CHAPTER 3

SYNTHESIS OF 3-AMINOCOUMARIN DERIVATIVES AS ANTI-HYPERGLYCEMIC AGENTS

3.1 Introduction

Diabetes is a serious metabolic disorder affecting more than 347 million people worldwide [1] with more than 90% suffering from type 2 diabetes (T2D).

Management of T2D can be achieved by controlling hyperglycemia through various targets but the major drawback in all these therapies is induction of hypoglycemia and weight gain. Hence newer approaches involve glucose-dependent insulin secretion (GDIS) for regulation of blood glucose levels to overcome these side-effects.

Glucagon-like peptide-1 (GLP-1), is the most potent insulinotropic hormone secreted by the intestinal L-cells in response to the food intake [2, 3]. It exhibits several biological effects including stimulation of insulin secretion and biosynthesis while inhibiting glucagon secretion and induces pancreatic β-cell proliferation each of which benefits in the control of glucose homeostasis without inducing hypoglycemia in patients with T2D [4, 5]. Thus GLP-1 has become a promising target for the treatment and management of T2D. This highly potent GLP-1 is rapidly degraded ($t_{½}$ ~ 1 min) by a serine protease dipeptidyl peptidase-IV (DPP-IV), *in vivo*, thereby rendering it pharmacologically inactive [6]. Even the synthetic GLP-1 agonists, although clinically efficacious have low bioavailability and most of them need to be dosed either subcutaneously or intravenously. Hence from medicinal chemistry point of view, inhibition of DPP-IV has gained importance as a new target for treatment of T2D which in turn will lead to increased half-life of GLP-1 and thereby enhancing the efficacy and potency of the incretin hormone. Also, GLP-1 is secreted in a glucose-dependent manner, hence hypoglycemia and pancreatic β-cell exhaustion, as in the case of other anti-hyperglycemic drugs, is not observed.

Some of the most potent DPP-IV inhibitors are as shown in the Figure 3.1.

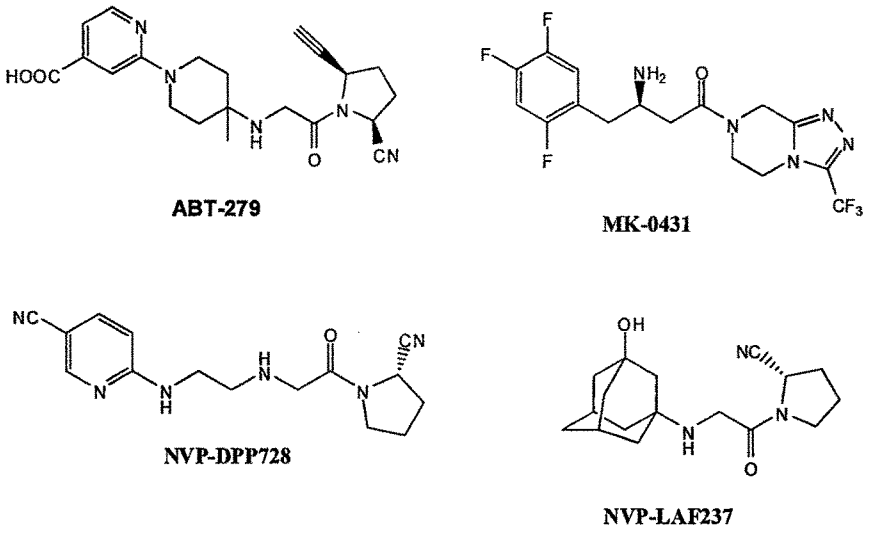
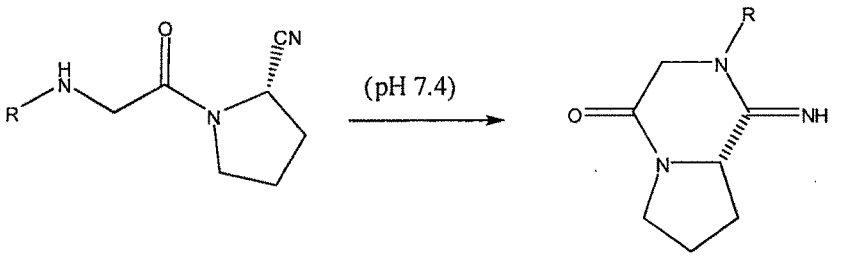


Figure 3.1: Some DPP-IV inhibitors

Amongst the first reported DPP-IV inhibitors is NVP-DPP728 [7], in a buffered aqueous medium (pH 7.4) gets converted to cyclic amidine, with $t_{½}$ ~ 48 h to > 70 days but has been reported to exist as a stable solid for months to year, as shown in Figure 3.2.



NVP-DPP728 **Cyclic amidine**

Figure 3.2: Intramolecular cyclization in a solution (pH 7.4)

But when adamantyl analogues were substituted at the P2 site, imidine formation was restricted due to stearic hindrance thereby rendering the molecule more potent (e.g. NVP-LAF237). Thus substitution of bulky groups at the P2 site is the key to increase the potency of the enzyme inhibitor.

In the previous chapter it was observed that substitution of sulphonamide derivatives at the P2 site acted as potent DPP-IV inhibitors. Hence, bulky heterocyclic molecules, like coumarin can be substituted at the P2 site, as shown in Figure 3.3, and its efficacy as DPP-IV enzyme inhibitor can be studied.

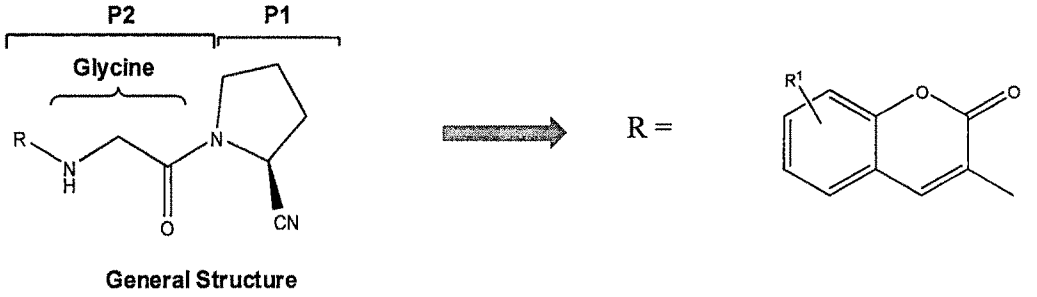


Figure 3.3: Designing of substituted 3-aminocoumarin derivatives as DPP-IV inhibitors.

Coumarin derivatives exhibit a wide spectrum of bioactivity. Both synthetic and naturally occurring coumarin derivatives have been widely studied for their various pharmacological activities. 3-amino coumarin derivatives have been reported for their antimicrobial and antioxidant activity [7] as well as anti-inflamatory properties [8]. They are also selective inhibitors MAO-B and acetylcholinesterase (AChE) for the treatment of Alzheimer's disease [9-11]. Recently, 3-aminocoumarin derivatives have been reported to show anti hyperglycemic activity [12, 13].

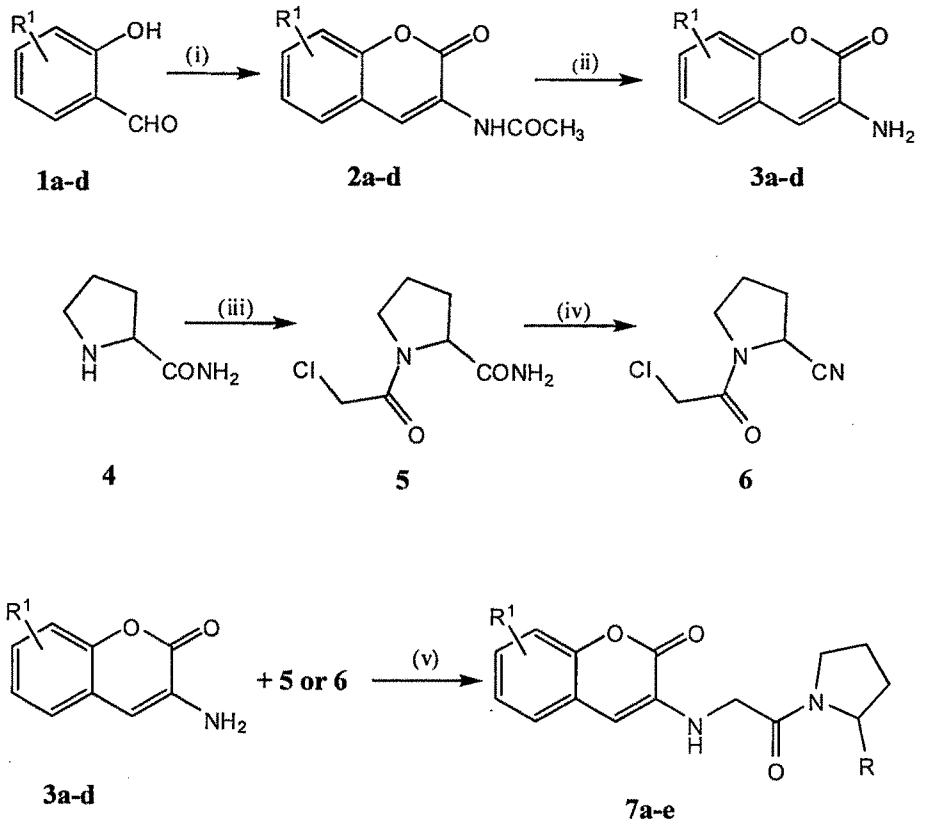
Synthesis and biological evaluation of 3-amino coumarin derivatives as DPP-IV inhibitors has been discussed in this chapter.

3.2 Results and Discussion

3.2.1 Chemistry

Classically, coumarin is synthesized by reaction of substituted salicylaldehyde with N-acetyl glycine in acetic anhydride, better known as Perkin reaction to yield substituted 3-acetamidocoumarin which on hydrolysis by methanolic HCl gives the desired substituted 3-aminocoumarin. Thus heating α-naphthaldehyde **1a**, N-acetylglycine and sodium acetate in acetic anhydride for seven hours at 100 °C yielded N-(2-oxo-2H-benzo[h]chromen-3-yl)acetamide **2a**, Scheme 3.1, confirmed from its IR spectrum (Figure 3.5.1) which shows bands at 3341, 1709, 1676 cm^{-1} for the –NH of amide, lactone carbonyl and amide carbonyl groups respectively while the ^1H NMR spectrum (Figure 3.5.2) shows two singlet's at δ 2.32 and 9.53 for the acetamido methyl group and amide -NH protons respectively. Figure 3.5.3 and Figure 3.5.4 shows ^{13}C NMR and ESI-MS spectrum of **2a**. Figures 3.6.1, 3.6.2, 3.6.3 and 3.6.4 show IR, ^1H NMR, ^{13}C NMR and ESI-MS spectra of compound **2b** respectively. Similarly, Figure nos. 3.7.1, 3.7.2, 3.7.3 and 3.7.4 shows the IR, ^1H NMR, ^{13}C NMR and ESI-MS spectra of **2d**. Further hydrolysis of **2a** by methanolic HCl gave free amine **3a** as confirmed from its IR spectrum (Figure 3.8.1) with bands at 3448 and 3366 cm^{-1} for the amino group and a strong band at 1713 cm^{-1} for the lactone carbonyl group and ^1H NMR spectrum (Figure 3.8.2) wherein a singlet at δ 4.44 for the two amino protons ($-NH_2$) and absence of signal for the $-CH_3$ of acetamide in the aliphatic region confirms the structure of **3a**. Figure 3.8.3 shows ^{13}C NMR spectrum and Figure 3.8.4 shows ESI-MS spectrum further confirm the structure of **3a**. Figures 3.9.1, 3.9.2, 3.9.3 and 3.9.4 show IR, ^1H NMR, ^{13}C

NMR and ESI-MS spectra of **3b** respectively while Figure 3.10.1, 3.10.2, 3.10.3 and 3.10.4 shows IR, ^{1}H NMR, ^{13}C NMR and ESI-MS spectra of **3d** thereby confirming their structures.



R = CN, R^1 = 7,8-Ph = a
= H = b
= 7-OH = c
= 8-OCH_3 = d
R = $CONH_2$, R^1 = H = e

Scheme 3.1: Reagents: (i) N-acetylglycine, $(CH_3CO)_2O$, CH_3COONa; (ii) CH_3OH-HCl; (iii) $ClCH_2COCl$, K_2CO_3, THF; (iv) TFAA, THF, NH_4HCO_3; (v) K_2CO_3, dry DMF.

On the other hand when chloroacetly chloride was added to a solution of L-proline amide **4** in THF, *(S)*-1-(2-chloroacetyl)pyrrolidine-2-carboxamide **5** is obtained, the structure of which was confirmed by IR spectrum which shows two bands at 1686, 1709 cm^{-1} for amides carbonyls, one of which disappears when **5** is dehydrated with trifluoroacetic anhydride (TFAA) to yield *(S)*-1-(2-chloroacetyl)pyrrolidine-2-carbonitrile **6**. The IR spectrum (**Figure 2.4.1**, **Chapter 2**) of **6** shows two strong bands at 2241 and 1656 cm^{-1} for the nitrile and amide carbonyl groups respectively while its ^{1}H NMR spectrum (**Figure 2.4.2**, **Chapter 2**) showed a singlet at δ 4.076 for the methylene protons and multiplet at δ 4.69-4.71 for -CH proton of the cyanopyrrolidide thus confirming its structure. Reaction of **3a-d** with **5** or **6** in DMF in presence of potassium carbonate as a base gave the compounds **7a-e** (Scheme 3.1). The IR spectrum of **7a** (Figure 3.11.1) showed two strong bands at 1709 and 1653 cm^{-1} for the lactone and amide groups respectively. ^{1}H NMR spectrum (Figure 3.11.2) showed multiplets from δ 2.28-2.41 for the four $-CH_2$ protons, a multiplet from 3.63 to 3.79 for two $-CH_2$ protons and another multiplet from δ 4.89-4.91 for the –CH proton of the cyanopyrrolidide; a doublet at δ 4.01 indicated the glycyl $-CH_2$ protons and multiplets from δ 6.99-8.18 indicated aromatic protons thereby confirming the formation of **7a.** Figures 3.11.3 and 3.11.4 shows ^{13}C NMR and ESI-MS spectra of **7a** which further supported its structure. The specific optical rotation of **7a** was found to be -272.11 due to the chirality of the cyanopyrrolidide system. Figures 3.12.1, 3.12.2, 3.12.3 and 3.12.4 shows IR, ^{1}H NMR, ^{13}C NMR and ESI-MS spectra of **7b** respectively thus confirming its structure. Figures 3.13.1, 3.13.2, 3.13.3 and 3.13.4 shows IR, ^{1}H NMR, ^{13}C NMR and ESI-MS spectra of **7c**

respectively and Figures 3.14.1, 3.14.2, 3.14.3 and 3.14.4 shows IR, ^{1}H NMR, ^{13}C NMR and ESI-MS spectra of **7d** respectively thus confirming their respective structures

3-amino-2H-chromen-2-one **3b** on reaction with *(S)*-1-(2-chloroacetyl)pyrrolidine-2-carboxamide **5** gave *(S)*-1-(2-(2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carboxamide **7e.** Its structure was also proved by its IR spectrum (Figure 3.15.1), ^{1}H NMR spectrum (Figure 3.15.2), ^{13}C NMR spectrum (Figure 3.15.3) and ESI-MS spectrum (Figure 3.15.4). Another compound **7g** was synthesized by reaction of 3-amino-7-hydroxy-2*H*-chromen-2-one **3c** with 2-chloro-1-morpholinoethanone, obtained by the reaction of morpholine with chloroacetyl chloride. The structures of all these compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR spectra and ESI-MS analyses.

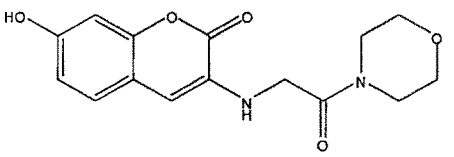


Figure 3.4: *Structure of 7-hydroxy-3-(2-morpholino-2-oxoethylamino)-2H-chromen-2-one 7g*

Figure 3.16.1 and 3.16.2 show IR and ^{1}H NMR of **7g** thus confirming the formation of **7g**.

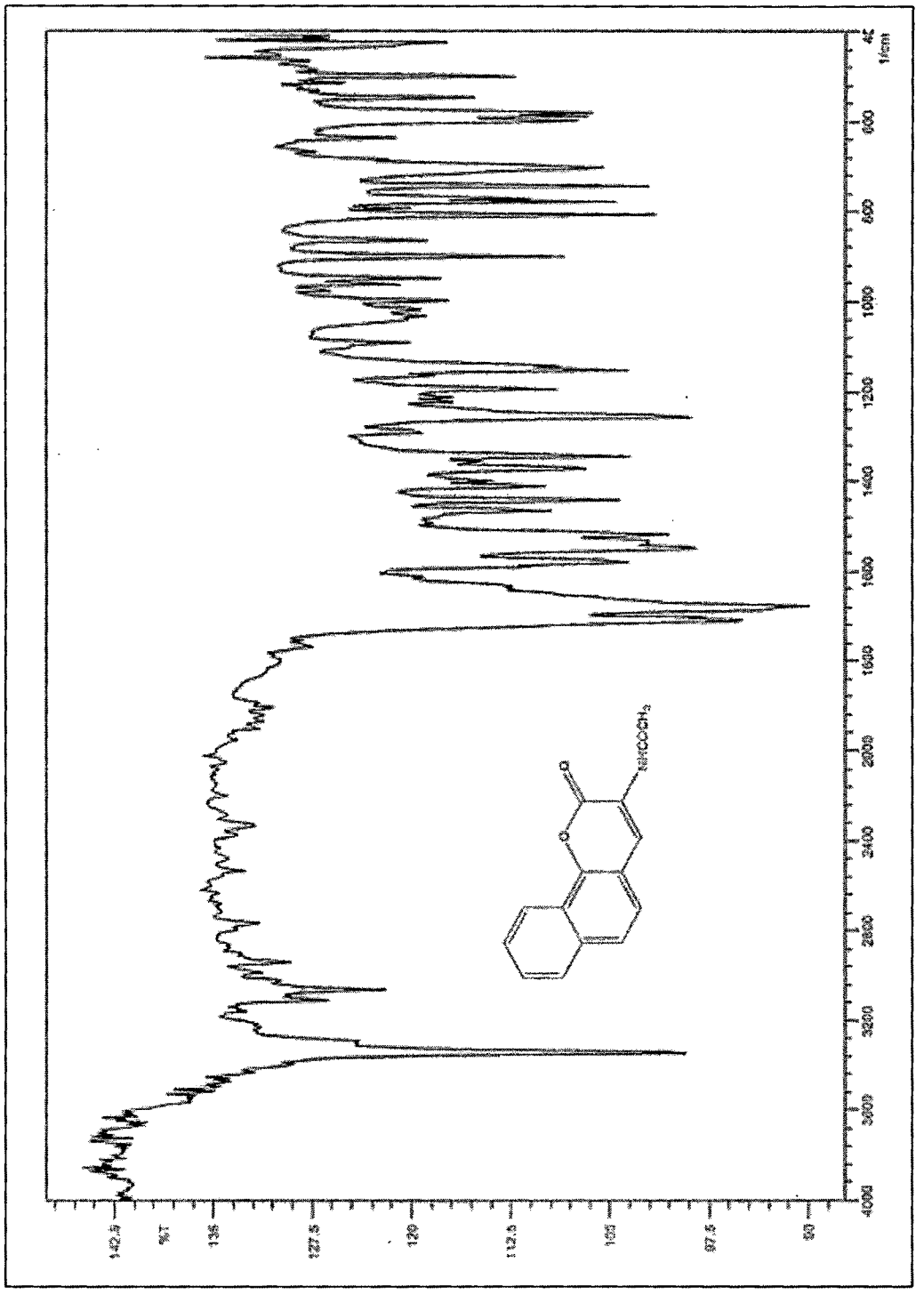


Figure 3.5.1: IR spectrum of N-(2-oxo-2H-benzo[h]chromen-3-yl)acetamide-acetyl-2H-benzo[h]chromen-2-one **2a**

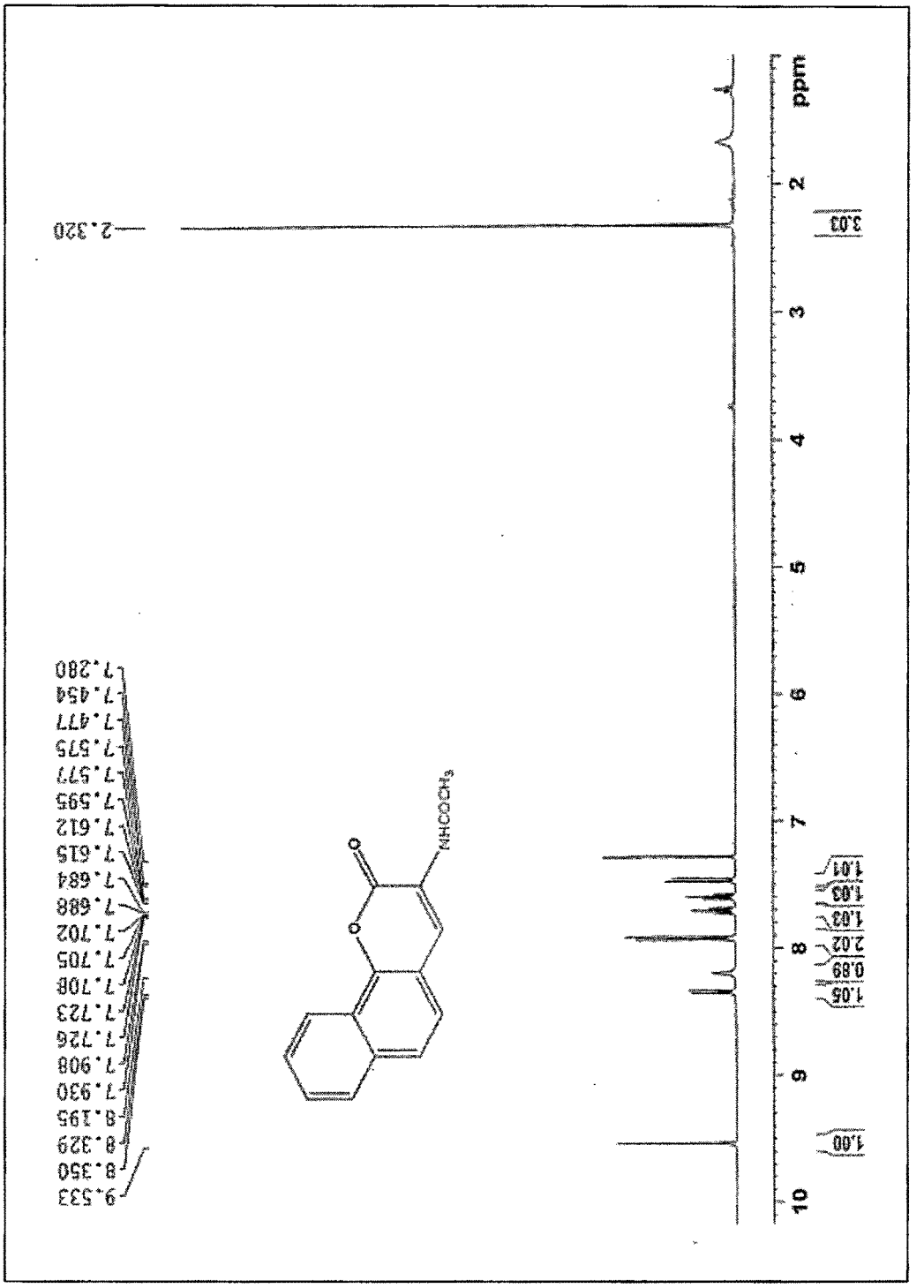


Figure 3.5.2: ^{1}H NMR spectrum of N-(2-oxo-2H-benzo[h]chromen-3-yl)acetamide-acetyl-2H-benzo[h]chromen-2-one **2a**

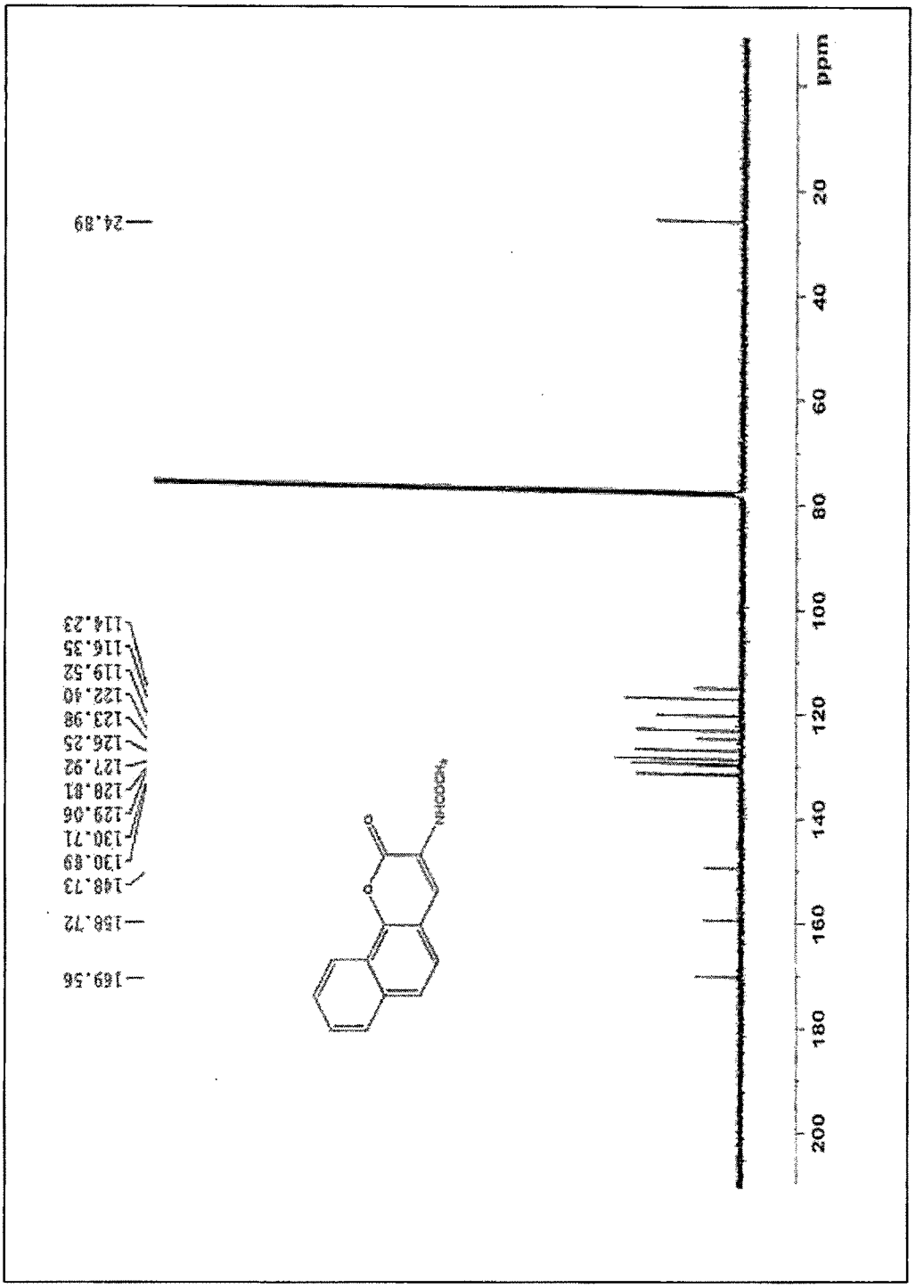


Figure 3.5.3: ^{13}C NMR spectrum of N-(2-oxo-2H-benzo[h]chromen-3-yl)acetamide-acetyl-2H-benzo[h]chromen-2-one **2a**

