Supporting Information to

Design and synthesis of novel pyridine-based compounds as potential PIM-1 kinase inhibitors, apoptosis, and autophagy inducers targeting MCF-7 cell lines: *In vitro* and *In vivo* studies.

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NMR Spectra

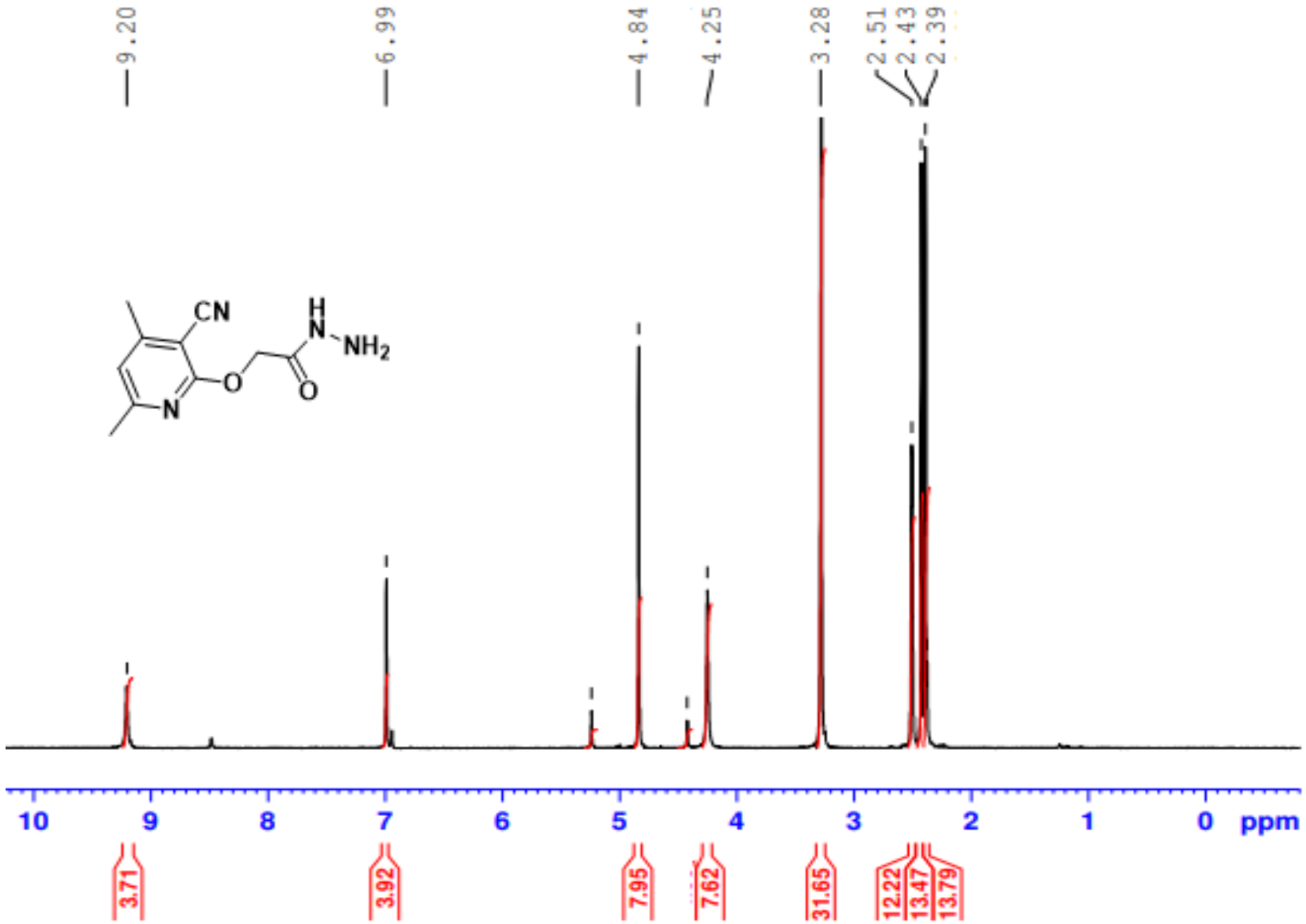


