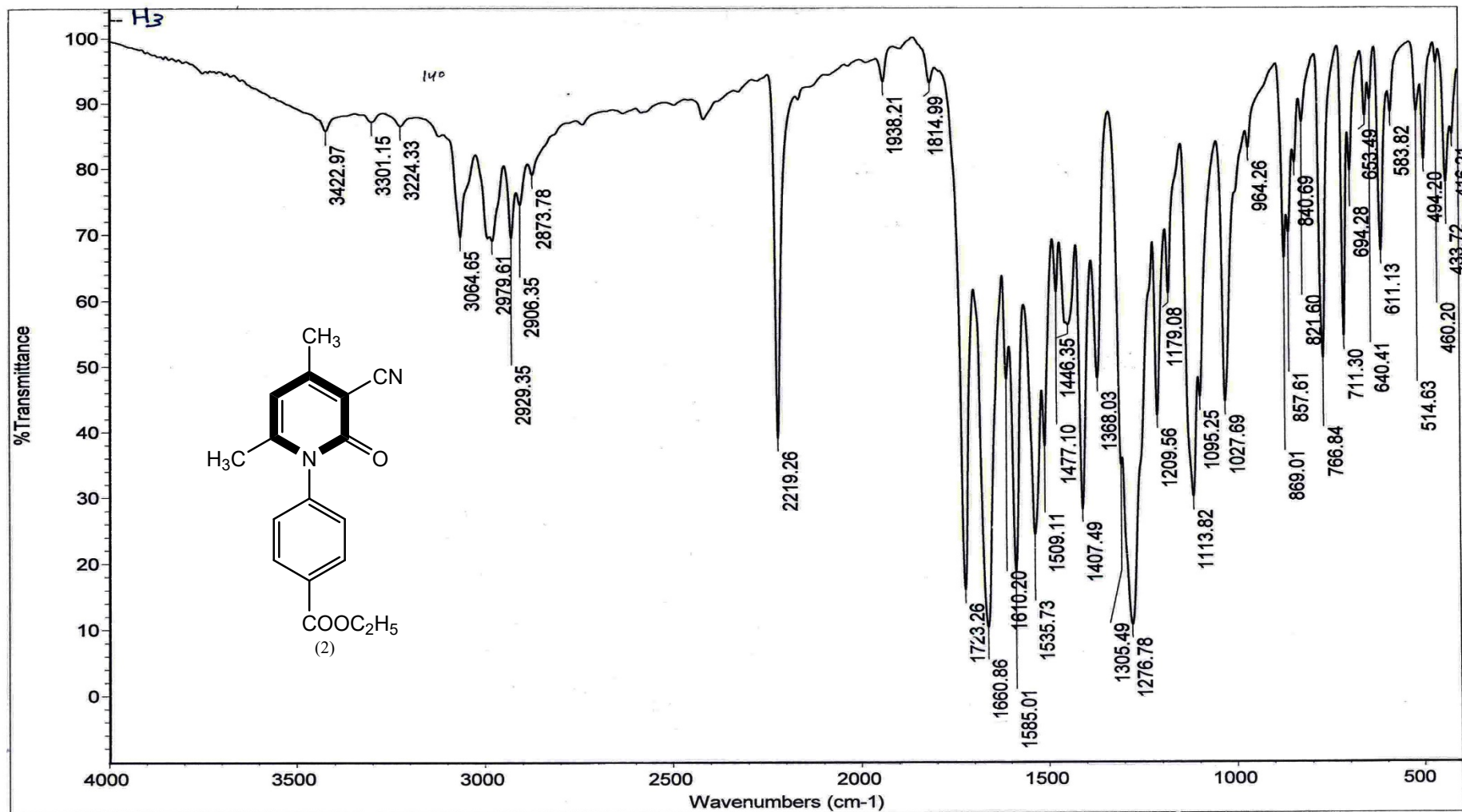


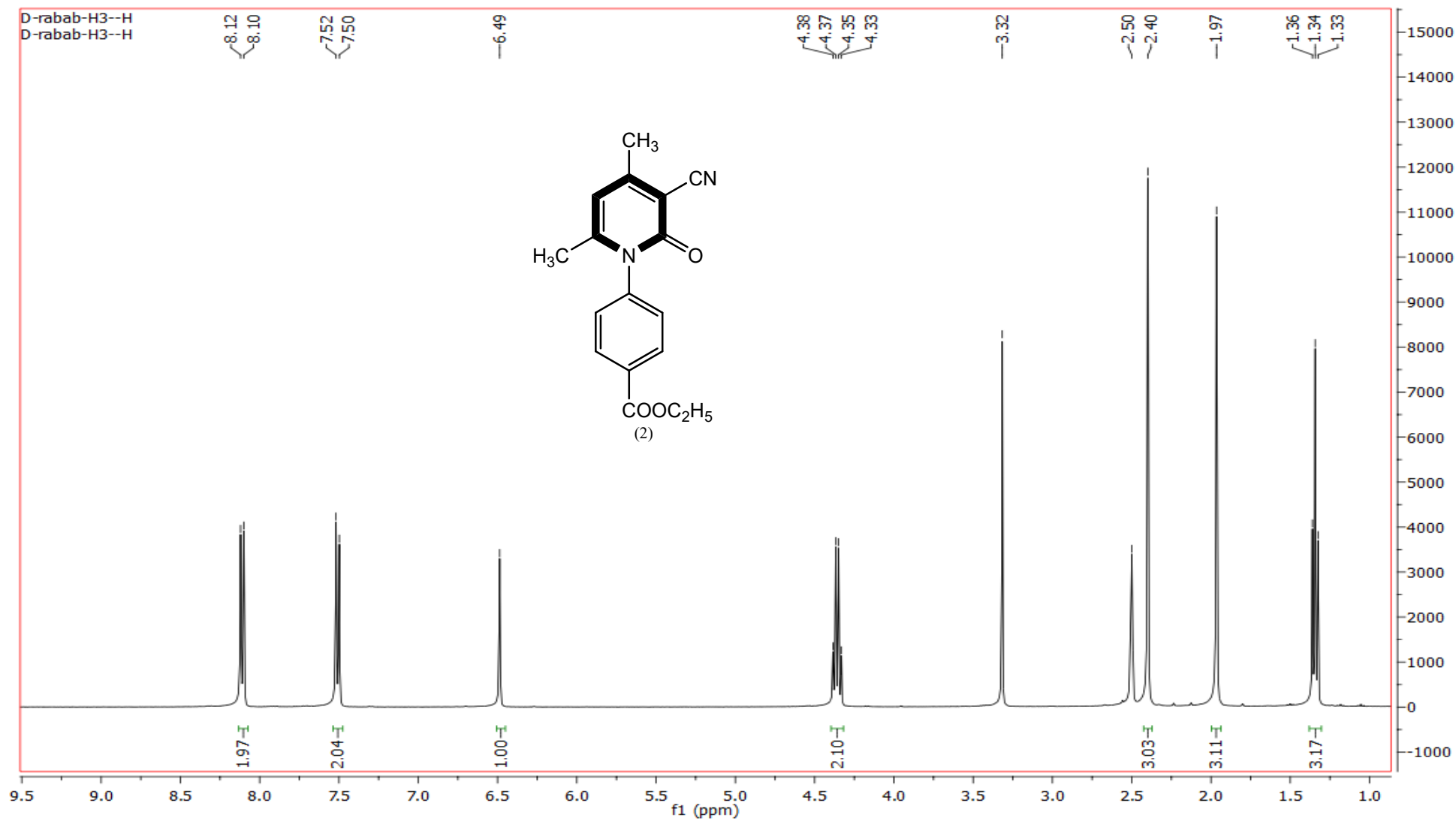
Evaluate the anti-proliferative activity of 2-oxo-pyridine and 1'*H*-spiro-pyridine derivatives as a new class of EGFR^{Wt} and VEGFR-2 inhibitors with apoptotic inducers

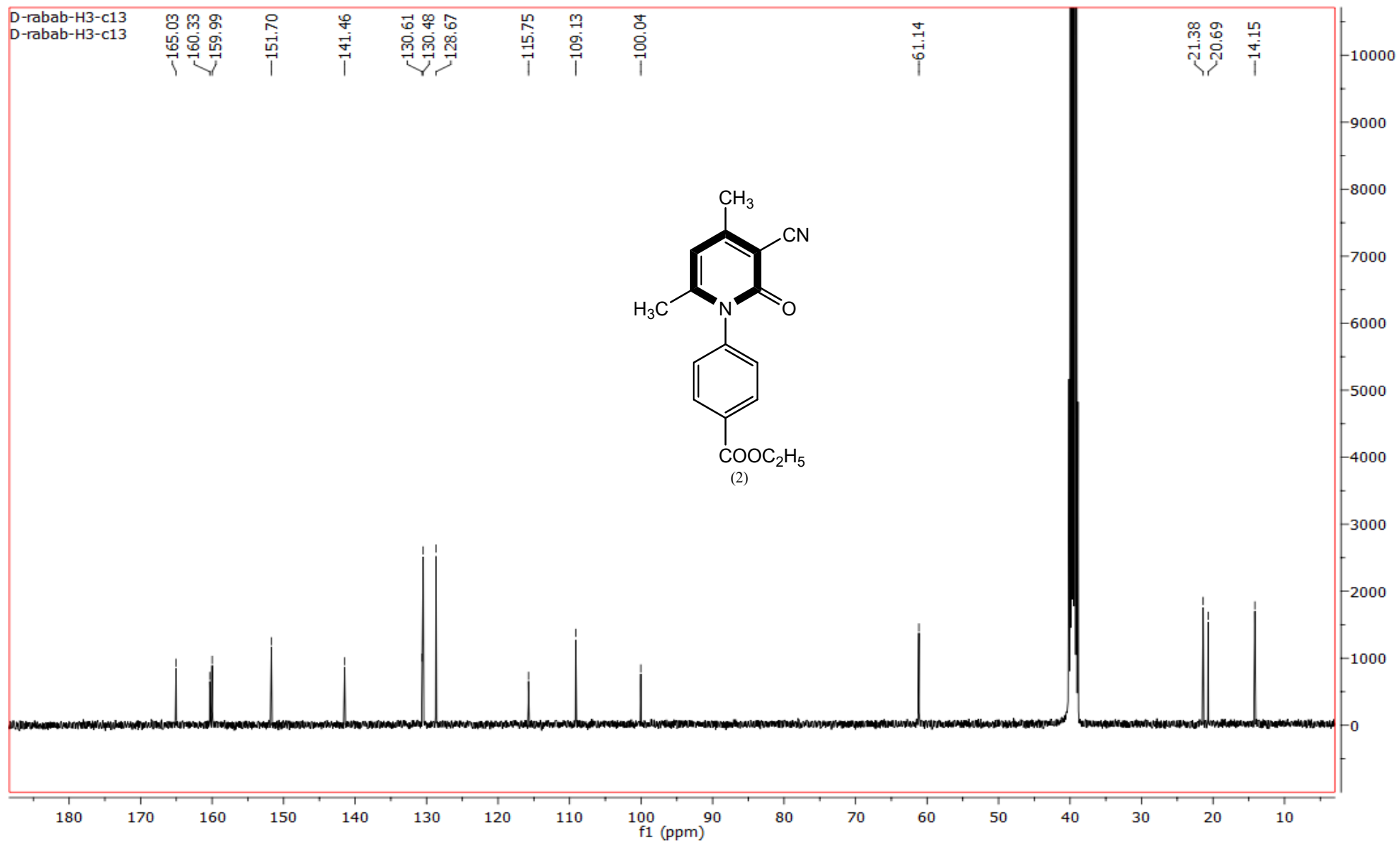
Reham R. Raslan¹, Yousry A. Ammar^{2,*}, Sawsan A. Fouad¹, Sadia A. Hessein¹, Nadia A. M. Shmiess¹, and Ahmed Ragab^{2,*}

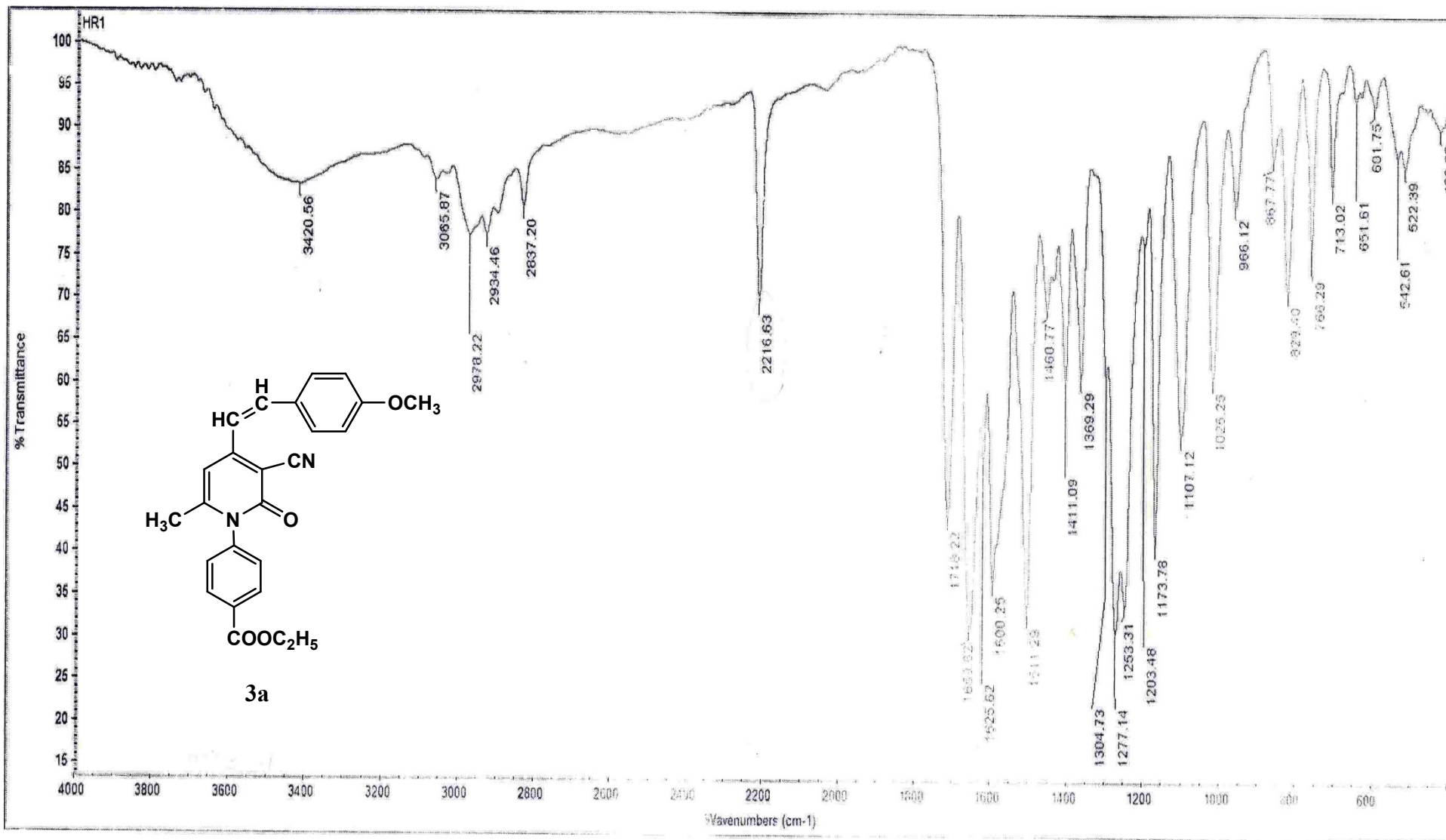
¹Department of Chemistry, Faculty of Science (Girls), Al-Azhar University, Nasr City, Cairo, Egypt.

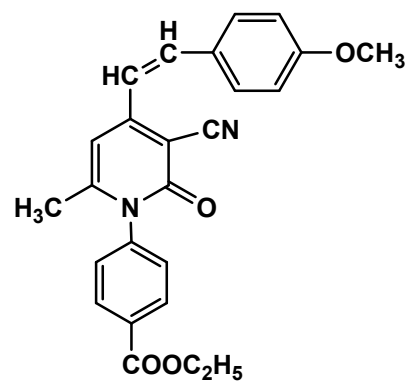
²Department of Chemistry, Faculty of Science (Boys), Al-Azhar University, Nasr City, 11884, Cairo, Egypt.



