

Design, green synthesis, and quorum sensing quenching potential of novel 2-oxo-pyridines containing thiophene/furan scaffold targeting lasr gene on *p. aeruginosa*

Yousry A. Ammar ^a, Ahmed Ragab ^{a,*}, M. A. Migahed ^b, S. Al-Sharbasy ^c, Mohamed A. Salem ^d, Omnia Kareem M. Riad ^e, Heba Mohammed Refat M. Selim ^{e,f,*}, Gehad A. Abd-elmaksoud ^c, Moustafa S. Abusaif ^a

^a Department of Chemistry, Faculty of Science (boys), Al-Azhar University, 11884 Nasr City, Cairo-Egypt

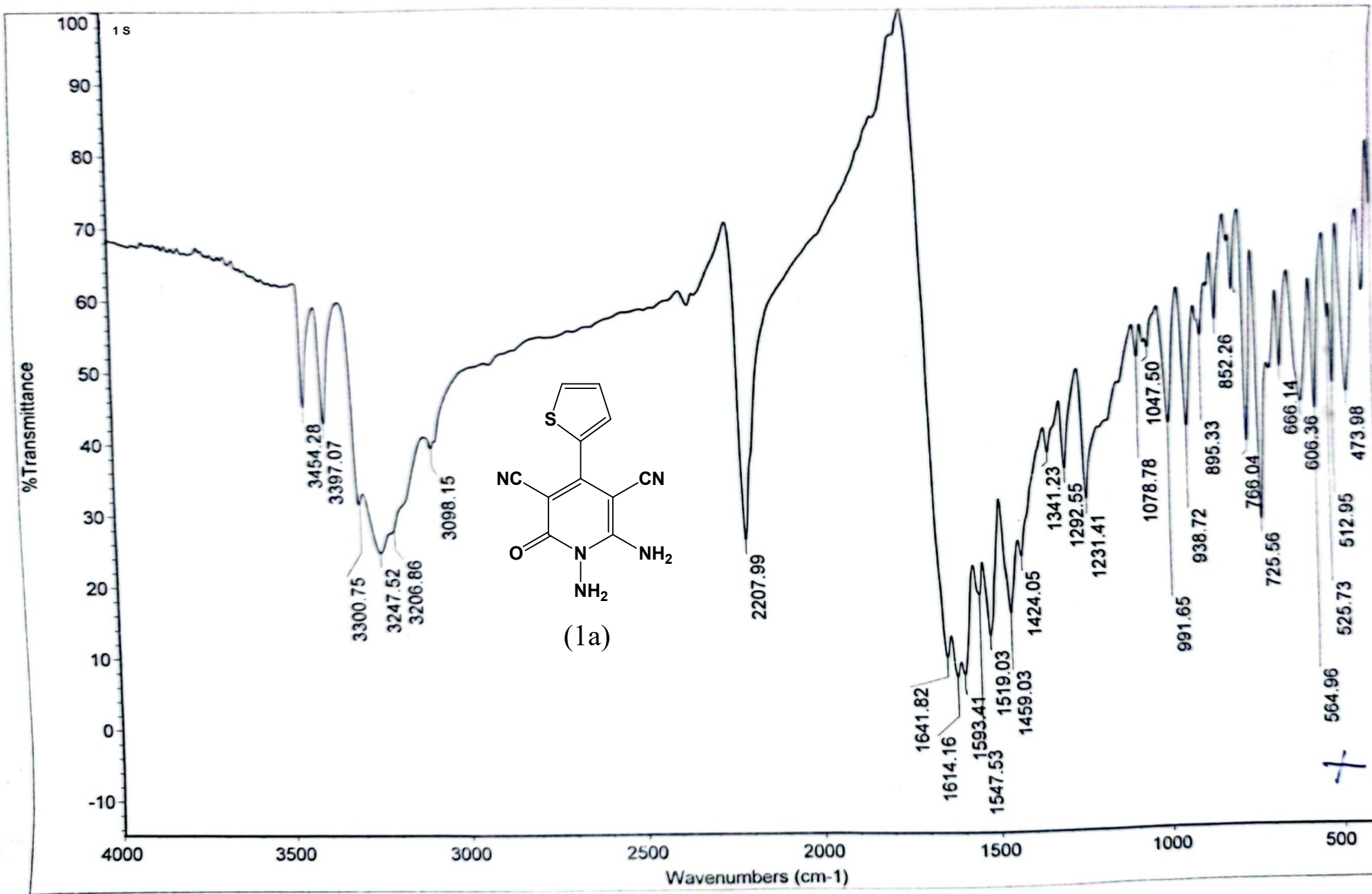
^b Egyptian Petroleum Research Institute (EPRI), 11727 Nasr City, Cairo-Egypt.

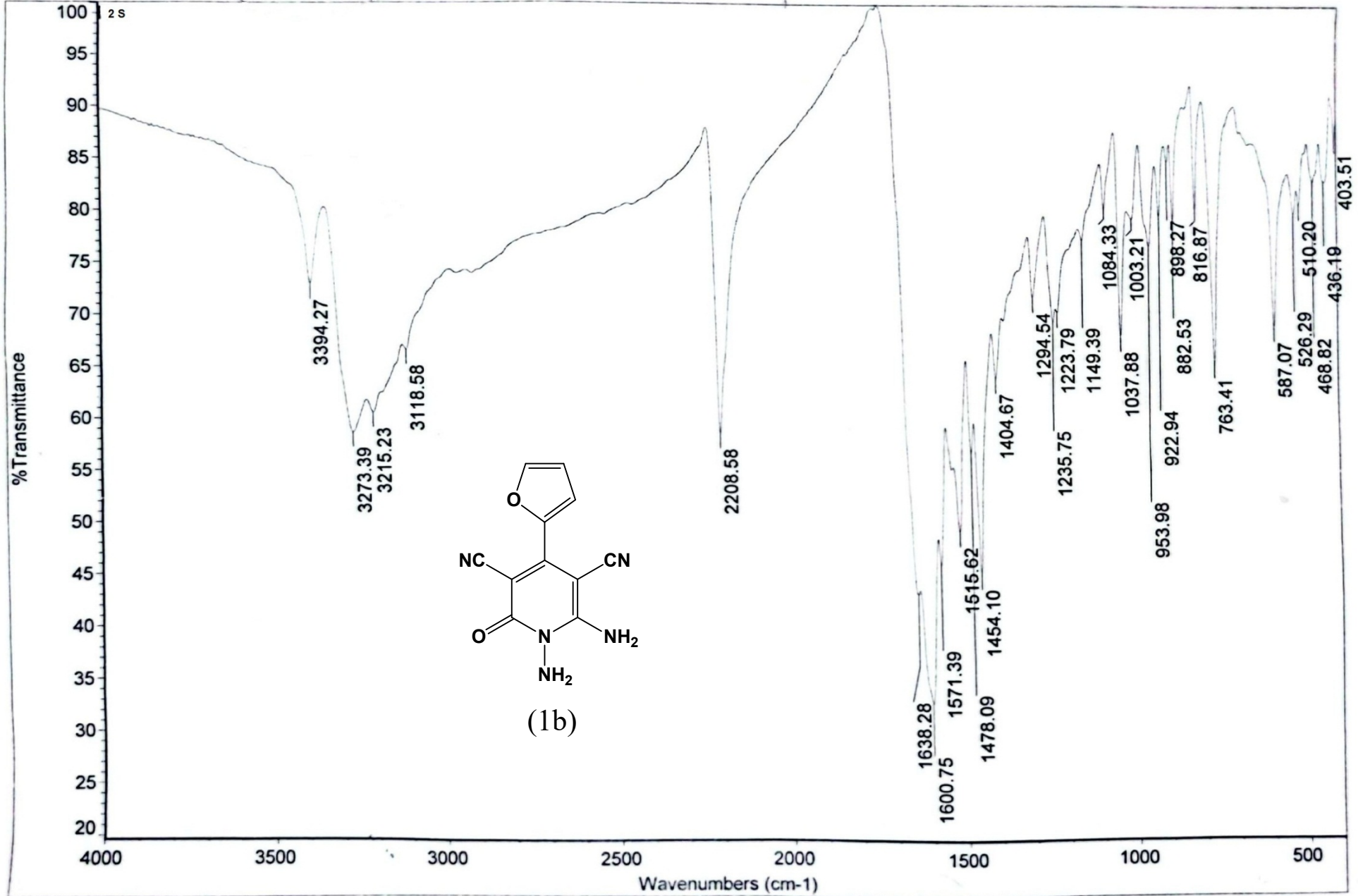
^c Department of Chemistry, Faculty of Science (girls), Al-Azhar University, 11884 Nasr City, Cairo-Egypt

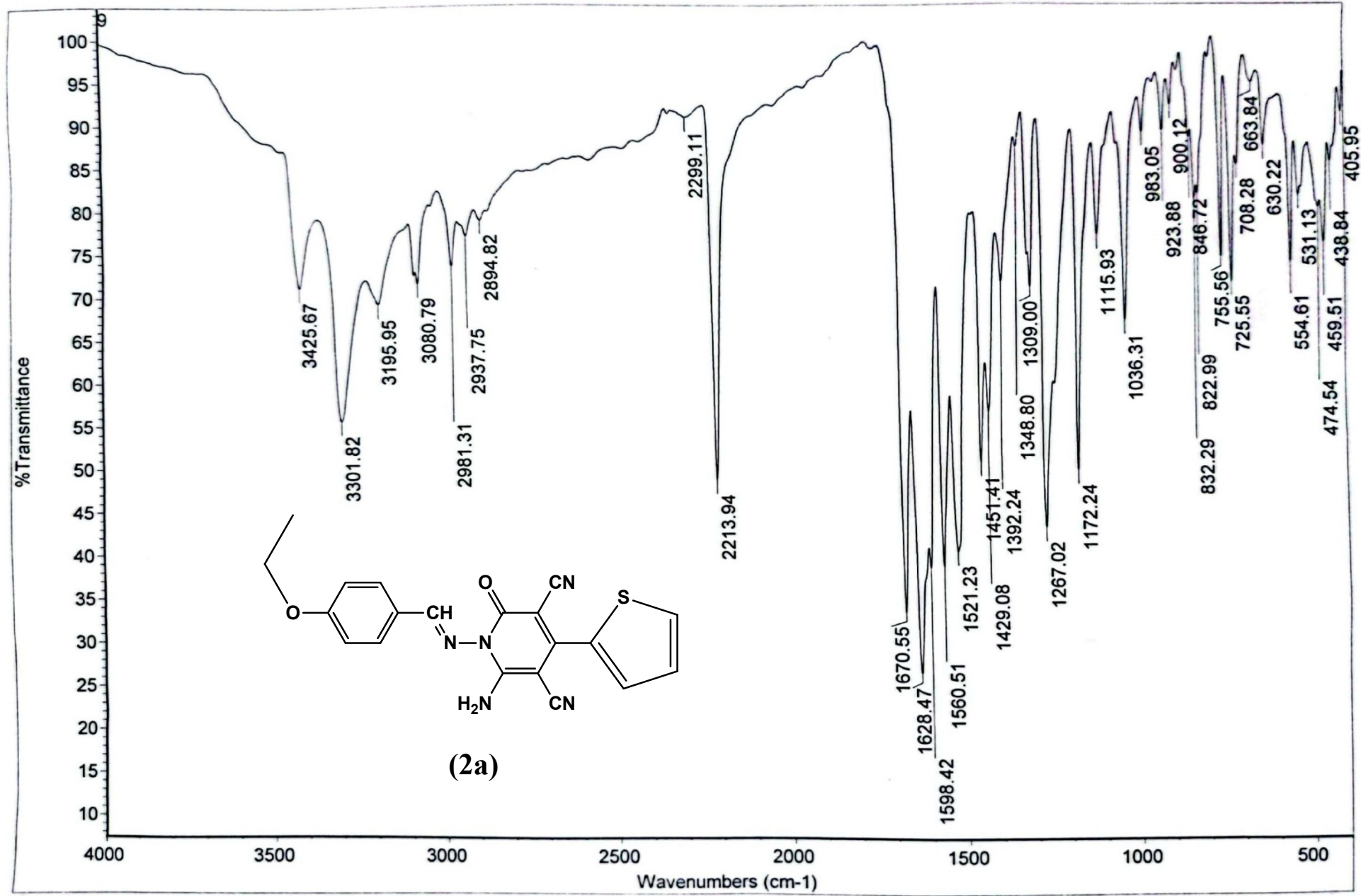
^d Department of Chemistry, Faculty of Science and Arts, King Khalid University, Mohail, Assir, Saudi Arabia

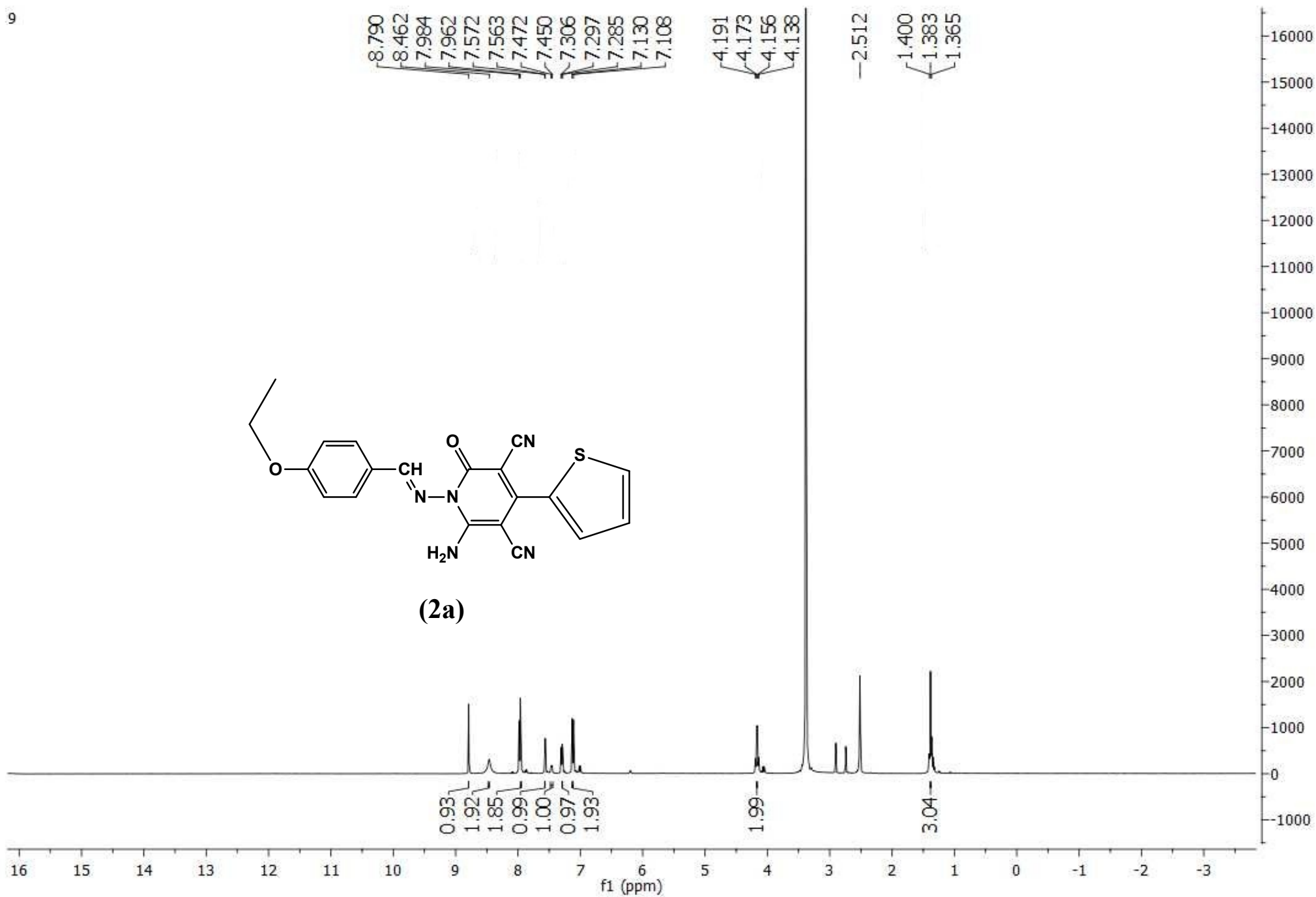
^e Department of Microbiology and Immunology, Faculty of Pharmacy (Girls), Al-Azhar University, Nasr City, Cairo, Egypt

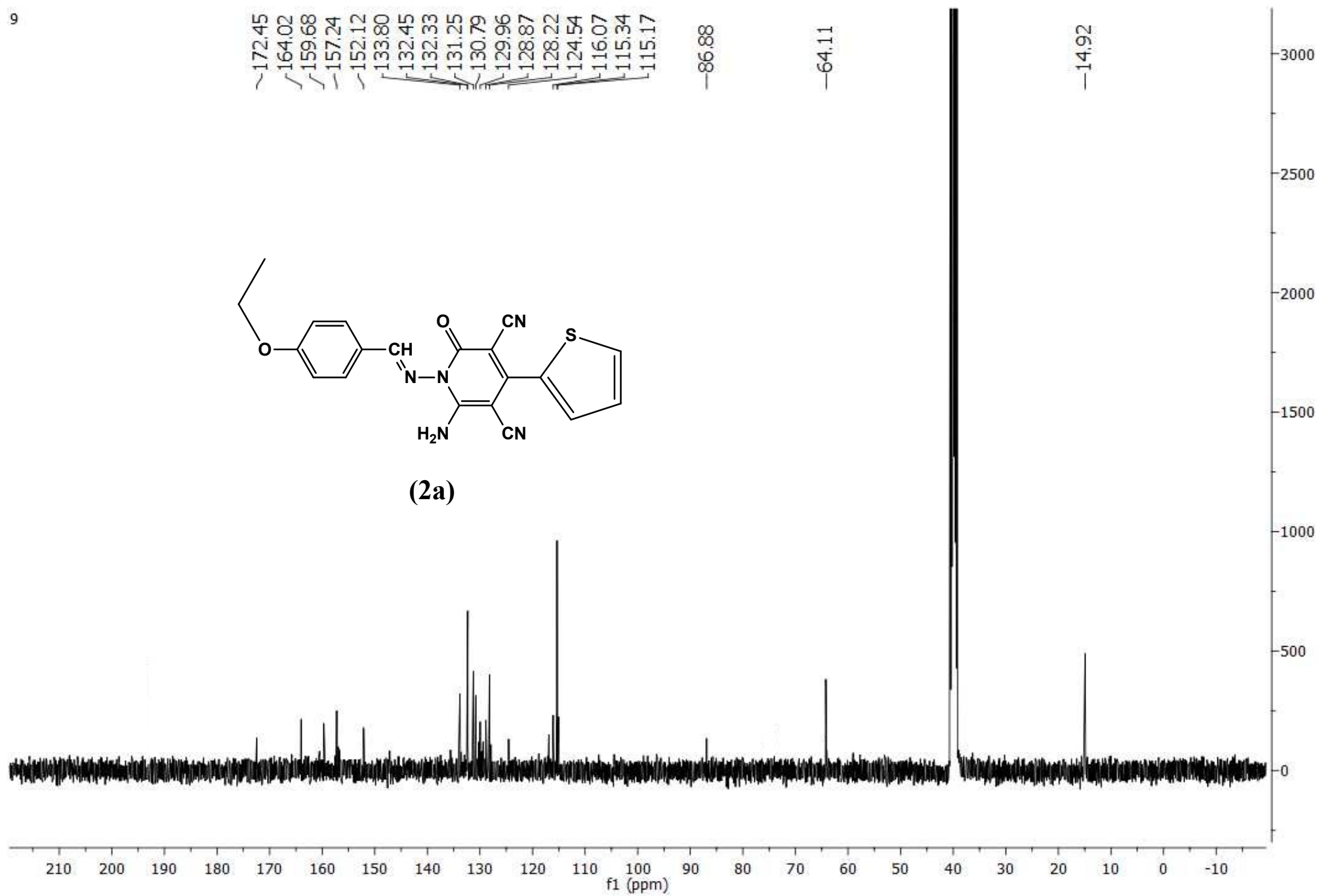
^f Department of Pharmaceutical Sciences, Faculty of Pharmacy, Al-Maarefa University, Diriyah, 13713, Riyadh, Saudi Arabia

