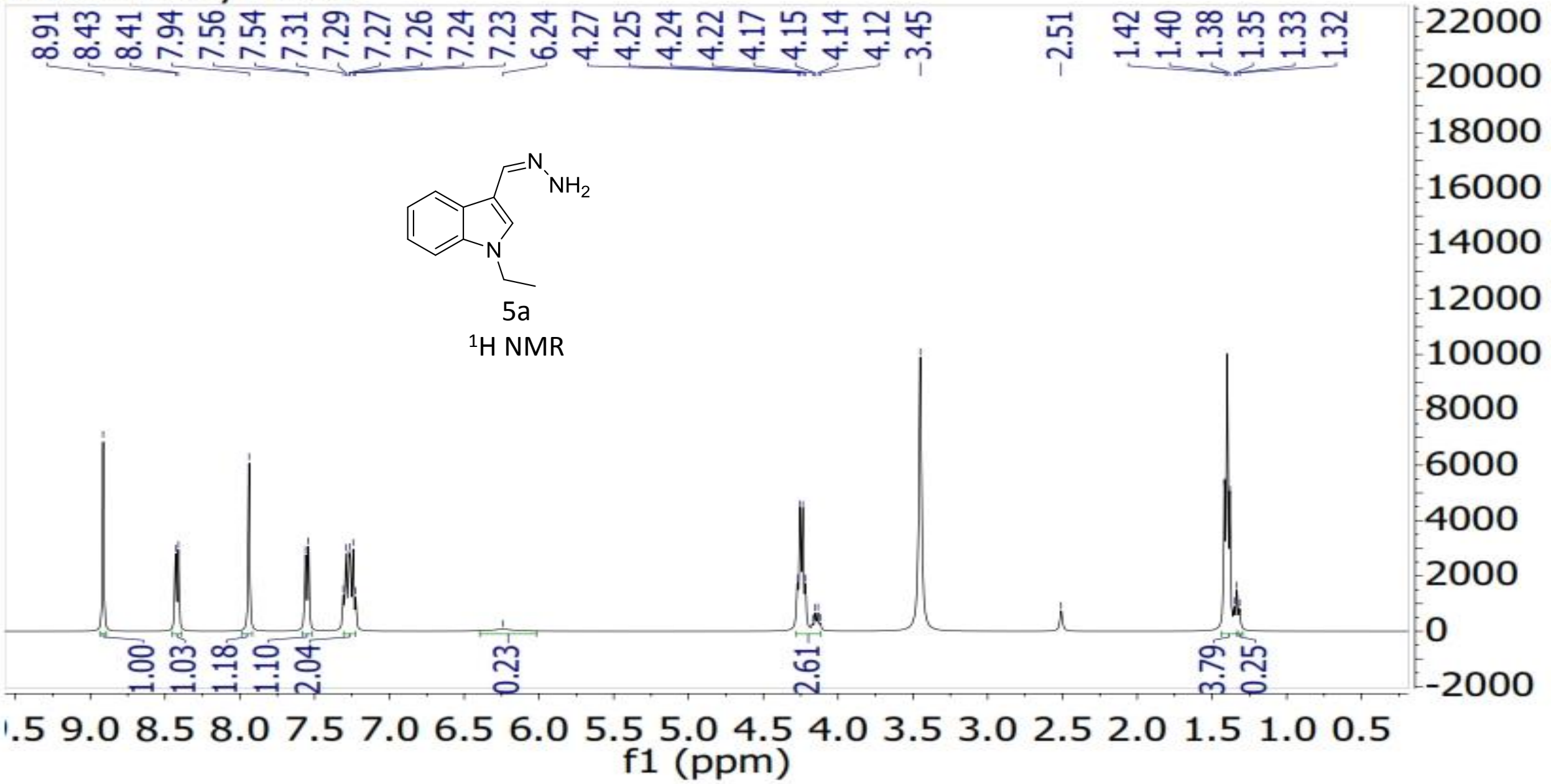
# Supporting Information

## Novel [(*N*-alkyl-3-indolylmethylene)hydrazono]oxindoles arrest cell cycle and induce cell apoptosis by inhibiting CDK2 and Bcl-2: Synthesis, biological evaluation and *in silico* studies

Tarfah Al-Warhi, Mahmoud F. Abo-Ashour, Hadia Almahli, Ohoud J. Alotaibi, Mohammad M. Al-Sanea, Ghada H. Al-Ansary, Hanaa Y. Ahmed, Mahmoud M. Elaasser, Wagdy M. Eldehna*, Hatem A. Abdel-Aziz

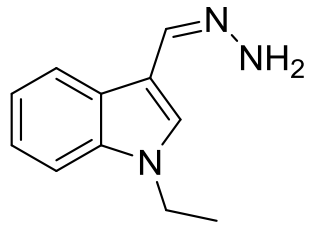
MEE-St-ethyl.1.fid —




5a

$^{1}$H NMR

MEE-St-ethyl.1.fid —

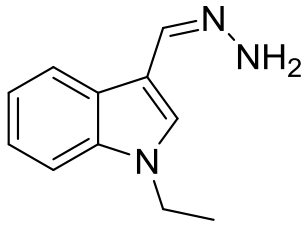



5a

$^{1}$H NMR

Aliphatic Zoom

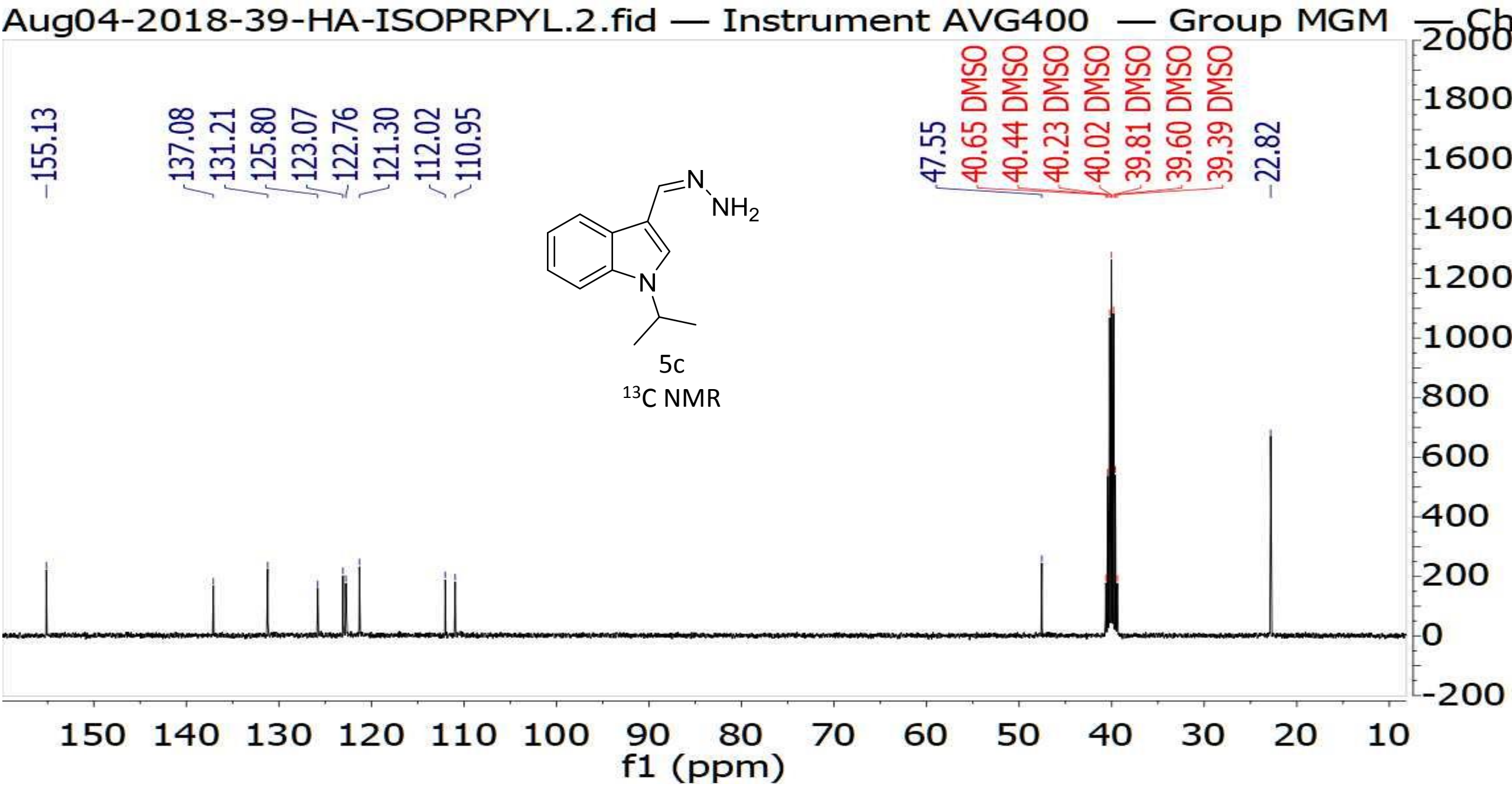
MEE-St-ethyl.1.fid —




5a

$^{1}$H NMR

Aromatic Zoom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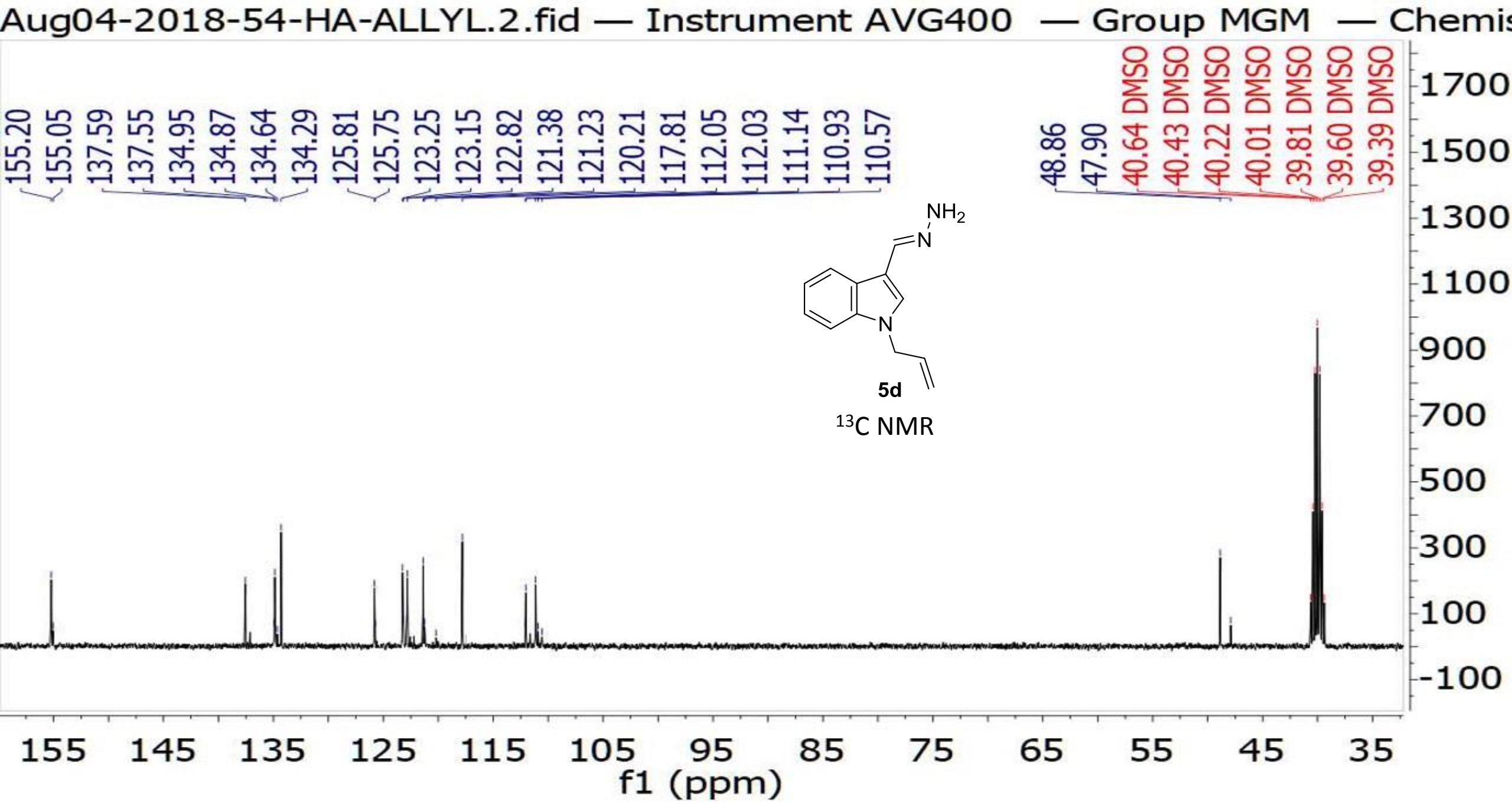
155.13
137.08
131.21
125.80
123.07
122.76
121.30
112.02
110.95
47.55
40.65 DMSO
40.44 DMSO
40.23 DMSO
40.02 DMSO
39.81 DMSO
39.60 DMSO
39.39 DMSO
22.82
N
NH2
N
5c
13C NMR
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)


Aug04-2018-54-HA-ALLYL.2.fid — Instrument AVG400 — Group MGM — Chemis
155.20
155.05
137.59
137.55
134.95
134.87
134.64
134.29
125.81
125.75
123.25
123.15
122.82
121.38
121.23
120.21
117.81
112.05
112.03
111.14
110.93
110.57
48.86
47.90
40.64 DMSO
40.43 DMSO
40.22 DMSO
40.01 DMSO
39.81 DMSO
39.60 DMSO
39.39 DMSO
NH2
N
N
5d
13C NMR
1700
1500
1300
1100
900
700
500
300
100
-100
155
145
135
125
115
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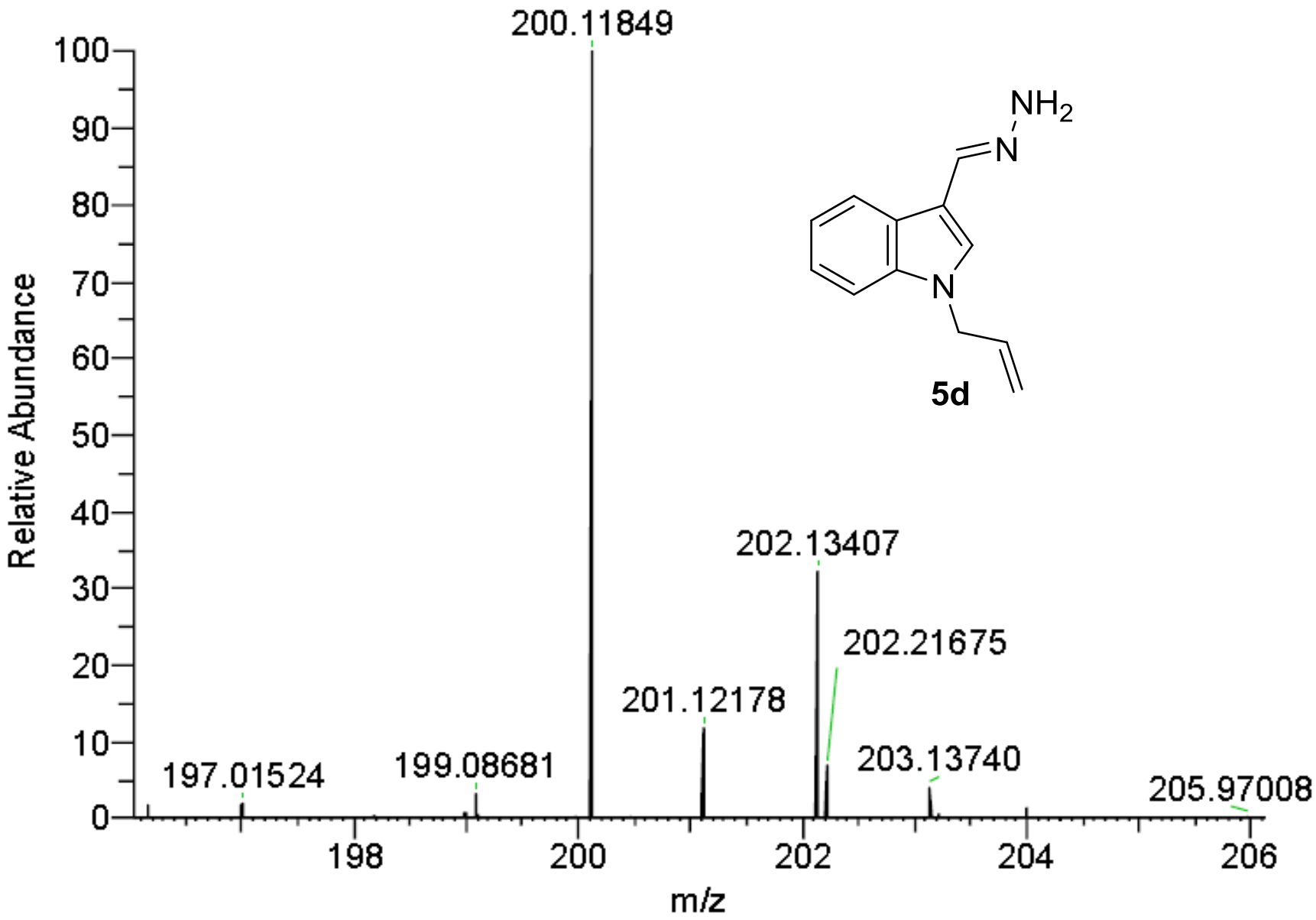
95
85
75
65
55
45
35
f1 (ppm)



