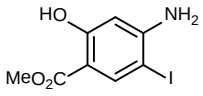
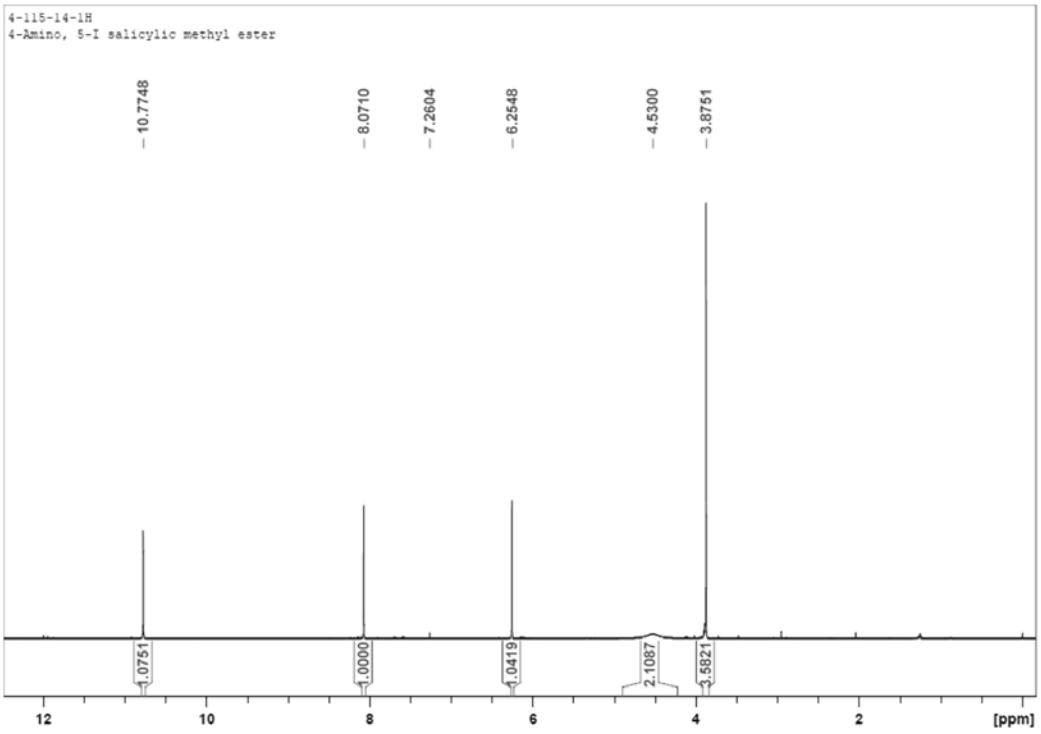
6.2013
3.8426
10
8
6
4
2
0
[ppm]


4-116b-4,5-diamino salicylic methyl
170.3546
158.4527
145.5827
124.7745
118.3762
102.1028
101.5922
77.2547
77.0007
76.7466
51.6040
200
150
100
50
0
[ppm]


HO
NH2
MeO2C
I

3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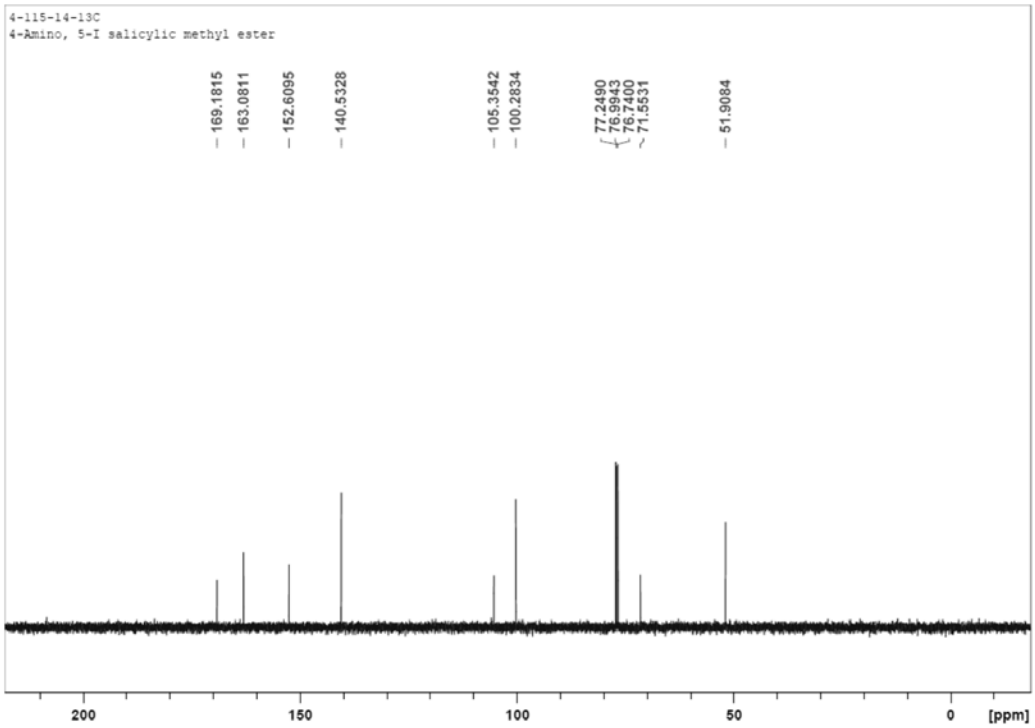
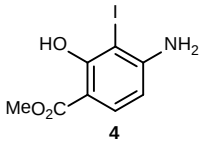
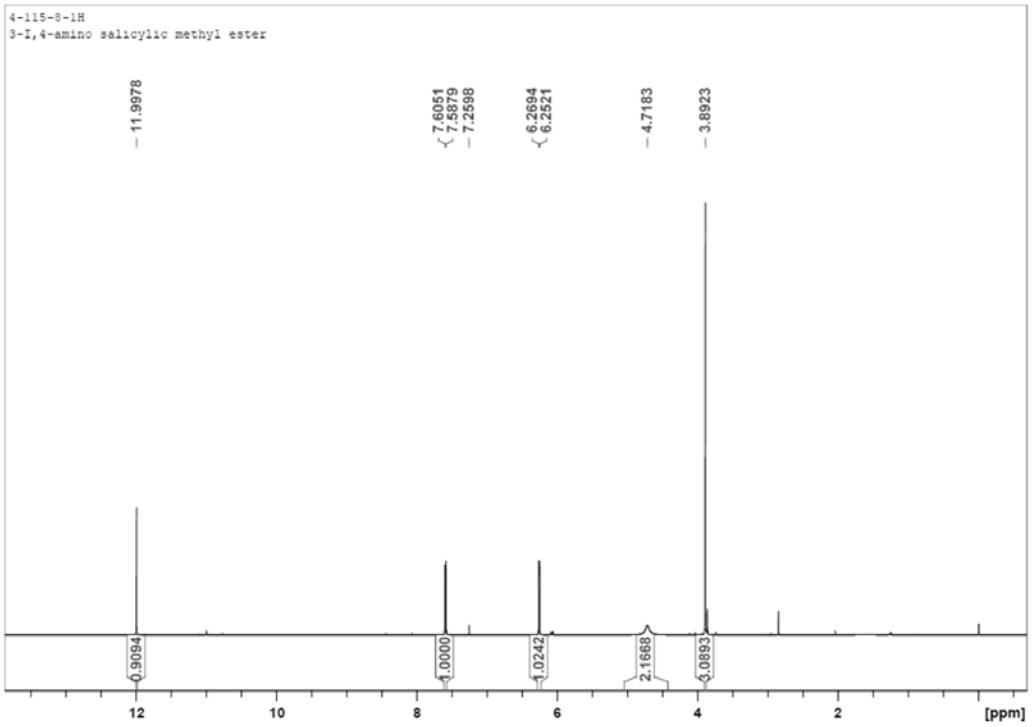
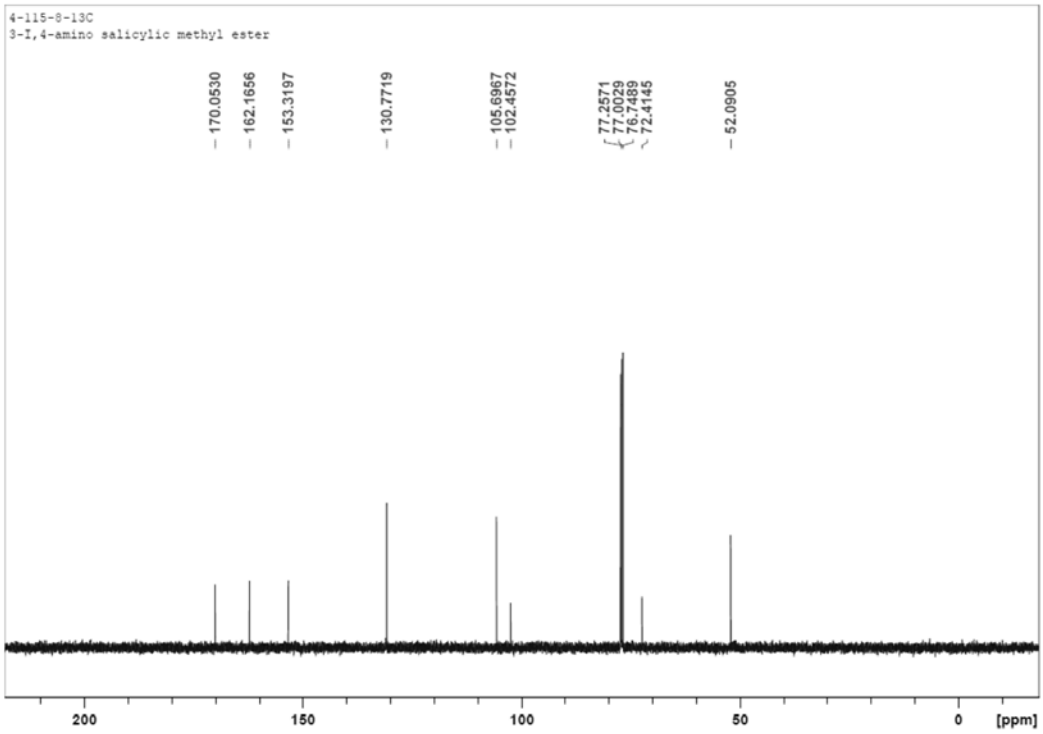
4-Amino, 5-I salicylic methyl ester
10.7748
8.0710
7.2604
6.2548
4.5300
3.8751
1.0751
1.0000
1.0419
2.1087
3.5821
12
10
8
6
4
2
[ppm]

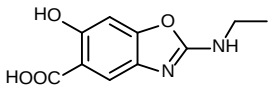

4-115-14-13C
4-Amino, 5-I salicylic methyl ester
169.1815
163.0811
152.6095
140.5328
105.3542
100.2834
77.2490
76.9943
76.7400
71.5531
51.9084
200
150
100
50
0
[ppm]


I
HO
NH2
MeO2C

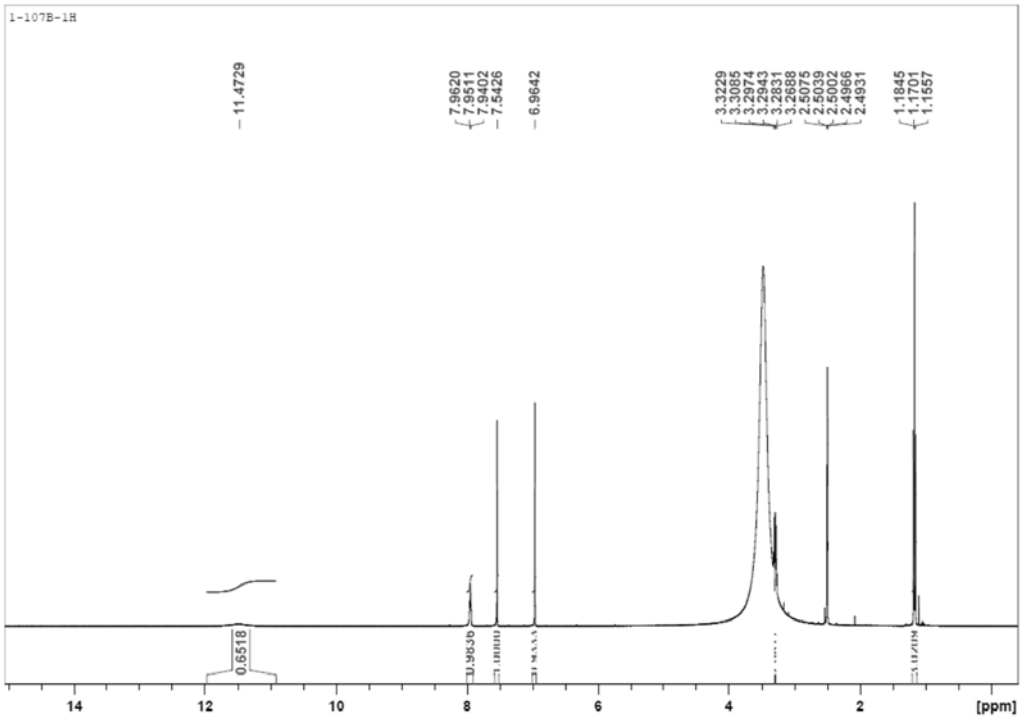
4

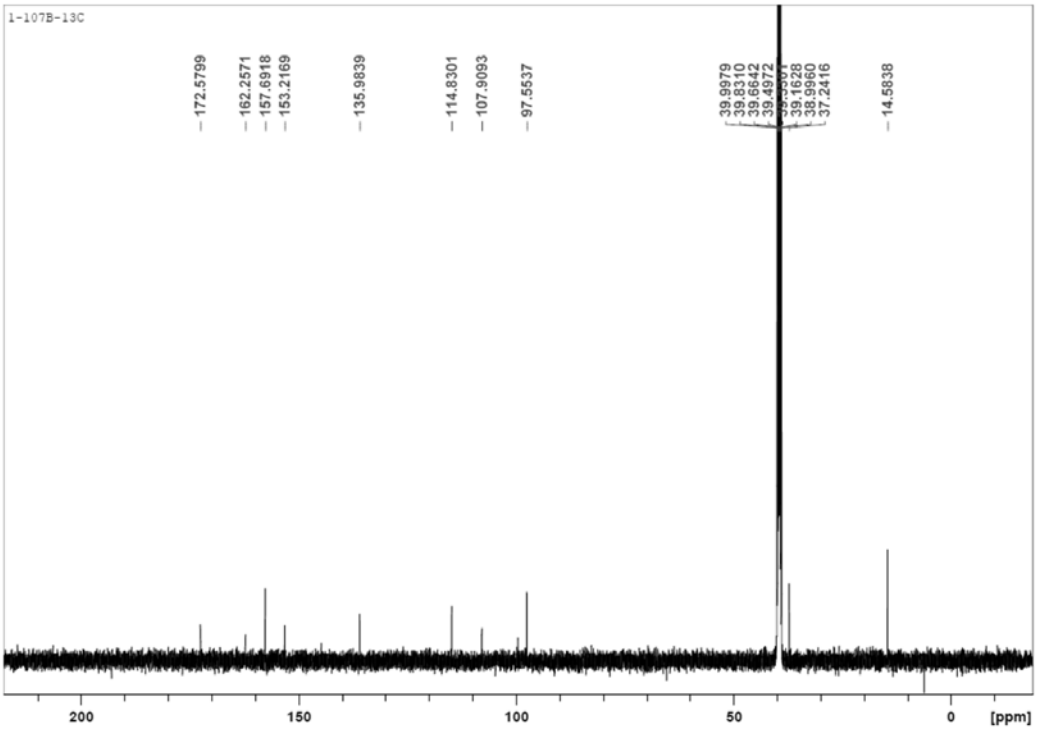

4-115-8-1H
3-I,4-amino salicylic methyl ester
11.9978
7.6051
7.5879
7.2598
6.2694
6.2521
4.7183
3.8923
0.9094
1.0000
1.0242
2.1668
3.0893
12
10
8
6
4
2
[ppm]


4-115-8-13C
3-I,4-amino salicylic methyl ester
170.0530
162.1656
153.3197
130.7719
105.6967
102.4572
77.2571
77.0029
76.7489
72.4145
52.0905
200
150
100
50
0
[ppm]


HO
O
NH
HOOC
N

5a


1-107B-1H
11.4729
7.9620
7.9511
7.9402
7.5426
6.9642
3.3229
3.3085
3.2974
3.2943
3.2831
3.2688
2.5075
2.5039
2.5002
2.4966
2.4931
1.1845
1.1701
1.1557
0.6518
14
12
10
8
6
4
2
[ppm]


1-107B-13C
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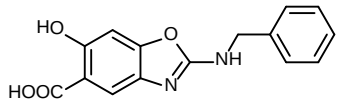
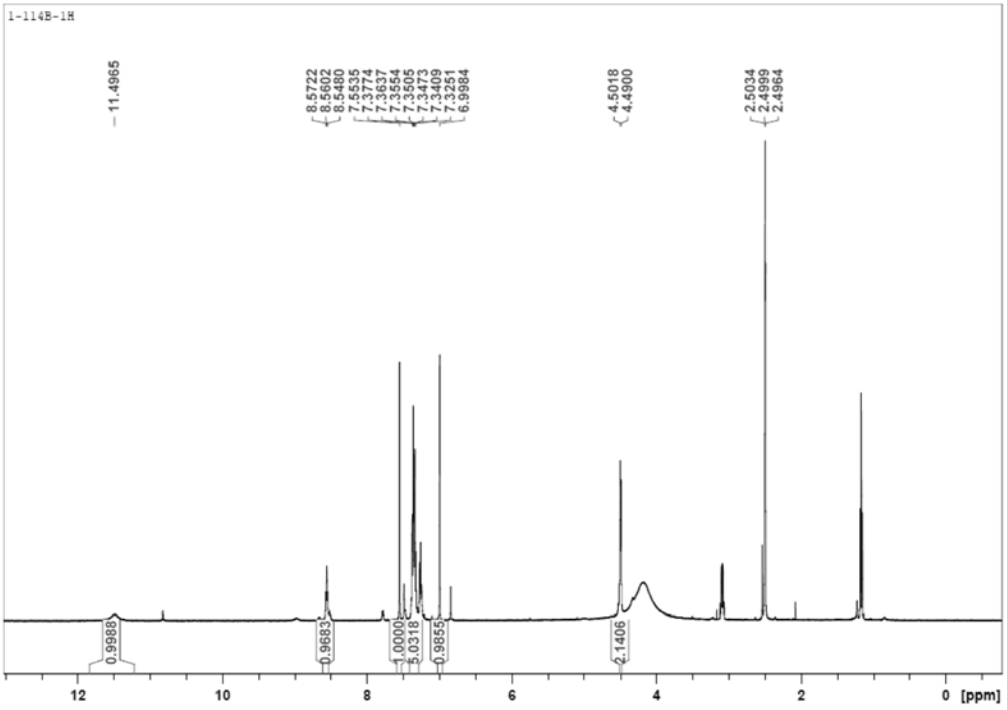
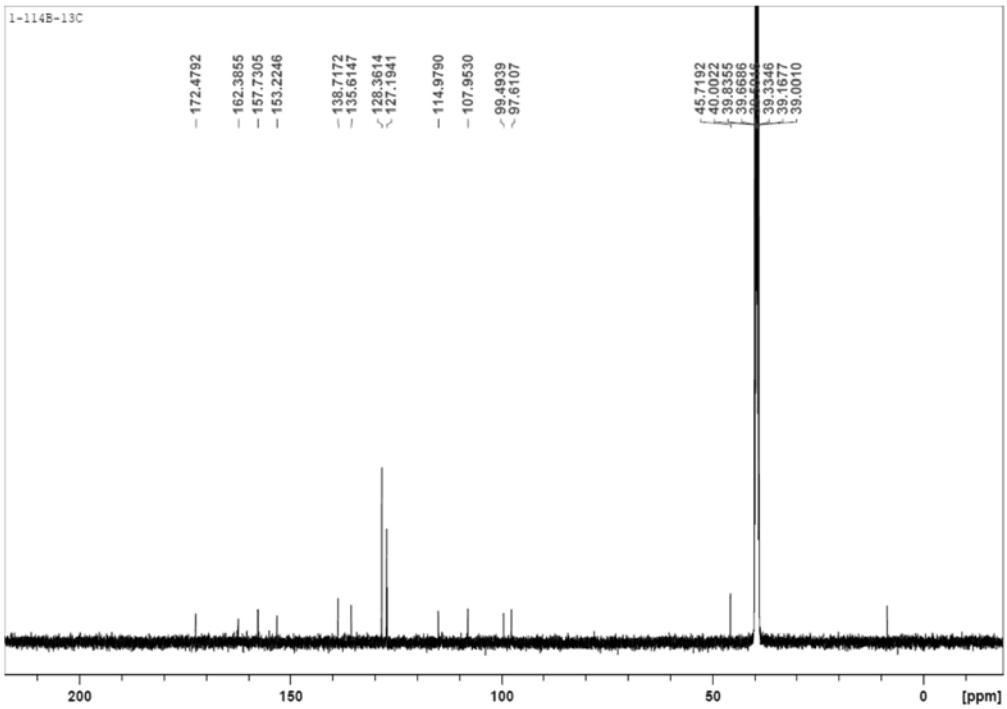
162.2571
157.6918
153.2169
135.9839
114.8301
107.9093
97.5537
39.9979
39.8310
39.6642
39.4972
39.1628
38.9960
37.2416
14.5838
200
150
100
50
0
[ppm]


HO
O
NH
HOOC
N

5b


1-114B-1H
11.4965
8.5722
8.5602
8.5480
7.5535
7.3774
7.3637
7.3554
7.3505
7.3473
7.3409
7.3251
6.9984
4.5018
4.4900
2.5034
2.4999
2.4964
0.9988
0.9683
1.0000
5.0318
0.9855
2.1406
12
10
8
6
4
2
0
[ppm]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128.3614
127.1941
114.9790
107.9530
99.4939
97.6107
45.7192
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39.8355
39.6686
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39.1677
39.0010
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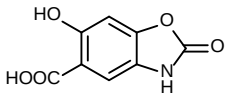
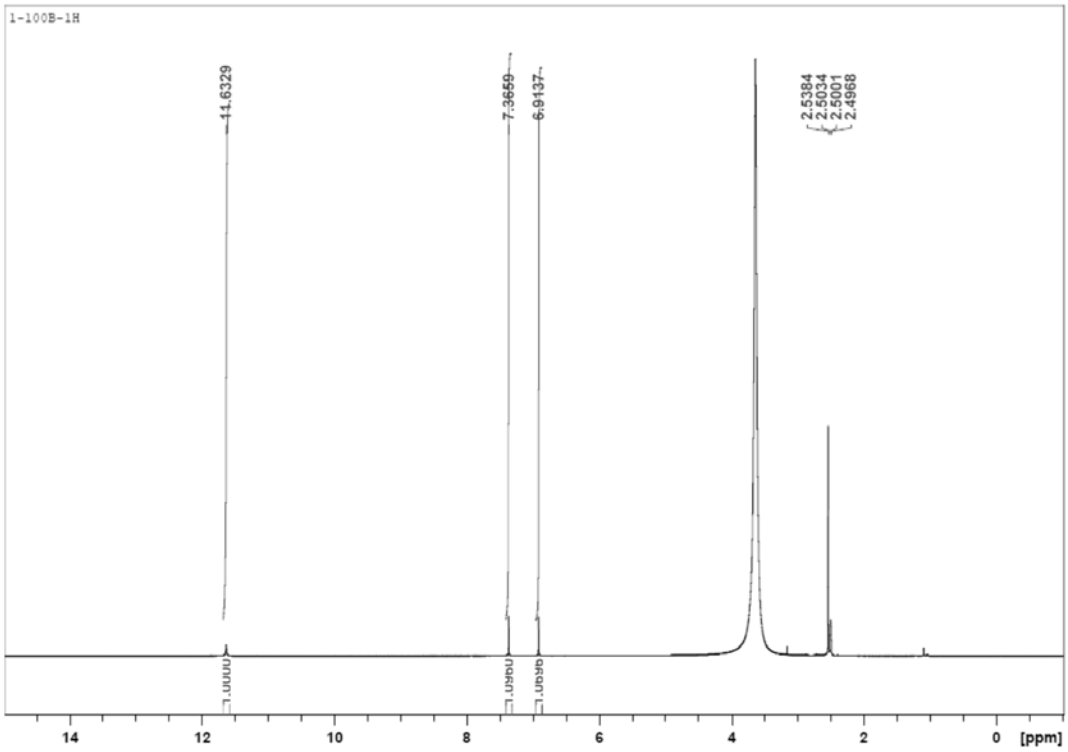
100
50
0
[ppm]


