Discovery of New Pyridine-Quinoline Hybrids as Competitive and Non-competitive PIM-1 Kinase Inhibitors with Apoptosis Induction and caspase 3/7 activation capabilities

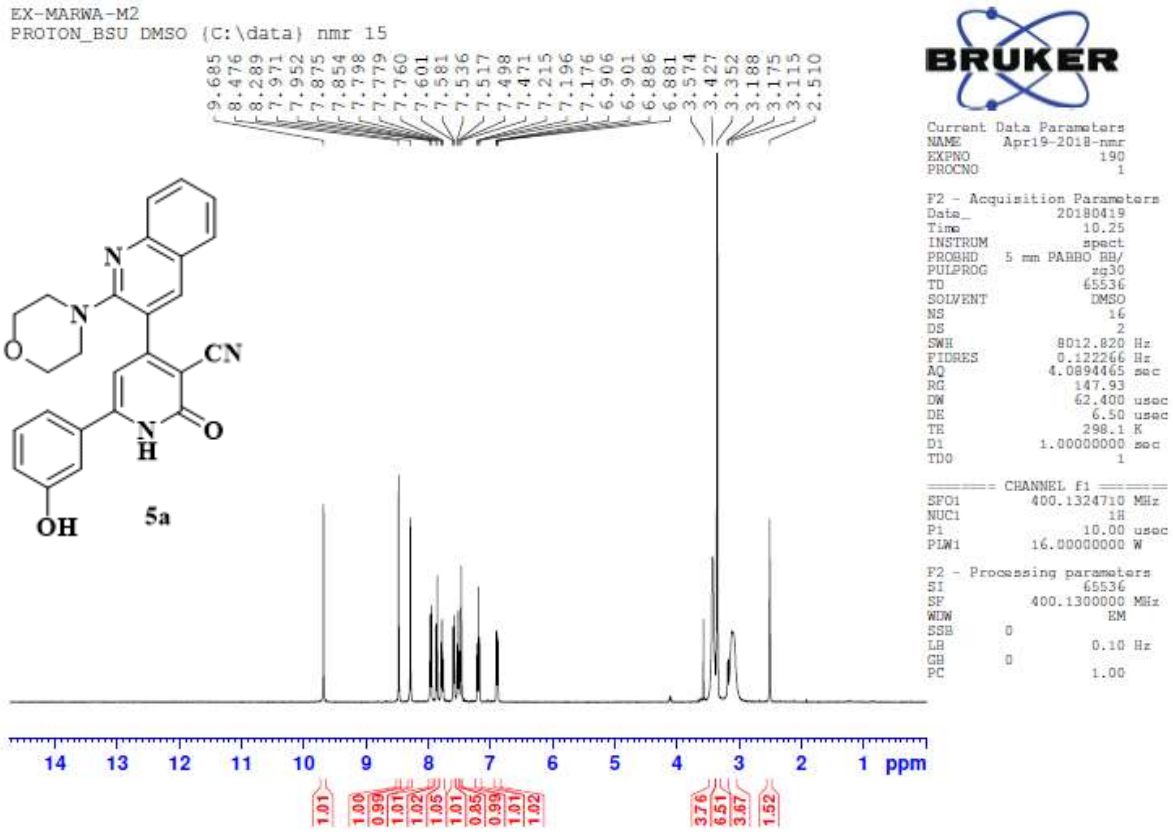
Mostafa M. M. El-Miligy[1]*, Marwa E. Abdelaziz[1]*, Salwa M. Fahmy[1], Tamer M. Ibrahim[3], Marwa M. Abu-Serie[2], Mona A. Mahran[1], Aly A. Hazzaa[1]

[1]Pharmaceutical Chemistry Department, Faculty of Pharmacy, Alexandria University, Alexandria 21521, Egypt

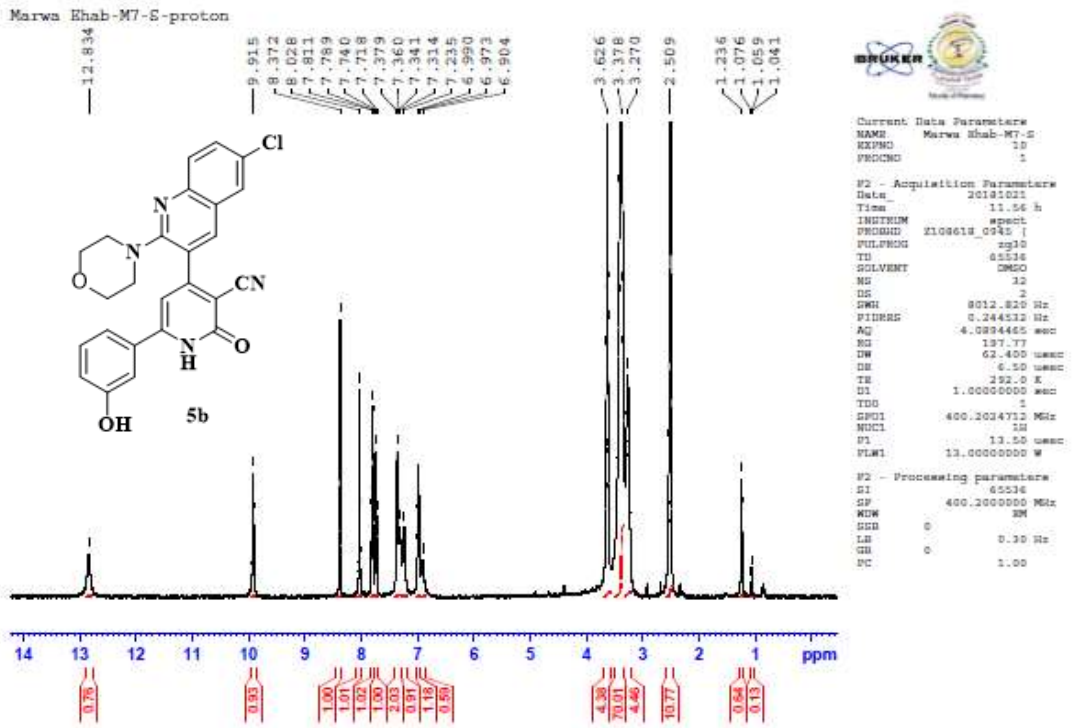
[2] Medical Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), City of Scientific Research and Technological Applications (SRTA-City), Alexandria 21934, Egypt

[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Kafrelsheikh University, Kafrelsheikh 33516, Egypt

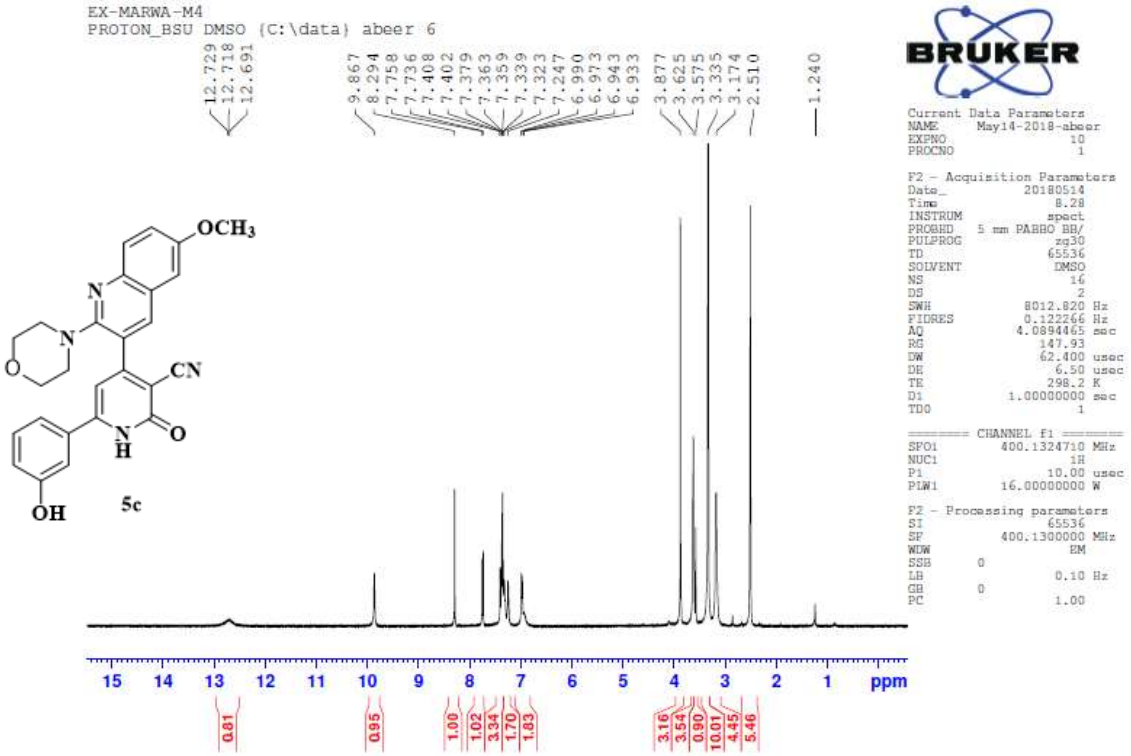
Scheme 1:



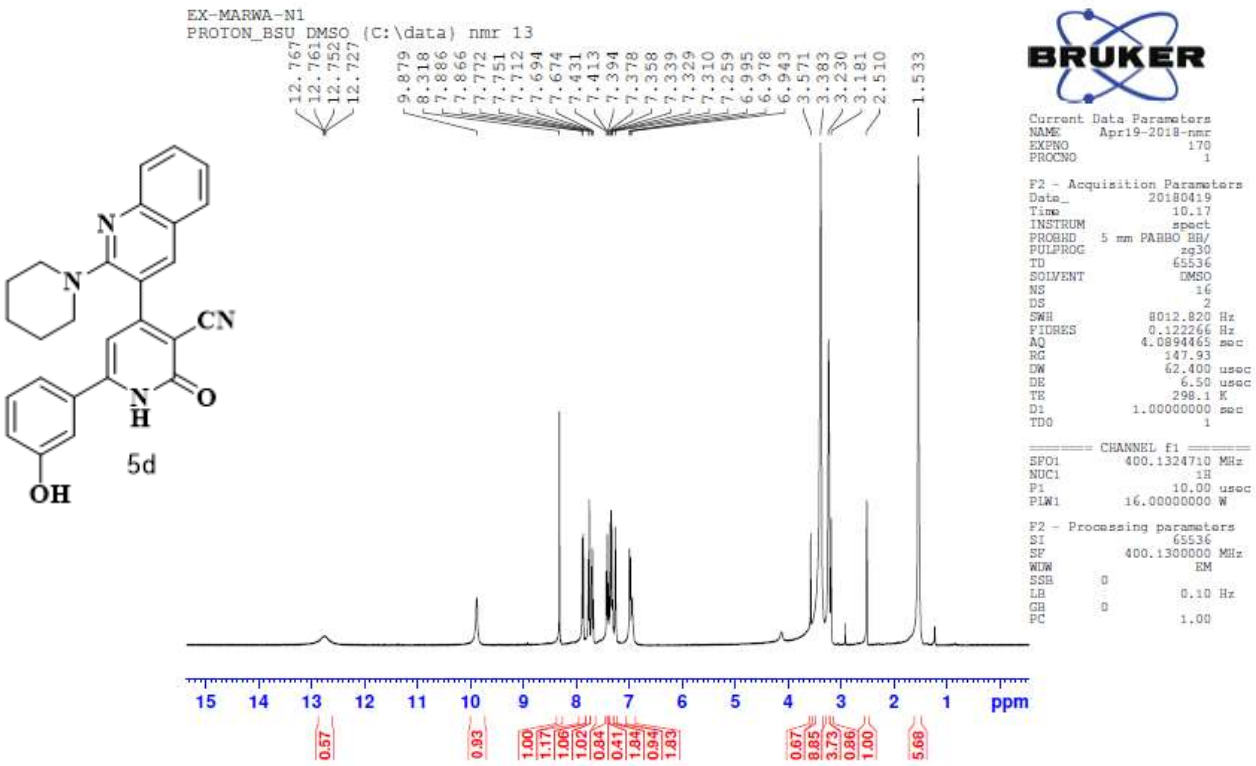
H^1-NMR spectra of compound 5a



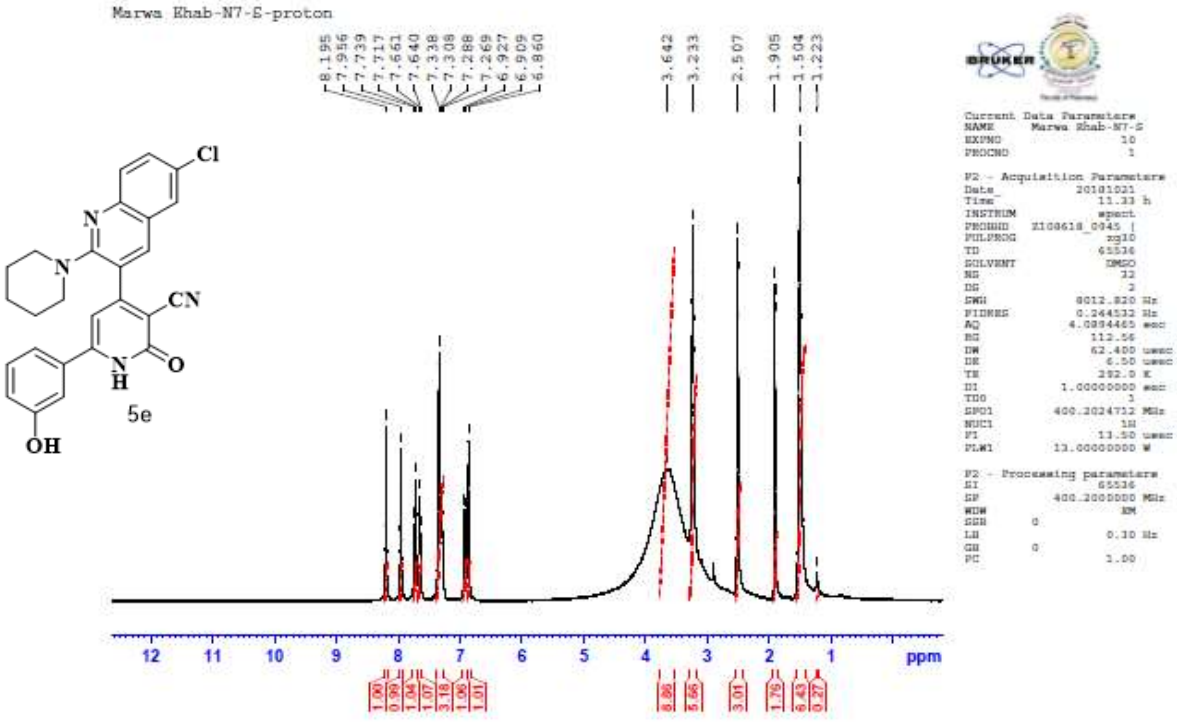
H^1-NMR spectra of compound 5b



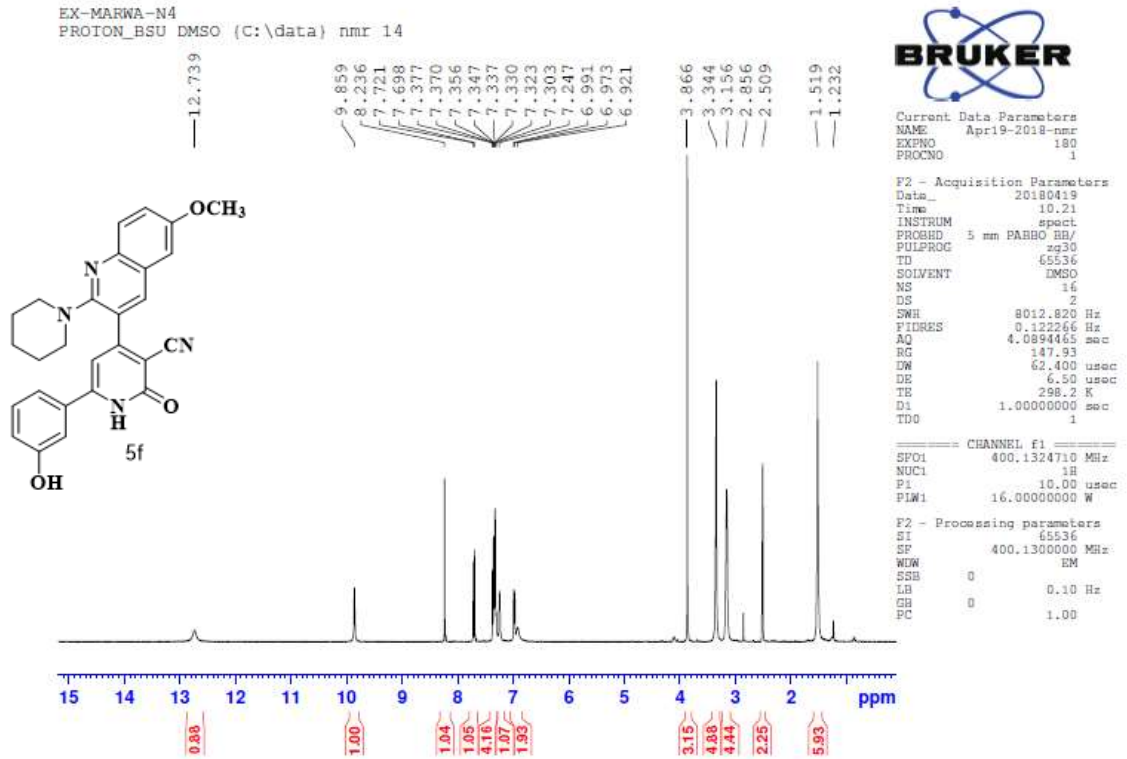
H^1-NMR spectra of compound 5c



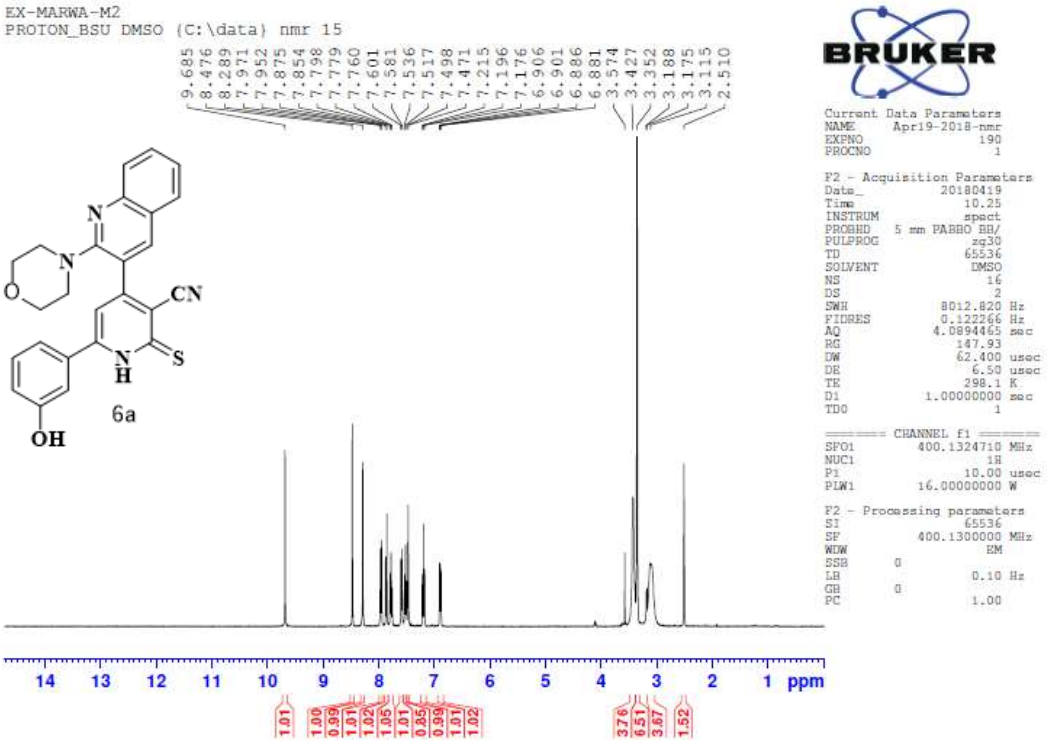
H[1]-NMR spectra of compound 5d



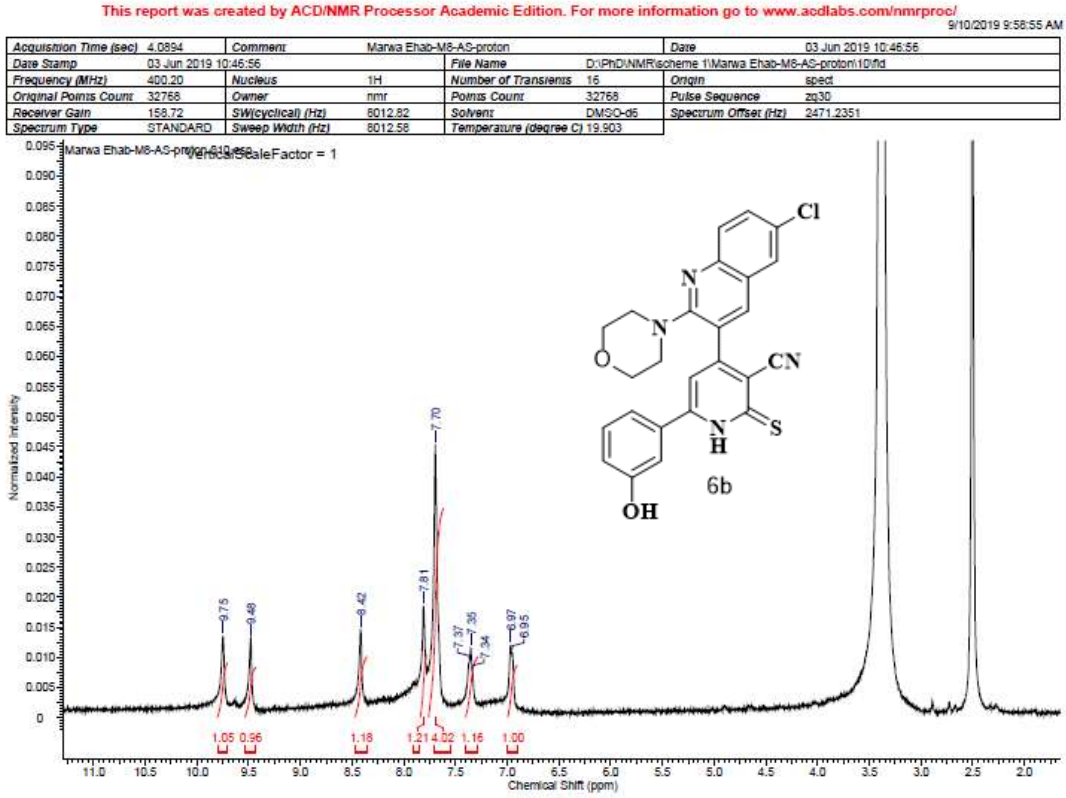
H[1]-NMR spectra of compound 5e



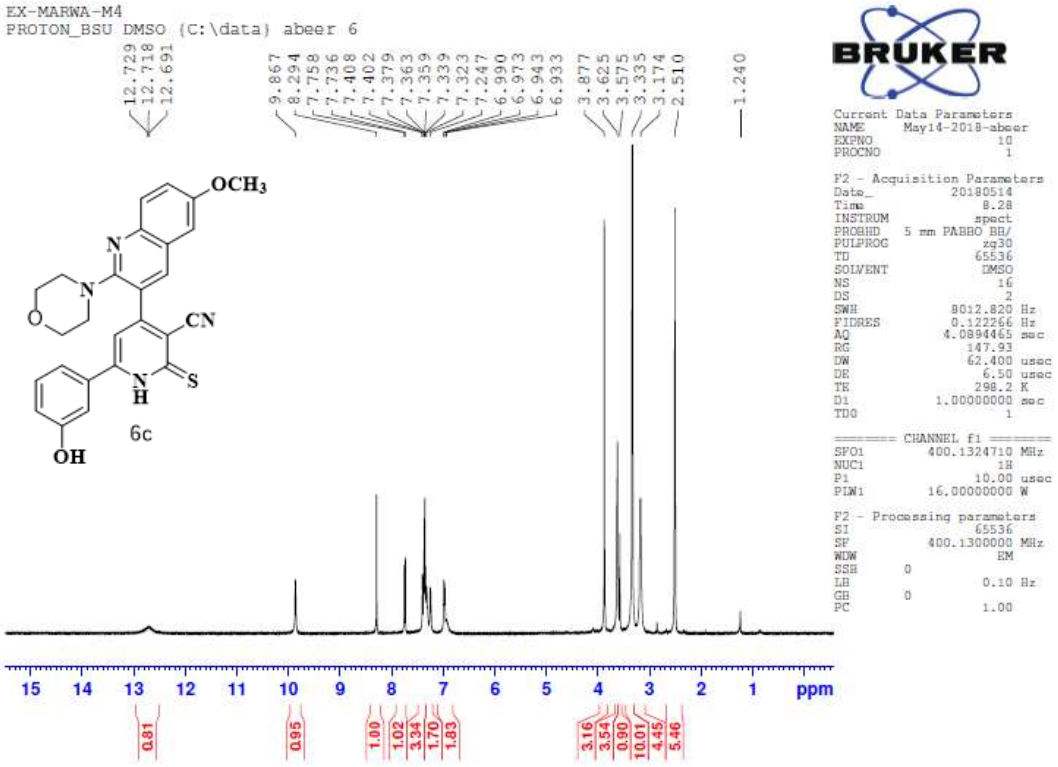
H[1]-NMR spectra of compound 5f



H[1]-NMR spectra of compound 6a



H[1]-NMR spectra of compound 6b



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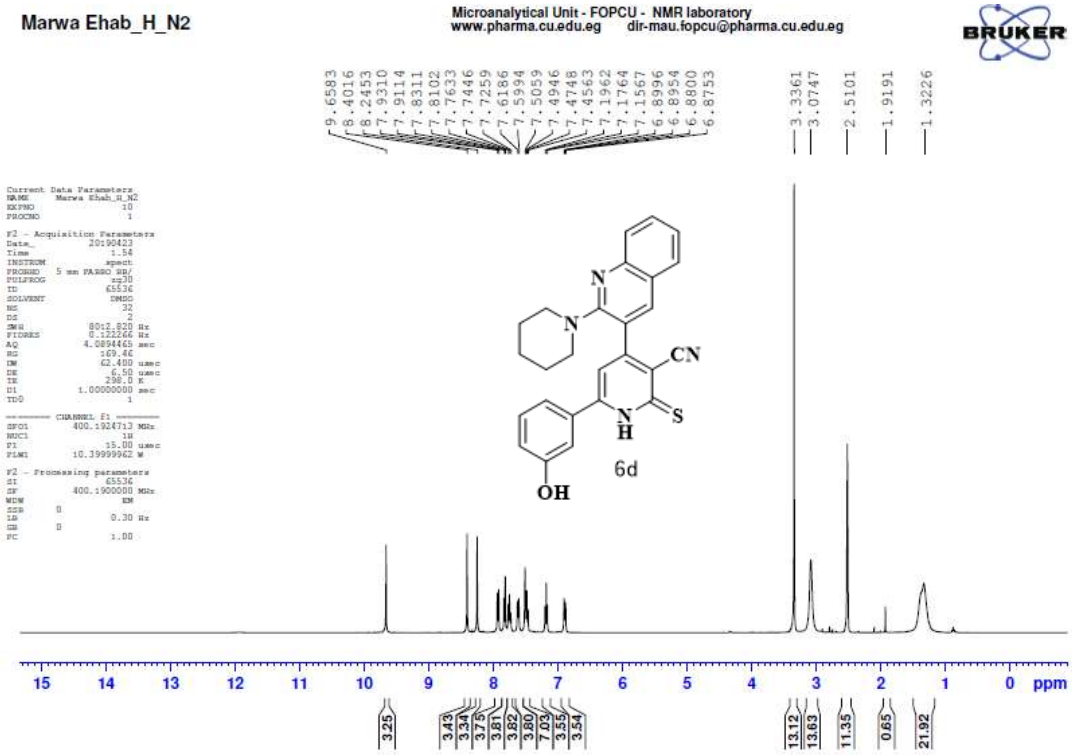
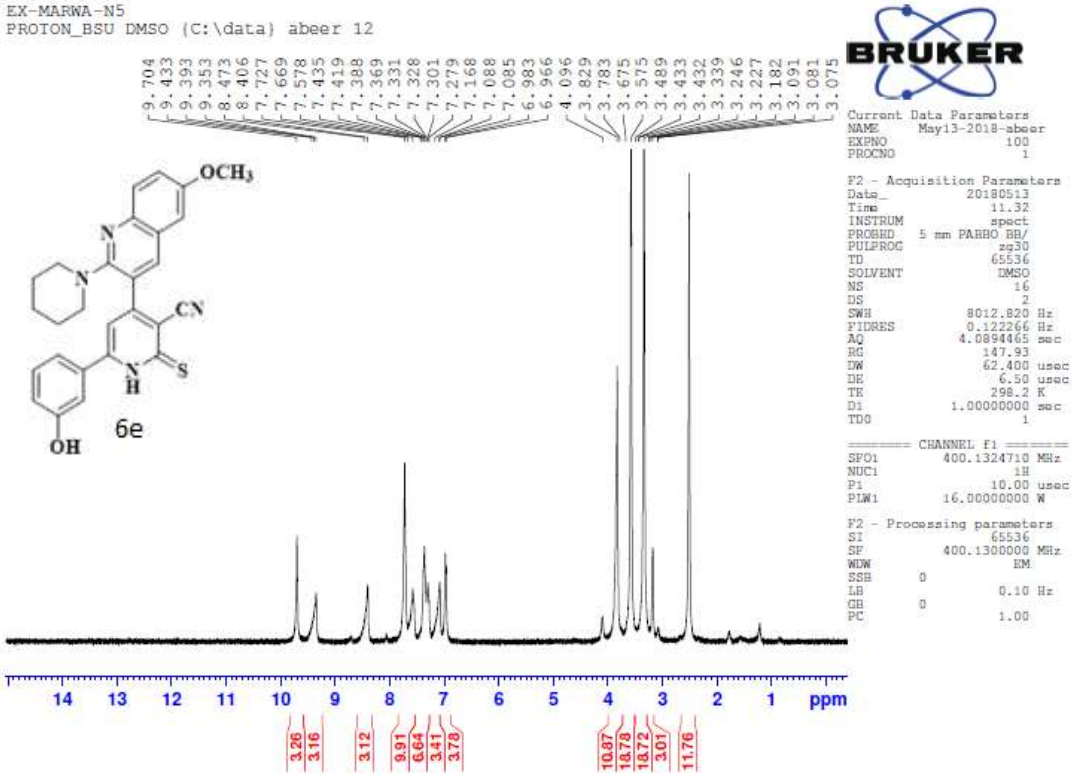
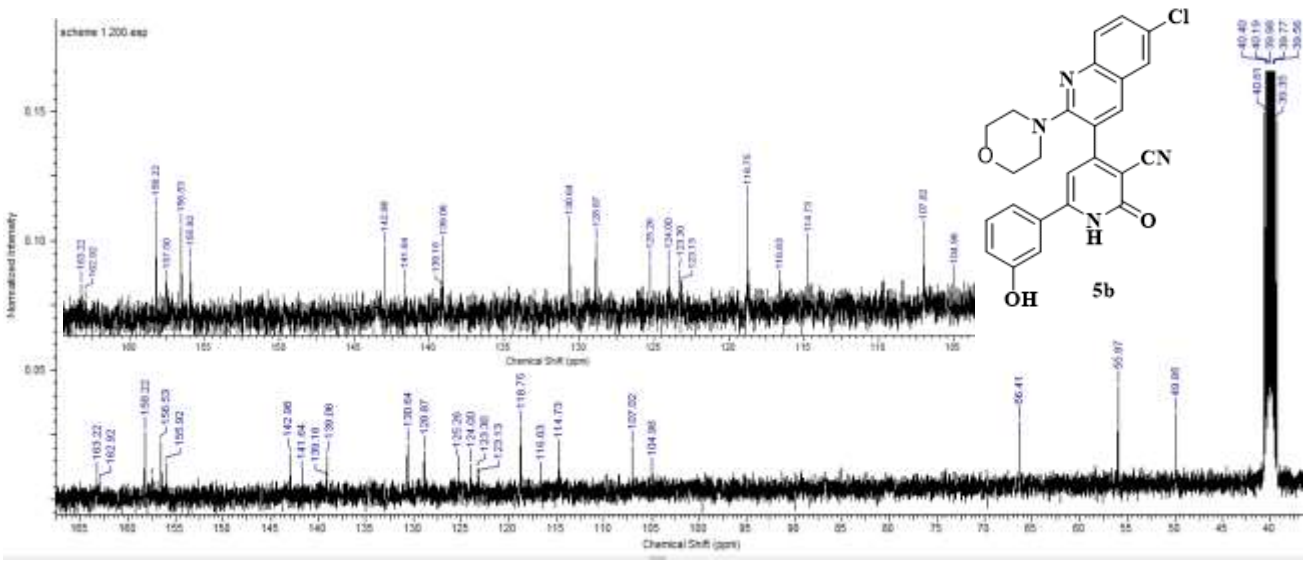