Fig.S1 : ^{1}H-NMR (DMSO-*d*$_6$)spectrum of compound 1

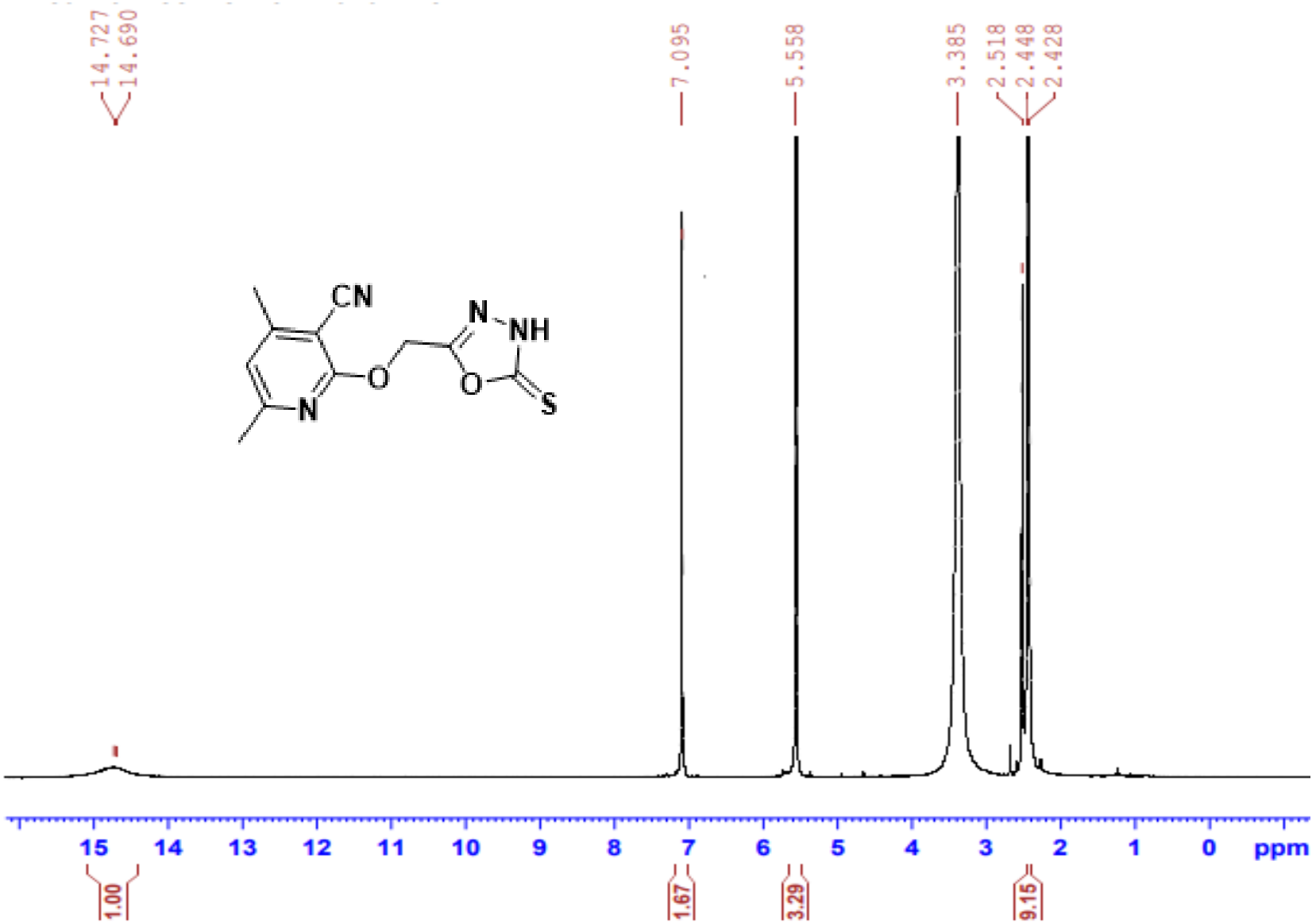


Fig.S2 : ^{1}H-NMR (DMSO-d$_{6}$)spectrum of compound 2

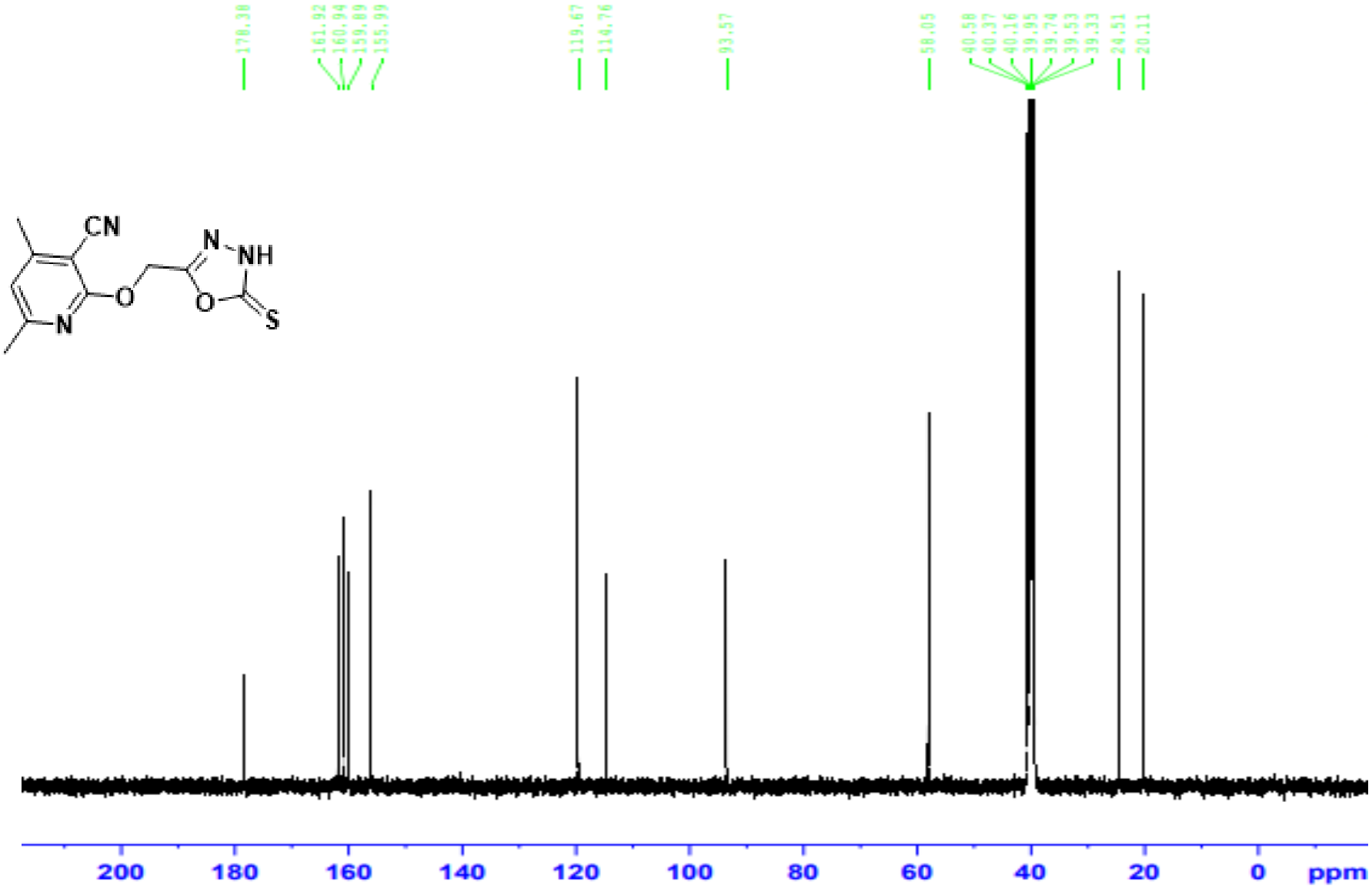


Fig.S3 : ^{13}C-NMR (DMSO-d$_6$)spectrum of compound 2

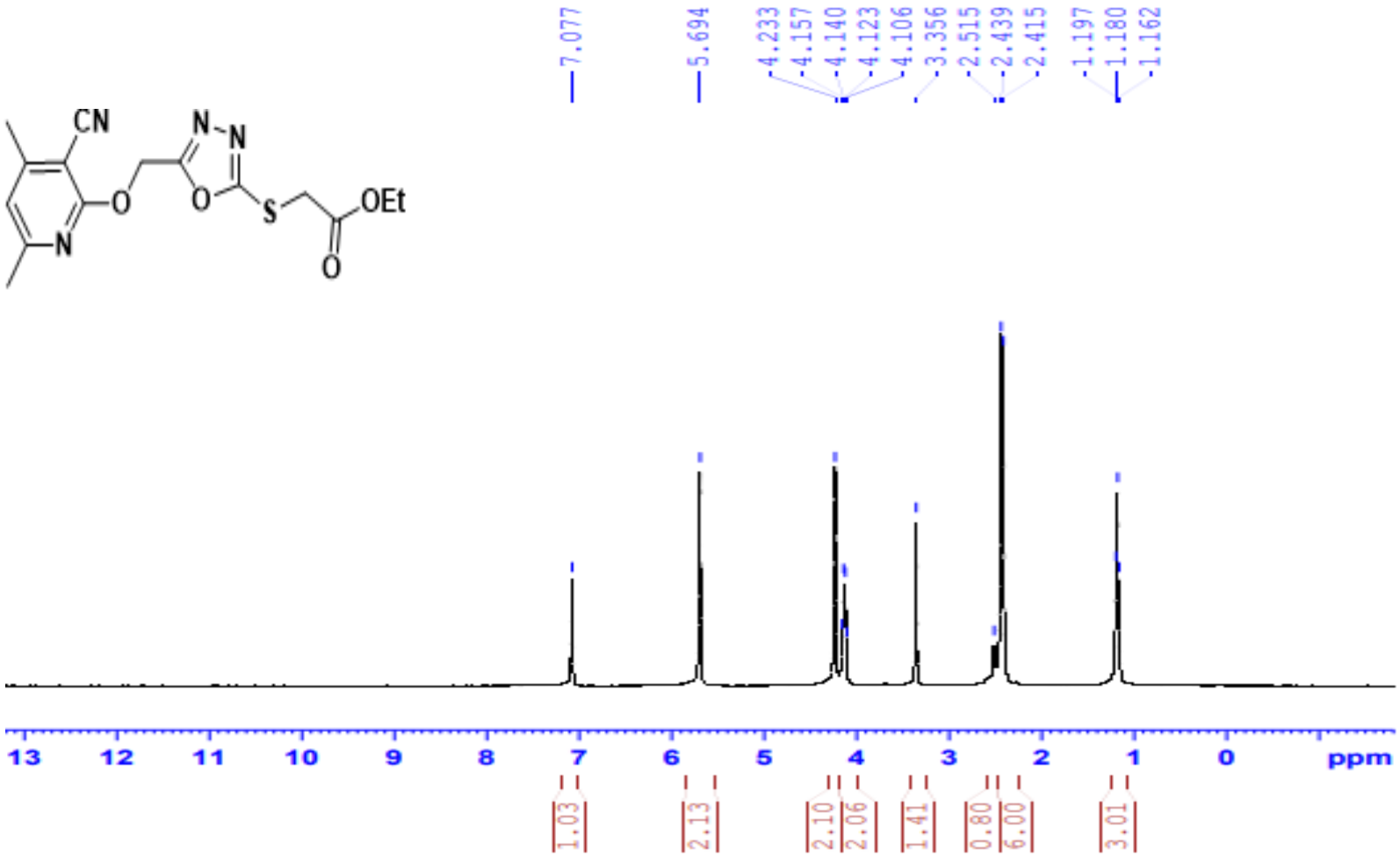
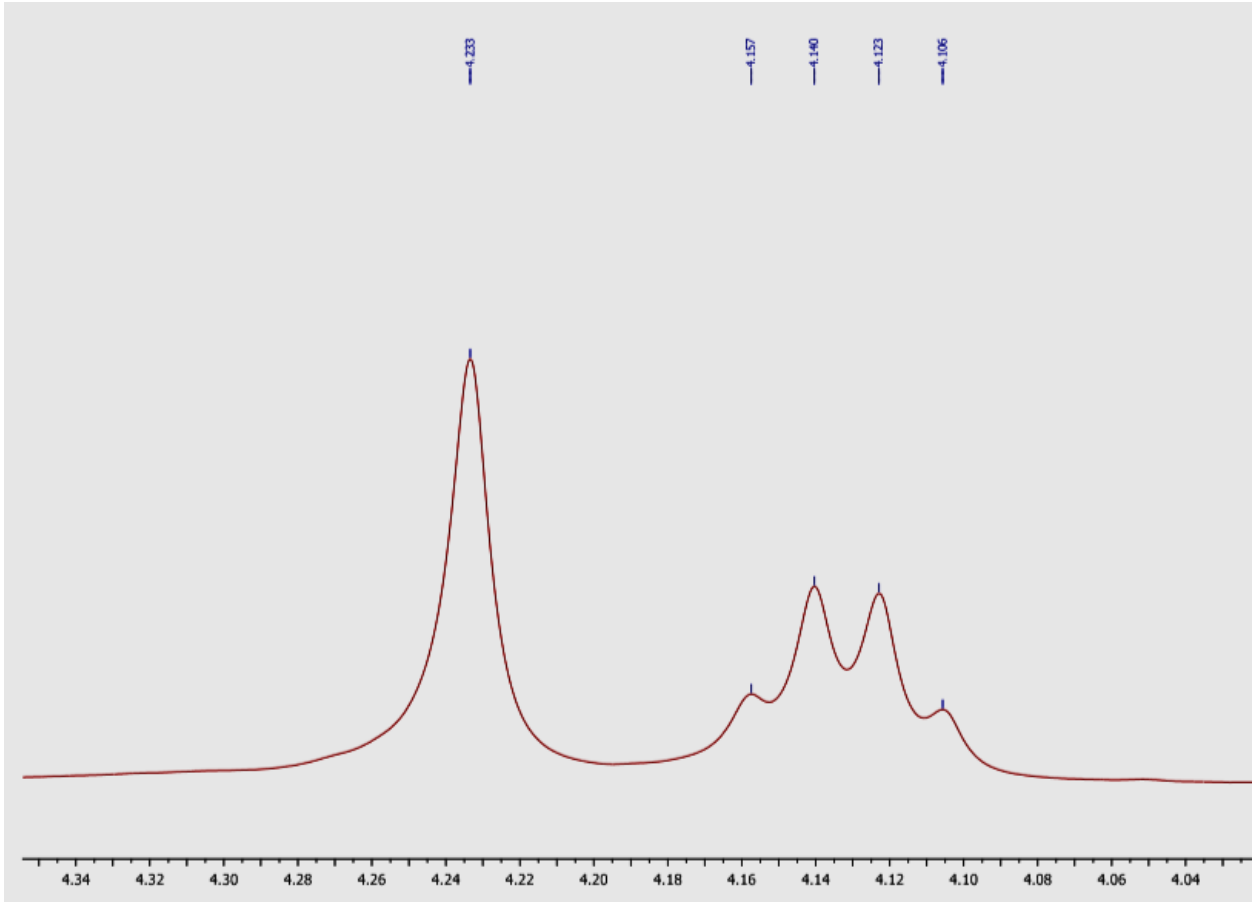


