**Supplementary material for**

**SYNTHESIS OF SOME NOVEL 5-(8-SUBSTITUTED-11H-INDOLO[3,2-c]ISOQUINOLIN-5-YLTHIO)-1ʹ,3ʹ,4ʹ-OXADIAZOL-2-AMINES BEARING THIAZOLIDINONES ANDAZETIDINONES AS POTENTIAL ANTIMICROBIAL, ANTIOXIDANT, ANTITUBERCULOSIS AND ANTICANCER AGENTS.**

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**Biological evaluation.**

1. **Antimicrobial activity**

The antimicrobial screening the synthesized compounds was tested against four bacteria and four fungal species using culture medium in a sterilized borosilicate test tubes and different bacterial strains incubated at 106 bacilli/mL concentration by the broth dilution method31-33 (concentrations 50, 25, 12.5, 6.25, 3.12 and 1.5 µg/mL), respectively. The optical density is measured at a wavelength of 655 nm of each sample and compared with streptomycin and fluconazole as standard drugs for antibacterial and antifungal activities, respectively. The precise of MIC values were obtained from the lowest concentration of the test compound. The bacterial and fungal growth were completely inhibited at this concentration.

1. **Antioxidant activity.**

**1,1-Diphenyl-2-picryl-hydrazil (DPPH) radical scavenging activity (RSA**)

The RSA of the test compounds in methanolic solution at concentrations 25, 50, 75 and 100 µg/mL containing freshly prepared DPPH solution (0.004 % w/v) was carried out and compared with those of standards by using reported method 34. All the test analyses were performed on three replicates were averaged. The results in percentage are expressed as the ratio of absorption decrease in the presence of test compounds and absorption of DPPH solution in the absence of test compounds at 517 nm on ELICO SL171 mini spect spectrometer. The percentage scavenging activity of the DPPH free radical was determined using the following equation:

DPPH radical scavenging (%) = [(Ac-As/Ac)] X 100

Where,

Ac is the absorbance of the control reaction and As is the absorbance of the sample or standards.

1. **Ferric ion (Fe3+) reducing antioxidant power (FRAP).**

Ferric ion (Fe3+) Reducing Antioxidant Power (FRAP): The reducing power of the synthesized compounds was determined according to the literature method35 using BHA, TBHQ and AA as standards. Different concentrations of samples (25, 50, 75 and 100 µg/mL) in DMSO (1 mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH=6.6) and potassium ferricyanide(2.5 mL,1 %). The mixture was incubated at 50 ºC for 20 min, after which 2.5 mL of trichloroacetic acid (2.5 mL, 10 %) was added to the mixture and centrifuged for 10 min at 1000 rpm. The upper layer solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%). Then the absorbance at 700 nm was measured in spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

**Data of 1,1-Diphenyl-2-picryl-hydrazil (DPPH) radical scavenging activity (RSA)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Compounds | 25 | 50 | 75 | 100 |
| 5a | 31.49 | 56.91 | 61.88 | 66.85 |
| 5b | 16.35 | 26.17 | 31.49 | 42.9 |
| 5c | 20.89 | 37.33 | 44.29 | 55.29 |
| 5d | 33.27 | 48.18 | 50.42 | 51.25 |
| 5e | 44.57 | 55.43 | 61.88 | 65.55 |
| 5f | 11.88 | 33.98 | 38.12 | 45.3 |
| 5g | 48.9 | 51.66 | 55.99 | 61.55 |
| 5h | 27.86 | 34.26 | 44.29 | 54.6 |
| 5i | 20.89 | 37.33 | 39.2 | 52.09 |
| 6a | 60.5 | 66.85 | 74.37 | 78.83 |
| 6b | 32.29 | 44.75 | 56.65 | 61.56 |
| 6c | 30.92 | 38.44 | 46.52 | 58.57 |
| 6d | 28.06 | 31.67 | 40.56 | 57.58 |
| 6e | 16.39 | 28.89 | 39.44 | 59.72 |
| 6f | 31.94 | 28.89 | 45 | 60.56 |
| 6g | 11.39 | 44.72 | 48.61 | 54.72 |
| 6h | 38.61 | 39.47 | 56.39 | 67.32 |
| 6g | 19.72 | 54.44 | 68.89 | 66.55 |
| 7a | 18.1 | 62.61 | 68.41 | 77.27 |
| 7b | 44.28 | 55.71 | 51.55 | 66.85 |
| 7c | 14.48 | 17.55 | 20.89 | 33.98 |
| 7d | 32.31 | 40.94 | 50.41 | 59.61 |
| 7e | 40.38 | 50.41 | 62.21 | 62.67 |
| 7f | 50.69 | 55.71 | 62.85 | 69.99 |
| 7g | 33.98 | 40.38 | 55.71 | 61.55 |
| 7h | 40.11 | 55.71 | 66.3 | 62.14 |
| 7i | 45.15 | 55.18 | 65.21 | 67.04 |
| 8a | 47.35 | 52.11 | 63.53 | 72.04 |
| 8b | 46.51 | 55.98 | 59.88 | 61.55 |
| 8c | 33.42 | 40.11 | 47.08 | 50.69 |
| 8d | 46.51 | 44.29 | 55.71 | 61.55 |
| 8e | 11.97 | 40.11 | 58.5 | 62.67 |
| 8f | 15.87 | 39.83 | 56.54 | 58.5 |
| 8g | 46.51 | 51.56 | 62.7 | 70.7 |
| 8h | 28.69 | 42.06 | 43.17 | 48.74 |
| 8i | 39.83 | 40.38 | 44.57 | 50.41 |
| BHA | 87.47 | 85.52 | 92.2 | 91.64 |
| TBHQ | 90.25 | 92.76 | 91.64 | 90.25 |
| AA | 88.86 | 88.02 | 94.71 | 93.59 |

**Data of Ferric ion (Fe3+) reducing antioxidant power (FRAP)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Compounds | 25 | 50 | 75 | 100 |
| 5a | 0.205 | 0.268 | 0.318 | 0.424 |
| 5b | 0.228 | 0.345 | 0.405 | 0.543 |
| 5c | 0.315 | 0.363 | 0.429 | 0.458 |
| 5d | 0.238 | 0.375 | 0.459 | 0.425 |
| 5e | 0.326 | 0.489 | 0.563 | 0.545 |
| 5f | 0.302 | 0.458 | 0.486 | 0.629 |
| 5g | 0.211 | 0.228 | 0.368 | 0.415 |
| 5h | 0.308 | 0.438 | 0.542 | 0.592 |
| 5i | 0.233 | 0.348 | 0.492 | 0.618 |
| 6a | 0.224 | 0.31 | 0.393 | 0.427 |
| 6b | 0.297 | 0.243 | 0.3 | 0.318 |
| 6c | 0.118 | 0.197 | 0.219 | 0.254 |
| 6d | 0.318 | 0.343 | 0.492 | 0.328 |
| 6e | 0.538 | 0.662 | 0.783 | 0.998 |
| 6f | 0.498 | 0.513 | 0.648 | 0.683 |
| 6g | 0.338 | 0.353 | 0.392 | 0.428 |
| 6h | 0.342 | 0.492 | 0.558 | 0.698 |
| 6i | 0.295 | 0.368 | 0.423 | 0.585 |
| 7a | 0.224 | 0.31 | 0.393 | 0.427 |
| 7b | 0.297 | 0.243 | 0.3 | 0.318 |
| 7c | 0.118 | 0.197 | 0.219 | 0.254 |
| 7d | 0.318 | 0.343 | 0.492 | 0.328 |
| 7e | 0.538 | 0.662 | 0.725 | 0.893 |
| 7f | 0.398 | 0.513 | 0.648 | 0.762 |
| 7g | 0.338 | 0.353 | 0.392 | 0.428 |
| 7h | 0.342 | 0.492 | 0.558 | 0.698 |
| 7i | 0.295 | 0.368 | 0.423 | 0.58 |
| 8a | 0.224 | 0.264 | 0.296 | 0.345 |
| 8b | 0.312 | 0.328 | 0.434 | 0.458 |
| 8c | 0.218 | 0.264 | 0.393 | 0.436 |
| 8d | 0.155 | 0.173 | 0.208 | 0.235 |
| 8e | 0.448 | 0.491 | 0.662 | 0.798 |
| 8f | 0.346 | 0.455 | 0.542 | 0.711 |
| 8g | 0.209 | 0.161 | 0.218 | 0.245 |
| 8h | 0.318 | 0.368 | 0.517 | 0.656 |
| 8i | 0.266 | 0.322 | 0.322 | 0.526 |
| BHA | 0.919 | 1.115 | 1.134 | 1.34 |
| TBHQ | 0.885 | 1.115 | 1.341 | 1.343 |
| AA | 0.7 | 0.838 | 0.991 | 1.158 |

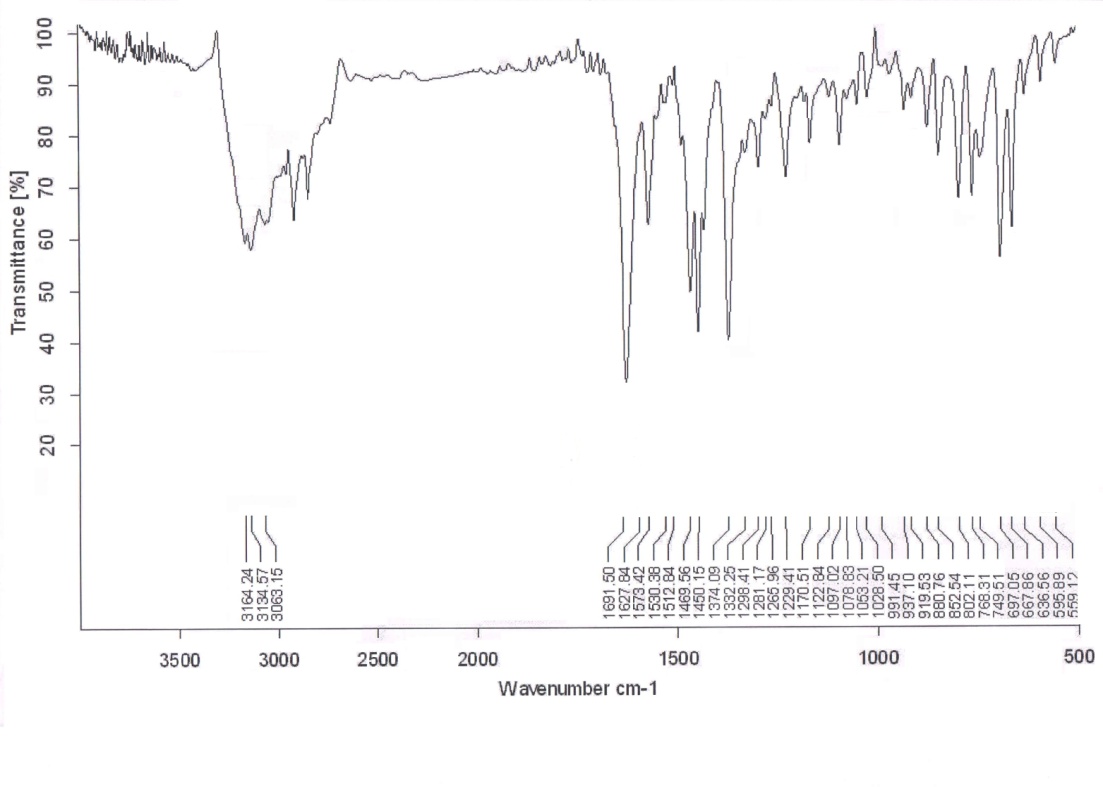
1. **Anti-tuberculosis activity**

Anti-tuberculosis activity of synthesized compounds was tested against *M. tuberculosis* (ATCC 27294) H37RV using alamar blue reagent. Serial diluted solutions (100, 50, 25, 12.5, 6.25, 3.12, 1.5 and 0.8 µg/mL) of the test compounds in dimethyl formamide (DMF), Rifampicin (1.0 μg/mL) as a positive control and DMF as negative control were used to determine the activity according to the literature (MABA) method 36.