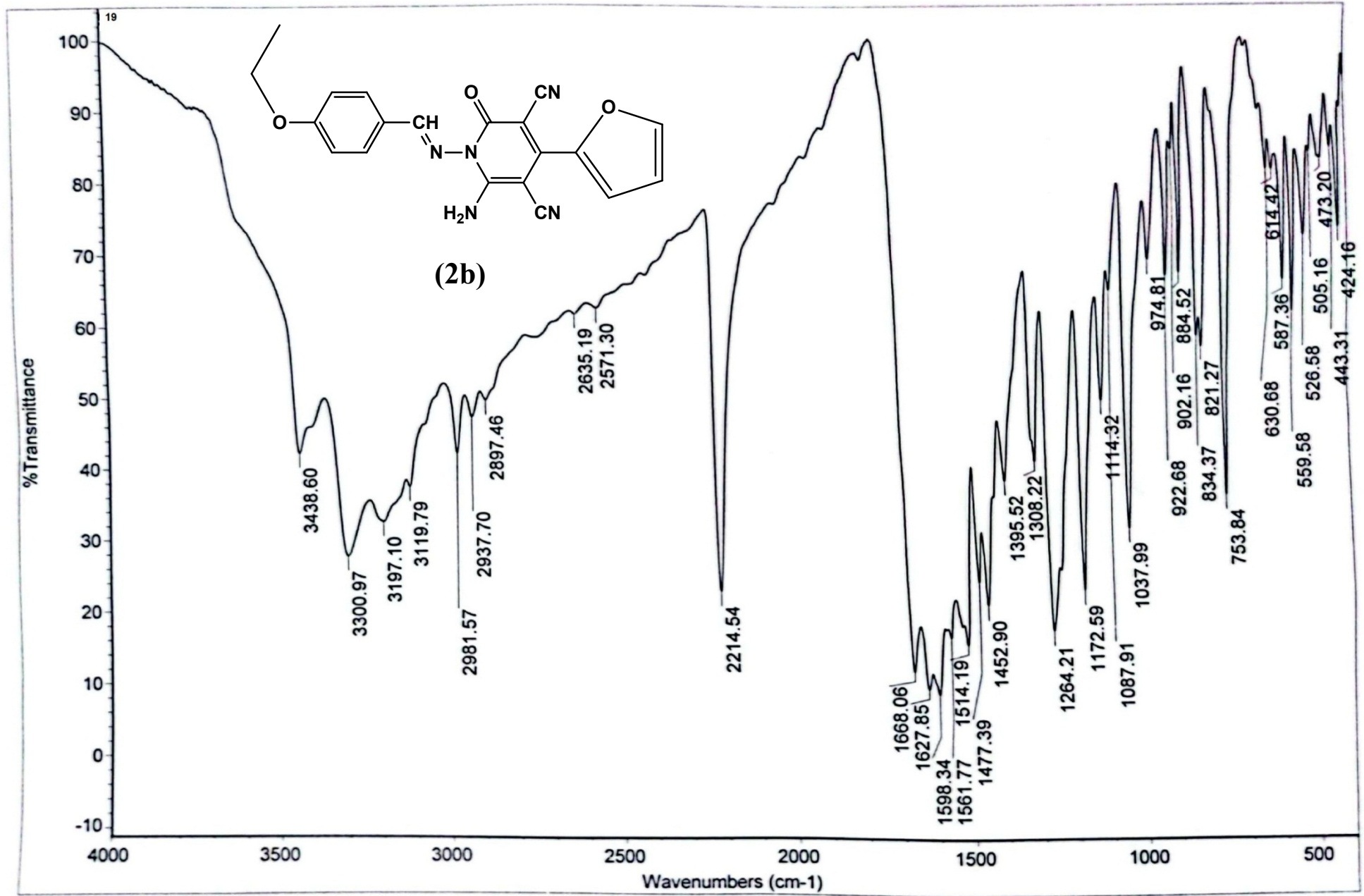


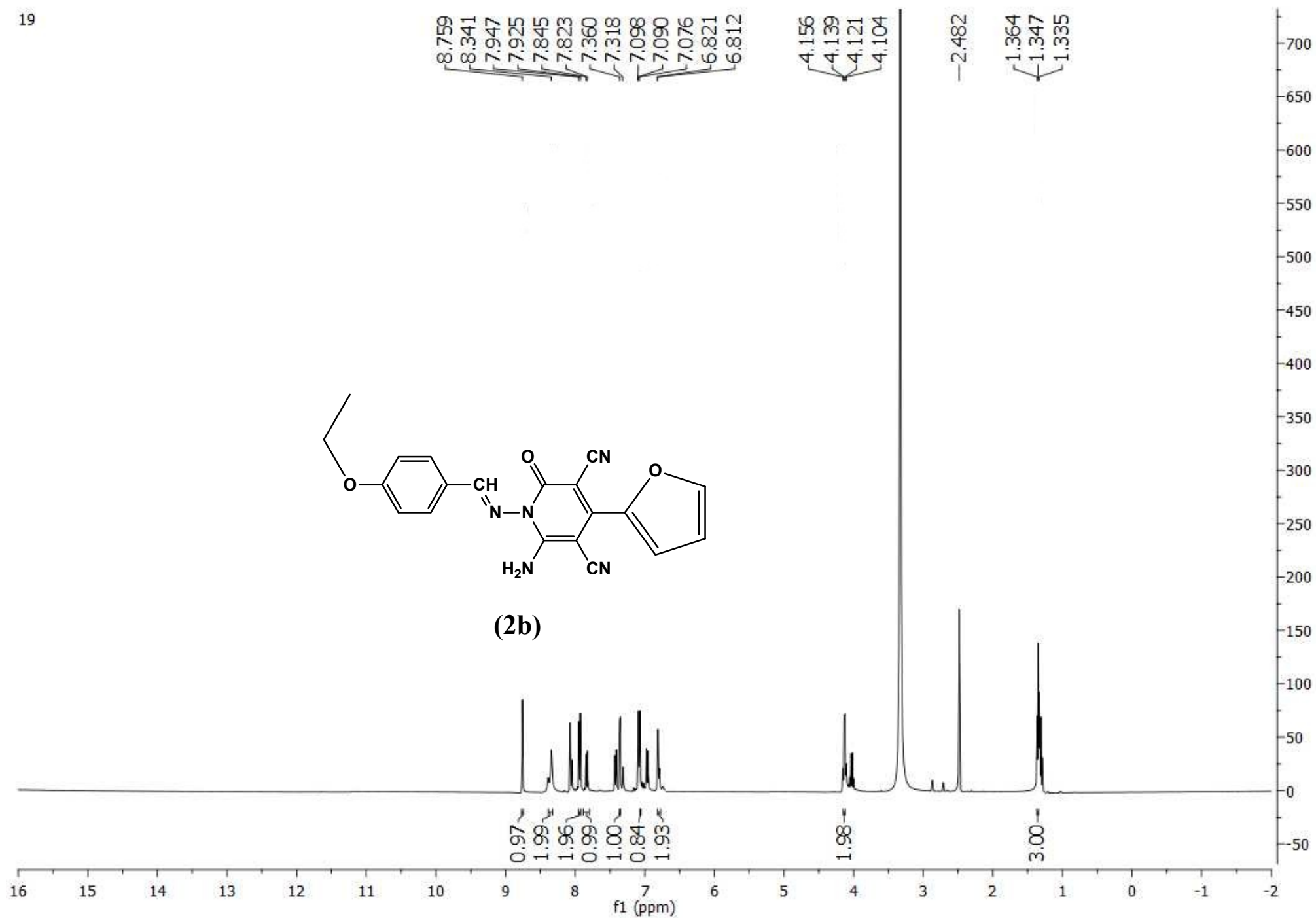


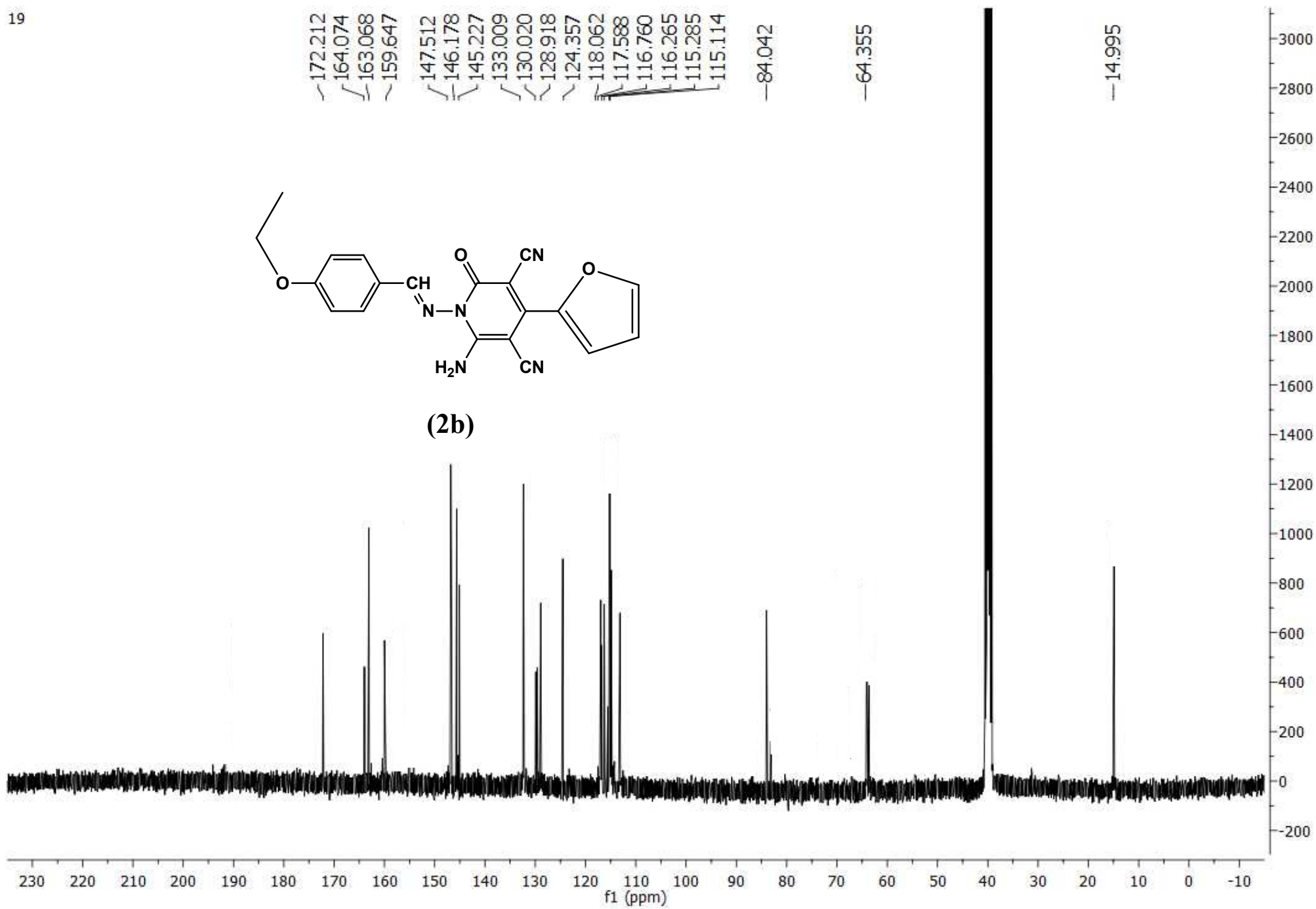


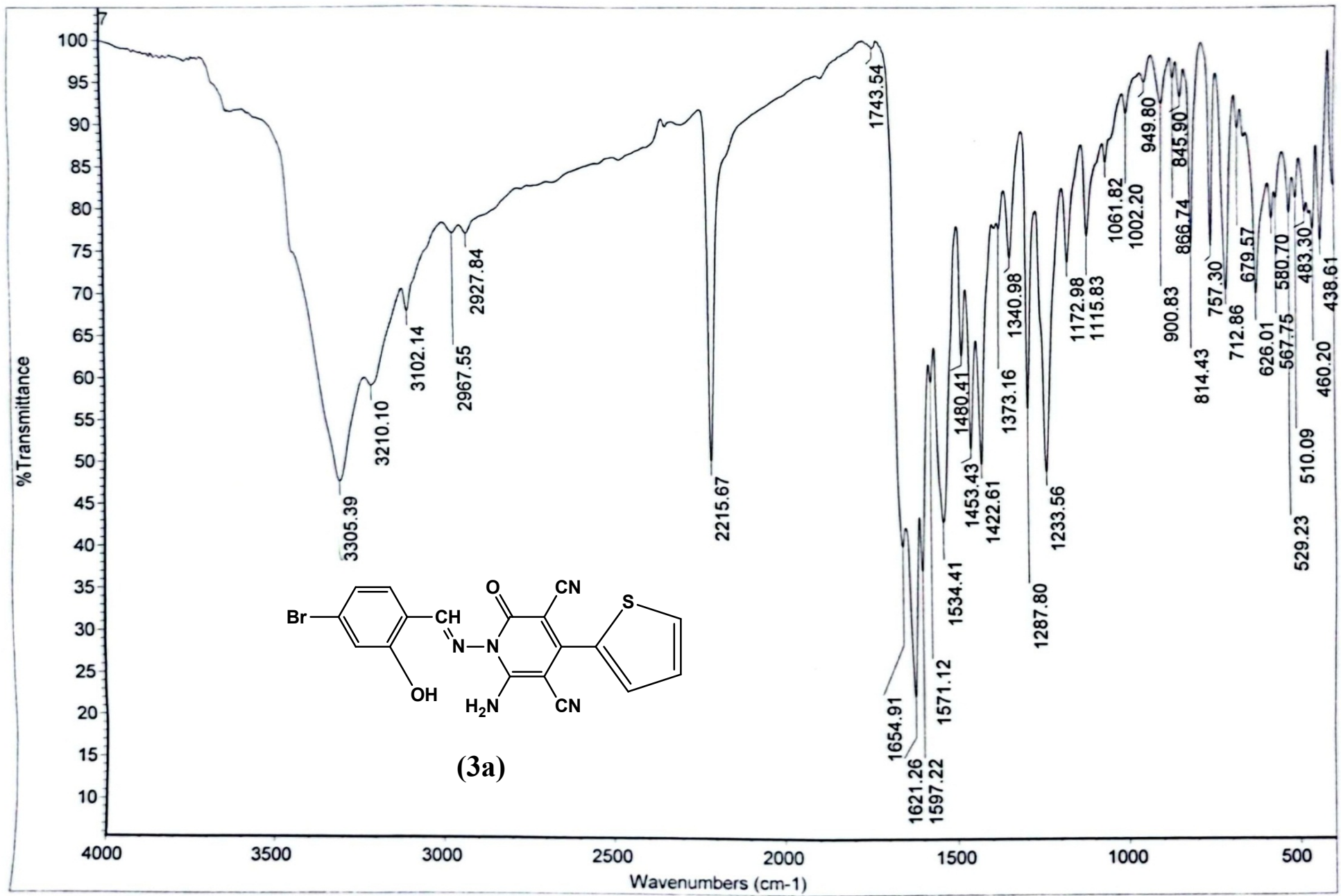


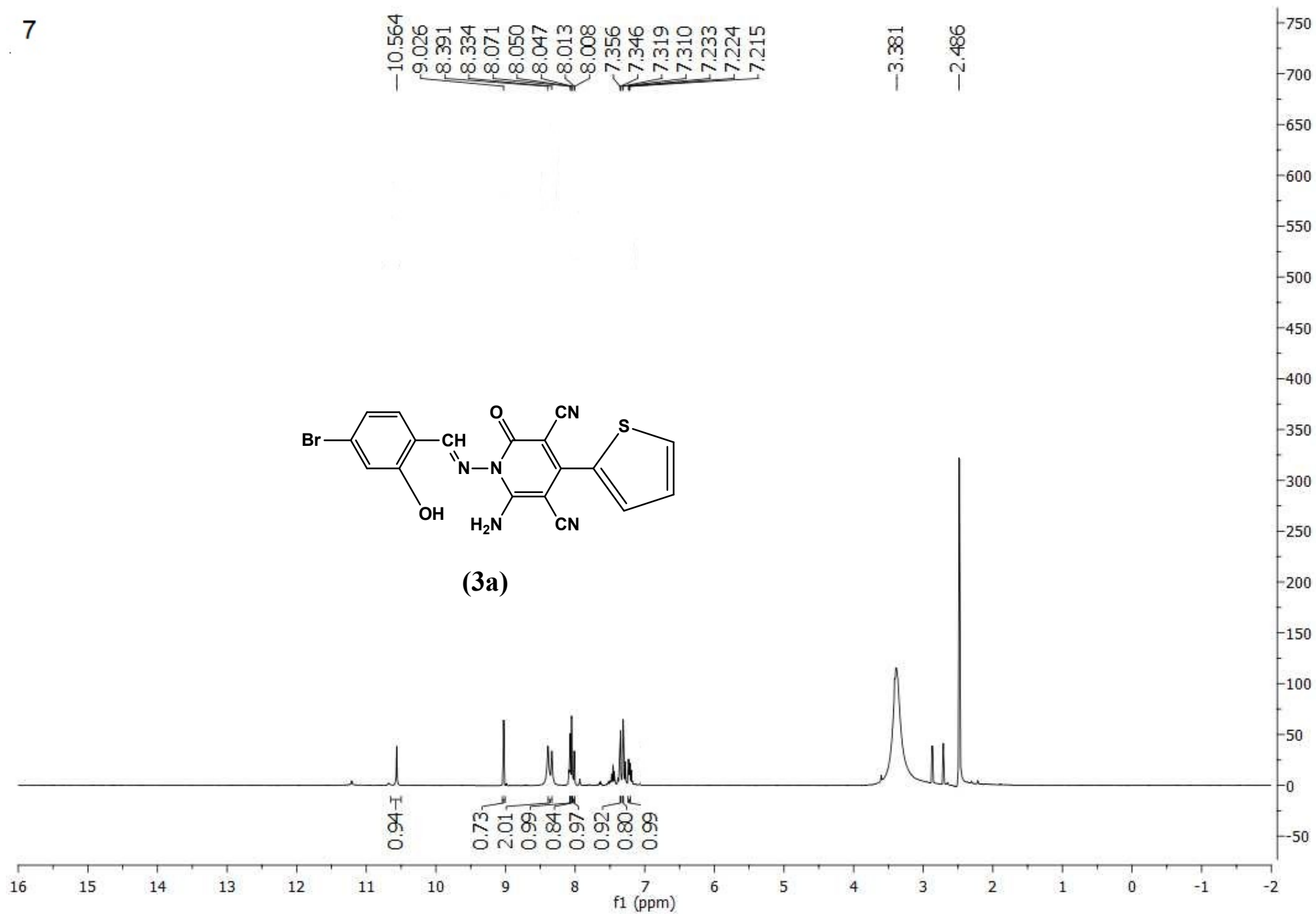


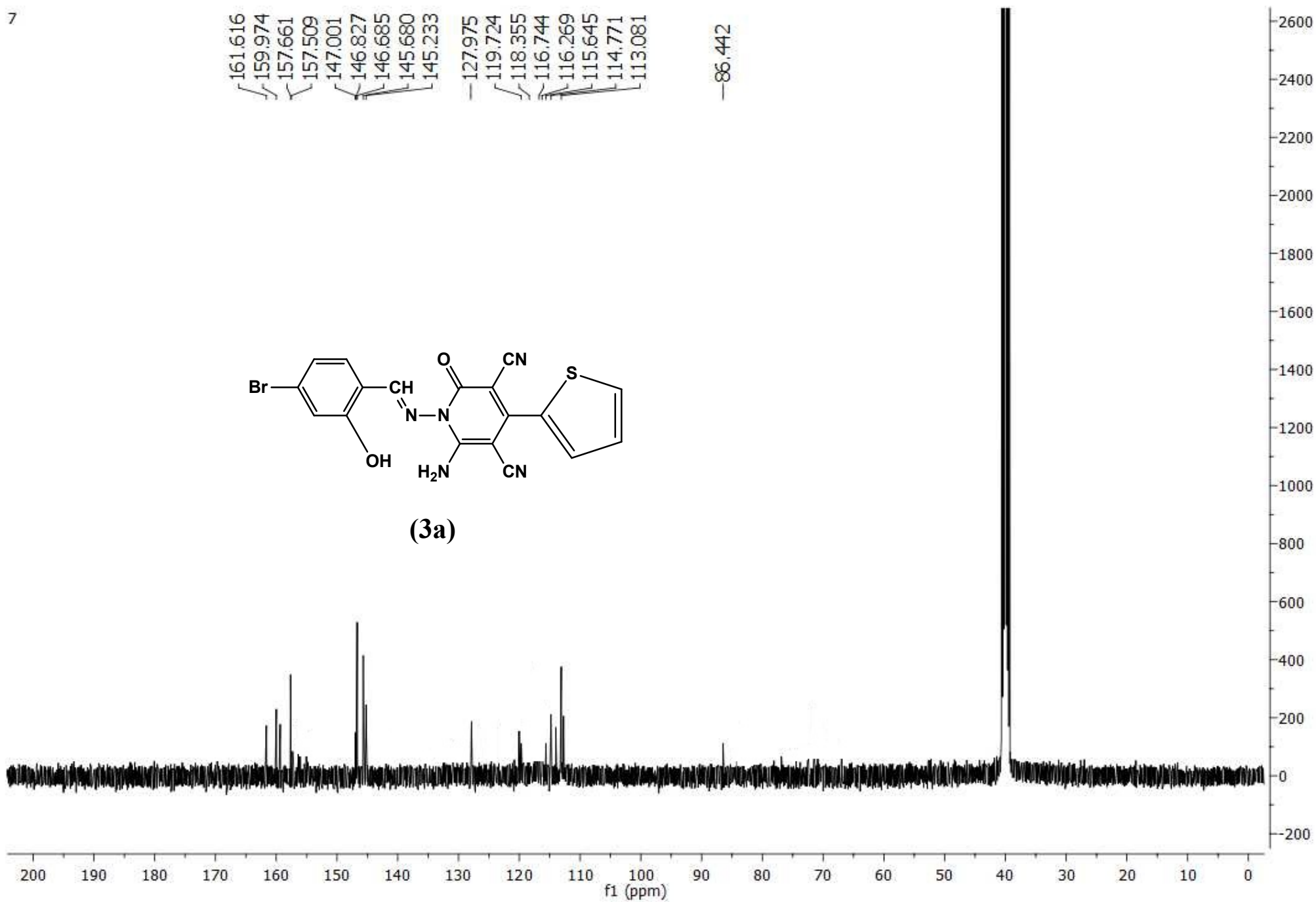


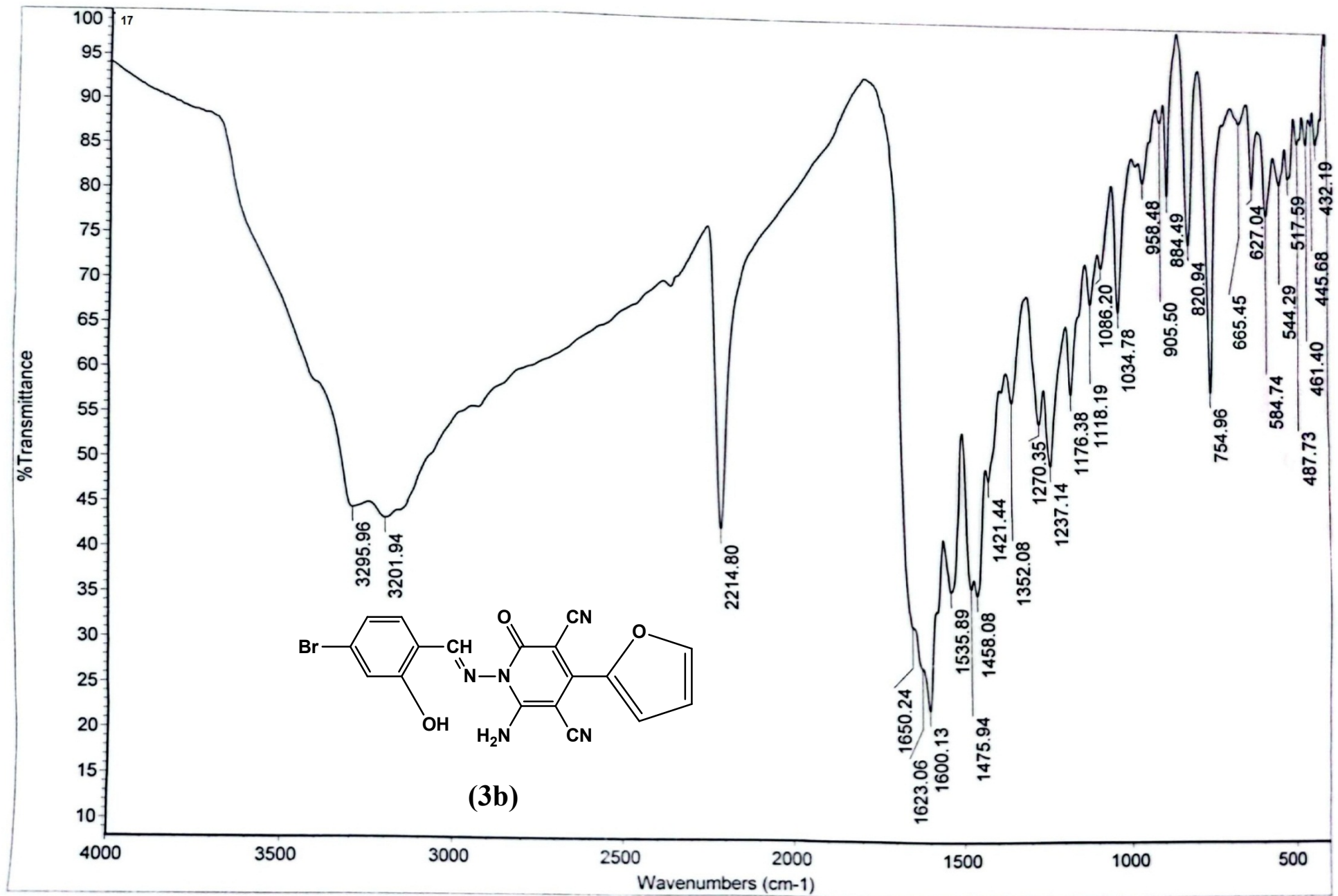


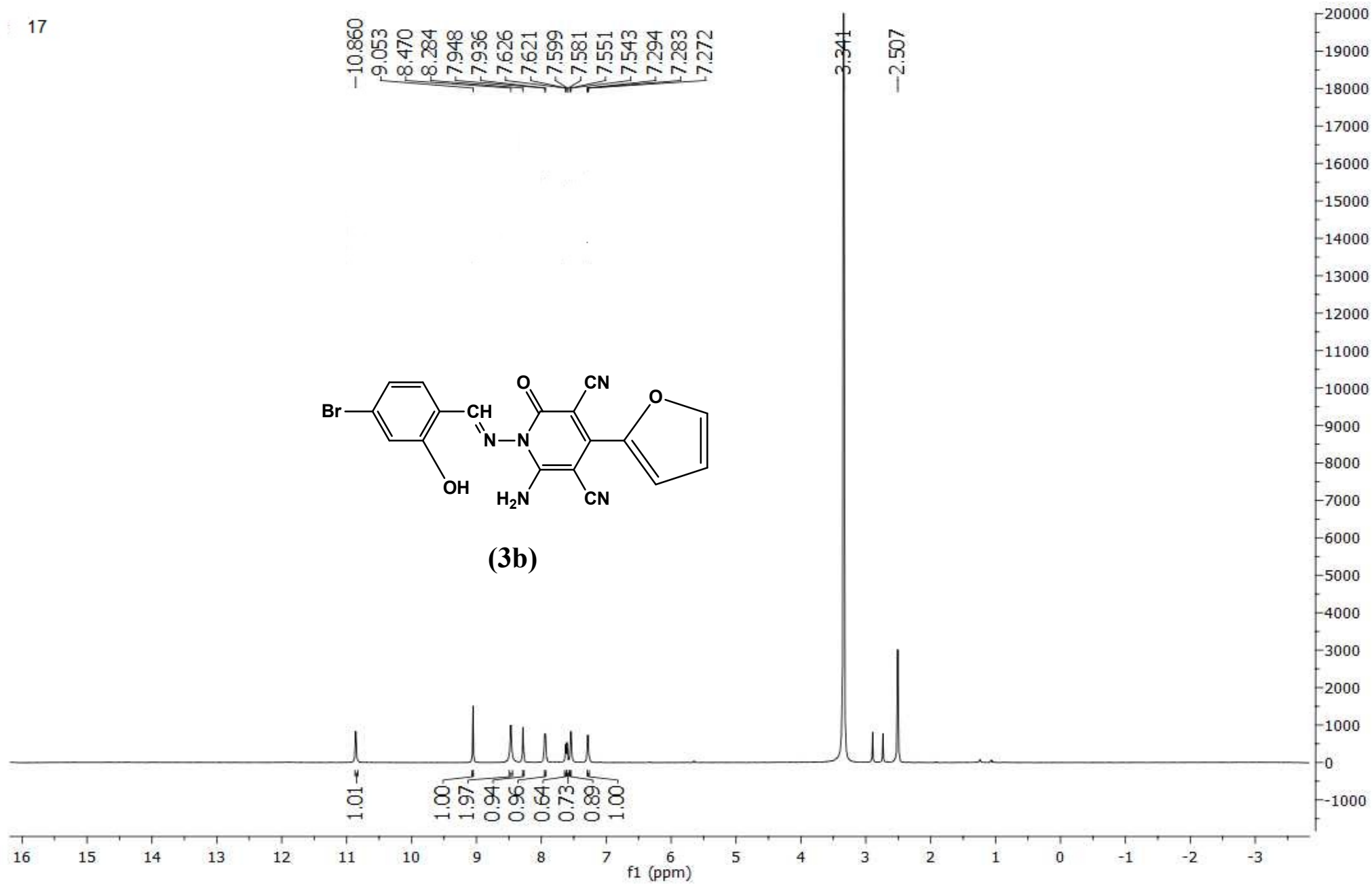




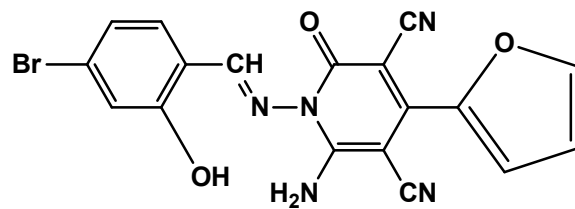




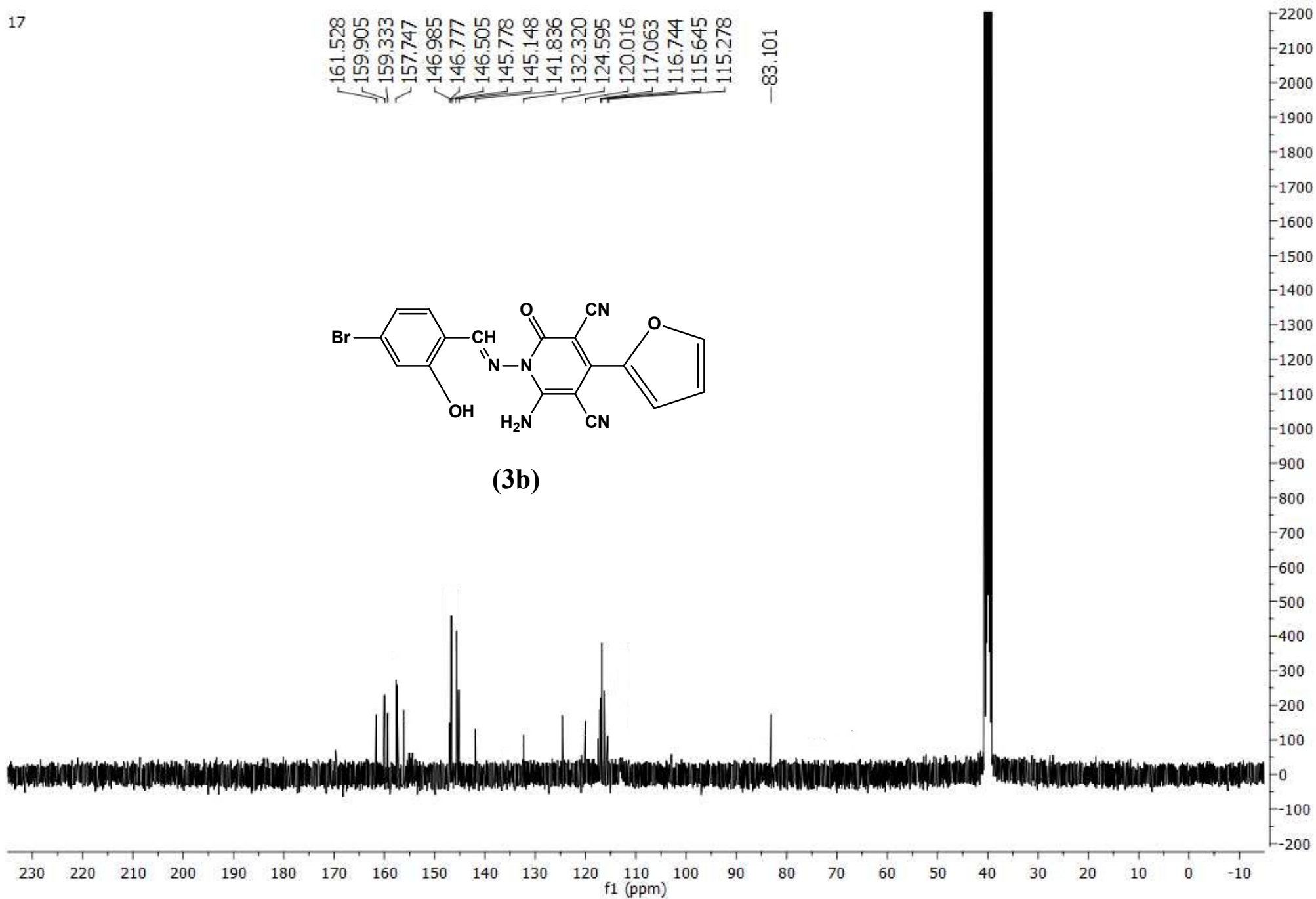


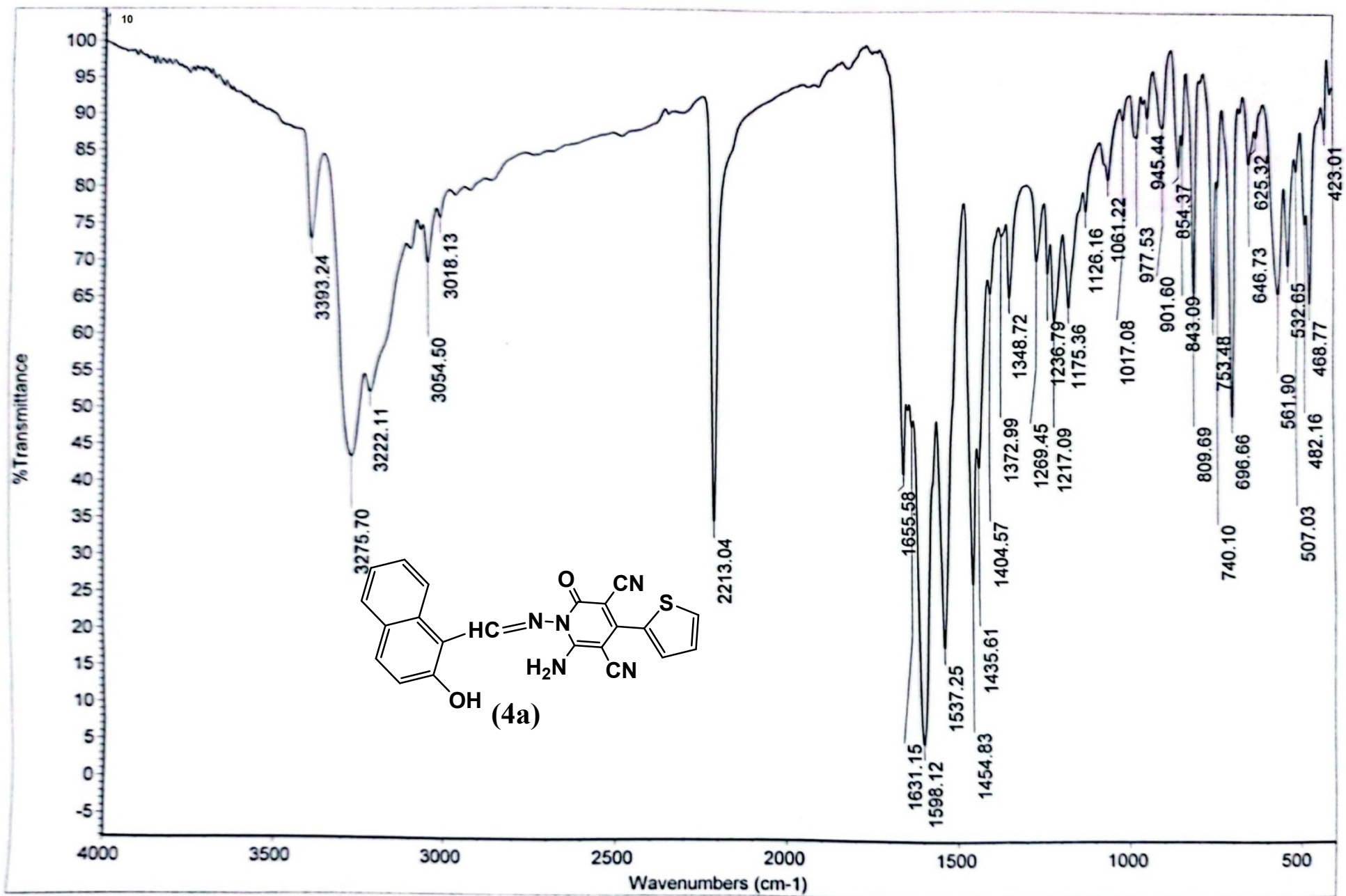


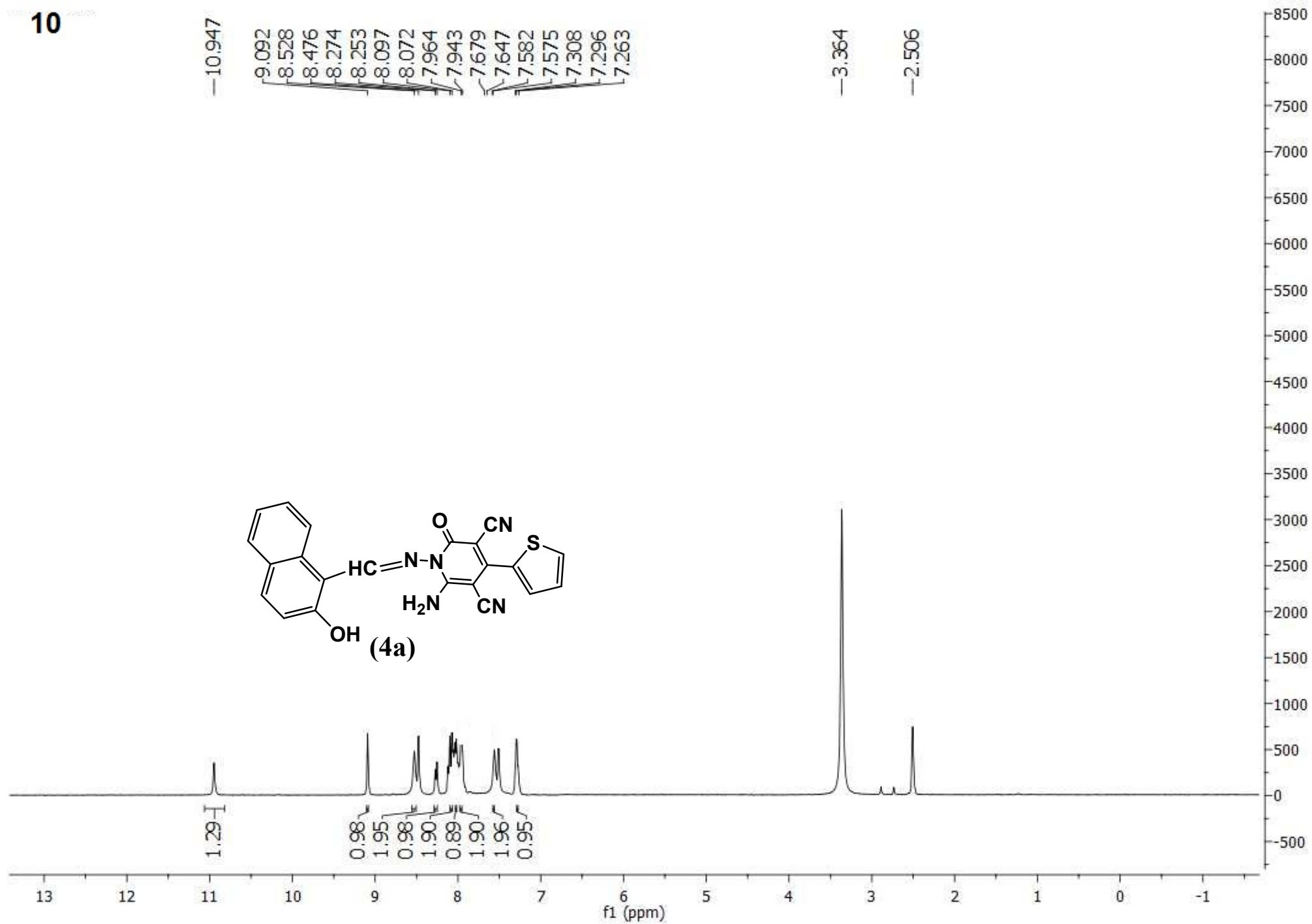
161.528
159.905
159.333
157.747
146.985
146.777
146.505
145.778
145.148
141.836
132.320
124.595
120.016
117.063
116.744
115.645
115.278
—83.101

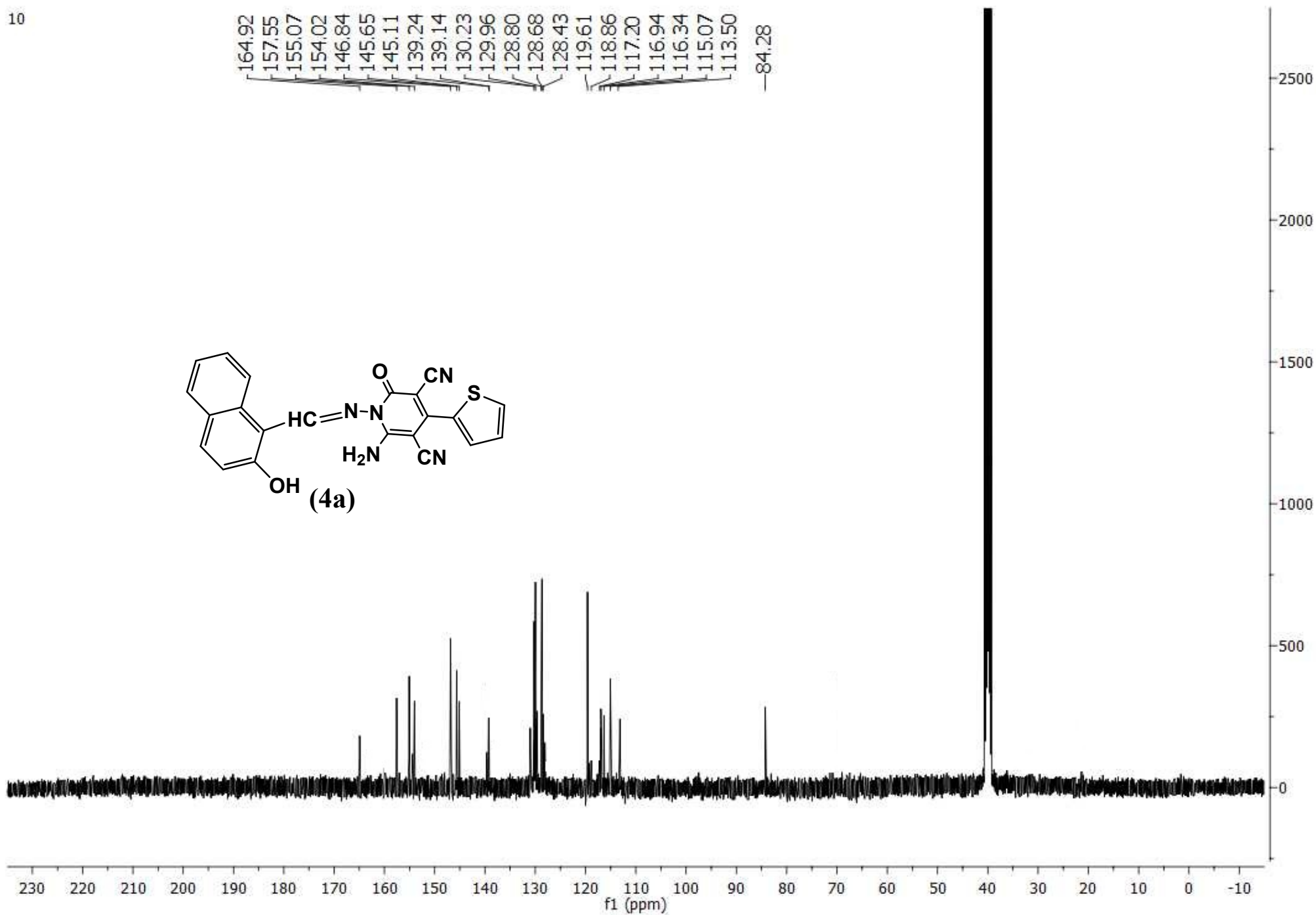


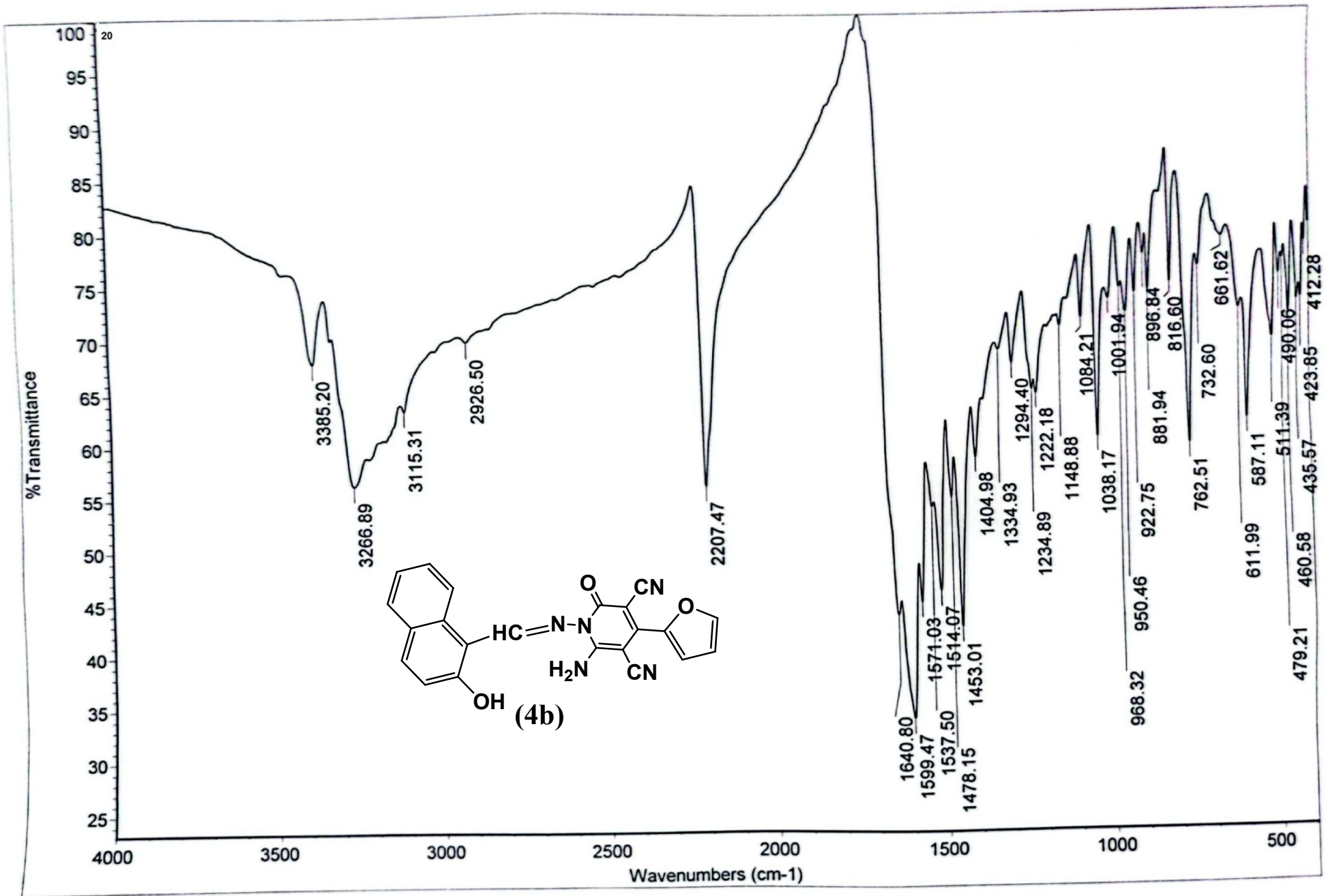
(3b)

