Design, Synthesis and Biological Evaluation of Substituted 2-amino-1,3-thiazine derivatives as Antituberculosis and Anti-Cancer agents

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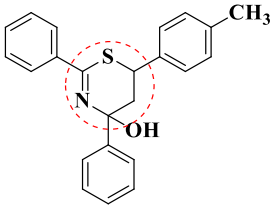
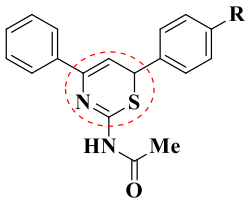
Supplementary Information (SI)

Figure 1: Some of the potent biological active compounds possessing thiazine core

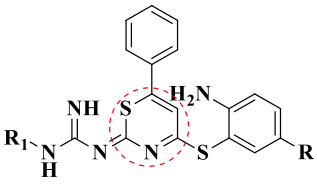


antifungal activity



R= H, Cl, NO_2, OCH_3, OH, NMe_2

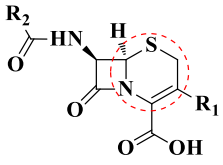
antimicrobial activity



R= R_1=H

R=H, R_1=phenyl

anti-inflammatory, analgesic and ulcerogenic activity

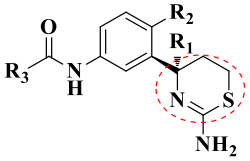


R_1= CH_3COOCH_2, CH_3, OCH_3, Cl

R_2= CH_2CN, $PhCH(CH_3)NH_2$,

Cephalosporin, beta-lactam antibiotics

anticancer against A-549, Bcap-37 cells

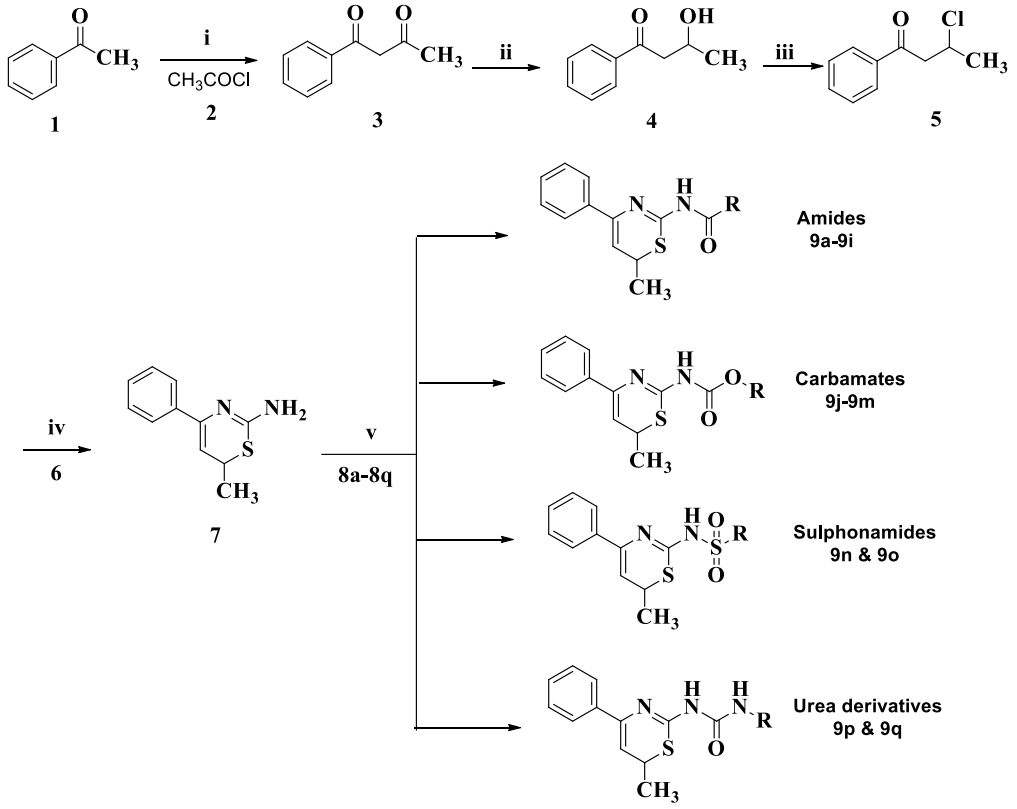


R_1= CH_3, R_2= F

R_3= CH_3, Ph

BACE 1 Inhibitors

Scheme 1: **Synthesis of 1,3-thiazine derivatives (9a-9q).**



Reagents and conditions: (i) LiHMDS, Toluene, 0 °C, 1 h; (ii) LiHMDS, $LiAlH_4$, THF, 0 °C, 1 h; (iii) $SOCl_2$, CH_2Cl_2, DMF, RT, 3 h; (iv) Thiourea (**6**), Ethanol, 75 °C, 8 h; (v) Compounds **8a-8s**, DIPEA, CH_2Cl_2, r.t.,, 2 hr. **8a**-Pivaloyl chloride; **8b**-cyclopropanecarbonyl chloride; **8c**-cyclopentanecarbonyl chloride; **8d**-cyclohexanecarbonyl chloride; **8e**-isobutyryl chloride; **8f**-2,2-dimethyl butyryl chloride; **8g**-propionyl chloride; **8h**-butyryl chloride; **8i**-benzoyl chloride; **8j**-methyl chloroformate; **8k**-ethyl chloroformate; **8l**-isopropyl chloroformate; **8m**-*tert*-butylchloroformate; **8n**-methanesulfonyl chloride; **8o**-4-(trifluoromethoxy)benzene-1-sulfonyl chloride; **8p**-2-F-phenyl isocynate; **8q**-2-Cl-Phenyl isocyanate.

Table 1: Anti tuberculosis, anti-cancer activity results of 1,3-thiazine derivatives 9a-9q

S.No	Compounds (R)	Anti-TB MIC in µg /mL	Anti-cancer Cytotoxicity % of inhibition at 25 µM[a]	
			MCF-7	EC-9706
1	**7**	> 25	15.42	> 100
Amides				
2	**9a (***t*-butyl)	6.25	22.46	18.46
3	**9b (**cyclopropyl)	1.26	1.73	6.12
4	**9c (**cyclopentyl)	>25	24.19	23.45
5	**9d (**cyclohexyl)	>25	10.55	12.94
6	**9e (**isopropyl)	25	20.50	26.18
7	**9f (**1-(2-methyl)butyl)	6.25	> 100	3.81
8	**9g (**Ethyl)	25	34.18	20.48
9	**9h (**Propyl)	1.56	5.48	9.12
10	**9i (**Phenyl)	0.78	16.48	20.92
Carbamates				
11	**9j (**methyl)	6.25	19.64	30.27
12	**9k (**ethyl)	6.25	5.83	> 100
13	**9l (**isopropyl)	>25	> 100	7.26
14	**9m (***t*-butyl)	3.12	2.26	5.18
Sulphonamides				
15	**9n (**Methyl)	>25	3.68	10.55
16	**9o (**4-OCF_3 phenyl)	6.25	7.69	> 100
Urea derivatives				
17	**9p (**2-F phenyl)	3.12	8.60	2.61
18	**9q (**2-Cl phenyl)	6.25	> 100	7.26
Standarad				
19	Isoniazid	0.72	ND	ND
20	Rifampicin	0.24	ND	ND
21	Ethambutol	7.64	ND	ND
22	Cisplatin	NA	4.12	7.10

[a]Inhibitory activity was assayed by exposure for 72 h substances and expressed as concentration required to inhibit tumor cell proliferation by 50 % (IC_{50}). ND means not done

Materials and methods

All the starting materials and reagents were procured from commercial suppliers and used with put purification. All new compounds were fully characterized. TLC analysis was performed using readily available TLC silica gel plates (Kieselgel 60 F254, Merck). IR (KBr) spectra was recorded as KBr pellets with Perkin–Elmer 400 FTIR spectrometer (υ_{max} in cm^{-1}). All synthesized compounds were purified by column chromatography using 230-400 silica gel. ^{1}H NMR spectra were recorded with 400 MHz. ^{13}C NMR spectra were recorded with 100 MHz and 125 MHz.

Synthesis of 1-phenyl-1,3-butanedione (3)

To a solution of compound **1** (15 g, 15 mmol) in toluene was added LiHMDS (38 mL 2M in THF, 187.5 mmol) at 0 °C and the RM was stirred for 30 min. To this RM was added acetyl chloride **2** (10.8 g, 137 mmol) at 0 °C and stirred for 30 min. After completion of SM (monitored by TLC), the RM was quenched with saturated aqueous NH_4Cl solution (200 mL) and extracted with ethyl acetate (3 x 200 mL). The combined organic layers were dried over Na_2SO_4 and purified by column chromatography (60-120 silica gel) using 10% ethyl acetate in hexane to afford pure compound 1-phenyl-1,3-butanedione **(3)** as white solid; Yield: 89 %; M.P: 54-58 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ: 16.18 (brs, 1H), 7.87-7.89 (m, 2H), 7.54 (m, 1H), 7.59 (t, J = 8.0 Hz, 2H), 6.18 (s, 1H), 2.20 (s, 3H).

Synthesis of 3-hydroxy-1-phenyl-1-butanone (4)

To a solution of 1-phenyl-1,3-butanedione **3** (2.3 mmol) in THF (10 mL) was added LiHMDS (2.5 mmol) at 0 °C and the reaction mass was stirred for 30 min at same temperature. To this RM was added $LiAlH_4$ (4.6 mmol, 1 M solution in THF) at 0 °C and stirred at the same temperature for 30 min. The reaction mixture was quenched with cold water (20 mL) and extracted with ethyl acetate (20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated over vacuum. The crude product was purified by coloumn chromatography using 30% ethyl acetate in hexane to afford pure product 3-hydroxy-1-phenyl-1-butanone **(4)**. Colorless liquid; Yield: 81 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.97 (d, J = 6.8 Hz, 2H), 7.61 (t, J = 8.0 Hz, 1H), 7.59 (t, J = 8.0 Hz, 2H), 4.43-4.41 (m, 1H), 3.31 (d, J = 3.0 Hz, 1H), 3.21-3.16 (dd, J = 3.0 ,8.8 Hz, 1H), 3.08-2.97 (m, 1H), 1.28 (d, J = 6.4 Hz, 3H).

Synthesis of 3-chloro-1-phenyl-1-butanone (5)

$SOCl_2$ (12.6 g, 122.6 mmol) was added to a solution of 3-hydroxy-1-phenyl-1-butanone **4** (10 g, 61.3 mmol) in CH_2Cl_2 at 0 $^{\circ}$C fallowed by 0.1 mL of DMF stirred the reaction mixture at room temperature for 3 h. After completion of reaction on TLC concentrated the reaction mixture and purified by column chromatography on silica gel (5 % ethyl acetate in pet ether) to afford pure product 3-chloro-1-phenyl-1-butanone **5** in 10 g. Colorless liquid; Yield: 89%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.97 (d, J = 6.8 Hz, 2H), 7.61 (t, J = 8.0 Hz, 1H), 7.59 (t, J = 8.0 Hz, 2H), 4.80-

4.65 (m, 1H), 3.62 (dd, *J* = 3.0 ,8.8 Hz, 1H), 3.29 (dd, *J* = 3.0 ,8.8 Hz, 1H), 1.64 (d, *J* = 6.4 Hz, 3H).

Synthesis of 6-Methyl-4-phenyl-6H-1,3-thiazin-2-amine (7)

Thiourea **6** (4.6 g, 60.4 mmol) was added to a solution of 3-chloro-1-phenyl-1-butanone **5** (10 g, 54.94 mmol) in ethanol at room temperature and heated the reaction mixture to 75 °C for 8 h. After completion of reaction on TLC concentrated the reaction mixture was quenched with 180 mL of 10 % aqueous citric acid. The RM was extracted with ethyl acetate (3 x 150 mL), the aqueous layer was basified with 10% aqueous bicarbonate solution and then extracted thewith ethyl acetate (3×150 mL). The combined organic layers were dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography on silica gel (20 % ethyl acetate in pet ether) to afford pure product 6-methyl-4-phenyl-2*H*-1,3-thiazin-2-amine **7** in 9.5 g. off white solid; Yield: 89 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.70–7.63 (m, 2H), 7.37–7.29 (m, 2H), 7.28 (t, *J* = 1.4 Hz, 1H), 5.54 (d, *J* = 5.7 Hz, 1H), 4.92 (brs, 2H), 3.84-3.72 (m, 1H), 1.44 (d, *J* = 6.9 Hz, 3H); IR (KBr, cm^{-1}): 3445, 3272, 3052, 1631, 1550, 1298, 752; HRMS (ESI): calcd for $C_{11}H_{13}N_2S$ $(M+H)^+$: 205.0799 found 205.0701.

General procedure for the synthesis of 9a-9q

To a solution of 6-methyl-4-phenyl-2*H*-1,3-thiazin-2-amine **7** (1.4 mmol) in 2-3 mL CH_2Cl_2 was added DIPEA at 0 °C maintained over 10 minutes, then acid chlorides **8a-8q** (1.76 mmol) was added and stirred the reaction mixture for 2 h at room temperature. After completion of reaction on TLC, the reaction mixture was quenched with cold ice water. Diluted with ethyl acetate, washed with aqueous bicarbonate solution, followed by brine solution organic layer was dried over Na_2SO_4 then concentrated and purified by column chromatography on silica gel (10 % ethyl acetate in pet ether) to afford pure compounds.

***N-(6-Methyl-4-phenyl-6H-1,3-thiazin-2-yl)pivalamide* (9a)**

The compound **9a** was prepared according to general procedure by utilizing pivaloyl chloride **8a**. Pale yellow solid; Yield: 92%; M.P: 103-106 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 7.59 (d, *J* = 7.3 Hz, 2H), 7.40–7.27 (m, 3H), 5.55 (d, *J* = 5.7 Hz, 1H), 3.77-3.66 (m, 1H), 1.45 (d, *J* = 6.8 Hz, 3H), 1.28 (s, 9H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 188.50 (CO), 163.58 (C2), 143.04 (C6), 135.86 (ArC), 130.91 (ArC), 129.84 (ArC), 129.53 (ArC), 128.14 (ArC), 122.78 (ArC), 113.93

(C5), 42.41 (tert-C), 35.54 (C4), 28.95 (<u>C</u>H_3), 21.99 (<u>C</u>H_3). **IR** (KBr, cm^{-1}): 3459 (NH str), 3256, 2967 (CH_3 str), 1673 (CO str), 1557 (Ar-C=C str), 1230 (C=N str), 1216 (C-S-C str); **HRMS** (ESI): calcd for $C_{16}H_{21}N_2OS$ $(M+H)^+$: 289.1375 found 289.1424.

***N-(6-Methyl-4-phenyl-6H-1,3-thiazin-2-yl)cyclopropane carboxamide* (9b)**

The compound **9b** was prepared according to general procedure by utilizing cyclopropane carbonyl chloride **8b**. Pale brown solid; Yield: 91 %; M.P: 130-133 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 7.56 (dd, J = 1.7, 7.9 Hz, 2H), 7.40–7.29 (m, 3H), 5.55 (d, J = 5.8 Hz, 1H), 3.82-377 (m, 1H), 1.88-1.74 (m, 1H), 1.47 (d, J = 7.0 Hz, 3H), 1.09 (td, J = 3.3, 4.6 Hz, 2H), 0.88 (dd, J = 3.0, 8.0 Hz, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ ppm: 189.82 (CO), 163.02 (C2), 143.44 (C6), 135.81 (ArC), 135.46 (ArC), 129.95 (ArC), 129.57 (ArC), 129.42 (ArC), 128.72 (ArC), 123.06 (C5), 30.39 (C4), 29.72 (<u>C</u>H_3), 21.72 (<u>C</u>H_2), 11.04 (<u>C</u>H_2). **IR** (KBr, cm^{-1}): 3254 (NH str), 3002, 2980, 2915 (CH_2 str), 1661 (CO str), 1616, 1598, 1552 (Ar-C=C str), 1229 (C-S-C str); **HRMS** (ESI): calcd for $C_{15}H_{17}N_2OS$ $(M+H)^+$: 273.1062 found 273.0961.

***N-(6-Methyl-4-phenyl-6H-1,3-thiazin-2-yl)cyclopentane carboxamide* (9c)**

The compound **9c** was prepared according to general procedure by utilizing cyclopentane carbonyl chloride **8c**.Pale yellow solid; Yield: 88%; M.P:85-88 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 7.63–7.57 (m, 2H), 7.39–7.29 (m, 3H), 5.57 (d, J = 5.8 Hz, 1H), 3.82-3.77 (m, 1H), 2.75 (p, J = 7.9 Hz, 1H), 1.98-1.84 (m, 4H), 1.81–1.70 (m, 2H), 1.60-1.50 (m, 2H), 1.45 (d, J = 7.0 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 174.51 (CO), 153.87 (C2), 140.66 (C6), 137.30 (ArC), 132.22 (ArC), 130.63 (ArC), 129.33 (ArC), 128.53 (ArC), 127.17 (ArC), 123.71 (C5), 45.03 (<u>C</u>H), 32.76 (C4), 30.66 (<u>C</u>H_2), 30.15 (<u>C</u>H_2), 22.37 (<u>C</u>H_3), 18.40 (<u>C</u>H_2). **IR** (KBr, cm^{-1}): 3436 (NH str), 2955 (CH_3 str), 2867 (CH_2 str), 1665 (CO str), 1560, 1491 (Ar-C=C str), 1444 (C=N str), 1222 (C-S-C str); **HRMS** (ESI): calcd for $C_{17}H_{21}N_2OS$ $(M+H)^+$: 301.1375 found 301.1354.

***N-(6-Methyl-4-phenyl-6H-1,3-thiazin-2-yl)cyclohexane carboxamide* (9d)**

The compound **9d** was prepared according to general procedure by utilizing cyclohexane carbonyl chloride **8d.** Pale brown solid; Yield: 89 %; M.P: 136-139 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 7.62–7.57 (m, 2H), 7.39–7.29 (m, 3H), 5.57 (d, J = 5.7 Hz, 1H), 3.82-3.77 (m, 1H),

2.36–2.20 (m, 1H), 1.92 (d, *J* = 8.2 Hz, 2H), 1.85–1.76 (m, 2H), 1.71–1.63 (m, 1H), 1.45 (d, *J* = 6.9 Hz, 6H), 1.35–1.21 (m, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) *δ* ppm: 174.38 (CO), 157.68 (C2), 143.68 (C6), 135.24 (ArC), 133.72 (ArC), 130.26 (ArC), 127.19 (ArC), 126.02 (ArC), 123.60 (ArC), 119.01 (C5), 45.28 (CH), 30.53 (C4), 28.03 (CH_2), 25.29 (CH_2), 22.68 (CH_2), 18.32 (CH_2), 16.11 (CH_3). **IR** (KBr, cm^{-1}): 3446 (NH str), 3043 (=CH str), 2973 (CH_3 str), 2925 (CH_2 str), 1667 (CO str), 1560 (Ar-C=C str), 1444 (C=N str), 1220 (C-S-C str); **HRMS** (ESI): calcd for $C_{18}H_{23}N_2OS$ $(M+H)^+$: 315.1531 found 315.1441.

***N-(6-Methyl-4-phenyl-6H-1,3-thiazin-2-yl)isobutyramide* (9e)**

The compound **9e** was prepared according to general procedure by utilizing isobutyryl chloride **8e.** Yield: 90 %; M.P: 128-131 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) *δ*: 7.63–7.54 (m, 2H), 7.39–7.30 (m, 3H), 5.57 (d, *J* = 5.7 Hz, 1H), 3.83-3.78 (m, 1H), 2.64-2.52 (m, 1H), 1.46 (d, *J* = 7.0 Hz, 3H), 1.21 (d, *J* = 6.9 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$) *δ* ppm: 180.12 (CO), 163.42 (C2), 140.17 (C6), 134.79 (ArC), 130.44 (ArC), 129.73 (ArC), 128.51 (ArC), 126.16 (ArC), 122.80 (ArC), 115.83 (C5), 40.13 ($C(CH_3)_2$), 30.19 (C4), 25.27 (CH_3), 23.75 (CH_3). **IR** (KBr, cm^{-1}): 3436 (NH str), 3041 (=CH str), 2971 (CH_3 str), 1676 (CO str), 1559, 1469 (Ar-C=C str), 1442 (C=N str), 1241 (C-S-C str); **HRMS** (ESI): calcd for $C_{15}H_{19}N_2OS$ $(M+H)^+$: 275.1218 found 275.1166.

***2,2-dimethyl-N-(6-methyl-4-phenyl-6H-1,3-thiazin-2-yl)-butanamide* (9f)**

The compound **9f** was prepared according to general procedure by utilizing 2,2-dimethyl butyryl chloride **8f.** Brown color liquid; Yield: 92 %; **^{1}H NMR** (400 MHz, $CDCl_3$) *δ*: 7.59 (d, *J* = 7.3 Hz, 2H), 7.40-7.32 (m, 3H), 5.55 (d, *J* = 5.8 Hz, 1H), 3.82–3.72 (m, 1H), 1.64 (q, *J* = 7.5 Hz, 2H), 1.56 (d, *J* = 6.8, 3H), 1.44 (s, 6H), 1.24 (s, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) *δ* ppm: 181.19 (CO), 165.23 (C2), 141.72 (C6), 136.46 (ArC), 133.42 (ArC), 128.61 (ArC), 127.02 (ArC), 126.66 (ArC), 123.79 (ArC), 117.17 (C5), 45.78 ($C(CH_3)_2$), 34.15 (CH_2), 32.45 (C4), 26.79 (CH_3), 24.13 (CH_3), 18.25 (CH_3). **IR** (KBr, cm^{-1}): 3422 (NH str), 2966 (CH_3 str), 2922 (CH_3 str), 1689 (CO str), 1552 (Ar-C=C str), 1473, 1445 (C=N str), 1223 (C-S-C str), 1133, 758; **HRMS** (ESI): calcd for $C_{17}H_{23}N_2OS$ $(M+H)^+$: 303.1531 found 303.1418.

***N-(6-methyl-4-phenyl-6H-1,3-thiazin-2-yl)propanamide* (9g)**

The compound **9g** was prepared according to general procedure by utilizing propionyl chloride **8g**.Brown color liquid; Yield: 85 %; **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 7.62-7.59 (m, 2H), 7.39–7.28 (m, 3H), 5.58 (d, J = 5.8 Hz, 1H), 3.86–3.70 (m, 1H), 2.49 (q, J = 7.5 Hz, 2H), 1.46 (d, J = 6.9 Hz, 3H), 1.20 (t, J = 7.5 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 175.12 (CO), 161.56 (C2), 145.73 (C6), 140.13 (ArC), 137.71 (ArC), 132.18 (ArC), 129.73 (ArC), 128.48 (ArC), 124.53 (ArC), 122.67 (C5), 38.10 ($\underline{C}H_2$), 26.78 ($\underline{C}H$), 24.40 ($\underline{C}H_3$), 20.70 ($\underline{C}H_3$). **IR** (KBr, cm^{-1}): 3433 (NH str), 3159 (=CH str), 2977 (CH_3 str), 2929 (CH_2 str), 1731 (CO str), 1561, 1492 (Ar-C=C str), 1226 (C=N str), 1104 (C-S-C str); **HRMS** (ESI): calcd for $C_{14}H_{17}N_2OS$ $(M+H)^+$: 261.1062 found 261.0999.

***N-(6-Methyl-4-phenyl-6H-1,3-thiazin-2-yl)butyramide* (9h)**

The compound **9h**was prepared according to general procedure by utilizing butyryl chloride **8h**. Brown colour liquid; Yield: 83 %; **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 7.62-7.59 (m, 2H), 7.39–7.28 (m, 3H), 5.58 (d, J = 5.8 Hz, 1H), 3.86–3.70 (m, 1H), 2.49 (q, J = 7.5 Hz, 2H), 1.46 (d, J = 6.9 Hz, 3H), 1.20 (t, J = 7.5 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 173.04 (CO), 169.00 (C2), 153.16 (C6), 142.34 (ArC), 142.12 (ArC), 130.17 (ArC), 129.60 (ArC), 128.09 (ArC), 124.98 (ArC), 124.72 (C5), 60.07 ($\underline{C}H_2$), 42.17 (C4), 28.95 ($\underline{C}H_3$), 22.02 ($\underline{C}H_2$), 21.92 ($\underline{C}H_3$). **IR** (KBr, cm^{-1}): 3434 (NH str), 2962 (CH_3 str), 2925 (CH_3 str), 1698 (CO str), 1557 (Ar-C=C str), 1259 (C=N str), 1152 (C-S-C str); **HRMS** (ESI): calcd for $C_{15}H_{19}N_2OS$ $(M+H)^+$: 275.1218 found 275.124.

