

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

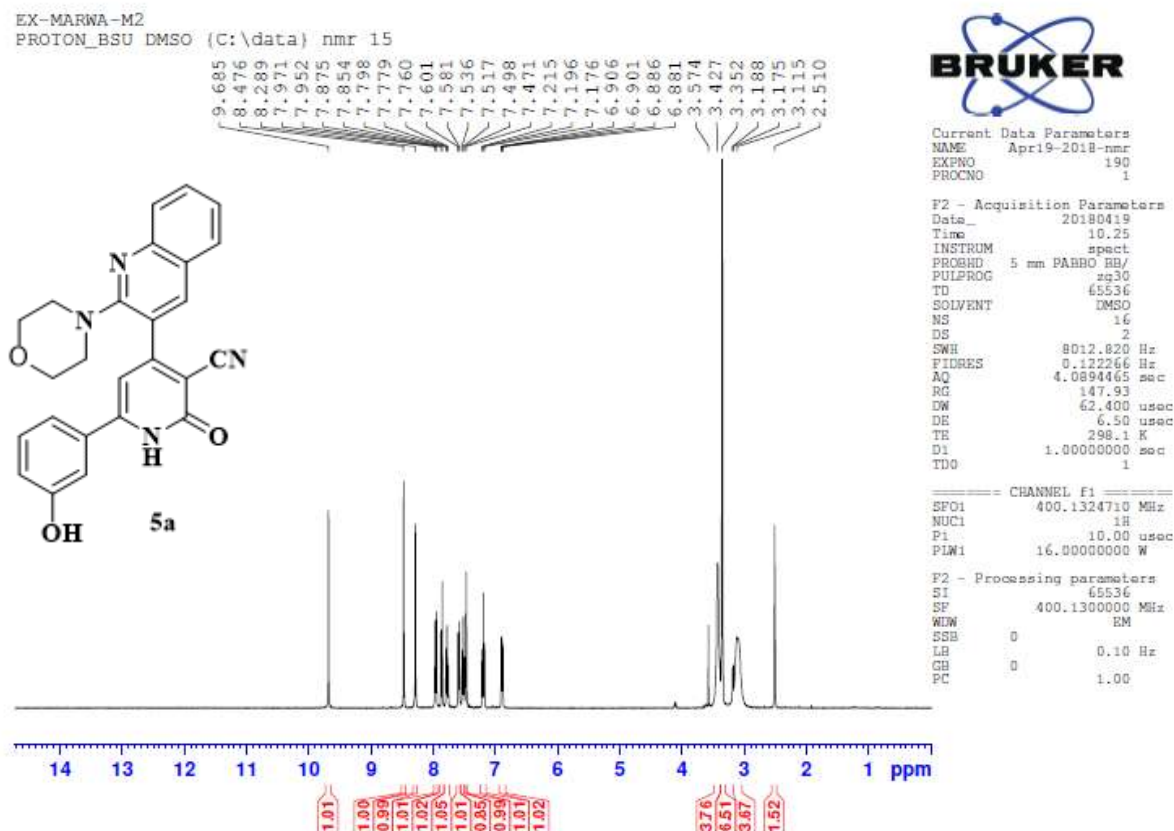
Mostafa M. M. El-Miligy^{1*}, Marwa E. Abdelaziz^{1*}, Salwa M. Fahmy¹, Tamer M. Ibrahim³,
Marwa M. Abu-Serie², Mona A. Mahran¹, Aly A. Hazzaa¹

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

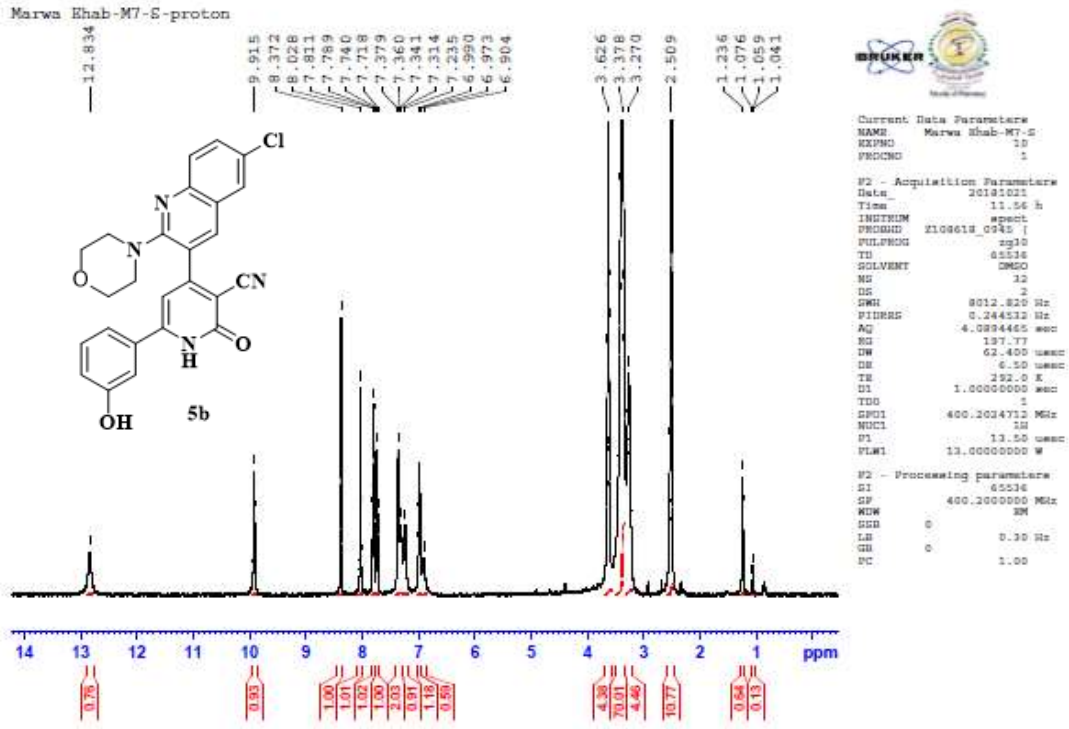
²Medical Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), City of Scientific Research and Technological Applications (SRTA-City), Alexandria 21934, Egypt

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

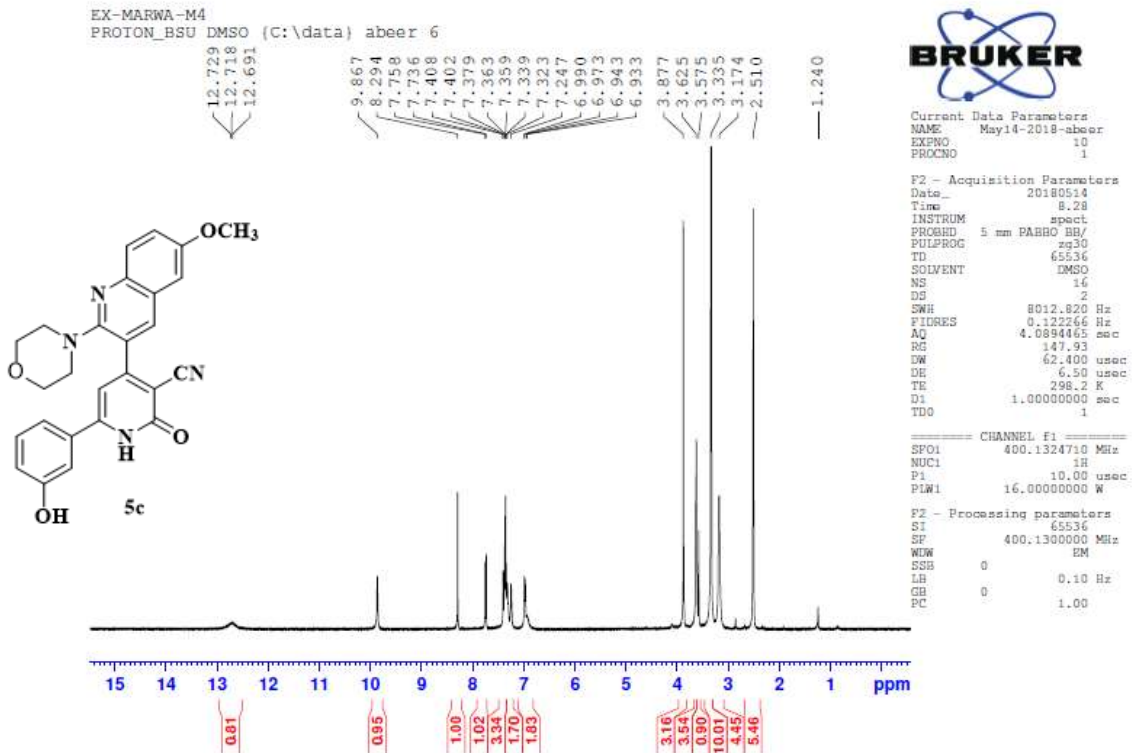
Scheme 1:



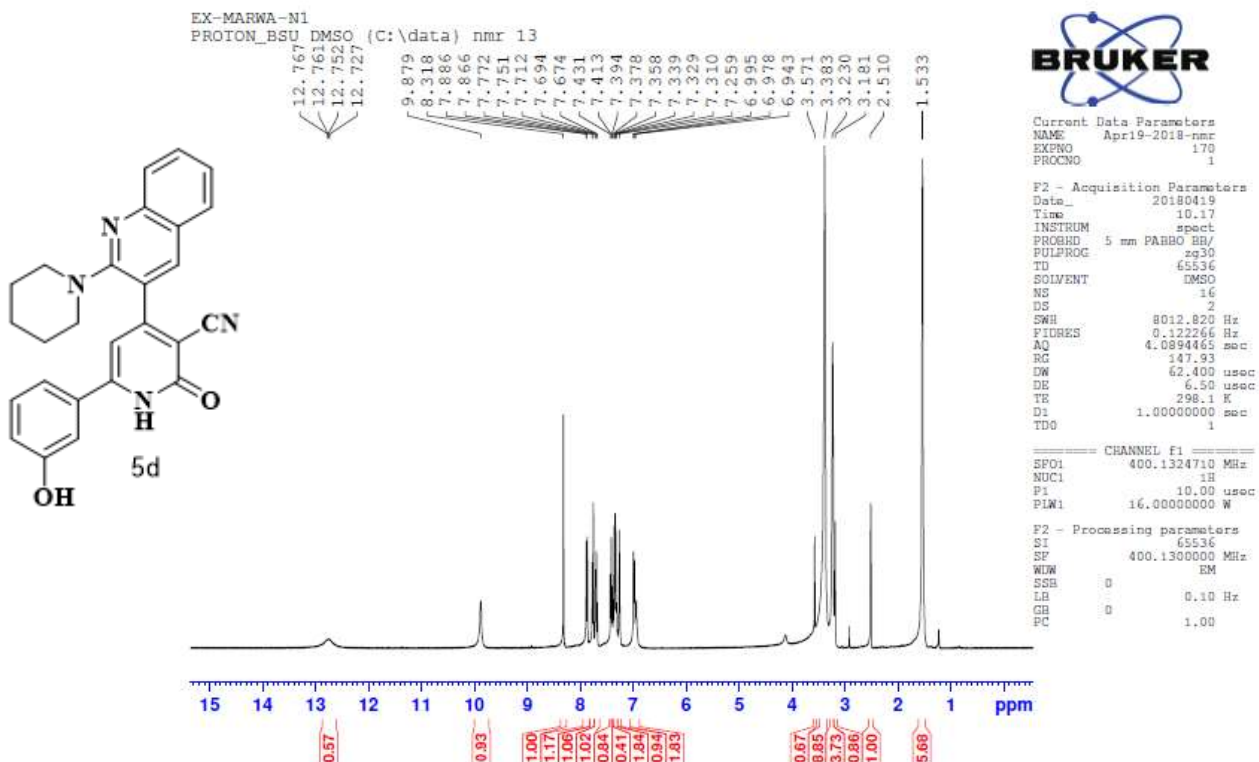
¹H-NMR spectra of compound 5a



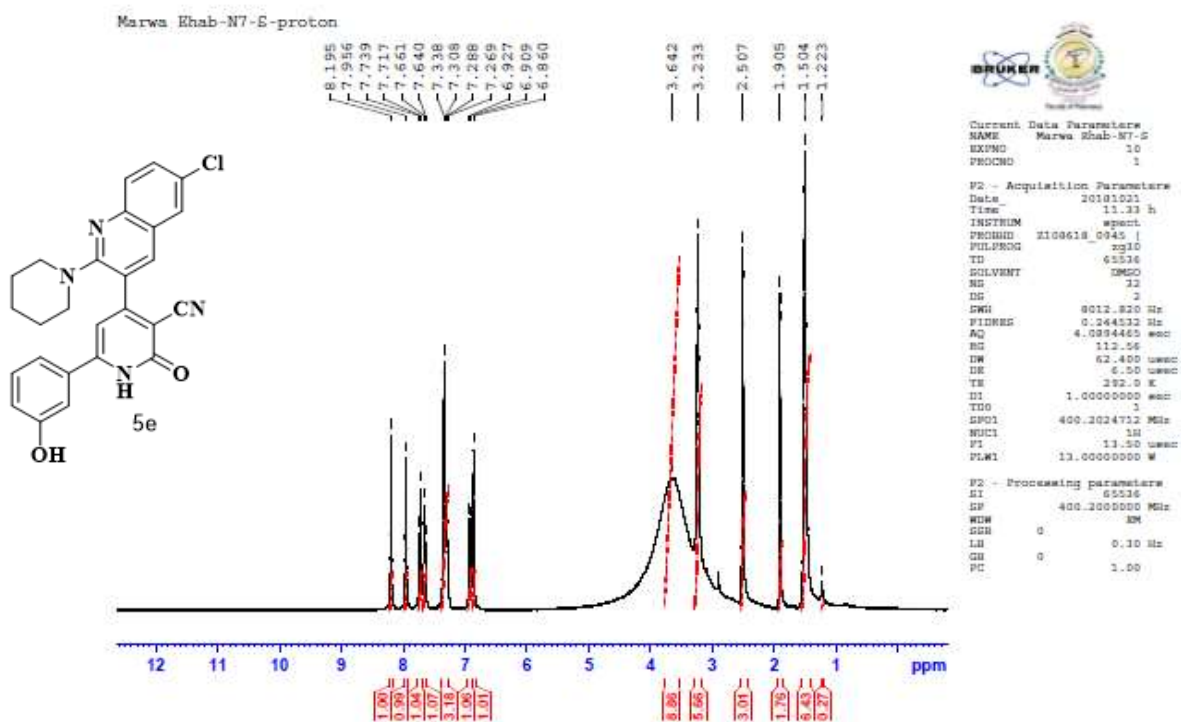
^1H -NMR spectra of compound 5b



^1H -NMR spectra of compound 5c

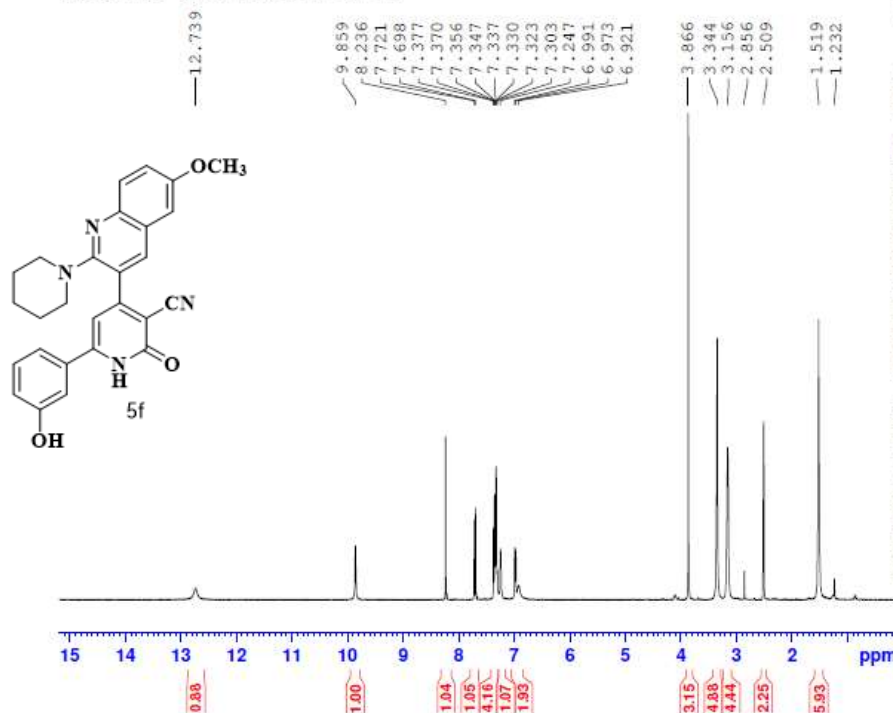


H¹-NMR spectra of compound 5d



H¹-NMR spectra of compound 5e

EX-MARWA-N4
 PROTON_BSU DMSO (C:\data) nmr 14



Current Data Parameters
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 PROCNO 1

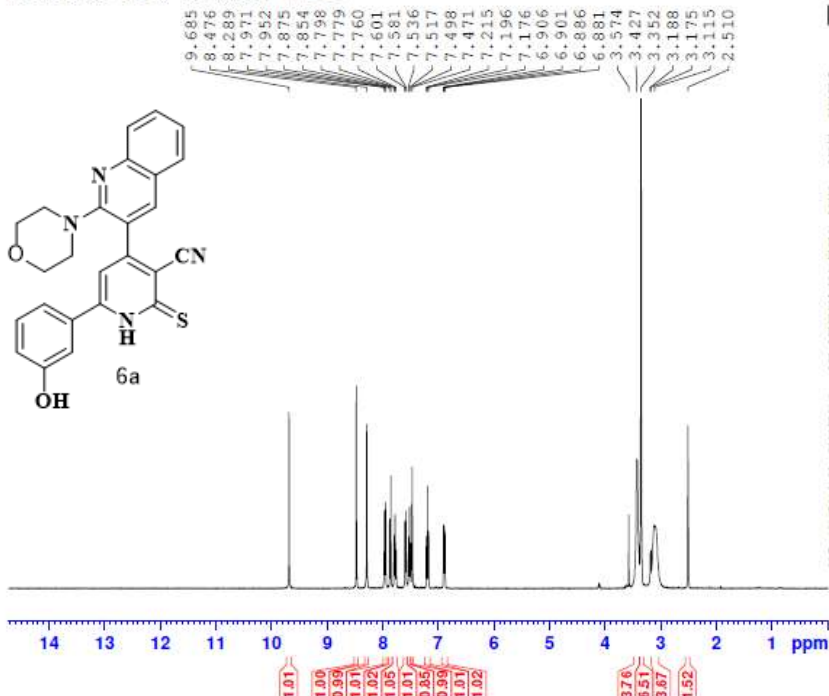
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 Time 10.21
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 PULPROG zg30
 TD 65536
 SOLVENT DMSO
 NS 16
 DS 2
 SWH 8012.820 Hz
 FIDRES 0.122266 Hz
 AQ 4.0894465 sec
 RG 147.93
 DW 62.400 usec
 DE 6.50 usec
 TE 298.2 K
 D1 1.00000000 sec
 TDO 1

==== CHANNEL f1 ====
 SFO1 400.1324710 MHz
 NUC1 1H
 P1 10.00 usec
 PLW1 16.00000000 W

F2 - Processing parameters
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 SF 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.10 Hz
 GB 0
 PC 1.00

^1H -NMR spectra of compound 5f

EX-MARWA-M2
 PROTON_BSU DMSO (C:\data) nmr 15



Current Data Parameters
 NAME Apr19-2018-nmr
 EXPNO 190
 PROCNO 1

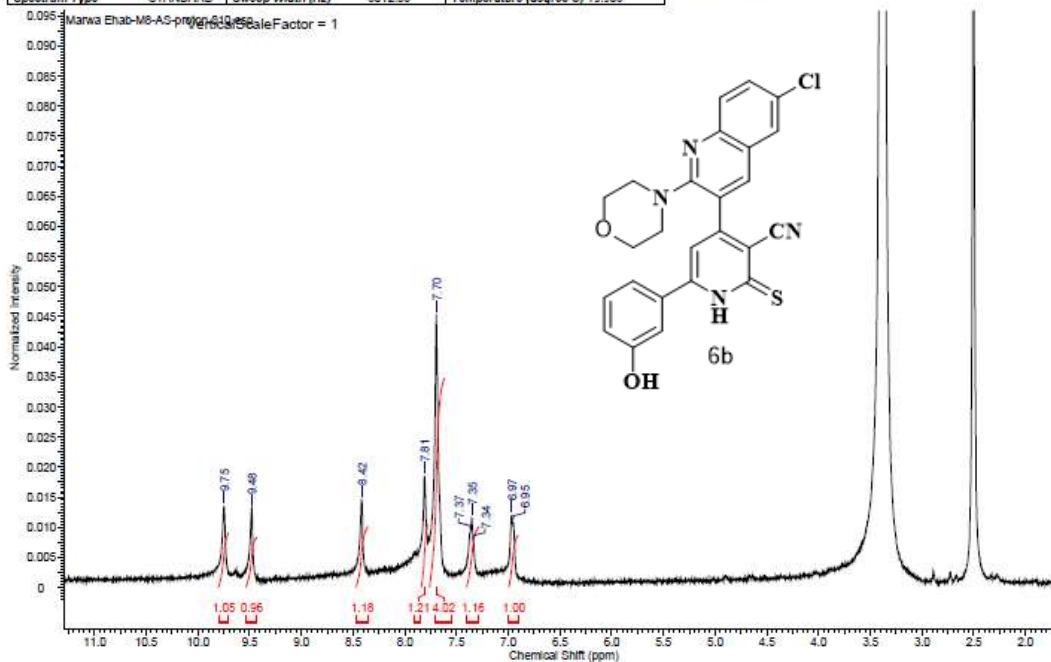
F2 - Acquisition Parameters
 Date_ 20180419
 Time 10.25
 INSTRUM spect
 PROBHD 5 mm PABBO BB/
 PULPROG zg30
 TD 65536
 SOLVENT DMSO
 NS 16
 DS 2
 SWH 8012.820 Hz
 FIDRES 0.122266 Hz
 AQ 4.0894465 sec
 RG 147.93
 DW 62.400 usec
 DE 6.50 usec
 TE 298.1 K
 D1 1.00000000 sec
 TDO 1

