Supplementary Material

Microwave-assisted synthesis of novel [1,4] oxazine derivatives as potent anti-bacterial and antioxidant agents

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Table of Contents

MATERIALS AND METHODS

All reagents were purchased from Merck and used without purification. Reactions were carried out in Microwave Digester (Anton paar Monowave 400). Melting points were measured on Ikon melting point apparatus and compared with reported values of known compounds. IR spectra were recorded on FTIR spectrometer (Perkin Elmer 1725X, Model: Spectrum Two FT-IR). Mass spectra were recorded on mass spectrophotometer (Advion expressions). NMR spectra were recorded with a Bruker spectrometer at 400 MHz (^{1}H NMR) and at 100 MHz (^{13}C NMR) in $CDCl_3$ as solvent and with TMS as internal standard; and chemical shifts are expressed as d/ppm.

Antibacterial activity:

In Vitro Antibacterial studies: All the synthesized compounds were screened for their anti-bacterial activity against two Gram-positive bacteria viz. *Bacillus subtilis* (BS) and *Staphylococcus aureus* (SA), and two Gram-negative bacteria viz. *Escherichia coli* (EC) and *Klebsiella pneumonia* (KP). Well diffusion method was used for the *in-vitro* anti-bacterial studies and the activity was determined by measuring the diameter of inhibition zones (mm); also, the Minimum inhibitory concentrations [MIC] were determined employing standard two-fold serial broth dilution method. 2mg/ml of DMSO concentration was used where DMSO was used as a negative control and Streptomycin was used as a positive control.

Anti-oxidant activity:

In Vitro antioxidant assays: The antioxidant activity of the sample was evaluated utilizing two separate assays: DPPH and FRAP. The antioxidant activity of the sample was tested using the two assays and compared with the standard Trolox. The experiments were carried out in triplicate and the results were averaged. The IC_{50} values for the standard and the sample were derived for the DPPH assay. The DPPH free-radical scavenging per-centage was calculated using the measured absorbance as follows:

DPPH scavenging activity (%) = $(A_{control} - A_{sample}) / A_{control} * 100$

where $A_{control}$ is the absorbance of the control (DPPH +methanol) and A_{sample} is the absorbance of the sample compound.

TheIC50 values were used to assess the antioxidant activity. For the FRAP assay, the absorbance of the reaction mixture was measured at 700 nm using a UV/Vis spectrophotometer. Greater absorbance indicated greater reducing power.

Table 2. Minimum Inhibition Zone (mm) of synthesized compounds

Compound (2mg/L)	Minimum Inhibition Zone (mm)			
	Gram Negative Bacteria		Gram-Positive Bacteria	
	KP	***EC***	***BS***	***SA***
4a	13	14	11	13
4b	12	10	14	14
4c	10	11	11	13
4d	16	10	18	14
4e	15	14	15	14
4f	18	16	17	13
4g	14	16	17	18
4h	13	13	15	-
4i	12	11	16	-
4j	14	19	19	14
4k	10	-	-	15
4l	13	12	13	14
Streptomycin	22	23	22	23

Table 3. Minimum Inhibition Concentration of synthesized compounds

Compound (2mg/L)	Minimum Inhibition Concentration (mg/L)			
	Gram Negative Bacteria		Gram-Positive Bacteria	
	KP	***EC***	***BS***	***SA***
4a	0.046	0.046	0.75	0.046
4b	0.375	0.75	0.187	0.187
4c	0.75	0.75	0.75	0.375
4d	0.046	0.75	0.023	0.187
4e	0.187	0.187	0.046	0.046
4f	0.023	0.046	0.046	0.046
4g	0.187	0.046	0.023	0.023
4h	0.046	0.046	0.187	-
4i	0.375	0.75	0.046	-
4j	0.187	0.005	0.005	0.187
4k	0.75	-	-	0.046
4l	0.046	0.375	0.046	0.046
Streptomycin	0.00729	0.00729	0.00729	0.00729

Table 4. IC_{50} values of the standard Trolox and the sample

Compound	IC_{50} (µg/ml)
4a	50.34
4b	54.29
4c	66.01
4d	43.98
4e	51.69
4f	77.48
4g	52.92
4h	68.12
4i	49.96
4j	68.03
4k	71.73
4l	48.35
Trolox	79.86

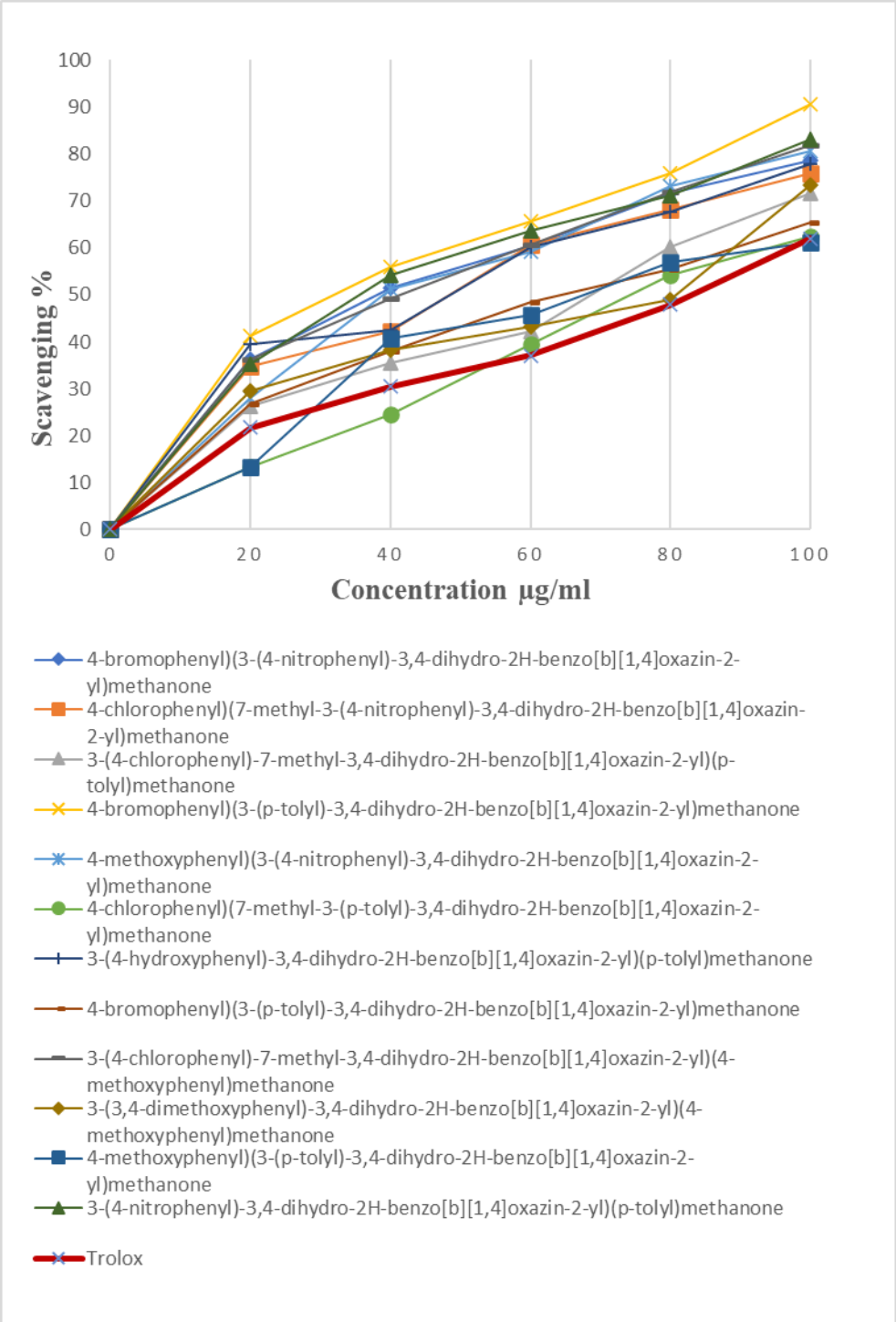


Figure 1. DPPH free-radical scavenging activity in the presence of different concentrations of Trolox and the synthesised oxazines.

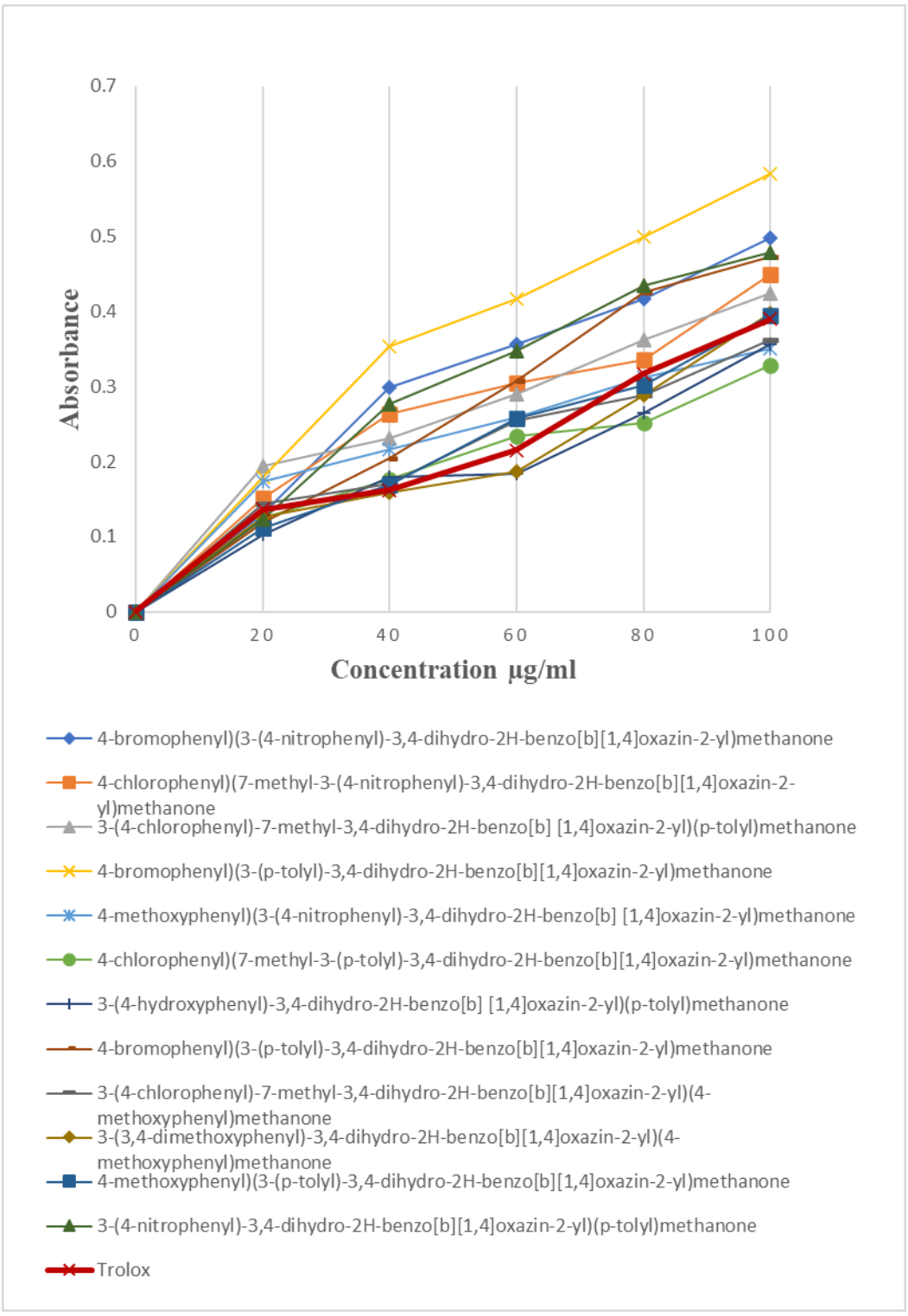
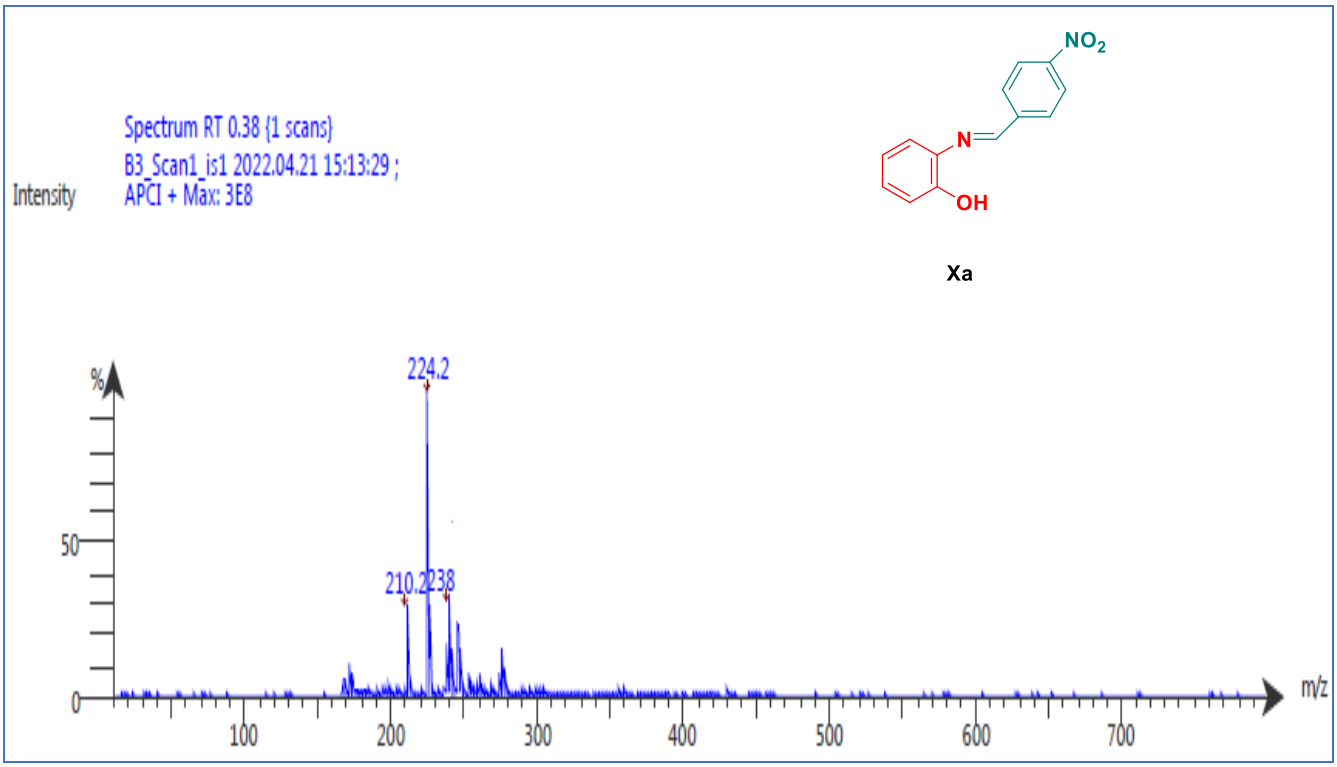
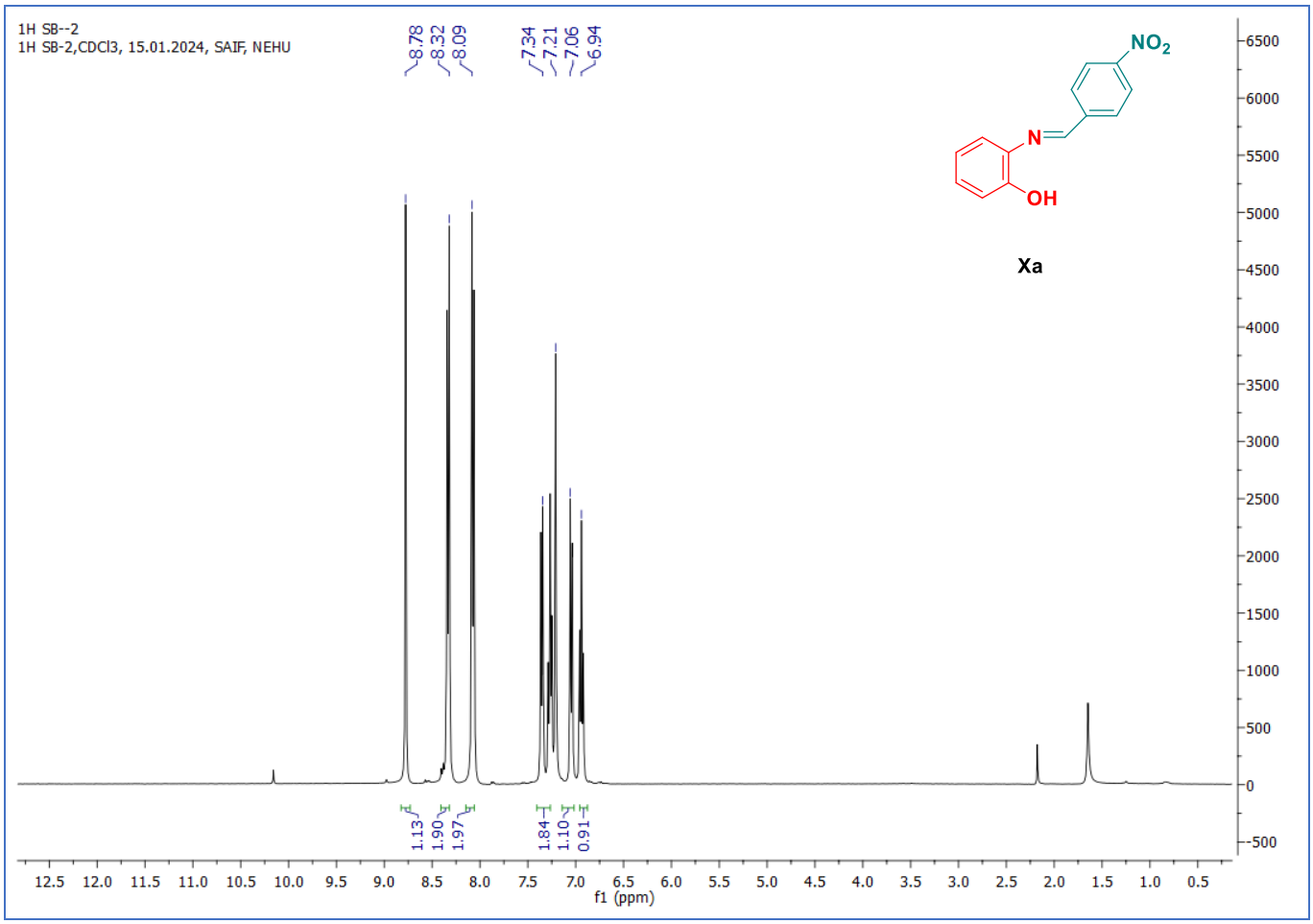


Figure 2. Antioxidant activity of standard (Trolox) and the synthesised oxazines using FRAP assay.