Fig.S4 : ^{1}H-NMR (DMSO-d$_6$)spectrum of compound 3



zoomed image

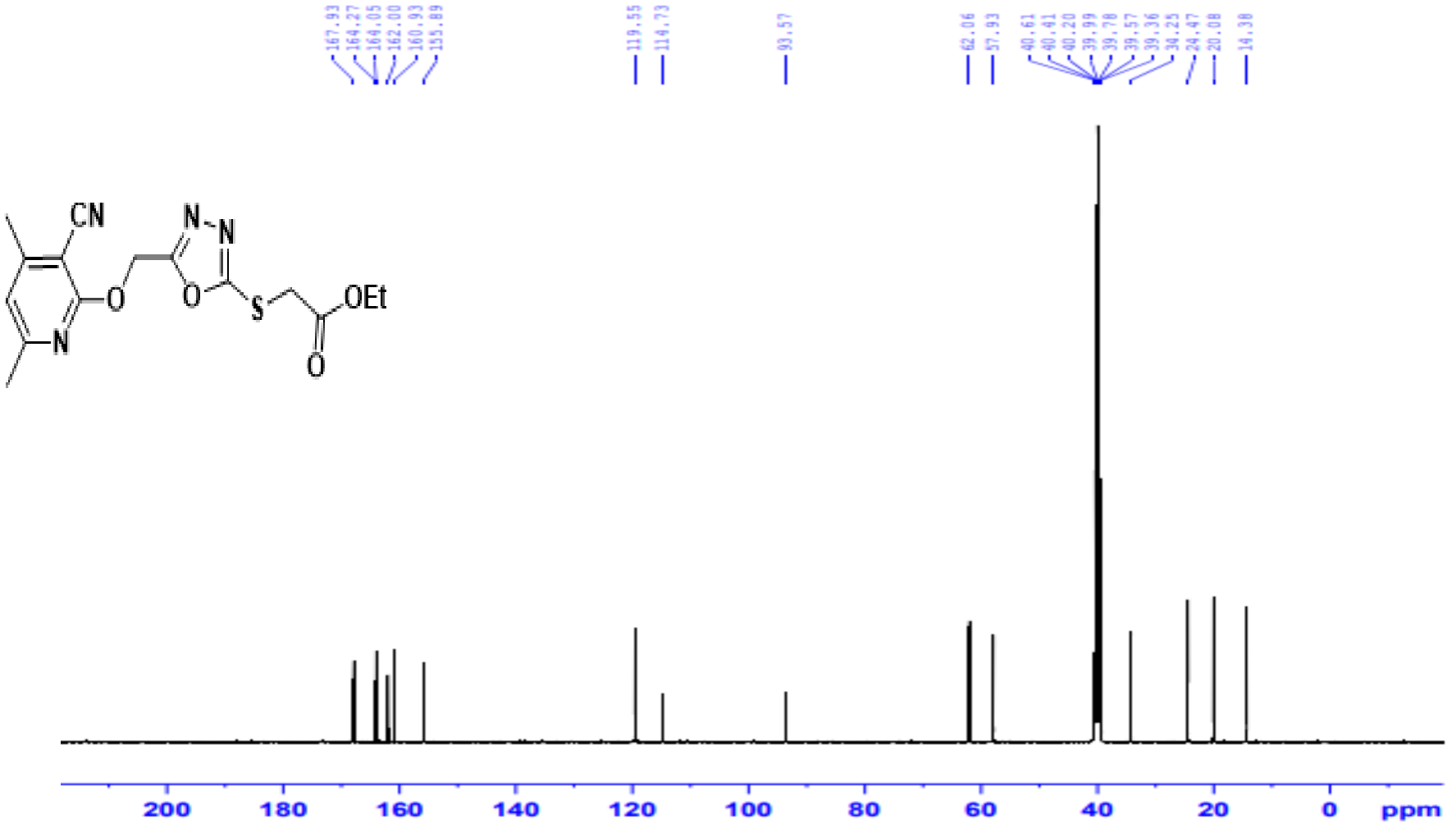


Fig.S5 : ^{13}C-NMR (DMSO-d$_6$)spectrum of compound 3

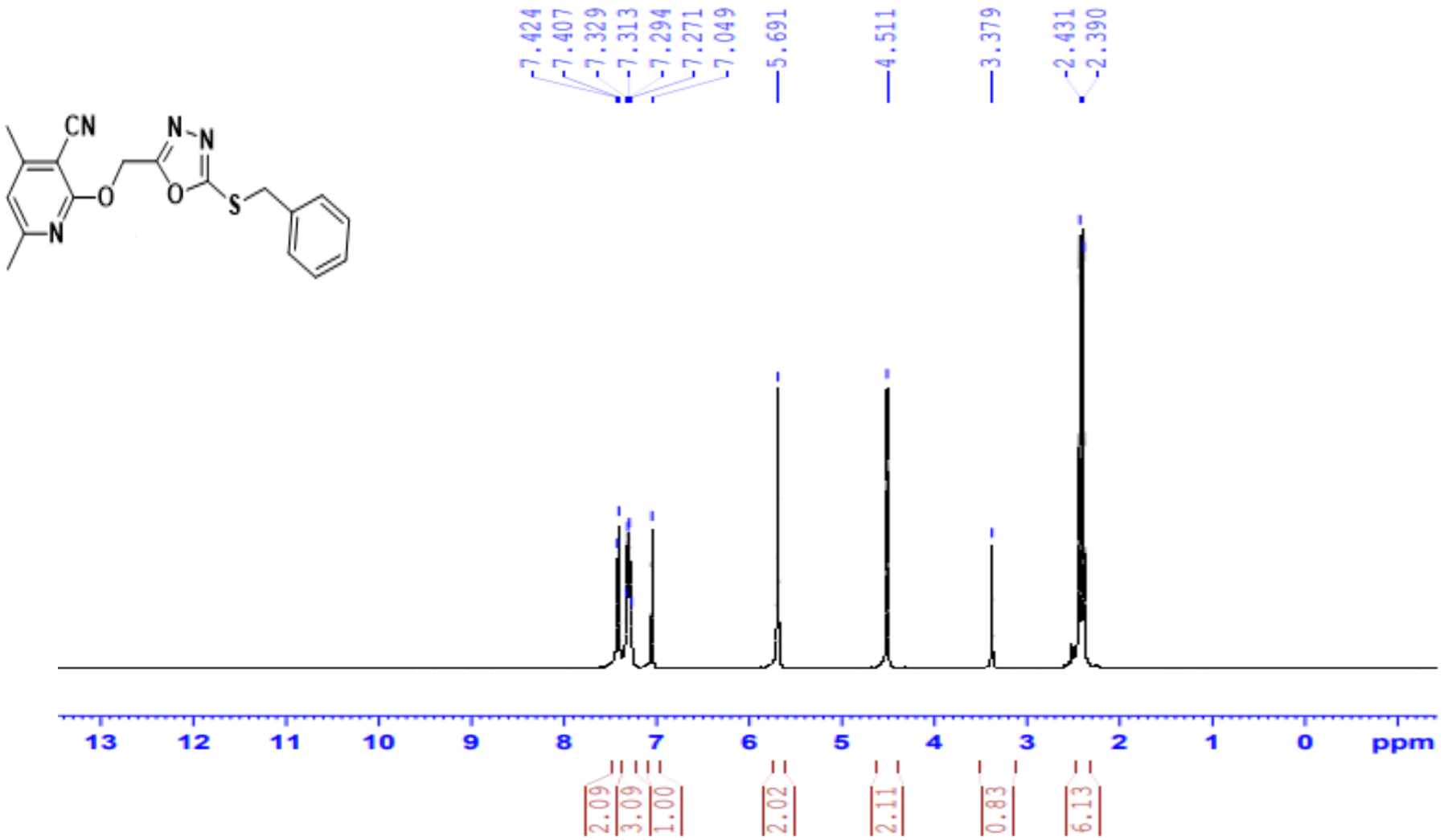
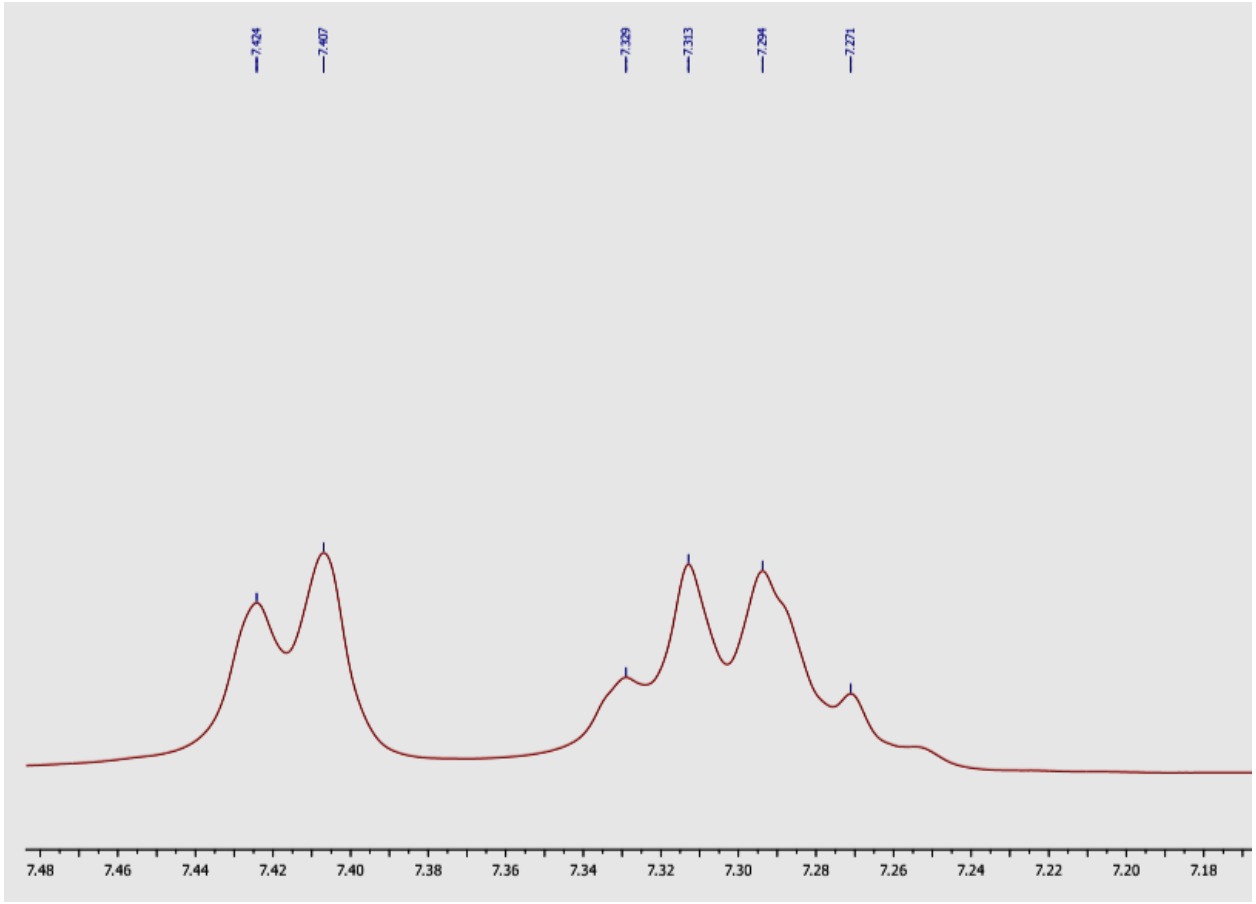


Fig.S6 : ^{1}H-NMR (DMSO-d$_6$)spectrum of compound 4



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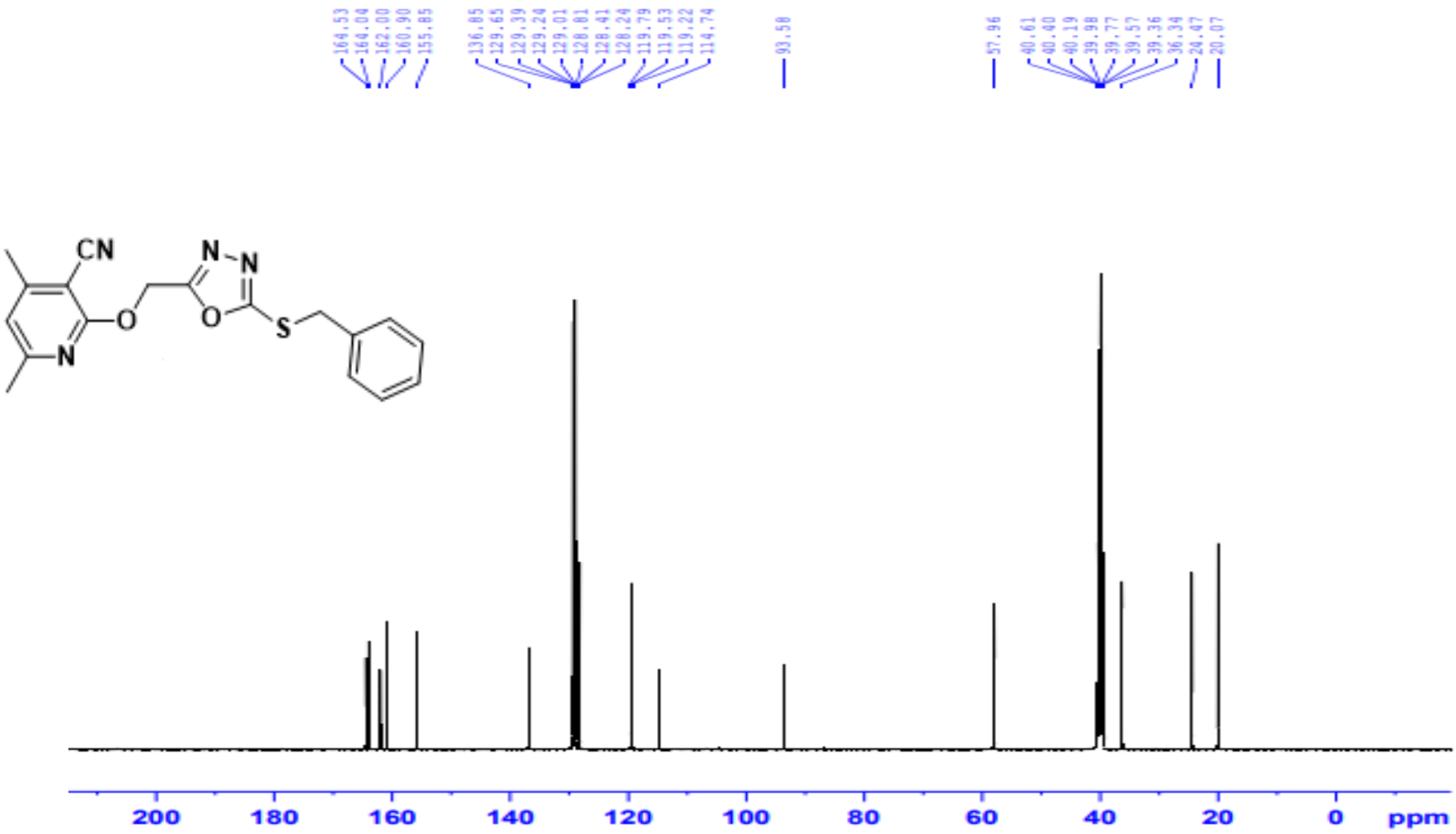


