

## Supporting Information

### Development of Novel Isatin Thiazolyl-pyrazoline Hybrids as Promising Antimicrobials in MDR pathogens

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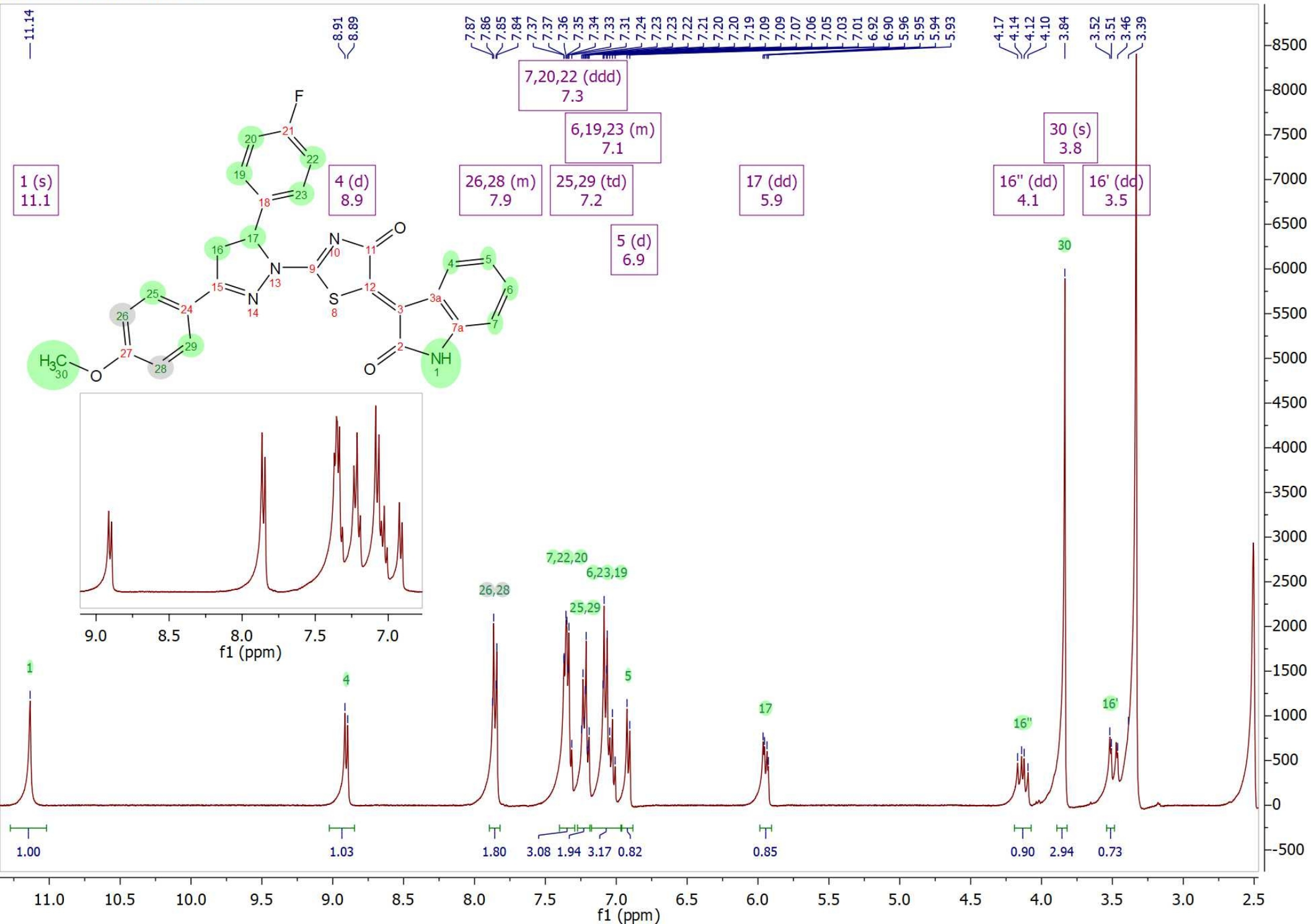
## 1. Antimicrobial XTT assay

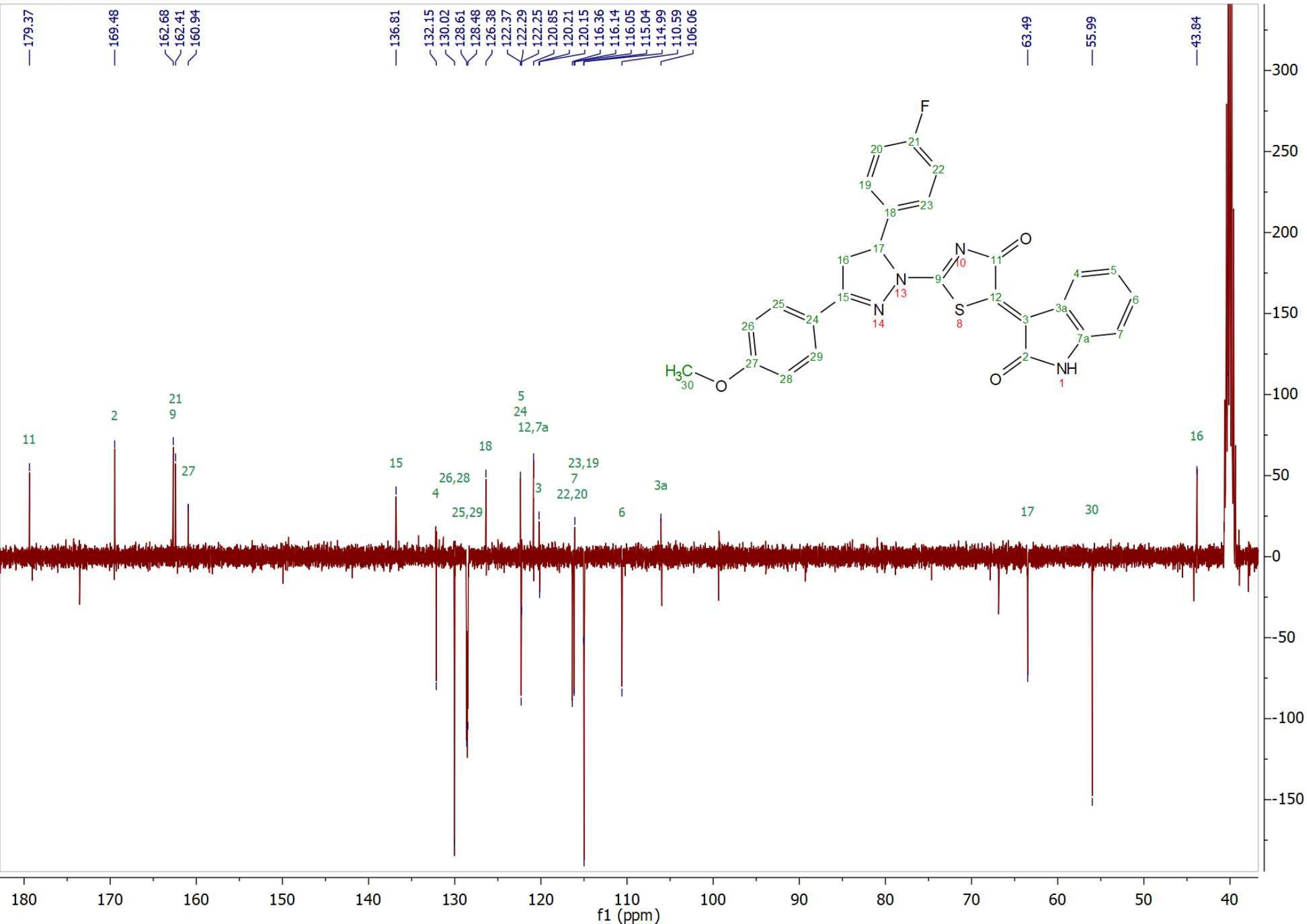
The antibacterial activity of the synthesized isatin derivatives was screened *in vitro*, using the broth microdilution calorimetric XTT reduction method, against 4 MDR strains viz; Methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC 700788) and Vancomycin-resistant *Enterococcus faecalis* (VRE, BAA-2365) strains as examples of Gram-positive bacteria; Carbapenem-resistant *Klebsiella pneumonia* (CRKP, ATCC BAA-2342) and Extended-spectrum Beta-lactamase *Escherichia coli* (ESBL-E, BAA-199) strains to represent Gram-negative bacteria. Additionally, the antifungal activity of the synthesized isatin derivatives was screened against an MDR strain viz; Fluconazole-resistant *Candida albicans* (FRCA, ATCC-MYA-1003A) strain as a yeast-like unicellular fungus.