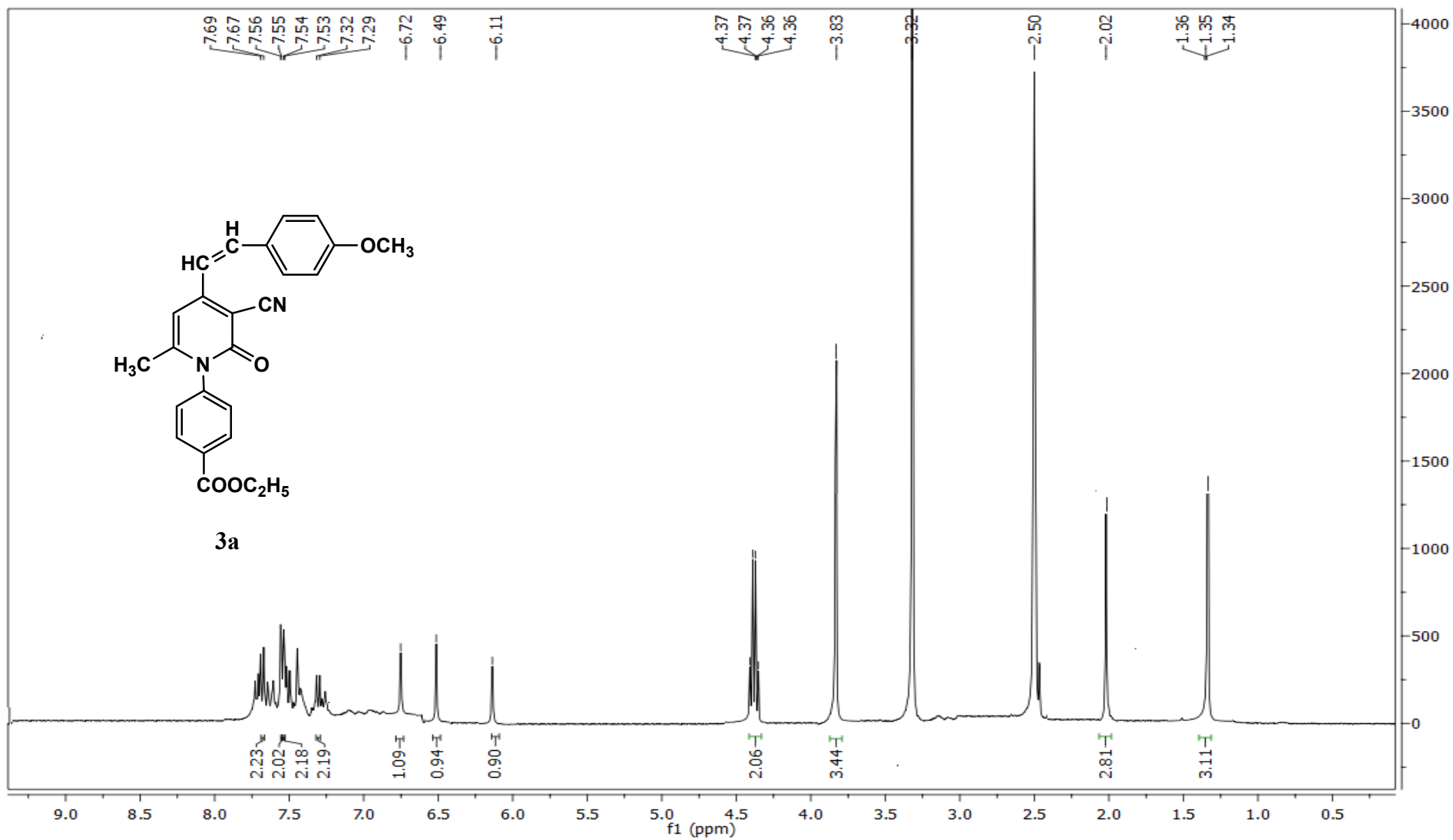


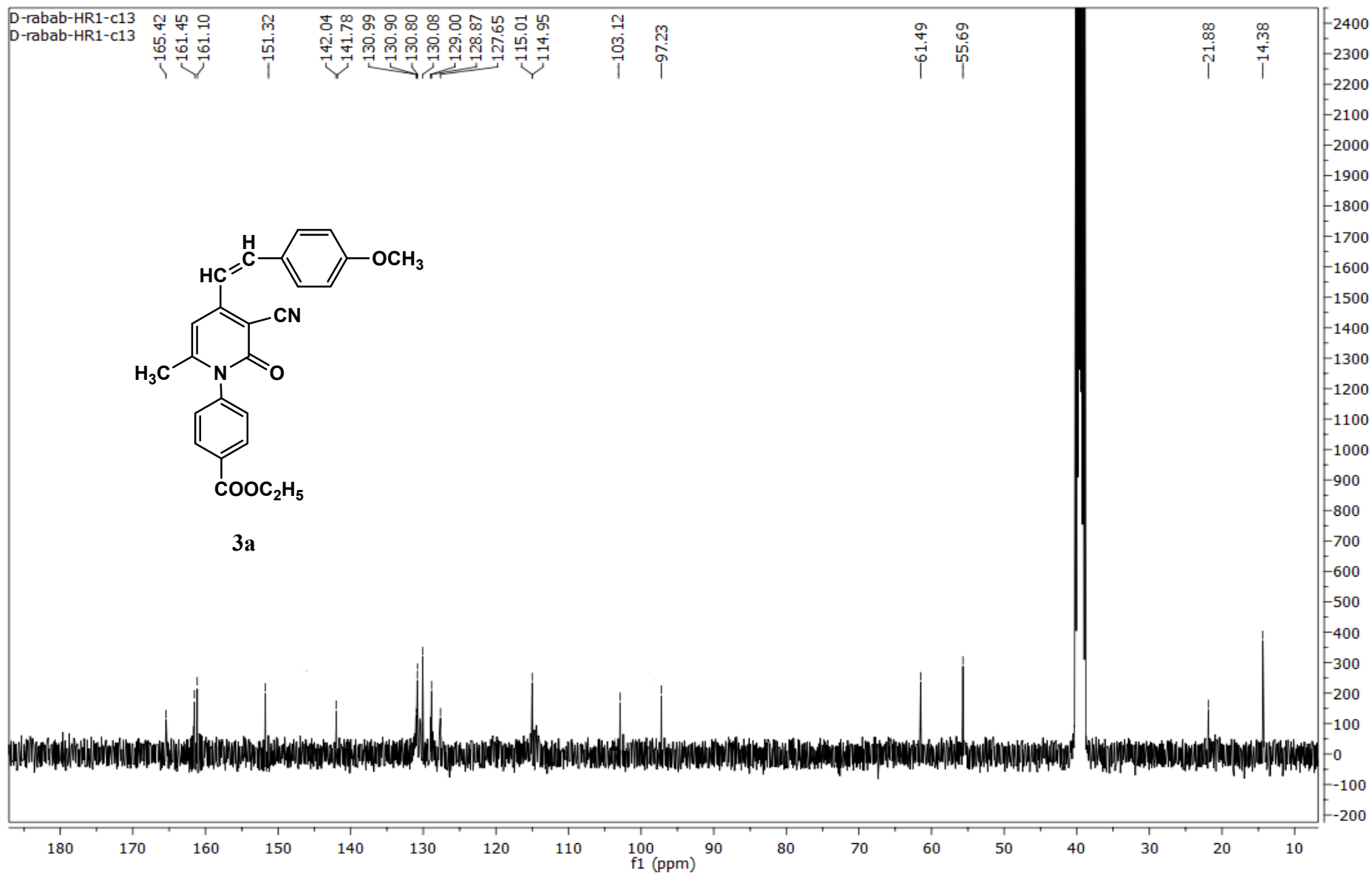


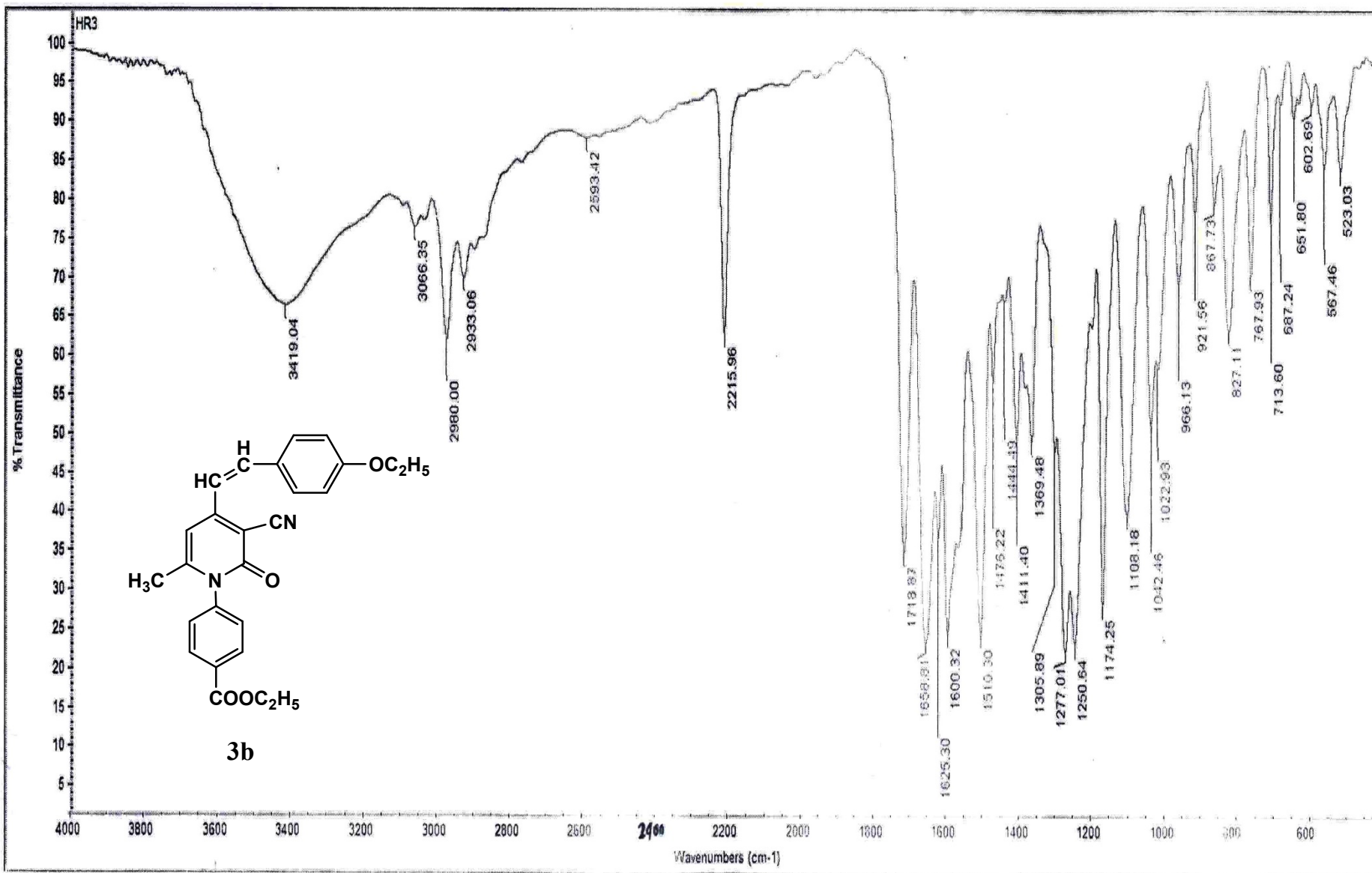


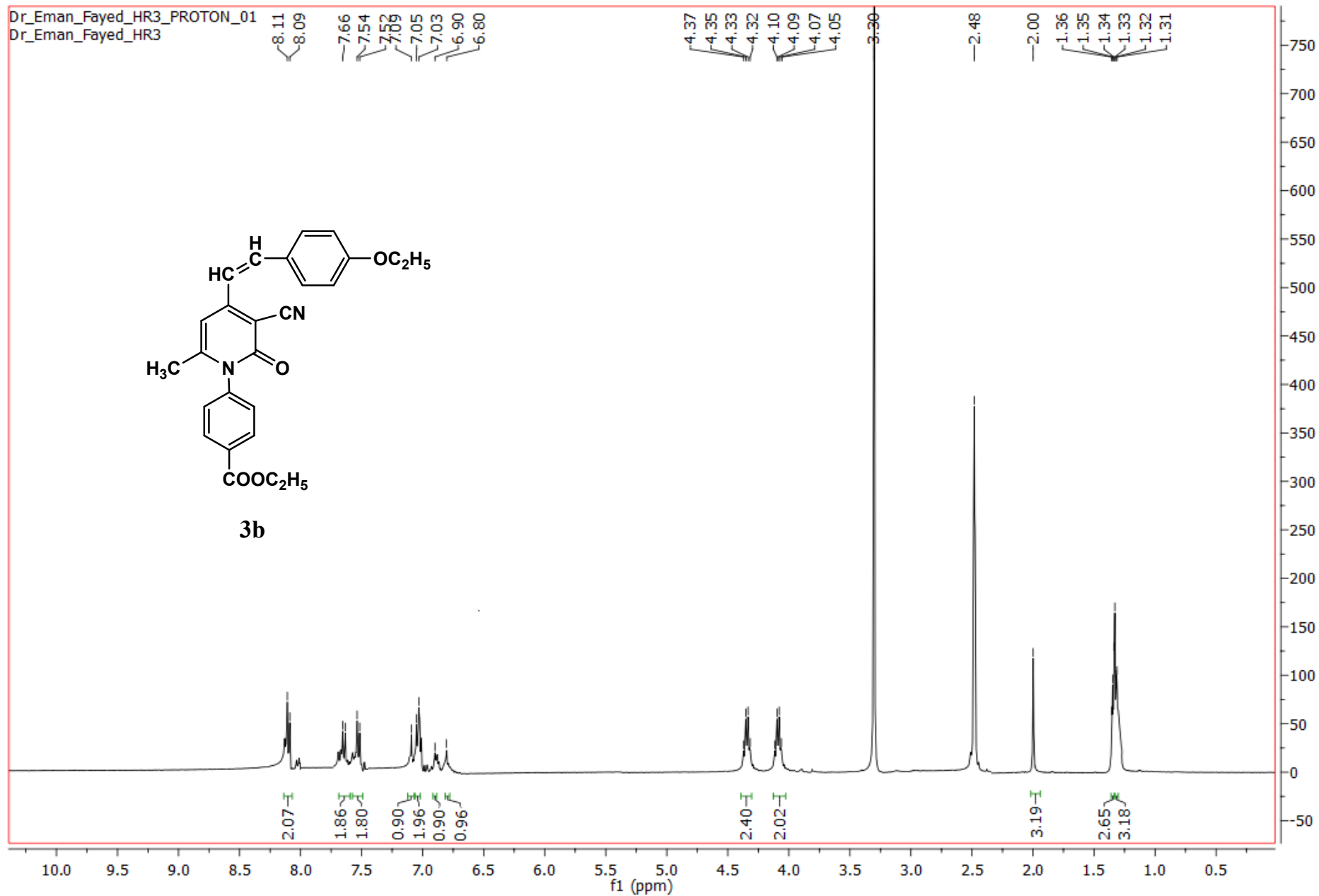


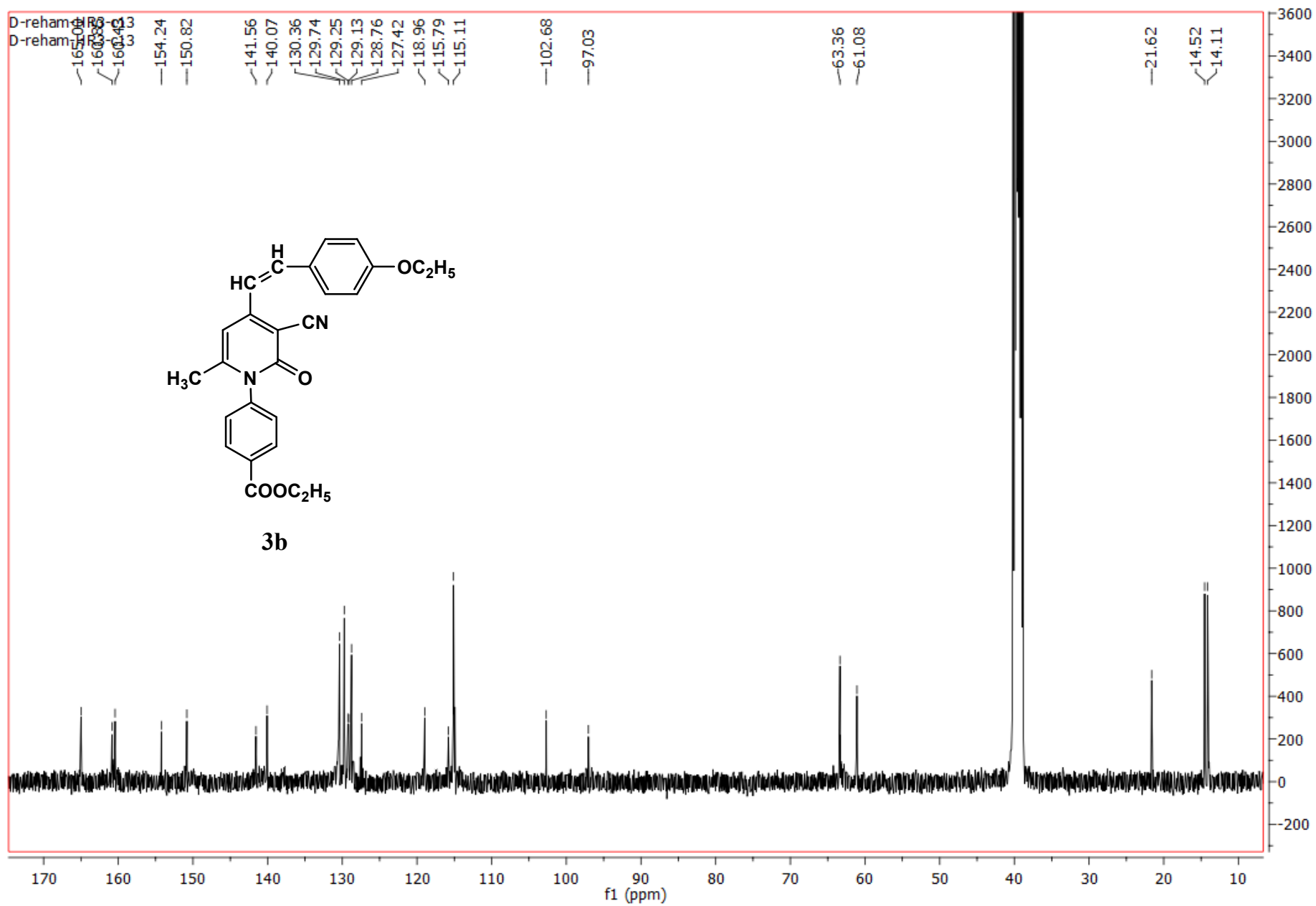
3a

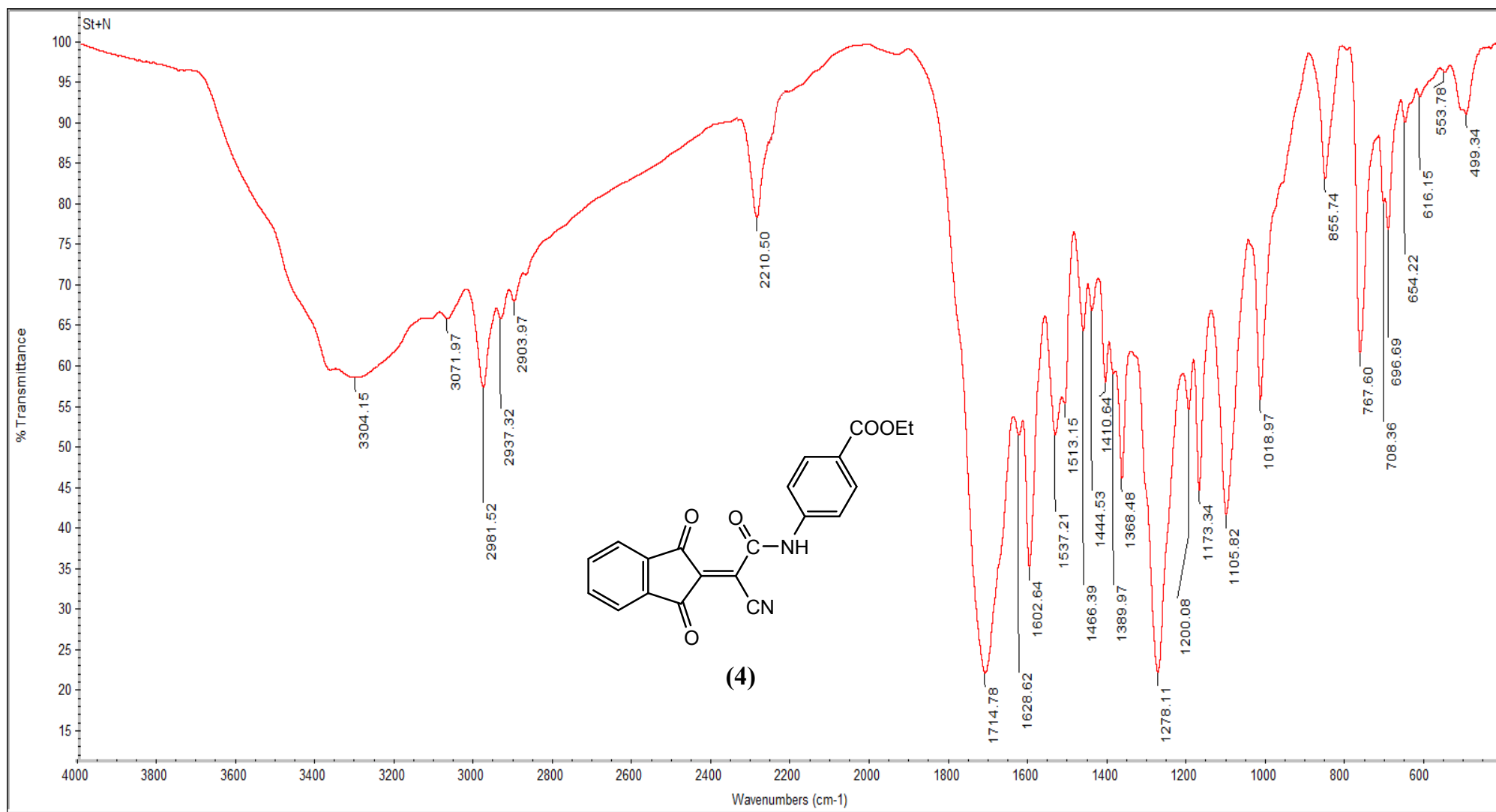


