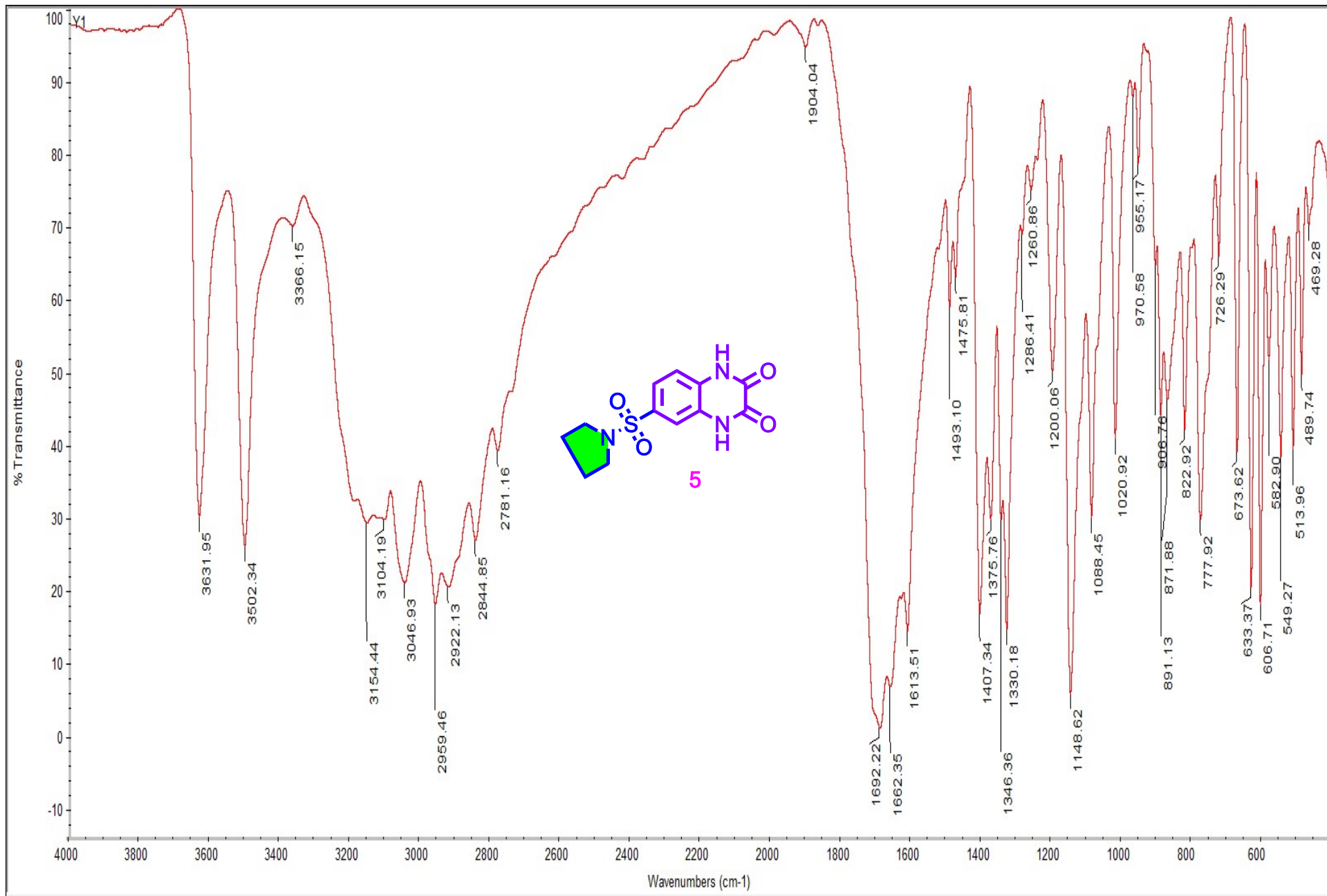


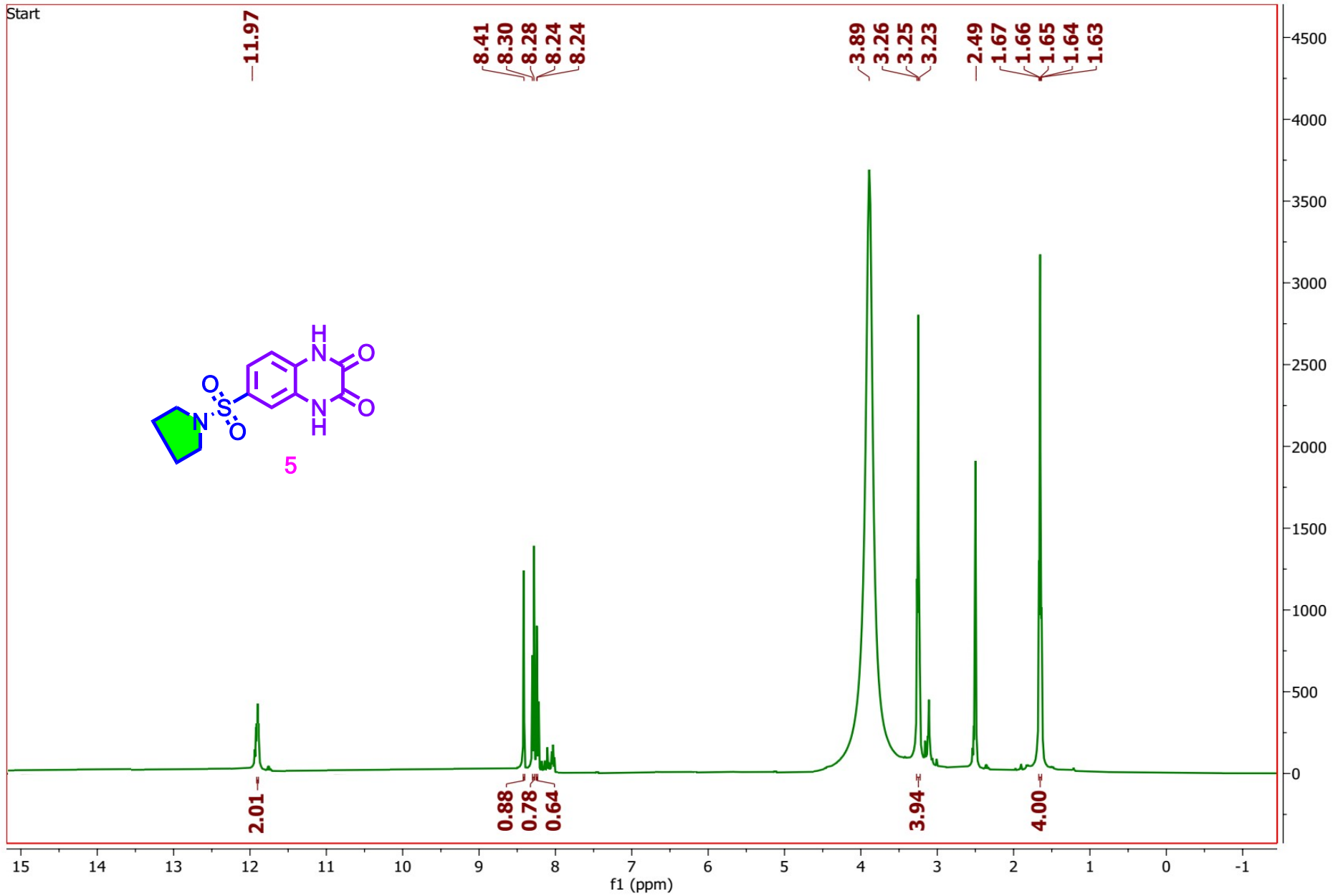
A new class of anti-proliferative activity and apoptotic inducer with molecular docking studies for a novel of 1,3-dithiolo[4,5-*b*]quinoxaline derivatives hybrid with a sulfonamide moiety

Mostafa A. Ismail ¹, Moustafa S. Abusaif ², Mohamed S. A. El-Gaby ^{2,*}, Yousry A. Ammar ^{2,*} and Ahmed Ragab ^{2,*}

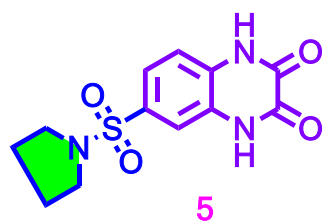
¹Chemistry Department, Faculty of Science, Al-Azhar University, Assiut, 71524, Egypt

²Chemistry Department, Faculty of Science (Boys), Al-Azhar University, Nasr City, Cairo, 11884, Egypt





C NMR



167.44

139.53

138.99

130.03

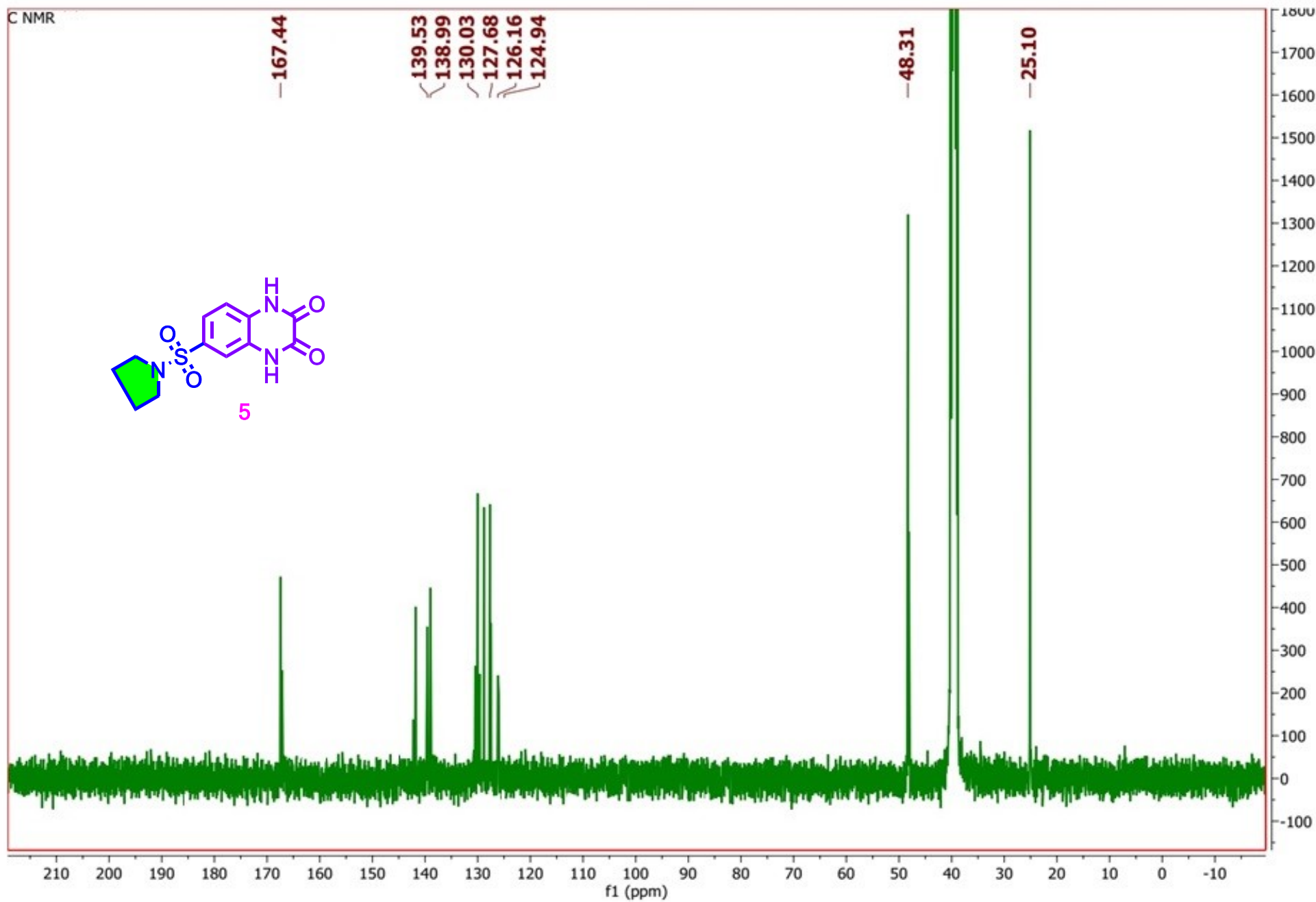
127.68

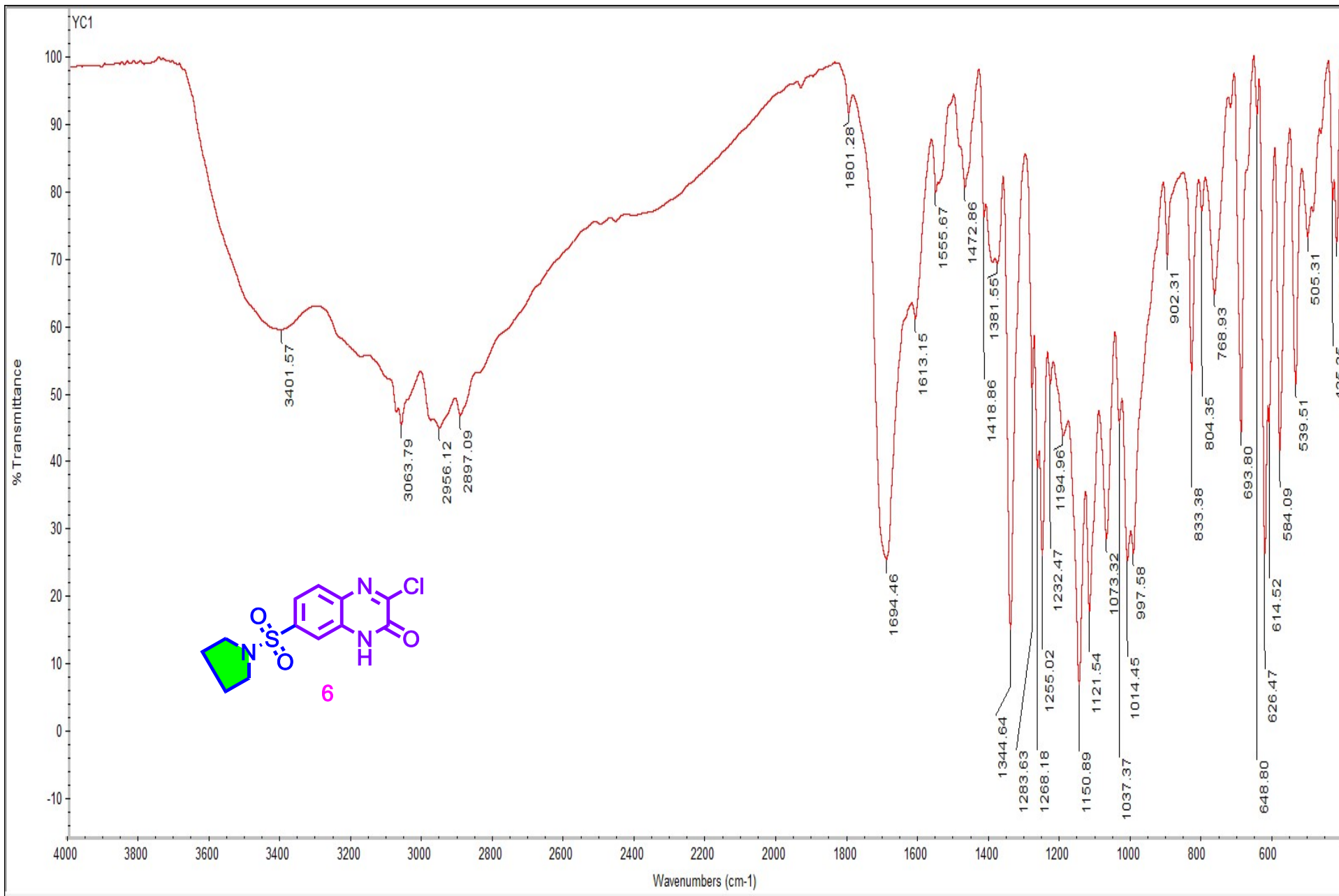
126.16

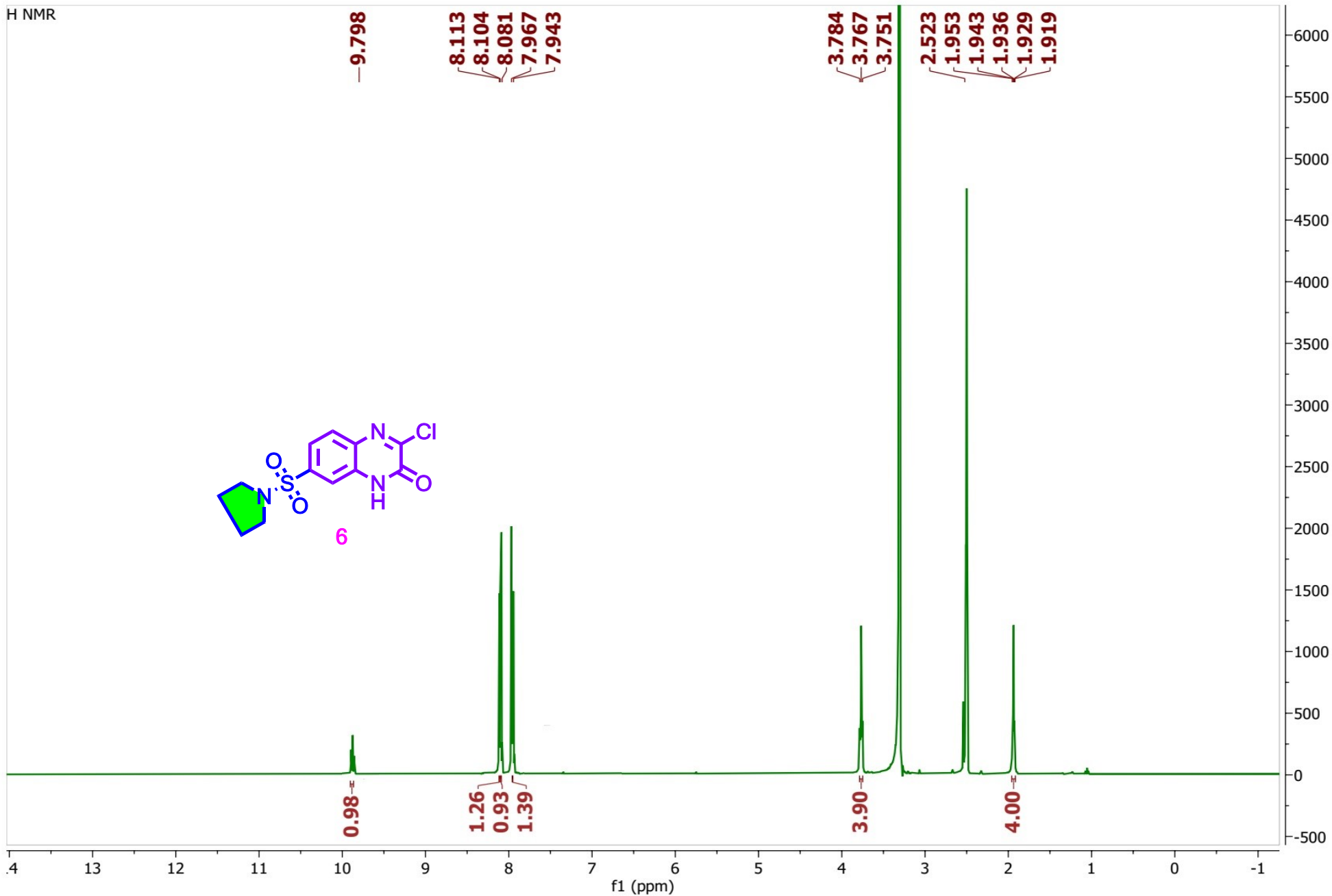
124.94

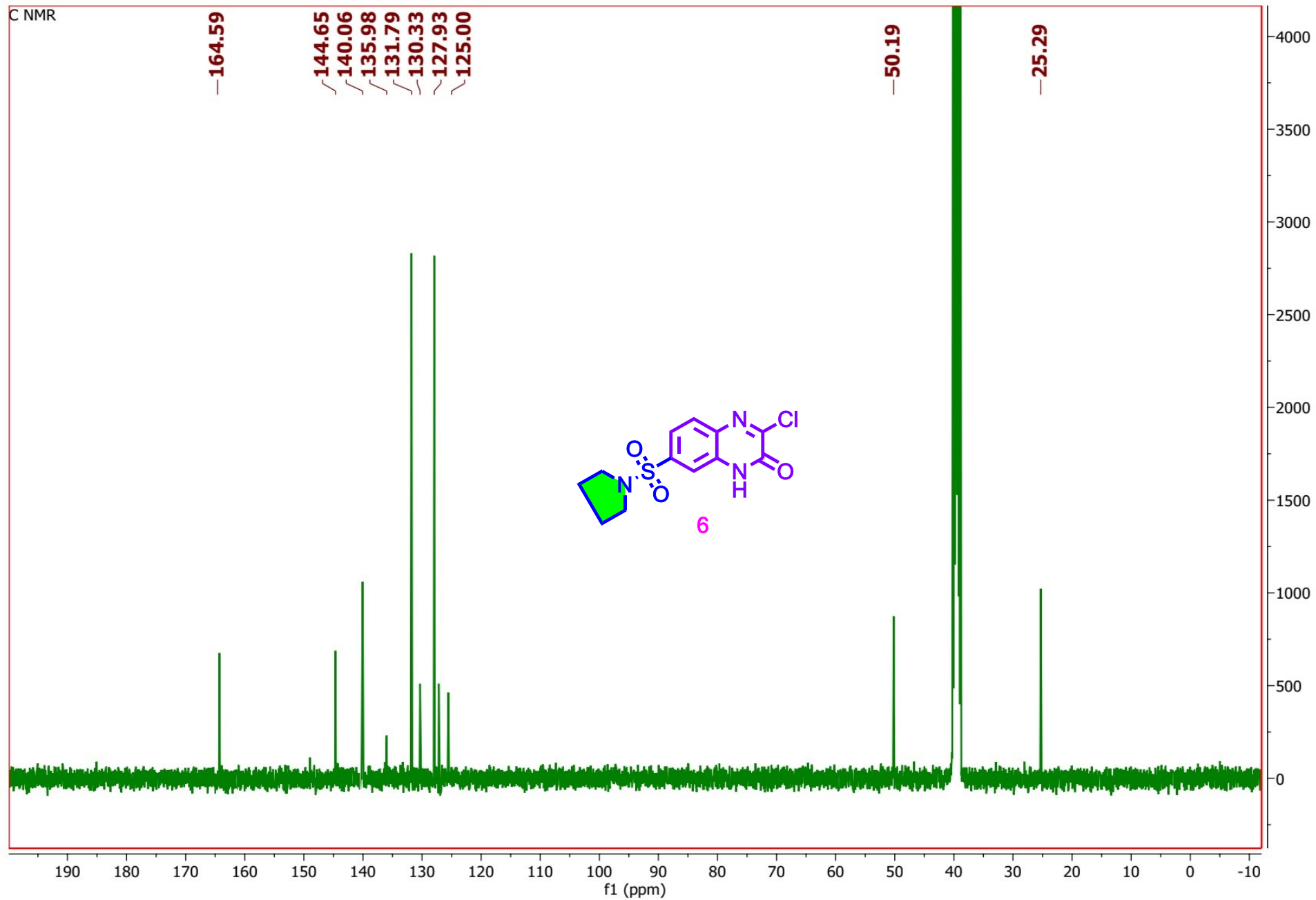
48.31

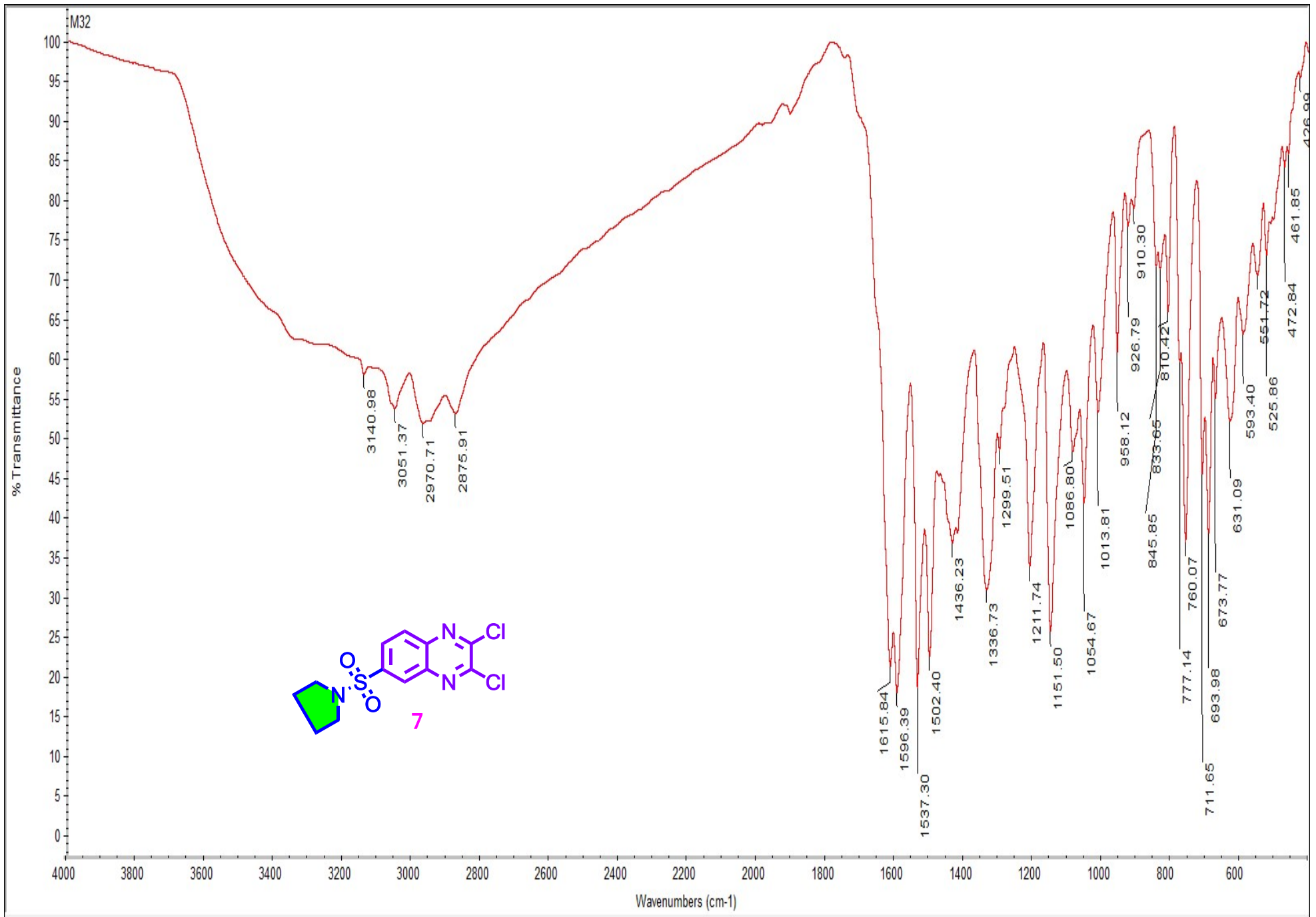
25.10



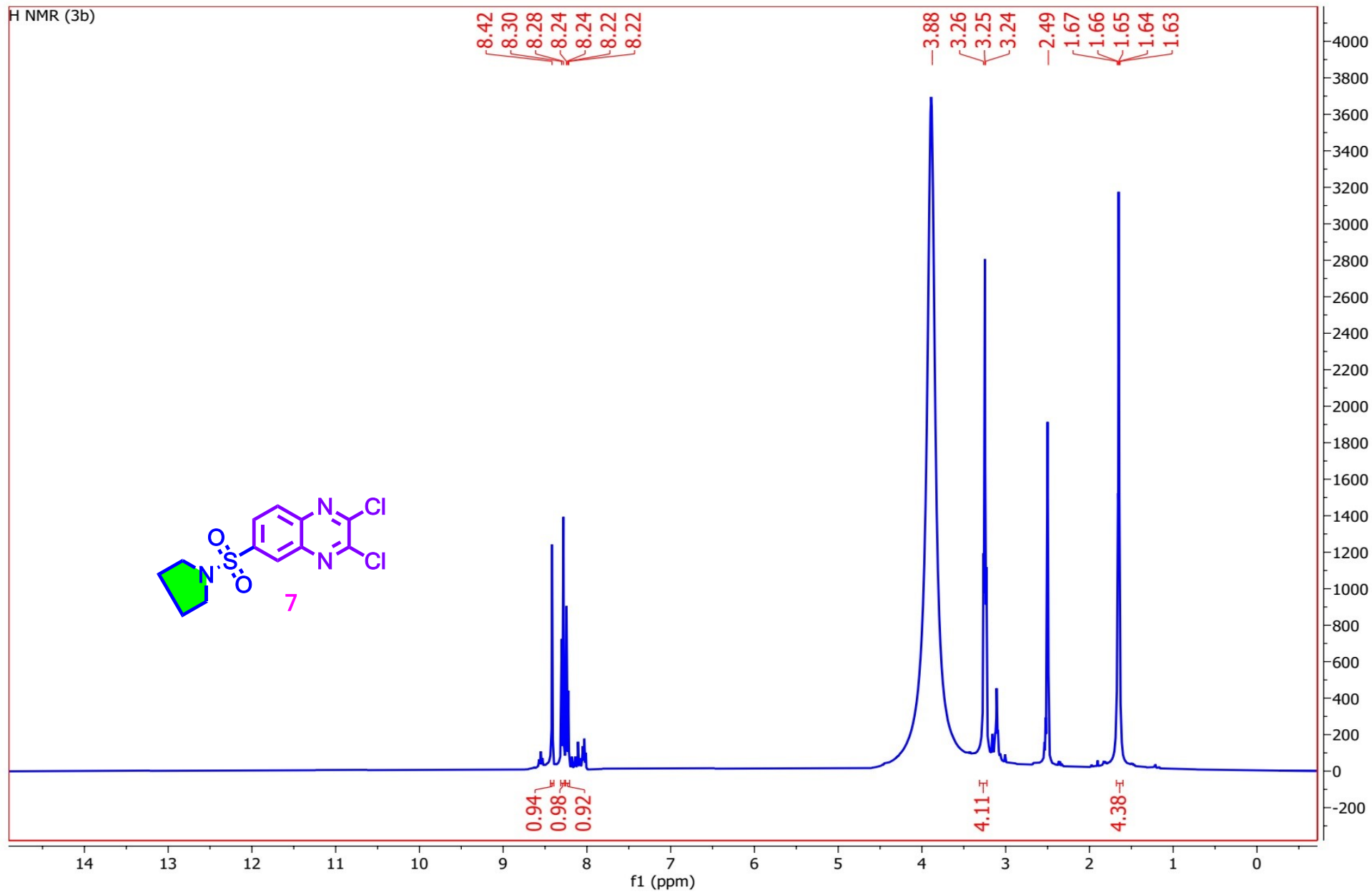








H NMR (3b)