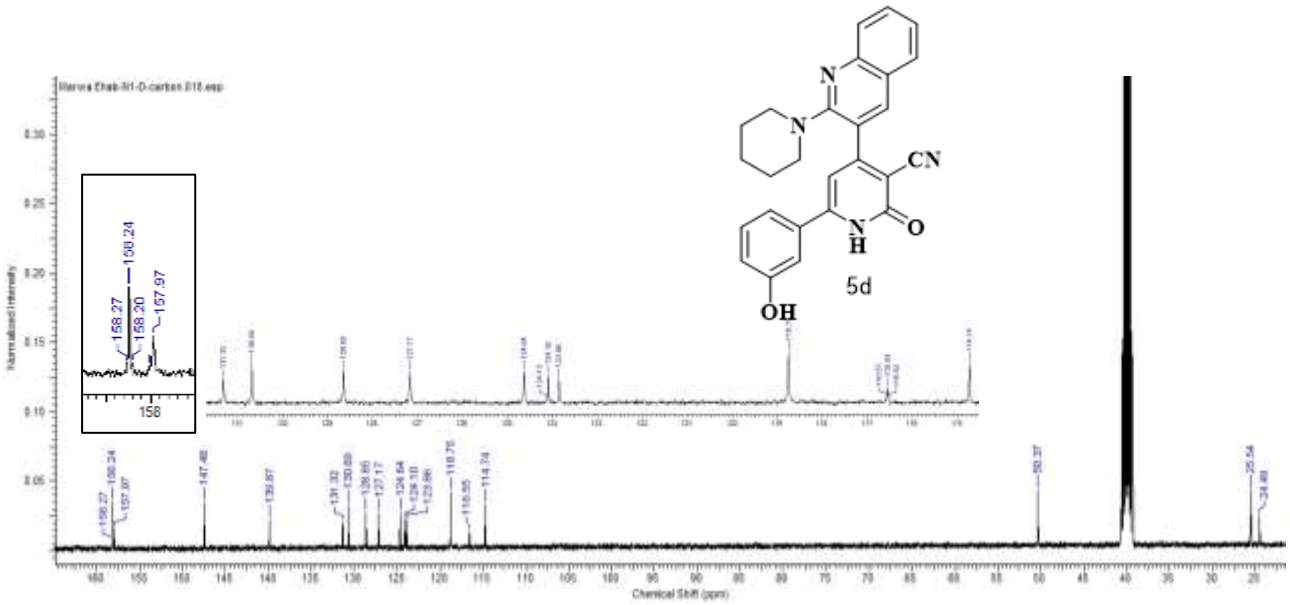
H^1-NMR spectra of compound 6d



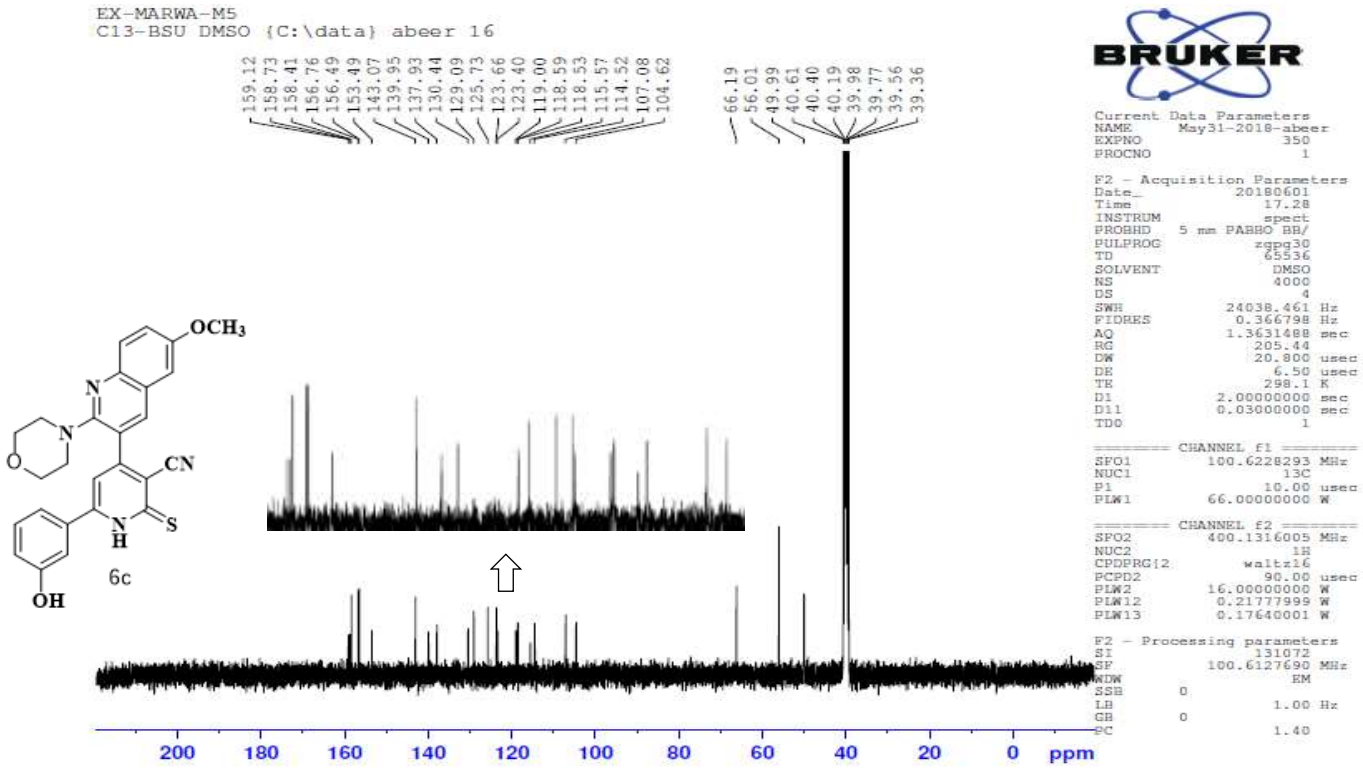
H^1-NMR spectra of compound 6e



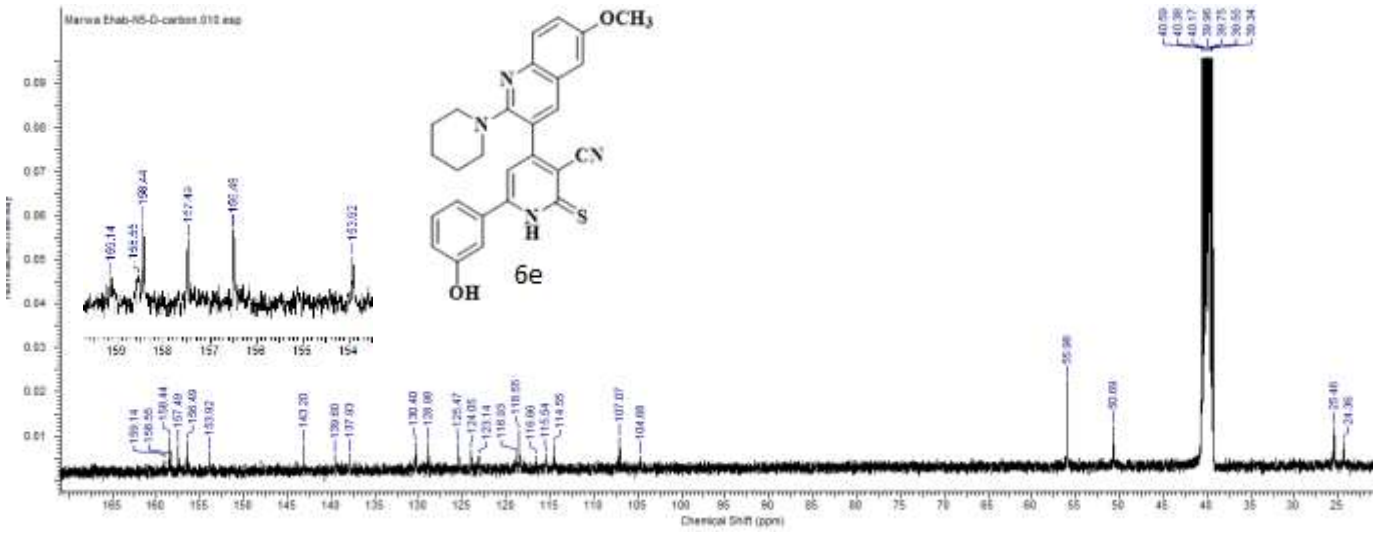
C^{13}- NMR spectra of compound 5b



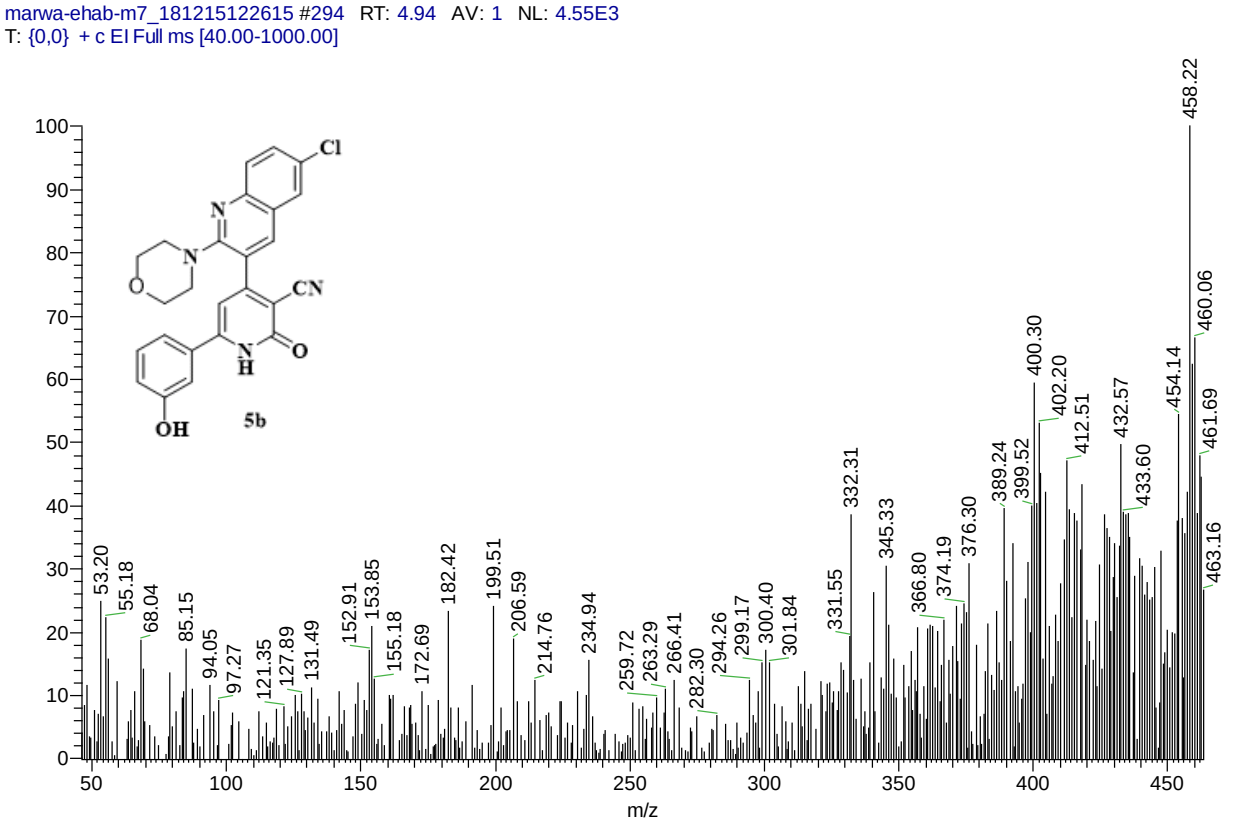
C^{13}- NMR spectra of compound 5d



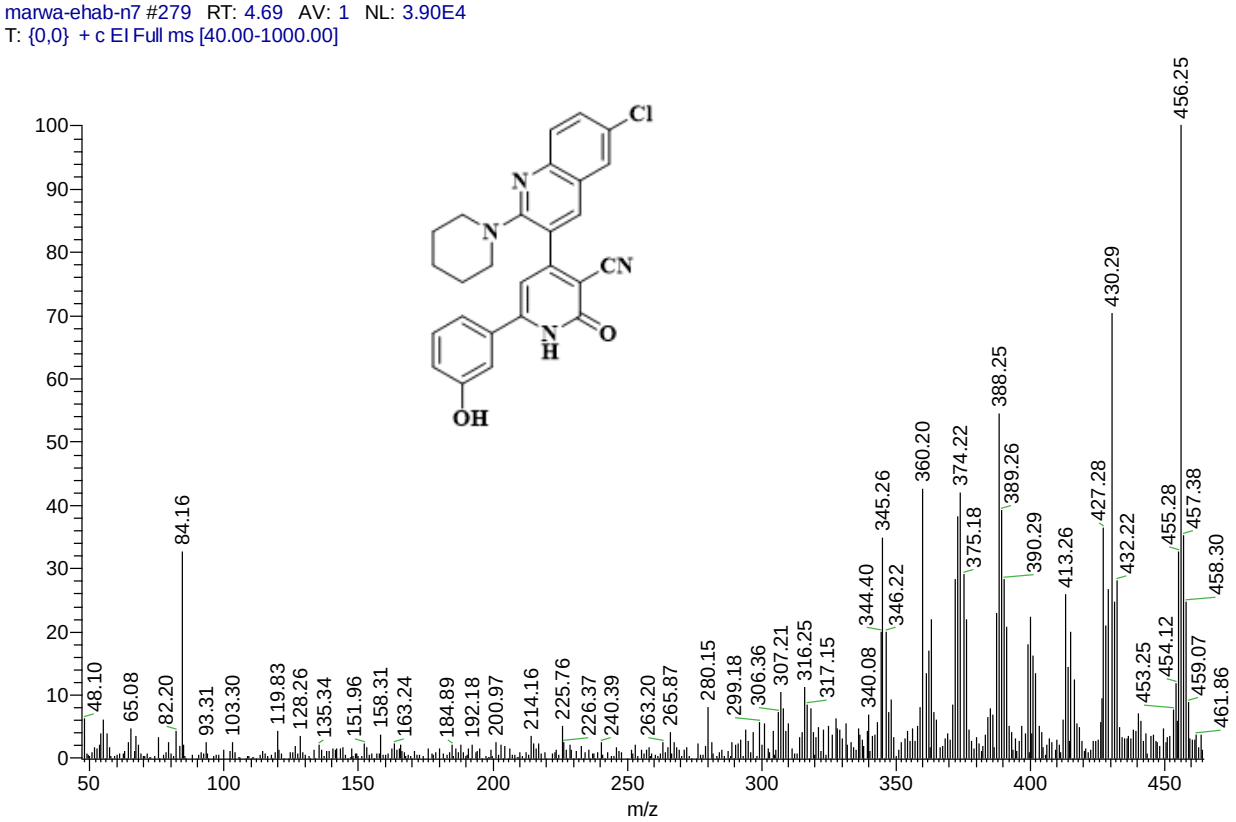
C^{13}- NMR spectra of compound 6c



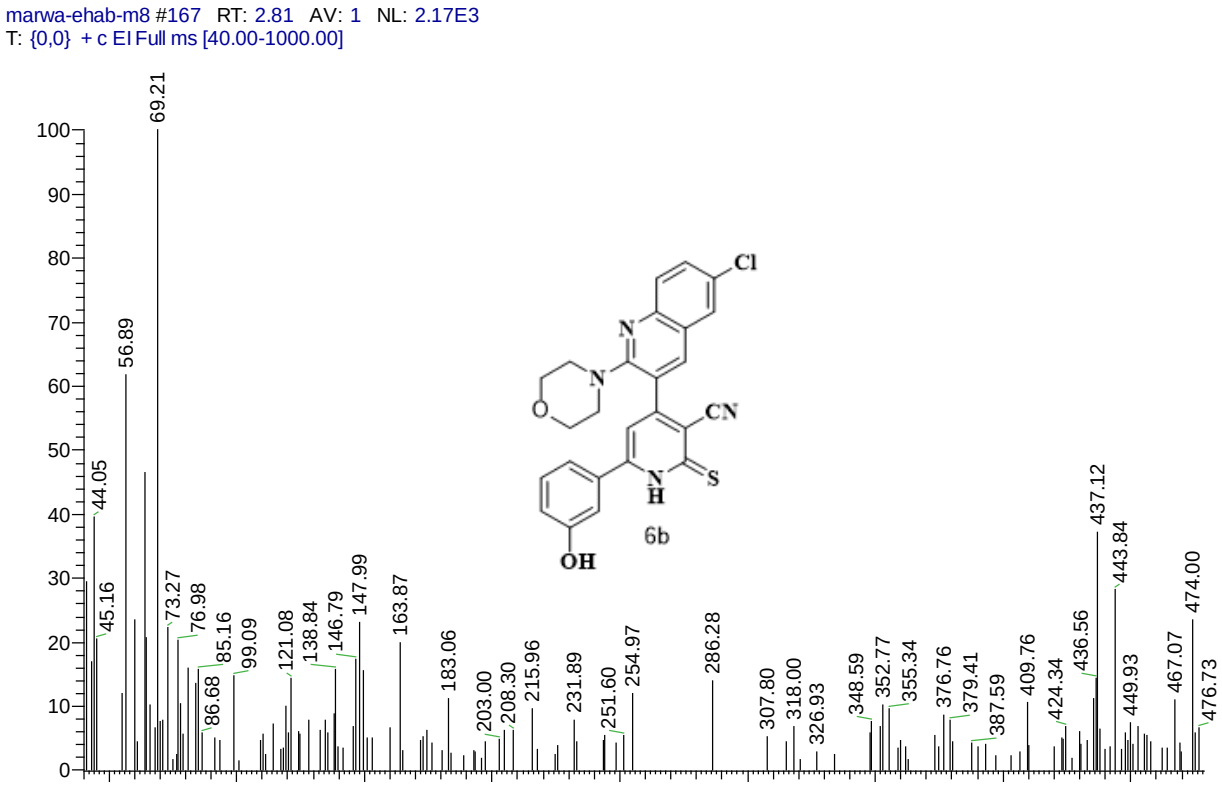
C^{13}- NMR spectra of compound 6e



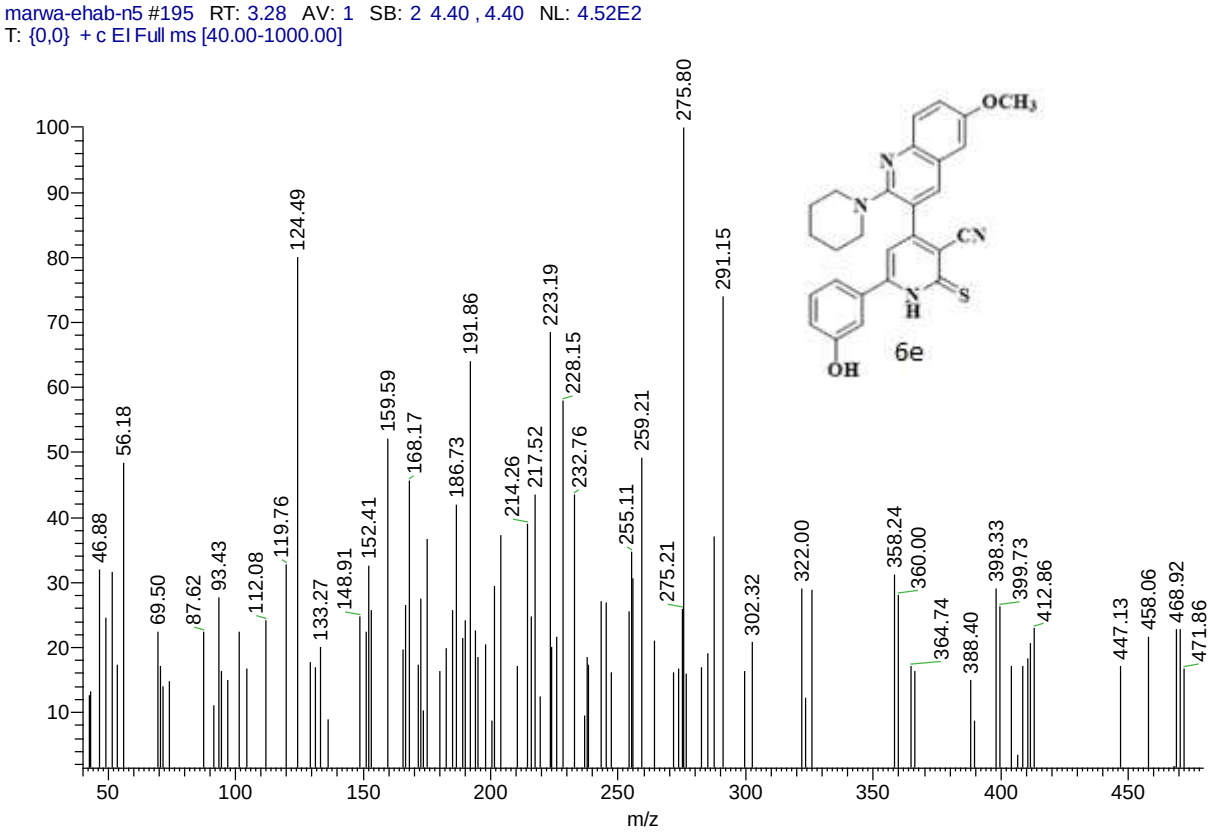
EI/MS spectrum of compound **5b**



EI/MS spectrum of compound **5e**



EI/MS spectrum of **compound 6b**



EI/MS spectrum of compound **6e**

Al-Azhar University
The Regional Center for Mycology and Biotechnology

Requester Data:

Name: Dr. Marwa Ehab Mohamed
Authority: Faculty of Pharmacy,
Alexandria University

Sample Data:

Eleven samples had been submitted for elemental analysis.

Analysis Report:

Sample Code	C%	H%	N%
CH_2	68.01	3.43	7.79
CH_5	68.56	3.88	7.42
M_1 5a	71.02	4.89	13.43
M_4 5c	68.94	5.12	12.59
M_7 5b	65.27	4.28	12.47
N_1 5d	74.19	5.39	13.53
N_4 5f	71.90	5.26	12.56
N_7 5e	68.60	4.80	12.49
O_2	70.69	3.84	11.97
O_5	68.80	3.85	10.79
O_8	64.89	3.32	10.85

INVESTIGATOR **DIRECTOR**

Al-Azhar University

The Regional Center for Mycology and Biotechnology

Requester Data:

Name: Dr. Marwa Ehab Mohamed
Authority: Faculty of Pharmacy,
Alexandria University

Sample Data:

Twenty samples had been submitted for elemental analysis.

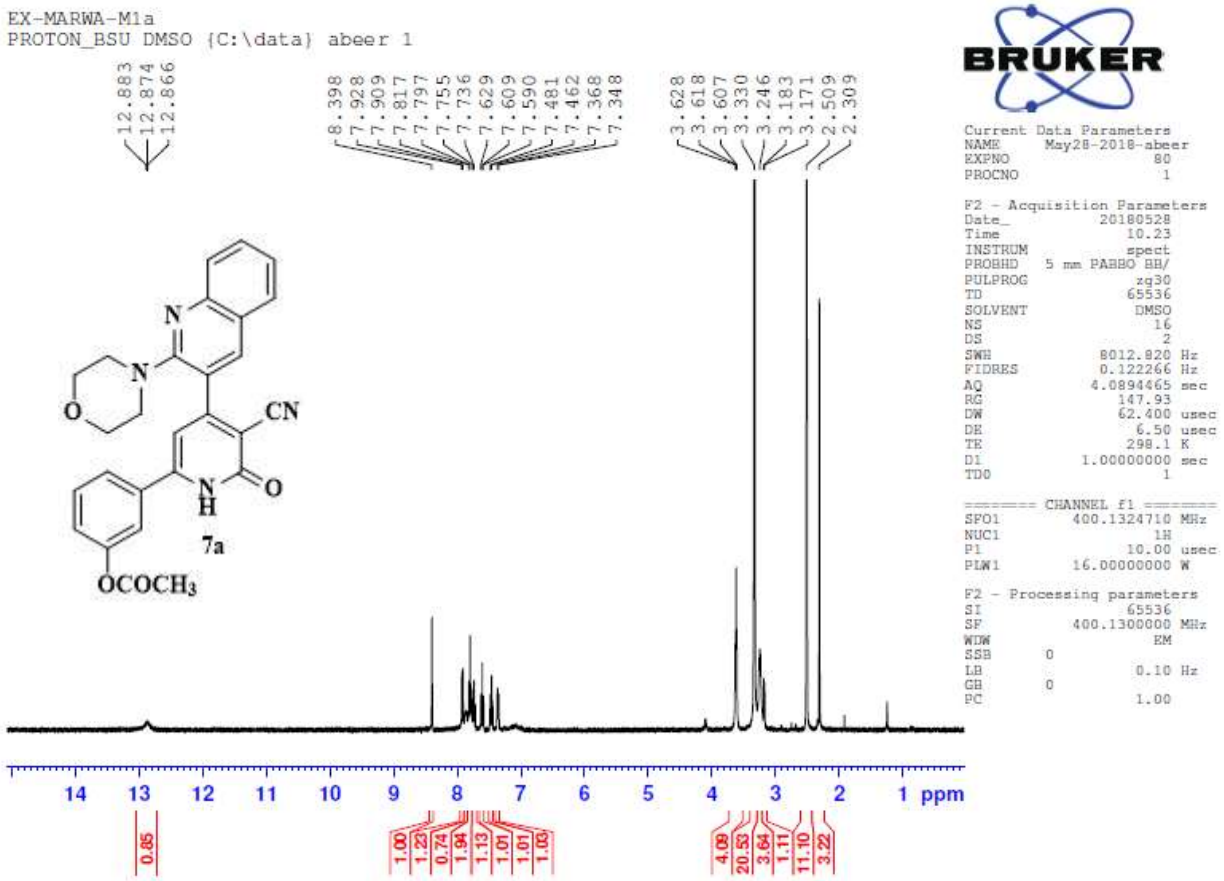
Analysis Report:

Sample Code	C%	H%	N%
M_1 eN	68.71	5.24	19.38
M_1 J	71.09	5.21	12.96
M_2 6a	68.42	4.75	12.94
M_4 e	70.38	5.84	11.50
M_4 k	69.87	5.68	11.79
M_5 6c	66.59	4.83	11.75
M_7 a	64.58	4.59	11.34
M_7 b	62.96	4.13	11.06
M_7 e	67.40	5.28	11.37
M_7 e N	63.26	4.69	17.58
M_7 f	63.44	4.21	11.39
M_7 h	66.81	4.62	11.94
M_7 J	66.26	4.69	11.72
M_7 k	66.49	4.88	11.69
M_9 6b	63.45	4.11	11.94
N_2 6d	71.47	5.28	13.02
N_5 6f	6e 43	5.34	12.13
O_1	49	4.06	15.95
O_4	68.95	4.32	14.67
O_7	64.71	3.19	14.62

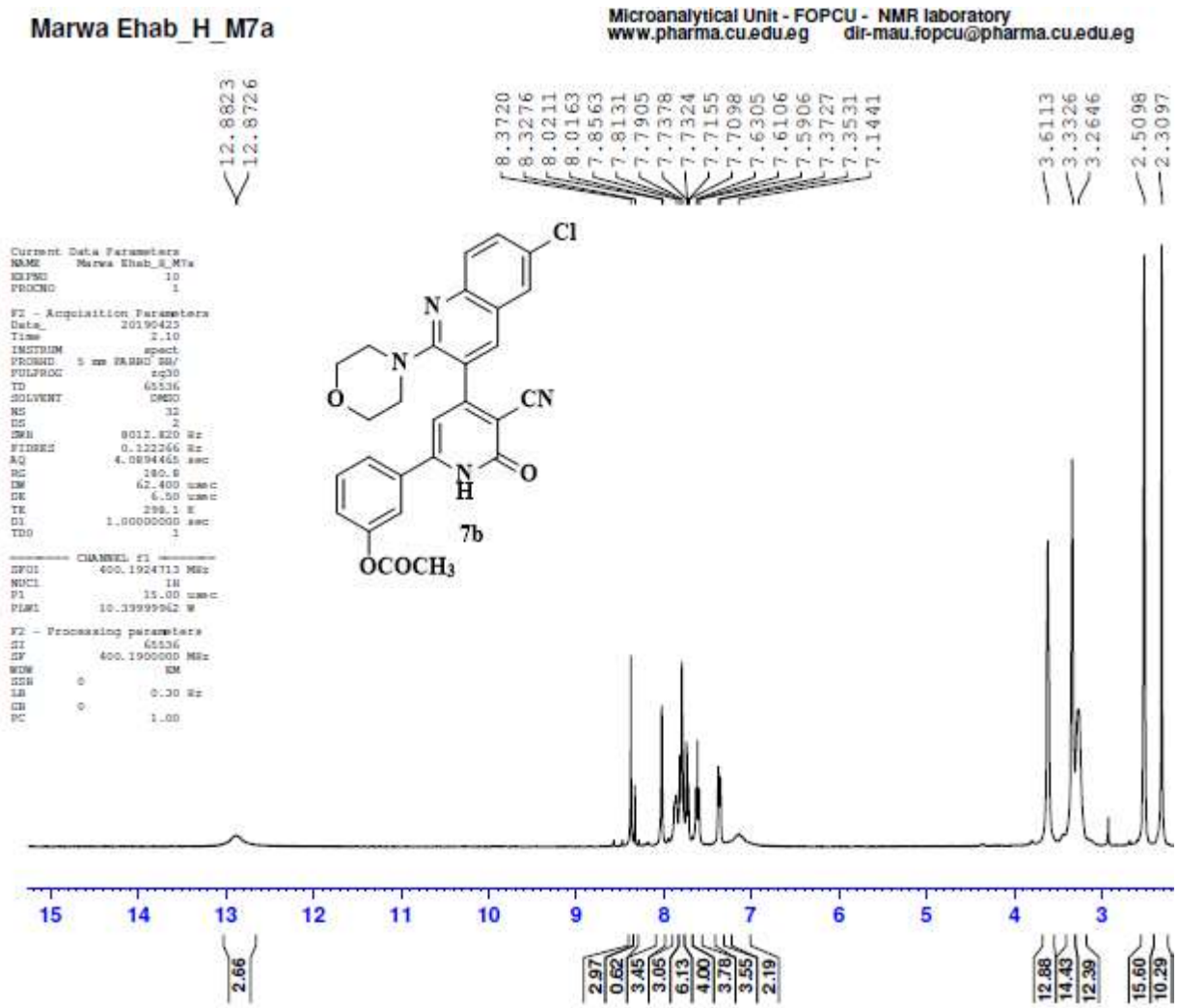
INVESTIGATOR

DIRECTOR

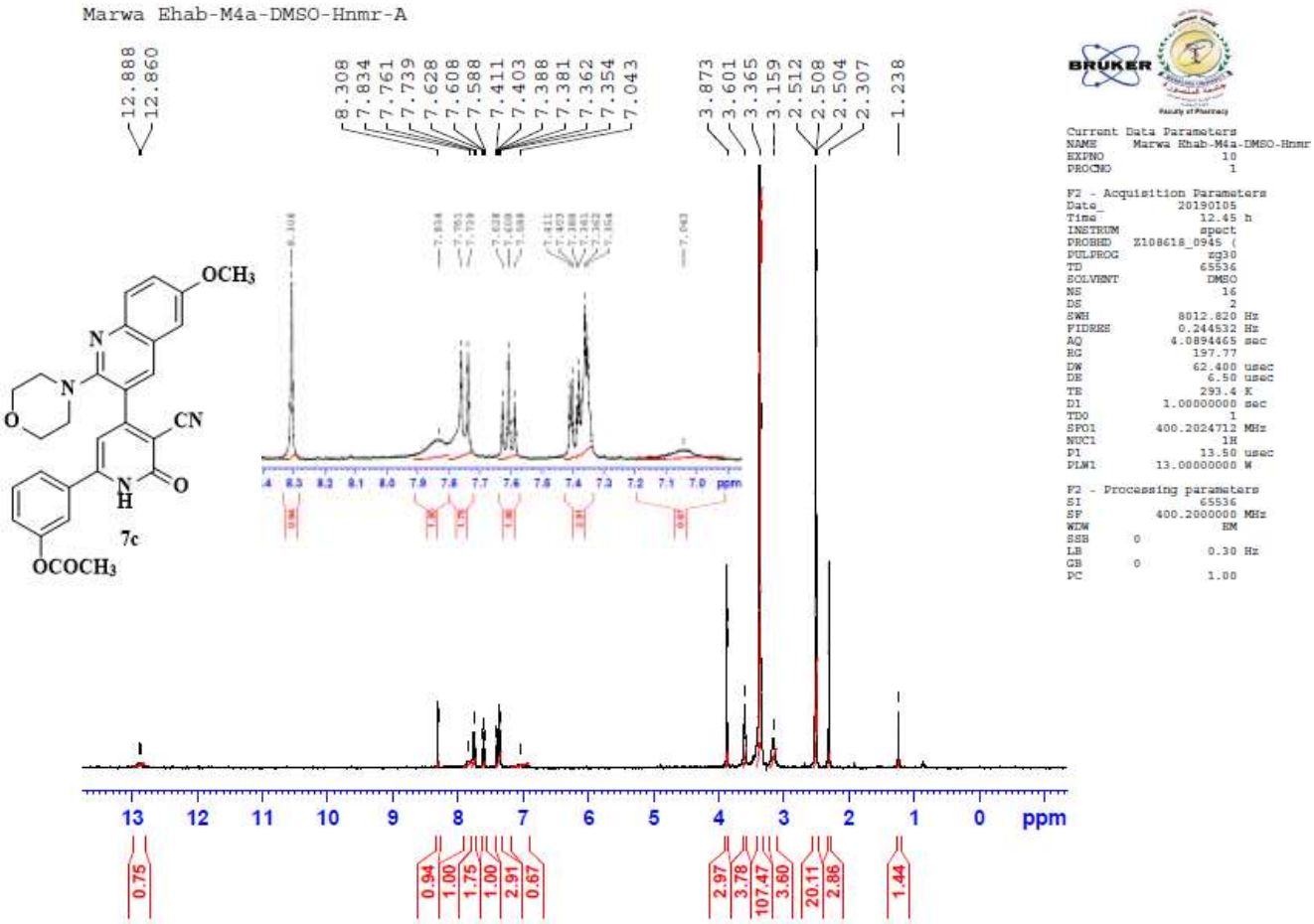
Scheme 2:



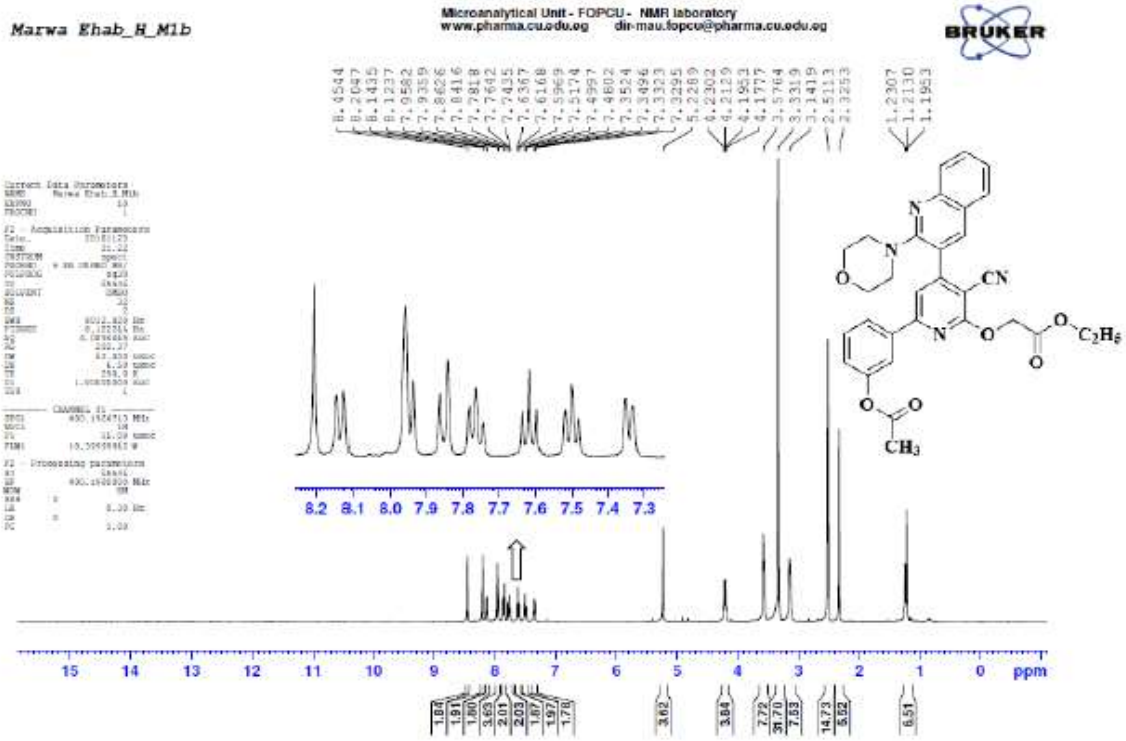
H[1]-NMR spectra of compound 7a



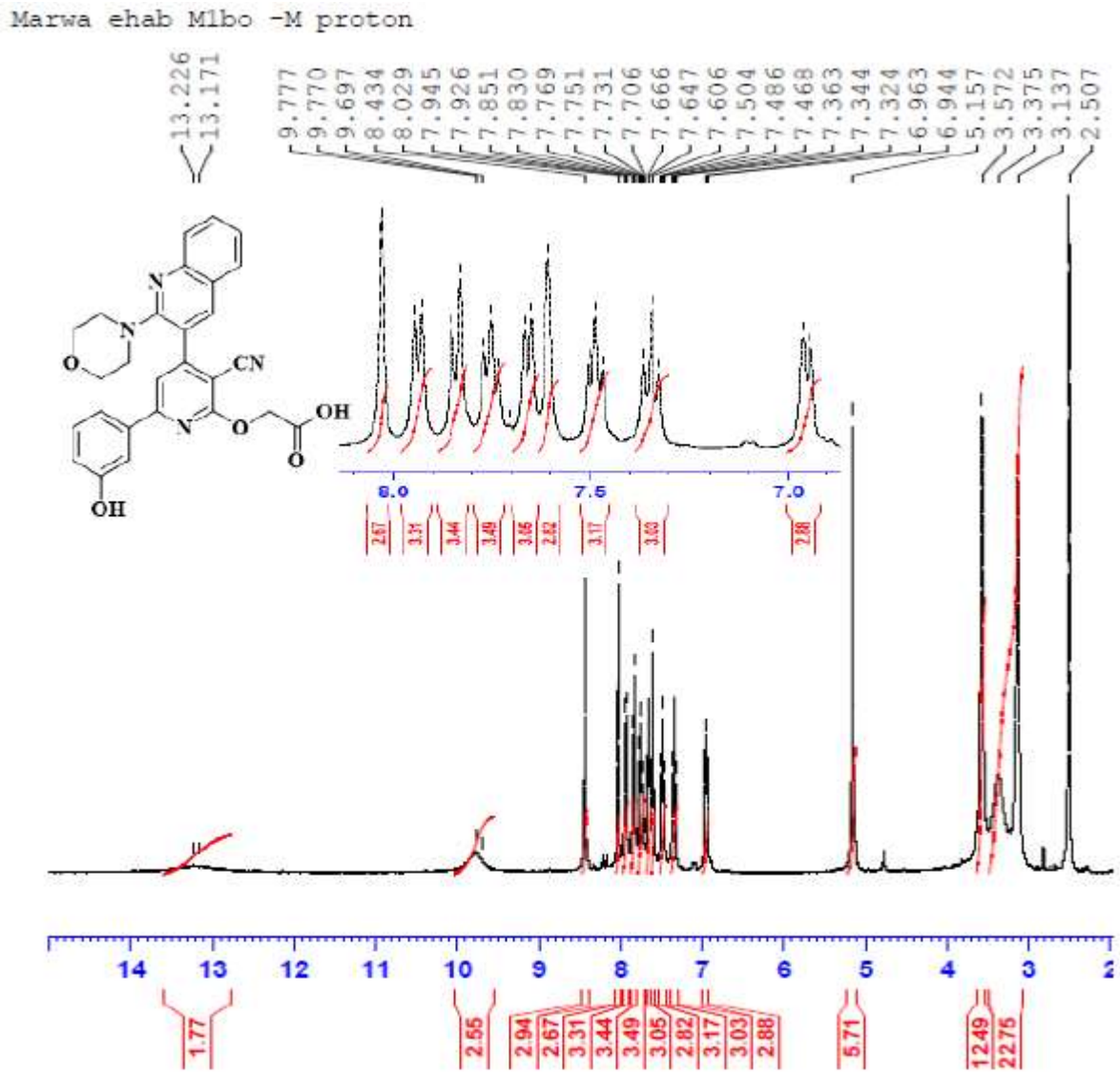
H[1]-NMR spectra of compound 7b



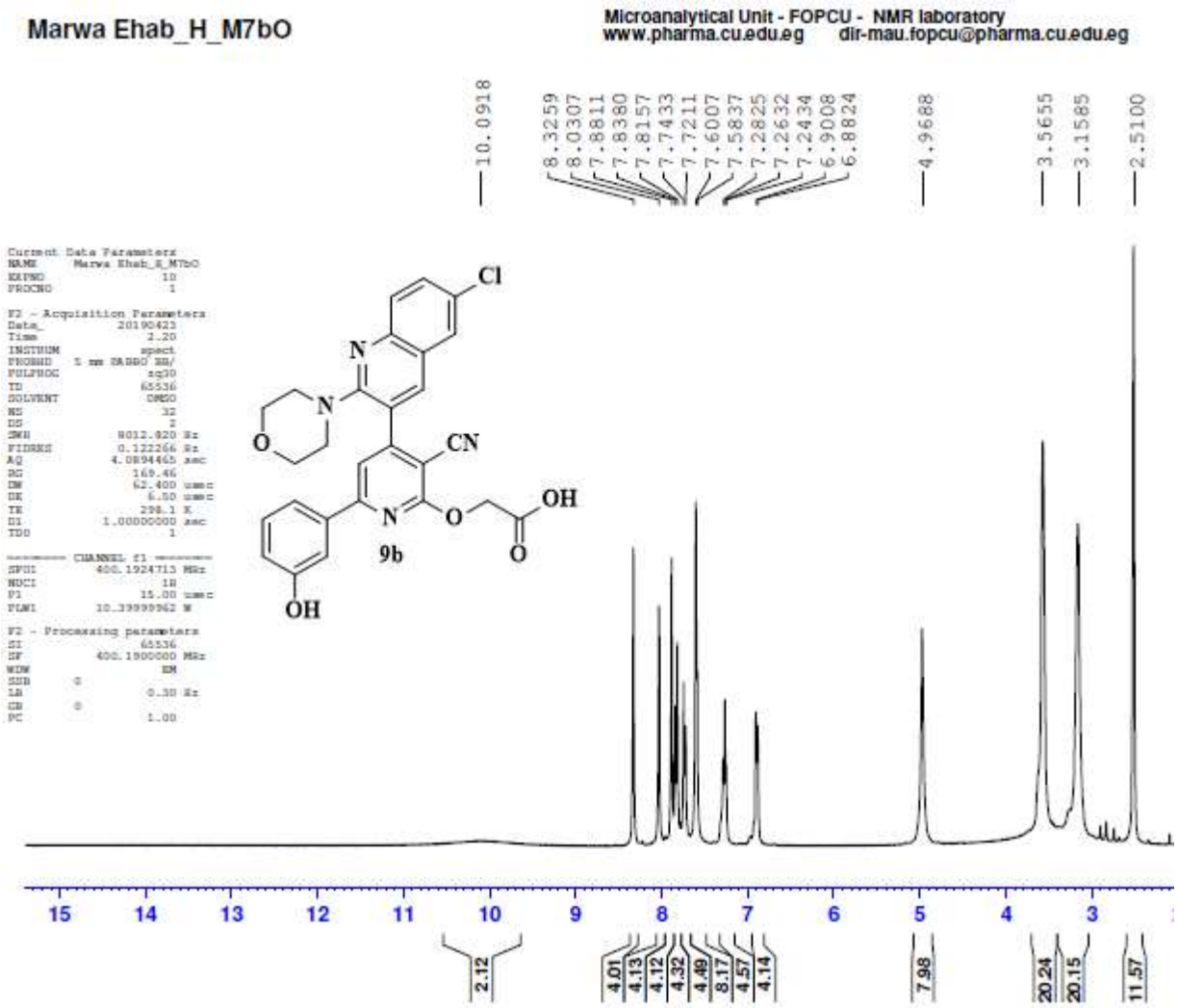
H[1]-NMR spectra of compound 7c



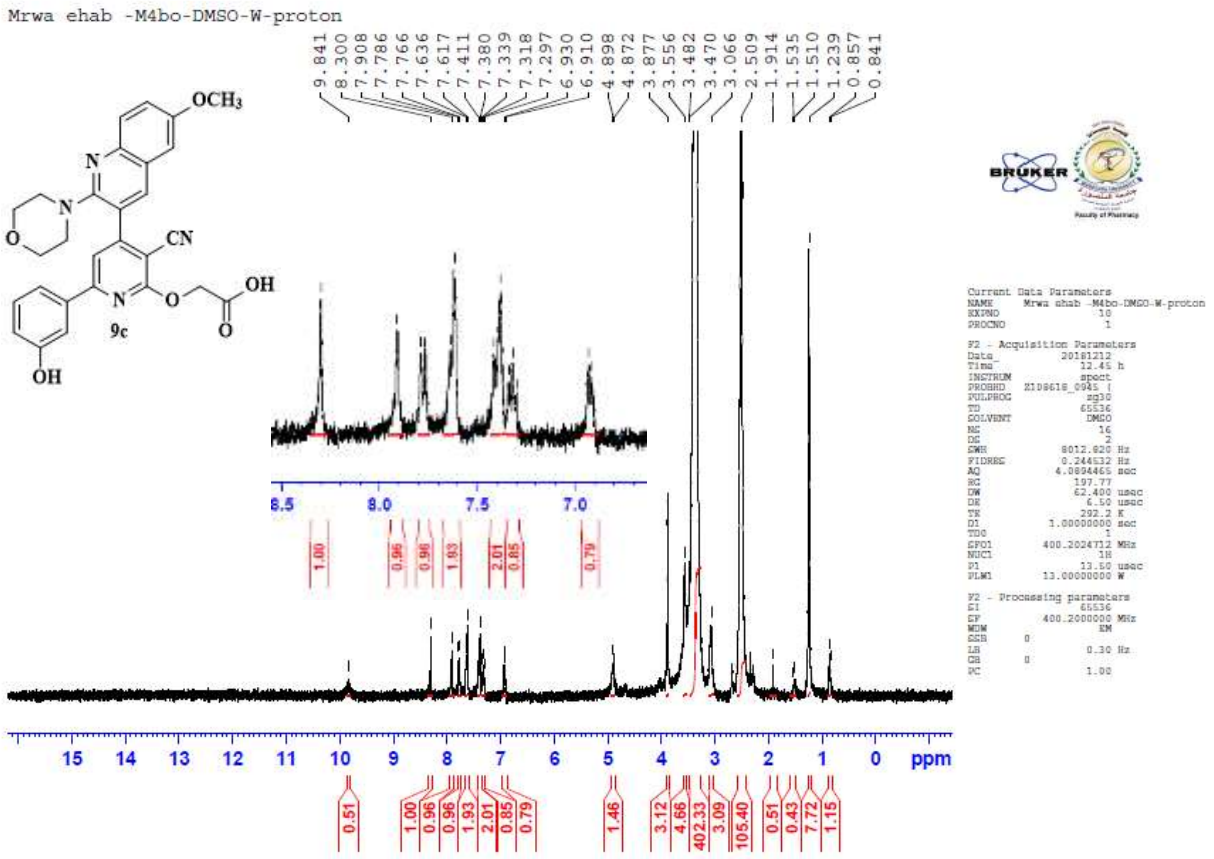
H[1]-NMR spectra of compound 8a



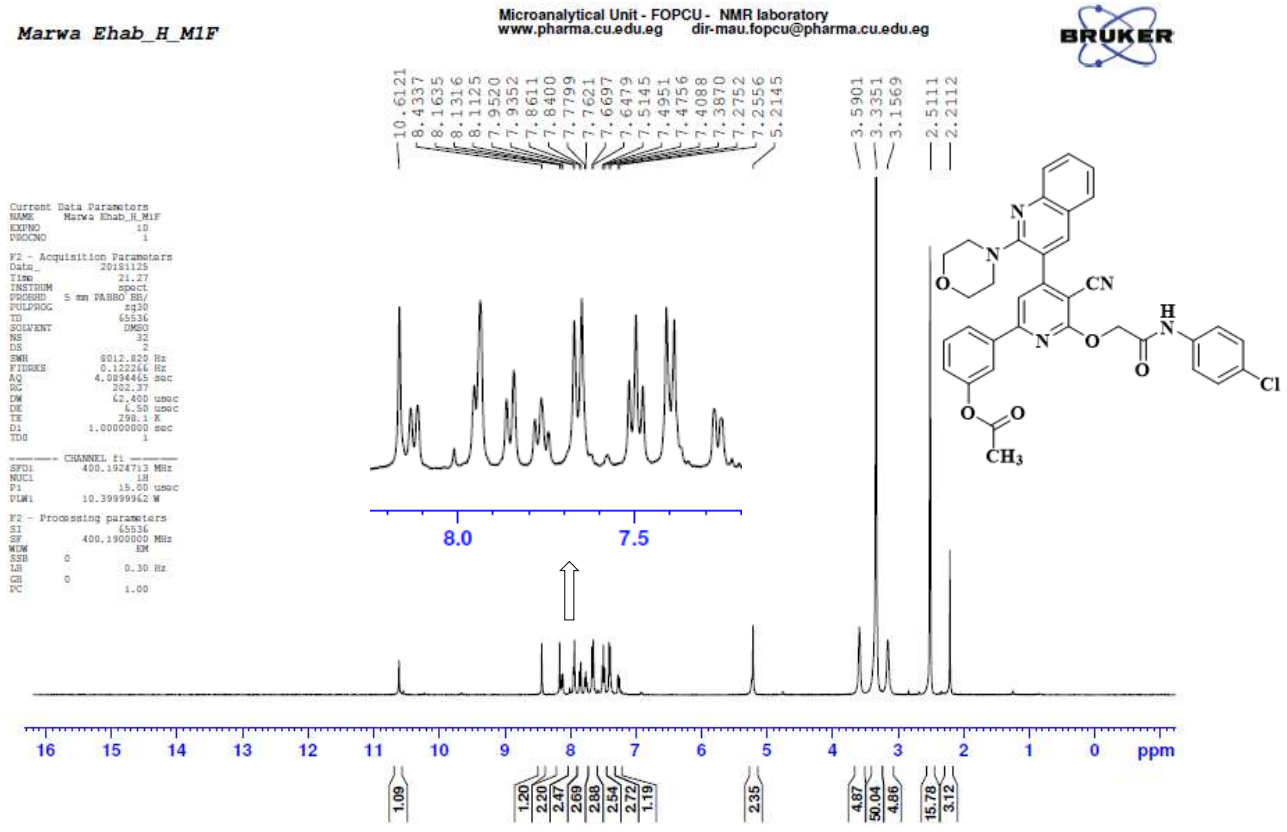
H[1]-NMR spectra of compound 9a



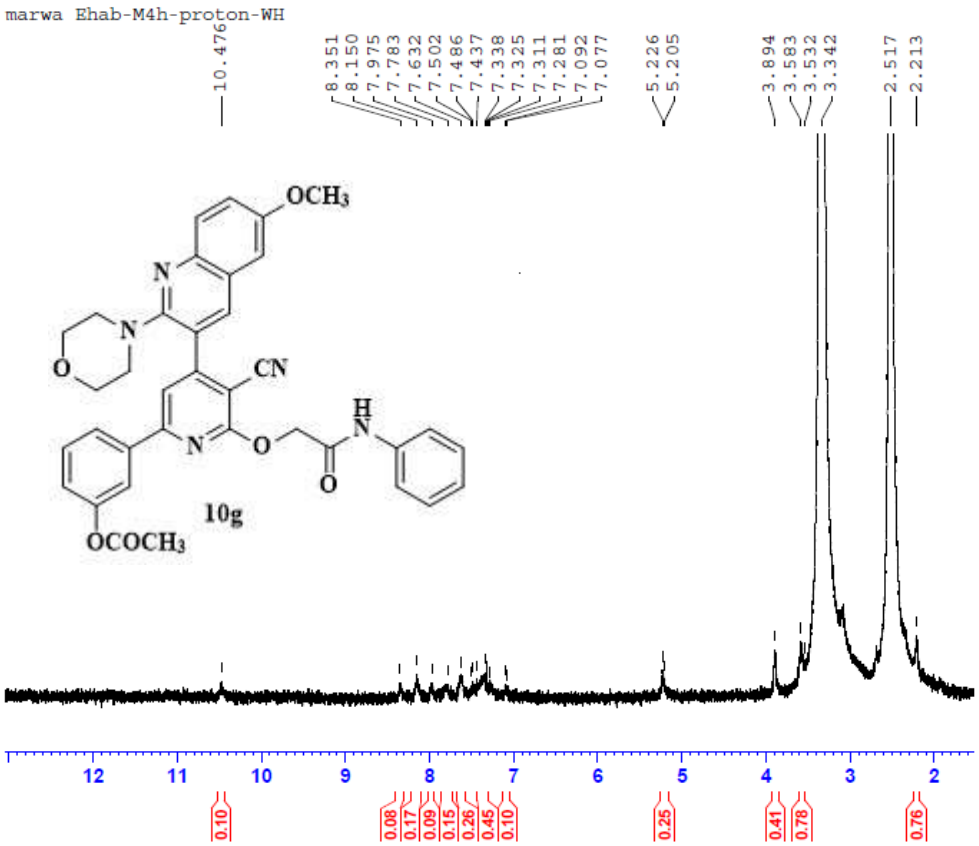
H[1]-NMR spectra of compound 9b



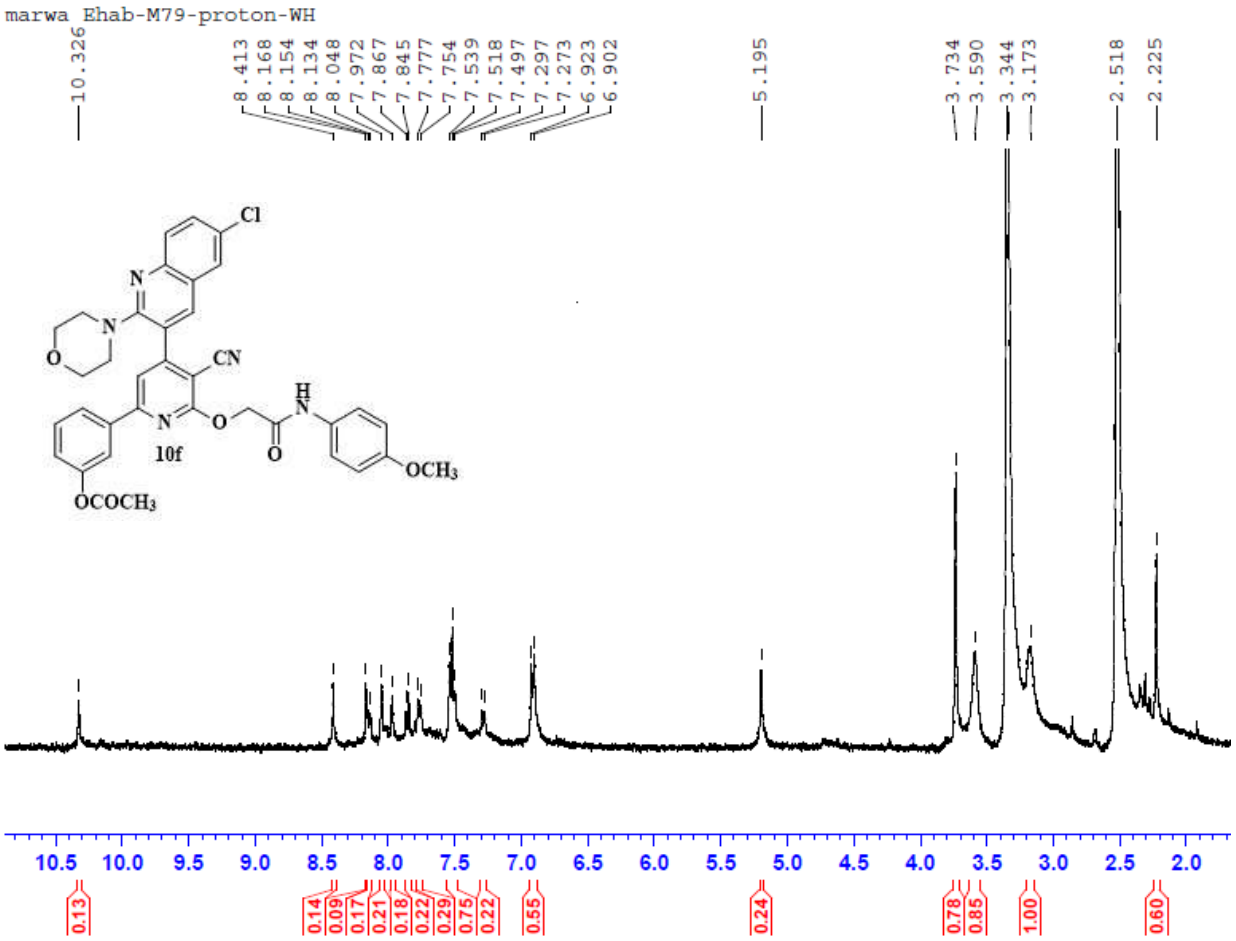
H[1]-NMR spectra of compound 9c



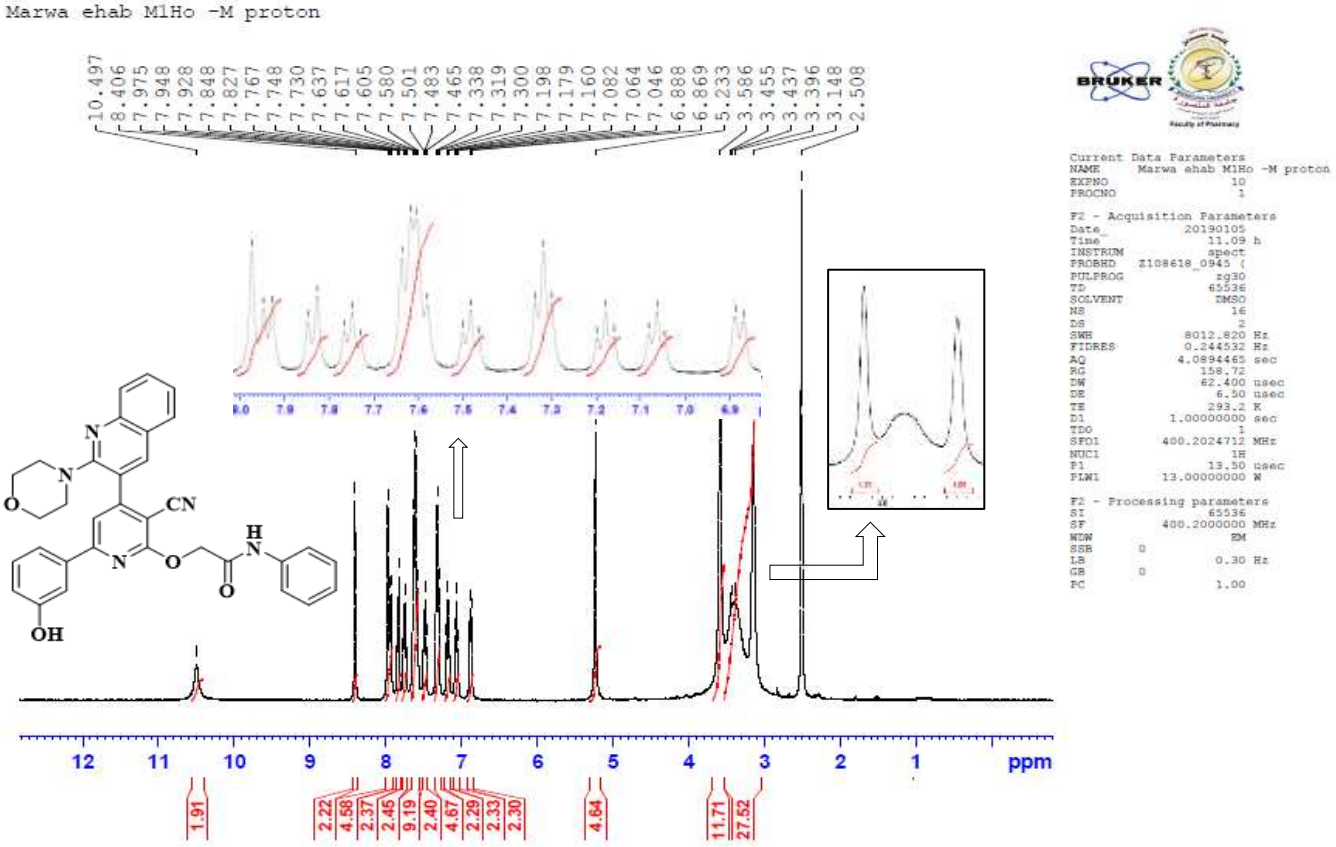
H[1]-NMR spectra of compound 10b



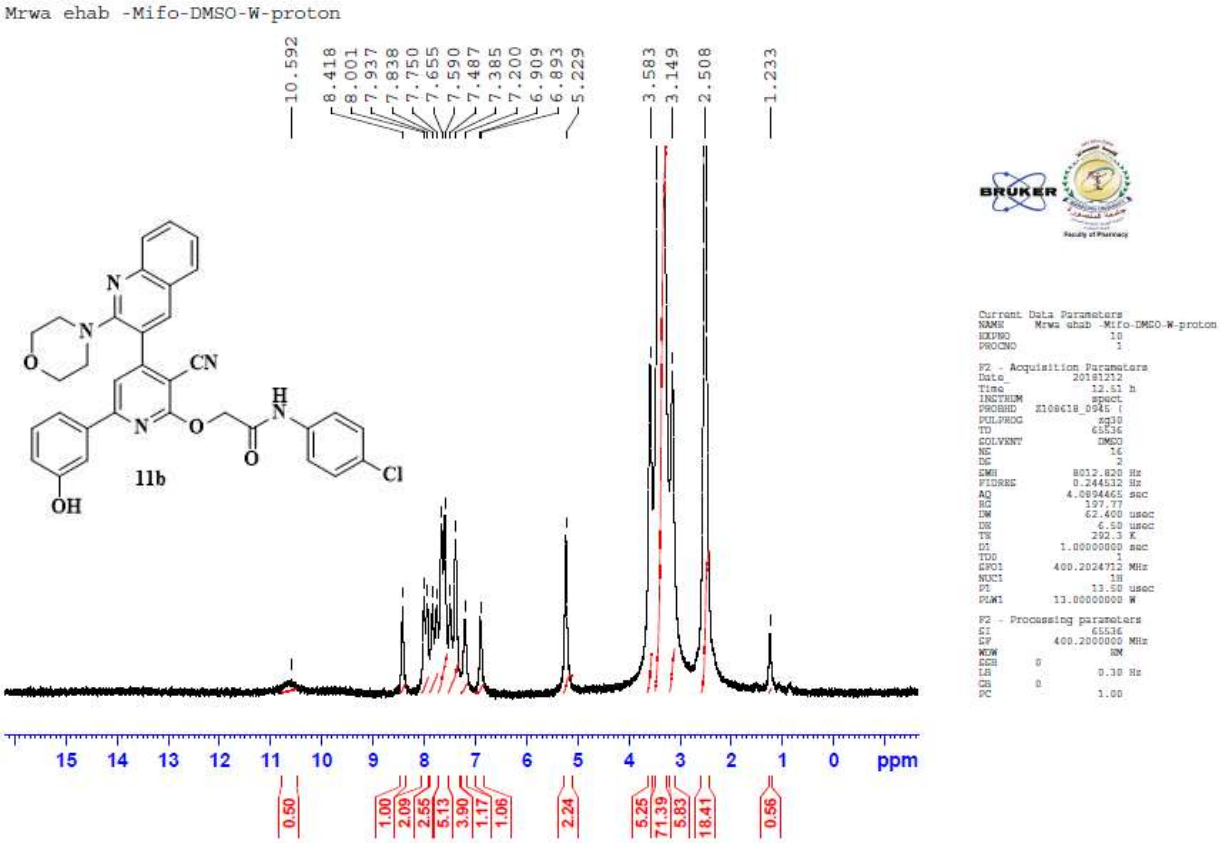
H[1]-NMR spectra of compound 10g



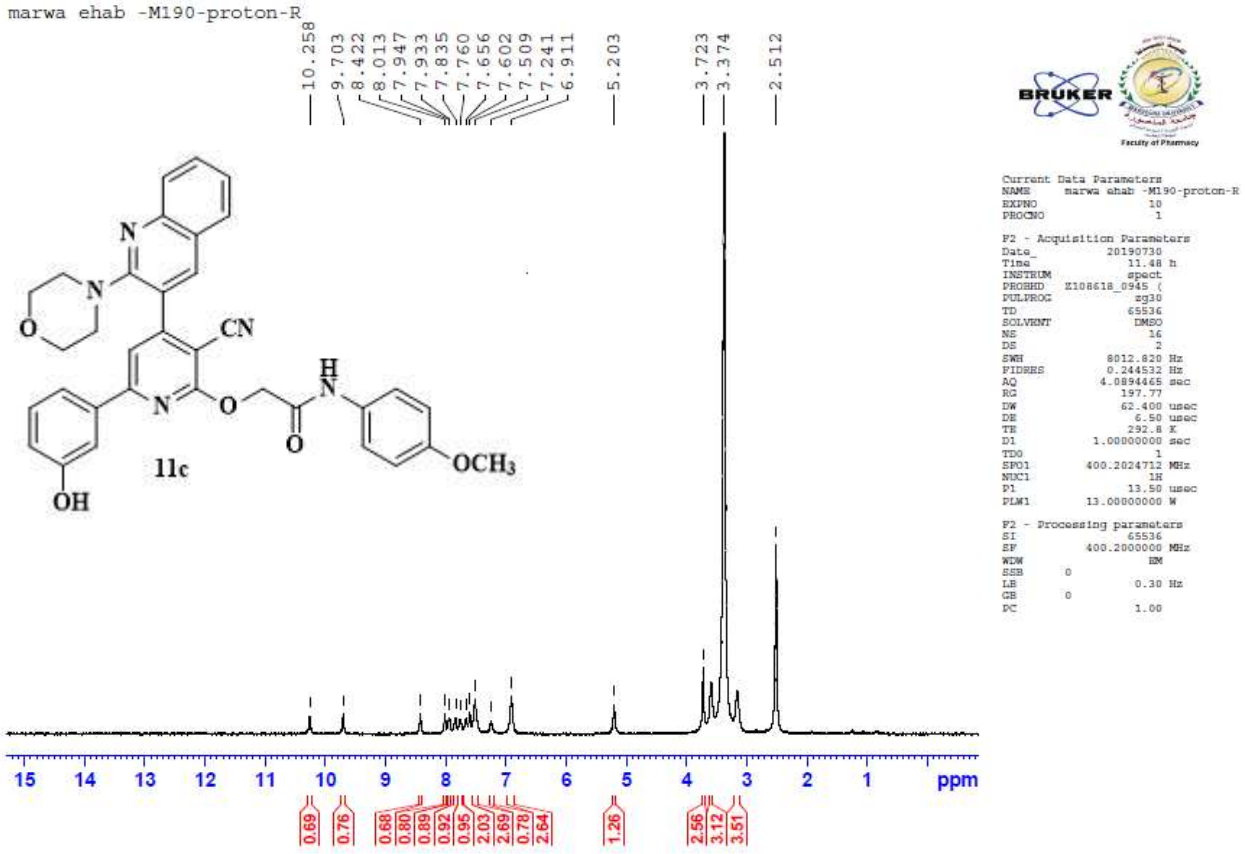
H^1-NMR spectra of compound 10f



H^1-NMR spectra of compound 11a



H[1]-NMR spectra of compound 11b



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PROCNO 1

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FIDRES 0.244532 Hz
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DE 6.50 usec
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NUC1 1H
P1 13.50 usec
PLW1 13.00000000 W