HO
O
O
HOOC
N
H

6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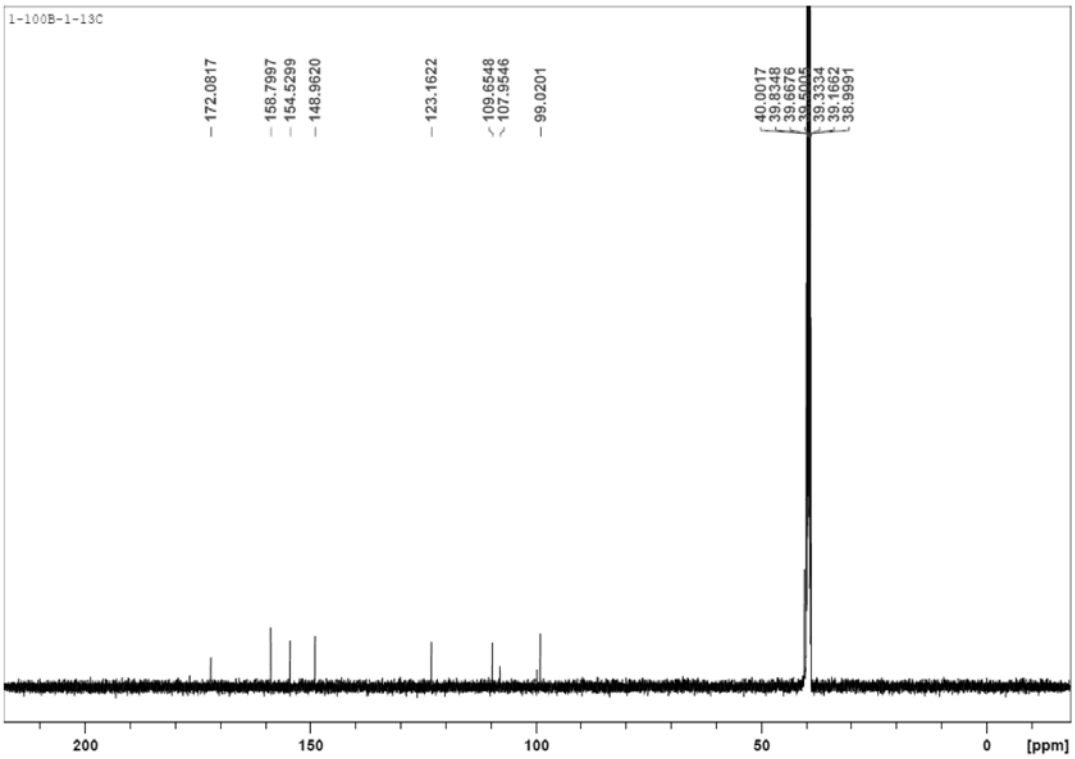
2.5001
2.4968
14
12
10
8
6
4
2
0
[ppm]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154.5299
148.9620
123.1622
109.6548
107.9546
99.0201
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39.8348
39.6676
39.3334
39.1662
38.9991
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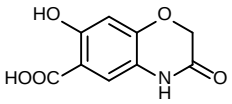
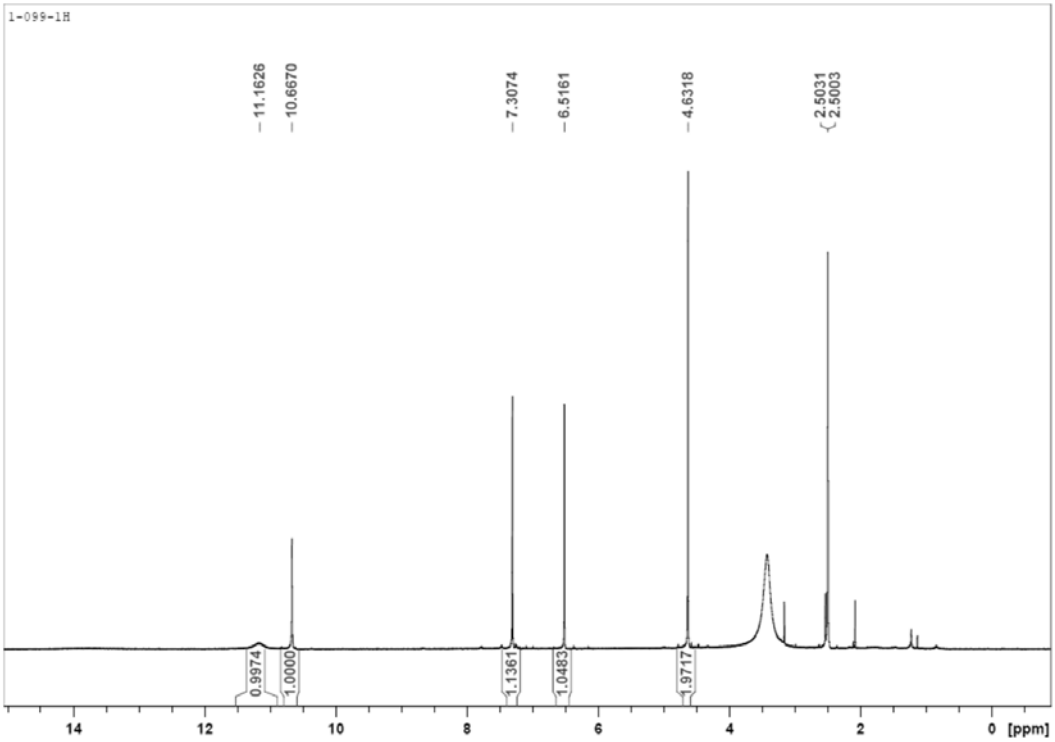
100
50
0
[ppm]


HO
O
HOOC
N
H
O

7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7.3074
6.5161
4.6318
2.5031
2.5003
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1.1361
1.0483
1.9717
14
12
10
8
6
4
2
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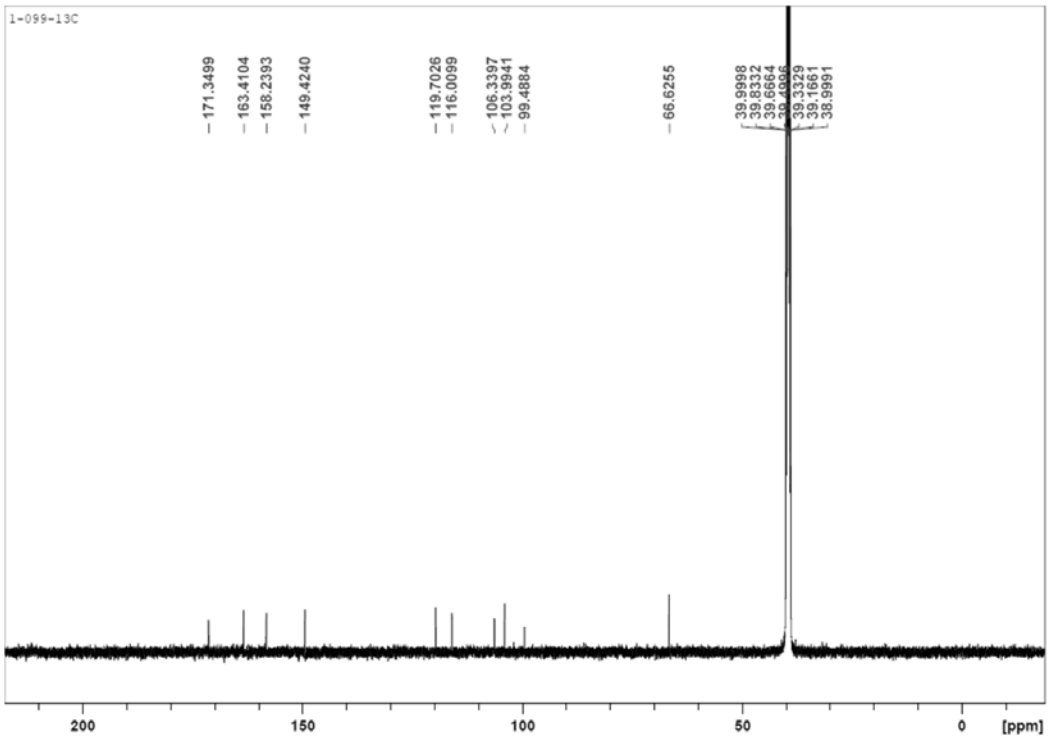
[ppm]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149.4240
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116.0099
106.3397
103.9941
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66.6255
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39.6664
39.4996
39.3329
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50
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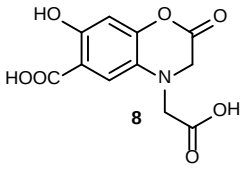
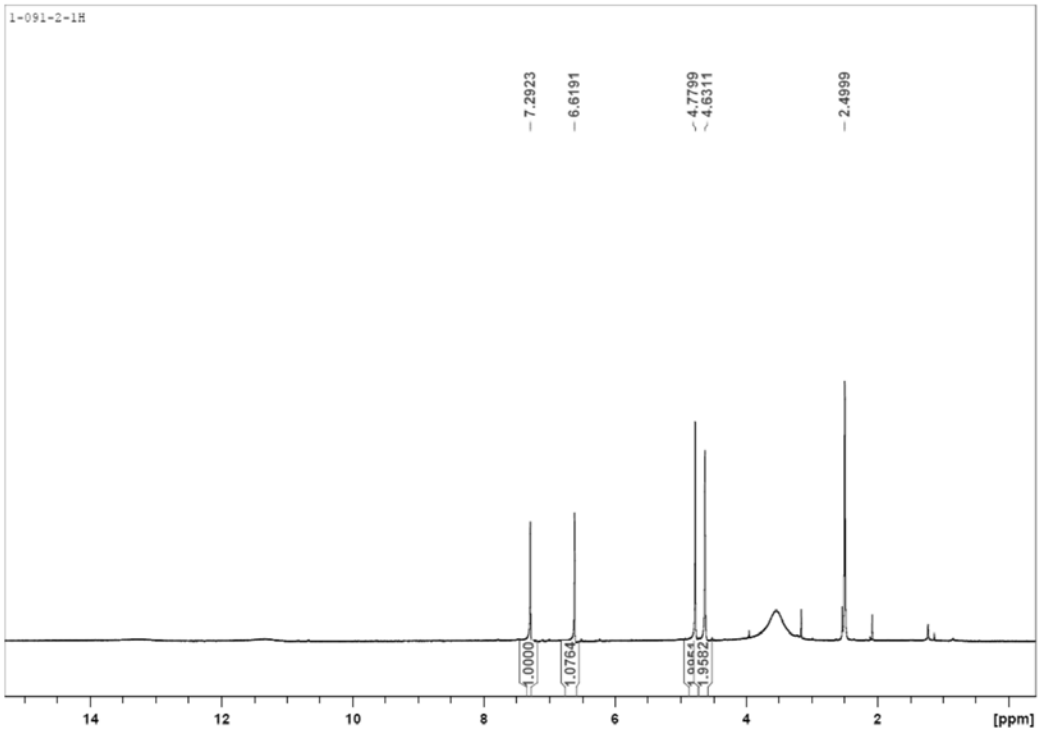
[ppm]


HO
O
O
HOOC
N
OH
O

8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1.0000
1.0764
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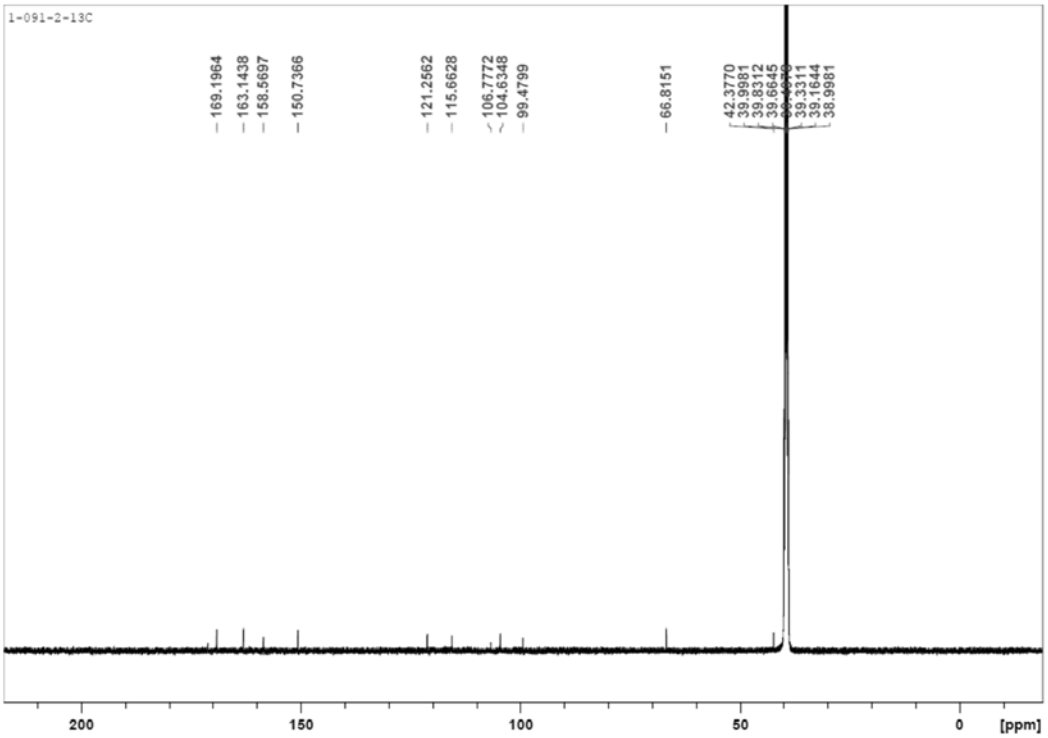
14
12
10
8
6
4
2
[ppm]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106.7772
104.6348
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42.3770
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39.3311
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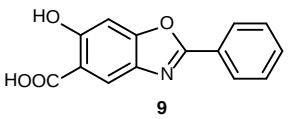
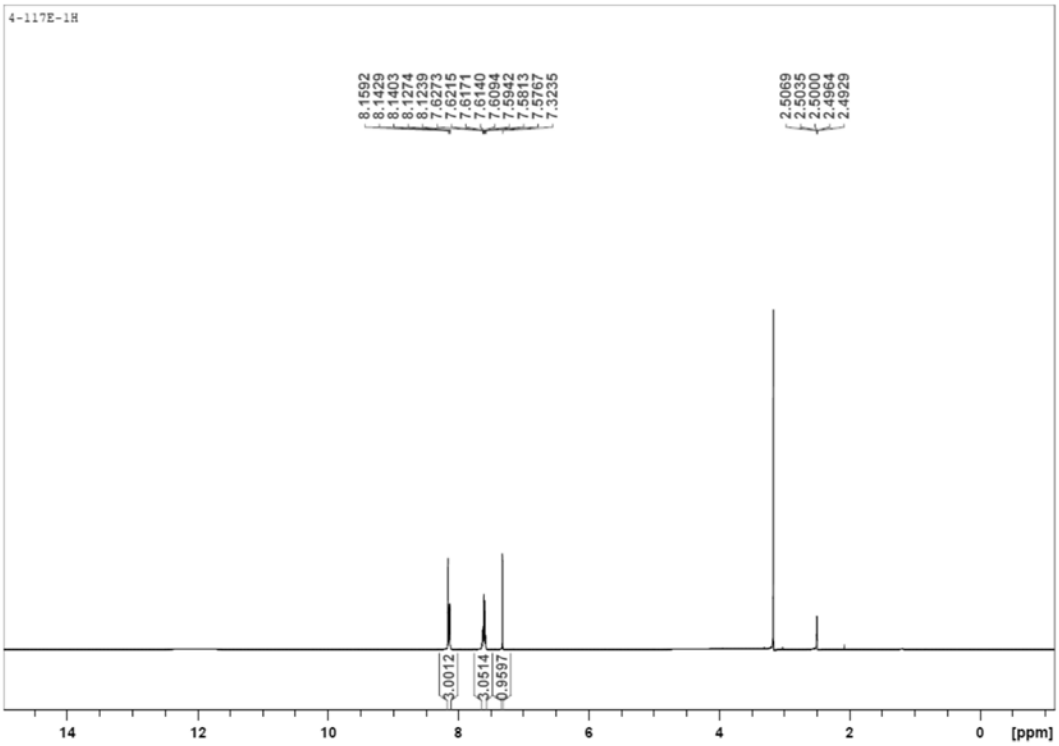

HO
O
HOOC
N

9


4-117E-1H
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8.1403
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8.1239
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7.6215
7.6171
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7.5813
7.5767
7.3235
2.5069
2.5035
2.5000
2.4964
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3.0012
3.0514
0.9597
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12
10
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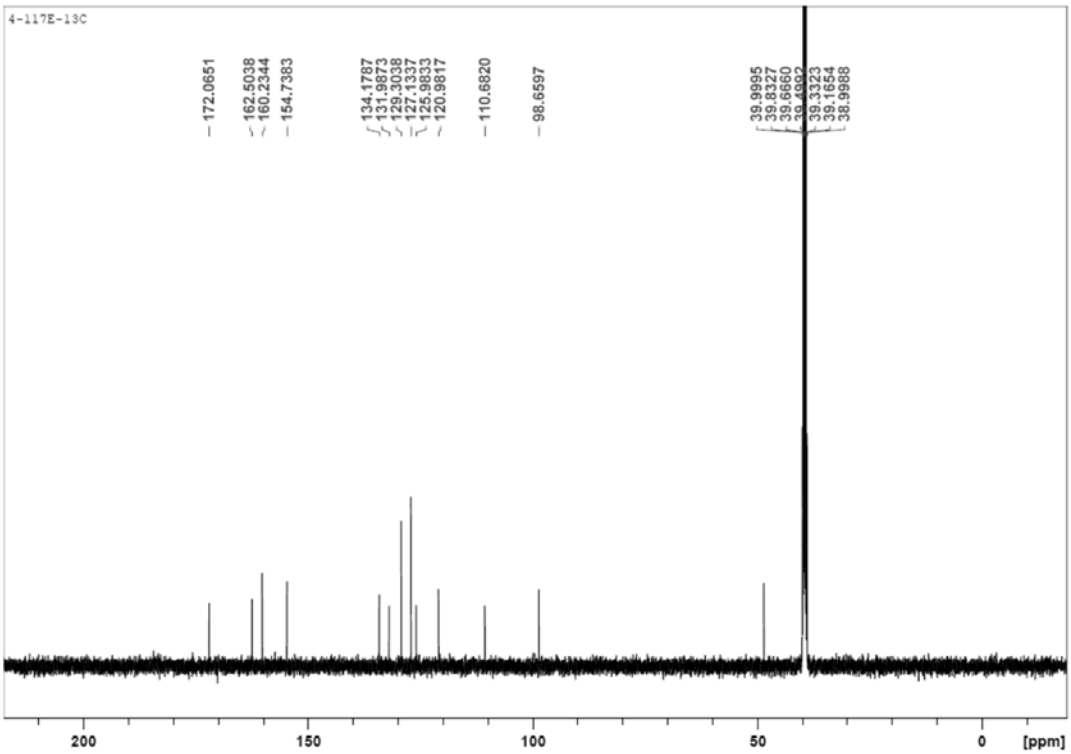
6
4
2
0
[ppm]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129.3038
127.1337
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98.6597
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39.4992
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39.1654
38.9988
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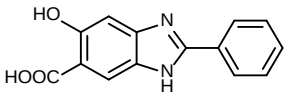
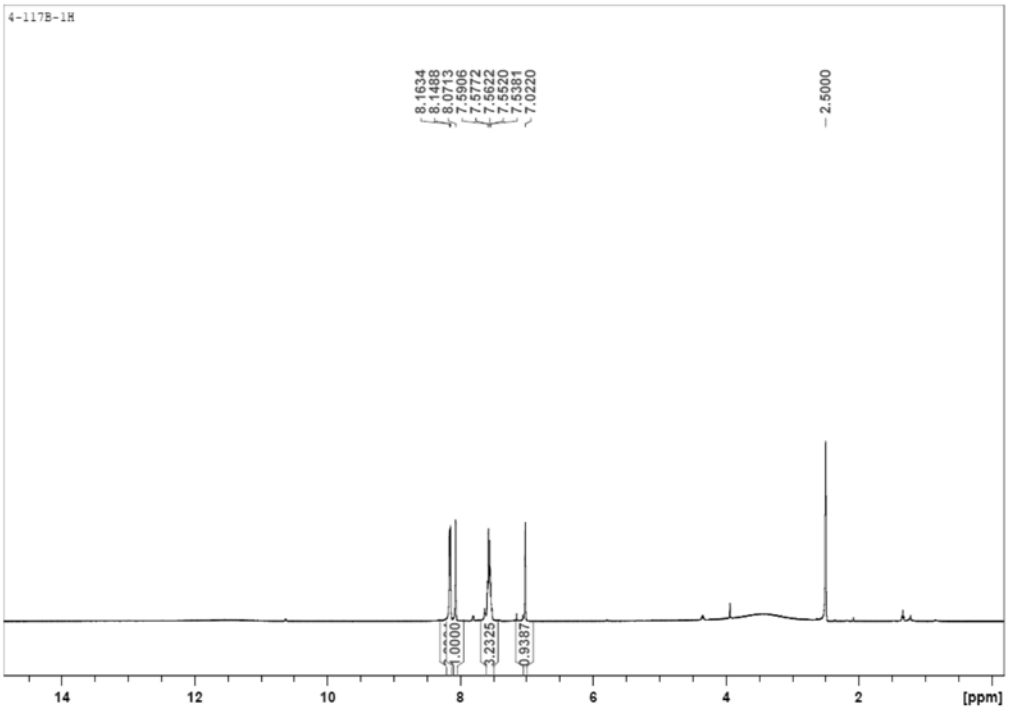
150
100
50
0
[ppm]


HO
N
HOOC
N
H

10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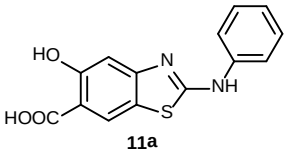
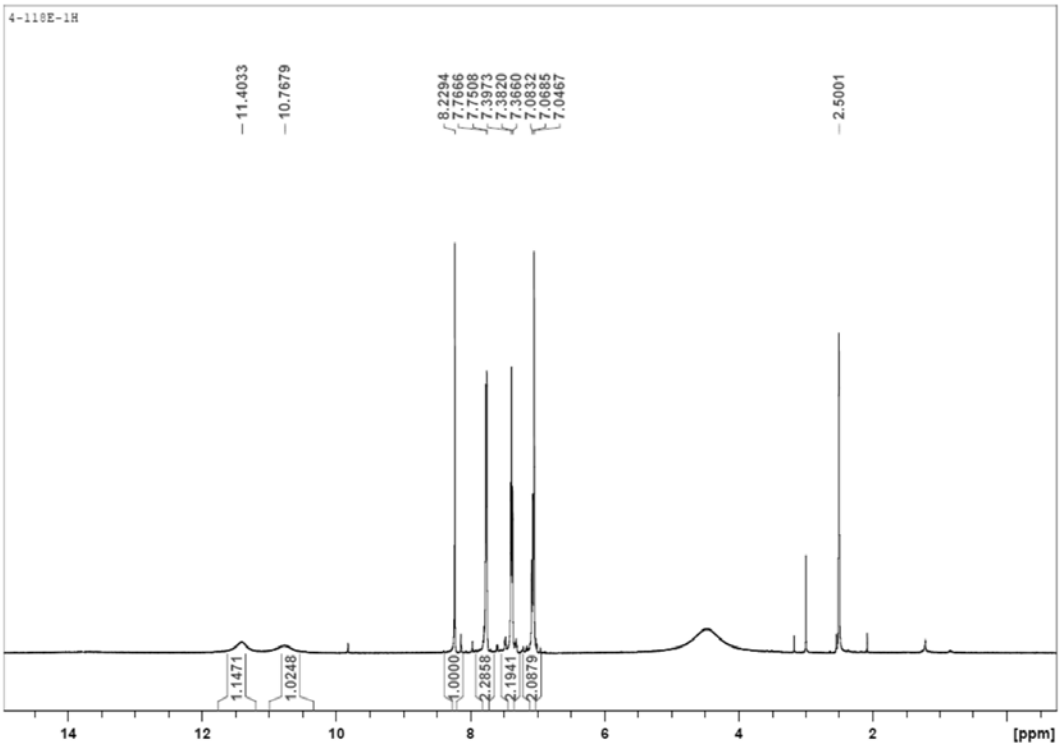
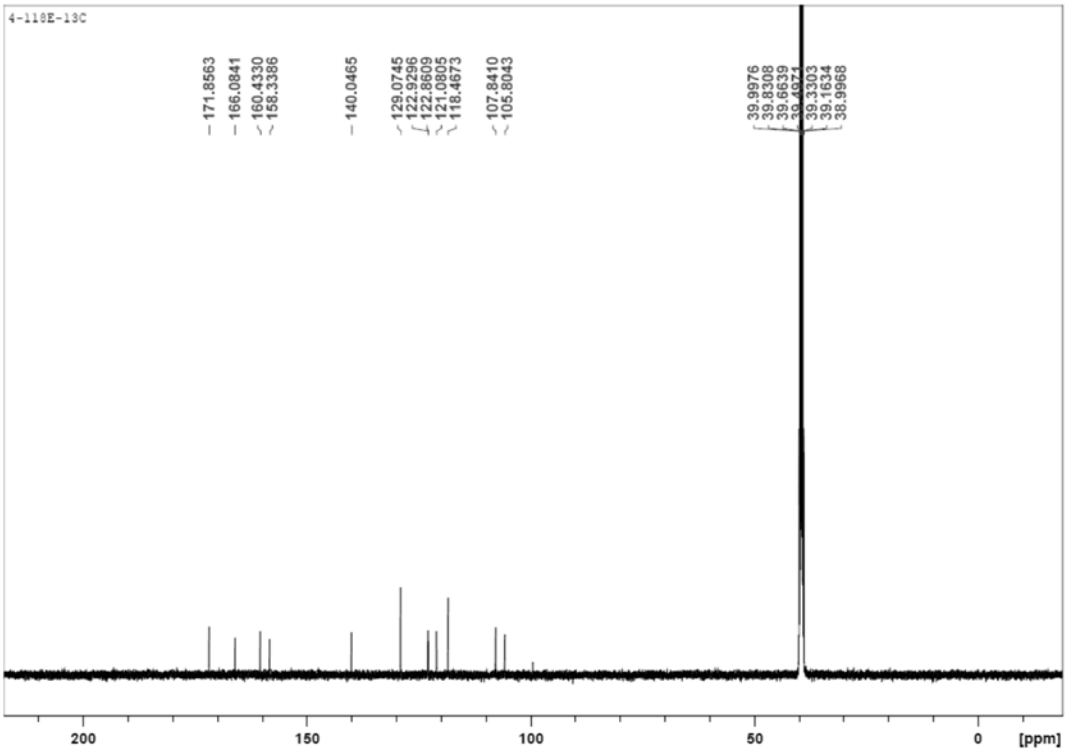
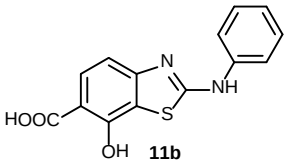
8.0713
7.5906
7.5772
7.5622
7.5520
7.5381
7.0220
2.5000
1.0000
3.2325
0.9387
14
12
10
8
6
4
2
[ppm]