Mass spectra of Xa



^{1}HNMR of Compounds Xa



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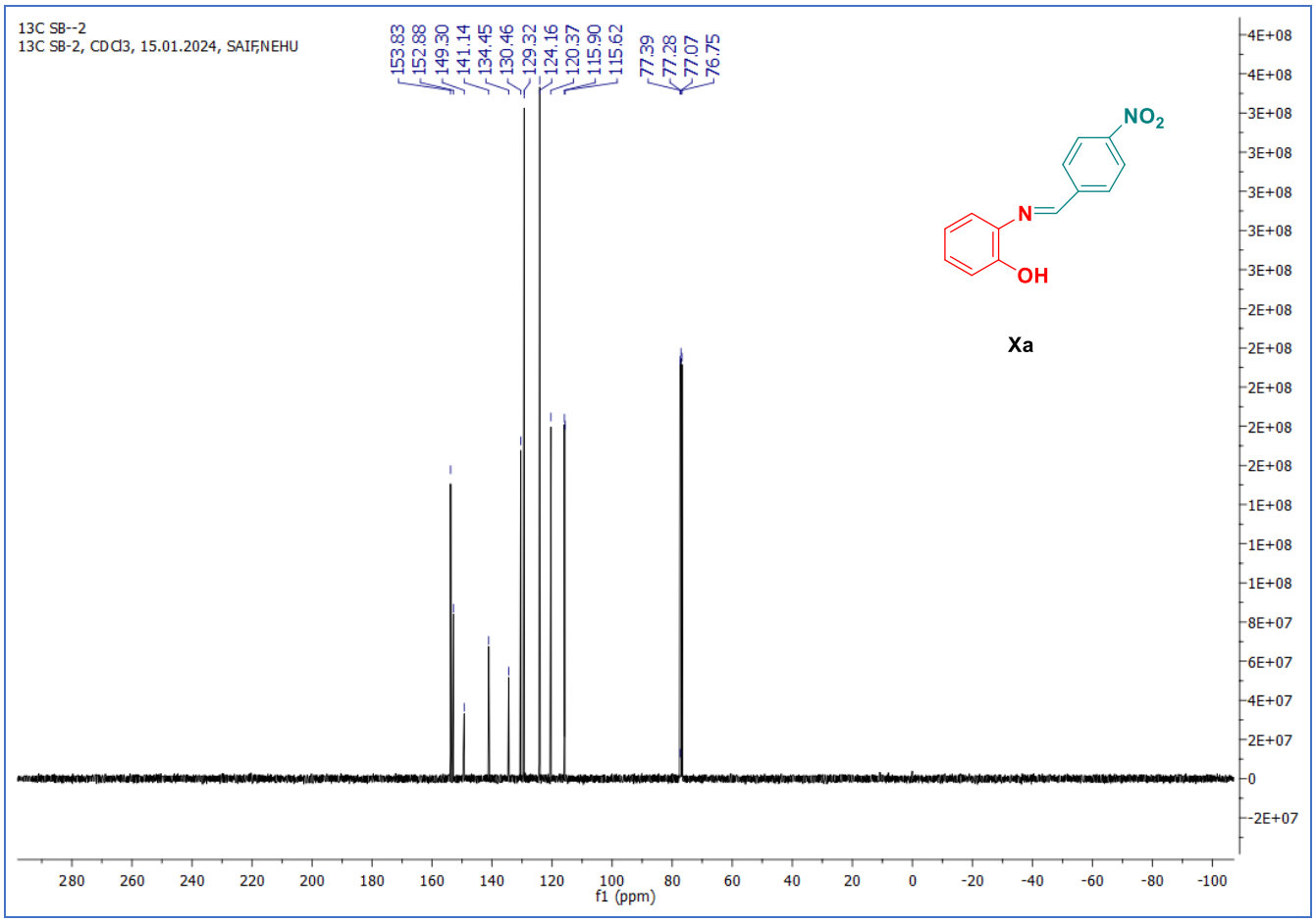
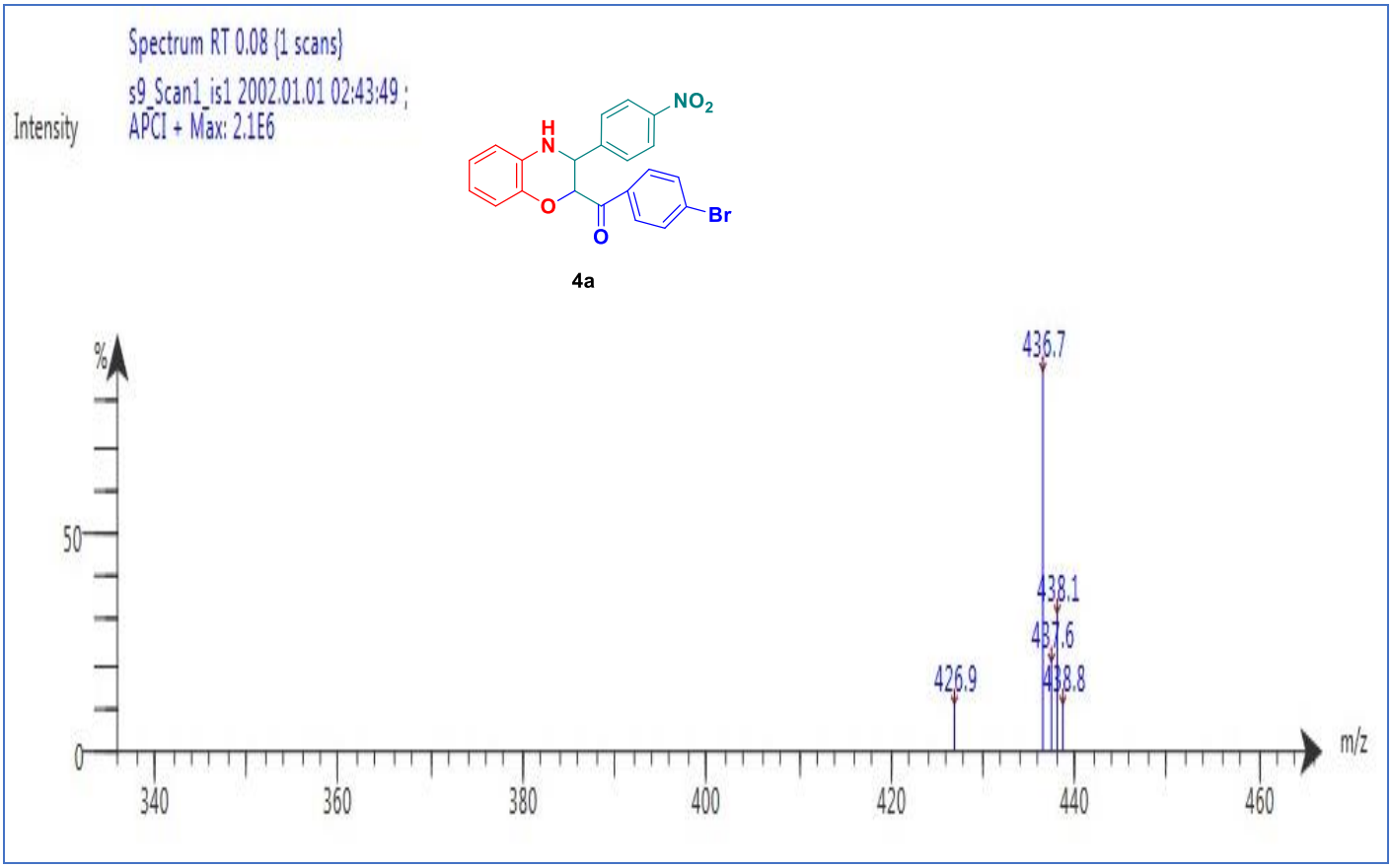


Xa

Mass spectra of 4a



4a

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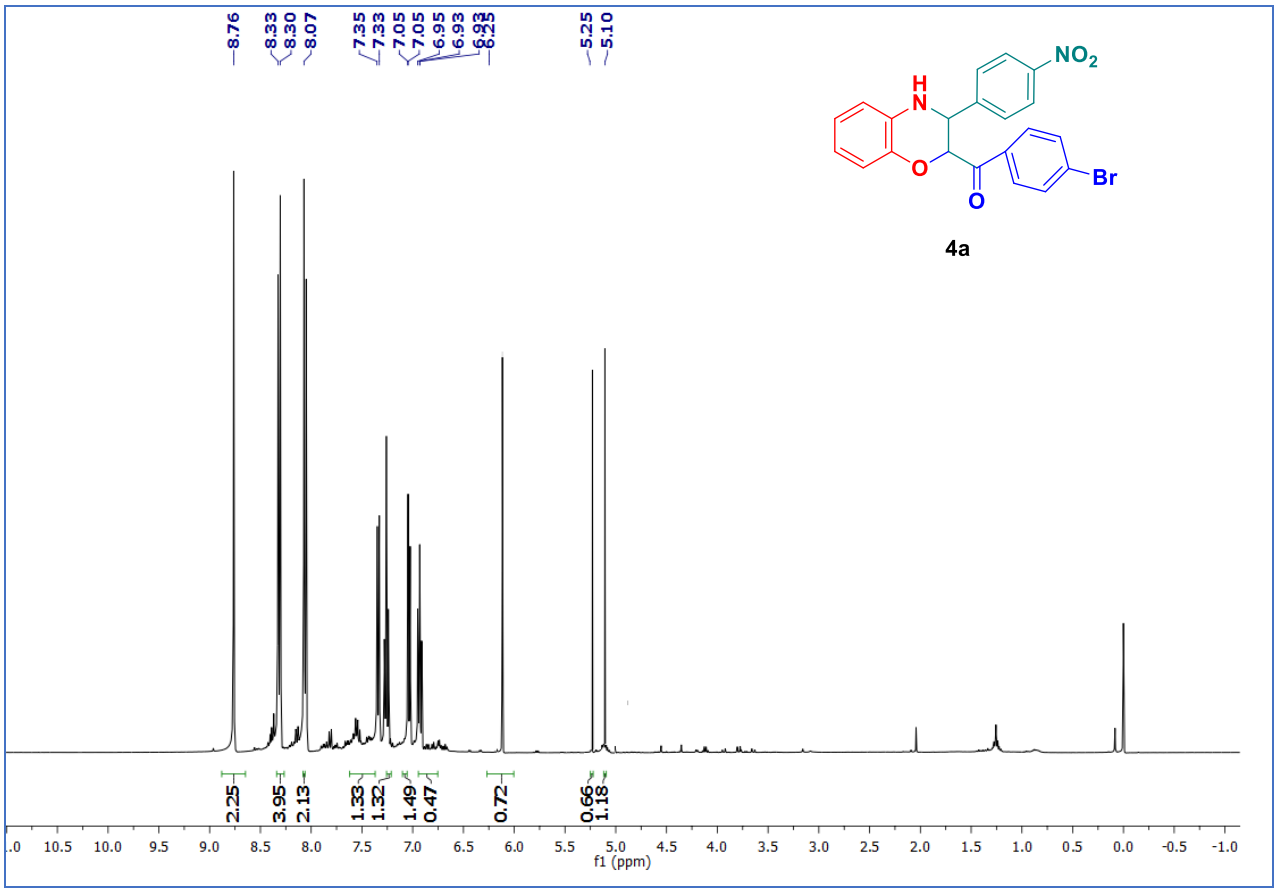

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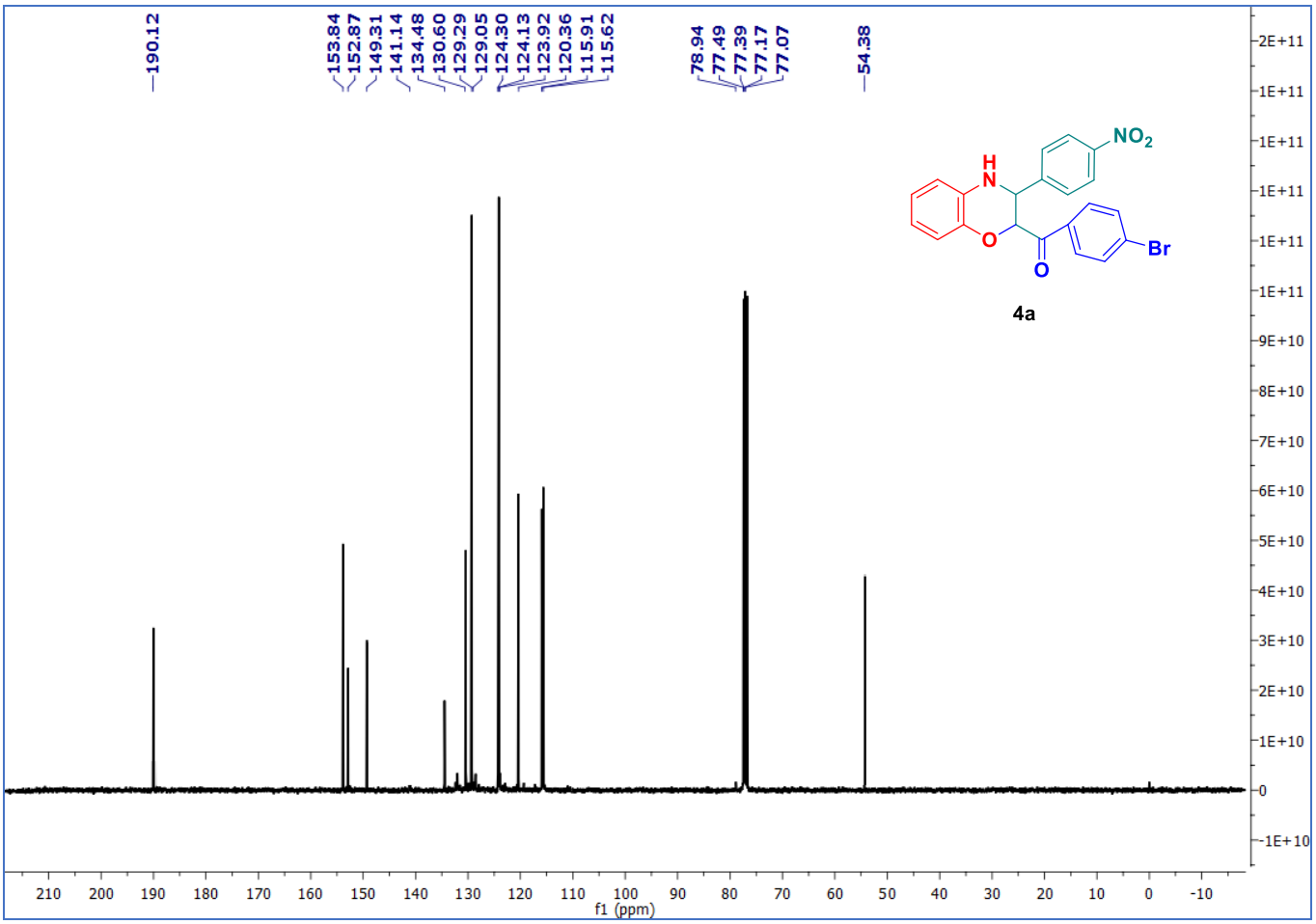

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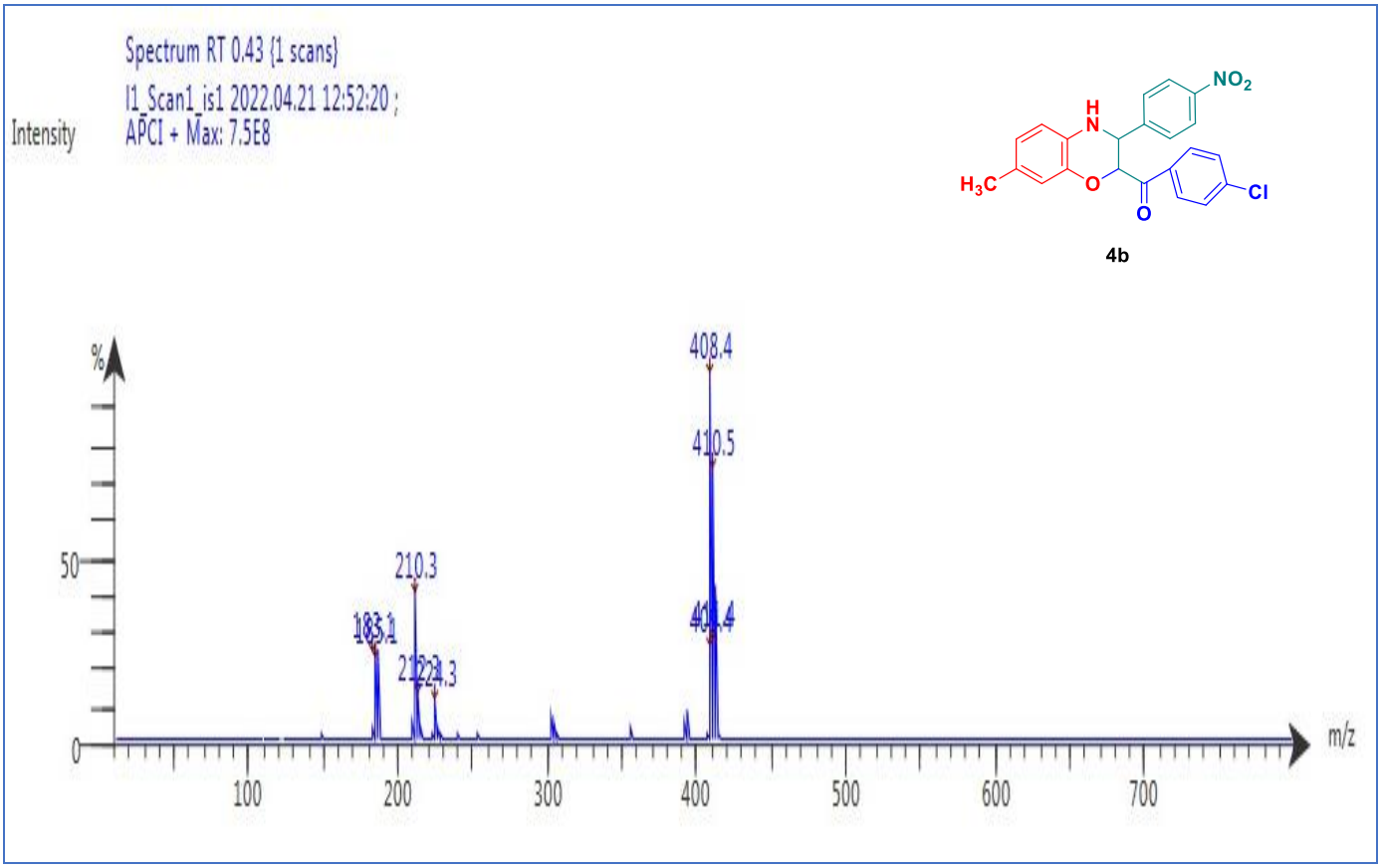