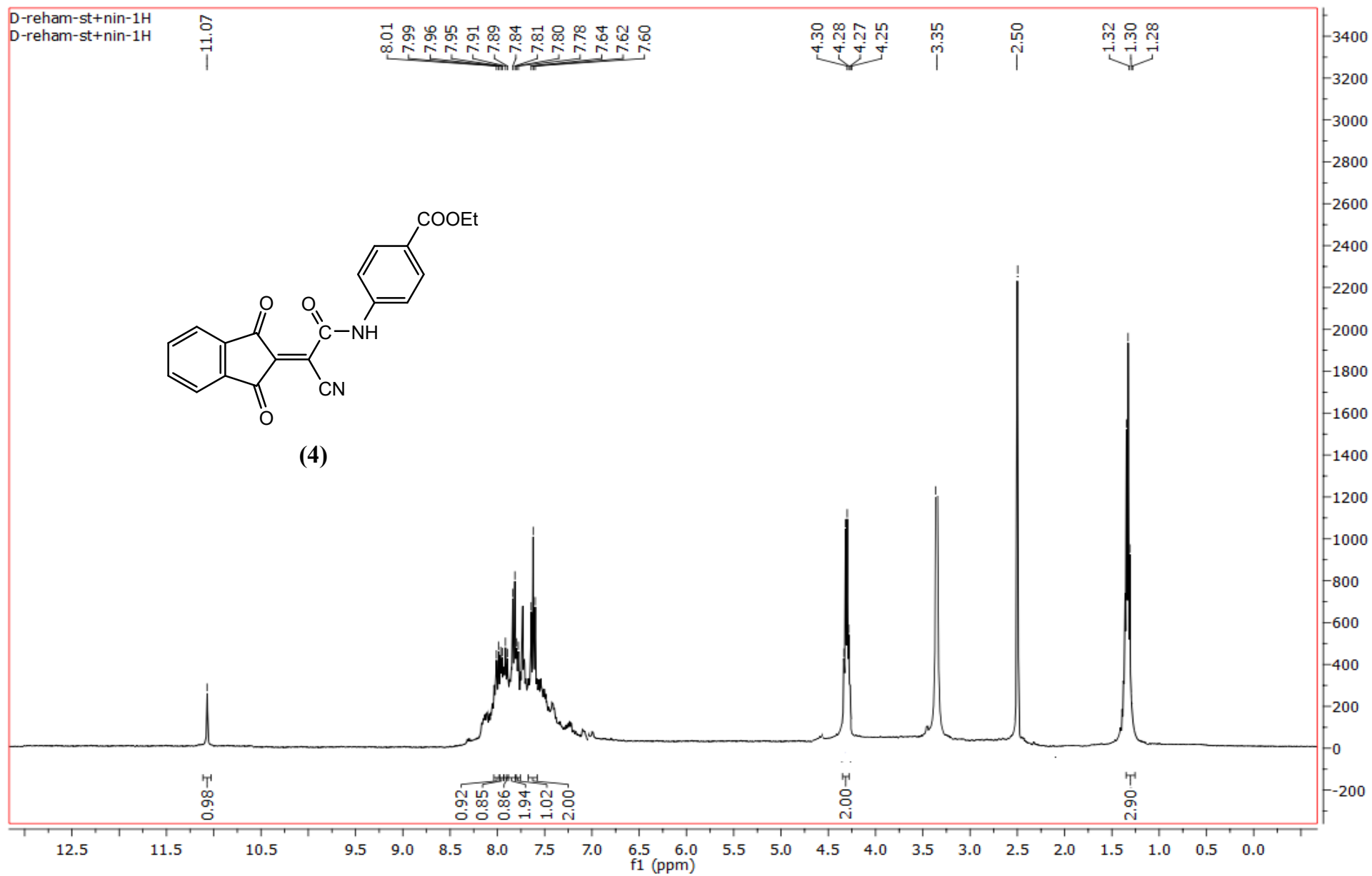


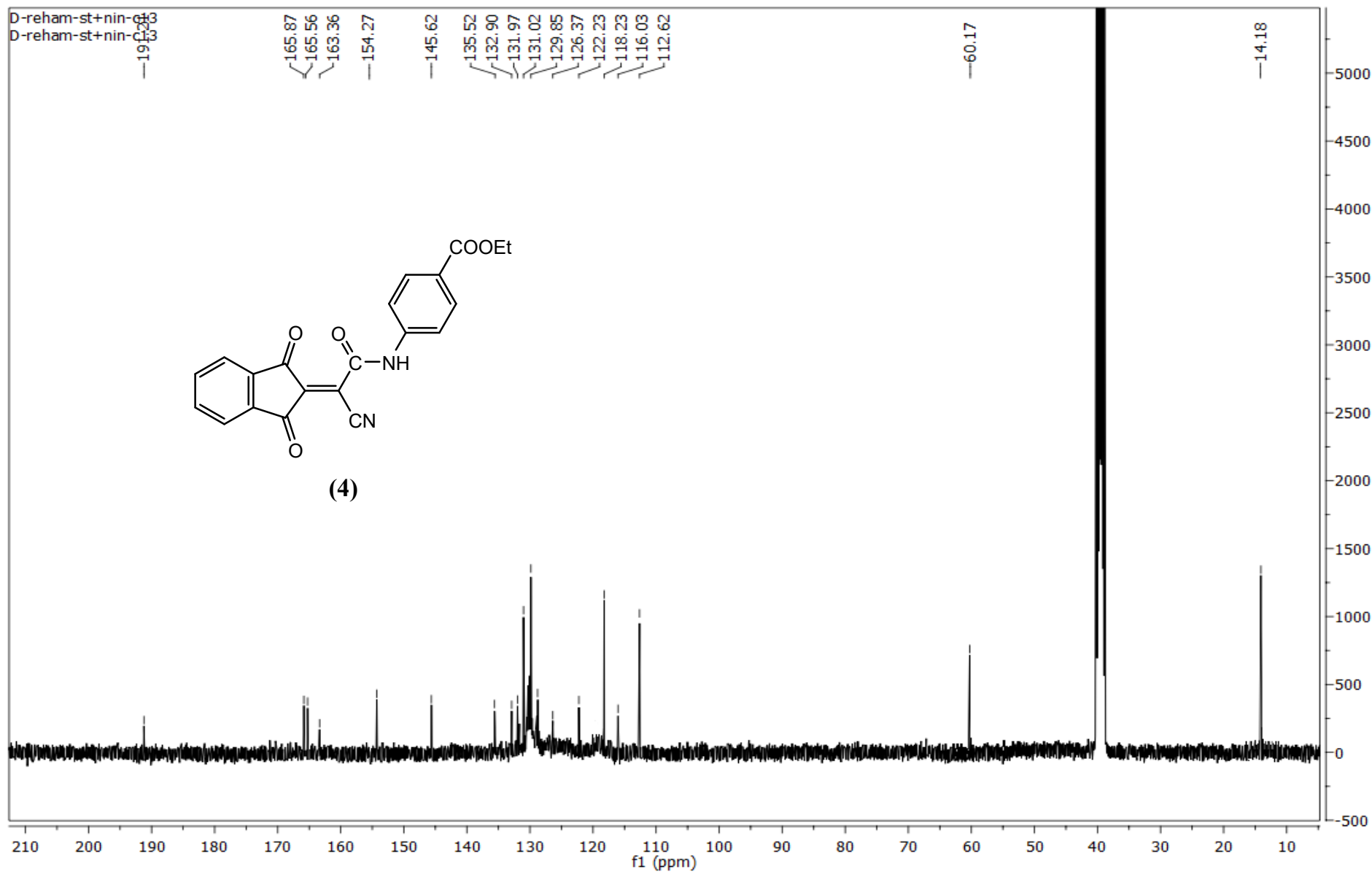


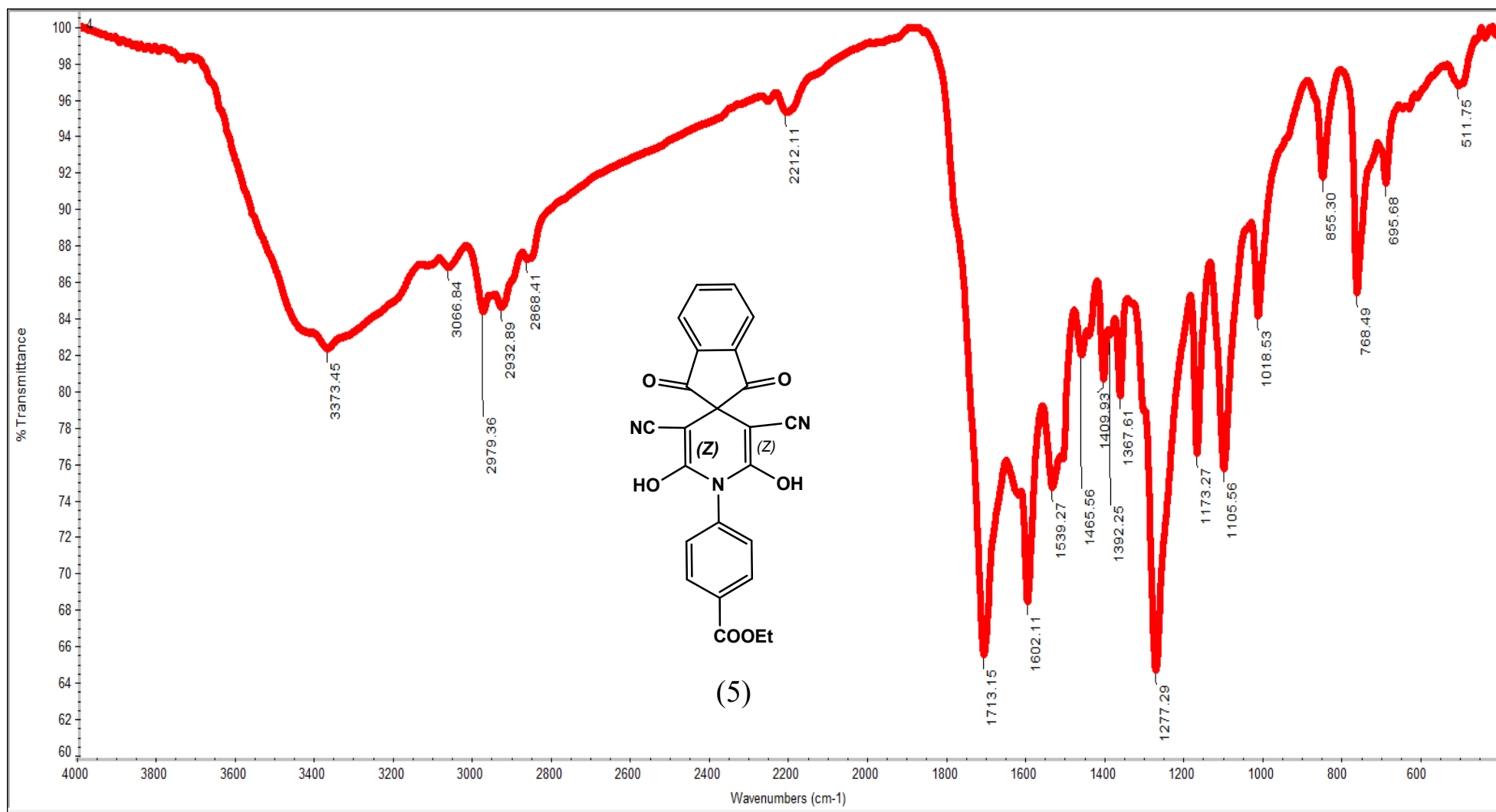




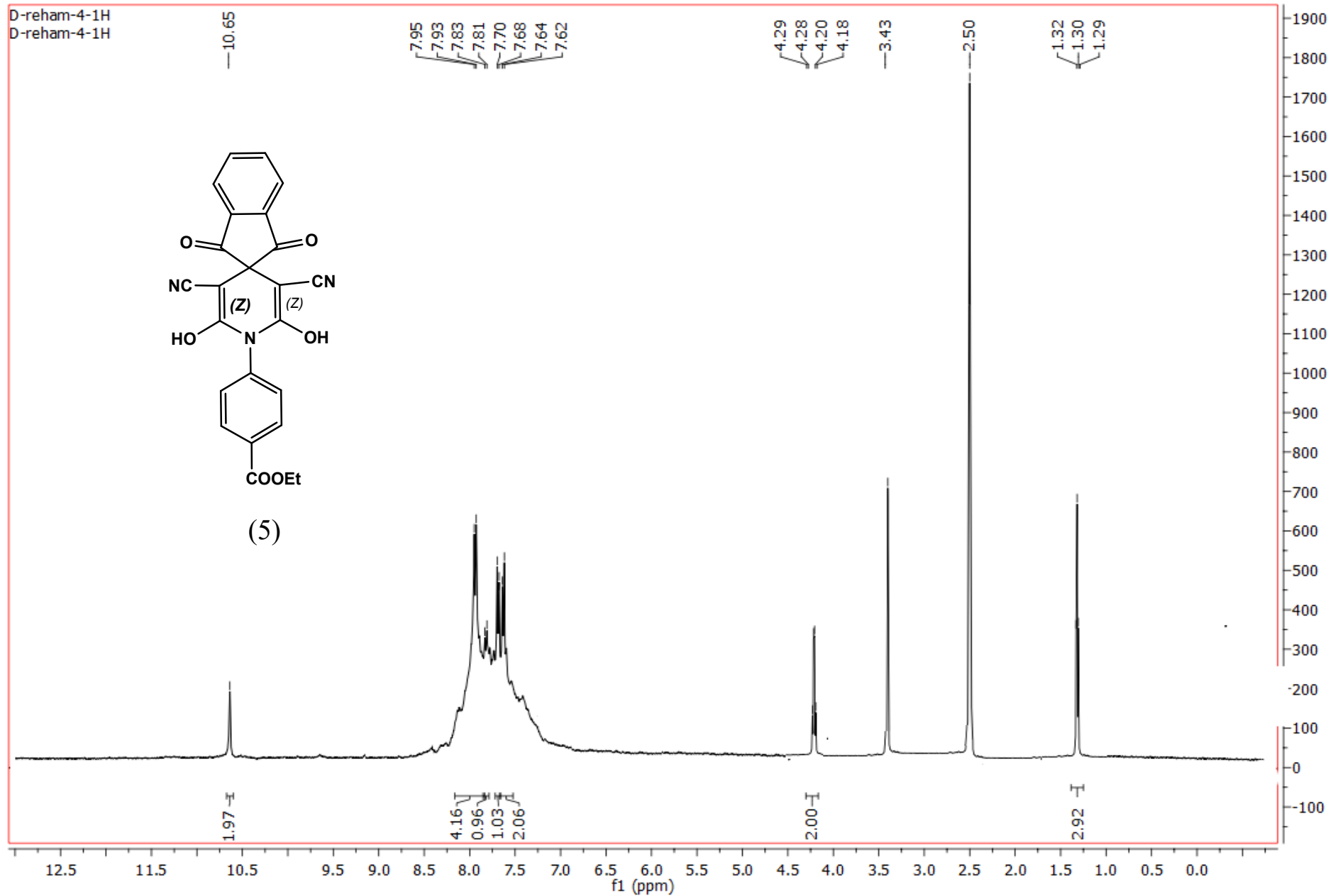
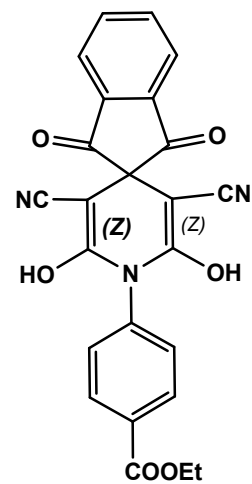




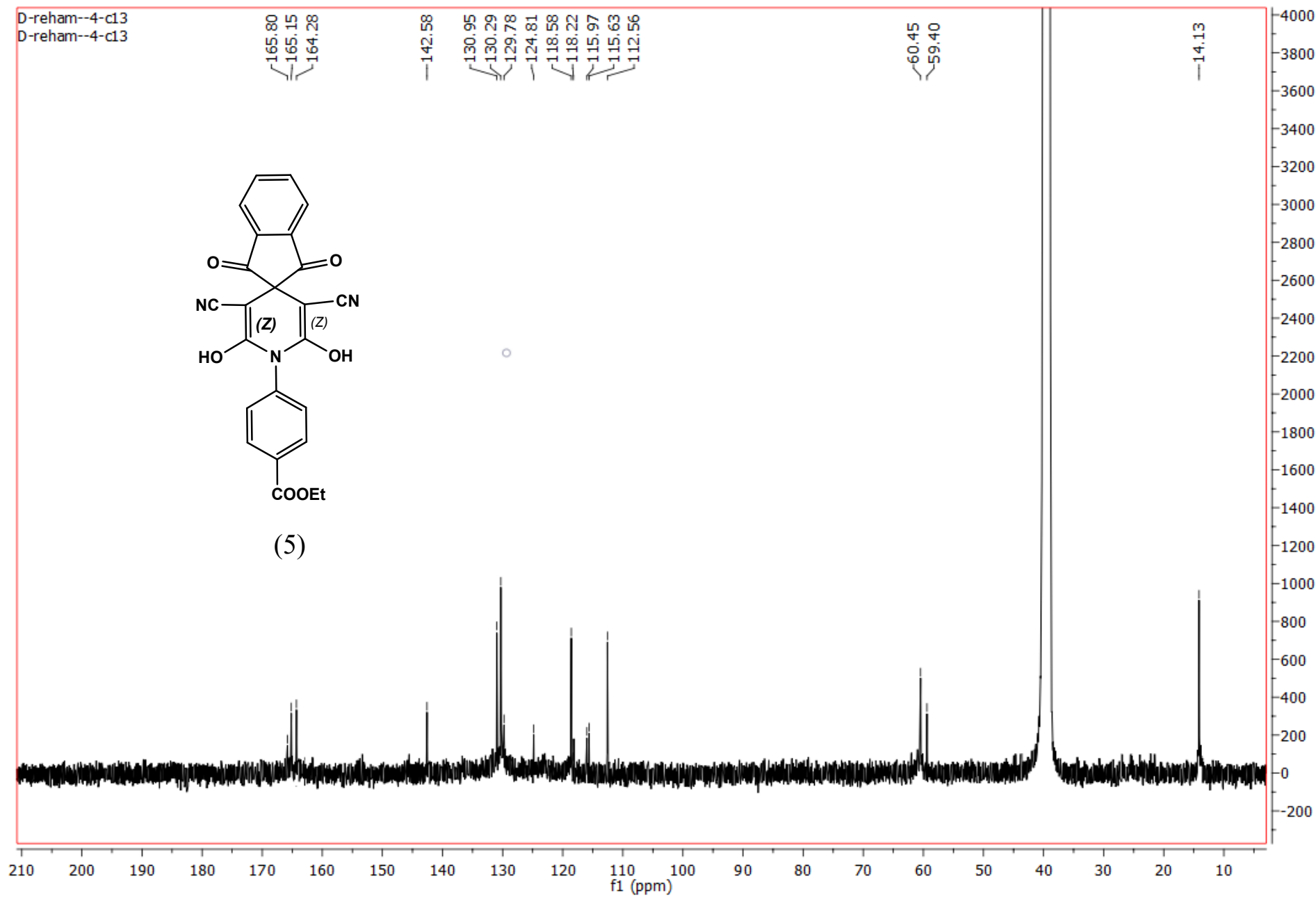
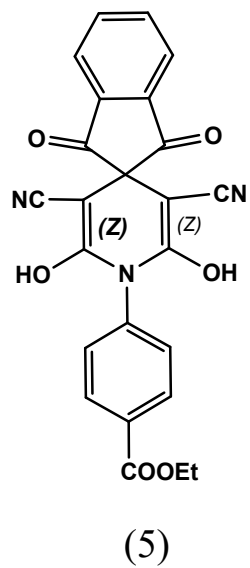