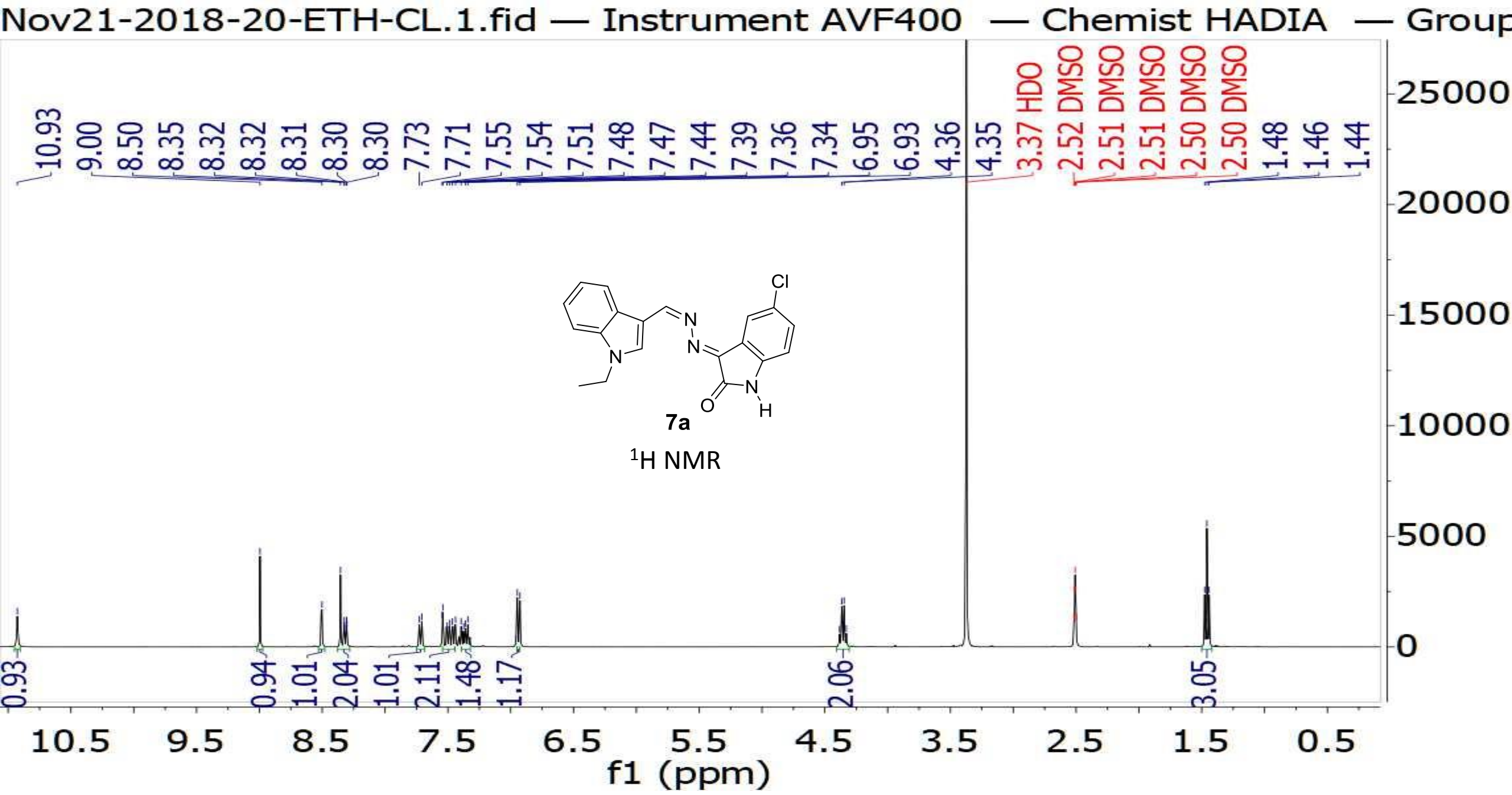




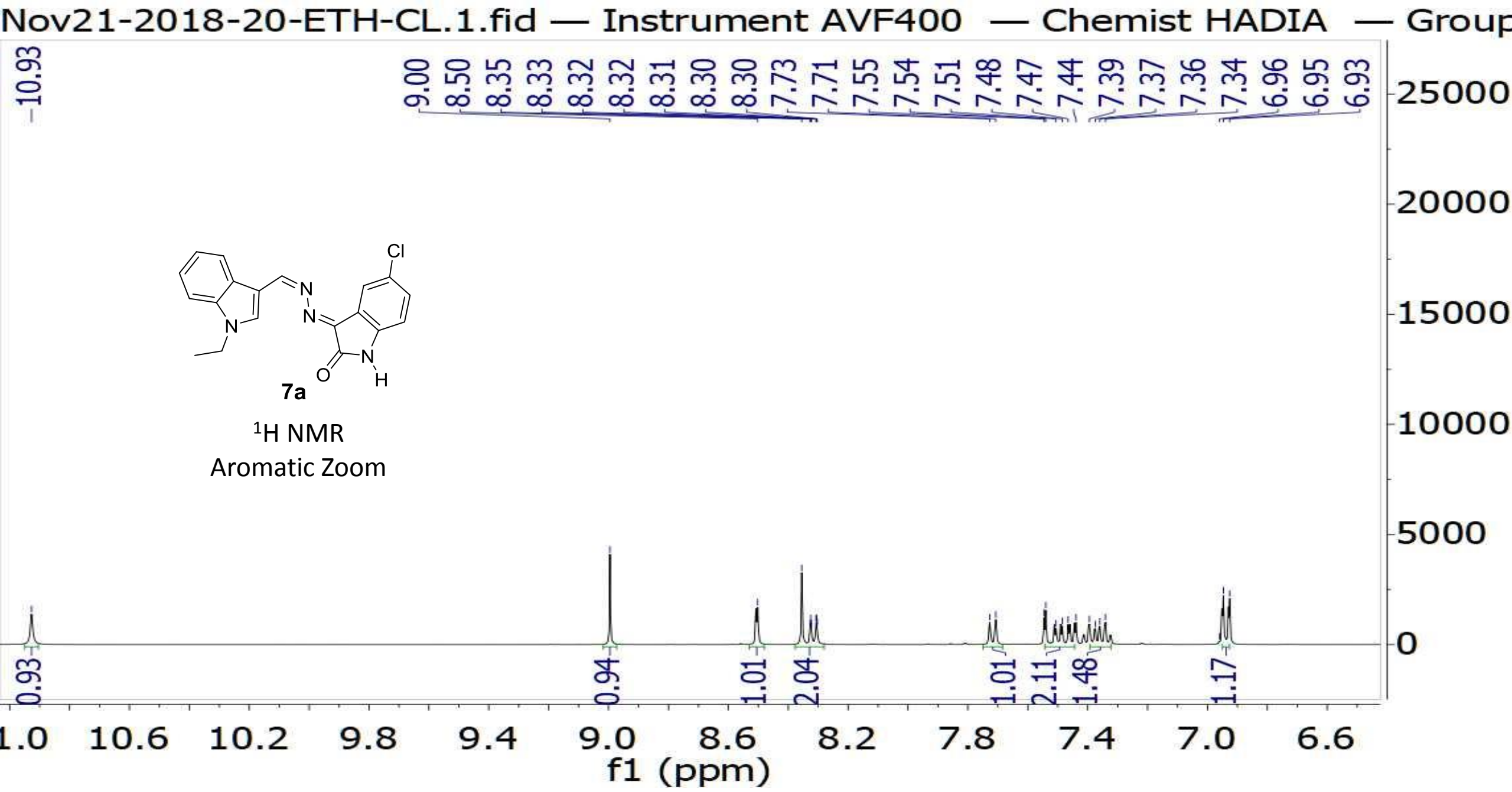
**5d**

NL: 1.12E6
ESI71805 #14-27 RT: 0.17-0.31 AV: 7 NL: 2.66E+007
T: FTMS {1,1} + p ESI Full ms [80.00-1600.00]

Measured Spectrum

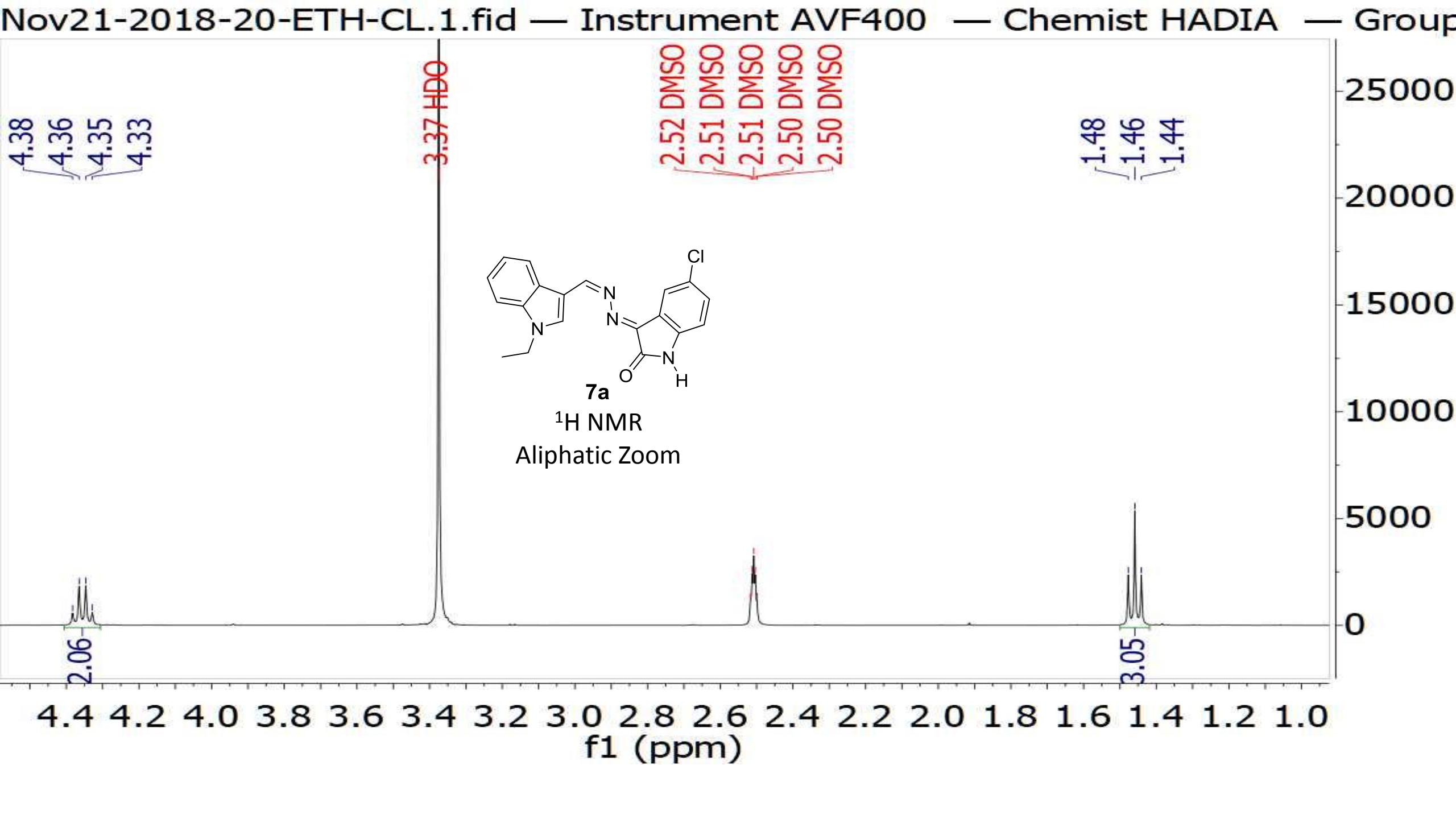

Nov21-2018-20-ETH-CL.1.fid — Instrument AVF400 — Chemist HADIA — Group
10.93
9.00
8.50
8.35
8.32
8.32
8.31
8.30
8.30
7.73
7.71
7.55
7.54
7.51
7.48
7.47
7.44
7.39
7.36
7.34
6.95
6.93
4.36
4.35
3.37 HDO
2.52 DMSO
2.51 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
1.48
1.46
1.44
Cl
N
N
N
O
N
H
7a
0.93
0.94
1.01
2.04
1.01
2.11
1.48
1.17
2.06
3.05
25000
20000
15000
10000
5000
0
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
f1 (ppm)