==== CHANNEL f1 ====
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 NUC1 1H
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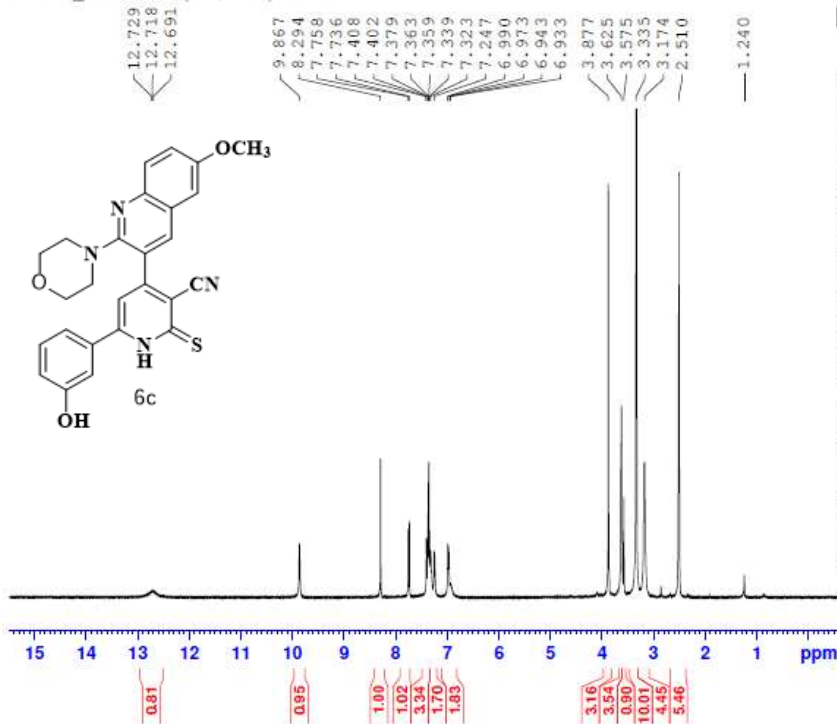
^1H -NMR spectra of compound 6a

Acquisition Time (sec)	4.0894	Comment	Marwa Ehab-M8-AS-proton	Date	03 Jun 2019 10:46:56
Date Stamp	03 Jun 2019 10:46:56	File Name	D:\PHD\NMR\Scheme 1\Marwa Ehab-M8-AS-proton10.fid	Origin	spect
Frequency (MHz)	400.20	Nucleus	1H	Number of Transients	16
Original Points Count	32768	Owner	nmr	Points Count	32768
Receiver Gain	158.72	SW(cyclical) (Hz)	8012.82	Solvent	DMSO-d6
Spectrum Type	STANDARD	Sweep Width (Hz)	8012.58	Temperature (degree C)	19.903
				Pulse Sequence	zg30
				Spectrum Offset (Hz)	2471.2351



^1H -NMR spectra of compound 6b

EX-MARWA-M4
PROTON_BSU DMSO (C:\data) aberer 6

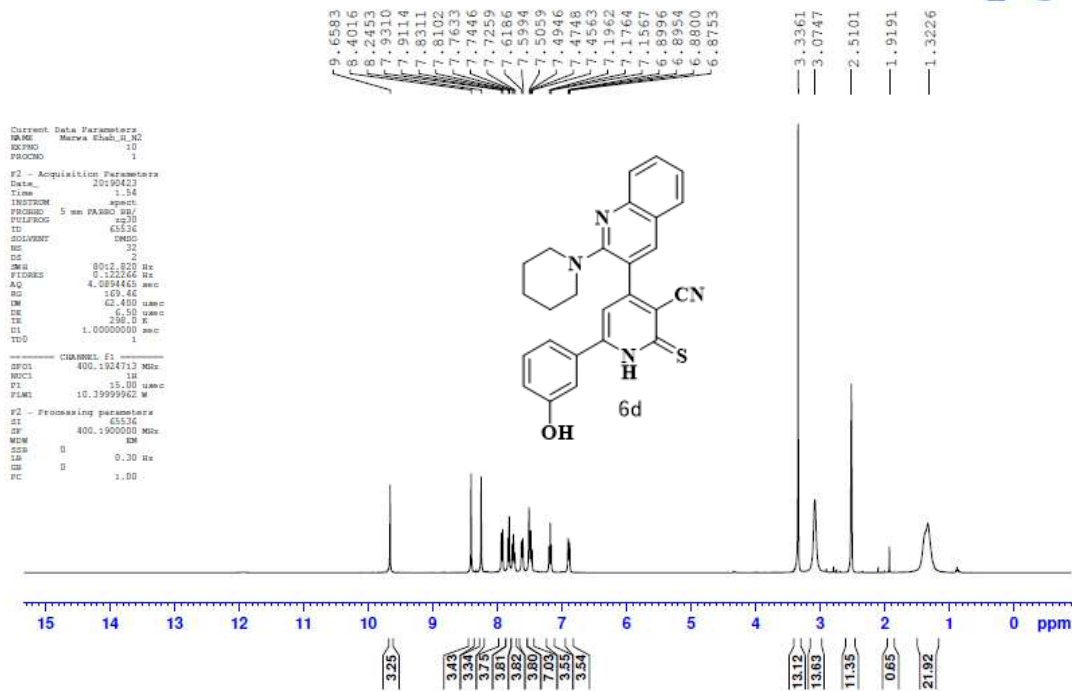


Current Data Parameters
NAME: May14-2018-aberer
EXPO: 10
PROCNO: 1

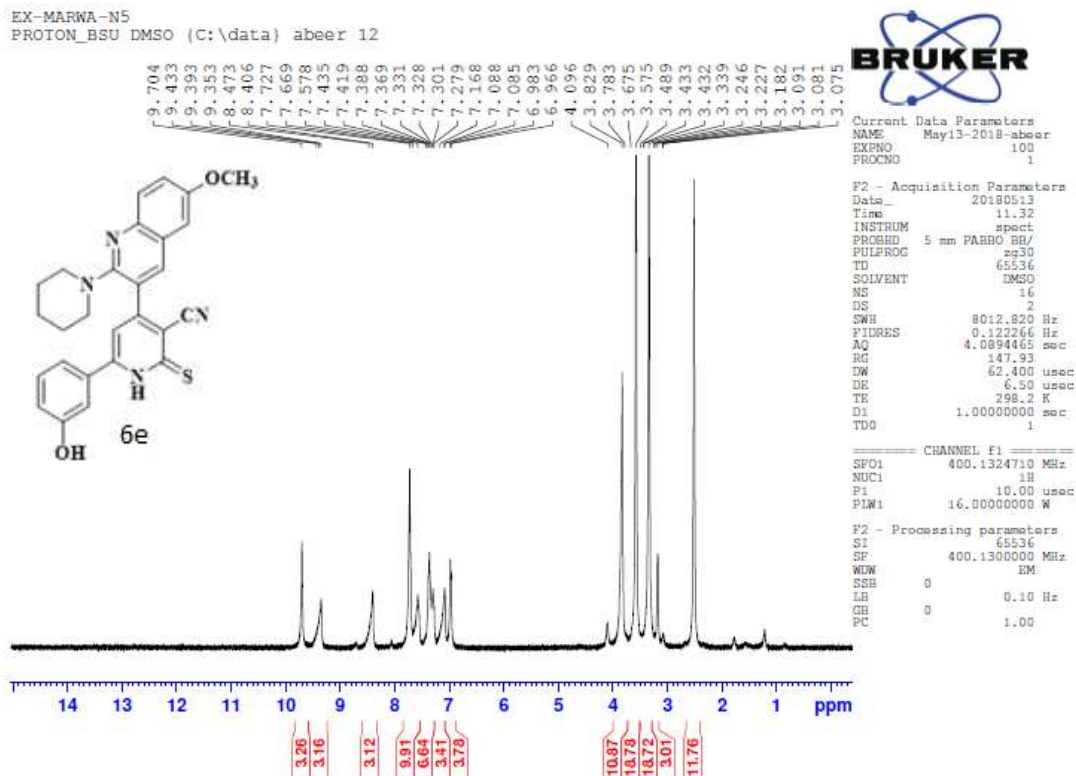
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PULPROG: zg30
TD: 65536
SOLVENT: DMSO
NS: 16
DS: 2
SWH: 8012.820 Hz
FIDRES: 0.122266 Hz
AQ: 4.0894465 sec
RG: 347.93
DW: 62.400 usec
DE: 6.50 usec
TE: 298.2 K
D1: 1.00000000 sec
TD0: 1

==== CHANNEL f1 =====
SFO1: 400.1324710 MHz
NUC1: 1H
P1: 10.00 usec
PIW1: 16.00000000 W
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SI: 65536
SF: 400.1300000 MHz
WDW: EM
SSB: 0
LB: 0.10 Hz
GB: 0
PC: 1.00

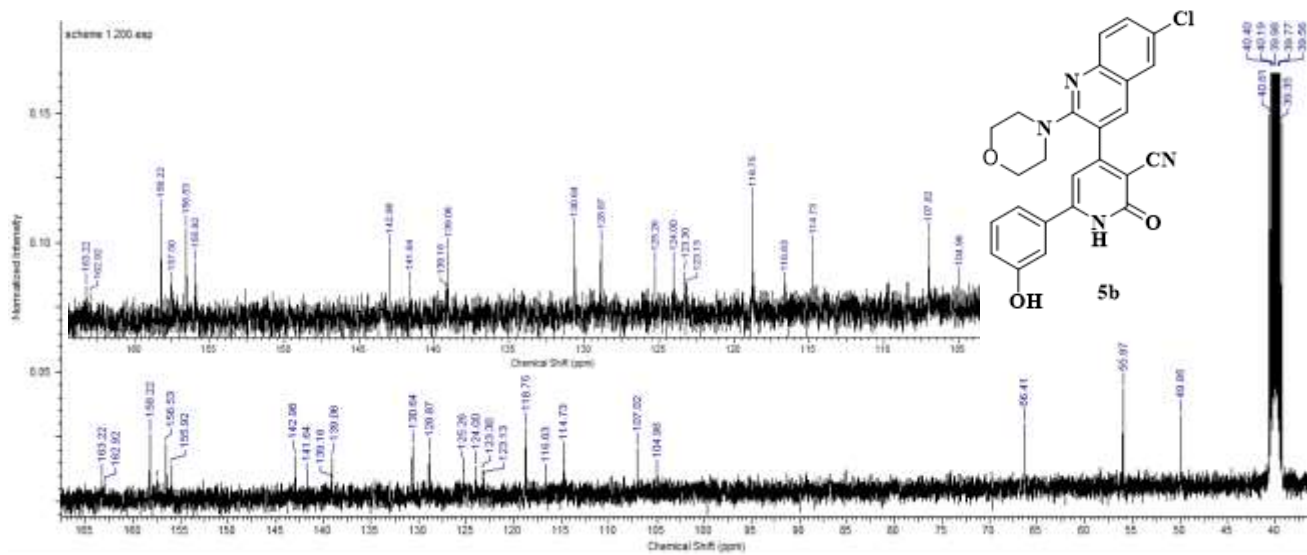
^1H -NMR spectra of compound 6c



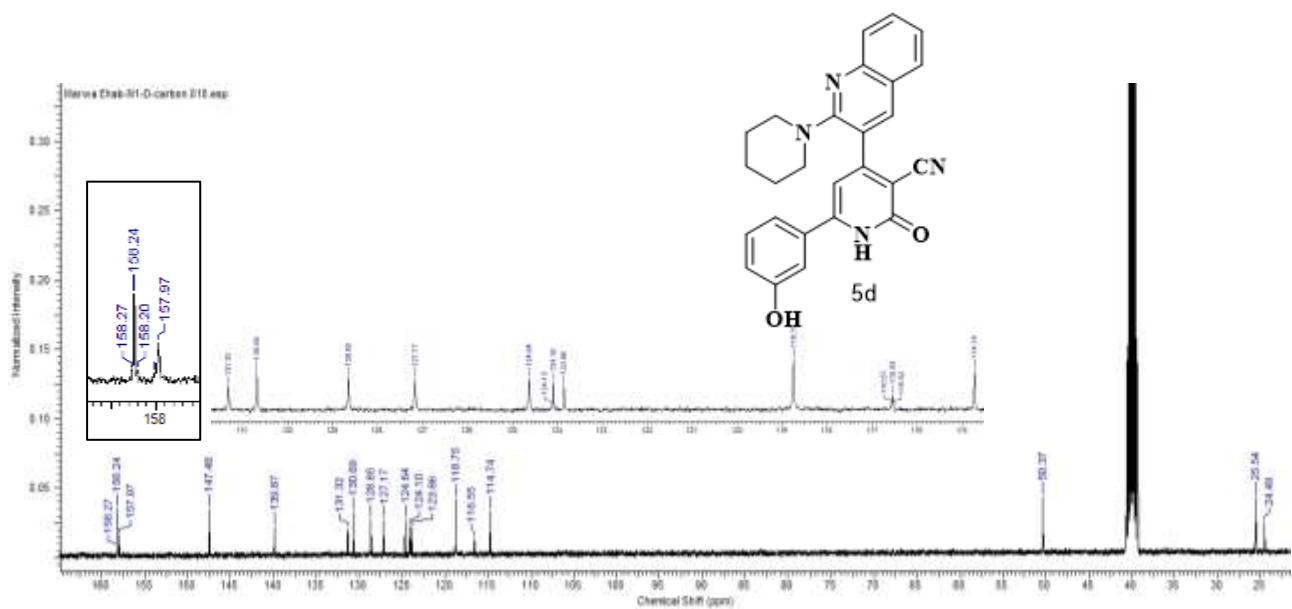
¹H-NMR spectra of compound 6d



¹H-NMR spectra of compound 6e



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



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

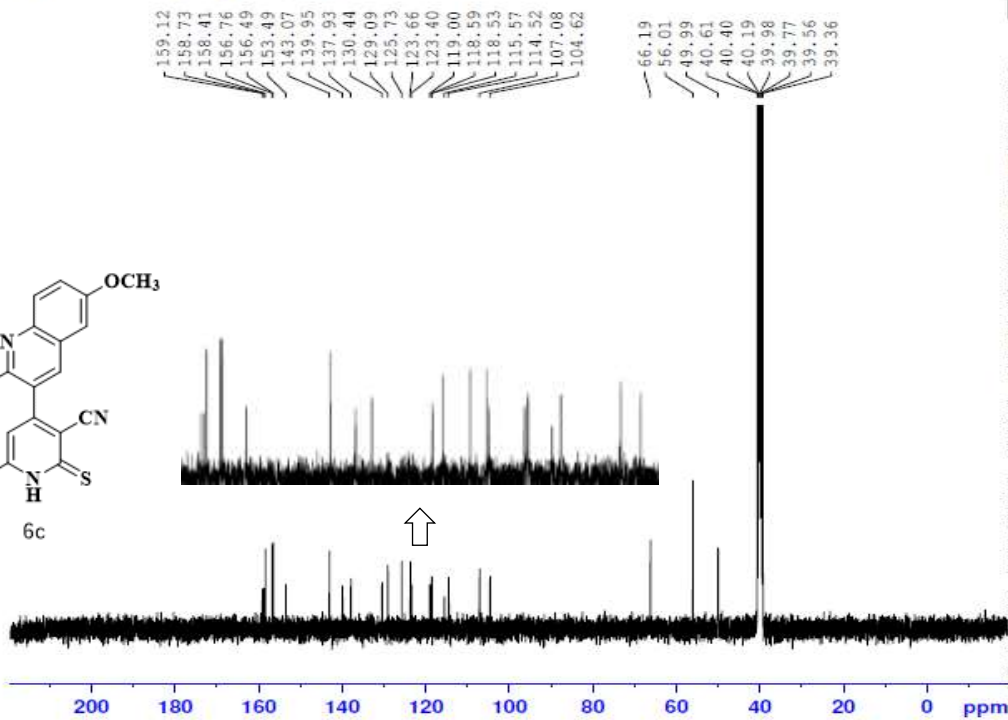
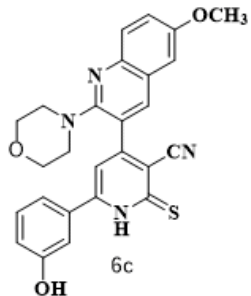
EX-MARWA-M5
 C13-BSU DMSO {C:\data} abeer 16



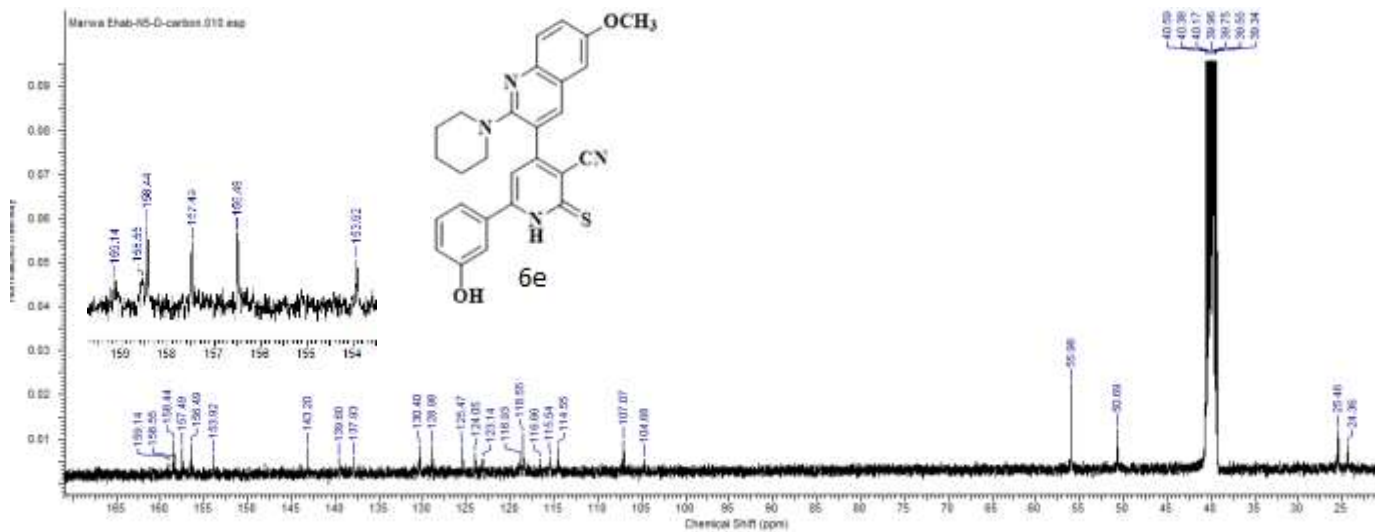
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 PROCNO 1
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 Time 17.28
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 SOLVENT DMSO
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 DS 4
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 FIDRES 0.366798 Hz
 AQ 1.3631488 sec
 RG 205.44
 DW 20.800 usec
 DE 6.50 usec
 TE 298.1 K
 D1 2.00000000 sec
 D11 0.03000000 sec
 TDO 1

CHANNEL F1
 SFO1 100.6228293 MHz
 NUC1 13C
 P1 10.00 usec
 PLW1 66.00000000 W
 CHANNEL F2
 SFO2 400.1316005 MHz
 NUC2 1H
 CPDPRG12 waltz16
 PCPD2 90.00 usec
 PLW2 16.00000000 W
 PLW12 0.21777999 W
 PLW13 0.17640001 W

F2 - Processing parameters
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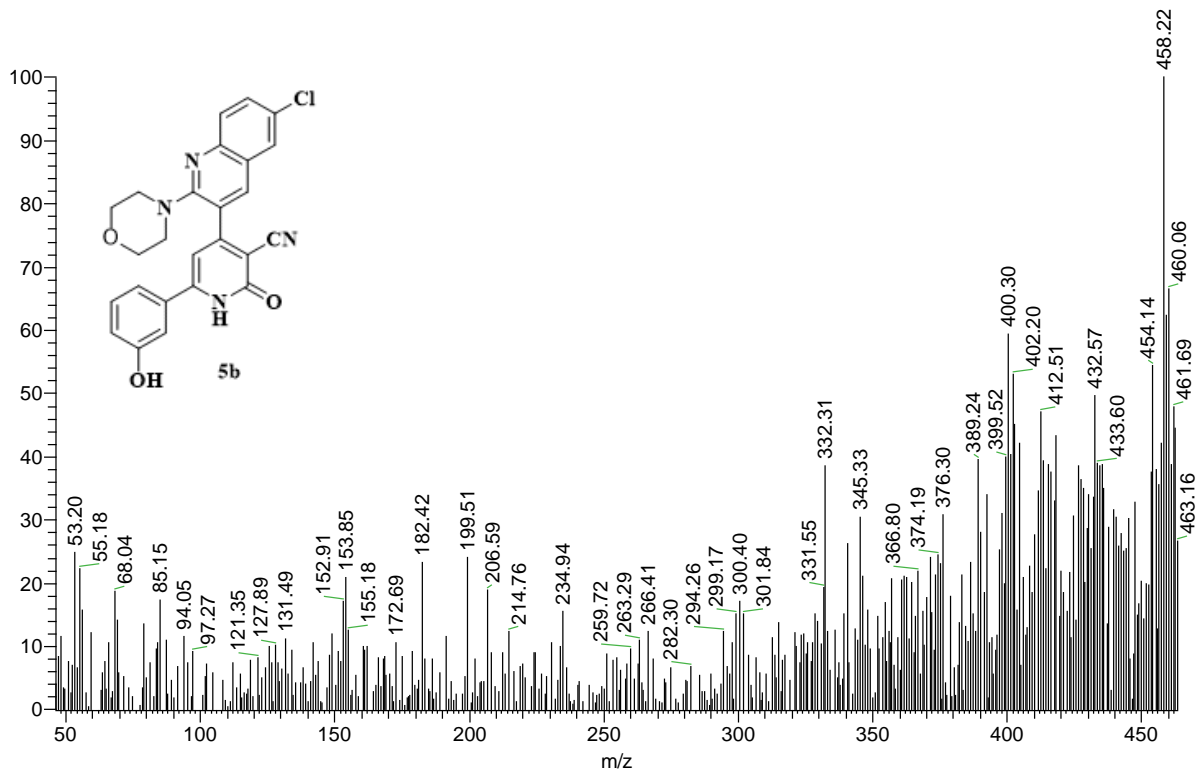


