

Design and Synthesis of Novel Uracil-Linked Schiff Bases as Dual HDAC II/Topo I Inhibitors with Apoptotic Potentials

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Ahmed A. Al-Karmalawy; E-mail: akarmalawy@acu.edu.eg

Supplementary Files

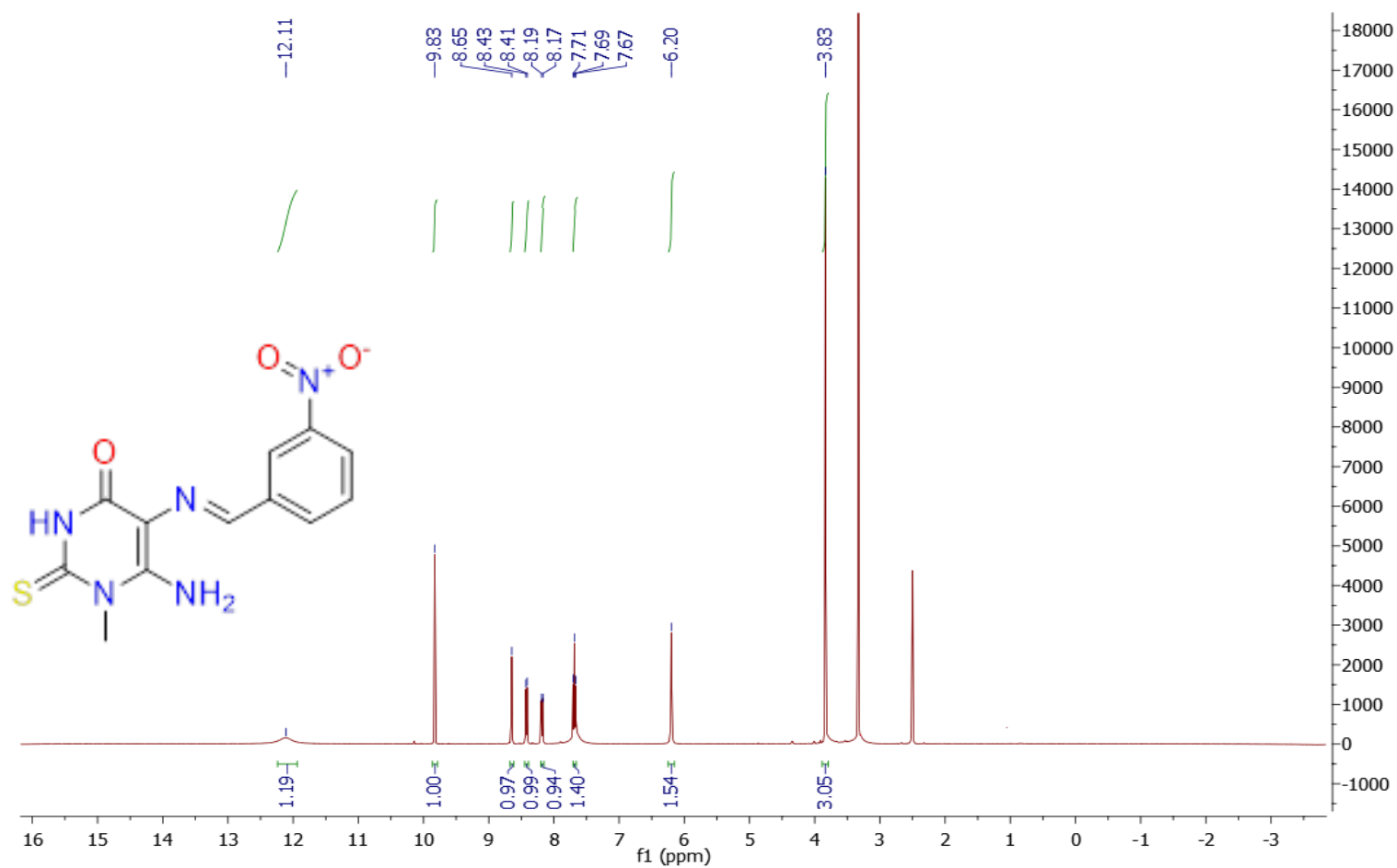
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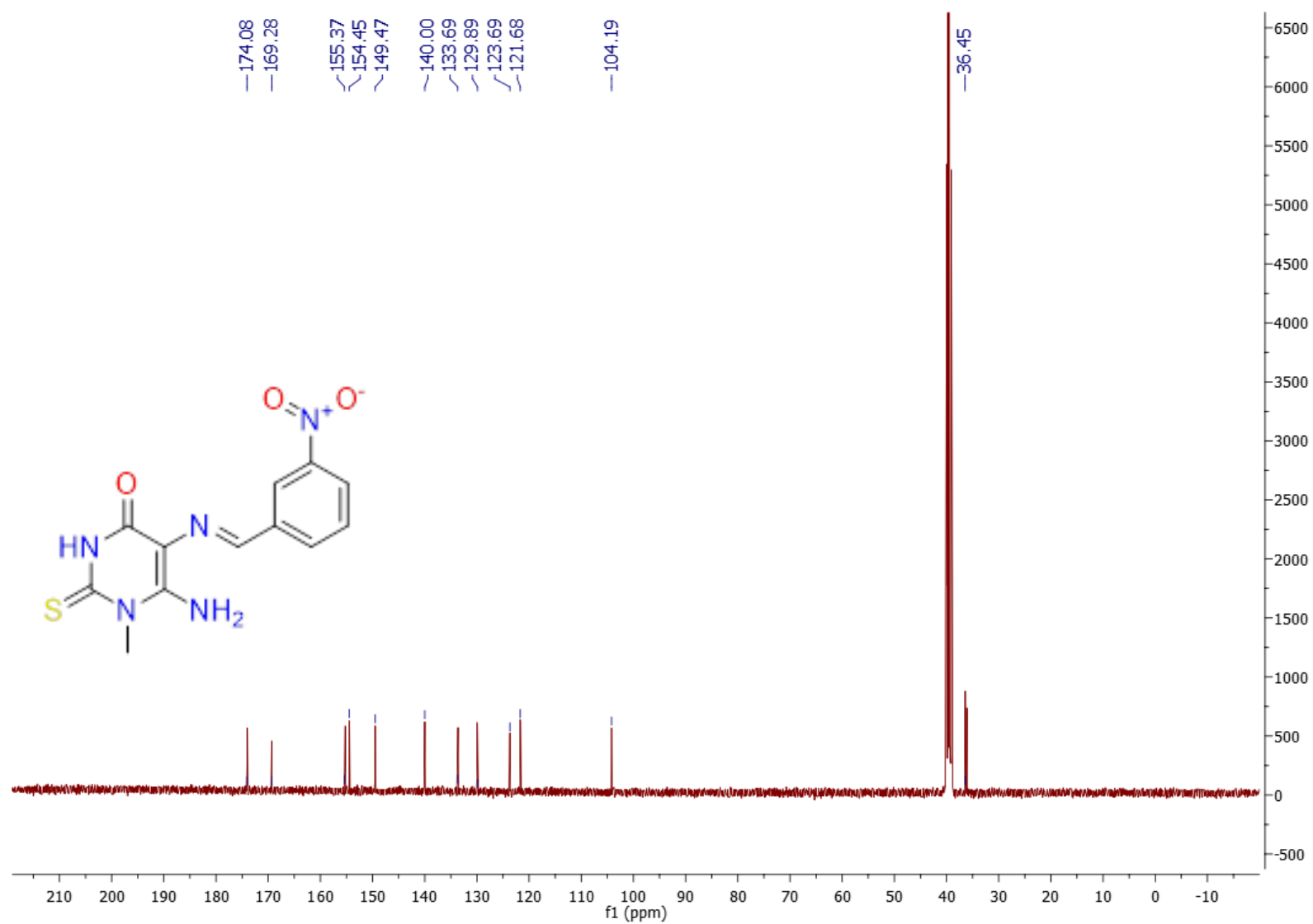
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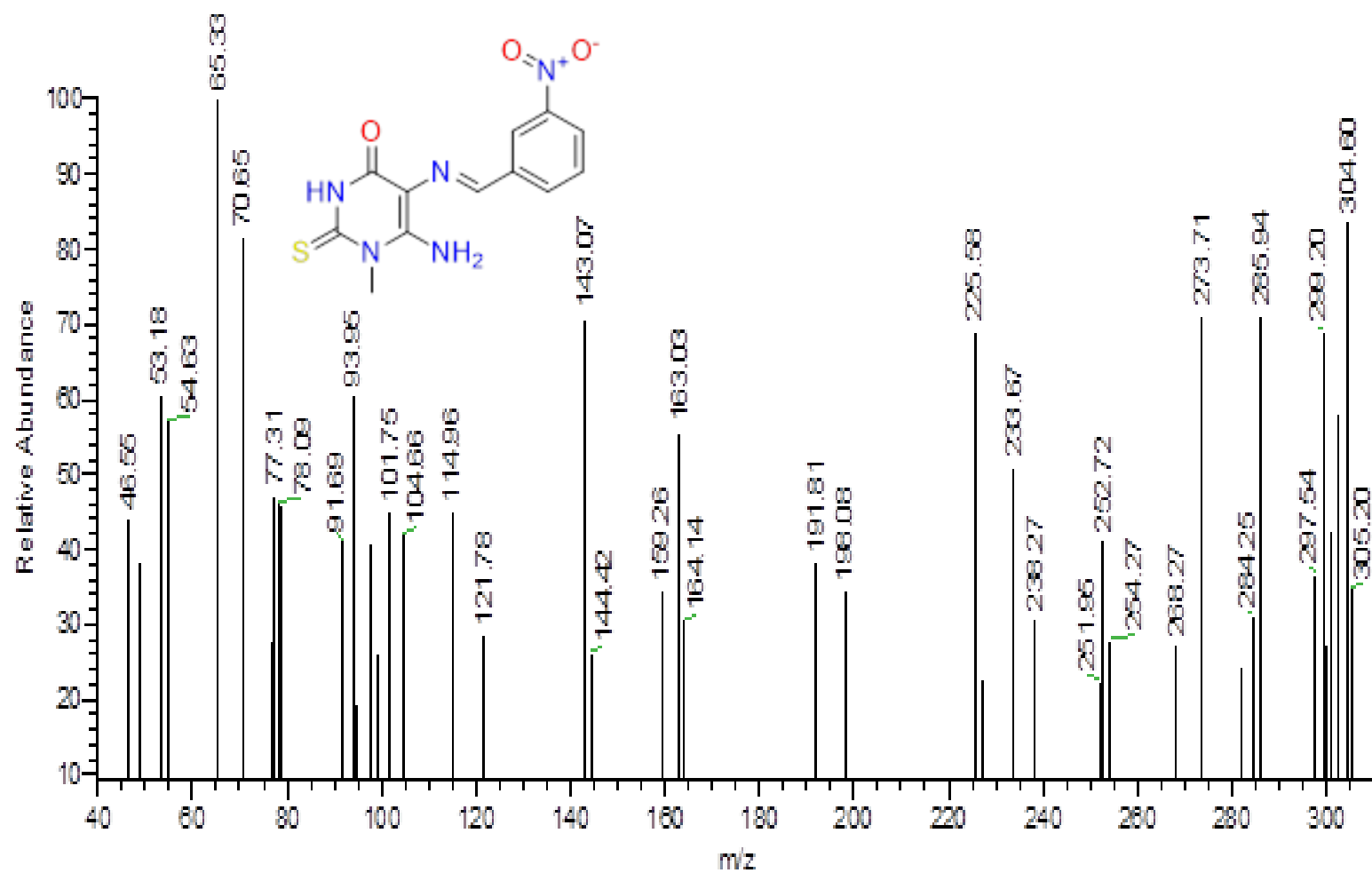
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1 Spectral Data

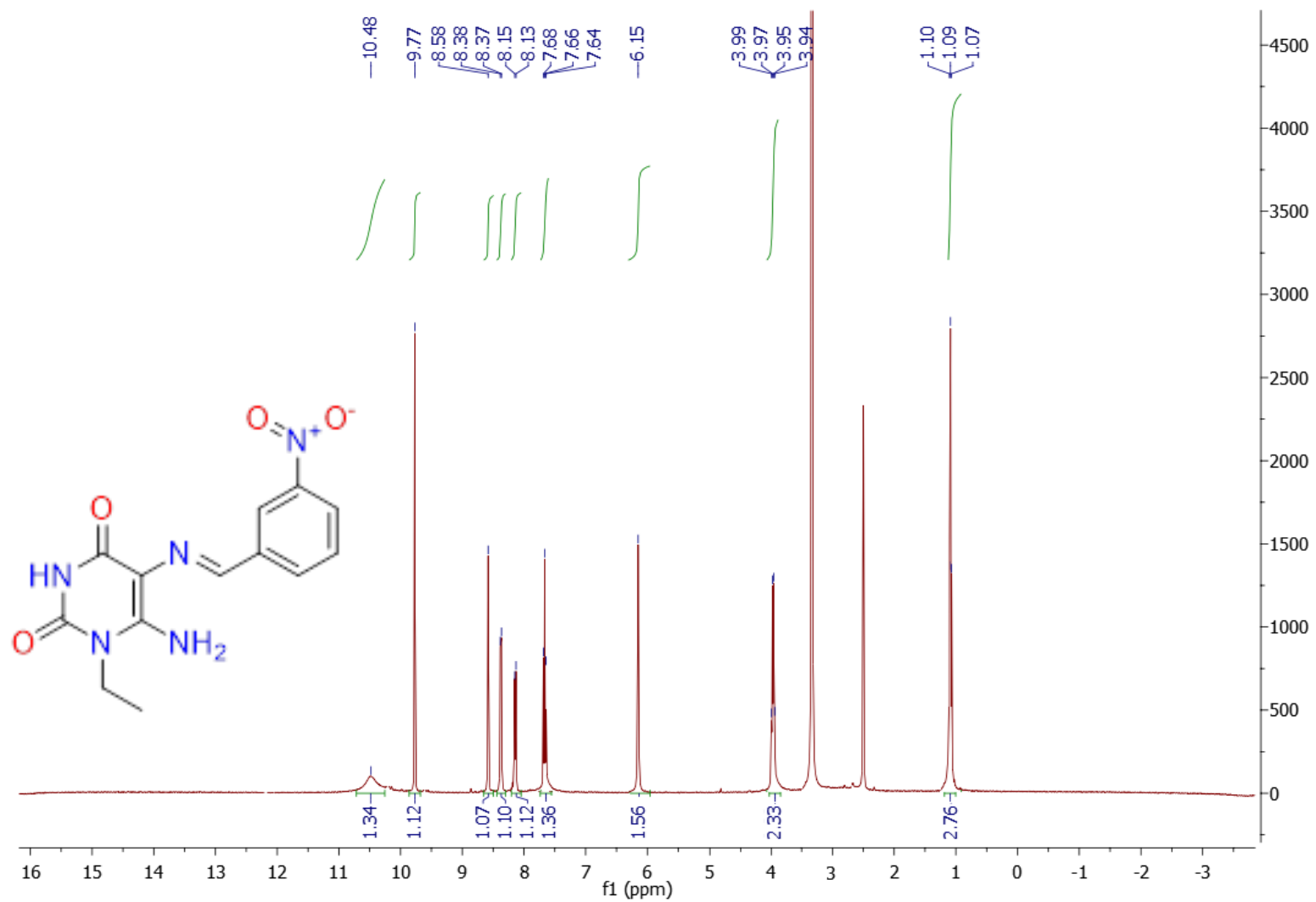
1.1 ^1H NMR, ^{13}C NMR and Mass spectra of 19

