Supplementary material

Antiproliferative ruthenium complexes containing curcuminoid ligands tested *in vitro* on human ovarian tumor cell line A2780, towards their capability to modulate the NF-κB transcription factor, FGF-2 growth factor and MMP-9 pathway

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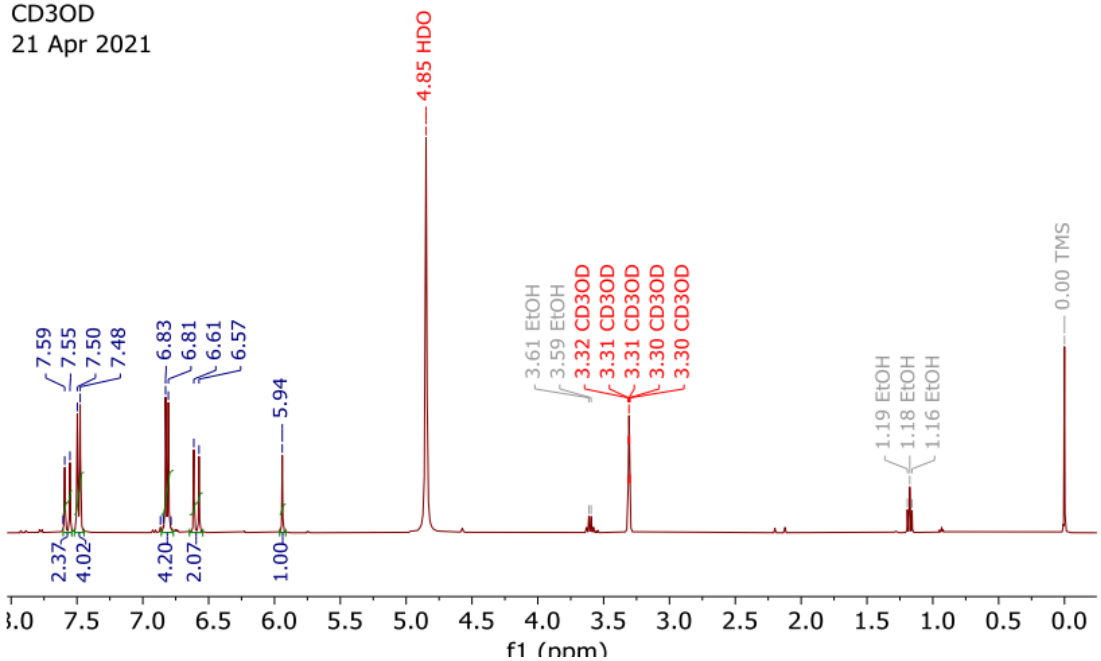
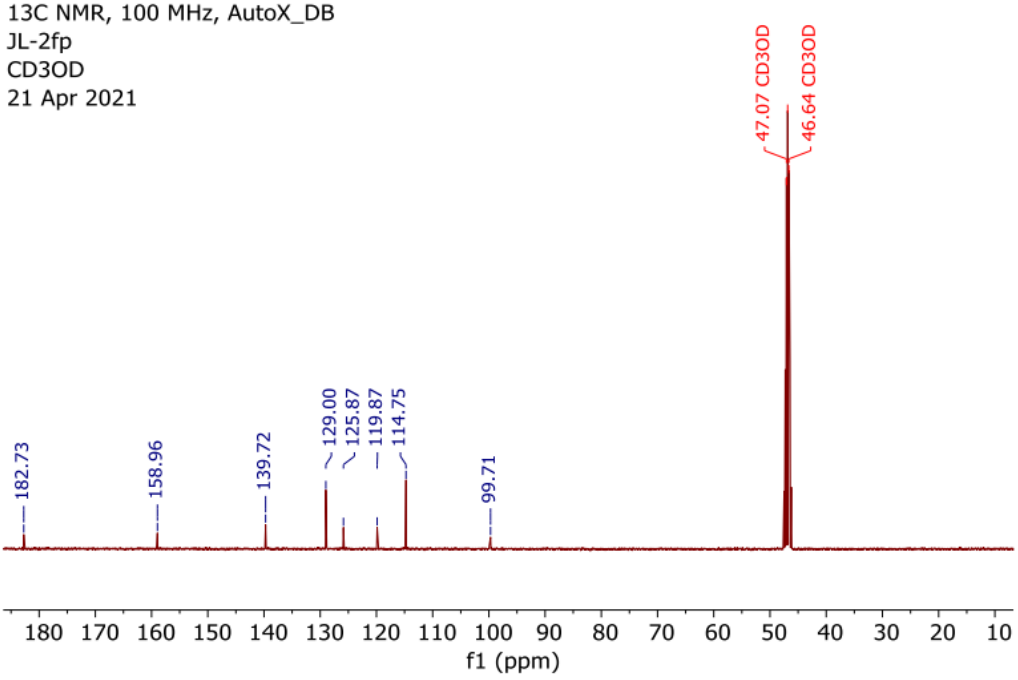
NMR, HRMS and IR spectra of free ligands and ruthenium complexes 1,2,3 and 4

NMR Spectrum of **BDMC**

PROTON_01
1H NMR, 400 MHz, AutoX_DB
JL-2fp
CD3OD
21 Apr 2021



CARBON_01
13C NMR, 100 MHz, AutoX_DB
JL-2fp
CD3OD
21 Apr 2021



NMR Spectrum of **Syringaldehyde curcumin**

PROTON_01
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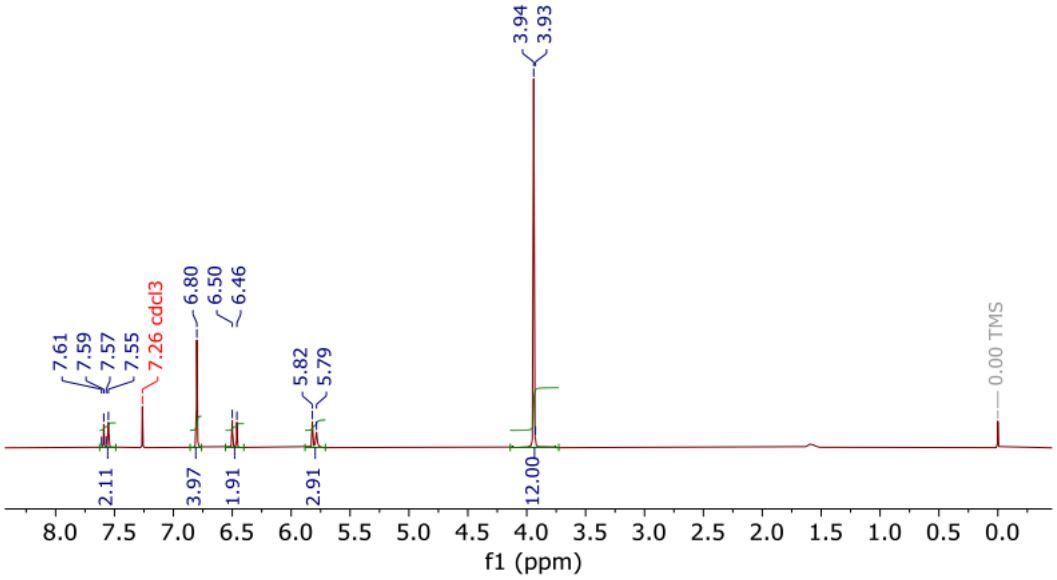
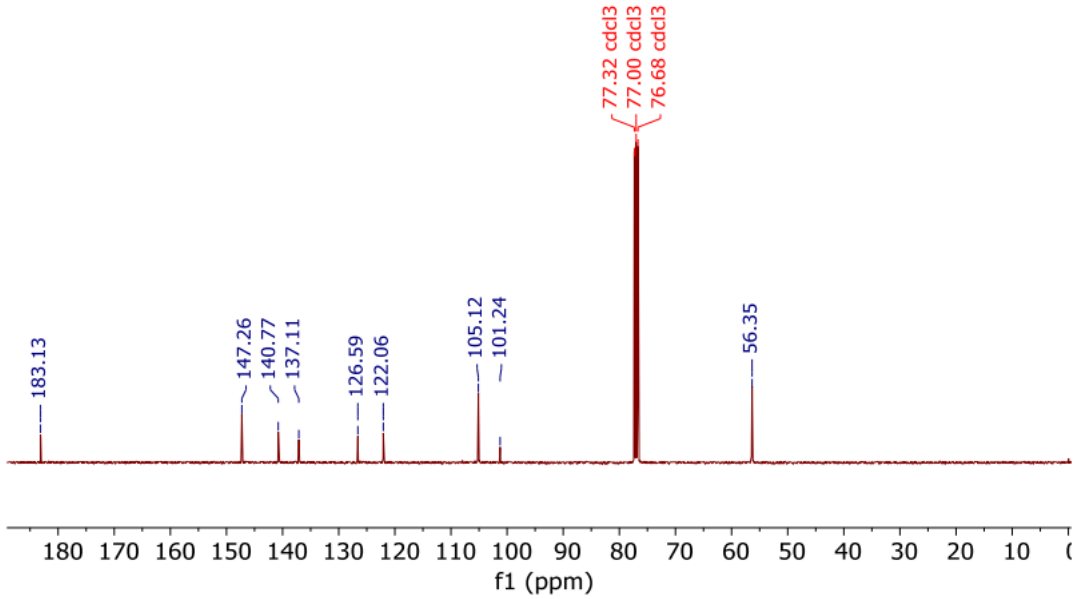
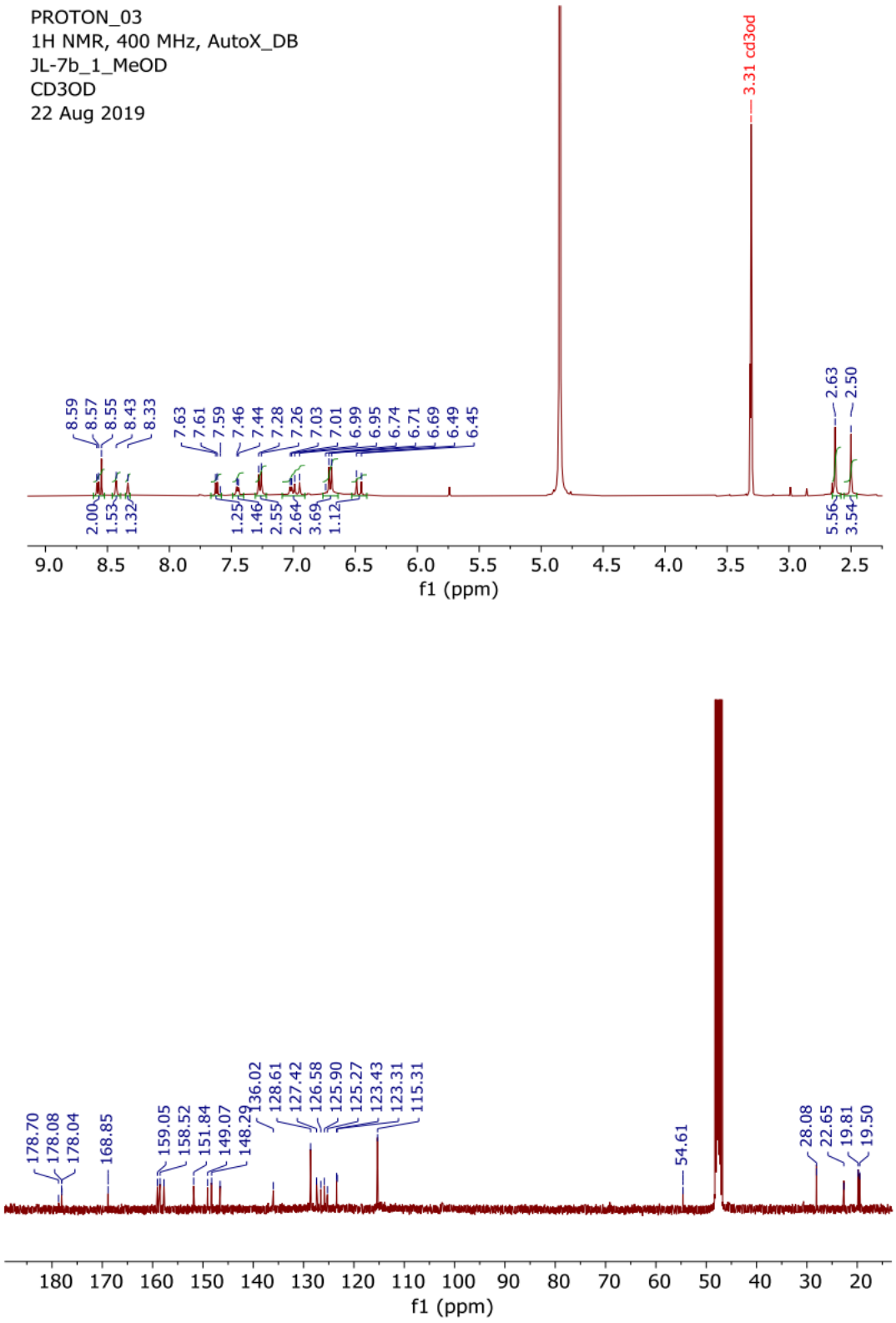
NM-167
CDCl3
10 Jun 2019