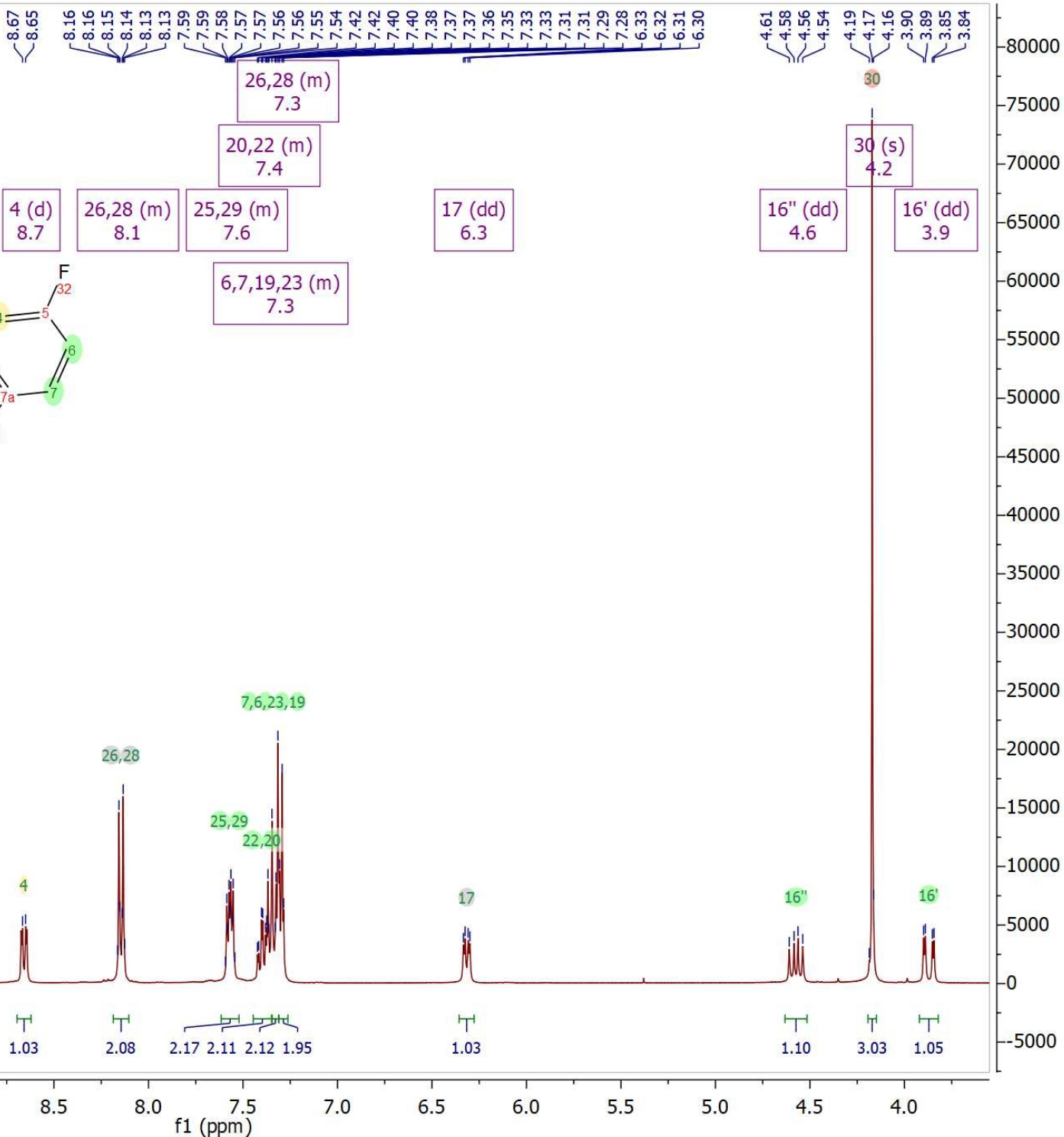
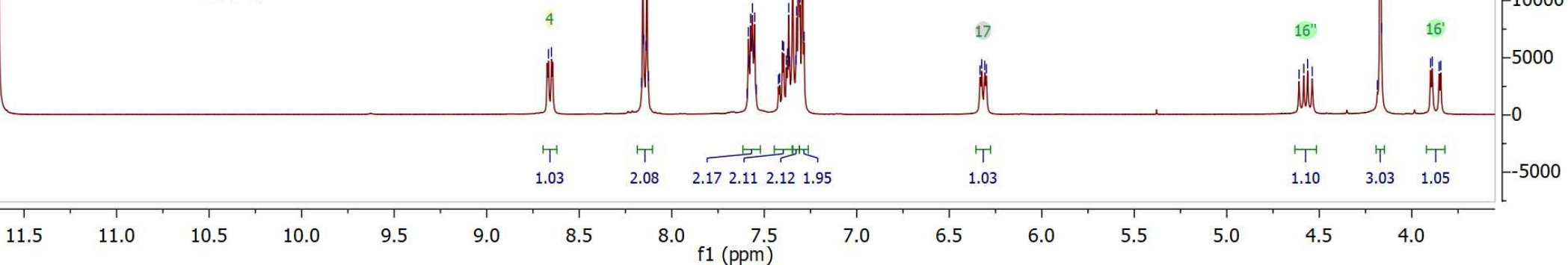
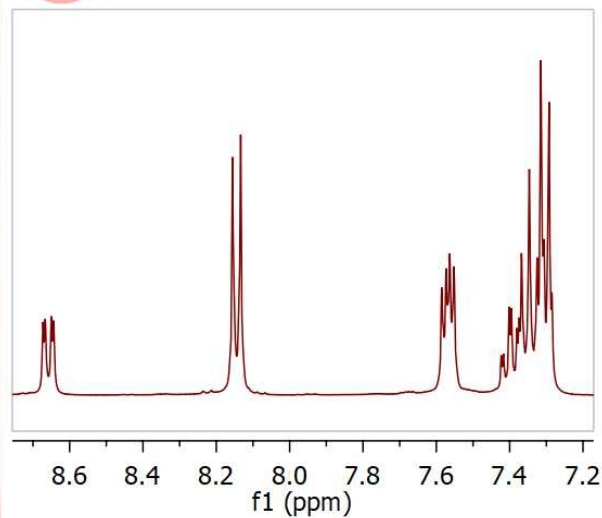
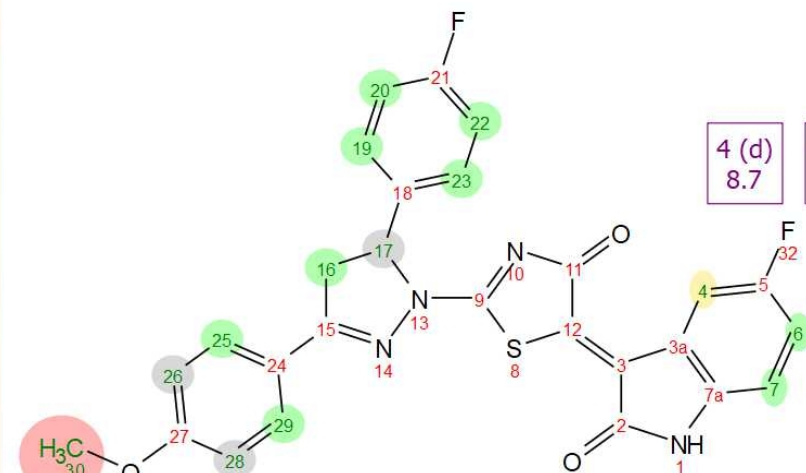
The calorimetric broth microdilution method using the XTT [2,3-bis(2-methoxy-4-nitro-5-sulfo-phenyl)-2H-tetrazolium-5-carboxanilide] reduction assay with minor modifications was used to determine the inhibitory percentages, and minimum inhibitory concentration (MIC) of the tested compounds against drug-resistant strains. All microbial strains were cultured overnight at 37 °C in Brain Heart Infusion (BHI) (Oxoid, UK). XTT (Sigma) was prepared in a saturated solution at 0.5 g/L in Ringer's lactate. The solution was sterilized through a 0.22- $\mu$ m-pore-size filter. The compounds were serially diluted in DMSO, and 50  $\mu$ L of each dilution at final concentrations of 1000–0.24  $\mu$ g/mL were added to the wells of a Microtiter plate (96 wells) containing 100  $\mu$ L TSB. Fifty microliters of adjusted microbial inoculum (10<sup>6</sup> CFU/mL) was added to each well, followed by the incubation of the Microtiter plates in the dark at 37 °C for 24 h. Subsequently, 100  $\mu$ L of freshly prepared XTT was added and the plates were incubated again for 1 h at 37 °C. Calorimetric variation in the XTT assay was measured using a Microtiter plate reader (BioTECK, USA) at 492 nm. Inhibition-mediated reduction of microbial viability was calculated by the following formula: % of inhibition =  $[1 - (\text{OD}_t/\text{OD}_c)] \times 100$ , where OD<sub>t</sub> is the mean optical density of the wells treated with the tested sample and OD<sub>c</sub> is the mean optical density of untreated wells. The relationship between the % of inhibition of microbial growth and sample concentration was plotted to obtain the inhibitory curve after treatment with the specified compound. The MIC was specified as the extract concentration that produced a 100% decrease in optical density compared with control growth results.

