Design, Synthesis, and Anti-mycobacterial Evaluation of 1,8-Naphthyridine-3-Carbonitrile Analogues

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Experimental

1. Materials and methods
Experimental:

Chemistry:

All chemical reagents and solvents are purchased from Aldrich, Alfa Aesar, Finar. The solvents and reagents were of LR grade. All the solvents were dried and distilled before use. Thin-layer chromatography (TLC) was carried out on aluminium-supported silica gel plates (Merck 60 F254) with visualization of components by UV light (254 nm). Column chromatography was carried out on silica gel (Merck 100-200 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz and 101 MHz respectively using a Bruker AV 400 spectrometer (Bruker CO., Switzerland) in CDCl$_3$ and DMSO-*d6* solution with tetramethylsilane as the internal standard and chemical shift values (δ) were given in ppm. ^{1}H NMR spectra were recorded in CDCl$_3$ or DMSO-*d6*. The following abbreviations are used to designate multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Melting points were determined on an electro thermal melting point apparatus (Stuart-SMP30) in open capillary tubes and are uncorrected. Mass spectra (ESI-MS) were recorded on Schimadzu LCMS 8040 MS/ESI mass spectrometer.

2. General procedure and analytical data:

2.1 Preparation of 2-hydroxy-1,8-naphthyridine-3-carbonitrile (3):

The intermediate, 2-hydroxy-1,8-naphthyridine-3-carbonitrile **3,** was prepared by triturating a mixture of 2-aminonicotinaldehyde **1,** (1 equiv), ethyl cyanoacetate **2,** (1 equiv), and piperidine (0.1 equiv), in a mortar and pestle at room temperature for about 1 h. Completion of reaction was marked by the change of state from solid to semi solid and reconversion to solid, which was also confirmed by TLC (petroleum ether/EtOAc, 10:2). The solid thus obtained was treated with water, filtered, and recrystallized from DMF–water mixture to give yellow solid compound **3.** The total percent yield was revealed to be (87 %) and (m.p. 310-311 °C, literature m.p. 315-317 ºC).[1]

2.2 Preparation of 2-chloro-1,8-naphthyridine-3-carbonitrile (4):

The compound 2-hydroxy-1,8-naphthyridine-3-carbonitrile **3,** (1 equiv), was refluxed with phosphorus oxychloride ($POCl_3$) (5 Volume), in the presence of catalytic amount of DMF at 120 ºC for 6 h. It was then cooled to room temperature and treated with ice water. The resulting solution was basified slowly under cooling with aqueous NaOH (40%). The separated product was filtered, washed with water, dried, and recrystallized from DMF–water mixture to give 2-chloro-1,8-naphthyridine-3-carbonitrile **4.** in 80 % yield as a brown solid. (m.p. 320-322 ºC, literature m.p. 325-326 ºC).[1]

2.3 Preparation of tert-butyl 4-(3-cyano-1,8-naphthyridin-2-yl) piperazine-1-carboxylate (5):

2-chloro-1,8-naphthyridine-3-carbonitrile **4** (1 equiv), treated with and N-Boc-piperazine (1 equiv), in the presence of K_2CO_3 (1.2 equiv). in DMF. reaction was carried out by conventional heating, at 120 ºC, for 4 h, the reaction was complete monitored by TLC, (petroleum ether/EtOAc, 10:3). The reaction was cooled to room temperature, diluted with a water and extracted with dichloromethane (3 x 50 mL), and filtered through anhydrous sodium sulphate, and concentrated under reduced pressure. to get tert-butyl 4-(3-cyano-1,8-naphthyridin-2-yl) piperazine-1-carboxylate **5.** (76 % yield) as solid. (m.p. 220-222 ºC), literature m.p. 226-227 ºC).[1]

2.4 Preparation of 2-(piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile (ANI-1):

The tert-butyl 4-(3-cyano-1,8-naphthyridin-2-yl) piperazine-1-carboxylate **5** (1 equiv), and 4.0 M HCl in dioxane (2 equiv), was added, in RB flask and the mixture stirred under argon at, 0-25 ºC, for 6 h. On completion of the reaction, as indicated by TLC, the reaction mixture was cooled to room temperature, diluted with a mixture of brine and water, and extracted with dichloromethane; filtered through anhydrous sodium sulphate, and concentrated under reduced pressure to afford 2-(piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile **(ANI-1)** as yellow solid. (75 % yield) as yellow solid. (m.p. 221-223 ºC, literature m.p. 224-225 ºC).[1]

2.5 Preparation of 2-(4-(2-chloroacetyl) piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile (ANI-2):

For synthesis of 2-(4-(2-chloroacetyl) piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile **ANI-2,** Dissolve 2-(piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile **ANI-1,** (1 equiv), and triethylamine (Et$_3$N) (1.5 equiv), as a base in dry dichloromethane (DCM) (15 volume), as solvent, In oven-dried, 50 mL, round bottomed flask. Cool the resulting solution to 0 °C and add chloroacetyl chloride (1.5 equiv), in DCM dropwise in 10 minutes. Stir the reaction mixture at 25 ºC for 4 h. Completion of reaction confirmed by thin layer chromatography (TLC) (petroleum ether/EtOAc, 10:2). After that Quench the reaction by water (25 mL). and Extract the mixture with DCM (20 mL × 3) and Finally dry the combined organic layer over Na$_2$SO$_4$ and concentrate under vacuum. Purify the crude product by column chromatography (petroleum ether/EtOAc, 10:2). And chloro pattern (1:3) in mass spectrometry confirming its formation of *2*-4-(2-chloroacetyl) piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile **ANI-2.** As Dark brown solid. The total percent yield was revealed to be (73 % yield) (m.p. 258-260 ºC).

2.6 General synthesis procedure for the synthesis of target molecules (ANC 1-14):

For substituted 2-(4-(phenylglycyl) piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile molecules **(ANC 1-14).** compound **(ANI-2),** (1 equiv), is subjected to reaction with substituted aniline (1.2 equiv), in the presence of Na$_2$CO$_3$ (1.5 equiv), and KI (0.5 equiv) in DMF as solvent. reaction was carried out at 120 ºC, for 4-8 h, After compilation of reaction (petroleum ether/EtOAc, 10:3). the mixture was poured into ice water and was extracted with ethyl acetate (20 mL × 3). The system was washed with brine. Then the organic layer was

dried over anhydrous Na_2SO_4 and concentrated in vacuo. The product was further purified by column chromatography with an appropriate ethyl acetate/petroleum ether mixture to provide the title compounds substituted 2-(4-(phenylglycyl) piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile molecules **(ANC 1-14).** As solid compounds. As solid compounds. analytical data for the final compounds is outlined in the experimental section in manuscript.

2.7 General synthesis procedure for the synthesis of target molecules (ANA 1-11):

For substituted 2-(4-(3-cyano-1,8-naphthyridin-2-yl) piperazin-1-yl)-N-phenylacetamide **(ANA 1-11).** compound **(ANI-2)** (1 equiv), is subjected to reaction with substituted N-phenylacetamide (1.2 equiv), in the presence of Na_2CO_3 (1.5 equiv), and KI (0.5 equiv) in DMF as solvent. reaction was carried out at 120 ºC, for 4-8 h, After compilation of reaction (petroleum ether/EtOAc, 10:3). the mixture was poured into ice water and was extracted with ethyl acetate (20 mL × 3). The system was washed with brine. Then the organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The product was further purified by column chromatography with an appropriate ethyl acetate/petroleum ether mixture to provide the title compounds substituted 2-(4-(3-cyano-1,8-naphthyridin-2-yl) piperazin-1-yl)-N-phenylacetamide **(ANA 1-11).** As solid compounds. As solid compounds. analytical data for the final compounds is outlined in the experimental section in manuscript.

2.7 General synthesis procedure for the synthesis of target molecule (ANA 12):

Target compound 2-(4-(5-nitrofuran-2-carbonyl) piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile **(ANA 12),** is synthesized via acid amine coupling reaction. mixture of compound 2-(piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile **ANI-I** (1 equiv), is subjected to an acid amine coupling reaction with 5-nitrofuran-2-carboxylic acid **6,** (1.2 equiv), in the presence of HOBt (1.2 equiv), EDC·HCl (1.2 equiv), and DIPEA (2 equiv), as base in DMF was stirred at 25 ºC, for 4 h, after completion of reaction monitor by TLC (petroleum ether/EtOAc, 10:3). after that ice-cold H_2O was added and the mixture was extracted with EtOAc The org layer was dried over Na_2SO_4 and concentrated *in vacuo* to provide the target compounds, 2-(4-(5-nitrofuran-2-carbonyl) piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile **ANA-12,** product as an red solid. (69 % yield) (m.p. 245-247 ºC). analytical data for the **ANA-12** compound is outlined in the experimental section in manuscript.

3. **Biology Experimental procedures:**

Anti-tubercular activity:

Anti-TB activity using Alamar Blue Dye:

Growth on Lowenstein Jensen (LJ) medium was suspended in sterile Middlebrook 7H9 broth supplemented with 0.2% glycerol and 10% OADC (oleate-albumin dextrose- catalase) enrichment and a 1:20 dilution used as the inoculum for MABA. All processing was conducted with suitable safety hoods. 200µl of sterile deionized water was added to sterile 96 well plate outer perimeter wells to minimize medium evaporation during incubation in the test wells. The 96 well plate supplied 100µl of the Middlebrook 7H9 broth, and compound serial dilution were made directly on the plate. The final drug concentrations tested were 25, 12.5, 6.25, 3.125, 1.56, and 0.78 µg/ml. Plates were covered and sealed with parafilm and incubated at 37 ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth. The MIC was defined as lowest drug concentration which prevented the colour change from blue to pink.

4. **Materials and methods for computational studies**

In-silico **predicted physicochemical parameters**

The physicochemical parameters of the designed compounds were *in silico* predicted using the Swiss ADME tool. The diverse parameters predicted were lipinski rule of five –Number of violations of Lipinski's rule of five. The rules are: mol MW - 130.0 – 725.0, log Po/w - –2.0 – 6.5, donor HB ≤ 5, accept HB ≤10 and maximum 4 violations. **Log Po/w**: Log of partition coefficient; **TPSA**: Topological polar surface area:TPSA < 140 is essential for good absorption; **ESOL:** Estimated aqueous solubility in mg/mL; **GI:** Gastrointestinal.

Table S1. Physico-chemical properties of the compounds as predicted through *In-silico.*

Entry	mol MW	Donor HB	Accept HB	Log Po/w	Rule of Five	TPSA	ESOL Class	GI absorption
ANI-1	239.28	1	4	1.92	0	64.84	Soluble	High
ANI-2	315.76	0	4	2.23	0	73.12	Soluble	High
ANC-1	372.42	1	4	2.67	0	85.15	Soluble	High
ANC-2	400.48	1	4	3.16	0	85.15	Moderately soluble	High
ANC-3	400.48	1	4	3.12	0	85.15	Moderately soluble	High
ANC-4	400.48	1	4	3.25	0	85.15	Moderately soluble	High
ANC-5	390.41	1	5	2.77	0	85.15	Moderately soluble	High
ANC-6	406.87	1	4	2.93	0	85.15	Moderately soluble	High
ANC-7	451.32	1	4	3.08	0	85.15	Moderately soluble	High
ANC-8	390.41	1	5	2.82	0	85.15	Moderately soluble	High
ANC-9	498.32	1	4	3.09	0	85.15	Moderately soluble	High
ANC-10	496.32	1	6	2.8	0	130.9	Moderately soluble	High
ANC-11	406.87	1	4	3.02	0	85.15	Moderately soluble	High
ANC-12	440.42	1	7	2.95	0	85.15	Moderately soluble	High
ANC-13	441.31	1	4	3.25	0	85.15	Moderately soluble	High
ANC-14	408.4	1	6	2.85	0	85.15	Moderately soluble	High

ANA-1	372.42	1	5	2.81	0	85.15	Soluble	High
ANA-2	400.48	1	5	3.15	0	85.15	Moderately soluble	High
ANA-3	400.48	1	5	2.99	0	85.15	Moderately soluble	High
ANA-4	390.41	1	6	2.85	0	85.15	Soluble	High
ANA-5	451.32	1	5	3.24	0	85.15	Moderately soluble	High
ANA-6	390.41	1	6	2.99	0	85.15	Soluble	High
ANA-7	417.42	1	7	2.47	0	130.97	Moderately soluble	High
ANA-8	440.42	1	8	2.63	0	85.15	Moderately soluble	High
ANA-9	408.4	1	7	2.85	0	85.15	Moderately soluble	High
ANA-10	417.42	1	7	2.24	0	130.97	Soluble	High
ANA-11	417.42	1	7	2.42	0	130.97	Soluble	High
ANA-12	378.34	0	7	1.98	0	132.08	Soluble	High

Description: Lipinski rule of five – Number of violations of Lipinski's rule of five. The rules are: mol MW - 130.0 – 725.0, log Po/w - – 2.0 – 6.5, donor HB ≤ 5, accept HB ≤10 and maximum 4 violations. **Log Po/w**:Log of partition coefficient; **TPSA**: Topological polar surface area:TPSA < 140 is essential for good absorption; **ESOL:** Estimated aqueous solubility in mg/mL; **GI:** Gastrointestinal.

Molecular docking studies

Docking studies of the significantly active compound were performed using the Glide module of Schrodinger software, Version 2020-4. Installed on Intel XenonW3565 processor and Ubuntu enterprise version 14.04 as an operating system. The selected target protein structure was retrieved from the RCSB protein data bank. (https://www.rcsb.org/structure/4TZK).[2]

Ligand preparation

The ligand used as an input for docking study was sketched in ChemDraw 16.0. Then, the ligand was incorporated into the workstation, and the energy was minimized using the OPLS3e (Optimized Potentials for Liquid Simulations) force field in the Ligprep module (Version 2019-1, Schrodinger) of the software. This minimization helps to allocate bond orders, the addition of hydrogens to the ligands, and conversion of 2D to 3D structure for the docking studies. The generated output file (Best conformations of the ligands) was further used for docking studies.[2]

Protein preparation

Protein preparation wizard (Version 2020-4, Schrodinger). Is the primary tool in Schrodinger to prepare and minimize the energy of protein. Hydrogen atoms were added to the protein, and charges were assigned. The Het states were generated using Epik at pH 7.0 ± 2.0. The protein was pre-processed, refined, modified by analyzing the workspace water molecules and other atoms. The critical water molecules remained the same, and the rest of the molecules apart from heteroatoms from the water was deleted. Finally, the protein was minimized using the OPLS3 force field. A grid was created by considering the co-crystal ligand, which was included in the active site of the selected protein target (PDB-4TZK). After the final step of docking with the co-crystal ligand in XP mode, root mean square deviation (RMSD) was checked to validate the protein.[2]

Receptor grid generation

A receptor grid was generated around the protein (PDB-4TZK) by choosing the inhibitory ligand (X-ray pose of the ligand in the protein). The centroid of the ligand was selected to create a grid box around it, and the Van der Waal radius of receptor atoms was scaled to 1.00 Å with a partial atomic charge of 0.25.[2]

Molecular dynamics studies

Molecular Dynamics (MD) simulation helps envisage the Protein-Ligand complex's (PLC) actions at the target's binding site region in the physiological conditions. MD was performed using the Desmond module of Schrödinger developed by D.E Shaw research group (Academic license, Version 2020-4). Through the system's builder panel; the orthorhombic simulation box was prepared with the Simple

Point-Charge (SPC) explicit water model in such a way that the minimum distance between the protein surface and the solvent surface is 10 Å. Protein-ligand docked complexes were solvated using the orthorhombic SPC water model. The solvated system was neutralized with counter ions, and physiological salt concentration was limited to 0.15 M. The receptor-ligand complex system was designated with the OPLS AA force field.[3]

The Reversible reference System Propagator Algorithms (RESPA) integrator. Nose-Hoover chain thermostat, and Martyna-Tobias-Klein barostat were used with two ps relaxation times. The equilibrated system was used for the final production of the MD simulation. The production of MD simulation was run for 100 ns at 300 K temperatures at 1.0 bar pressure with NPT (Isothermal-Isobaric ensemble, constant temperature, constant pressure, constant number of particles) ensemble. with the default settings of relaxation before simulation. The MD simulation was run by using the MD simulation tool, simulation time setup to 100 ns. Further, for viewing the trajectories and creating a movie, out file used. CMS file was imported, and the movie was exported with high resolution (1280 × 1024) with improved quality. During the MD simulation, the trajectory was written with 1000 frames. To understand the stability of the complex during MD simulation, the protein backbone frames were aligned to the backbone of the initial frame. Finally, the analysis of the simulation interaction diagram was achieved after loading the outacts file and selected Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) in the analysis type to obtain the mentioned plots.[3]

5. ^{1}H NMR and ^{13}C NMR spectras of final compounds (ANI 1-2, ANC 1-14 and ANA 1-12):
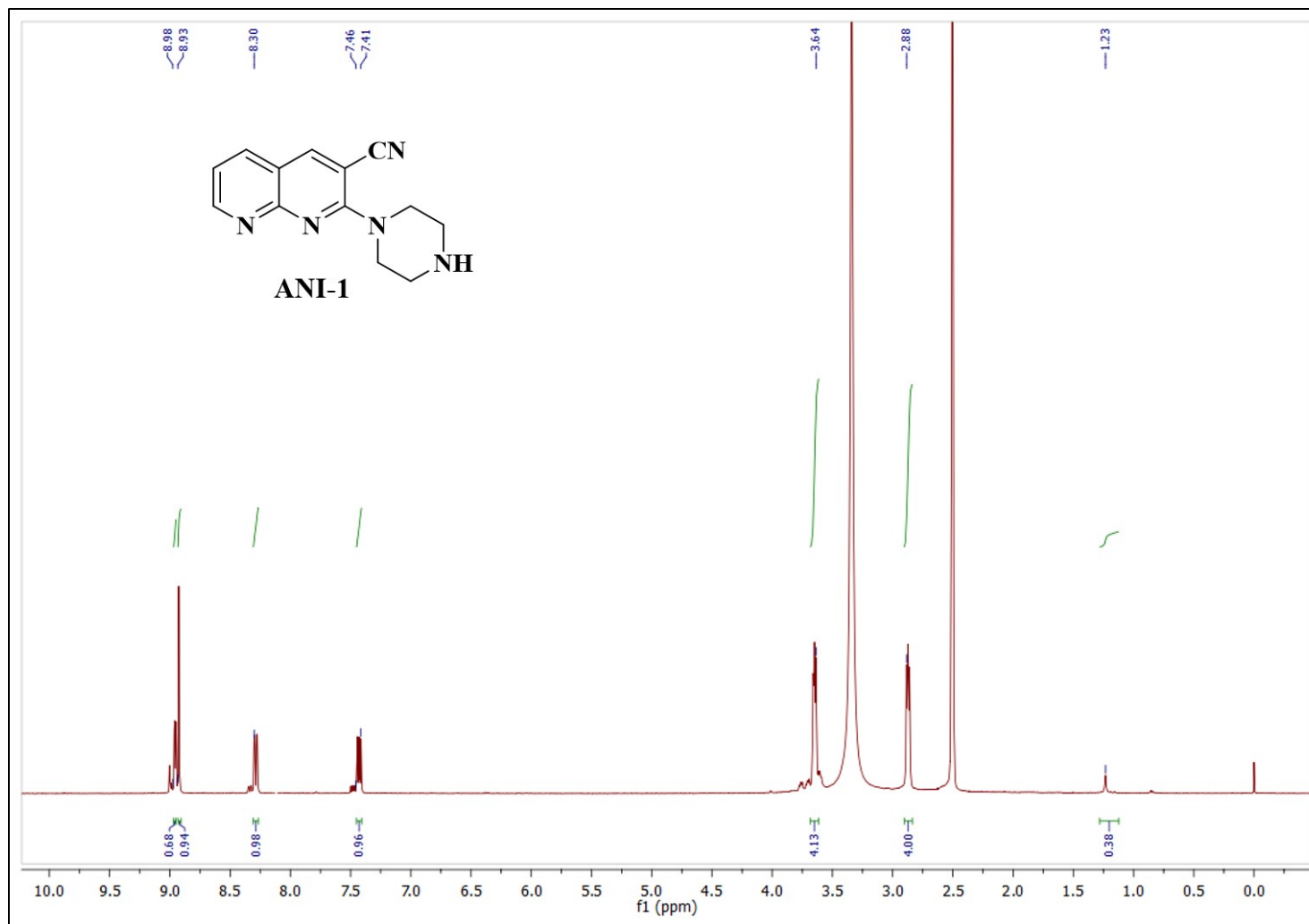
^{1}**H NMR** spectrum of compound **ANI-1** (DMSO-d_6 400 MHz)
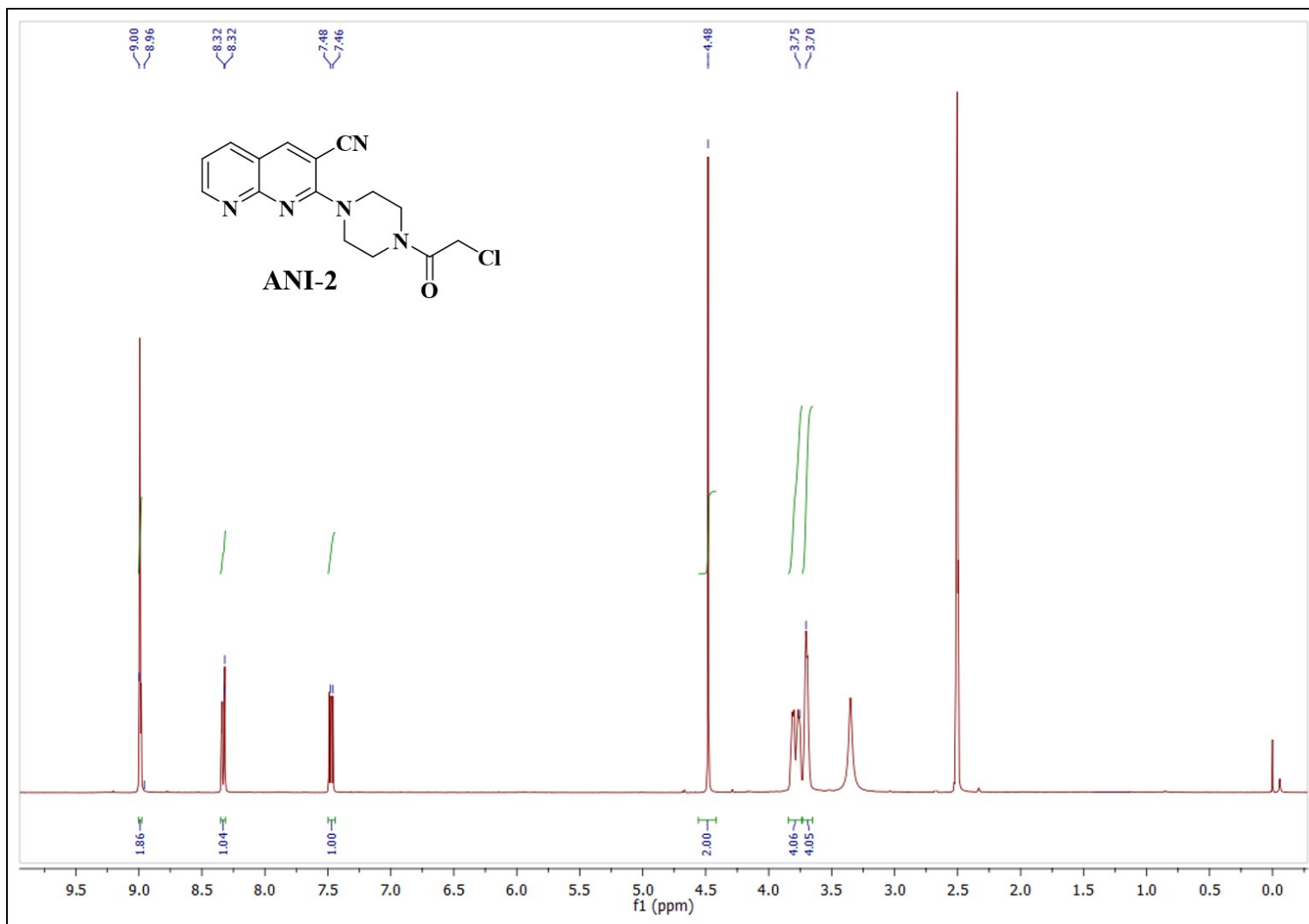
^{1}H NMR spectrum of compound **ANI-2** (DMSO-d_6 400 MHz)

165.55
159.23
156.76
155.73
153.92
149.24
138.48
121.22
118.38
117.41
98.91
49.18
48.27
42.56

CN

ANI-2

Cl

O

170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)
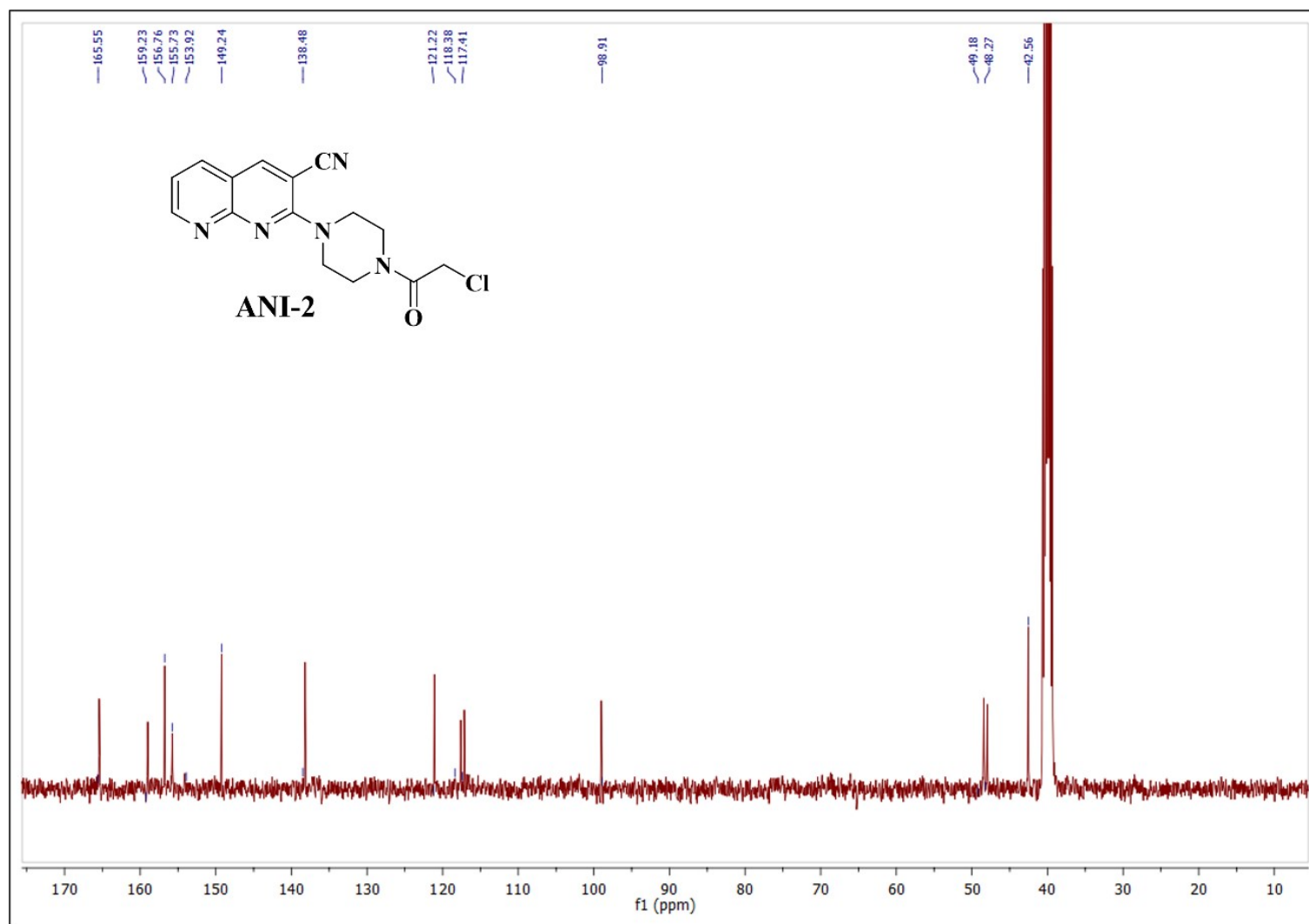
^{13}C NMR spectrum of compound ANI-2 (DMSO-d_6 101 MHz)
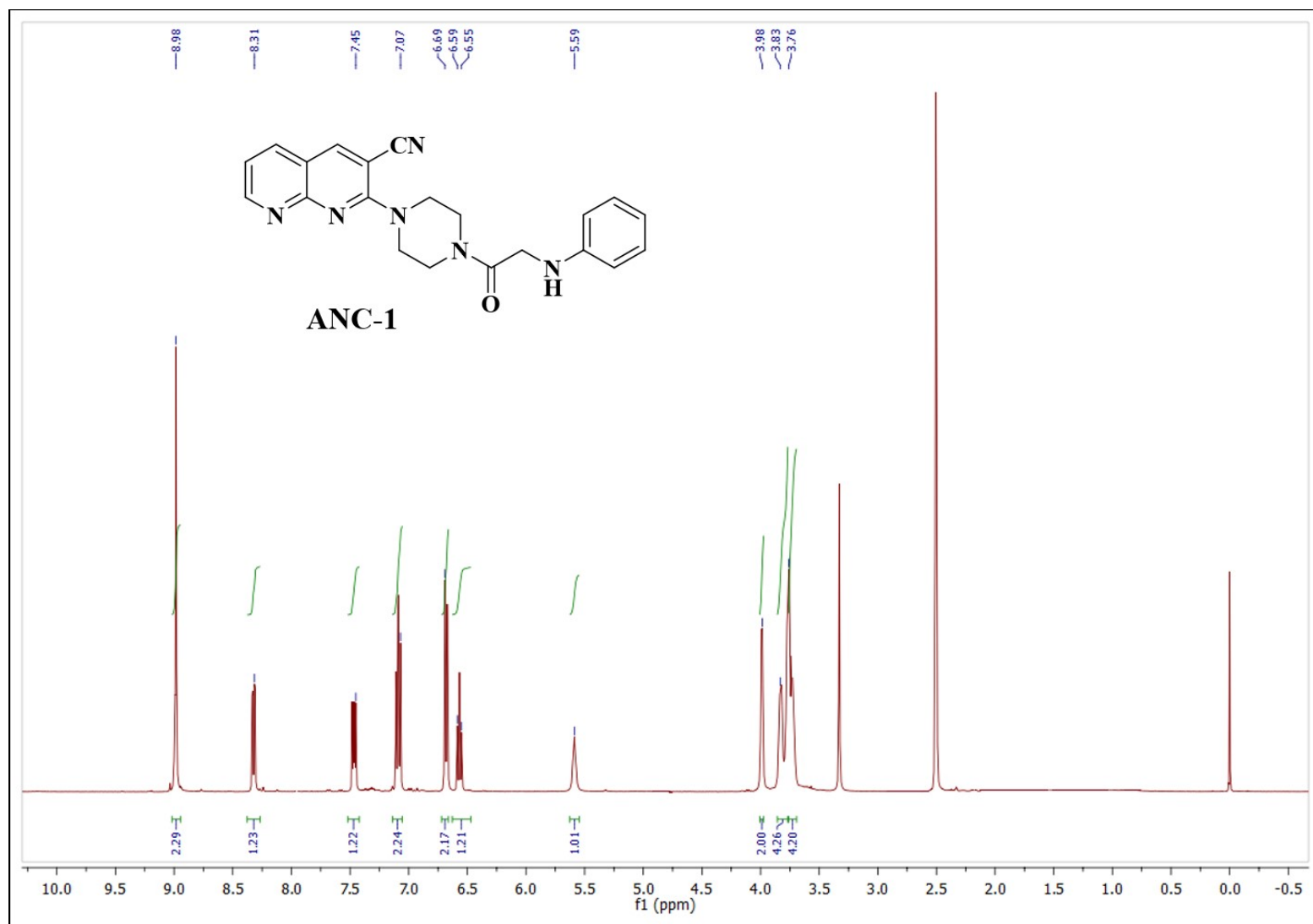
^{1}**H NMR** spectrum of compound **ANC-1 (**DMSO-d$_6$ 400 MHz)
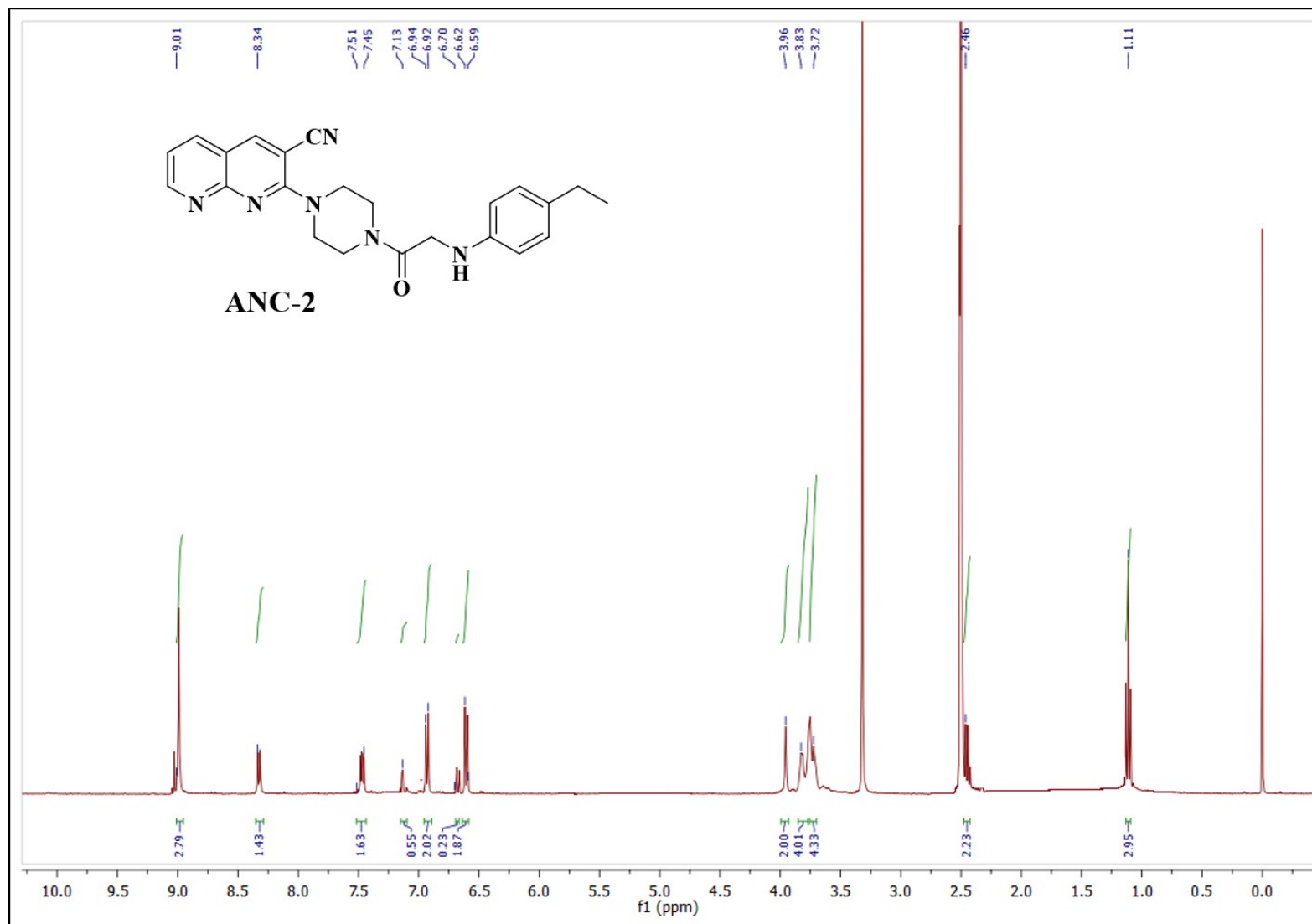
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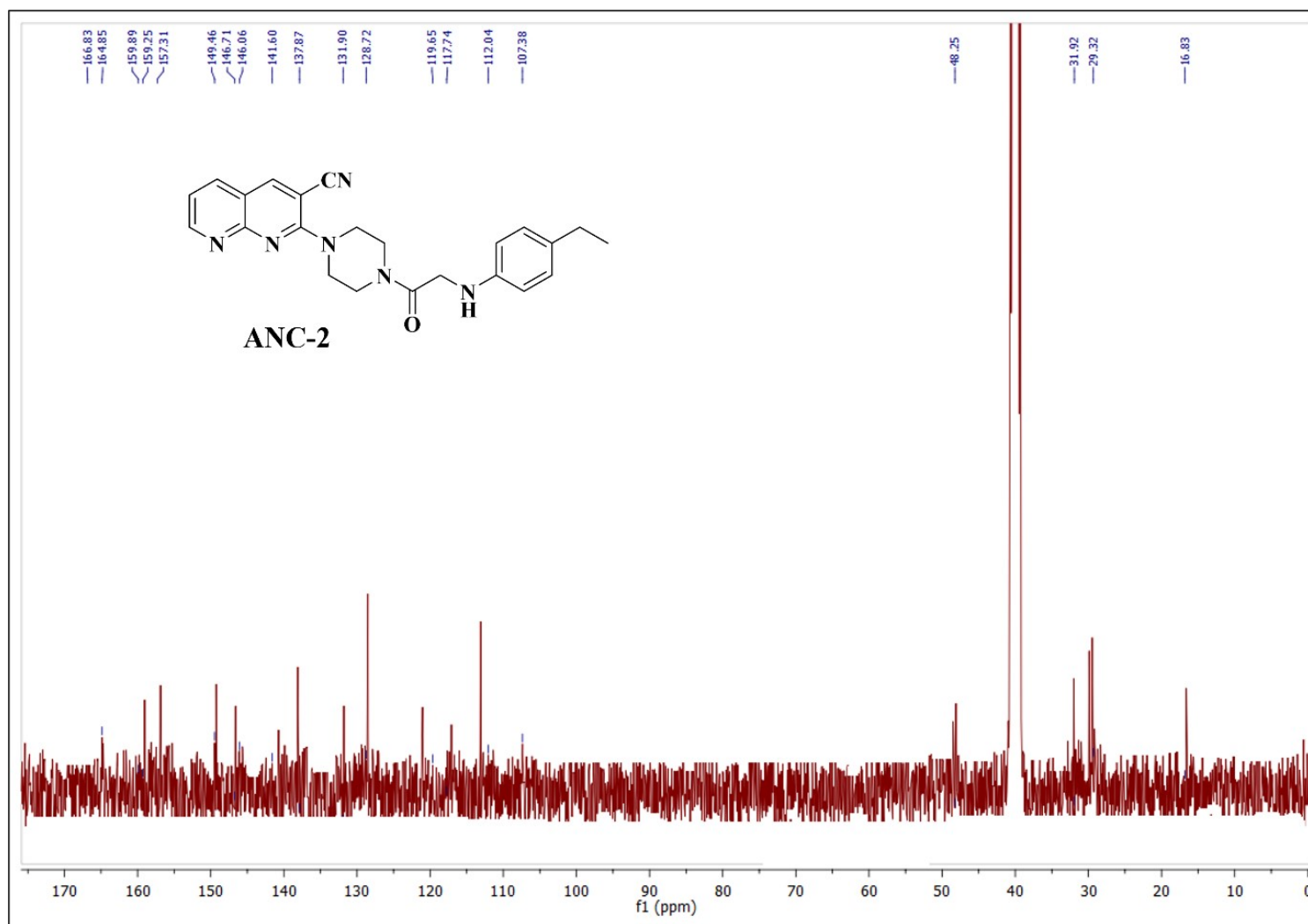
^{13}**C NMR** spectrum of compound **ANC-2** (DMSO-d_6 101 MHz)
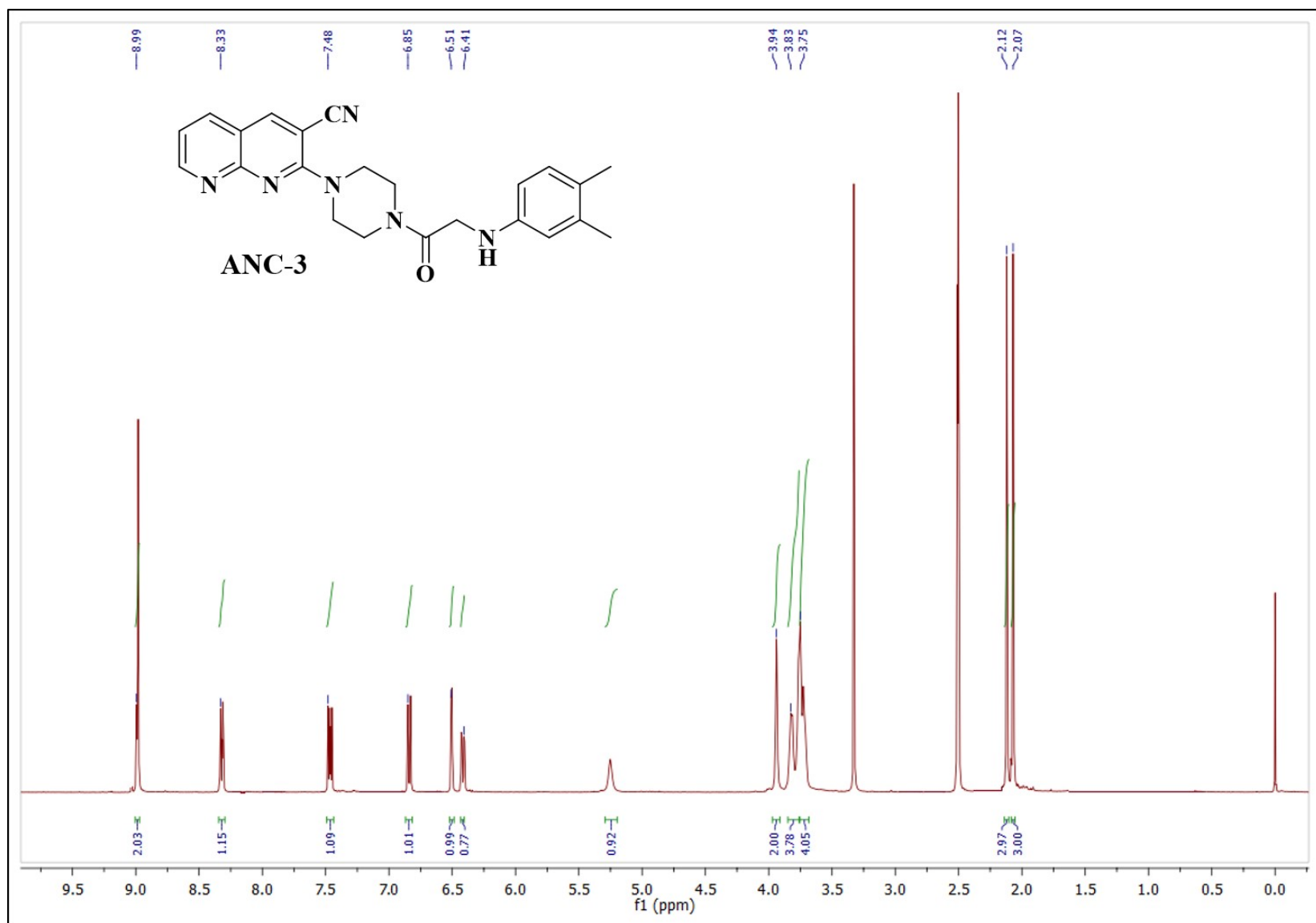
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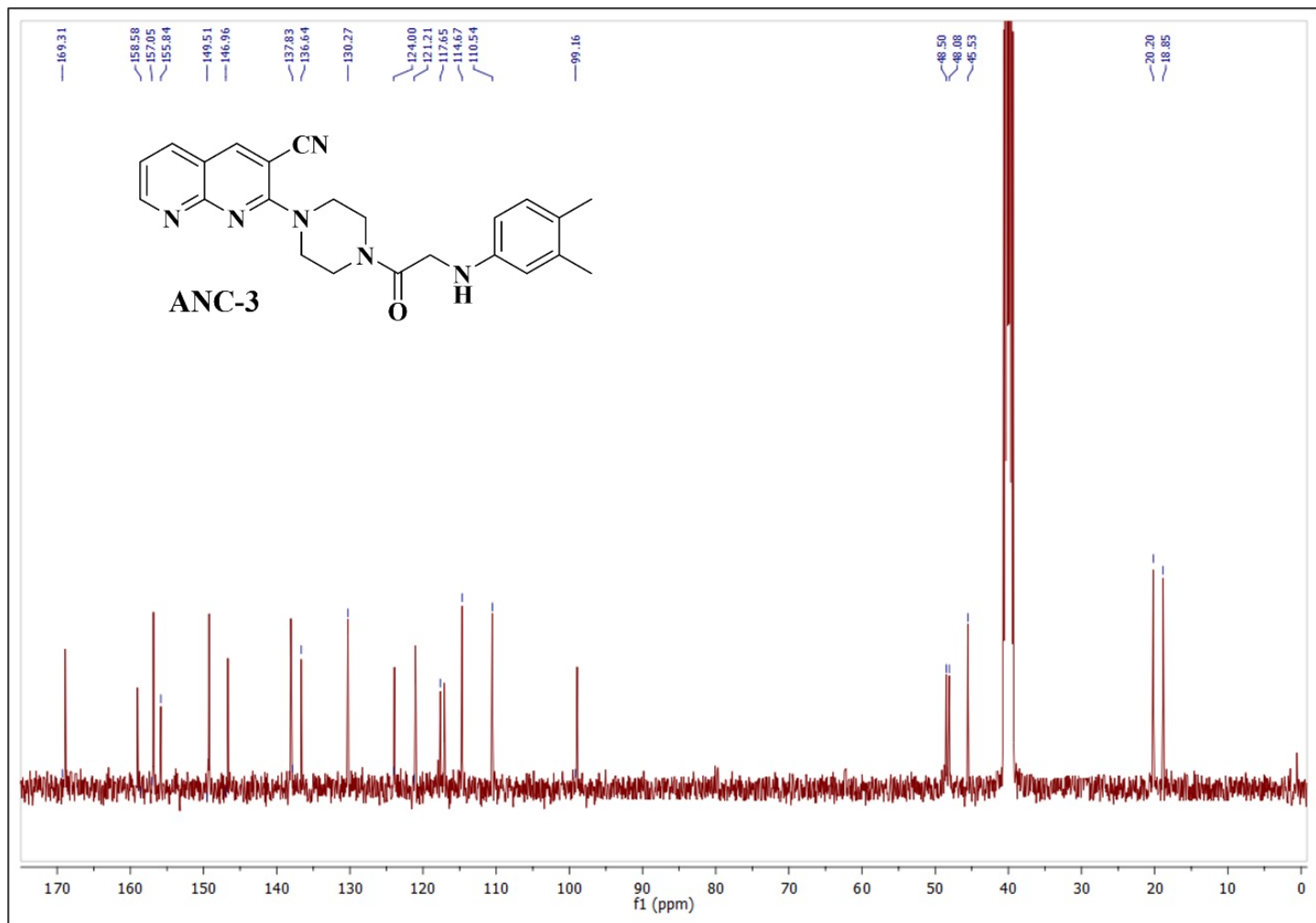
ANC-3