¹³C- NMR spectra of compound 6c



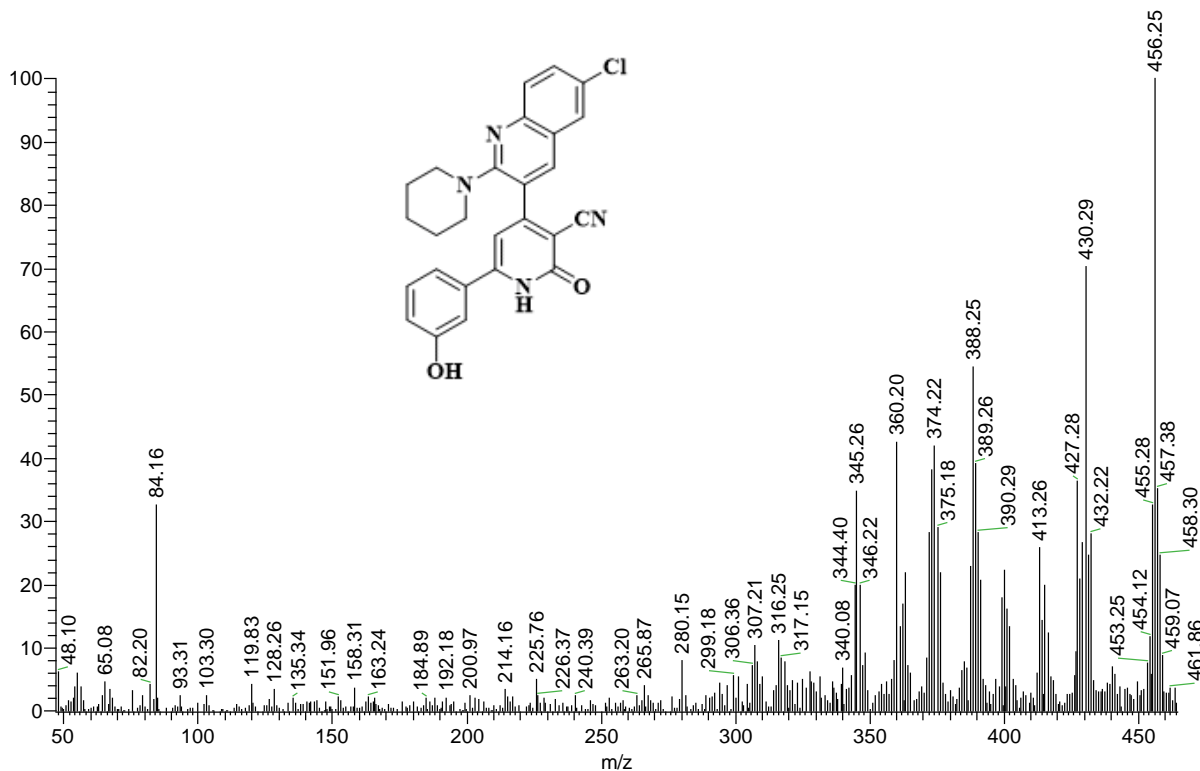
¹³C- NMR spectra of compound 6e

marwa-ehab-m7_181215122615 #294 RT: 4.94 AV: 1 NL: 4.55E3
T: {0,0} + c EI Full ms [40.00-1000.00]



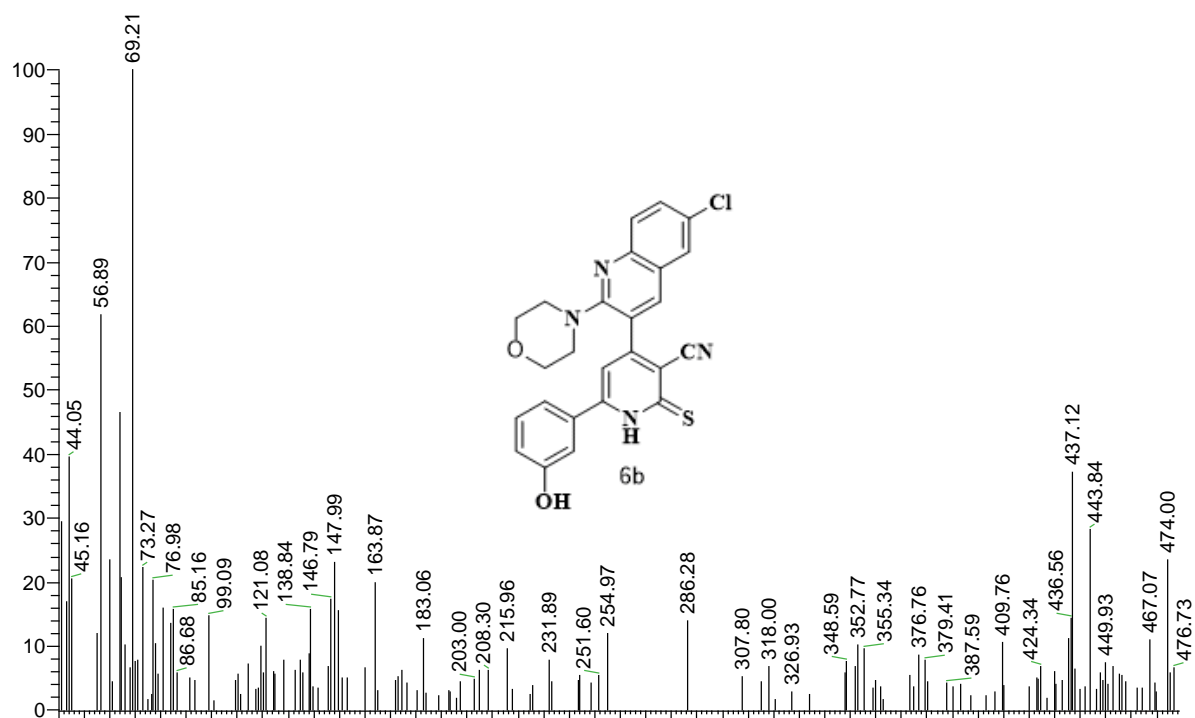
EI/MS spectrum of compound **5b**

marwa-ehab-n7 #279 RT: 4.69 AV: 1 NL: 3.90E4
T: {0,0} + c EI Full ms [40.00-1000.00]



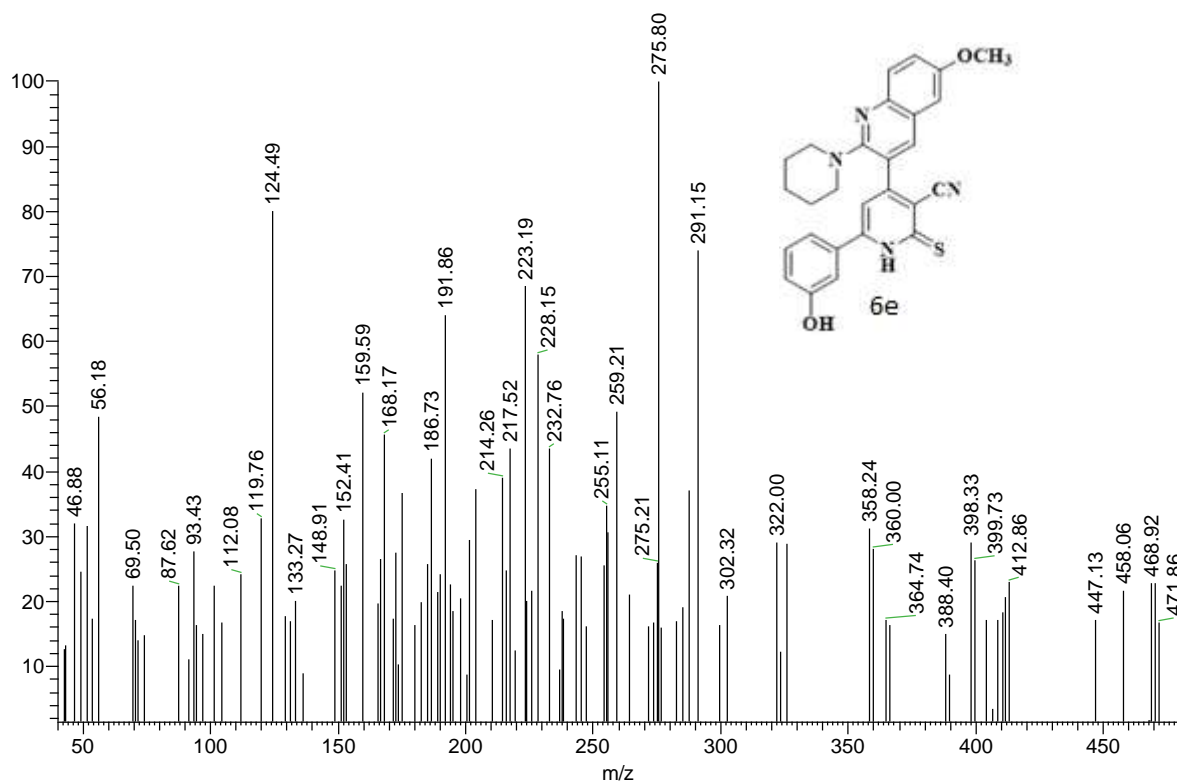
EI/MS spectrum of compound **5e**

marwa-ehab-m8 #167 RT: 2.81 AV: 1 NL: 2.17E3
T: {0,0} + c EI Full ms [40.00-1000.00]



EI/MS spectrum of compound 6b

marwa-ehab-n5 #195 RT: 3.28 AV: 1 SB: 2 4.40, 4.40 NL: 4.52E2
T: {0,0} + c EI Full ms [40.00-1000.00]



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 ₃	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





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 ₃ 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 ₈ 6b	63.45	4.11	11.94
N ₂ 6d	71.47	5.28	13.02
N ₅ 6f	63.43	5.34	12.13
O ₁	66.49	4.06	15.95
O ₄	68.95	4.32	14.67
O ₇	64.71	3.19	14.62

INVESTIGATOR

M. Ehab

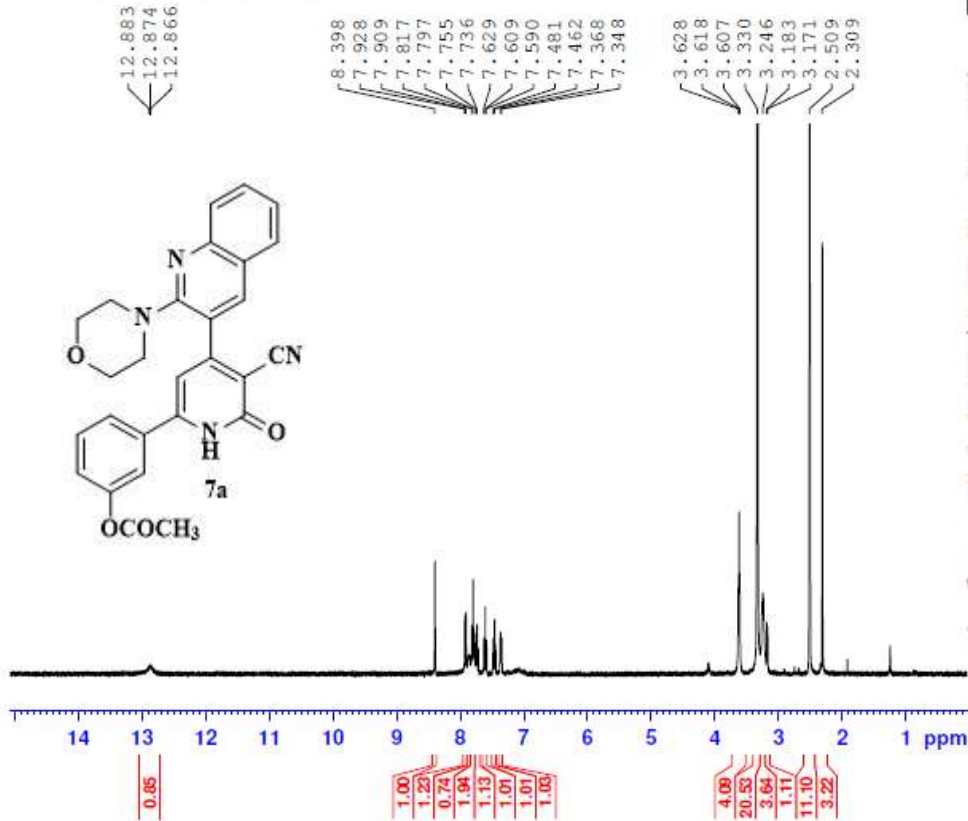


DIRECTOR

H. Shal

Scheme 2:

EX-MARWA-M1a
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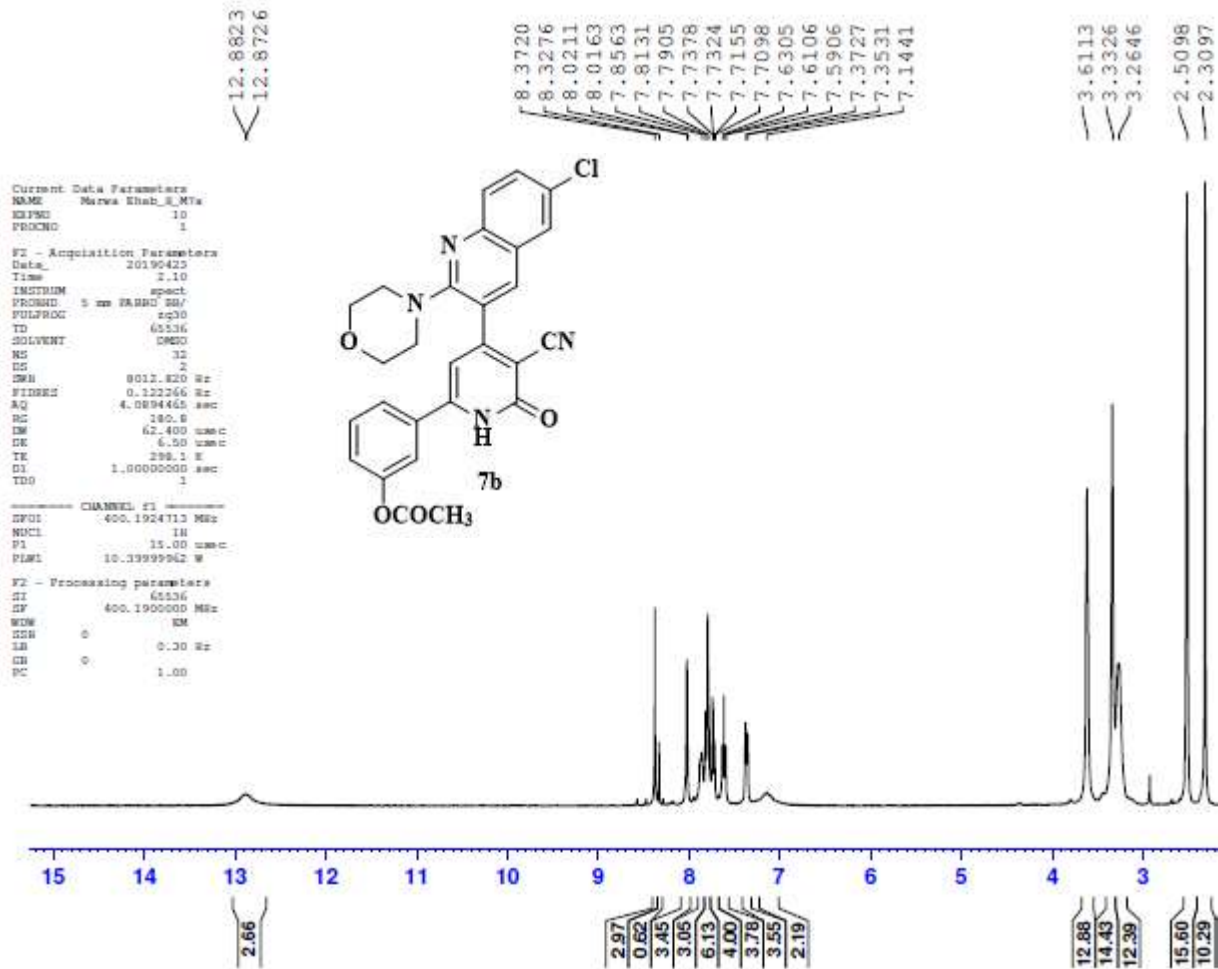
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 PULPROG zg30
 TD 65536
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 NS 16
 DS 2
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 DE 6.50 usec
 TE 298.1 K
 DI 1.0000000 sec
 TDO 1

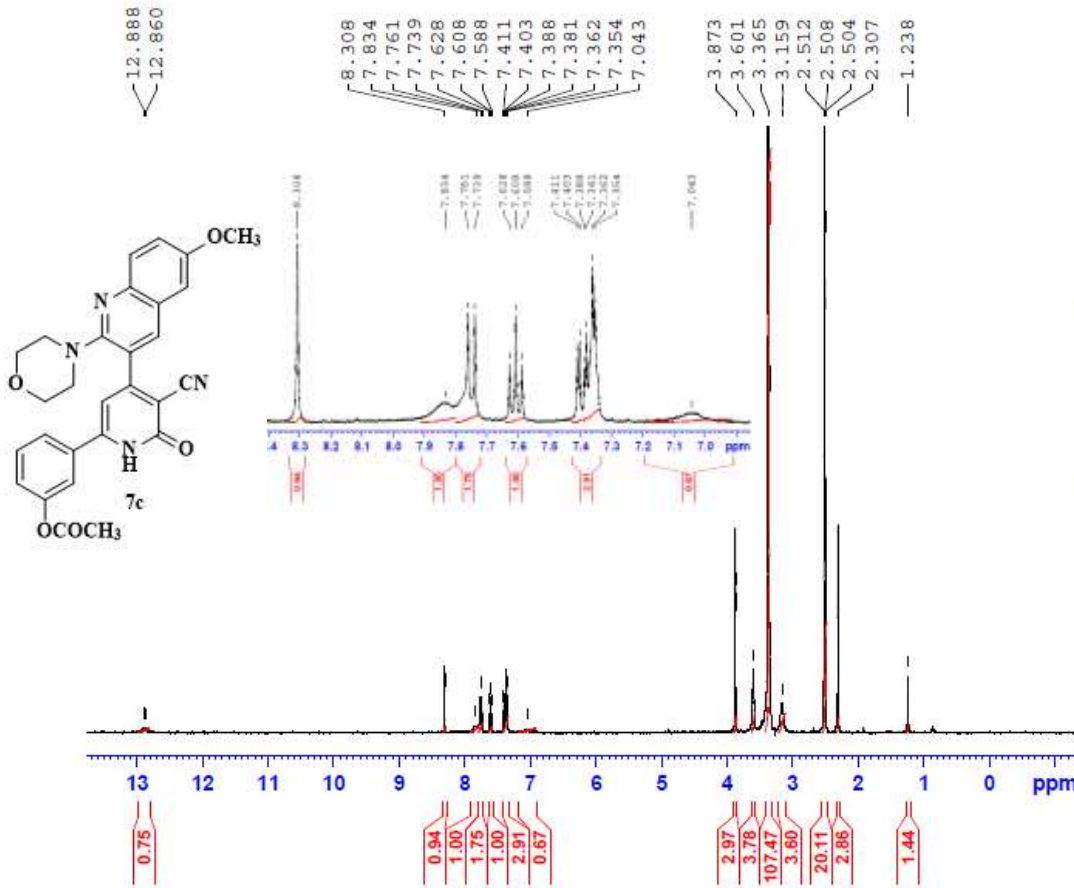
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 PLW1 16.00000000 W

F2 - Processing parameters
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 SF 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.10 Hz
 GB 0
 PC 1.00

¹H-NMR spectra of compound 7a



H¹-NMR spectra of compound 7b



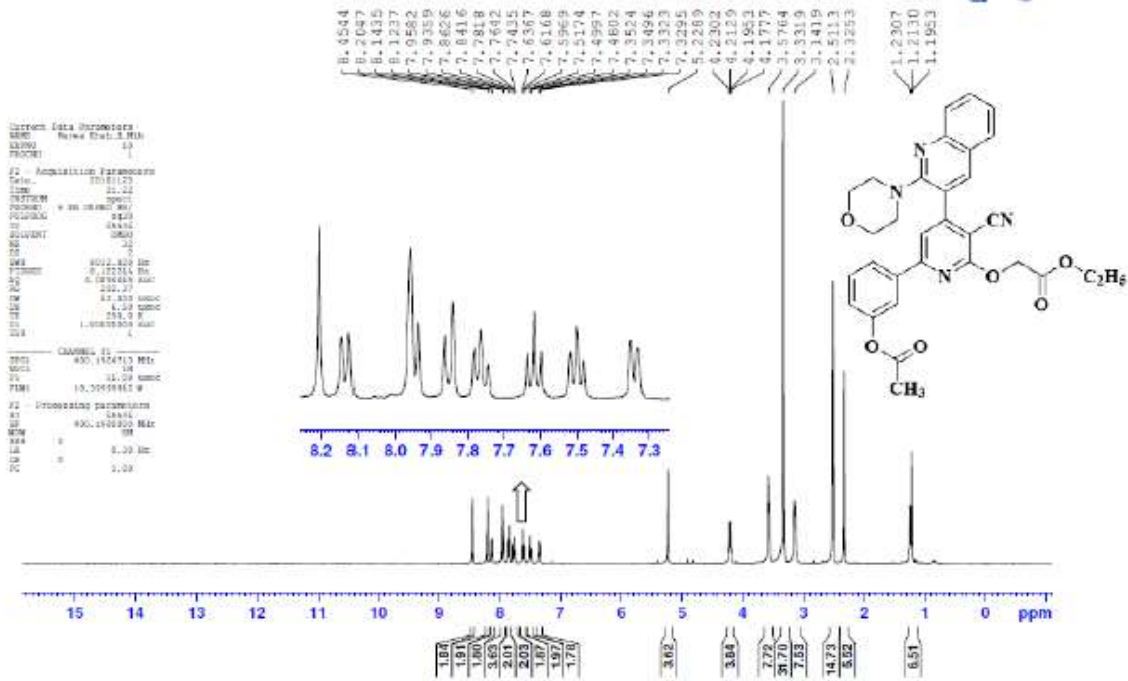
BRUKER
Faculty of Pharmacy

Current Data Parameters
 NAME Marwa Ehab-M4a-DMSO-Hnmr
 EXPRNO 10
 PROCNO 1

F2 - Acquisition Parameters
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 Time 12.45 h
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 PULPROG zg30
 TD 65536
 SOLVENT DMSO
 NS 16
 DS 2
 SWH 8012.820 Hz
 FIDRES 0.244832 Hz
 AQ 4.0894465 sec
 RG 197.77
 DW 62.400 usec
 DE 6.50 usec
 TE 293.4 K
 D1 1.00000000 sec
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 NUC1 1H
 F1 13.50 usec
 PLM1 13.00000000 W