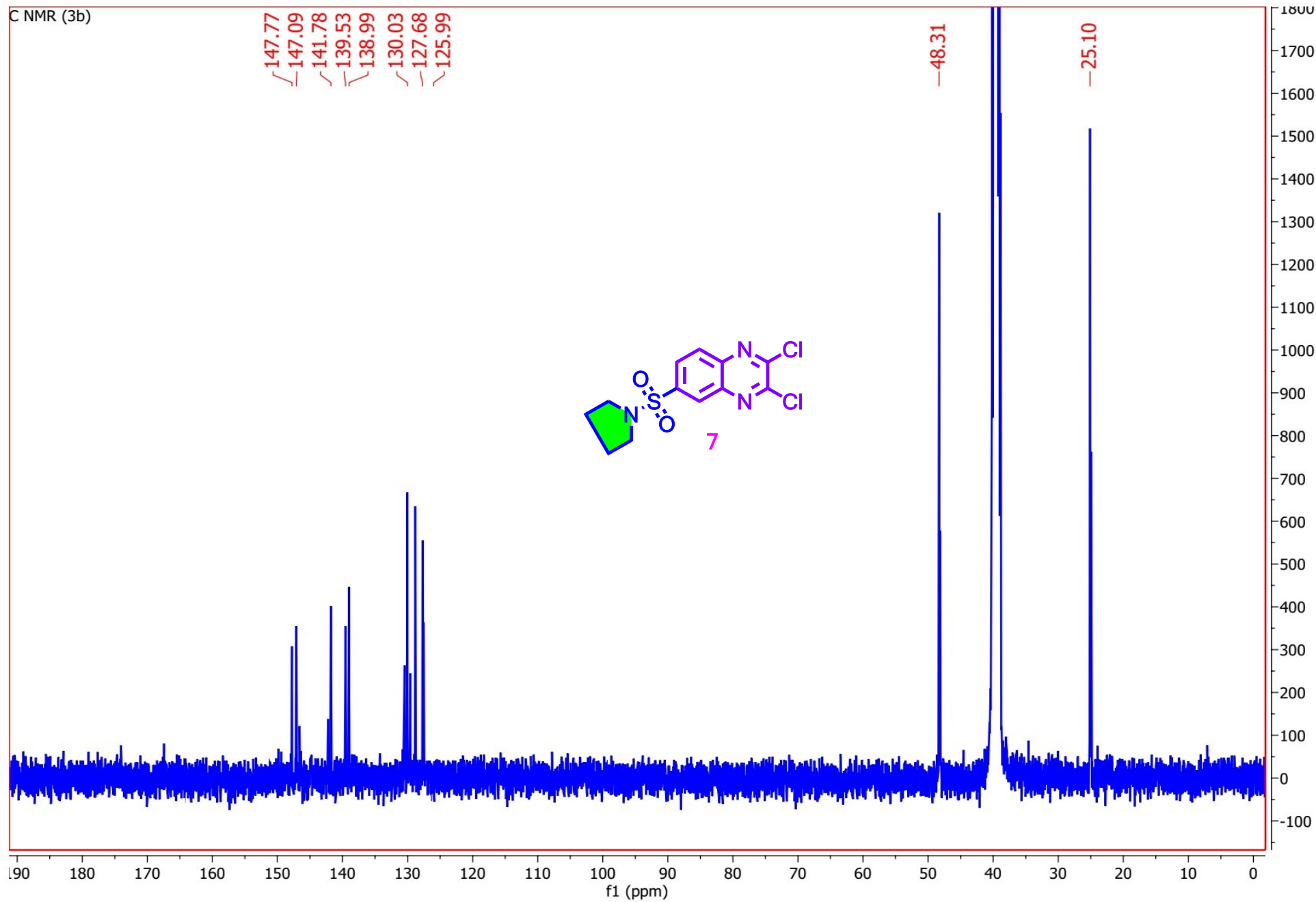
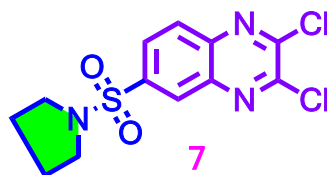


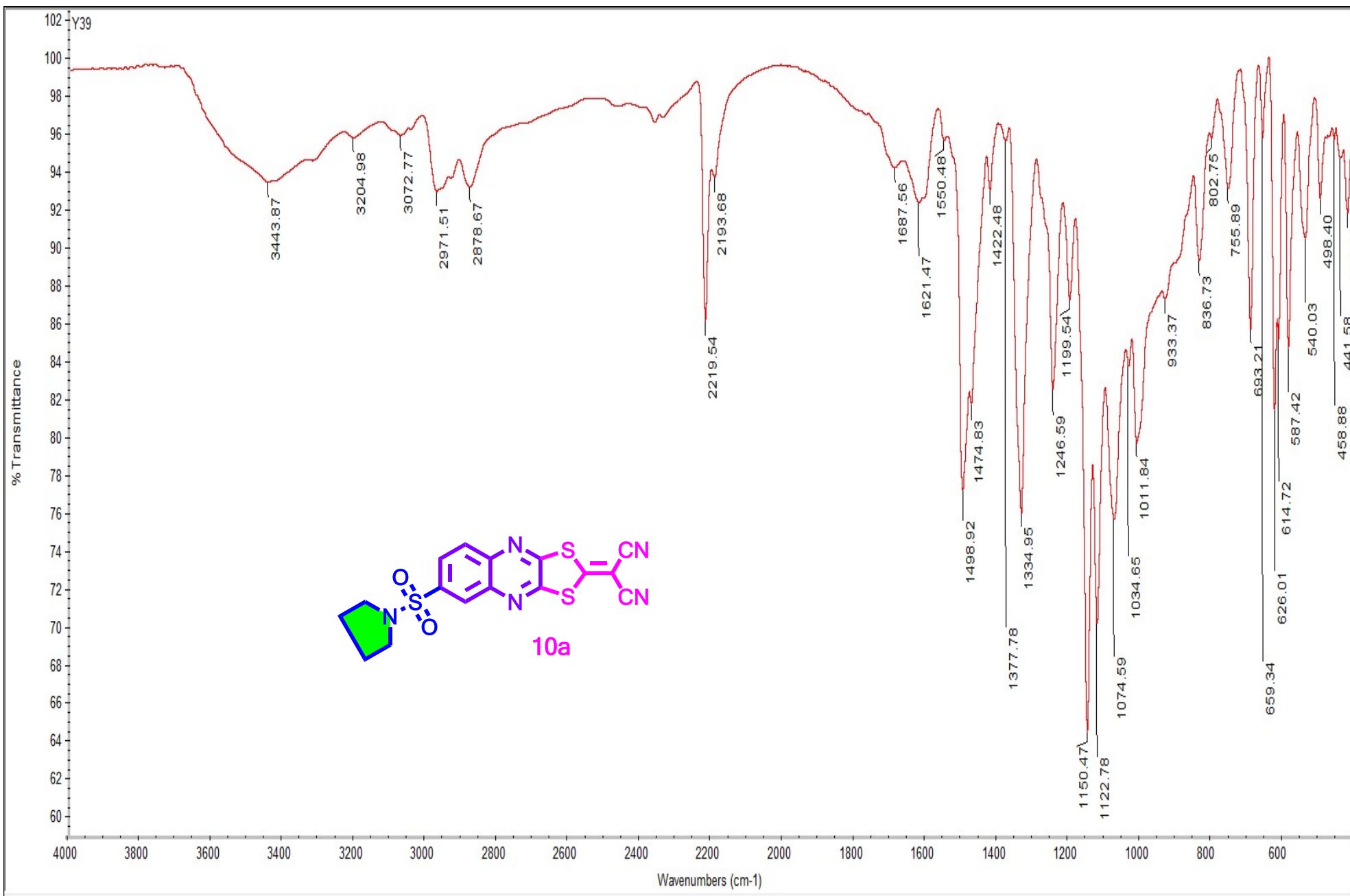
C NMR (3b)

147.77
147.09
141.78
139.53
138.99
130.03
127.68
125.99

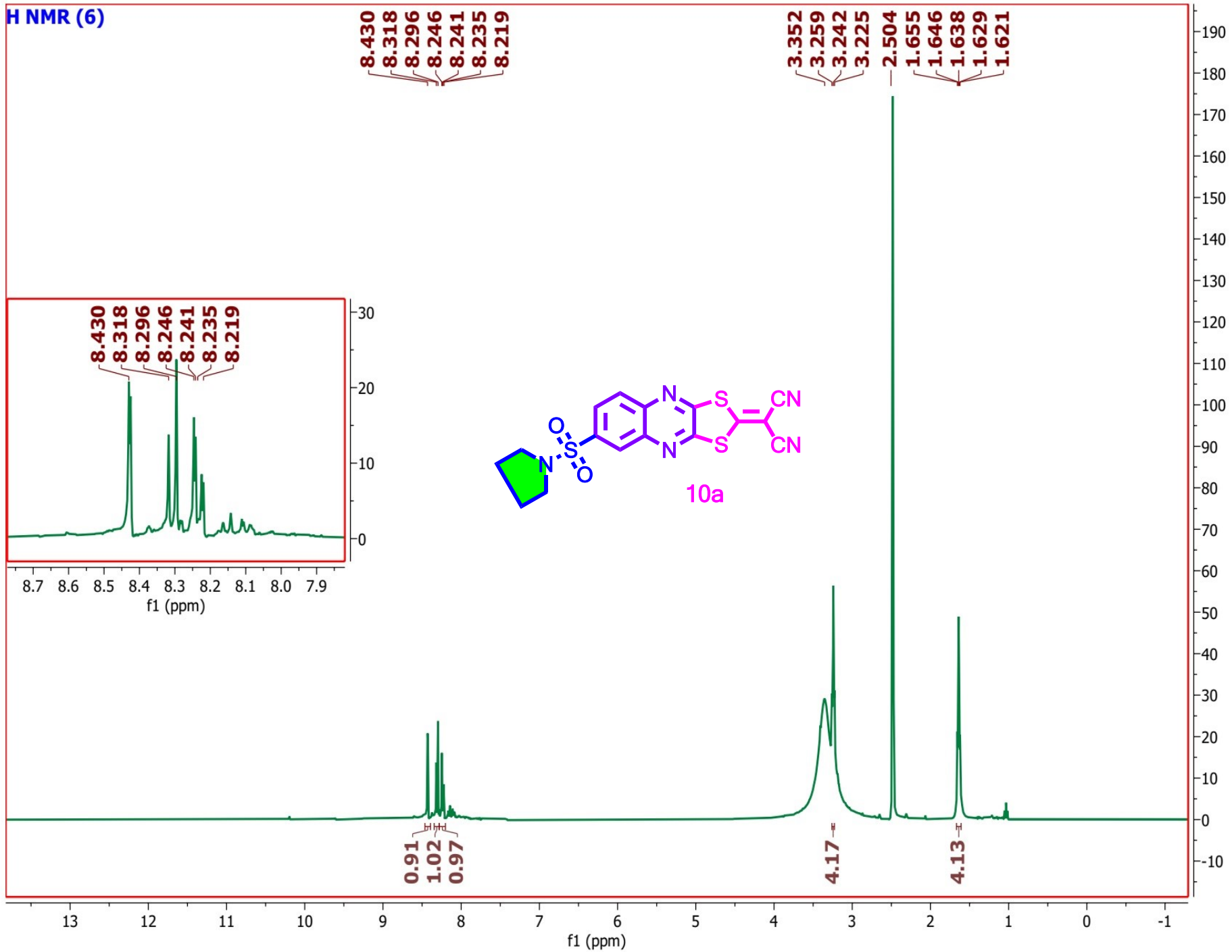
48.31

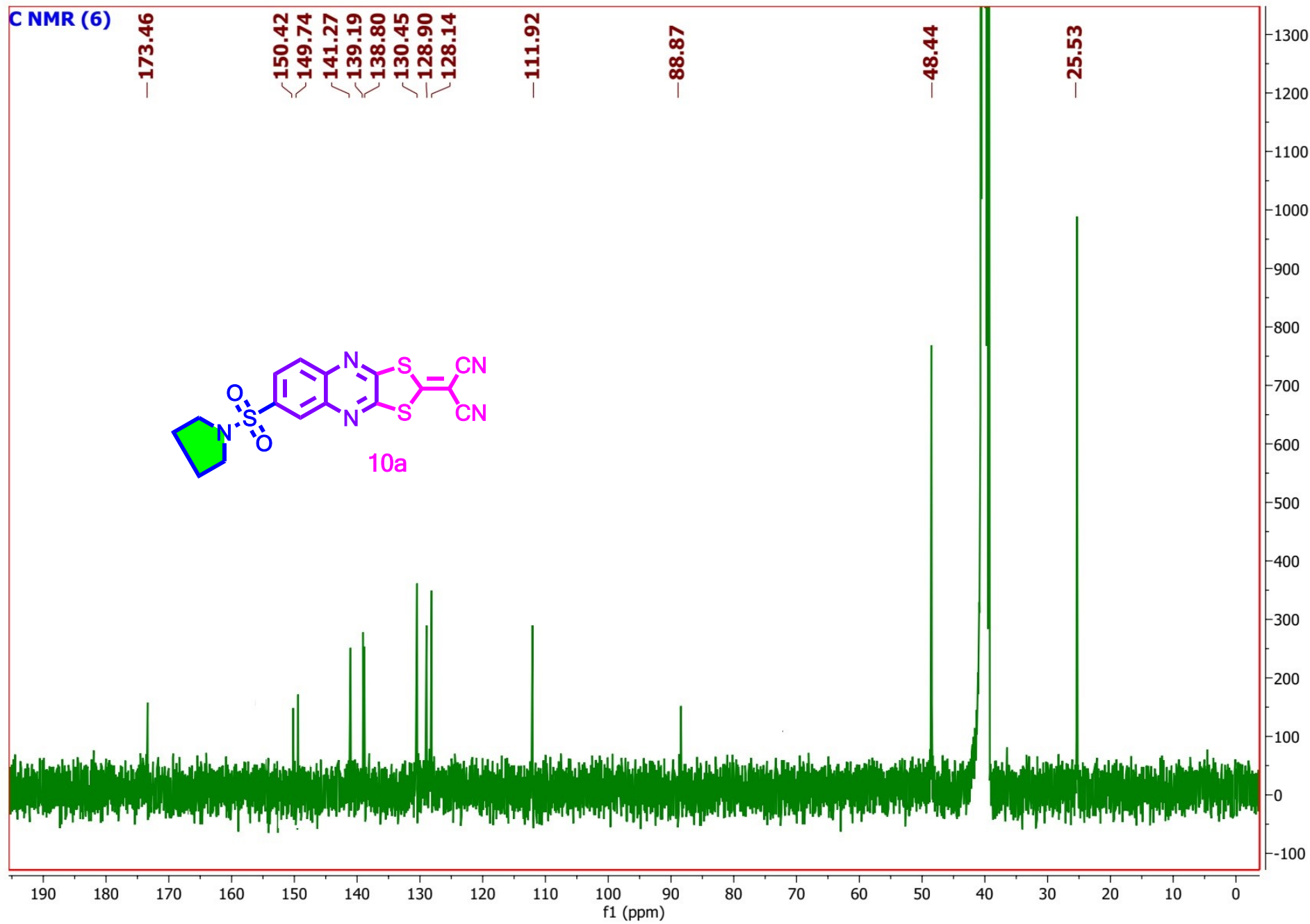
25.10





H NMR (6)

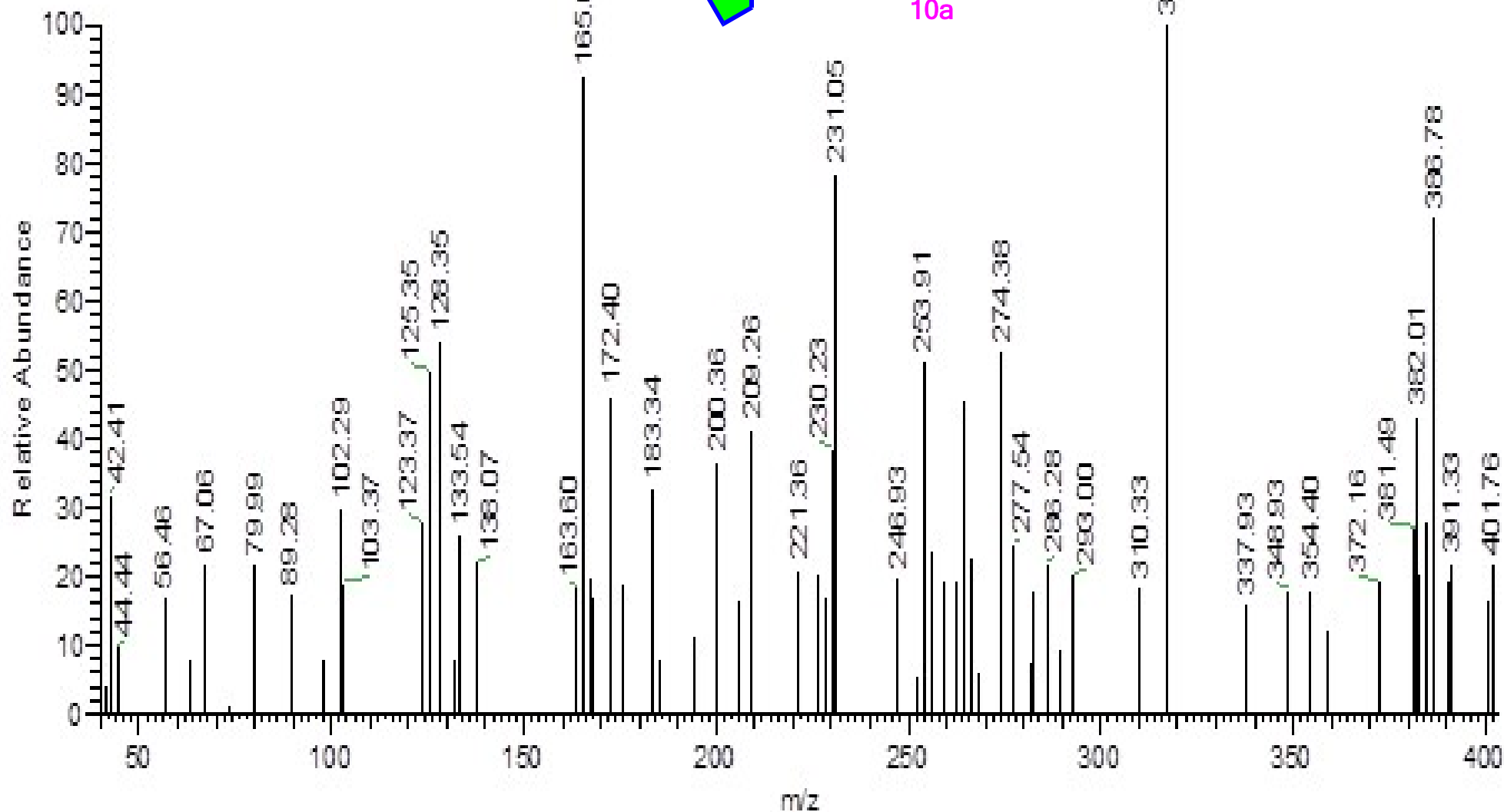
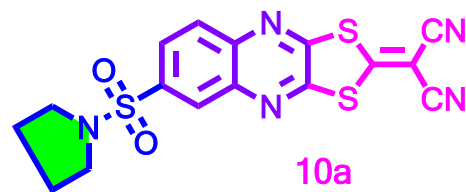


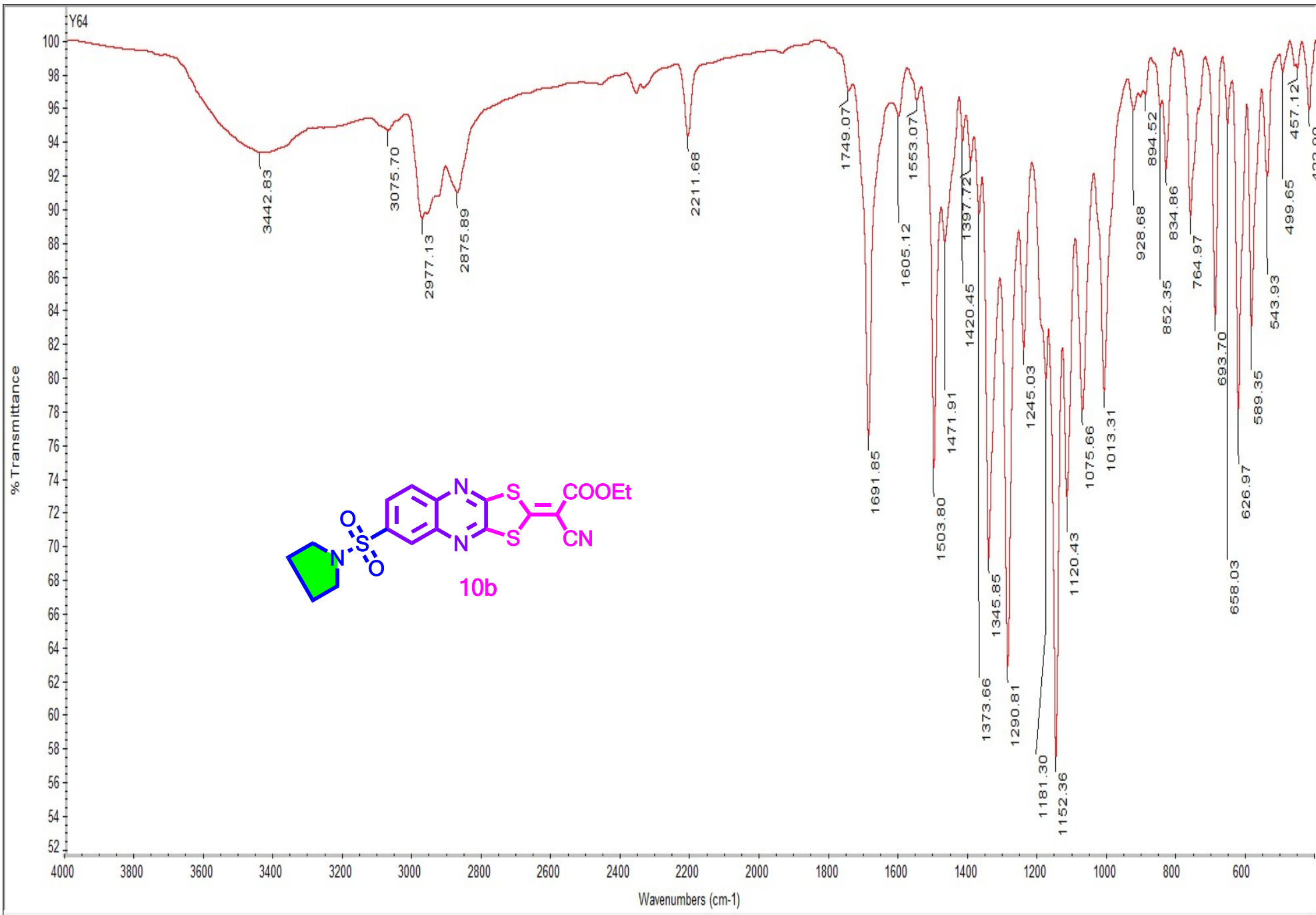


Time (min)

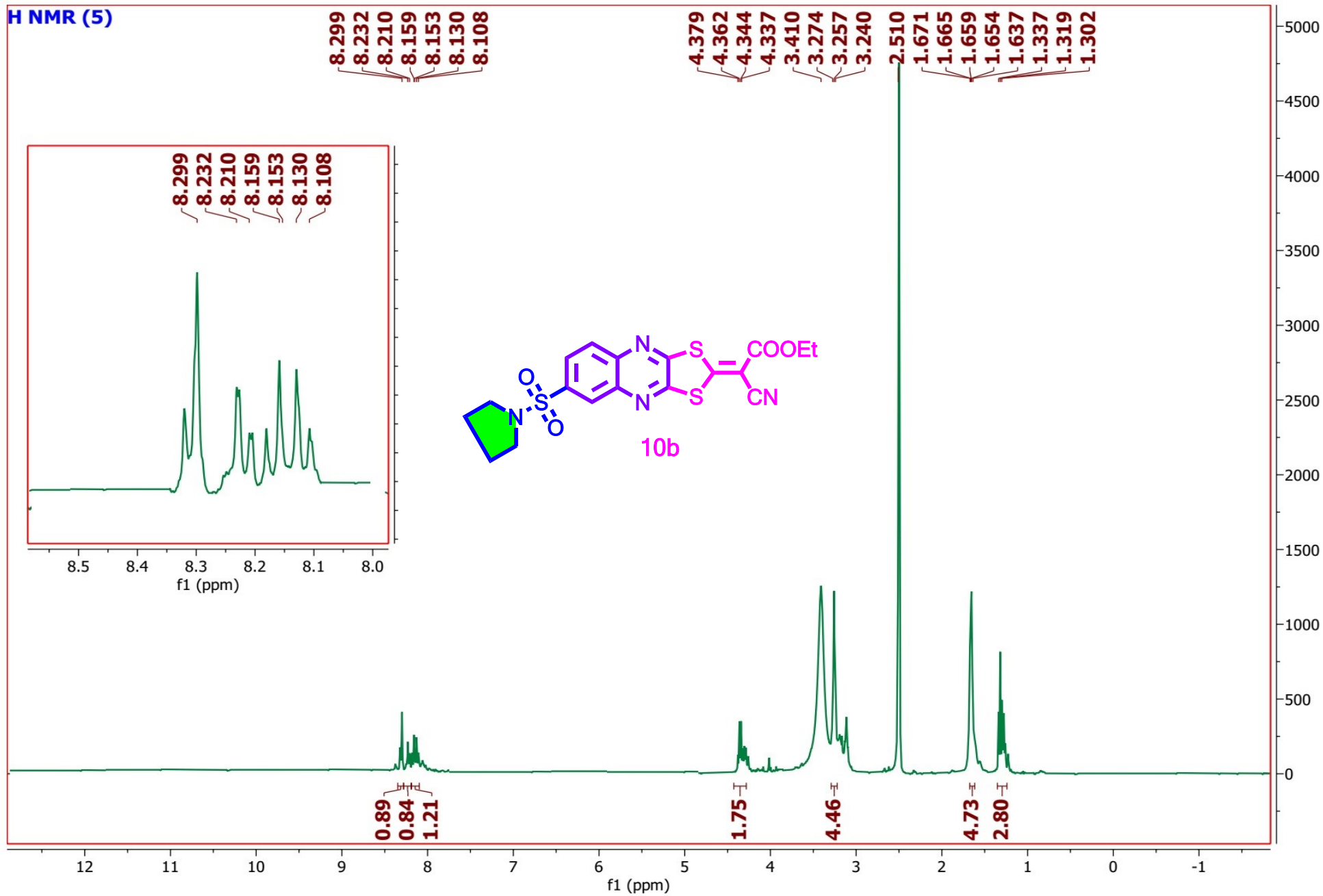
ahmed-10a#43 RT: 0.74 AV: 1 SB: 28 1.21-1.34 , 0.87-1.14 NL: 5.50E2

T: + cEI Full ms [40.00-1000.00]





H NMR (5)



C NMR (5)