^{13}C NMR spectrum of compound ANC-3 (DMSO-d_6 101 MHz)
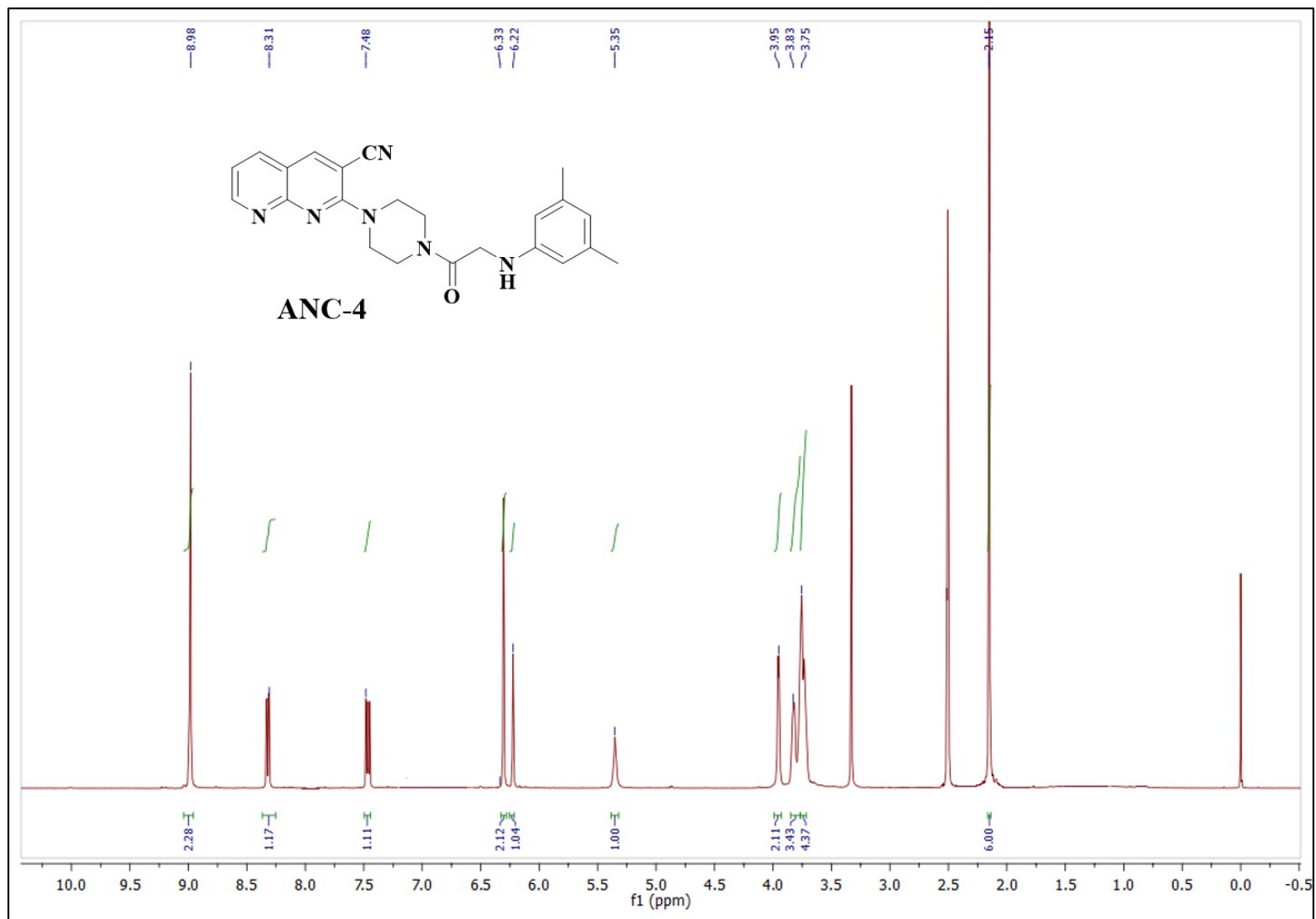
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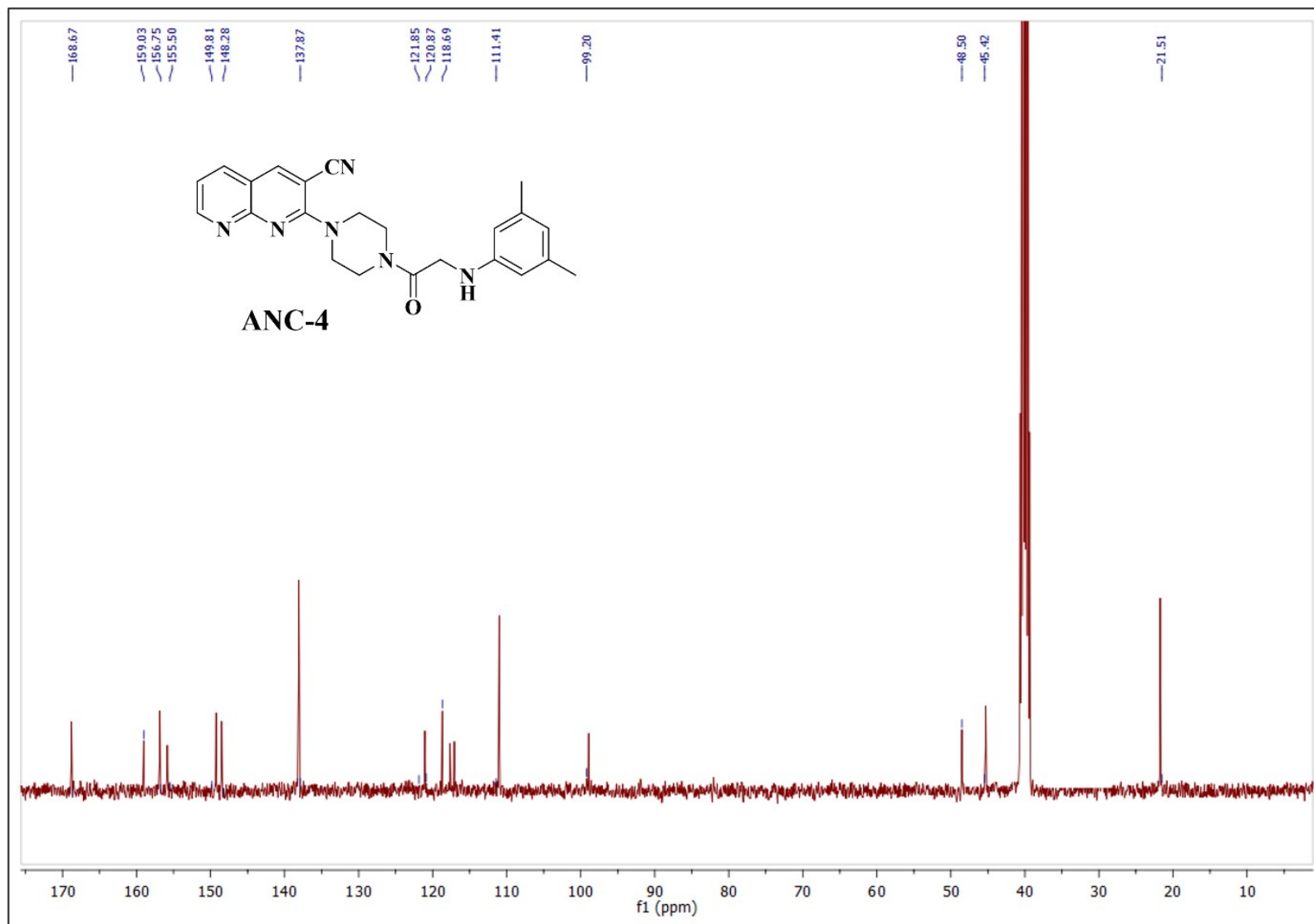
^{13}**C NMR** spectrum of compound **ANC-4** (DMSO-d_6 101 MHz)
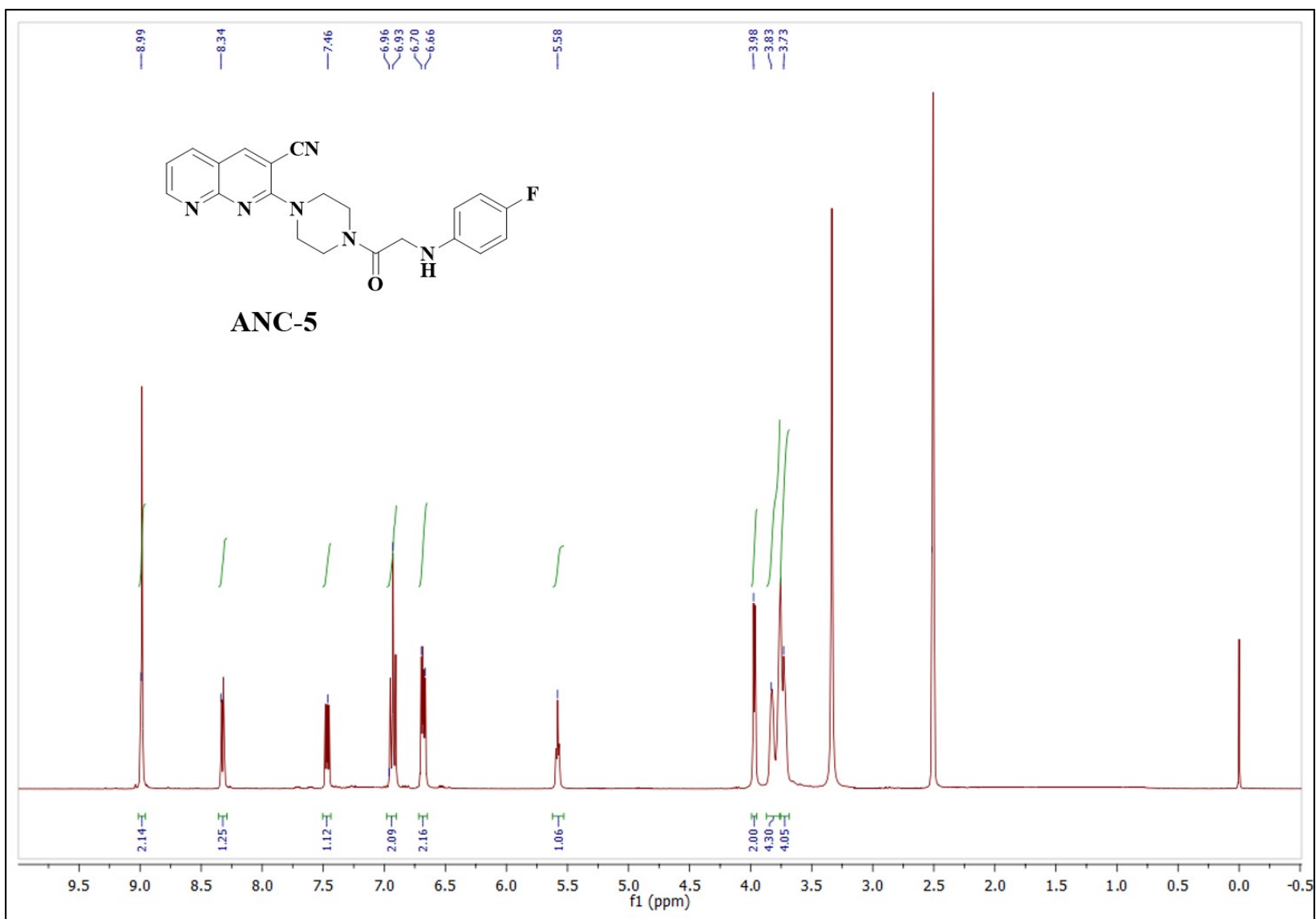
ANC-5

^{1}**H NMR** spectrum of compound **ANC-5** (DMSO-d_6 400 MHz)
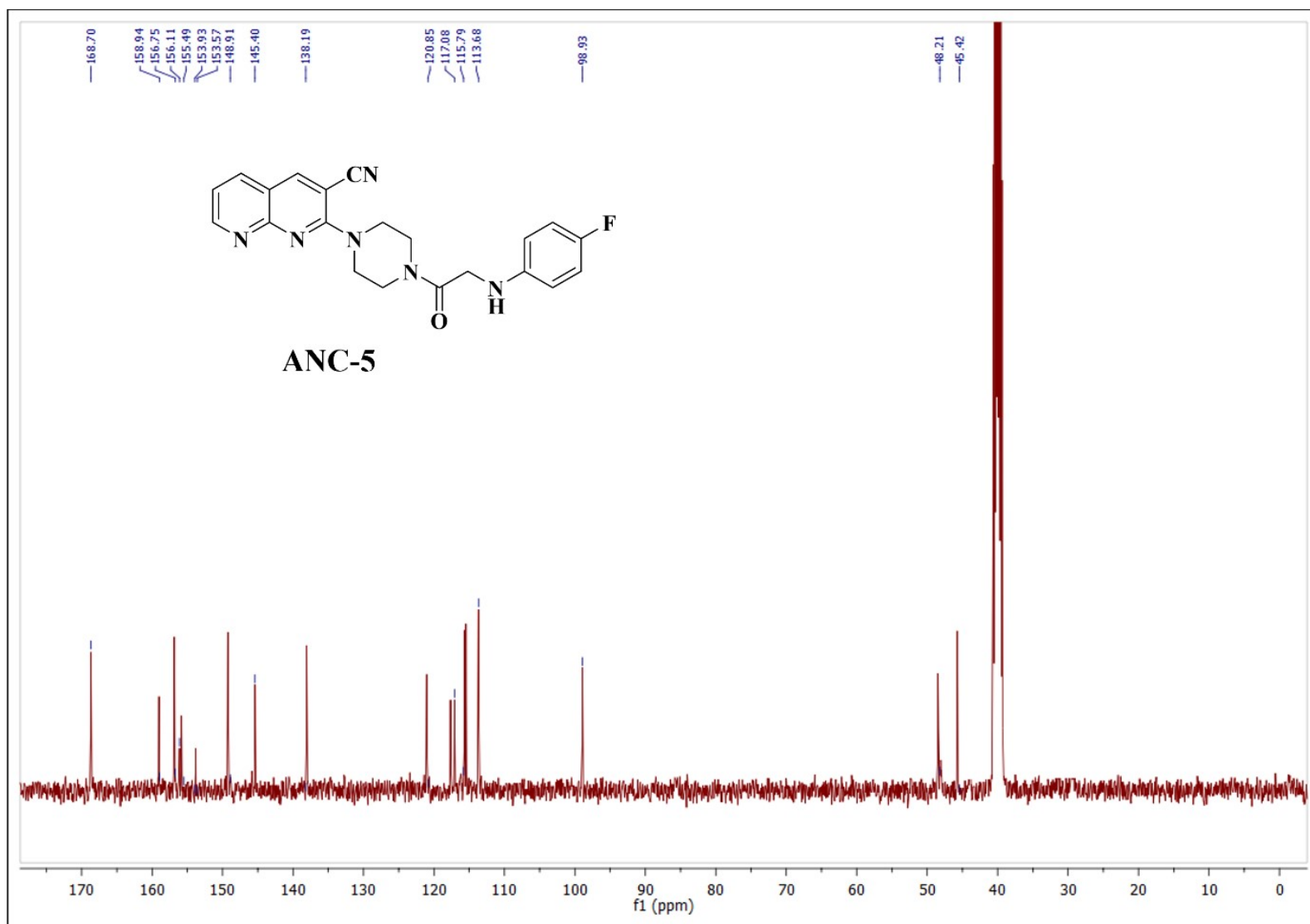
ANC-5

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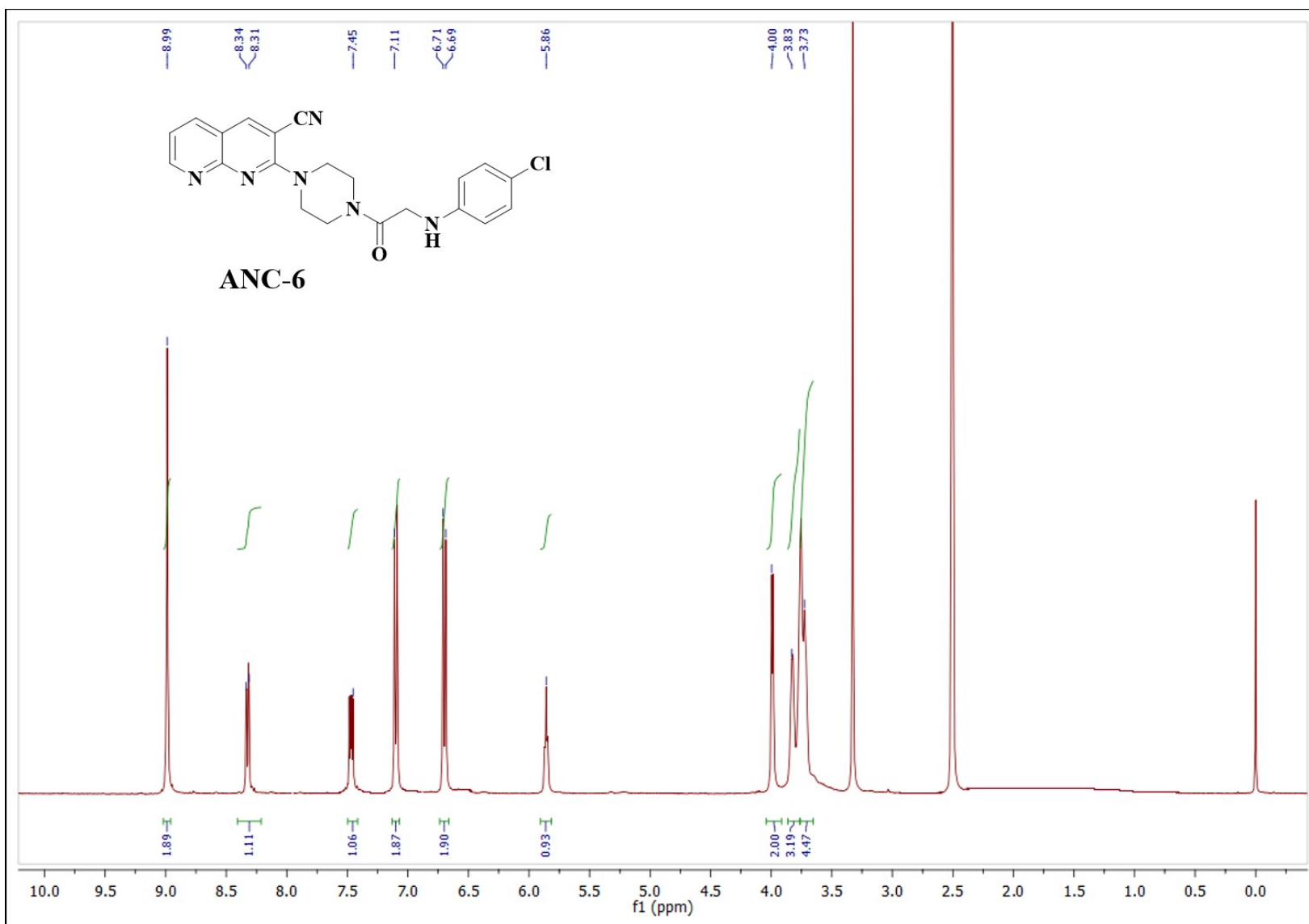
ANC-6

^{1}**H NMR** spectrum of compound **ANC-6** (DMSO-d_6 400 MHz)
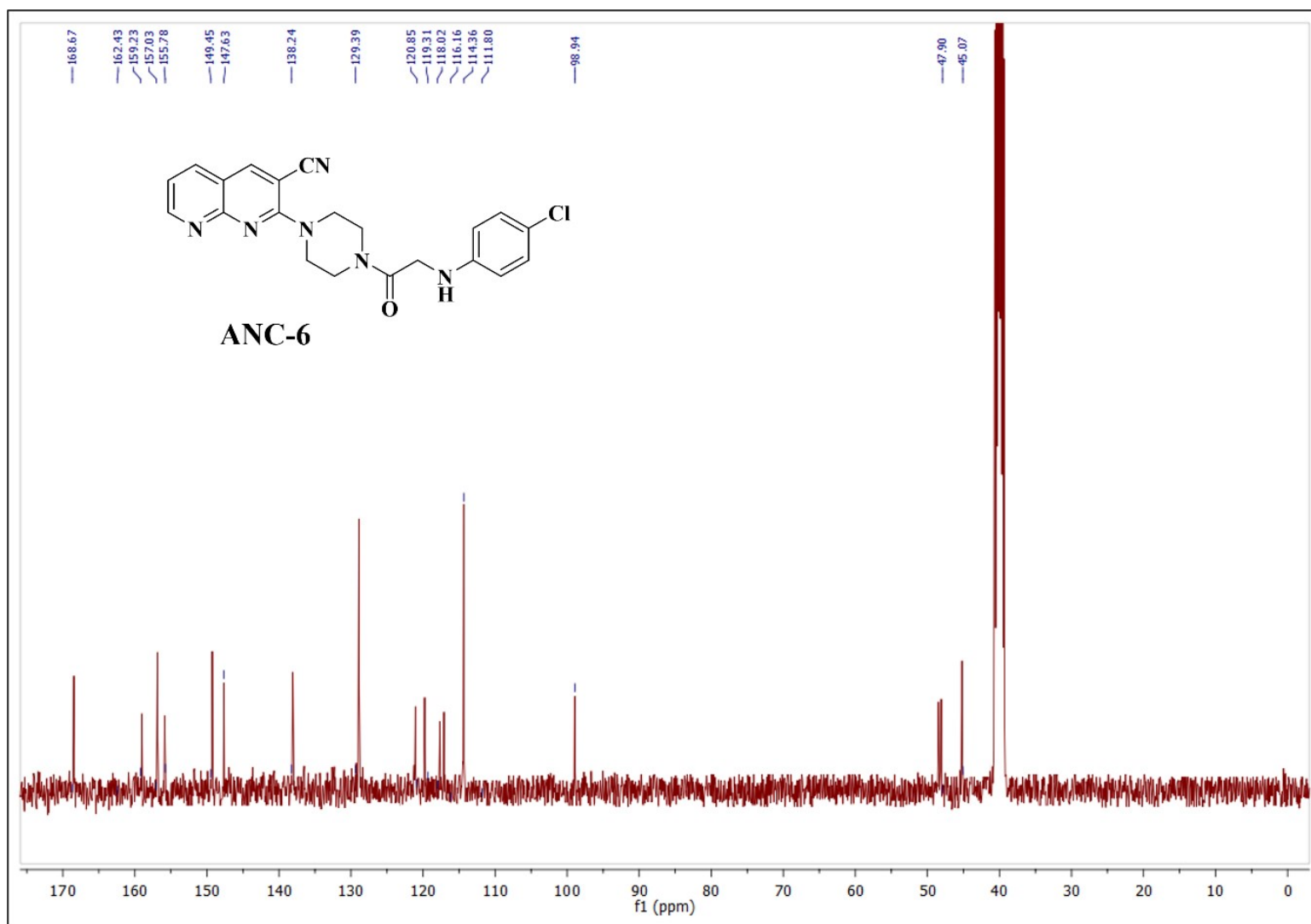
^{13}C NMR spectrum of compound ANC-6 (DMSO-d_6 101 MHz)
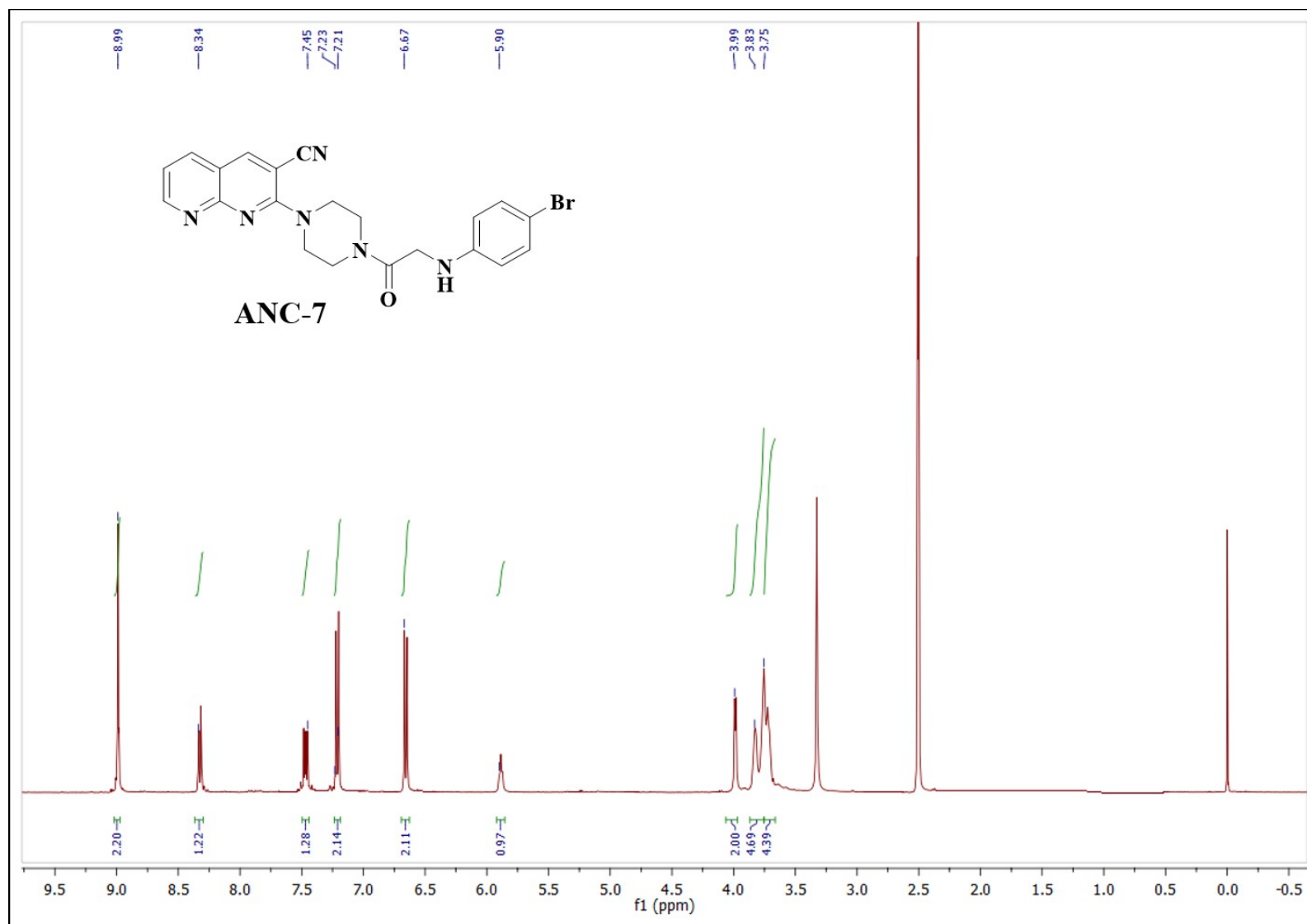
^{1}**H NMR** spectrum of compound **ANC-7 (**DMSO-d_6 400 MHz)
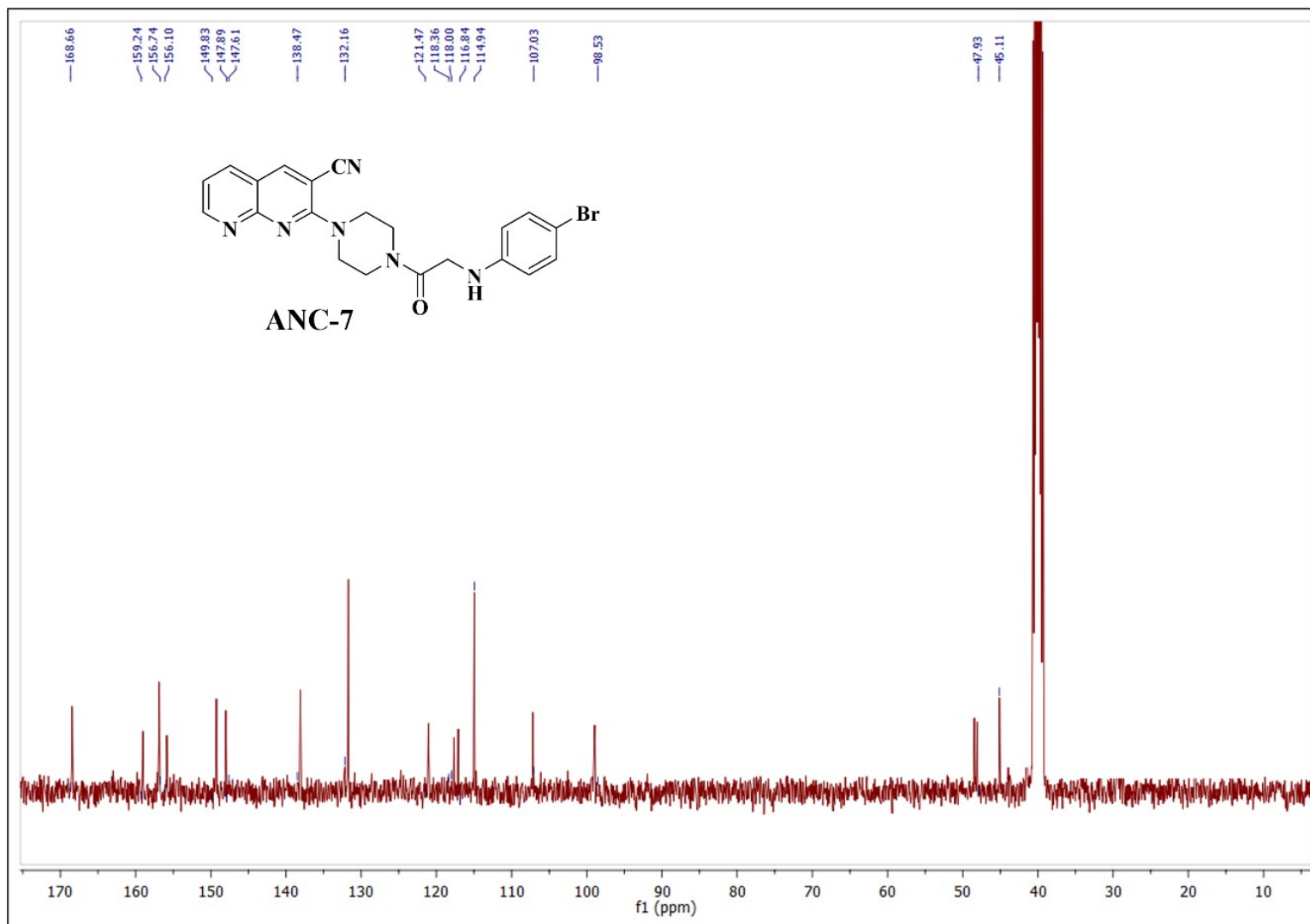
^{13}**C NMR** spectrum of compound **ANC-7** (DMSO-d_6 101 MHz)
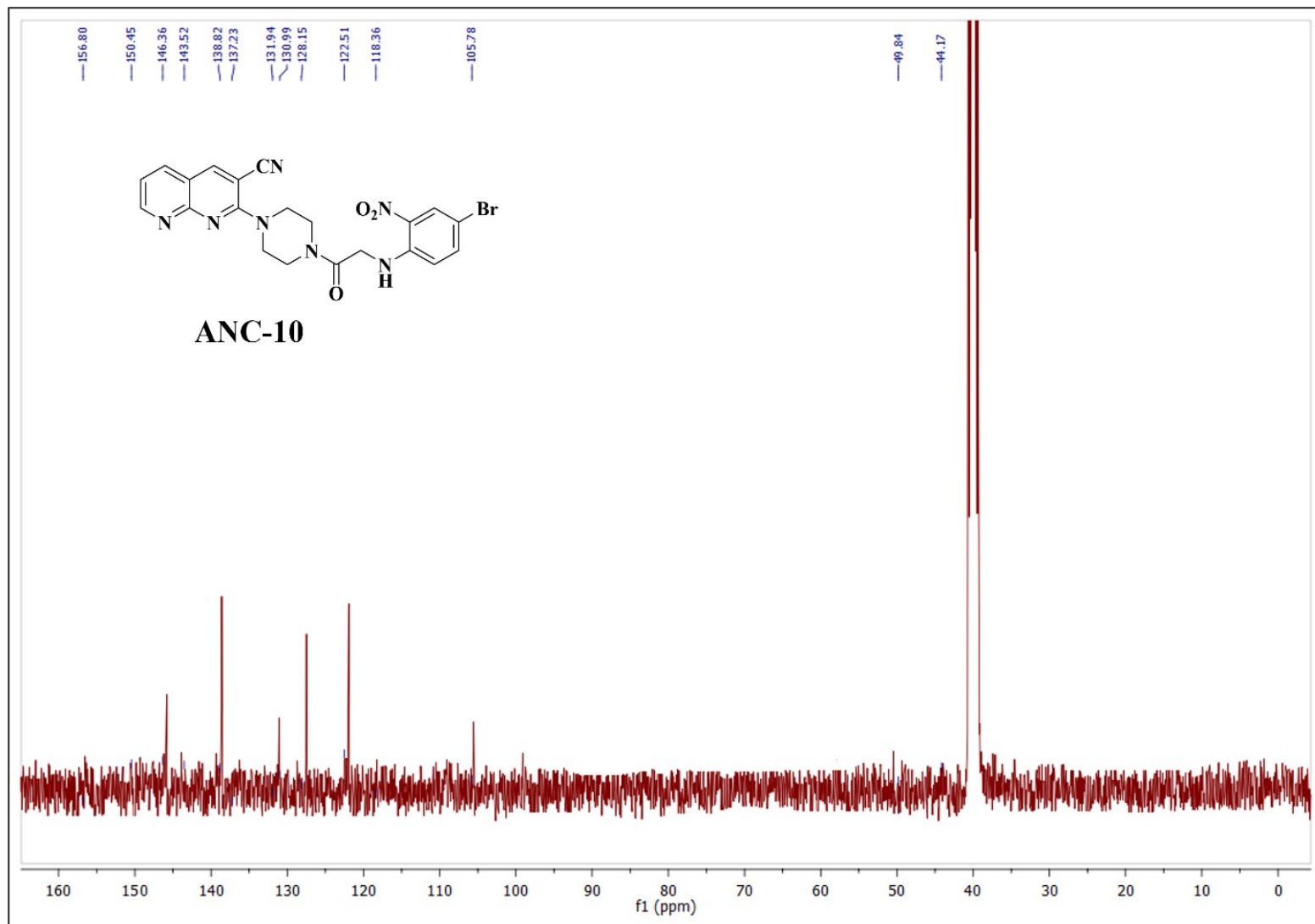
13C NMR spectrum of compound **ANC-10** (DMSO-d_6 101 MHz)
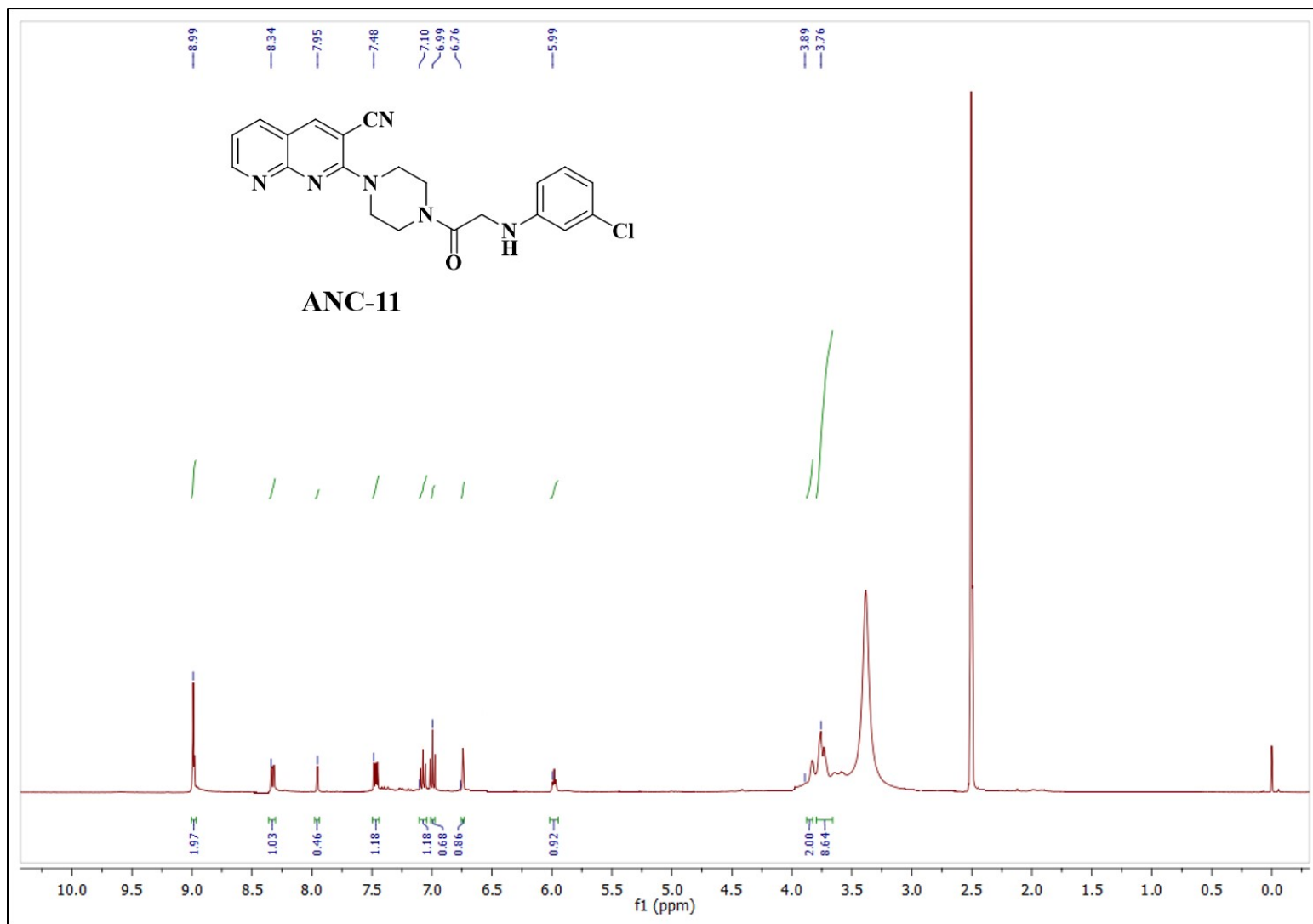
ANC-11