Fig.S7 : ^{13}C-NMR (DMSO-d$_6$)spectrum of compound 4

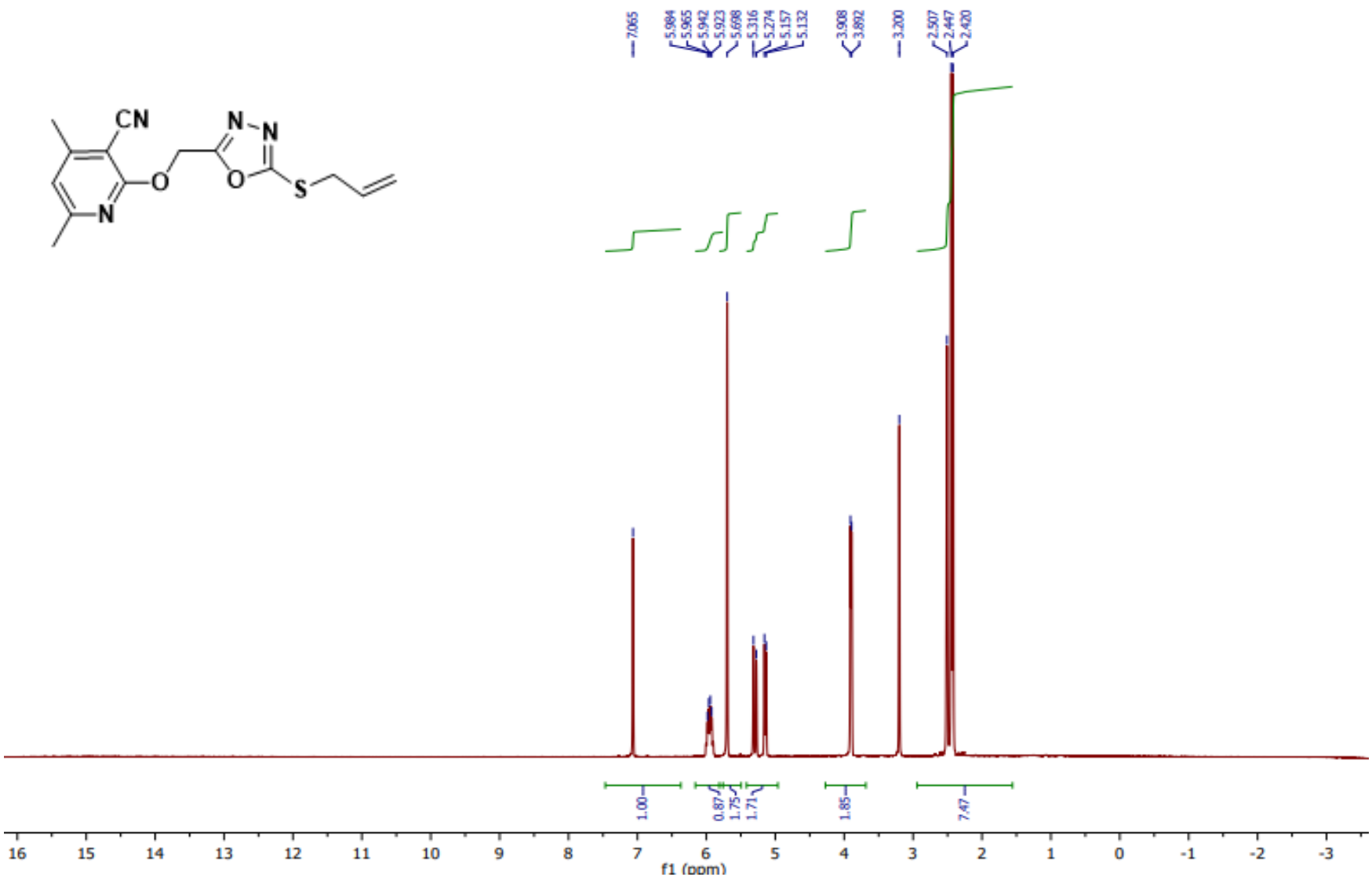
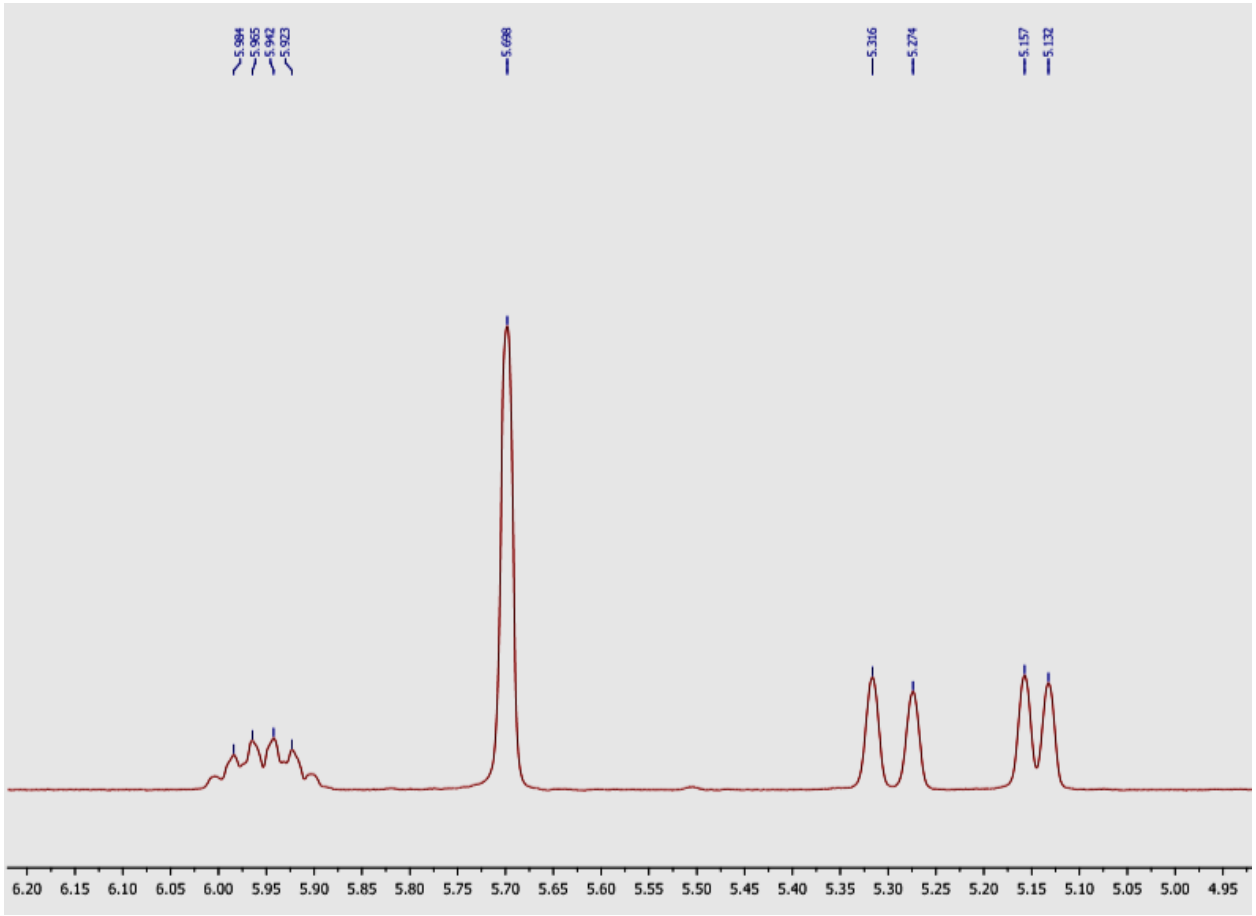


Fig.S8 : ^{1}H-NMR (DMSO-d$_6$)spectrum of compound 5



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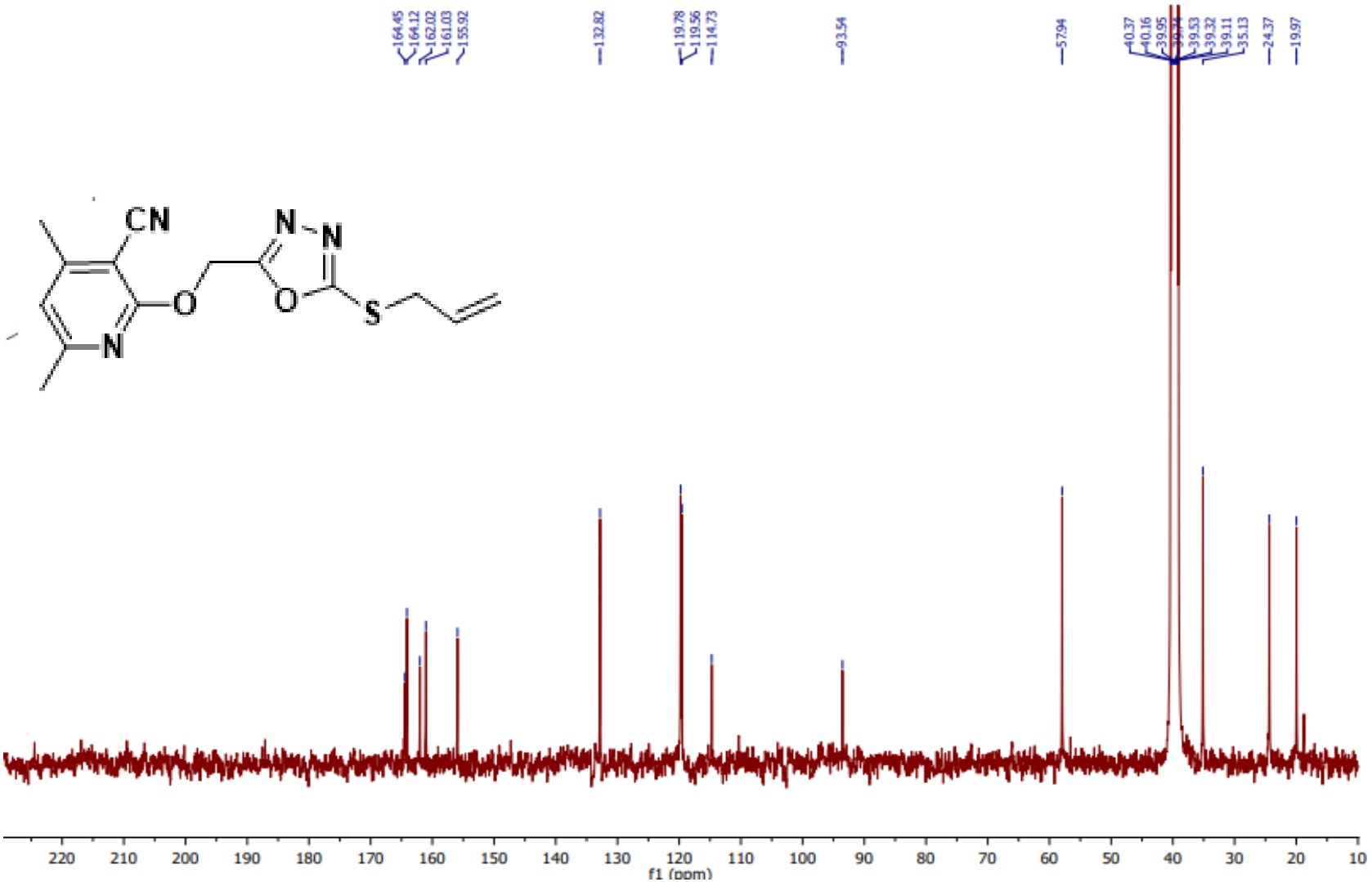


