

# **Exploration of VEGFR-2 inhibition activity of Phthalazine derivatives: Design, synthesis, cytotoxicity, ADMET, molecular docking and dynamic simulation**

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## 5.1. Biological testing

### 5.1.1. *In vitro* anti-cancer activity

Our derivatives were tested against two cell lines, HepG2 and MCF-7 using MTT colorimetric assay. Cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and 100 $\mu$ g/ml streptomycin at 37 °C in a 5% CO<sub>2</sub> incubator. The cell lines were seeded in a 96-well plate at a density of 1.0 x 10<sup>4</sup> cells / well at 37 °C for 48 h under 5% CO<sub>2</sub>. After incubation the cells were treated with different concentrations of synthesized compounds and incubated for 24 h. After 24 h of drug treatment, 20  $\mu$ l of MTT solution at 5mg/ml was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100  $\mu$ l was added into each well to dissolve the purple formazan formed. The colorimetric assay was measured and recorded at absorbance of 570 nm using a plate reader (EXL 800, USA). The relative cell viability in percentage was calculated as (A<sub>570</sub> of treated samples/A<sub>570</sub> of untreated sample) X 100. Results for IC<sub>50</sub> values of the active compounds are summarized in Table 1 [[35-37](#)].

### 5.1.2. *In vitro* VEGFR-2 assay

The VEGFR-2 kinase activity was measured using an anti-phosphotyrosine antibody with the Alpha Screen system (PerkinElmer, USA) according to manufacturer's instructions [38]. Enzyme reactions were performed in 50 mM Tris-HCl pH 7.5, 5 mM MnCl<sub>2</sub>, 5 mM MgCl<sub>2</sub>, 0.01% Tween-20 and 2 mM DTT, containing 10 μM ATP, 0.1 μg/mL biotinylated poly-GluTyr (4:1) and 0.1 nM of VEGFR-2 (Millipore, UK). Prior to catalytic initiation with ATP, the tested compounds at final concentrations ranging from 0-300 μg/mL and enzyme were incubated for 5 min at room temperature. The reactions were quenched by the addition of 25 μL of 100 mM EDTA, 10 μg/mL Alpha Screen streptavidine donor beads and 10 μg/mL acceptor beads in 62.5 mM HEPES pH 7.4, 250 mM NaCl, and 0.1% BSA. The plate was left to incubate in the dark overnight and then analyzed using an ELISA reader (PerkinElmer, USA). Control reactions were conducted with wells containing substrate and enzyme without compounds, while wells containing biotinylated poly-GluTyr (4:1) and the enzyme without ATP were used as basal controls. The percent inhibition was calculated by comparing the compounds treated to control incubations. The concentration of the test compound causing 50% inhibition (IC<sub>50</sub>) was determined from the concentration-inhibition response curve (triplicate determinations). Sorafenib (Sigma-Aldrich, USA) was used as a standard VEGFR-2 inhibitor for comparison.

## 5.2. Docking studies

VEGFR-2 (PDB ID 4ASD) [40] was used by Molsoft program to carry out all docking studies. Each experiment used the VEGFR-2 receptor complexes with the Ligand downloaded from the Brookhaven Protein Databank. The compounds were drawn as a 3D structure and their energies were minimized. The ligand was extracted from the binding site and the compounds discussed herein were docked into the active site.

## 5.3. Molecular dynamics simulation

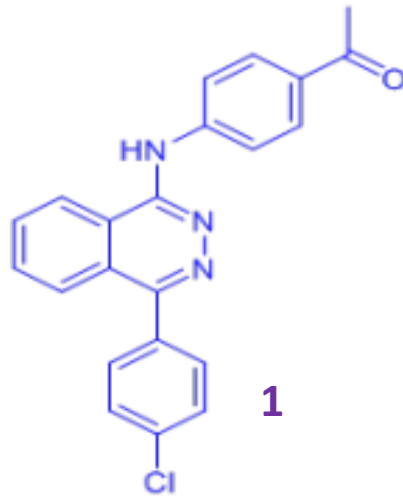
The highly active derivatives **2g**, **3a**, **4a** and **5b** were simulated using molecular dynamics (MD) in VEGFR-2 With the help of GAFF2 [40,41]. The molecular dynamics utilizes Amber's MM/GBSA.py script and the trajectory, the receptor-ligand binding energy was calculated [40]. The force field AMBERff14SB for the protein, ligand force fields were produced [43].

## 5.4. ADMET Profile

*In silico* ADMET profile of the highly active derivatives **2g** and **4a** was predicted using pkCSM descriptor algorithm procedures [44].

10.689  
8.204  
8.186  
8.167  
8.097  
8.077  
8.035  
8.014  
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4.854

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2.512



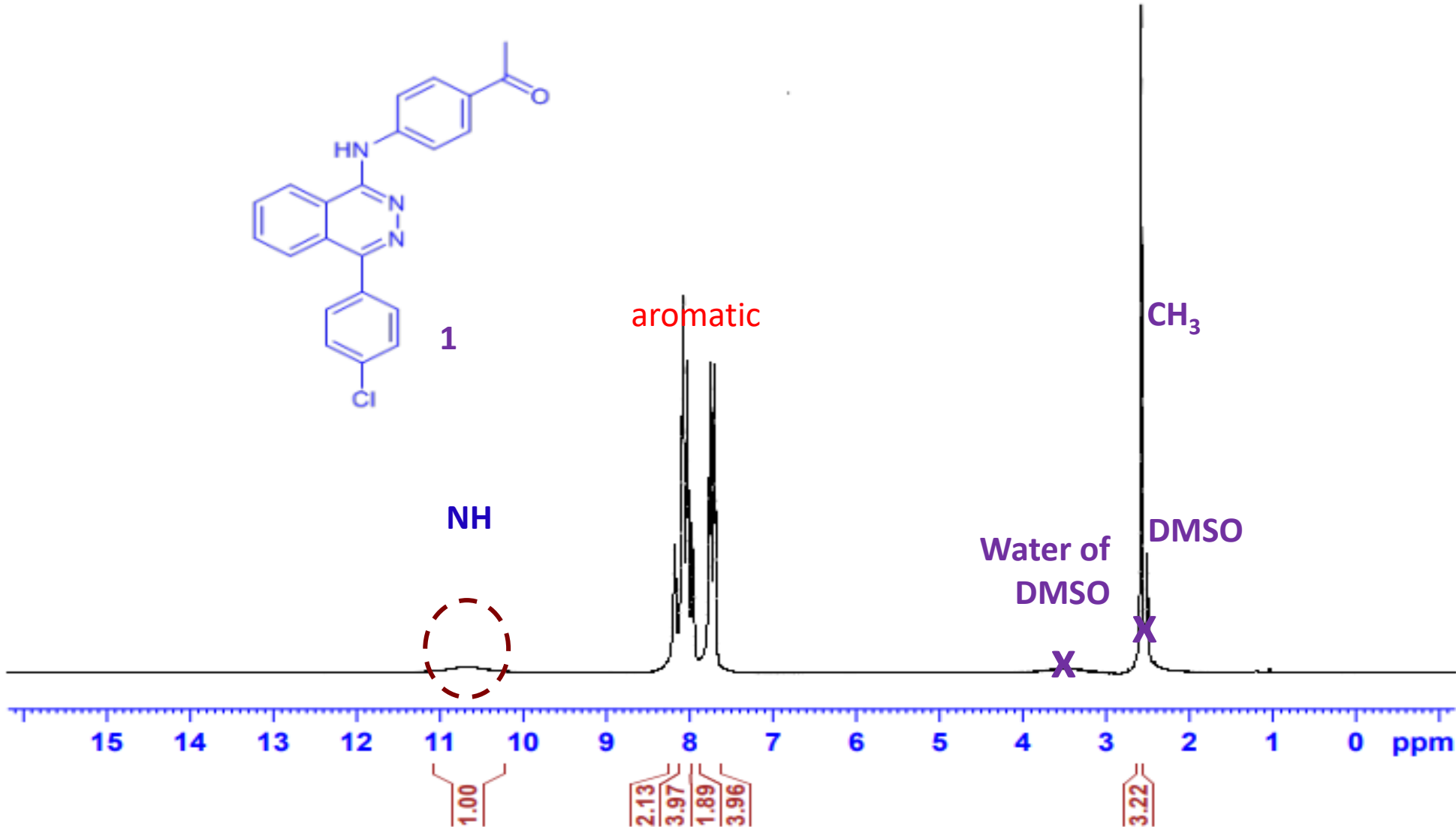
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CH<sub>3</sub>

Water of  
DMSO

DMSO



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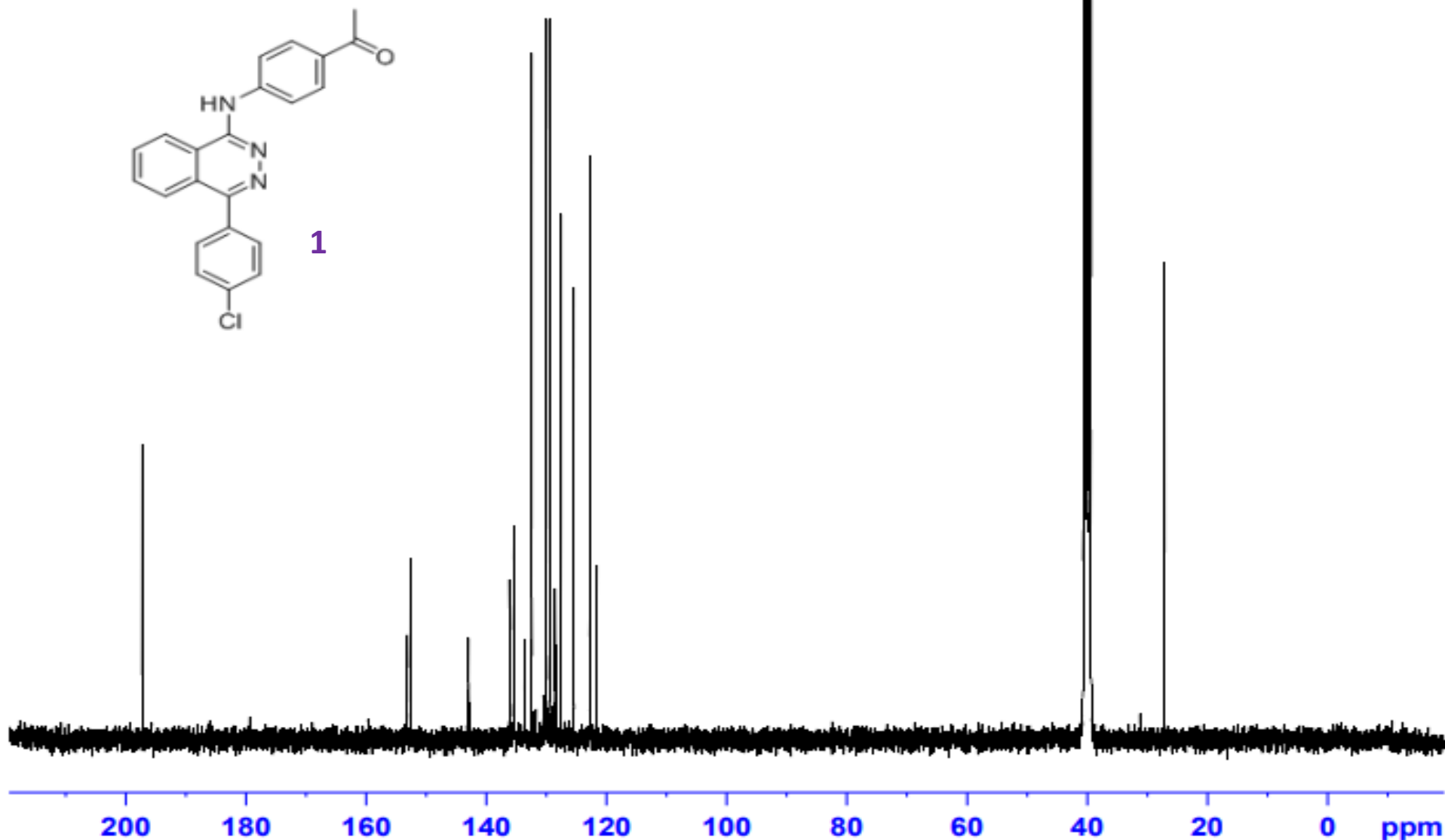
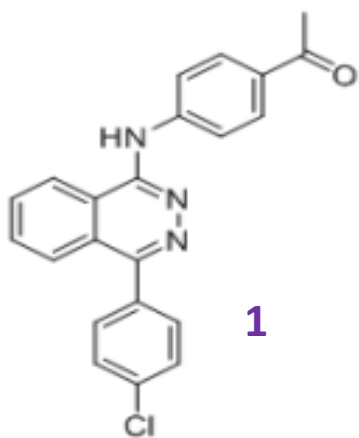
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135.39  
133.62  
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131.69  
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129.12  
128.55  
127.57  
125.45  
122.74  
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39.57  
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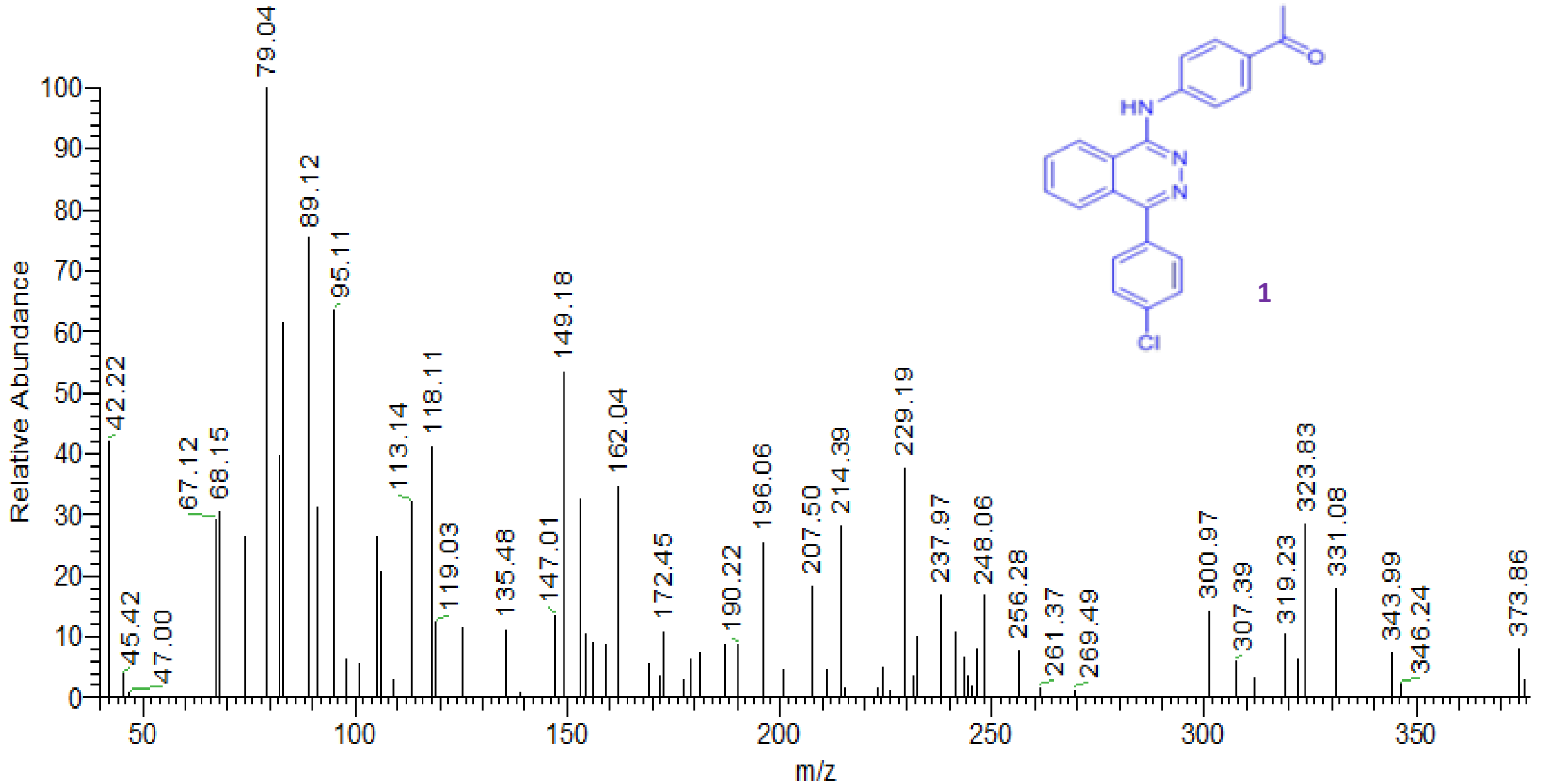
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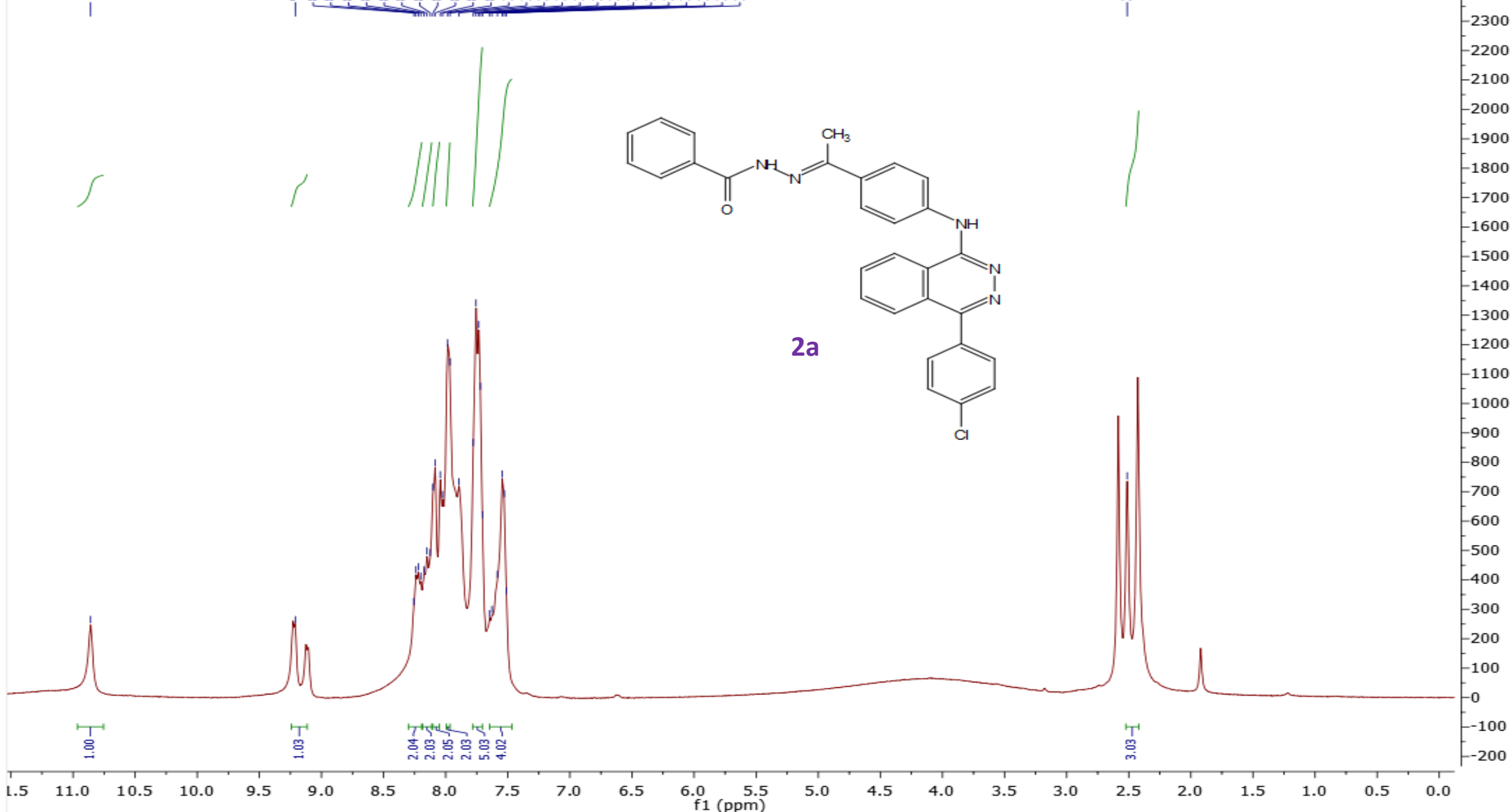
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Mohammed Dahab-FD8-Hnmr-RR-10-64

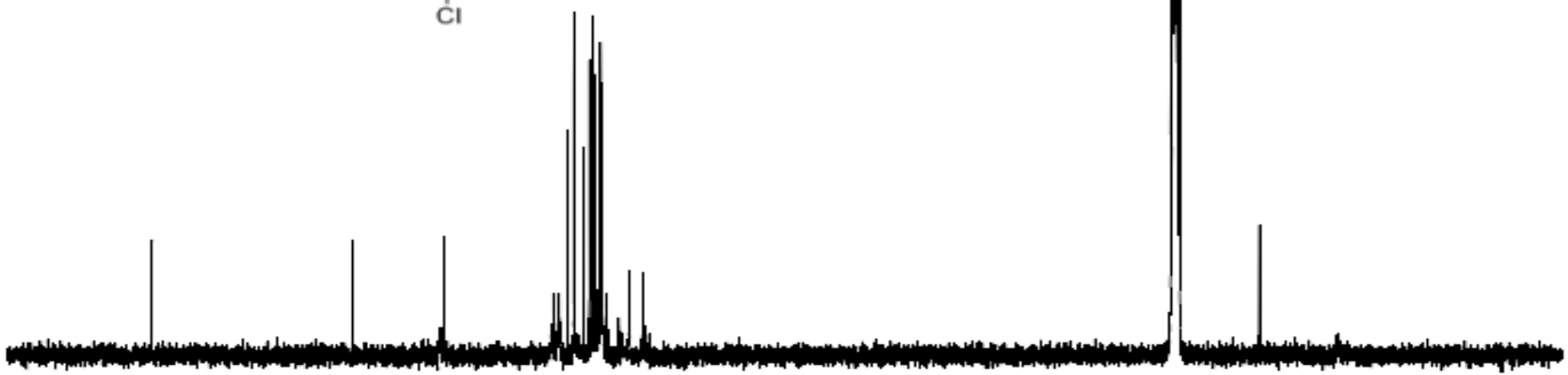
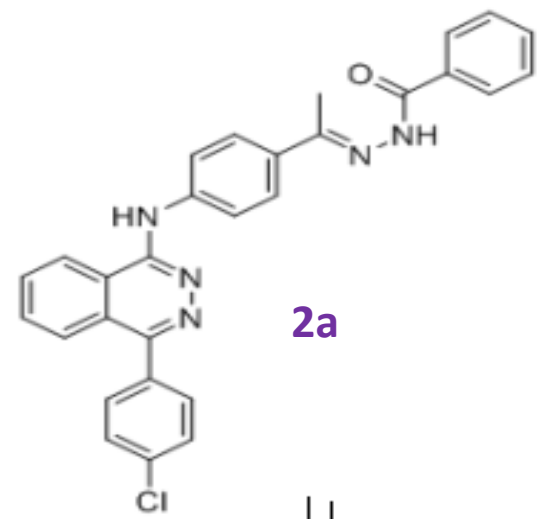




197.11

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130.90  
129.92  
129.45  
129.34  
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128.24  
127.98  
127.42  
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125.44  
123.80  
121.71  
121.38

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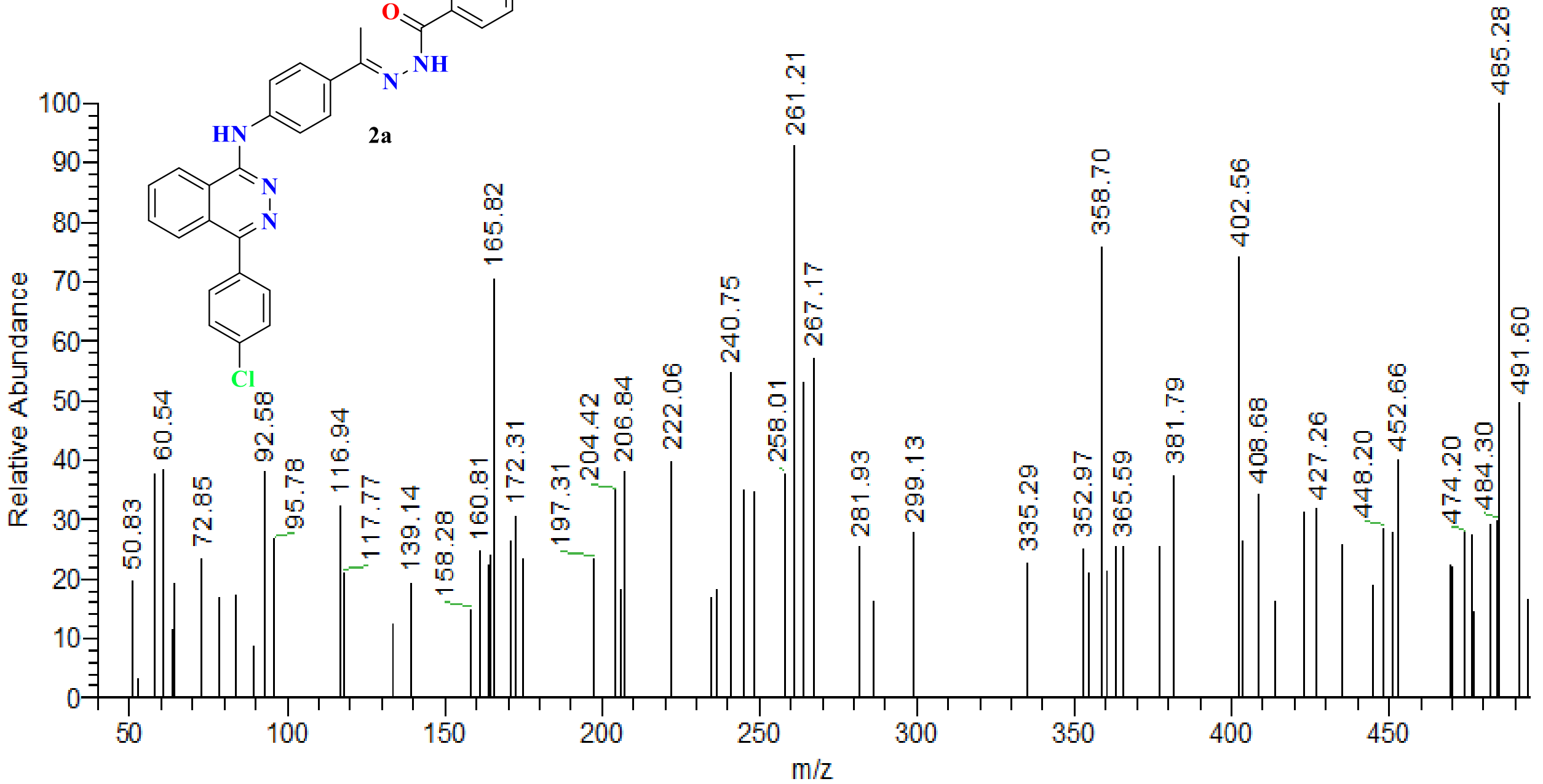
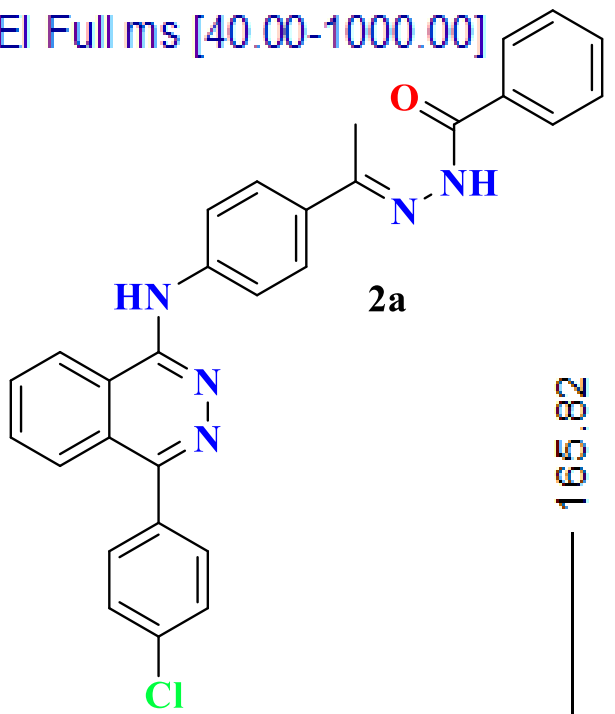
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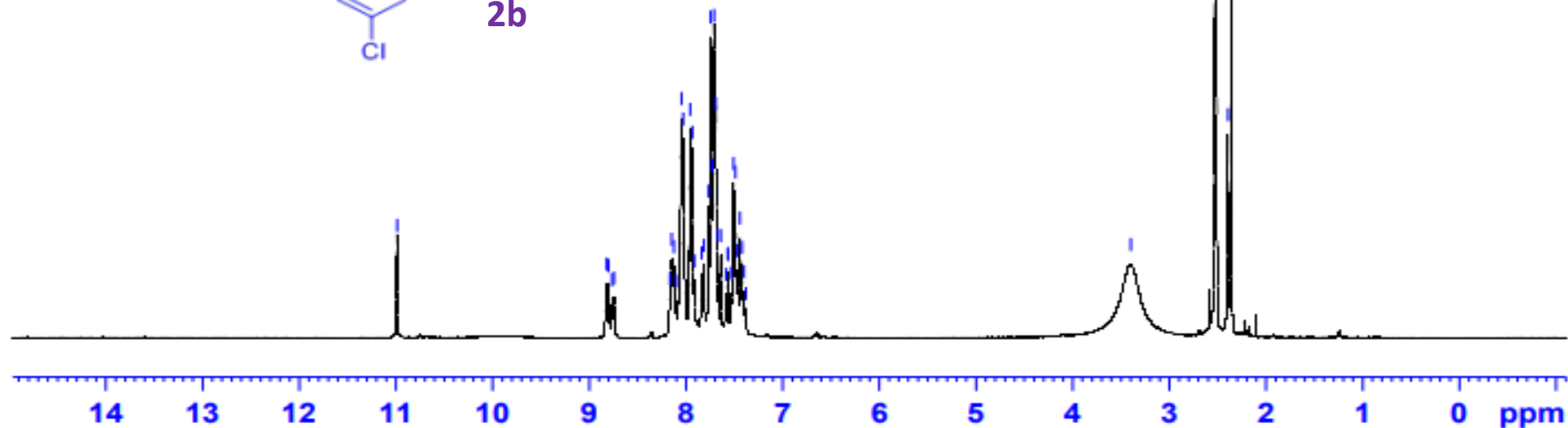
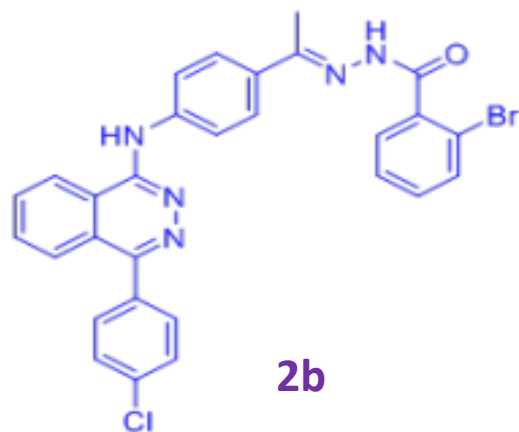
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8.102  
8.045  
8.028  
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7.934  
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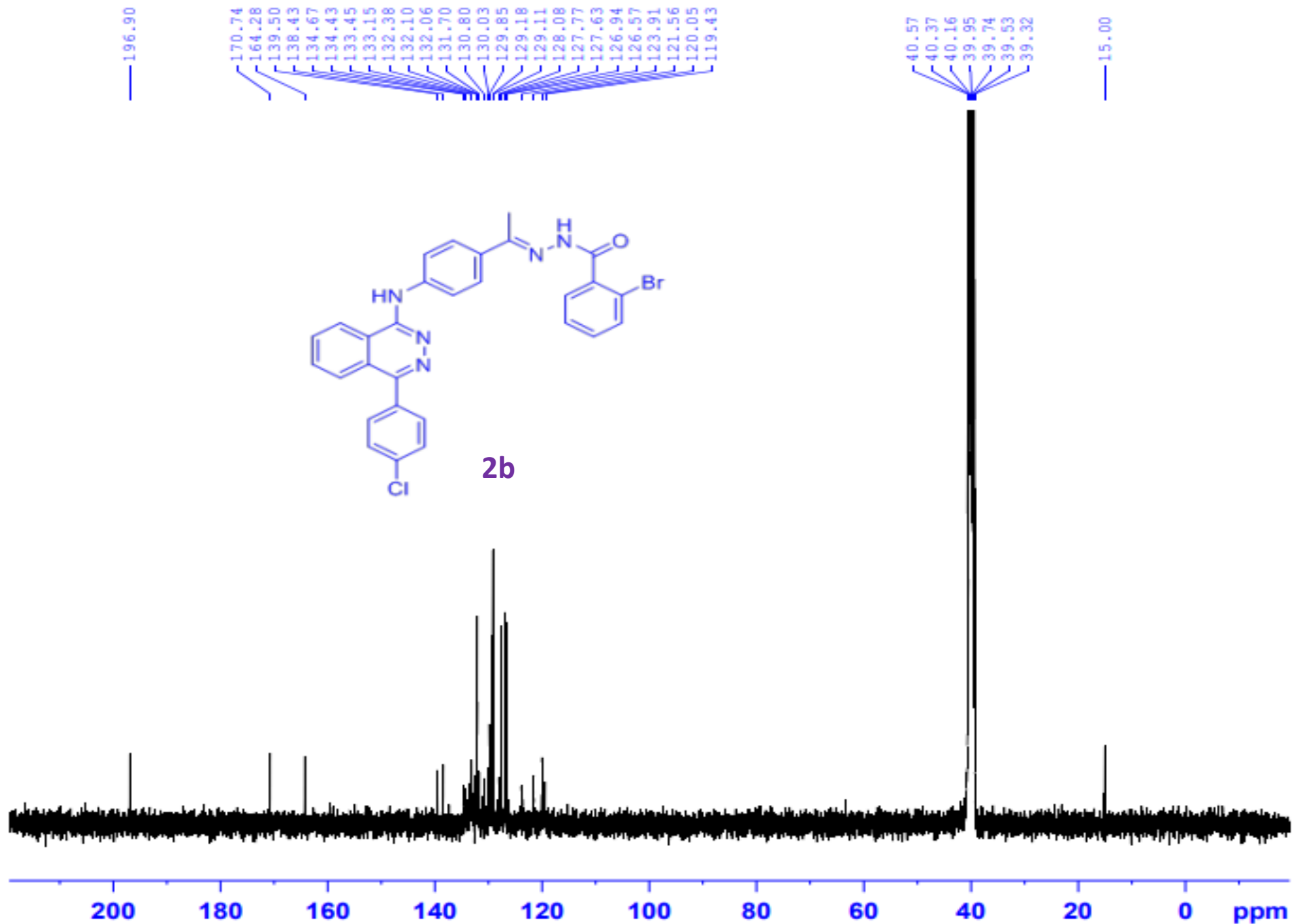
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1.90

6.16  
4.27  
3.03

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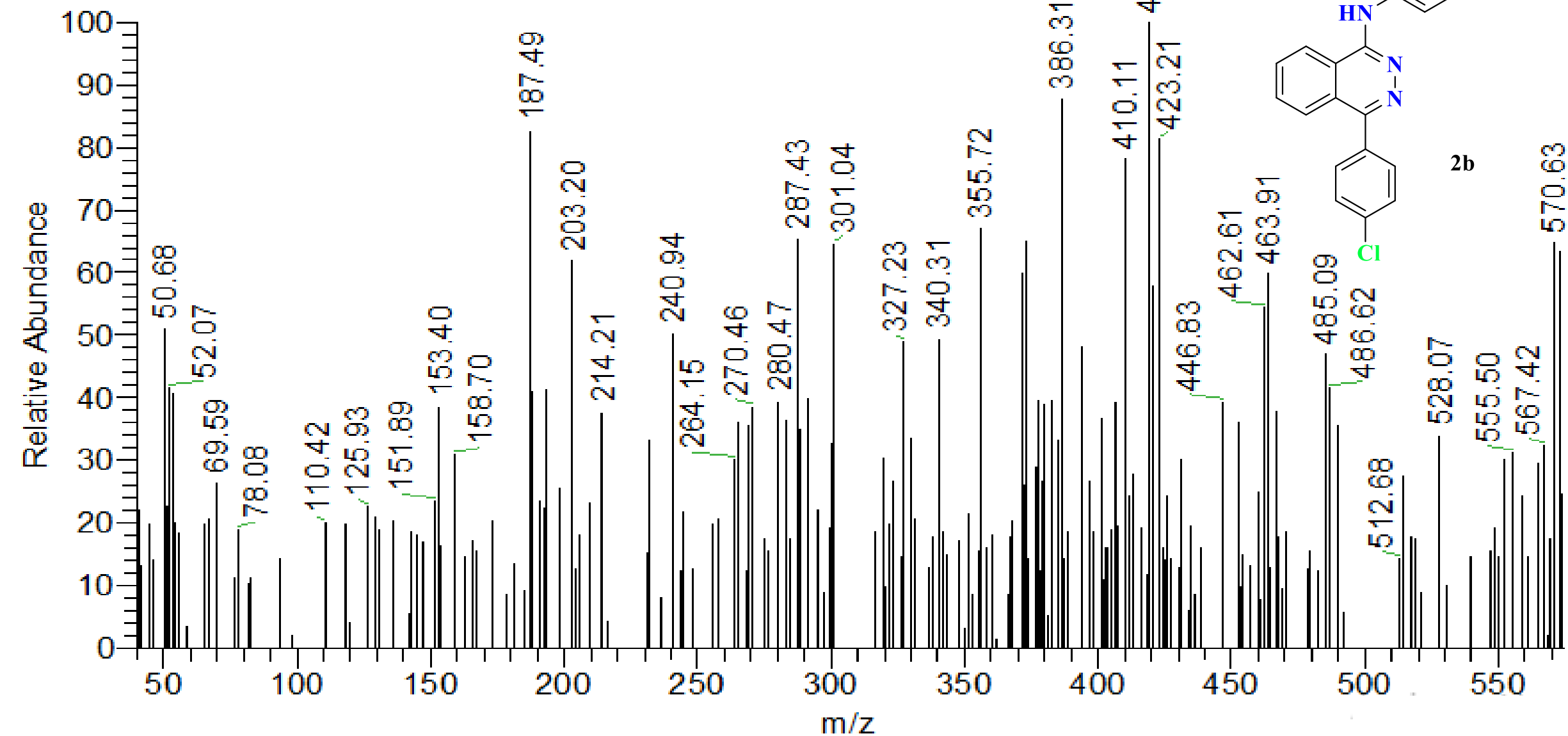
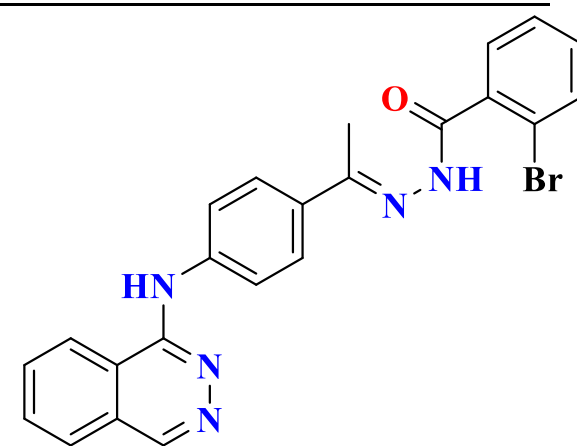
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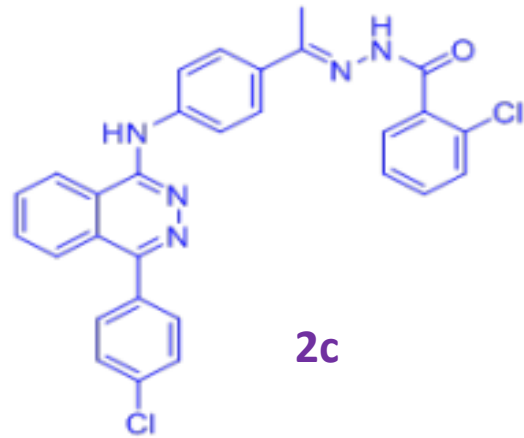
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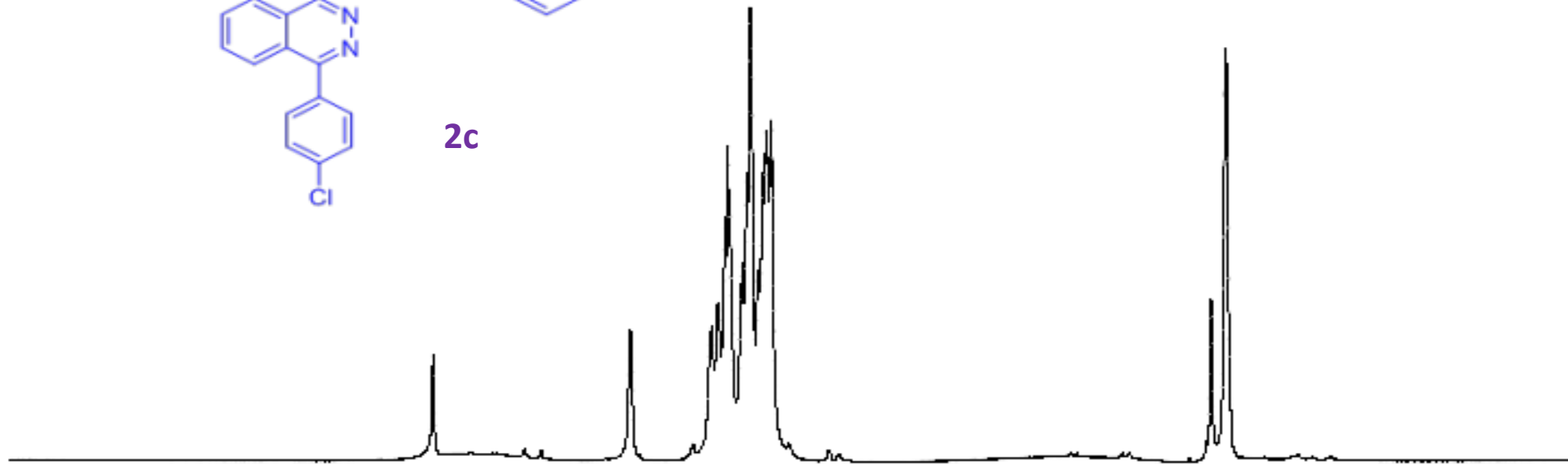
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 7.925  
 7.801  
 7.783  
 7.733  
 7.693  
 7.613  
 7.591  
 7.549  
 7.516  
 7.498  
 7.455

2.509  
 2.351  
 2.328



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1.88

1.87

4.63

6.18

10.58

12.02

1.41

6.12

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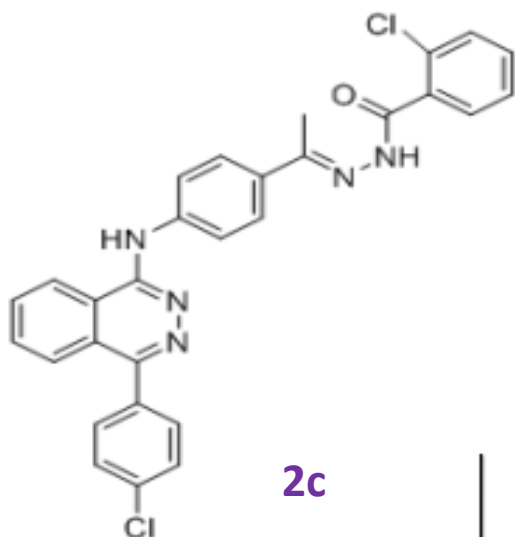
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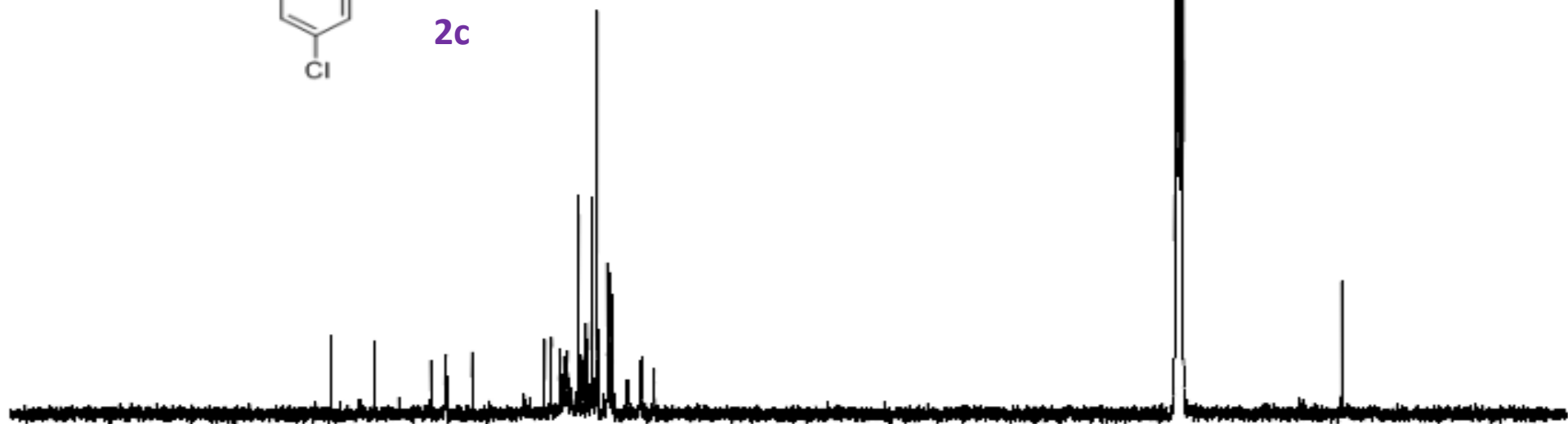
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15.03



2c



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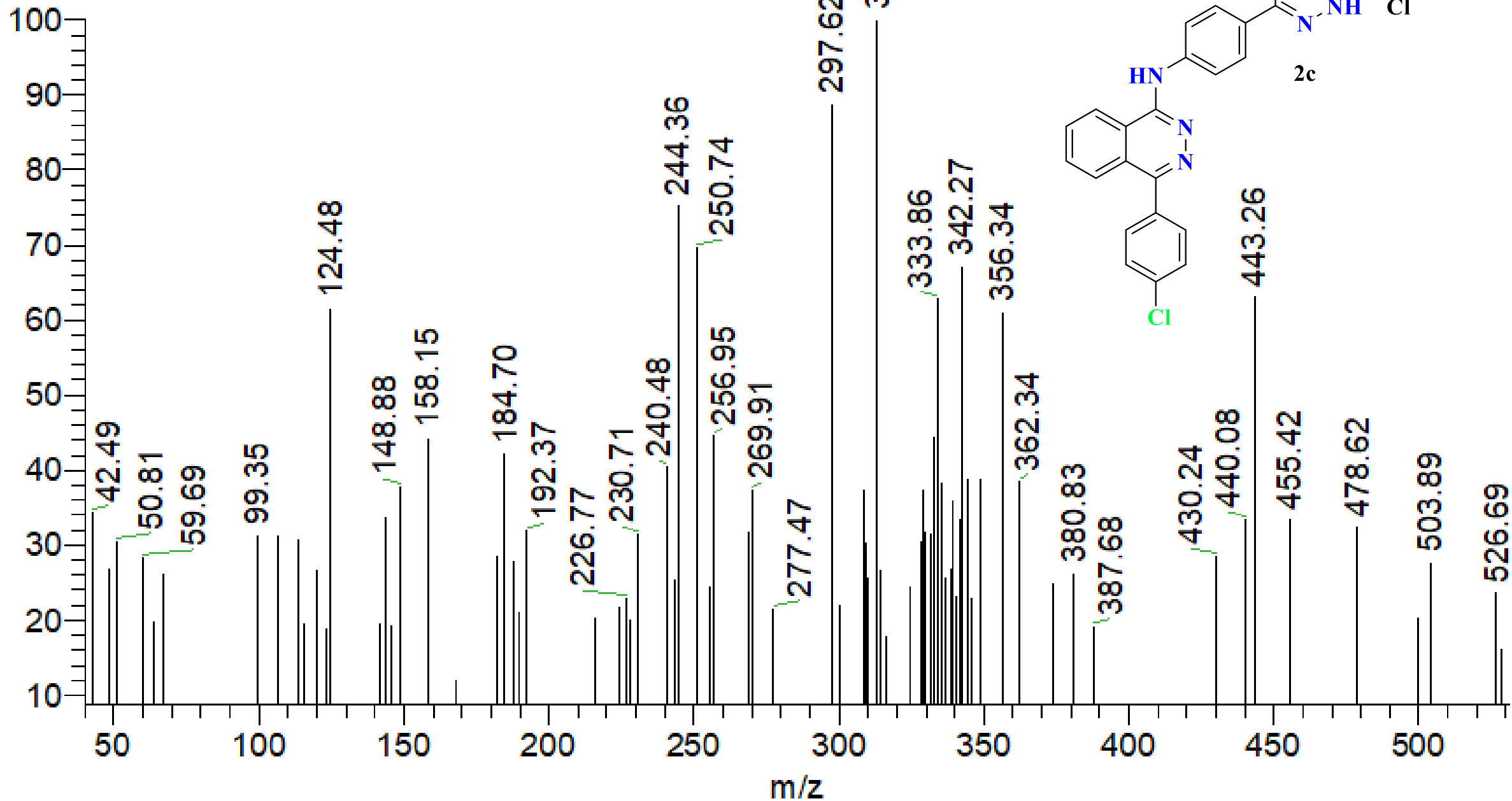
Faculty of Pharmacy

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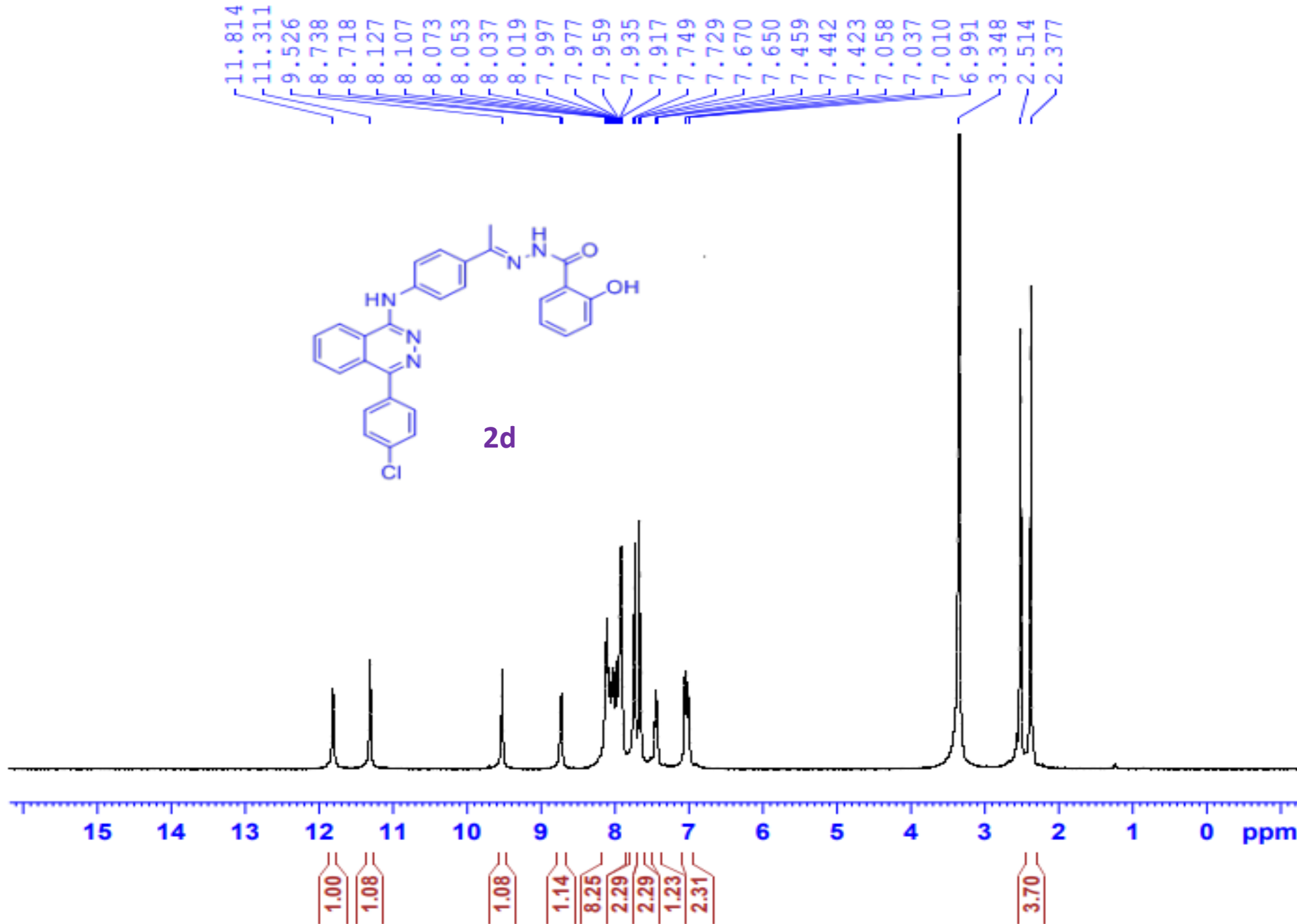
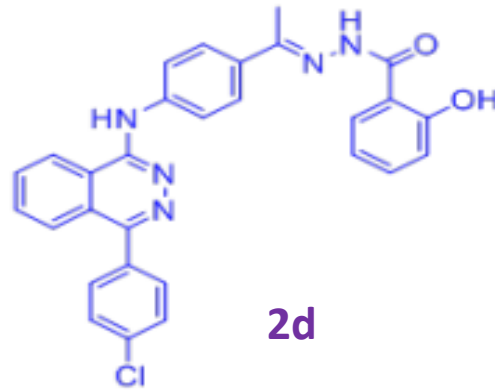
Relative Abundance







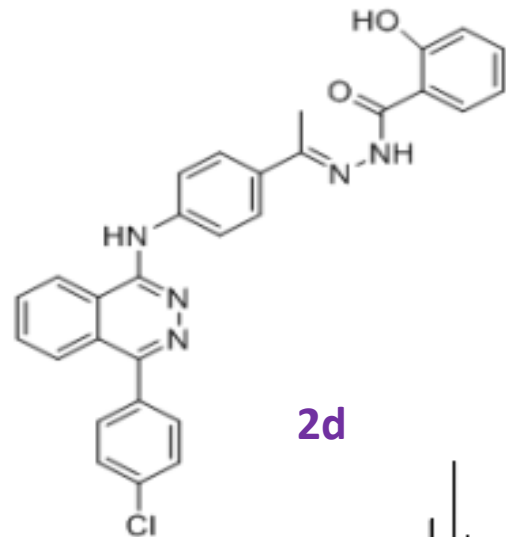
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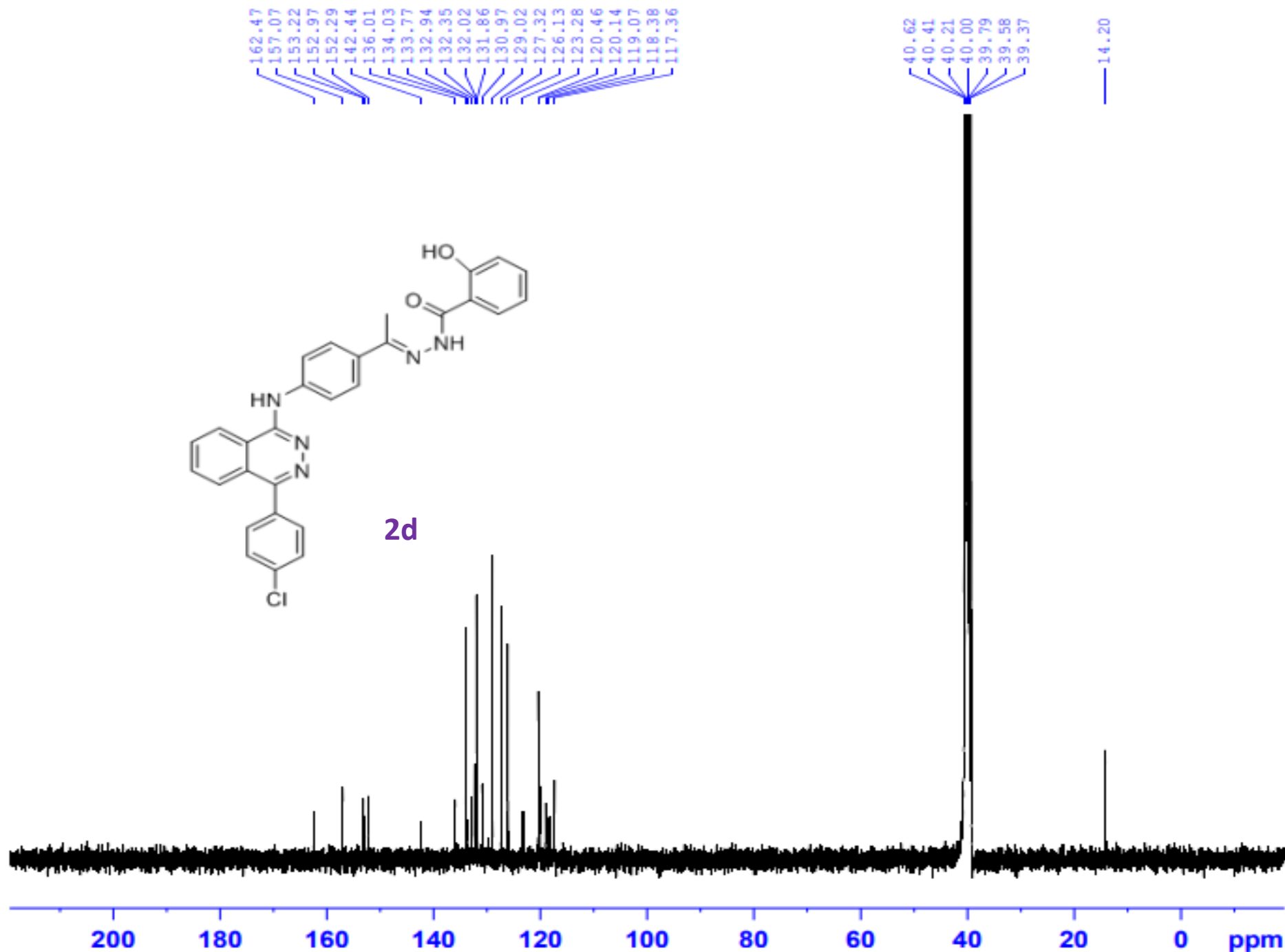
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2d



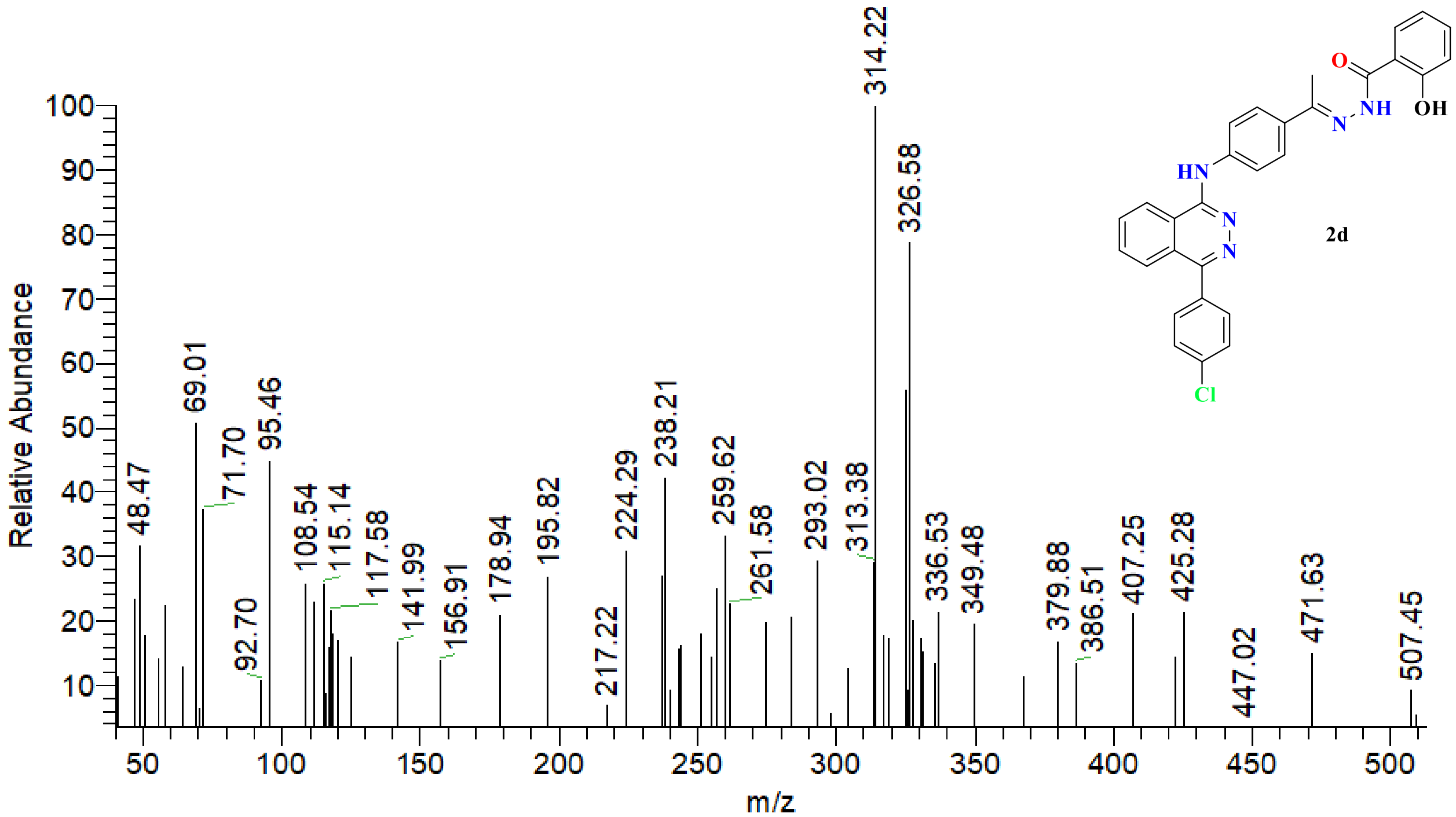
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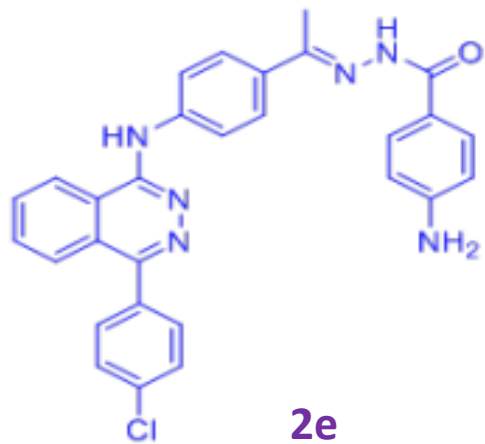
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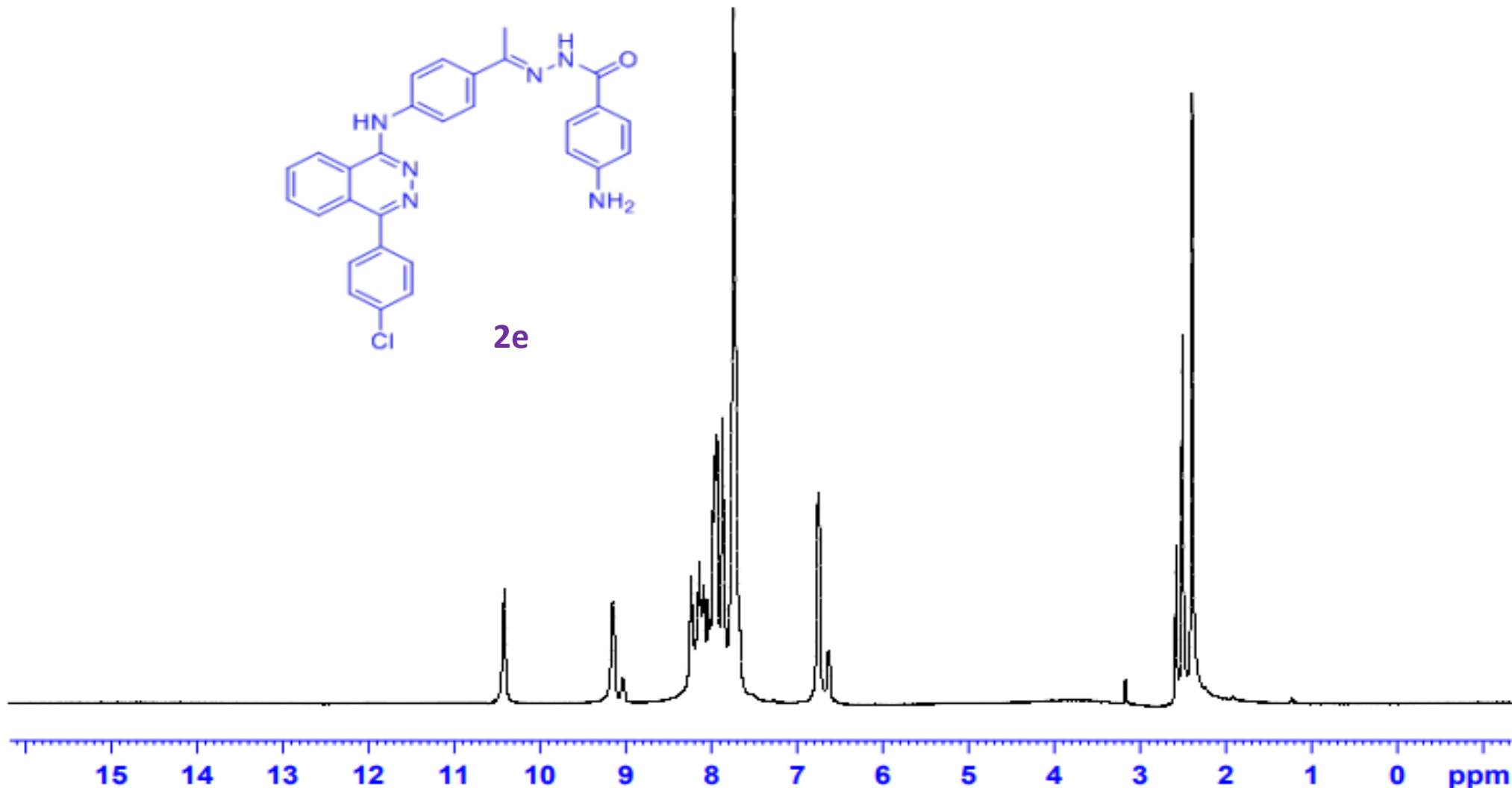
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8.146  
8.129  
8.110  
8.090  
8.046  
7.993  
7.961  
7.940  
7.877  
7.857  
7.747  
6.763  
6.746  
6.643  
6.624

3.178  
2.586  
2.512  
2.398



1.00  
1.02  
0.25  
0.27  
2.22  
4.34  
2.28  
7.82  
1.83  
0.42

0.63  
3.26

Current Data Parameters  
NAME Mohammed Dahab-FD10-Hnmr-RR  
EXPNO 10  
PROCNO 1

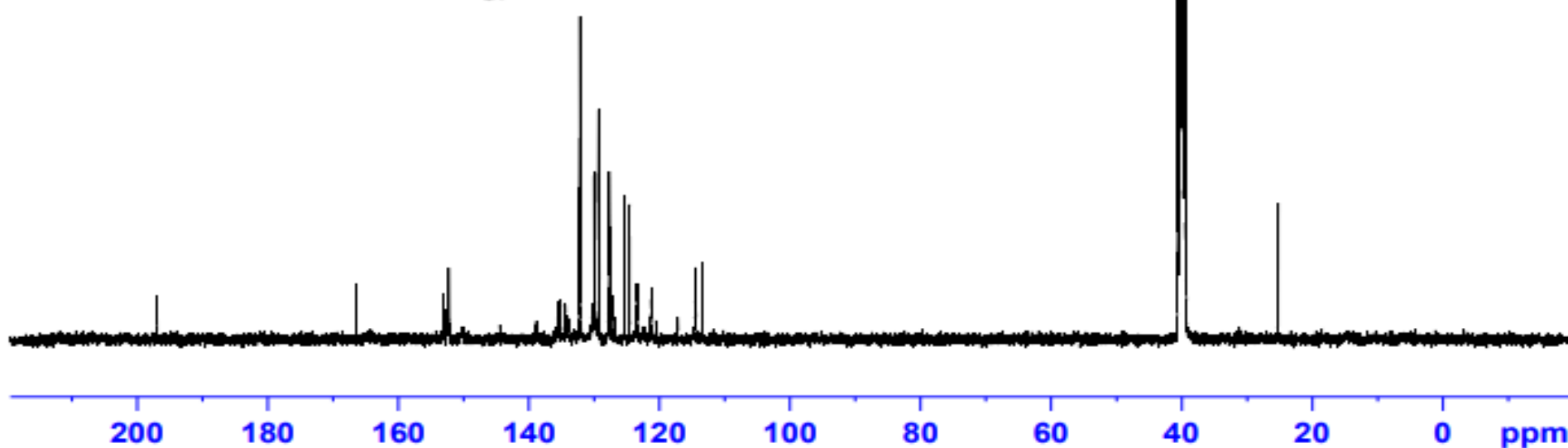
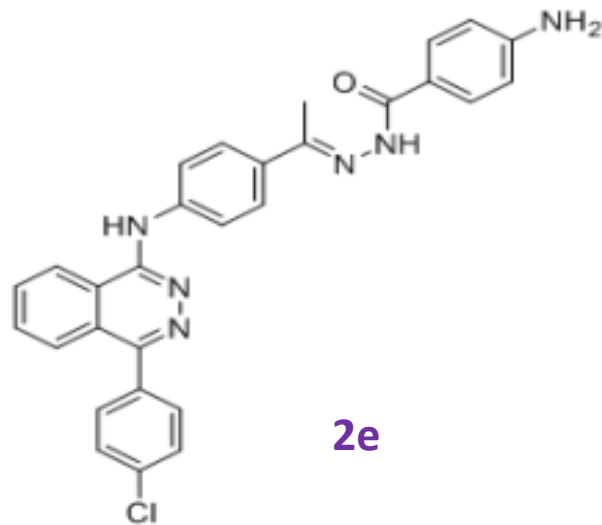
F2 - Acquisition Parameters  
Date\_ 20230131  
Time\_ 11.30 h  
INSTRUM spect  
PROBHD 2108618\_0945 (4  
PULPROG zg30  
TD 65536  
SOLVENT DMSO  
NS 16  
DS 2  
SWH 8012.820 Hz  
FIDRES 0.244532 Hz  
RG 158.72  
AQ 4.0894465 sec  
RG 158.72  
DW 62.400 usec  
DE 6.50 usec  
TE 301.6 K  
D1 1.00000000 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

197.04

166.48  
153.12  
152.64  
152.47  
152.29  
144.50  
138.93  
135.51  
135.20  
134.47  
134.23  
133.89  
132.23  
130.26  
130.07  
129.83  
129.38  
129.26  
127.90  
127.68  
127.25  
126.83  
125.37  
124.64  
123.48  
121.22  
120.50  
117.24  
114.48  
113.47

40.61  
40.40  
40.19  
39.98  
39.77  
39.57  
39.36  
25.42

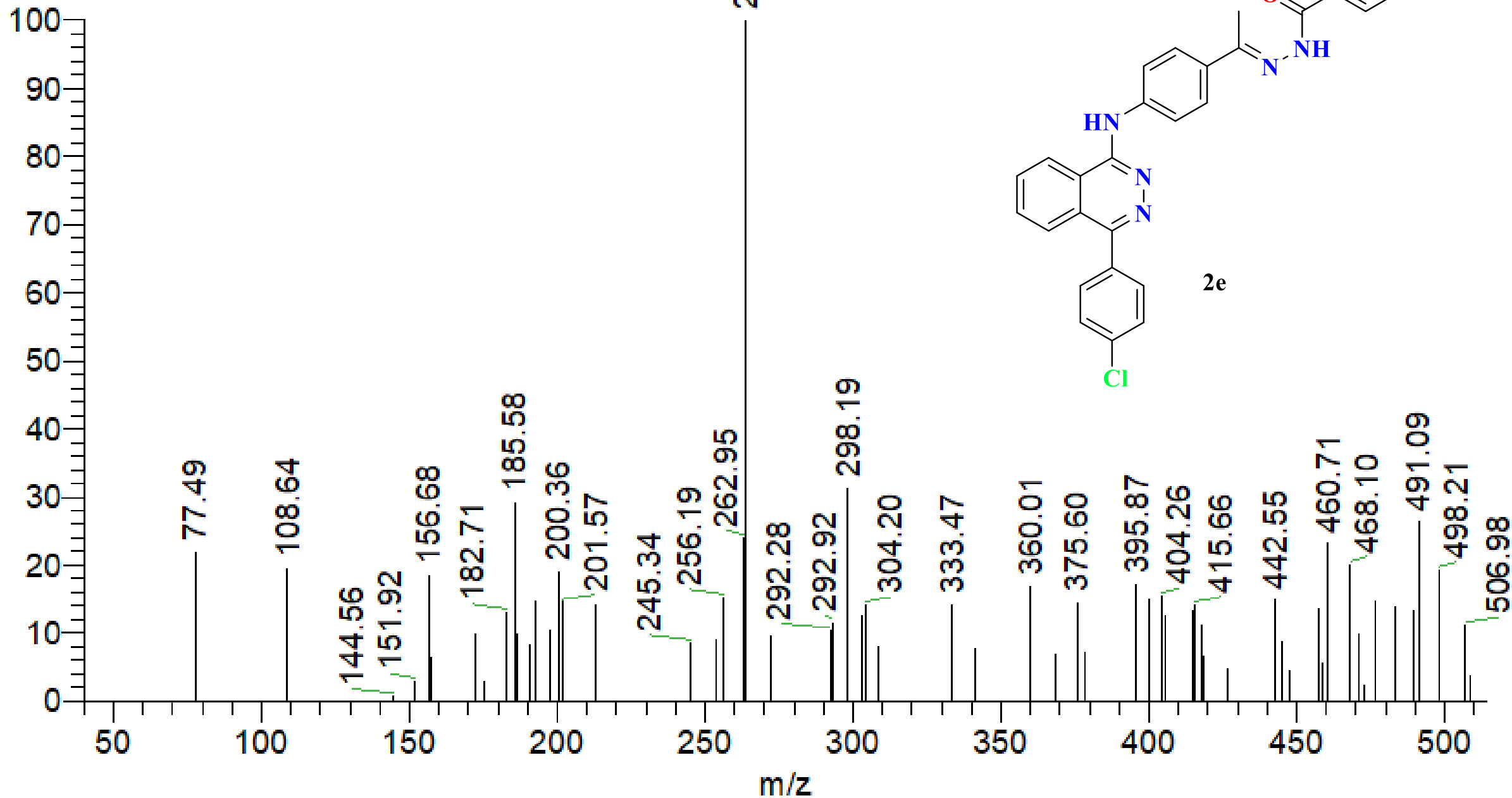


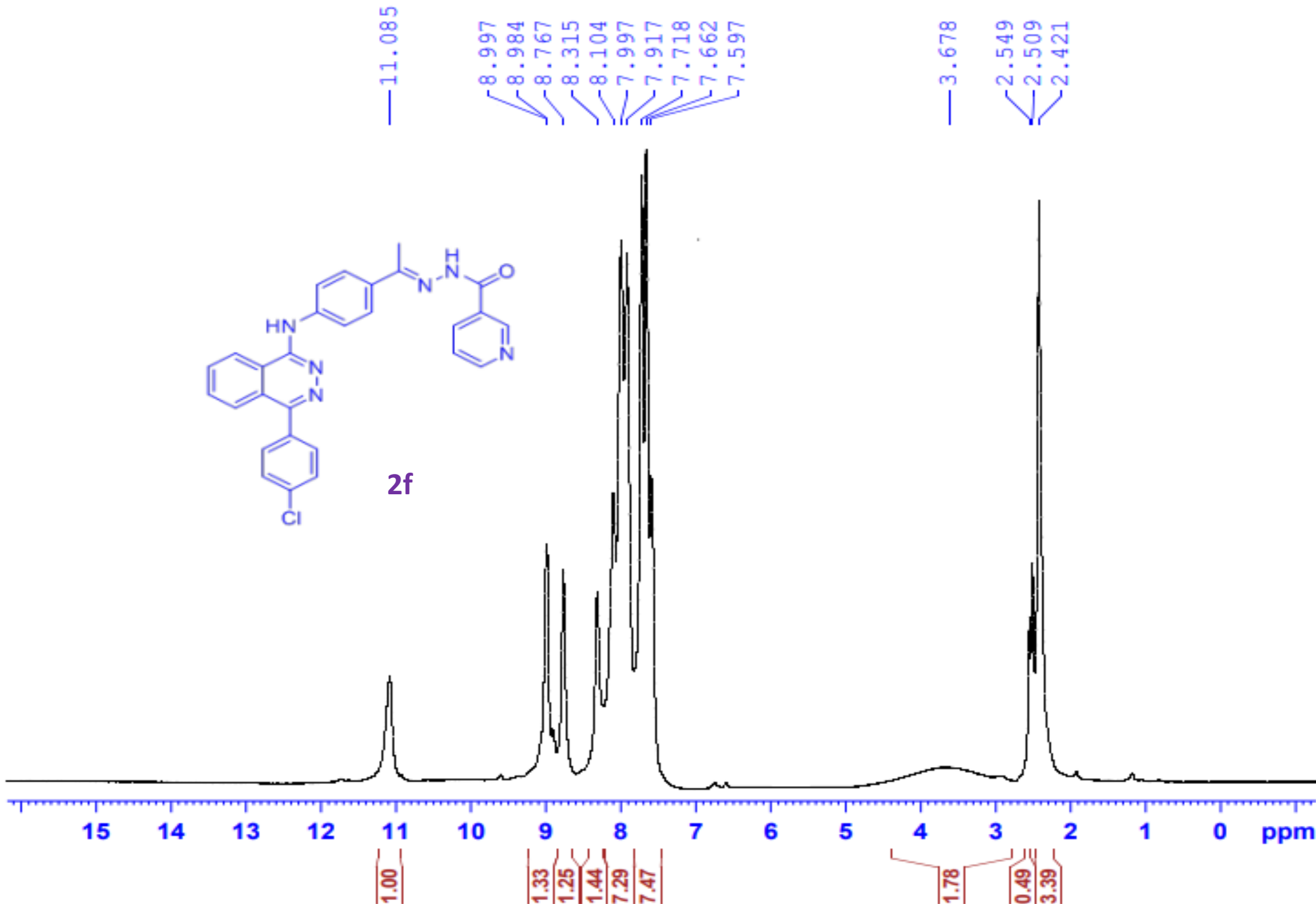
Current Data Parameters  
NAME Hatem Abdel-aziz-VI e-C13-RR  
EXPNO 10  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20230621  
Time\_ 12.05 h  
INSTRUM spect  
PROBHD z108618\_0945 (   
PULPROG zgpg30  
TD 65536  
SOLVENT DMSO  
NS 2200  
DS 4  
SWH 24038.461 Hz  
FIDRES 0.733596 Hz  
AQ 1.3631488 sec  
RG 197.77  
DW 20.800 usec  
DE 6.50 usec  
TE 296.8 K  
D1 2.00000000 sec  
D11 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  
CPDPRG[2] waltz16  
PCPD2 90.00 usec  
PLW2 13.00000000 W  
PLW12 0.29249999 W  
PLW13 0.14713000 W