HO
N
NH
HOOC
S

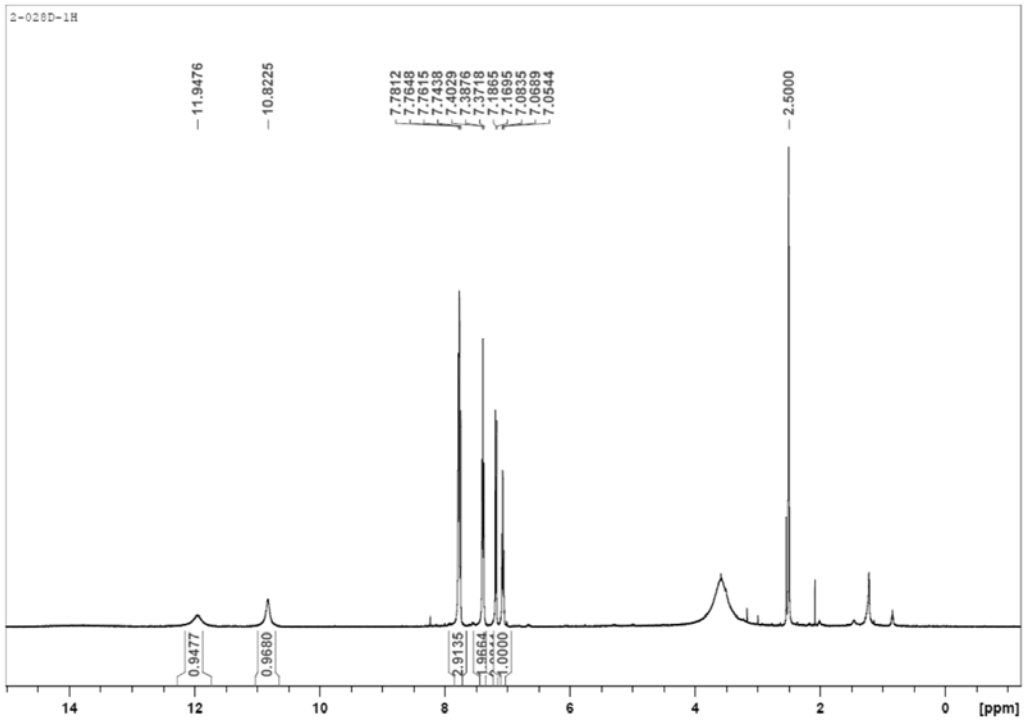
11a

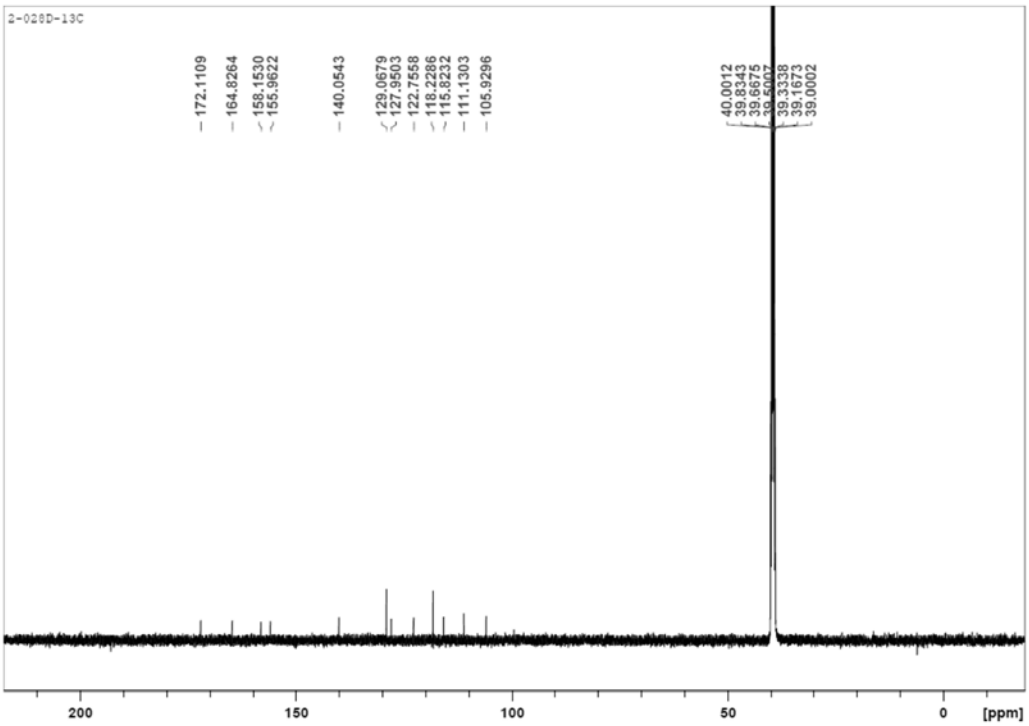

4-118E-1H
11.4033
10.7679
8.2294
7.7666
7.7508
7.3973
7.3820
7.3660
7.0832
7.0685
7.0467
2.5001
1.1471
1.0248
1.0000
2.2858
2.1941
2.0879
14
12
10
8
6
4
2
[ppm]

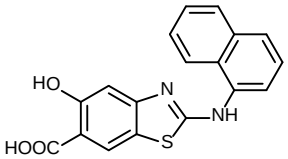

4-118E-13C
171.8563
166.0841
160.4330
158.3386
140.0465
129.0745
122.9296
122.8609
121.0805
118.4673
107.8410
105.8043
39.9976
39.8308
39.6639
39.4971
39.3303
39.1634
38.9968
200
150
100
50
0
[ppm]


N
NH
S
HOOC
OH

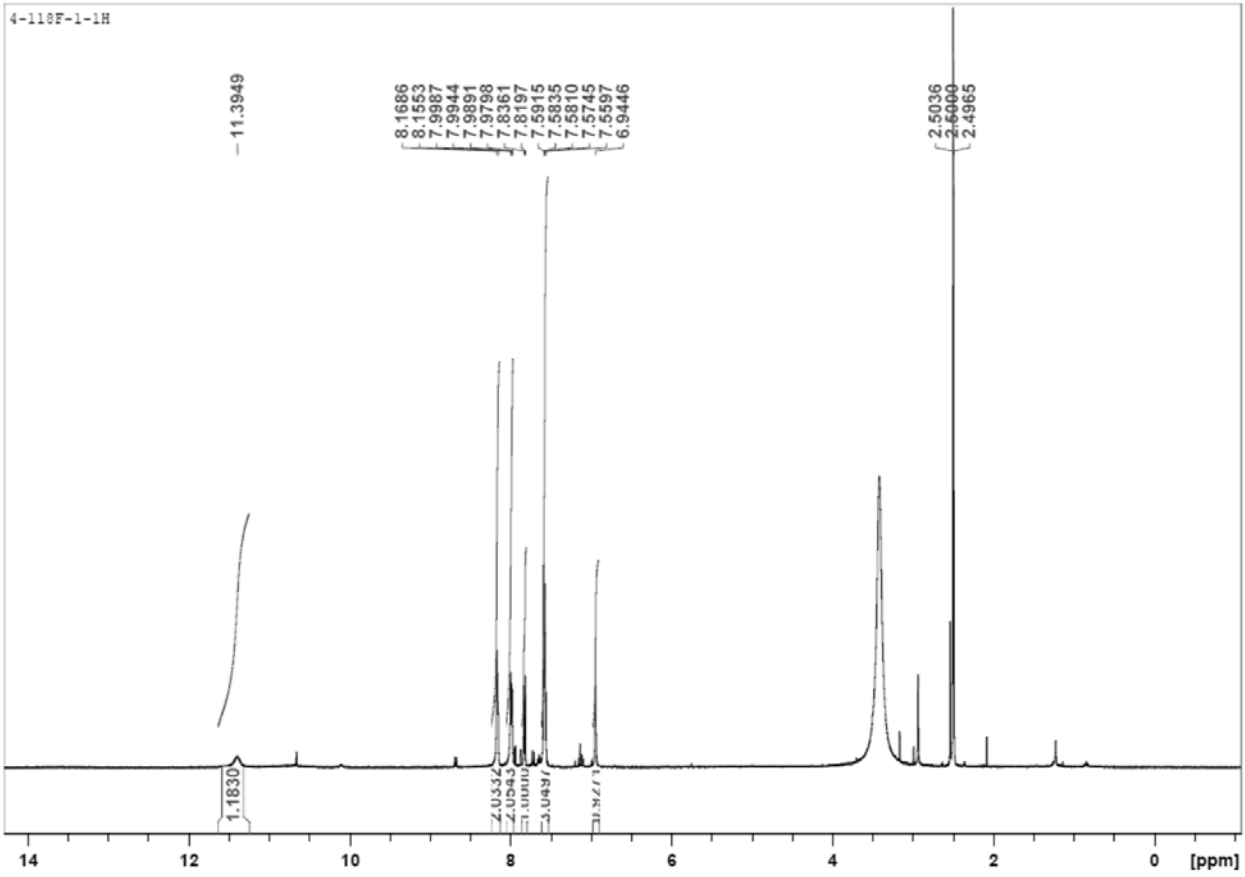
11b

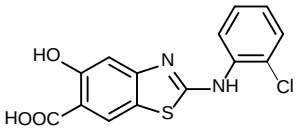

2-028D-1H
11.9476
10.8225
7.7812
7.7648
7.7615
7.7438
7.4029
7.3876
7.3718
7.1865
7.1695
7.0835
7.0689
7.0544
2.5000
0.9477
0.9680
2.9135
1.9664
1.0000
14
12
10
8
6
4
2
0
[ppm]


2-028D-13C
172.1109
164.8264
158.1530
155.9622
140.0543
129.0679
127.9503
122.7558
118.2286
115.8232
111.1303
105.9296
40.0012
39.8343
39.6675
39.5007
39.3338
39.1673
39.0002
200
150
100
50
0
[ppm]

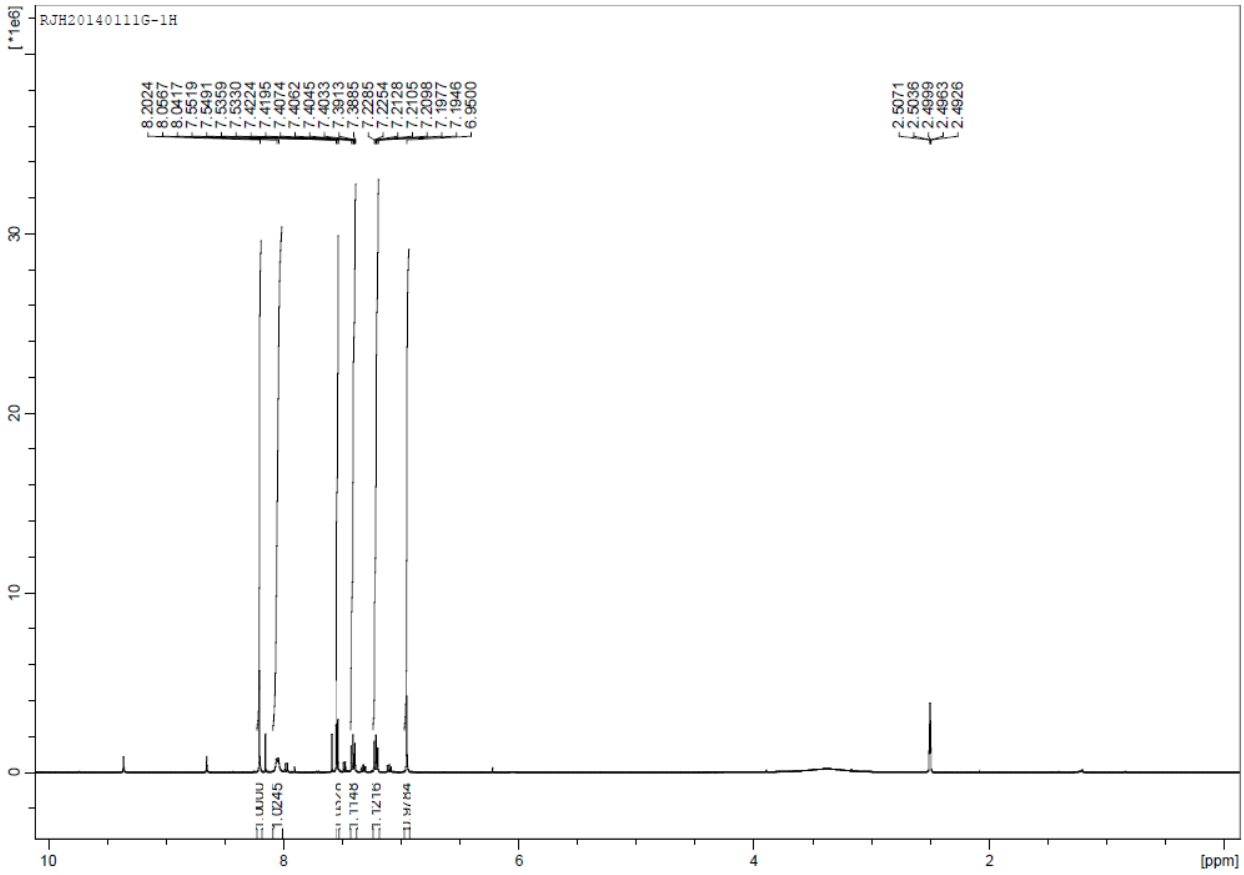

HO
N
NH
S
HOOC

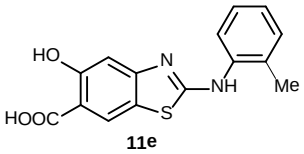
11c


4-110F-1-1H
11.3949
8.1686
8.1553
7.9987
7.9944
7.9891
7.9798
7.8361
7.8197
7.5915
7.5835
7.5810
7.5745
7.5597
6.9446
2.5036
2.6000
2.4965
1.1830
14
12
10
8
6
4
2
0
[ppm]

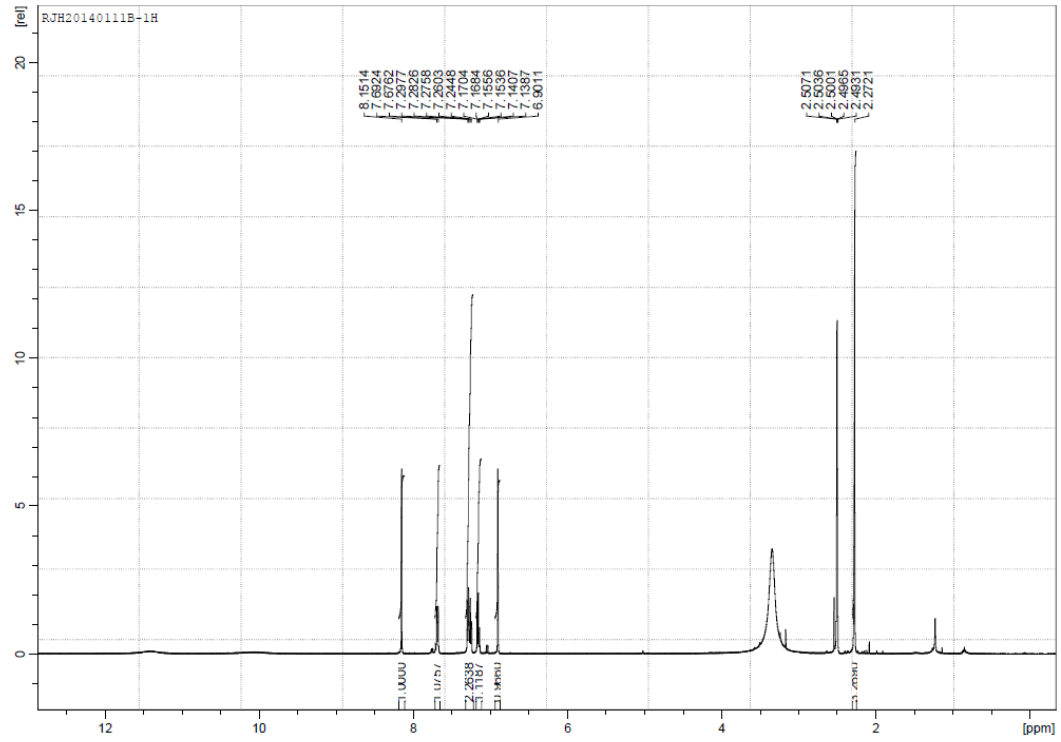

HO
N
NH
Cl
HOOC
S

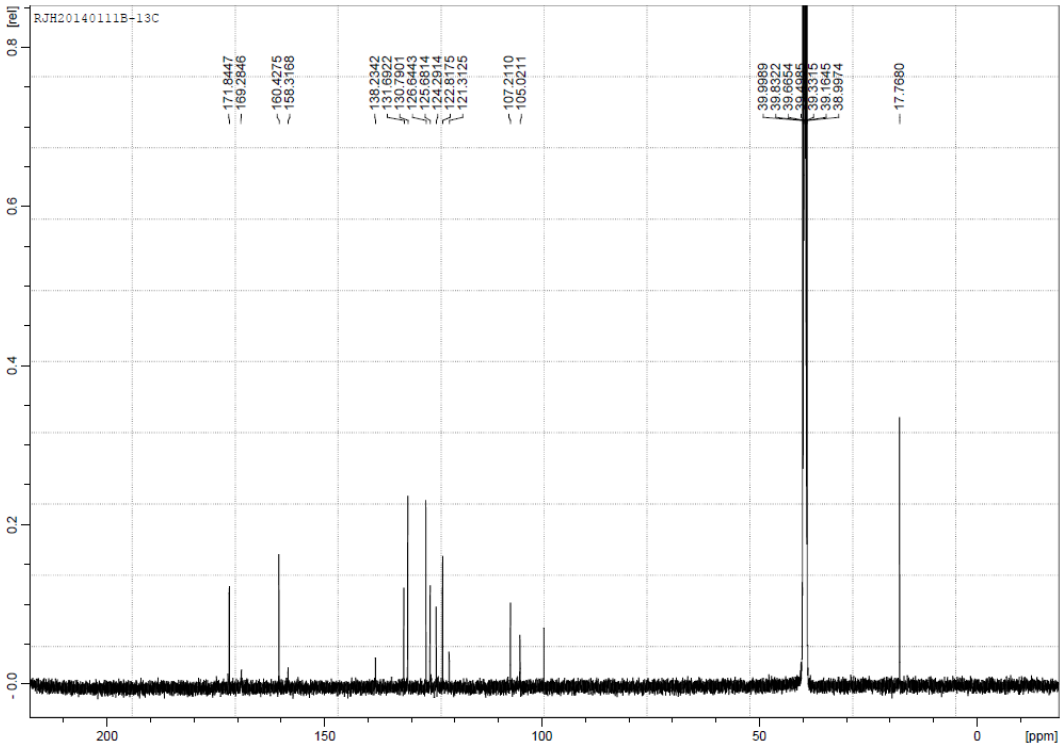
11d

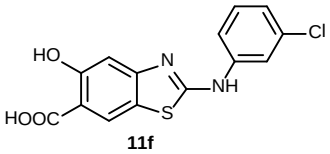

RJH20140111G-1H
[*1e6]
8.2024
8.0567
8.0417
7.5519
7.5491
7.5359
7.5330
7.4224
7.4195
7.4074
7.4062
7.4045
7.4033
7.3913
7.3885
7.2285
7.2254
7.2128
7.2105
7.2098
7.1977
7.1946
6.9500
2.5071
2.5036
2.4999
2.4963
2.4926
1.0000
1.0245
1.1148
1.7216
30
20
10
0
10
8
6
4
2
[ppm]