**Anticancer activity**

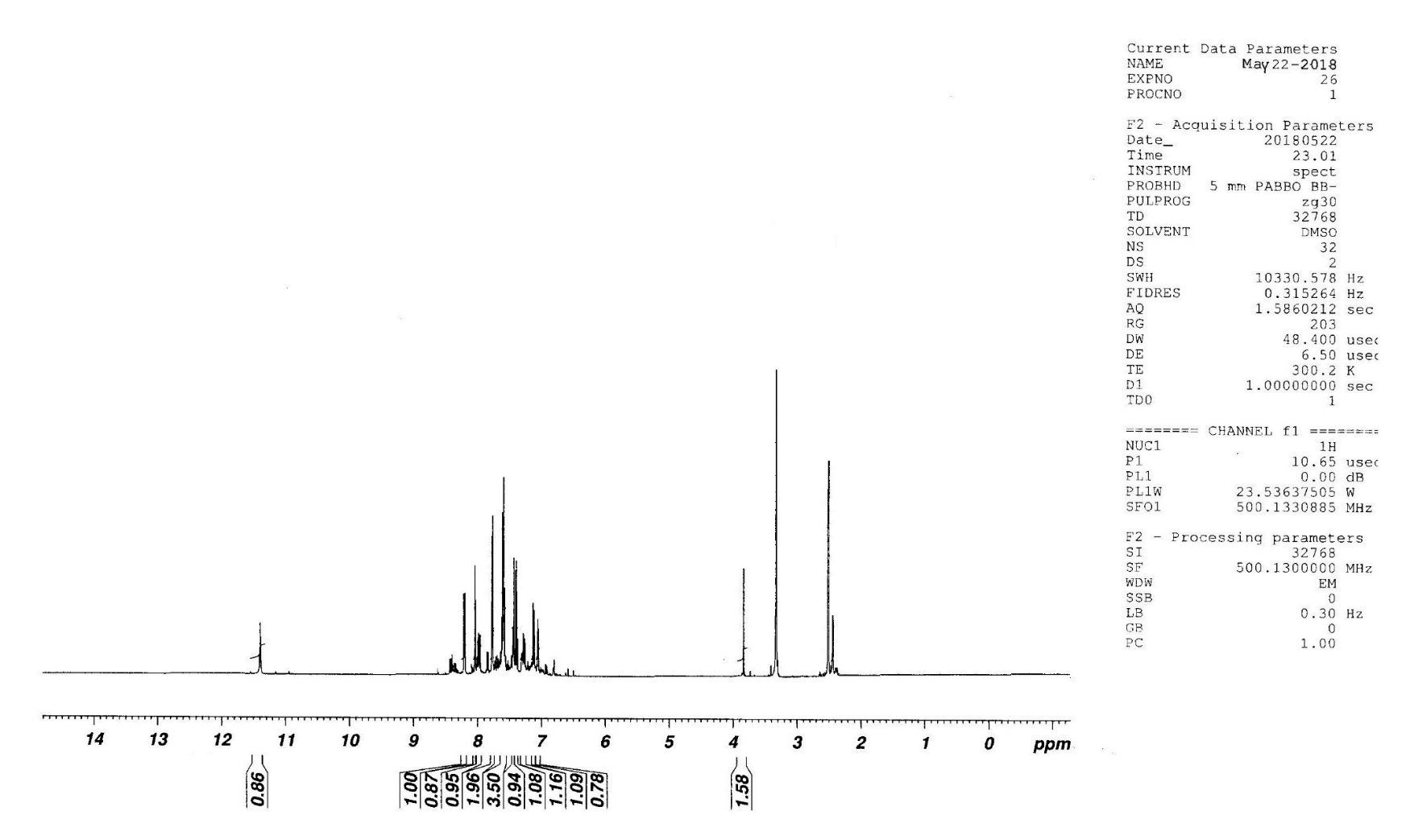
The anticancer activity of synthesized compounds was tested against three different human cancer cell lines including MCF-7 (breast carcinoma), A-549(lung carcinoma) and HeLa (Cervical carcinoma) cancer cell lines. The synthesized compounds were diluted in Dimethyl Sulfoxide (DMSO) at various concentrations (10, 5, 2.5 and 1.25 μg ML-1) and assessed using 3-(4, 5-dimethyl-2-yl-2, 5-diphenyl tetrazolium bromide (MTT assay)37. Anticancer activity was determined for cells treated various concentrations of the tested compounds, the untreated cells (negative control) and Doxorubicin (positive control). A statistical significance was tested between sample and negative control using independent t-test by SPSS 12 program. The concentration of test compounds required to kill 50% of cell population (IC50) were determined by non-linear regression analysis. Cytotoxic activity was expressed as the mean IC50 of three independent experiments.

**5. IR, 1H, 13C-NMR and MS Spectra of all c(2-6 and 8) series synthesized compounds**

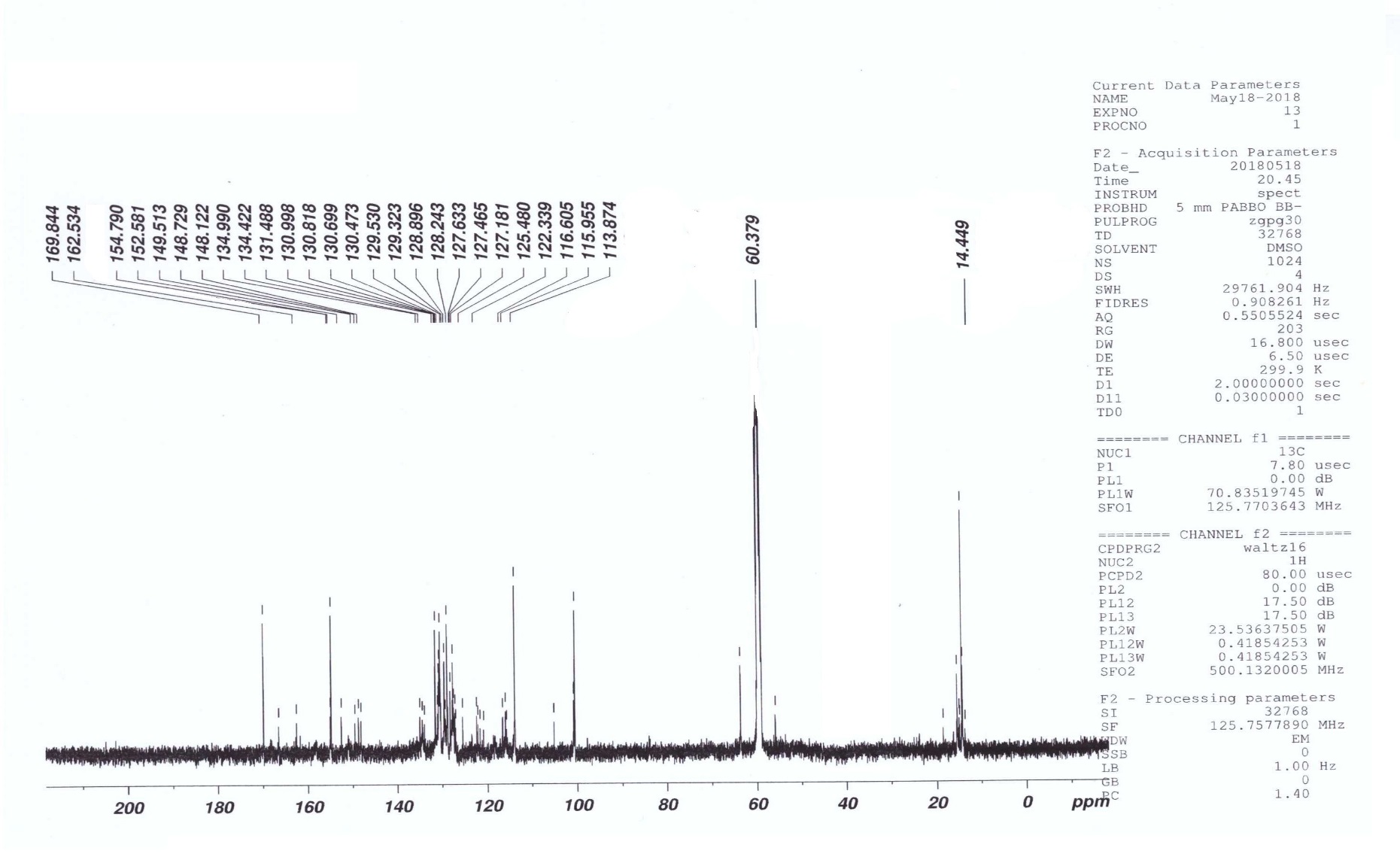




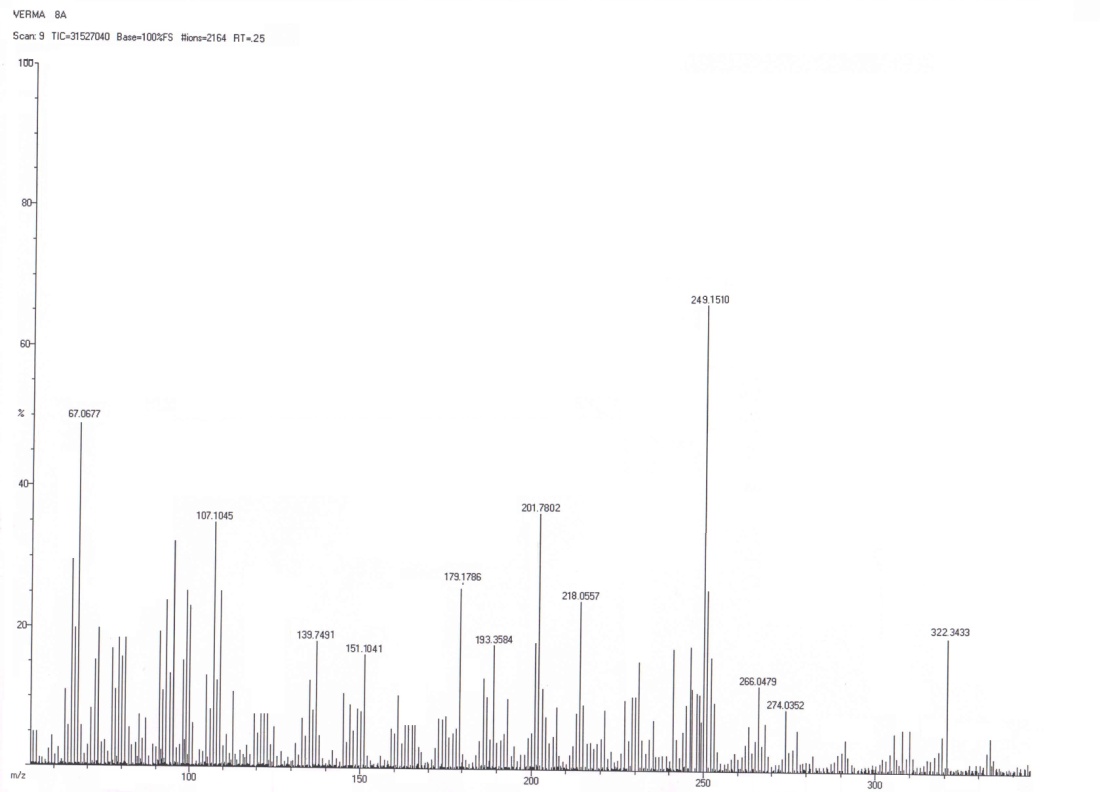
**Figure S1:** IR spectrum for ethyl (11*H*-indolo[3,2-c]isoquinolin-5-ylthio)formate (2c)