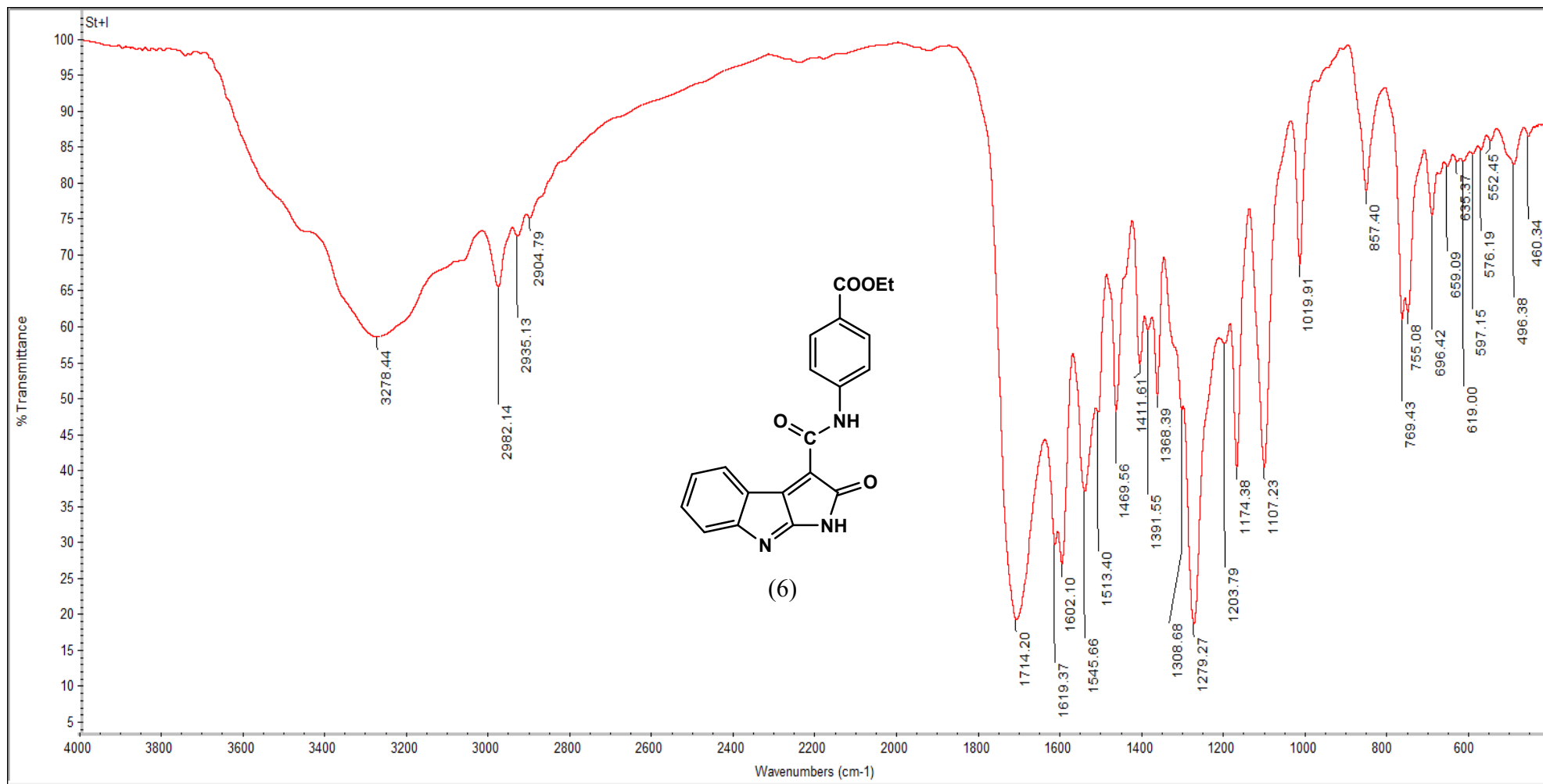


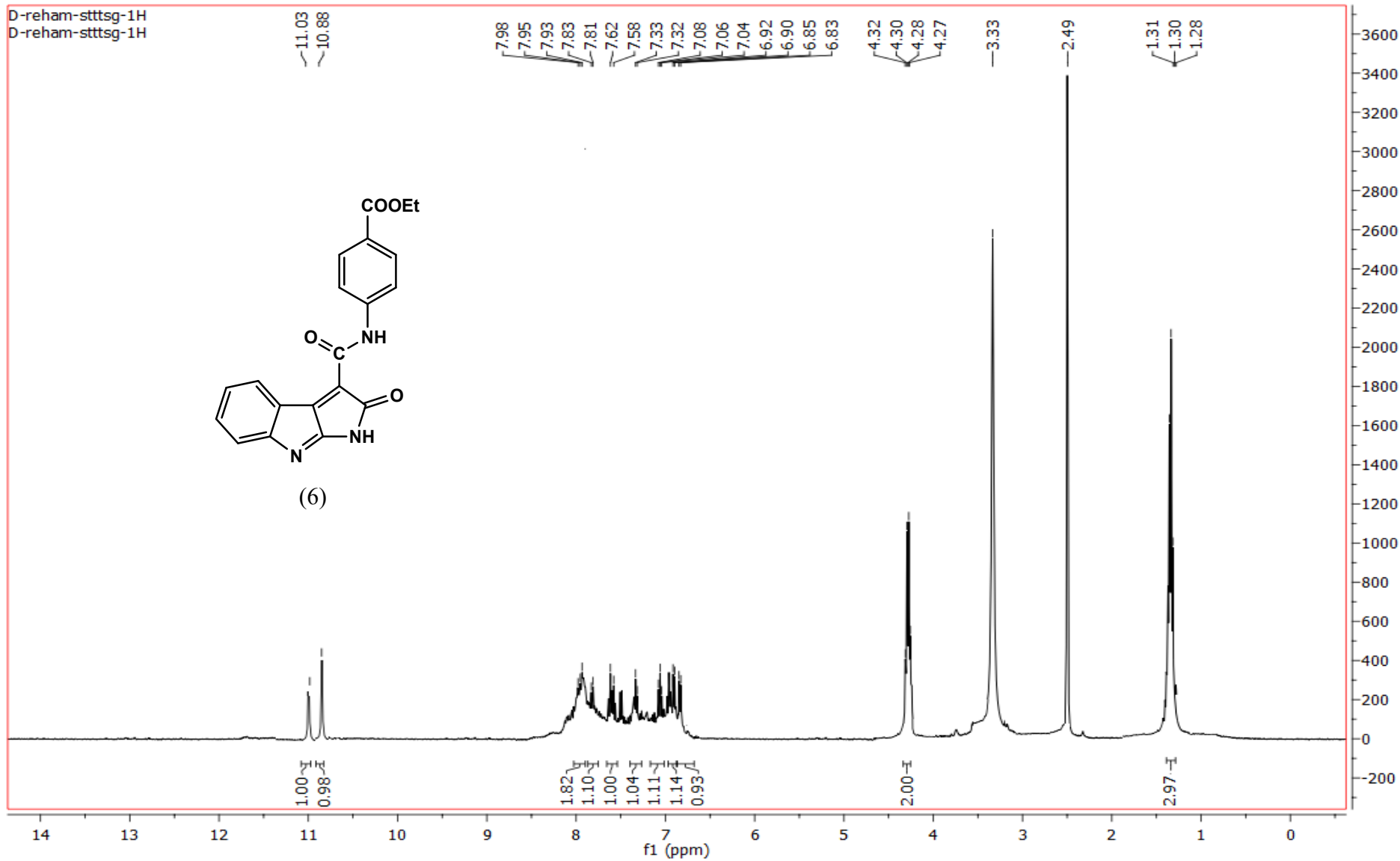
D-reham-4-1H
D-reham-4-1H

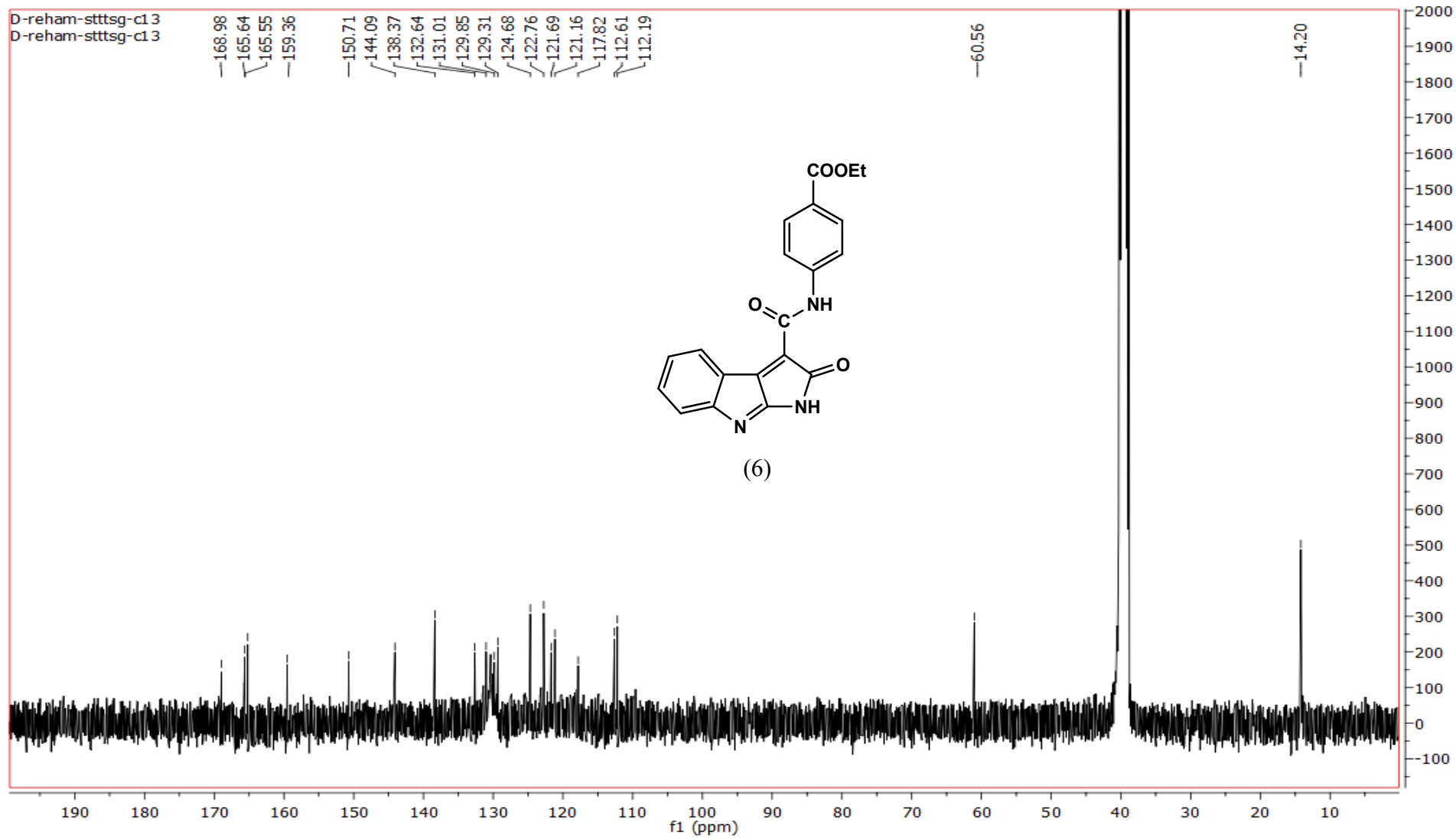


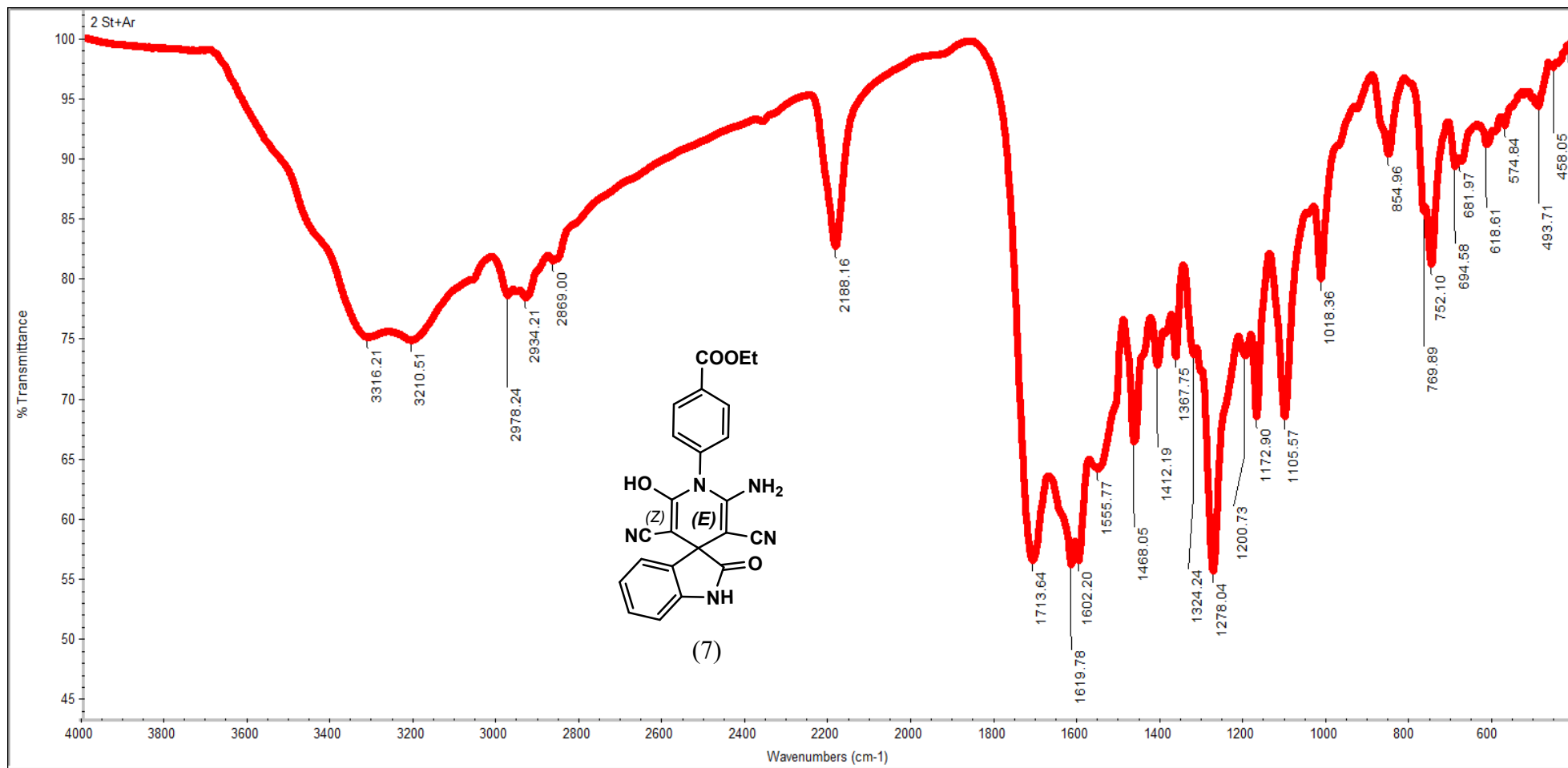
D-reham--4-cl3
D-reham--4-cl3



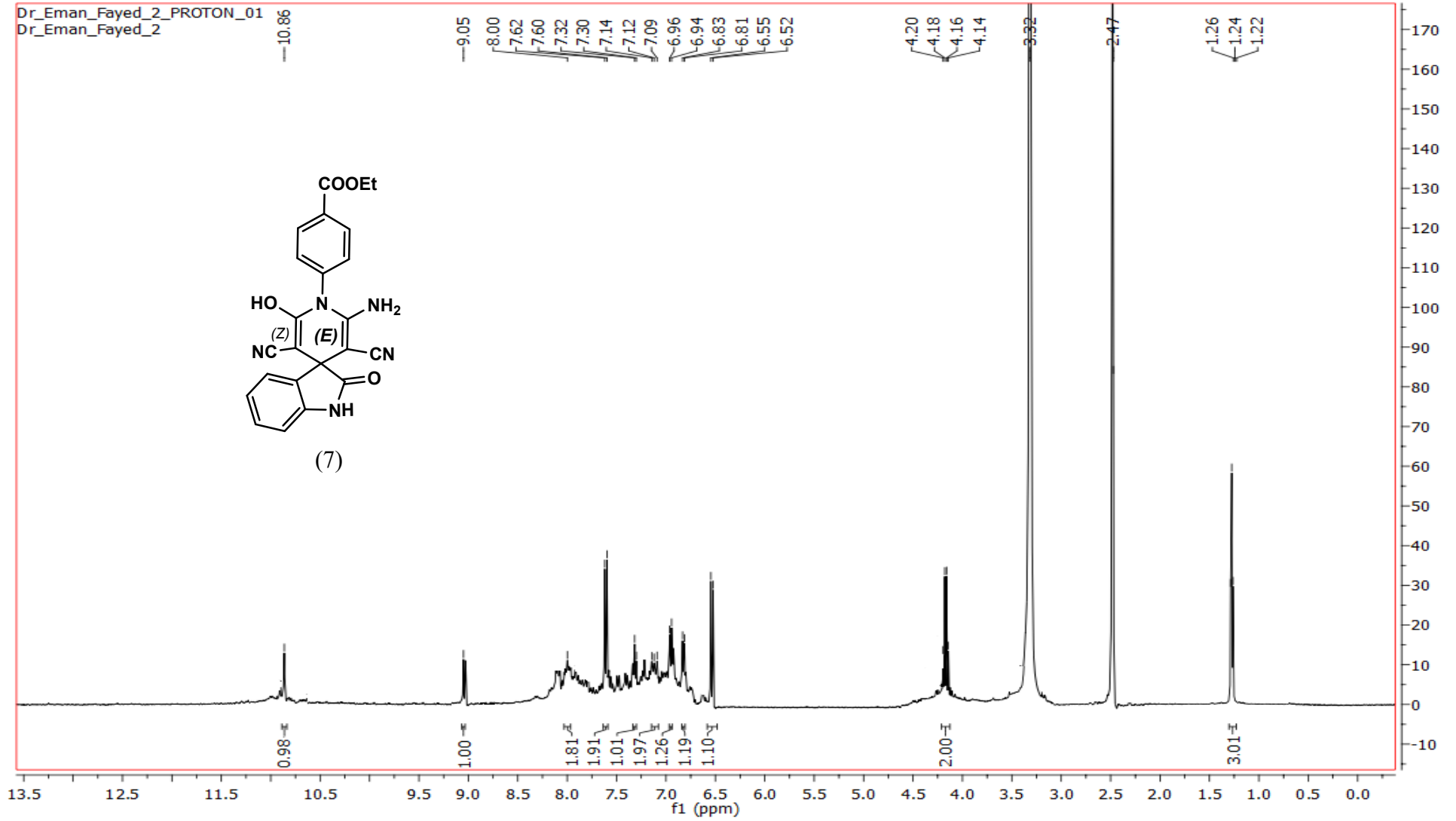
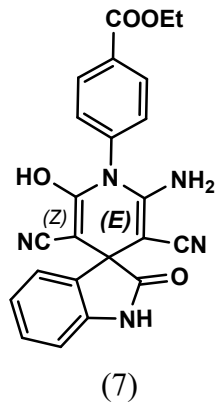




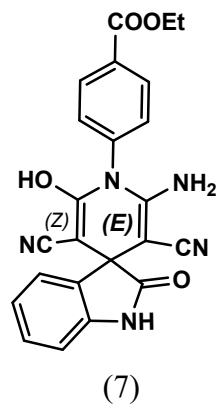




Dr_Eman_Fayed_2_PROTON_01
Dr_Eman_Fayed_2



D-reham-2-c13
D-reham-2-c13



169.47
166.35
165.62
153.90
144.57
133.82
133.11
131.49
129.79
125.49
122.18
121.63
118.71
116.51
115.77
113.10
110.02
103.68