HO
N
NH
Me
HOOC
S

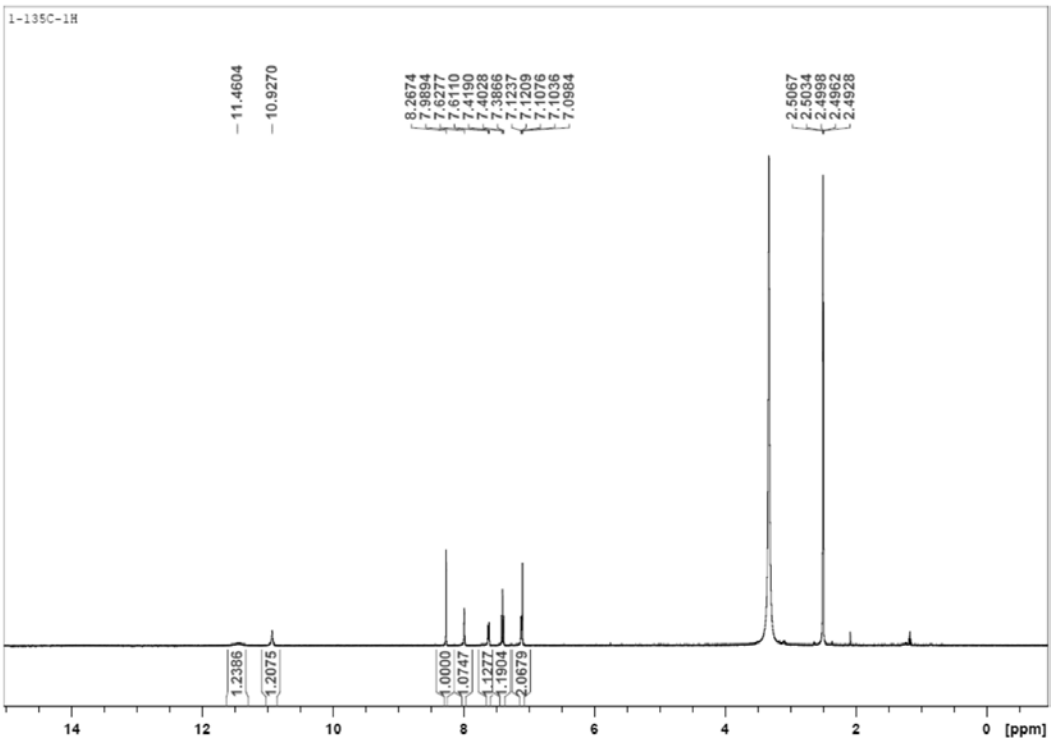
11e

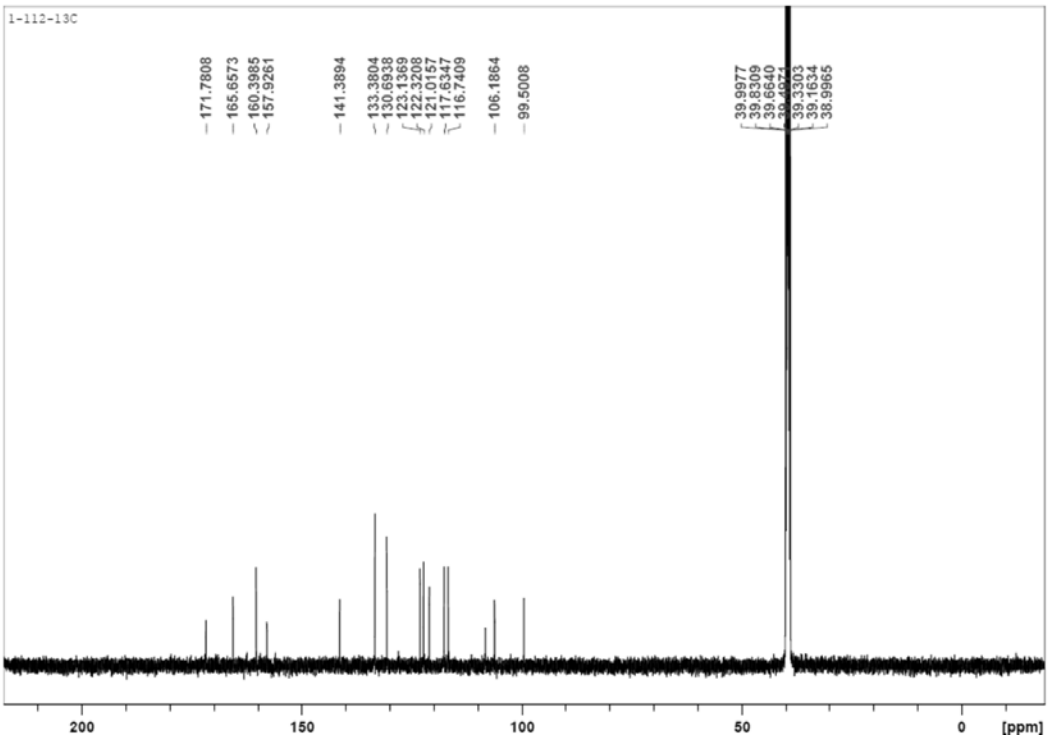

RJH20140111B-1H
[rel]
8.1514
7.6764
7.6562
7.2977
7.2826
7.2758
7.2603
7.2448
7.1704
7.1684
7.1556
7.1536
7.1407
7.1387
6.9011
2.5071
2.5036
2.5001
2.4965
2.4831
2.2721
[ppm]

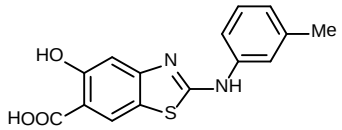

RJH20140111B-13C
[rel]
171.8447
169.2846
160.4275
158.3168
138.2342
131.6922
130.7901
126.6443
125.6814
124.2914
122.8175
121.3125
107.2110
105.0211
39.9989
39.8322
39.6654
39.4986
39.3315
39.1645
38.9974
17.7680
[ppm]


HO
HOOC
N
S
NH
Cl

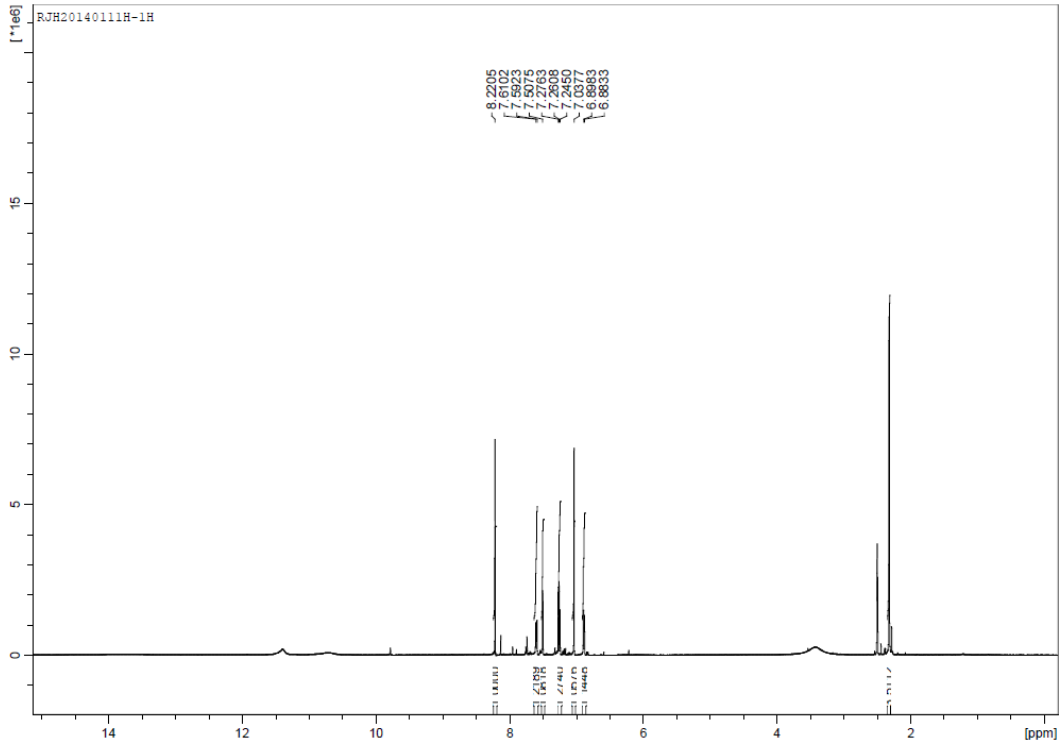
11f

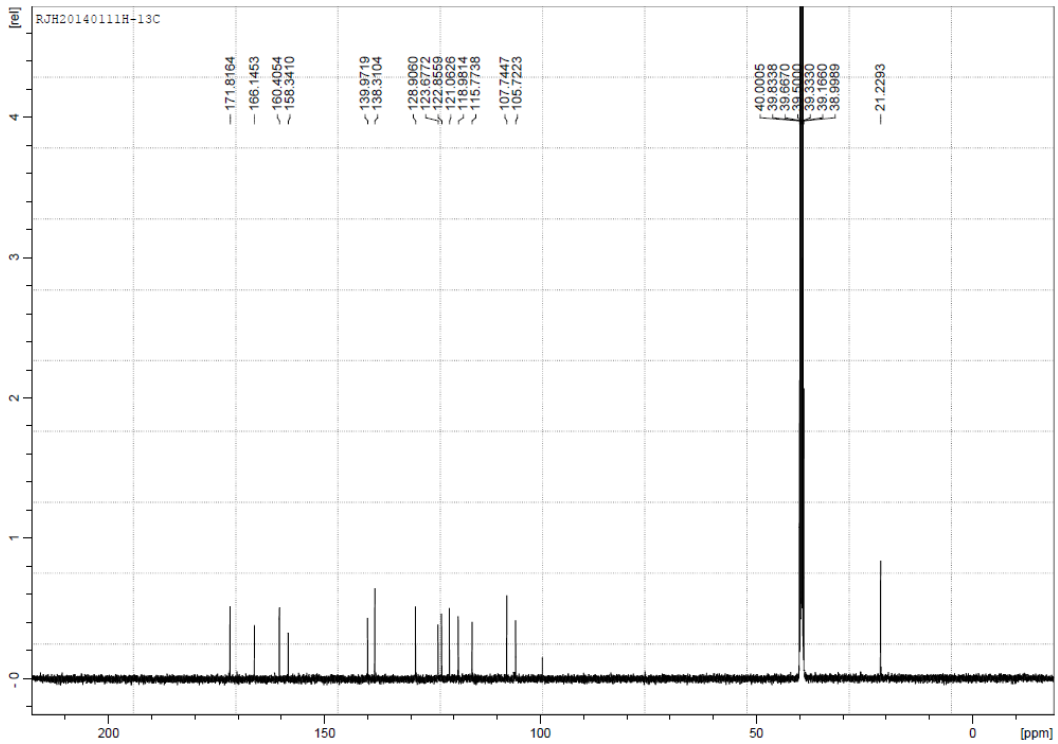

1-135C-1H
11.4604
10.9270
8.2674
7.9894
7.6277
7.6110
7.4190
7.4028
7.3866
7.1237
7.1209
7.1076
7.1036
7.0984
2.5067
2.5034
2.4998
2.4962
2.4928
1.2386
1.2075
1.0000
1.0747
1.1277
1.1904
2.0679
14
12
10
8
6
4
2
0
[ppm]

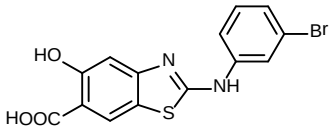

1-112-13C
171.7808
165.6573
160.3985
157.9261
141.3894
133.3804
130.6938
123.1369
122.3208
121.0157
117.6347
116.7409
106.1864
99.5008
39.9977
39.8309
39.6640
39.4971
39.3303
39.1634
38.9965
200
150
100
50
0
[ppm]


HO
N
Me
NH
HOOC
S

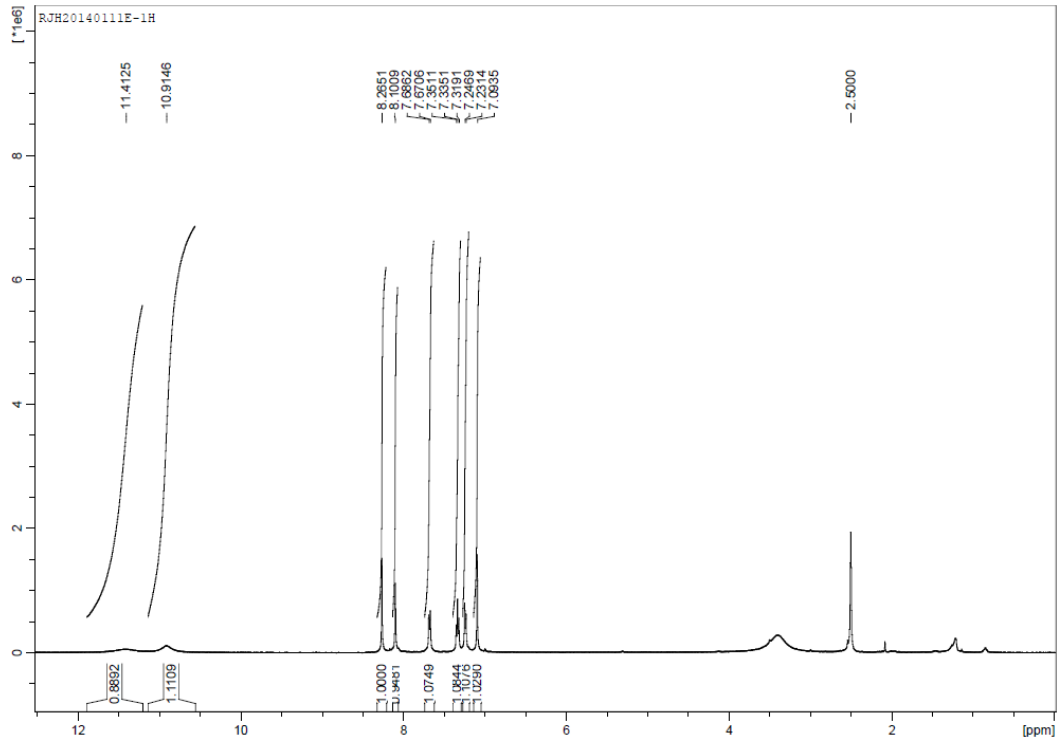
11g

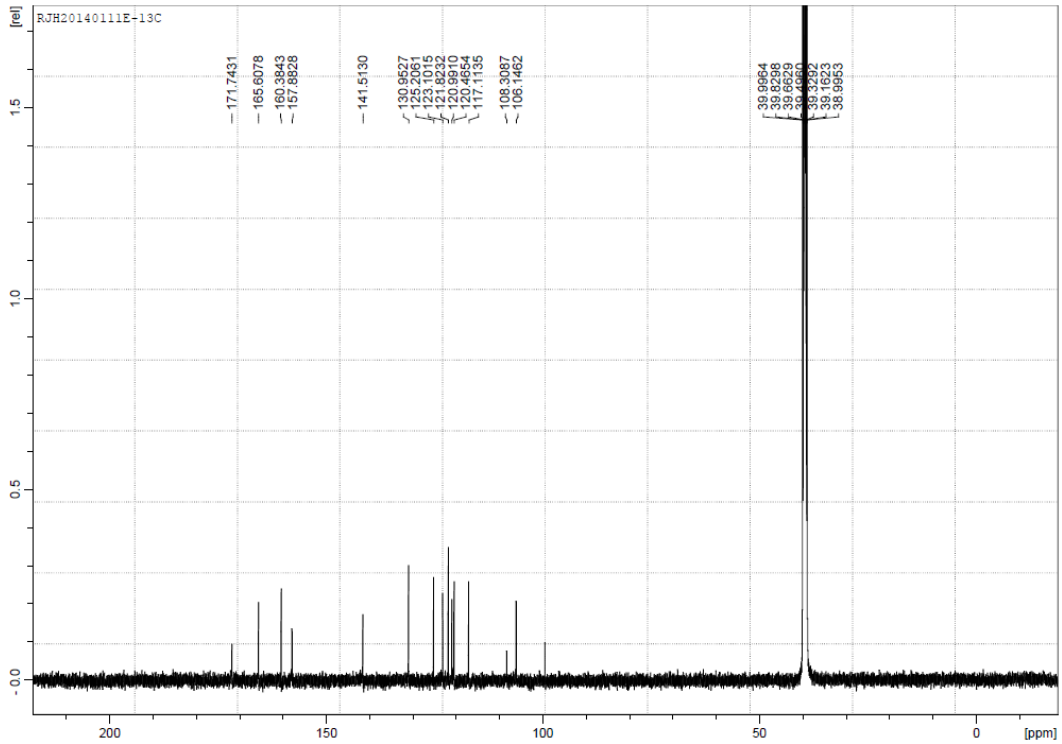

RJH20140111H-1H
[*1e6]
8.2205
7.6102
7.5823
7.5075
7.2763
7.2608
7.2450
7.0077
6.8983
6.8833
[ppm]

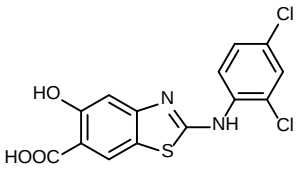

RJH20140111H-13C
[rel]
171.8164
166.1453
160.4054
158.3410
139.9719
138.3104
128.9060
123.6772
122.8559
121.0626
118.9814
115.7738
107.7447
105.7223
40.0005
39.8338
39.6670
39.5000
39.3330
39.1660
38.9989
21.2293
[ppm]


HO
HOOC
N
S
NH
Br

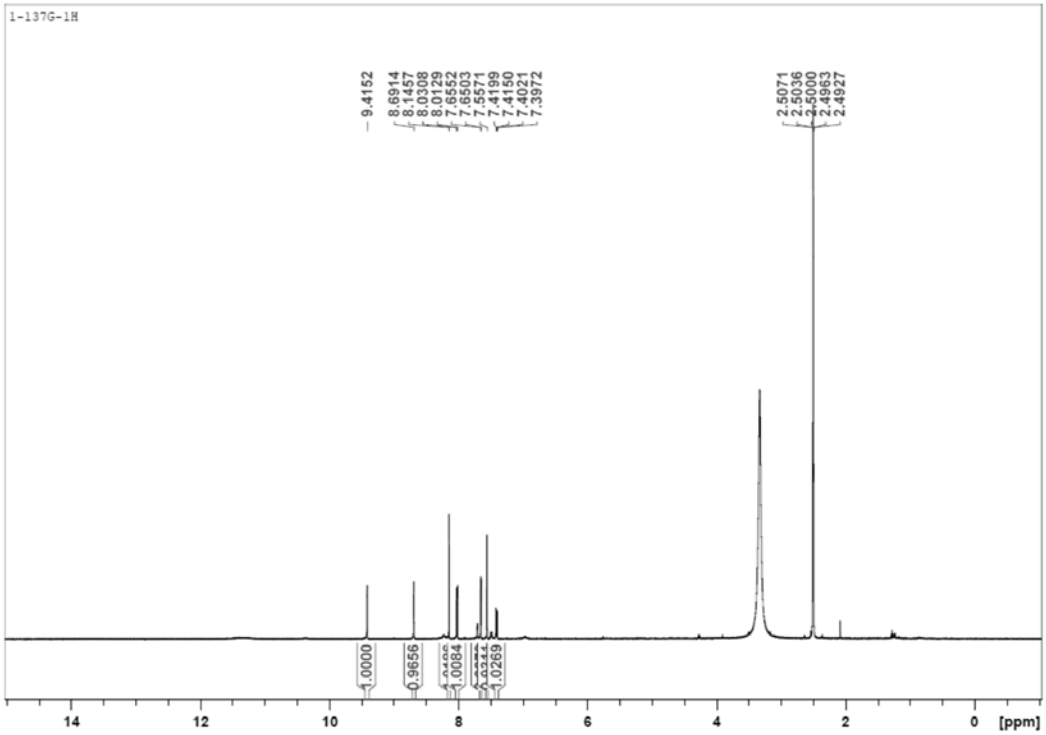
11h

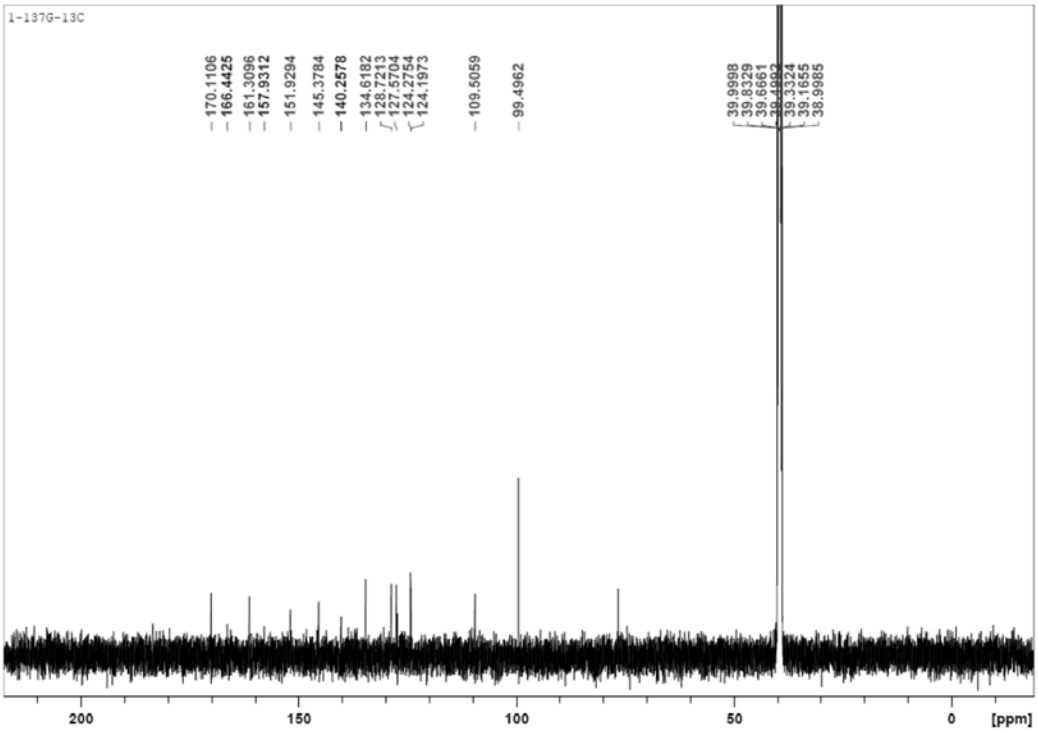

RJH20140111E-1H
[*1e6]
11.4125
10.9146
8.2651
8.1009
7.6862
7.6706
7.3551
7.3511
7.3191
7.2469
7.2314
7.0935
2.5000
0.8892
1.1109
1.0000
0.9451
1.0749
1.0844
1.0076
1.0290
[ppm]

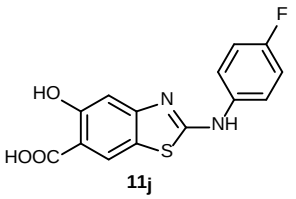

RJH20140111E-13C
[rel]
171.7431
165.6078
160.3843
157.8828
141.5130
130.9527
125.2061
123.1015
121.8232
120.9910
120.4654
117.1135
108.3087
106.1462
39.9964
39.8298
39.6629
39.4960
39.3292
39.1623
38.9953
[ppm]


Cl
HO
N
NH
Cl
HOOC
S

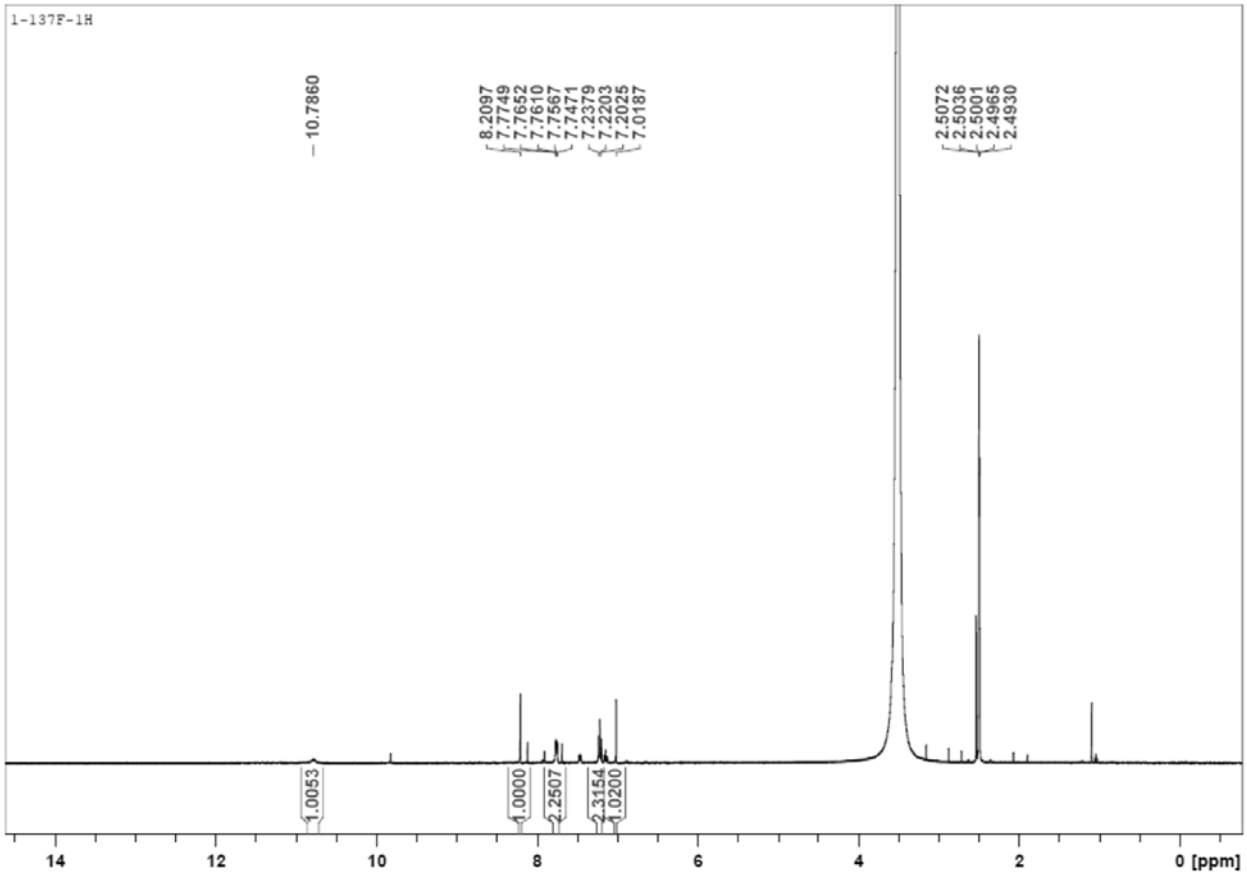
11i

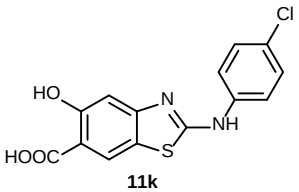

1-137G-1H
9.4152
8.6914
8.1457
8.0308
8.0129
7.6552
7.6503
7.5571
7.4199
7.4150
7.4021
7.3972
2.5071
2.5036
2.5000
2.4963
2.4927
1.0000
0.9656
1.0084
1.0269
14
12
10
8
6
4
2
0
[ppm]