CARBON_01
13C NMR, 100 MHz, AutoX_DB
NM-167
CDCl3
11 Jun 2019

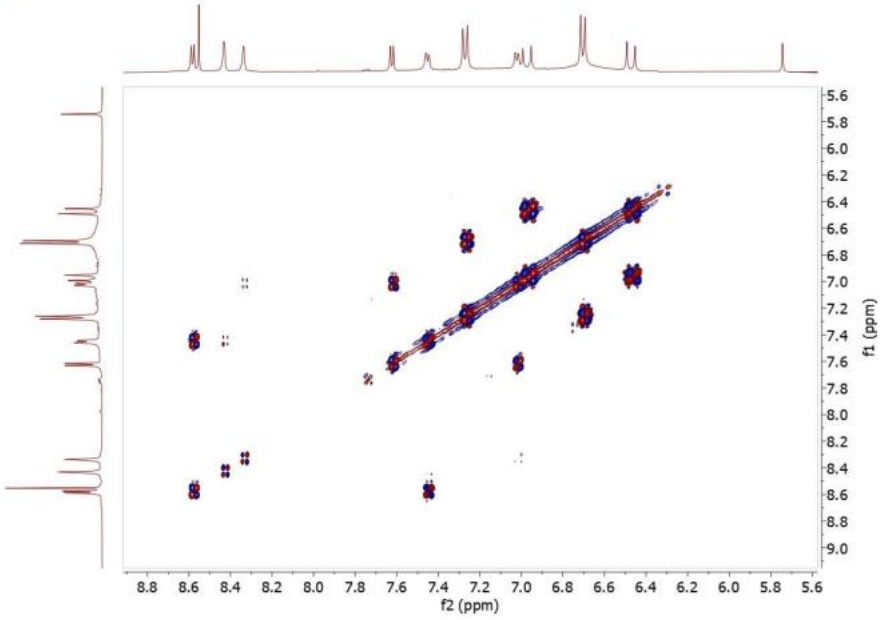


NMR Spectrum of complex 1

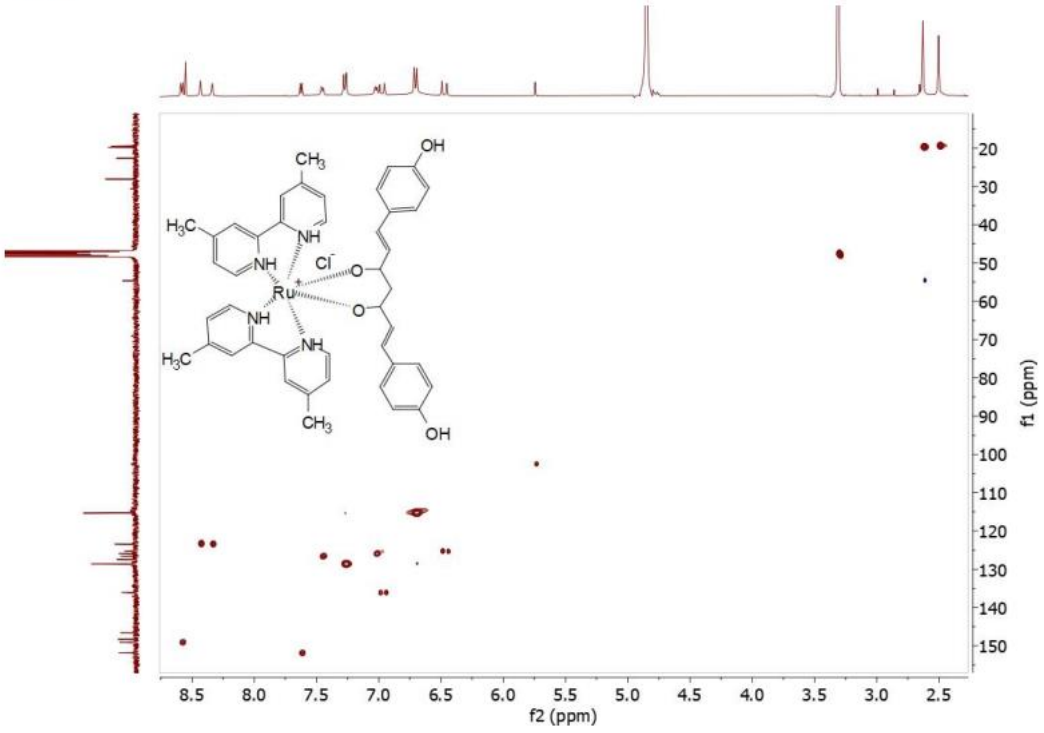


2D NMR measurements confirming the structure of complex **1**

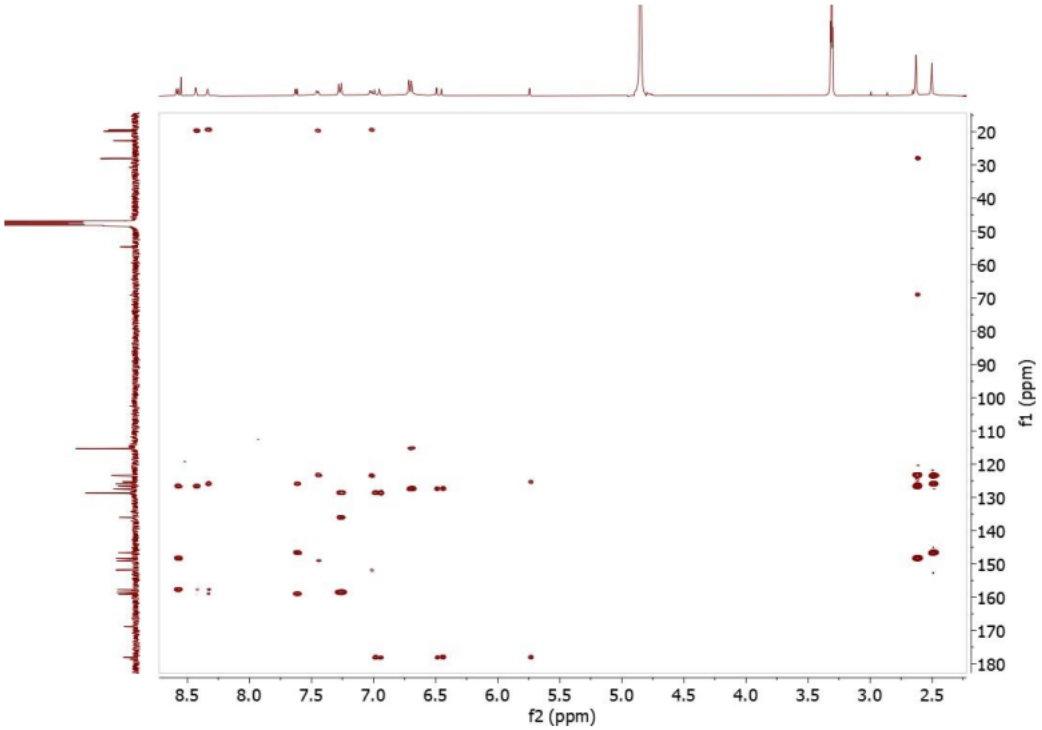
DQCOSY



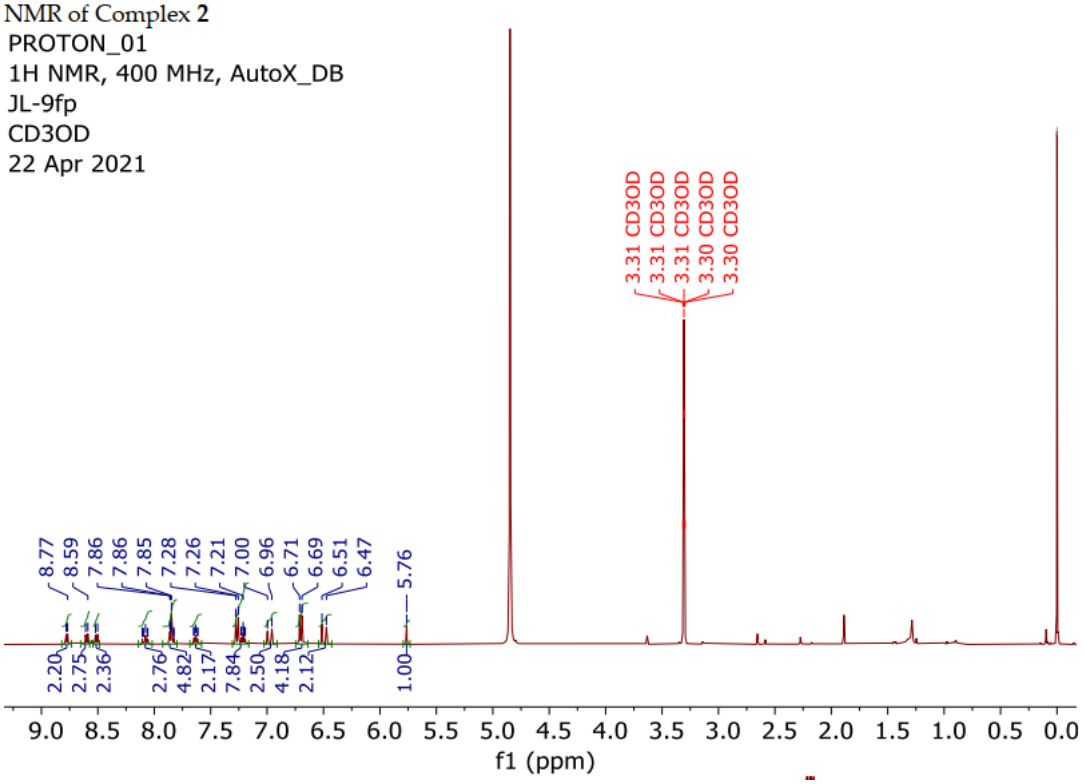
HSQC

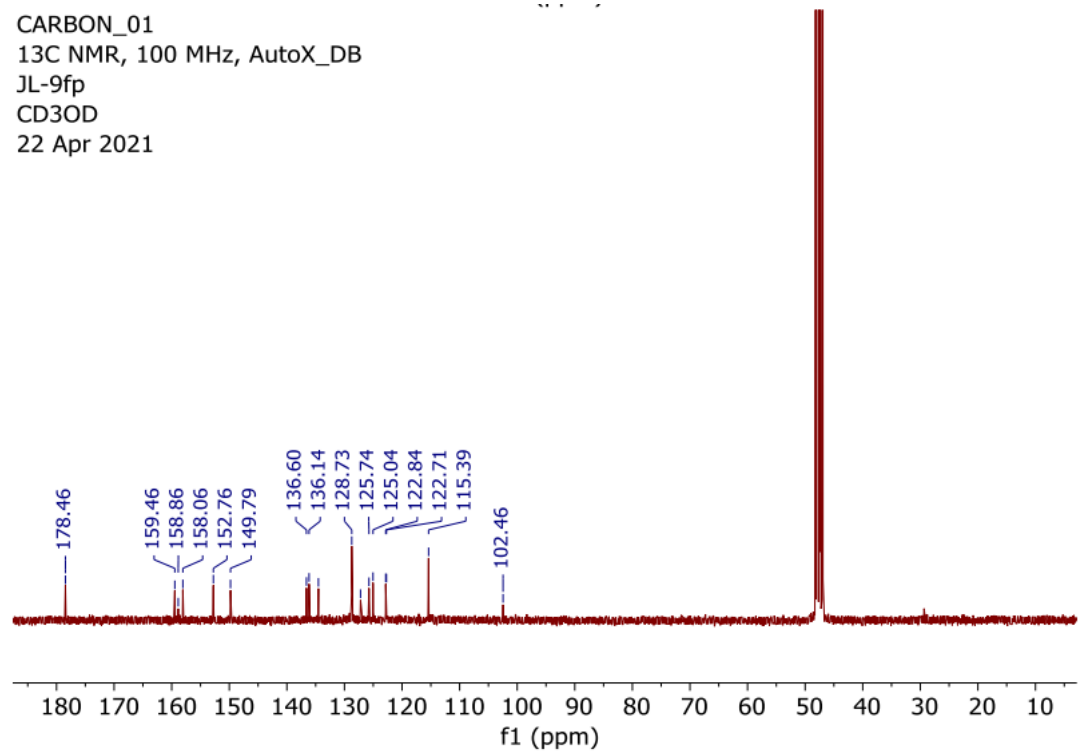


HMBC


