Supporting Information

Design, Synthesis and Cytotoxicity Screening of New Thiazole derivatives as Potential Anticancer Agents through VEGFR-2 Inhibition

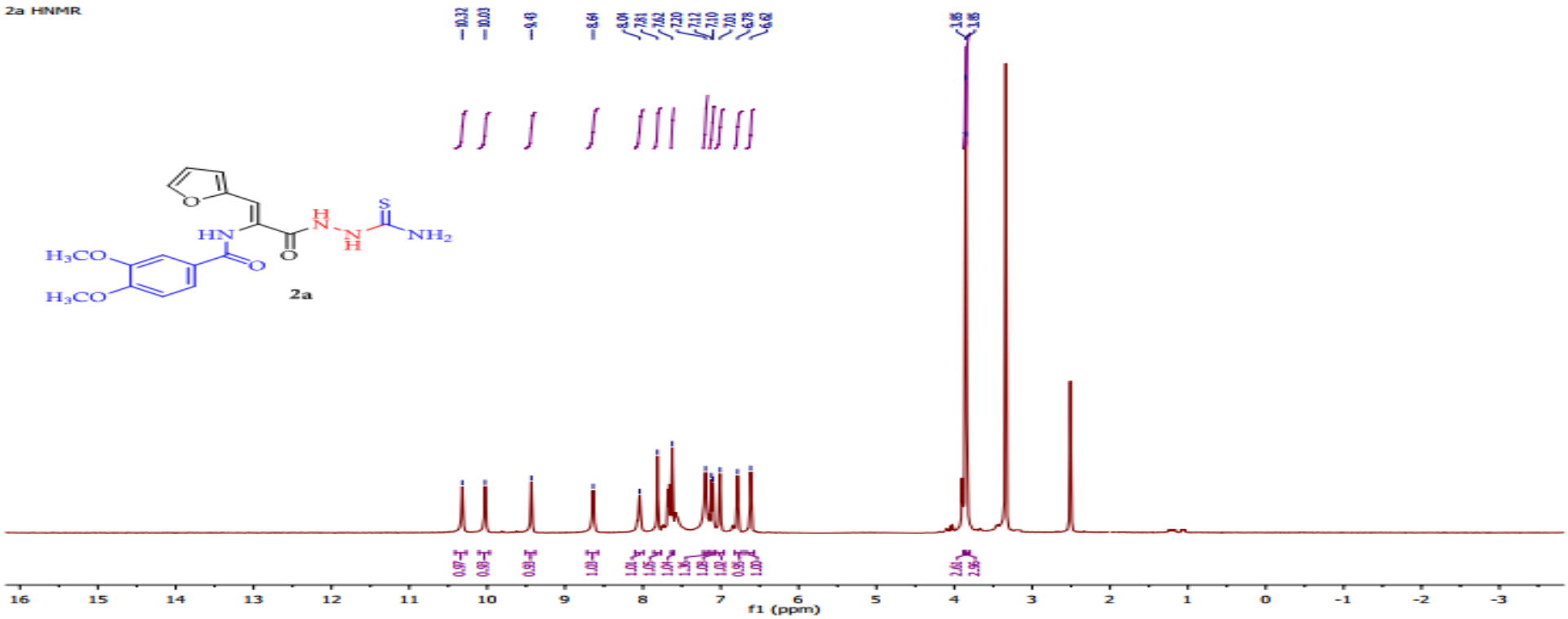

Figure S1: ^{1}H-NMR spectrum of compound **2a**

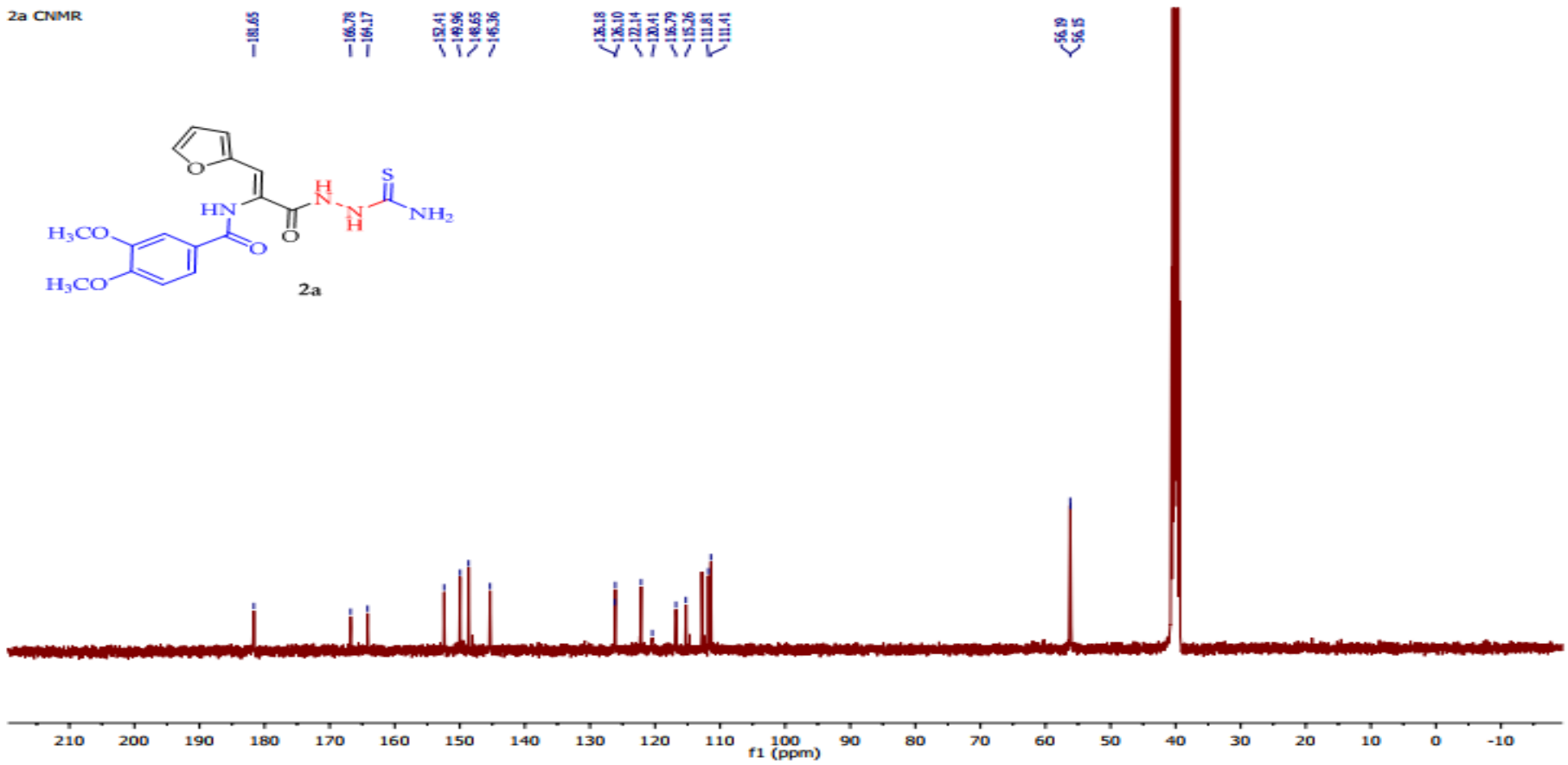

Figure S2: ^{13}C-NMR spectrum of compound **2a**

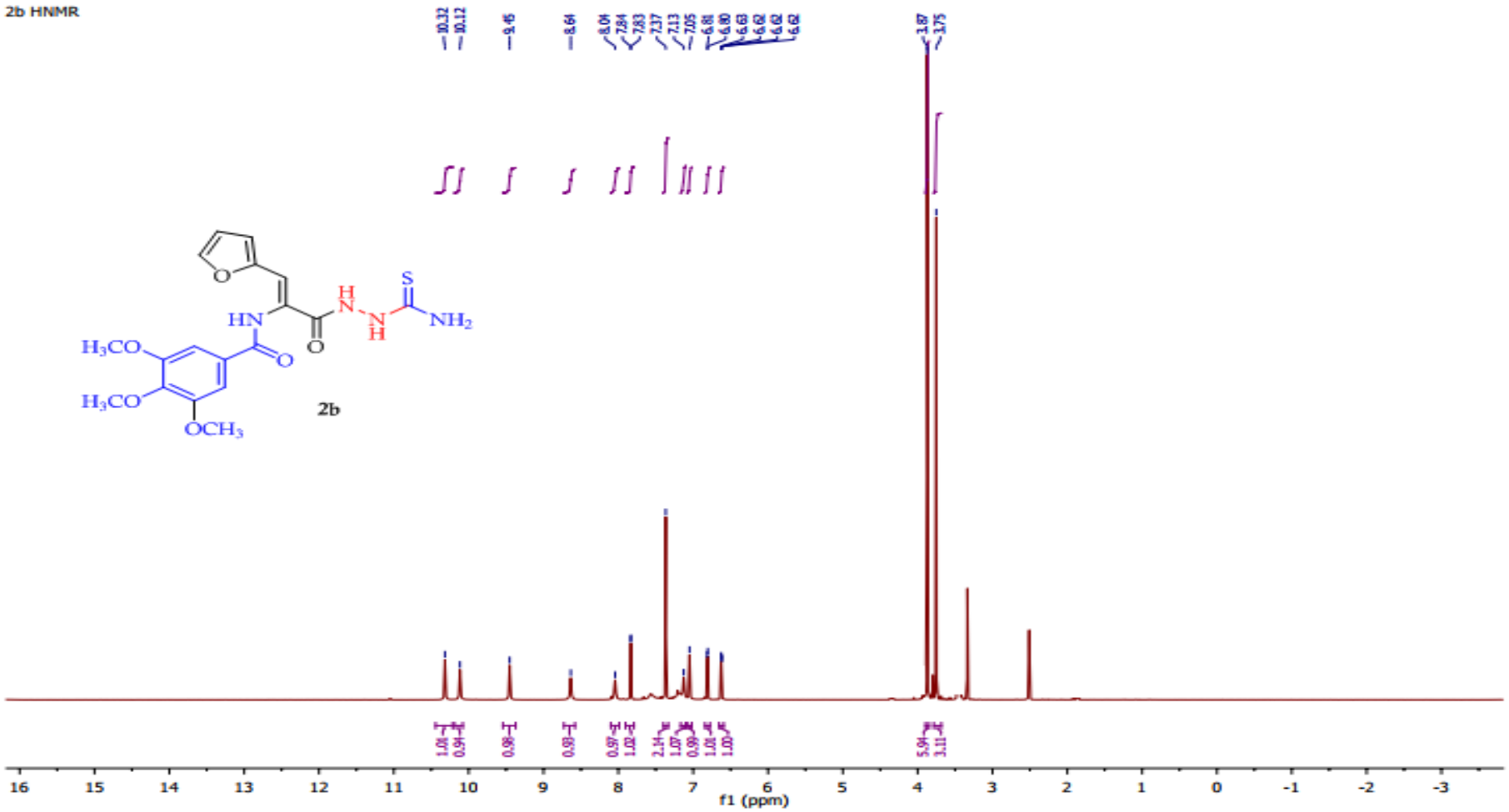

Figure S3: ^{1}H-NMR spectrum of compound **2b**

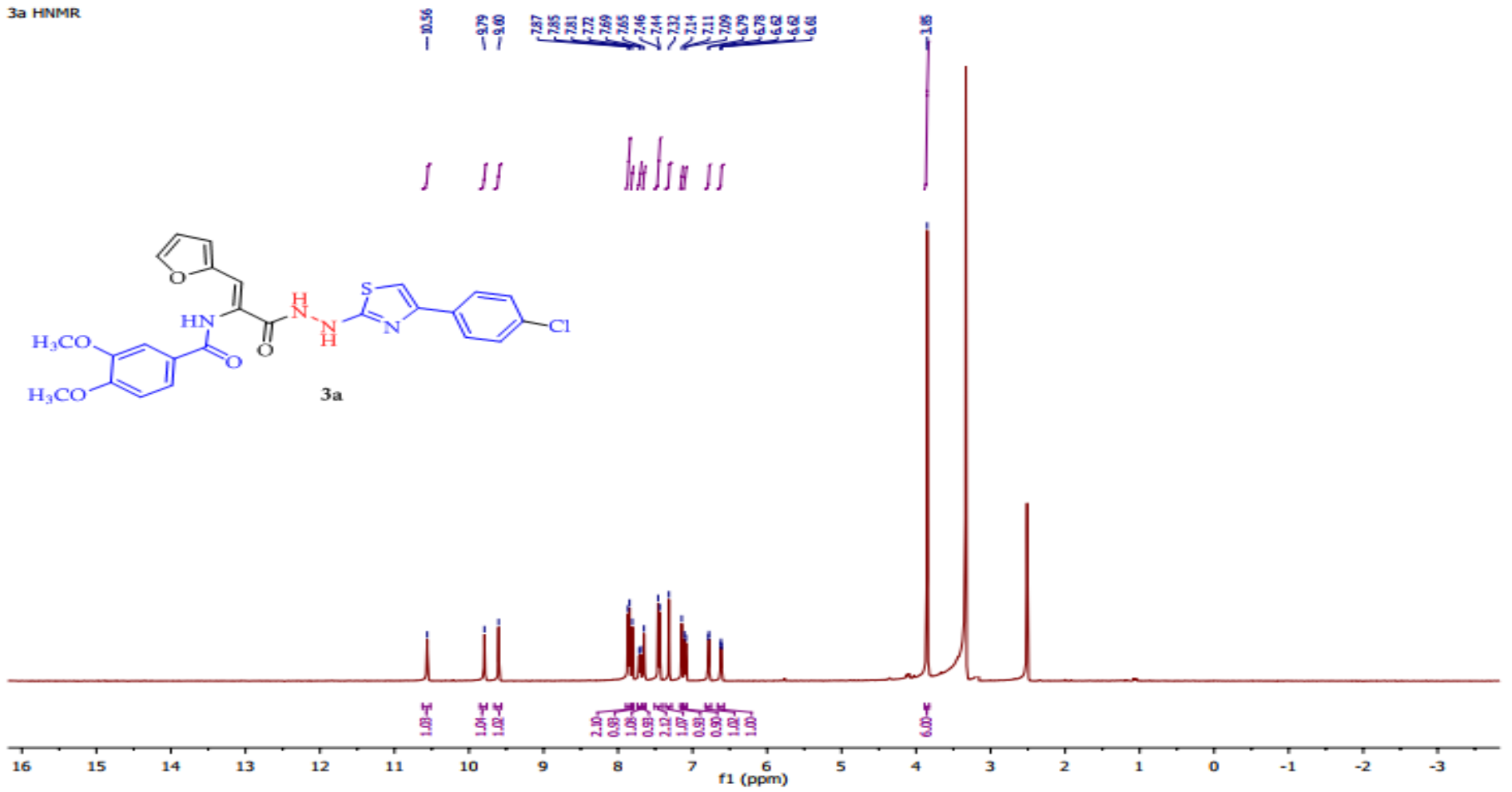

Figure S4: [1]H-NMR spectrum of compound **3a**

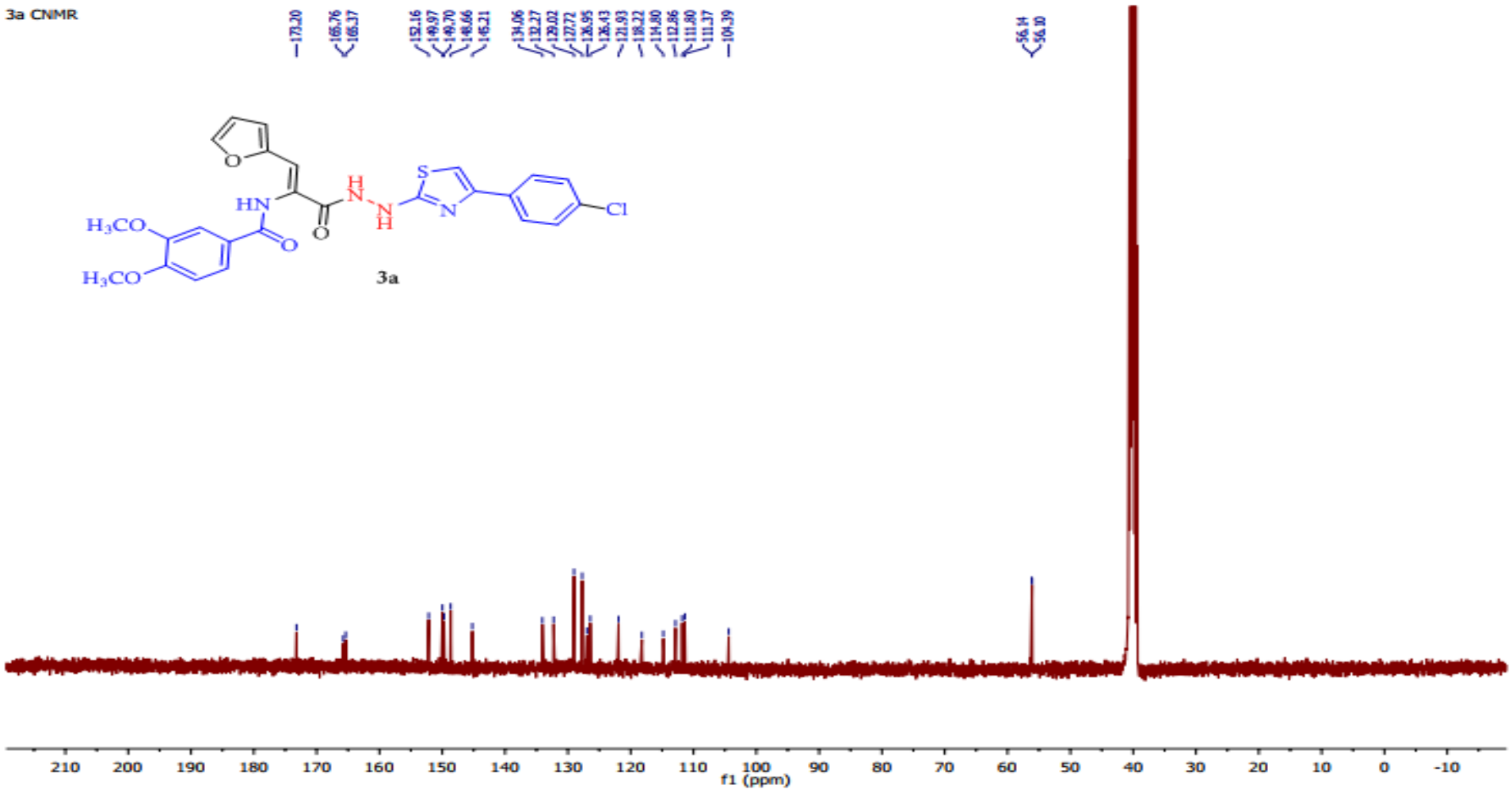

Figure S5: ^{13}C-NMR spectrum of compound **3a**

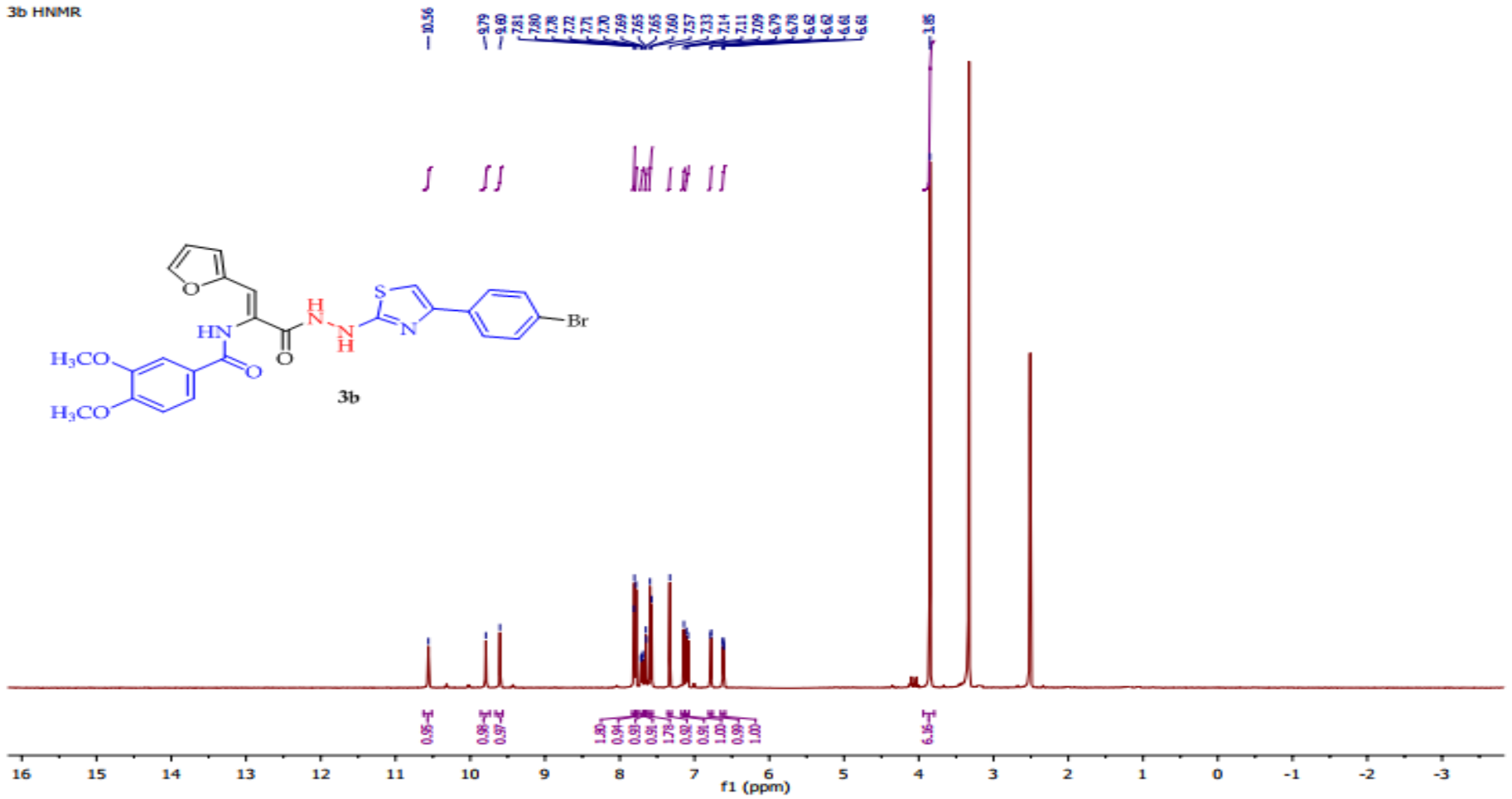

Figure S6: [1]H-NMR spectrum of compound **3b**

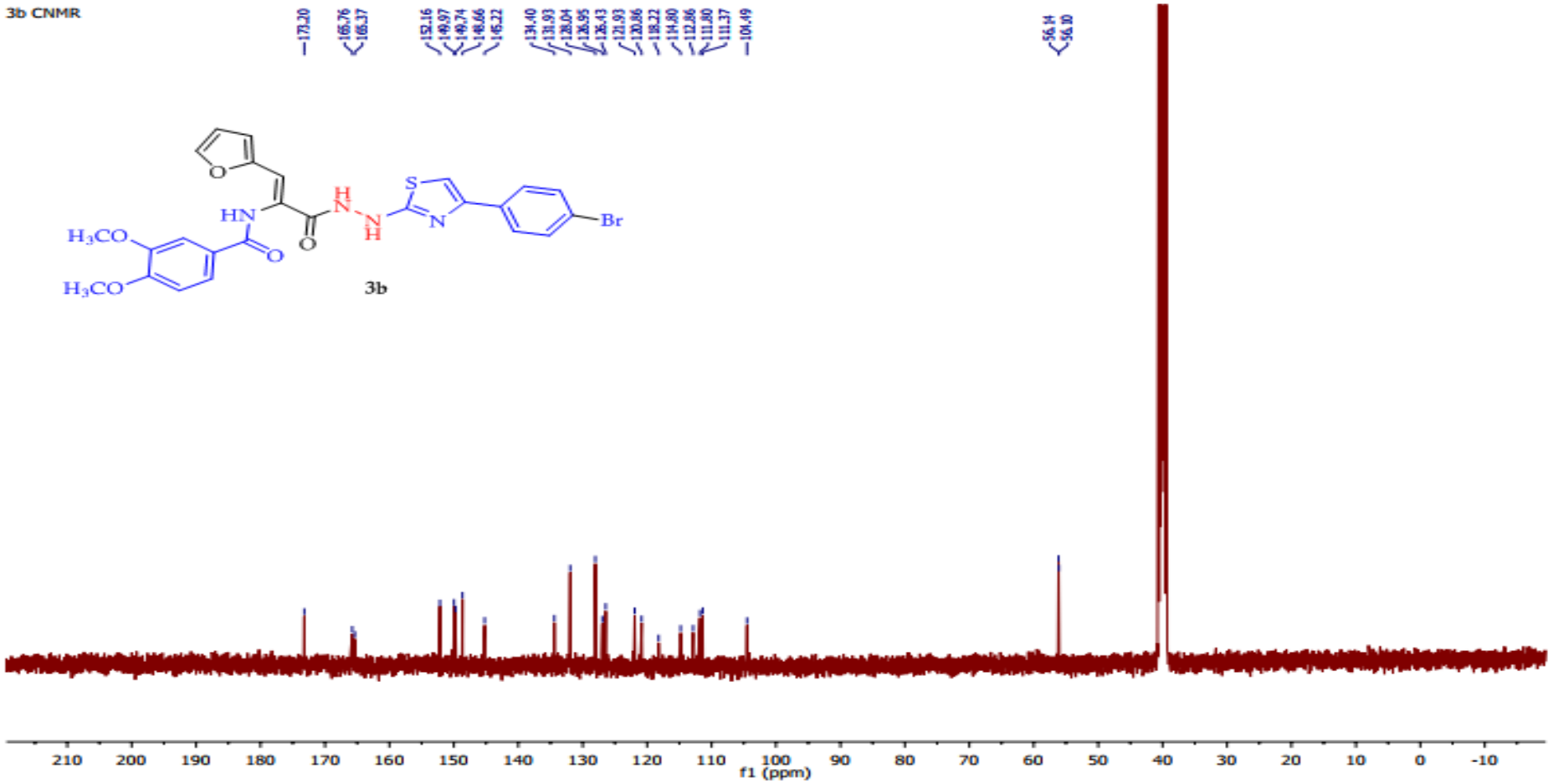

Figure S7: ^{13}C-NMR spectrum of compound **3b**

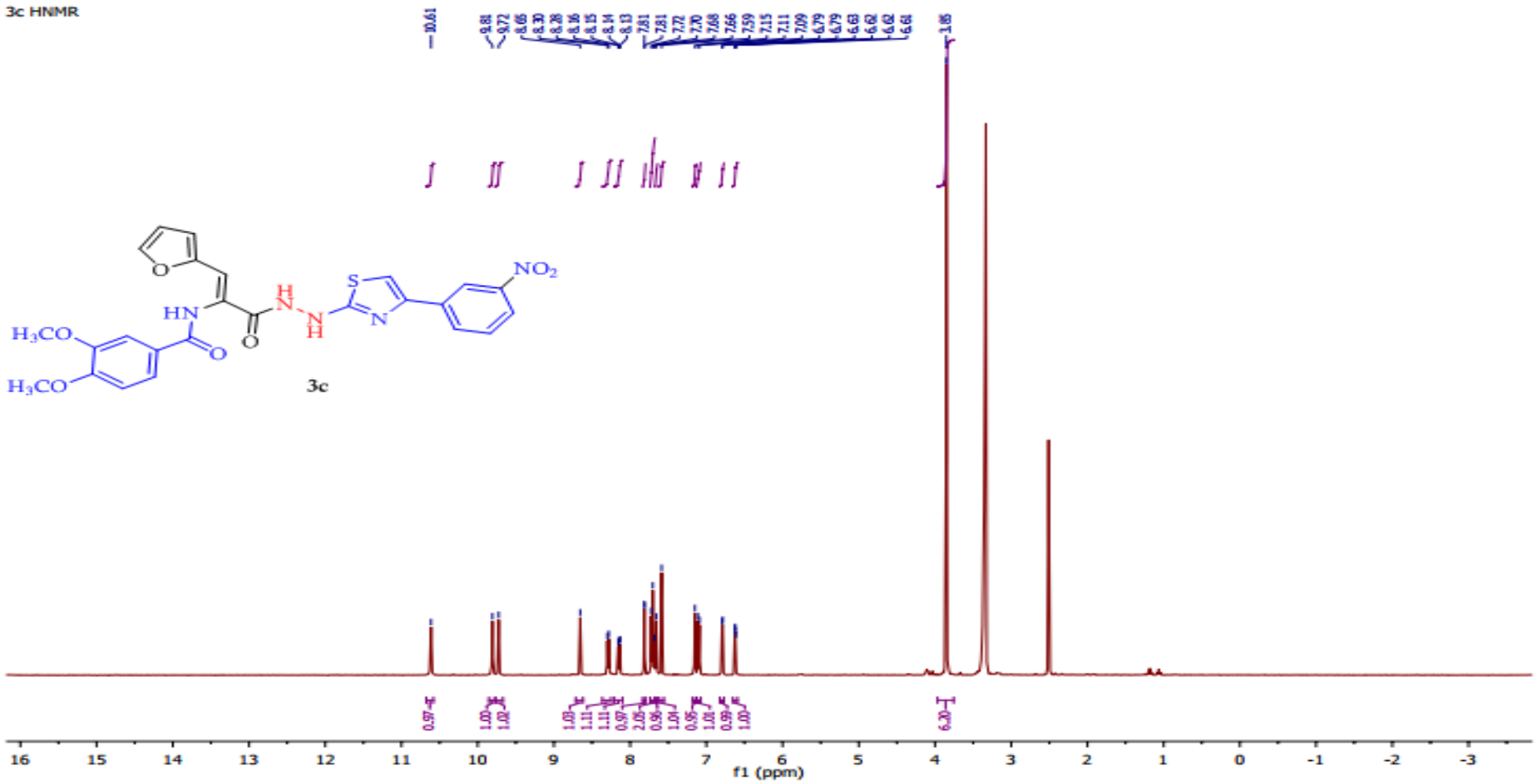

Figure S8: [1]H-NMR spectrum of compound **3c**

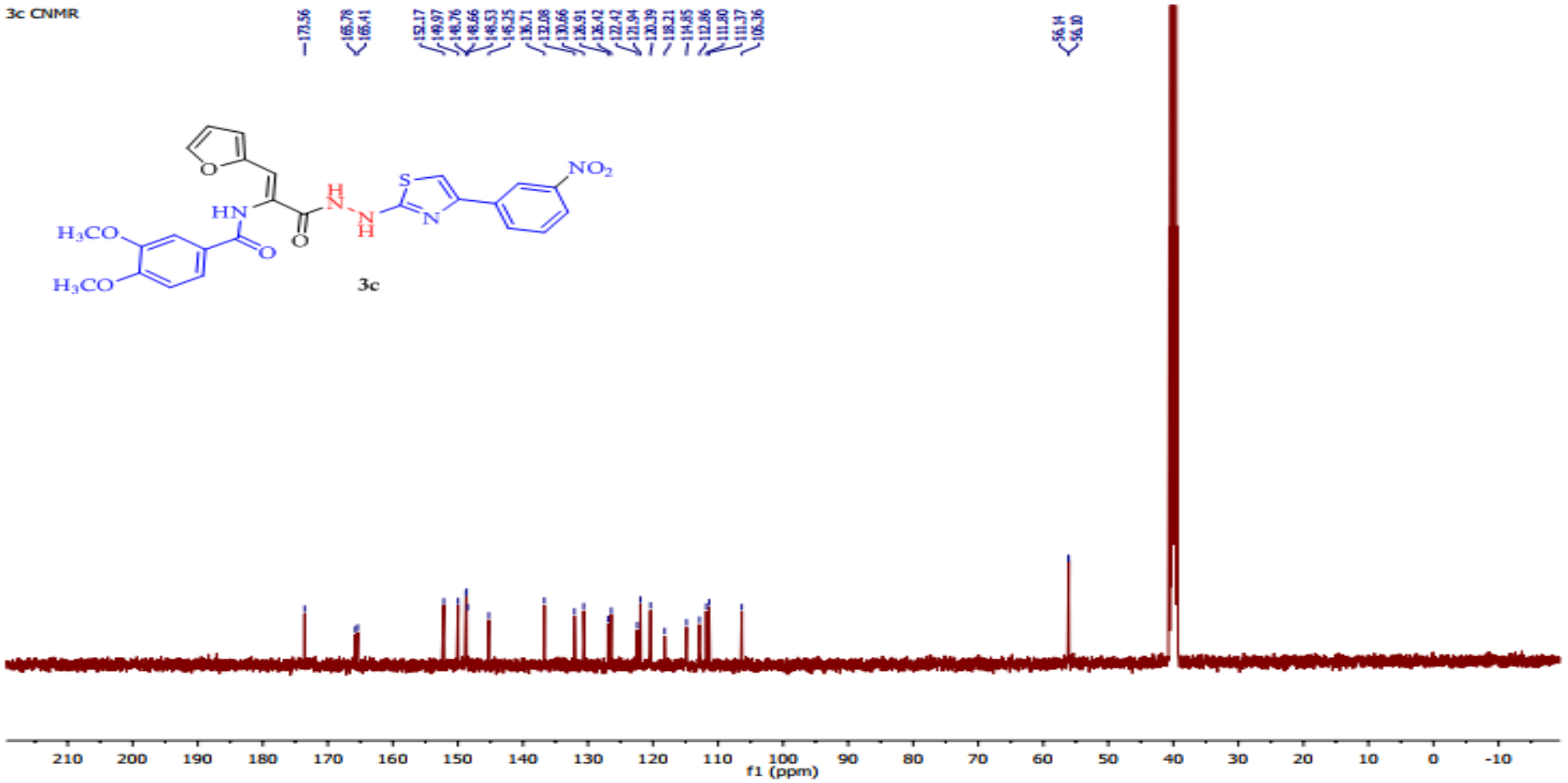