***N-(6-Methyl-4-phenyl-6H-1,3-thiazin-2-yl)benzamide* (9i)**

The compound **9i** was prepared according to general procedure by utilizing benzoyl chloride **8i**. Off white solid; Yield: 85%; M.P: 91-95 °C.; **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 12.20 (brm 1H), 8.22-8.18 (m, 2H), 7.57–7.49 (m, 3H), 7.46–7.39 (m, 5H), 5.53 (d, J = 5.5 Hz, 1H), 4.00–3.89 (m, 1H), 1.62–1.49 (m, 6H);); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 178.87 (CO), 168.90 (C2), 154.32 (C6), 142.54 (ArC), 142.34 (ArC), 140.71 (ArC), 128.82 (ArC), 128.54 (ArC), 128.50 (ArC), 128.35 (ArC), 126.66 (ArC), 126.64 (ArC), 124.92 (ArC), 124.46 (ArC), 124.09 (ArC), 122.88 (C5), 23.01 ($\underline{C}H$), 21.53 ($\underline{C}H_3$), **IR** (KBr, cm^{-1}): 3435 (NH str), 3058 (=CH str), 2959

(CH_3 str), 1654 (CO str), 1598, 1538 (Ar-C=C str), 1370 (C=N str), 1275 (C-S-C str); **HRMS** (ESI): calcd for $C_{18}H_{17}N_2OS$ $(M+H)^+$: 309.1062 found 309.1106.

***Methyl-6-methyl-4-phenyl-6H-1,3-thiazin-2-ylcarbamate* (9j)**

The compound **9j** was prepared according to general procedure by utilizing methyl-chloro formate **8j**. Brown color liquid; Yield: 88 %;**^{1}H NMR** (400 MHz, $CDCl_3$) δ: 7.52–7.49 (m, 2H, ArH), 7.40–7.38 (m, 4H, ArH), 5.45 (d, J = 5.5 Hz, 1H), 3.95–3.91 (m, 1H), 3.79 (s, 3H), 1.56 (d, J = 7.0 Hz, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ ppm: 166.12 (C6), 162.45 (CO), 155.40 (ArC), 131.22 (ArC), 130.47 (ArC), 128.06 (ArC), 127.11 (ArC), 126.16 (ArC), 124.74 (ArC), 120.45 (C5), 45.12 (O<u>C</u>H$_3$), 29.81 (<u>C</u>4), 25.46 (<u>C</u>H$_3$); **IR** (KBr, cm^{-1}): 3429 (NH str), 2978 (CH_3 str), 2928 (CH_3 str), 1732 (CO str), 1566, 1493 (Ar-C=C str), 1374 (C=N str), 1195 (C-S-C str), 1105 (C-O-C str); **HRMS** (ESI): calcd for $C_{13}H_{15}N_2O_2S$ $(M+H)^+$: 263.0854 found 263.0811.

***Ethyl-6-methyl-4-phenyl-6H-1,3-thiazin-2-ylcarbamate* (9k)**

The compound **9k** was prepared according to general procedure by utilizing ethyl chloroformate **8k**. Brown color liquid; Yield: 90 %; **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 7.53-7.48 (m, 2H), 7.42-7.39 (m, 3H), 5.48 (d, J = 5.4 Hz, 1H), 4.20 (q, J = 7.1 Hz, 2H), 3.96 (brs, 1H), 1.56 (d, J = 7.0 Hz, 3H), 1.33 (t, J = 7.1 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 163.18 (C6), 158.12 (CO), 149.67 (ArC), 133.43 (ArC), 128.53 (ArC), 127.50 (ArC), 126.79 (ArC), 123.24 (ArC), 122.56 (ArC), 118.91 (C5), 45.39 (O<u>C</u>H$_2$), 27.43 (<u>C</u>4), 24.60 (<u>C</u>H$_3$), 21.07 (<u>C</u>H$_3$); **IR** (KBr, cm^{-1}): 3436 (NH str), 2924 (CH_3 str), 2853 (CH_2 str), 1725 (CO str), 1636, 1558 (Ar-C=C str), 1440 (C=N str), 1260 (C-S-C str); **LC-MS** (+ESI): calcd for $C_{14}H_{19}N_2O_3S$ $(M+H_2O+H)^+$: 295.111; Found 295.2.

***Isopropyl-6-methyl-4-phenyl-6H-1,3-thiazin-2-ylcarbamate* (9l)**

The compound **9l** was prepared according to general procedure by utilizing isopropyl chloroformate **8l**. Off white solid; Yield: 90 %; M.P: 122-125 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 7.53-7.48 (m, 2H), 7.42-7.39 (m, 3H), 5.48 (d, J = 5.6 Hz, 1H), 5.02-4.96 (q, J = 4.5 Hz, 1H), 3.88-3.77 (m, 1H), 1.56 (d, J = 7.0 Hz, 3H), 1.34 (d, J = 6.8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 164.76 (C6), 159.14 (CO), 150.29 (ArC), 130.82 (ArC), 129.47 (ArC), 127.43 (ArC), 126.15 (ArC), 124.40 (ArC), 123.58 (ArC), 119.40 (C5), 48.37 (O<u>C</u>H), 28.42 (<u>C</u>H), 25.61 (<u>C</u>H$_3$), 21.07 (<u>C</u>H$_3$); **IR** (KBr, cm^{-1}): 3435 (NH str), 3045 (=CH str), 2923 (CH_3 str), 2954 (CH

str), 1733 (CO str), 1558, 1486 (Ar-C=C str), 1445 (C=N str), 1220 (C-S-C str); **HRMS** (ESI): calcd for $C_{15}H_{19}N_2O_2S$ $(M+H)^+$: 291.1167 found 291.1308.

Tert-butyl-6-methyl-4-phenyl-6H-1,3-thiazin-2-ylcarbamate **(9m)**

The compound **9m** was prepared according to general procedure by utilizing tertbutyl chloro formate **8m**. Brown color liquid; Yield: 90 %; **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 7.53-7.48 (m, 2H), 7.42-7.39 (m, 3H), 5.48 (d, J = 5.6 Hz, 1H), 3.82-3.77 (m, 1H),1.54-1.42 (m, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 166.97 (C2), 159.76 (C6), 151.10 (ArC), 131.48 (ArC), 129.19 (ArC), 127.49 (ArC), 126.25 (ArC), 124.89 (ArC), 121.78 (ArC), 119.89 (C5), 50.40 ($O\underline{C}(CH_3)_3$), 30.18 ($\underline{C}H$), 26.78 ($\underline{C}H_3)_3$, 24.18 ($\underline{C}H_3$), 21.07 ($\underline{C}H_3$); **IR** (KBr, cm^{-1}): 3436 (NH str), 3052 (=CH str), 2975 (CH_3 str), 2924 (CH str), 1731 (CO str), 1617, 1567, 1481 (Ar-C=C str), 1368 (=CN str), 1239 (C-S-C str), 755. **LC-MS** (ESI): calcd for $C_{16}H_{21}N_2O_2S$ $(M+H)^+$: 305.13 found 305.71

N-(6-methyl-4-phenyl-6H-1,3-thiazin-2-yl)methanesulfonamide **(9n)**

The compound **9n** was prepared according to general procedure utilizing methane sulfonyl chloride 8**n** brown colour liquid. Yield: 82 %; **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 9.81 (brs, 1H), 7.47–7.26 (m, 5H), 5.69 (d, J = 5.2 Hz, 1H), 4.01-3.97 (m, 1H), 3.10 (s, 3H), 1.57 (d, J =7.0 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 163.85 (C2), 154.49 (C6), 143.44 (ArC), 133.32 (ArC), 130.02 (ArC), 129.63 (ArC), 125.02 (ArC), 115.40 (C5), 113.54 (ArC), 30.02 (CH_3), 25.81 (C4), 15.40 (CH_3); **IR** (KBr, cm^{-1}): 3402 (NH str), 3058 (=CH str), 2928 (CH_3 str), 1557, 1505 (Ar-C=C str), 1429 (C=N str), 1331 (C=N str), 1287 (C-S-C str); **Mass** (ESI): calcd for $C_{12}H_{15}N_2O_2S_2$ $(M+H)^+$: 283.06; Found 283.0

N-(6-Methyl-4-phenyl-6H-1,3-thiazin-2-yl)-4-(trifluoromethoxy)benzenesulfonamide **(9o)**

The compound **9o** was prepared according to general procedure by utilizing 4-(trifluoro methoxy)benzene-1-sulfonyl chloride **8o**. Brown solid; **Yield:** 76 %;. **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 9.77 (brs, 1H), 8.06–7.99 (m, 2H), 7.50–7.40 (m, 5H), 7.36–7.30 (m, 2H), 5.46 (d, J = 5.2 Hz, 1H), 3.97-3.86 (m, 1H), 1.54 (d, J =7.0 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ ppm: 167.37 (C2), 162.81 (C6), 159.90 (ArC), 155.40 (ArC), 131.22 (ArC), 130.47 (ArC), 128.56 (ArC), 128.06 (ArC), 127.11 (ArC), 126.95 (ArC), 126.44 (ArC), 124.89 (ArC), 124.10 (ArC),

121.40 (C5), 29.84 (C4), 22.48 (CH_3); **IR** (KBr, cm^{-1}): 3434 (NH str), 3053 (=CH str), 2987 (CH_3 str), 2926 (CH str), 1552 (Ar-C=C str), 1258 (C=N str), 1151 (C-S-C str); **Mass** (ESI): calcd for $C_{18}H_{14}N_2O_3S_2F_3$ $(M-H)^+$: 427.04; Found 427.1

***1-(2-fluorophenyl)-3-(6-methyl-4-phenyl-6H-1,3-thiazin-2-yl)urea* (9p)**

The compound **9p** was prepared according to general procedure by utilizing 2-fluoro phenyl iso cyanate **8p**. Yield: 82 %; M.P: 81-83 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 11.32 (brs, 1H), 10.37 (brs, 1H), 8.19–8.17 (m, 1H), 7.62 (d, J = 8.0 Hz, 2H), 7.36 (d, J = 8.0 Hz, 3H), 7.26-7.10 (m, 3H), 5.79 (d, J = 4.0 Hz, 1H), 3.97–3.95 (m, 1H), 1.38 (m, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm: 166.03 (C2), 162.59 (CO), 158.83 (ArC), 151.21 (ArC), 148.53 (C6), 141.83 (ArC), 140.20 (ArC), 139.92 (ArC), 132.70 (ArC), 128.68 (ArC), 128.27 (ArC), 120.00 (C5), 113.26 (ArC), 21.57 (C4), 20.49 (CH_3); **IR** (KBr, cm^{-1}): 3459 (NH str), 3084 (=CH str), 2964 (CH_3 str), 1684, 1547 (Ar-C=C str), 1254 (C=N str), 1162 (C-S-C str). **Mass** (ESI): calcd for $C_{18}H_{16}N_3OSF$ $(M)^+$: 341.10; Found 341.0.

***1-(2-chlorophenyl)-3-(6-methyl-4-phenyl-6H-1,3-thiazin-2-yl)urea* (9q)**

The compound **9q** was prepared according to general procedure by utilizing 2-chloro phenyl iso cyanate **8q**.Yield: 82 %; M.P: 81-83 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 11.79 (brs, 1H), 10.80 (brs, 1H), 7.66–7.35 (m, 9H), 5.63 (d, J = 4.0 Hz, 1H), 3.97–3.95 (m, 1H), 1.38 (m, 3H). **^{13}C NMR** (125 MHz, $CDCl_3$) δ ppm: 195.81 (CO), 153.51 (C2), 144.57 (C6), 144.33 (ArC), 140.79 (ArC), 138.07 (ArC), 137.29 (ArC), 136.42 (ArC), 133.03 (ArC), 132.45 (ArC), 130.42 (ArC), 130.23 (ArC), 129.45 (ArC), 128.69 (ArC), 127.93 (C5), 29.84 (C4), 21.93 (CH_3); **IR** (KBr, cm^{-1}): 3434 (NH str), 3072 (=CH str), 2926 (CH_3 str), 1552 (Ar-C=C str), 1258 (C=N str), 1151 (C-S-C str); **LC-MS** (ESI): calcd for $C_{18}H_{17}N_3OSCl$ $(M+H)^+$: 358.08; Found 358.0.

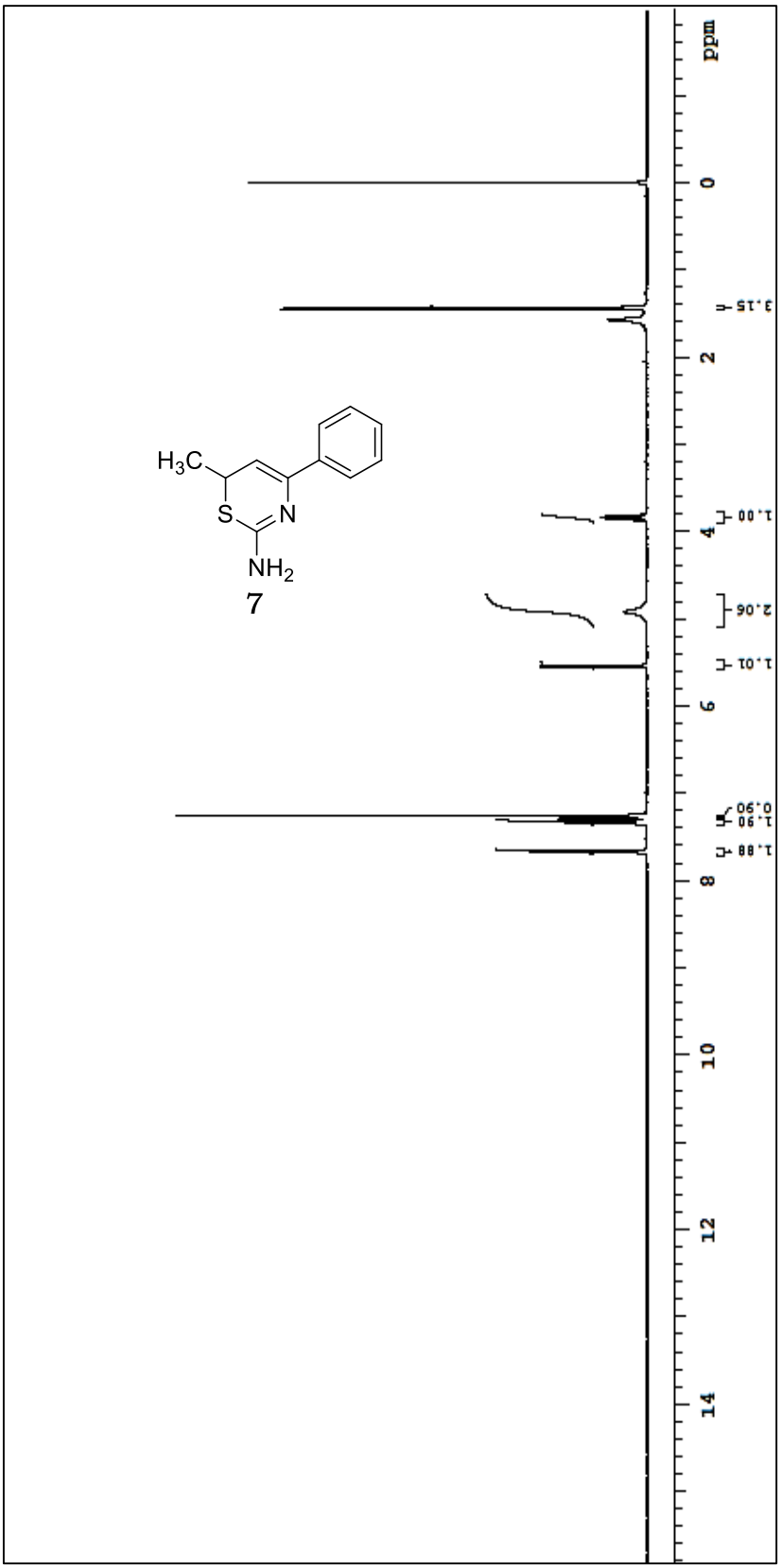
Invitro MTB screening

All the synthesized compounds were dissolved in 100% DMSO and stored as frozen stocks at concentrations of 50, 25, 12.5, 6.25, 3.13, 1.56, and 0.78 µg/mL using Middle-brook 7H11 agar medium. Inoculum was prepared by inoculating frozen stocks into 10 ml 7H11 mycobacterial culture medium supplemented with Oleic Albumin Dextrose Catalase (OADC) (1%) final concentration, and 0.05% Tween 80 For growth evaluation in the (MGIT) 960 instrument was

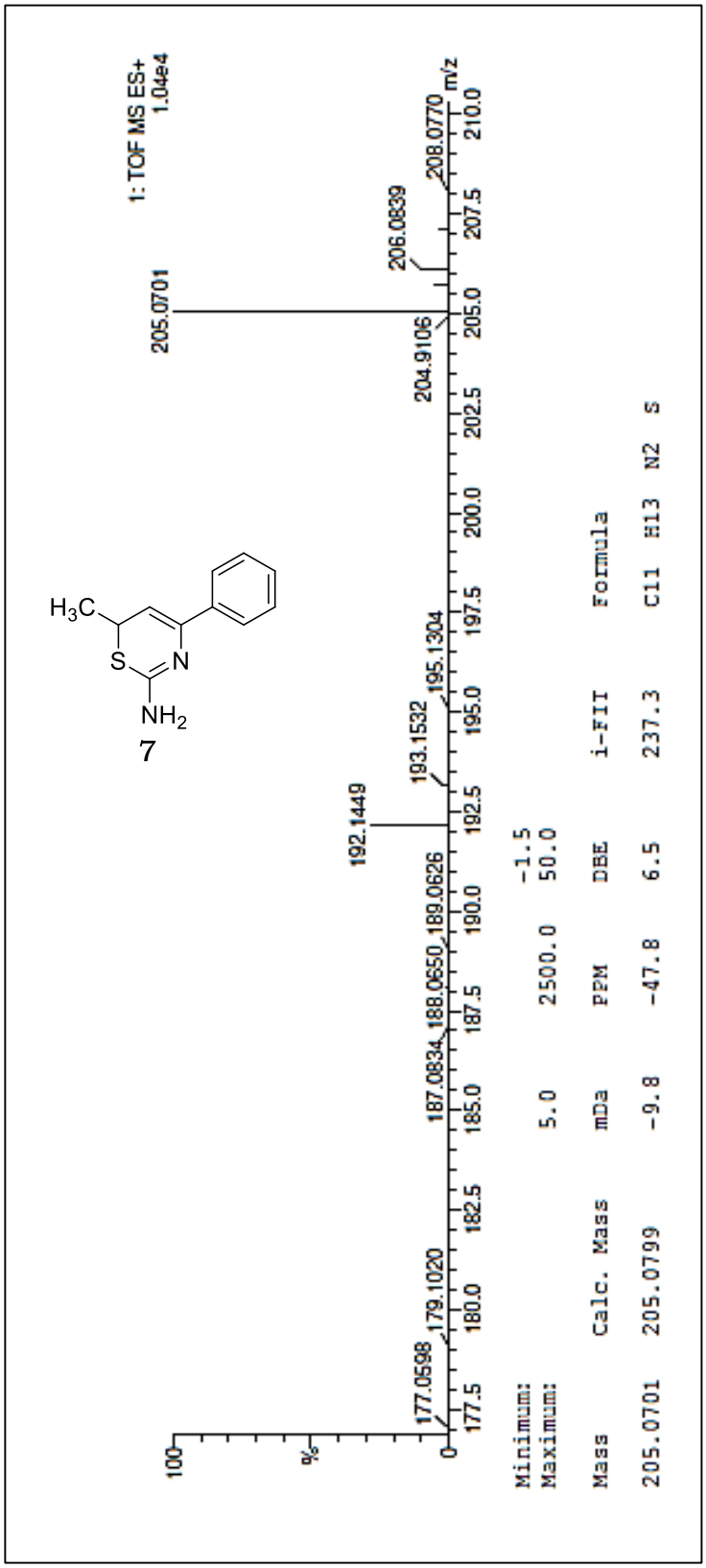
used. Mycobacterial growth is monitored through changes in oxygen consumption which in turn changes fluorescence. Serially two-fold diluted compounds (0.1 ml in DMSO) were added to the 7H11 culture medium contained in Mycobacteria Growth Inhibition Tube (MGIT) tube with the final DMSO concentration not exceeding 1.2 %. 5 µL of this bacterial suspension was spotted onto 7H11 agar tubes containing different concentrations of the drug as discussed above. The tubes were incubated at 37 °C, and final readings (as MIC in µg/mL) were determined after 28 days.

Cytotoxicity

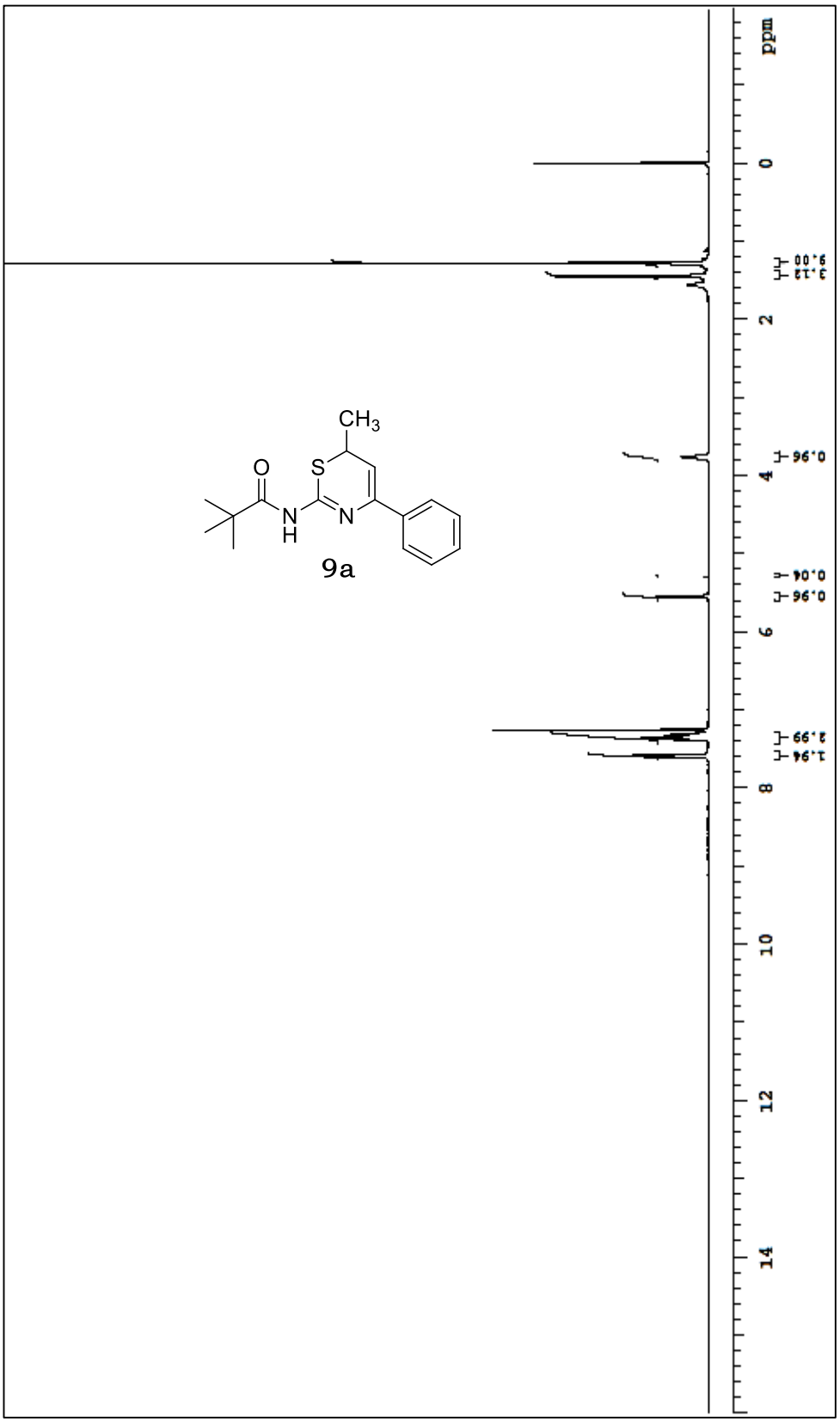
Invitro anti-cancer activity of the test compounds was tested using MTT colorimetric assay as per ATCC protocol.[4,5] Cell lines used in the present includes MCF-7 derived from human breast cancer cell line cells (ATCC No. HTB-28), EC-9706 derived from human esophageal cancer cell lines (ATCC No. HTB-39) which were procured from American Type Culture Collection, Manassas, VA, USA. Cisplatin was used as the standard drug in the assay. MCF-7 supplemented with 10 % new born calf serum (NBCS), 100 IU/mL penicillin, 100 mg/ml streptomycin and 2 mM-glutamine. Cell lines were maintained at 37 ^{0}C in a humidified 5 % CO_2 incubator (Thermo scientific). EC-9706 was maintained in DMEM medium supplemented with 10 % new born calf serum, along with 1 % non-essential amino acids, 0.2 % sodium bicarbonate, 1 % sodium pyruvate and 1 % antibiotic mixture (10, 000 U penicillin and 10 mg streptomycin per mL). Cell lines were processed by initial trypsinization to detach the adhered cells and followed by centrifugation to get cell pellet. Fresh media was added to the pellet to make a cell count using haemocytometer and plate 100 µL of media with cells ranging from 5,000-6,000 per well in a 96-well plate. The plate was incubated overnight in CO_2 incubator for the cells to adhere and regain its shape. After 24 h cells were treated with the test compounds at 25 µM diluted using the media to deduce the percentage inhibition on cancer cells and human normal cells. The cells were incubated for 48 h to assay the effect of the test compounds on different cell lines. Zero hour reading was noted down with untreated cells and also control with 1 % DMSO to subtract further from the 48hr reading. After 48 h incubation, cells were treated by MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dissolved in PBS (5 mg/ml) and incubated for 3-4 hr at 37 ˚C. The formazan crystals thus formed were dissolved in 100 µL of DMSO and the viability was measured at 540 nm on a multimode reader (spectra max).