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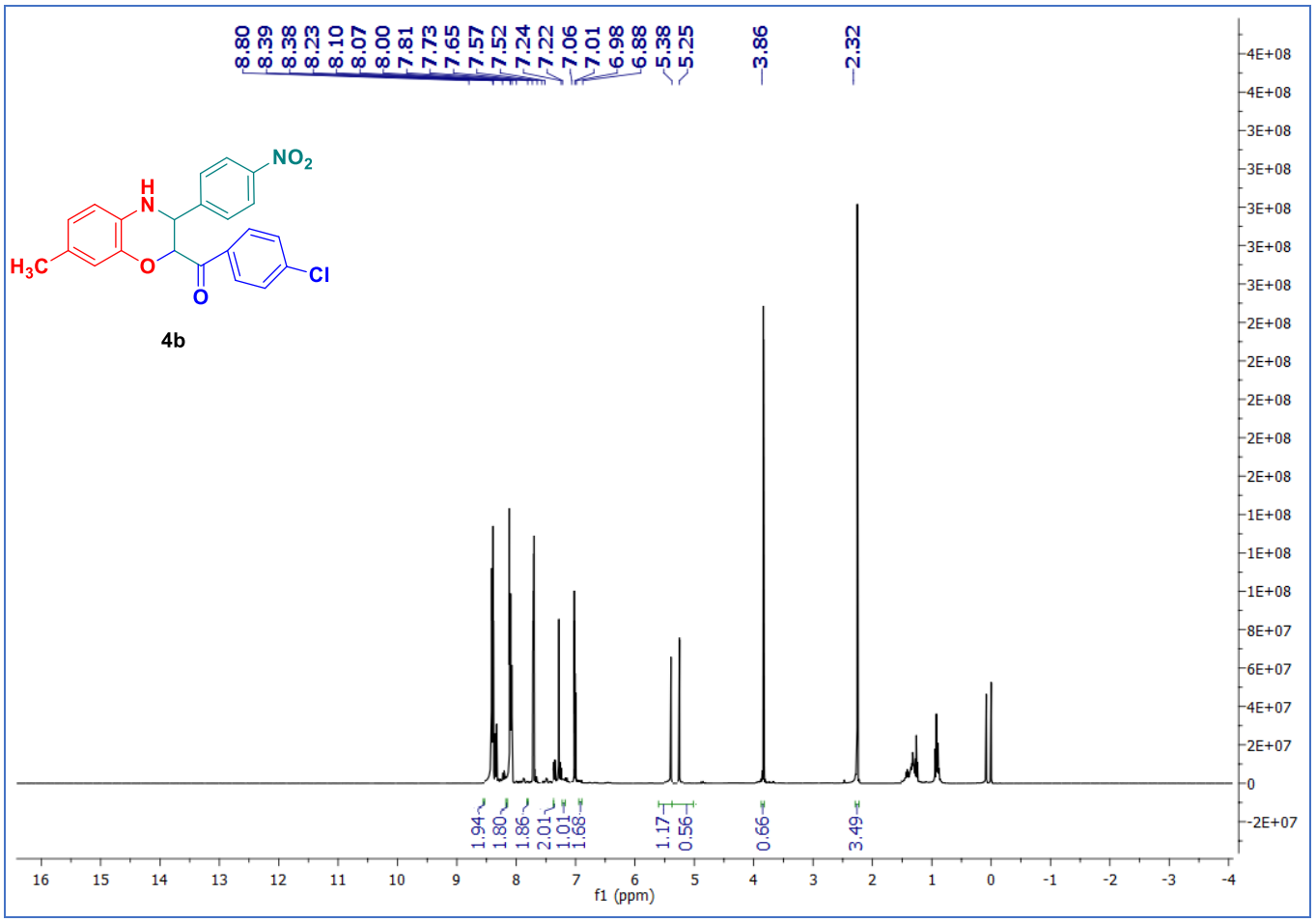

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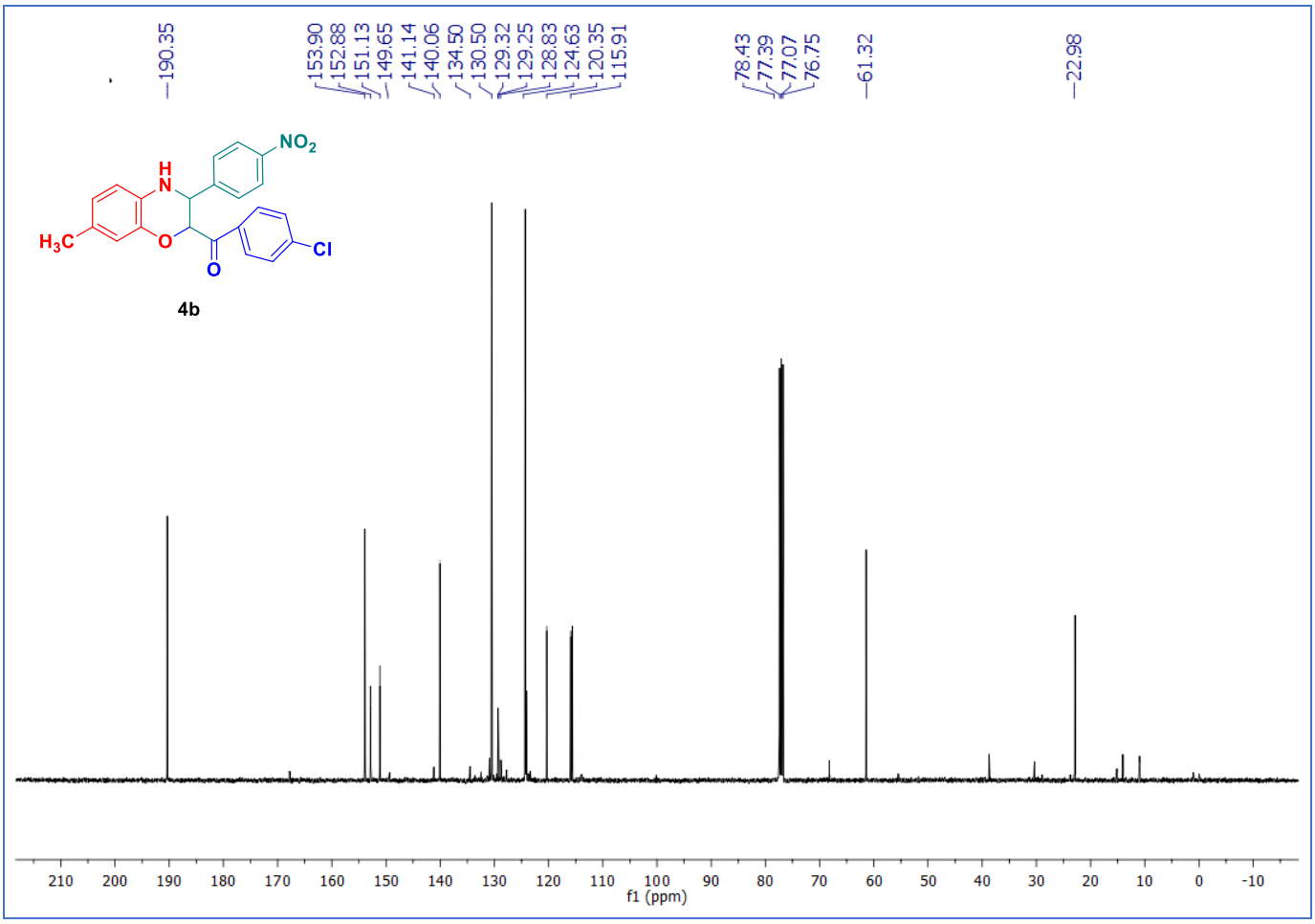

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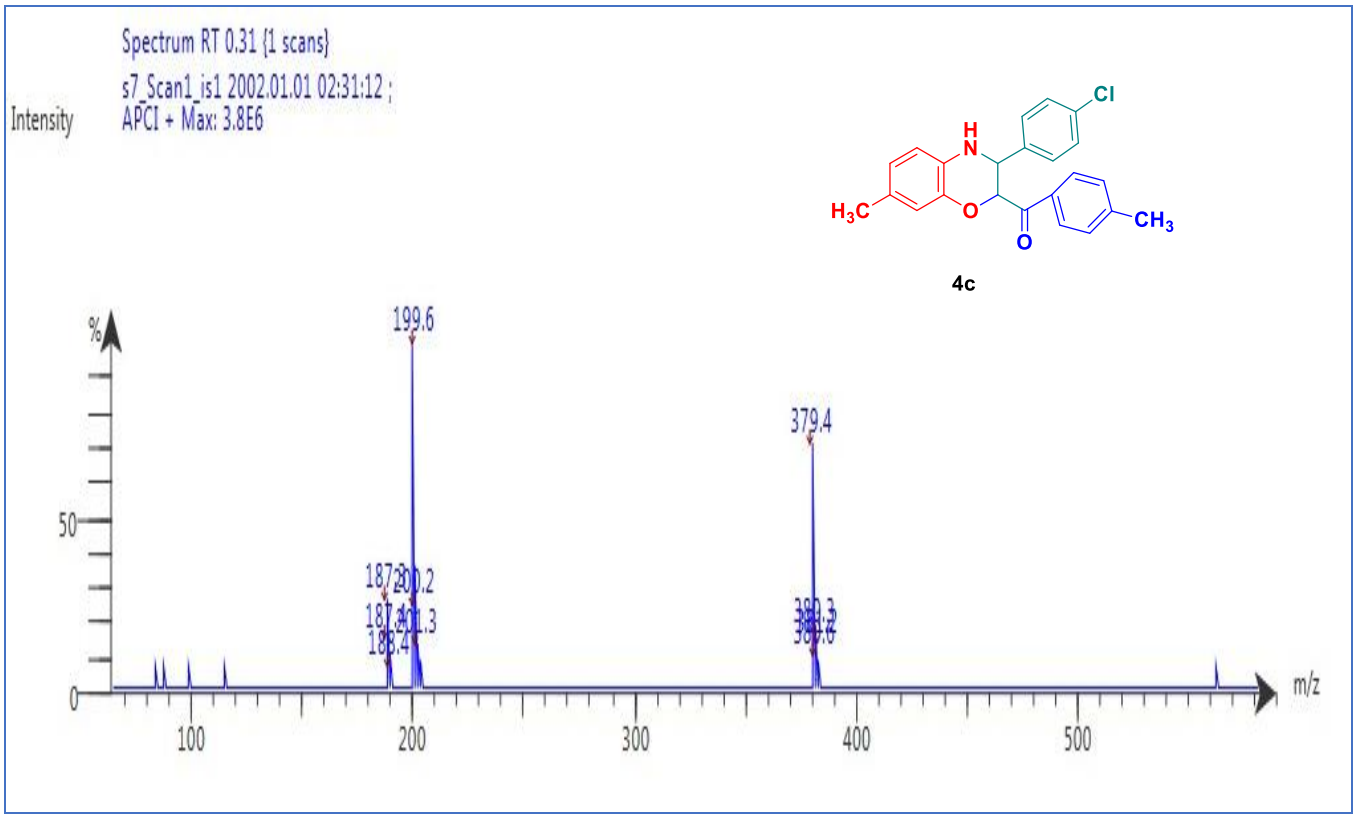

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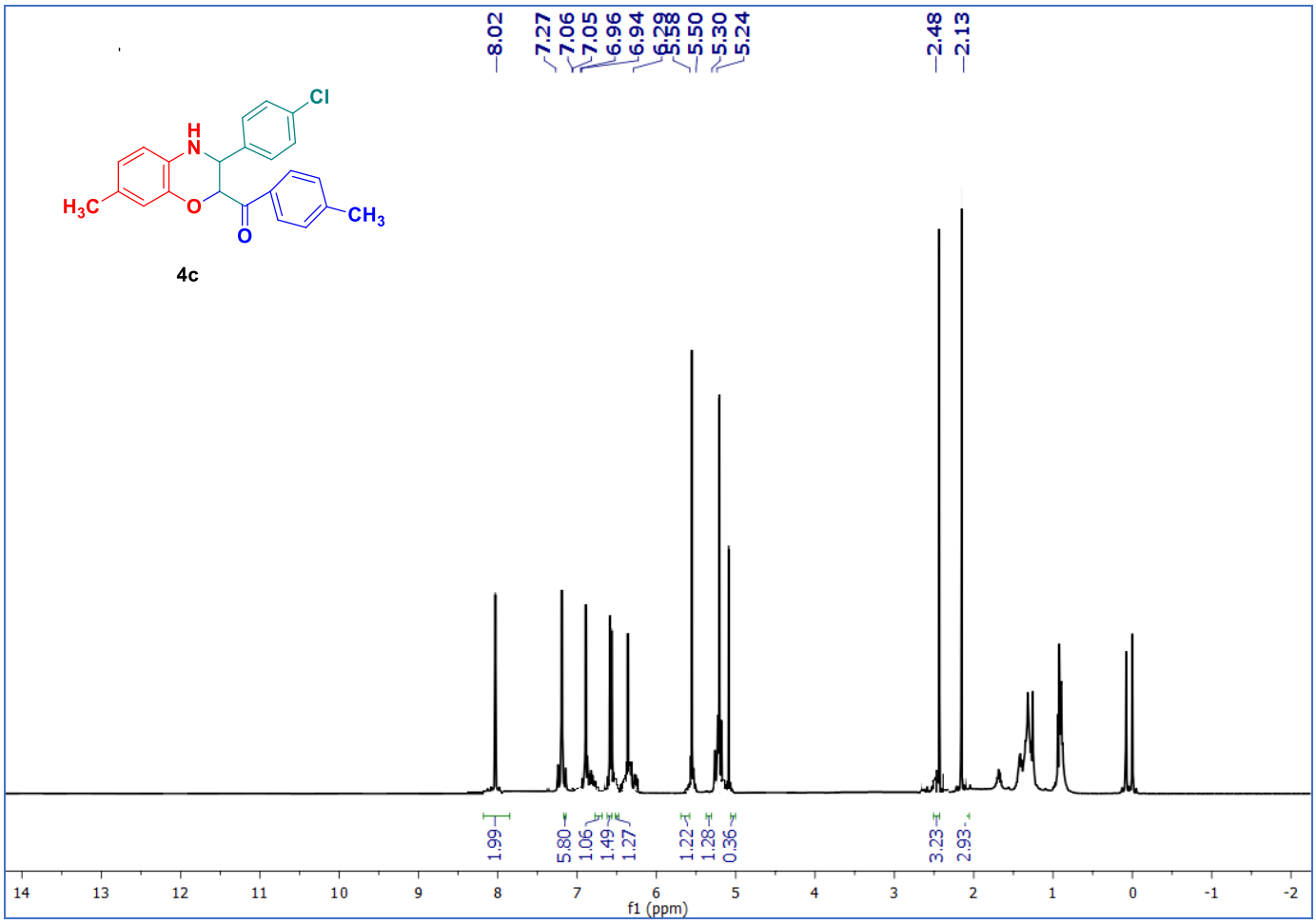

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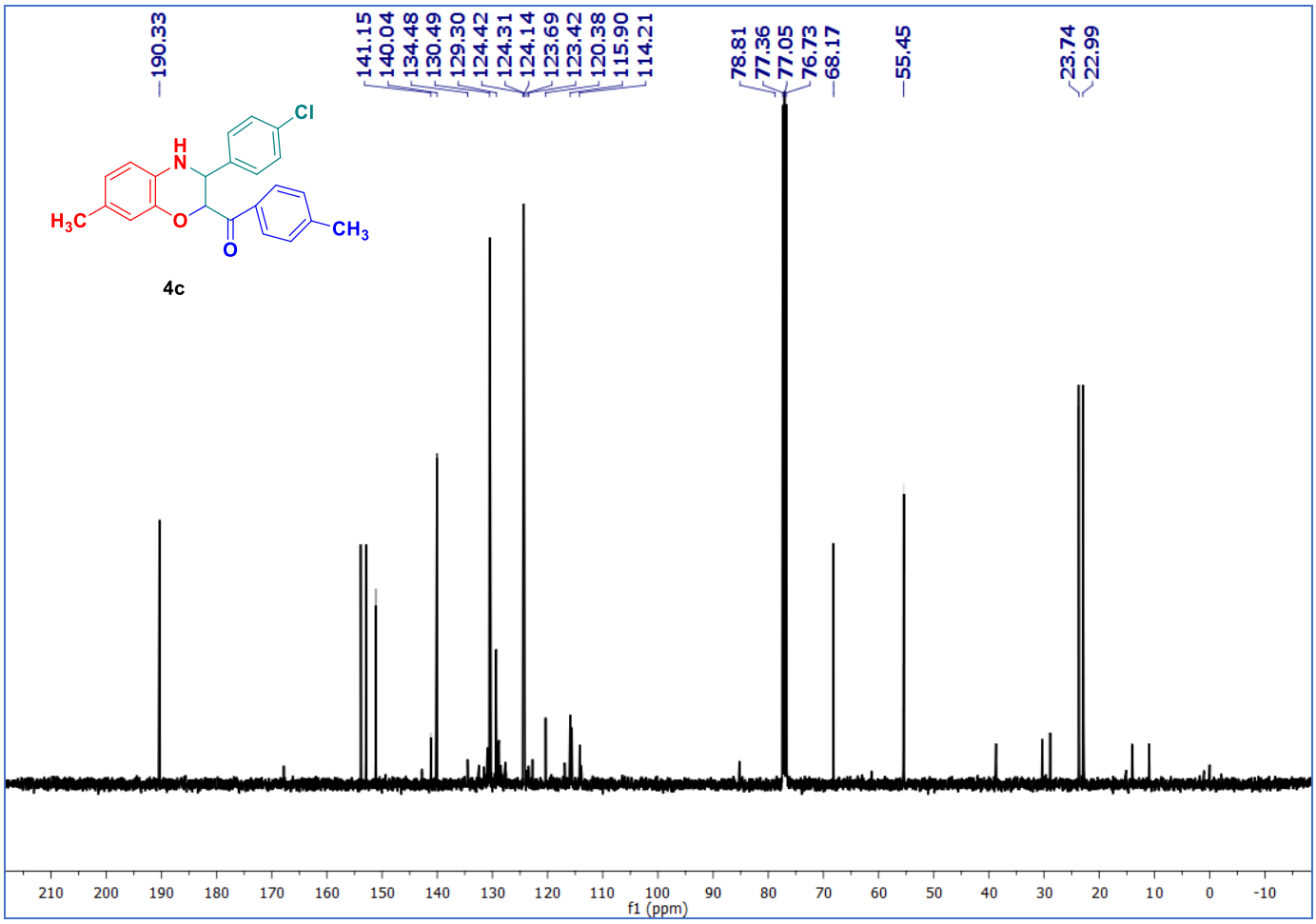