F2 - Processing parameters  
SI 32768  
SF 100.6303700 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.40

Relative Abundance





Current Data Parameters  
 NAME Mohammed Dahab-FD7-Hnmr-RR  
 EXPNO 10  
 PROCNO 1

F2 - Acquisition Parameters  
 Date\_ 20230131  
 Time\_ 11.18 h  
 INSTRUM spect  
 PROBHD z108618\_0945 (zq30)  
 PULPROG zg30  
 TD 65536  
 SOLVENT DMSO  
 NS 16  
 DS 2  
 SWH 8012.820 Hz  
 FIDRES 0.244532 Hz  
 AQ 4.0894465 sec  
 RG 68.17  
 DW 62.400 usec  
 DE 6.50 usec  
 TE 302.0 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



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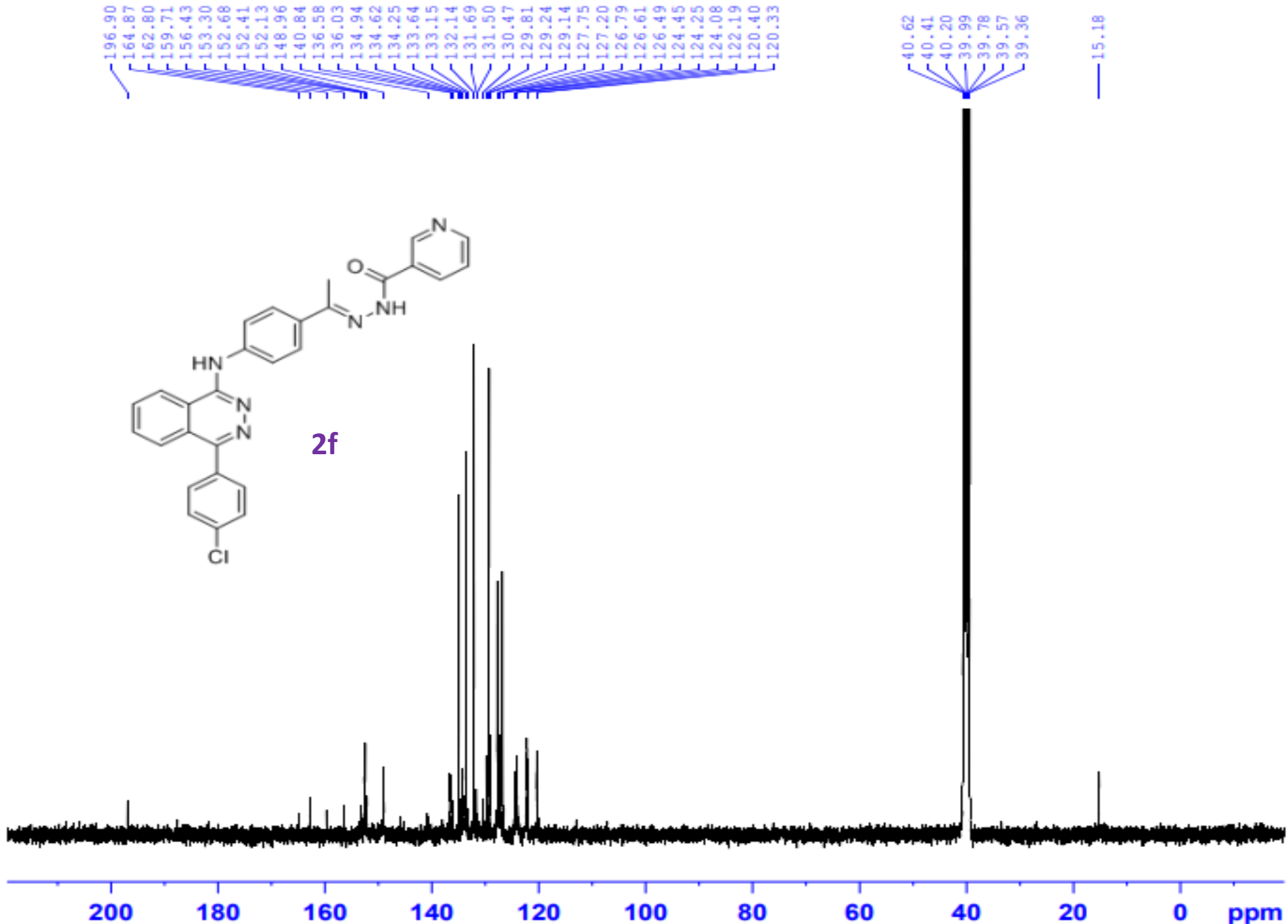
Current Data Parameters  
NAME Hatem Abdel-aziz-VI f-C13-RR  
EXPNO 10  
PROCNO 1

F2 - Acquisition Parameters

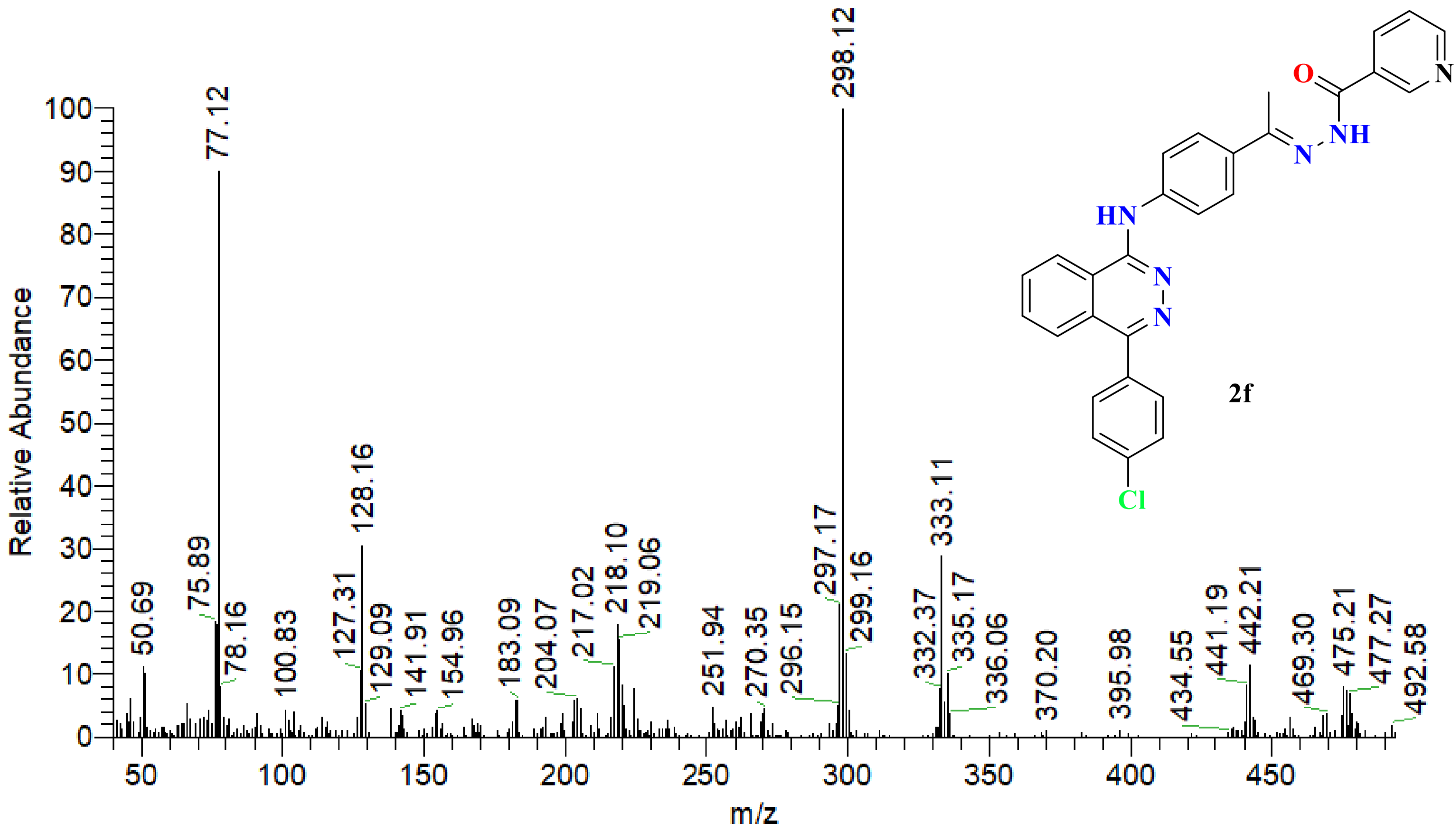
Date 20230620  
Time 1.00 h  
INSTRUM spect  
PROBHD Z108618\_0945 (   
PULPROG zgpg30  
TD 65536  
SOLVENT DMSO  
NS 2200  
DS 4  
SMH 24038.461 Hz  
FIDRES 0.733596 Hz  
AQ 1.3631488 sec  
RG 197.77  
DW 20.800 usec  
DE 6.50 usec  
TE 0 K  
D1 2.00000000 sec  
D11 0.03000000 sec  
TDO 1  
SFO1 100.6404331 MHz  
NUC1 13C  
P1 10.00 usec  
PLW1 47.00000000 W  
SFO2 400.2016008 MHz  
NUC2 1H  
CPDPRG[2] waltz16  
PCPD2 90.00 usec  
PLW2 13.00000000 W  
PLW12 0.29249999 W  
PLW13 0.14713000 W

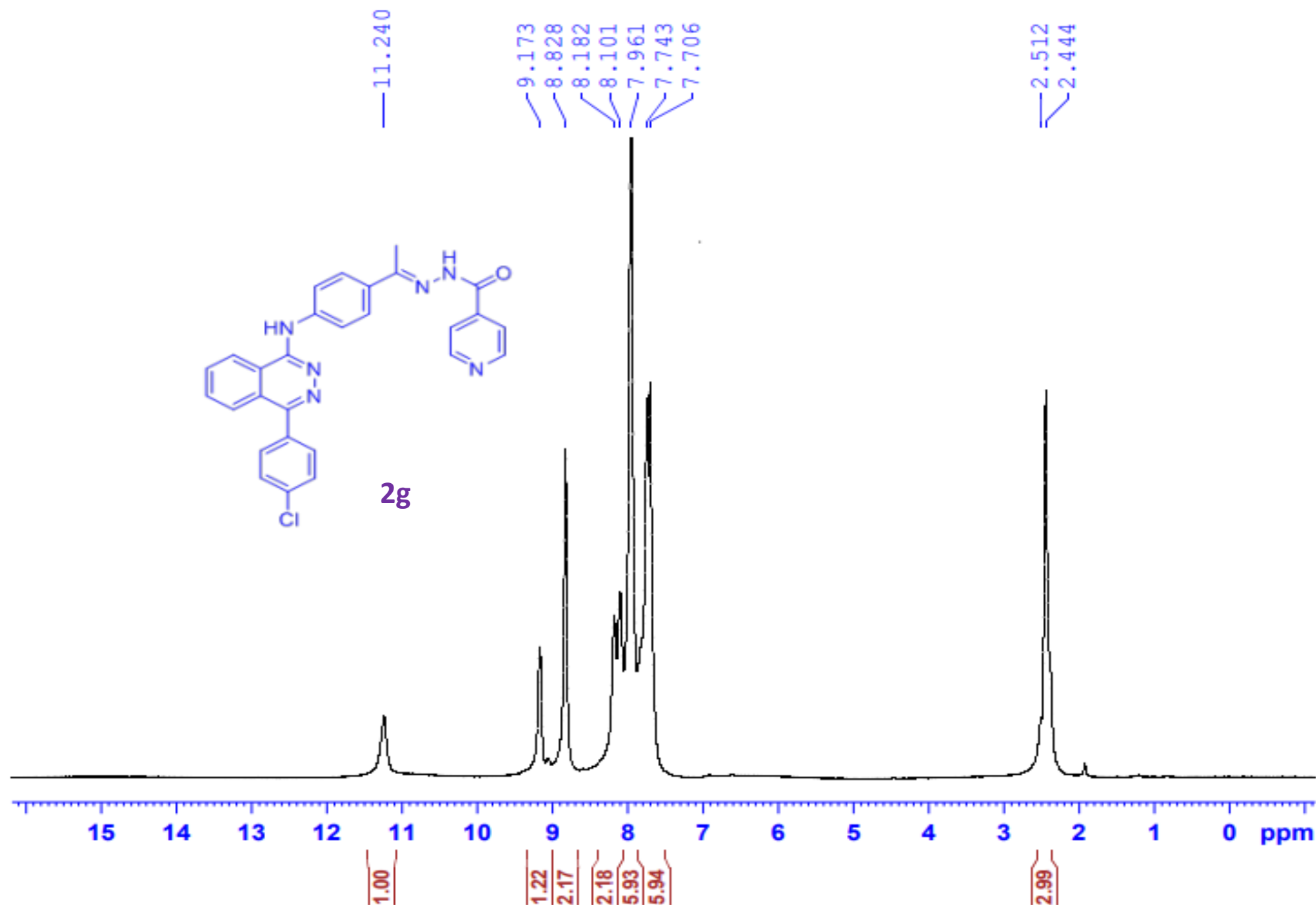
F2 - Processing parameters

SI 32768  
SF 100.6303700 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.40





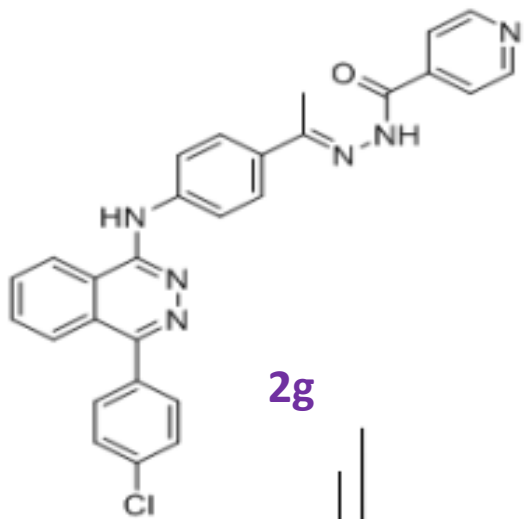
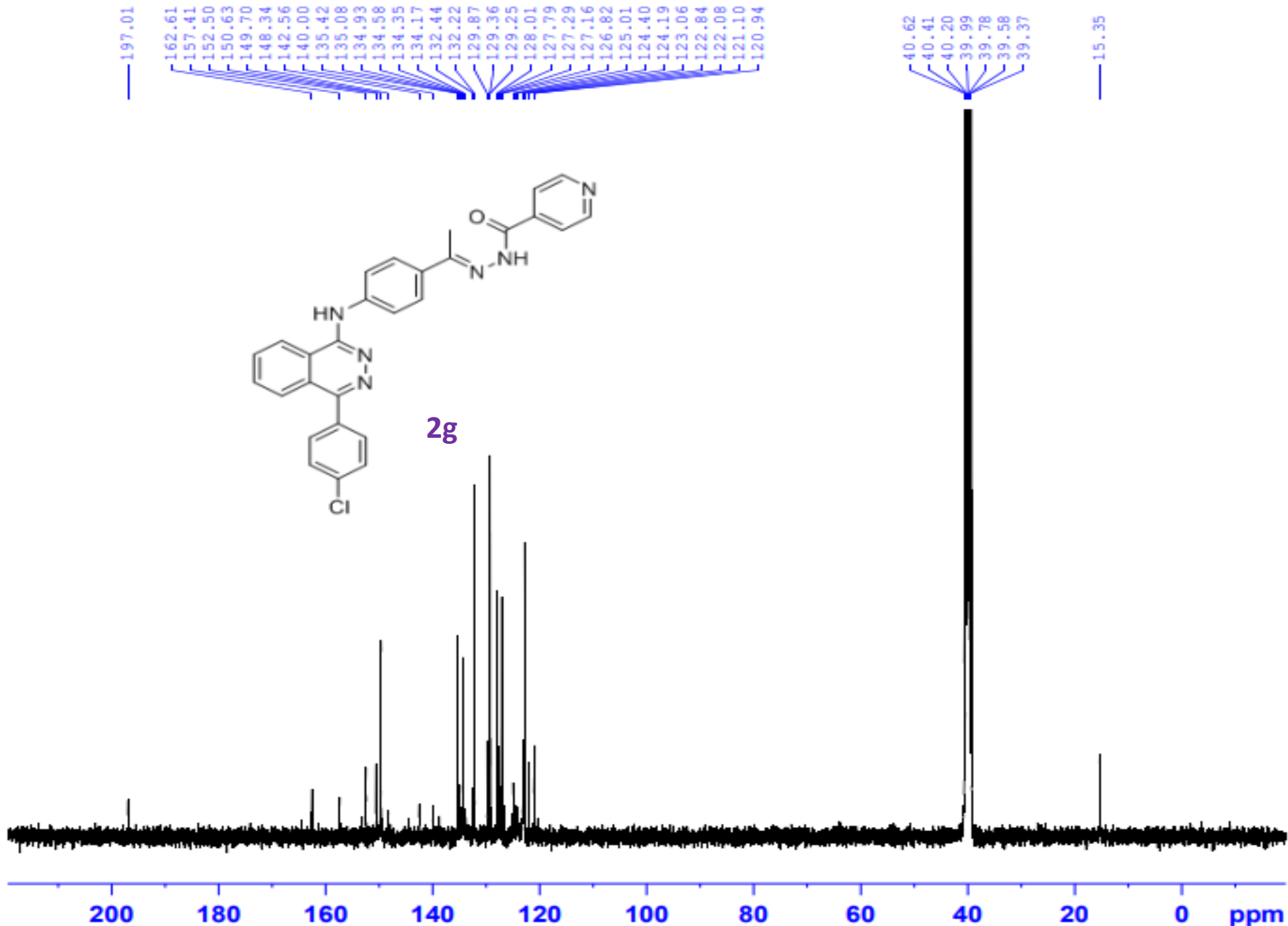




Current Data Parameters  
 NAME Mohammed Dahab-FD6-Hnmr-RR  
 EXPNO 10  
 PROCNO 1

F2 - Acquisition Parameters  
 Date\_ 20230131  
 Time\_ 11.10 h  
 INSTRUM spect  
 PROBHD 2108618\_0945 (   
 PULPROG zg30  
 TD 65536  
 SOLVENT DMSO  
 NS 16  
 DS 2  
 SWH 8012.820 Hz  
 FIDRES 0.244532 Hz  
 AQ 4.0894465 sec  
 RG 78.59  
 DW 62.400 usec  
 DE 6.50 usec  
 TE 302.3 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



2g



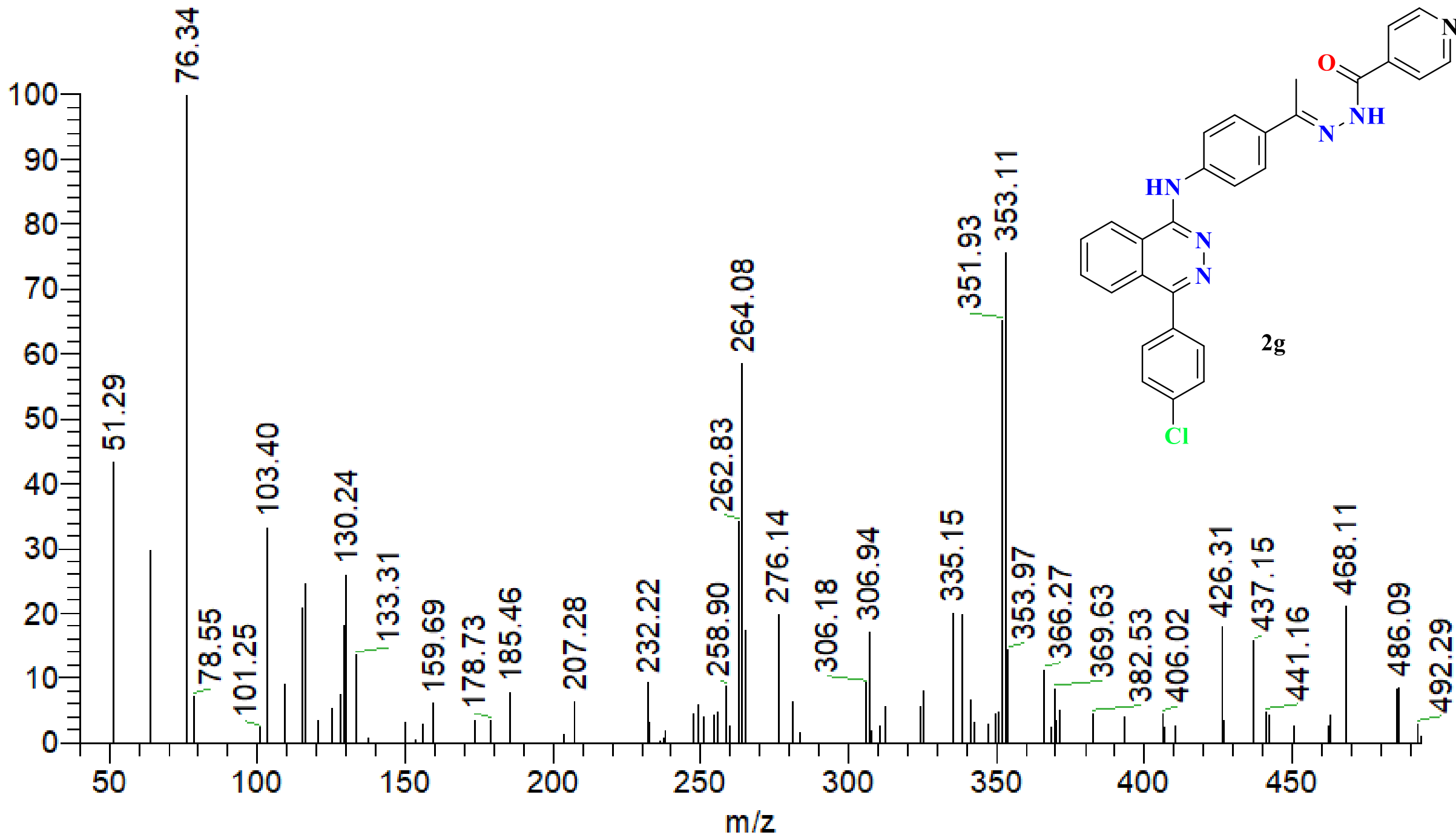
Current Data Parameters  
 NAME Hatem Abdel-aziz-VI g-C13-RR  
 EXPNO 10  
 PROCNO 1

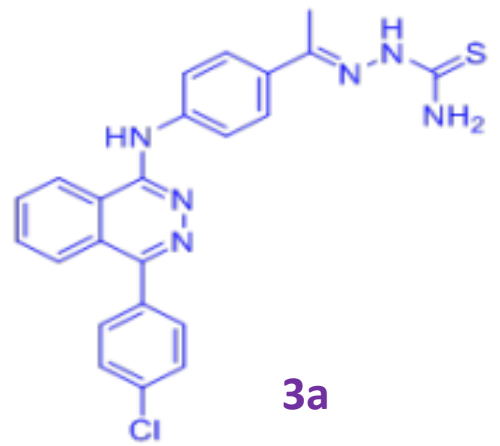
F2 - Acquisition Parameters  
 Date 20230619  
 Time 20.41 h  
 INSTRUM spect  
 PROBHD E108618\_0945 (zggg30)  
 PULPROG zgpg30  
 TD 65536  
 SOLVENT DMSO  
 NS 2200  
 DS 4  
 SWH 24038.461 Hz  
 FIDRES 0.733596 Hz  
 AQ 1.3631488 sec  
 RG 197.77  
 DW 20.800 usec  
 DE 6.50 usec  
 TE 0 K

D1 2.00000000 sec  
 D11 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  
 CPDPRG[2] waltz16  
 PCPD2 90.00 usec  
 PLW2 13.00000000 W  
 PLW12 0.29249999 W  
 PLW13 0.14713000 W

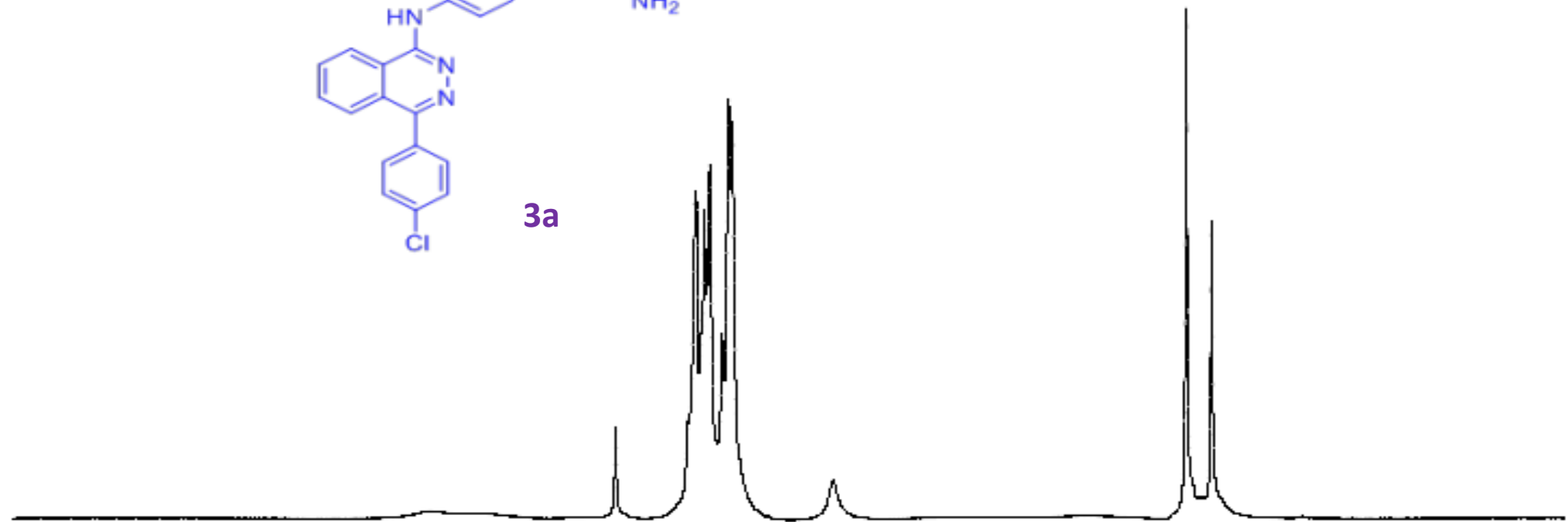
F2 - Processing parameters  
 SI 32768  
 SF 100.6303700 MHz  
 MDW EM  
 SSB 0  
 LB 1.00 Hz  
 GB 0  
 PC 1.40

Relative Abundance





11.110  
 9.037  
 9.019  
 8.204  
 8.127  
 8.107  
 8.072  
 8.054  
 8.021  
 8.000  
 7.957  
 7.820  
 7.801  
 7.743  
 7.714  
 7.698  
 6.551  
 2.513  
 2.235



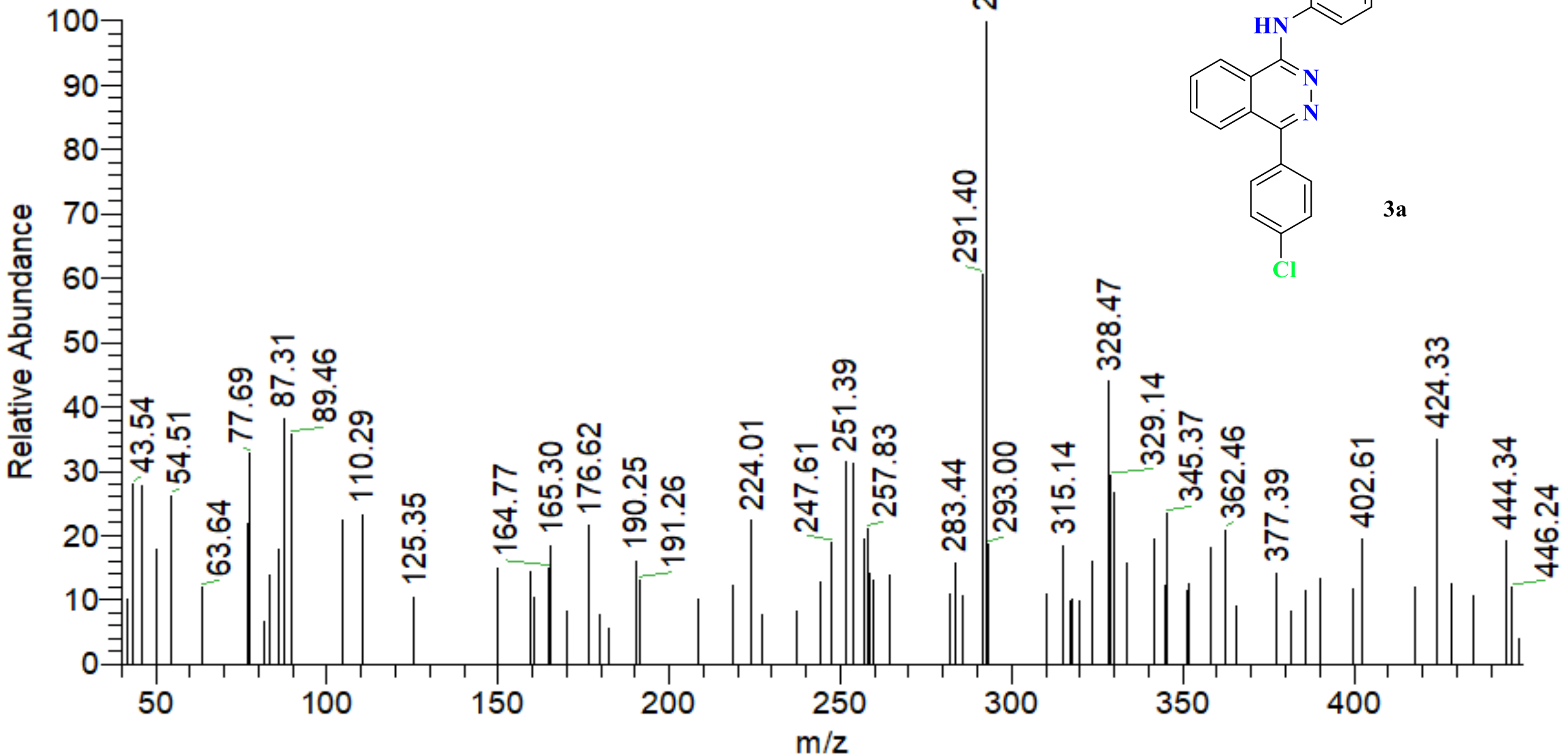
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm

1.07  
 1.09  
 1.98  
 1.99  
 2.08  
 2.03  
 3.97  
 1.99  
 2.68  
 3.03

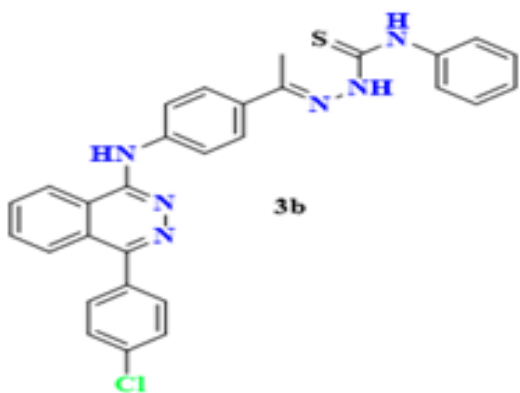
Current Data Parameters  
 NAME Mohammed Dahab-FD15-Hnmr-RN  
 EXPNO 10  
 PROCNO 1

F2 - Acquisition Parameters  
 Date\_ 20230131  
 Time\_ 11.40 h  
 INSTRUM spect  
 PROBHD 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 99.3  
 DW 62.400 usec  
 DE 6.50 usec  
 TE 302.1 K  
 D1 1.00000000 sec  
 TD0 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



166.02  
159.72  
152.87  
145.83  
142.18  
139.84  
138.84  
135.52  
134.80  
134.50  
134.40  
134.29  
134.20  
134.20  
132.27  
132.15  
131.69  
129.37  
129.27  
129.13  
128.32  
127.88  
127.82  
127.23  
127.12  
126.88  
126.61  
124.83  
124.06  
122.59  
121.17  
120.85  
115.26  
113.54



40.58  
40.37  
40.16  
39.95  
39.74  
39.53  
39.32

12.24



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Current Data Parameters  
NAME Mohamed Dahab-XVb-C13-RR  
EXPNO 10  
PROCNO 1

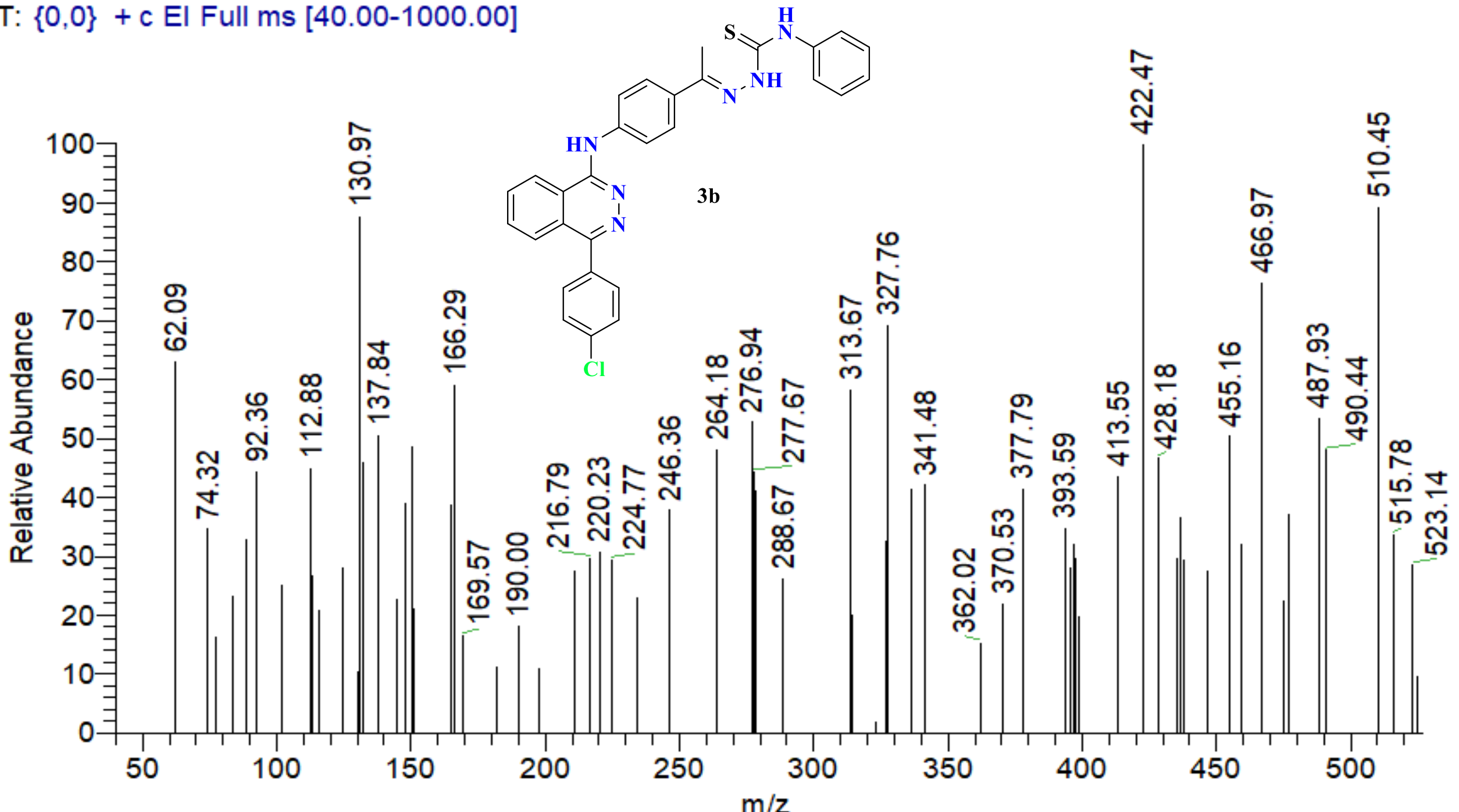
F2 - Acquisition Parameters

Date\_ 20230712  
Time\_ 9.47 h  
INSTRUM spect  
PROBHD z108618\_0945 (   
PULPROG zgpg30  
TD 65536  
SOLVENT DMSO  
NS 2200  
DS 4  
SWH 24038.461 Hz  
FIDRES 0.733596 Hz  
AQ 1.3631488 sec  
RG 197.77  
DW 20.800 usec  
DE 6.50 usec  
TE 293.4 K  
D1 2.00000000 sec  
D11 0.03000000 sec  
TD0 1  
SFO1 100.6404331 MHz  
NUC1 13C  
P1 10.00 usec  
PLW1 47.00000000 W  
SFO2 400.2016008 MHz  
NUC2 1H  
CPDPRG[2] waltz16  
PCPD2 90.00 usec  
PLW2 13.00000000 W  
PLW12 0.29249999 W  
PLW13 0.14713000 W

F2 - Processing parameters

SI 32768  
SF 100.6303700 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.40

200 180 160 140 120 100 80 60 40 20 0 ppm





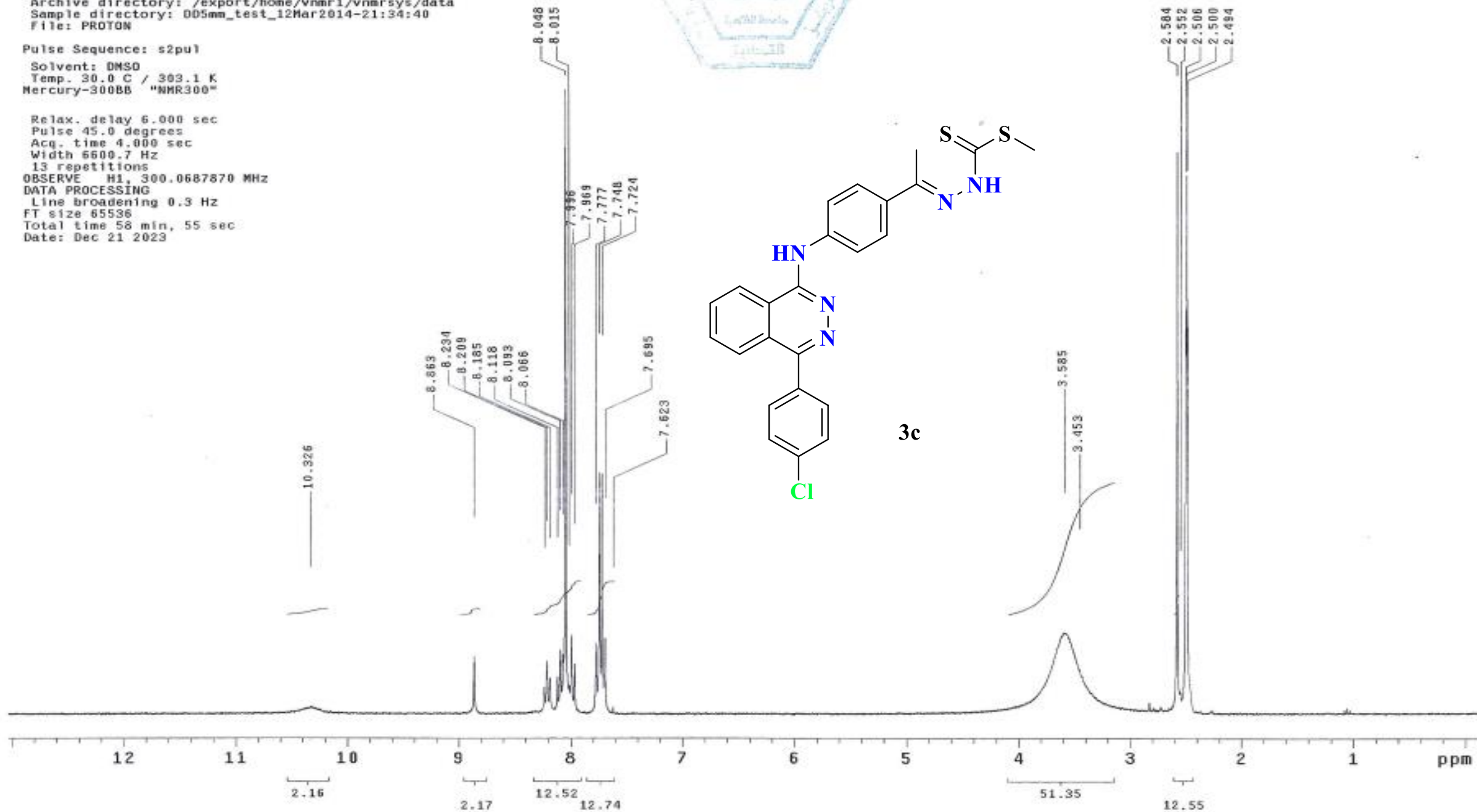
HatemHusein-VIIC-DMSO-H1

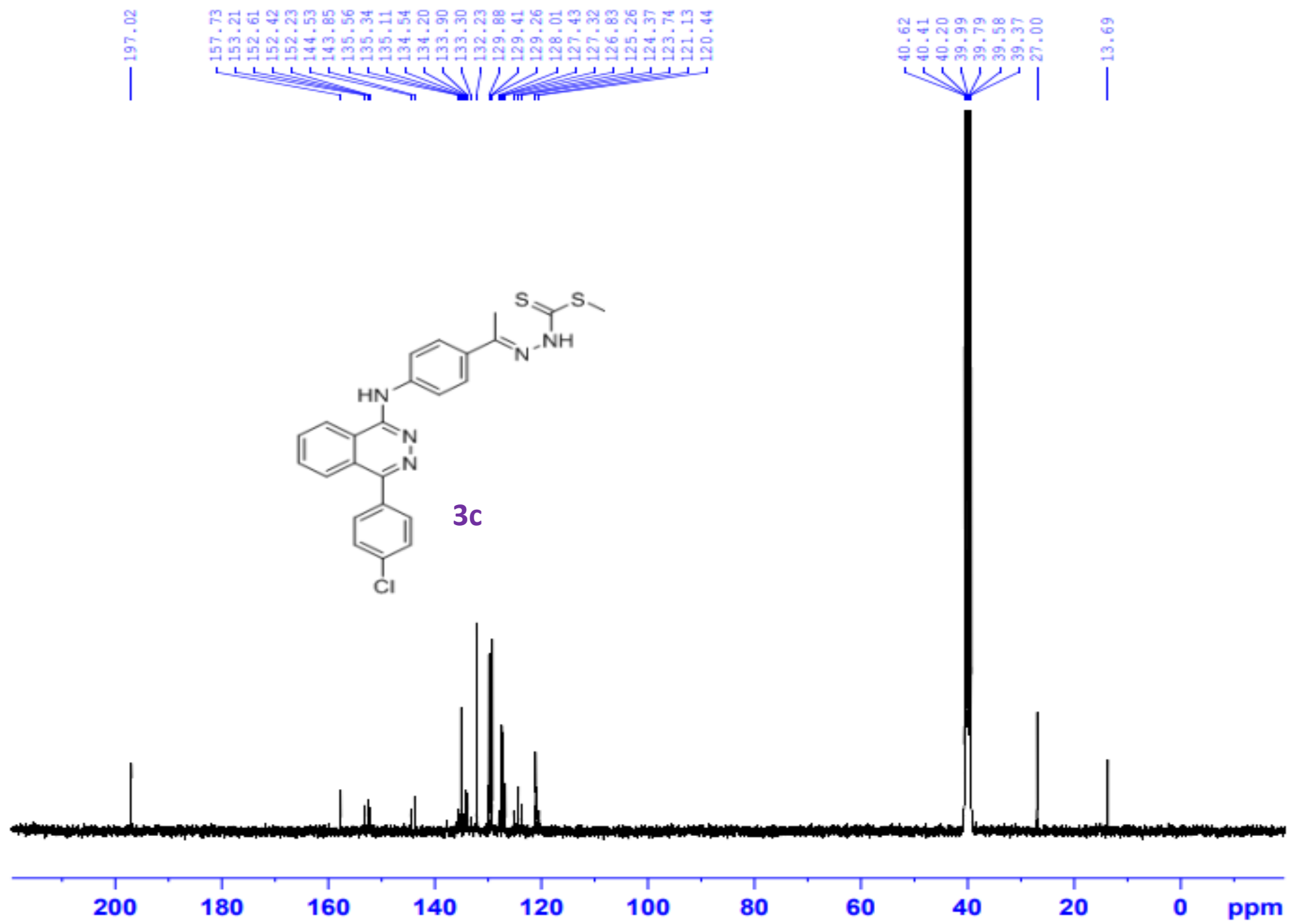
Archive directory: /export/home/vnmr1/vnmrsys/data  
Sample directory: 005mm\_test\_12Mar2014-21:34:40  
File: PROTON

Pulse Sequence: s2pu1

Solvent: DMSO  
Temp. 30.0 C / 303.1 K  
Mercury-300BB "NMR300"

Relax. delay 6.000 sec  
Pulse 45.0 degrees  
Acq. time 4.000 sec  
Width 6600.7 Hz  
13 repetitions  
OBSERVE H1, 300.0687870 MHz  
DATA PROCESSING  
Line broadening 0.3 Hz  
FT size 65536  
Total time 58 min, 55 sec  
Date: Dec 21 2023

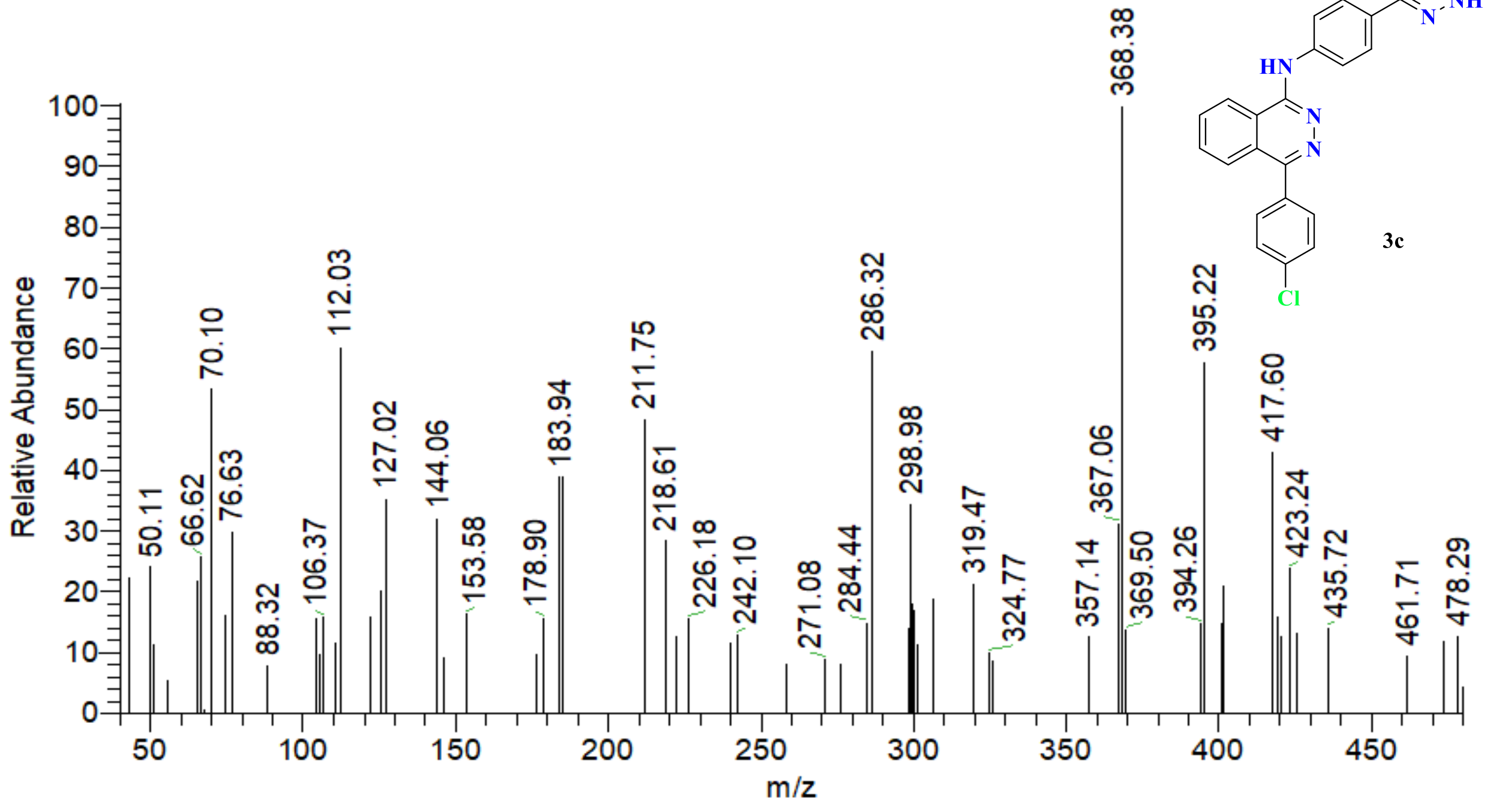




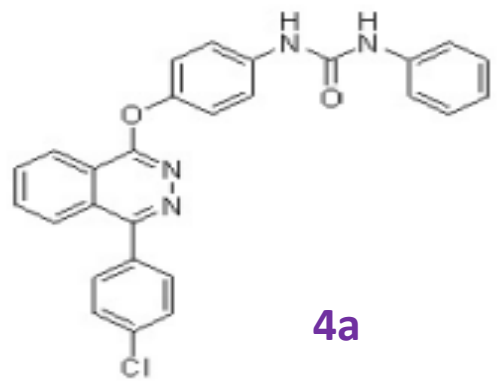
Current Data Parameters  
 NAME Hatem Abdel-aziz-VII a-C13-RR  
 EXPNO 10  
 PROCNO 1

F2 - Acquisition Parameters  
 Date\_ 20230621  
 Time 4.49 h  
 INSTRUM spect  
 PROBHD 2108618\_0945 ( )  
 PULPROG zgpg30  
 TD 65536  
 SOLVENT DMSO  
 NS 2200  
 DS 4  
 SWH 24038.461 Hz  
 FIDRES 0.733596 Hz  
 AQ 1.3631488 sec  
 RG 197.77  
 DW 20.800 usec  
 DE 6.50 usec  
 TE 297.0 K  
 D1 2.00000000 sec  
 D11 0.03000000 sec  
 TD0 1  
 SFO1 100.6404331 MHz  
 NUC1 13C  
 P1 10.00 usec  
 PLW1 47.00000000 W  
 SFO2 400.2016008 MHz  
 NUC2 1H  
 CPDPRG[2] waltz16  
 PCPD2 90.00 usec  
 PLW2 13.00000000 W  
 PLW12 0.29249999 W  
 PLW13 0.14713000 W

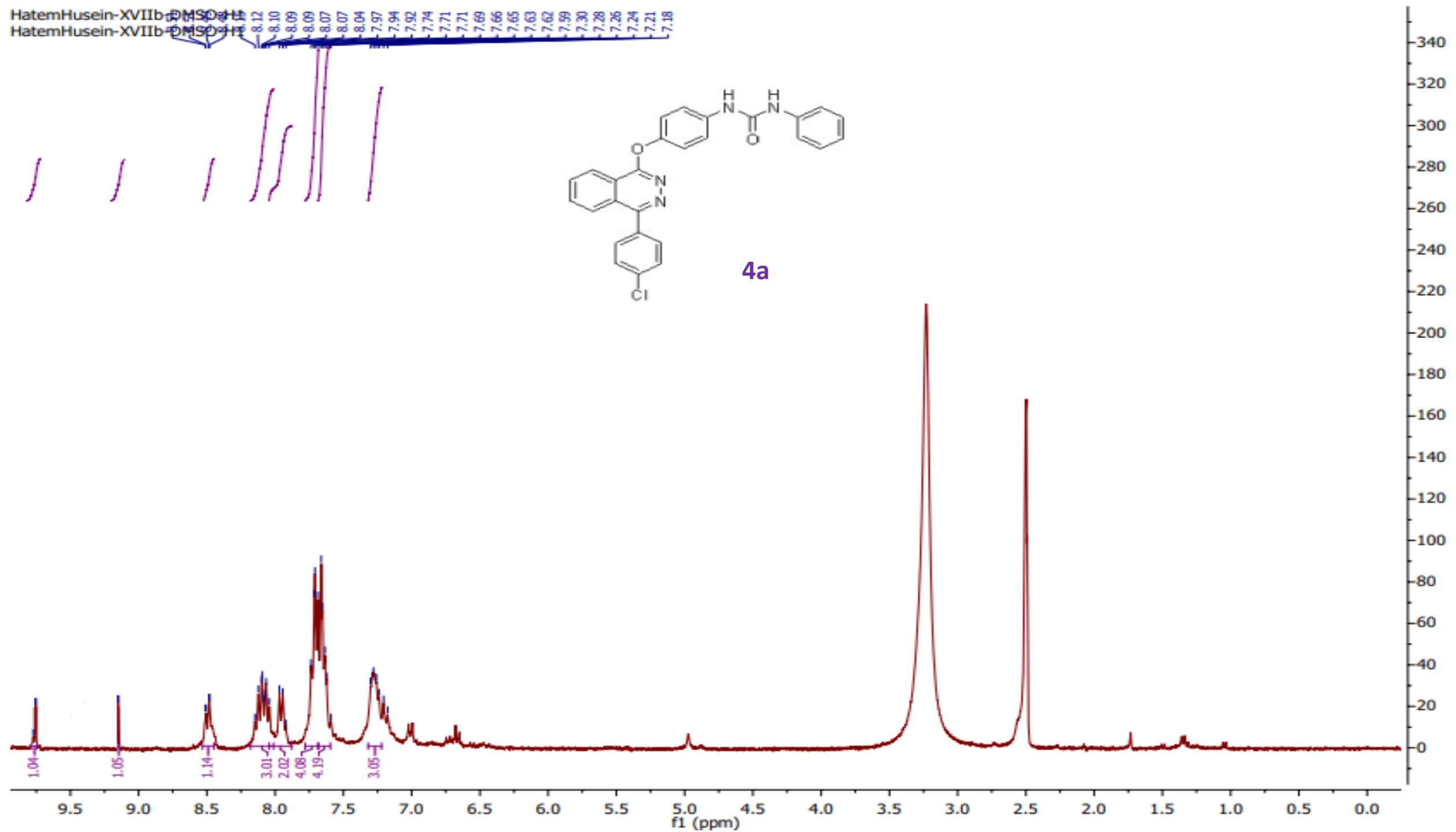
F2 - Processing parameters  
 SI 32768  
 SF 100.6303700 MHz  
 MDW EM  
 SSB 0  
 LB 1.00 Hz  
 GB 0  
 PC 1.40



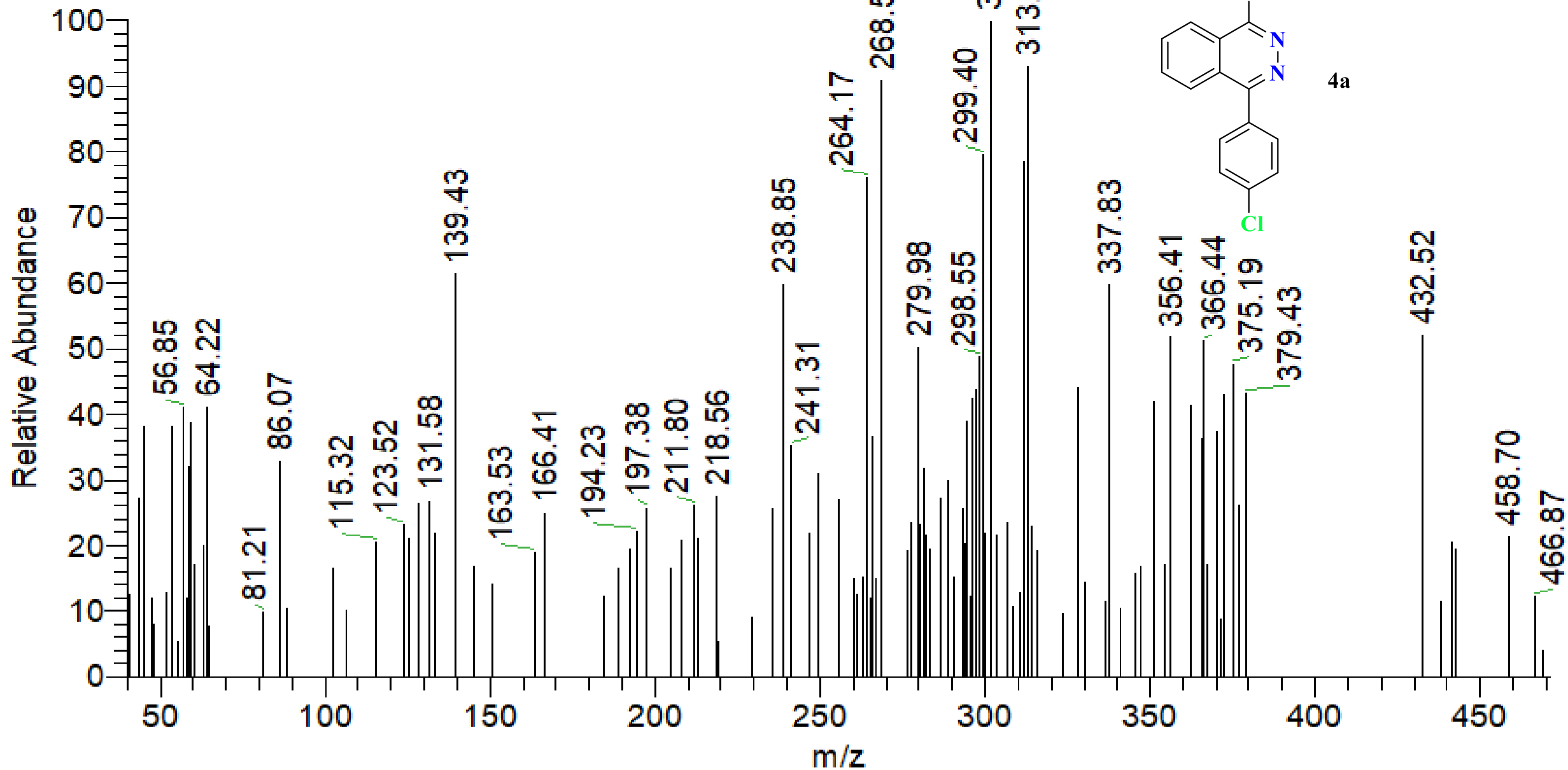
HatemHusein-XVIIb-DMSO-d<sub>6</sub>-H<sub>2</sub>O  
HatemHusein-XVIIb-DMSO-d<sub>6</sub>-H<sub>2</sub>O



4a

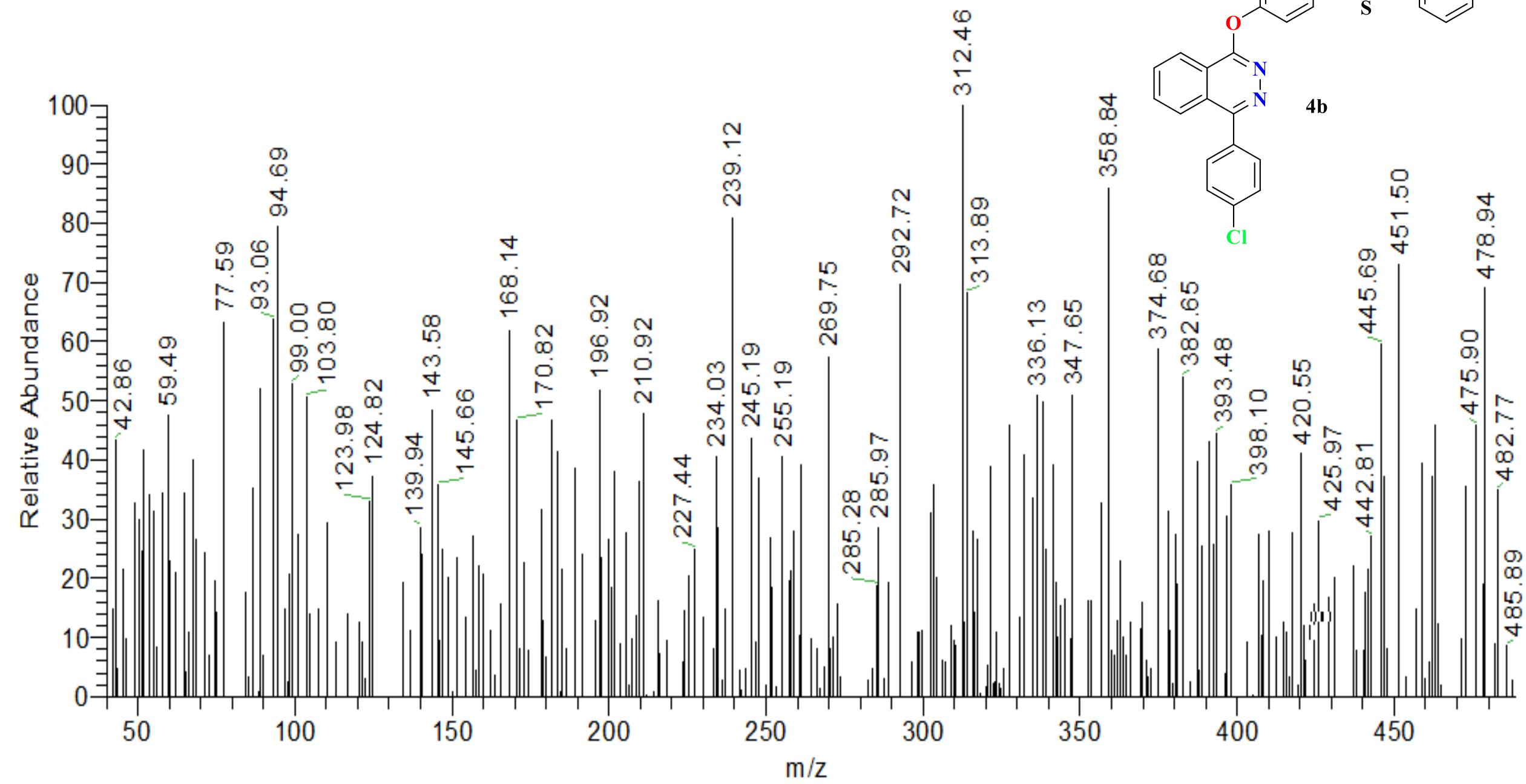


T: {0,0} + c EI Full ms [40.00-1000.00]



hahem-XVIIIB #48-64 RT: 0.82-1.09 AV: 17 SB: 26 1.21-1.34 , 0.87-1.14 NL: 5.03E1

T: {0,0} + c EI Full ms [40.00-1000.00]



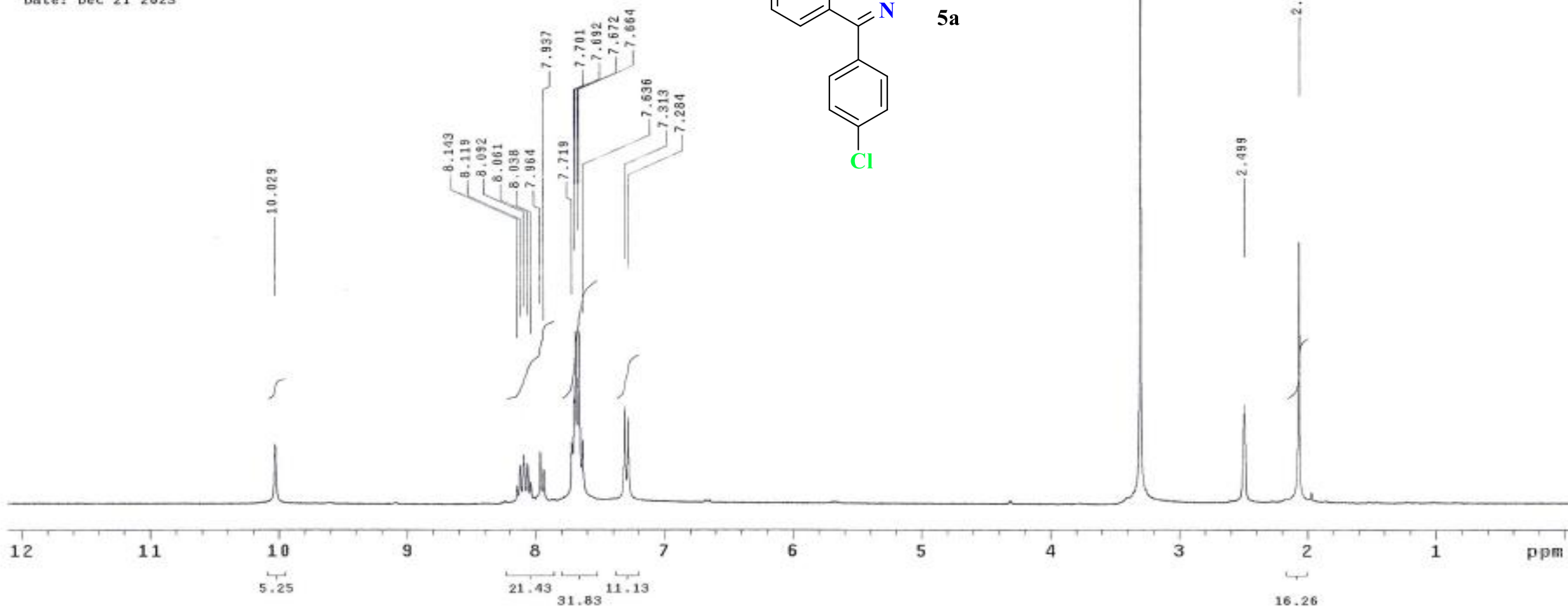
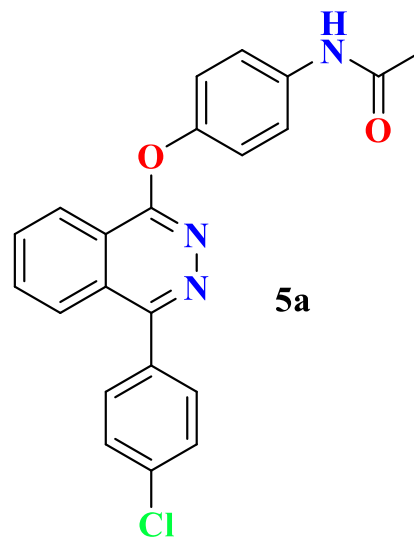
HatemHusein-XIXa-DMSO-H1

Archive directory: /export/home/vnmr1/vnmrsys/data  
Sample directory: DD5am\_test\_12Mar2014-21:34:40  
File: PROTON

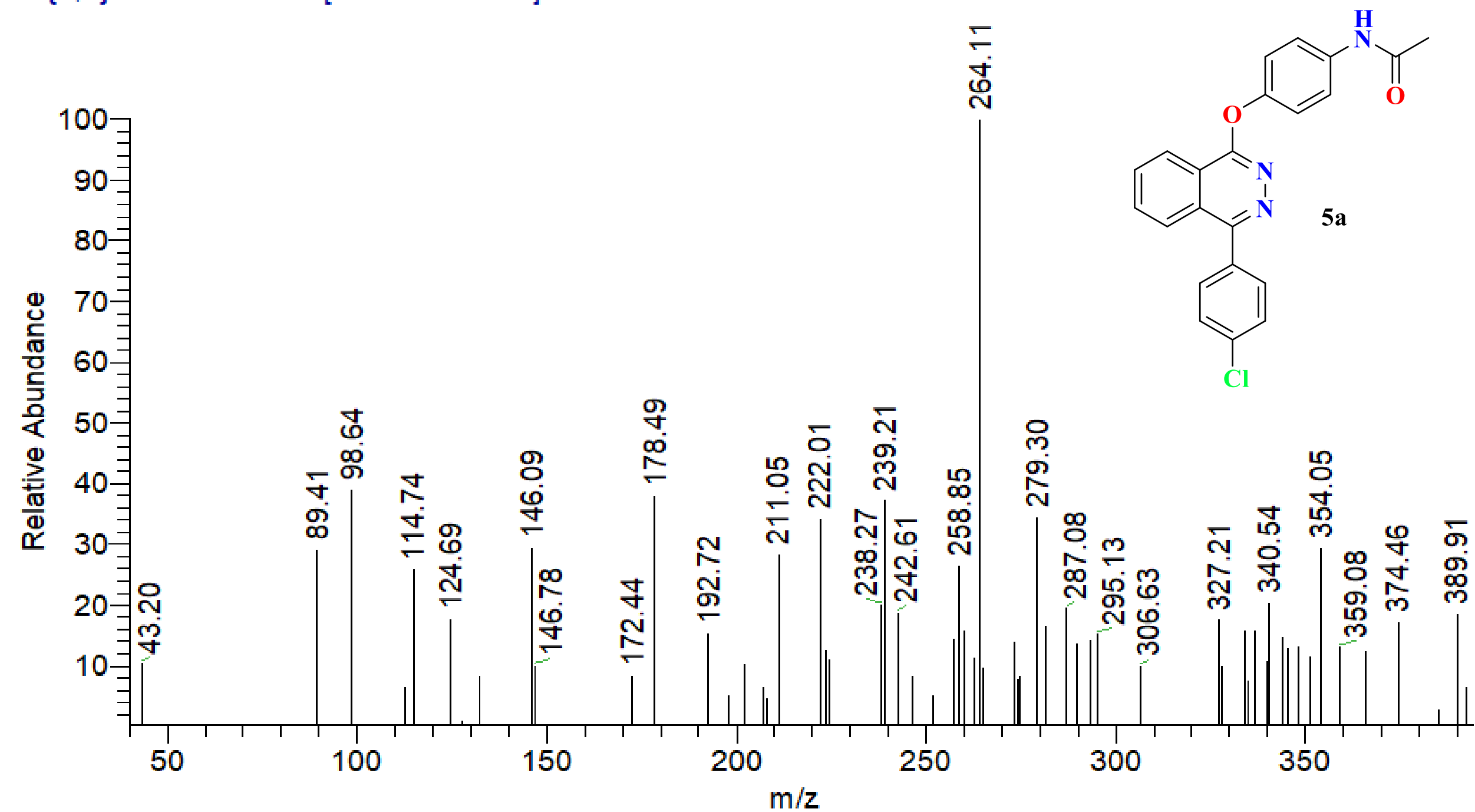
Pulse Sequence: s2pu1

Solvent: DMSO  
Temp. 30.0 C / 303.1 K  
Mercury-300BB "NMR300"

Relax. delay 6.000 sec  
Pulse 45.0 degrees  
Acq. time 4.000 sec  
Width 6600.7 Hz  
7 repetitions  
OBSERVE H1, 300.0687870 MHz  
DATA PROCESSING  
Line broadening 0.1 Hz  
FT size 65536  
Total time 58 min, 55 sec  
Date: Dec 21 2023