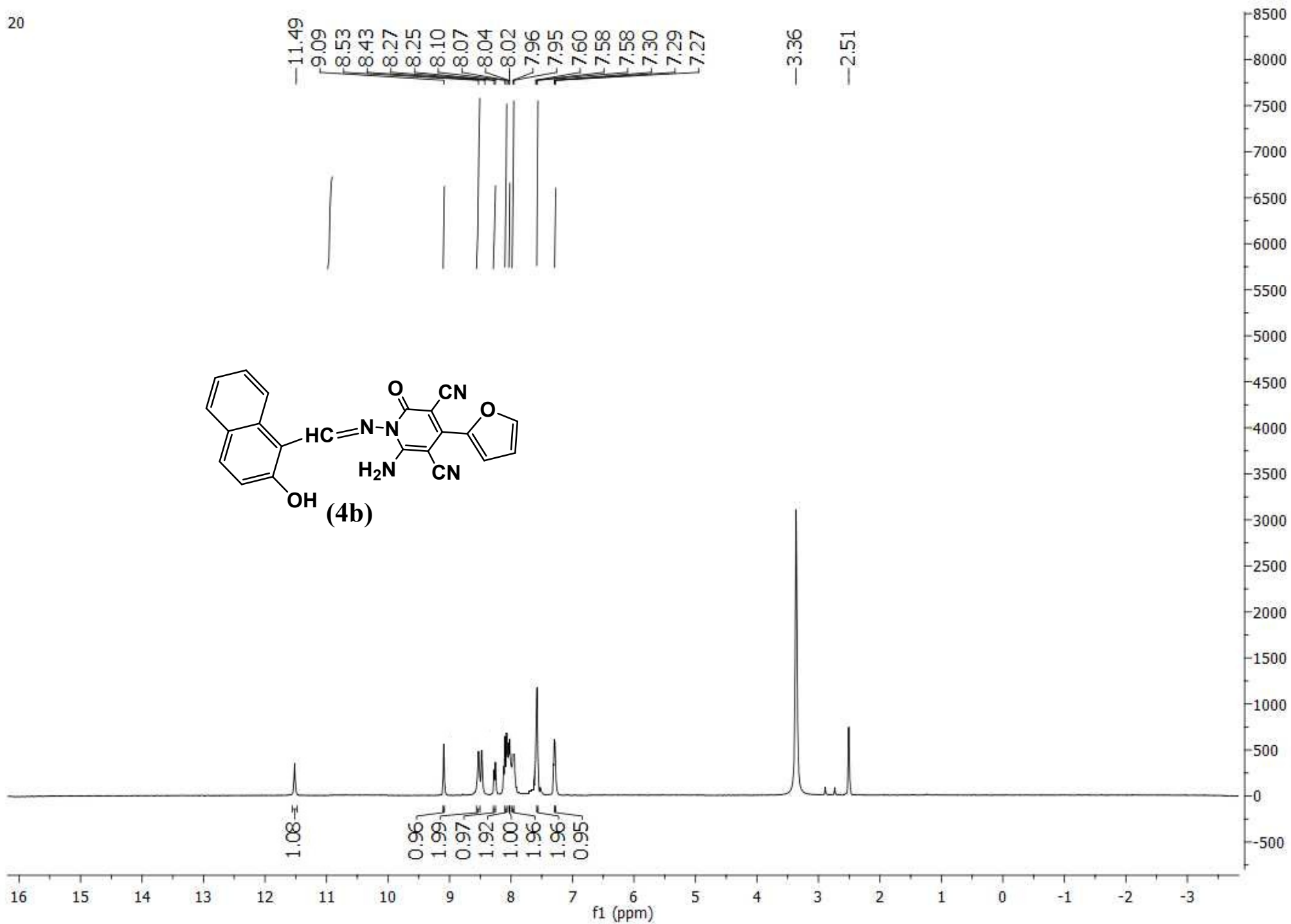


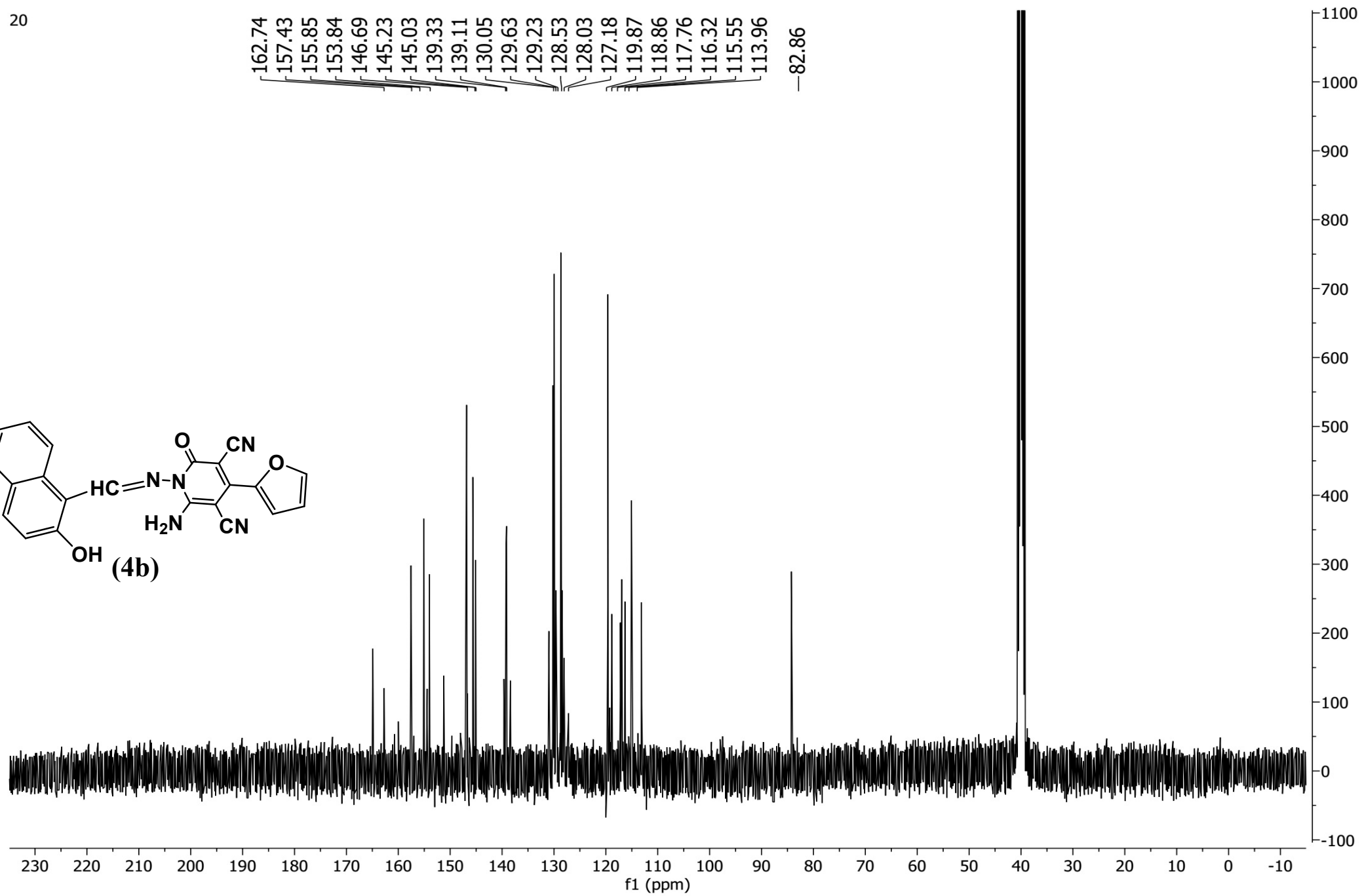
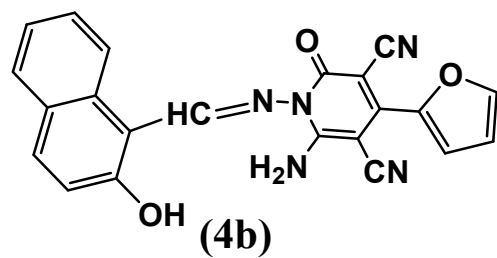