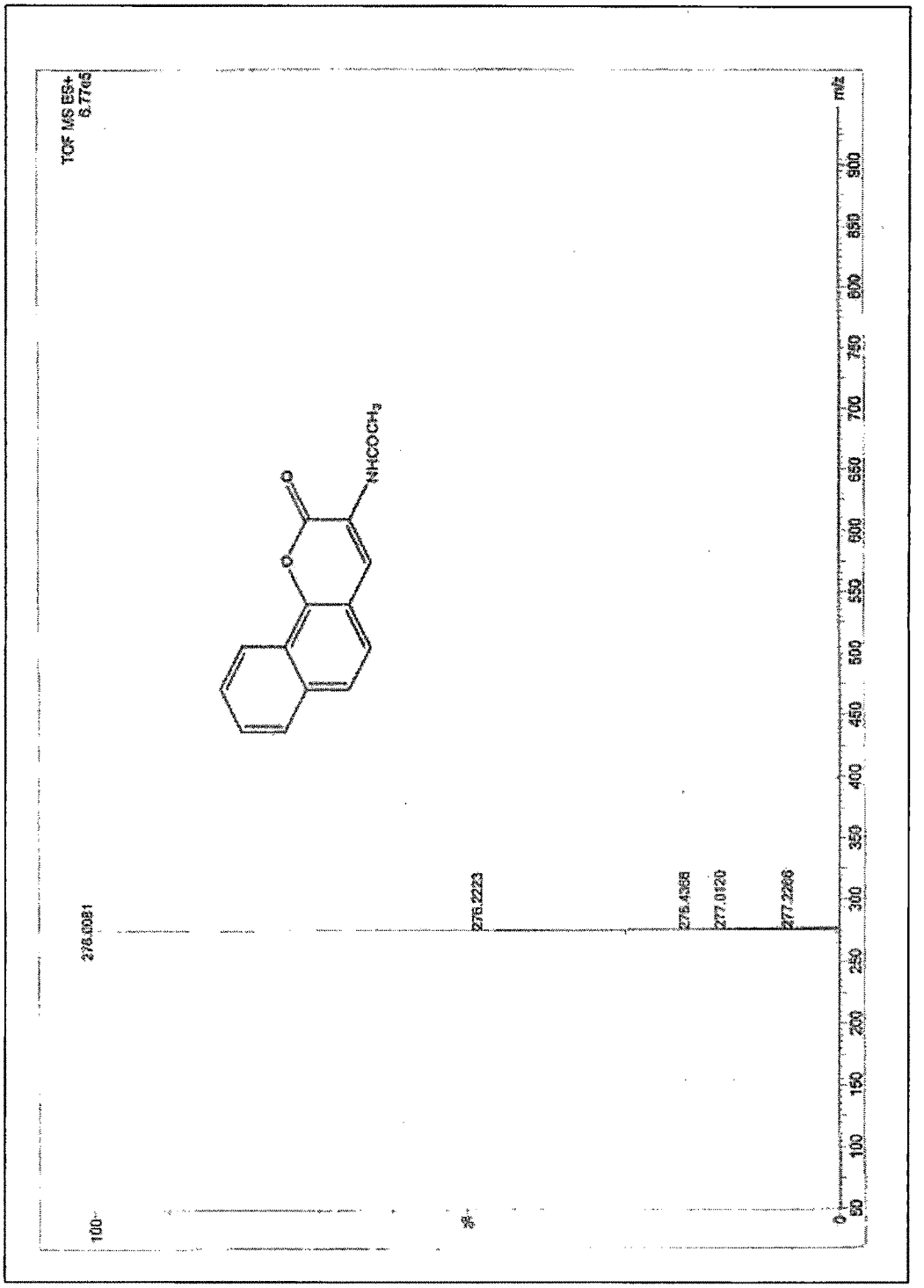


Figure 3.5.4: ESI-MS spectrum of N-(2-oxo-2H-benzo[h]chromen-3-yl)acetamide-acetyl-2H-benzo[h]chromen-2-one **2a**

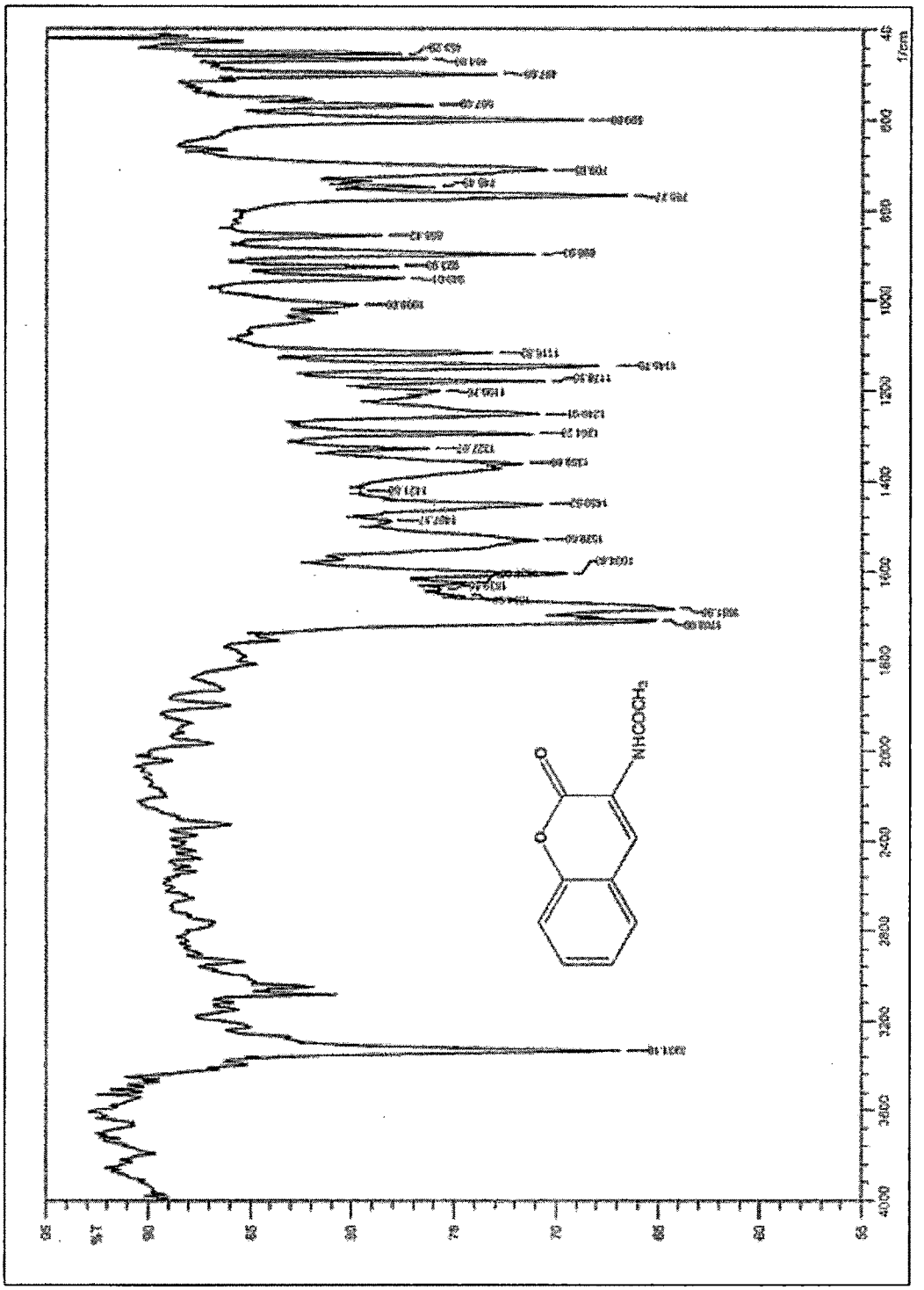


Figure 3.6.1: IR spectrum of N-(2-oxo-2H-chromen-3-yl)acetamide **2b**

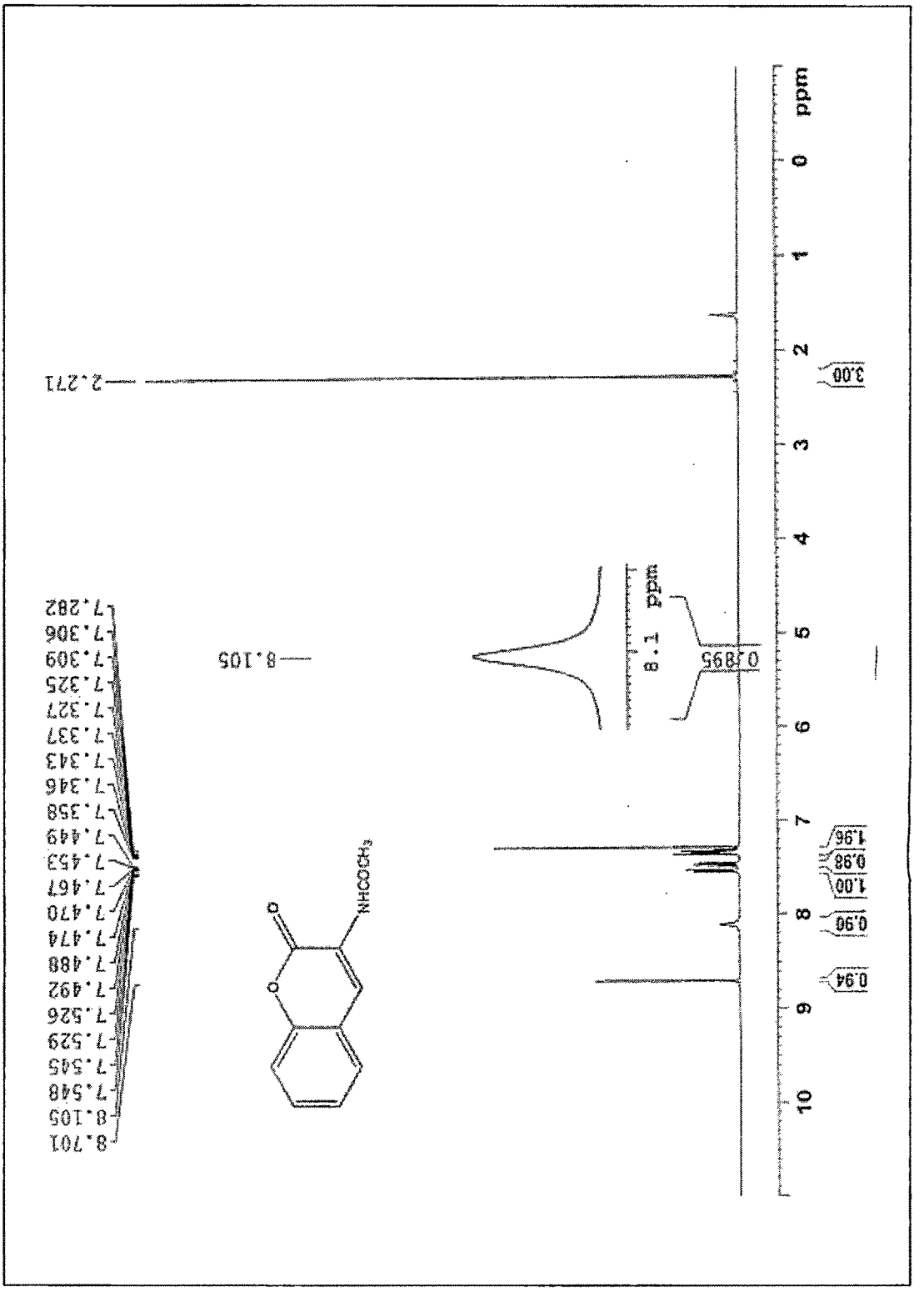


Figure 3.6.2: ^{1}H NMR spectrum of N-(2-oxo-2H-chromen-3-yl)acetamide **2b**

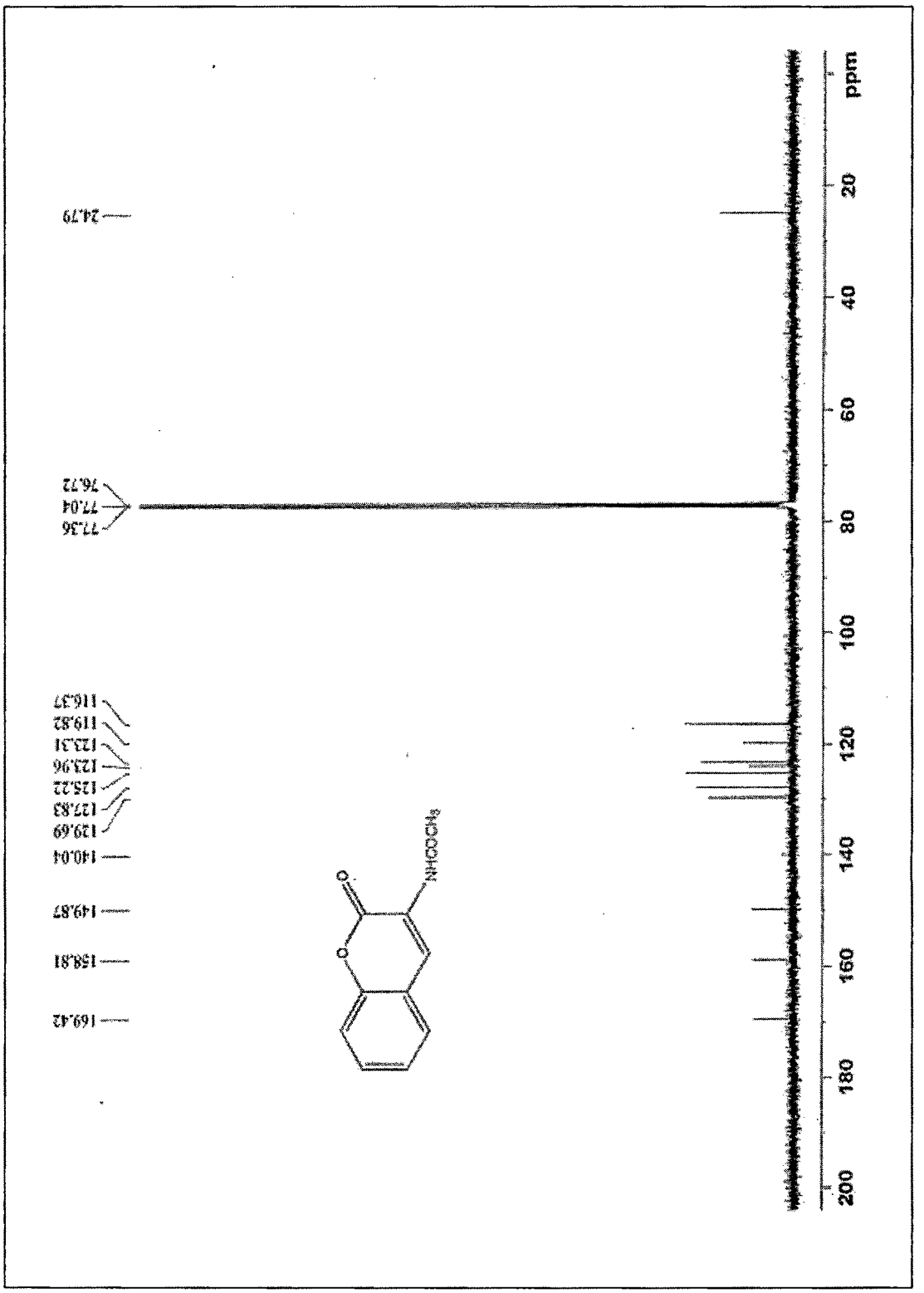


Figure 3.6.3: ^{13}C NMR spectrum of N-(2-oxo-2H-chromen-3-yl)acetamide **2b**

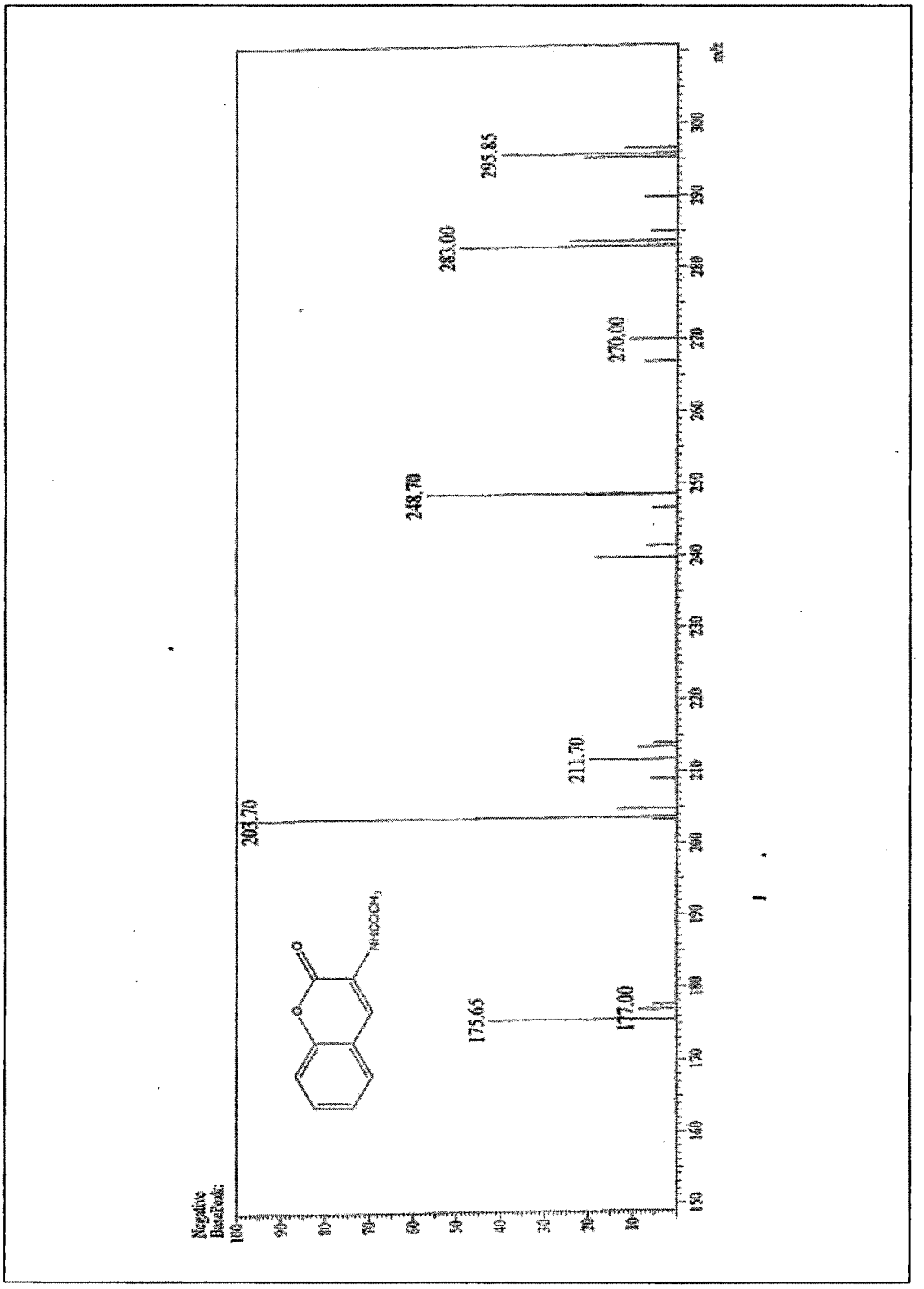


Figure 3.6.4: ESI-MS spectrum of N-(2-oxo-2H-chromen-3-yl)acetamide **2b**

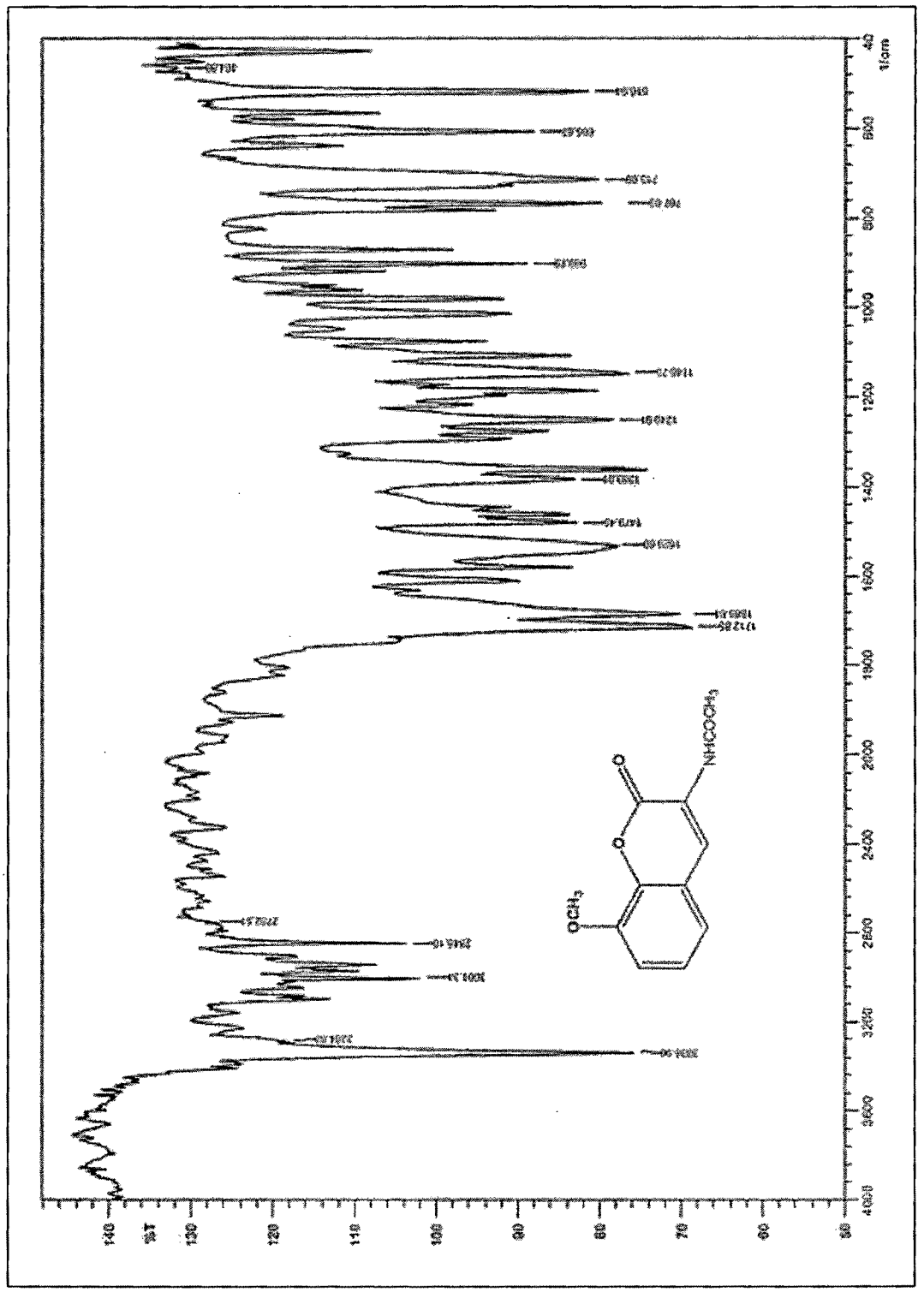


Figure 3.7.1: IR spectrum of N-(8-methoxy-2-oxo-2H-chromen-3-yl)acetamide **2d**

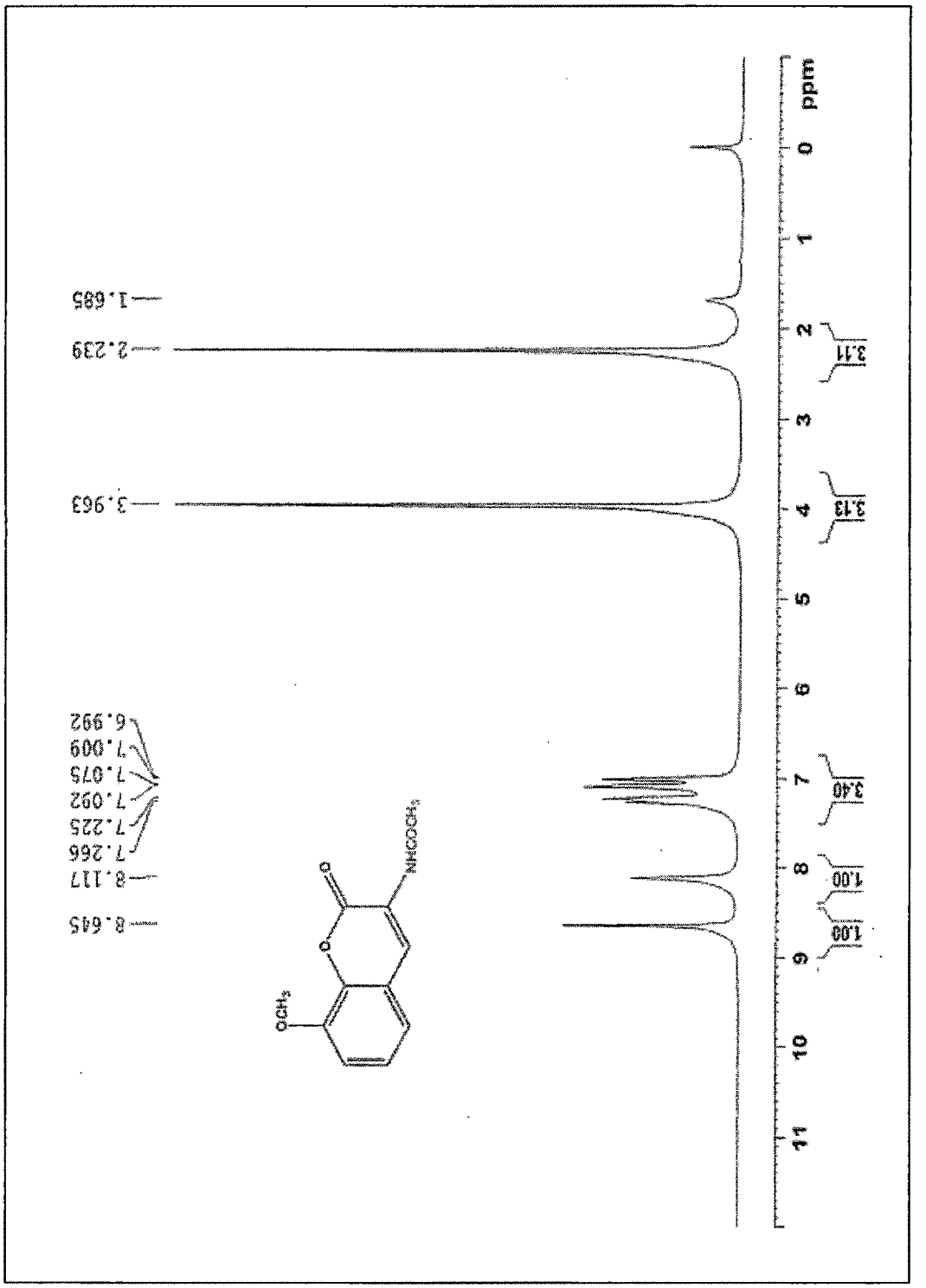


Figure 3.7.2: ^{1}H NMR spectrum of N-(8-methoxy-2-oxo-2H-chromen-3-yl)acetamide **2d**