1-137G-13C
170.1106
166.4425
161.3096
157.9312
151.9294
145.3784
140.2578
134.6182
128.7213
127.5704
124.2754
124.1973
109.5059
99.4962
39.9998
39.8329
39.6661
39.3324
39.1655
38.9985
200
150
100
50
0
[ppm]

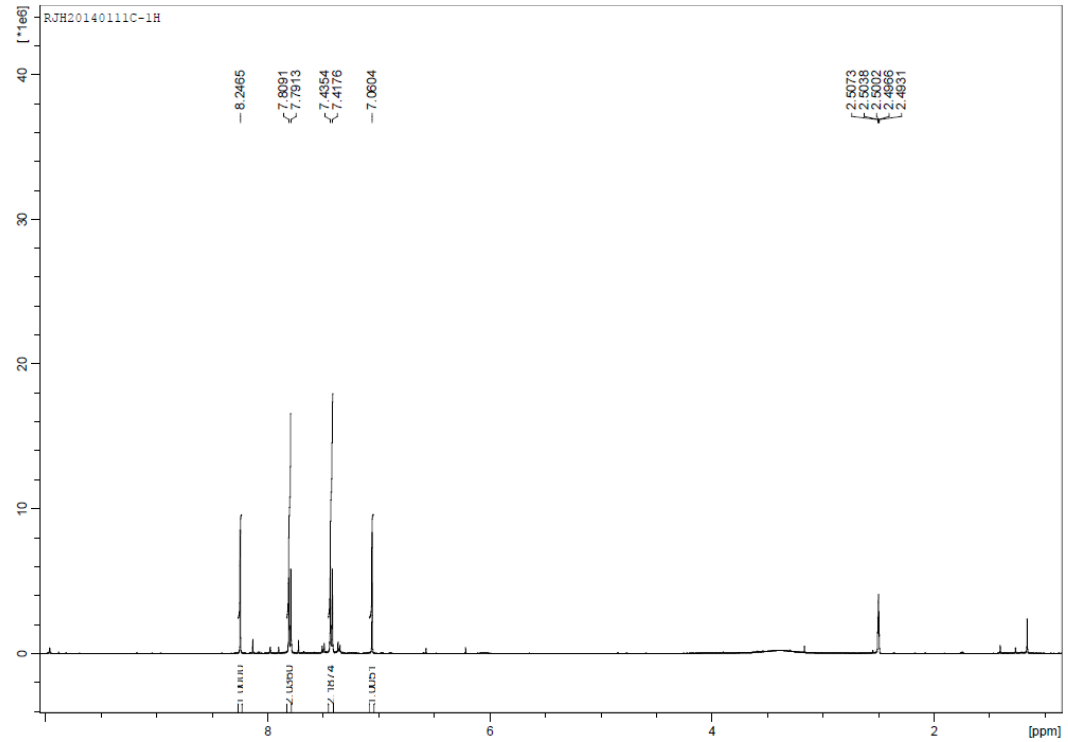

F
HO
N
NH
HOOC
S

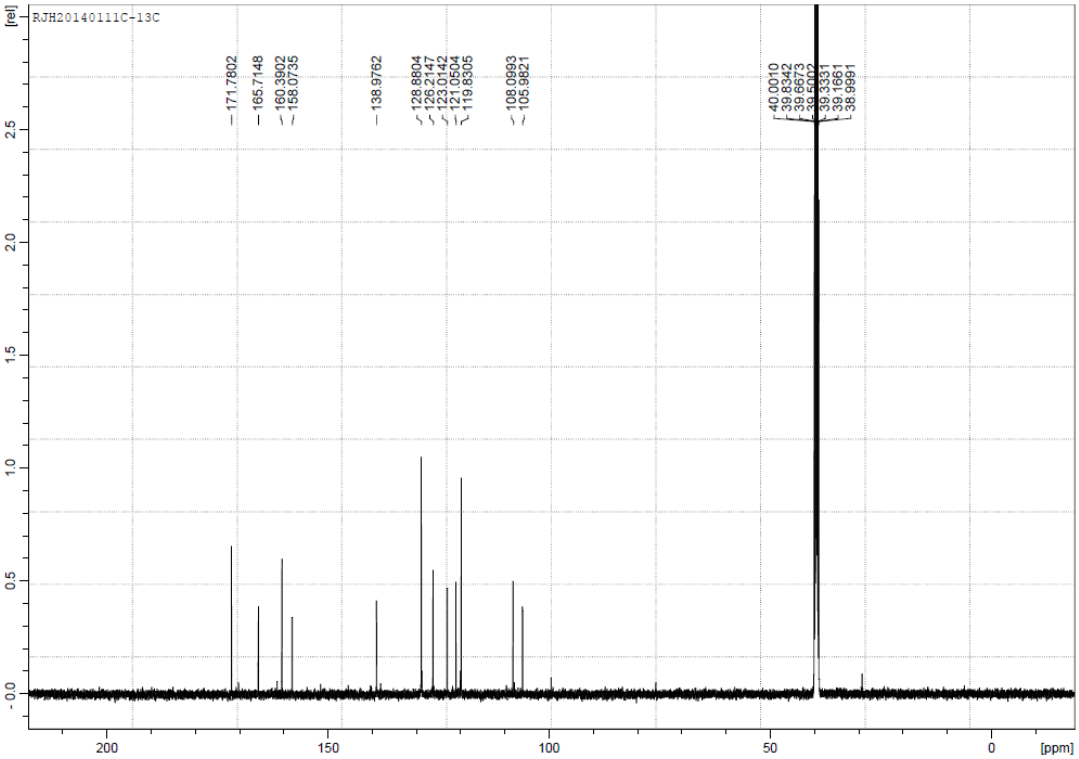
11j

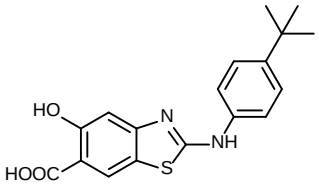

1-137F-1H
10.7860
8.2097
7.7749
7.7652
7.7610
7.7567
7.7471
7.2379
7.2203
7.2025
7.0187
2.5072
2.5036
2.5001
2.4965
2.4930
1.0053
1.0000
2.2507
2.3154
1.0200
14
12
10
8
6
4
2
0 [ppm]


Cl
HO
N
NH
HOOC
S

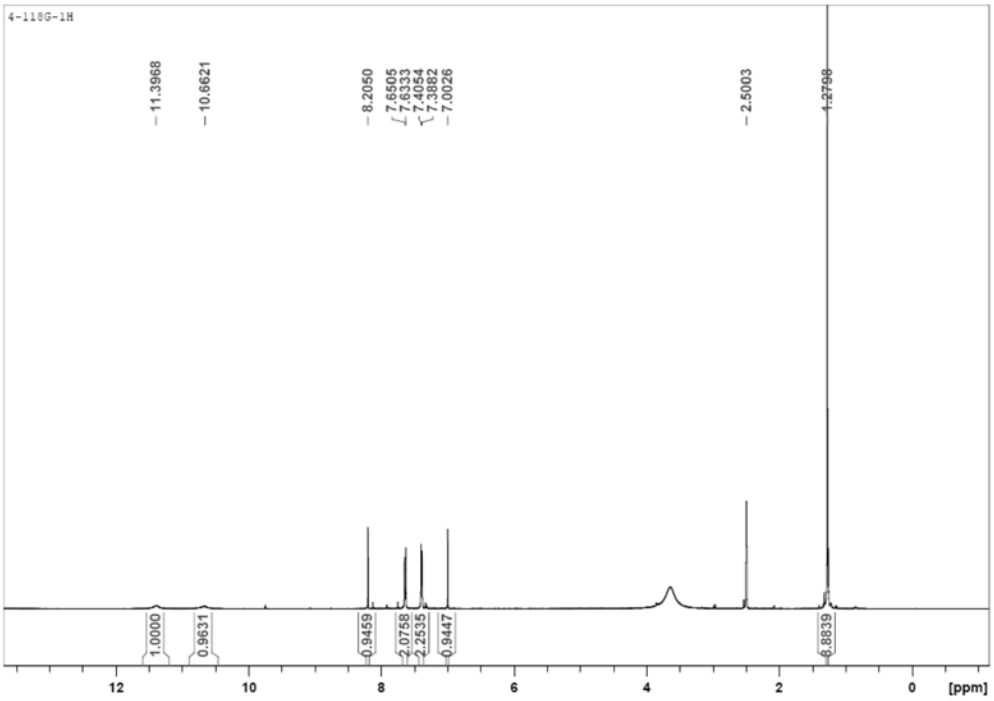
11k

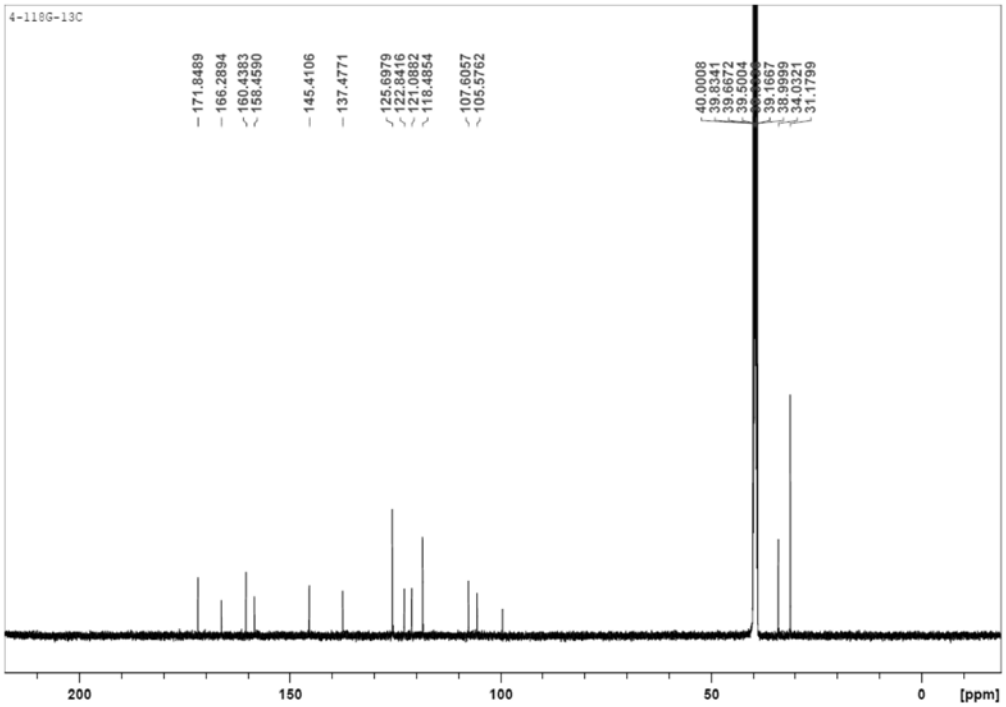

RJH20140111C-1H
[*1e6]
8.2465
7.8091
7.7913
7.4354
7.4176
7.0604
2.5073
2.5038
2.5002
2.4966
2.4931
[ppm]

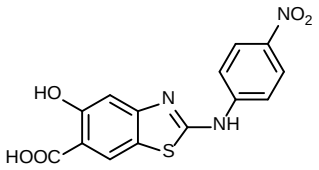

RJH20140111C-13C
[rel]
171.7802
165.7148
160.3902
158.0735
138.9762
128.8804
126.2147
123.0142
121.0504
119.8305
108.0993
105.9821
40.0010
39.8342
39.6673
39.5002
39.3331
39.1661
38.9991
[ppm]


HO
N
NH
HOOC
S

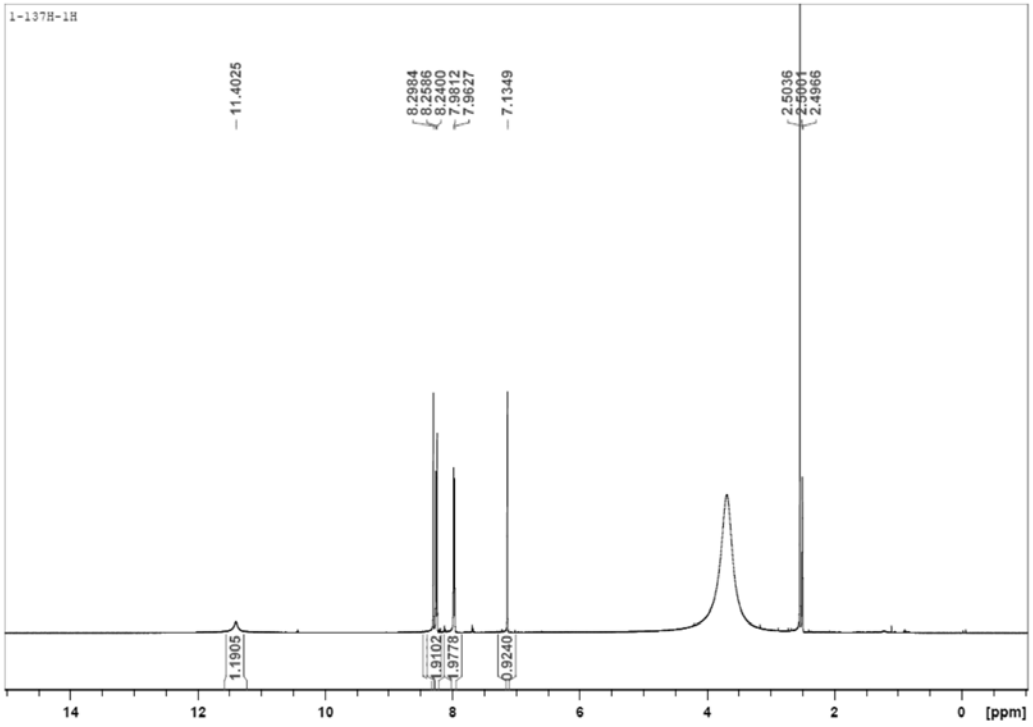
11l


4-118G-1H
11.3968
10.6621
8.2050
7.6505
7.6333
7.4054
7.3882
7.0026
2.5003
1.2798
1.0000
0.9631
0.9459
2.0758
2.2535
0.9447
6.8839
12
10
8
6
4
2
0
[ppm]


4-118G-13C
171.8489
166.2894
160.4383
158.4590
145.4106
137.4771
125.6979
122.8416
121.0882
118.4854
107.6057
105.5762
40.0008
39.8341
39.6672
39.5004
39.1667
38.9999
34.0321
31.1799
200
150
100
50
0
[ppm]


NO2
HO
N
NH
HOOC
S

11m


1-137H-1H
11.4025
8.2984
8.2586
8.2400
7.9812
7.9627
7.1349
2.5036
2.5001
2.4966
1.1905
1.9102
1.9778
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8
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4
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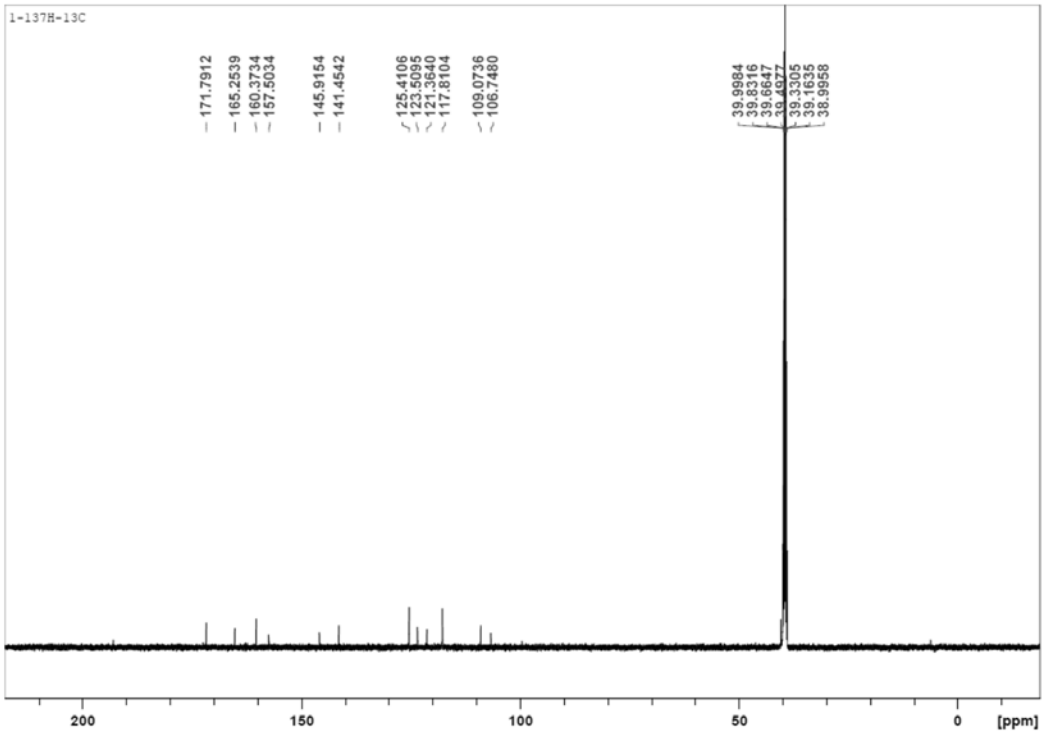
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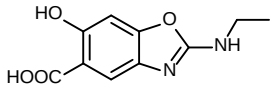
[ppm]

Copies of LC-MS Spectra of compounds **5-11**

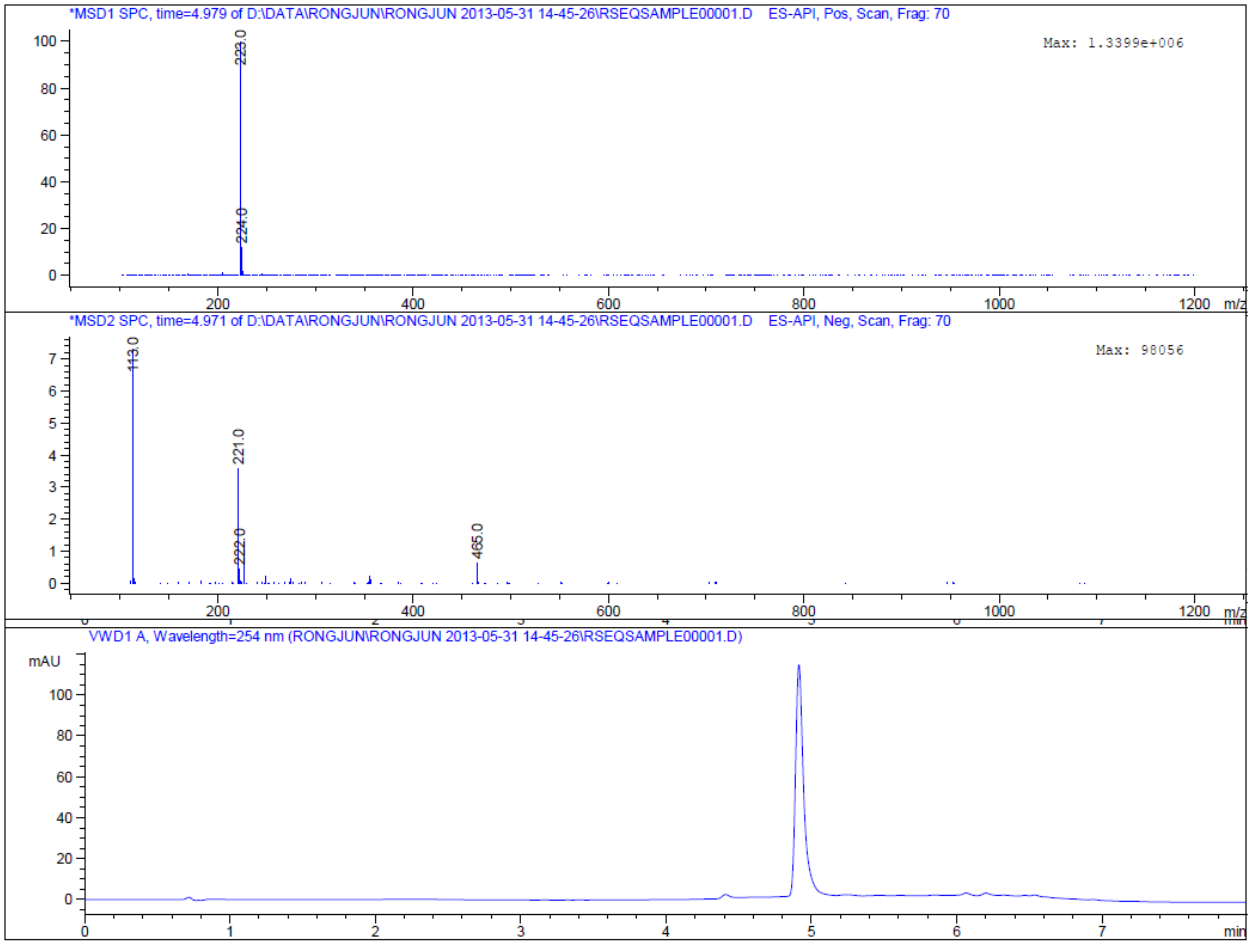


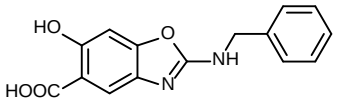
5a

Chemical Formula: $C_{10}H_{10}N_2O_4$

Exact Mass: 222.0641

Molecular Weight: 222.1974





5b

Chemical Formula: $C_{15}H_{12}N_2O_4$

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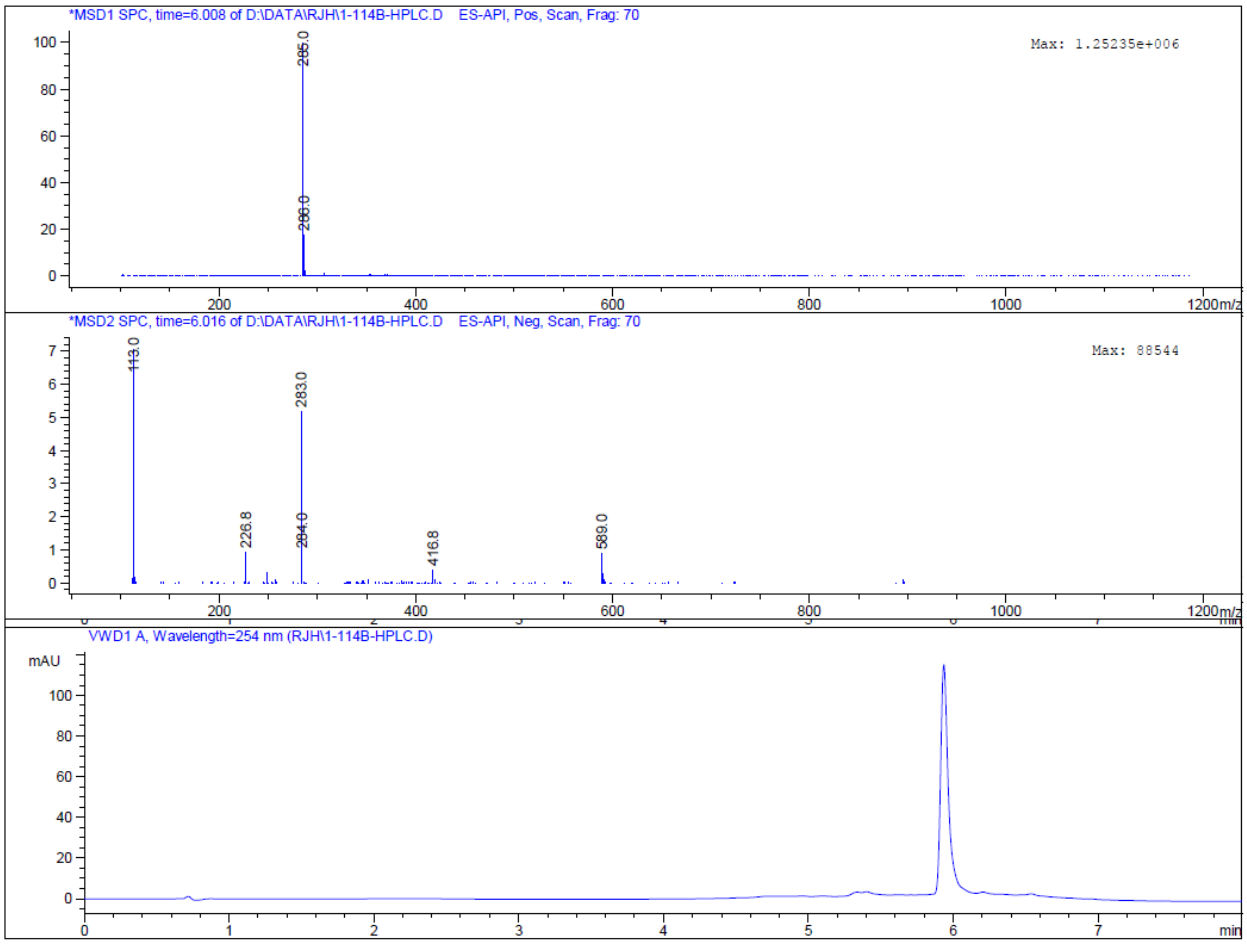
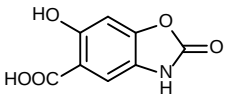
Molecular Weight: 284.2668