20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
f1 (ppm)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f2 (ppm)

NMR of Complex **2**





NMR of Complex **3**

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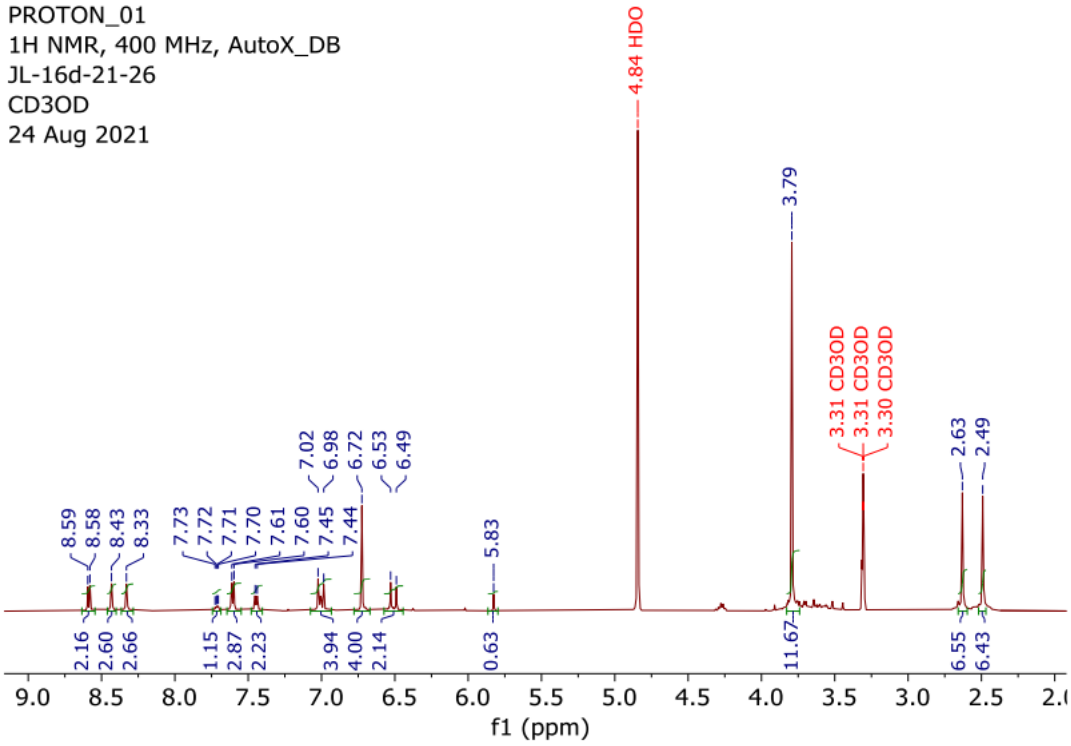
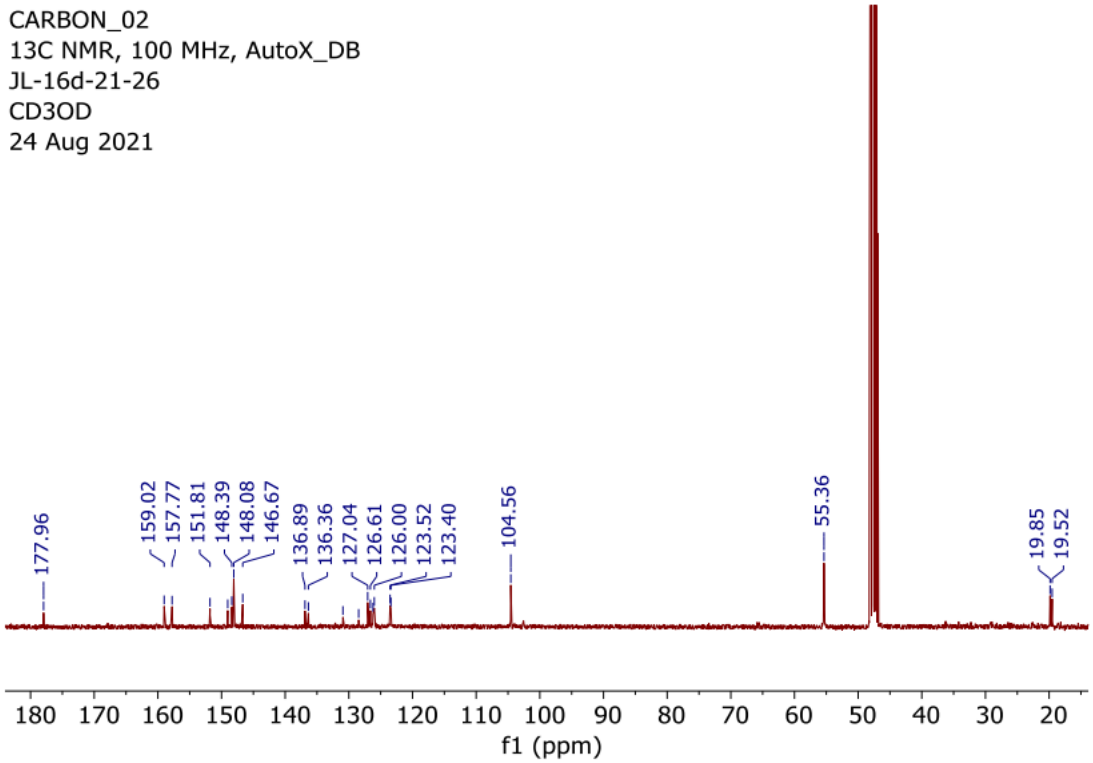
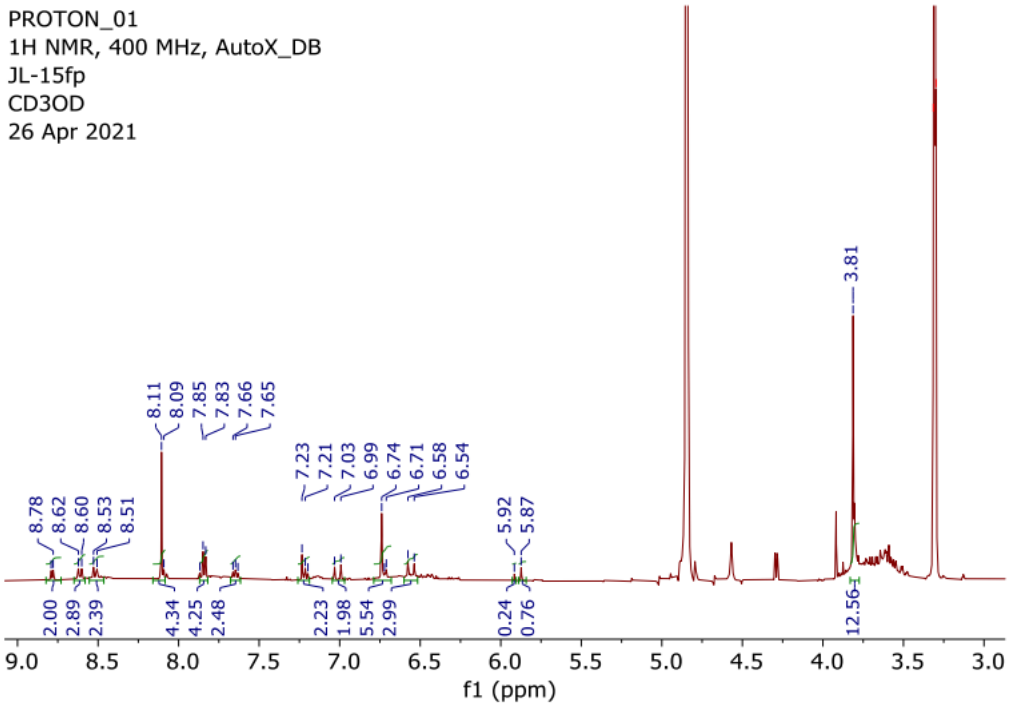
JL-16d-21-26
CD3OD
24 Aug 2021