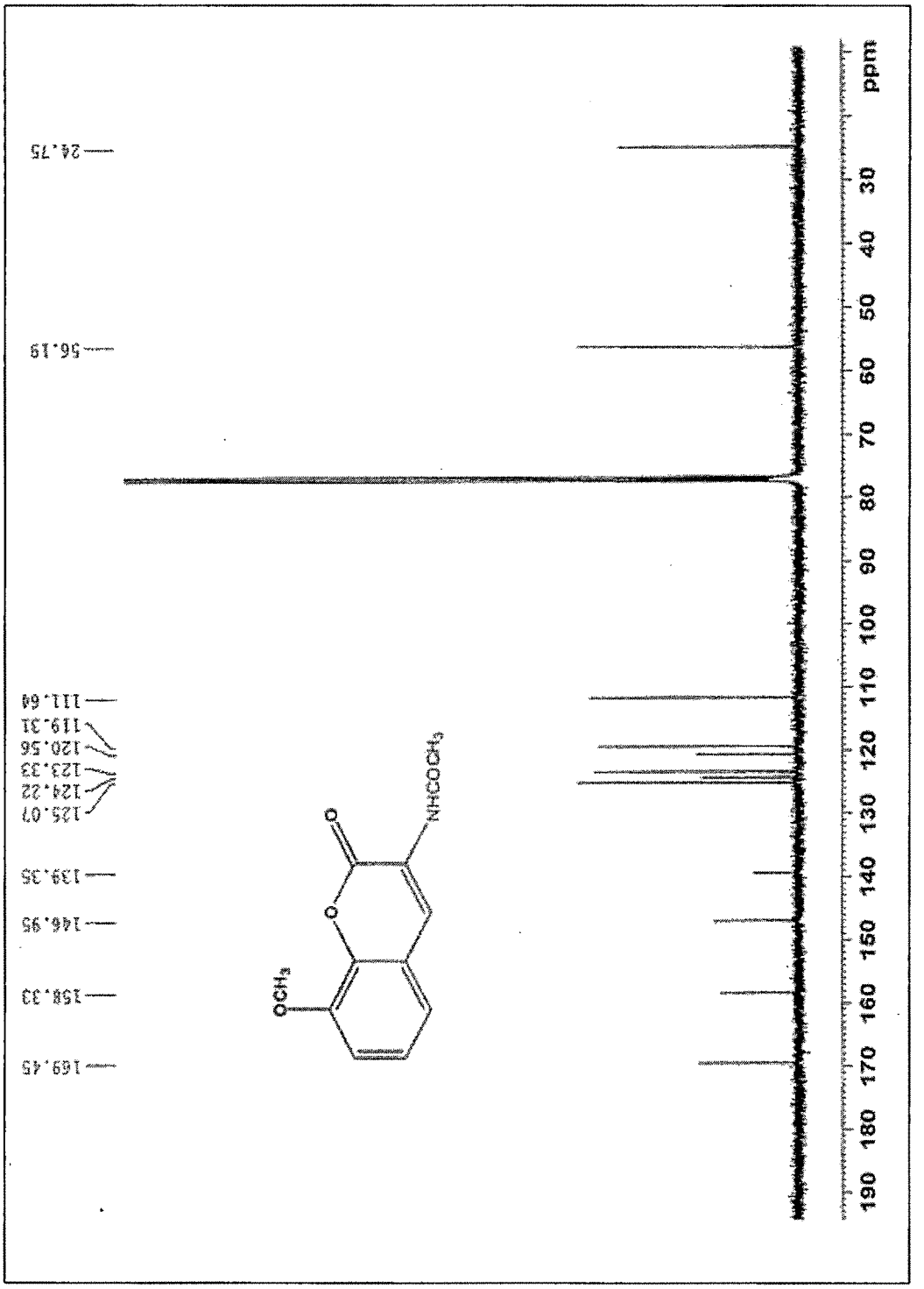


Figure 3.7.3: ^{13}C NMR spectrum of N-(8-methoxy-2-oxo-2H-chromen-3-yl)acetamide **2d**

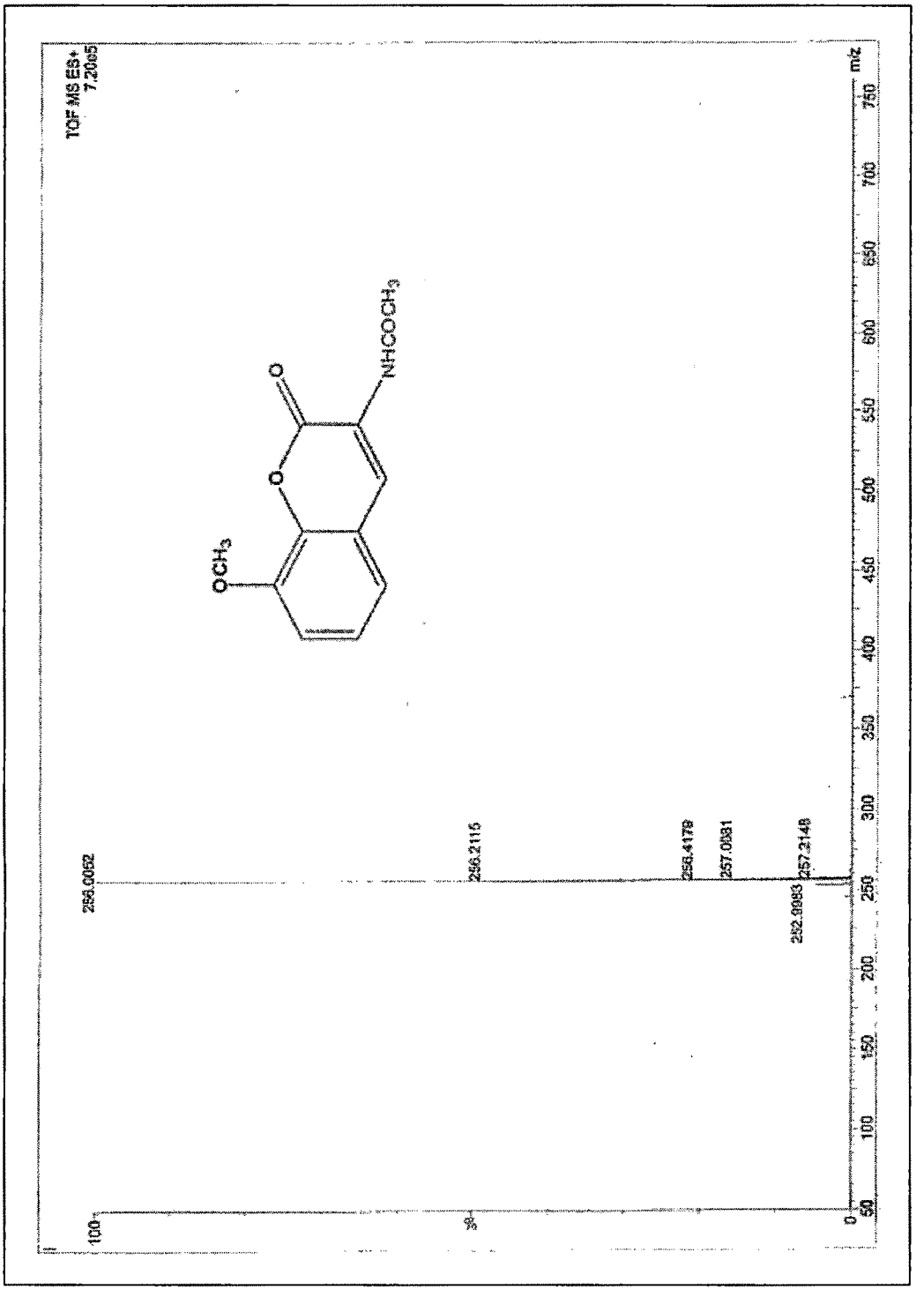


Figure 3.7.4: ESI-MS spectrum of N-(8-methoxy-2-oxo-2H-chromen-3-yl)acetamide **2d**