59.94

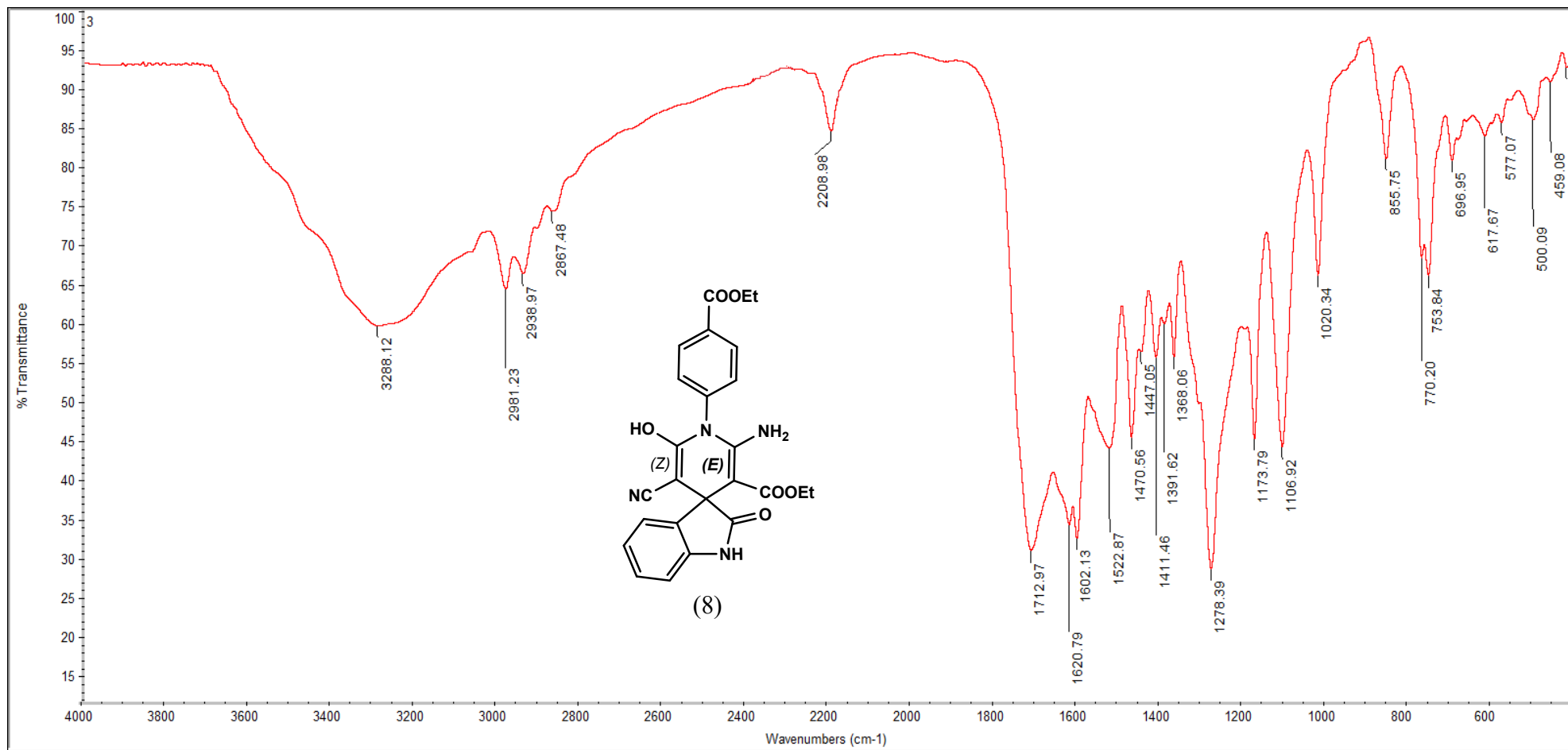
49.07

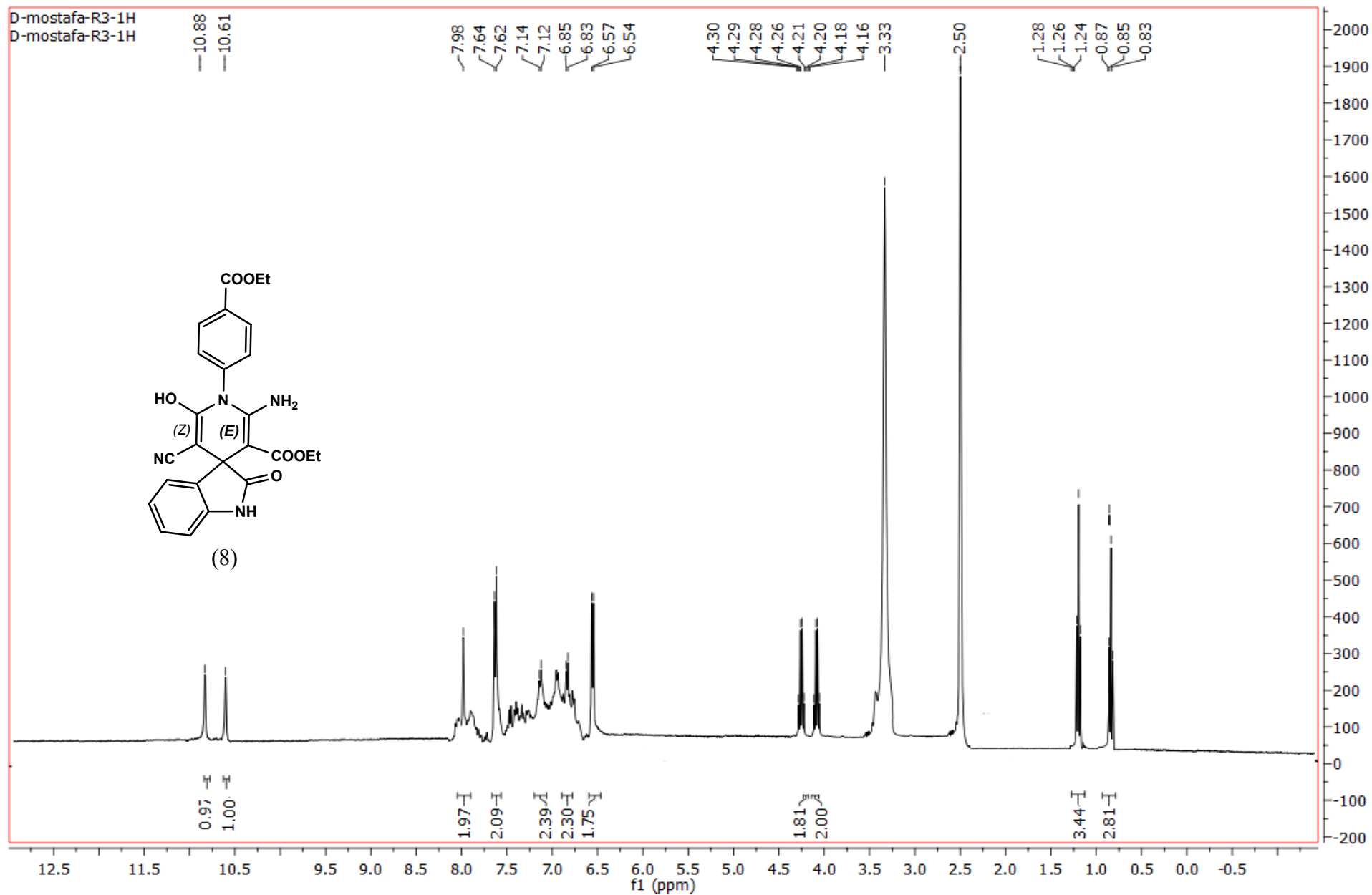
14.65

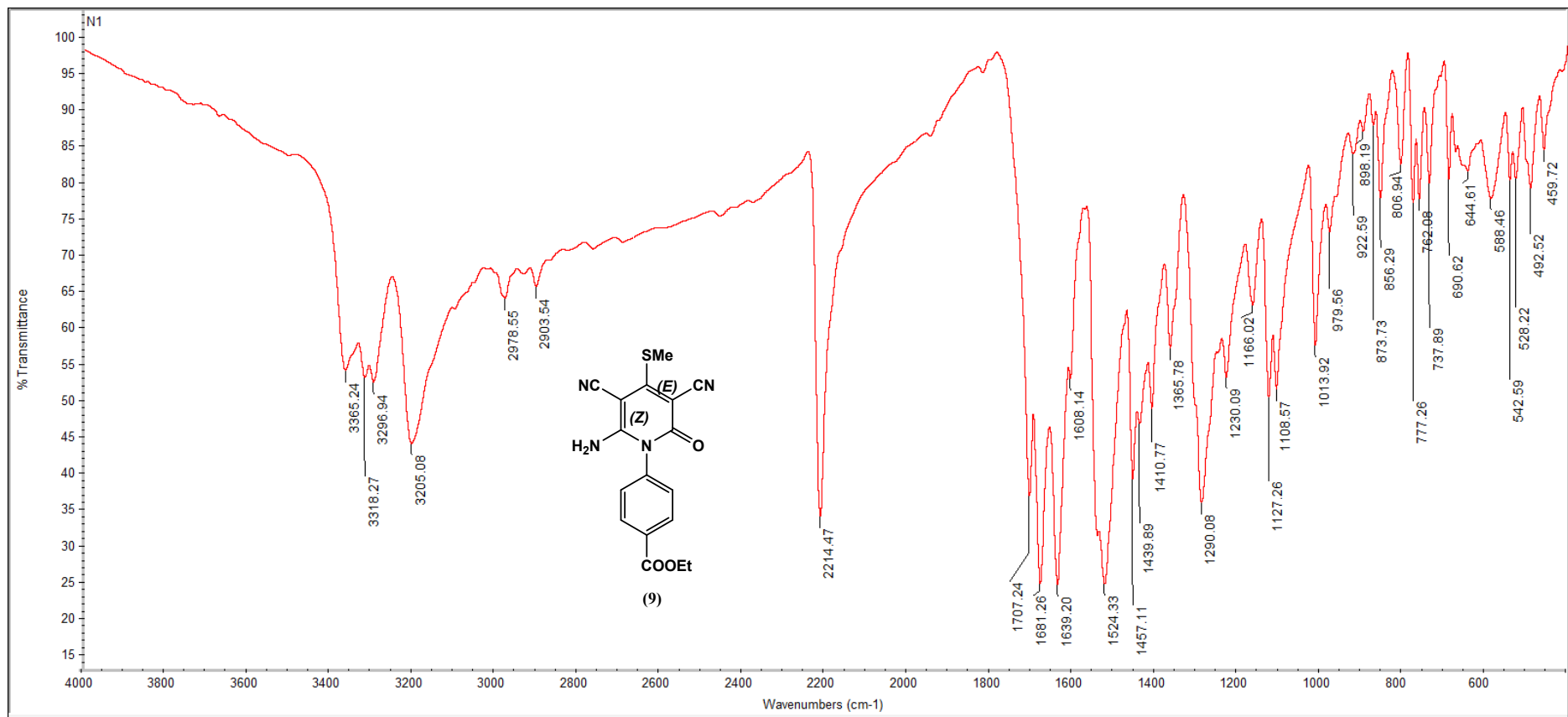
3500
3000
2500
2000
1500
1000
500
0

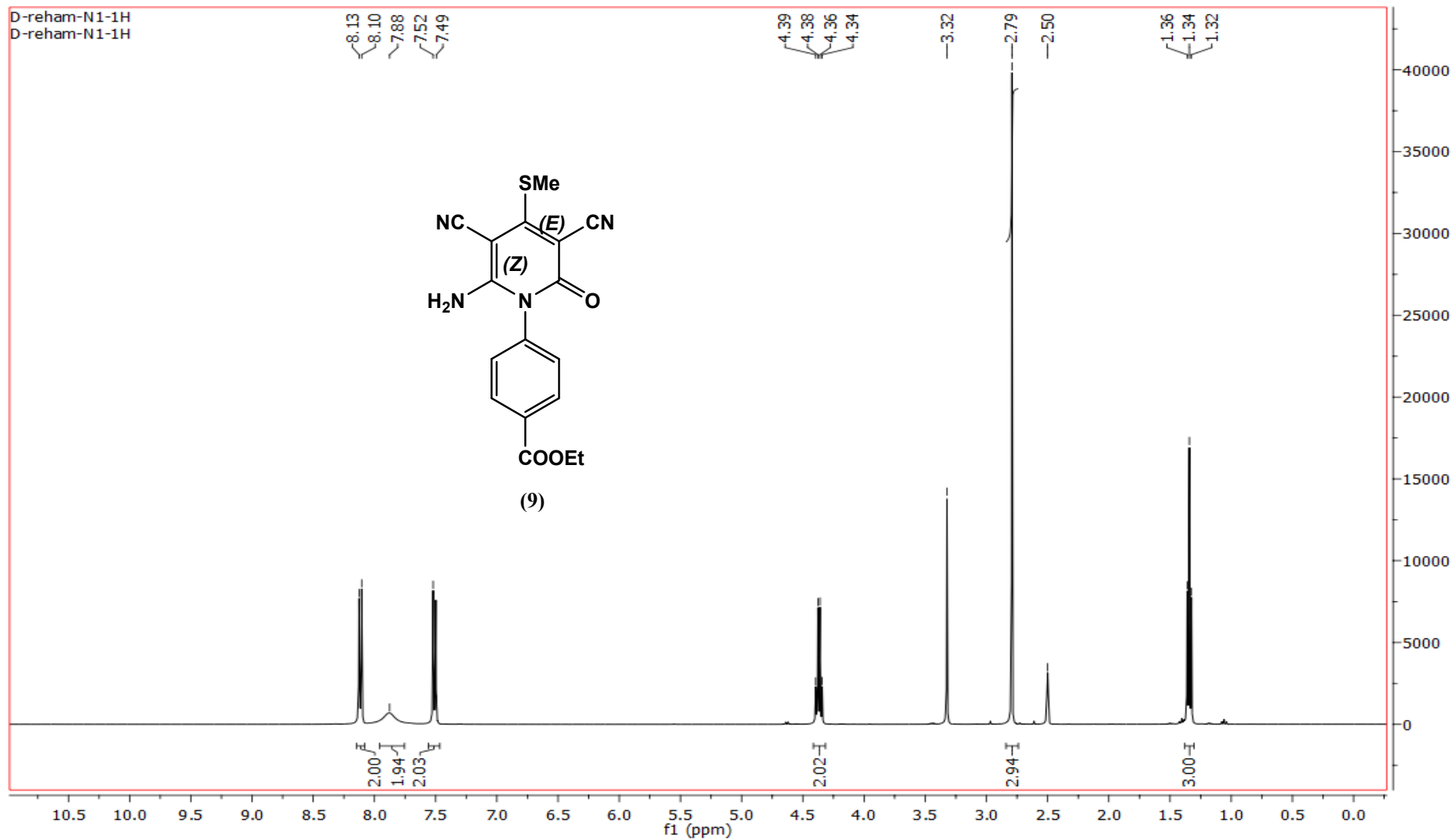
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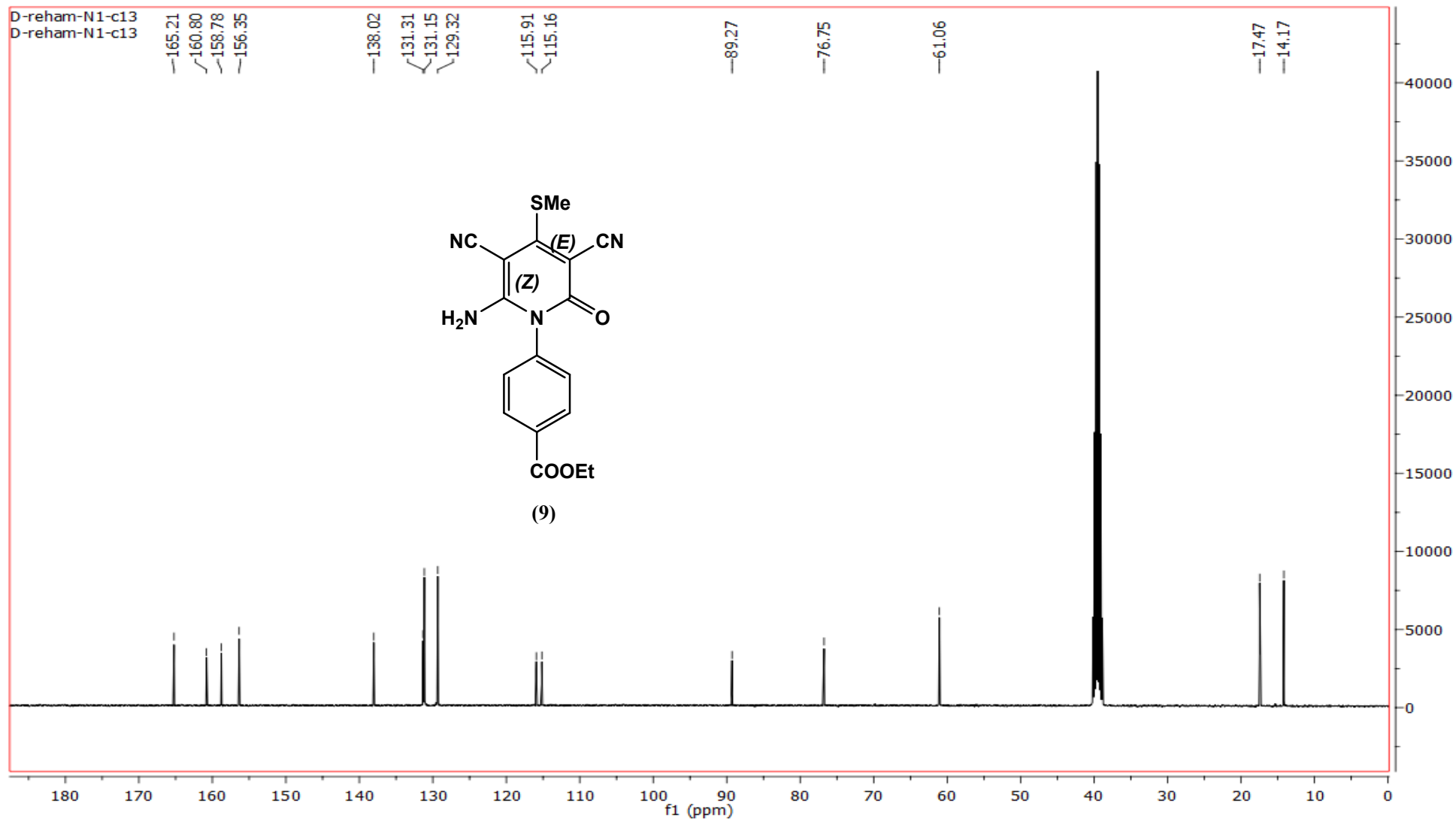
f1 (ppm)

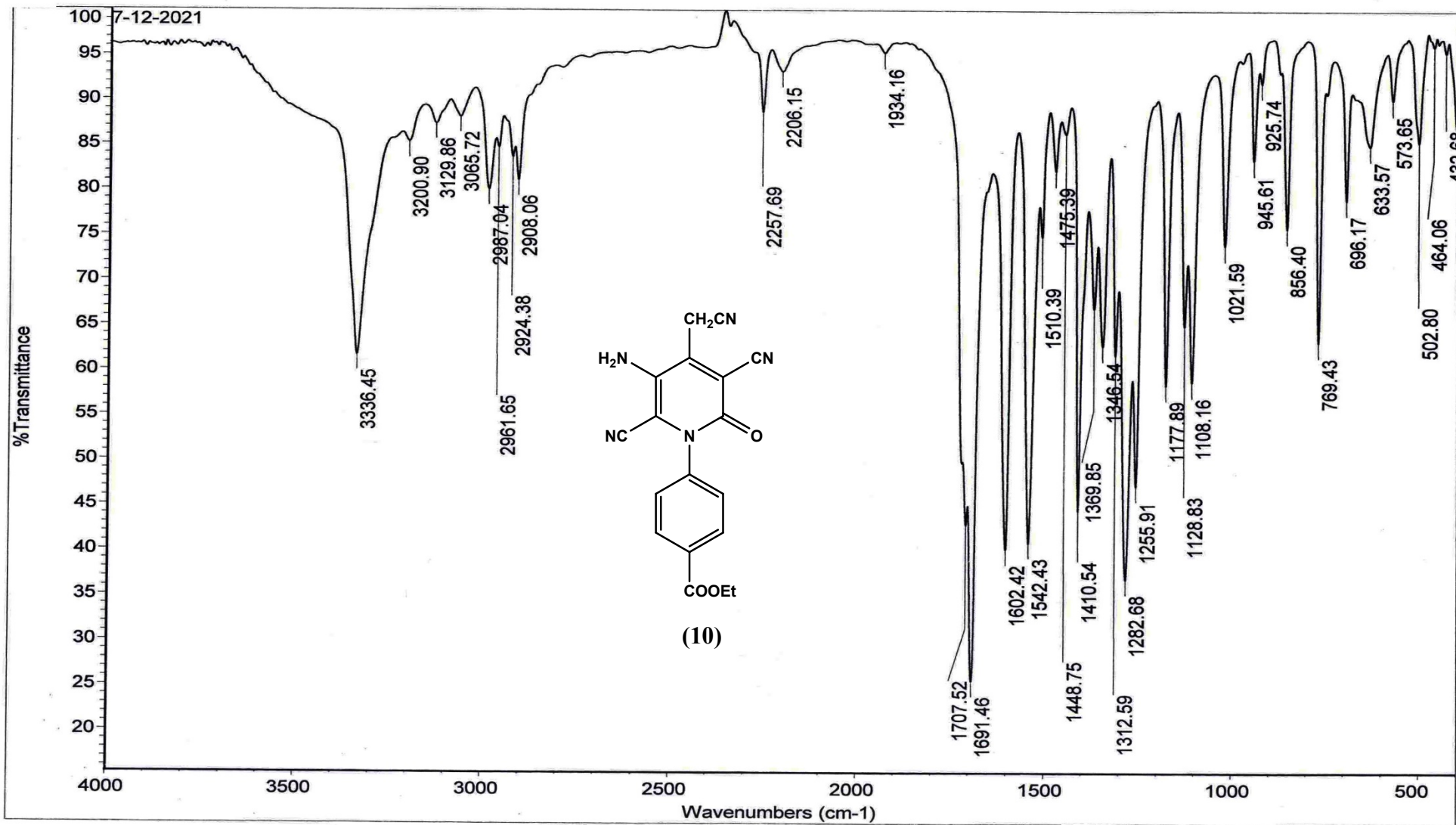




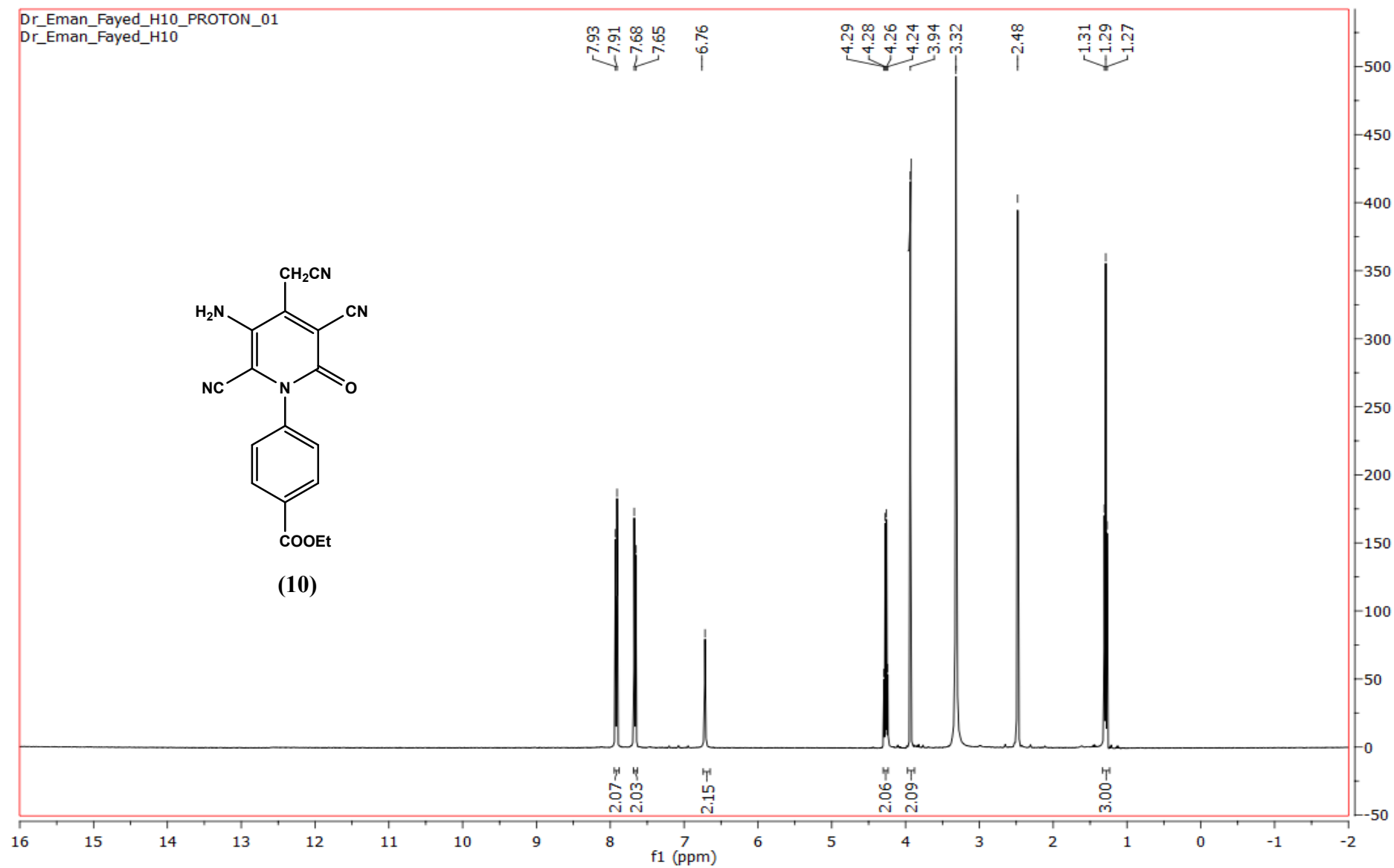
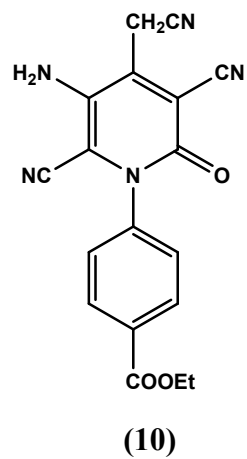