167.57
165.86

151.26
149.95

138.16
137.17
136.68

126.40
125.06
122.33

114.45

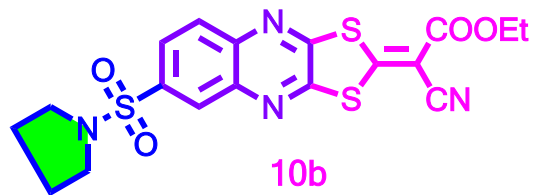
92.39

62.72

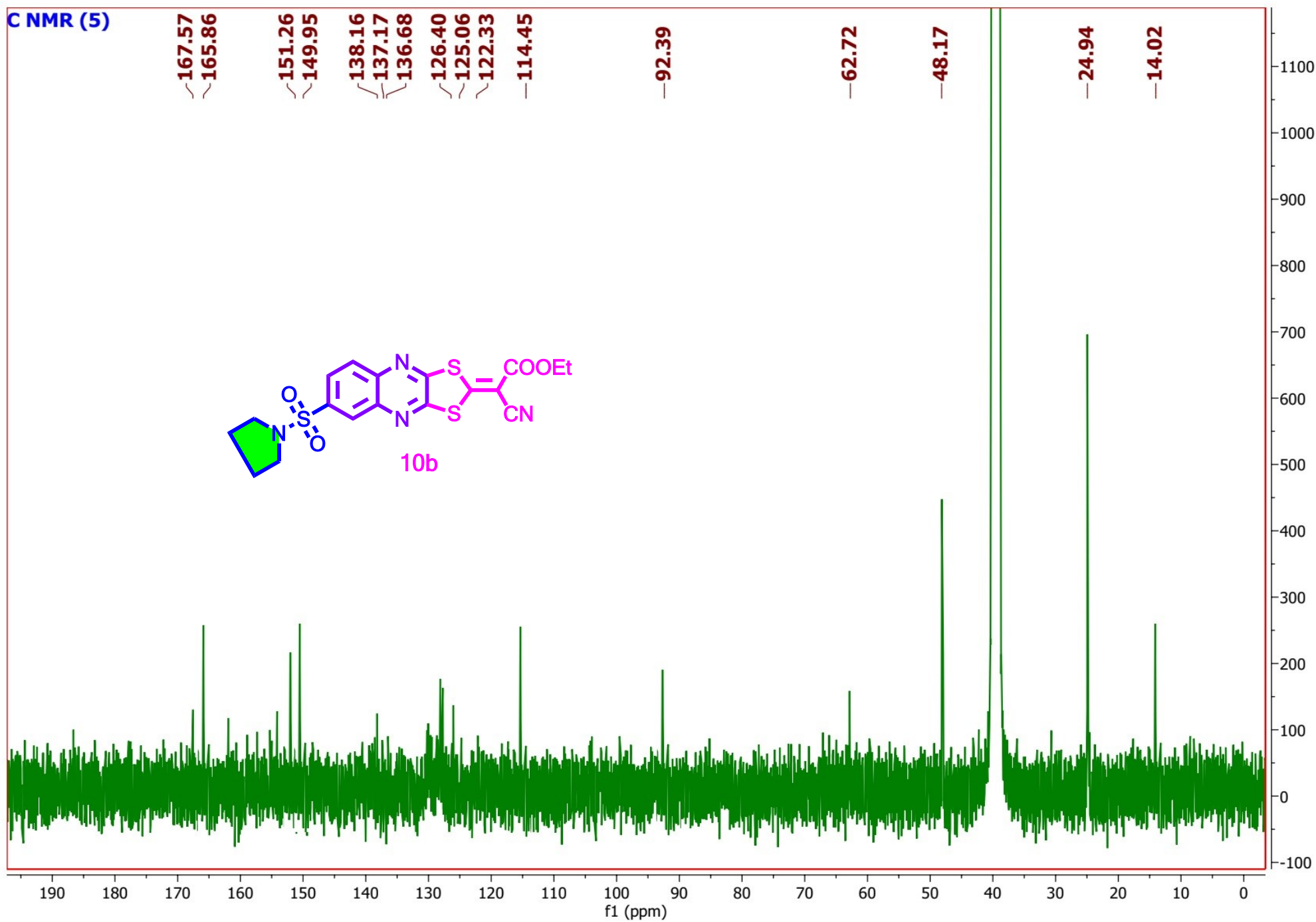
48.17

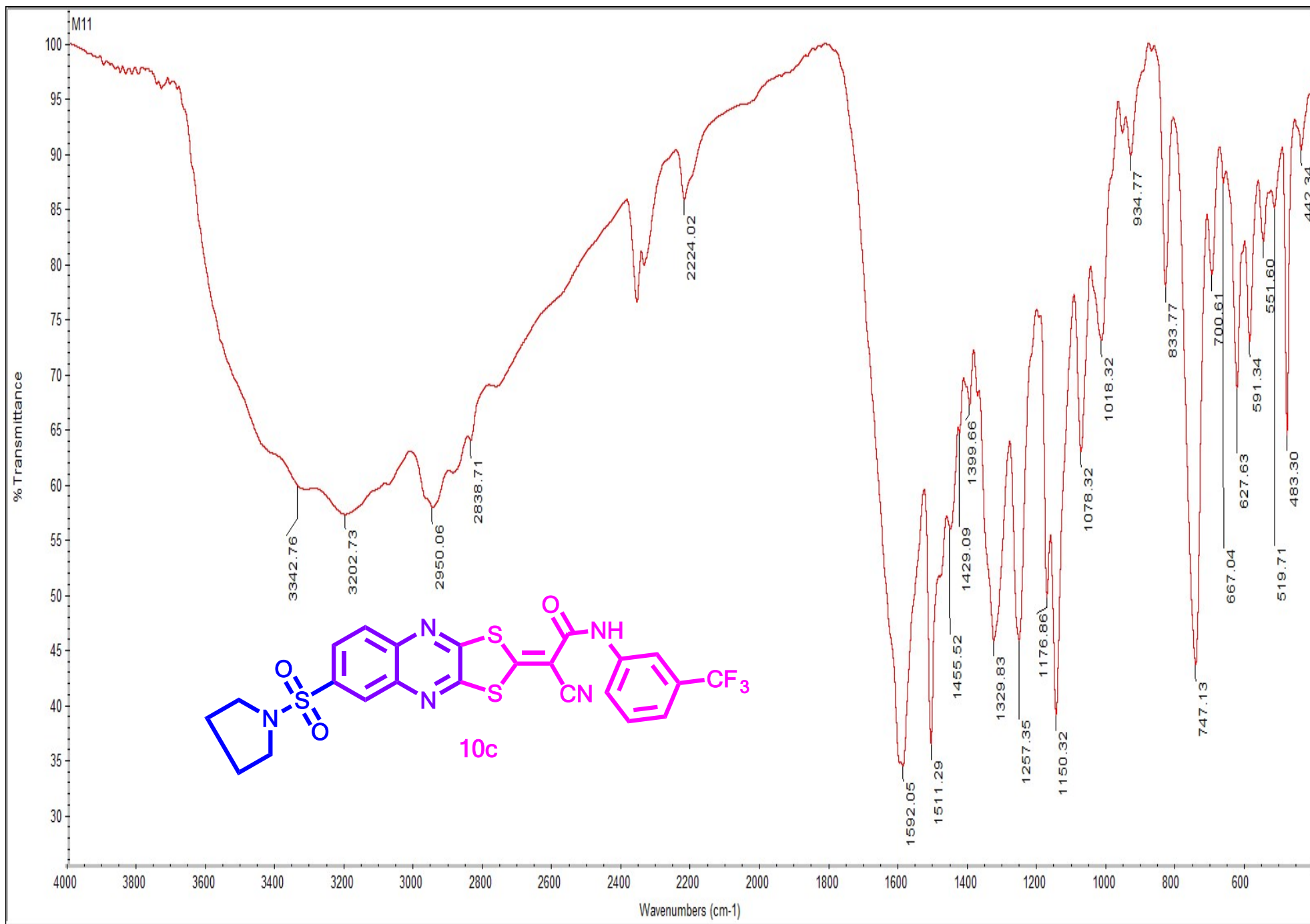
24.94

14.02

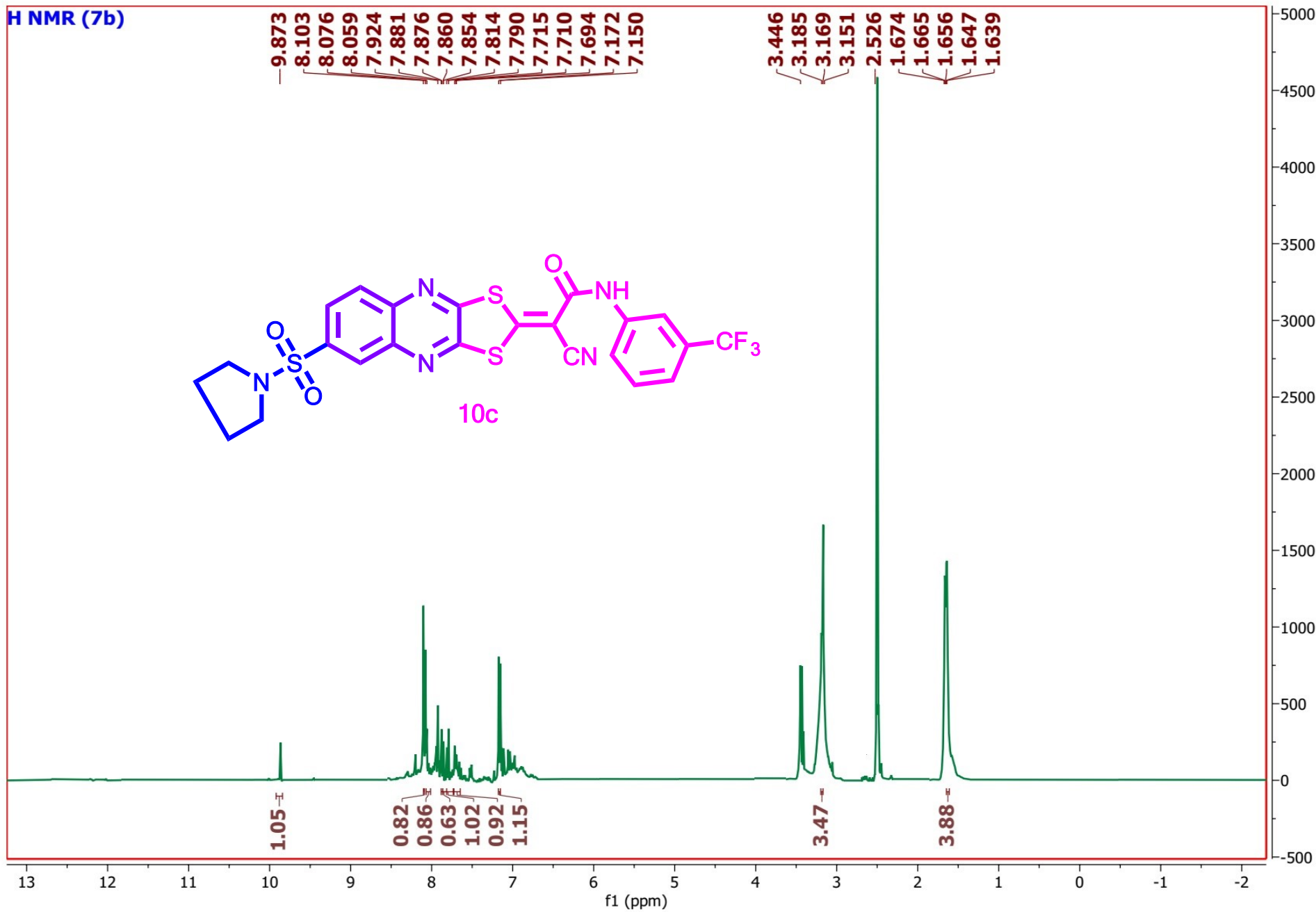
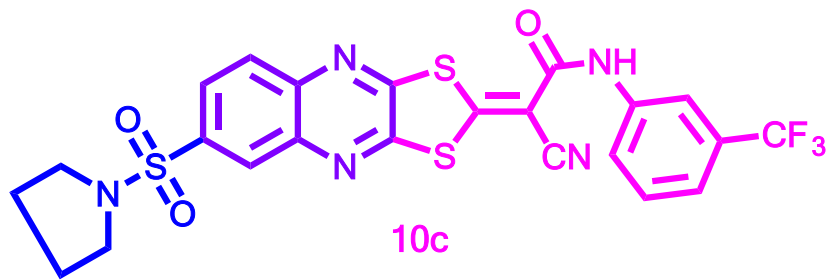


10b

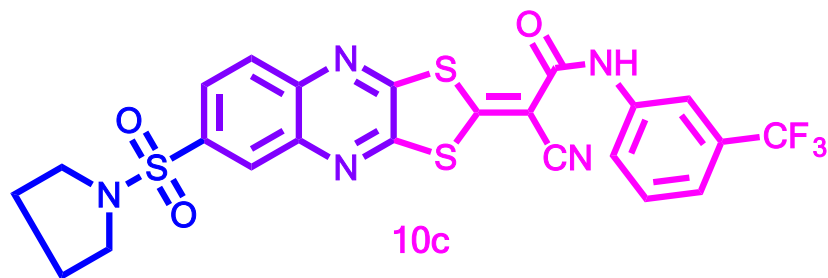




H NMR (7b)



C NMR (7b)

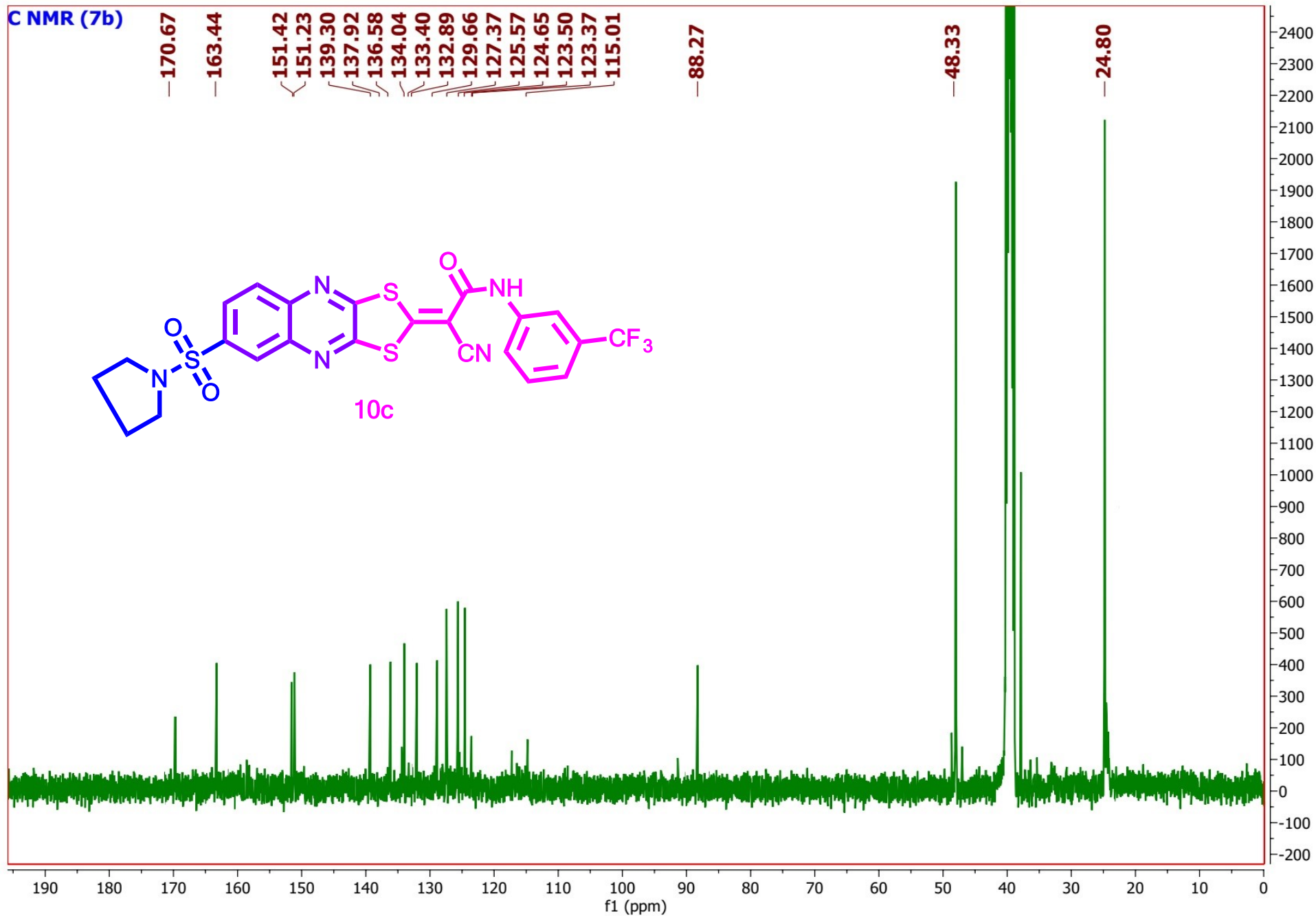


170.67
163.44
151.42
151.23
139.30
137.92
136.58
134.04
133.40
132.89
129.66
127.37
125.57
124.65
123.50
123.37
115.01

88.27

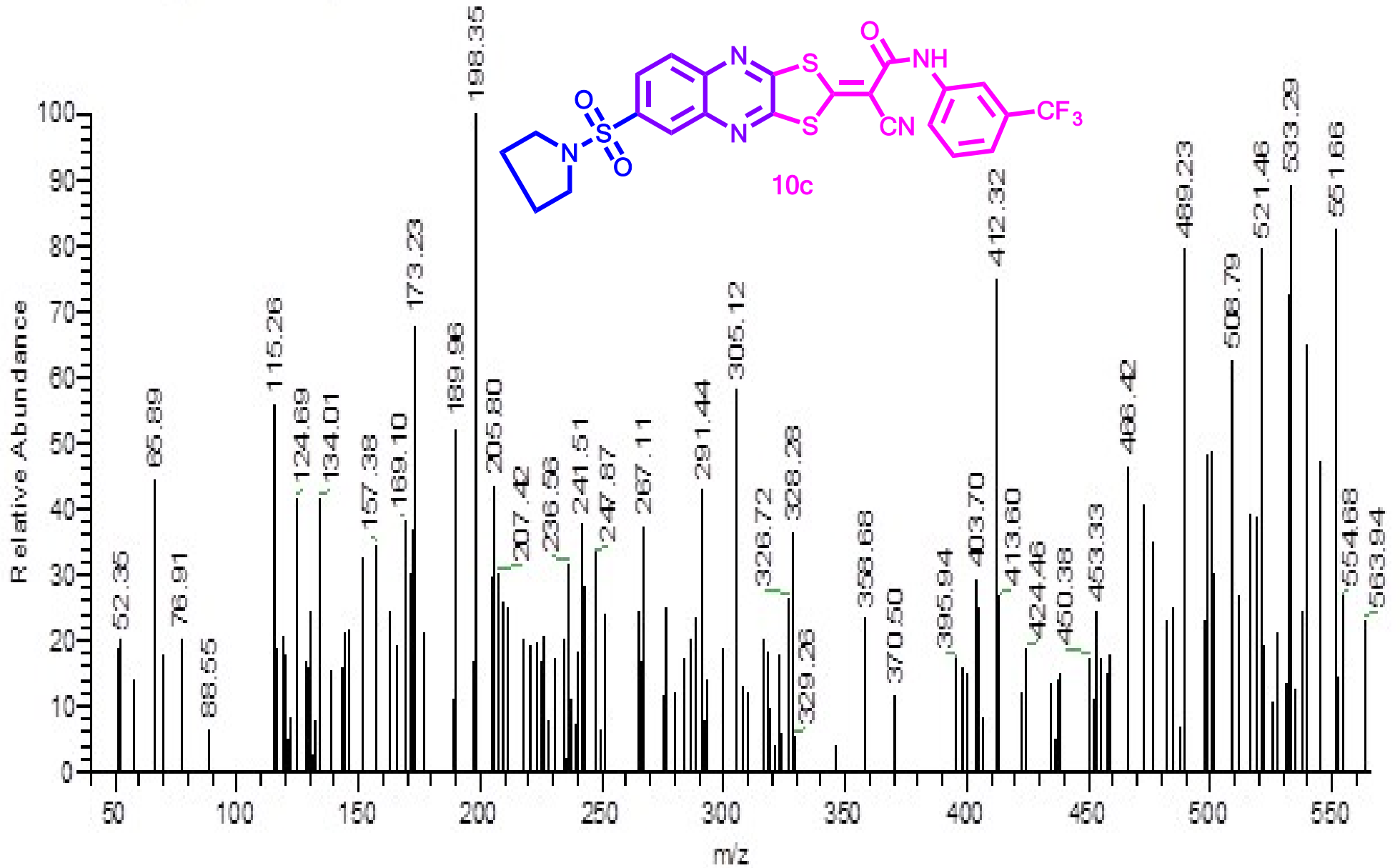
48.33

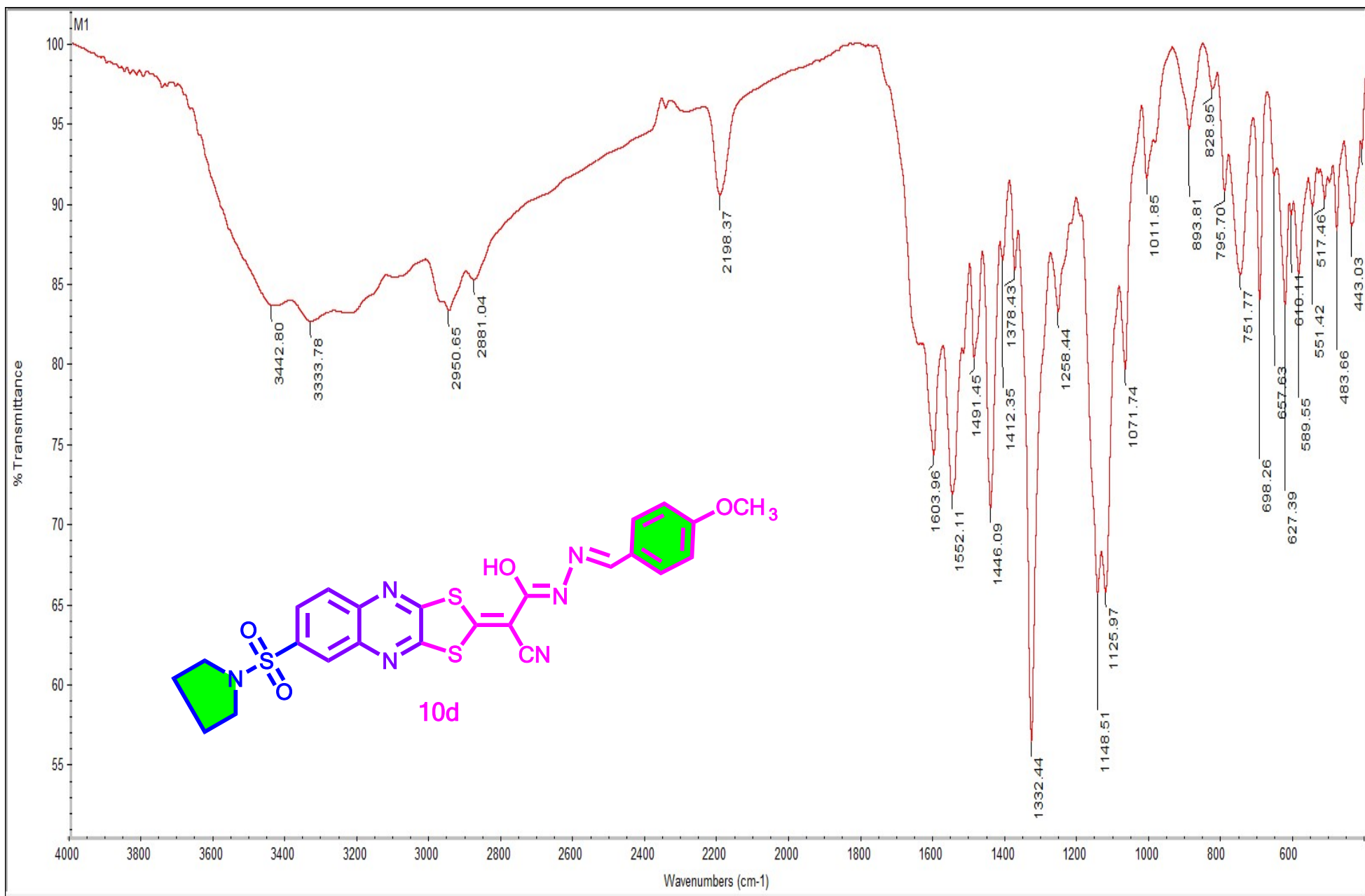
24.80



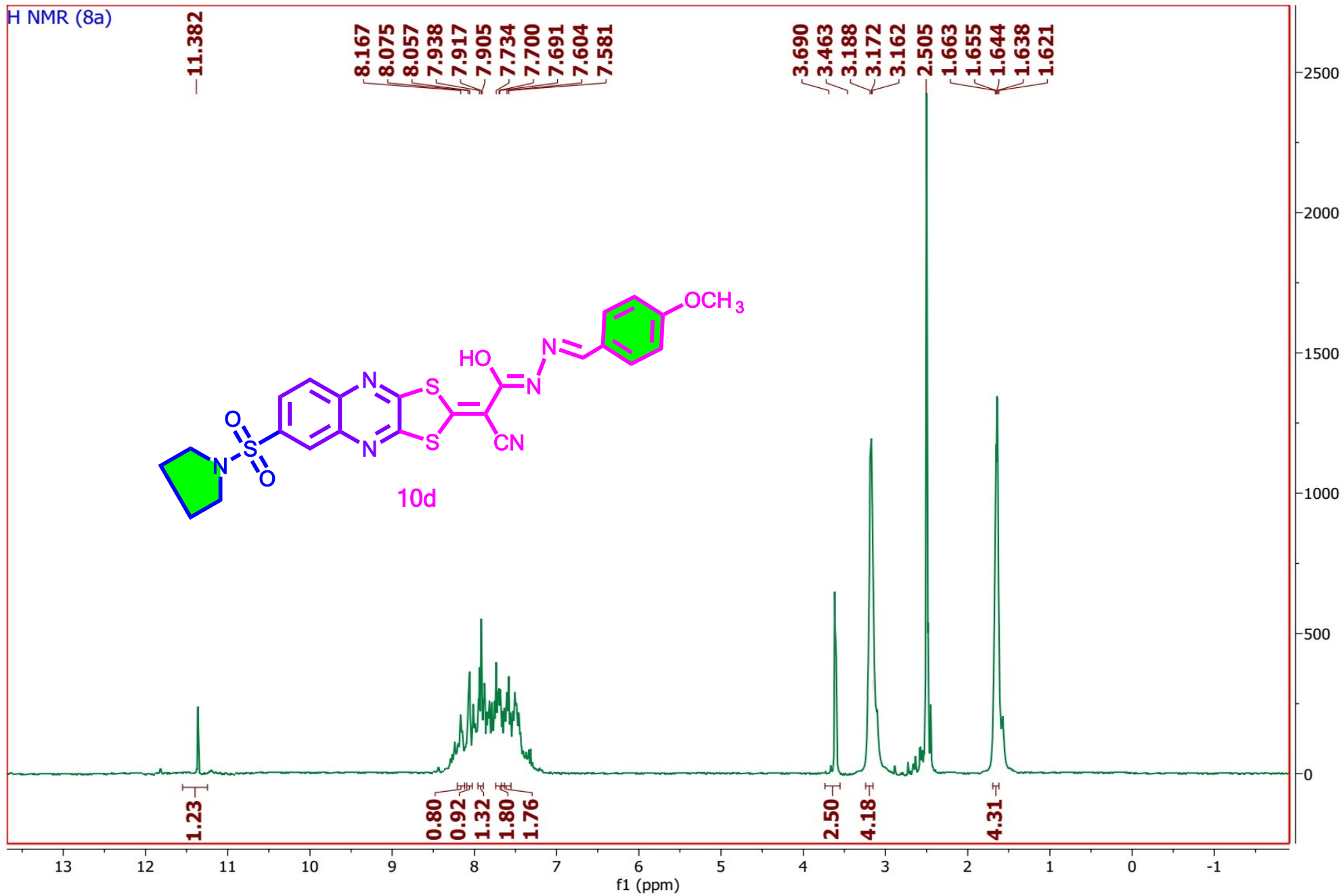
Time (min)

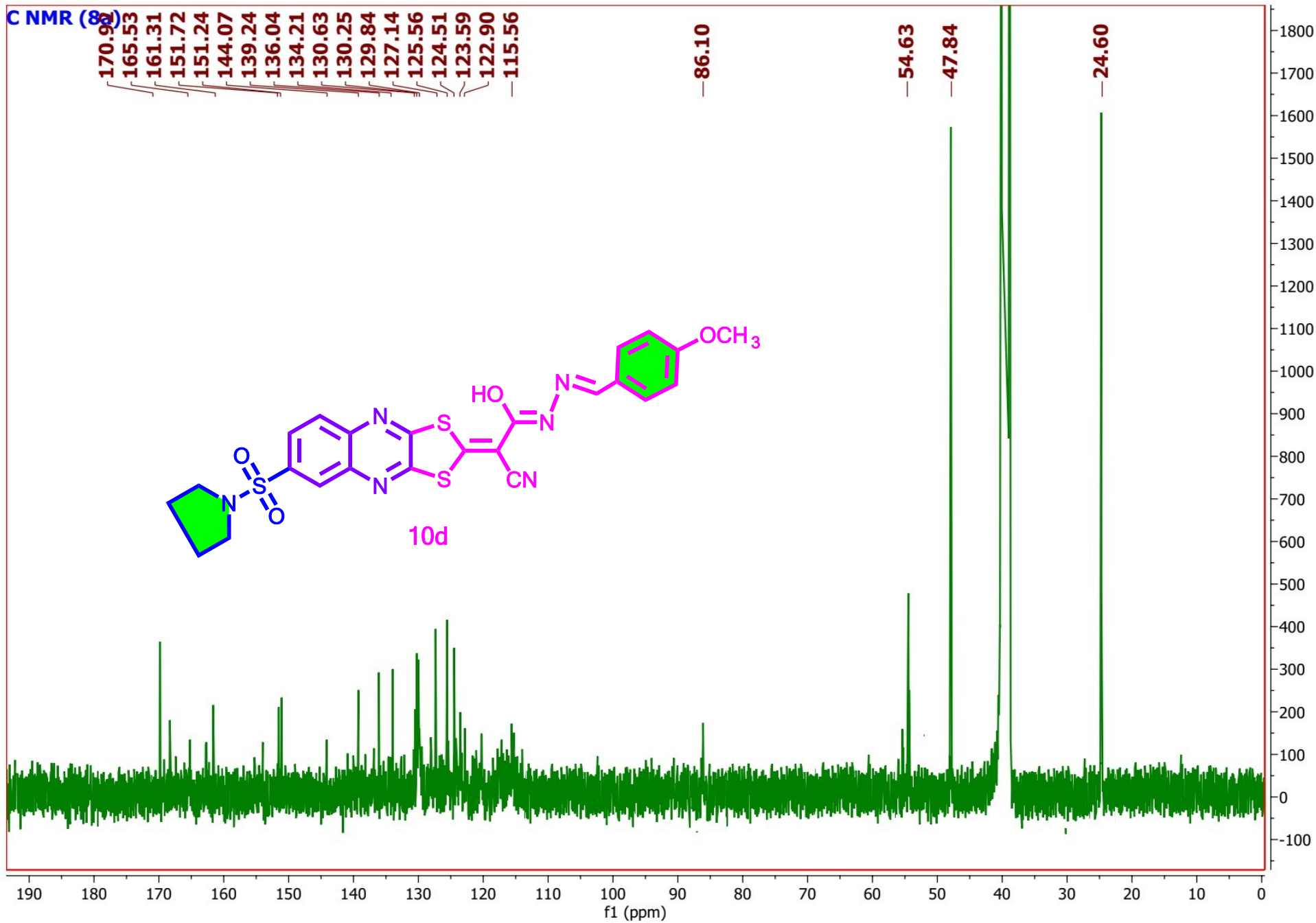
ahmed-10c#100-101 RT: 1.69-1.71 AV: 2 SB: 28 1.21-1.34, 0.87-1.14 NL: 2.12E2
T: + cEI Full ms [40.00-1000.00]