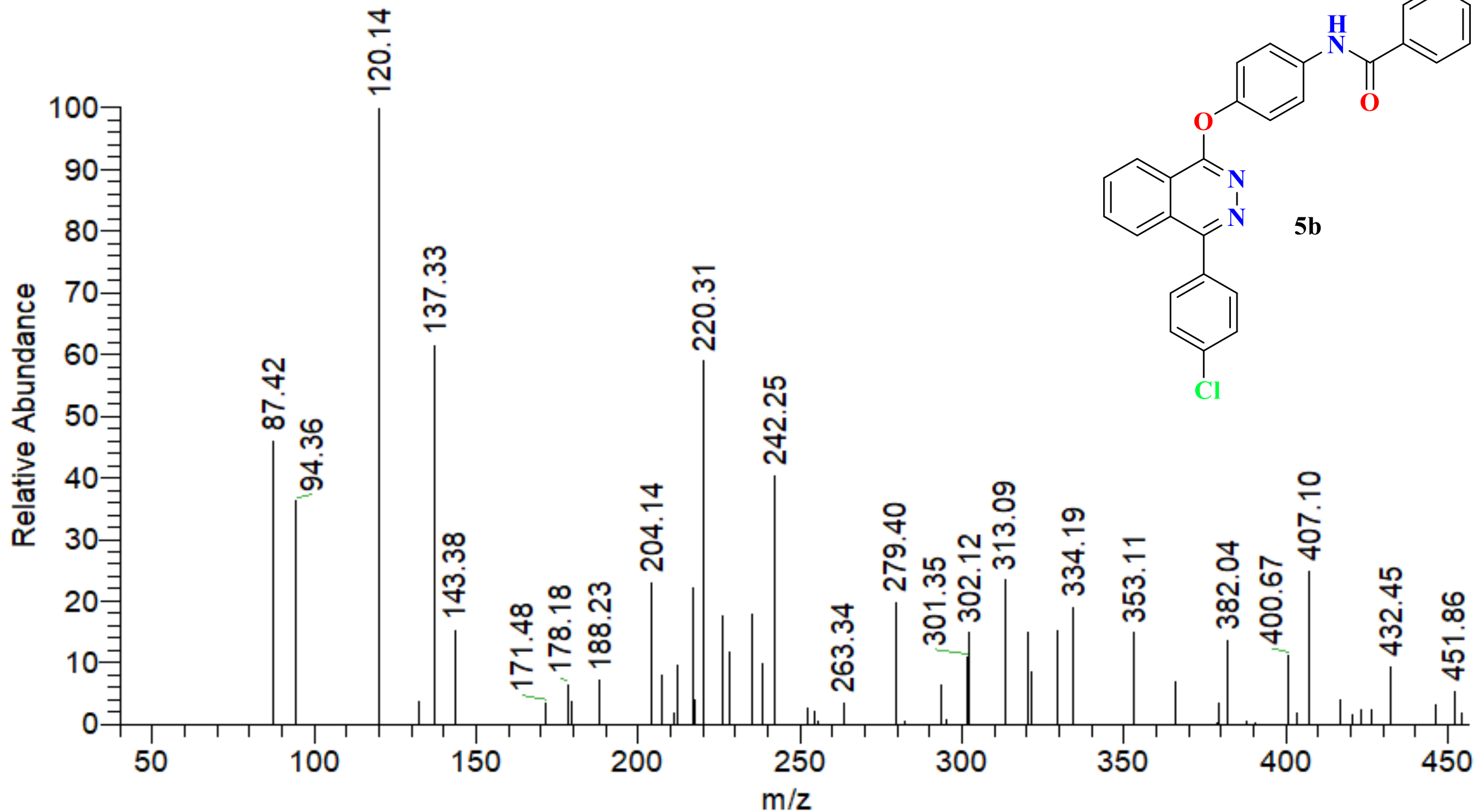


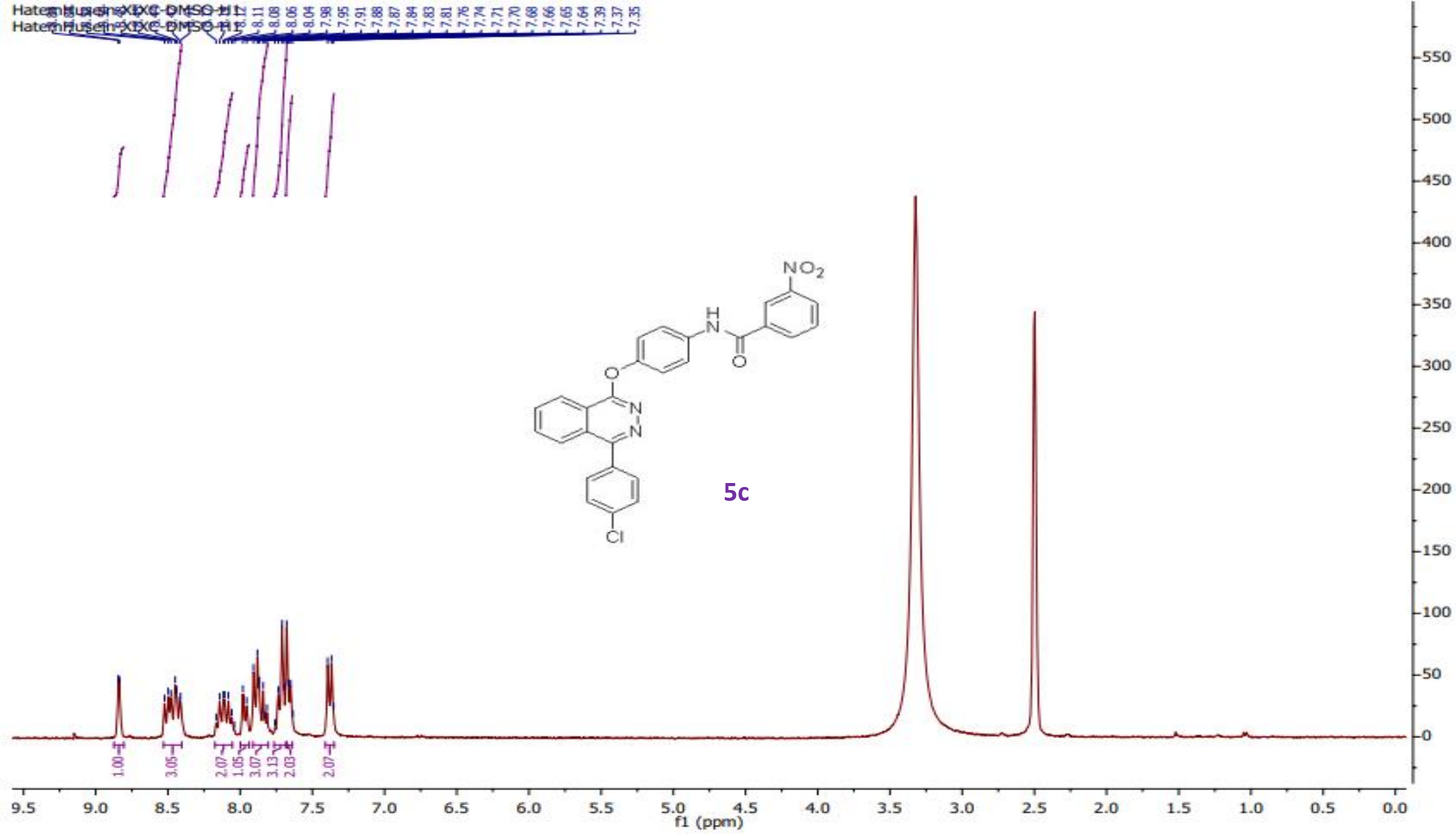
T: {0,0} + c EI Full ms [40.00-1000.00]

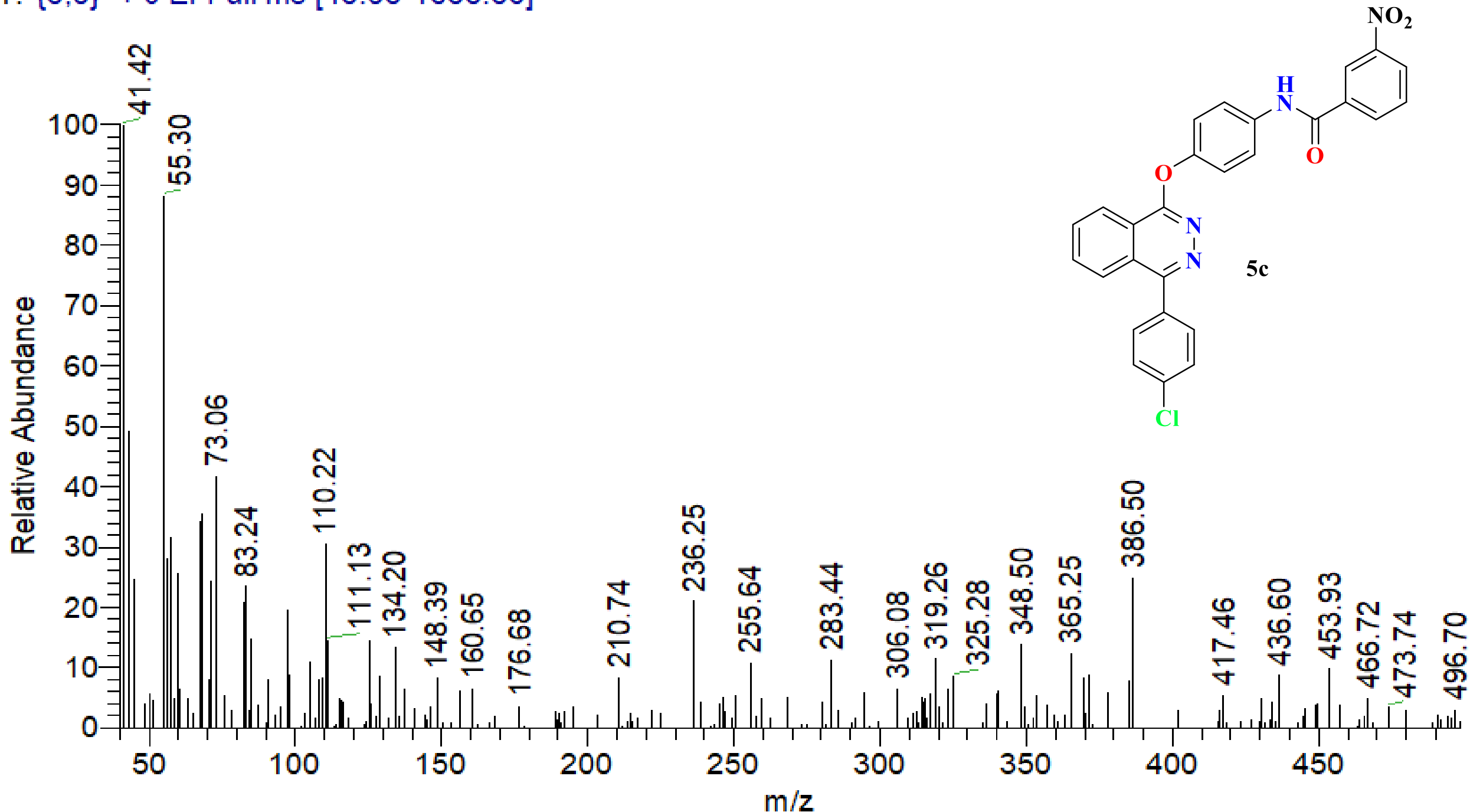




T: {0,0} + c EI Full ms [40.00-1000.00]



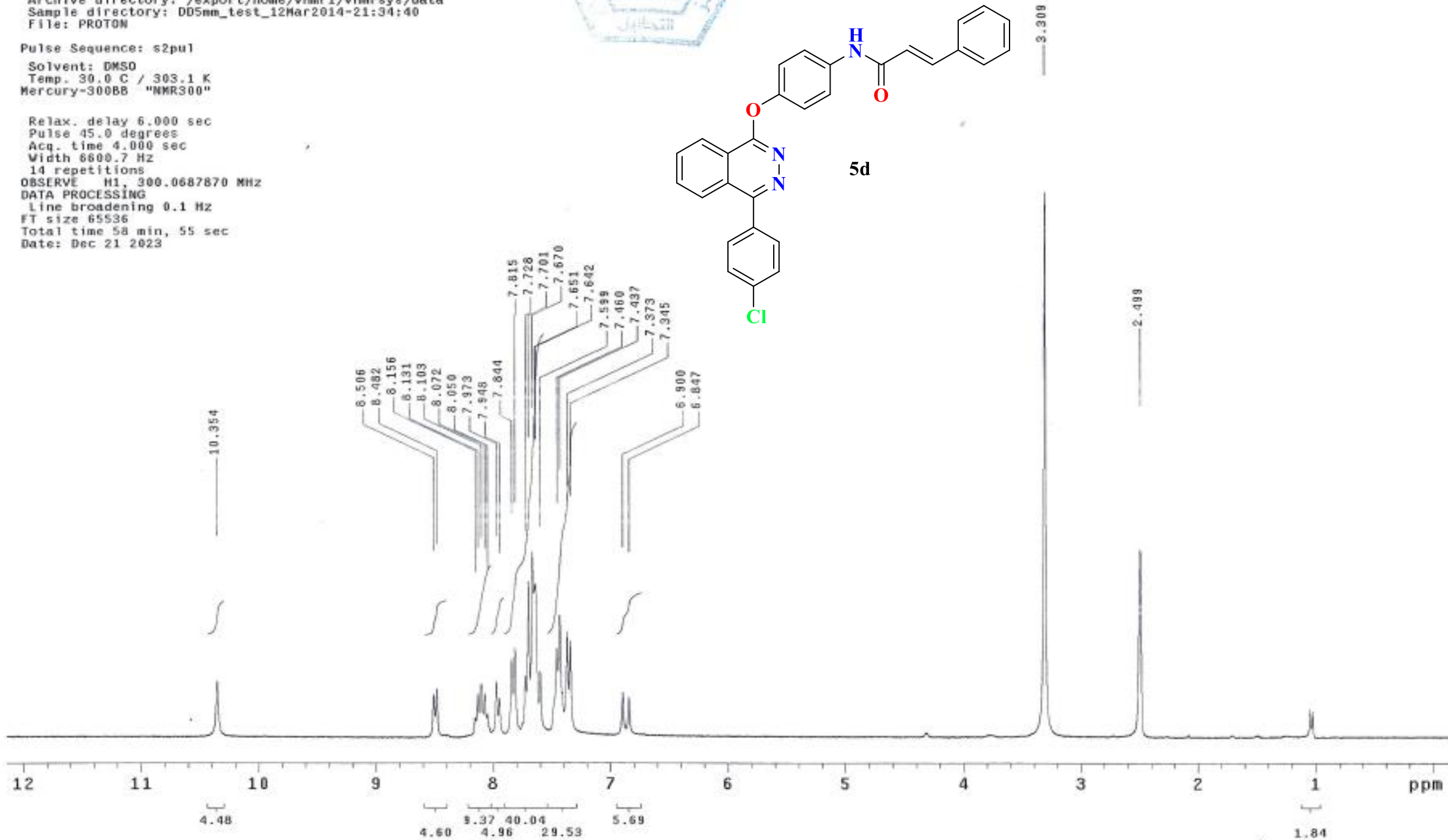
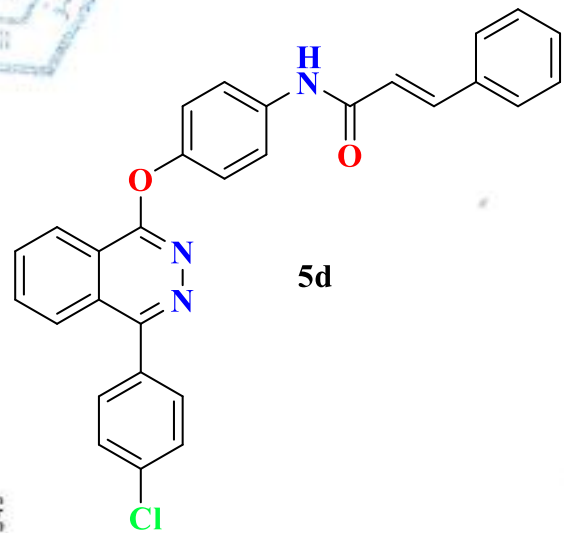


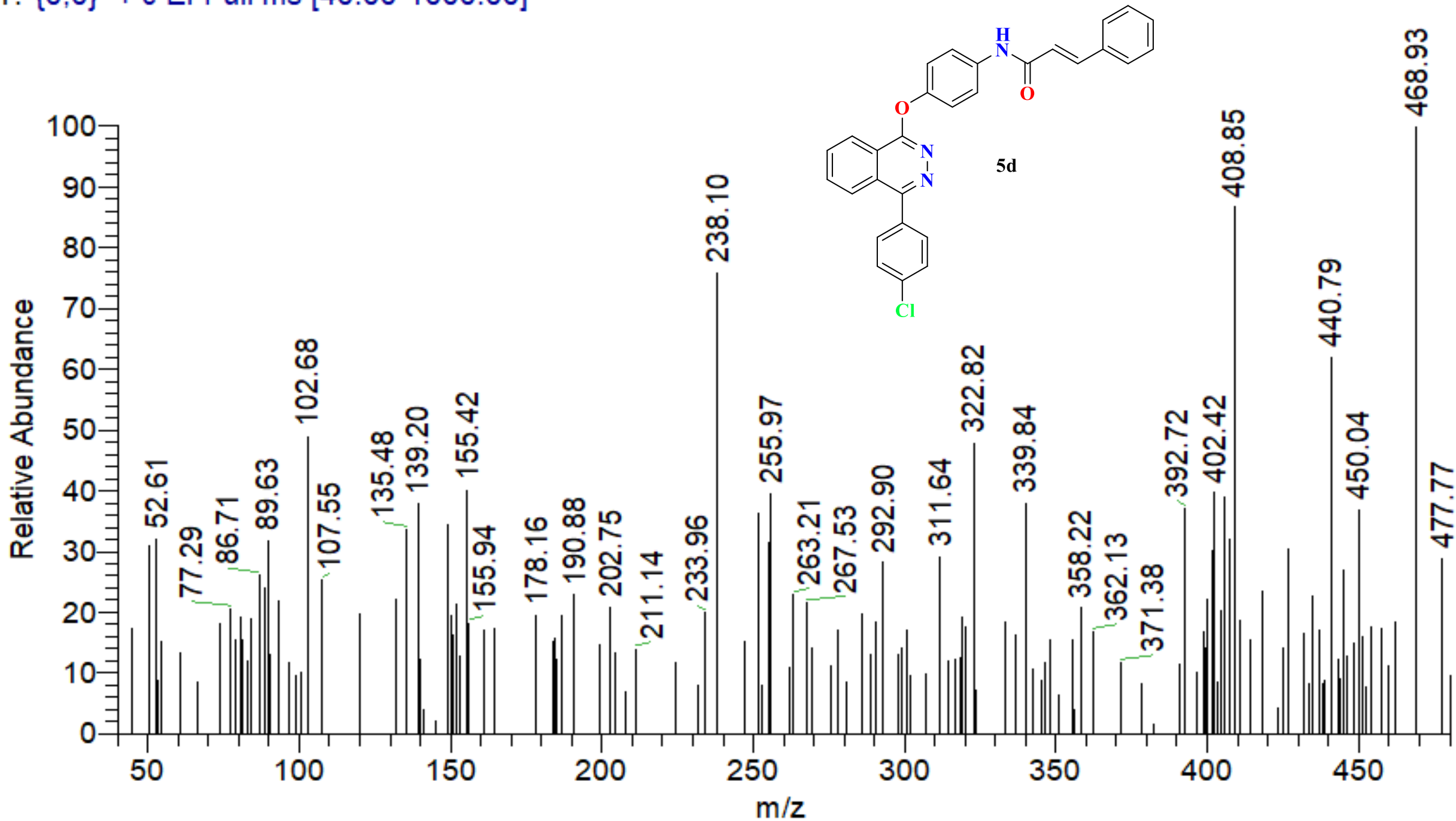


Archive directory: /export/home/vnmr1/vnmrsys/data  
Sample directory: DD5mm\_test\_12Mar2014-21:34:40  
File: PROTON

Pulse Sequence: s2pu1  
Solvent: DMSO  
Temp. 30.0 C / 303.1 K  
Mercury-300BB "NMR300"

Relax. delay 6.000 sec  
Pulse 45.0 degrees  
Acq. time 4.000 sec  
Width 6600.7 Hz  
14 repetitions  
OBSERVE H1, 300.0687870 MHz  
DATA PROCESSING  
Line broadening 0.1 Hz  
FT size 65536  
Total time 58 min, 55 sec  
Date: Dec 21 2023

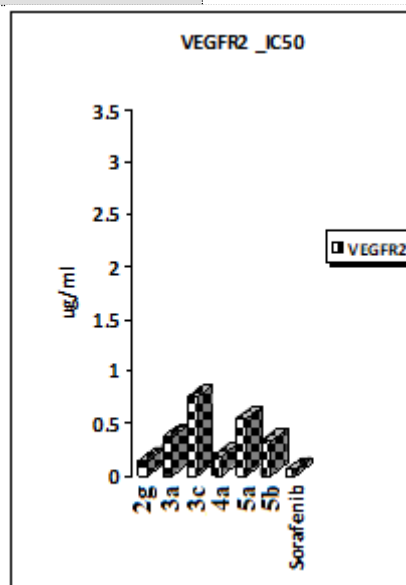




|            |                      |        |      |
|------------|----------------------|--------|------|
| Researcher | : Dr.Mohamed Dahab   | email: | mob. |
| Assay      | : VEGFR2 inh.assay   |        |      |
| Samples    | : 16 compounds       |        |      |
| Cell lines | : ---                |        |      |
| Ref.       | : ---                |        |      |
| Date       | : 11-10-2023         |        |      |
| Reader     | : Tecan Spark Reader |        |      |
| Kit used   | : ---.               |        |      |
| Solvent    | : DMSO               |        |      |

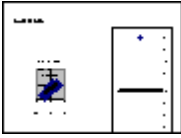
## Lab Report

| ser | Compound  | VEGFR2                 | SD<br>± |
|-----|-----------|------------------------|---------|
|     | code      | IC <sub>50</sub><br>uM |         |
| 1   | 2g        | 0.1479                 | 0.005   |
| 2   | 3a        | 0.3745                 | 0.014   |
| 3   | 3c        | 0.8921                 | 0.127   |
| 4   | 4a        | 0.1955                 | 0.007   |
| 5   | 5a        | 0.5482                 | 0.02    |
| 6   | 5b        | 0.331                  | 0.012   |
| *** | Sorafenib | 0.0591                 | 0.002   |





| code      | IC50 | conc | log | %inh | T2 | T1 | ΔT | RFU2  | RFU1 | ΔRFU  | slope | K.Activity |
|-----------|------|------|-----|------|----|----|----|-------|------|-------|-------|------------|
| EC        |      |      |     | 0    | 30 | 0  | 30 | 100   | 0    | 100   | 3.333 | 120        |
| Sorafenib |      | 100  | 2   | 95.6 | 30 | 0  | 30 | 4.43  | 0    | 4.43  | 3.333 | 5.316      |
|           |      | 10   | 1   | 89.6 | 30 | 0  | 30 | 10.39 | 0    | 10.39 | 3.333 | 12.468     |
|           |      | 1    | 0   | 75.9 | 30 | 0  | 30 | 24.11 | 0    | 24.11 | 3.333 | 28.932     |
|           |      | 0.1  | -1  | 57.4 | 30 | 0  | 30 | 42.57 | 0    | 42.57 | 3.333 | 51.084     |
|           |      | 0.01 | -2  | 30.8 | 30 | 0  | 30 | 69.18 | 0    | 69.18 | 3.333 | 83.016     |
| EC        |      |      |     | 0    | 30 | 0  | 30 | 100   | 0    | 100   | 3.333 | 120        |







## Viability assay

**Test code: T-9-023-8**

**Institute / Researcher: prof.Dr. hatim**

**Experiment :** functional assay (MTT)  
(viability/cytotoxicity)

**samples number :** 35

**experiment design : effect against HepG2 and Mcf7 cells**

### laboratory comments:

#### References

Slater, T. et al. (1963) Biochem. Biophys. Acta 77:383.

van de Loosdrecht, A.A., et al. J. Immunol. Methods 174: 311-320, 1994.

Alley, M.C., et al. Cancer Res. 48: 589-601, 1988.

## **Viability assay**

### **MTT protocol**

#### **Determination of sample cytotoxicity on cells (MTT protocol)**

- 1- the 96 well tissue culture plate was inoculated with  $1 \times 10^5$  cells / ml (100 ul / well) and incubated at 37°C for 24 hours to develop a complete monolayer sheet.
- 2- Growth medium was decanted from 96 well micro titer plates after confluent sheet of cells were formed, cell monolayer was washed twice with wash media.
- 3- two-fold dilutions of tested sample was made in RPMI medium with 2% serum (maintenance medium).
- 4- 0.1 ml of each dilution was tested in different wells leaving 3 wells as control, receiving only maintenance medium.
- 5- Plate was incubated at 37°C and examined. Cells were checked for any physical signs of toxicity, e.g. partial or complete loss of the monolayer, rounding, shrinkage, or cell granulation.
- 6- MTT solution was prepared (5mg/ml in PBS) (BIO BASIC CANADA INC).
- 8- 20ul MTT solution were added to each well. Place on a shaking table, 150rpm for 5 minutes, to thoroughly mix the MTT into the media.
- 9) Incubate (37C, 5% CO<sub>2</sub>) for 4 hours to allow the MTT to be metabolized.
- 10) Dump off the media. (dry plate on paper towels to remove residue if necessary).

11) Resuspend formazan (MTT metabolic product) in 200ul DMSO. Place on a shaking table, 150rpm for 5 minutes, to thoroughly mix the formazan into the solvent.

12) Read optical density at 560nm and subtract background at 620nm. Optical density should be directly correlated with cell quantity.

### **Morphological assay**

- ❖ Large-scale, morphological changes that occur at the cell surface, or in the cytoskeleton, can be followed and related to cell viability.
- ❖ Damage can be identified by large decreases in volume secondary to losses in protein and intracellular ions of due to altered permeability to sodium or potassium.
- ❖ Necrotic cells: nuclear swelling, chromatin flocculation, loss of nuclear basophilia
- ❖ Apoptotic cells: cell shrinkage, nuclear condensation, nuclear fragmentation

| ID   | uM    | O.D   |       |       | Mean O.D | ±SE      | Viability % | Toxicity %  | IC50 ± SD   |
|------|-------|-------|-------|-------|----------|----------|-------------|-------------|-------------|
| Mcf7 | ----- | 0.758 | 0.753 | 0.748 | 0.753    | 0.002887 | 100         | 0           | uM          |
| Vla  | 10    | 0.042 | 0.047 | 0.044 | 0.044333 | 0.001453 | 5.887560868 | 94.11243913 | 1.55 ± 0.01 |
|      | 5     | 0.04  | 0.037 | 0.04  | 0.039    | 0.001    | 5.179282869 | 94.82071713 |             |
|      | 2.5   | 0.095 | 0.104 | 0.111 | 0.103333 | 0.004631 | 13.72288623 | 86.27711377 |             |
|      | 1.25  | 0.387 | 0.368 | 0.392 | 0.382333 | 0.007311 | 50.77467906 | 49.22532094 |             |
|      | 0.625 | 0.744 | 0.748 | 0.735 | 0.742333 | 0.003844 | 98.583444   | 1.416555998 |             |
|      | 0.312 | 0.75  | 0.748 | 0.747 | 0.748333 | 0.000882 | 99.38025675 | 0.619743249 |             |
| Vlb  | 10    | 0.044 | 0.042 | 0.044 | 0.043333 | 0.000667 | 5.754758743 | 94.24524126 | 0.98 ± 0.01 |
|      | 5     | 0.05  | 0.043 | 0.047 | 0.046667 | 0.002028 | 6.197432492 | 93.80256751 |             |
|      | 2.5   | 0.1   | 0.107 | 0.123 | 0.11     | 0.006807 | 14.60823373 | 85.39176627 |             |
|      | 1.25  | 0.231 | 0.247 | 0.23  | 0.236    | 0.005508 | 31.34130146 | 68.65869854 |             |
|      | 0.625 | 0.576 | 0.58  | 0.559 | 0.571667 | 0.006438 | 75.91854803 | 24.08145197 |             |
|      | 0.312 | 0.719 | 0.684 | 0.708 | 0.703667 | 0.010333 | 93.44842851 | 6.551571492 |             |
| Vlc  | 10    | 0.036 | 0.033 | 0.037 | 0.035333 | 0.001202 | 4.692341744 | 95.30765826 | 1.74 ± 0.01 |
|      | 5     | 0.063 | 0.058 | 0.066 | 0.062333 | 0.002333 | 8.277999115 | 91.72200089 |             |
|      | 2.5   | 0.174 | 0.163 | 0.182 | 0.173    | 0.005508 | 22.9747676  | 77.0252324  |             |
|      | 1.25  | 0.461 | 0.489 | 0.453 | 0.467667 | 0.010914 | 62.10712705 | 37.89287295 |             |
|      | 0.625 | 0.746 | 0.758 | 0.751 | 0.751667 | 0.00348  | 99.8229305  | 0.1770695   |             |
|      | 0.312 | 0.755 | 0.749 | 0.753 | 0.752333 | 0.001764 | 99.91146525 | 0.08853475  |             |
| Vld  | 10    | 0.04  | 0.04  | 0.04  | 0.04     | 0        | 5.312084993 | 94.68791501 | 3.71 ± 0.07 |
|      | 5     | 0.251 | 0.249 | 0.263 | 0.254333 | 0.004372 | 33.77600708 | 66.22399292 |             |
|      | 2.5   | 0.46  | 0.453 | 0.478 | 0.463667 | 0.007446 | 61.57591855 | 38.42408145 |             |
|      | 1.25  | 0.682 | 0.691 | 0.668 | 0.680333 | 0.006692 | 90.34971226 | 9.650287738 |             |
|      | 0.625 | 0.71  | 0.704 | 0.724 | 0.712667 | 0.005925 | 94.64364763 | 5.356352368 |             |
|      | 0.312 | 0.746 | 0.744 | 0.753 | 0.747667 | 0.002728 | 99.291722   | 0.708277999 |             |
| Vle  | 10    | 0.057 | 0.062 | 0.058 | 0.059    | 0.001528 | 7.835325365 | 92.16467463 | 0.71 ± 0.02 |
|      | 5     | 0.043 | 0.047 | 0.041 | 0.043667 | 0.001764 | 5.799026118 | 94.20097388 |             |
|      | 2.5   | 0.04  | 0.039 | 0.042 | 0.040333 | 0.000882 | 5.356352368 | 94.64364763 |             |
|      | 1.25  | 0.094 | 0.099 | 0.126 | 0.106333 | 0.009939 | 14.12129261 | 85.87870739 |             |
|      | 0.625 | 0.328 | 0.319 | 0.346 | 0.331    | 0.007937 | 43.95750332 | 56.04249668 |             |
|      | 0.312 | 0.674 | 0.642 | 0.65  | 0.655333 | 0.009615 | 87.02965914 | 12.97034086 |             |

| ID   | uM    | O.D   |       |       | Mean O.D | ±SE      | Viability % | Toxicity %  | IC50 ± SD   |
|------|-------|-------|-------|-------|----------|----------|-------------|-------------|-------------|
| Mcf7 | ----- | 0.758 | 0.753 | 0.748 | 0.753    | 0.002887 | 100         | 0           | uM          |
| Vlf  | 10    | 0.036 | 0.037 | 0.037 | 0.036667 | 0.000333 | 4.869411244 | 95.13058876 | 0.78 ± 0.01 |
|      | 5     | 0.035 | 0.038 | 0.036 | 0.036333 | 0.000882 | 4.825143869 | 95.17485613 |             |
|      | 2.5   | 0.111 | 0.107 | 0.116 | 0.111333 | 0.002603 | 14.78530323 | 85.21469677 |             |
|      | 1.25  | 0.118 | 0.129 | 0.101 | 0.116    | 0.008145 | 15.40504648 | 84.59495352 |             |
|      | 0.625 | 0.389 | 0.356 | 0.372 | 0.372333 | 0.009528 | 49.44665781 | 50.55334219 |             |
|      | 0.312 | 0.757 | 0.751 | 0.751 | 0.753    | 0.002    | 100         | 0           |             |
| Vlg  | 10    | 0.074 | 0.055 | 0.069 | 0.066    | 0.005686 | 8.764940239 | 91.23505976 | 0.15 ± 0    |
|      | 5     | 0.03  | 0.058 | 0.063 | 0.050333 | 0.010269 | 6.684373617 | 93.31562638 |             |
|      | 2.5   | 0.044 | 0.042 | 0.04  | 0.042    | 0.001155 | 5.577689243 | 94.42231076 |             |
|      | 1.25  | 0.038 | 0.032 | 0.035 | 0.035    | 0.001732 | 4.648074369 | 95.35192563 |             |
|      | 0.625 | 0.033 | 0.036 | 0.032 | 0.033667 | 0.001202 | 4.471004869 | 95.52899513 |             |
|      | 0.312 | 0.058 | 0.07  | 0.062 | 0.063333 | 0.003528 | 8.410801239 | 91.58919876 |             |
|      | 0.156 | 0.189 | 0.163 | 0.204 | 0.185333 | 0.011977 | 24.61266047 | 75.38733953 |             |
|      | 0.078 | 0.639 | 0.61  | 0.628 | 0.625667 | 0.008452 | 83.08986277 | 16.91013723 |             |
| Vlla | 10    | 0.057 | 0.088 | 0.073 | 0.072667 | 0.00895  | 9.650287738 | 90.34971226 | 0.18 ± 0    |
|      | 5     | 0.069 | 0.066 | 0.07  | 0.068333 | 0.001202 | 9.074811864 | 90.92518814 |             |
|      | 2.5   | 0.064 | 0.069 | 0.072 | 0.068333 | 0.002333 | 9.074811864 | 90.92518814 |             |
|      | 1.25  | 0.047 | 0.038 | 0.04  | 0.041667 | 0.002728 | 5.533421868 | 94.46657813 |             |
|      | 0.625 | 0.031 | 0.037 | 0.035 | 0.034333 | 0.001764 | 4.559539619 | 95.44046038 |             |
|      | 0.312 | 0.044 | 0.051 | 0.059 | 0.051333 | 0.004333 | 6.817175741 | 93.18282426 |             |
|      | 0.156 | 0.381 | 0.364 | 0.395 | 0.38     | 0.008963 | 50.46480744 | 49.53519256 |             |
|      | 0.078 | 0.666 | 0.683 | 0.692 | 0.680333 | 0.007623 | 90.34971226 | 9.650287738 |             |
| Vllb | 10    | 0.026 | 0.024 | 0.028 | 0.026    | 0.001155 | 3.452855246 | 96.54714475 | 0.87 ± 0.02 |
|      | 5     | 0.042 | 0.058 | 0.063 | 0.054333 | 0.006333 | 7.215582116 | 92.78441788 |             |
|      | 2.5   | 0.094 | 0.099 | 0.082 | 0.091667 | 0.005044 | 12.17352811 | 87.82647189 |             |
|      | 1.25  | 0.194 | 0.206 | 0.222 | 0.207333 | 0.00811  | 27.53430722 | 72.46569278 |             |
|      | 0.625 | 0.416 | 0.447 | 0.424 | 0.429    | 0.009292 | 56.97211155 | 43.02788845 |             |
|      | 0.312 | 0.748 | 0.752 | 0.755 | 0.751667 | 0.002028 | 99.8229305  | 0.1770695   |             |
| Vllc | 10    | 0.05  | 0.05  | 0.062 | 0.054    | 0.004    | 7.171314741 | 92.82868526 | 0.2 ± 0     |
|      | 5     | 0.052 | 0.038 | 0.041 | 0.043667 | 0.004256 | 5.799026118 | 94.20097388 |             |
|      | 2.5   | 0.073 | 0.089 | 0.072 | 0.078    | 0.005508 | 10.35856574 | 89.64143426 |             |
|      | 1.25  | 0.077 | 0.08  | 0.064 | 0.073667 | 0.00491  | 9.783089863 | 90.21691014 |             |
|      | 0.625 | 0.11  | 0.084 | 0.095 | 0.096333 | 0.007535 | 12.79327136 | 87.20672864 |             |
|      | 0.312 | 0.183 | 0.192 | 0.165 | 0.18     | 0.007937 | 23.90438247 | 76.09561753 |             |
|      | 0.156 | 0.38  | 0.392 | 0.375 | 0.382333 | 0.005044 | 50.77467906 | 49.22532094 |             |
|      | 0.078 | 0.684 | 0.699 | 0.716 | 0.699667 | 0.009244 | 92.91722001 | 7.082779991 |             |

| ID   | uM    | O.D   |       |       | Mean<br>O.D | ±SE      | Viability % | Toxicity %  | IC50<br>± SD   |
|------|-------|-------|-------|-------|-------------|----------|-------------|-------------|----------------|
| Mcf7 | ----- | 0.758 | 0.753 | 0.748 | 0.753       | 0.002887 | 100         | 0           | uM             |
| IXa  | 10    | 0.022 | 0.019 | 0.02  | 0.020333    | 0.000882 | 2.700309872 | 97.29969013 | 1.16 ±<br>0.01 |
|      | 5     | 0.022 | 0.018 | 0.02  | 0.02        | 0.001155 | 2.656042497 | 97.3439575  |                |
|      | 2.5   | 0.037 | 0.038 | 0.039 | 0.038       | 0.000577 | 5.046480744 | 94.95351926 |                |
|      | 1.25  | 0.329 | 0.333 | 0.316 | 0.326       | 0.005132 | 43.2934927  | 56.7065073  |                |
|      | 0.625 | 0.694 | 0.722 | 0.687 | 0.701       | 0.010693 | 93.09428951 | 6.905710491 |                |
|      | 0.312 | 0.737 | 0.748 | 0.751 | 0.745333    | 0.004256 | 98.98185038 | 1.018149624 |                |
| IXb  | 10    | 0.046 | 0.044 | 0.045 | 0.045       | 0.000577 | 5.976095618 | 94.02390438 | 0.74 ±<br>0.01 |
|      | 5     | 0.038 | 0.027 | 0.03  | 0.031667    | 0.003283 | 4.20540062  | 95.79459938 |                |
|      | 2.5   | 0.047 | 0.047 | 0.043 | 0.045667    | 0.001333 | 6.064630367 | 93.93536963 |                |
|      | 1.25  | 0.039 | 0.041 | 0.028 | 0.036       | 0.004041 | 4.780876494 | 95.21912351 |                |
|      | 0.625 | 0.382 | 0.365 | 0.39  | 0.379       | 0.007371 | 50.33200531 | 49.66799469 |                |
|      | 0.312 | 0.733 | 0.719 | 0.746 | 0.732667    | 0.007796 | 97.29969013 | 2.700309872 |                |
| IXc  | 10    | 0.022 | 0.024 | 0.021 | 0.022333    | 0.000882 | 2.965914121 | 97.03408588 | 0.83 ±<br>0.02 |
|      | 5     | 0.028 | 0.023 | 0.025 | 0.025333    | 0.001453 | 3.364320496 | 96.6356795  |                |
|      | 2.5   | 0.022 | 0.034 | 0.028 | 0.028       | 0.003464 | 3.718459495 | 96.2815405  |                |
|      | 1.25  | 0.091 | 0.128 | 0.153 | 0.124       | 0.018009 | 16.46746348 | 83.53253652 |                |
|      | 0.625 | 0.483 | 0.446 | 0.461 | 0.463333    | 0.010745 | 61.53165117 | 38.46834883 |                |
|      | 0.312 | 0.756 | 0.741 | 0.748 | 0.748333    | 0.004333 | 99.38025675 | 0.619743249 |                |
| IXd  | 10    | 0.038 | 0.042 | 0.04  | 0.04        | 0.001155 | 5.312084993 | 94.68791501 | 0.16 ± 0       |
|      | 5     | 0.04  | 0.038 | 0.038 | 0.038667    | 0.000667 | 5.135015494 | 94.86498451 |                |
|      | 2.5   | 0.038 | 0.046 | 0.043 | 0.042333    | 0.002333 | 5.621956618 | 94.37804338 |                |
|      | 1.25  | 0.044 | 0.04  | 0.036 | 0.04        | 0.002309 | 5.312084993 | 94.68791501 |                |
|      | 0.625 | 0.058 | 0.043 | 0.052 | 0.051       | 0.004359 | 6.772908367 | 93.22709163 |                |
|      | 0.312 | 0.055 | 0.059 | 0.071 | 0.061667    | 0.004807 | 8.189464365 | 91.81053564 |                |
|      | 0.156 | 0.241 | 0.204 | 0.227 | 0.224       | 0.010786 | 29.74767596 | 70.25232404 |                |
|      | 0.078 | 0.661 | 0.632 | 0.658 | 0.650333    | 0.009207 | 86.36564852 | 13.63435148 |                |
| IXe  | 10    | 0.052 | 0.039 | 0.048 | 0.046333    | 0.003844 | 6.153165117 | 93.84683488 | 0.88 ±<br>0.03 |
|      | 5     | 0.055 | 0.042 | 0.046 | 0.047667    | 0.003844 | 6.330234617 | 93.66976538 |                |
|      | 2.5   | 0.11  | 0.107 | 0.125 | 0.114       | 0.005568 | 15.13944223 | 84.86055777 |                |
|      | 1.25  | 0.153 | 0.168 | 0.17  | 0.163667    | 0.005364 | 21.7352811  | 78.2647189  |                |
|      | 0.625 | 0.452 | 0.479 | 0.561 | 0.497333    | 0.032774 | 66.04692342 | 33.95307658 |                |
|      | 0.312 | 0.757 | 0.751 | 0.75  | 0.752667    | 0.002186 | 99.95573263 | 0.044267375 |                |

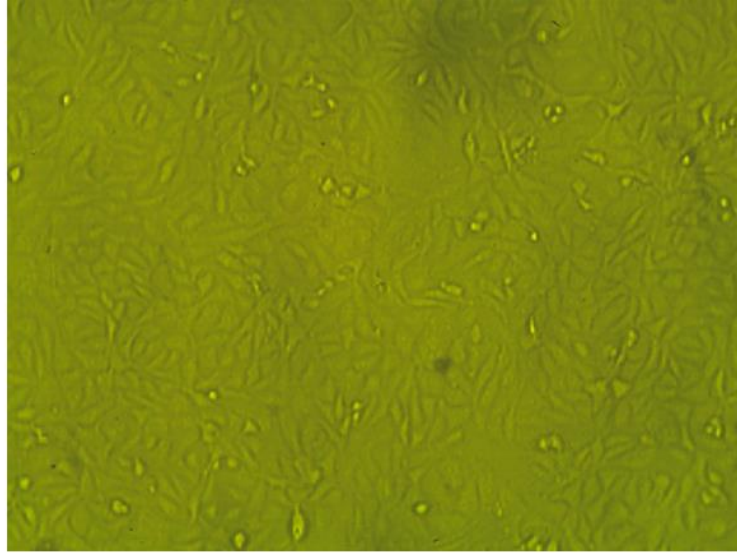
| ID   | uM    | O.D   |       |       | Mean O.D | ±SE      | Viability % | Toxicity %  | IC50 ± SD   |
|------|-------|-------|-------|-------|----------|----------|-------------|-------------|-------------|
| Mcf7 | ----- | 0.758 | 0.753 | 0.748 | 0.753    | 0.002887 | 100         | 0           | uM          |
| IXf  | 10    | 0.05  | 0.047 | 0.048 | 0.048333 | 0.000882 | 6.418769367 | 93.58123063 | 0.11 ± 0.01 |
|      | 5     | 0.044 | 0.046 | 0.049 | 0.046333 | 0.001453 | 6.153165117 | 93.84683488 |             |
|      | 2.5   | 0.04  | 0.043 | 0.048 | 0.043667 | 0.002333 | 5.799026118 | 94.20097388 |             |
|      | 1.25  | 0.042 | 0.037 | 0.039 | 0.039333 | 0.001453 | 5.223550243 | 94.77644976 |             |
|      | 0.625 | 0.04  | 0.044 | 0.041 | 0.041667 | 0.001202 | 5.533421868 | 94.46657813 |             |
|      | 0.312 | 0.072 | 0.068 | 0.061 | 0.067    | 0.003215 | 8.897742364 | 91.10225764 |             |
|      | 0.156 | 0.182 | 0.153 | 0.166 | 0.167    | 0.008386 | 22.17795485 | 77.82204515 |             |
|      | 0.078 | 0.452 | 0.489 | 0.471 | 0.470667 | 0.010682 | 62.50553342 | 37.49446658 |             |
| IXg  | 10    | 0.039 | 0.045 | 0.04  | 0.041333 | 0.001856 | 5.489154493 | 94.51084551 | 0.06 ± 0.01 |
|      | 5     | 0.042 | 0.047 | 0.04  | 0.043    | 0.002082 | 5.710491368 | 94.28950863 |             |
|      | 2.5   | 0.038 | 0.044 | 0.044 | 0.042    | 0.002    | 5.577689243 | 94.42231076 |             |
|      | 1.25  | 0.028 | 0.034 | 0.035 | 0.032333 | 0.002186 | 4.29393537  | 95.70606463 |             |
|      | 0.625 | 0.022 | 0.021 | 0.028 | 0.023667 | 0.002186 | 3.142983621 | 96.85701638 |             |
|      | 0.312 | 0.026 | 0.038 | 0.03  | 0.031333 | 0.003528 | 4.161133245 | 95.83886676 |             |
|      | 0.156 | 0.166 | 0.193 | 0.182 | 0.180333 | 0.007839 | 23.94864985 | 76.05135015 |             |
|      | 0.078 | 0.378 | 0.361 | 0.39  | 0.376333 | 0.008413 | 49.97786631 | 50.02213369 |             |
| IXh  | 10    | 0.063 | 0.066 | 0.061 | 0.063333 | 0.001453 | 8.410801239 | 91.58919876 | 0.08 ± 0.01 |
|      | 5     | 0.057 | 0.06  | 0.066 | 0.061    | 0.002646 | 8.100929615 | 91.89907039 |             |
|      | 2.5   | 0.064 | 0.063 | 0.061 | 0.062667 | 0.000882 | 8.32226649  | 91.67773351 |             |
|      | 1.25  | 0.058 | 0.05  | 0.059 | 0.055667 | 0.002848 | 7.392651616 | 92.60734838 |             |
|      | 0.625 | 0.072 | 0.06  | 0.084 | 0.072    | 0.006928 | 9.561752988 | 90.43824701 |             |
|      | 0.312 | 0.088 | 0.079 | 0.091 | 0.086    | 0.003606 | 11.42098274 | 88.57901726 |             |
|      | 0.156 | 0.127 | 0.162 | 0.14  | 0.143    | 0.010214 | 18.99070385 | 81.00929615 |             |
|      | 0.078 | 0.399 | 0.416 | 0.382 | 0.399    | 0.009815 | 52.98804781 | 47.01195219 |             |
| IXi  | 10    | 0.047 | 0.044 | 0.041 | 0.044    | 0.001732 | 5.843293493 | 94.15670651 | 0.86 ± 0.01 |
|      | 5     | 0.058 | 0.073 | 0.088 | 0.073    | 0.00866  | 9.694555113 | 90.30544489 |             |
|      | 2.5   | 0.092 | 0.118 | 0.102 | 0.104    | 0.007572 | 13.81142098 | 86.18857902 |             |
|      | 1.25  | 0.138 | 0.119 | 0.12  | 0.125667 | 0.006173 | 16.68880035 | 83.31119965 |             |
|      | 0.625 | 0.539 | 0.512 | 0.548 | 0.533    | 0.010817 | 70.78353254 | 29.21646746 |             |
|      | 0.312 | 0.731 | 0.748 | 0.716 | 0.731667 | 0.009244 | 97.166888   | 2.833111996 |             |
| XIIa | 10    | 0.05  | 0.05  | 0.05  | 0.05     | 4.91E-18 | 6.640106242 | 93.35989376 | 0.17 ± 0    |
|      | 5     | 0.05  | 0.055 | 0.05  | 0.051667 | 0.001667 | 6.861443116 | 93.13855688 |             |
|      | 2.5   | 0.055 | 0.055 | 0.055 | 0.055    | 0        | 7.304116866 | 92.69588313 |             |
|      | 1.25  | 0.048 | 0.039 | 0.04  | 0.042333 | 0.002848 | 5.621956618 | 94.37804338 |             |
|      | 0.625 | 0.033 | 0.032 | 0.037 | 0.034    | 0.001528 | 4.515272244 | 95.48472776 |             |
|      | 0.312 | 0.084 | 0.123 | 0.104 | 0.103667 | 0.01126  | 13.76715361 | 86.23284639 |             |
|      | 0.156 | 0.261 | 0.299 | 0.283 | 0.281    | 0.011015 | 37.31739708 | 62.68260292 |             |
|      | 0.078 | 0.658 | 0.62  | 0.619 | 0.632333 | 0.012837 | 83.97521027 | 16.02478973 |             |

| ID   | uM    | O.D   |       |       | Mean O.D | ±SE      | Viability % | Toxicity %  | IC50 ± SD   |
|------|-------|-------|-------|-------|----------|----------|-------------|-------------|-------------|
| Mcf7 | ----- | 0.758 | 0.753 | 0.748 | 0.753    | 0.002887 | 100         | 0           | uM          |
| XIIb | 10    | 0.047 | 0.06  | 0.05  | 0.052333 | 0.00393  | 6.949977866 | 93.05002213 | 0.08 ± 0.01 |
|      | 5     | 0.055 | 0.05  | 0.05  | 0.051667 | 0.001667 | 6.861443116 | 93.13855688 |             |
|      | 2.5   | 0.047 | 0.051 | 0.049 | 0.049    | 0.001155 | 6.507304117 | 93.49269588 |             |
|      | 1.25  | 0.02  | 0.019 | 0.022 | 0.020333 | 0.000882 | 2.700309872 | 97.29969013 |             |
|      | 0.625 | 0.027 | 0.032 | 0.033 | 0.030667 | 0.001856 | 4.072598495 | 95.92740151 |             |
|      | 0.312 | 0.038 | 0.056 | 0.041 | 0.045    | 0.005568 | 5.976095618 | 94.02390438 |             |
|      | 0.156 | 0.148 | 0.172 | 0.152 | 0.157333 | 0.007424 | 20.89420097 | 79.10579903 |             |
|      | 0.078 | 0.416 | 0.392 | 0.441 | 0.416333 | 0.014146 | 55.28995131 | 44.71004869 |             |
| XIIc | 10    | 0.055 | 0.055 | 0.055 | 0.055    | 0        | 7.304116866 | 92.69588313 | 0.12 ± 0.01 |
|      | 5     | 0.054 | 0.057 | 0.057 | 0.056    | 0.001    | 7.436918991 | 92.56308101 |             |
|      | 2.5   | 0.055 | 0.056 | 0.054 | 0.055    | 0.000577 | 7.304116866 | 92.69588313 |             |
|      | 1.25  | 0.072 | 0.068 | 0.077 | 0.072333 | 0.002603 | 9.606020363 | 90.39397964 |             |
|      | 0.625 | 0.101 | 0.081 | 0.093 | 0.091667 | 0.005812 | 12.17352811 | 87.82647189 |             |
|      | 0.312 | 0.111 | 0.093 | 0.136 | 0.113333 | 0.012468 | 15.05090748 | 84.94909252 |             |
|      | 0.156 | 0.251 | 0.286 | 0.266 | 0.267667 | 0.010138 | 35.54670208 | 64.45329792 |             |
|      | 0.078 | 0.489 | 0.465 | 0.444 | 0.466    | 0.013    | 61.88579017 | 38.11420983 |             |
| XIId | 10    | 0.05  | 0.05  | 0.05  | 0.05     | 4.91E-18 | 6.640106242 | 93.35989376 | 1.25 ± 0.05 |
|      | 5     | 0.074 | 0.066 | 0.071 | 0.070333 | 0.002333 | 9.340416113 | 90.65958389 |             |
|      | 2.5   | 0.152 | 0.119 | 0.124 | 0.131667 | 0.010269 | 17.4856131  | 82.5143869  |             |
|      | 1.25  | 0.341 | 0.389 | 0.372 | 0.367333 | 0.014051 | 48.78264719 | 51.21735281 |             |
|      | 0.625 | 0.679 | 0.671 | 0.702 | 0.684    | 0.009292 | 90.83665339 | 9.163346614 |             |
|      | 0.312 | 0.746 | 0.755 | 0.75  | 0.750333 | 0.002603 | 99.645861   | 0.354139    |             |
| XIIe | 10    | 0.054 | 0.055 | 0.055 | 0.054667 | 0.000333 | 7.259849491 | 92.74015051 | 0.95 ± 0.01 |
|      | 5     | 0.054 | 0.067 | 0.058 | 0.059667 | 0.003844 | 7.923860115 | 92.07613988 |             |
|      | 2.5   | 0.062 | 0.058 | 0.061 | 0.060333 | 0.001202 | 8.012394865 | 91.98760514 |             |
|      | 1.25  | 0.163 | 0.182 | 0.15  | 0.165    | 0.009292 | 21.9123506  | 78.0876494  |             |
|      | 0.625 | 0.651 | 0.637 | 0.667 | 0.651667 | 0.008667 | 86.54271802 | 13.45728198 |             |
|      | 0.312 | 0.754 | 0.742 | 0.756 | 0.750667 | 0.004372 | 99.69012838 | 0.309871625 |             |
| XIIf | 10    | 0.026 | 0.023 | 0.024 | 0.024333 | 0.000882 | 3.231518371 | 96.76848163 | 0.17 ± 0    |
|      | 5     | 0.022 | 0.028 | 0.025 | 0.025    | 0.001732 | 3.320053121 | 96.67994688 |             |
|      | 2.5   | 0.021 | 0.027 | 0.026 | 0.024667 | 0.001856 | 3.275785746 | 96.72421425 |             |
|      | 1.25  | 0.052 | 0.047 | 0.044 | 0.047667 | 0.002333 | 6.330234617 | 93.66976538 |             |
|      | 0.625 | 0.062 | 0.04  | 0.053 | 0.051667 | 0.006386 | 6.861443116 | 93.13855688 |             |
|      | 0.312 | 0.088 | 0.064 | 0.076 | 0.076    | 0.006928 | 10.09296149 | 89.90703851 |             |
|      | 0.156 | 0.306 | 0.28  | 0.291 | 0.292333 | 0.007535 | 38.82248783 | 61.17751217 |             |
|      | 0.078 | 0.718 | 0.683 | 0.699 | 0.7      | 0.010116 | 92.96148738 | 7.038512616 |             |



| ID    | uM    | O.D   |       |       | Mean O.D | ±SE      | Viability % | Toxicity %  | IC50 ± SD   |
|-------|-------|-------|-------|-------|----------|----------|-------------|-------------|-------------|
| Mcf7  | ----- | 0.758 | 0.753 | 0.748 | 0.753    | 0.002887 | 100         | 0           | uM          |
| XIIg  | 10    | 0.023 | 0.02  | 0.02  | 0.021    | 0.001    | 2.788844622 | 97.21115538 | 0.05 ± 0.01 |
|       | 5     | 0.022 | 0.021 | 0.025 | 0.022667 | 0.001202 | 3.010181496 | 96.9898185  |             |
|       | 2.5   | 0.022 | 0.021 | 0.021 | 0.021333 | 0.000333 | 2.833111996 | 97.166888   |             |
|       | 1.25  | 0.02  | 0.026 | 0.024 | 0.023333 | 0.001764 | 3.098716246 | 96.90128375 |             |
|       | 0.625 | 0.048 | 0.052 | 0.044 | 0.048    | 0.002309 | 6.374501992 | 93.62549801 |             |
|       | 0.312 | 0.109 | 0.093 | 0.127 | 0.109667 | 0.009821 | 14.56396636 | 85.43603364 |             |
|       | 0.156 | 0.188 | 0.192 | 0.214 | 0.198    | 0.008083 | 26.29482072 | 73.70517928 |             |
|       | 0.078 | 0.377 | 0.358 | 0.361 | 0.365333 | 0.005897 | 48.51704294 | 51.48295706 |             |
| XIV   | 10    | 0.025 | 0.03  | 0.028 | 0.027667 | 0.001453 | 3.67419212  | 96.32580788 | 0.17 ± 0    |
|       | 5     | 0.025 | 0.03  | 0.029 | 0.028    | 0.001528 | 3.718459495 | 96.2815405  |             |
|       | 2.5   | 0.049 | 0.044 | 0.036 | 0.043    | 0.003786 | 5.710491368 | 94.28950863 |             |
|       | 1.25  | 0.093 | 0.11  | 0.111 | 0.104667 | 0.00584  | 13.89995573 | 86.10004427 |             |
|       | 0.625 | 0.104 | 0.116 | 0.113 | 0.111    | 0.003606 | 14.74103586 | 85.25896414 |             |
|       | 0.312 | 0.163 | 0.148 | 0.136 | 0.149    | 0.00781  | 19.7875166  | 80.2124834  |             |
|       | 0.156 | 0.216 | 0.193 | 0.256 | 0.221667 | 0.018406 | 29.43780434 | 70.56219566 |             |
|       | 0.078 | 0.668 | 0.634 | 0.651 | 0.651    | 0.009815 | 86.45418327 | 13.54581673 |             |
| XVa   | 10    | 0.02  | 0.022 | 0.02  | 0.020667 | 0.000667 | 2.744577247 | 97.25542275 | 0.95 ± 0    |
|       | 5     | 0.021 | 0.023 | 0.022 | 0.022    | 0.000577 | 2.921646746 | 97.07835325 |             |
|       | 2.5   | 0.022 | 0.022 | 0.024 | 0.022667 | 0.000667 | 3.010181496 | 96.9898185  |             |
|       | 1.25  | 0.197 | 0.2   | 0.175 | 0.190667 | 0.007881 | 25.32093847 | 74.67906153 |             |
|       | 0.625 | 0.583 | 0.563 | 0.594 | 0.58     | 0.009074 | 77.0252324  | 22.9747676  |             |
|       | 0.312 | 0.756 | 0.751 | 0.752 | 0.753    | 0.001528 | 100         | 0           |             |
| XVb   | 10    | 0.033 | 0.028 | 0.03  | 0.030333 | 0.001453 | 4.02833112  | 95.97166888 | 0.16 ± 0    |
|       | 5     | 0.032 | 0.034 | 0.031 | 0.032333 | 0.000882 | 4.29393537  | 95.70606463 |             |
|       | 2.5   | 0.033 | 0.03  | 0.035 | 0.032667 | 0.001453 | 4.338202745 | 95.66179726 |             |
|       | 1.25  | 0.036 | 0.027 | 0.03  | 0.031    | 0.002646 | 4.11686587  | 95.88313413 |             |
|       | 0.625 | 0.021 | 0.028 | 0.024 | 0.024333 | 0.002028 | 3.231518371 | 96.76848163 |             |
|       | 0.312 | 0.019 | 0.026 | 0.02  | 0.021667 | 0.002186 | 2.877379371 | 97.12262063 |             |
|       | 0.156 | 0.294 | 0.256 | 0.282 | 0.277333 | 0.011215 | 36.83045595 | 63.16954405 |             |
|       | 0.078 | 0.671 | 0.699 | 0.666 | 0.678667 | 0.010269 | 90.12837539 | 9.871624613 |             |
| XVIIa | 10    | 0.042 | 0.038 | 0.041 | 0.040333 | 0.001202 | 5.356352368 | 94.64364763 | 0.18 ± 0    |
|       | 5     | 0.025 | 0.023 | 0.024 | 0.024    | 0.000577 | 3.187250996 | 96.812749   |             |
|       | 2.5   | 0.022 | 0.027 | 0.026 | 0.025    | 0.001528 | 3.320053121 | 96.67994688 |             |
|       | 1.25  | 0.024 | 0.033 | 0.03  | 0.029    | 0.002646 | 3.85126162  | 96.14873838 |             |
|       | 0.625 | 0.031 | 0.037 | 0.036 | 0.034667 | 0.001856 | 4.603806994 | 95.39619301 |             |
|       | 0.312 | 0.083 | 0.074 | 0.098 | 0.085    | 0.007    | 11.28818061 | 88.71181939 |             |
|       | 0.156 | 0.341 | 0.318 | 0.309 | 0.322667 | 0.009528 | 42.85081895 | 57.14918105 |             |
|       | 0.078 | 0.738 | 0.712 | 0.728 | 0.726    | 0.007572 | 96.41434263 | 3.585657371 |             |

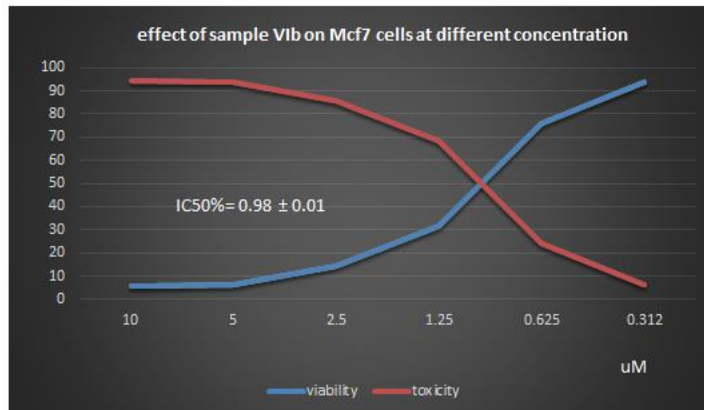
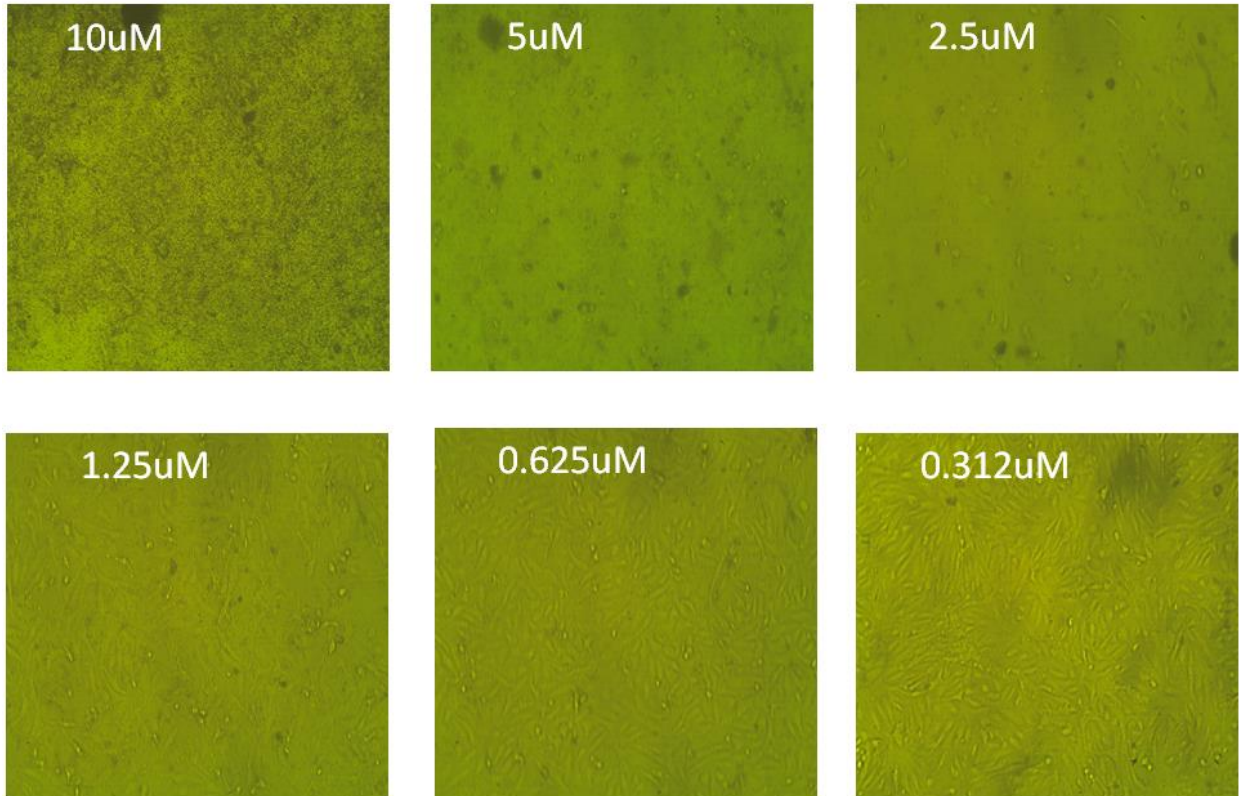
| ID    | uM    | O.D   |       |       | Mean O.D | ±SE      | Viability % | Toxicity %  | IC50 ± SD   |
|-------|-------|-------|-------|-------|----------|----------|-------------|-------------|-------------|
| Mcf7  | ----- | 0.758 | 0.753 | 0.748 | 0.753    | 0.002887 | 100         | 0           | uM          |
| XVIIb | 10    | 0.022 | 0.019 | 0.022 | 0.021    | 0.001    | 2.788844622 | 97.21115538 | 1.52 ± 0.01 |
|       | 5     | 0.02  | 0.018 | 0.023 | 0.020333 | 0.001453 | 2.700309872 | 97.29969013 |             |
|       | 2.5   | 0.027 | 0.029 | 0.026 | 0.027333 | 0.000882 | 3.629924745 | 96.37007525 |             |
|       | 1.25  | 0.426 | 0.417 | 0.438 | 0.427    | 0.006083 | 56.7065073  | 43.2934927  |             |
|       | 0.625 | 0.752 | 0.755 | 0.746 | 0.751    | 0.002646 | 99.73439575 | 0.26560425  |             |
|       | 0.312 | 0.758 | 0.751 | 0.75  | 0.753    | 0.002517 | 100         | 0           |             |
| XIXa  | 10    | 0.04  | 0.043 | 0.047 | 0.043333 | 0.002028 | 5.754758743 | 94.24524126 | 0.15 ± 0    |
|       | 5     | 0.028 | 0.03  | 0.026 | 0.028    | 0.001155 | 3.718459495 | 96.2815405  |             |
|       | 2.5   | 0.046 | 0.044 | 0.041 | 0.043667 | 0.001453 | 5.799026118 | 94.20097388 |             |
|       | 1.25  | 0.044 | 0.048 | 0.045 | 0.045667 | 0.001202 | 6.064630367 | 93.93536963 |             |
|       | 0.625 | 0.037 | 0.036 | 0.038 | 0.037    | 0.000577 | 4.913678619 | 95.08632138 |             |
|       | 0.312 | 0.118 | 0.132 | 0.102 | 0.117333 | 0.008667 | 15.58211598 | 84.41788402 |             |
|       | 0.156 | 0.222 | 0.194 | 0.2   | 0.205333 | 0.008511 | 27.26870297 | 72.73129703 |             |
|       | 0.078 | 0.626 | 0.573 | 0.594 | 0.597667 | 0.015409 | 79.37140328 | 20.62859672 |             |
| XIXb  | 10    | 0.054 | 0.048 | 0.049 | 0.050333 | 0.001856 | 6.684373617 | 93.31562638 | 0.15 ± 0    |
|       | 5     | 0.046 | 0.055 | 0.055 | 0.052    | 0.003    | 6.905710491 | 93.09428951 |             |
|       | 2.5   | 0.05  | 0.043 | 0.056 | 0.049667 | 0.003756 | 6.595838867 | 93.40416113 |             |
|       | 1.25  | 0.036 | 0.034 | 0.037 | 0.035667 | 0.000882 | 4.736609119 | 95.26339088 |             |
|       | 0.625 | 0.033 | 0.027 | 0.025 | 0.028333 | 0.002404 | 3.76272687  | 96.23727313 |             |
|       | 0.312 | 0.03  | 0.032 | 0.044 | 0.035333 | 0.004372 | 4.692341744 | 95.30765826 |             |
|       | 0.156 | 0.184 | 0.155 | 0.168 | 0.169    | 0.008386 | 22.4435591  | 77.5564409  |             |
|       | 0.078 | 0.687 | 0.621 | 0.646 | 0.651333 | 0.019238 | 86.49845064 | 13.50154936 |             |
| XIXc  | 10    | 0.062 | 0.06  | 0.058 | 0.06     | 0.001155 | 7.96812749  | 92.03187251 | 0.18 ± 0    |
|       | 5     | 0.066 | 0.057 | 0.056 | 0.059667 | 0.00318  | 7.923860115 | 92.07613988 |             |
|       | 2.5   | 0.035 | 0.039 | 0.036 | 0.036667 | 0.001202 | 4.869411244 | 95.13058876 |             |
|       | 1.25  | 0.029 | 0.025 | 0.028 | 0.027333 | 0.001202 | 3.629924745 | 96.37007525 |             |
|       | 0.625 | 0.074 | 0.081 | 0.066 | 0.073667 | 0.004333 | 9.783089863 | 90.21691014 |             |
|       | 0.312 | 0.12  | 0.11  | 0.153 | 0.127667 | 0.012991 | 16.9544046  | 83.0455954  |             |
|       | 0.156 | 0.287 | 0.315 | 0.265 | 0.289    | 0.014468 | 38.37981408 | 61.62018592 |             |
|       | 0.078 | 0.725 | 0.701 | 0.736 | 0.720667 | 0.010333 | 95.70606463 | 4.29393537  |             |
| XIXd  | 10    | 0.064 | 0.062 | 0.058 | 0.061333 | 0.001764 | 8.14519699  | 91.85480301 | 1.12 ± 0.02 |
|       | 5     | 0.077 | 0.05  | 0.062 | 0.063    | 0.00781  | 8.366533865 | 91.63346614 |             |
|       | 2.5   | 0.173 | 0.145 | 0.162 | 0.16     | 0.008145 | 21.24833997 | 78.75166003 |             |
|       | 1.25  | 0.285 | 0.314 | 0.303 | 0.300667 | 0.008452 | 39.9291722  | 60.0708278  |             |
|       | 0.625 | 0.737 | 0.688 | 0.714 | 0.713    | 0.014154 | 94.68791501 | 5.312084993 |             |
|       | 0.312 | 0.755 | 0.746 | 0.757 | 0.752667 | 0.003383 | 99.95573263 | 0.044267375 |             |



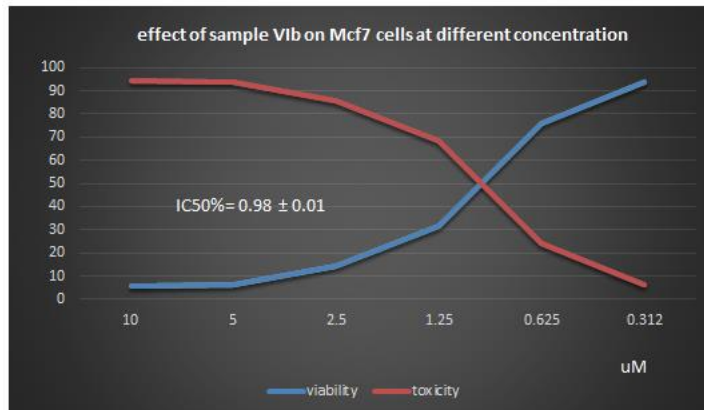
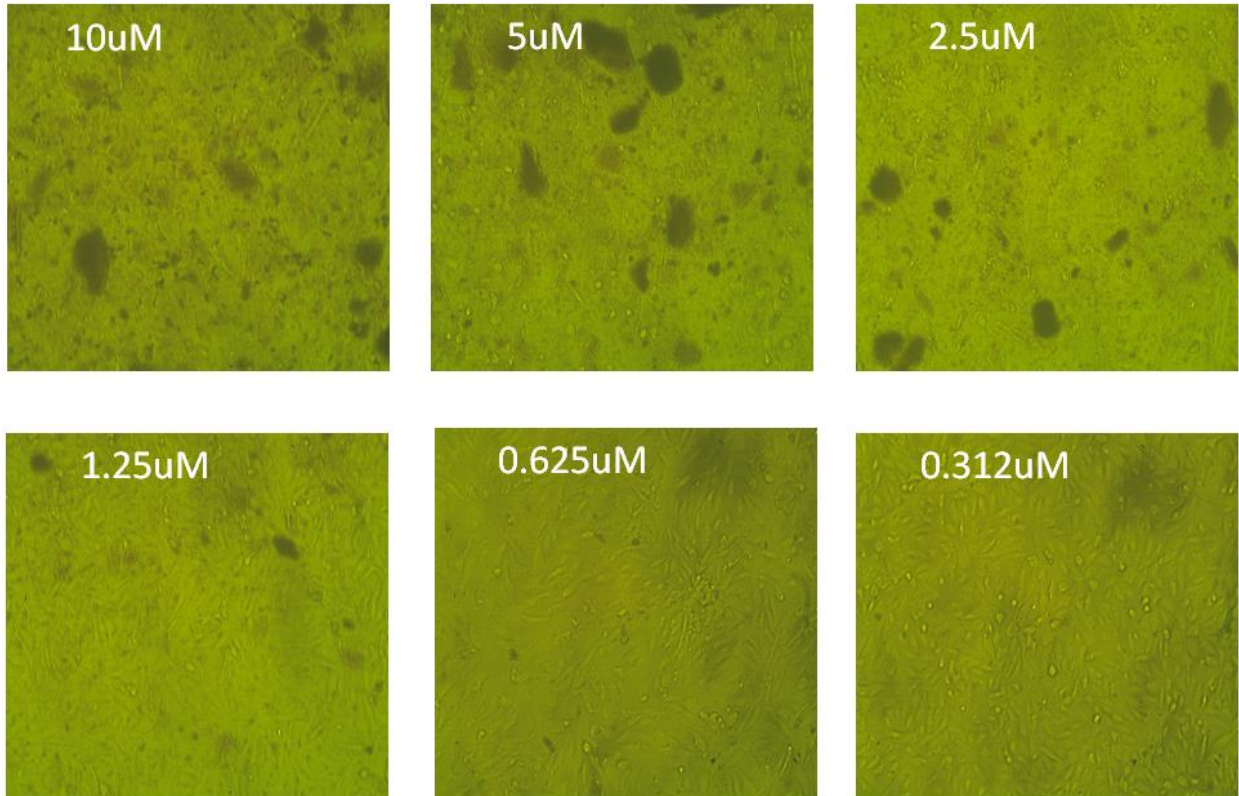
**control**  
**Mcf7 cells**

|                      |   |
|----------------------|---|
| Organism :           | <i>Homo sapiens</i> , human   |
| Tissue :             | mammary gland, breast; derived from metastatic site: pleural effusion |
| Cell Type :          | epithelial  |
| Culture Properties : | adherent  |
| Disease :            | adenocarcinoma  |
| ATCC :               | HTB-22  |

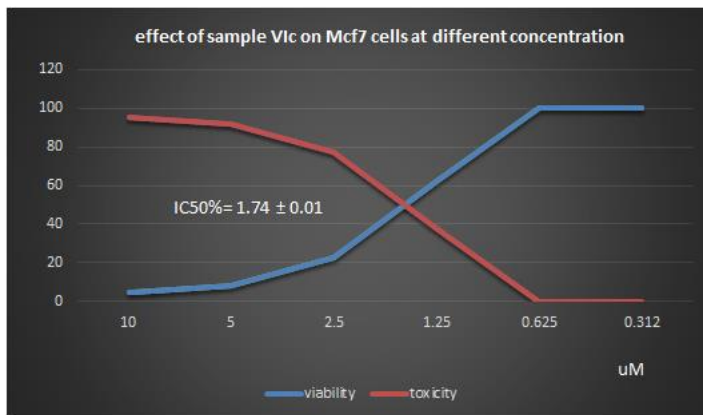
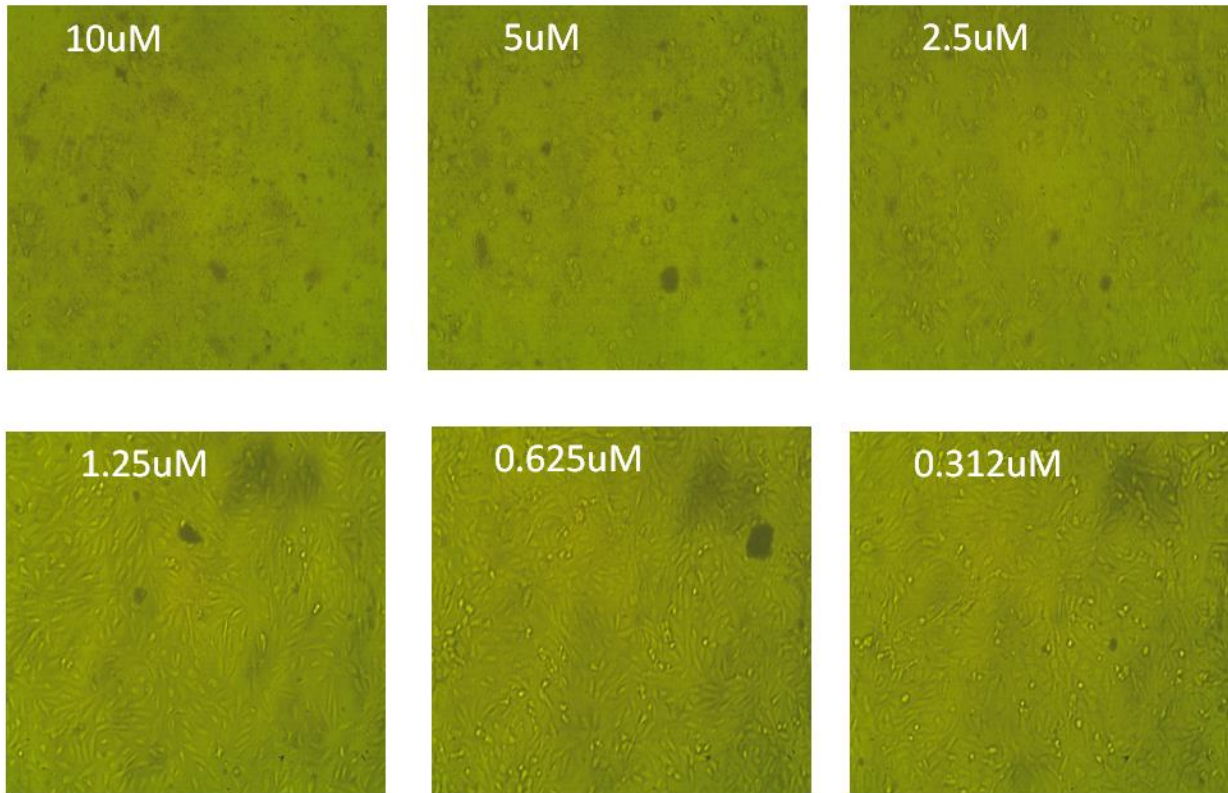
## Effect of sample VIa on Mcf7 cells at different concentration