¹H NMR spectrum of compound **ANC-11 (**DMSO-d_6 400 MHz)

The peak labels from left to right: 168.38, 162.46, 158.95, 157.32, 156.14, 151.10, 149.48, 149.25, 145.07, 137.86, 134.06, 130.34, 121.49, 118.66, 117.09, 113.64, 112.99, 112.42, 98.56, 48.89, 44.79
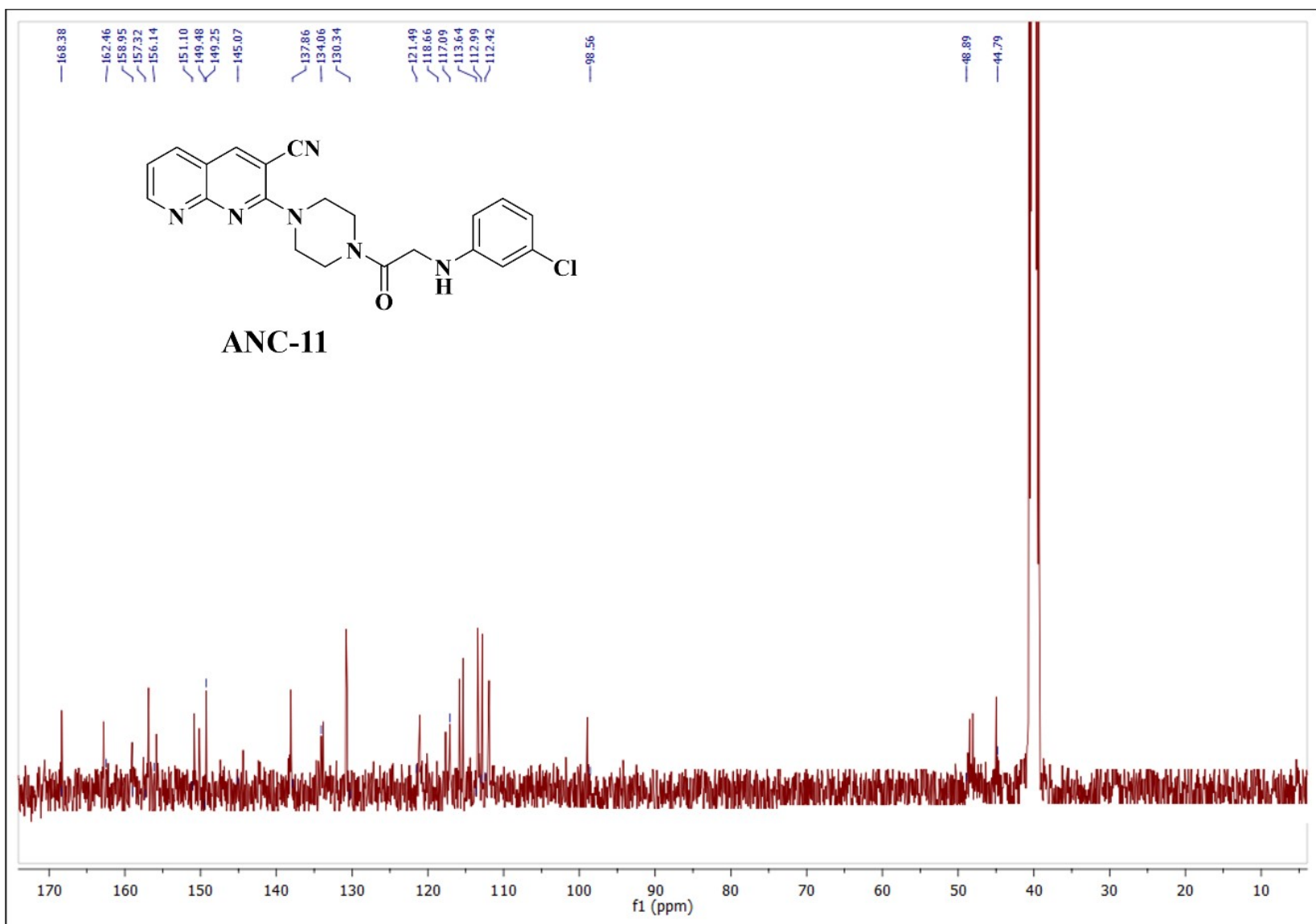
ANC-11

^{13}**C NMR** spectrum of compound **ANC-11** (DMSO-d_6 101 MHz)
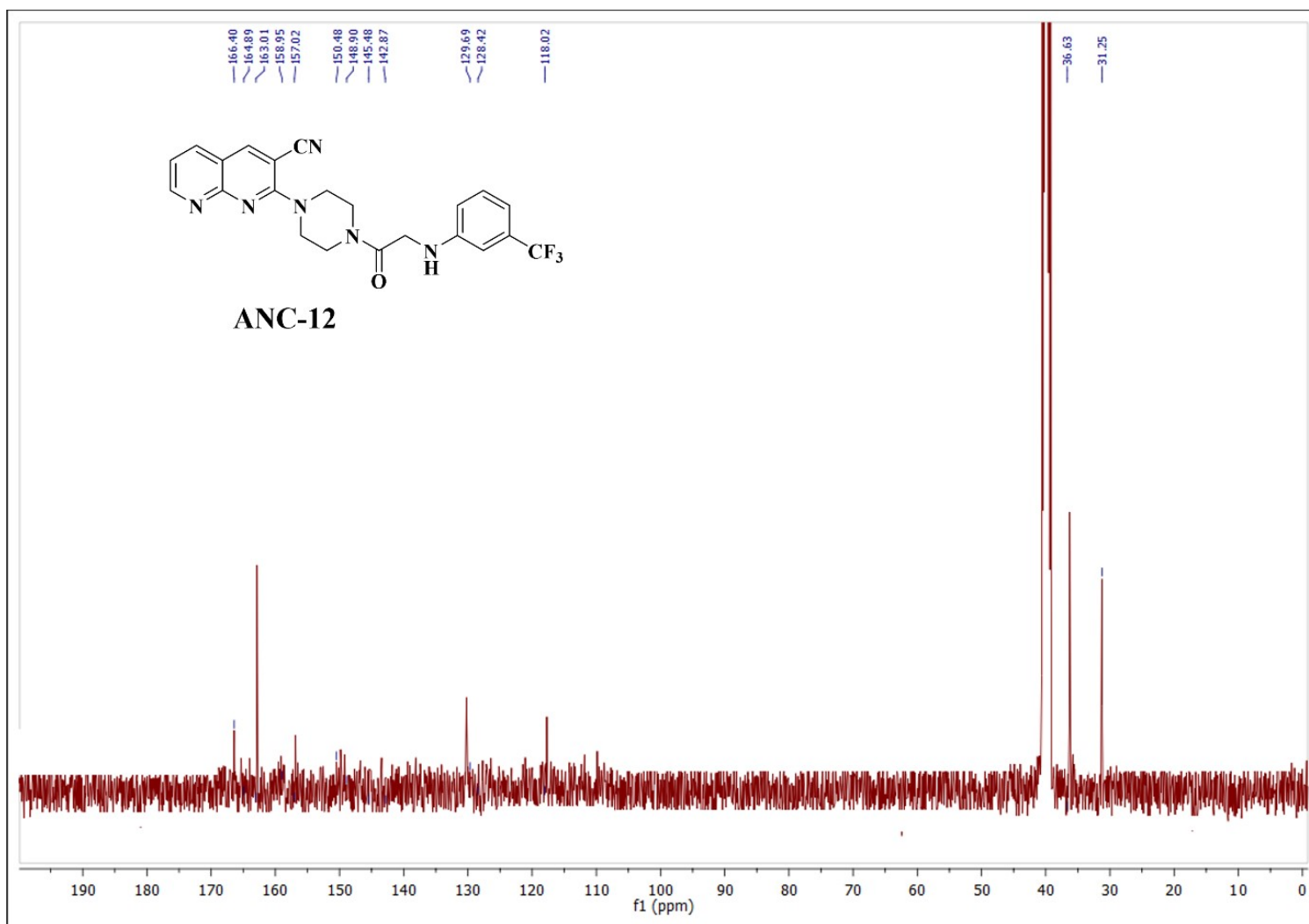
ANC-12

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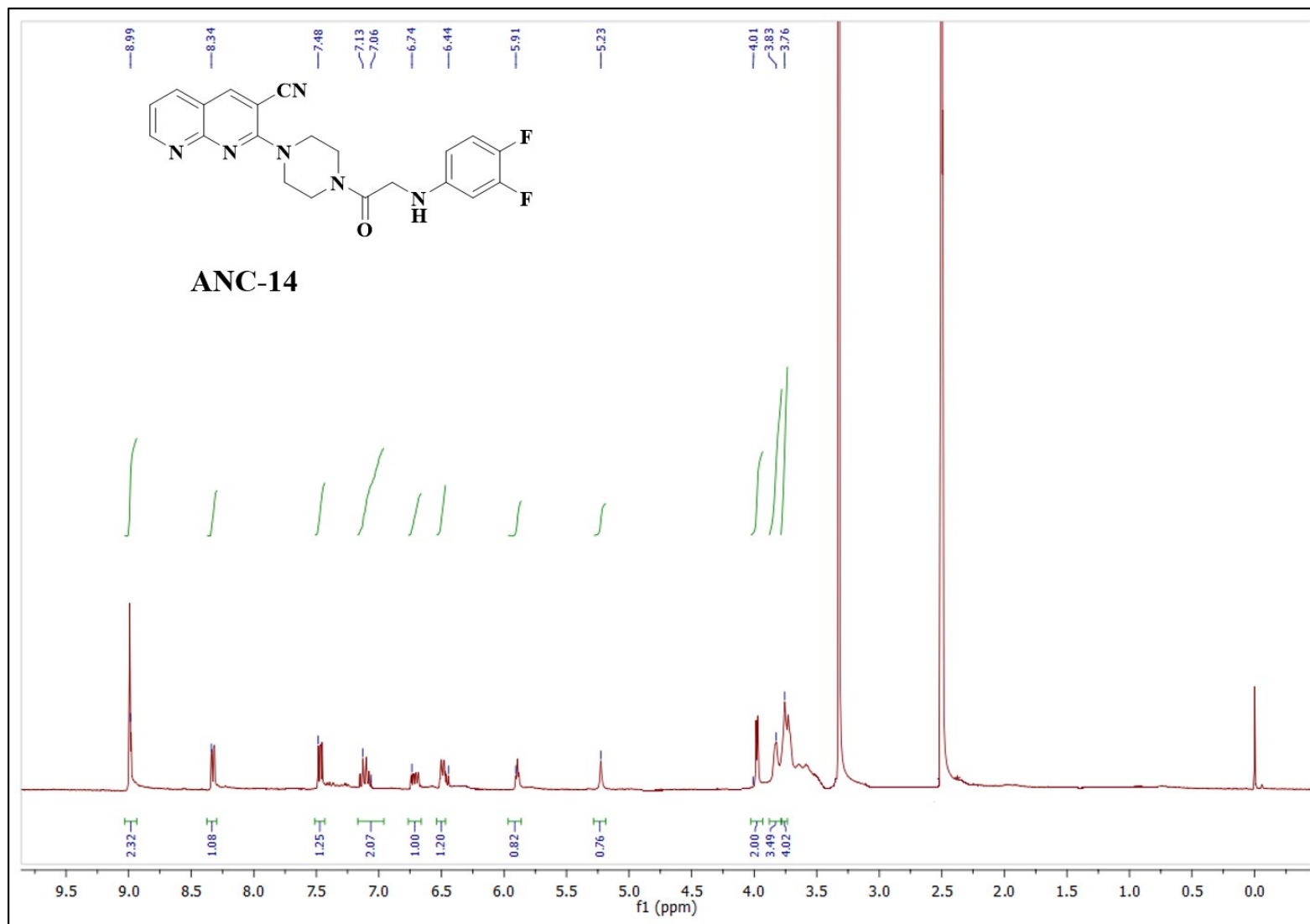
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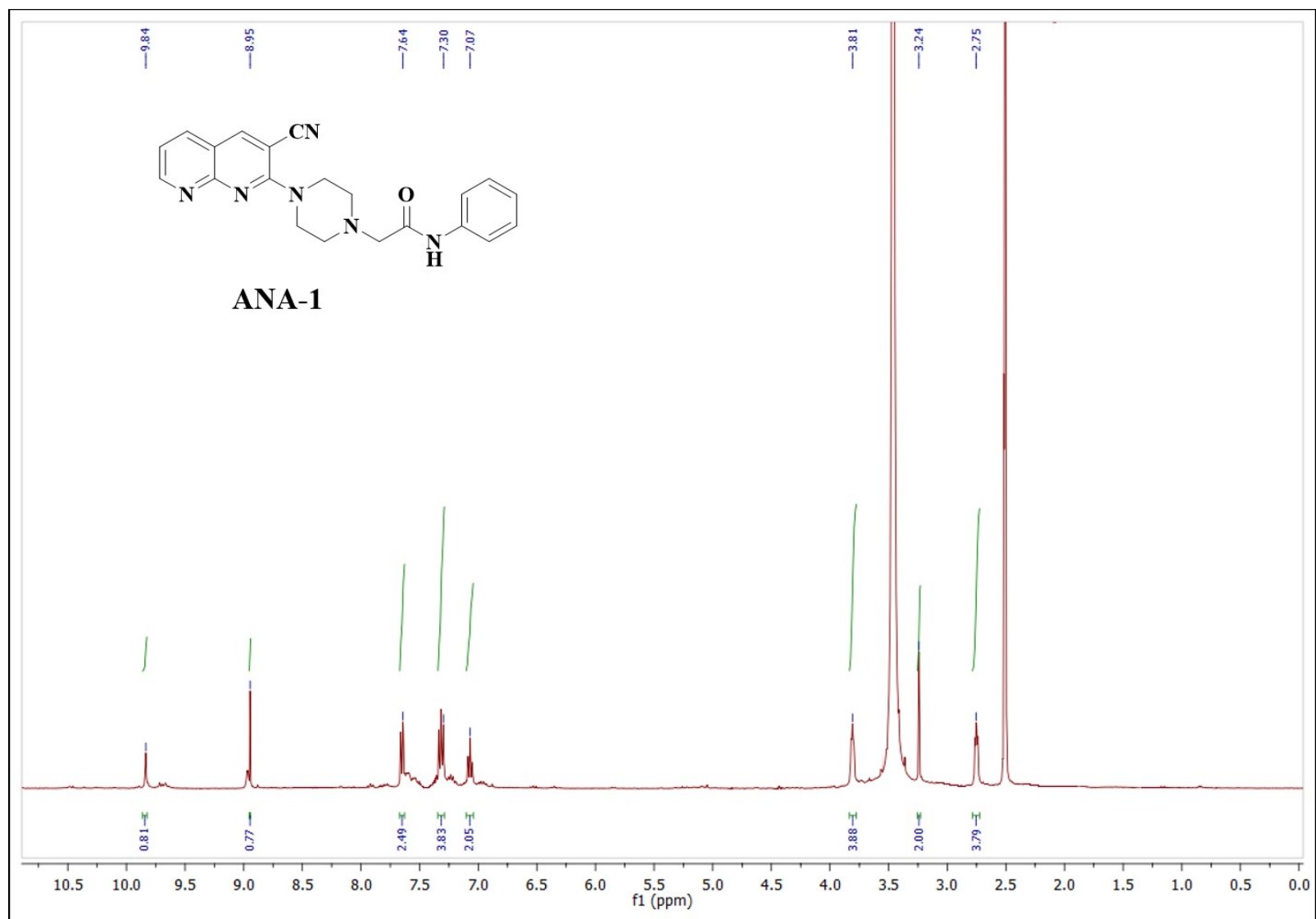
¹H NMR spectrum of compound **ANA-1** (DMSO-d_6 400 MHz)
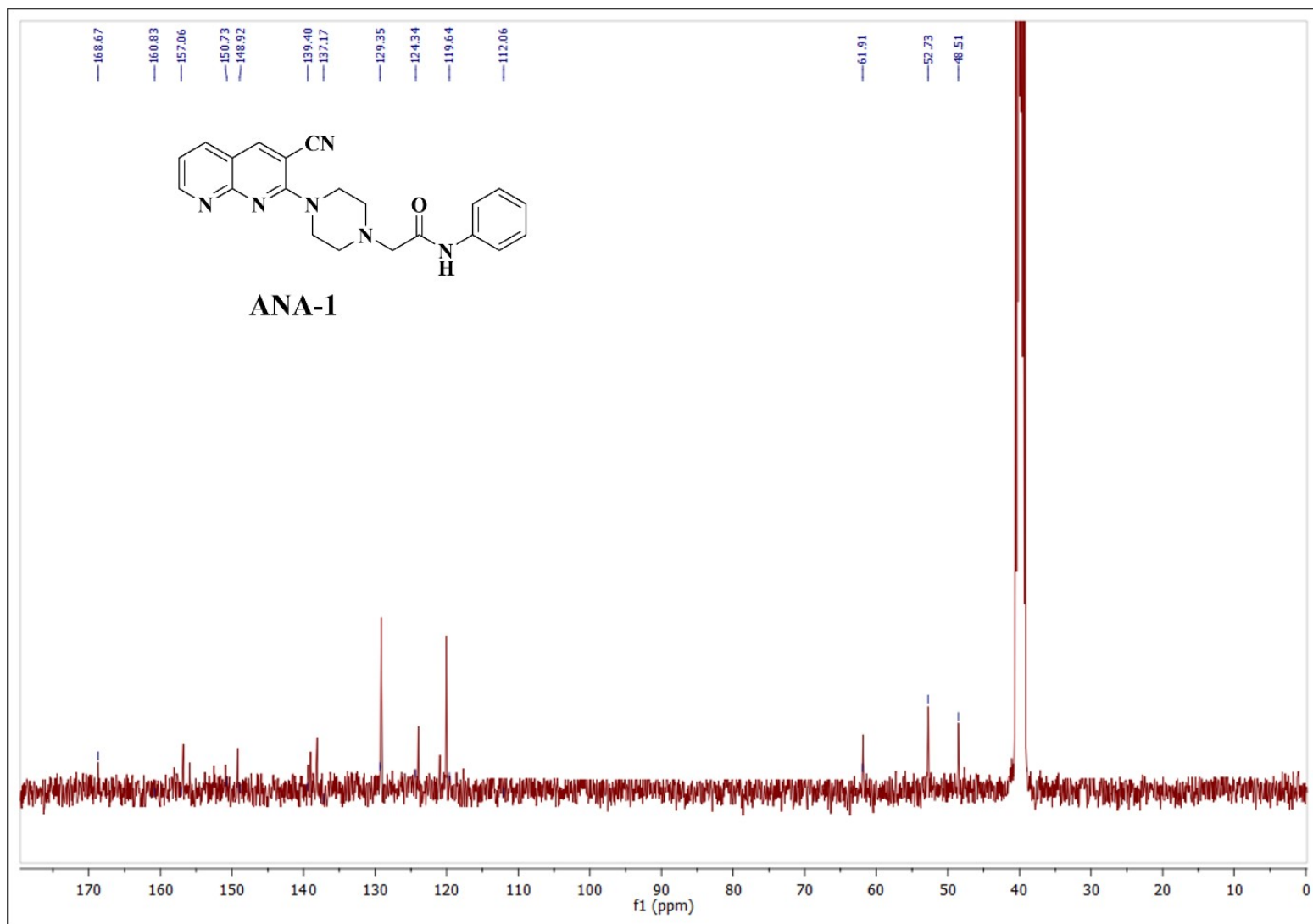
ANA-1

^{13}C NMR spectrum of compound ANA-1 (DMSO-d_6 101 MHz)
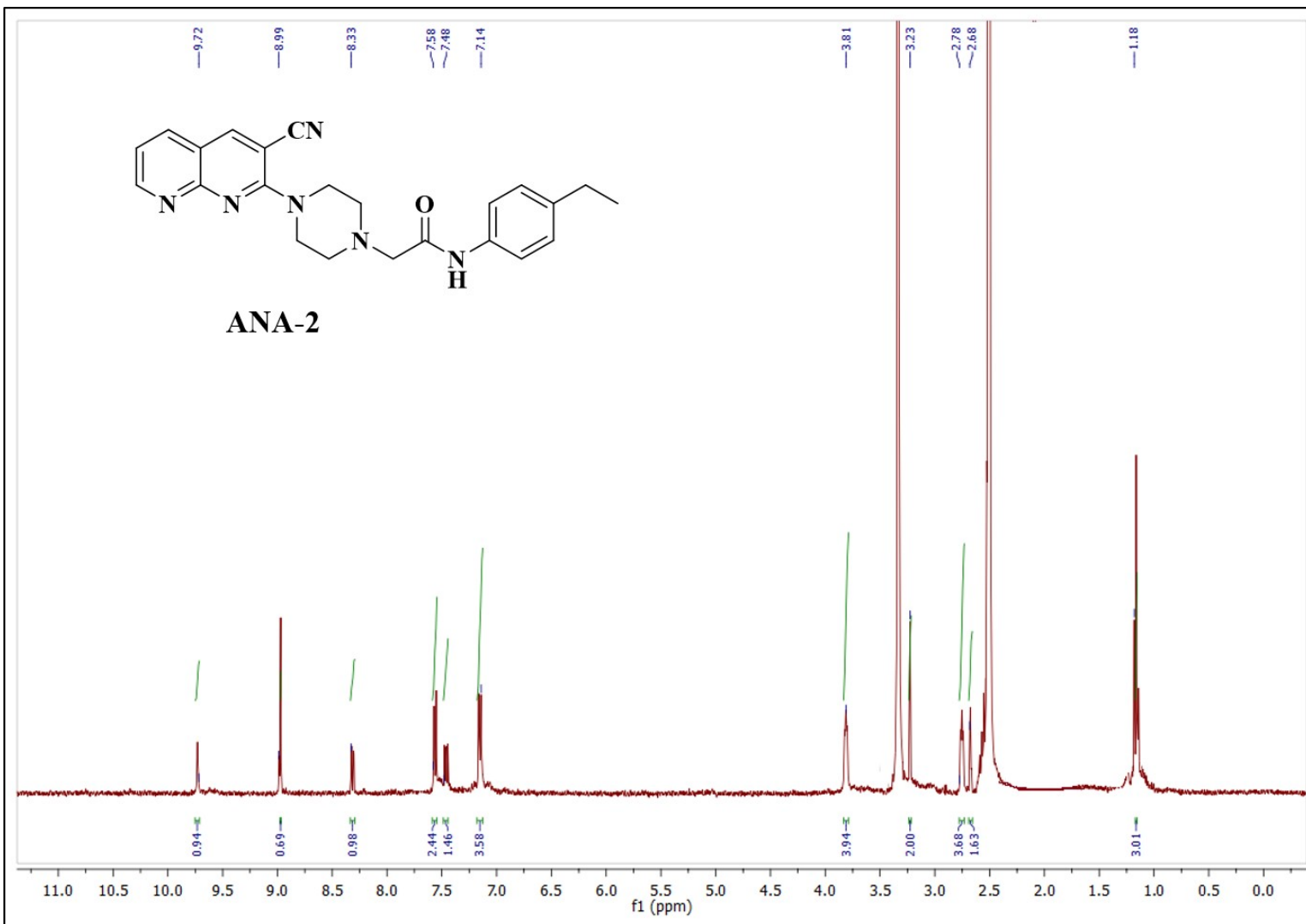
ANA-2

^{1}**H NMR** spectrum of compound **ANA-2** (DMSO-d_6 400 MHz)

Peaks: 168.67, 162.99, 159.58, 156.41, 151.73, 149.20, 146.38, 138.49, 137.54, 131.91, 121.84, 120.85, 117.09, 115.26, 110.78, 61.45, 52.88, 48.94, 36.26, 31.24
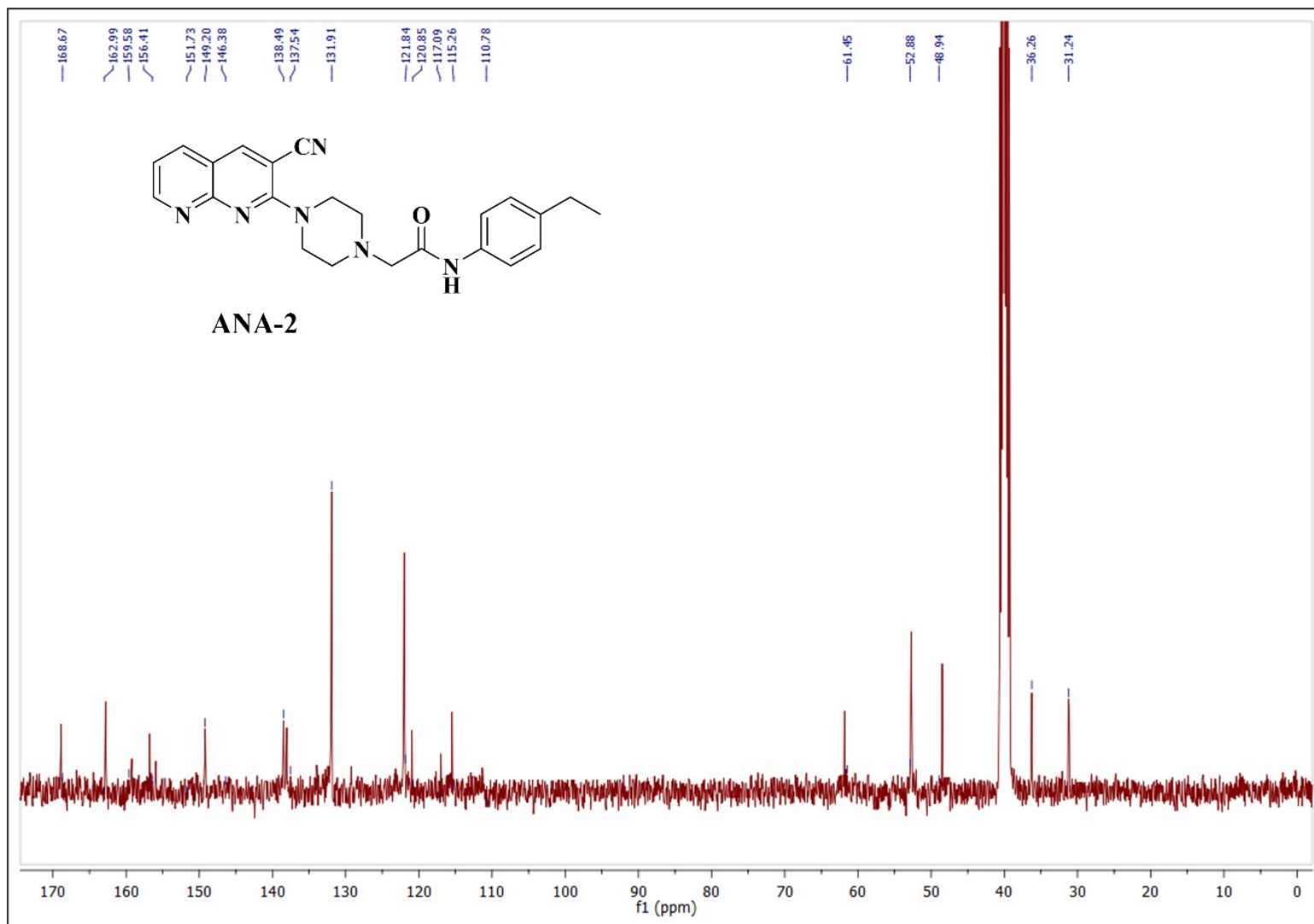
ANA-2