Fig.S9 : ^{13}C-NMR (DMSO-d$_6$)spectrum of compound 5

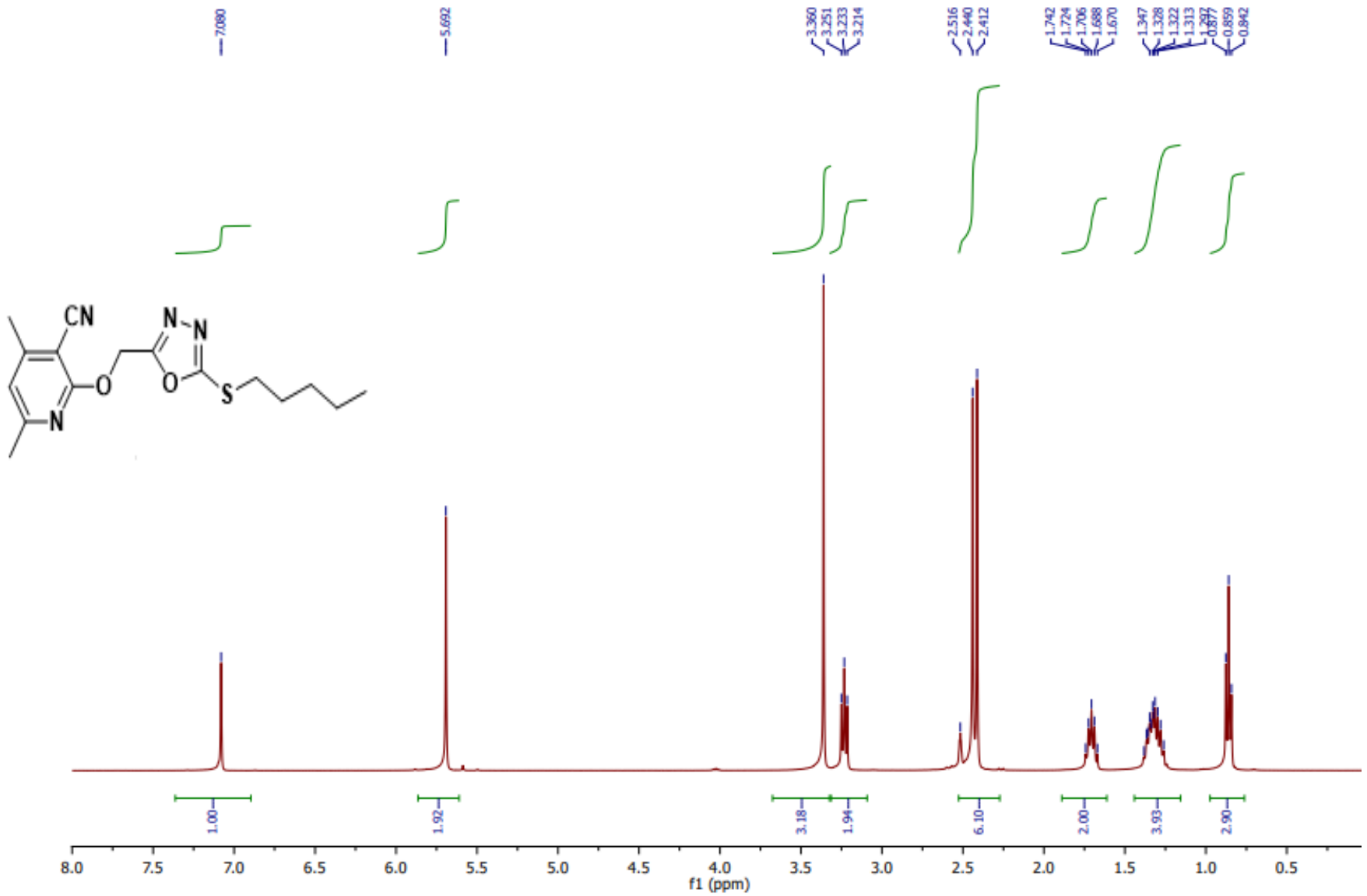
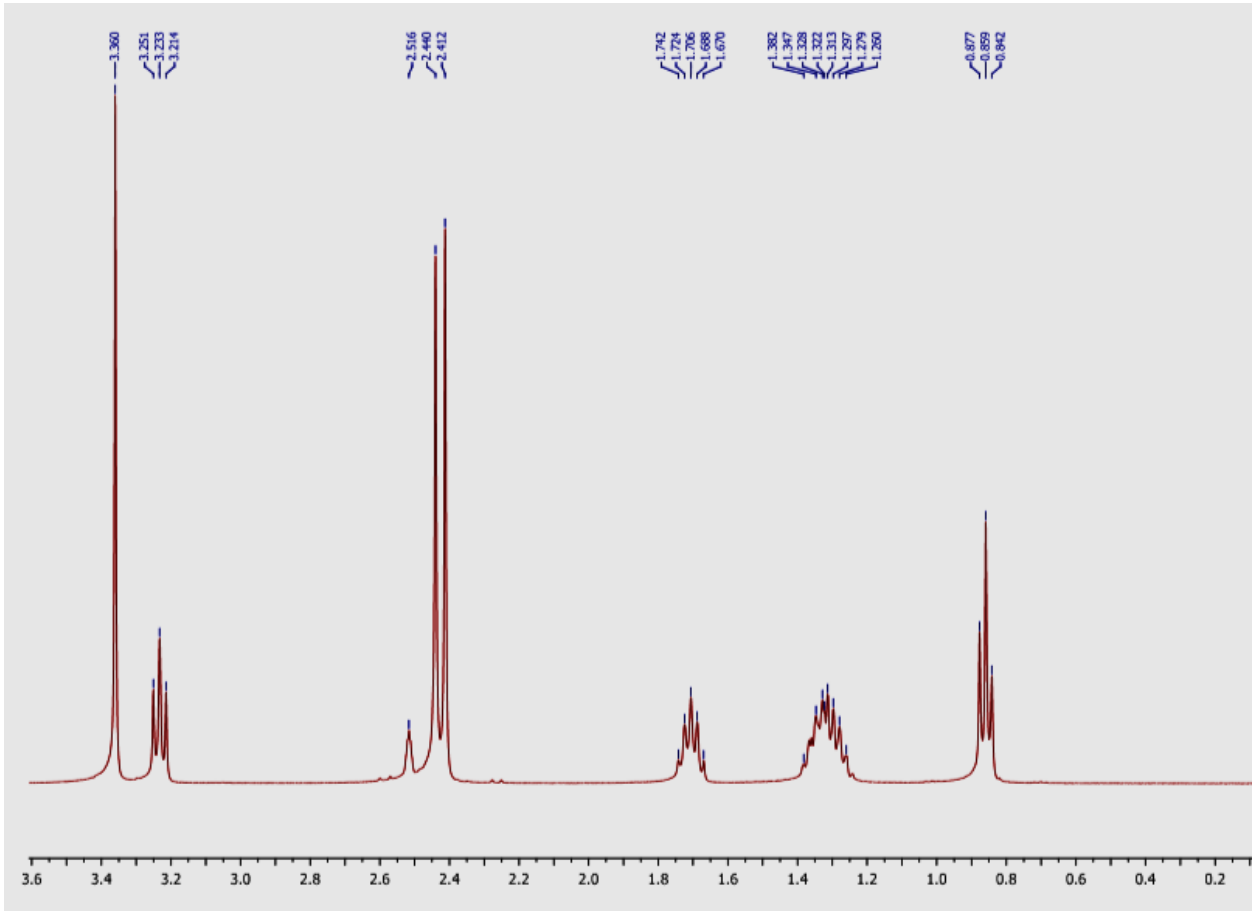


Fig.S10 : ^{1}H-NMR (DMSO-d$_6$)spectrum of compound 6



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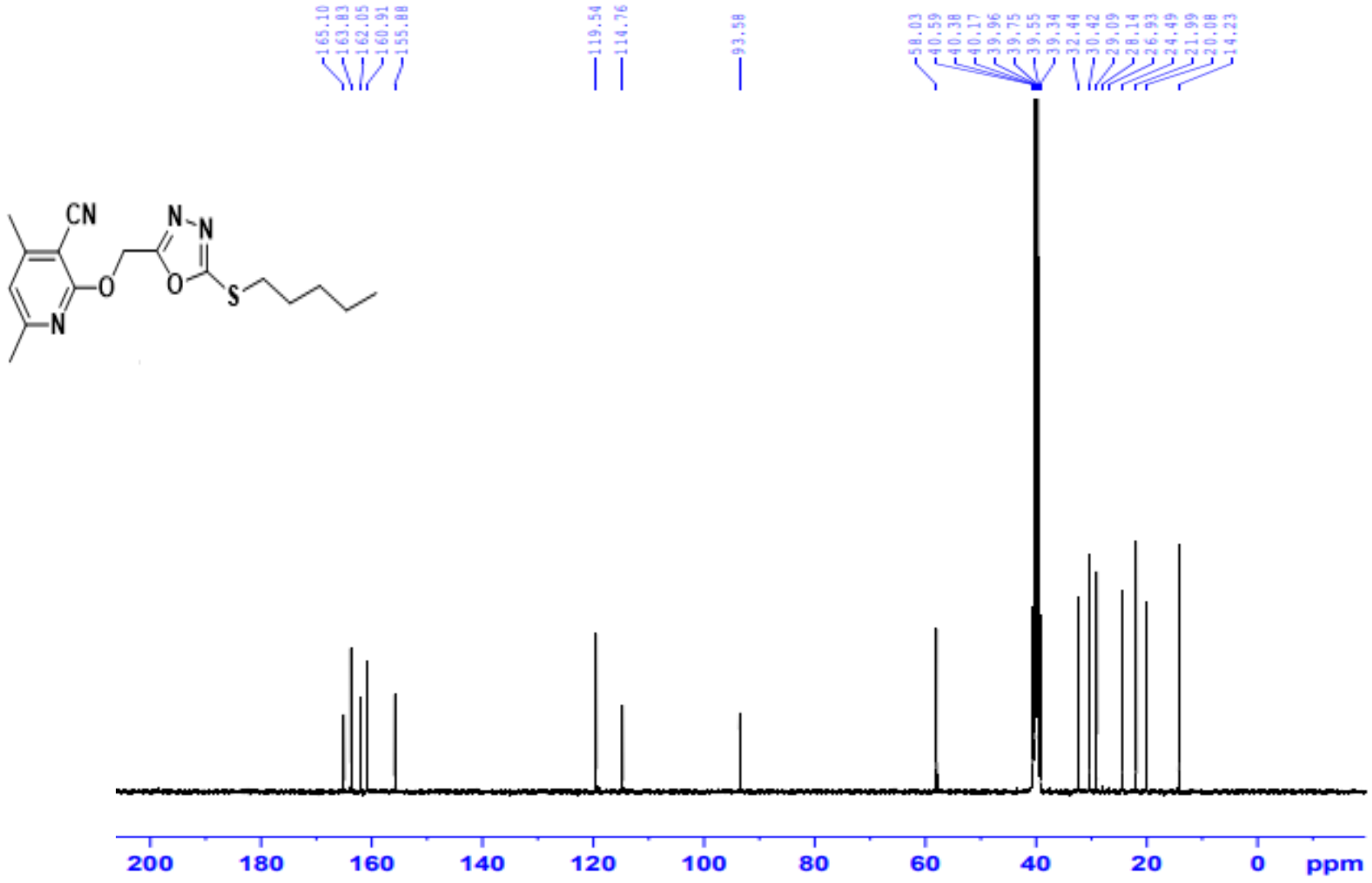