Figure S9: ^{13}C-NMR spectrum of compound **3c**

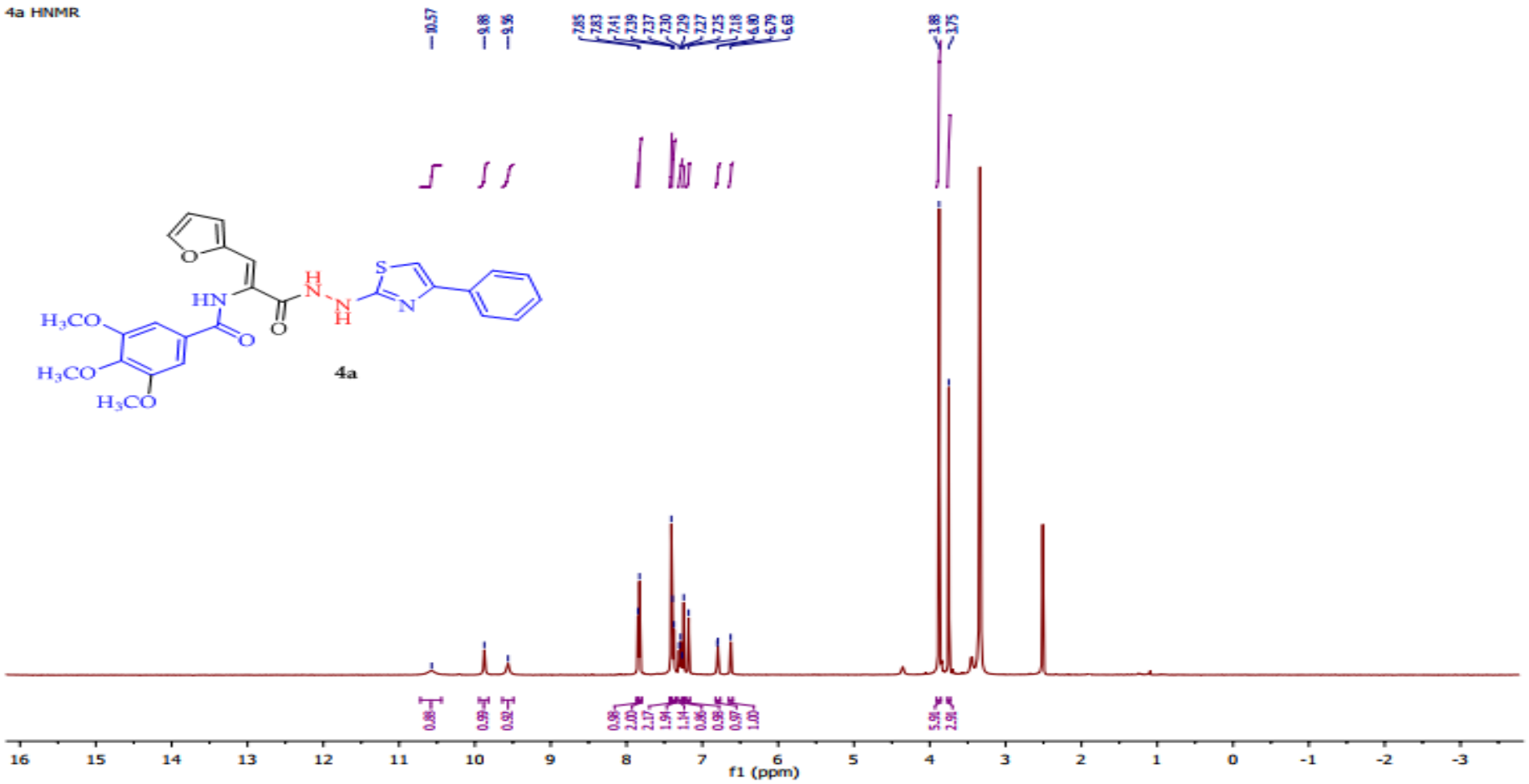

Figure S10: [1]H-NMR spectrum of compound **4a**

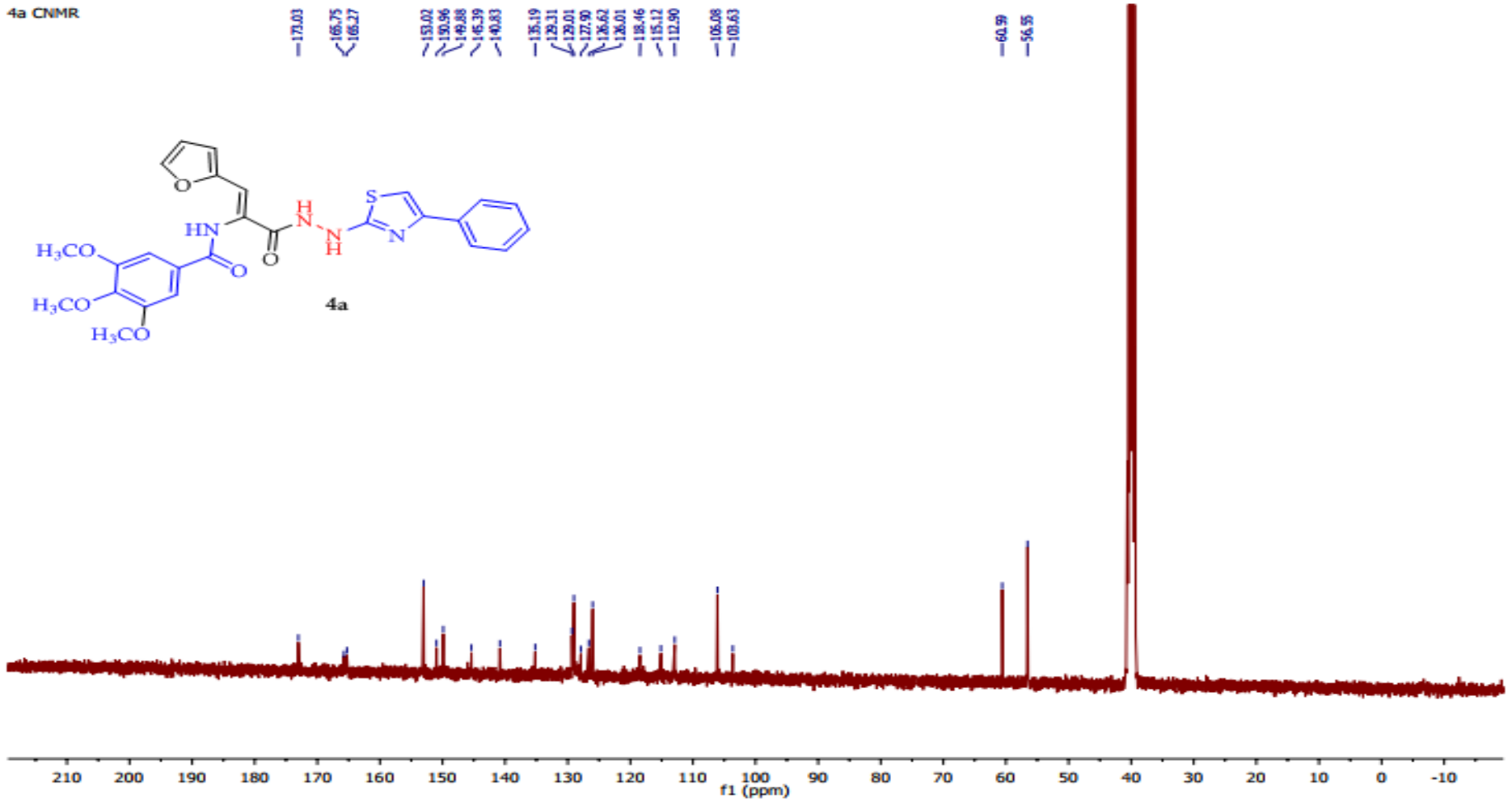

Figure S11: ^{13}C-NMR spectrum of compound **4a**

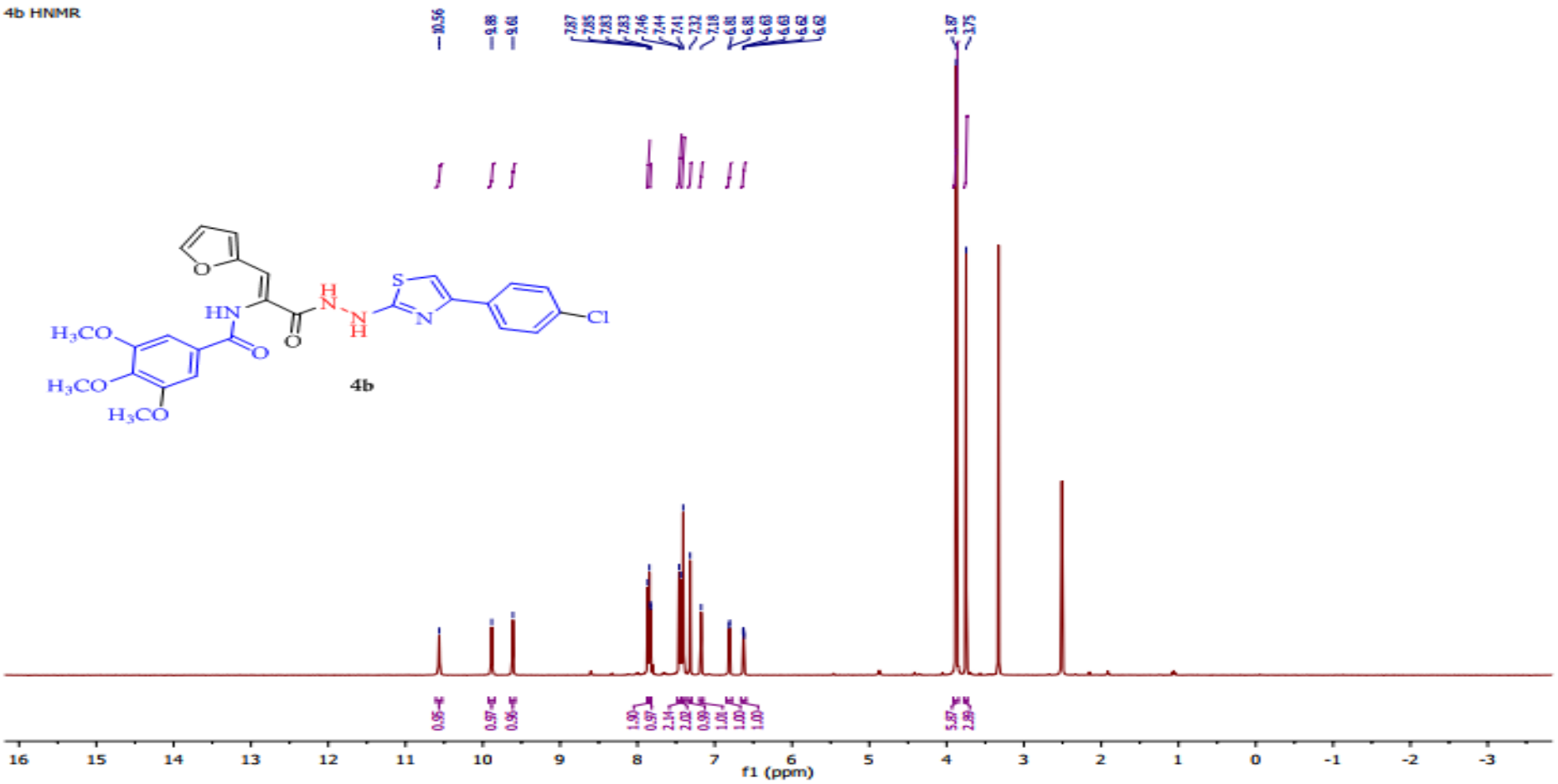

Figure S12: [1]H-NMR spectrum of compound **4b**

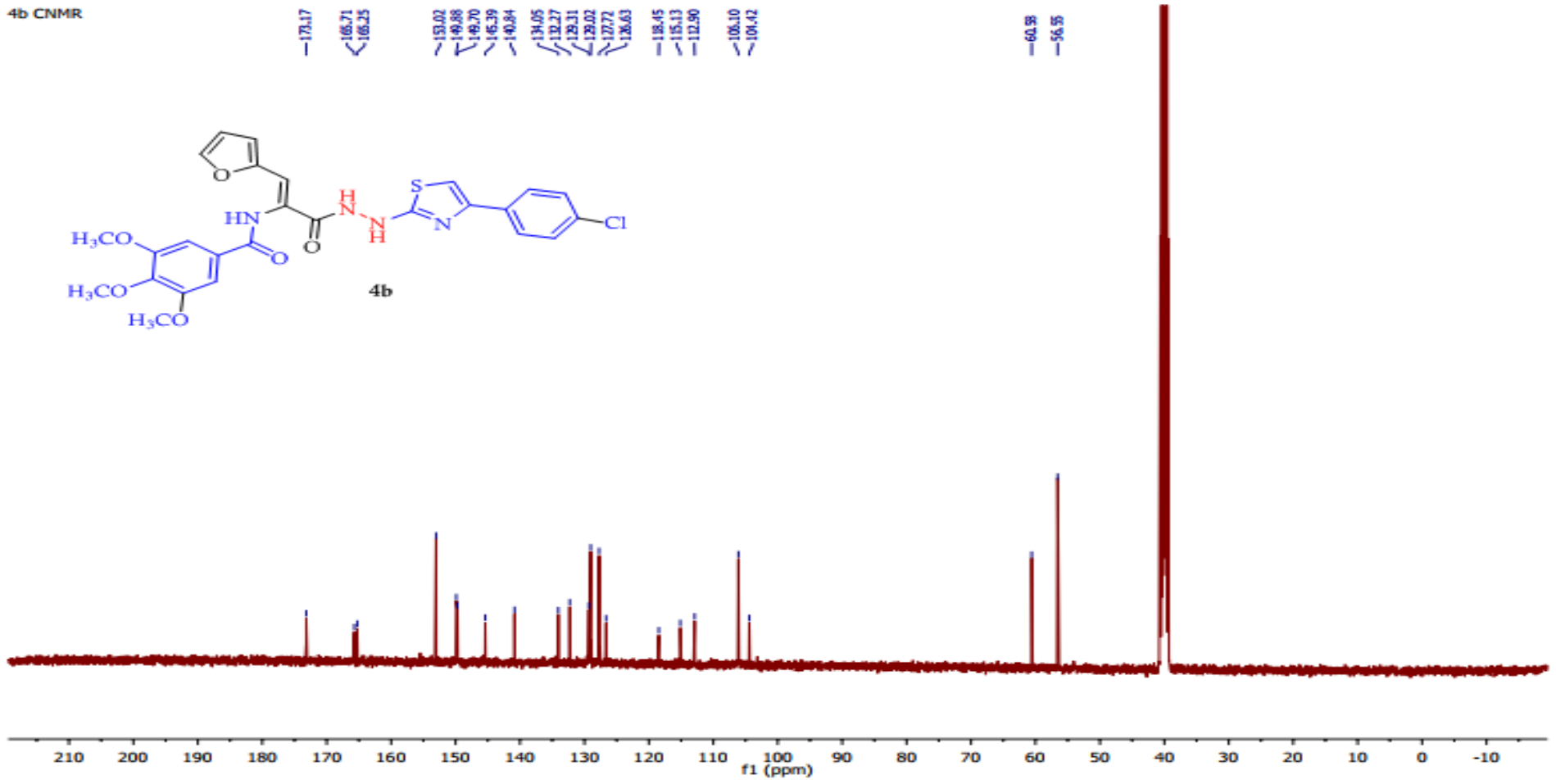

Figure S13: ^{13}C-NMR spectrum of compound **4b**

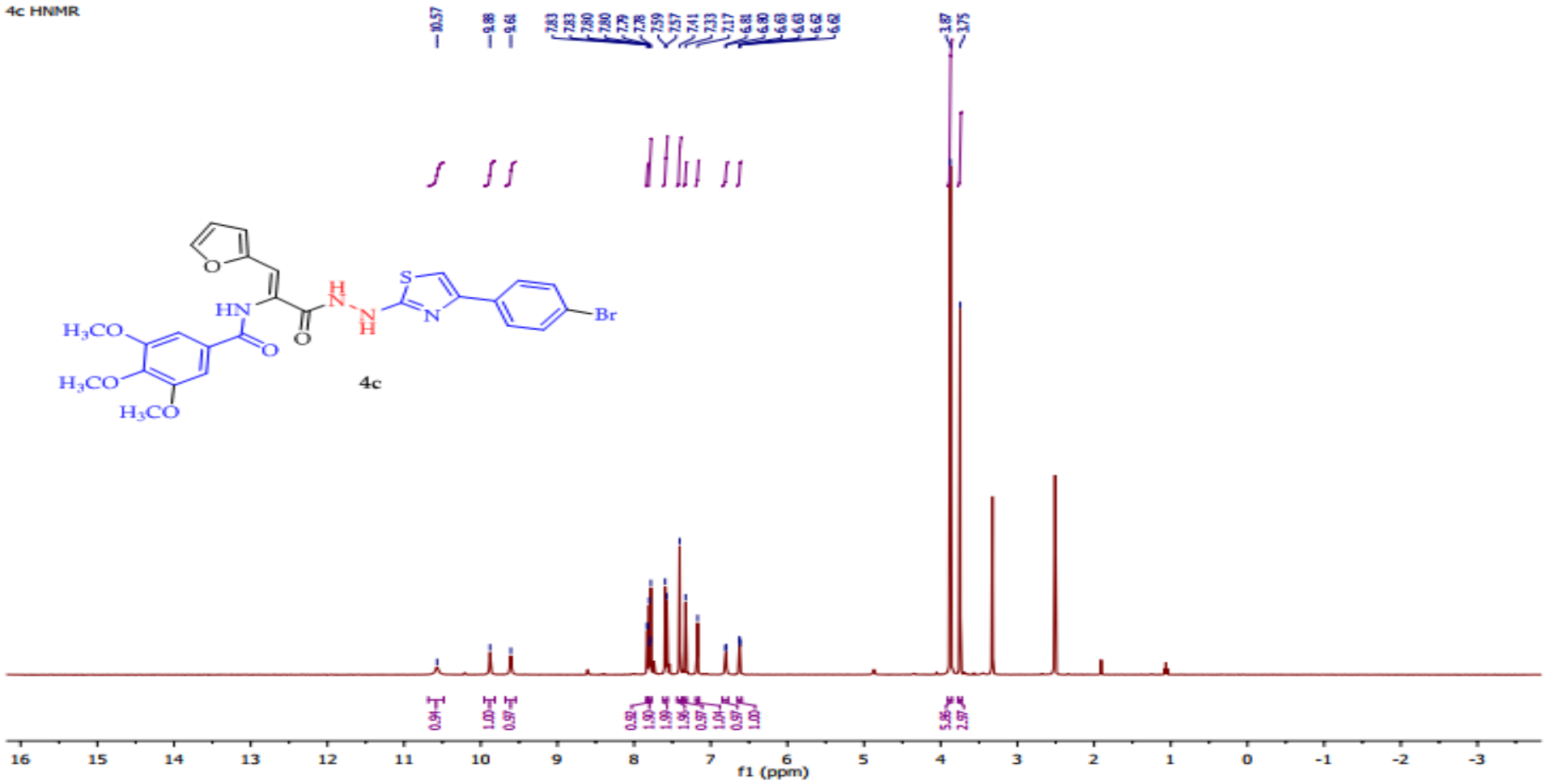

Figure S14: [1]H-NMR spectrum of compound **4c**

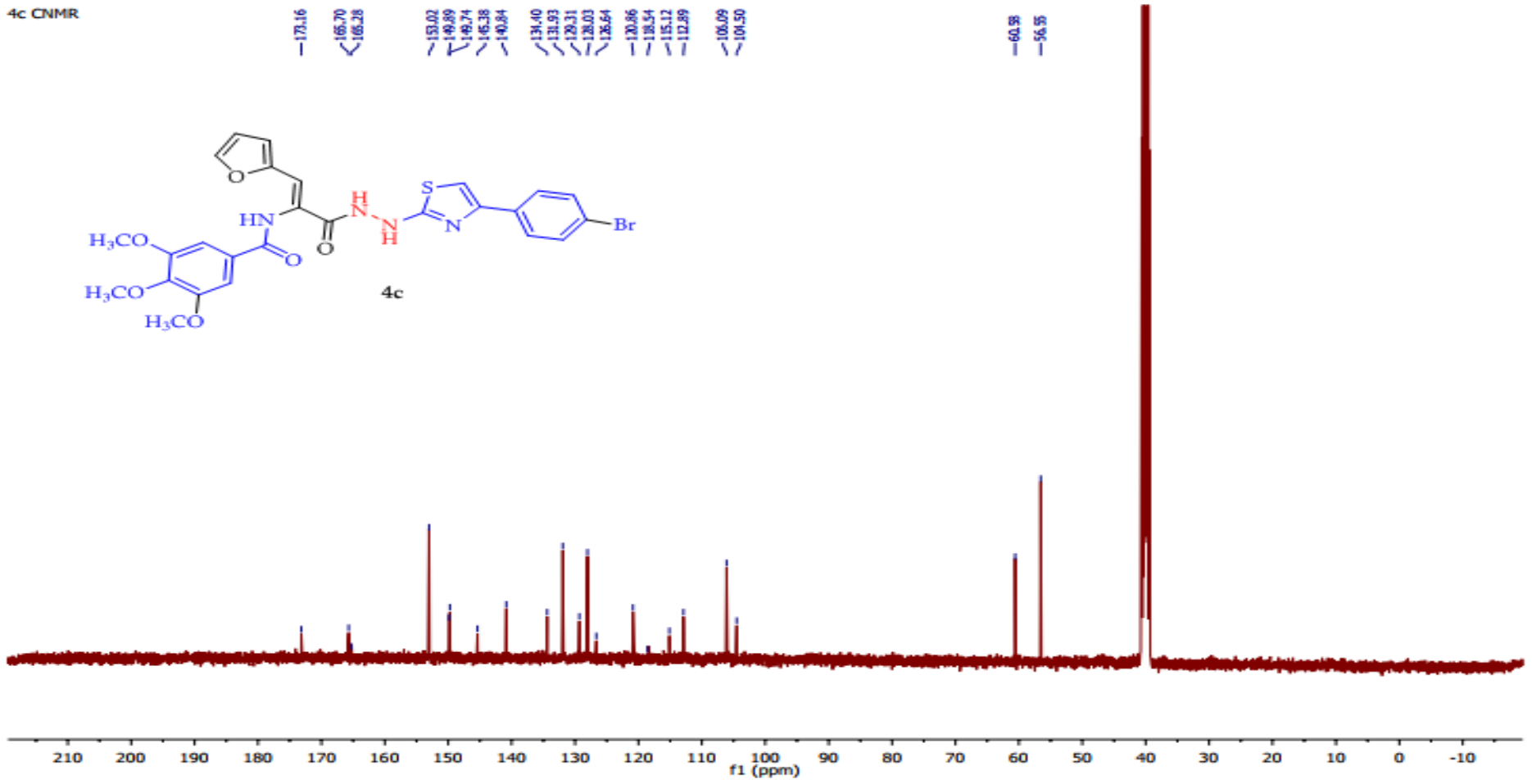

Figure S15: ^{13}C-NMR spectrum of compound **4c**

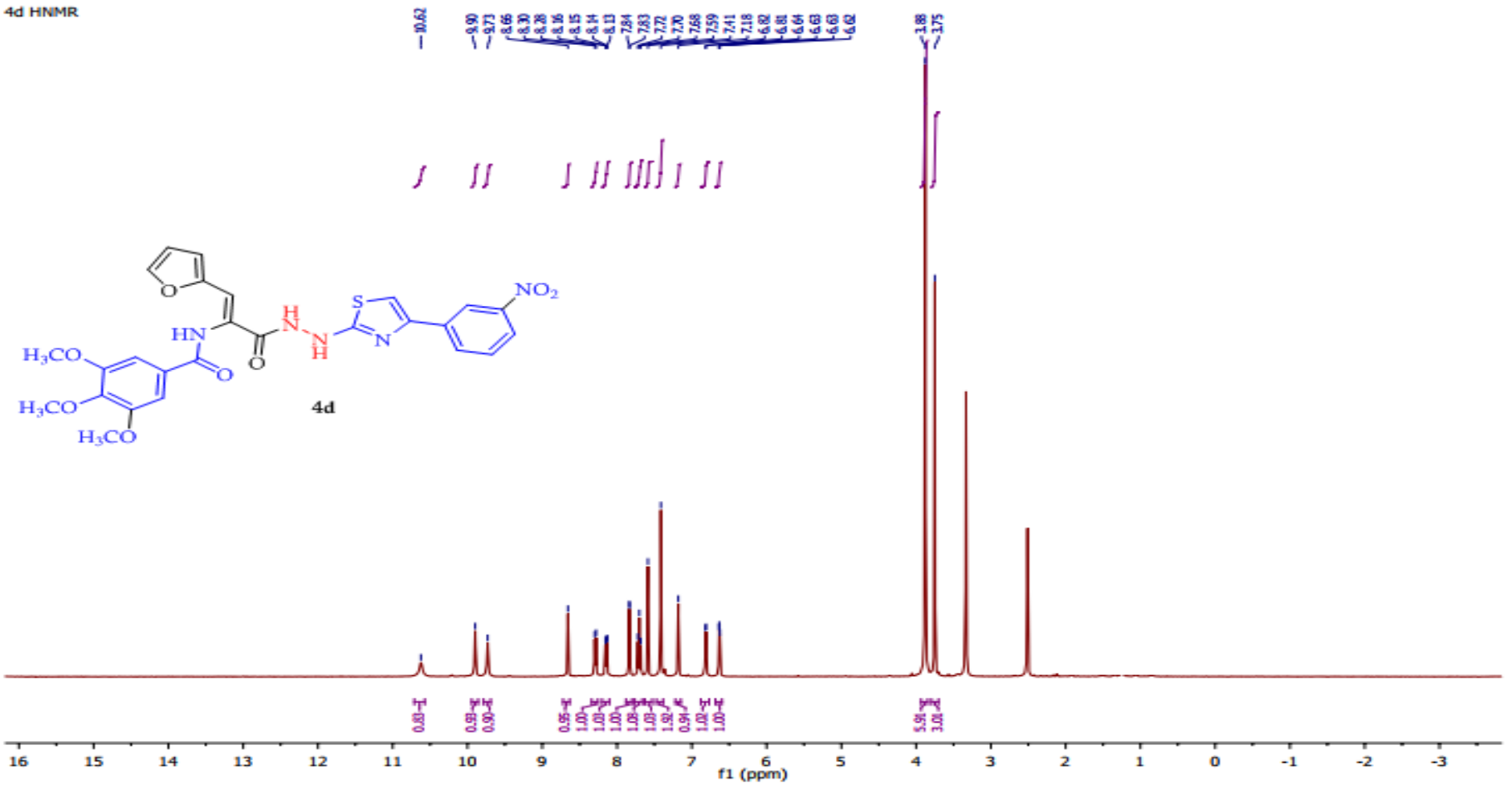

Figure S16: [1]H-NMR spectrum of compound **4d**

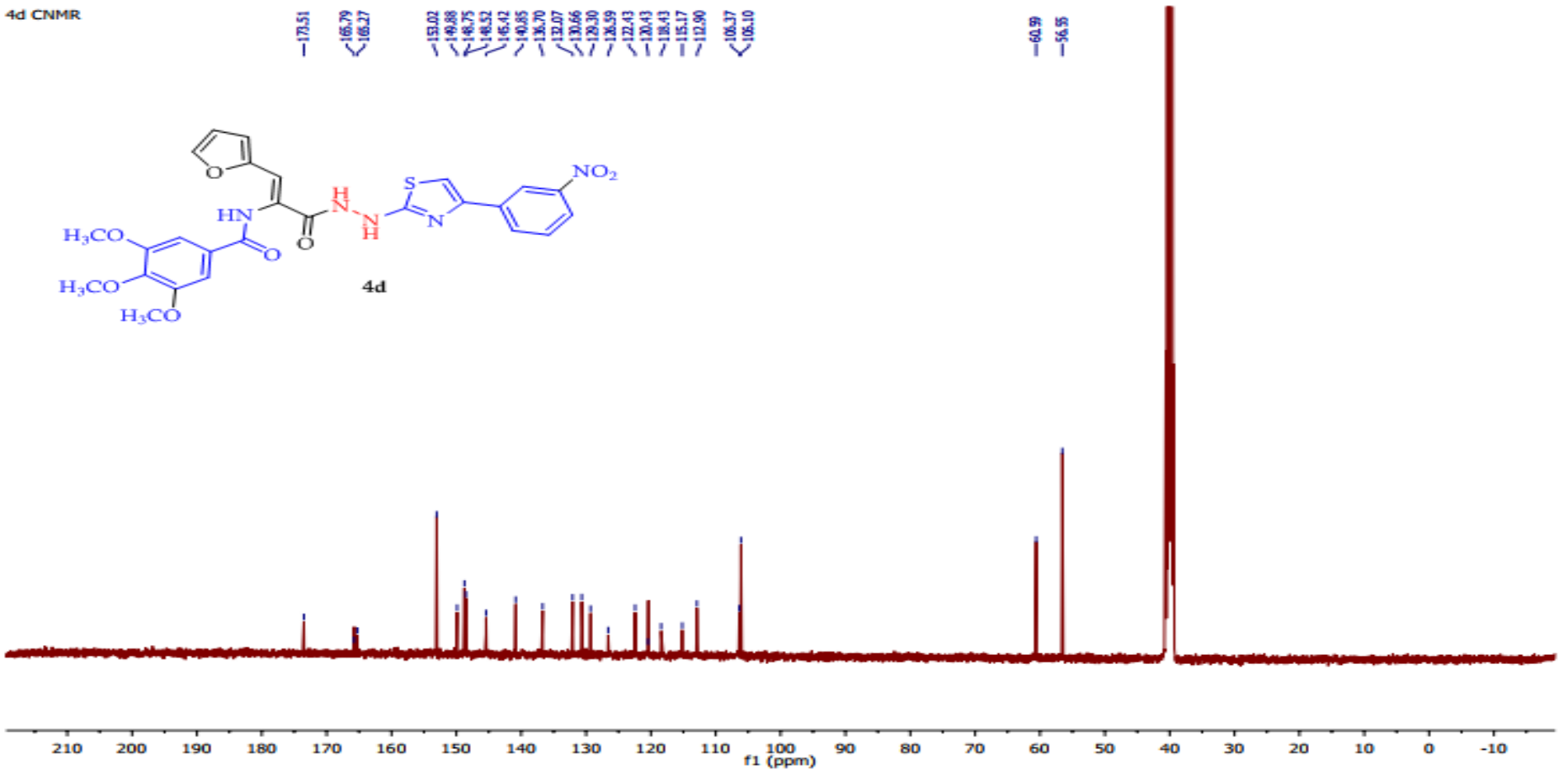

Figure S17: ^{13}C-NMR spectrum of compound **4d**

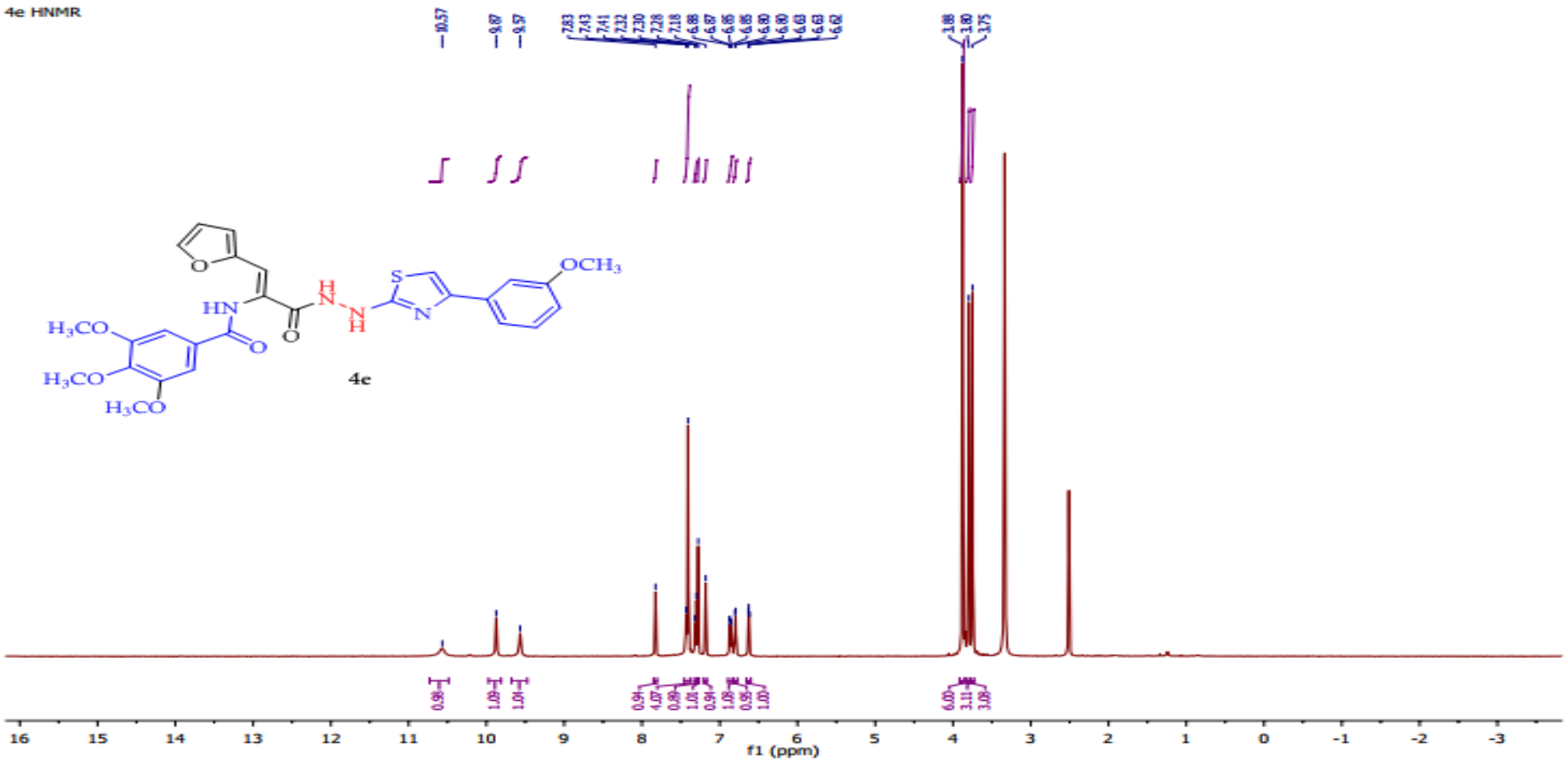

Figure S18: [1]H-NMR spectrum of compound **4e**

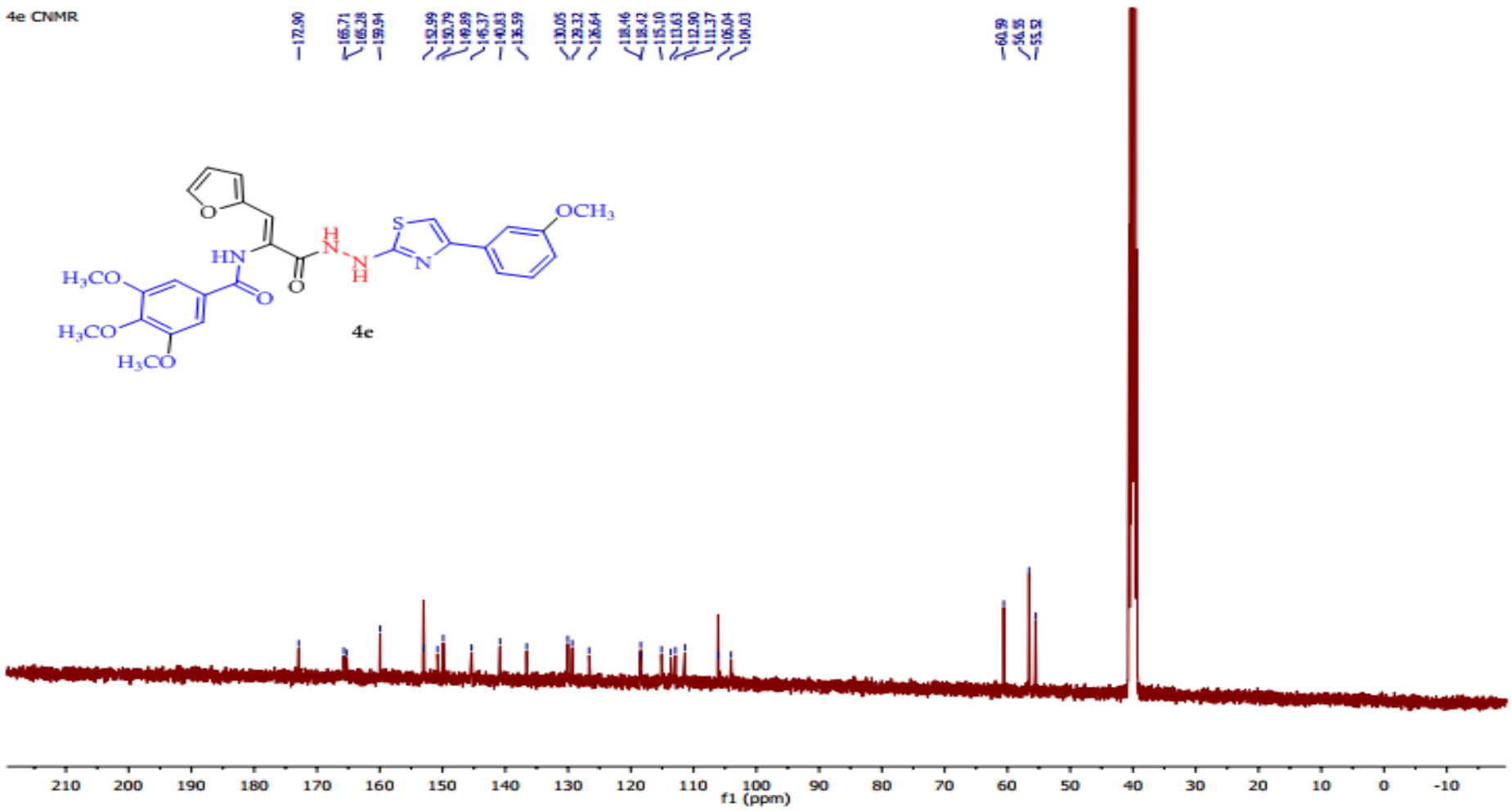

Figure S19: ^{13}C-NMR spectrum of compound **4e**