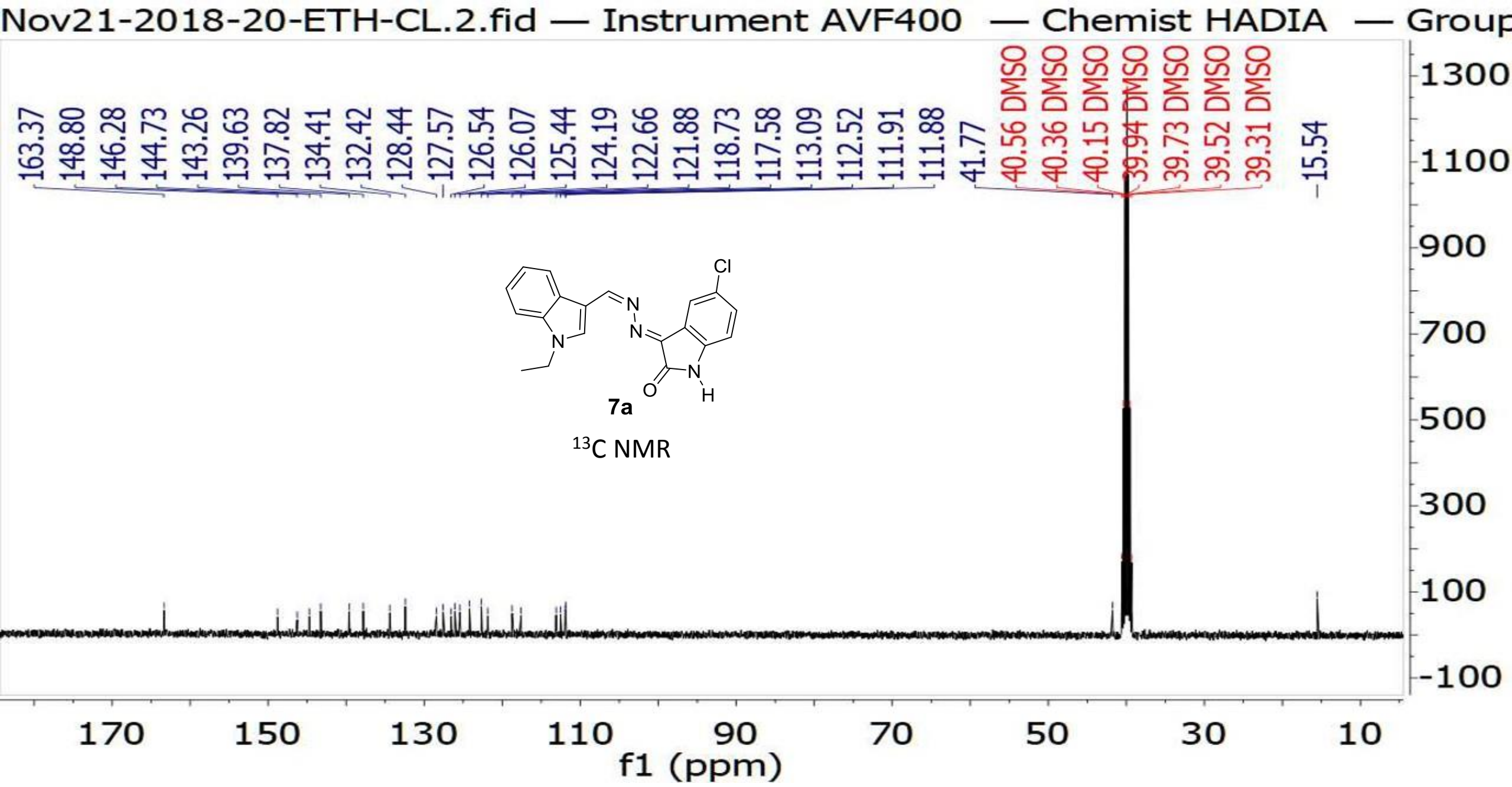
$^{1}$H NMR

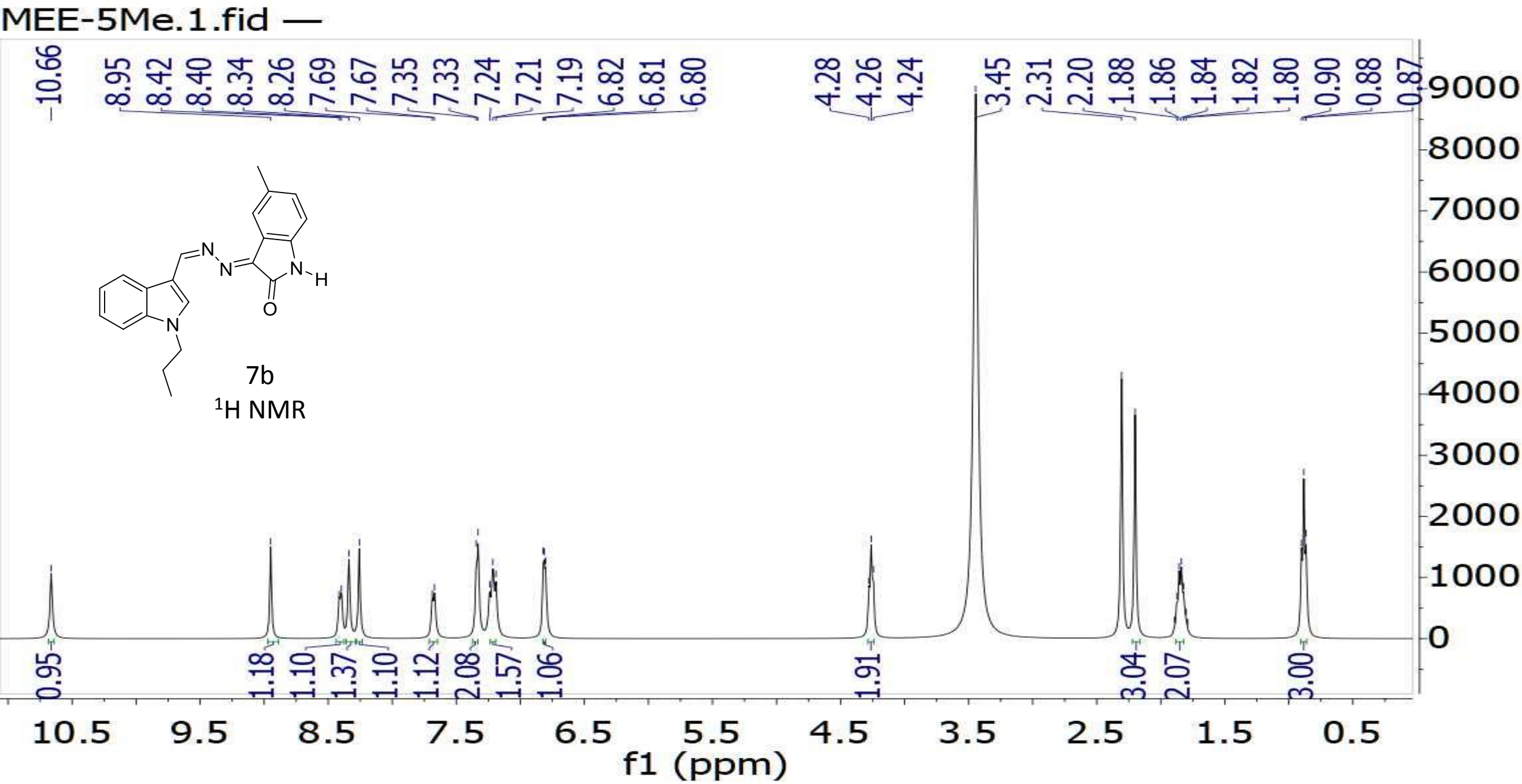


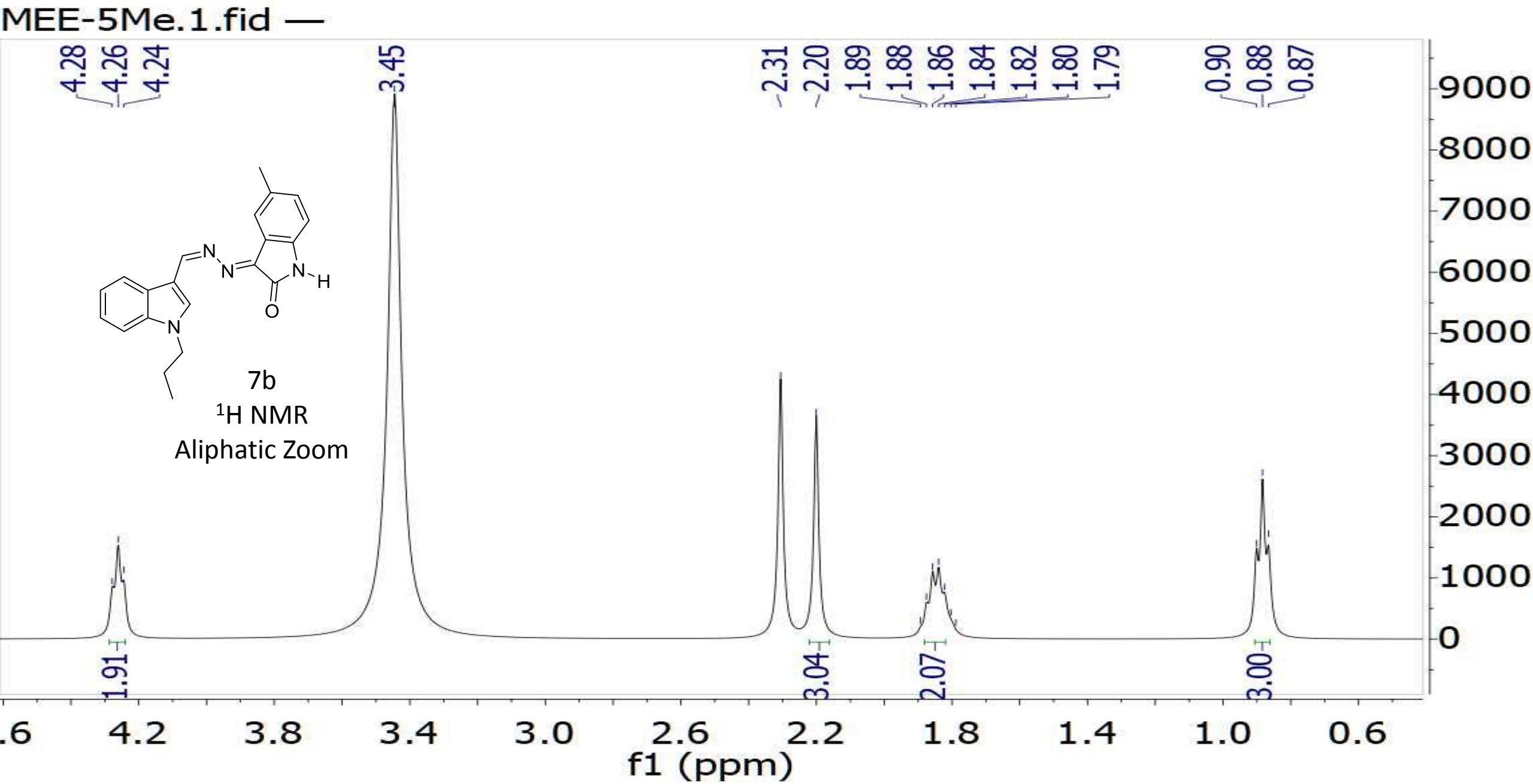

$^{1}$H NMR

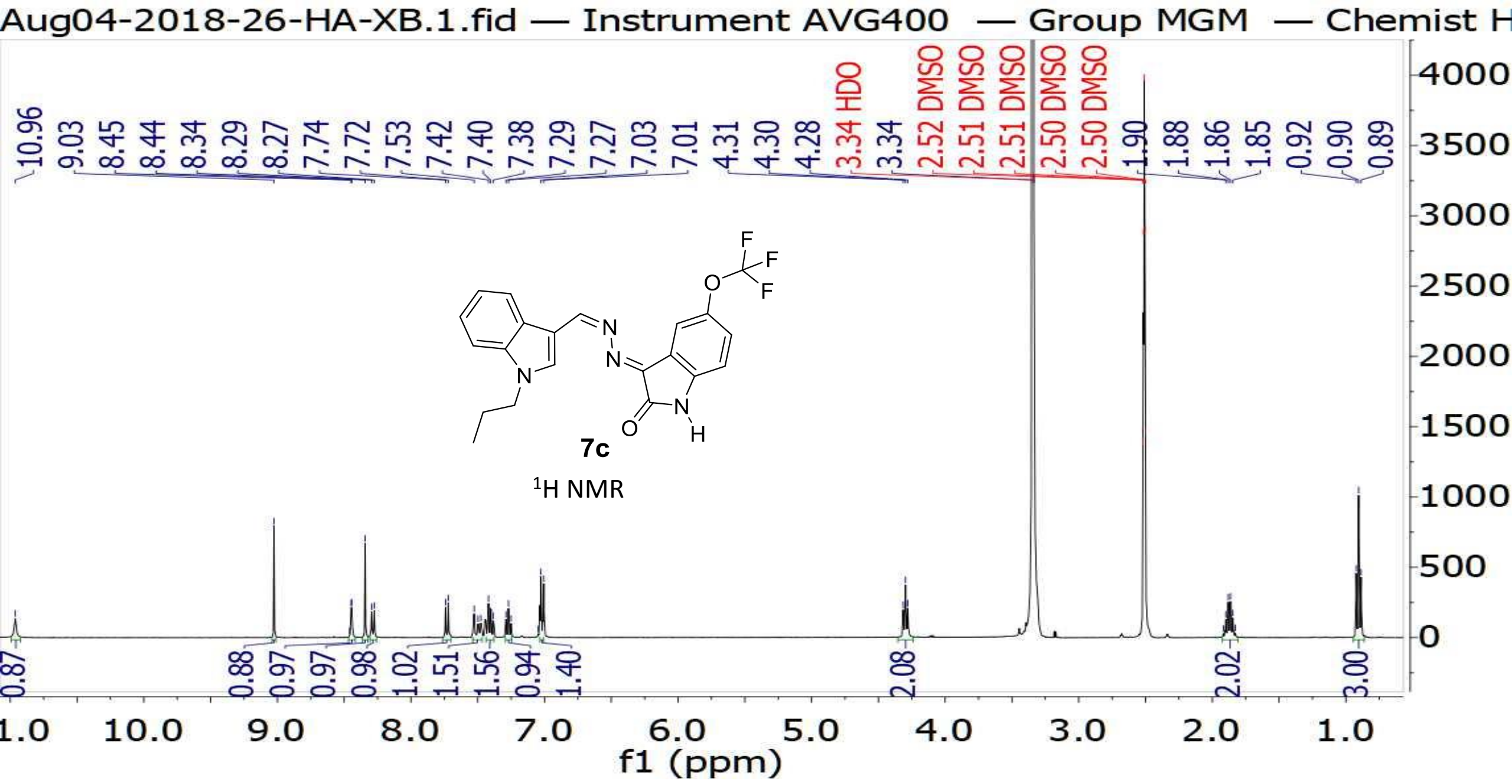
Aromatic Zoom

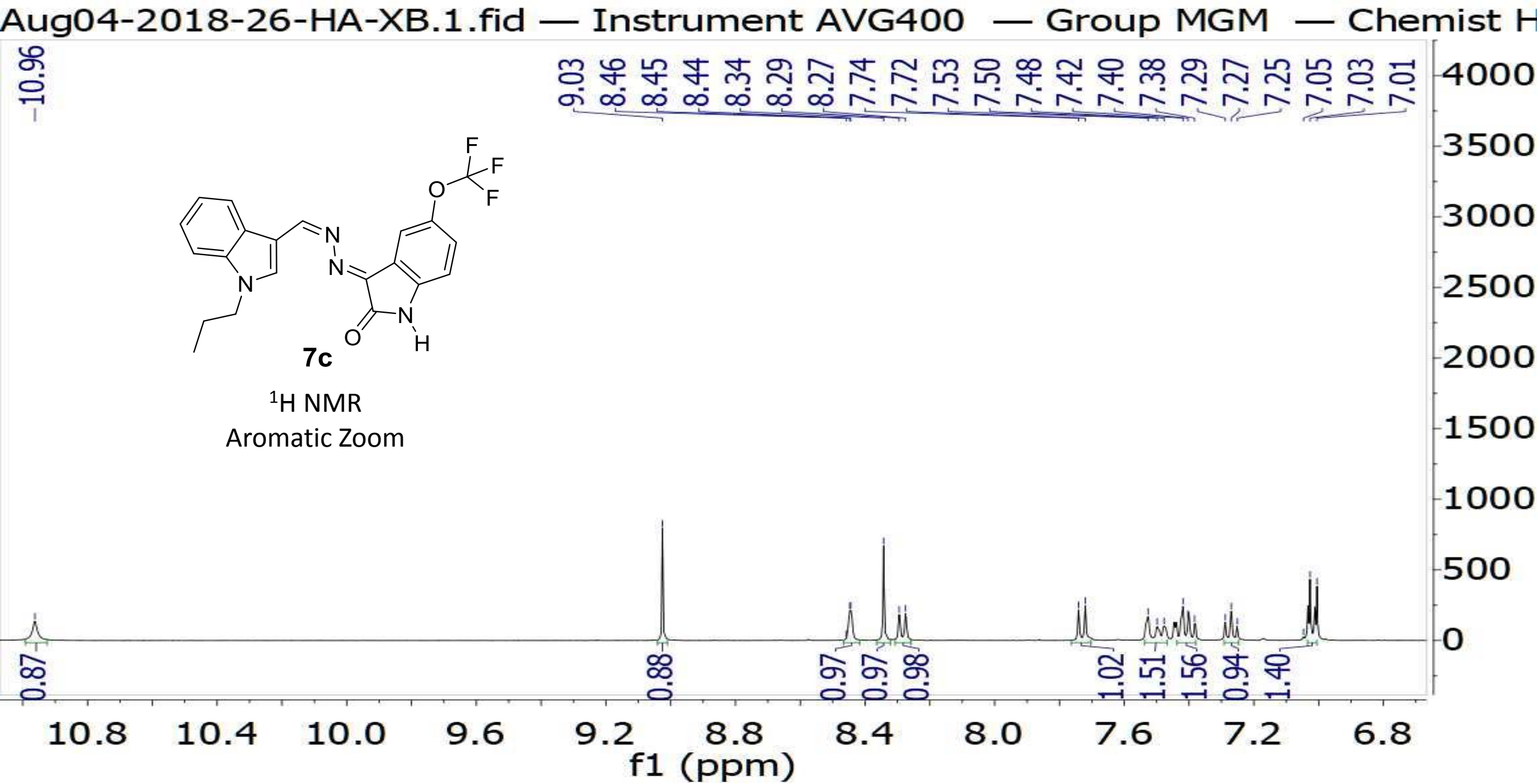

Nov21-2018-20-ETH-CL.1.fid — Instrument AVF400 — Chemist HADIA — Group
4.38
4.36
4.35
4.33
3.37 HDO
2.52 DMSO
2.51 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
1.48
1.46
1.44
Cl
N
N
N
O
H
7a
1H NMR
Aliphatic Zoom
25000
20000
15000
10000
5000
0
2.06
3.05
4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0
f1 (ppm)


Nov21-2018-20-ETH-CL.2.fid — Instrument AVF400 — Chemist HADIA — Group
163.37
148.80
146.28
144.73
143.26
139.63
137.82
134.41
132.42
128.44
127.57
126.54
126.07
125.44
124.19
122.66
121.88
118.73
117.58
113.09
112.52
111.91
111.88
41.77
40.56 DMSO
40.36 DMSO
40.15 DMSO
39.94 DMSO
39.73 DMSO
39.52 DMSO
39.31 DMSO
15.54
Cl
N
N
N
O
N
H
7a
13C NMR
1300
1100
900
700
500
300
100
-100
170
150
130
110
90
70
50
30
10
f1 (ppm)


MEE-5Me.1.fid
10.66
8.95
8.42
8.40
8.34
8.26
7.69
7.67
7.35
7.33
7.24
7.21
7.19
6.82
6.81
6.80
4.28
4.26
4.24
3.45
2.31
2.20
1.88
1.86
1.84
1.82
1.80
0.90
0.88
0.87
N
N
N
H
O
N
7b
$^1$H NMR
0.95
1.18
1.10
1.37
1.10
1.12
2.08
1.57
1.06
1.91
3.04
2.07
3.00
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
f1 (ppm)

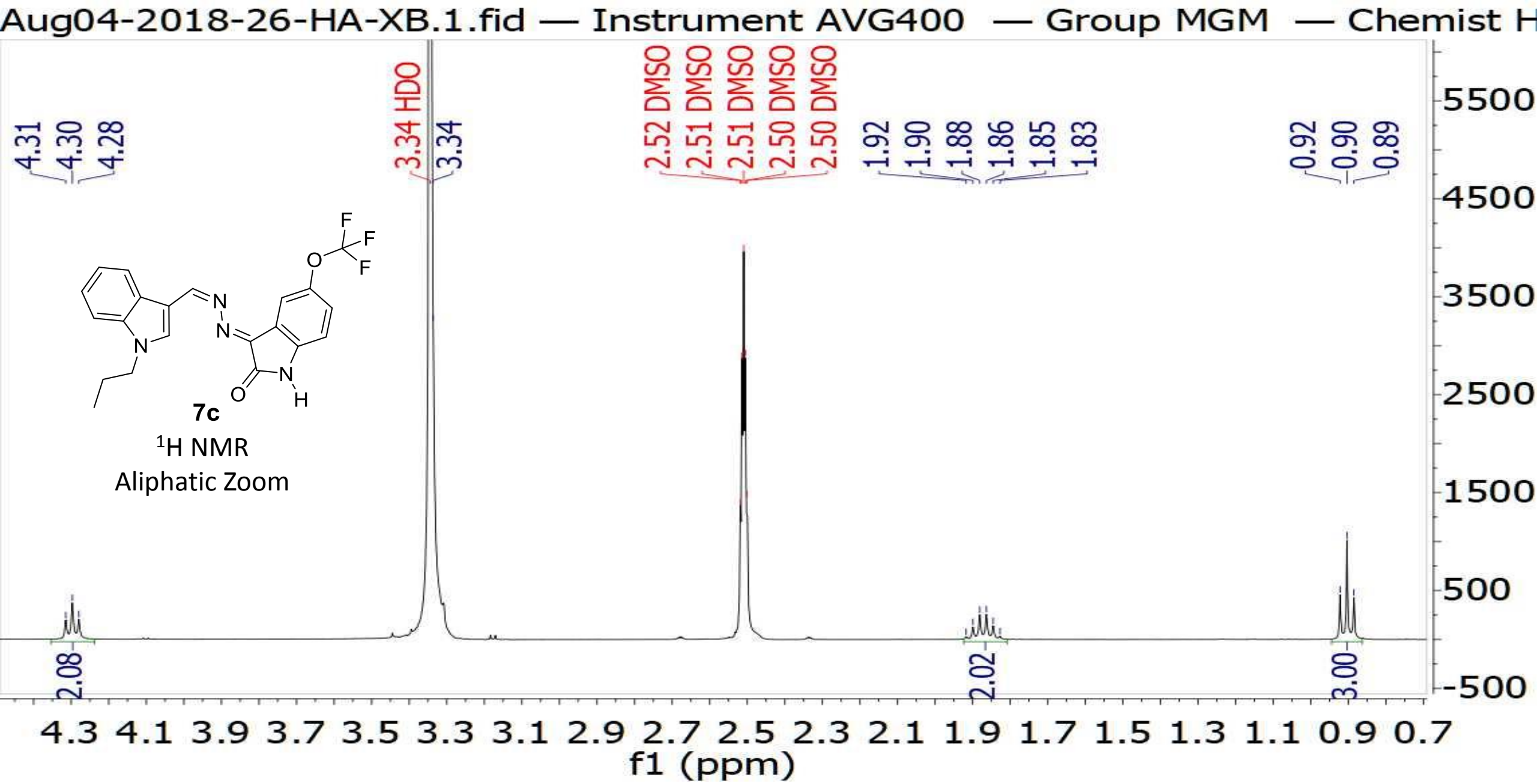

MEE-5Me.1.fid
4.28
4.26
4.24
3.45
2.31
2.20
1.89
1.88
1.86
1.84
1.82
1.80
1.79
0.90
0.88
0.87
7b
1H NMR
Aliphatic Zoom
1.91
3.04
2.07
3.00
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
4.2
3.8
3.4
3.0
2.6
2.2
1.8
1.4
1.0
0.6
f1 (ppm)


Aug04-2018-26-HA-XB.1.fid — Instrument AVG400 — Group MGM — Chemist H
10.96
9.03
8.45
8.44
8.34
8.29
8.27
7.74
7.72
7.53
7.42
7.40
7.38
7.29
7.27
7.03
7.01
4.31
4.30
4.28
3.34 HDO
3.34
2.52 DMSO
2.51 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
1.90
1.88
1.86
1.85
0.92
0.90
0.89
F
F
F
O
N
N
N
H
O
7c
1H NMR
0.87
0.88
0.97
0.97
0.98
1.02
1.51
1.56
0.94
1.40
2.08
2.02
3.00
4000
3500
3000
2500
2000
1500
1000
500
0
1.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
f1 (ppm)




$^{1}$H NMR

Aromatic Zoom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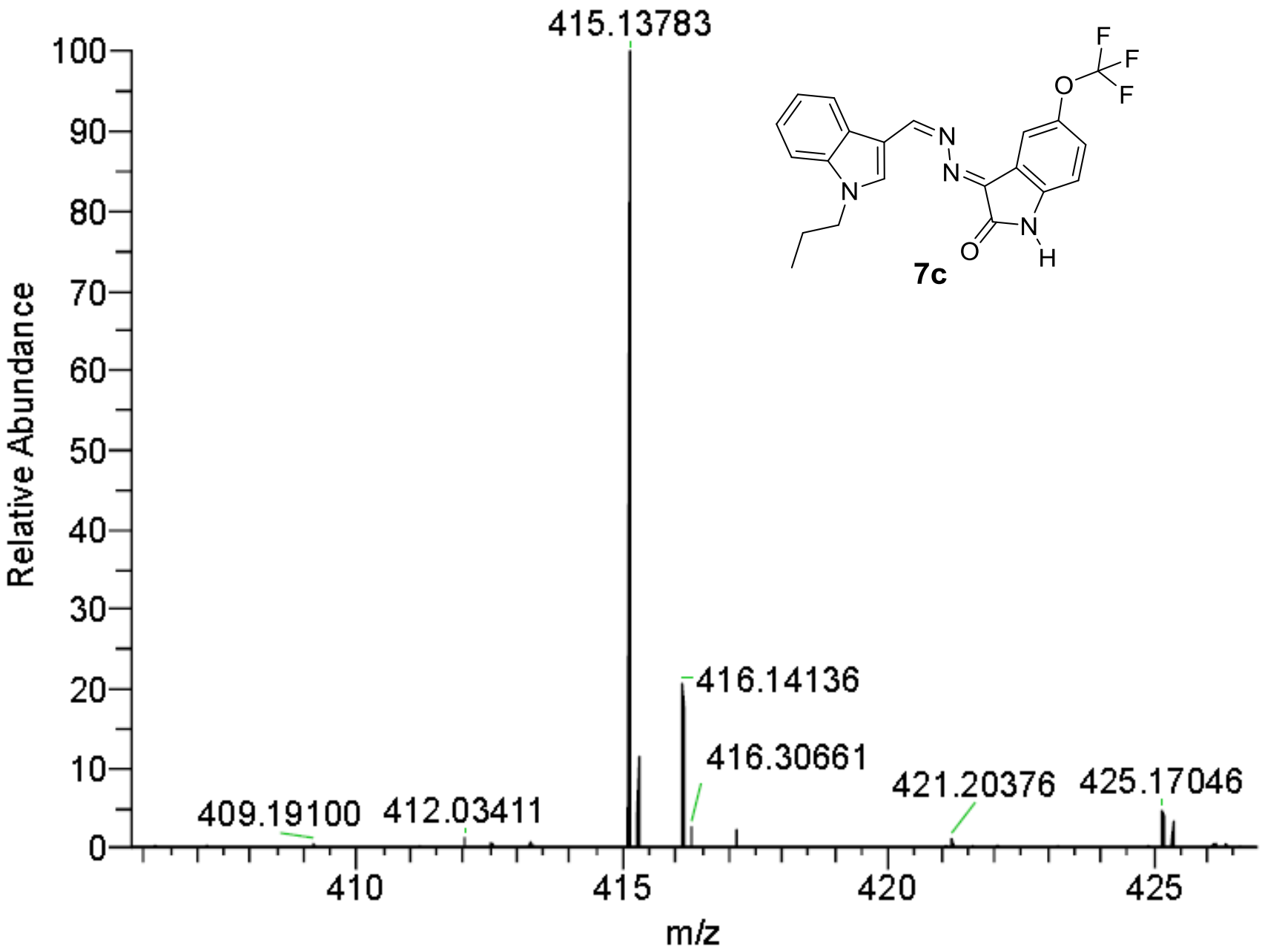
4.31
4.30
4.28
3.34 HDO
3.34
2.52 DMSO
2.51 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
1.92
1.90
1.88
1.86
1.85
1.83
0.92
0.90
0.89
F
F
F
O
N
N
N
H
O
7c
1H NMR
Aliphatic Zoom
2.08
2.02
3.00
5500
4500
3500
2500
1500
500
-500
4.3 4.1 3.9 3.7 3.5 3.3 3.1 2.9 2.7 2.5 2.3 2.1 1.9 1.7 1.5 1.3 1.1 0.9 0.7
f1 (ppm)





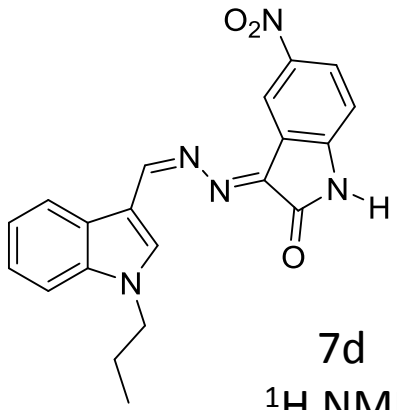


**7c**

NL: 6.71E5
ESI71802 #14-27 RT: 0.17-0.31 AV: 7 NL: 2.55E+007
T: FTMS {1,1} + p ESI Full ms [80.00-1600.00]