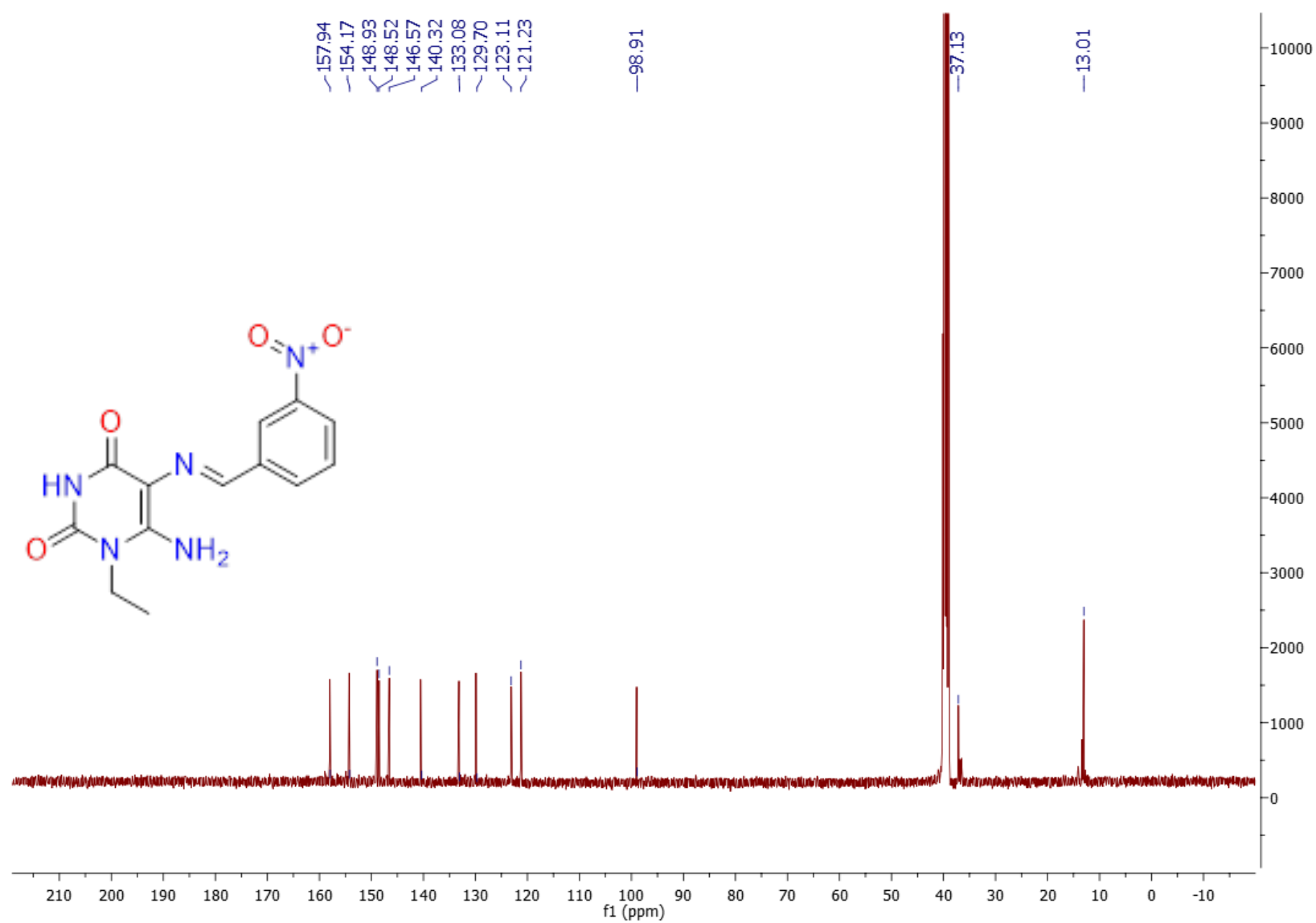


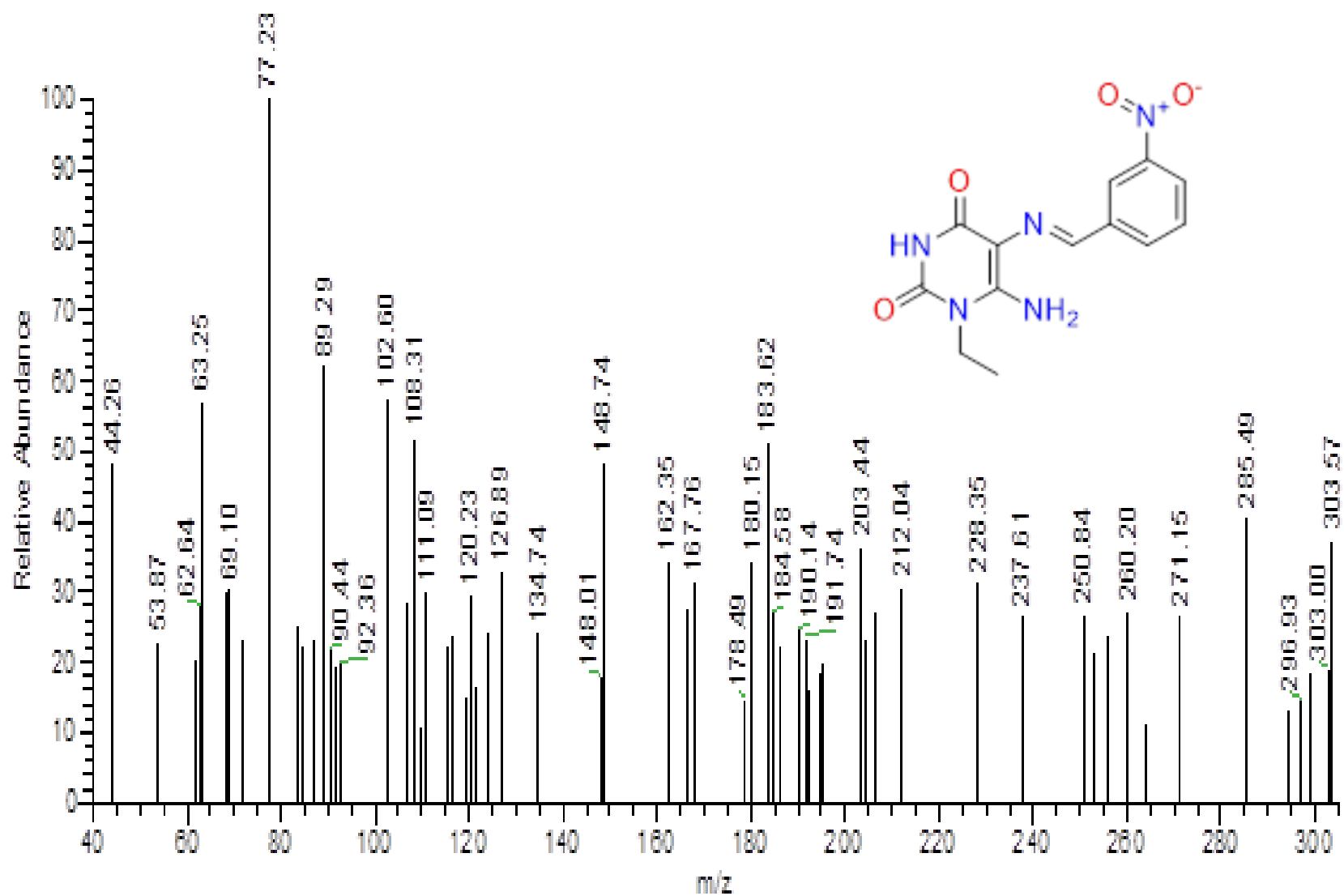




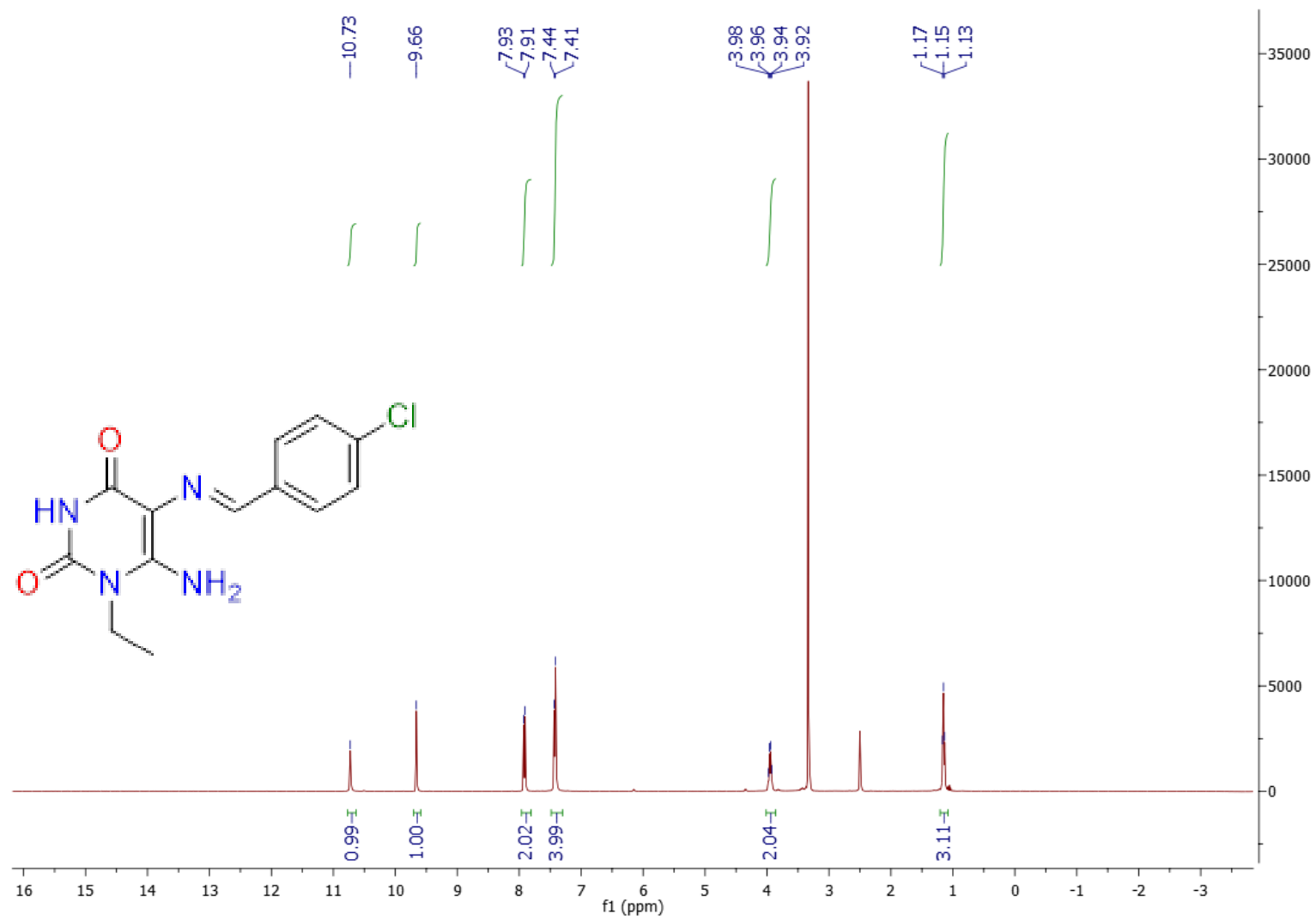
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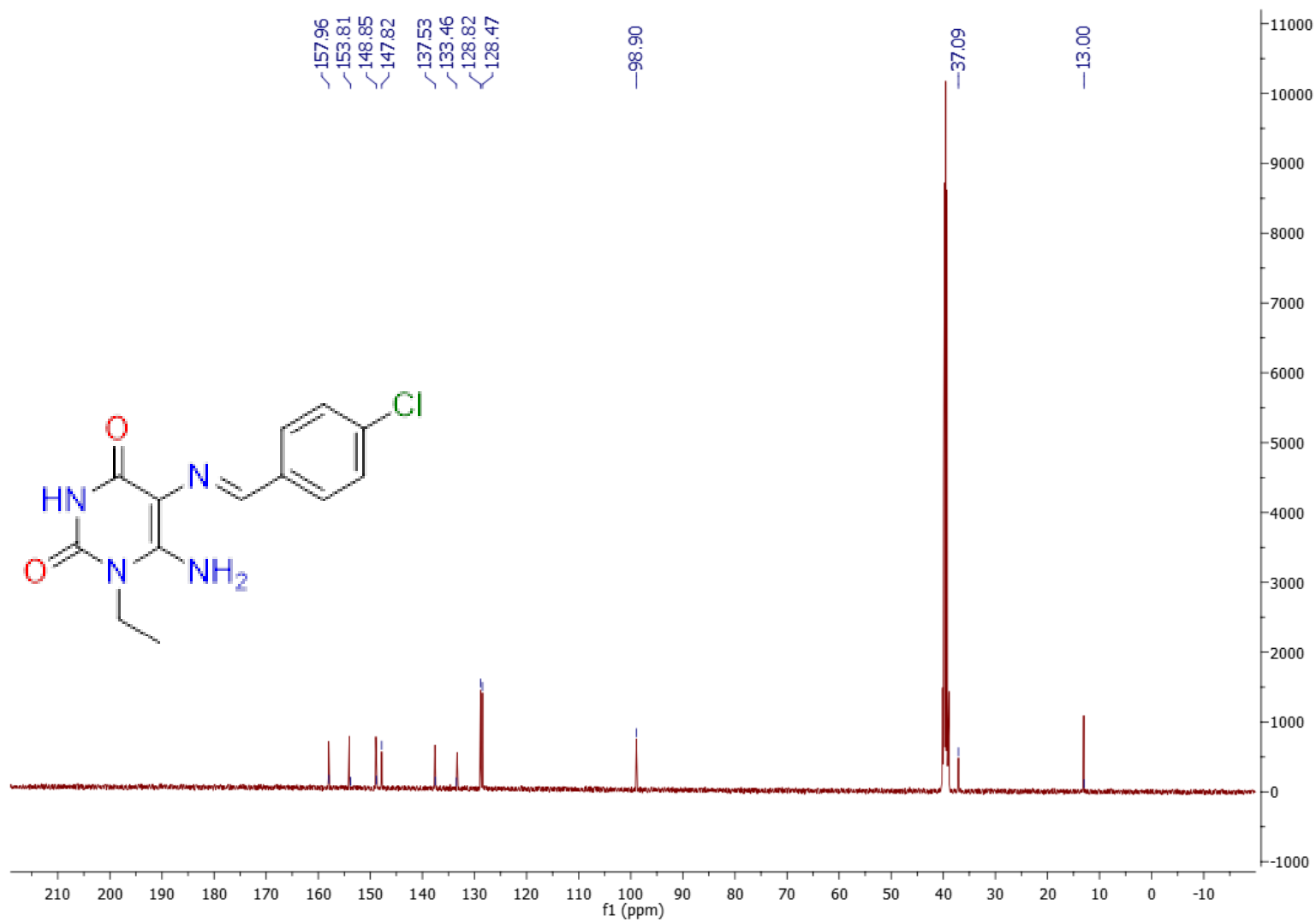


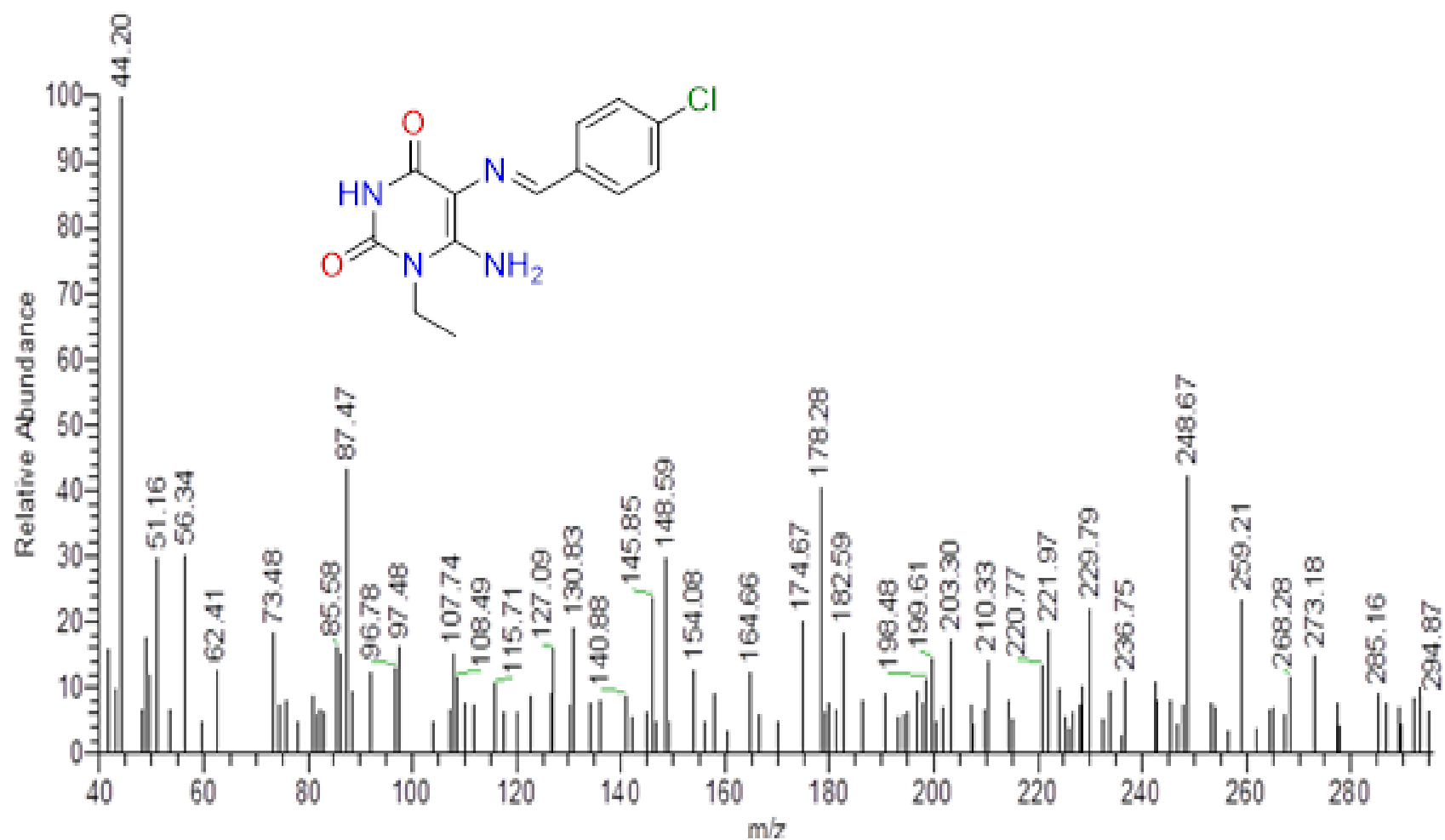




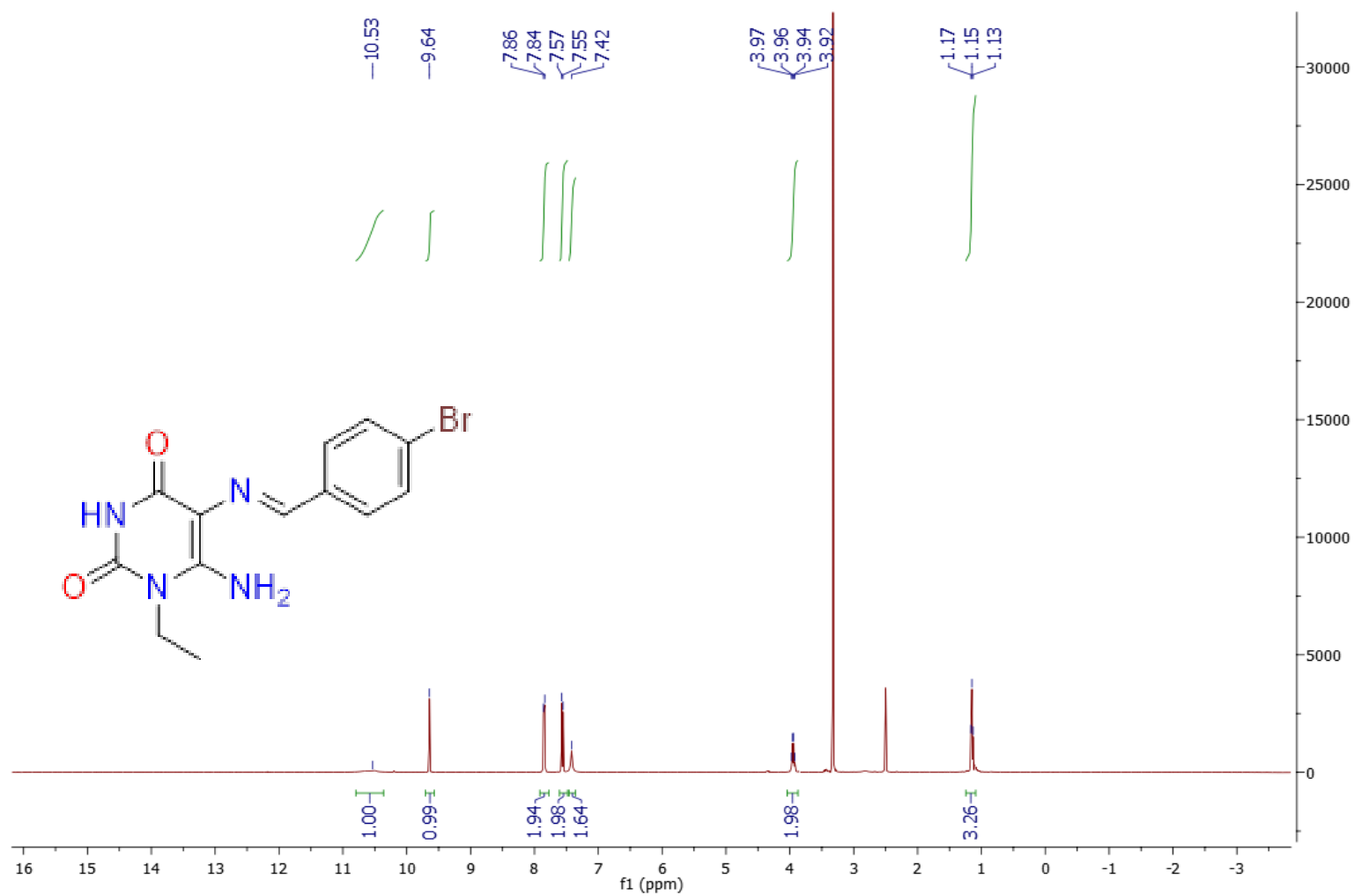
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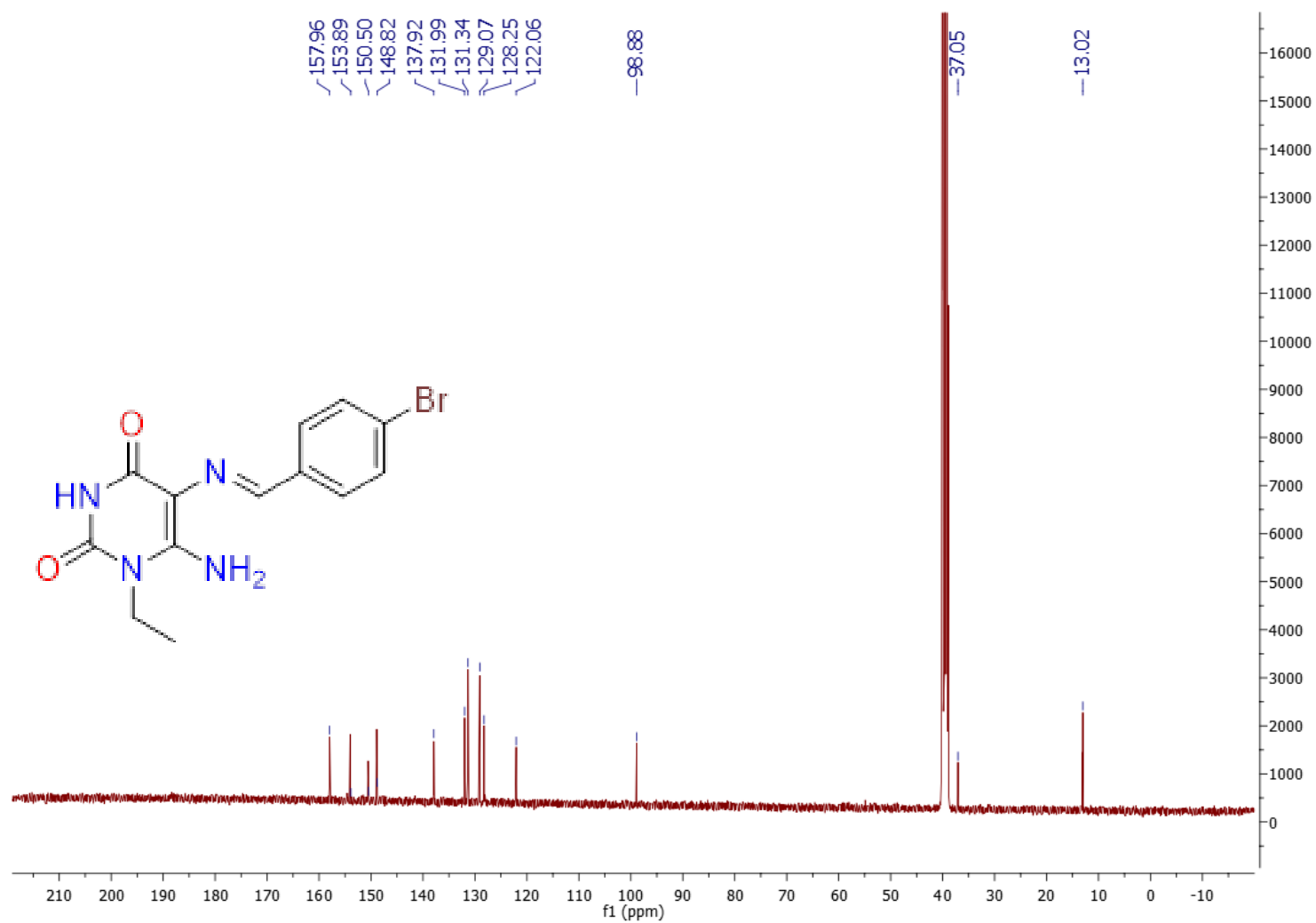