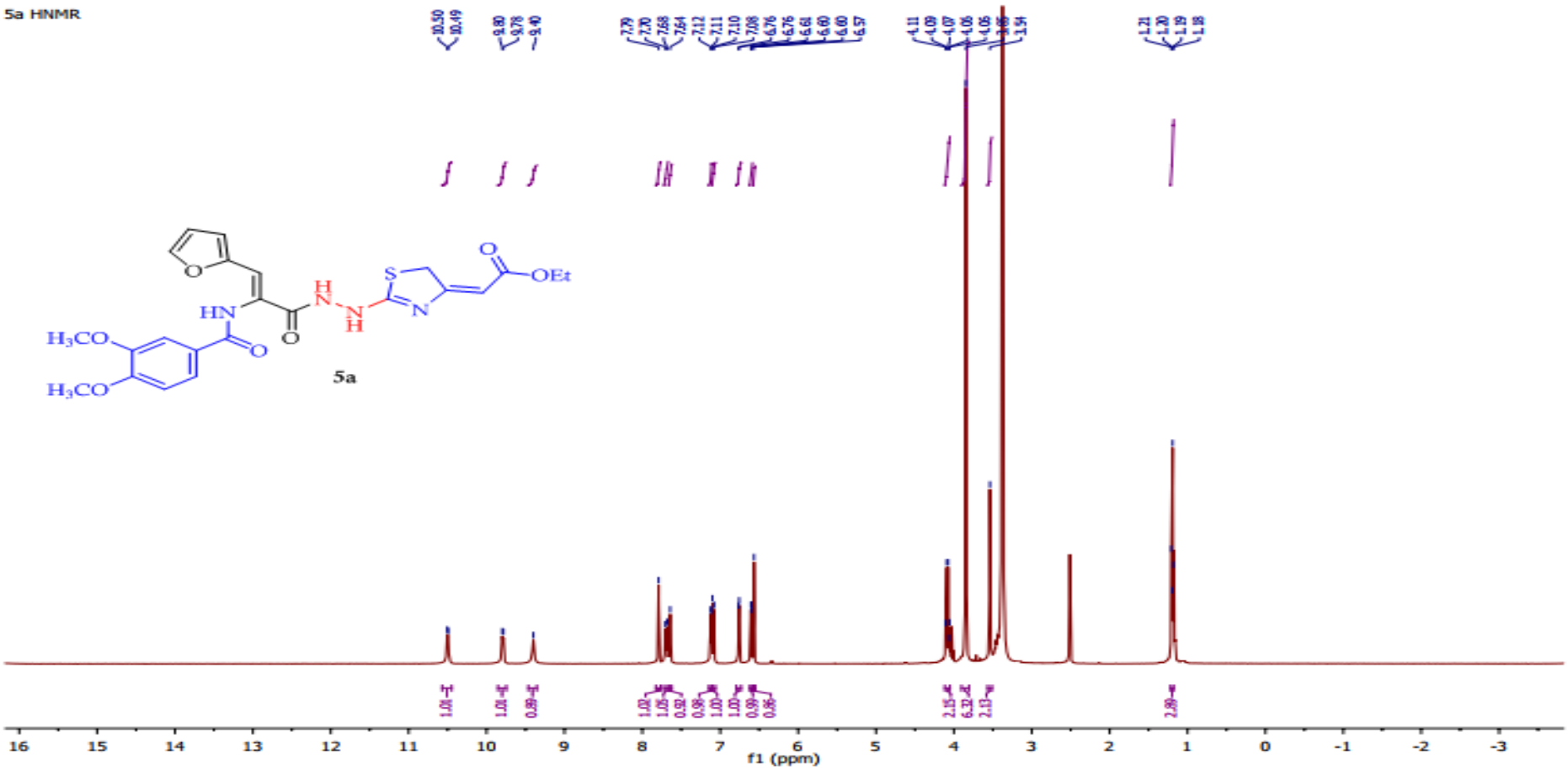

Figure S20: [1]H-NMR spectrum of compound **5a**

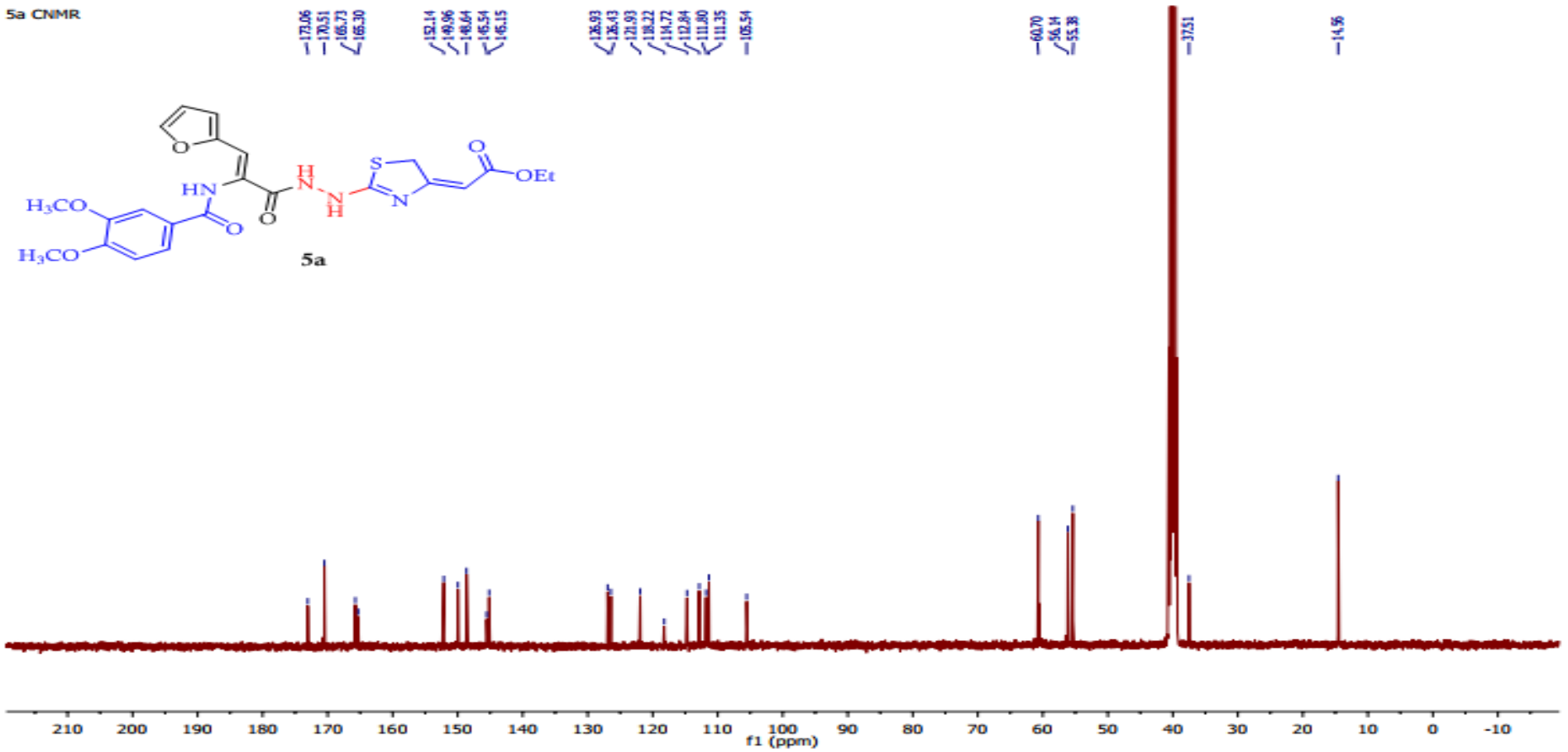

Figure S21: ^{13}C-NMR spectrum of compound **5a**

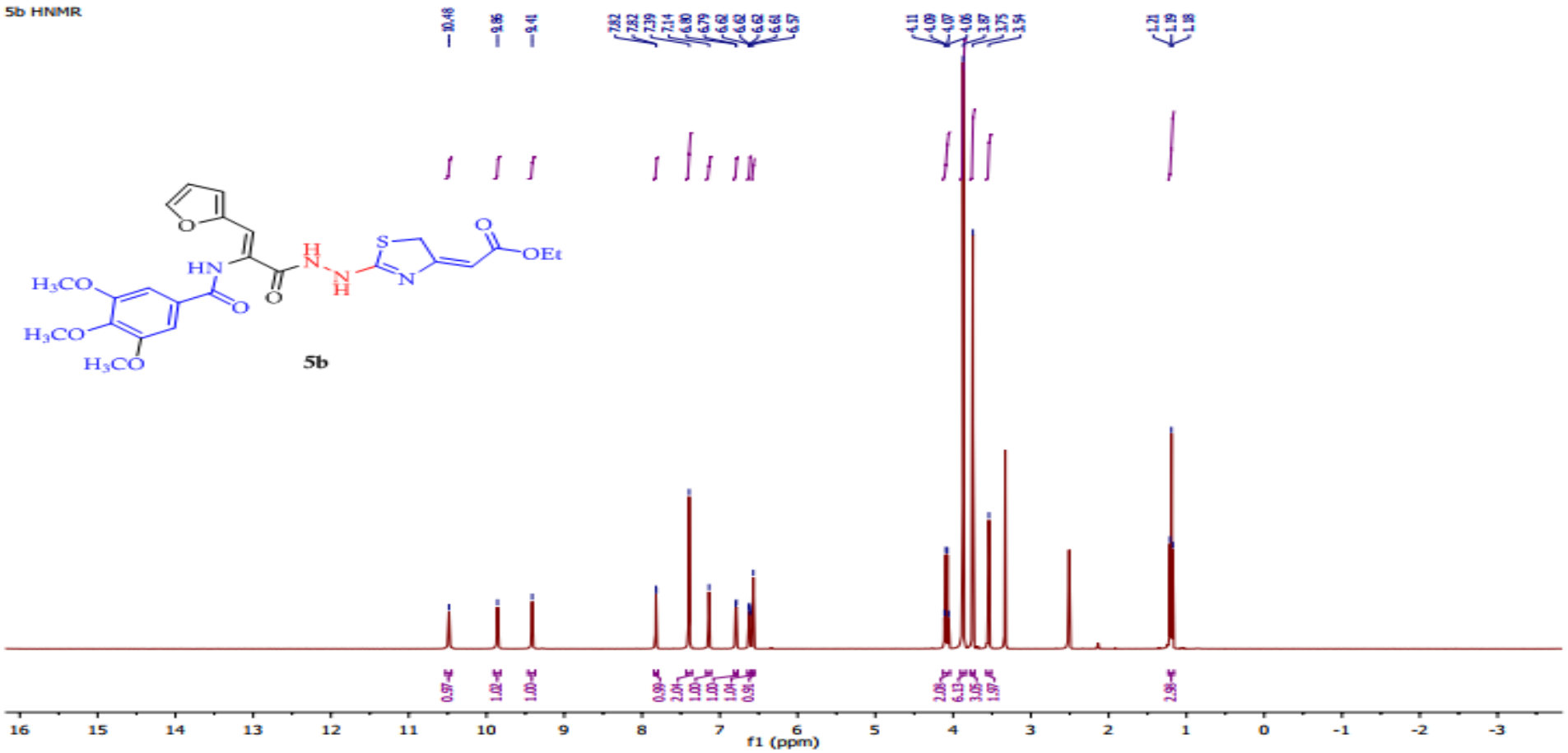

Figure S22: [1]H-NMR spectrum of compound **5b**

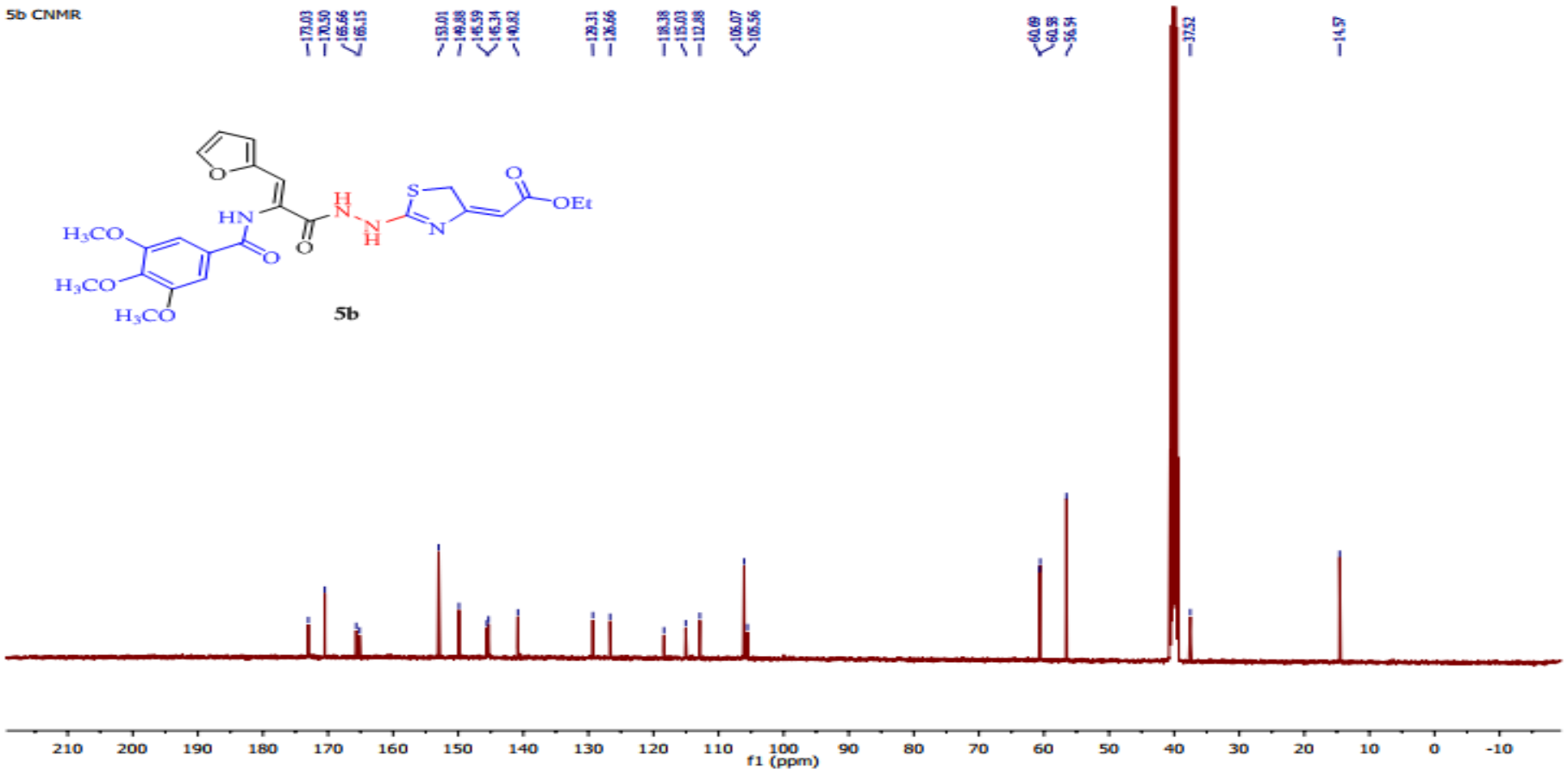

Figure S23: ^{13}C-NMR spectrum of compound **5b**

4.1.1. Chemistry: General

Melting points were determined in open capillaries tube using Electrothermal Digital melting point apparatus and were uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were obtained with a Bruker 400 MHz DRX-Avance NMR spectrometer, peaks positions are given in ppm downfield from tetramethylsilane (TMS) as the internal standard. Elemental analyses were performed on Elementar, Vario El, Microanalytical unit, Cairo, Egypt and were found within ±0.4% of the theoretical values.

4.2. Biological studies

4.2.1. Cytotoxic activity evaluation