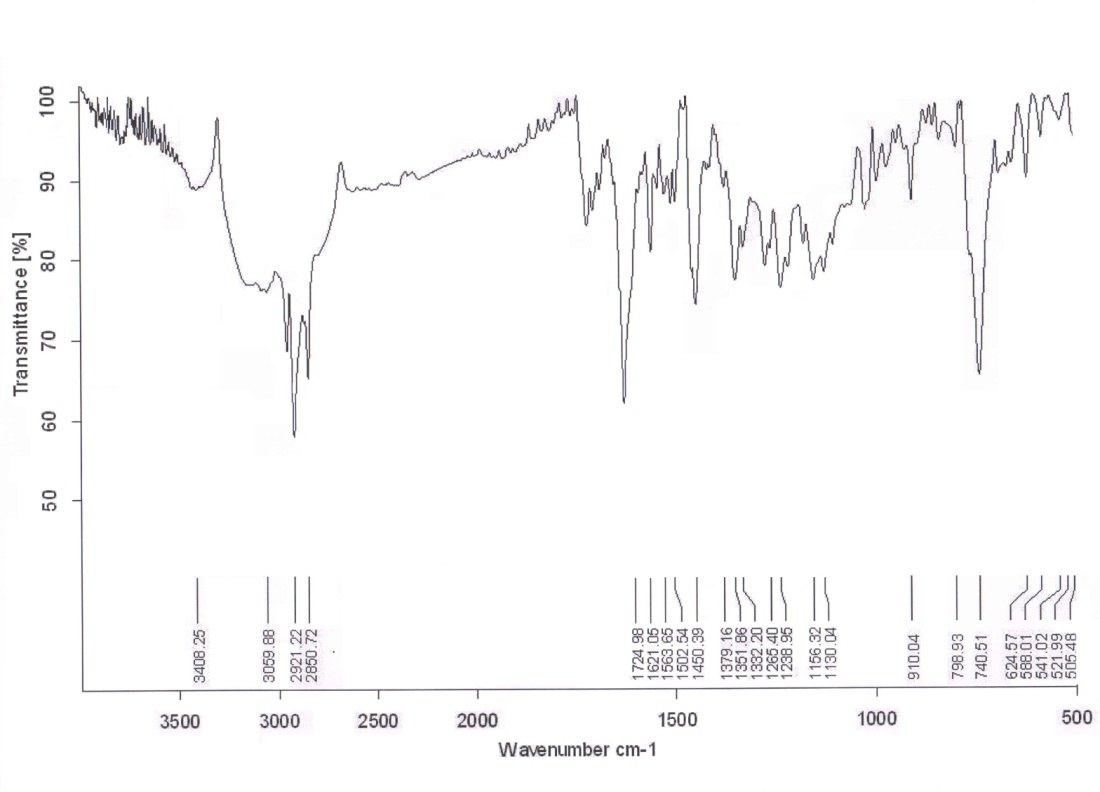
**Figure S2:** 1H NMR for ethyl (11*H*-indolo[3,2-c]isoquinolin-5-ylthio)formate (2c)