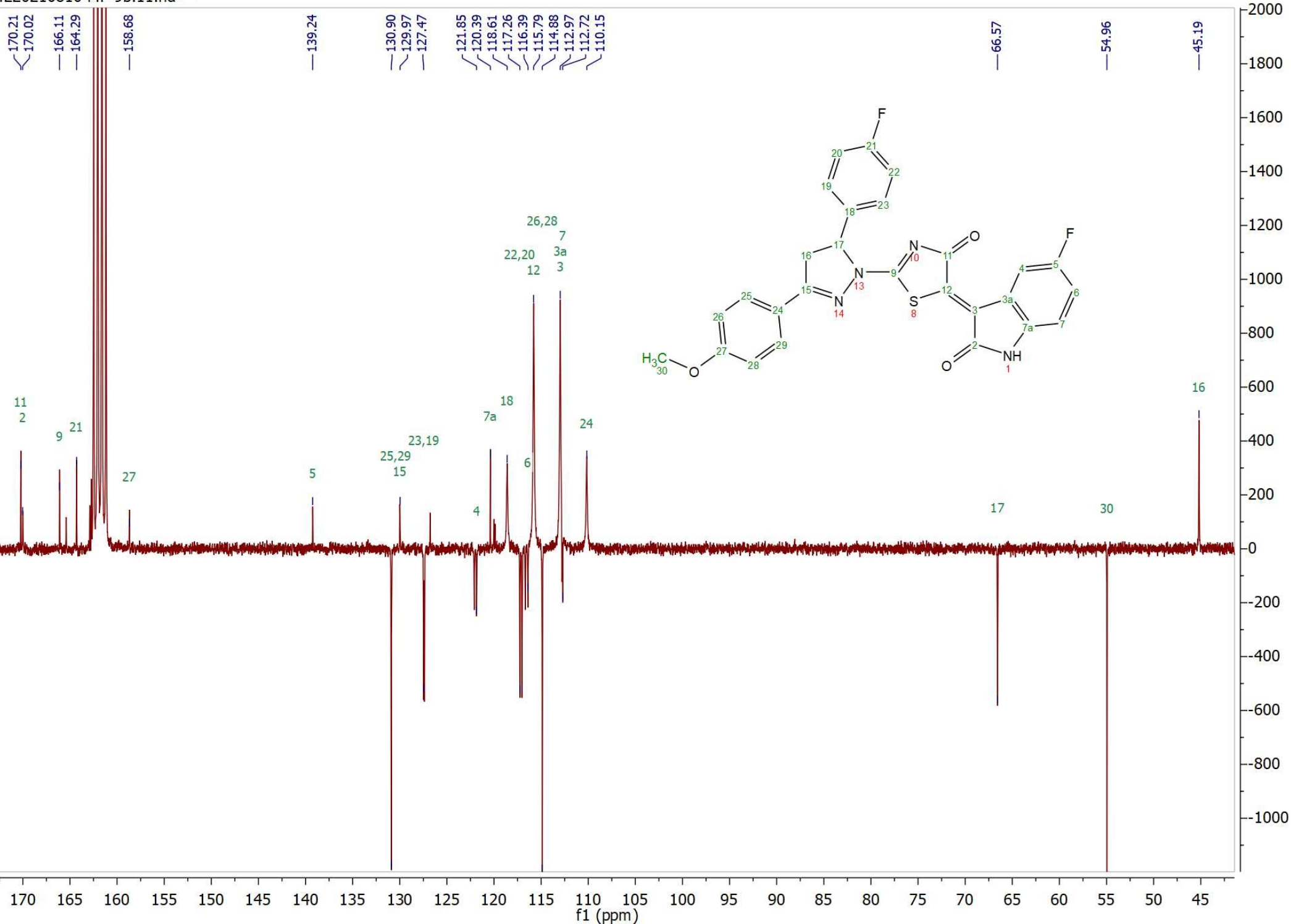
## 2. Cytotoxicity SRB assay

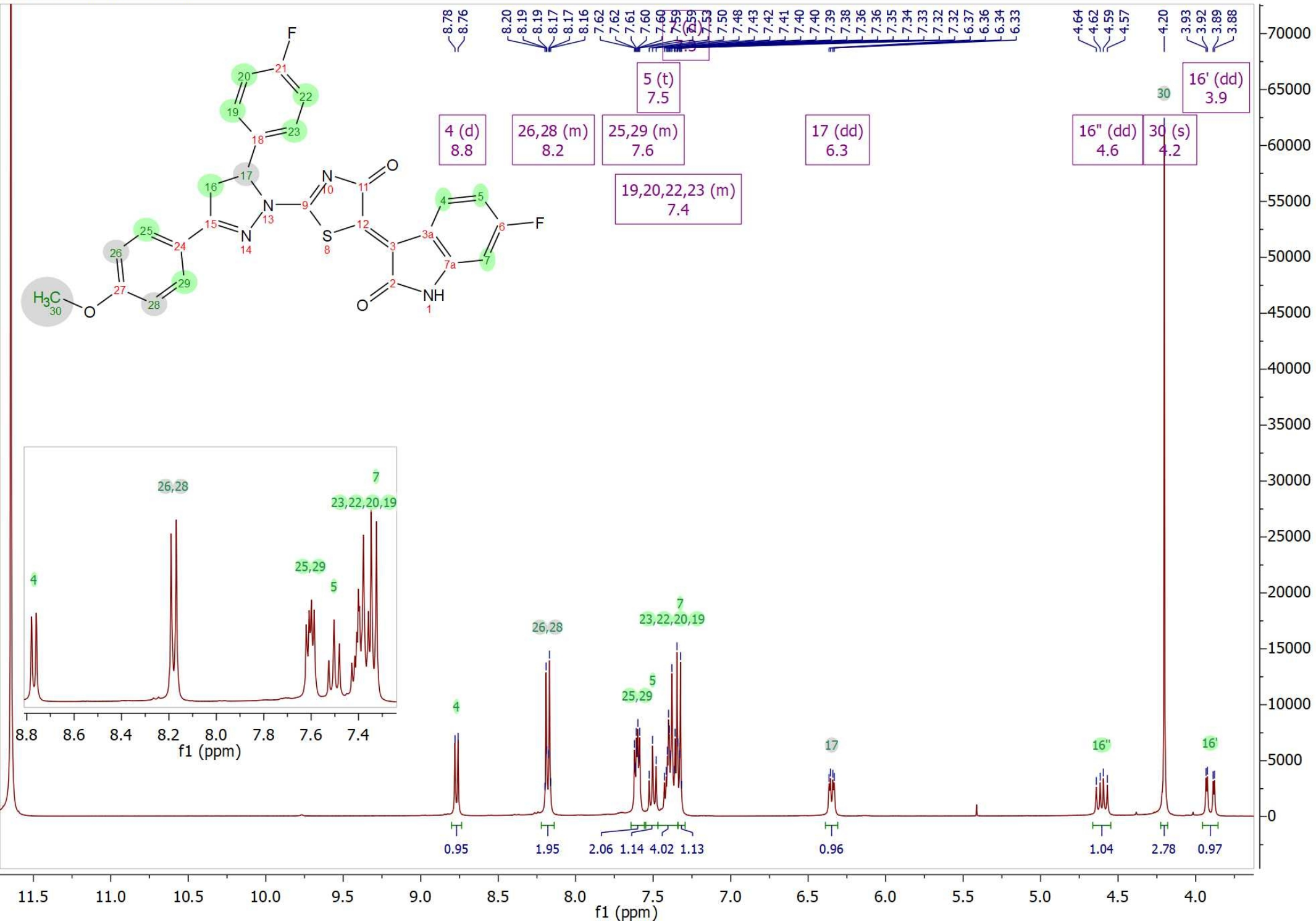
The examined human lung fibroblast MRC-5 cell line has been obtained from American Type Culture Collection (ATCC). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37 °C in humidified atmosphere containing 5% CO<sub>2</sub>. Cells at a concentration of  $0.50 \times 10^6$  were grown in a 25 cm<sup>2</sup> flask in 5 ml of culture medium. The cytotoxic activity of compounds **9f** and **10h** was measured *in vitro* using the Sulfo-Rhodamine-B stain (SRB) assay. Briefly, cells were plated out in 96-well microtiter plate ( $0.5 \times 10^4$  cells/well) and incubated for 24 h before treatment with the tested hybrids to allow cells to attach to the bottom of the well 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 were added to the cells (three wells were prepared for each individual dose). Cells were incubated with the hybrids for 48 h at 37°C and in atmosphere of 5% CO<sub>2</sub>. 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 log drug concentration is plotted to get the survival curve for each cell line.