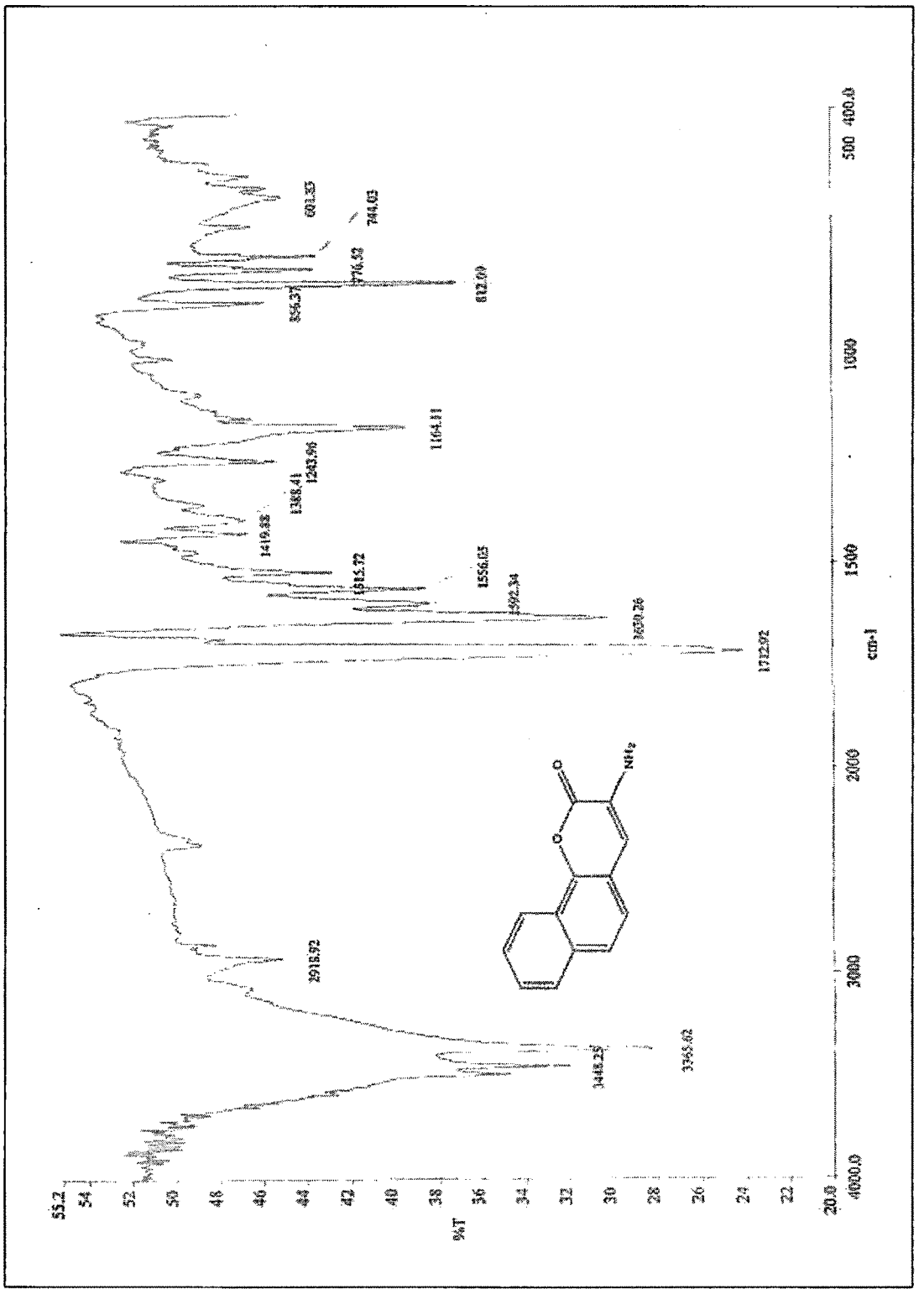


Figure 3.8.1: IR spectrum of 3-amino-2H-benzo[h]chromen-2-one **3a**

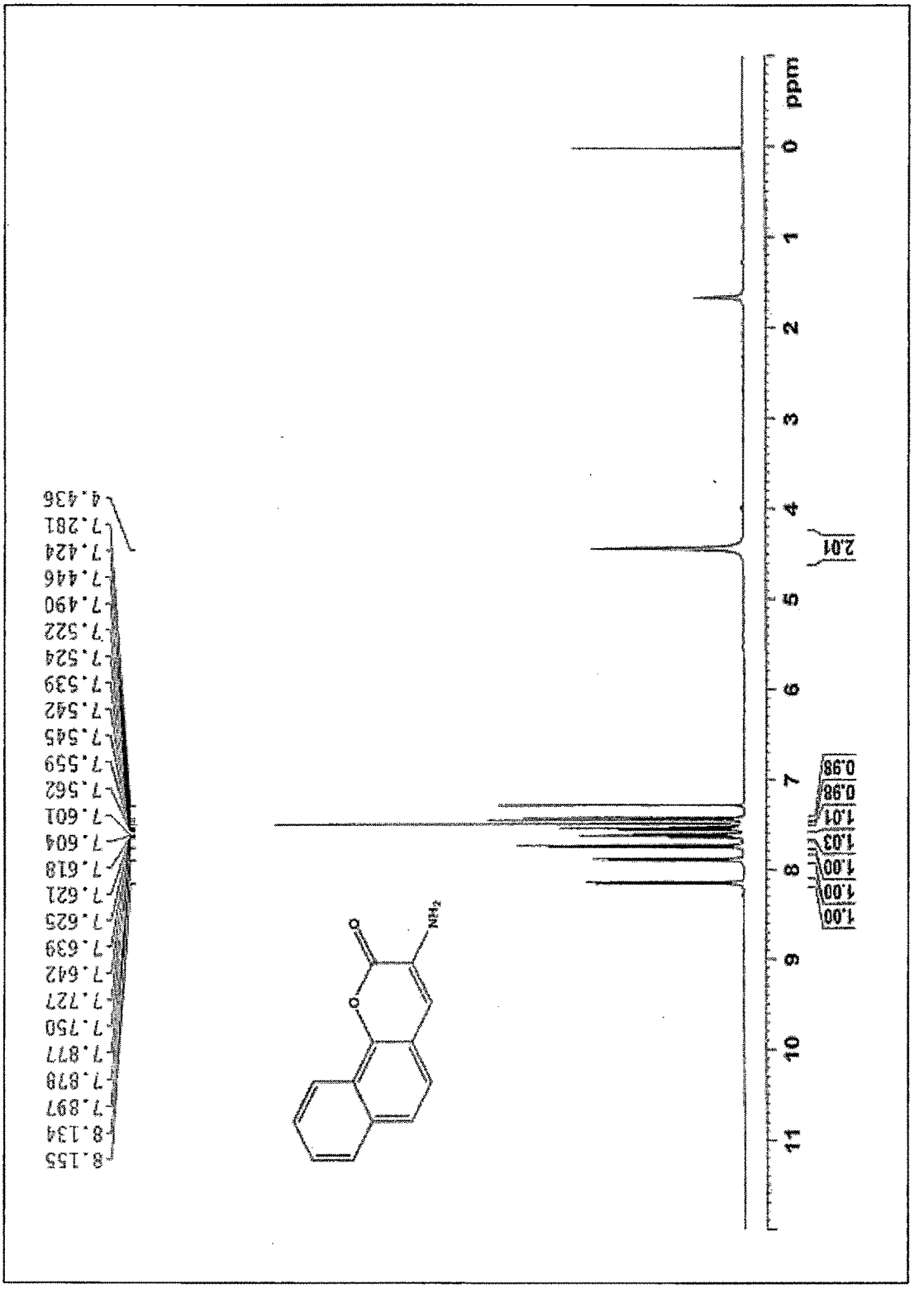


Figure 3.8.2: ^{1}H NMR spectrum of 3-amino-2H-benzo[h]chromen-2-one **3a**

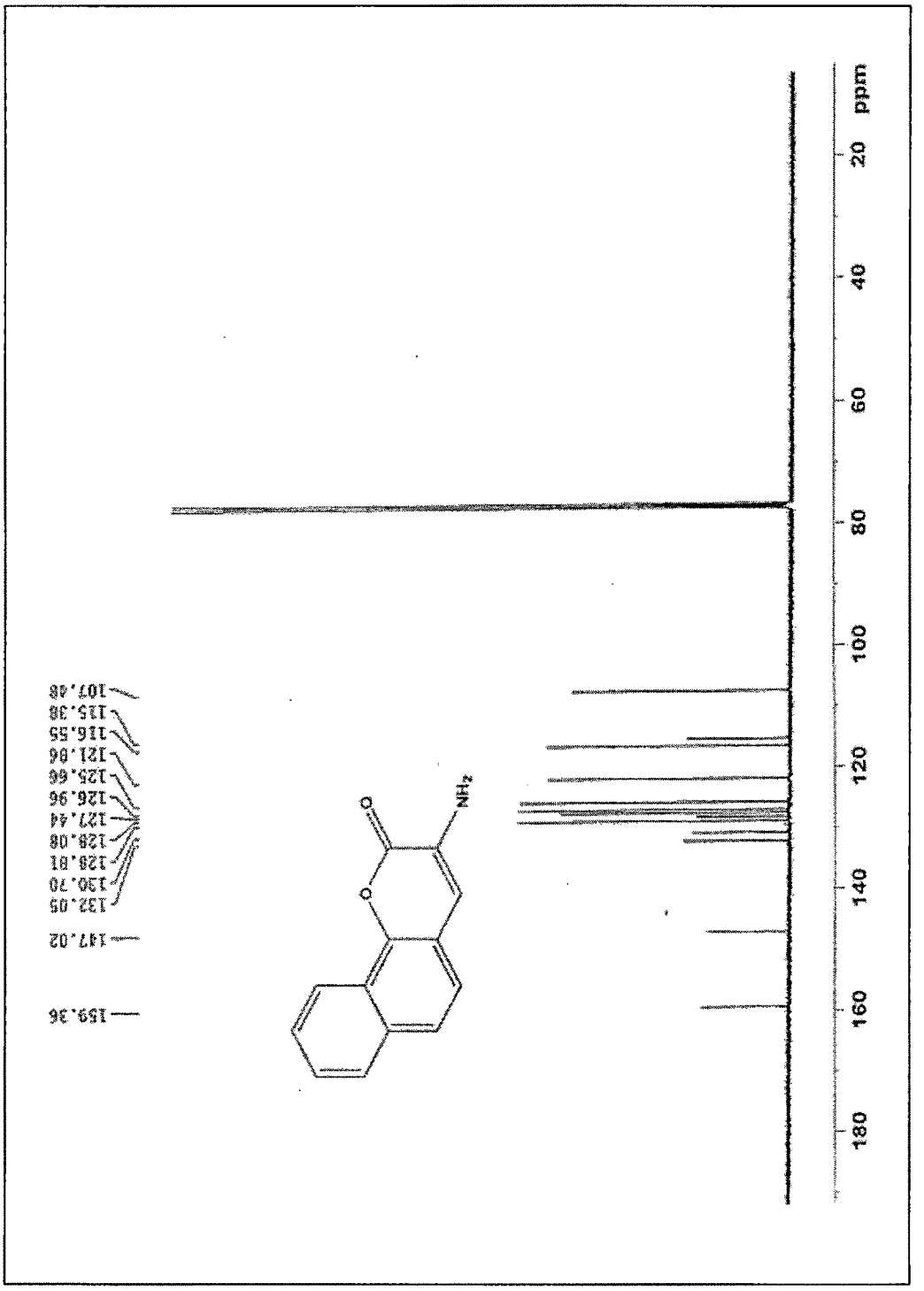


Figure 3.8.3: ^{13}C NMR spectrum of 3-amino-2H-benzo[h]chromen-2-one **3a**

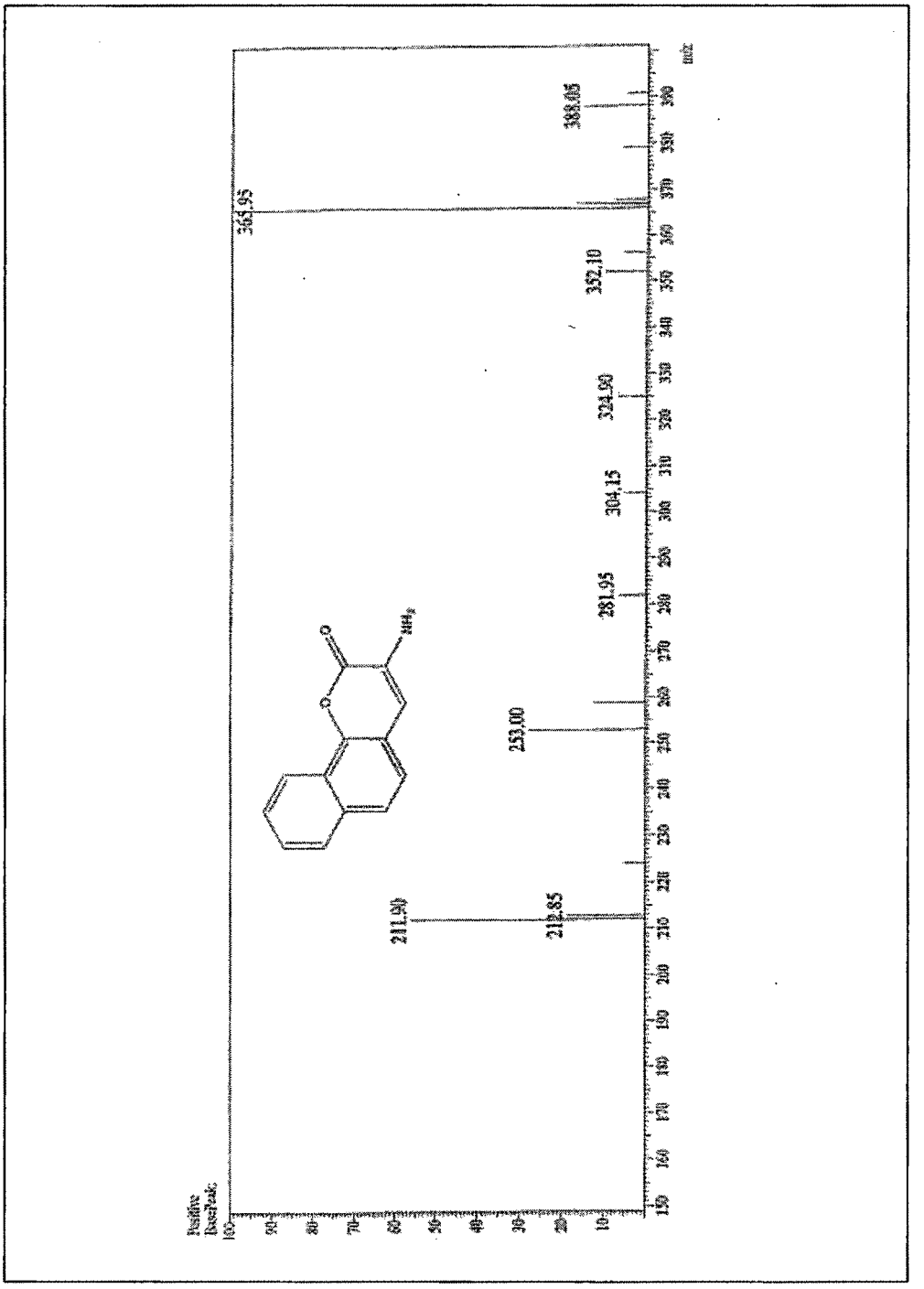


Figure 3.8.4: ESI-MS spectrum of 3-amino-2H-benzo[h]chromen-2-one **3a**

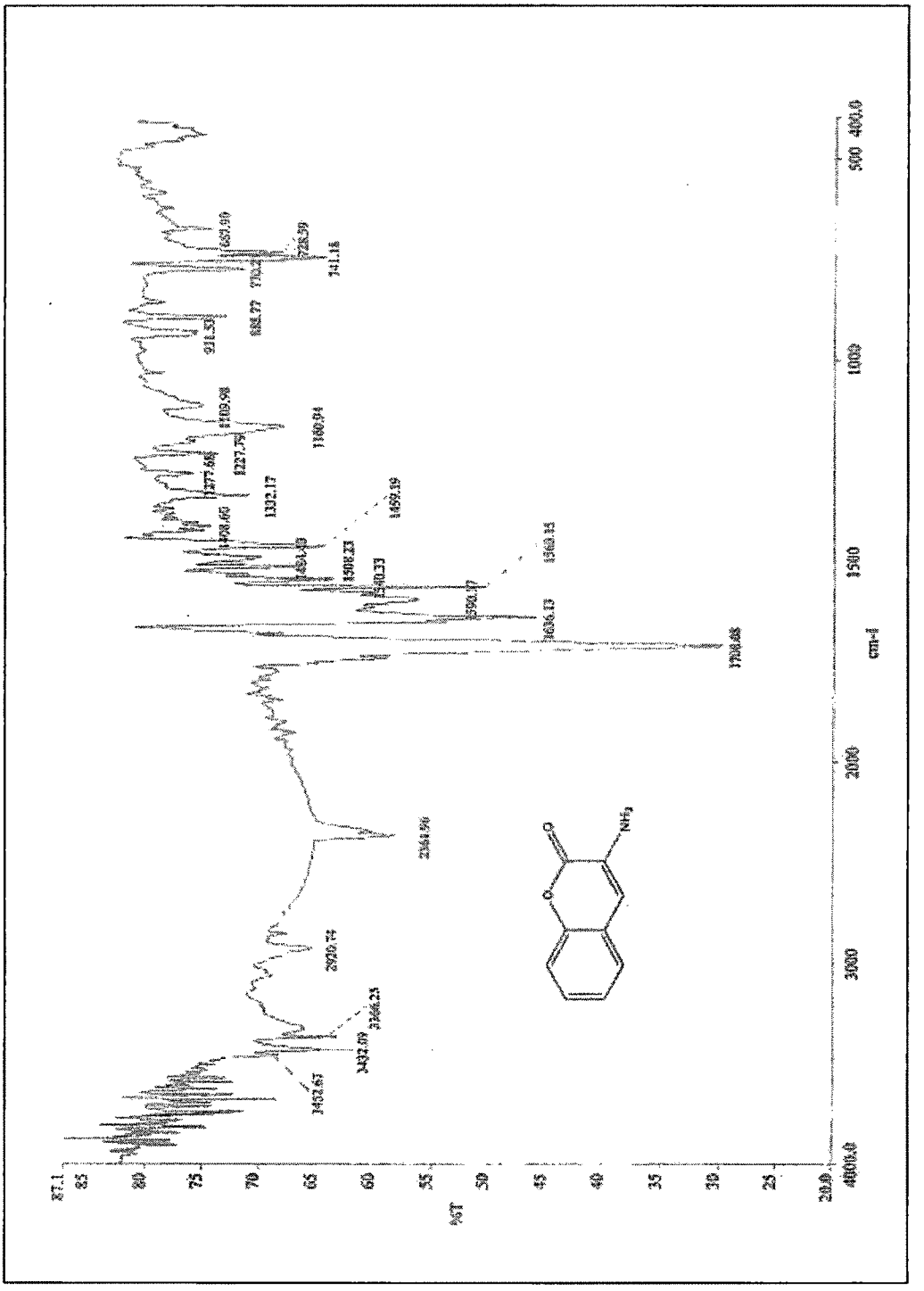


Figure 3.9.1: IR spectrum of 3-amino-2H-chromen-2-one **3b**

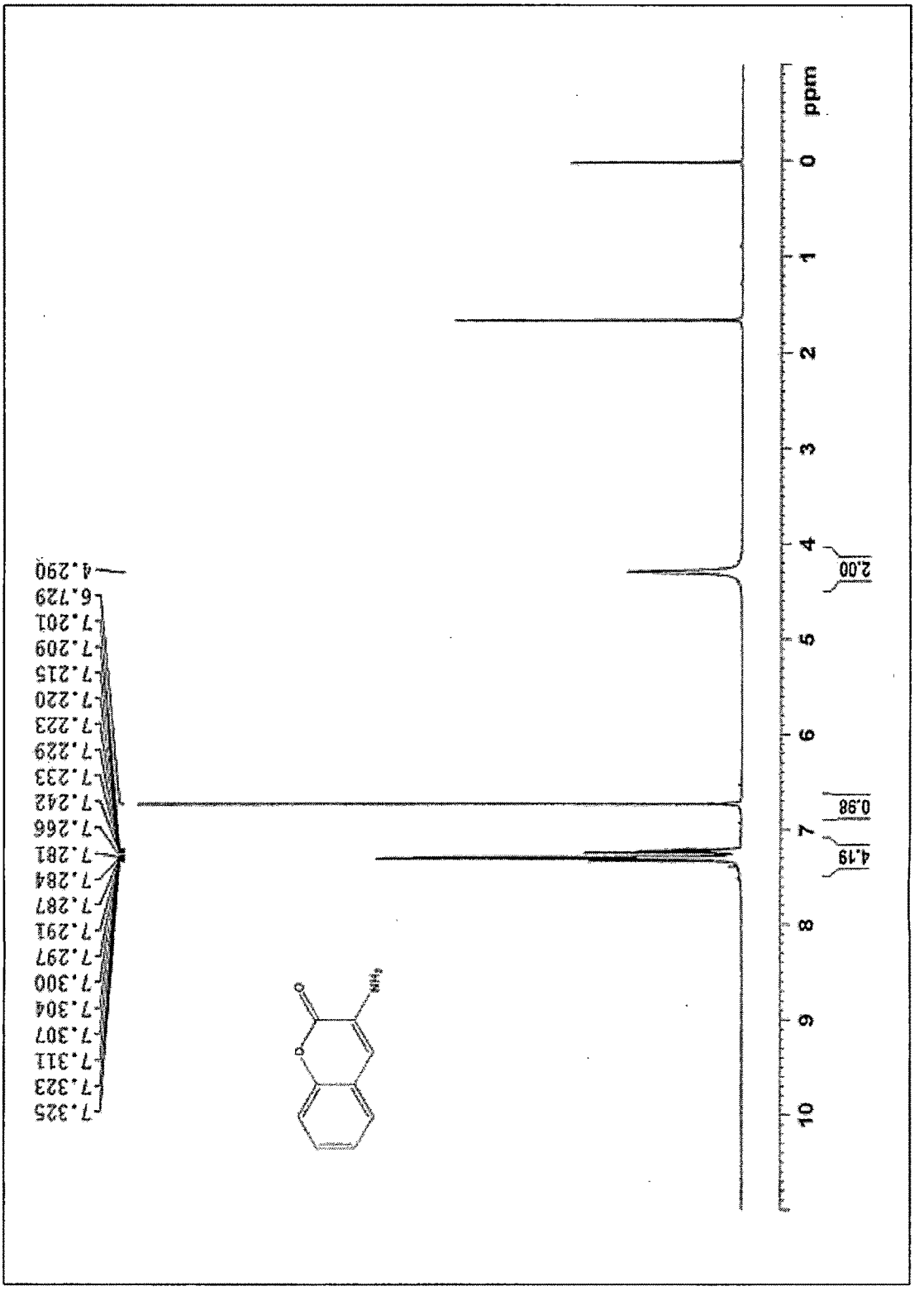


Figure 3.9.2: ^{1}H NMR spectrum of 3-amino-2H-chromen-2-one **3b**

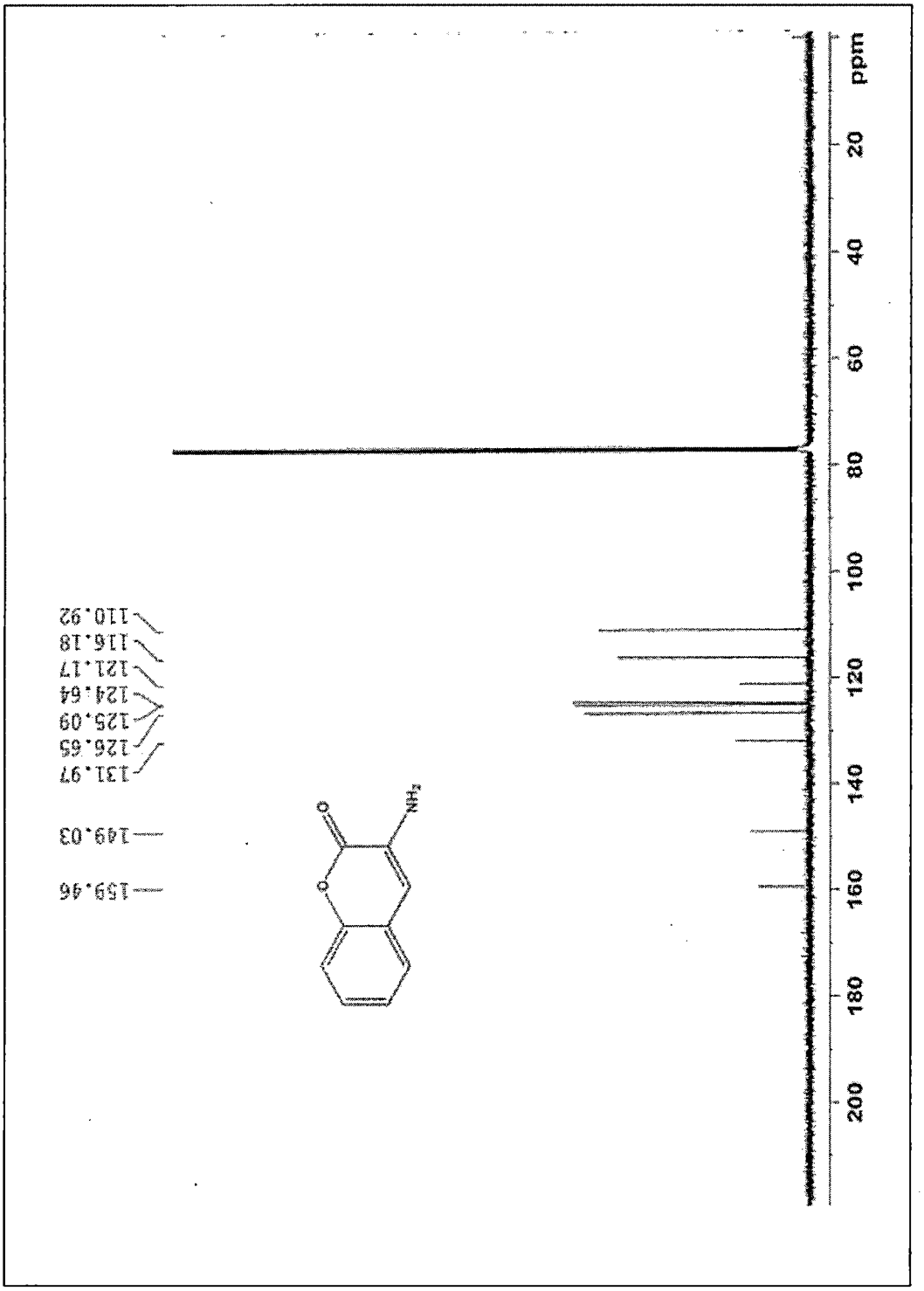


Figure 3.9.3: ^{13}C NMR spectrum of 3-amino-2H-chromen-2-one **3b**

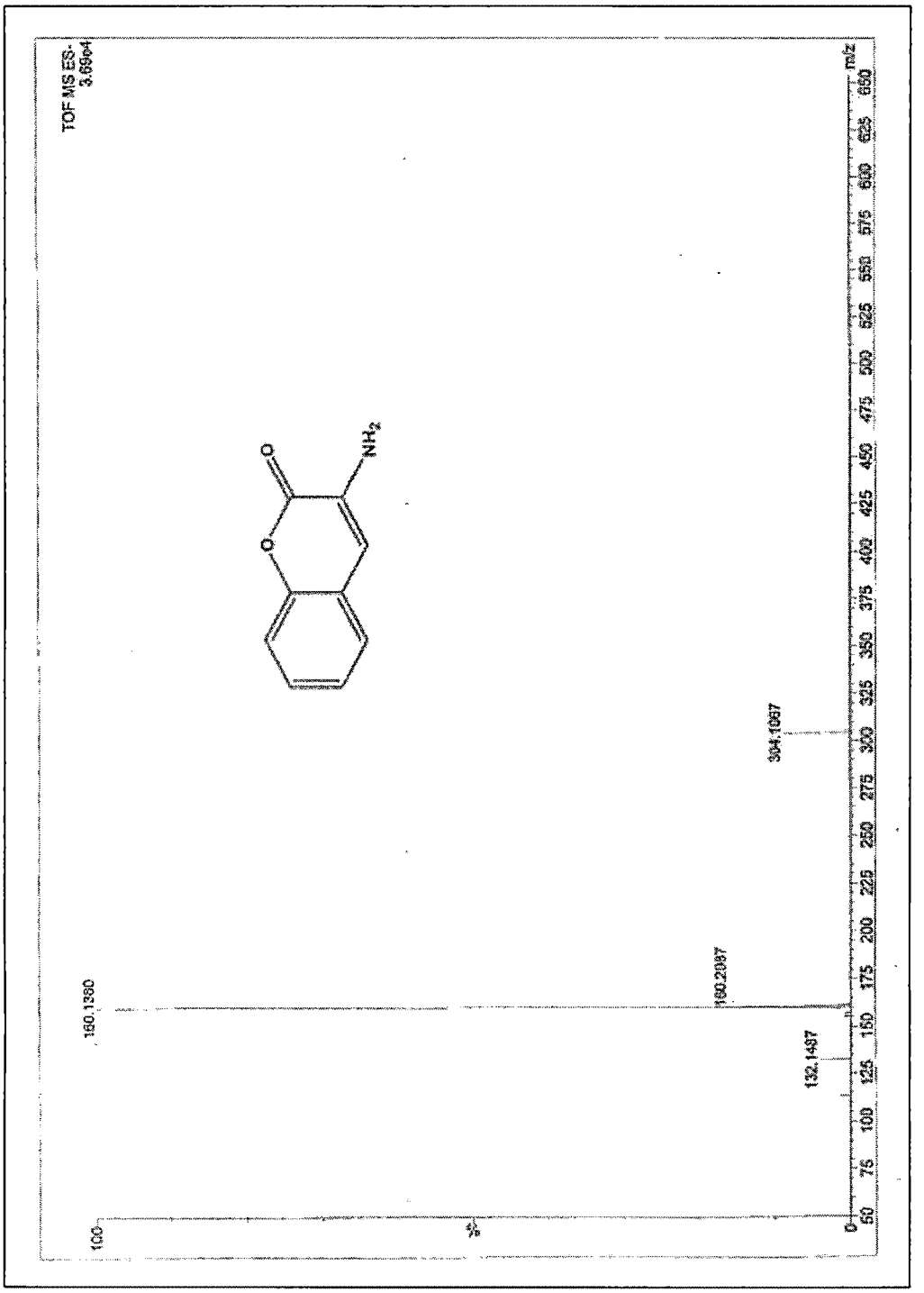


Figure 3.9.4: ESI-MS spectrum of 3-amino-2H-chromen-2-one **3b**

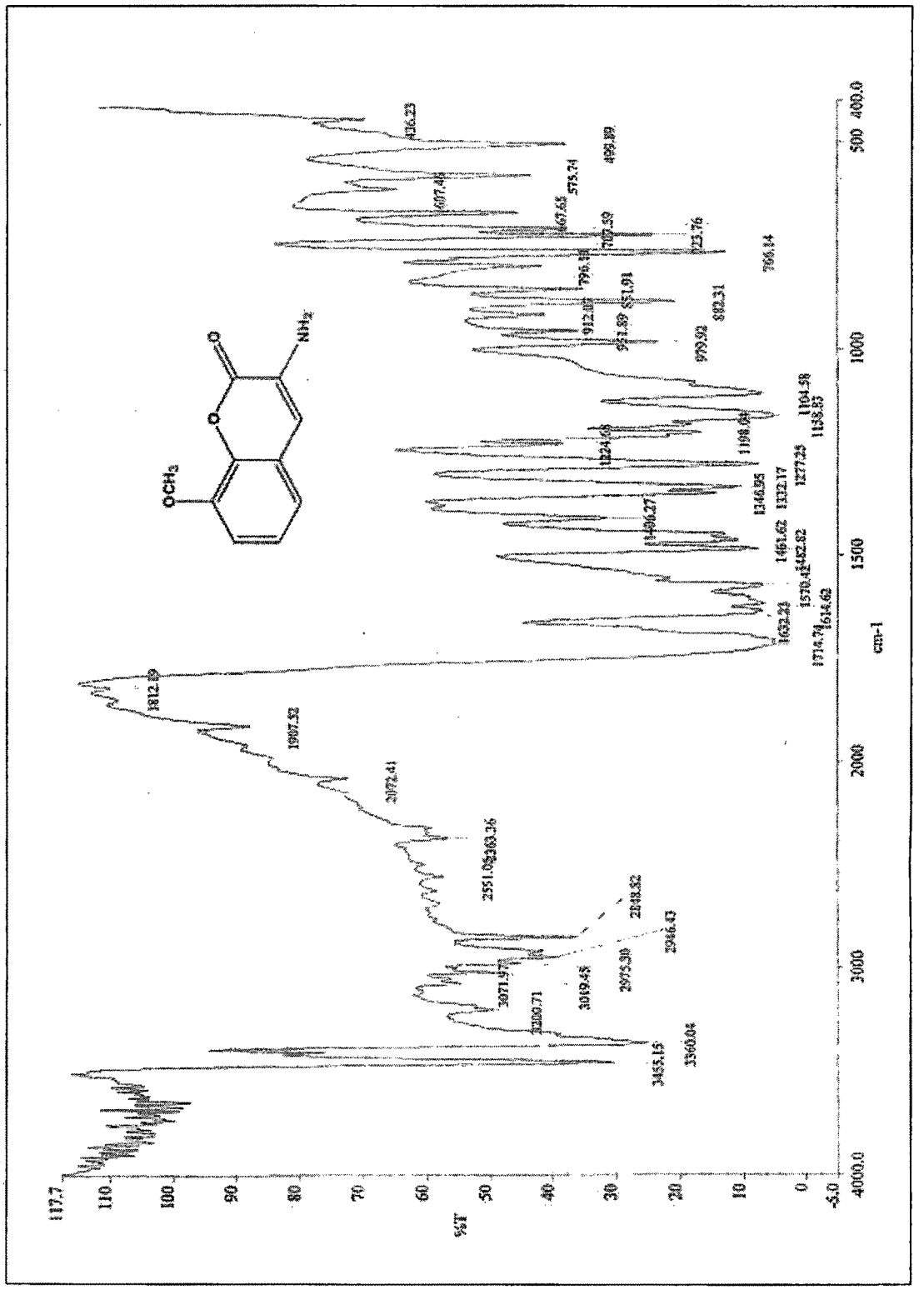


Figure 3.10.1: IR spectrum of 3-amino-8-methoxy-2H-chromen-2-one **3d**

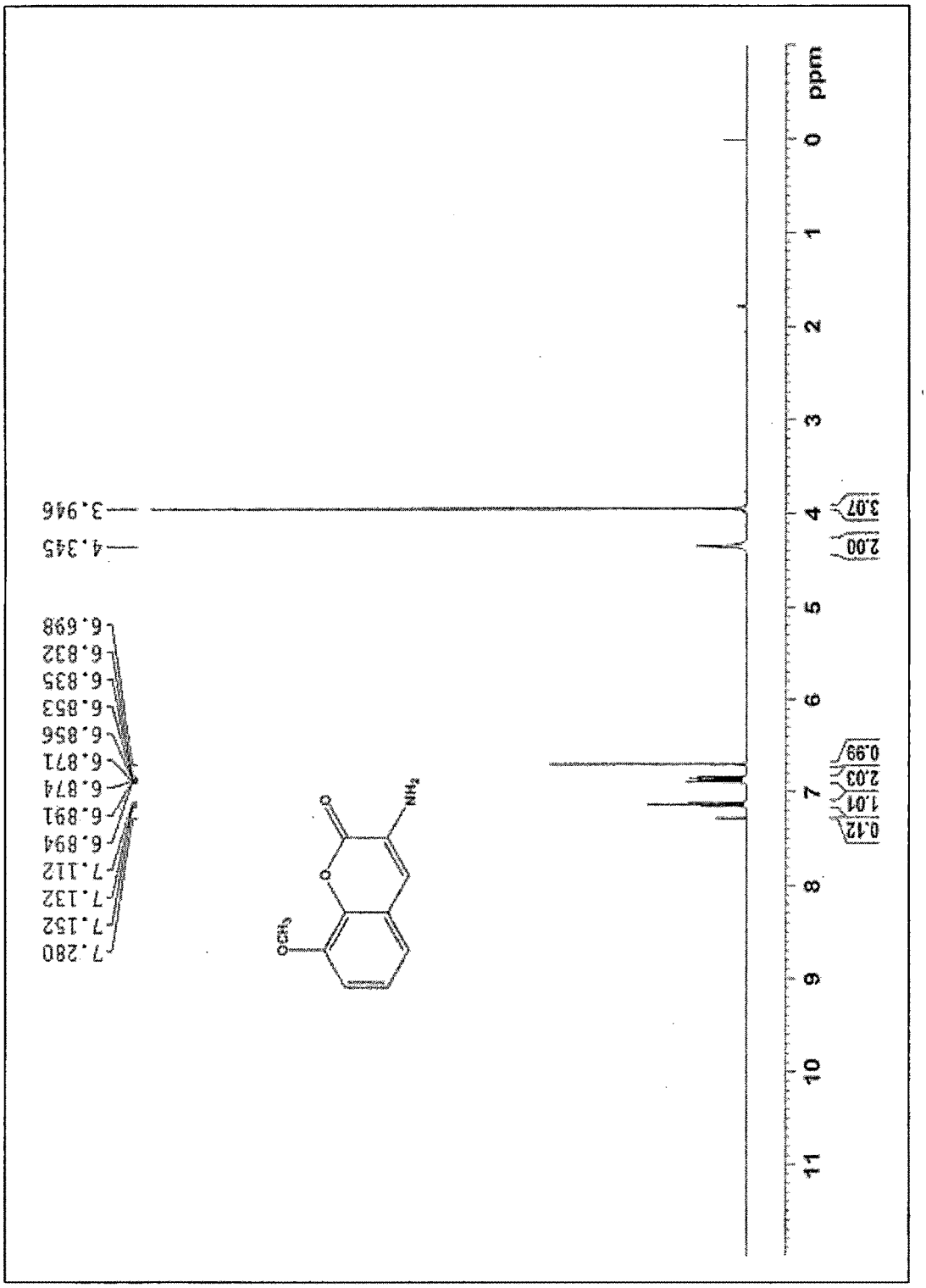


Figure 3.10.2: ^{1}H NMR spectrum of 3-amino-8-methoxy-2H-chromen-2-one **3d**

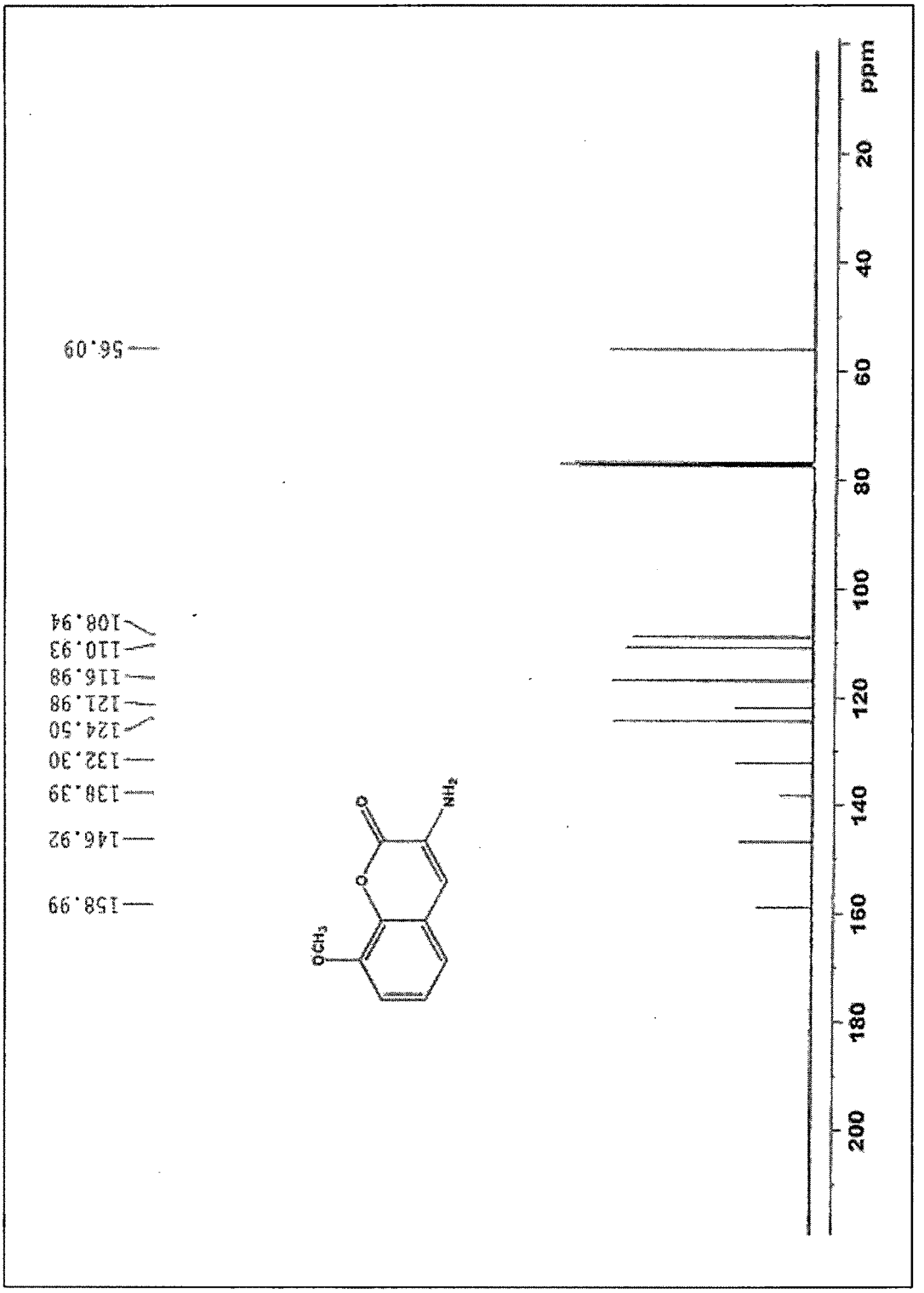


Figure 3.10.3: ^{13}C NMR spectrum of 3-amino-8-methoxy-2H-chromen-2-one **3d**

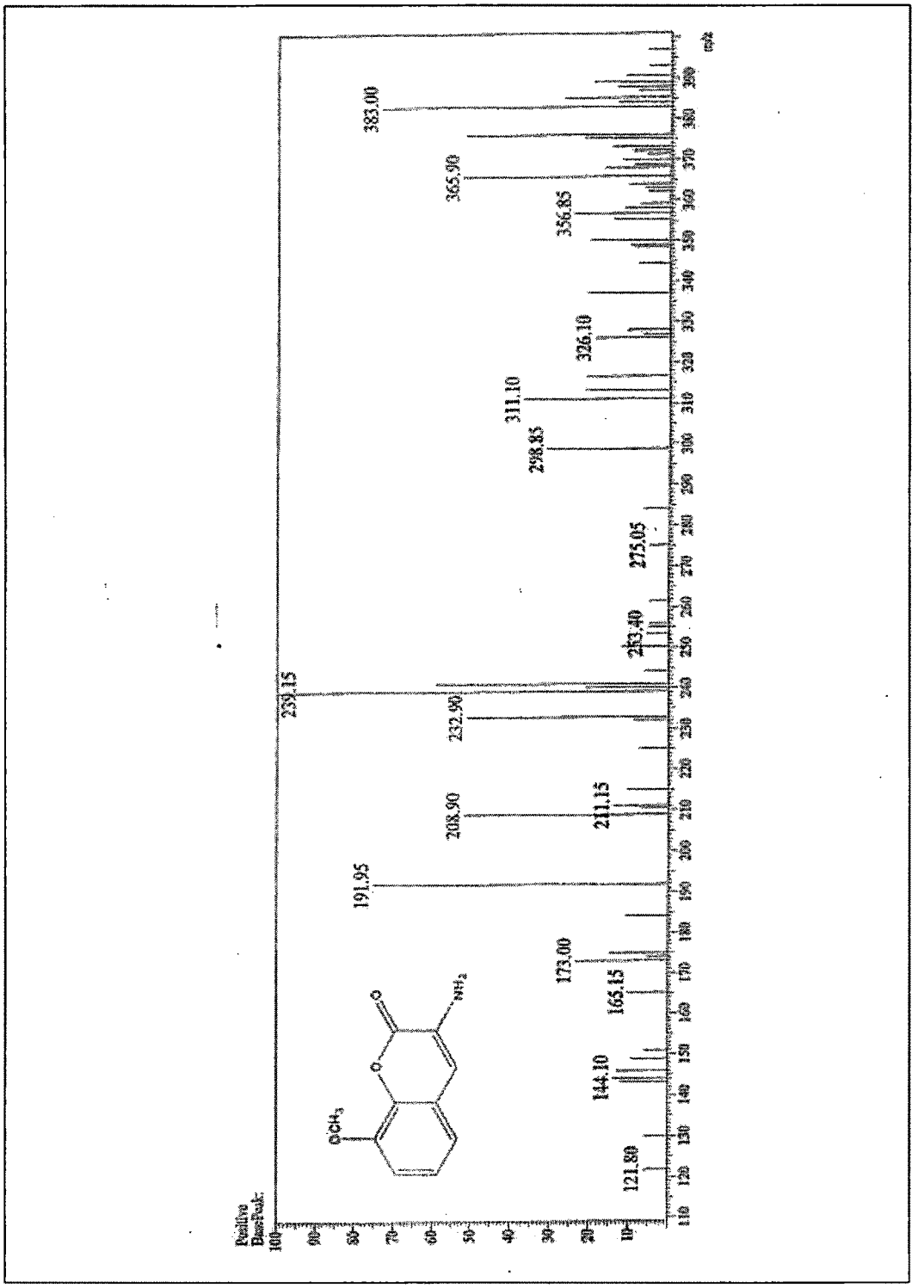


Figure 3.10.4: ESI-MS spectrum of 3-amino-8-methoxy-2H-chromen-2-one **3d**

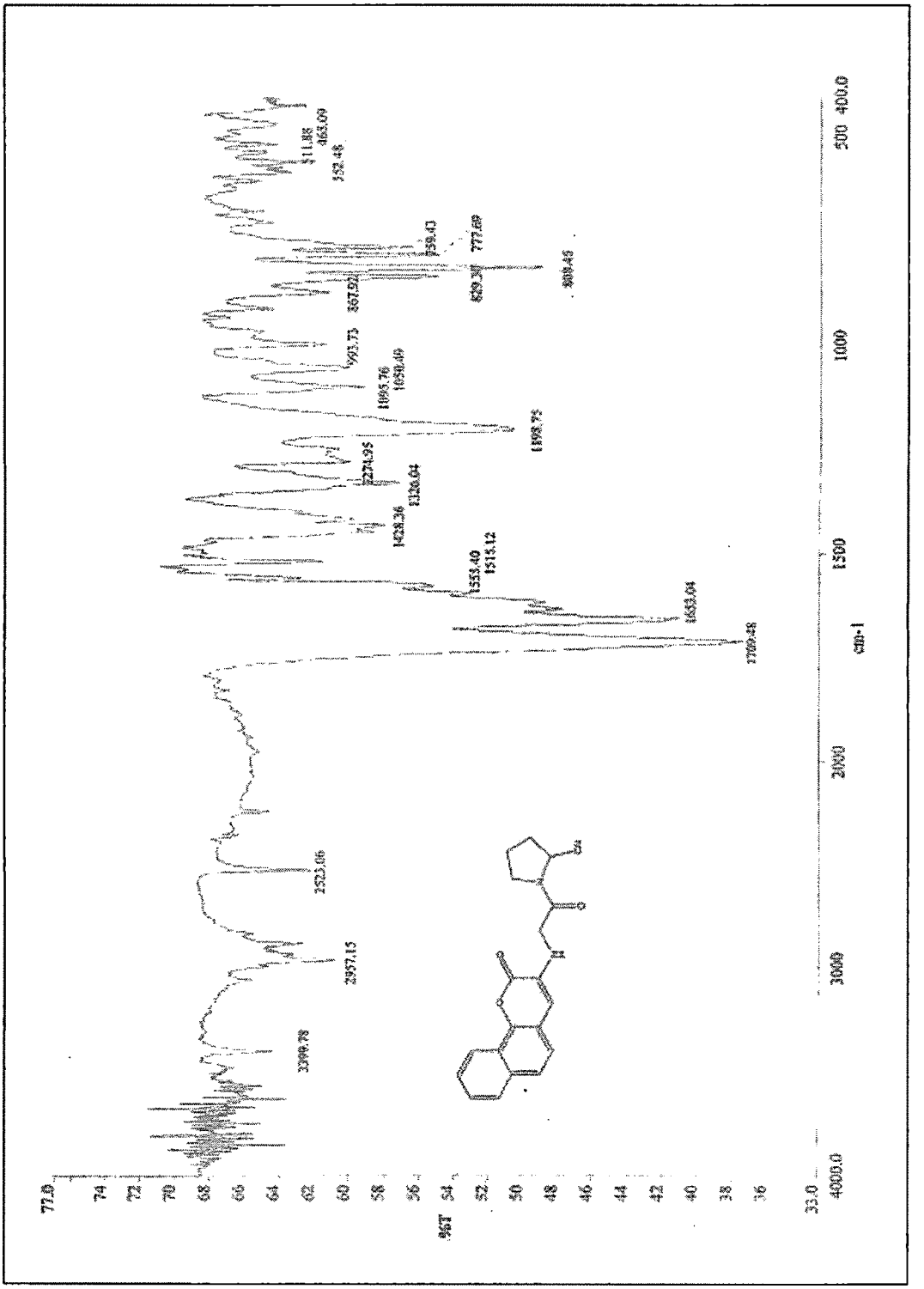


Figure 3.11.1: IR spectrum of *(S)*-1-(2-(2-oxo-2H-benzo[h]chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile **7a**