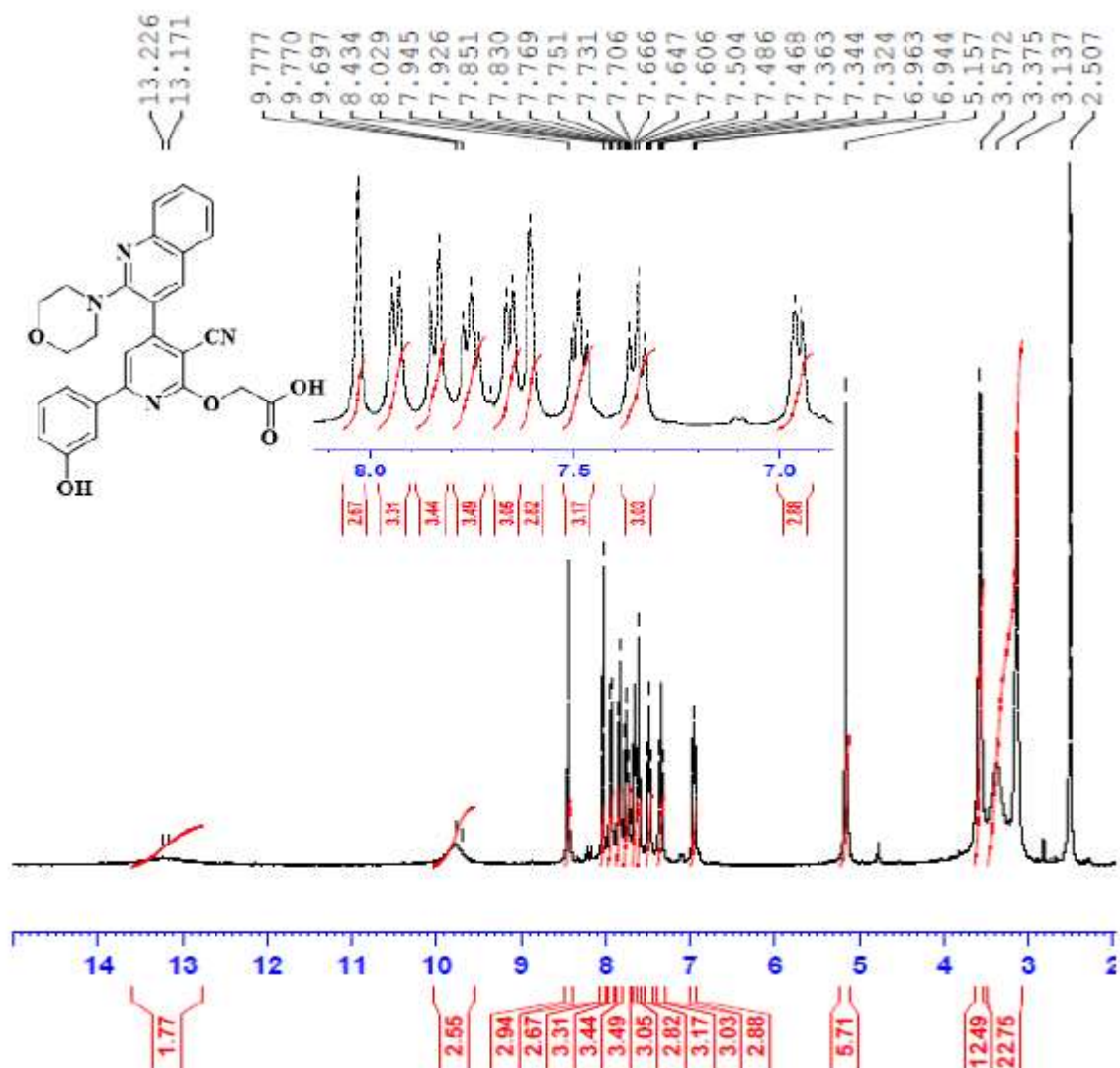
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¹H-NMR spectra of compound 7c

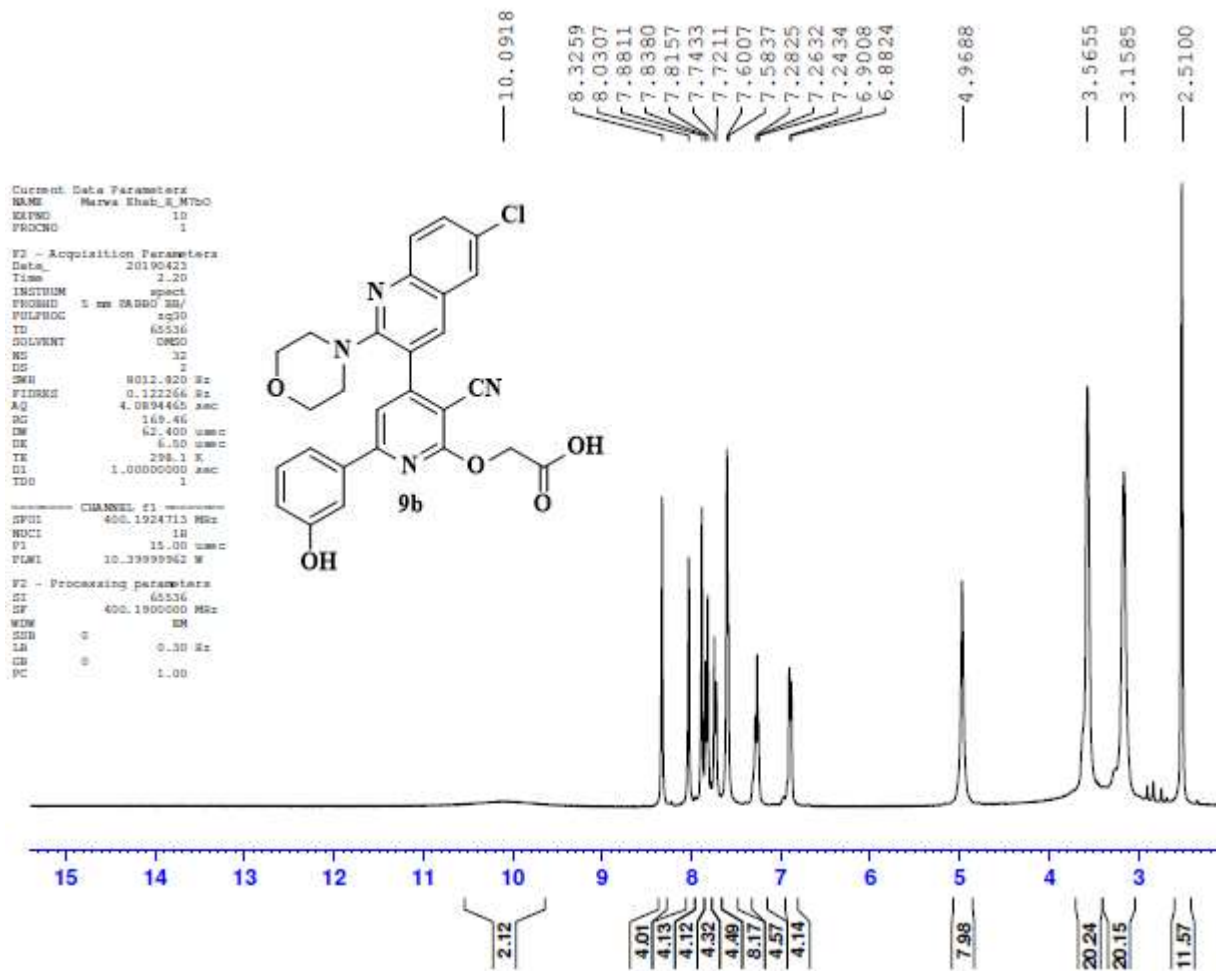


¹H-NMR spectra of compound 8a

Marwa ehab Mlbo -M proton

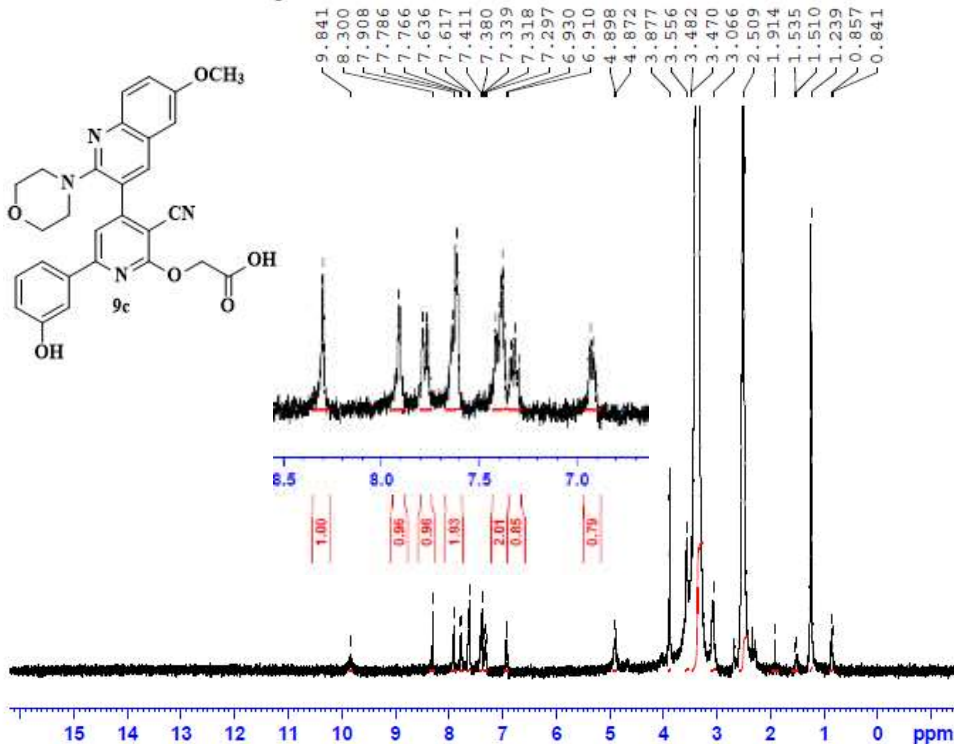


¹H-NMR spectra of compound 9a



¹H-NMR spectra of compound 9b

Mrwa ehab -M4bo-DMSO-W-proton



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 EXPNO 10
 PROCNO 1

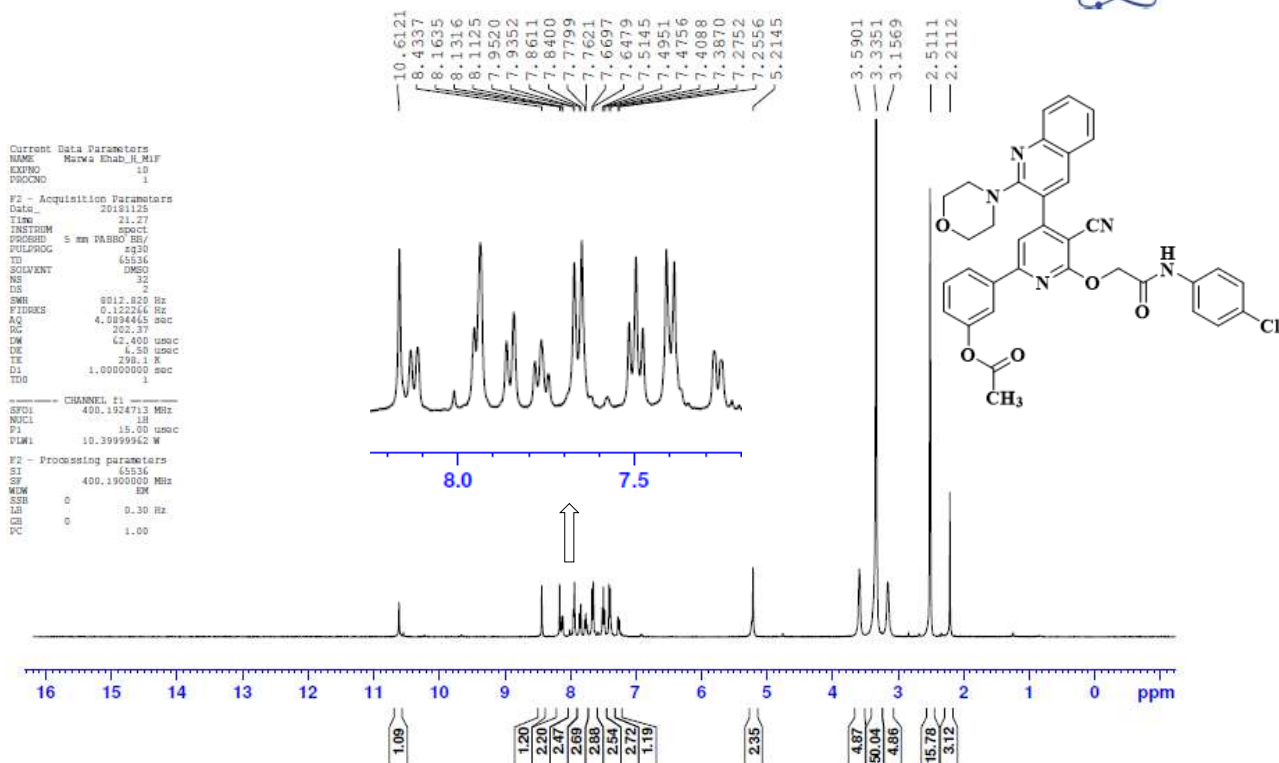
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 SOLVENT DMSO
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 RG 197.77
 DW 62.400 usec
 DE 6.50 usec
 TE 292.3 K
 D1 1.00000000 sec
 SFO1 400.2024113 MHz
 NUC1 1H
 P1 13.00 usec
 PL1 13.00000000 W

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¹H-NMR spectra of compound 9c

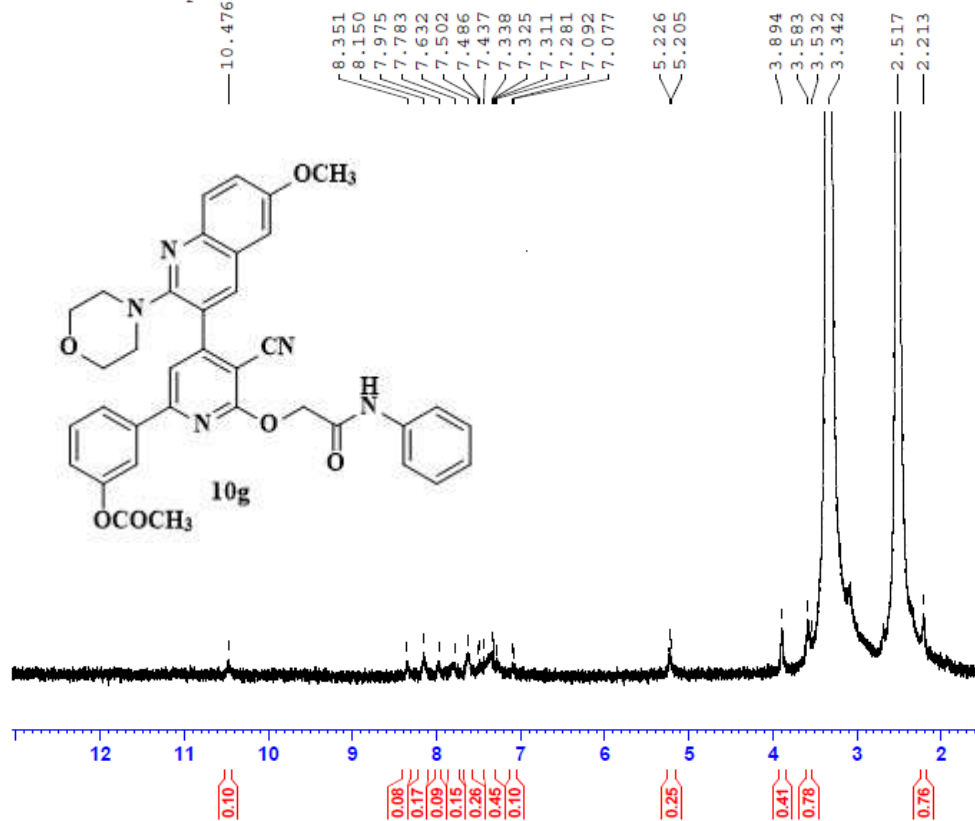
Marwa Ehab_H_MIF

Microanalytical Unit - FOPCU - NMR laboratory
www.pharma.cu.edu.eg dir-mau.fopcu@pharma.cu.edu.eg