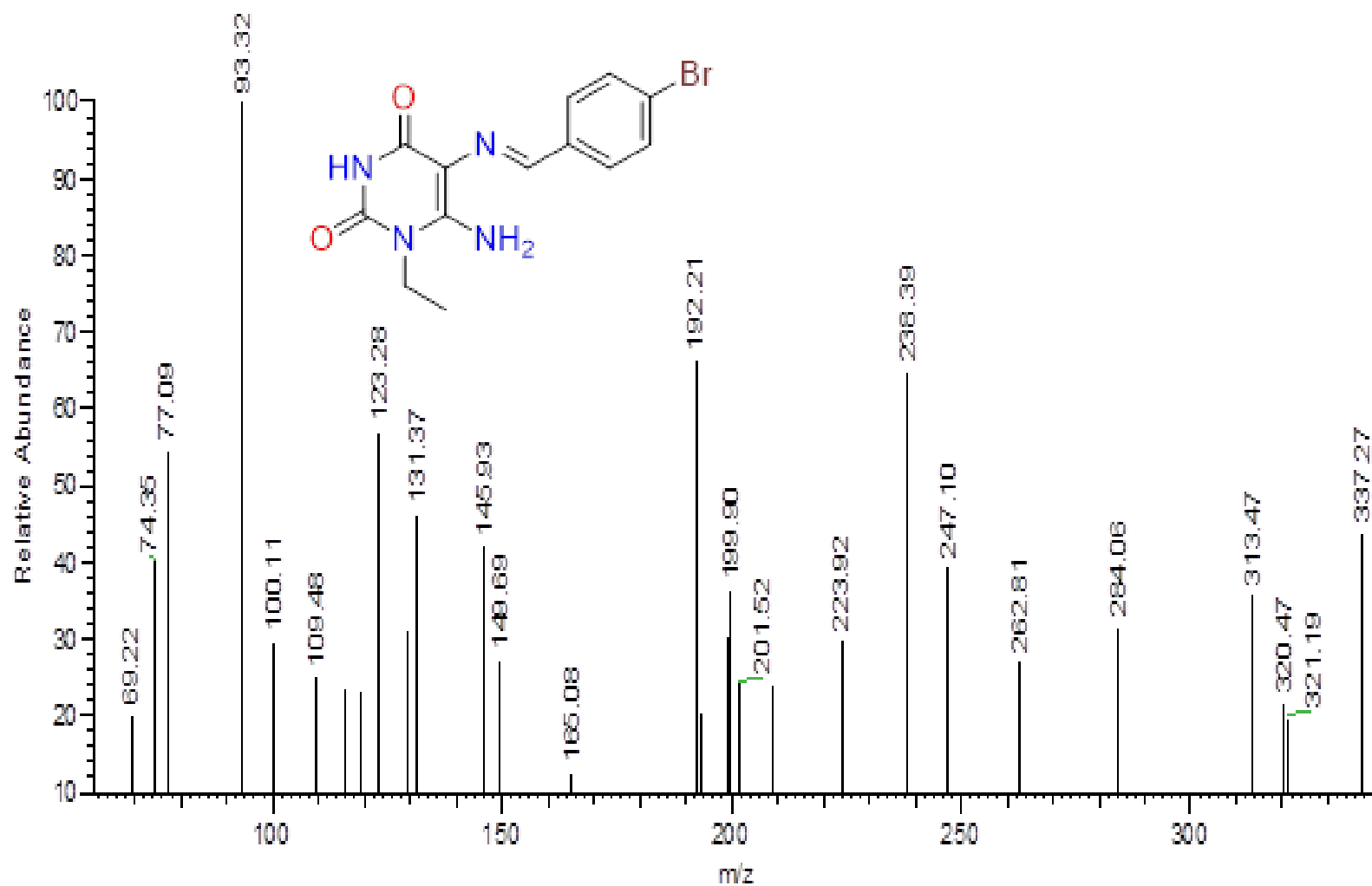




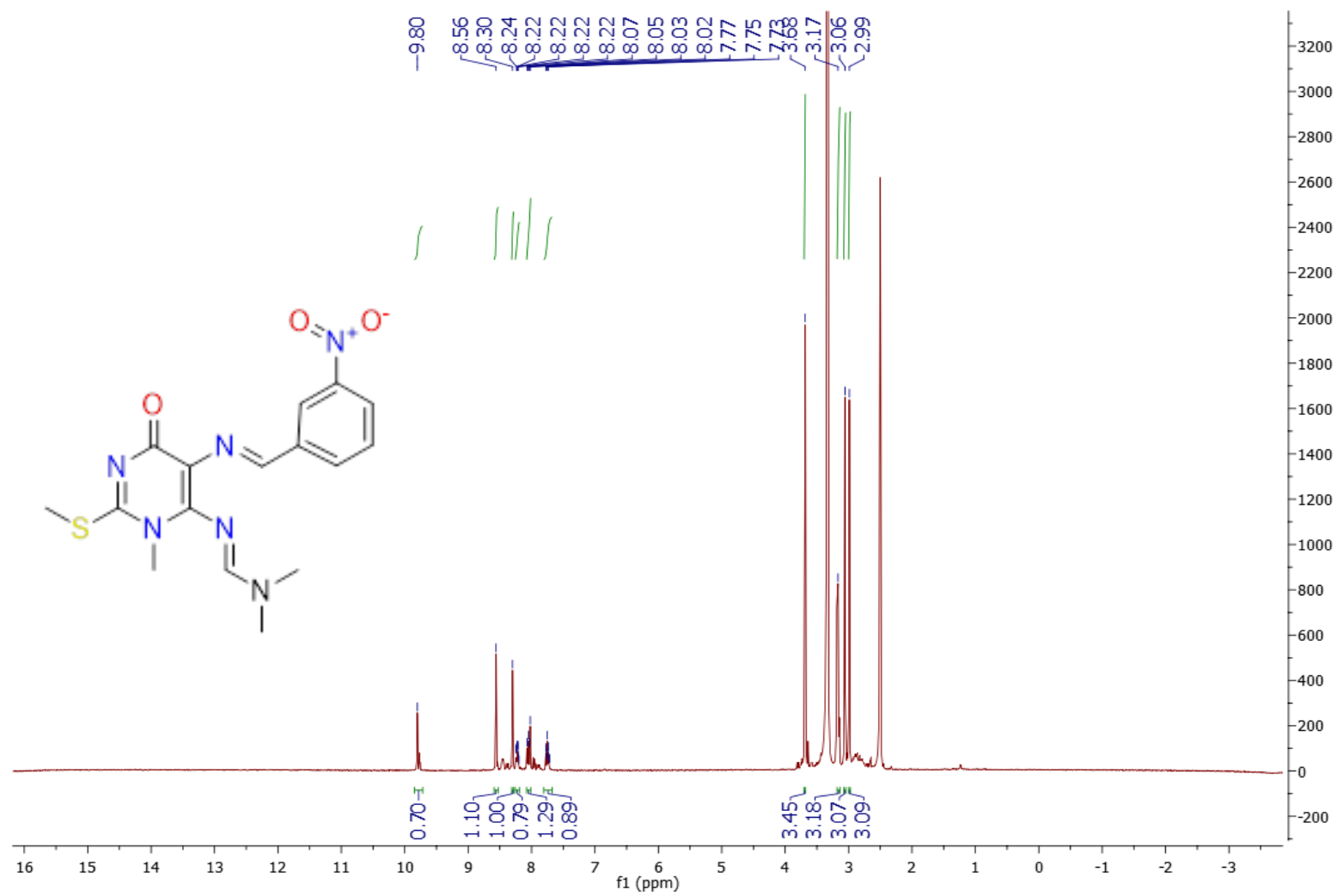
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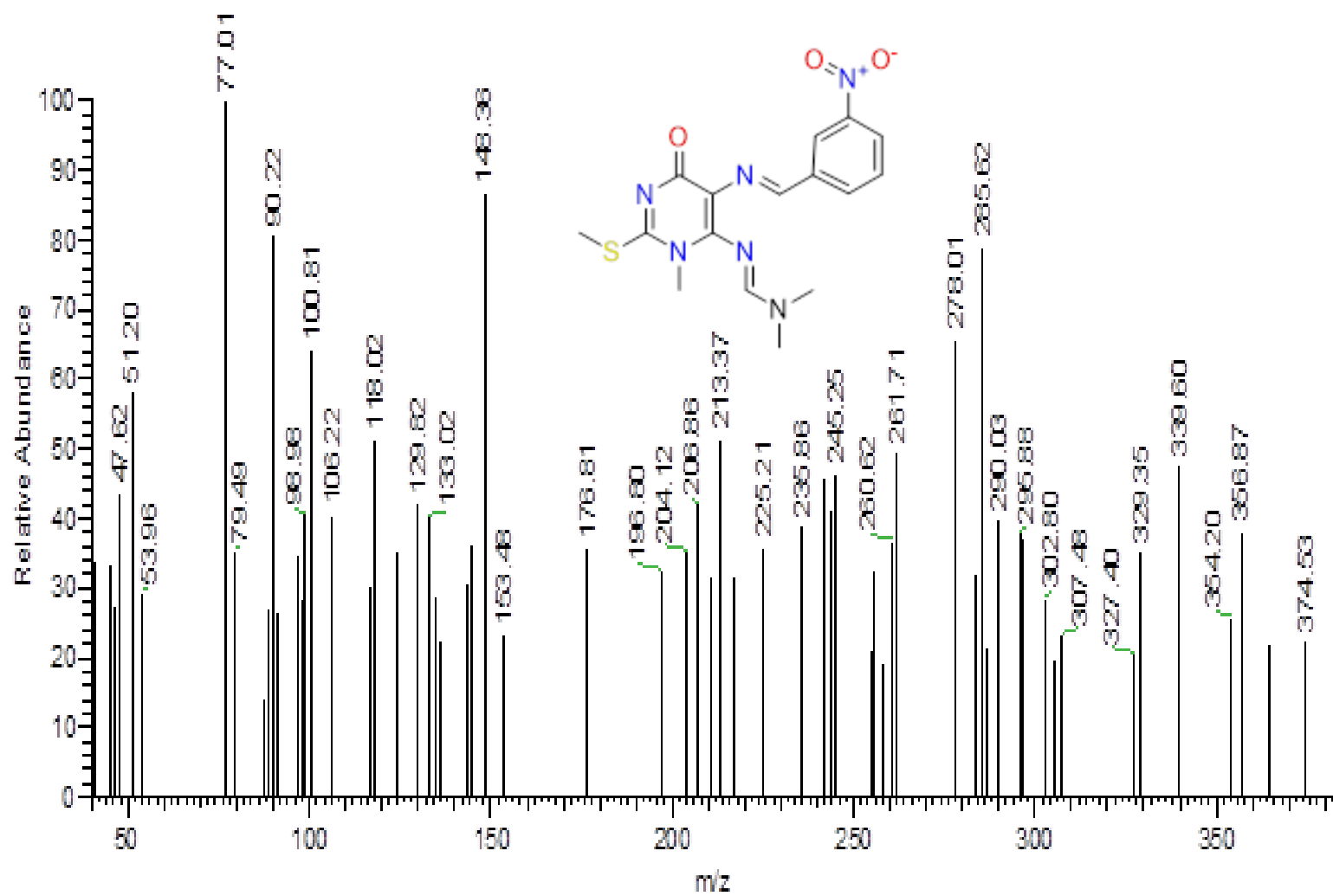




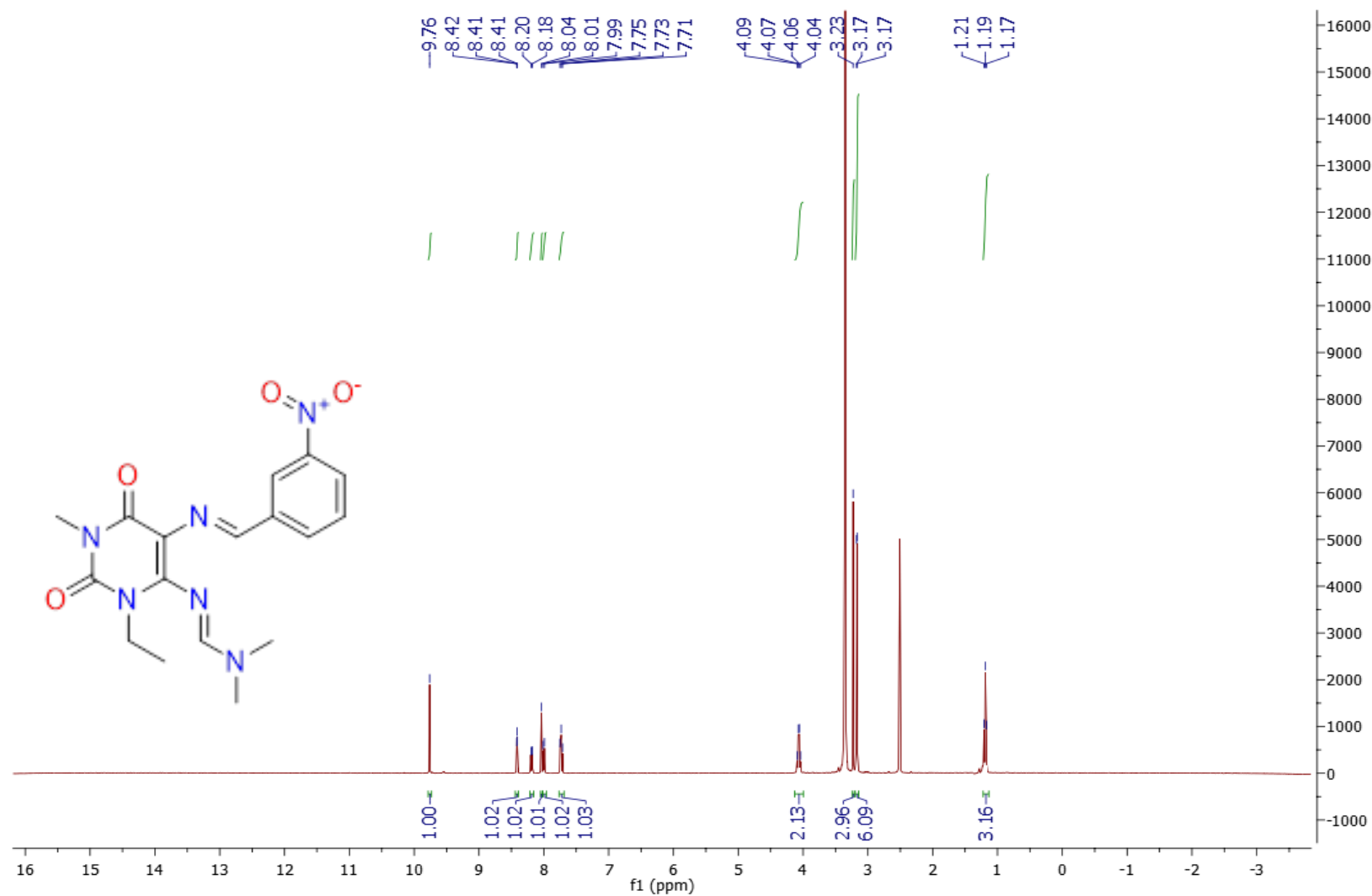


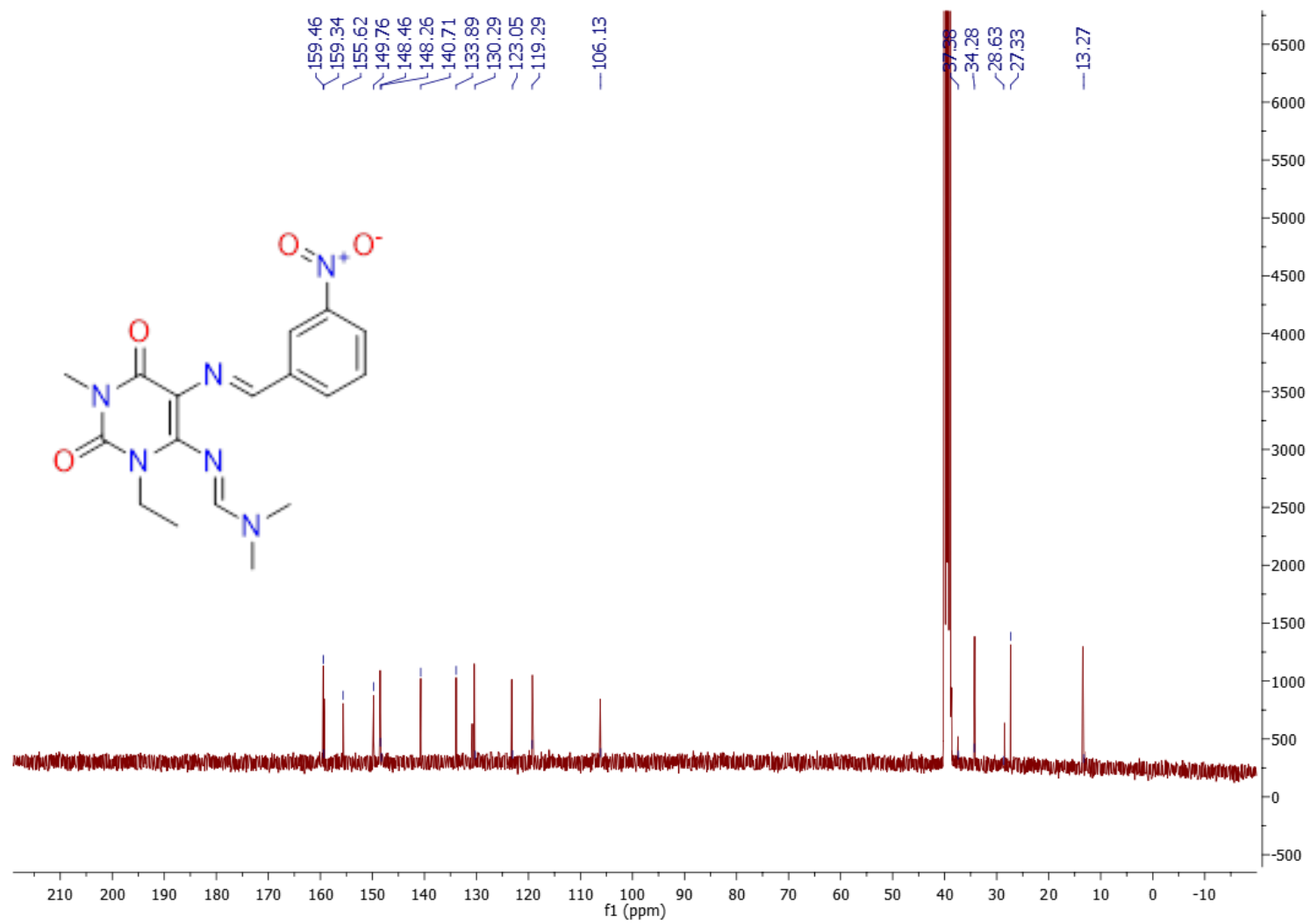
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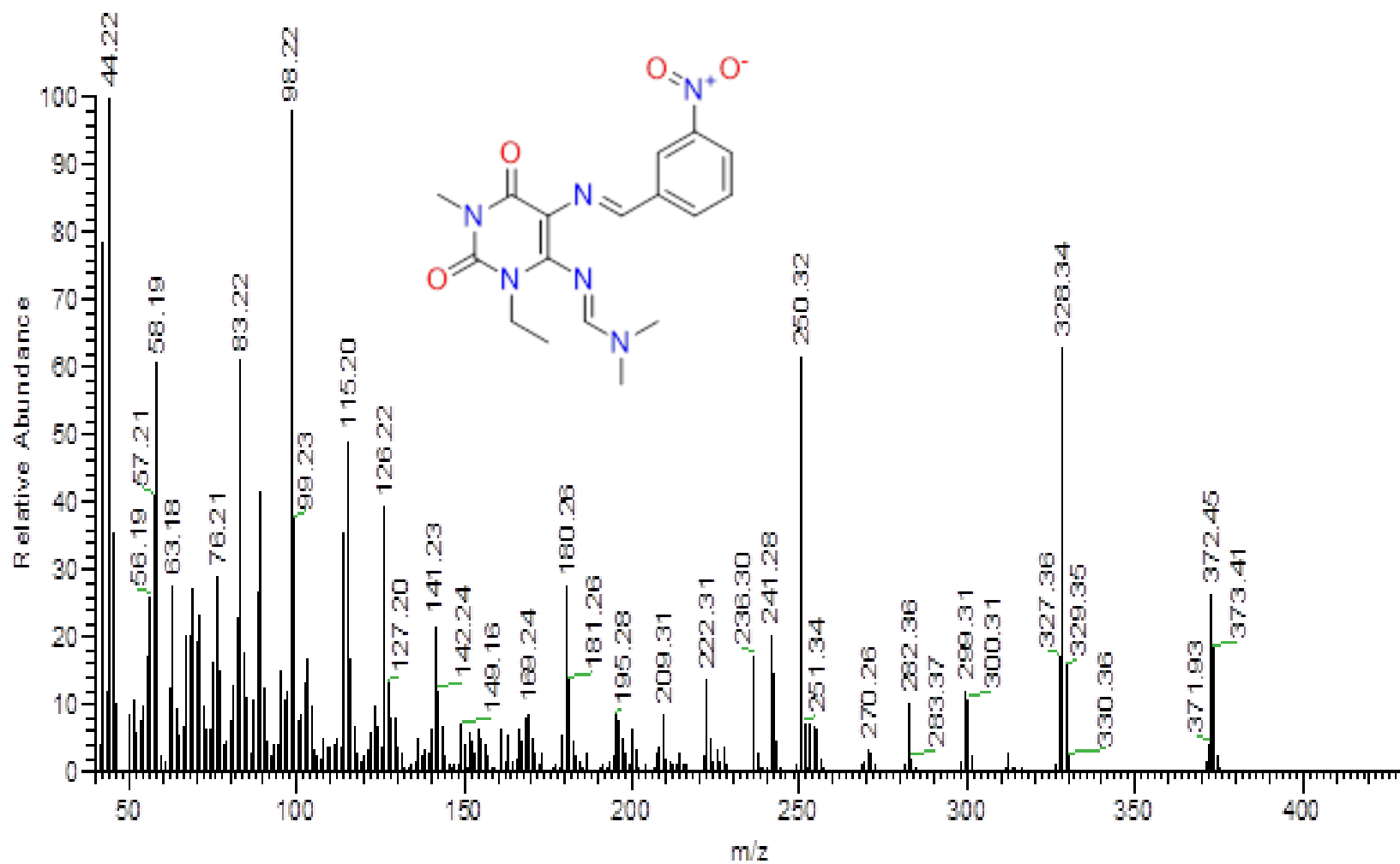




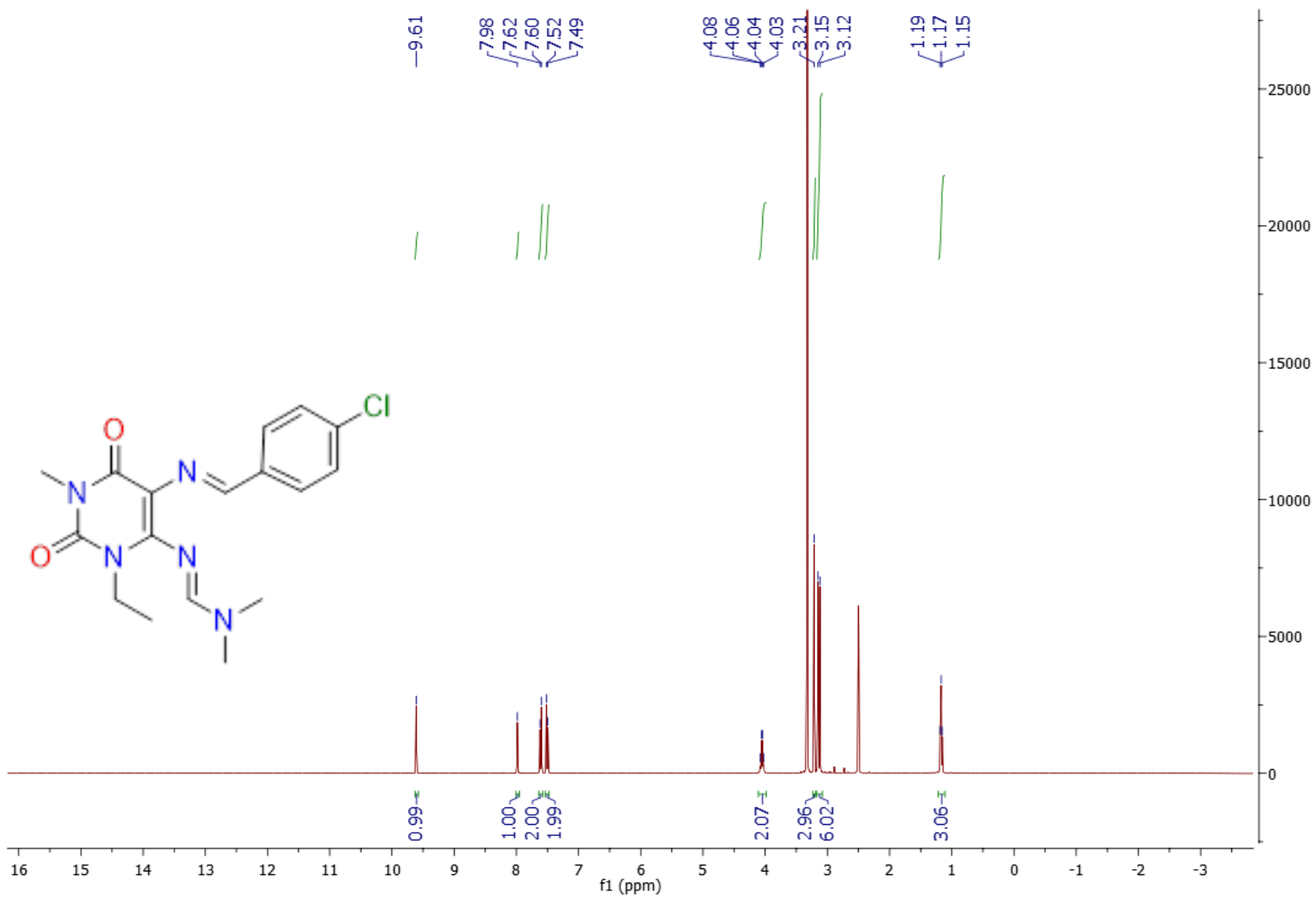
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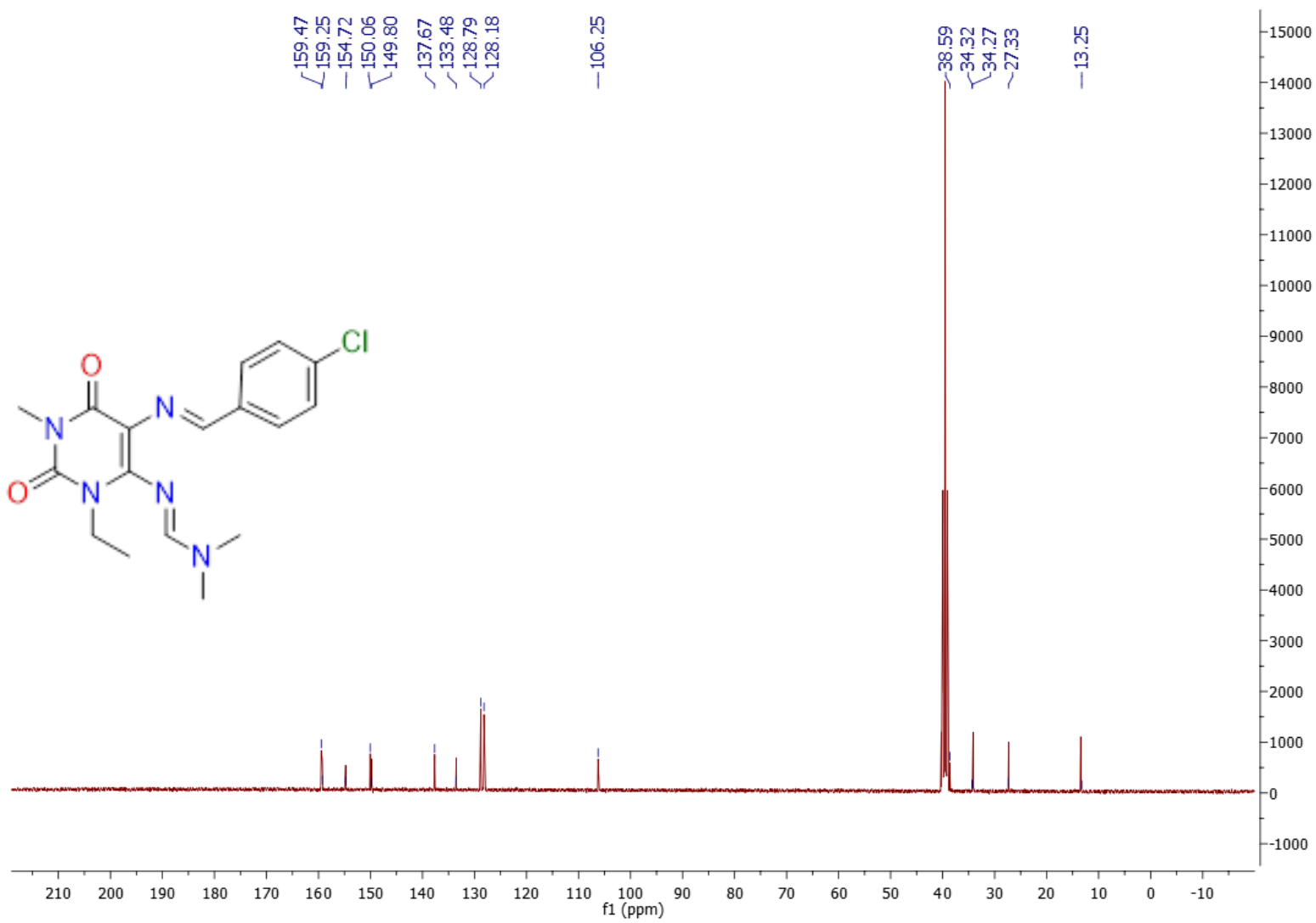