Measured Spectrum

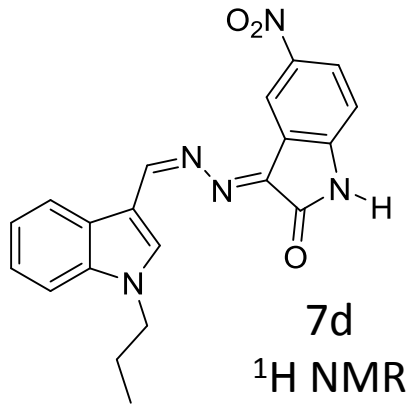
MEE-NO2.1.fid —



7d

$^1$H NMR

MEE-NO2.1.fid

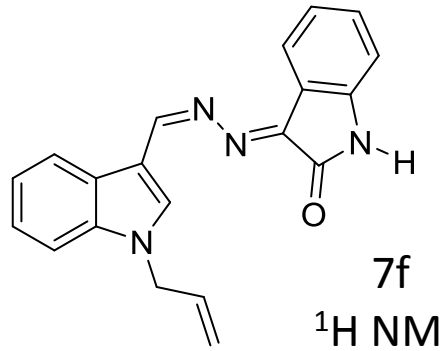


7d

$^1$H NMR

Aromatic Zoom

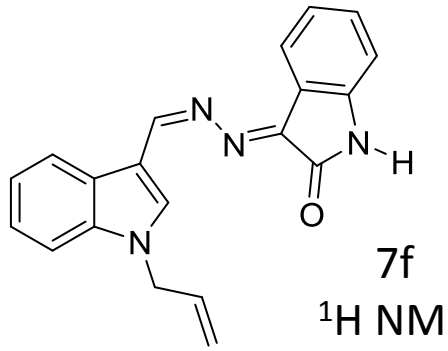
MEE-allyl-H.1.fid —



7f

$^{1}$H NMR

MEE-allyl-H.1.fid

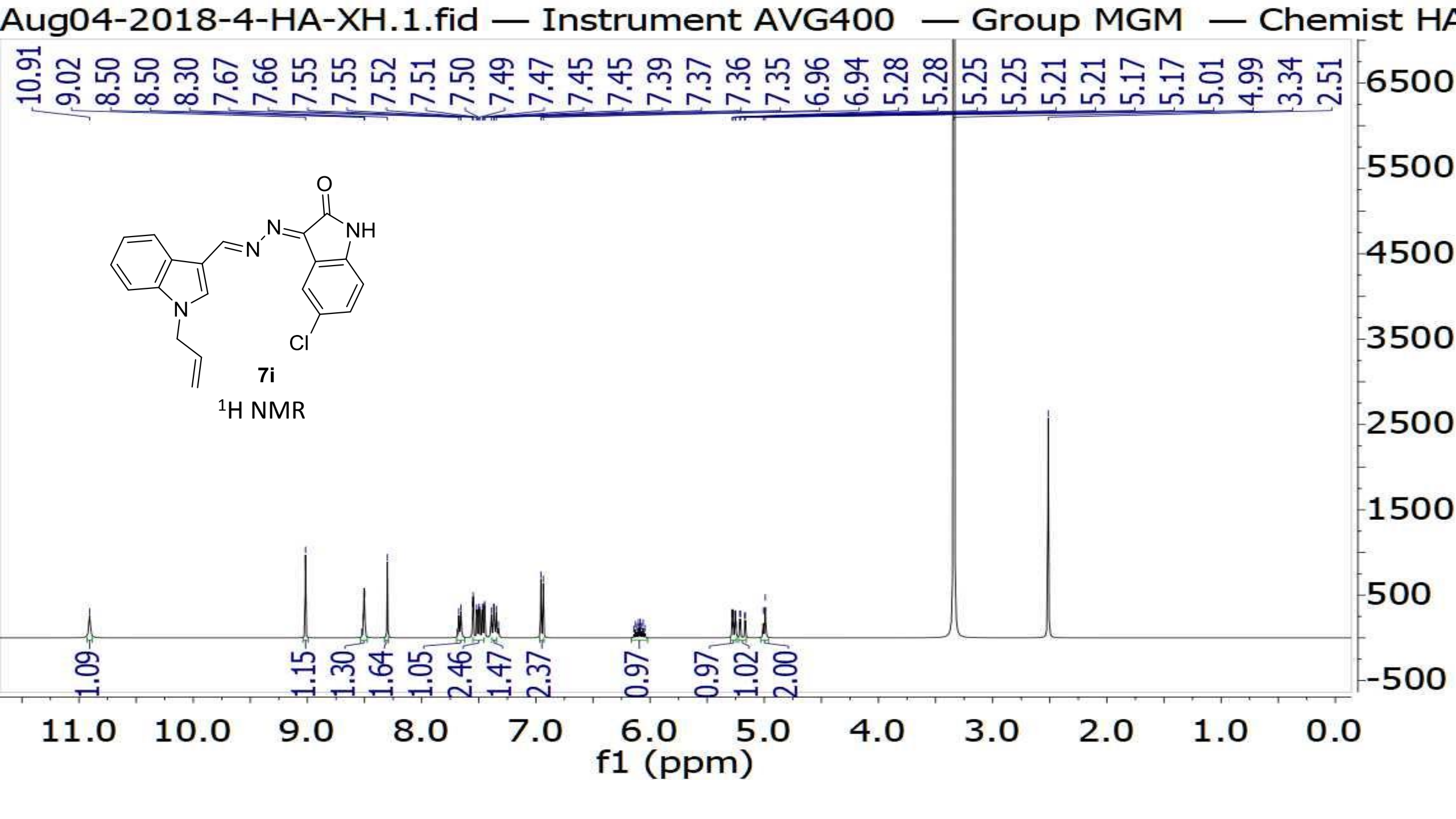


7f

$^{1}$H NMR

Aromatic Zoom

f1 (ppm)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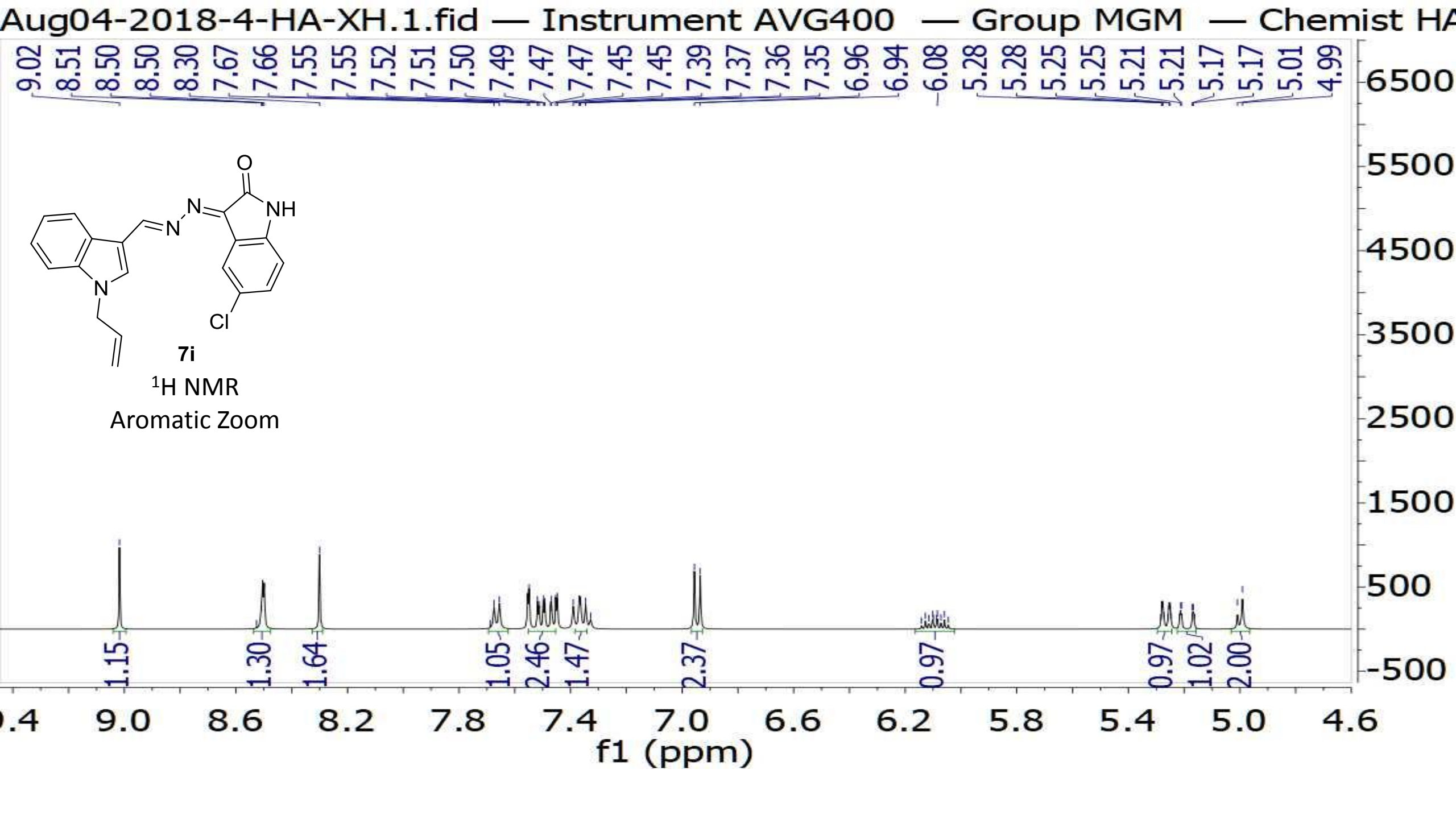
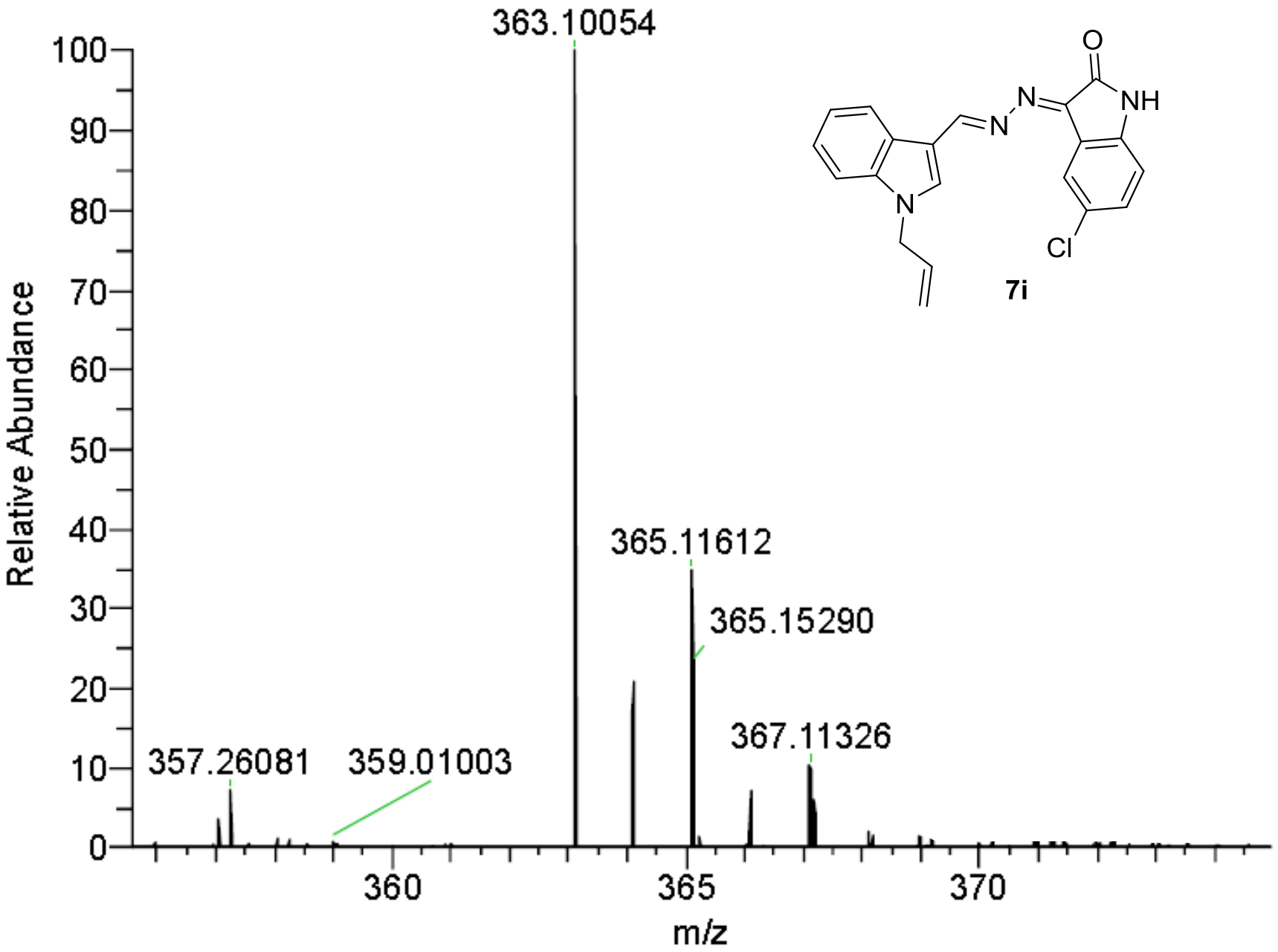
10.91
9.02
8.50
8.50
8.30
7.67
7.66
7.55
7.55
7.52
7.51
7.50
7.49
7.47
7.45
7.45
7.39
7.37
7.36
7.35
6.96
6.94
5.28
5.28
5.25
5.25
5.21
5.21
5.17
5.17
5.01
4.99
3.34
2.51
O
N
N
NH
N
Cl
7i
1H NMR
1.09
1.15
1.30
1.64
1.05
2.46
1.47
2.37
0.97
0.97
1.02
2.00
6500
5500
4500
3500
2500
1500
500
-500
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)


Aug04-2018-4-HA-XH.1.fid — Instrument AVG400 — Group MGM — Chemist HA
9.02 8.51 8.50 8.50 8.30 7.67 7.66 7.55 7.55 7.52 7.51 7.50 7.49 7.47 7.47 7.45 7.45 7.39 7.37 7.36 7.35 6.96 6.94 6.08 5.28 5.28 5.25 5.25 5.21 5.21 5.17 5.17 5.01 4.99
O
NH
N
N
N
Cl
7i
1H NMR
Aromatic Zoom
1.15 1.30 1.64 1.05 2.46 1.47 2.37 0.97 0.97 1.02 2.00
6500 5500 4500 3500 2500 1500 500 -500
9.4 9.0 8.6 8.2 7.8 7.4 7.0 6.6 6.2 5.8 5.4 5.0 4.6
f1 (ppm)



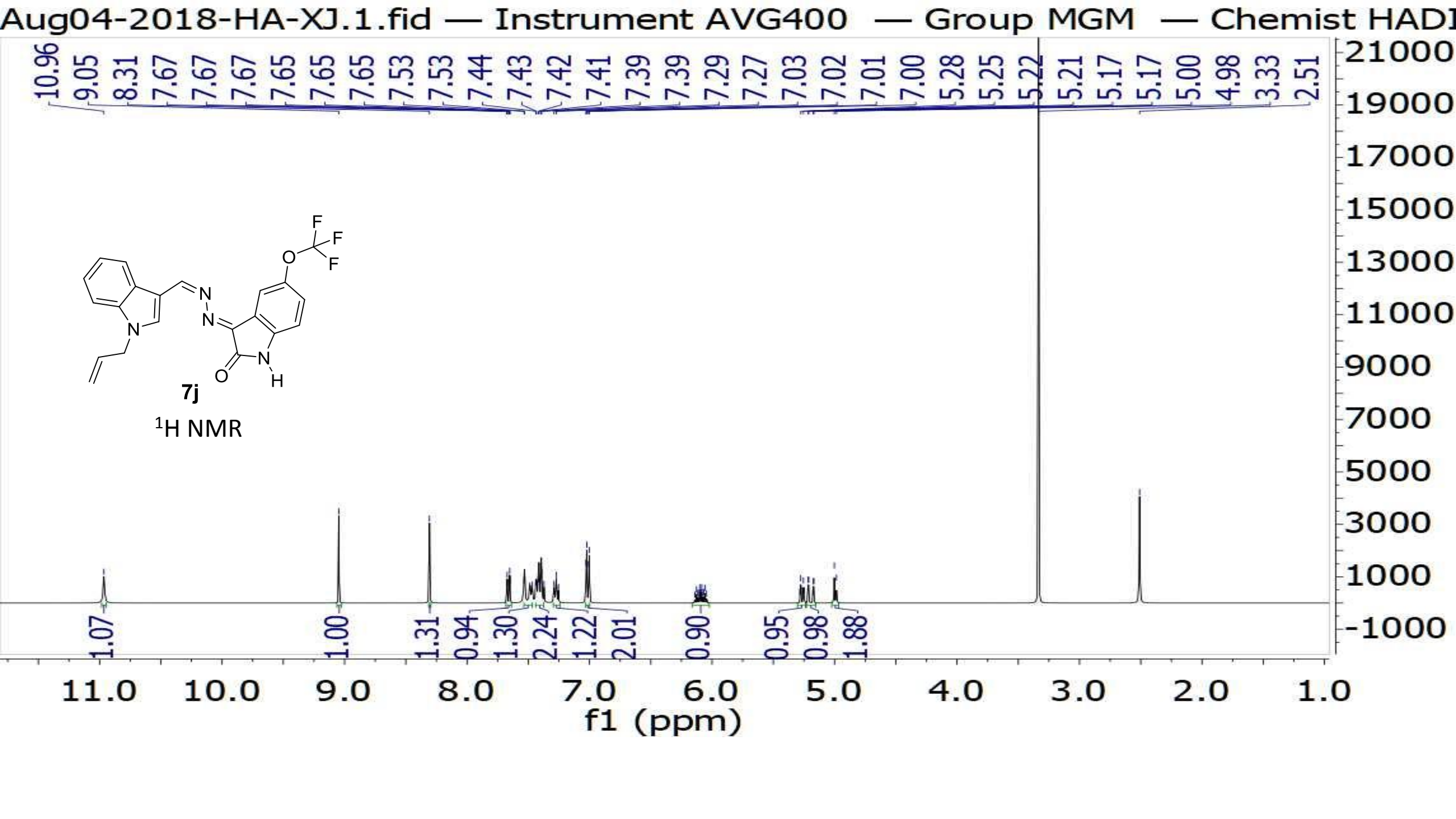




**7i**

NL: 7.58E5
ESI71804 #14-27 RT: 0.17-0.31 AV: 7 NL: 2.72E+007
T: FTMS {1,1} + p ESI Full ms [80.00-1600.00]