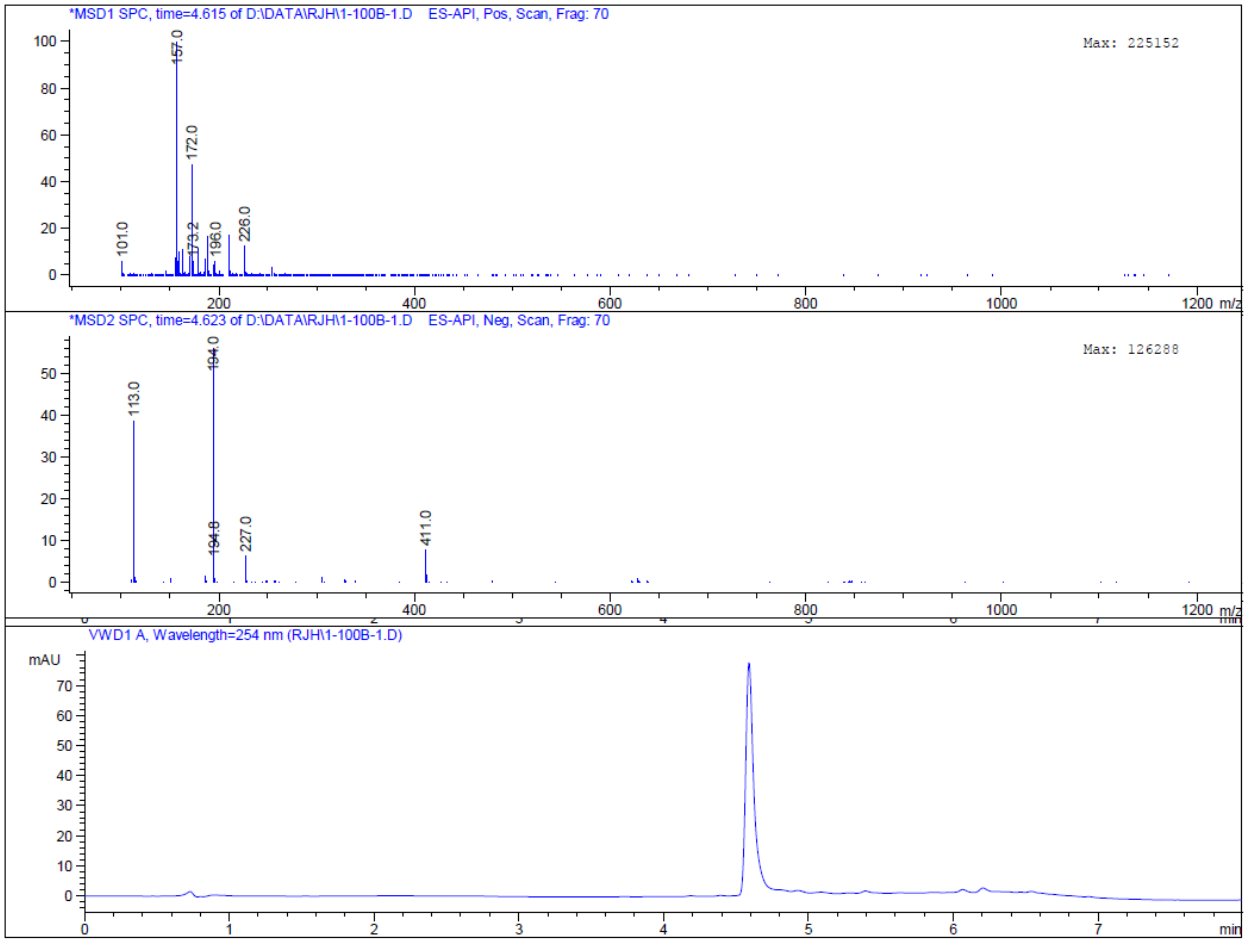


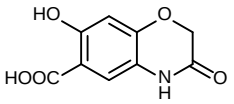
6

Chemical Formula: $C_8H_5NO_5$

Exact Mass: 195.0168

Molecular Weight: 195.1290





7

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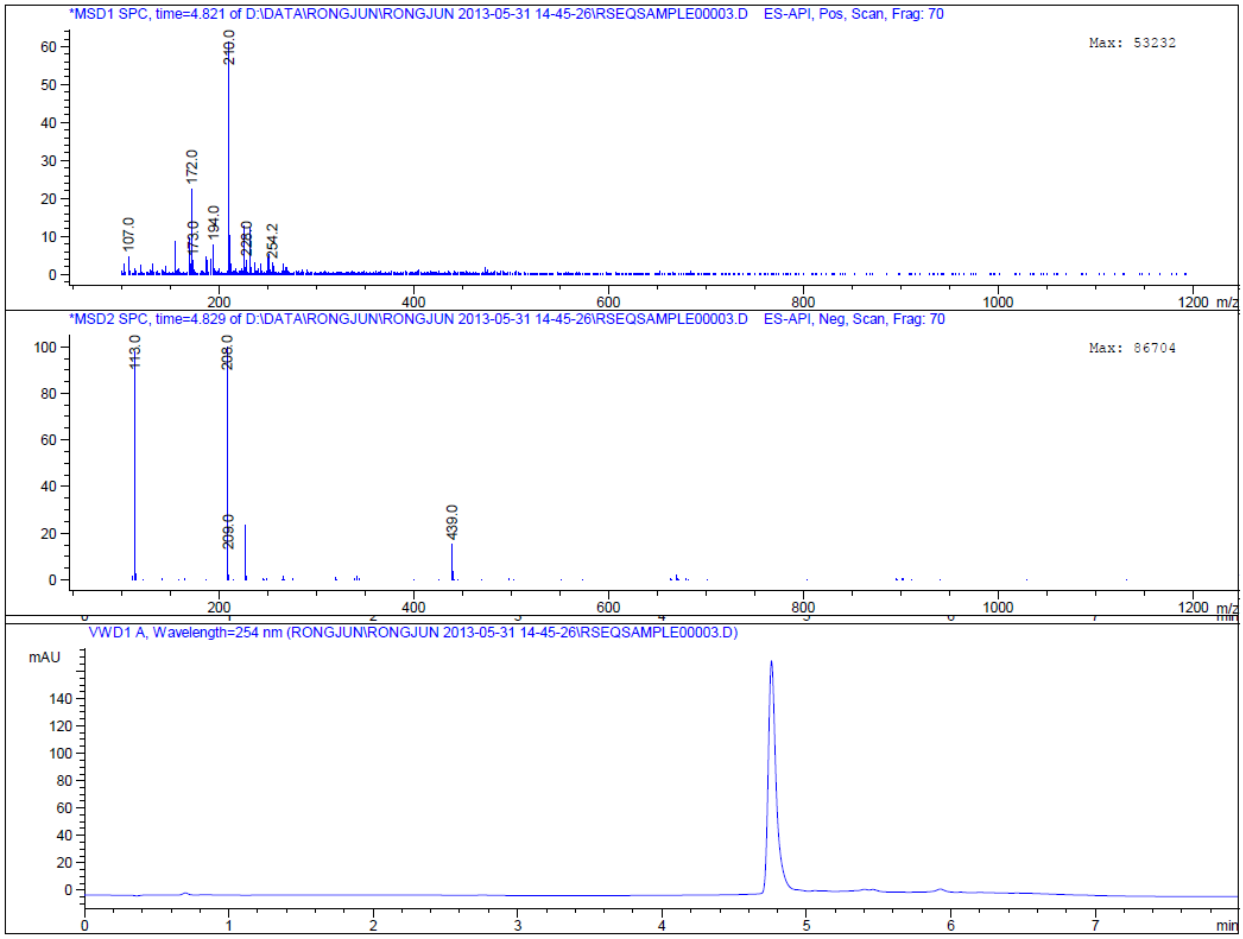
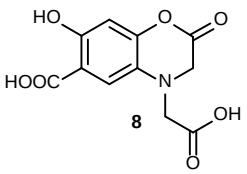
Exact Mass: 209.0324

Molecular Weight: 209.1556



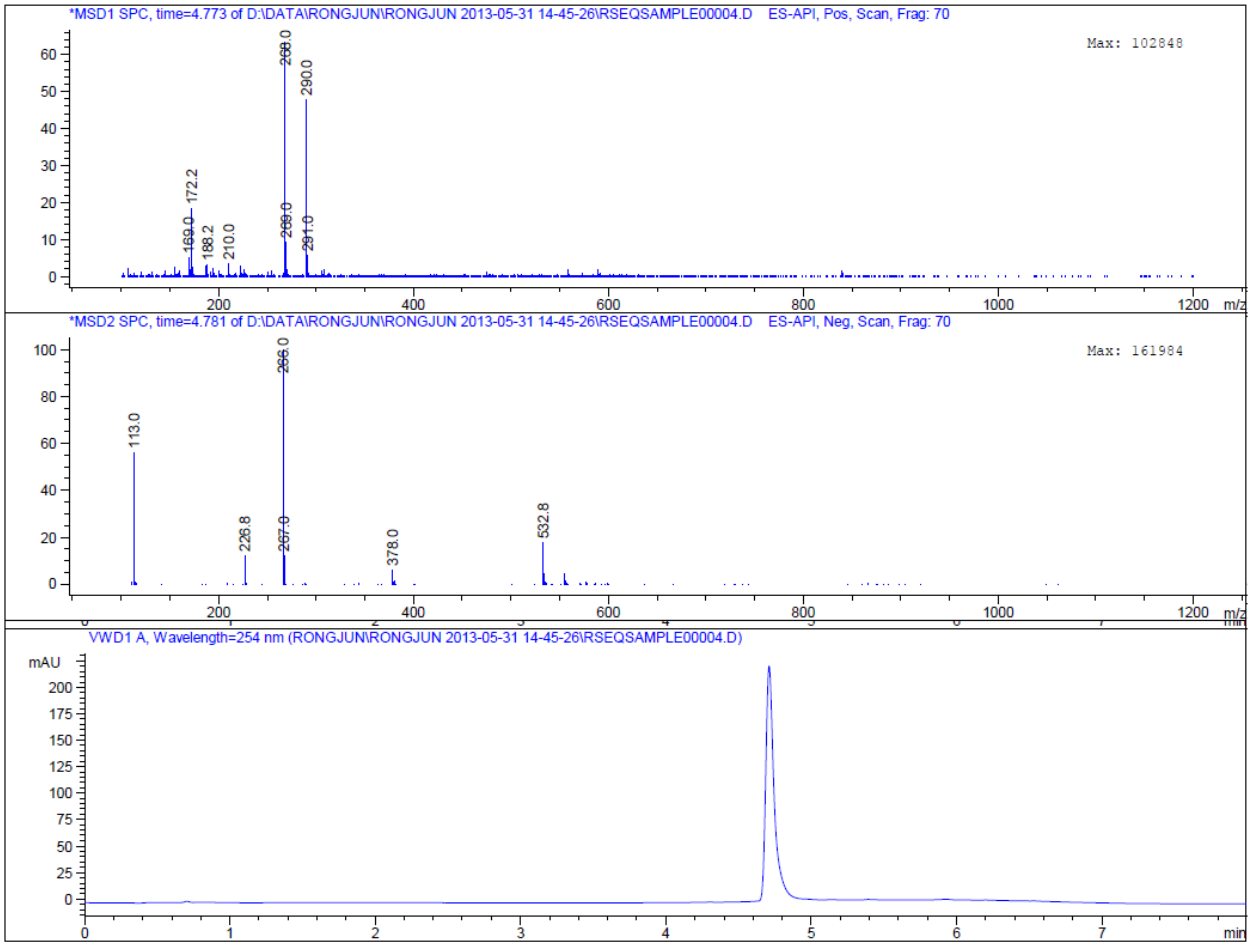


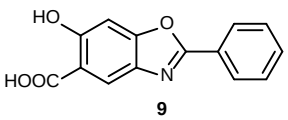
8

Chemical Formula: $C_{11}H_9NO_7$

Exact Mass: 267.0379

Molecular Weight: 267.1917



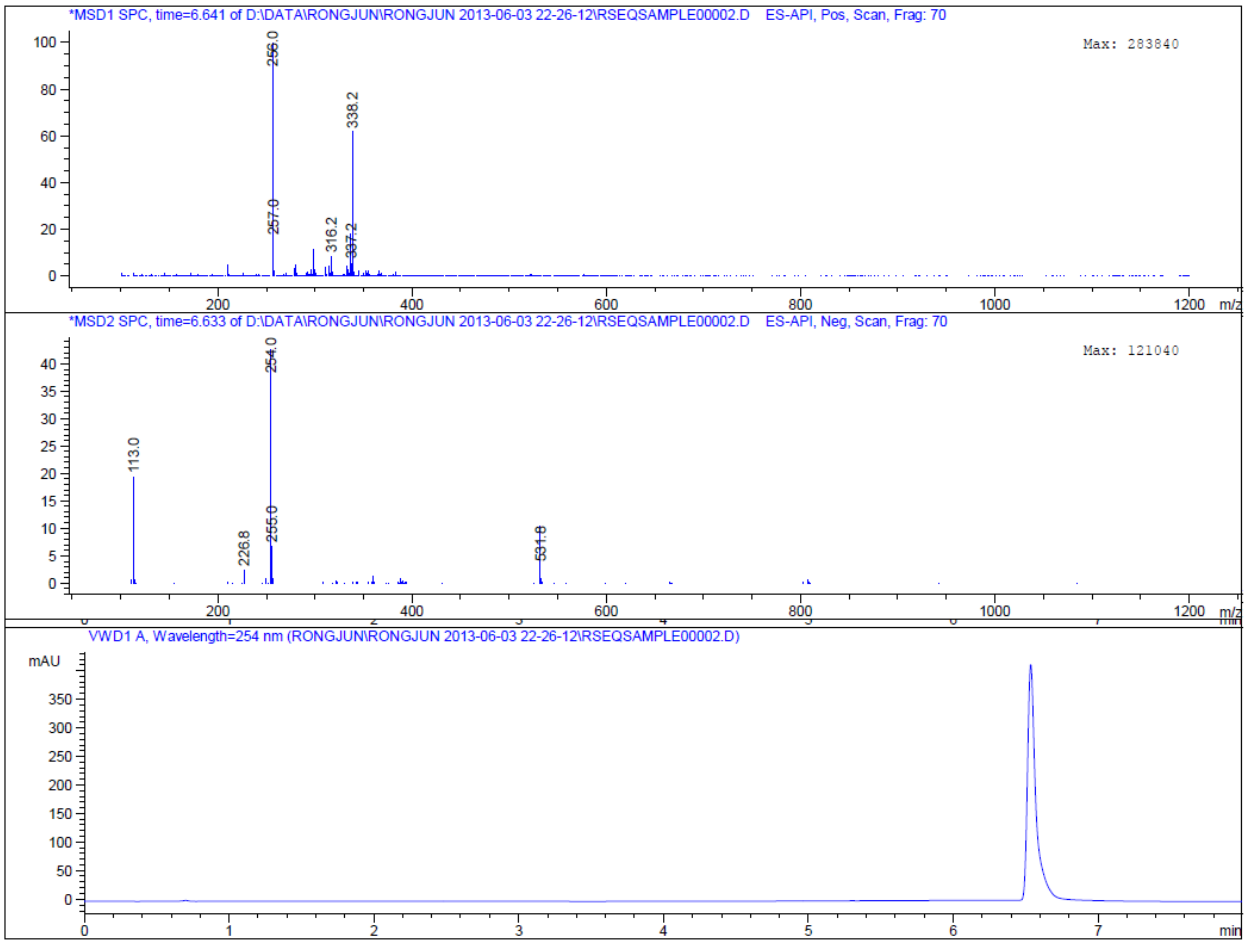


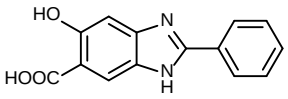
9

Chemical Formula: $C_{14}H_9NO_4$

Exact Mass: 255.0532

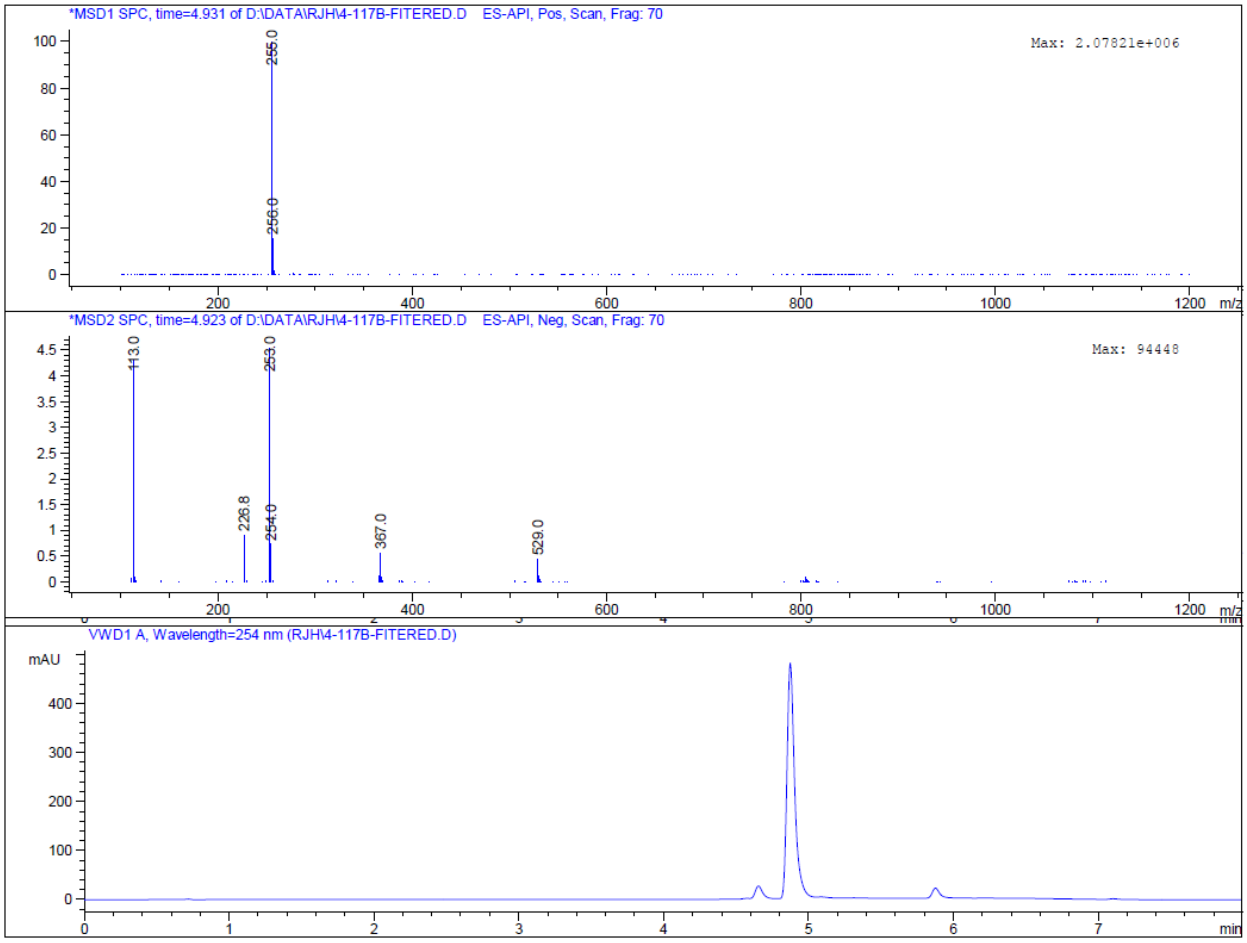
Molecular Weight: 255.2256

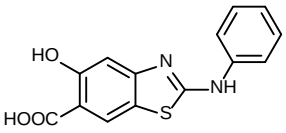




10

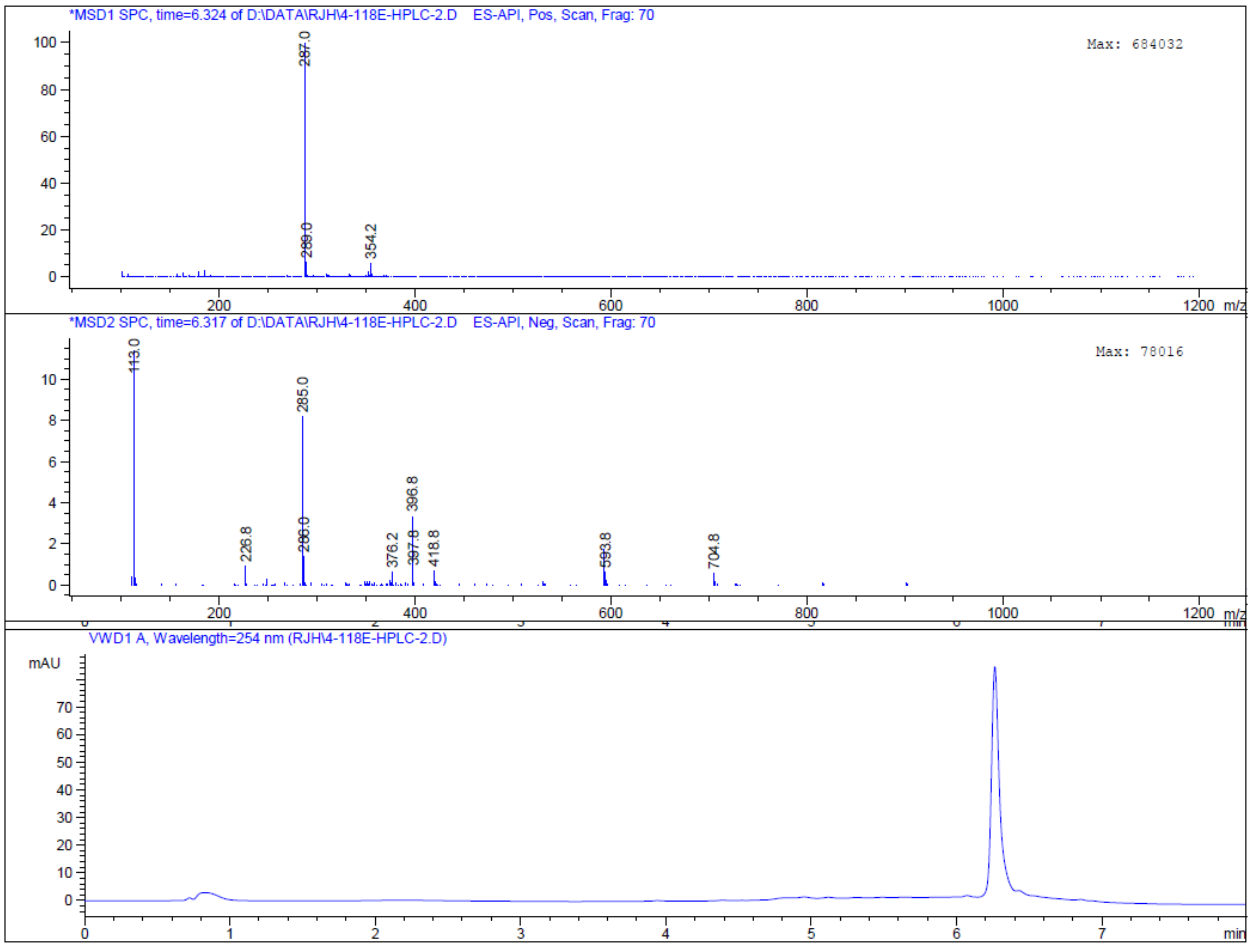
Chemical Formula: $C_{14}H_{10}N_2O_3$
Exact Mass: 254.0691
Molecular Weight: 254.2408