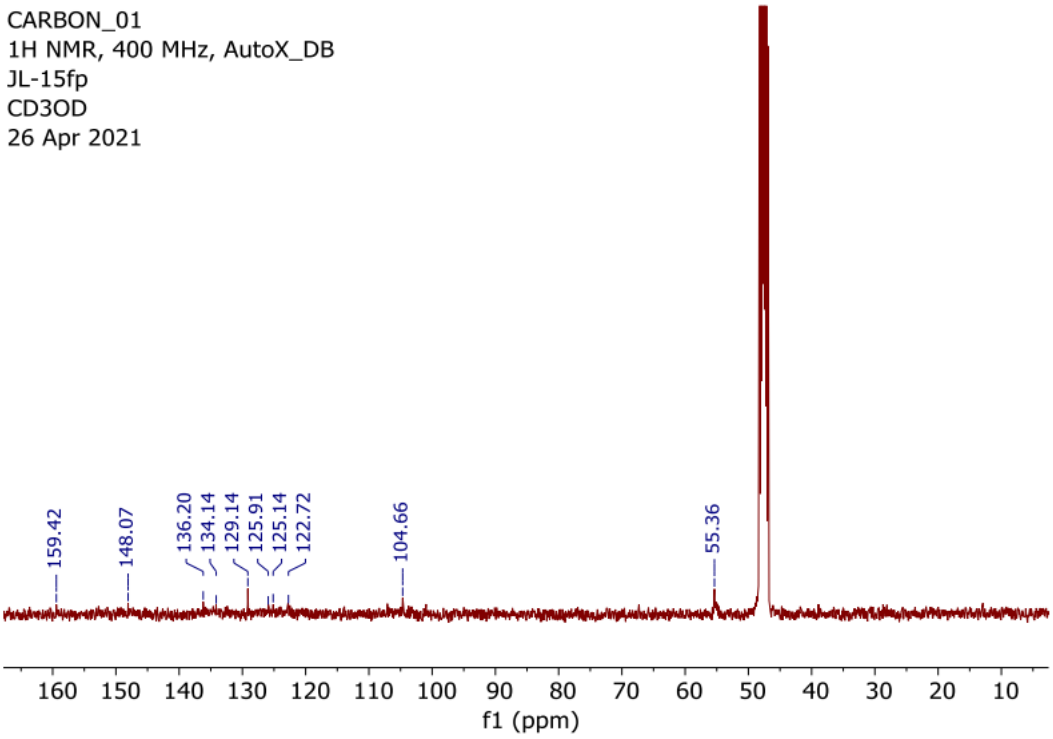


CARBON_02
13C NMR, 100 MHz, AutoX_DB
JL-16d-21-26
CD3OD
24 Aug 2021



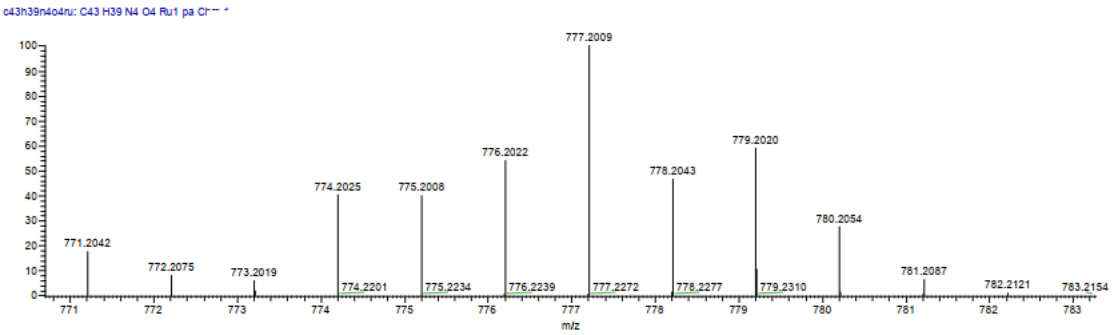
NMR of Complex 4

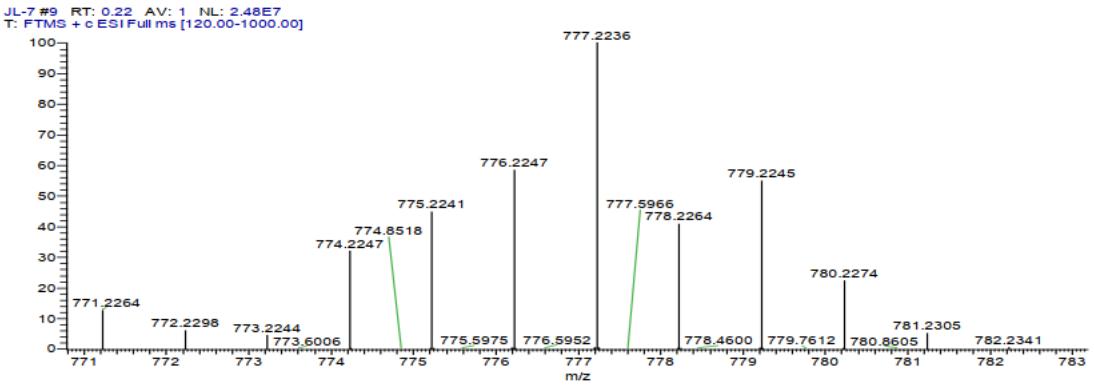


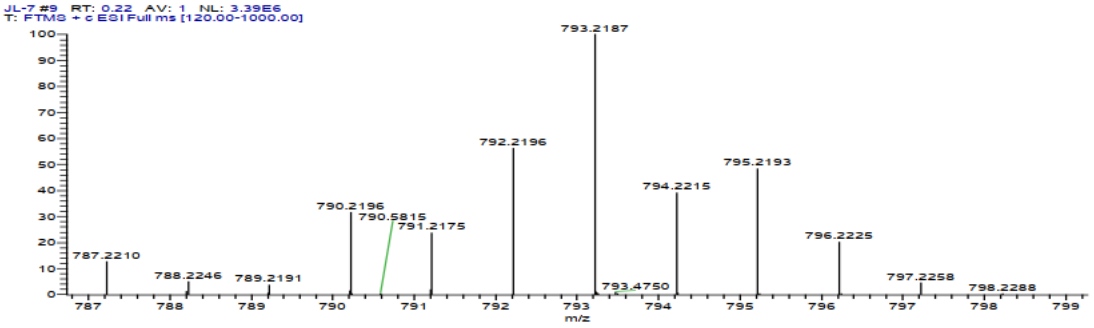


HR-MS data

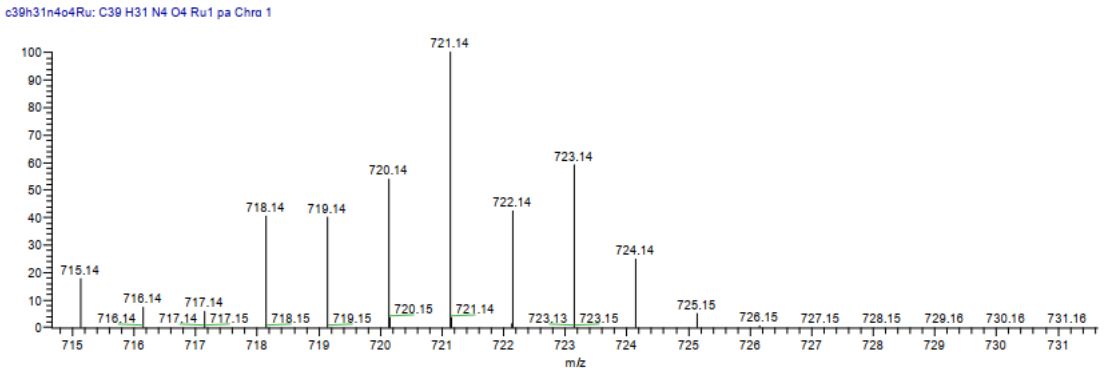
Complex 1

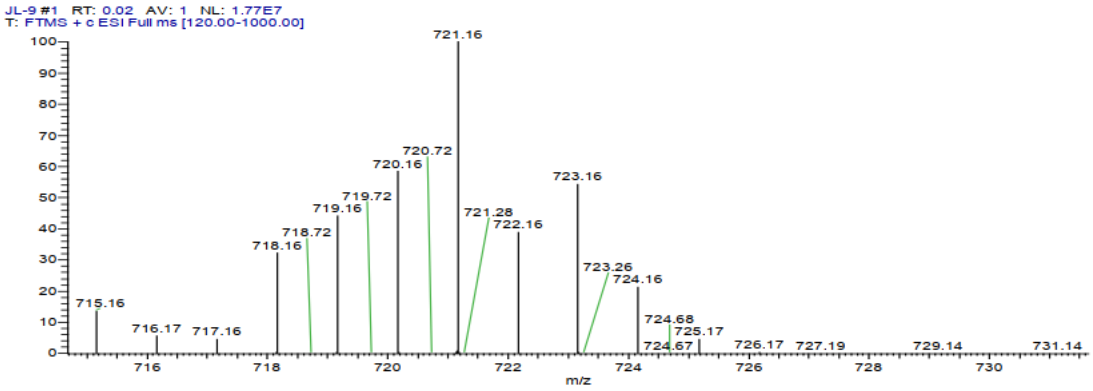




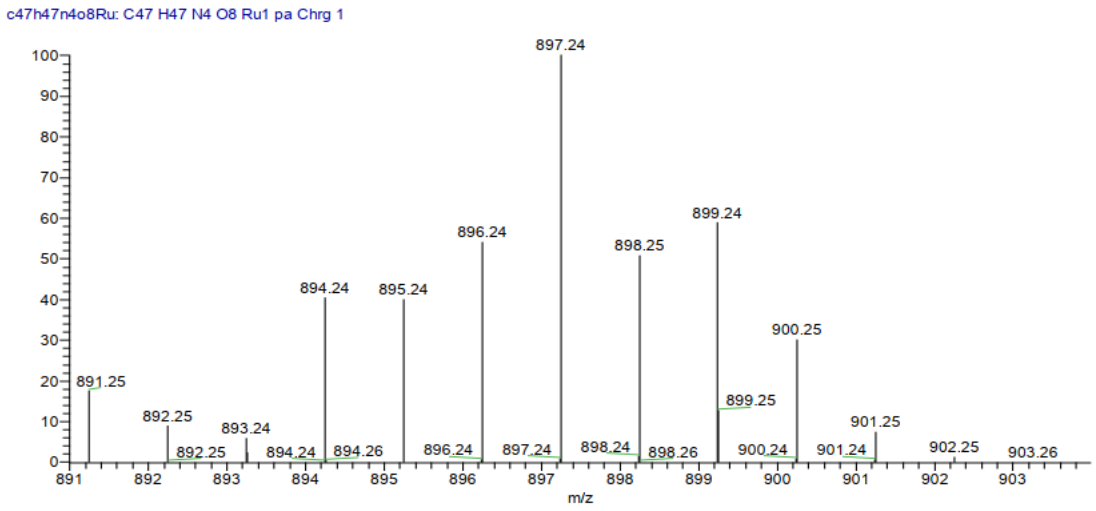


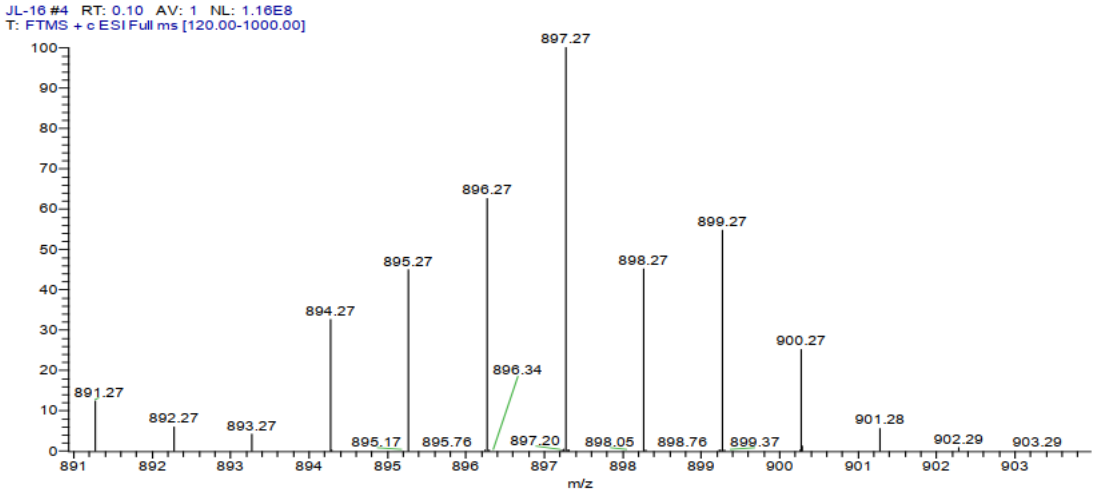
Complex 2


c39h31n4o4Ru: C39 H31 N4 O4 Ru1 pa Chrg 1
m/z

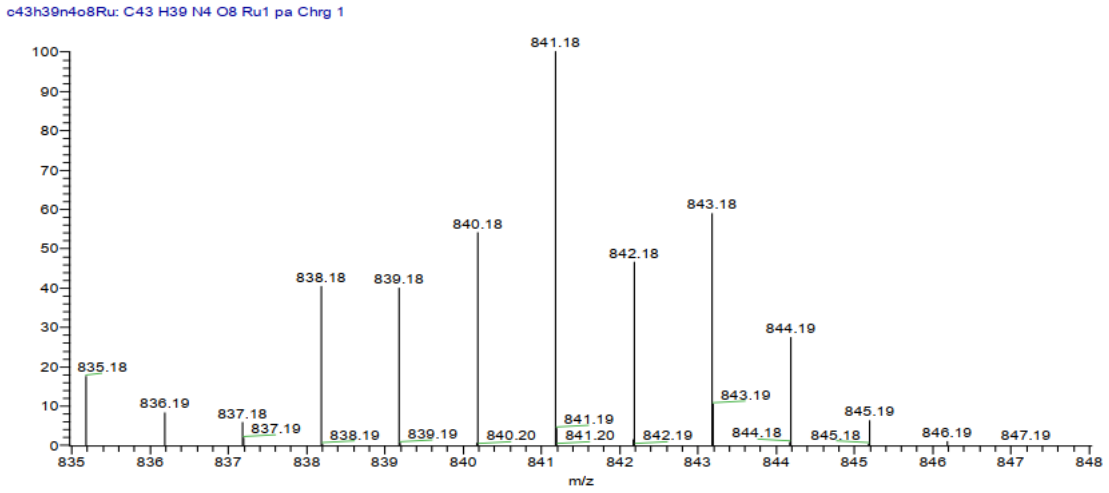

JL-9 #1 RT: 0.02 AV: 1 NL: 1.77E7
T: FTMS + c ESI Full ms [120.00-1000.00]
m/z

Complex 3


c47h47n4o8Ru: C47 H47 N4 O8 Ru1 pa Chrg 1
m/z


JL-16 #4 RT: 0.10 AV: 1 NL: 1.16E8