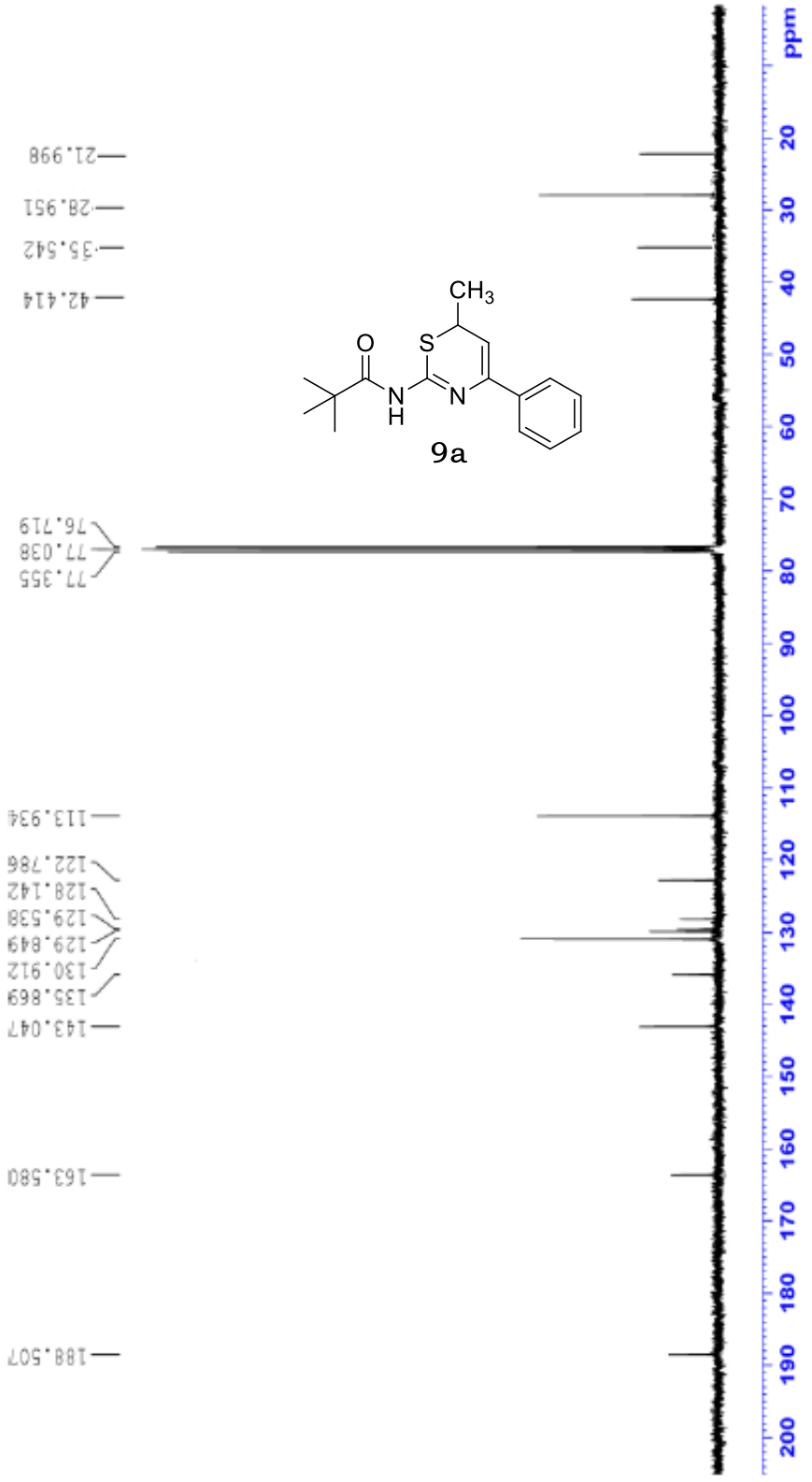
^{1}H NMR spectrum of compound 7



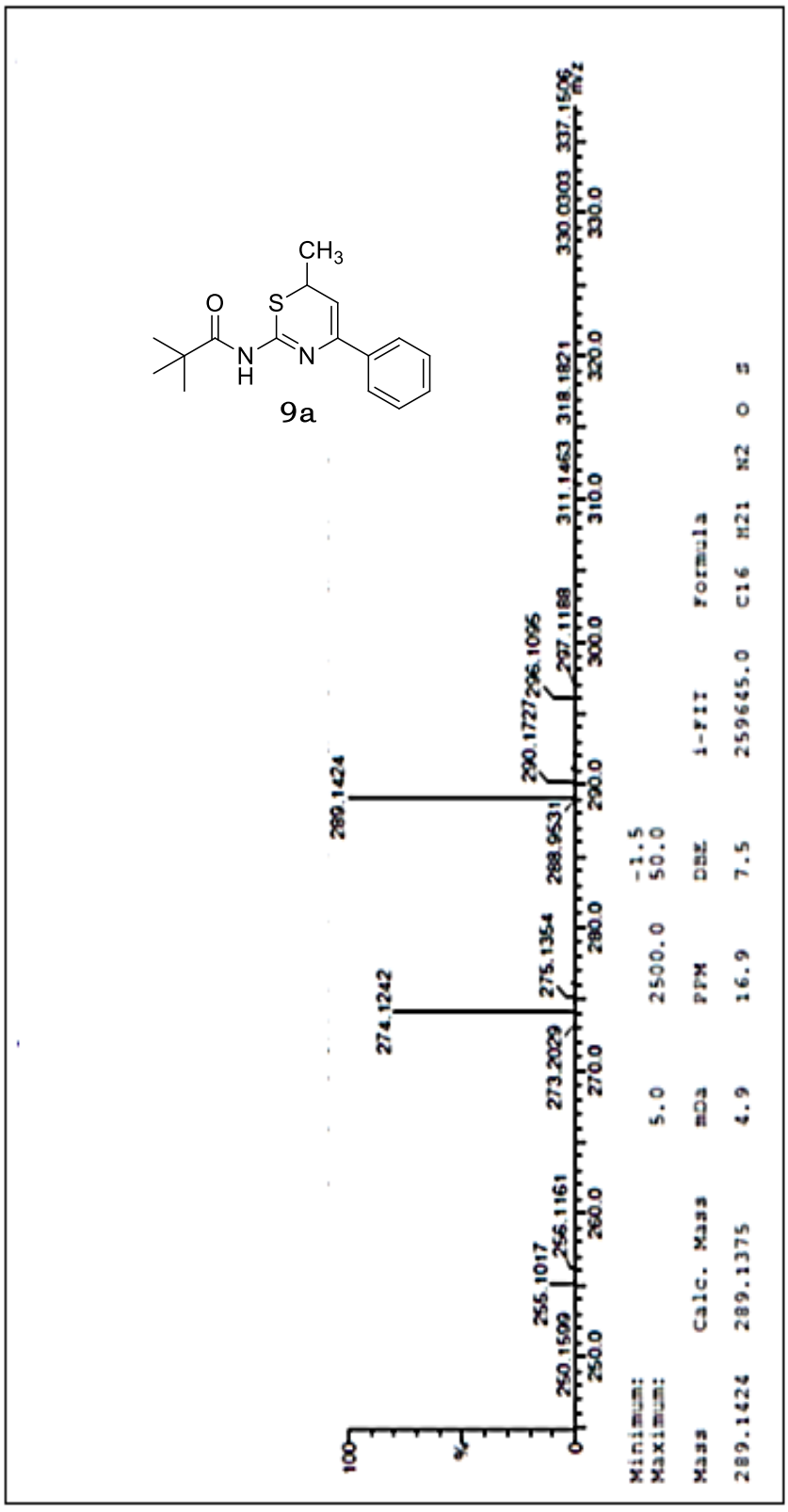
HRMS spectrum of compound 7



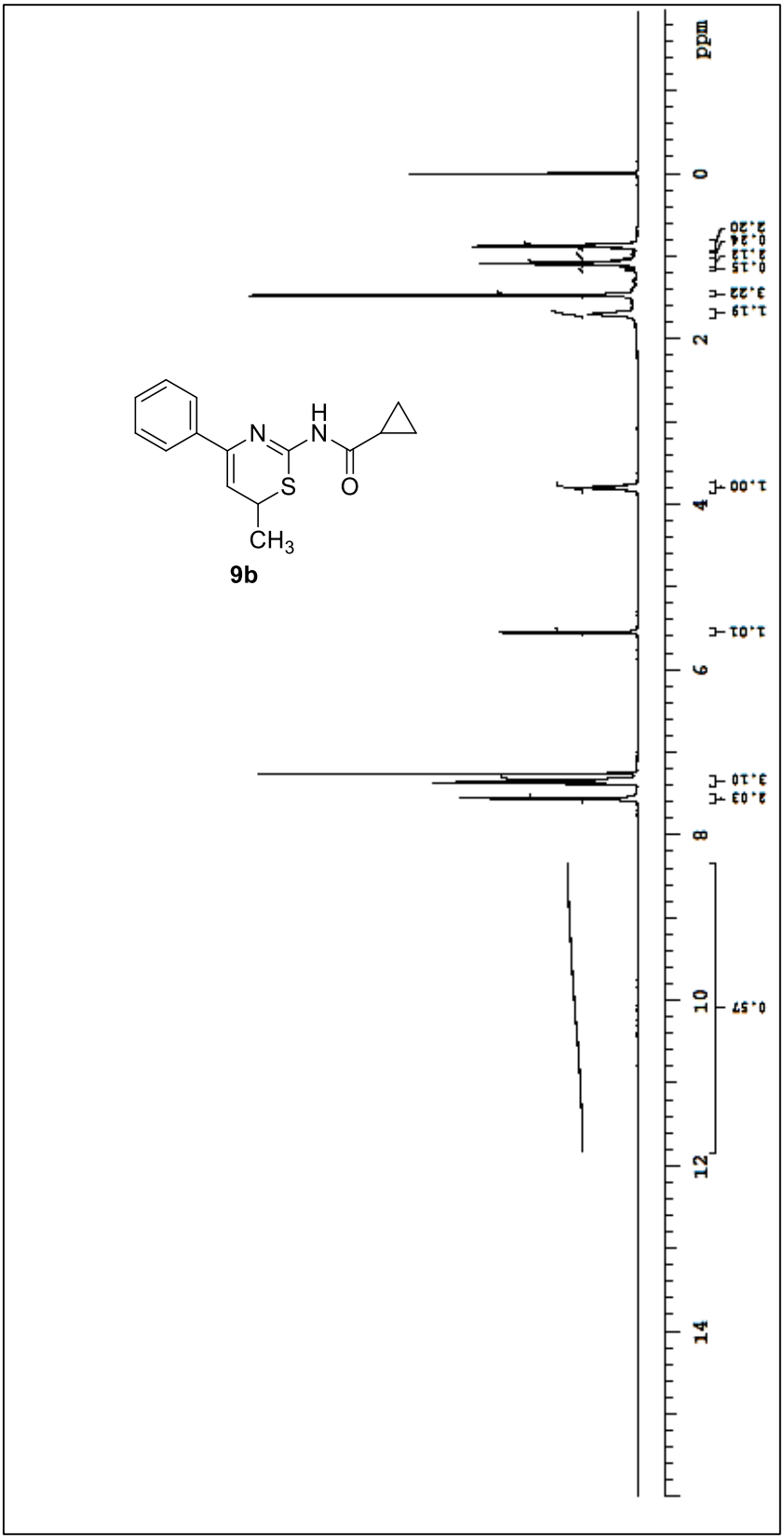
^{1}H NMR spectrum of compound 9a



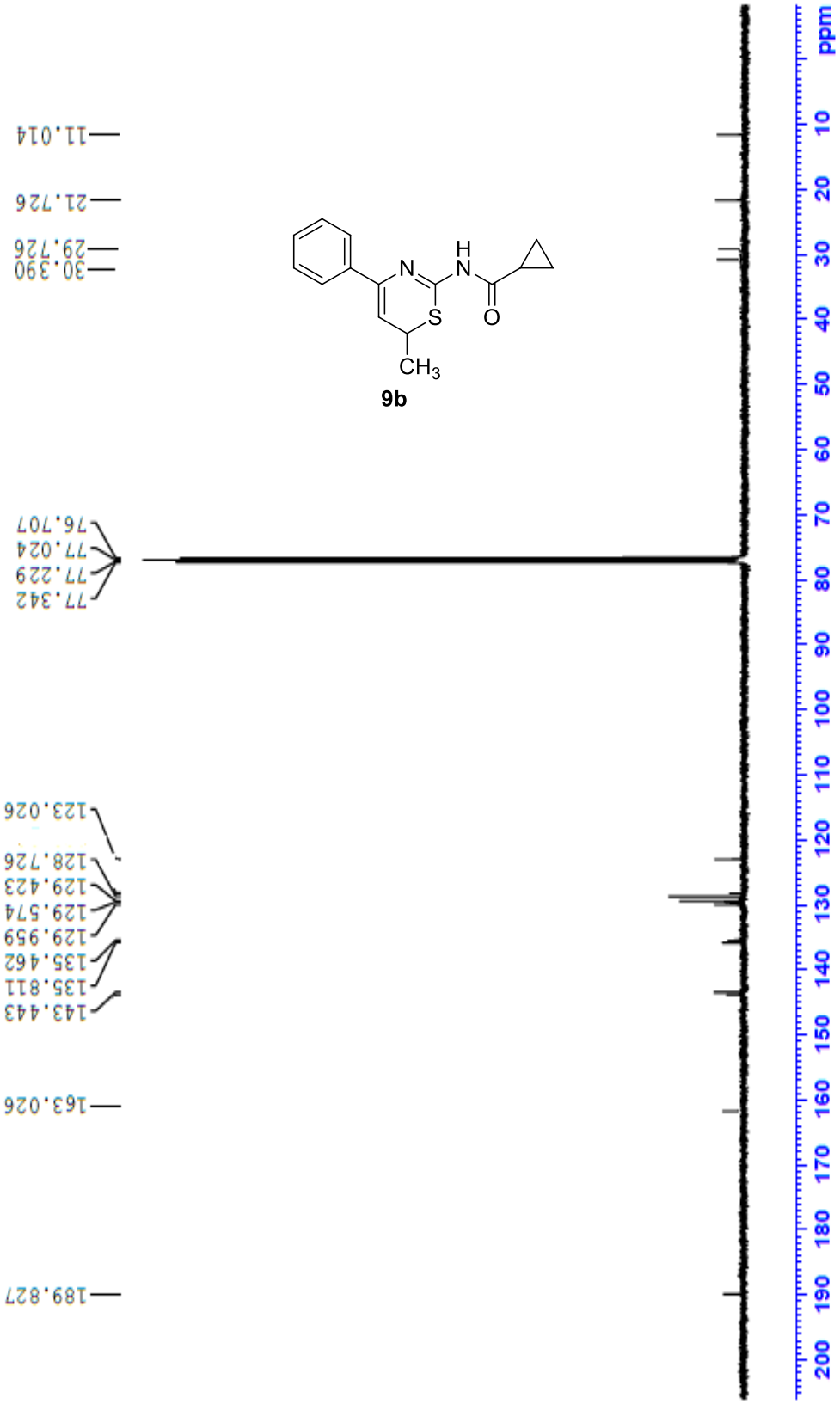
^{13}C NMR spectrum of compound 9a



HRMS spectrum of compound 9a

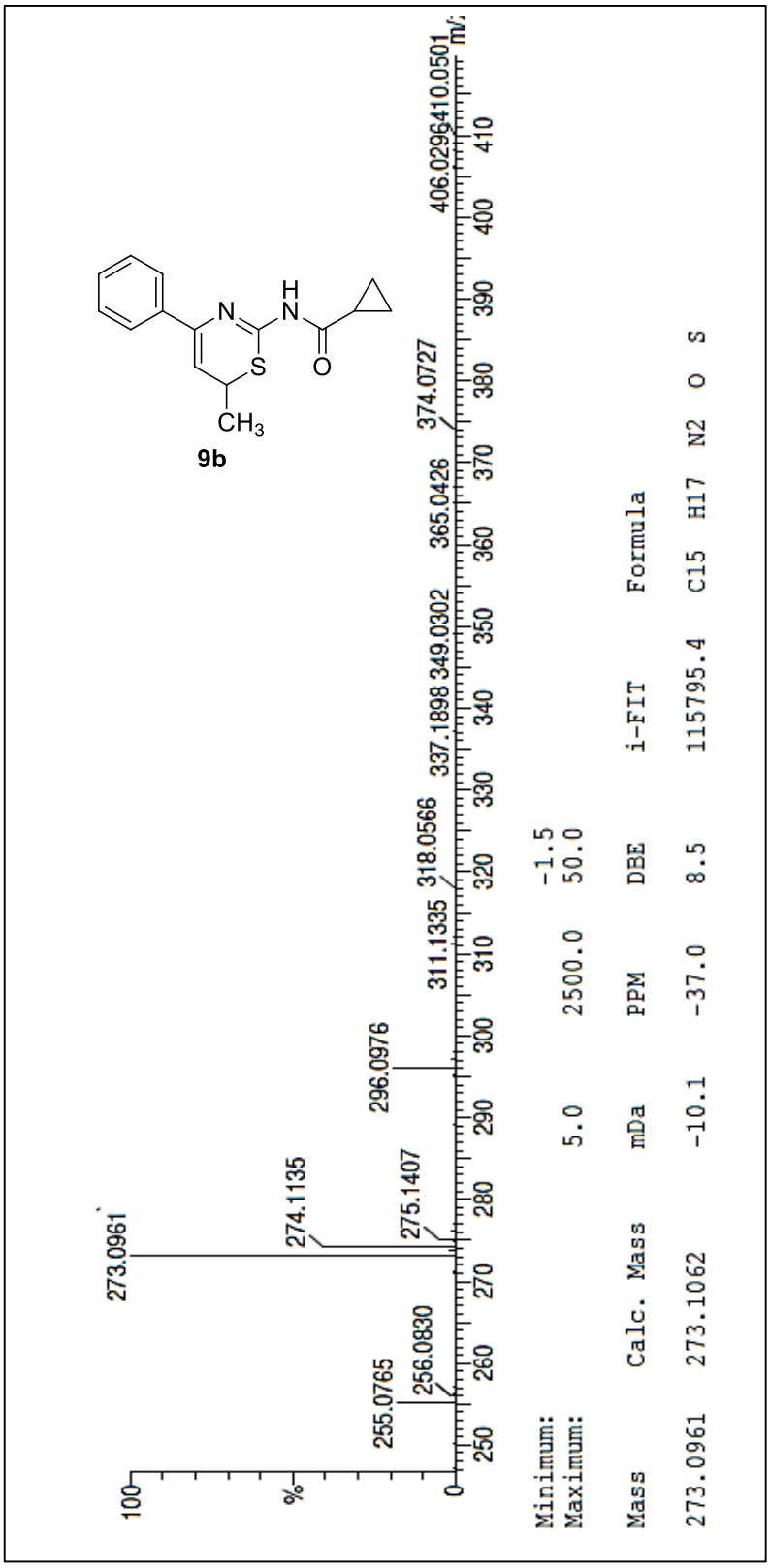


^{1}H NMR spectrum of compound 9b

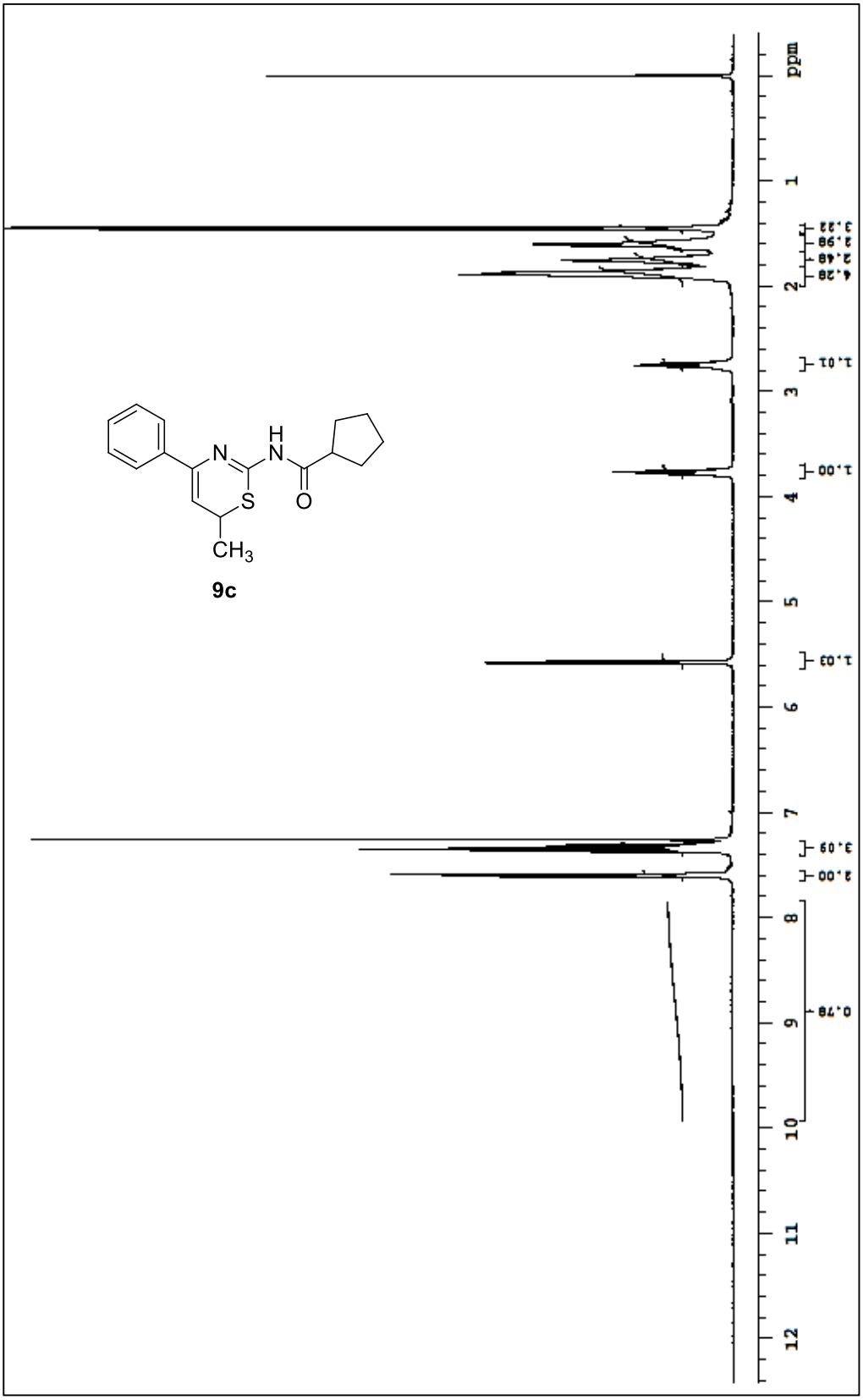


9b

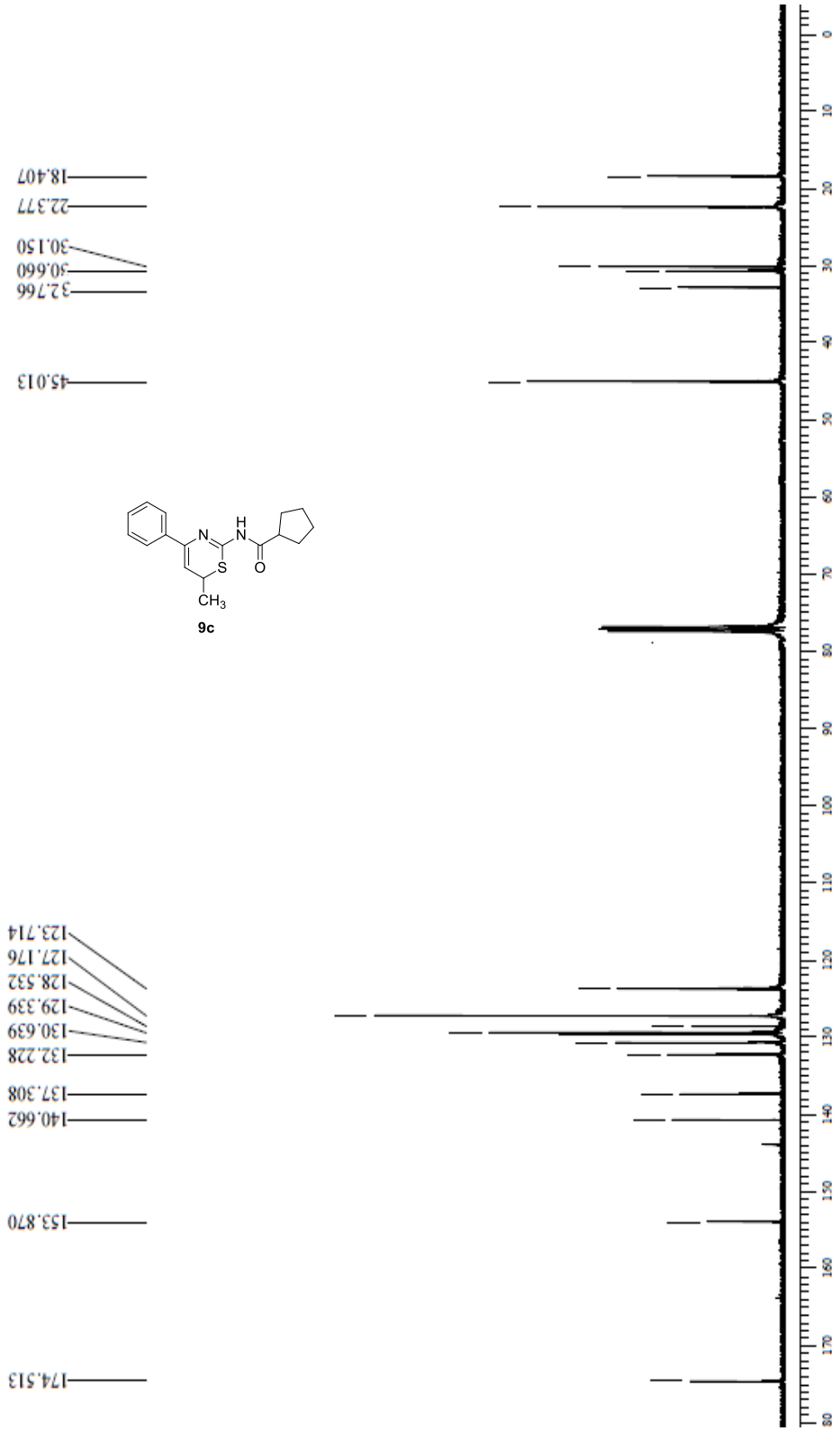
^{13}C NMR spectrum of compound 9b



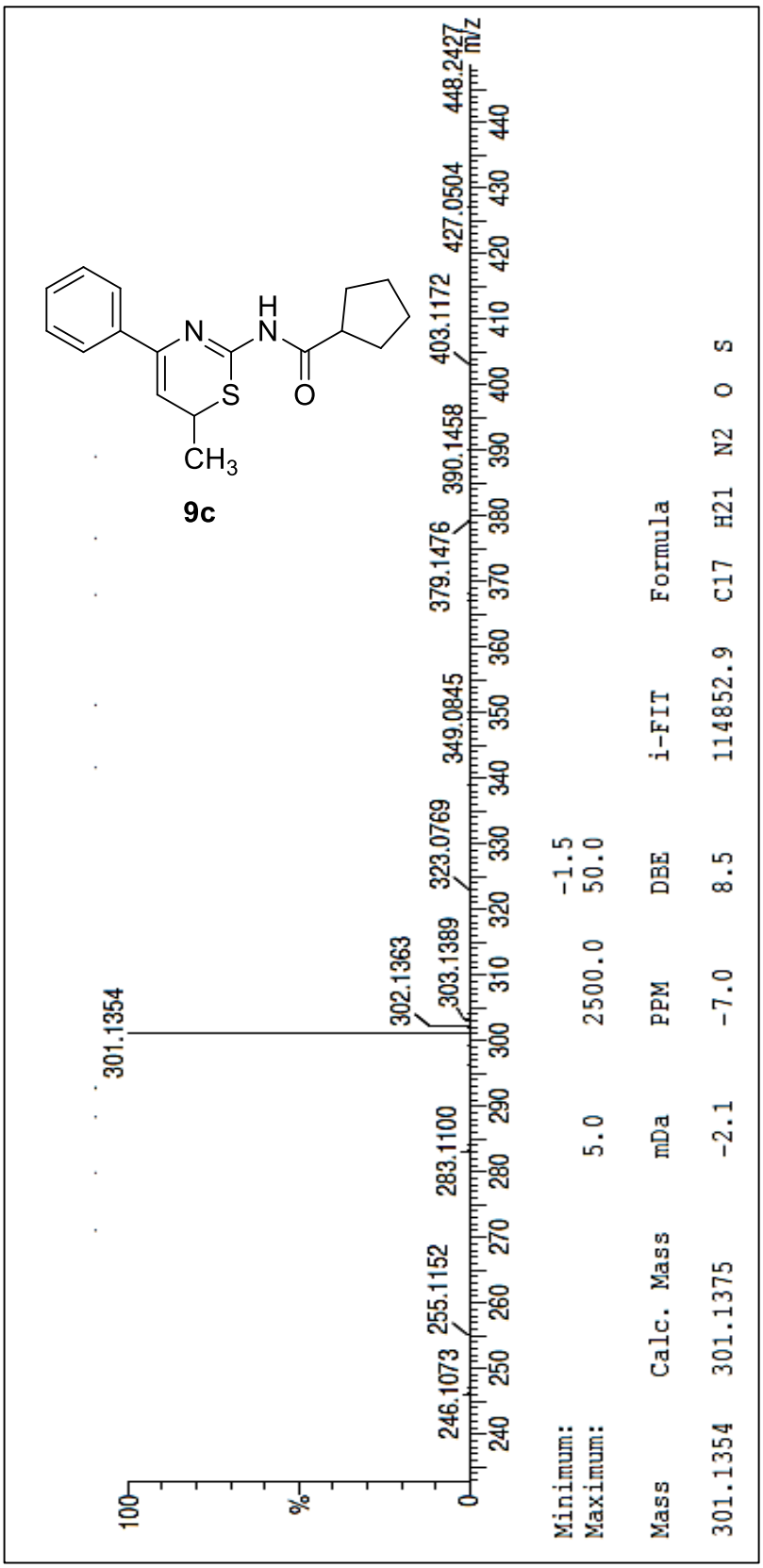
HRMS spectrum of compound 9b