Fig.S11 : ^{13}C-NMR (DMSO-d$_6$)spectrum of compound 6

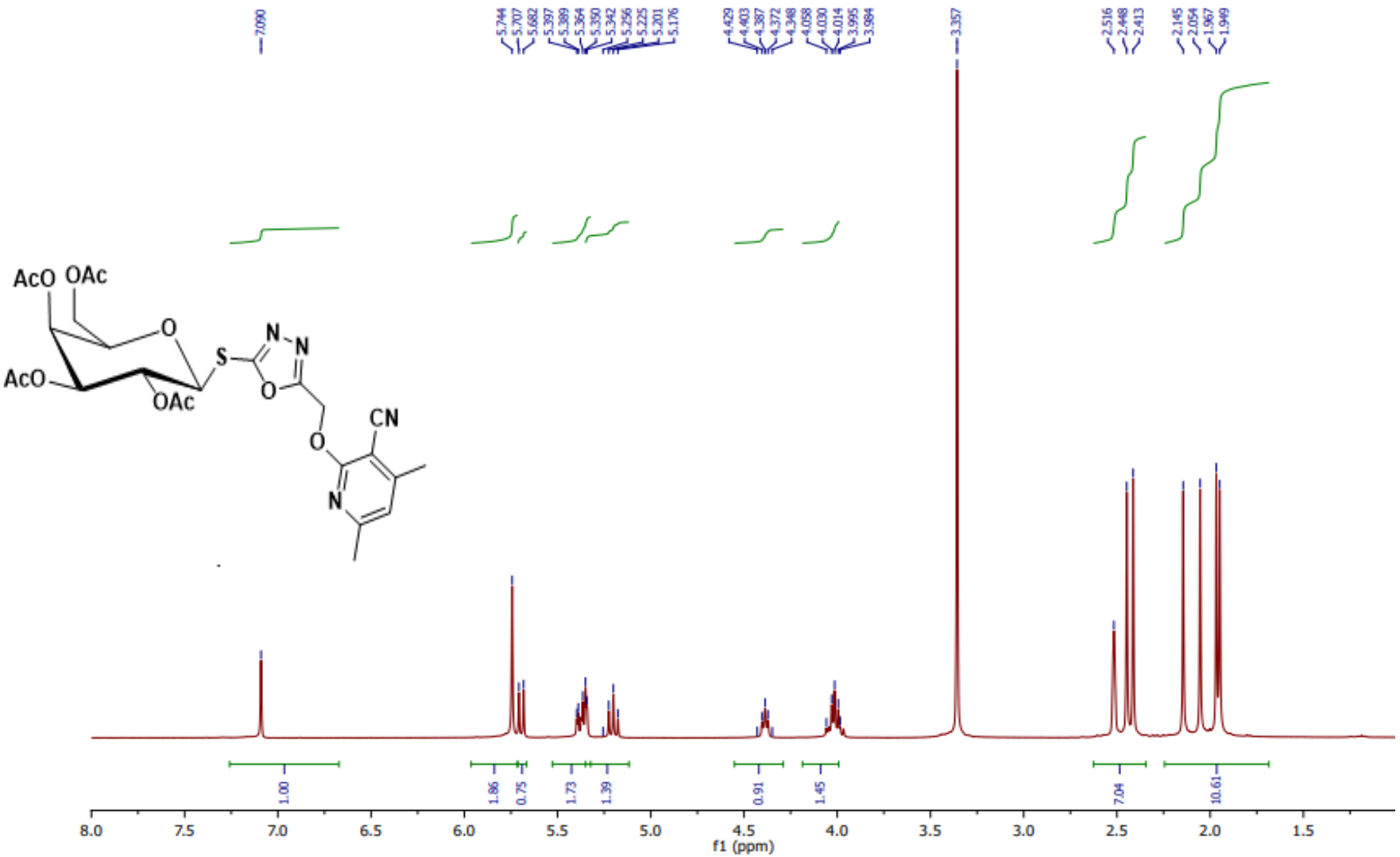
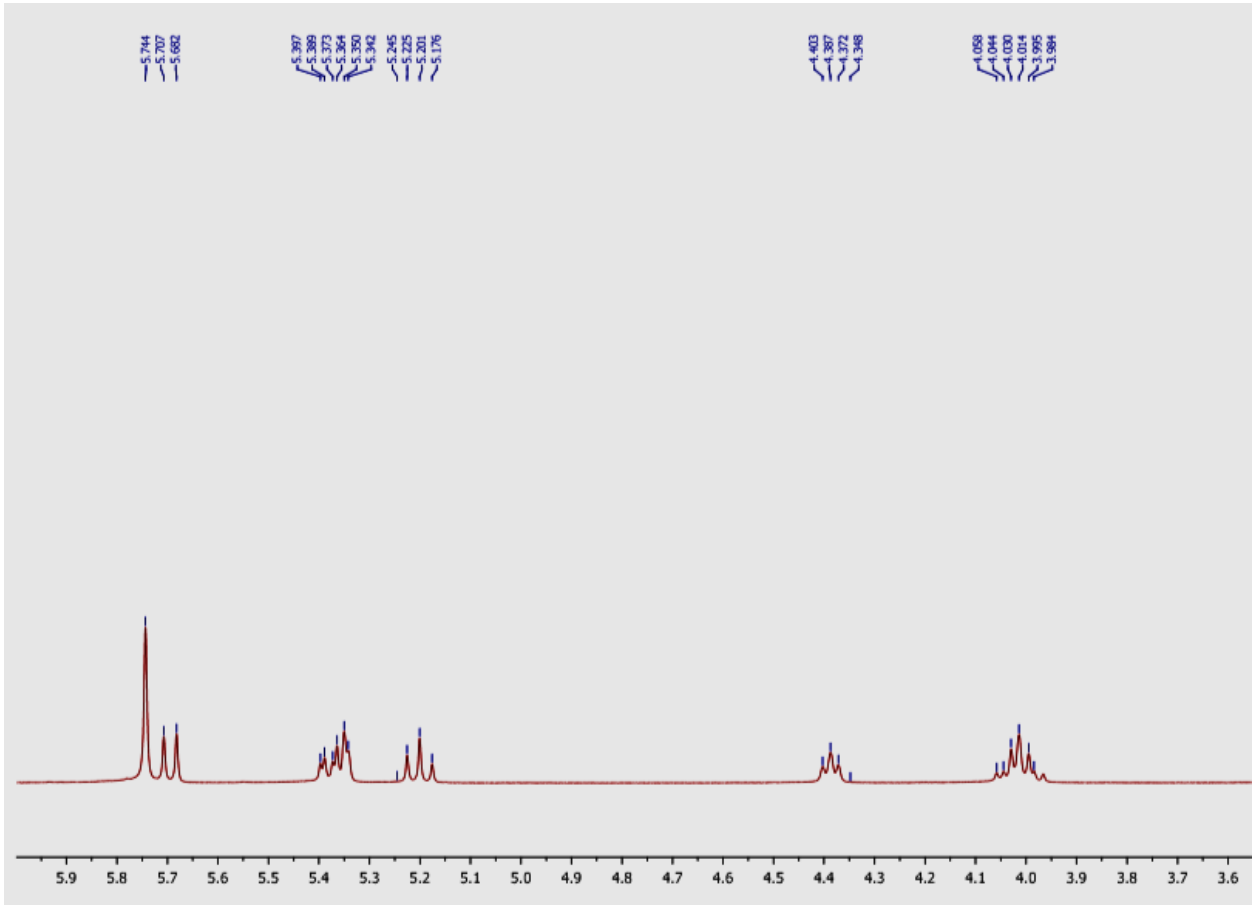


Fig.S12 : ^{1}H-NMR (DMSO-d$_6$)spectrum of compound 8



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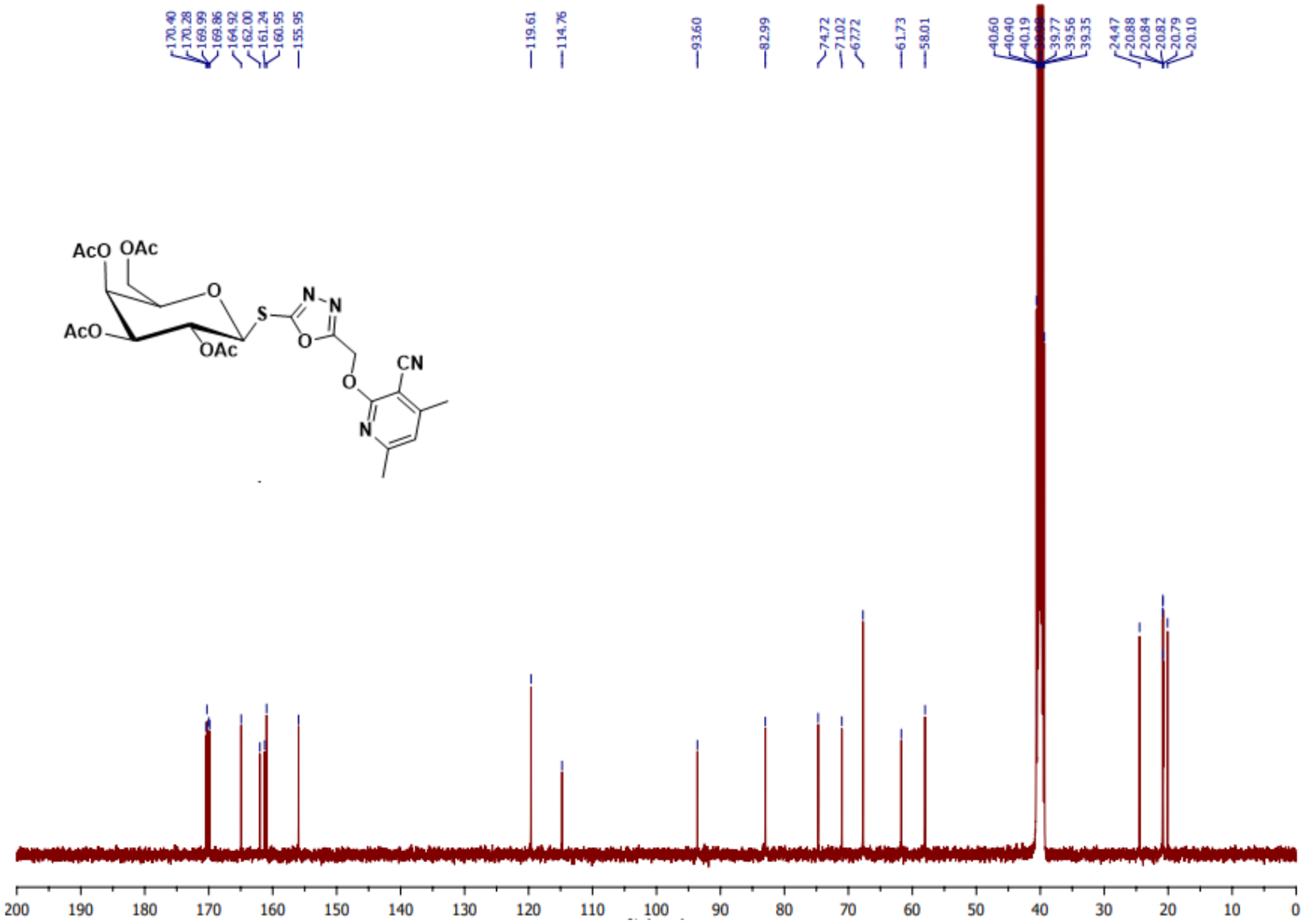