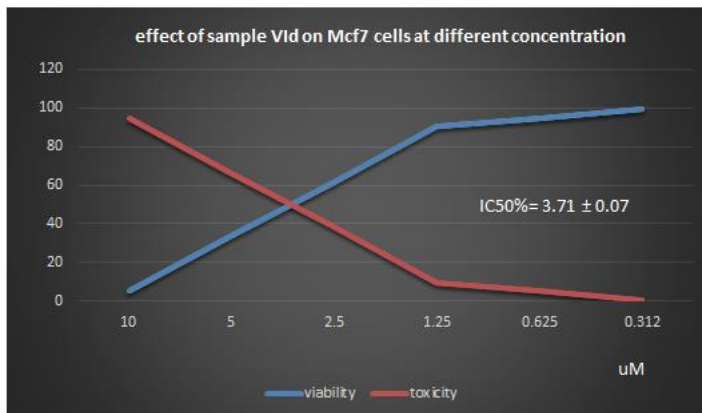
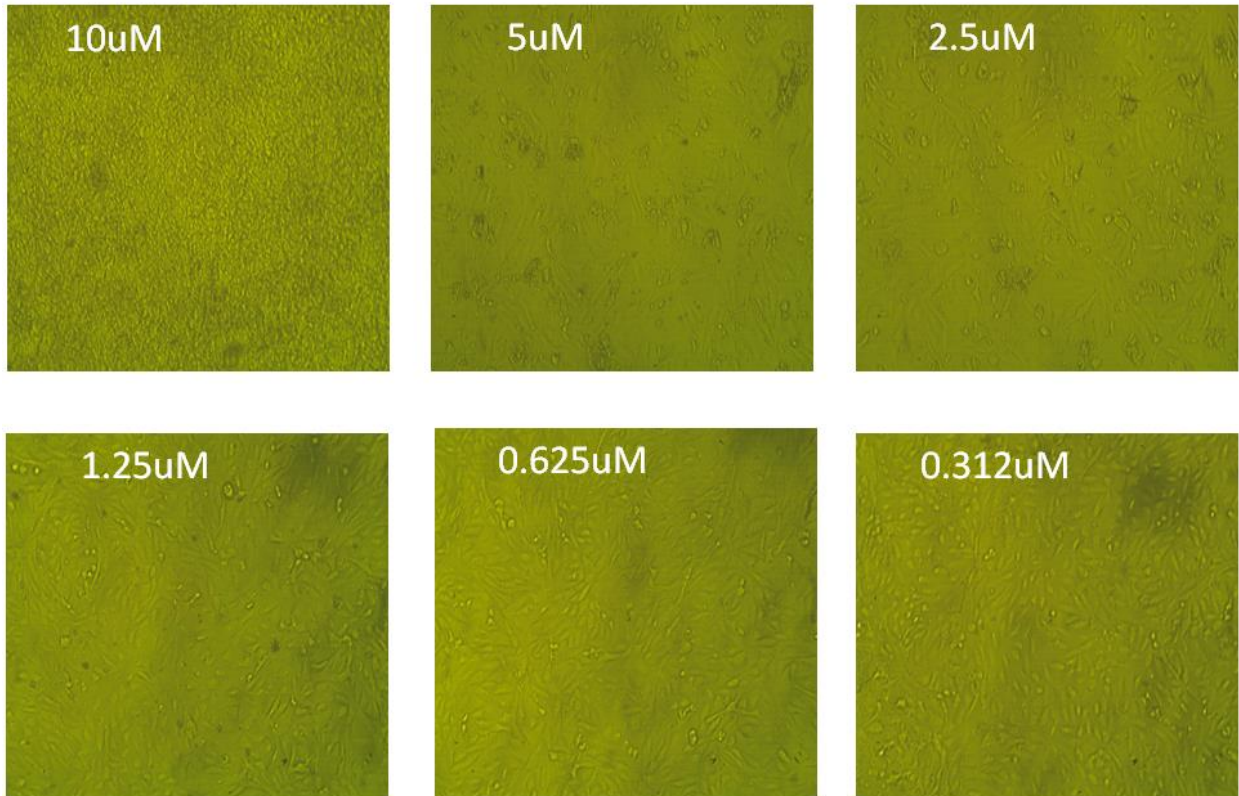
## Effect of sample V1b on MCF7 cells at different concentration



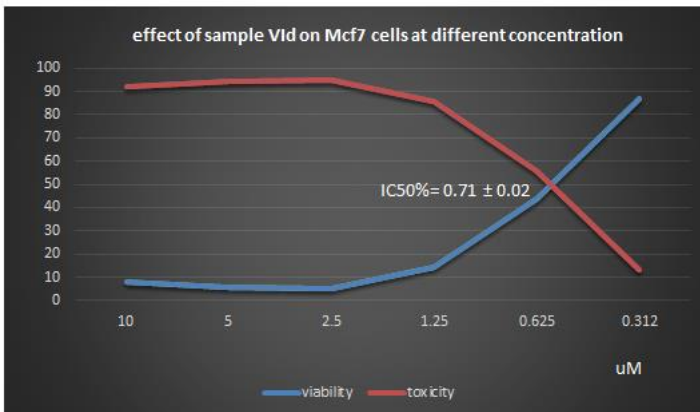
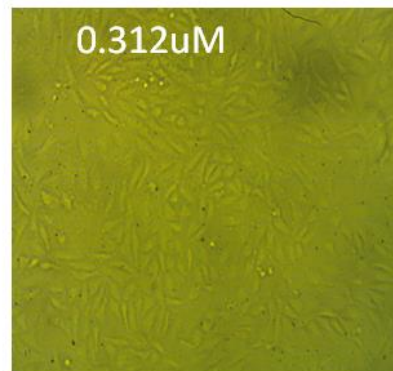
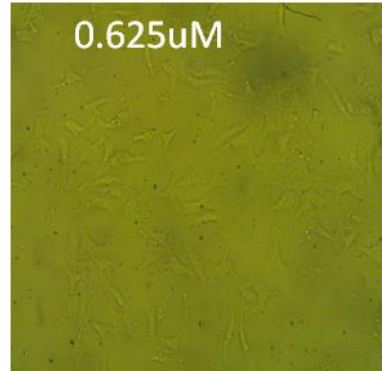
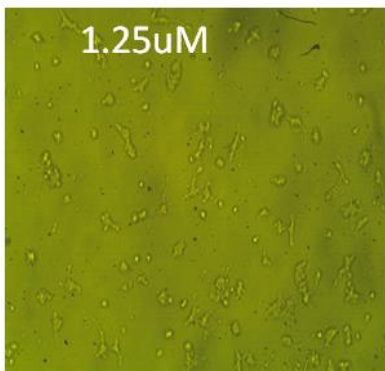
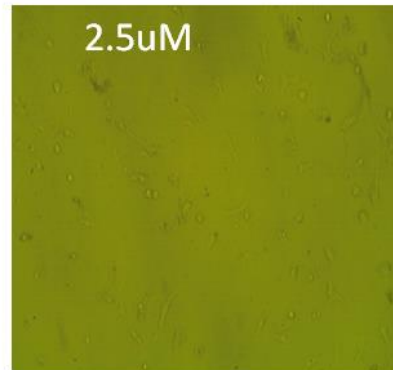
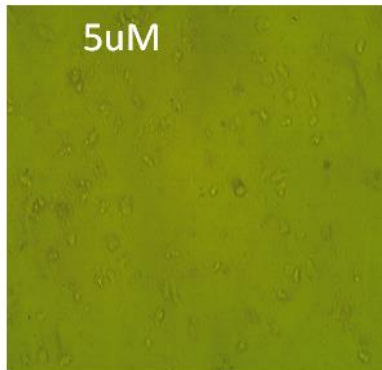
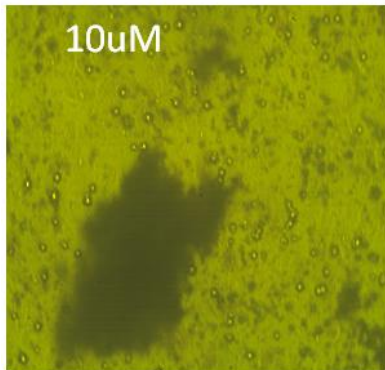
## Effect of sample VIc on Mcf7 cells at different concentration



## Effect of sample VId on Mcf7 cells at different concentration

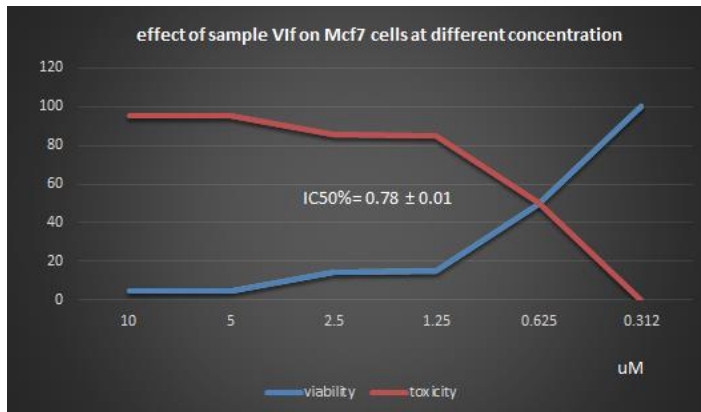
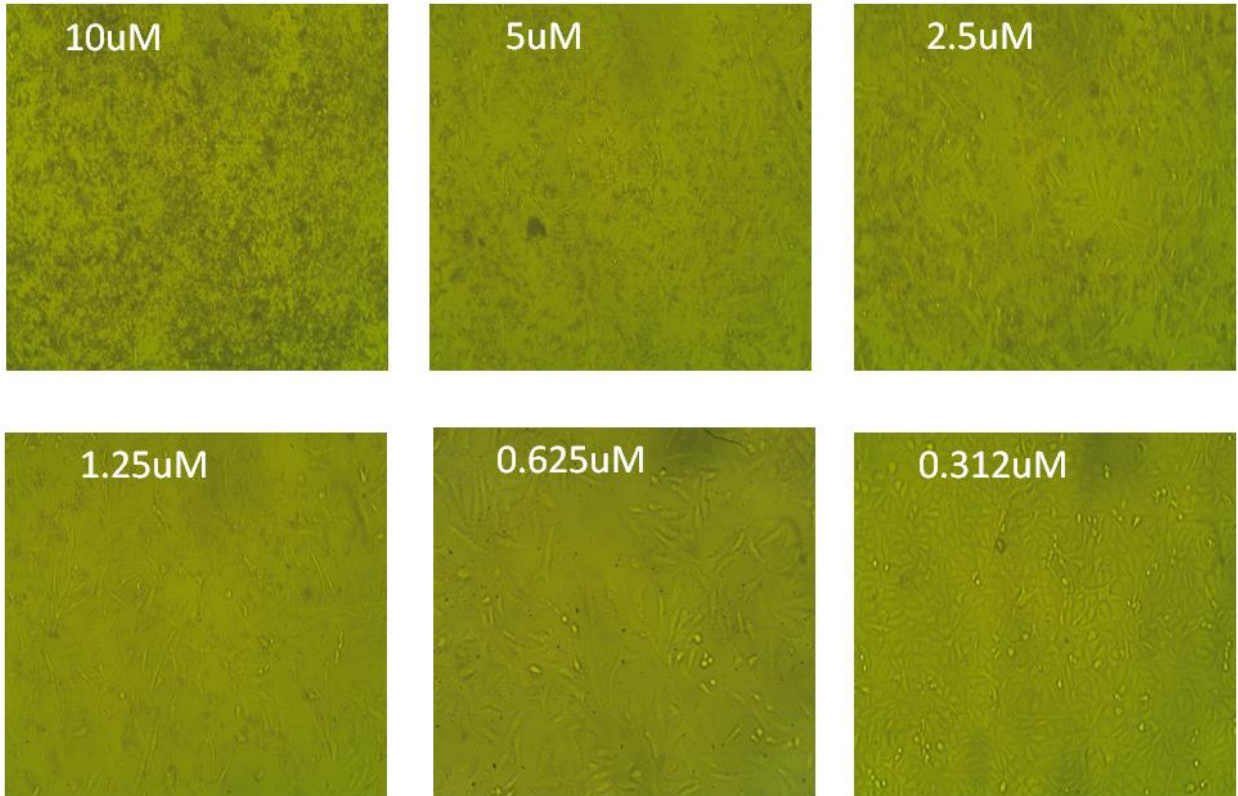


## Effect of sample VIe on Mcf7 cells at different concentration

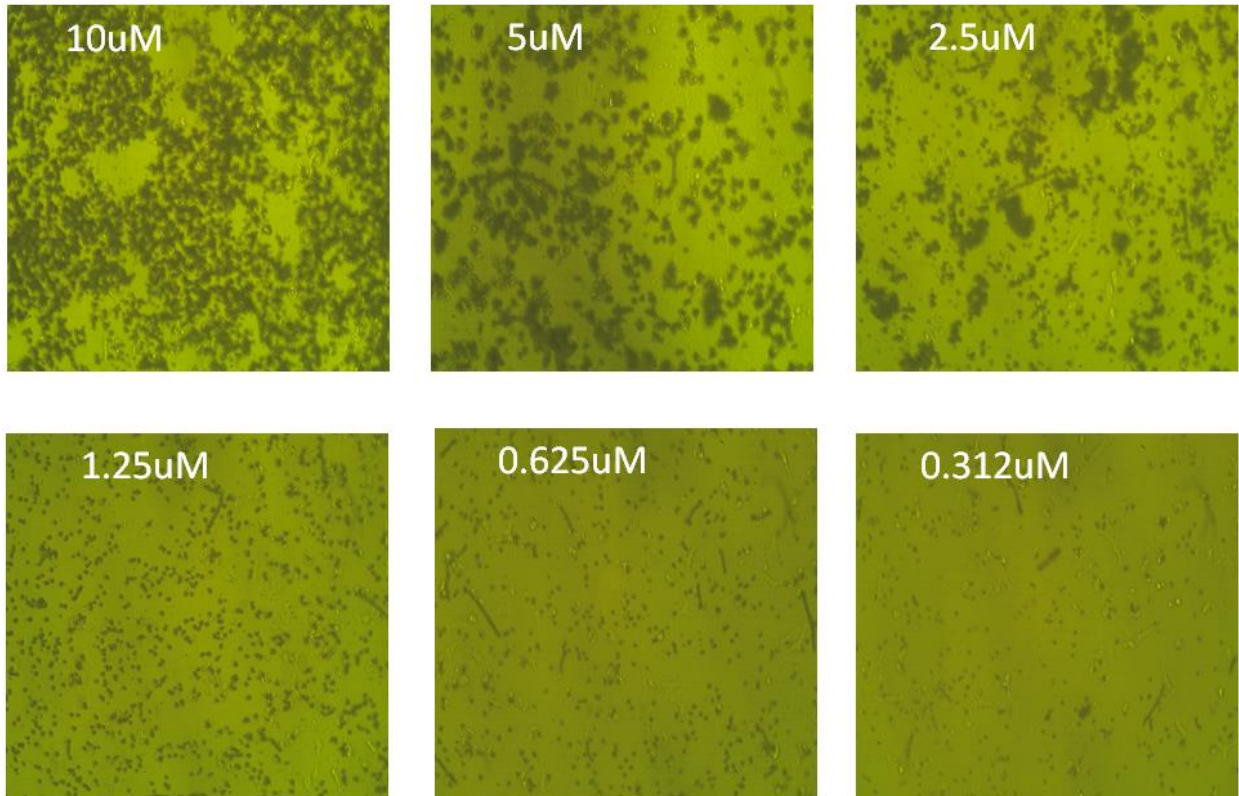




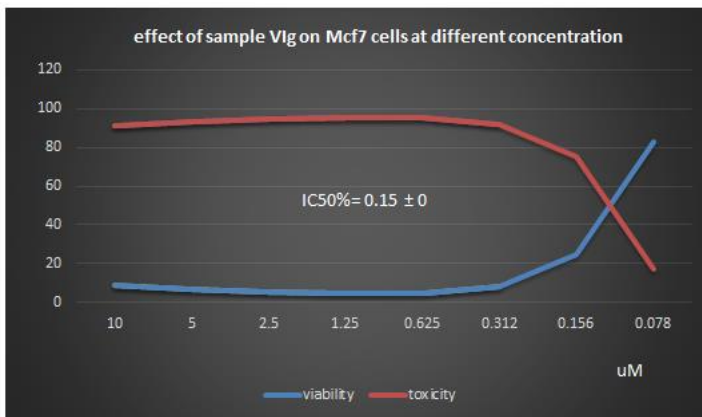
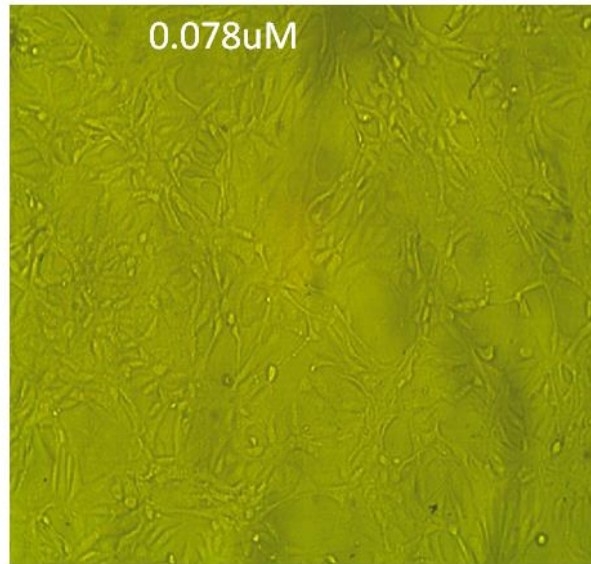
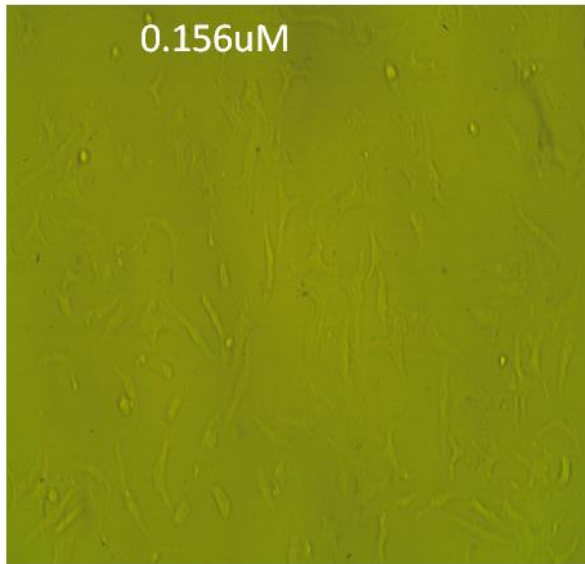
## Effect of sample Vif on Mcf7 cells at different concentration



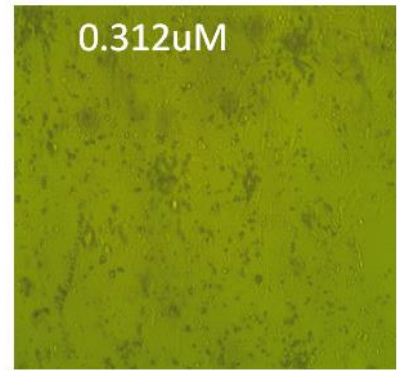
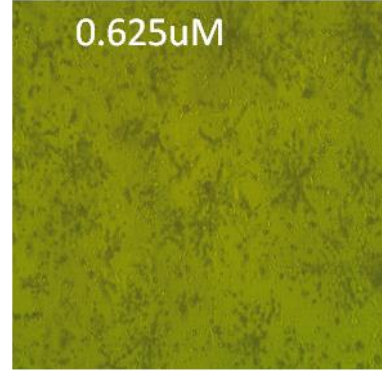
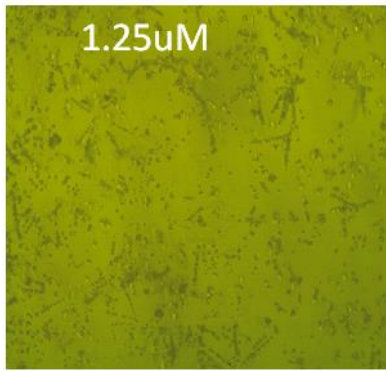
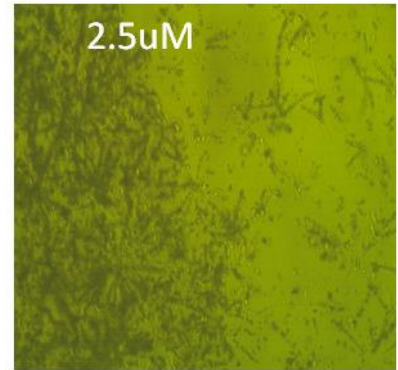
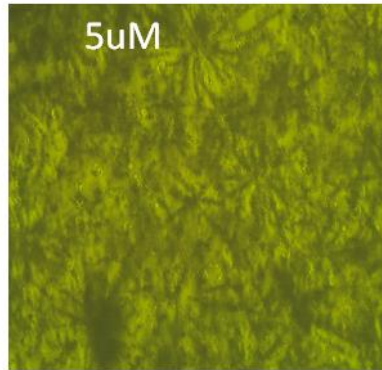
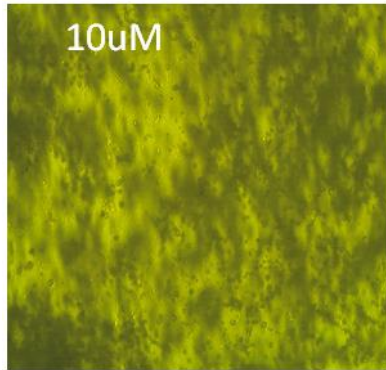
## Effect of sample VIg on Mcf7 cells at different concentration



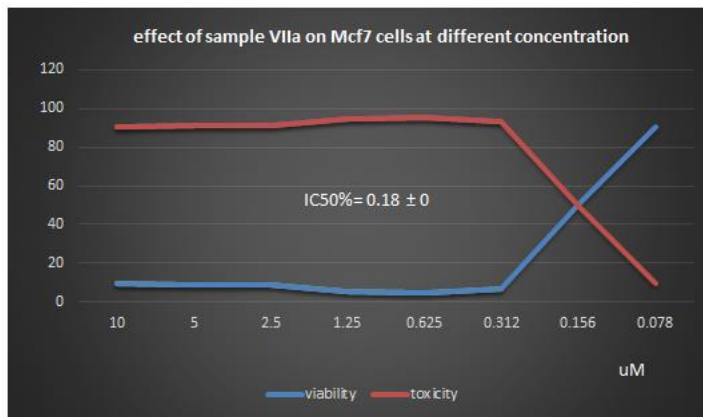
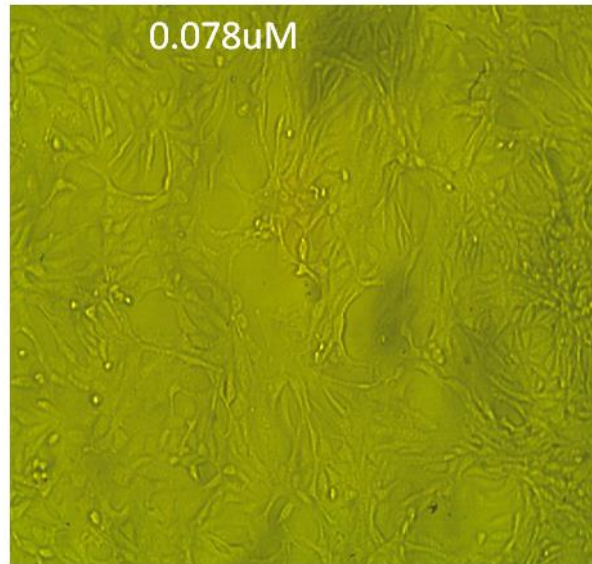
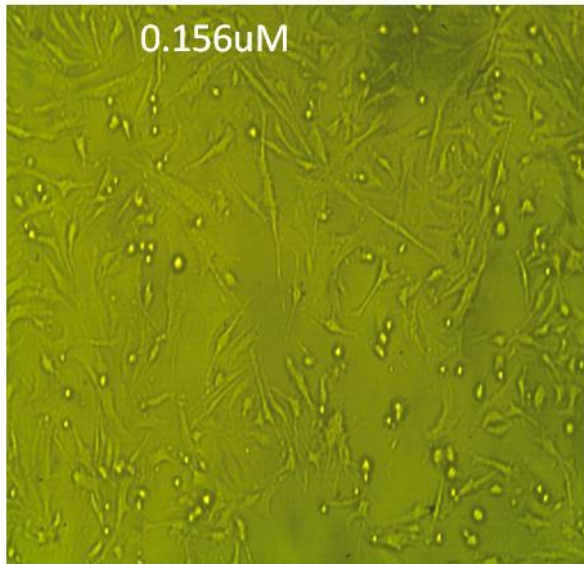
## Effect of sample VIg on Mcf7 cells at different concentration



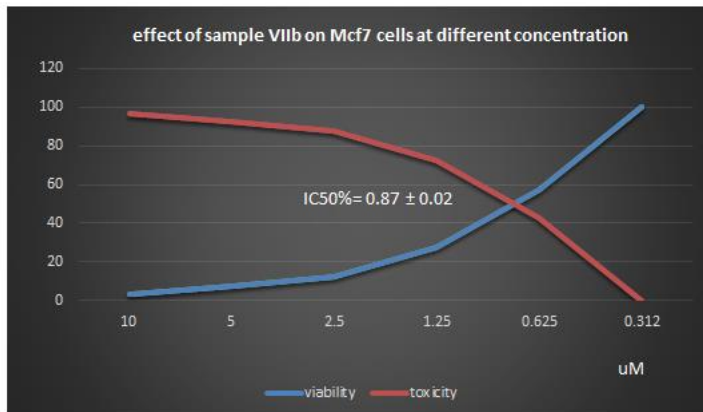
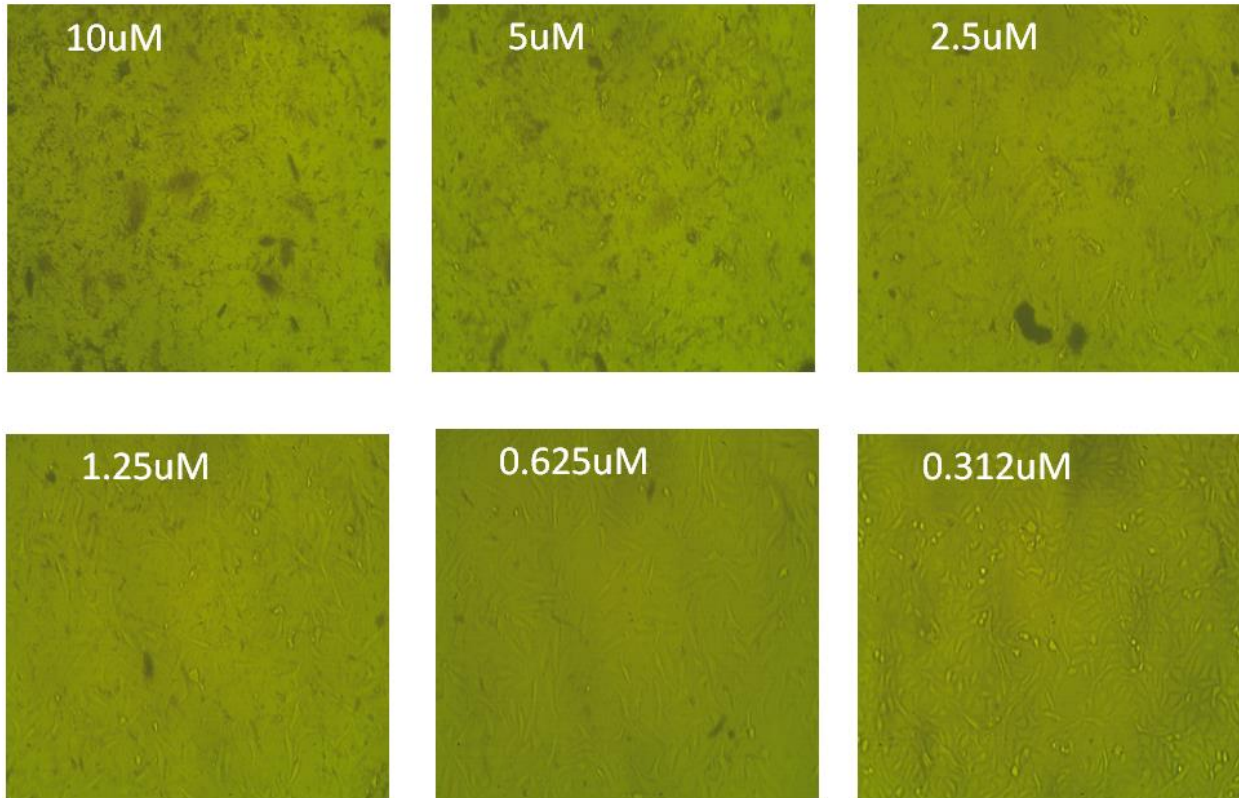
## Effect of sample VIIa on MCF7 cells at different concentration



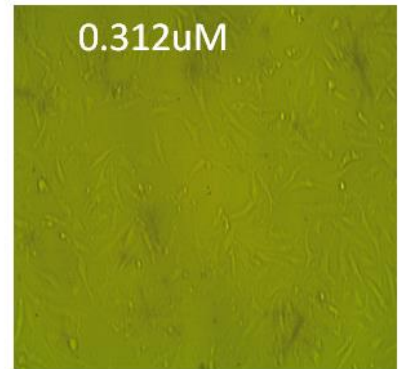
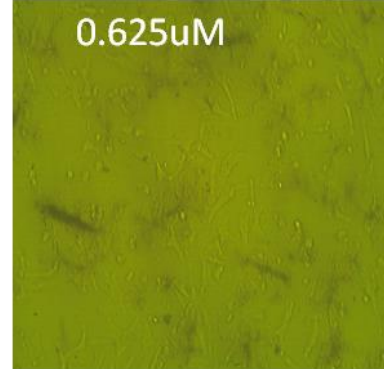
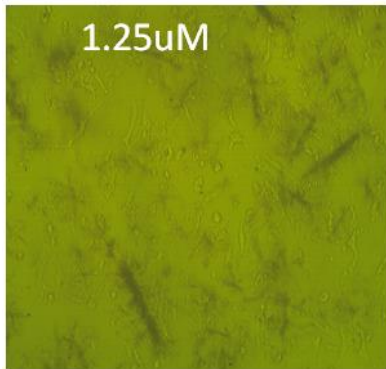
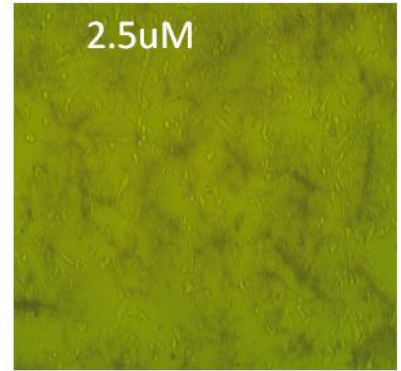
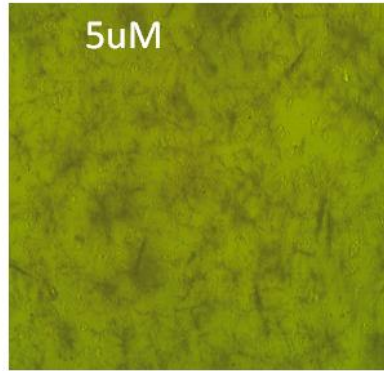
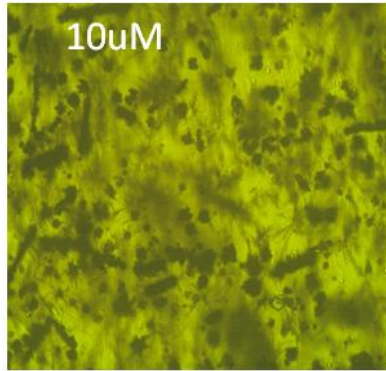
## Effect of sample VIIa on Mcf7 cells at different concentration



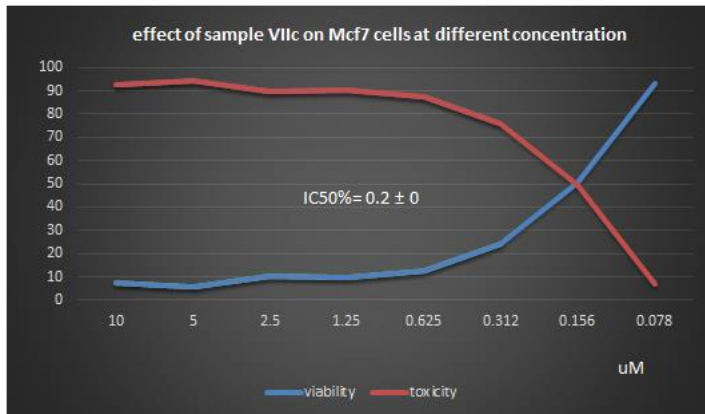
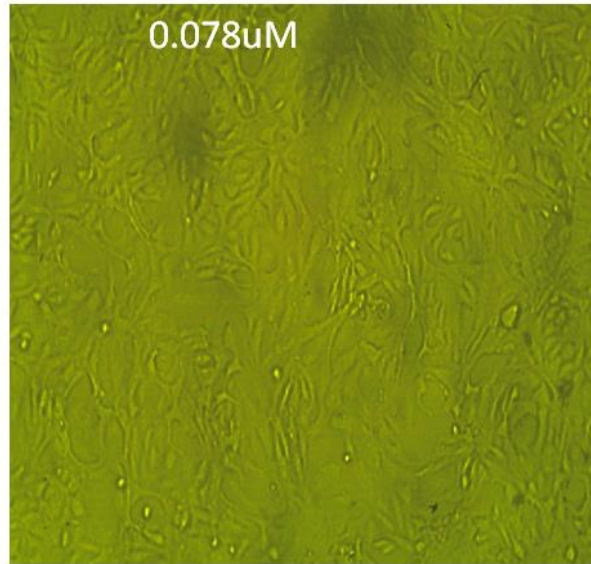
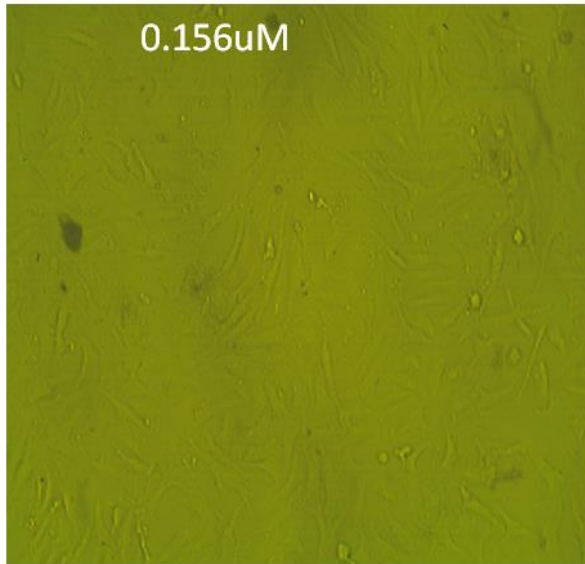
## Effect of sample VIIb on Mcf7 cells at different concentration



## Effect of sample VIIc on MCF7 cells at different concentration

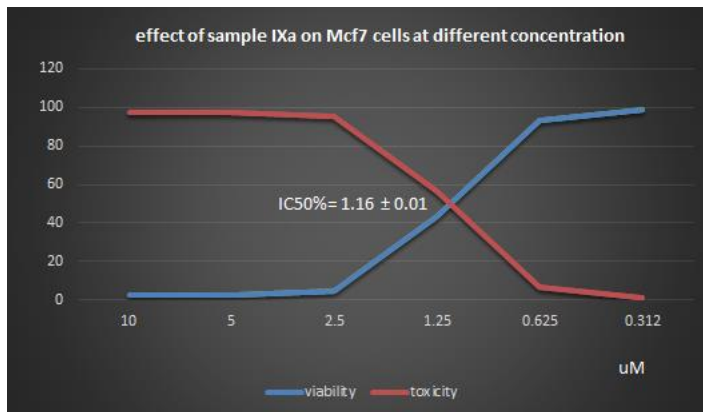
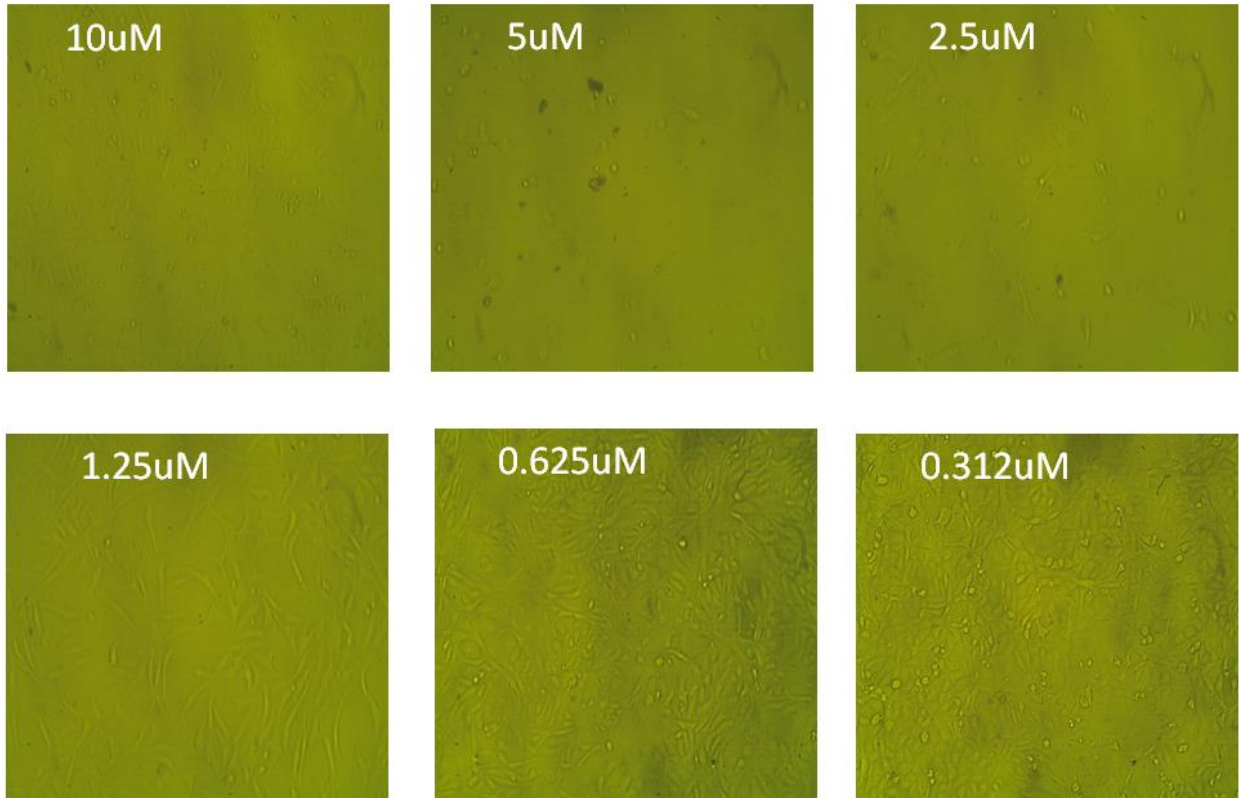


## Effect of sample VIIc on MCF7 cells at different concentration

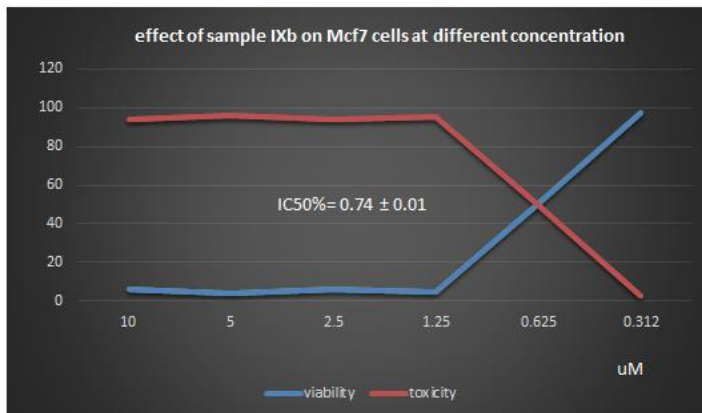
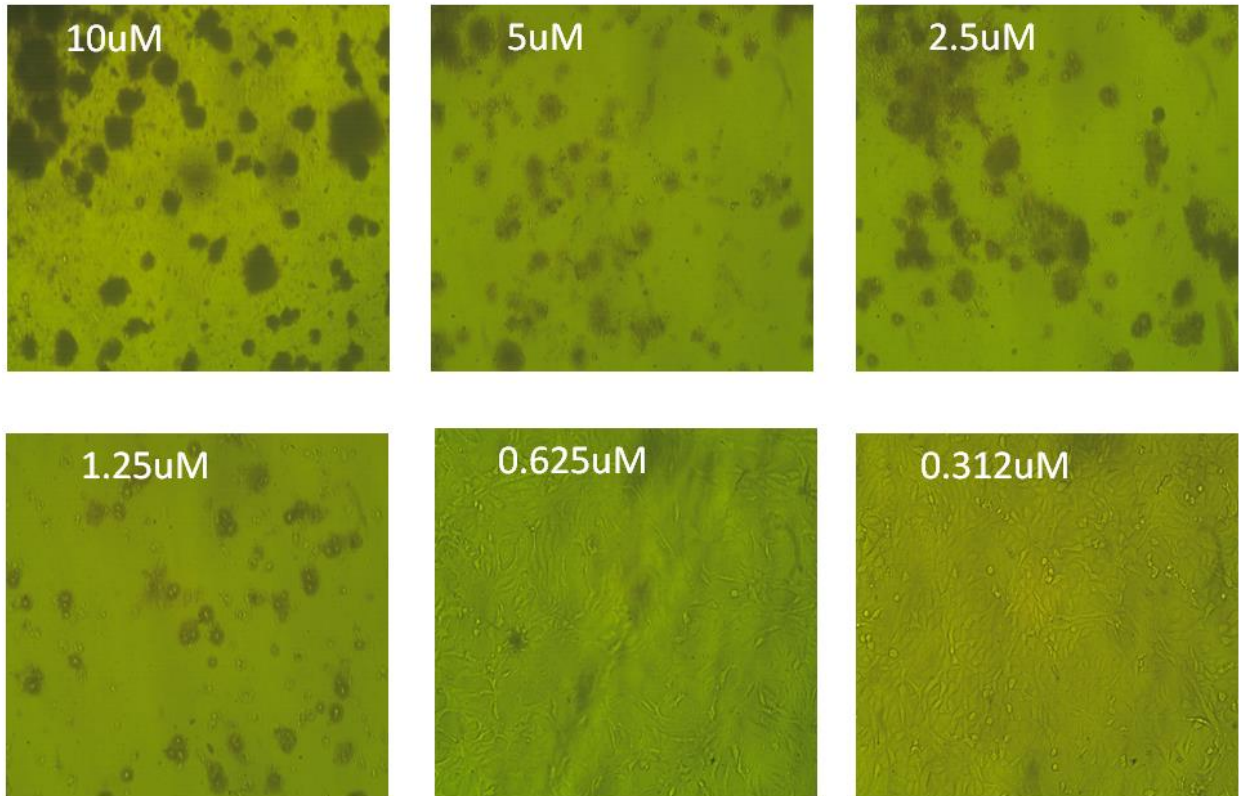




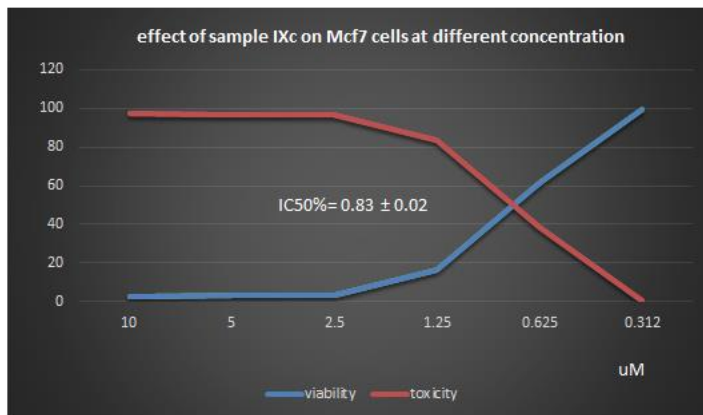
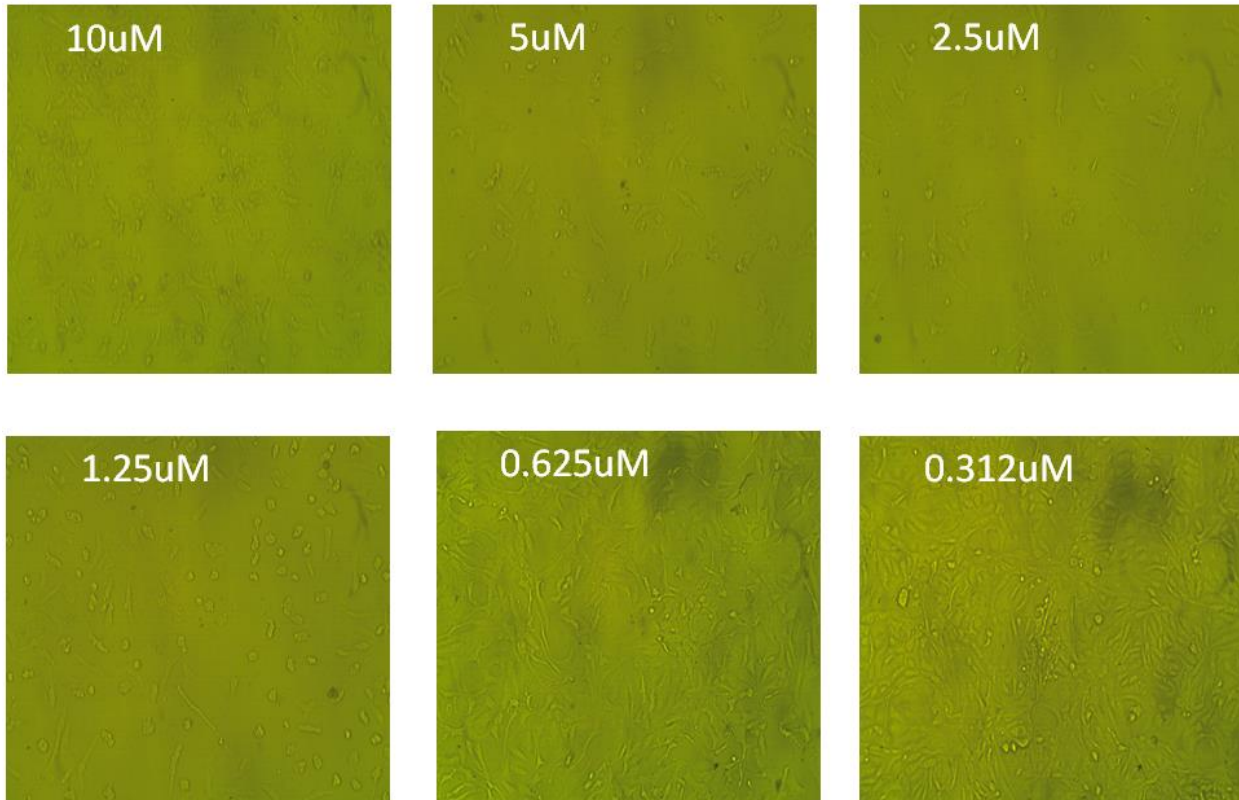
## Effect of sample IXa on Mcf7 cells at different concentration



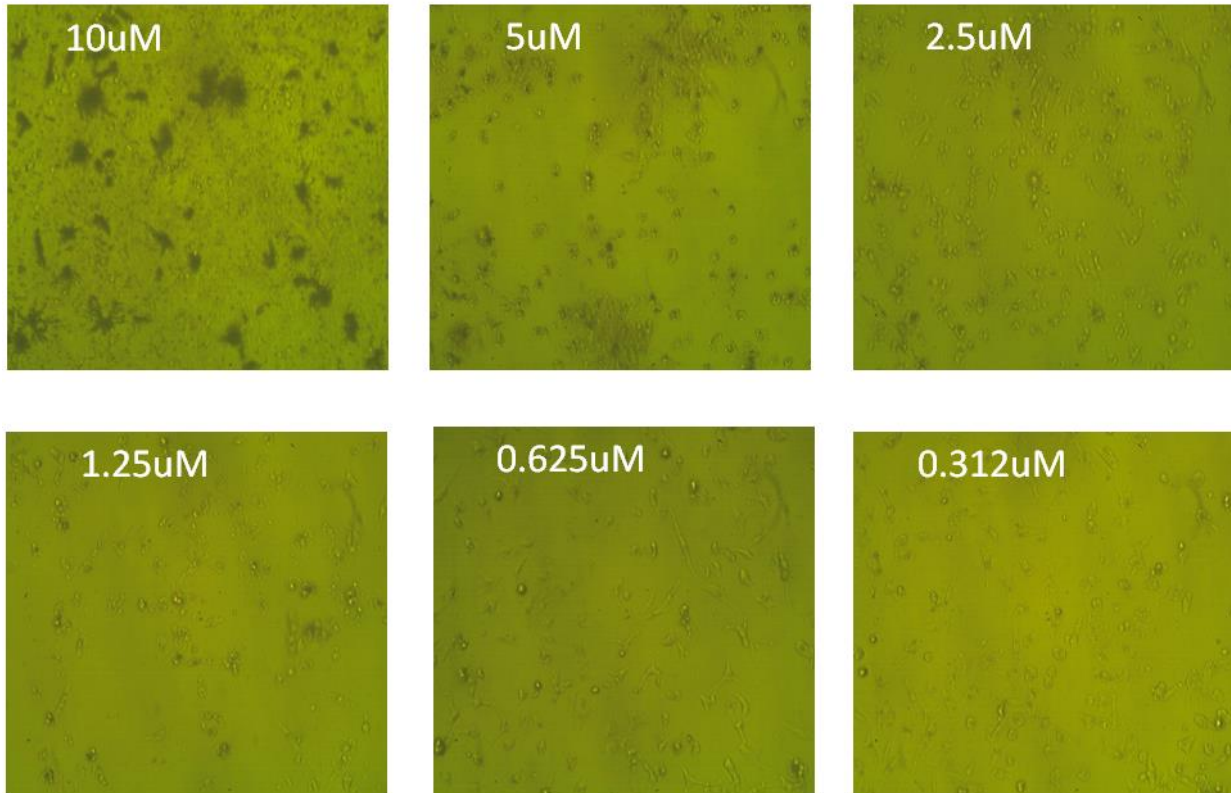
## Effect of sample IXb on Mcf7 cells at different concentration



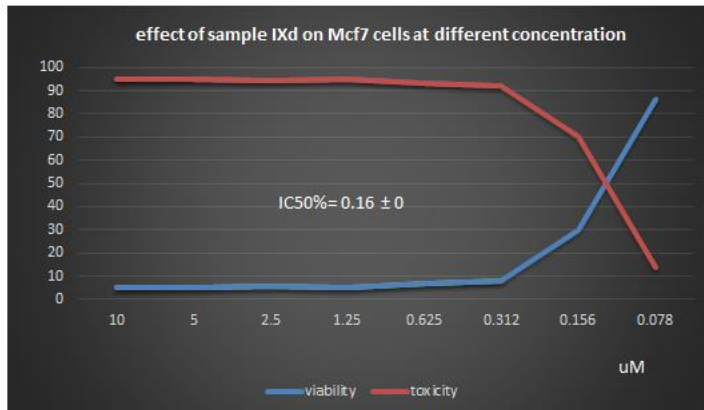
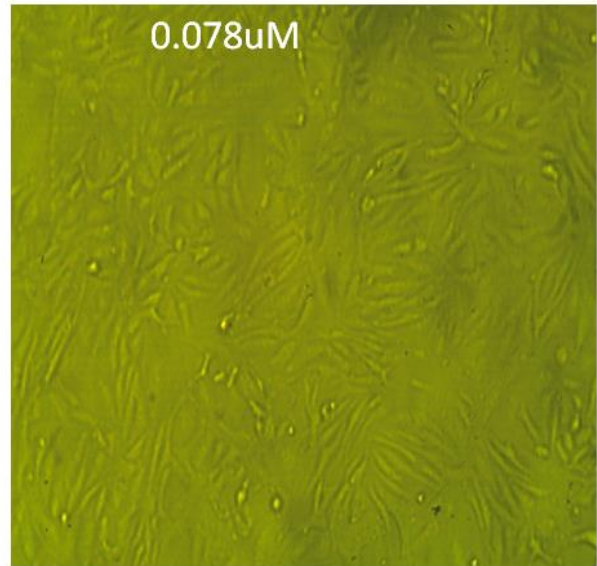
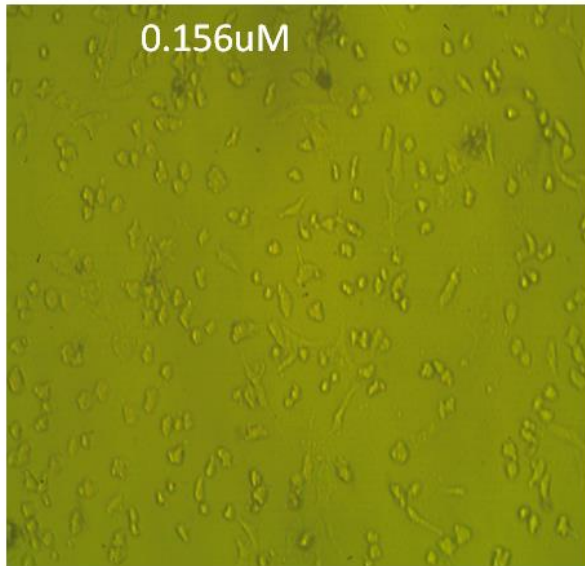
## Effect of sample IXc on Mcf7 cells at different concentration



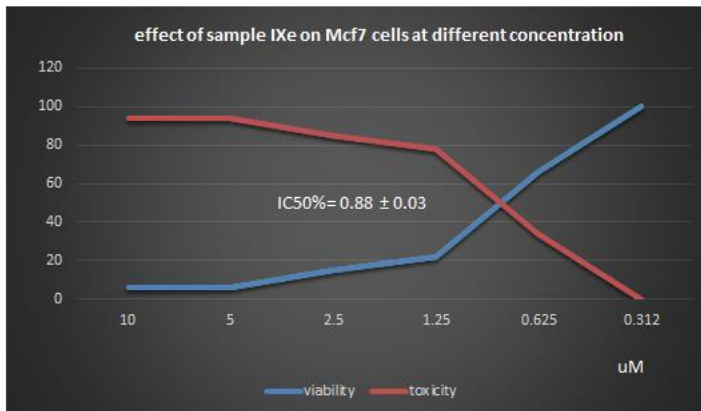
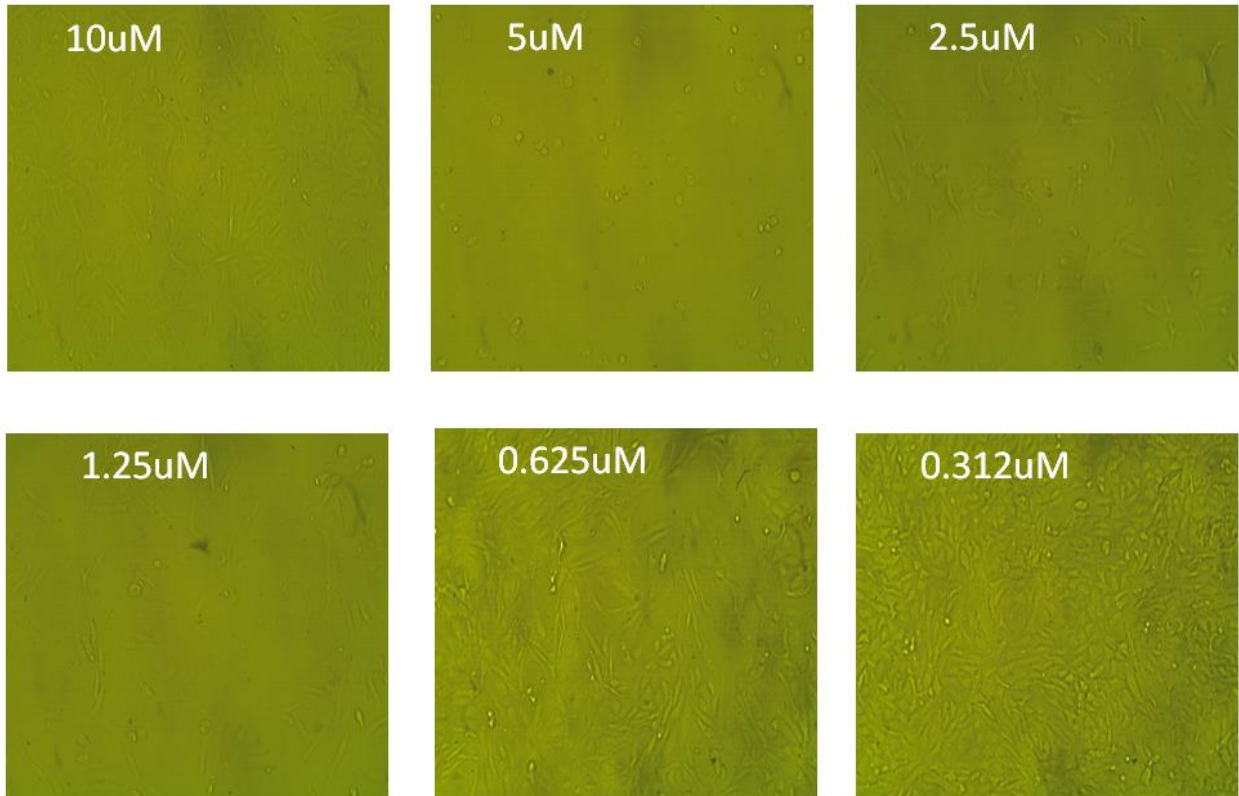
## Effect of sample IXd on Mcf7 cells at different concentration



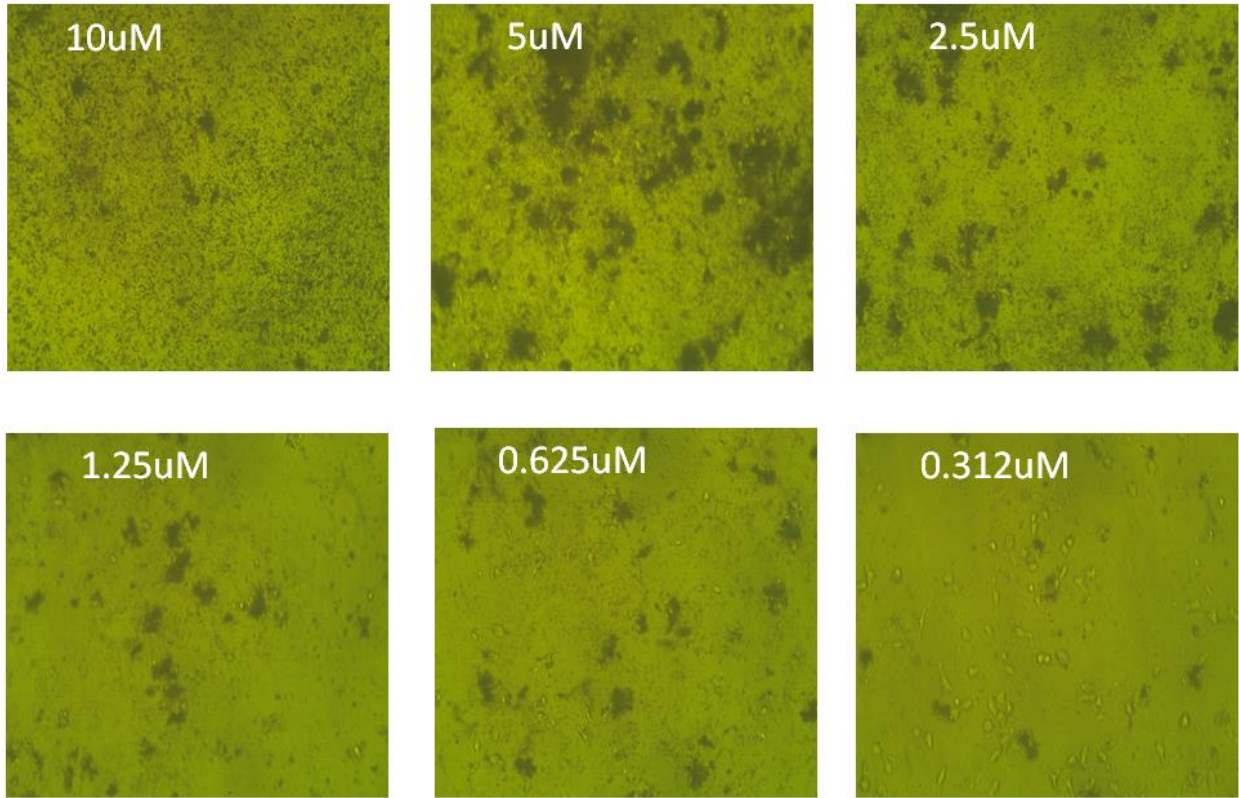
## Effect of sample IXd on MCF7 cells at different concentration



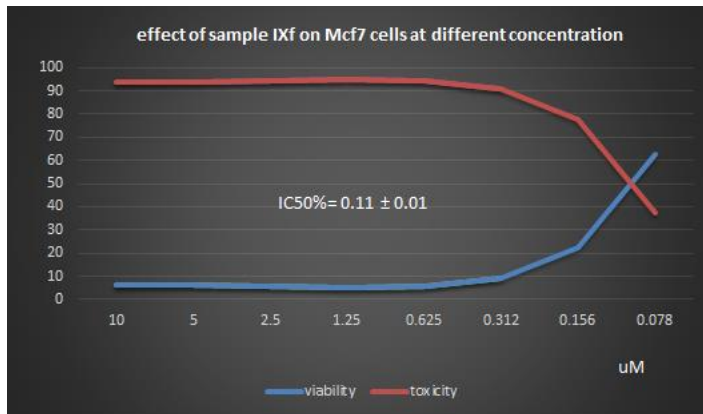
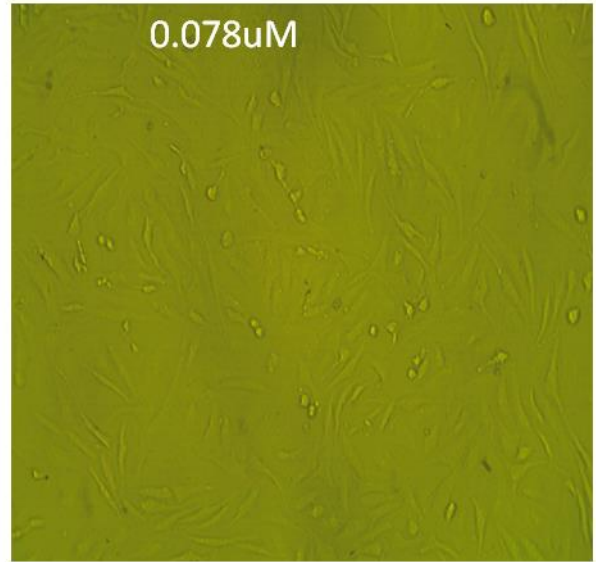
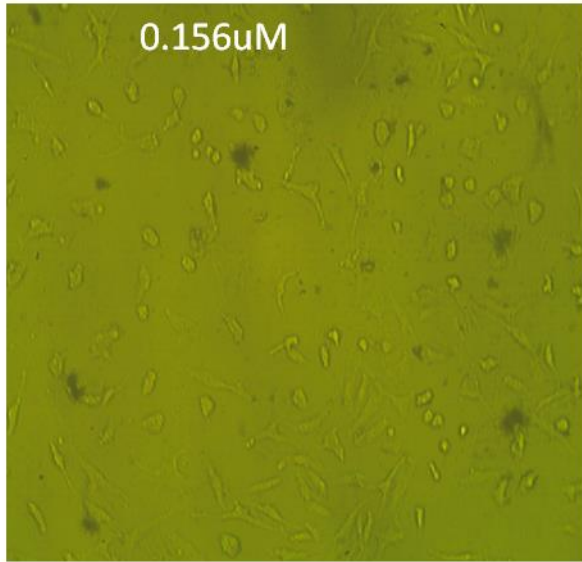
## Effect of sample IXe on Mcf7 cells at different concentration



## Effect of sample IXf on Mcf7 cells at different concentration

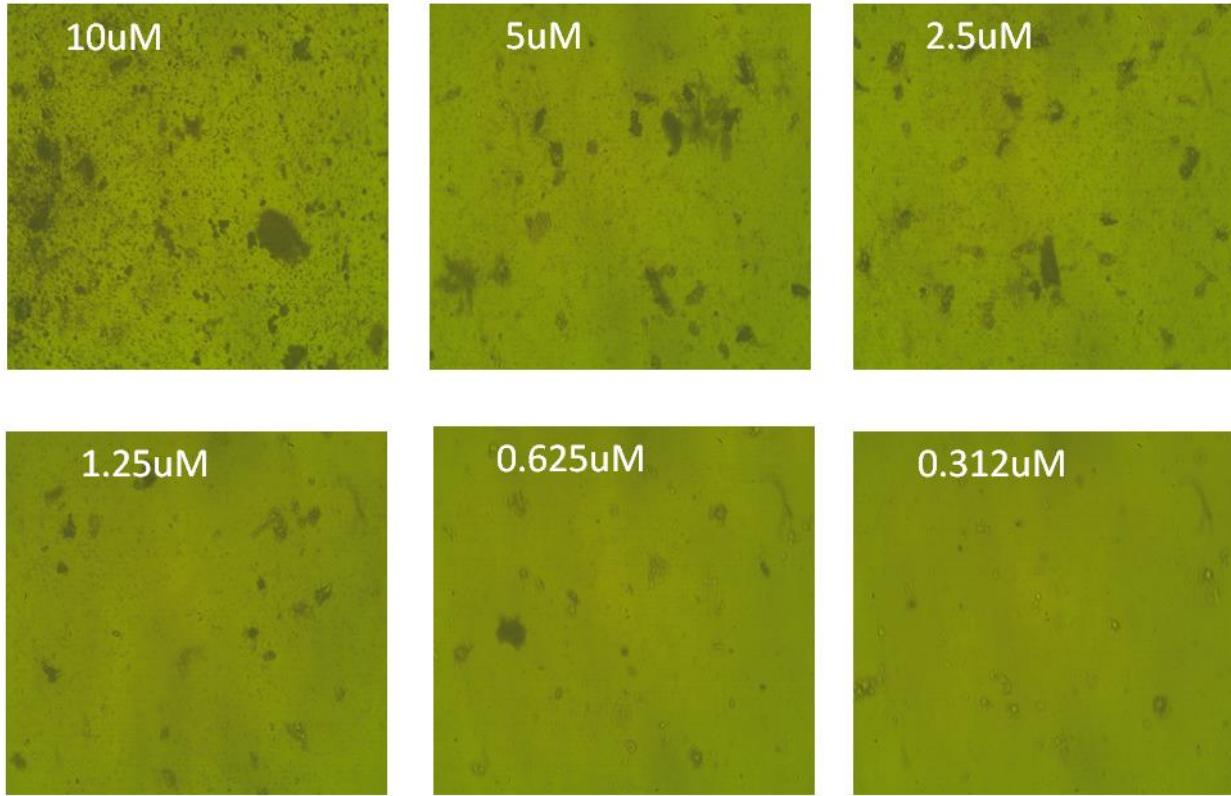


## Effect of sample IXf on Mcf7 cells at different concentration

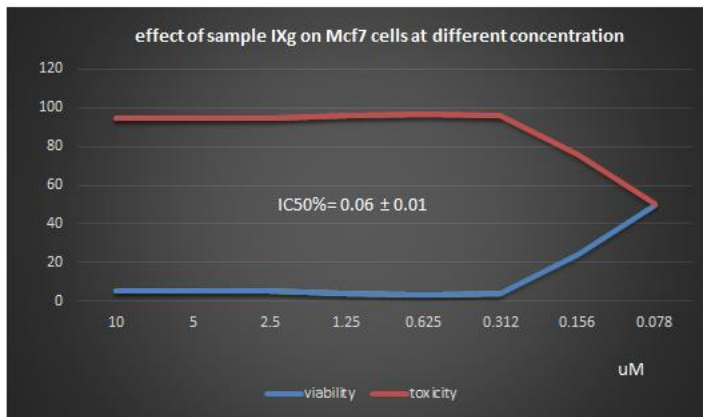
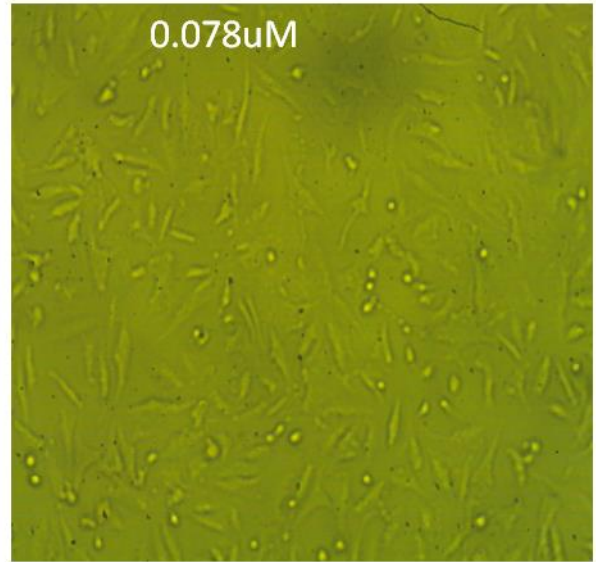
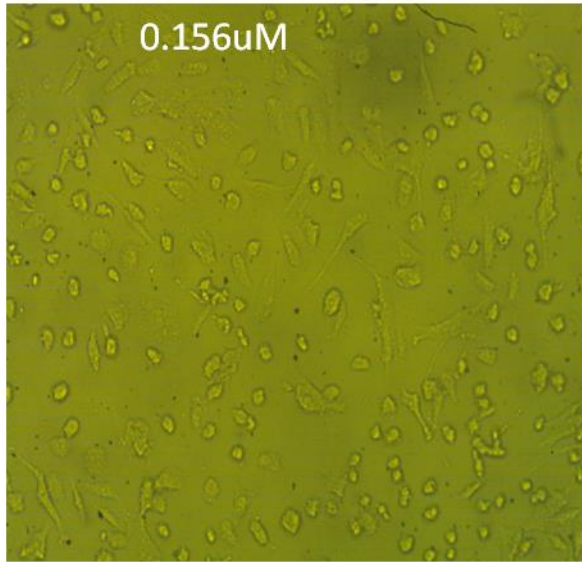




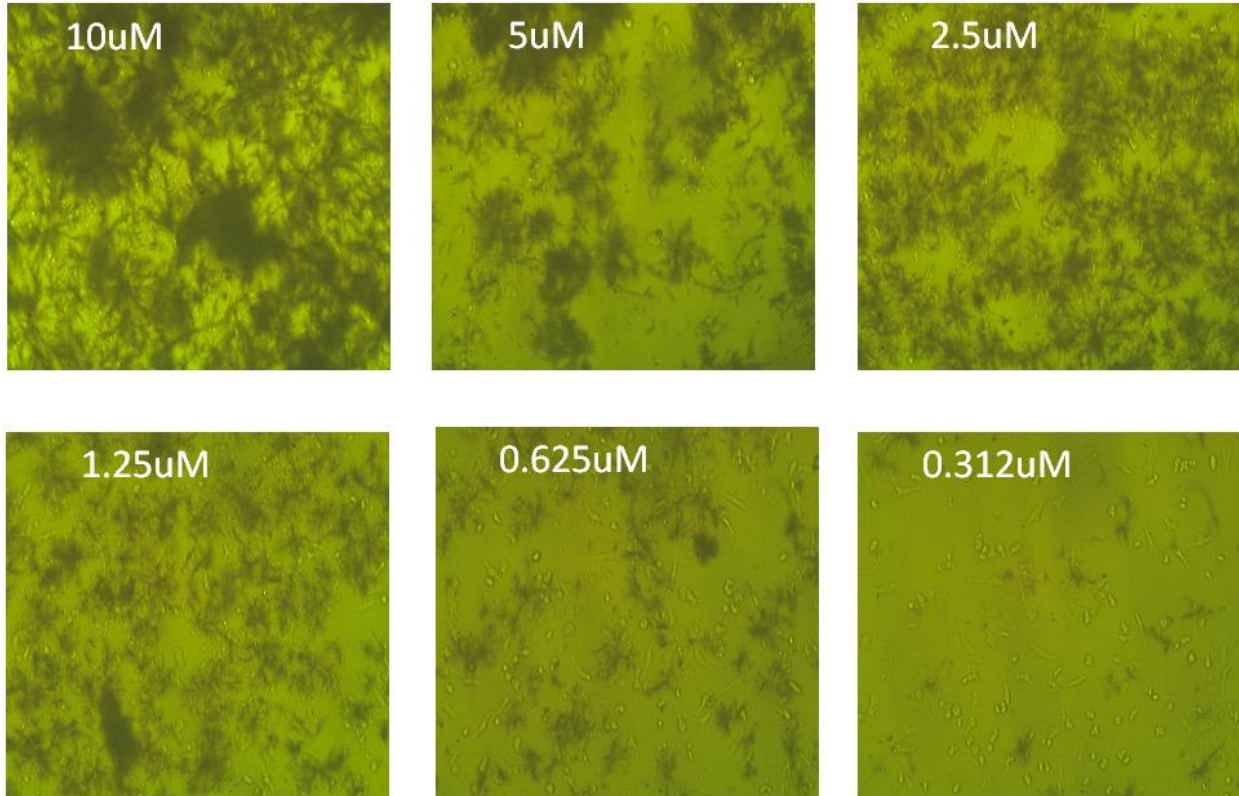
## Effect of sample IXg on Mcf7 cells at different concentration



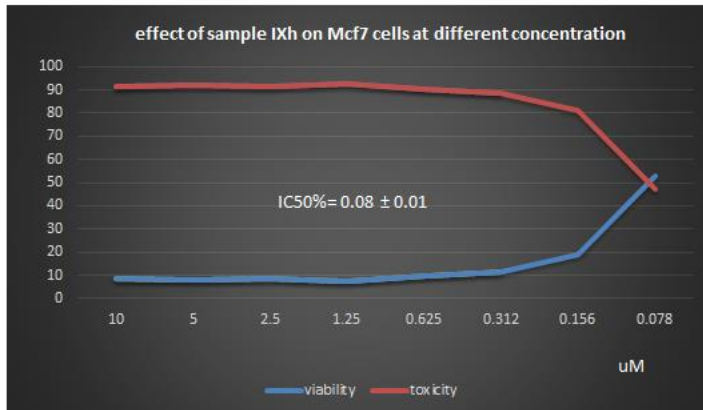
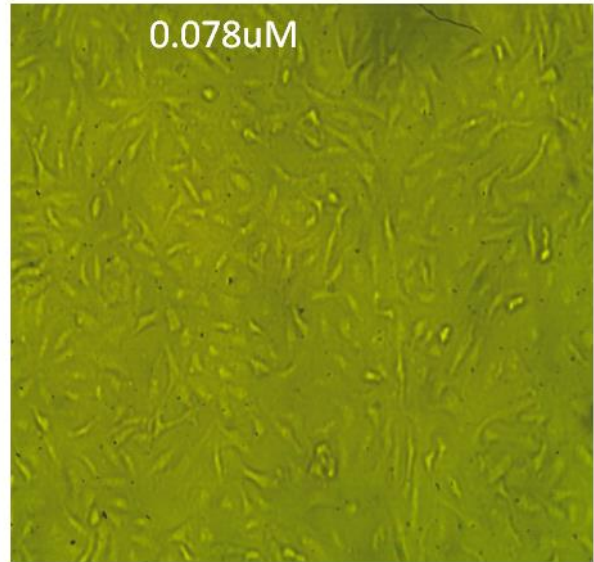
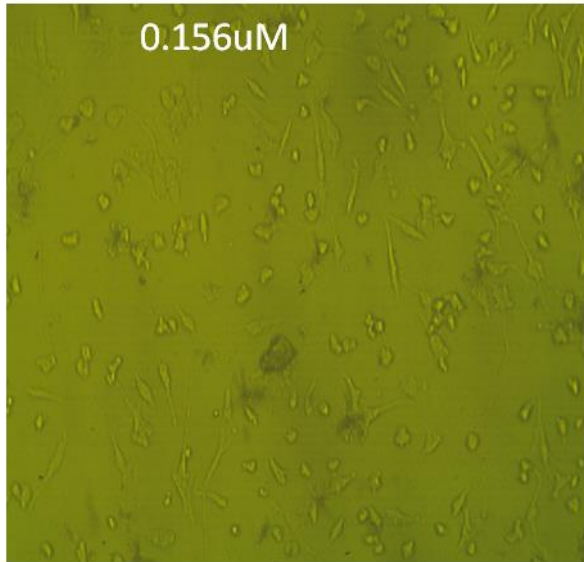
## Effect of sample IXg on Mcf7 cells at different concentration