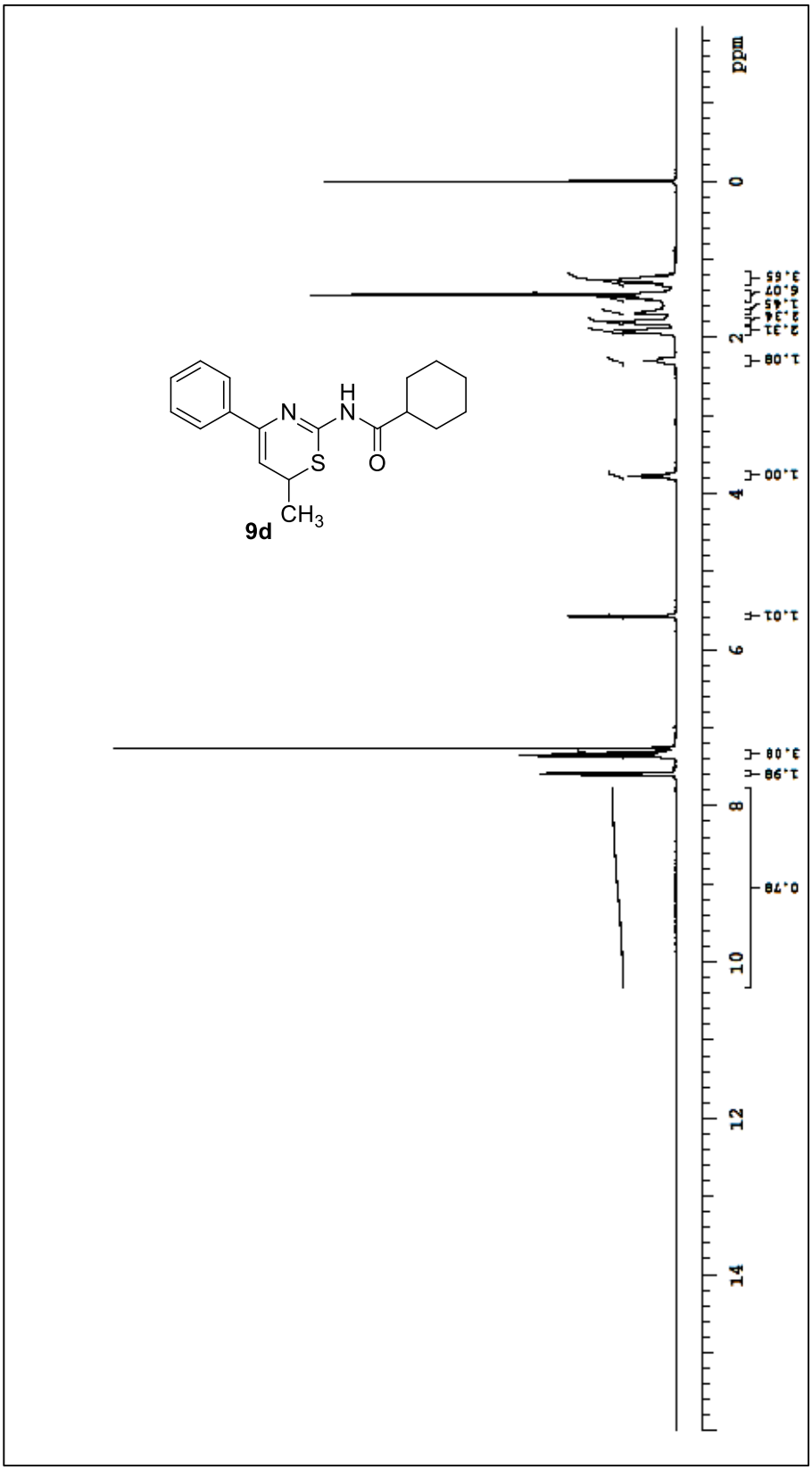
^{1}H NMR spectrum of compound 9c



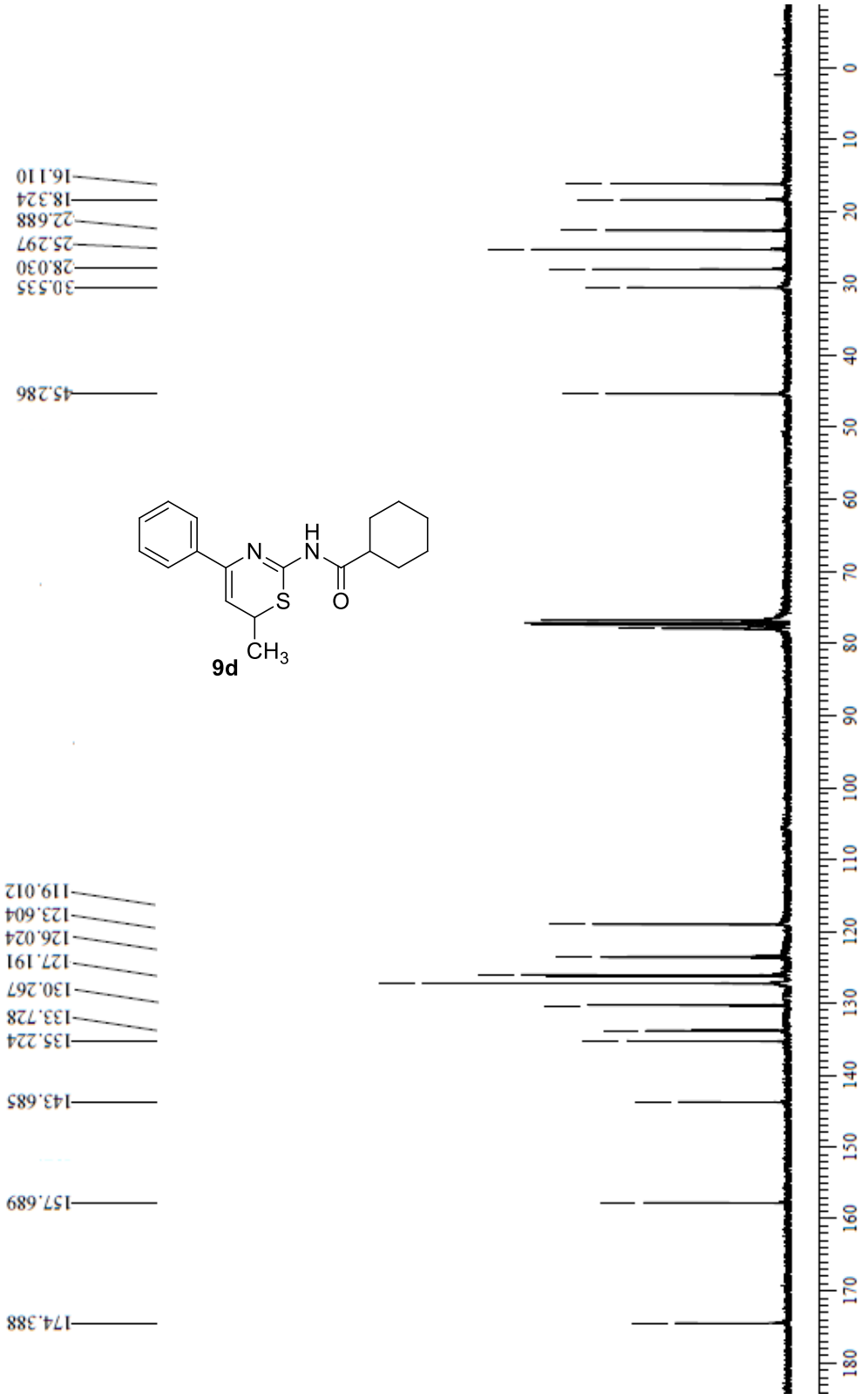
^{13}C NMR spectrum of **9c**



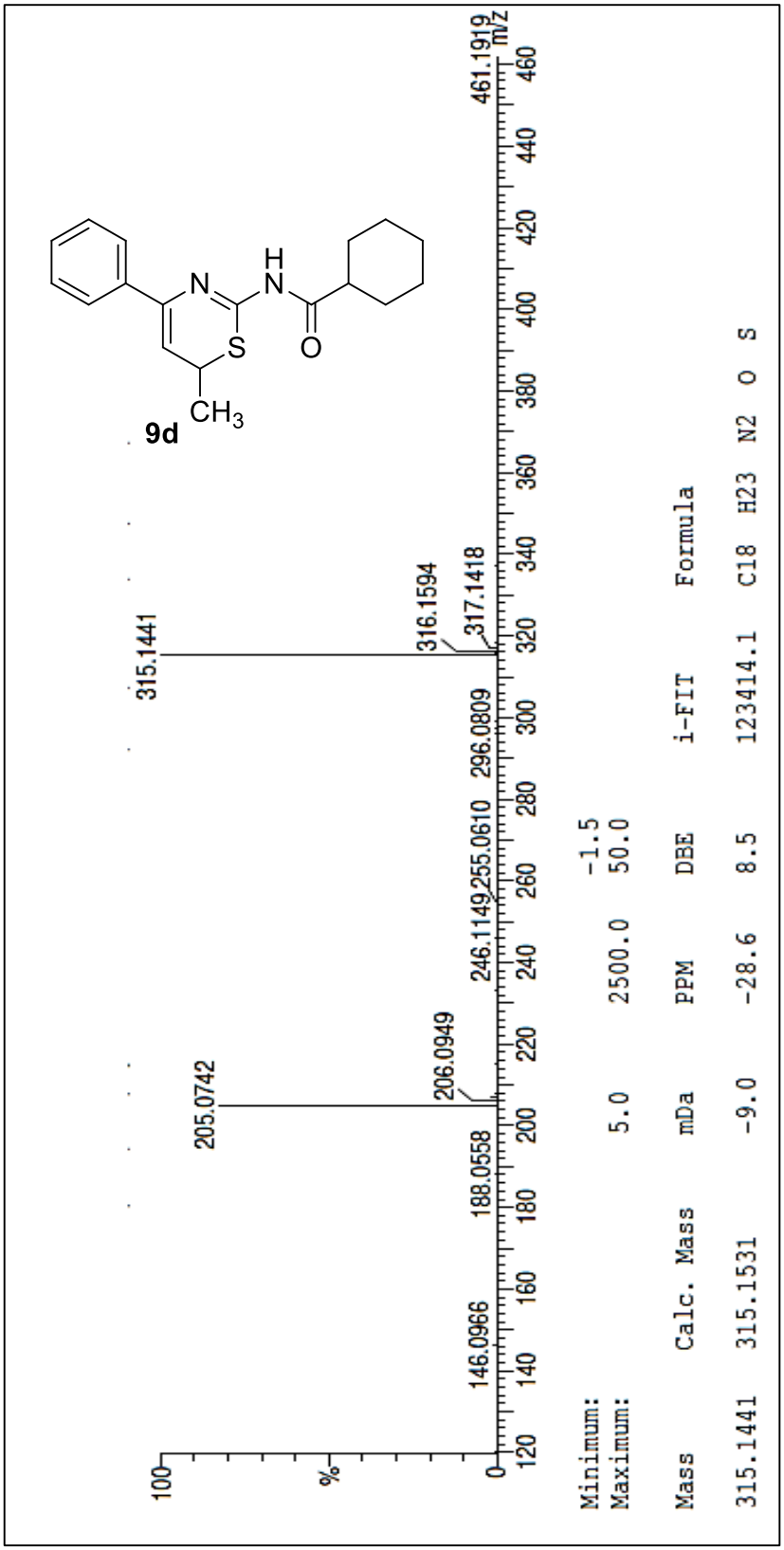
HRMS spectrum of compound 9c



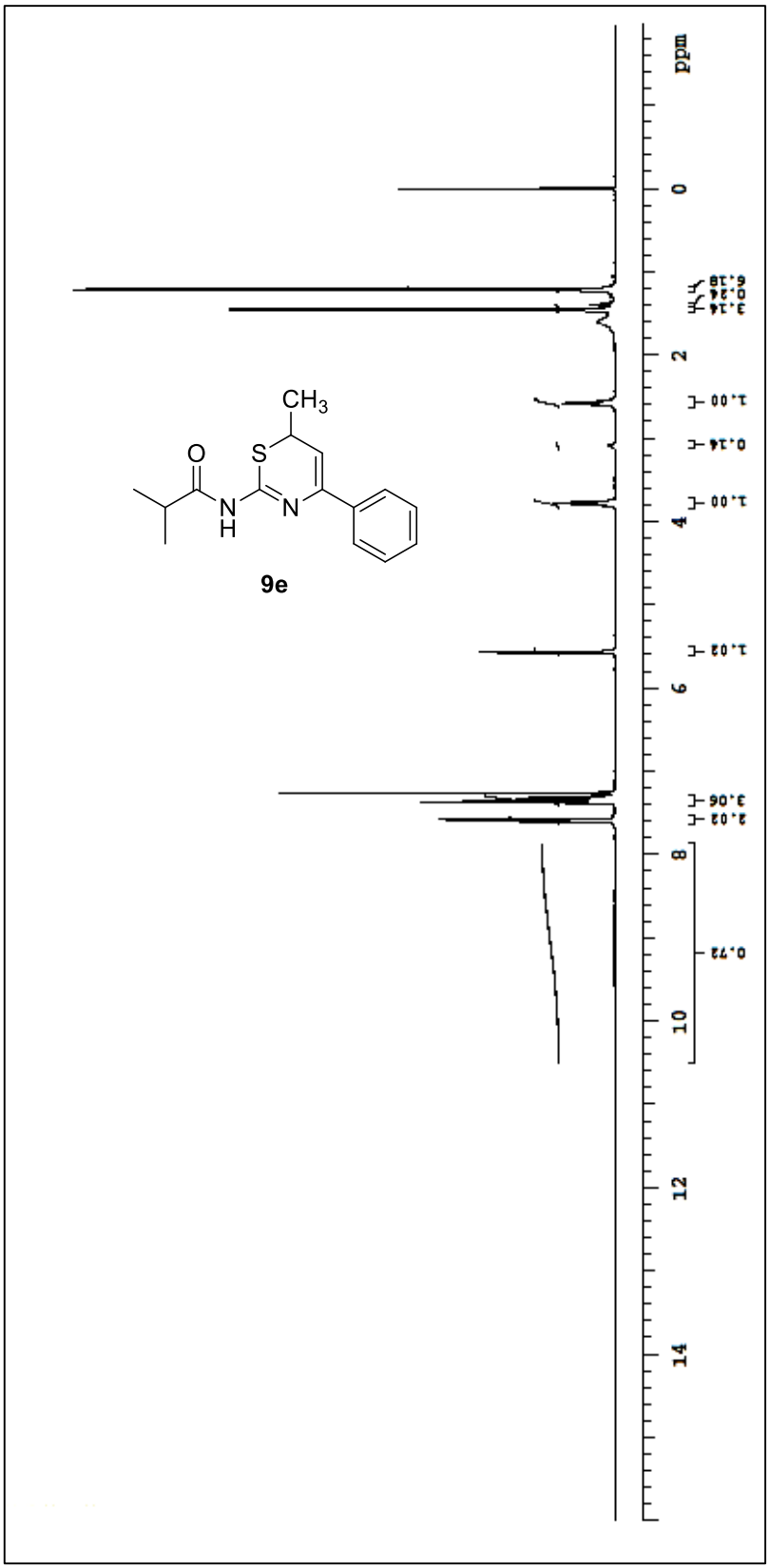
^{1}H NMR spectrum of compound 9d



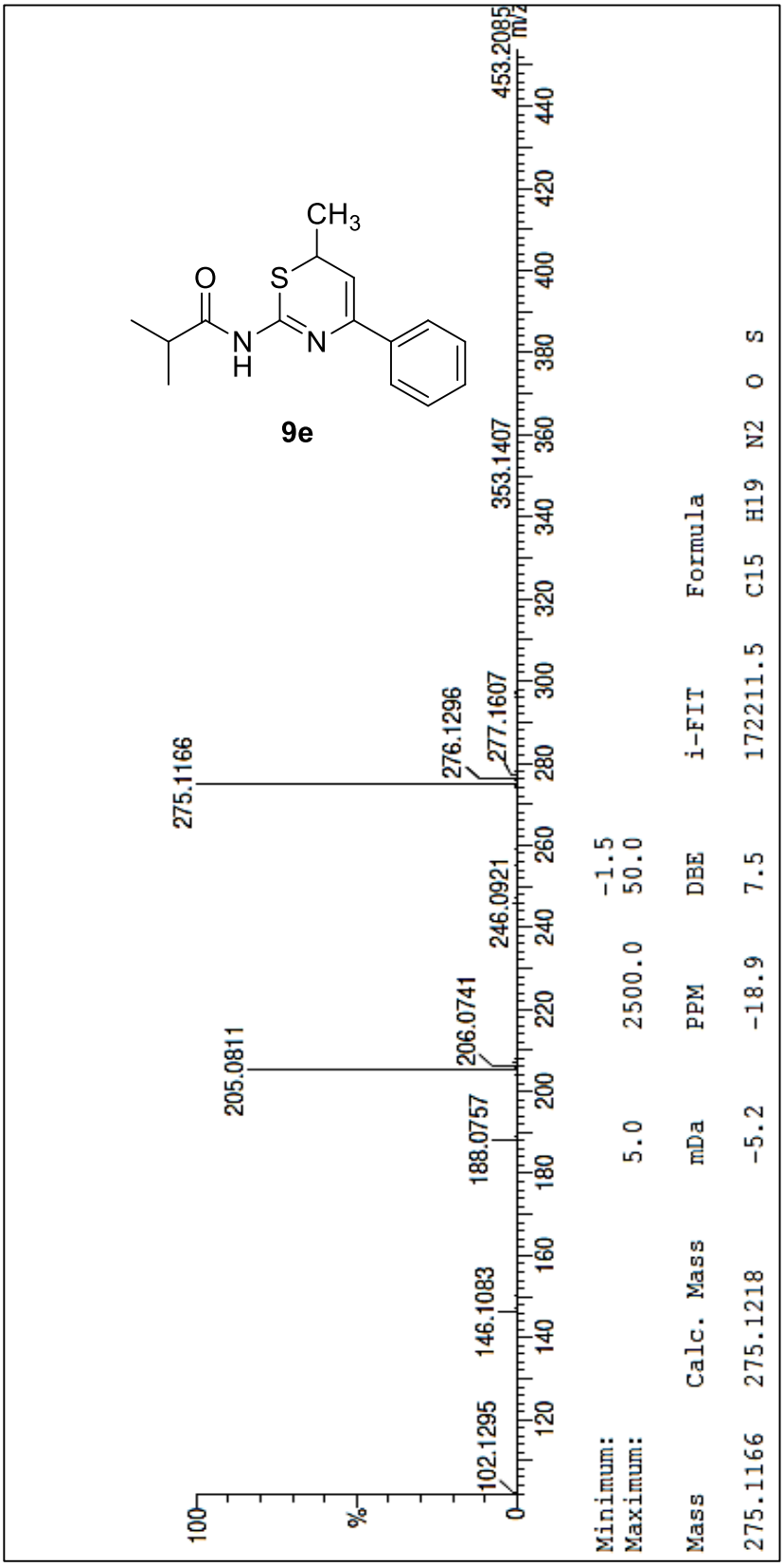
^{13}C NMR spectrum of compound 9d



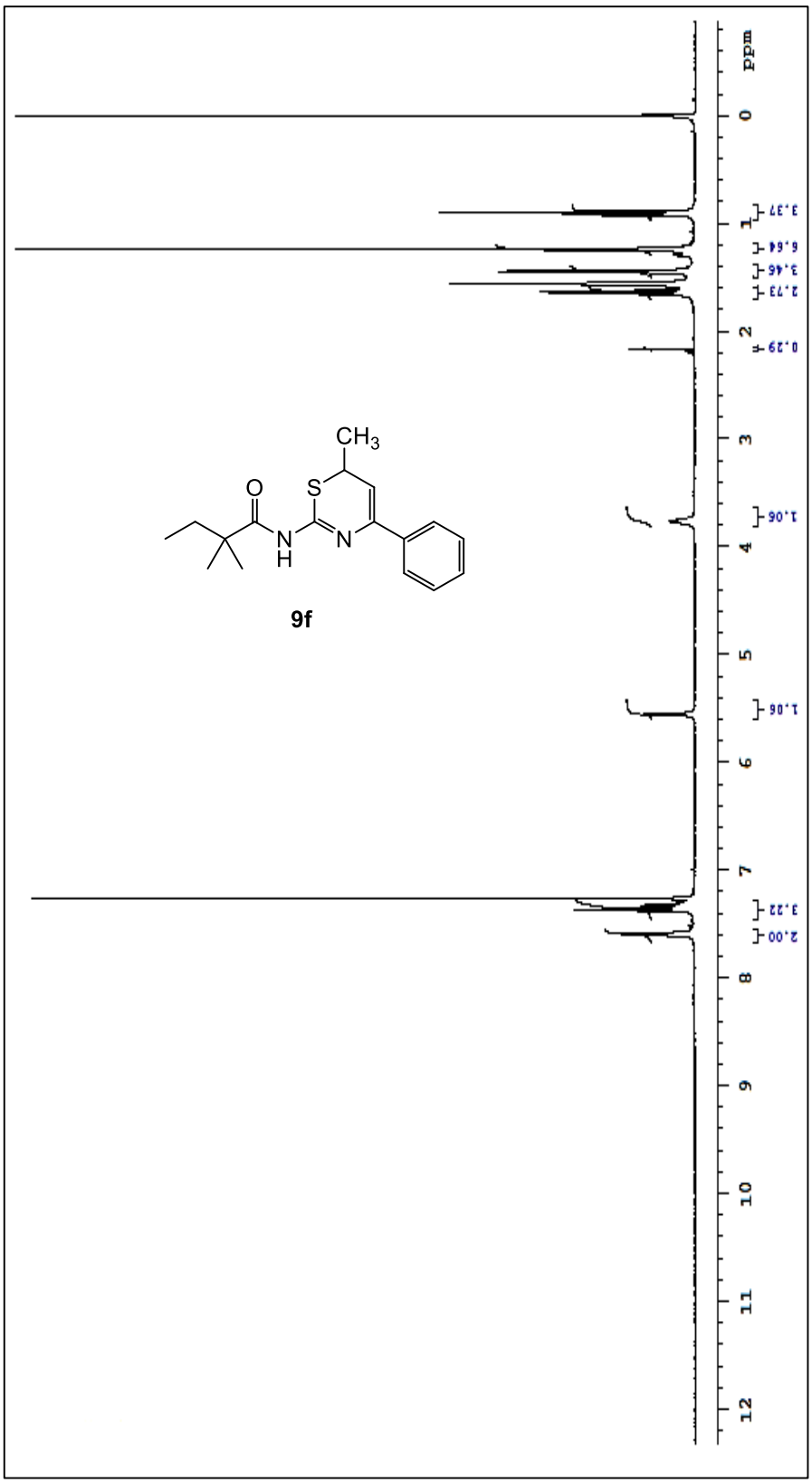
HRMS spectrum of compound 9d



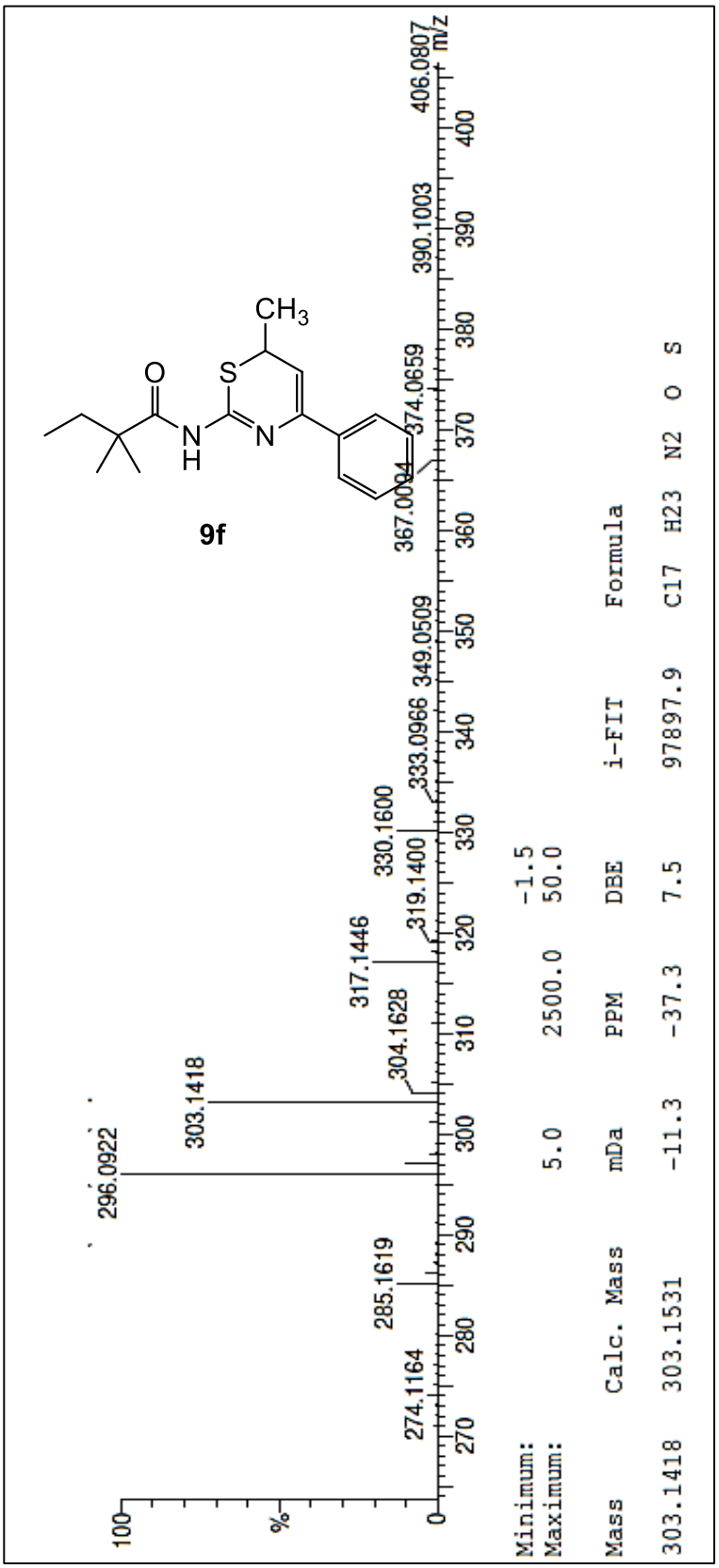
^{1}H NMR Spectrum of compound 9e



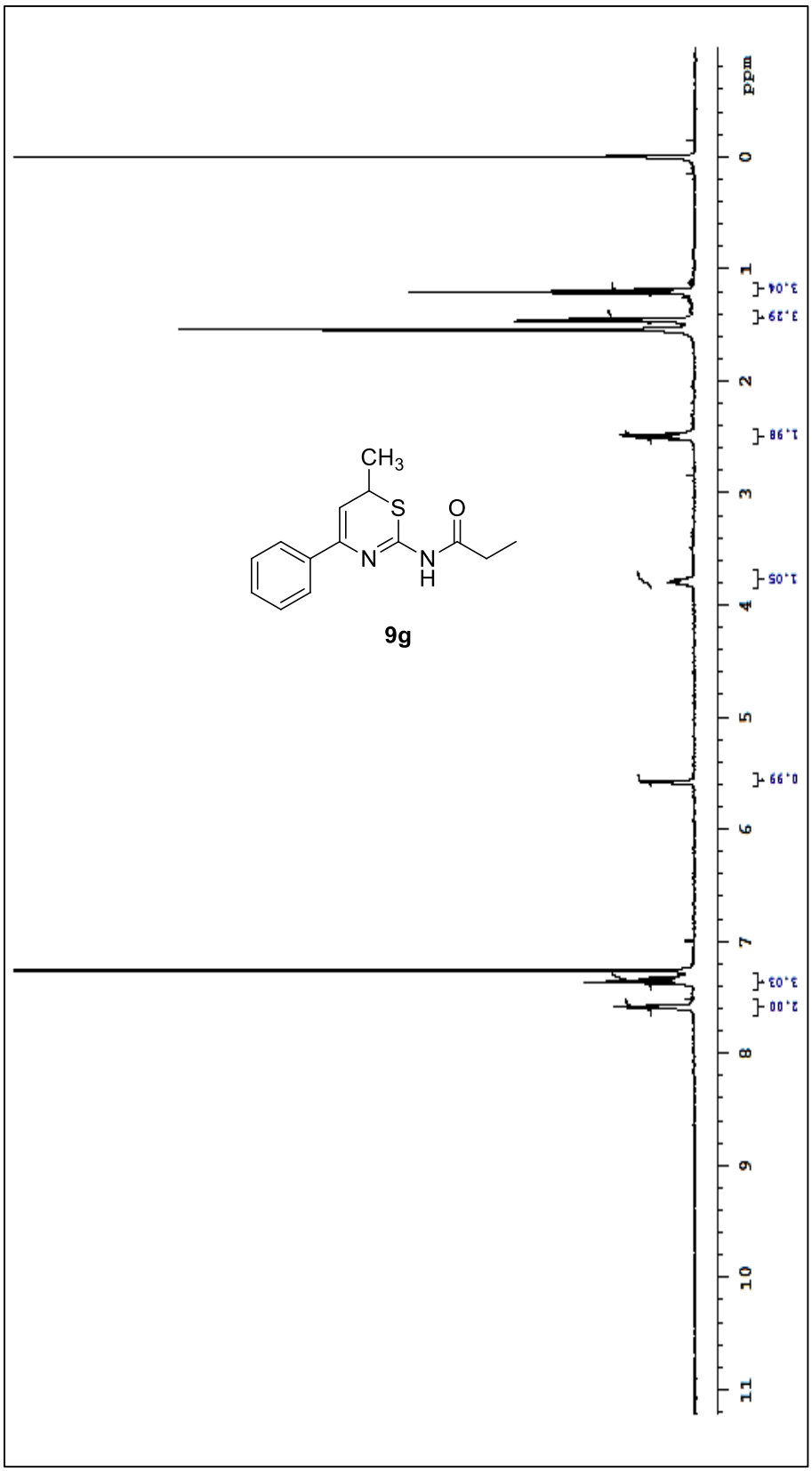
HRMS spectrum of compound 9e