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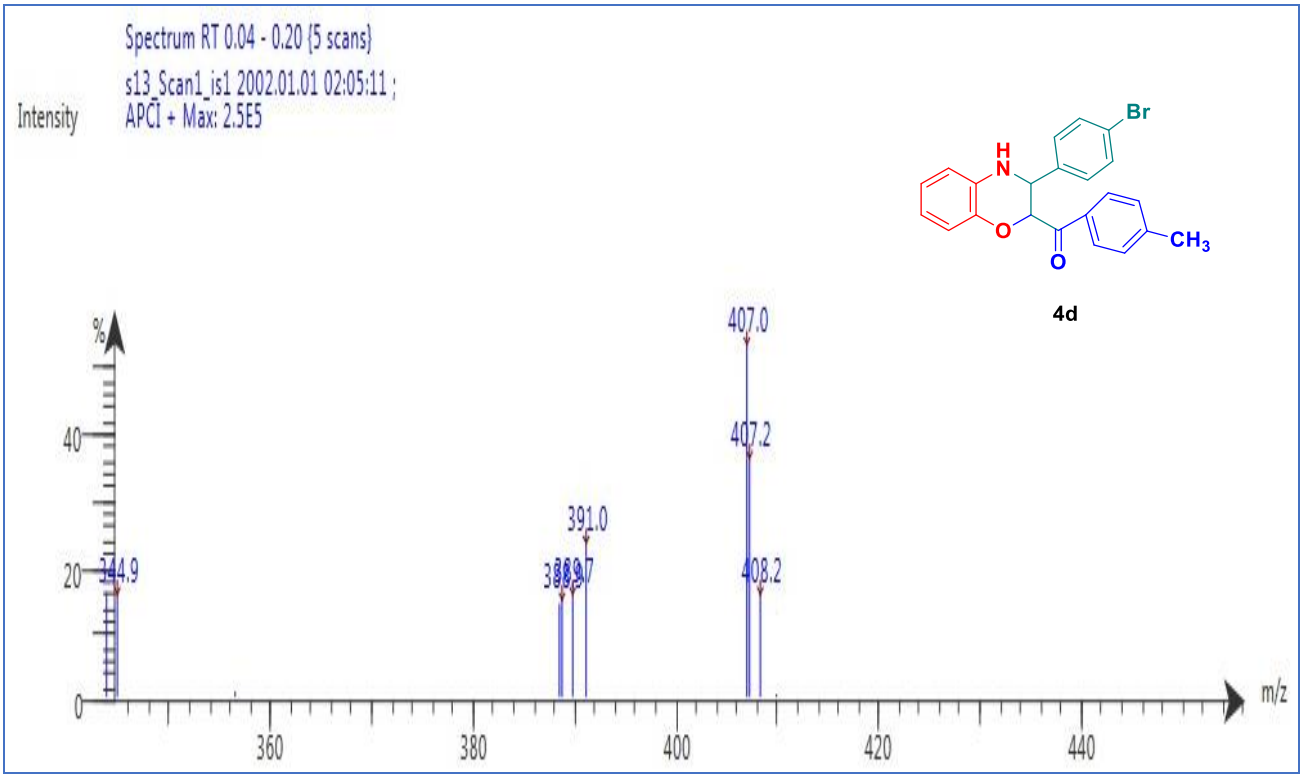

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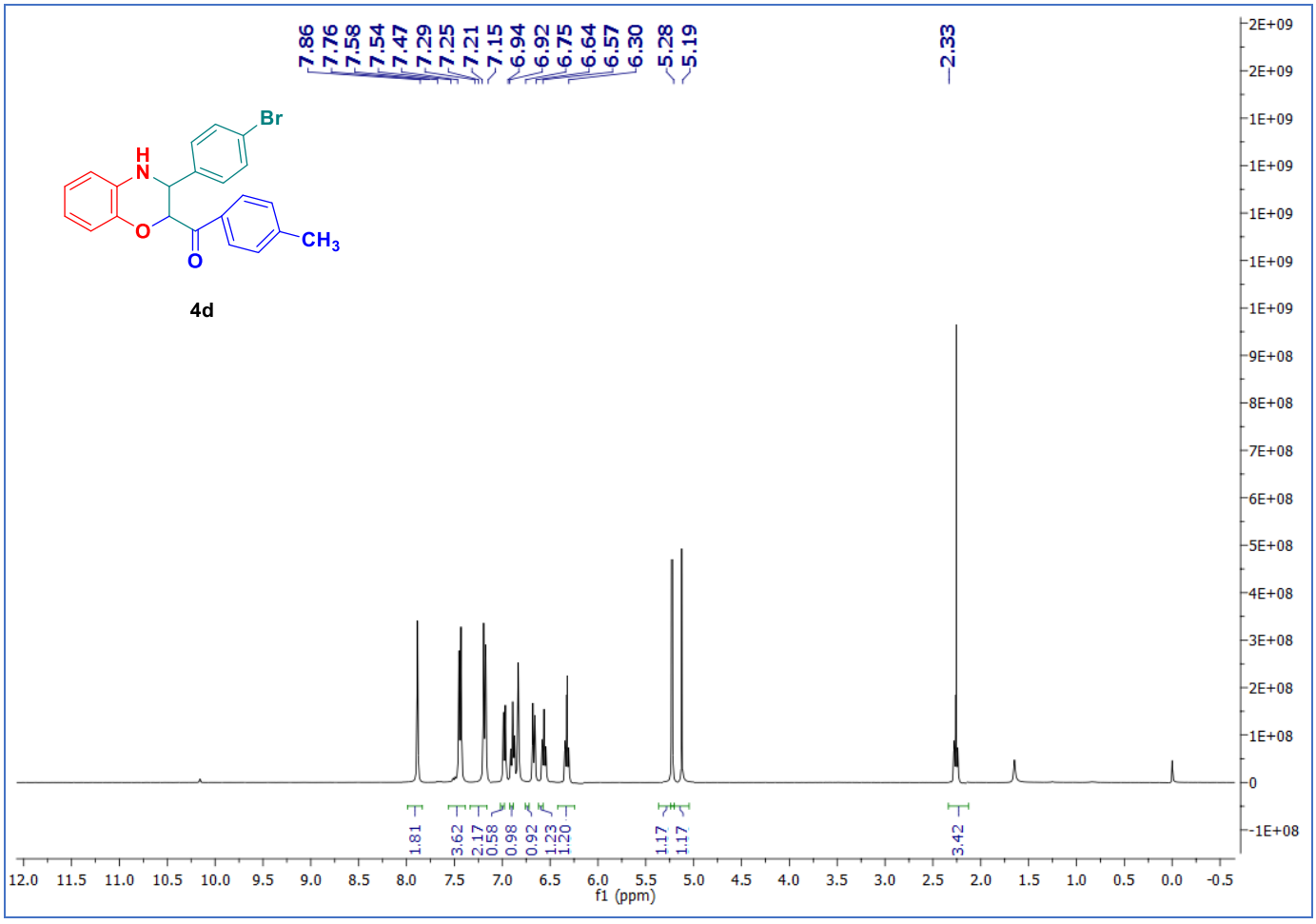

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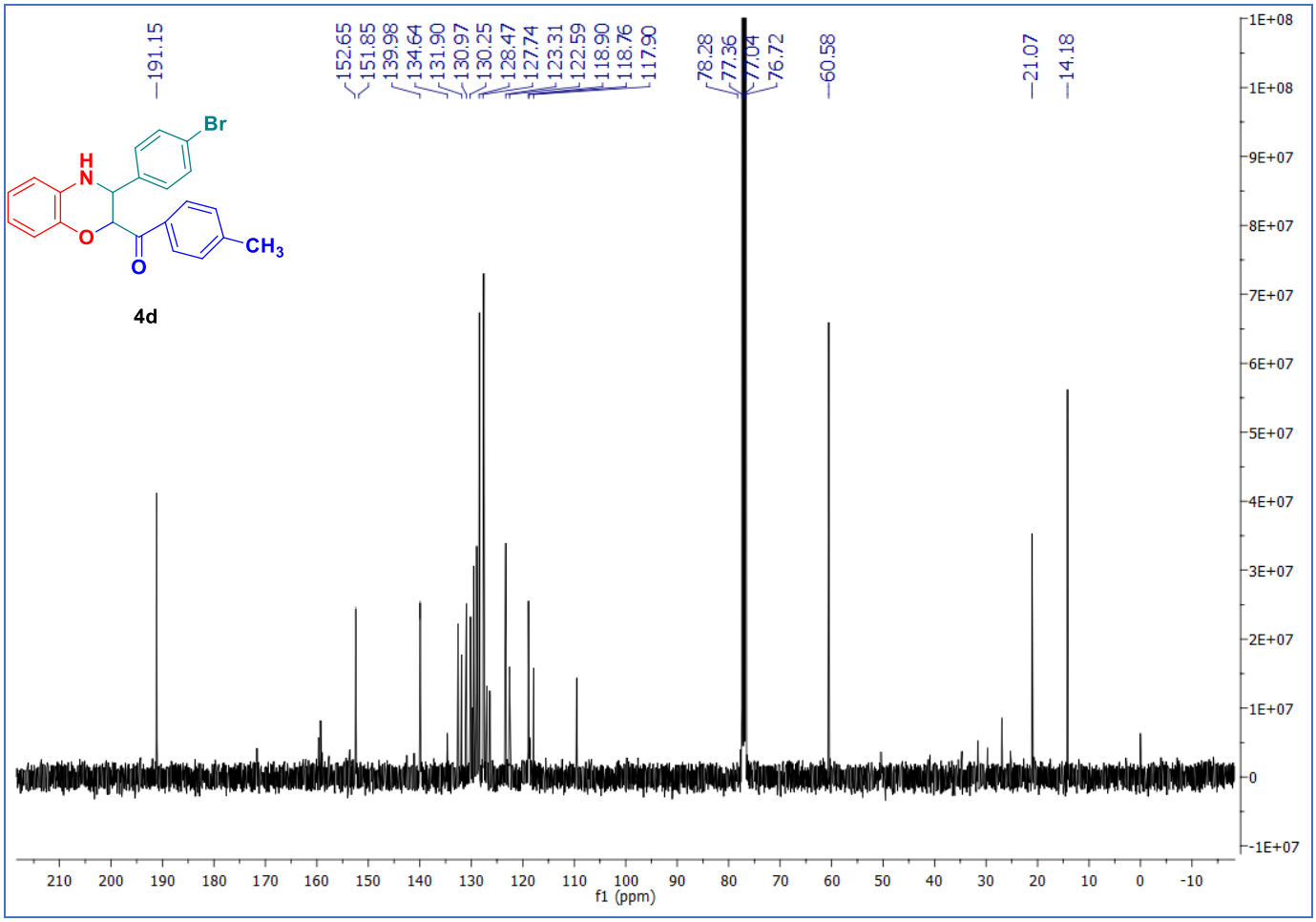

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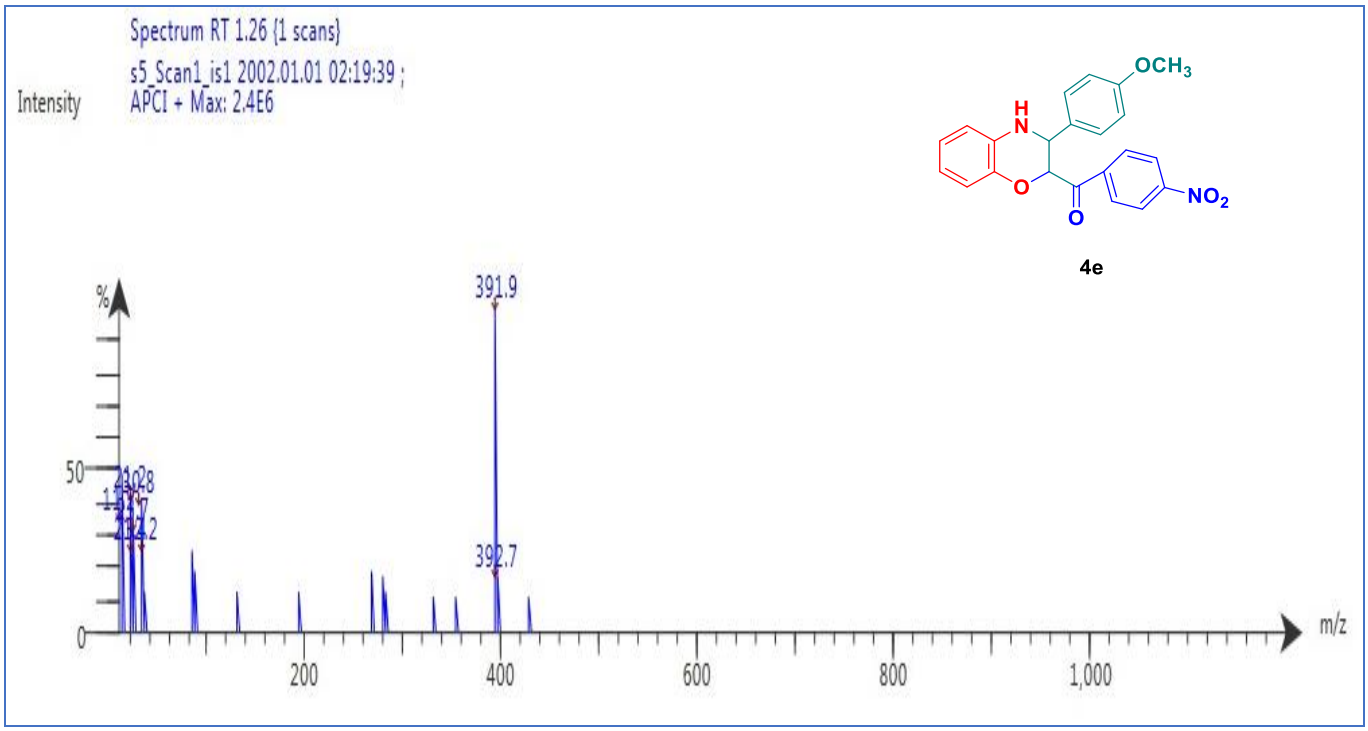

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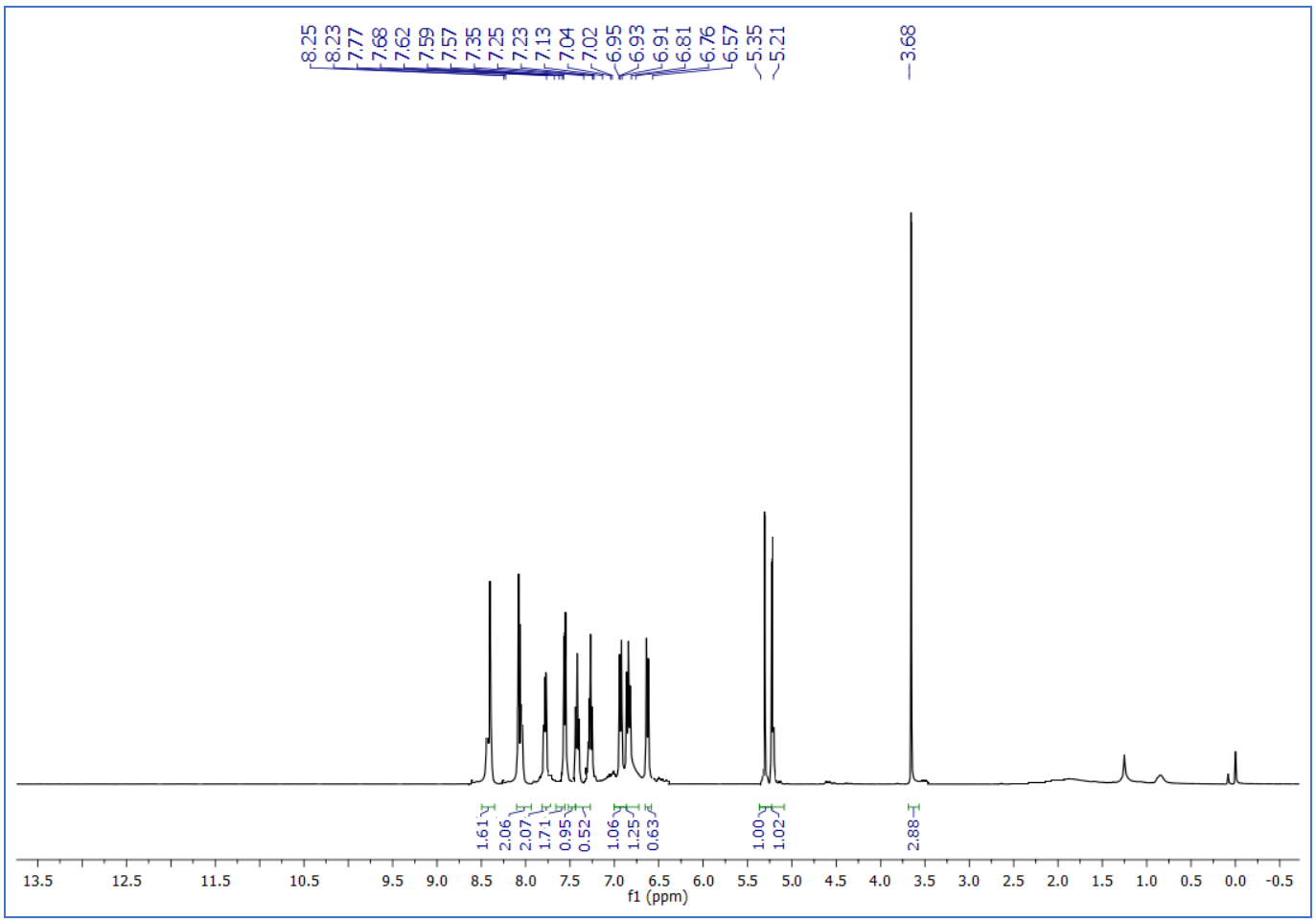