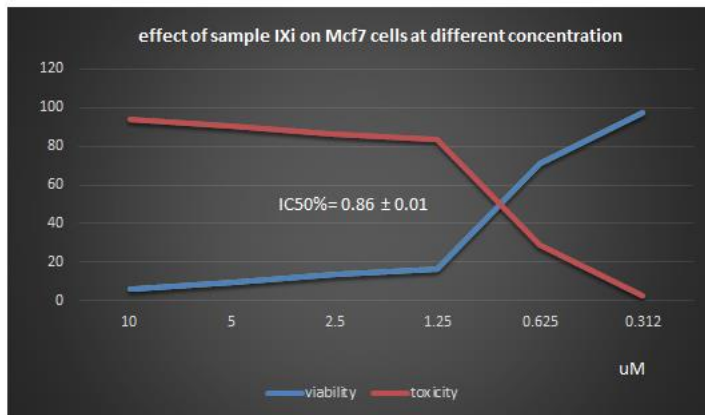
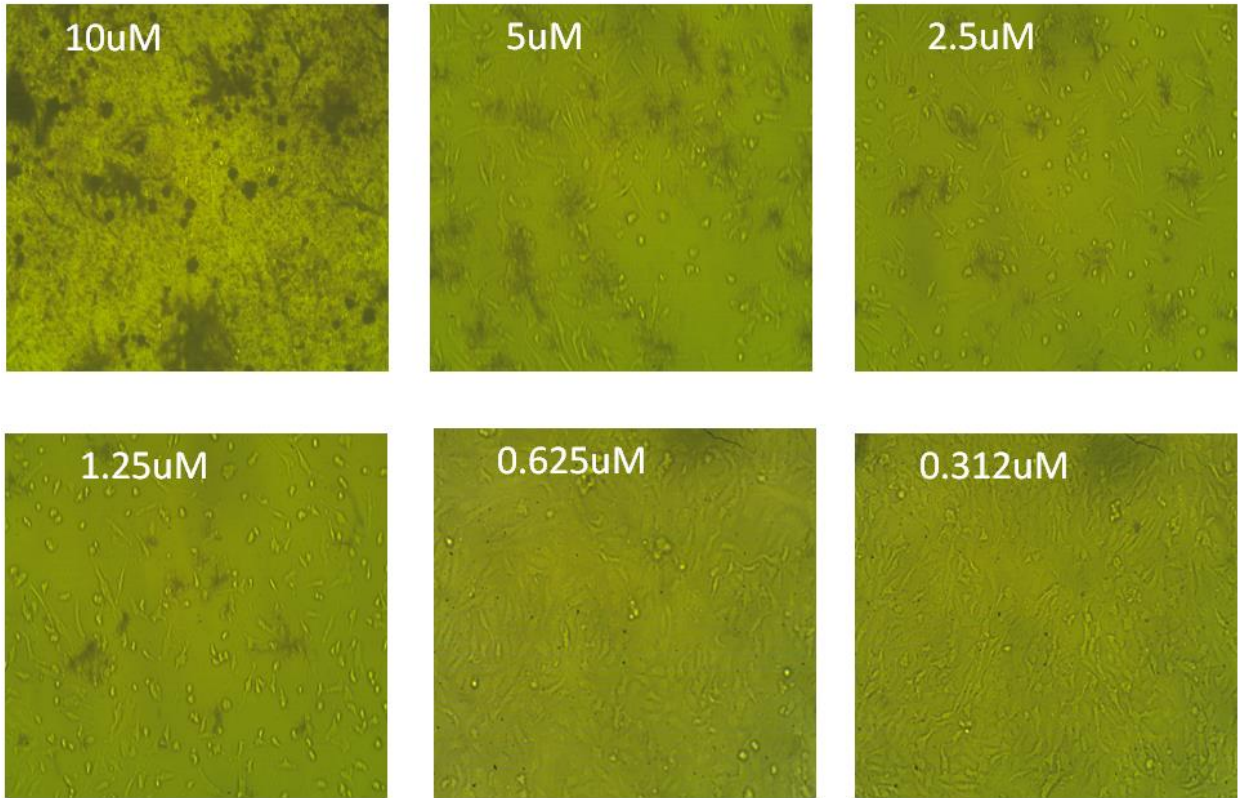
## Effect of sample IXh on Mcf7 cells at different concentration



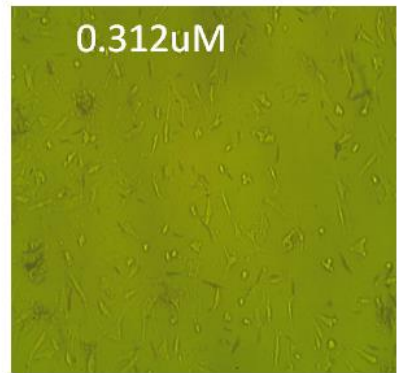
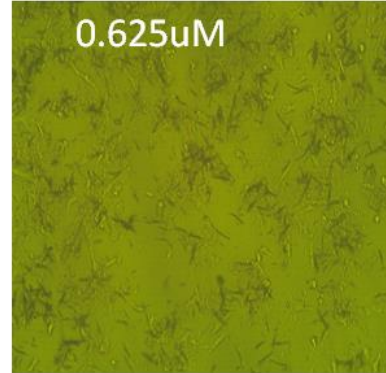
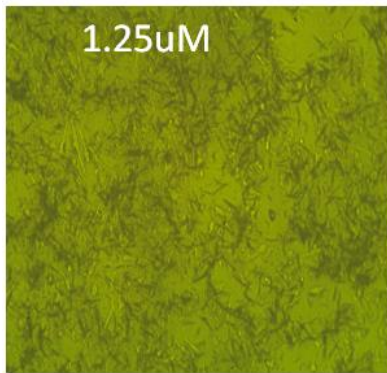
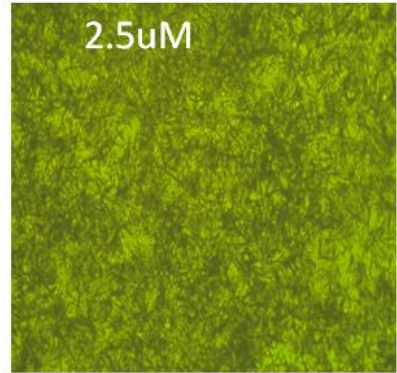
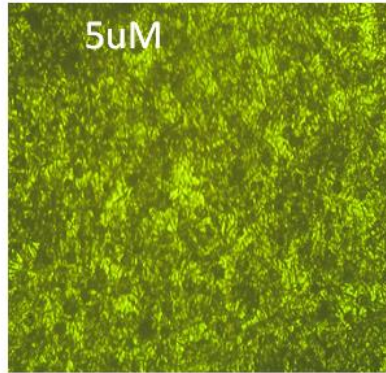
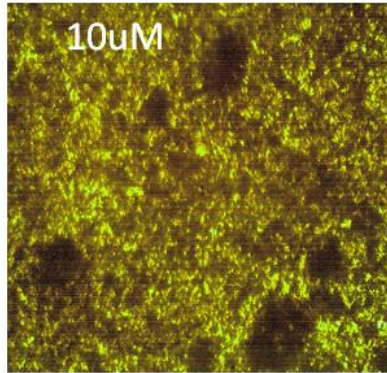
## Effect of sample IXh on Mcf7 cells at different concentration



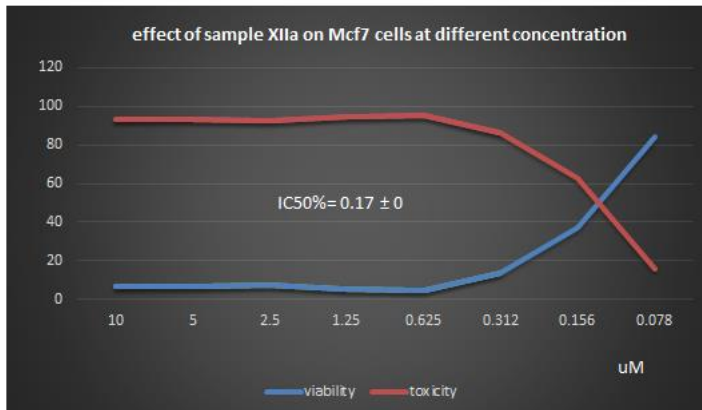
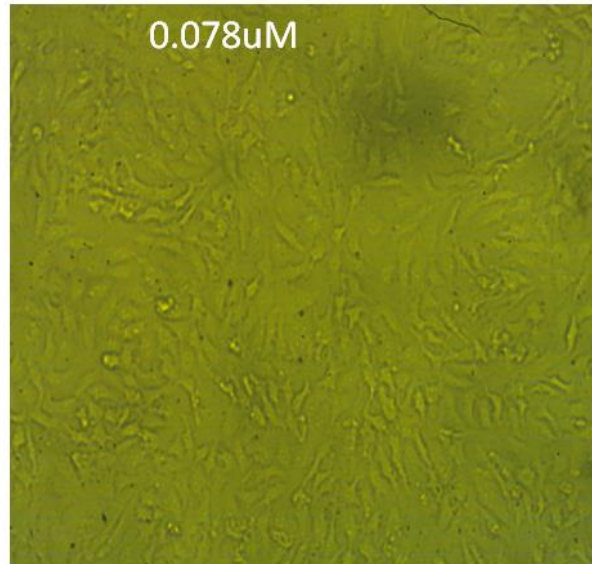
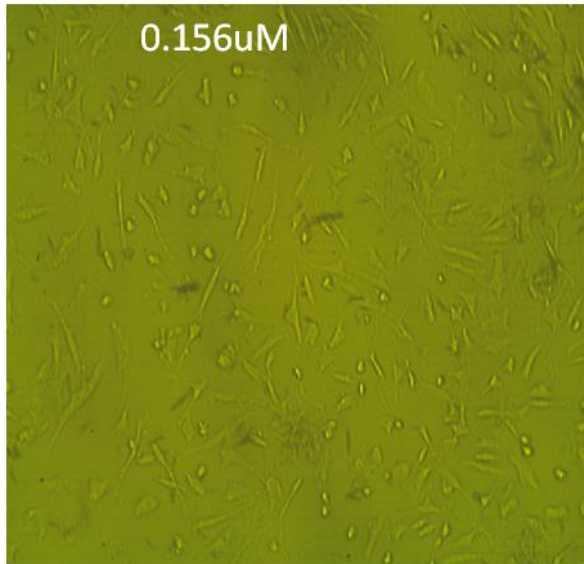
## Effect of sample IXi on Mcf7 cells at different concentration



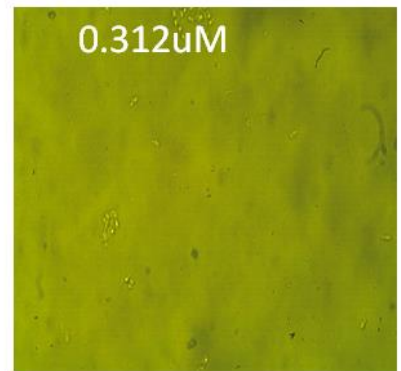
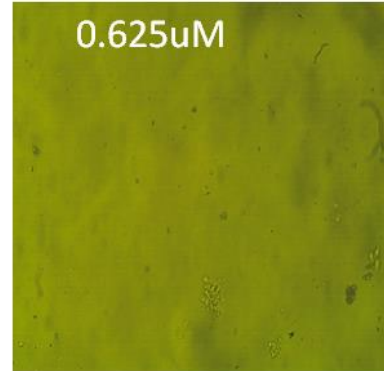
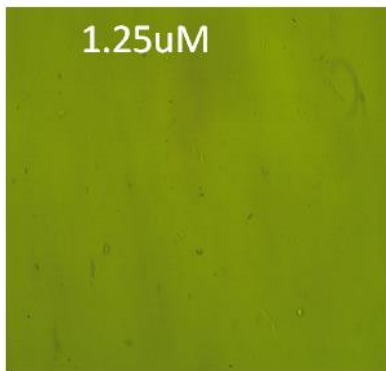
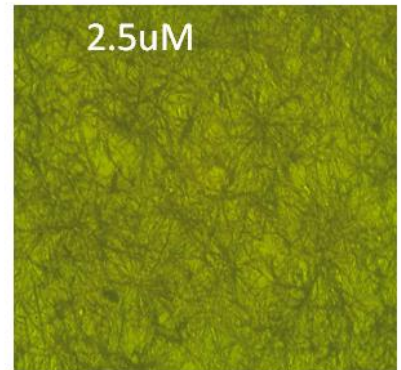
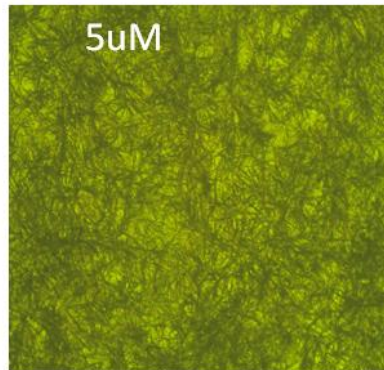
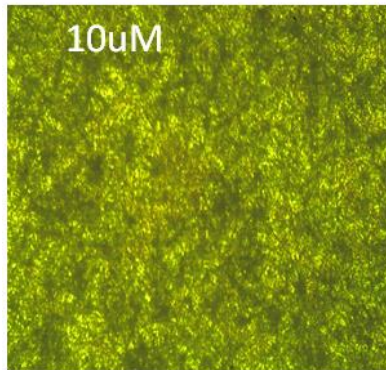
## Effect of sample Xlla on Mcf7 cells at different concentration



## Effect of sample Xlla on Mcf7 cells at different concentration

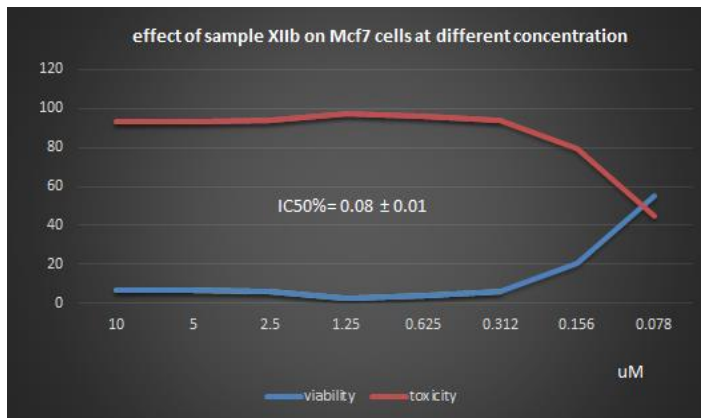
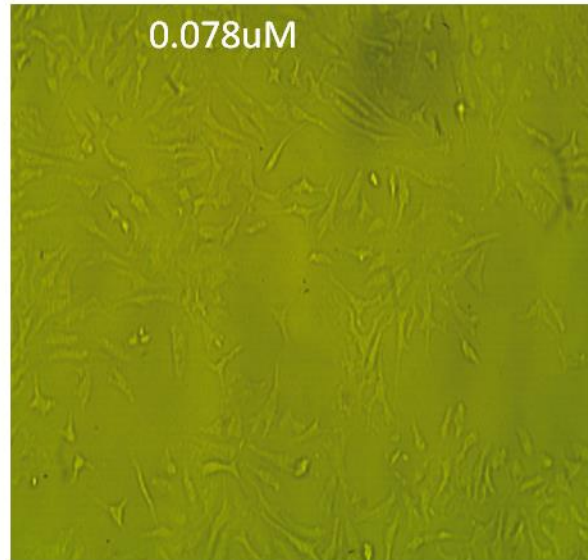
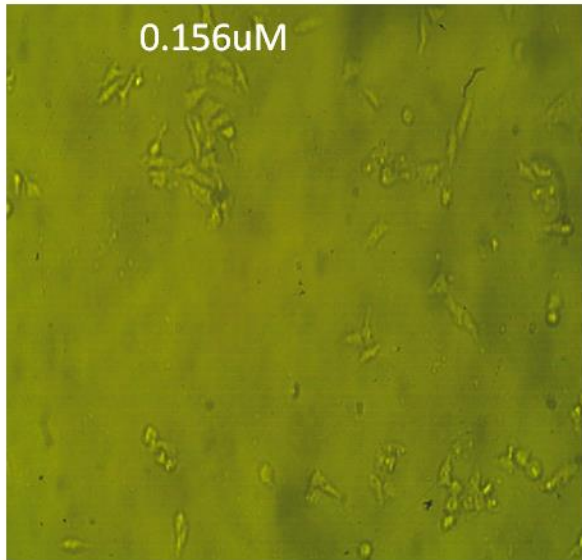


## Effect of sample XIIb on Mcf7 cells at different concentration

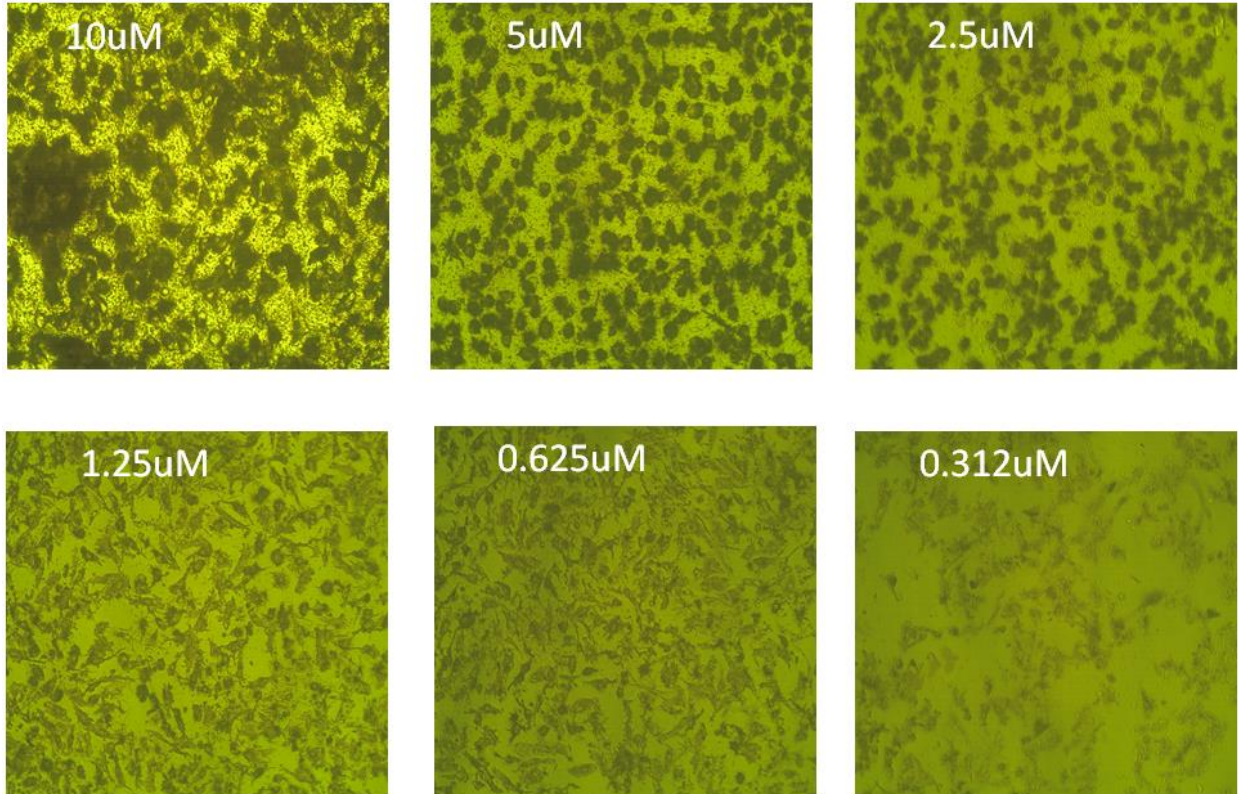




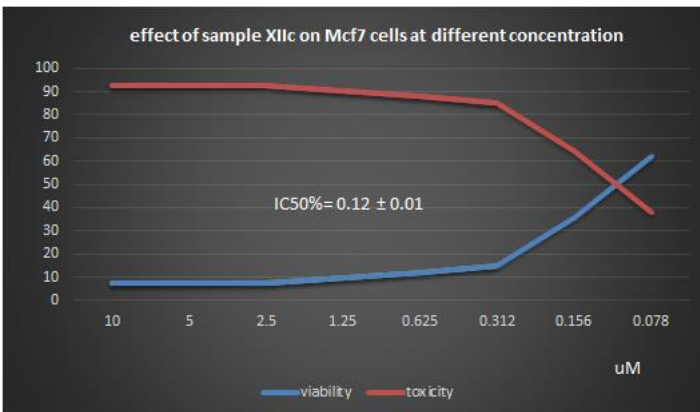
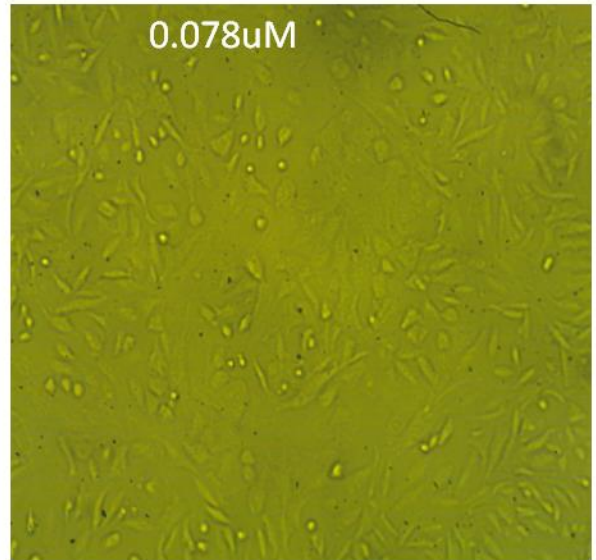
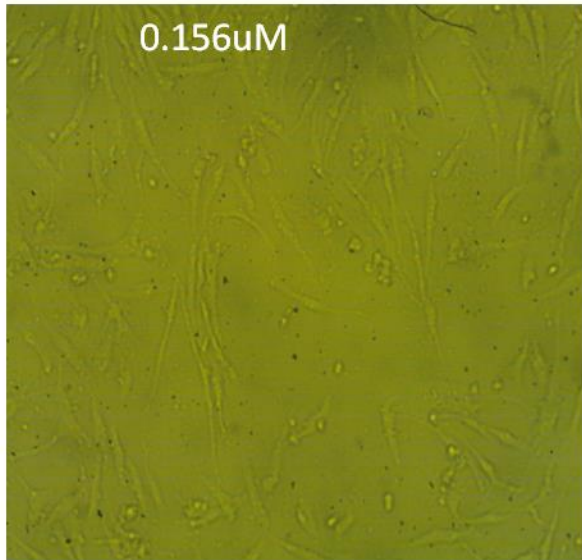
## Effect of sample XIIb on MCF7 cells at different concentration



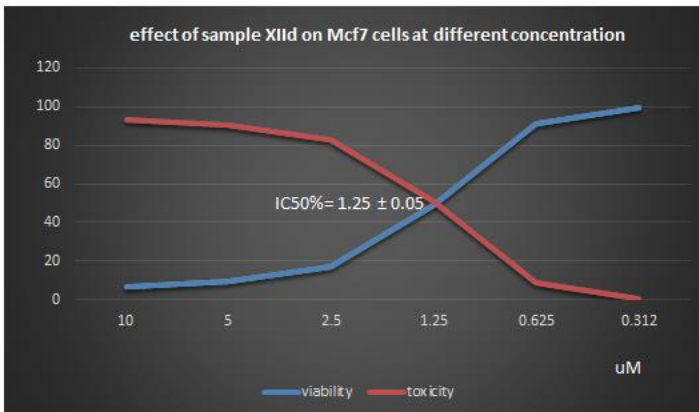
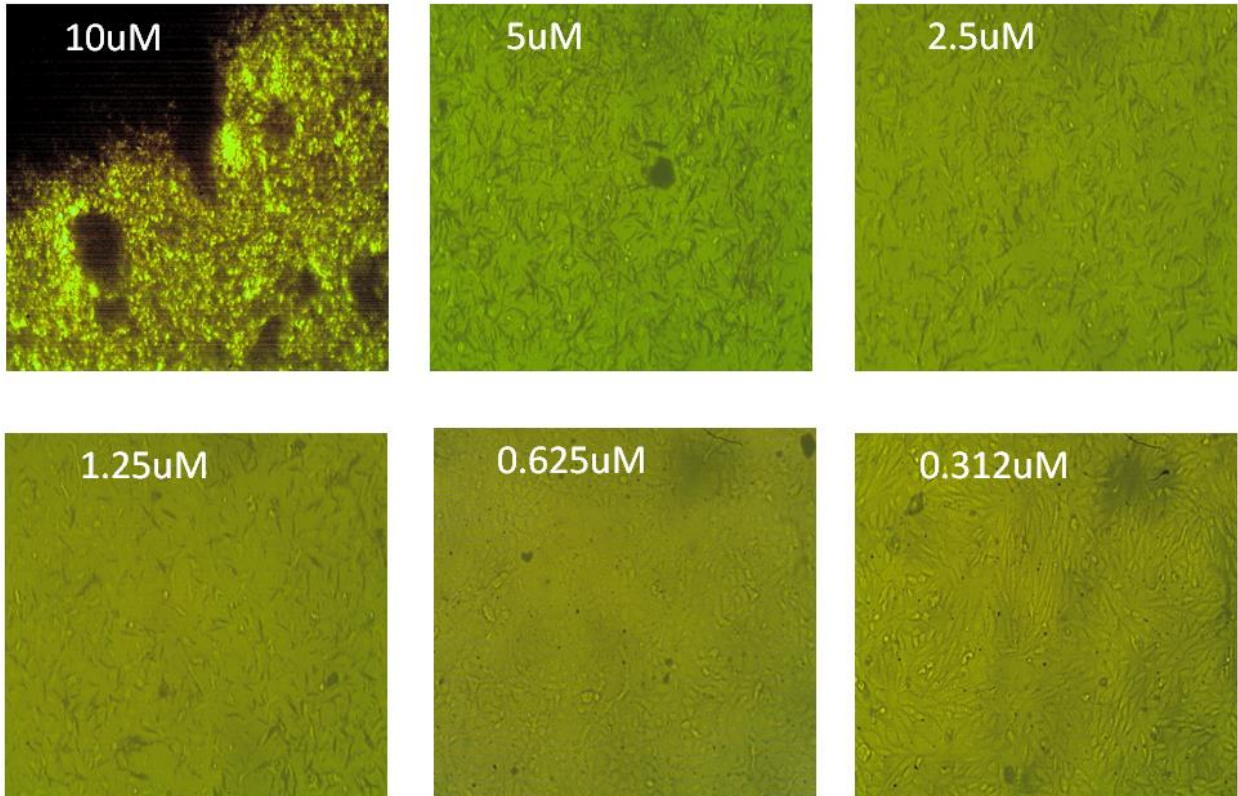
## Effect of sample XIIc on MCF7 cells at different concentration



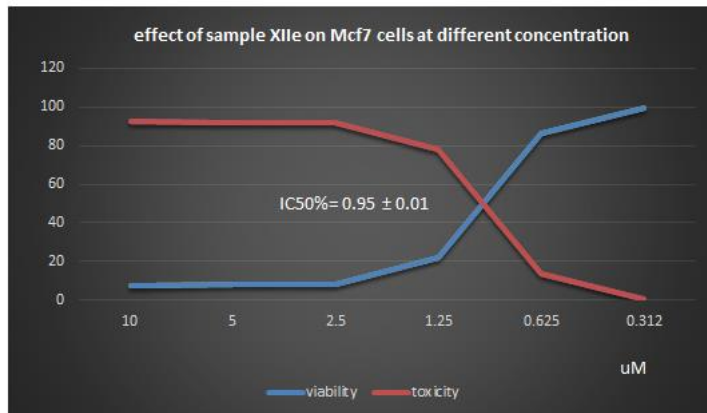
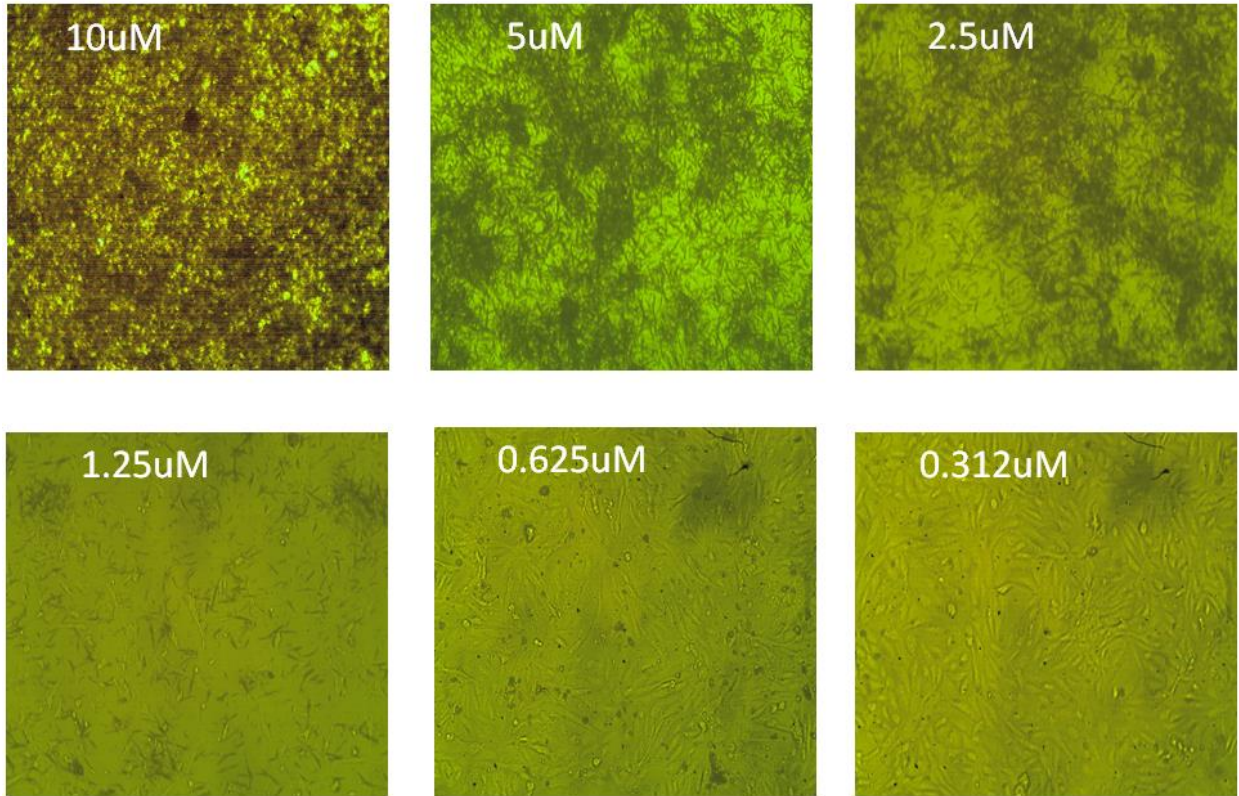
## Effect of sample XIIc on Mcf7 cells at different concentration



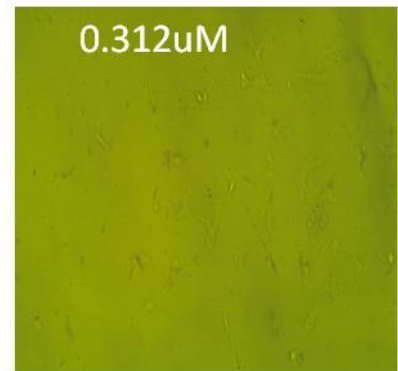
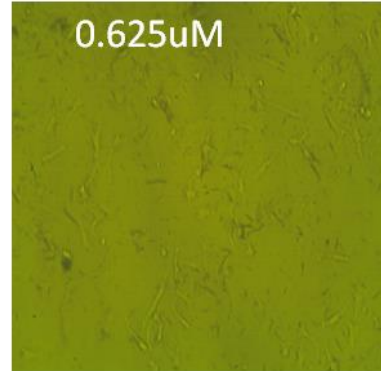
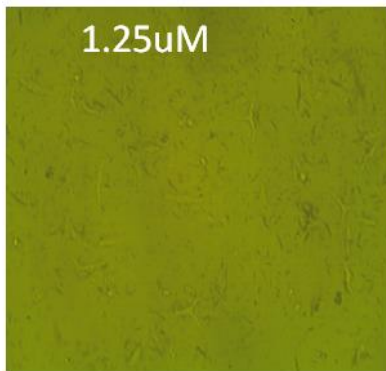
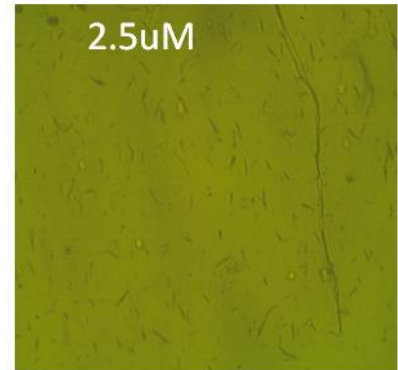
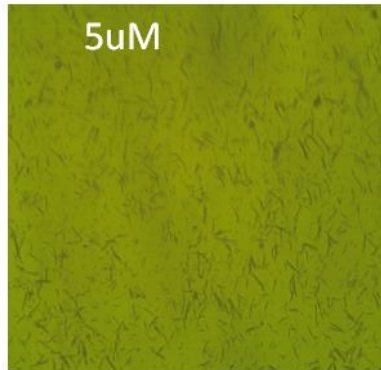
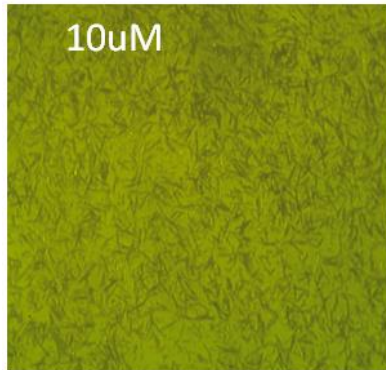
## Effect of sample XIId on MCF7 cells at different concentration



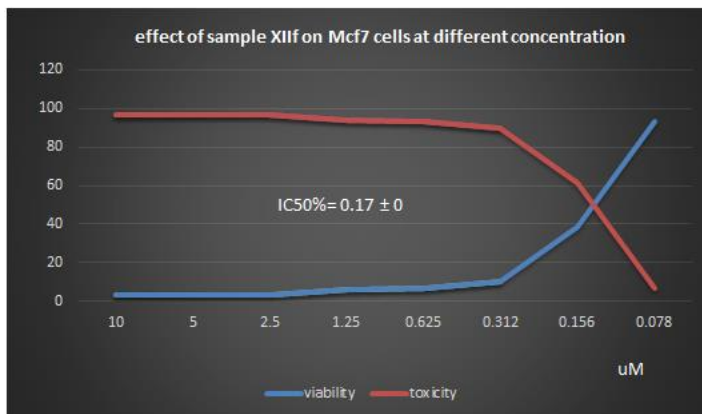
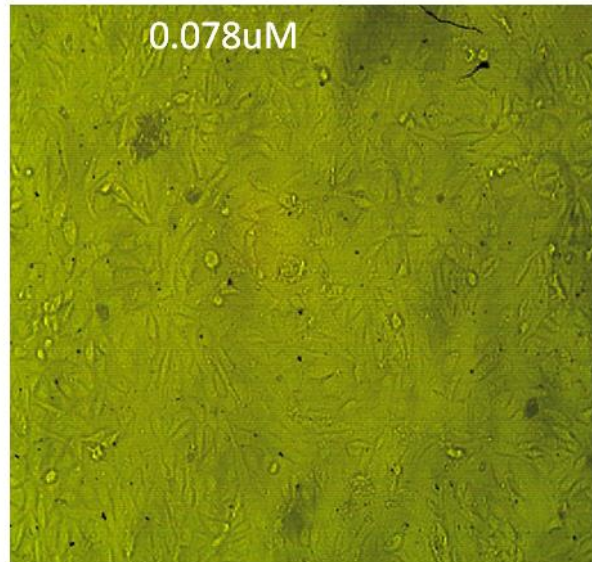
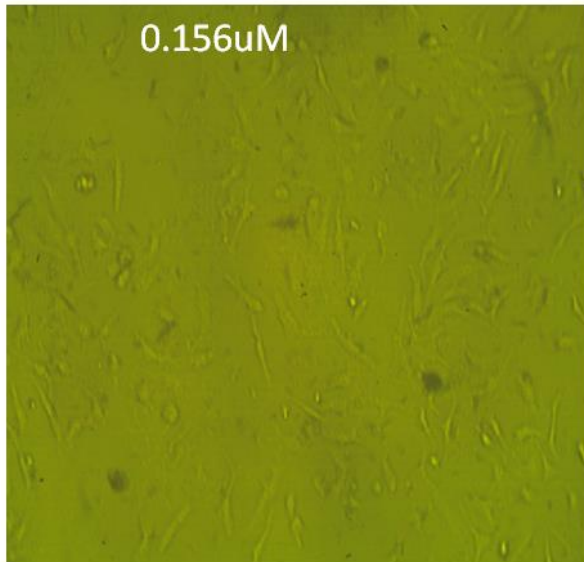
## Effect of sample XIIE on MCF7 cells at different concentration



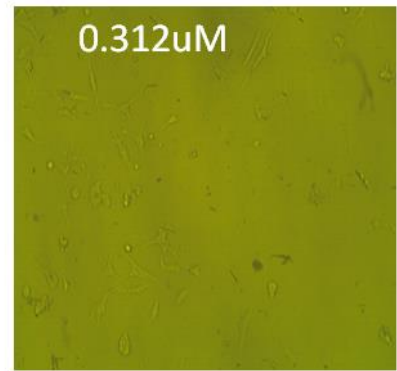
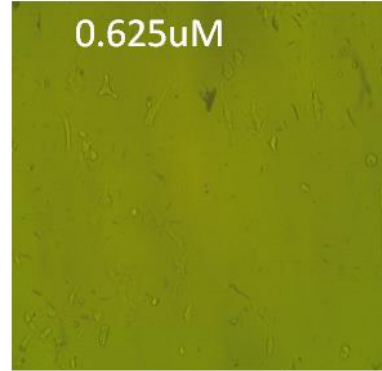
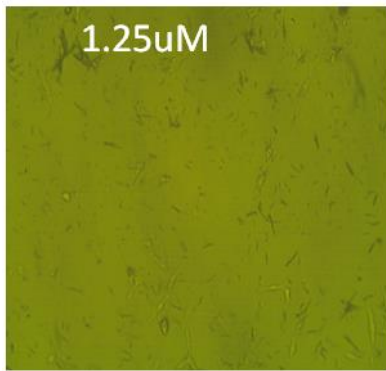
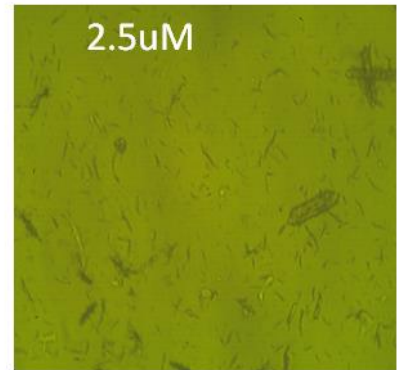
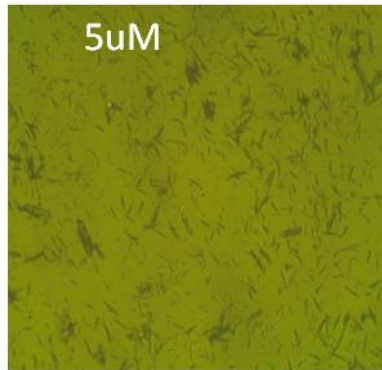
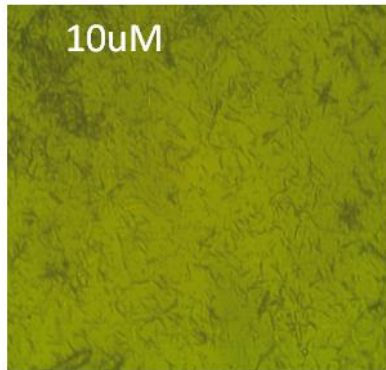
## Effect of sample XIIf on Mcf7 cells at different concentration



## Effect of sample XIIIf on Mcf7 cells at different concentration

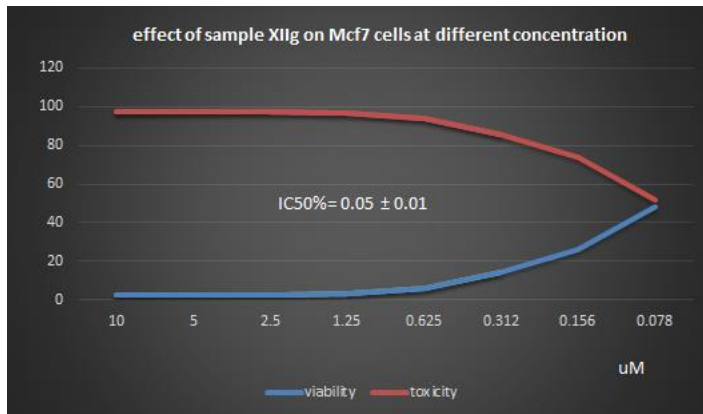
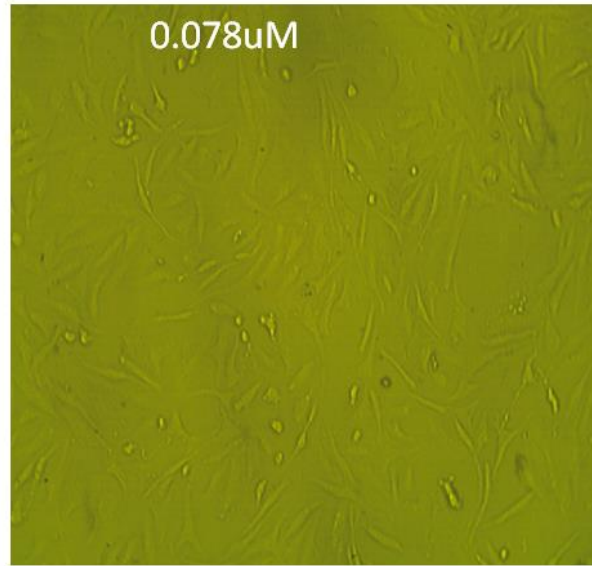
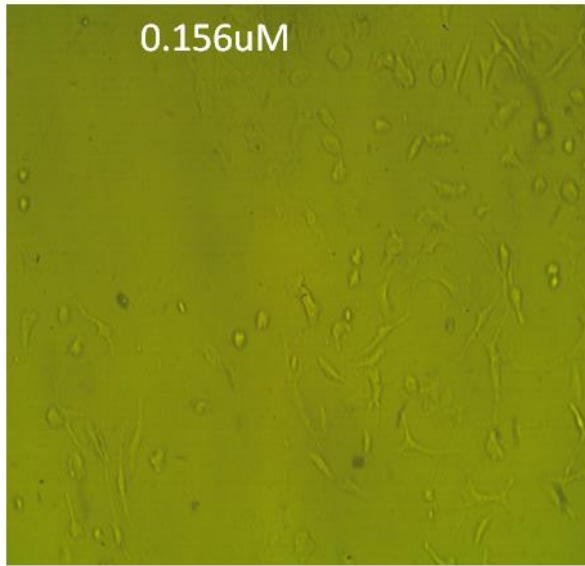


## Effect of sample XIlg on MCF7 cells at different concentration

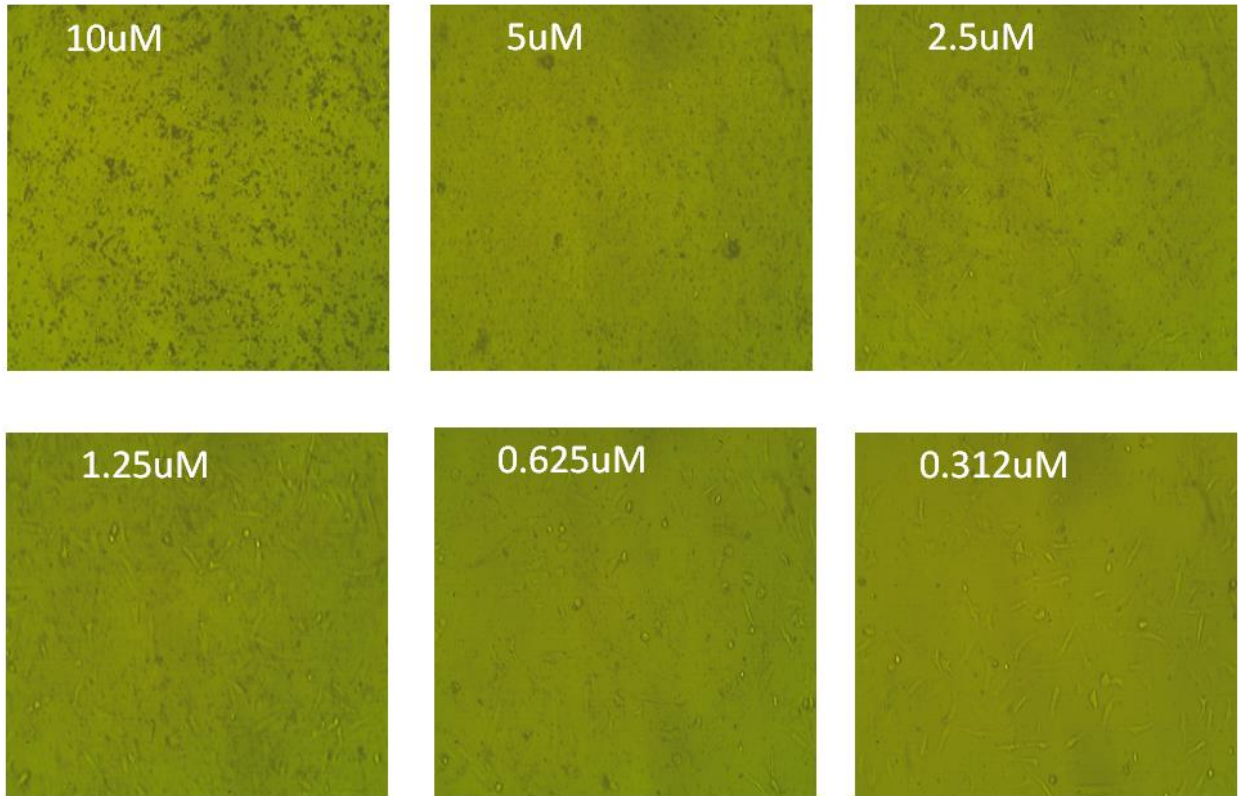




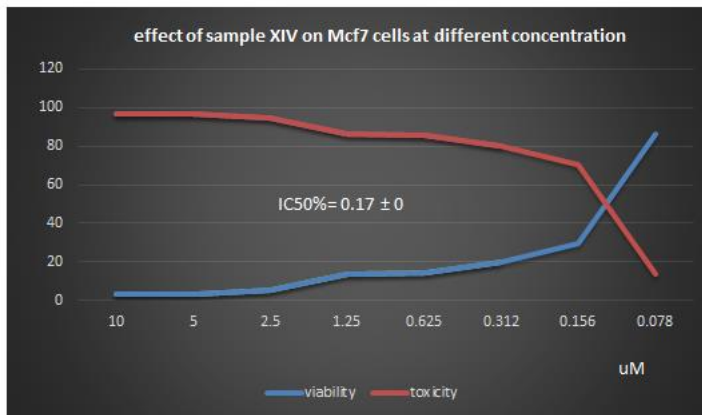
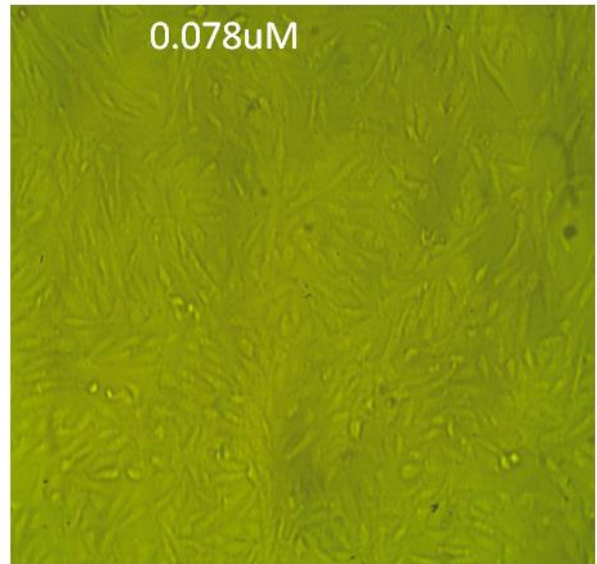
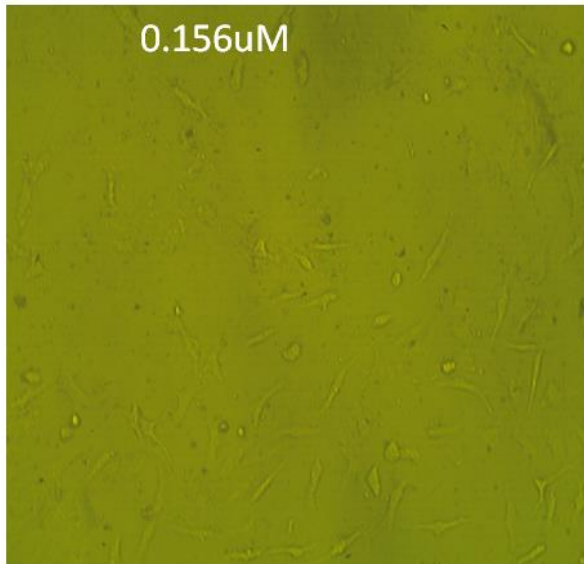
## Effect of sample XIIg on Mcf7 cells at different concentration



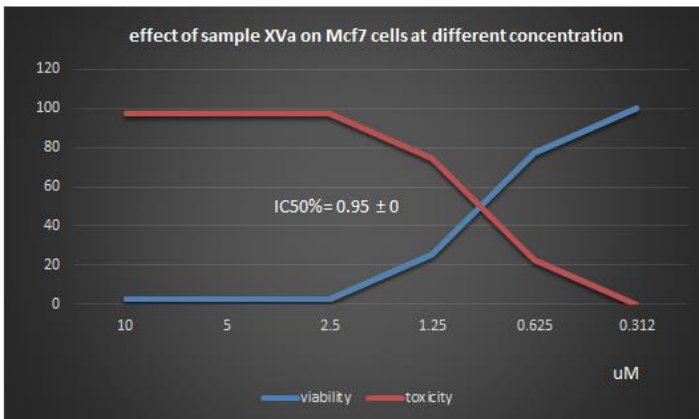
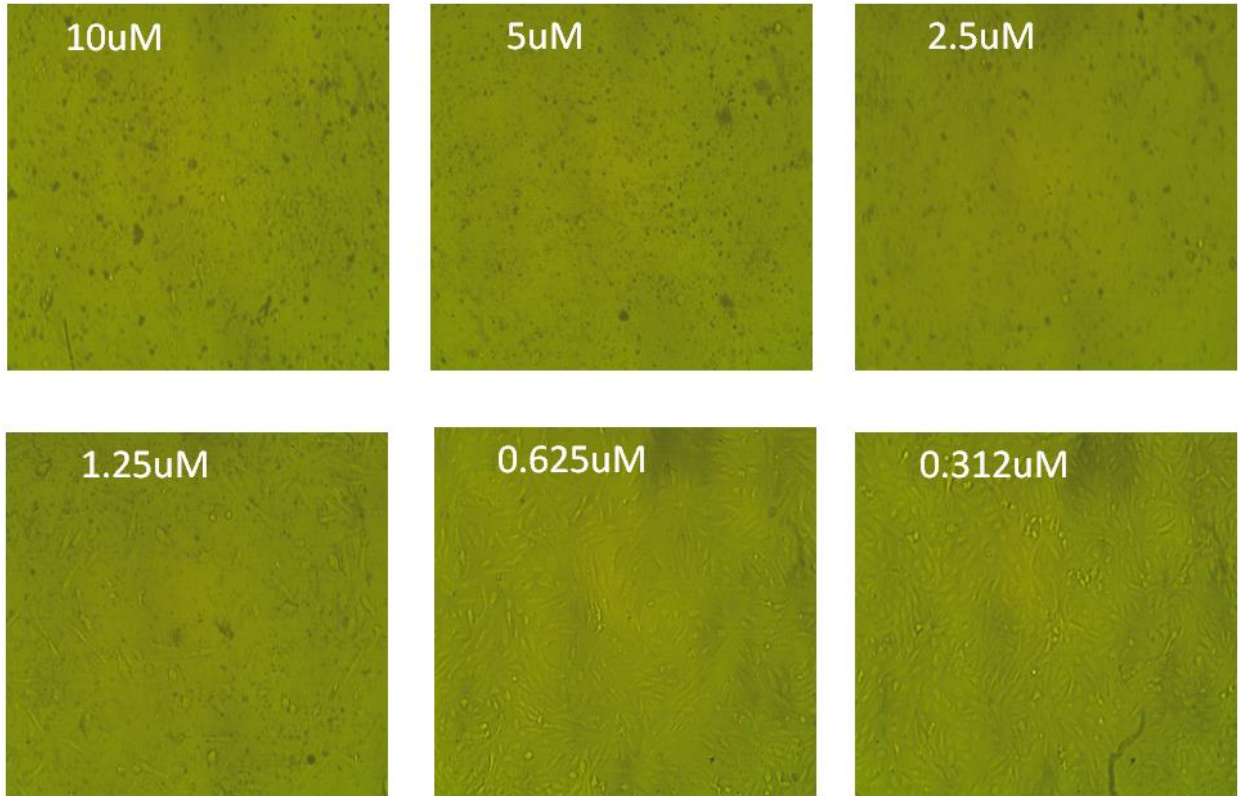
## Effect of sample XIV on Mcf7 cells at different concentration



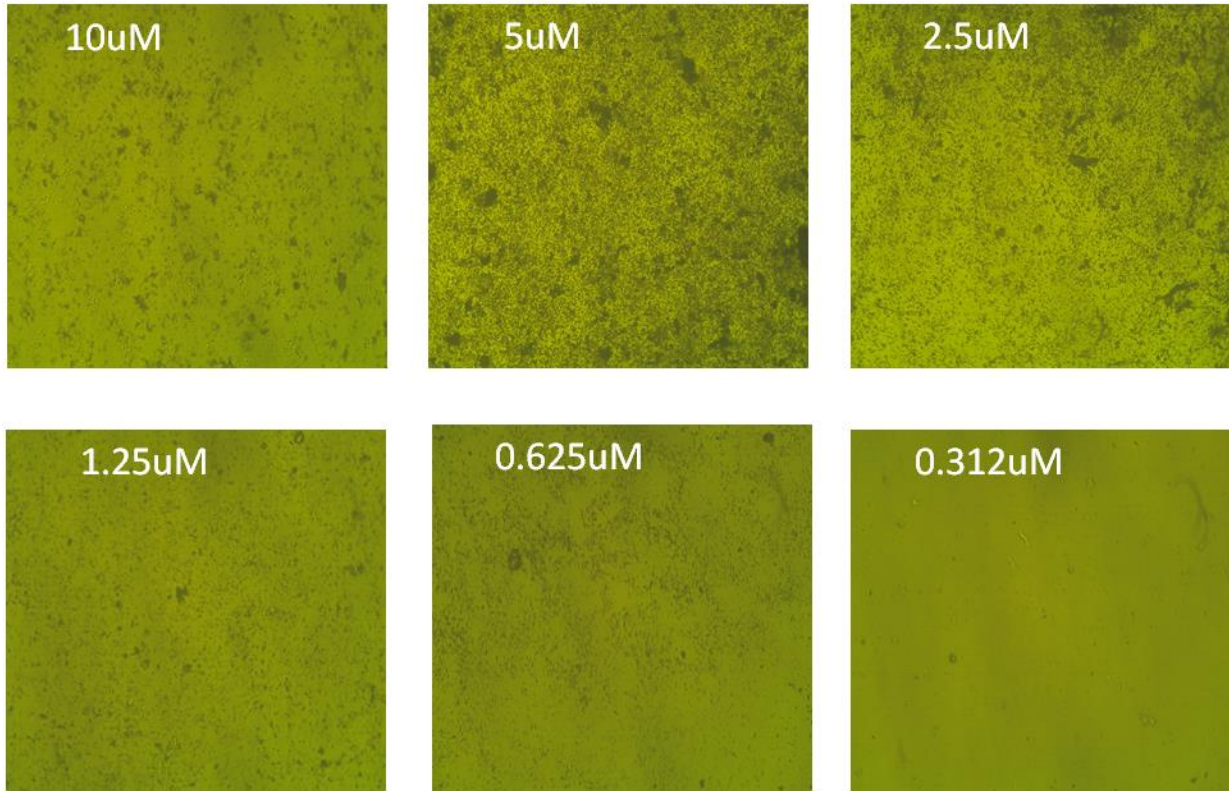
## Effect of sample XIV on MCF7 cells at different concentration



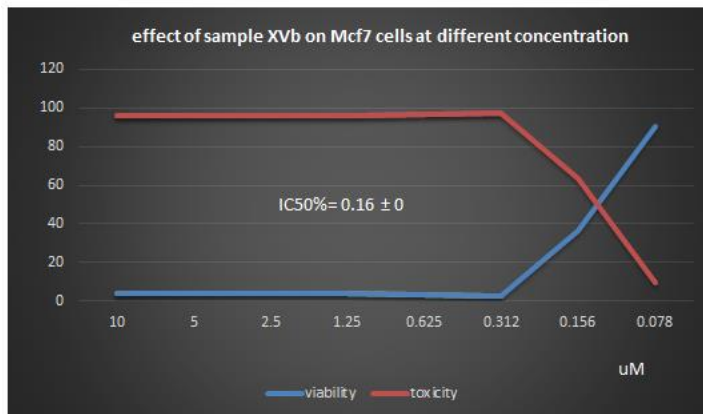
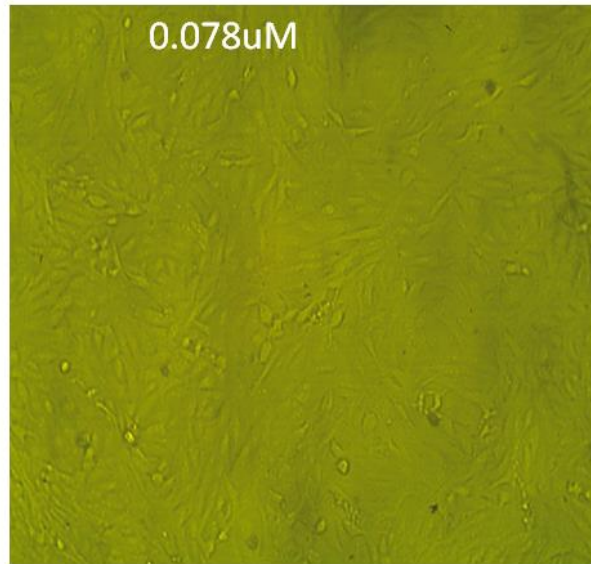
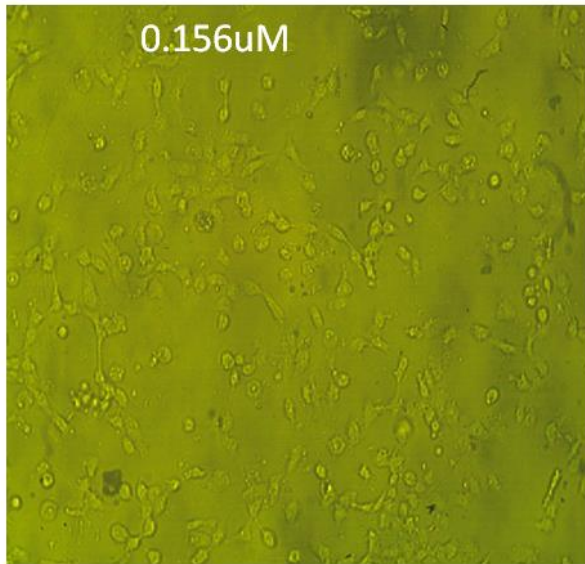
## Effect of sample XVa on Mcf7 cells at different concentration



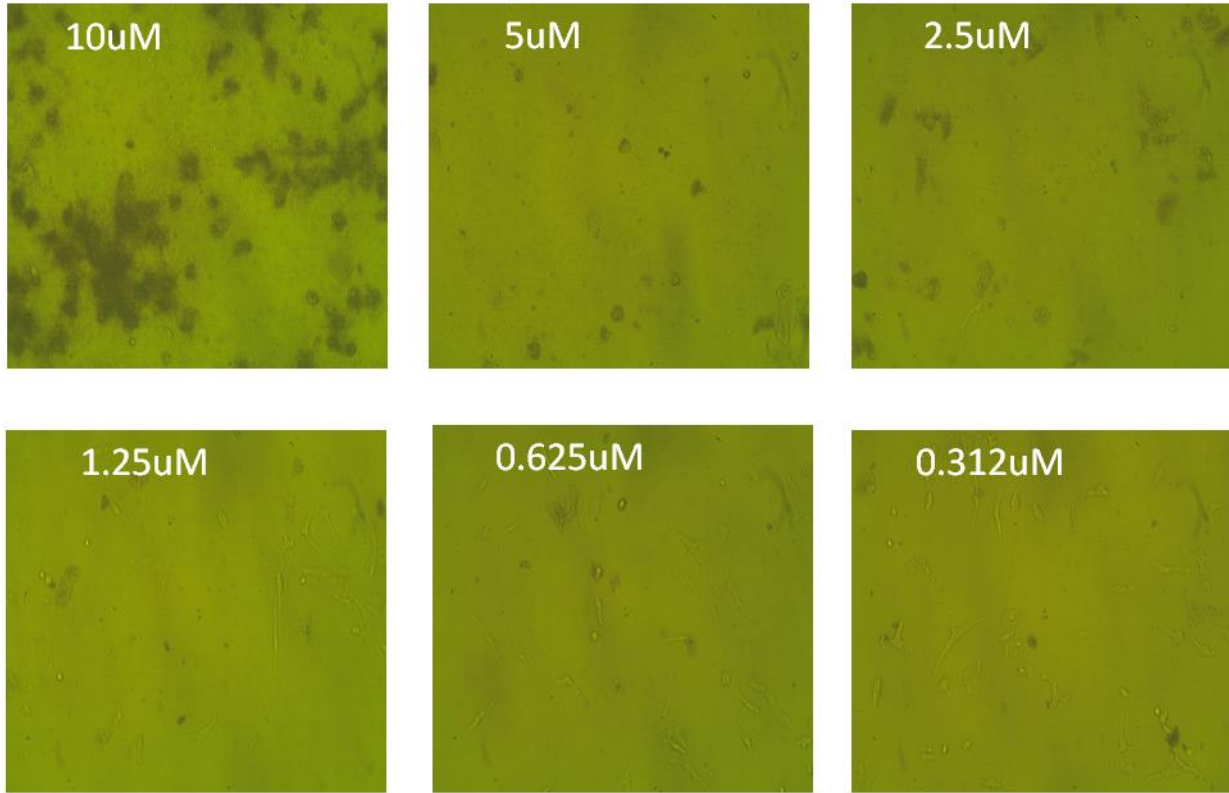
## Effect of sample XVb on MCF7 cells at different concentration



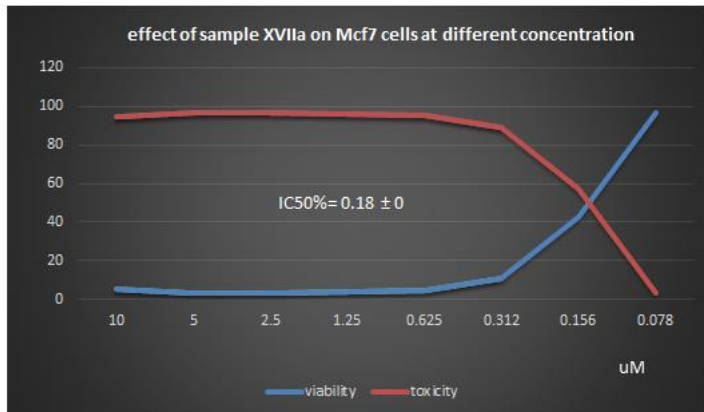
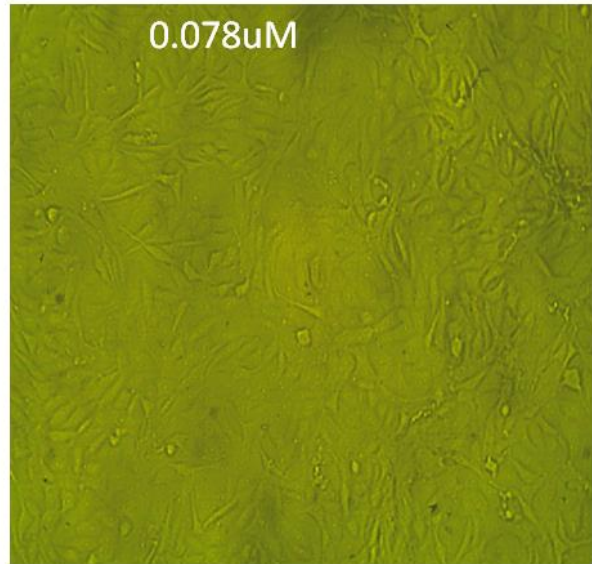
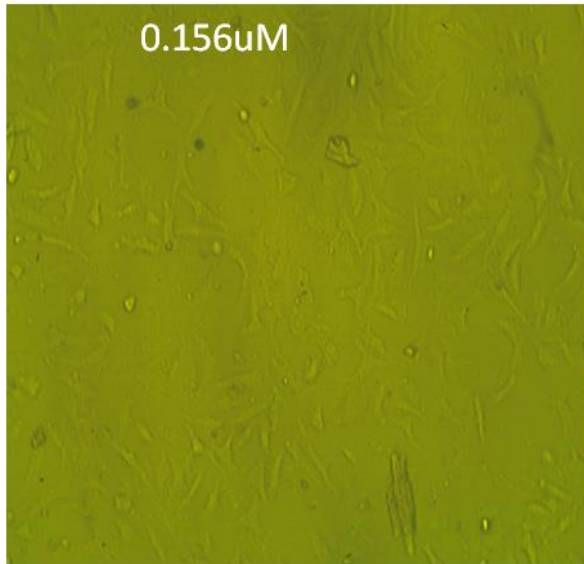
## Effect of sample XVb on Mcf7 cells at different concentration



## Effect of sample XVIIa on Mcf7 cells at different concentration

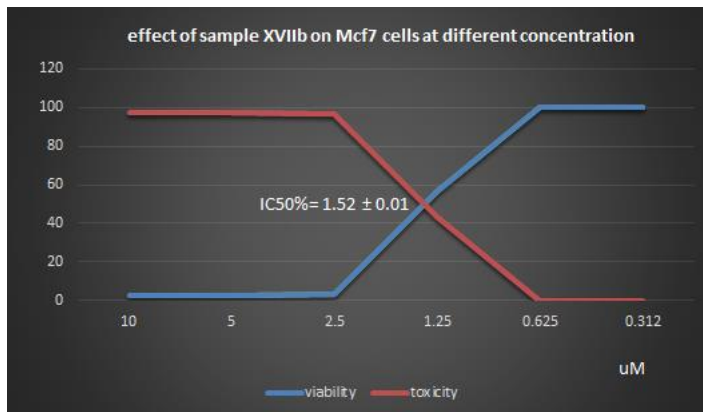
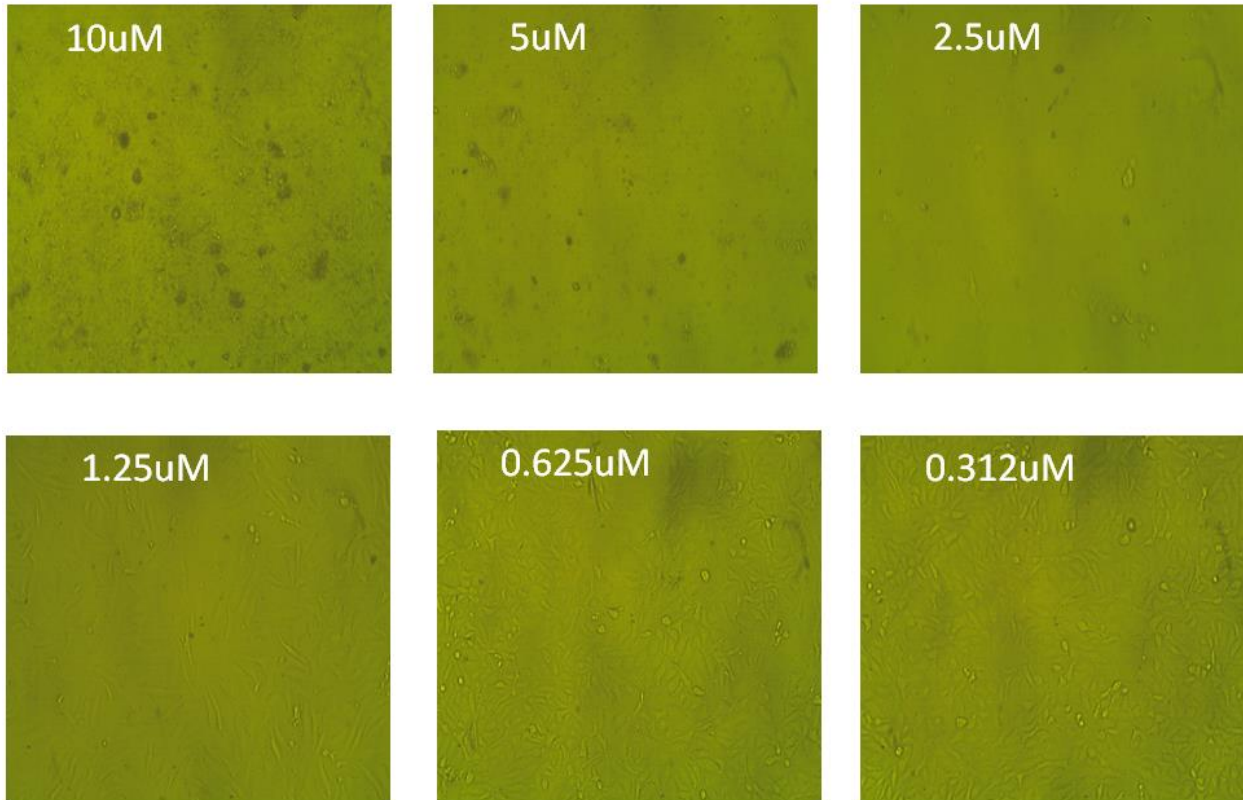


## Effect of sample XVIIa on MCF7 cells at different concentration

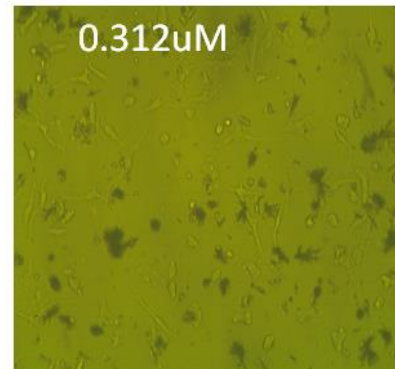
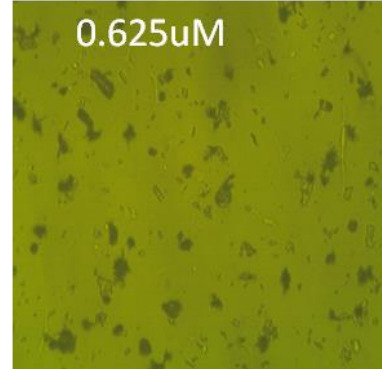
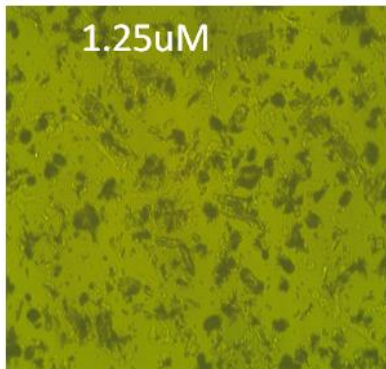
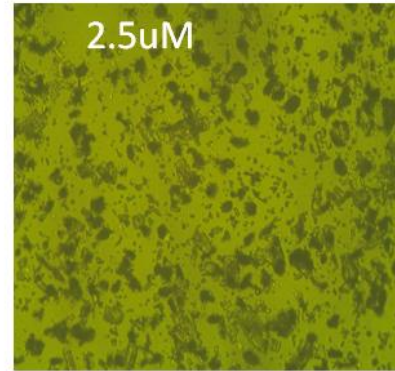
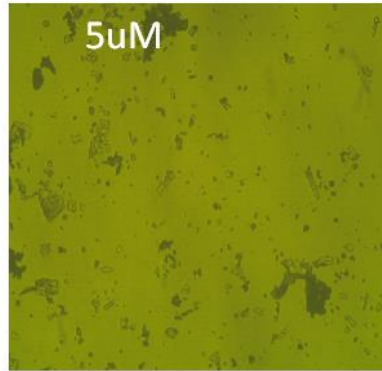
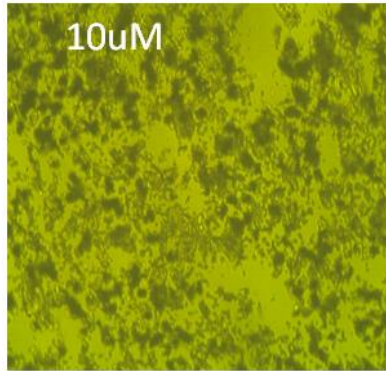




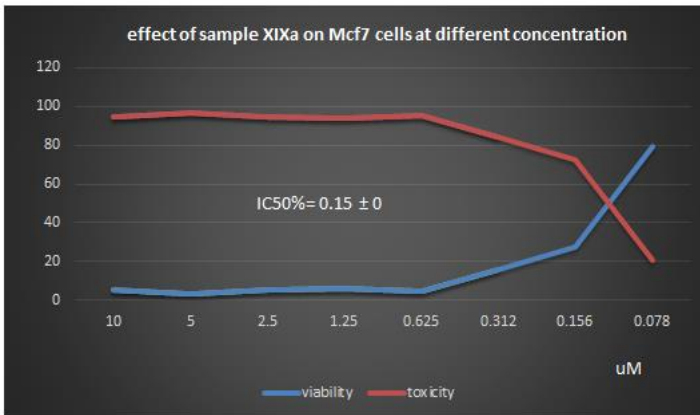
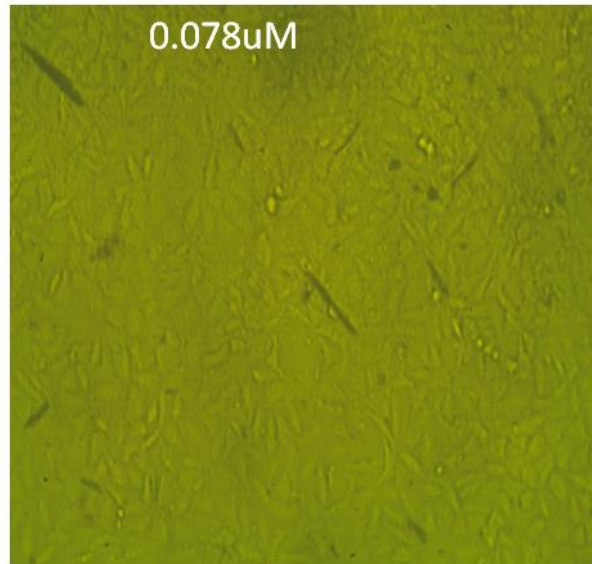
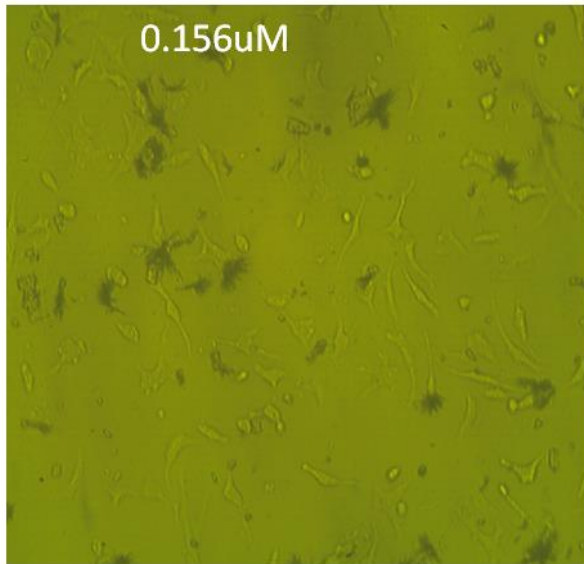
## Effect of sample XVIIb on MCF7 cells at different concentration