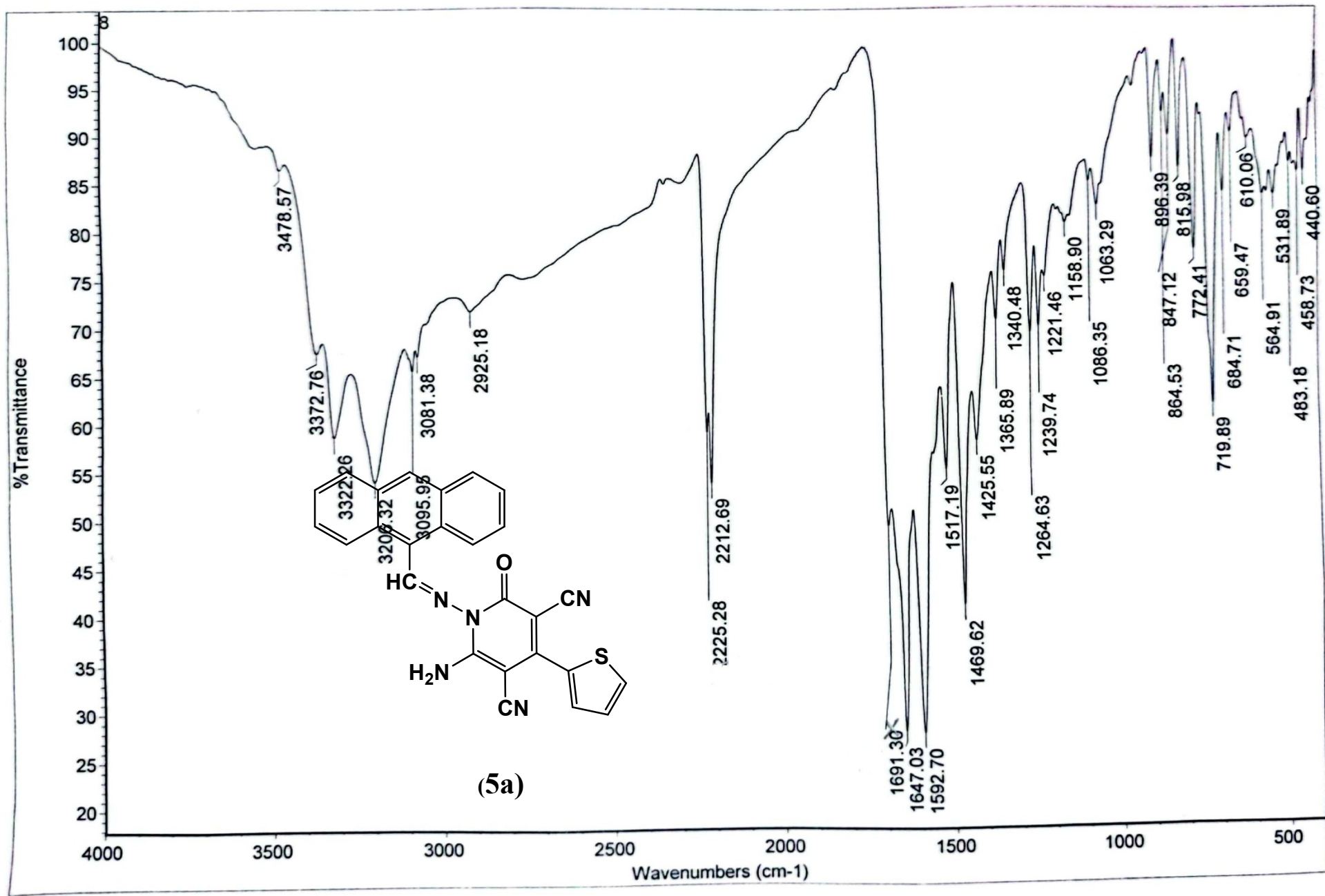


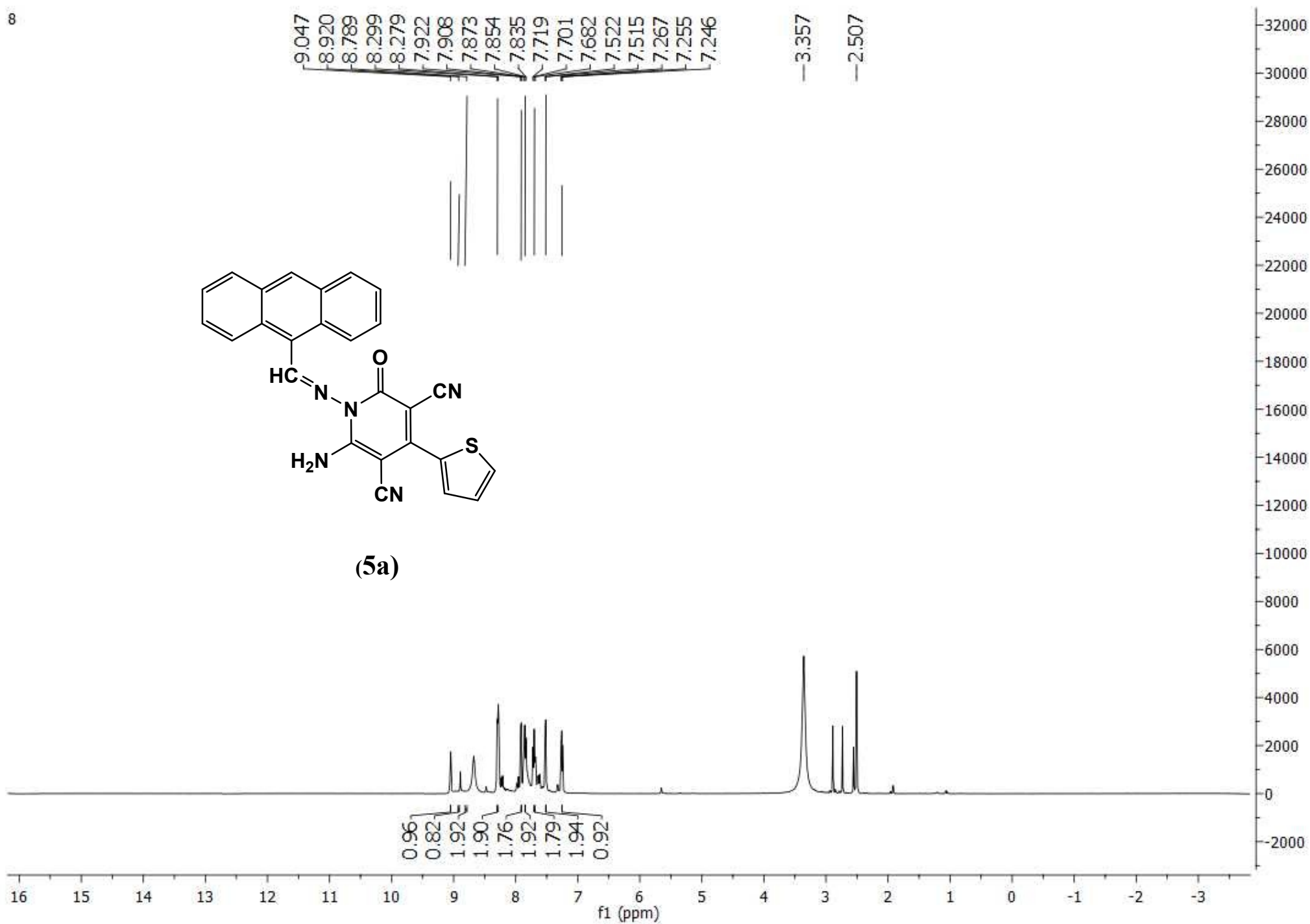


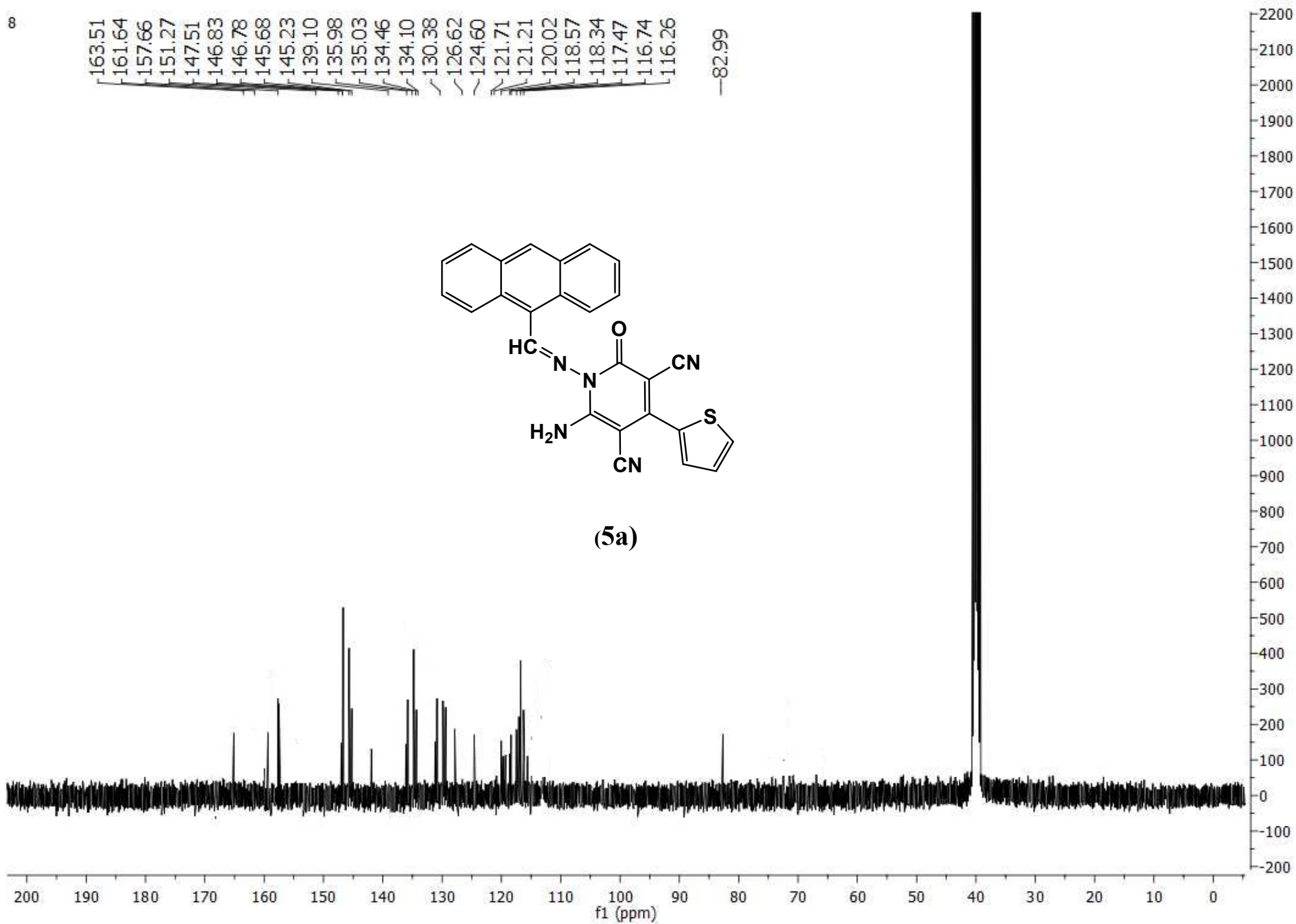


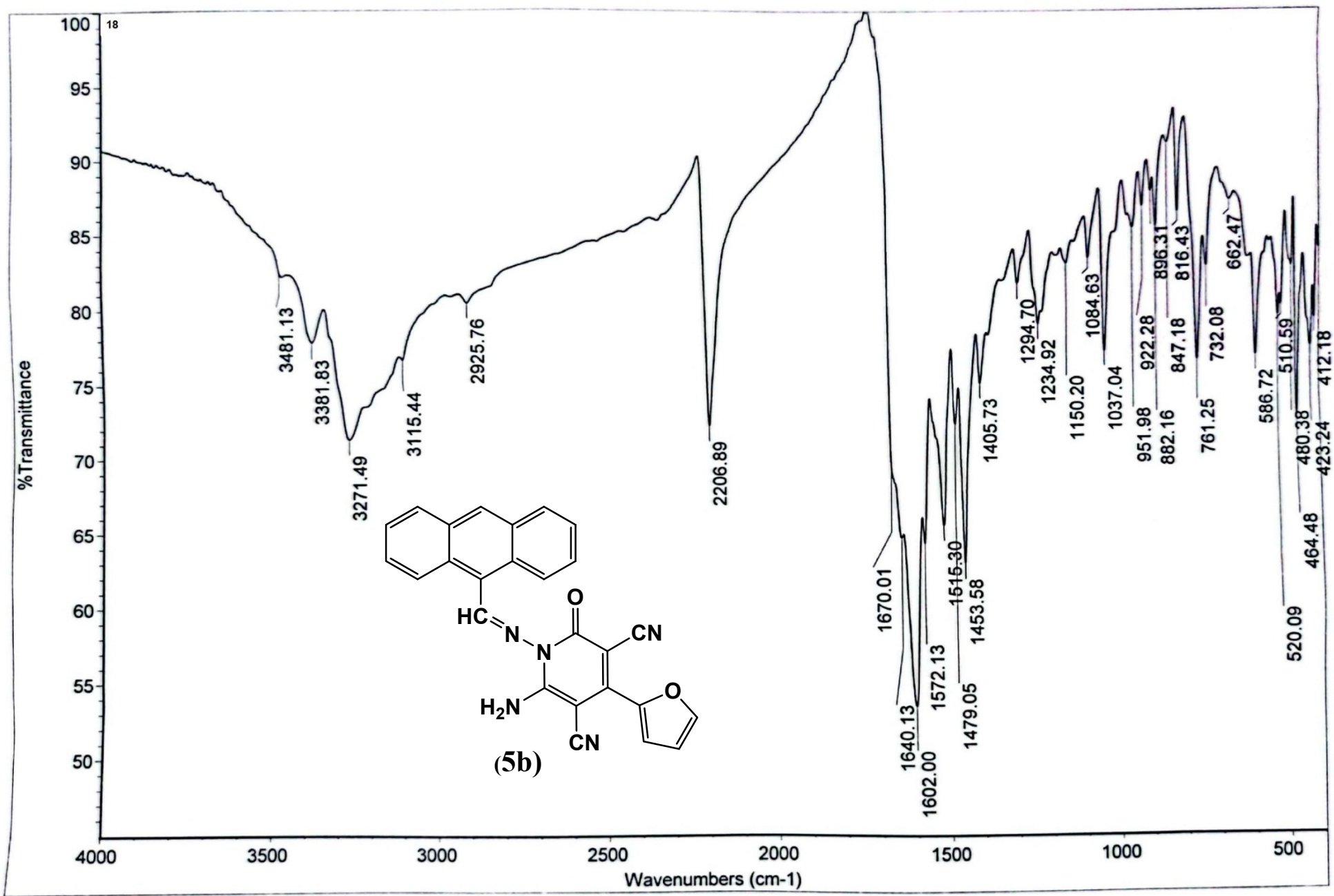


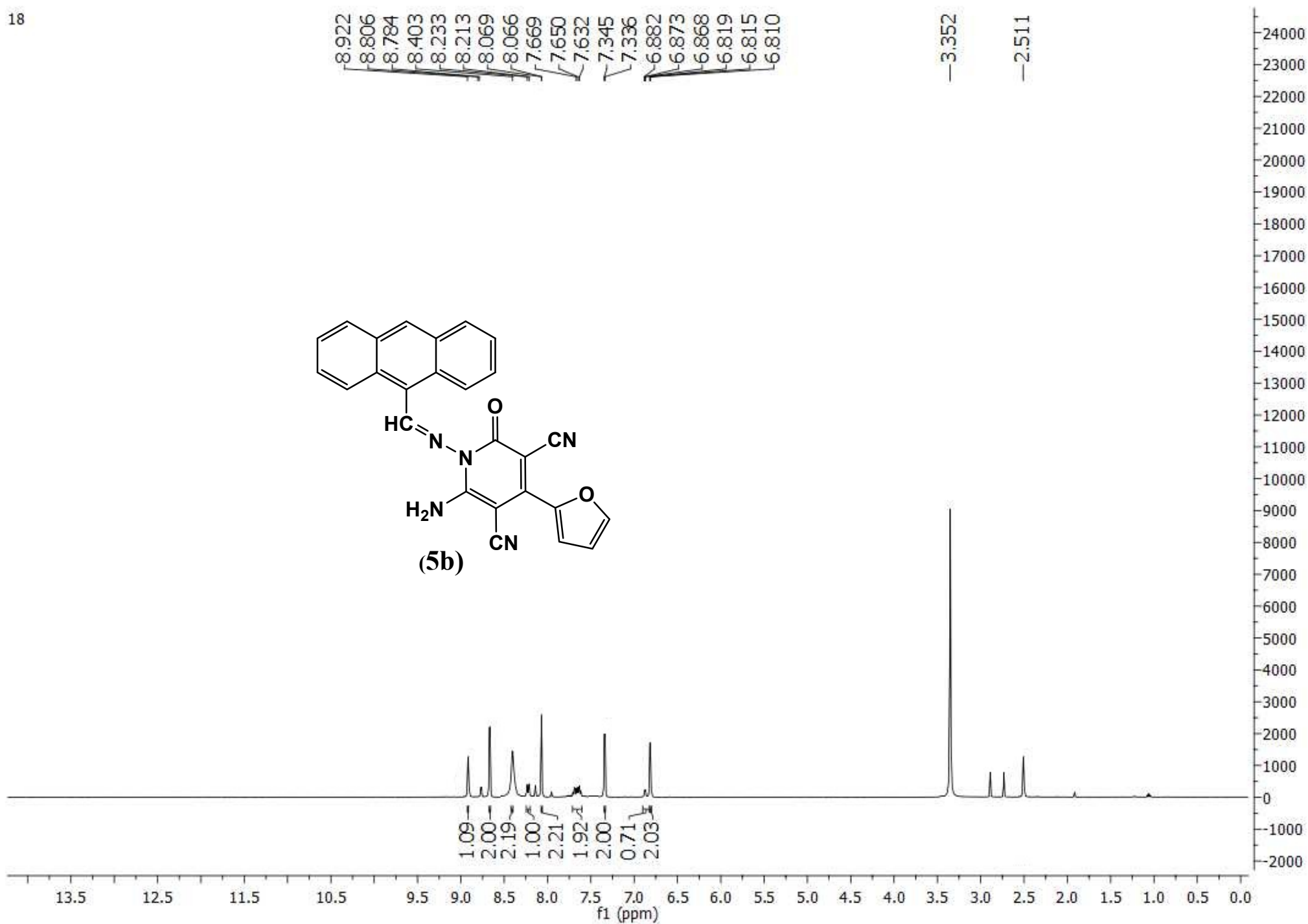


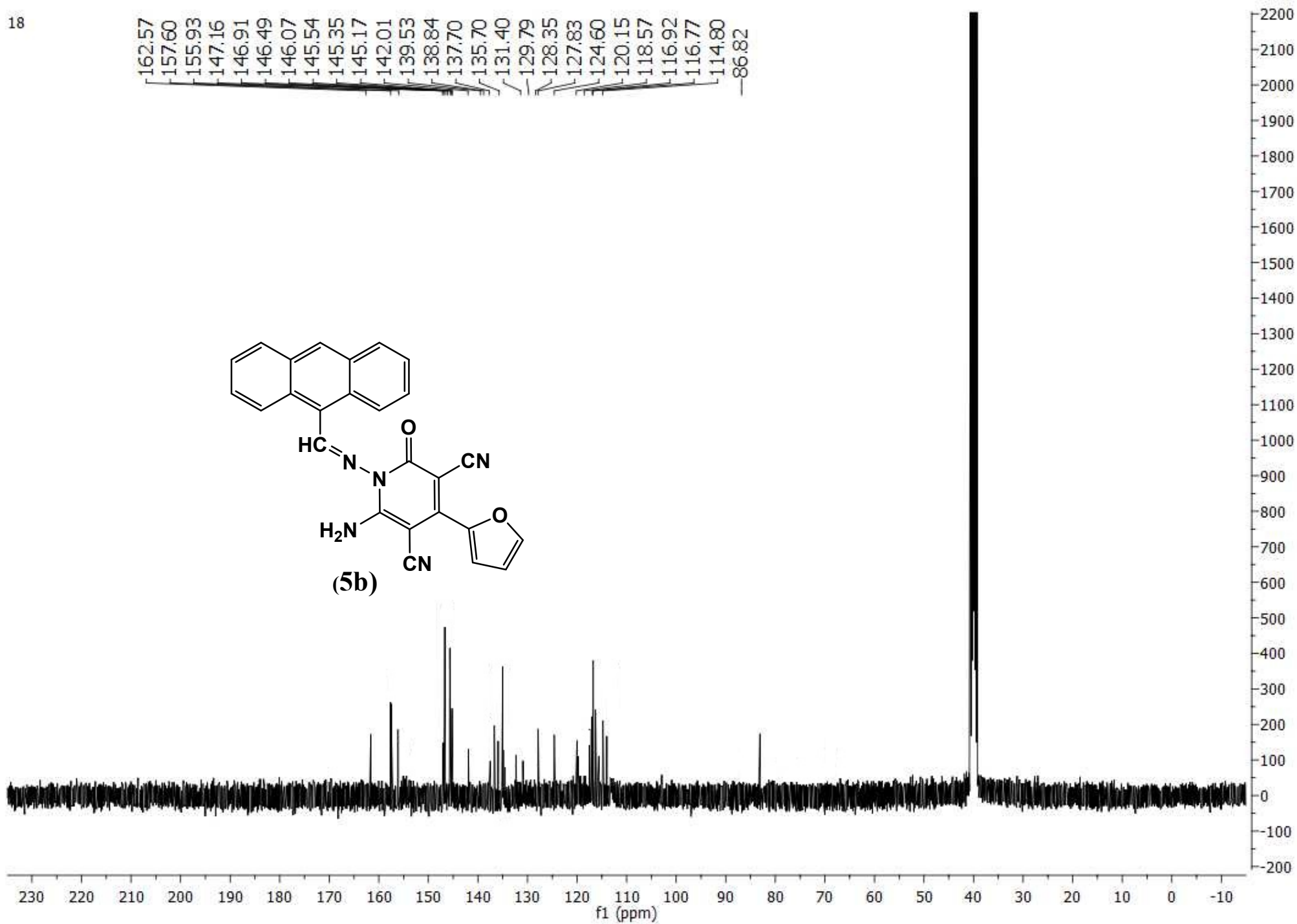


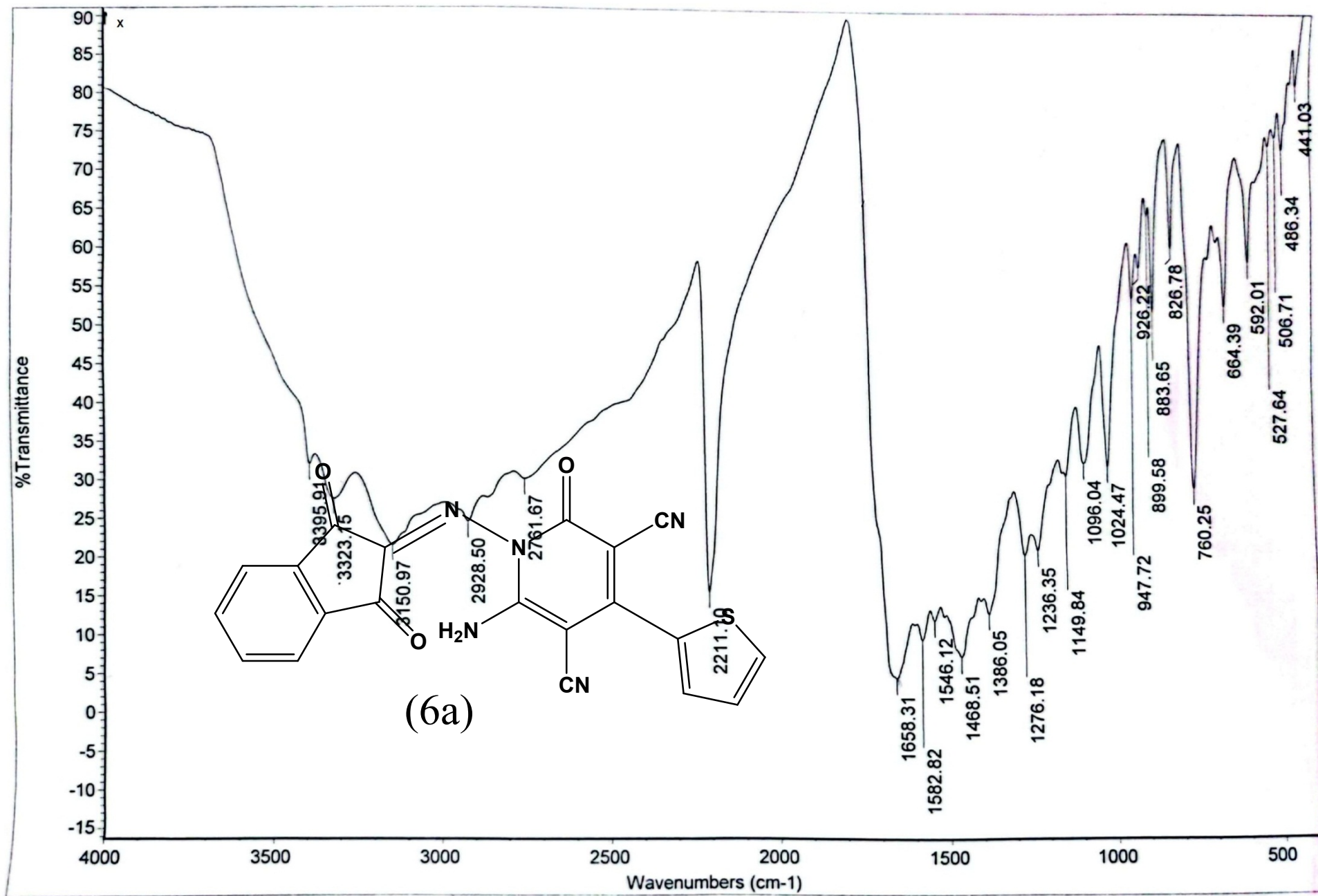




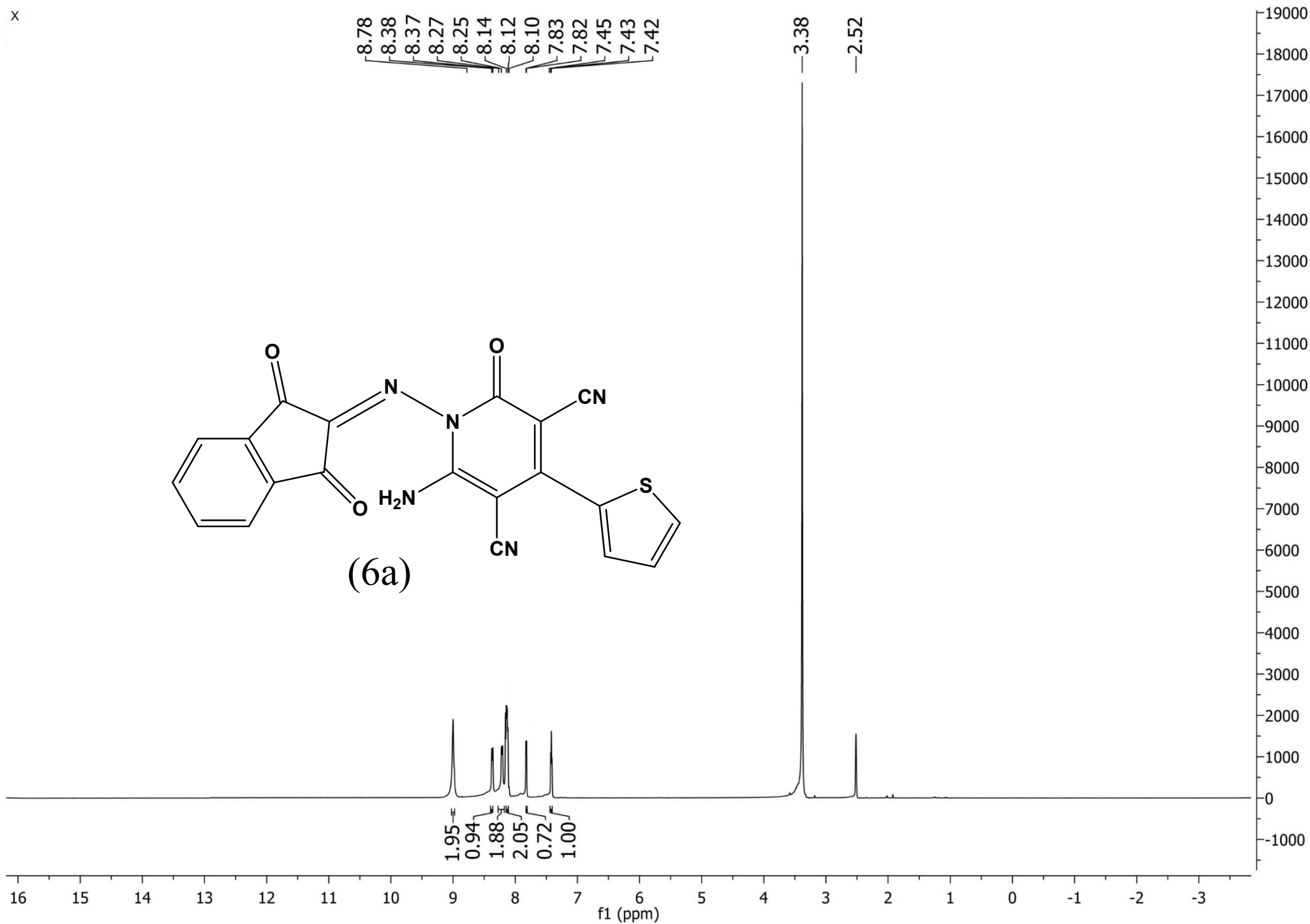








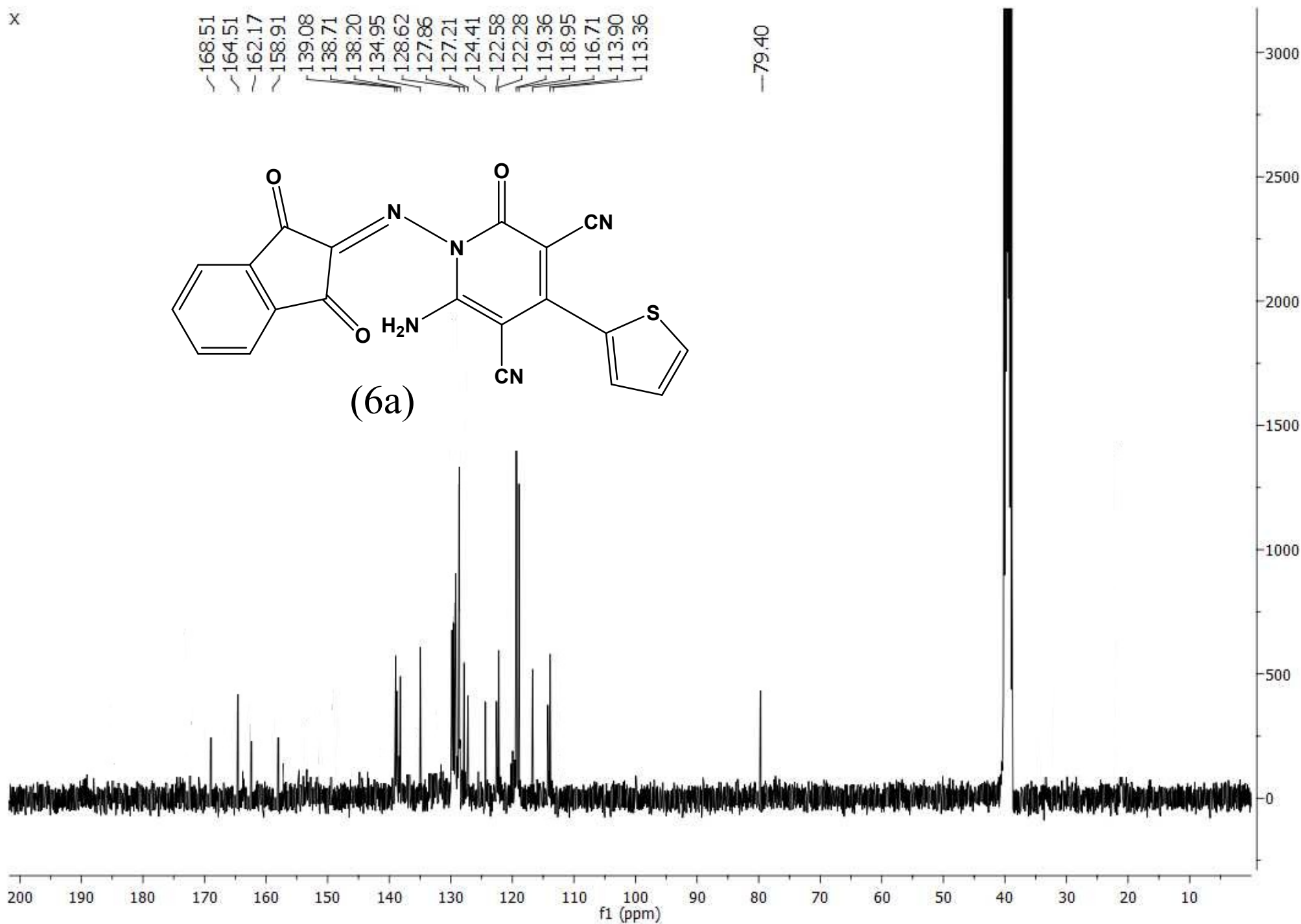
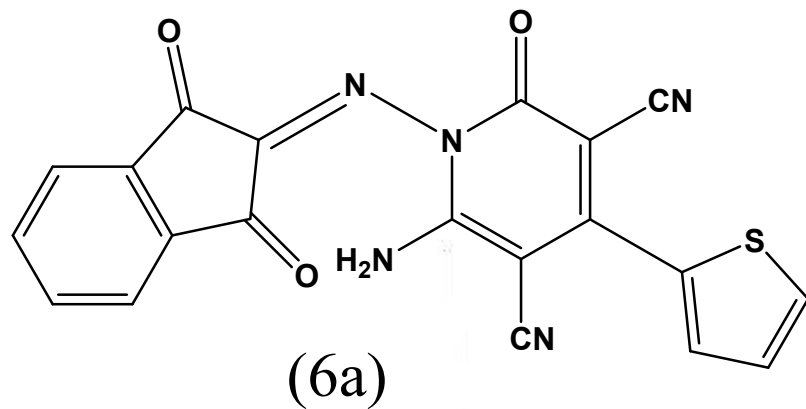
x

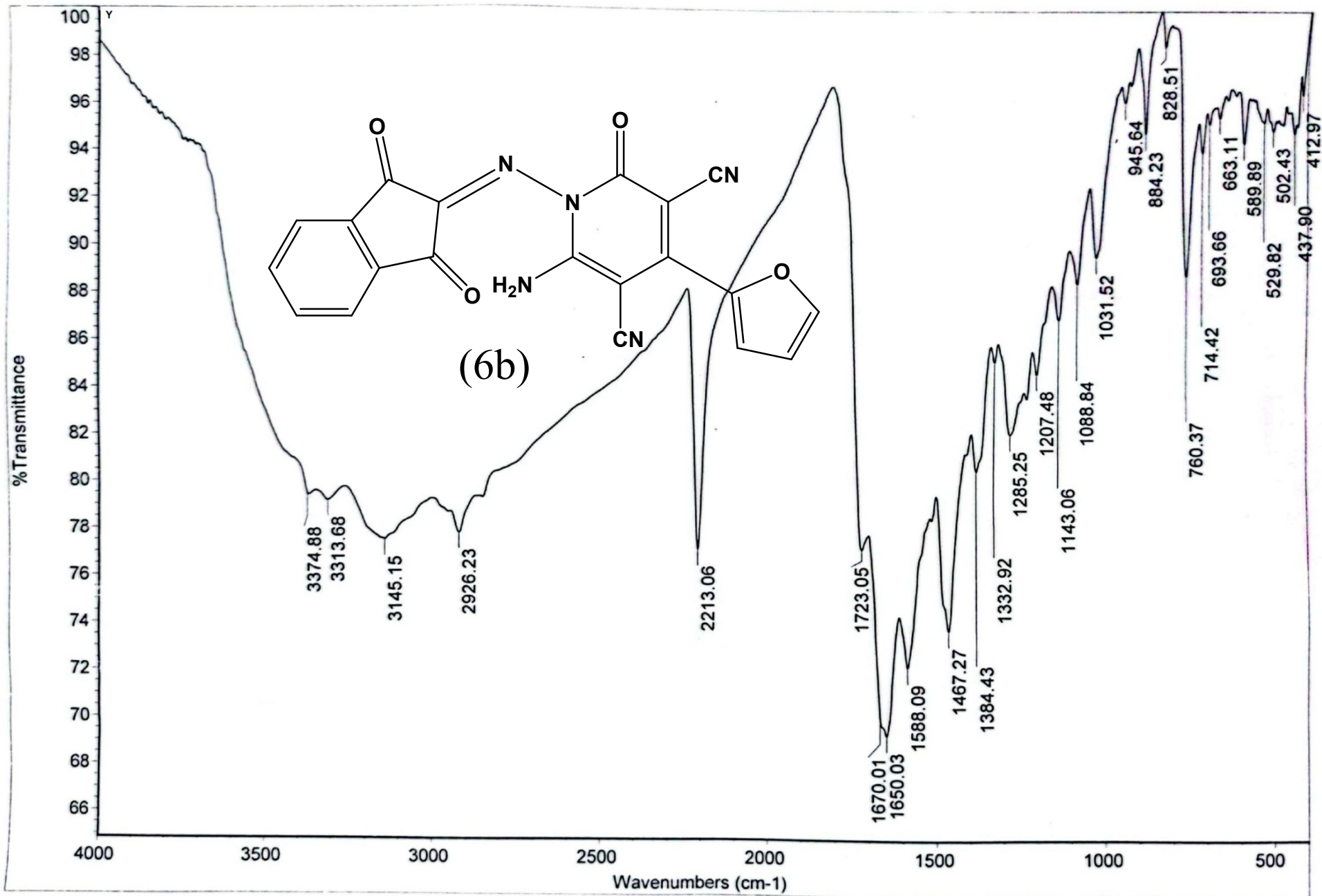


x

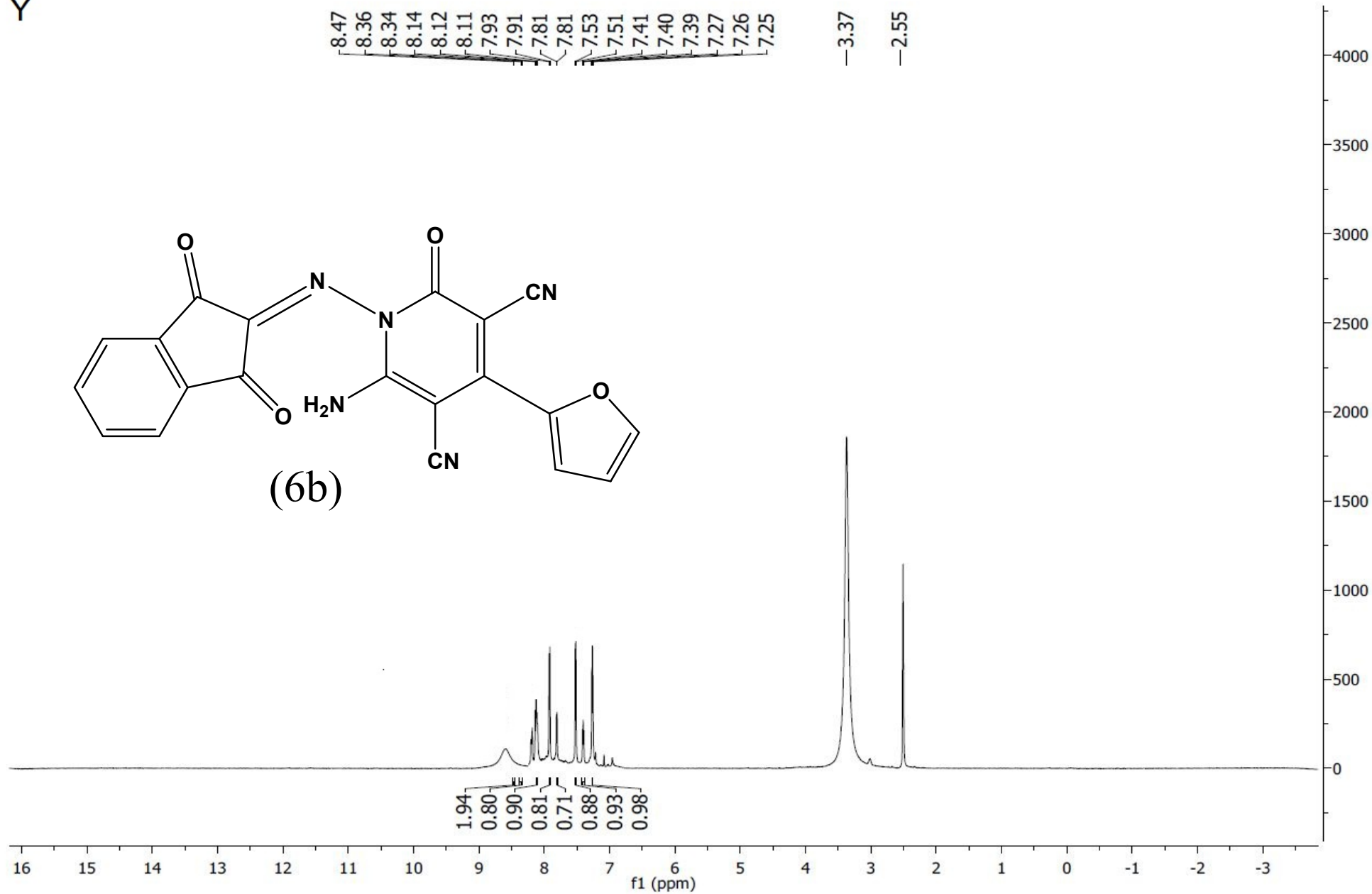
~168.51
~164.51
~162.17
~158.91
139.08
138.71
138.20
134.95
128.62
127.86
127.21
124.41
122.58
122.28
119.36
118.95
116.71
113.90
113.36

—79.40

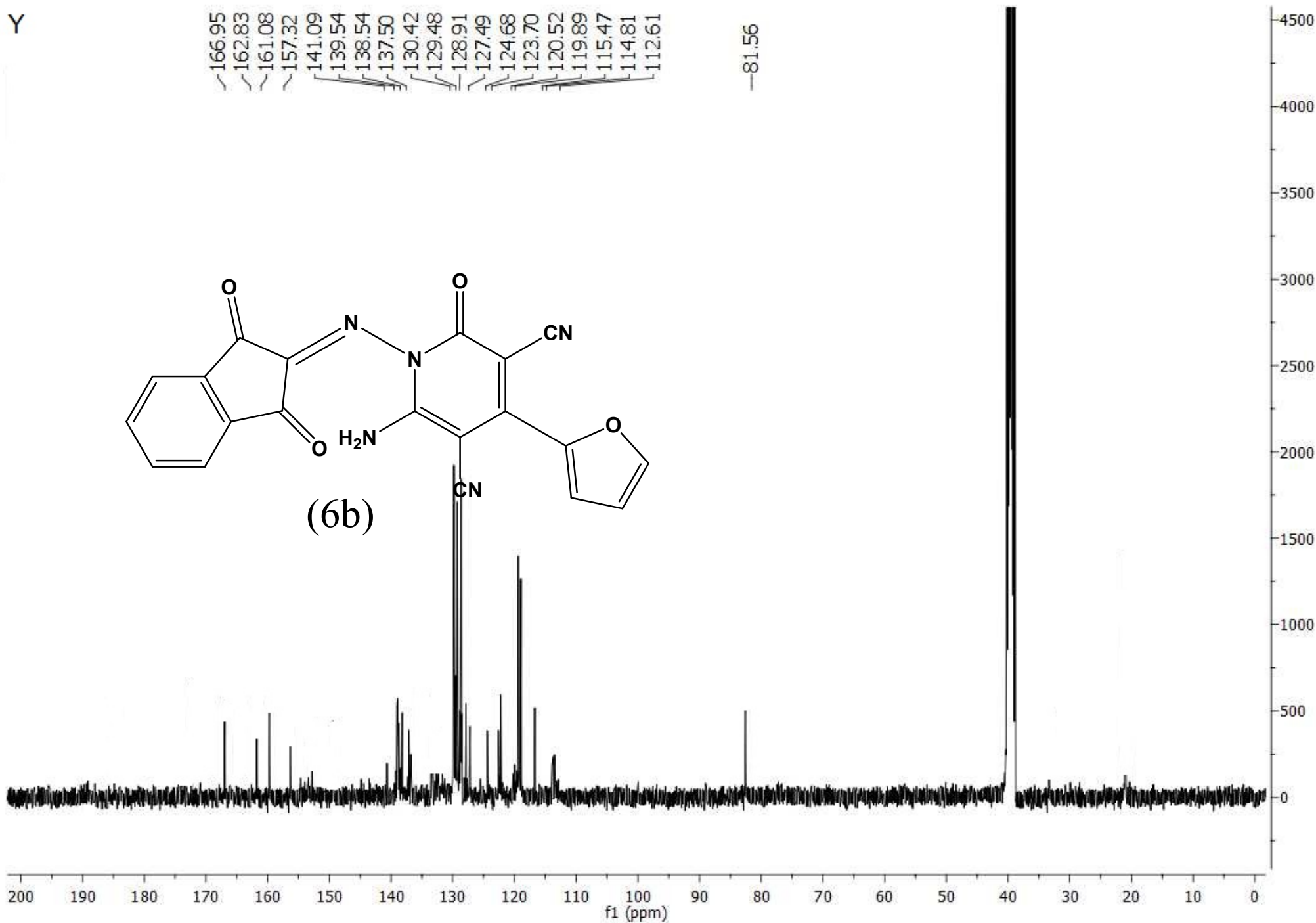


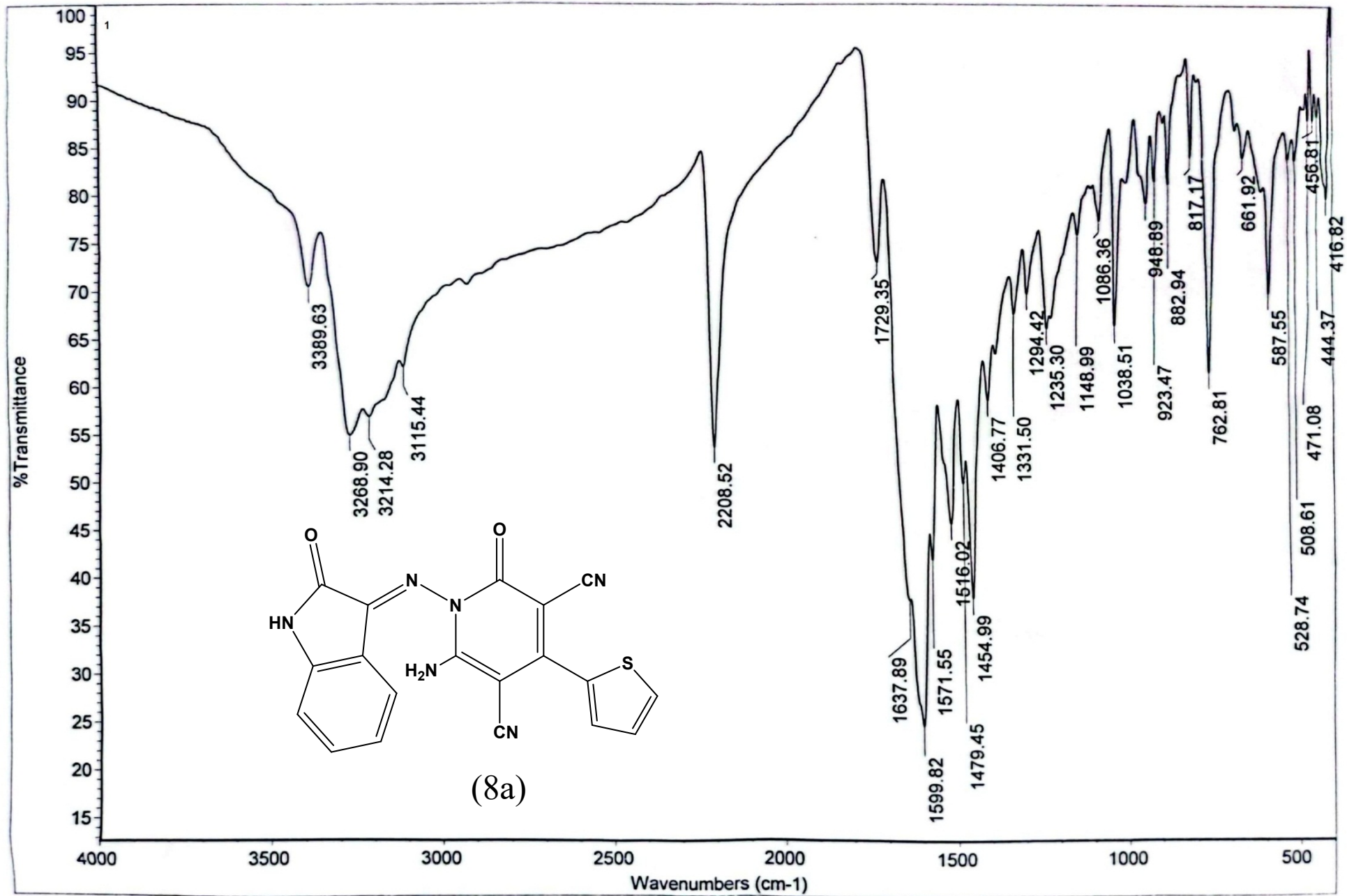


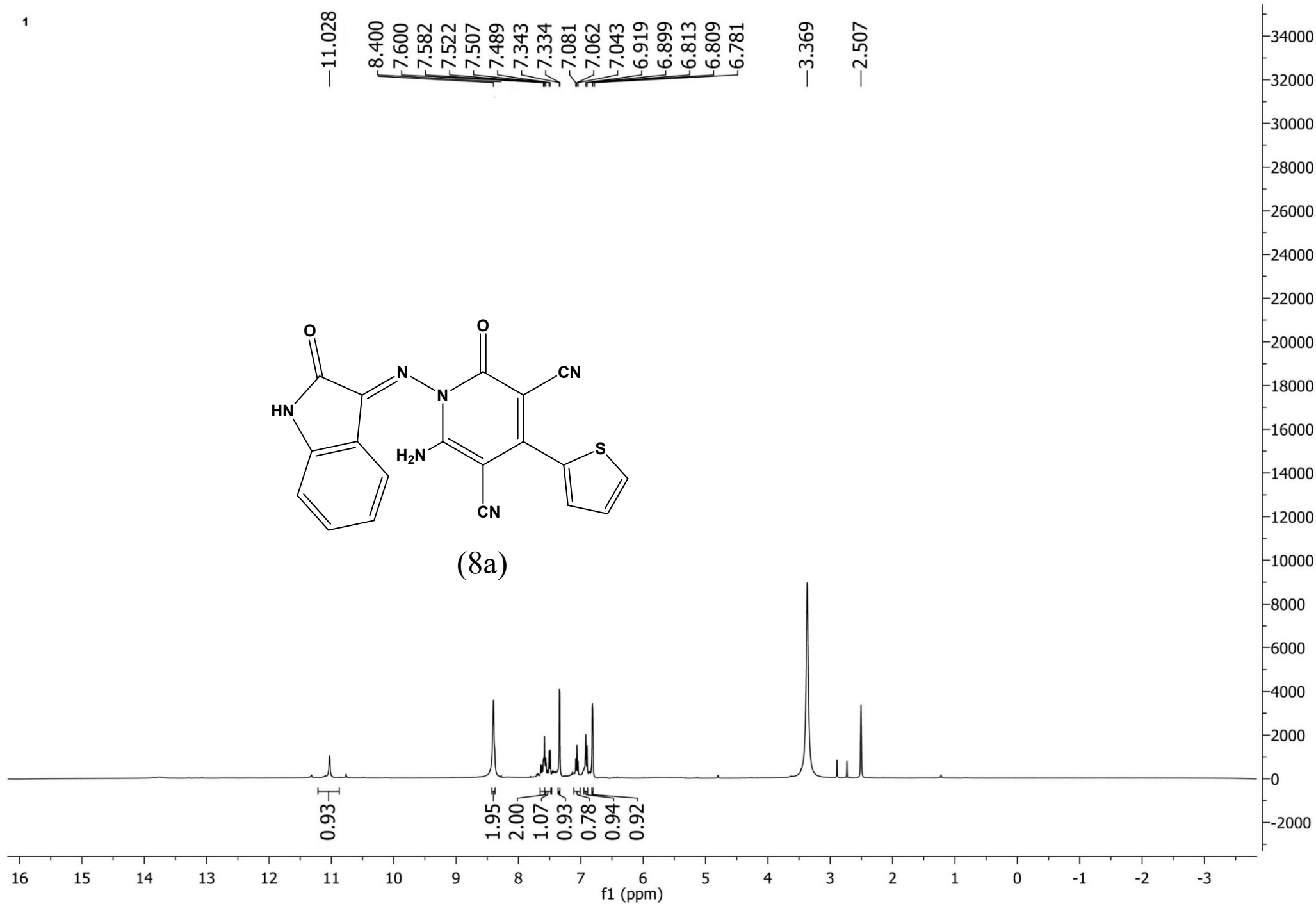
Y



Y



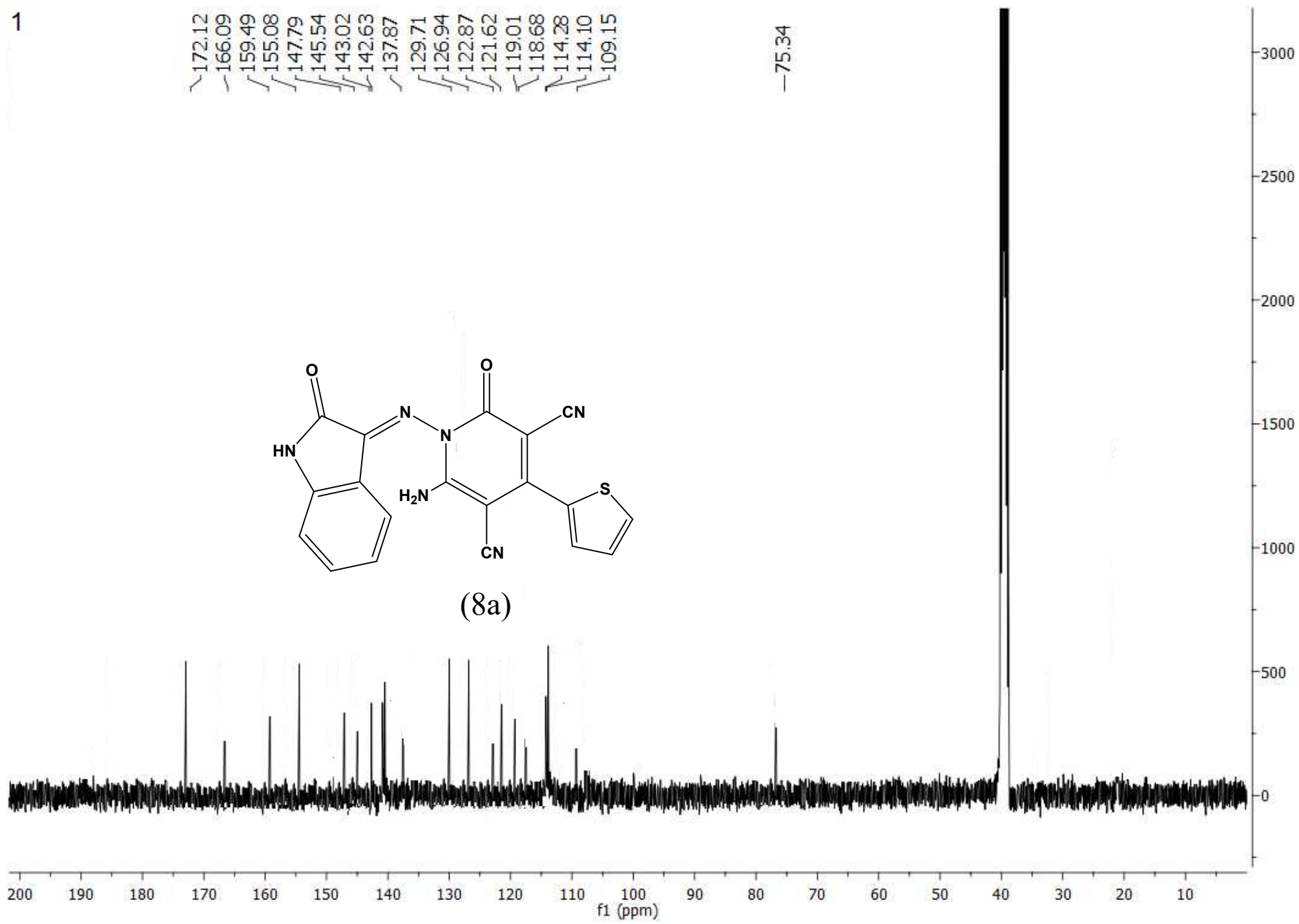
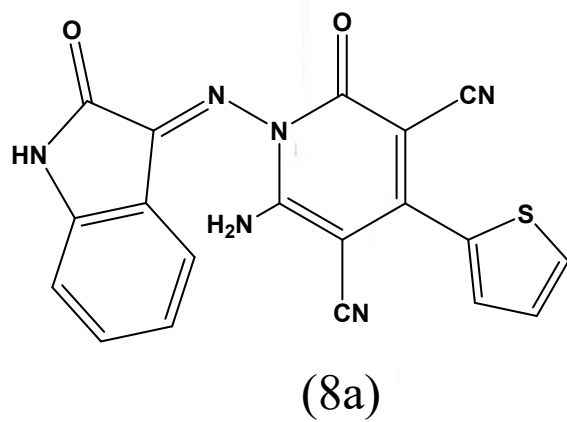