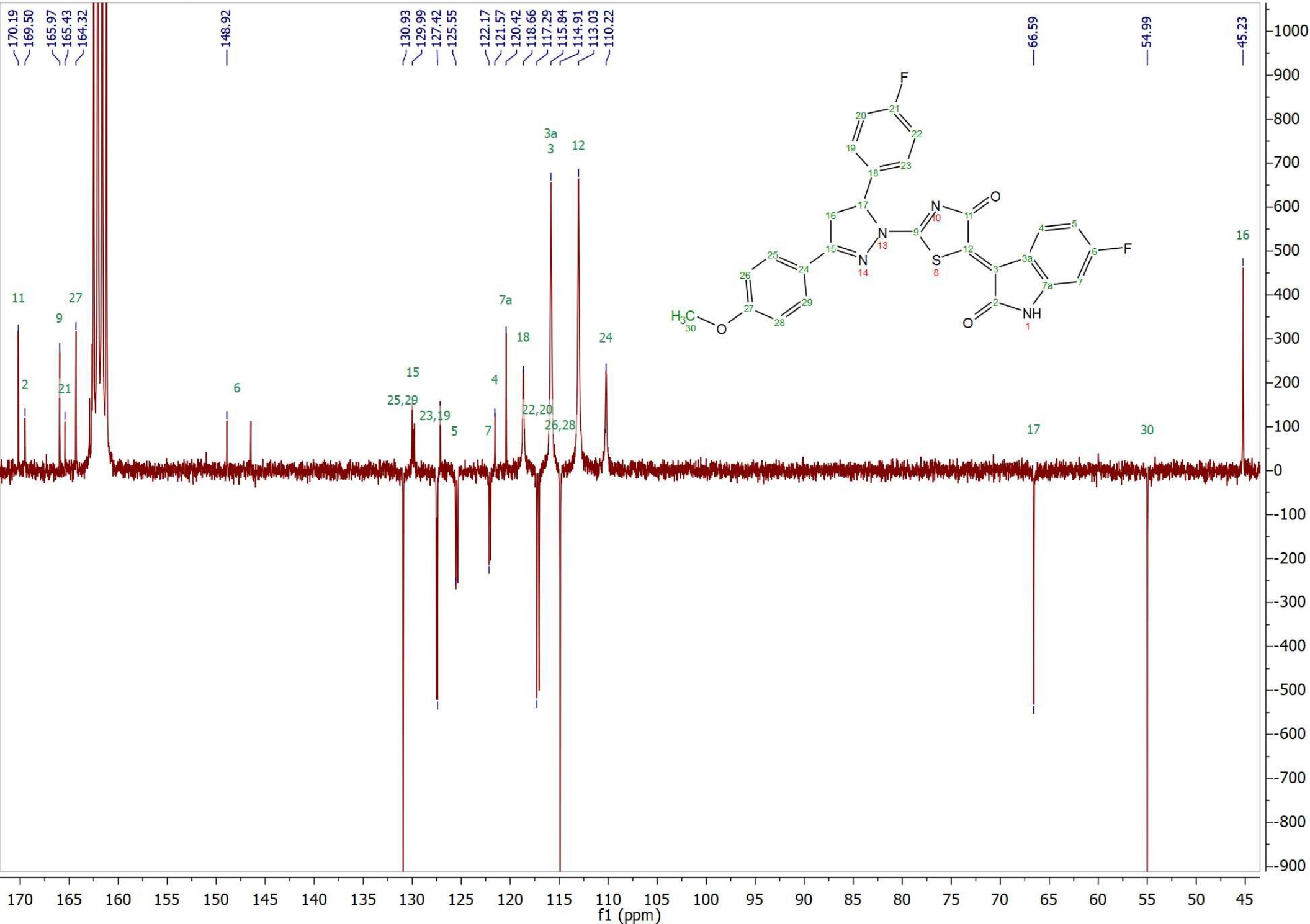


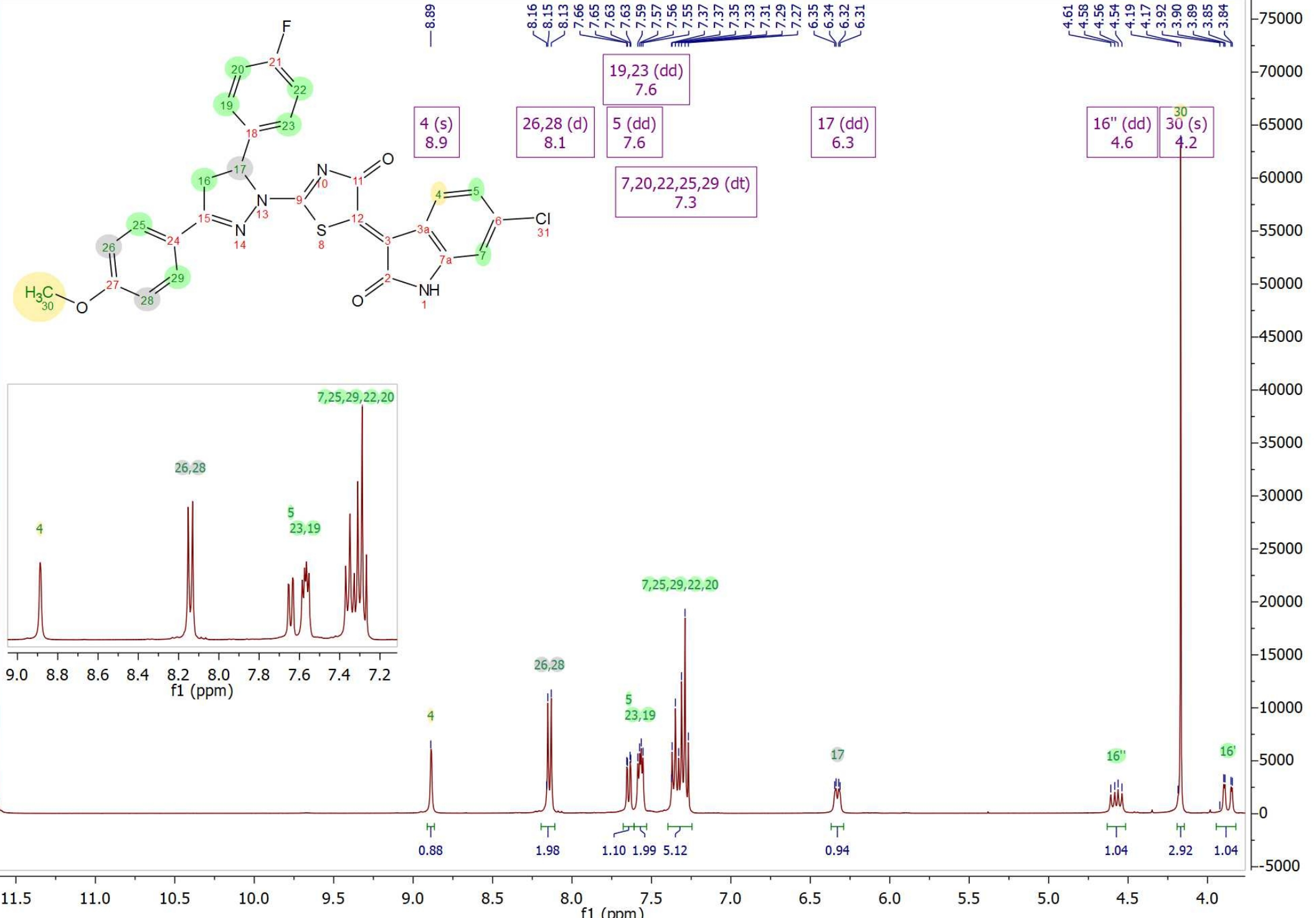


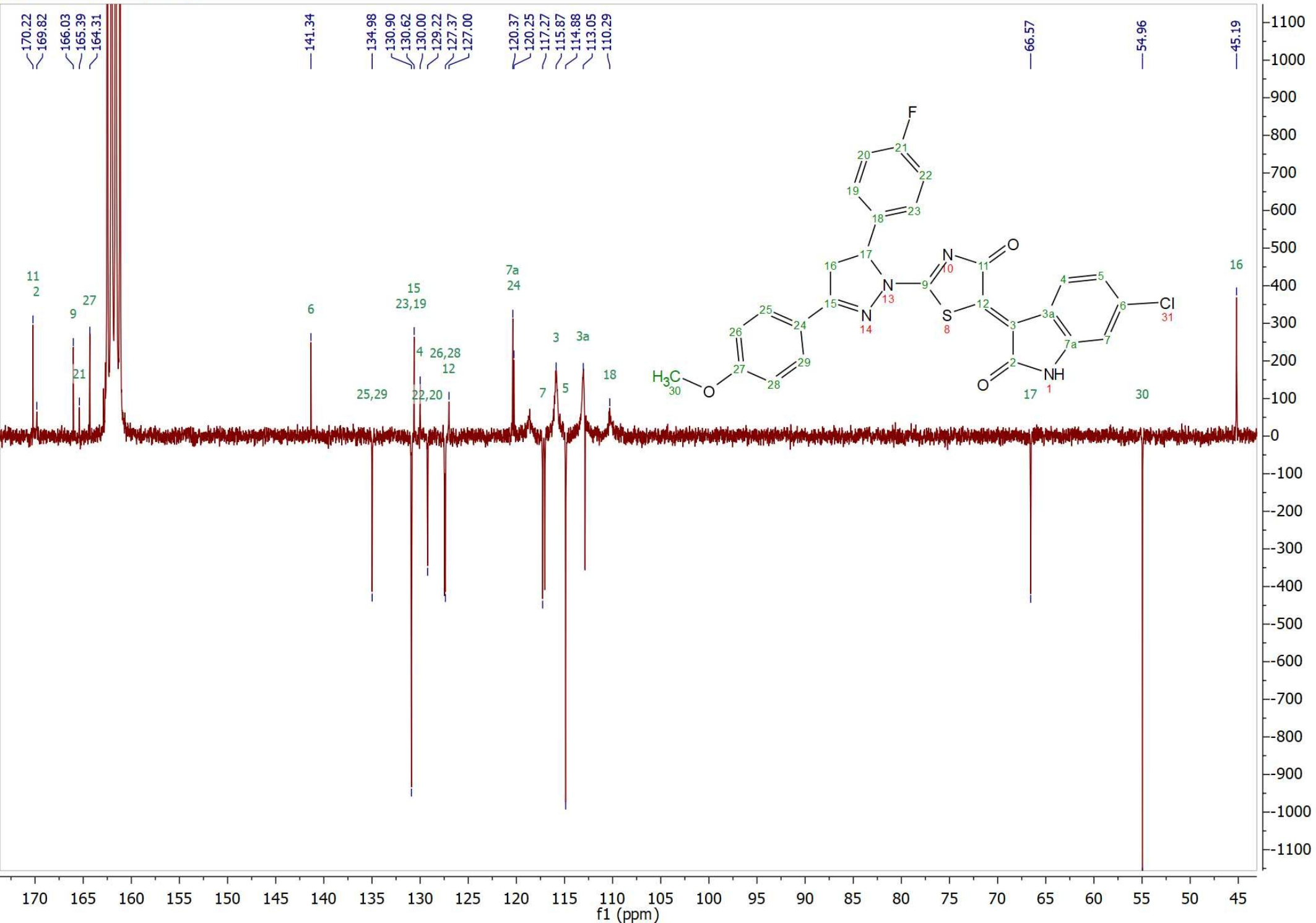


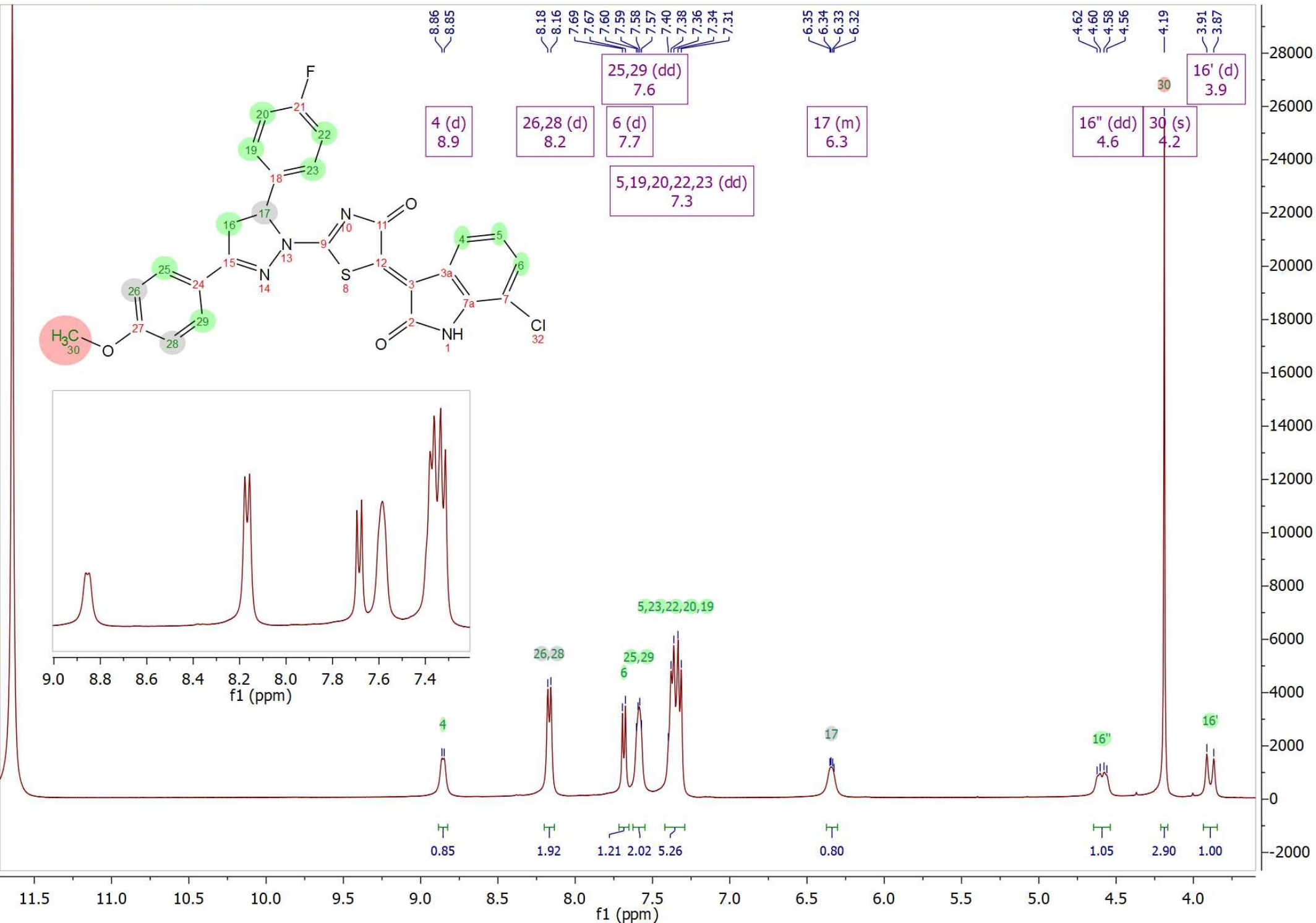


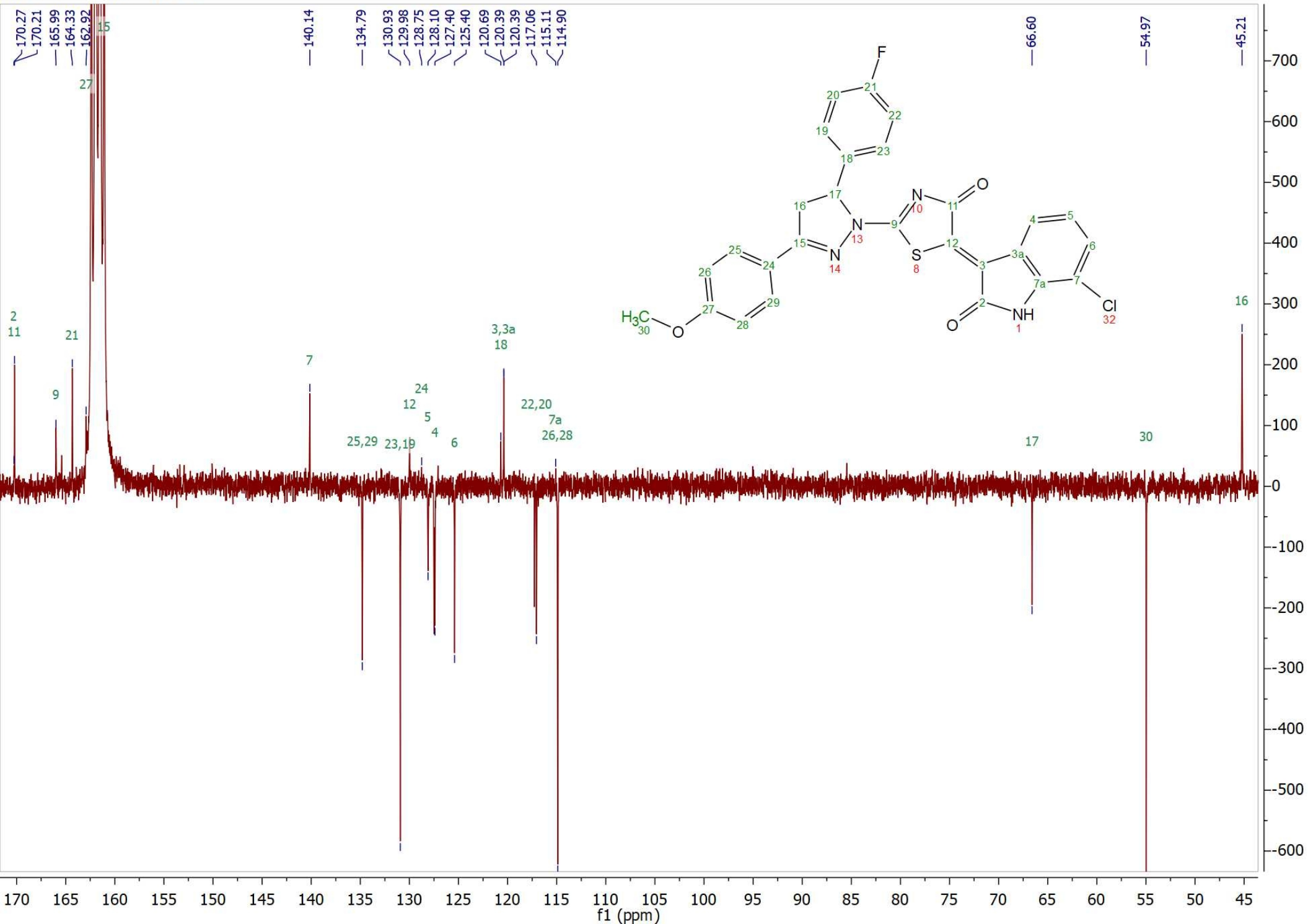