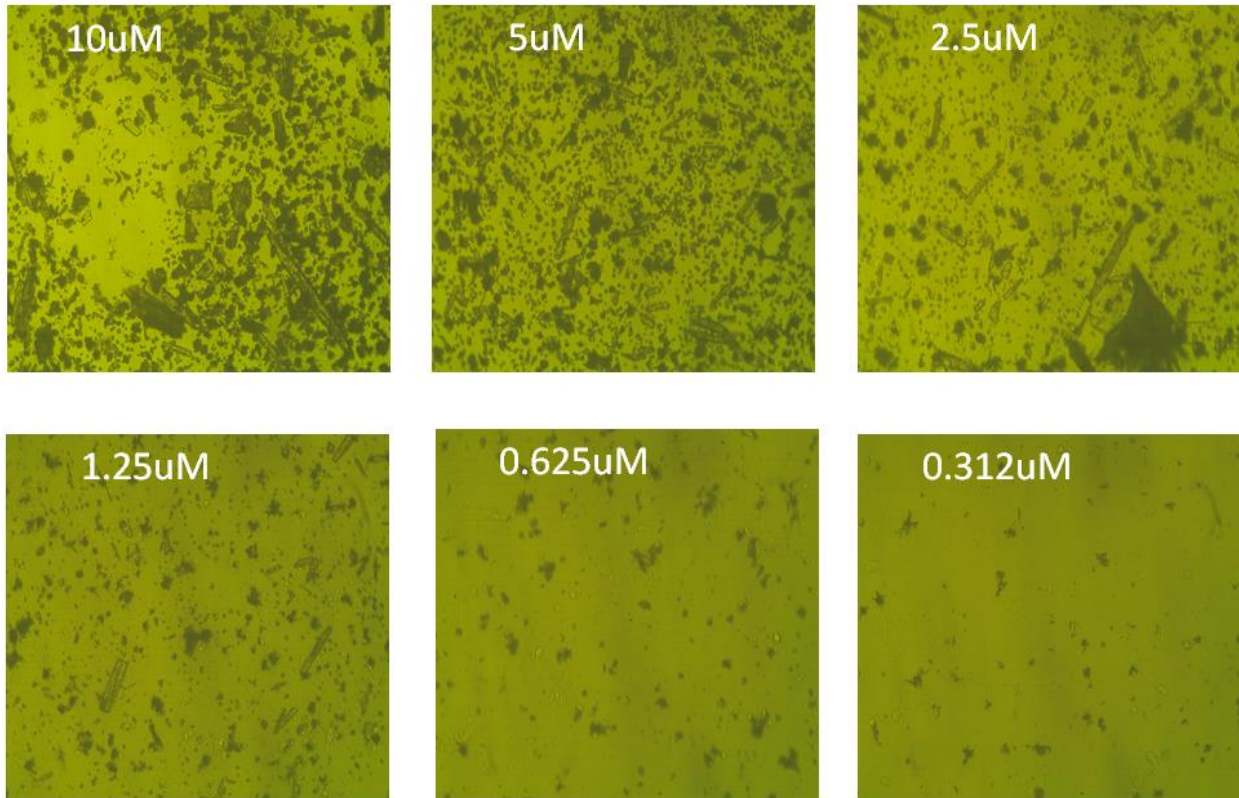
## Effect of sample XIXa on Mcf7 cells at different concentration



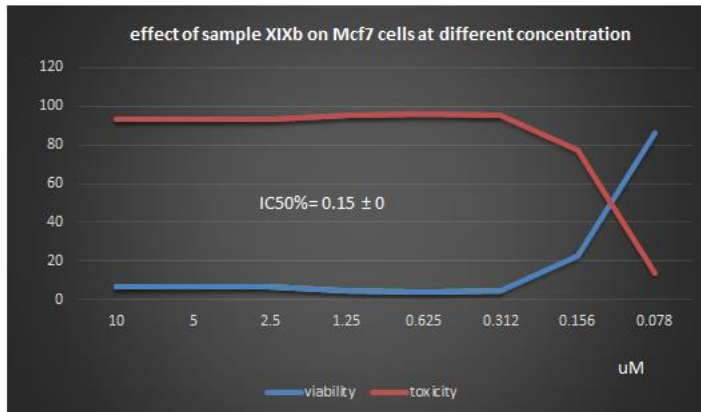
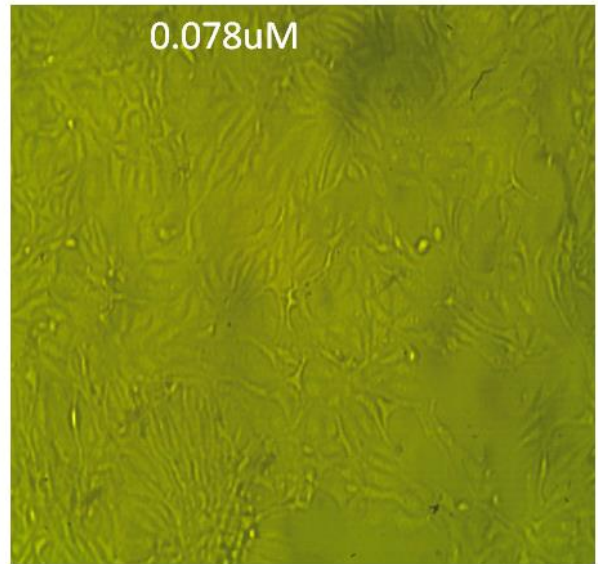
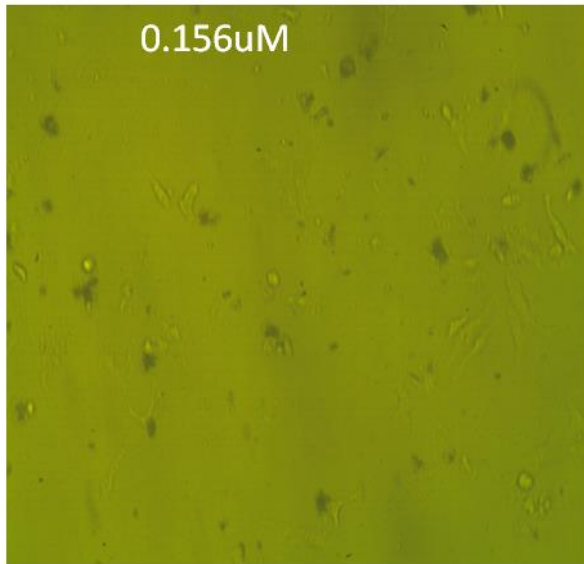
## Effect of sample XIXa on Mcf7 cells at different concentration



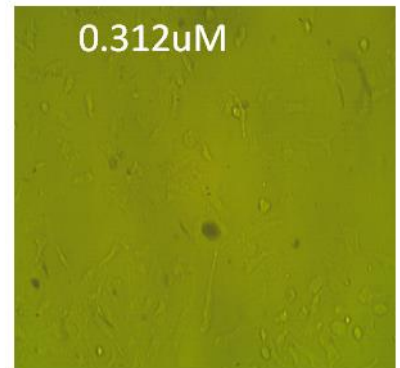
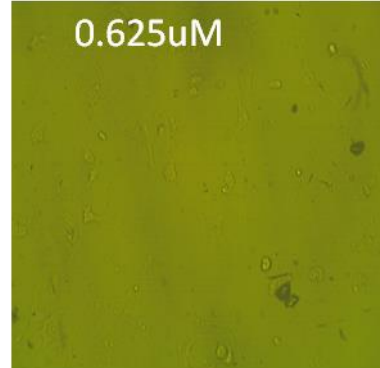
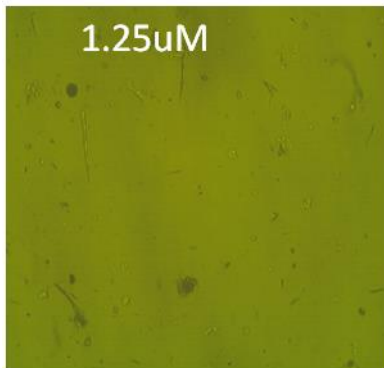
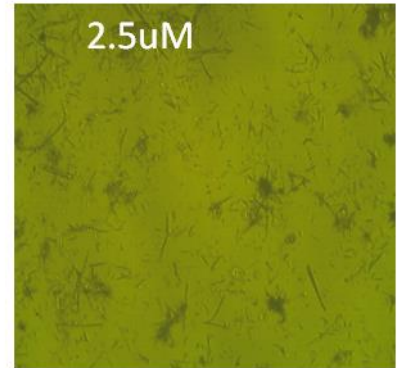
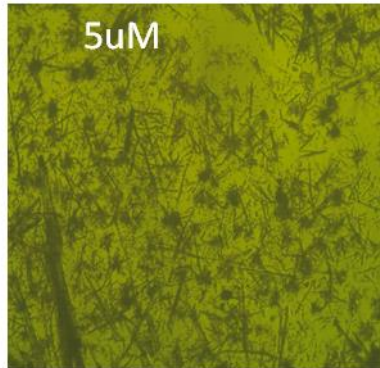
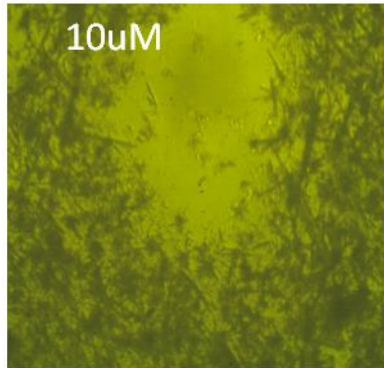
## Effect of sample XIXb on Mcf7 cells at different concentration



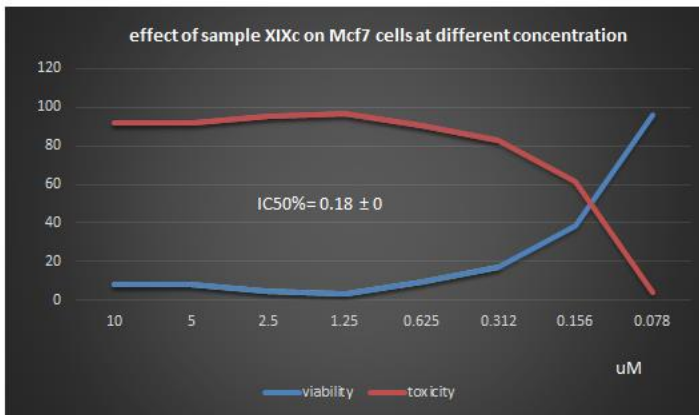
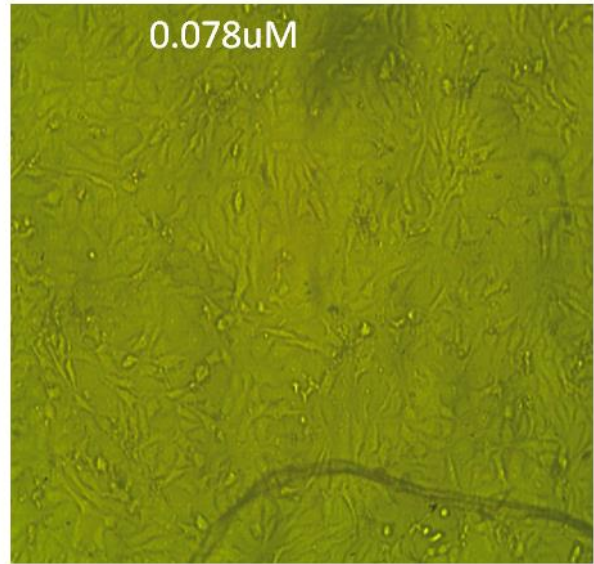
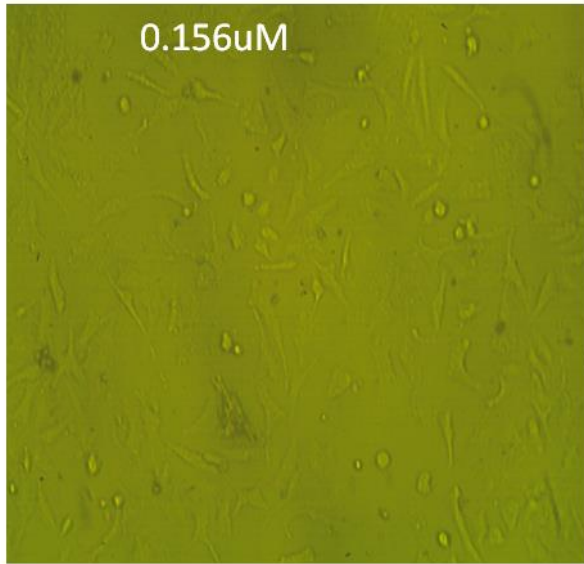
## Effect of sample XIXb on MCF7 cells at different concentration



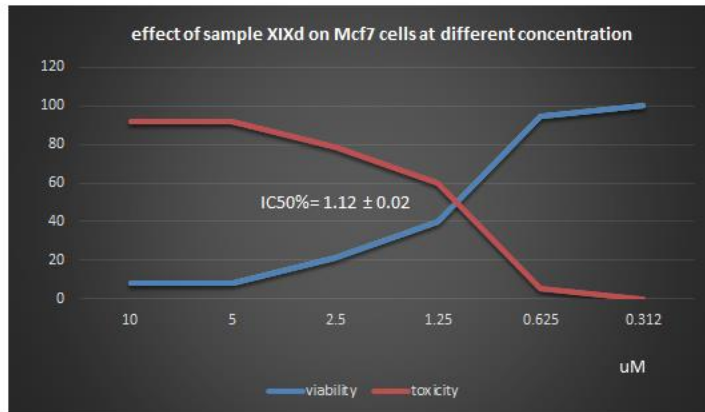
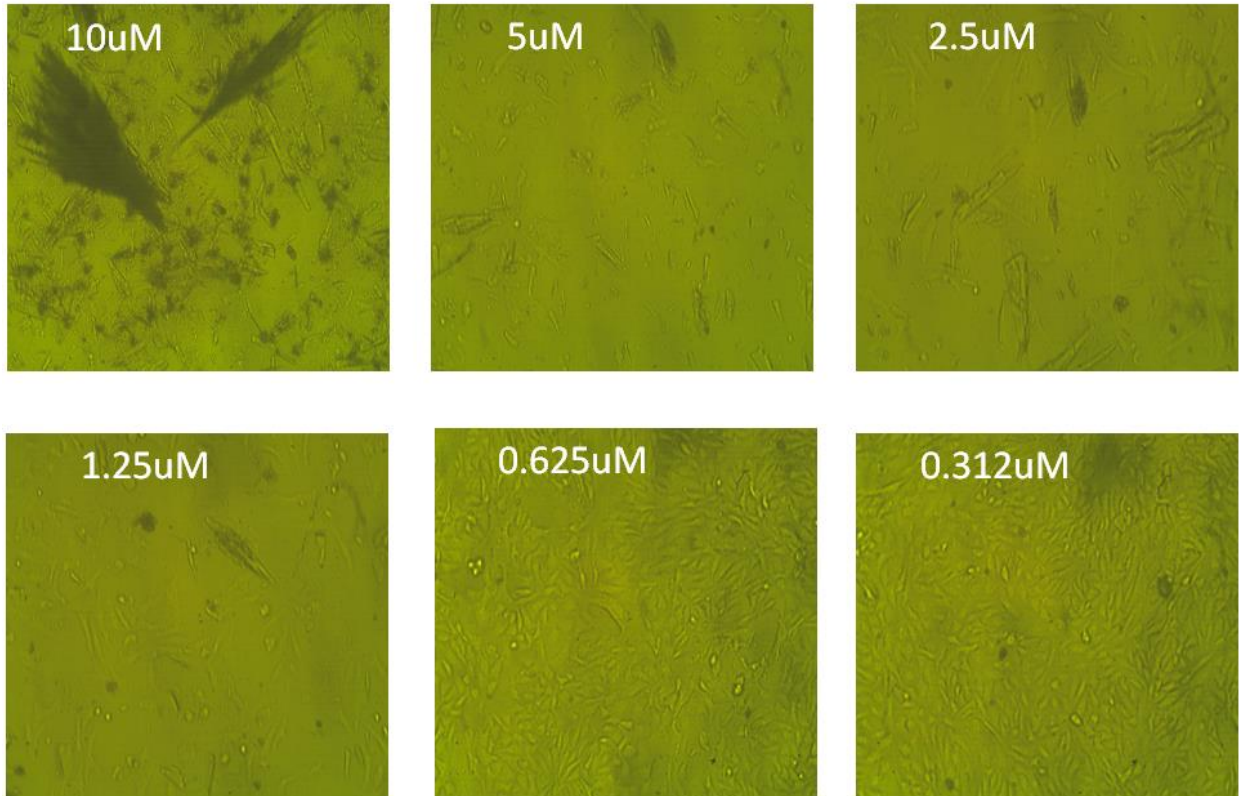
## Effect of sample XIXc on MCF7 cells at different concentration



## Effect of sample XIXc on Mcf7 cells at different concentration



## Effect of sample XIXd on Mcf7 cells at different concentration





| ID    | uM    | O.D   |       |       | Mean O.D | ±SE      | Viability % | Toxicity %  | IC50 ± SD   |
|-------|-------|-------|-------|-------|----------|----------|-------------|-------------|-------------|
| HepG2 | ----- | 0.688 | 0.705 | 0.698 | 0.697    | 0.004933 | 100         | 0           | uM          |
| Vla   | 10    | 0.025 | 0.024 | 0.02  | 0.023    | 0.001528 | 3.299856528 | 96.70014347 | 0.77 ± 0.01 |
|       | 5     | 0.019 | 0.02  | 0.018 | 0.019    | 0.000577 | 2.725968436 | 97.27403156 |             |
|       | 2.5   | 0.036 | 0.023 | 0.029 | 0.029333 | 0.003756 | 4.208512673 | 95.79148733 |             |
|       | 1.25  | 0.092 | 0.088 | 0.1   | 0.093333 | 0.003528 | 13.39072214 | 86.60927786 |             |
|       | 0.625 | 0.35  | 0.324 | 0.344 | 0.339333 | 0.00786  | 48.68483979 | 51.31516021 |             |
|       | 0.312 | 0.692 | 0.711 | 0.679 | 0.694    | 0.009292 | 99.56958393 | 0.430416069 |             |
| Vlb   | 10    | 0.031 | 0.033 | 0.03  | 0.031333 | 0.000882 | 4.495456719 | 95.50454328 | 0.63 ± 0.01 |
|       | 5     | 0.03  | 0.028 | 0.034 | 0.030667 | 0.001764 | 4.399808704 | 95.6001913  |             |
|       | 2.5   | 0.026 | 0.034 | 0.027 | 0.029    | 0.002517 | 4.160688666 | 95.83931133 |             |
|       | 1.25  | 0.083 | 0.088 | 0.077 | 0.082667 | 0.00318  | 11.8603539  | 88.1396461  |             |
|       | 0.625 | 0.153 | 0.127 | 0.163 | 0.147667 | 0.010729 | 21.18603539 | 78.81396461 |             |
|       | 0.312 | 0.574 | 0.599 | 0.612 | 0.595    | 0.01115  | 85.36585366 | 14.63414634 |             |
| Vlc   | 10    | 0.022 | 0.023 | 0.022 | 0.022333 | 0.000333 | 3.204208513 | 96.79579149 | 0.59 ± 0.01 |
|       | 5     | 0.026 | 0.021 | 0.026 | 0.024333 | 0.001667 | 3.491152559 | 96.50884744 |             |
|       | 2.5   | 0.023 | 0.047 | 0.03  | 0.033333 | 0.007126 | 4.782400765 | 95.21759923 |             |
|       | 1.25  | 0.044 | 0.032 | 0.038 | 0.038    | 0.003464 | 5.451936872 | 94.54806313 |             |
|       | 0.625 | 0.183 | 0.21  | 0.199 | 0.197333 | 0.007839 | 28.31181253 | 71.68818747 |             |
|       | 0.312 | 0.542 | 0.573 | 0.561 | 0.558667 | 0.009025 | 80.15303682 | 19.84696318 |             |
| Vld   | 10    | 0.03  | 0.023 | 0.046 | 0.033    | 0.006807 | 4.734576758 | 95.26542324 | 2.18 ± 0.03 |
|       | 5     | 0.095 | 0.125 | 0.111 | 0.110333 | 0.008667 | 15.82974653 | 84.17025347 |             |
|       | 2.5   | 0.274 | 0.259 | 0.26  | 0.264333 | 0.004842 | 37.92443807 | 62.07556193 |             |
|       | 1.25  | 0.592 | 0.628 | 0.604 | 0.608    | 0.010583 | 87.23098996 | 12.76901004 |             |
|       | 0.625 | 0.689 | 0.7   | 0.699 | 0.696    | 0.003512 | 99.85652798 | 0.143472023 |             |
|       | 0.312 | 0.674 | 0.688 | 0.692 | 0.684667 | 0.005457 | 98.23051172 | 1.769488283 |             |
| Vle   | 10    | 0.063 | 0.057 | 0.055 | 0.058333 | 0.002404 | 8.369201339 | 91.63079866 | 1.19 ± 0.04 |
|       | 5     | 0.1   | 0.094 | 0.137 | 0.110333 | 0.013445 | 15.82974653 | 84.17025347 |             |
|       | 2.5   | 0.166 | 0.142 | 0.126 | 0.144667 | 0.011624 | 20.75561932 | 79.24438068 |             |
|       | 1.25  | 0.318 | 0.347 | 0.296 | 0.320333 | 0.014769 | 45.95887135 | 54.04112865 |             |
|       | 0.625 | 0.622 | 0.578 | 0.713 | 0.637667 | 0.039751 | 91.48732664 | 8.512673362 |             |
|       | 0.312 | 0.674 | 0.688 | 0.692 | 0.684667 | 0.005457 | 98.23051172 | 1.769488283 |             |

| ID    | uM    | O.D   |       |       | Mean O.D | ±SE      | Viability % | Toxicity %  | IC50 ± SD   |
|-------|-------|-------|-------|-------|----------|----------|-------------|-------------|-------------|
| HepG2 | ----- | 0.688 | 0.705 | 0.698 | 0.697    | 0.004933 | 100         | 0           | uM          |
| Vlf   | 10    | 0.024 | 0.021 | 0.022 | 0.022333 | 0.000882 | 3.204208513 | 96.79579149 | 0.75 ± 0.01 |
|       | 5     | 0.02  | 0.026 | 0.021 | 0.022333 | 0.001856 | 3.204208513 | 96.79579149 |             |
|       | 2.5   | 0.052 | 0.043 | 0.05  | 0.048333 | 0.002728 | 6.93448111  | 93.06551889 |             |
|       | 1.25  | 0.093 | 0.074 | 0.081 | 0.082667 | 0.005548 | 11.8603539  | 88.1396461  |             |
|       | 0.625 | 0.344 | 0.368 | 0.332 | 0.348    | 0.010583 | 49.92826399 | 50.07173601 |             |
|       | 0.312 | 0.642 | 0.663 | 0.652 | 0.652333 | 0.006064 | 93.59158297 | 6.408417025 |             |
| Vlg   | 10    | 0.035 | 0.036 | 0.041 | 0.037333 | 0.001856 | 5.356288857 | 94.64371114 | 0.26 ± 0.01 |
|       | 5     | 0.033 | 0.03  | 0.031 | 0.031333 | 0.000882 | 4.495456719 | 95.50454328 |             |
|       | 2.5   | 0.028 | 0.024 | 0.03  | 0.027333 | 0.001764 | 3.921568627 | 96.07843137 |             |
|       | 1.25  | 0.042 | 0.033 | 0.036 | 0.037    | 0.002646 | 5.308464849 | 94.69153515 |             |
|       | 0.625 | 0.051 | 0.069 | 0.044 | 0.054667 | 0.007446 | 7.843137255 | 92.15686275 |             |
|       | 0.312 | 0.243 | 0.266 | 0.23  | 0.246333 | 0.010525 | 35.34194165 | 64.65805835 |             |
|       | 0.156 | 0.568 | 0.594 | 0.572 | 0.578    | 0.008083 | 82.92682927 | 17.07317073 |             |
|       | 0.078 | 0.665 | 0.689 | 0.671 | 0.675    | 0.007211 | 96.84361549 | 3.156384505 |             |
| VIIa  | 10    | 0.046 | 0.055 | 0.052 | 0.051    | 0.002646 | 7.317073171 | 92.68292683 | 0.18 ± 0    |
|       | 5     | 0.057 | 0.048 | 0.049 | 0.051333 | 0.002848 | 7.364897178 | 92.63510282 |             |
|       | 2.5   | 0.055 | 0.062 | 0.053 | 0.056667 | 0.002728 | 8.130081301 | 91.8699187  |             |
|       | 1.25  | 0.053 | 0.056 | 0.051 | 0.053333 | 0.001453 | 7.651841224 | 92.34815878 |             |
|       | 0.625 | 0.055 | 0.057 | 0.047 | 0.053    | 0.003055 | 7.604017217 | 92.39598278 |             |
|       | 0.312 | 0.056 | 0.054 | 0.067 | 0.059    | 0.004041 | 8.464849354 | 91.53515065 |             |
|       | 0.156 | 0.321 | 0.348 | 0.304 | 0.324333 | 0.012811 | 46.53275945 | 53.46724055 |             |
|       | 0.078 | 0.652 | 0.68  | 0.666 | 0.666    | 0.008083 | 95.55236729 | 4.447632712 |             |
| VIIb  | 10    | 0.034 | 0.025 | 0.03  | 0.029667 | 0.002603 | 4.256336681 | 95.74366332 | 1.53 ± 0.06 |
|       | 5     | 0.026 | 0.041 | 0.036 | 0.034333 | 0.00441  | 4.925872788 | 95.07412721 |             |
|       | 2.5   | 0.162 | 0.183 | 0.126 | 0.157    | 0.016643 | 22.5251076  | 77.4748924  |             |
|       | 1.25  | 0.344 | 0.37  | 0.331 | 0.348333 | 0.011465 | 49.976088   | 50.023912   |             |
|       | 0.625 | 0.552 | 0.569 | 0.542 | 0.554333 | 0.007881 | 79.53132473 | 20.46867527 |             |
|       | 0.312 | 0.688 | 0.695 | 0.69  | 0.691    | 0.002082 | 99.13916786 | 0.860832138 |             |
| VIIc  | 10    | 0.055 | 0.055 | 0.054 | 0.054667 | 0.000333 | 7.843137255 | 92.15686275 | 0.2 ± 0     |
|       | 5     | 0.056 | 0.054 | 0.055 | 0.055    | 0.000577 | 7.890961263 | 92.10903874 |             |
|       | 2.5   | 0.043 | 0.056 | 0.051 | 0.05     | 0.003786 | 7.173601148 | 92.82639885 |             |
|       | 1.25  | 0.027 | 0.023 | 0.025 | 0.025    | 0.001155 | 3.586800574 | 96.41319943 |             |
|       | 0.625 | 0.048 | 0.052 | 0.04  | 0.046667 | 0.003528 | 6.695361071 | 93.30463893 |             |
|       | 0.312 | 0.118 | 0.145 | 0.104 | 0.122333 | 0.012032 | 17.55141081 | 82.44858919 |             |
|       | 0.156 | 0.347 | 0.371 | 0.355 | 0.357667 | 0.007055 | 51.31516021 | 48.68483979 |             |
|       | 0.078 | 0.684 | 0.705 | 0.683 | 0.690667 | 0.007172 | 99.09134385 | 0.908656145 |             |

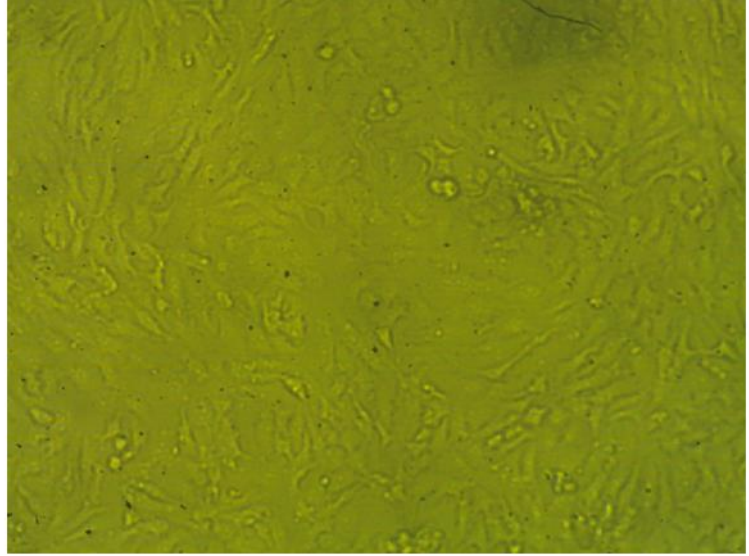
| ID    | uM    | O.D   |       |       | Mean O.D | ±SE      | Viability % | Toxicity %  | IC50 ± SD   |
|-------|-------|-------|-------|-------|----------|----------|-------------|-------------|-------------|
| HepG2 | ----- | 0.688 | 0.705 | 0.698 | 0.697    | 0.004933 | 100         | 0           | uM          |
| IXa   | 10    | 0.017 | 0.018 | 0.017 | 0.017333 | 0.000333 | 2.486848398 | 97.5131516  | 1.27 ± 0.03 |
|       | 5     | 0.022 | 0.019 | 0.023 | 0.021333 | 0.001202 | 3.06073649  | 96.93926351 |             |
|       | 2.5   | 0.177 | 0.182 | 0.159 | 0.172667 | 0.006984 | 24.77283596 | 75.22716404 |             |
|       | 1.25  | 0.337 | 0.369 | 0.342 | 0.349333 | 0.009939 | 50.11956002 | 49.88043998 |             |
|       | 0.625 | 0.624 | 0.66  | 0.653 | 0.645667 | 0.01102  | 92.63510282 | 7.364897178 |             |
|       | 0.312 | 0.69  | 0.684 | 0.697 | 0.690333 | 0.003756 | 99.04351985 | 0.956480153 |             |
| IXb   | 10    | 0.035 | 0.033 | 0.028 | 0.032    | 0.002082 | 4.591104735 | 95.40889527 | 0.15 ± 0    |
|       | 5     | 0.032 | 0.038 | 0.035 | 0.035    | 0.001732 | 5.021520803 | 94.9784792  |             |
|       | 2.5   | 0.043 | 0.029 | 0.036 | 0.036    | 0.004041 | 5.164992826 | 94.83500717 |             |
|       | 1.25  | 0.033 | 0.036 | 0.027 | 0.032    | 0.002646 | 4.591104735 | 95.40889527 |             |
|       | 0.625 | 0.044 | 0.038 | 0.035 | 0.039    | 0.002646 | 5.595408895 | 94.4045911  |             |
|       | 0.312 | 0.052 | 0.044 | 0.044 | 0.046667 | 0.002667 | 6.695361071 | 93.30463893 |             |
|       | 0.156 | 0.283 | 0.254 | 0.274 | 0.270333 | 0.00857  | 38.78527021 | 61.21472979 |             |
|       | 0.078 | 0.542 | 0.579 | 0.521 | 0.547333 | 0.016954 | 78.52702056 | 21.47297944 |             |
| IXc   | 10    | 0.022 | 0.019 | 0.018 | 0.019667 | 0.001202 | 2.821616451 | 97.17838355 | 0.8 ± 0     |
|       | 5     | 0.018 | 0.019 | 0.019 | 0.018667 | 0.000333 | 2.678144429 | 97.32185557 |             |
|       | 2.5   | 0.037 | 0.026 | 0.032 | 0.031667 | 0.00318  | 4.543280727 | 95.45671927 |             |
|       | 1.25  | 0.197 | 0.168 | 0.188 | 0.184333 | 0.00857  | 26.44667623 | 73.55332377 |             |
|       | 0.625 | 0.316 | 0.346 | 0.321 | 0.327667 | 0.00928  | 47.01099952 | 52.98900048 |             |
|       | 0.312 | 0.628 | 0.669 | 0.647 | 0.648    | 0.011846 | 92.96987088 | 7.030129125 |             |
| IXd   | 10    | 0.03  | 0.026 | 0.036 | 0.030667 | 0.002906 | 4.399808704 | 95.6001913  | 0.29 ± 0.01 |
|       | 5     | 0.033 | 0.035 | 0.031 | 0.033    | 0.001155 | 4.734576758 | 95.26542324 |             |
|       | 2.5   | 0.032 | 0.038 | 0.036 | 0.035333 | 0.001764 | 5.069344811 | 94.93065519 |             |
|       | 1.25  | 0.034 | 0.03  | 0.03  | 0.031333 | 0.001333 | 4.495456719 | 95.50454328 |             |
|       | 0.625 | 0.032 | 0.041 | 0.046 | 0.039667 | 0.004096 | 5.691056911 | 94.30894309 |             |
|       | 0.312 | 0.314 | 0.296 | 0.305 | 0.305    | 0.005196 | 43.758967   | 56.241033   |             |
|       | 0.156 | 0.633 | 0.675 | 0.656 | 0.654667 | 0.012143 | 93.92635103 | 6.073648972 |             |
|       | 0.078 | 0.689 | 0.701 | 0.694 | 0.694667 | 0.00348  | 99.66523195 | 0.334768054 |             |
| IXe   | 10    | 0.018 | 0.019 | 0.016 | 0.017667 | 0.000882 | 2.534672406 | 97.46532759 | 1.25 ± 0.02 |
|       | 5     | 0.026 | 0.042 | 0.038 | 0.035333 | 0.004807 | 5.069344811 | 94.93065519 |             |
|       | 2.5   | 0.077 | 0.094 | 0.083 | 0.084667 | 0.004978 | 12.14729794 | 87.85270206 |             |
|       | 1.25  | 0.34  | 0.362 | 0.333 | 0.345    | 0.008737 | 49.49784792 | 50.50215208 |             |
|       | 0.625 | 0.643 | 0.687 | 0.672 | 0.667333 | 0.012914 | 95.74366332 | 4.256336681 |             |
|       | 0.312 | 0.686 | 0.699 | 0.695 | 0.693333 | 0.003844 | 99.47393592 | 0.526064084 |             |

| ID    | uM    | O.D   |       |       | Mean O.D | ±SE      | Viability % | Toxicity %  | IC50 ± SD   |
|-------|-------|-------|-------|-------|----------|----------|-------------|-------------|-------------|
| HepG2 | ----- | 0.688 | 0.705 | 0.698 | 0.697    | 0.004933 | 100         | 0           | uM          |
| IXf   | 10    | 0.026 | 0.023 | 0.025 | 0.024667 | 0.000882 | 3.538976566 | 96.46102343 | 0.15 ± 0.01 |
|       | 5     | 0.024 | 0.024 | 0.022 | 0.023333 | 0.000667 | 3.347680536 | 96.65231946 |             |
|       | 2.5   | 0.027 | 0.023 | 0.025 | 0.025    | 0.001155 | 3.586800574 | 96.41319943 |             |
|       | 1.25  | 0.026 | 0.026 | 0.025 | 0.025667 | 0.000333 | 3.682448589 | 96.31755141 |             |
|       | 0.625 | 0.027 | 0.032 | 0.027 | 0.028667 | 0.001667 | 4.112864658 | 95.88713534 |             |
|       | 0.312 | 0.088 | 0.105 | 0.094 | 0.095667 | 0.004978 | 13.7254902  | 86.2745098  |             |
|       | 0.156 | 0.163 | 0.199 | 0.153 | 0.171667 | 0.013968 | 24.62936394 | 75.37063606 |             |
|       | 0.078 | 0.563 | 0.587 | 0.534 | 0.561333 | 0.015322 | 80.53562889 | 19.46437111 |             |
| IXg   | 10    | 0.023 | 0.032 | 0.02  | 0.025    | 0.003606 | 3.586800574 | 96.41319943 | 0.06 ± 0.01 |
|       | 5     | 0.025 | 0.021 | 0.027 | 0.024333 | 0.001764 | 3.491152559 | 96.50884744 |             |
|       | 2.5   | 0.022 | 0.024 | 0.021 | 0.022333 | 0.000882 | 3.204208513 | 96.79579149 |             |
|       | 1.25  | 0.019 | 0.02  | 0.022 | 0.020333 | 0.000882 | 2.917264467 | 97.08273553 |             |
|       | 0.625 | 0.018 | 0.017 | 0.019 | 0.018    | 0.000577 | 2.582496413 | 97.41750359 |             |
|       | 0.312 | 0.02  | 0.017 | 0.018 | 0.018333 | 0.000882 | 2.630320421 | 97.36967958 |             |
|       | 0.156 | 0.137 | 0.162 | 0.188 | 0.162333 | 0.014723 | 23.29029173 | 76.70970827 |             |
|       | 0.078 | 0.35  | 0.332 | 0.368 | 0.35     | 0.010392 | 50.21520803 | 49.78479197 |             |
| IXh   | 10    | 0.054 | 0.06  | 0.055 | 0.056333 | 0.001856 | 8.082257293 | 91.91774271 | 0.19 ± 0    |
|       | 5     | 0.055 | 0.059 | 0.054 | 0.056    | 0.001528 | 8.034433286 | 91.96556671 |             |
|       | 2.5   | 0.051 | 0.056 | 0.058 | 0.055    | 0.002082 | 7.890961263 | 92.10903874 |             |
|       | 1.25  | 0.05  | 0.057 | 0.053 | 0.053333 | 0.002028 | 7.651841224 | 92.34815878 |             |
|       | 0.625 | 0.042 | 0.038 | 0.05  | 0.043333 | 0.003528 | 6.217120995 | 93.78287901 |             |
|       | 0.312 | 0.217 | 0.175 | 0.188 | 0.193333 | 0.012414 | 27.73792444 | 72.26207556 |             |
|       | 0.156 | 0.296 | 0.325 | 0.318 | 0.313    | 0.008737 | 44.90674319 | 55.09325681 |             |
|       | 0.078 | 0.588 | 0.594 | 0.556 | 0.579333 | 0.011795 | 83.1181253  | 16.8818747  |             |
| IXi   | 10    | 0.04  | 0.037 | 0.038 | 0.038333 | 0.000882 | 5.49976088  | 94.50023912 | 0.8 ± 0.02  |
|       | 5     | 0.046 | 0.052 | 0.057 | 0.051667 | 0.00318  | 7.412721186 | 92.58727881 |             |
|       | 2.5   | 0.066 | 0.047 | 0.068 | 0.060333 | 0.006692 | 8.656145385 | 91.34385462 |             |
|       | 1.25  | 0.11  | 0.152 | 0.14  | 0.134    | 0.01249  | 19.22525108 | 80.77474892 |             |
|       | 0.625 | 0.326 | 0.376 | 0.361 | 0.354333 | 0.014814 | 50.83692013 | 49.16307987 |             |
| XIIa  | 10    | 0.046 | 0.066 | 0.073 | 0.061667 | 0.00809  | 8.847441416 | 91.15255858 | 0.27 ± 0    |
|       | 5     | 0.053 | 0.049 | 0.06  | 0.054    | 0.003215 | 7.74748924  | 92.25251076 |             |
|       | 2.5   | 0.074 | 0.062 | 0.071 | 0.069    | 0.003606 | 9.899569584 | 90.10043042 |             |
|       | 1.25  | 0.077 | 0.058 | 0.069 | 0.068    | 0.005508 | 9.756097561 | 90.24390244 |             |
|       | 0.625 | 0.046 | 0.068 | 0.082 | 0.065333 | 0.010477 | 9.3735055   | 90.6264945  |             |
|       | 0.312 | 0.263 | 0.281 | 0.264 | 0.269333 | 0.00584  | 38.64179818 | 61.35820182 |             |
|       | 0.156 | 0.593 | 0.572 | 0.586 | 0.583667 | 0.006173 | 83.7398374  | 16.2601626  |             |
|       | 0.078 | 0.687 | 0.7   | 0.701 | 0.696    | 0.004509 | 99.85652798 | 0.143472023 |             |

| ID    | uM    | O.D   |       |       | Mean O.D | ±SE      | Viability % | Toxicity %  | IC50 ± SD   |
|-------|-------|-------|-------|-------|----------|----------|-------------|-------------|-------------|
| HepG2 | ----- | 0.688 | 0.705 | 0.698 | 0.697    | 0.004933 | 100         | 0           | uM          |
| XIIb  | 10    | 0.065 | 0.059 | 0.063 | 0.062333 | 0.001764 | 8.943089431 | 91.05691057 | 0.17 ± 0    |
|       | 5     | 0.066 | 0.066 | 0.064 | 0.065333 | 0.000667 | 9.3735055   | 90.6264945  |             |
|       | 2.5   | 0.063 | 0.059 | 0.059 | 0.060333 | 0.001333 | 8.656145385 | 91.34385462 |             |
|       | 1.25  | 0.019 | 0.019 | 0.018 | 0.018667 | 0.000333 | 2.678144429 | 97.32185557 |             |
|       | 0.625 | 0.018 | 0.02  | 0.019 | 0.019    | 0.000577 | 2.725968436 | 97.27403156 |             |
|       | 0.312 | 0.019 | 0.02  | 0.022 | 0.020333 | 0.000882 | 2.917264467 | 97.08273553 |             |
|       | 0.156 | 0.313 | 0.297 | 0.334 | 0.314667 | 0.010713 | 45.14586322 | 54.85413678 |             |
|       | 0.078 | 0.626 | 0.618 | 0.638 | 0.627333 | 0.005812 | 90.0047824  | 9.995217599 |             |
| XIIc  | 10    | 0.05  | 0.05  | 0.05  | 0.05     | 4.91E-18 | 7.173601148 | 92.82639885 | 0.17 ± 0.01 |
|       | 5     | 0.047 | 0.05  | 0.048 | 0.048333 | 0.000882 | 6.93448111  | 93.06551889 |             |
|       | 2.5   | 0.038 | 0.036 | 0.042 | 0.038667 | 0.001764 | 5.547584888 | 94.45241511 |             |
|       | 1.25  | 0.028 | 0.03  | 0.026 | 0.028    | 0.001155 | 4.017216643 | 95.98278336 |             |
|       | 0.625 | 0.033 | 0.046 | 0.041 | 0.04     | 0.003786 | 5.738880918 | 94.26111908 |             |
|       | 0.312 | 0.209 | 0.126 | 0.197 | 0.177333 | 0.025899 | 25.44237207 | 74.55762793 |             |
|       | 0.156 | 0.297 | 0.326 | 0.301 | 0.308    | 0.009074 | 44.18938307 | 55.81061693 |             |
|       | 0.078 | 0.544 | 0.497 | 0.521 | 0.520667 | 0.013569 | 74.70109995 | 25.29890005 |             |
| XIIId | 10    | 0.022 | 0.025 | 0.021 | 0.022667 | 0.001202 | 3.25203252  | 96.74796748 | 0.18 ± 0    |
|       | 5     | 0.019 | 0.02  | 0.022 | 0.020333 | 0.000882 | 2.917264467 | 97.08273553 |             |
|       | 2.5   | 0.018 | 0.019 | 0.02  | 0.019    | 0.000577 | 2.725968436 | 97.27403156 |             |
|       | 1.25  | 0.018 | 0.017 | 0.018 | 0.017667 | 0.000333 | 2.534672406 | 97.46532759 |             |
|       | 0.625 | 0.019 | 0.017 | 0.02  | 0.018667 | 0.000882 | 2.678144429 | 97.32185557 |             |
|       | 0.312 | 0.126 | 0.146 | 0.157 | 0.143    | 0.009074 | 20.51649928 | 79.48350072 |             |
|       | 0.156 | 0.331 | 0.285 | 0.303 | 0.306333 | 0.013383 | 43.95026303 | 56.04973697 |             |
|       | 0.078 | 0.569 | 0.614 | 0.583 | 0.588667 | 0.013296 | 84.45719751 | 15.54280249 |             |
| XIIe  | 10    | 0.055 | 0.055 | 0.056 | 0.055333 | 0.000333 | 7.93878527  | 92.06121473 | 0.79 ± 0.01 |
|       | 5     | 0.054 | 0.067 | 0.05  | 0.057    | 0.005132 | 8.177905308 | 91.82209469 |             |
|       | 2.5   | 0.048 | 0.053 | 0.058 | 0.053    | 0.002887 | 7.604017217 | 92.39598278 |             |
|       | 1.25  | 0.037 | 0.049 | 0.051 | 0.045667 | 0.004372 | 6.551889048 | 93.44811095 |             |
|       | 0.625 | 0.413 | 0.446 | 0.428 | 0.429    | 0.009539 | 61.54949785 | 38.45050215 |             |
|       | 0.312 | 0.684 | 0.698 | 0.692 | 0.691333 | 0.004055 | 99.18699187 | 0.81300813  |             |
| XIIIf | 10    | 0.024 | 0.028 | 0.026 | 0.026    | 0.001155 | 3.730272597 | 96.2697274  | 0.92 ± 0.02 |
|       | 5     | 0.021 | 0.025 | 0.026 | 0.024    | 0.001528 | 3.443328551 | 96.55667145 |             |
|       | 2.5   | 0.019 | 0.022 | 0.021 | 0.020667 | 0.000882 | 2.965088474 | 97.03491153 |             |
|       | 1.25  | 0.213 | 0.256 | 0.233 | 0.234    | 0.012423 | 33.57245337 | 66.42754663 |             |
|       | 0.625 | 0.397 | 0.425 | 0.401 | 0.407667 | 0.008743 | 58.48876136 | 41.51123864 |             |
|       | 0.312 | 0.663 | 0.675 | 0.695 | 0.677667 | 0.009333 | 97.22620756 | 2.773792444 |             |

| ID    | uM    | O.D   |       |       | Mean O.D | ±SE      | Viability % | Toxicity %  | IC50 ± SD   |
|-------|-------|-------|-------|-------|----------|----------|-------------|-------------|-------------|
| HepG2 | ----- | 0.688 | 0.705 | 0.698 | 0.697    | 0.004933 | 100         | 0           | uM          |
| XIIg  | 10    | 0.044 | 0.042 | 0.048 | 0.044667 | 0.001764 | 6.408417025 | 93.59158297 | 1.88 ± 0.04 |
|       | 5     | 0.041 | 0.045 | 0.037 | 0.041    | 0.002309 | 5.882352941 | 94.11764706 |             |
|       | 2.5   | 0.173 | 0.198 | 0.163 | 0.178    | 0.010408 | 25.53802009 | 74.46197991 |             |
|       | 1.25  | 0.518 | 0.536 | 0.499 | 0.517667 | 0.010682 | 74.27068388 | 25.72931612 |             |
|       | 0.625 | 0.687 | 0.698 | 0.694 | 0.693    | 0.003215 | 99.42611191 | 0.573888092 |             |
|       | 0.312 | 0.691 | 0.694 | 0.706 | 0.697    | 0.004583 | 100         | 0           |             |
| XIV   | 10    | 0.03  | 0.032 | 0.033 | 0.031667 | 0.000882 | 4.543280727 | 95.45671927 | 1.69 ± 0.03 |
|       | 5     | 0.05  | 0.048 | 0.046 | 0.048    | 0.001155 | 6.886657102 | 93.1133429  |             |
|       | 2.5   | 0.184 | 0.208 | 0.192 | 0.194667 | 0.007055 | 27.92922047 | 72.07077953 |             |
|       | 1.25  | 0.352 | 0.377 | 0.361 | 0.363333 | 0.007311 | 52.12816834 | 47.87183166 |             |
|       | 0.625 | 0.659 | 0.685 | 0.679 | 0.674333 | 0.00786  | 96.74796748 | 3.25203252  |             |
|       | 0.312 | 0.688 | 0.691 | 0.698 | 0.692333 | 0.002963 | 99.33046389 | 0.669536107 |             |
| XVa   | 10    | 0.028 | 0.034 | 0.031 | 0.031    | 0.001732 | 4.447632712 | 95.55236729 | 1.58 ± 0.02 |
|       | 5     | 0.044 | 0.044 | 0.038 | 0.042    | 0.002    | 6.025824964 | 93.97417504 |             |
|       | 2.5   | 0.046 | 0.072 | 0.06  | 0.059333 | 0.007513 | 8.512673362 | 91.48732664 |             |
|       | 1.25  | 0.403 | 0.441 | 0.425 | 0.423    | 0.011015 | 60.68866571 | 39.31133429 |             |
|       | 0.625 | 0.682 | 0.675 | 0.699 | 0.685333 | 0.007126 | 98.32615973 | 1.673840268 |             |
|       | 0.312 | 0.693 | 0.702 | 0.695 | 0.696667 | 0.002728 | 99.95217599 | 0.047824008 |             |
| XVb   | 10    | 0.05  | 0.05  | 0.055 | 0.051667 | 0.001667 | 7.412721186 | 92.58727881 | 0.88 ± 0.06 |
|       | 5     | 0.032 | 0.037 | 0.035 | 0.034667 | 0.001453 | 4.973696796 | 95.0263032  |             |
|       | 2.5   | 0.036 | 0.034 | 0.039 | 0.036333 | 0.001453 | 5.212816834 | 94.78718317 |             |
|       | 1.25  | 0.175 | 0.194 | 0.035 | 0.134667 | 0.050134 | 19.32089909 | 80.67910091 |             |
|       | 0.625 | 0.463 | 0.479 | 0.454 | 0.465333 | 0.007311 | 66.76231468 | 33.23768532 |             |
|       | 0.312 | 0.698 | 0.684 | 0.688 | 0.69     | 0.004163 | 98.99569584 | 1.004304161 |             |
| XVIIa | 10    | 0.026 | 0.045 | 0.03  | 0.033667 | 0.005783 | 4.830224773 | 95.16977523 | 0.09 ± 0.01 |
|       | 5     | 0.032 | 0.033 | 0.041 | 0.035333 | 0.002848 | 5.069344811 | 94.93065519 |             |
|       | 2.5   | 0.046 | 0.026 | 0.037 | 0.036333 | 0.005783 | 5.212816834 | 94.78718317 |             |
|       | 1.25  | 0.036 | 0.046 | 0.044 | 0.042    | 0.003055 | 6.025824964 | 93.97417504 |             |
|       | 0.625 | 0.037 | 0.035 | 0.039 | 0.037    | 0.001155 | 5.308464849 | 94.69153515 |             |
|       | 0.312 | 0.052 | 0.047 | 0.048 | 0.049    | 0.001528 | 7.030129125 | 92.96987088 |             |
|       | 0.156 | 0.13  | 0.119 | 0.143 | 0.130667 | 0.006936 | 18.747011   | 81.252989   |             |
|       | 0.078 | 0.372 | 0.409 | 0.387 | 0.389333 | 0.010745 | 55.85844094 | 44.14155906 |             |

| ID    | uM    | O.D   |       |       | Mean O.D | ±SE      | Viability % | Toxicity %  | IC50 ± SD   |
|-------|-------|-------|-------|-------|----------|----------|-------------|-------------|-------------|
| HepG2 | ----- | 0.688 | 0.705 | 0.698 | 0.697    | 0.004933 | 100         | 0           | uM          |
| XVIIb | 10    | 0.02  | 0.018 | 0.02  | 0.019333 | 0.000667 | 2.773792444 | 97.22620756 | 1.63 ± 0.03 |
|       | 5     | 0.046 | 0.033 | 0.032 | 0.037    | 0.004509 | 5.308464849 | 94.69153515 |             |
|       | 2.5   | 0.109 | 0.142 | 0.126 | 0.125667 | 0.009528 | 18.02965088 | 81.97034912 |             |
|       | 1.25  | 0.387 | 0.416 | 0.391 | 0.398    | 0.009074 | 57.10186514 | 42.89813486 |             |
|       | 0.625 | 0.672 | 0.687 | 0.666 | 0.675    | 0.006245 | 96.84361549 | 3.156384505 |             |
|       | 0.312 | 0.689 | 0.679 | 0.695 | 0.687667 | 0.004667 | 98.66092779 | 1.339072214 |             |
| XIXa  | 10    | 0.046 | 0.045 | 0.052 | 0.047667 | 0.002186 | 6.838833094 | 93.16116691 | 0.26 ± 0    |
|       | 5     | 0.055 | 0.042 | 0.038 | 0.045    | 0.005132 | 6.456241033 | 93.54375897 |             |
|       | 2.5   | 0.057 | 0.035 | 0.044 | 0.045333 | 0.006386 | 6.504065041 | 93.49593496 |             |
|       | 1.25  | 0.04  | 0.052 | 0.057 | 0.049667 | 0.005044 | 7.12577714  | 92.87422286 |             |
|       | 0.625 | 0.083 | 0.072 | 0.089 | 0.081333 | 0.004978 | 11.66905787 | 88.33094213 |             |
|       | 0.312 | 0.229 | 0.267 | 0.24  | 0.245333 | 0.011289 | 35.19846963 | 64.80153037 |             |
|       | 0.156 | 0.572 | 0.578 | 0.604 | 0.584667 | 0.009821 | 83.88330942 | 16.11669058 |             |
|       | 0.078 | 0.687 | 0.699 | 0.692 | 0.692667 | 0.00348  | 99.3782879  | 0.621712099 |             |
| XIXb  | 10    | 0.033 | 0.024 | 0.028 | 0.028333 | 0.002603 | 4.06504065  | 95.93495935 | 0.22 ± 0    |
|       | 5     | 0.019 | 0.023 | 0.02  | 0.020667 | 0.001202 | 2.965088474 | 97.03491153 |             |
|       | 2.5   | 0.023 | 0.027 | 0.021 | 0.023667 | 0.001764 | 3.395504543 | 96.60449546 |             |
|       | 1.25  | 0.042 | 0.033 | 0.027 | 0.034    | 0.004359 | 4.87804878  | 95.12195122 |             |
|       | 0.625 | 0.084 | 0.071 | 0.099 | 0.084667 | 0.00809  | 12.14729794 | 87.85270206 |             |
|       | 0.312 | 0.218 | 0.239 | 0.222 | 0.226333 | 0.006438 | 32.4725012  | 67.5274988  |             |
|       | 0.156 | 0.366 | 0.39  | 0.382 | 0.379333 | 0.007055 | 54.42372071 | 45.57627929 |             |
|       | 0.078 | 0.662 | 0.687 | 0.659 | 0.669333 | 0.008876 | 96.03060736 | 3.969392635 |             |
| XIXc  | 10    | 0.044 | 0.044 | 0.041 | 0.043    | 0.001    | 6.169296987 | 93.83070301 | 1.37 ± 0.14 |
|       | 5     | 0.026 | 0.046 | 0.036 | 0.036    | 0.005774 | 5.164992826 | 94.83500717 |             |
|       | 2.5   | 0.021 | 0.029 | 0.026 | 0.025333 | 0.002333 | 3.634624582 | 96.36537542 |             |
|       | 1.25  | 0.342 | 0.377 | 0.365 | 0.361333 | 0.010269 | 51.84122429 | 48.15877571 |             |
|       | 0.625 | 0.662 | 0.07  | 0.684 | 0.472    | 0.2011   | 67.71879484 | 32.28120516 |             |
|       | 0.312 | 0.703 | 0.692 | 0.687 | 0.694    | 0.004726 | 99.56958393 | 0.430416069 |             |
| XIXd  | 10    | 0.018 | 0.019 | 0.018 | 0.018333 | 0.000333 | 2.630320421 | 97.36967958 | 1.26 ± 0.03 |
|       | 5     | 0.017 | 0.017 | 0.018 | 0.017333 | 0.000333 | 2.486848398 | 97.5131516  |             |
|       | 2.5   | 0.084 | 0.093 | 0.077 | 0.084667 | 0.004631 | 12.14729794 | 87.85270206 |             |
|       | 1.25  | 0.263 | 0.249 | 0.27  | 0.260667 | 0.006173 | 37.39837398 | 62.60162602 |             |
|       | 0.625 | 0.562 | 0.539 | 0.541 | 0.547333 | 0.007356 | 78.52702056 | 21.47297944 |             |
|       | 0.312 | 0.709 | 0.684 | 0.69  | 0.694333 | 0.007535 | 99.61740794 | 0.382592061 |             |

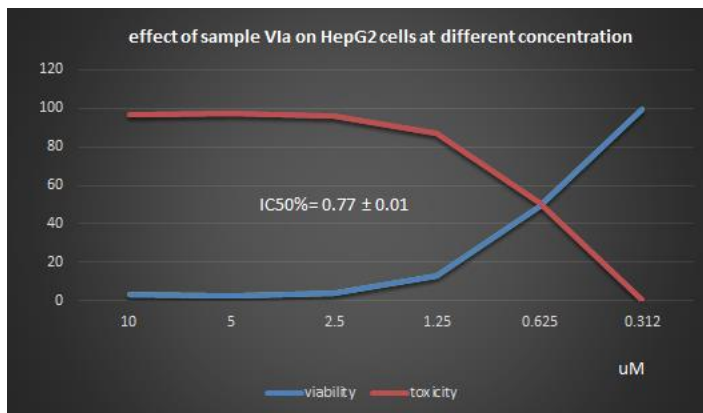
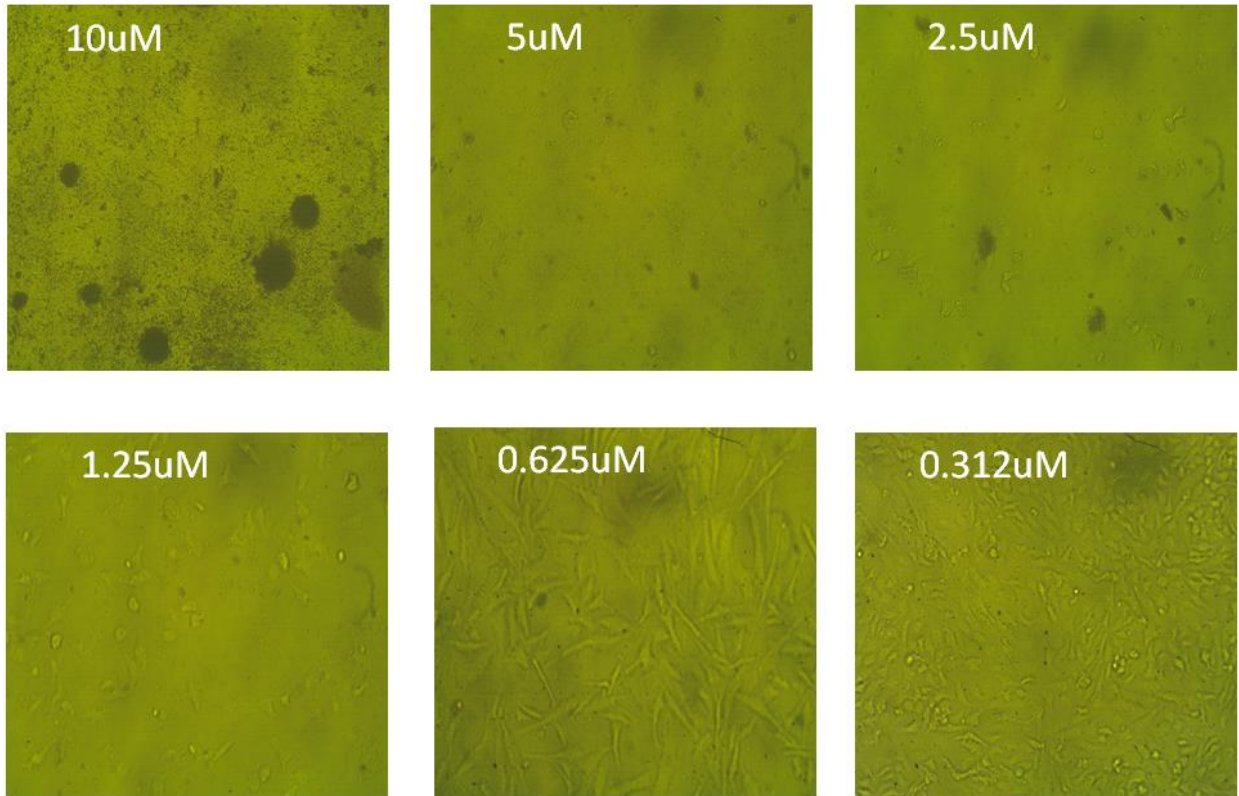


**control**  
**HepG2 cells**

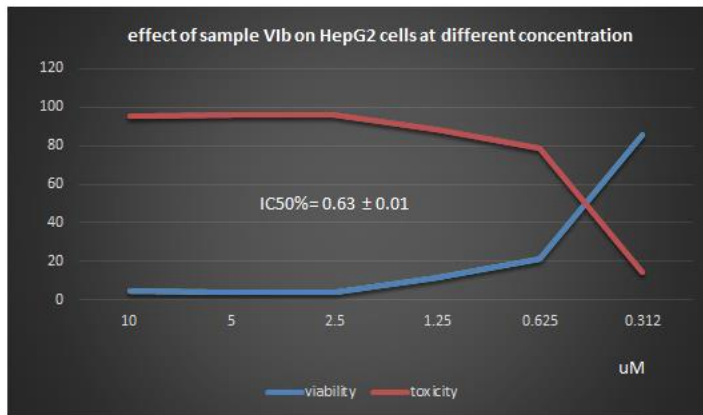
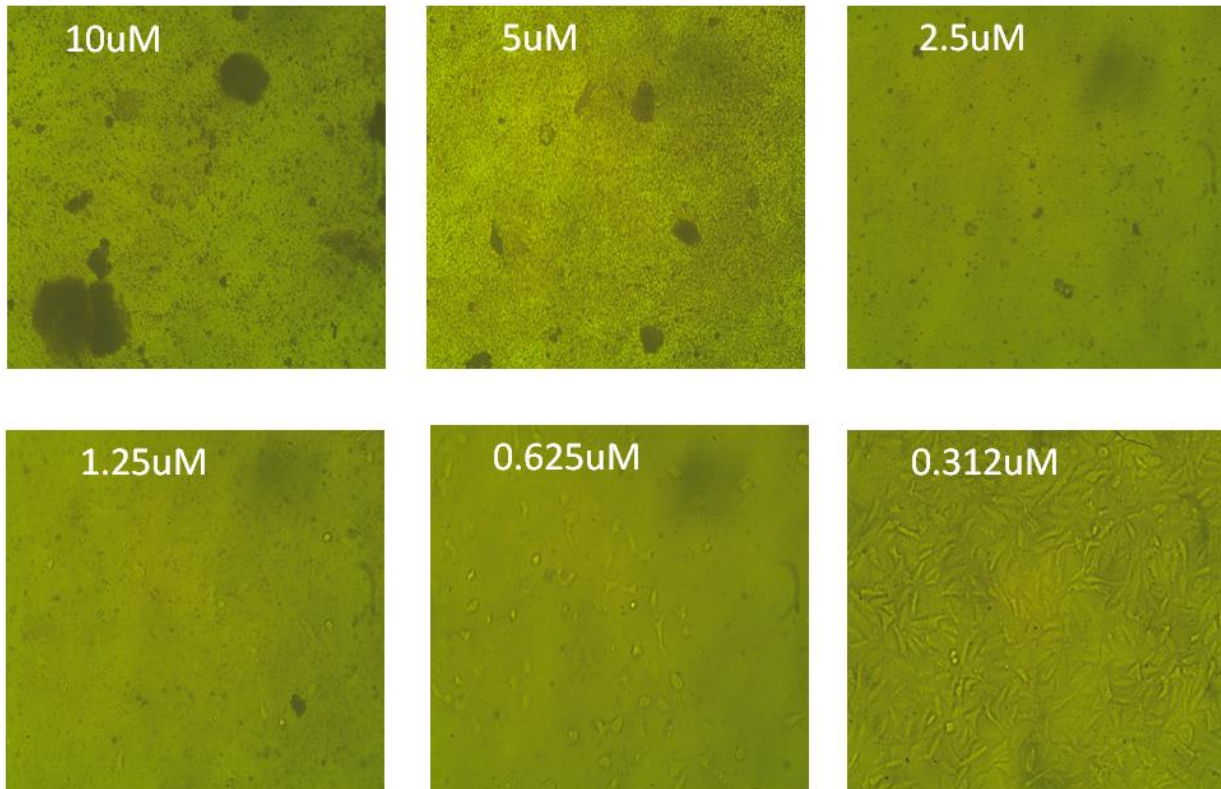
|                      |                             |
|----------------------|-----------------------------|
| Organism :           | <i>Homo sapiens</i> , human |
| Tissue :             | liver                       |
| Cell Type :          | epithelial                  |
| Culture Properties : | adherent                    |
| Disease :            | hepatocellular carcinoma    |
| ATCC :               | HB-8065                     |



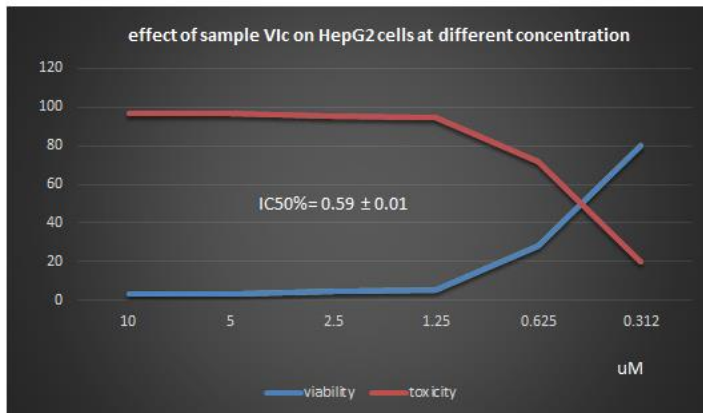
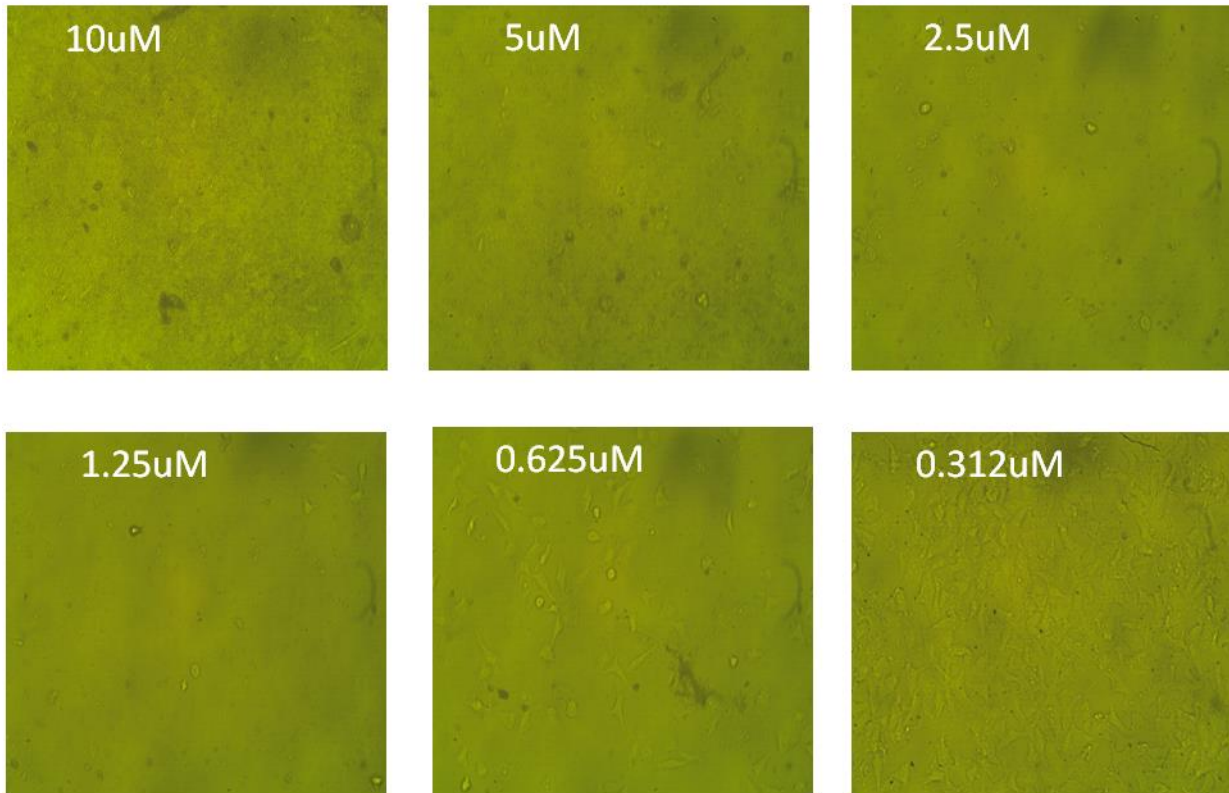
## Effect of sample VIa on HepG2 cells at different concentration



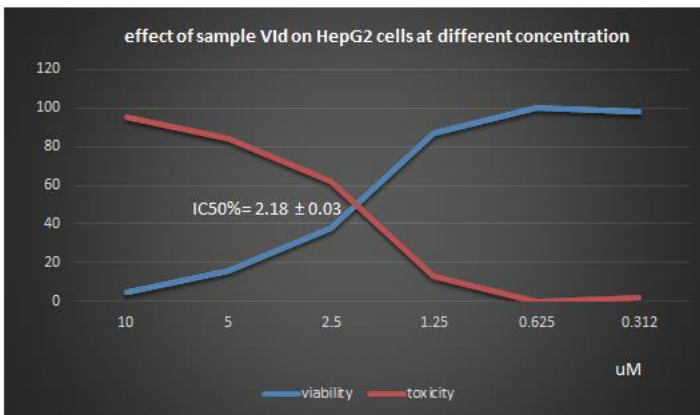
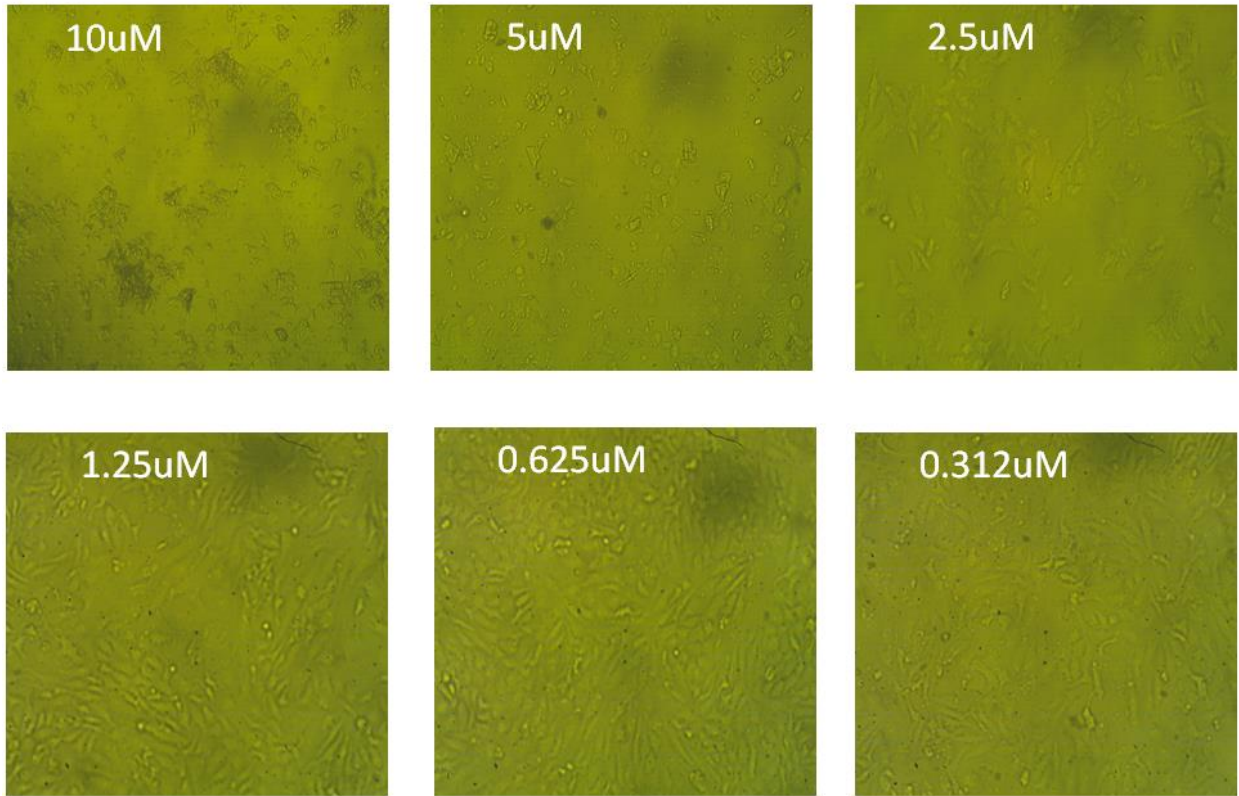
## Effect of sample V1b on HepG2 cells at different concentration



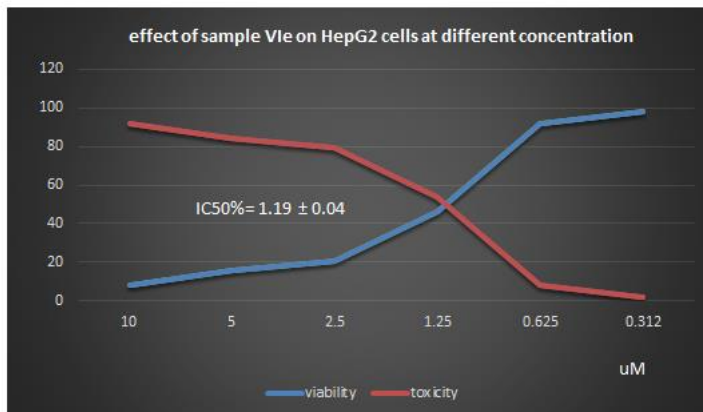
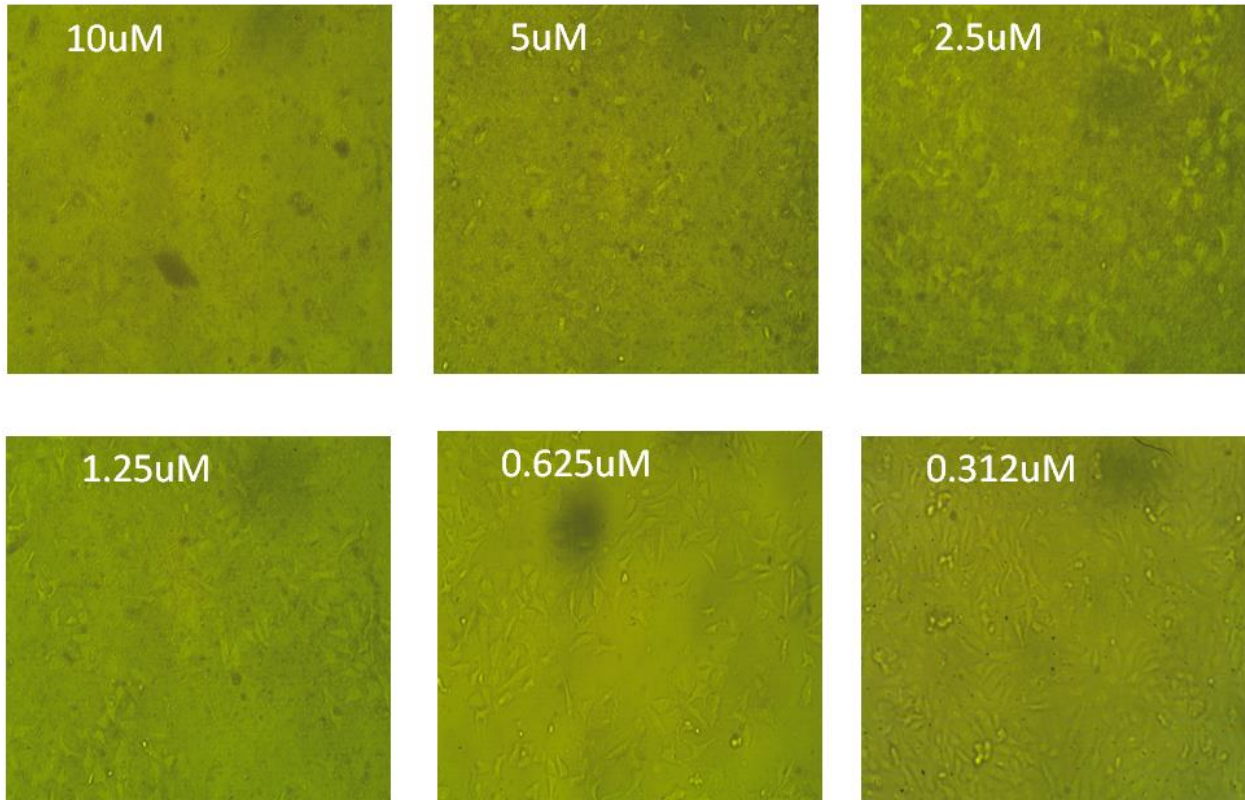
## Effect of sample Vlc on HepG2 cells at different concentration