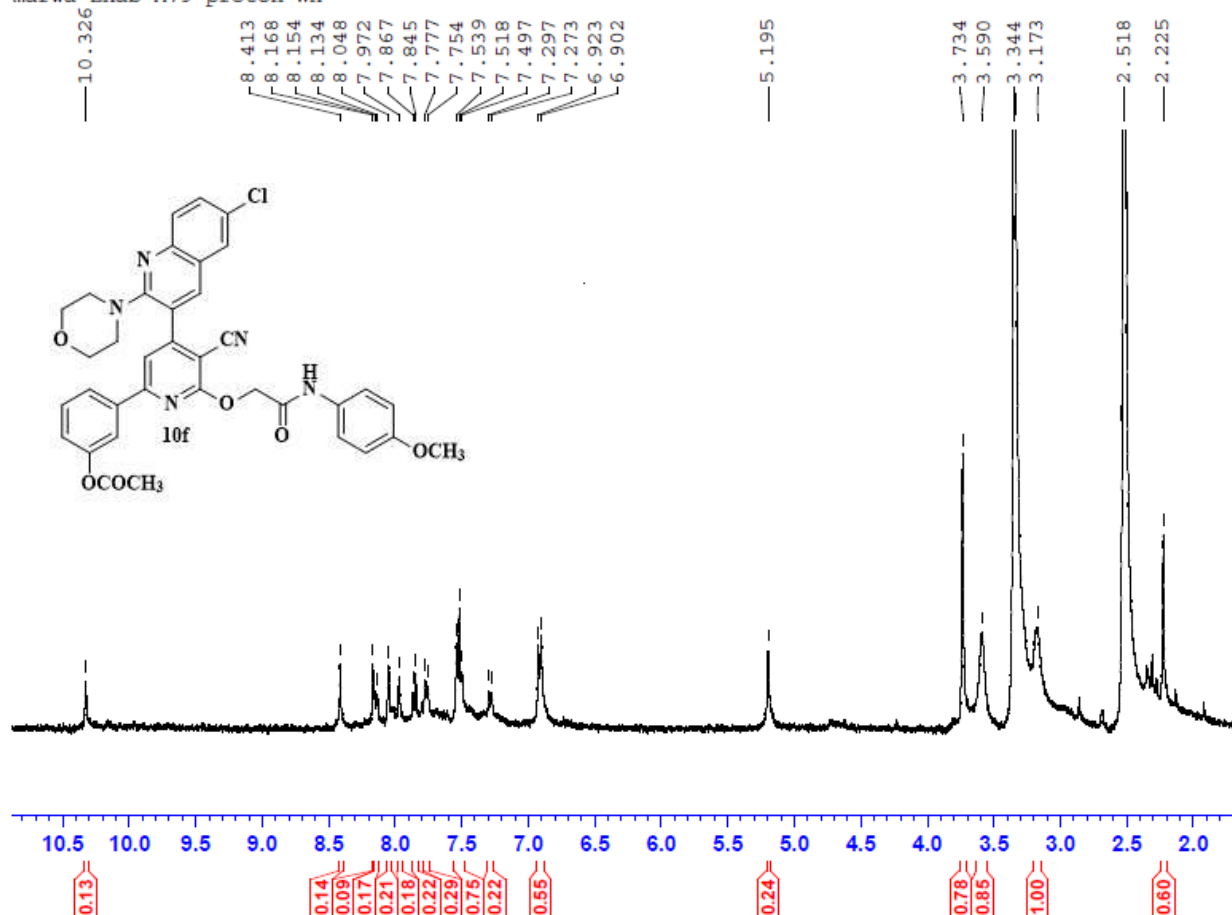
¹H-NMR spectra of compound 10b

marwa Ehab-M4h-proton-WH



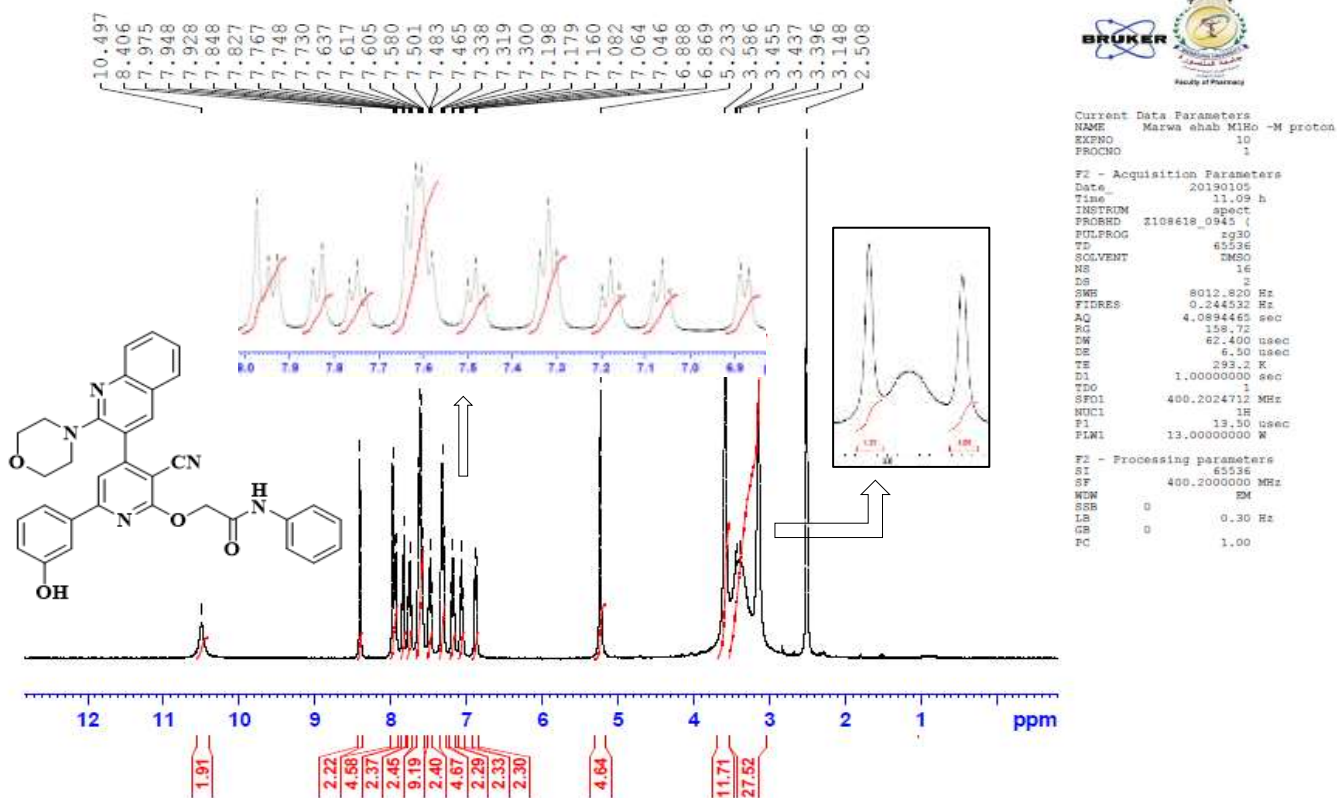
H^1 -NMR spectra of compound 10g

marwa Ehab-M79-proton-WH



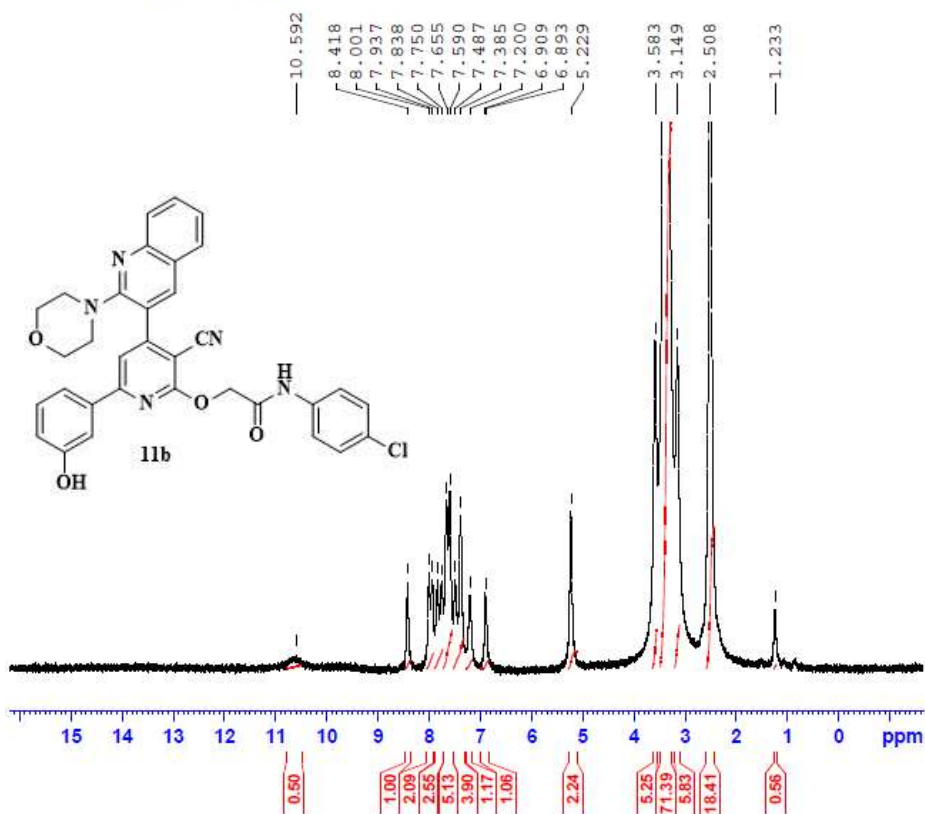
H¹-NMR spectra of compound 10f

Marwa ehab MIHo -M proton



H¹-NMR spectra of compound 11a

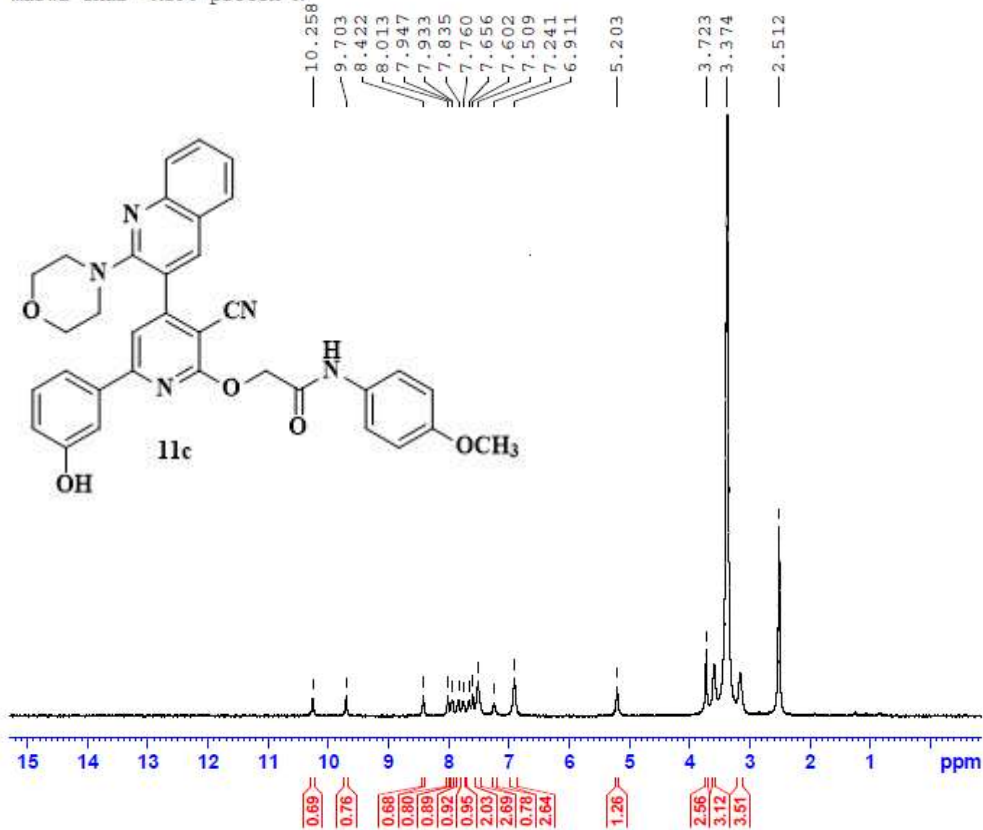
Mrwa ehab -Mifo-DMSO-W-proton



Current Data Parameters
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PROCNO 1
F2 - Acquisition Parameters
Date 20181212
Time 12.51 h
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PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244512 Hz
AQ 4.8894465 sec
RG 197.77
DM 62.400 usec
DE 6.50 usec
TE 292.3 K
D8 1.00000000 sec
TDC 1
SFO1 400.2024712 MHz
NUC1 1H
PT 13.50 usec
PL1 13.00000000 W
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CF 400.2000000 MHz
KEW 0
CGB 0
IE 0 0.10 Hz
GB 0
PC 1.00

^1H -NMR spectra of compound 11b

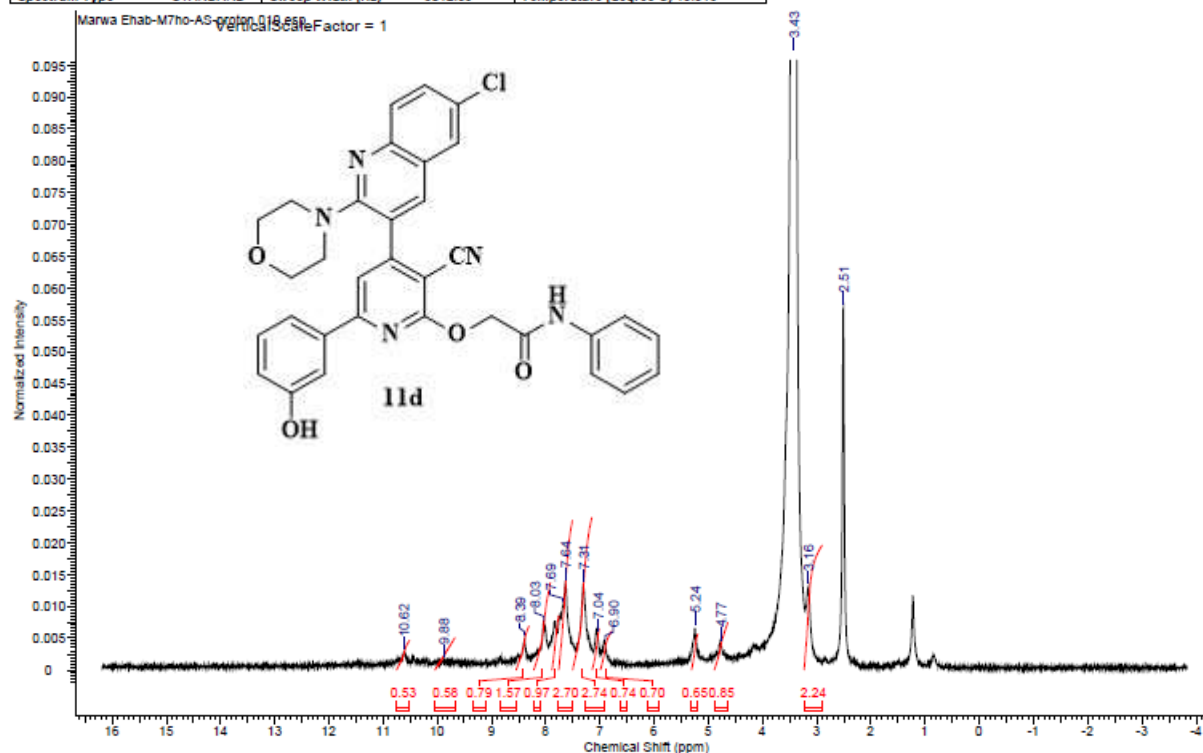
marwa ehab -M190-proton-R



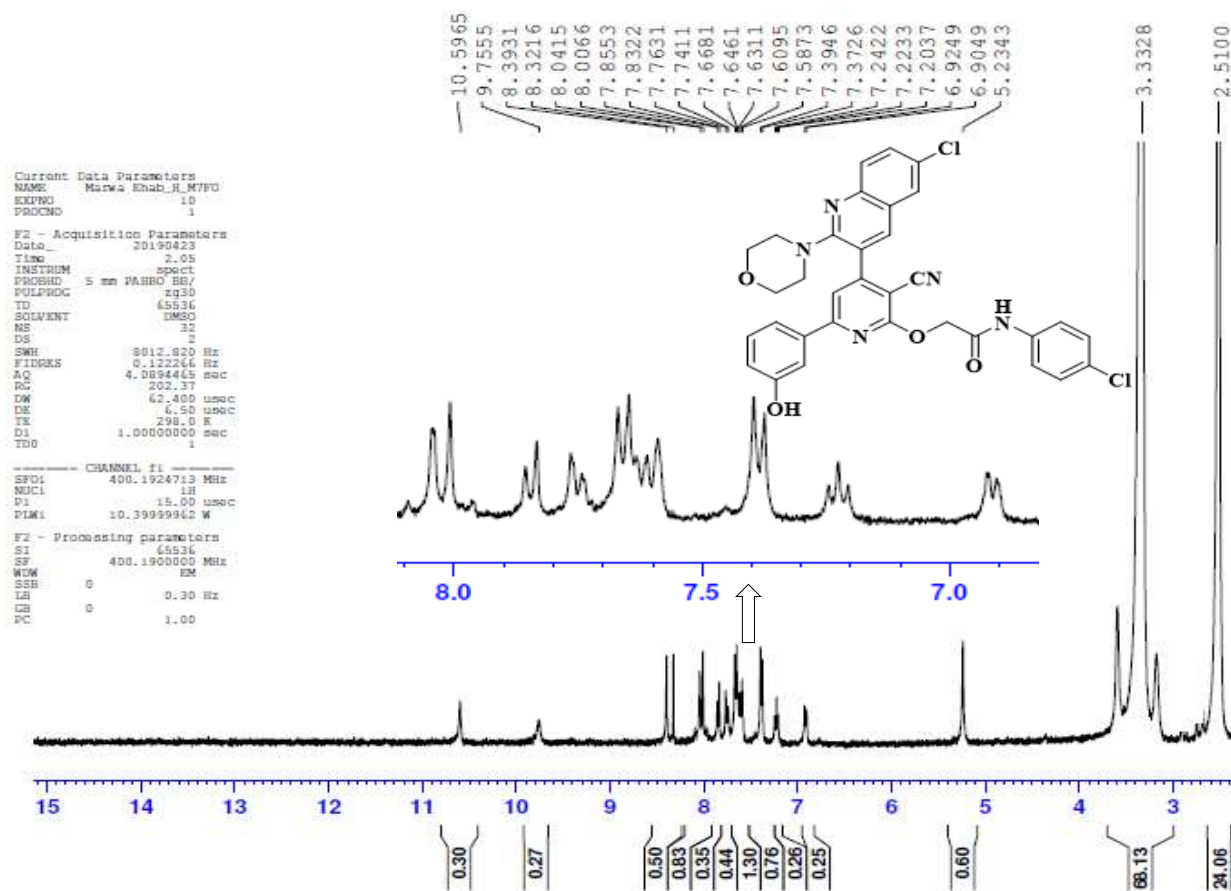
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PROCNO 1
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Time 11.48 h
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PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 4.0894465 sec
RG 197.77
DW 62.400 usec
DE 6.50 usec
TE 292.8 K
D1 1.00000000 sec
TEO 1
SFO1 400.2024712 MHz
NUC1 1H
P1 13.50 usec
PLW1 13.00000000 W
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SF 400.2000000 MHz
WVW RM
SFB 0
LB 0.30 Hz
GB 0
PC 1.00

H¹-NMR spectra of compound 11c

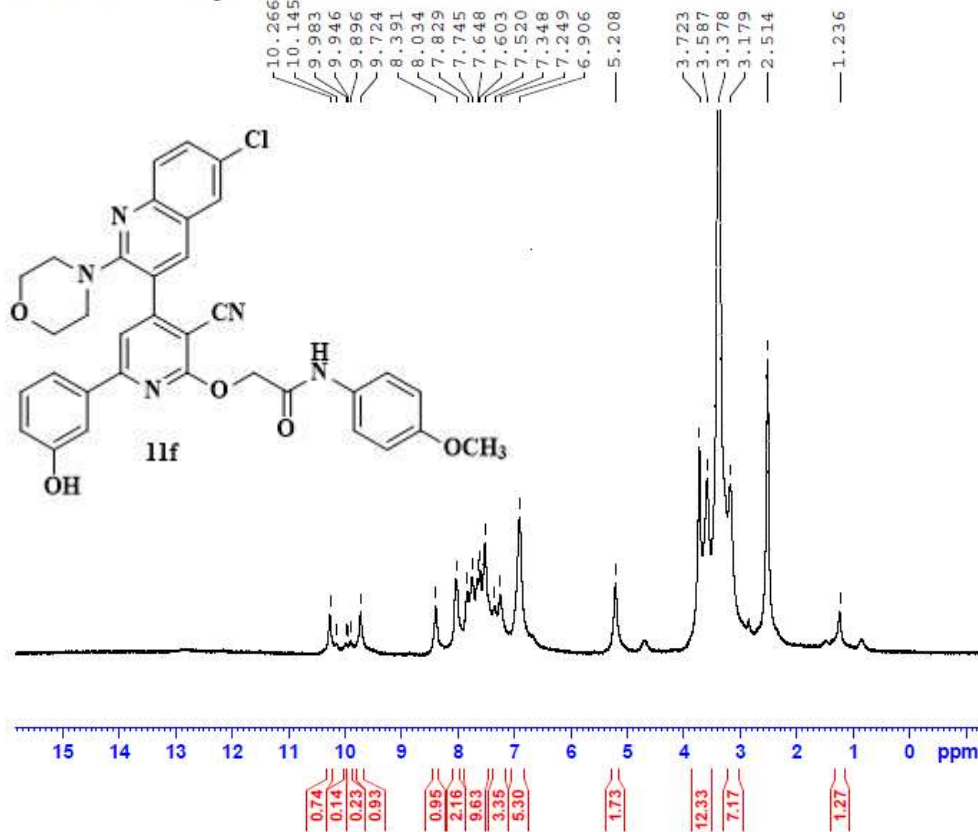
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Original Points Count	32768	Owner	nmr	Points Count	32768
Receiver Gain	112.56	SW(cyclical) (Hz)	8012.82	Solvent	DMSO-d6
Spectrum Type	STANDARD	Sweep Width (Hz)	8012.58	Temperature (degree C)	19.913
				Spectrum Offset (Hz)	2471.2351



¹H-NMR spectra of compound 11d

H¹-NMR spectra of compound 11e

marwa ehab -M790-proton-R



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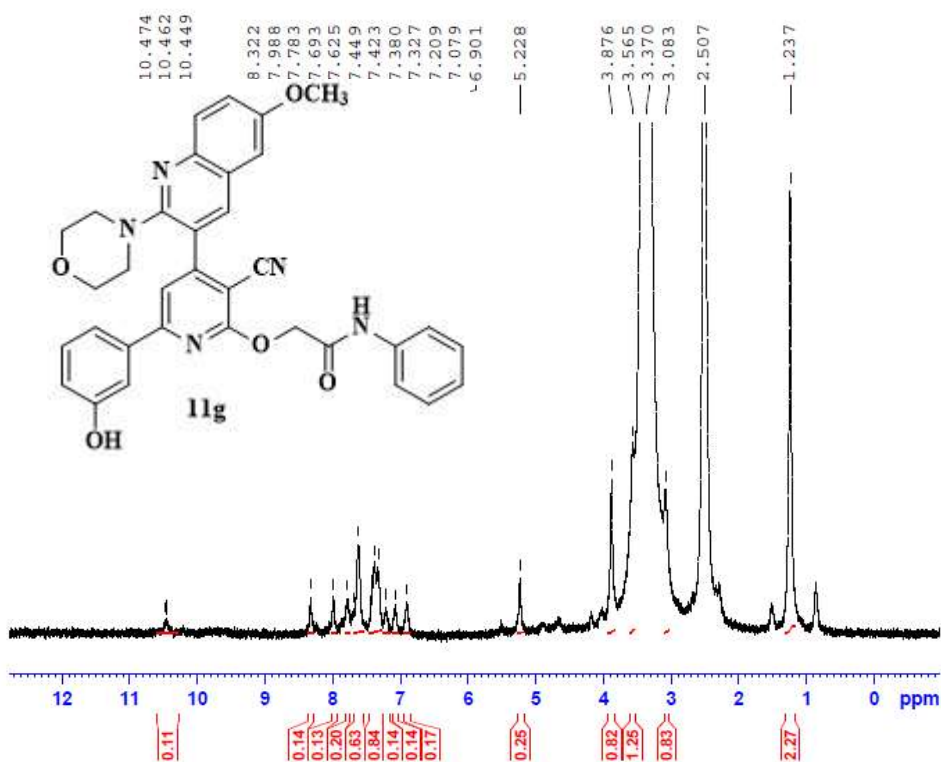
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 PROCNO 1

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 PULPROG zg30
 TD 65536
 SOLVENT DMSO
 NS 16
 DS 2
 SWH 8012.820 Hz
 FIDRES 0.244532 Hz
 AQ 4.0894465 sec
 RG 158.72
 EC 158.72
 DW 62.400 usec
 DE 6.50 usec
 TE 293.1 K
 D1 1.00000000 sec
 TDO 1
 SFO1 400.2024712 MHz
 NUC1 1H
 P1 13.50 usec
 PLW1 13.00000000 W

F2 - Processing parameters
 SI 65536
 SF 400.2000000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

H¹-NMR spectra of compound 11f

Marwa ehab M4Ho -M proton



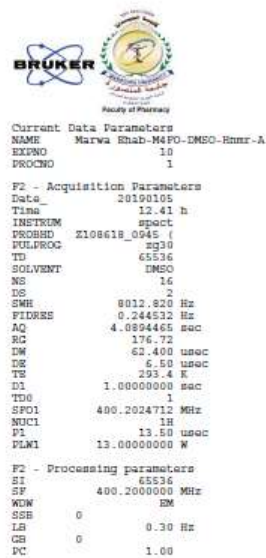
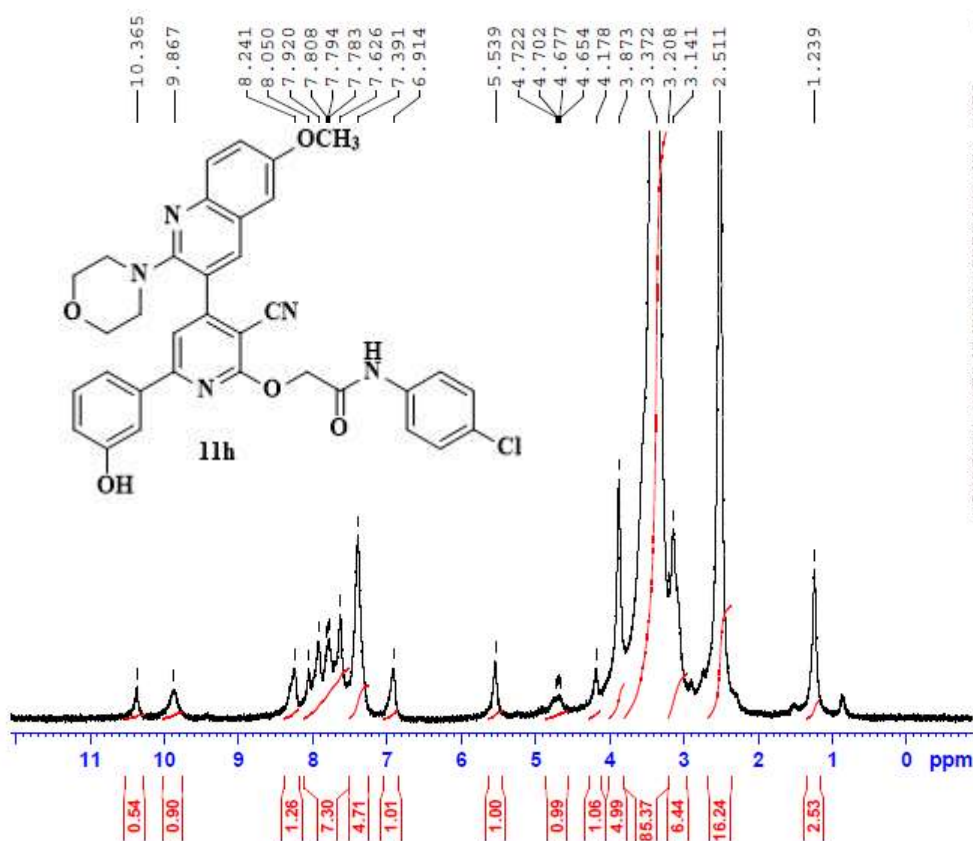
BRUKER
 Faculty of Pharmacy