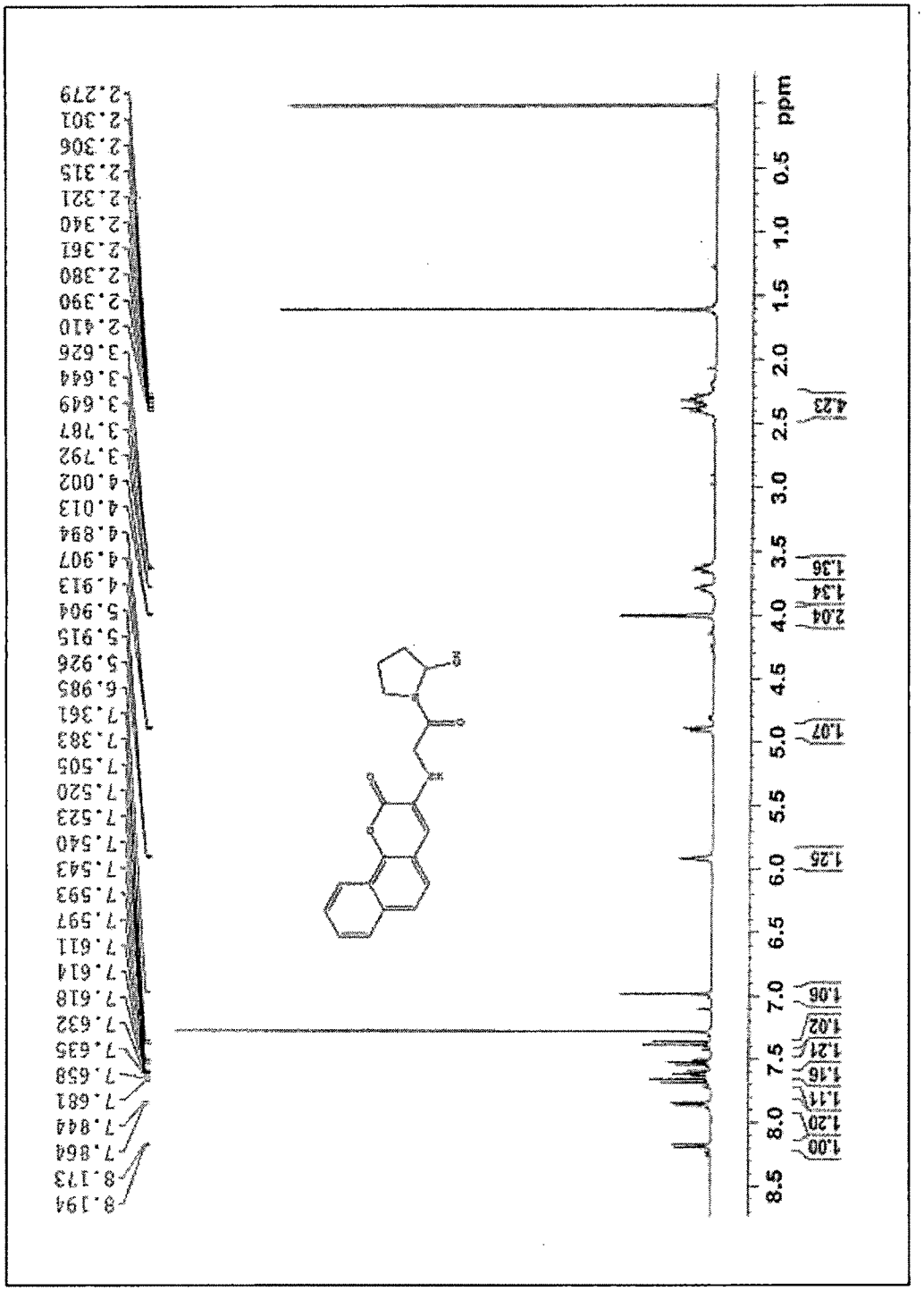


Figure 3.11.2: ^{1}H NMR spectrum of *(S)*-1-(2-(2-oxo-2H-benzo[h]chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile **7a**

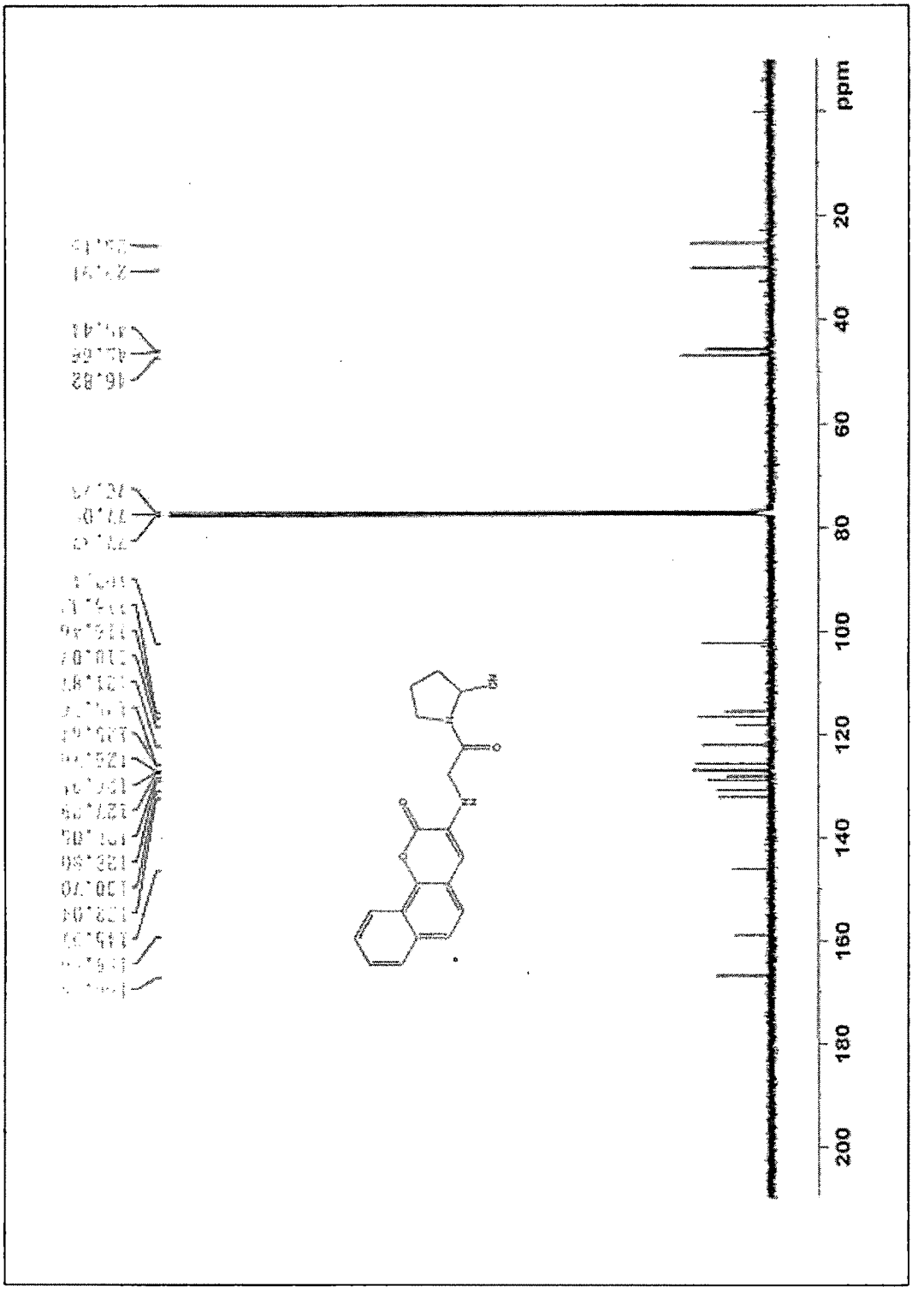


Figure 3.11.3: ^{13}C NMR spectrum of *(S)*-1-(2-(2-oxo-2H-benzo[h]chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile **7a**

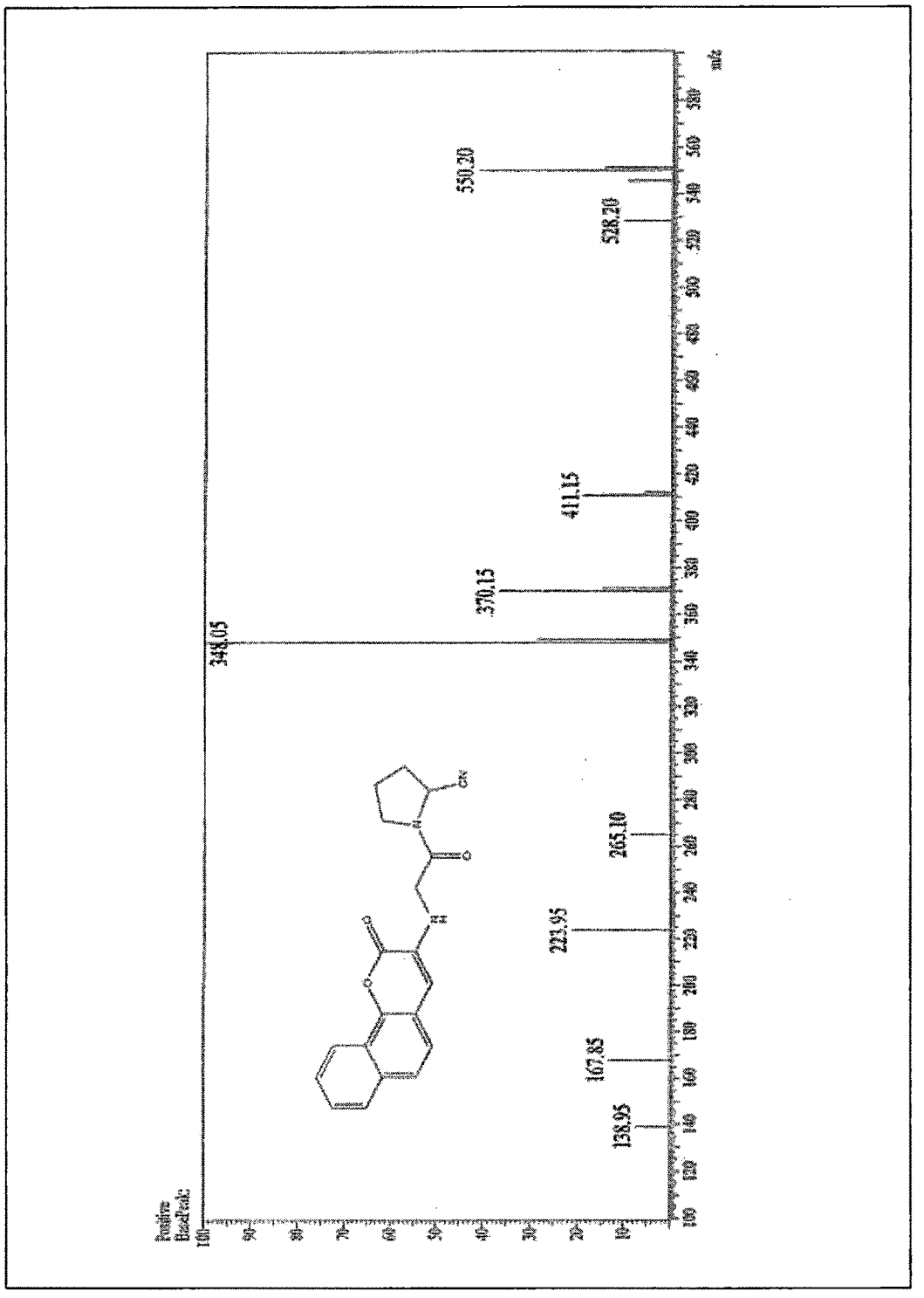


Figure 3.11.4: ESI-MS spectrum of (*S*)-1-(2-(2-oxo-2H-benzo[h]chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile **7a**

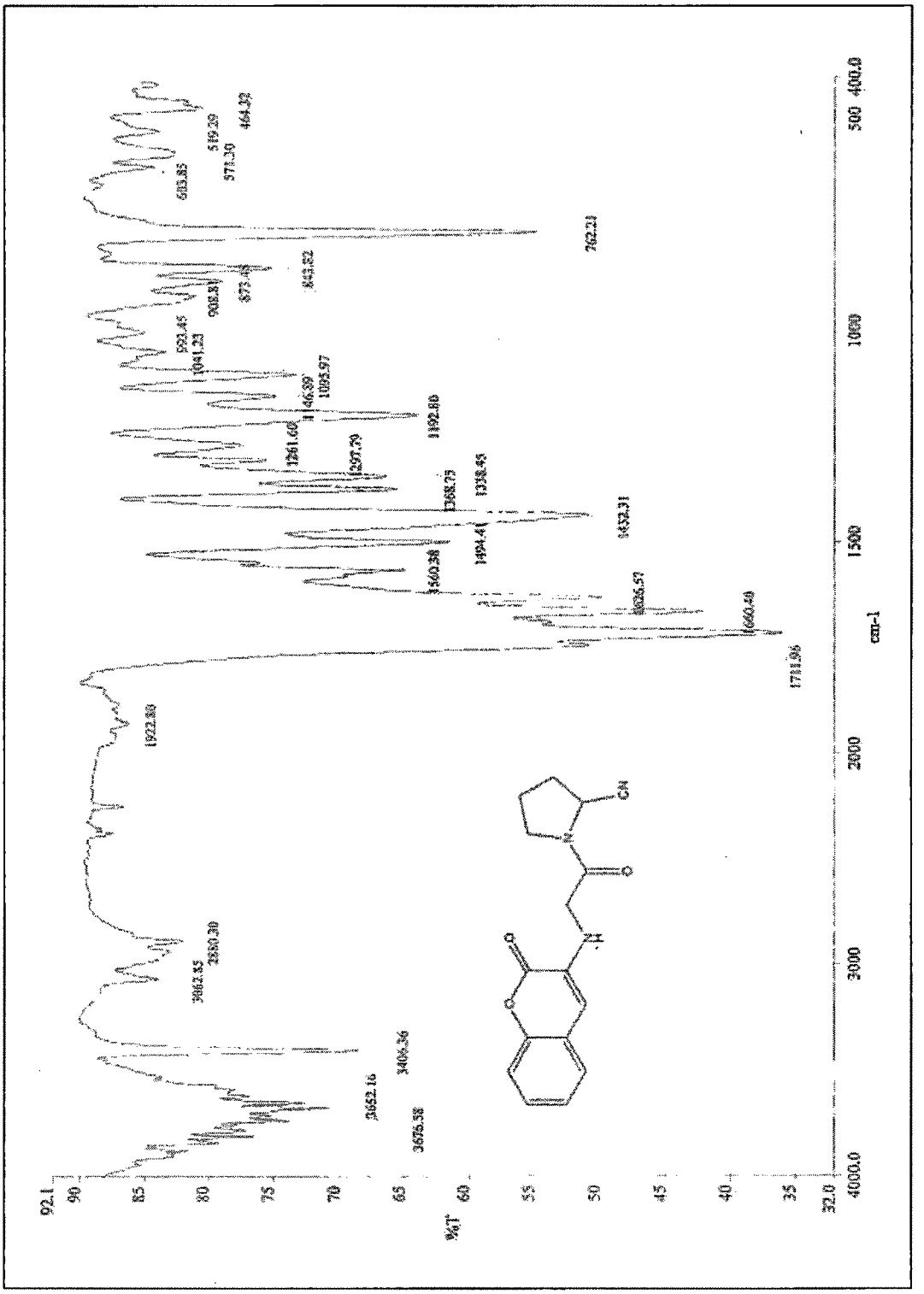


Figure 3.12.1: IR spectrum of (*S*)-1-(2-(2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile **7b**

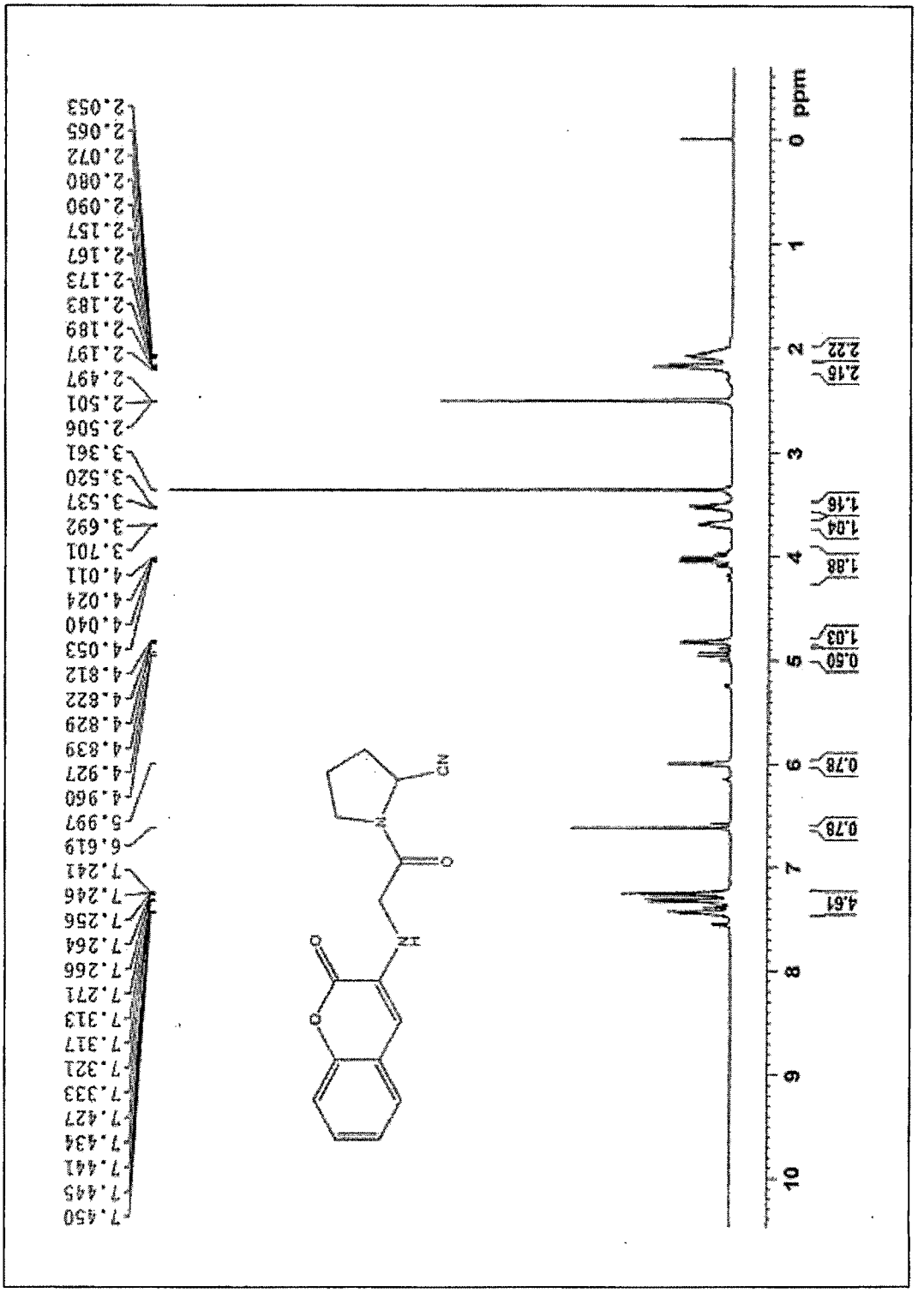


Figure 3.12.2: ^{1}H NMR spectrum of (*S*)-1-(2-(2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile **7b**

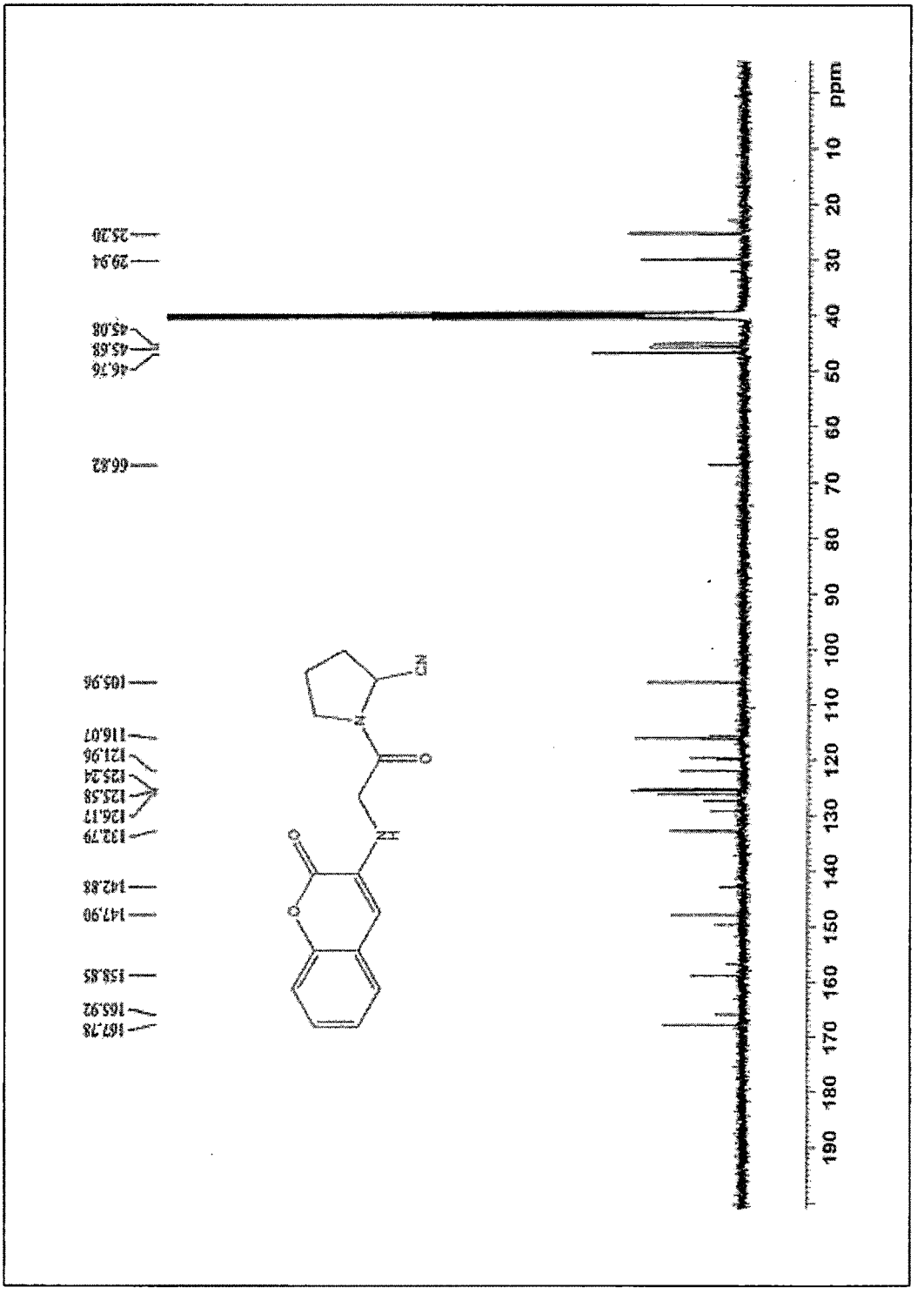


Figure 3.12.3: ^{13}C NMR spectrum of (*S*)-1-(2-(2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile **7b**

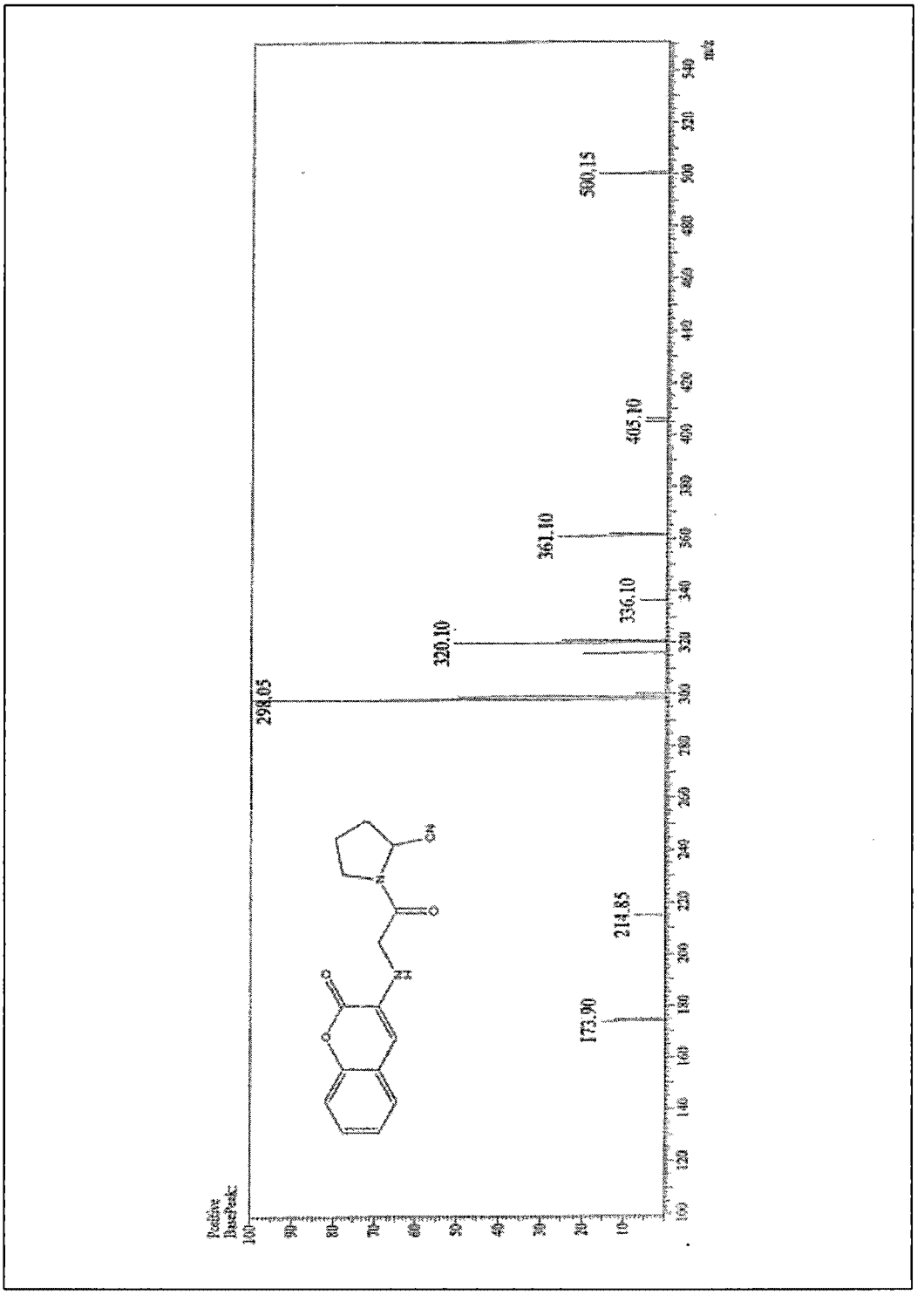


Figure 3.12.4: ESI-MS spectrum of (*S*)-1-(2-(2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile **7b**