**Figure S3**: 13C NMR for ethyl (11*H*-indolo[3,2-c]isoquinolin-5-ylthio)formate (2c)

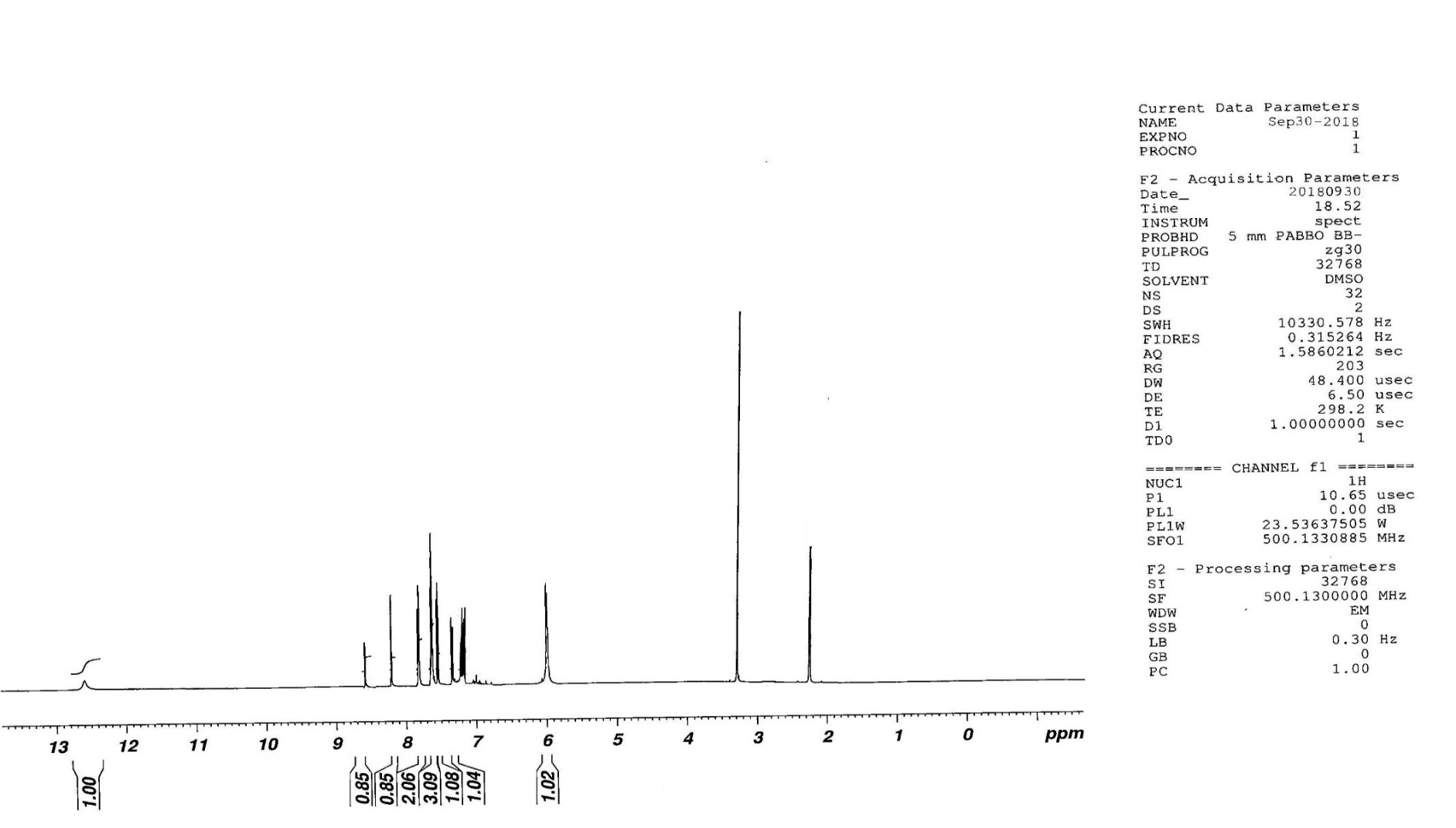


**Figure S4**: Mass spectrum for ethyl (11*H*-indolo[3,2-c]isoquinolin-5-ylthio)formate (2c)

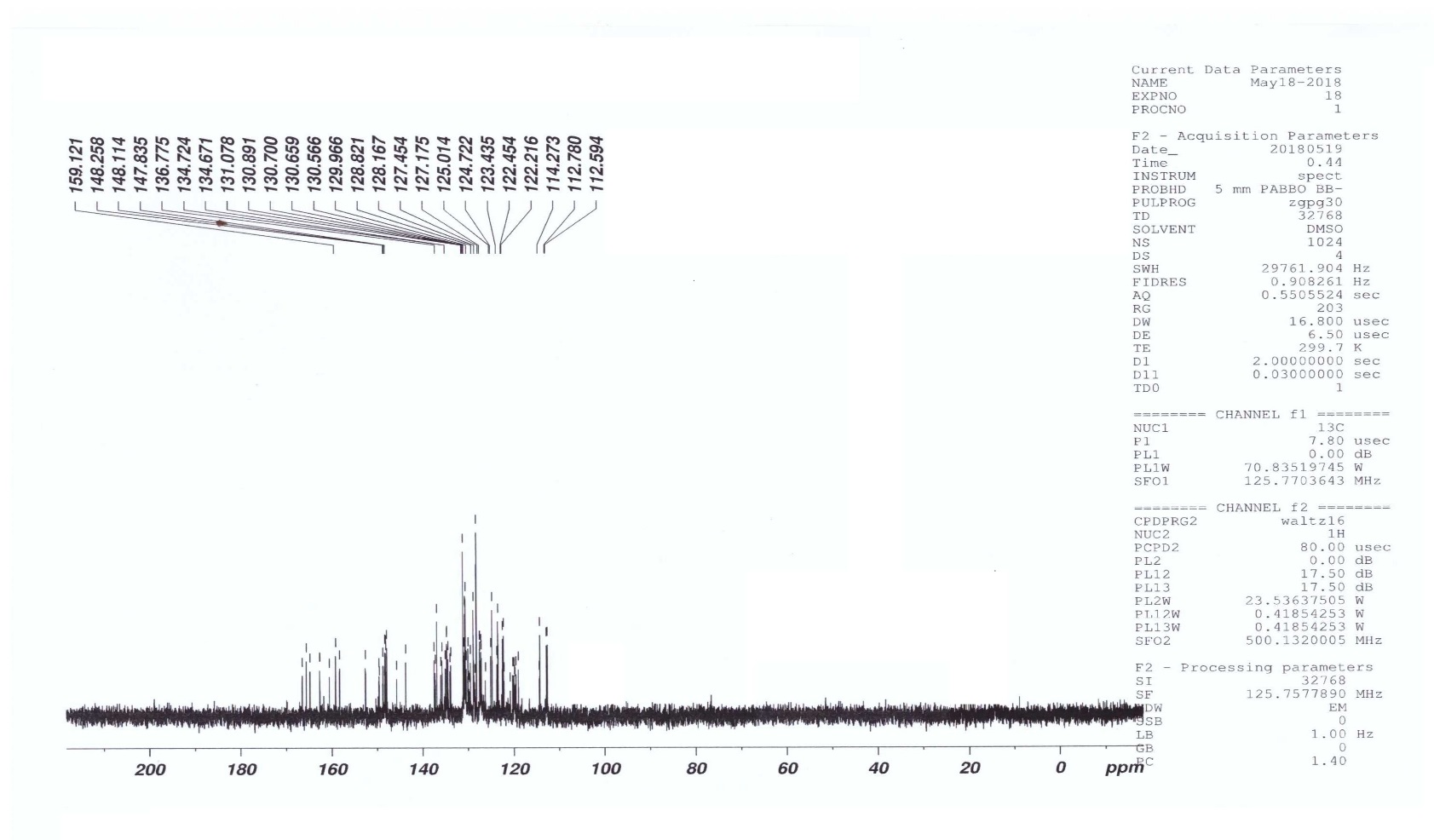




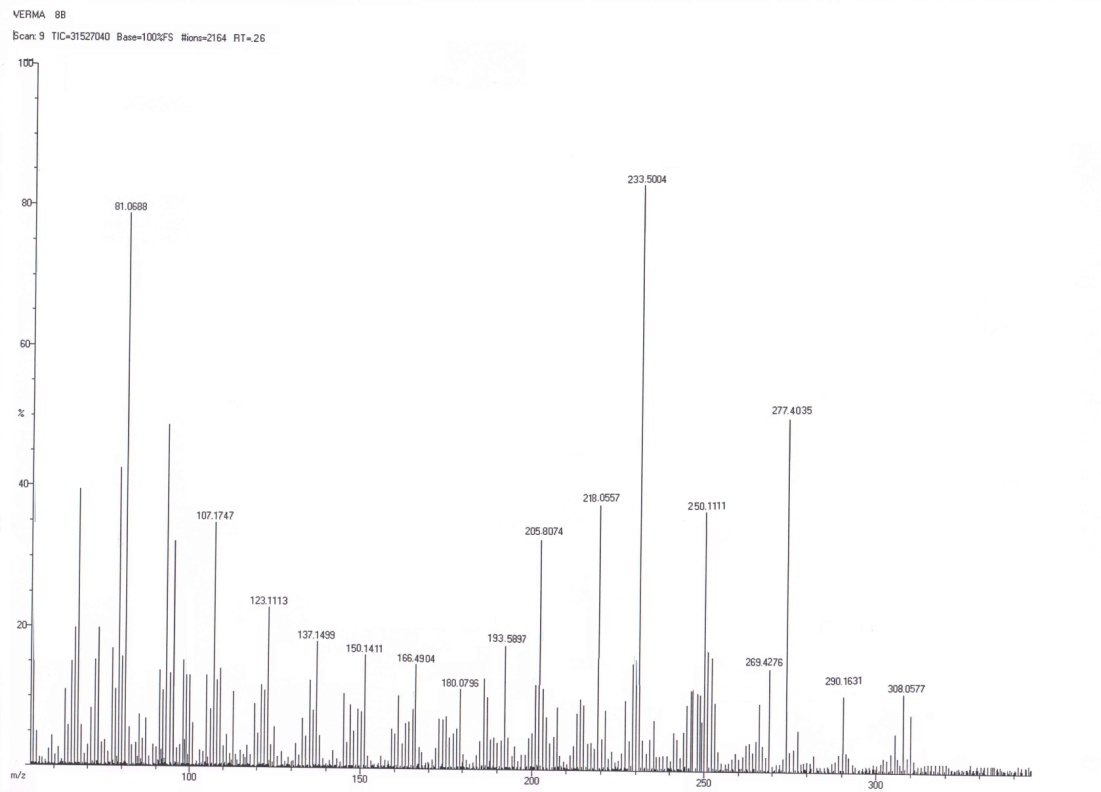
**Figure S5:** IR spectrum for ethyl (11*H*-indolo[3,2-c]isoquinolin-5ylthio)carbohydrazide (3c)



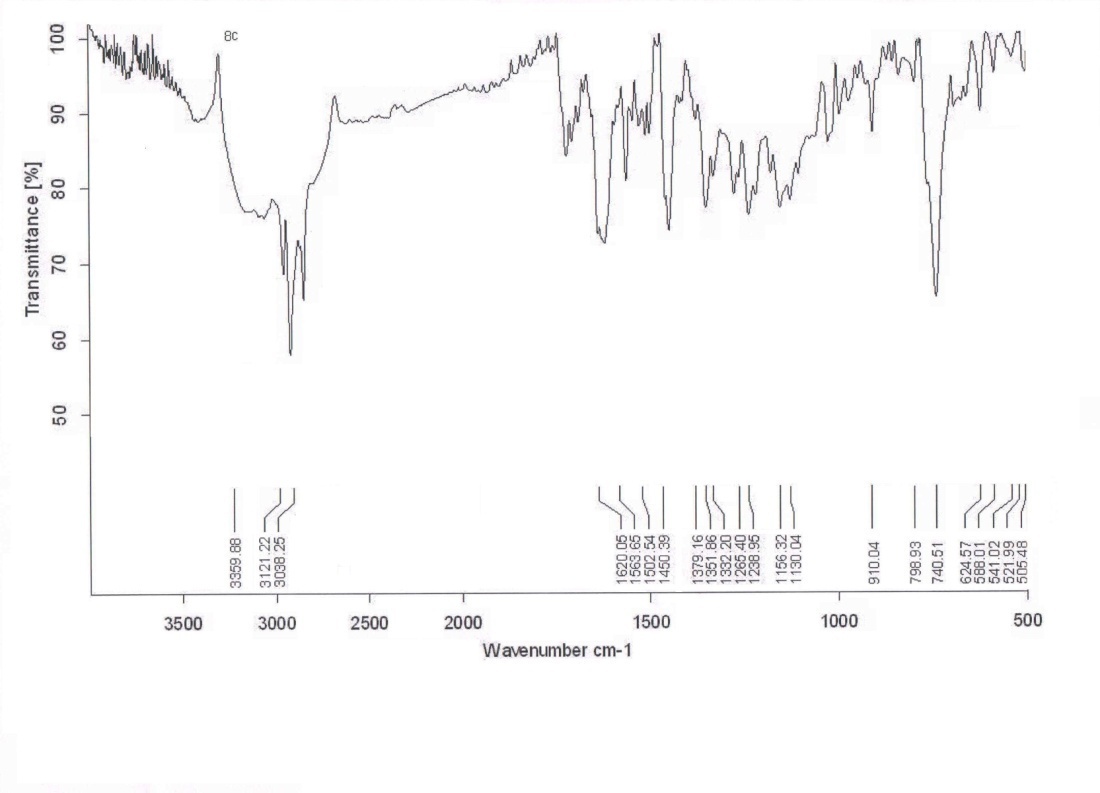
**Figure S6:** 1H NMR for ethyl (11*H*-indolo[3,2-c]isoquinolin-5ylthio)carbohydrazide (3c)