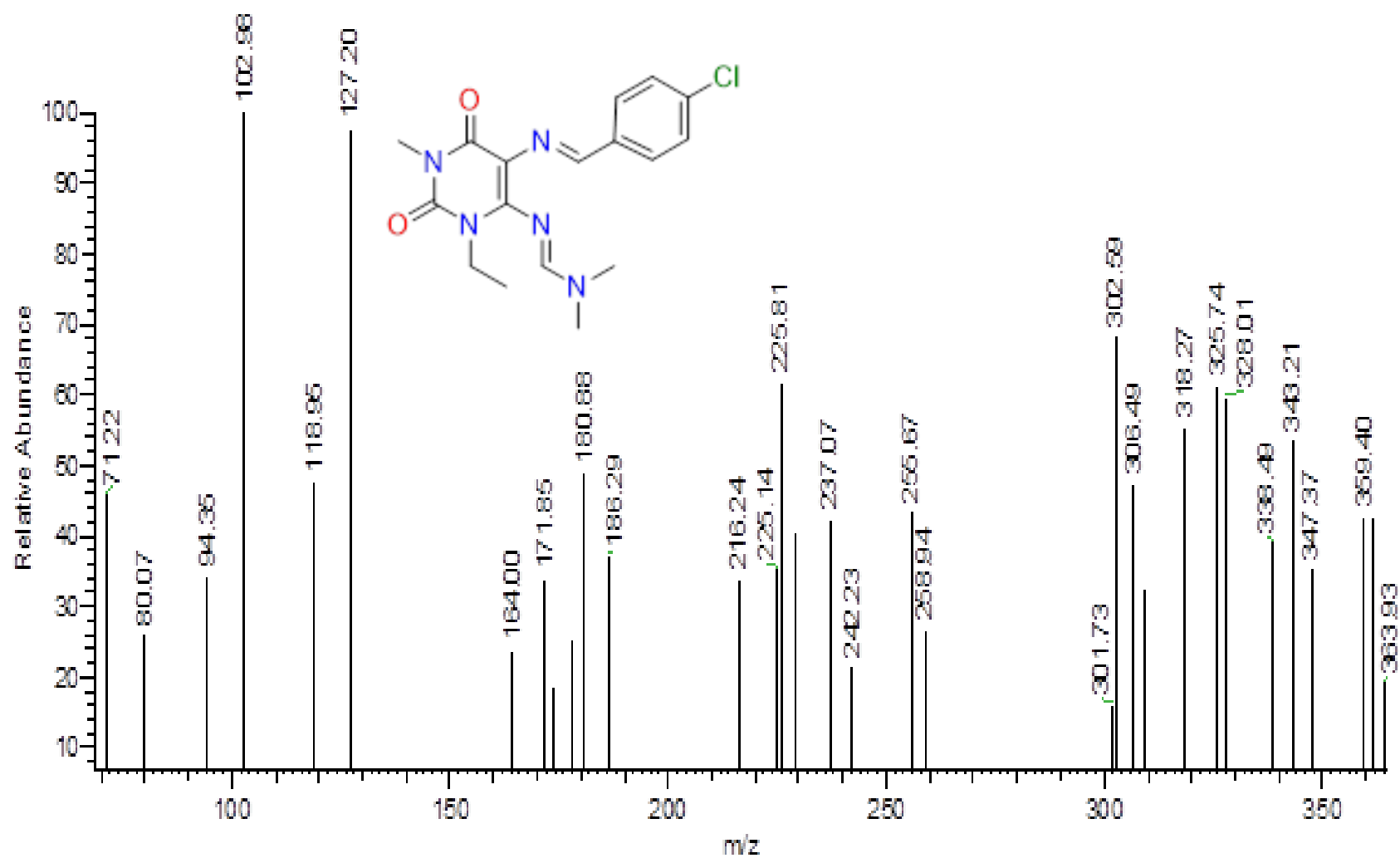




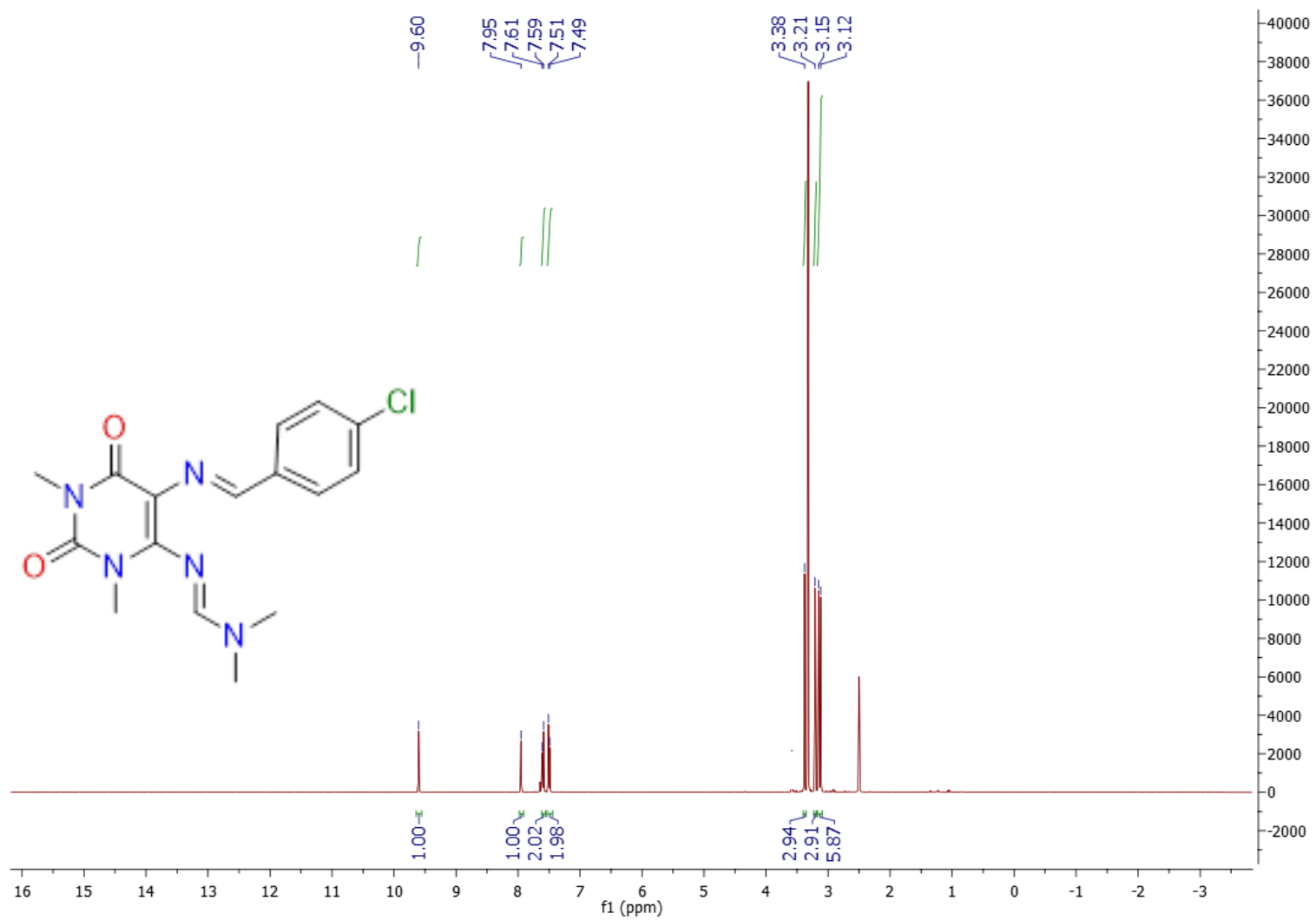
1.7 ^1H NMR, ^{13}C NMR and Mass spectra of 26

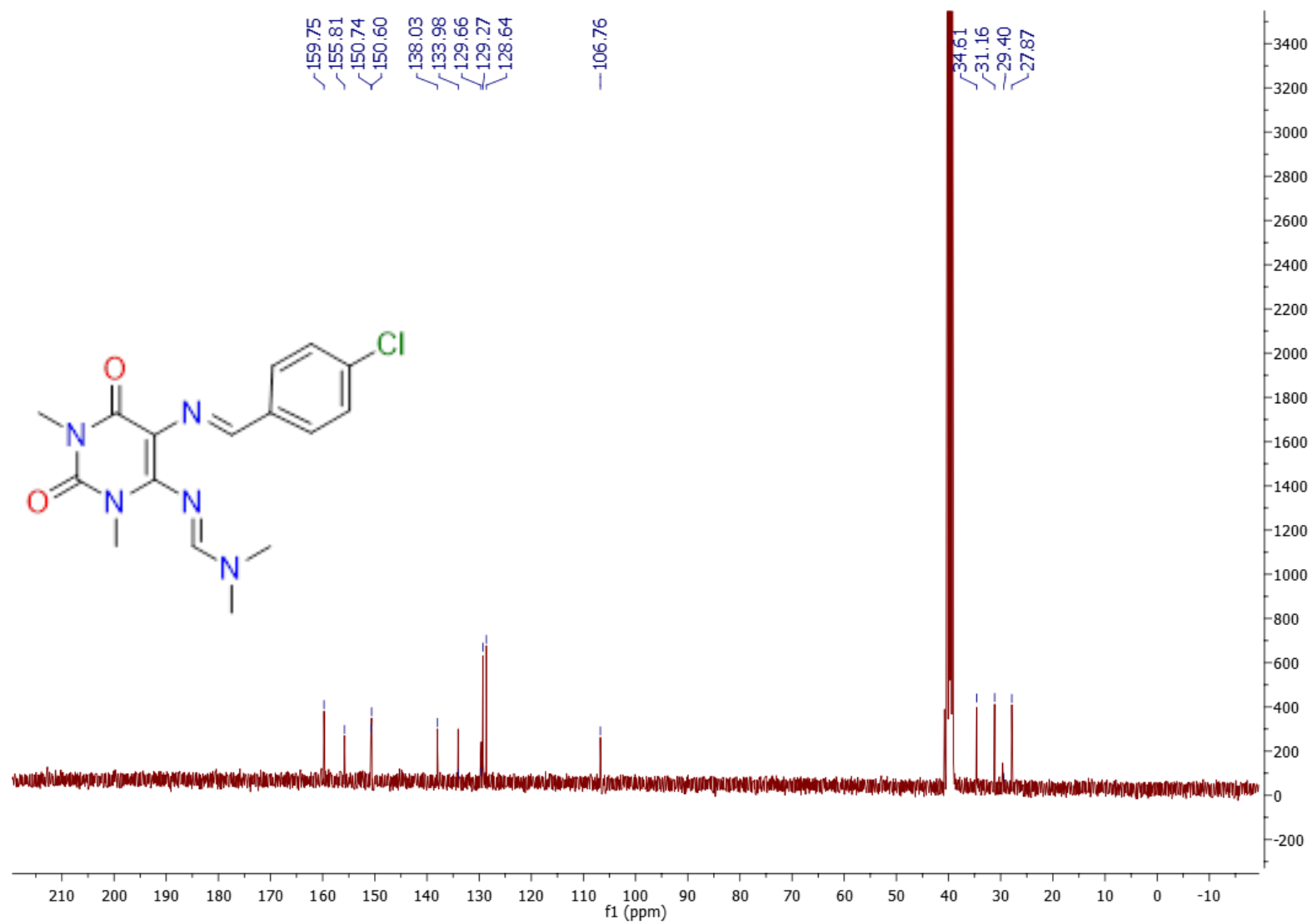


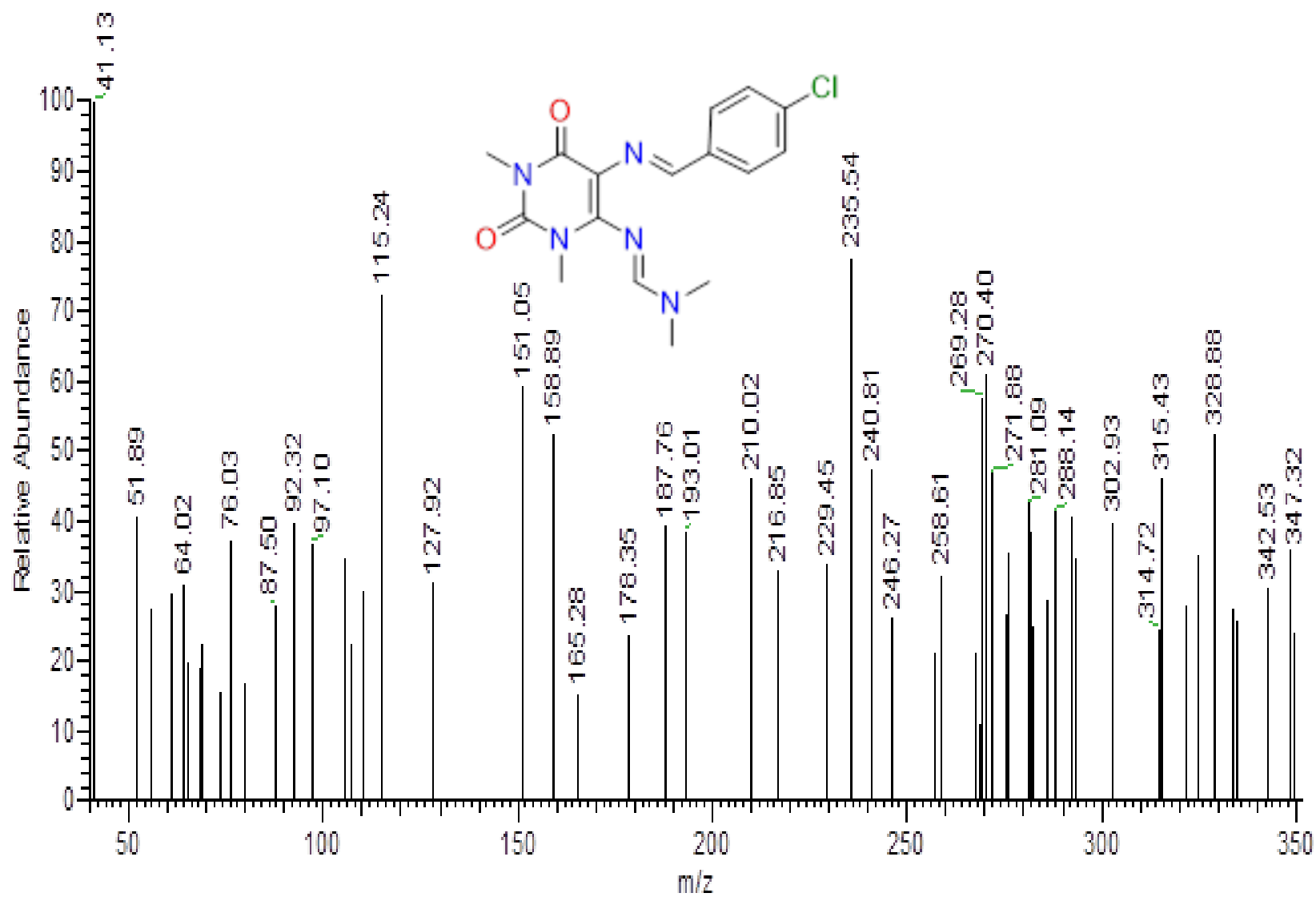




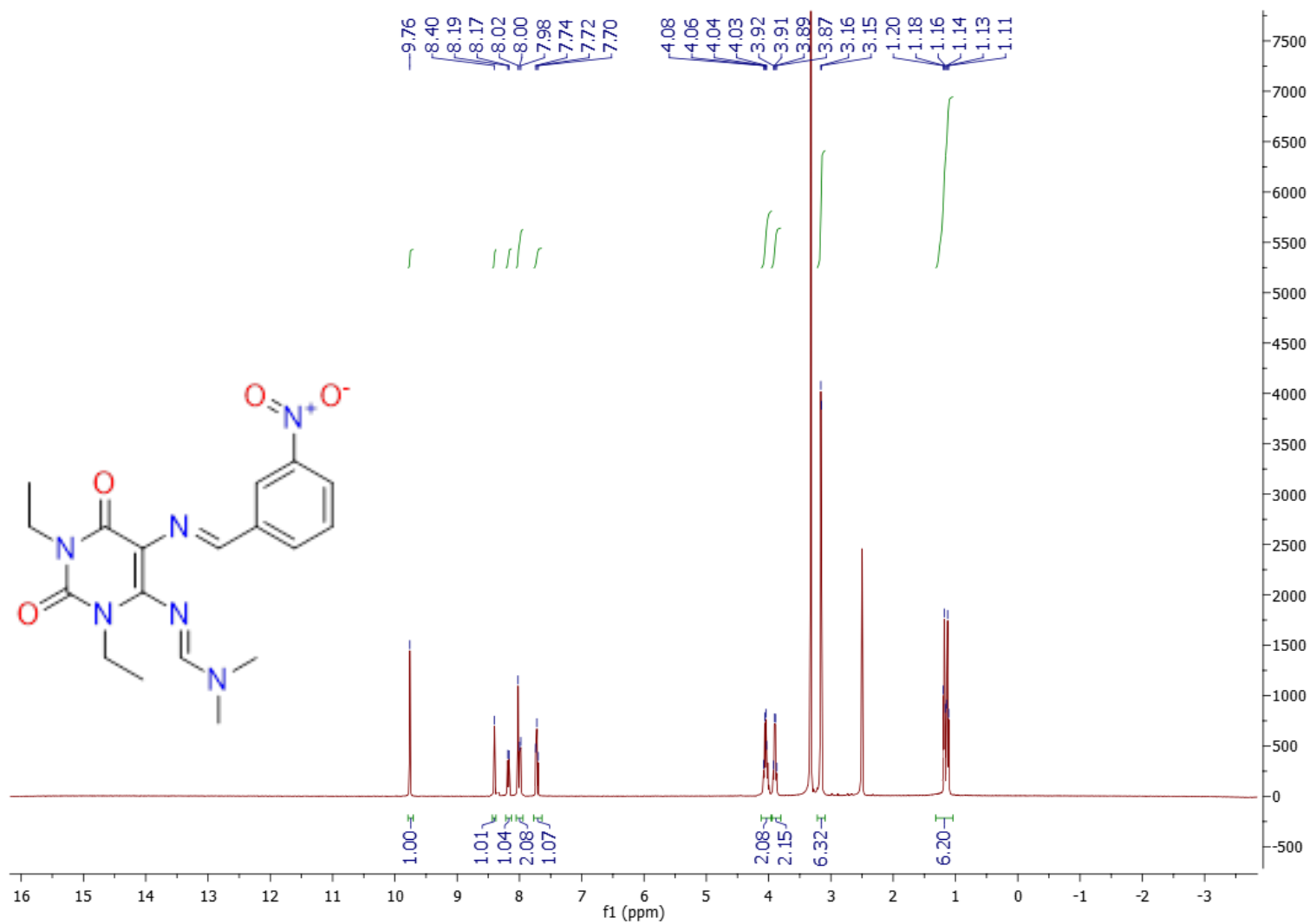
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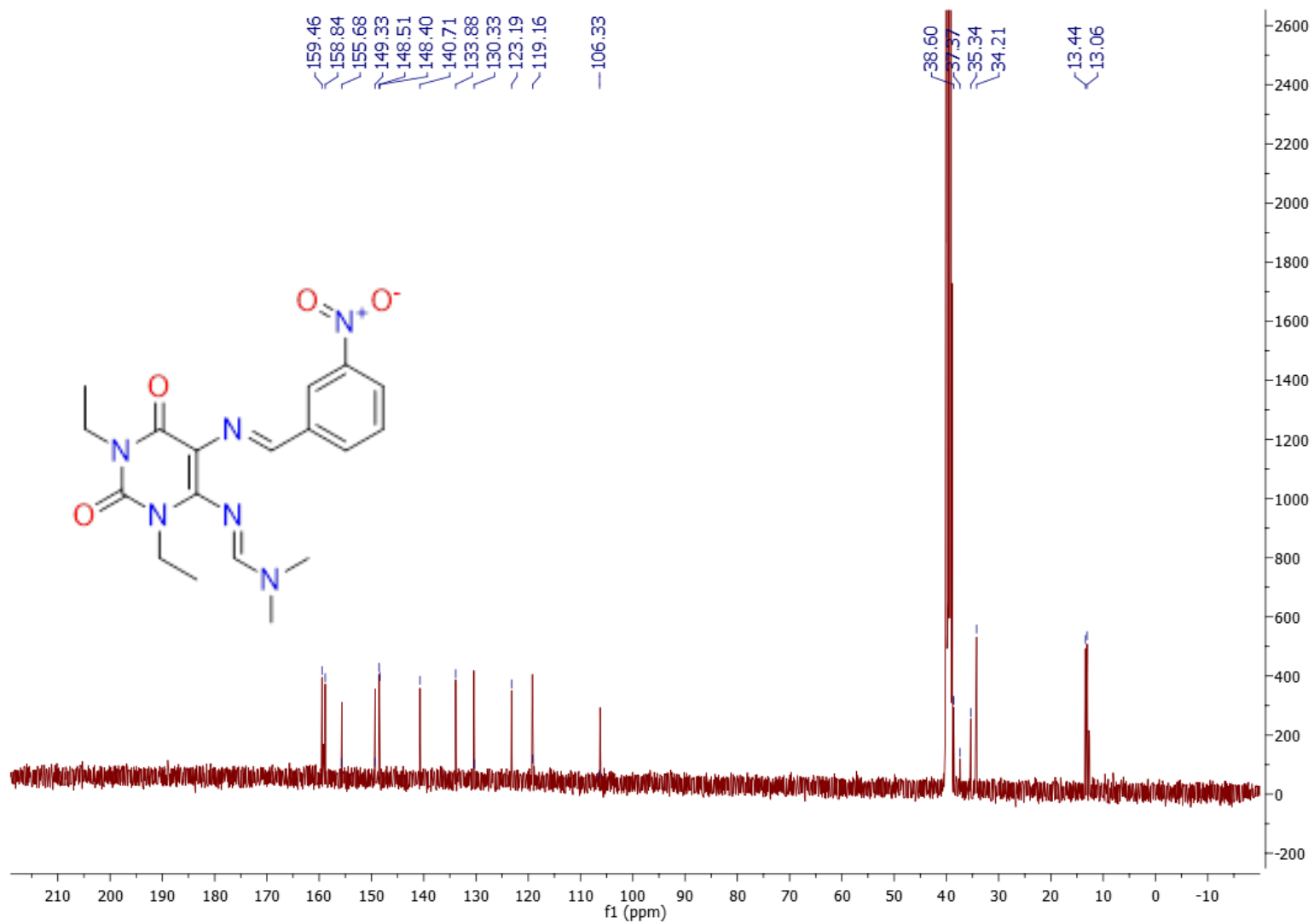