Measured Spectrum


Aug04-2018-HA-XJ.1.fid — Instrument AVG400 — Group MGM — Chemist HADI
10.96
9.05
8.31
7.67
7.67
7.67
7.65
7.65
7.65
7.53
7.53
7.44
7.43
7.42
7.41
7.39
7.39
7.29
7.27
7.03
7.02
7.01
7.00
5.28
5.25
5.22
5.21
5.17
5.17
5.00
4.98
3.33
2.51
F
F
F
O
N
N
N
O
N
H
7j
1H NMR
1.07
1.00
1.31
0.94
1.30
2.24
1.22
2.01
0.90
0.95
0.98
1.88
21000
19000
17000
15000
13000
11000
9000
7000
5000
3000
1000
-1000
11.0
10.0
9.0
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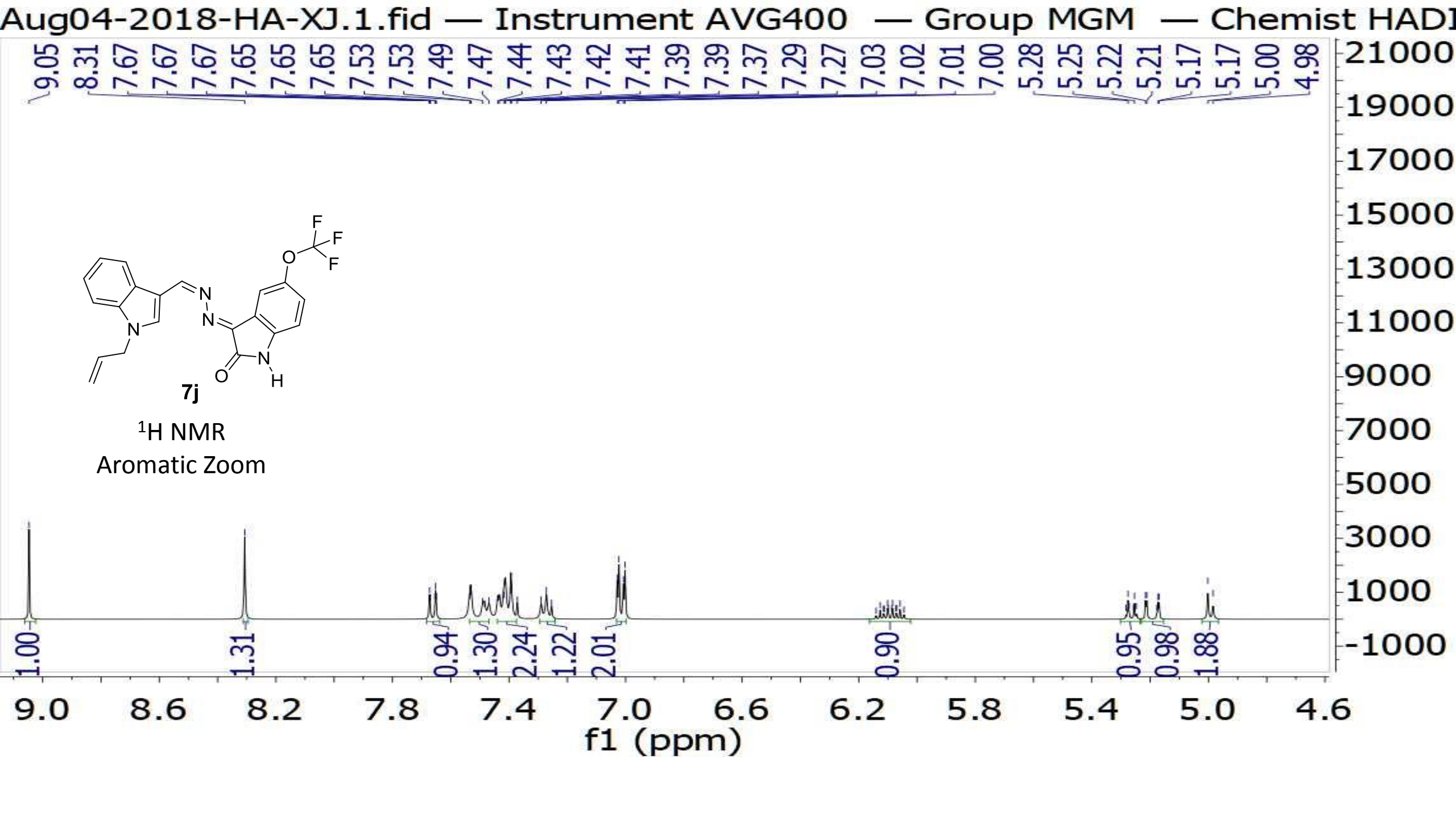
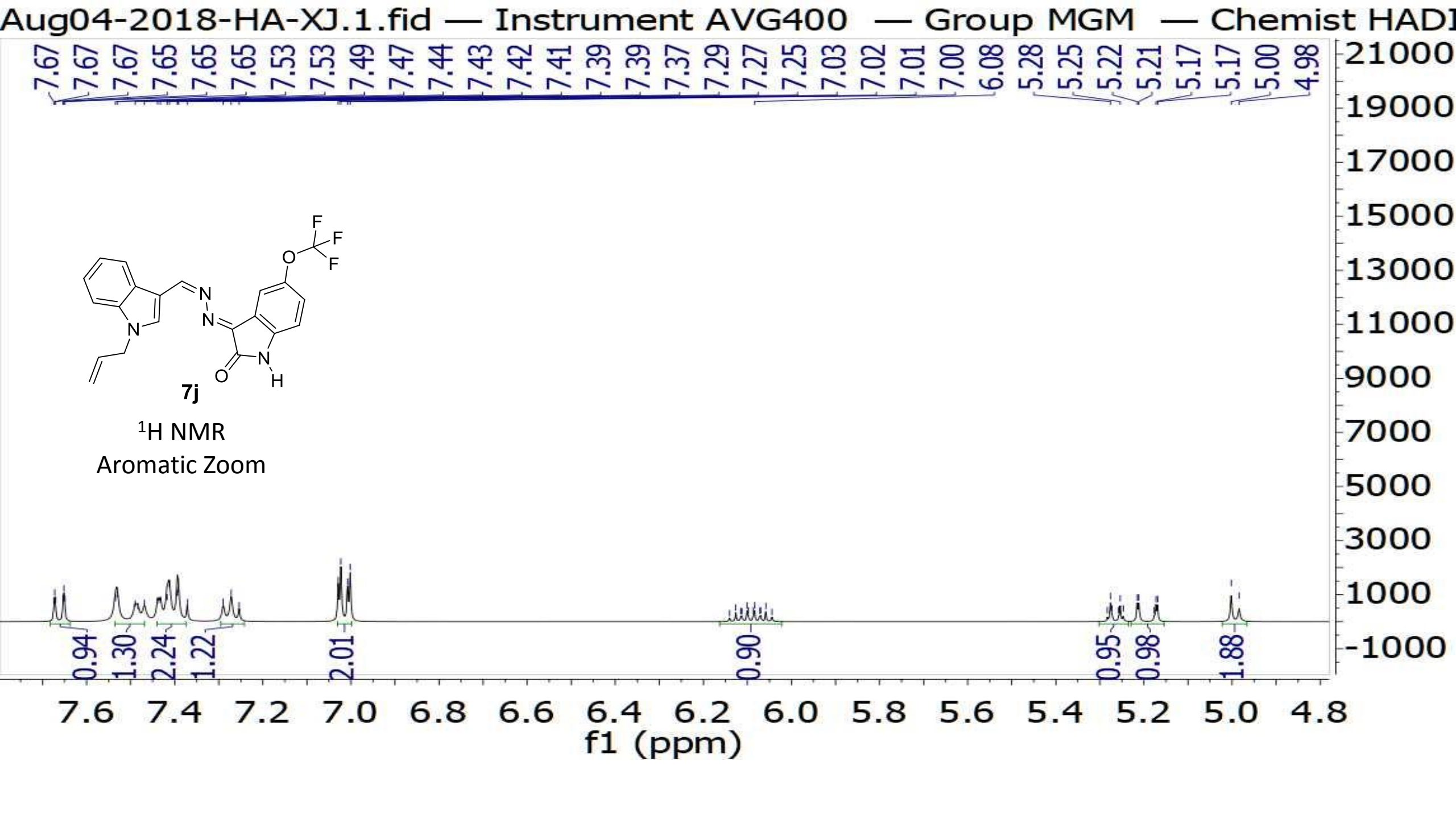
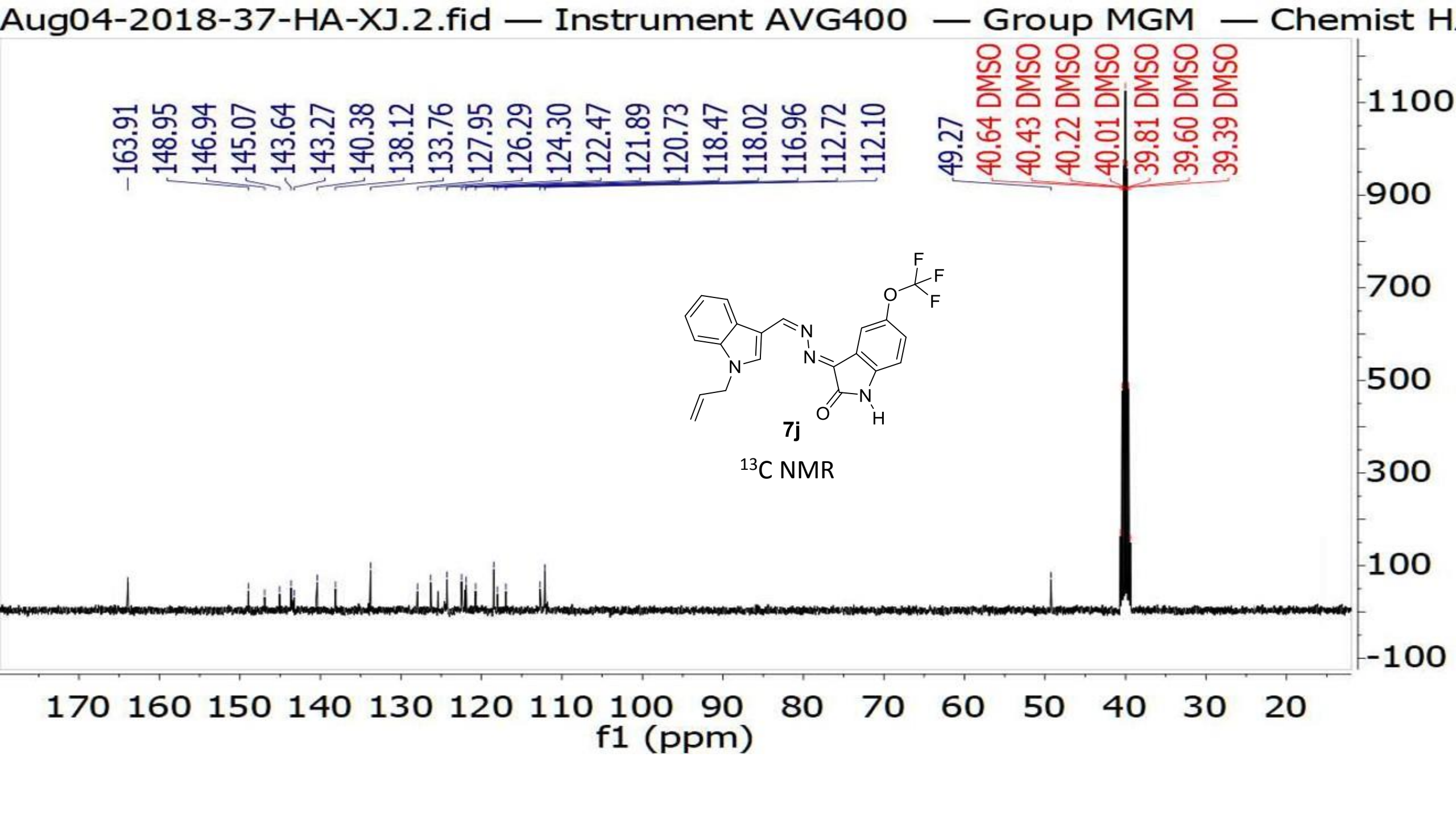
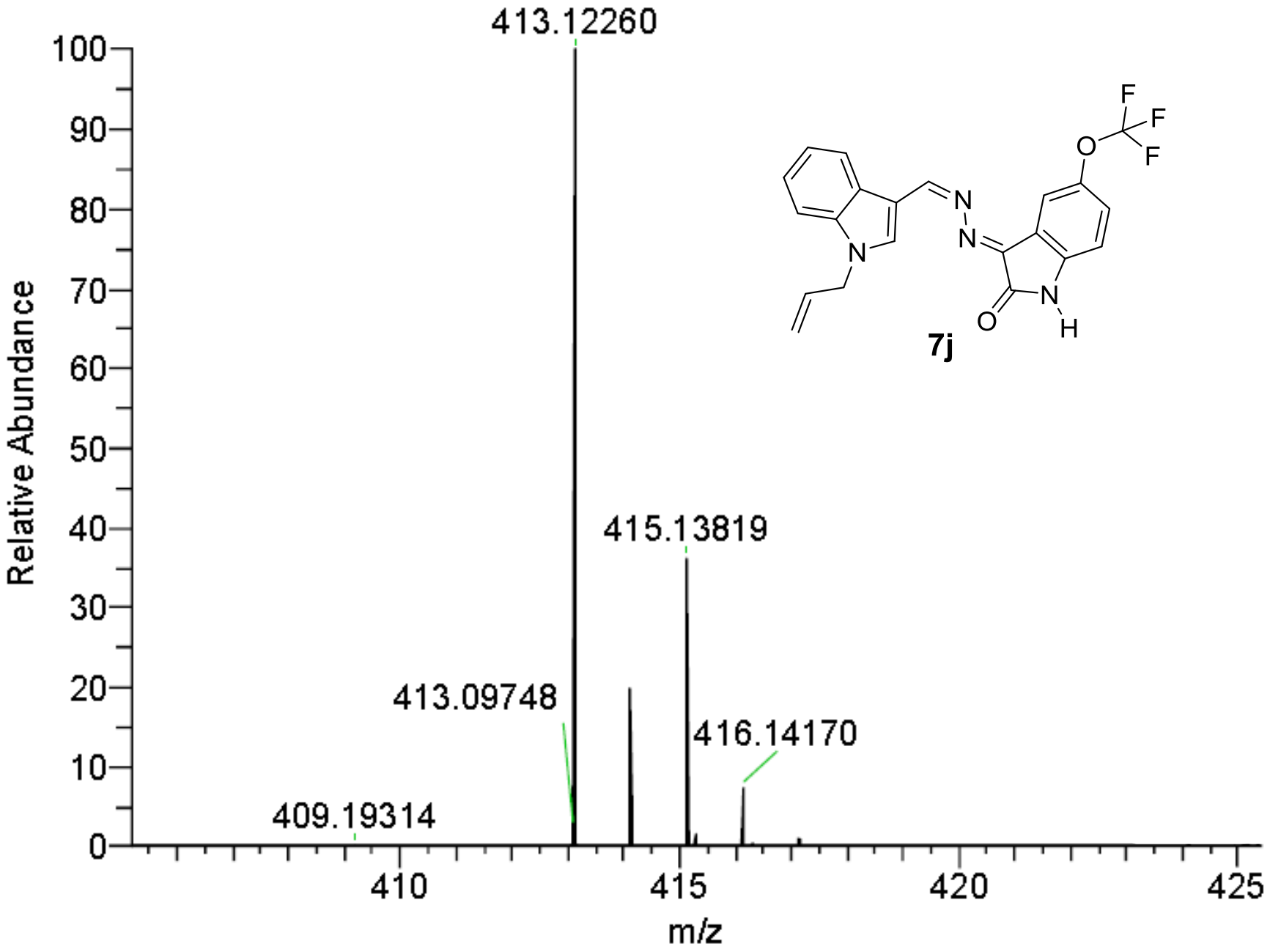
4.0
3.0
2.0
1.0
f1 (ppm)


Aug04-2018-HA-XJ.1.fid — Instrument AVG400 — Group MGM — Chemist HADI
9.05 8.31 7.67 7.67 7.67 7.65 7.65 7.65 7.53 7.53 7.49 7.47 7.44 7.43 7.42 7.41 7.39 7.39 7.37 7.29 7.27 7.03 7.02 7.01 7.00 5.28 5.25 5.22 5.21 5.17 5.17 5.00 4.98
F F F O N N N H O
7j
1H NMR
Aromatic Zoom
1.00 1.31 0.94 1.30 2.24 1.22 2.01 0.90 0.95 0.98 1.88
21000 19000 17000 15000 13000 11000 9000 7000 5000 3000 1000 -1000
9.0 8.6 8.2 7.8 7.4 7.0 6.6 6.2 5.8 5.4 5.0 4.6
f1 (ppm)


Aug04-2018-HA-XJ.1.fid — Instrument AVG400 — Group MGM — Chemist HADI
7.67 7.67 7.67 7.65 7.65 7.65 7.53 7.53 7.49 7.47 7.44 7.43 7.42 7.41 7.39 7.39 7.37 7.29 7.27 7.25 7.03 7.02 7.01 7.00 6.08 5.28 5.25 5.22 5.21 5.17 5.17 5.00 4.98
21000
19000
17000
15000
13000
11000
9000
7000
5000
3000
1000
-1000
F
F
F
O
N
N
N
O
N
H
7j
1H NMR
Aromatic Zoom
0.94
1.30
2.24
1.22
2.01
0.90
0.95
0.98
1.88
7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8
f1 (ppm)


Aug04-2018-37-HA-XJ.2.fid — Instrument AVG400 — Group MGM — Chemist H
163.91
148.95
146.94
145.07
143.64
143.27
140.38
138.12
133.76
127.95
126.29
124.30
122.47
121.89
120.73
118.47
118.02
116.96
112.72
112.10
49.27
40.64 DMSO
40.43 DMSO
40.22 DMSO
40.01 DMSO
39.81 DMSO
39.60 DMSO
39.39 DMSO
F
F
F
O
N
N
N
O
N
H
7j
13C NMR
1100
900
700
500
300
100
-100
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)







**7j**

NL: 2.54E6
ESI71803 #14-28 RT: 0.17-0.3 AV: 7 NL: 2.15E+007
T: FTMS {1,1} + p ESI Full ms [80.00-1600.00]

Measured Spectrum

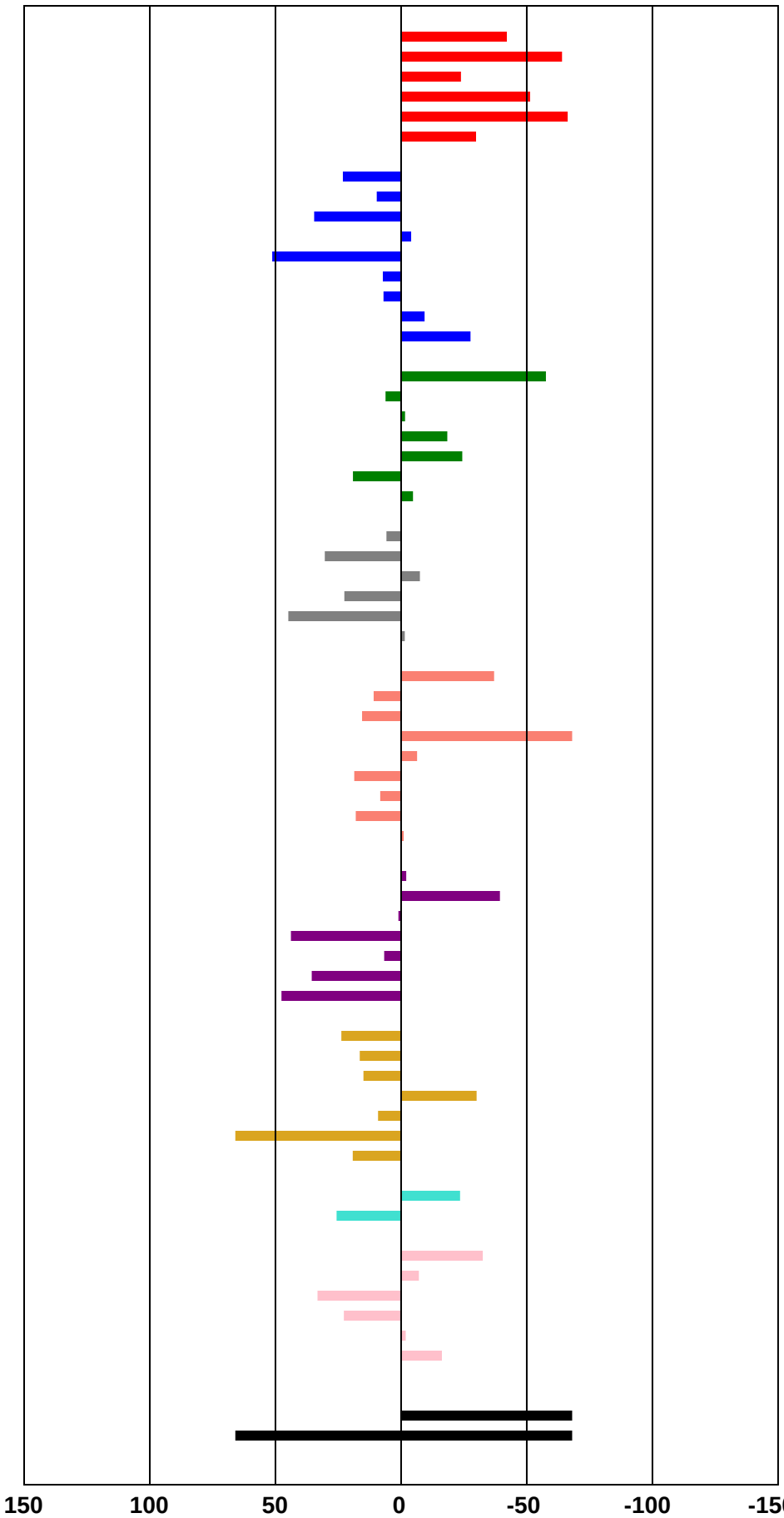
# Developmental Therapeutics Program

## One Dose Mean Graph

| | | |
|---|---|---|
| **NSC:** D-795311 / 1 | **Conc:** 1.00E-5 Molar | **Test Date:** Jan 17, 2017 |
| **Experiment ID:** 1701OS42 | | **Report Date:** Feb 18, 2017 |