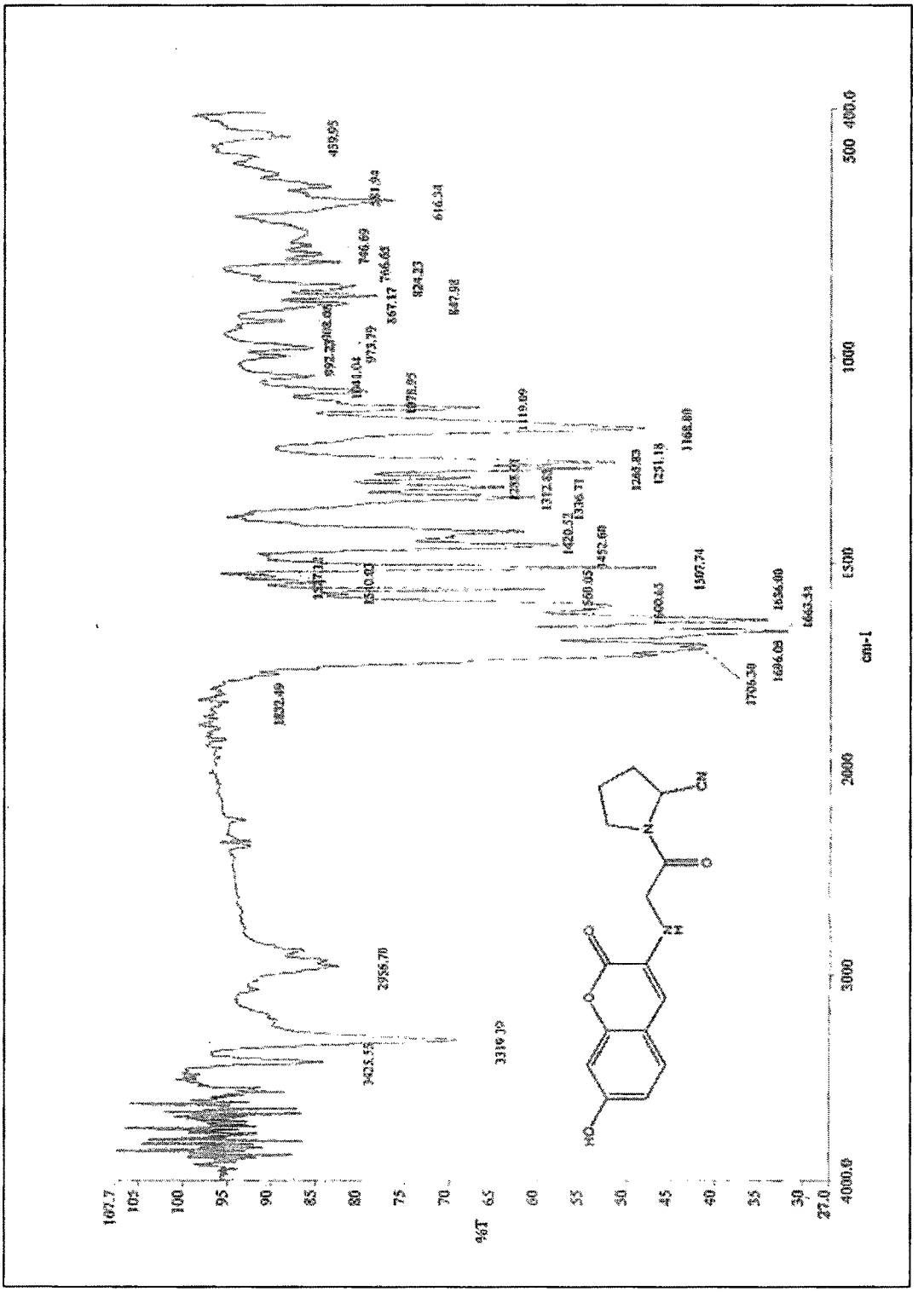


Figure 3.13.1: IR spectrum of (*S*)-1-(2-(7-hydroxy-2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile **7c**

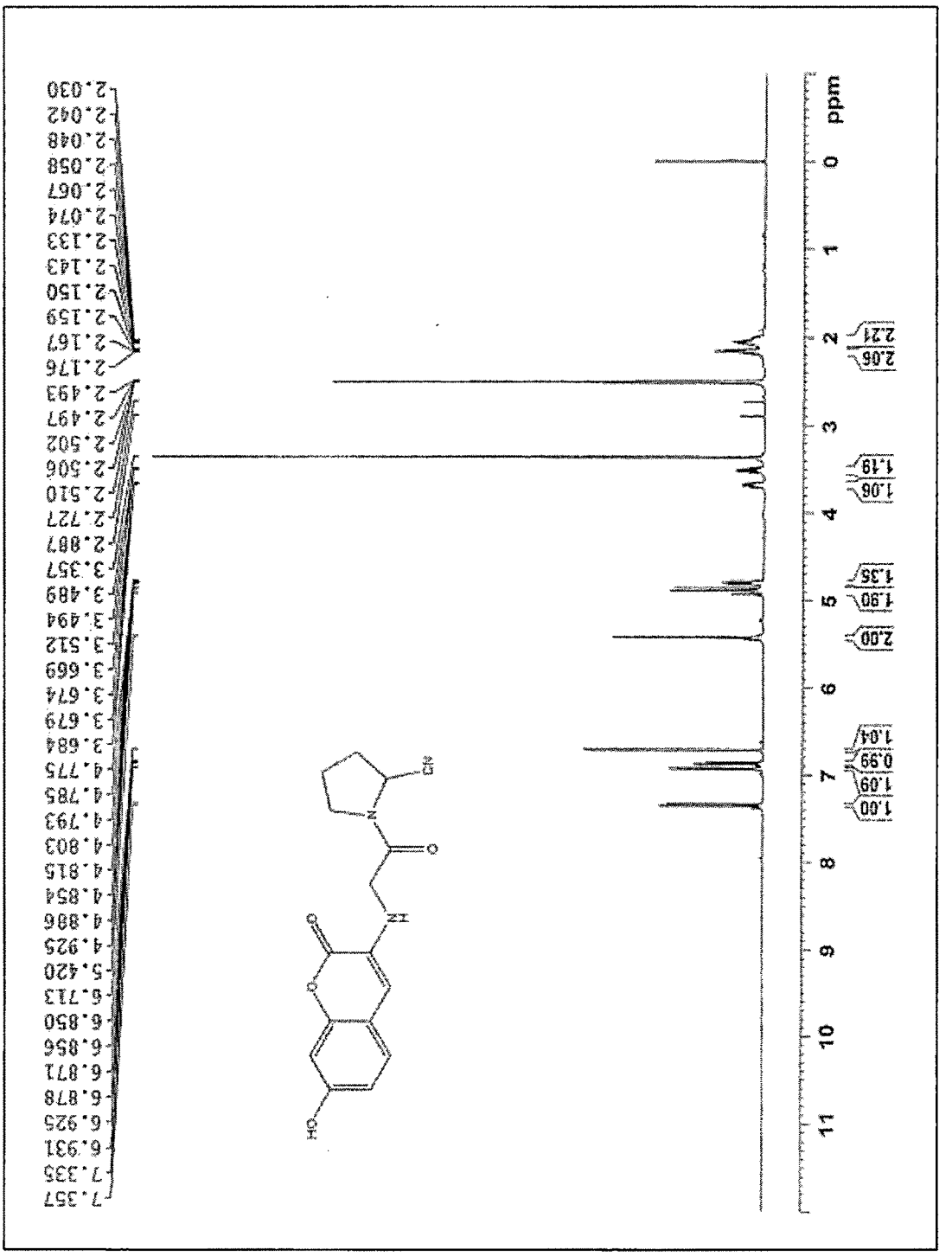


Figure 3.13.2: ^{1}H NMR spectrum of (*S*)-1-(2-(7-hydroxy-2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile **7c**

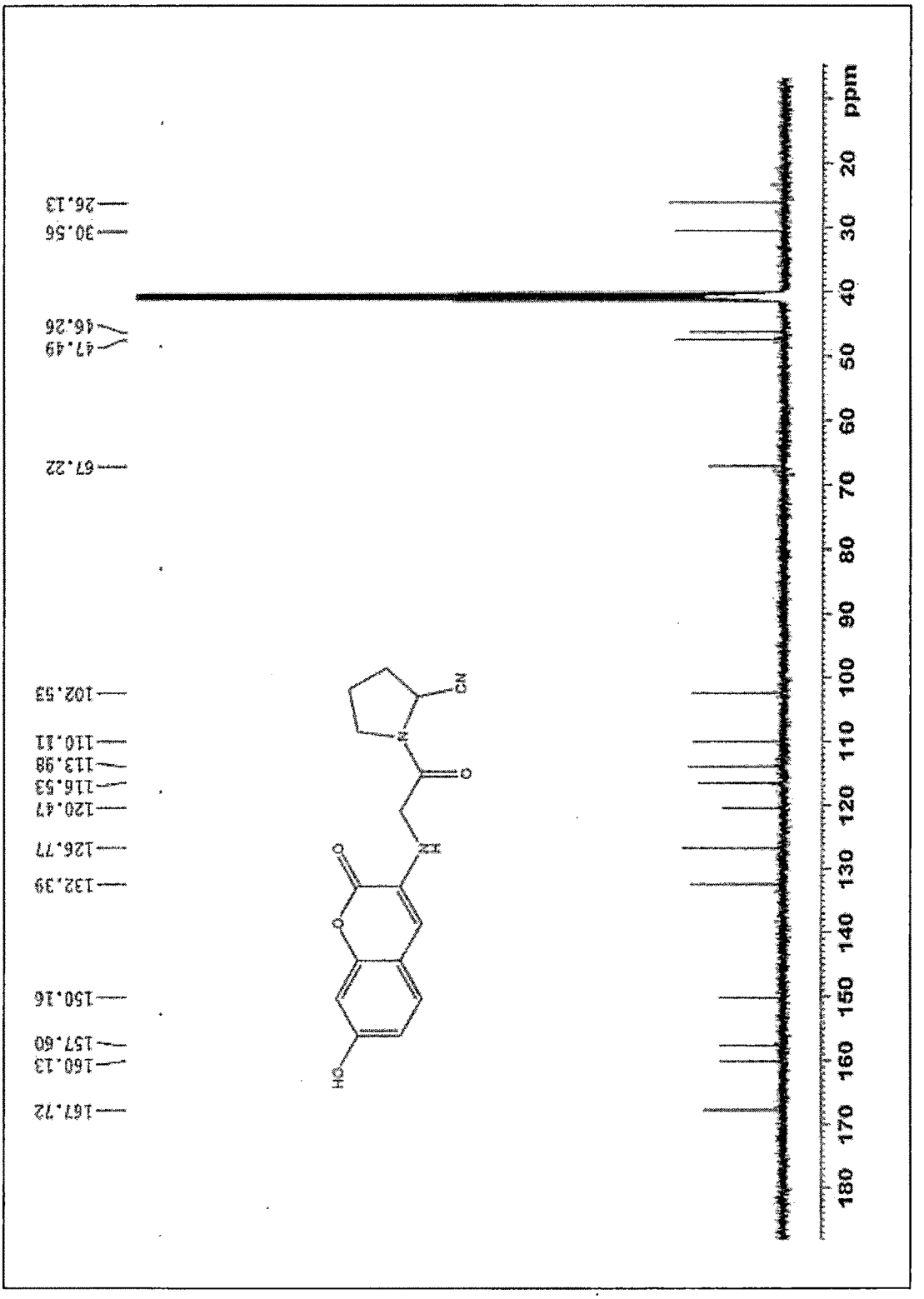


Figure 3.13.3: ^{13}C NMR spectrum of (*S*)-1-(2-(7-hydroxy-2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile **7c**

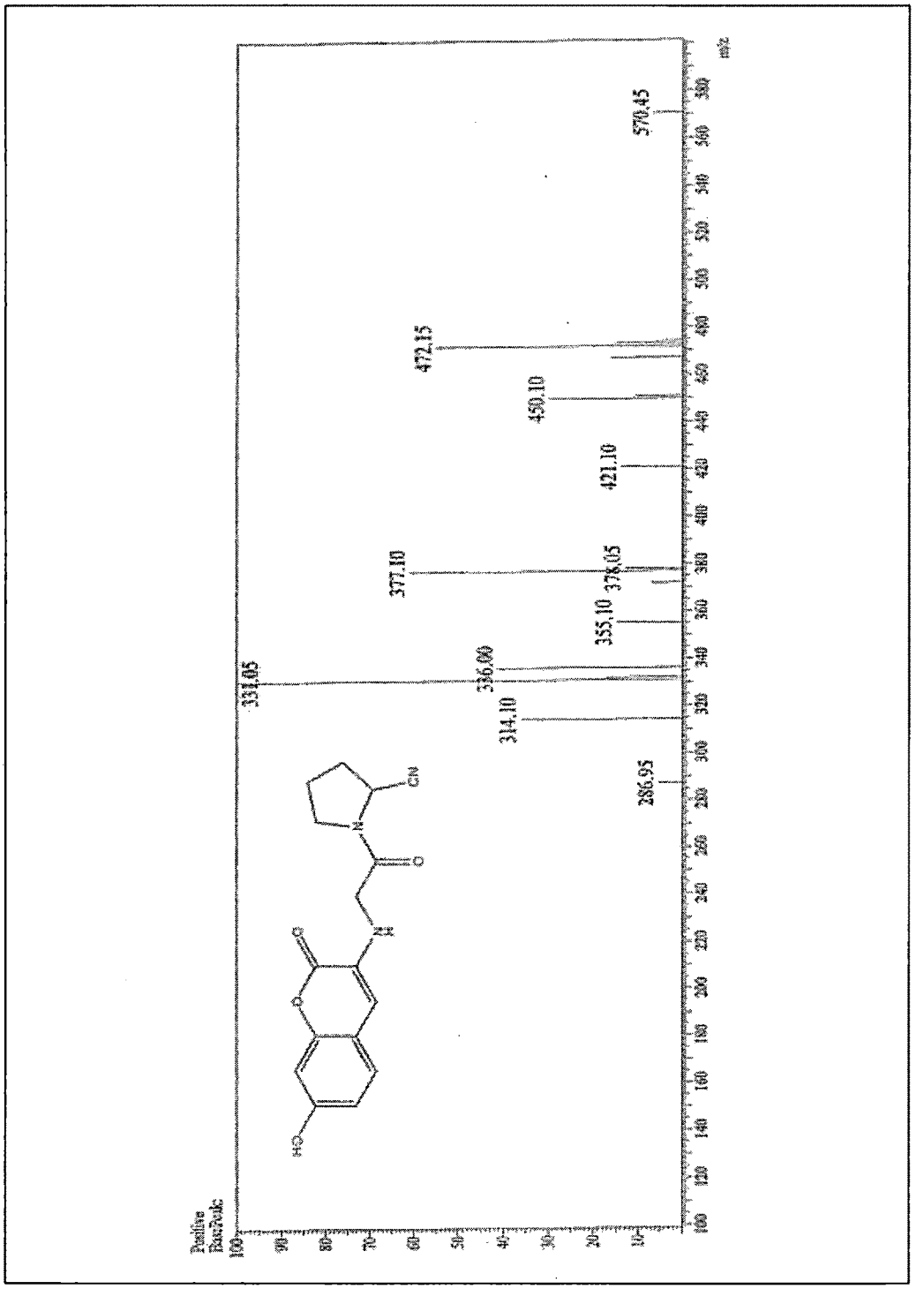


Figure 3.13.4: ESI-MS spectrum of (*S*)-1-(2-(7-hydroxy-2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile **7c**

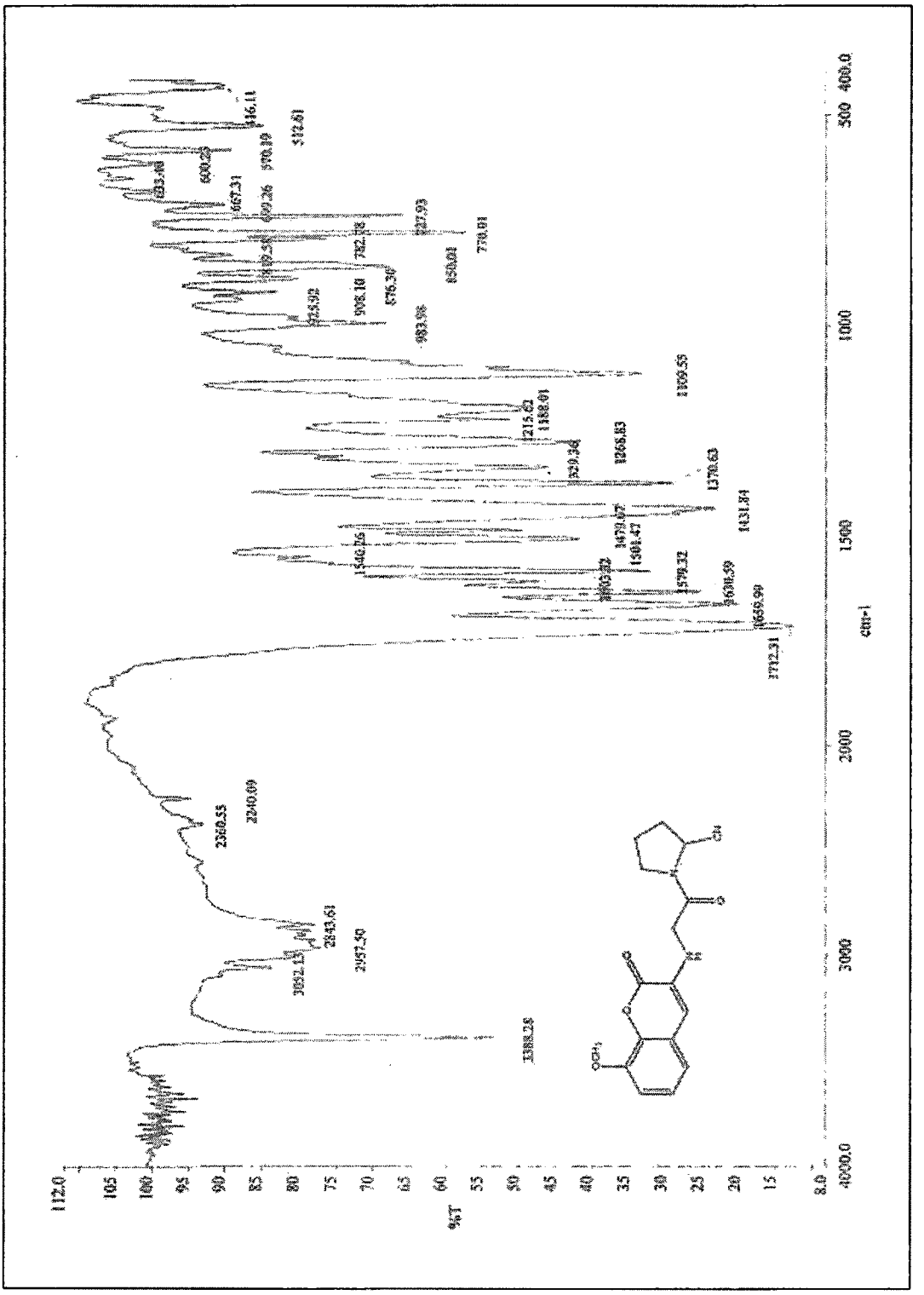


Figure 3.14.1: IR spectrum of (*S*)-1-(2-(8-methoxy-2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile **7d**

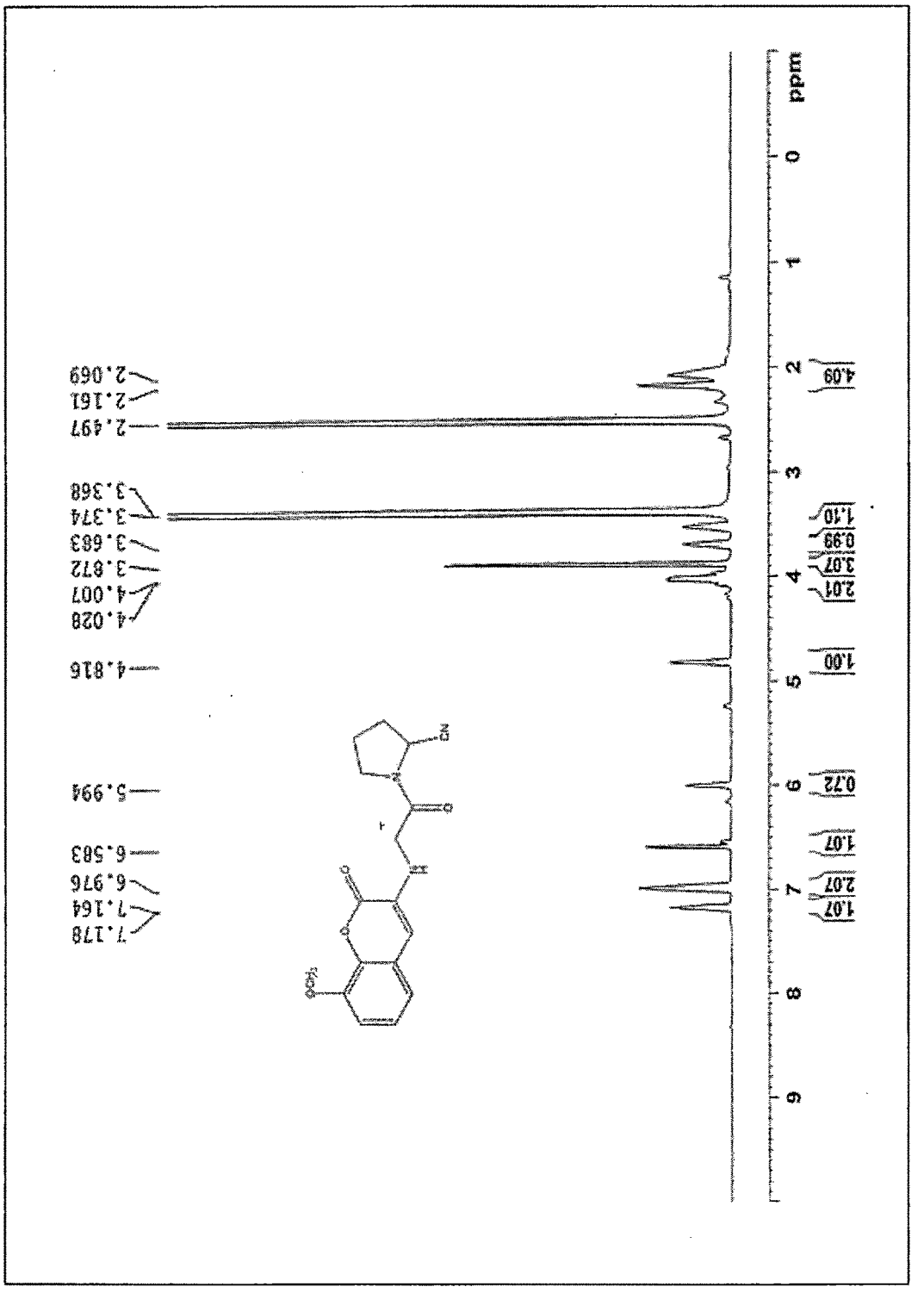


Figure 3.14.2: ^{1}H NMR spectrum of (*S*)-1-(2-(8-methoxy-2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile **7d**

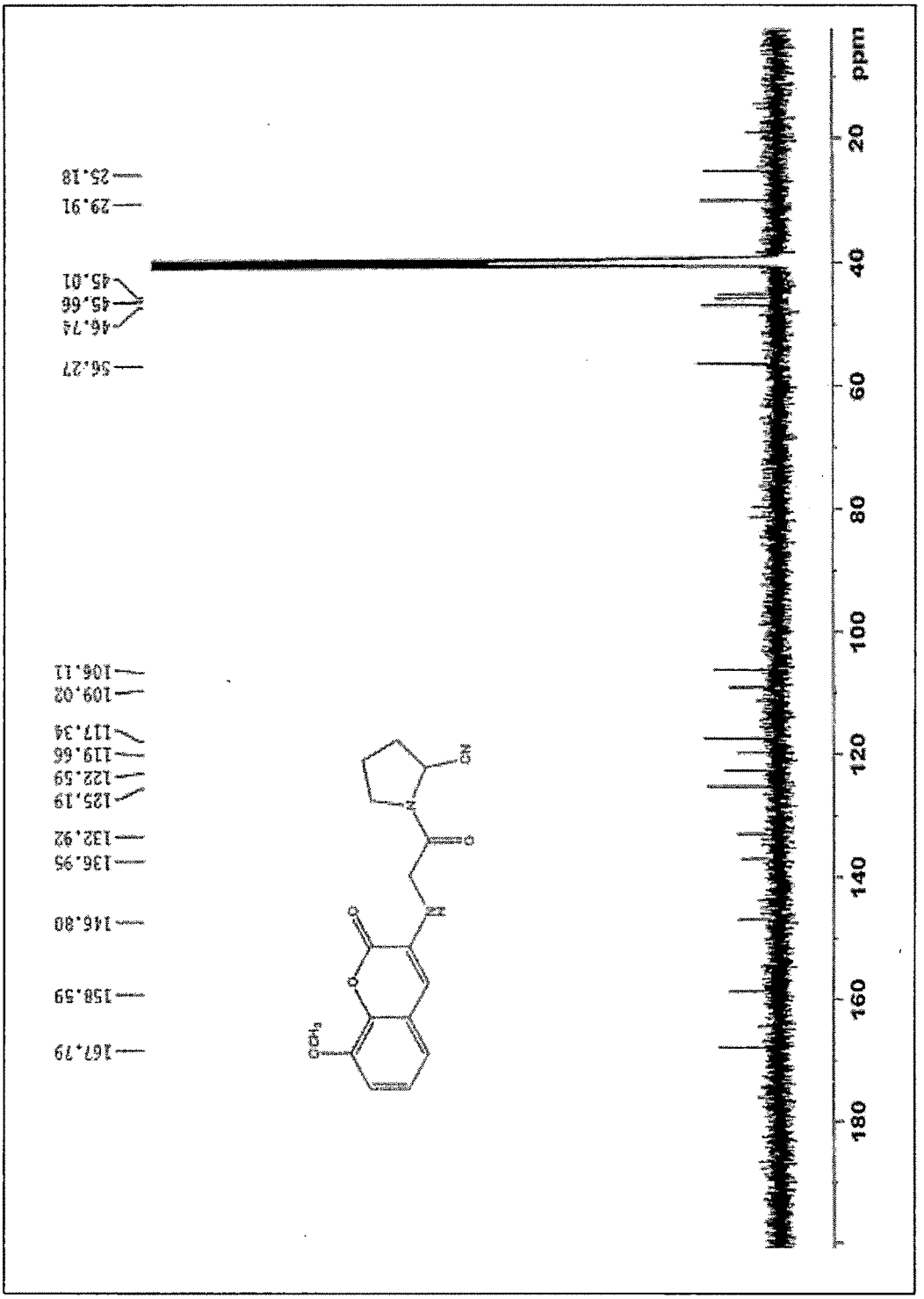


Figure 3.14.3: ^{13}C NMR spectrum of (*S*)-1-(2-(8-methoxy-2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile **7d**