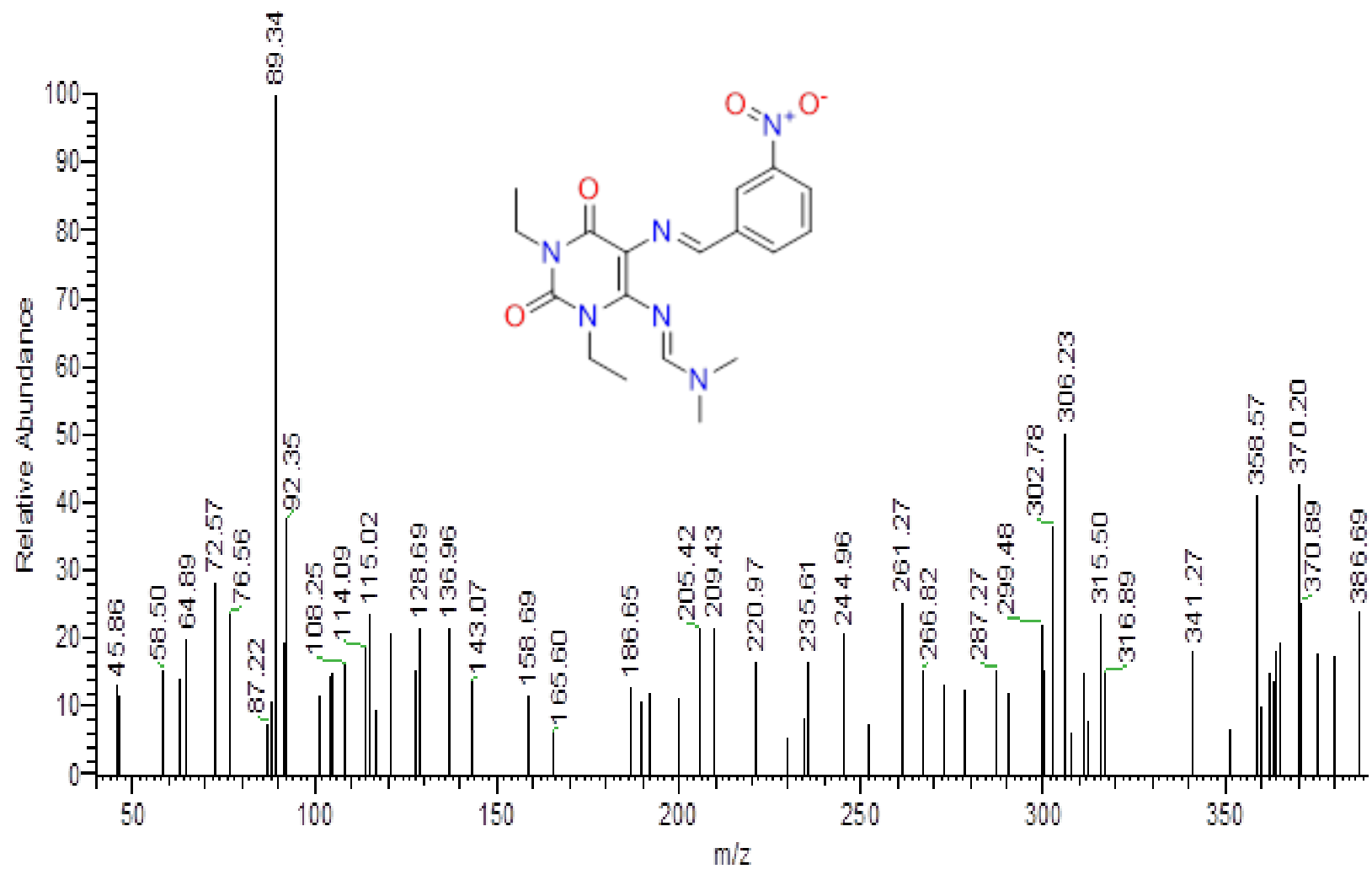




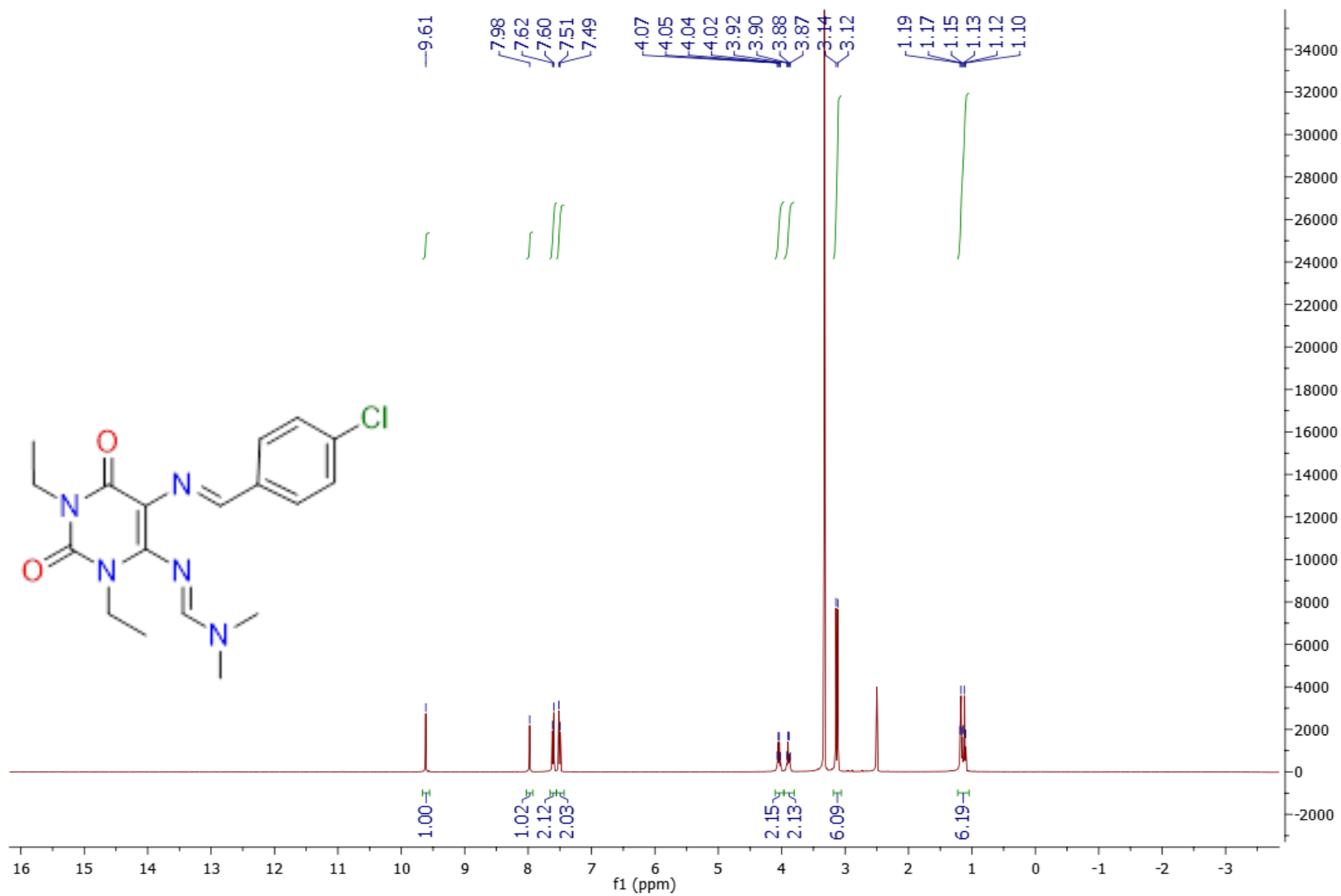
1.9 ^1H NMR, ^{13}C NMR and Mass spectra of 28

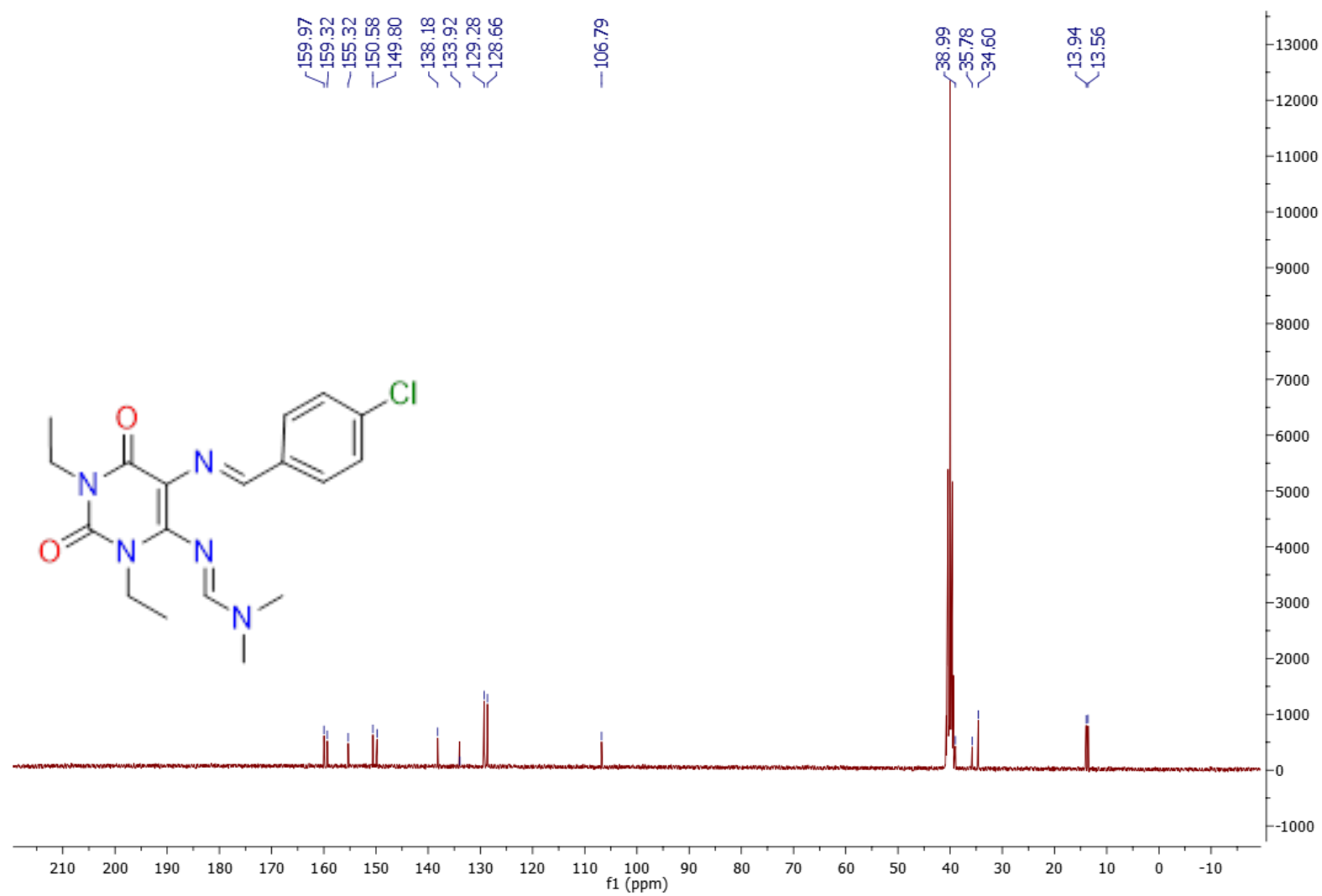


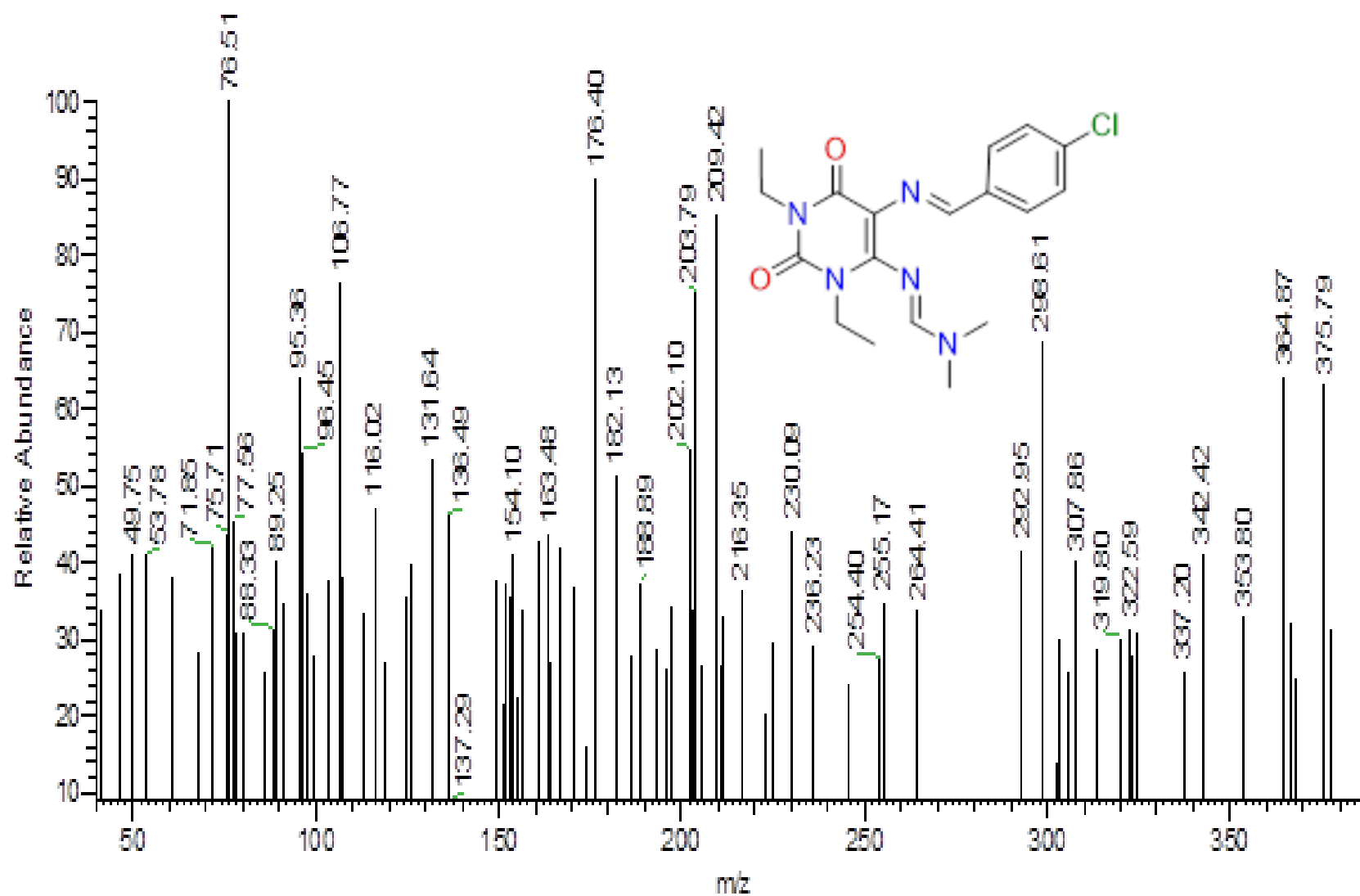




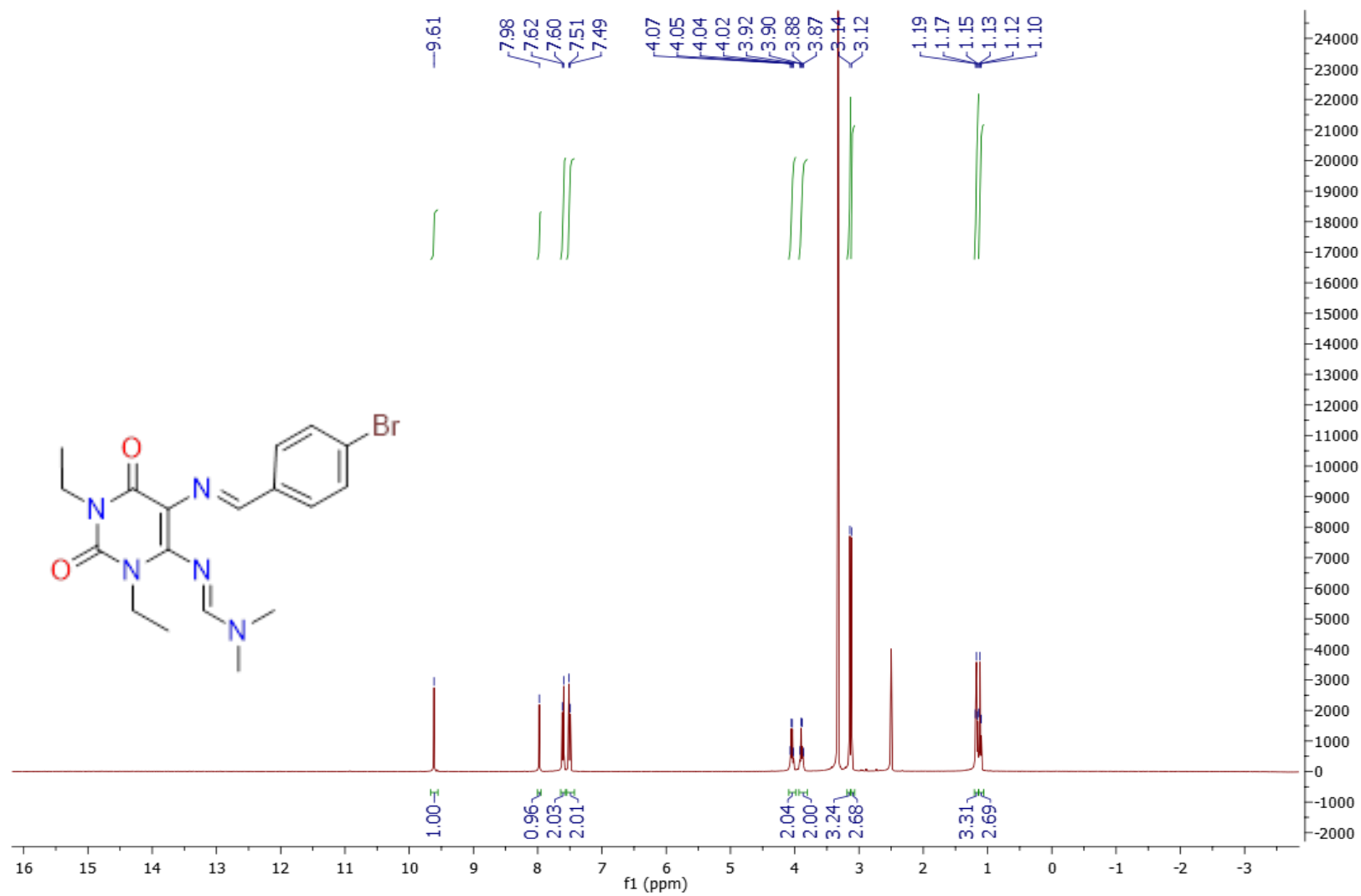
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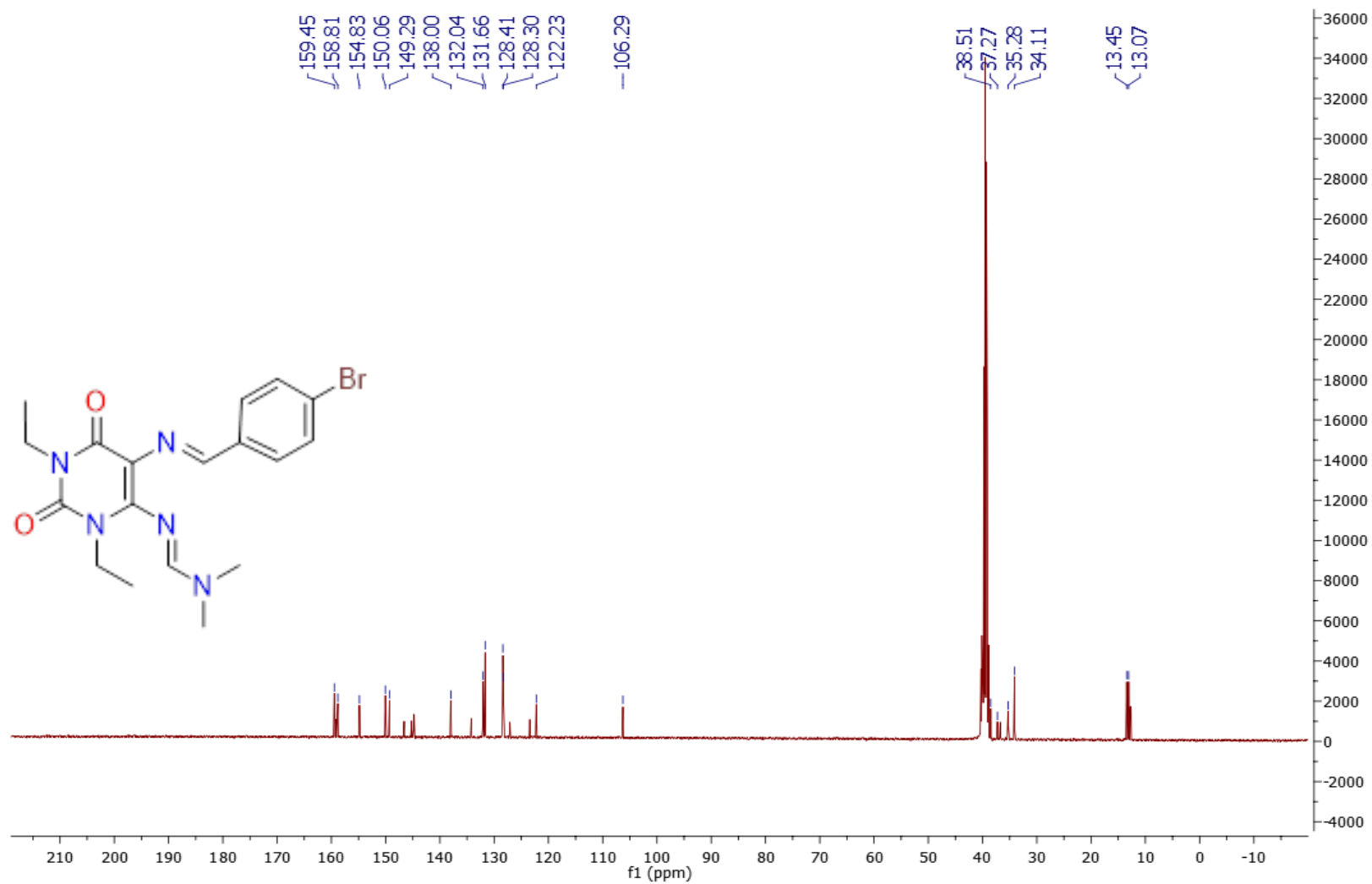