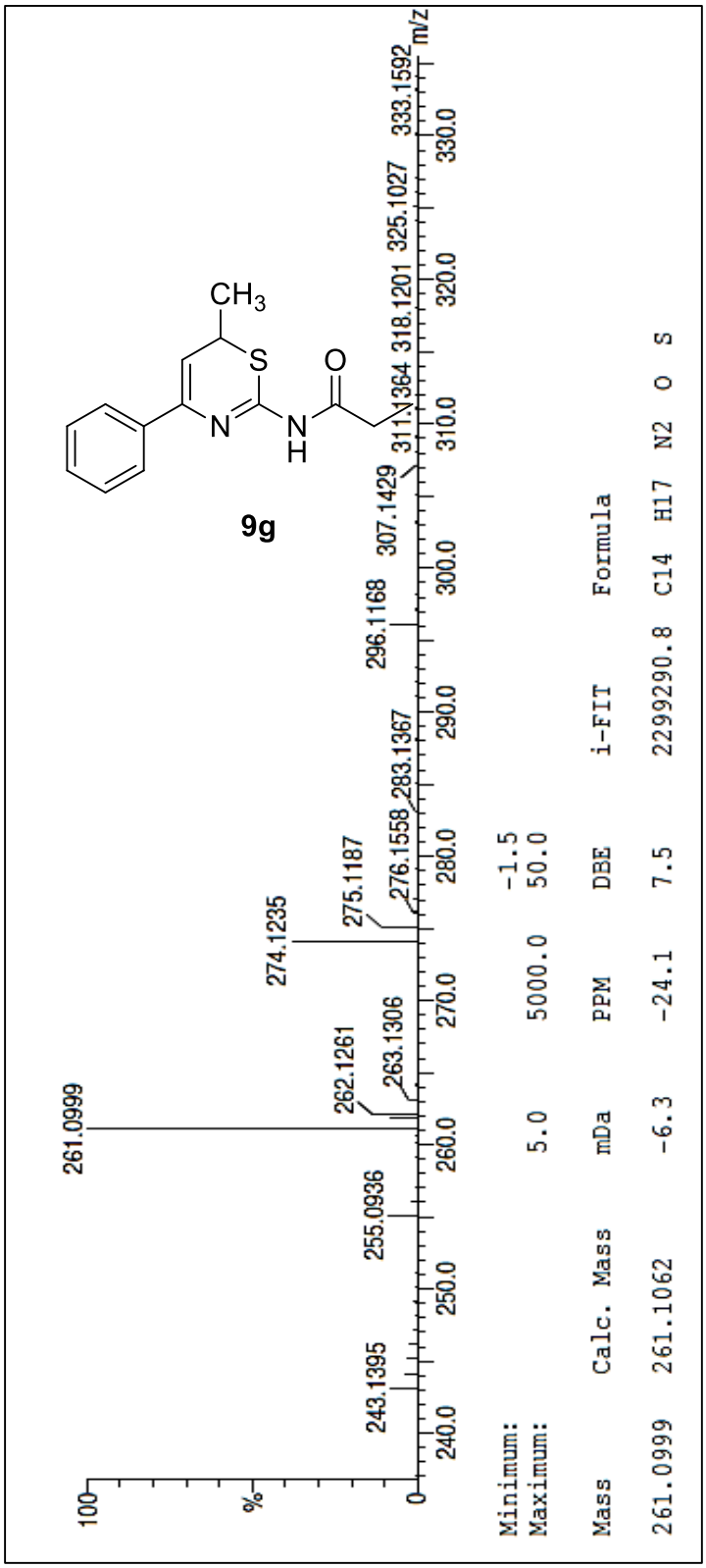
^{1}H NMR spectrum of compound 9f



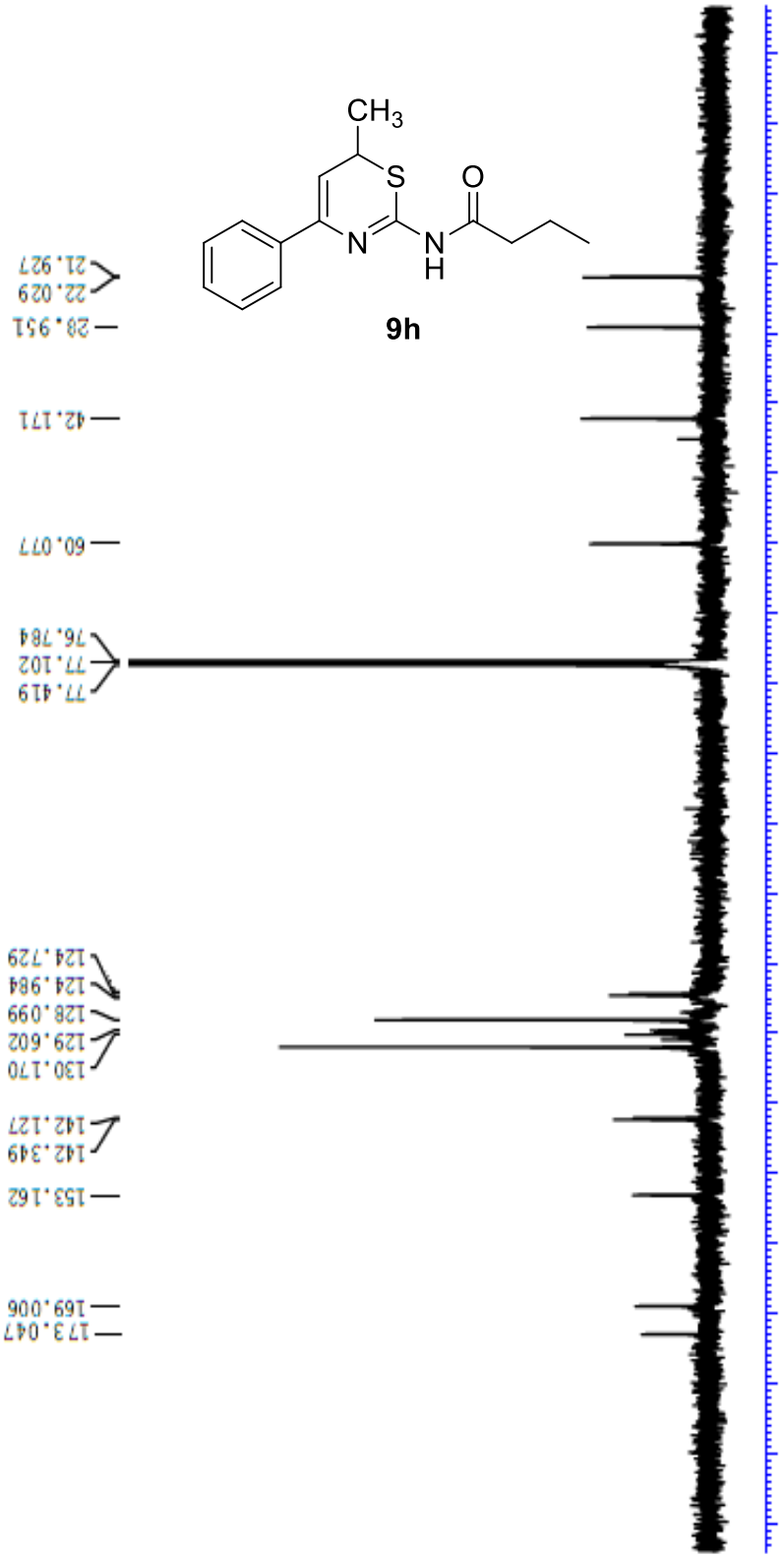
HRMS spectrum compound 9f



^{1}H NMR spectrum of compound 9g



HRMS spectrum compound 9g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N
H
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77.419
124.729
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128.099
129.602
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142.127
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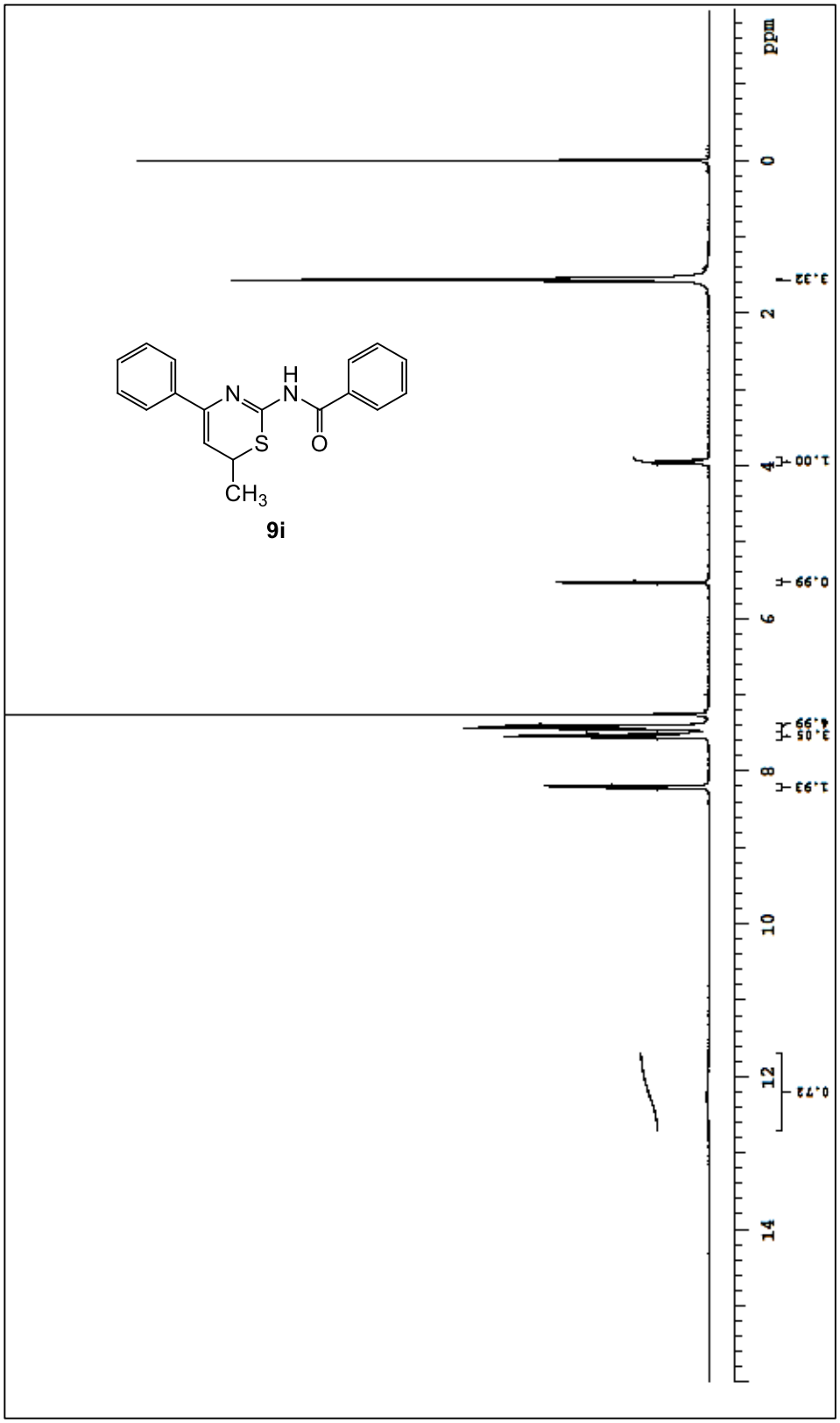
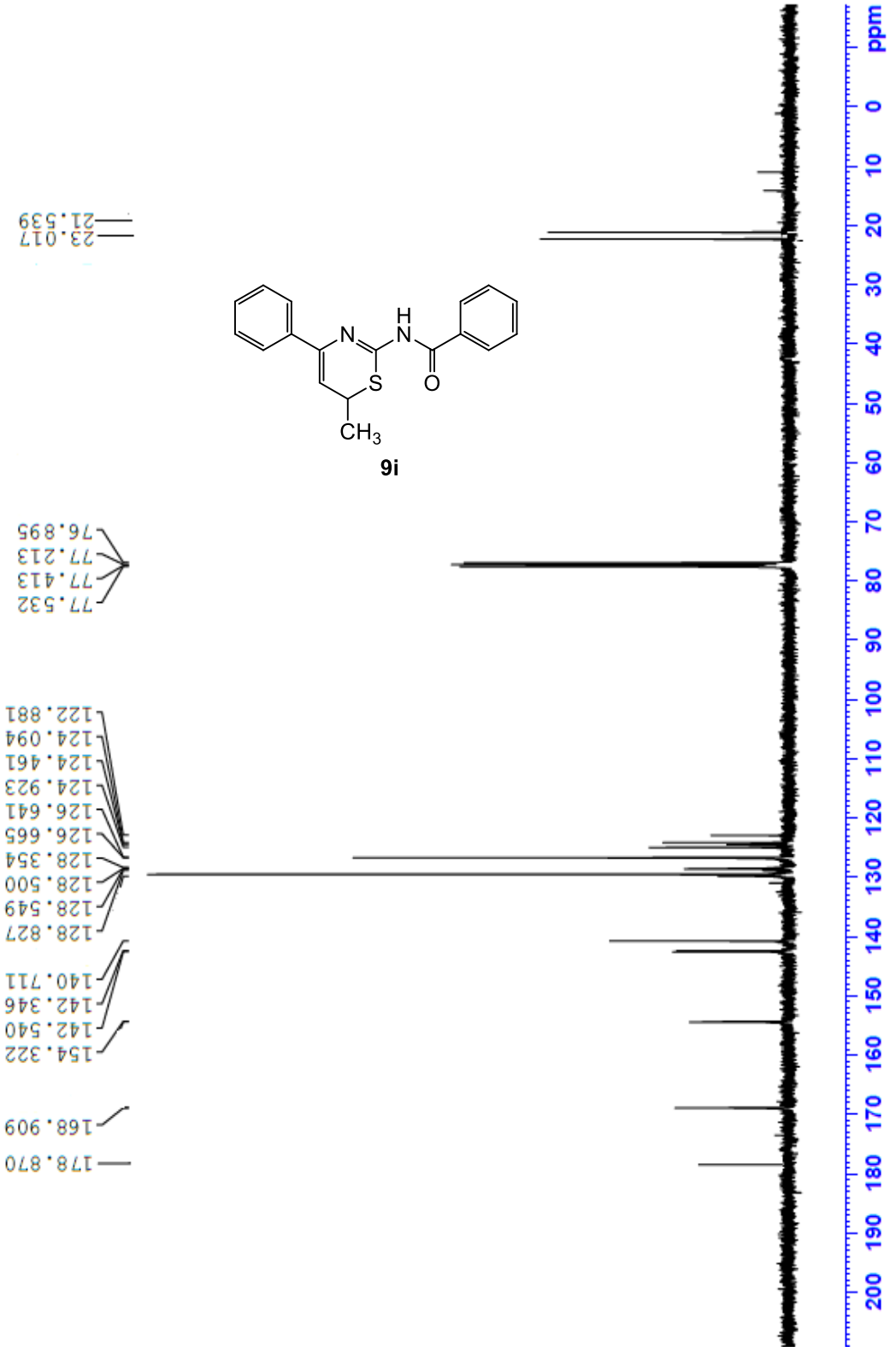
173.047