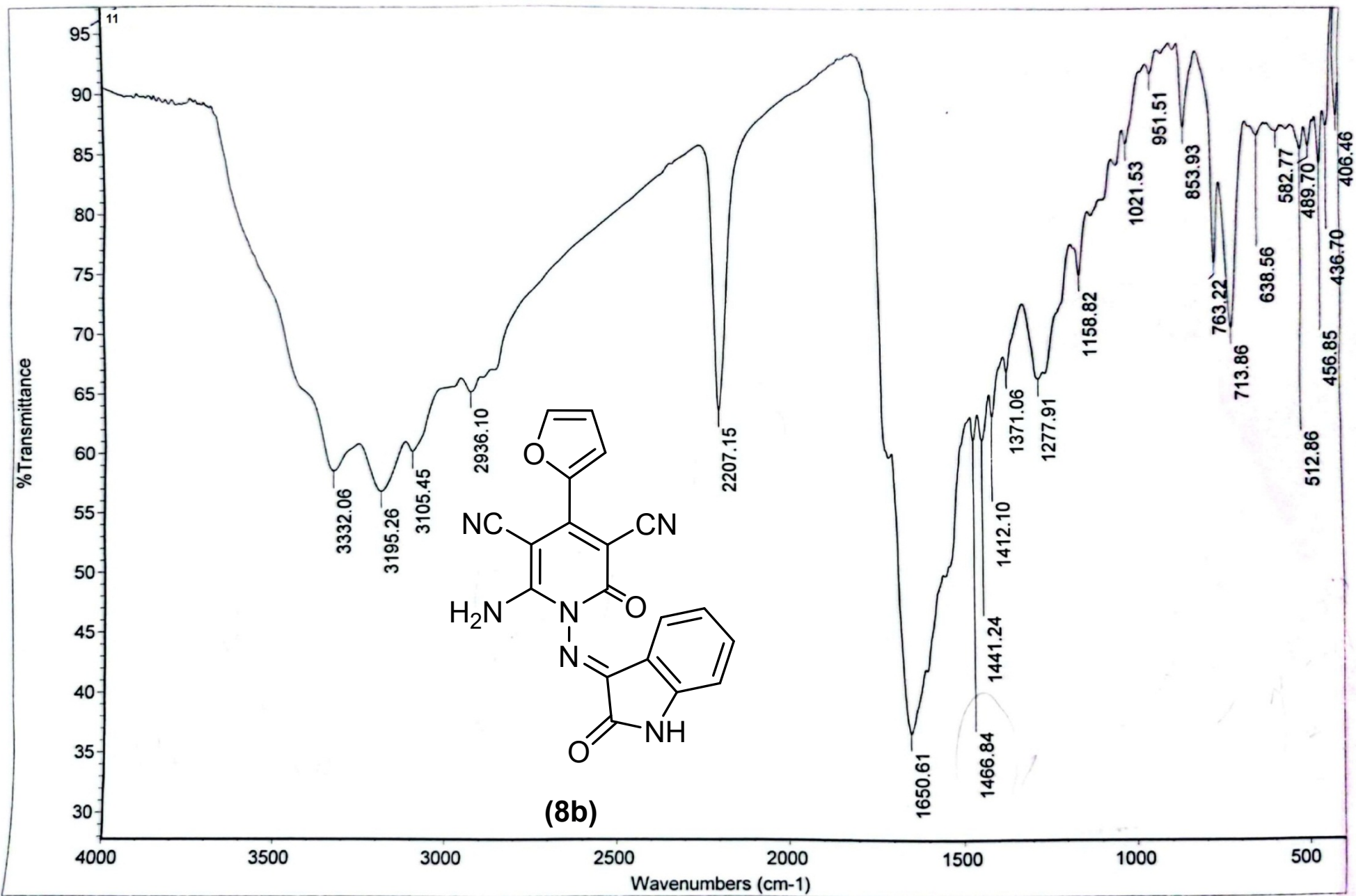


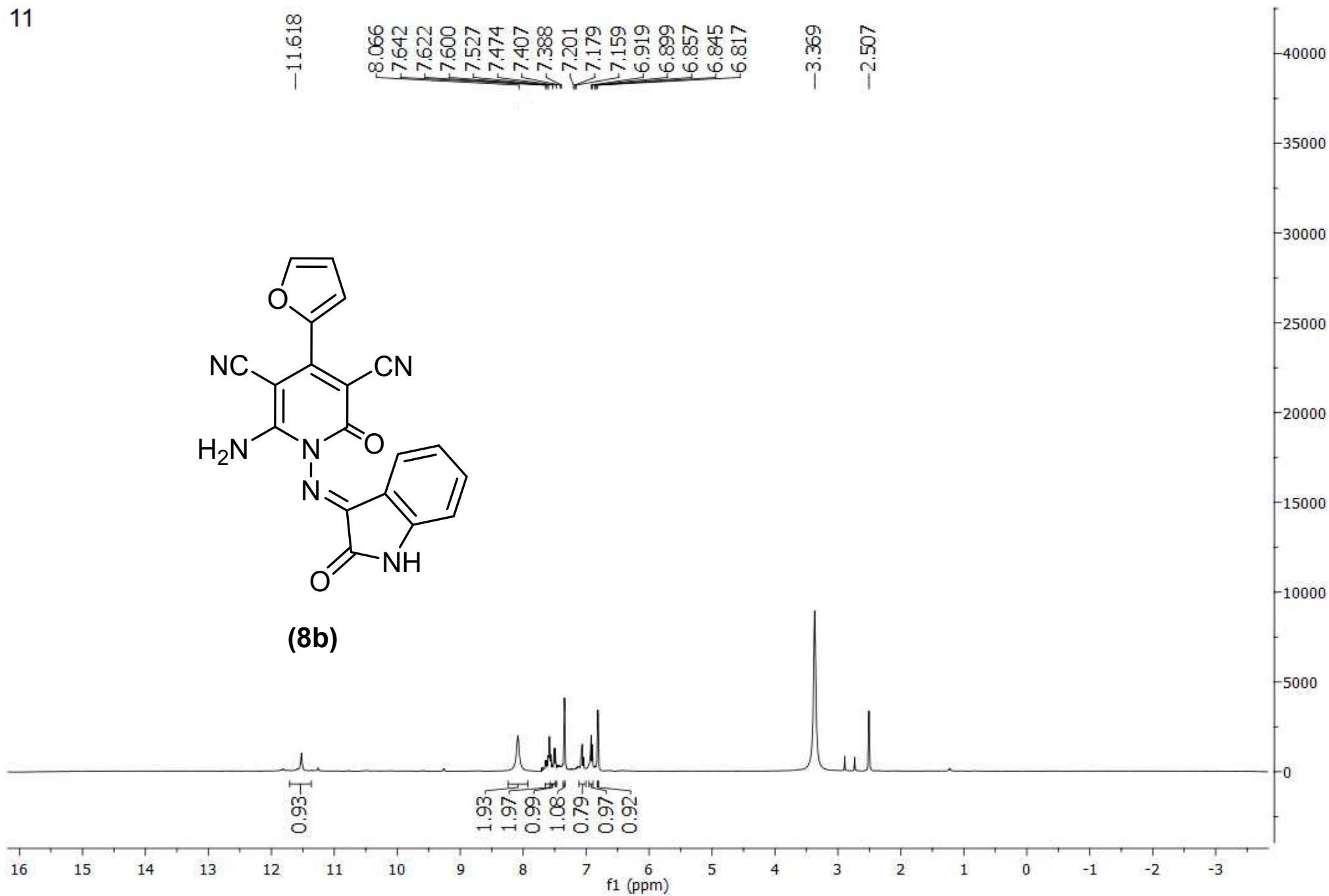
1

172.12
166.09
159.49
155.08
147.79
145.54
143.02
142.63
137.87
129.71
126.94
122.87
121.62
119.01
118.68
114.28
114.10
109.15

-75.34



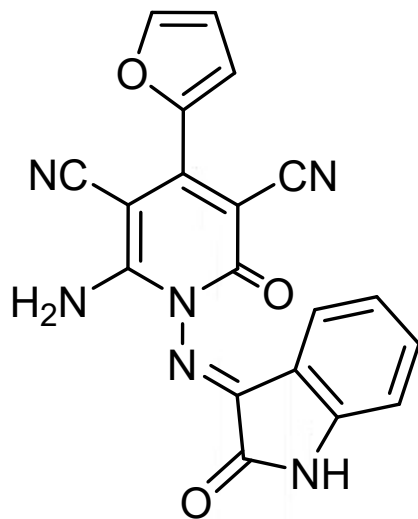




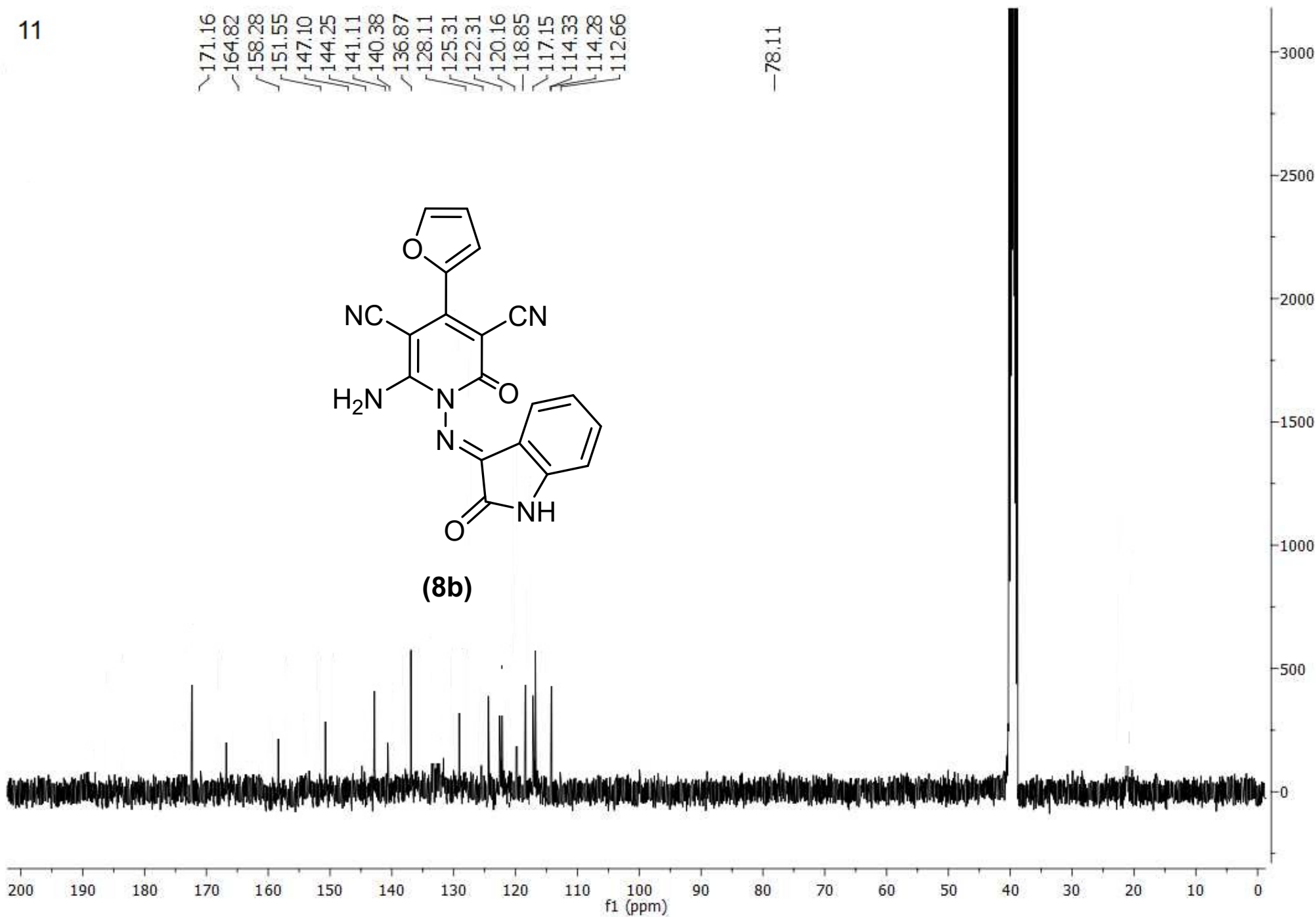
11

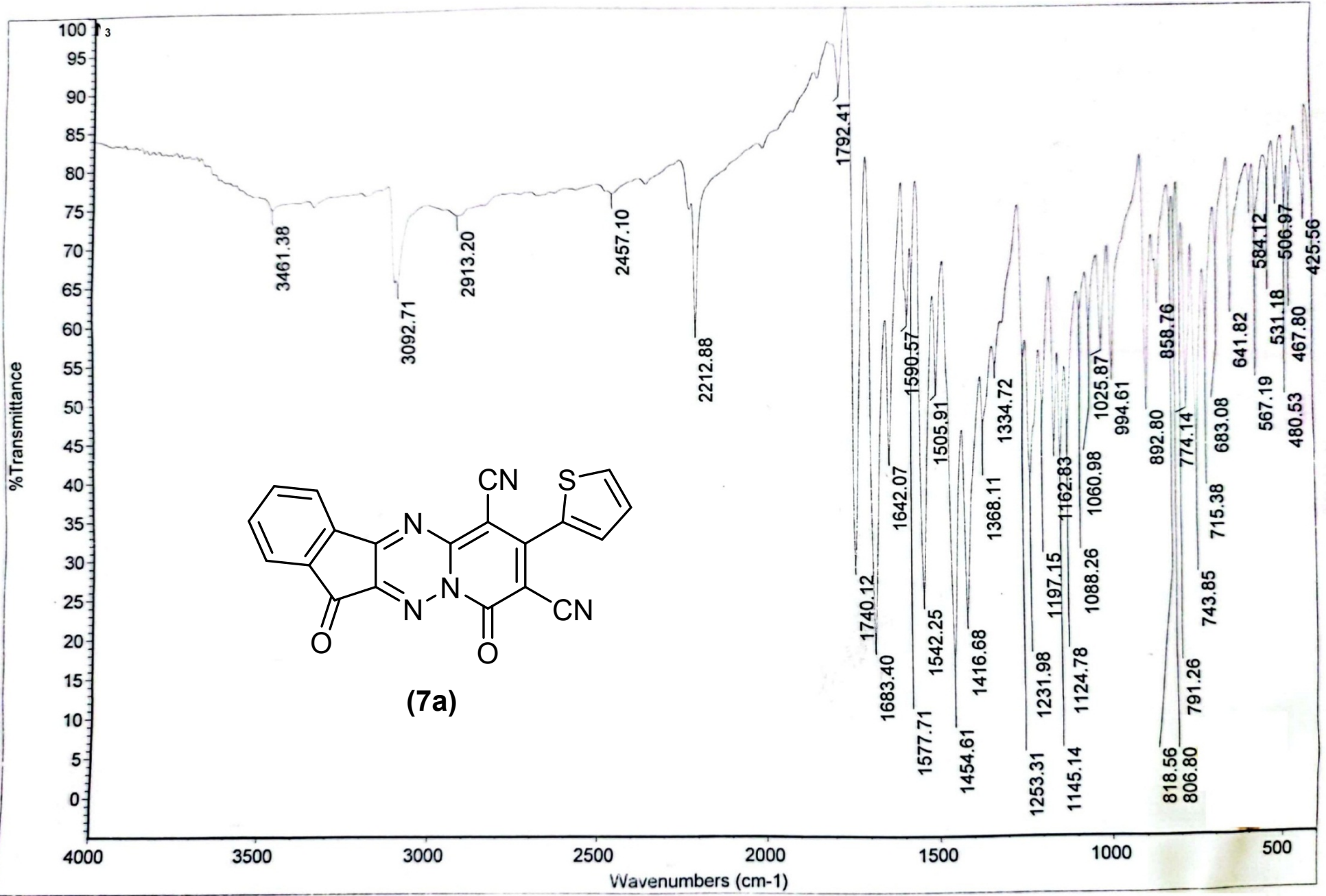
171.16
164.82
158.28
151.55
147.10
144.25
141.11
140.38
136.87
128.11
125.31
122.31
120.16
118.85
117.15
114.33
114.28
112.66

-78.11

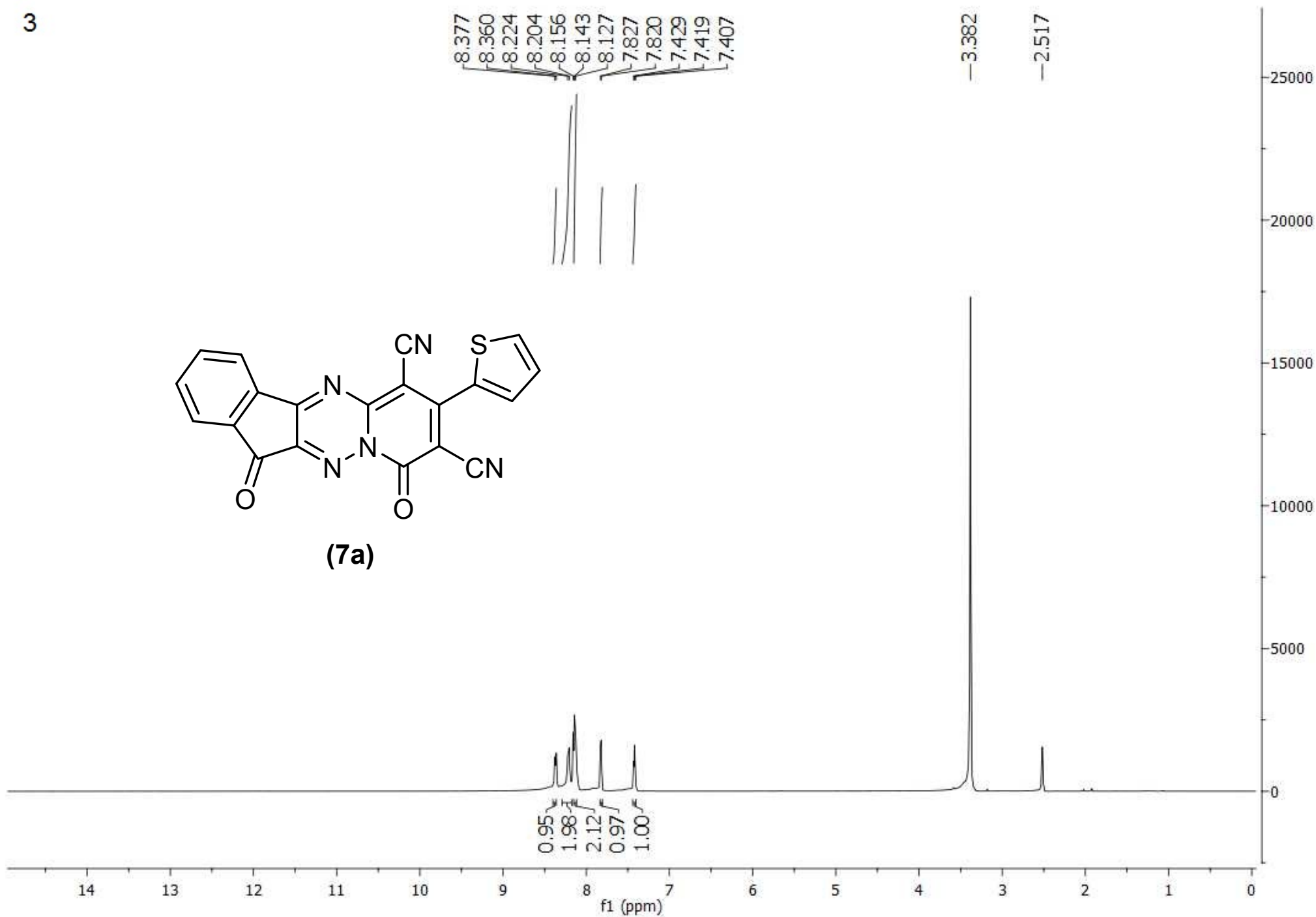


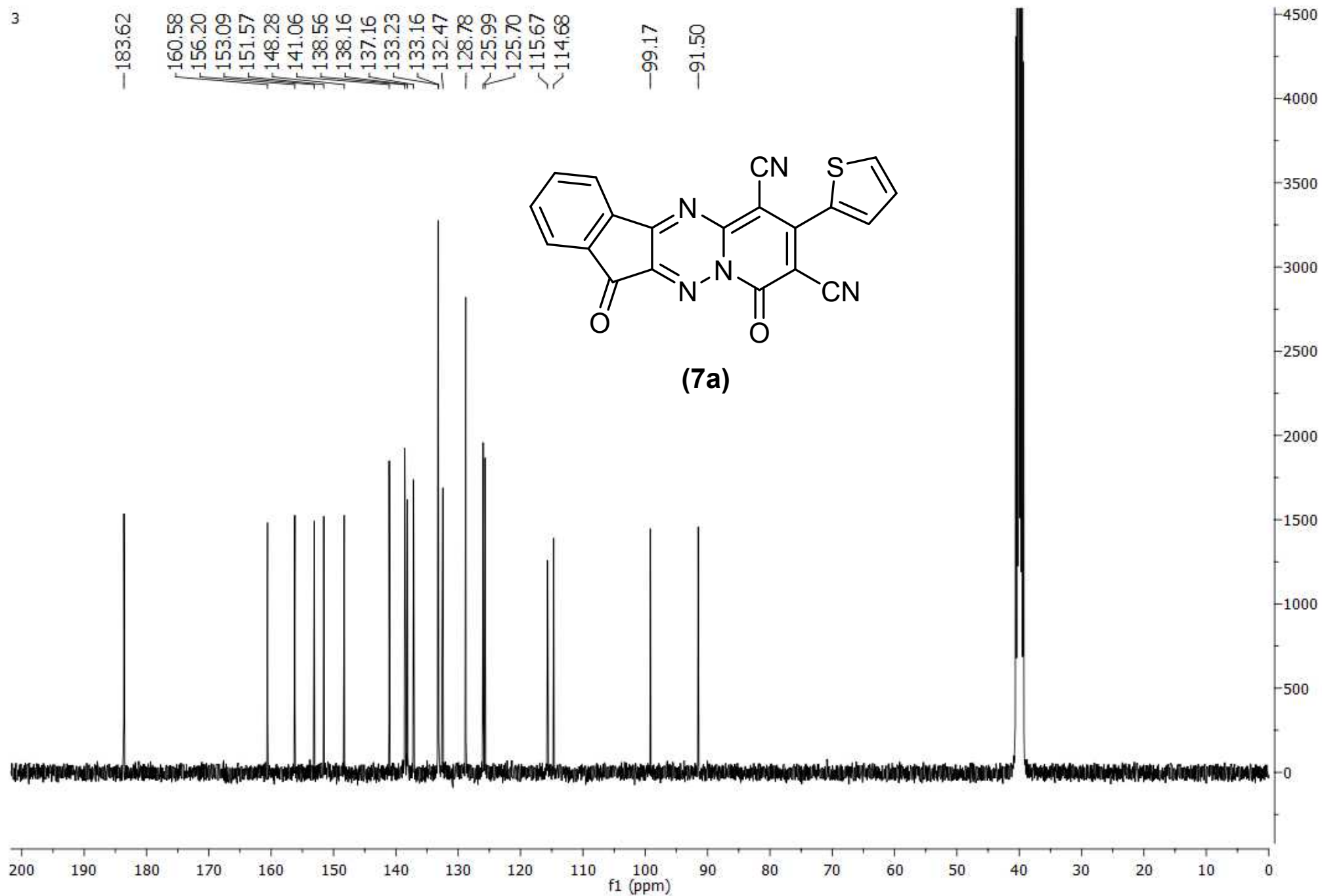
(8b)