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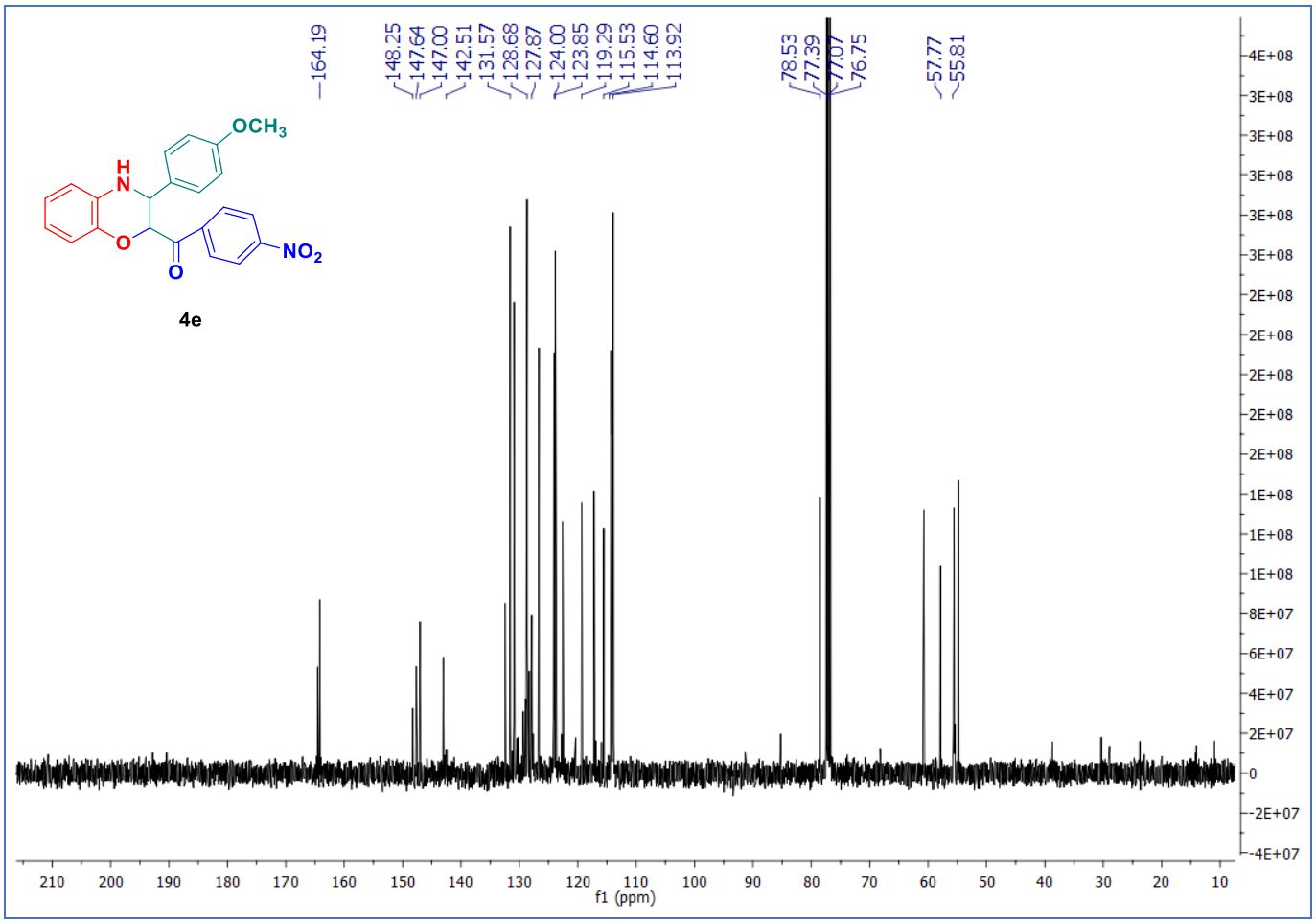

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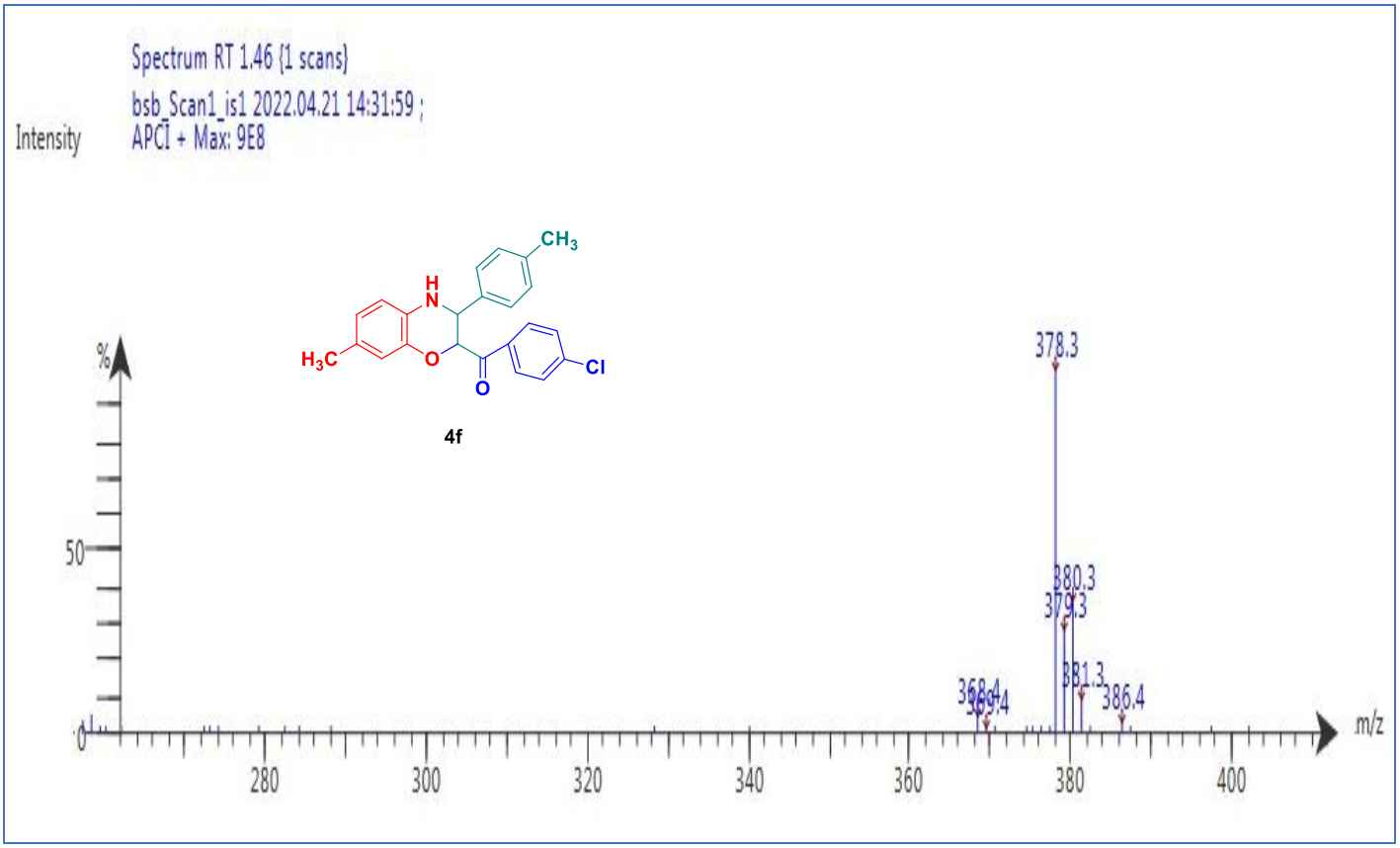

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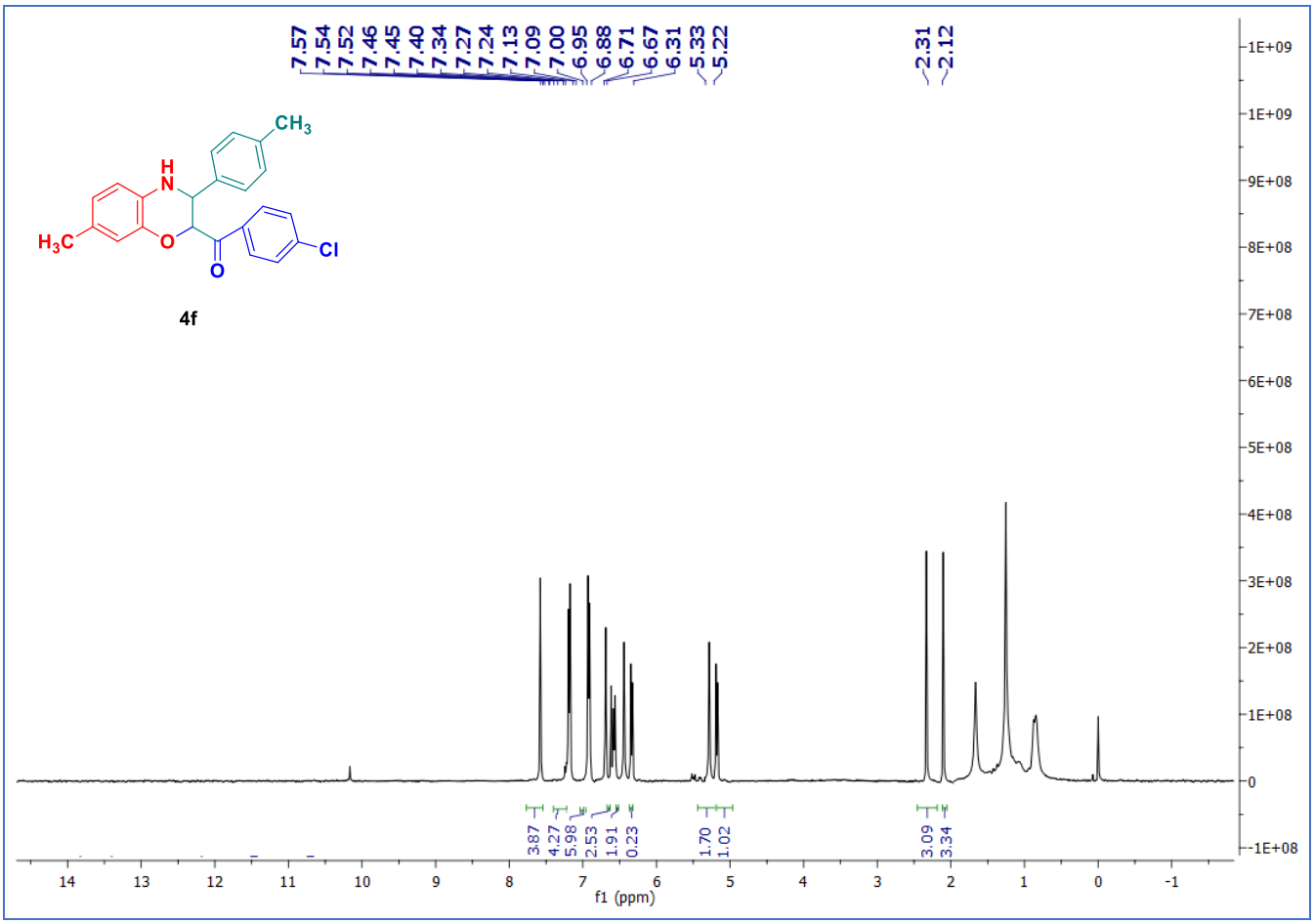

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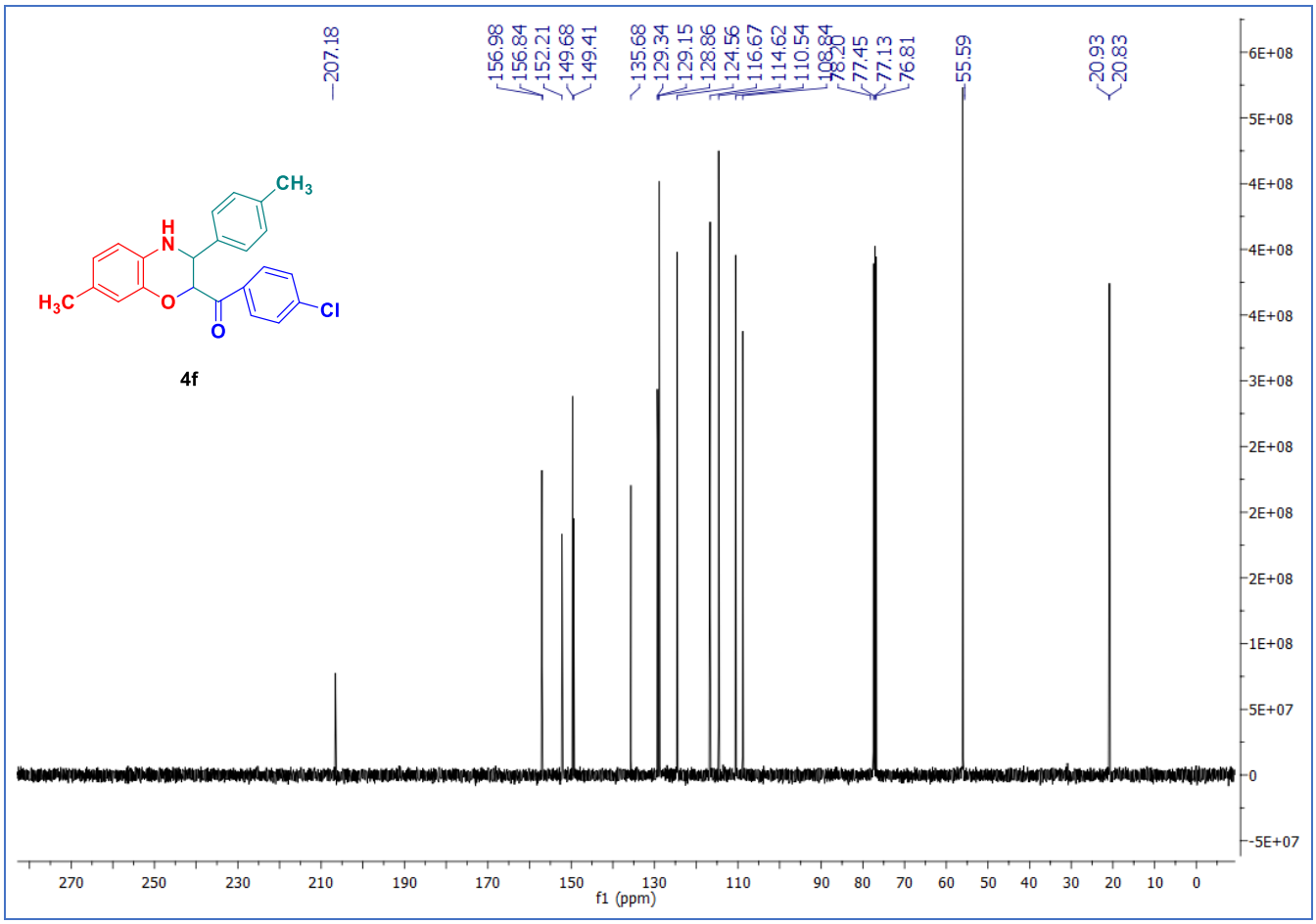

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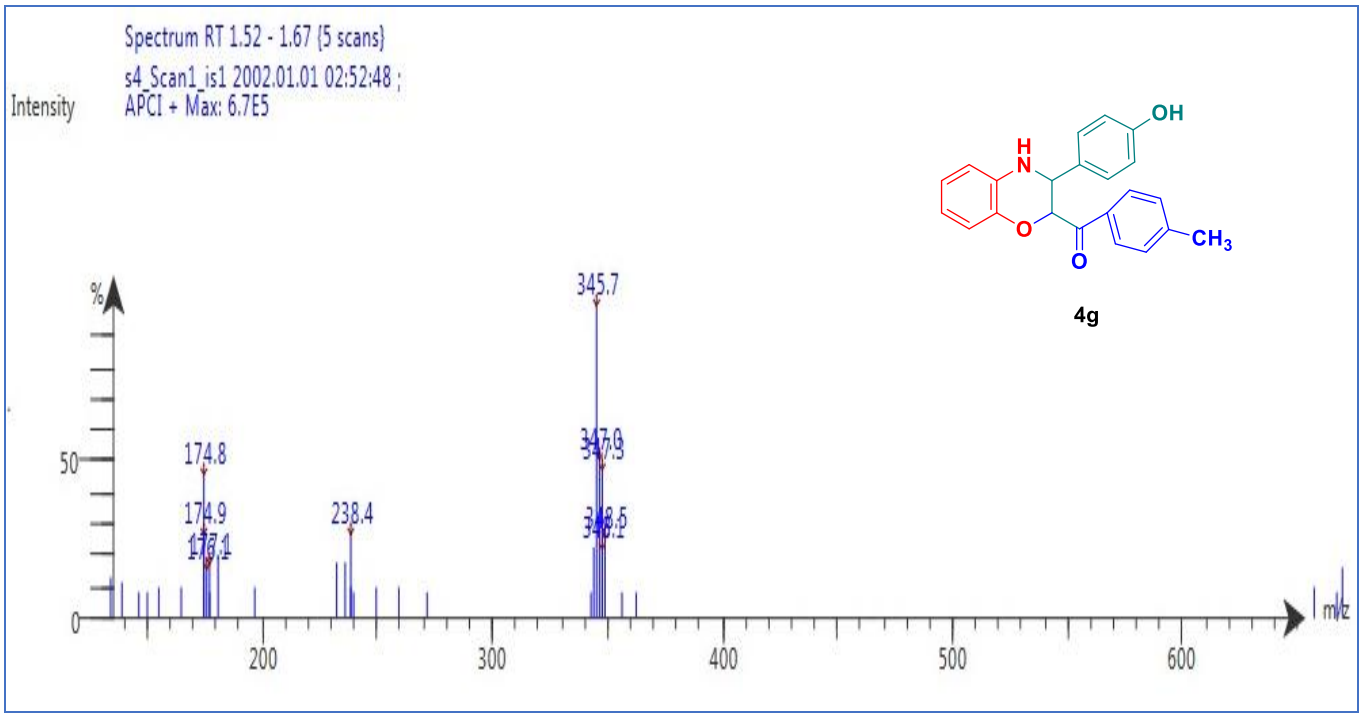

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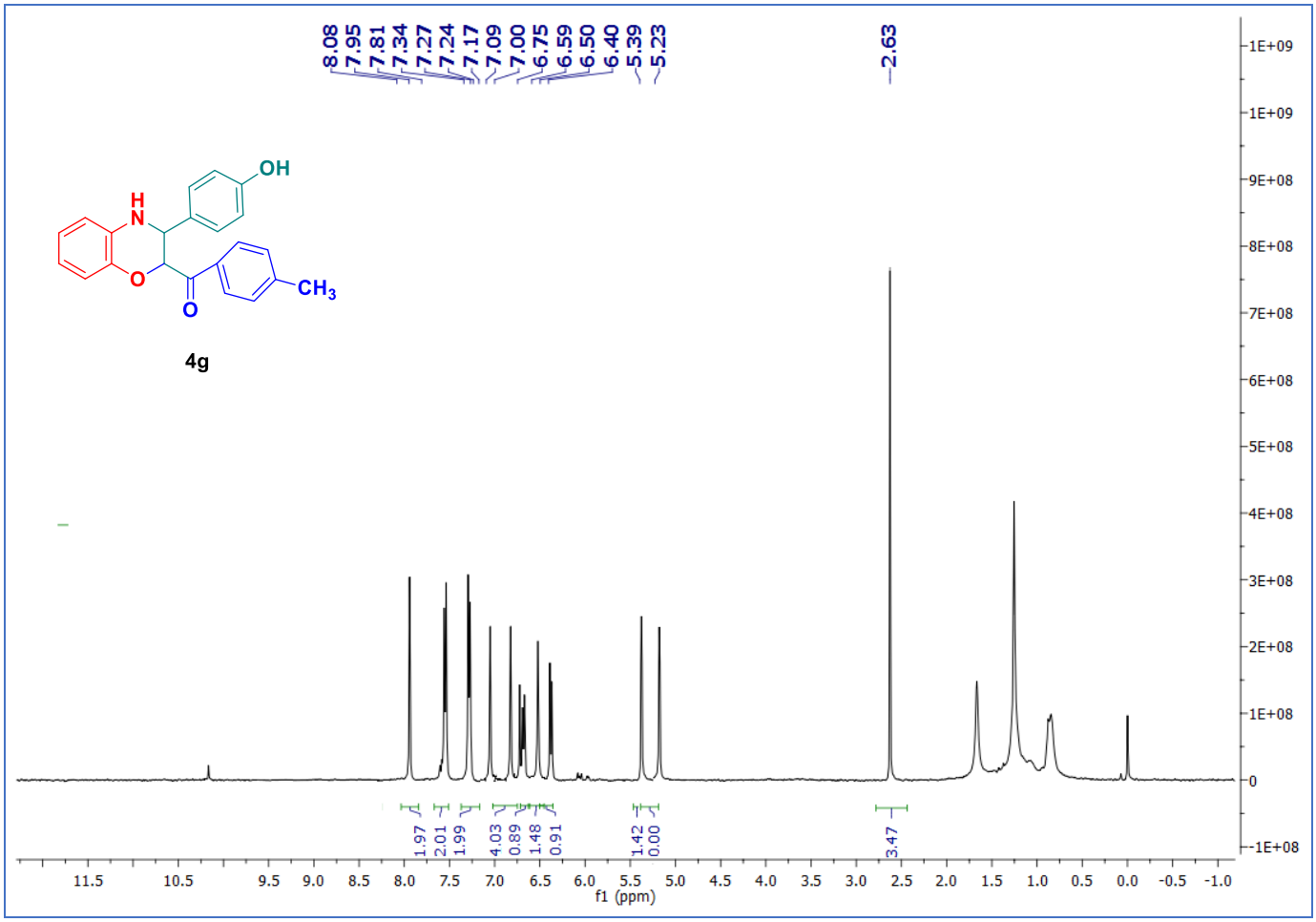