F2 - Processing parameters
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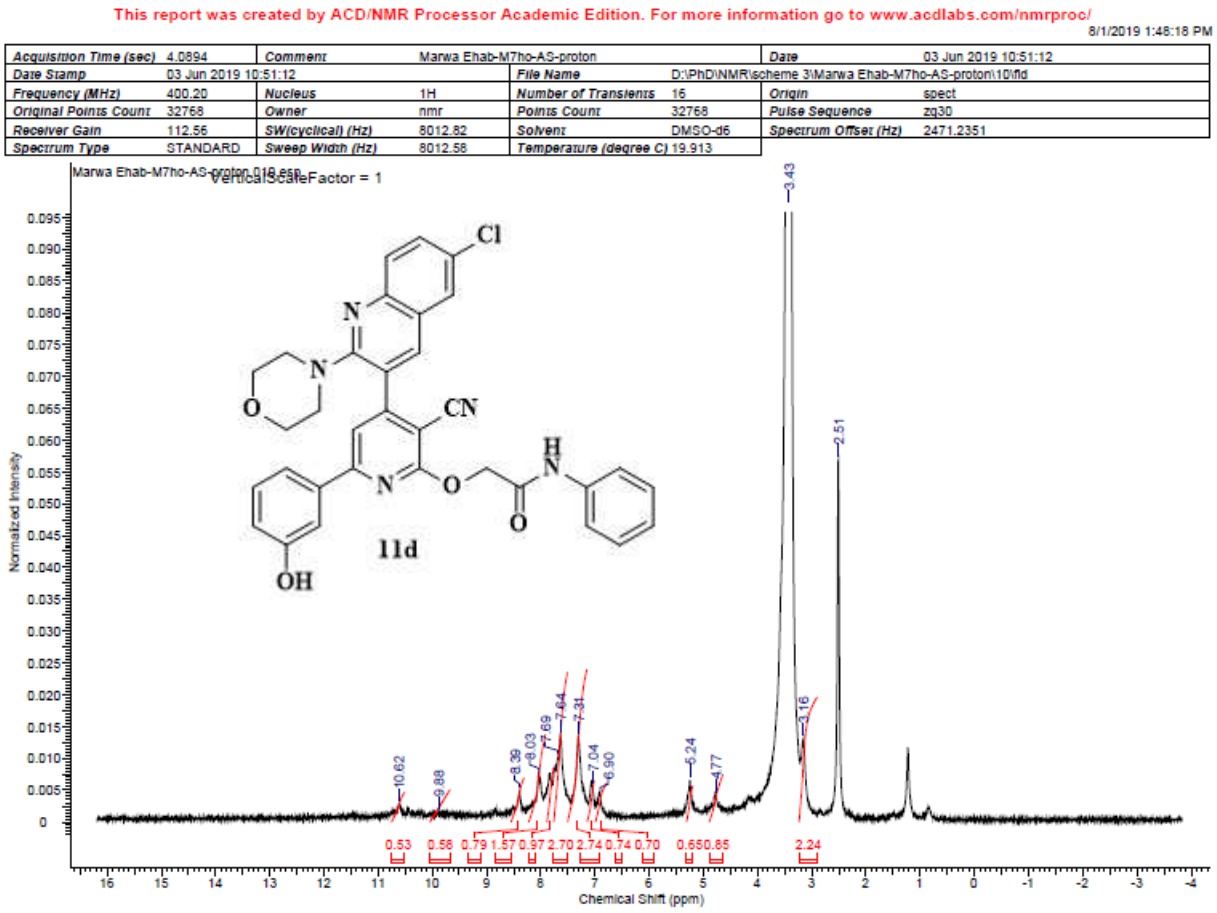
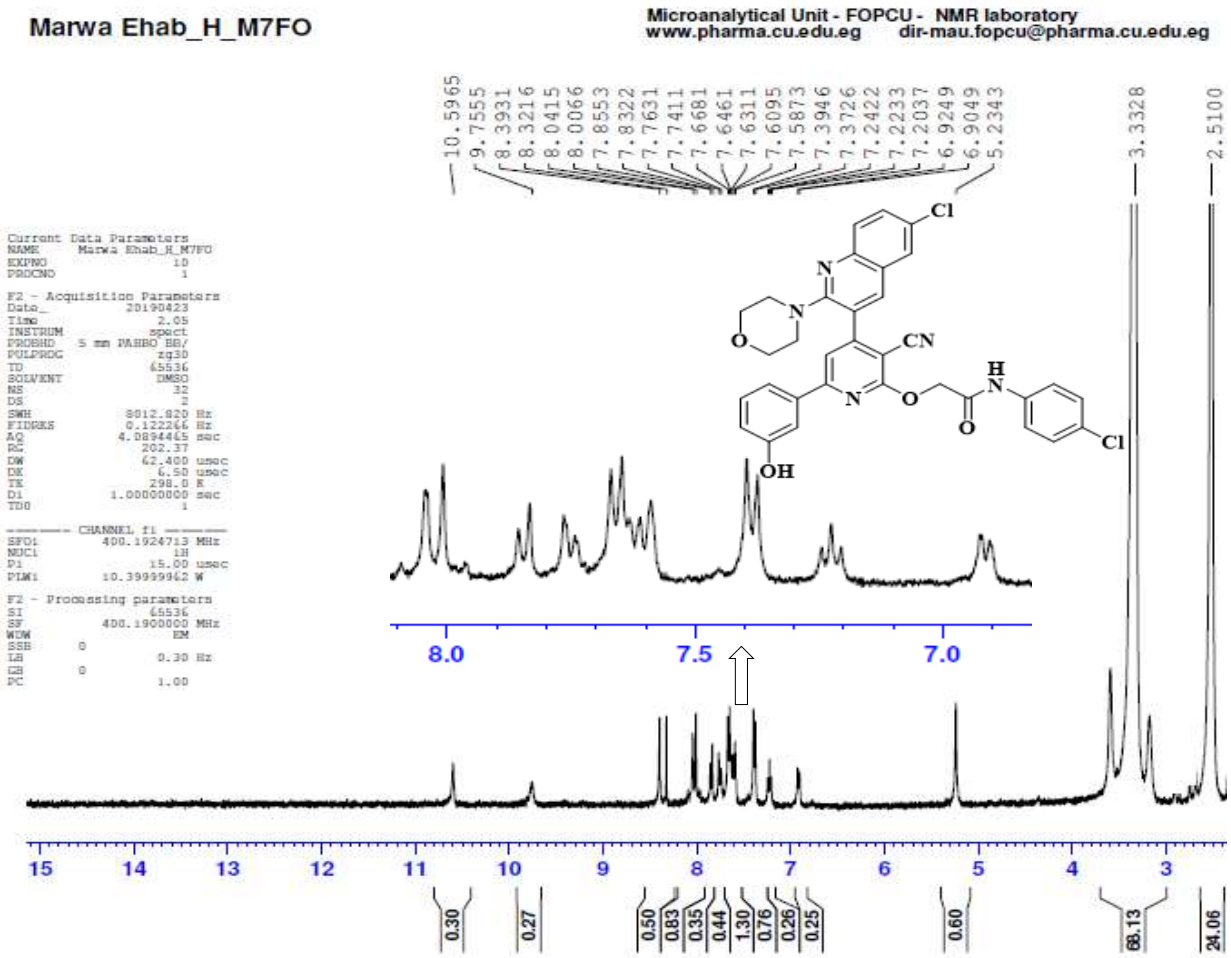
GB 0
PC 1.00