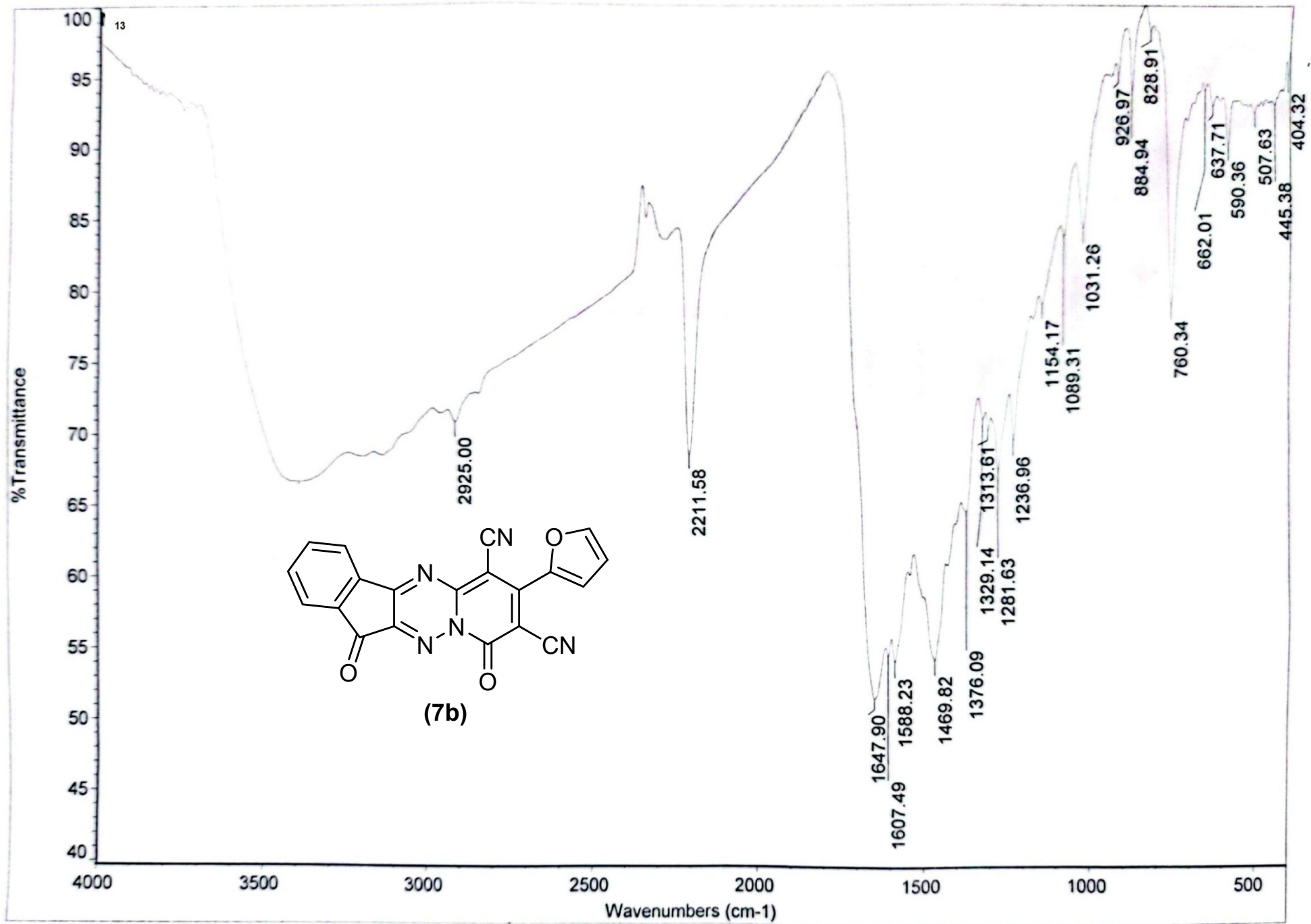


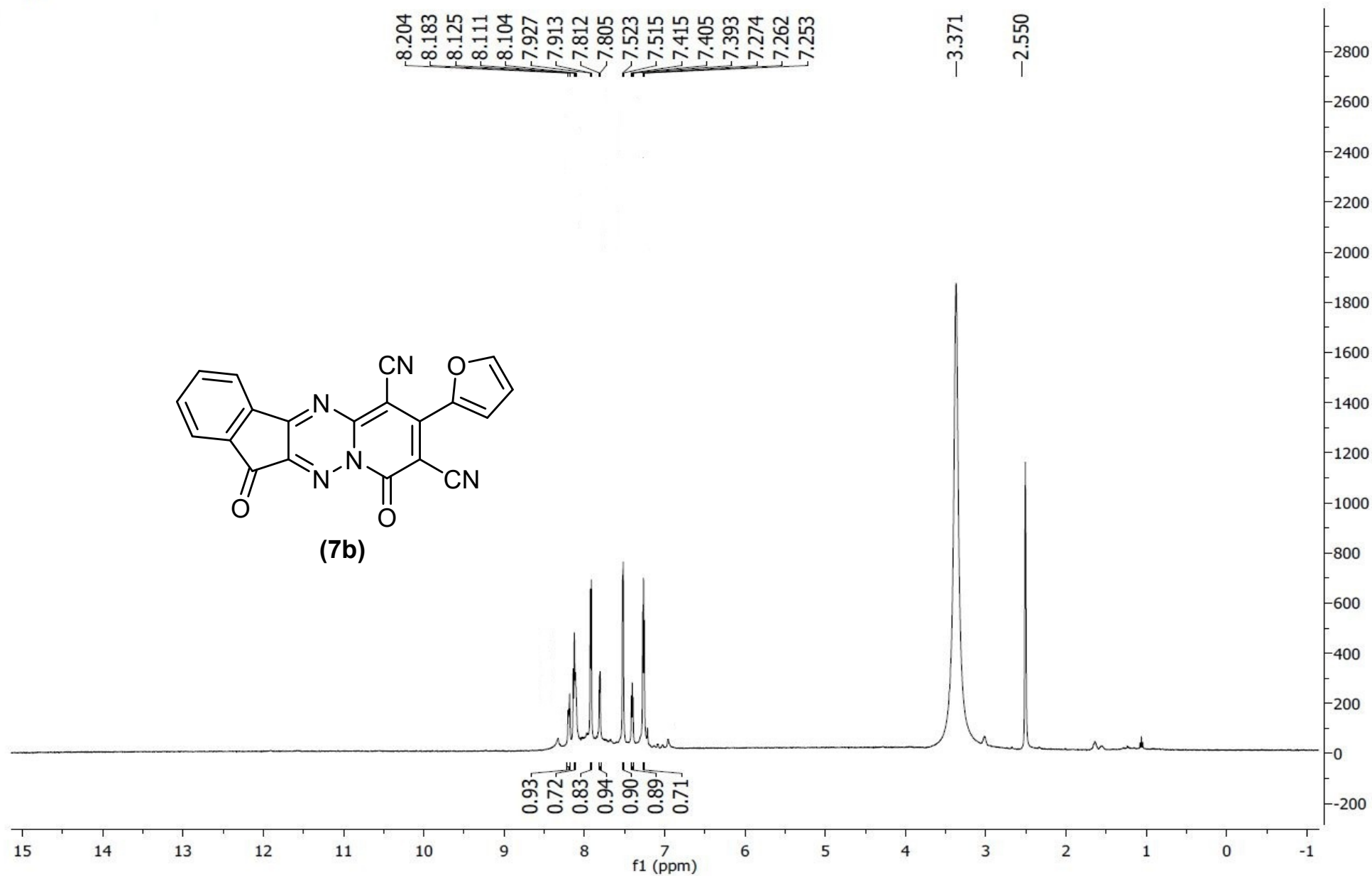


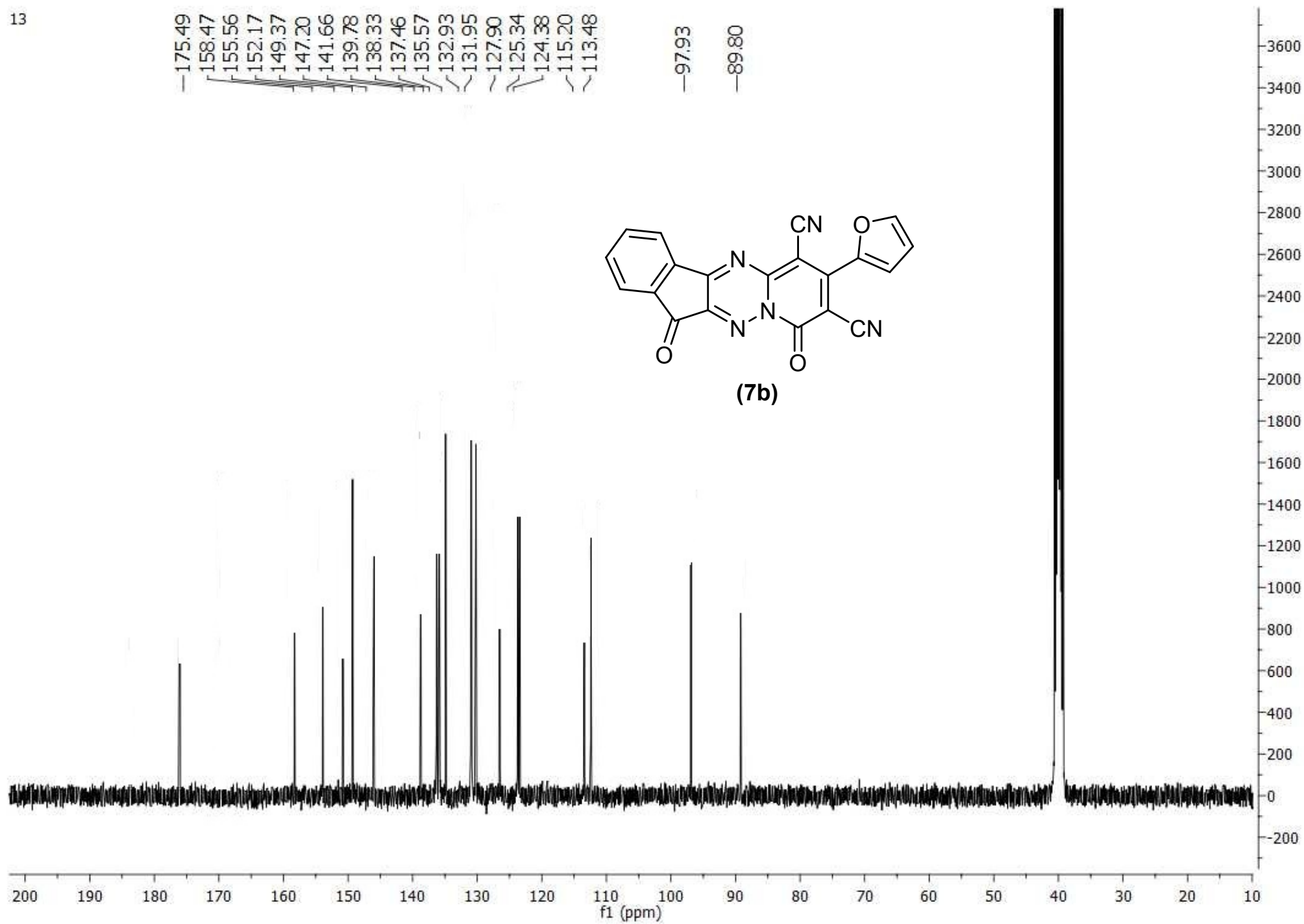
3

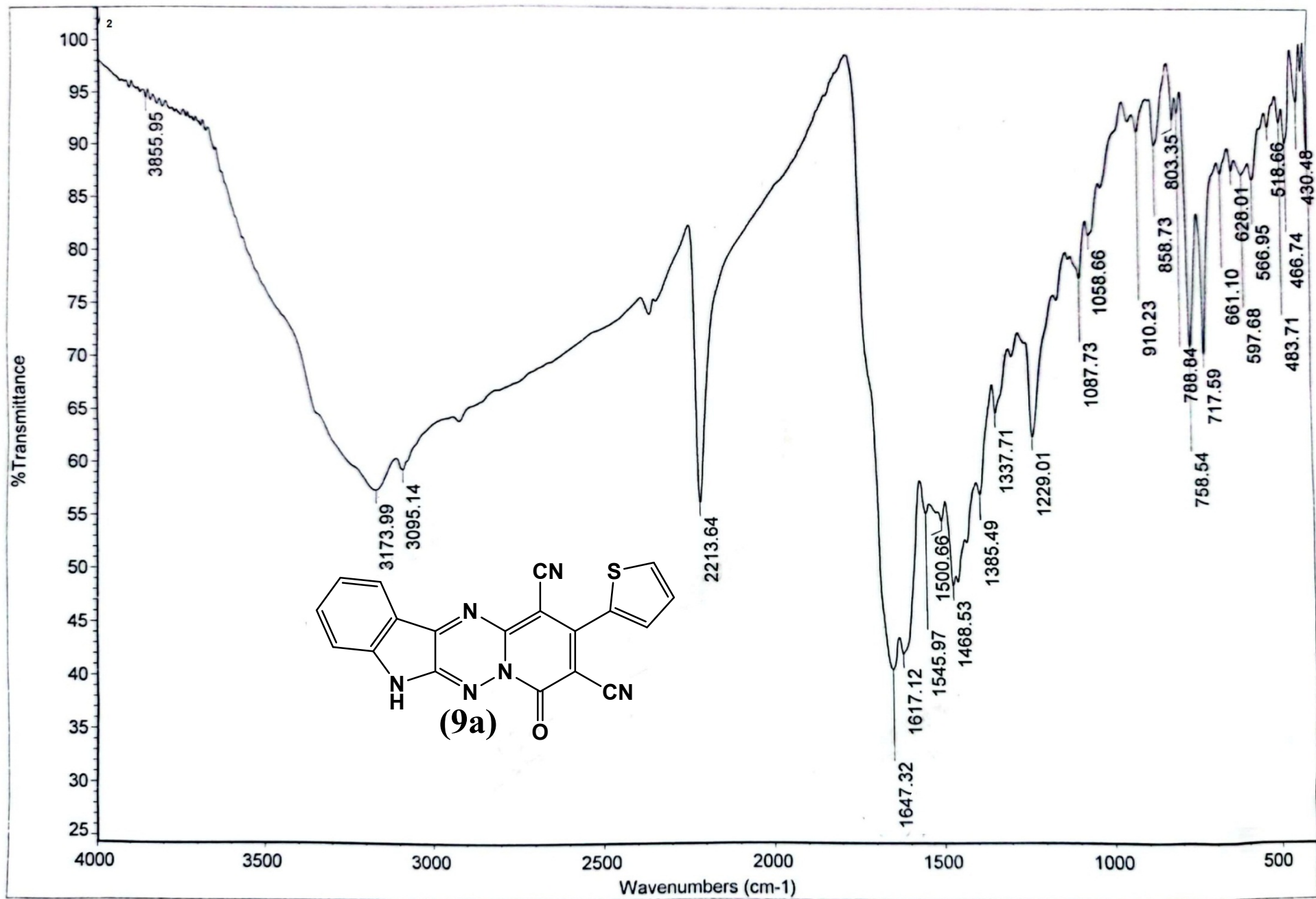


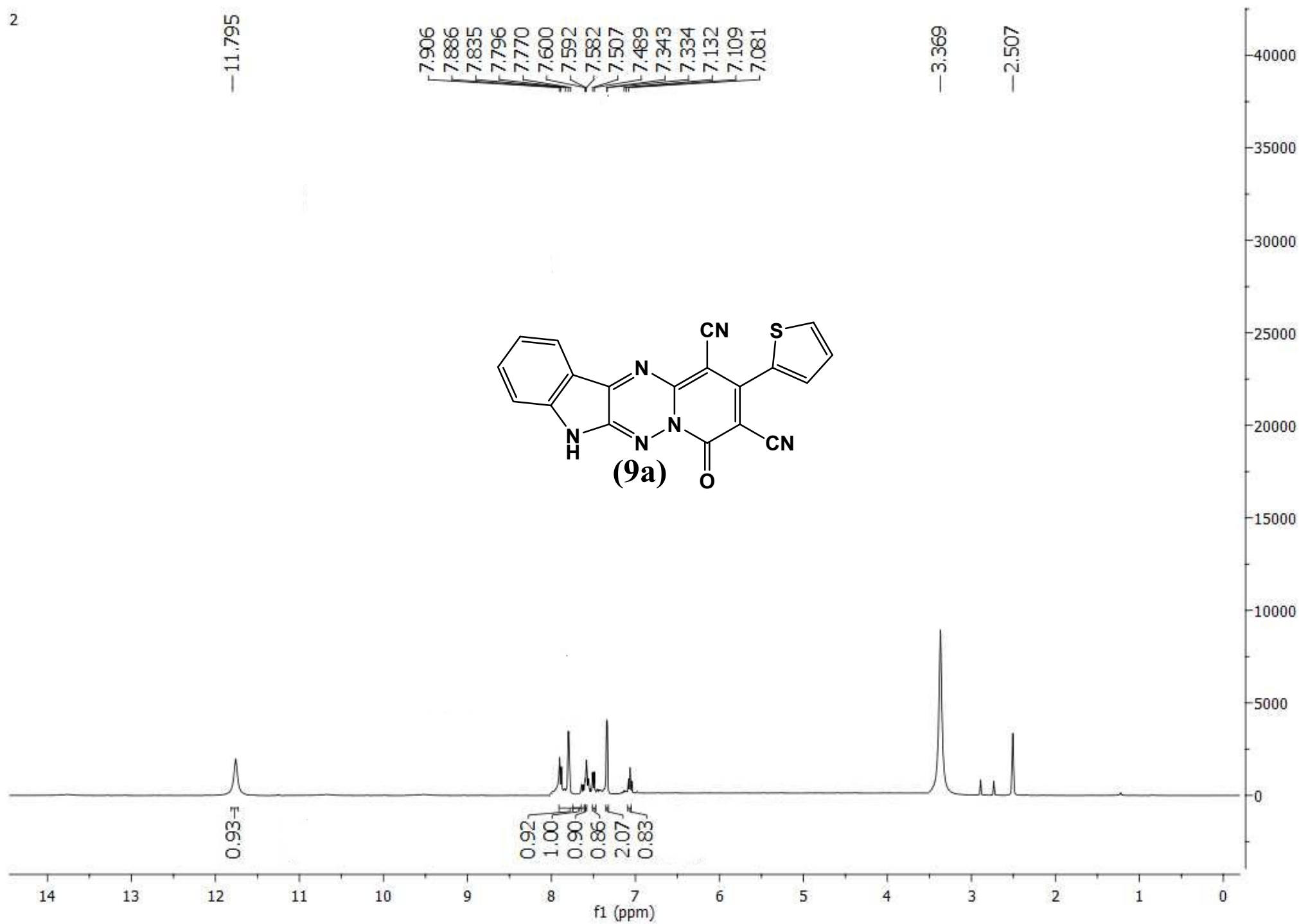


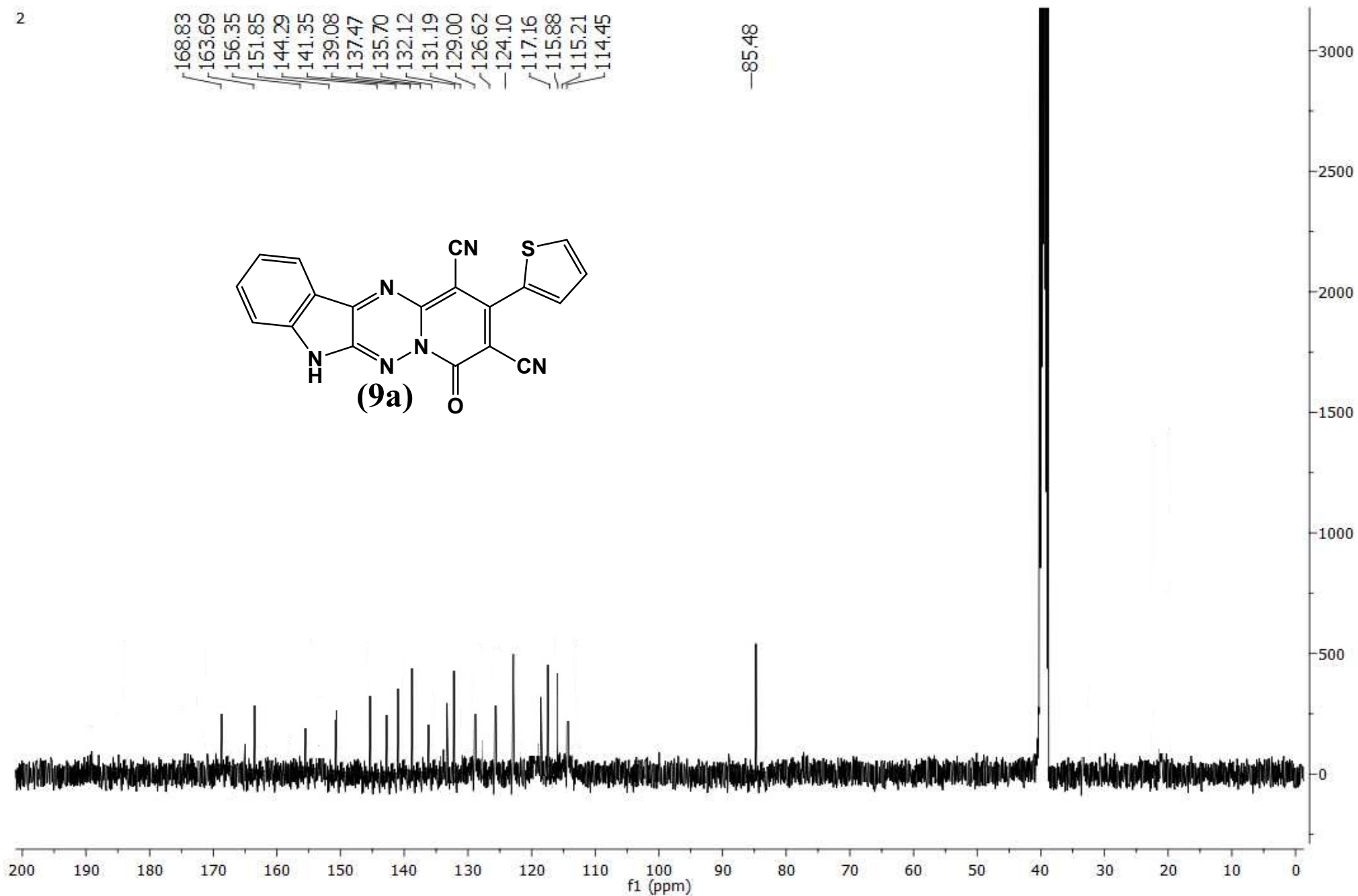


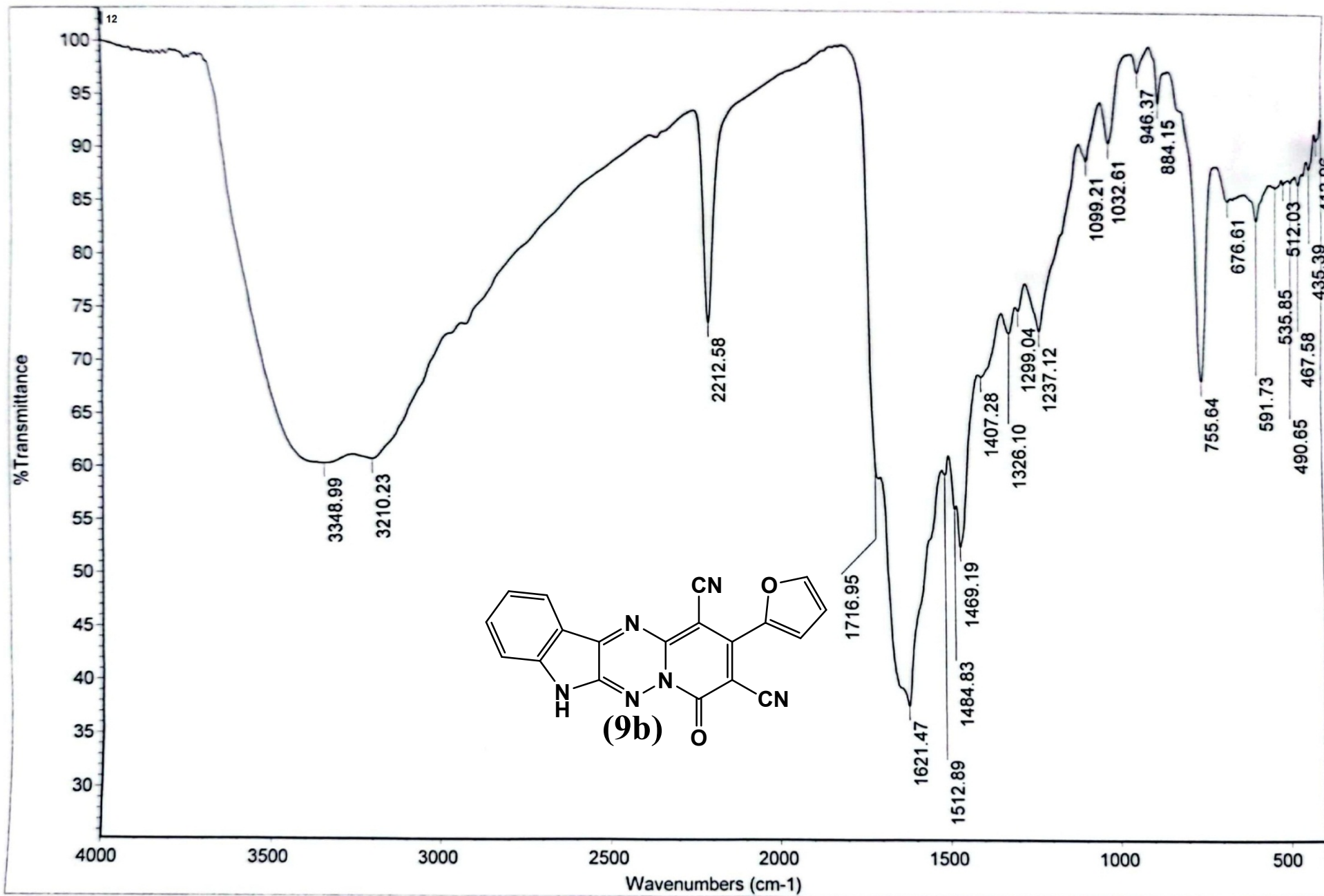


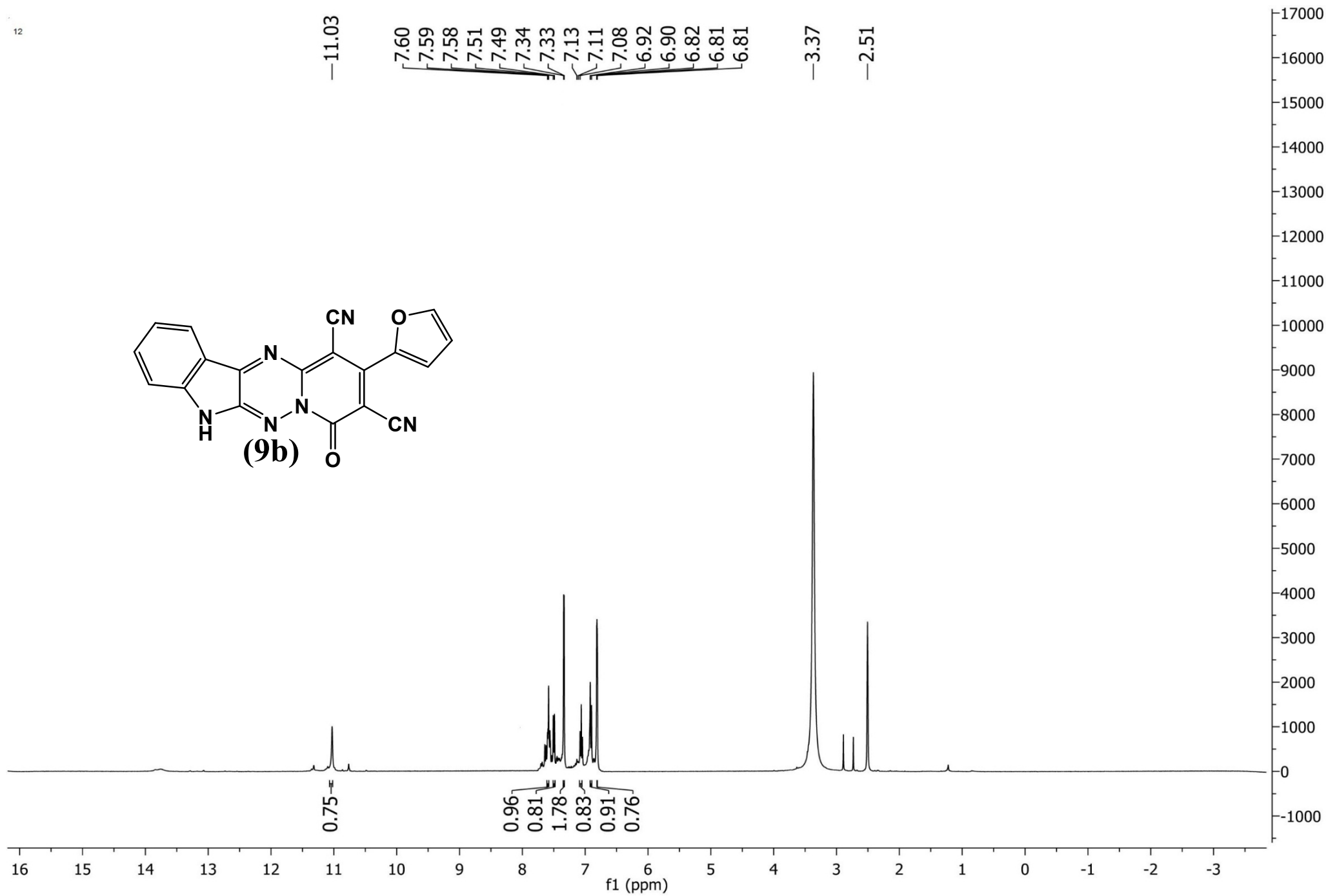












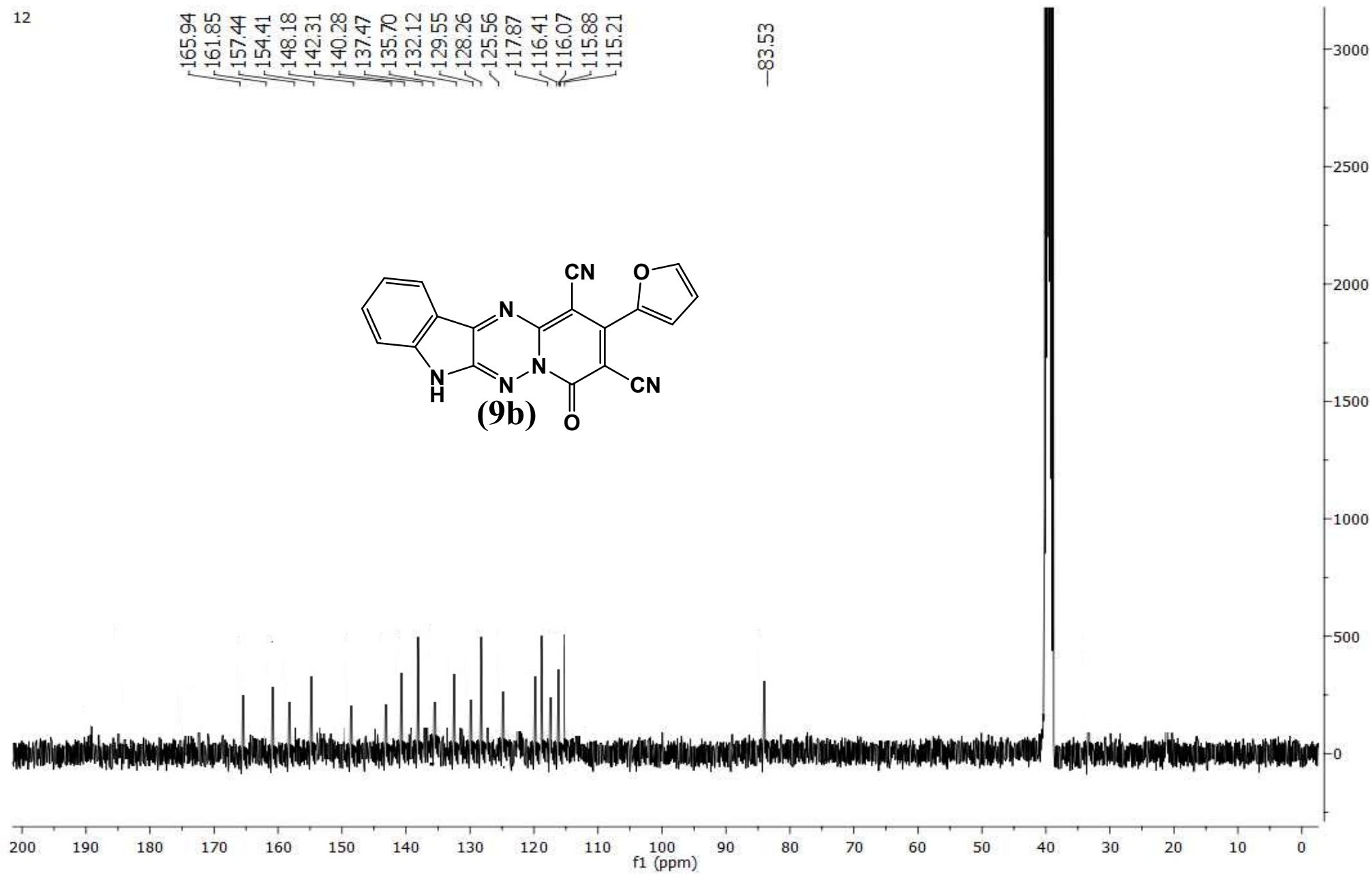


Table (s1): The raw data of inhibition zone diameters mean \pm SD expressed by (mm) of all synthesized derivatives 2a-9b against different pathogenic microorganisms

Comp. no.	Gram +ve		Gram -ve				Fungi
			Enterobacteriaceae		Non-Enterobacteriaceae		
	MS	MR	EC	K	PS	AC	C
2a	18	13	13	25	18	22	16
	19	13	13	26	19	22	17
	18	14	14	25	18	23	16
Mean	18.3	13.4	13.3	25.3	18.3	22.3	16.3
SD	0.5773503	0.57735	0.57735	0.57735	0.57735	0.57735	0.57735
2b	16	13	17	21	18	22	18
	16	13	17	21	18	23	19
	17	14	18	22	19	23	18
Mean	16.3	13.3	17.3	21.3	18.3	22.6	18.3
SD	0.5773503	0.57735	0.57735	0.57735	0.57735	0.57735	0.57735
3a	20	15	15	20	20	20	16
	21	16	15	21	20	21	17
	20	15	16	20	21	20	16
Mean	20.3	15.3	15.3	20.3	20.3	20.3	16.3
SD	0.5773503	0.57735	0.57735	0.57735	0.57735	0.57735	0.57735
3b	23	20	16	20	18	20	14
	24	21	17	20	18	21	14
	23	20	17	21	19	22	15
Mean	23.3	20.3	16.7	20.3	18.3	21	14.3
SD	0.5773503	0.57735	0.57735	0.57735	0.57735	1	0.57735
4a	15	15	17	20	18	23	17
	15	15	17	21	18	23	16
	16	16	18	20	19	24	17
Mean	15.3	15.3	17.3	20.3	18.3	23.3	16.6
SD	0.5773503	0.57735	0.57735	0.57735	0.57735	0.57735	0.57735
4b	20	16	17	20	15	20	12
	21	17	18	21	16	21	13
	20	16	18	20	15	20	14
Mean	20.3	16.3	17.6	20.3	15.3	20.3	13

SD	0.5773503	0.57735	0.57735	0.57735	0.57735	0.57735	1
5a	18	14	17	20	15	20	15
	18	15	18	21	16	21	15
	19	14	17	20	15	22	16
Mean	18.3	14.3	17.3	20.3	15.3	21	15.3
SD	0.5773503	0.57735	0.57735	0.57735	0.57735	1	0.57735
5b	18	16	17	22	16	20	15
	18	17	17	23	16	21	16
	19	16	16	22	17	22	15
Mean	18.3	16.3	16.6	22.3	16.3	21	15.3
SD	0.5773503	0.57735	0.57735	0.57735	0.57735	1	0.57735
6a	18	18	17	20	17	30	15
	19	19	18	20	18	31	16
	18	18	17	21	17	30	15
Mean	18.3	18.3	17.3	20.3	17.3	30.3	15.3
SD	0.5773503	0.57735	0.57735	0.57735	0.57735	0.57735	0.57735
6b	17	16	18	20	18	15	15
	18	17	18	20	18	16	16
	17	16	19	21	19	17	16
Mean	17.3	16.3	18.3	20.3	18.3	16	15.6
SD	0.5773503	0.57735	0.57735	0.57735	0.57735	1	0.57735
7a	20	17	14	20	12	26	15
	20	18	15	18	13	27	15
	21	17	15	19	12	26	16
Mean	20.3	17.3	14.6	19	12.3	26.3	15.3
SD	0.5773503	0.57735	0.57735	1	0.57735	0.57735	0.57735
SE	0.3333333	0.3333333	0.3333333	0.57735	0.3333333	0.3333333	0.3333333
7b	15	NO ZONE	15	20	15	25	12
	15		15	20	16	26	13
	16		16	21	17	25	14
Mean	15.3		15.3	20.3	16	25.3	13
SD	0.5773503		0.57735	0.57735	1	0.57735	1
8a	20	15	15	18	15	23	15
	21	16	15	18	15	23	16
	20	15	16	19	16	24	15
Mean	20.3	15.3	15.3	18.3	15.3	23.3	15.3

Table S2: Percentage of inhibitory effect of different MICs of synthesized compounds on biofilm formation in various microorganisms:

	Methicillin resistant <i>S. aureus</i>			<i>E. coli</i>			<i>P. aeruginosa</i>			<i>C. albicans</i>		
	½ MIC	¼ MIC	1/8 MIC	½ MIC	¼ MIC	1/8 MIC	½ MIC	¼ MIC	1/8 MIC	½ MIC	¼ MIC	1/8 MIC
6b	74.1±0.45	71.3±0.5	61±0.9	68.2±1.3	65.7±0.9	63.8±1.6	57.6±1.7	50.9±2.5	45.4±1.3	76.9±0.2	76.1±0.45	75±0.51
3a	74.6±0.5	70.8±0.45	61.5±0.45	69.9±0.45	66.2±0.45	63.8±0.45	35.9±2.51	10±0.90	0.7±0.45	76.7±0.51	67.8±1.32	55.1±2.77
2a	71.3±0.5	45±0.45	27.6±8.79	67.9±0.70	56.1±0.70	46.2±0.95	78.4±1.04	77.6±0.55	1.6±2.23	75.6±0.46	74.4±0.9	17.6±0.92
3b	70.2±0.72	68.3±0.79	59.9±2.60	52.3±1.65	43.5±0.45	43±0.9	74.9±0.70	47.1±1.48	10.1±3.32	62.7±1.89	29.4±1.53	20.7±0.85
2b	73.7±1.20	68.8±0.72	64.7±1.85	65.1±0.70	64.6±0.72	63.2±0.288	72.5±1.53	37.6±2.85	3.1±2.41	75.6±1.32	66.5±0.61	29.7±1.50
8a	71.8±0.5	69.1±0.70	55.5±1.86	52.3±1.26	38.2±0.66	10.3±2.36	81.2±0.70	82.9±1.15	26.5±5.08	70.1±1.17	47.3±1.33	15.152±5.26

Table S3: effect of 1/8 MIC comp. on expression lasR gene that control the QS in *P. aeruginosa*

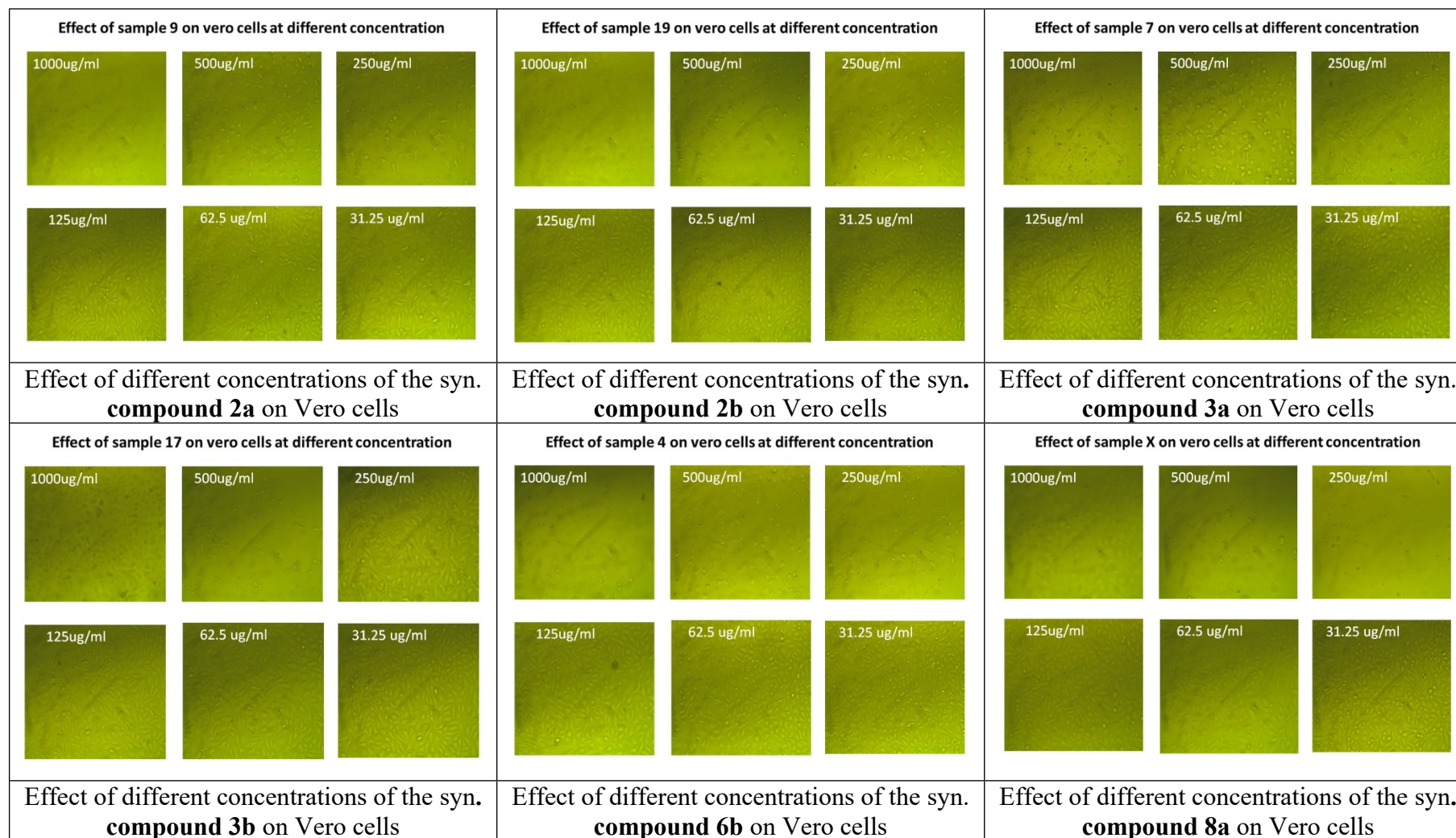
		Ct of lasR gene	Ct of housekeeping gene	Δ Ct	$\Delta\Delta$ Ct	$2^{-\Delta\Delta$ Ct}
lasR gene expression before treatment	control	25.6	25	0.6	0	1
	2a	25.5	24.5	1	0.4	0.8
	2b	25.1	24.4	0.7	0.1	0.9
	3a	25.2	24.4	0.8	0.2	0.9
	3b	25.4	24.6	0.8	0.2	0.9
	6a	25.7	24.7	1	0.4	0.8
	6b	25.8	24.4	1.4	0.8	0.6
	Doxycycline	26	24.1	1.9	1.3	0.4

Table S4: displayed the Cytotoxicity and Viability assay

ID	ug/ml	O.D			Mean O.D	±SE	Viability %	Toxicity %	IC50 ± SD
vero	-----	0.732	0.697	0.725	0.718	0.010693	100	0	ug
6b	1000	0.027	0.031	0.024	0.027333	0.002028	3.806870938	96.19312906	207.87 ± 5.14
	500	0.086	0.08	0.093	0.086333	0.003756	12.02414113	87.97585887	
	250	0.217	0.243	0.229	0.229667	0.007513	31.98700093	68.01299907	
	125	0.658	0.682	0.688	0.676	0.009165	94.15041783	5.849582173	
	62.5	0.724	0.711	0.712	0.715667	0.004177	99.67502321	0.324976787	
	31.25	0.713	0.72	0.694	0.709	0.007767	98.74651811	1.253481894	
3a	1000	0.02	0.017	0.022	0.019667	0.001453	2.739090065	97.26090994	344.27 ± 3.81
	500	0.132	0.119	0.153	0.134667	0.009905	18.75580316	81.24419684	
	250	0.463	0.46	0.482	0.468333	0.006888	65.22748375	34.77251625	
	125	0.728	0.722	0.698	0.716	0.009165	99.72144847	0.278551532	
	62.5	0.709	0.725	0.717	0.717	0.004619	99.86072423	0.139275766	
	31.25	0.724	0.699	0.712	0.711667	0.007219	99.11792015	0.882079851	
2a	1000	0.017	0.019	0.018	0.018	0.000577	2.506963788	97.49303621	230.57 ± 6.52
	500	0.138	0.174	0.155	0.155667	0.010398	21.68059424	78.31940576	
	250	0.296	0.32	0.302	0.306	0.007211	42.6183844	57.3816156	
	125	0.684	0.702	0.673	0.686333	0.008452	95.58960074	4.410399257	
	62.5	0.722	0.719	0.707	0.716	0.004583	99.72144847	0.278551532	
	31.25	0.713	0.724	0.712	0.716333	0.003844	99.76787372	0.232126277	
3b	1000	0.023	0.052	0.048	0.041	0.009074	5.710306407	94.28969359	335.3 ± 7.95
	500	0.089	0.126	0.104	0.106333	0.010745	14.80965645	85.19034355	
	250	0.462	0.45	0.479	0.463667	0.008413	64.57753018	35.42246982	
	125	0.713	0.719	0.722	0.718	0.002646	100	0	
	62.5	0.695	0.716	0.713	0.708	0.006557	98.60724234	1.39275766	

	31.25	0.727	0.711	0.716	0.718	0.004726	100	0	
2b	1000	0.016	0.019	0.018	0.017667	0.000882	2.460538533	97.53946147	231.82 ± 2.21
	500	0.096	0.074	0.068	0.079333	0.008511	11.04921077	88.95078923	
	250	0.315	0.297	0.322	0.311333	0.007446	43.36118849	56.63881151	
	125	0.683	0.709	0.708	0.7	0.008505	97.49303621	2.506963788	
	62.5	0.718	0.699	0.723	0.713333	0.007311	99.35004643	0.649953575	
	31.25	0.715	0.719	0.72	0.718	0.001528	100	0	
8a	1000	0.022	0.017	0.02	0.019667	0.001453	2.739090065	97.26090994	175.17 ± 3.49
	500	0.048	0.032	0.039	0.039667	0.004631	5.524605385	94.47539461	
	250	0.044	0.062	0.05	0.052	0.005292	7.242339833	92.75766017	
	125	0.699	0.701	0.684	0.694667	0.005364	96.75023213	3.249767874	
	62.5	0.674	0.699	0.703	0.692	0.009074	96.37883008	3.621169916	
	31.25	0.722	0.714	0.705	0.713667	0.00491	99.39647168	0.603528319	

Figure S1: Effect of different concentrations of the synthesized compound on Vero cells.