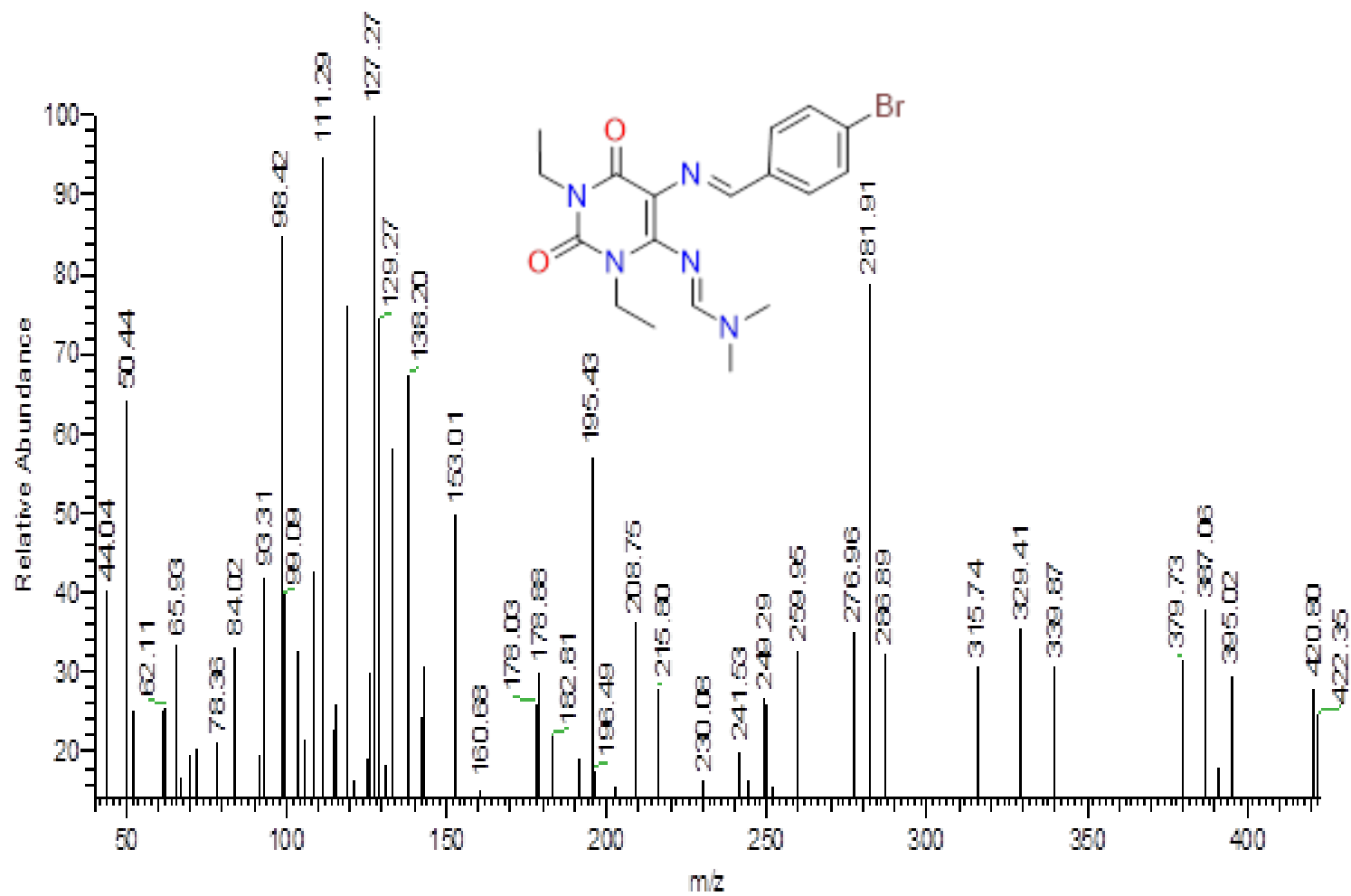




1.11 ^1H NMR, ^{13}C NMR and Mass spectra of 30

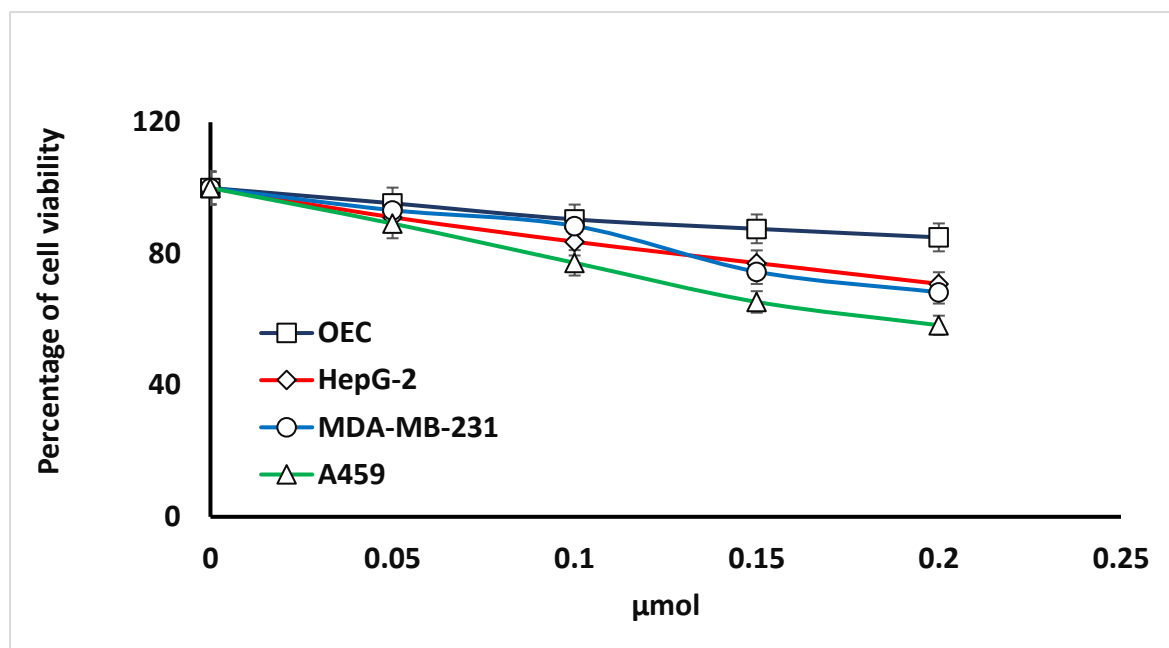




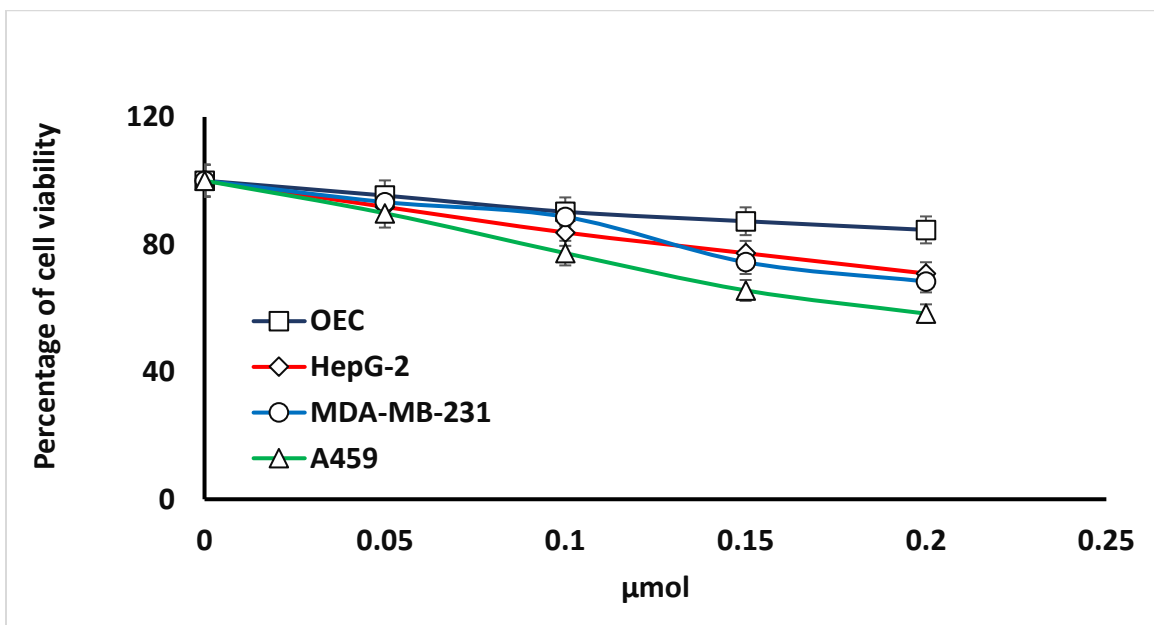


2 Cytotoxicity Evaluation

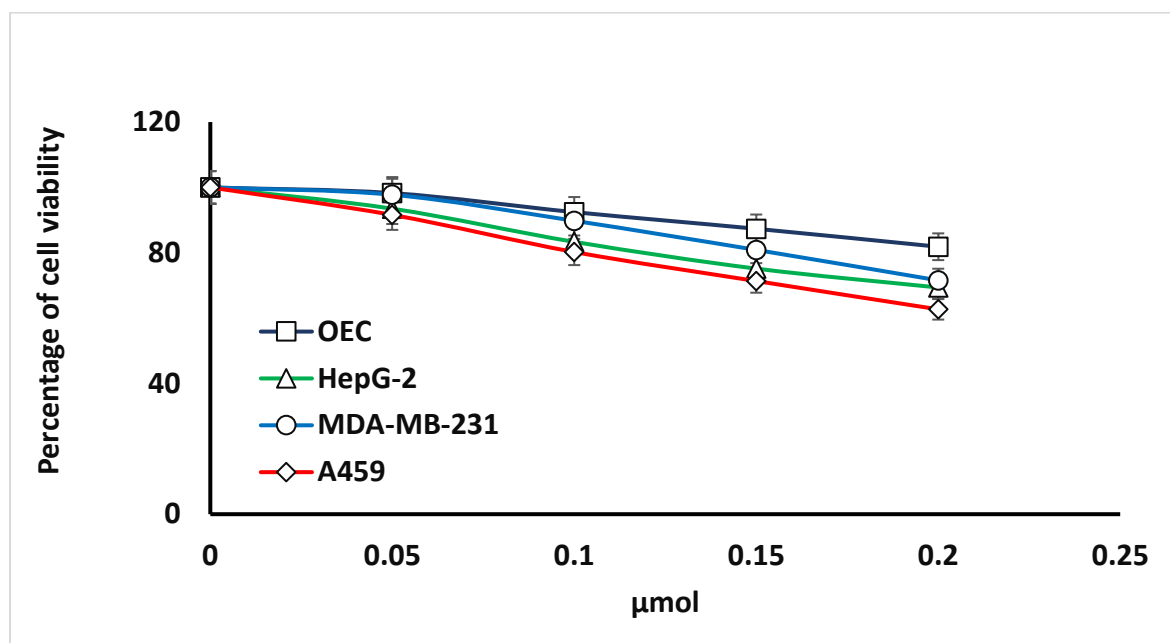
2.1 Cytotoxicity Evaluation of 19



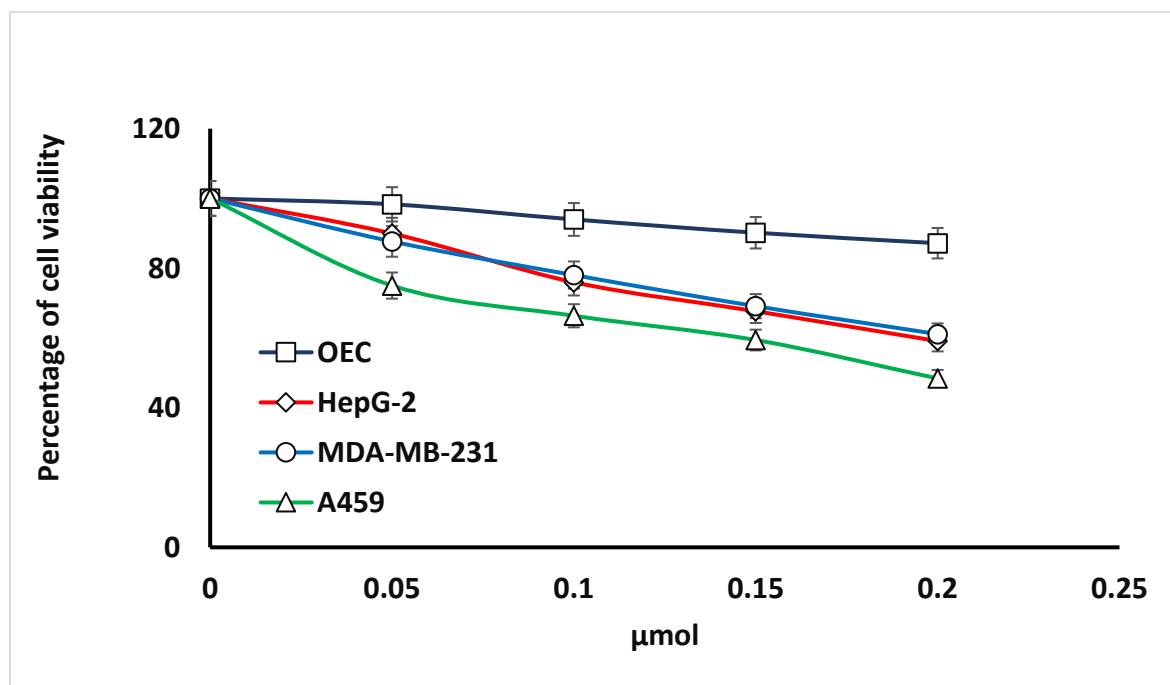
2.2 Cytotoxicity Evaluation of 20



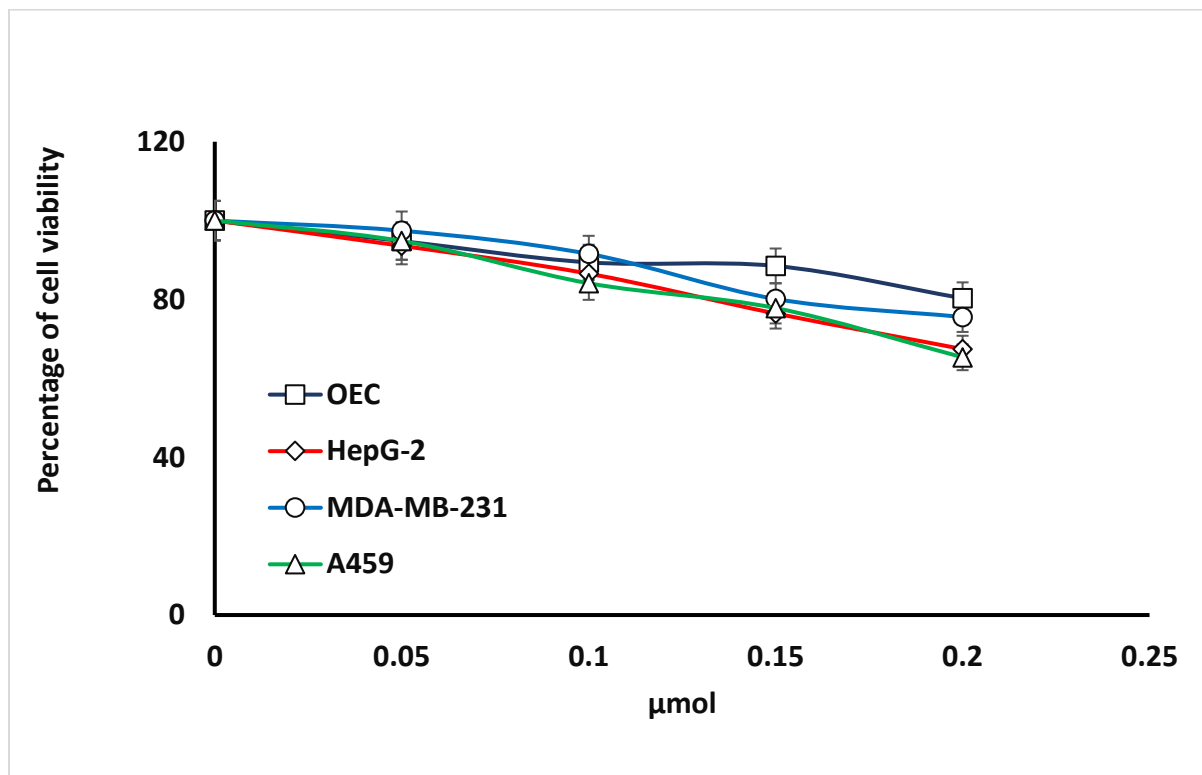
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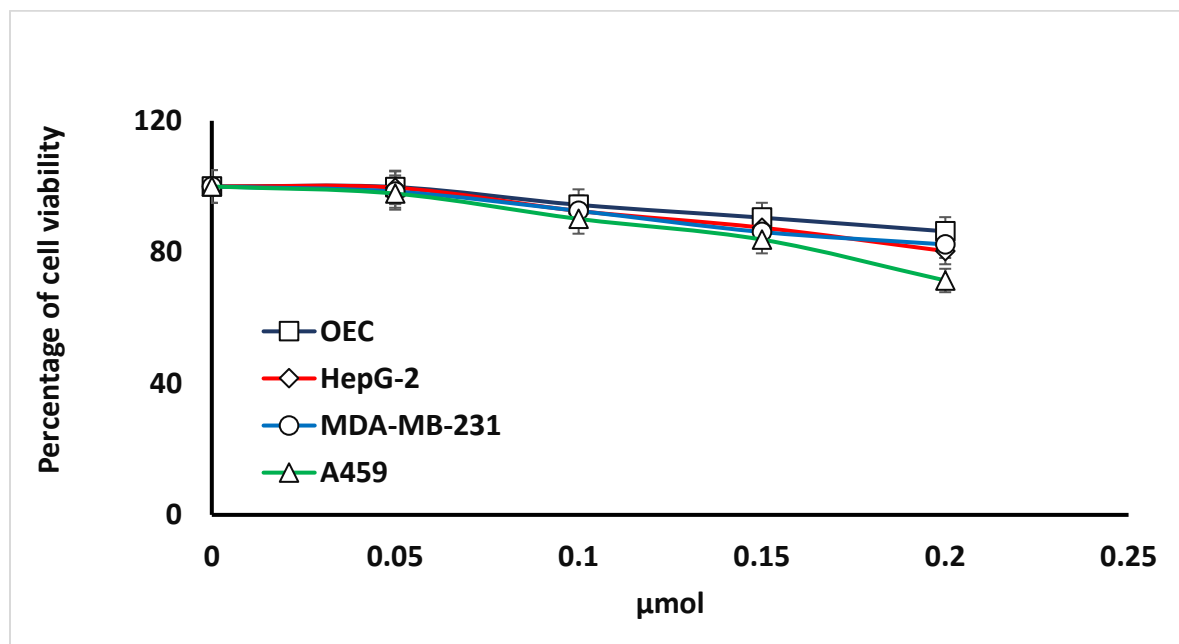
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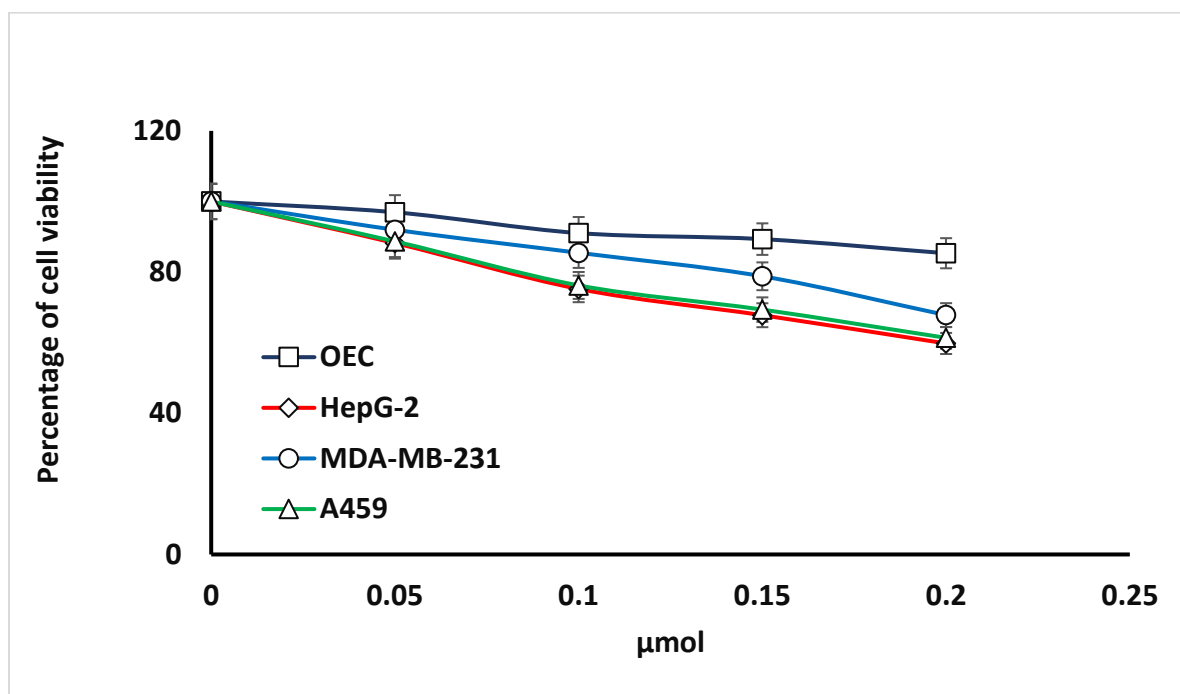
2.5 Cytotoxicity Evaluation of 23