^{13}C NMR spectrum of compound ANA-2 (DMSO-d_6 101 MHz)
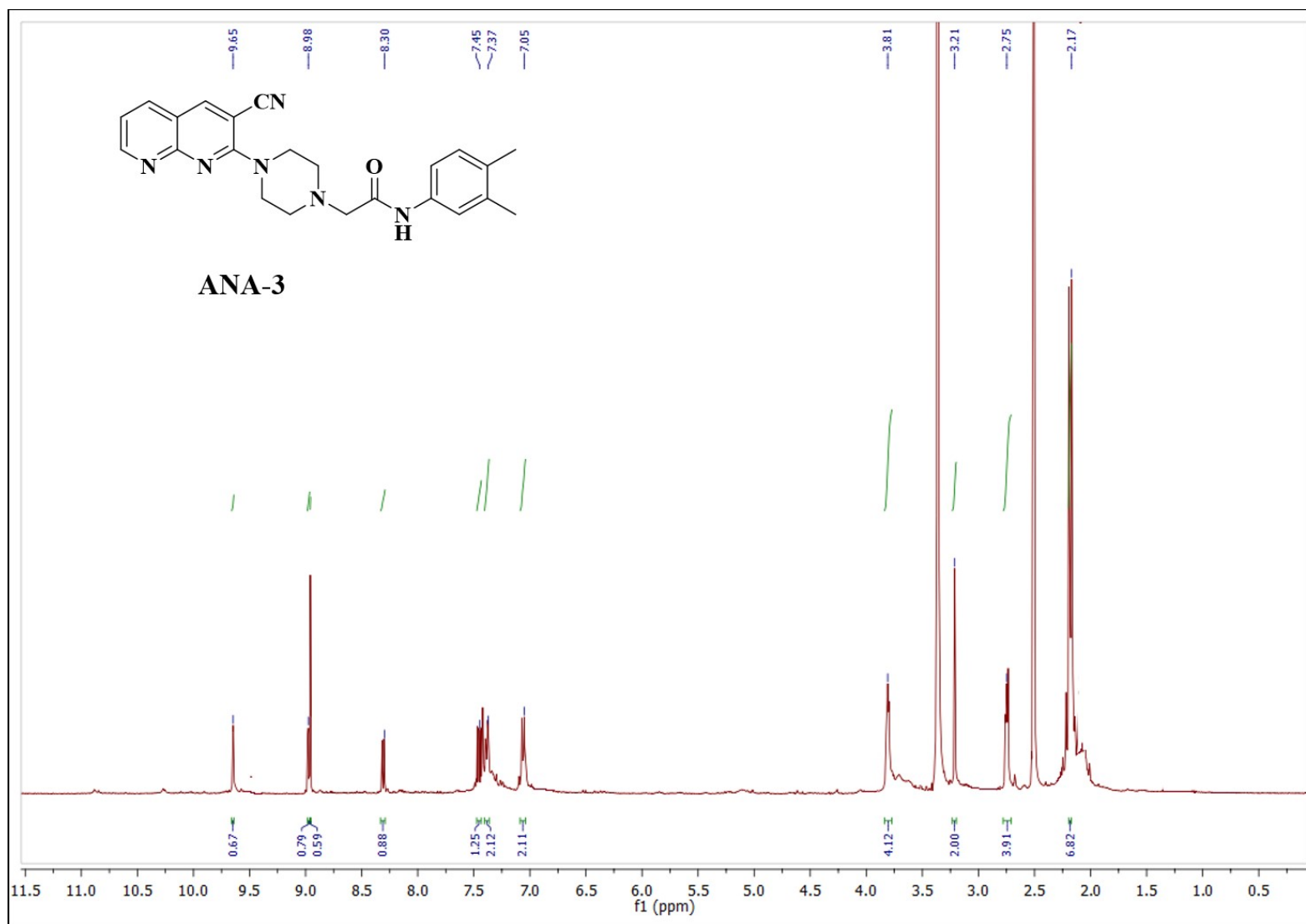
¹H NMR spectrum of compound ANA-3 (DMSO-*d₆* 400 MHz)
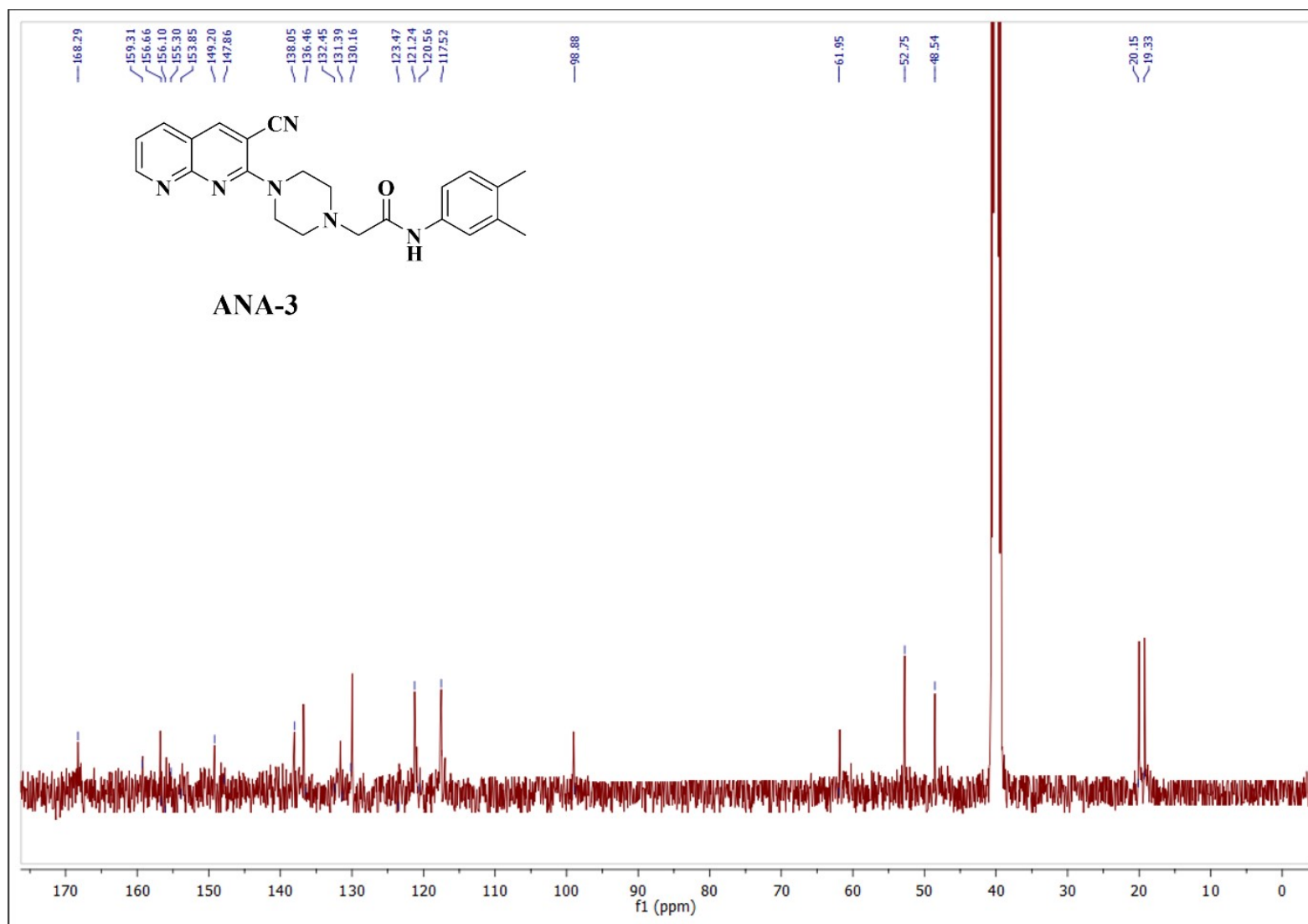
^{13}**C NMR** spectrum of compound **ANA-3** (DMSO-d_6 101 MHz)
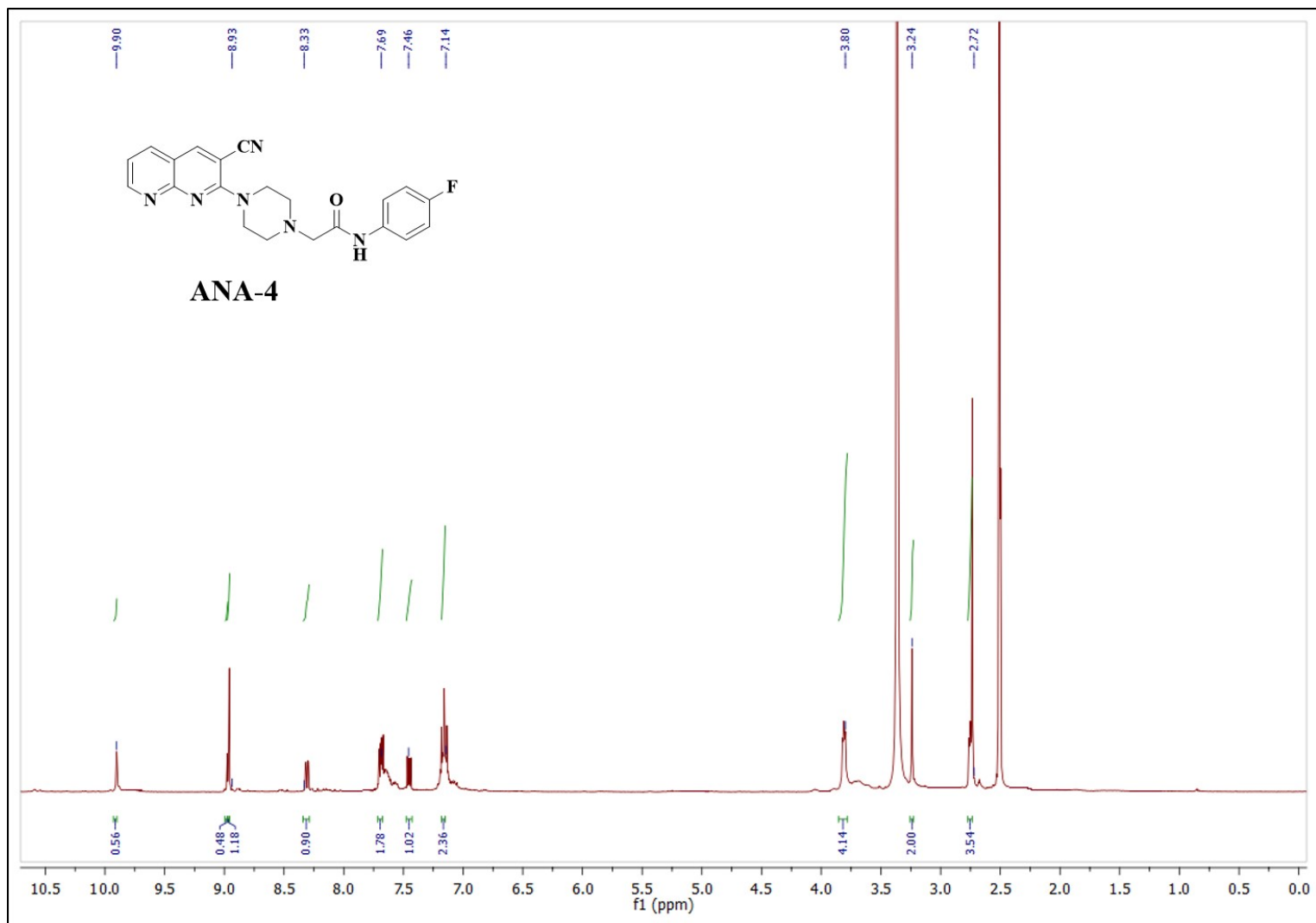
1H NMR spectrum of compound **ANA-4 (**DMSO-d_6 400 MHz)
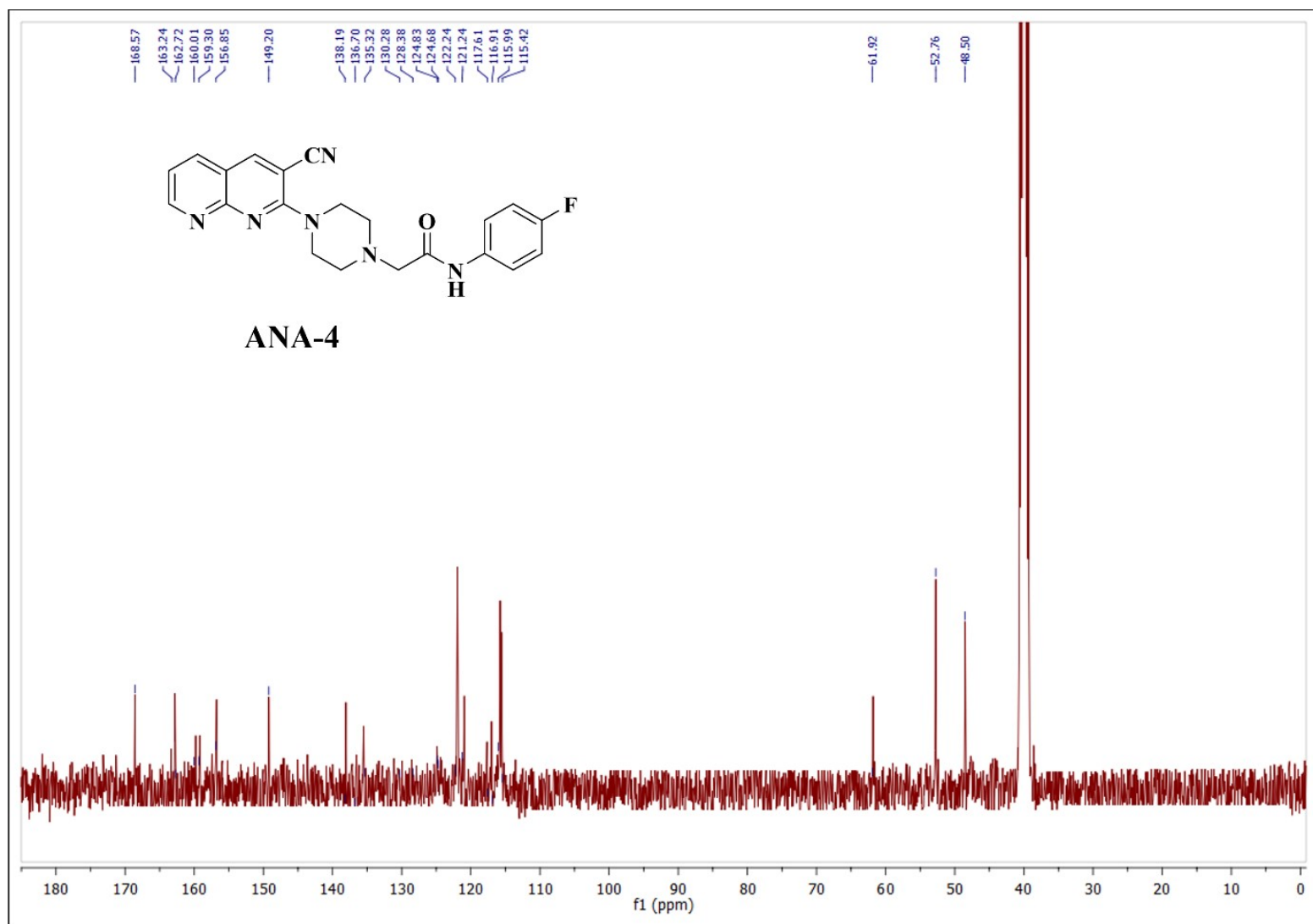
^{13}C NMR spectrum of compound ANA-4 (DMSO-d_6 101 MHz)
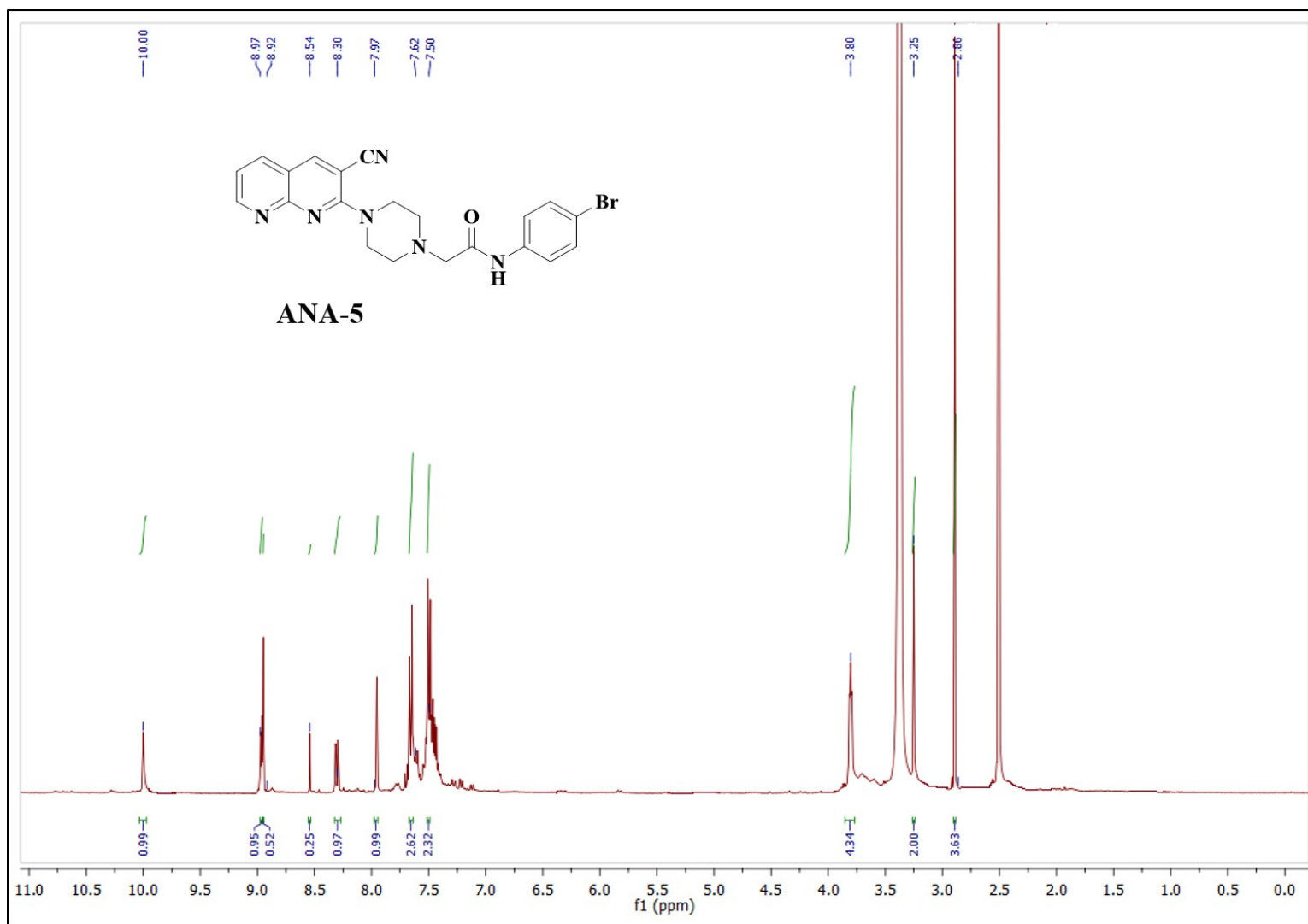
¹H NMR spectrum of compound **ANA-5** (DMSO-d_6 400 MHz)
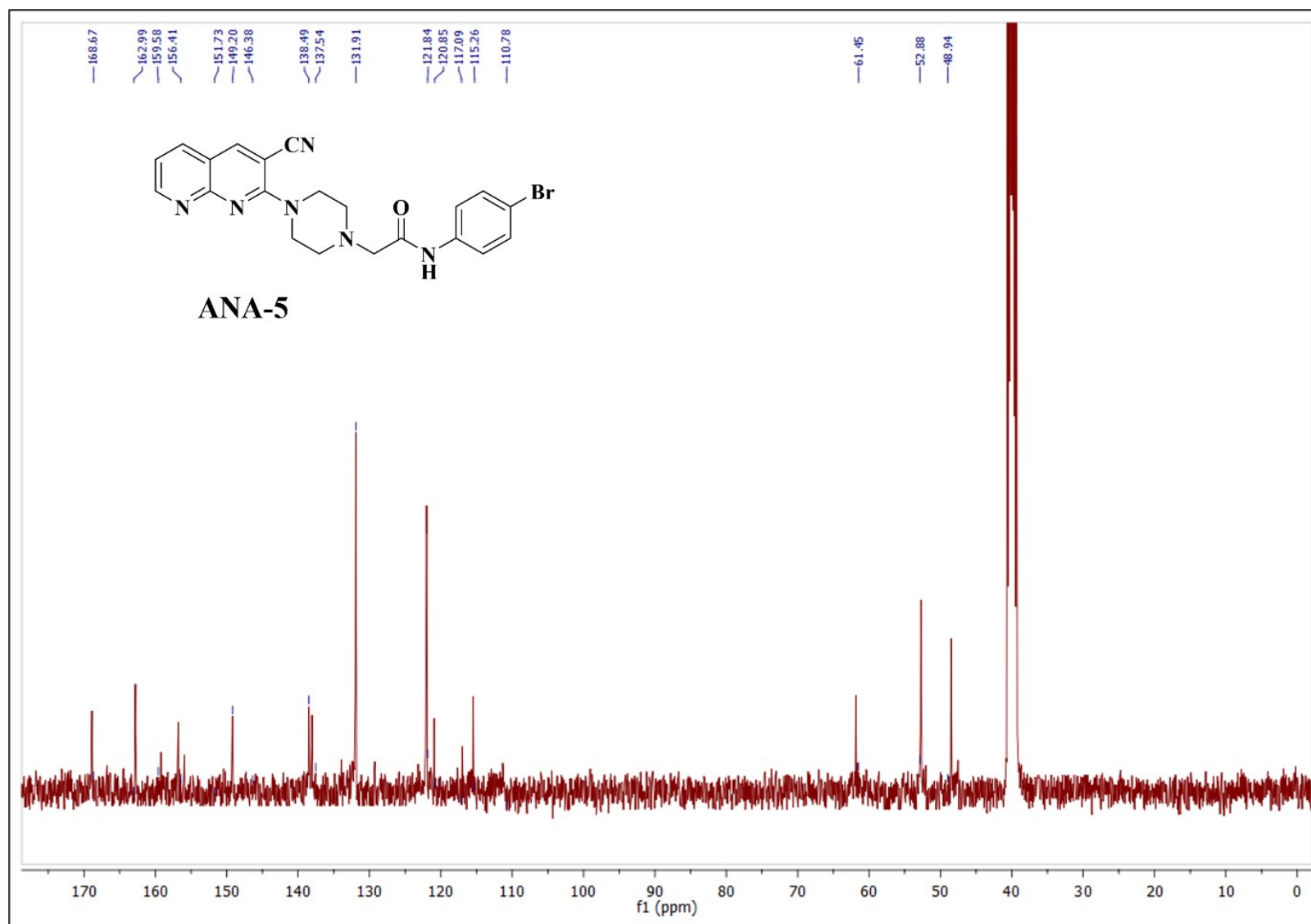
^{13}**C NMR** spectrum of compound **ANA-5** (DMSO-d_6 101 MHz)
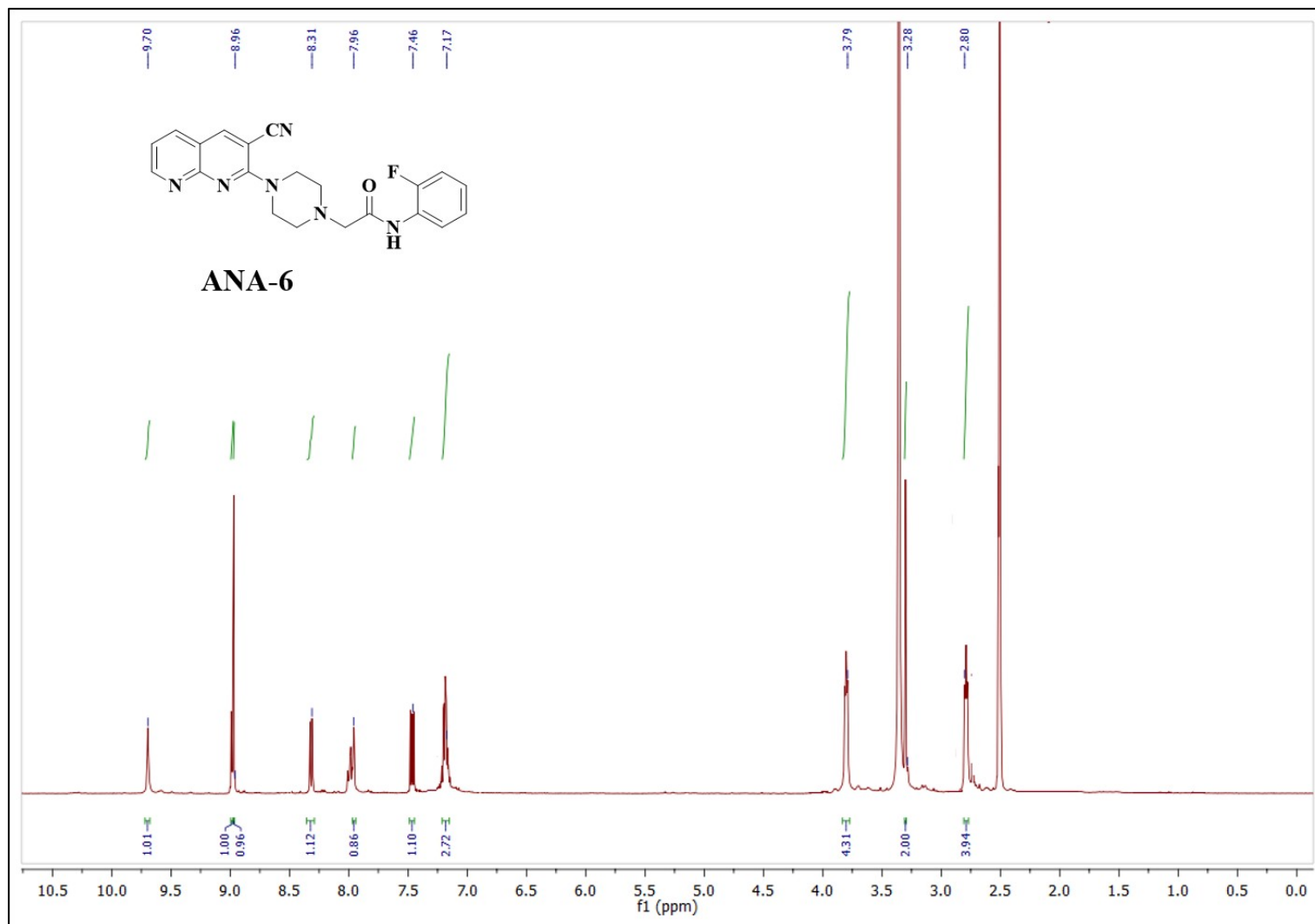
^{1}**H NMR** spectrum of compound **ANA-6 (**DMSO-d_6 400 MHz)
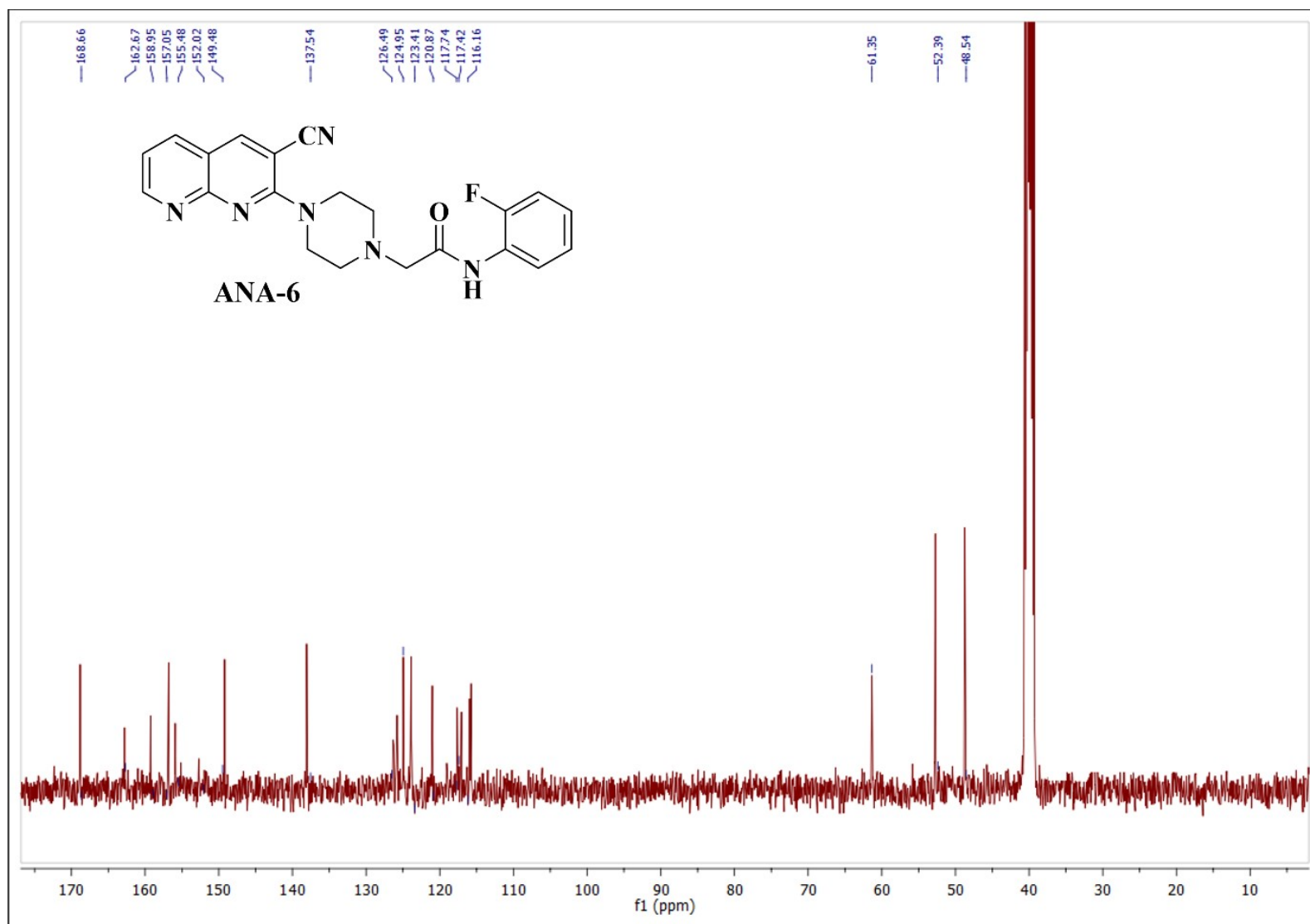
^{13}C NMR spectrum of compound ANA-6 (DMSO-d_6 101 MHz)
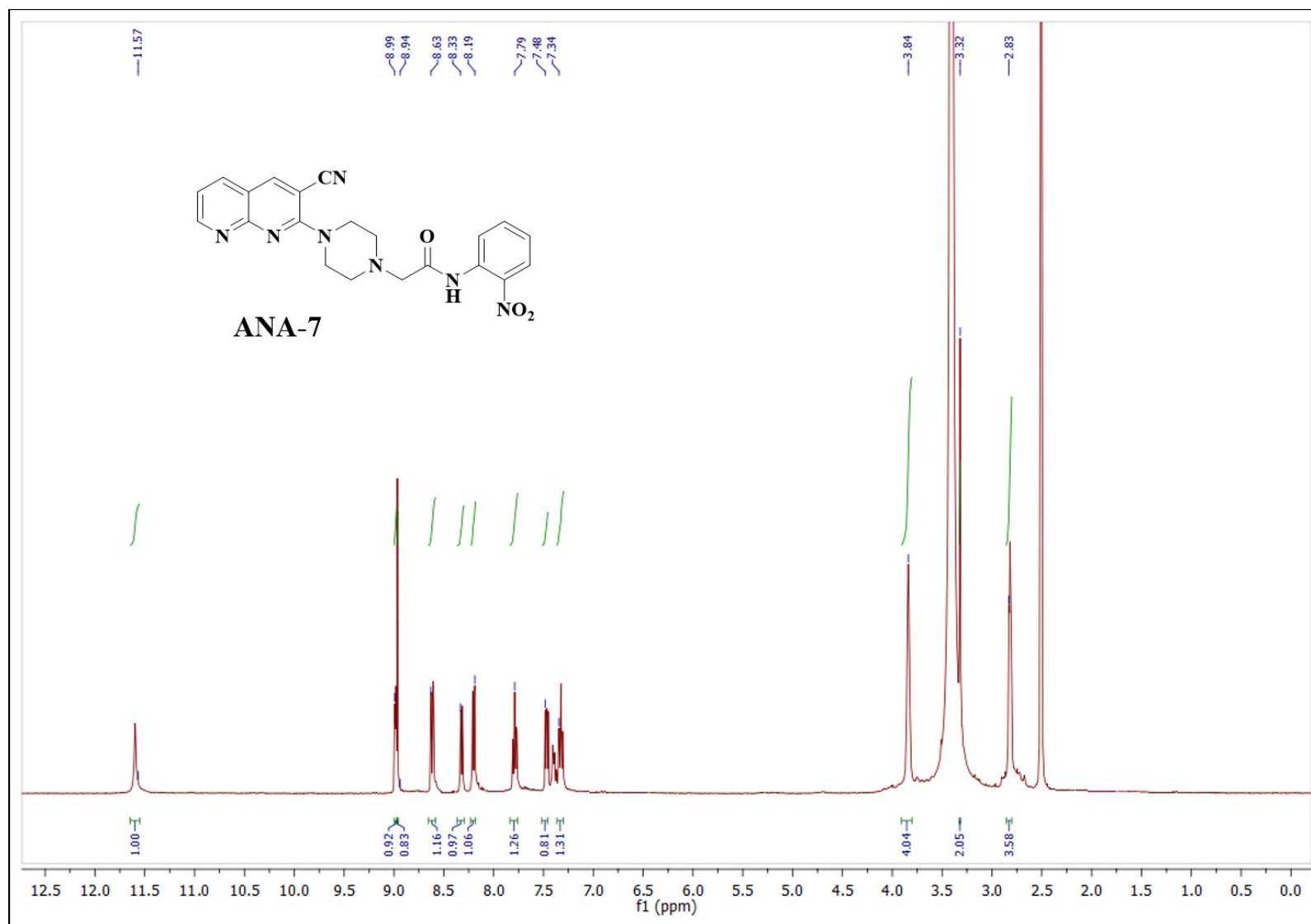
ANA-7