| Panel/Cell Line | Growth Percent |
|---|---|
| Leukemia | |
| CCRF-CEM | -0.65 |
| HL-60(TB) | -22.61 |
| K-562 | 17.61 |
| MOLT-4 | -9.90 |
| RPMI-8226 | -24.86 |
| SR | 11.60 |
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 63.93 |
| EKVX | 50.45 |
| HOP-62 | 75.35 |
| HOP-92 | 37.42 |
| NCI-H226 | 92.04 |
| NCI-H23 | 48.03 |
| NCI-H322M | 47.76 |
| NCI-H460 | 32.08 |
| NCI-H522 | 13.81 |
| Colon Cancer | |
| COLO 205 | -16.20 |
| HCC-2998 | 46.95 |
| HCT-116 | 39.85 |
| HCT-15 | 23.04 |
| HT29 | 17.08 |
| KM12 | 59.92 |
| SW-620 | 36.69 |
| CNS Cancer | |
| SF-268 | 46.59 |
| SF-295 | 71.12 |
| SF-539 | 33.94 |
| SNB-19 | 63.34 |
| SNB-75 | 85.63 |
| U251 | 39.96 |
| Melanoma | |
| LOX IMVI | 4.42 |
| MALME-3M | 51.68 |
| M14 | 56.29 |
| MDA-MB-435 | -26.64 |
| SK-MEL-2 | 35.06 |
| SK-MEL-28 | 59.40 |
| SK-MEL-5 | 49.07 |
| UACC-257 | 58.80 |
| UACC-62 | 40.39 |
| Ovarian Cancer | |
| IGROV1 | 39.38 |
| OVCAR-3 | 2.10 |
| OVCAR-4 | 41.83 |
| OVCAR-5 | 84.60 |
| OVCAR-8 | 47.49 |
| NCI/ADR-RES | 76.32 |
| SK-OV-3 | 88.36 |
| Renal Cancer | |
| 786-0 | 64.53 |
| A498 | 57.21 |
| ACHN | 55.74 |
| RXF 393 | 11.38 |
| SN12C | 49.93 |
| TK-10 | 106.72 |
| UO-31 | 60.00 |
| Prostate Cancer | |
| PC-3 | 17.96 |
| DU-145 | 66.43 |
| Breast Cancer | |
| MCF7 | 8.92 |
| MDA-MB-231/ATCC | 34.35 |
| HS 578T | 74.02 |
| BT-549 | 63.53 |
| T-47D | 39.59 |
| MDA-MB-468 | 25.19 |
| Mean | 41.08 |
| Delta | 67.72 |
| Range | 133.36 |

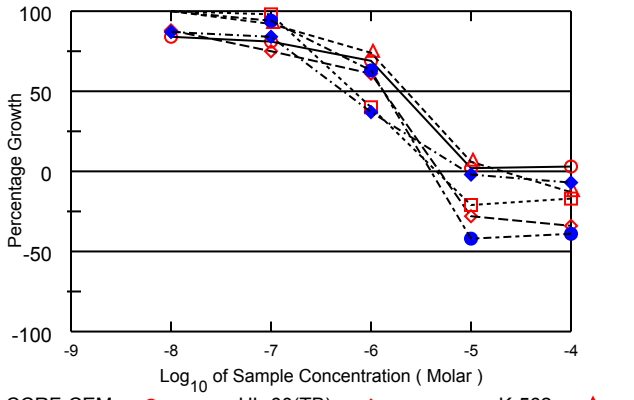
**Mean Growth Percent - Growth Percent**



**National Cancer Institute Developmental Therapeutics Program**
**Dose Response Curves**

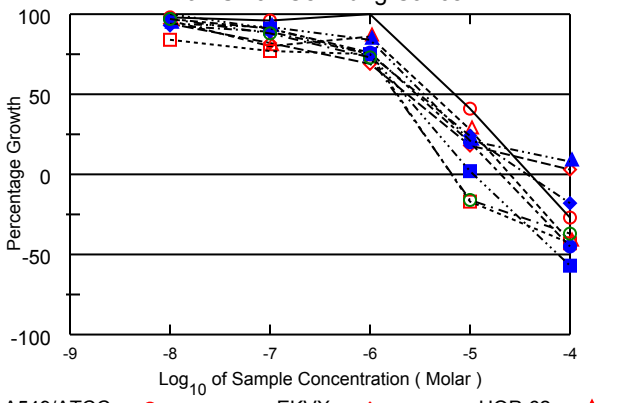
| NSC: D - 795311 / 1 | SSPL: 0YVT | EXP. ID: 1702NS56 |
|---|---|---|
| Report Date: March 20, 2017 | Test Date: February 13, 2017 | |

## Leukemia



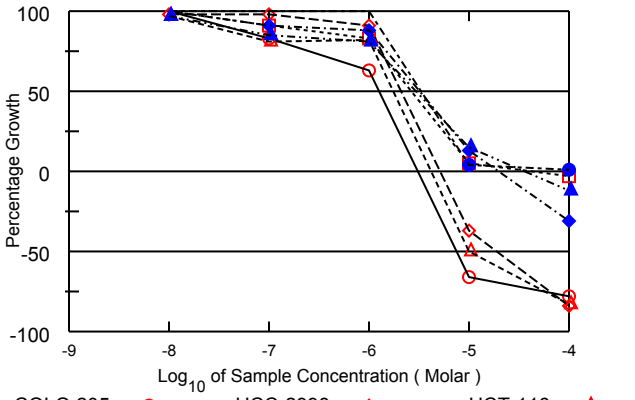

CCRF-CEM, HL-60(TB), K-562, MOLT-4, RPMI-8226, SR

## Non-Small Cell Lung Cancer



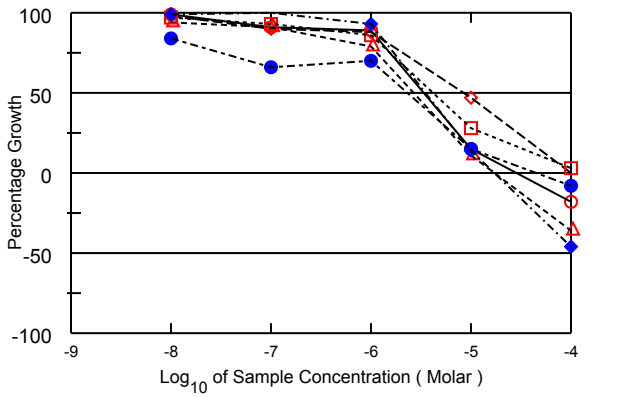

A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522

## Colon Cancer



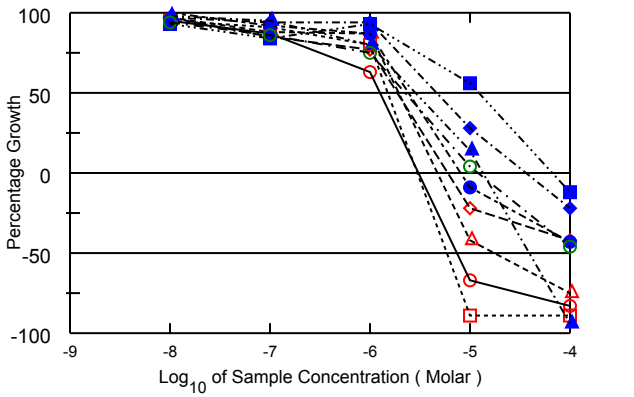

COLO 205, HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620

## CNS Cancer



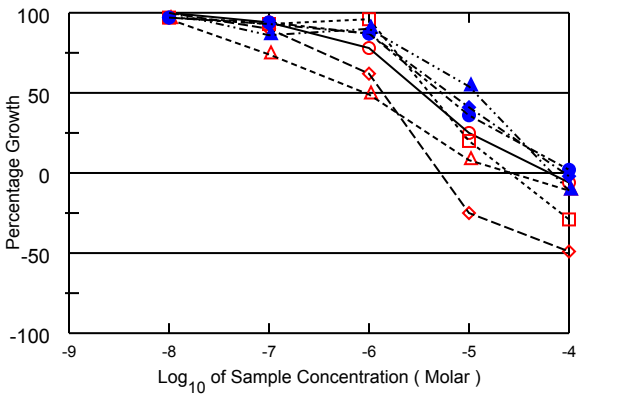

SF-268, SF-295, SF-539, SNB-19, SNB-75, U251

## Melanoma



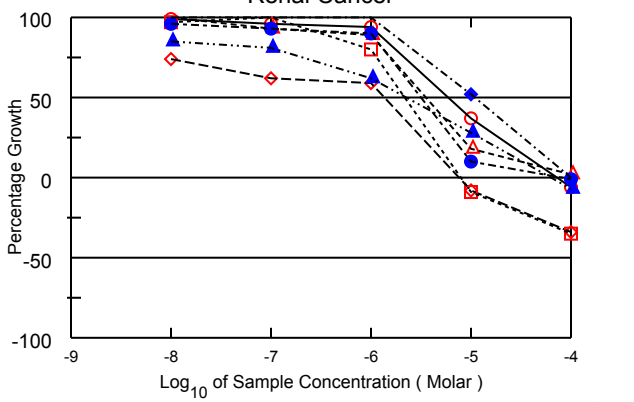

LOX IMVI, MALME-3M, M14, MDA-MB-435, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257, UACC-62

## Ovarian Cancer



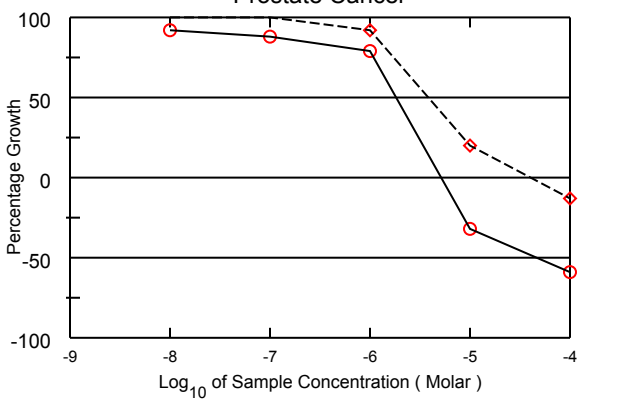

IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, NCI/ADR-RES, SK-OV-3

## Renal Cancer



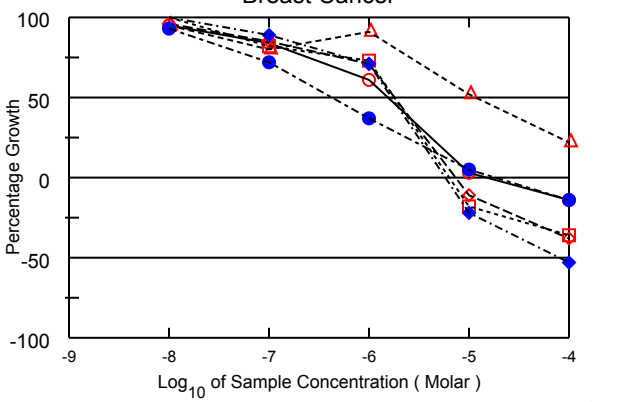

786-0, A498, ACHN, RXF 393, SN12C, TK-10, UO-31

## Prostate Cancer




PC-3, DU-145

## Breast Cancer




MCF7, MDA-MB-231/, HS 578T, BT-549, T-47D, MDA-MB-468

# National Cancer Institute Developmental Therapeutics Program
# In-Vitro Testing Results

| NSC : D - 795311 / 1 | Experiment ID : 1702NS56 | Test Type : 08 | Units : Molar |
|---|---|---|---|
| Report Date : March 20, 2017 | Test Date : February 13, 2017 | QNS : | MC : |
| COMI : Prop-Me | Stain Reagent : SRB Dual-Pass Related | SSPL : 0YVT | |

| | | Log10 Concentration | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time | Mean Optical Densities | | | | | Percent Growth | | | | | | | | |
| Panel/Cell Line | Zero | Ctrl | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | GI50 | TGI | LC50 |
| Leukemia | | | | | | | | | | | | | | | |
| CCRF-CEM | 0.504 | 2.705 | 2.354 | 2.280 | 2.031 | 0.551 | 0.564 | 84 | 81 | 69 | 2 | 3 | 1.94E-6 | > 1.00E-4 | > 1.00E-4 |
| HL-60(TB) | 0.668 | 2.267 | 2.078 | 1.875 | 1.636 | 0.479 | 0.442 | 88 | 75 | 61 | -28 | -34 | 1.31E-6 | 4.80E-6 | > 1.00E-4 |
| K-562 | 0.211 | 1.584 | 1.582 | 1.481 | 1.221 | 0.289 | 0.185 | 100 | 92 | 74 | 6 | -13 | 2.22E-6 | 2.05E-5 | > 1.00E-4 |
| MOLT-4 | 0.683 | 2.487 | 2.504 | 2.445 | 1.413 | 0.539 | 0.567 | 101 | 98 | 40 | -21 | -17 | 6.82E-7 | 4.54E-6 | > 1.00E-4 |
| RPMI-8226 | 0.610 | 2.131 | 2.147 | 2.044 | 1.568 | 0.352 | 0.374 | 101 | 94 | 63 | -42 | -39 | 1.33E-6 | 3.96E-6 | > 1.00E-4 |
| SR | 0.236 | 0.707 | 0.645 | 0.630 | 0.409 | 0.231 | 0.221 | 87 | 84 | 37 | -2 | -7 | 5.21E-7 | 8.72E-6 | > 1.00E-4 |
| Non-Small Cell Lung Cancer | | | | | | | | | | | | | | | |
| A549/ATCC | 0.570 | 2.212 | 2.174 | 2.144 | 2.291 | 1.240 | 0.414 | 98 | 96 | 105 | 41 | -27 | 7.18E-6 | 3.96E-5 | > 1.00E-4 |
| EKVX | 0.660 | 2.099 | 2.011 | 1.836 | 1.656 | 0.913 | 0.703 | 94 | 82 | 69 | 18 | 3 | 2.36E-6 | > 1.00E-4 | > 1.00E-4 |
| HOP-62 | 0.685 | 1.425 | 1.393 | 1.274 | 1.323 | 0.891 | 0.397 | 96 | 80 | 86 | 28 | -42 | 4.17E-6 | 2.50E-5 | > 1.00E-4 |
| HOP-92 | 1.070 | 1.700 | 1.601 | 1.554 | 1.542 | 0.891 | 0.608 | 84 | 77 | 75 | -17 | -43 | 1.87E-6 | 6.57E-6 | > 1.00E-4 |
| NCI-H226 | 0.980 | 2.464 | 2.418 | 2.332 | 2.111 | 1.283 | 0.543 | 97 | 91 | 76 | 20 | -45 | 2.95E-6 | 2.06E-5 | > 1.00E-4 |
| NCI-H23 | 0.590 | 1.794 | 1.714 | 1.665 | 1.496 | 0.884 | 0.487 | 93 | 89 | 75 | 24 | -18 | 3.13E-6 | 3.82E-5 | > 1.00E-4 |
| NCI-H322M | 0.868 | 1.921 | 1.859 | 1.832 | 1.748 | 1.086 | 0.947 | 94 | 92 | 84 | 21 | 8 | 3.42E-6 | > 1.00E-4 | > 1.00E-4 |
| NCI-H460 | 0.268 | 2.685 | 2.718 | 2.475 | 2.073 | 0.305 | 0.115 | 101 | 91 | 75 | 2 | -57 | 2.17E-6 | 1.06E-5 | 7.57E-5 |
| NCI-H522 | 1.072 | 2.422 | 2.375 | 2.262 | 2.058 | 0.899 | 0.671 | 97 | 88 | 73 | -16 | -37 | 1.81E-6 | 6.59E-6 | > 1.00E-4 |
| Colon Cancer | | | | | | | | | | | | | | | |
| COLO 205 | 0.429 | 1.122 | 1.148 | 1.007 | 0.864 | 0.144 | 0.097 | 104 | 83 | 63 | -66 | -78 | 1.25E-6 | 3.06E-6 | 7.46E-6 |
| HCC-2998 | 0.490 | 1.692 | 1.672 | 1.672 | 1.585 | 0.309 | 0.081 | 98 | 98 | 91 | -37 | -84 | 2.09E-6 | 5.14E-6 | 1.90E-5 |
| HCT-116 | 0.226 | 1.957 | 1.902 | 1.633 | 1.645 | 0.113 | 0.038 | 97 | 81 | 82 | -50 | -83 | 1.75E-6 | 4.17E-6 | 9.96E-6 |
| HCT-15 | 0.466 | 2.733 | 2.747 | 2.520 | 2.341 | 0.572 | 0.453 | 101 | 91 | 83 | 5 | -3 | 2.63E-6 | 4.22E-5 | > 1.00E-4 |
| HT29 | 0.332 | 1.747 | 1.942 | 1.839 | 1.771 | 0.394 | 0.351 | 114 | 107 | 102 | 4 | 1 | 3.40E-6 | > 1.00E-4 | > 1.00E-4 |
| KM12 | 0.427 | 2.174 | 2.201 | 2.016 | 1.970 | 0.653 | 0.295 | 102 | 91 | 88 | 13 | -31 | 3.22E-6 | 1.97E-5 | > 1.00E-4 |
| SW-620 | 0.359 | 2.293 | 2.229 | 1.995 | 1.933 | 0.650 | 0.315 | 97 | 85 | 81 | 15 | -12 | 2.97E-6 | 3.53E-5 | > 1.00E-4 |
| CNS Cancer | | | | | | | | | | | | | | | |
| SF-268 | 0.687 | 2.032 | 2.025 | 1.908 | 1.878 | 0.886 | 0.562 | 99 | 91 | 89 | 15 | -18 | 3.33E-6 | 2.80E-5 | > 1.00E-4 |
| SF-295 | 0.827 | 2.650 | 2.611 | 2.467 | 2.428 | 1.683 | 0.821 | 98 | 90 | 88 | 47 | . | 8.41E-6 | 9.66E-5 | > 1.00E-4 |
| SF-539 | 1.072 | 2.757 | 2.655 | 2.604 | 2.409 | 1.257 | 0.684 | 94 | 91 | 79 | 11 | -36 | 2.69E-6 | 1.71E-5 | > 1.00E-4 |
| SNB-19 | 0.424 | 1.474 | 1.438 | 1.404 | 1.330 | 0.723 | 0.453 | 97 | 93 | 86 | 28 | 3 | 4.24E-6 | > 1.00E-4 | > 1.00E-4 |
| SNB-75 | 0.917 | 1.745 | 1.611 | 1.464 | 1.495 | 1.044 | 0.846 | 84 | 66 | 70 | 15 | -8 | 2.31E-6 | 4.60E-5 | > 1.00E-4 |
| U251 | 0.476 | 1.883 | 1.876 | 1.878 | 1.782 | 0.678 | 0.259 | 99 | 100 | 93 | 14 | -46 | 3.51E-6 | 1.73E-5 | > 1.00E-4 |
| Melanoma | | | | | | | | | | | | | | | |
| LOX IMVI | 0.263 | 1.556 | 1.494 | 1.387 | 1.079 | 0.087 | 0.046 | 95 | 87 | 63 | -67 | -83 | 1.26E-6 | 3.06E-6 | 7.41E-6 |
| MALME-3M | 0.758 | 1.163 | 1.152 | 1.101 | 1.071 | 0.593 | 0.437 | 97 | 85 | 77 | -22 | -42 | 1.88E-6 | 6.03E-6 | > 1.00E-4 |
| M14 | 0.396 | 1.549 | 1.510 | 1.412 | 1.395 | 0.229 | 0.099 | 97 | 88 | 87 | -42 | -75 | 1.92E-6 | 4.70E-6 | 1.72E-5 |
| MDA-MB-435 | 0.386 | 2.087 | 1.996 | 1.932 | 1.740 | 0.041 | 0.042 | 95 | 91 | 80 | -89 | -89 | 1.50E-6 | 2.96E-6 | 5.85E-6 |
| SK-MEL-2 | 1.239 | 2.493 | 2.520 | 2.387 | 2.326 | 1.126 | 0.705 | 102 | 92 | 87 | -9 | -43 | 2.42E-6 | 8.03E-6 | > 1.00E-4 |
| SK-MEL-28 | 0.626 | 1.875 | 1.843 | 1.803 | 1.801 | 0.982 | 0.490 | 97 | 94 | 94 | 28 | -22 | 4.70E-6 | 3.69E-5 | > 1.00E-4 |
| SK-MEL-5 | 0.615 | 2.983 | 2.933 | 2.859 | 2.512 | 0.949 | 0.035 | 98 | 95 | 80 | 14 | -94 | 2.86E-6 | 1.35E-5 | 3.90E-5 |
| UACC-257 | 1.416 | 2.586 | 2.507 | 2.404 | 2.503 | 2.073 | 1.246 | 93 | 84 | 93 | 56 | -12 | 1.23E-5 | 6.66E-5 | > 1.00E-4 |
| UACC-62 | 0.893 | 2.838 | 2.723 | 2.558 | 2.345 | 0.975 | 0.480 | 94 | 86 | 75 | 4 | -46 | 2.24E-6 | 1.21E-5 | > 1.00E-4 |
| Ovarian Cancer | | | | | | | | | | | | | | | |
| IGROV1 | 0.661 | 2.072 | 2.107 | 1.991 | 1.756 | 1.020 | 0.619 | 103 | 94 | 78 | 25 | -6 | 3.38E-6 | 6.31E-5 | > 1.00E-4 |
| OVCAR-3 | 0.432 | 1.481 | 1.558 | 1.380 | 1.079 | 0.323 | 0.221 | 107 | 90 | 62 | -25 | -49 | 1.36E-6 | 5.11E-6 | > 1.00E-4 |
| OVCAR-4 | 0.767 | 1.624 | 1.587 | 1.401 | 1.190 | 0.835 | 0.680 | 96 | 74 | 49 | 8 | -11 | 9.35E-7 | 2.56E-5 | > 1.00E-4 |
| OVCAR-5 | 0.665 | 1.872 | 1.836 | 1.784 | 1.826 | 0.907 | 0.475 | 97 | 93 | 96 | 20 | -29 | 4.04E-6 | 2.58E-5 | > 1.00E-4 |
| OVCAR-8 | 0.682 | 2.479 | 2.422 | 2.365 | 2.254 | 1.336 | 0.721 | 97 | 94 | 87 | 36 | 2 | 5.41E-6 | > 1.00E-4 | > 1.00E-4 |
| NCI/ADR-RES | 0.579 | 2.027 | 1.976 | 1.931 | 1.838 | 1.174 | 0.568 | 97 | 93 | 87 | 41 | -2 | 6.39E-6 | 8.99E-5 | > 1.00E-4 |
| SK-OV-3 | 0.895 | 1.897 | 1.904 | 1.756 | 1.799 | 1.440 | 0.793 | 101 | 86 | 90 | 54 | -11 | 1.17E-5 | 6.71E-5 | > 1.00E-4 |
| Renal Cancer | | | | | | | | | | | | | | | |
| 786-0 | 0.707 | 2.492 | 2.473 | 2.414 | 2.378 | 1.372 | 0.662 | 99 | 96 | 94 | 37 | -6 | 5.94E-6 | 7.12E-5 | > 1.00E-4 |
| A498 | 1.494 | 2.251 | 2.055 | 1.964 | 1.942 | 1.368 | 0.992 | 74 | 62 | 59 | -8 | -34 | 1.37E-6 | 7.50E-6 | > 1.00E-4 |
| ACHN | 0.430 | 1.874 | 1.921 | 1.771 | 1.719 | 0.690 | 0.463 | 103 | 93 | 89 | 18 | 2 | 3.56E-6 | > 1.00E-4 | > 1.00E-4 |
| RXF 393 | 0.919 | 1.528 | 1.510 | 1.532 | 1.405 | 0.838 | 0.600 | 97 | 101 | 80 | -9 | -35 | 2.17E-6 | 7.94E-6 | > 1.00E-4 |
| SN12C | 0.422 | 1.597 | 1.553 | 1.513 | 1.483 | 0.545 | 0.416 | 96 | 93 | 90 | 10 | -1 | 3.20E-6 | 7.59E-5 | > 1.00E-4 |
| TK-10 | 0.882 | 1.906 | 2.006 | 2.101 | 2.163 | 1.418 | 0.887 | 110 | 119 | 125 | 52 | . | 1.11E-5 | > 1.00E-4 | > 1.00E-4 |
| UO-31 | 0.771 | 1.790 | 1.639 | 1.596 | 1.408 | 1.052 | 0.715 | 85 | 81 | 62 | 28 | -7 | 2.28E-6 | 6.18E-5 | > 1.00E-4 |
| Prostate Cancer | | | | | | | | | | | | | | | |
| PC-3 | 0.394 | 1.376 | 1.296 | 1.261 | 1.169 | 0.269 | 0.163 | 92 | 88 | 79 | -32 | -59 | 1.83E-6 | 5.17E-6 | 4.74E-5 |
| DU-145 | 0.465 | 1.781 | 1.830 | 1.795 | 1.682 | 0.733 | 0.404 | 104 | 101 | 92 | 20 | -13 | 3.88E-6 | 4.06E-5 | > 1.00E-4 |
| Breast Cancer | | | | | | | | | | | | | | | |
| MCF7 | 0.548 | 2.600 | 2.494 | 2.269 | 1.797 | 0.614 | 0.473 | 95 | 84 | 61 | 3 | -14 | 1.54E-6 | 1.55E-5 | > 1.00E-4 |
| MDA-MB-231/ATCC | 0.642 | 1.427 | 1.399 | 1.308 | 1.200 | 0.574 | 0.397 | 96 | 85 | 71 | -11 | -38 | 1.81E-6 | 7.40E-6 | > 1.00E-4 |
| HS 578T | 1.159 | 2.155 | 2.105 | 1.959 | 2.064 | 1.672 | 1.374 | 95 | 80 | 91 | 52 | 22 | 1.13E-5 | > 1.00E-4 | > 1.00E-4 |
| BT-549 | 1.264 | 2.058 | 2.064 | 1.914 | 1.843 | 1.036 | 0.809 | 101 | 82 | 73 | -18 | -36 | 1.79E-6 | 6.33E-6 | > 1.00E-4 |
| T-47D | 0.865 | 1.530 | 1.481 | 1.343 | 1.109 | 0.899 | 0.744 | 93 | 72 | 37 | 5 | -14 | 4.16E-7 | 1.84E-5 | > 1.00E-4 |
| MDA-MB-468 | 0.732 | 1.367 | 1.392 | 1.298 | 1.186 | 0.569 | 0.344 | 104 | 89 | 71 | -22 | -53 | 1.69E-6 | 5.78E-6 | 7.94E-5 |