Docking simulation figures

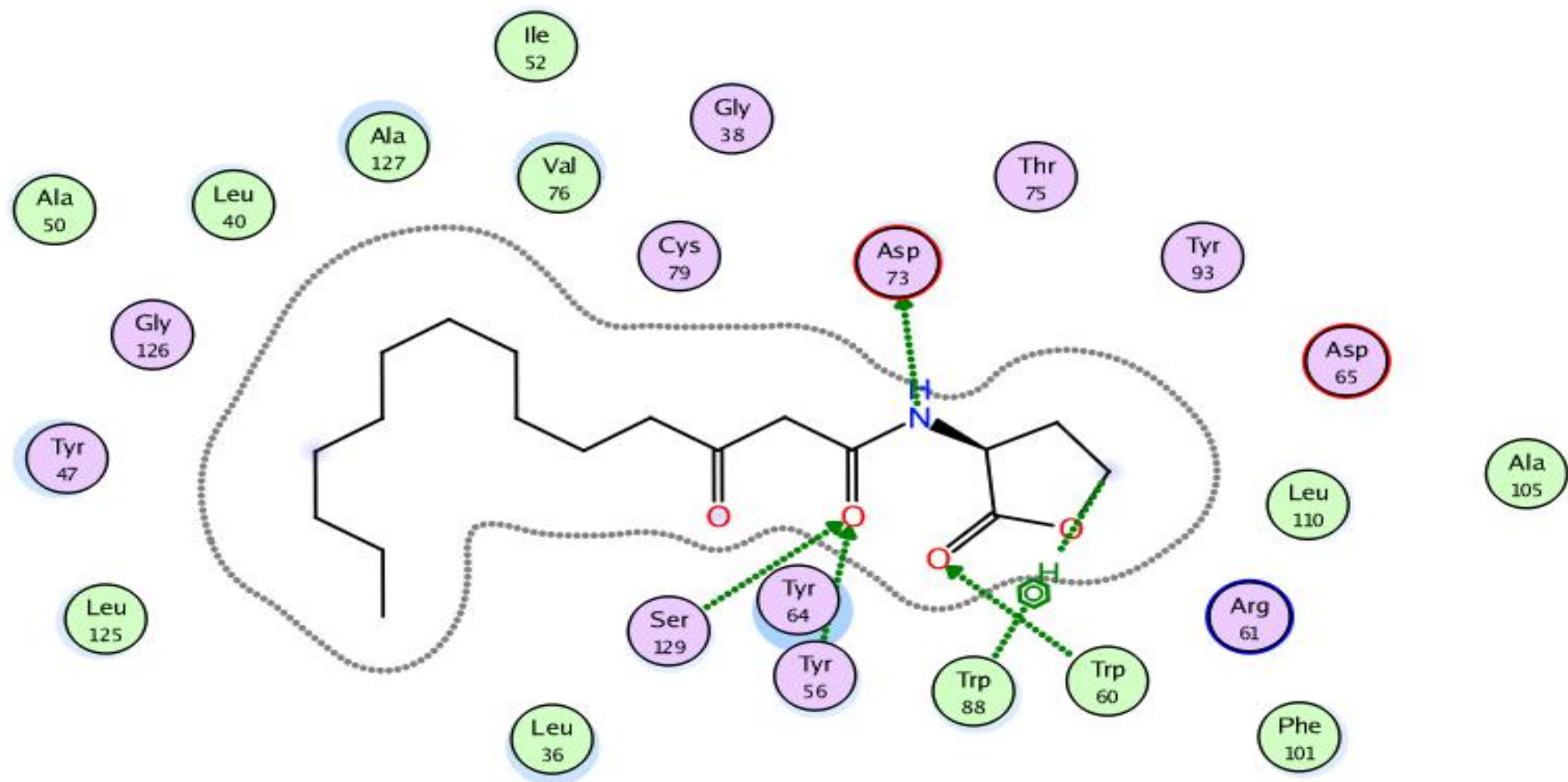


Figure S2: 2D structure of Docking pose and ligand interaction of compound **Co-crystallized ligand** inside the active site of LasR (PDB: 6MVN) in *P. aeruginosa* (UCBPP-PA14)

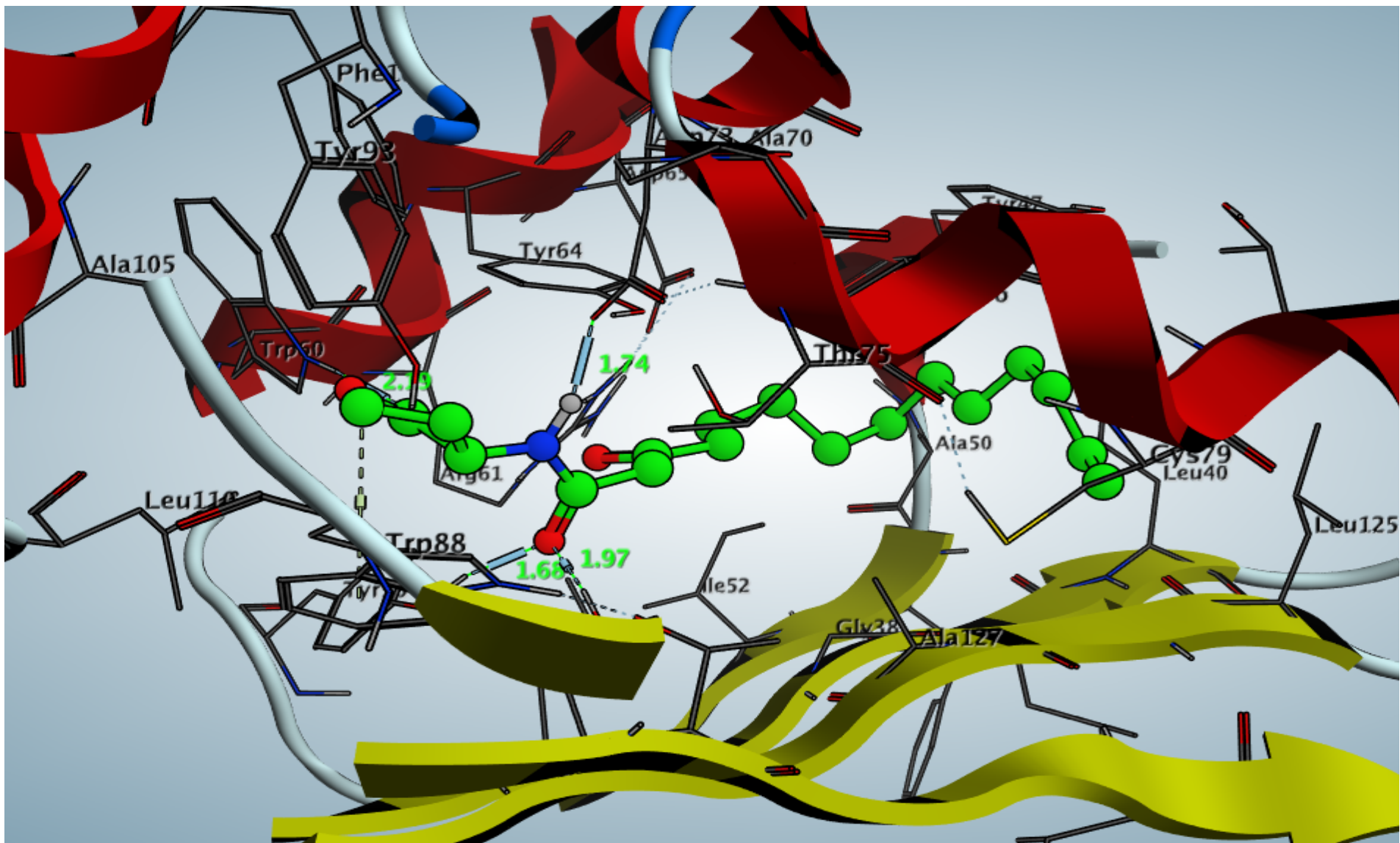


Figure S3: 3D structure of Docking pose and ligand interaction of compound **Co-crystallized ligand** inside the active site of LasR (PDB: 6MVN) in *P. aeruginosa* (UCBPP-PA14)

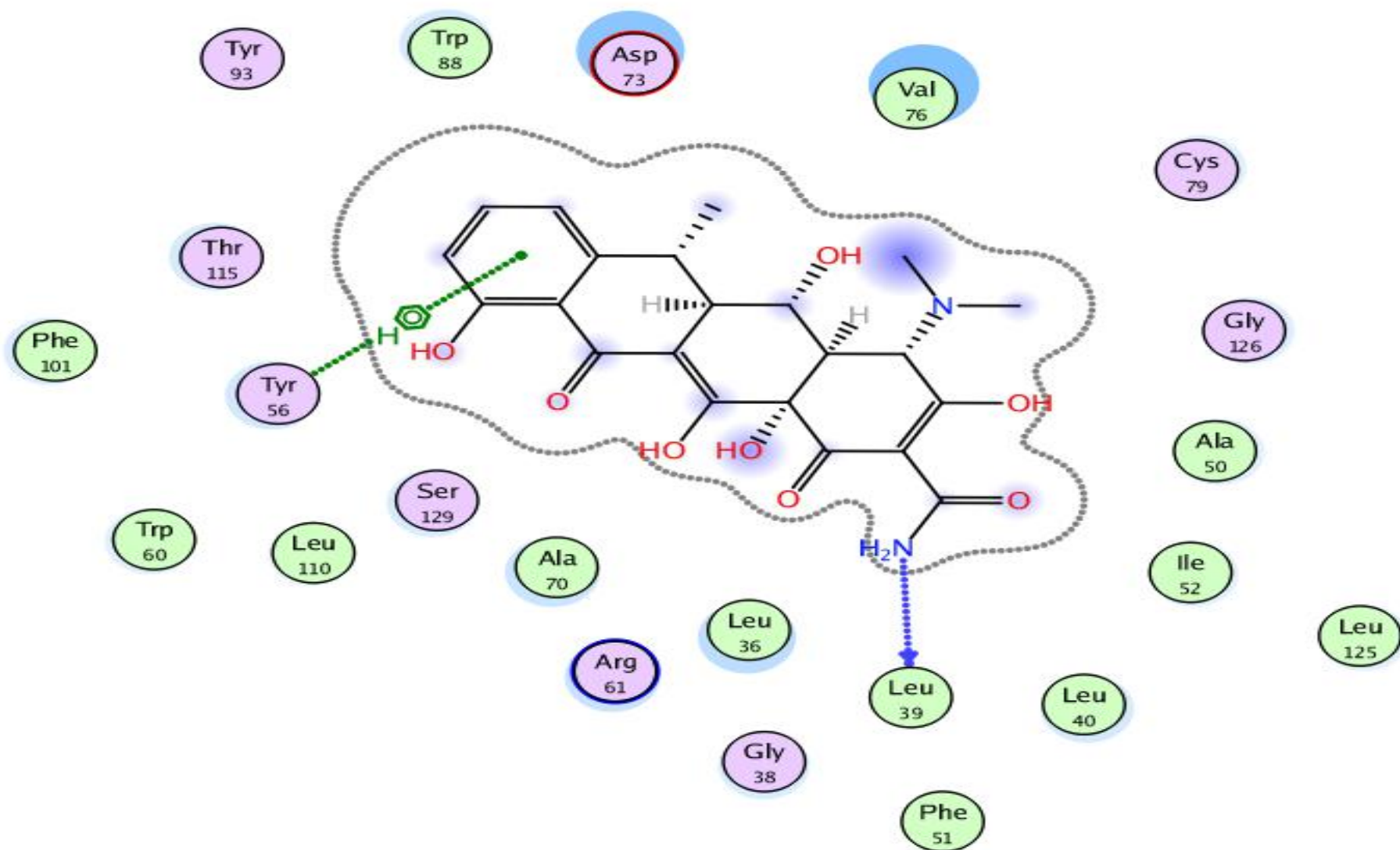


Figure S4: 2D structure of Docking pose and ligand interaction of compound **Doxycycline** inside the active site of LasR (PDB: 6MVN) in *P. aeruginosa* (UCBPP-PA14)

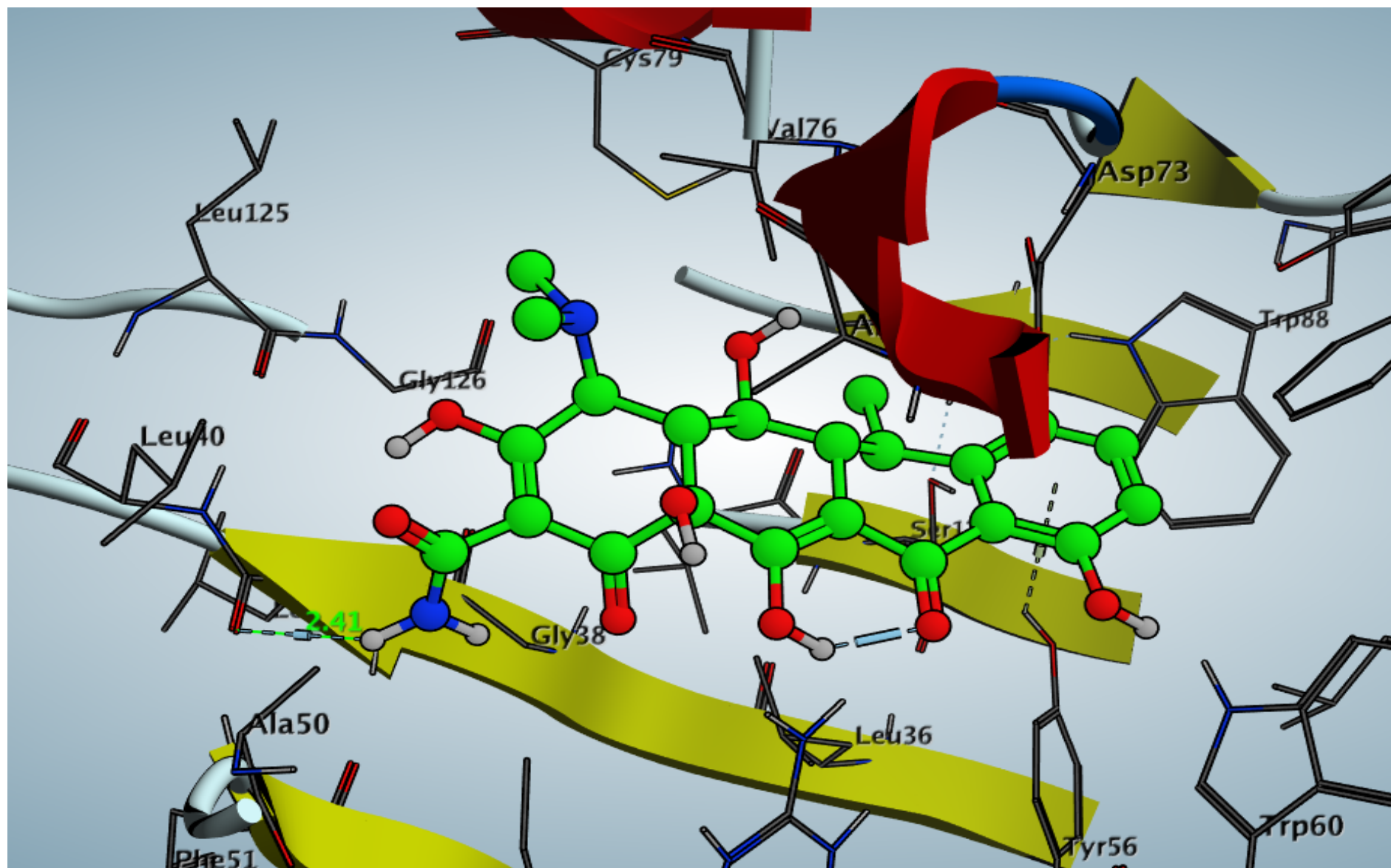


Figure S5: 3D structure of Docking pose and ligand interaction of compound **Doxycycline** inside the active site of LasR (PDB: 6MVN) in *P. aeruginosa* (UCBPP-PA14)

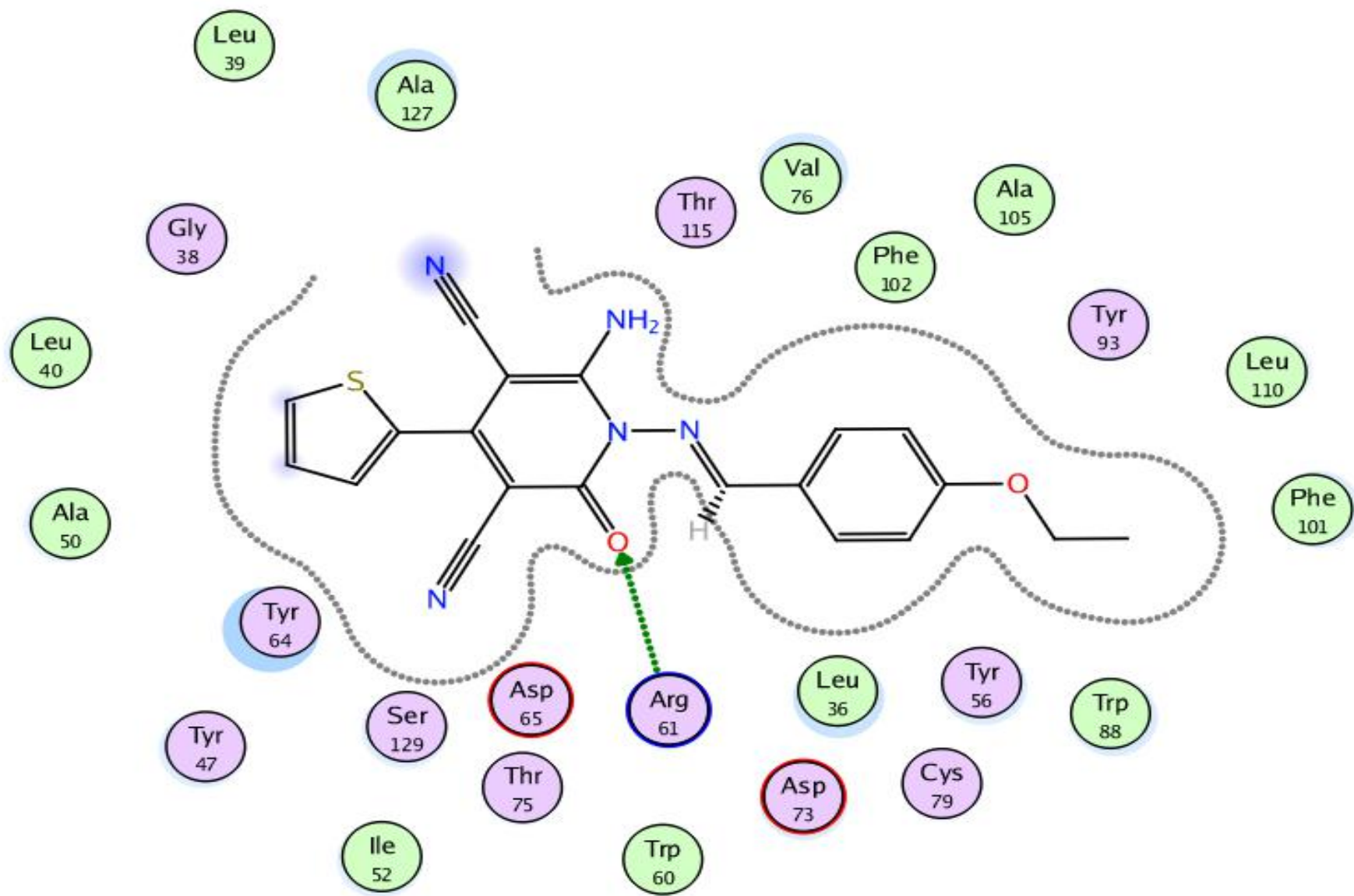


Figure S6: 2D structure of Docking pose and ligand interaction of compound **2a** inside the active site of LasR (PDB: 6MVN) in *P. aeruginosa* (UCBPP-PA14)

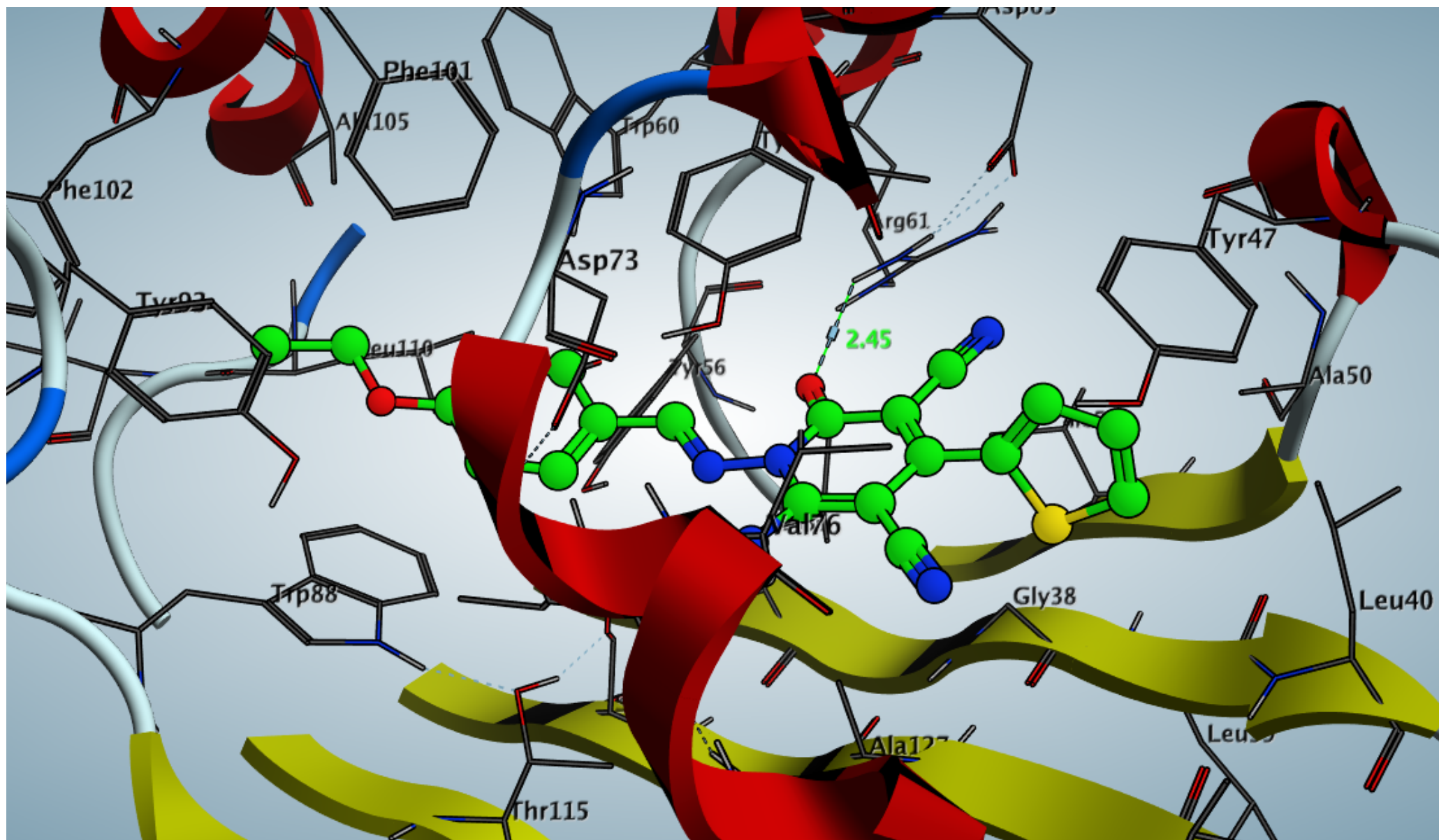


Figure S7: 3D structure of Docking pose and ligand interaction of compound **2a** inside the active site of LasR (PDB: 6MVN) in *P. aeruginosa* (UCBPP-PA14)

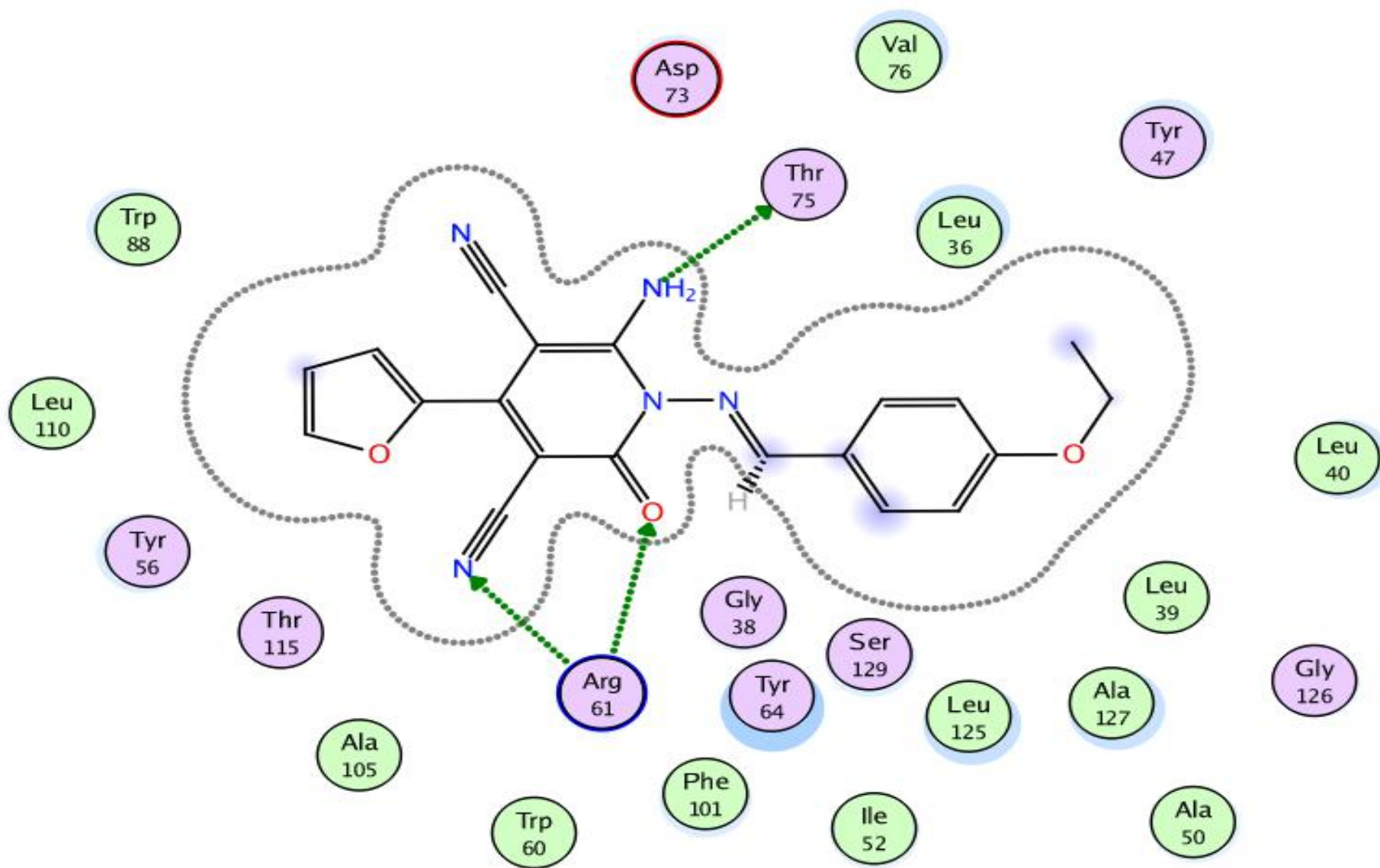


Figure S8: 2D structure of Docking pose and ligand interaction of compound **2b** inside the active site of LasR (PDB: 6MVN) in *P. aeruginosa* (UCBPP-PA14)