^{13}C NMR of 9h



^{1}H NMR spectrum of compound 9i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77.213
76.895
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124.094
124.461
124.923
126.641
126.665
128.354
128.500
128.549
128.827
140.711
142.346
142.540
154.322
168.909
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ppm
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10
20
30
40
50
60
70
80
90
100
110
120
130
140
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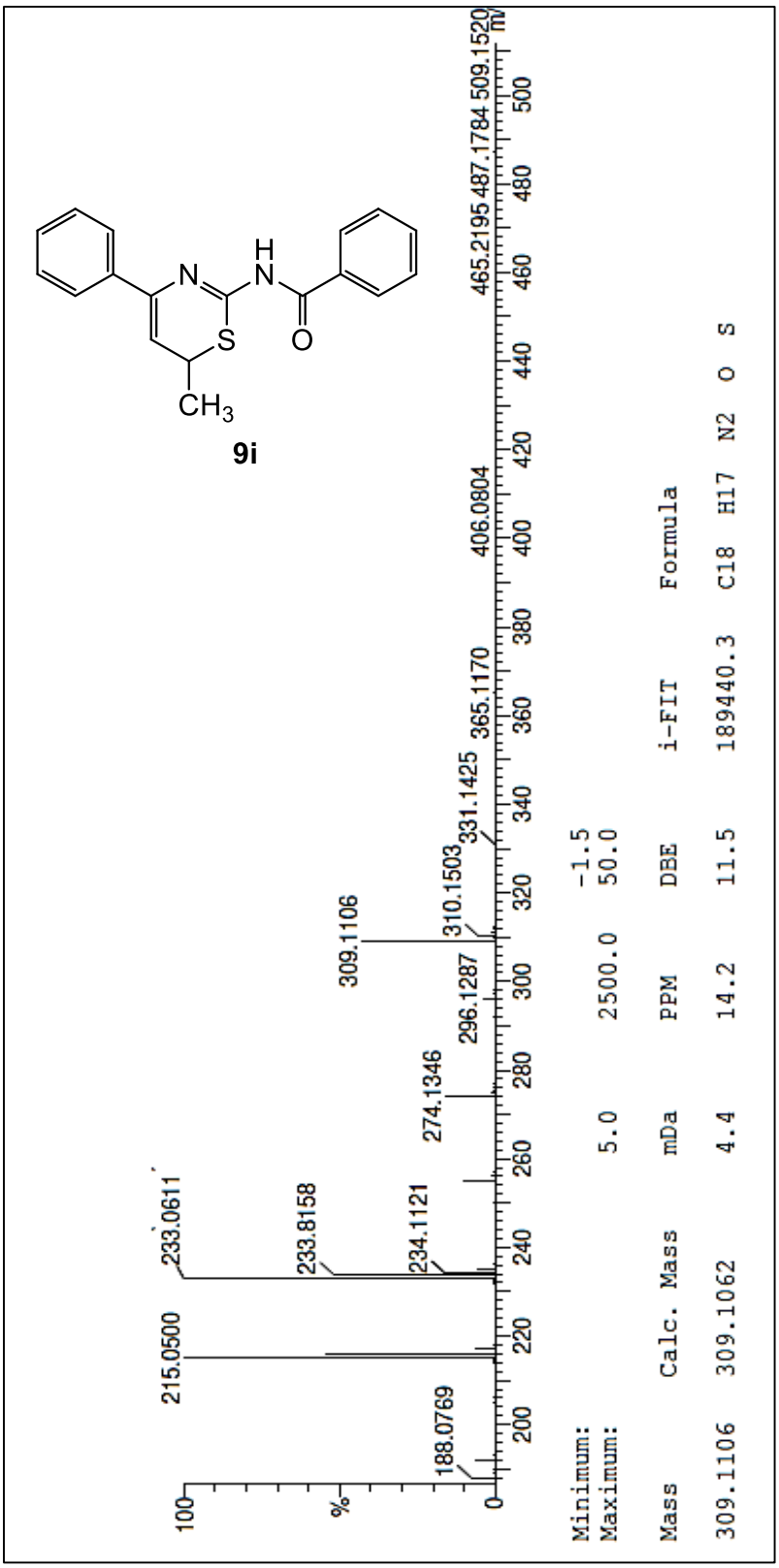
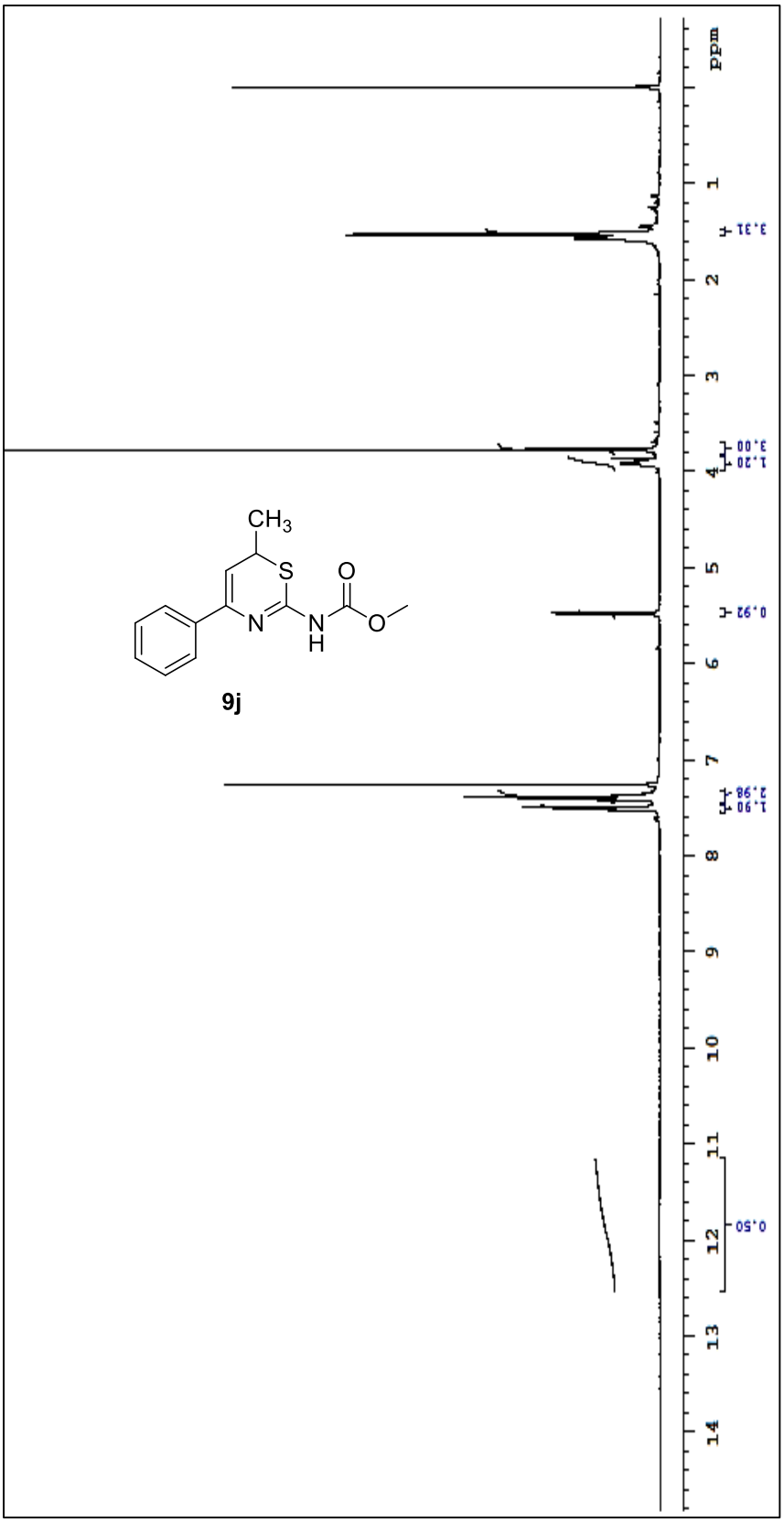
170
180
190
200
N
H
N
S
O
CH3
9i