^{1}**H NMR** spectrum of compound **ANA-7 (**DMSO-d_6 400 MHz)
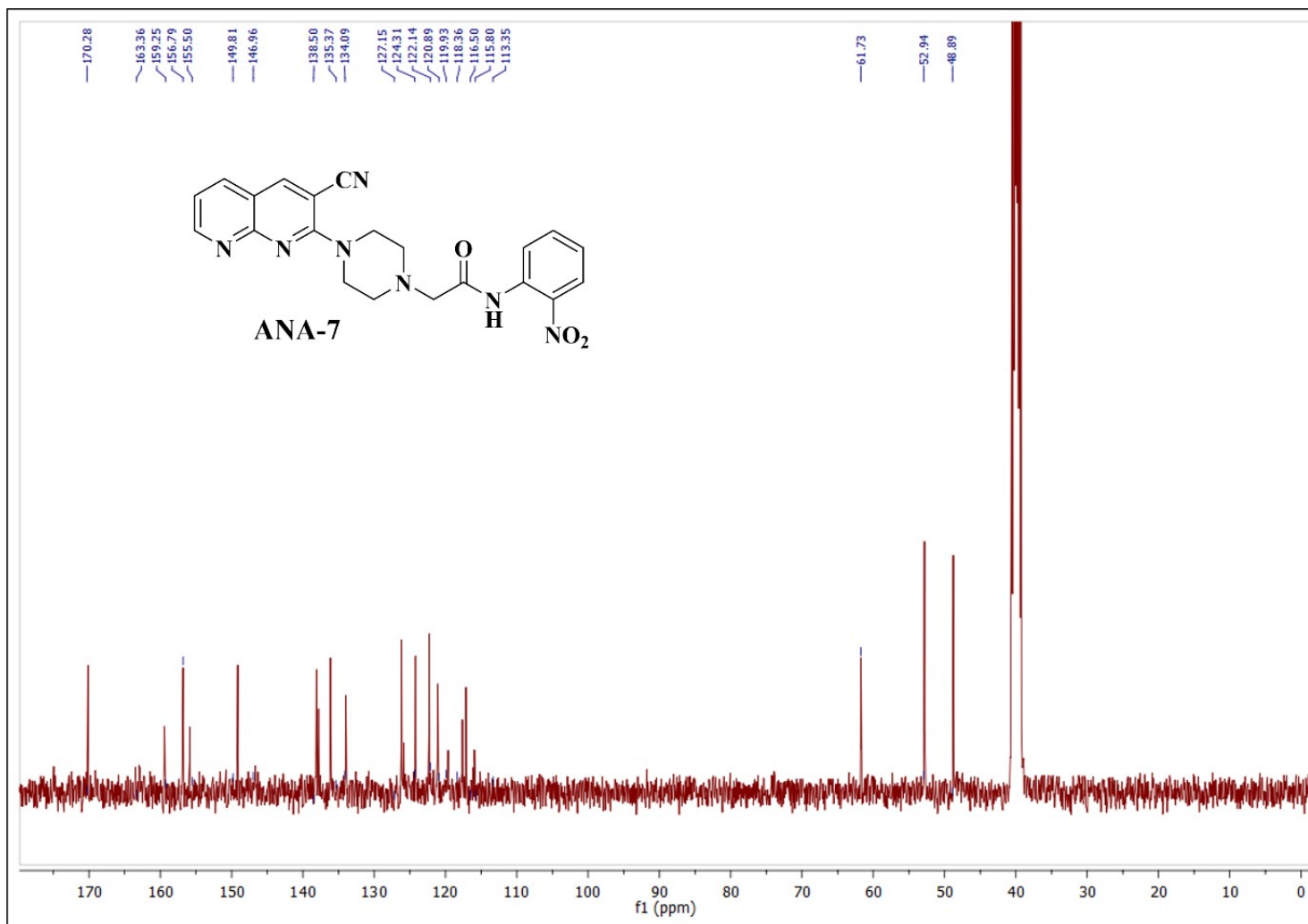
¹³C NMR spectrum of compound **ANA-7** (DMSO-*d₆* 101 MHz)
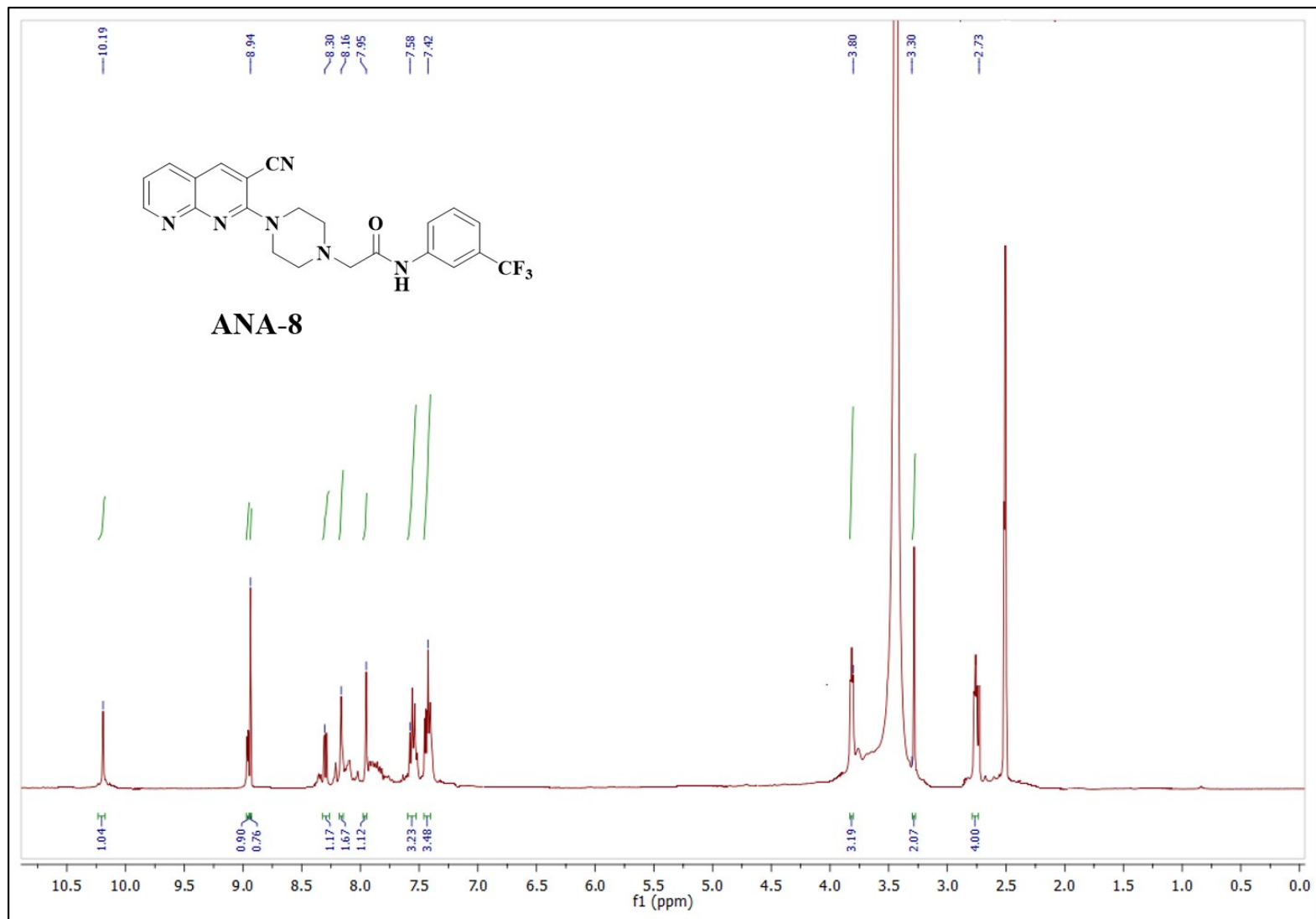
^{1}**H NMR** spectrum of compound **ANA-8 (**DMSO-d_6 400 MHz)
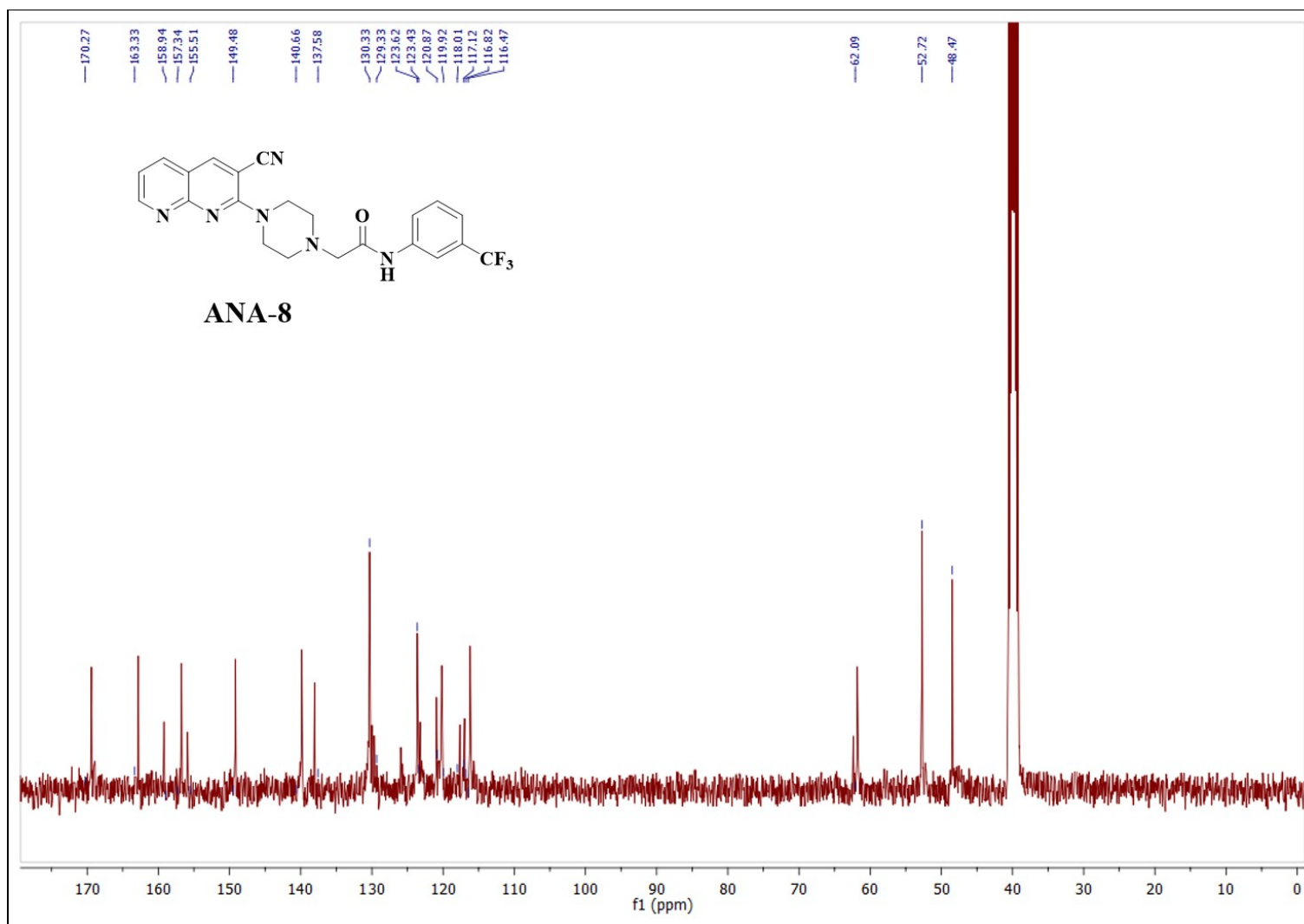
^{13}C **NMR** spectrum of compound **ANA-8** (DMSO-d_6 101 MHz)
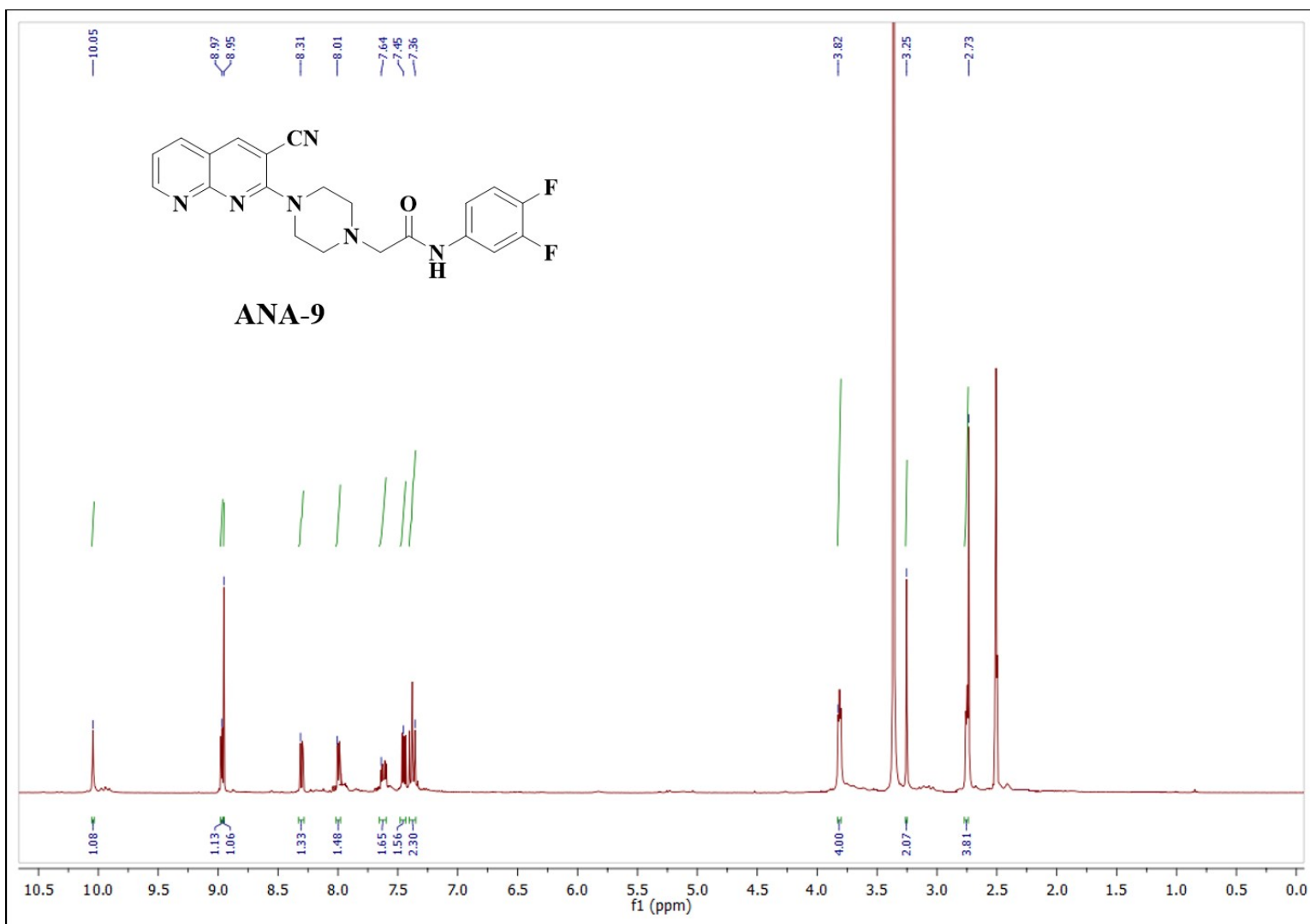
^{1}**H NMR** spectrum of compound **ANA-9 (**DMSO-d_6 400 MHz)
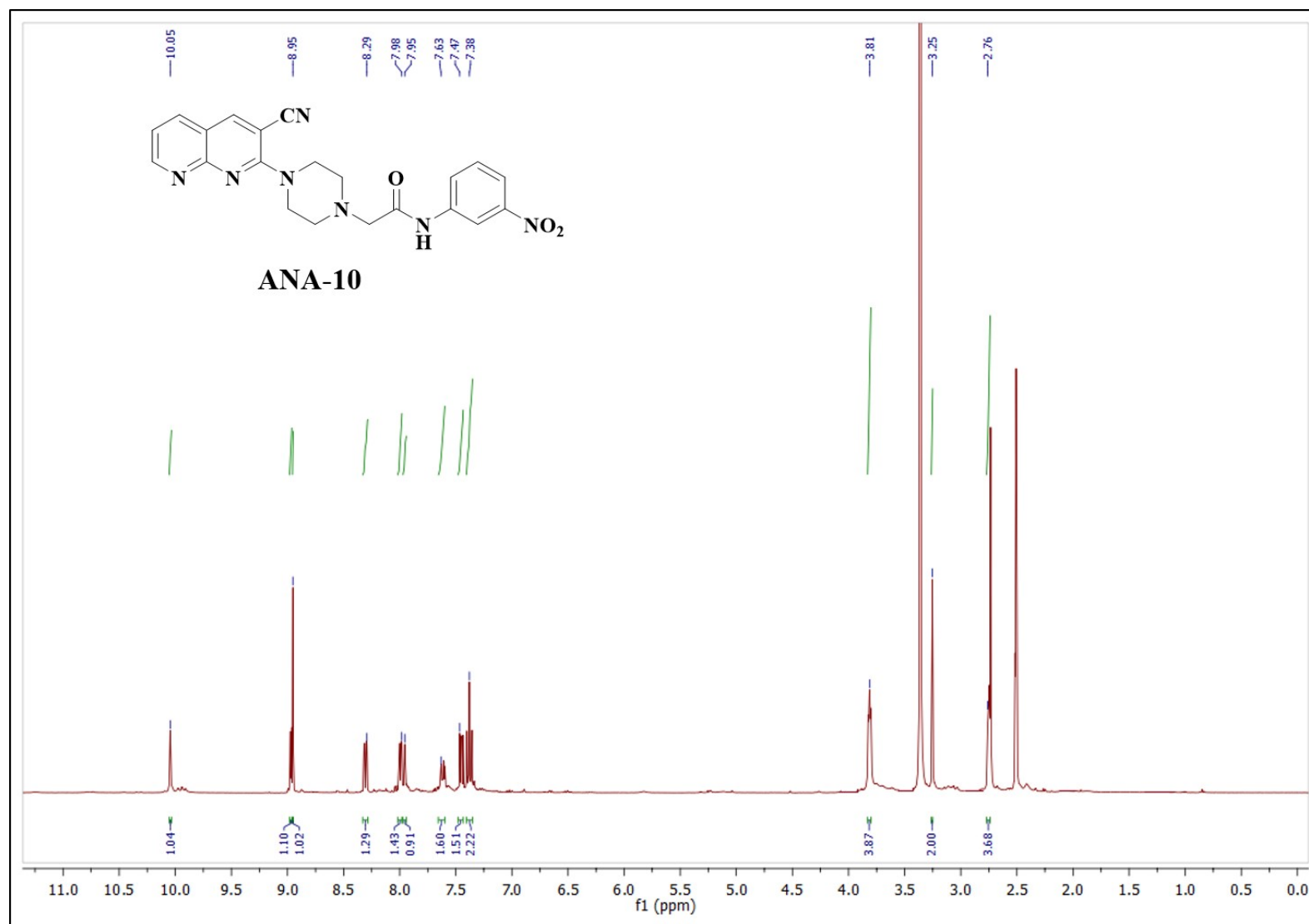
^{1}**H NMR** spectrum of compound **ANA-10** (DMSO-d_6 400 MHz)

ANA-11

^{1}H NMR spectrum of compound ANA-11 (DMSO-d$_6$ 400 MHz)
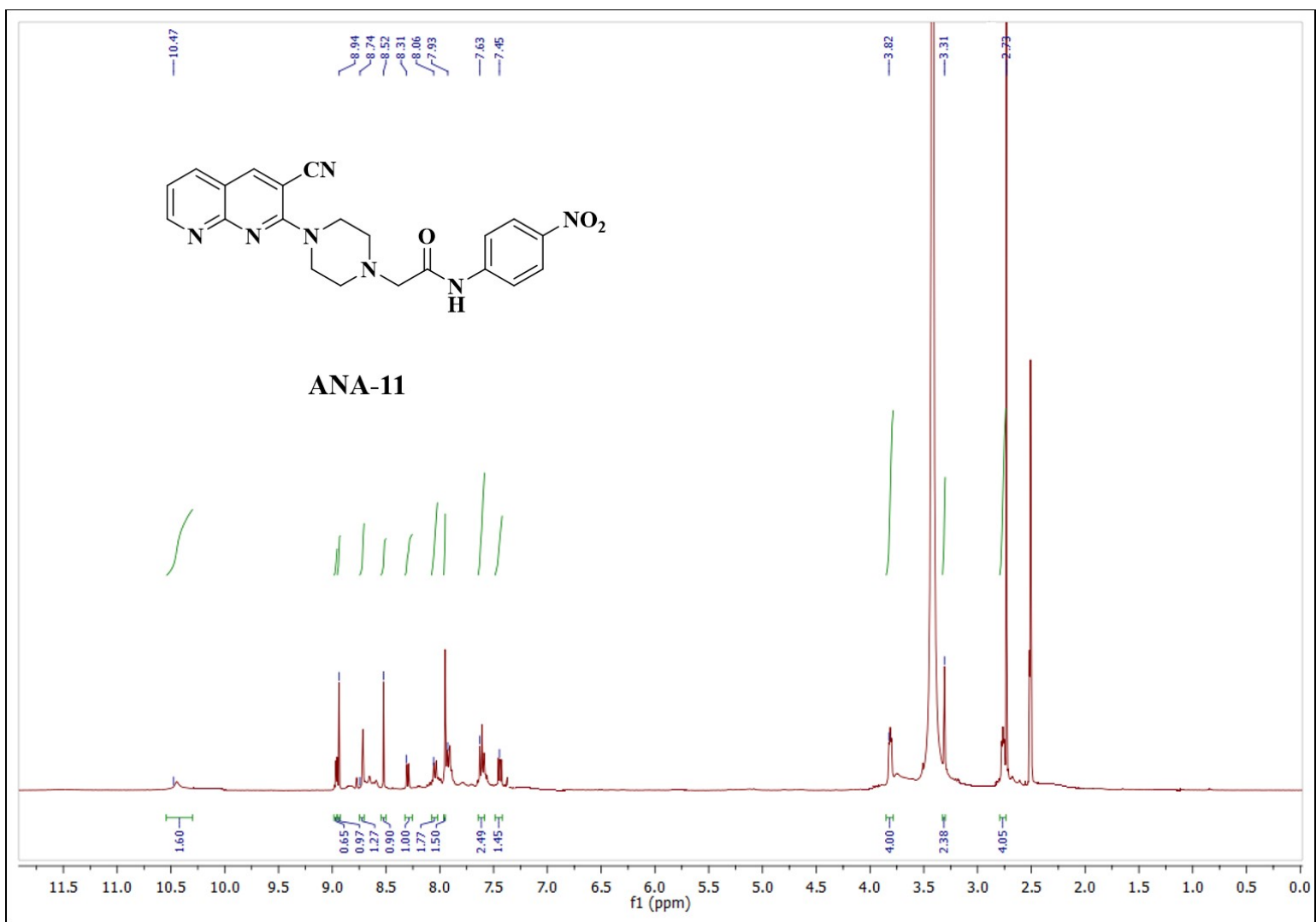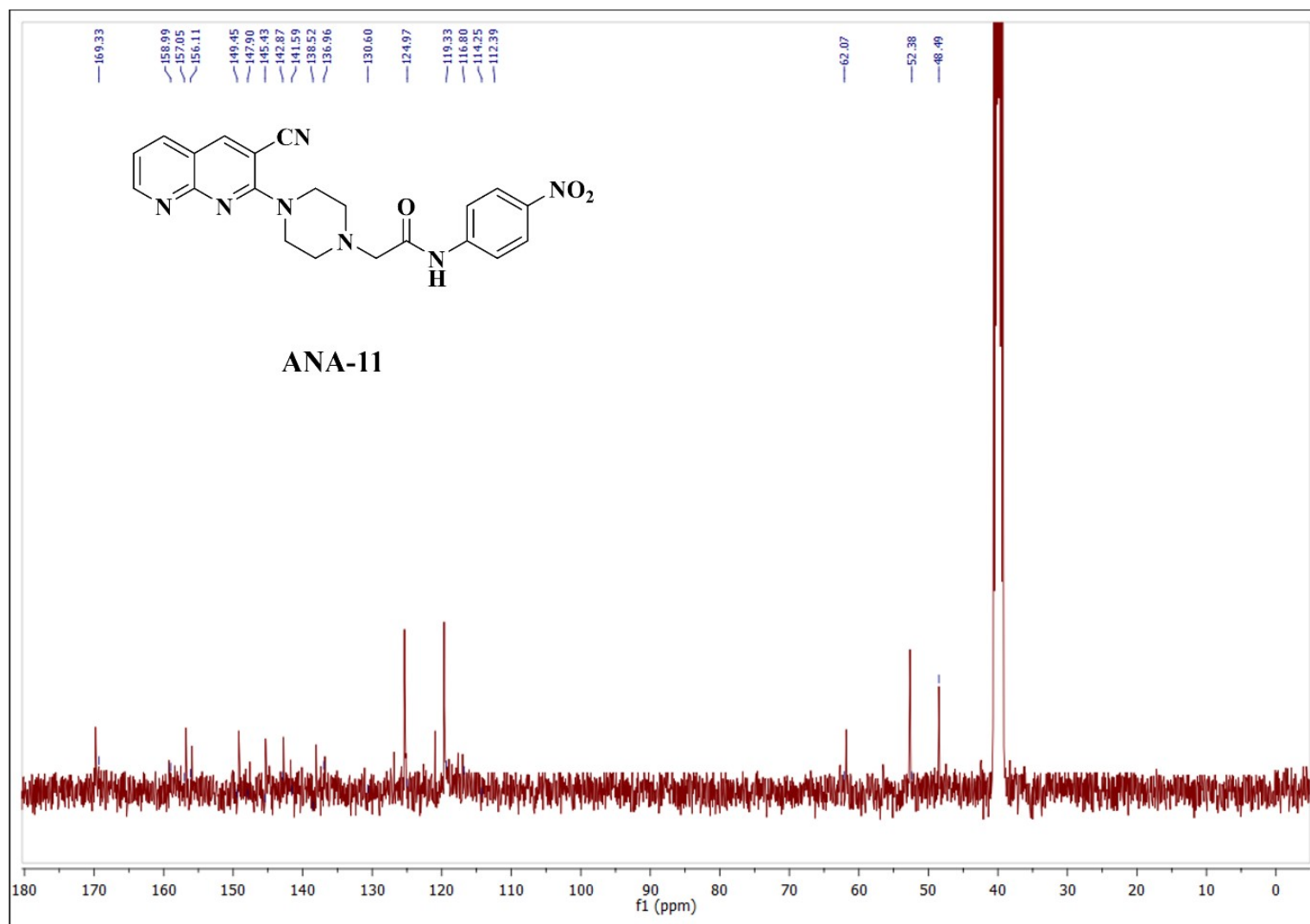
^{13}C NMR spectrum of compound ANA-11 (DMSO-d_6 101 MHz)
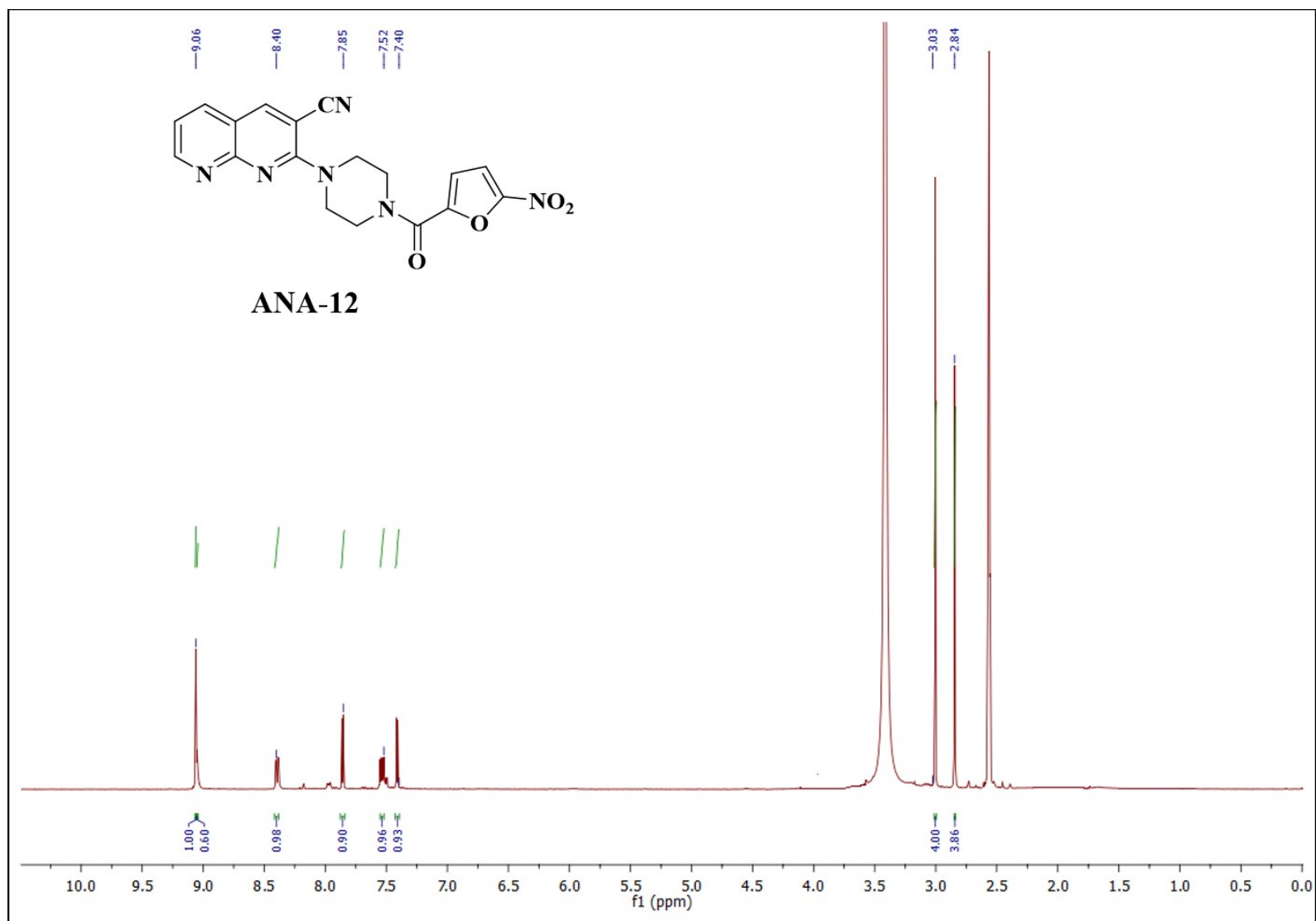
^{1}**H NMR** spectrum of compound **ANA-12** (DMSO-d_6 400 MHz)
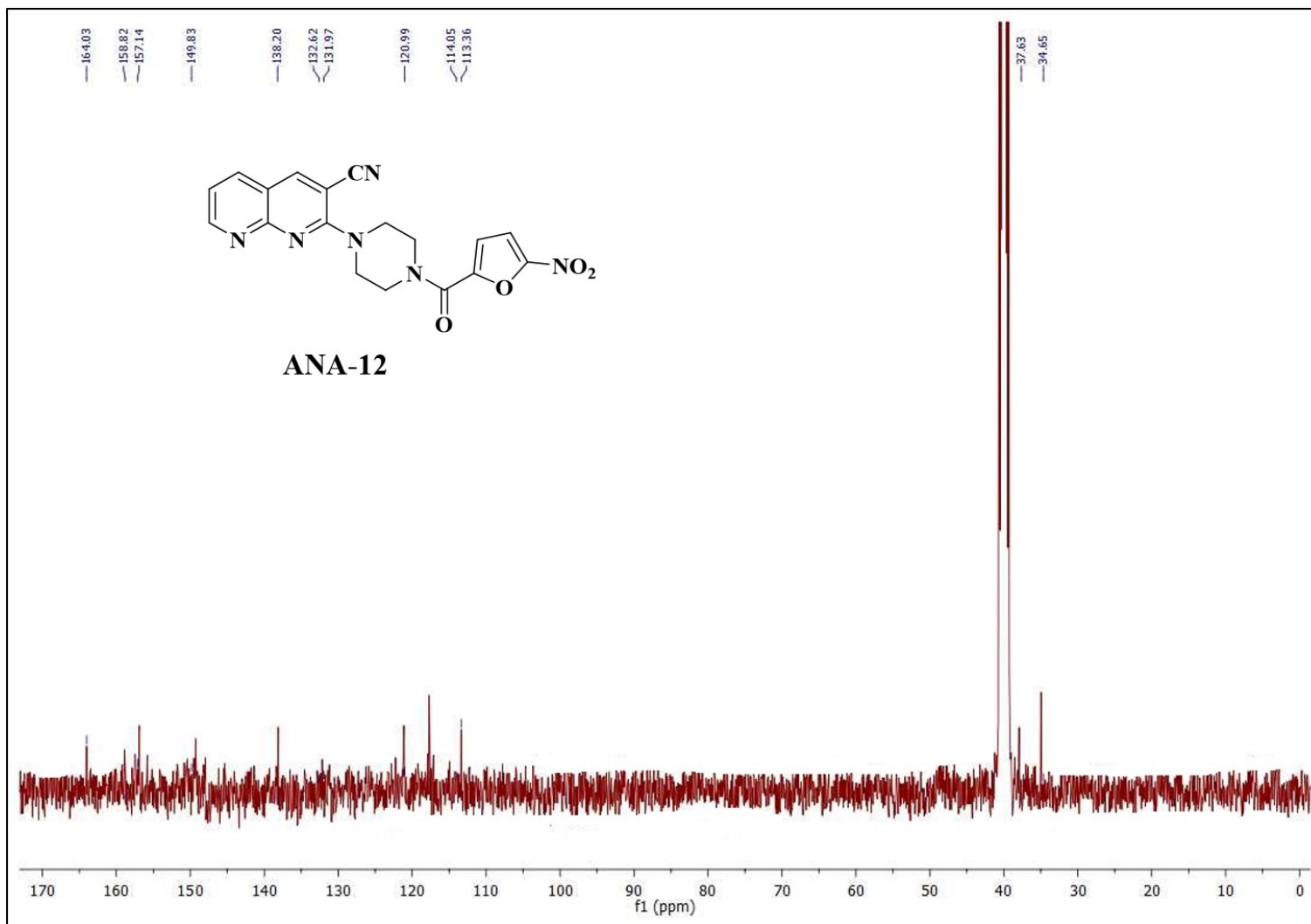
13C NMR spectrum of compound **ANA-12** (DMSO-d_6 101 MHz)

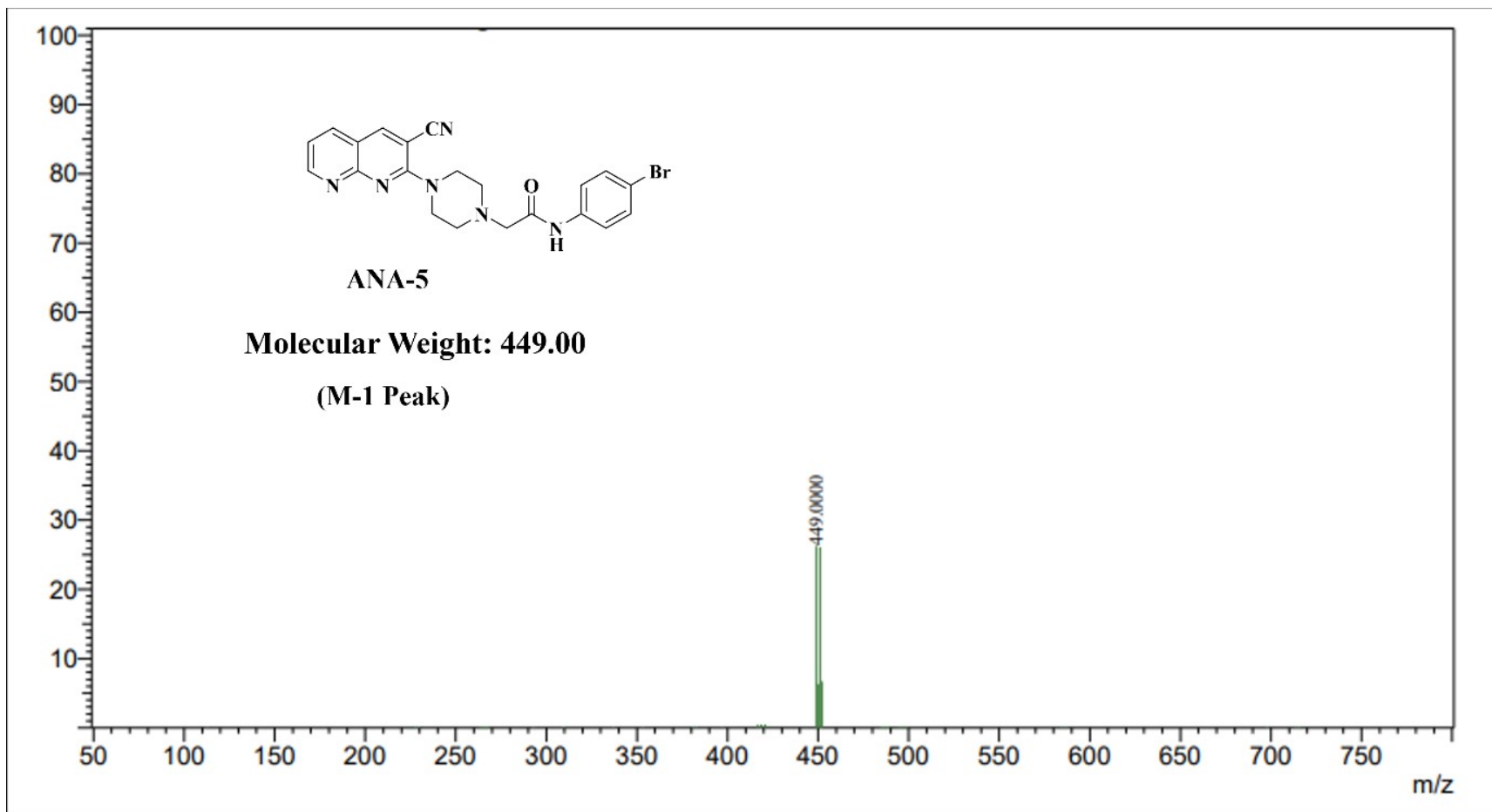## 6. Mass spectra of final compounds (ANI 1-2, ANC 1-14 and ANA 1-12):
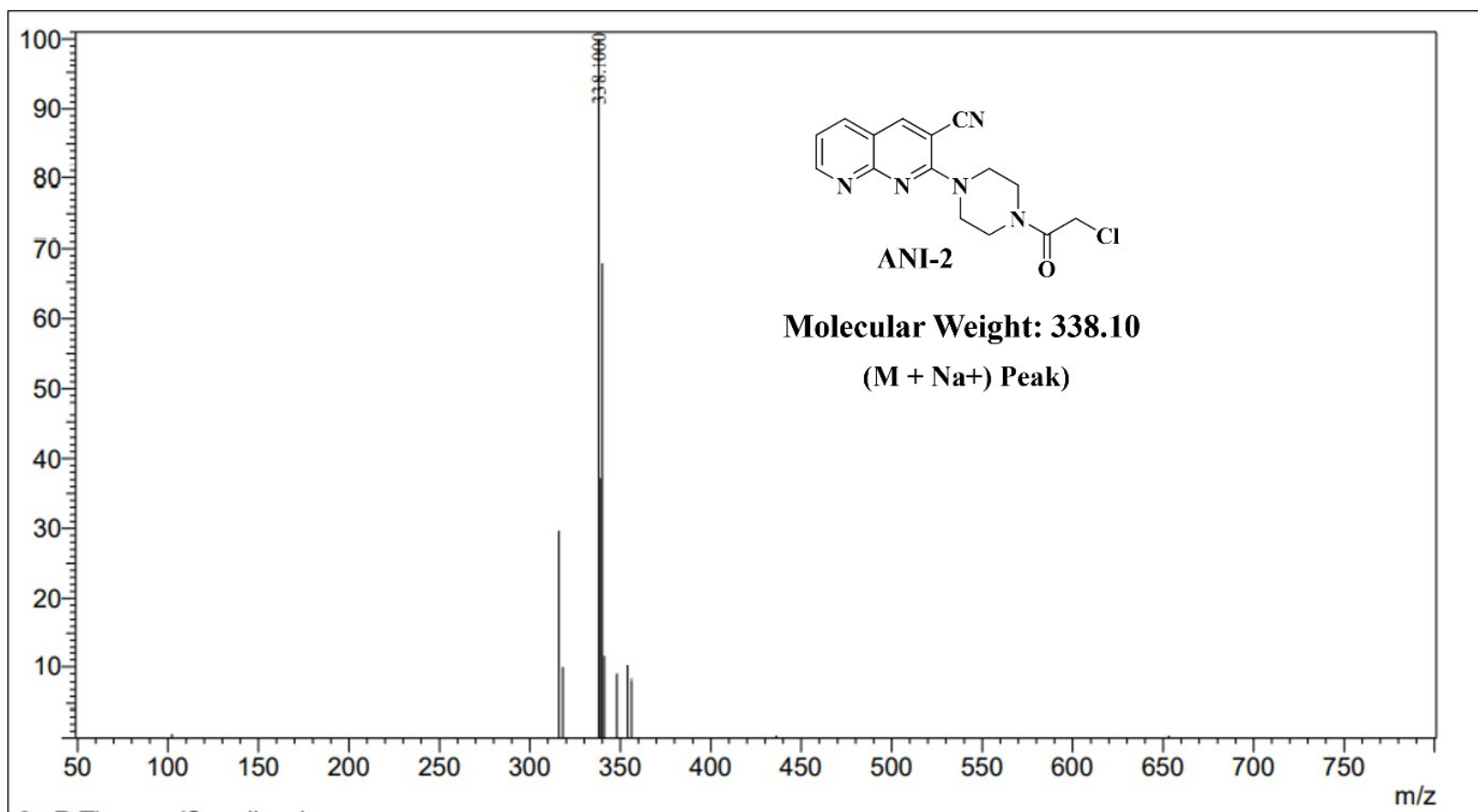
Mass spectrum of compound ANI-2
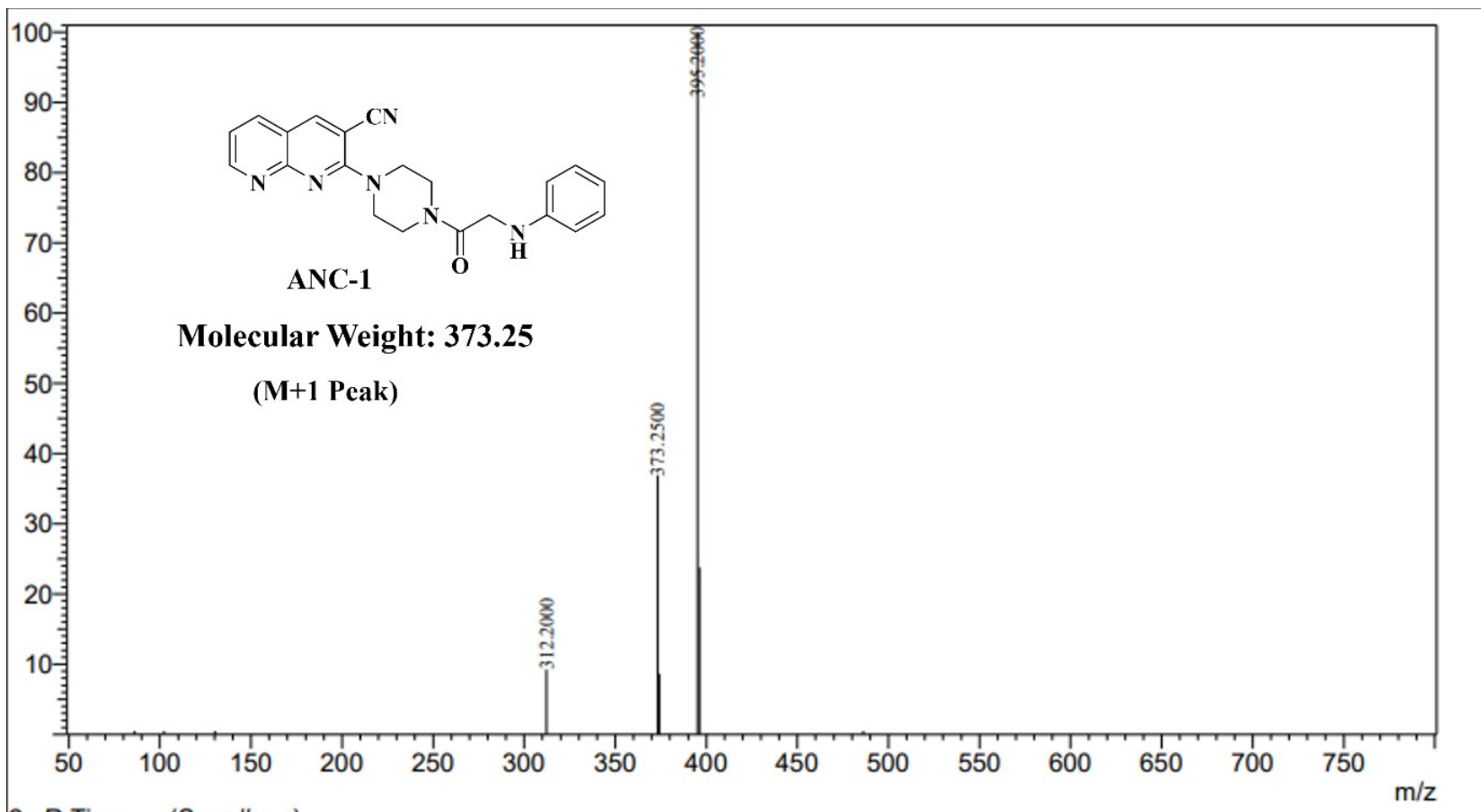
Mass spectrum of compound ANC-1
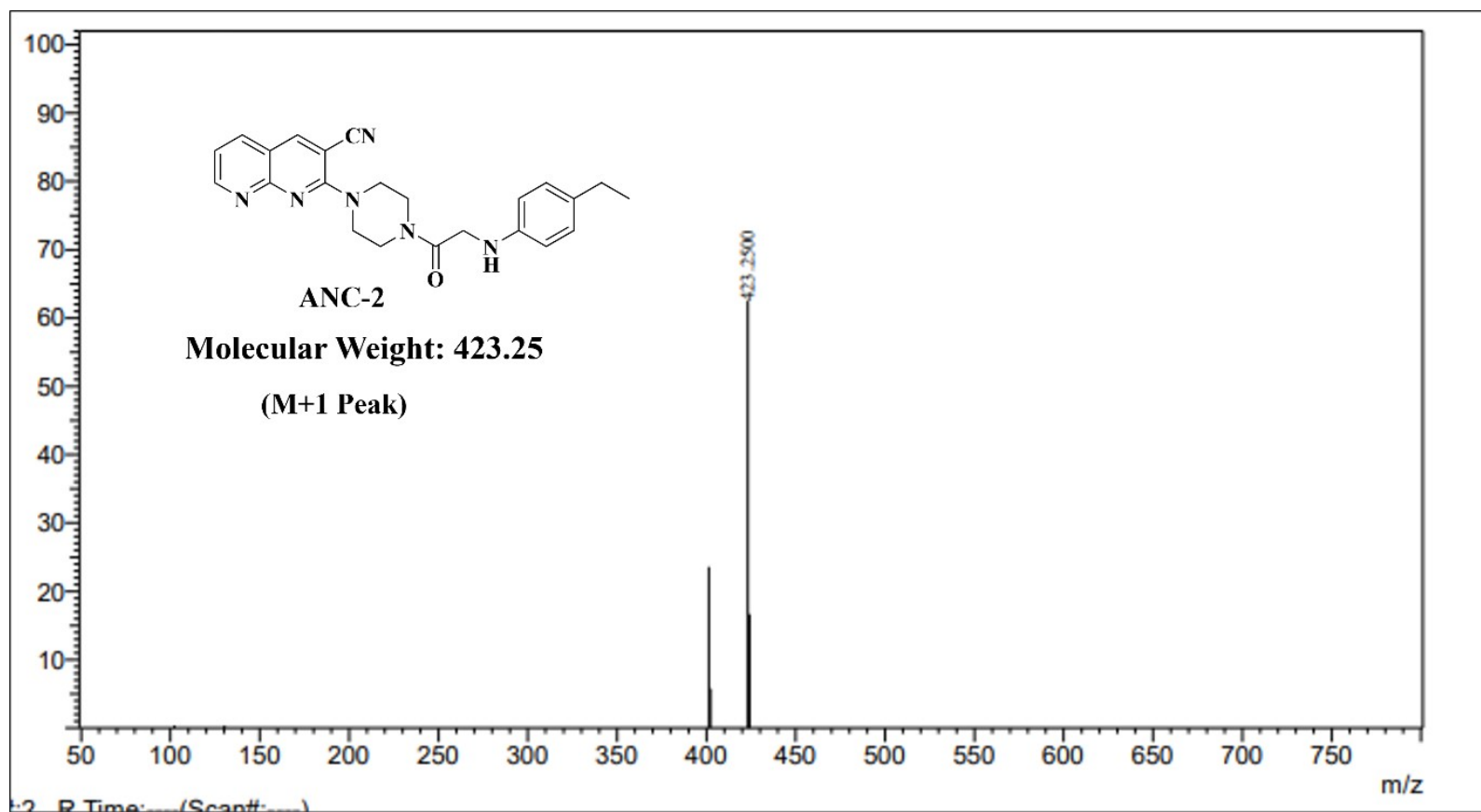
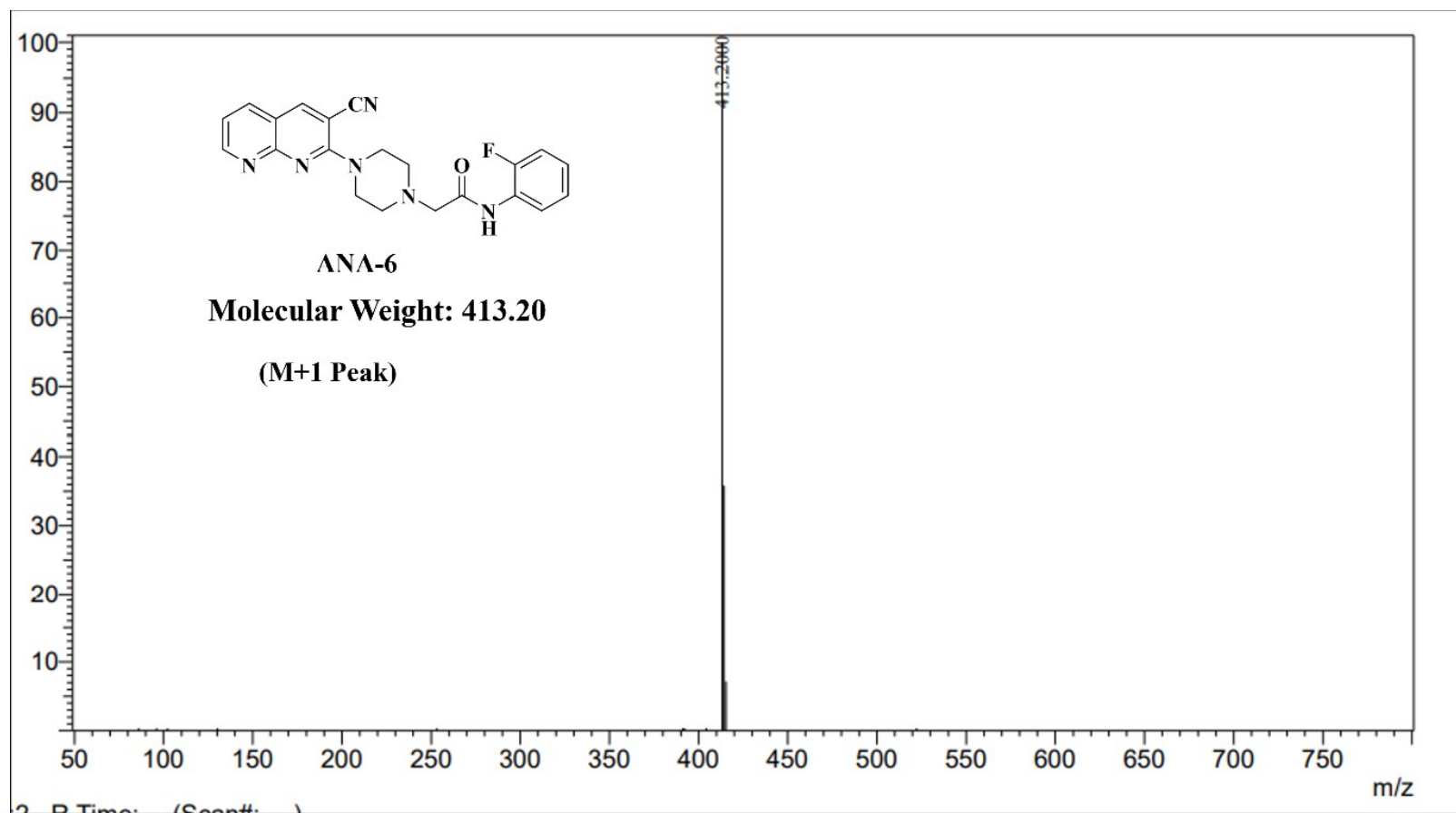**Mass spectrum of compound ANC-2**
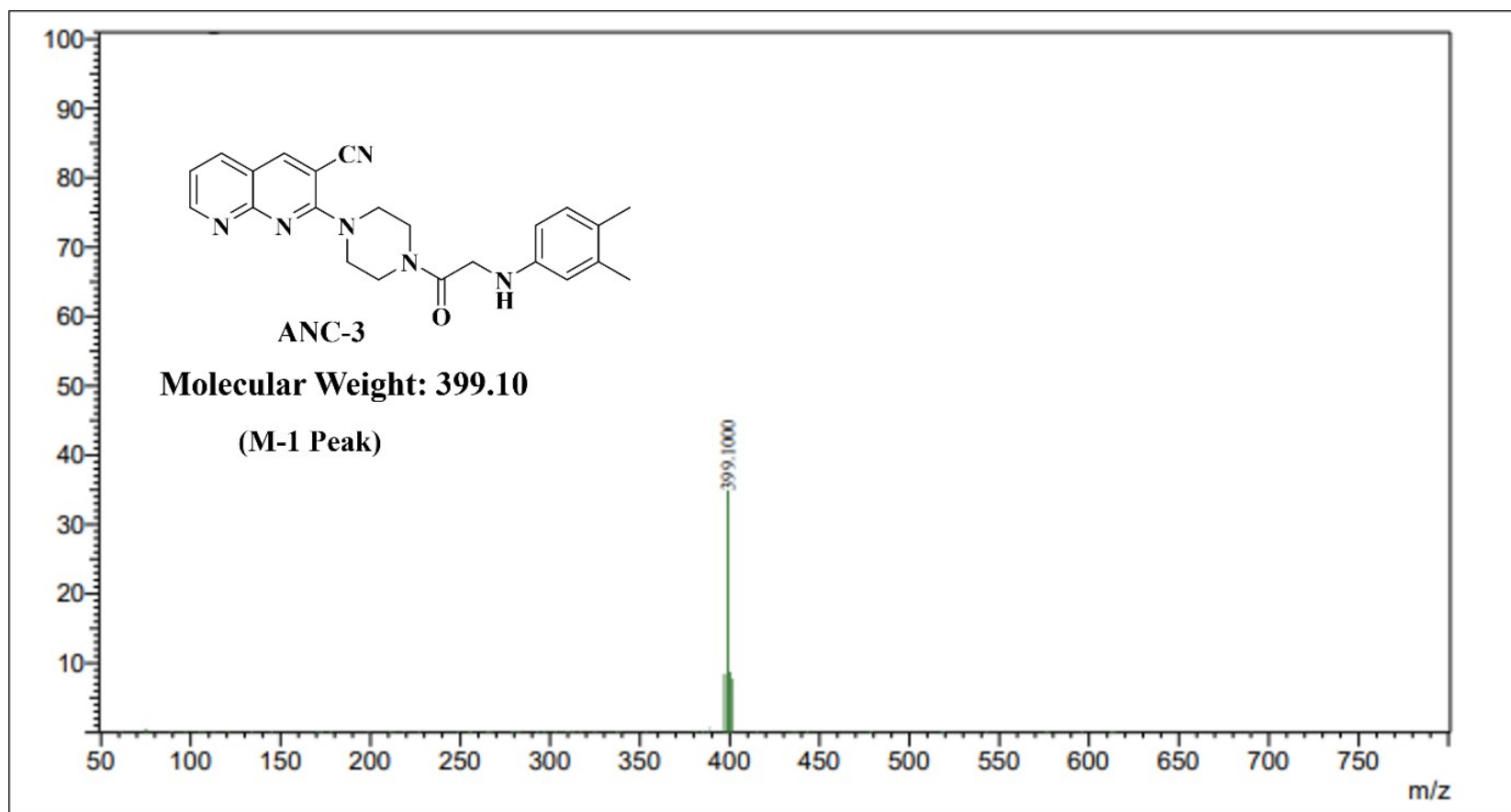
Mass spectrum of compound ANC-3