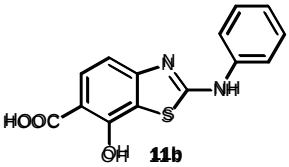




11a

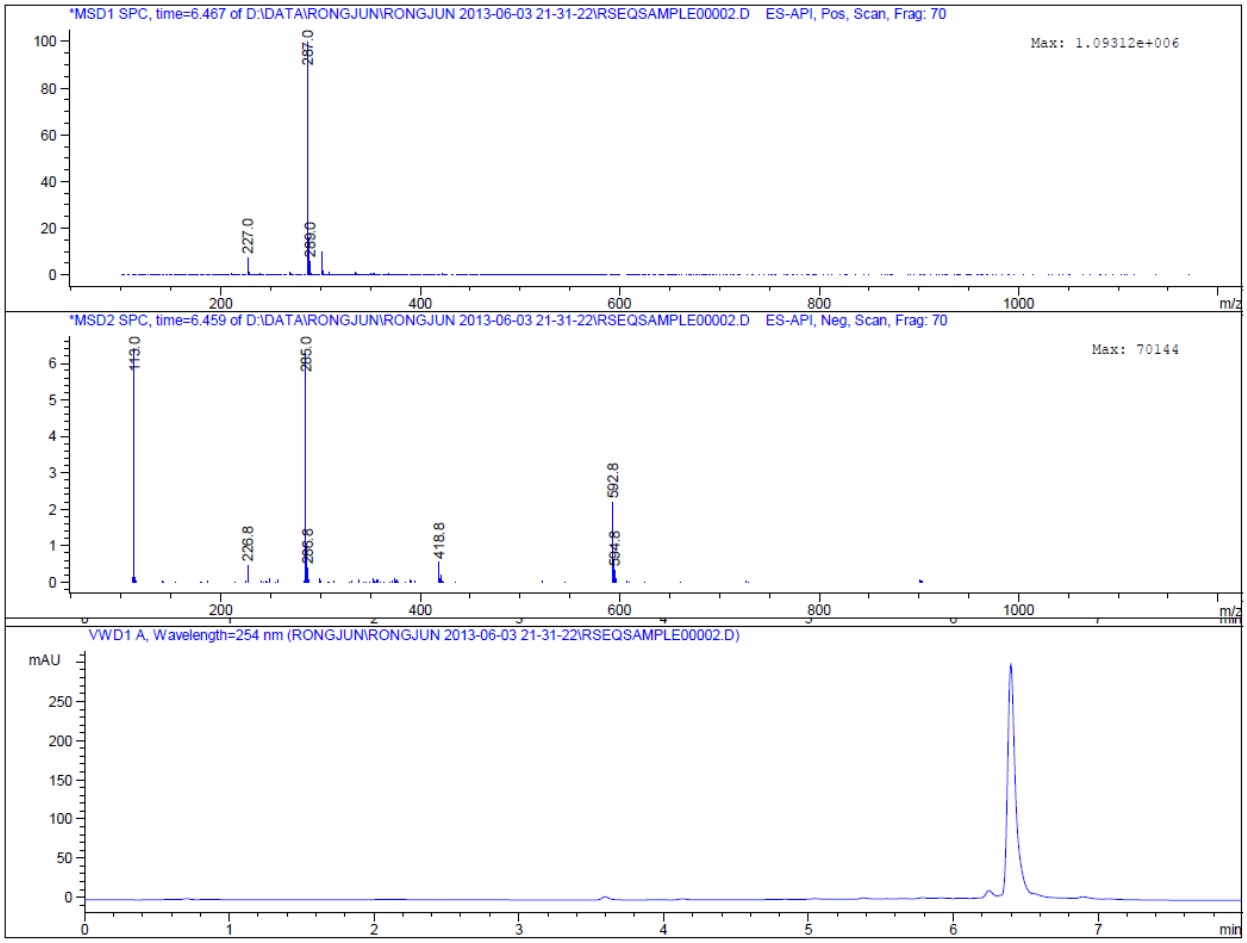
Chemical Formula: $C_{14}H_{10}N_2O_3S$
Exact Mass: 286.0412
Molecular Weight: 286.3058

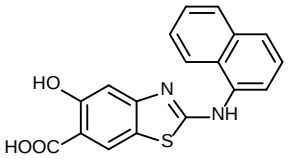




11b

Chemical Formula: $C_{14}H_{10}N_2O_3S$
Exact Mass: 286.0412
Molecular Weight: 286.3058



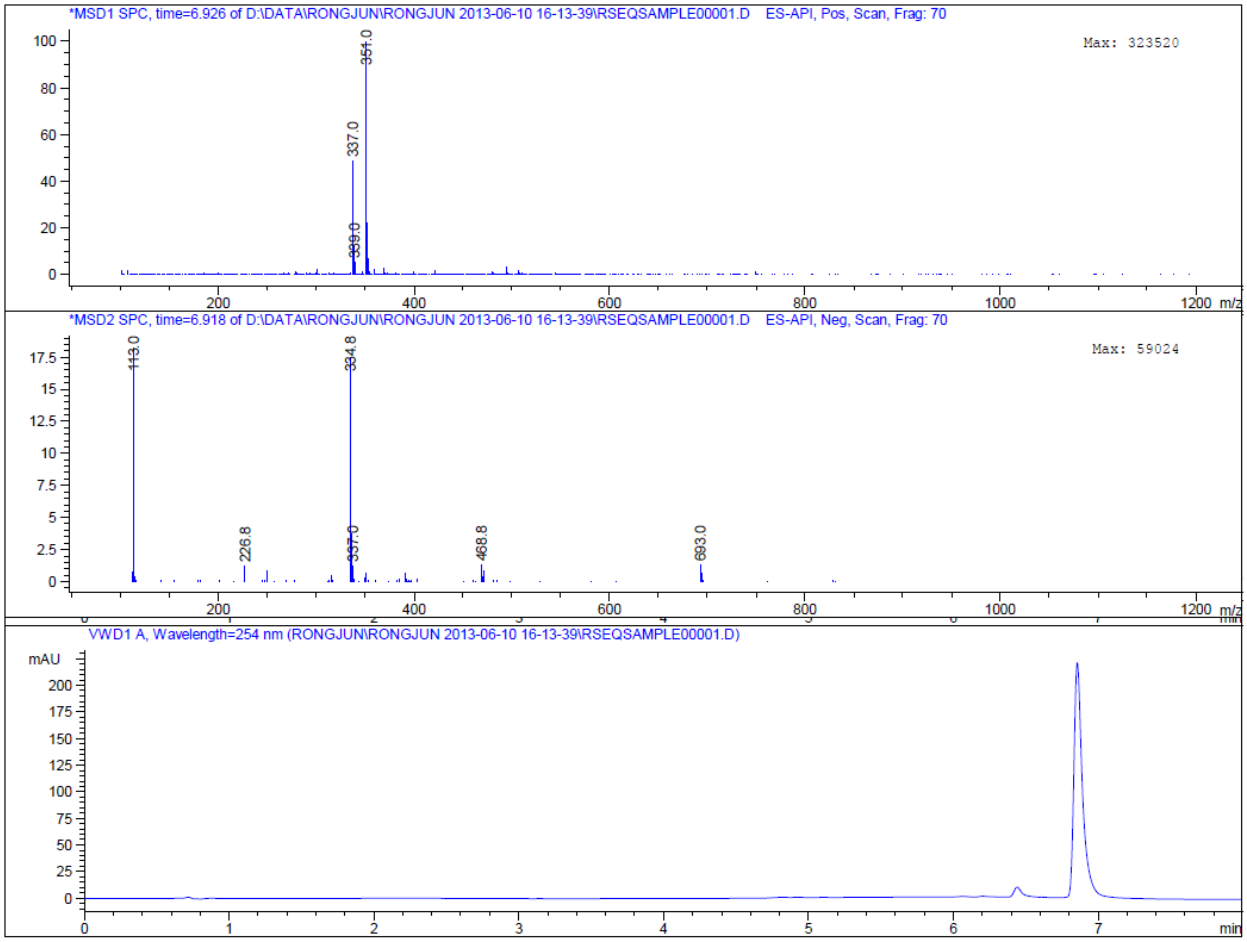


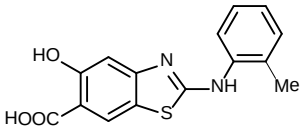
11c

Chemical Formula: $C_{18}H_{12}N_2O_3S$

Exact Mass: 336.0569

Molecular Weight: 336.3645



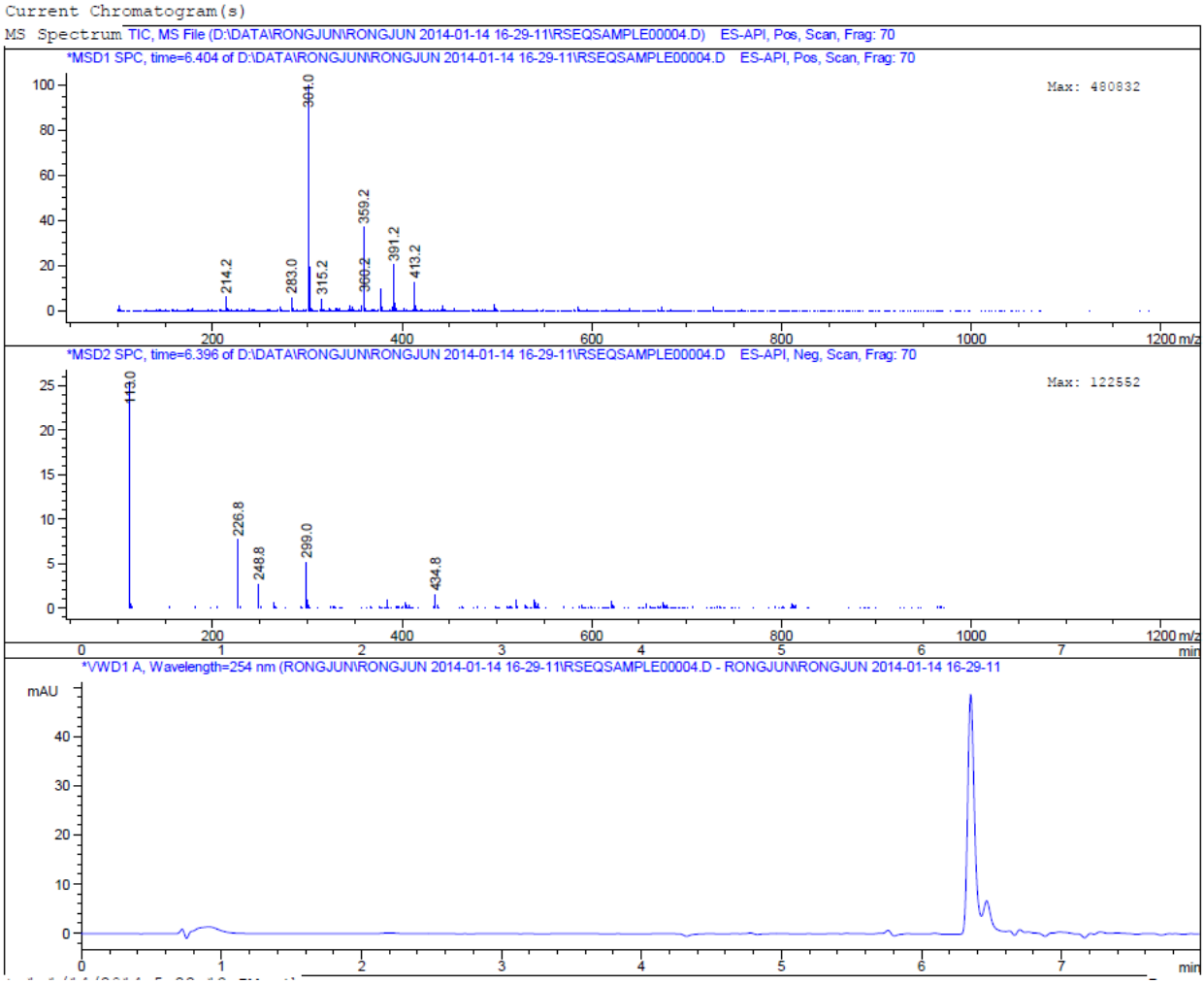


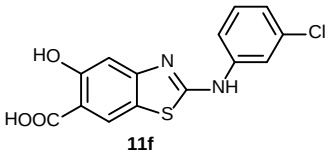
11e

Chemical Formula: $C_{15}H_{12}N_2O_3S$

Exact Mass: 300.0569

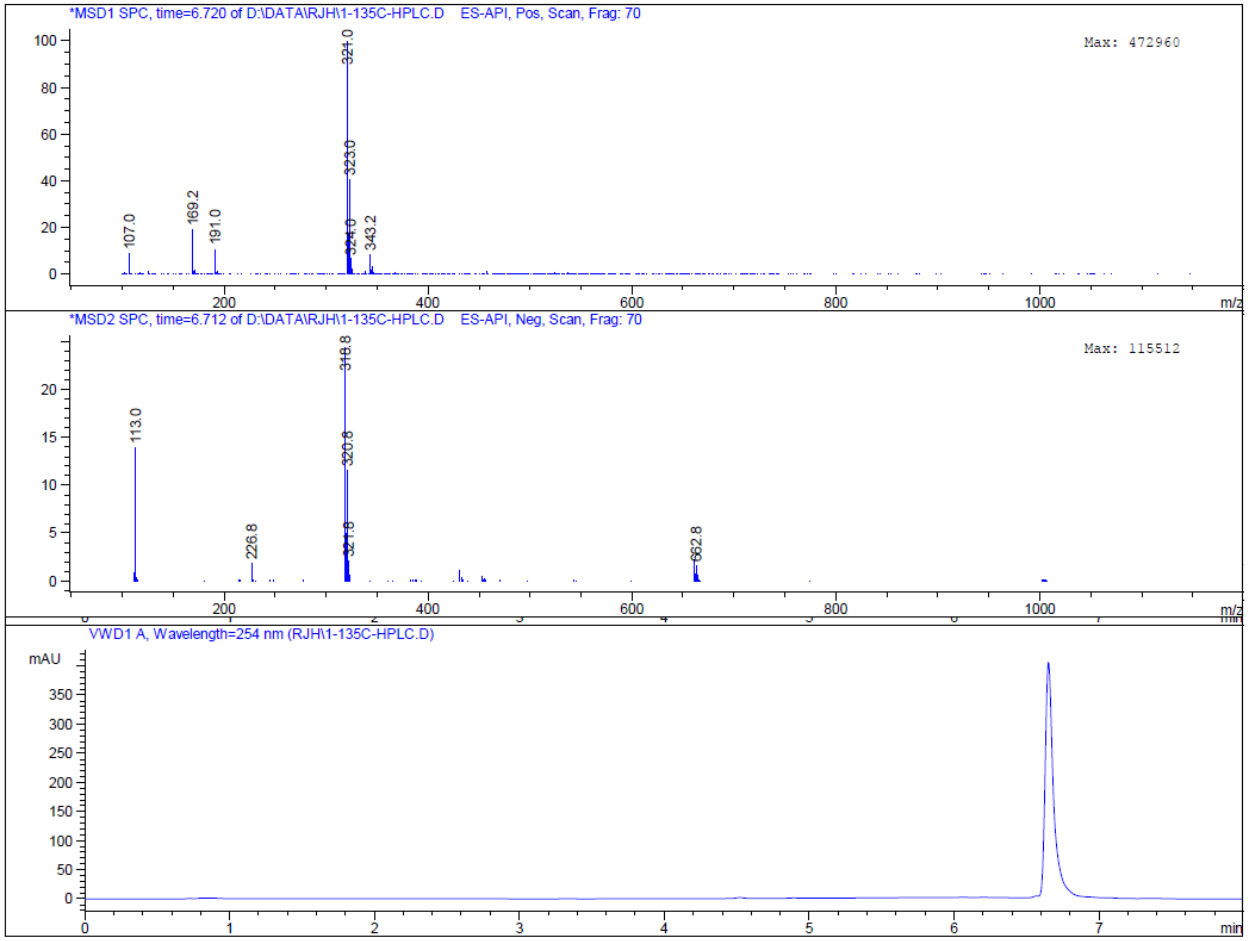
Molecular Weight: 300.3324

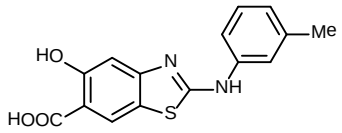




11f

Chemical Formula: $C_{14}H_9ClN_2O_3S$
Exact Mass: 320.0022
Molecular Weight: 320.7509