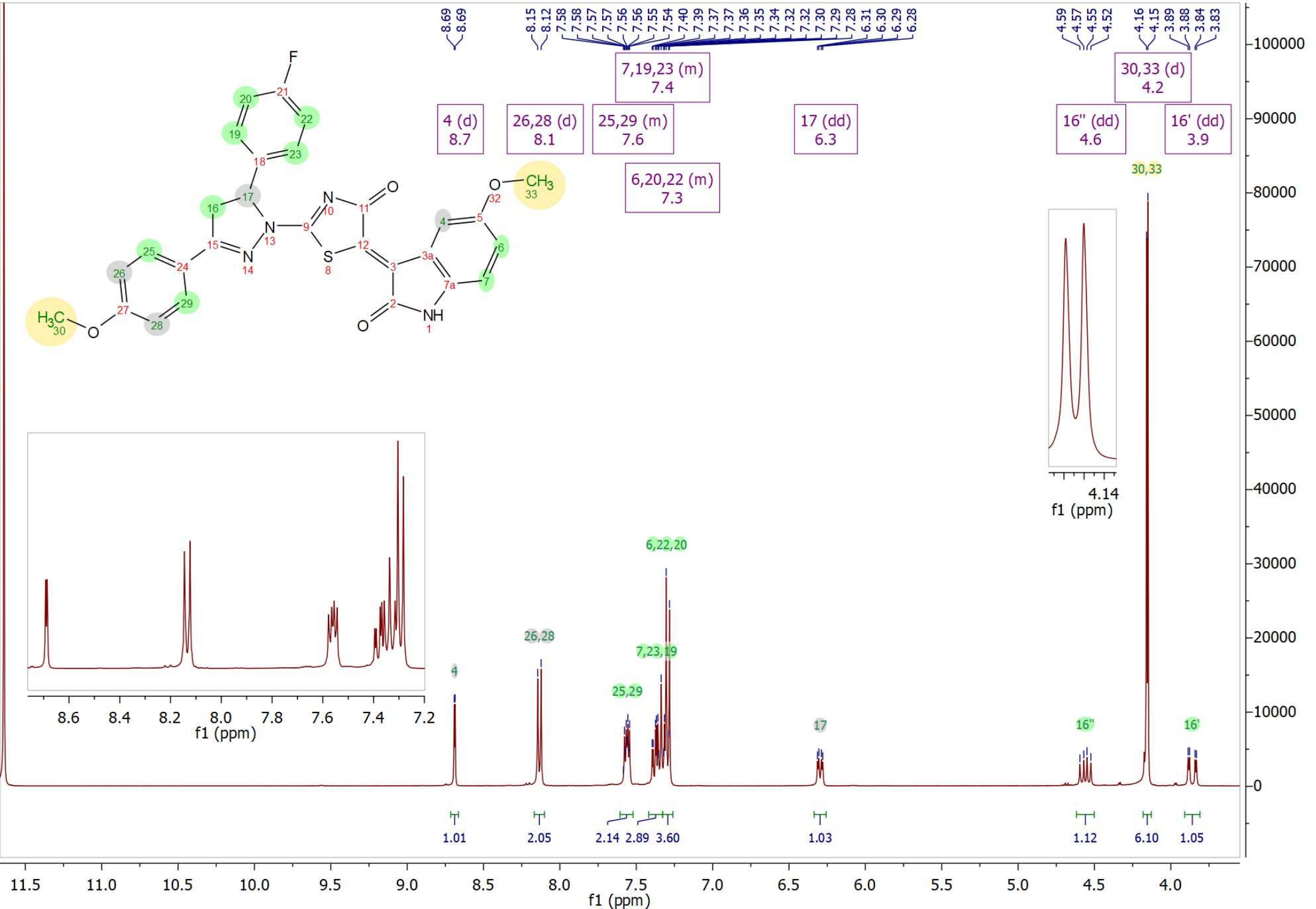


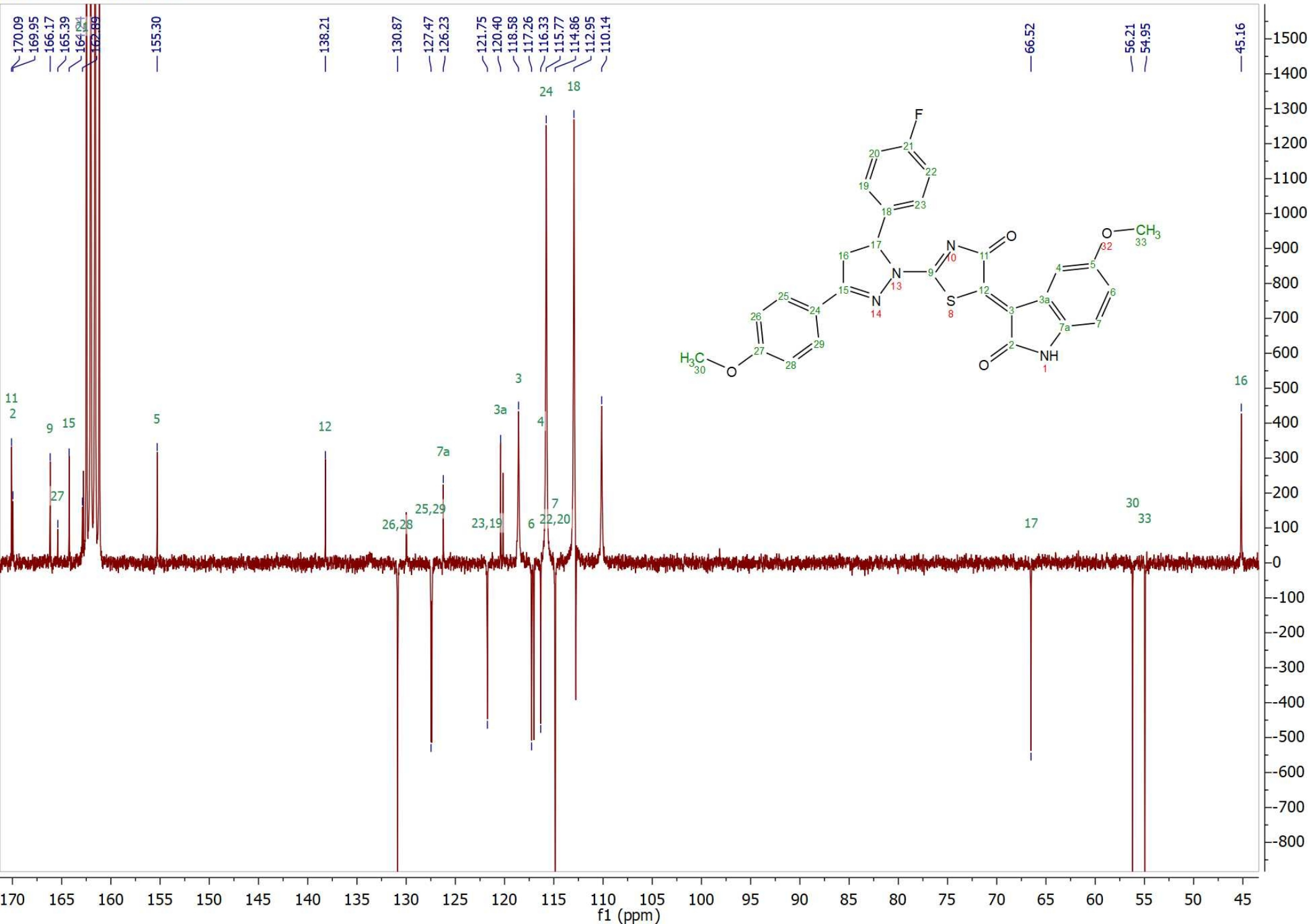


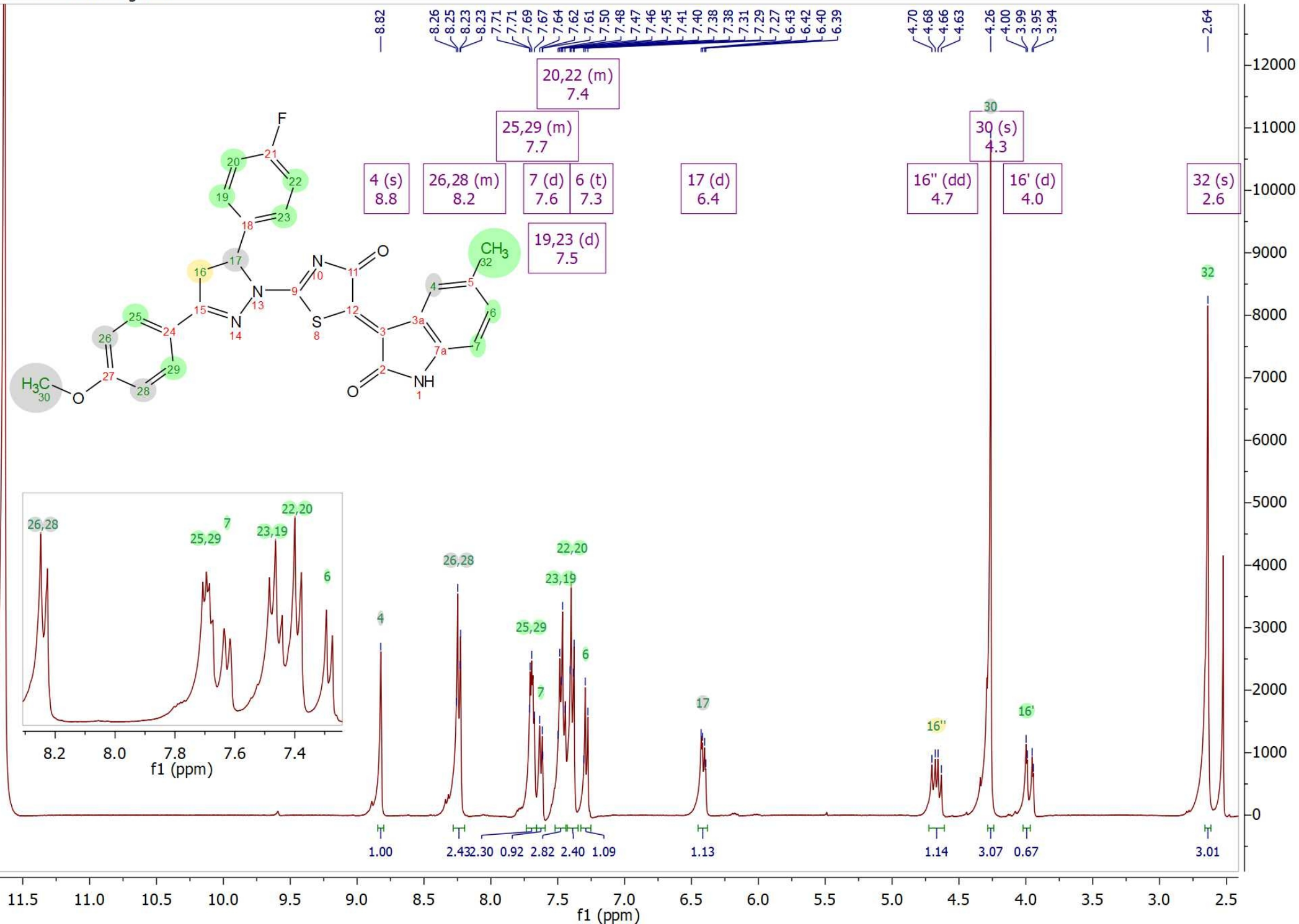




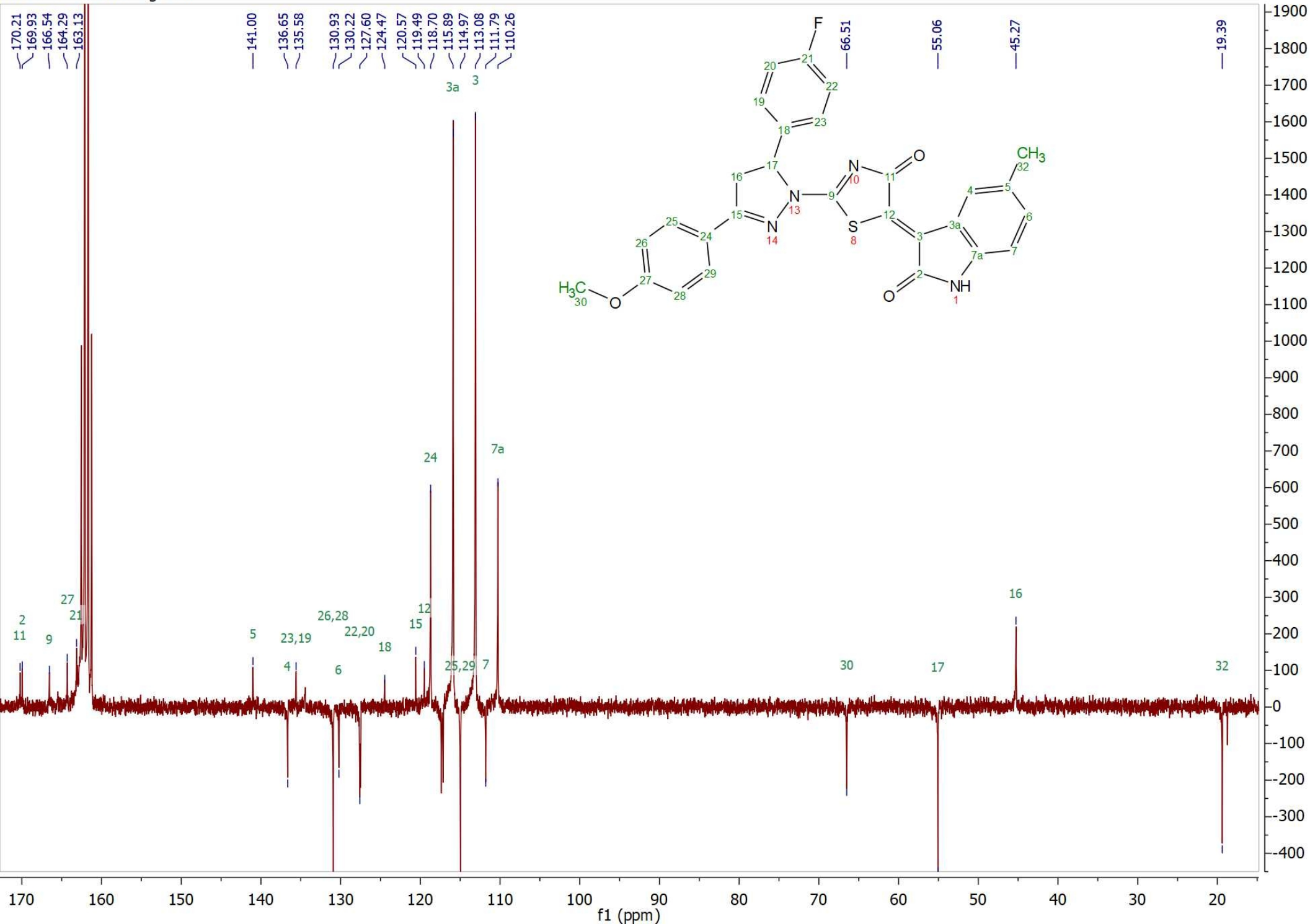