Current Data Parameters
 NAME Marwa ehab M4Ho -M proton
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 PROCNO 1

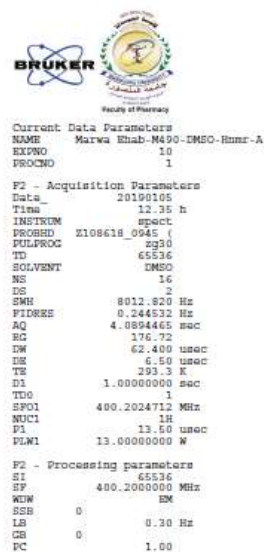
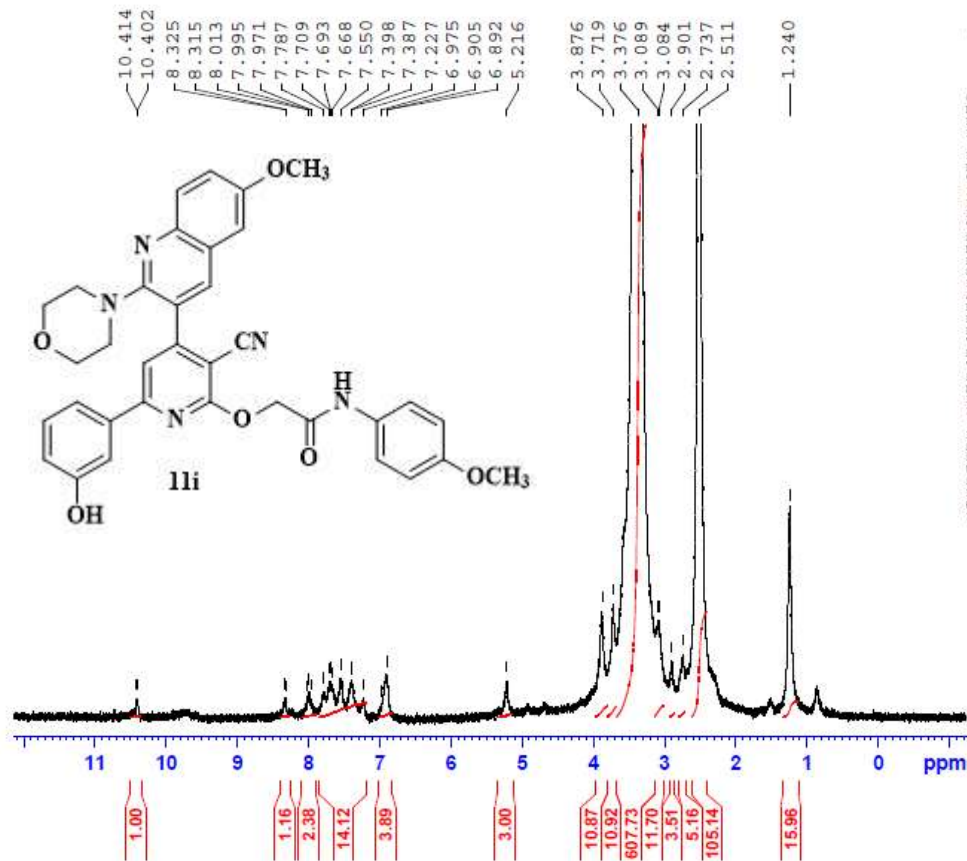
F2 - Acquisition Parameters
 Date 20190105
 Time 11.05 h
 INSTRUM spect
 PROBRD Z108618_0945 (
 PULPROG zg30
 TD 65536
 SOLVENT DMSO
 NS 16
 DS 2
 SWH 8012.820 Hz
 FIDRES 0.244532 Hz
 AQ 4.0894465 sec
 RG 176.72
 EC 176.72
 DW 62.400 usec
 DE 6.50 usec
 TE 293.4 K
 D1 1.00000000 sec
 TDO 1
 SFO1 400.2024712 MHz
 NUC1 1H
 P1 13.50 usec
 PLW1 13.00000000 W

F2 - Processing parameters
 SI 65536
 SF 400.2000000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

H¹-NMR spectra of compound 11g

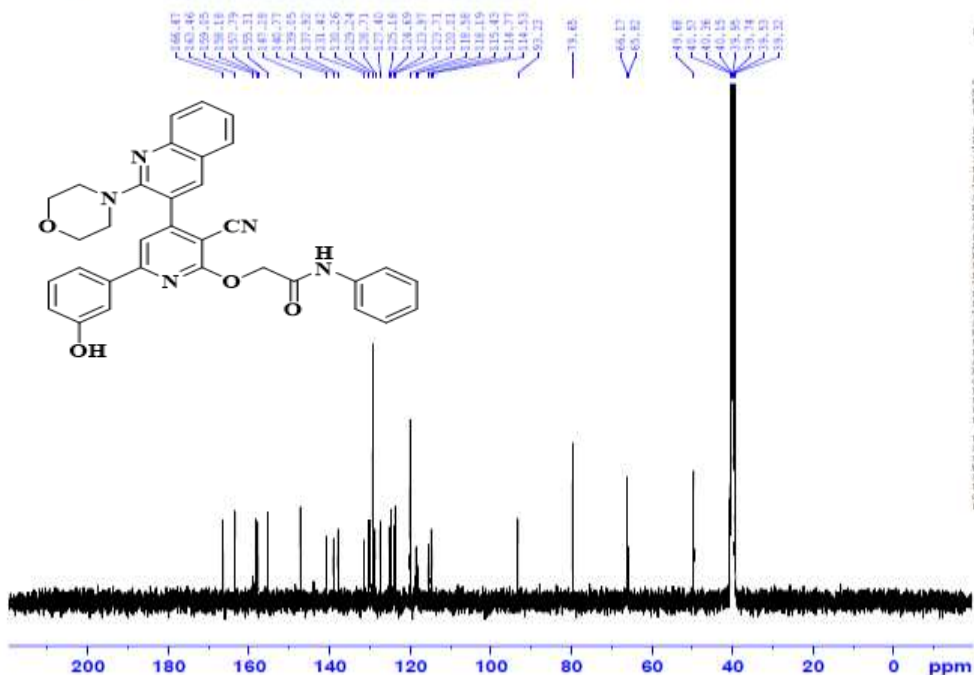


H¹-NMR spectra of compound 11h



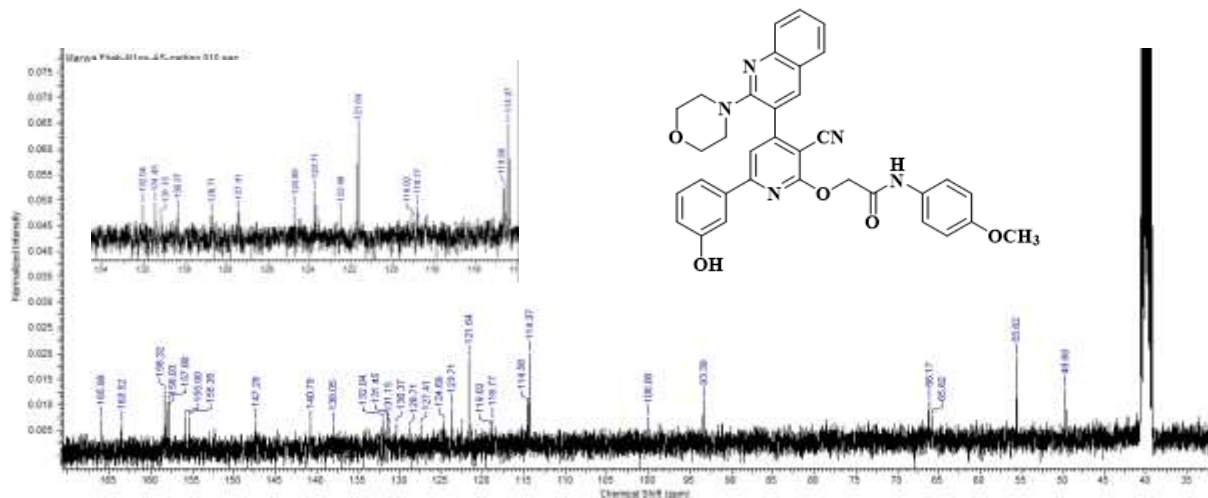
H¹-NMR spectra of compound 11i

Marwa ehab Miho -M carbon



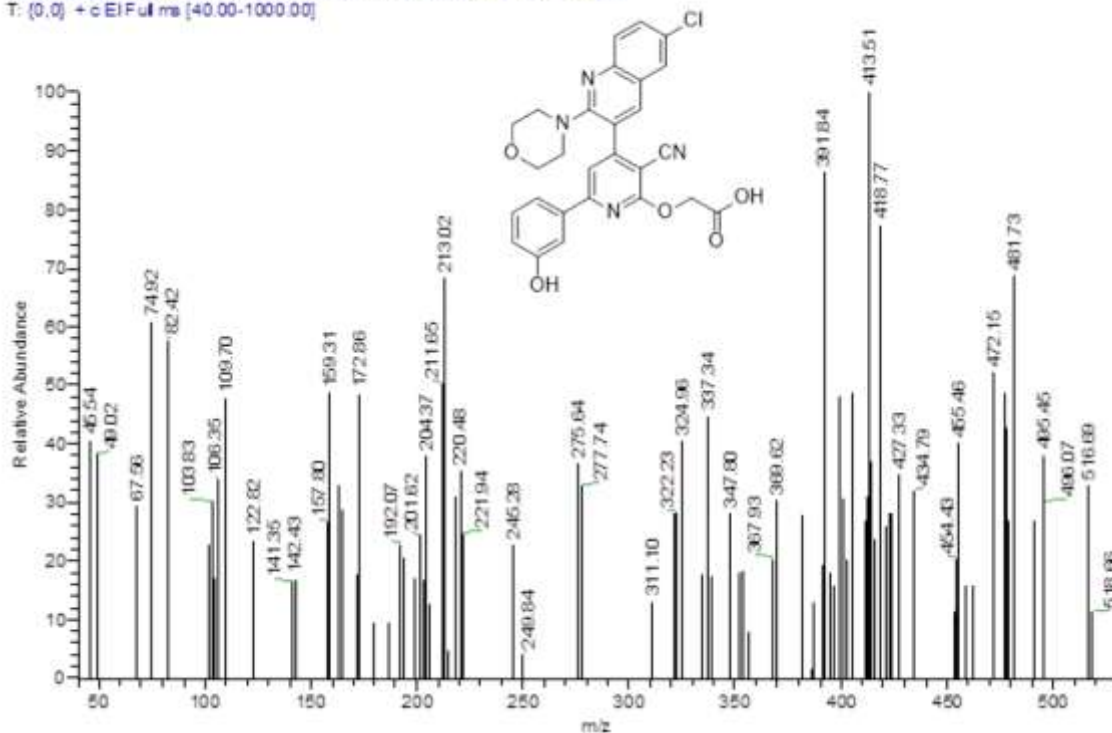
Current Data Parameters
 NAME Marwa ehab Miho -M carbon
 EXPNO 10
 PROCNO 1
 F2 - Acquisition Parameters
 Date_ 20190109
 Time 11:27 A
 INSTRUM spect
 PROBRD siusw18 0945 J
 PULPROG zgpg30
 TD 65536
 SCLVNT DMSO
 NS 1024
 DS 4
 SWH 24038.461 Mc
 FIDRES 0.732598 Hz
 AQ 1.5631488 sec
 RG 197.77
 OW 20.000 usec
 DE 4.50 usec
 TE 303.2 K
 D1 2.0000000 sec
 D11 0.0300000 sec
 TDC 1
 SFO1 100.6404231 MHz
 NUC1 13C
 P1 10.00 usec
 PLW1 47.0000000 W
 SFO2 400.2014000 MHz
 NUC2 1H
 CDEPRG12 waltz16
 PCPD2 90.00 usec
 PLW2 13.0000000 W
 PLW3 0.2924999 W
 PLW4 0.14713000 W
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 SF 100.6203700 MHz
 WDW EM
 SSB 0
 LB 1.00 Hz
 GB 0
 EC 1.40

¹³C-NMR spectra of compound 11a



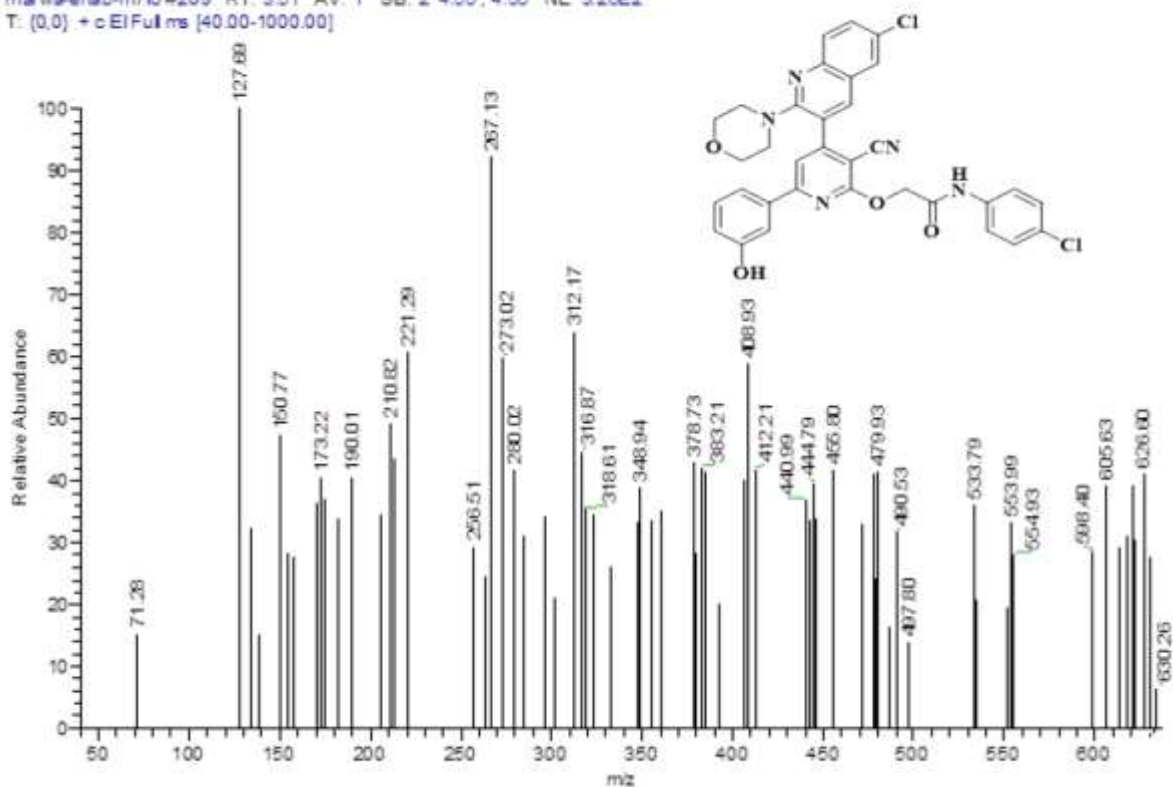
¹³C-NMR spectra of compound 11c

marwa-ehab-m7b #133 RT: 2.24 AV: 1 SB: 2 4.66, 4.69 NL: 3.61E2
T: (0,0) +c EI Full ms [40.00-1000.00]



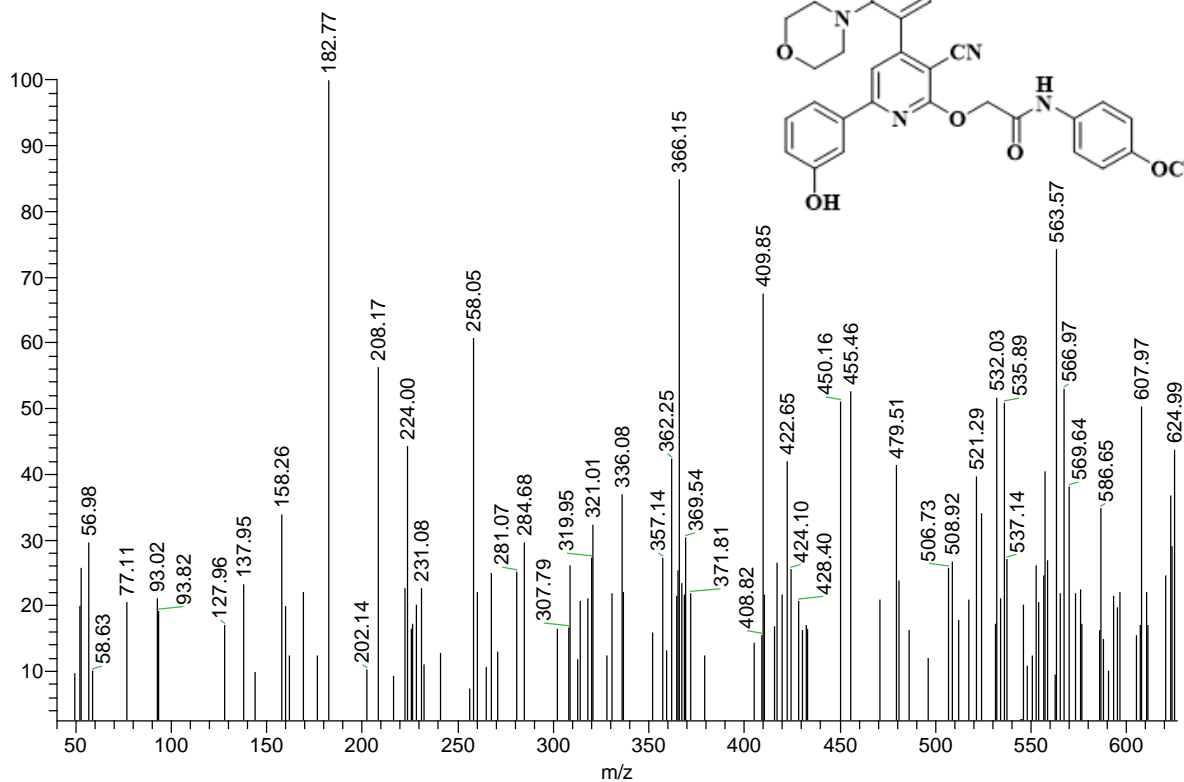
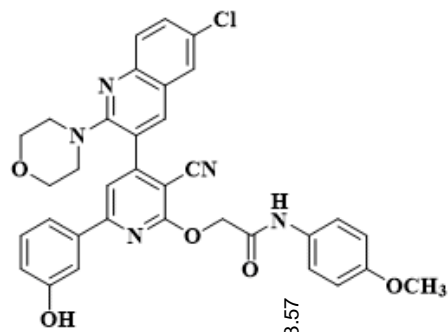
Mass spectrum for compound 9a

marwa-ehab-m7b #209 RT: 3.51 AV: 1 SB: 2 4.66, 4.66 NL: 3.28E2
T: (0,0) +c EI Full ms [40.00-1000.00]



Mass spectrum for compound 11e

marwa-ehab-m790 #220 RT: 3.70 AV: 1 SB: 2 4.55, 4.55 NL: 4.51E2
T: {0,0} + c EI Full ms [40.00-1000.00]



Mass spectrum for compound 11f



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 cN	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

M. Ehab



DIRECTOR

H. Shalaby

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:

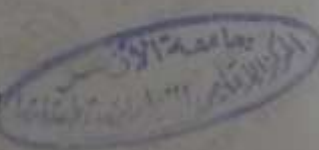
Twenty samples had been submitted for elemental analysis.