**Figure S7**: 13C NMR for ethyl (11*H*-indolo[3,2-c]isoquinolin-5ylthio)carbohydrazide (3c)

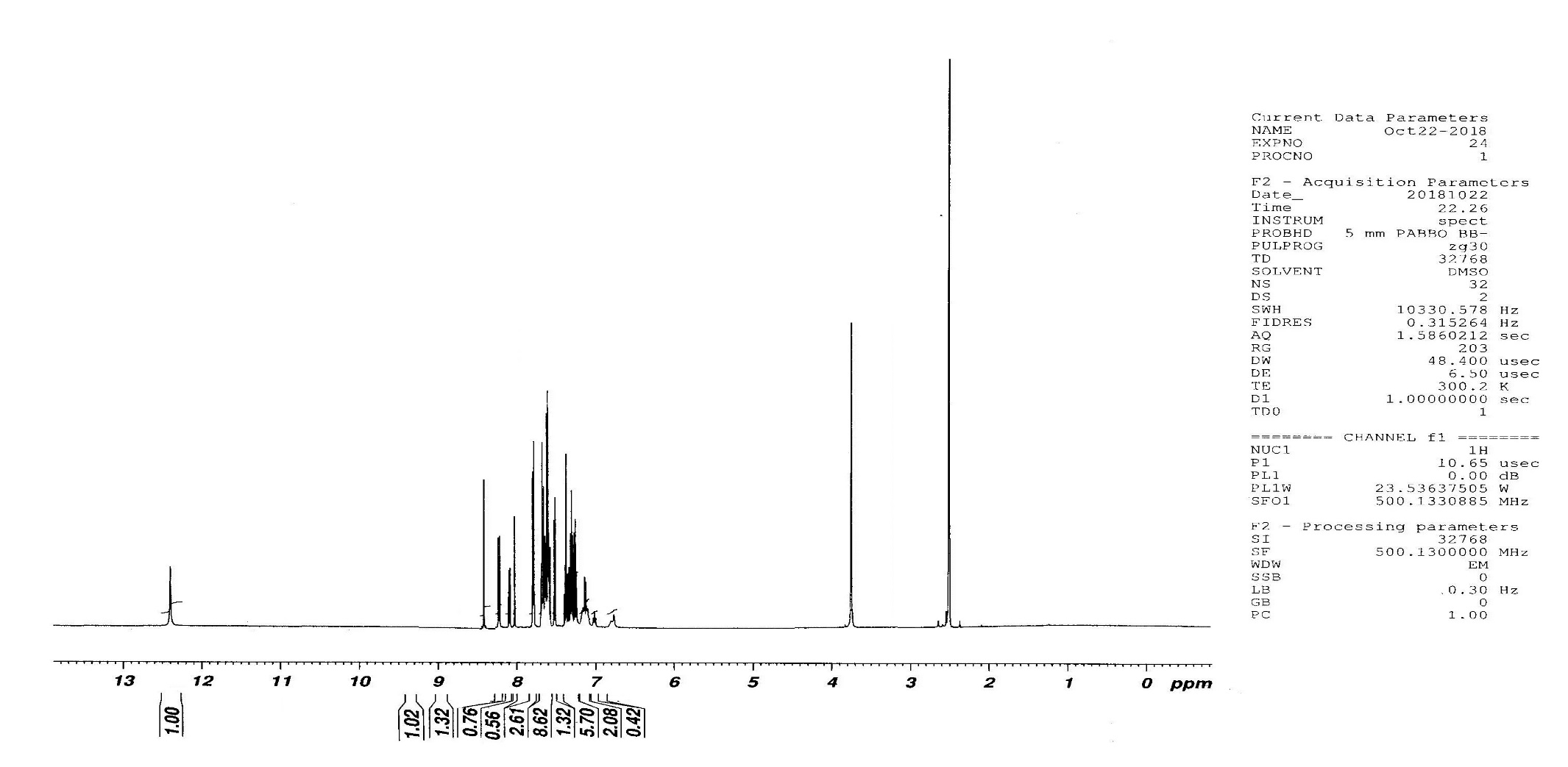


**Figure S8**: Mass spectrum for ethyl (11*H*-indolo[3,2-c]isoquinolin-5ylthio)carbohydrazide (3c)

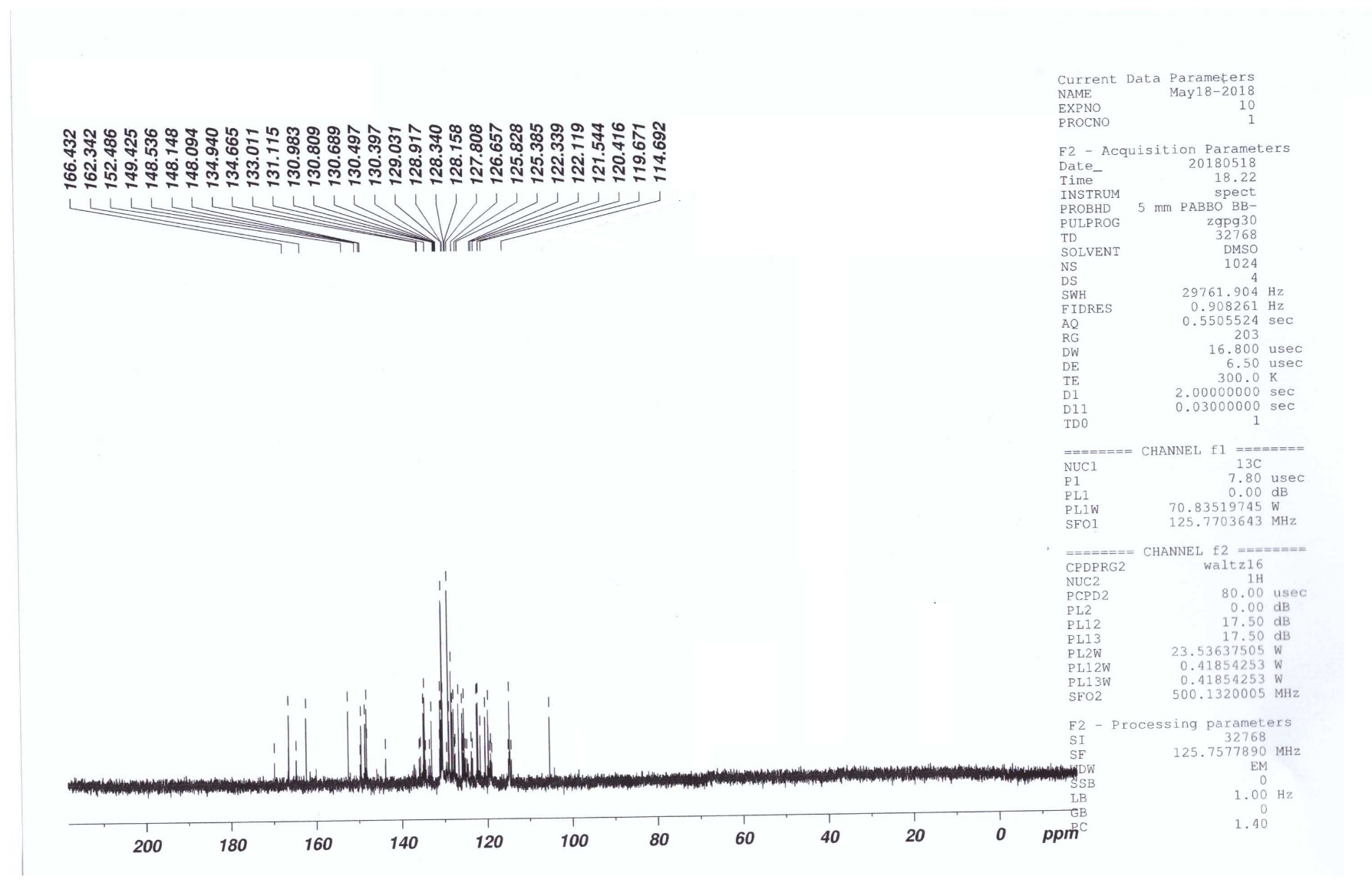




**Figure S9**: IR spectrum for 5-(8-Substituted 11*H*-indolo[3,2-c]isoquinolin-5-ylthio)-1,3,4-oxadiazol-2-amine (4c) .

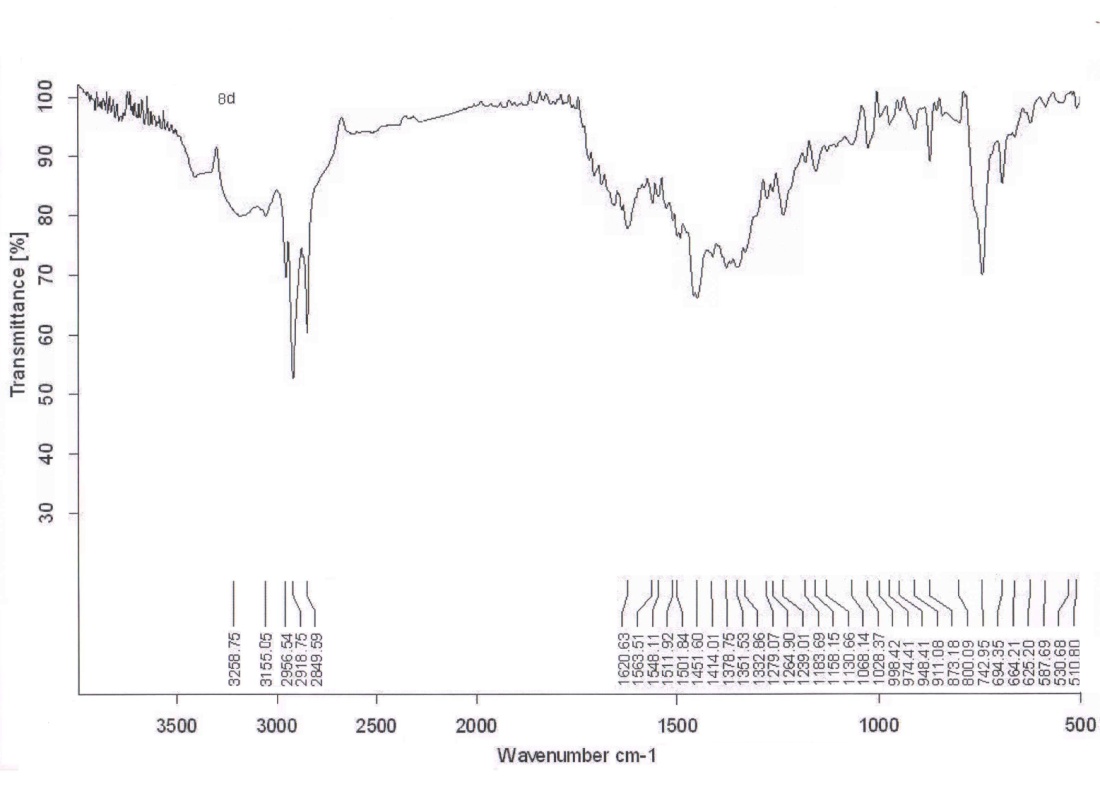


**Figure S10**: 1H NMR spectrum for 5-(8-Substituted 11*H*-indolo[3,2-c]isoquinolin-5-ylthio)-1,3,4-oxadiazol-2-amine (4c)



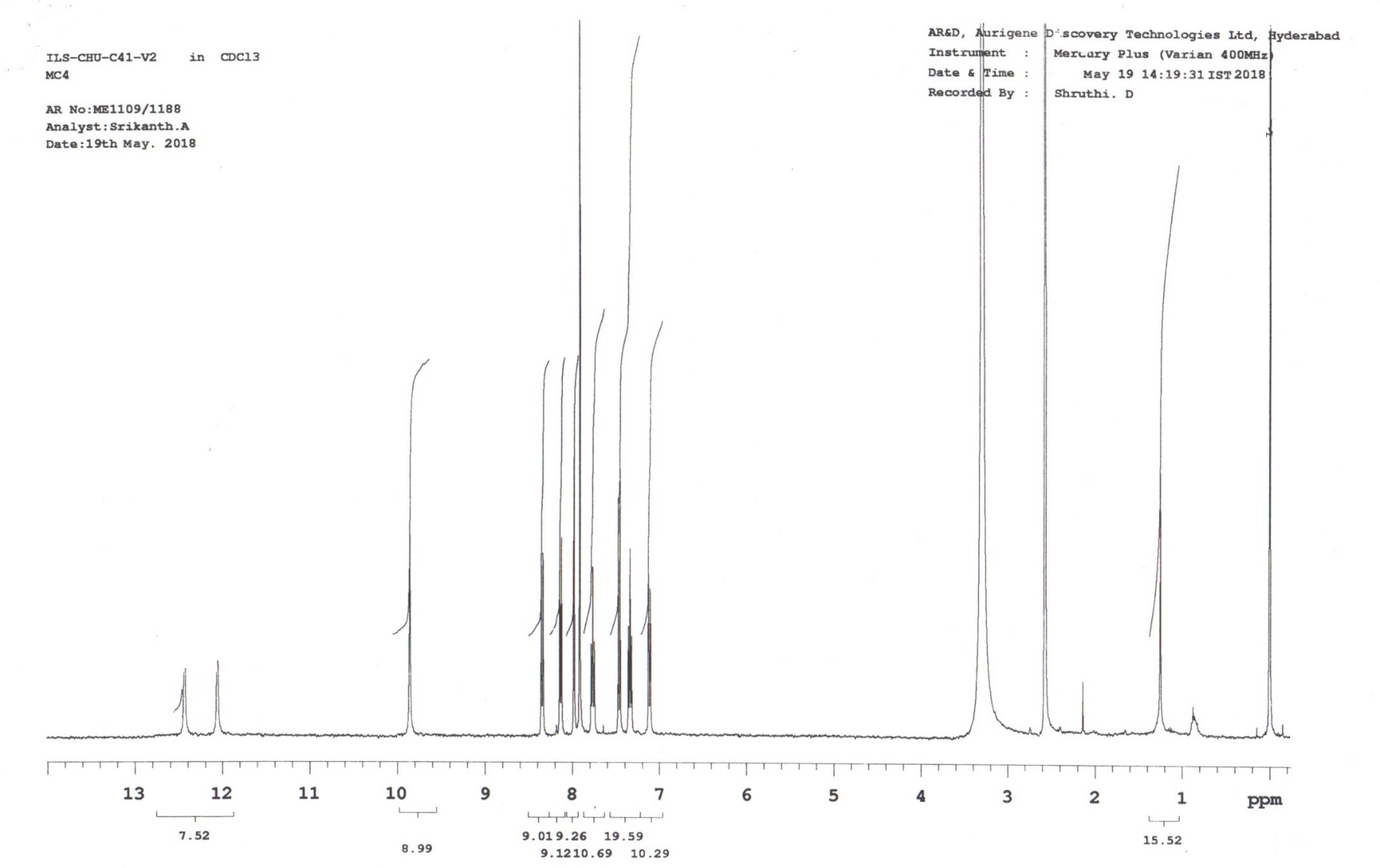
**Figure S11**:13C NMR spectrum for 5-(8-Substituted 11*H*-indolo[3,2-c]isoquinolin-5-ylthio)-1,3,4-oxadiazol-2-amine (**4c**)