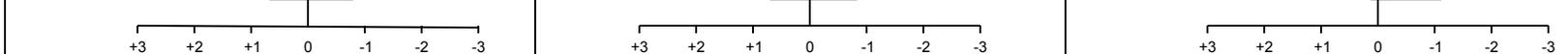
National Cancer Institute Developmental Therapeutics Program

Mean Graphs

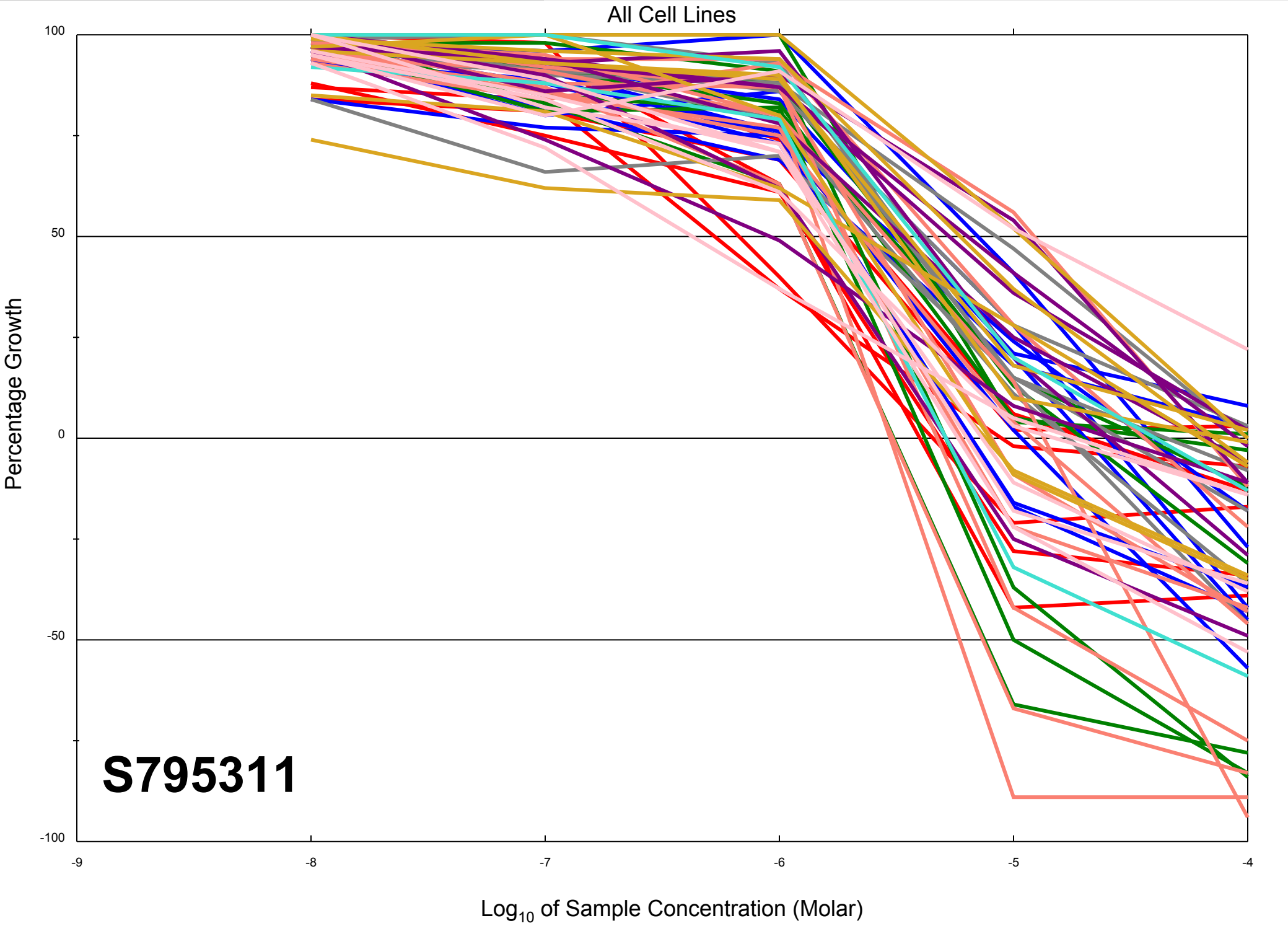
NSC : D - 795311/1 | Units :Molar | SSPL :0YVT | EXP. ID :1702NS56

Report Date :March 20, 2017 | Test Date :February 13, 2017

| Panel/Cell Line | $Log_{10}$GI50 | GI50 | $Log_{10}$TGI | TGI | $Log_{10}$LC50 | LC50 |
|---|---|---|---|---|---|---|
| Leukemia | | | | | | |
| CCRF-CEM | -5.71 | | > -4.00 | | > -4.00 | |
| HL-60(TB) | -5.88 | | -5.32 | | > -4.00 | |
| K-562 | -5.65 | | -4.69 | | > -4.00 | |
| MOLT-4 | -6.17 | | -5.34 | | > -4.00 | |
| RPMI-8226 | -5.88 | | -5.40 | | > -4.00 | |
| SR | -6.28 | | -5.06 | | > -4.00 | |
| Non-Small Cell Lung Cancer | | | | | | |
| A549/ATCC | -5.14 | | -4.40 | | > -4.00 | |
| EKVX | -5.63 | | > -4.00 | | > -4.00 | |
| HOP-62 | -5.38 | | -4.60 | | > -4.00 | |
| HOP-92 | -5.73 | | -5.18 | | > -4.00 | |
| NCI-H226 | -5.53 | | -4.69 | | > -4.00 | |
| NCI-H23 | -5.50 | | -4.42 | | > -4.00 | |
| NCI-H322M | -5.47 | | > -4.00 | | > -4.00 | |
| NCI-H460 | -5.66 | | -4.97 | | -4.12 | |
| NCI-H522 | -5.74 | | -5.18 | | > -4.00 | |
| Colon Cancer | | | | | | |
| COLO 205 | -5.90 | | -5.51 | | -5.13 | |
| HCC-2998 | -5.68 | | -5.29 | | -4.72 | |
| HCT-116 | -5.76 | | -5.38 | | -5.00 | |
| HCT-15 | -5.58 | | -4.37 | | > -4.00 | |
| HT29 | -5.47 | | > -4.00 | | > -4.00 | |
| KM12 | -5.49 | | -4.71 | | > -4.00 | |
| SW-620 | -5.53 | | -4.45 | | > -4.00 | |
| CNS Cancer | | | | | | |
| SF-268 | -5.48 | | -4.55 | | > -4.00 | |
| SF-295 | -5.08 | | -4.02 | | > -4.00 | |
| SF-539 | -5.57 | | -4.77 | | > -4.00 | |
| SNB-19 | -5.37 | | > -4.00 | | > -4.00 | |
| SNB-75 | -5.64 | | -4.34 | | > -4.00 | |
| U251 | -5.45 | | -4.76 | | > -4.00 | |
| Melanoma | | | | | | |
| LOX IMVI | -5.90 | | -5.51 | | -5.13 | |
| MALME-3M | -5.73 | | -5.22 | | > -4.00 | |
| M14 | -5.72 | | -5.33 | | -4.77 | |
| MDA-MB-435 | -5.82 | | -5.53 | | -5.23 | |
| SK-MEL-2 | -5.62 | | -5.10 | | > -4.00 | |
| SK-MEL-28 | -5.33 | | -4.43 | | > -4.00 | |
| SK-MEL-5 | -5.54 | | -4.87 | | -4.41 | |
| UACC-257 | -4.91 | | -4.18 | | > -4.00 | |
| UACC-62 | -5.65 | | -4.92 | | > -4.00 | |
| Ovarian Cancer | | | | | | |
| IGROV1 | -5.47 | | -4.20 | | > -4.00 | |
| OVCAR-3 | -5.87 | | -5.29 | | > -4.00 | |
| OVCAR-4 | -6.03 | | -4.59 | | > -4.00 | |
| OVCAR-5 | -5.39 | | -4.59 | | > -4.00 | |
| OVCAR-8 | -5.27 | | > -4.00 | | > -4.00 | |
| NCI/ADR-RES | -5.19 | | -4.05 | | > -4.00 | |
| SK-OV-3 | -4.93 | | -4.17 | | > -4.00 | |
| Renal Cancer | | | | | | |
| 786-0 | -5.23 | | -4.15 | | > -4.00 | |
| A498 | -5.86 | | -5.12 | | > -4.00 | |
| ACHN | -5.45 | | > -4.00 | | > -4.00 | |
| RXF 393 | -5.66 | | -5.10 | | > -4.00 | |
| SN12C | -5.50 | | -4.12 | | > -4.00 | |
| TK-10 | -4.96 | | > -4.00 | | > -4.00 | |
| UO-31 | -5.64 | | -4.21 | | > -4.00 | |
| Prostate Cancer | | | | | | |
| PC-3 | -5.74 | | -5.29 | | -4.32 | |
| DU-145 | -5.41 | | -4.39 | | > -4.00 | |
| Breast Cancer | | | | | | |
| MCF7 | -5.81 | | -4.81 | | > -4.00 | |
| MDA-MB-231/ATCC | -5.74 | | -5.13 | | > -4.00 | |
| HS 578T | -4.95 | | > -4.00 | | > -4.00 | |
| BT-549 | -5.75 | | -5.20 | | > -4.00 | |
| T-47D | -6.38 | | -4.74 | | > -4.00 | |
| MDA-MB-468 | -5.77 | | -5.24 | | -4.10 | |
| _MID | -5.59 | | -4.69 | | -4.12 | |
| Delta | 0.79 | | 0.84 | | 1.11 | |
| Range | 1.47 | | 1.53 | | 1.23 | |



| National Cancer Institute Developmental Therapeutics Program<br>Dose Response Curves | NSC : D - 795311/1 | SSPL:0YVT | EXP. ID:1702NS56 |
|---|---|---|---|
| | Report Date:March 20, 2017 | Test Date:February 13, 2017 | |



## Biological Evaluations

### *In vitro anti-proliferative activity assay*

The three examined cancer cell lines (non-small cell lung A-549, Breast MDA-MB-231 and colon HCT-116 cancer cells) were obtained from American Type Culture Collection (ATCC). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37 $^{o}$C in humidified atmosphere containing 5% $CO_2$. Cells at a concentration of 0.50 x $10^6$ were grown in a 25 $cm^2$ flask in 5 ml of culture medium.

The anti-proliferative activity of the tested compounds was measured *in vitro* using the Sulfo-Rhodamine-B stain (SRB) assay. Briefly, Cells were inoculated in 96-well microtiter plate ($5X10^4$ cells/ well) for 24 h before treatment with the tested compounds to allow attachment of cell to the wall of the plate. Tested compounds were dissolved in DMSO at 1 mg/ml immediately before use and diluted to the appropriate volume just before addition to the cell culture. Different concentrations of tested compounds, doxorubicin and sorafenib were added to the cells (three wells were prepared for each individual dose). Cells were incubated with the compounds for 48 h at 37°C and in atmosphere of 5% $CO_2$. After 48 h cells were fixed, washed, and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an ELISA reader. The relation between percent of surviving fraction and drug concentration is plotted to get the survival curve for each cell line. The concentration required for 50% inhibition of cell viability ($IC_{50}$) was calculated and the results are given in Table 1. The results were compared to the effect of the reference drug doxorubicin.

### *NCI-in vitro antitumor activity towards 59 cancer cell lines*

The cytotoxicity assays were performed at National Cancer Institute (NCI), Bethesda, USA (against 59 cell lines). The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96 well microtiter plates in 100 µ at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 $^{o}$C, 5% $CO_2$, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two

plates of each cell line were fixed *in situ* with trichloroacetic acid (TCA), to represent a measurement of the cell population for each cell line at the time of drug addition ($T_z$). Experimental drugs were solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/ml gentamicin. Additional four, 10-fold or ½ log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations. Triplicate wells were prepared for each individual dose. Following drug addition, the plates were incubated for an additional 48 h at 37 $^{o}$C, 5% $CO_2$, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 $^{o}$C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero (*Tz*), control growth (*C*), and test growth in the presence of drug at the five concentration levels ($T_i$)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as:

$[(T_i - T_z) / (C - T_z)]$ x 100 for concentrations for which $T_i \geq T_z$

$[(T_i - T_z) / T_z]$ x 100 for concentrations for which $T_i < T_z$

Three dose response parameters were calculated for each experimental compound: Growth inhibition of 50% ($GI_{50}$) was calculated when $[(T_i - T_z) / (C - T_z)]$ x 100 = 50. The compound concentration resulting in total growth inhibition (TGI) was calculated when $T_i = T_z$. The $LC_{50}$

indicating a net loss of cells following treatment was calculated when $[(T_i - T_z) / T_z] \times 100 = -50$.