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

INVESTIGATOR

M. Elaissi

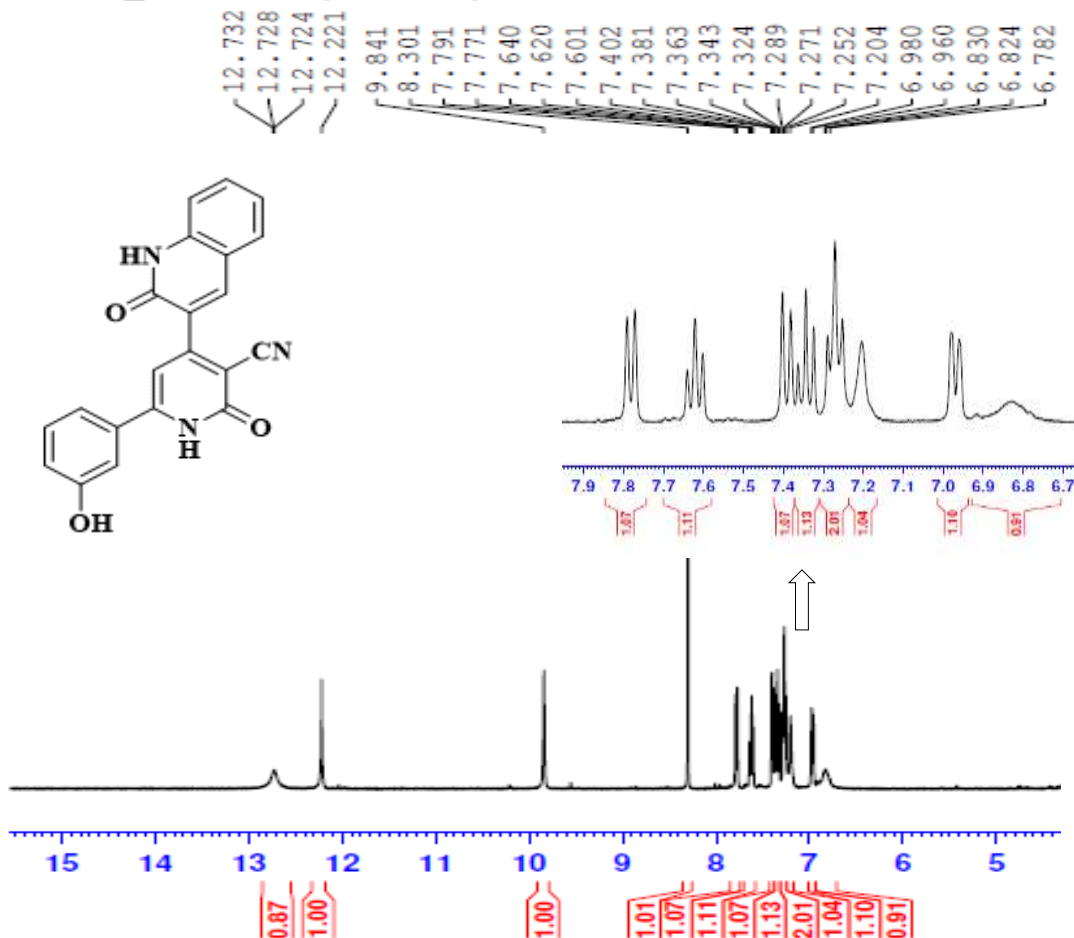


DIRECTOR

G. Sherb

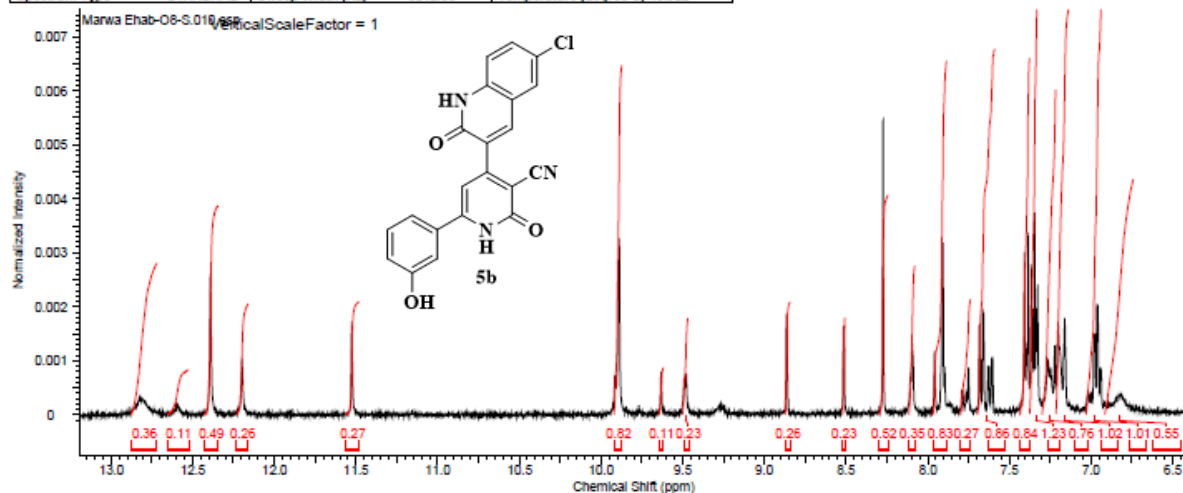
Scheme 3:

MARWA EHAB-O2
 PROTON_BSU DMSO {C:\data} aber 6

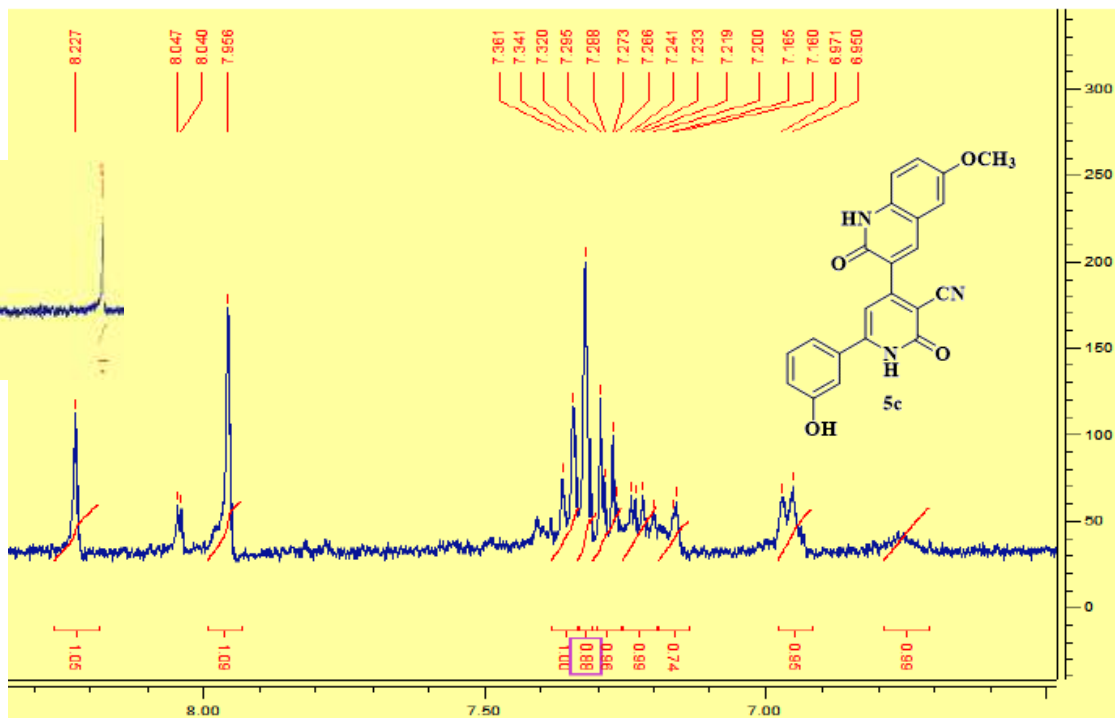


¹H-NMR spectra of compound 5a

Acquisition Time (sec)	Comment	Marwa Ehab-O8-S-proton	Date
4.0894			21 Oct 2018 11:44:32
Date Stamp	21 Oct 2018 11:44:32	File Name	D:\PhD\NMR\Scheme 2\Marwa Ehab-O8-S\10.fid
Frequency (MHz)	400.20	Nucleus	¹ H
Original Points Count	32768	Owner	nmr
Receiver Gain	175.72	Number of Transients	32
Spectrum Type	STANDARD	Points Count	32768
		Solvent	DMSO-d6
		Temperature (degree C)	18.922
		Spectrum Offset (Hz)	2471.2351

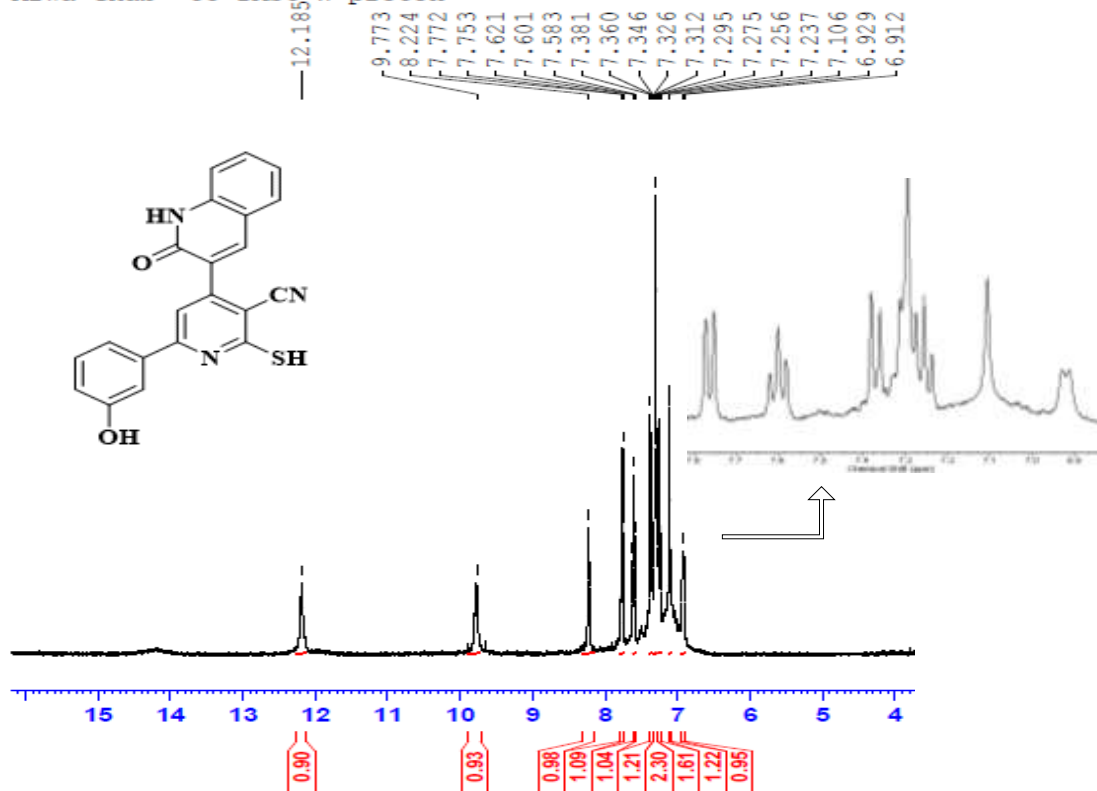


¹H-NMR spectra of compound 5b



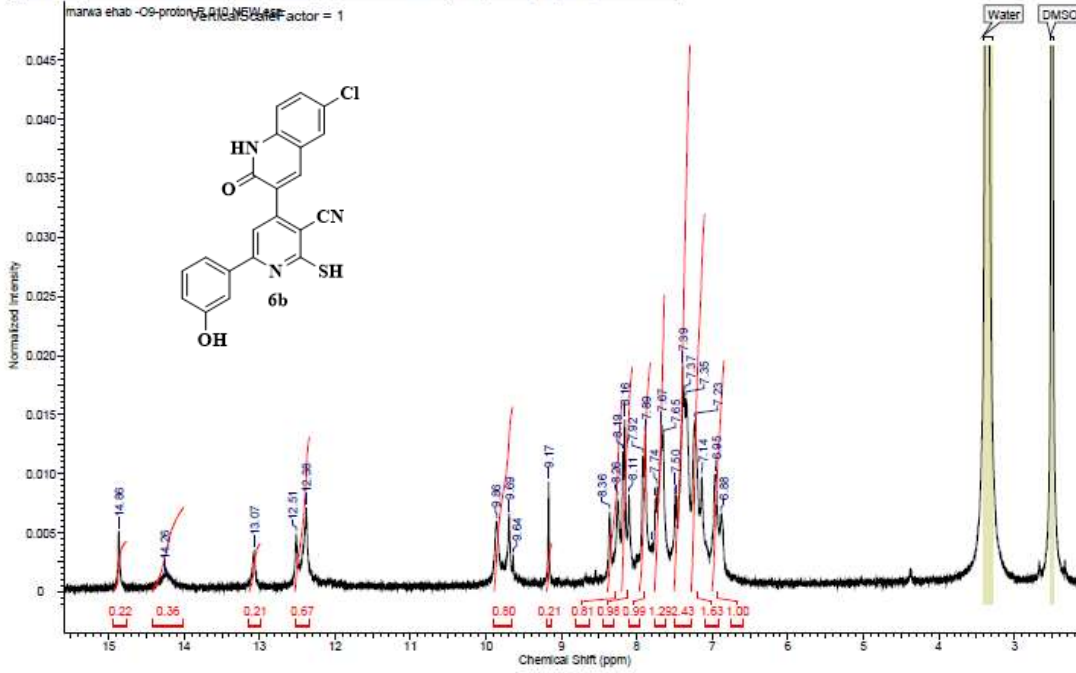
H¹-NMR spectra of compound 5c

Mrwa ehab -03-DMSO-W-proton



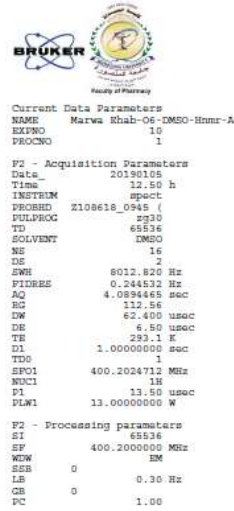
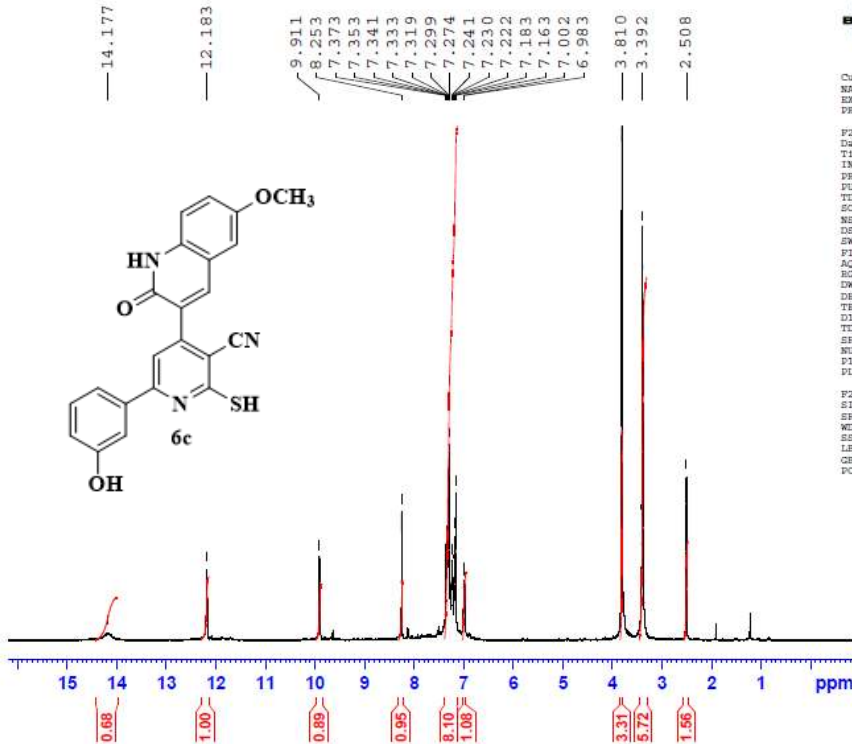
H¹-NMR spectra of compound 6a

Acquisition Time (sec)	4.0894	Comment	marwa ehav -O9-proton-R	Date	30 Jul 2019 11:33:52
Date Stamp	30 Jul 2019 11:33:52	File Name	D:\PhD\NMR\Scheme 2\marwa ehav -O9-proton-R10.fid	Origin	spect
Frequency (MHz)	400.20	Nucleus	¹ H	Number of Transients	16
Original Points Count	32768	Owner	nmr	Points Count	32768
Receiver Gain	197.77	SW(cyclical) (Hz)	8012.82	Solvent	DMSO-d6
Spectrum Type	STANDARD	Sweep Width (Hz)	8012.58	Temperature (degree C)	19.723
				Pulse Sequence	zg30
				Spectrum Offset (Hz)	2471.2351



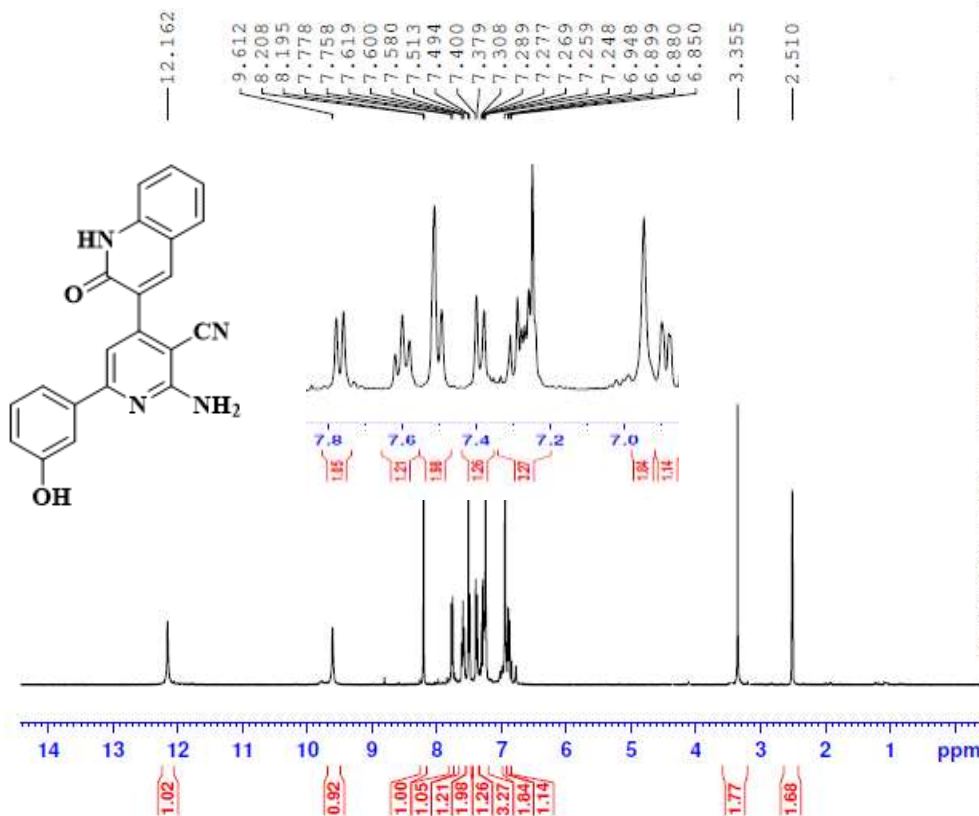
¹H-NMR spectra of compound 6b

Marwa Ehab-O6-DMSO-Hnmr-A



¹H-NMR spectra of compound 6c

EX-MARWA-01
 PROTON_BSU DMSO {C:\data} nmr 13



Current Data Parameters
 NAME Dec24-2017-nmr
 EXPNO 50
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20171224
 Time 10.16
 INSTRUM spect
 PROBHD 5 mm PABBO BH/
 PULPROG zg30
 TD 65536
 SOLVENT DMSO
 NS 16
 DS 2
 SWH 8012.820 Hz
 FIDRES 0.122266 Hz
 AQ 4.0894465 sec
 RG 147.93
 DW 62.400 usec
 DE 6.50 usec
 TE 298.1 K
 D1 1.00000000 sec
 TDO 1

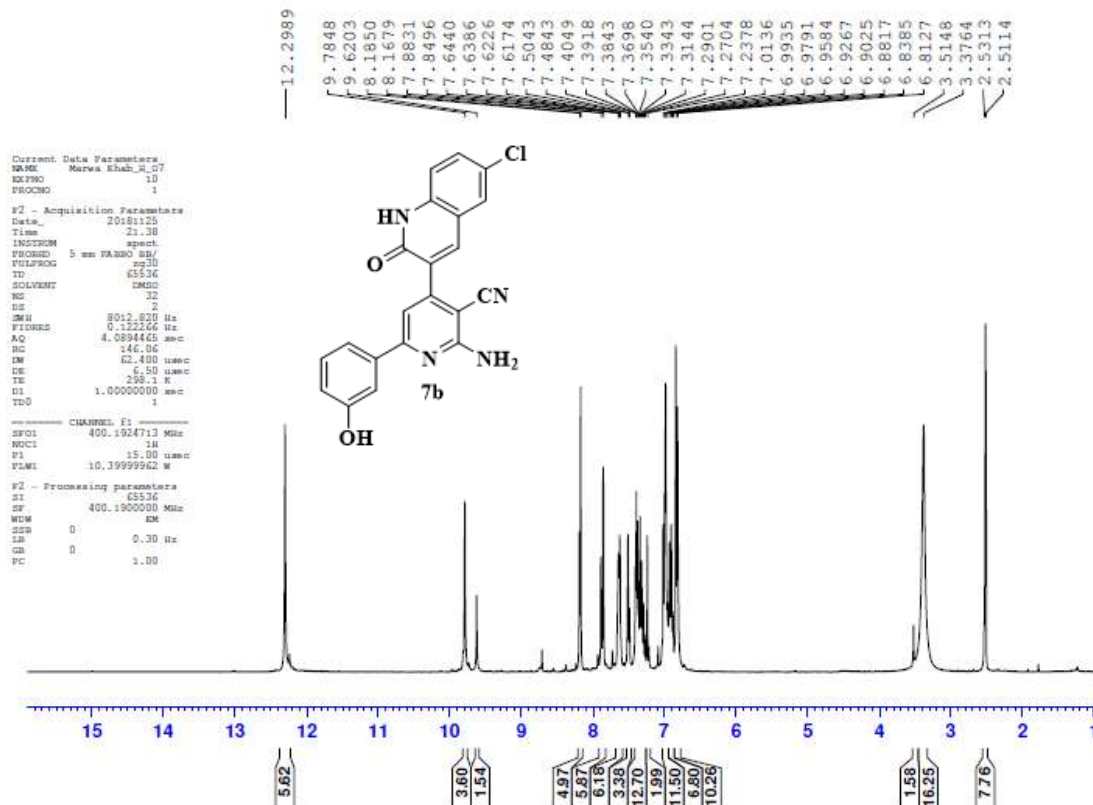
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 NUC1 1H
 P1 10.00 usec
 PLW1 16.00000000 W

F2 - Processing parameters
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 SF 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.10 Hz
 GB 0
 PC 1.00