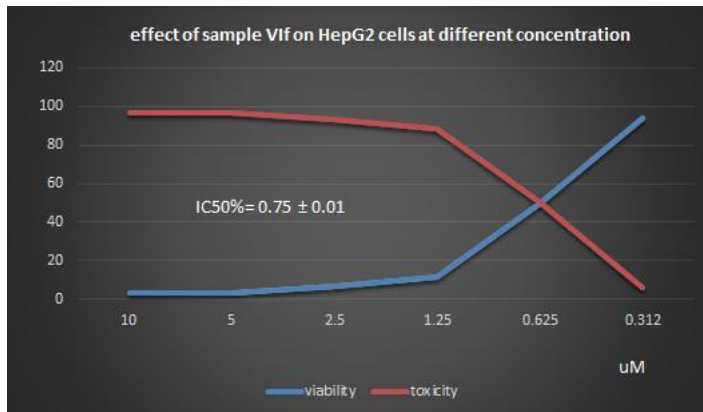
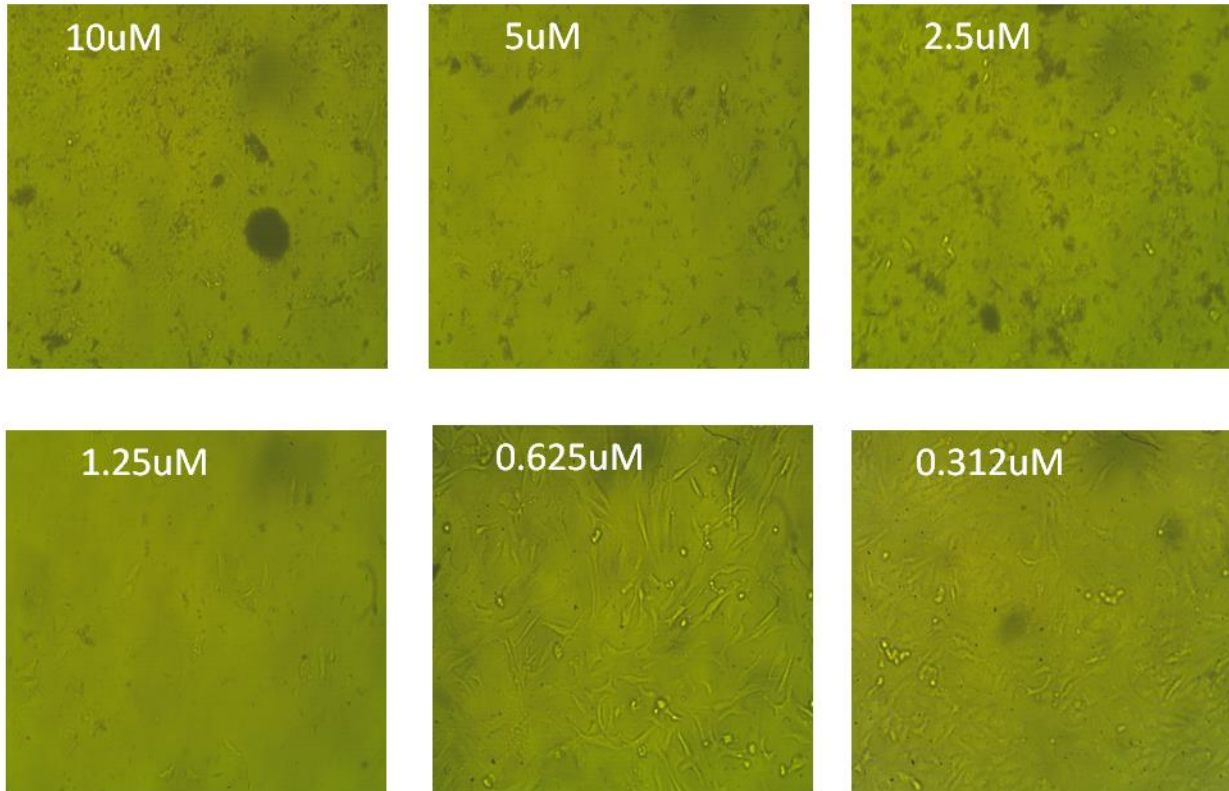
## Effect of sample VId on HepG2 cells at different concentration



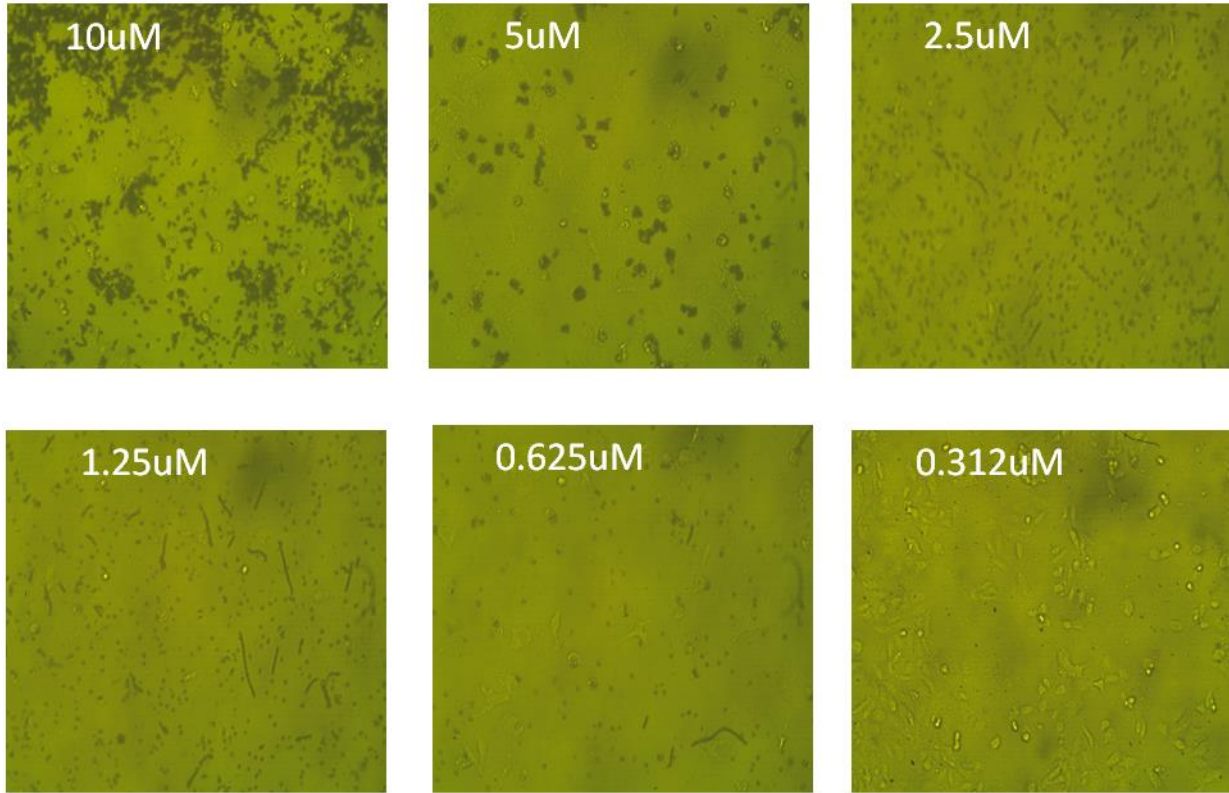
## Effect of sample VIe on HepG2 cells at different concentration



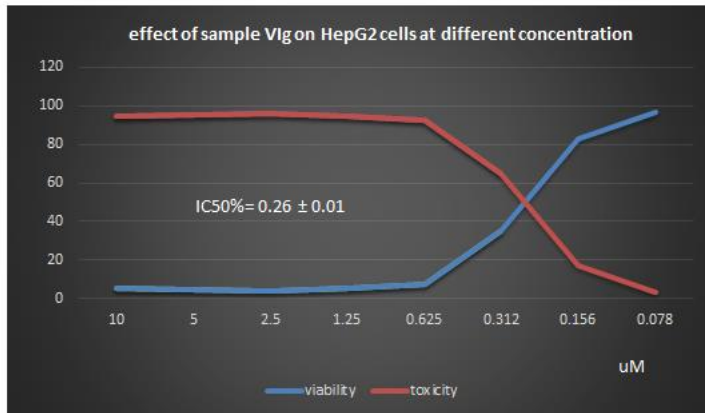
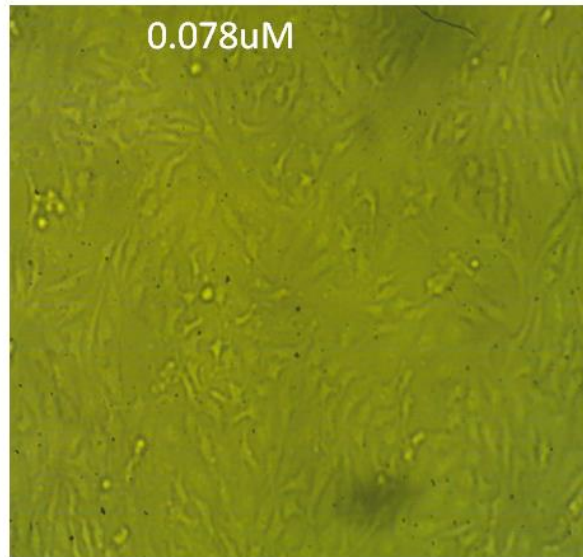
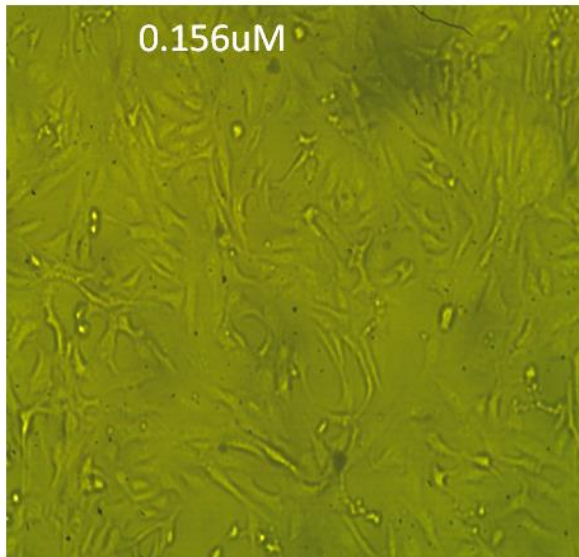
## Effect of sample Vif on HepG2 cells at different concentration



## Effect of sample VIg on HepG2 cells at different concentration

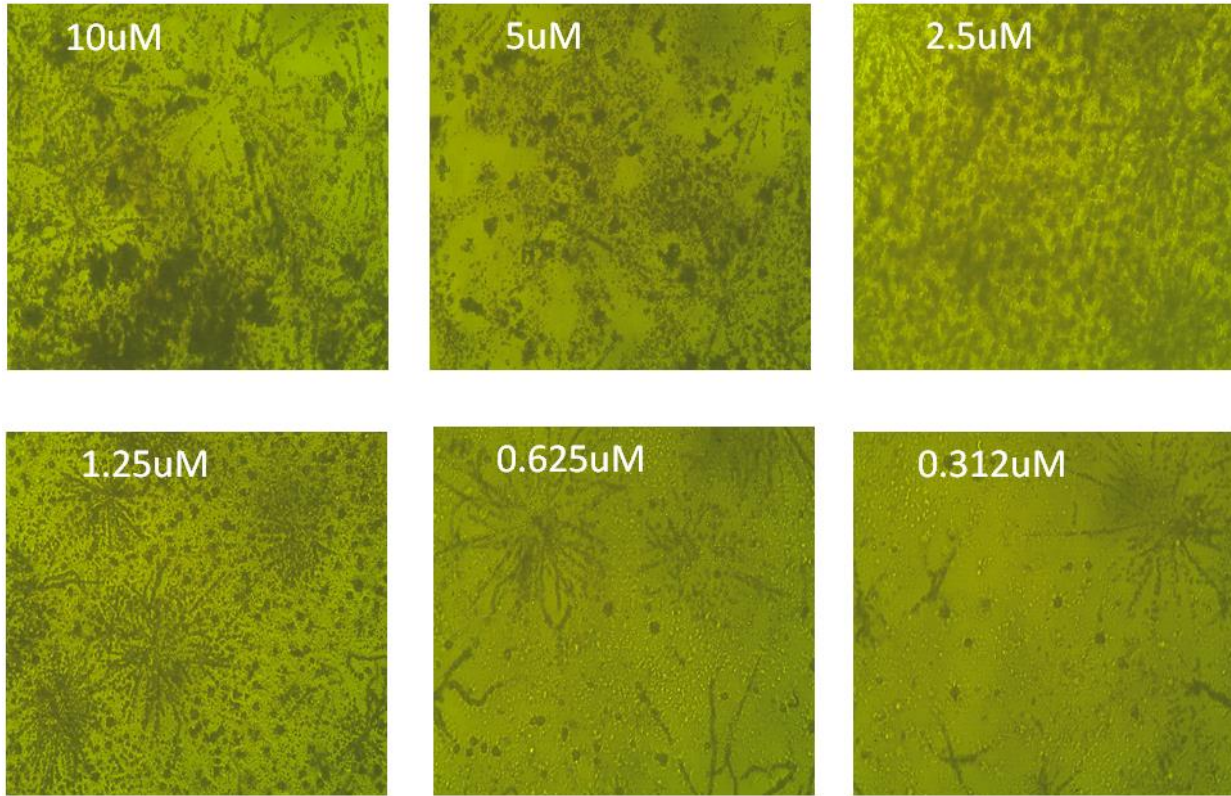


## Effect of sample VIg on HepG2 cells at different concentration

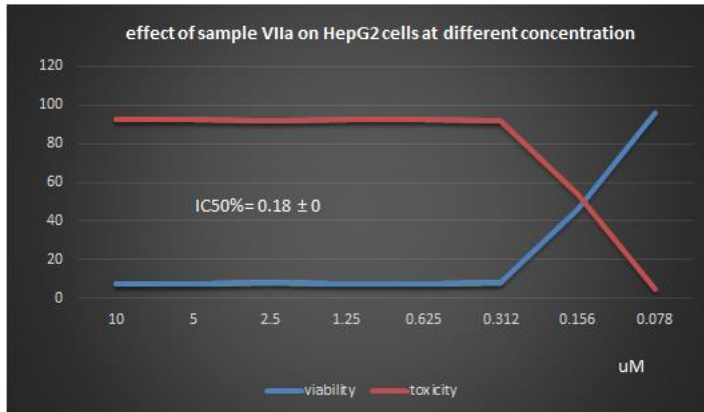
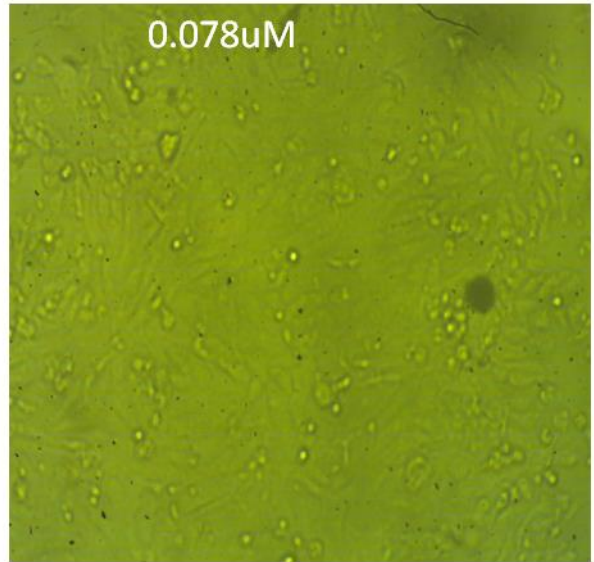
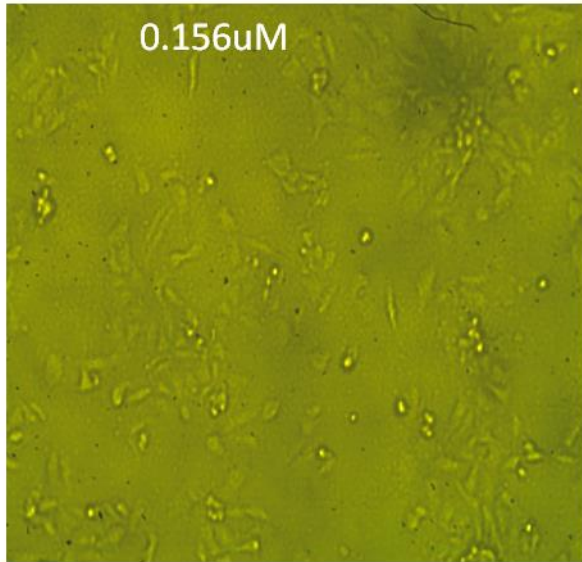




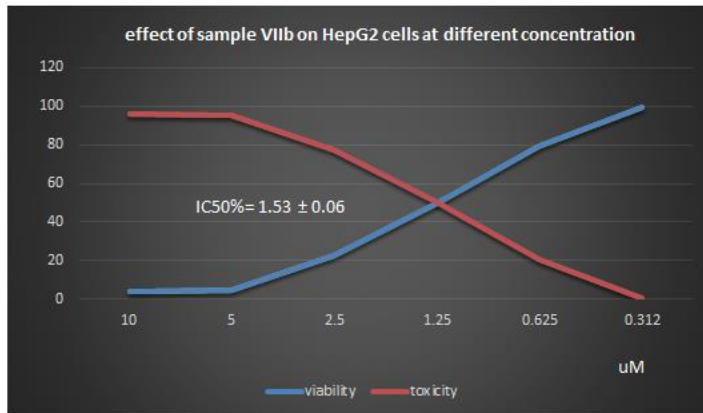
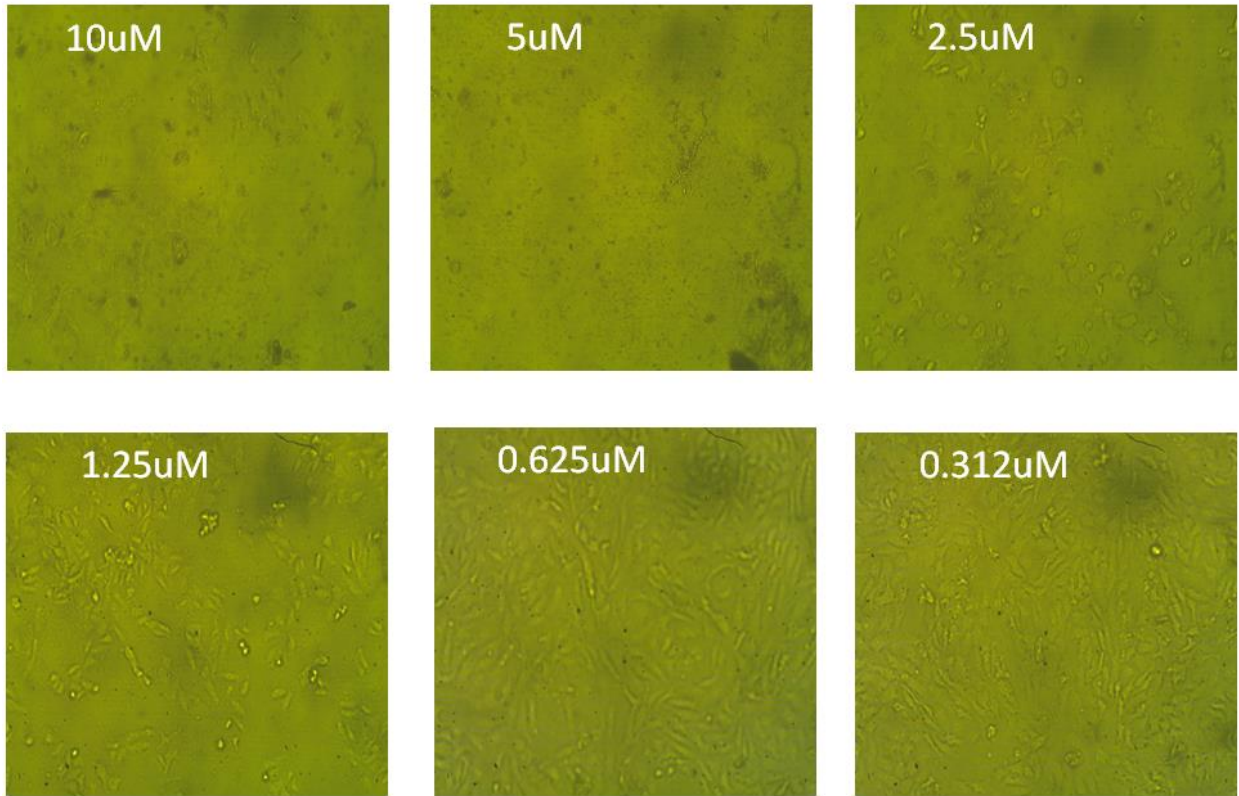
## Effect of sample VIIa on HepG2 cells at different concentration



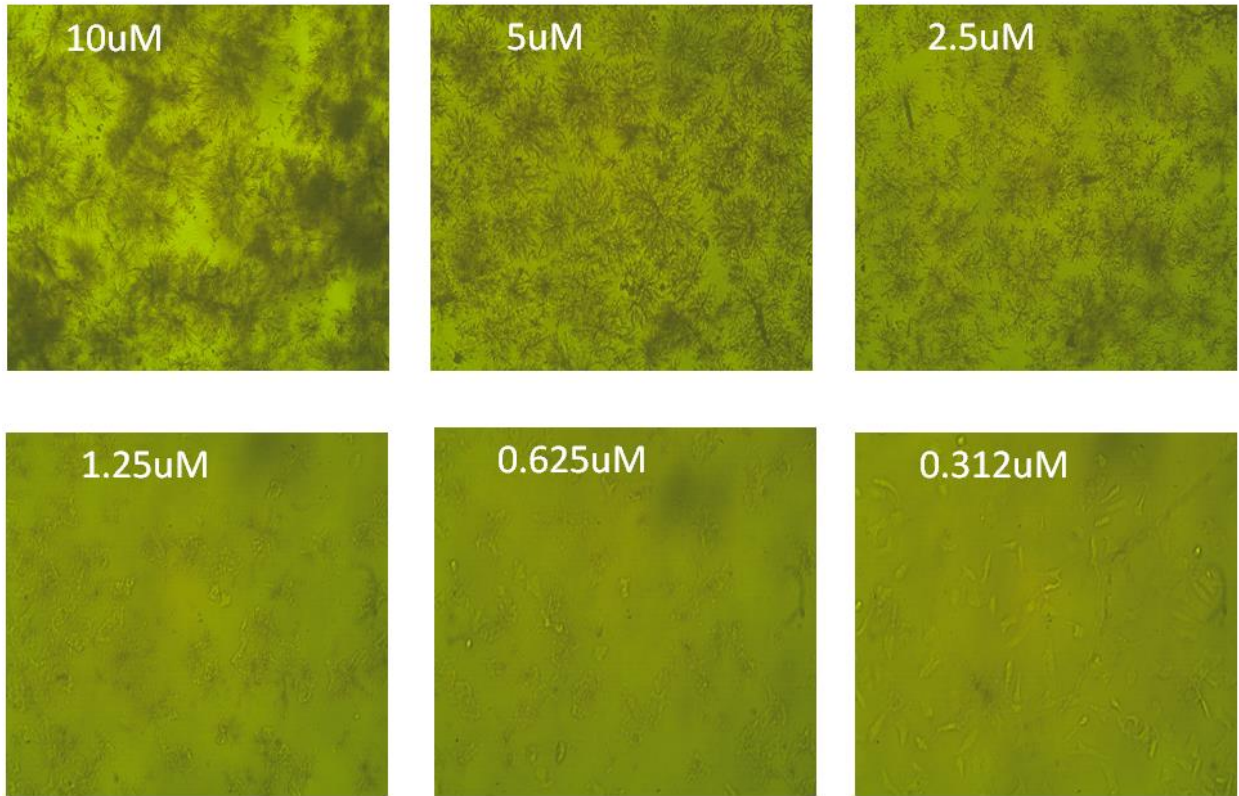
## Effect of sample VIIa on HepG2 cells at different concentration



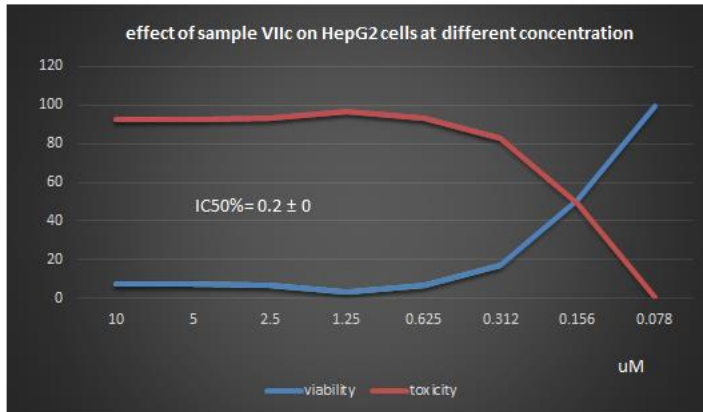
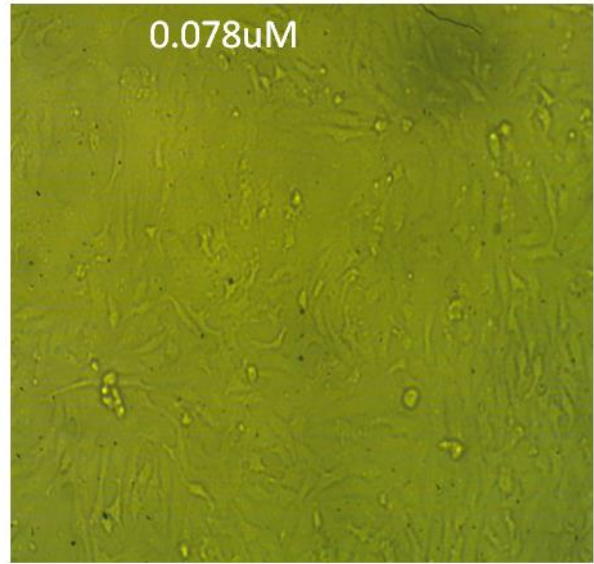
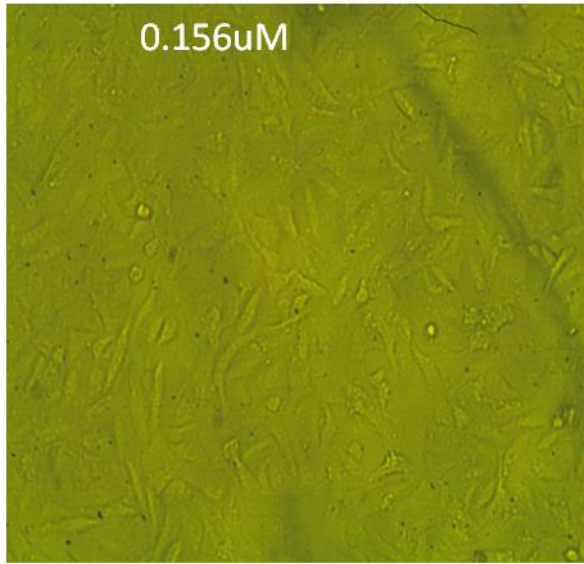
## Effect of sample VIIb on HepG2 cells at different concentration



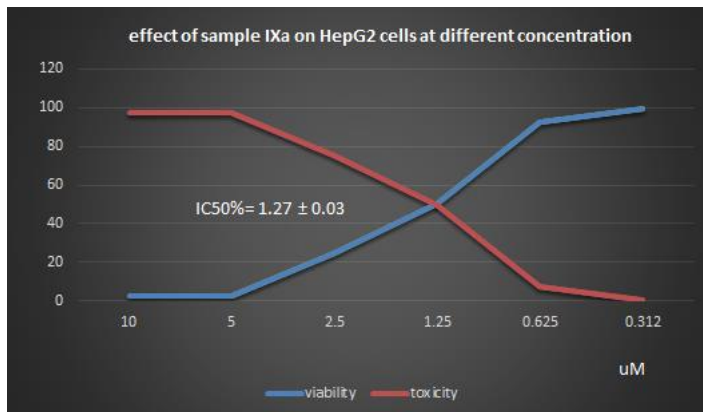
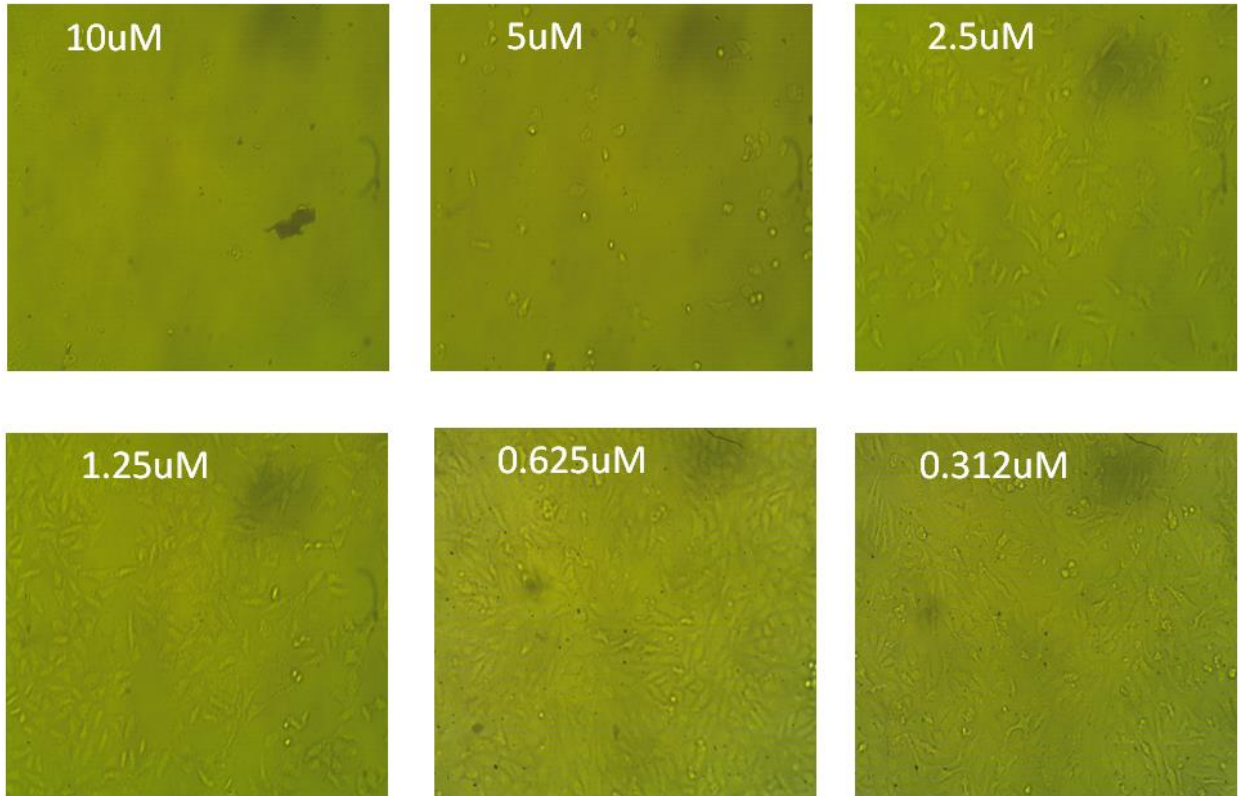
## Effect of sample VIIc on HepG2 cells at different concentration



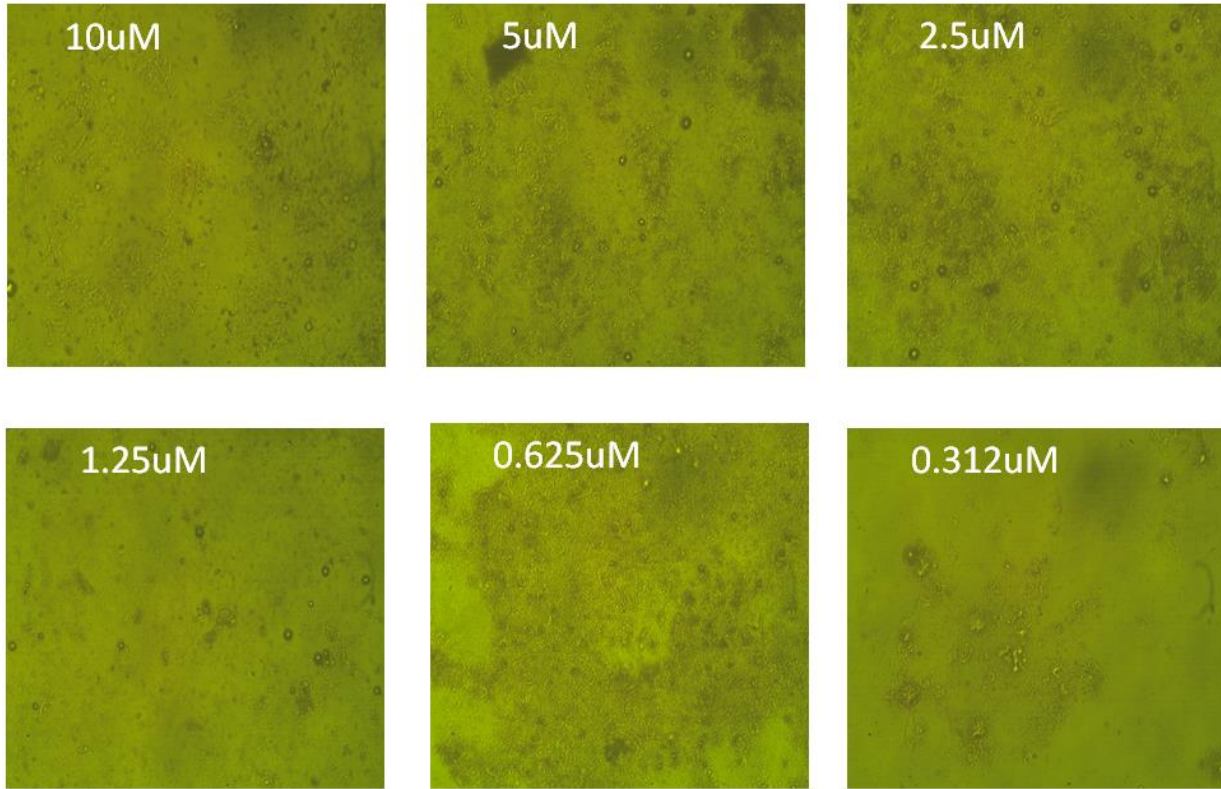
## Effect of sample VIIc on HepG2 cells at different concentration



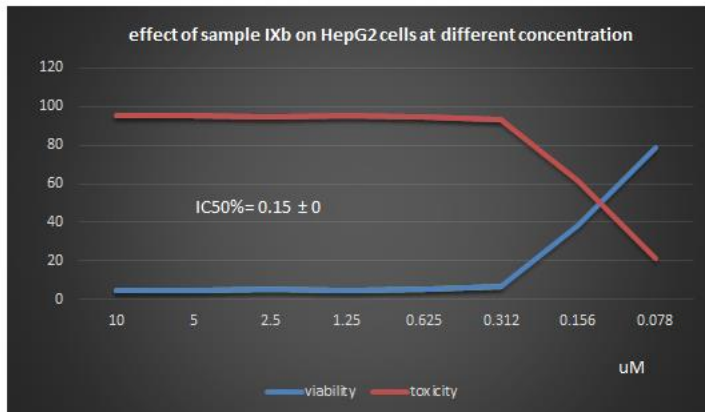
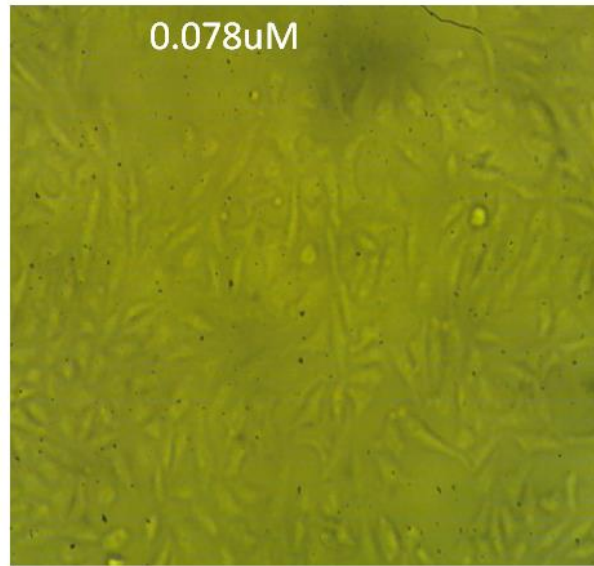
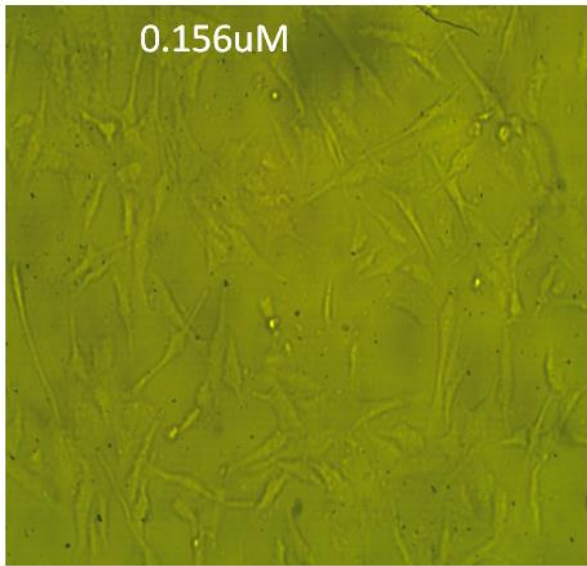
## Effect of sample IXa on HepG2 cells at different concentration



## Effect of sample IXb on HepG2 cells at different concentration

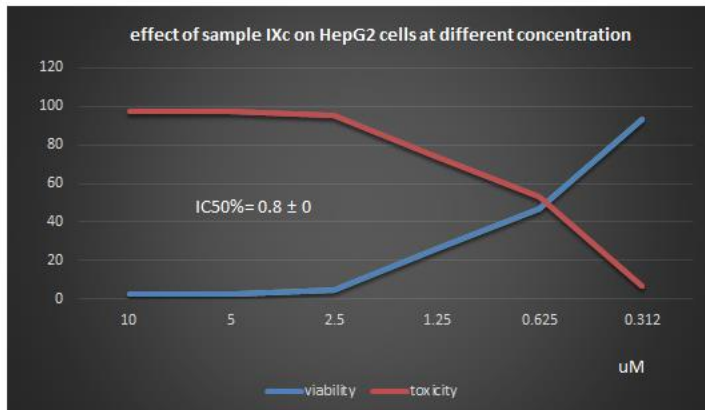
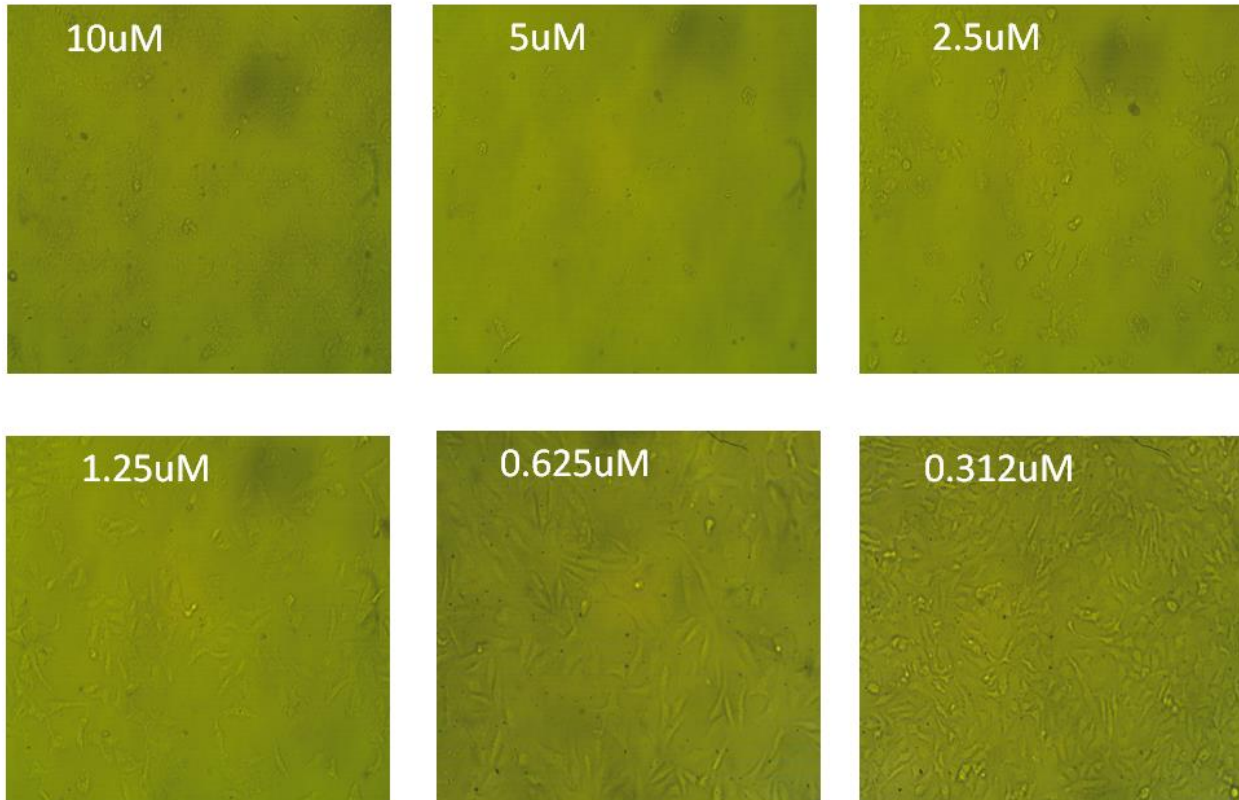


## Effect of sample IXb on HepG2 cells at different concentration

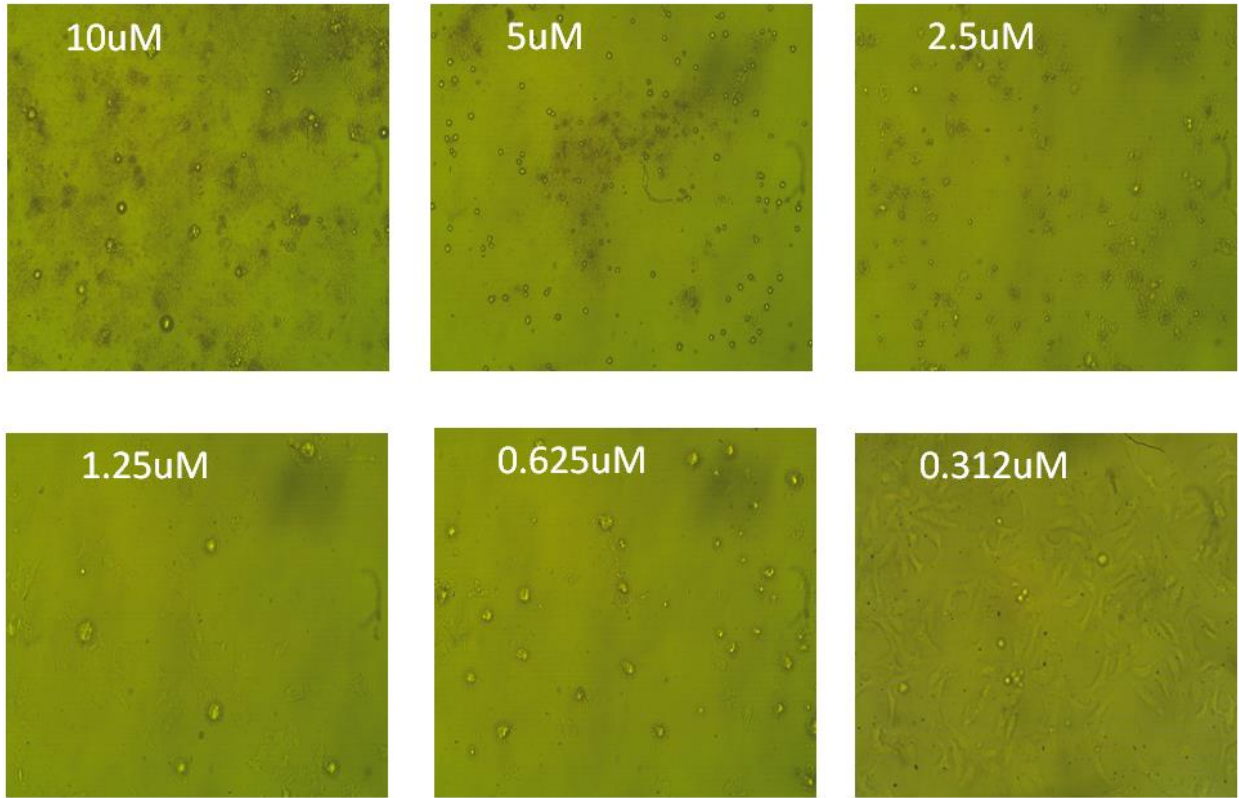




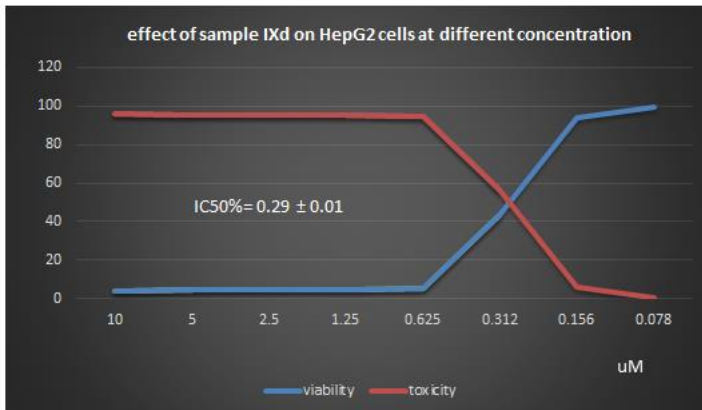
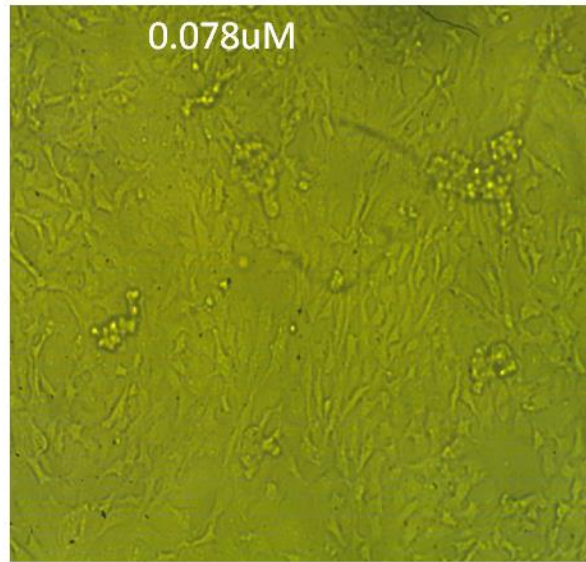
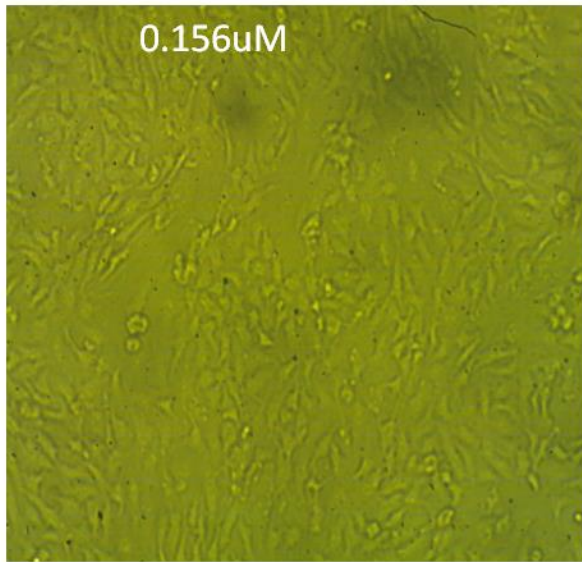
## Effect of sample IXc on HepG2 cells at different concentration



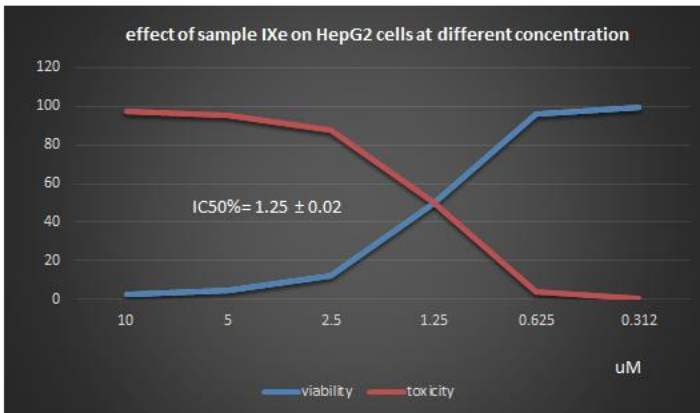
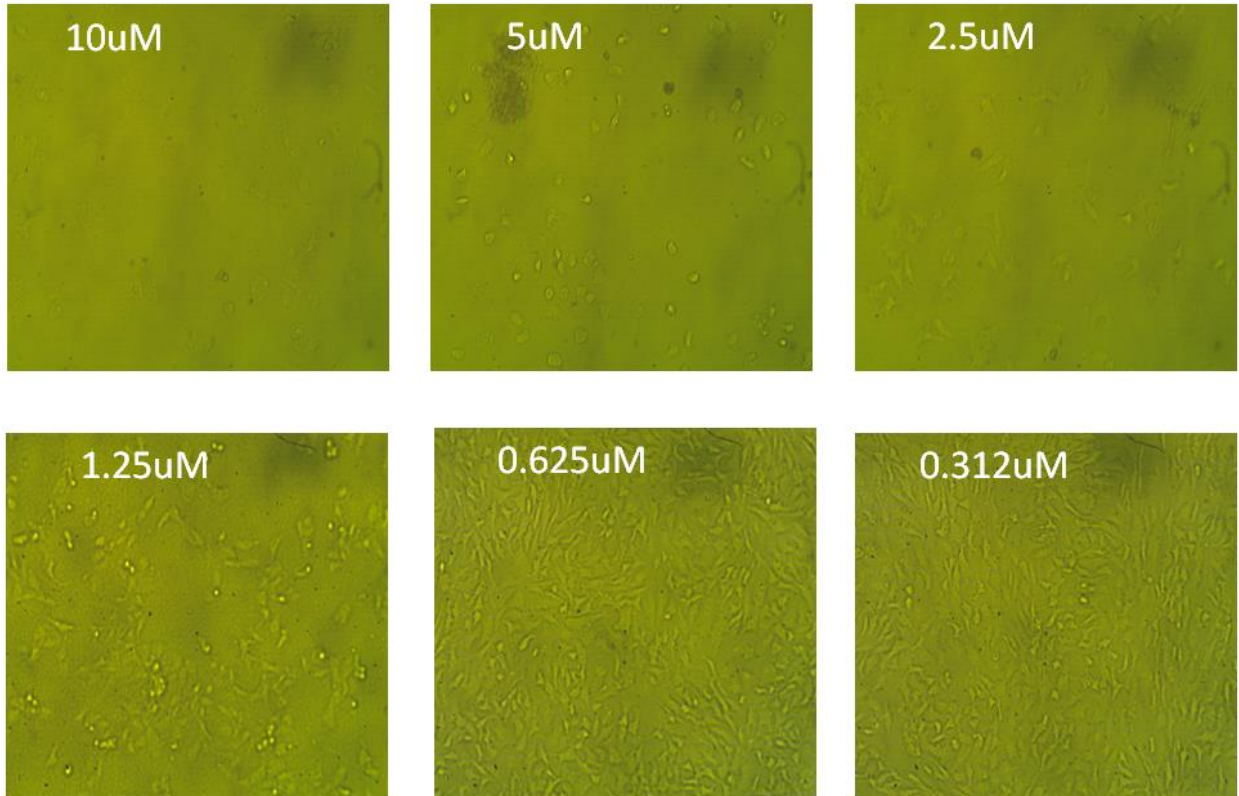
## Effect of sample IXd on HepG2 cells at different concentration



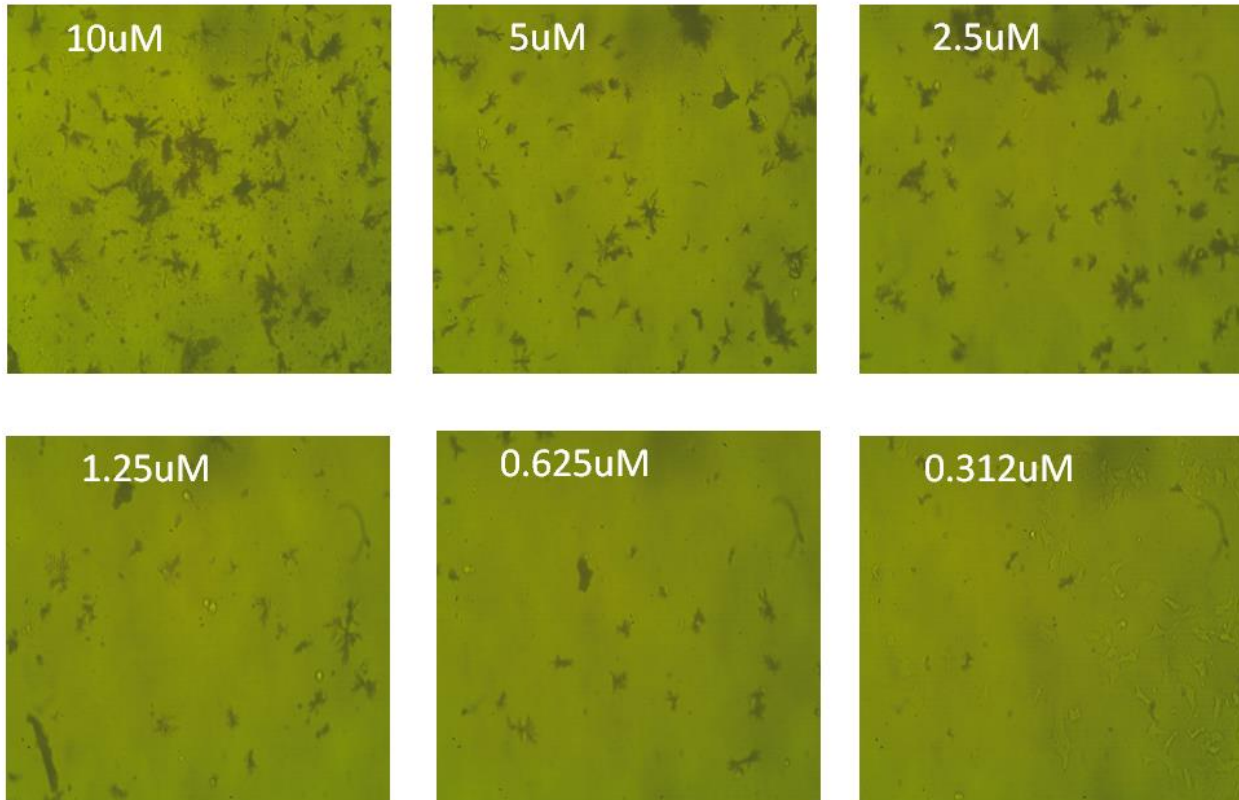
## Effect of sample IXd on HepG2 cells at different concentration



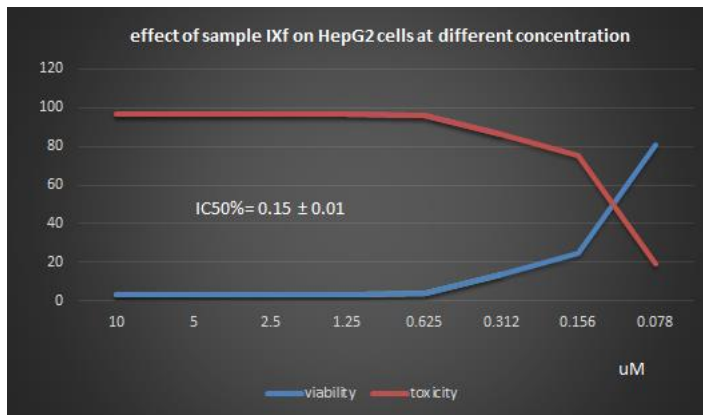
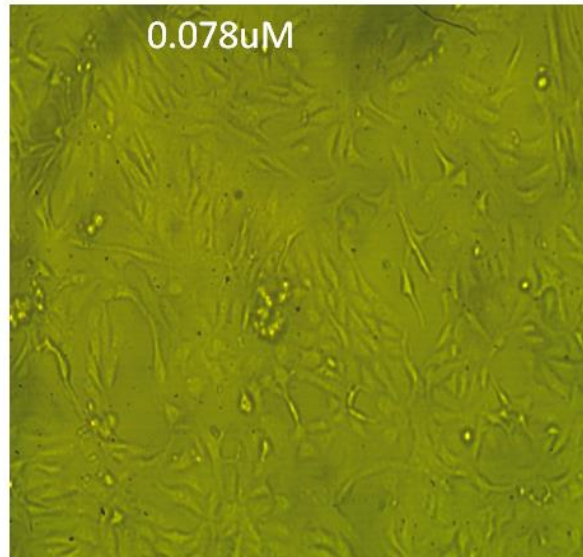
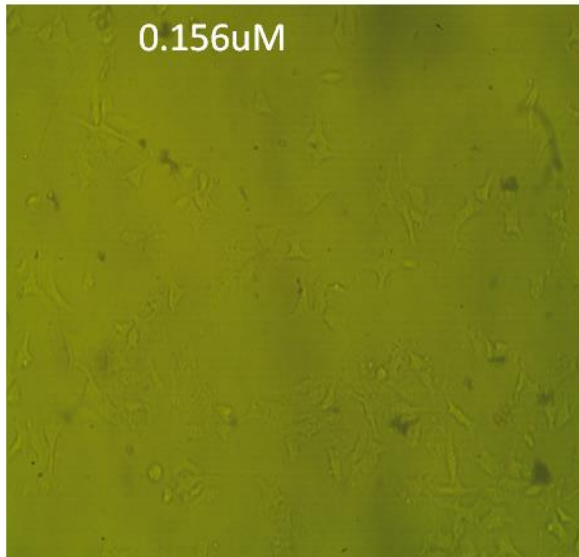
## Effect of sample IXe on HepG2 cells at different concentration



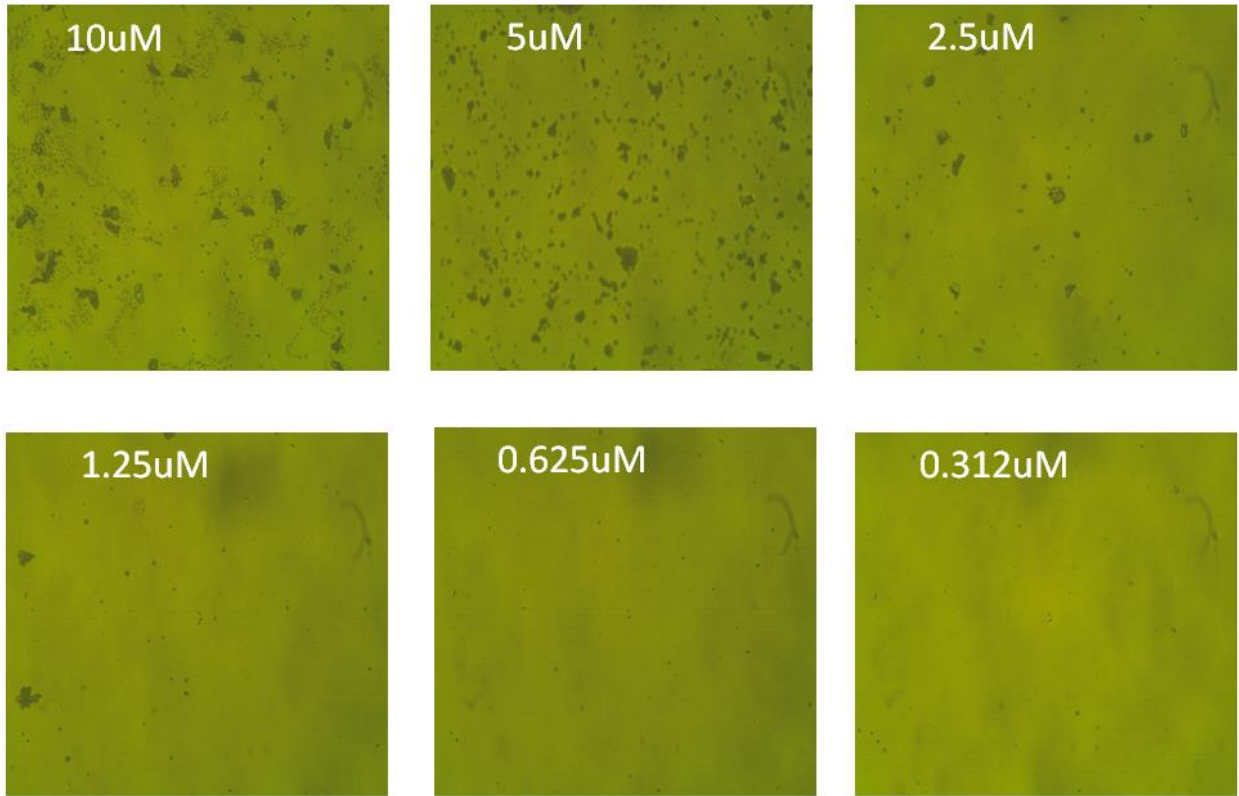
## Effect of sample IXf on HepG2 cells at different concentration



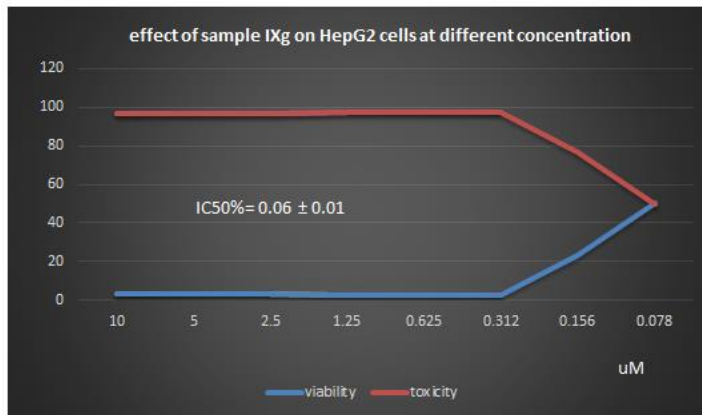
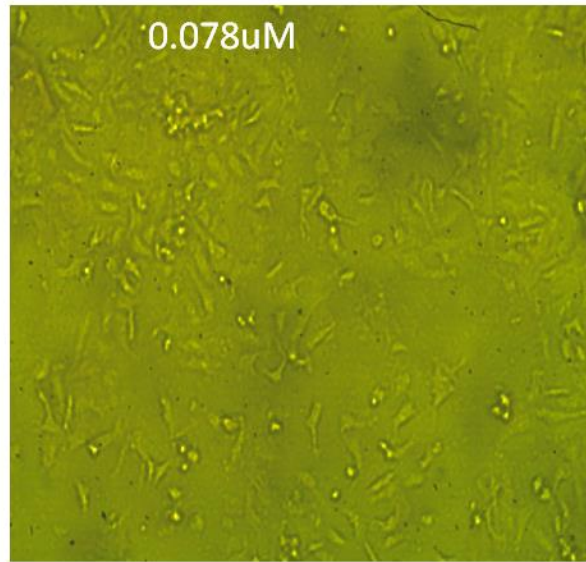
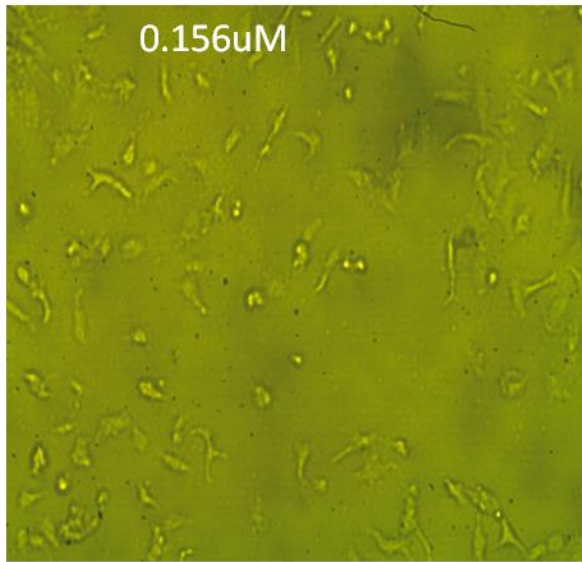
## Effect of sample IXf on HepG2 cells at different concentration



## Effect of sample IXg on HepG2 cells at different concentration

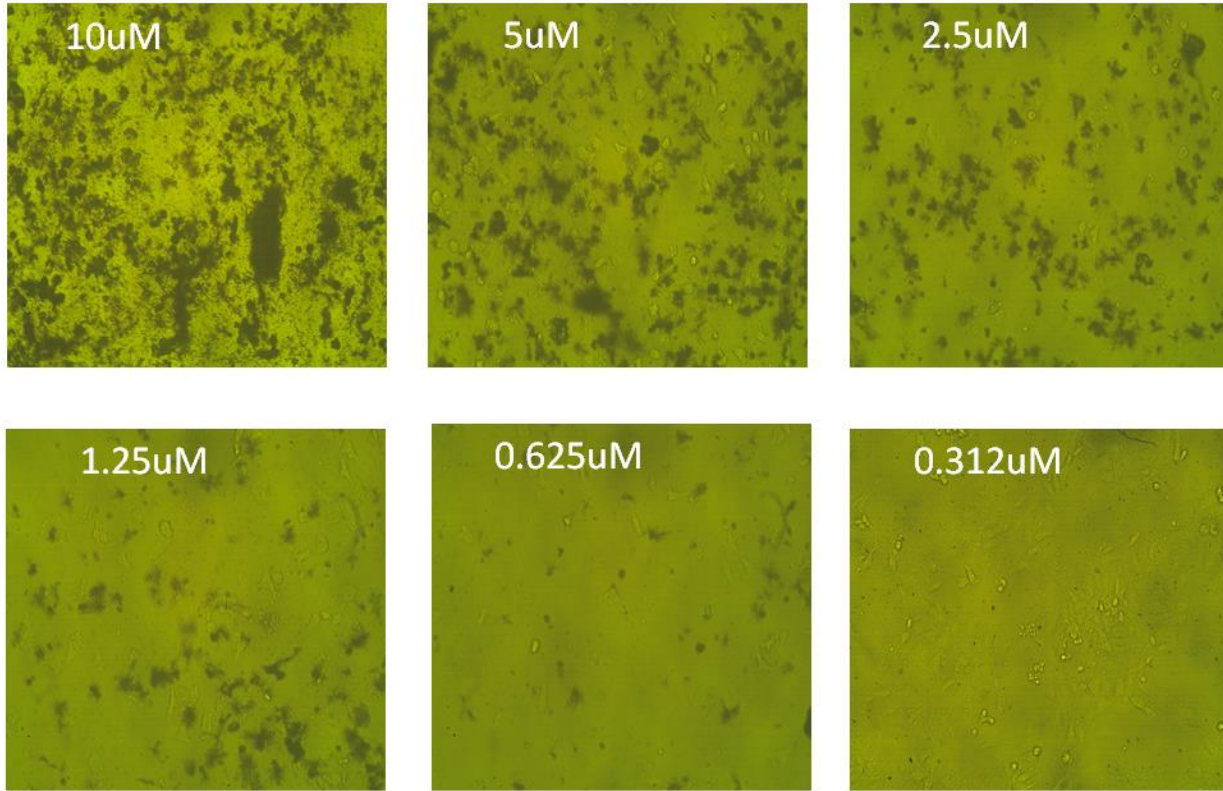


## Effect of sample IXg on HepG2 cells at different concentration

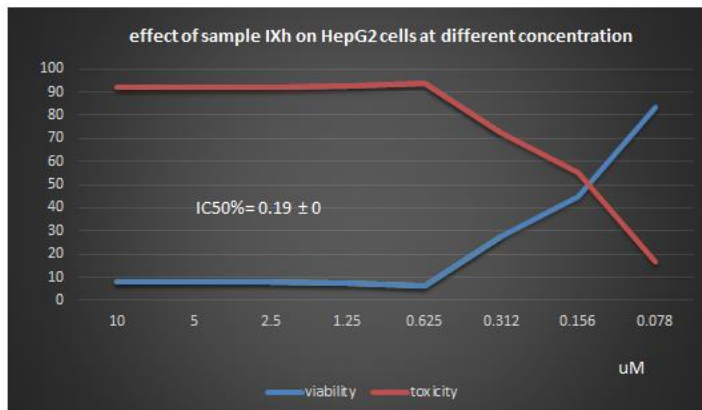
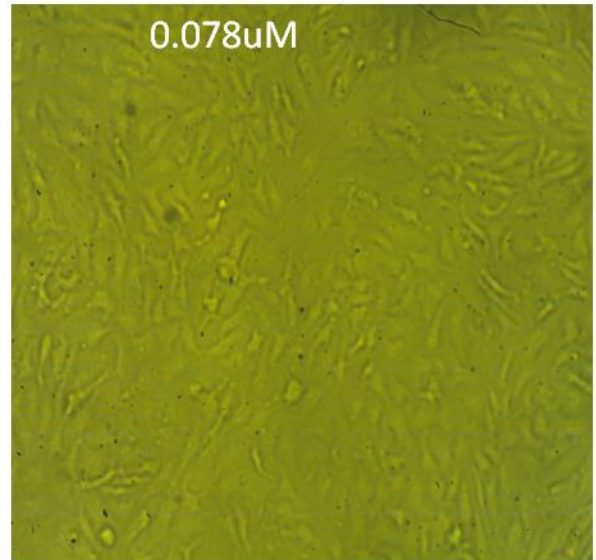
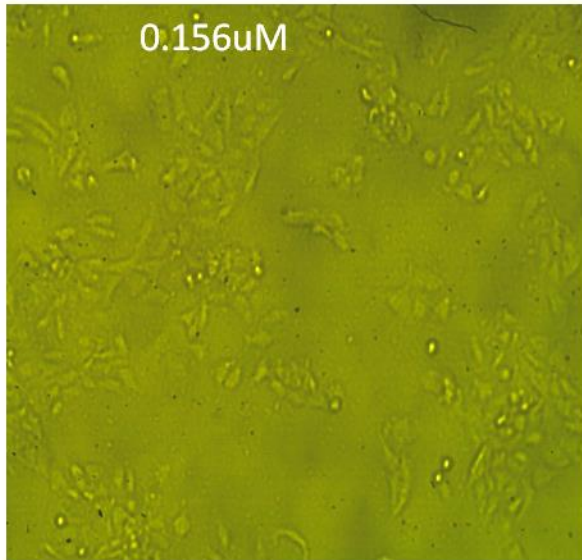




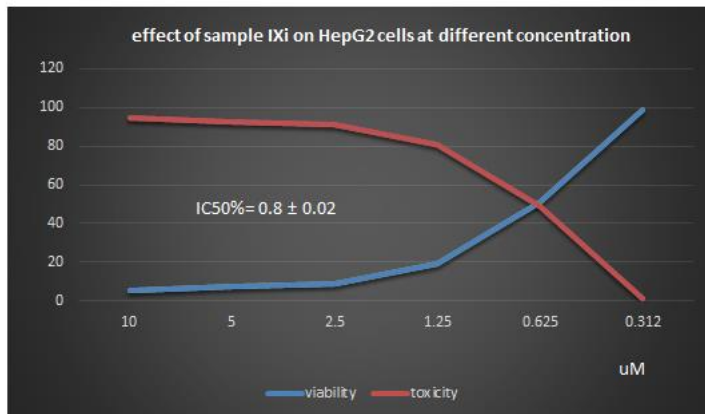
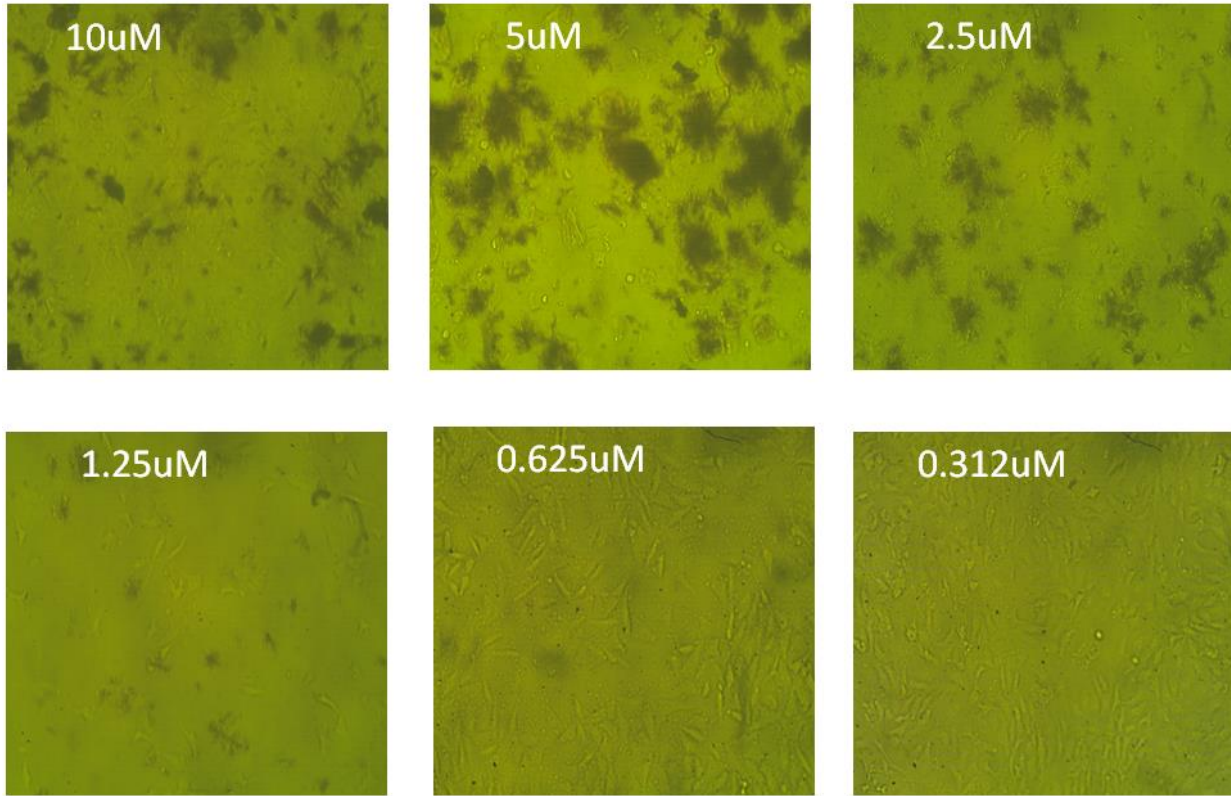
## Effect of sample IXh on HepG2 cells at different concentration