**Figure S12:** Mass spectrum 5-(8-Substituted 11*H*-indolo[3,2-c]isoquinolin-5-ylthio)-1,3,4-oxadiazol-2-amine (**4c**)





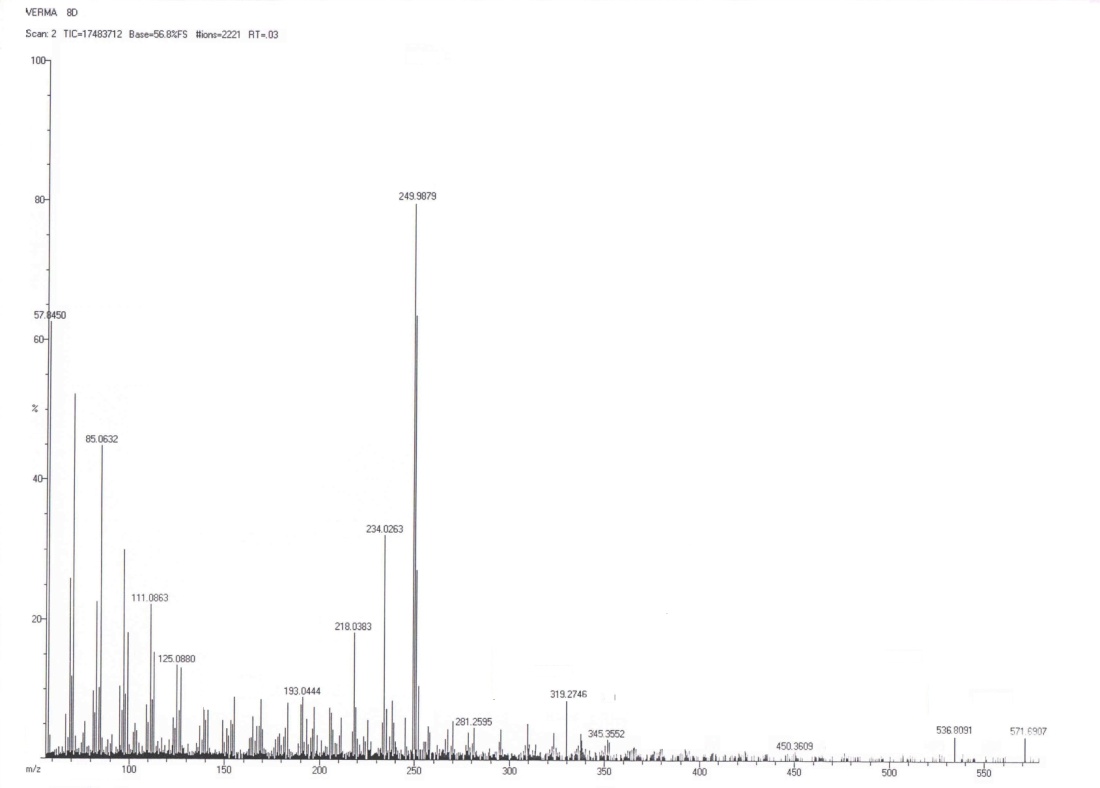
**Figure S13:** IR spectrum for 5-(11*H*-indolo[3,2-c]isoquinolin-5-ylthio)-N-{(5-chloro-2-phenyl-1*H*-indol-3-yl)methylene}-1,3,4-oxadiazol-2-amine (5c).

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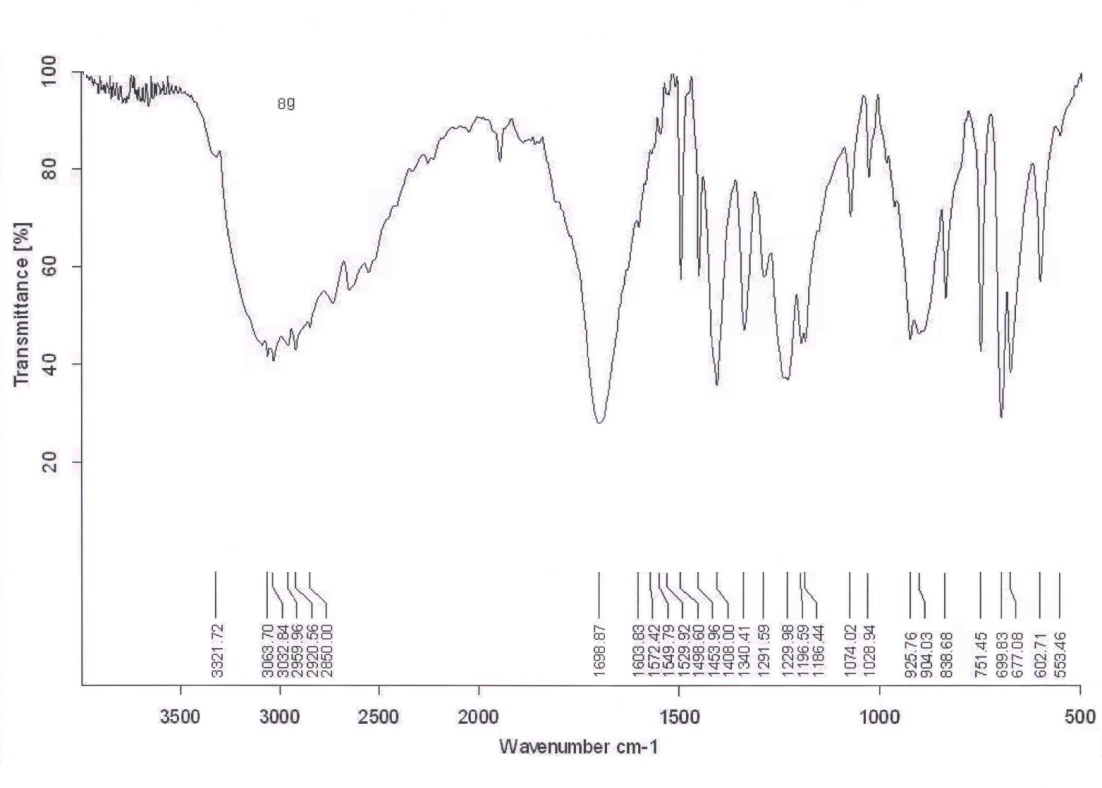
**Figure S14:** 1H NMR spectrum for 5-(11*H*-indolo[3,2-c]isoquinolin-5-ylthio)-N-{(5-chloro-2-phenyl-1*H*-indol-3-yl)methylene}-1,3,4-oxadiazol-2-amine (5c).

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**Figure S15:** 13C NMR spectrum for 5-(11*H*-indolo[3,2-c]isoquinolin-5-ylthio)-N-{(5-chloro-2-phenyl-1*H*-indol-3-yl)methylene}-1,3,4-oxadiazol-2-amine (5c).

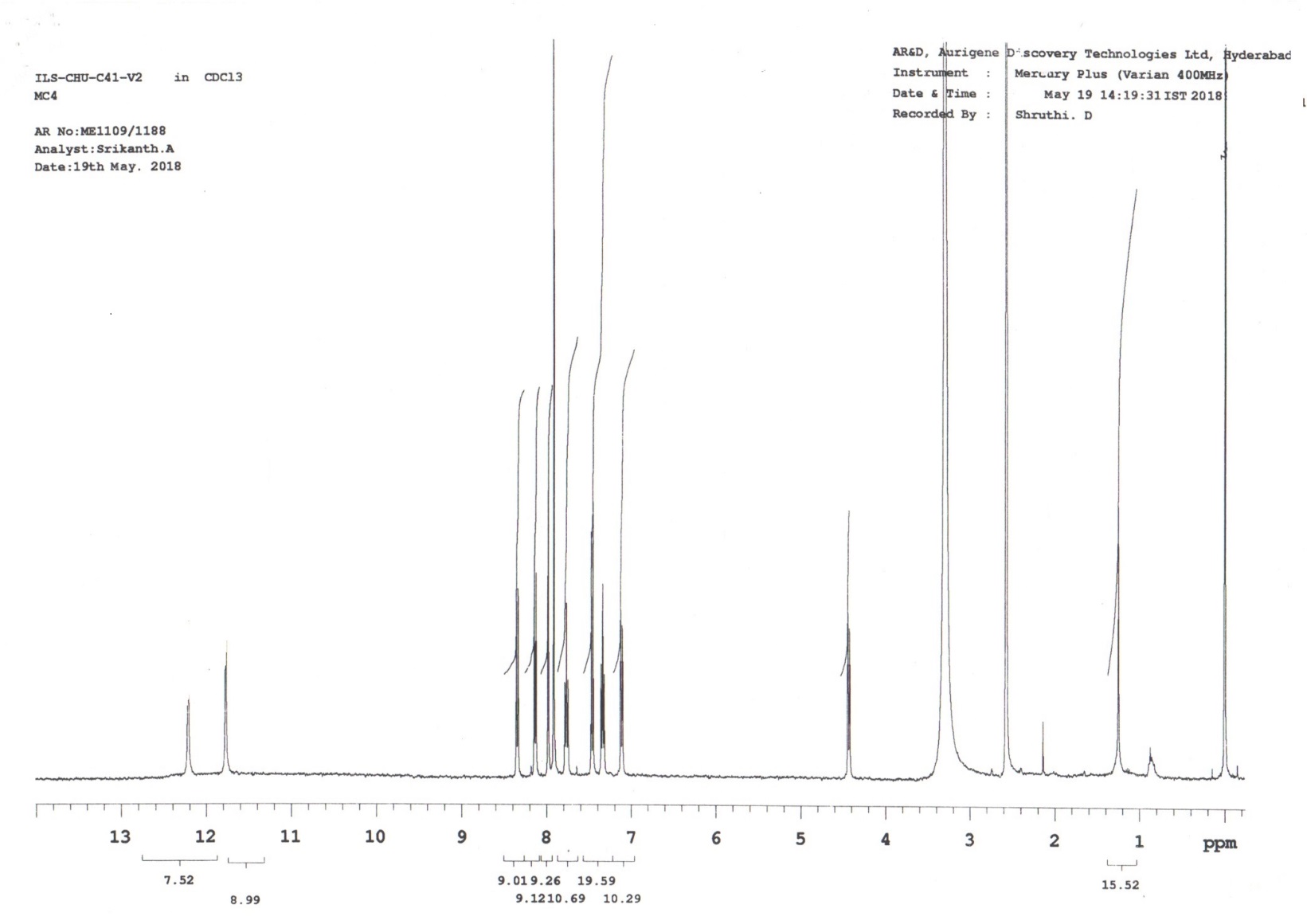
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**Figure S16:** Massspectrum for for 5-(11*H*-indolo[3,2-c]isoquinolin-5-ylthio)-N-{(5-chloro-2-phenyl-1*H*-indol-3-yl)methylene}-1,3,4-oxadiazol-2-amine (5c).