Fig.S13 : ^{13}C-NMR (DMSO-d$_6$)spectrum of compound 8

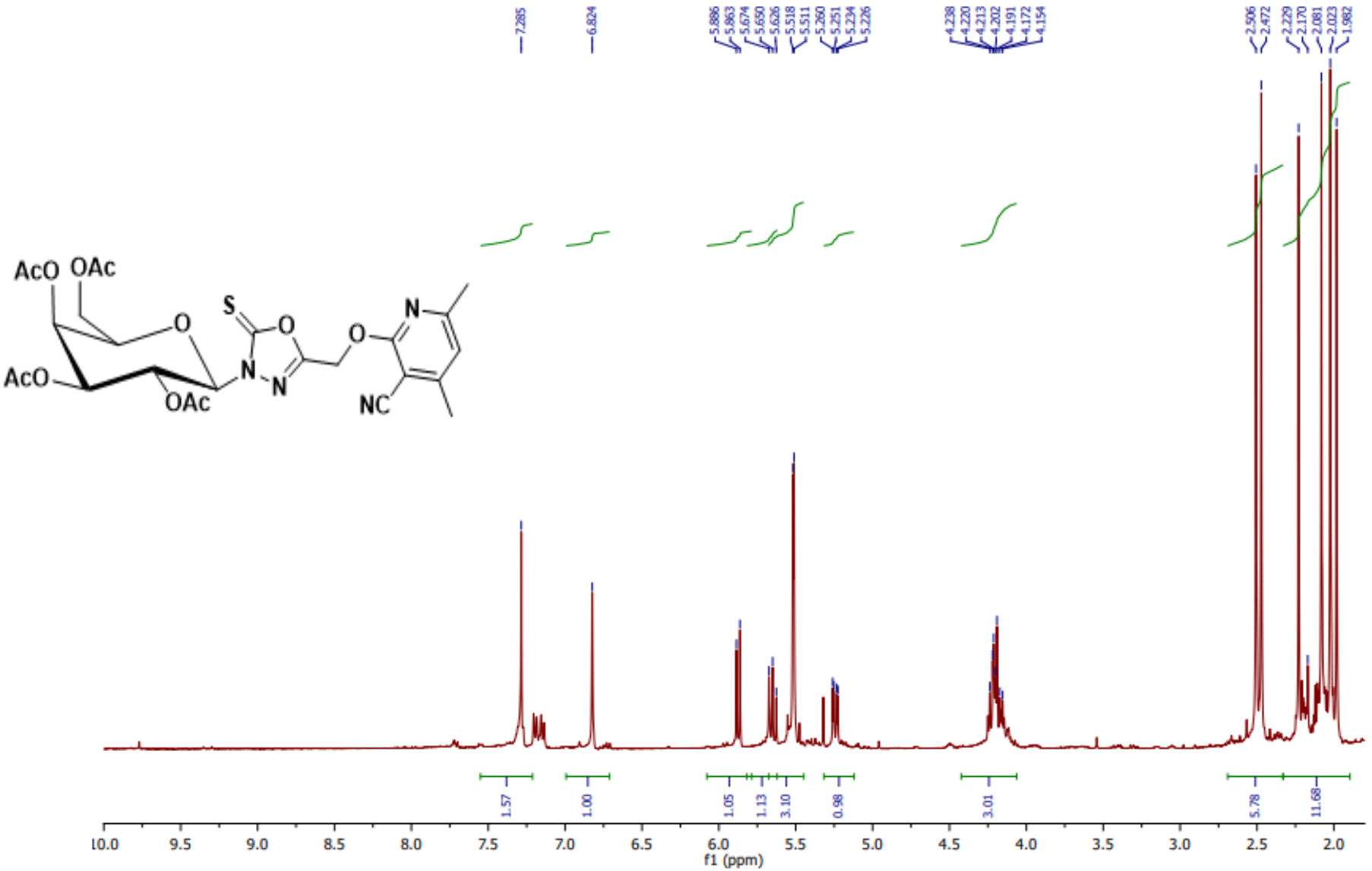
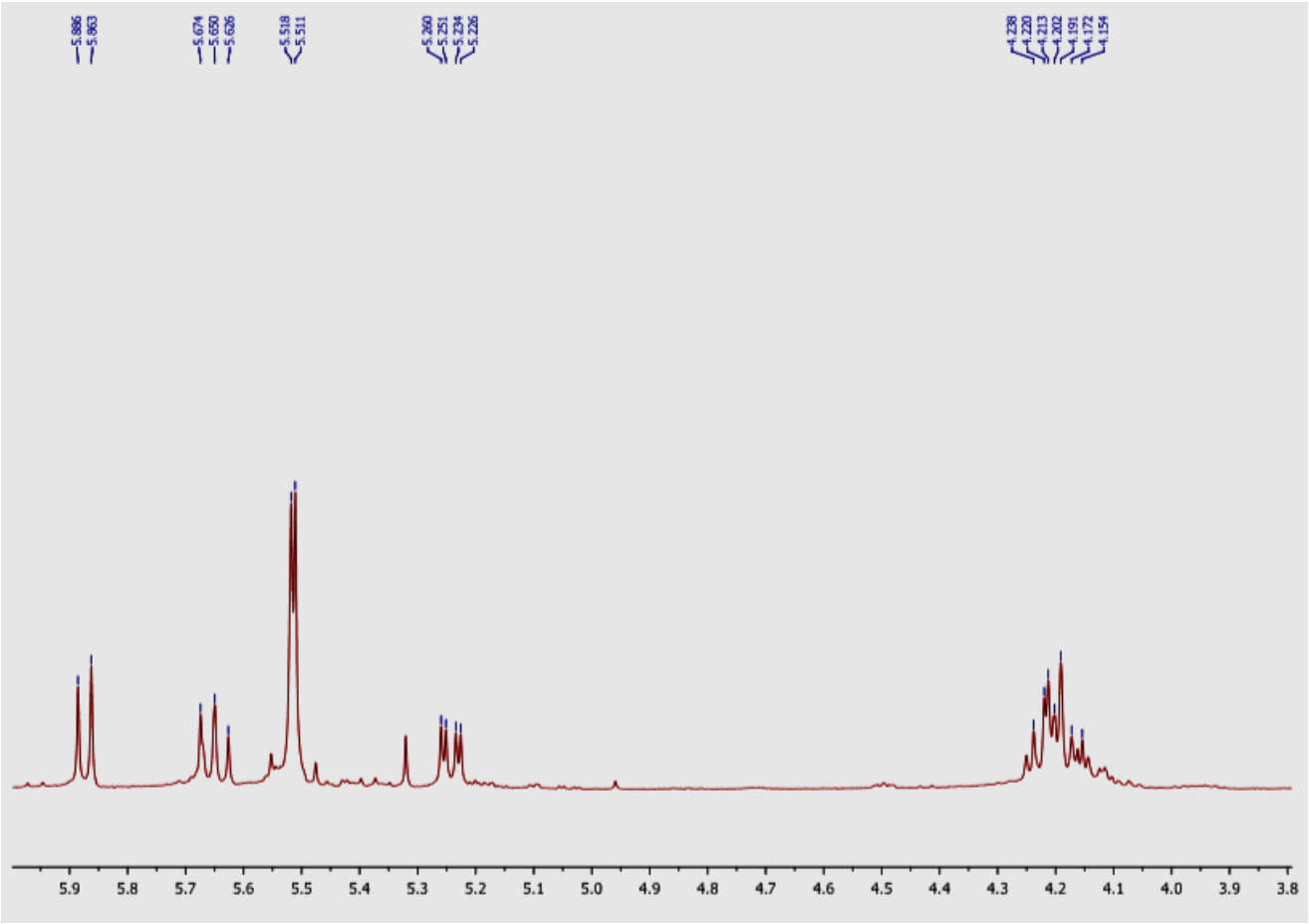


Fig. S14: ^{1}H-NMR ($CDCl_3$-d_6)spectrum of compound 9



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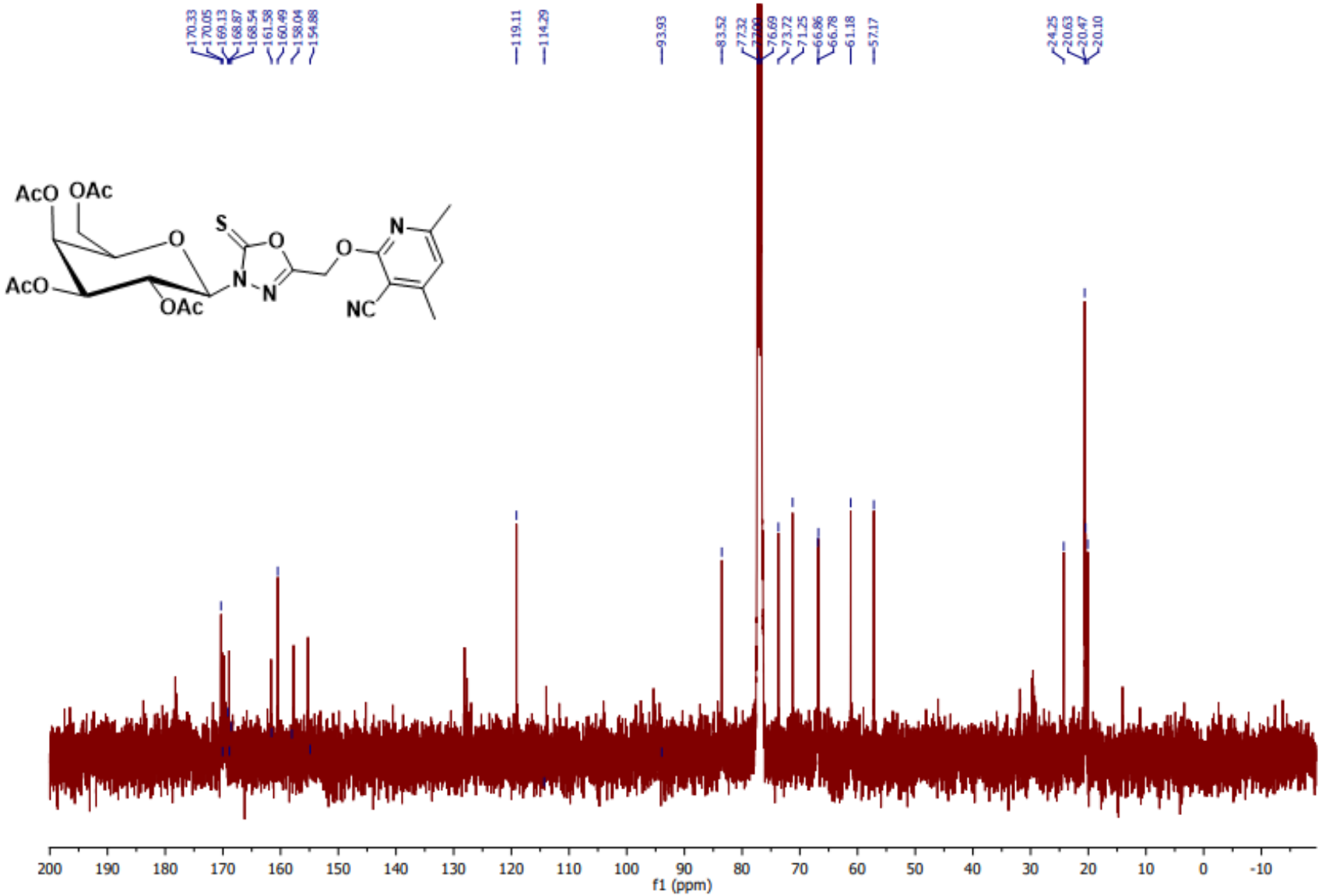