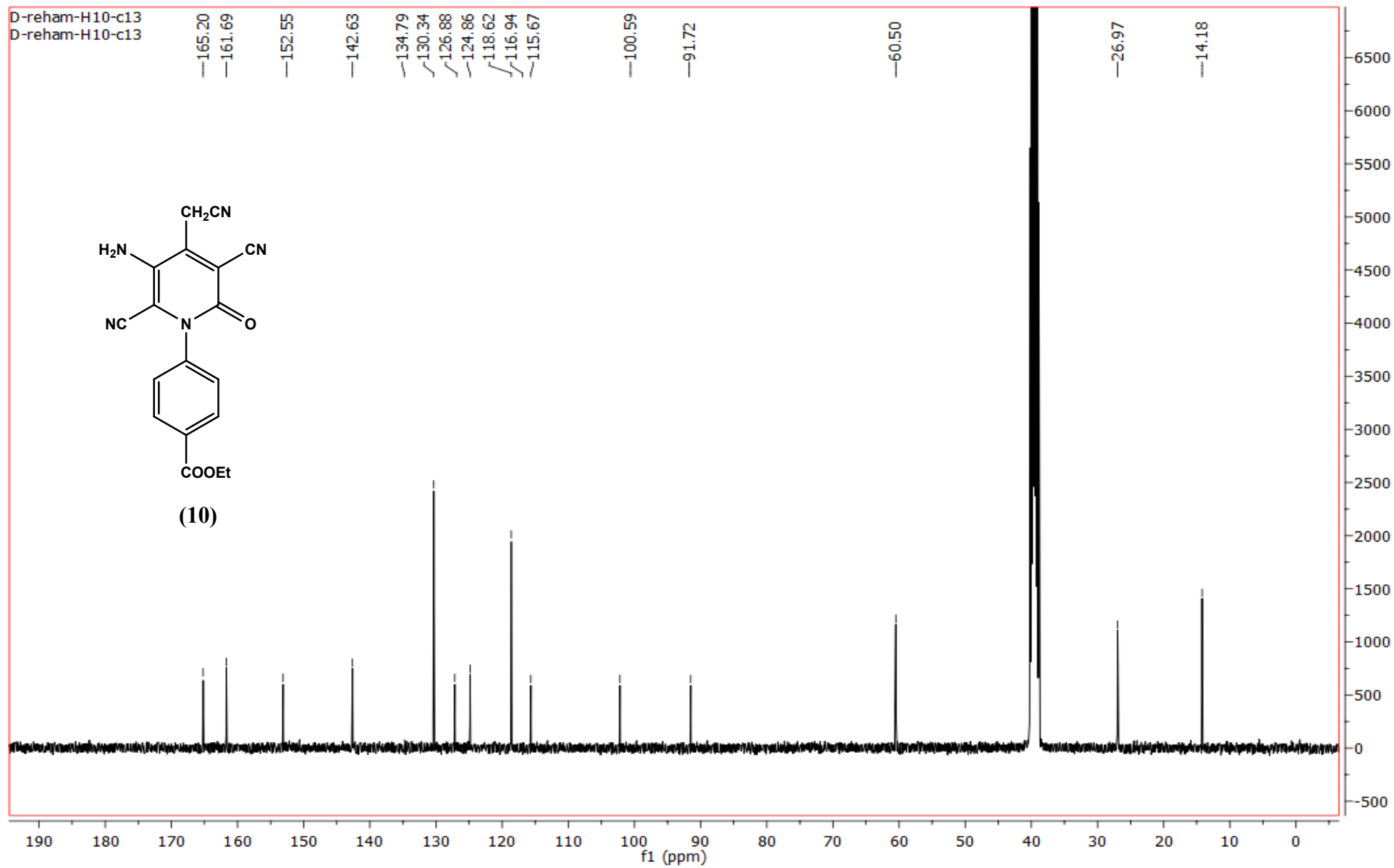


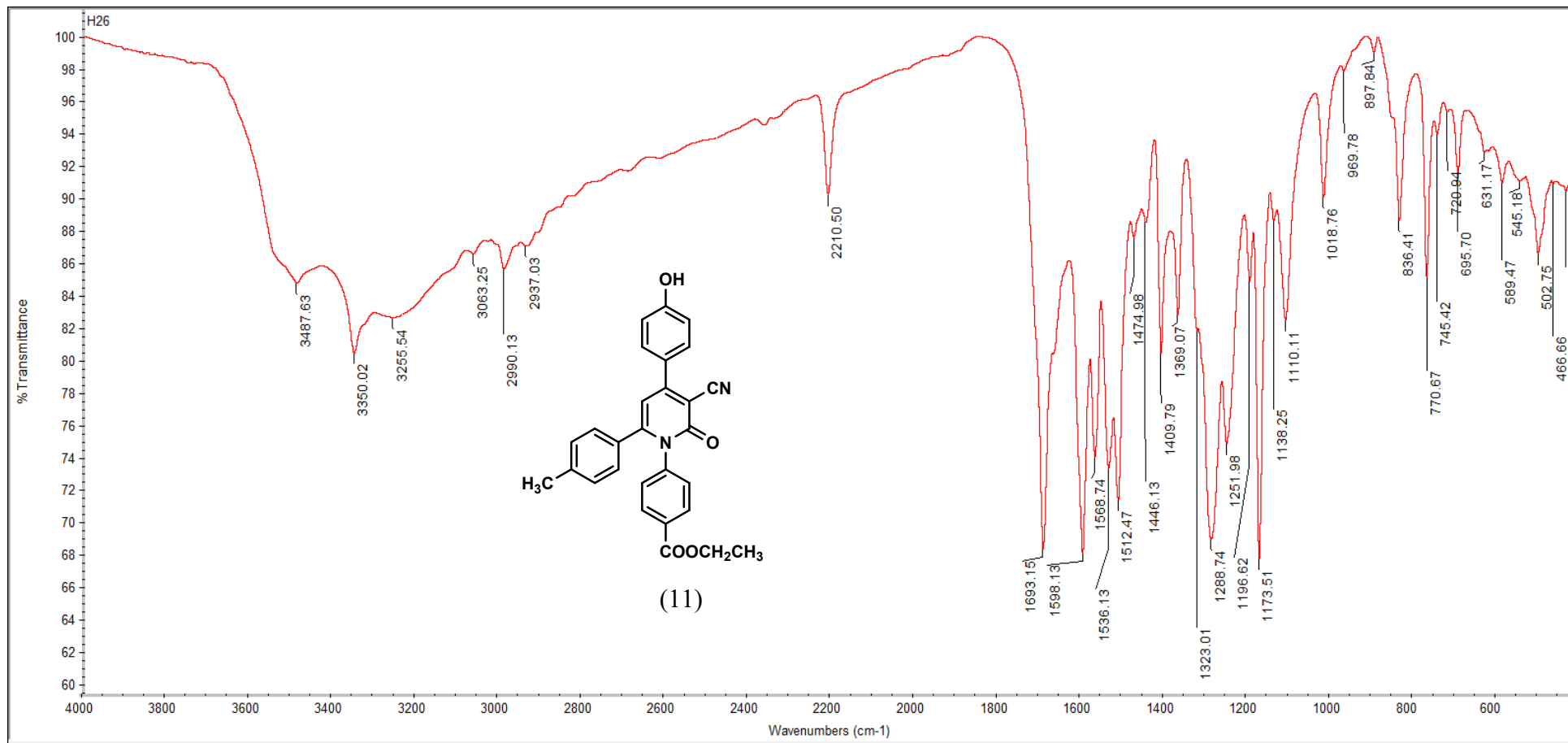




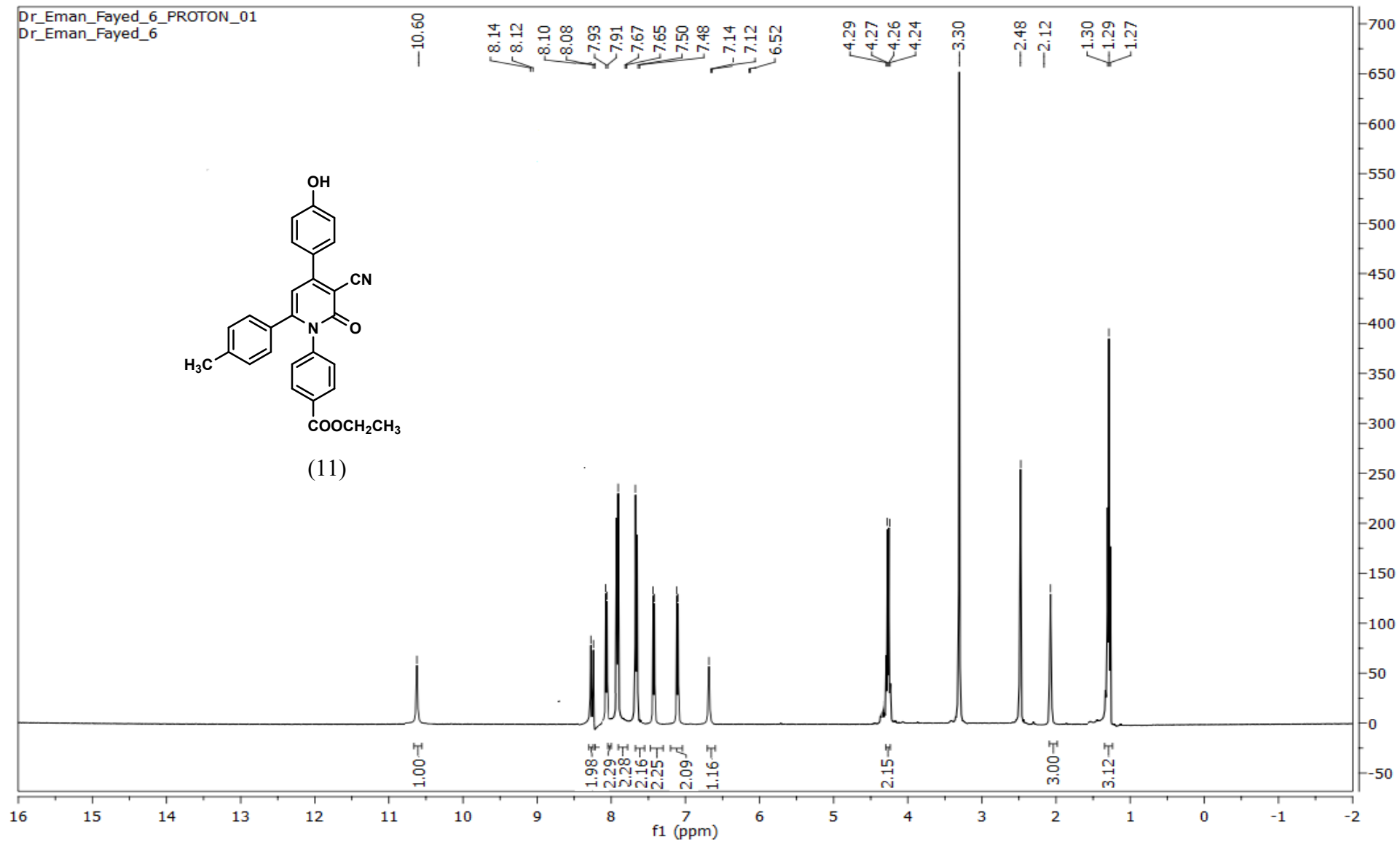
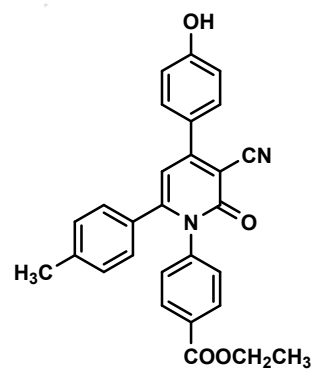
Dr_Eman_Fayed_H10_PROTON_01
Dr_Eman_Fayed_H10

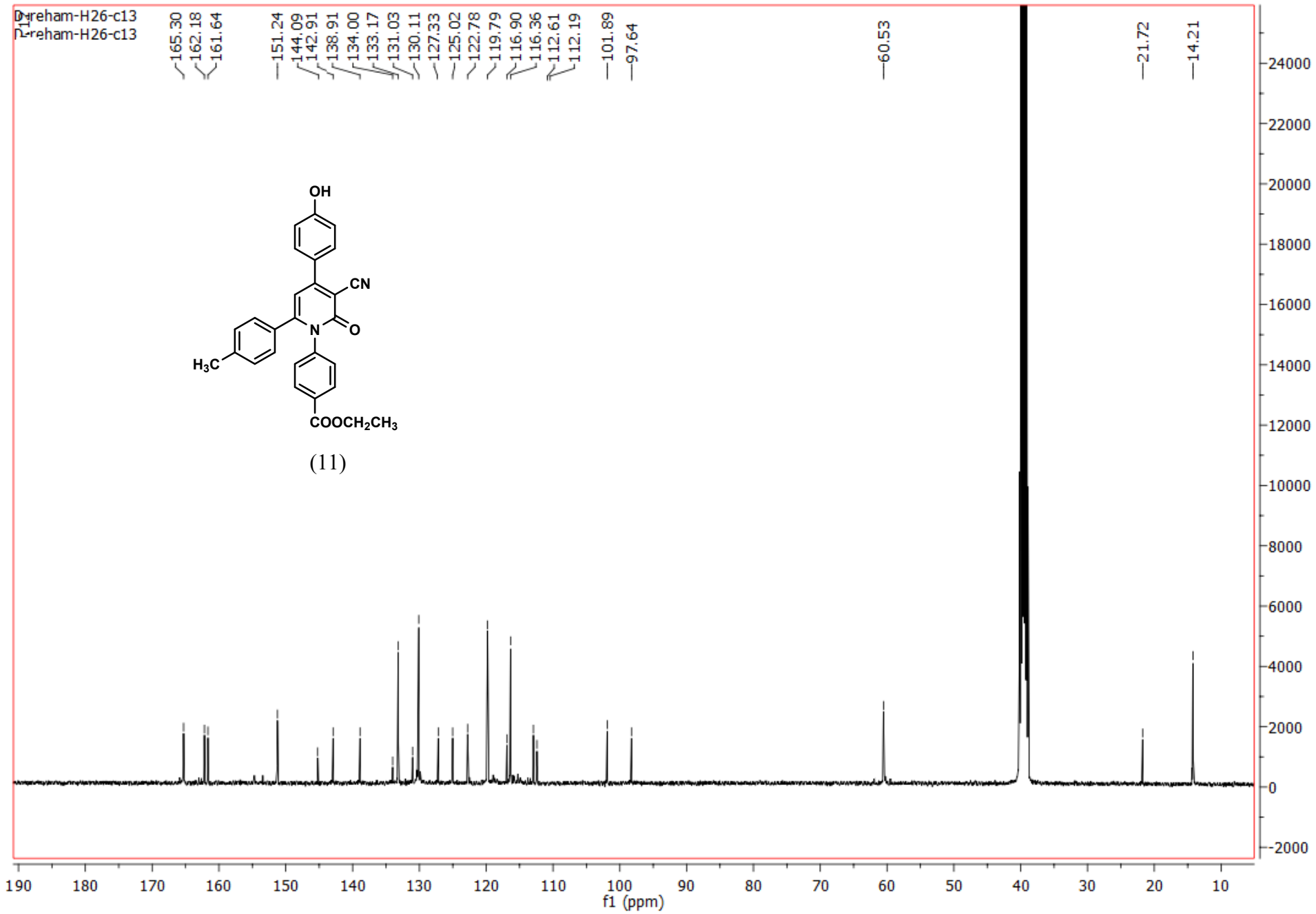






Dr_Eman_Fayed_6_PROTON_01
Dr_Eman_Fayed_6





Biological evaluation raw materials

Average of Relative viability of cells (%)

Conc.(μ M)	HePG-2	Caco-2
DOX		
100	6.3	15.1
50	11.2	24.7
25	14.1	36.8
12.5	28.3	42.6
6.25	45.8	62.0
3.125	57.6	78.3
1.56	71.2	100
2		
100	36.4	34.6
50	51.2	45.1
25	64.7	57.9
12.5	76.1	70.2
6.25	90.3	91.4
3.125	100	100
1.56	100	100
3a		
100	47.4	49.4
50	58.1	60.2
25	72.6	73.8
12.5	83.5	89.5
6.25	96.0	100
3.125	100	100
1.56	100	100
3b		
100	40.7	47.8
50	53.1	55.2
25	72.0	73.6
12.5	90.3	85.4
6.25	100	97.7
3.125	100	100
1.56	100	100
4		
100	26.5	33.9
50	37.9	44.0
25	50.0	52.1
12.5	64.2	70.6
6.25	78.6	85.2

3.125	96.5	100
1.56	100	100
5		
100	13.0	8.3
50	18.6	16.9
25	29.7	26.7
12.5	38.1	40.1
6.25	64.5	59.2
3.125	77.2	79.6
1.56	98.5	95.3
6		
100	35.6	34.6
50	42.7	48.3
25	56.3	60.7
12.5	68.1	73.9
6.25	90.2	87.5
3.125	100	100
1.56	100	100
7		
100	10.7	8.6
50	17.9	16.1
25	23.5	24.9
12.5	37.7	33.6
6.25	61.3	50.4
3.125	69.3	72.7
1.56	94.2	96.0
8		
100	7.8	18.3
50	16.1	27.1
25	26.3	33.4
12.5	33.9	40.6
6.25	58.6	69.2
3.125	74.5	85.9
1.56	88.2	100
9		
100	30.2	38.9
50	42.7	53.1
25	56.1	64.8
12.5	67.5	77.6
6.25	79.4	98.5
3.125	99.8	100
1.56	100	100
10		
100	24.5	29.3
50	35.1	44.7

25	50.9	59.1
12.5	63.6	64.0
6.25	71.8	95.4
3.125	90.2	100
1.56	100	100
11		
100	18.3	23.7
50	25.6	30.2
25	33.1	39.1
12.5	50.4	55.3
6.25	67.9	73.8
3.125	88.0	90.4
1.56	100	100
100	21.3	26.1
50	32.8	38.3

Lab Report

Ser	Sample			RT-PCR Results Fold Change		
	code	cells	IC50	Bax	Bcl2	
	1	5	Caco2	9.78	4.508	
2	7	Caco2	7.83	7.508	0.194	
3	8	Caco2	13.61	4.188	0.275	
4	Staurosporine	Caco2	5.07	7.648	0.321	
5	control	Caco2	---	1	1	

Quantitation Report

Experiment Information

Run Name	Run 2022-08-12 (1)
Run Start	2022-08-12 01:55:02 PM
Run Finish	2022-08-12 04:27:17 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'-TGTGTCCACGGCGCAATCATC-3'.

bcl2 : F 5'-ATGTGTGTGGAGACCGTCAA-3',

bcl2 : R 5'-GCCGTACAGTCCACAAAGG-3'.

GAPDH : F 5'- GTCTCCTGACTTCAACAGCG-3'

GAPDH : R 5'- ACCACCCTGTTGCTGTAGCCAA-3'

Sample			Bax							
Ser	code	Conc	Control cells			Test cells				FLD
			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=1.849
1	5		22.07	35.25	13.18	22.18	32.91	10.73	-2.45	4.508
2	7		22.07	35.25	13.18	22.29	32.19	9.9	-3.28	7.508
3	8		22.07	35.25	13.18	21.94	32.79	10.85	-2.33	4.188
4	Staurosporine		22.07	35.25	13.18	22.36	32.23	9.87	-3.31	7.648
5	control		22.07	35.25	13.18	22.07	35.25	13.18	0	1

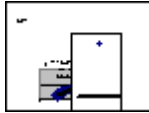
Sample			bcl2							
Ser	code	Conc	Control cells			Test cells				FLD
			GAPDH	bcl2	Δ CTC	GAPDH	bcl2	Δ CTE	$\Delta\Delta$ CT	$2^{\Delta\Delta$ CT
			HC	TC	TC-HC	HE	TE	TE-HE	Δ CTE- Δ CTC	E=1.849
1	5		22.07	27.49	5.42	22.18	28.95	6.77	1.35	0.436
2	7		22.07	27.49	5.42	21.94	30.03	8.09	2.67	0.194
3	8		22.07	27.49	5.42	22.29	29.81	7.52	2.1	0.275
4	Staurosporine		22.07	27.49	5.42	22.36	29.63	7.27	1.85	0.321
5	control		22.07	27.49	5.42	22.07	27.49	5.42	0	1


Lab Report

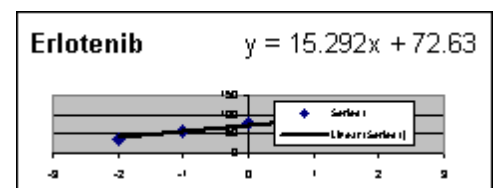
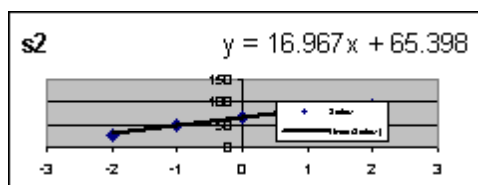
ser	Compound		EGFR		SD ±
	code	MW g/mol	IC50 uM		
1	7	293	0.124		0.009
***	Erlotinib	393.436	0.033		0.002

Detailed Results

EGFR

code	IC50	conc	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
7		100	2	93.03	30	0	30	6.97	0	6.97	3.3333	8.36408
		10	1	88.41	30	0	30	11.59	0	11.59	3.3333	13.9081
		1	0	68.08	30	0	30	31.92	0	31.92	3.3333	38.3044
		0.1	-1	50.14	30	0	30	49.86	0	49.86	3.3333	59.8326
		0.01	-2	27.33	30	0	30	72.67	0	72.67	3.3333	87.2049
	EC				0	30	0	30	100	0	100	3.3333

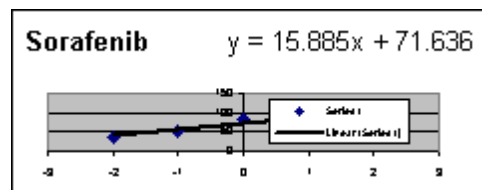
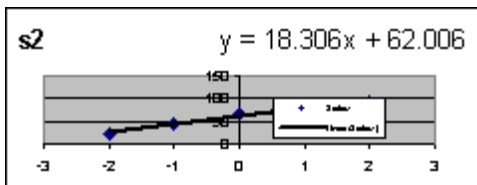
code	IC50	conc	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
Erlotinib		100	2	95.73	30	0	30	4.27	0	4.27	3.3333	5.12405
		10	1	92.85	30	0	30	7.15	0	7.15	3.3333	8.58009
		1	0	80.06	30	0	30	19.94	0	19.94	3.3333	23.9282
		0.1	-1	57.63	30	0	30	42.37	0	42.37	3.3333	50.8445
		0.01	-2	36.88	30	0	30	63.12	0	63.12	3.3333	75.7448
	EC				0	30	0	30	100	0	100	3.3333



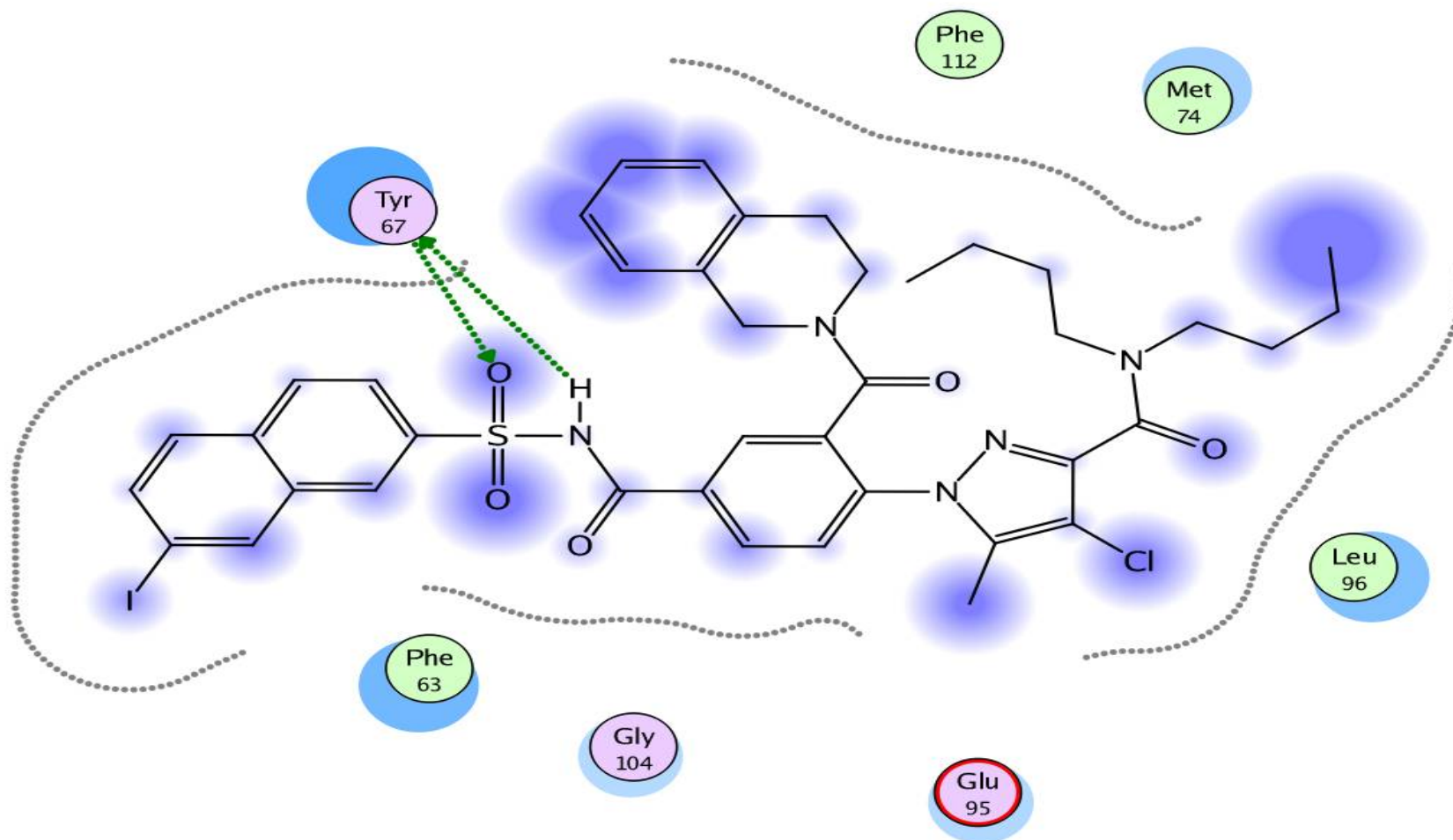
ser	Compound		VEGFR2		SD ±
	code	MW g/mol	IC50 uM		
1	7	293	0.221		0.009
***	Sorafenib	464.8	0.043		0.002