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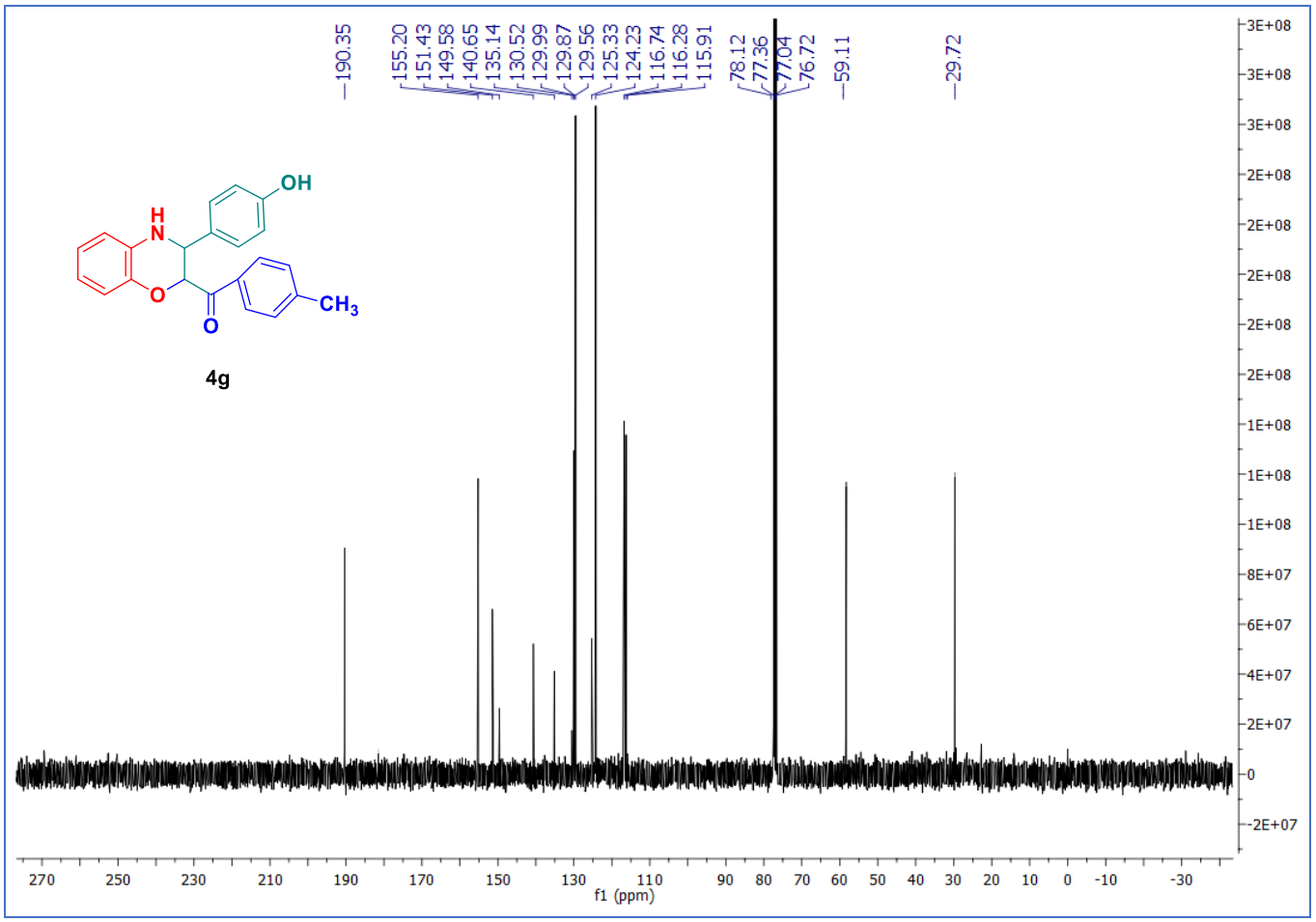
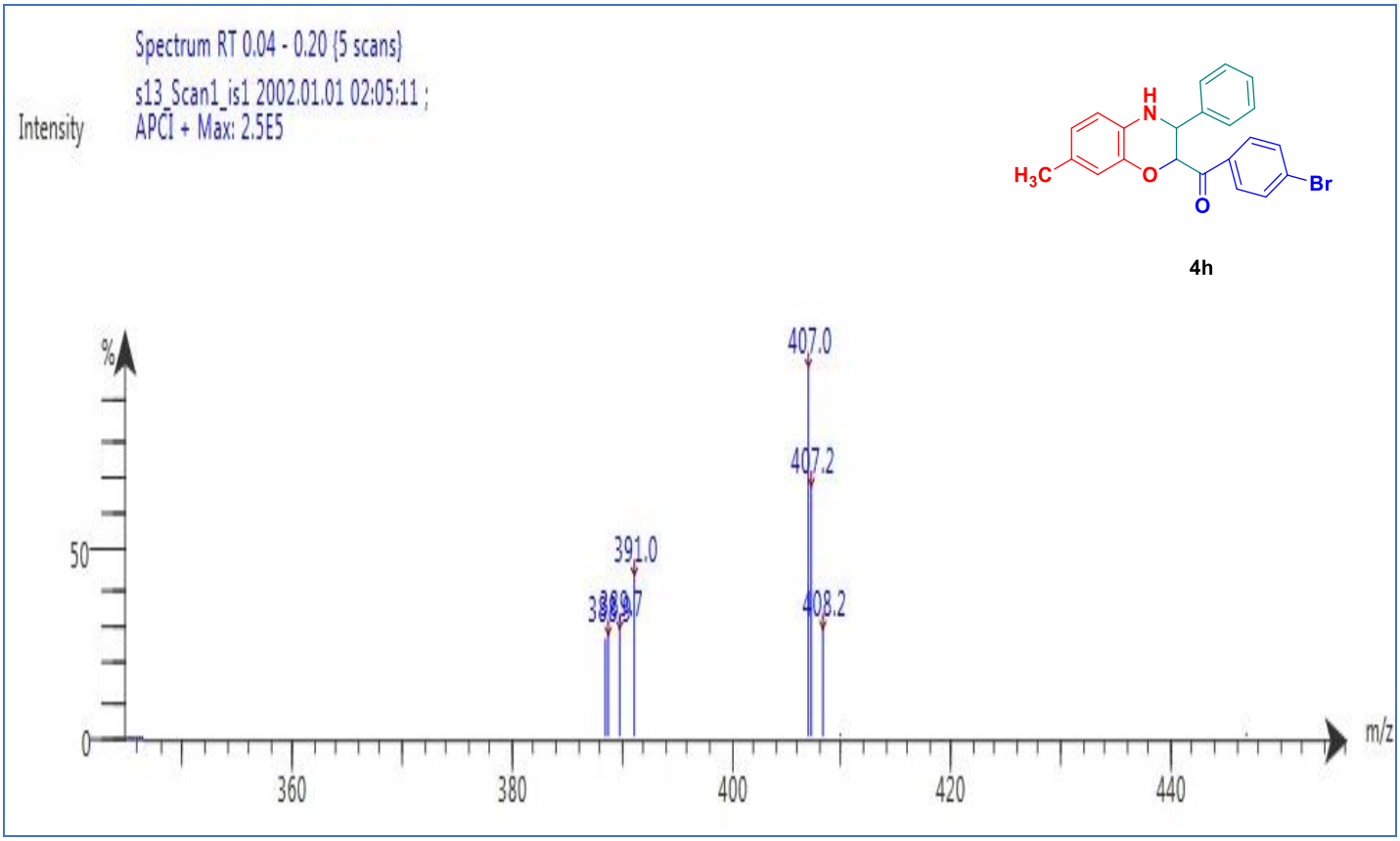


4g

Mass spectra of 4h



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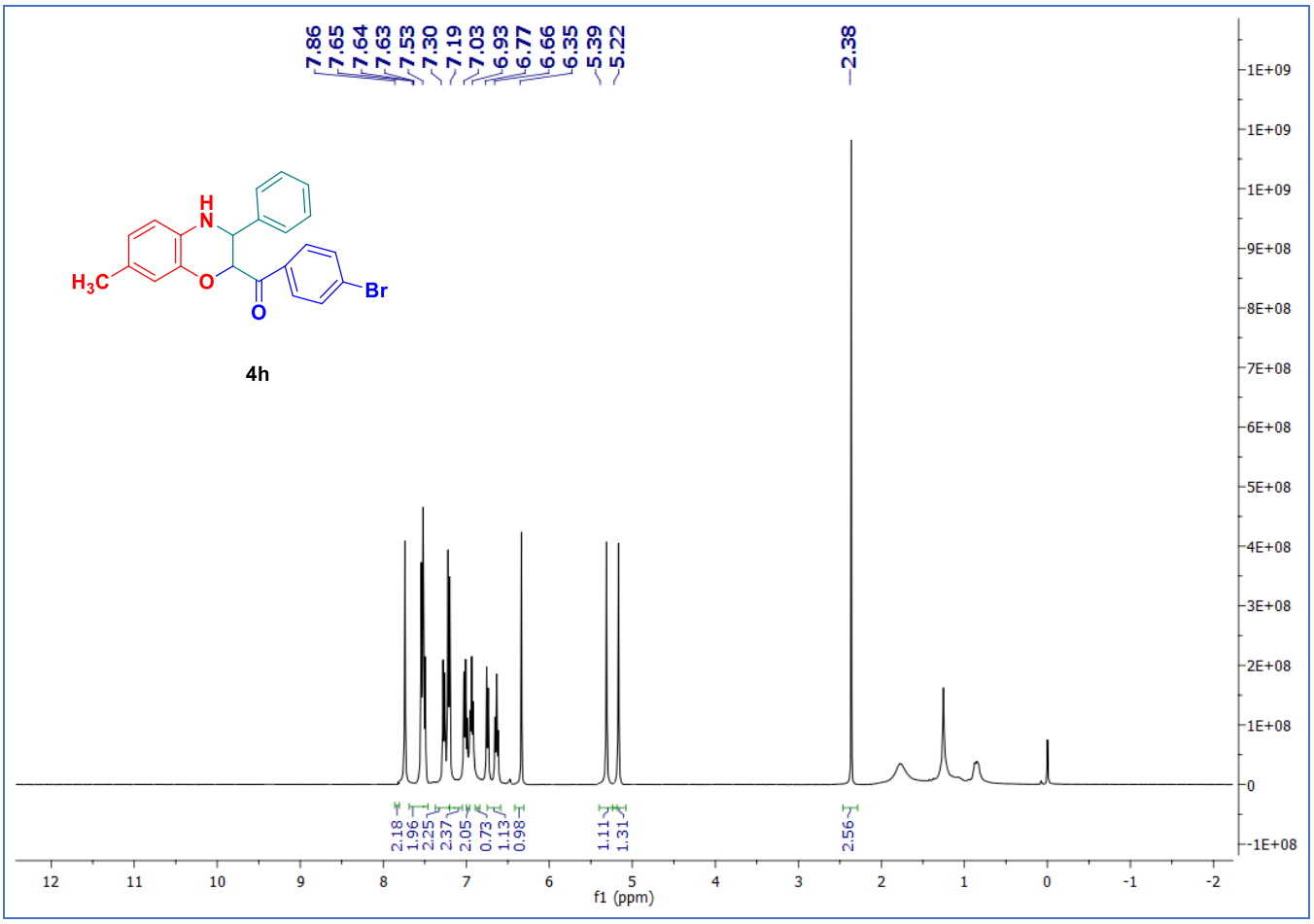

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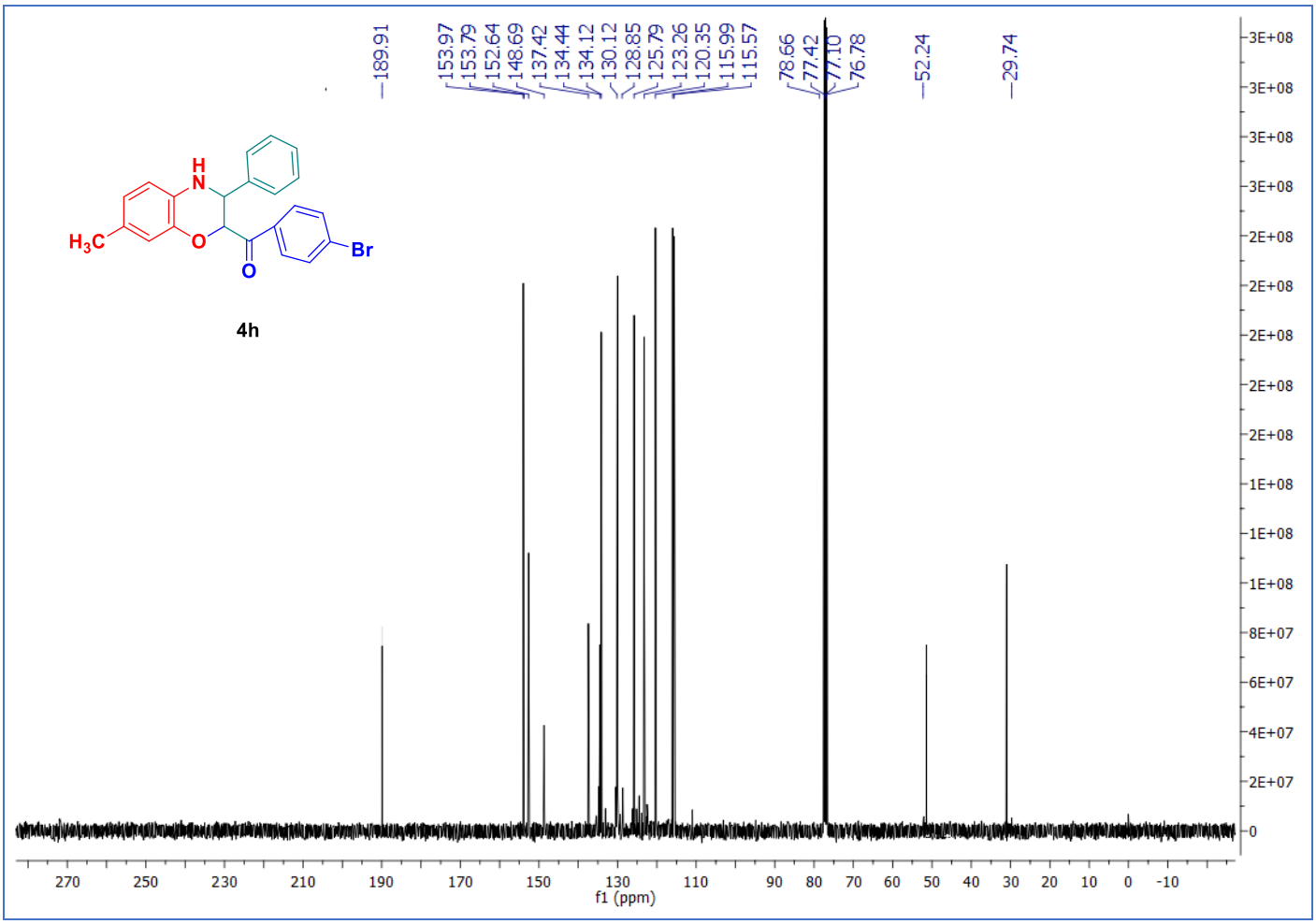

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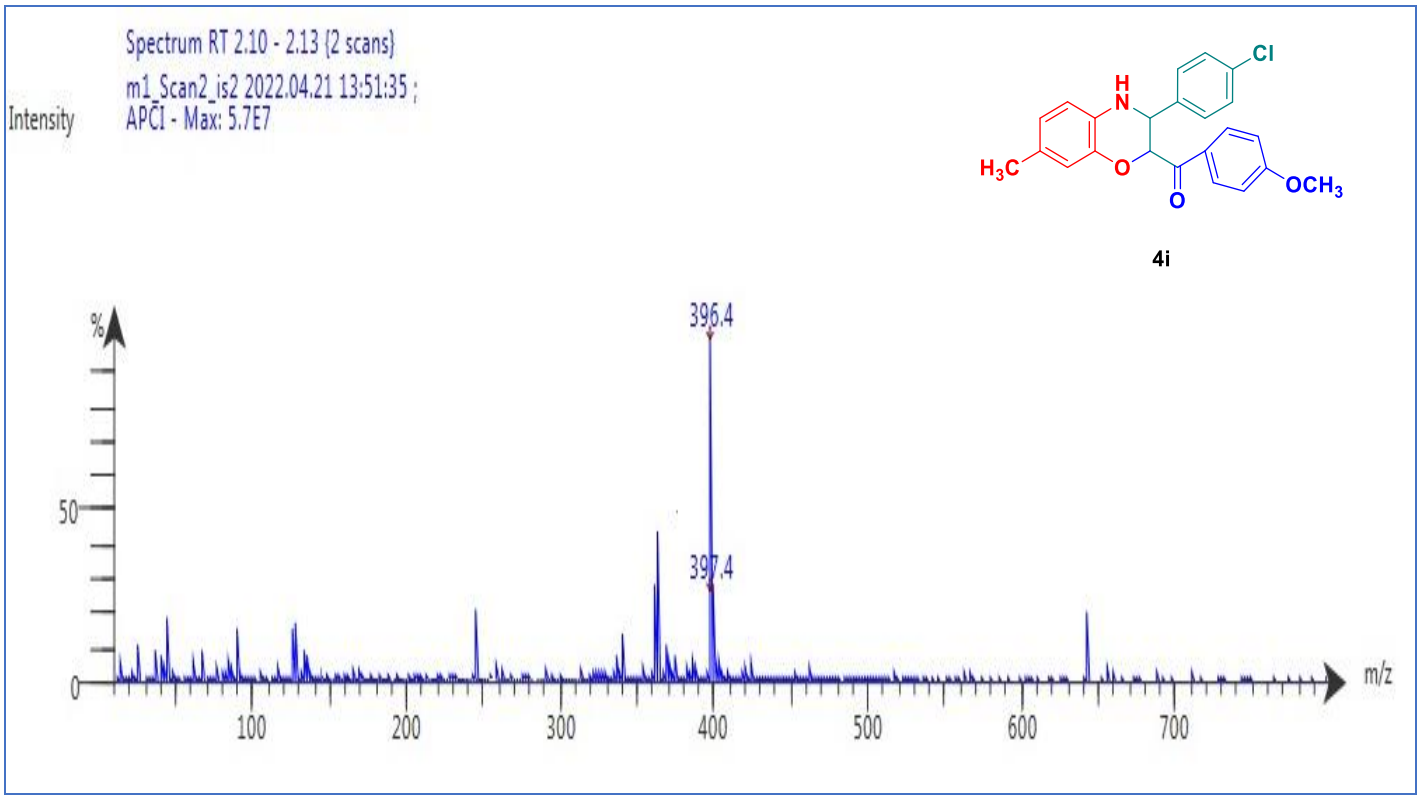