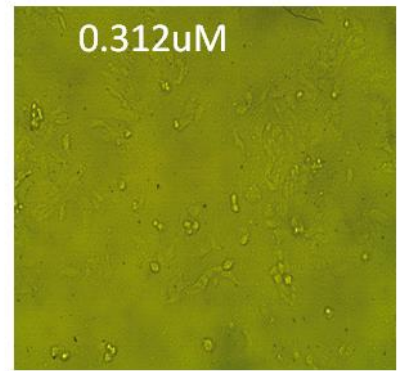
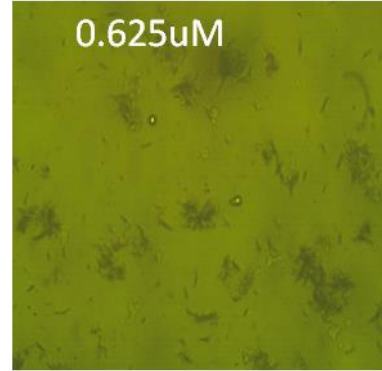
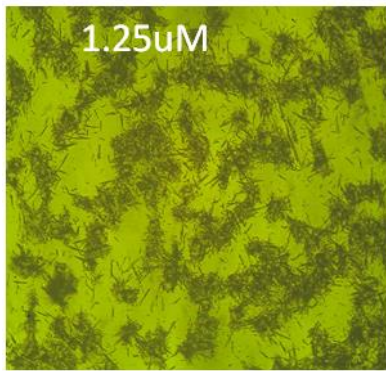
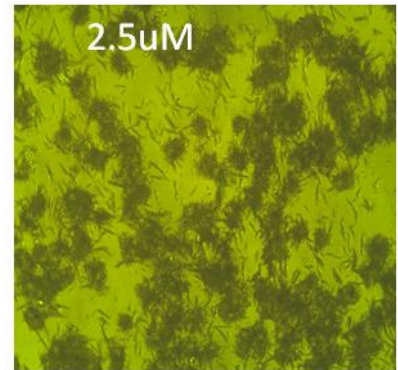
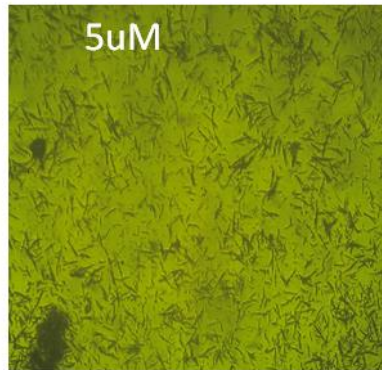
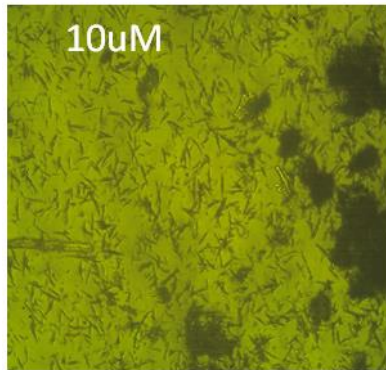
## Effect of sample IXh on HepG2 cells at different concentration



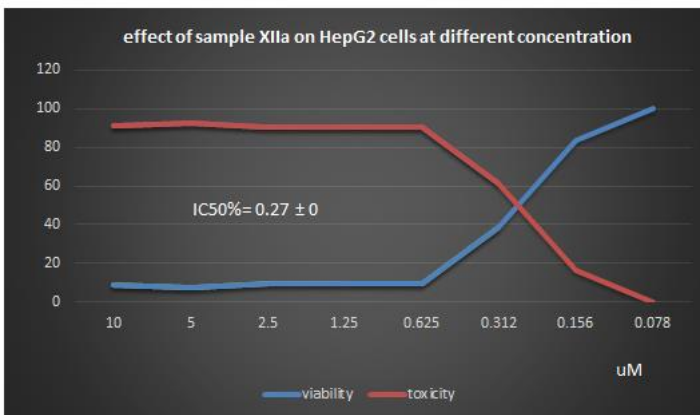
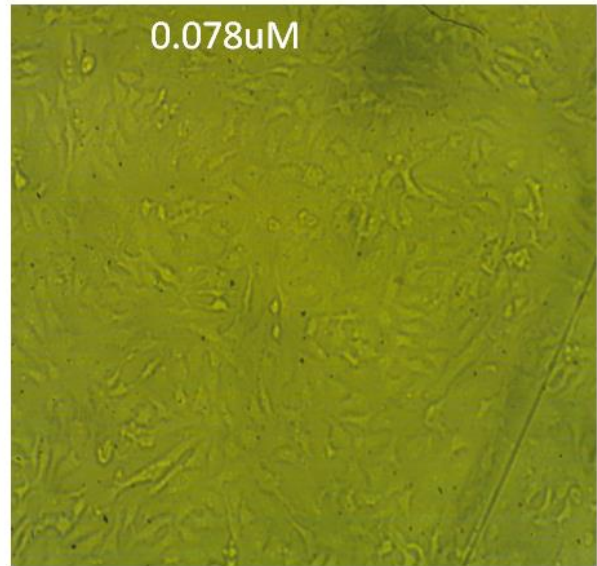
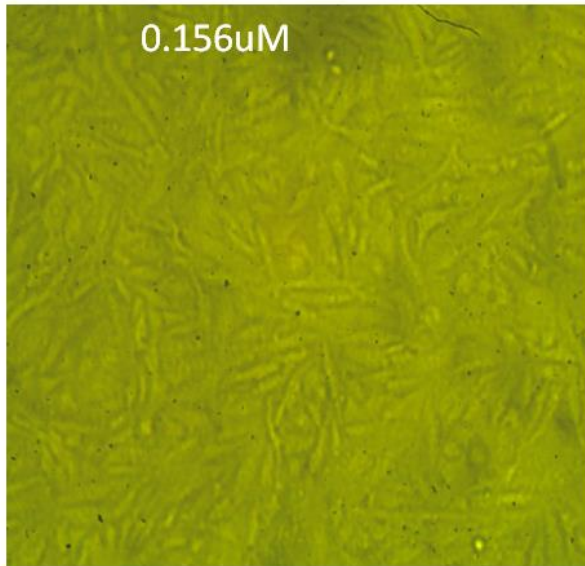
## Effect of sample IXi on HepG2 cells at different concentration



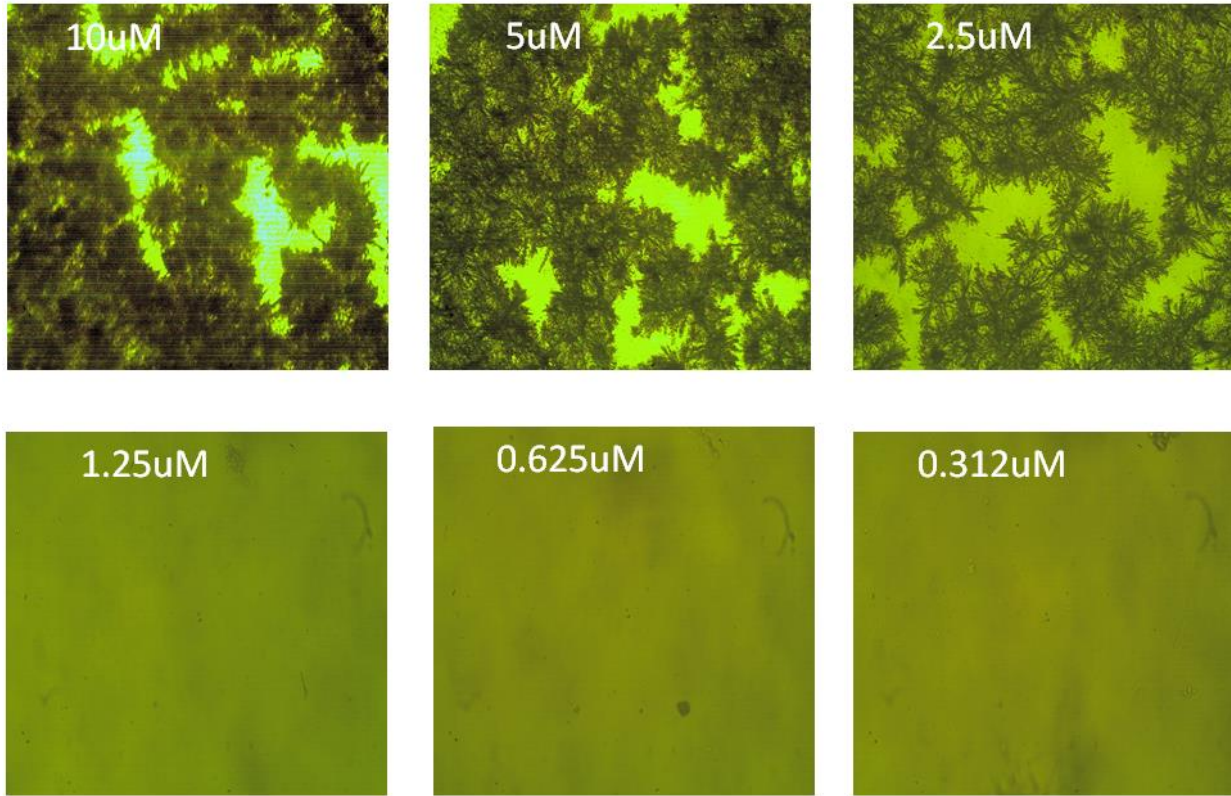
## Effect of sample Xlla on HepG2 cells at different concentration



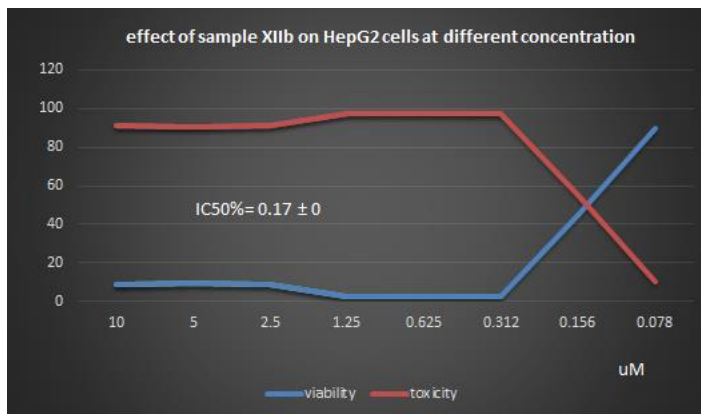
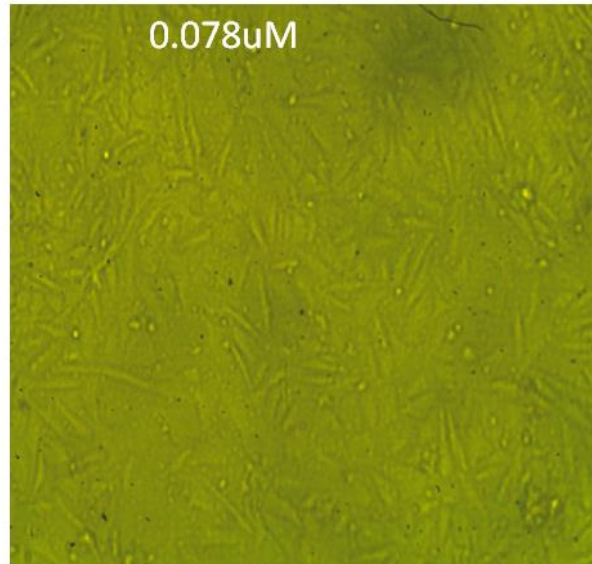
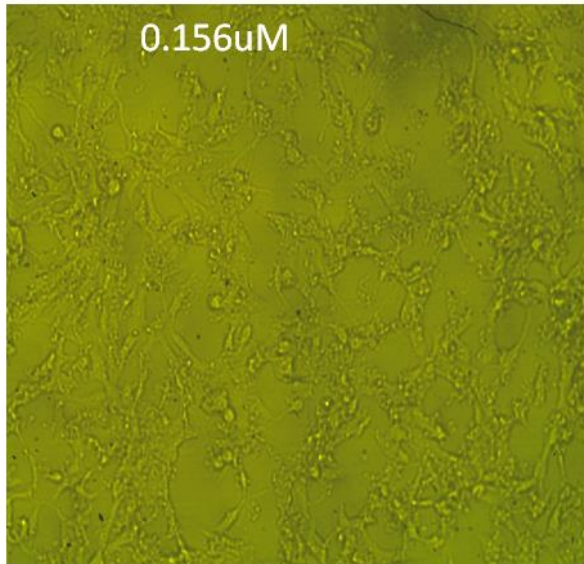
## Effect of sample Xlla on HepG2 cells at different concentration



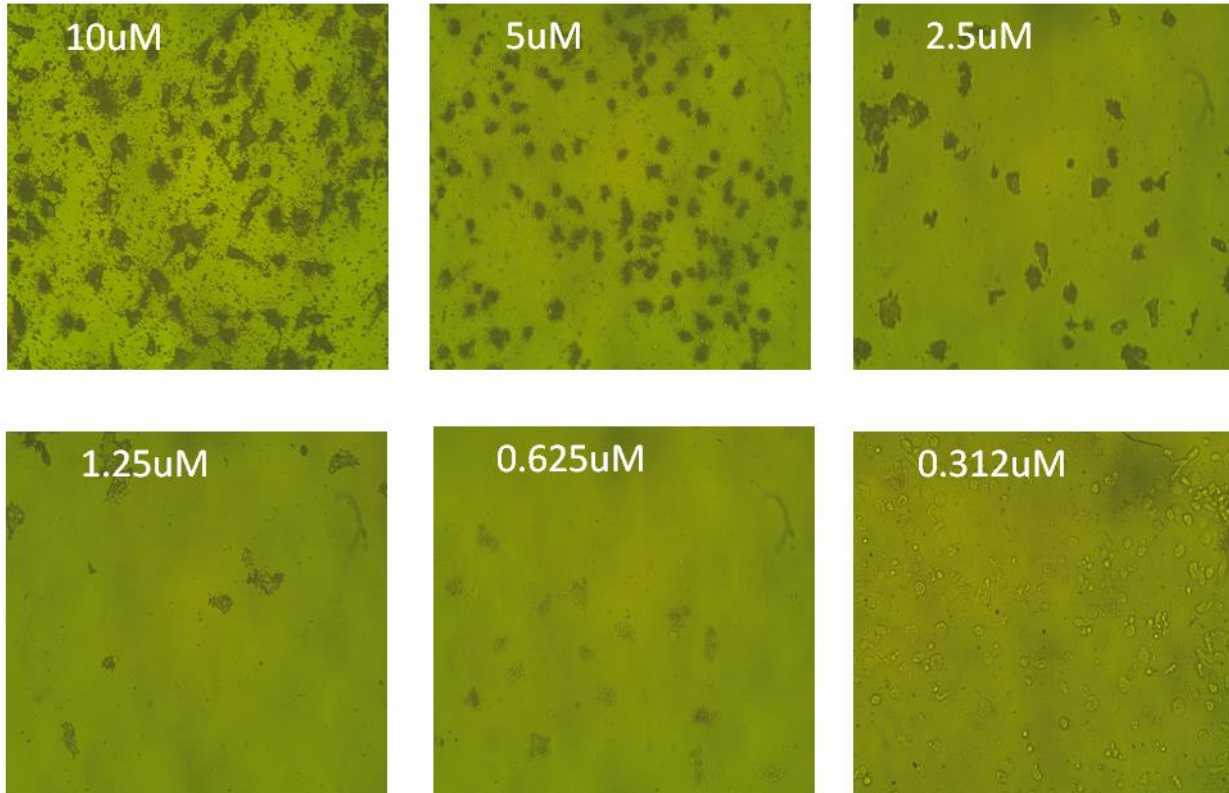
## Effect of sample XIIb on HepG2 cells at different concentration



## Effect of sample XIIb on HepG2 cells at different concentration

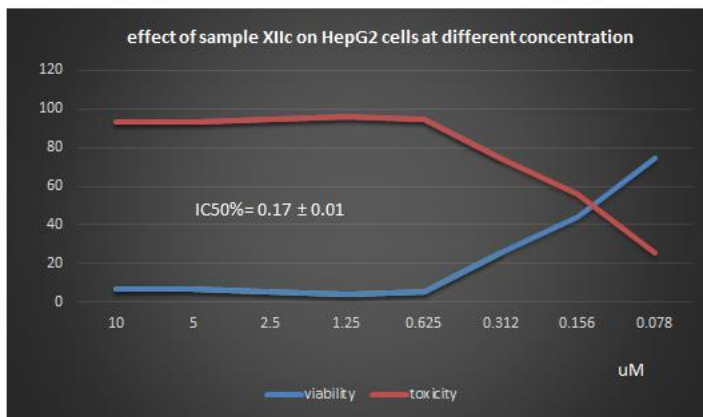
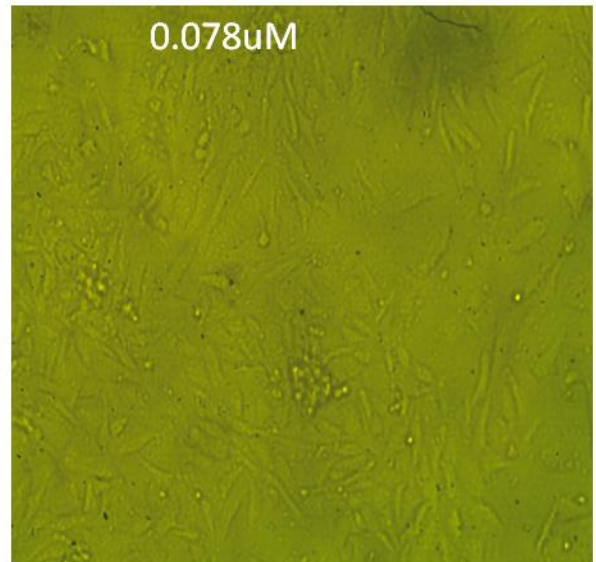
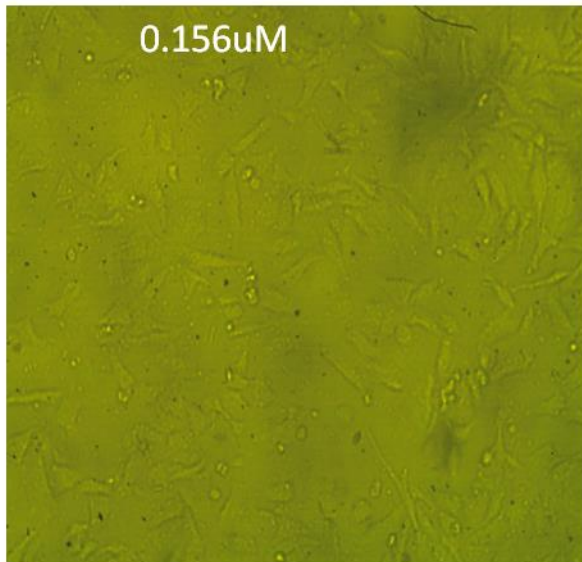


## Effect of sample XIIc on HepG2 cells at different concentration

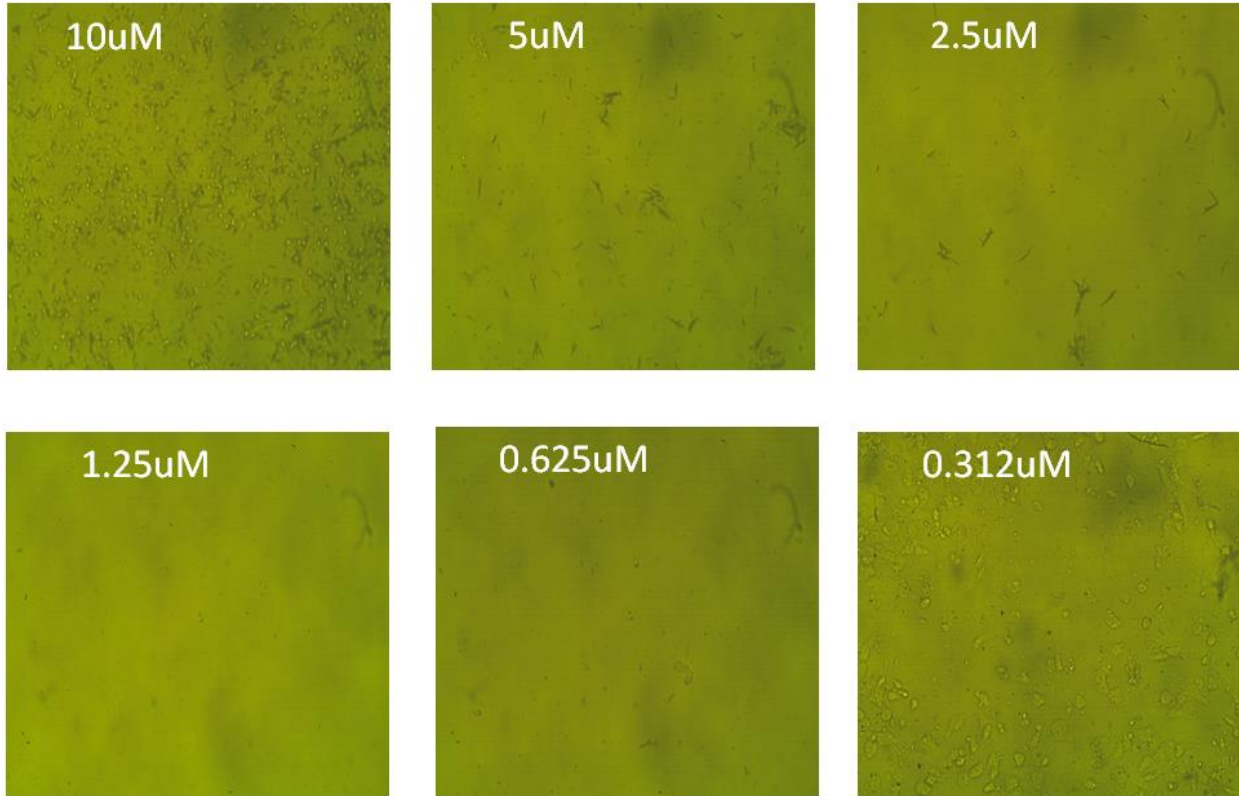




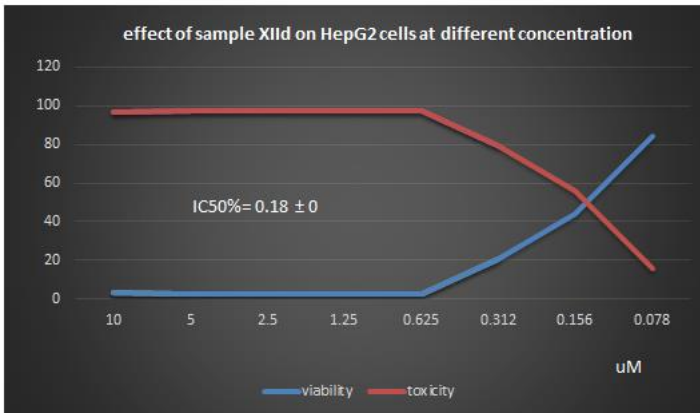
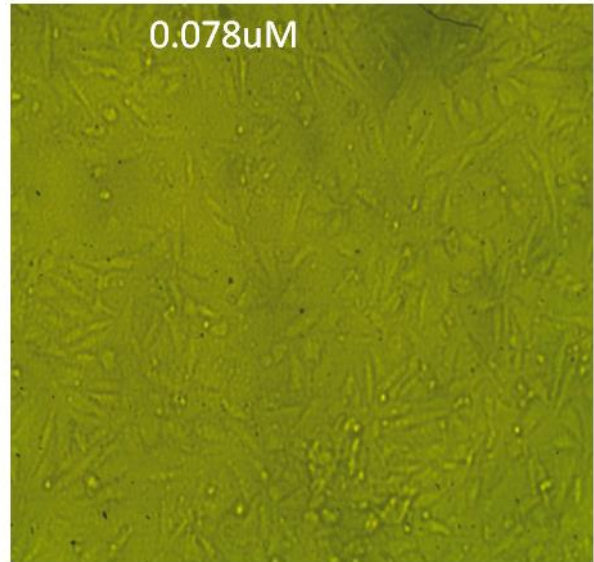
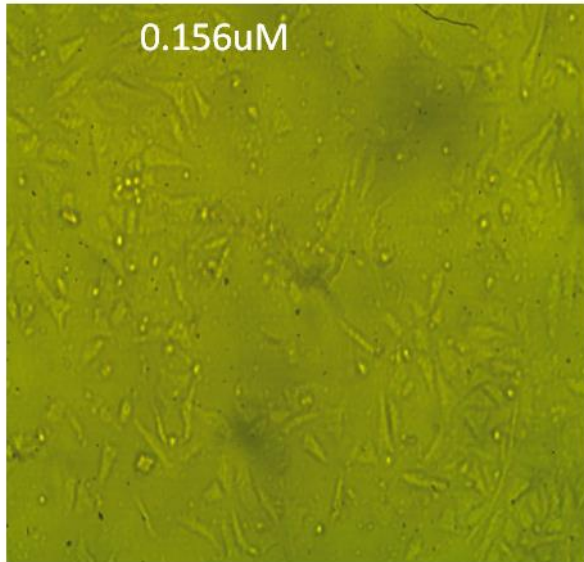
## Effect of sample XIIc on HepG2 cells at different concentration



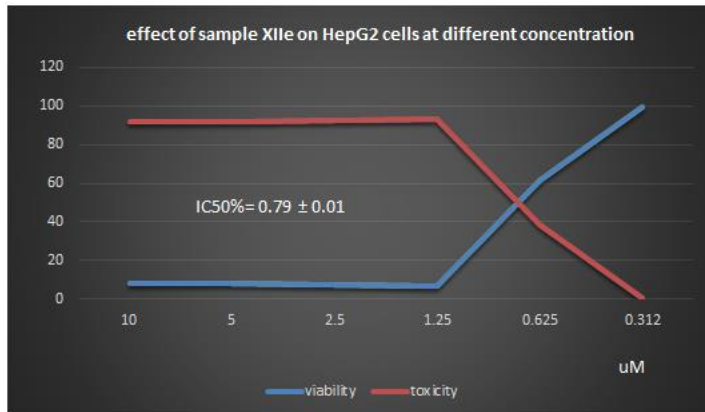
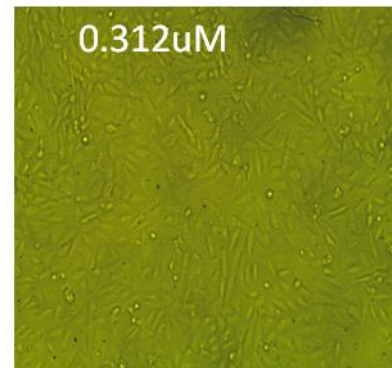
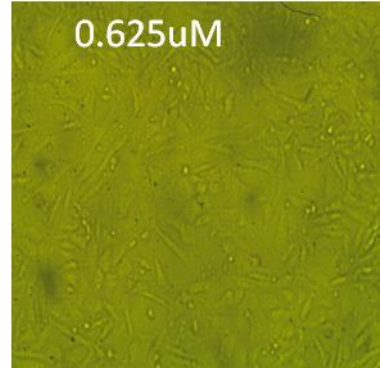
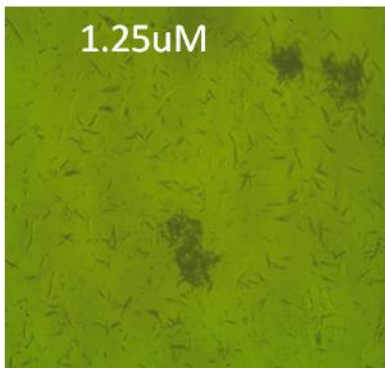
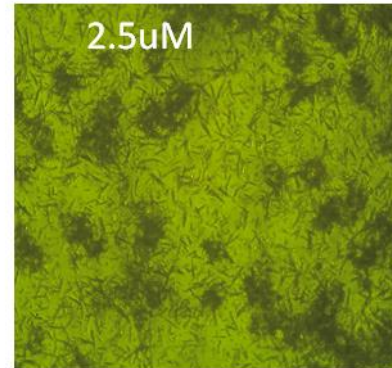
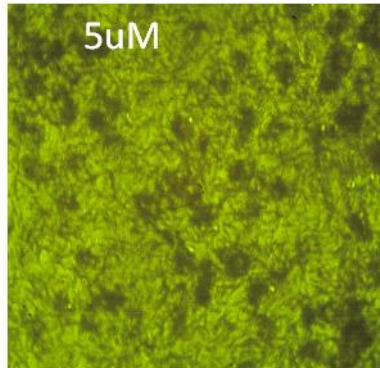
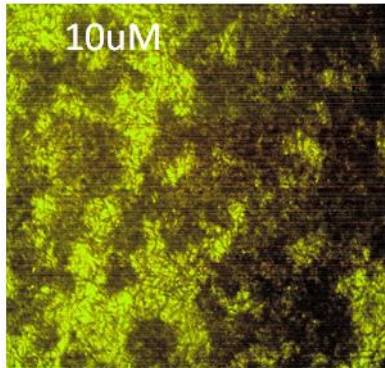
## Effect of sample XIId on HepG2 cells at different concentration



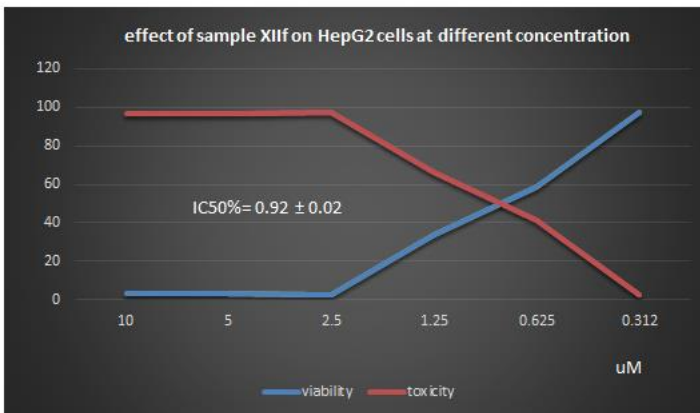
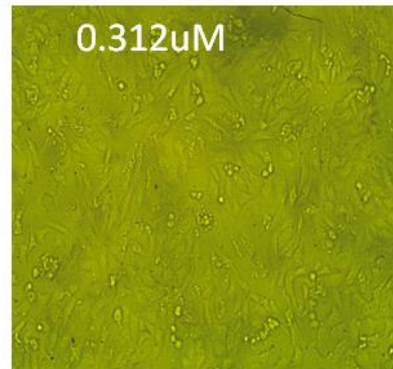
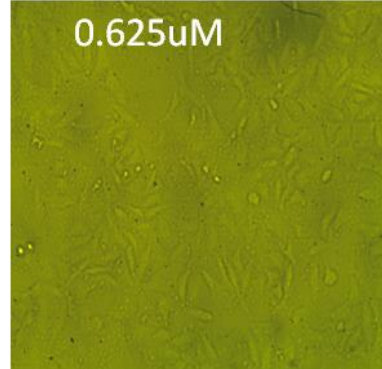
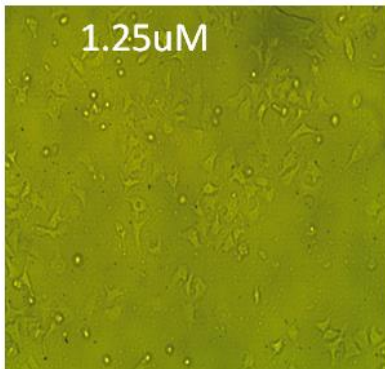
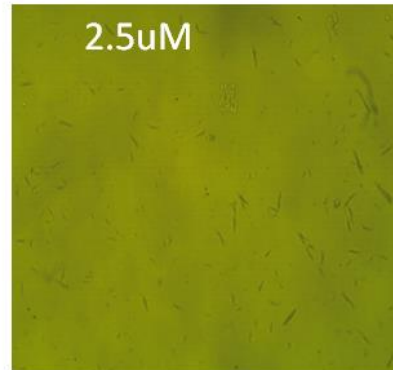
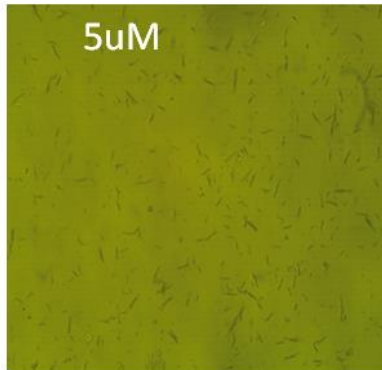
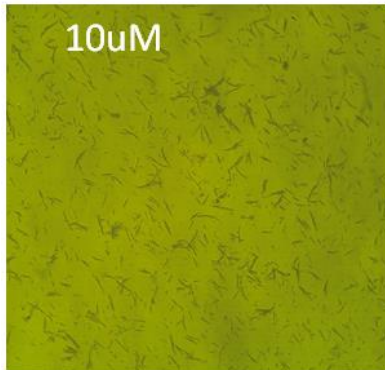
## Effect of sample XIId on HepG2 cells at different concentration



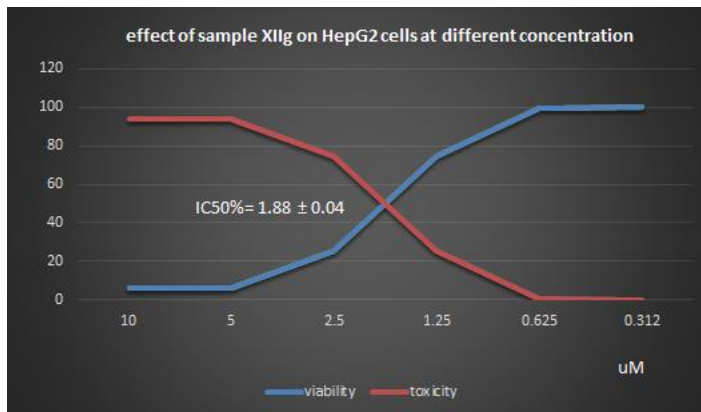
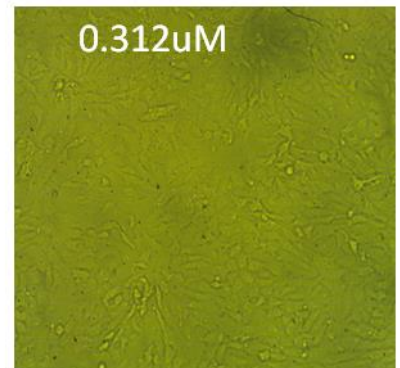
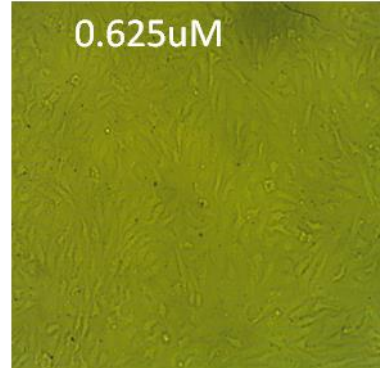
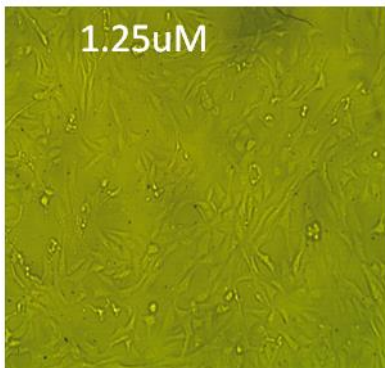
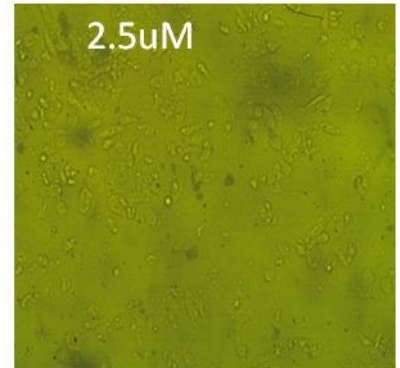
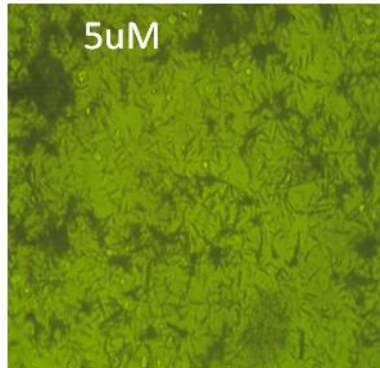
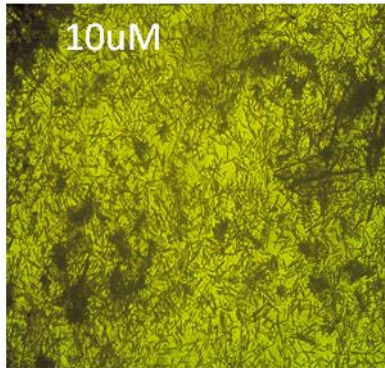
## Effect of sample XIle on HepG2 cells at different concentration



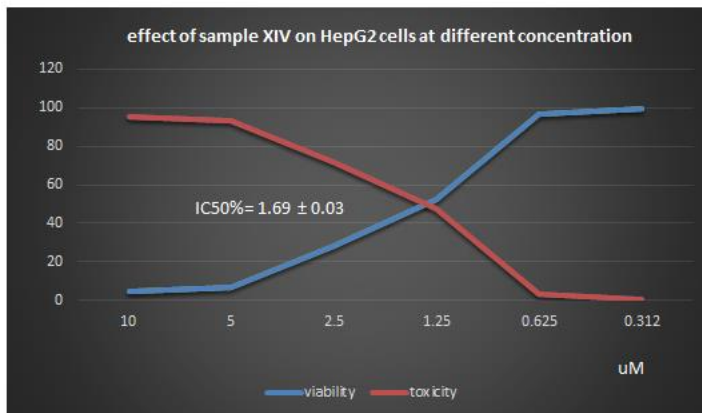
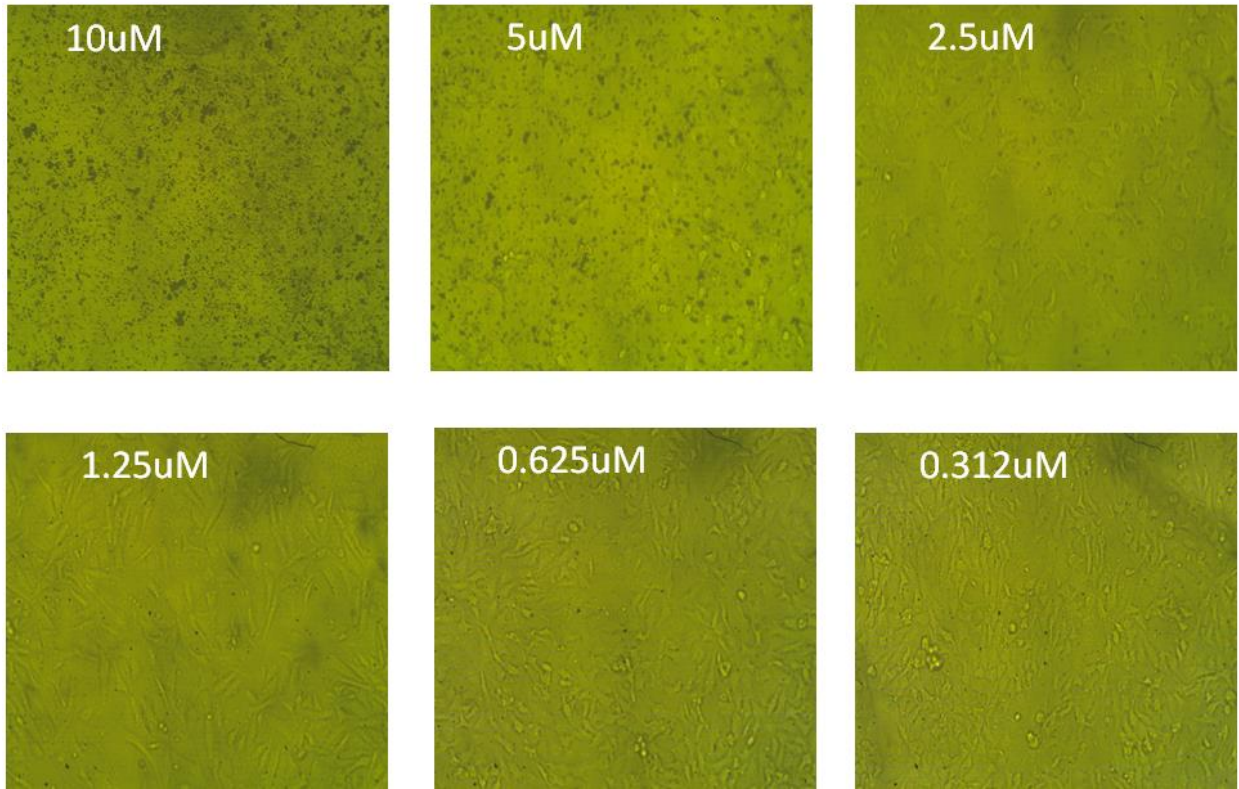
## Effect of sample XIIf on HepG2 cells at different concentration



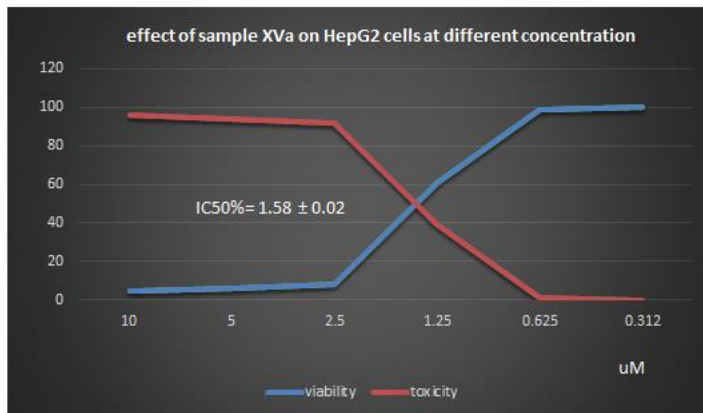
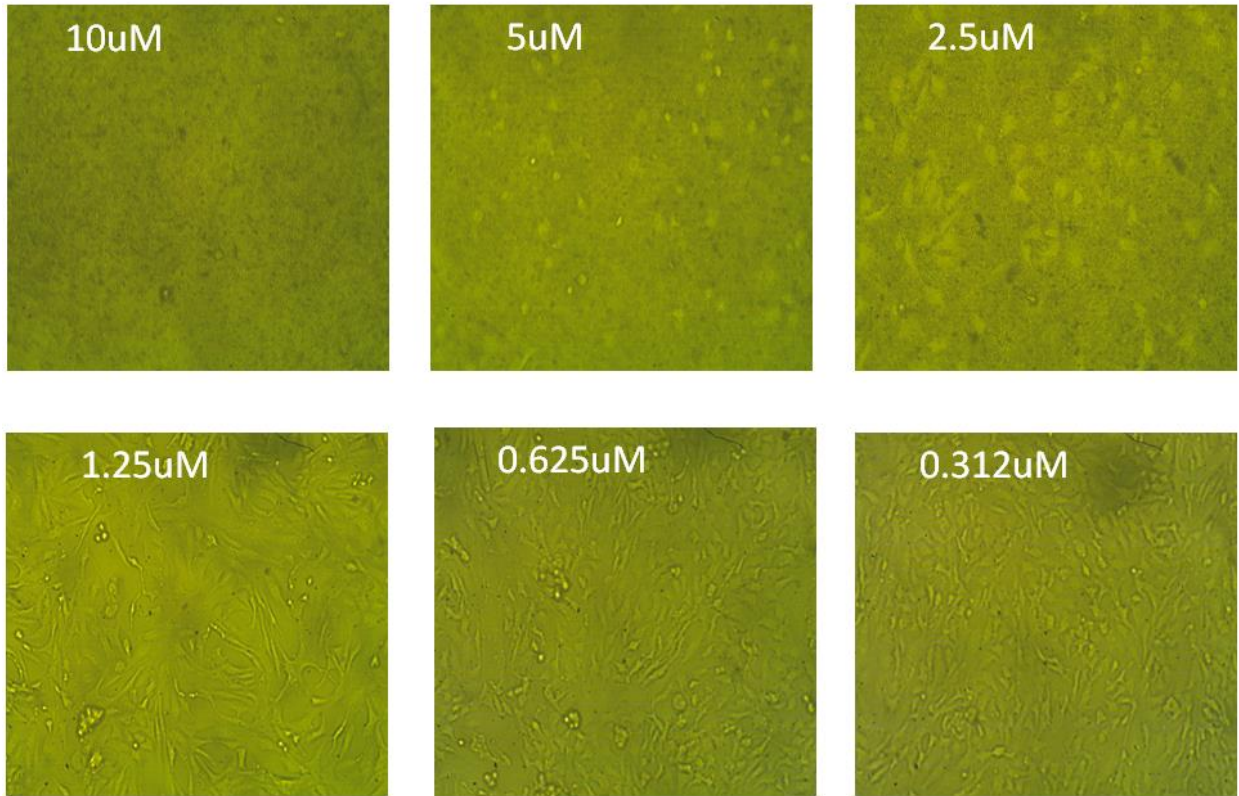
## Effect of sample XIlg on HepG2 cells at different concentration



## Effect of sample XIV on HepG2 cells at different concentration

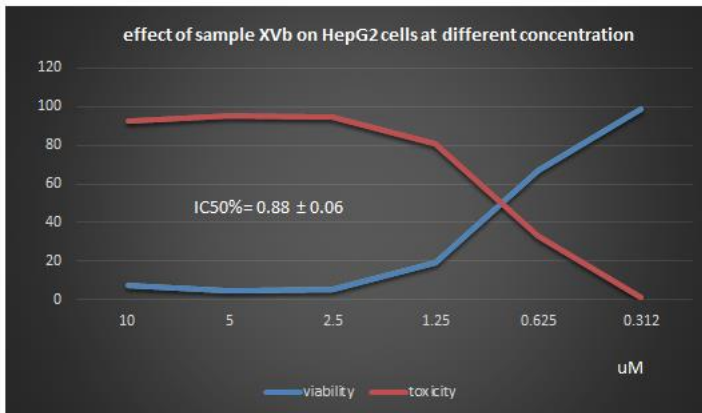
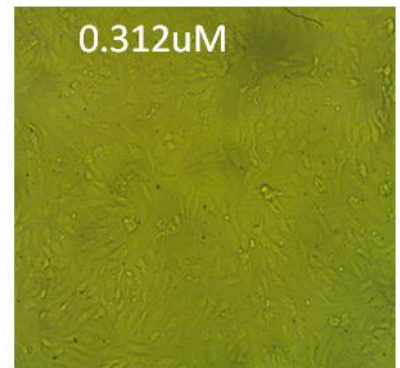
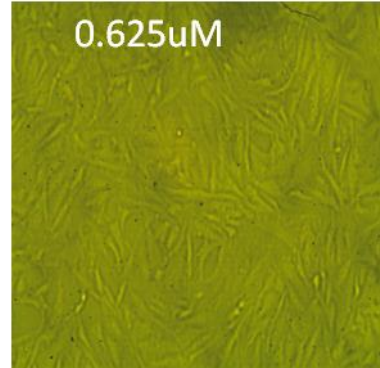
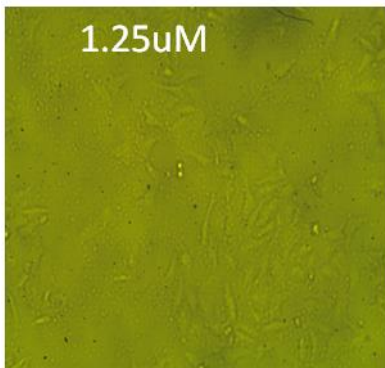
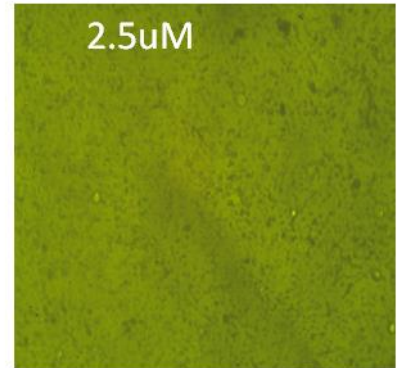
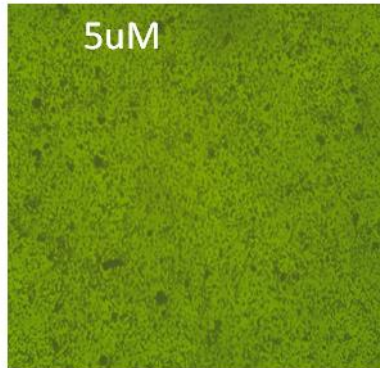
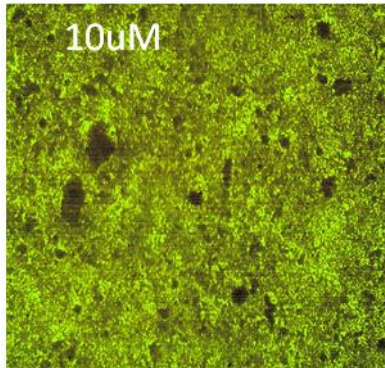


## Effect of sample XVa on HepG2 cells at different concentration

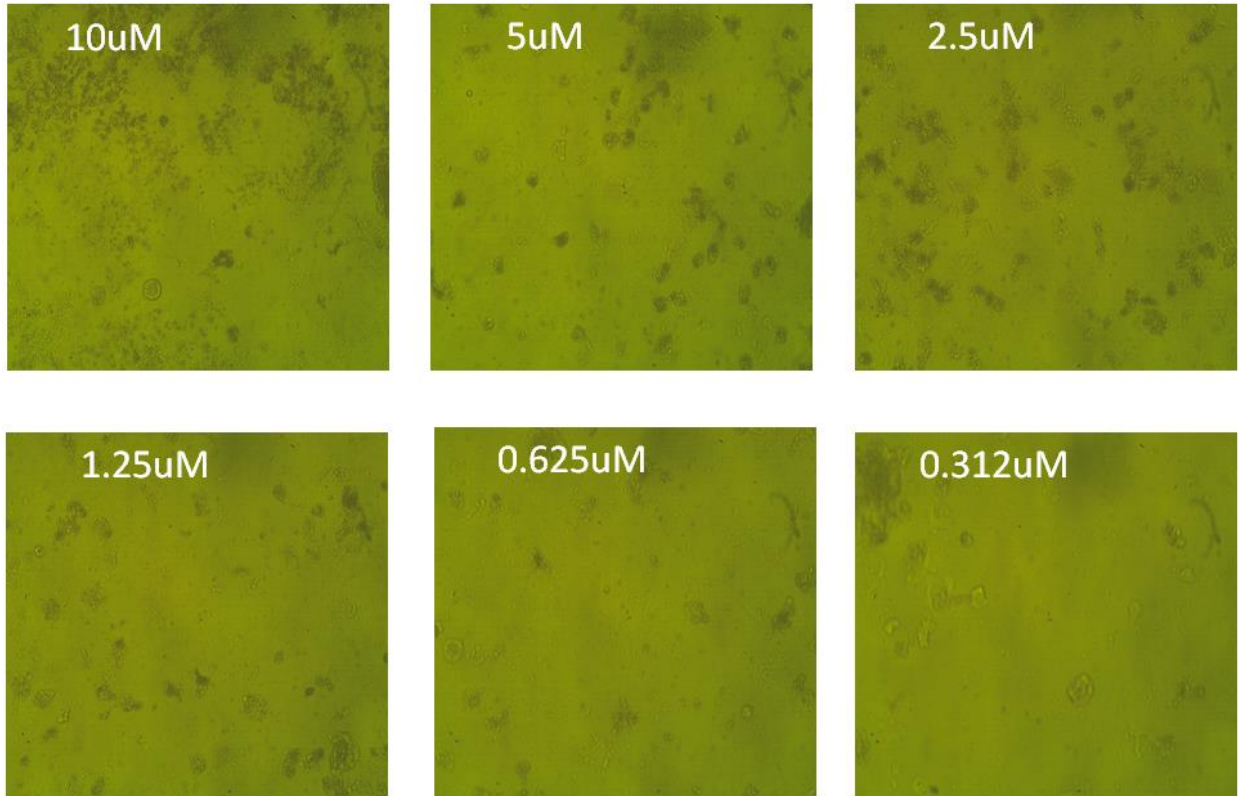




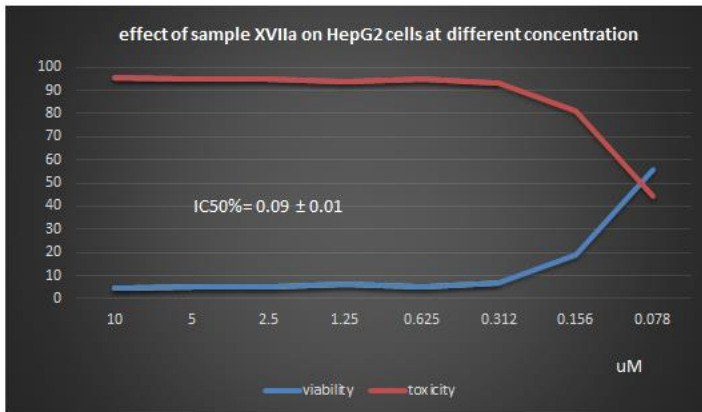
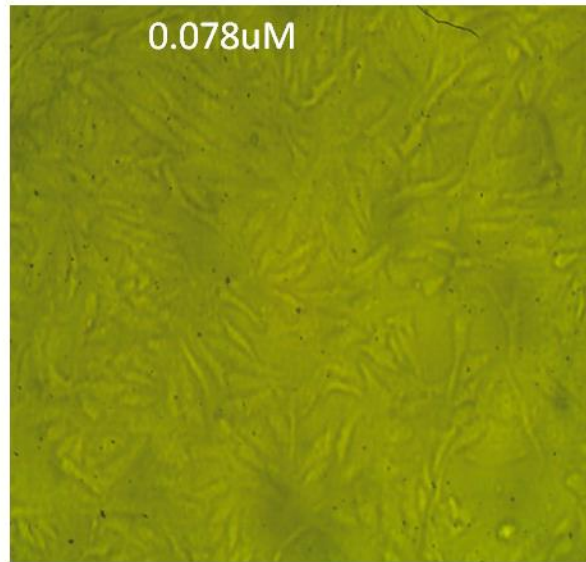
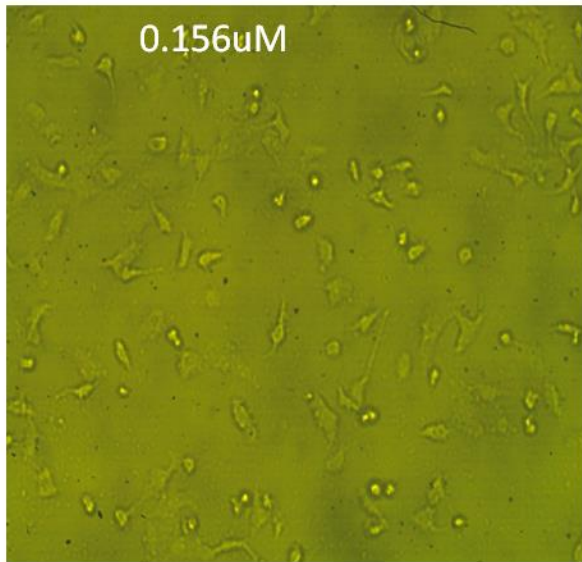
## Effect of sample XVb on HepG2 cells at different concentration



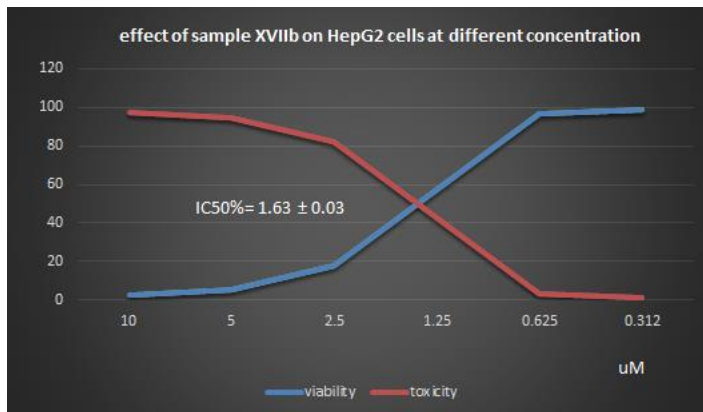
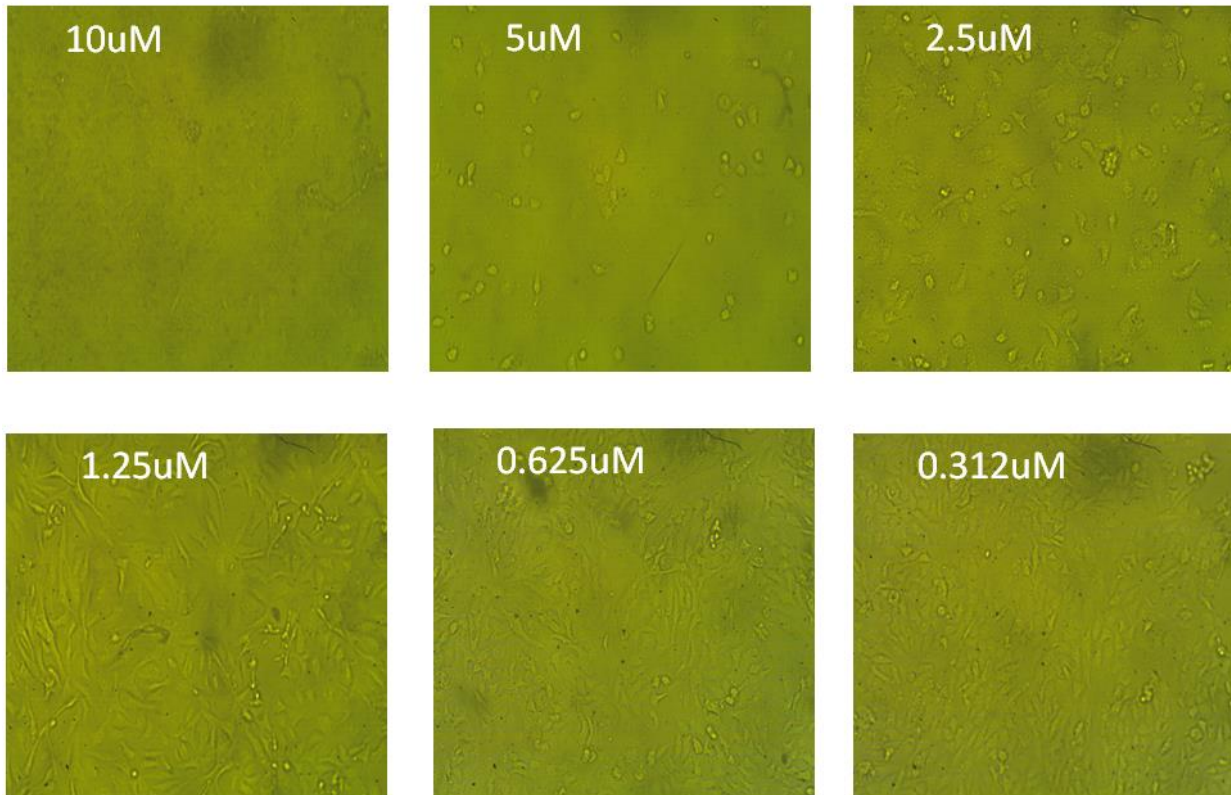
## Effect of sample XVIIa on HepG2 cells at different concentration



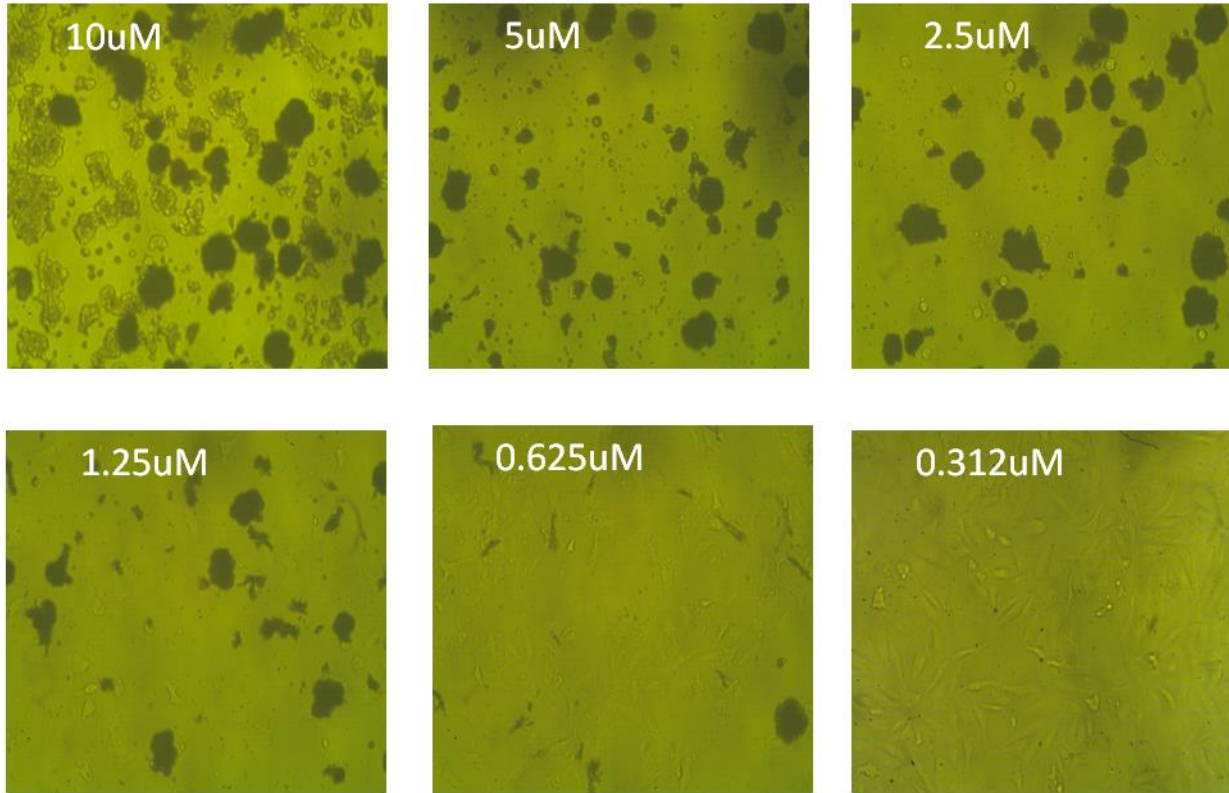
## Effect of sample XVIIa on HepG2 cells at different concentration



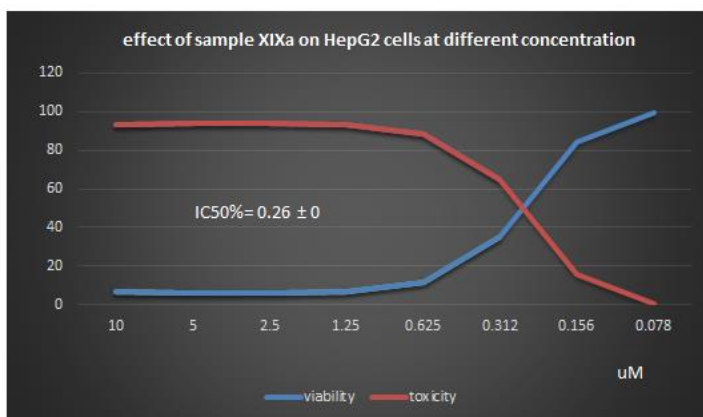
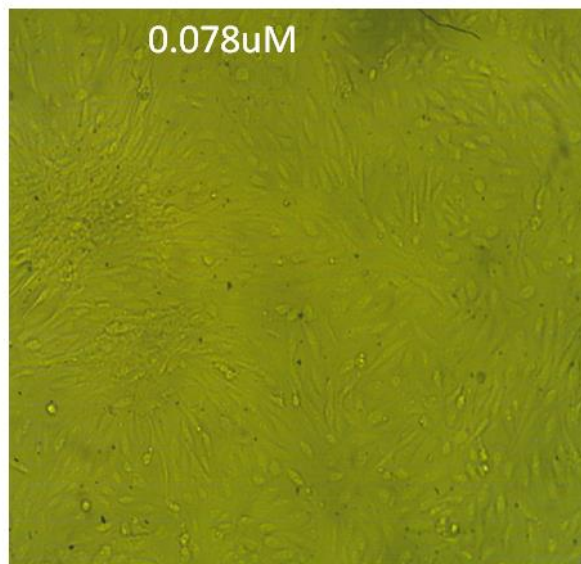
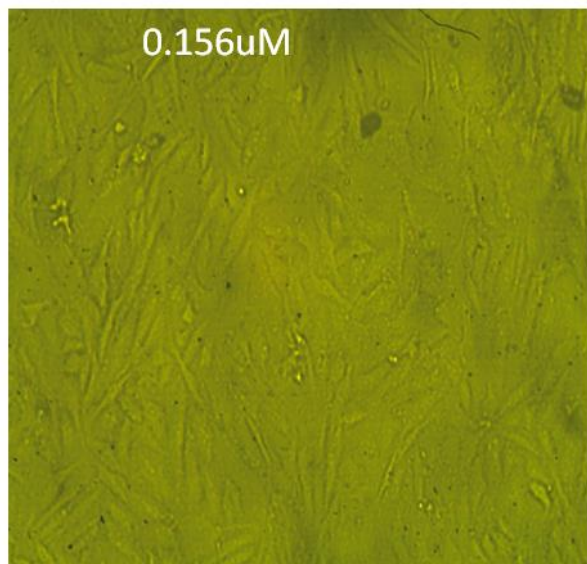
## Effect of sample XVIIb on HepG2 cells at different concentration



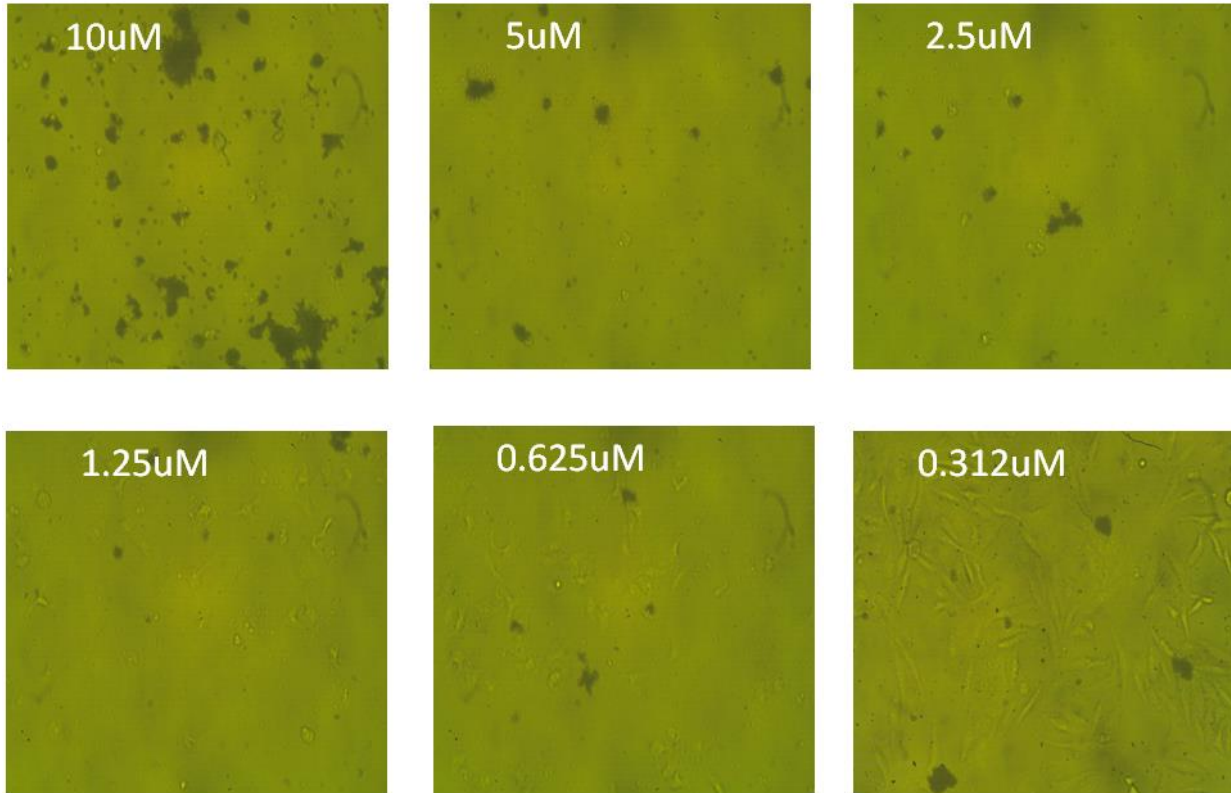
## Effect of sample XIXa on HepG2 cells at different concentration



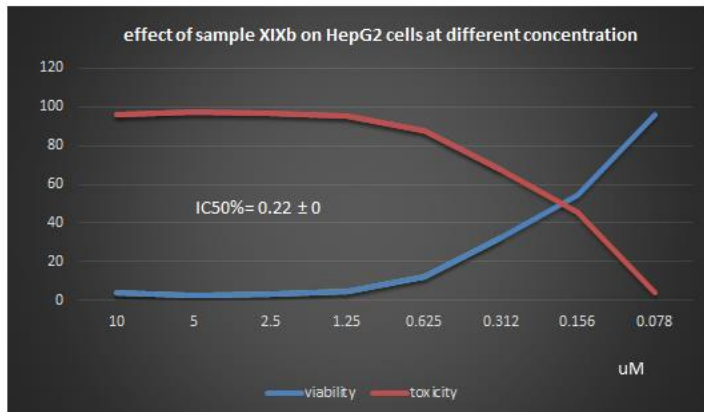
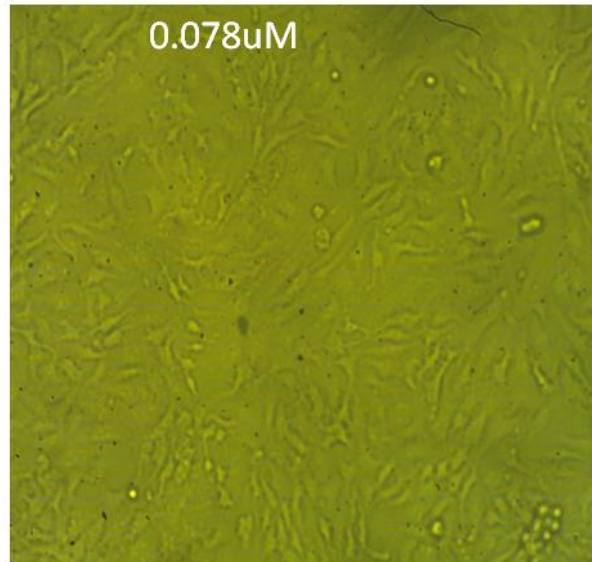
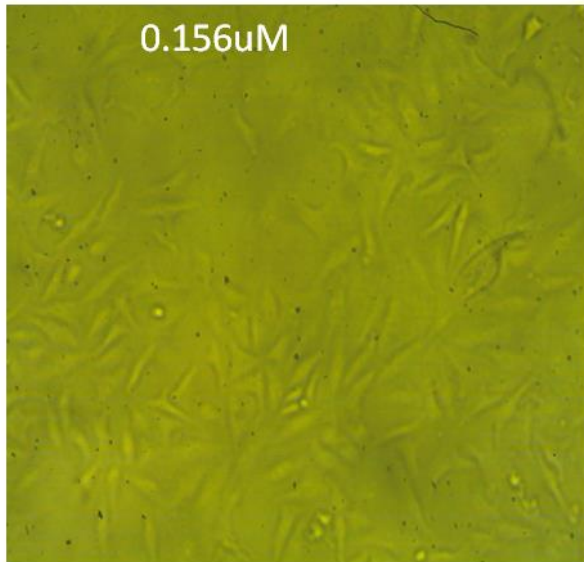
## Effect of sample XIXa on HepG2 cells at different concentration



## Effect of sample XIXb on HepG2 cells at different concentration

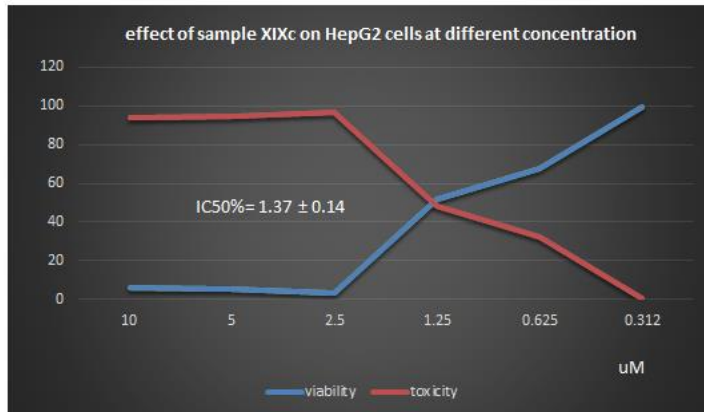
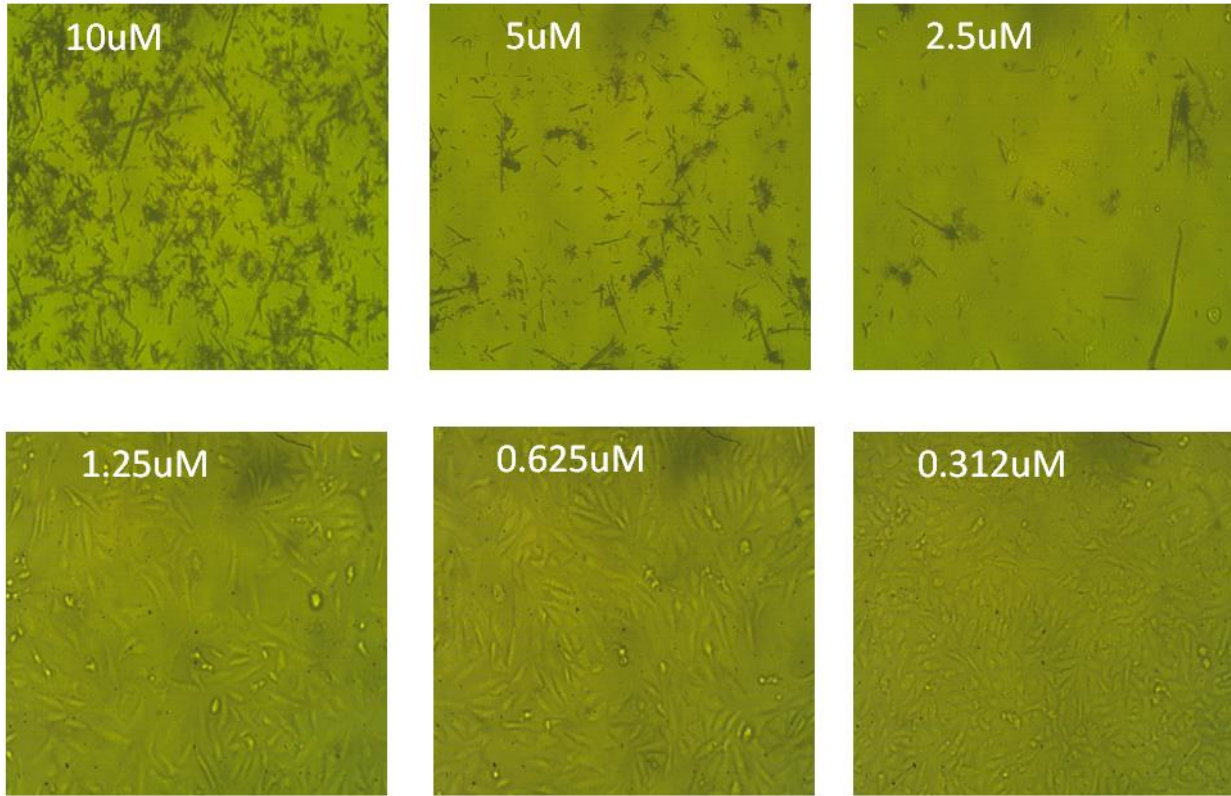


## Effect of sample XIXb on HepG2 cells at different concentration





## Effect of sample XIXc on HepG2 cells at different concentration



## Effect of sample XIXd on HepG2 cells at different concentration