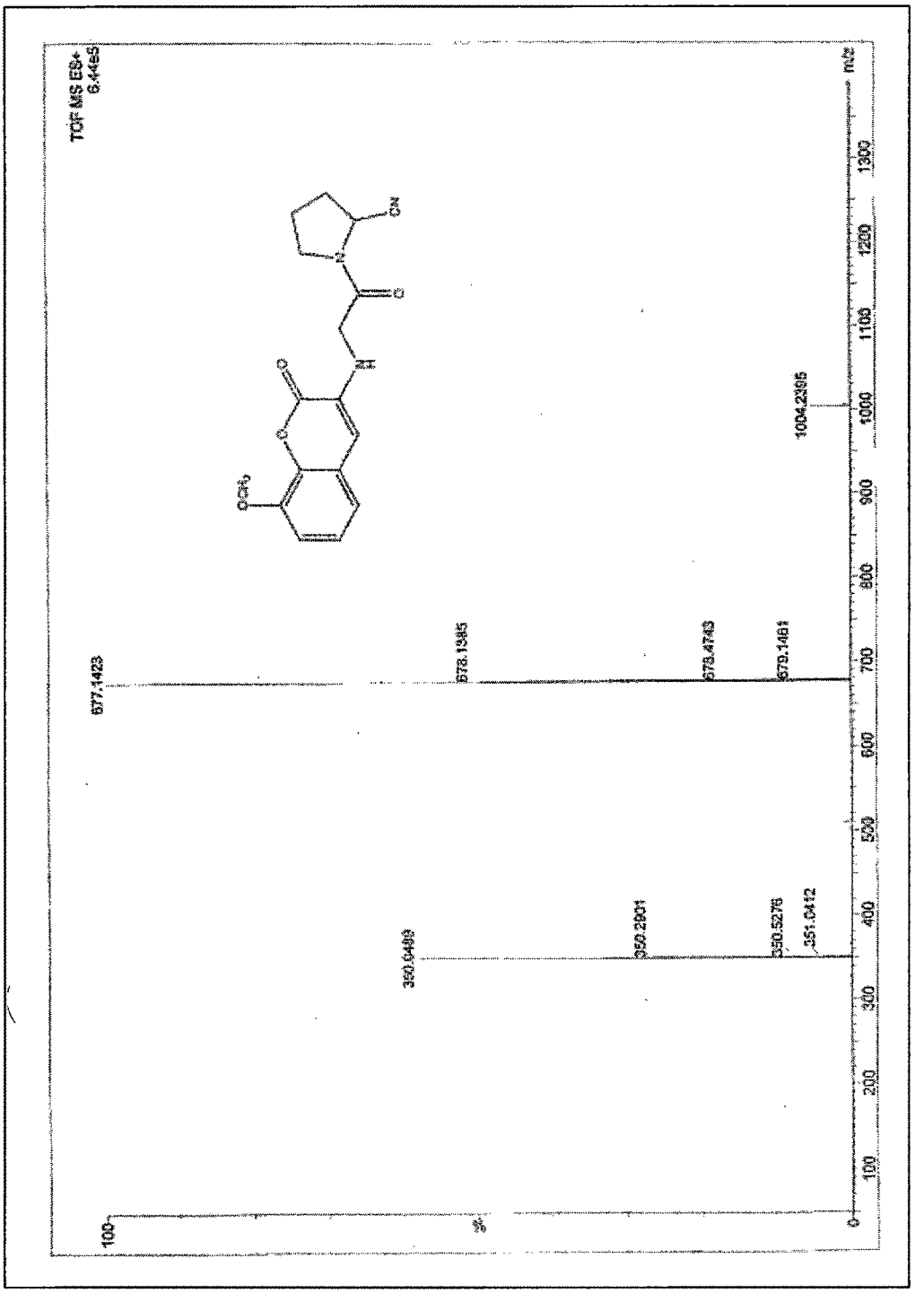


Figure 3.14.4: ESI-MS spectrum of (*S*)-1-(2-(8-methoxy-2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile **7d**

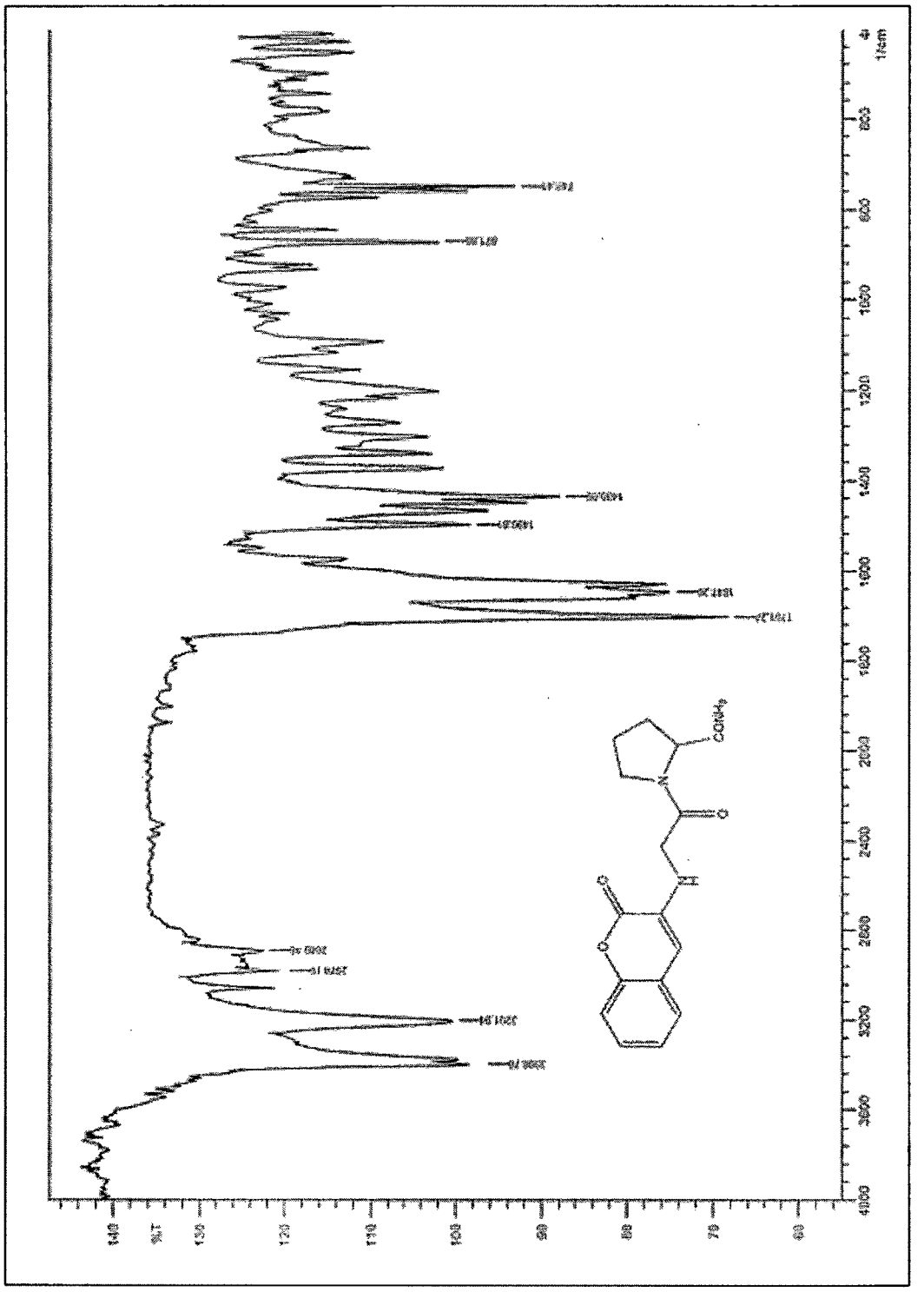


Figure 3.15.1: IR spectrum of (*S*)-1-(2-(2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carboxamide **7e**

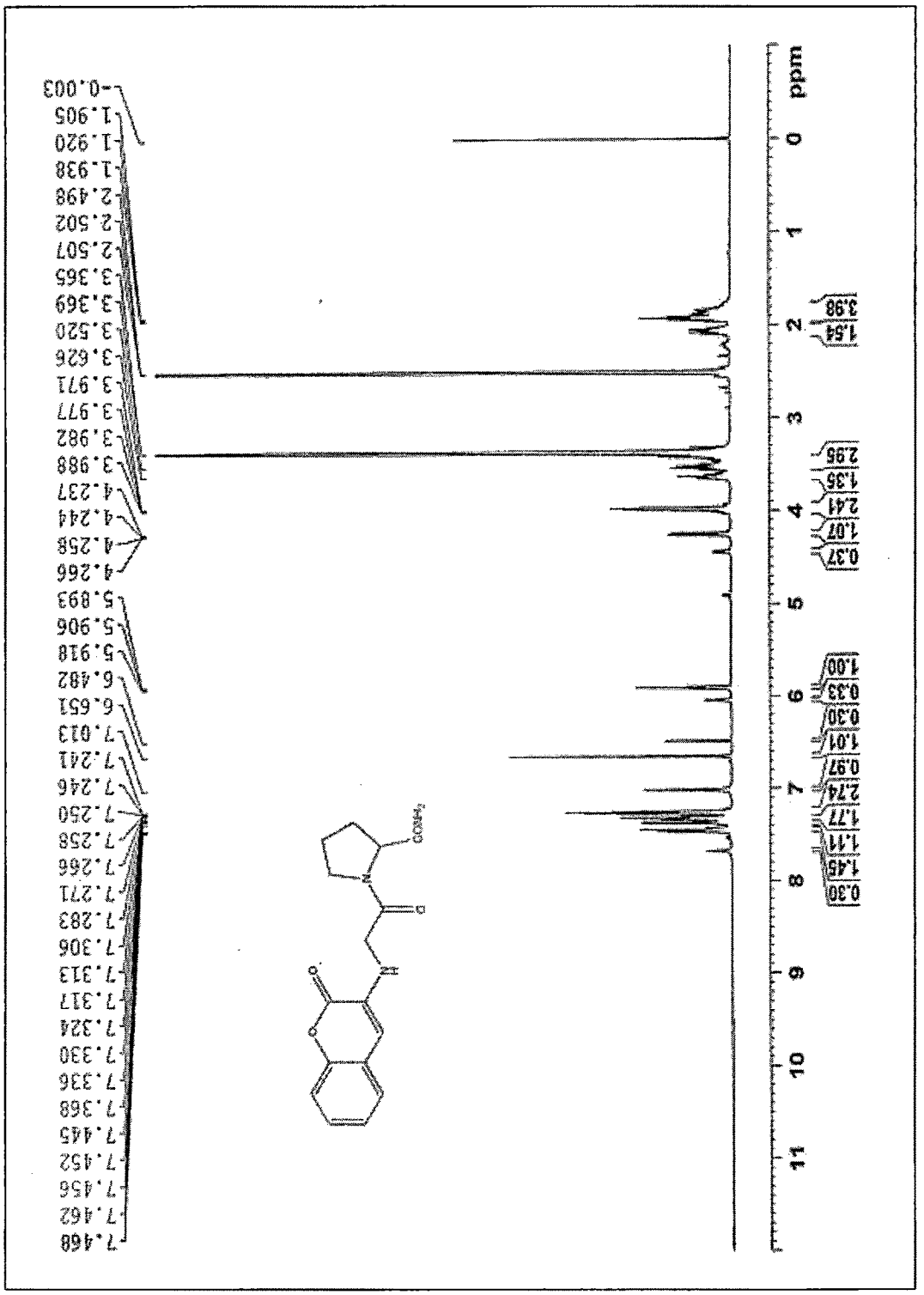


Figure 3.15.2: ^{1}H NMR spectrum of (*S*)-1-(2-(2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carboxamide **7e**

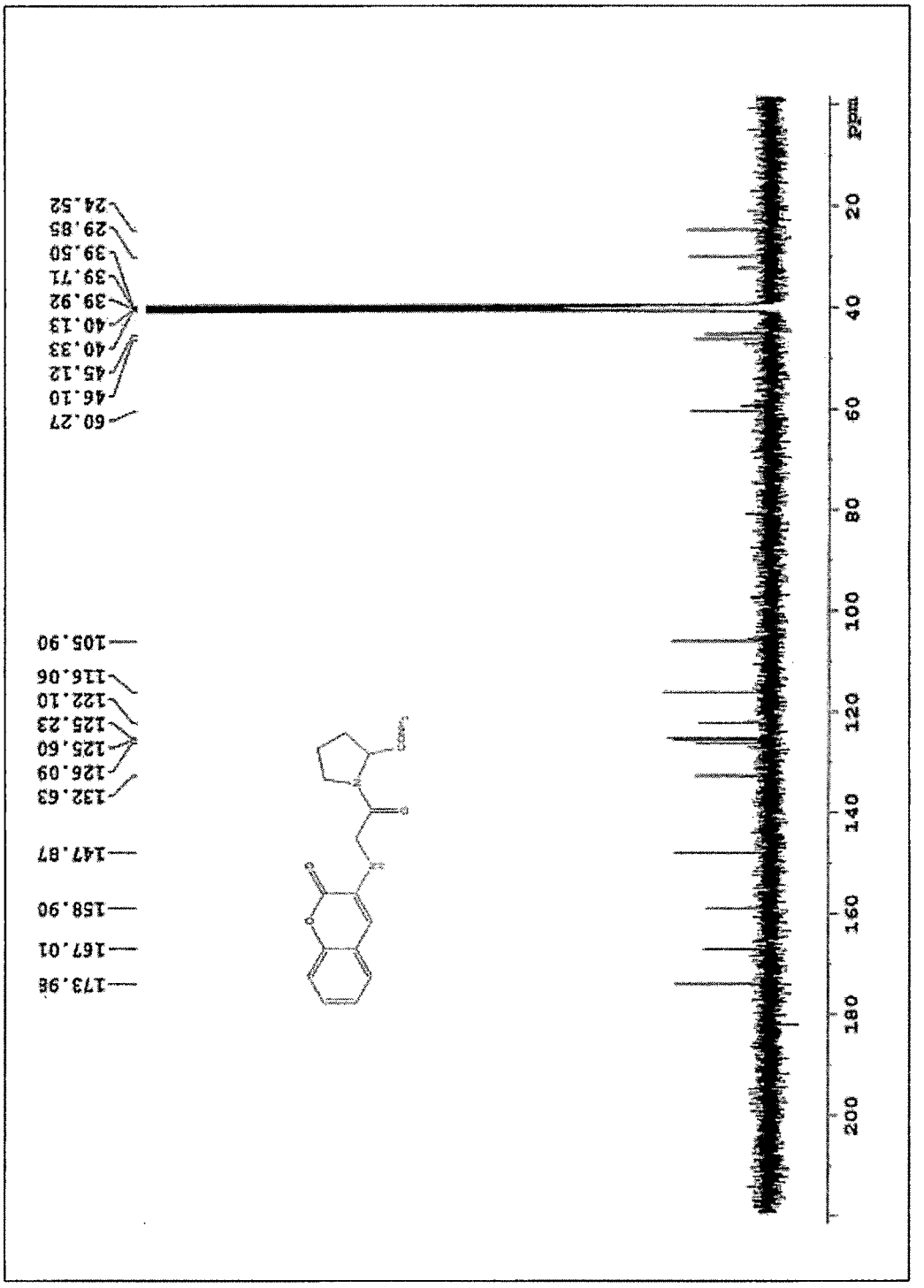


Figure 3.15.3: ^{13}C NMR spectrum of (*S*)-1-(2-(2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carboxamide **7e**

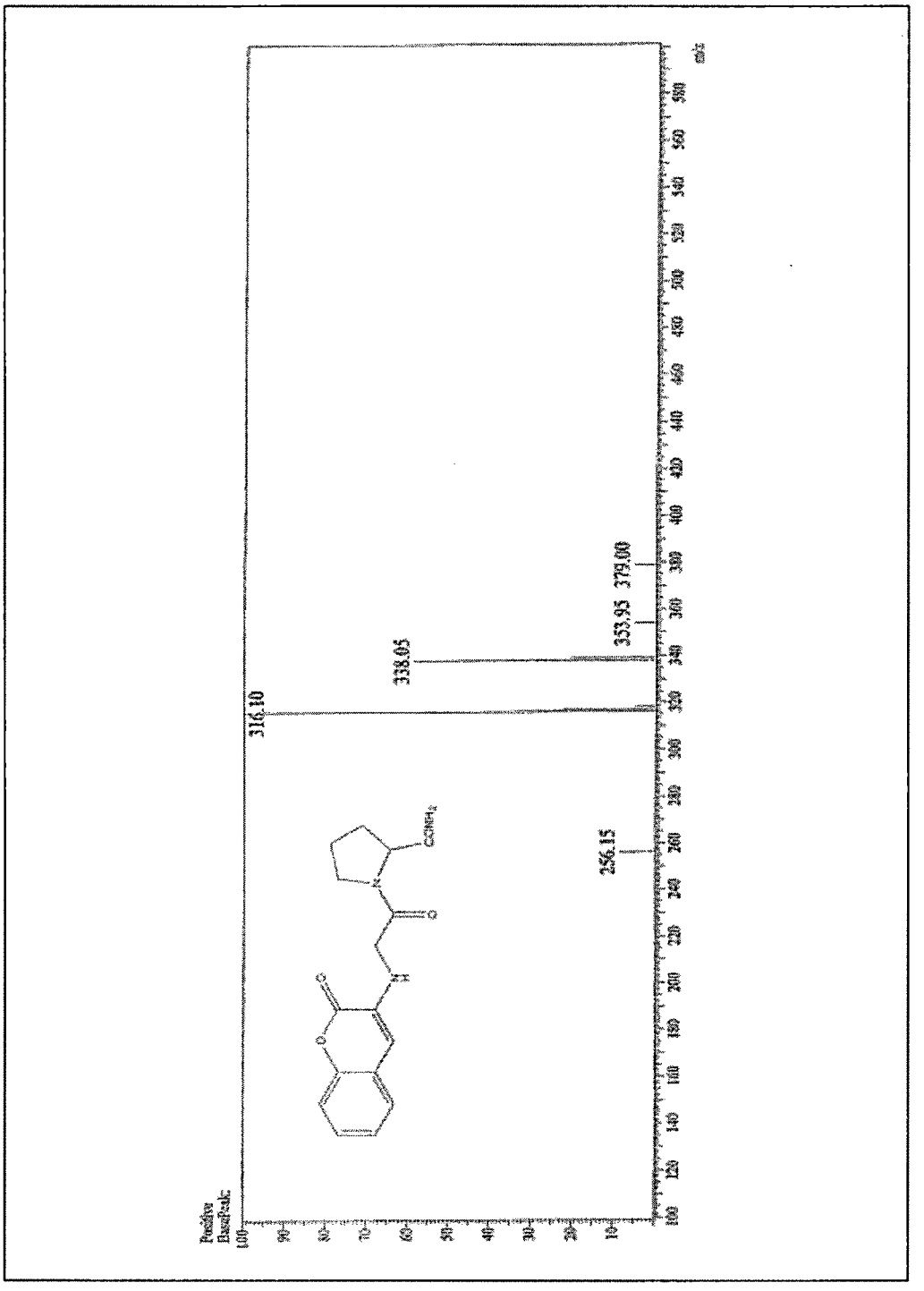


Figure 3.15.4: ESI-MS spectrum of (*S*)-1-(2-(2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carboxamide **7e**

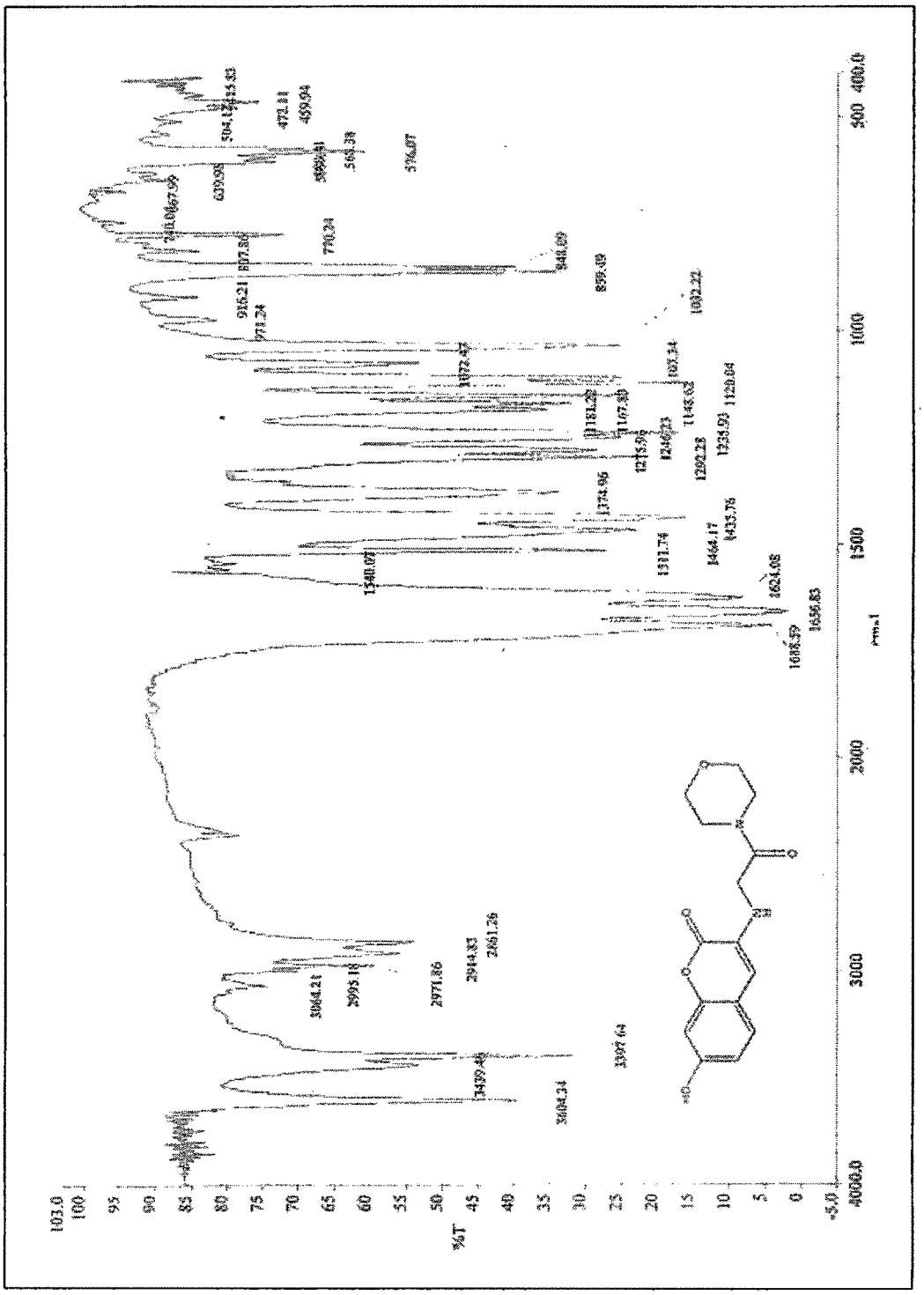


Figure 3.16.1: IR spectrum of 7-hydroxy-3-(2-morpholino-2-oxoethylamino)-2H-chromen-2-one **7g**

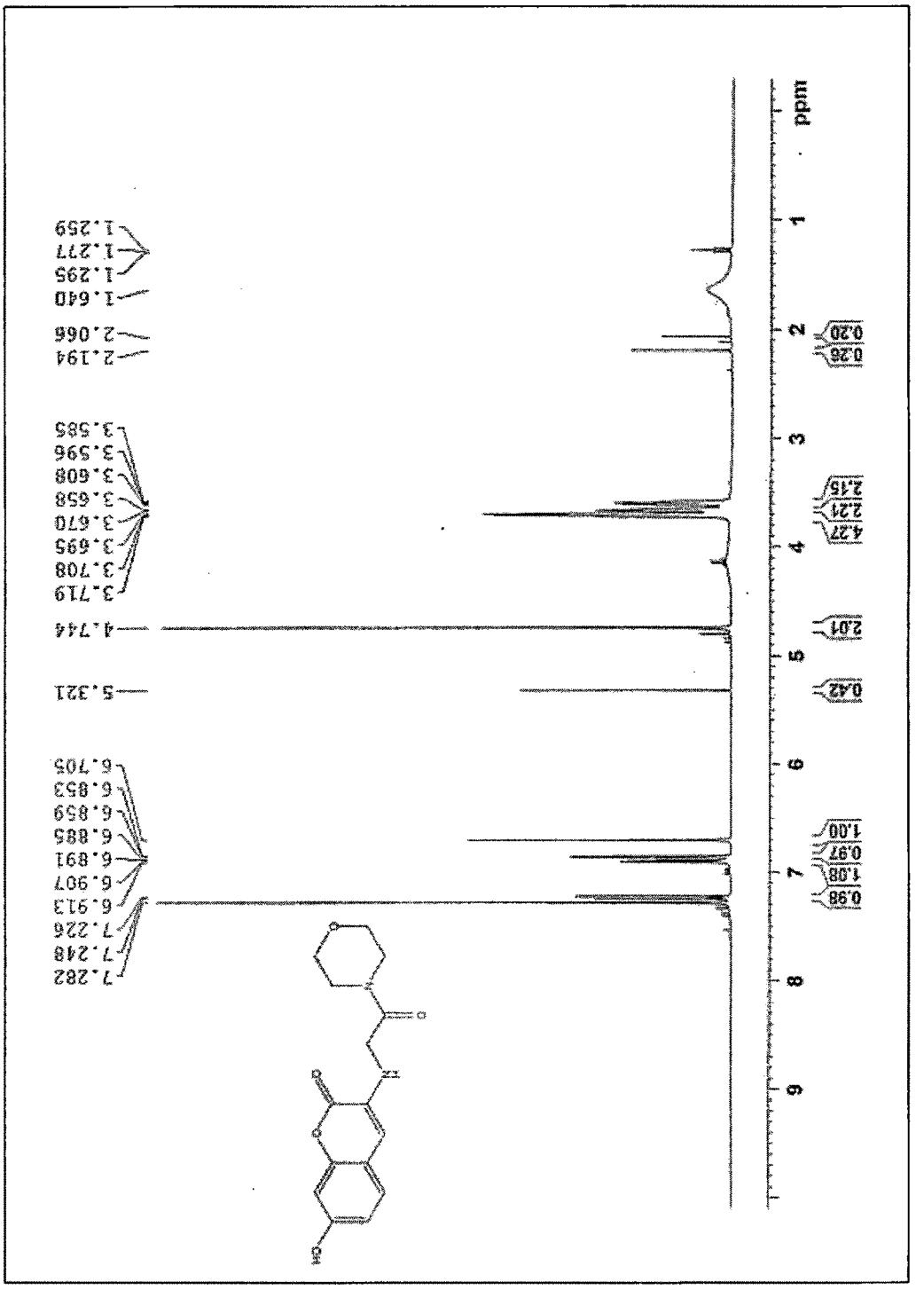
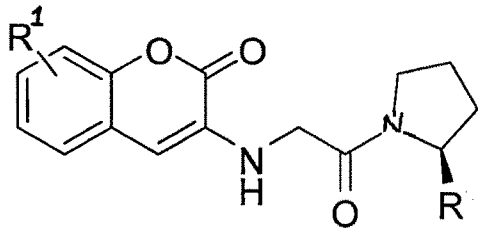


Figure 3.16.2: ^{1}H NMR spectrum of 7-hydroxy-3-(2-morpholino-2-oxoethylamino)-2H-chromen-2-one **7g**

3.2.2 Biological evaluation:

Compounds **7a-g** were then tested for their in-vitro DPP-IV inhibition using human recombinant DPP-IV enzyme procured form Prospec (enz-375-b), substrate : H-Gly-Pro-AMC procured from Enzo life science (Lot No. : 01221304) and assay buffer was prepared in-house consisting TrisHCl (50 mM), EDTA (1mM), sodium chloride (100mM) in deionized water having pH 7.5. DPP-IV inhibition assay uses fluorogenic substrate, Gly-Pro-Aminomethylcoumarin (AMC), to measure DPP-IV activity. DPP-IV activity was measured by mixing reagents in 96-well plate (order of addition of reagents: assay buffer, enzyme, solvent/test sample and finally substrate). Both the enzyme and 96 well-plate were incubated for 30 minutes. Cleavage of the peptide bond by DPP-IV releases the free AMC group, resulting in fluorescence that was analyzed using an excitation wavelength of 360 nm and emission wavelength of 450 nm and % inhibition of the test compounds was calculated as shown in Table 3.1.