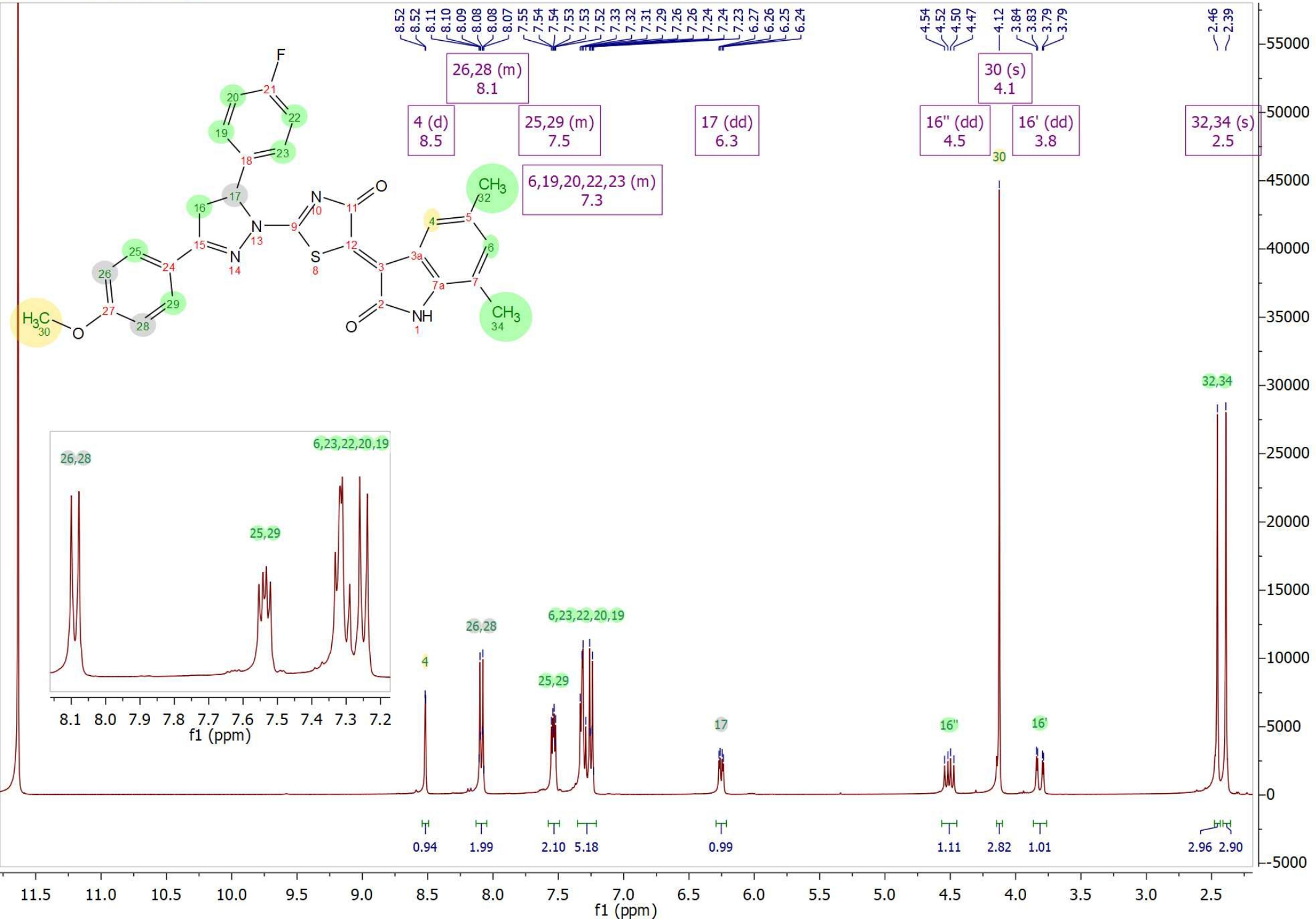


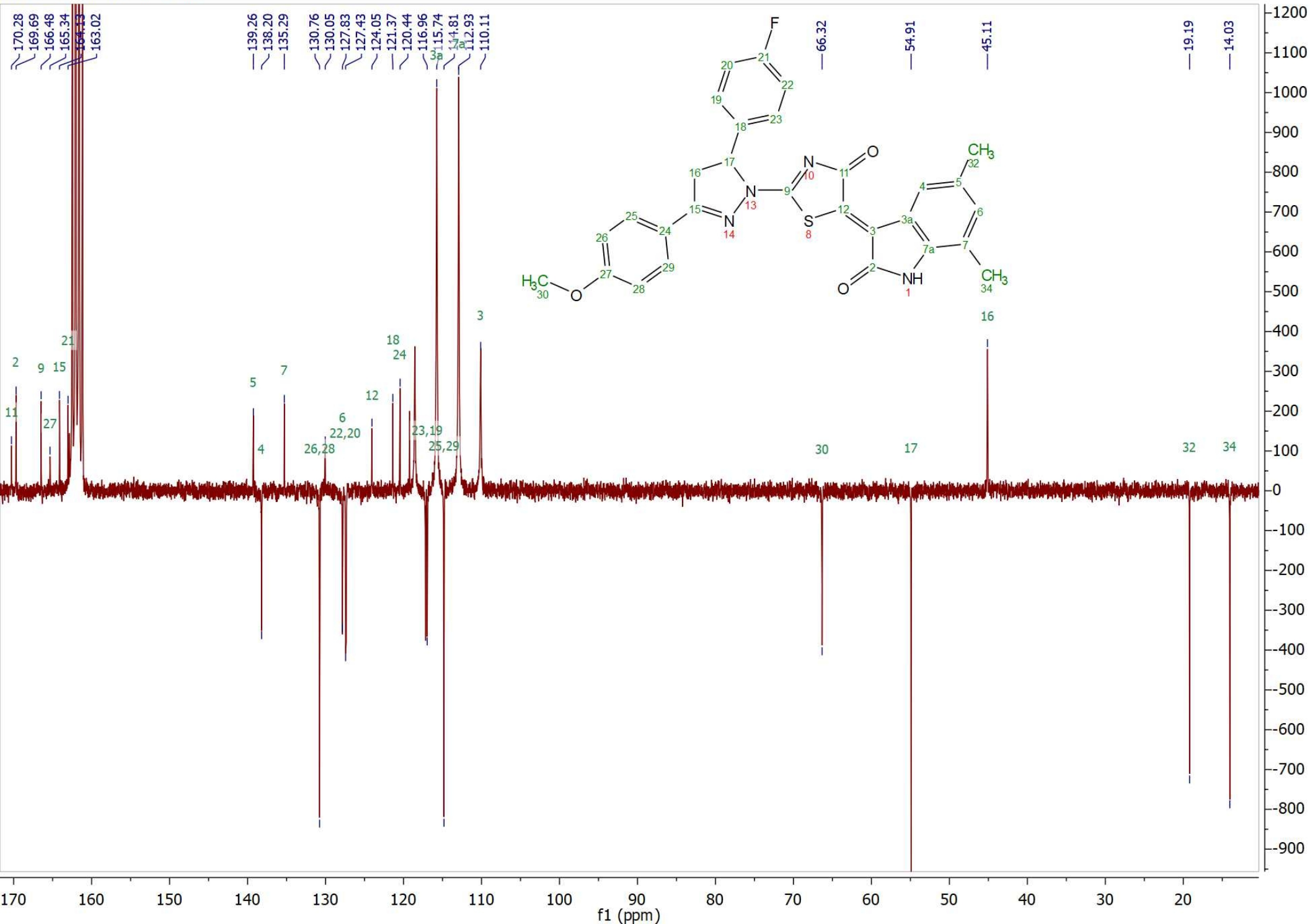


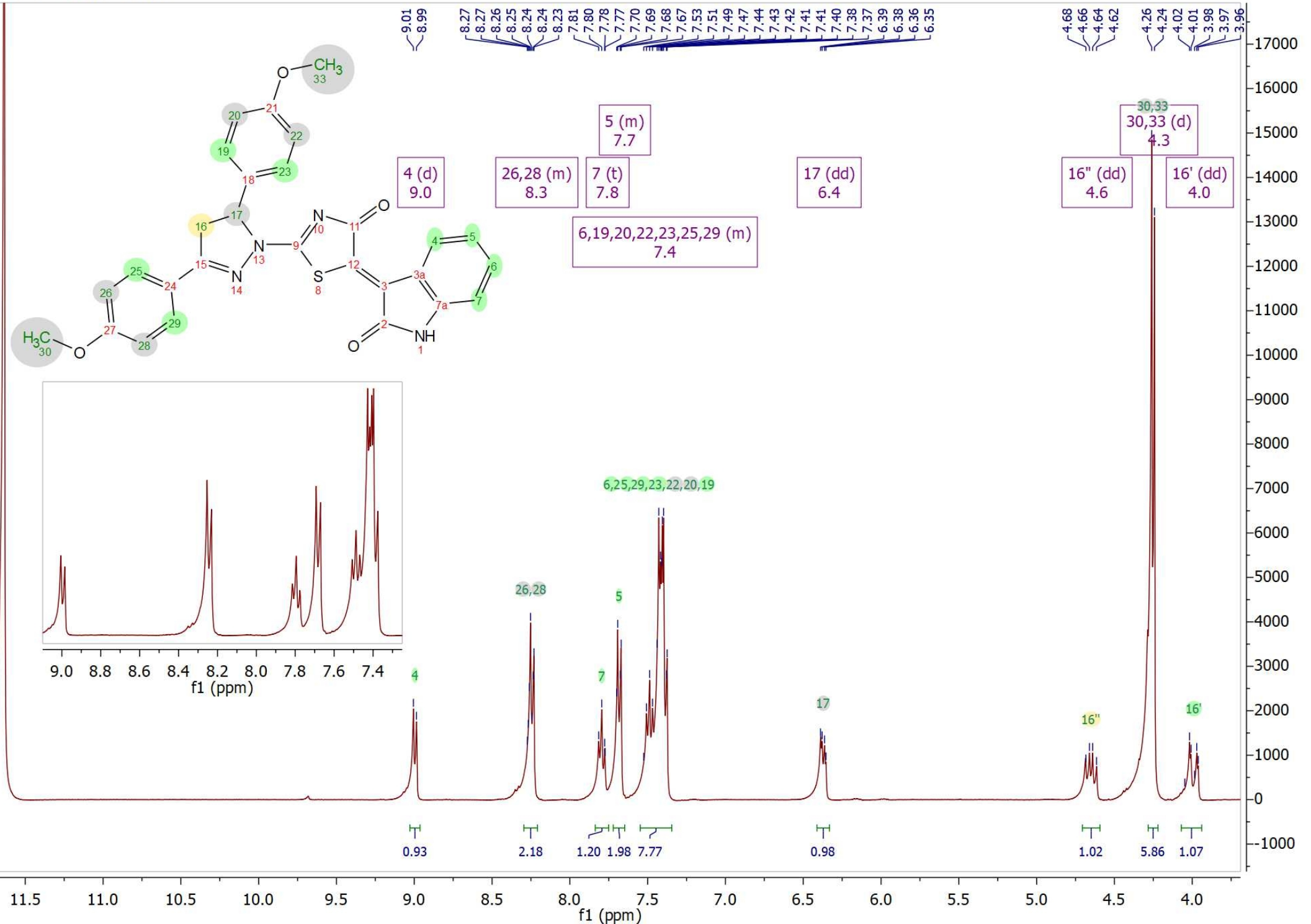


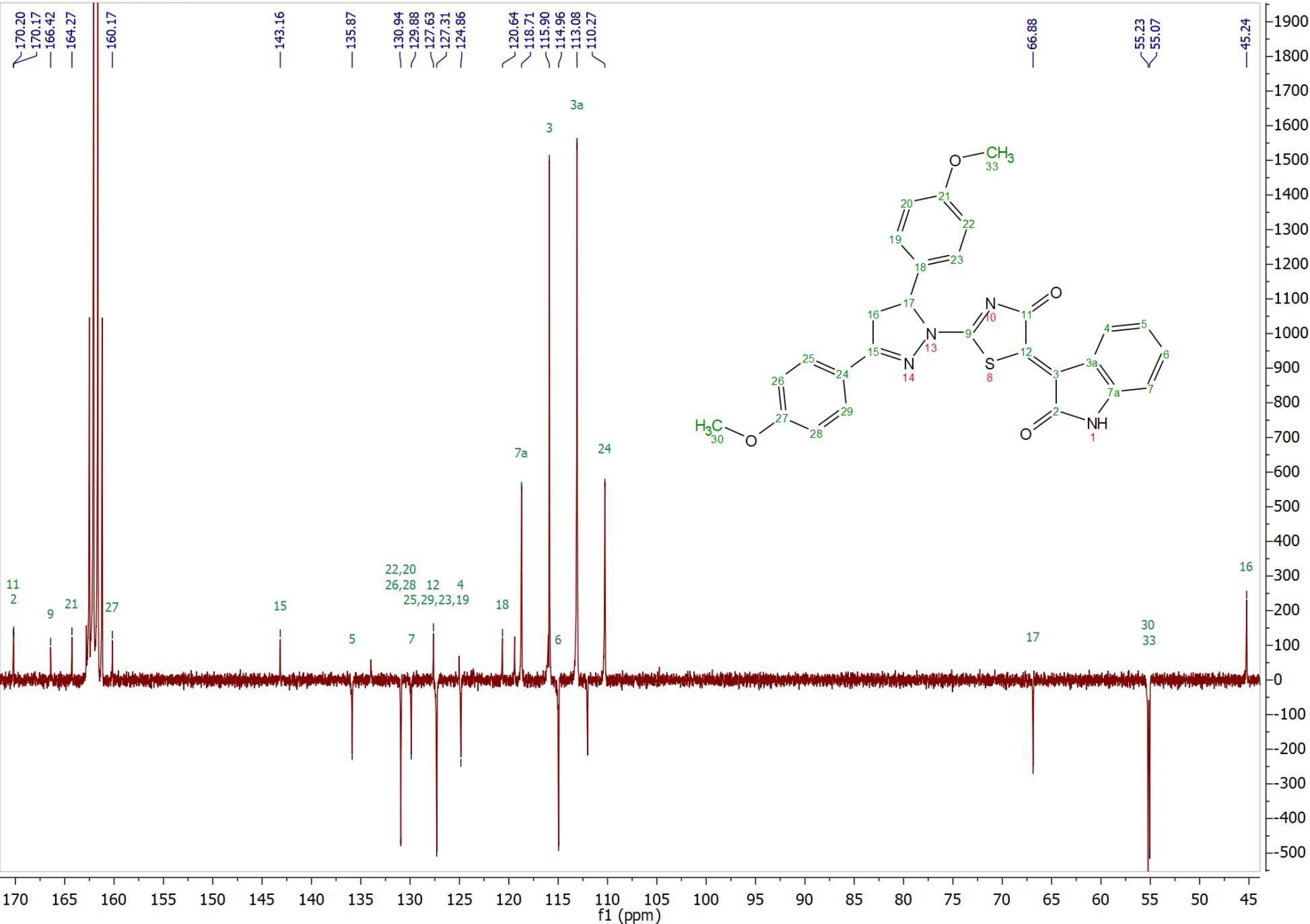


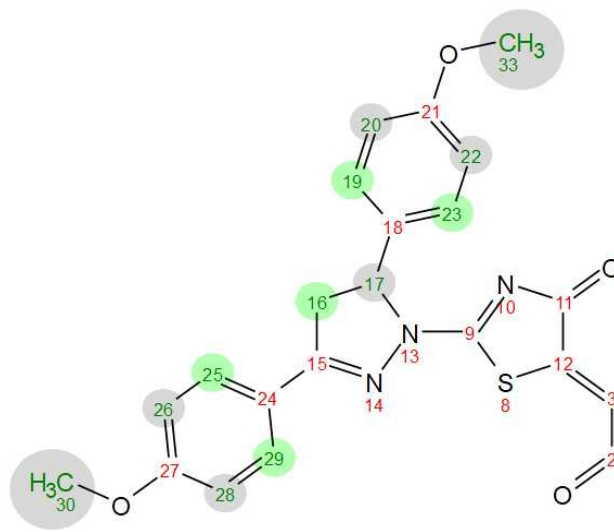