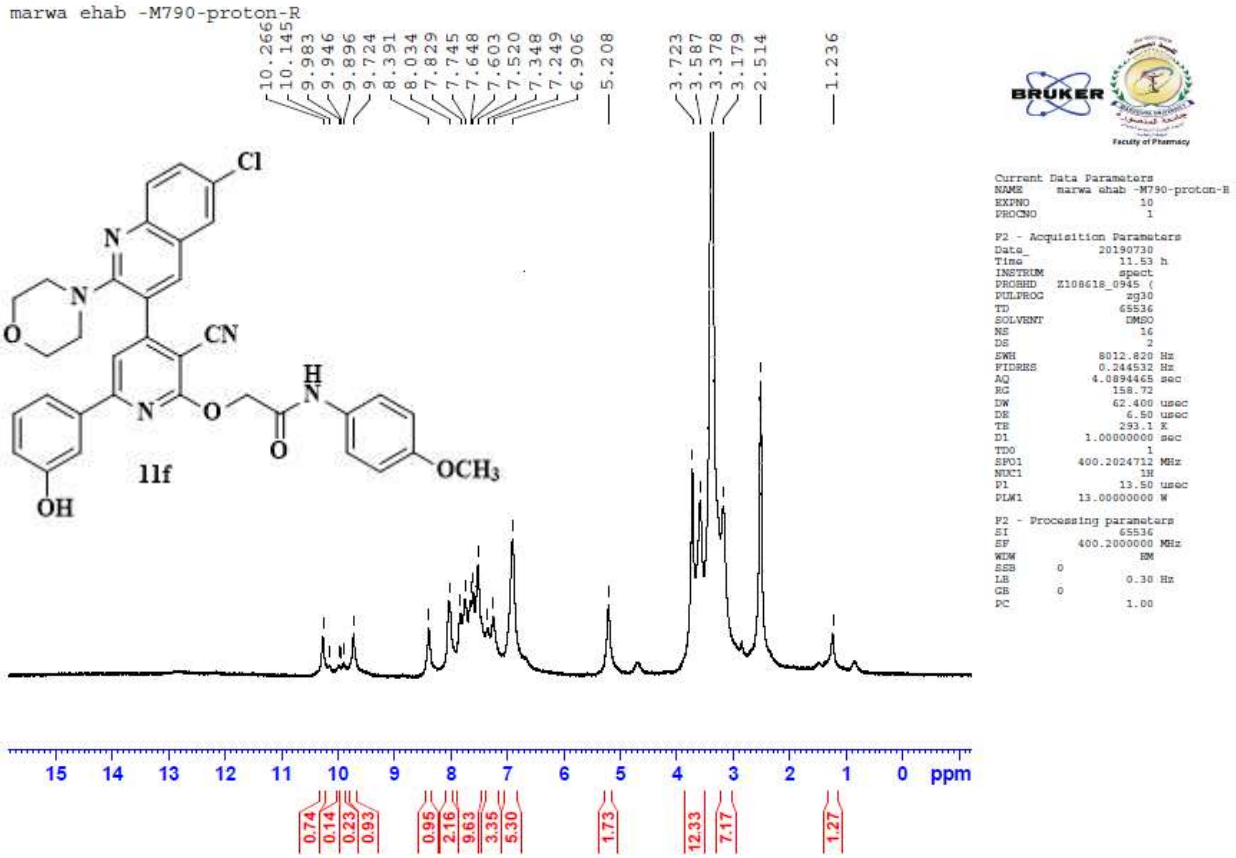
H[1]-NMR spectra of compound 11c



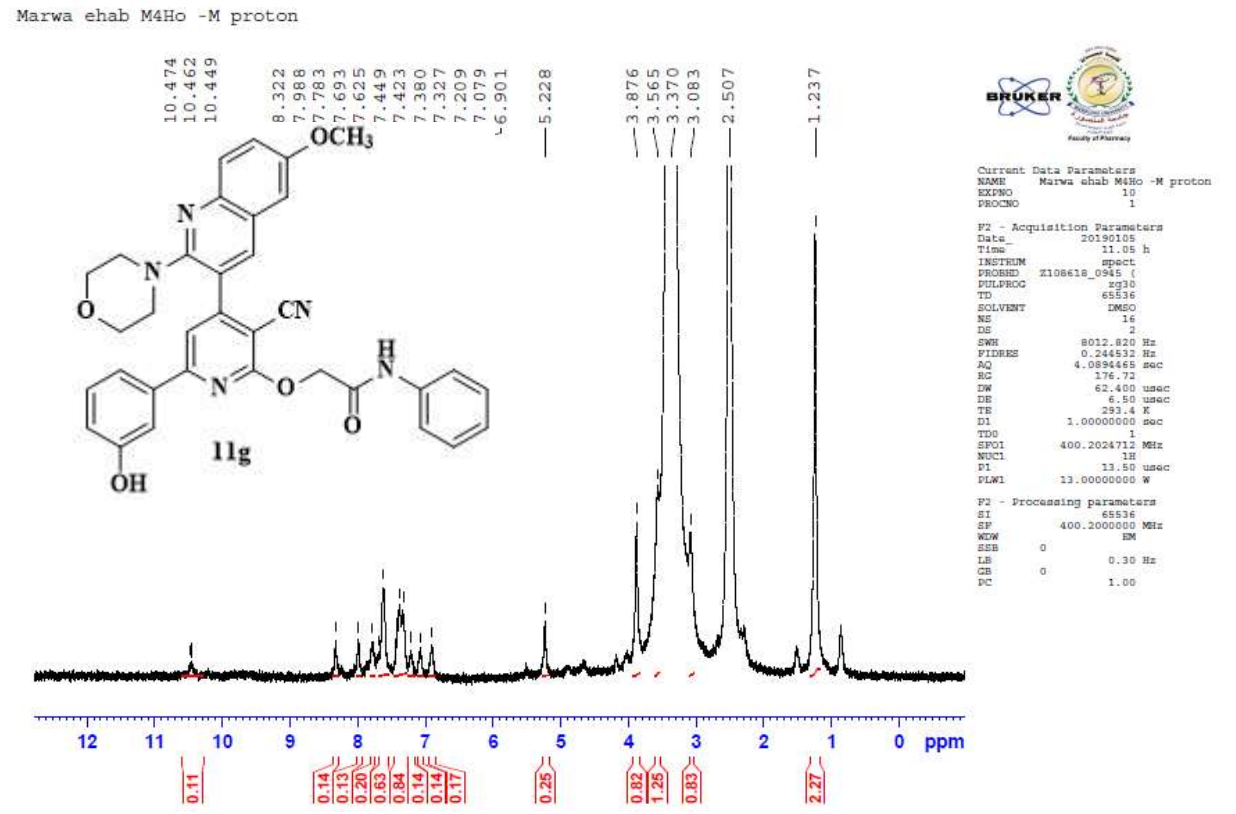
H^1-NMR spectra of compound 11d



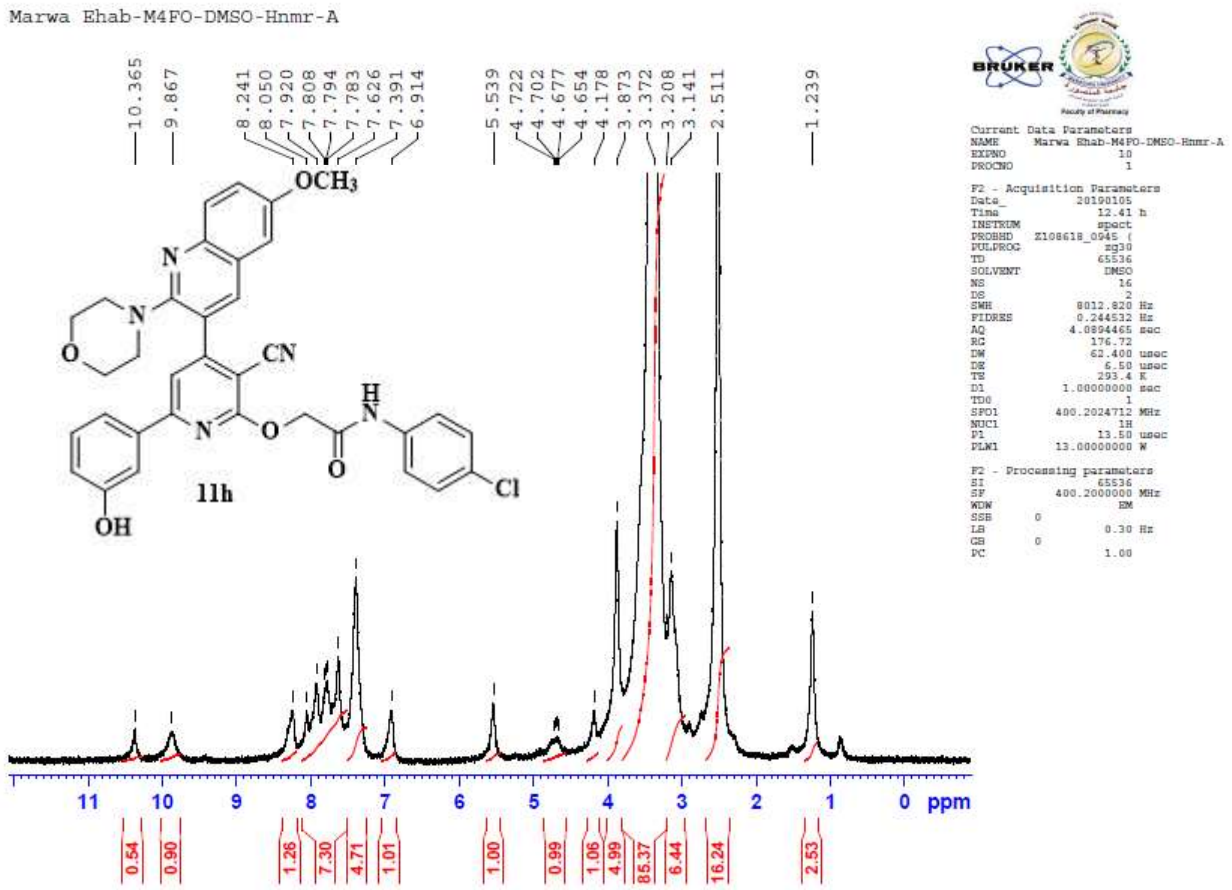
H^1-NMR spectra of compound 11e



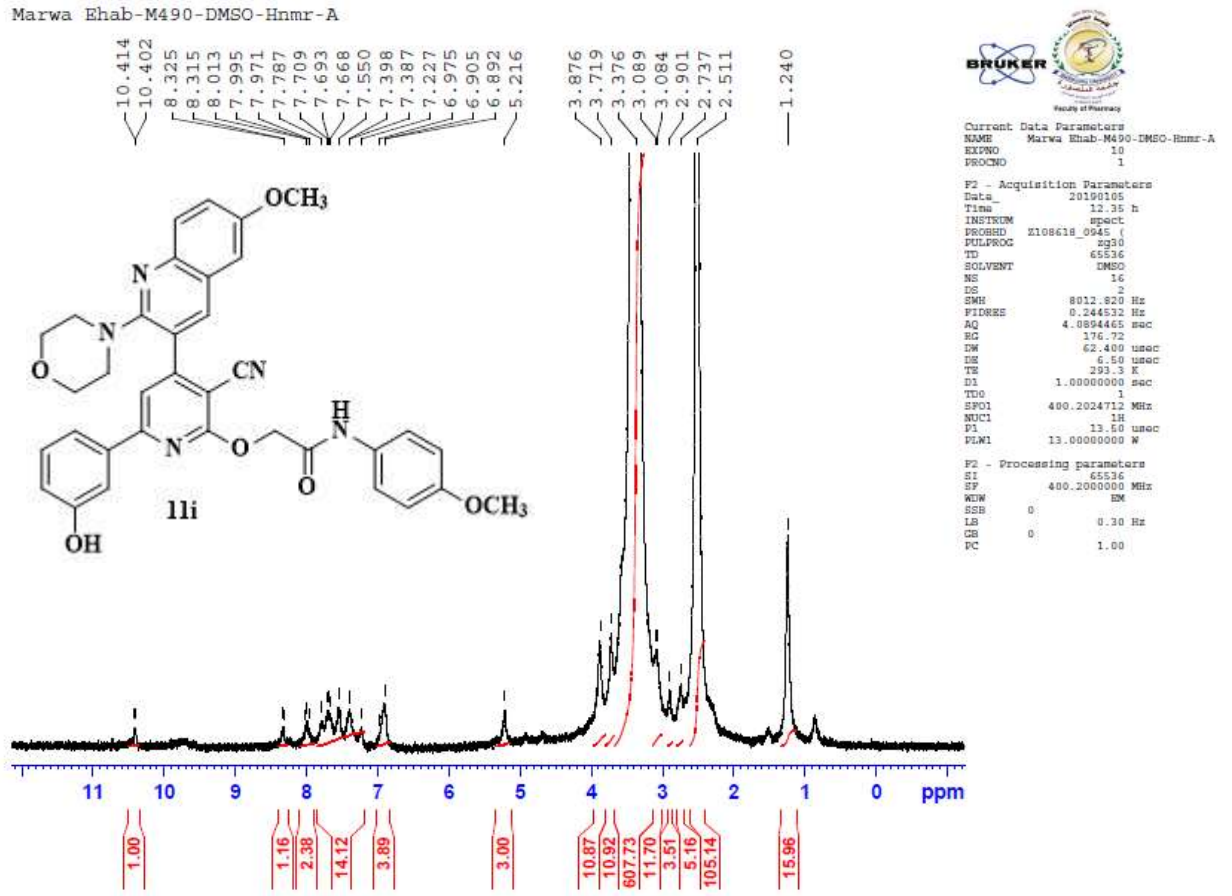
H[1]-NMR spectra of compound 11f



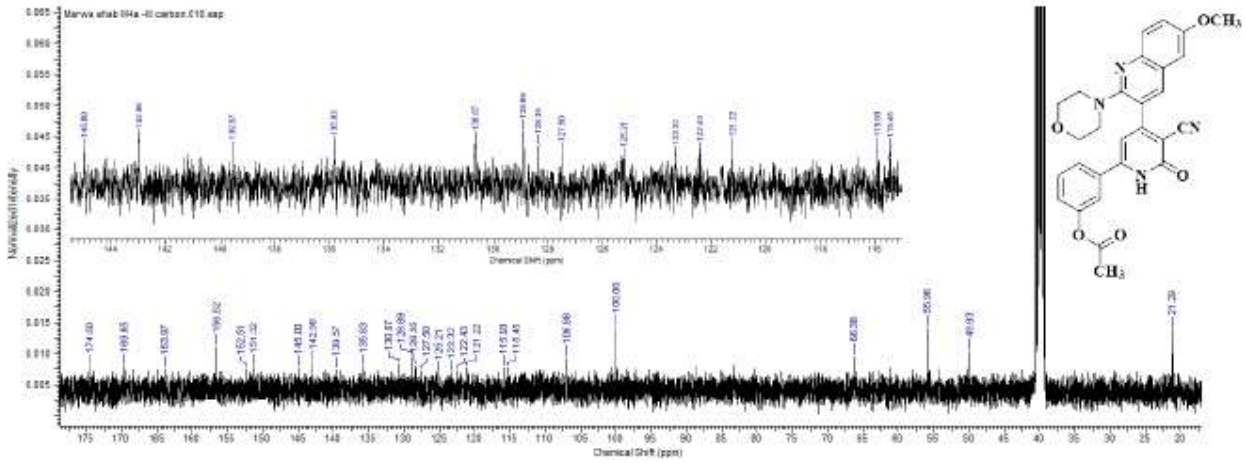
H[1]-NMR spectra of compound 11g



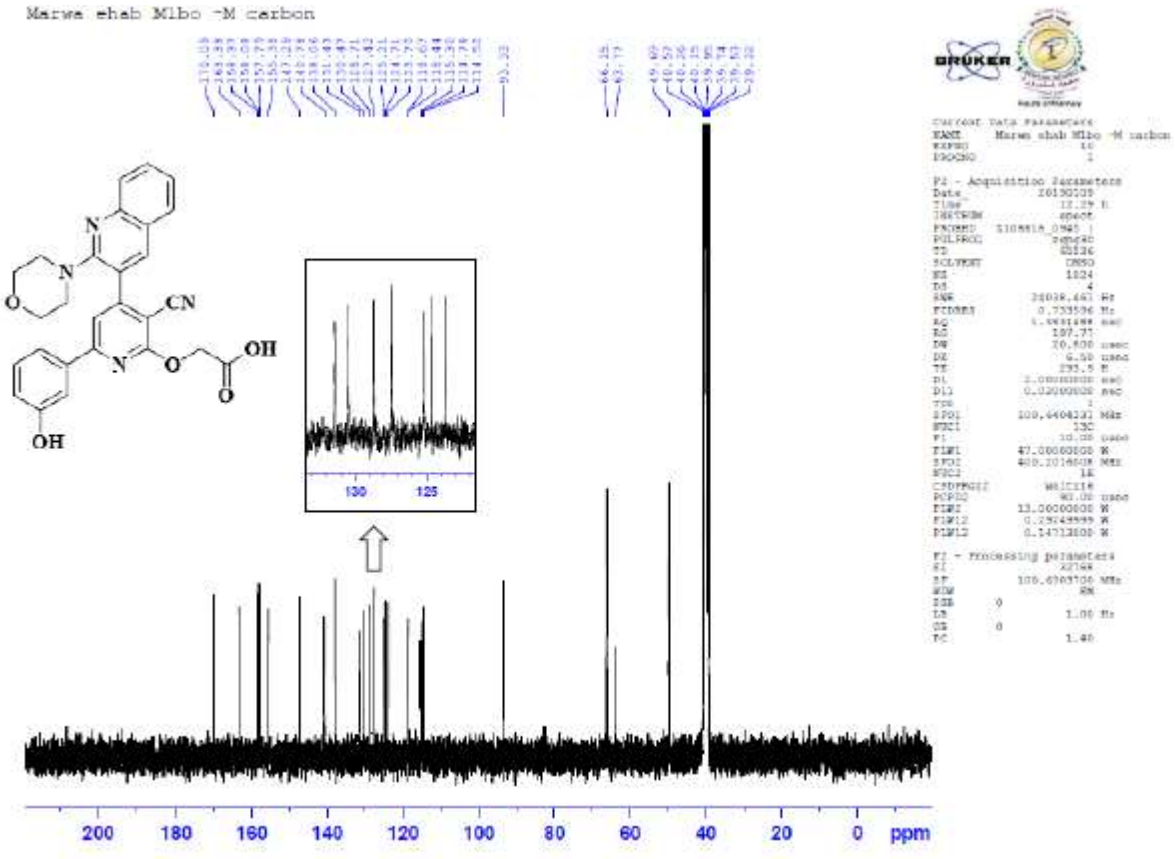
H[1]-NMR spectra of compound 11h



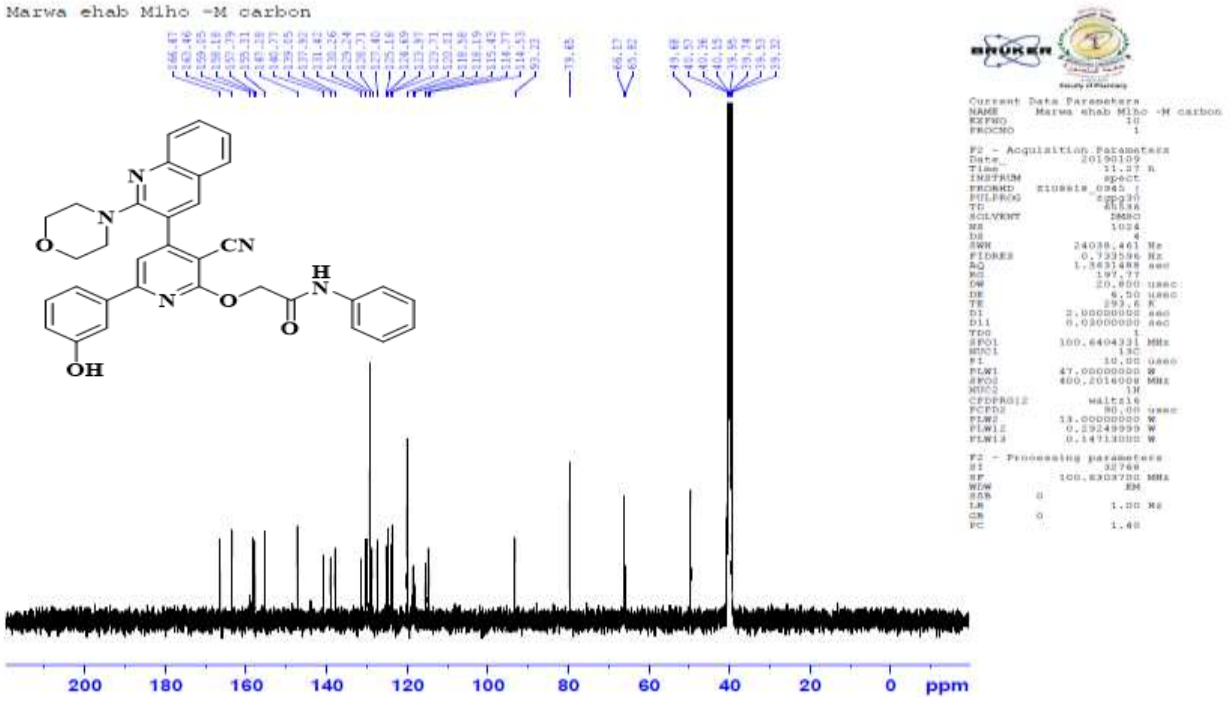
H[1]-NMR spectra of compound 11i



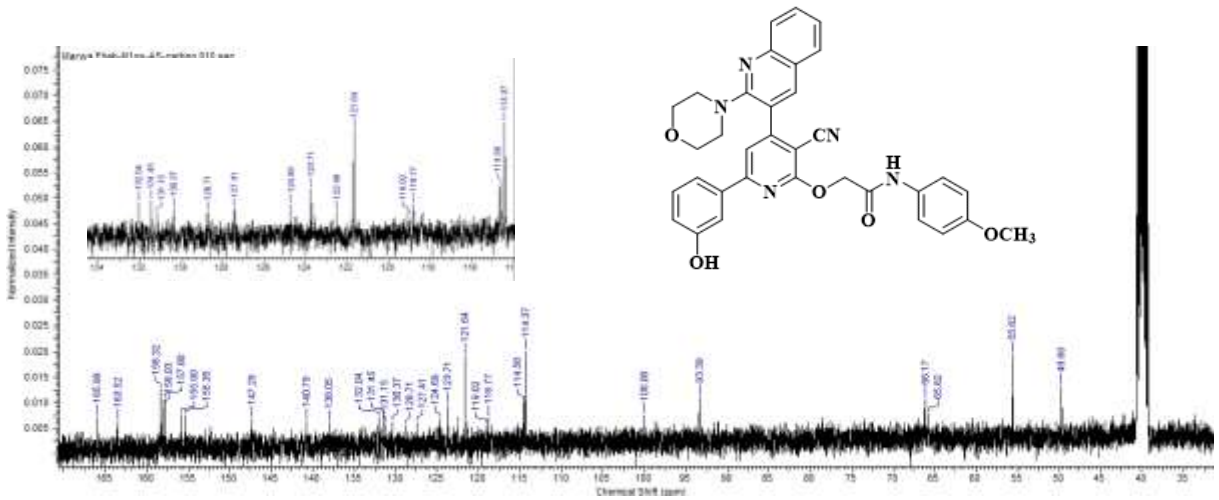
C^{13}-NMR spectra of compound 7c



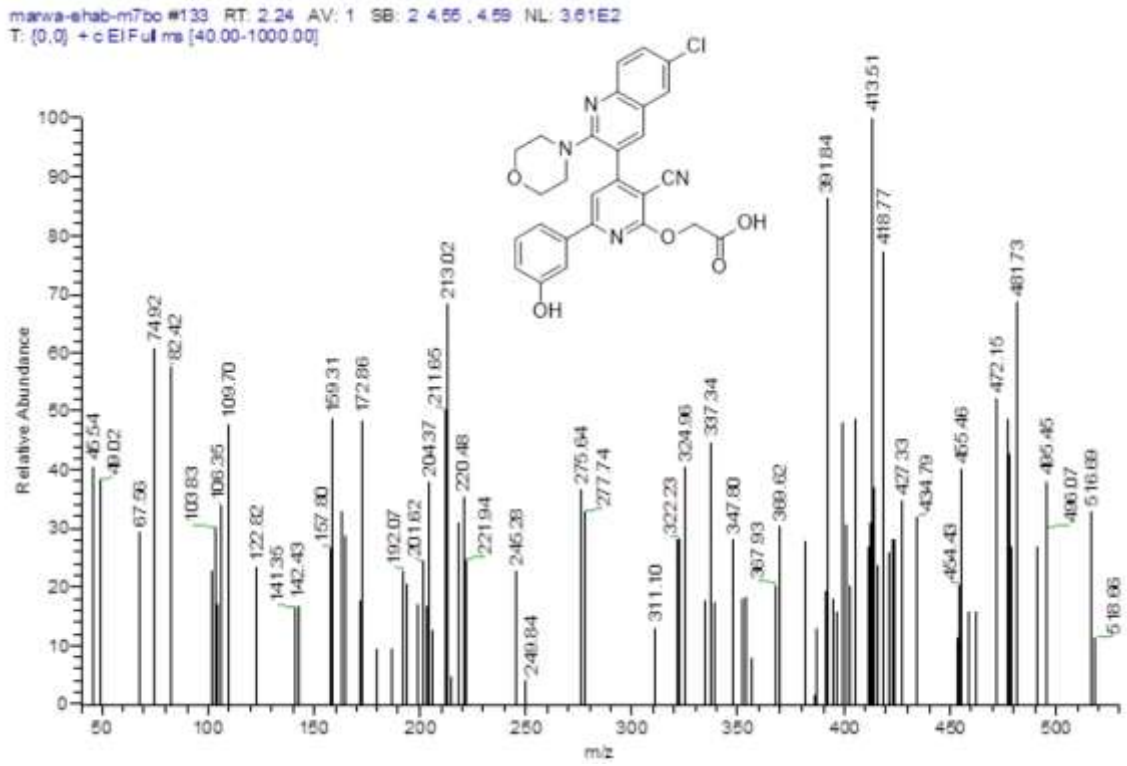
C^{13}-NMR spectra of compound 9a



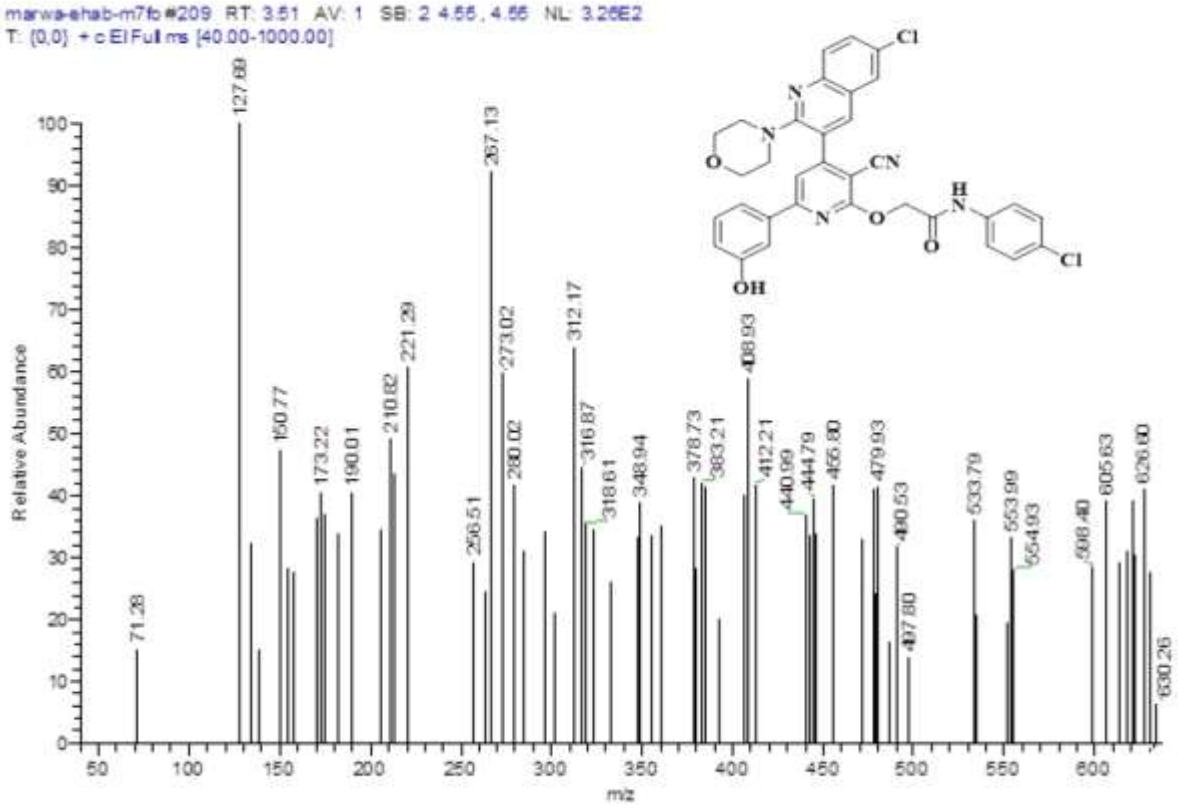
C^{13}-NMR spectra of compound 11a



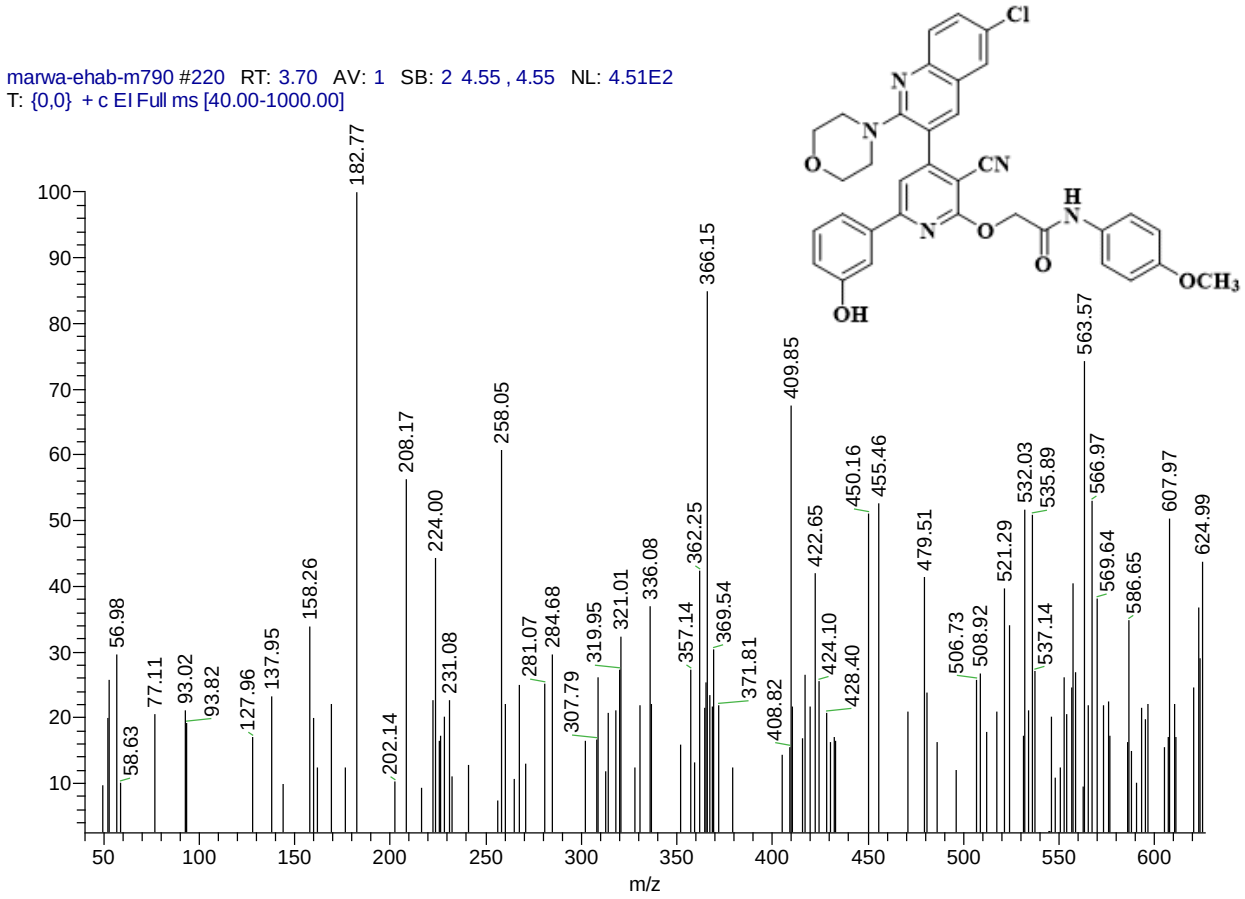
C^{13}-NMR spectra of compound 11c



Mass spectrum for compound 9a



Mass spectrum for compound 11e



Mass spectrum for compound 11f

Al-Azhar University

The Regional Center for Mycology and Biotechnology

Requester Data:

Name: Dr. Marwa Ehab Mohamed
Authority: Faculty of Pharmacy,
Alexandria University

Sample Data:

Twenty samples had been submitted for elemental analysis.

Analysis Report:

Sample Code	C%	H%	N%
M_1 eN	68.71	5.24	19.38
M_1 J	71.09	5.21	12.96
M_2	68.42	4.75	12.94
M_4 e	70.38	5.84	11.50
M_4 k	69.87	5.68	11.79
M_5	66.59	4.83	11.75
M_7 a **7b**	64.58	4.59	11.34
M_7 b **9b**	62.96	4.13	11.06
M_7 e	67.40	5.28	11.37
M_7 e N	63.26	4.69	17.58
M_7 f **11e**	63.44	4.21	11.39
M_7 h **11d**	66.81	4.62	11.94
M_7 J	66.26	4.69	11.72
M_7 k	66.49	4.88	11.69
M_8	63.45	4.11	11.94
N_2	71.47	5.28	13.02
N_5	69.43	5.34	12.13
O_1	71.49	4.06	15.95
O_4	68.95	4.32	14.67
O_7	64.71	3.19	14.62

INVESTIGATOR **DIRECTOR**

جامعة الأزهر
Al-Azhar University
المركز الإقليمي للفطريات وتطبيقاتها
The Regional Center for Mycology and Biotechnology

Requester Data:

Name: Dr. Marwa Ehab Abdel-Aziz
Authority: Faculty of Pharmacy,
Alexandria University

Sample Data:

Seven samples had been submitted for elemental analysis.

Analysis Report:

Sample Code	C%	H%	N%
M_1 go 11c	69.32	5.13	12.18
M_1 ko	71.50	5.49	12.61
M_4 d4o	68.94	4.97	16.27
M_7 do	67.91	4.38	11.49
M_7 d2o	61.53	3.98	17.08
M_7 d3o	64.89	4.58	15.35
M_7 go 11f	65.47	4.63	11.45

INVESTIGATOR **DIRECTOR**

Authority: Faculty of Pharmacy,
Alexandria University

Sample Data:

Twenty samples had been submitted for elemental analysis.

Analysis Report:

Sample Code	C%	H%	N%
M1 a 7a	69.30	4.94	12.29
M1 b 9a	67.49	4.83	11.88
M1 d	72.86	4.85	12.40
M1 e	71.88	5.78	12.23
M1 f 11b	67.13	4.60	12.06
M1 h 11a	70.89	4.96	12.31
M1 d_2	65.41	4.29	18.06
M1 d_3	68.98	4.57	16.24
M1 d_4	70.43	4.89	17.09
M4 a 7c	67.54	5.01	11.49
M4 b 9c	65.90	4.89	11.24
M4 d	70.51	5.13	11.57
M4 f 11h	65.92	4.67	11.52
M4 g 11i	68.34	5.24	11.49
M4 h 11g	69.26	4.86	11.23
M4 d_2	64.31	4.57	16.79
M4 d_3	67.52	5.04	15.45
M4 eN	66.54	5.37	17.83
O_3	68.43	4.65	12.99
O_6	66.59	4.83	12.17

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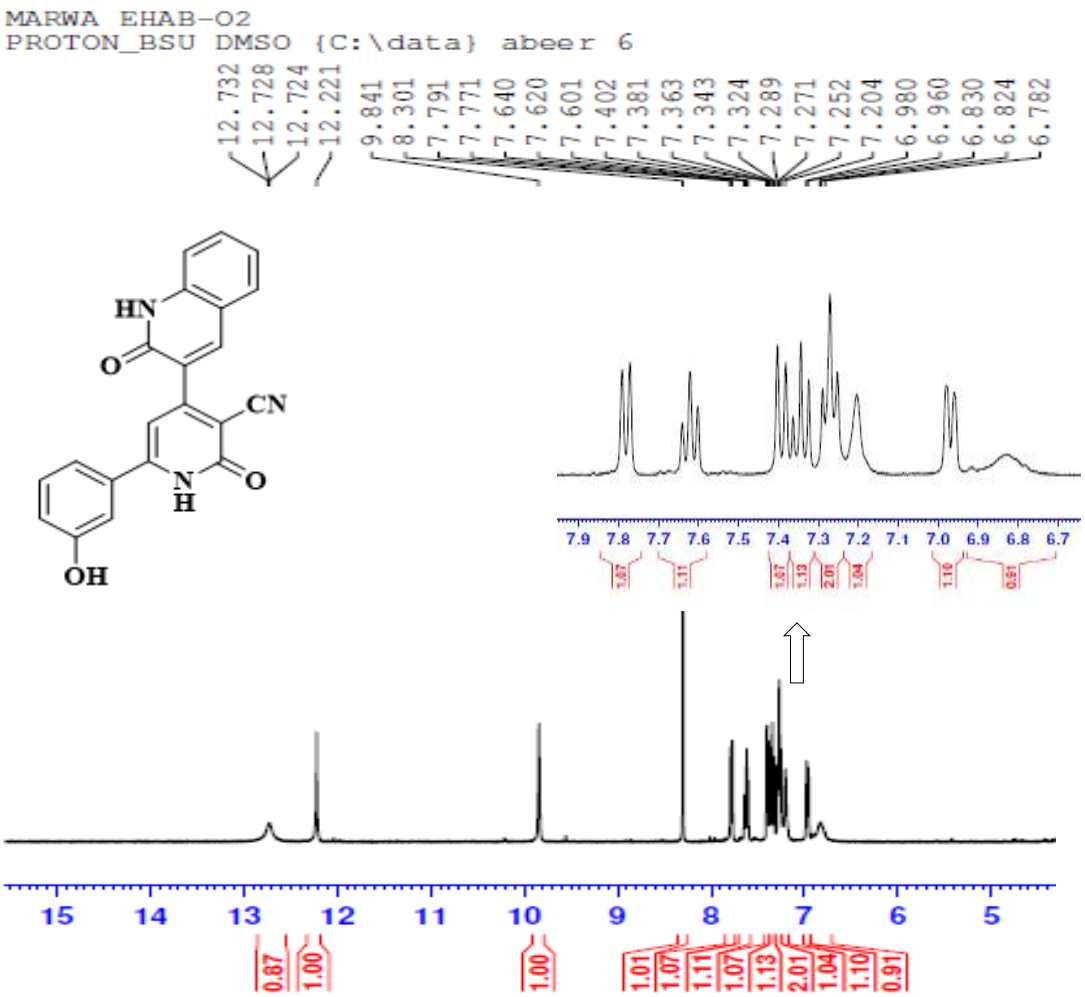
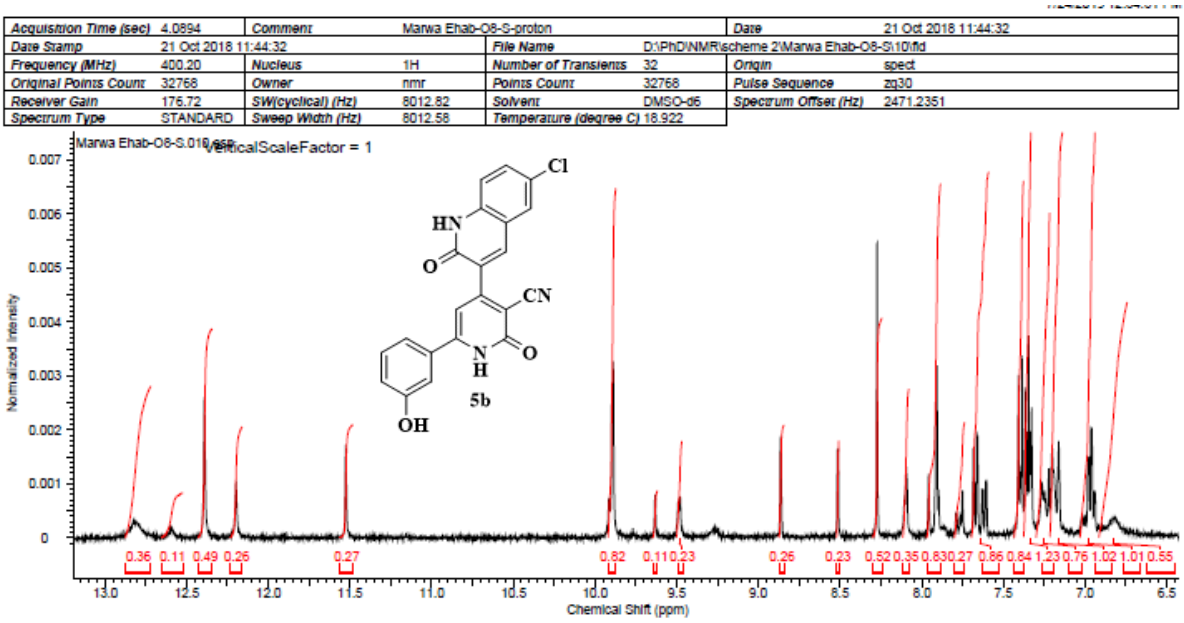
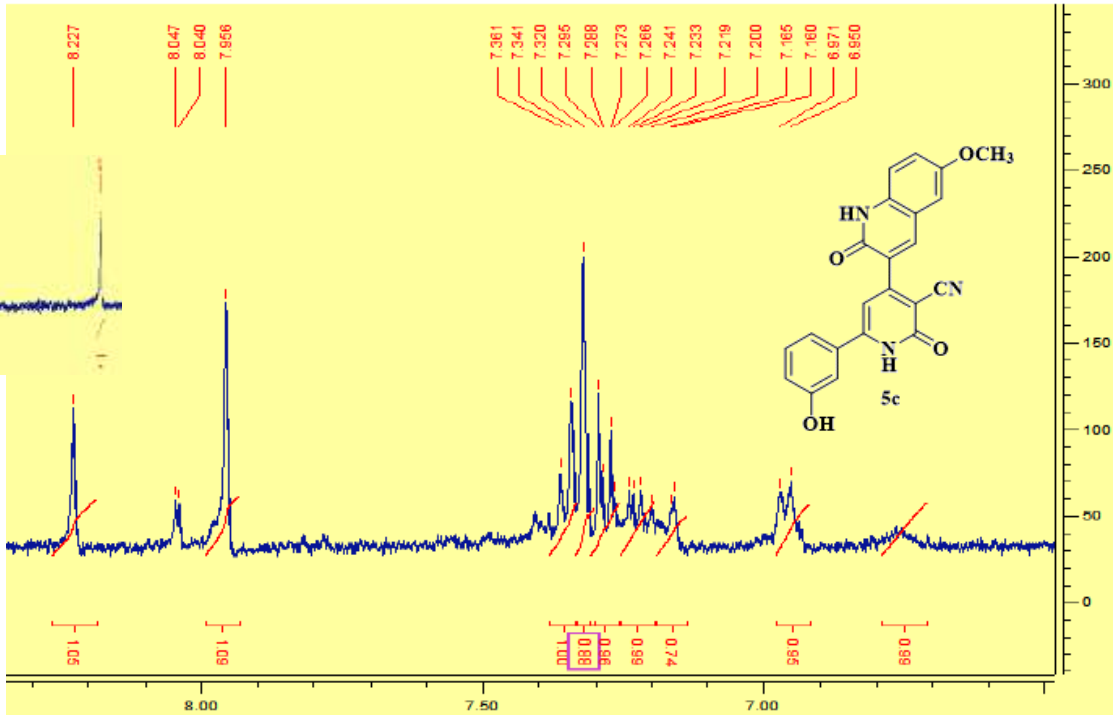
Scheme 3:



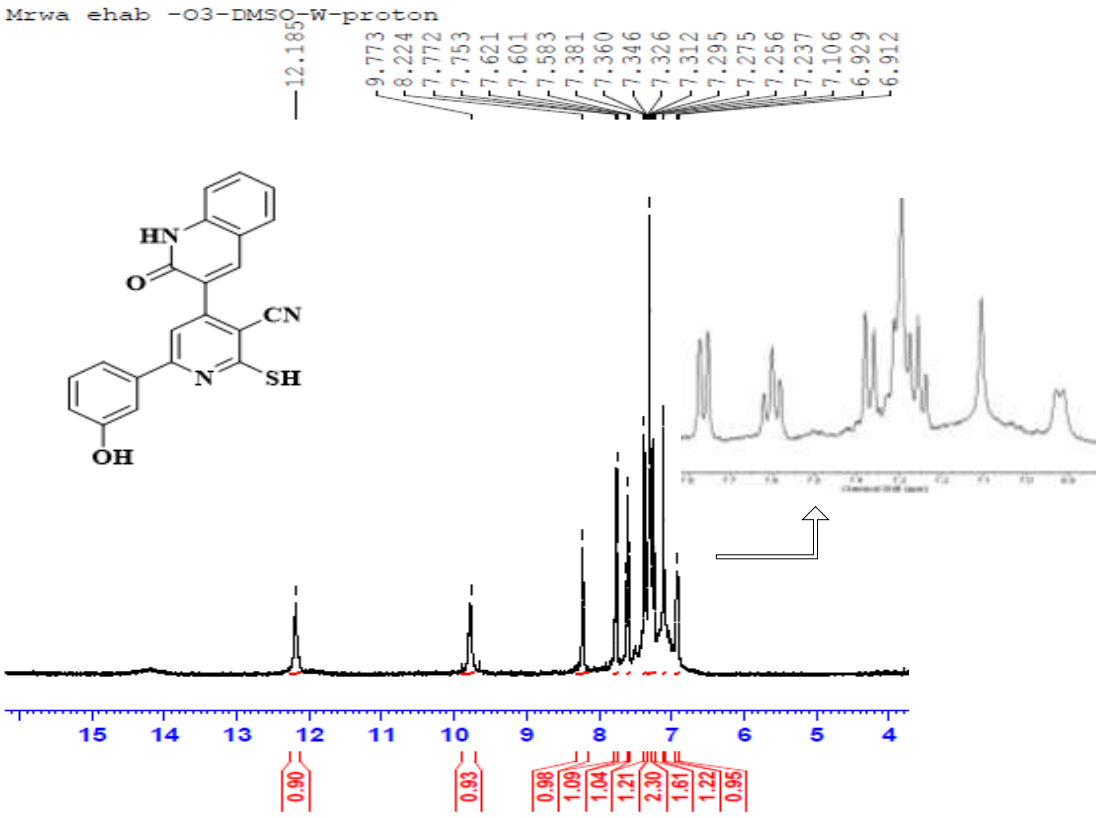
H^1-NMR spectra of compound 5a



H^1-NMR spectra of compound 5b



H[1]-NMR spectra of compound 5c



H[1]-NMR spectra of compound 6a

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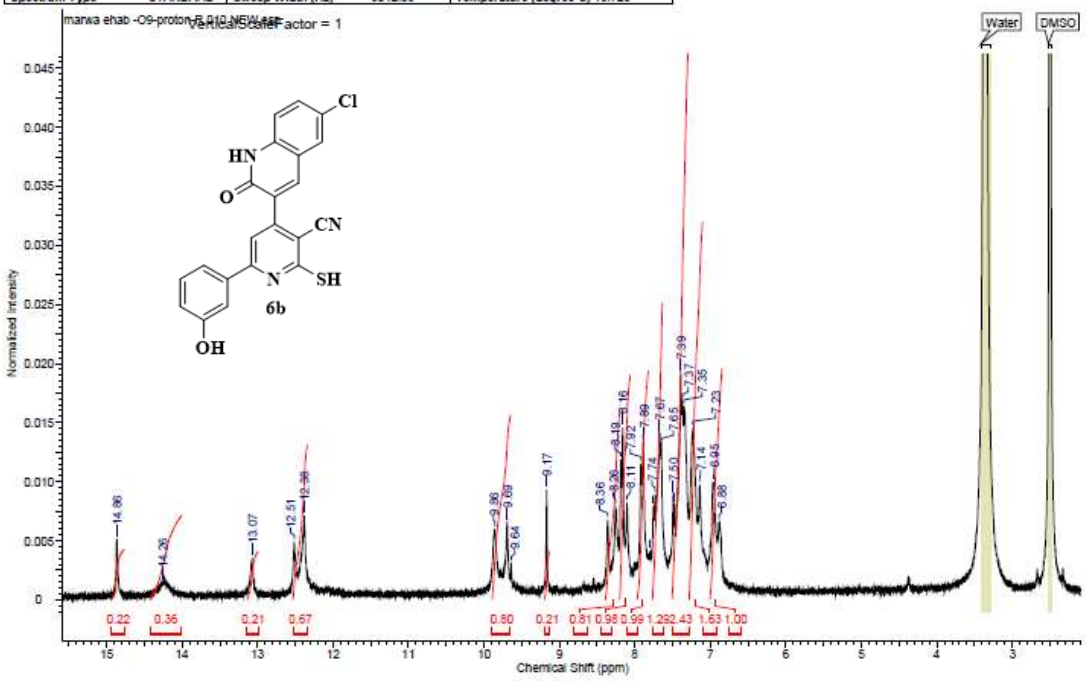
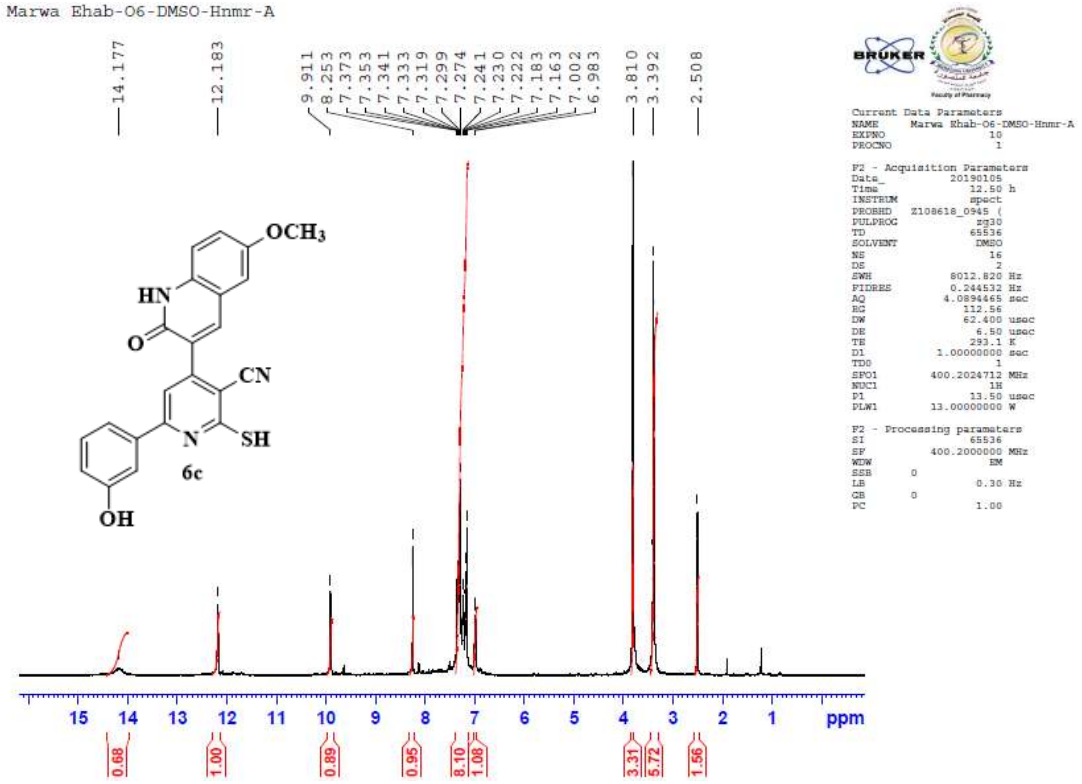
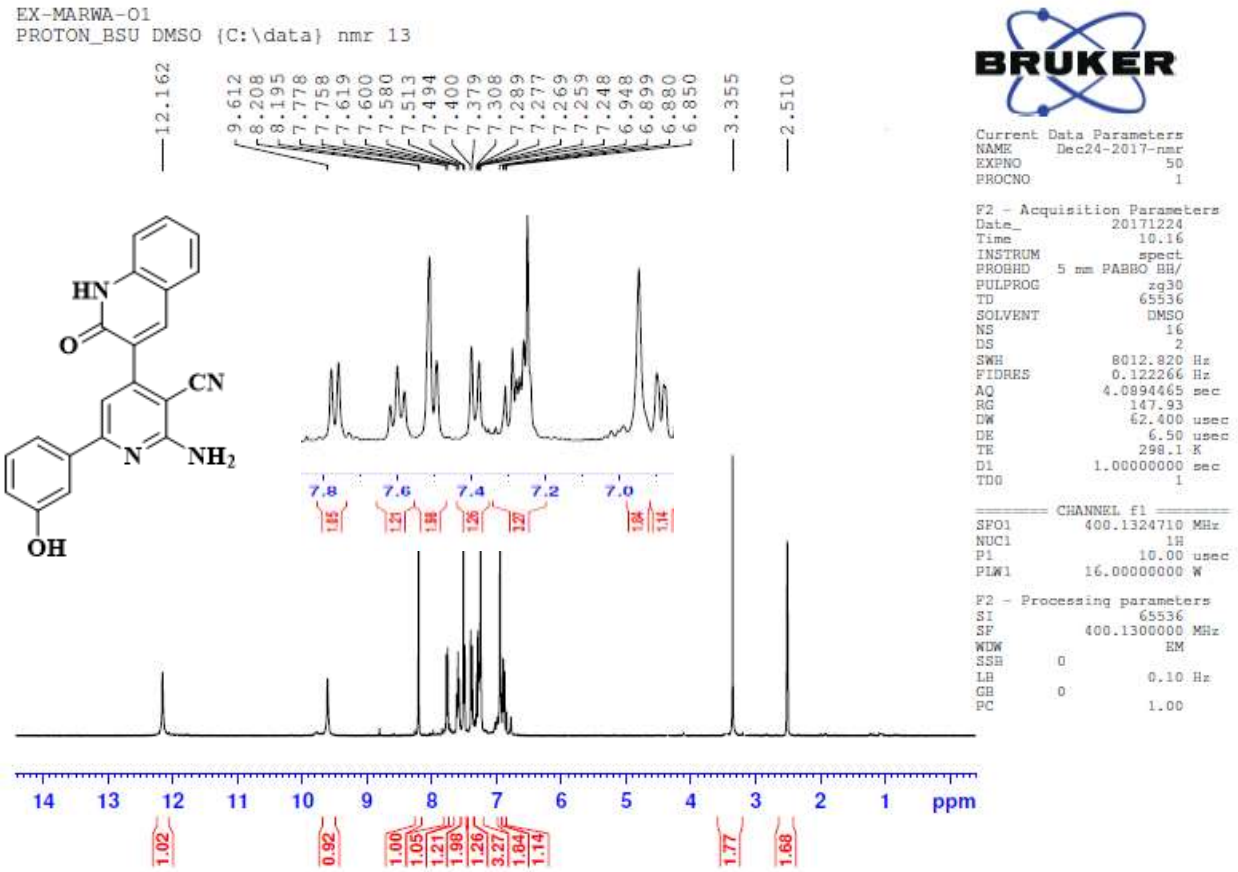


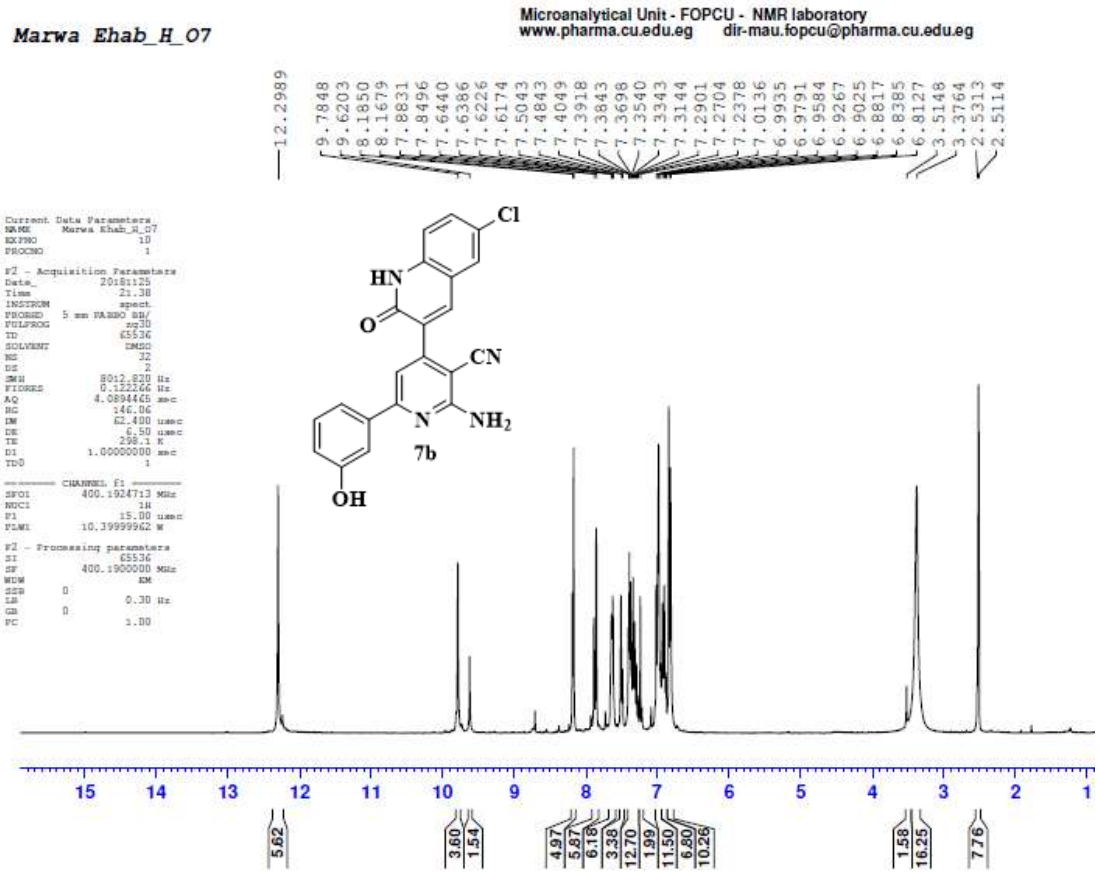
H[1]-NMR spectra of compound 6b



H[1]-NMR spectra of compound 6c



H[1]-NMR spectra of compound 7a



H[1]-NMR spectra of compound 7b

7/24/2019 1:25:43 PM

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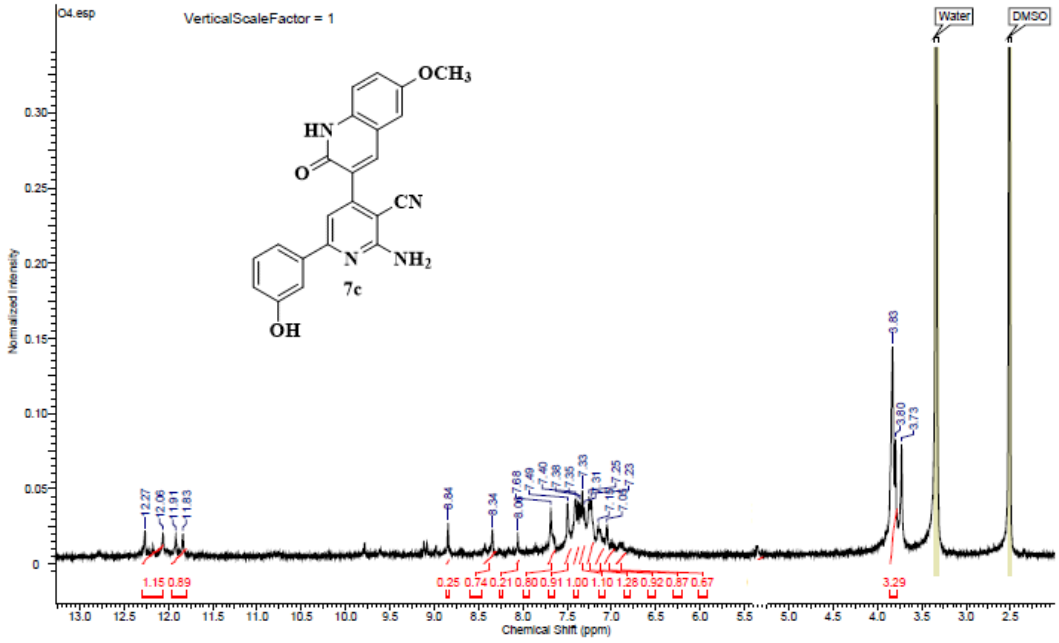
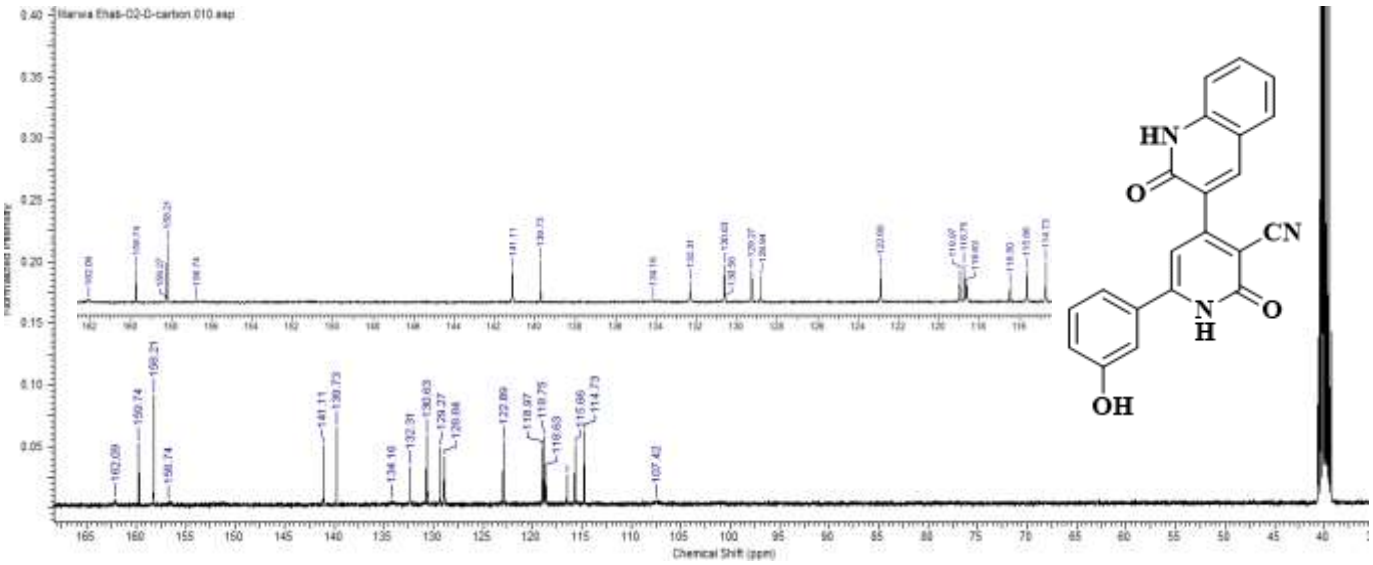
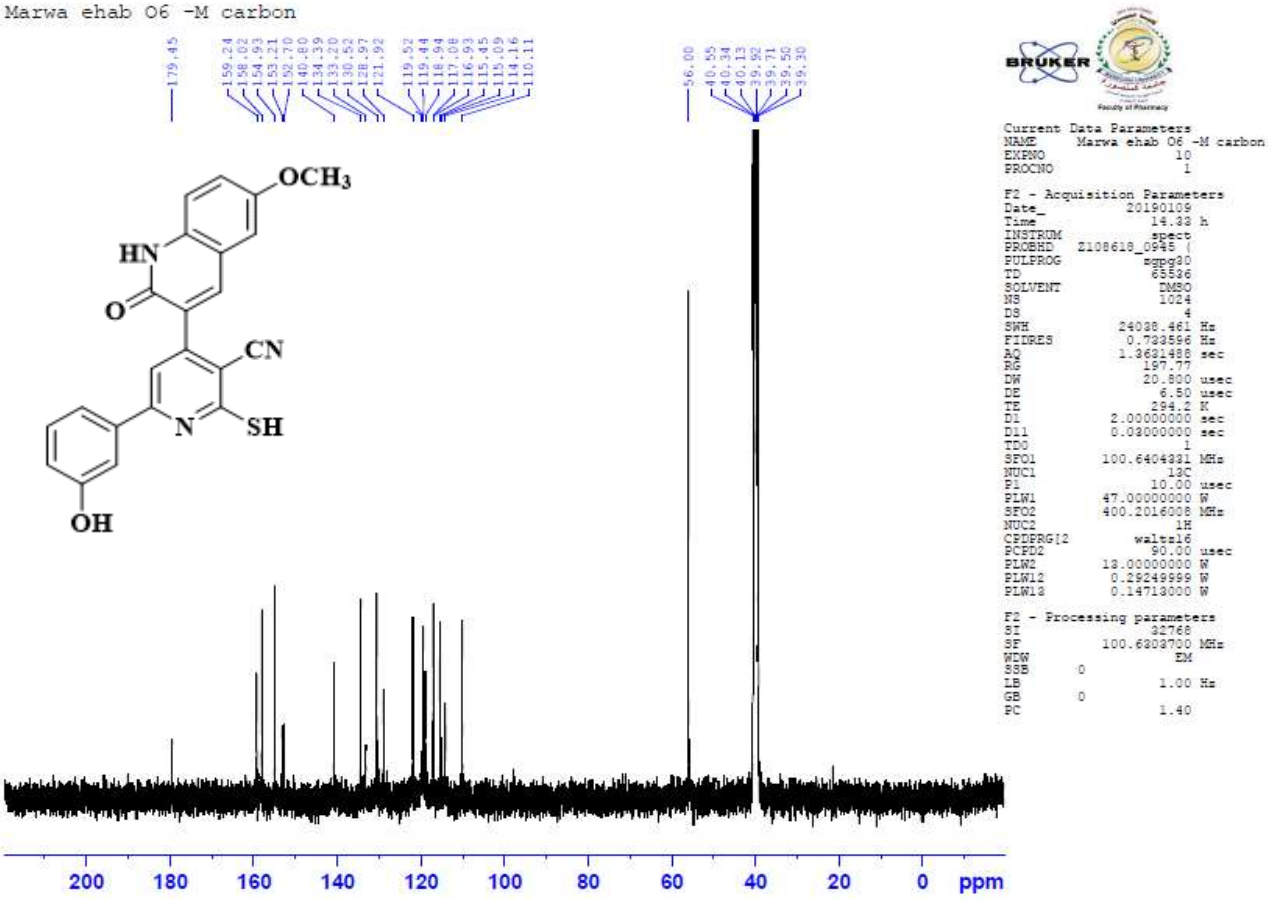