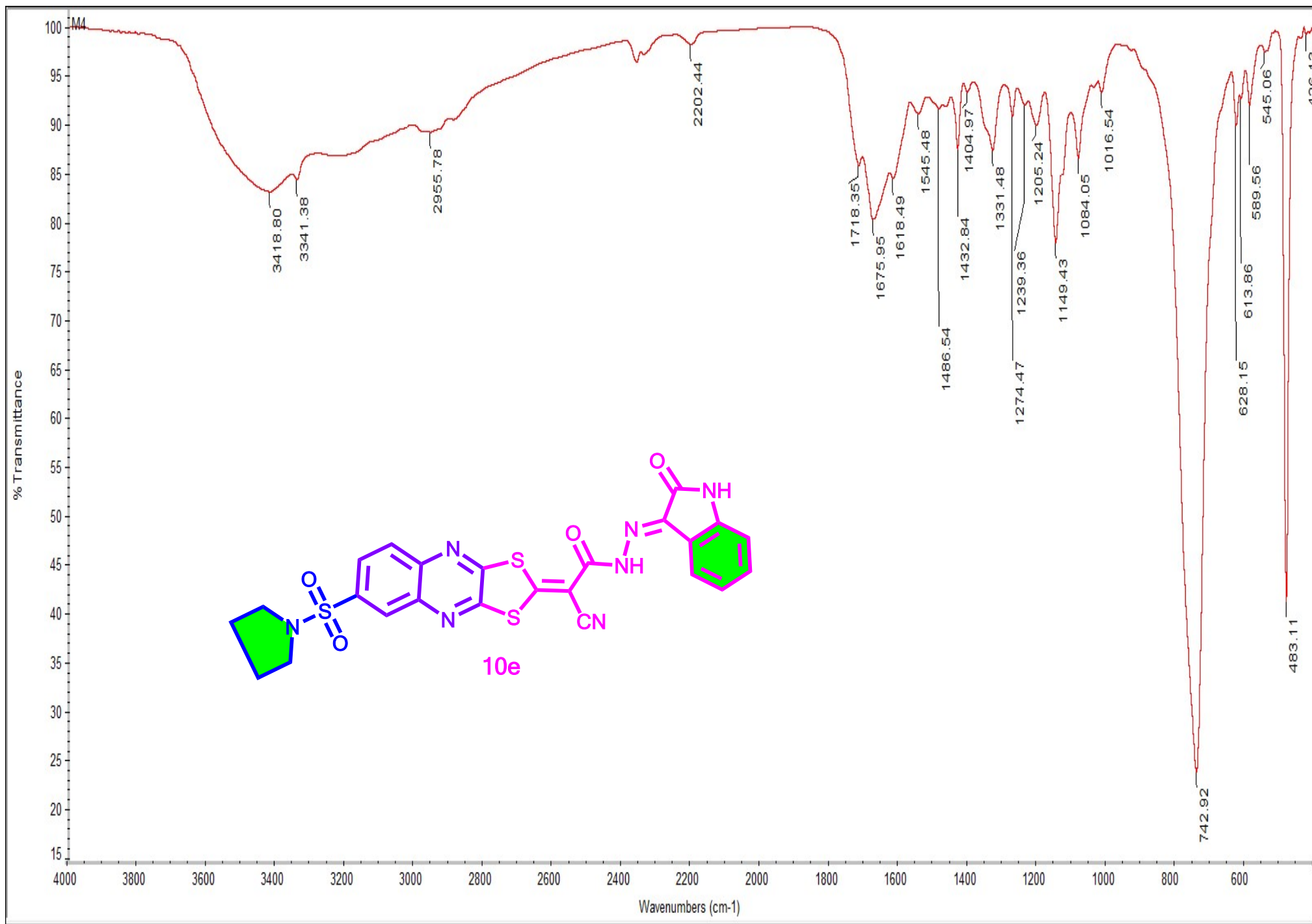




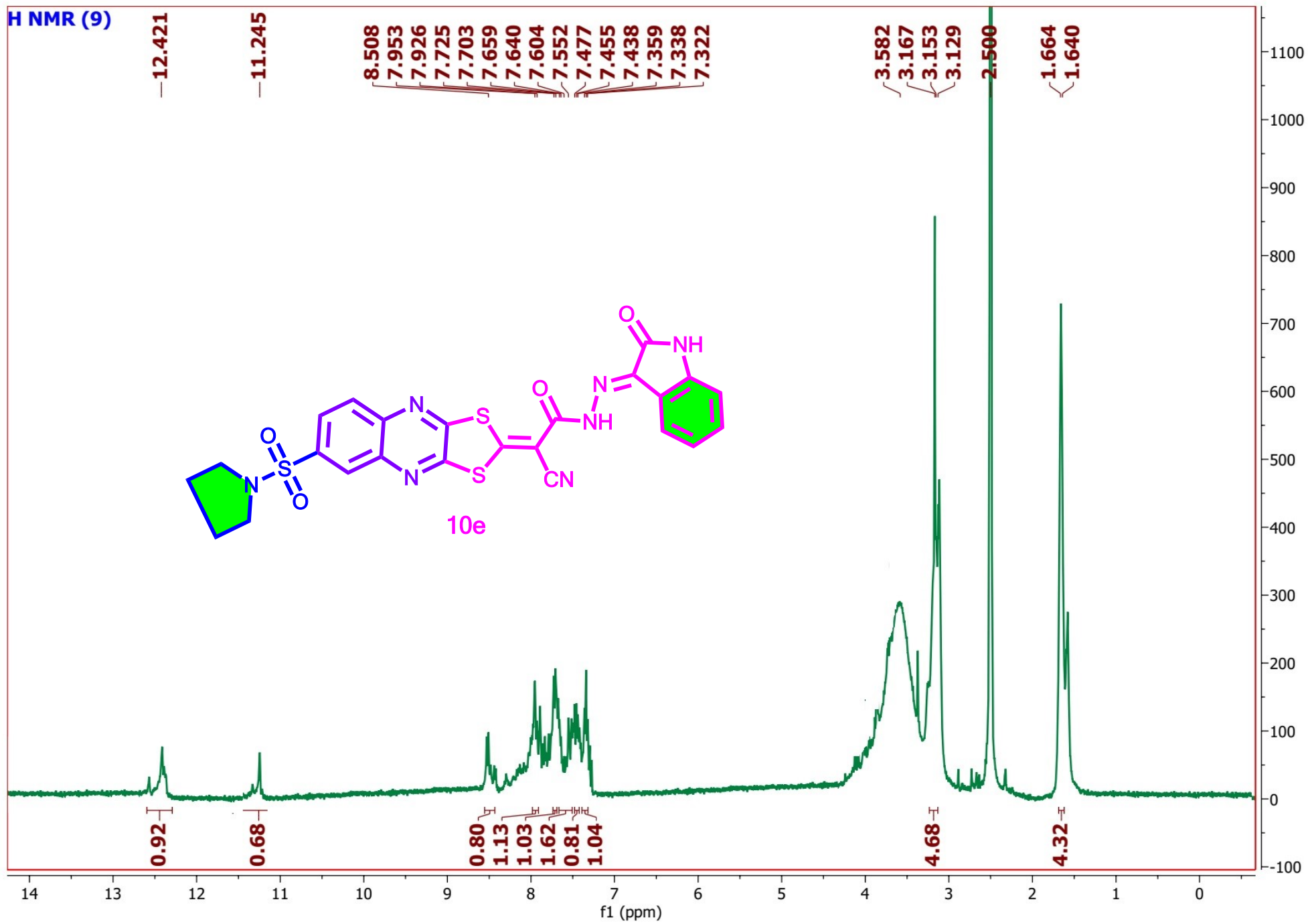
H NMR (8a)

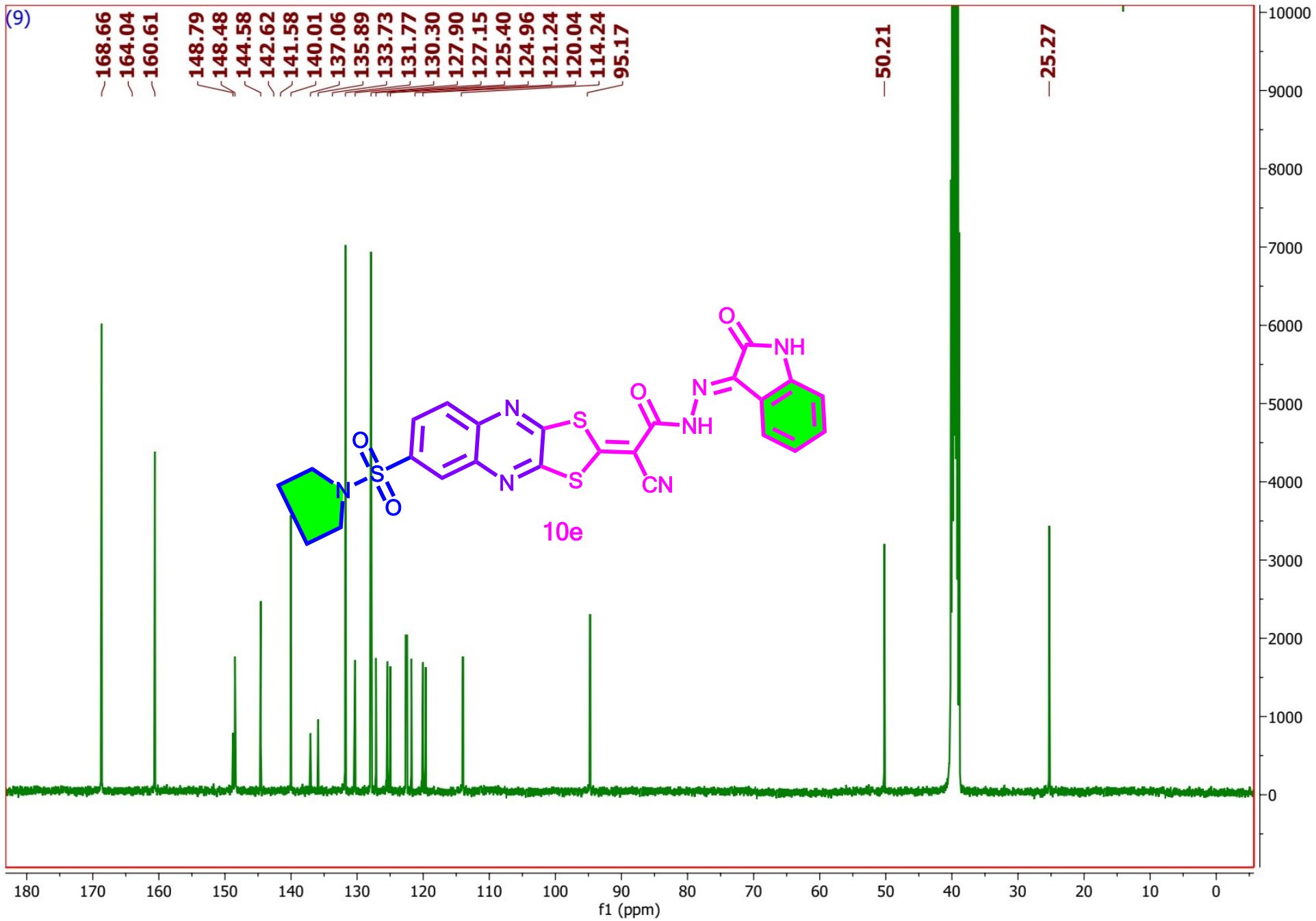


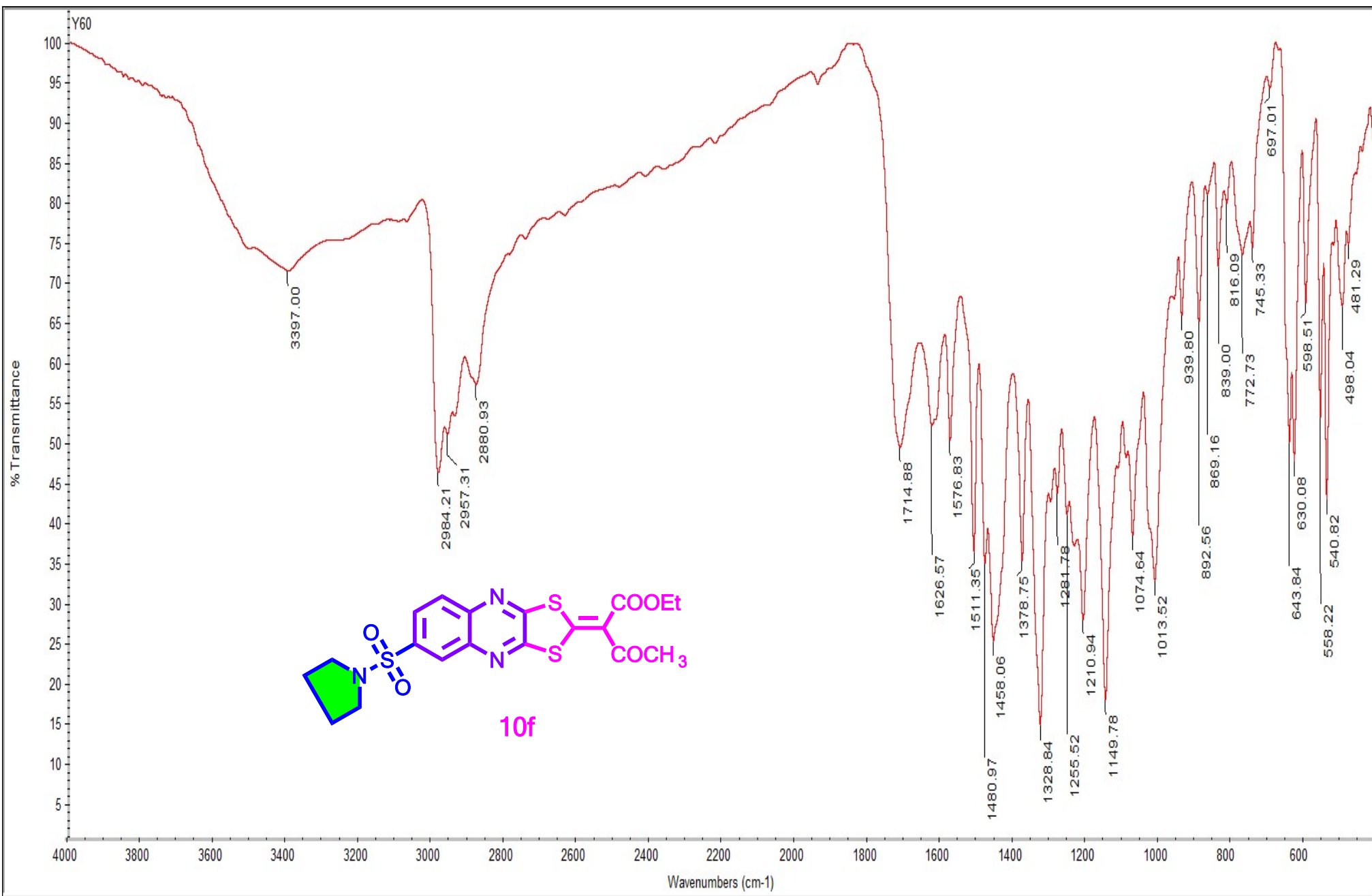




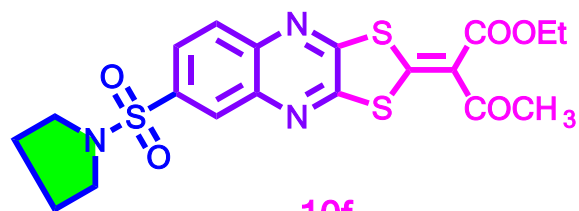
H NMR (9)







H NMR (4)



10f

8.024
7.895
7.874
7.855
7.850
7.833
7.829

4.565
4.550
4.529
4.514
3.334
3.201
3.184
3.167
2.495
2.491
1.657
1.649
1.641
1.632
1.623
1.436
1.419
1.401

0.95
1.03
1.01

2.80

4.22

2.46

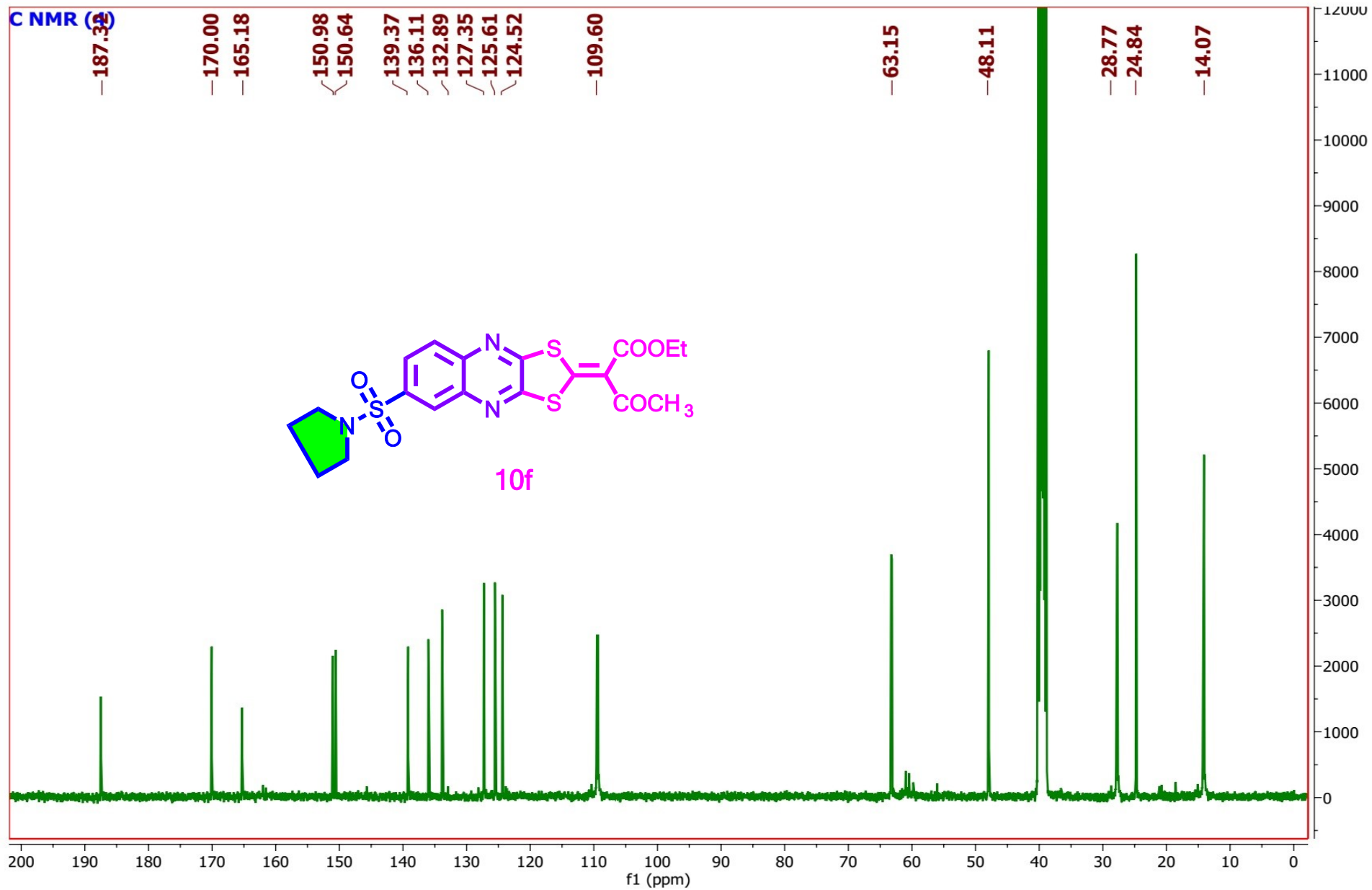
4.48

3.70

12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2

f1 (ppm)

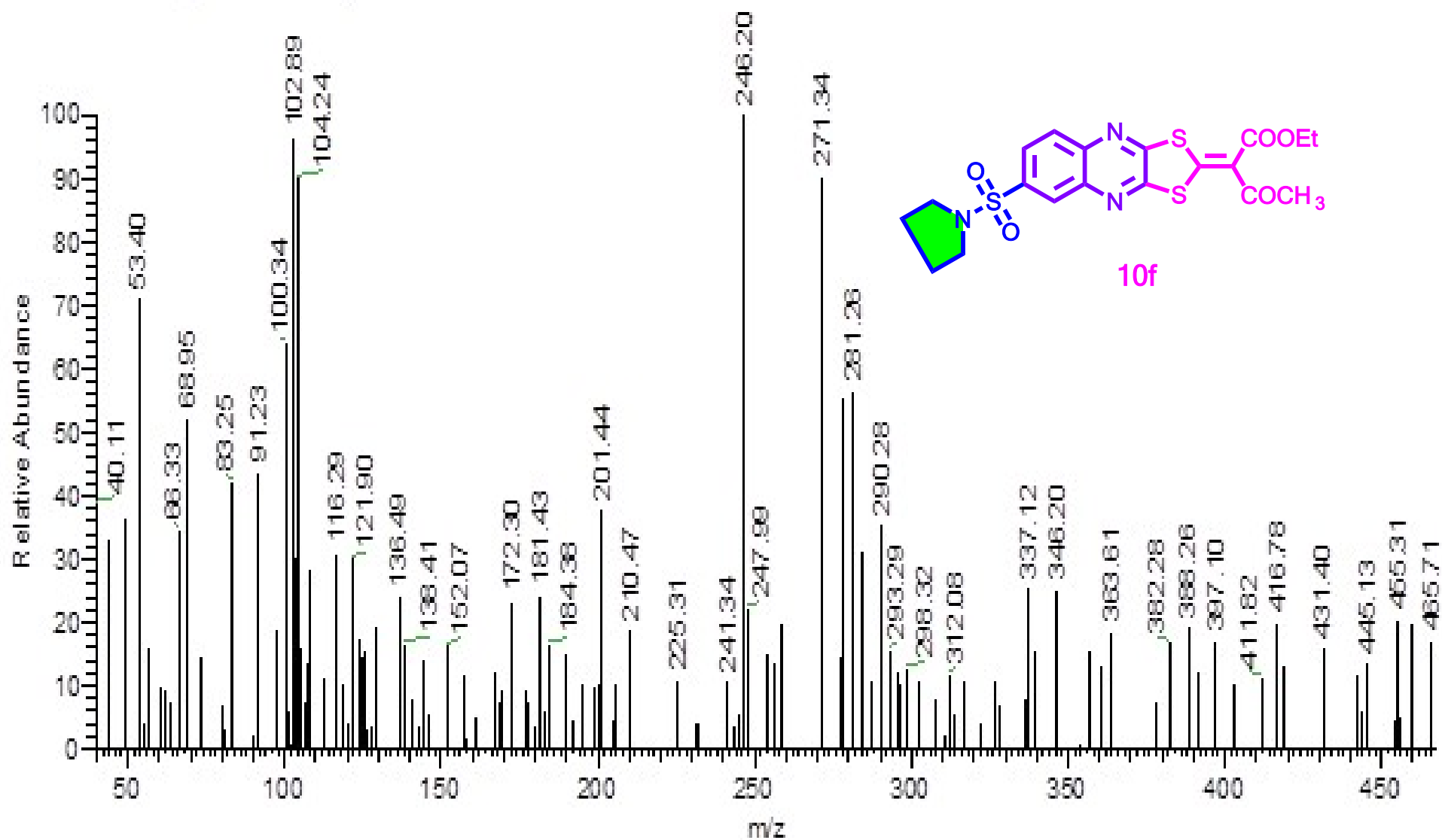
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000

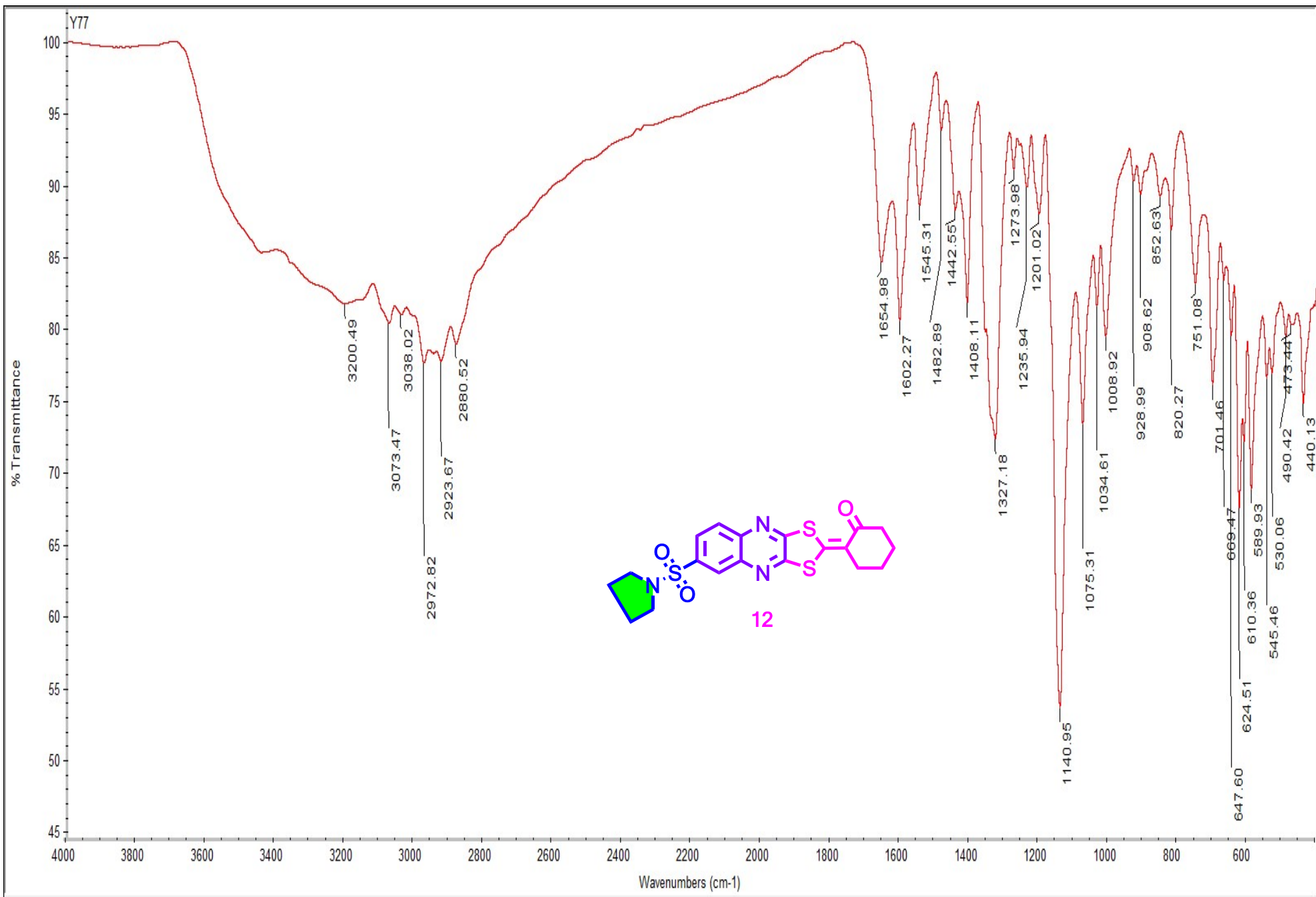


TIME (min)

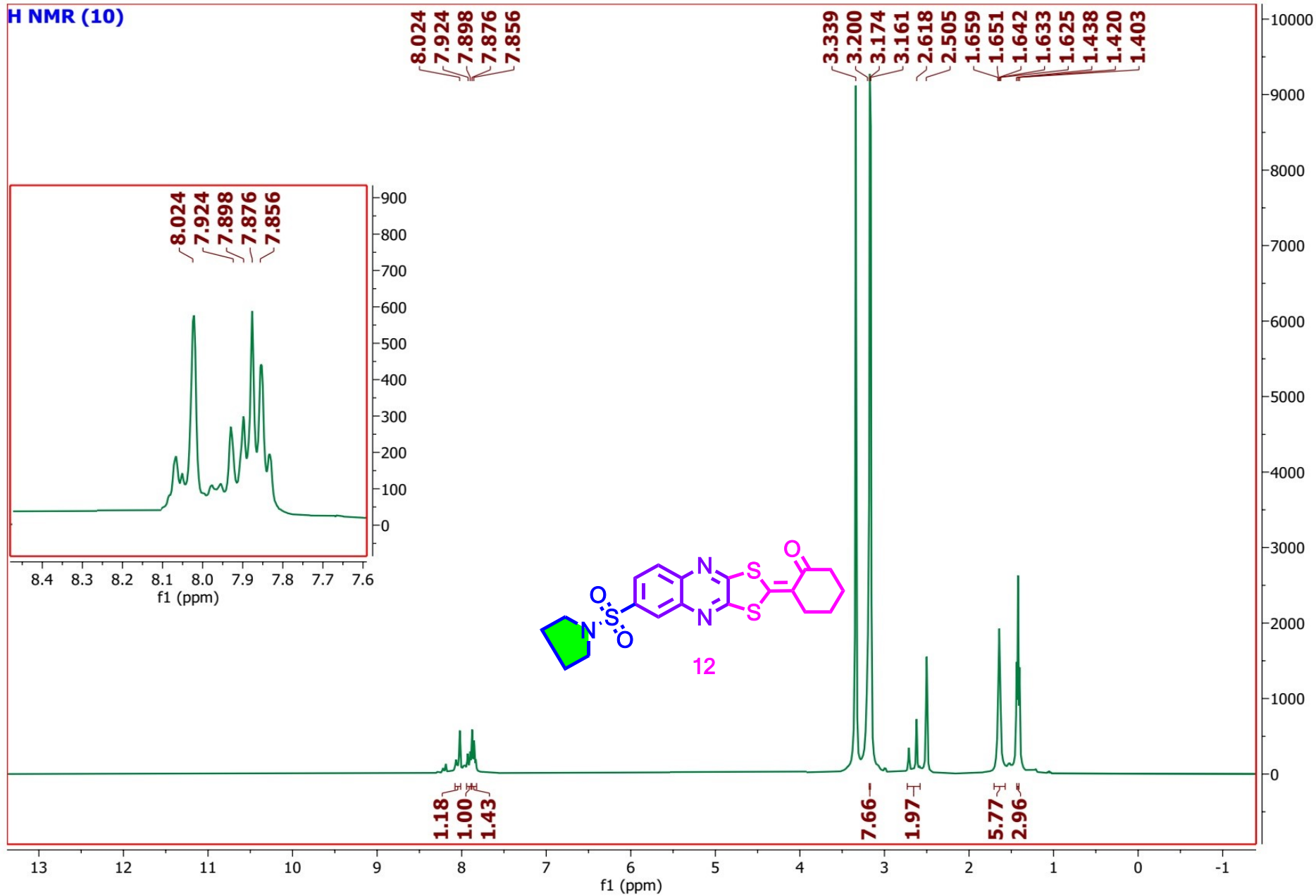
ahmed-10f#148-151 RT: 2.49-2.54 AV: 4 SB: 20 2.38-2.58, 2.34-2.44 NL: 201E2

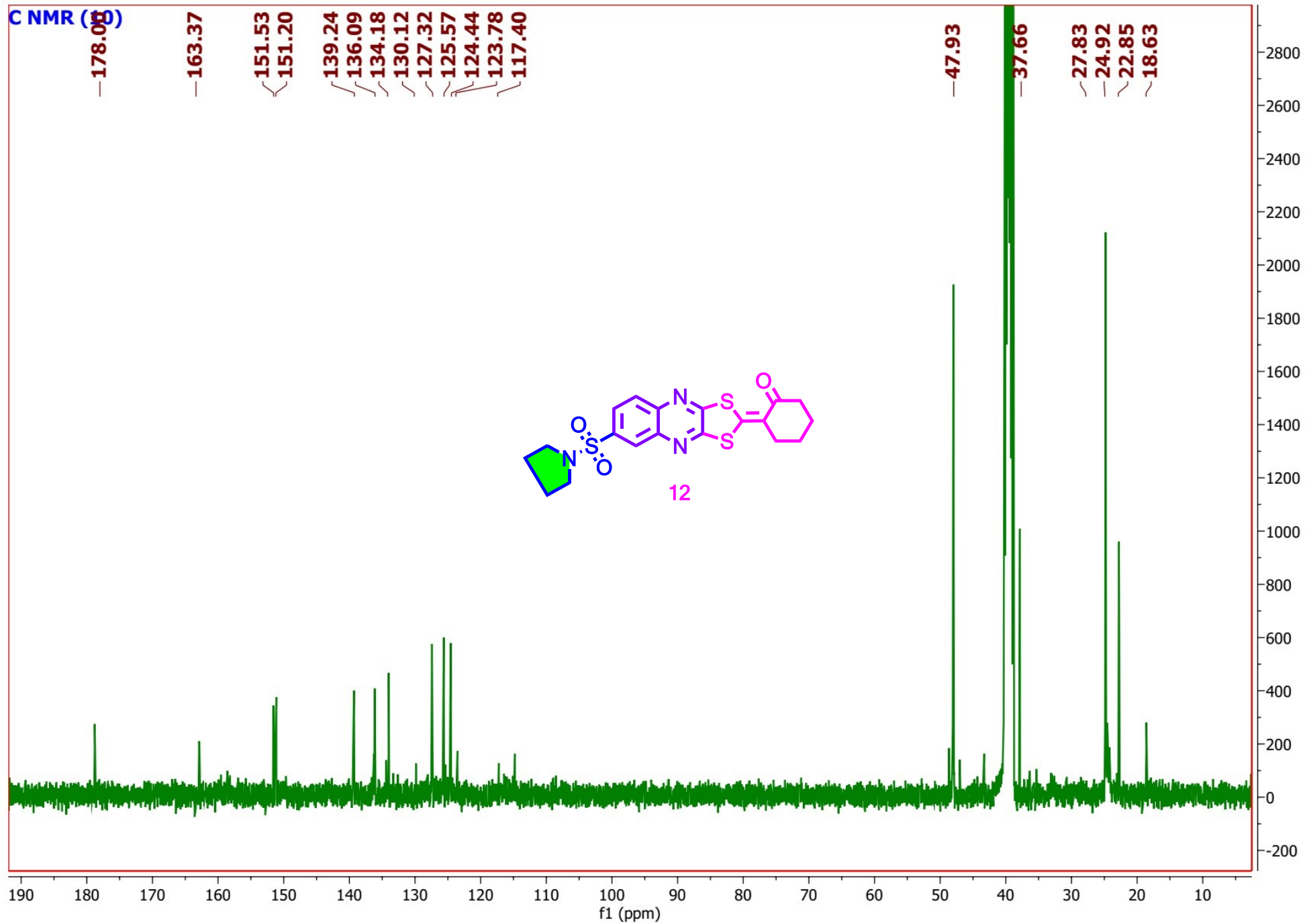
T: + cEI Full ms [40.00-1000.00]