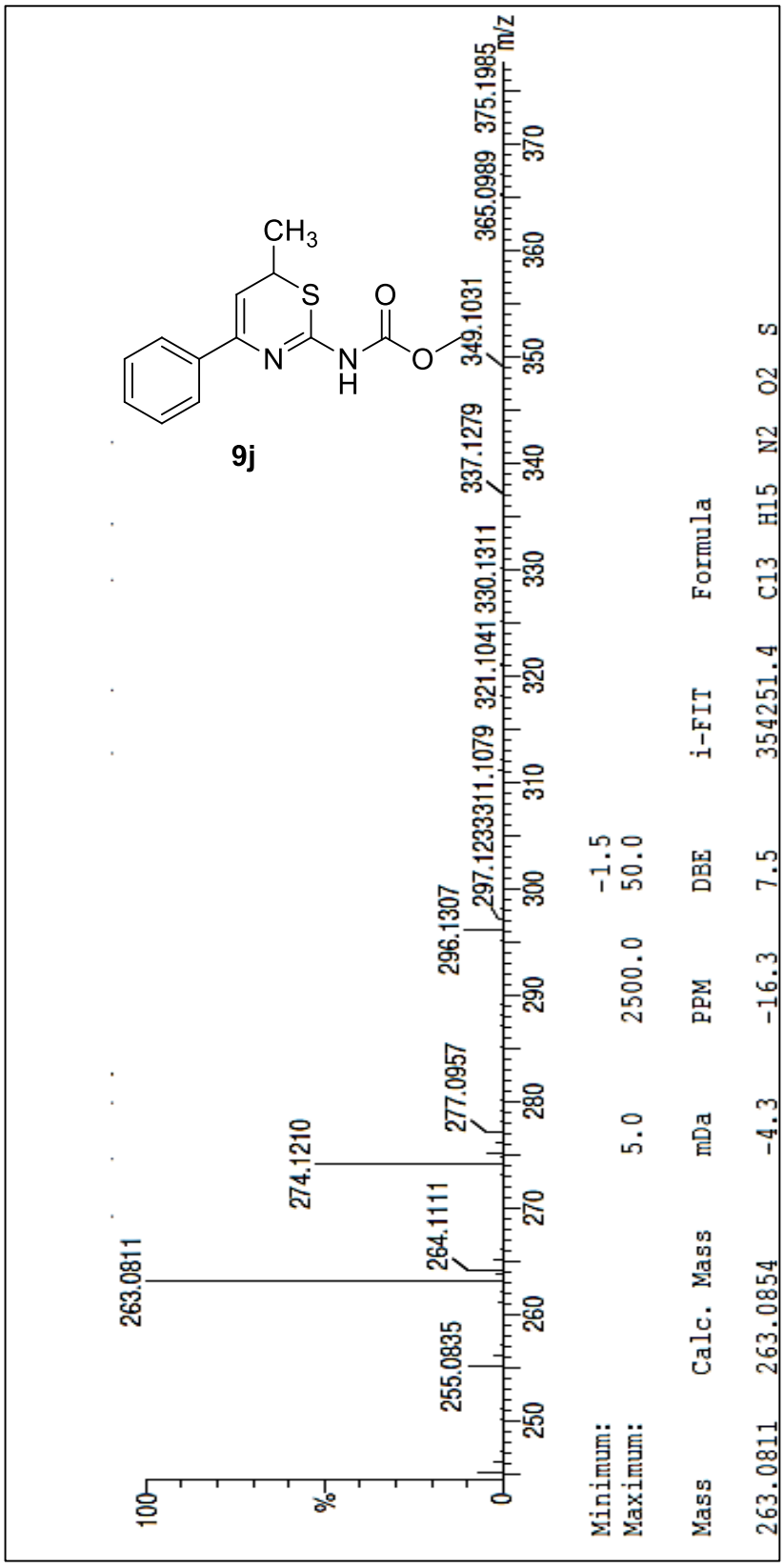
^{13}C NMR of 9i



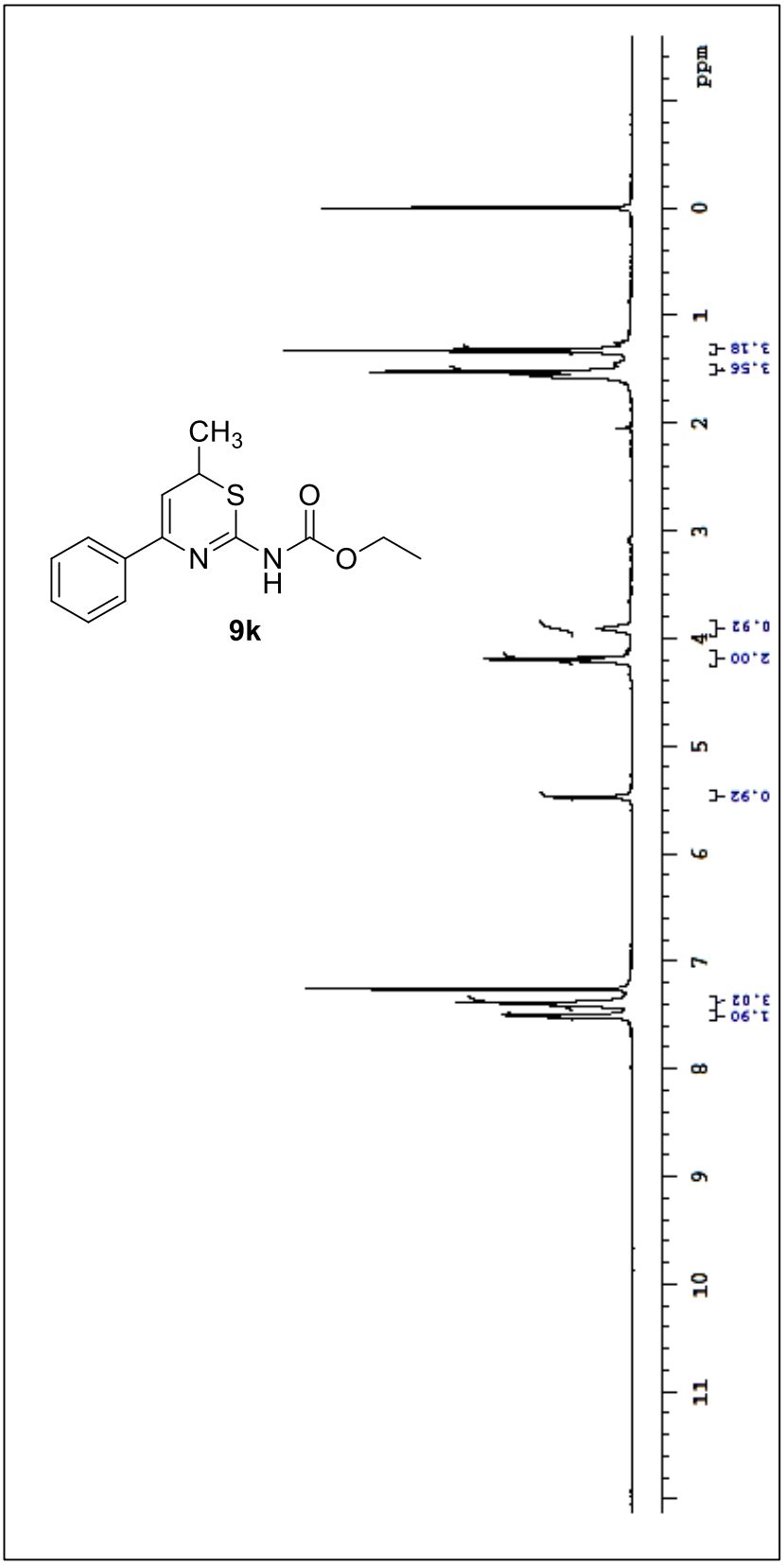
HRMS spectrum of compound 9i



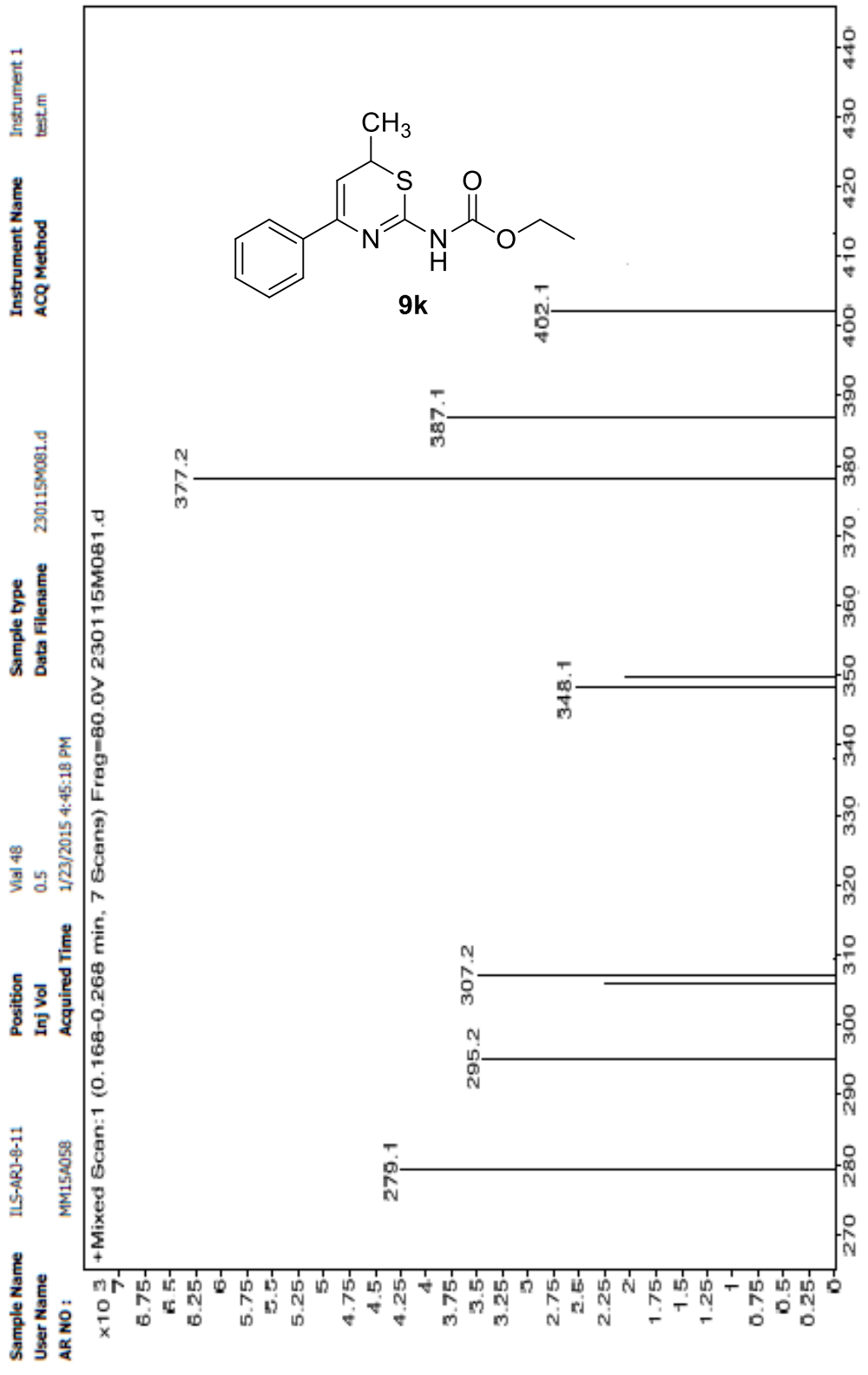
^{1}H NMR spectrum of compound 9j



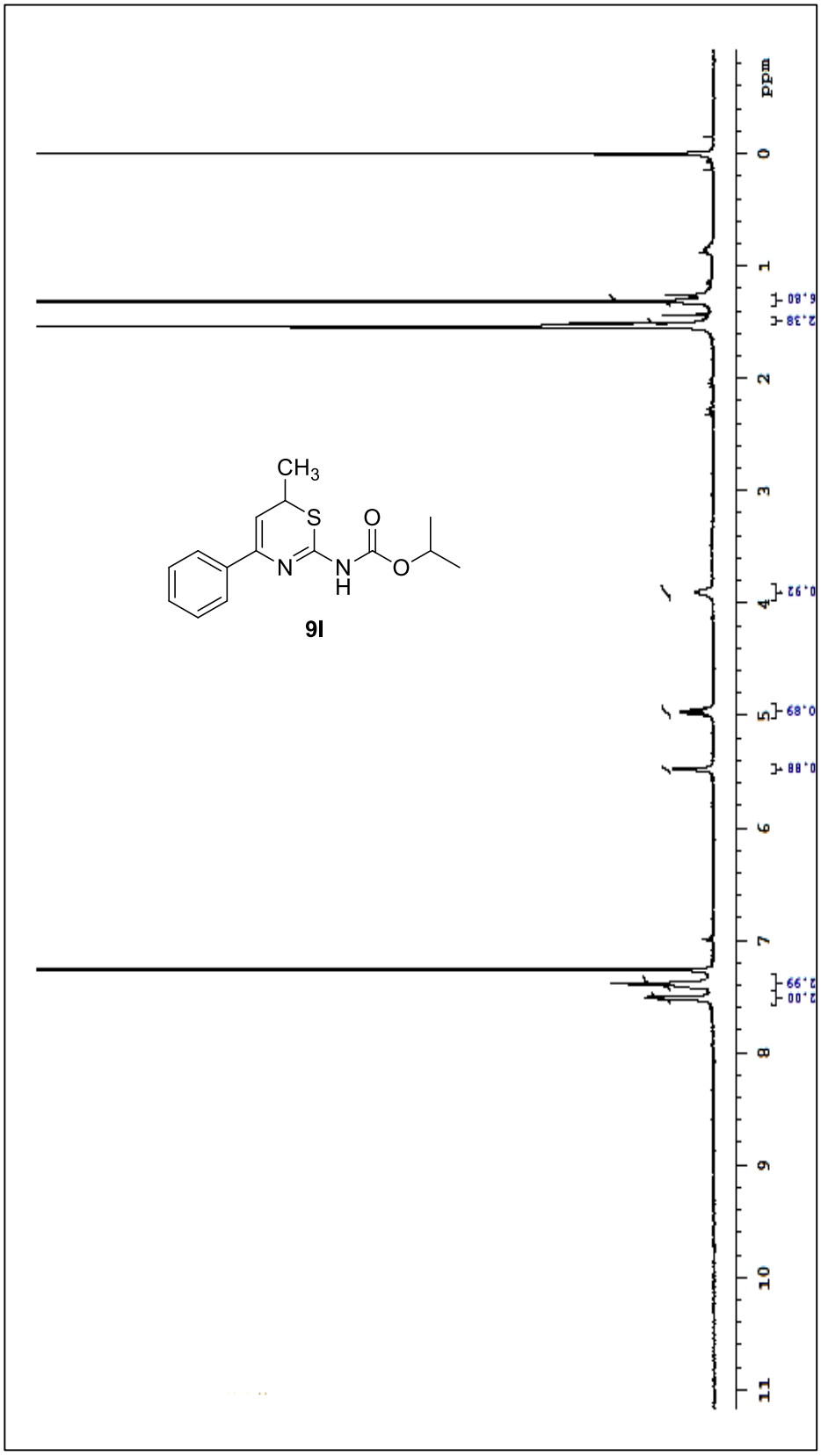
HRMS spectrum of compound 9j



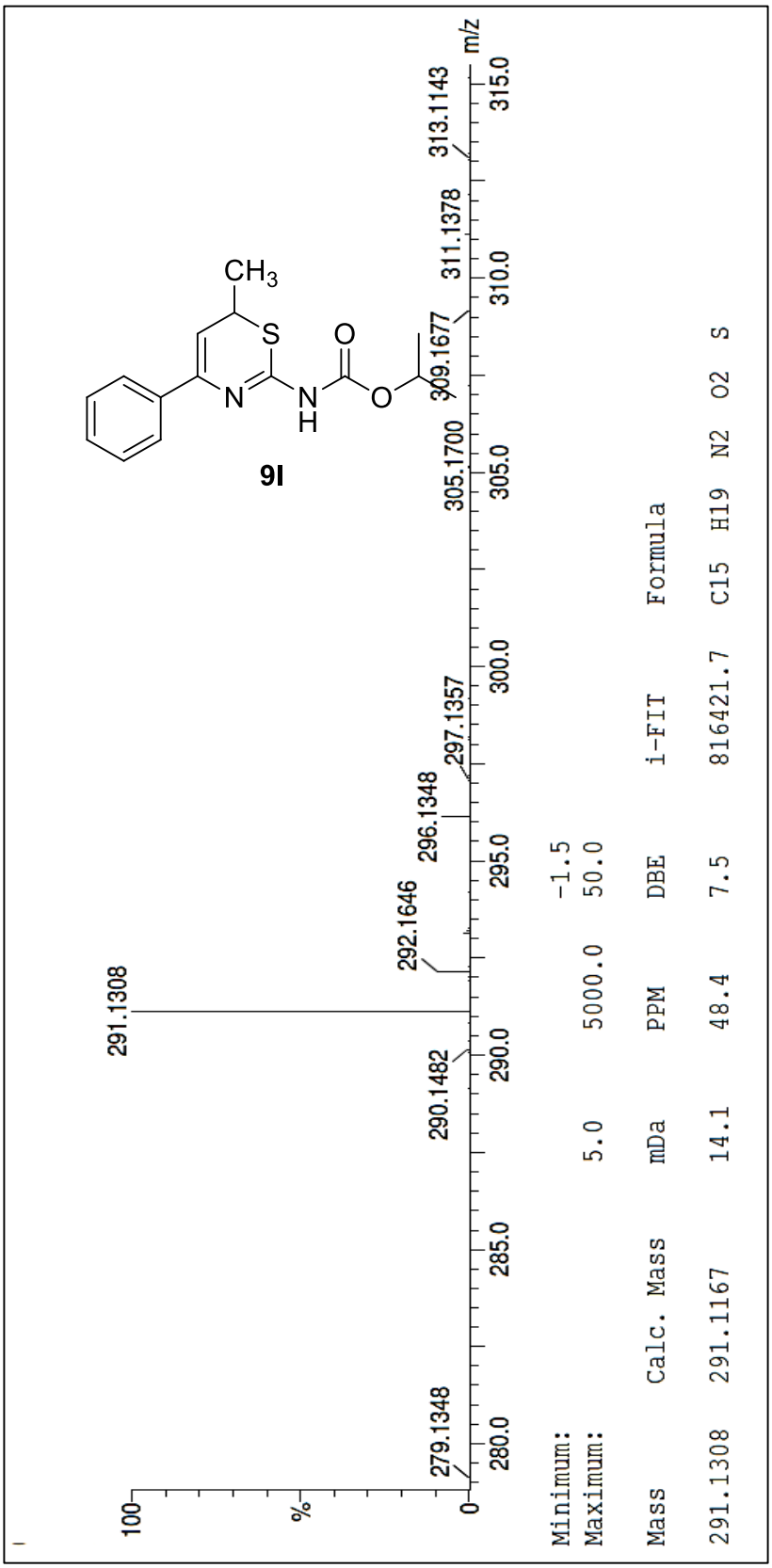
^{1}H NMR spectrum of compound 9k



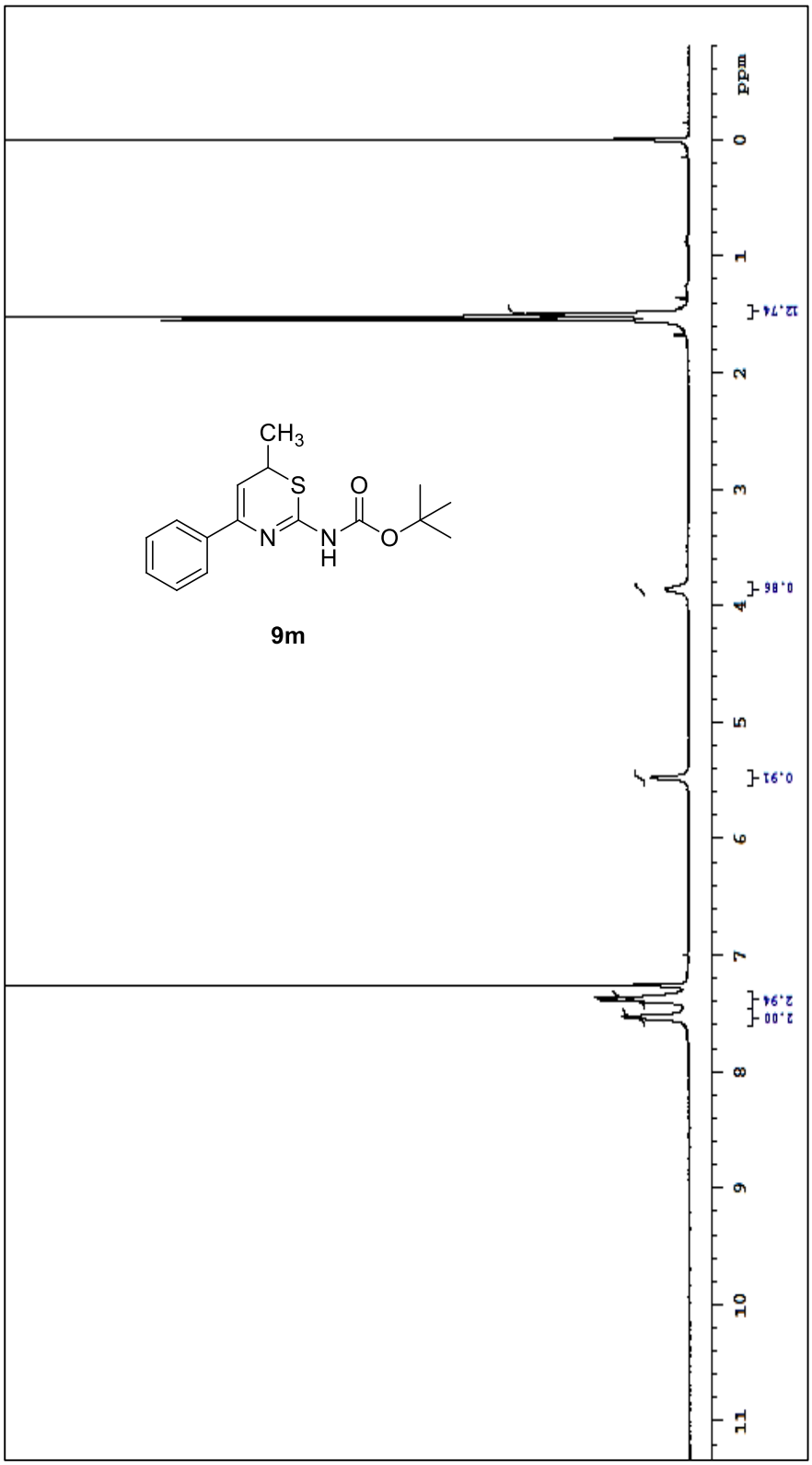
LC-MS spectrum of 9k



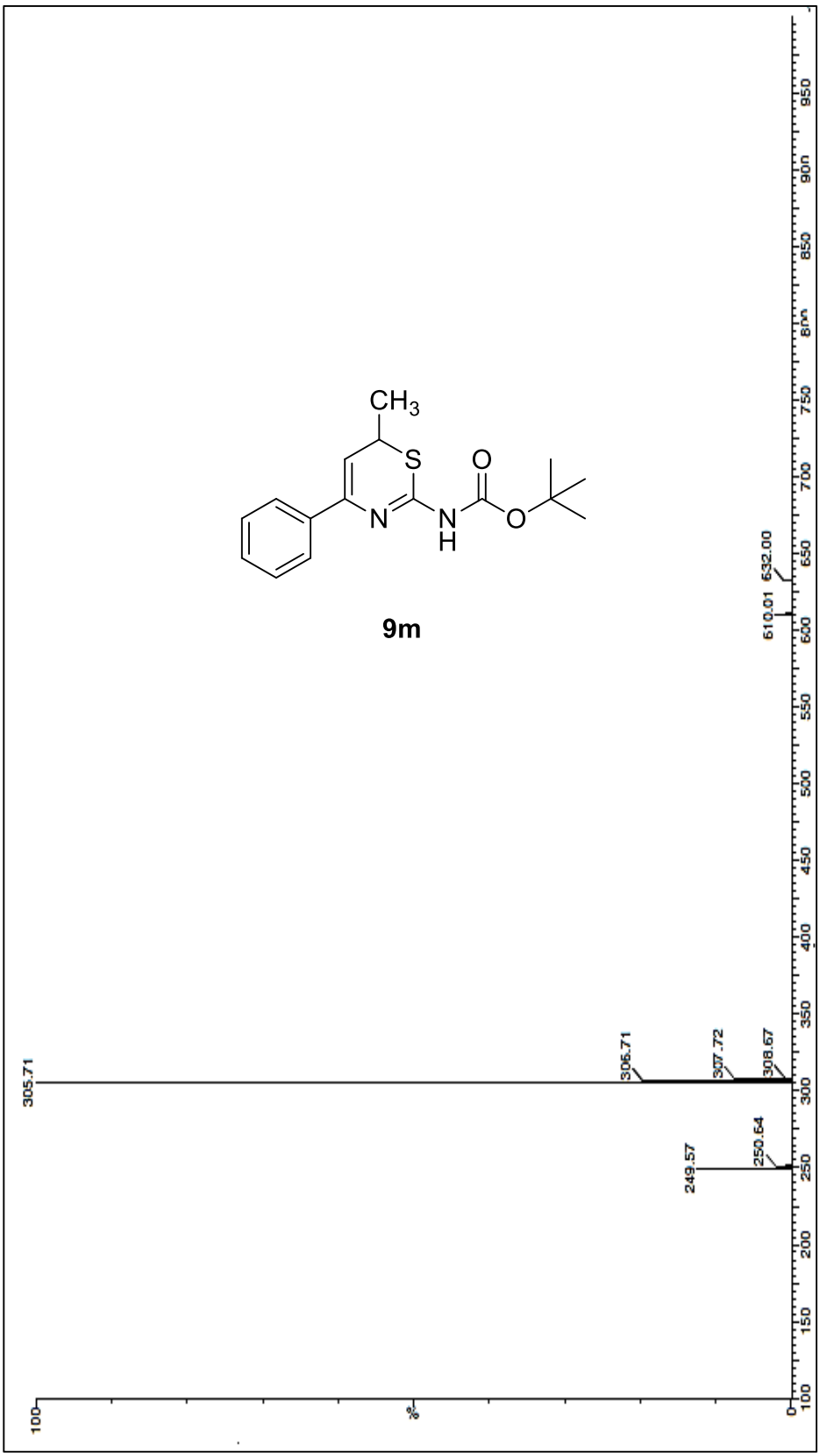
^{1}H NMR spectrum of compound 9l



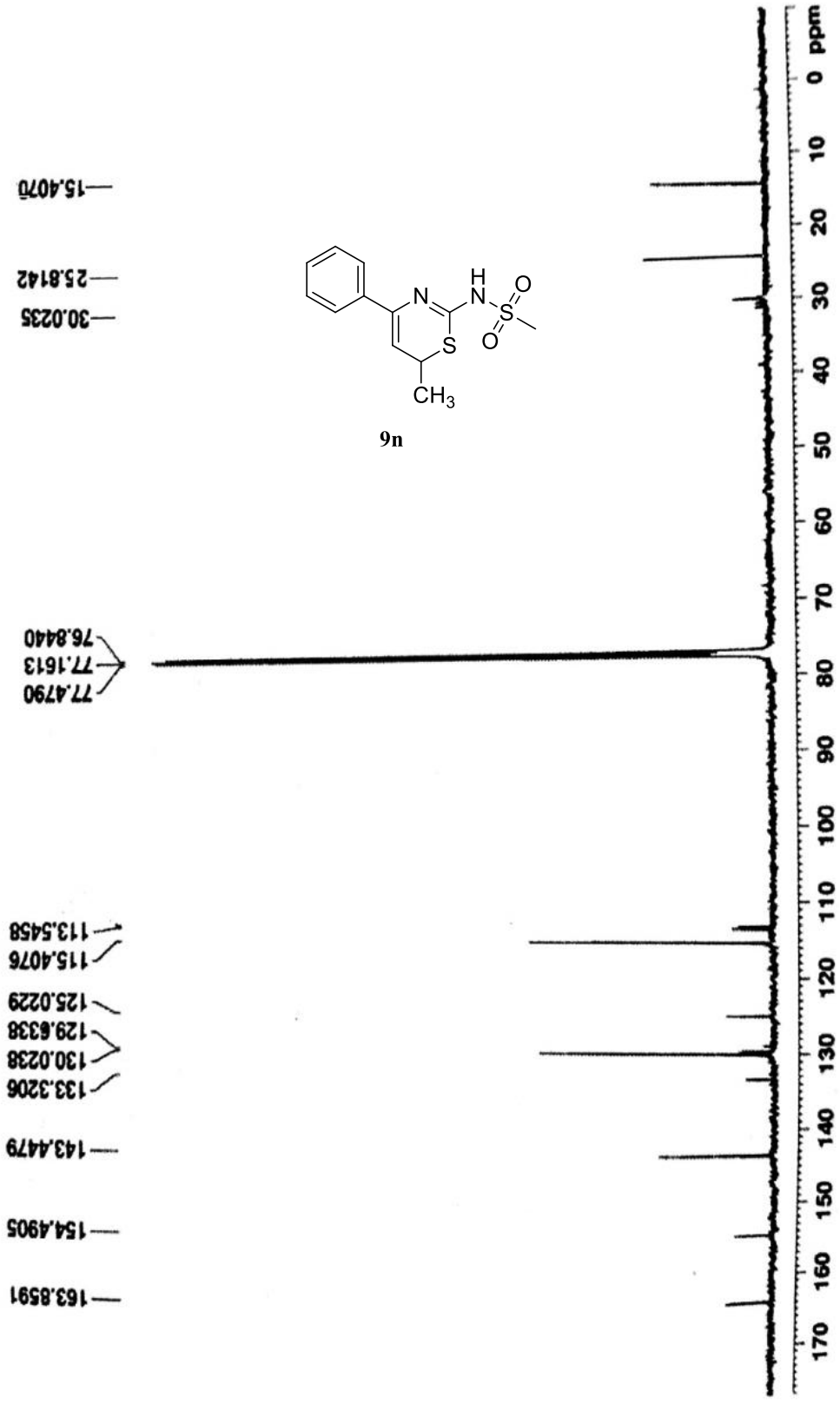
HRMS spectrum of compound 9l



^{1}H NMR spectrum of compound 9m

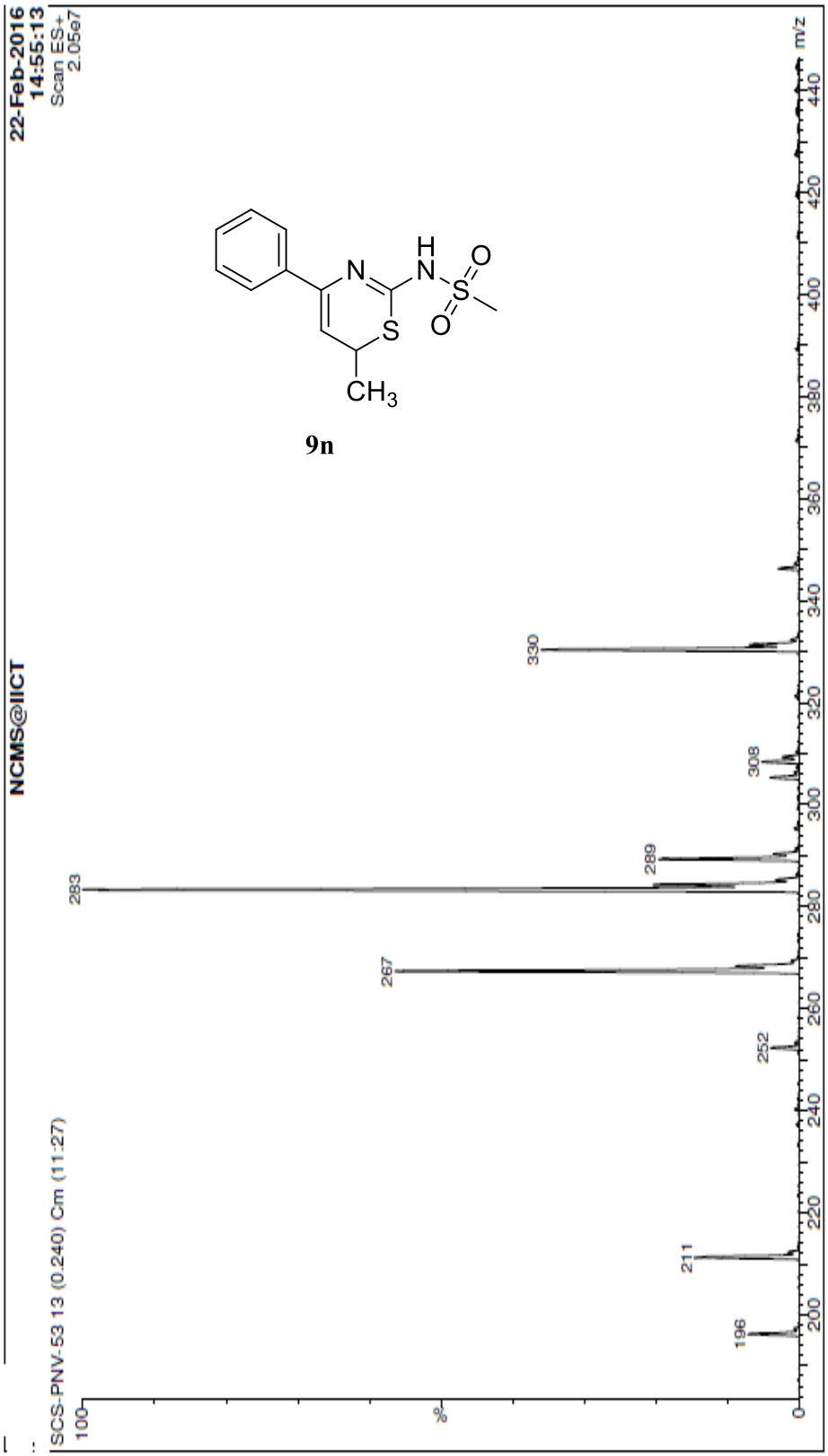


LC-MS spectrum of compound 9m



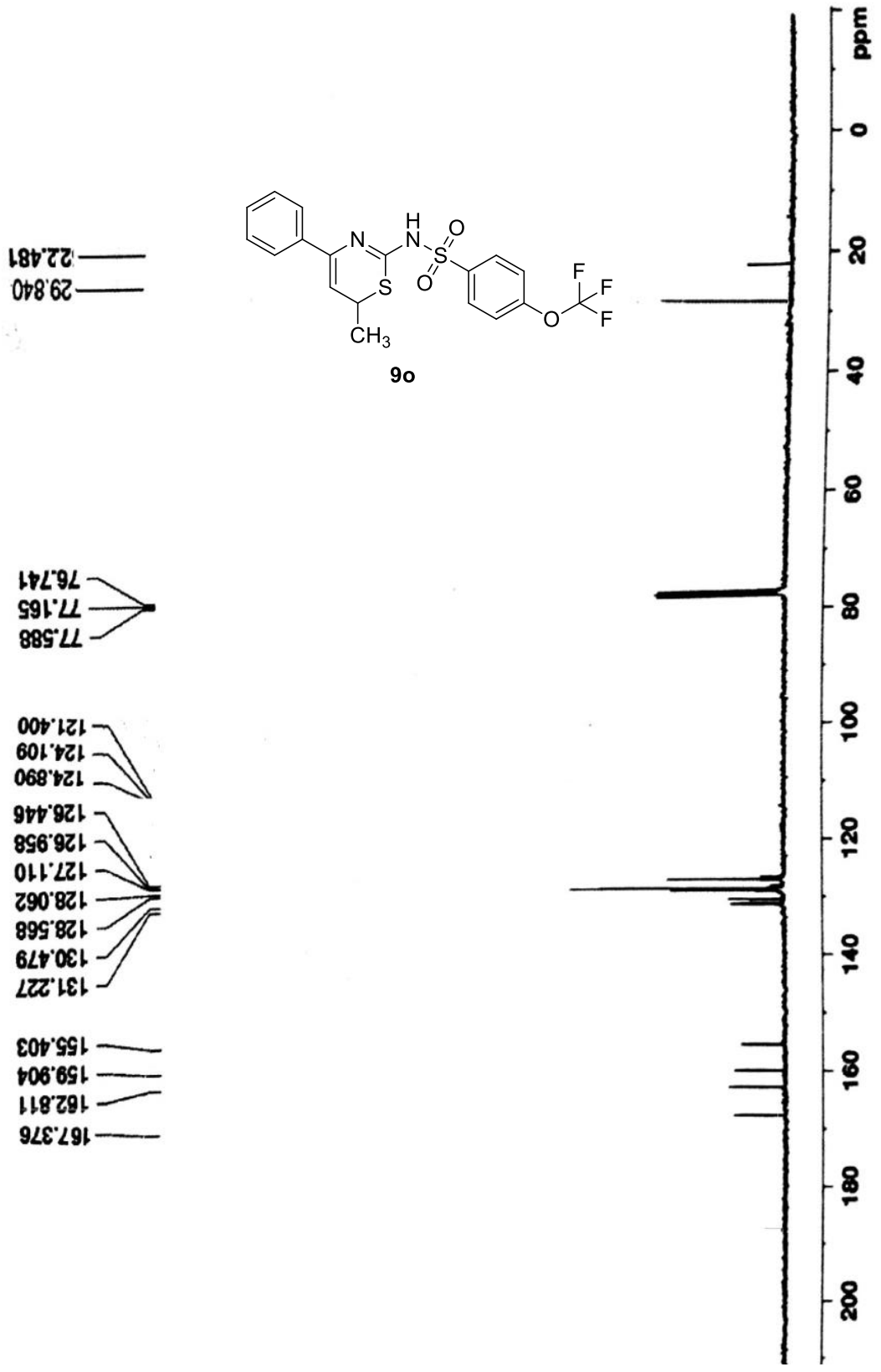
9n

^{13}C NMR of 9n



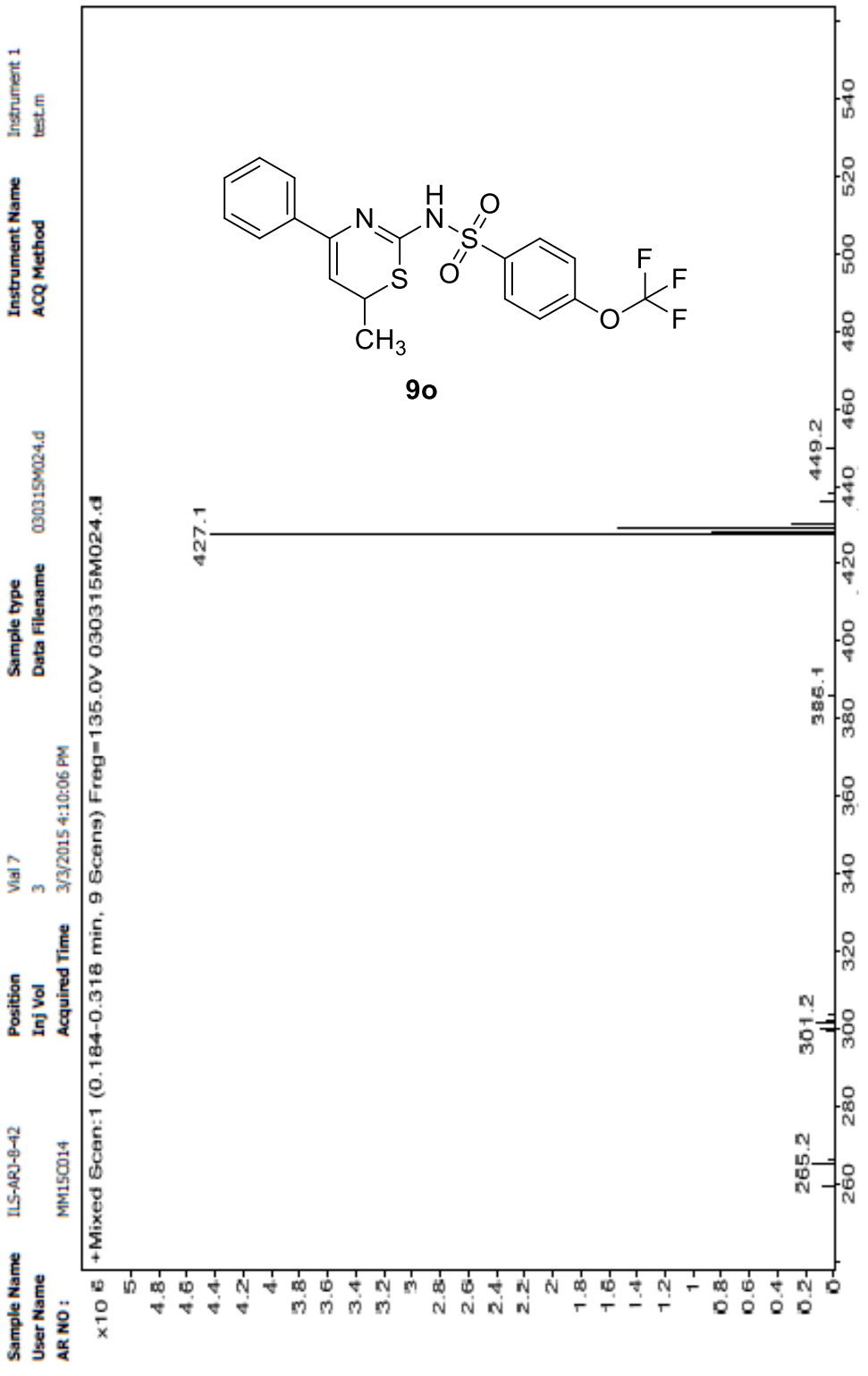
9n

Mass spectrum of compound of 9n

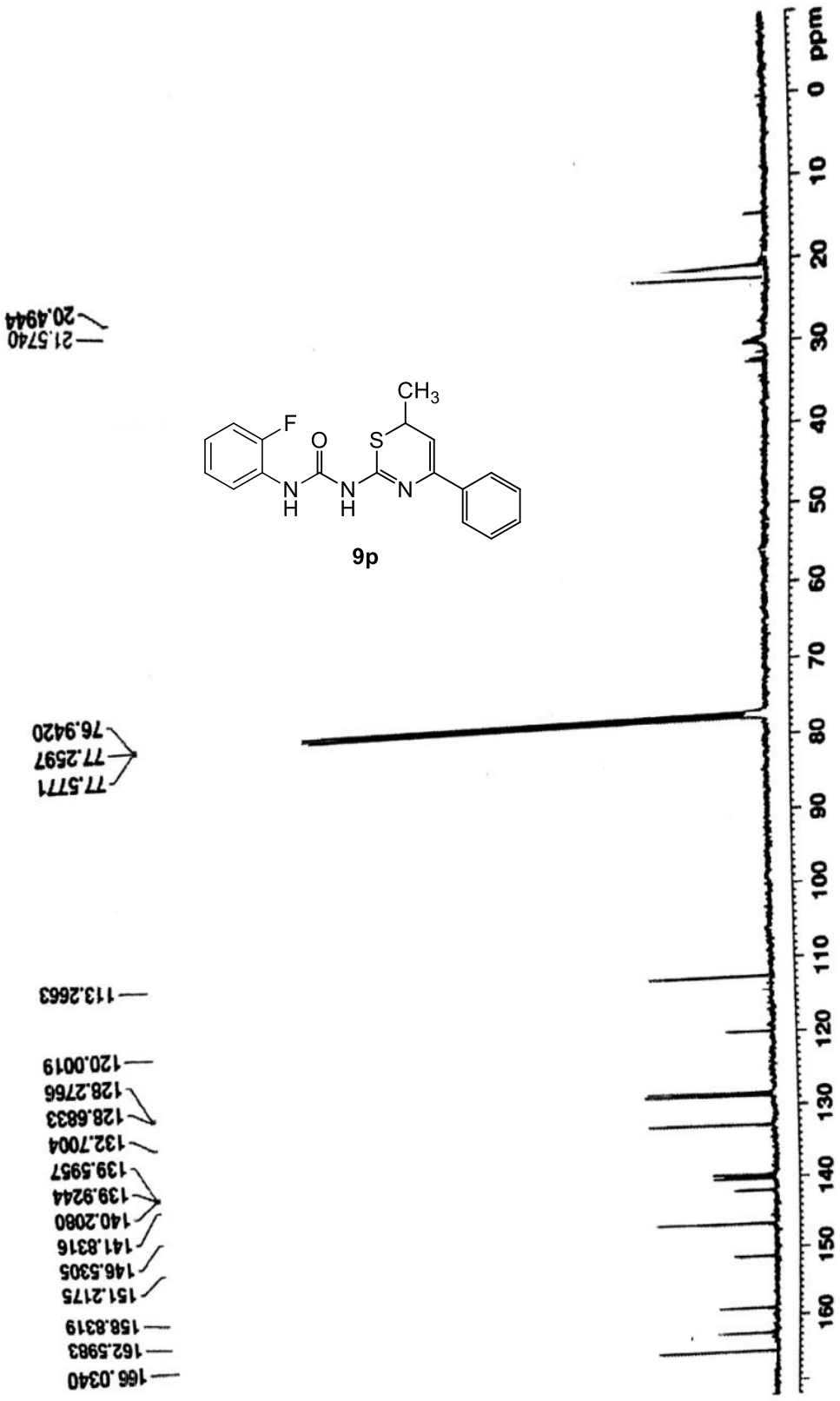