H[1]-NMR spectra of compound 7c



C[13]-NMR spectra of compound 5a

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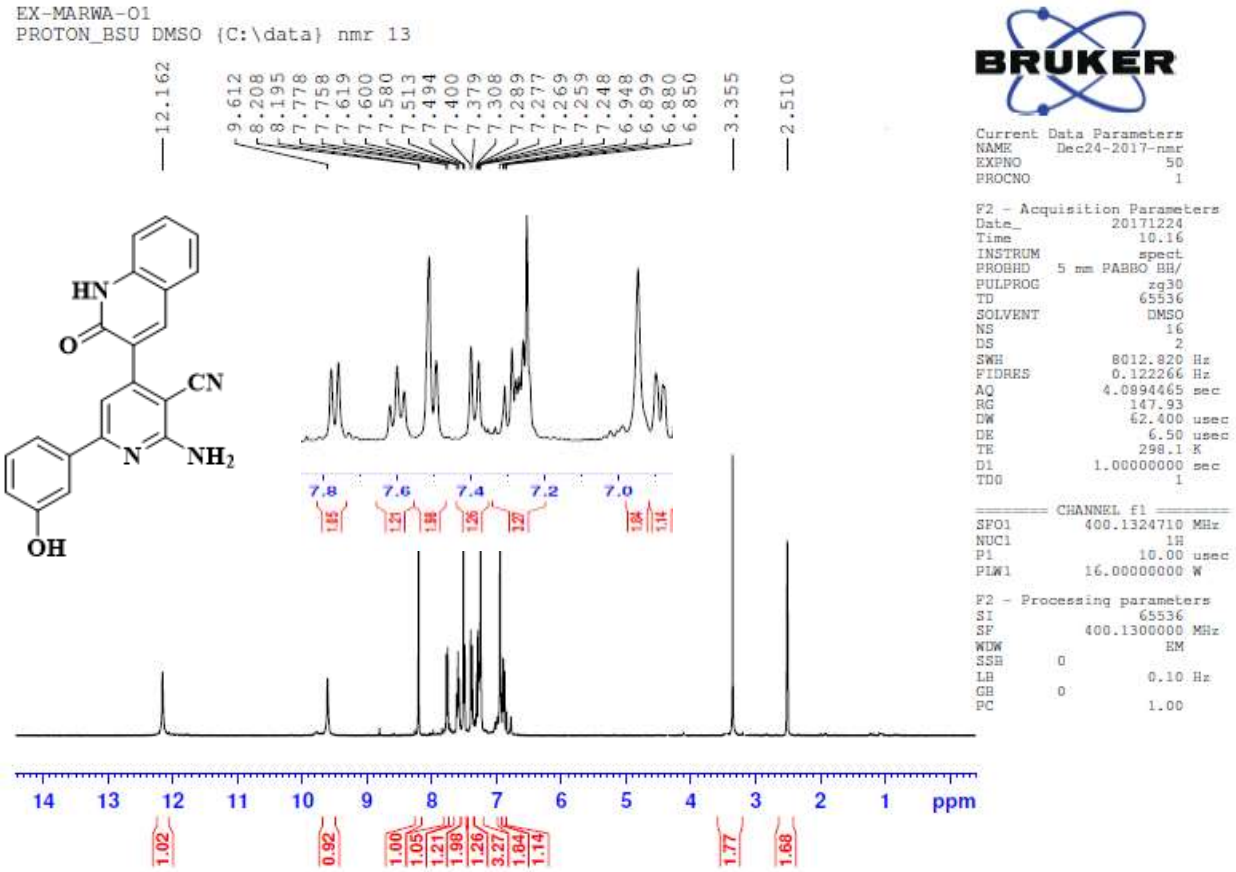
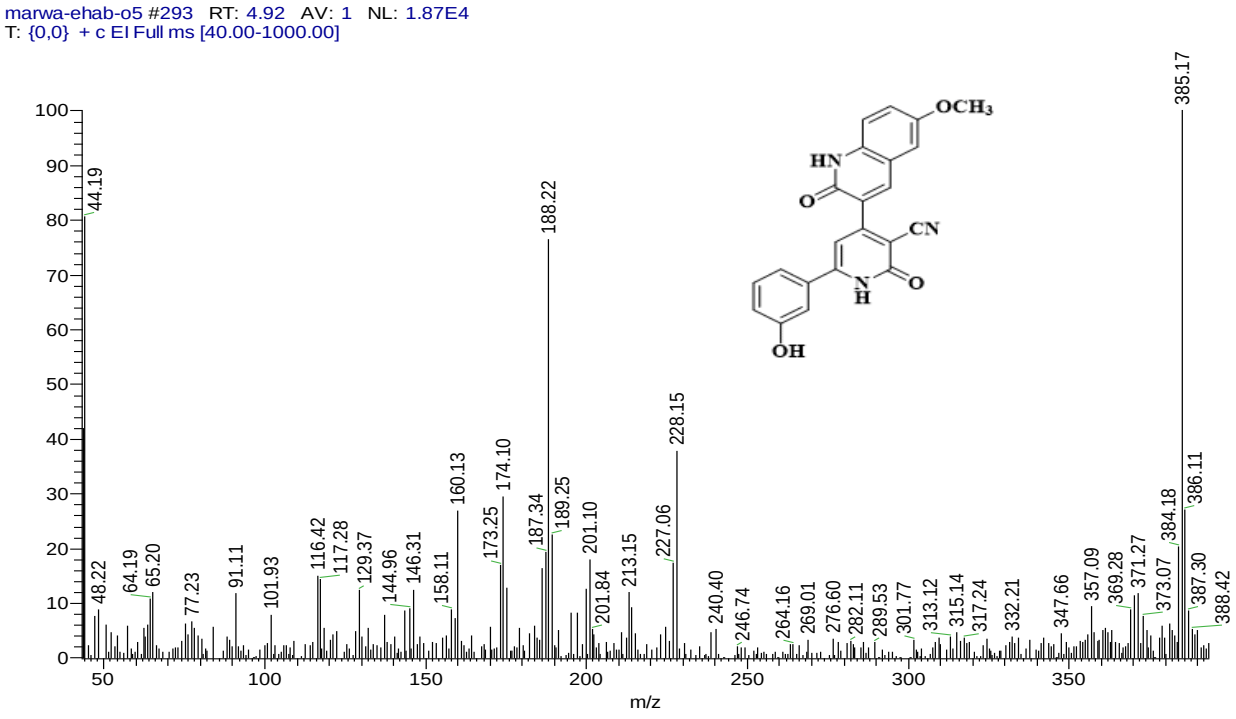
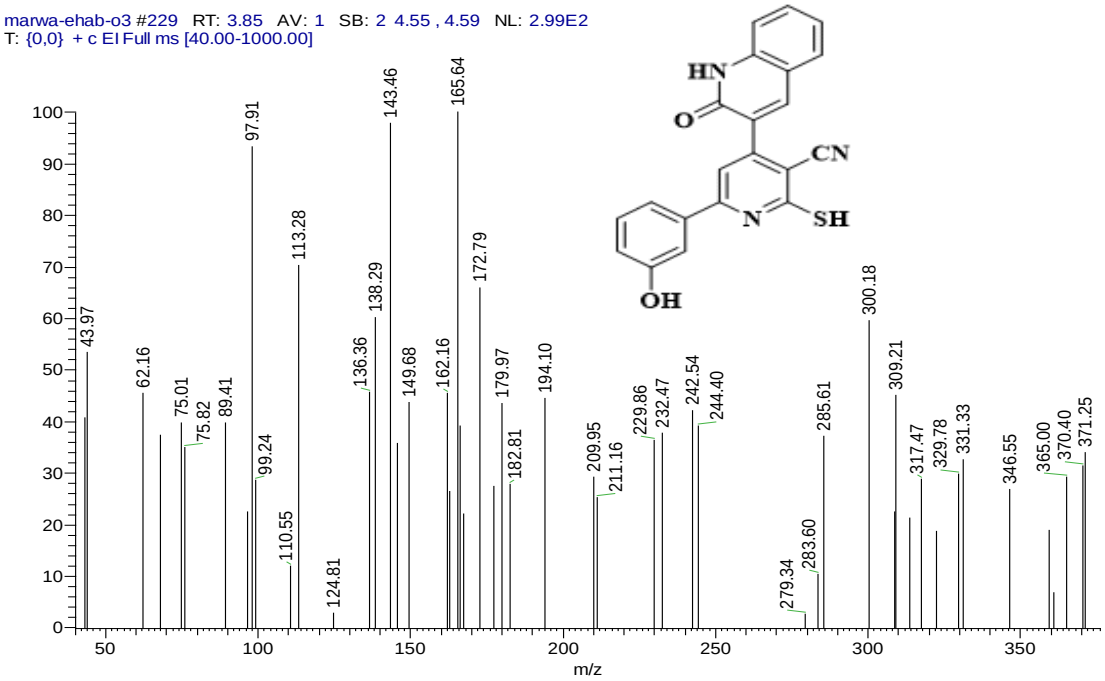
C^{13}-NMR spectra of compound 6c



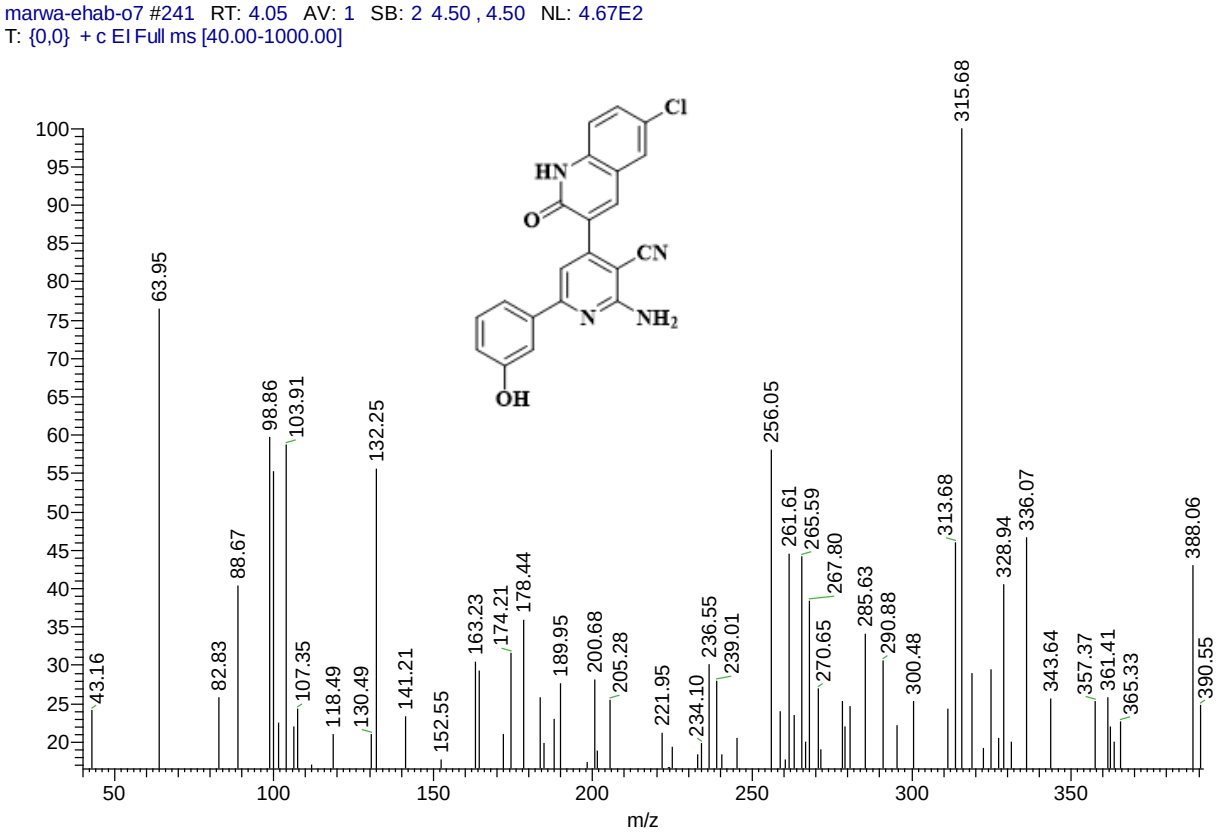
C[13]-NMR spectra of compound 7a



EI/MS spectrum of compound 5c



EI/MS spectrum of compound 6a



EI/MS spectrum of compound 7b

Al-Azhar University
The Regional Center for Mycology and Biotechnology

Requester Data:

Name: Dr. Marwa Ehab Mohamed
Authority: Faculty of Pharmacy,
Alexandria University

Sample Data:

Eleven samples had been submitted for elemental analysis.

Analysis Report:

Sample Code	C%	H%	N%
CH_2	68.01	3.43	7.79
CH_5	68.56	3.88	7.42
M_1	71.02	4.89	13.43
M_4	68.94	5.12	12.59
M_7	65.27	4.28	12.47
N_1	74.19	5.39	13.53
N_4	71.90	5.26	12.56
N_7	68.60	4.80	12.49
O_2 **5a**	70.69	3.84	11.97
O_5 **5c**	68.80	3.85	10.79
O_8 **5b**	64.89	3.32	10.85

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Al-Azhar University

The Regional Center for Mycology and Biotechnology

Requester Data:

Name: Dr. Marwa Ehab Mohamed
Authority: Faculty of Pharmacy,
Alexandria University

Sample Data:

Twenty samples had been submitted for elemental analysis.

Analysis Report:

Sample Code	C%	H%	N%
M_1 eN	68.71	5.24	19.38
M_1 J	71.09	5.21	12.96
M_2	68.42	4.75	12.94
M_4 e	70.38	5.84	11.50
M_4 k	69.87	5.68	11.79
M_5	66.59	4.83	11.75
M_7 a	64.58	4.59	11.34
M_7 b	62.96	4.13	11.06
M_7 e	67.40	5.28	11.37
M_7 e N	63.26	4.69	17.58
M_7 f	63.44	4.21	11.39
M_7 h	66.81	4.62	11.94
M_7 J	66.26	4.69	11.72
M_7 k	66.49	4.88	11.69
M_8	63.45	4.11	11.94
N_3	71.47	5.28	13.02
N_5	69.43	5.34	12.13
O_1 **7a**	71.49	4.06	15.95
O_4 **7c**	68.95	4.32	14.67
O_7 **7b**	64.71	3.19	14.62

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المركز الإقليمي للفطريات وتطبيقاتها
The Regional Center for Mycology and Biotechnology

Requester Data:

Name: Dr. Marwa Ehab Abdel-Aziz
Authority: Faculty of Pharmacy,
Alexandria University

Sample Data:

Nine samples had been submitted for elemental analysis.

Analysis Report:

Sample Code	C%	H%	N%
M_3	73.68	5.12	13.89
M_3 r	70.69	5.23	16.75
M_6	71.43	5.24	12.97
N_3	76.71	5.70	13.96
N_6	74.52	5.68	12.59
N_8	65.89	4.65	11.76
O_3 **6a**	67.69	3.75	11.48
O_6 **6c**	65.70	3.98	10.60
O_9 **6b**	62.41	3.16	10.44

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Table 1: Anticancer activities IC_{50} (µM) of the tested compounds against human cancer cells, In vitro cytotoxicity and selectivity index (SI) values of the tested compounds.

Cpd No	Wi-38		HepG-2		Caco-2		PC-3		NFS-60	
	EC_{50}(µM)	EC_{100}(µM)	IC_{50}(µM)	SI	IC_{50}(µM)	SI	IC_{50}(µM)	SI	IC_{50}(µM)	SI
5a	0.0603±0.04	0.0192±0.003	0.0344±0.006	1.753	0.0388±0.008	1.554	0.0716±0.0.4	0.842	0.03162±0.0001	1.907
5b	0.171±0.002	0.048±0.0001	**0.00528±0.0001**	32.386	**0.0453±0.001**	3.775	**0.0261±0.004**	6.552	**0.00932±0.004**	18.348
5c	0.717±0.002	0.361±0.014	**0.0076±0.0001**	94.342	**0.0361±0.0001**	19.861	**0.0272±0.003**	26.360	**0.0181±0.001**	39.613
5d	0.0299±0.0001	0.0239±0.0003	0.0897±0.013	0.333	0.0386±0.001	0.775	0.05341±0.004	0.560	0.07126±0.003	0.420
5e	0.0276±0.001	0.0234±0.001	0.03612±0.001	0.764	0.0517±0.005	0.534	0.0409±0.001	0.675	0.06267±0.027	0.440
5f	0.0298±0.002	0.0258±0.008	0.0378±0.01	0.788	0.0508±0.004	0.587	0.0451±0.0001	0.661	0.04283±0.0003	0.696
6a	0.181±0.005	0.0785±0.003	0.074±0.002	2.446	0.104±0.004	1.740	0.127±0.002	1.425	0.135±0.002	1.341
6b	0.138±0.002	0.0493±0.001	0.0466±0.001	2.961	0.0552±0.003	2.500	0.119±0.014	1.160	0.204±0.006	0.676
6c	0.376±0.001	0.177±0.011	0.0848±0.002	4.434	0.0999±0.003	3.764	0.1008±0.0001	3.730	0.145±0.015	2.593
6d	0.759±0.025	0.212±0.009	0.0876±0.002	8.664	0.0708±0.003	10.720	0.135±0.011	5.622	0.217±0.022	3.498
6e	0.155±0.003	0.0277±0.0001	**0.0122±0.0001**	12.705	**0.027±0.002**	5.741	**0.0429±0.003**	3.613	**0.0539±0.0001**	2.876
7a	0.174±0.005	0.102±0.01	0.0495±0.0001	3.515	0.0799±0.003	2.178	0.140±0.02	1.243	0.145±0.002	1.200
7b	0.140±0.009	0.110±0.002	0.1016±0.001	1.378	0.119±0.016	1.176	0.135±0.002	1.037	0.107±0.001	1.308
7c	0.137±0.0001	0.094±0.005	0.1421±0.001	0.964	0.127±0.014	1.079	0.101±0.001	1.356	0.153±0.034	0.895
9a	0.159±0.004	0.0254±0.002	0.0782±0.002	2.033	0.0739±0.0001	2.152	0.0847±0.003	1.877	0.0923±0.0001	1.723
9b	0.171±0.001	0.134±0.01	0.0454±0.003	3.767	0.0552±0.002	3.098	0.0973±0.008	1.757	0.380±0.01	0.450
9c	0.276±0.002	0.0754±0.003	0.0867±0.003	3.183	0.0526±0.0001	5.247	0.0462±0.002	5.974	0.0961±0.002	2.872

Cpd No	Wi-38		HepG-2		Caco-2		PC-3		NFS-60	
	EC_{50}(µM)	EC_{100}(µM)	IC_{50}(µM)	SI	IC_{50}(µM)	SI	IC_{50}(µM)	SI	IC_{50}(µM)	SI
11a	0.144±0.001	0.0906±0.012	0.336±0.022	0.429	0.158±0.002	0.911	0.158±0.026	0.911	0.126±0.006	1.143
11b	0.337±0.024	0.00232±0.001	0.0715±0.001	4.713	0.063±0.002	5.349	0.130±0.011	2.592	0.181±0.007	1.862
11c	0.101±0.001	0.0483±0.004	0.281±0.007	0.359	0.100±0.014	1.010	0.159±0.007	0.635	0.110±0.002	0.918
11d	0.107±0.001	0.0455±0.002	0.0458±0.001	2.336	0.0521±0.002	2.054	0.0594±0.001	1.801	0.0927±0.0001	1.154
11e	0.085±0.003	0.0178±0.0001	0.0416±0.004	2.043	0.0311±0.006	2.733	0.0444±0.004	1.914	0.0532±0.002	1.598
11f	0.181±0.013	0.0347±0.003	0.0363±0.001	4.986	0.0344±0.003	5.262	0.0476±0.0001	3.803	0.0998±0.011	1.814
11g	0.186±0.01	0.0450±0.005	0.0628±0.001	2.962	0.0626±0.001	2.971	0.0839±0.003	2.217	0.103±0.009	1.806
11h	0.166±0.003	0.0182±0.001	0.074±0.002	2.243	0.0829±0.003	2.002	0.0709±0.0001	2.341	0.0781±0.007	2.125
11i	0.258±0.031	0.148±0.0001	0.0486±0.001	5.309	0.0347±0.003	7.435	0.0634±0.002	4.069	0.523±0.04	0.493
13a	0.0255±0.004	0.0145±0.0001	**0.00238±0.0001**	10.714	**0.0228±0.001**	1.118	**0.0200±0.003**	1.275	**0.009±0.0001**	2.833
13b	0.0486±0.016	0.017±0.002	0.0309±0.001	1.573	0.04836±0.005	1.005	0.0388±0.003	1.253	0.03373±0.002	1.441
13c	0.0348±0.003	0.0242±0.001	**0.00377±0.0001**	9.231	**0.0337±0.006**	1.033	**0.0225±0.002**	1.547	**0.0088±0.0001**	3.955
14a	0.299±0.004	0.134±0.003	**0.0127±0.003**	23.543	**0.0141±0.004**	21.206	**0.0168±0.0001**	17.798	**0.0232±0.002**	12.888
14b	0.196±0.012	0.153±0.005	0.125±0.002	1.568	0.146±0.019	1.342	0.140±0.004	1.400	0.135±0.003	1.452
14c	0.166±0.009	0.0972±0.005	0.0557±0.003	2.980	0.0842±0.005	1.971	0.0626±0.0001	2.652	0.0965±0.004	1.720
15a	0.202±0.003	0.0663±0.003	0.108±0.007	1.870	0.191±0.019	1.058	0.242±0.037	0.835	0.247±0.016	0.818
15b	0.264±0.006	0.169±0.009	0.299±0.008	0.883	0.194±0.002	1.361	0.196±0.061	1.347	0.323±0.007	0.817
15c	0.121±0.002	0.101±0.005	0.107±0.011	1.131	0.141±0.021	0.858	0.143±0.013	0.846	0.122±0.001	0.992
11a	0.144±0.001	0.0906±0.012	0.336±0.022	0.429	0.158±0.002	0.911	0.158±0.026	0.911	0.126±0.006	1.143
11b	0.337±0.024	0.00232±0.001	0.0715±0.001	4.713	0.063±0.002	5.349	0.130±0.011	2.592	0.181±0.007	1.862
11c	0.101±0.001	0.0483±0.004	0.281±0.007	0.359	0.100±0.014	1.010	0.159±0.007	0.635	0.110±0.002	0.918
11d	0.107±0.001	0.0455±0.002	0.0458±0.001	2.336	0.0521±0.002	2.054	0.0594±0.001	1.801	0.0927±0.0001	1.154

Cytotoxicity screening

Cytotoxicity of the tested compounds was evaluated using normal human lung fibroblast Wi-38 cell line compared to currently used anticancer drug (doxorubicin). Firstly, Wi-38 cell line was subcultured in DMEM medium-contained 10% fetal bovine serum (FBS), then, seeded as $5x10^3$ cells per well in 96-well cell culture plate and incubated at 37ºC in 5% CO_2 incubator. Serial concentrations of these compounds and doxorubicin (Dox) were incubated with Wi-38 cells for 72 h after 24 h for cell attachment. MTT method was used for assaying cell viability[25]. Twenty microliters of 5 mg/ml MTT (Sigma, USA) was added to each well and the plate was incubated at 37ºC for 3 h. Then MTT solution was removed, 100 µl DMSO was used and the absorbance of each well was measured with a microplate reader (BMG LabTech, Germany) at 570 nm. The effective concentration (IC_{50}) and safe dose (EC_{100}) values of the tested compounds that cause 50% and 100% cell viability were estimated by the Graphpad Instat software.

Determination of the anticancer activity

Anticancer effect of the above-mentioned compounds was evaluated using four human cancer cell lines. Colon cancer cells (Caco-2) were maintained in DMEM (Lonza, USA) containing 10% FBS while myeloid leukemia cell line (NFS-60), liver cancer cell line (HepG-2) and prostate cancer cell line (PC-3) were cultured in RPMI-1640 (Lonza, USA) supplemented with 10% FBS. All cancer cells ($5x10^3$ cells/well) were seeded in sterile 96-well plates. After 24h, serial concentrations of the tested compounds and Dox were incubated with four cancer cell lines for 72 h at 37ºC in 5% CO_2 incubator. MTT method was done as described above. The Graphpad Instat software was used for calculating the half maximal inhibitory concentration (IC_{50}) values. Furthermore, phase contrast inverted microscope with a digital camera (Olympus, Japan) was used for investigating cellular morphological changes before and after treatment with the most effective and safest anticancer compounds.

Flow cytometric analysis of apoptosis

The IC_{50} of the most effective compounds and Dox was incubated for 72 h with HepG2, Caco-2, PC-3 and NFS-60 cell lines. After trypsinization, the untreated and treated cells were incubated with annexin V/PI for 15 min. Then cells were fixed and incubated with streptavidin-fluorescein (5 µg/mL) for 15 min. The apoptosis-dependent anticancer effect was determined by quantification of annexin-stained apoptotic cells using the FITC signal detector (FL1) against the phycoerythrin emission signal detector (FL2).

Caspase 3/7 activation assay

The Apo-ONE ® caspase 3/7 kit following the manufacturer's instructions was used for quantifying the percentage of caspase 3/7 activation. The fluorescence substrate present in the kit was cut by caspases to produce fluorescence signal. This signal was measured by the fluorescence omega microplate reader (BMG LabTech, Germany) at 490 nm excitation and 520 nm emission.

Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical significance was estimated by the multiple comparisons Tukey post-hoc analysis of variance (ANOVA) using the SPSS16 program. When $p < 0.05$, the differences were marked as statistically significant.

PIM-1 and PIM-2 kinase inhibitory activity

The most active anticancer compounds were tested for their ability to *in-vitro* inhibit PIM-1 and PIM-2 kinase utilizing PIM-1 and PIM-2 Kinase Assay Kit – Promega Corporation catalog #V4032, following the manufacturer's instructions [26]