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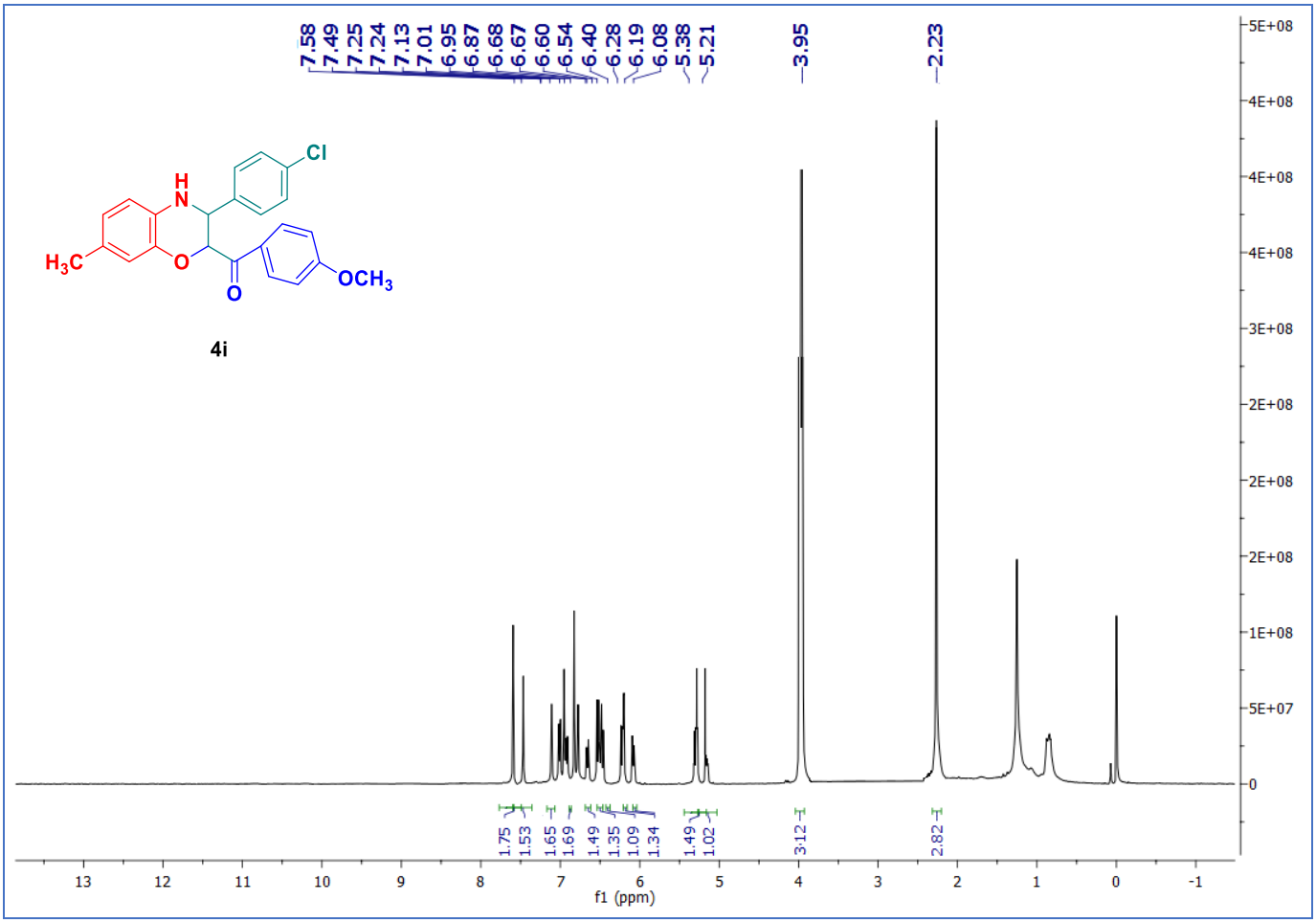

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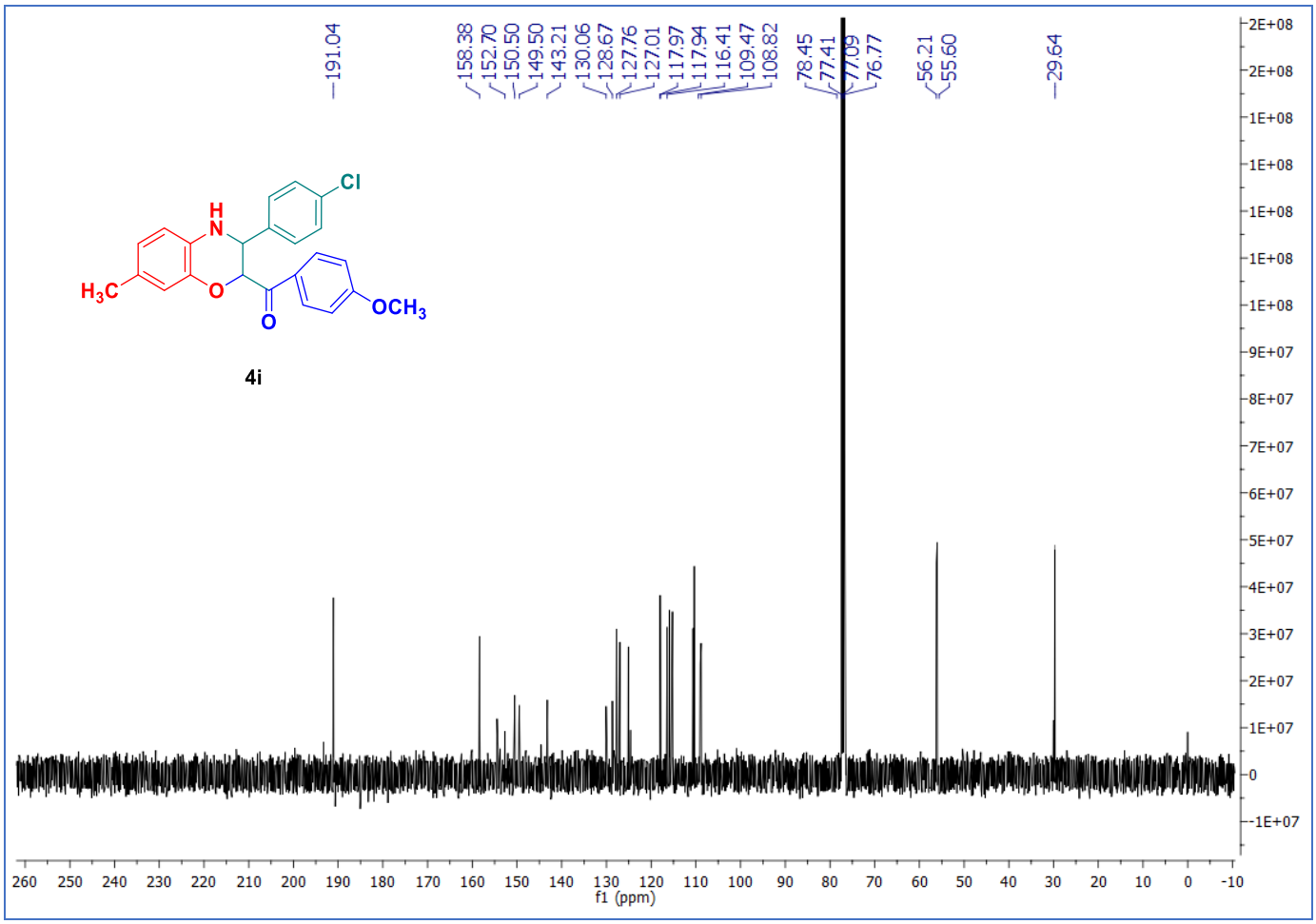

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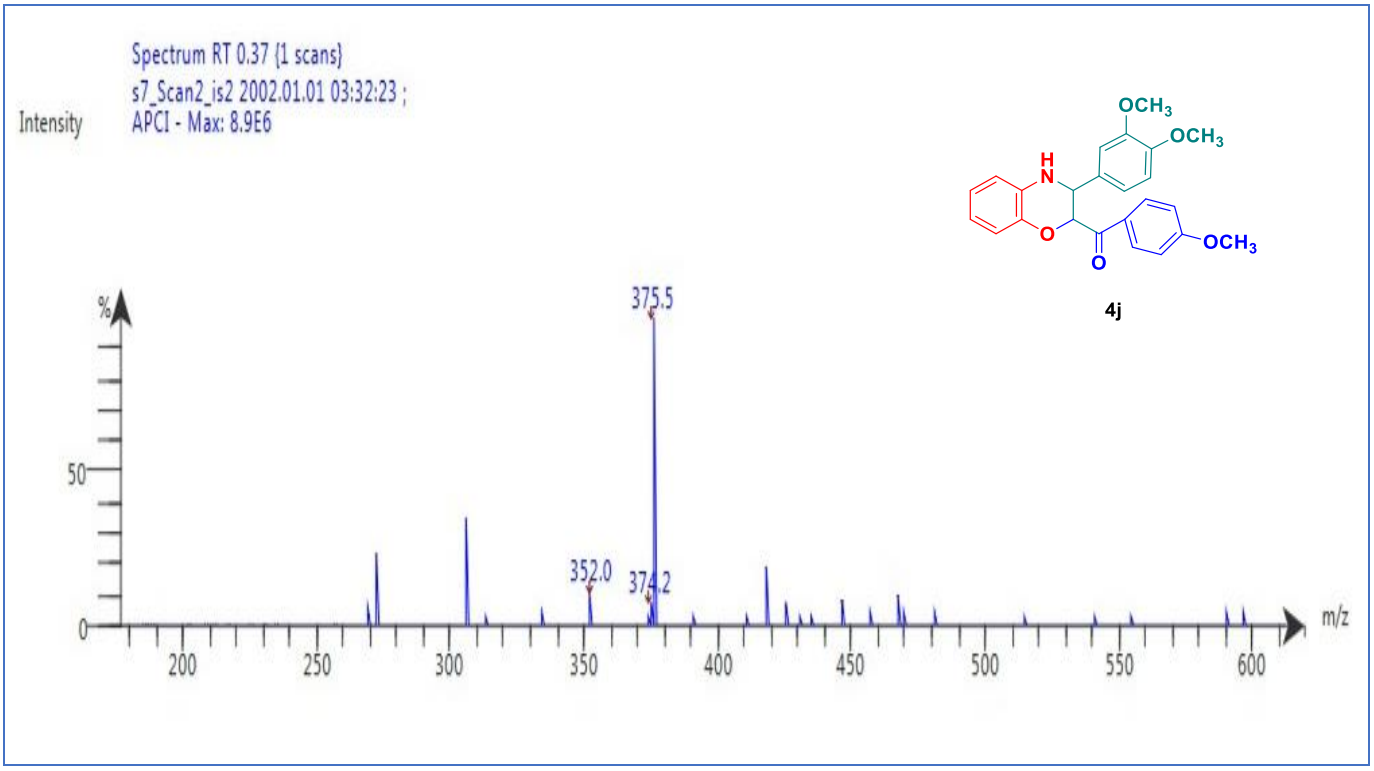

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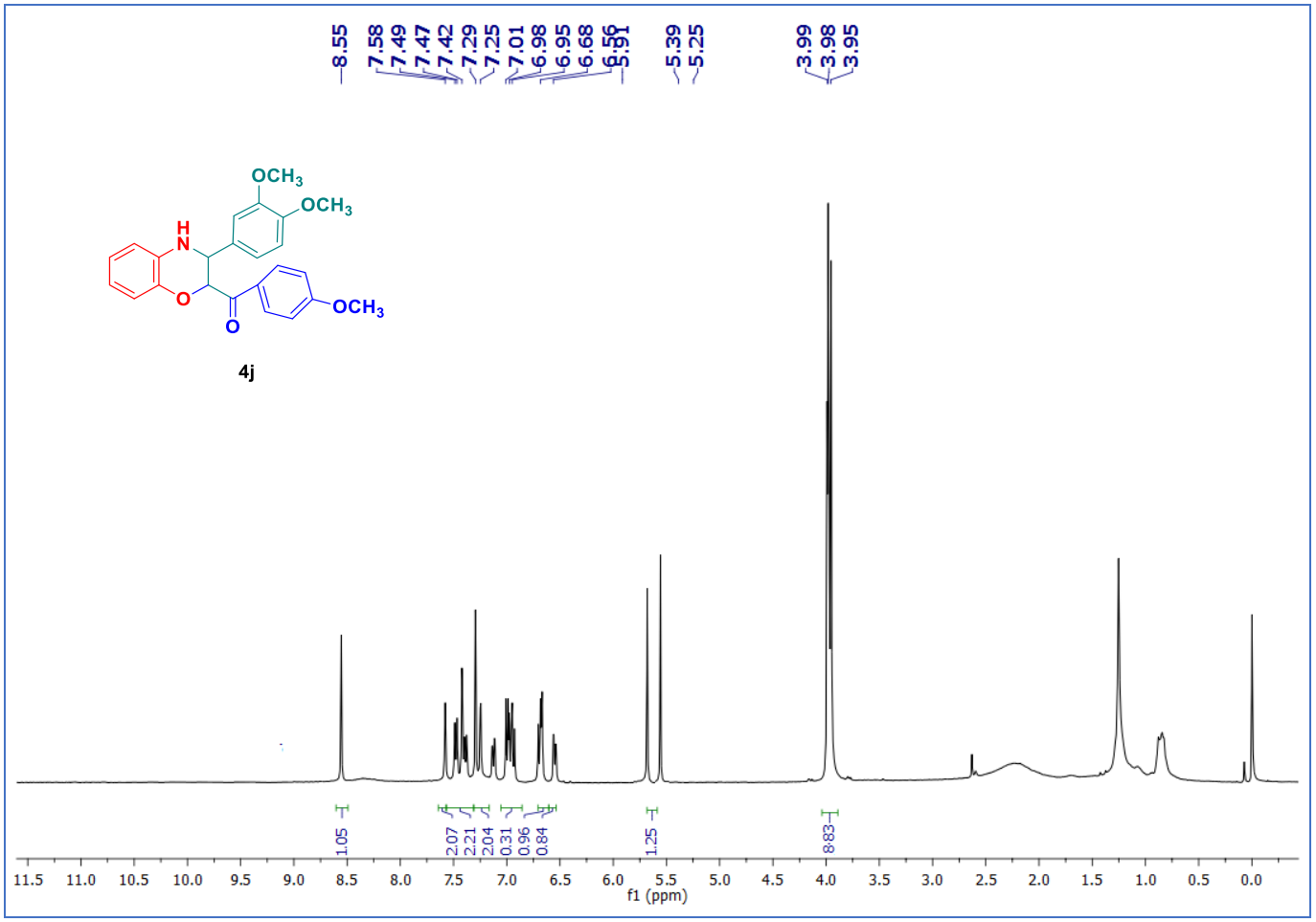

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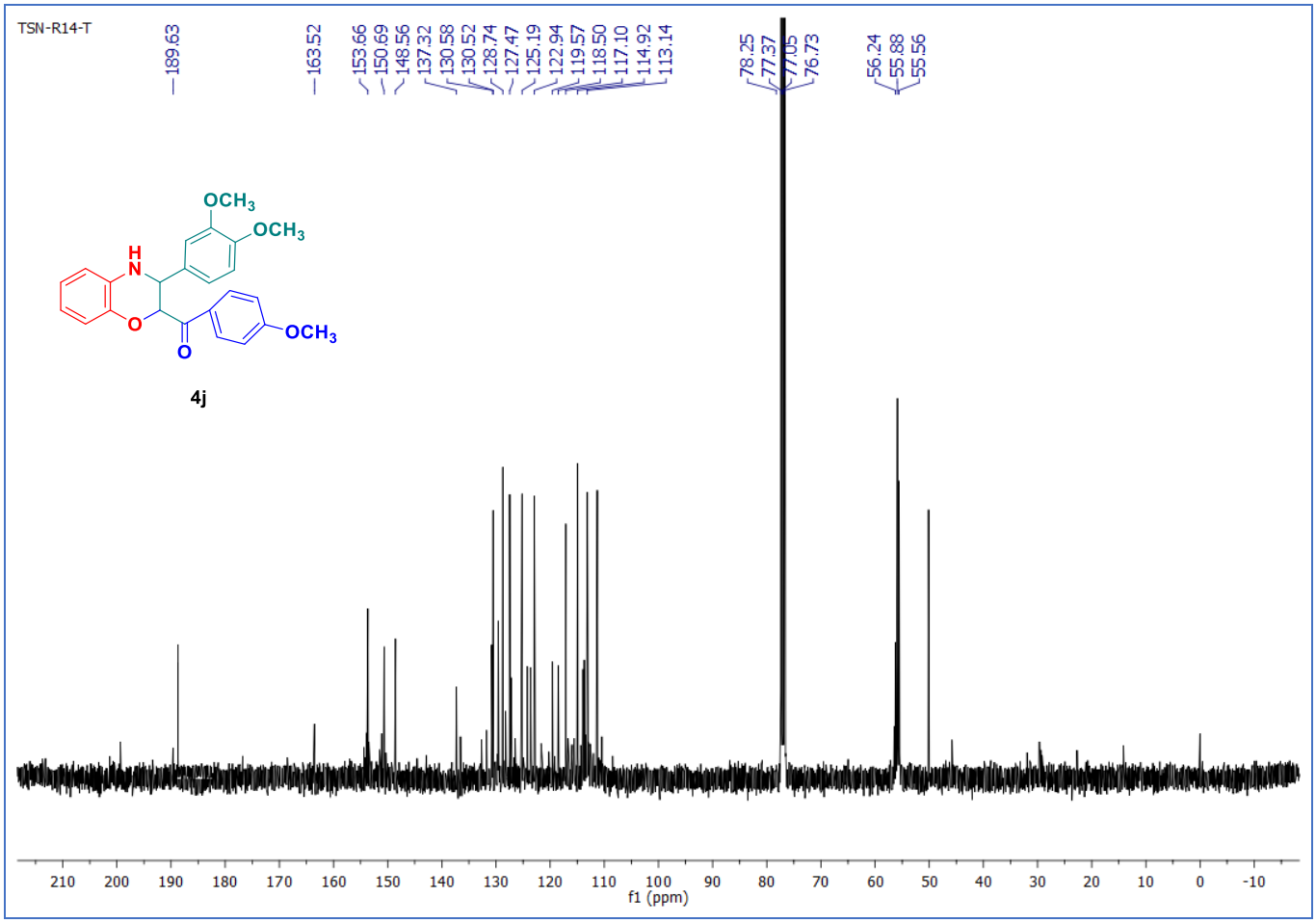