ANC-4

Molecular Weight: 423.20

(M+1 Peak)

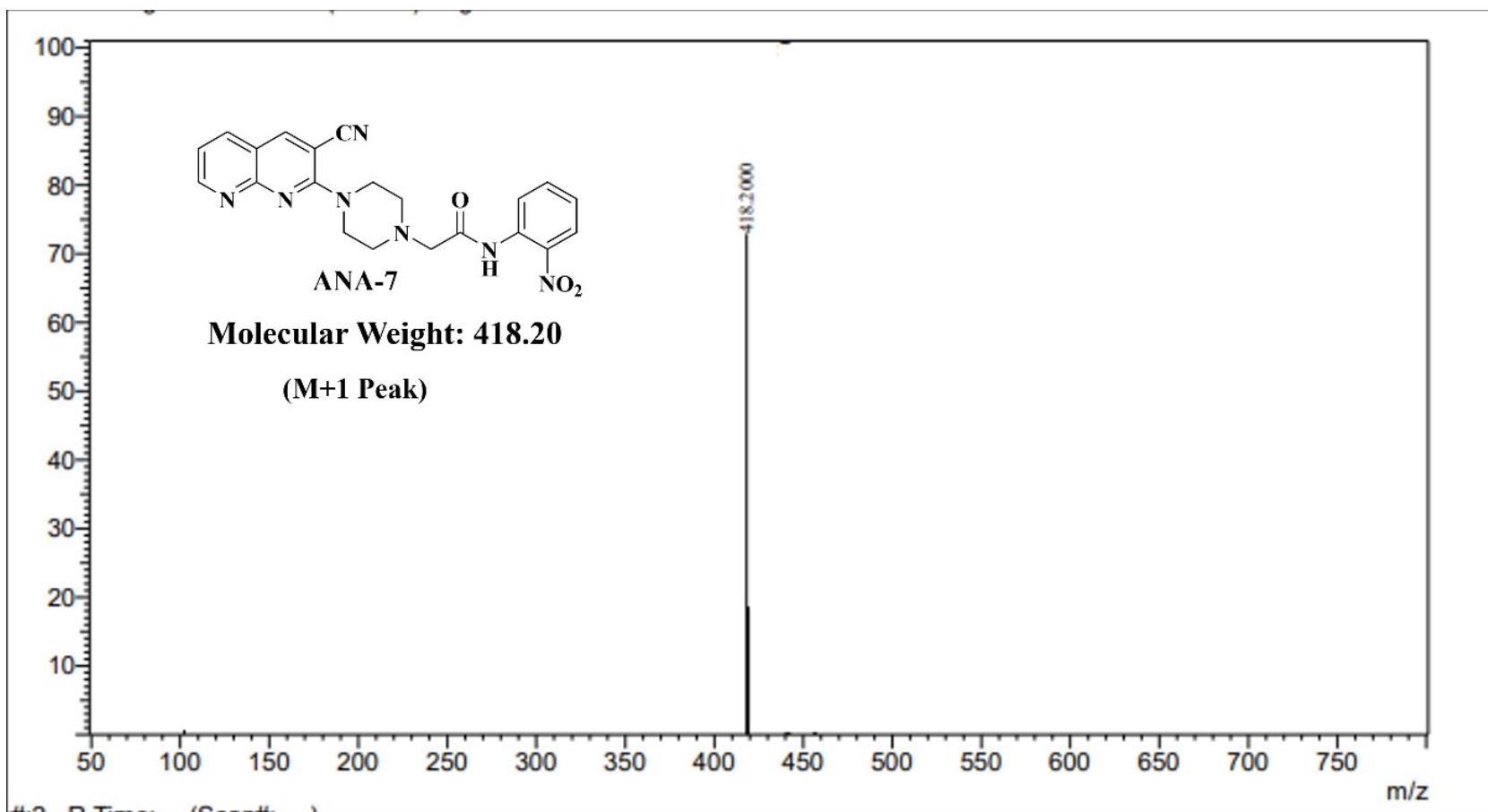Mass spectrum of compound ANC-4

ANC-5

Molecular Weight: 389.05

(M-1 Peak)
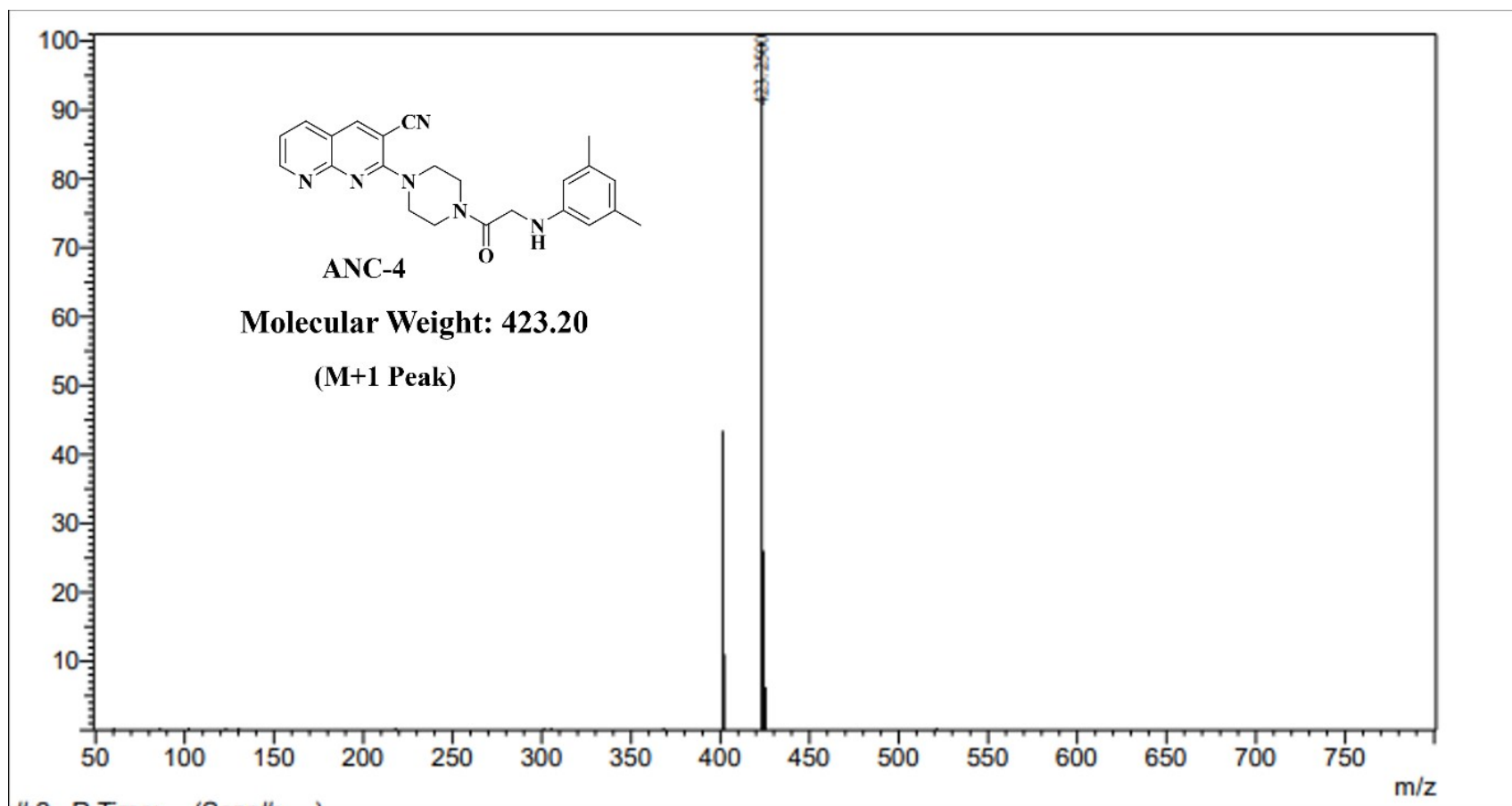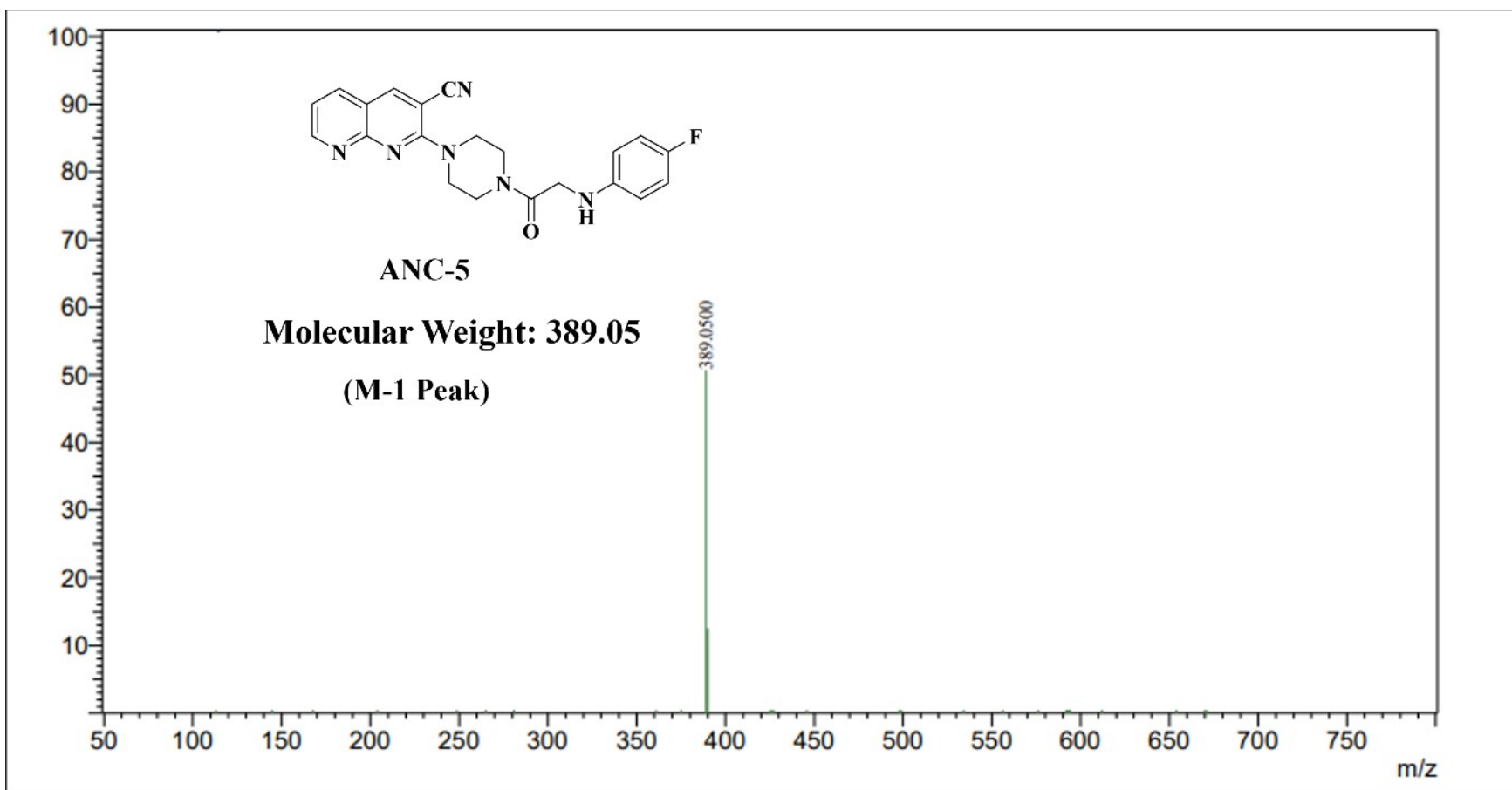
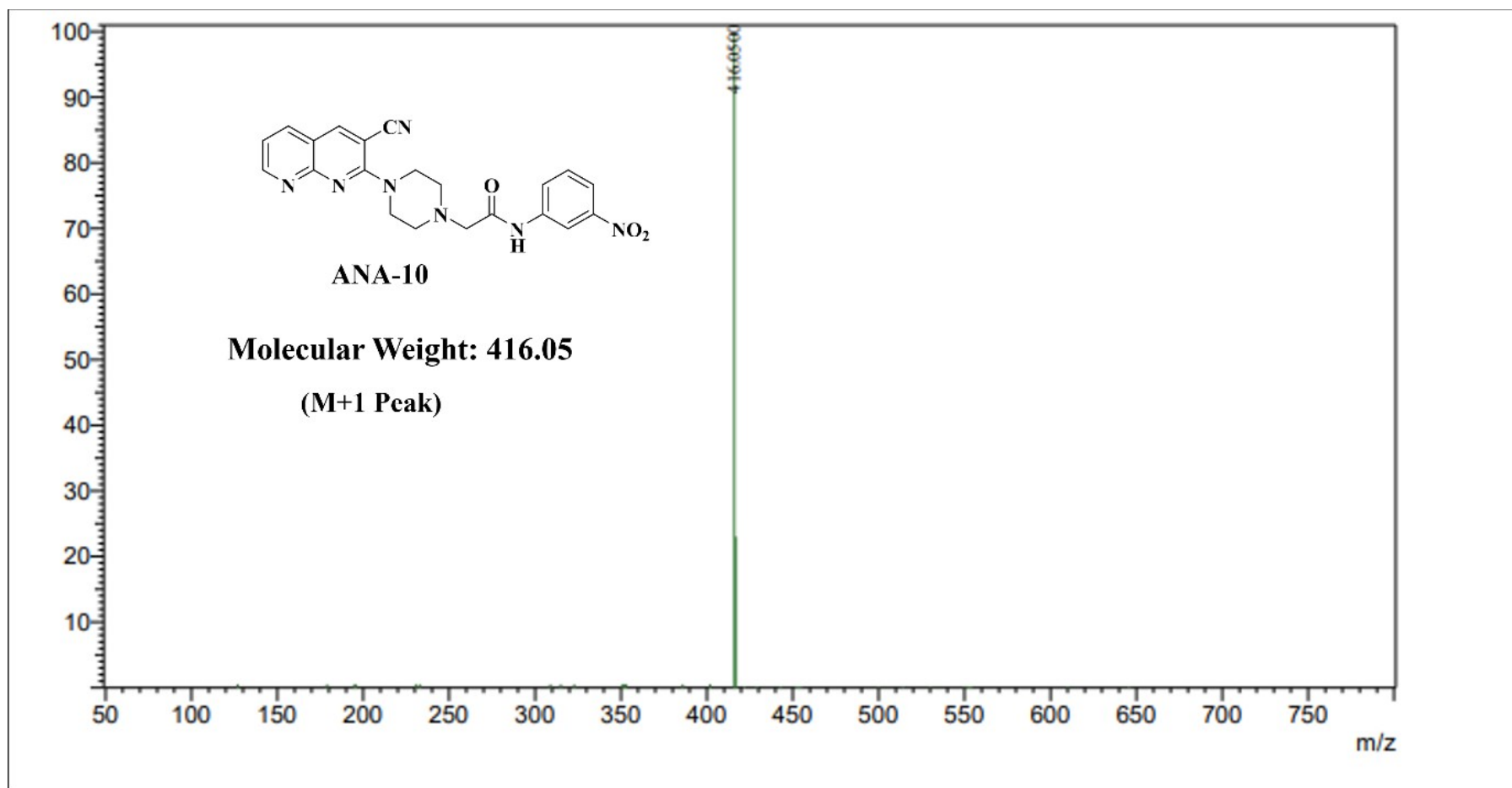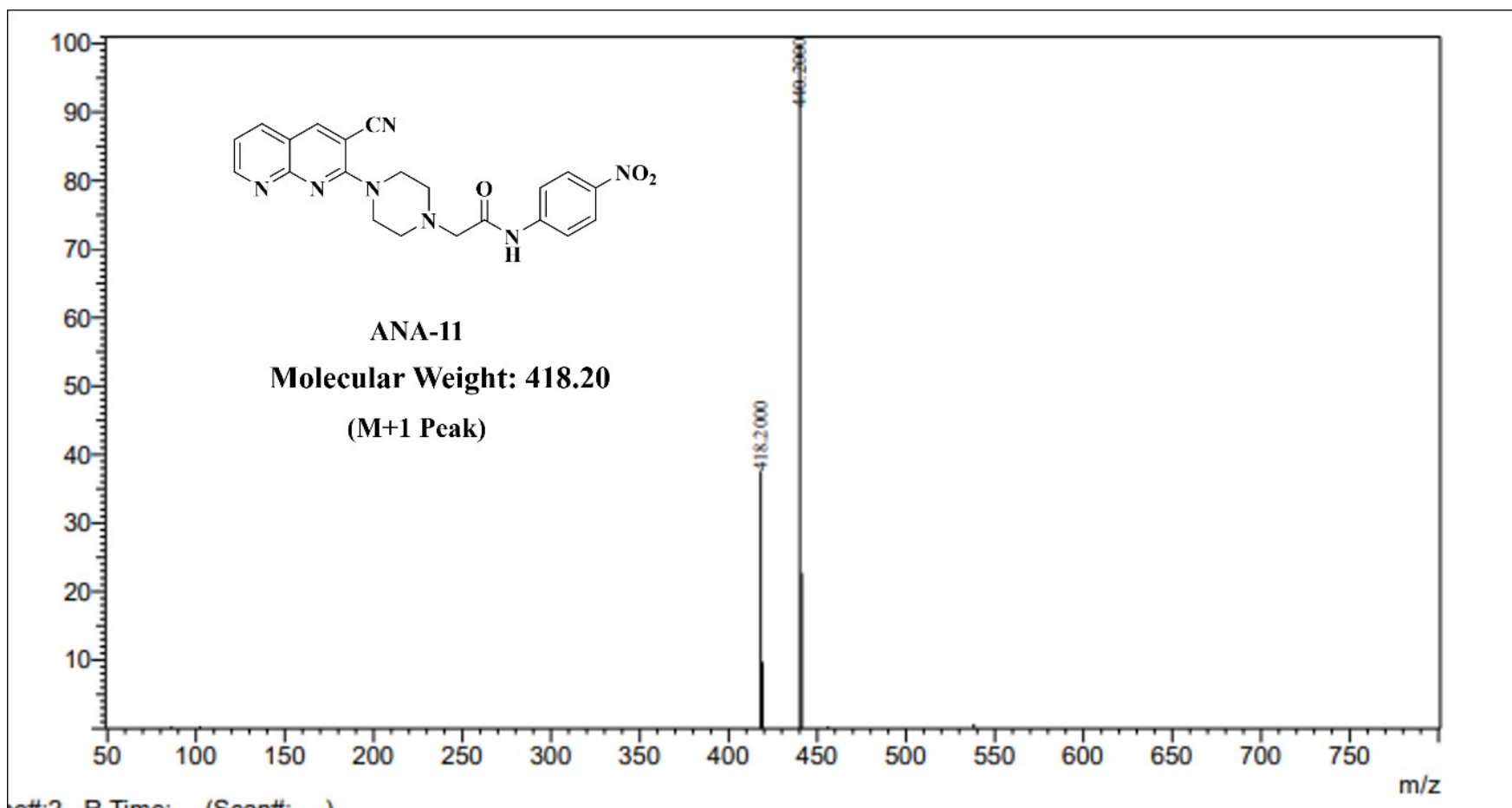Mass spectrum of compound ANC-5
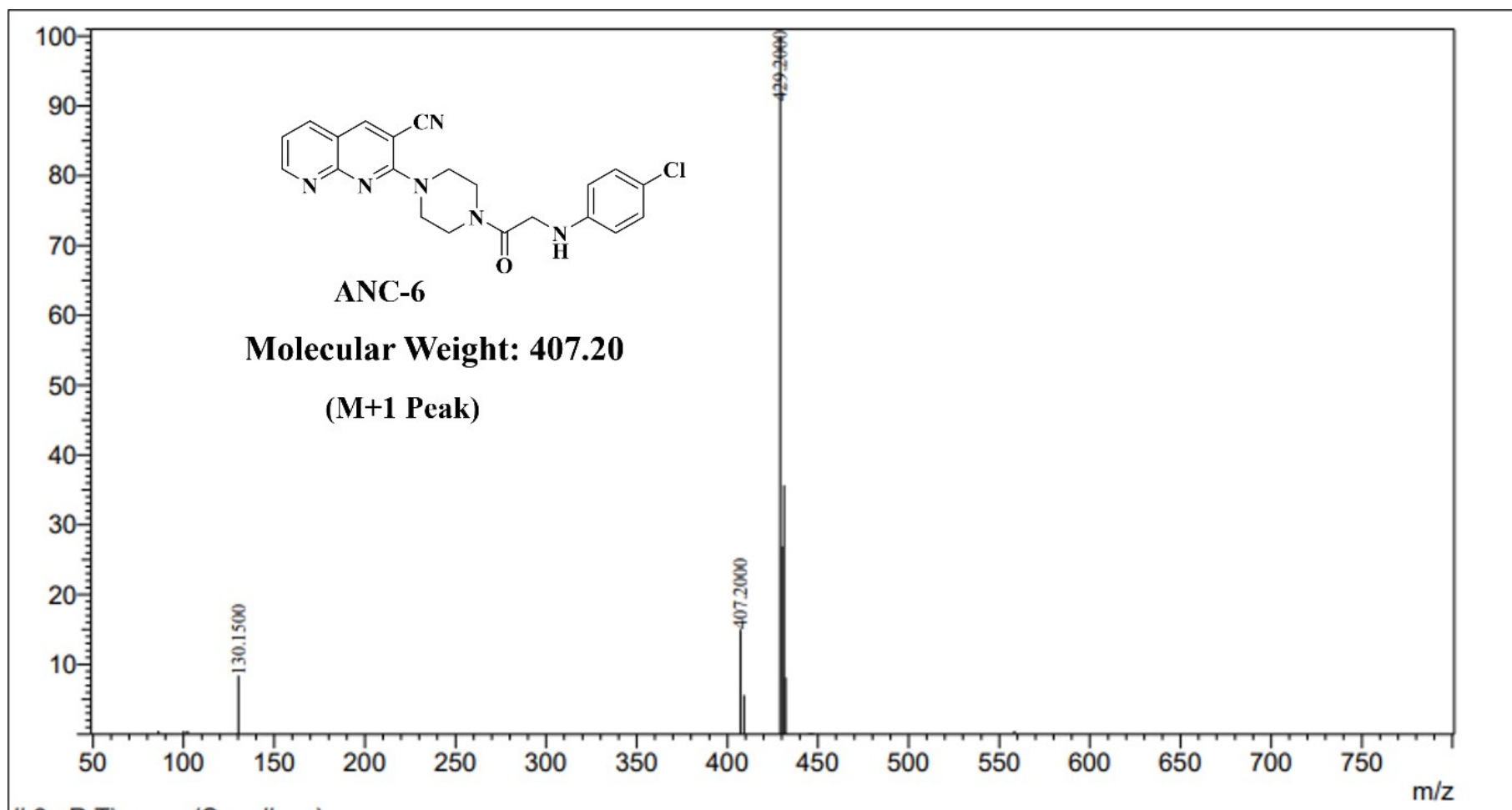
Mass spectrum of compound ANC-6
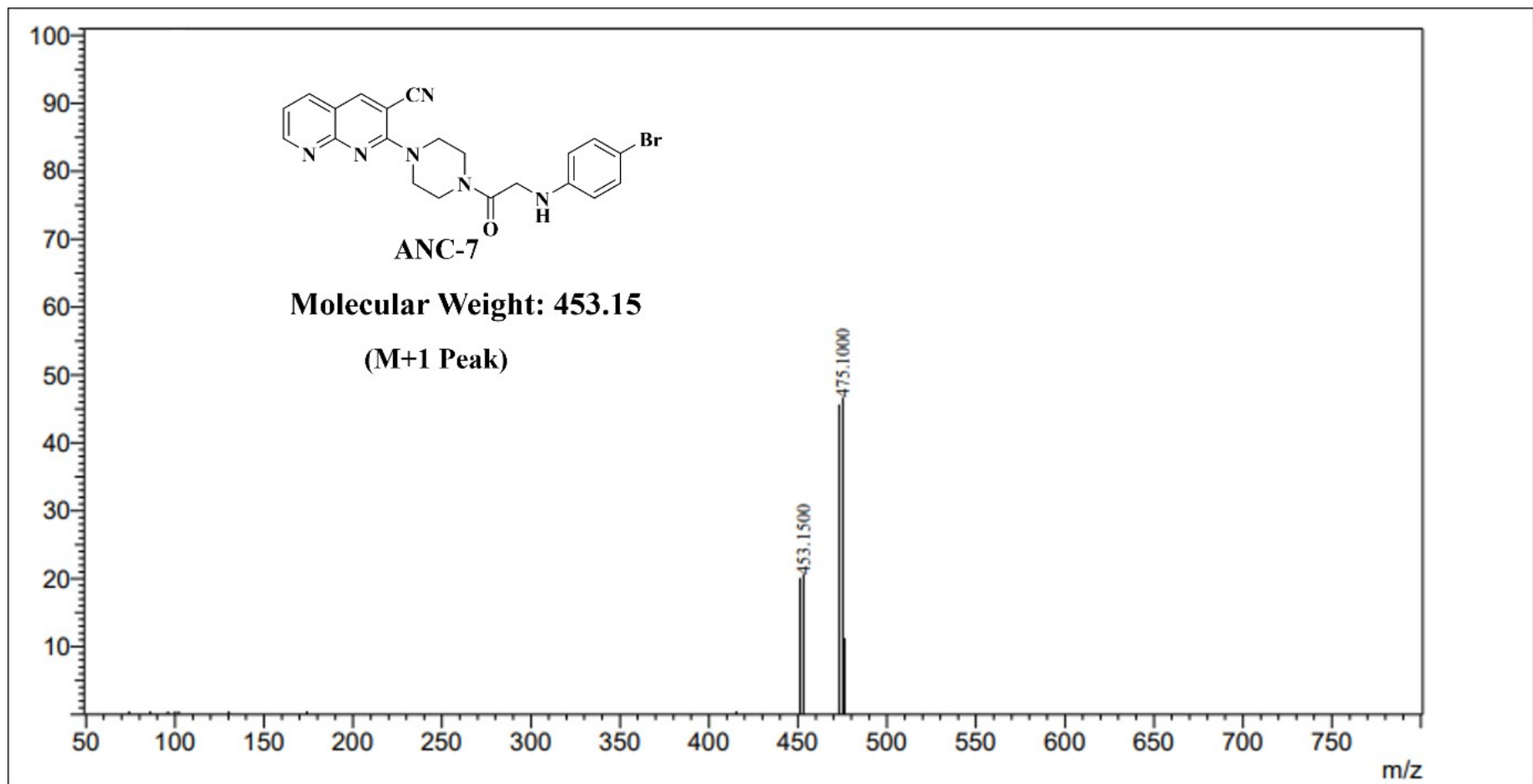
Mass spectrum of compound ANC-7
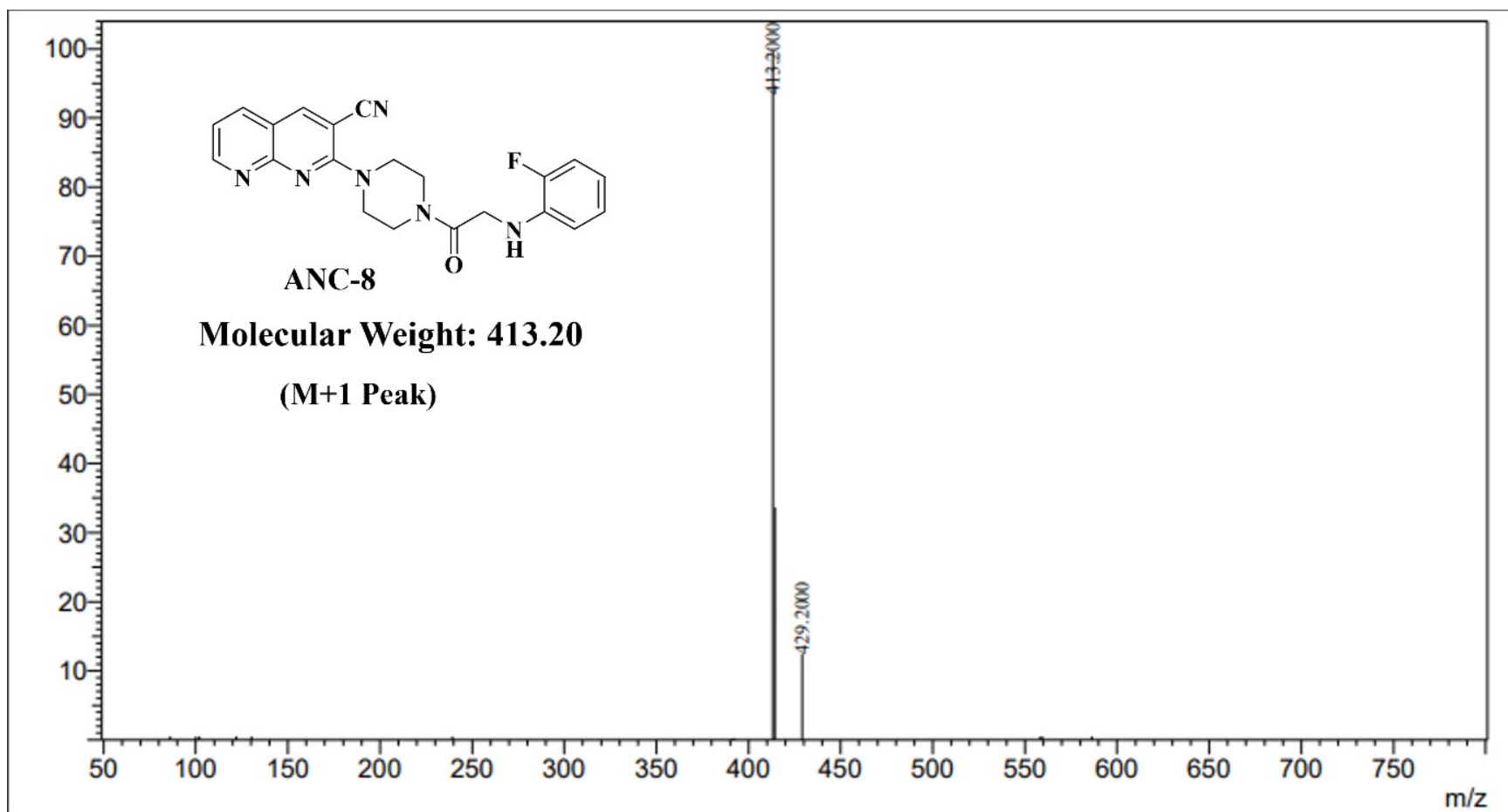
Mass spectrum of compound ANC-8