### *4.2.4. Cell Cycle Analysis*

T-47D cells were treated with conjugate **8a** for 24 h (at its $IC_{50}$ concentration), and then cells were washed twice with ice-cold phosphate buffered saline (PBS). Subsequently, the treated cells were collected by centrifugation, fixed in ice-cold 70% (*v*/*v*) ethanol, washed with PBS, re-suspended with 100 µg/mL RNase, stained with 40 µg/mL PI, and analyzed by flow cytometry using FACS Calibur (Becton Dickinson, BD, Franklin Lakes, NJ, USA). The cell cycle distributions were calculated using CellQuest software 5.1 (Becton Dickinson).

### *Annexin V-FITC Apoptosis Assay*

Phosphatidylserine externalization was assayed using Annexin V-FITC/PI apoptosis detection kit (BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions. T-47D cells were cultured to a monolayer then treated with conjugate **8a** at its $IC_{50}$ concentration. Cells were then harvested via trypsinization, and rinsed twice in PBS followed by binding buffer. Moreover, cells were re-suspended in 100 µL of binding buffer with the addition of 1 µL of FITC-Annexin V (Becton Dickinson BD Pharmingen™, Heidelberg, Germany) followed by an incubation period of 30 min at 4 °C. Cells were then rinsed in binding buffer and resuspended in 150 µL of binding buffer with the addition of 1 µL of DAPI (1 µg/µL in PBS) (Invitrogen, Life Technologies, Darmstadt, Germany). Cells were then analyzed using the flow cytometer BD FACS Canto II (BD Biosciences, San Jose, CA, USA) and the results were interpreted with FlowJo7.6.4 software (Tree Star, Ashland, OR, USA).

### *CDK2 inhibition assay*

The kinase was assayed with histone H1 in the presence of 15 µM ATP, 0.05 µCi [$\gamma$-$^{33}$P]ATP and of the test compound in a final volume of 10 µL, all in a reaction buffer (60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, 2.5 µg / 50 µl $PEG_{20.000}$). The reactions were stopped by adding 5 µL of 3 % aq. $H_3PO_4$. Aliquots were spotted onto P-81 phosphocellulose (Whatman), washed 3× with 0.5 % aq. $H_3PO_4$ and finally air-dried. Kinase inhibition was quantified using a FLA-7000 digital image analyzer (Fujifilm).

*5.1.2.1. 1-Ethyl-3-(hydrazonomethyl)-1H-indole* (**5a**)

Yield 64 %, MP: 139-140 °C; $^{1}$H NMR $\delta$ *ppm*: 1.32, 1.38 (t, 3H, *J* = 8 Hz, *N*-$CH_2$-C$\underline{H}_3$), 4.12, 4.22 (t, 2H, *J* = 8 Hz, *N*-C$\underline{H}_2$-$CH_3$), 6.24 (brs, 2H, $NH_2$), 7.23-7.31 (m, 2H, H-5 and H-6 of indol), 7.54 (d, 1H, *J* = 8 Hz, H-7 of indol), 7.94 (s, 1H, H-2 of indol), 8.41 (d, 1H, *J* = 8 Hz, H-4 of indol), 8.91 (s, 1H, -C$\underline{H}$=N-); IR: 3378 ($NH_2$); Anal. calcd. For $C_{11}H_{13}N_3$: C, 70.56; H, 7.00; N, 22.44; Found C, 70.32; H, 6.97; N, 22.61.

*5.1.2.2. 3-(Hydrazonomethyl)-1-isopropyl-1H-indole* (**5c**)

Yield 57 %, MP: 186-188°C; $^{1}$H NMR $\delta$ *ppm*: 1.51 (d, 6H, -CH(C$\underline{H}_3$)$_2$), 4.77-4.85 (m, 1H, *N*-CH), 7.06 (brs, 2H, $NH_2$), 7.20-7.30 (m, 2H, H-5 and H-6 of indol), 7.60 (d, 1H, *J* = 8 Hz, H-7 of indol), 8.07 (s, 1H, H-2 of indol), 8.36 (d, 1H, *J* = 8 Hz, H-4 of indol), 8.89 (s, 1H, -C$\underline{H}$=N-); $^{13}$C NMR $\delta$ *ppm*: 22.82 (-CH(C$\underline{H}_3$)$_2$), 47.55 (*N*-CH),, 110.95, 112.02, 121.30, 122.76, 123.07, 125.80, 131.21, 137.08, 155.13; IR: 3342 ($NH_2$); Anal. calcd. For $C_{12}H_{15}N_3$: C, 71.61; H, 7.51; N, 20.88; Found C, 71.87; H, 7.50; N, 20.79.

*5.1.2.3. 1-Allyl-3-(hydrazonomethyl)-1H-indole* (**5d**)

Yield 73 %, MP: 177-180°C; $^{1}$H NMR $\delta$ *ppm*: 4.90 (d, 2H, *J*= 8 Hz, *N*-$CH_2$), 5.10-5.11 (m, 1H, oliefinic H), 5.20-5.24 (m, 1H, oliefinic H), 6.01-6.11 (m, 1H, oliefinic H), 7.06 (brs, 2H, $NH_2$), 7.20-7.30 (m, 2H, Ar-H), 7.52-7.59 (m, 1H, Ar-H), 7.92-7.96 (m, 1H, Ar-H), 8.37-8.40 (m, 1H, Ar-H), 8.89-8.91 (m, 1H, Ar-H); $^{13}$C NMR $\delta$ *ppm*: 47.90, 48.86 (N-CH2), 110.57, 110.93, 111.14, 112.03, 112.05, 117.81, 120.21, 121.23, 121.38, 122.82, 123.15, 123.25, 125.75, 125.81, 134.29, 134.64, 134.87, 134.95, 137.55, 137.59, 155.05, 155.20; IR: 3362 ($NH_2$); HRMS (ESI) for $C_{12}H_{14}N_3$, calcd 200.11822, found 200.11849 $[M+H]^+$; Anal. calcd. For $C_{12}H_{13}N_3$: C, 72.33; H, 6.58; N, 21.09; Found C, 72.20; H, 6.55; N, 21.15.

*5.1.3.1. 5-Chloro-3-(((1-ethyl-1H-indol-3-yl)methylene)hydrazono)indolin-2-one* (**7**)

Yield 78 %, MP: 202-203 °C; $^{1}$H NMR $\delta$ *ppm*: 1.44 (t, 3H, *J* = 8 Hz, -$CH_2$-C$\underline{H}_3$), 4.33 (q, 2H, *J* = 8 Hz, $CH_2$), 6.93-6.96 (m, 1H, Ar-H), 7.34-7.39 (m, 1H, Ar-H), 7.45-7.55 (m, 2H, Ar-H), 7.71 (d, 1H, *J* = 8 Hz, Ar-H), 8.30-8.35 (m, 2H, Ar-H), 8.50 (s, 1H, Ar-H), 9.00 (s, 1H, Ar-H), 10.93 (s, 1H, NH); $^{13}$C NMR $\delta$ *ppm*: 15.54 ($CH_3$), 41.77 ($CH_2$), 111.88, 111.91, 112.52, 113.09,

117.58, 118.73, 121.88, 122.66, 124.19, 125.44, 126.07, 126.54, 127.57, 128.44, 132.42, 134.41, 137.82, 139.63, 143.26, 144.73, 146.28, 148.80, 163.37 (C=O); IR: 3401 (NH), 1698 (C=O); Anal. calcd. For $C_{19}H_{15}ClN_4O$: C, 65.05; H, 4.31; N, 15.97; Found C, 65.18; H, 4.30; N, 16.05.

*5.1.3.2. 5-Methyl-3-(((1-propyl-1H-indol-3-yl)methylene)hydrazono)indolin-2-one* (**8a**)

Yield 74 %, MP: 195-197 °C; $^1$H NMR $\delta$ *ppm*: 0.87 (t, 3H, *J* = 8 Hz, -$CH_2C\underline{H_3}$), 1.79-189 (m, 2H, -$C\underline{H_2}CH_3$), 2.20 (s, 3H, $CH_3$ of isatin), 4.22 (t, 2H, *J* = 8 Hz, *N*-$CH_2$), 6.80 (m, 1H, Ar-H), 7.19 (t, 1H, *J* = 8 Hz, Ar-H), 7.33 (d, 2H, *J* = 8 Hz, Ar-H), 7.67 (d, 1H, *J* = 8 Hz, Ar-H), 8.26 (s, 1H, Ar-H), 8.34 (s, 1H, Ar-H), 8.40 (d, 1H, *J* = 8 Hz, Ar-H), 8.95 (s, 1H, Ar-H), 10.66 (s, 1H, NH); IR: 3375 (NH), 1701 (C=O); Anal. calcd. For $C_{21}H_{20}N_4O$: C, 73.23; H, 5.85; N, 16.27; Found C, 72.97; H, 5.88; N, 16.32.

*5.1.3.3. 3-(((1-propyl-1H-indol-3-yl)methylene)hydrazono)-5-(trifluoromethoxy)indolin-2-one* (**8b**)

Yield 77 %, MP: 189-190 °C; $^1$H NMR $\delta$ *ppm*: 0.89 (t, 3H, *J* = 8 Hz, -$CH_2C\underline{H_3}$), 1.83-192 (m, 2H, -$C\underline{H_2}CH_3$), 4.28 (t, 2H, *J* = 8 Hz, *N*-$CH_2$), 7.01-7.05 (m, 1H, Ar-H), 7.25 (t, 1H, *J* = 8 Hz, Ar-H), 7.38 (t, 1H, *J* = 8 Hz, Ar-H), 7.48 (t, 1H, *J* = 8 Hz, Ar-H), 7.72 (d, 1H, *J* = 8 Hz, Ar-H), 8.27 (d, 1H, *J* = 8 Hz, Ar-H), 8.34 (s, 1H, Ar-H), 8.44-4.46 (m, 1H, Ar-H), 9.03 (s, 1H, Ar-H), 10.96 (s, 1H, NH); $^{13}$C NMR $\delta$ *ppm*: 11.51 (-$CH_3$), 23.23 ($CH_3$-$\underline{C}H_2$), 48.37 (*N*-$CH_2$), 111.78, 114.15, 116.06, 119.48, 120.68, 122.40, 123.91, 125.35, 126.26, 127.78, 128.72, 131.61, 133.46, 133.98, 136.11, 140.60, 143.58, 163.98 (C=O); IR: 3391 (NH), 1699 (C=O); HRMS (ESI) for $C_{21}H_{18}F_3N_4O_2$, calcd 415.13764, found 415.13783 $[M+H]^+$; Anal. calcd. For $C_{21}H_{17}F_3N_4O_2$: C, 60.87; H, 4.14; N, 13.52; Found C, 60.69; H, 4.11; N, 13.50.

*5.1.3.4. 5-Nitro-3-(((1-propyl-1H-indol-3-yl)methylene)hydrazono)indolin-2-one* (**8c**)

Yield 83 %, MP: 247-250°C; $^1$H NMR $\delta$ *ppm*: 0.86-.92 (m, 3H, -$CH_2C\underline{H_3}$), 1.78-191 (m, 2H, -$C\underline{H_2}CH_3$), 4.18,426 (t, 2H, *J* = 8 Hz, *N*-$CH_2$), 7.07 (d, 0.5H, *J* = 8 Hz, Ar-H), 7.19-7.29 (m, 1.5H, Ar-H), 7.39 (d, 1H, *J* = 8 Hz, Ar-H), 7.56, 7.70 (d, 1H, *J* = 8 Hz, Ar-H), 7.94 (s, 1H, Ar-H), 8.29-8.47 (m, 2H, Ar-H), 8.87 (s, 1H, Ar-H), 9.06, 9.36 (s, 1H, Ar-H), 11.45, 11.65 (s, 1H, NH); IR: 3396 (NH), 1710 (C=O); Anal. calcd. For $C_{20}H_{17}N_5O_3$: C, 63.99; H, 4.56; N, 18.66; Found C, 64.21; H, 4.54; N, 18.60.

*5.1.3.5. 3-(( (1-Isopropyl-1H-indol-3-yl)methylene)hydrazono)indolin-2-one* (**9**)

Yield 78 %, MP: 260-261°C; $^{1}$H NMR $\delta$ *ppm*: 1.53 (brs, 6H, -CH(C$\underline{H}_3$)$_2$), 4.85 (brs, 1H, *N*-CH), 6.87-6.97 (m, 2H, Ar-H), 7.07-7.23 (m, 2H, Ar-H), 7.30-7.46 (m, 2H, Ar-H), 7.70-7.95 (m, 1H, Ar-H), 7.36-7.46 (m, 1H, Ar-H), 8.74-8.94 (m, 1H, Ar-H), 9.55 (s, 1H, Ar-H), 10.55 (s, 1H, NH); IR: 3391 (NH), 1700 (C=O); Anal. calcd. For $C_{20}H_{18}N_4O$: C, 72.71; H, 5.49; N, 16.96; Found C, 72.60; H, 5.48; N, 17.02.

*5.1.3.6. 3-(((1-Allyl-1H-indol-3-yl)methylene)hydrazono)indolin-2-one* (**10a**)

Yield 80 %, MP: 220-222 °C; $^{1}$H NMR $\delta$ *ppm*: 4.97 (d, 2H, *J* = 8 Hz, *N*-CH$_2$), 5.15-5.19 (m, 1H, Ar-H), 5.24-5.27 (m, 1H, Ar-H), 6.04-6.13 (m, 1H, Ar-H), 6.77 (d, 0.5H, *J* = 8 Hz, Ar-H), 6.92 (d, 1H, *J* = 8 Hz, Ar-H), 7.07 (t, 1H, *J* = 8 Hz, Ar-H), 7.36-7.38 (m, 2H, Ar-H), 7.62-7.64 (m, 1H, Ar-H), 7.68 (d, 0.5H, *J* = 8 Hz, Ar-H), 8.26 (s, 1H, Ar-H), 8.31-8.35 (m, 1H, Ar-H), 8.37 (d, 1H, *J* = 8 Hz, Ar-H), 8.96 (s, 1H, Ar-H), 10.78 (s, 1H, NH); IR: 3410 (NH), 1701 (C=O); Anal. calcd. For $C_{20}H_{16}N_4O$: C, 73.15; H, 4.91; N, 17.06; Found C, 73.27; H, 4.95; N, 16.95.

*5.1.3.7. 3-(((1-Allyl-1H-indol-3-yl)methylene)hydrazono)-5-fluoroindolin-2-one* (**10b**)

Yield 77 %, MP: 235-237 °C; $^{1}$H NMR $\delta$ *ppm*: 4.99-5.03 (m, 2H, *N*-CH$_2$), 5.15-5.27 (m, 2H, Ar-H), 6.05-6.12 (m, 1H, Ar-H), 6.91-6.94 (m, 1H, Ar-H), 7.31-7.36 (m, 3H, Ar-H), 8.15-8.19 (m, 1H, Ar-H), 8.28-8.34 (m, 2H, Ar-H), 8.98-9.03 (m, 2H, Ar-H), 10.91 (s, 1H, NH); $^{13}$C NMR $\delta$ *ppm*: 47.79 (CH$_2$), 112.58, 114.55, 115.61, 115.87, 116.79, 117.71, 118.39, 121.33, 121.56, 122.76, 124.16, 125.52, 127.86, 130.11, 133.85, 142.32, 146.46, 159.21, 164.01 (C=O); IR: 3394 (NH), 1697 (C=O); HRMS (ESI) for $C_{20}H_{16}FN_4O$, calcd 347.13027, found 347.13019 $[M+H]^+$; Anal. calcd. For $C_{20}H_{15}FN_4O$: C, 69.35; H, 4.37, N, 16.18; Found C, 69.51; H, 4.39, N, 16.25.

*5.1.3.8. 3-(((1-Allyl-1H-indol-3-yl)methylene)hydrazono)-7-fluoroindolin-2-one* (**10c**)

Yield 82 %, MP: 208-210 °C; $^{1}$H NMR $\delta$ *ppm*: 4.98 (d, 2H, *J* = 8 Hz, *N*-CH$_2$), 5.15-5.21(m, 1H, Ar-H), 5.24-5.28 (m, 1H, Ar-H), 6.04-6.14 (m, 1H, Ar-H), 7.03-7.09 (m, 2H, Ar-H), 7.37-7.40 (m, 5H, Ar-H), 8.30 (s, 1H, Ar-H), 8.99 (s, 1H, Ar-H), 11.55 (s, 1H, NH); $^{13}$C NMR $\delta$ *ppm*: 49.52 (CH$_2$), 112.04, 118.39, 118.79, 121.68, 121.86, 121.95, 122.90, 123.85, 123.91, 124.08, 124.90, 130.38, 132.63, 132.77, 133.84, 137.99, 141.29, 144.75, 163.58 (C=O); IR: 3380 (NH),

1702 (C=O); Anal. calcd. For $C_{20}H_{15}FN_4O$: C, 69.35; H, 4.37, N, 16.18; Found C, 69.21; H, 4.32, N, 16.15.

*5.1.3.9. 3-(((1-Allyl-1H-indol-3-yl)methylene)hydrazono)-5-chloroindolin-2-one* (**10d**)

Yield 75 %, MP: 267-269 °C; $^1$H NMR $\delta$ *ppm*: 4.99 (d, 2H, *J* = 8 Hz, *N*-$CH_2$), 5.16-5.22 (m, 1H, Ar-H), 5.25-5.28 (m, 1H, Ar-H), 6.05-6.14 (m, 1H, Ar-H), 6.94 (d, 2H, *J* = 8 Hz, Ar-H), 7.33-7.39 (m, 1H, Ar-H), 7.45-7.55 (m, 2H, Ar-H), 7.66-7.69 (m, 1H, Ar-H), 8.30(s, 1H, Ar-H), 8.50-8.53 (m, 1H, Ar-H), 9.02 (s, 1H, Ar-H), 10.91 (s, 1H, NH); $^{13}$C NMR $\delta$ *ppm*: 49.26 ($CH_2$), 112.11, 112.53, 113.12, 117.59, 118.45, 121.86, 122.70, 124.24, 126.10, 127.62, 128.44, 131.35, 132.50, 133.80, 137.73, 140.02, 142.13, 143.35, 163.17 (C=O); IR: 3401 (NH), 1699 (C=O); HRMS (ESI) for $C_{20}H_{16}ClN_4O$, calcd 363.10072, found 363.10054 $[M+H]^+$; Anal. calcd. For $C_{20}H_{15}ClN_4O$: C, 66.21; H, 4.17, N, 15.44; Found C, 66.34; H, 4.13, N, 15.39.

*5.1.3.10. 3-(((1-Allyl-1H-indol-3-yl)methylene)hydrazono)-5-(trifluoromethoxy)indolin-2-one* (**10e**)

Yield 80 %, MP: 228-229 °C; $^1$H NMR $\delta$ *ppm*: 4.98 (d, 2H, *J* = 8 Hz, *N*-$CH_2$), 5.17-5.22 (m, 1H, Ar-H), 5.25-5.28 (m, 1H, Ar-H), 6.04-6.14 (m, 1H, Ar-H), 7.0-7.03 (m, 2H, Ar-H), 7.25-7.29 (m, 1H, Ar-H), 7.37-7.7.44 (m, 2H, Ar-H), 7.47-7.53 (m, 1H, Ar-H), 7.65-7.67 (m, 1H, Ar-H), 8.31(s, 1H, Ar-H), 9.05 (s, 1H, Ar-H), 10.98 (s, 1H, NH); $^{13}$C NMR (DMSO-$d_6$) $\delta$ *ppm*: 49.27 ($CH_2$), 112.10, 112.72, 116.96, 118.02, 118.47, 120.73, 121.89, 122.47, 124.30, 126.29, 127.95, 133.76, 138.12, 140.38, 143.27, 143.64, 145.07, 146.94, 148.95, 163.91 (C=O); IR: 3398 (NH), 1711 (C=O); HRMS (ESI) for $C_{21}H_{16}F_3N_4O_2$, calcd 413.12199, found 413.12260 $[M+H]^+$; Anal. calcd. For $C_{21}H_{15}F_3N_4O_2$: C, 61.17; H, 3.67; N, 13.59; Found C, 61.01; H, 3.63; N, 13.55.