Detailed results

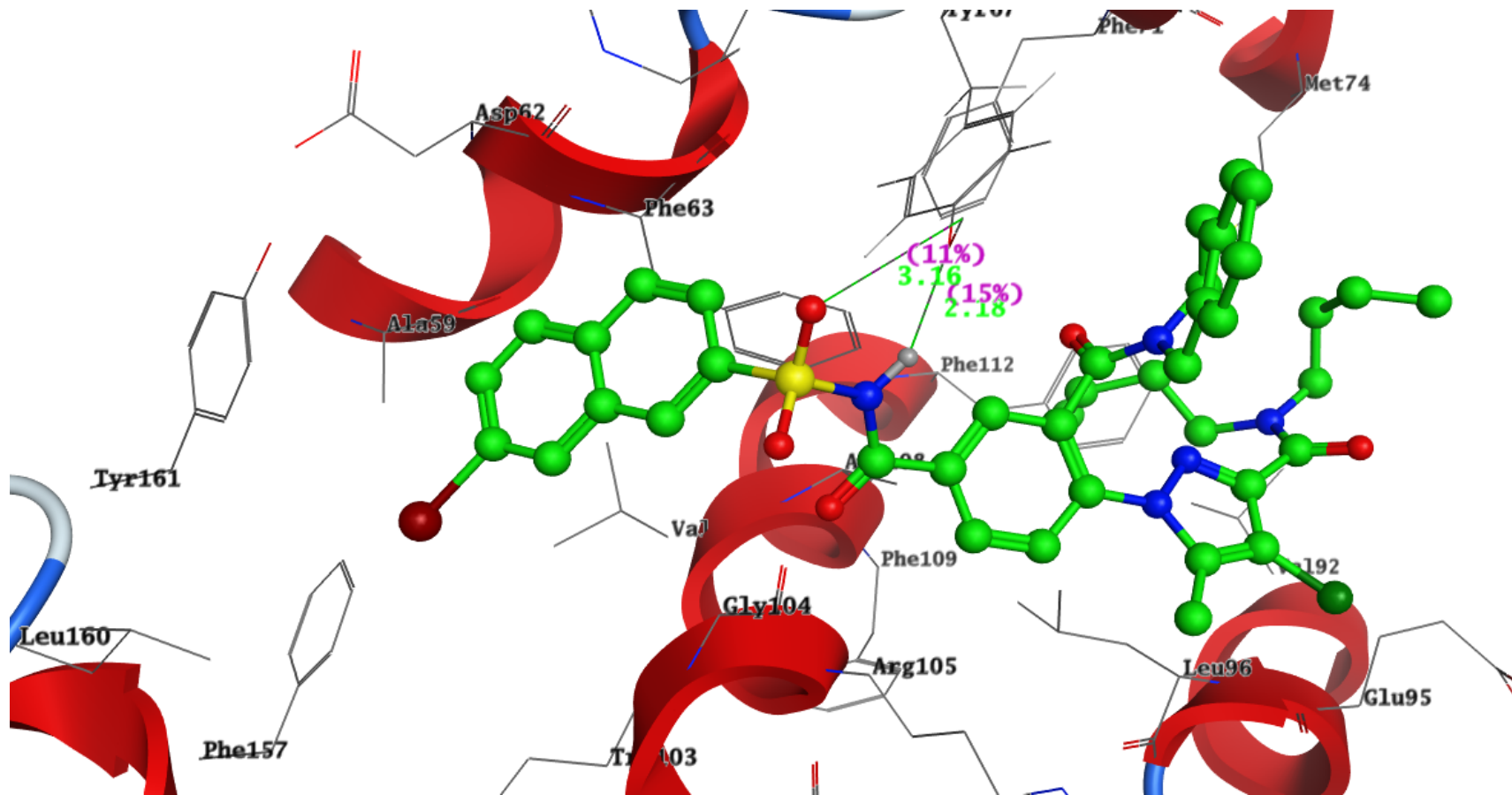
VEGFR2												
code	IC50	conc	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
7		100	2	92.44	30	0	30	7.56	0	7.56	3.3333	9.07209
		10	1	87.03	30	0	30	12.97	0	12.97	3.3333	15.5642
		1	0	64.12	30	0	30	35.88	0	35.88	3.3333	43.0564
		0.1	-1	44.03	30	0	30	55.97	0	55.97	3.3333	67.1647
		0.01	-2	22.41	30	0	30	77.59	0	77.59	3.3333	93.1089
EC				0	30	0	30	100	0	100	3.3333	120
code	IC50	conc	log	%inh	T2	T1	ΔT	RFU2	RFU1	ΔRFU	slope	K.Activity
Sorafenib		100	2	95.93	30	0	30	4.07	0	4.07	3.3333	4.88405
		10	1	92.08	30	0	30	7.92	0	7.92	3.3333	9.5041
		1	0	80.89	30	0	30	19.11	0	19.11	3.3333	22.9322
		0.1	-1	53.47	30	0	30	46.53	0	46.53	3.3333	55.8366
		0.01	-2	35.81	30	0	30	64.19	0	64.19	3.3333	77.0288
EC				0	30	0	30	100	0	100	3.3333	120



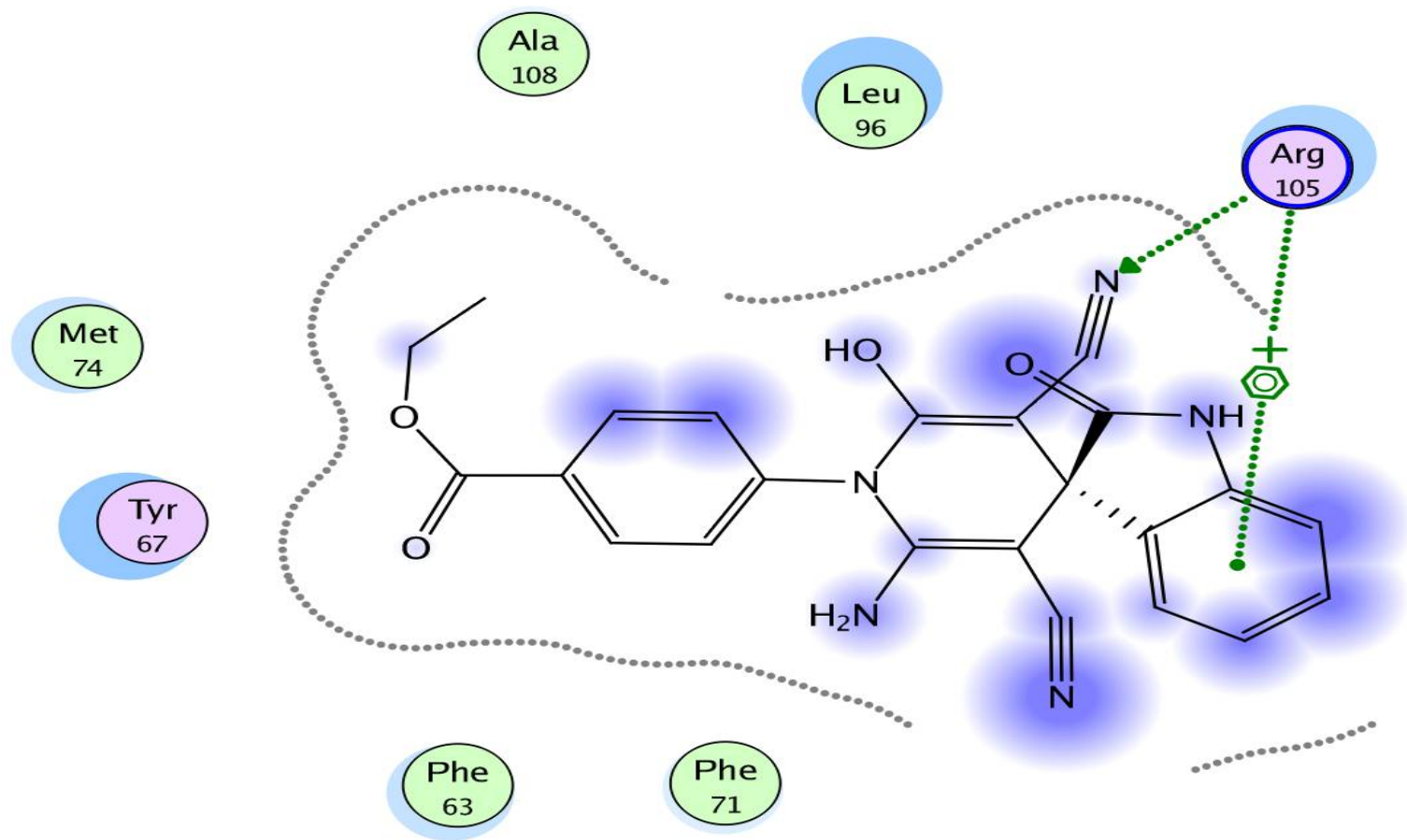
Docking
simulation
figures



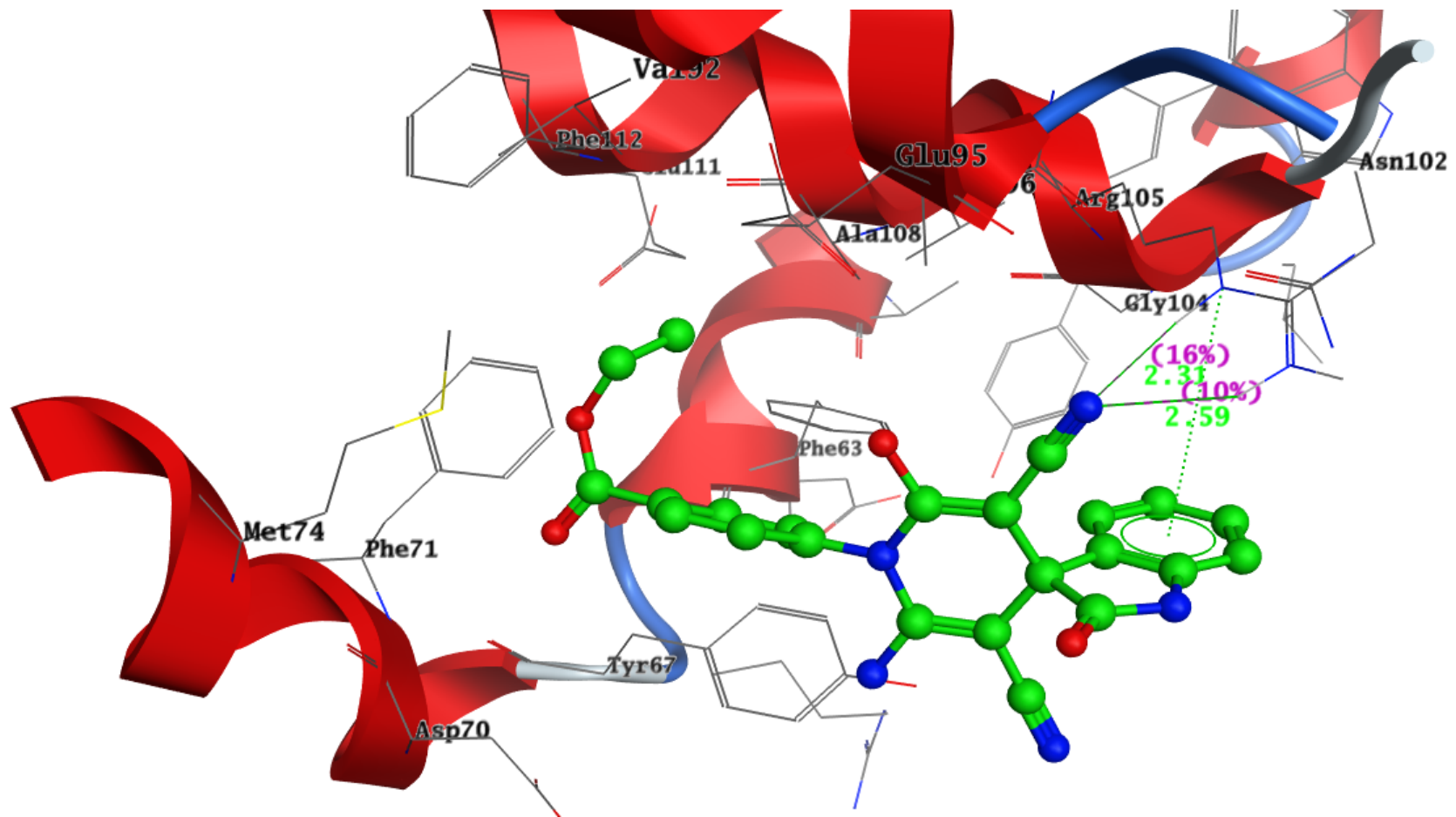
2D binding mode of co-crystallized ligand inside Bcl-2 binding pocket (PDB: 4AQ3)



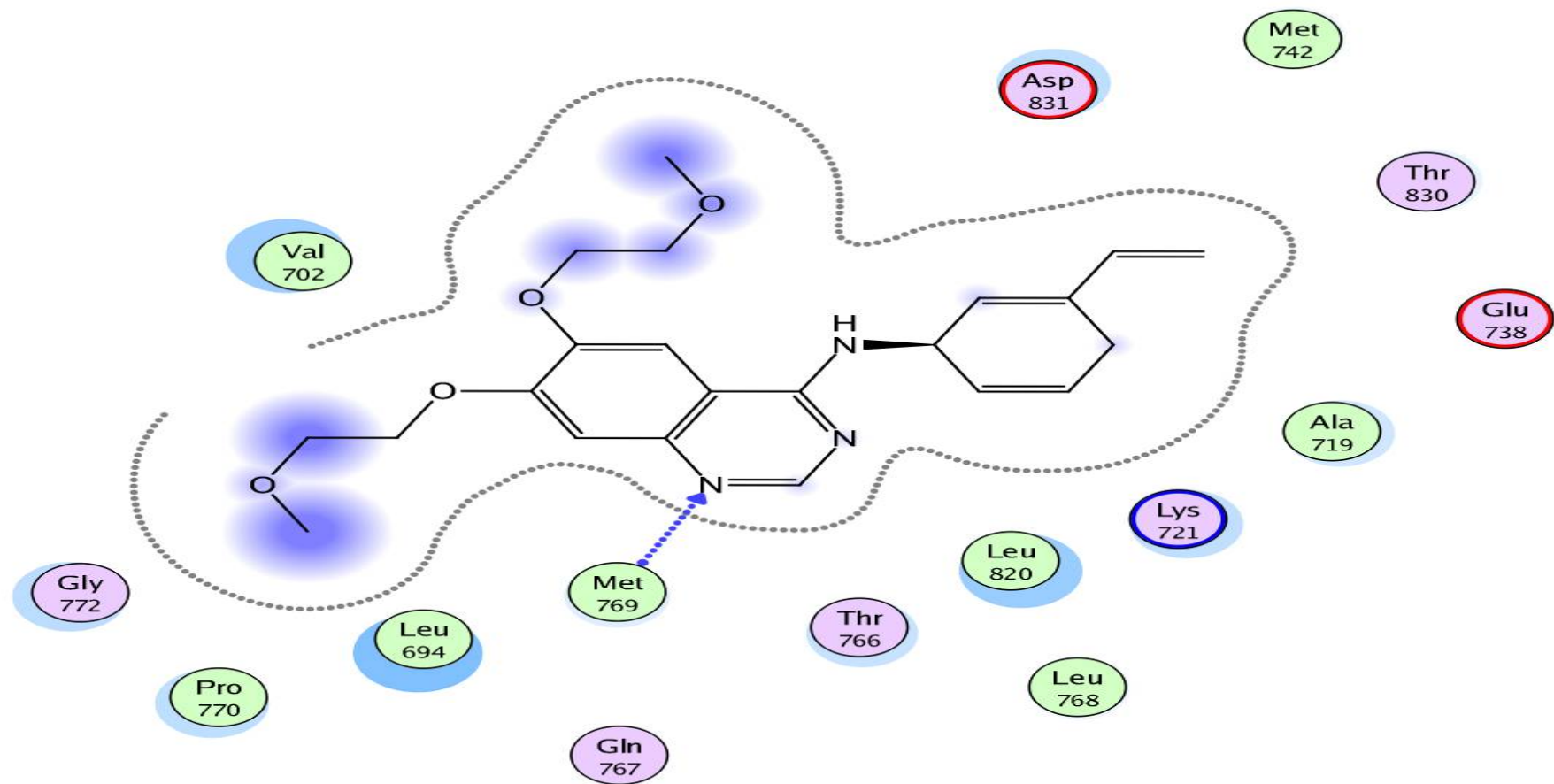
3D binding mode of co-crystallized ligand inside Bcl-2 binding pocket (PDB: 4AQ3)



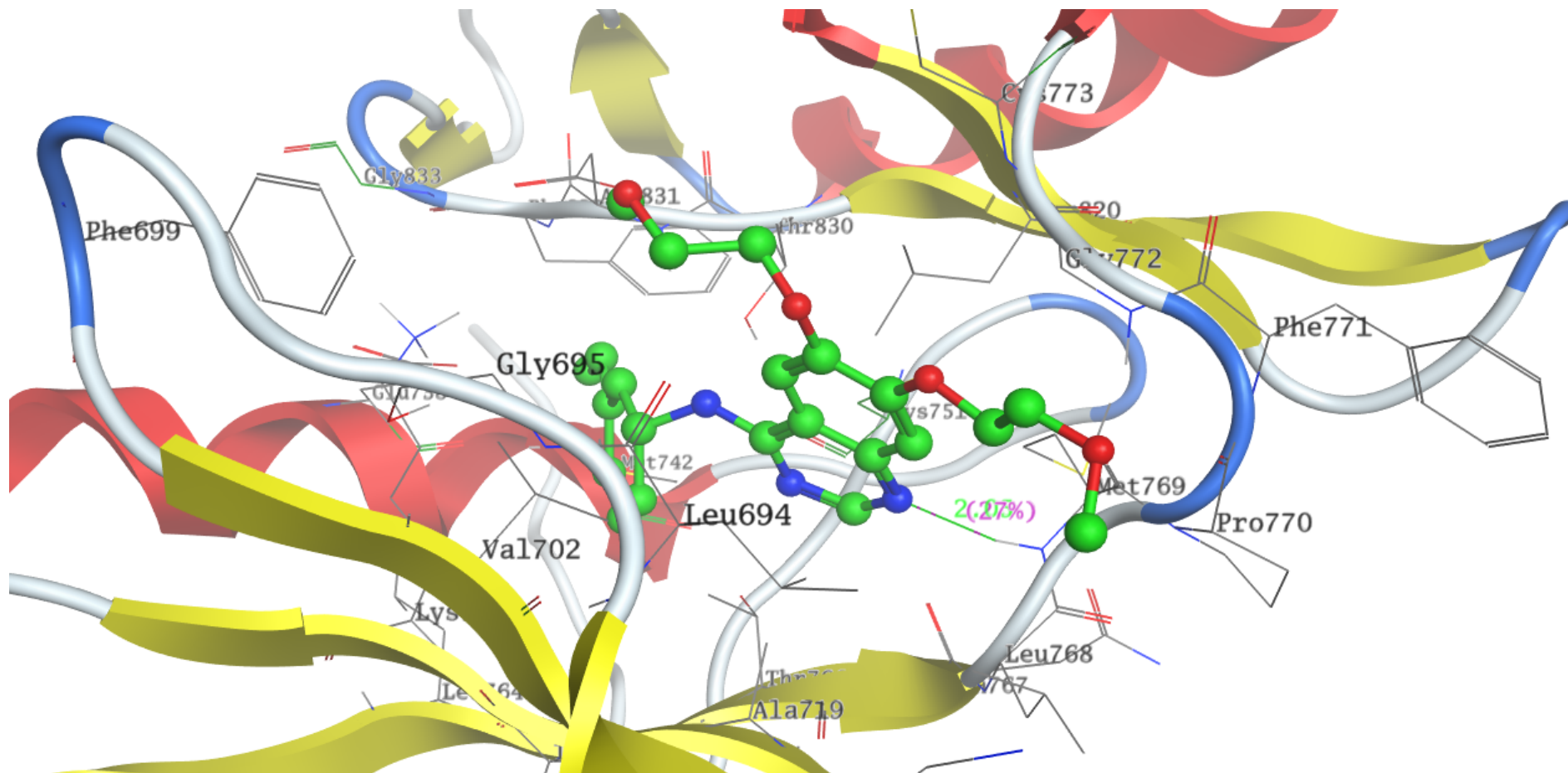
2D binding mode of 2-oxo-1'H-spiro-indoline-3,4'-pyridine 7 inside Bcl-2 binding pocket (**PDB: 4AQ3**)