To measure the cytotoxic actvity of the newly synthesized thiazolyl derivatives **3a-5b** in breast carcinoma (MDA-MB-231) cell line, cell viability assay was assessed using MTT assay method. Cells at density of 1 x 10^4 were seeded in a 96-well plate at 37 ^{o}C for 24 h under 5% CO_2. After incubation, the cells were treated with different concentrations of the test compounds **3a-5b** and incubated for 24 h, then 20 µl of MTT solution at 5 mg/mL was applied and incubated for 4 h at 37 °C. Dimethyl sulphoxide (DMSO) in volume of 100 µl was added to each well to dissolve the purple formazan that had formed. The color intensity of the formazan product, which represents the growth condition of the cells, is quantified by using an ELISA plate reader (EXL 800, USA) at 570 nm absorbance. The experimental conditions were carried out with at least three replicates, and the experiments were repeated at least three times.

4.2.2. VEGFR-2 inhibitions Assay

Compounds **4b, 4d** and **4d** were evaluated for their VEGFR-2 inhibitory activity according to manufacturer's instructions using VEGFR2 (KDR) Kinase Assay Kit *Catalog #* 40325.

4.2.3. Cell cycle analysis of compound 4d

Cell cycle analysis in MDA-MB-231 cells was investigated using fluorescent Annexin V-FITC/ PI detection kit (*BioVision* EZCell^{TM} Cell Cycle Analysis Kit Catalog #K920) by flow cytometry assay. MDA-MB-231 cells at a density of 2 × 10^5 per well were harvested and washed twice in PBS. After that, the cells were incubated at 37 °C and 5% CO_2. Afterwards, the medium was incubated with the tested compound **4d** at

its IC_{50} (μM) for 48 h, washed twice in PBS, fixed with 70% ethanol, rinsed again with PBS. Afterward, medium was stained with DNA fluorochrome PI for 15 min at 37 °C. The samples were immediately analyzed using Facs Calibur flow cytometer (Becton and Dickinson, Heidelberg, Germany).

4.2.4. Apoptosis assay for compound 4d