T: FTMS + c ESI Full ms [120.00-1000.00]
m/z

Complex 4


c43h39n4o8Ru: C43 H39 N4 O8 Ru1 pa Chrg 1
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840.18
843.18
842.18
838.18
839.18
844.19
835.18
843.19
836.19
845.19
837.18
837.19
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841.20
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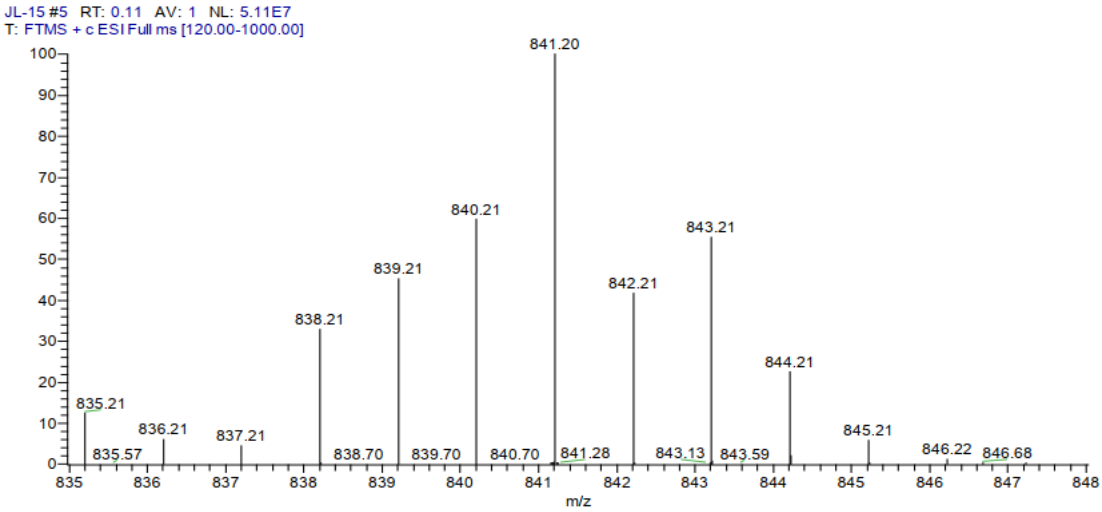
844.18
845.18
846.19
847.19
m/z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T: FTMS + c ESI Full ms [120.00-1000.00]
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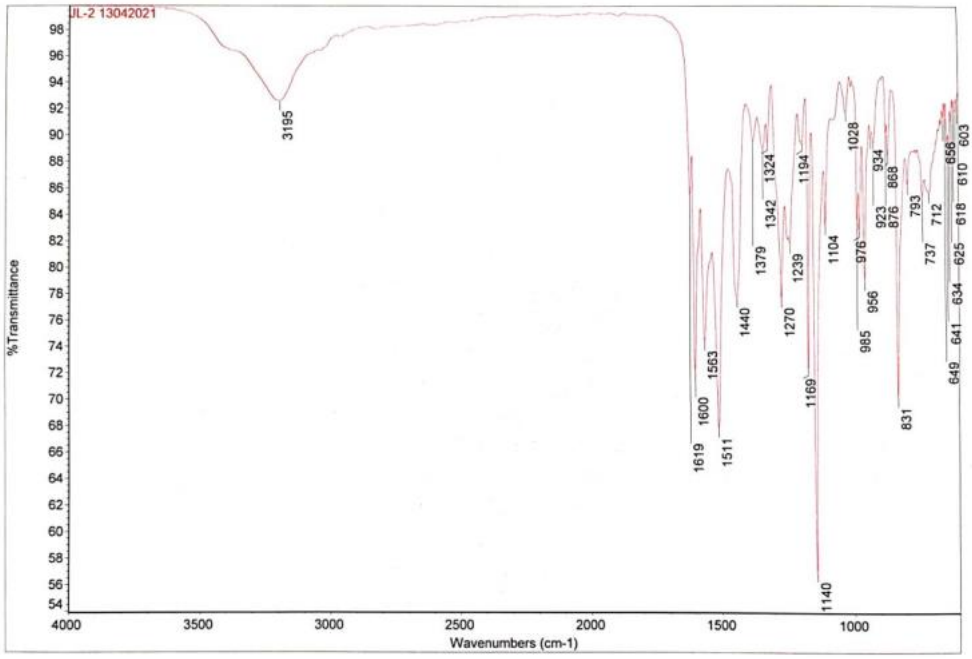
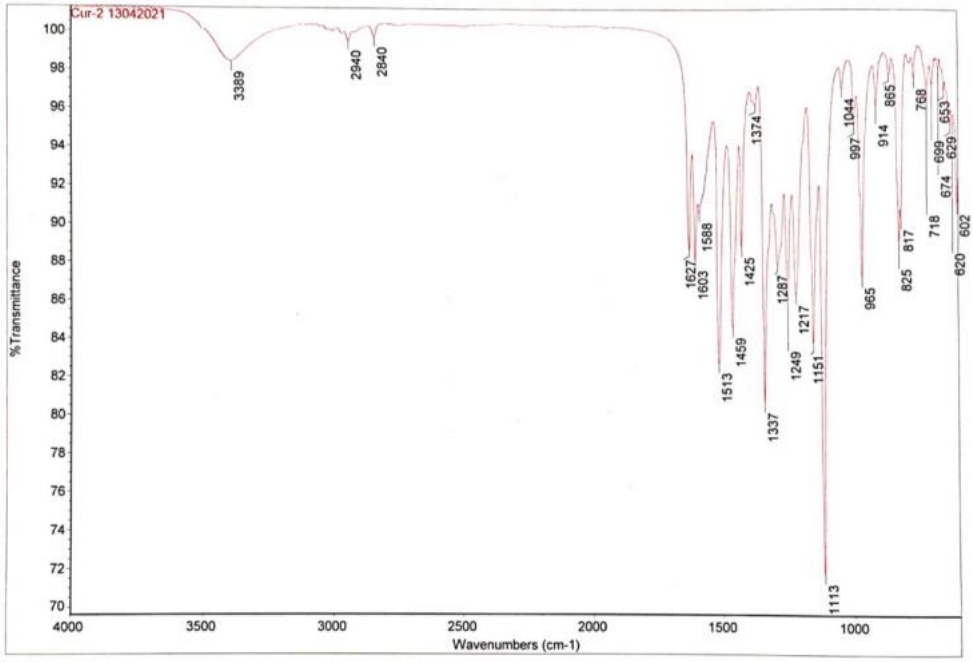
836.21
845.21
837.21
846.22
846.68
835.57
838.70
839.70
840.70
841.28
843.13
843.59
m/z