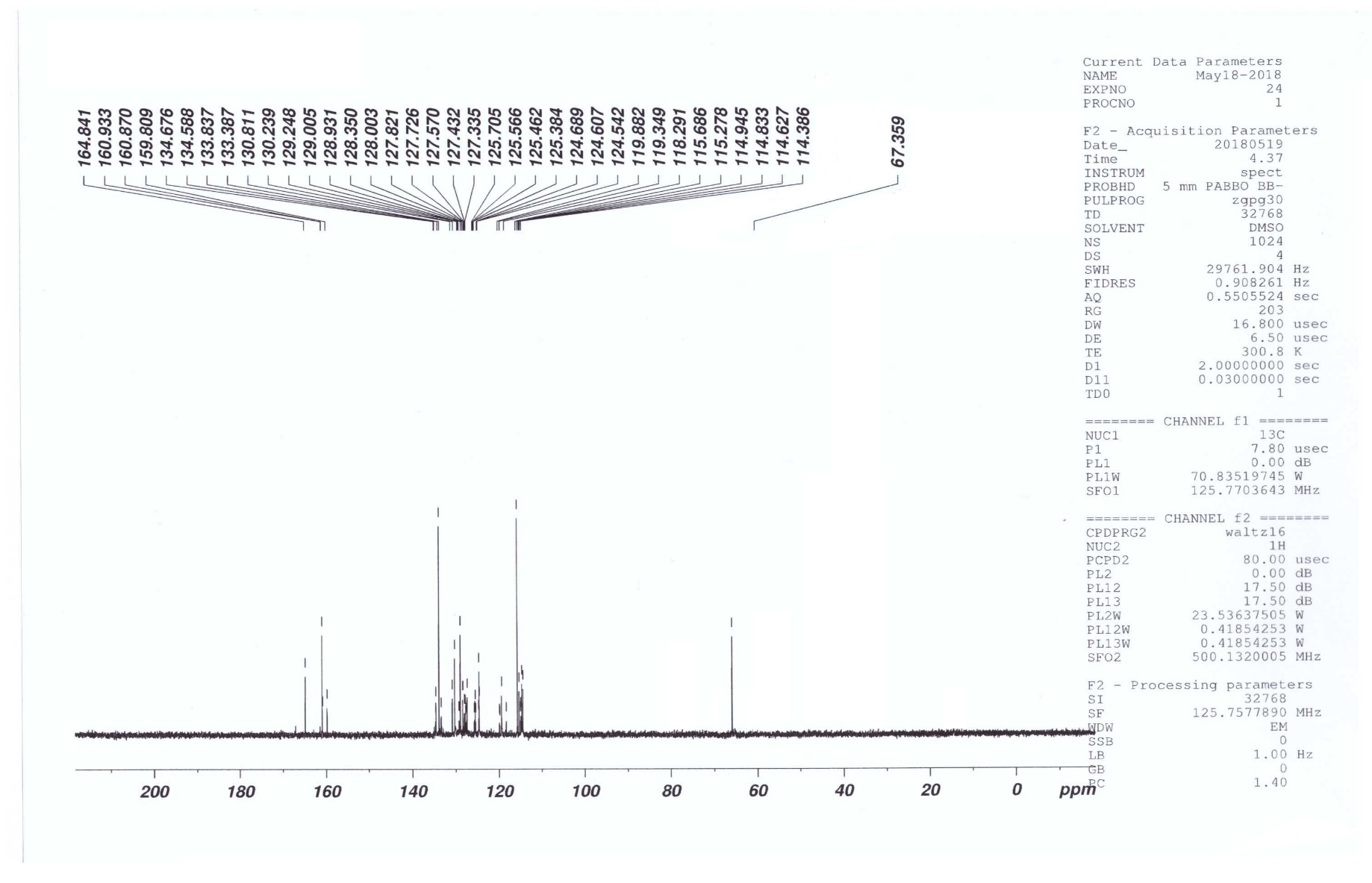
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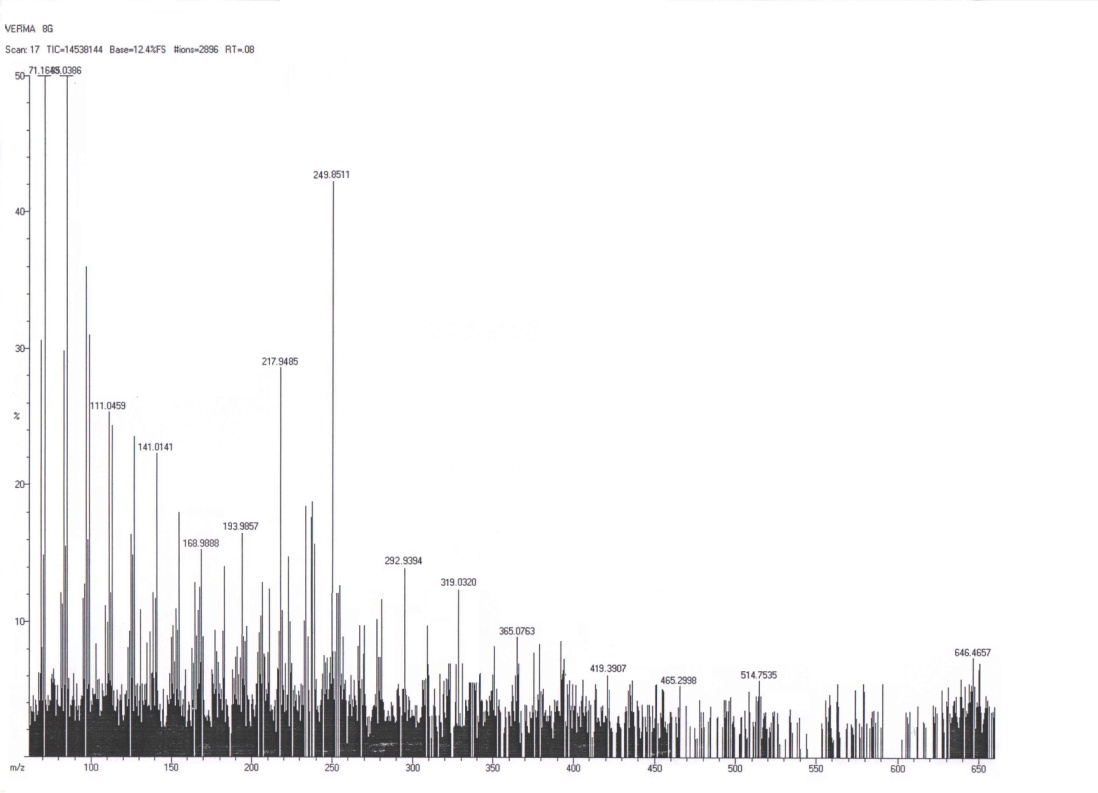
**Figure S17:** IRspectrum for 3-{5-(11*H*-indolo[3,2-c]isoquinolin-5-ylthio)-1,3,4-oxadiazol-2-yl}-2-(5-chloro2-phenyl-1*H*-indol-3-yl)thiazolidin-4-one (6c)



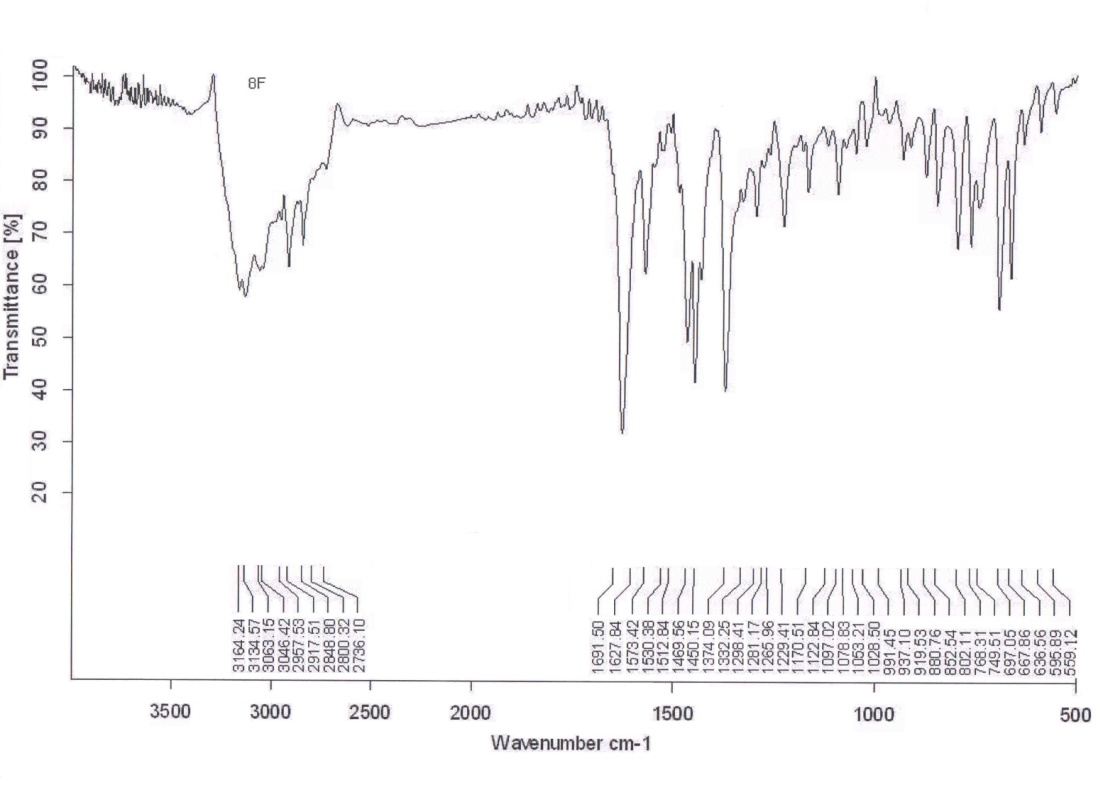
**Figure S18:** 1H NMR spectrum for 3-{5-(11*H*-indolo[3,2-c]isoquinolin-5-ylthio)-1,3,4-oxadiazol-2-yl}-2-(5-chloro2-phenyl-1*H*-indol-3-yl)thiazolidin-4-one (6c)

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**Figure S19:** 13C NMR spectrum for 3-{5-(11*H*-indolo[3,2-c]isoquinolin-5-ylthio)-1,3,4-oxadiazol-2-yl}-2-(5-chloro2-phenyl-1*H*-indol-3-yl)thiazolidin-4-one (6c)