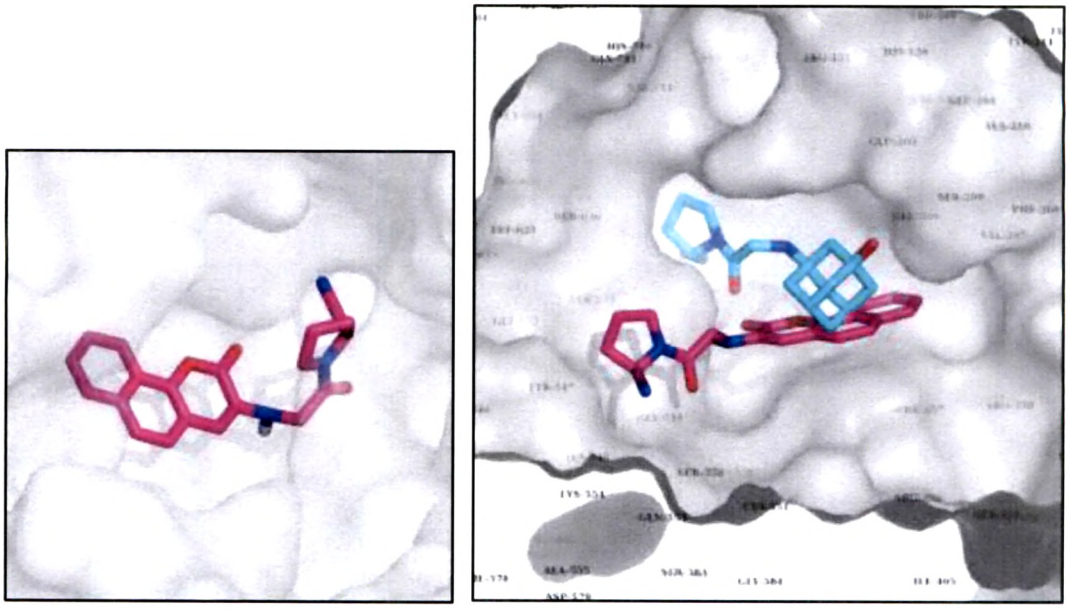
Compound	R^1	R'	% Inhibition of DPP-IV at 10μM
7a	7,8-Ph	CN	84.43
7b	H	CN	36.36
7c	7-OH	CN	34.23
7d	8-OCH_3	CN	17.01
7e	H	$CONH_2$	18.01
7g	7-OH	Instead of proline, morpholine ring substituted.	36.36

Table 3.1: DPP-IV inhibition by compounds **7a-g** at 10μM concentration

Vildagliptin (NVP-LAF237), was used as a standard for the assay with an IC_{50} of 2.9 nM. Amongst all the test samples, compound **7a** was found to be the most potent with an IC_{50} of 3.16 μM. For compounds 7b-g as the % inhibition of DPP-IV were less than 50% at 10 μM conc., their IC_{50} values weren't determined

3.2.3 Docking studies:

AutoDock Vina [15] was used for the docking studies. To perform the docking studies, binding site residues of the A chain of DPP-IV (PDB ID: 3W2T) [16] at a distance of 4.5 Å from vildagliptin were selected. The affinity for the compound **7a** was -8.4 kcal/mol while that of vildagliptin was shown to be -6.7 kcal/mol. LigPlot [17] was used to observe the interaction of the ligand with the binding site residues (Figure 3.18). Pymol [18] was used to visualize the protein and the docked compounds **7a** and **NVP-LAF237** as seen in Figure 3.17.



A B

Figure 3.17: (**A**) Interaction of **7a** at the active site of DPP-IV; (**B**) Comparison of binding of **7a** and **NVP-LAF237** at the active site of DPP-IV.

Figure 3.18.1 shows Ligplot of vildagliptin (**NVP-LAF237**) while 3.18.2 shows the Ligplot of **7a**.

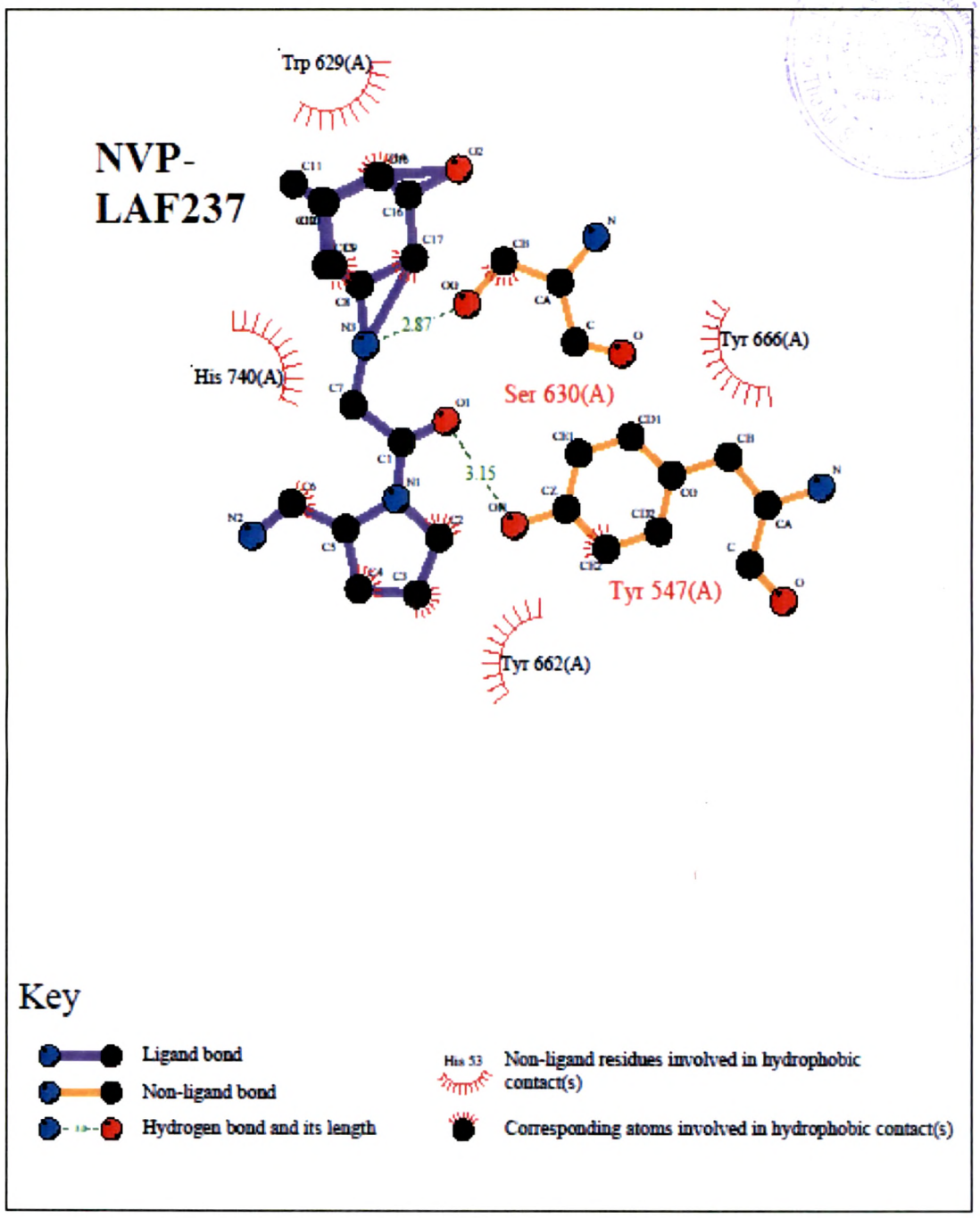


Figure 3.18.1: Ligplot of **NVP-LAF237** (vildagliptin).

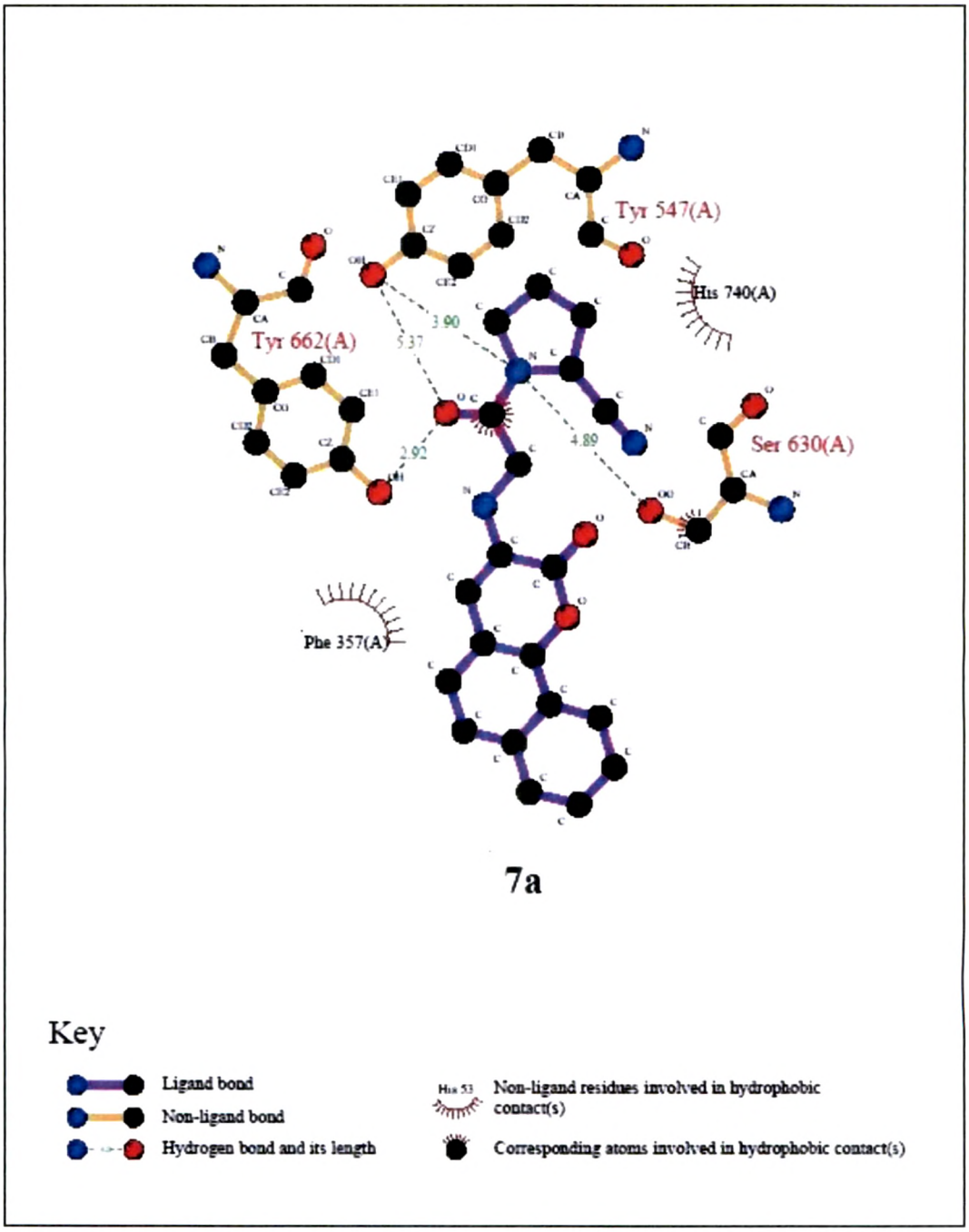


Figure 3.18.2: Ligplot of 1-(2-(2-oxo-2H-benzo[h]chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile **7a**.

3.3 Conclusion

While pharmacophore (cyanopyrrolidine) remained constant, it was expected that 3-amino-7-hydroxy-2*H*-chromen-2-one derivative **7c,** mimicking hydroxyl adamantyl group would be most potent molecule in the series but to our surprise it was observed that substituted 3-aminocoumarin derivatives **7c**, **7d** were less potent than the non-substituted derivative **7b** as seen in table 3.1. Yet the most ste-rically rigid, 3-aminocoumarin derivative **7a** was found to be the most potent of all the molecules synthesised in the series with an IC_{50} of 3.16 μM.

Replacement of hydroxyl adamantyl amine group in vildagliptin by various substituted 3-aminocoumarins does not lead to greater enzyme inhibition which can be attributed to different interaction at the binding site of DPP-IV, as can be seen in Figure 3.17 wherein binding interactions of **7a** and **NVP-LAF237** can be seen.

Hence, it can be concluded that more ste-rically rigid substituent at the P2 site does lead to better DPP-IV inhibition.

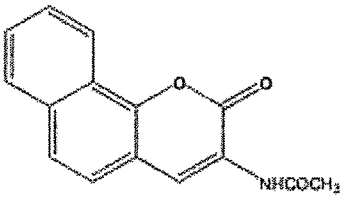
3.4 Experimental

Reagent grade chemicals and solvents were purchased from commercial supplier and used after purification. TLC was performed on silica gel F254 plates (Merck). Acme's silica gel (60-120 mesh) was used for column chromatographic purification. Melting points are uncorrected and were measured in open capillary tubes, using a Rolex melting point apparatus. IR spectra were recorded as KBr pellets on Perkin Elmer RX 1 spectrometer. ^{1}H NMR and ^{13}C NMR spectral data were recorded on Advance Bruker 400 spectrometer (400 MHz) with $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal standard. *J* values are in Hz. Mass spectra were determined by ESI-MS, using a Shimadzu LCMS 2020 apparatus. All reactions were carried out under nitrogen atmosphere. Methanol was used as solvent for determining SOR using AKruss Optronic instrument.

General method for the synthesis of compounds 2a-d:

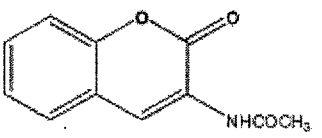
To a stirring solution of substituted salicylaldehyde **1a-d** (1.0 mmol) in acetic anhydride (5.0 mmol) N-acetylglycine (1.0 mmol) and sodium acetate (4.0 mmol) were added and the resulting mixture was heated at 100-110 °C for 7 hours or till the completion of reaction as monitored by TLC. On completion of reaction it was cooled to room temperature, water (20 mL) was added and the resulting solid was filtered, dried and then recrystallized from absolute ethanol to give the product as crystalline solid.

***N-(2-oxo-2H-benzo[h]chromen-3-yl)acetamide-acetyl-2H-benzo[h]chromen-2-one* 2a:**



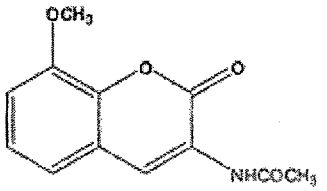
Yield: 35 %; pale pink solid; m.p.: 238-240 °C; IR (KBr): 3340, 3105, 3059, 1709, 1676, 1578, 1547, 1254, 1142, 804 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.32 (s, 3H), 7.47 (d, 1H, J = 9.2 Hz), 7.58-7.62 (m, 1H), 7.68-7.73 (m, 1H), 7.92 (d, 2H, J = 8.8 Hz), 8.20 (s, 1H), 8.34 (d, 1H, J = 8.4 Hz), 9.53 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 24.89, 114.23, 116.35, 119.52, 122.40, 123.98, 126.25, 127.92, 128.81, 129.06, 130.71, 130.89, 148.73, 158.72, 169.56; $C_{15}H_{11}NO_3$; ESI-MS: *m/z* 276.0 $[M+Na]^+$.

***N-(2-oxo-2H-chromen-3-yl)acetamide* 2b:**



Yield: 32%; white solid; m.p.: 198-200 °C (Lit.[19] 201.5 °C, Lit.[20] 206 °C); IR (KBr): 3331, 3080, 3044, 2934, 2322, 1709, 1682, 1605, 1530, 1450, 1360, 1294, 1250, 1179, 1146, 897, 766, 708, 600 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.27 (s, 3H), 7.30-7.36 (m, 2H), 7.45-7.49 (m, 1H), 7.54 (dd, 1H, J_1 = 7.6 Hz, J_2 = 1.2 Hz), 8.11 (s, 1H), 8.70 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 24.79, 116.37, 119.82, 123.31, 123.96, 125.22, 127.83, 129.69, 140.04, 149.87, 158.81, 169.42; $C_{11}H_9NO_3$; ESI-MS: *m/z* 203.7 $[M+H]^+$.

***N-(8-methoxy-2-oxo-2H-chromen-3-yl)acetamide* 2d:**

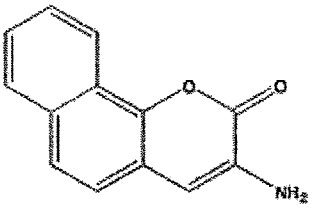


Yield: 41%; yellow solid; m.p.: 240-242 °C (Lit.[21] 237-239 °C); IR (KBr): 3337, 3098, 3001, 2969, 2943, 2845, 1713, 1684, 1609, 1580, 1535, 1479, 1460, 1383, 1360, 1250, 1184, 1146, 1105, 1074, 1015, 980, 781, 714, 517 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.24 (s, 3H), 3.96 (s, 3H), 6.99-7.27 (m, 3H), 8.12 (s, 1H), 8.65 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 24.75, 56.19, 111.64, 119.31, 120.56, 123.33, 124.22, 125.07, 139.35, 146.95, 158.33, 169.45; $C_{12}H_{11}NO_4$; ESI-MS: *m/z* 256.01 $[M+Na]^+$.

General method for the synthesis of compounds 3a-d:

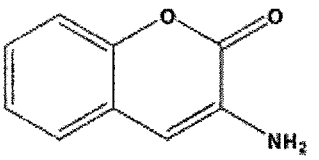
To a solution of compound **5a-d** (1.0 mmol) in methanol (10 mL), conc. HCl (0.5 mL) was added and the resulting solution was refluxed for an hour. On completion of reaction it was cooled to room temperature, concentrated to a small volume and then neutralized with saturated sodium bicarbonate solution to yield crude compound which on purification by column chromatography using silica gel as stationary phase and ethylacetate : petroleum ether (15:85) as the eluent gave pure crystalline product.

***3-amino-2H-benzo[h]chromen-2-one* 3a:**



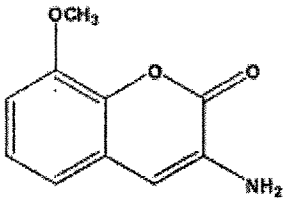
Yield: 50%; yellow solid; m.p.: 150-152 °C; IR (KBr): 3448, 3366, 2919, 1713, 1630, 1592, 1556, 1515, 1244, 1164, 856, 812 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 4.44 (s, 2H), 7.44 (d, 1H, J = 8.8 Hz), 7.49 (s, 1H), 7.52-7.56 (m, 1H), 7.60-7.64 (m, 1H), 7.74 (d, 1H, J = 9.2 Hz), 7.88-7.90 (m, 1H), 8.14 (d, 1H, J = 8.4 Hz); ^{13}C NMR (400 MHz, $CDCl_3$): δ 107.48, 115.38, 116.55, 121.86, 125.66, 126.96, 127.44, 128.08, 128.81, 130.70, 132.05, 147.02, 159.36; $C_{13}H_9NO_2$; ESI-MS: *m/z* 211.9 $[M+H]^+$.

***3-amino-2H-chromen-2-one* 3b:**



Yield: 45%; pale yellow solid; m.p.: 128-130 °C (Lit.[20] 132-135 °C); IR (KBr): 3432, 3366, 2921, 2362, 1708, 1636, 1590, 1560, 1459, 1161, 889, 770, 741 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 4.29 (s, 2H), 6.73 (s, 1H), 7.20-7.33 (m, 4H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 110.92, 116.18, 121.17, 124.64, 125.09, 126.65, 131.97, 149.03, 159.46; $C_9H_7NO_2$; ESI-MS: *m/z* 184.0 $[M+Na]^+$.

***3-amino-8-methoxy-2H-chromen-2-one* 3d:**

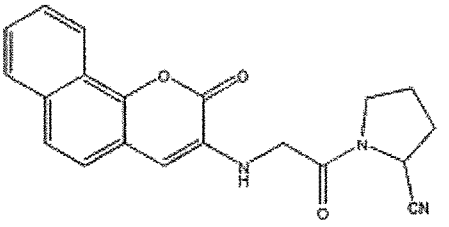


Yield: 52%; yellow solid; m.p.: 116-118 °C (Lit.[22] 124-126 °C); IR (KBr): 3455, 3360, 2946, 2849, 1715, 1623, 1615, 1570, 1483, 1332, 1277, 1159, 1105, 979, 882, 766 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.93 (s, 3H), 4.34 (s, 2H), 6.70 (s, 1H), 6.83-6.89 (m, 2H), 7.13 (t, 1H, J = 8.0 Hz); ^{13}C NMR (400 MHz, $CDCl_3$): δ 56.09, 108.94, 110.93, 116.98, 121.98, 124.50, 132.30, 138.39, 146.92, 158.99; $C_{10}H_9NO_3$; ESI-MS: *m/z* 191.95 $[M+H]^+$.

General method for the synthesis of compounds 7a-g:

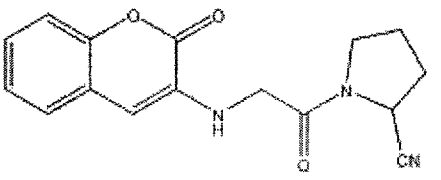
A mixture of compound **3a-d** (1.0 mmol), compound **5** or **6** (1.1 mmol) and anhydrous potassium carbonate (5.0 mmol) in dimethyl formamide (DMF) (5.0 mL) was heated at 80-85 °C for about four hours. On completion of reaction, as monitored on TLC the reaction mixture was poured onto ice cold water and stirred. The resulting solid that separated out was then filtered and dried to yield crude product which was purified by column chromatography using silica gel as stationary phase and ethylacetate : petroleum ether (70:30) as the eluent to give pure product.

***(S)-1-(2-(2-oxo-2H-benzo[h]chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile* 7a:**



Yield: 10%; pale brown solid; $[\alpha]_D$ = -272.11; m.p.: 115-117 °C; IR (KBr): 3400, 2957, 2523, 1709, 1653, 1555, 1515, 1199, 808, 778 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.28-2.41 (m, 4H), 3.63-3.65 (m, 1H), 3.78-3.79 (m, 1H), 4.01 (d, 2H, *J* = 4.4 Hz), 4.89-4.91 (m, 1H), 5.90-5.93 (m, 1H), 6.99 (s, 1H), 7.36-7.38 (m, 1H), 7.52-7.68 (m, 3H), 7.85 (d, 1H, *J* = 8.0 Hz), 8.18 (d, 1H, *J* = 8.4 Hz); ^{13}C NMR (400 MHz, $CDCl_3$): δ 25.15, 29.91, 45.44, 45.66, 46.82, 102.14, 115.33, 116.46, 118.07, 121.87, 125.56, 125.64, 126.76, 126.91, 127.09, 128.05, 128.80, 130.70, 132.04, 145.97, 158.86, 166.75; $C_{20}H_{17}N_3O_3$; ESI-MS: *m/z* 348.05 $[M+H]^+$.

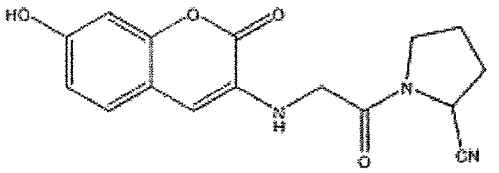
***(S)-1-(2-(2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile* 7b:**



Yield: 20%; pale yellow solid; $[\alpha]_D$ = -92.93; m.p.: 236-238 °C; IR (KBr): 3406, 3063, 2880, 1712, 1660, 1627, 1432, 1193, 844, 762 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.05-2.20 (m, 4H), 3.50-3.56 (m, 1H), 3.67-3.73 (m, 1H), 3.97-4.01 (m, 2H), 4.80-4.84 (m, 1H), 6.00 (t, 1H, *J* = 5.2 Hz), 6.62 (s, 1H), 7.22-7.46 (m, 4H); ^{13}C NMR (400 MHz,

DMSO-d_6): δ 25.20, 29.94, 45.08, 45.68, 46.76, 66.82, 105.96, 116.07, 121.96, 125.24, 125.58, 126.17, 132.79, 142.88, 147.90, 158.85, 165.92, 167.78; $C_{16}H_{15}N_3O_3$; ESI-MS: *m/z* 298.05 $[M+H]^+$.

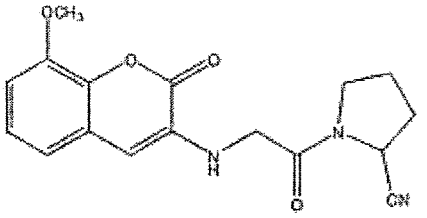
***(S)-1-(2-(7-hydroxy-2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile* 7c:**



Yield: 25%; yellow solid; $[\alpha]_D$ = -147.53; m.p.: 158-160 °C; IR (KBr): 3426, 3319, 2957, 1706, 1696, 1664, 1636, 1508, 1251, 1169, 1119, 848, 824, 616 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.00-2.08 (m, 2H), 2.13-2.18 (m, 2H), 3.47-3.53 (m, 1H), 3.65-3.70 (m, 1H), 4.78-4.82 (m, 1H), 4.85-4.93 (m, 2H), 5.42 (s, 2H), 6.71 (s, 1H), 6.86 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.8 Hz), 6.93 (d, 1H, J = 2.4 Hz), 7.35 (d, 1H, J = 8.8 Hz); ^{13}C NMR (400 MHz, DMSO-d_6): δ 26.13, 30.56, 46.26, 47.49, 67.22, 102.53, 110.11, 113.98, 116.53, 120.47, 126.77, 132.39, 150.16, 157.60, 160.13, 167.72; $C_{16}H_{15}N_3O_4$; ESI-MS: *m/z* 331.05 $[M+NH_4]^+$.

(S)-1-(2-(8-methoxy-2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carbonitrile

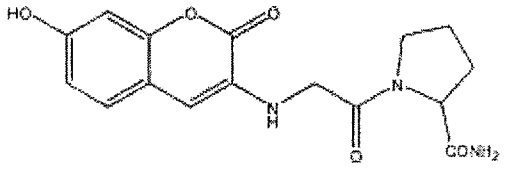
7d:



Yield: 25%; pale yellow solid; $[\alpha]_D$ = -98.80; m.p.: 205-207 °C; IR (KBr): 3388, 3052, 2958, 2844, 1712, 1660, 1631, 1579, 1432, 1371, 1329, 1269, 1110, 984, 850, 770, 728 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.07-2.16 (m, 4H), 3.50 (s, 1H), 3.68 (s, 1H), 3.87 (s, 3H), 4.01-4.03 (m, 2H), 4.82 (m, 1H), 5.99 (s, 1H), 6.58 (s, 1H), 6.98-7.18 (m, 3H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 25.18, 29.91, 45.01, 45.66, 46.74, 56.27, 106.11, 109.02, 117.34, 119.66, 122.59, 125.19, 132.92, 136.95, 146.80, 158.59, 167.79; $C_{17}H_{17}N_3O_4$; ESI-MS: *m/z* 350.05 $[M+Na]^+$.

(S)-1-(2-(2-oxo-2H-chromen-3-ylamino)acetyl)pyrrolidine-2-carboxamide ***7e***

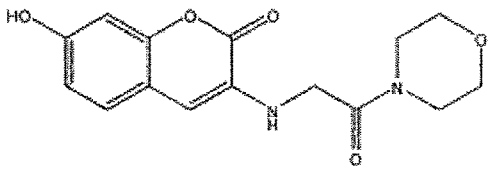
(exists as a mixture of rotamers (3:1):



Yield: 28%; white solid; m.p.: 262-264 °C; IR (KBr): 3397, 3395, 3202, 3053, 2978, 2889, 1701, 1647, 1628, 1466, 1447, 1435, 1373, 1339, 1302, 1269, 1202, 872, 758, 748 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.84-2.10 (m, 4H), 3.47-3.56 (m, 1H), 3.59-3.67 (m, 1H), 3.97-3.99 (m, 2H), 4.24-4.27 (m, 0.74H), 4.40-4.45 (m, 0.26H), 5.91 (t, 0.75H, *J* = 5.2 Hz), 6.04 (t, 0.25H, *J* = 5.2 Hz), 6.48 (s, 0.23H), 6.65 (s, 0.77H), 7.01 (s, 1H), 7.23-

7.47 (m, 4H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 24.52, 29.85, 45.12, 46.10, 60.27, 105.90, 116.06, 122.10, 125.23, 125.60, 126.09, 132.63, 147.87, 158.90, 167.01, 173.98; $C_{16}H_{17}N_3O_4$; ESI-MS: *m/z* 316.10 $[M+H]^+$.

***7-hydroxy-3-(2-morpholino-2-oxoethylamino)-2H-chromen-2-one* 7g:**



Yield: 20%; yellow solid; m.p.: 164-166 °C; IR (KBr): 3604, 3398, 2972, 2915, 2861, 1689, 1657, 1624, 1512, 1464, 1436, 1375, 1292, 1236, 1236, 1149, 1120, 1032, 859, 848, 770, 576 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.59-3.61 (m, 2H), 3.66-3.67 (m, 2H), 3.70-3.72 (m, 4H), 4.74 (s, 2H), 6.71 (s, 1H), 6.85-6.86 (m, 1H), 6.89-6.91 (m, 1H), 7.23-7.25 (m, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 18.91, 42.02, 45.06, 56.52, 66.31, 66.41, 66.48, 101.67, 106.93, 109.55, 113.20, 115.58, 125.98, 131.45, 149.34, 156.90, 159.37, 166.27, 167.22; $C_{15}H_{16}N_2O_5$; ESI-MS: *m/z* 327.03 $[M+Na]^+$.

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