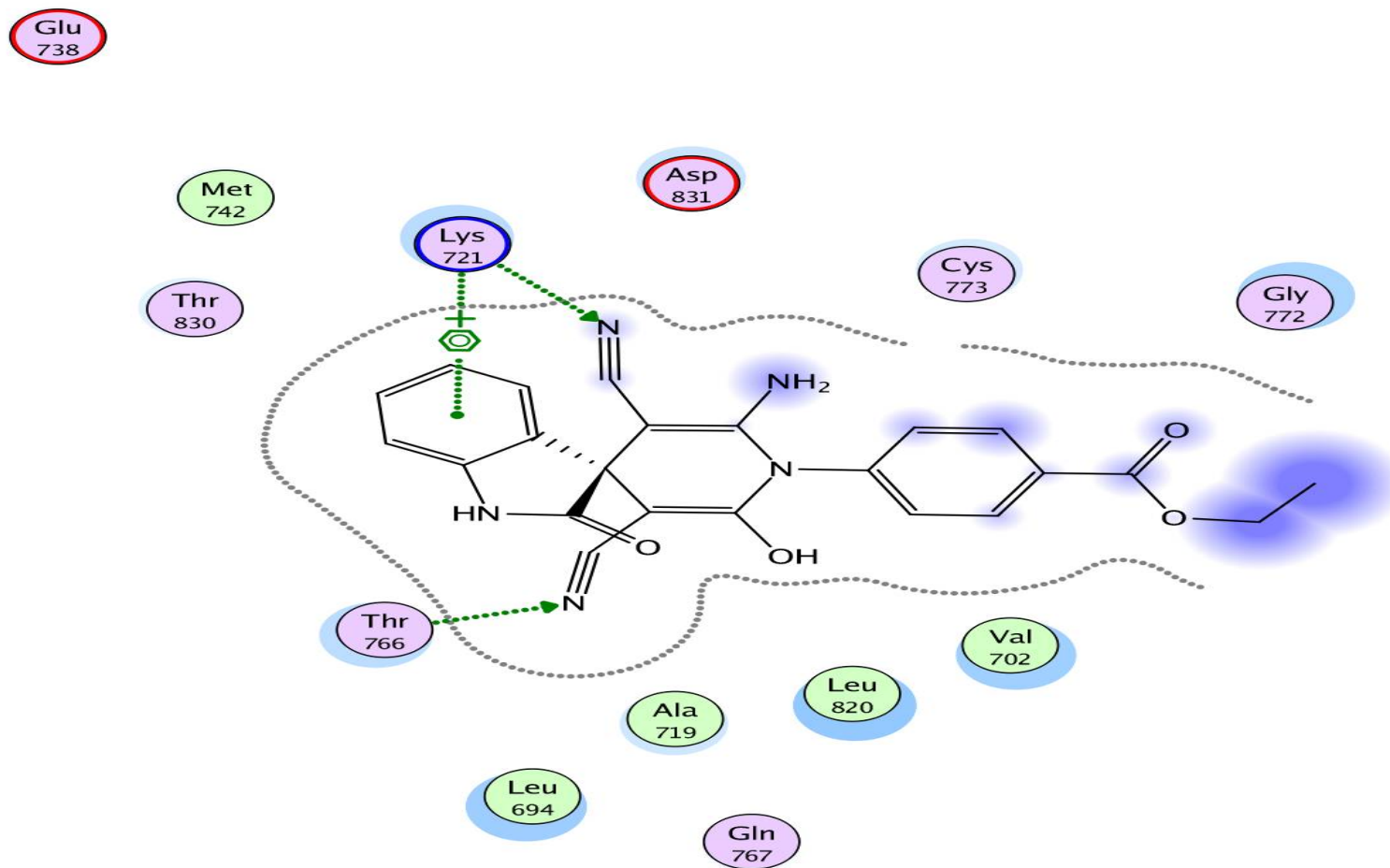
3D binding mode of 2-oxo-1'H-spiro-indoline-3,4'-pyridine 7 inside Bcl-2 binding pocket (PDB: 4AQ3)



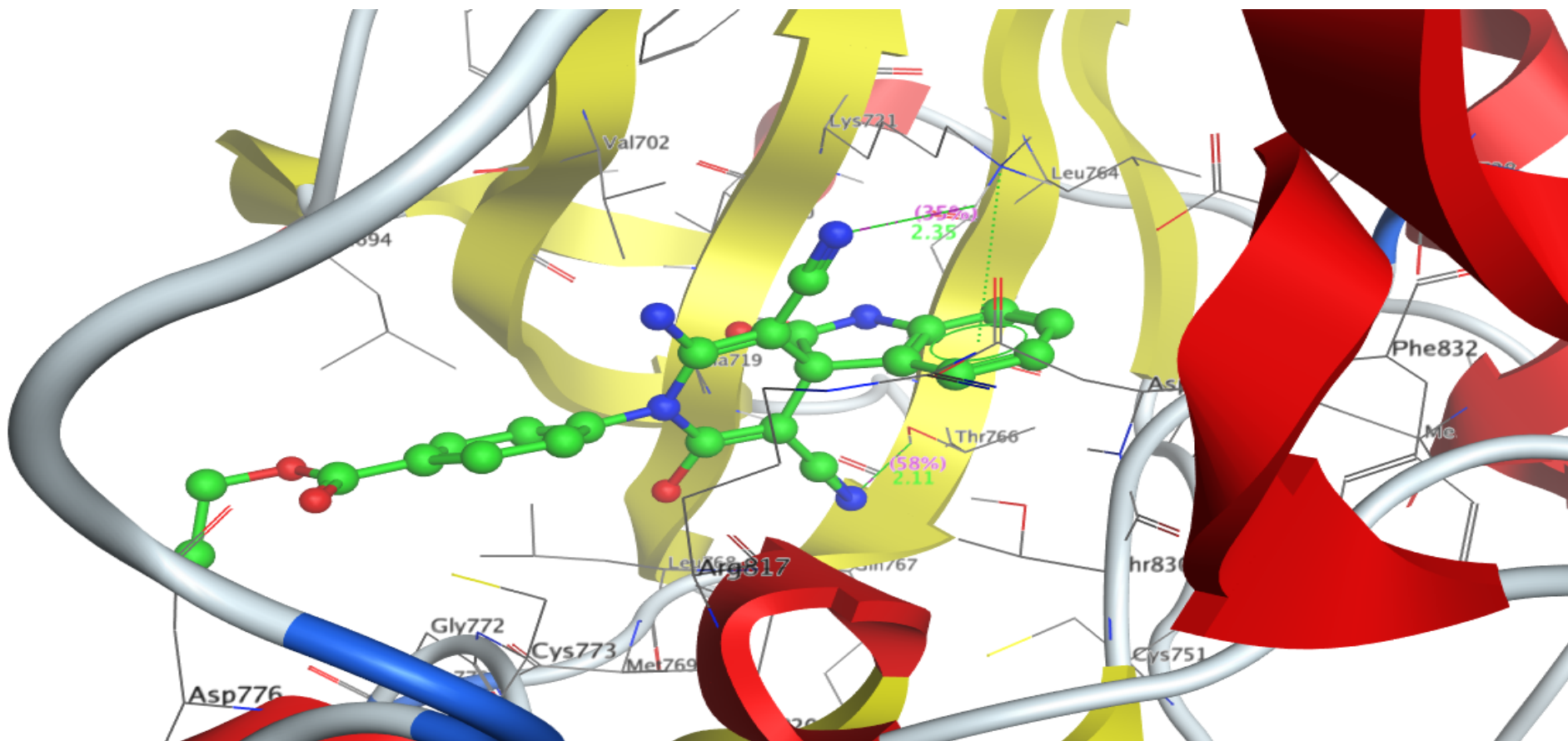
2D binding mode of co-crystallized ligand inside the EGFR binding pocket (PDB: 1M17)



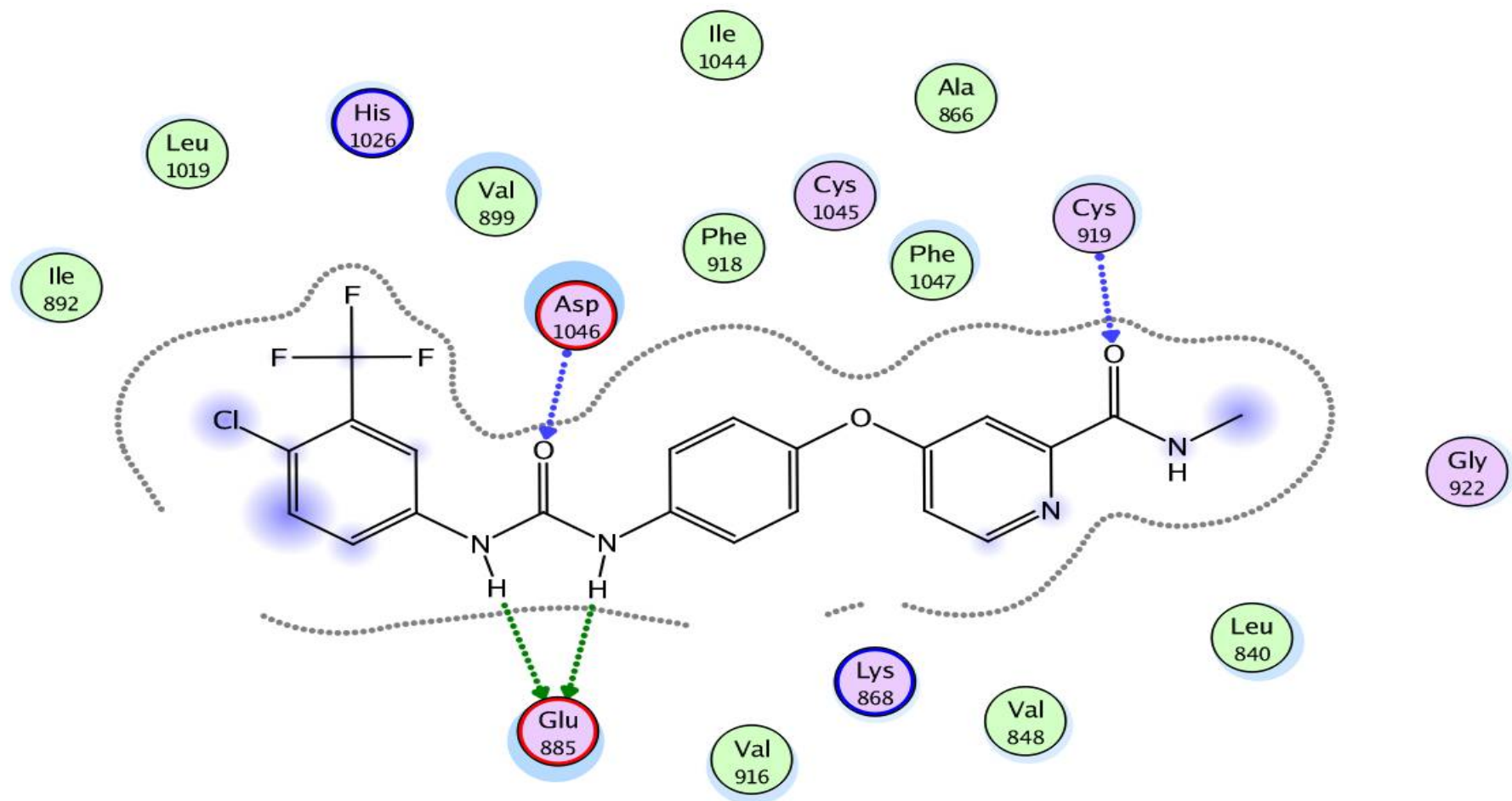
3D binding mode of co-crystallized ligand inside the EGFR binding pocket (PDB: 1M17)



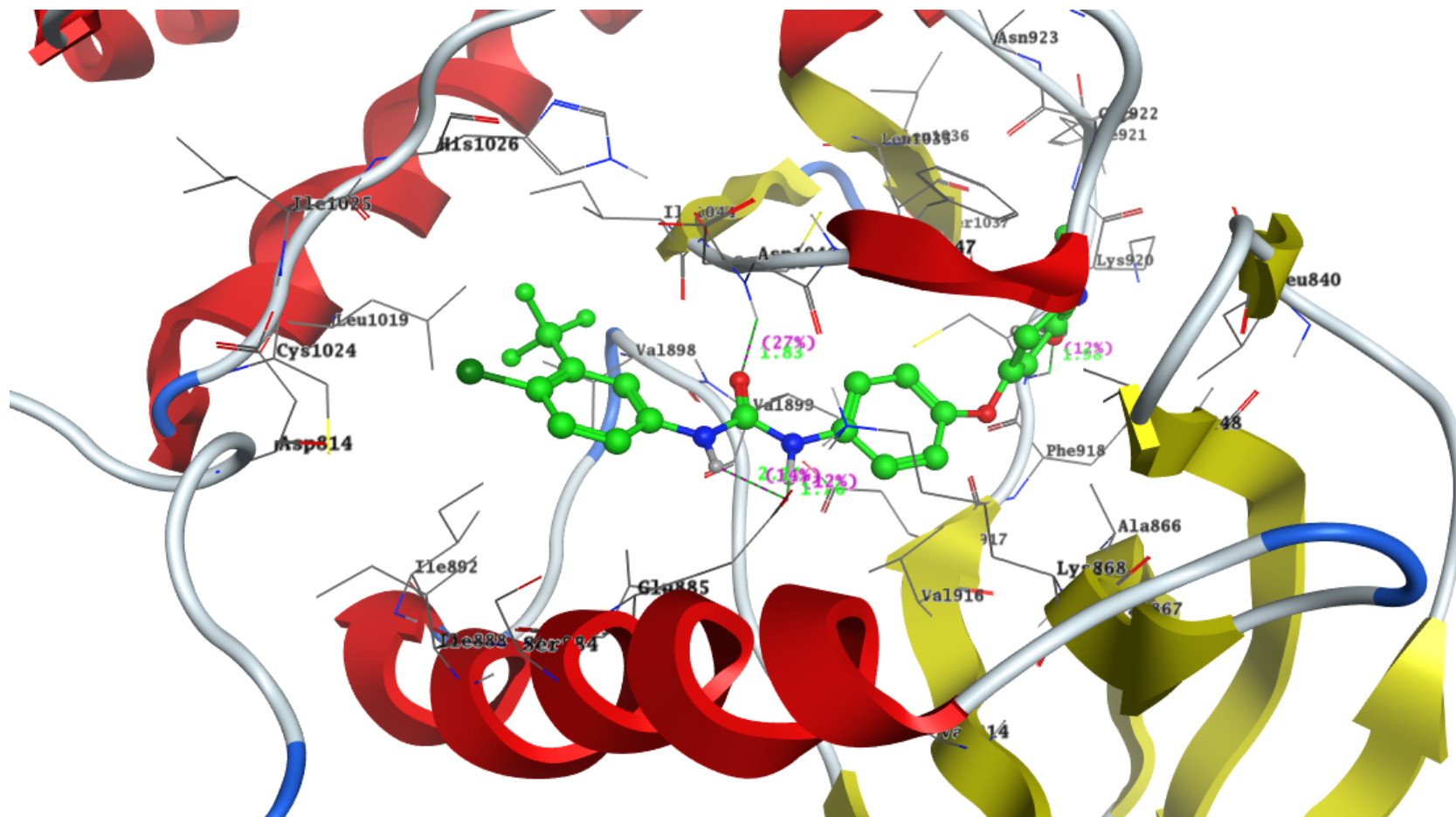
2D binding mode of 2-oxo-1H-spiro-indoline-3,4'-pyridine 7 inside the EGFR binding pocket (PDB: 1M17)



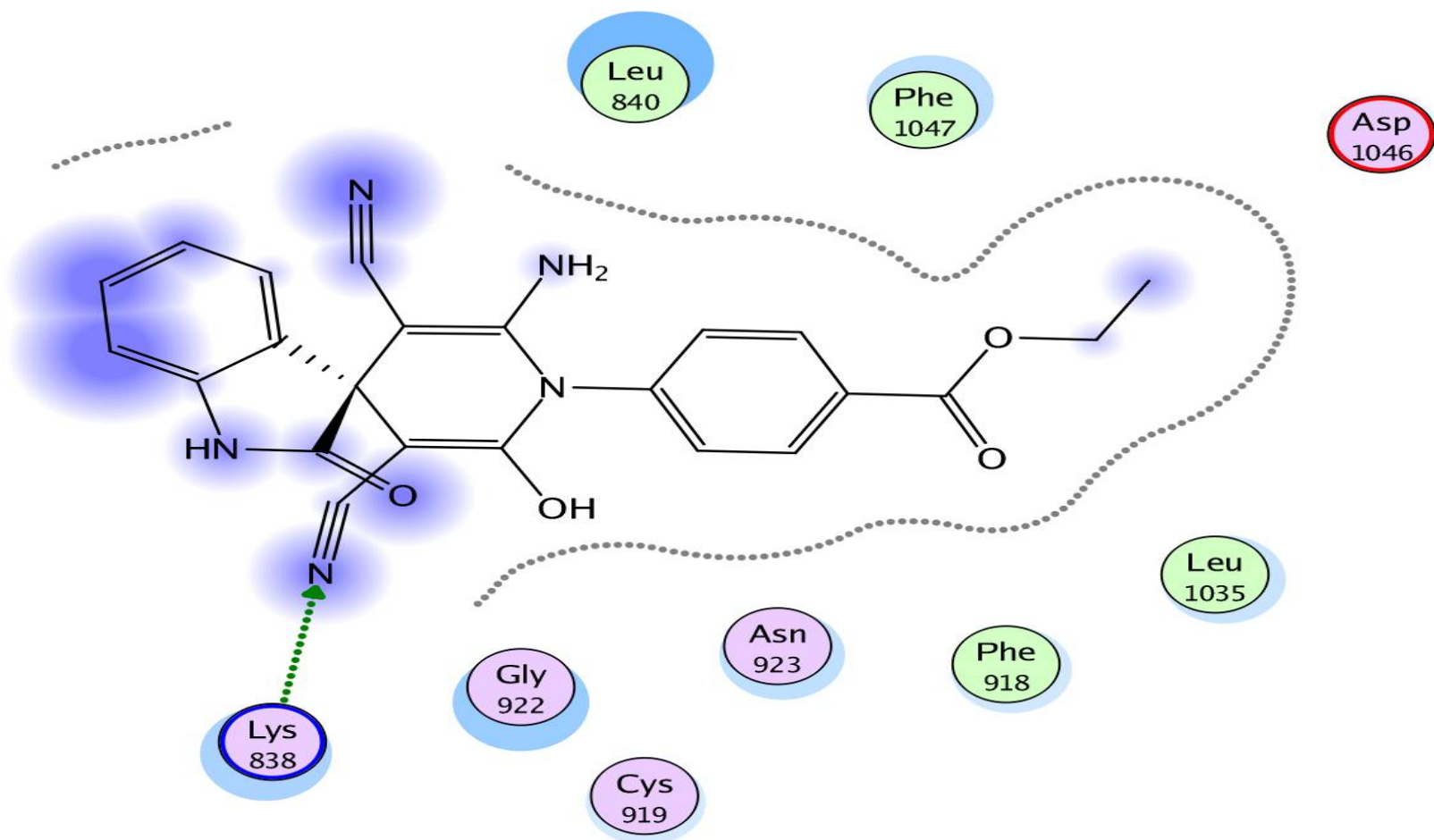
3D binding mode of 2-oxo-1'H-spiro-indoline-3,4'-pyridine 7 inside the EGFR binding pocket (PDB: 1M17)



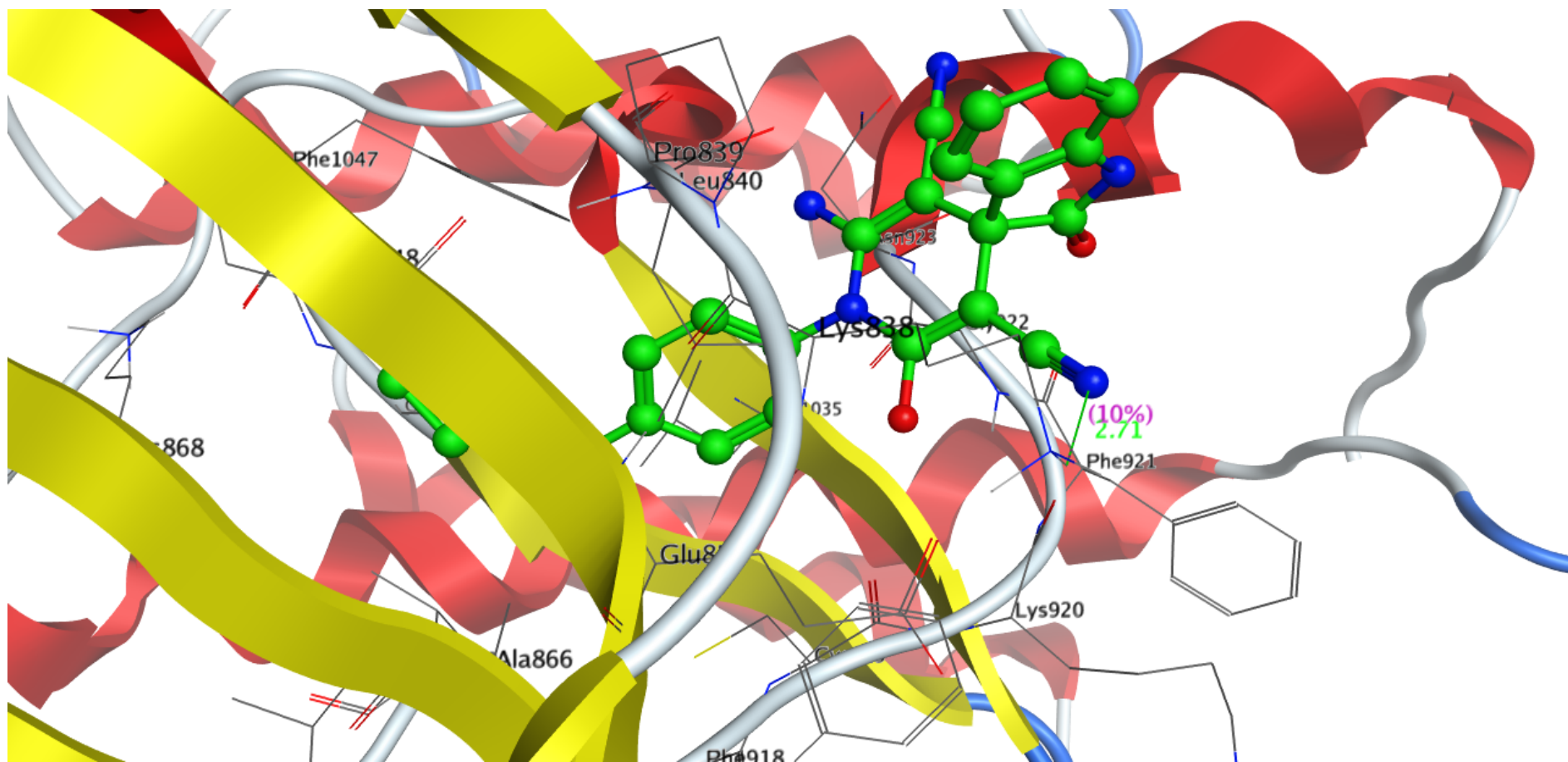
2D binding mode of co-crystallized ligand inside the VEGFR-2 binding pocket (**PDB: 4ASD**)



3D binding mode of co-crystallized ligand inside the VEGFR-2 binding pocket (PDB: 4ASD)



2D binding mode of 2-oxo-1'H-spiro-indoline-3,4'-pyridine **7** inside the VEGFR-2 binding pocket (**PDB**: 4ASD)



3D binding mode of 2-oxo-1'H-spiro-indoline-3,4'-pyridine **7** inside the VEGFR-2 binding pocket (**PDB: 4ASD**)