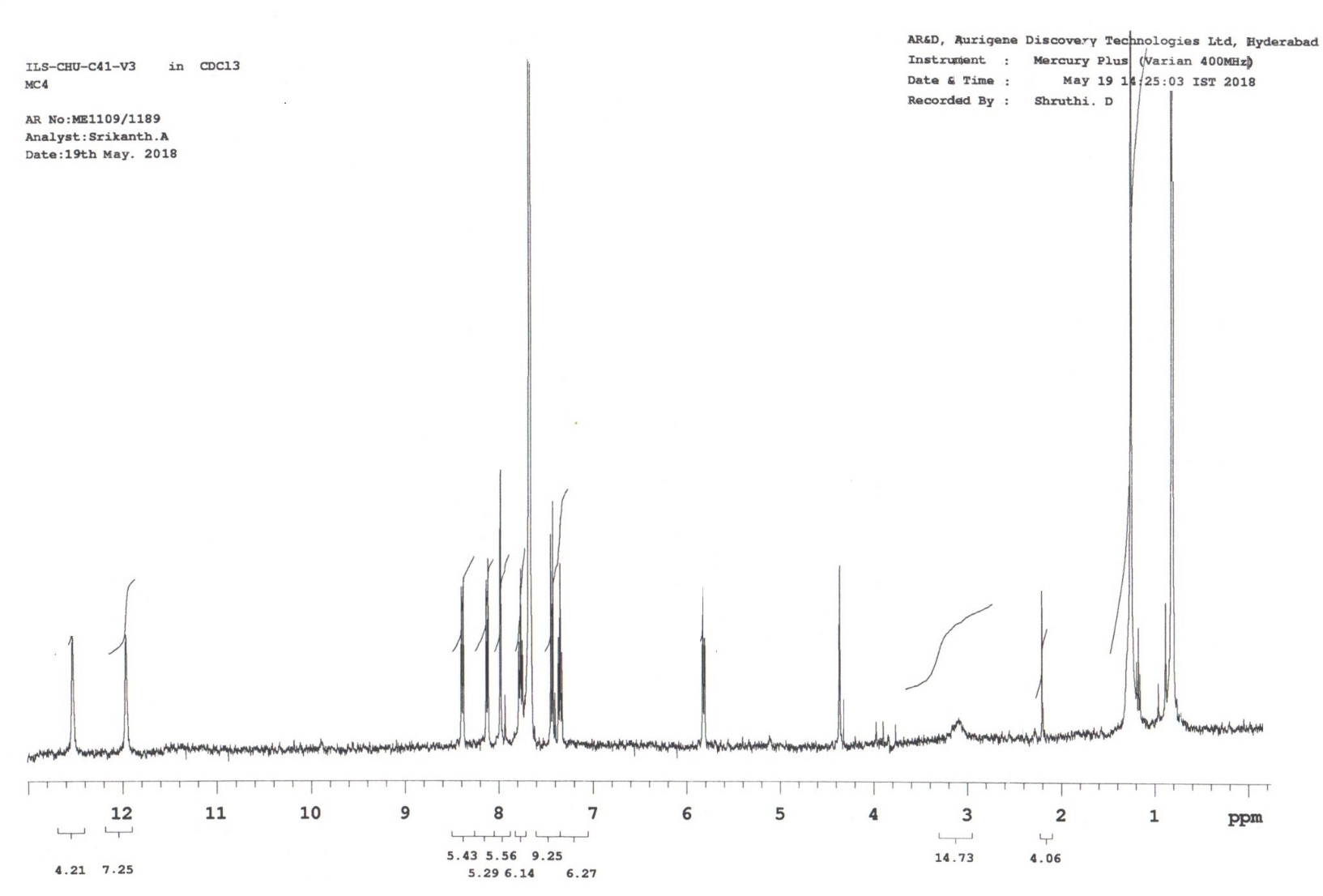
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**Figure S20:** Mass spectrum for 3-{5-(11*H*-indolo[3,2-c]isoquinolin-5-ylthio)-1,3,4-oxadiazol-2-yl}-2-(5-chloro2-phenyl-1*H*-indol-3-yl)thiazolidin-4-one (6c)

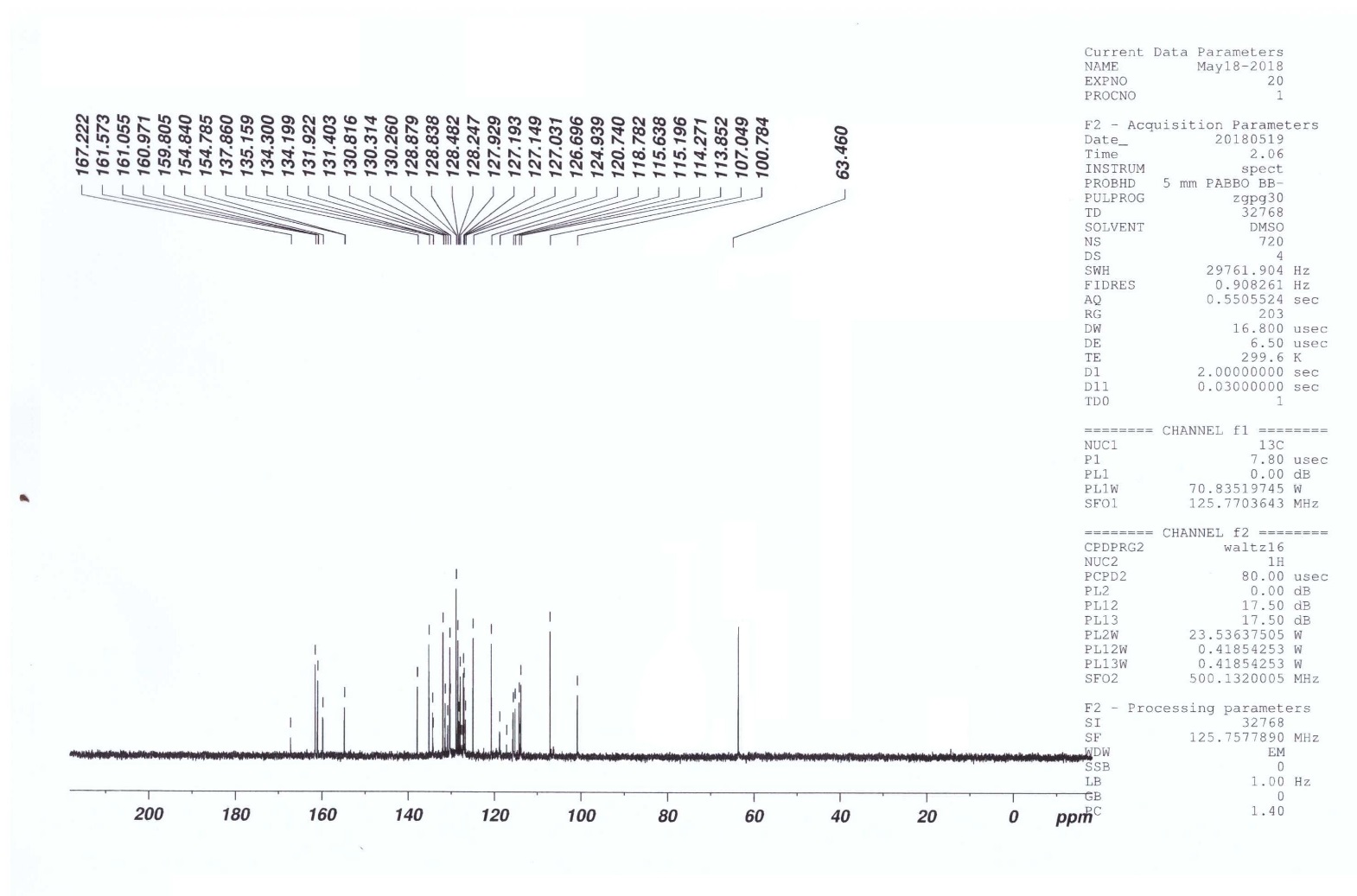
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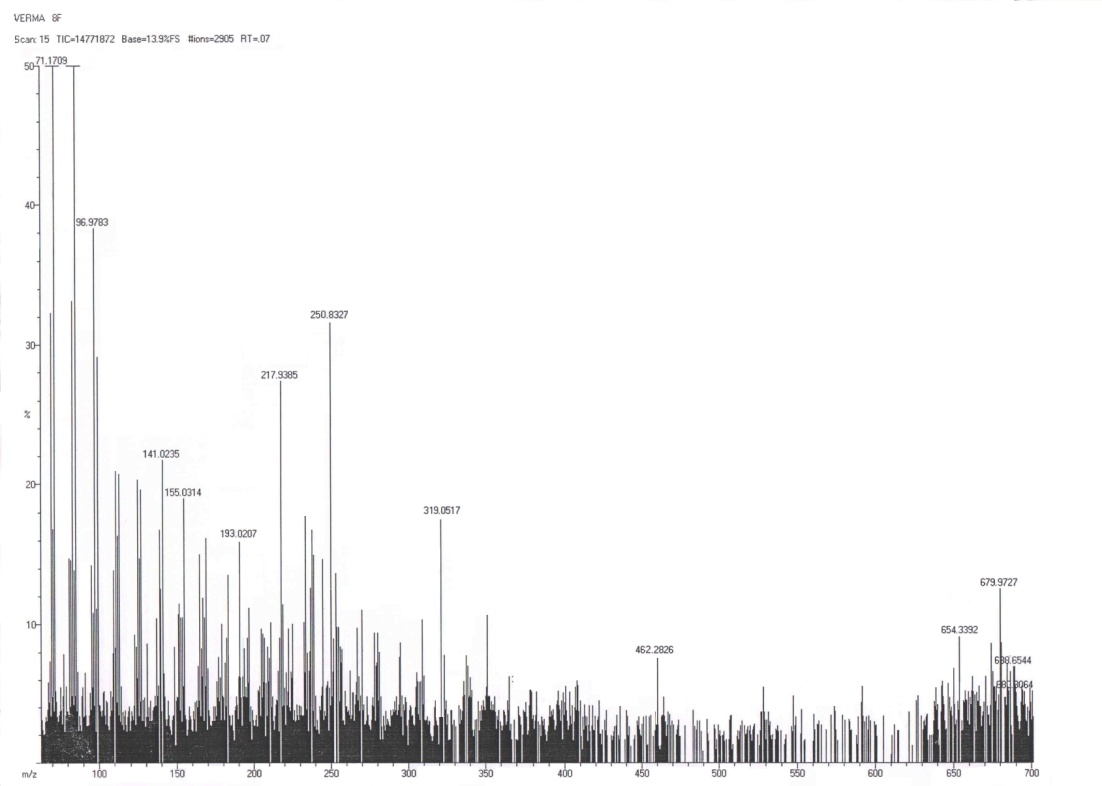
**Figure S21:** IRspectrum for 1-{5-(11*H*-indolo[3,2-c]isoquinolin-5-ylthio)-1,3,4-oxadiazol-2-yl}-4-(5-chloro-2-phenyl-1*H*-indol-3-yl)-3-phenylazetidin-2-one (8c)

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**Figure S22:** 1H NMR spectrum for 1-{5-(11*H*-indolo[3,2-c]isoquinolin-5-ylthio)-1,3,4-oxadiazol-2-yl}-4-(5-chloro-2-phenyl-1*H*-indol-3-yl)-3-phenylazetidin-2-one (8c)



**Figure S23:** 13C NMR spectrum for 1-{5-(11*H*-indolo[3,2-c]isoquinolin-5-ylthio)-1,3,4-oxadiazol-2-yl}-4-(5-chloro-2-phenyl-1*H*-indol-3-yl)-3-phenylazetidin-2-one (8c)



**Figure S24:** Mass spectrum 1-{5-(11*H*-indolo[3,2-c]isoquinolin-5-ylthio)-1,3,4-oxadiazol-2-yl}-4-(5-chloro-2-phenyl-1*H*-indol-3-yl)-3-phenylazetidin-2-one (8c)