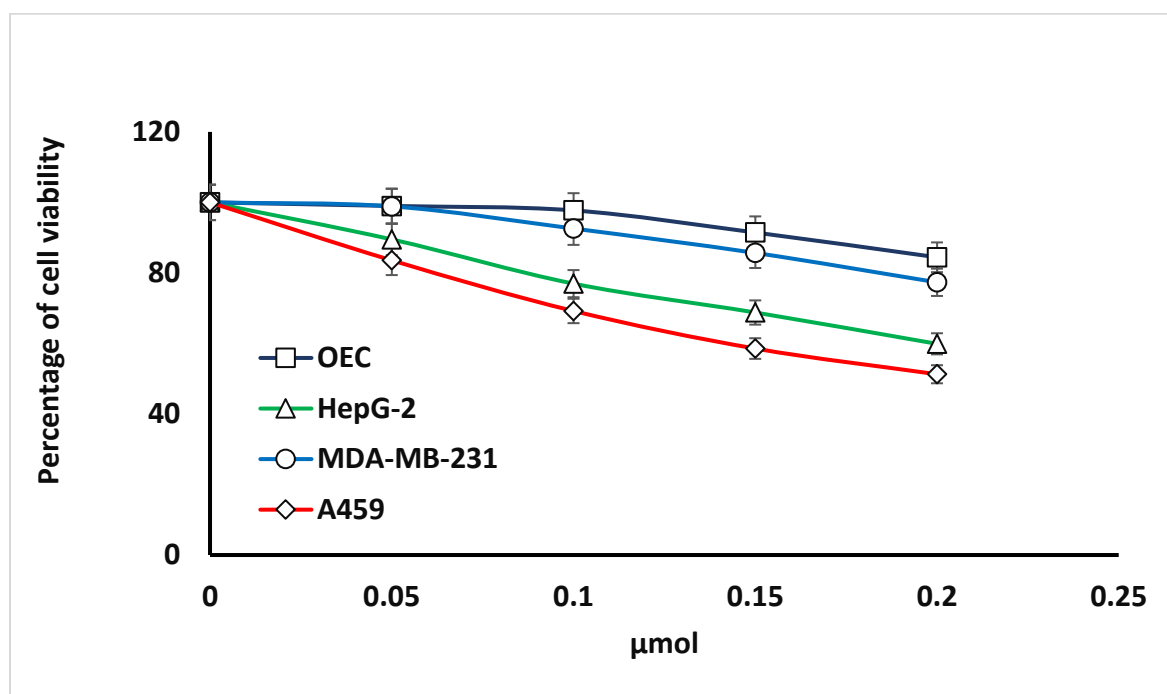
2.6 Cytotoxicity Evaluation of 24



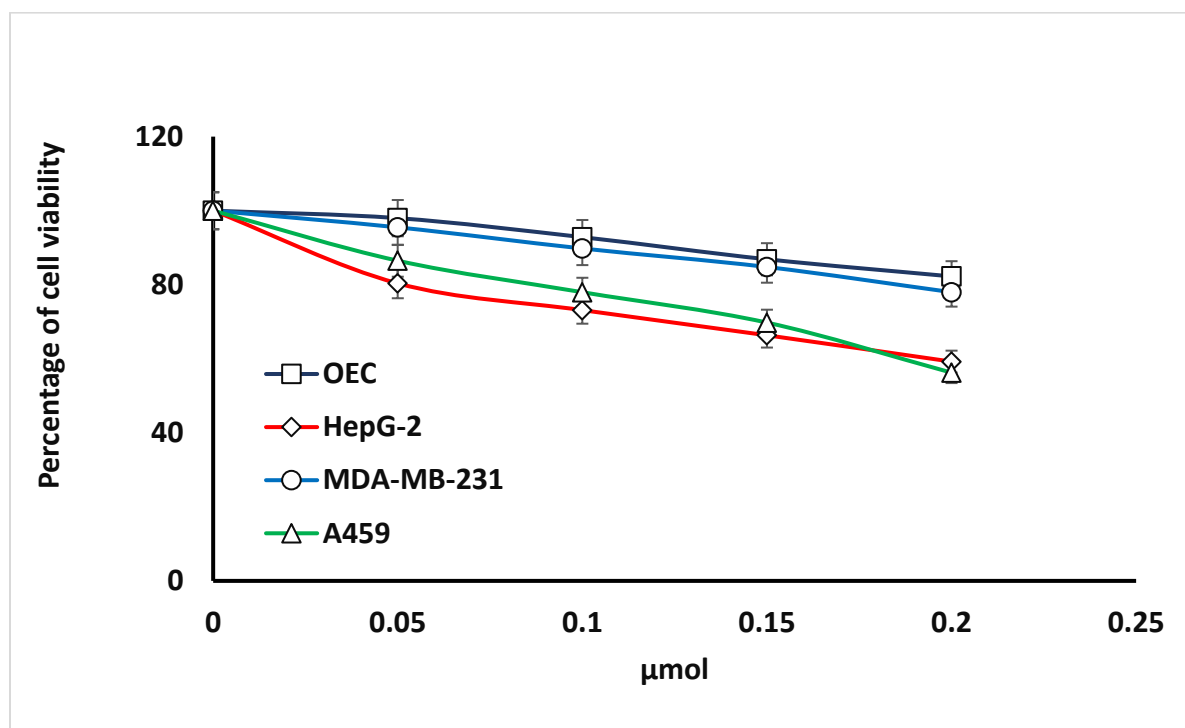
2.7 Cytotoxicity Evaluation of 25



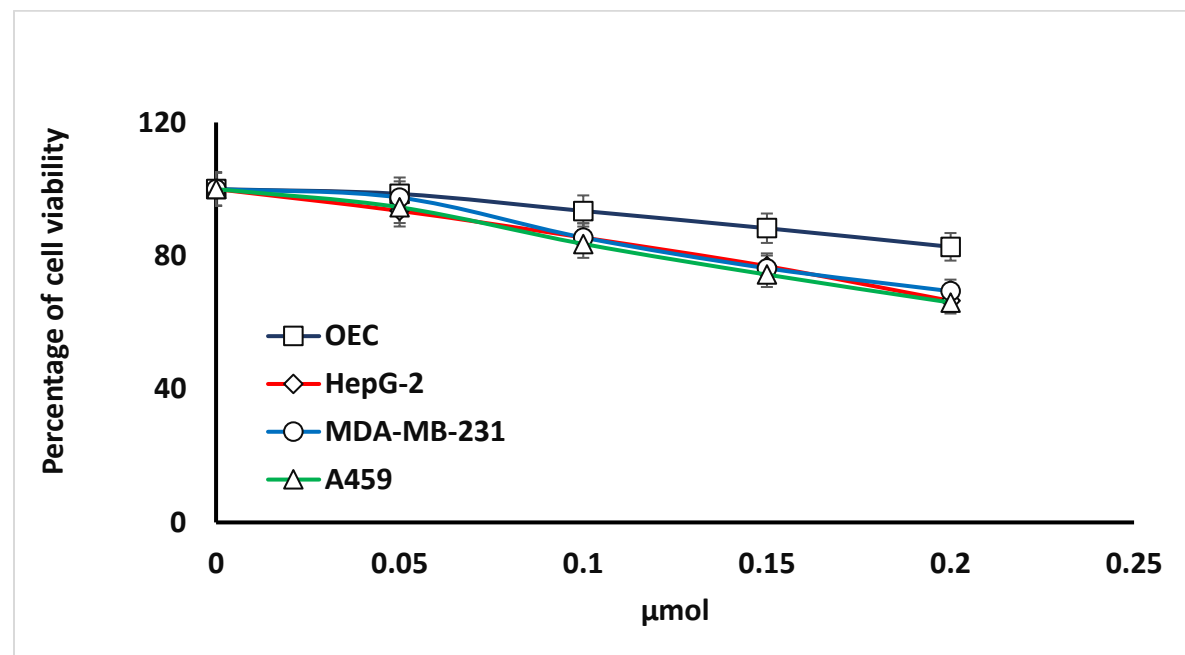
2.8 Cytotoxicity Evaluation of 26



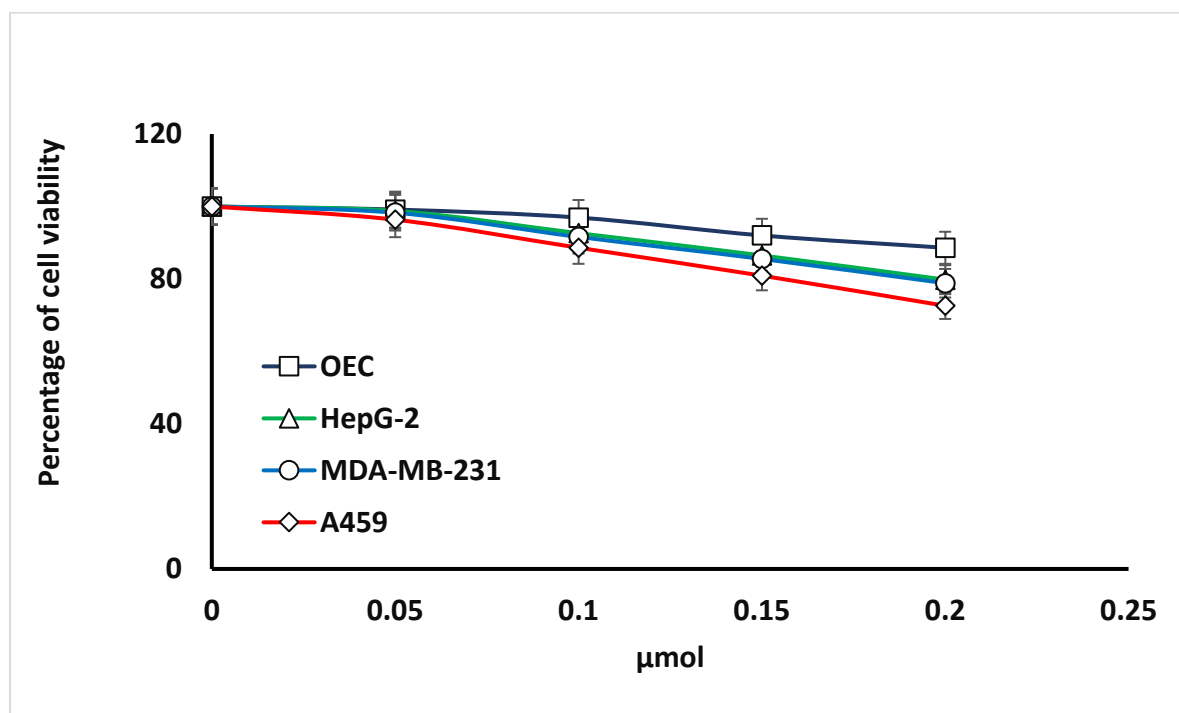
2.9 Cytotoxicity Evaluation of 27



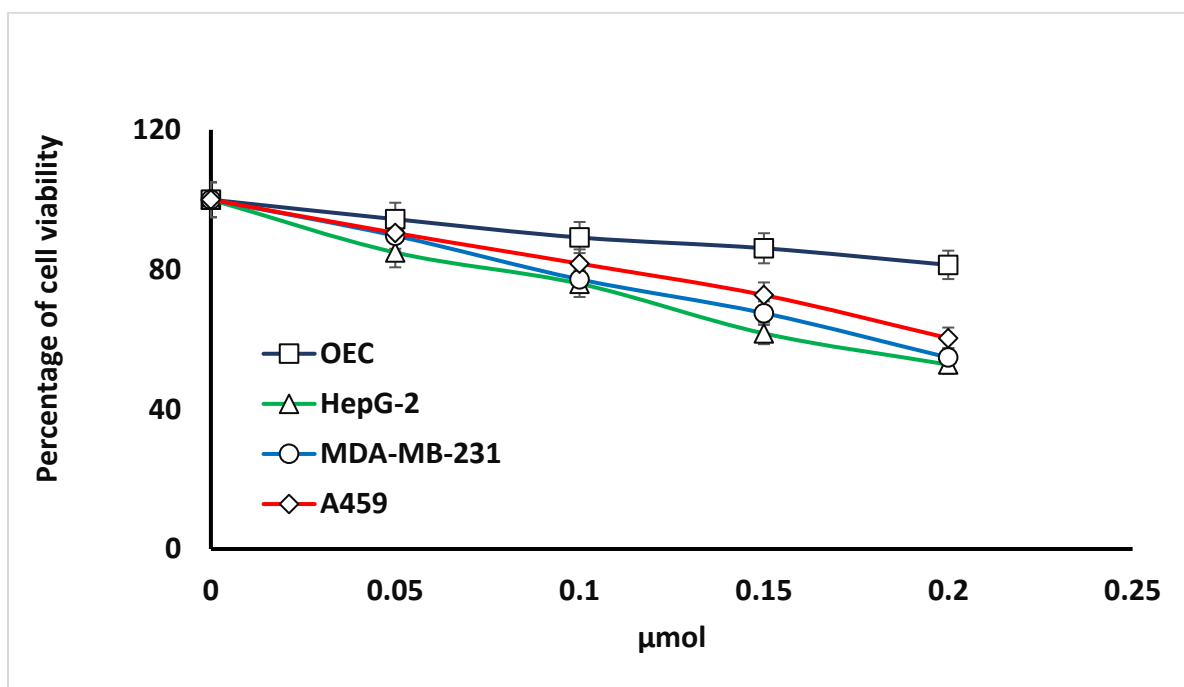
2.10 Cytotoxicity Evaluation of 28



2.11 Cytotoxicity Evaluation of 29



2.12 Cytotoxicity Evaluation of 30



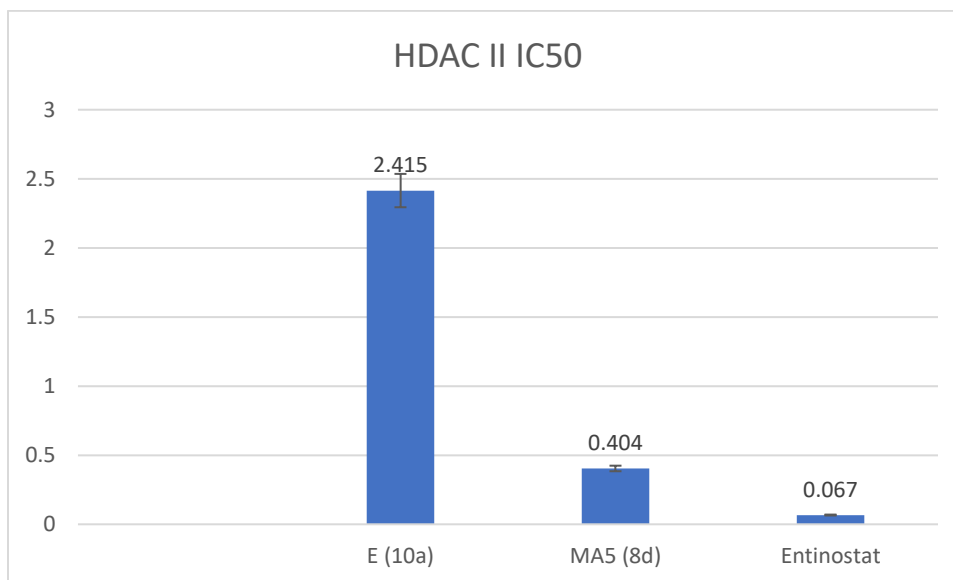
3 Enzyme Inhibition Assay

3.1 HDAC II

Researcher : Dr. Samar elkalyoubi email: s.elkalyoubi@hotmail.com mob. 01119952620
Date : 02-06-2022 Ash.elsayed@gmail.com 01024686495
Samples : 04 compounds.
Cell line : ---
Reference : ---
Date : 02/06/2022
Kit used : ---
Reader : BIOLINE ELISA READER wl 450 nm
Solvent : DMSO

Lab Report

ser	Compound		HDACx IC50 ug/ml	SD ±
	code	MW g/mol	HDAC2	
1	E (25)	372	2.415	0.11
4	MA5 (22)	336	0.404	0.02
***	Entinostat	376	0.067	0.003



Detailed results

HDAC2

code	IC50	conc	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
E		100	2	80.9	30	0	30	19.05	0	19.05	3.33333	22.86002
		10	1	64.1	30	0	30	35.93	0	35.93	3.33333	43.11604
		1	0	40.6	30	0	30	59.42	0	59.42	3.33333	71.30407
		0.1	-1	20.4	30	0	30	79.61	0	79.61	3.33333	95.5321
		0.01	-2	7.54	30	0	30	92.46	0	92.46	3.33333	110.9521
EC				0	30	0	30	100	0	100	3.3333333	120
code	IC50	conc.uM	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
1A1		100	2	74.5	30	0	30	25.46	0	25.46	3.3333	30.55231
		10	1	45.4	30	0	30	54.59	0	54.59	3.3333	65.50866
		1	0	17.6	30	0	30	82.37	0	82.37	3.3333	98.84499
		0.1	-1	8.48	30	0	30	91.52	0	91.52	3.3333	109.8251
		0.01	-2	5.23	30	0	30	94.77	0	94.77	3.3333	113.7251
EC				0	30	0	30	100	0	100	3.3333333	120
code	IC50	conc.uM	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
EthXY		100	2	92.5	30	0	30	7.53	0	7.53	3.33333	9.036009
		10	1	80.5	30	0	30	19.54	0	19.54	3.33333	23.44802
		1	0	68.9	30	0	30	31.08	0	31.08	3.33333	37.29604
		0.1	-1	42.6	30	0	30	57.42	0	57.42	3.33333	68.90407
		0.01	-2	27.3	30	0	30	72.69	0	72.69	3.33333	87.22809
EC				0	30	0	30	100	0	100	3.3333333	120
code	IC50	conc.uM	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
MA5		100	2	88.9	30	0	30	11.06	0	11.06	3.33333	13.27201
		10	1	77.2	30	0	30	22.83	0	22.83	3.33333	27.39603
		1	0	56	30	0	30	43.96	0	43.96	3.33333	52.75205
		0.1	-1	37.8	30	0	30	62.15	0	62.15	3.33333	74.58007
		0.01	-2	23.5	30	0	30	76.53	0	76.53	3.33333	91.83609
EC				0	30	0	30	100	0	100	3.3333333	120
code	IC50	conc.uM	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
Entinostat		100	2	93.5	30	0	30	6.46	0	6.46	3.33333	7.752008
		10	1	83.2	30	0	30	16.82	0	16.82	3.33333	20.18402
		1	0	72.3	30	0	30	27.66	0	27.66	3.33333	33.19203

EC	0.1	-1	51.5	30	0	30	48.53	0	48.53	3.33333	58.23606
	0.01	-2	35.7	30	0	30	64.25	0	64.25	3.33333	77.10008
			0	30	0	30	100	0	100	3.3333333	120

3.2 Topoisomerase I

Researcher : Dr.Samar elkalyoubi email: s.elkalyoubi@hotmail.com mob. 01119952620

Assay : **TOPO I** Ash.elsayed@gmail.com 01024686495

Samples : 04 compounds

Ref. : ---

Date : 02-06-2022

Reader : ROBONIK EIA READER wl 450 nm

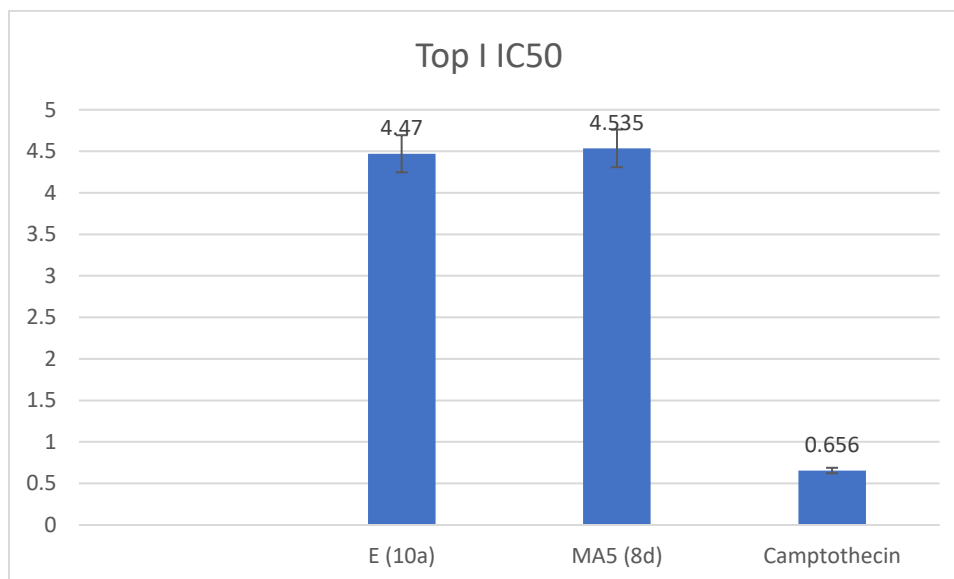
Cell line : ---

Kit used : DNA Topoisomerase 1 Kit

Solvent : DMSO

Lab Report

ser	Compound			TOP1 α	SD \pm
	code	MW	IC50	IC50 ug/ml	
1	E (25)			4.47	0.215
4	MA5 (22)			4.535	0.218
***	Camptothecin			0.656	0.044



Detailed results

TOPO I

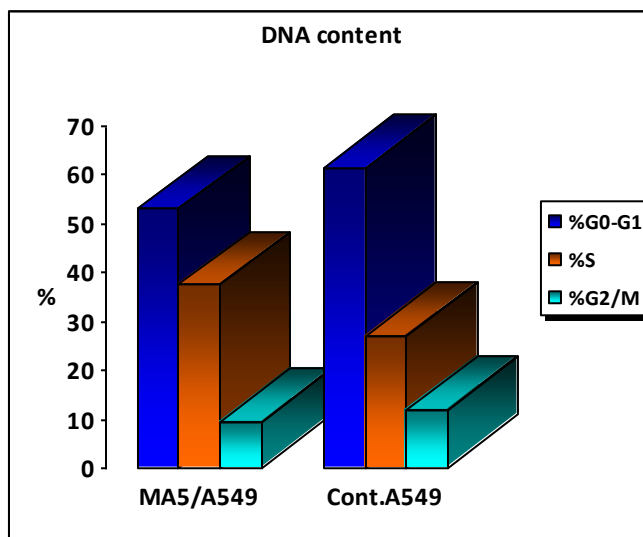
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E		100	2	81.8	30	0	30	18.23	0	18.23	3.33333	21.87602
		10	1	57.3	30	0	30	42.66	0	42.66	3.33333	51.19205
		1	0	27.4	30	0	30	72.56	0	72.56	3.33333	87.07209
		0.1	-1	13.4	30	0	30	86.55	0	86.55	3.33333	103.8601
		0.01	-2	7.31	30	0	30	92.69	0	92.69	3.33333	111.2281
EC				0	30	0	30	100	0	100	3.3333333	120
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
1A1		100	2	66.4	30	0	30	33.56	0	33.56	3.3333	40.2724
		10	1	43.2	30	0	30	56.82	0	56.82	3.3333	68.18468
		1	0	28.3	30	0	30	71.69	0	71.69	3.3333	86.02886
		0.1	-1	12	30	0	30	87.99	0	87.99	3.3333	105.5891
		0.01	-2	3.53	30	0	30	96.47	0	96.47	3.3333	115.7652
EC				0	30	0	30	100	0	100	3.3333333	120
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
EthXY		100	2	88.2	30	0	30	11.75	0	11.75	3.33333	14.10001
		10	1	65.2	30	0	30	34.82	0	34.82	3.33333	41.78404
		1	0	40.2	30	0	30	59.75	0	59.75	3.33333	71.70007
		0.1	-1	17.4	30	0	30	82.63	0	82.63	3.33333	99.1561
		0.01	-2	7.23	30	0	30	92.77	0	92.77	3.33333	111.3241
EC				0	30	0	30	100	0	100	3.3333333	120
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
MA5		100	2	84	30	0	30	16.01	0	16.01	3.33333	19.21202
		10	1	60.6	30	0	30	39.41	0	39.41	3.33333	47.29205
		1	0	22.1	30	0	30	77.85	0	77.85	3.33333	93.42009
		0.1	-1	10.6	30	0	30	89.43	0	89.43	3.33333	107.3161
		0.01	-2	3.31	30	0	30	96.69	0	96.69	3.33333	116.0281
EC				0	30	0	30	100	0	100	3.3333333	120
code	IC50	conc.uM	log conc	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
CPT		100	2	92.1	30	0	30	7.93	0	7.93	3.33333	9.51601
		10	1	77.1	30	0	30	22.85	0	22.85	3.33333	27.42003
		1	0	53.3	30	0	30	46.71	0	46.71	3.33333	56.05206
		0.1	-1	30.5	30	0	30	69.51	0	69.51	3.33333	83.41208
		0.01	-2	15.3	30	0	30	84.69	0	84.69	3.33333	101.6281
EC				0	30	0	30	100	0	100	3.3333333	120

4 Apoptosis induction and cell cycle analysis

Researcher	: Dr. Samar Ali	email: s.elkalyoubi@hotmail.com	mob. 01119952620
Assay	: Cell Cycle Analysis		
Samples	: 02 samples		
cell line	: A549		
Ref.	: ---		

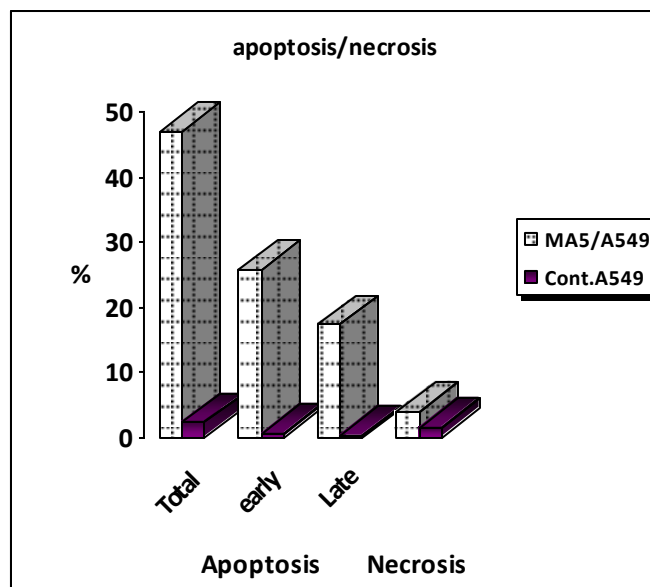
Lab Report

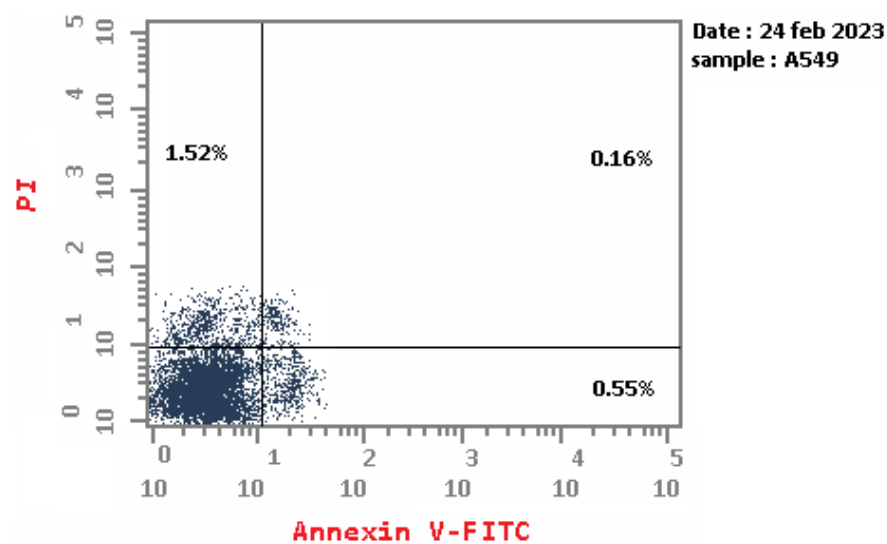
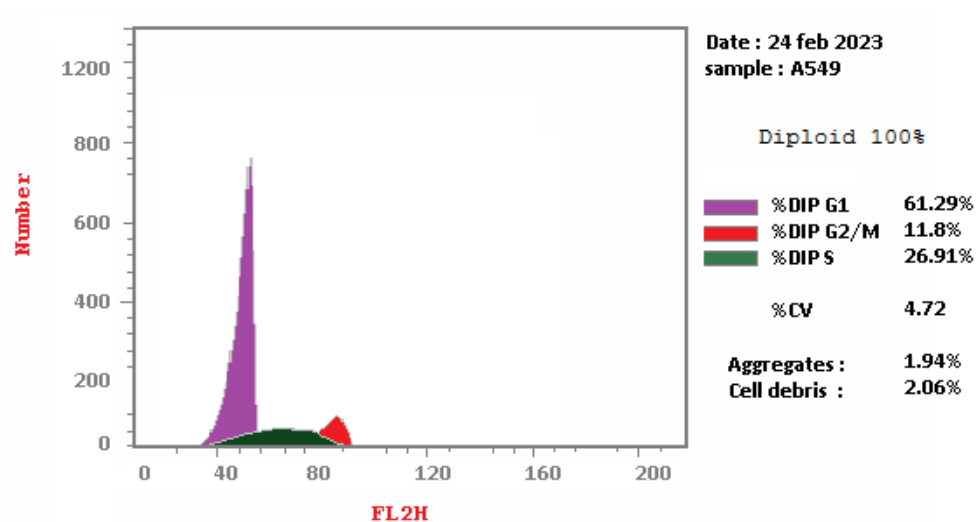
ser	Sample		DNA content			
	code	IC50 uM	%G0-G1	%S	%G2/M	Comment
1	MA5 (22)/A549	---	52.91	37.44	9.65	cell growth arrest@ G0-G1
2	Cont.A549	---	61.29	26.91	11.8	---