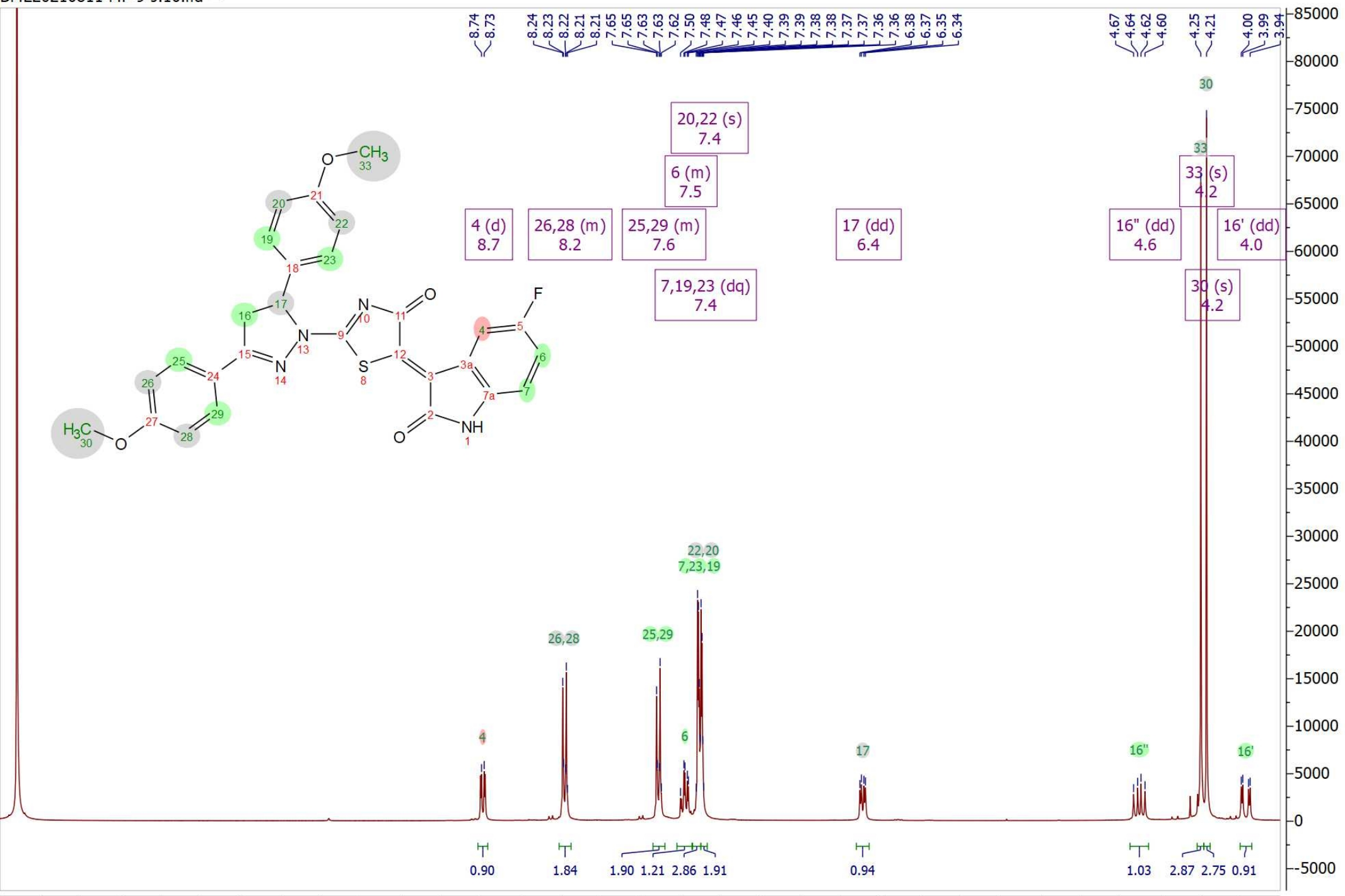






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20,22 (s) 7.4  
 6 (m) 7.5  
 4 (d) 8.7  
 26,28 (m) 8.2  
 25,29 (m) 7.6  
 17 (dd) 6.4  
 7,19,23 (dq) 7.4  
 16'' (dd) 4.6  
 33 (s) 4.2  
 16' (dd) 4.0  
 30 (s) 4.2



11.5, 11.0, 10.5, 10.0, 9.5, 9.0, 8.5, 8.0, 7.5, 7.0, 6.5, 6.0, 5.5, 5.0, 4.5, 4.0

f1 (ppm)