H NMR (10)

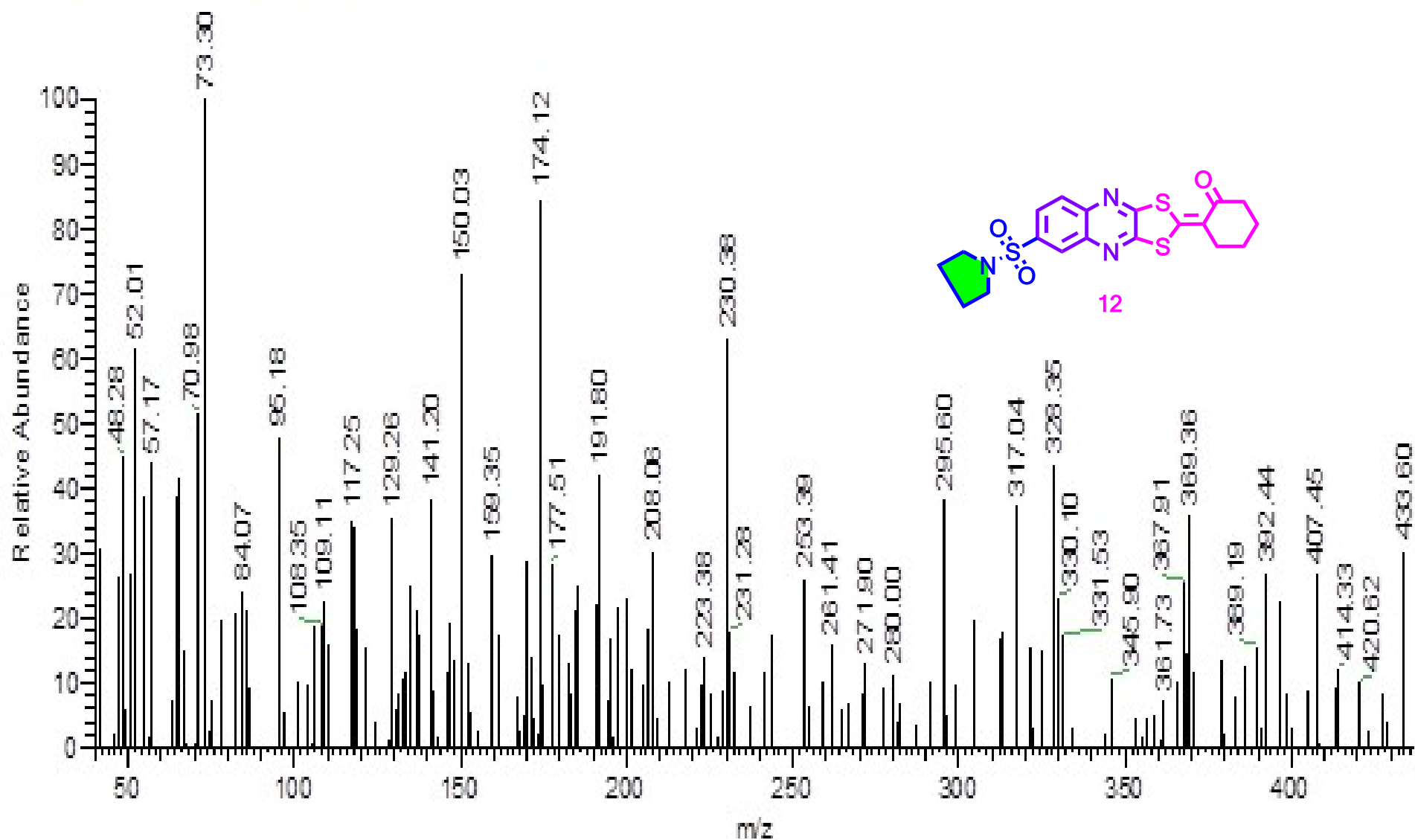


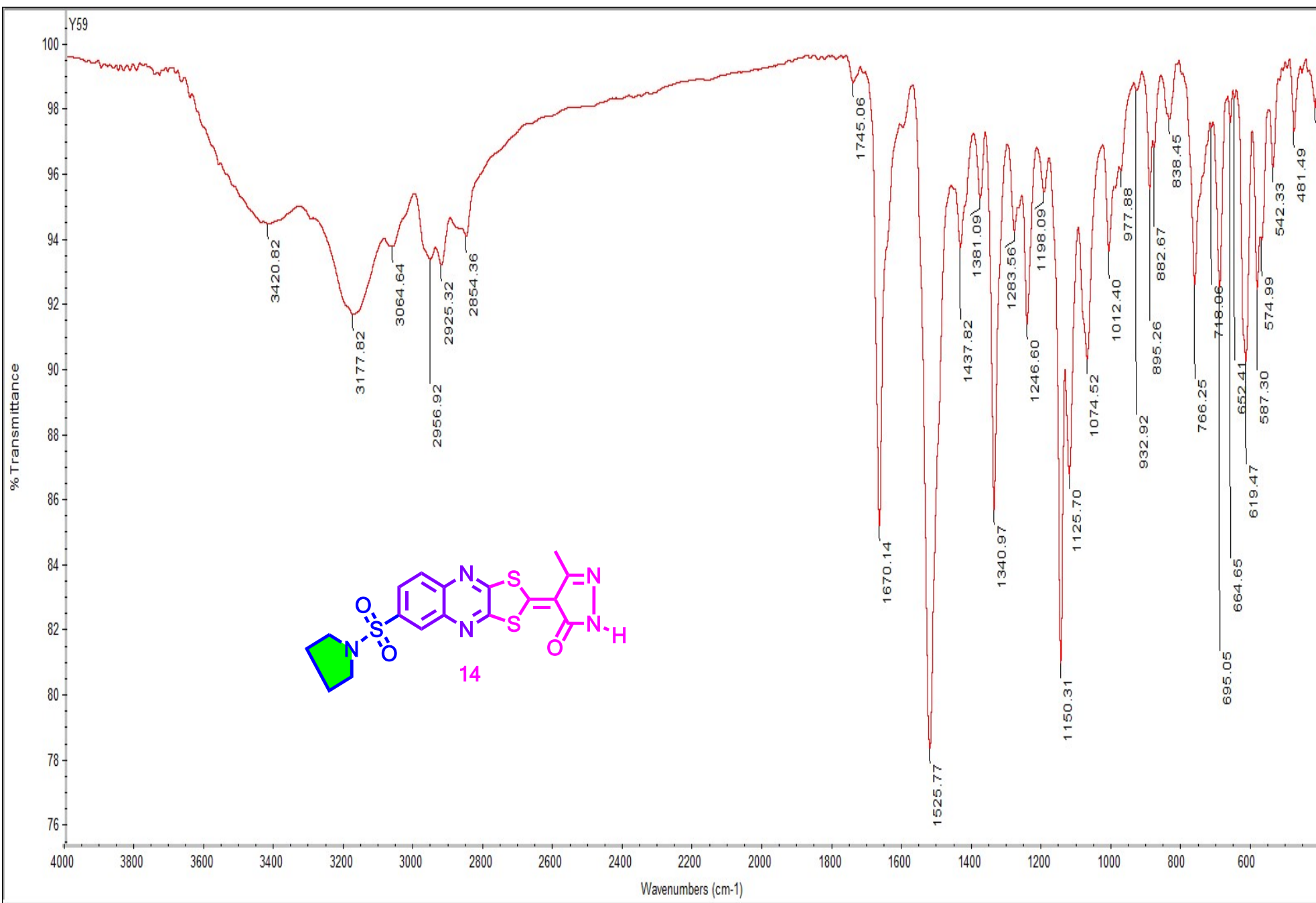


Time (min)

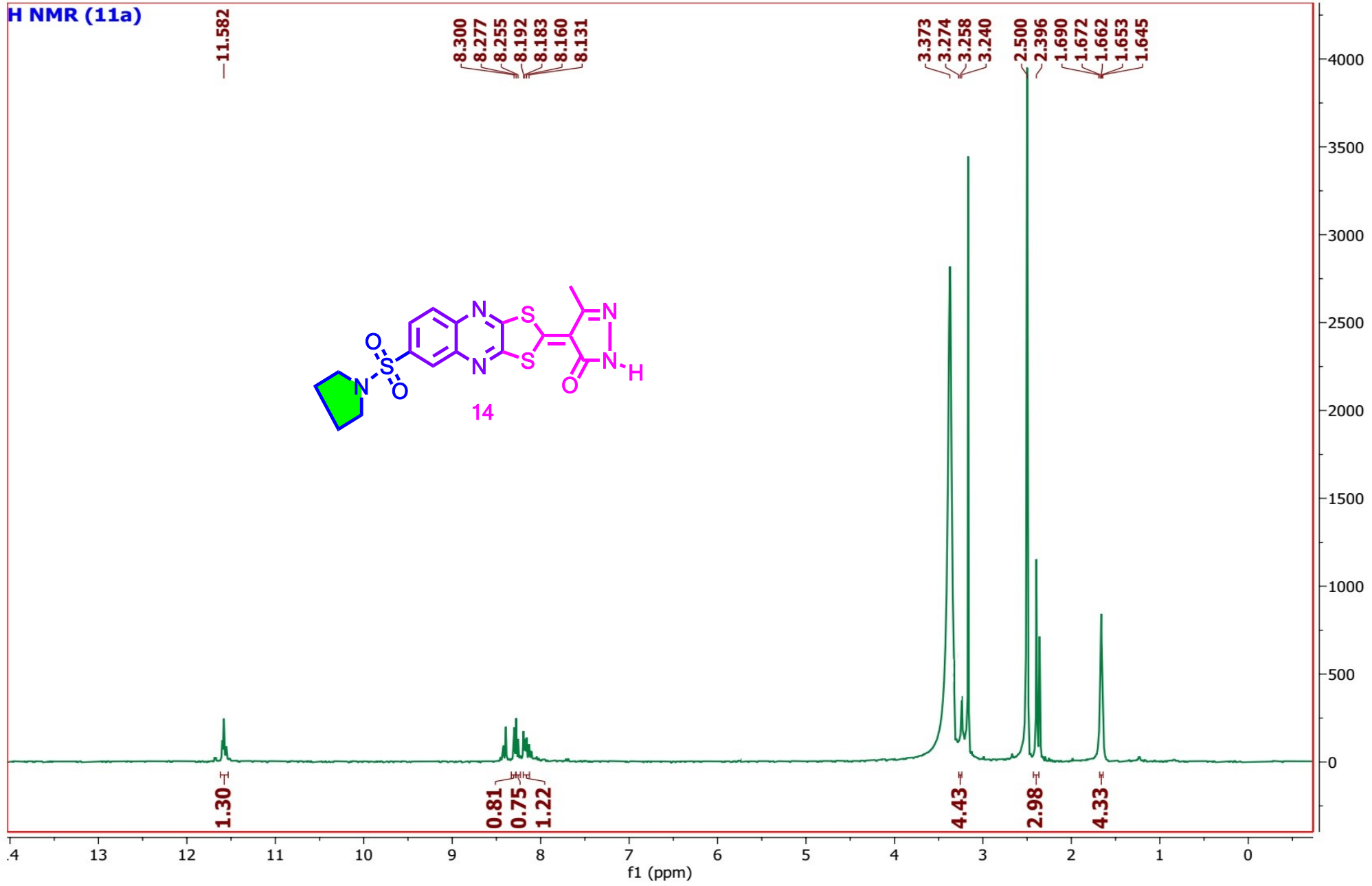
ahmed-12 #87-95 RT: 1.47-1.61 AV: 9 SB: 26 1.21-1.34 , 0.87-1.14 NL: 1.20E2

T: + cEI Full ms [40.00-1000.00]

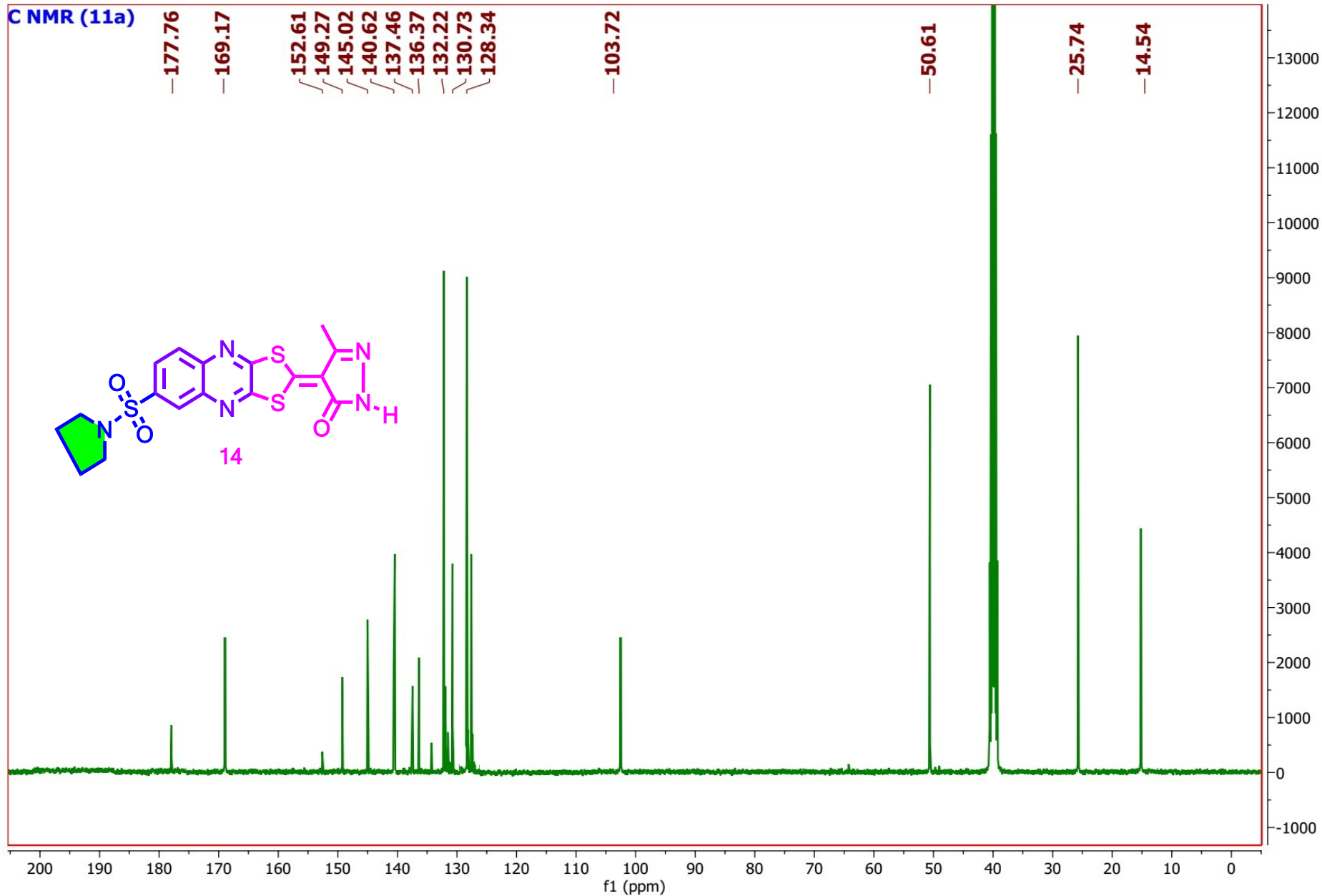


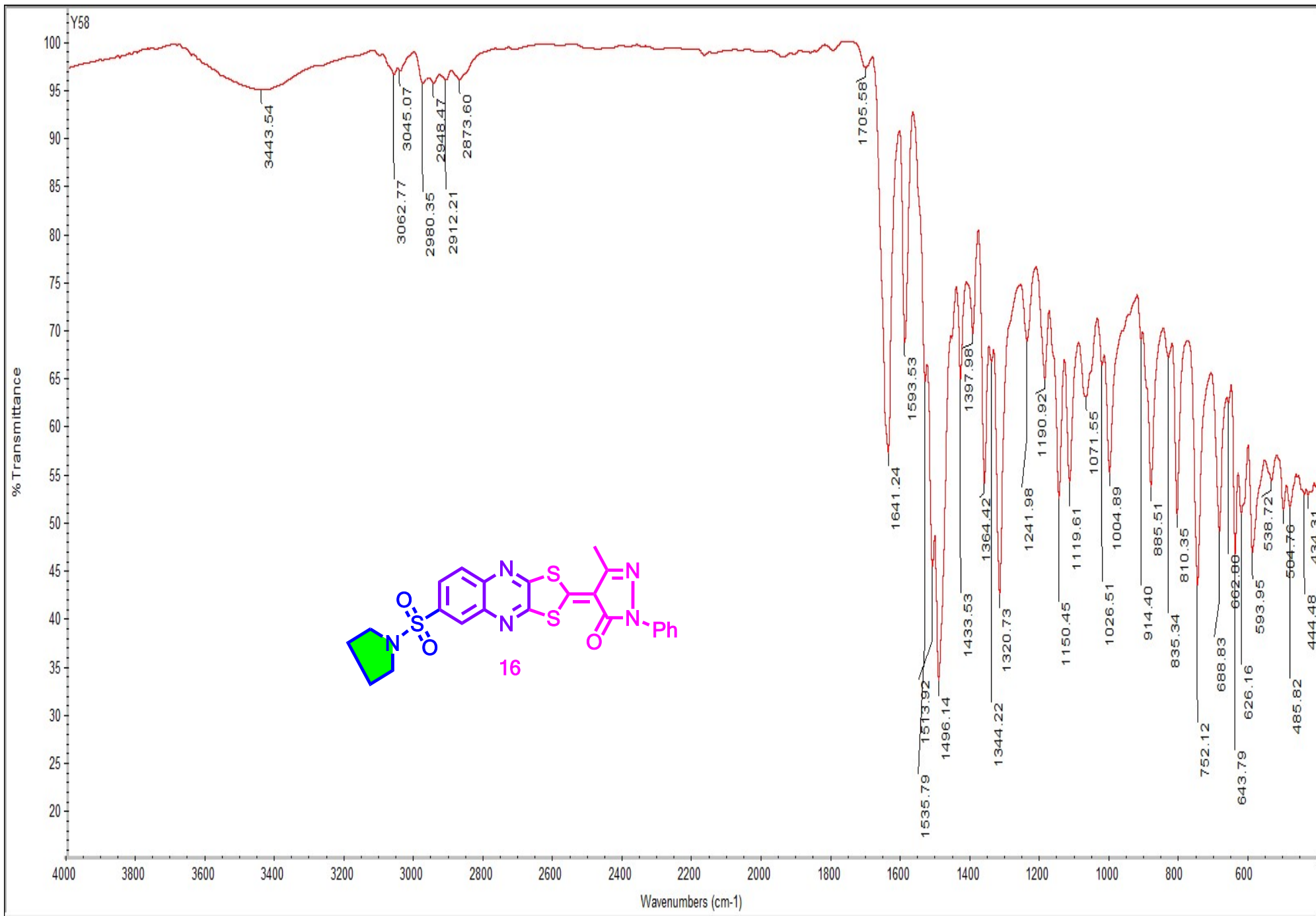


H NMR (11a)



C NMR (11a)

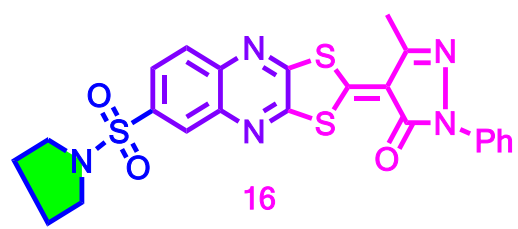




H NMR (11b)

8.303
8.180
8.159
8.124
8.107
7.927
7.907
7.470
7.452
7.430
7.279
7.226
7.206
7.201

3.385
3.256
3.220
3.130
2.500
2.442
1.683
1.675
1.666
1.658
1.649

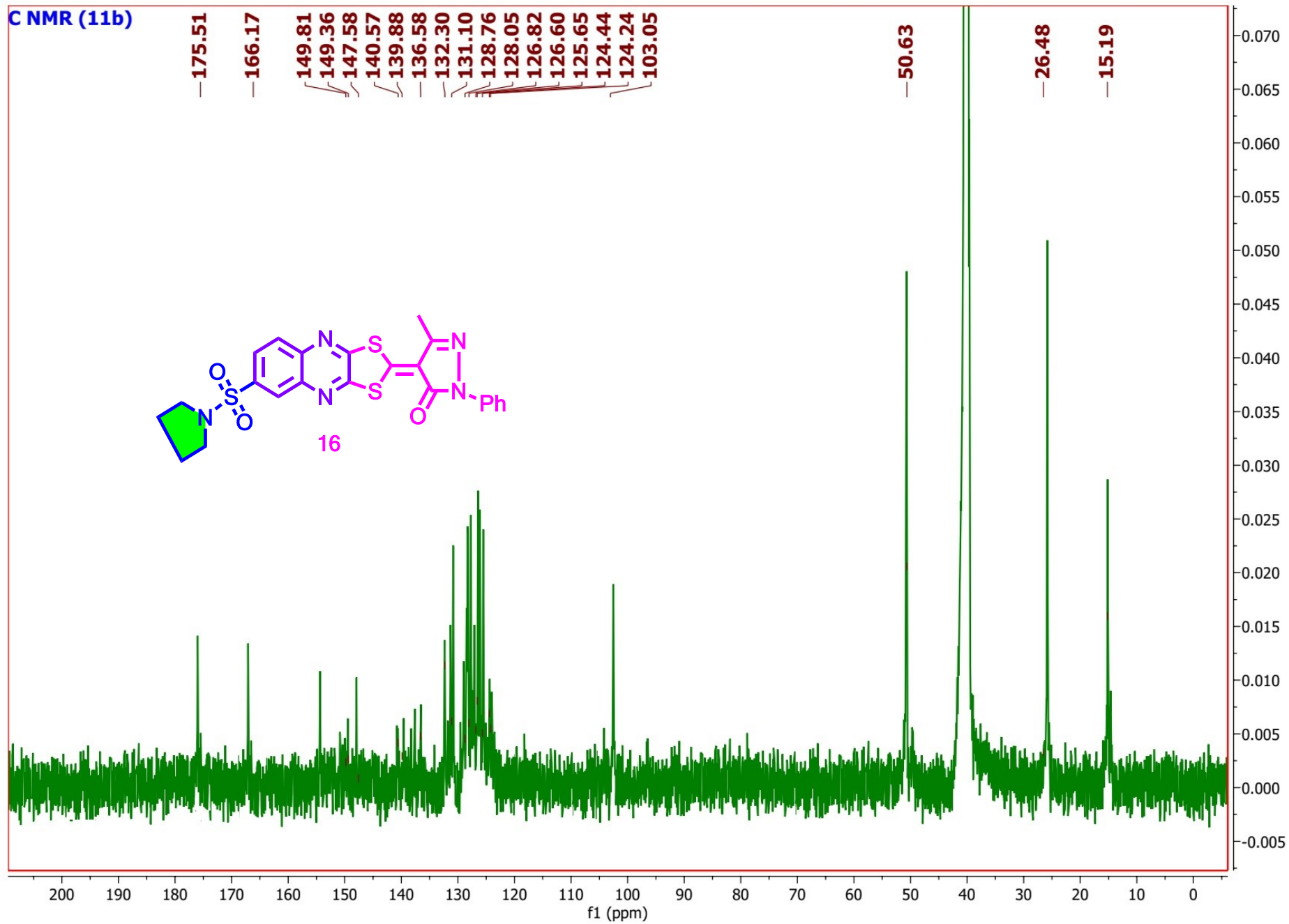


1.24
1.33
1.87
1.18
1.91
0.96

4.40
2.62
4.46

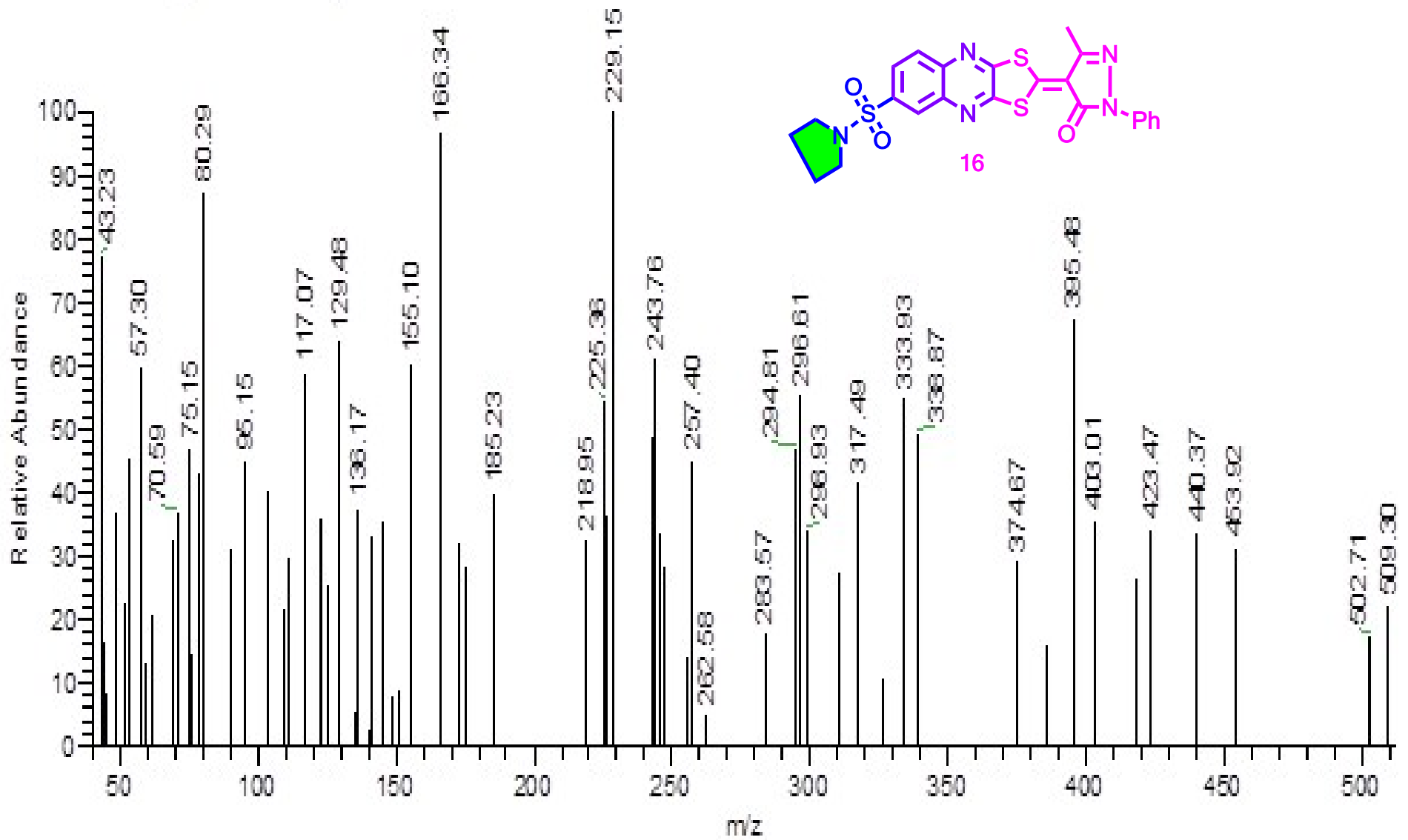
4 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1
f1 (ppm)

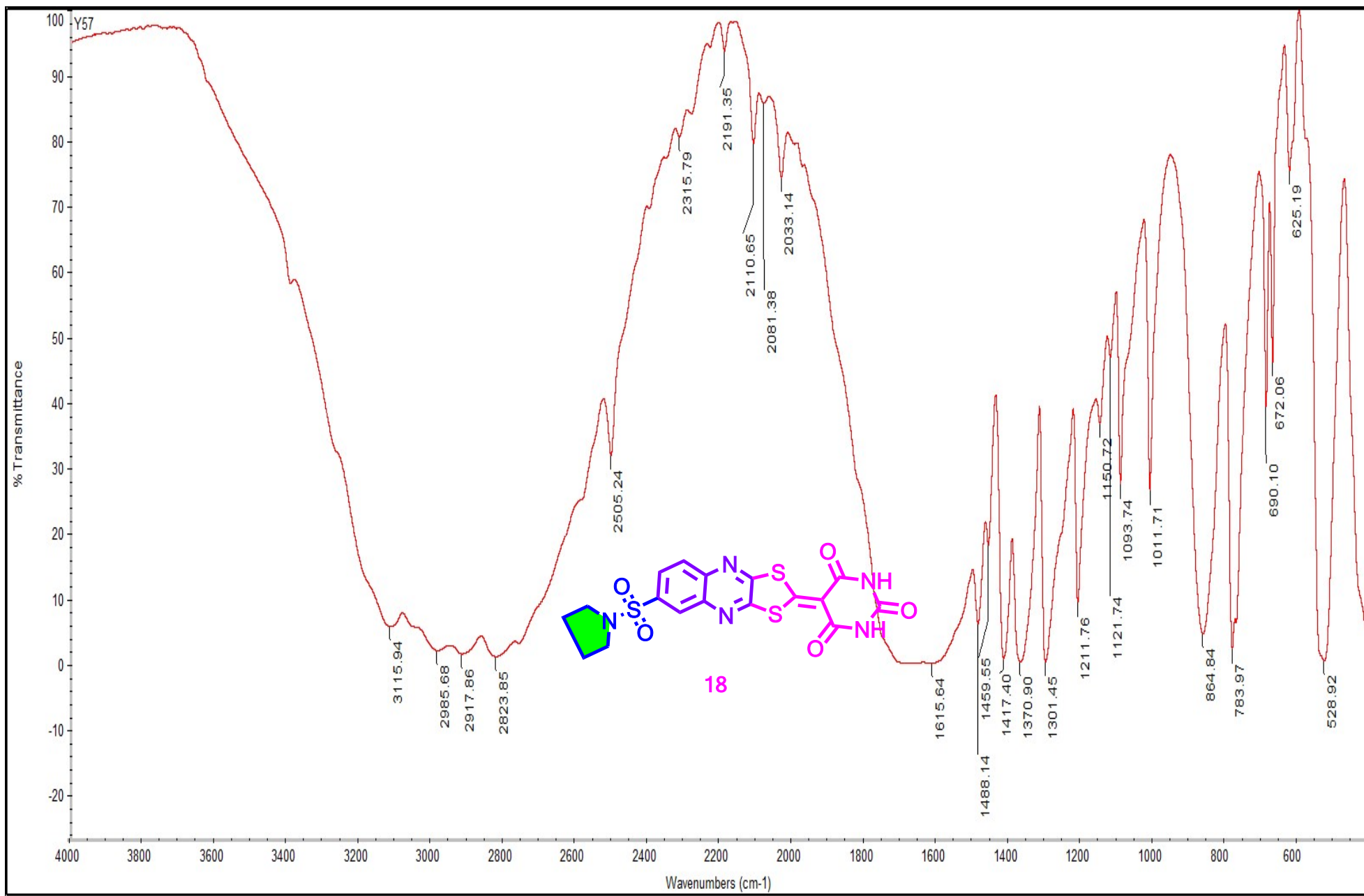
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200