IR Spectra

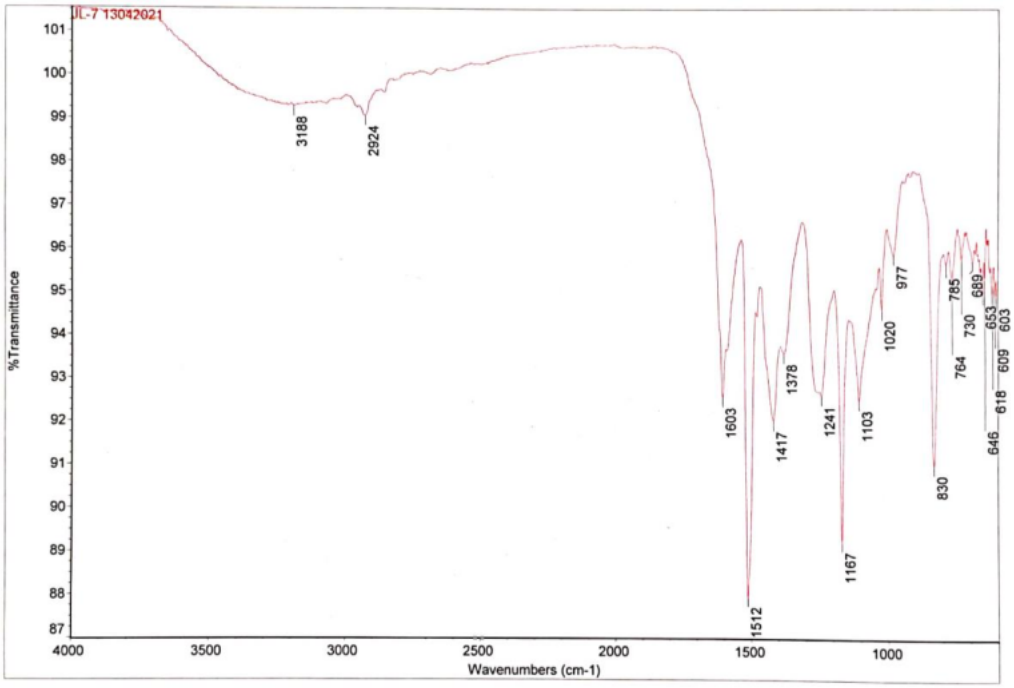
L1 - BDMC



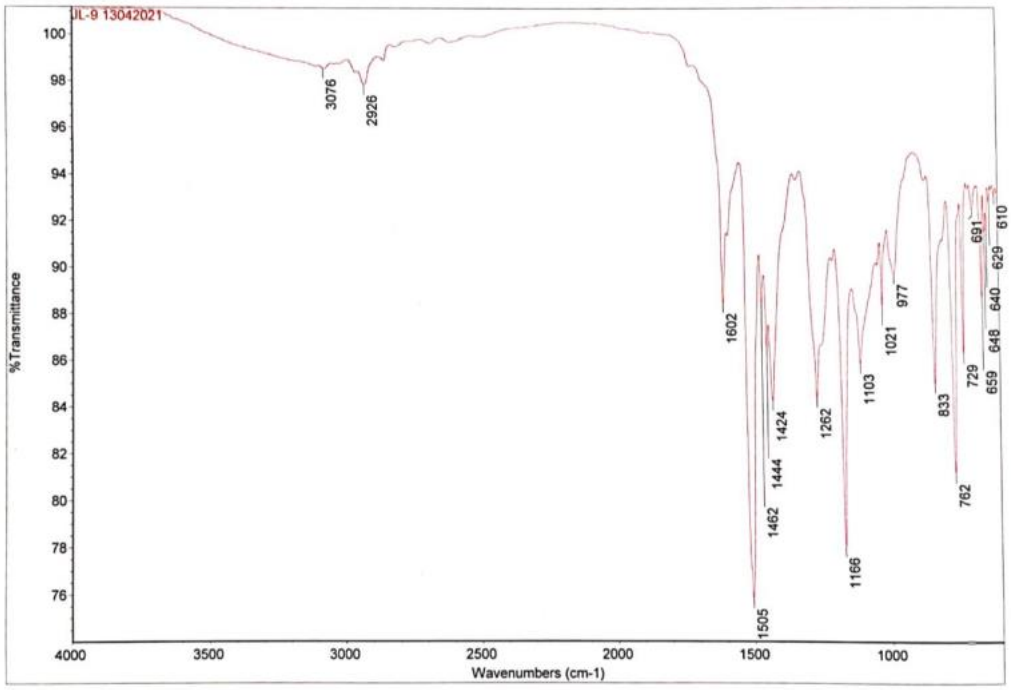
L2 - Syringaldehyde curcumin



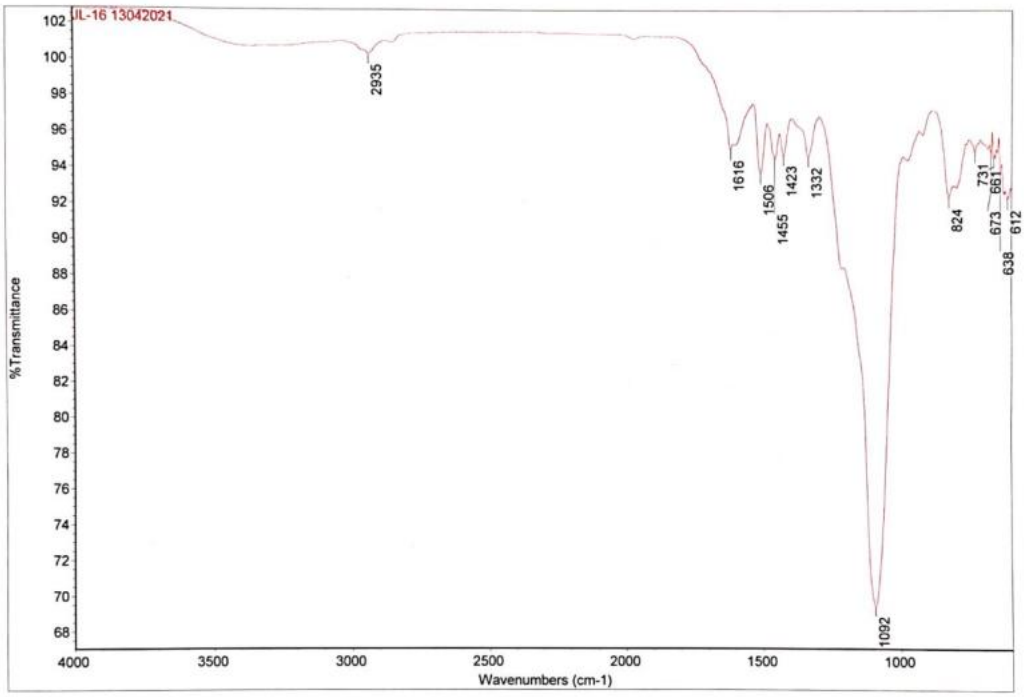
Complex **1**



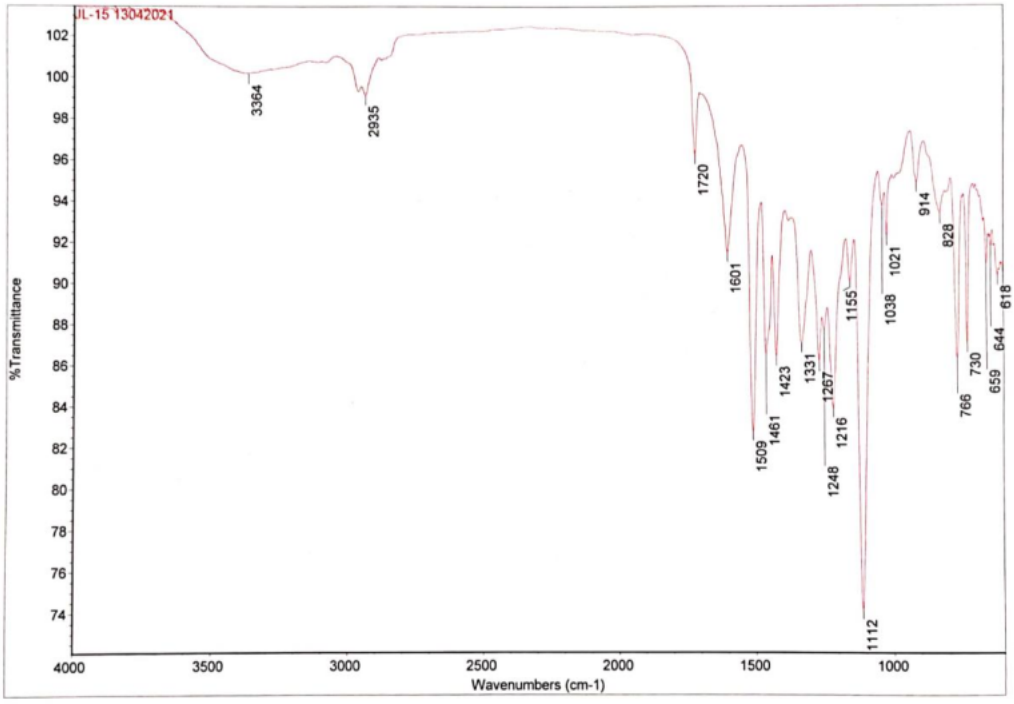
Complex **2**



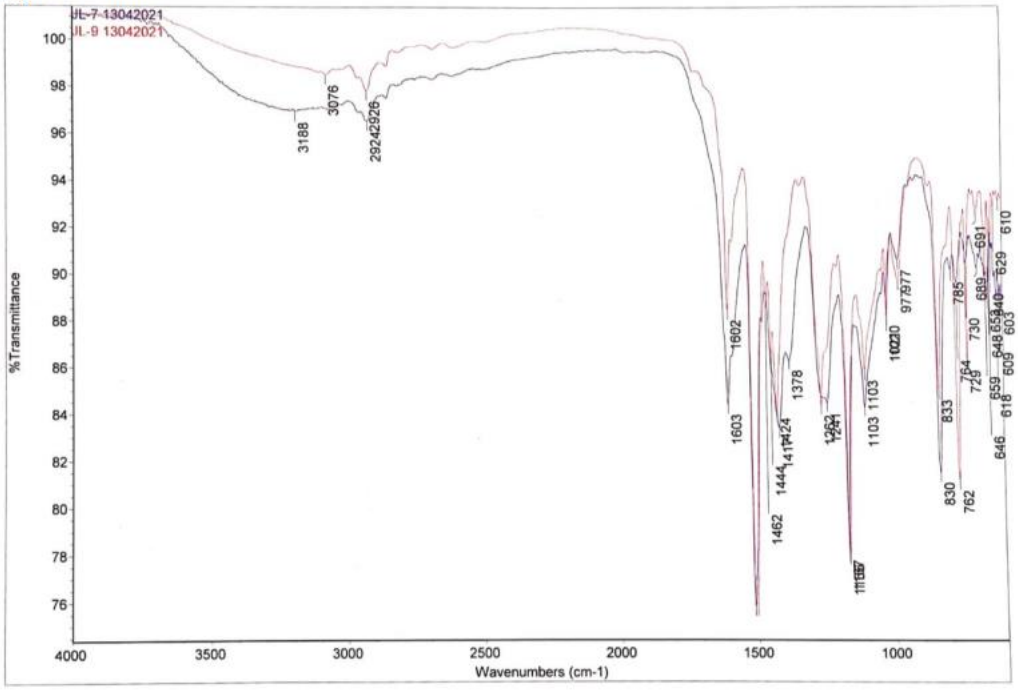
Complex **3**



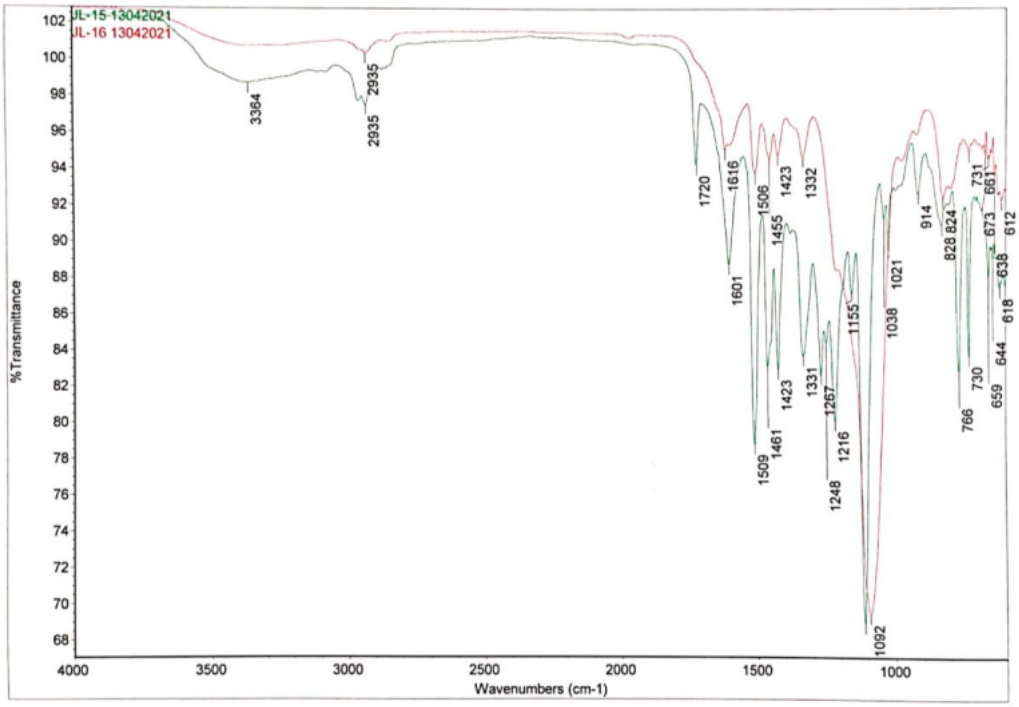
Complex **4**



Complexes **1** and **2**



Complexes **3** and **4**



Complexes **1** and **3**

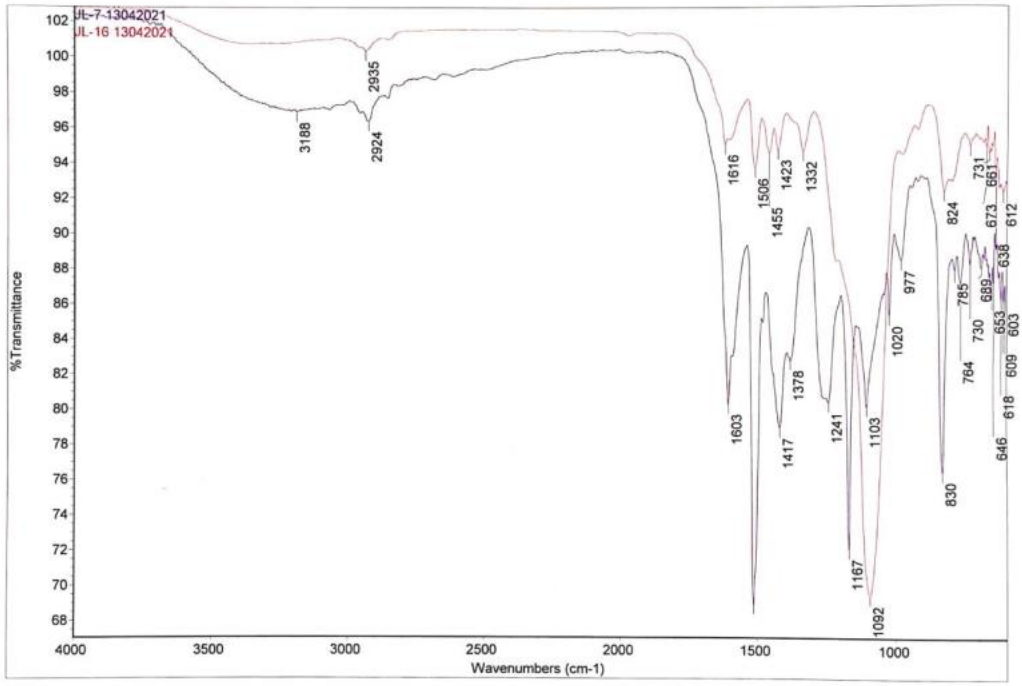
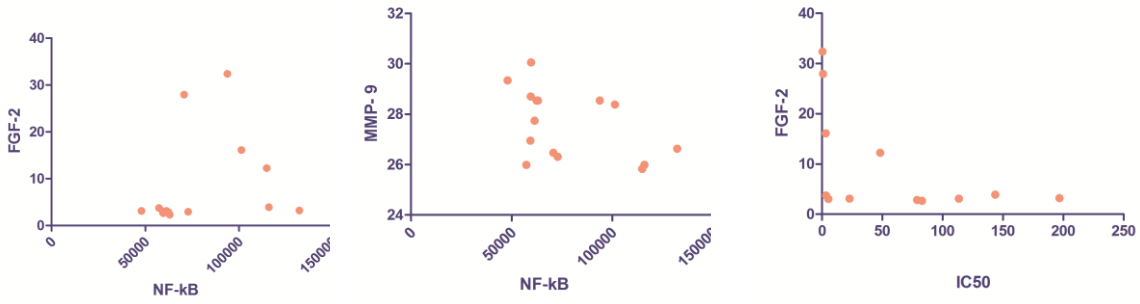


Table S1 Eigenvalues provided by the Principal component analysis, based on active variables NF-κB, FGF-2 and MMP-9, in correlation with IC50 values reflecting cytotoxicity.

Variable	Eigenvalue	% total variance	Cumulative Eigenvalue	Cumulative %
NF-κB	1.804357	45.10894	1.804357	45.1089
FGF-2	1.331217	33.28042	3.135574	78.3894
MMP-9	0.706521	17.66303	3.842095	96.0524
IC_{50}	0.157905	3.94761	4.000000	100.0000

Table S2 Correlation matrix of factor coordinates generated by PCA, corresponding to the variations of IC_{50}, NF-κB, FGF-2 and MMP-9 in A2780 cells following the time-dependent treatment with the series of novel synthesized ruthenium complexes **1-4**.

Variable	Factor 1	Factor 2	Factor 3	Factor 4
NF-κB	0.764701	0.506585	-0.325586	0.229341
FGF-2	-0.351237	0.881138	-0.241306	-0.204939
MMP-9	-0.630277	-0.387184	-0.668951	0.073109
IC_{50}	0.836046	-0.385063	-0.307882	-0.240753



(**a**) (**b**) (**c**)

Figure S1 The intracellular activated NF-κB p65 in treated A2780 cells (**a**) correlates well with FGF-2 secreted by the cells (nonparametric Spearman correlation coefficient r = 0.4665, p value 0.0398), and (**b**) increases inversely proportional to MMP-9 (r = -0.4592, p value 0.0425). As well, it is a significant negative correlation between (**c**) the compounds cytotoxicity and FGF-2
(r = -0.5604, p value 0.0290).

Stability studies of complexes 1-4 in methanol and stock solution (ethanol:water; 1:20)