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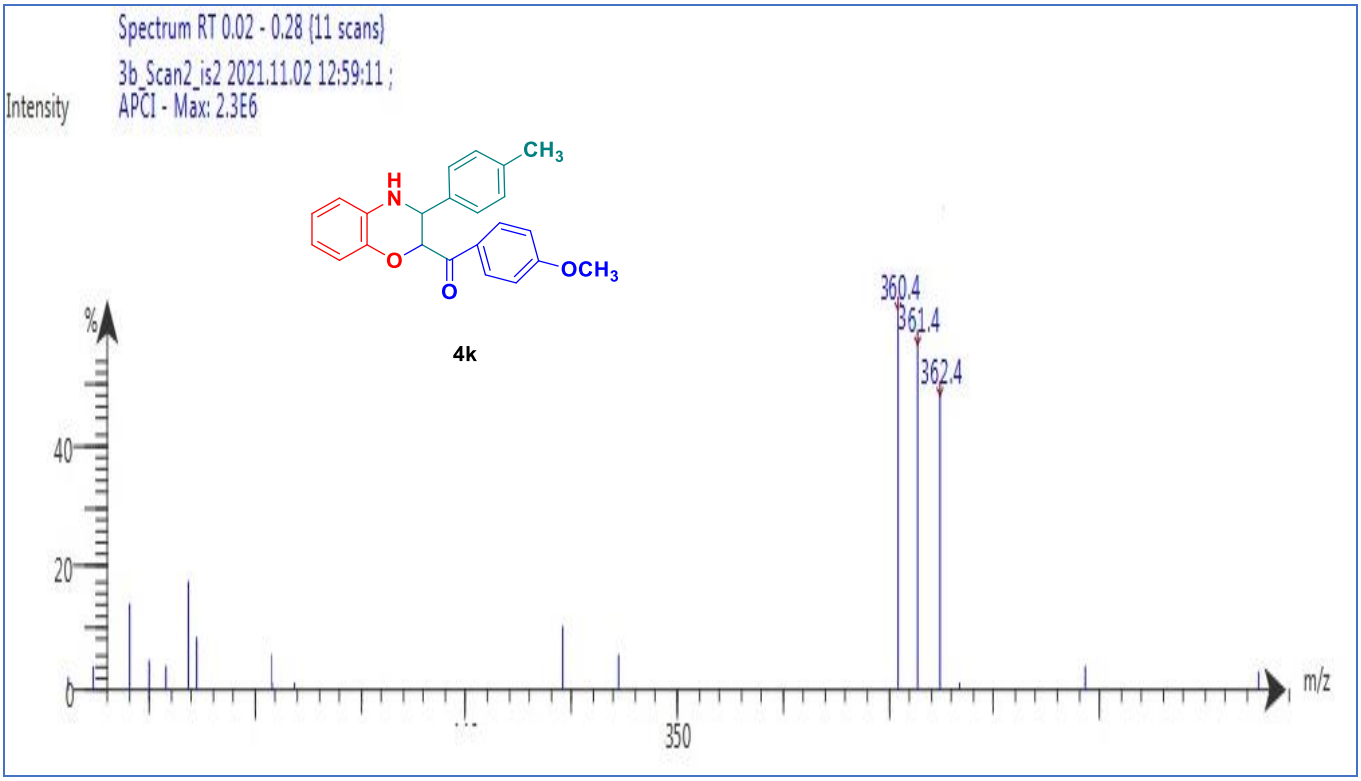
Mass Spectra of 4k



4k

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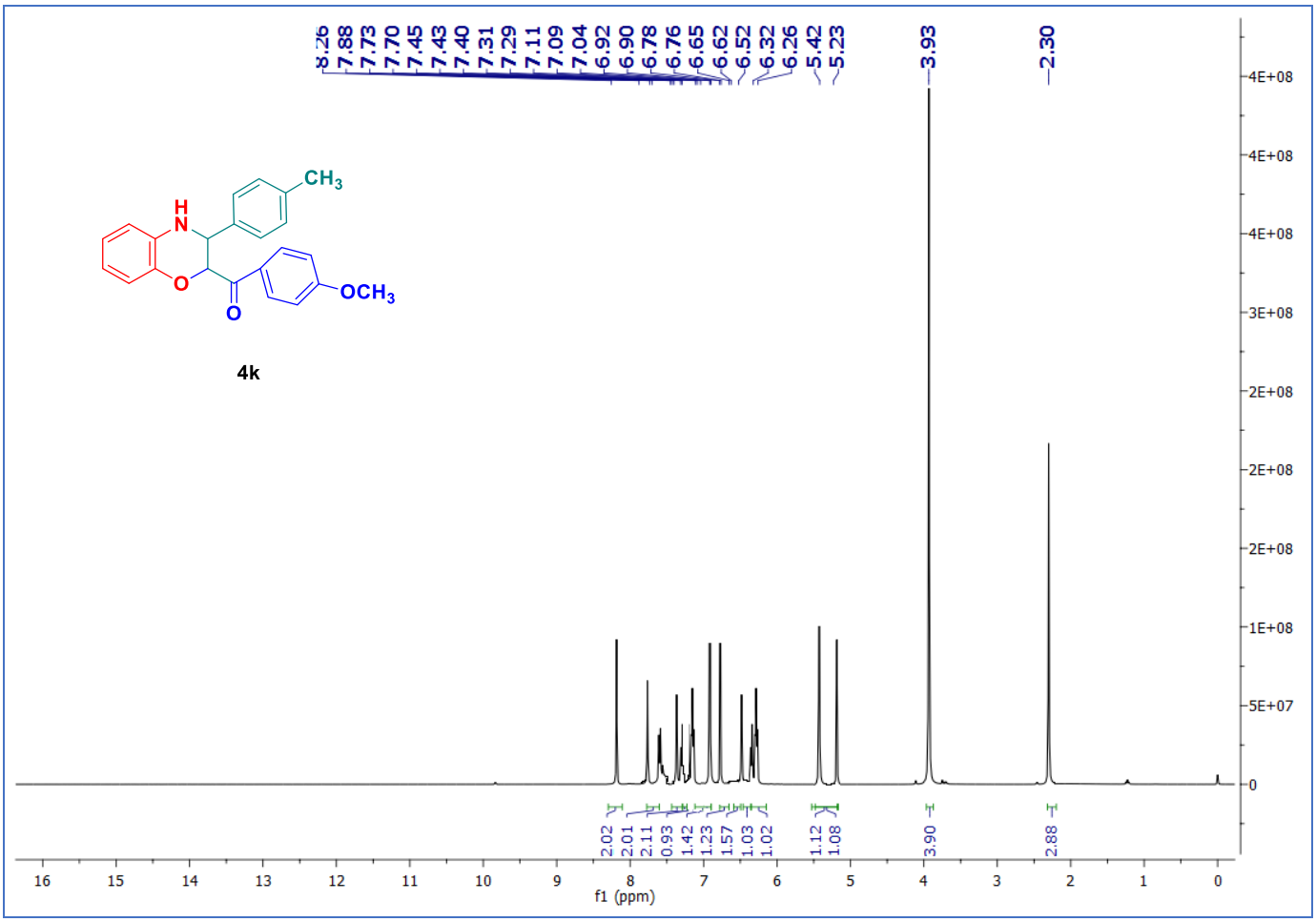

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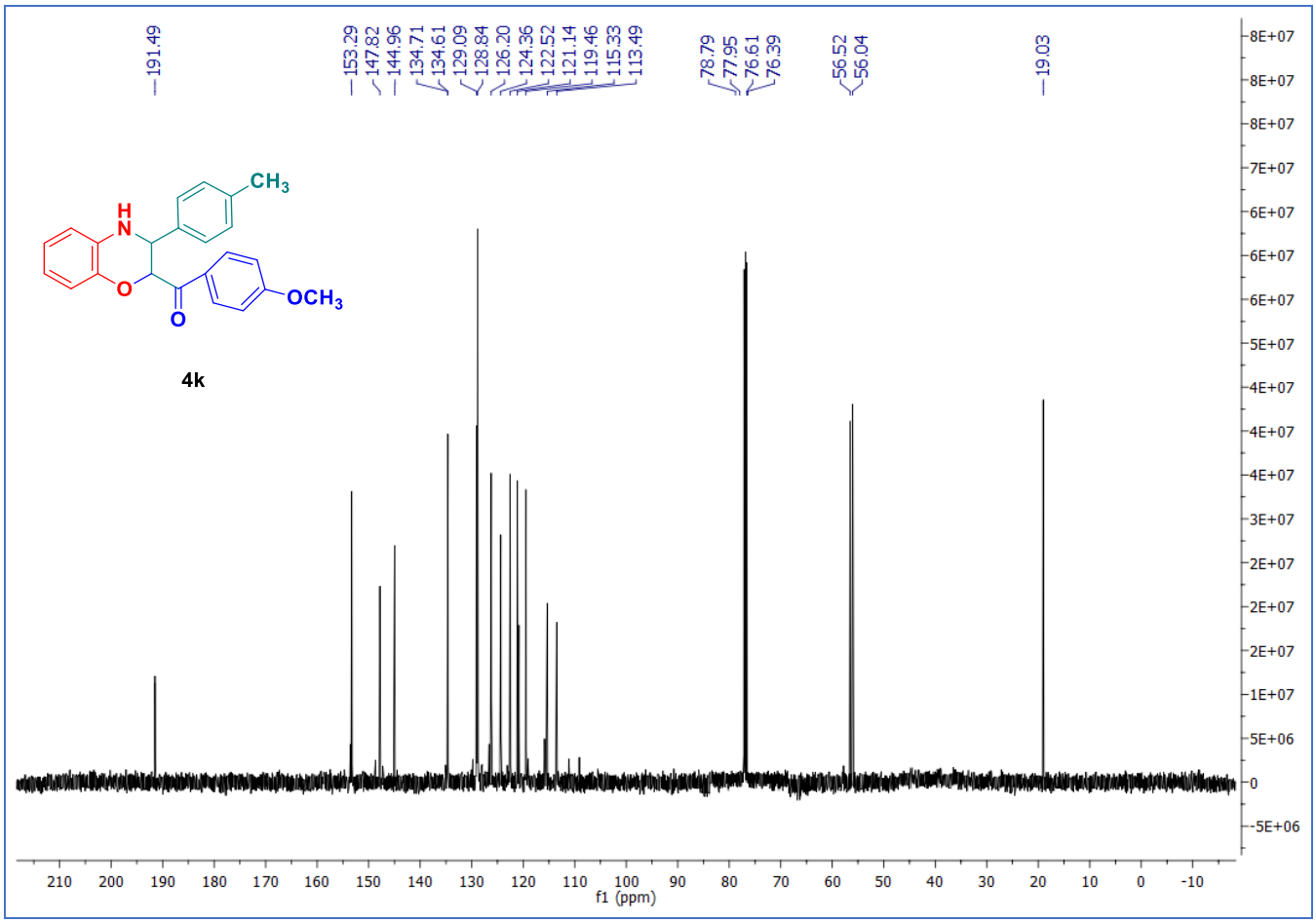

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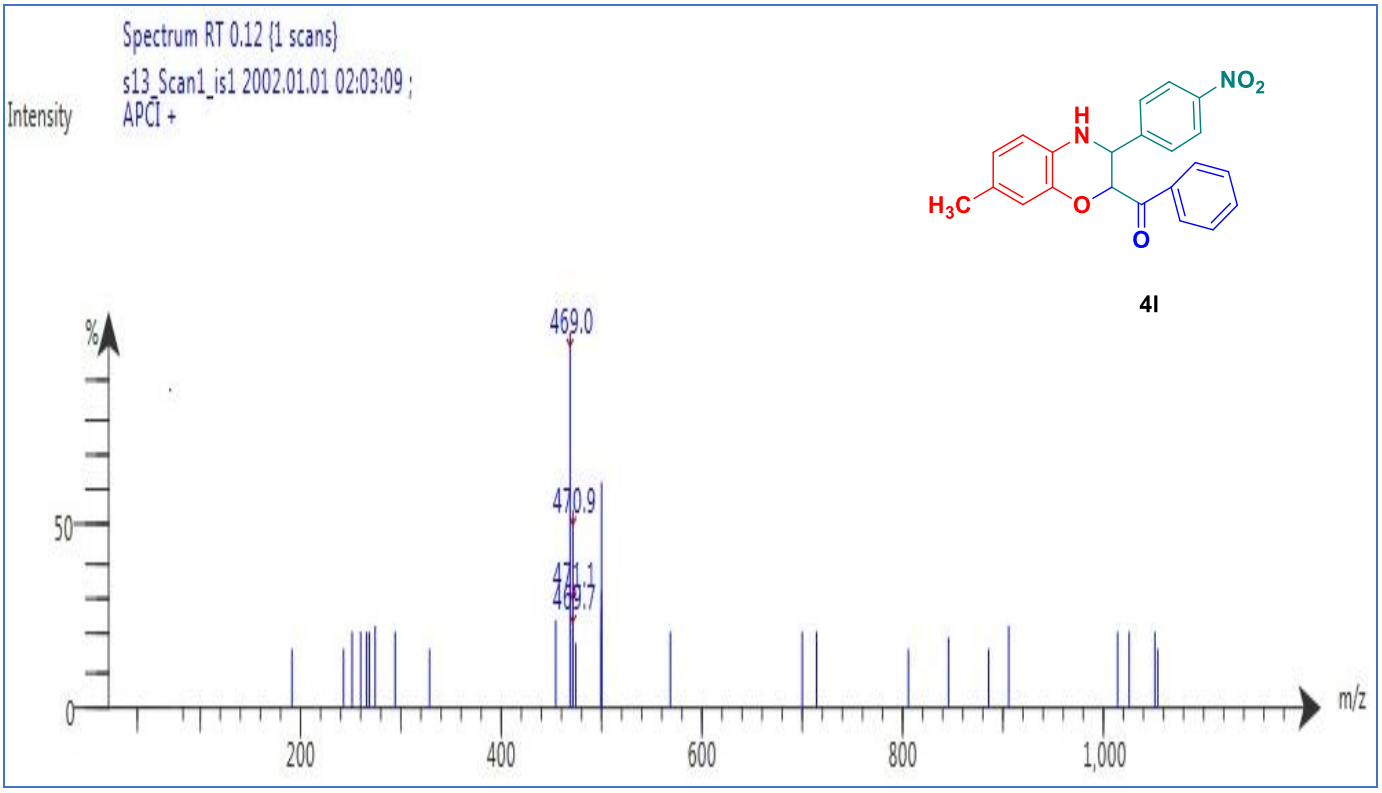

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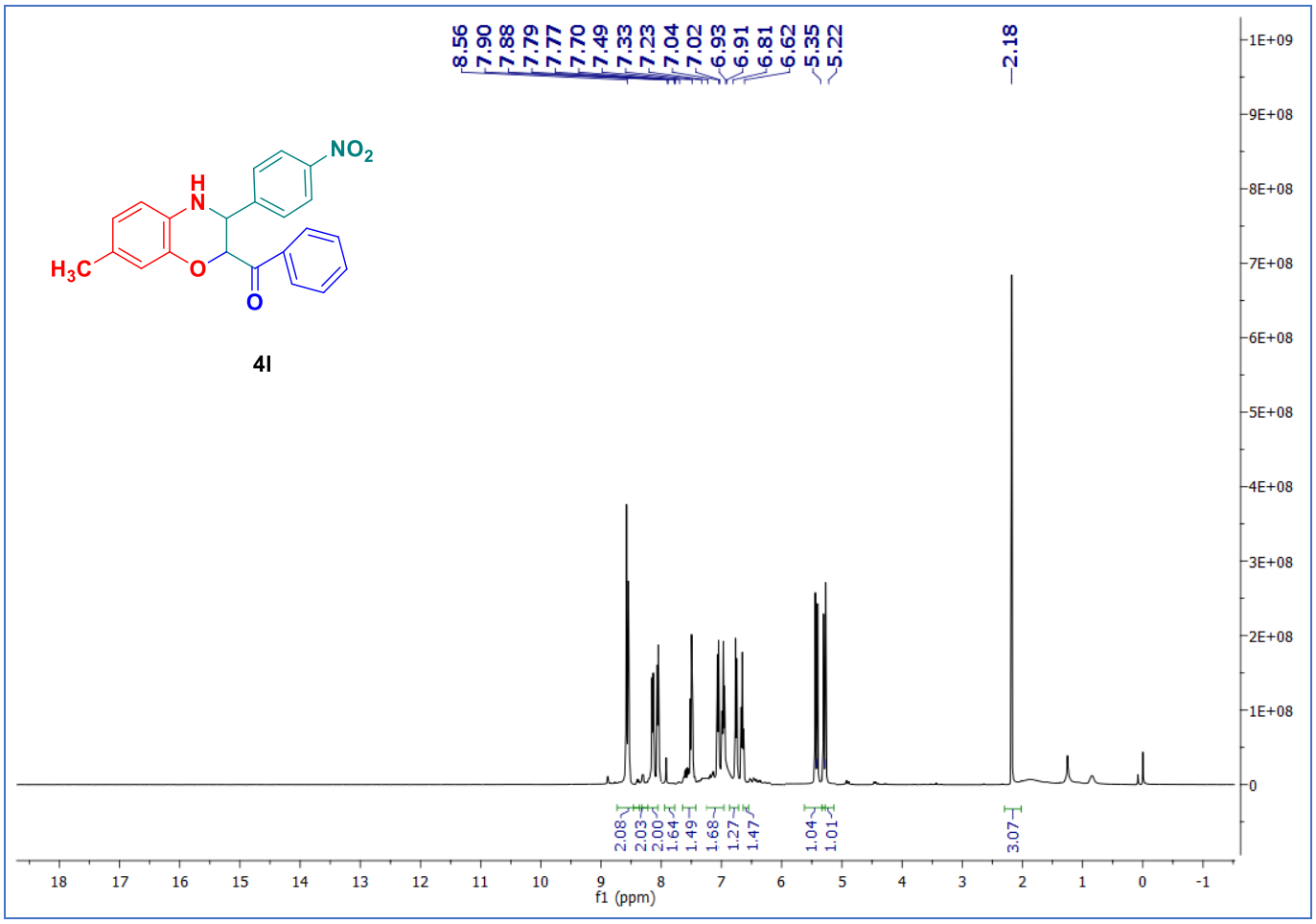

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