Apoptosis in MDA-MB-231 cells was investigated using fluorescent Annexin V-FITC/ PI detection kit (*BioVision* Annexin V-FITC Apoptosis Detection Kit, Catalog #: K101) by flow cytometry assay. MDA-MB-231cells at a density of 2×10^5 per well were treated with compound **4d** at its IC_{50} (μM) for 48 h, then the cells were harvested and stained with Annexin V-FITC/ PI dye for 15 min in the dark at 37 °C. The samples were immediately analyzed using *FACS Calibur* flow cytometer (Becton and Dickinson, Heidelberg, Germany).

4.2.5. Measurement of mitochondrial membrane potential (MMP) for compound 9

MMP was measured by FACS analysis using abcam ab113852TMRE Mitochondrial Membrane Potential Assay Kit with DiOC2(3) staining using MitoProbe™ DiOC2(3) Assay Kit for Flow Cytometry (M34150). After treatment with compound **4d** at its IC_{50} concentration, cells were stained with DiOC2(3) dye for 30 min in the incubator, then harvested and washed with PBS. DiOC2(3)-stained cells were represented with Annexin binding buffer and incubated at 37 ^oC for 15 min. The samples were immediately analyzed using by cell Quest software.

4.2.6. Effect on p53

p53 activity in MDA-MB-231 cells were detected in the presence of compound **4d** at its IC_{50} concentration (μM). The level of the tumor suppressor gene p53 was assessed using p53 ELISA kit. The procedure of the used kits was done according to the manufacturer's instructions. Briefly, Cell lysates were prepared from control and MDA-MB-231 cells (2.5×10^5/mL) treated with IC_{50} concentration of compound **4d**. Then equal amounts of cell lysates were loaded and probed with specific antibodies. The samples were measured at 450 nm in ROBONEK P2000 ELISA reader. Analysis was confirmed with three different sets of extracts. All experiments were done in triplicates.

4.2.7. Molecular docking study

Molecular docking study was performed using MOE software program (MOE 2015.10). The VEGFR-2 crystal structure (PDB code: 4ASD) was obtained from protein data bank. All molecular docking calculations and docking studies into the active site of 4ASD of compound **4d** were carried out using Molecular Operating Environment (MOE 2015.10) software. Energy minimizations were performed with a root mean standard deviation (RMSD) gradient of 0.05 kcal/mol^{-1} Å^{-1} with an MMFF94X forced field and the partial charges were calculated. The 3D structures of the ligands were built using MOE and subjected to the following procedure: (i) 3D protonation of the structure (ii) hide of the hydrogen (iii) selecting the least energetic conformer. Also, the targets 4ASD were prepared for docking studies as follows; (i) water molecules were discarded; (ii) hydrogen atoms were added to the enzyme (iii) MOE alpha site finder was used for the active site search, (iv) docking of the targeted ligands.