Mass spectrum of compound ANC-9

ANC-11

Molecular Weight: 405.00

(M-1 Peak)
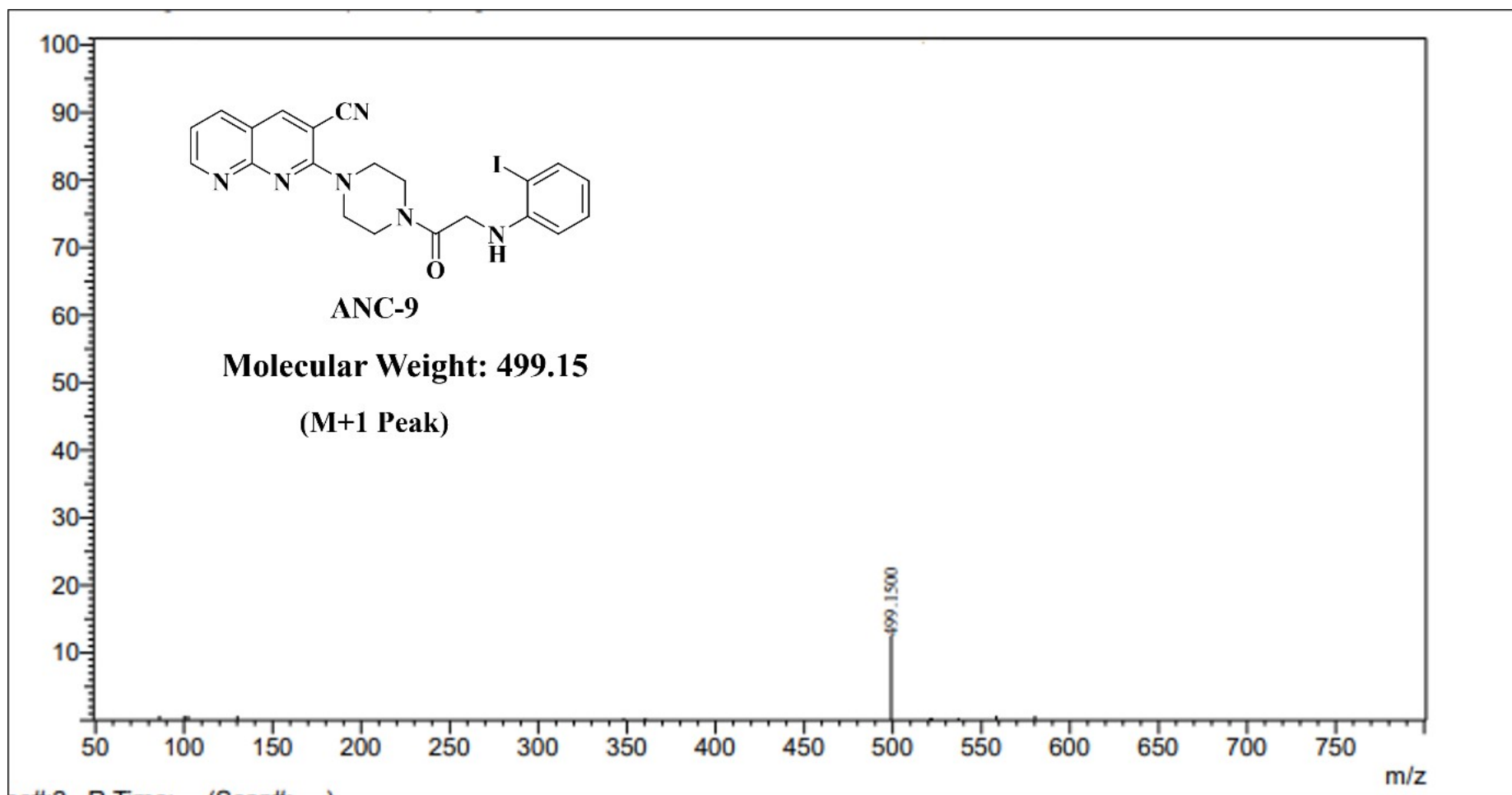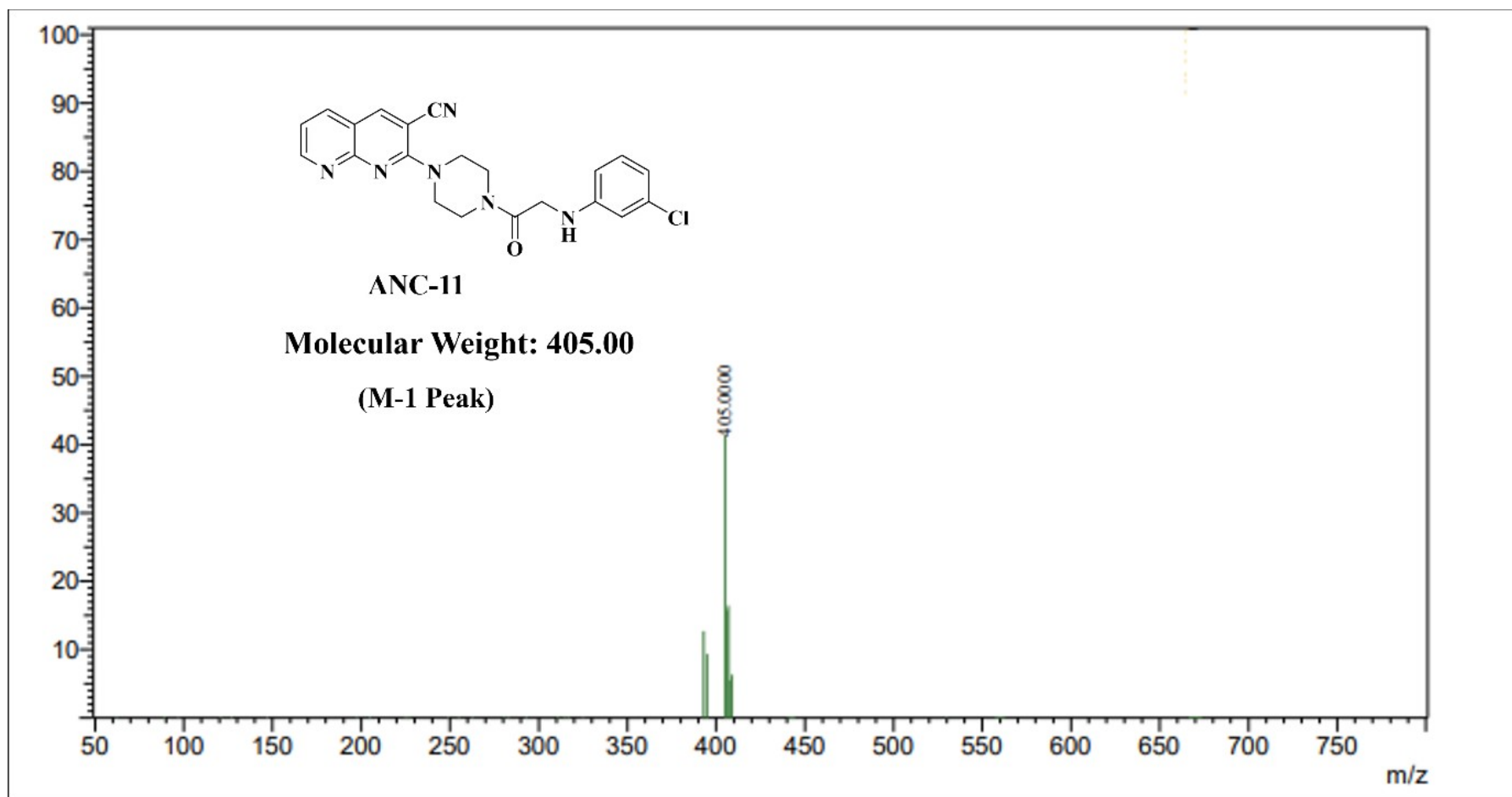
Mass spectrum of compound ANC-10
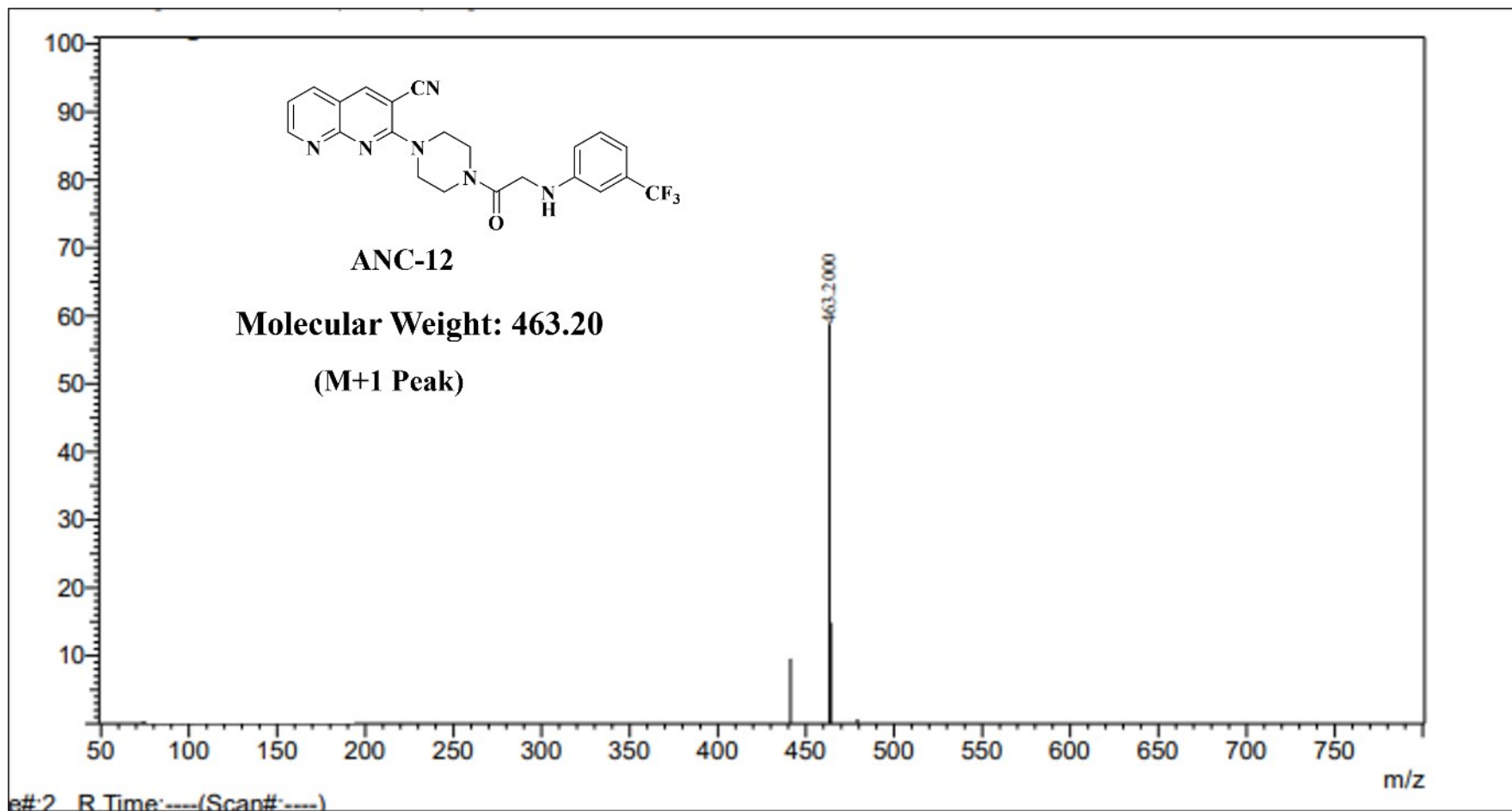
Mass spectrum of compound ANC-12
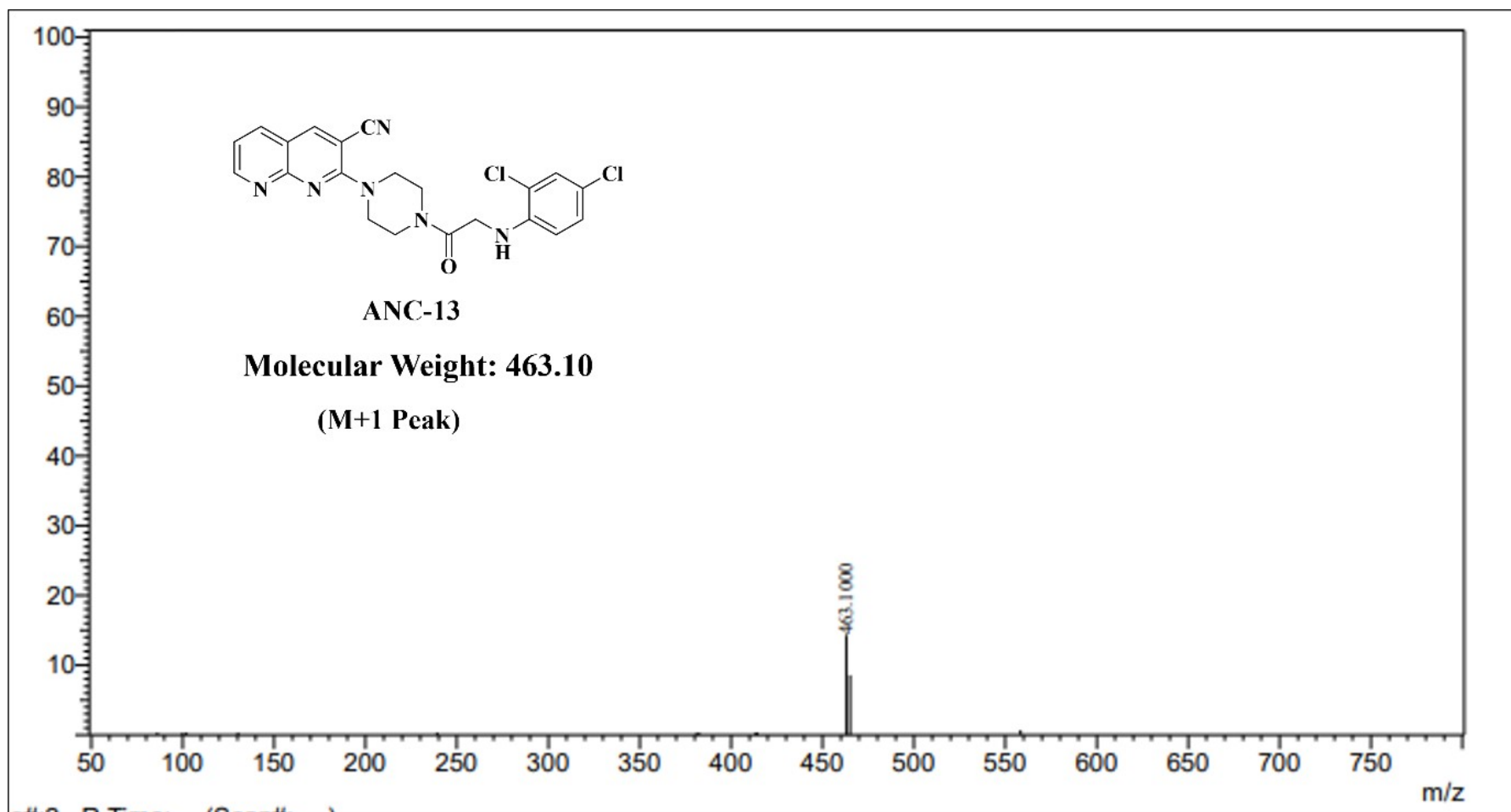
Mass spectrum of compound ANC-13
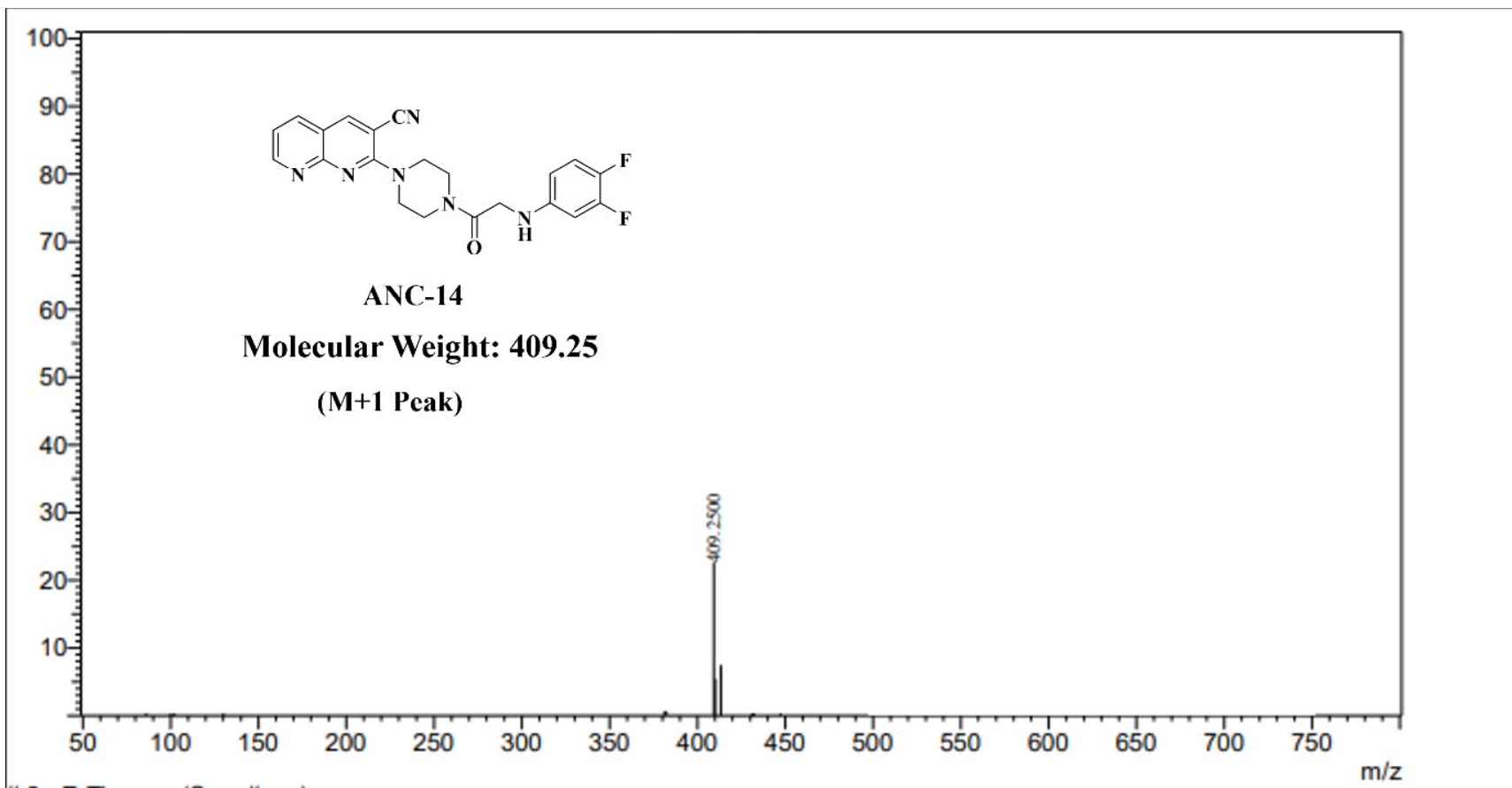
Mass spectrum of compound ANC-14

Mass spectrum of compound ANA-1

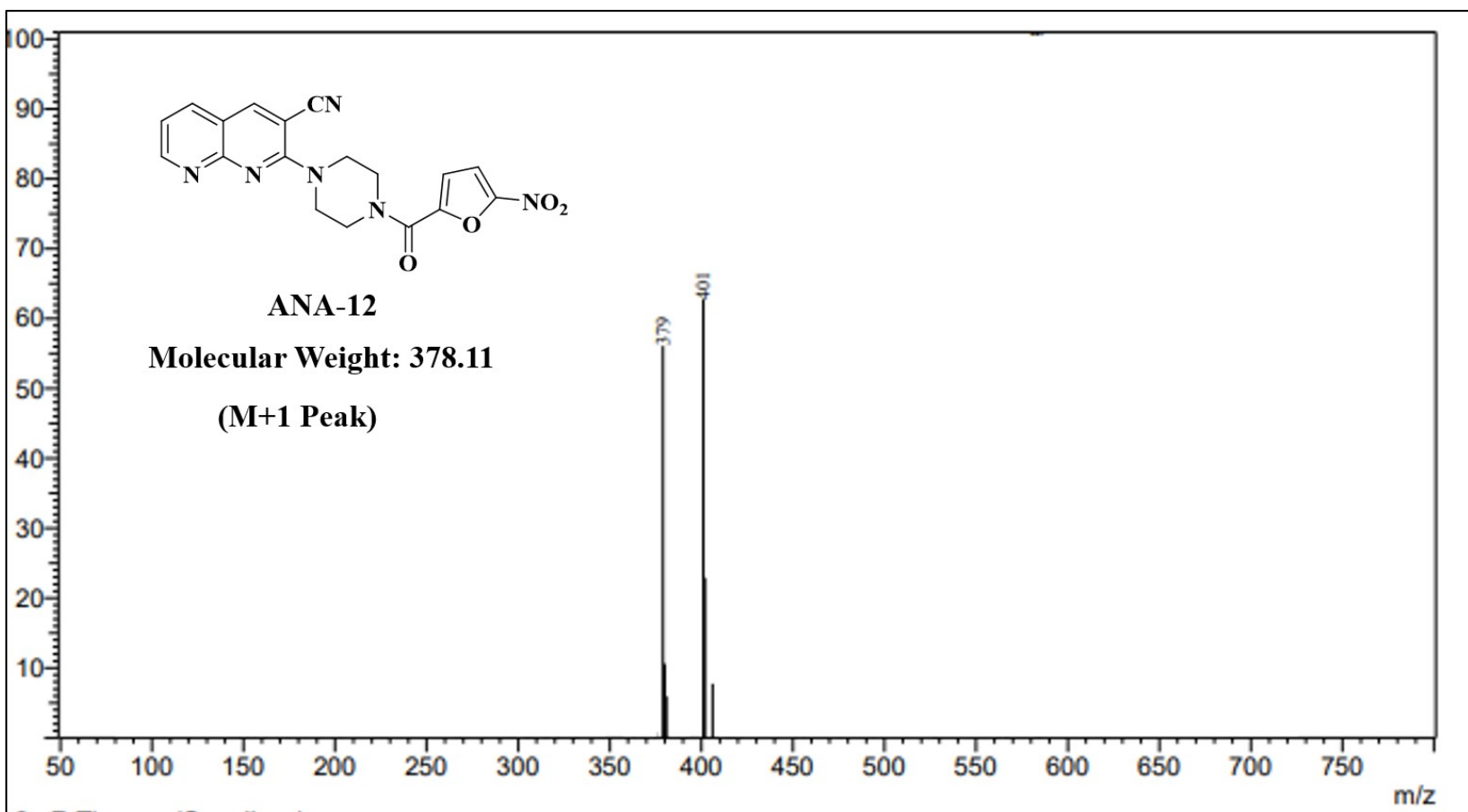ANA-2
Molecular Weight: 423.25

(M+1 Peak)
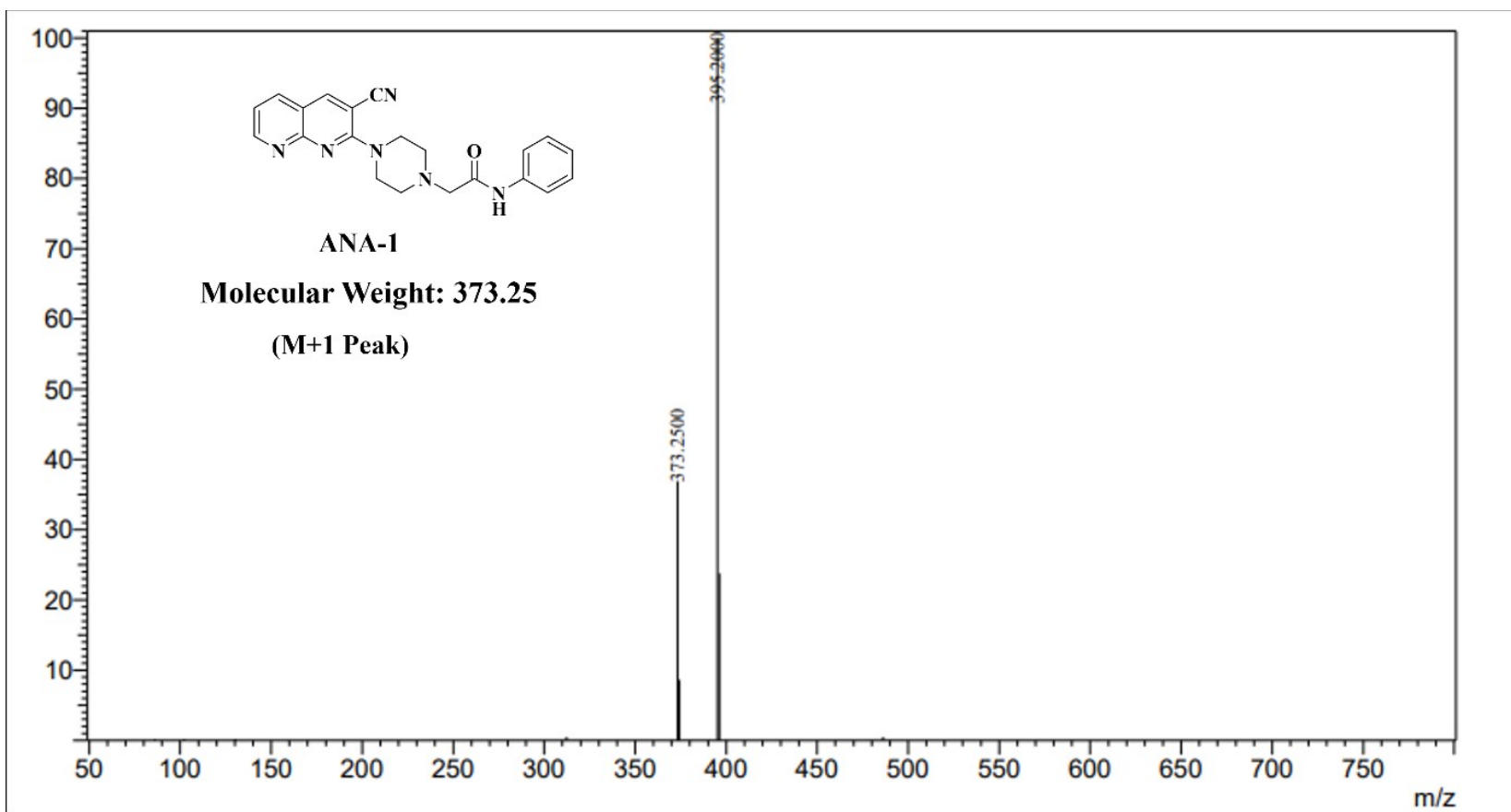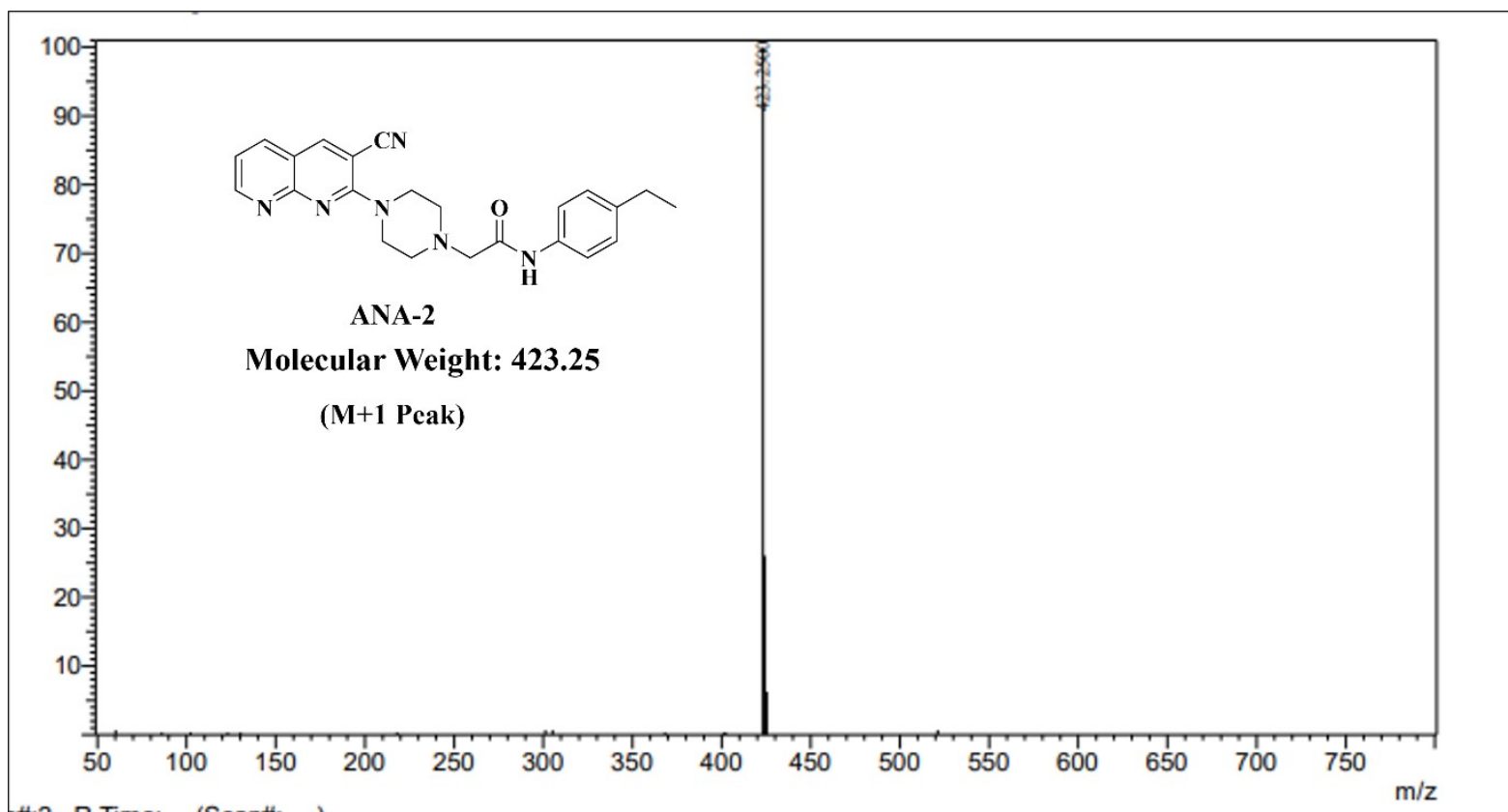
Mass spectrum of compound ANA-2
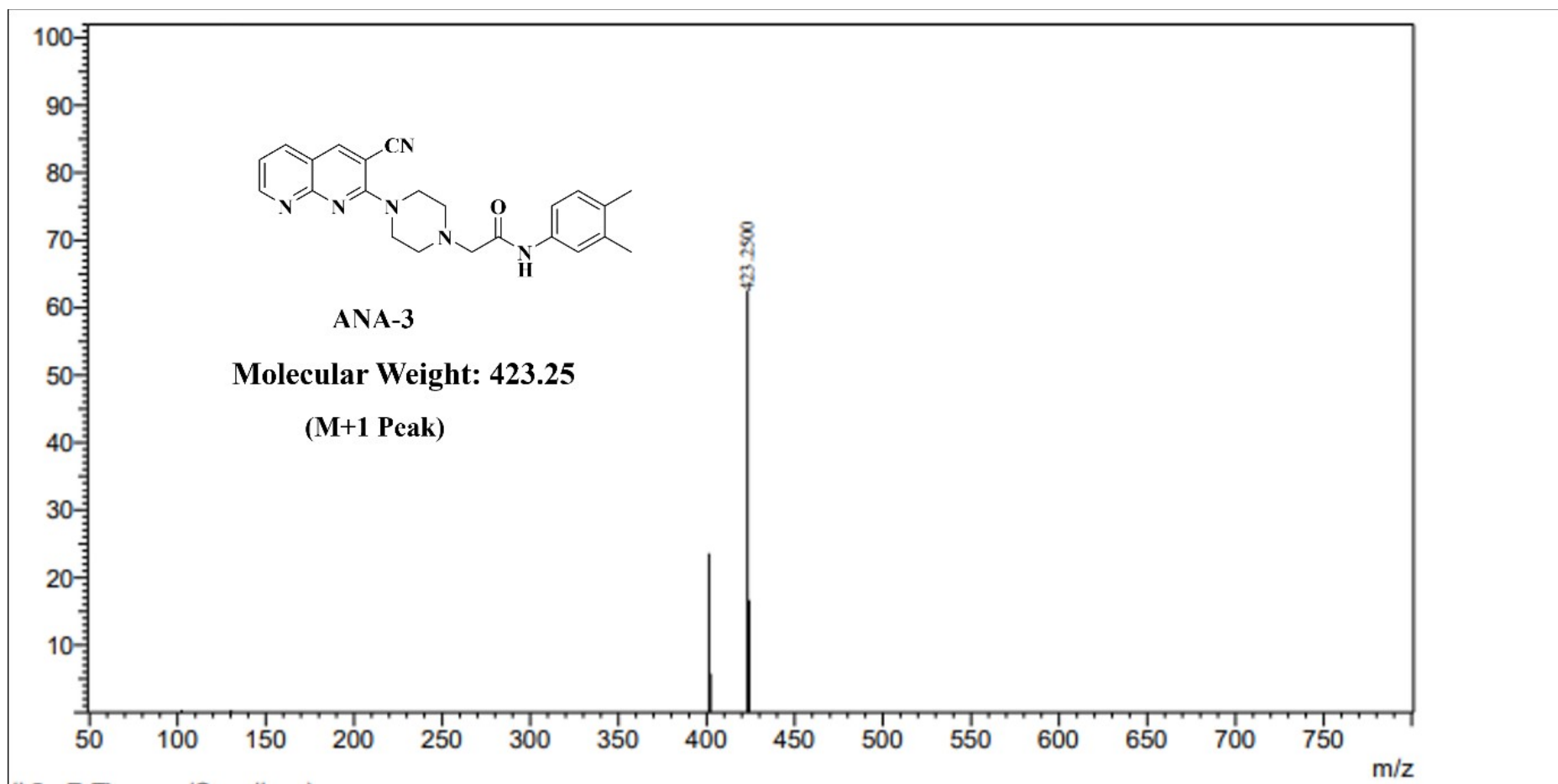
Mass spectrum of compound ANA-3
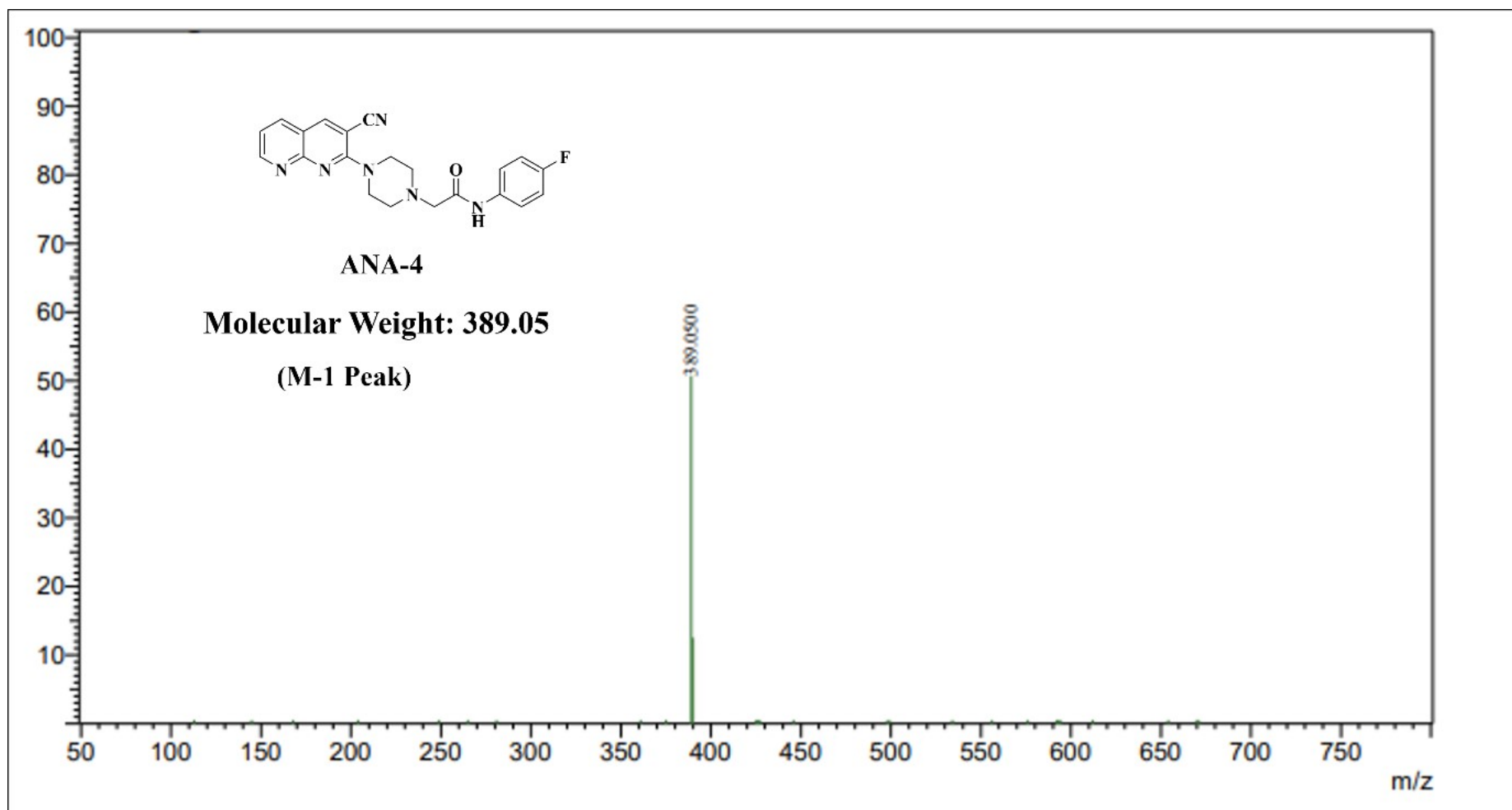
Mass spectrum of compound ANA-4

ANA-5

Molecular Weight: 449.00

(M-1 Peak)

Mass spectrum of compound ANA-5

ANA-6

Molecular Weight: 413.20

(M+1 Peak)