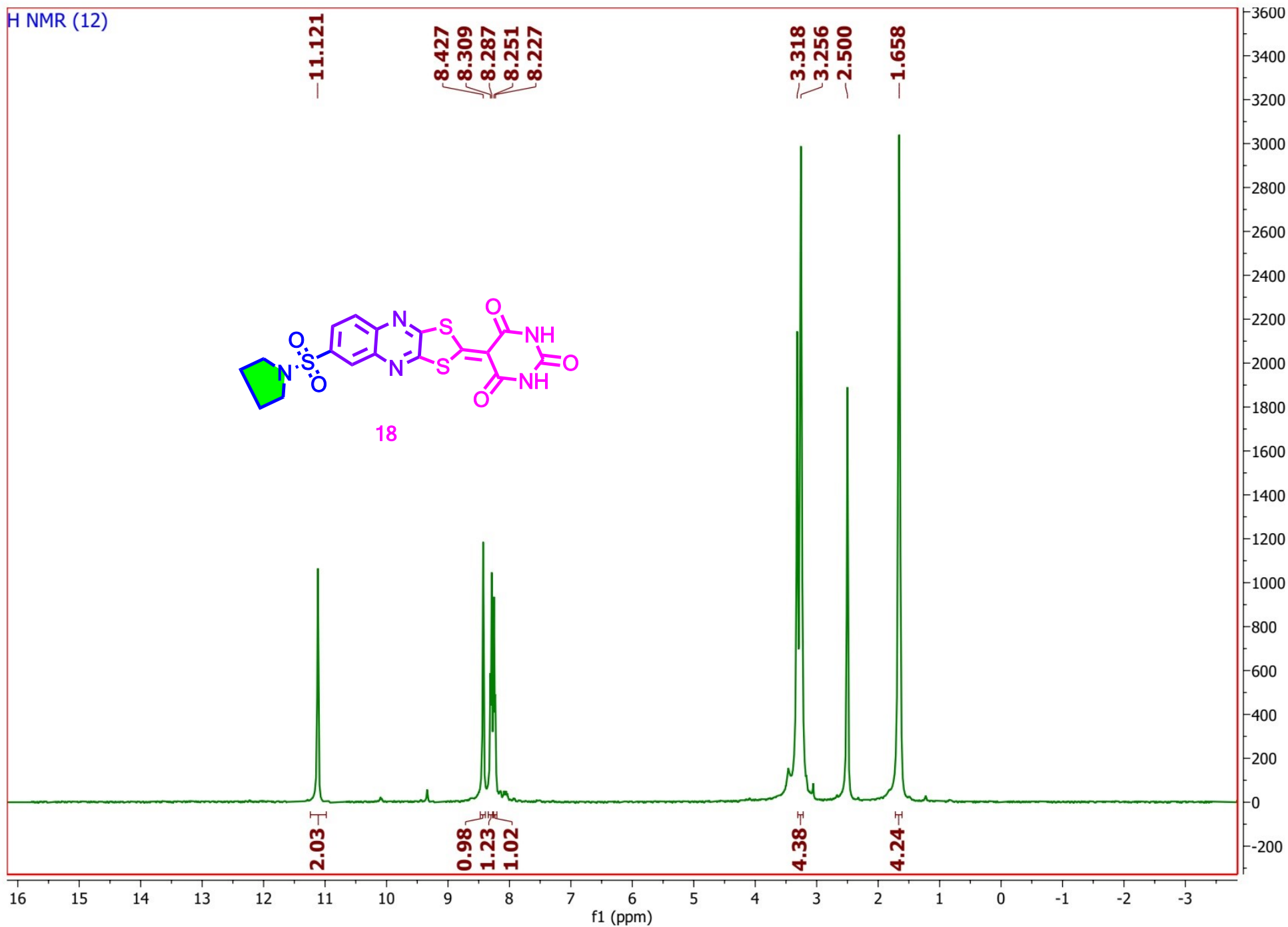
ahmed-18 #158-159 RT: 2.63-2.68 AV: 4 SB: 17 2.81-2.90, 2.61-2.78 NL: 8.77E1

T: + cEI Full ms [40.00-1000.00]





H NMR (12)



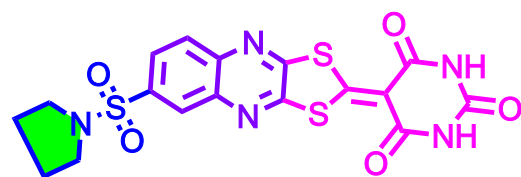
C NMR (12)

168.13
160.41
157.97
152.13
147.81
147.37
142.15
140.00
139.44
136.03
130.30
128.91
127.79

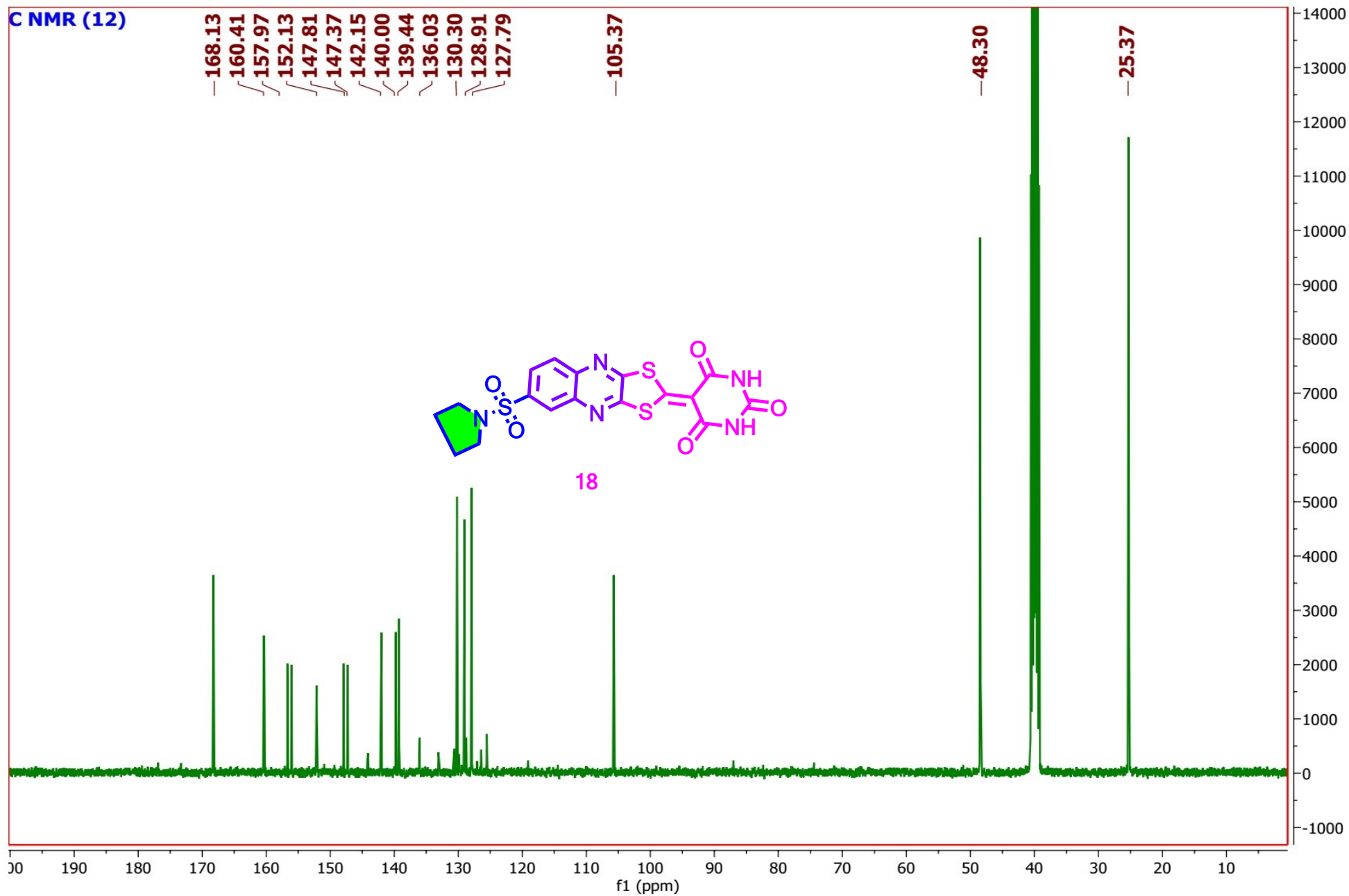
105.37

48.30

25.37



18



**Biological evaluation
supplementary material**

Cytotoxicity assay

Materials and methods

Cell line

Hepatocellular carcinoma (HEPG-2) , Mammary gland breast cancer (MCF-7) Colorectal carcinoma Colon cancer (HCT-116), Human lung fibroblast (WI38) and Breast cancer (MDA-MB-231). The cell lines were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt.

Doxorubicin was used as a standard anticancer drug for comparison.

Chemical reagents

The reagents RPMI-1640 medium , MTT and DMSO (sigma co., St. Louis, USA), Fetal Bovine serum (GIBCO, UK) .

MTT assay

The cell lines mentioned above were used to determine the inhibitory effects of compounds on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. Cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and 100µg/ml streptomycin at 37 C in a 5% CO₂ incubator. The cell lines were seeds in a 96-well plate at a density of 1.0x10⁴ cells/well. at 37 C for 48 h under 5% CO₂. After incubation the cells were treated with different concentration of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µl of MTT solution at 5mg/ml was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100 µl is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800 , USA). The relative cell viability in percentage was calculated as (A₅₇₀ of treated samples/A₅₇₀ of untreated sample) X 100.

Average of Relative viability of cells (%)

| Conc. (μ M) | HePG-2 | HCT-116 | MCF-7 |
|------------------|-------------|-------------|-------------|
| DOX | | | |
| 100 | 6.3 | 7.1 | 6.2 |
| 50 | 11.2 | 13.9 | 10.9 |
| 25 | 14.1 | 18.7 | 14.3 |
| 12.5 | 28.3 | 31.4 | 26.9 |
| 6.25 | 45.8 | 47.9 | 41.5 |
| 3.125 | 57.6 | 60.5 | 58.4 |
| 1.56 | 71.2 | 73.8 | 69.1 |
| 10b | | | |
| 100 | 48.2 | 50.1 | 45.4 |
| 50 | 59.3 | 61.9 | 58.6 |
| 25 | 72.1 | 74.2 | 71.8 |
| 12.5 | 84.5 | 96.5 | 85.1 |
| 6.25 | 100 | 100 | 98.5 |
| 3.125 | 100 | 100 | 100 |
| 1.56 | 100 | 100 | 100 |
| 7 | | | |
| 100 | 36.4 | 29.8 | 31.5 |
| 50 | 48.1 | 44.7 | 42.6 |
| 25 | 63.8 | 59.3 | 50.7 |
| 12.5 | 74.2 | 64.1 | 60.3 |
| 6.25 | 95.3 | 95.4 | 77.4 |
| 3.125 | 100 | 100 | 98.9 |
| 1.56 | 100 | 100 | 100 |
| 14 | | | |
| 100 | 44.7 | 45.8 | 43.2 |
| 50 | 56.1 | 54.9 | 58.5 |

| | | | |
|--------------|-------------|-------------|-------------|
| 25 | 69.3 | 72.3 | 70.3 |
| 12.5 | 79.6 | 83.4 | 81.7 |
| 6.25 | 97.5 | 98.7 | 98.1 |
| 3.125 | 100 | 100 | 100 |
| 1.56 | 100 | 100 | 100 |
| 10e | | | |
| 100 | 36.0 | 29.5 | 23.7 |
| 50 | 46.5 | 34.6 | 37.8 |
| 25 | 55.7 | 43.0 | 46.3 |
| 12.5 | 67.5 | 56.8 | 58.1 |
| 6.25 | 94.9 | 69.7 | 76.5 |
| 3.125 | 100 | 88.3 | 95.0 |
| 1.56 | 100 | 100 | 100 |
| 12 | | | |
| 100 | 8.8 | 7.6 | 4.7 |
| 50 | 10.4 | 11.3 | 9.1 |
| 25 | 21.5 | 24.7 | 16.3 |
| 12.5 | 38.7 | 34.1 | 22.8 |
| 6.25 | 59.2 | 55.9 | 38.6 |
| 3.125 | 70.3 | 74.6 | 57.5 |
| 1.56 | 91.6 | 88.5 | 67.8 |
| 18 | | | |
| 100 | 48.9 | 55.9 | 40.3 |
| 50 | 60.4 | 76.5 | 52.7 |
| 25 | 73.1 | 91.2 | 72.1 |
| 12.5 | 89.2 | 100 | 95.8 |
| 6.25 | 100 | 100 | 100 |
| 3.125 | 100 | 100 | 100 |
| 1.56 | 100 | 100 | 100 |
| 16 | | | |
| 100 | 56.1 | 54.3 | 49.6 |
| 50 | 76.2 | 72.5 | 61.5 |
| 25 | 87.5 | 84.7 | 73.2 |
| 12.5 | 100 | 97.6 | 95.8 |
| 6.25 | 100 | 100 | 100 |

| | | | |
|--------------|-------------|-------------|-------------|
| 3.125 | 100 | 100 | 100 |
| 1.56 | 100 | 100 | 100 |
| 10c | | | |
| 100 | 21.5 | 7.9 | 6.9 |
| 50 | 28.3 | 16.1 | 14.6 |
| 25 | 37.0 | 28.7 | 22.0 |
| 12.5 | 50.1 | 37.8 | 31.8 |
| 6.25 | 71.8 | 61.4 | 55.3 |
| 3.125 | 92.1 | 74.6 | 64.0 |
| 1.56 | 100 | 89.2 | 73.8 |
| 10f | | | |
| 100 | 22.3 | 15.0 | 8.9 |
| 50 | 40.1 | 27.1 | 16.2 |
| 25 | 48.8 | 33.7 | 21.6 |
| 12.5 | 60.7 | 43.1 | 37.4 |
| 6.25 | 80.2 | 64.2 | 59.5 |
| 3.125 | 98.5 | 76.5 | 74.1 |
| 1.56 | 100 | 100 | 95.2 |
| 10a | | | |
| 100 | 50.7 | 51.1 | 48.7 |
| 50 | 61.4 | 63.7 | 59.2 |
| 25 | 75.8 | 78.9 | 68.3 |
| 12.5 | 88.6 | 92.6 | 89.5 |
| 6.25 | 100 | 100 | 99.6 |
| 3.125 | 100 | 100 | 100 |
| 1.56 | 100 | 100 | 100 |
| 10d | | | |
| 100 | 29.4 | 20.6 | 11.6 |
| 50 | 41.6 | 29.7 | 18.1 |
| 25 | 53.7 | 36.9 | 30.3 |
| 12.5 | 65.3 | 53.5 | 44.0 |
| 6.25 | 77.1 | 74.2 | 61.2 |
| 3.125 | 98.5 | 97.8 | 82.5 |
| 1.56 | 100 | 100 | 99.4 |

Average of Relative viability of cells (%)

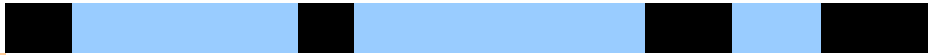
| Conc. (μ M) | WI38 | MDA-231 |
|------------------|-------------|-------------|
| DOX | | |
| 100 | 7.8 | 4.1 |
| 50 | 14.5 | 11.8 |
| 25 | 21.2 | 16.2 |
| 12.5 | 34.0 | 27.3 |
| 6.25 | 50.3 | 36.1 |
| 3.125 | 64.9 | 52.6 |
| 1.56 | 87.4 | 61.7 |
| 10c | | |
| 100 | 43.0 | 12.9 |
| 50 | 55.4 | 18.4 |
| 25 | 67.5 | 26.5 |
| 12.5 | 80.8 | 37.8 |
| 6.25 | 96.1 | 68.3 |
| 3.125 | 100 | 77.1 |
| 1.56 | 100 | 95.0 |
| 10f | | |
| 100 | 36.1 | 3.7 |
| 50 | 48.2 | 8.1 |
| 25 | 60.1 | 13.8 |
| 12.5 | 71.7 | 20.9 |
| 6.25 | 94.5 | 32.6 |
| 3.125 | 100 | 49.2 |
| 1.56 | 100 | 57.5 |
| 12 | | |
| 100 | 47.9 | 8.2 |
| 50 | 61.3 | 14.9 |
| 25 | 70.8 | 23.4 |
| 12.5 | 86.4 | 34.0 |


| | | |
|--------------|-------------|-------------|
| 6.25 | 99.7 | 55.7 |
| 3.125 | 100 | 69.8 |
| 1.56 | 100 | 88.6 |

Lab Report

| ser | Compound | | EGFR | SD ± |
|-----|--------------------|-------------|--------------|---------|
| | code | MW g/mol | IC50 uM | |
| 1 | 12/M10 | 419.53 | 0.19 | 0.009 |
| * | Erlotenib | 393.436 | 0.037 | 0.002 |
| ** | Doxorubicin | 543.52 | 0.349 | 0.016 |

Detailed Results



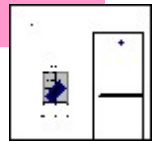
| EGFR | | | | | | | | | | | | |
|---|------|------|-----|-------|----|----|----|-------|------|-------|--------|------------|
| code | IC50 | conc | log | %inh | T2 | T1 | ΔT | RFU2 | RFU1 | ΔRFU | slope | K.Activity |
| 12/M10 | | 100 | 2 | 90.98 | 30 | 0 | 30 | 9.02 | 0 | 9.02 | 3.3333 | 10.8241 |
|  | | 10 | 1 | 85.41 | 30 | 0 | 30 | 14.59 | 0 | 14.59 | 3.3333 | 17.5082 |
| | | 1 | 0 | 66.96 | 30 | 0 | 30 | 33.04 | 0 | 33.04 | 3.3333 | 39.6484 |
| | | 0.1 | -1 | 42.45 | 30 | 0 | 30 | 57.55 | 0 | 57.55 | 3.3333 | 69.0607 |

| | | | | | | | | | | | |
|----|------|----|-------|----|---|----|-------|---|-------|--------|---------|
| | 0.01 | -2 | 26.36 | 30 | 0 | 30 | 73.64 | 0 | 73.64 | 3.3333 | 88.3689 |
| EC | | | 0 | 30 | 0 | 30 | 100 | 0 | 100 | 3.3333 | 120 |



| code | IC50 | conc | log | %inh | T2 | T1 | ΔT | RFU2 | RFU1 | ΔRFU | slope | K.Activity |
|------|------|------|-----|------|----|----|----|------|------|------|-------|------------|
|------|------|------|-----|------|----|----|----|------|------|------|-------|------------|

| | | | | | | | | | | | | |
|------------------|--|-----|---|-------|----|---|----|------|---|------|--------|---------|
| Erlotenib | | 100 | 2 | 95.65 | 30 | 0 | 30 | 4.35 | 0 | 4.35 | 3.3333 | 5.22005 |
|------------------|--|-----|---|-------|----|---|----|------|---|------|--------|---------|



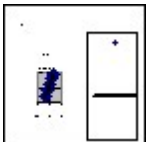
| | | | | | | | | | | | | |
|--|--|------|----|-------|----|---|----|-------|---|-------|--------|---------|
| | | 10 | 1 | 92.48 | 30 | 0 | 30 | 7.52 | 0 | 7.52 | 3.3333 | 9.02409 |
| | | 1 | 0 | 78.93 | 30 | 0 | 30 | 21.07 | 0 | 21.07 | 3.3333 | 25.2843 |
| | | 0.1 | -1 | 57.82 | 30 | 0 | 30 | 42.18 | 0 | 42.18 | 3.3333 | 50.6165 |
| | | 0.01 | -2 | 35.63 | 30 | 0 | 30 | 64.37 | 0 | 64.37 | 3.3333 | 77.2448 |

| | | | | | | | | | | | | |
|----|--|--|--|---|----|---|----|-----|---|-----|--------|-----|
| EC | | | | 0 | 30 | 0 | 30 | 100 | 0 | 100 | 3.3333 | 120 |
|----|--|--|--|---|----|---|----|-----|---|-----|--------|-----|



| code | IC50 | conc | log | %inh | T2 | T1 | ΔT | RFU2 | RFU1 | ΔRFU | slope | K.Activity |
|------|------|------|-----|------|----|----|----|------|------|------|-------|------------|
|------|------|------|-----|------|----|----|----|------|------|------|-------|------------|

| | | | | | | | | | | | | |
|------------|--|-----|---|-------|----|---|----|------|---|------|--------|---------|
| DOX | | 100 | 2 | 90.61 | 30 | 0 | 30 | 9.39 | 0 | 9.39 | 3.3333 | 11.2681 |
|------------|--|-----|---|-------|----|---|----|------|---|------|--------|---------|



| | | | | | | | | | | | | |
|--|--|------|----|-------|----|---|----|-------|---|-------|--------|---------|
| | | 10 | 1 | 77.59 | 30 | 0 | 30 | 22.41 | 0 | 22.41 | 3.3333 | 26.8923 |
| | | 1 | 0 | 61.16 | 30 | 0 | 30 | 38.84 | 0 | 38.84 | 3.3333 | 46.6085 |
| | | 0.1 | -1 | 37.48 | 30 | 0 | 30 | 62.52 | 0 | 62.52 | 3.3333 | 75.0248 |
| | | 0.01 | -2 | 23.16 | 30 | 0 | 30 | 76.84 | 0 | 76.84 | 3.3333 | 92.2089 |

| | | | | | | | | | | | | |
|----|--|--|--|---|----|---|----|-----|---|-----|--------|-----|
| EC | | | | 0 | 30 | 0 | 30 | 100 | 0 | 100 | 3.3333 | 120 |
|----|--|--|--|---|----|---|----|-----|---|-----|--------|-----|



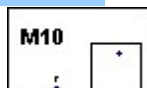
| | |
|------------------|-----------------------|
| M10 | $y = 17.22x + 62.432$ |
| Erlotenib | $y = 15.47x + 72.102$ |
| DOX | $y = 17.501x + 58$ |

Lab Report

| Ser | Sample | | EGFR (L858R) | SD ± |
|-----|-----------|--------------|--------------|---------|
| | code | M.W g/mol | IC50 uM | |
| 1 | 12/M10 | 419.53 | 0.121 | 0.007 |
| *** | Erlotenib | 393.436 | 0.026 | 0.001 |

Detailed results

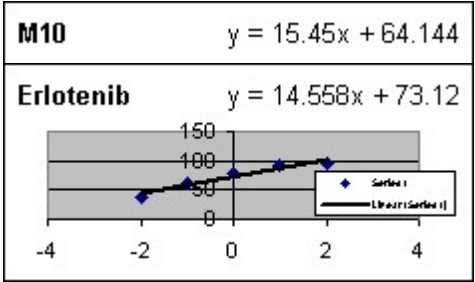
| EGFR-L858R | | | | | | | | | | | | |
|------------|------|------|-----|------|----|----|----|-------|------|-------|-------|------------|
| code | IC50 | conc | log | %inh | T2 | T1 | ΔT | RFU2 | RFU1 | ΔRFU | slope | K.Activity |
| 12/ M10 | | 100 | 2 | 91 | 30 | 0 | 30 | 8.68 | 0 | 8.68 | 3.333 | 10.416 |
| | | 10 | 1 | 87 | 30 | 0 | 30 | 17.45 | 0 | 17.45 | 4.333 | 16.10769 |
| | | 1 | 0 | 61 | 30 | 0 | 30 | 38.66 | 0 | 38.66 | 3.333 | 46.392 |
| | | 0.1 | -1 | 48 | 30 | 0 | 30 | 52.09 | 0 | 52.09 | 3.333 | 62.50801 |
| | | 0.01 | -2 | 33 | 30 | 0 | 30 | 66.54 | 0 | 66.54 | 3.333 | 79.84801 |
| EC | | | | 0 | 30 | 0 | 30 | 100 | 0 | 100 | 3.333 | 120 |



| code | IC50 | conc.ng/ml | log conc | %inh | T2 | T1 | ΔT | RFU2 | RFU1 | ΔRFU | slope | K.Activity |
|-----------|------|------------|----------|------|----|----|----|-------|------|-------|-------|------------|
| Erlotenib | | 100 | 2 | 95 | 30 | 0 | 30 | 4.82 | 0 | 4.82 | 3.333 | 5.784001 |
| | | 10 | 1 | 93 | 30 | 0 | 30 | 9.61 | 0 | 9.61 | 4.333 | 8.87077 |
| | | 1 | 0 | 79 | 30 | 0 | 30 | 21.08 | 0 | 21.08 | 3.333 | 25.296 |



| | | | | | | | | | | | |
|----|------|----|----|----|---|----|-------|---|-------|-------|----------|
| | 0.1 | -1 | 60 | 30 | 0 | 30 | 39.79 | 0 | 39.79 | 3.333 | 47.748 |
| | 0.01 | -2 | 39 | 30 | 0 | 30 | 61.38 | 0 | 61.38 | 3.333 | 73.65601 |
| EC | | | 0 | 30 | 0 | 30 | 100 | 0 | 100 | 3.333 | 120 |

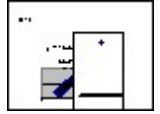


Lab Report

| ser | Compound | | VEGFR2 | | SD ± |
|-----|-----------|-------------|------------|--|---------|
| | code | MW g/mol | IC50 uM | | |
| 1 | 12/M10 | 488 | 0.42 | | 0.021 |
| * | sorafenib | 464.825 | 0.035 | | 0.002 |

VEGFR2

| code | IC50 | conc | log | %inh | T2 | T1 | ΔT | RFU2 | RFU1 | ΔRFU | slope | K.Activity |
|--------|------|------|-----|-------|----|----|----|-------|------|-------|--------|------------|
| 12/M10 | | 100 | 2 | 91.38 | 30 | 0 | 30 | 8.62 | 0 | 8.62 | 3.3333 | 10.3441 |
| | | 10 | 1 | 78.92 | 30 | 0 | 30 | 21.08 | 0 | 21.08 | 3.3333 | 25.2963 |



| | | | | | | | | | | | |
|----|------|----|-------|----|---|----|-------|---|-------|--------|---------|
| | 1 | 0 | 57.04 | 30 | 0 | 30 | 42.96 | 0 | 42.96 | 3.3333 | 51.5525 |
| | 0.1 | -1 | 36.88 | 30 | 0 | 30 | 63.12 | 0 | 63.12 | 3.3333 | 75.7448 |
| | 0.01 | -2 | 20.41 | 30 | 0 | 30 | 79.59 | 0 | 79.59 | 3.3333 | 95.509 |
| EC | | | 0 | 30 | 0 | 30 | 100 | 0 | 100 | 3.3333 | 120 |

| code | IC50 | conc | log | %inh | T2 | T1 | ΔT | RFU2 | RFU1 | ΔRFU | slope | K.Activity |
|------|------|------|-----|------|----|----|----|------|------|------|-------|------------|
|------|------|------|-----|------|----|----|----|------|------|------|-------|------------|

| | | | | | | | | | | | | |
|-----------|--|-----|---|-------|----|---|----|------|---|------|--------|---------|
| Sorafenib | | 100 | 2 | 95.14 | 30 | 0 | 30 | 4.86 | 0 | 4.86 | 3.3333 | 5.83206 |
|-----------|--|-----|---|-------|----|---|----|------|---|------|--------|---------|



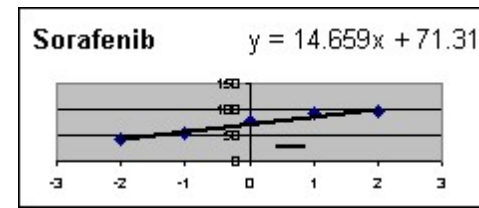
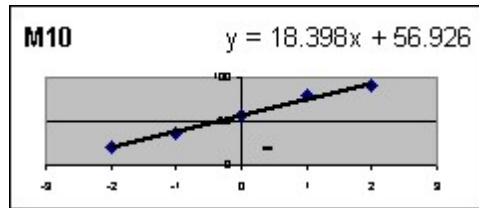
| | | | | | | | | | | | |
|--|----|---|-------|----|---|----|------|---|------|--------|--------|
| | 10 | 1 | 91.68 | 30 | 0 | 30 | 8.32 | 0 | 8.32 | 3.3333 | 9.9841 |
|--|----|---|-------|----|---|----|------|---|------|--------|--------|

| | | | | | | | | | | | |
|--|---|---|-------|----|---|----|-------|---|-------|--------|---------|
| | 1 | 0 | 75.08 | 30 | 0 | 30 | 24.92 | 0 | 24.92 | 3.3333 | 29.9043 |
|--|---|---|-------|----|---|----|-------|---|-------|--------|---------|

| | | | | | | | | | | | |
|--|-----|----|-------|----|---|----|-------|---|-------|--------|---------|
| | 0.1 | -1 | 53.93 | 30 | 0 | 30 | 46.07 | 0 | 46.07 | 3.3333 | 55.2846 |
|--|-----|----|-------|----|---|----|-------|---|-------|--------|---------|

| | | | | | | | | | | | |
|--|------|----|-------|----|---|----|-------|---|-------|--------|---------|
| | 0.01 | -2 | 40.72 | 30 | 0 | 30 | 59.28 | 0 | 59.28 | 3.3333 | 71.1367 |
|--|------|----|-------|----|---|----|-------|---|-------|--------|---------|

| | | | | | | | | | | | |
|----|--|--|---|----|---|----|-----|---|-----|--------|-----|
| EC | | | 0 | 30 | 0 | 30 | 100 | 0 | 100 | 3.3333 | 120 |
|----|--|--|---|----|---|----|-----|---|-----|--------|-----|



Lab Report

| Ser | Sample | | | RT-PCR Results | | |
|-----|-----------|-------|---------------|----------------|-------|-------|
| | | | | Fold Change | | |
| | code | cells | IC50 ug/ml | Bax | bcl2 | p53 |
| 1 | 12/MCF7 | --- | --- | 3.792 | 0.368 | 4.976 |
| 2 | Cont.MCF7 | --- | --- | 1 | 1 | 1 |

Detailed results



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 F + 61 2 9736 1364
 W www.corbettlifescience.com

Quantitation Report

Experiment Information

| | |
|-------------------------|-----------------------------|
| Run Name | Run 2022-10-29 (1) |
| Run Start | 2022-10-29 01:44:12 PM |
| Run Finish | 2022-10-29 04:26:38 PM |
| Operator | ERA |
| Notes | --- |
| Run On Software Version | Rotor-Gene 1.7.87 |
| Run Signature | The Run Signature is valid. |
| Gain Green | 10. |

| | |
|-------------|------|
| Gain Yellow | 9.33 |
|-------------|------|

Quantitation data

This report generated by Rotor-Gene 6000 Series Software 1.7 (Build 87)
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 ISO 9001:2000 (Reg. No. QEC21313)

Primers

primers

Bax : F 5'- TCAGGATGCGTCCACCAAGAAG-3',

Bax : R 5'- TGTGTCCACGGCGGCAATCATC-3'.