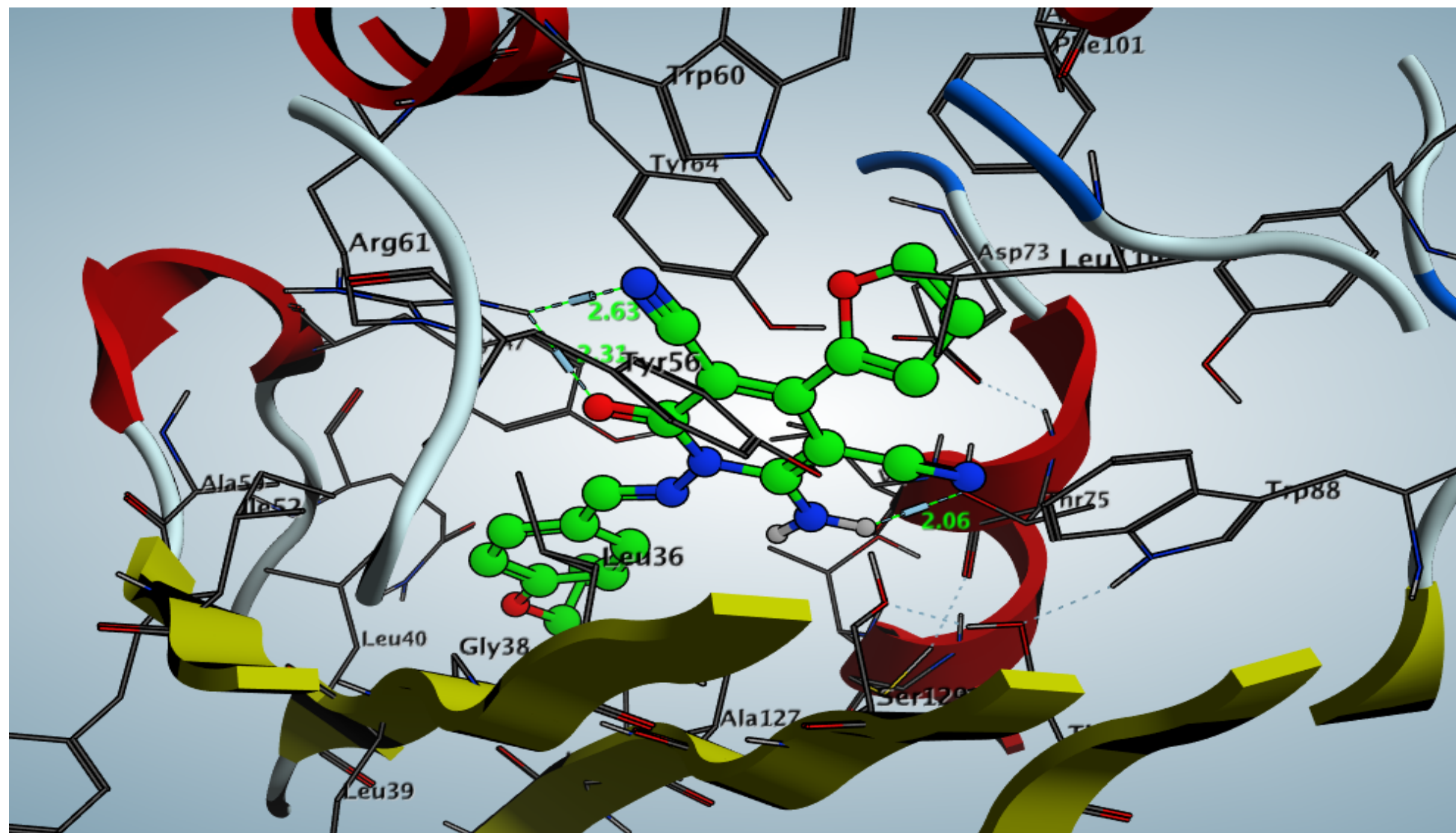


Figure S9: 3D structure of Docking pose and ligand interaction of compound **2b** inside the active site of LasR (PDB: 6MVN) in *P. aeruginosa* (UCBPP-PA14)

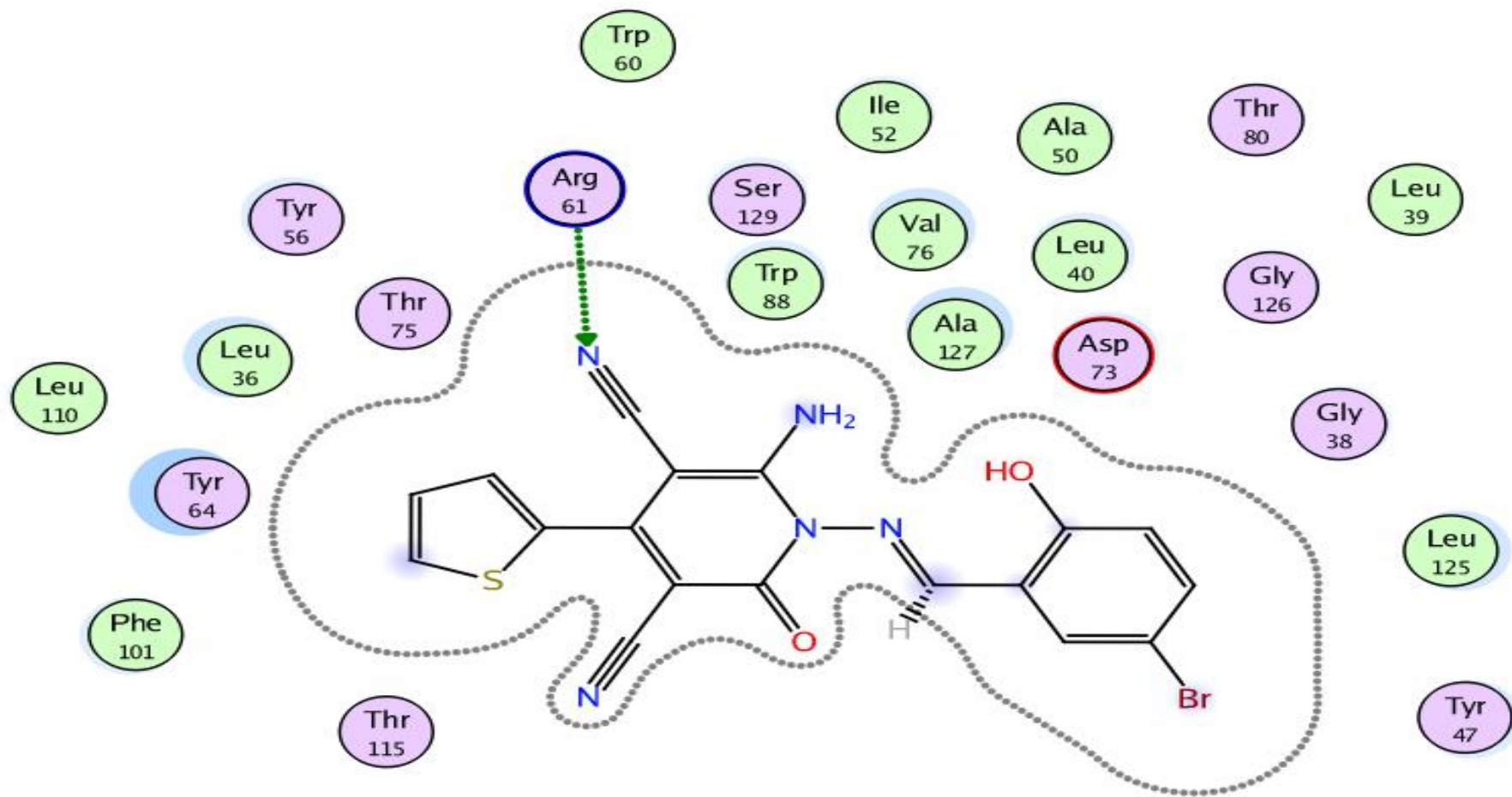


Figure S10: 2D structure of Docking pose and ligand interaction of compound **3a** inside the active site of LasR (PDB: 6MVN) in *P. aeruginosa* (UCBPP-PA14)

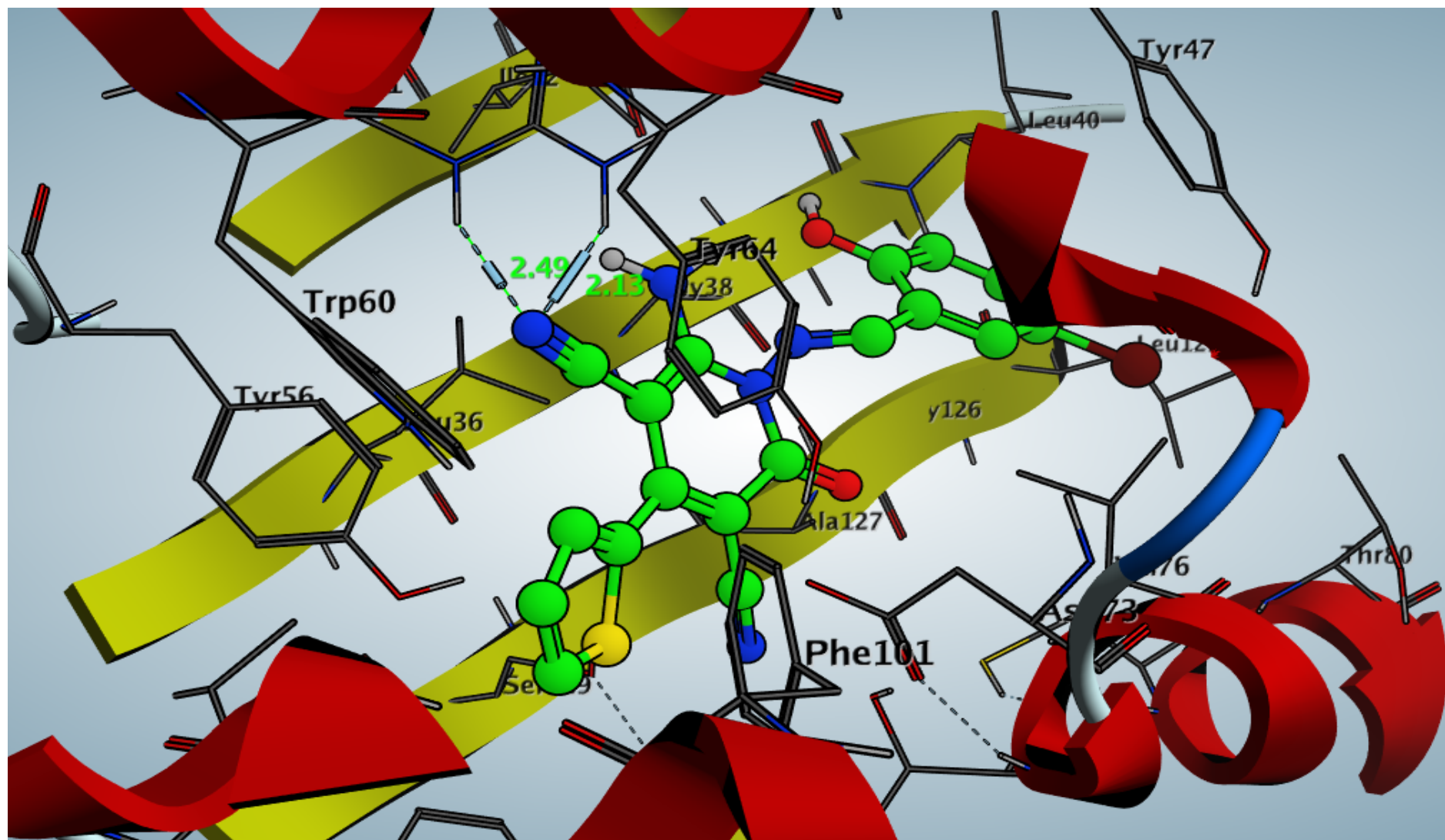


Figure S11: 3D structure of Docking pose and ligand interaction of compound **3a** inside the active site of LasR (PDB: 6MVN) in *P. aeruginosa* (UCBPP-PA14)

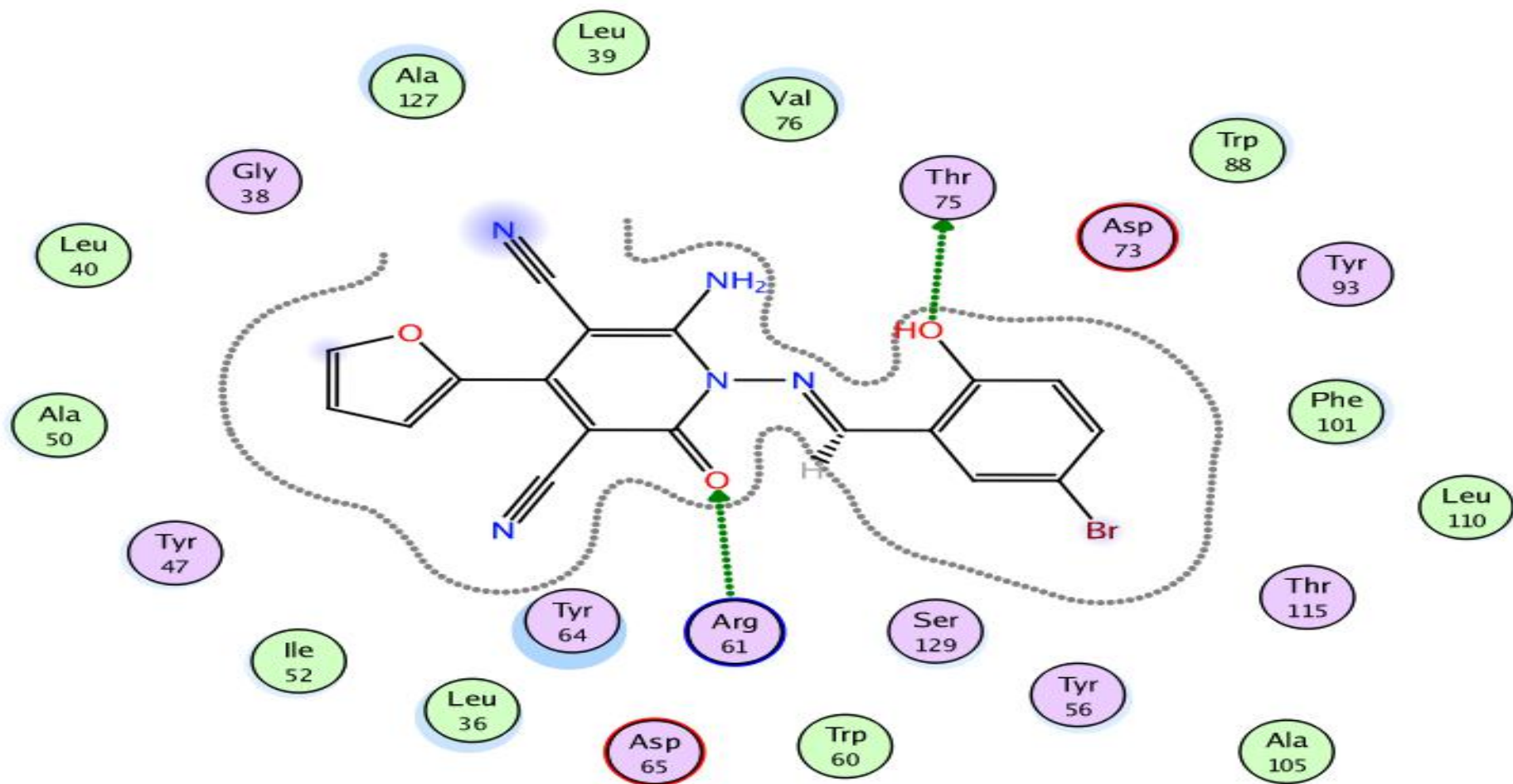


Figure S12: 2D structure of Docking pose and ligand interaction of compound **3b** inside the active site of LasR (PDB: 6MVN) in *P. aeruginosa* (UCBPP-PA14)

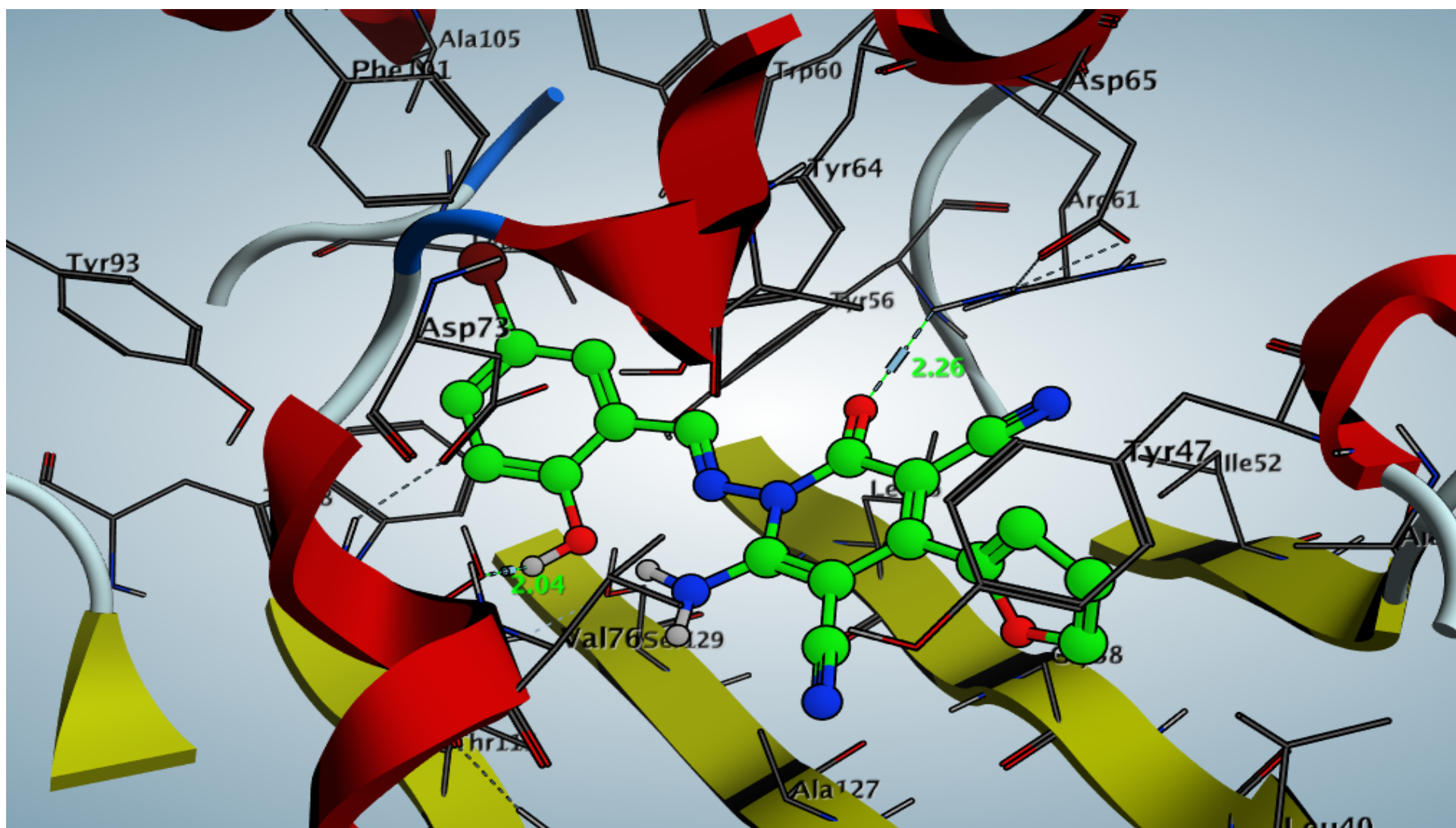


Figure S13: 3D structure of Docking pose and ligand interaction of compound **3b** inside the active site of LasR (PDB: 6MVN) in *P. aeruginosa* (UCBPP-PA14)

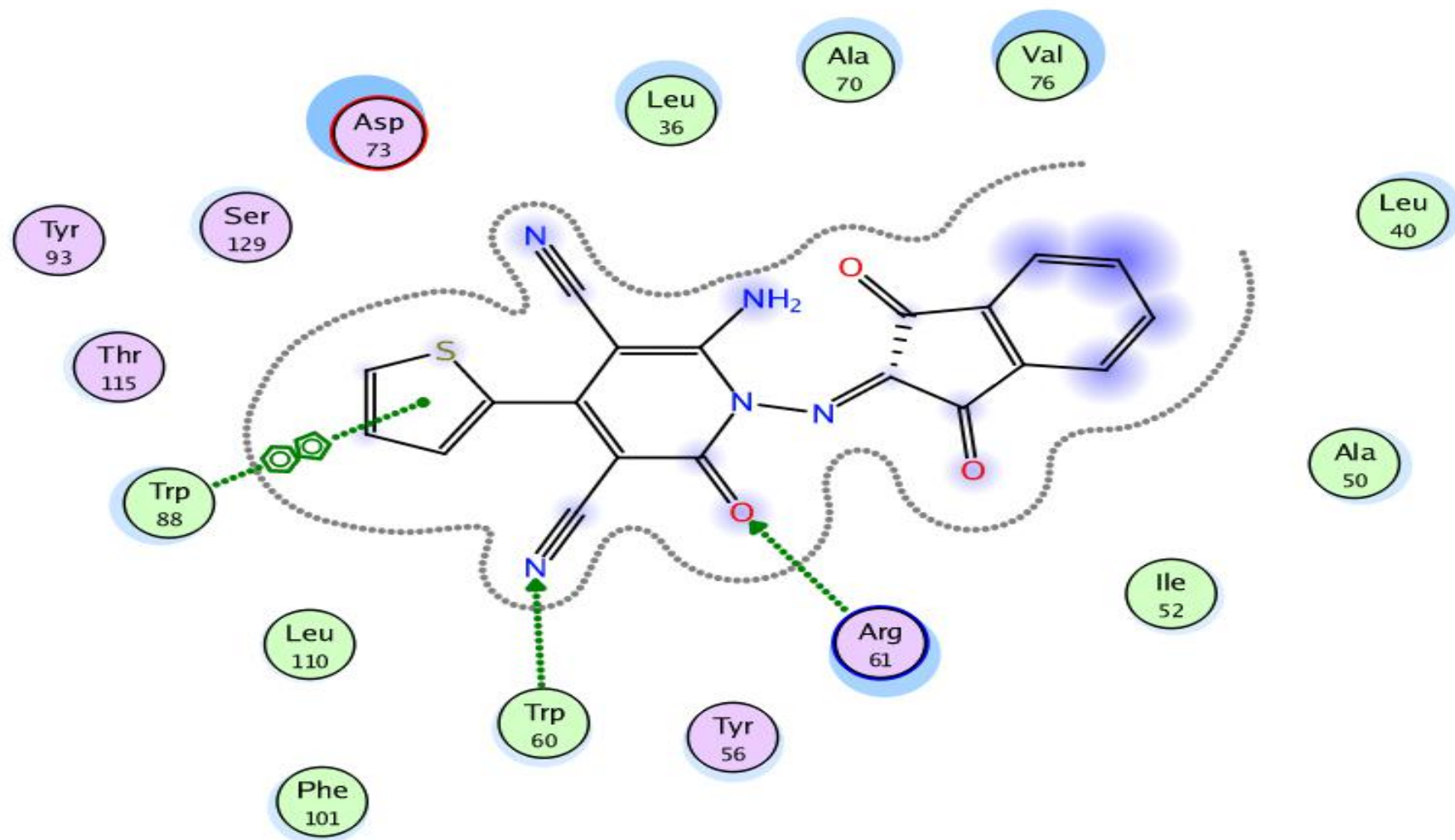


Figure S14: 2D structure of Docking pose and ligand interaction of compound **6a** inside the active site of LasR (PDB: 6MVN) in *P. aeruginosa* (UCBPP-PA14)

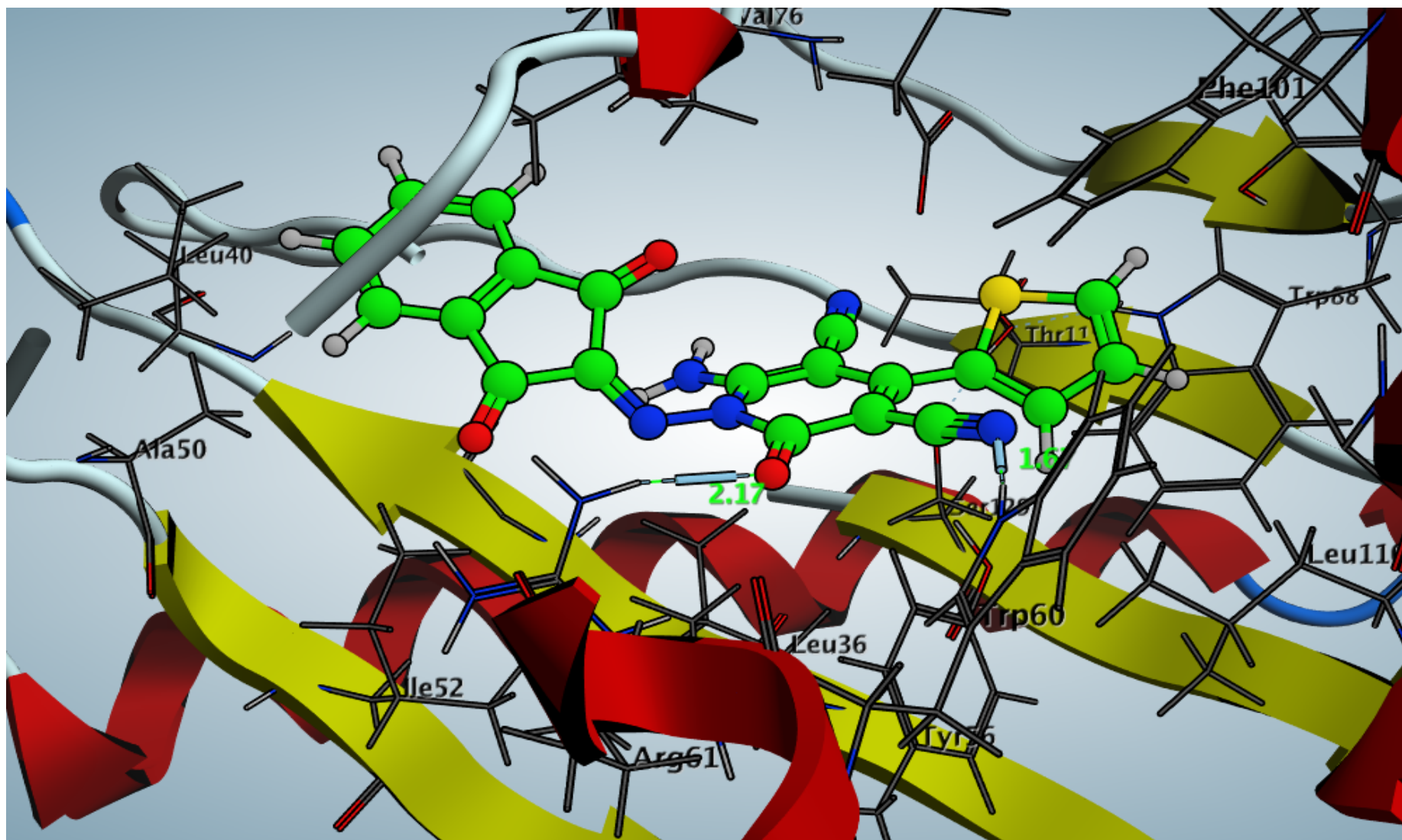


Figure S15: 3D structure of Docking pose and ligand interaction of compound **6a** inside the active site of LasR (PDB: 6MVN) in *P. aeruginosa* (UCBPP-PA14)

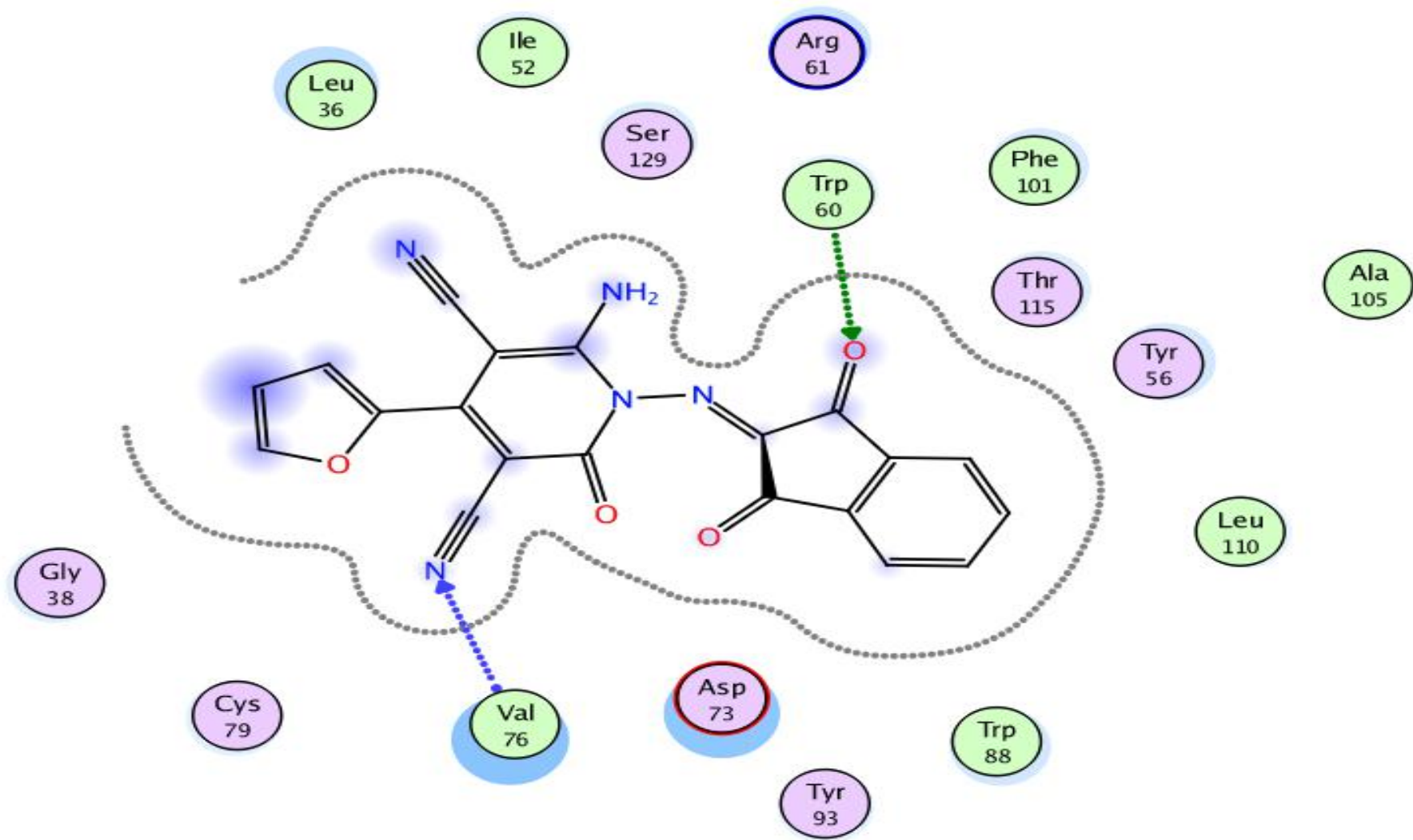


Figure S16: 2D structure of Docking pose and ligand interaction of compound **6b** inside the active site of LasR (PDB: 6MVN) in *P. aeruginosa* (UCBPP-PA14)

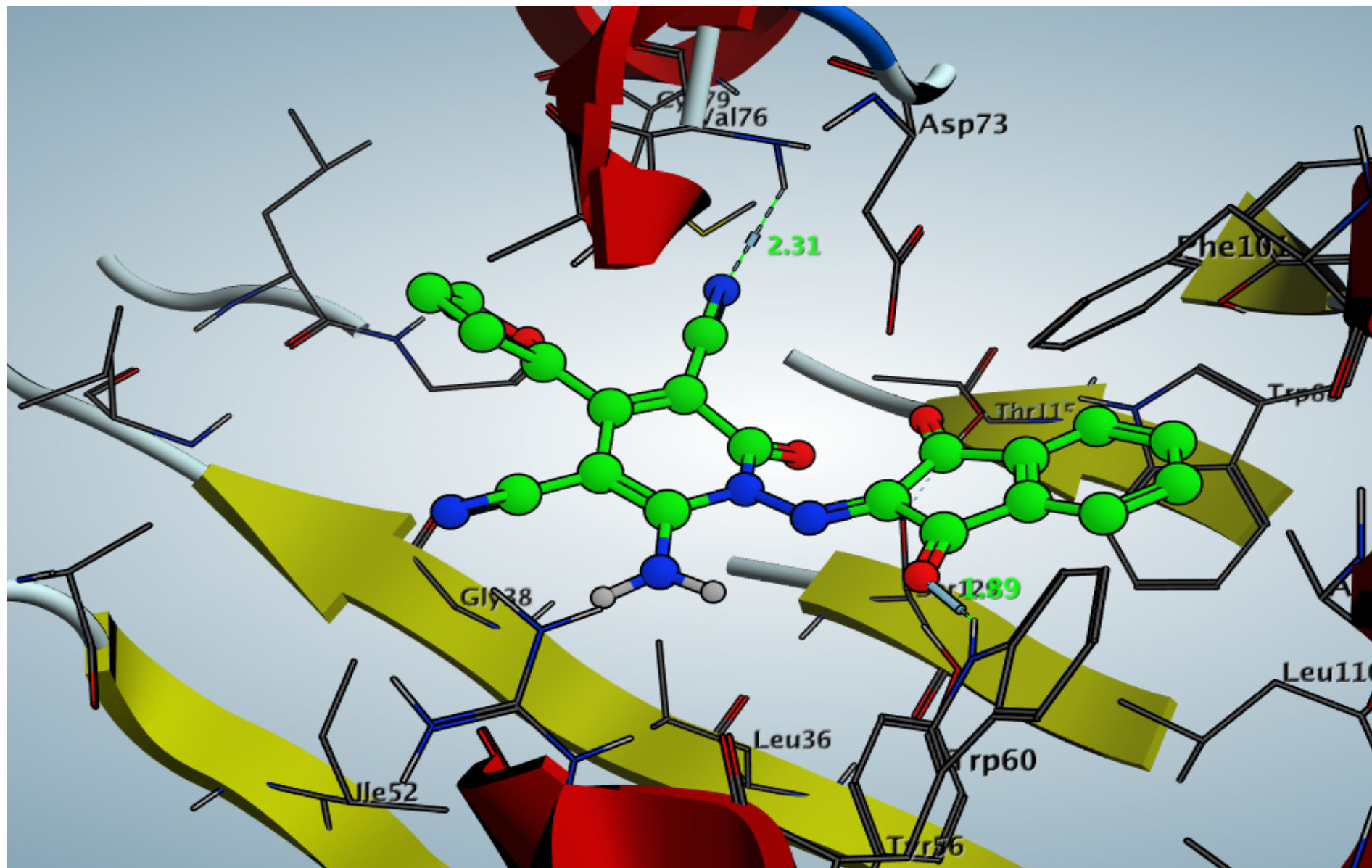


Figure S17: 3D structure of Docking pose and ligand interaction of compound **6b** inside the active site of LasR (PDB: 6MVN) in *P. aeruginosa* (UCBPP-PA14)