413.200

Mass spectrum of compound ANA-6

Mass spectrum of compound ANA-7
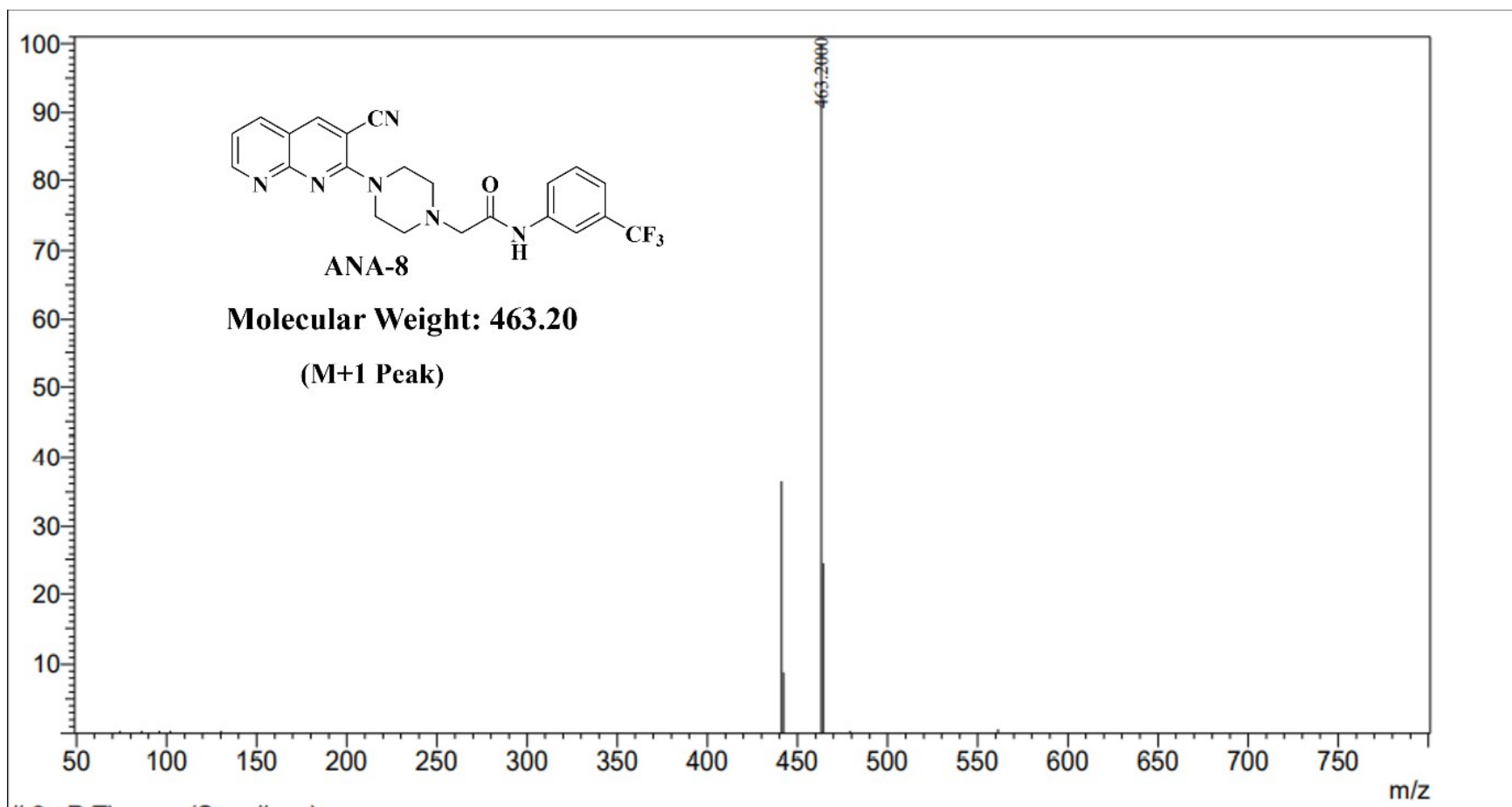
ANA-8

Molecular Weight: 463.20

(M+1 Peak)

Mass spectrum of compound ANA-8

ANA-10

Molecular Weight: 416.05

(M+1 Peak)

Mass spectrum of compound ANA-10

ANA-11

Molecular Weight: 418.20

(M+1 Peak)

Mass spectrum of compound ANA-11

ANA-12

Molecular Weight: 378.11

(M+1 Peak)

Mass spectrum of compound ANA-12

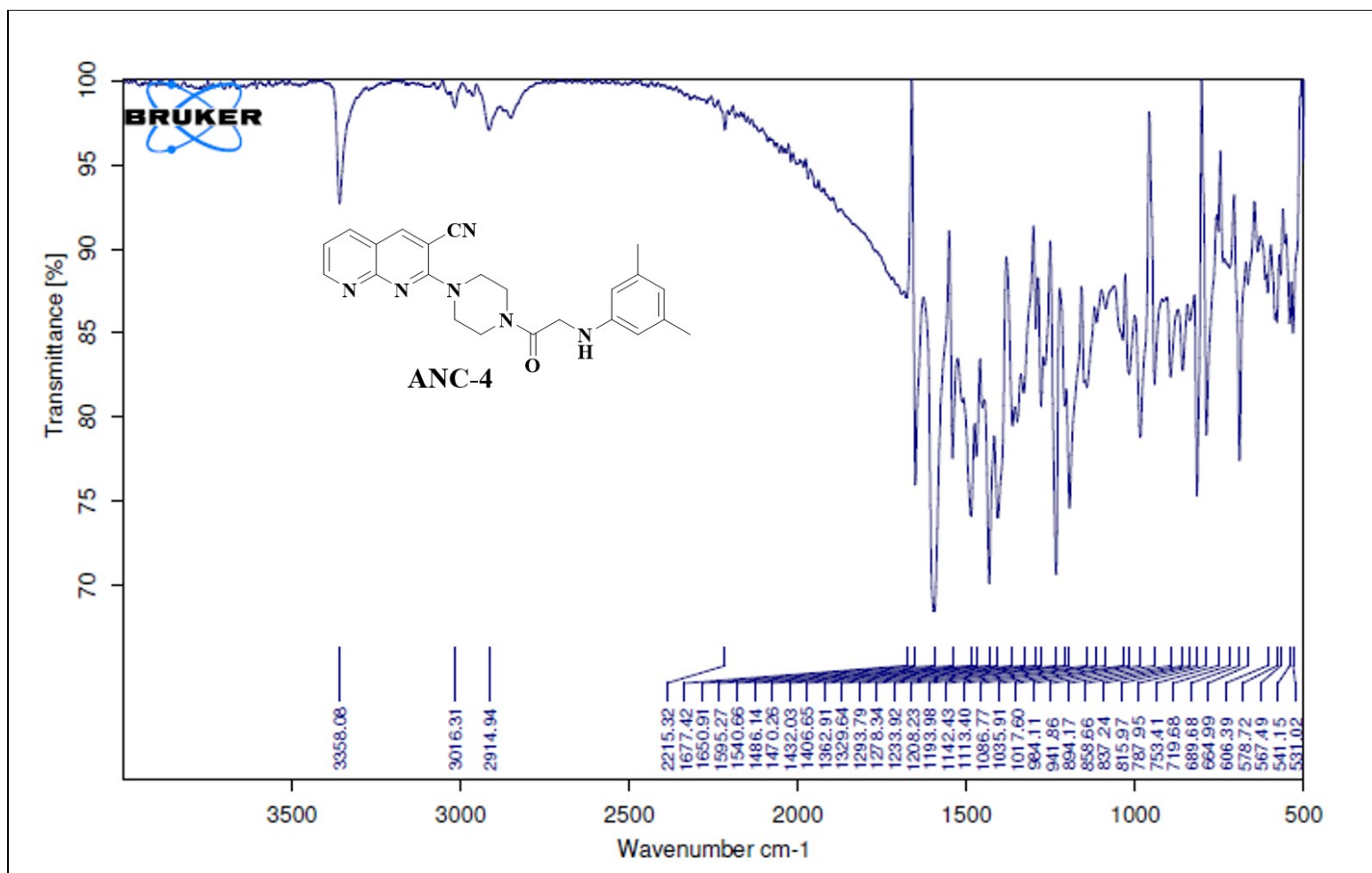## 7. IR spectra of final compounds:
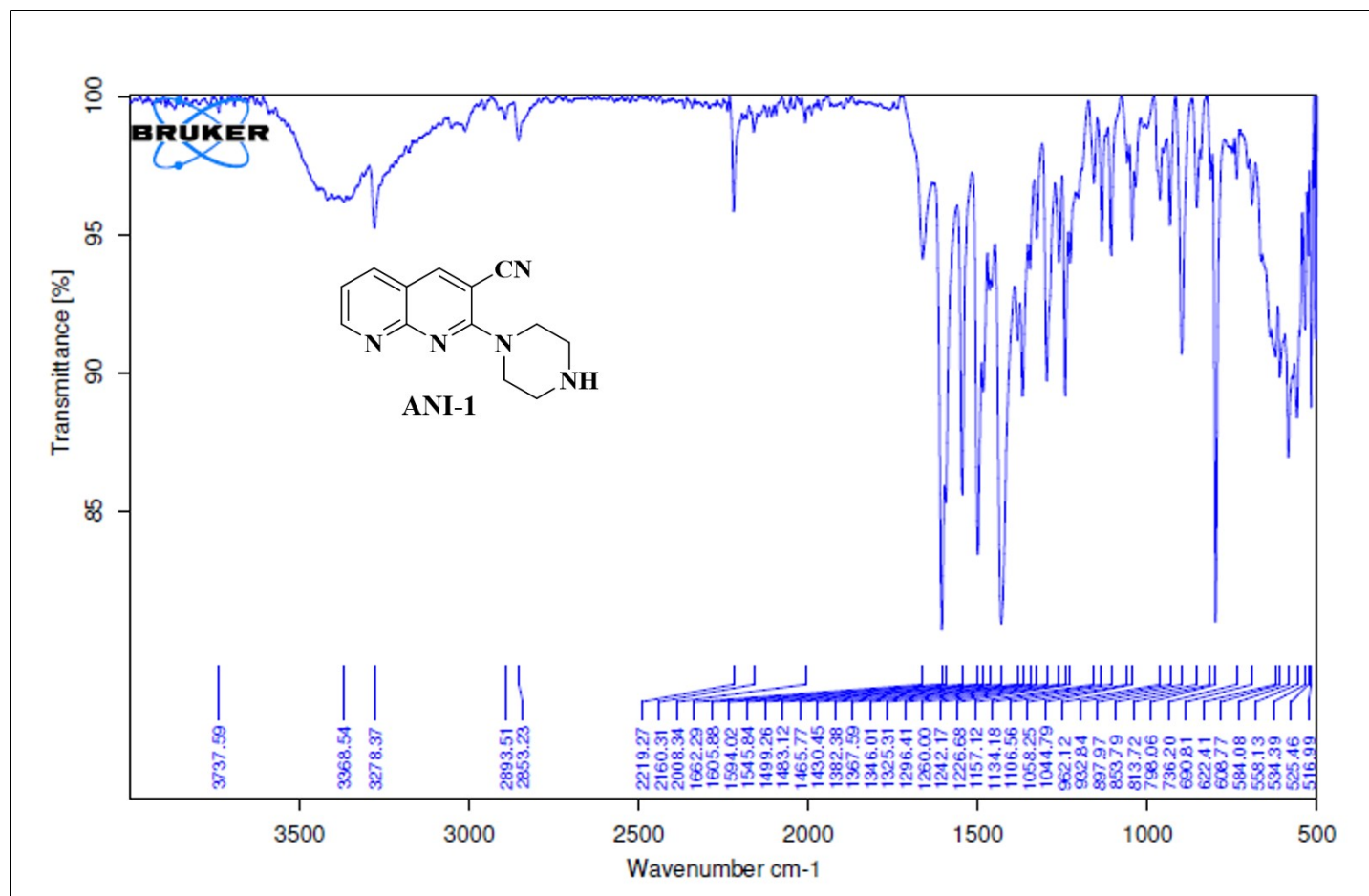
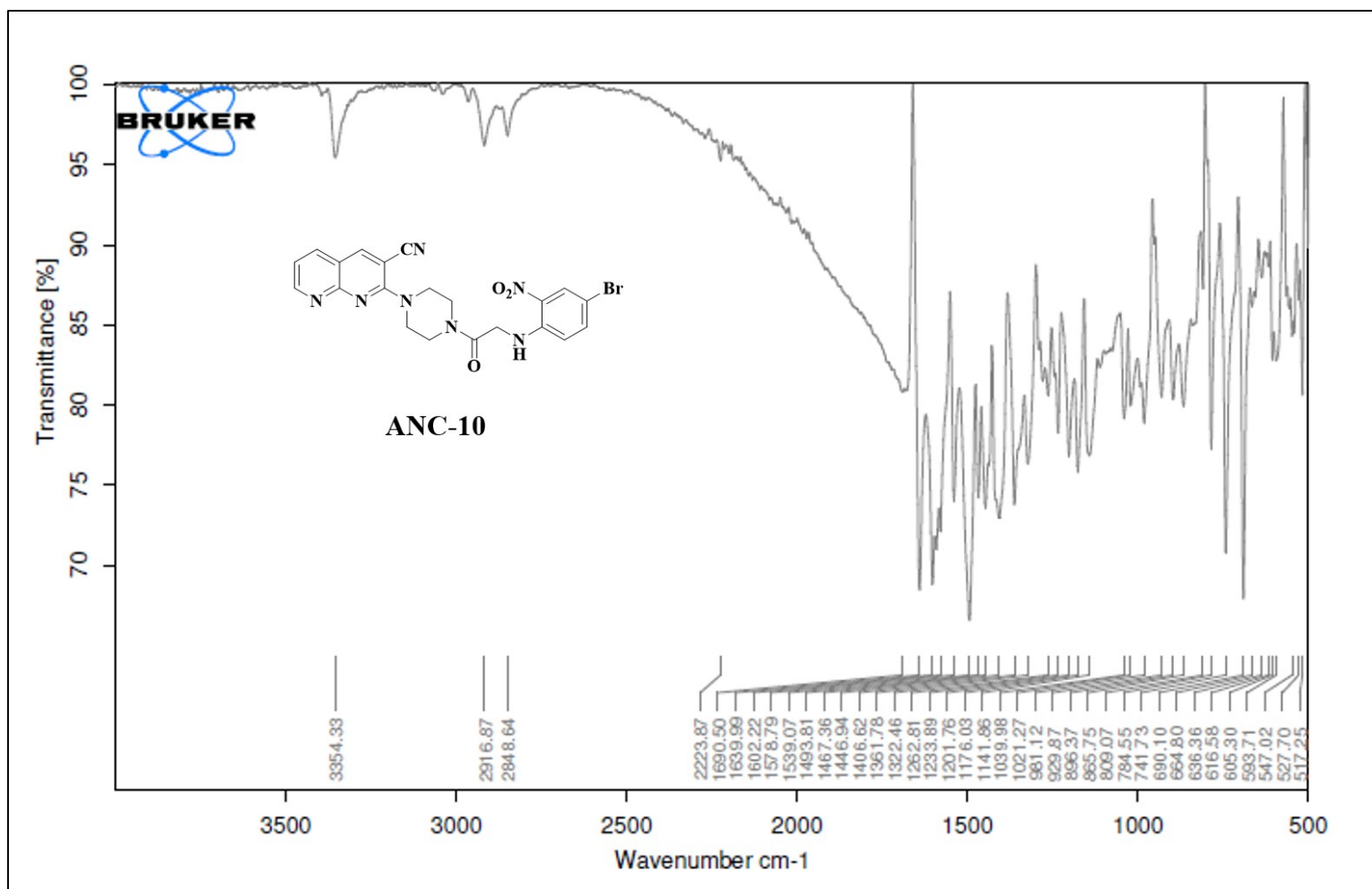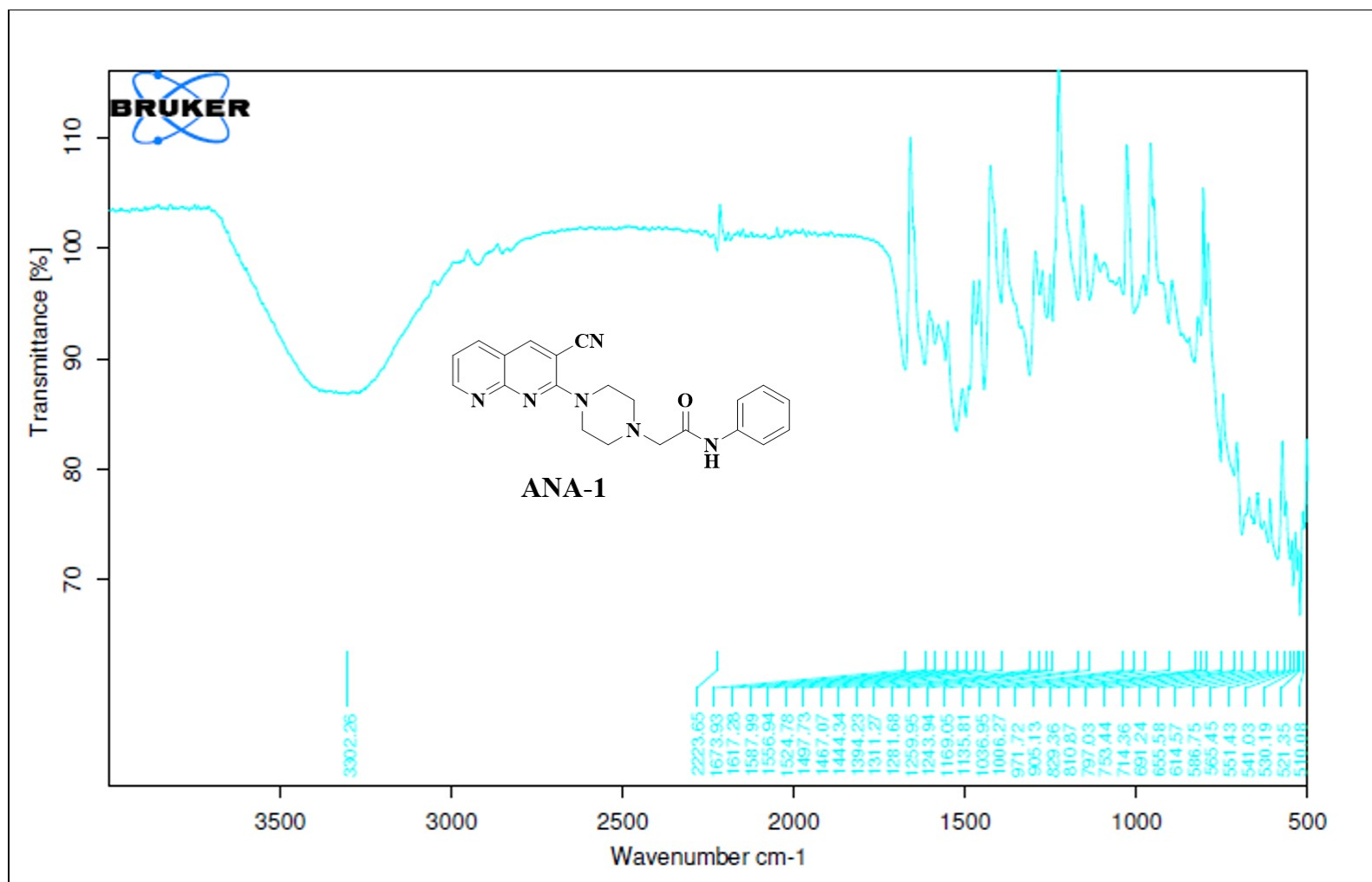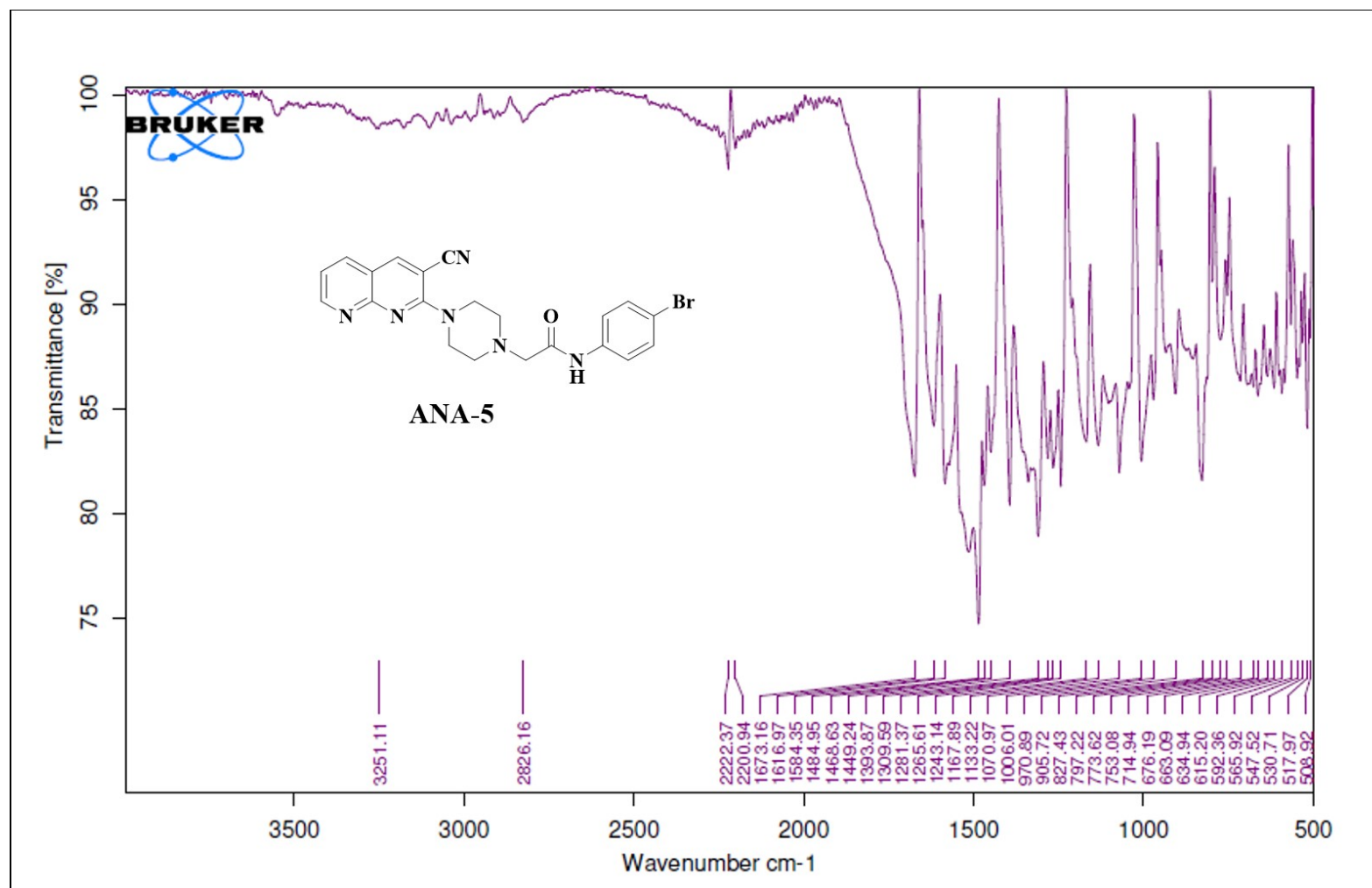IR spectrum of compound ANI-1
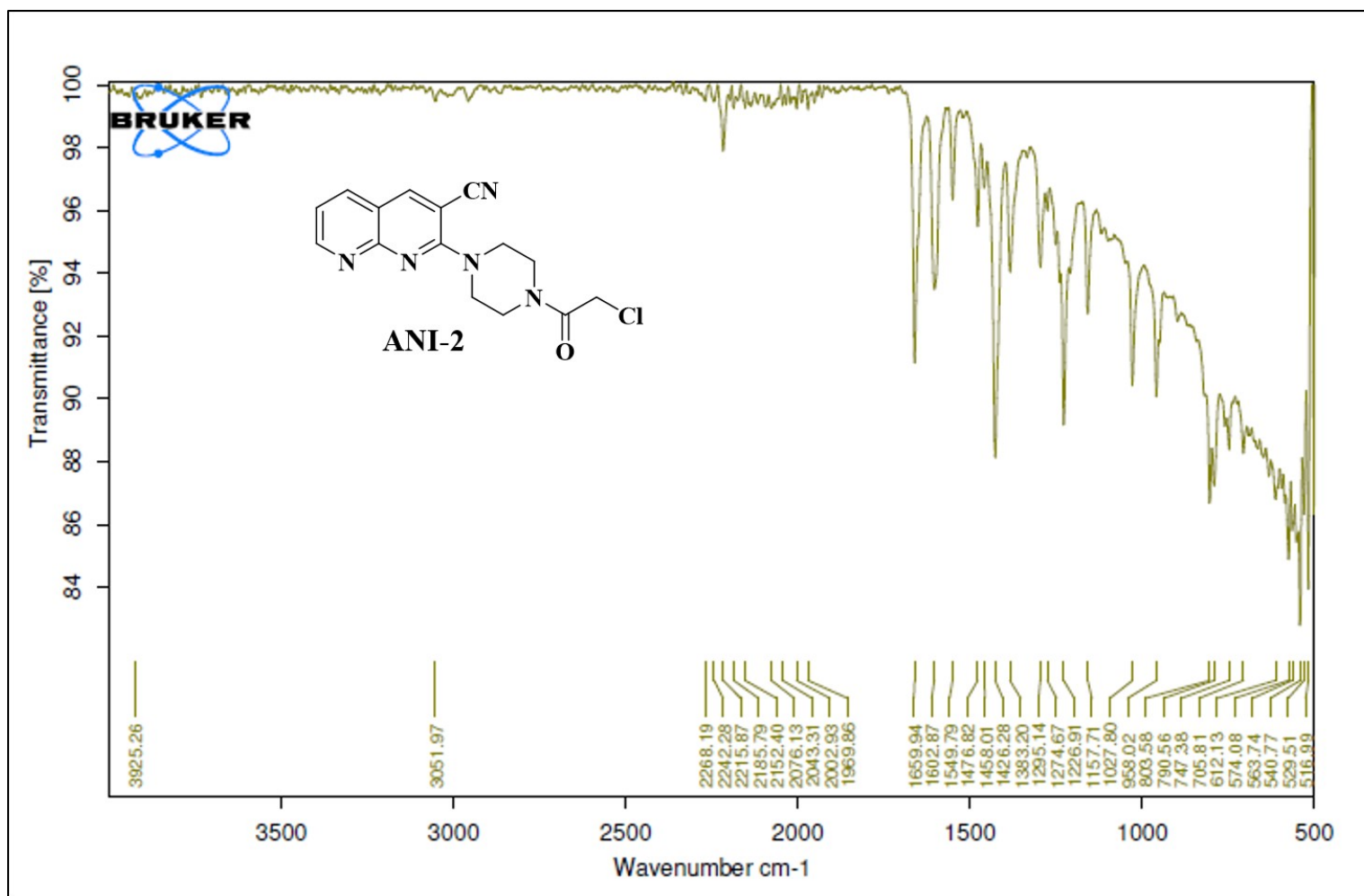
IR spectrum of compound ANI-2
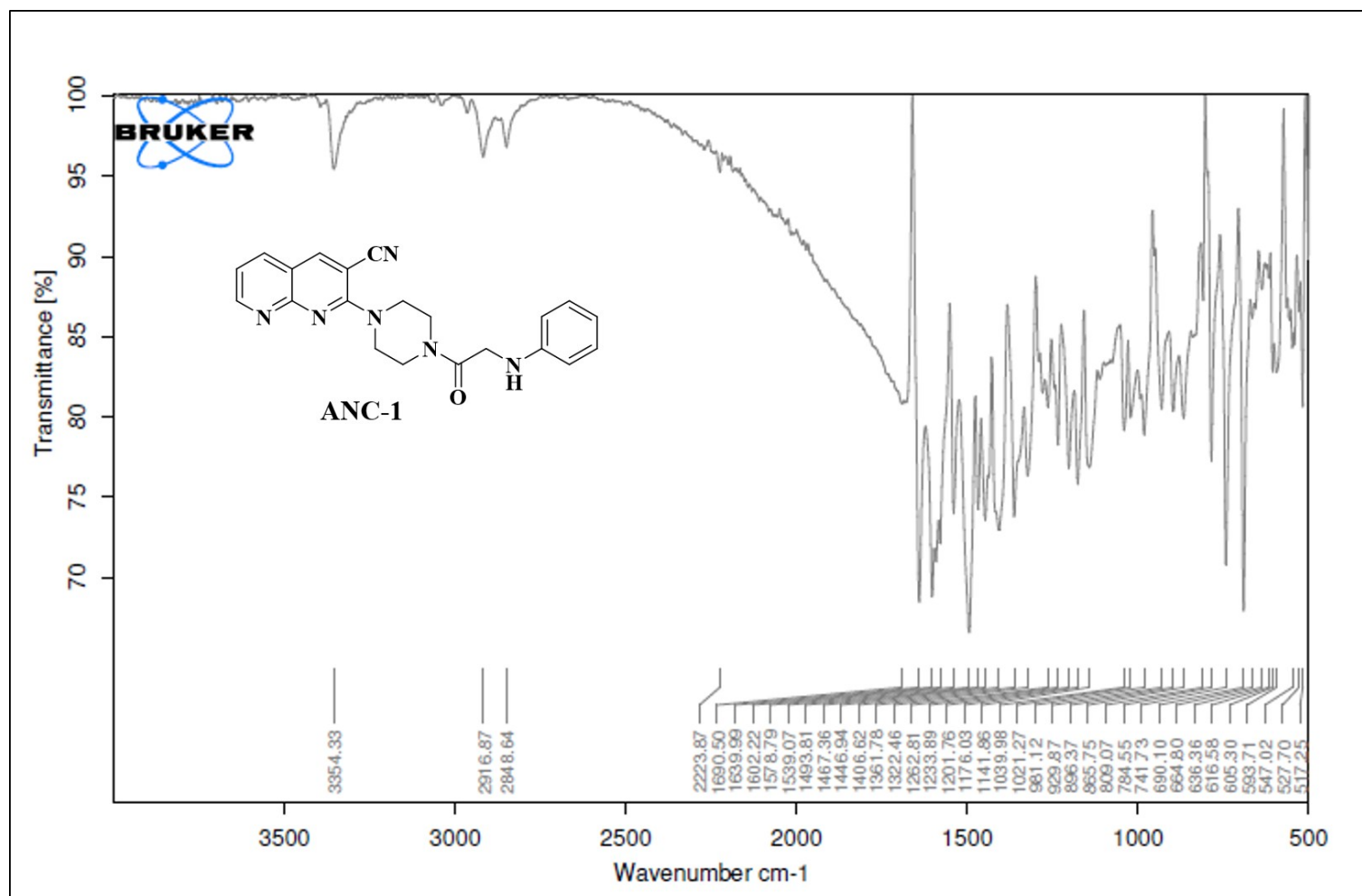
IR spectrum of compound ANC-1
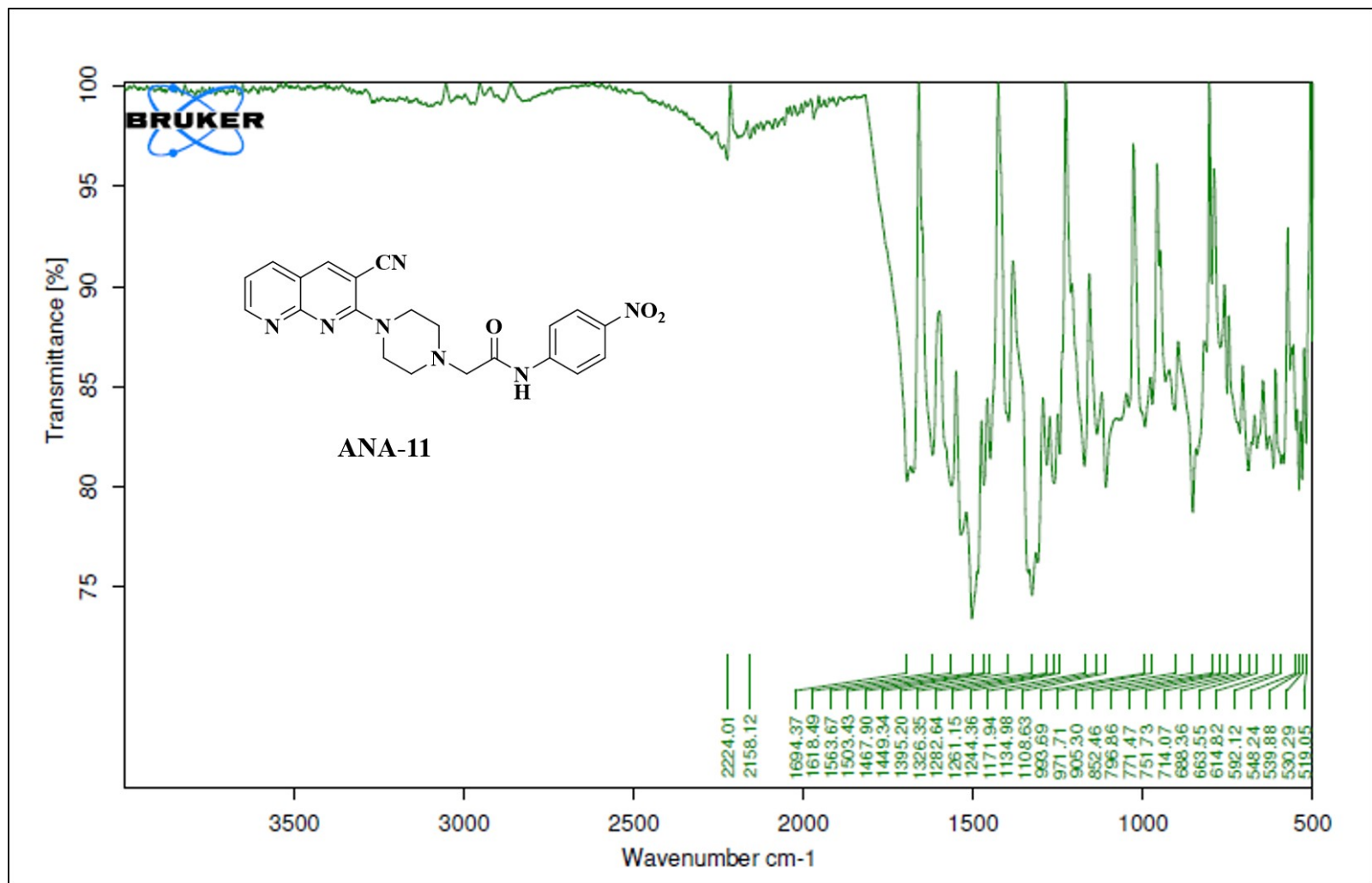
IR spectrum of compound ANC-4

IR spectrum of compound ANC-10

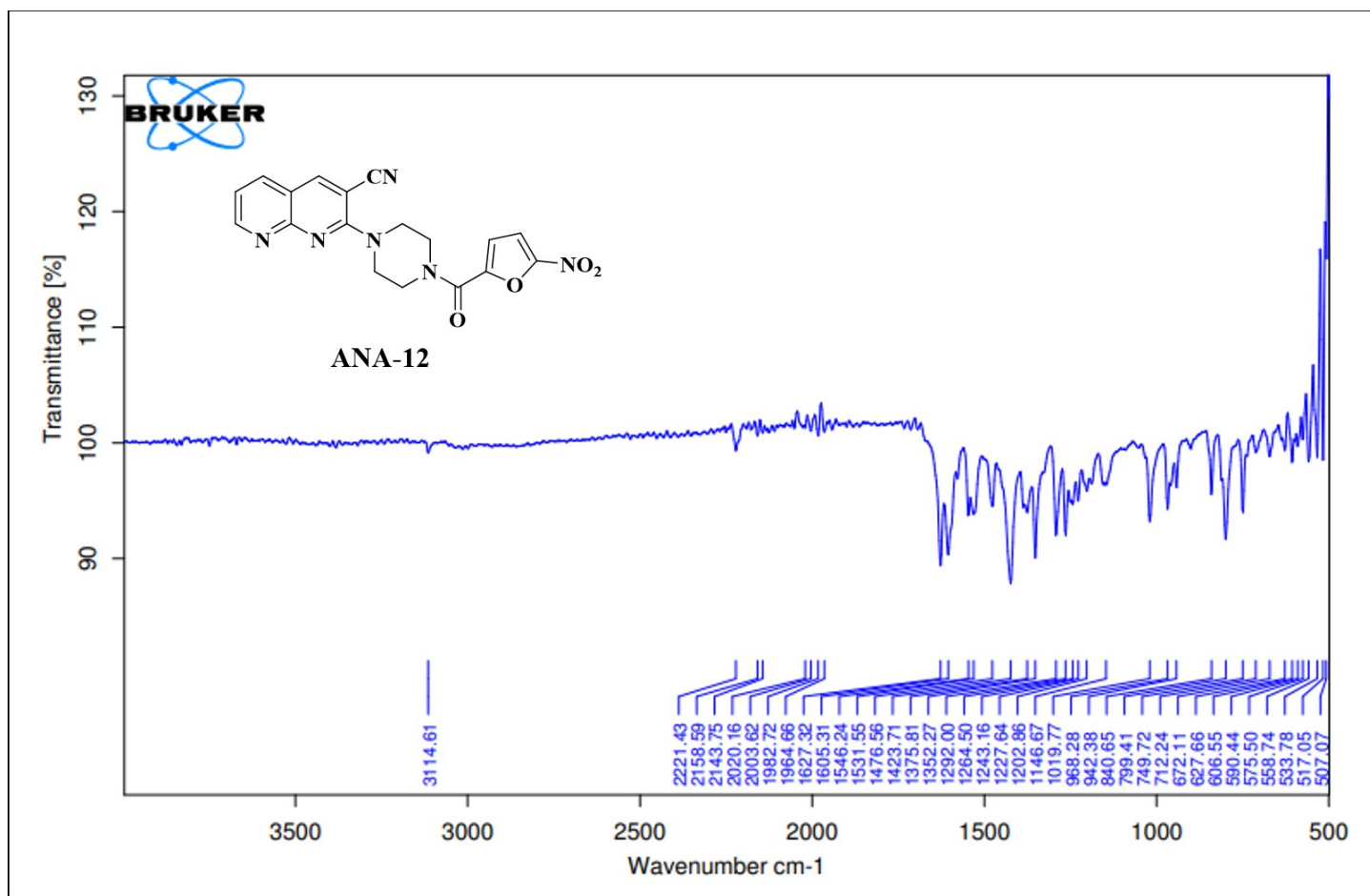**IR spectrum of compound ANA-1**

IR spectrum of compound ANA-5

IR spectrum of compound ANA-11

IR spectrum of compound ANA-12

8. References:

1. R. Mahesh, and R. V. Perumal, Microwave assisted synthesis of 2-(4-substituted piperazin-1-yl)-1, 8-naphthyridine-3-carbonitrile as a new class of serotonin 5-HT3 receptor antagonists, *Bioorg. Med. Chem. Lett.,* 2004, **14**(20), 5179-5181.
2. Schrodinger, 2020-4. (n.d.). Schrödinger Release Version 2020-4: Schrödinger, LLC, New York, NY 2019.
3. Schrödinger Release 2020-4: Desmond Molecular Dynamics System, D. E. Shaw Research, New York, NY, 2020. Maestro-Desmond Interoperability Tools, Schrödinger, New York, NY 2020 (n.d.).