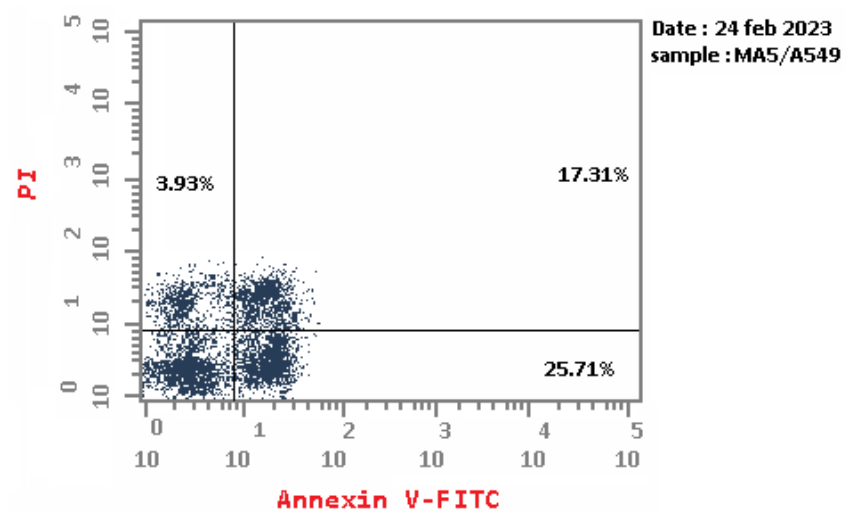
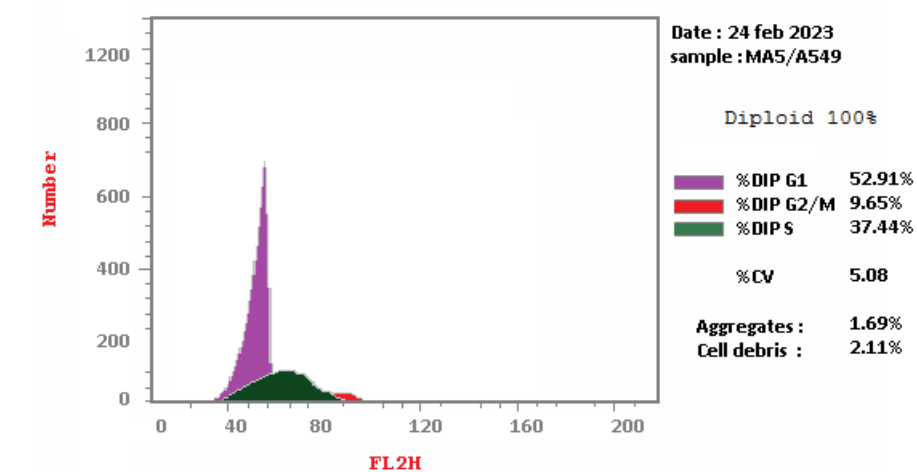


Detailed results

s	code	conc	Apoptosis			Necrosis
			Total	Early	Late	
1	MA5/A549	---	46.95	25.71	17.31	3.93
2	Cont.A549	---	2.23	0.55	0.16	1.52





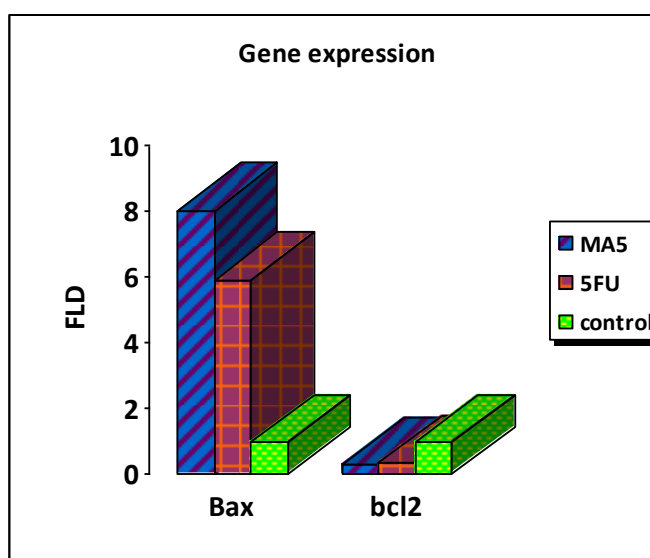


5 Apoptotic gene expression

Researcher	: Dr.Samar Ali	email: s.elkalyoubi@hotmail.com	mob. 01119952620
Assay	: RT-PCR		
Samples	: 01 Samples		
Cell lines	: A549		

Lab Report

Ser	Sample			RT-PCR	
				Fold Change	
	code	MW	IC50	A549	
				Bax	bcl2
1	MA5 (22)	---	---	8.0127	0.2854
2	5FU	---	---	5.909	0.3463
3	control	---	---	1	1





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Quantitation Report

5.1.1.1 Experiment Information

Run Name	Run 2023-02-24 (1)
Run Start	2023-02-24 03:42:25 PM
Run Finish	2023-02-24 06:21:09 PM
Operator	ERA
Notes	---
Run On Software Version	Rotor-Gene 1.7.87
Run Signature	The Run Signature is valid.
Gain Green	10.
Gain Yellow	9.33

Quantitation data



This report generated by Rotor-Gene 6000 Series Software 1.7 (Build 87)
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ISO 9001:2000 (Reg. No. QEC21313)

primers

Bax : F 5'- TCAGGATGCGTCCACCAAGAAG-3',
Bax : R 5'- TGTGTCCACGGCGGCAATCATC-3'.

Bcl2 : F 5'-ATCGCCCTGTGGATGACTGAGT -3',
Bcl2 : R 5'- GCCAGGAGAAATCAAACAGAGGC-3'.

GAPDH : F 5'- GTCTCCTCTGACTTCAACAGCG-3'
GAPDH : R 5'- ACCACCCTGTTGCTGTAGCCAA-3'

Sample	Bax		
	Control cells	Test cells	FLD

Ser	code	Conc	GAPDH	Bax	Δ CTC	GAPDH	Bax	Δ CTE	$\Delta\Delta$ CT	$2^{\Delta\Delta CT}$
			HC	TC	TC-HC	HE	TE	TE-HE	Δ CTE- Δ CTC	E=1.866
1	MA5		22.37	33.97	11.6	22.29	30.61	8.32	-3.28	8.0127
2	5FU		22.37	33.97	11.6	21.97	30.77	8.8	-2.8	5.909
3	control		22.37	33.97	11.6	22.37	33.97	11.6	0	1

Sample	Bcl2		
	Control cells	Test cells	FLD

Ser	code	Conc	GAPDH	Bcl2	Δ CTC	GAPDH	Bcl2	Δ CTE	$\Delta\Delta$ CT	$2^{\Delta\Delta CT}$
			HC	TC	TC-HC	HE	TE	TE-HE	Δ CTE- Δ CTC	E=1.866
1	MA5		22.37	27.61	5.24	22.29	29.54	7.25	2.01	0.2854
2	5FU		22.37	27.61	5.24	21.97	28.91	6.94	1.7	0.3463
3	control		22.37	27.61	5.24	22.37	27.61	5.24	0	1

6 *In silico* Studies

6.1 Table S1: Docking results with HDAC II

Compound	S score Kcal/mol	H-bond interaction	Pi interaction
19	-5.7179	PHE210, LEU276, TYR308, Zinc binding	-
22	-8.8980	HIS145, ASP181, Zinc binding	HIS183, PHE210
25	-9.6062	In the proximity of Zinc ion	PHE210, LEU276
28	-6.1625	Zinc binding	PHE155
Vorinostat	-10.7656	ASP104, GLY154, CYS156, Zinc binding	PHE155, HIS183, PHE210
Tubastatin A	-9.1402	GLY154, TYR308, Zinc binding	-

6.2 Figure S1: 3D interaction pattern of compound 28 within the active site of HDAC II

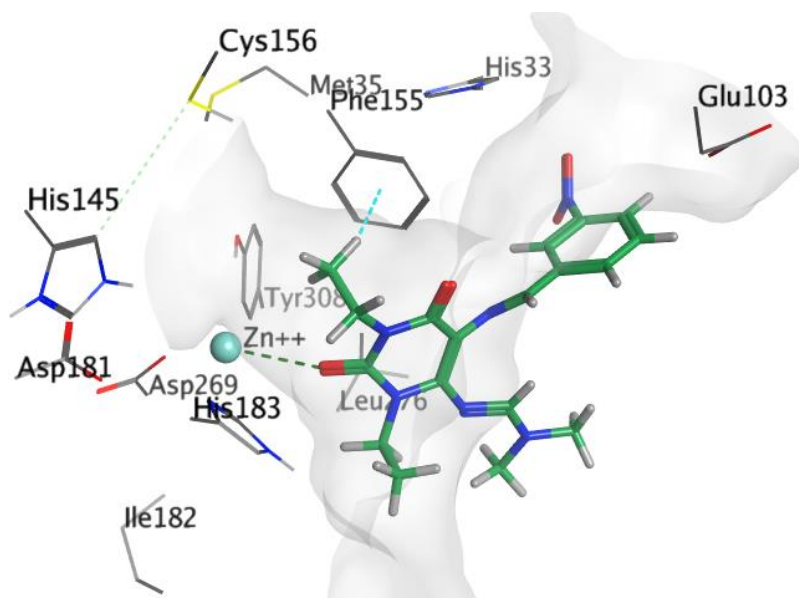


Figure 1: 3D interaction pattern of compound 28 within the active site of HDAC II

6.3 Figure S2: Flexible alignment of Vorinostat, 22, and compound 25

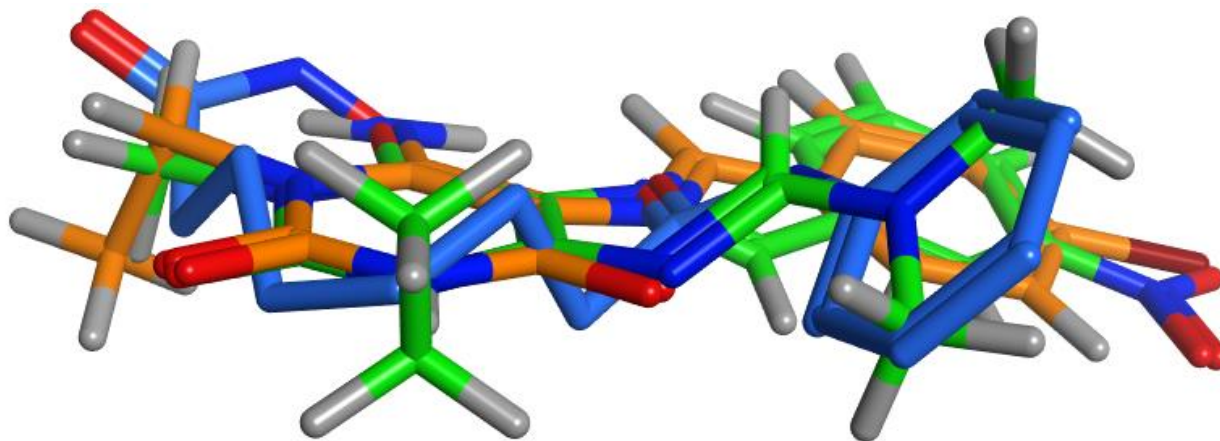


Figure 2: Flexible alignment of the co-crystallized ligand (Vorinostat: blue color), compound 22 (orange color), and compound 25 (green color) where $S = -75.86$ Kcal/mol.

6.4 Table S2: Docking results with Topo I.

Compound	S score Kcal/mol	H-bond interaction	<i>Pi</i> interaction
19	-7.2366	ASN722, THR718, HIS632, DG12	DG10
22	-6.5171	THR718, DG12	DT10
25	-7.7835	-	DT10, DA113
28	-8.1707	-	DT10, DA113
Topotecan	-8.4601	THR718, DG12	-
camptothecin	-6.9865	THR718, DG12	-

6.5 Figure S3: 3D interaction pattern of compound 28 within the active site of Topo I

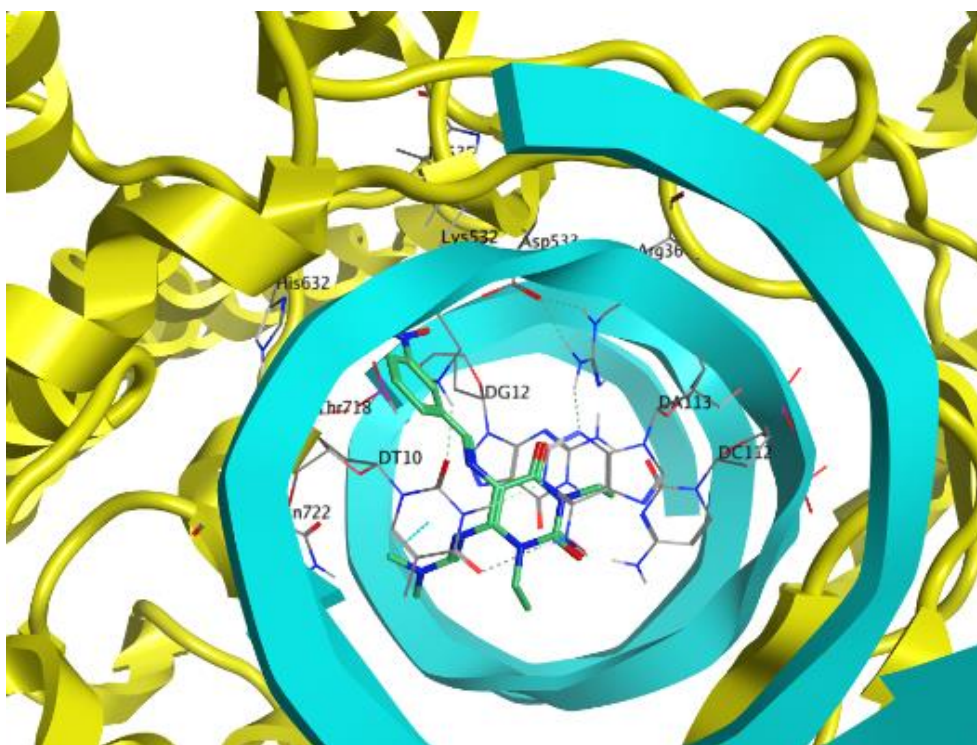


Figure 3:: 3D interaction pattern of compound 28 within the active site of Topo I

6.6 Figure S4: Flexible alignment of the Topotecan, 22, and 25

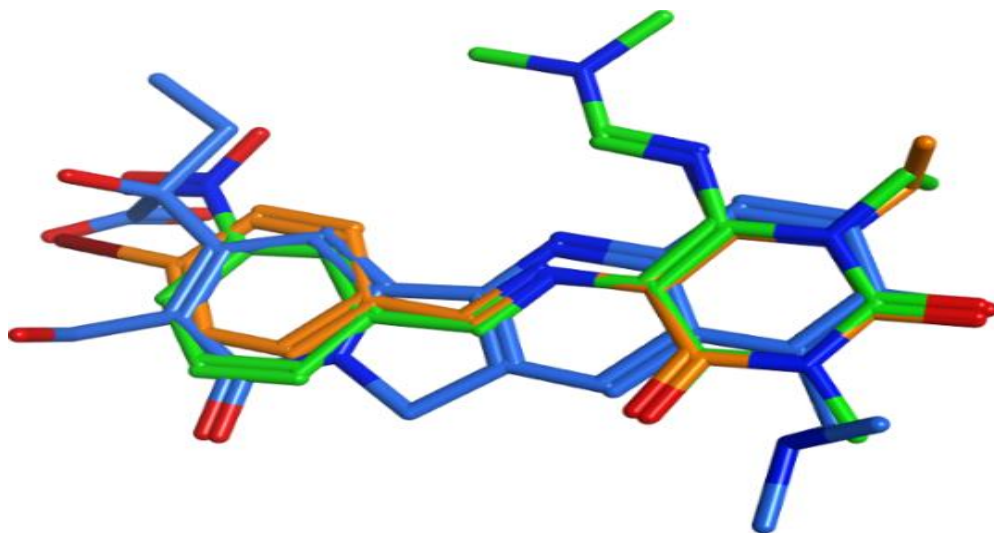


Figure 4: Flexible alignment of the co-crystallized ligand (Topotecan: blue color), compound 22 (orange color), and compound 25 (green color) where $S = -126.23$ Kcal/mol.

6.7 Figure S5: GIT absorption and brain penetration of 22 and 25

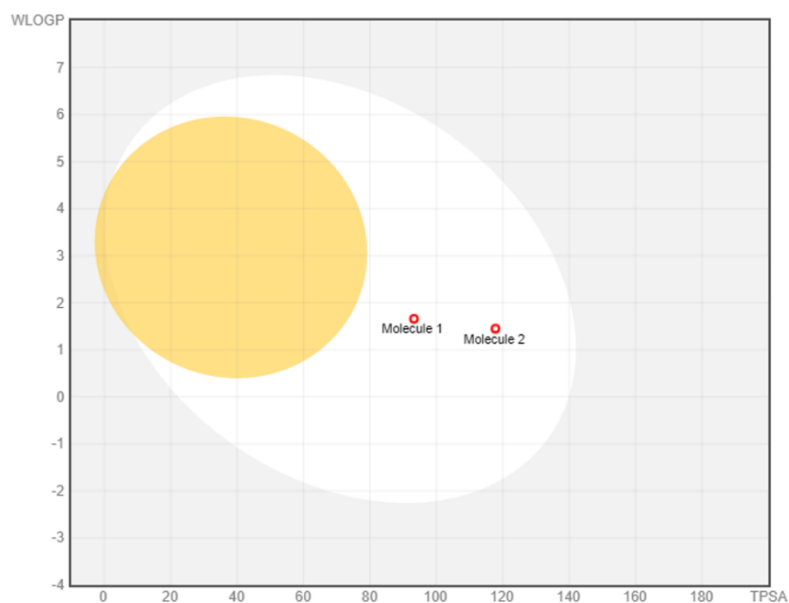
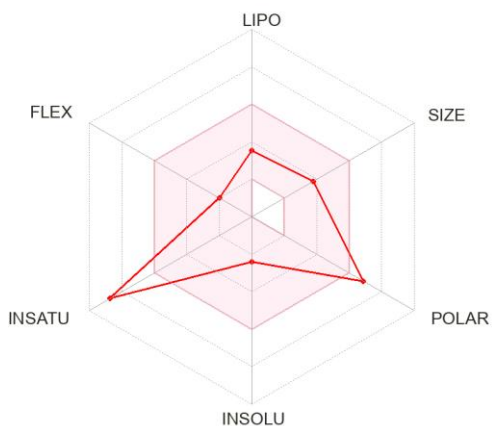


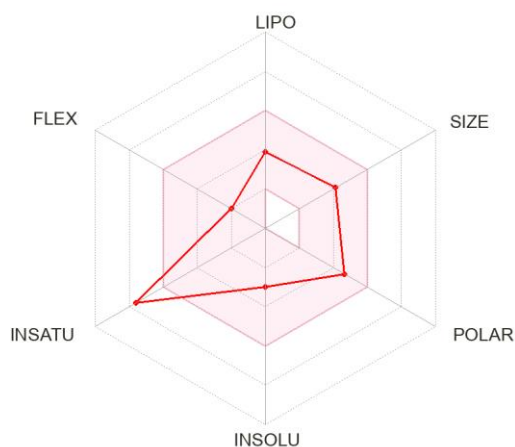
Figure 5: A Boiled-Egg graph predicting gastrointestinal absorption and brain penetration of **22** and **25**; BOILED-Egg's yolk region: BBB permeation; BOILED-Egg's white region: GI absorption.

6.8 Figure S6: Bioavailability radar of the representative compounds 19, 22, 25, and 28

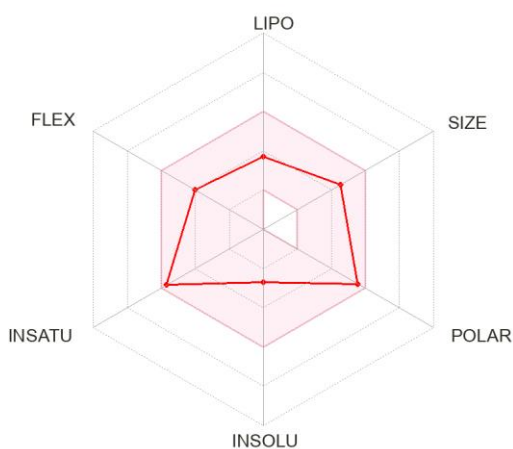
19



22



25



28

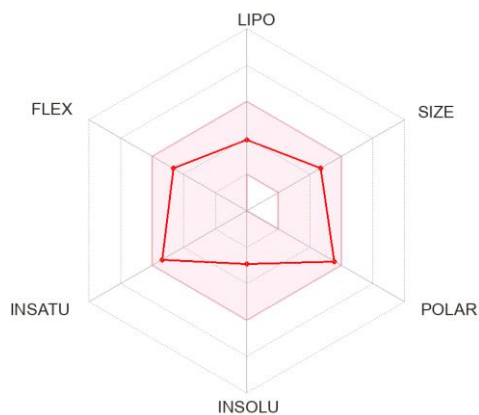
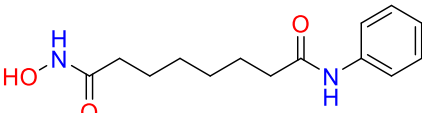
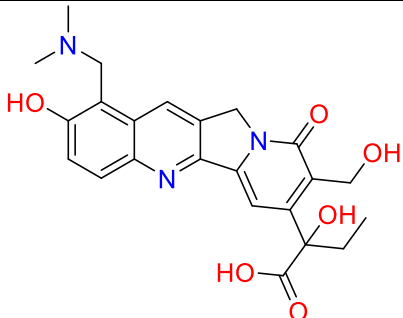


Figure 6: Bioavailability radar of the representative compounds **19**, **22**, **25**, and **28** assessed using the swissADME web tool. The pink area is the optimal range for each particular property for the tested compounds [LIPO = lipophilicity; SIZE = size as molecular weight; POLAR = polarity as TPSA (topological polar surface area); INSOLU = insolubility in water by log S scale; INSATU = insaturation as per fraction of carbons in the sp^3 hybridization and FLEX = flexibility as per rotatable bonds). INSATU = insaturation as per fraction of carbons in the sp^3 hybridization and FLEX = flexibility as per rotatable bonds).

6.9 Table S3: Pharmacokinetic parameters of for 19, 22, 25, 28, 5-FU, and methotrexate

Compound	GI	BBB	P-gp	CYP inhibition				
	absorption	permeant	substrate	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
19	Low	No	No	No	No	No	No	No
22	High	No	No	No	No	No	No	No
25	High	No	No	No	No	No	No	No
28	High	No	No	No	No	No	No	No
5-Fluorouracil	High	No	No	No	No	No	No	No
Methotrexate	Low	No	Yes	No	No	No	No	No

6.10 Table S4: The target enzymes used for docking studies

Enzymes (PDB)	Resolution	co-crystallized ligand	Structure of the co-crystallized ligand	RMSD	Energy score (S) (Kcal/mol)
4LXZ	1.85 Å	SAHA		1.2565	-10.7656
1K4T	2.10 Å	TTG		1.0734	-7.8879