Bcl2 : F 5'- ATCGCCCTGTGGATGACTGAGT-3',

Bcl2 : R 5'- GCCAGGAGAAATCAAACAGAGGC -3'.

p53 : F 5'- GTTTGAGGACCTTCGACCAGCT-3',

p53 : R 5'- CAACGTACCAGGAGCCACTCTT-3'.

GAPDH : F 5'- GTCTCCTCTGACTTCAACAGCG-3'

GAPDH : R 5'- ACCACCCTGTTGCTGTAGCCAA-3'



| Sample | | | Bax | | | | | | | |
|--------|-----------|------|---------------|-------|--------------|------------|-------|--------------|----------------------------|----------------------|
| | | | Control cells | | | Test cells | | | | FLD |
| Ser | code | Conc | GAPDH | Bax | Δ CTC | GAPDH | Bax | Δ CTE | $\Delta\Delta$ CT | $2^{\Delta\Delta$ CT |
| | | | HC | TC | TC-HC | HE | TE | TE-HE | Δ CTE- Δ CTC | E=1.881 |
| 1 | 12/MCF7 | | 22.39 | 33.64 | 11.25 | 22.15 | 31.29 | 9.14 | -2.11 | 3.7928 |
| 2 | Cont.MCF7 | | 22.39 | 33.64 | 11.25 | 22.39 | 33.64 | 11.25 | 0 | 1 |

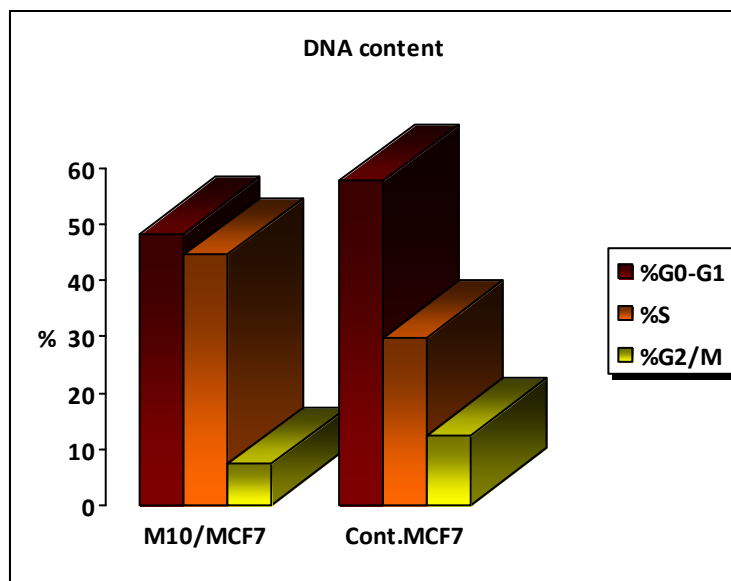
| Sample | | | Bcl2 | | | | | | | |
|--------|-----------|------|---------------|-------|--------------|------------|-------|--------------|----------------------------|----------------------|
| | | | Control cells | | | Test cells | | | | FLD |
| Ser | code | Conc | GAPDH | Bcl2 | Δ CTC | GAPDH | Bcl2 | Δ CTE | $\Delta\Delta$ CT | $2^{\Delta\Delta$ CT |
| | | | HC | TC | TC-HC | HE | TE | TE-HE | Δ CTE- Δ CTC | E=1.881 |
| 1 | 12/MCF7 | | 22.39 | 27.41 | 5.02 | 22.15 | 28.75 | 6.6 | 1.58 | 0.3685 |
| 2 | Cont.MCF7 | | 22.39 | 27.41 | 5.02 | 22.39 | 27.41 | 5.02 | 0 | 1 |

| Sample | | | p53 | | | | | | | |
|--------|-----------|------|---------------|-------|--------------|------------|-------|--------------|----------------------------|----------------------|
| | | | Control cells | | | Test cells | | | | FLD |
| Ser | code | Conc | GAPDH | p53 | Δ CTC | GAPDH | p53 | Δ CTE | $\Delta\Delta$ CT | $2^{\Delta\Delta$ CT |
| | | | HC | TC | TC-HC | HE | TE | TE-HE | Δ CTE- Δ CTC | E=1.881 |
| 1 | 12/MCF7 | | 22.39 | 27.41 | 5.02 | 22.15 | 28.75 | 6.6 | 1.58 | 0.3685 |
| 2 | Cont.MCF7 | | 22.39 | 27.41 | 5.02 | 22.39 | 27.41 | 5.02 | 0 | 1 |

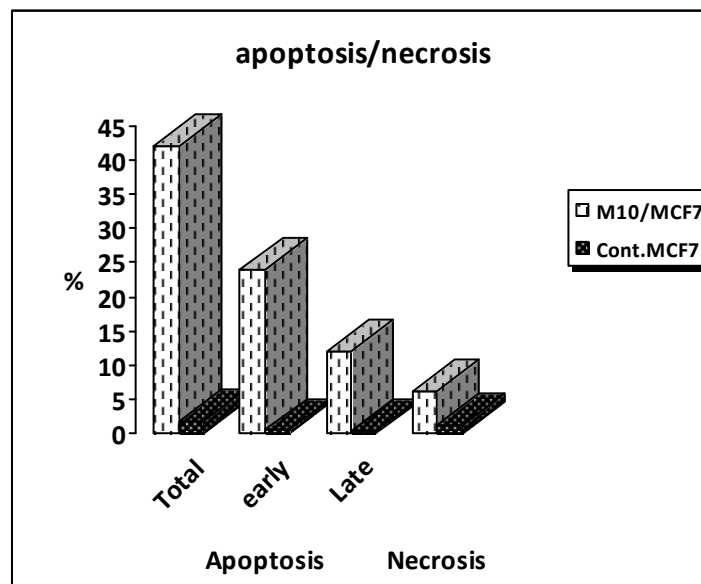
| | | HC | TC | TC-HC | HE | TE | TE-HE | Δ CTE- Δ CTC | E=1.881 |
|---|-----------|-------|-------|-------|-------|-------|-------|----------------------------|---------|
| 1 | 12/MCF7 | 22.39 | 32.85 | 10.46 | 22.15 | 30.07 | 7.92 | -2.54 | 4.9768 |
| 2 | Cont.MCF7 | 22.39 | 32.85 | 10.46 | 22.39 | 32.85 | 10.46 | 0 | 1 |

Lab Report

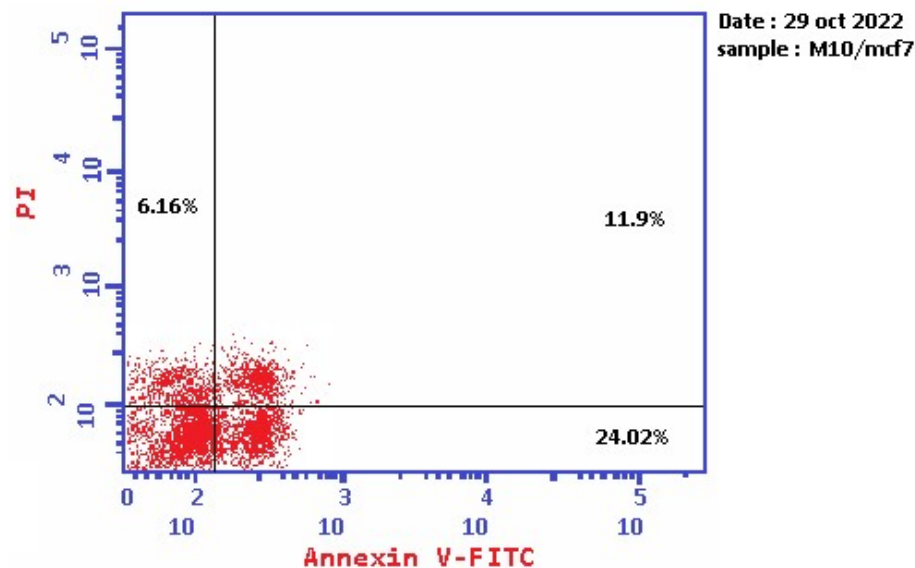
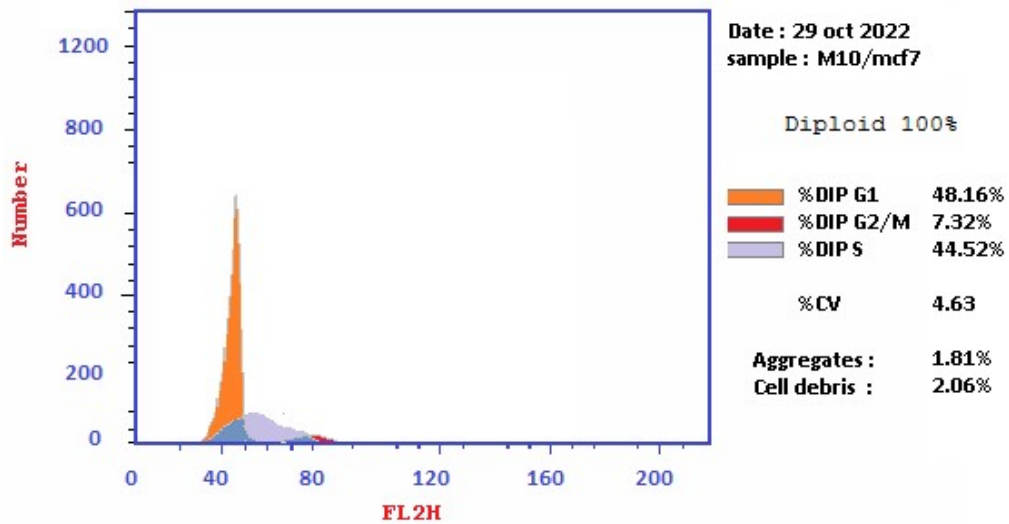
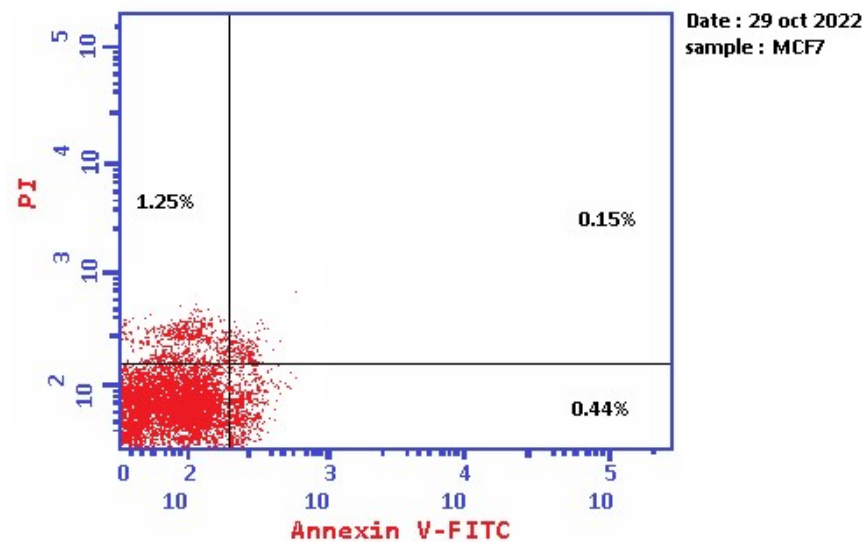
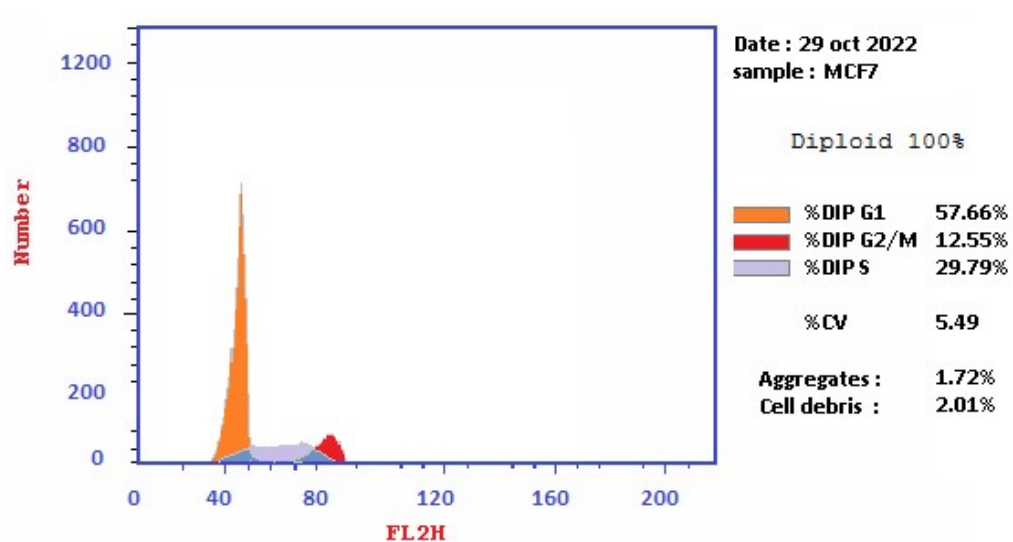
| ser | Sample | | DNA content | | | |
|-----|-----------|------------|-------------|-------|-------|-----------------------------|
| | code | IC50 uM | %G0-G1 | %S | %G2/M | Comment |
| 1 | M10/MCF7 | 3.82 | 48.16 | 44.52 | 7.32 | cell growth arrest@ S phase |
| 2 | Cont.MCF7 | --- | 57.66 | 29.79 | 12.55 | --- |



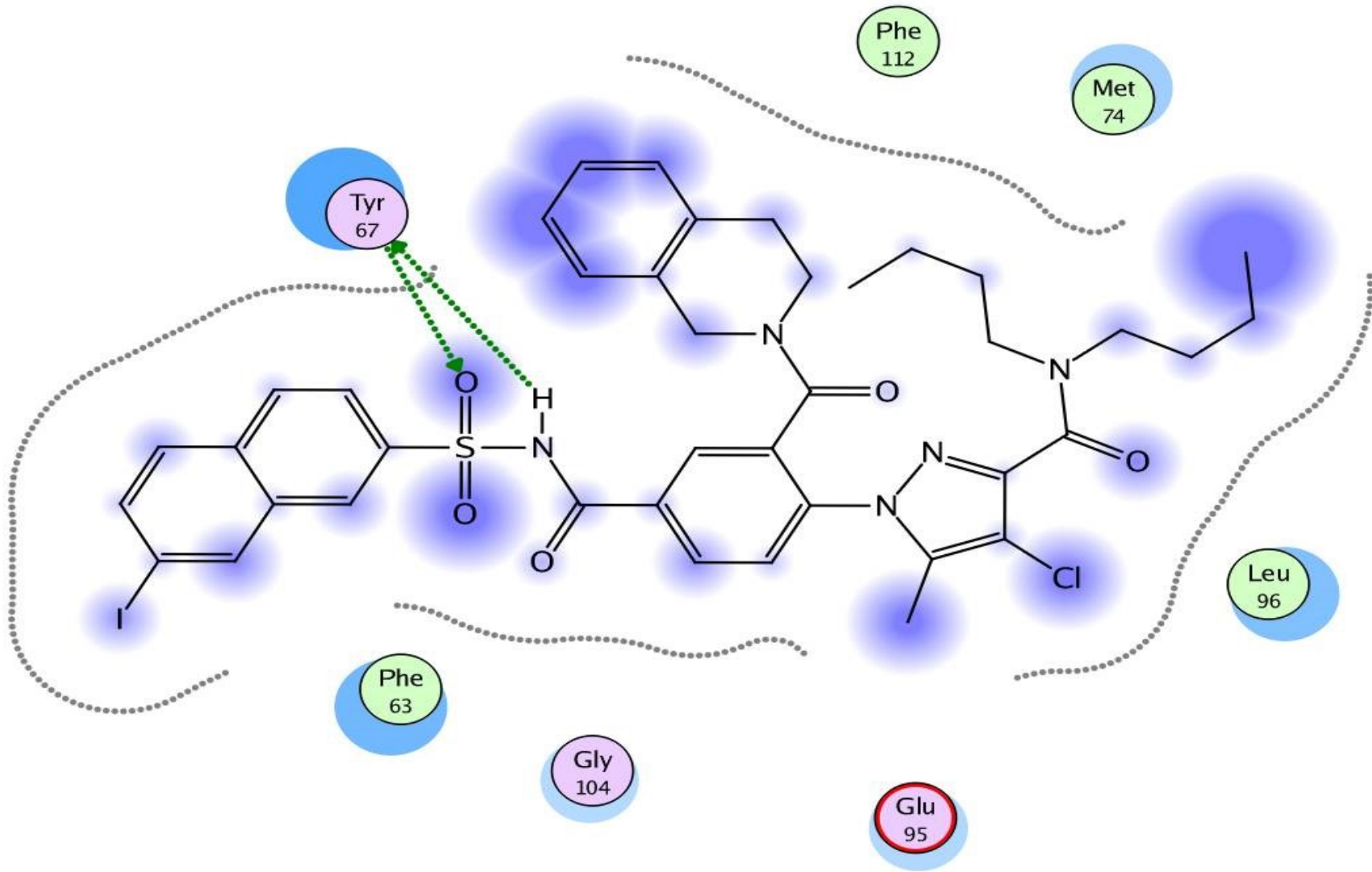
| s | code | conc | Apoptosis | | | Necrosis |
|---|--------------|------|-----------|-------|------|----------|
| | | | Total | Early | Late | |
| 1 | 12= M10/MCF7 | | 42.08 | 24.02 | 11.9 | 6.16 |
| 2 | Cont.MCF7 | | 1.84 | 0.44 | 0.15 | 1.25 |



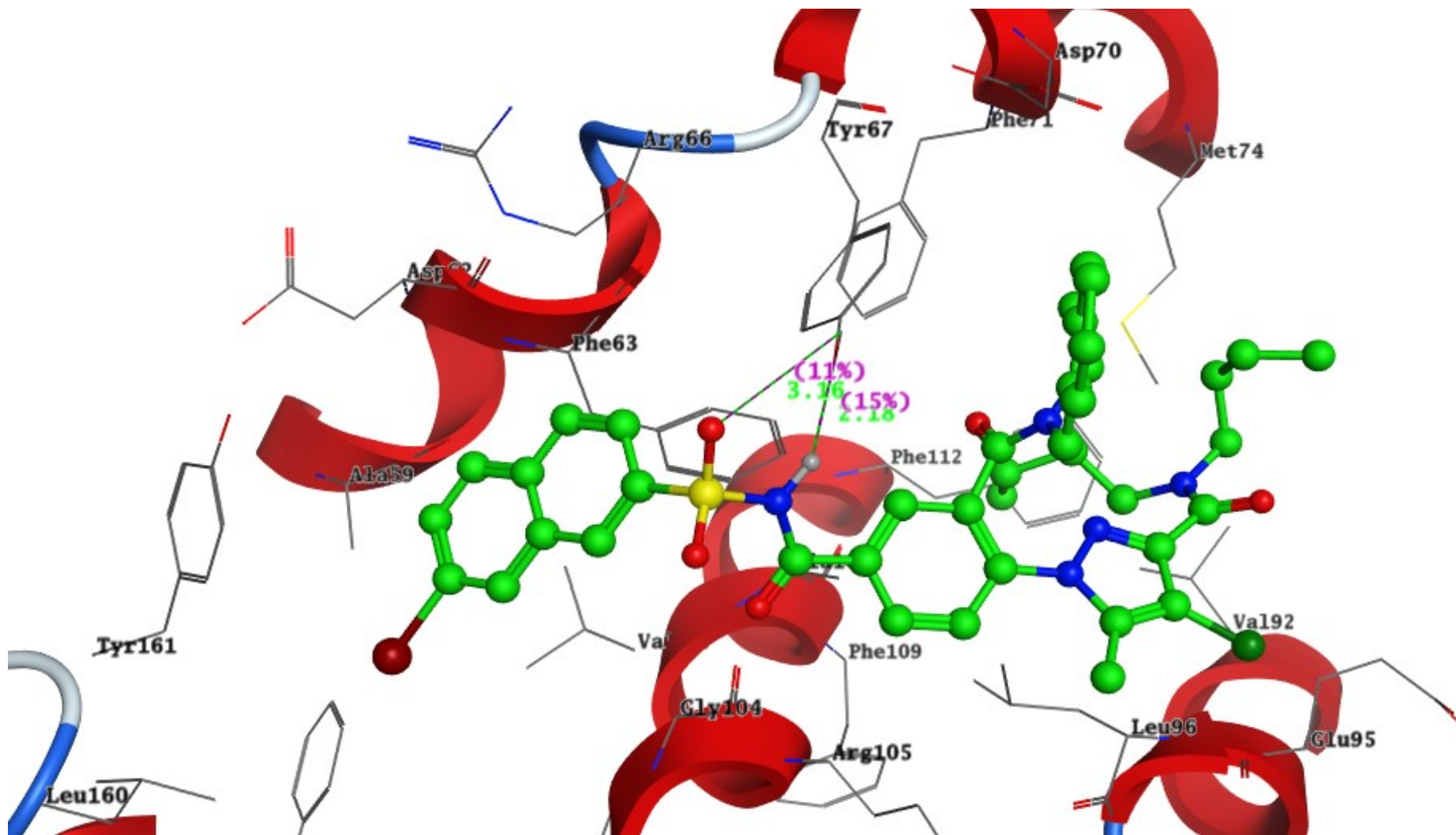
Detailed results



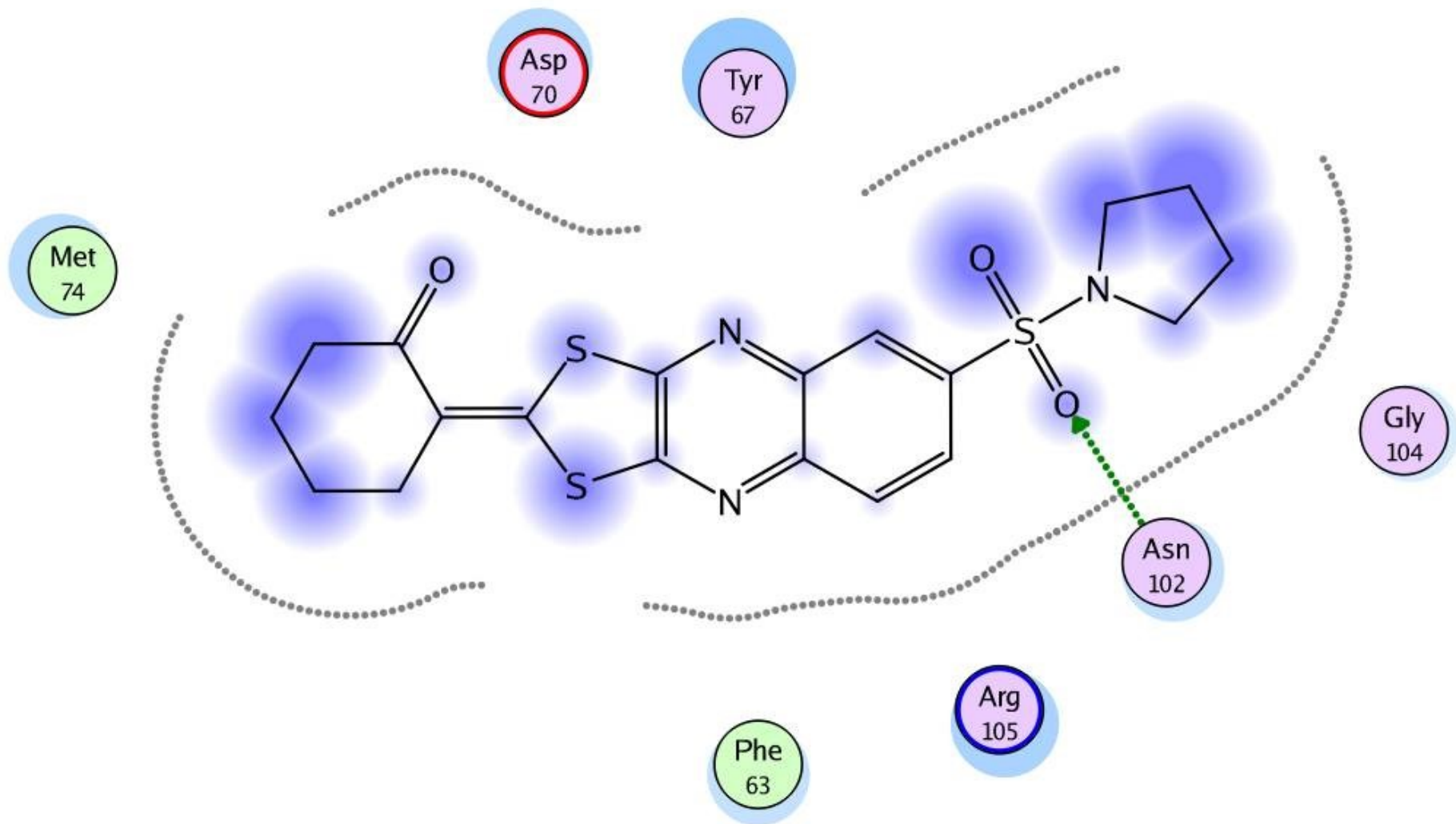
Docking simulation
figures



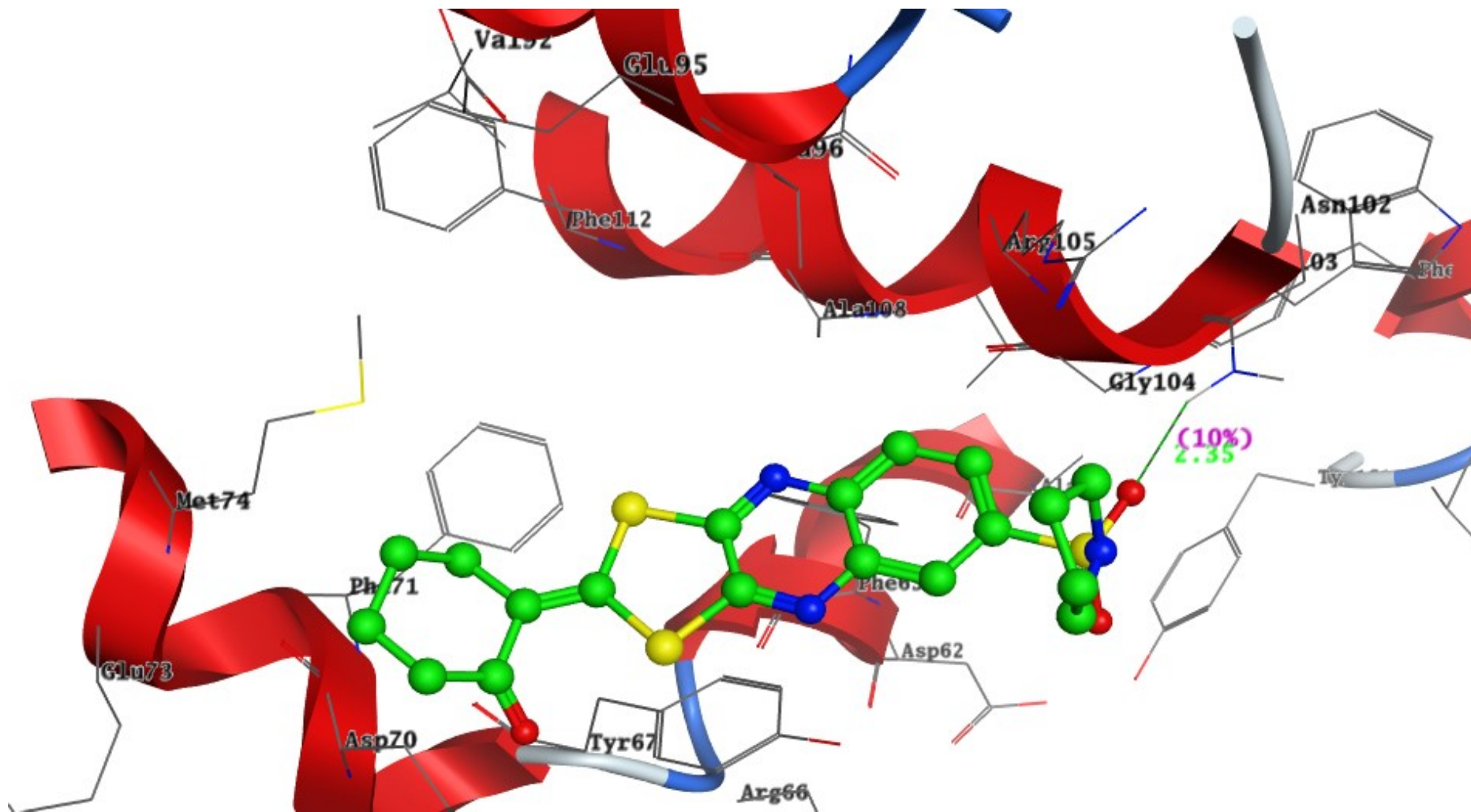
2D binding mode of co-crystallized ligand inside Bcl-2 binding pocket (**PDB**: 4AQ3)