H¹-NMR spectra of compound 7a

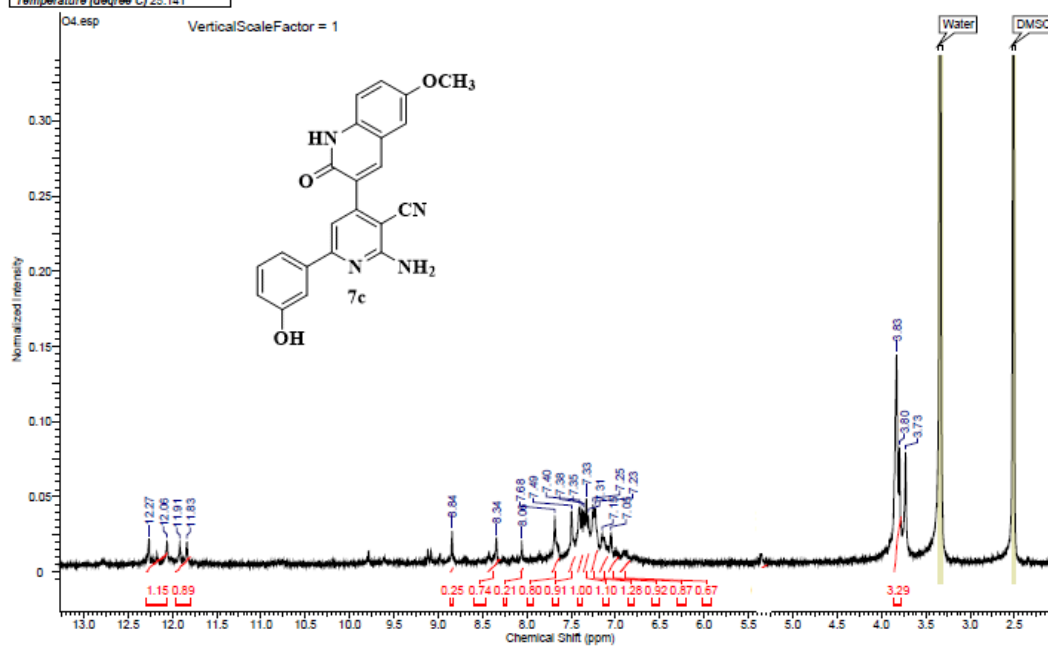
Marwa Ehab_H_07

Microanalytical Unit - FOPCU - NMR laboratory
 www.pharma.cu.edu.eg dir-mau.fopcu@pharma.cu.edu.eg

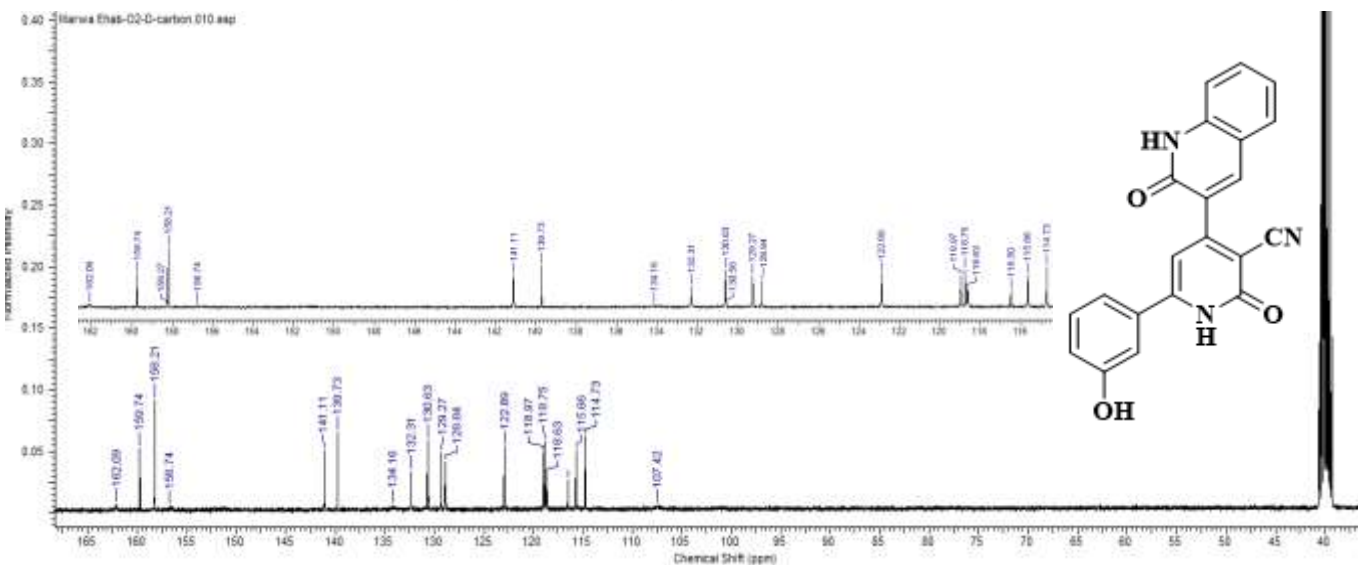


H¹-NMR spectra of compound 7b

Acquisition Time (sec)	4.0894	Comment	EX-MARVAEHAS-04 PROTON BSU DMSO (C:\data) aber 11		
Date	14 Aug 2018 10:27:44	Date Stamp	14 Aug 2018 10:27:44		
File Name	D:\PHD\NMR\Scheme 2\04\fid	Frequency (MHz)	400.13	Nucleus	¹ H
Number of Transients	16	Origin	spec1	Original Points Count	32768
Points Count	32768	Pulse Sequence	zg30	Receiver Gain	147.83
Solvent	DMSO-d6	Spectrum Offset (Hz)	2470.9668	Sweep Width (Hz)	8012.58
Temperature (degree C)	25.141	Spectrum Type	STANDARD		

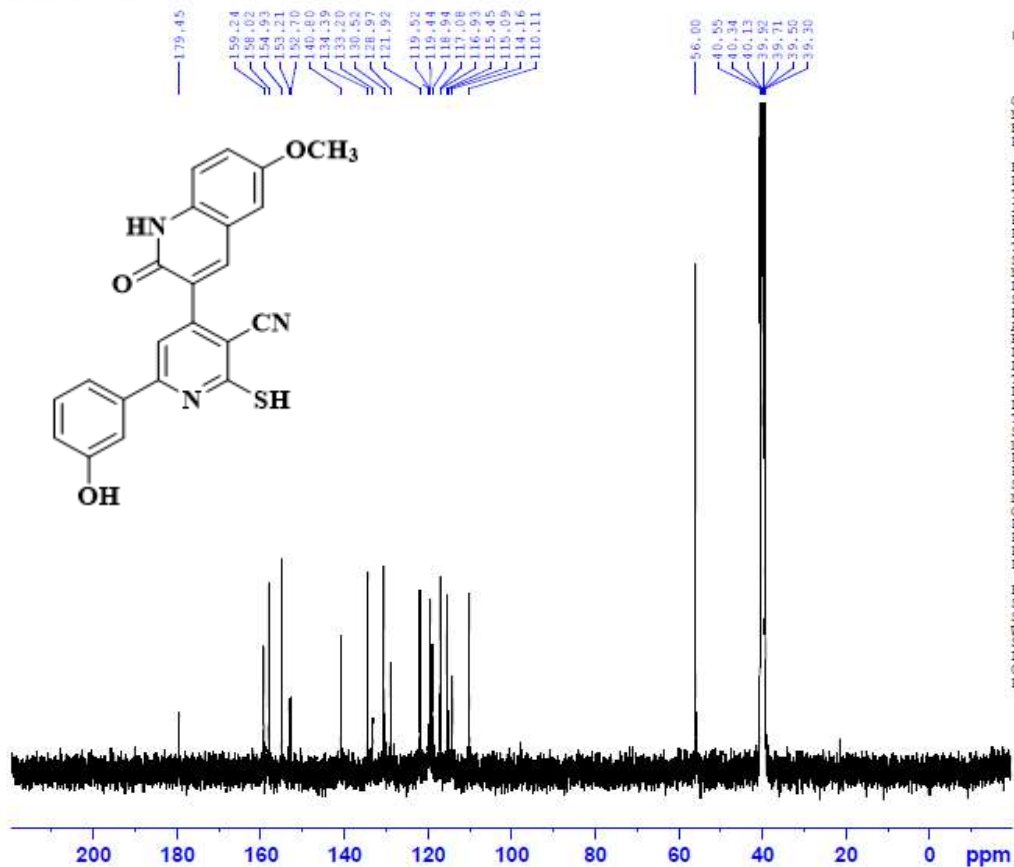


¹H-NMR spectra of compound 7c



¹³C-NMR spectra of compound 5a

Marwa ehab O6 -M carbon



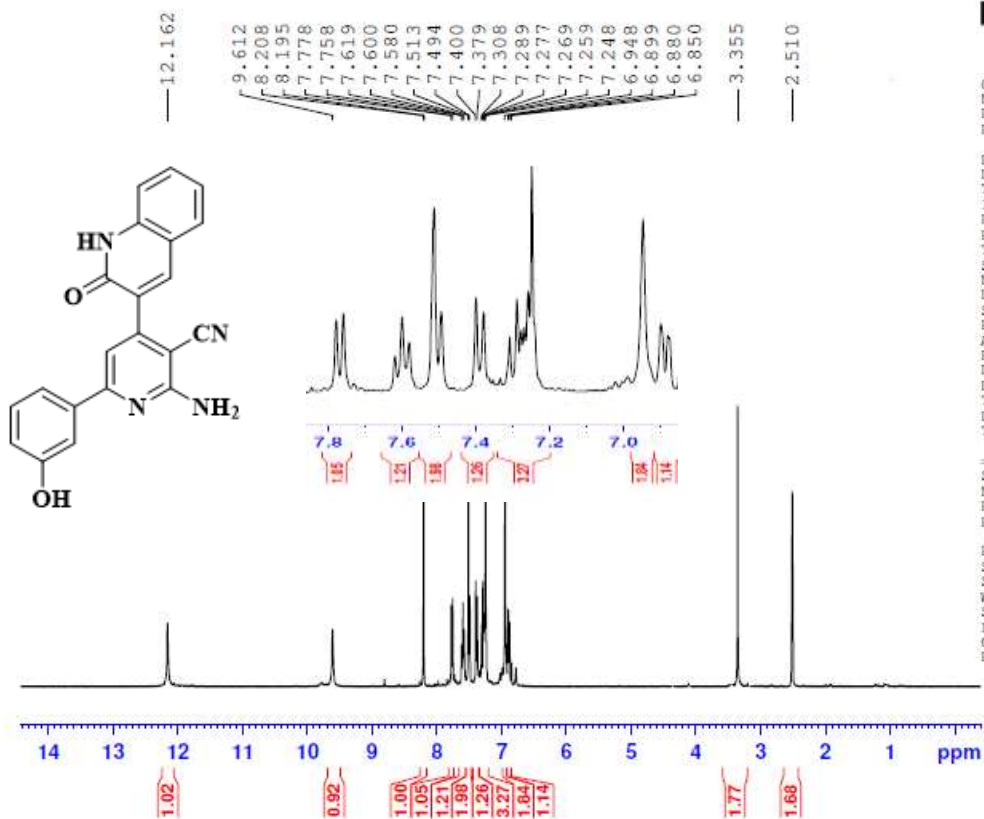
Current Data Parameters
 NAME Marwa ehab O6 -M carbon
 EXPNO 10
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20190109
 Time 14:33 h
 INSTRUM spect
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 PULPROG zgpg30
 TD 65536
 SOLVENT DMSO
 NS 1024
 DS 4
 SWH 24098.461 Hz
 FIDRES 0.733596 Hz
 AQ 1.3621488 sec
 RG 197.77
 DW 20.800 usec
 DE 6.50 usec
 TE 299.2 K
 DL 2.00000000 sec
 DLI 0.03000000 sec
 TDO 1
 SFO1 100.6404331 MHz
 NUC1 13C
 P1 10.00 usec
 PLW1 47.00000000 W
 SFO2 400.2016008 MHz
 NUC2 1H
 CPDPRG12 waltz16
 PCPD2 90.00 usec
 PLW2 13.00000000 W
 PLW12 0.29249999 W
 PLW13 0.14713000 W

F2 - Processing parameters
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 SF 100.6202700 MHz
 WDW EM
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.40

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

EX-MARWA-01
 PROTON_BSU DMSO {C:\data} nmr 13



Current Data Parameters
 NAME Dec24-2017-nmr
 EXPNO 50
 PROCNO 1

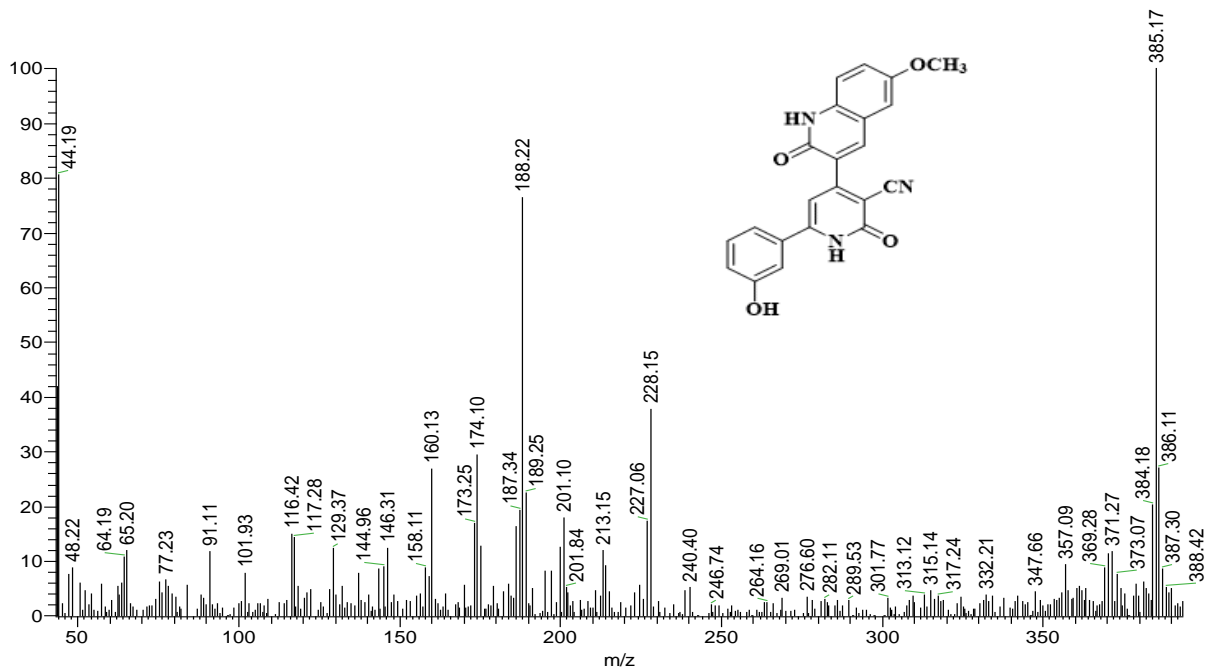
F2 - Acquisition Parameters
 Date_ 20171224
 Time 10.16
 INSTRUM spect
 PROBHD 5 mm PABBO BB/
 PULPROG zg30
 TD 65536
 SOLVENT DMSO
 NS 16
 DS 2
 SWH 8012.820 Hz
 FIDRES 0.122266 Hz
 AQ 4.0994465 sec
 RG 147.93
 DW 62.400 usec
 DE 6.50 usec
 TE 298.1 K
 D1 1.0000000 sec
 TD0 1

CHANNEL f1
 SFO1 400.1324710 MHz
 NUC1 1H
 P1 10.00 usec
 PLW1 16.00000000 W

F2 - Processing parameters
 SI 65536
 SF 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.10 Hz
 CB 0
 PC 1.00

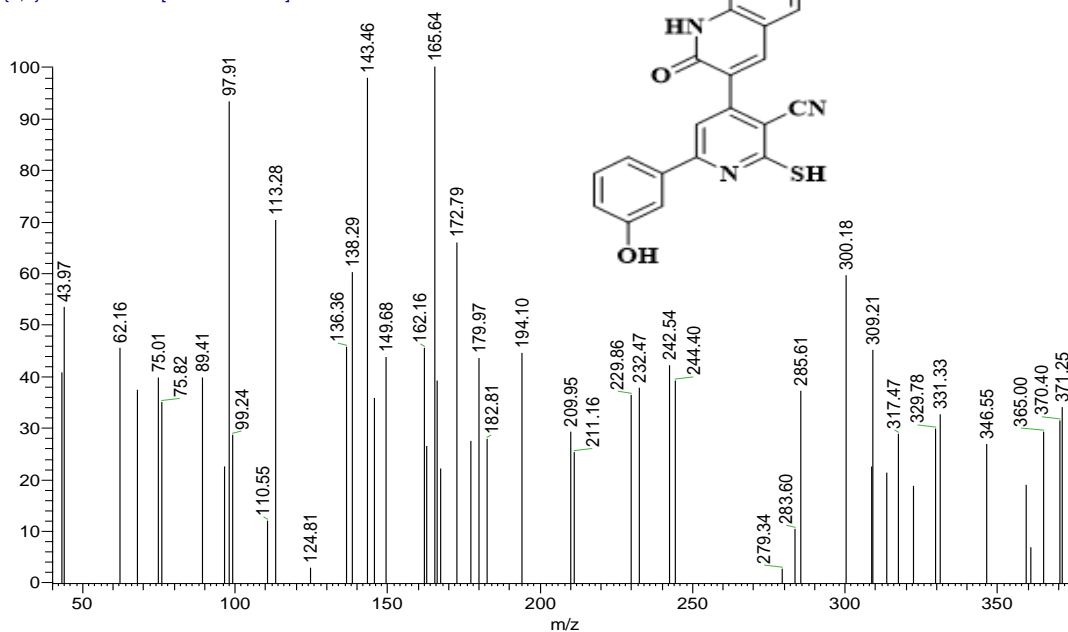
¹³C-NMR spectra of compound 7a

marwa-ehab-o5 #293 RT: 4.92 AV: 1 NL: 1.87E4
 T: (0,0) + c EI Full ms [40.00-1000.00]



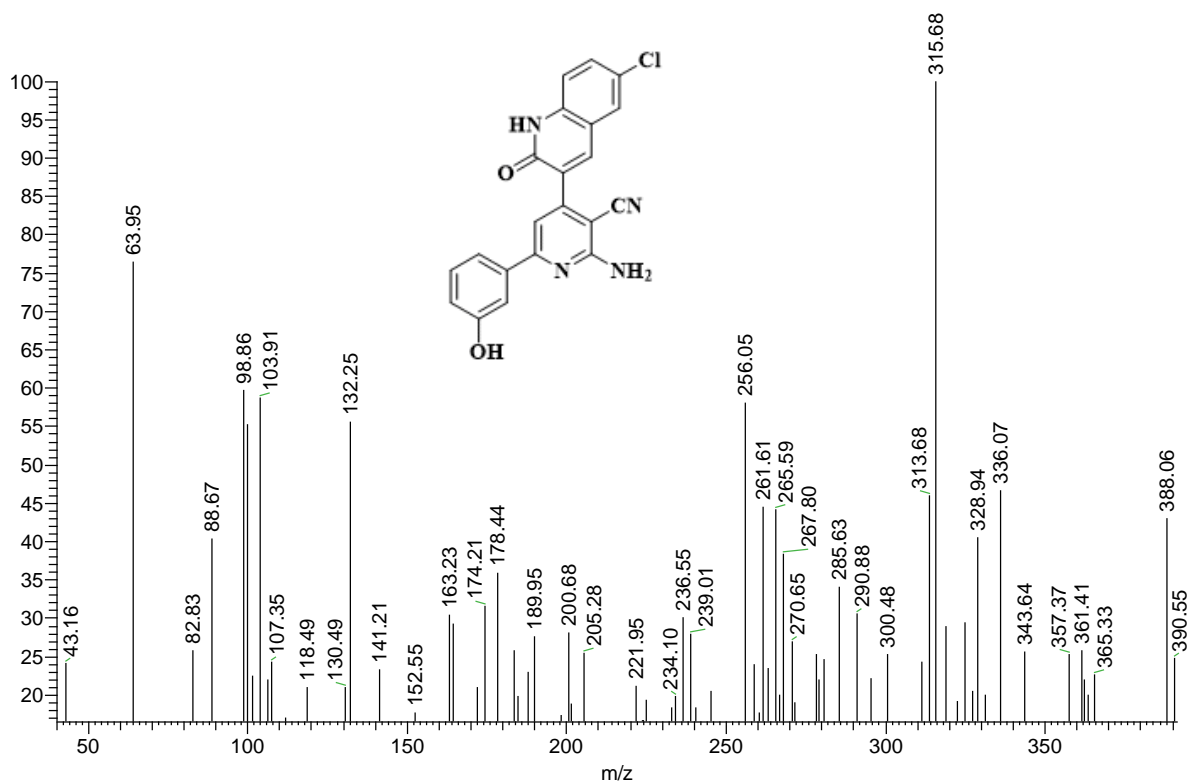
EI/MS spectrum of compound 5c

marwa-ehab-o3 #229 RT: 3.85 AV: 1 SB: 2 4.55 , 4.59 NL: 2.99E2
T: (0,0) + c EI Full ms [40.00-1000.00]



EI/MS spectrum of compound 6a

marwa-ehab-o7 #241 RT: 4.05 AV: 1 SB: 2 4.50 , 4.50 NL: 4.67E2
T: (0,0) + c EI Full ms [40.00-1000.00]



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 ₃	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

INVESTIGATOR

DIRECTOR





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 cN	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 ₂	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

INVESTIGATOR

M. Ehab



DIRECTOR

H. Shab

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:

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

INVESTIGATOR

Mr. Elsayed

DIRECTOR

(Dr. S. El-Sayed)

Table 1: Anticancer activities IC₅₀ (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 (μ M)	EC ₁₀₀ (μ M)	IC ₅₀ (μ M)	SI	IC ₅₀ (μ M)	SI	IC ₅₀ (μ M)	SI	IC ₅₀ (μ M)	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 5×10^3 cells per well in 96-well cell culture plate and incubated at 37°C in 5% CO₂ 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 viability²⁵. Twenty microliters of 5 mg/ml MTT (Sigma, USA) was added to each well and the plate was incubated at 37°C 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 (IC₅₀) and safe dose (EC₁₀₀) 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 (5×10^3 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 37°C in 5% CO₂ incubator. MTT method was done as described above. The Graphpad Instat software was used for calculating the half maximal inhibitory concentration (IC₅₀) 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 IC₅₀ 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 \pm 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 instructions²⁶