Complex 1 in methanol

```
Operator:             PC19216812\hp
Created:              13. 6. 2022 11:45:22
Spectrophotometer:    GENESYS 10S UV-Vis
Serial number:        2L9P364001
Firmware:             4.003
Baseline:             13. 6. 2022 11:45:22
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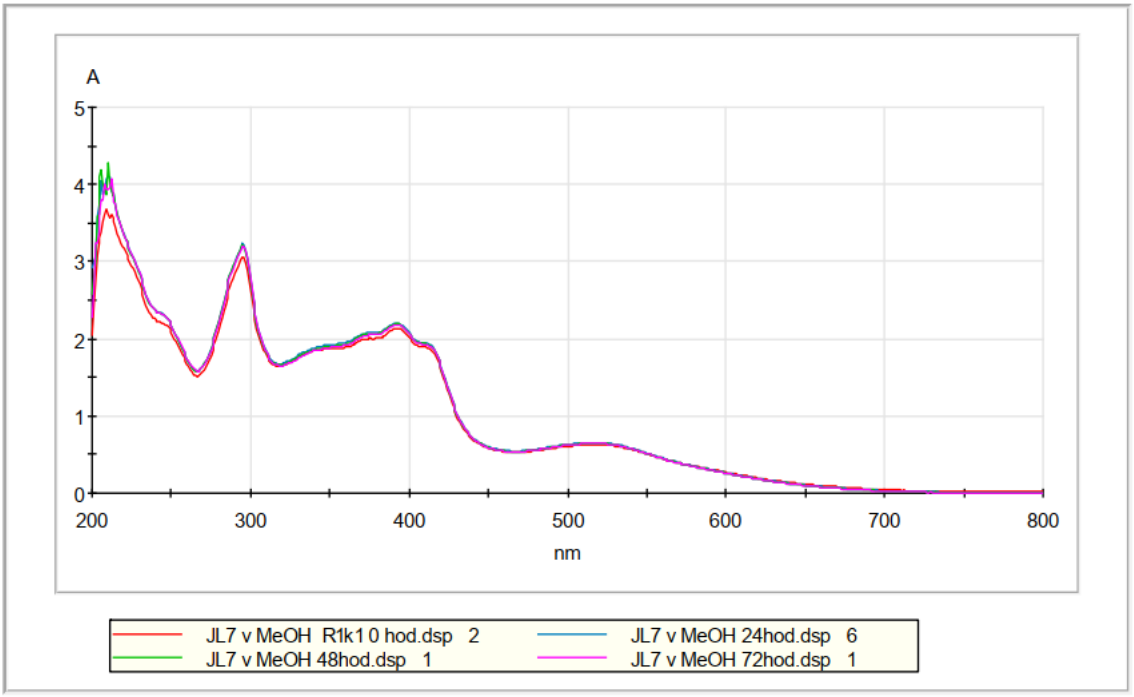

Complex 2 in Methanol

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Serial number:         2L9P364001
Firmware:              4.003
Baseline:              14. 6. 2022 11:55:03
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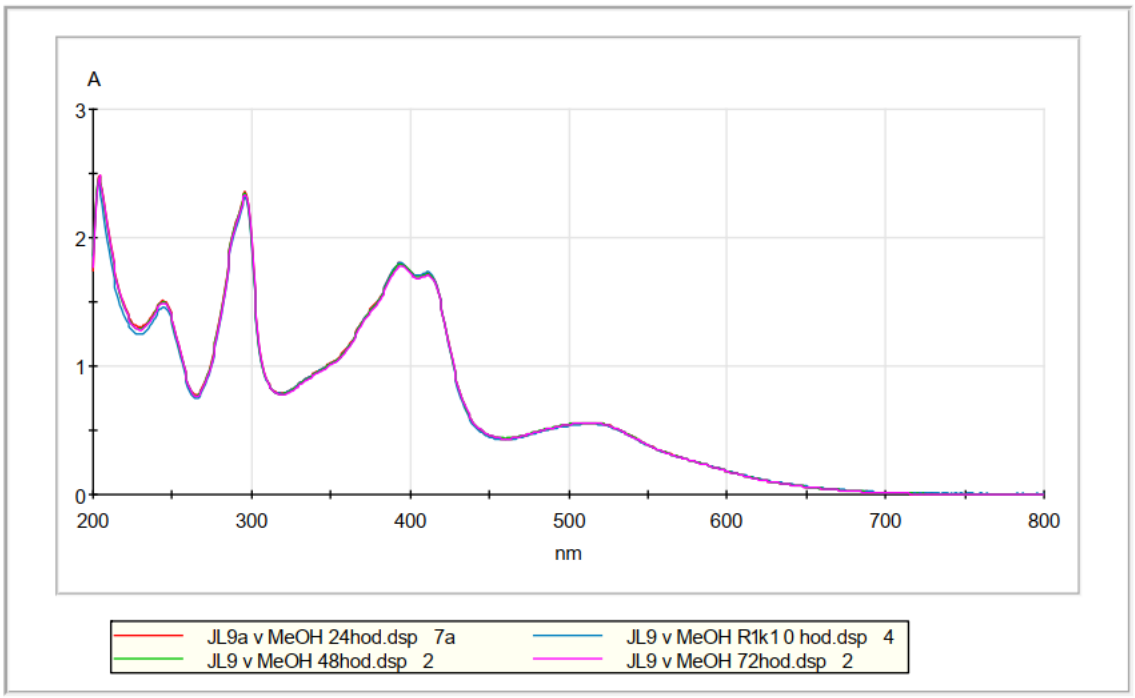

Complex 3 in Methanol

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Spectrophotometer:     GENESYS 10S UV-Vis
Serial number:         2L9P364001
Firmware:              4.003
Baseline:              13. 6. 2022 12:06:12
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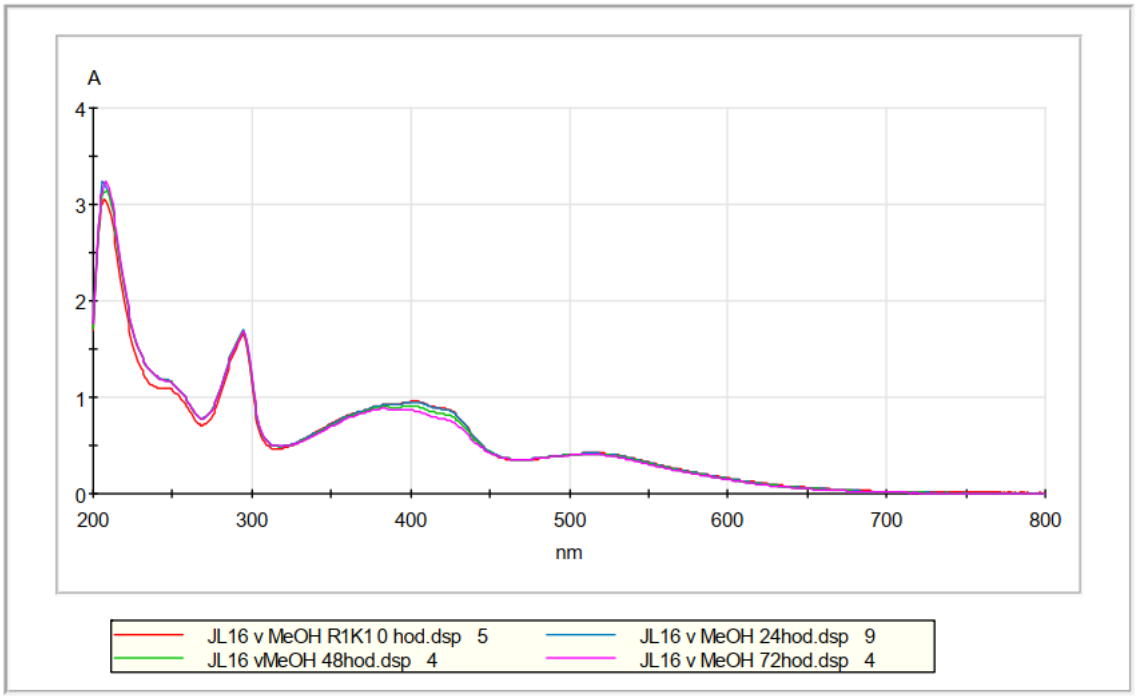

Complex 4 in Methanol

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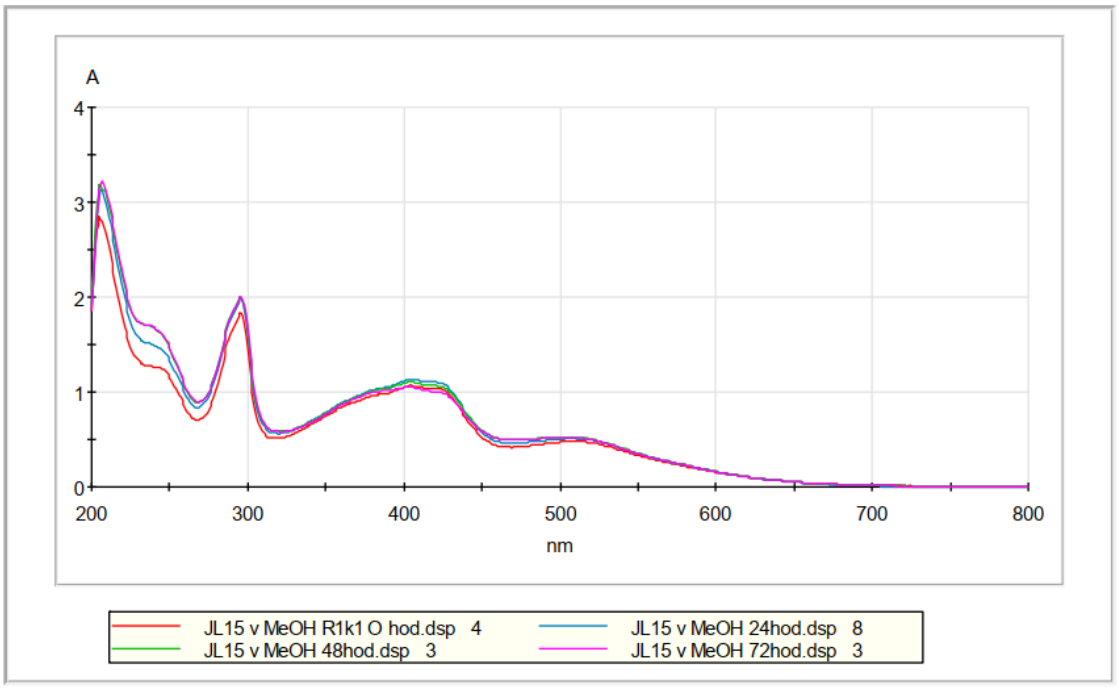

Complex 1 in ethanol + water

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Operator:              PC19216812\hp
Created:               13. 6. 2022 12:14:49
Spectrophotometer:     GENESYS 10S UV-Vis
Serial number:         2L9P364001
Firmware:              4.003
Baseline:              13. 6. 2022 12:14:49
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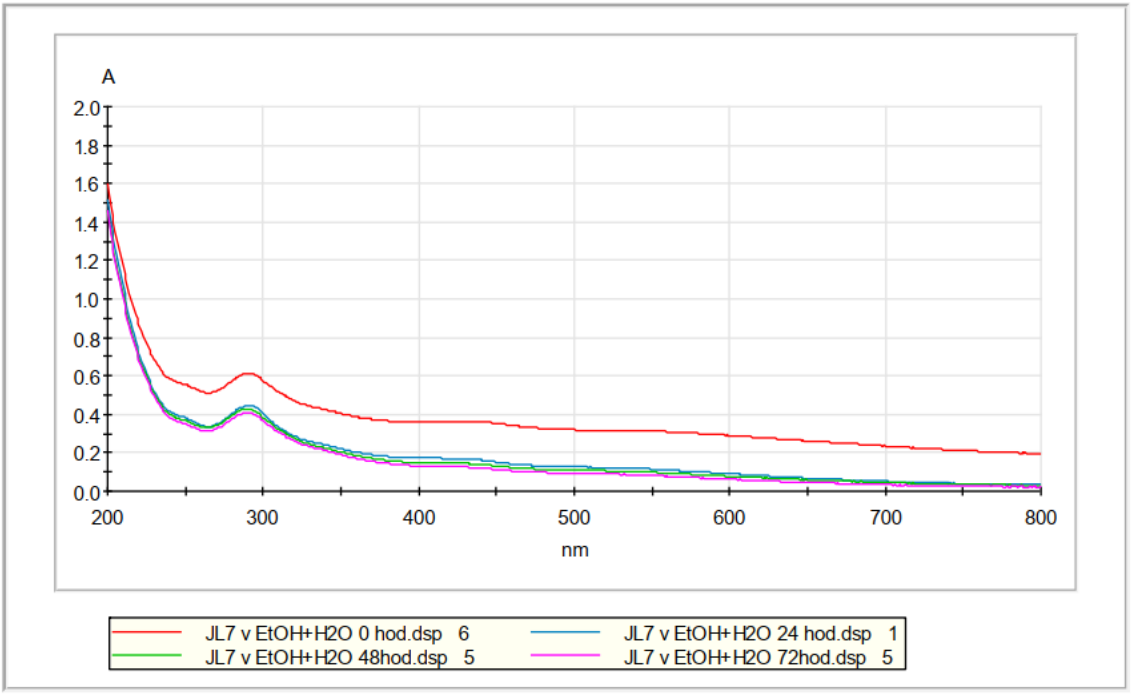

Complex 2 in ethanol + water

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Serial number:         2L9P364001
Firmware:              4.003
Baseline:              13. 6. 2022 12:26:36
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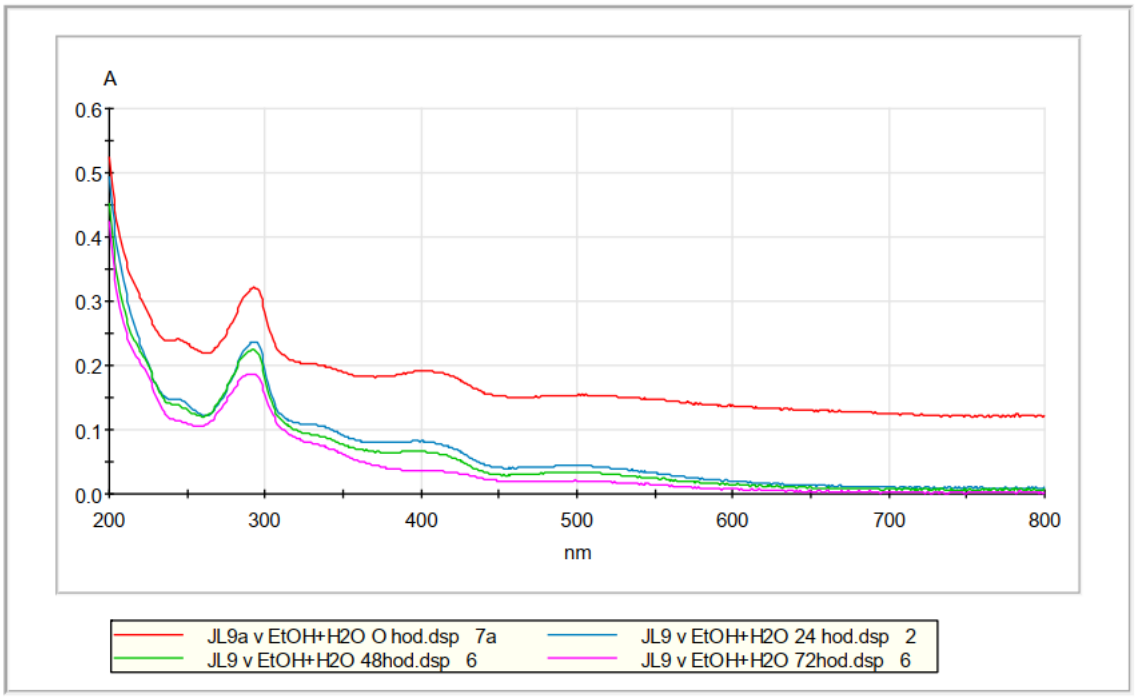

Complex 3 in ethanol + water

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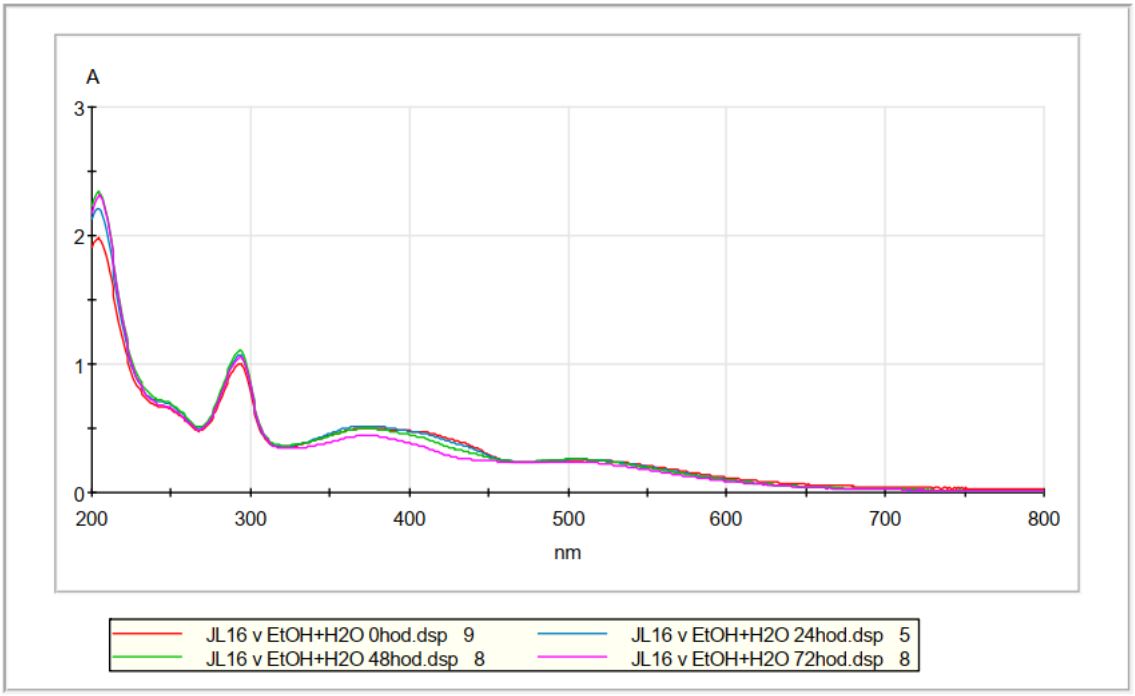

Complex 4 in ethanol + water

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Description:          8
Operator:             PC19216812\hp
Created:              13. 6. 2022 12:30:02
Spectrophotometer:    GENESYS 10S UV-Vis
Serial number:        2L9P364001
Firmware:             4.003
Baseline:             13. 6. 2022 12:30:02
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