9o

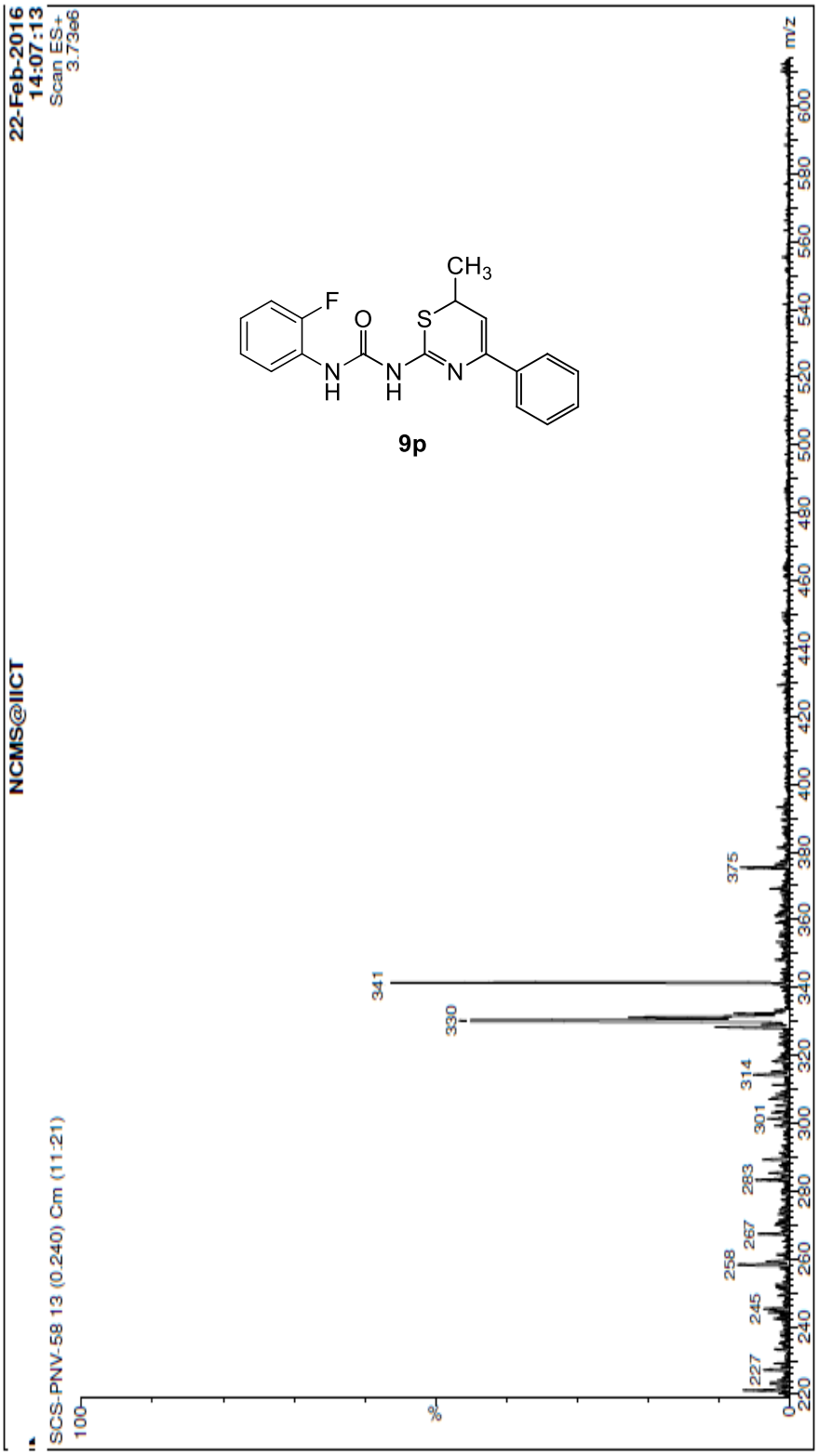
^{13}C NMR of 9o



Mass spectrum of compound of 9o

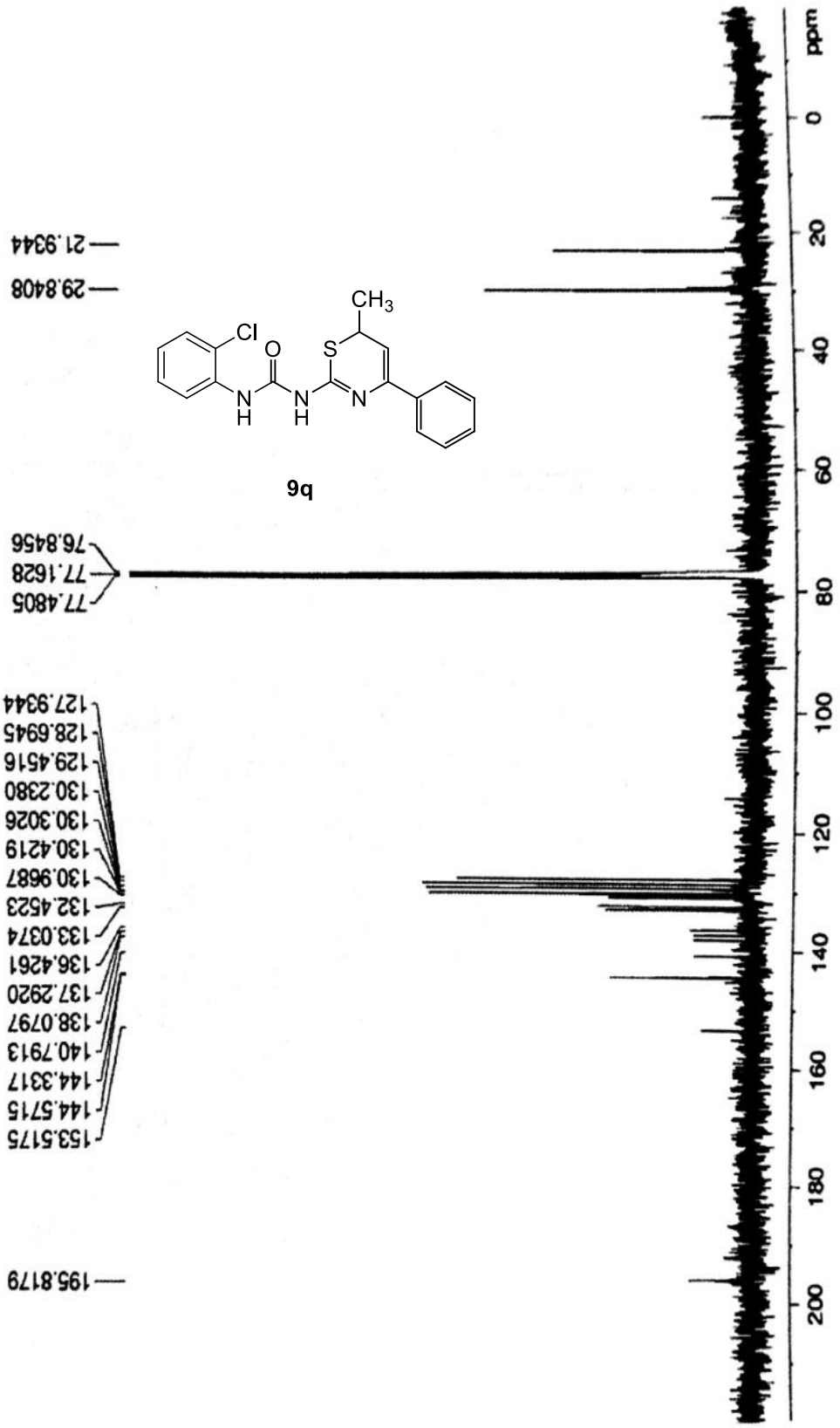

20.4944
21.5740
CH3
F
O
S
N
H
N
H
N
9p
76.9420
77.2597
77.5771
113.2663
120.0019
128.2766
128.6833
132.7004
139.5957
139.9244
140.2080
141.8316
146.5305
151.2175
158.8319
162.5983
166.0340
0 ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160

^{13}C NMR of 9p

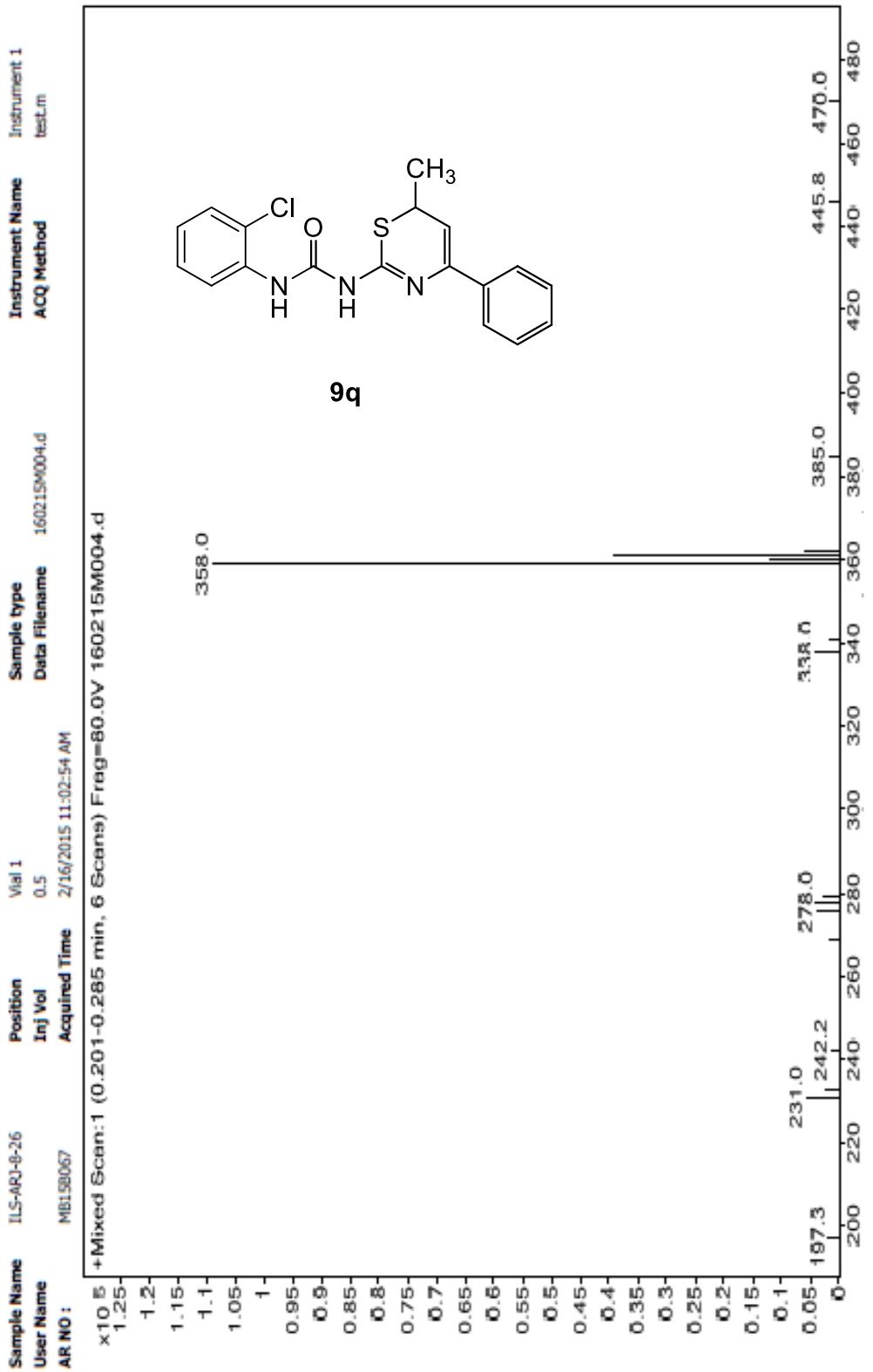


9p

Mass spectrum of compound of 9p


21.9344
29.8408
CH3
Cl
O
S
N
H
N
H
N
9q
76.8456
77.1628
77.4805
127.9344
128.6945
129.4516
130.2380
130.3026
130.4219
130.9687
132.4523
133.0374
136.4261
137.2920
138.0797
140.7913
144.3317
144.5715
153.5175
195.8179
ppm
0
20
40
60
80
100
120
140
160
180
200

^{13}C NMR of 9q



Mass spectrum of compound of 9q

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