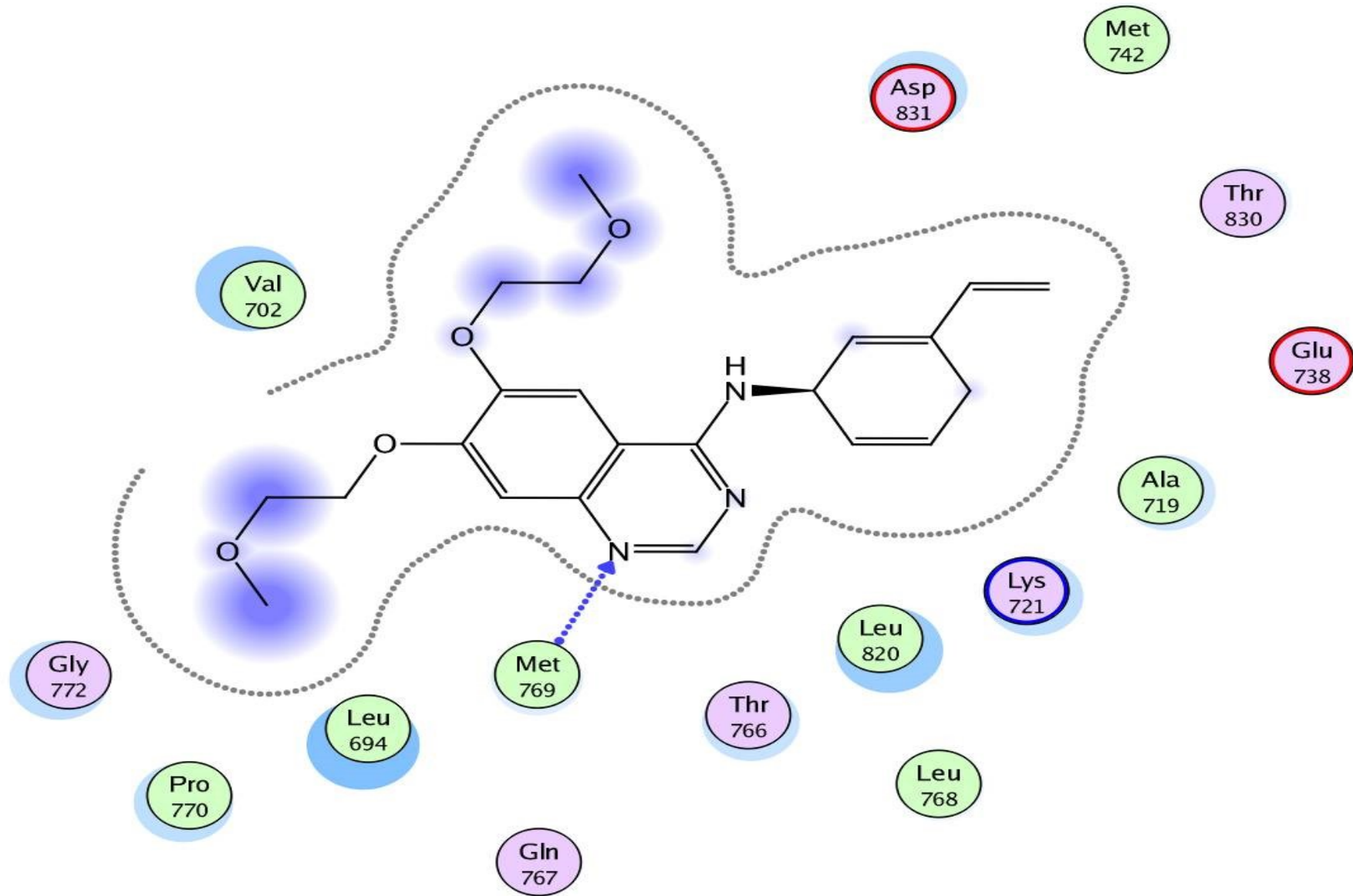
3D binding mode of co-crystallized ligand inside Bcl-2 binding pocket (PDB: 4AQ3)



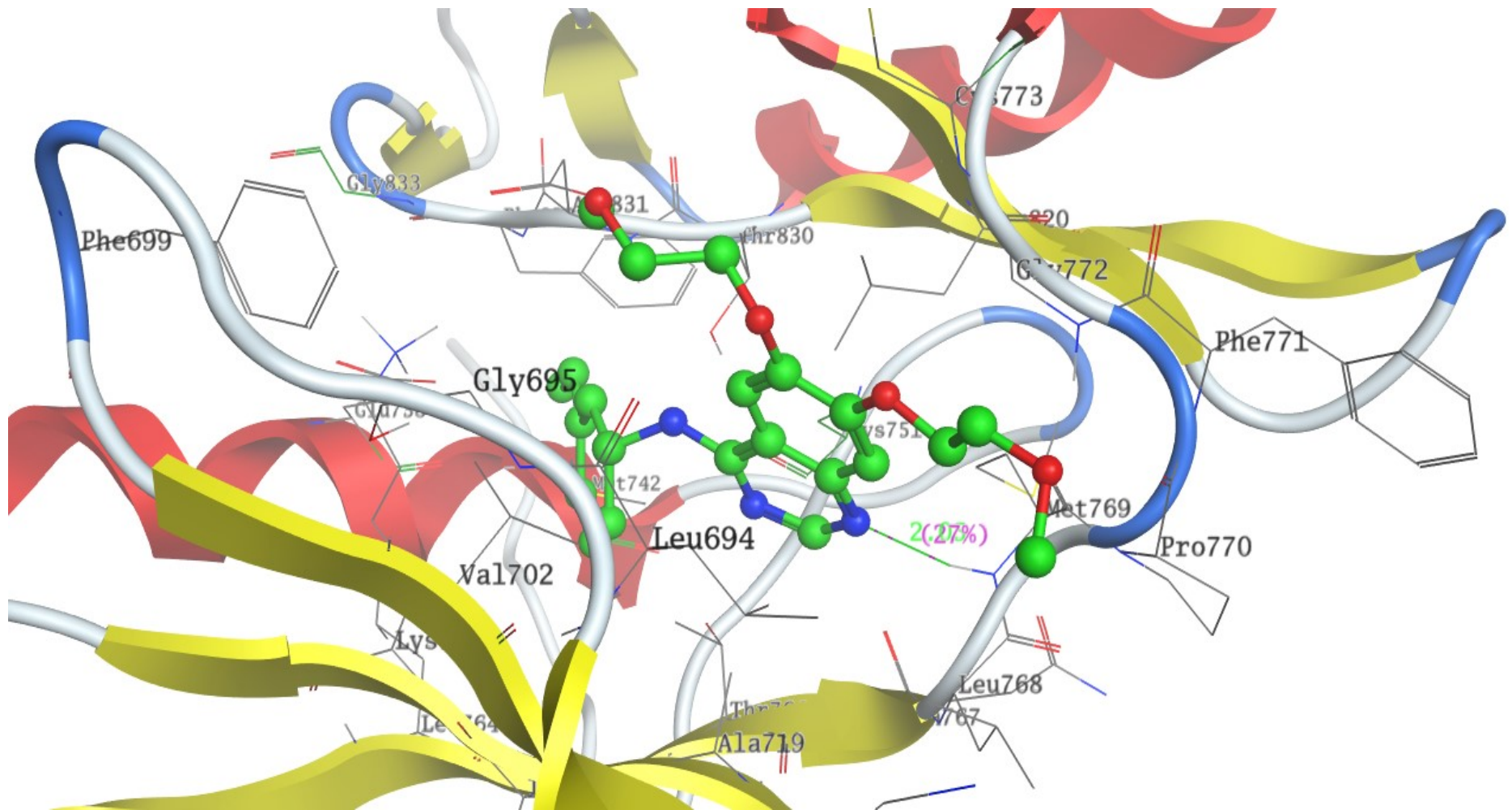
2D binding mode of compound **12** inside Bcl-2 binding pocket (PDB: 4AQ3)



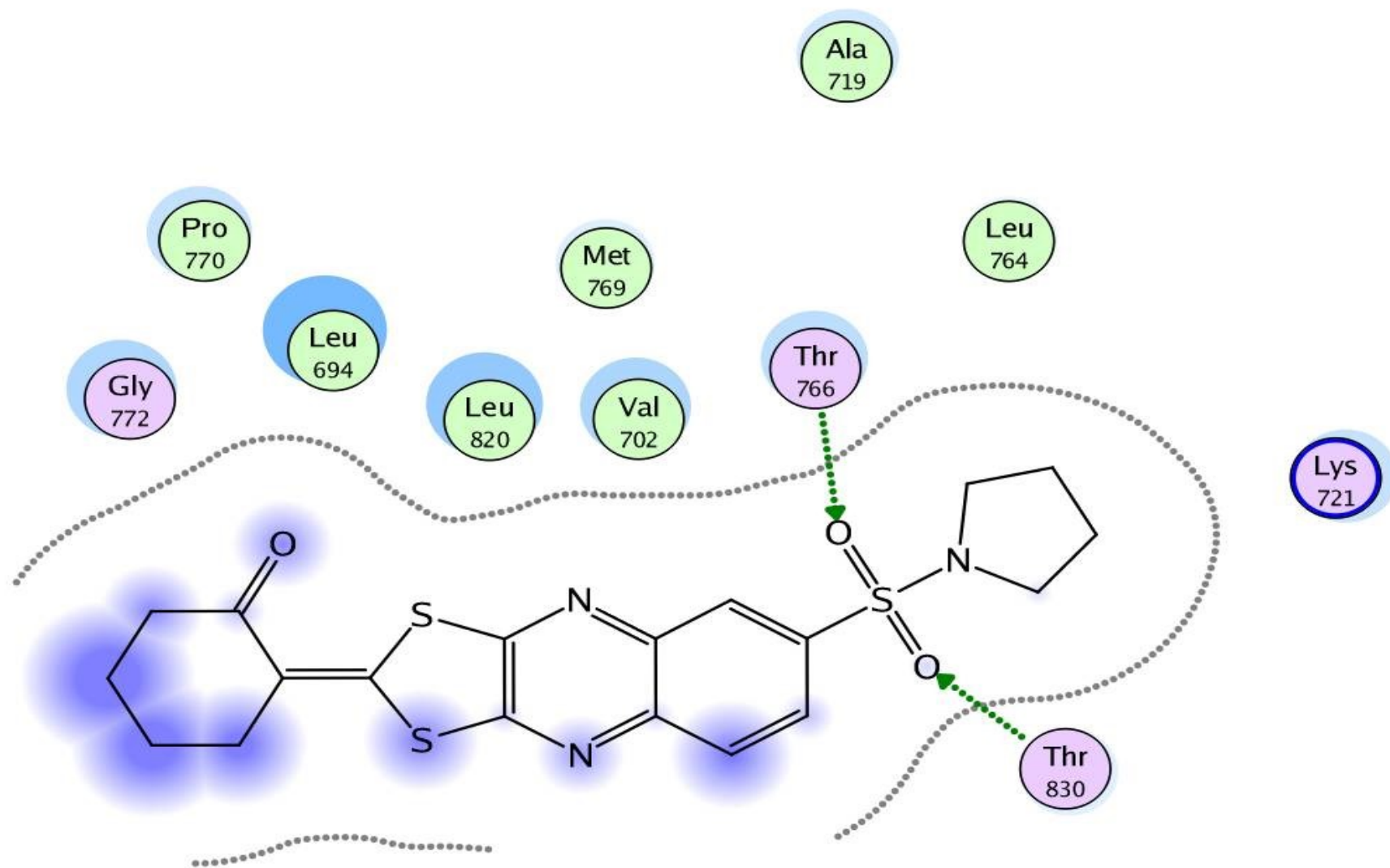
3D binding mode of compound 12 inside Bcl-2 binding pocket (PDB: 4AQ3)



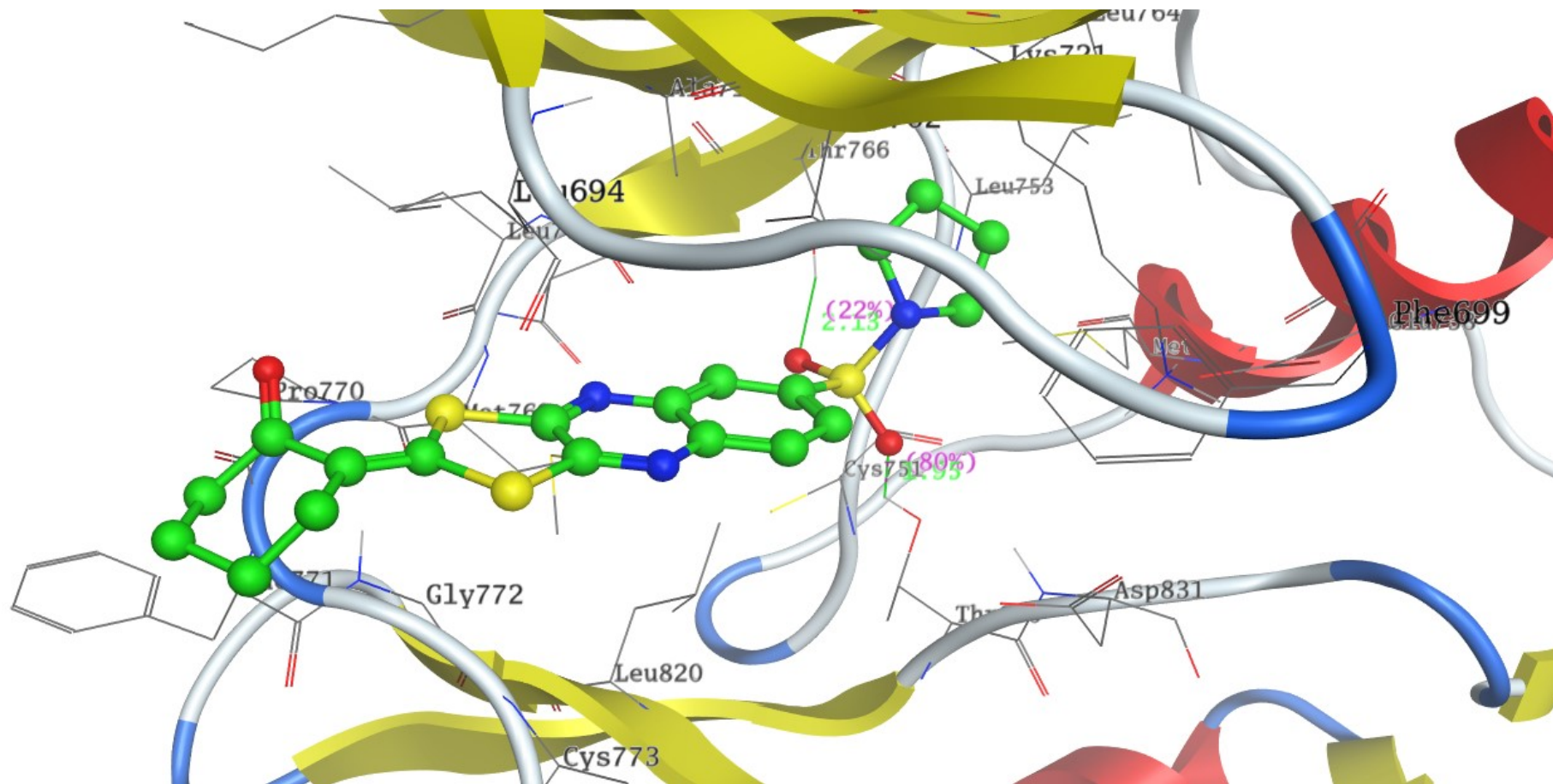
2D binding mode of co-crystallized ligand inside the EGFR binding pocket (PDB: 1M17)



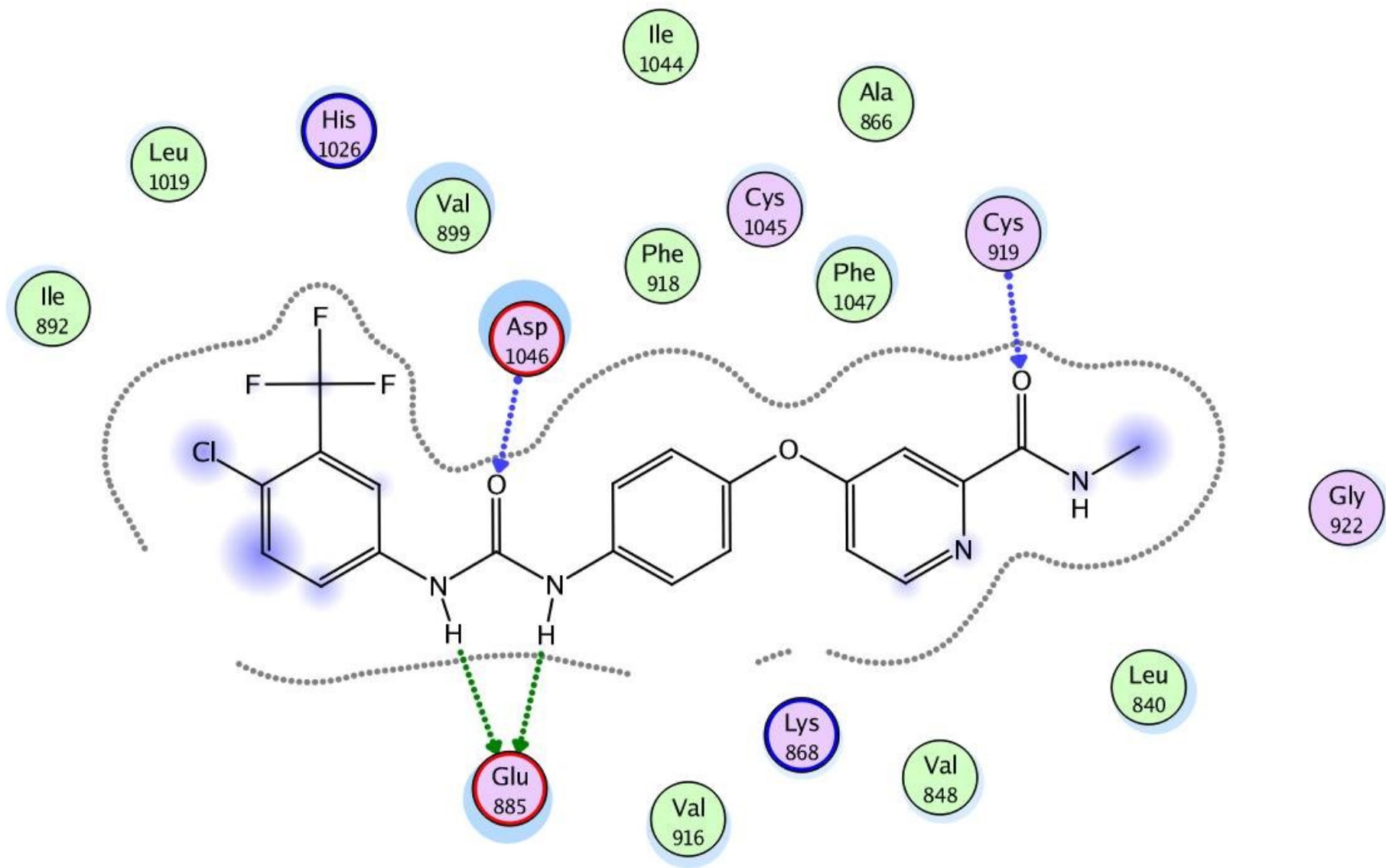
3D binding mode of co-crystallized ligand inside the EGFR binding pocket (PDB: 1M17)



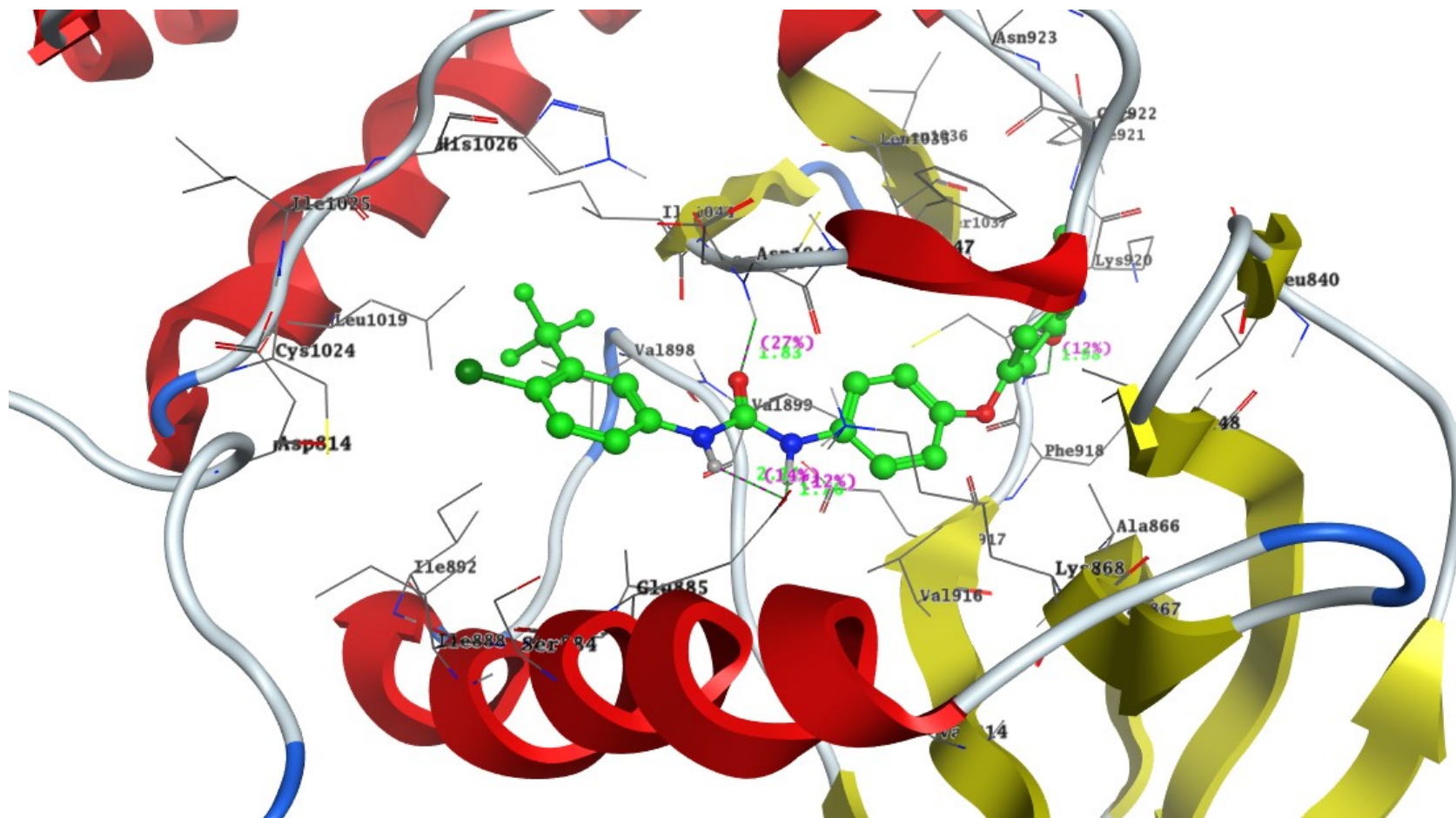
2D binding mode of compound **12** inside the EGFR binding pocket (PDB: 1M17)



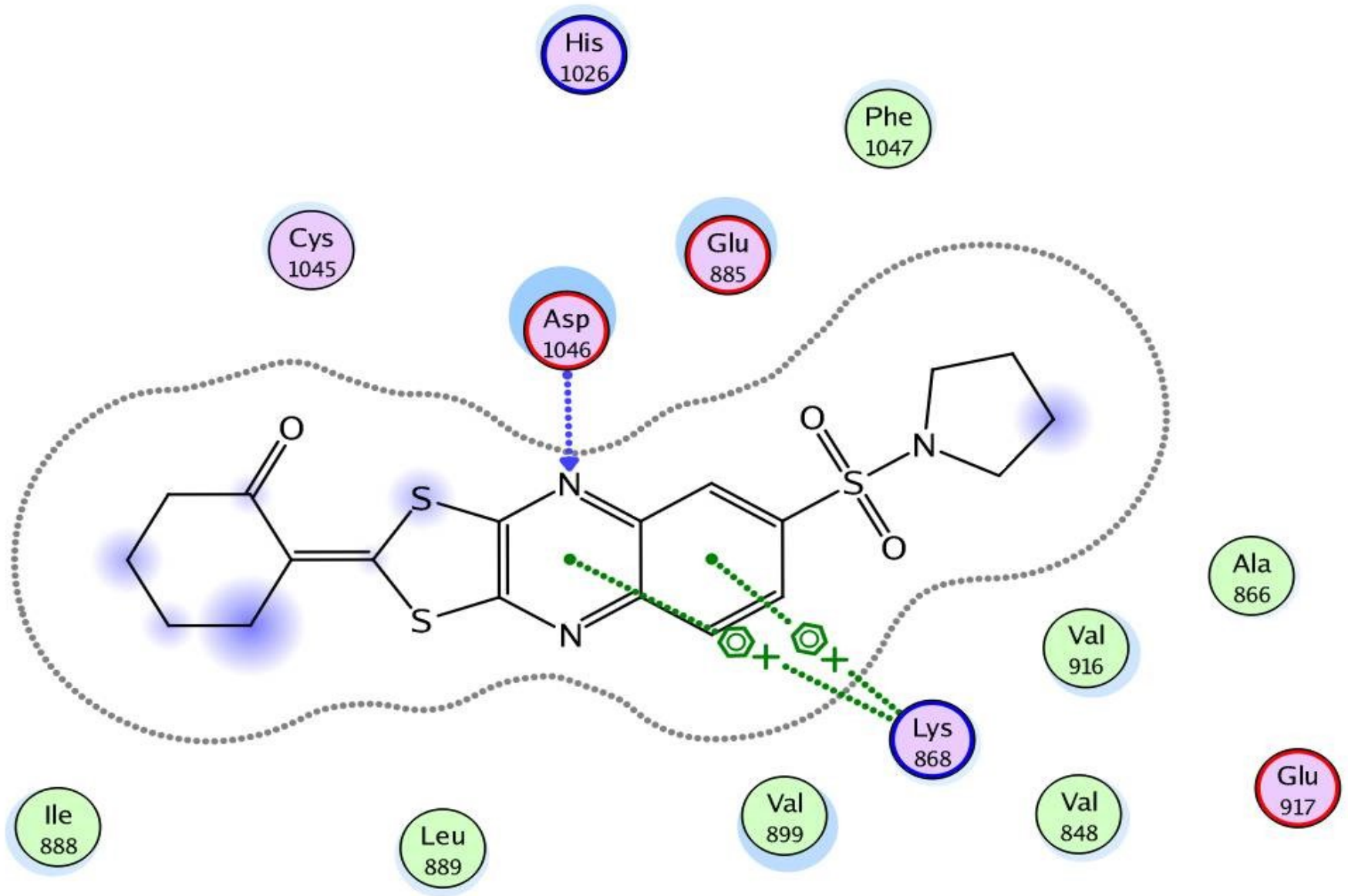
3D binding mode of compound **12** inside the EGFR binding pocket (PDB: 1M17)



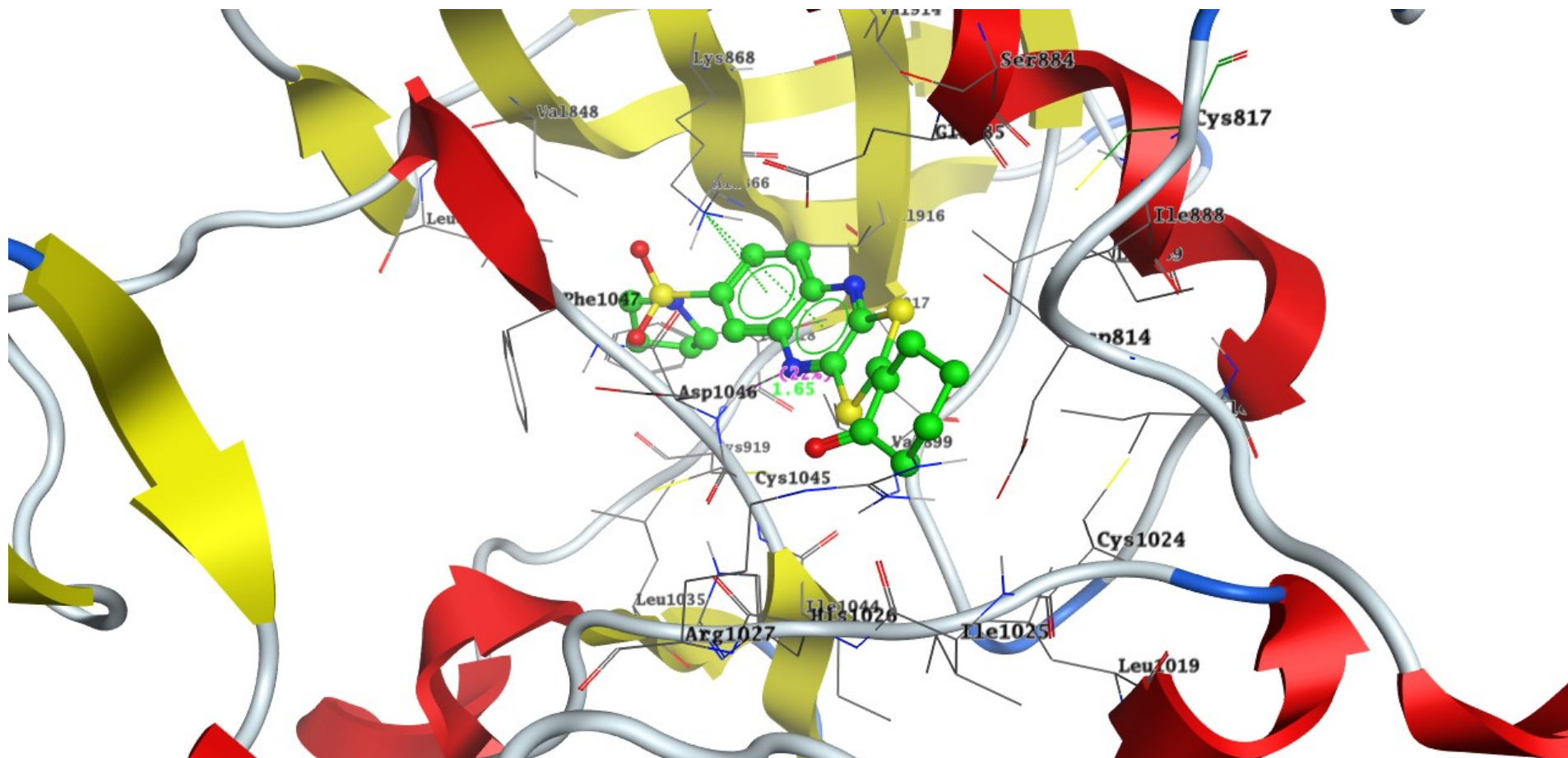
2D binding mode of co-crystallized ligand inside the VEGFR-2 binding pocket (PDB: 4ASD)



3D binding mode of co-crystallized ligand inside the VEGFR-2 binding pocket (PDB: 4ASD)



2D binding mode of compound **12** inside the VEGFR-2 binding pocket (PDB: 4ASD)



3D binding mode of compound 12 inside the VEGFR-2 binding pocket (PDB: 4ASD)