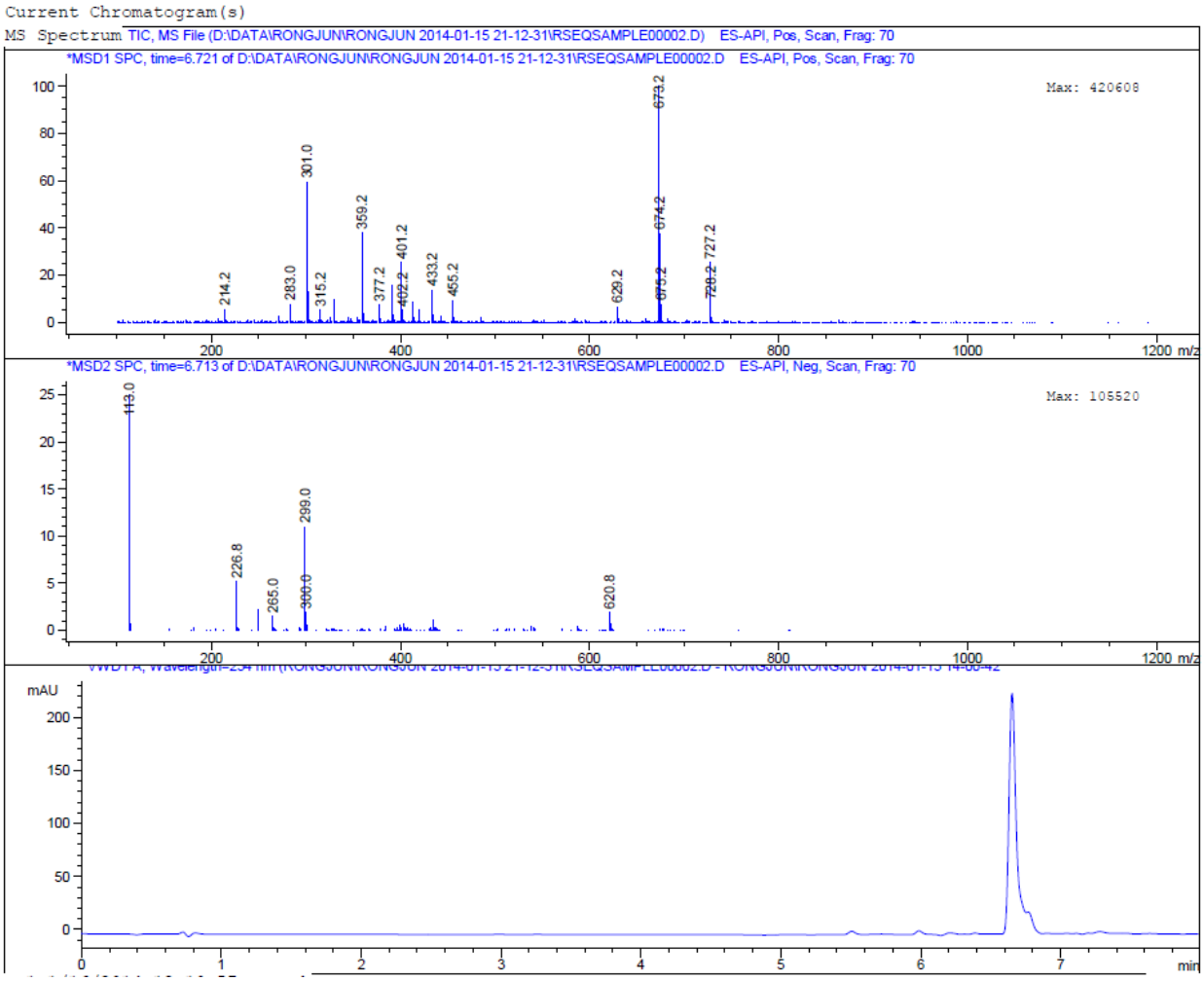


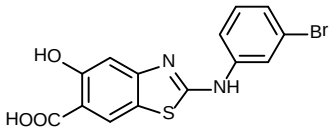
11g

Chemical Formula: $C_{15}H_{12}N_2O_3S$

Exact Mass: 300.0569

Molecular Weight: 300.3324



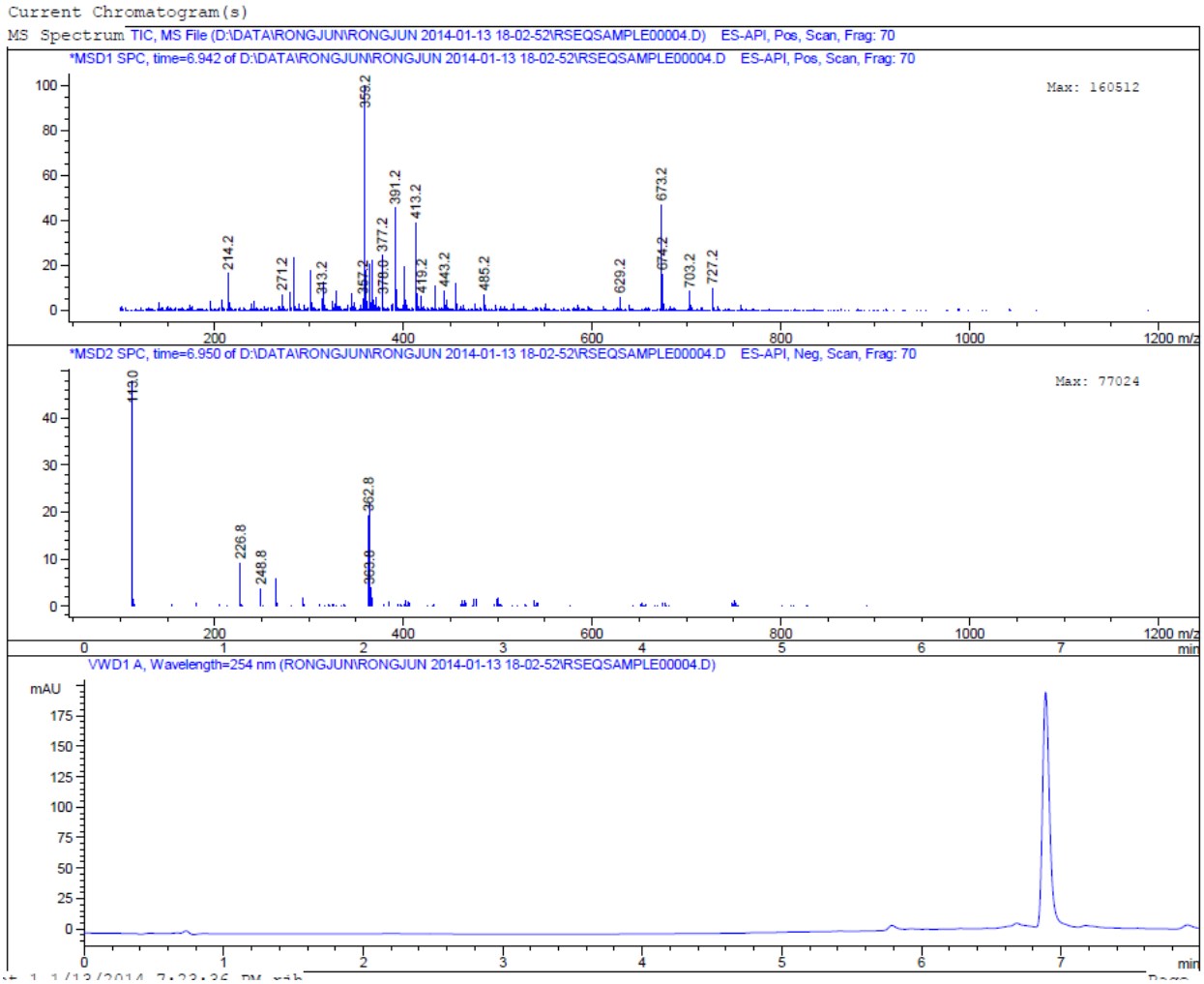


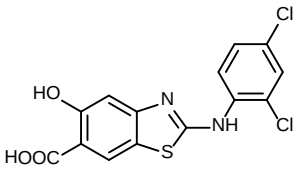
11h

Chemical Formula: $C_{14}H_9BrN_2O_3S$

Exact Mass: 363.9517

Molecular Weight: 365.2019



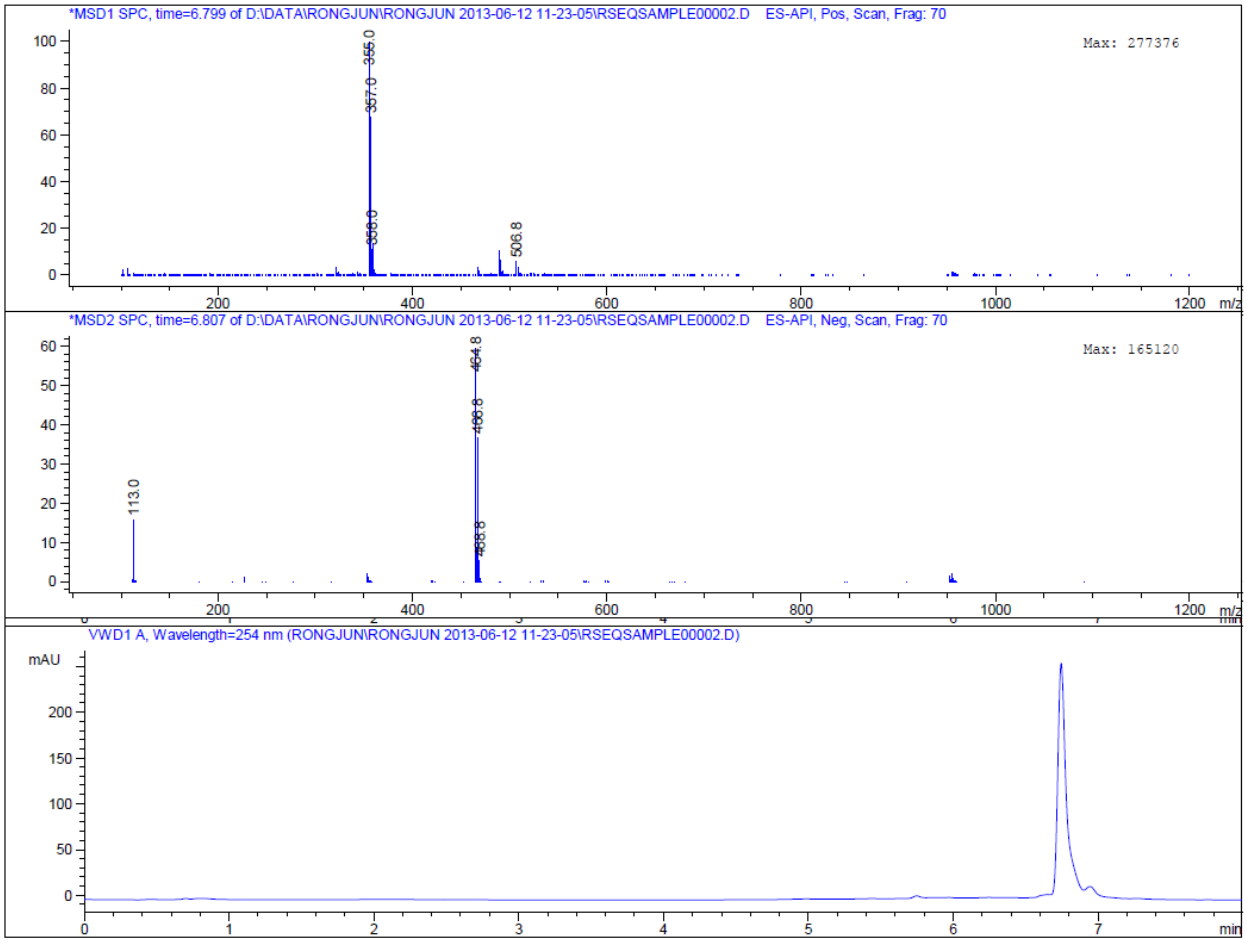


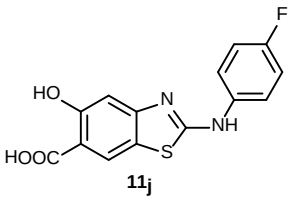
11i

Chemical Formula: $C_{14}H_8Cl_2N_2O_3S$

Exact Mass: 353.9633

Molecular Weight: 355.1959



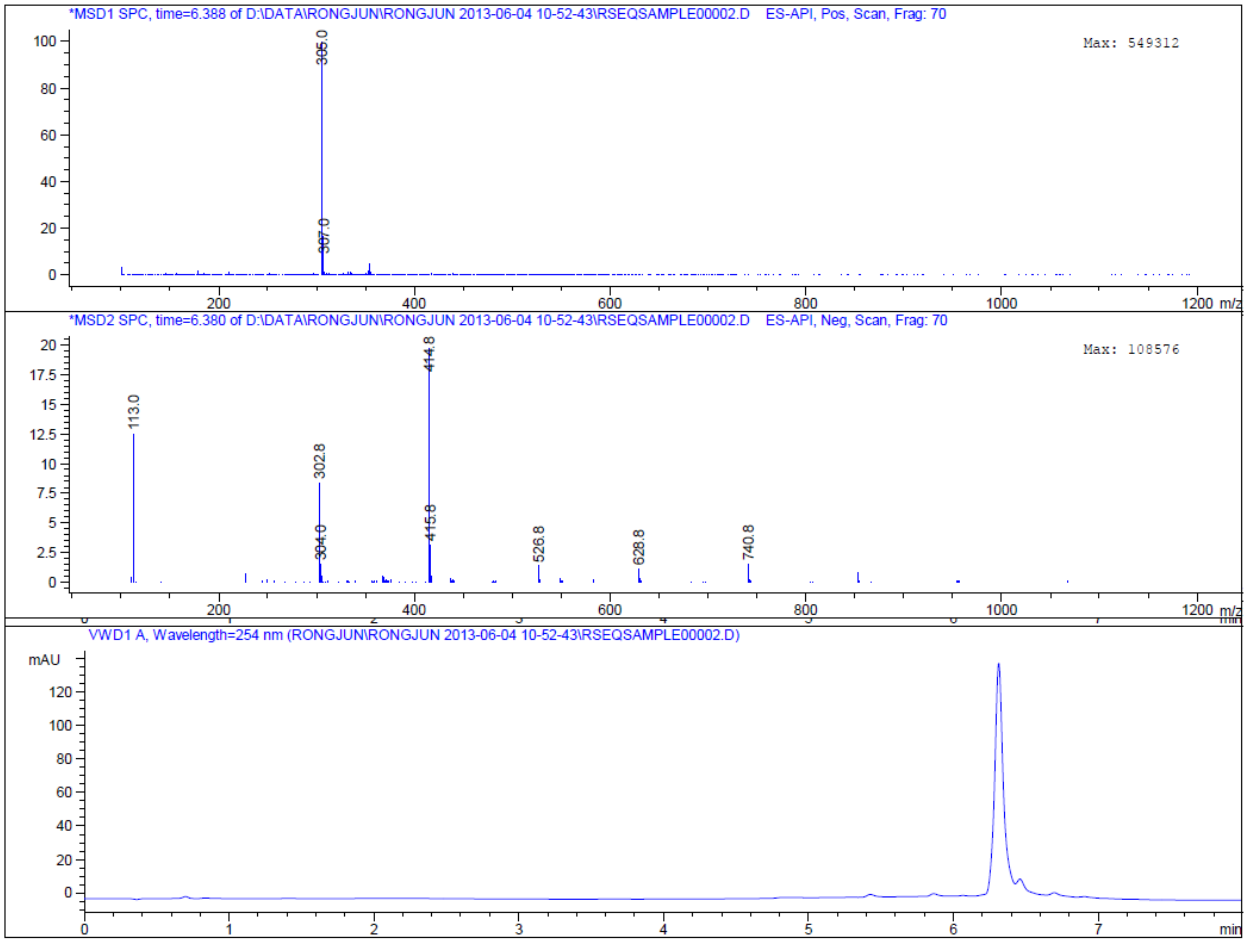


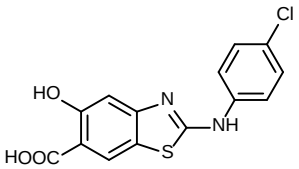
11j

Chemical Formula: $C_{14}H_9FN_2O_3S$

Exact Mass: 304.0318

Molecular Weight: 304.2963



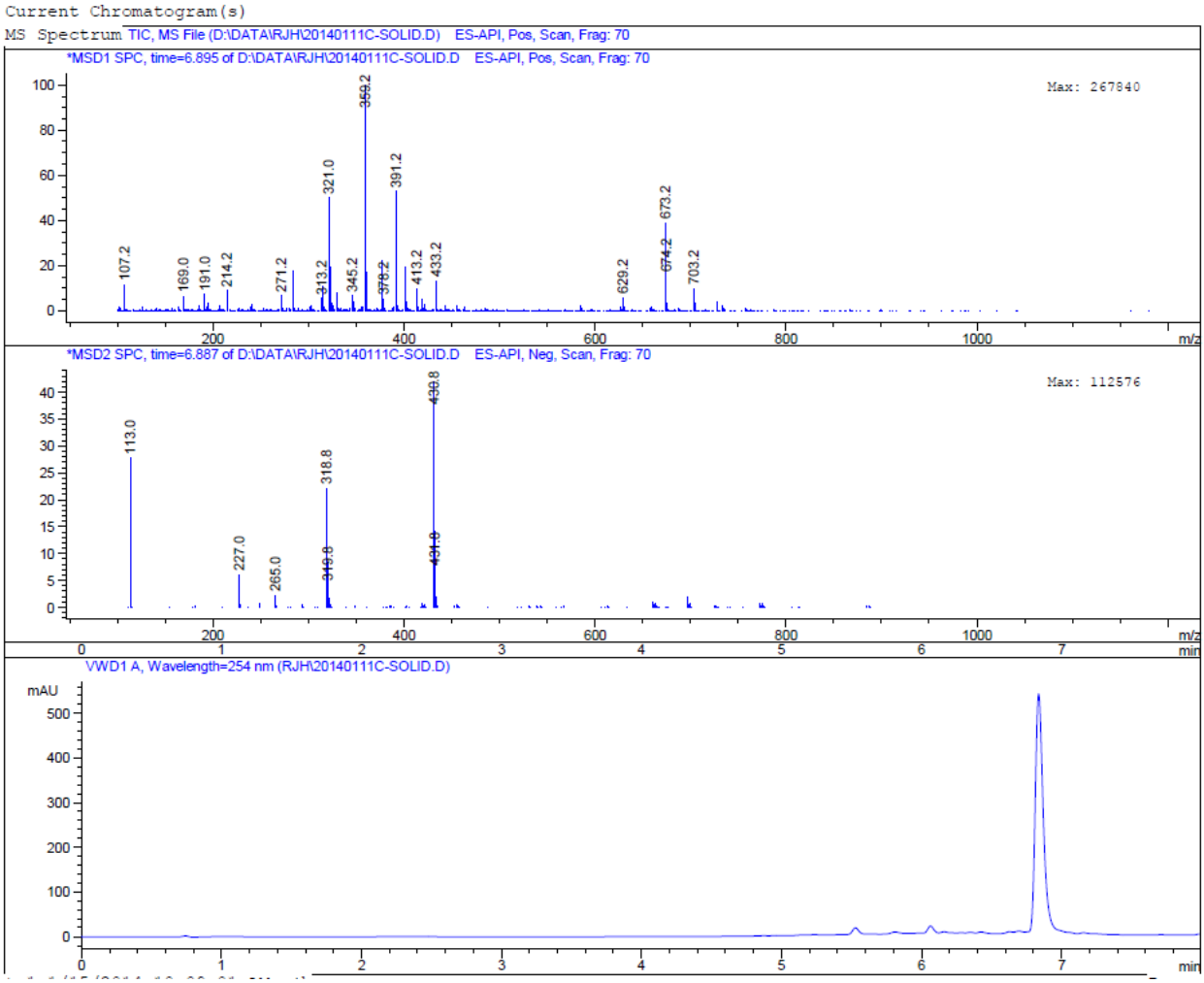


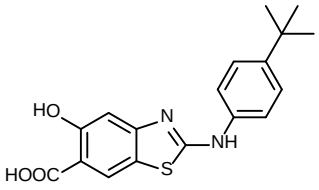
11k

Chemical Formula: $C_{14}H_9ClN_2O_3S$

Exact Mass: 320.0022

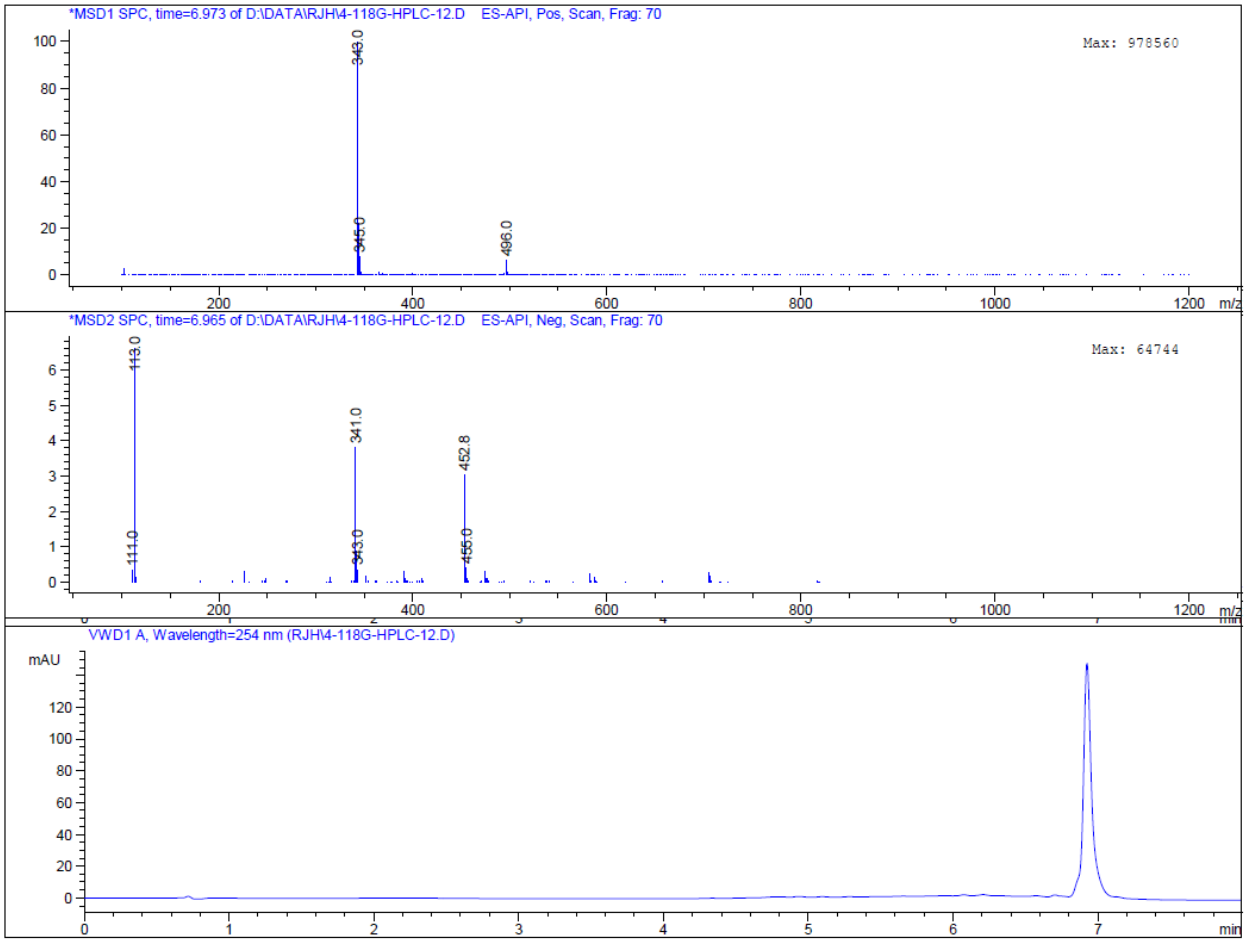
Molecular Weight: 320.7509

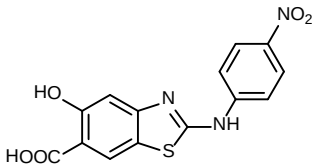




11l

Chemical Formula: $C_{18}H_{18}N_2O_3S$
Exact Mass: 342.1038
Molecular Weight: 342.4121



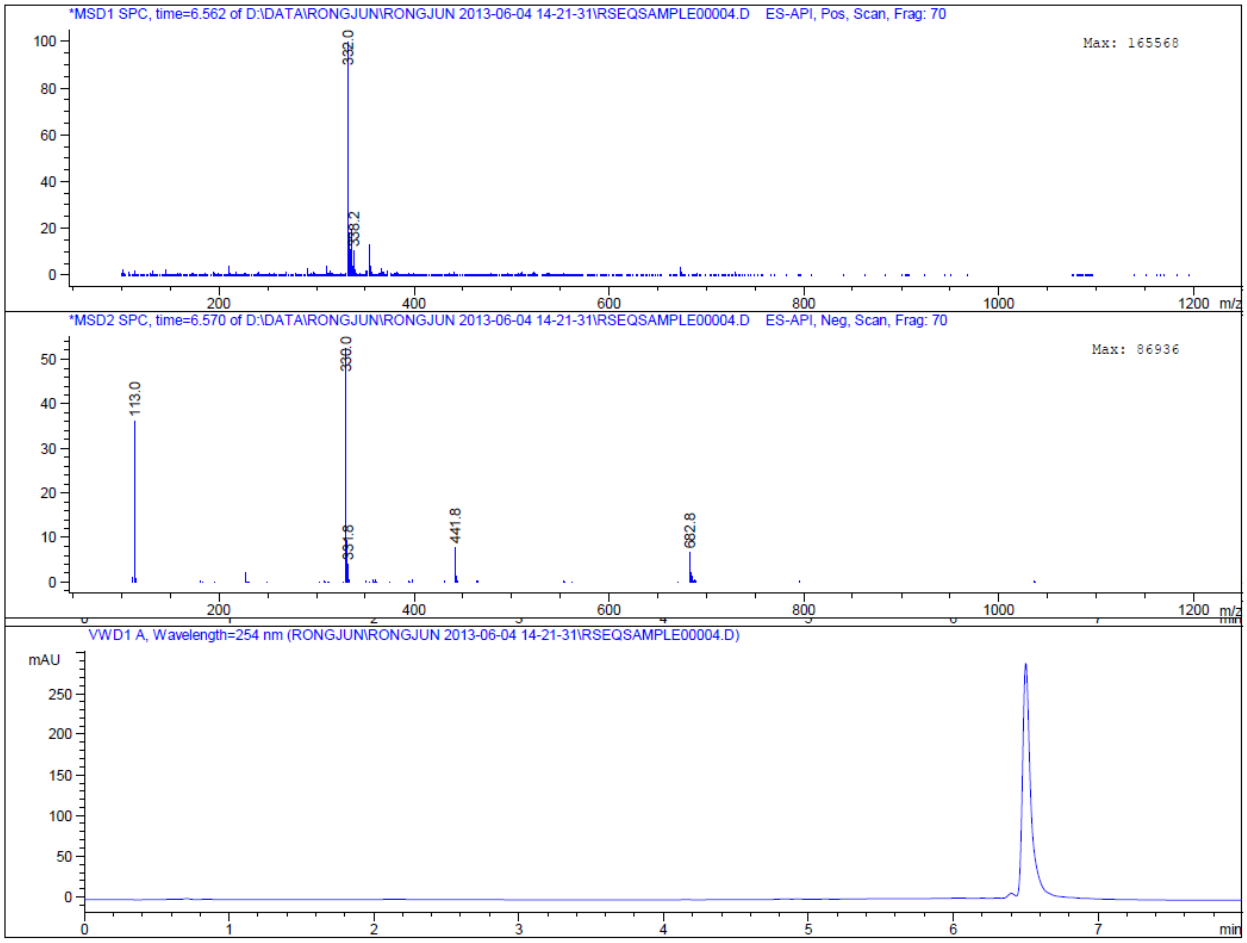


11m

Chemical Formula: $C_{14}H_9N_3O_5S$

Exact Mass: 331.0263

Molecular Weight: 331.3034



mPTPB inhibition studies for compounds **5-11,** expression and purification of recombinant mPTPB, and cellular studies of **11h**

The inhibition assays were performed on 96-well plates at 25°C in 50 mM 3,3-dimethylglutarate buffer, pH 7.0, containing 1 mM EDTA with an ionic strength of 0.15M adjusted by NaCl. The reaction was started by the addition of 50 µl of the enzyme to 150 µl of reaction mixture containing *p*-nitrophenyl phosphate (*p*NPP) and various concentrations of the inhibitor [final concentration of mPTPB: 20 nM, final concentration of *p*NPP: 3 mM (the K_m value)]. The reaction was quenched after 10 min by the addition of 50 µl of 5N NaOH. The absorbance at 405 nm was detected by a Spectra MAX340 microplate spectrophotometer (Molecular Devices). IC_{50} values were calculated by fitting the absorbance at 405 nm *versus* inhibitor concentration to the following equation:

$AI/A0=IC_{50}/(IC_{50}+[I])$, where AI is the absorbance at 405 nm of the sample in the presence of inhibitor; A0 is the absorbance at 405 nm in the absence of inhibitor; and [I] is the concentration of the inhibitor.

Expression and purification of recombinant mPTPB were described previously.[5] Briefly, pET28b-mPTPB (a generous gift from Dr. Christoph Grunder, University of California, Berkeley) was used to transform into E.coli BL21/DE3 and grown in LB medium containing 50 µg/ml kanamycin at 37°C to an OD600 of 0.5. Following the addition of IPTG to a final concentration of 20 µM, the culture was incubated at 20°C with shaking for additional 16 hr. The cells were harvested by centrifugation at 5000 rpm for 5 min at 4°C. The bacterial cell pellets were resuspended in 20 mM Tris, pH 7.9, 500 mM NaCl, 5 mM imidazole, and were lysed by passage through a French press cell at 1,200 p.s.i. twice. Cellular debris was removed by centrifugation at 16,000 rpm for 30 min at 4°C. The protein was purified form the supernatant using standard procedures of Ni-nitrilotriacetic acid-agarose (Qiagen) affinity purification. The protein eluted from Ni-NTA column was concentrated with an Amicon Ultra centrifugal filter device (Millipore) and the buffer was changed to 20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA and 1 mM DTT. Protein concentration was determined using the Bradford dye binding assay (Bio-Rad) diluted according to the manufacturer's recommendations with bovine serum albumin as standard. The purified mPTPB were made to 20% glycerol and stored at -20°C.

For cellular studies, mPTPB transfected Raw264.7 mouse macrophages were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS (Invitrogen), penicillin (50 units/mL), and streptomycin (50 μg/mL) under a humidified atmosphere containing 5% CO_2 at 37°C. Transfected Raw264.7 cells were seeded in a 12-well plate at a density of 4 x 10^4 cells/well. The following day cells were treated with mPTPB inhibitor **11h** for 1 h, then stimulated with IFN-γ (200 U/ml) for 1 h. Subsequently, the cells were washed with ice-cold phosphate buffered saline, and lysed with lysis buffer on ice for 30 min. Cell lysate was then cleared by centrifuging at 13,000 rpm for 15 min. The phosphorylations of Erk1/2 and Akt were detected by Western blotting.

Procedures for molecular modeling studies of **11h** in complex with mPTPB

The 3D-structure of **11h** was built and energy-minimized in Chem3D, and the coordinates of mPTPB were taken from the mPTPB•OMTS complex structure (PDBID: 2OZ5). Both ligand and receptor were pre-docking processed in AutoDockTools 1.4.6, such as merge non-polar hydrogens, add Gasteiger charges, set rotatable bond for ligand, add solvation parameter for receptor, and so on. The docking space was visually set around the catalytic active site, the energy grid size was set to 46× 60 ×46 points with 0.375Å spacing on each axis, and the energy grid maps for each atom type (i.e. A, C, HD, N, NA, OA, SA, Br), as well as the electrostatics and de-solvation maps were calculated using the AutoGrid 4. The molecular dockings were carried out using AutoDock 4.2.5 program, the optimal binding conformation was determined by LGALS (Lamarckian Genetic Algorithm with Local Search) algorithm. 500 separate docking runs were performed; the binding conformations were clustered and ranked according to the calculated binding free energy. The binding mode analyses were performed in AutoDockTools 1.4.6 by visual inspections and energy comparisons.

References

1. G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew, A. J. Olson, *J. Comput. Chem.* **1998,** *19*, 1639-1662.

2. C. Grundner, D. Perrin, R. H. van Huijsduijnen, D. Swinnen, J. Gonzalez, C. L. Gee, T. N. Wells, T. Alber, *Structure* **2007,** *15*, 499-509.

3. D. Barford, A. J. Flint, N. K. Tonks, *Science* **1994**, *263*, 1397-1404.

4. J. Yuvaniyama, J. M. Denu, J. E. Dixon, M. A. Saper, *Science* **1996**, *272*, 1328-1331.

5. B. Zhou, Y. He, X. Zhang, J. Xu, Y. Luo, Y. Wang, S. G. Franzblau, Z. Yang, R. J. Chan, Y. Liu, J. Zheng, Z.-Y. Zhang, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 4573-4578.