Fig.S15 : ^{13}C-NMR ($CDCl_3$-d_6)spectrum of compound 9

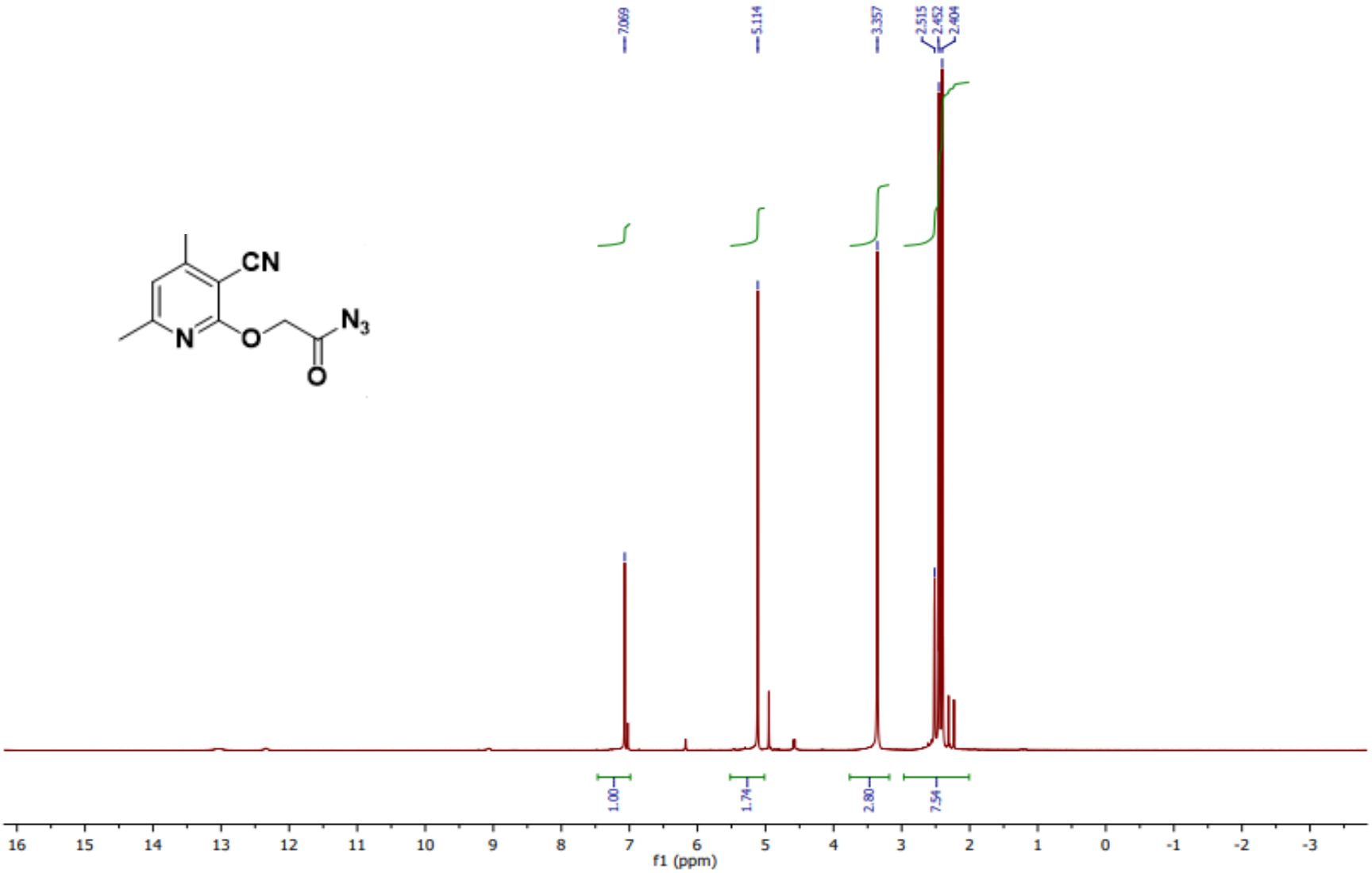


Fig.S16 : ^{1}H-NMR (DMSO-d$_6$)spectrum of compound 10

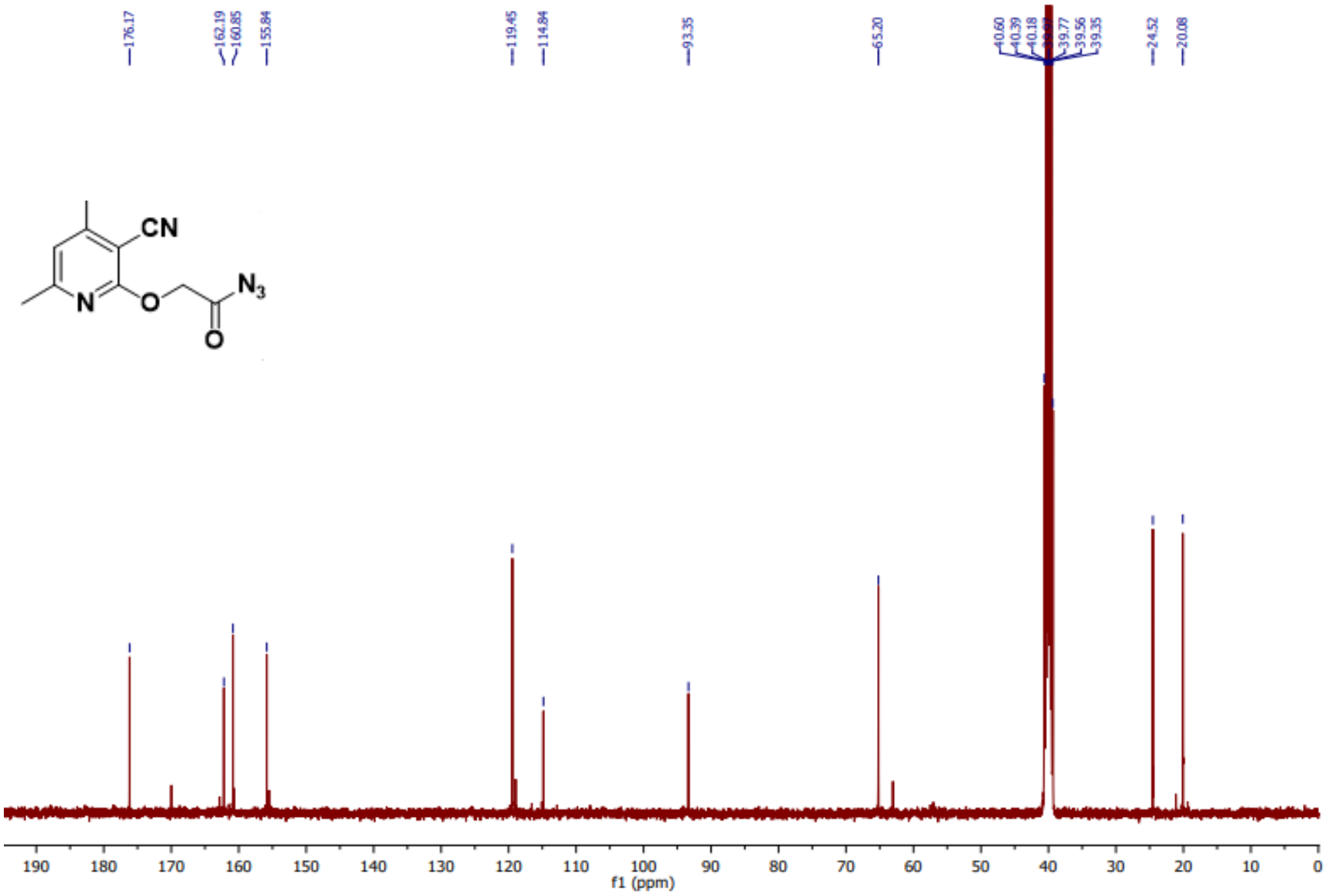


Fig. S17: ^{13}C-NMR (DMSO-d$_6$)spectrum of compound 10

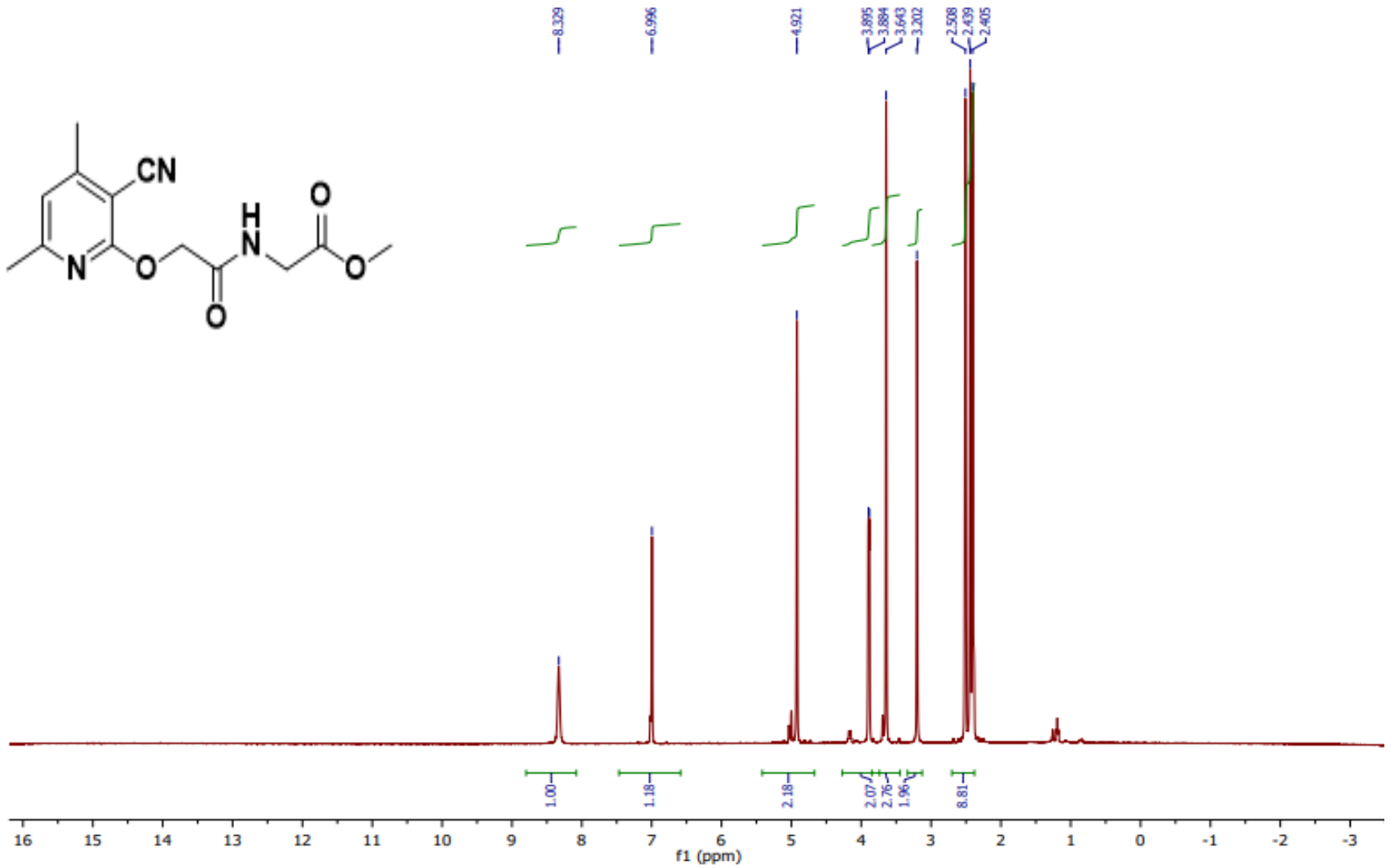


Fig.S18 : ^{1}H-NMR (DMSO-d$_6$)spectrum of compound 11

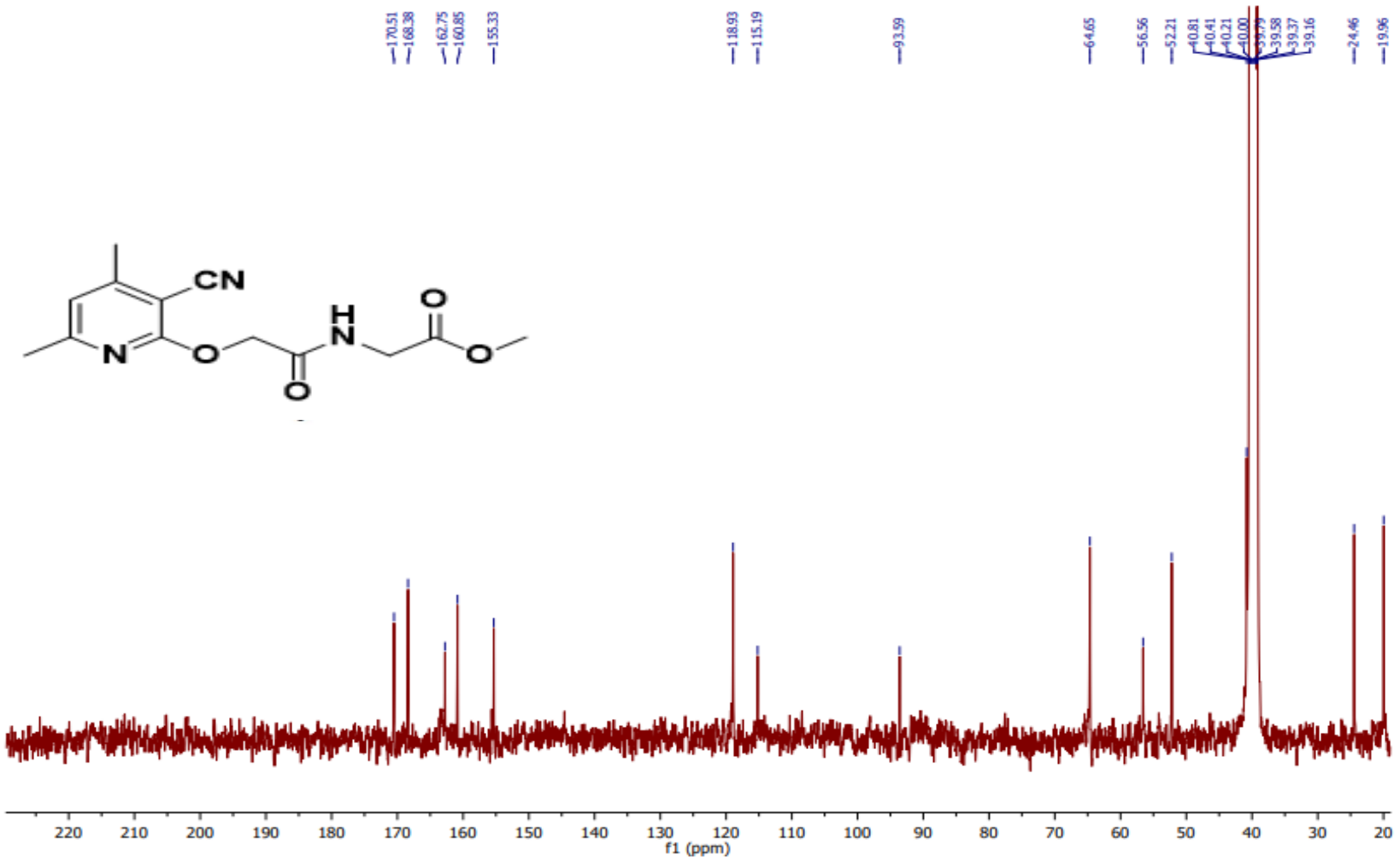


Fig.S19 : ^{13}C-NMR (DMSO-d$_6$)spectrum of compound 11

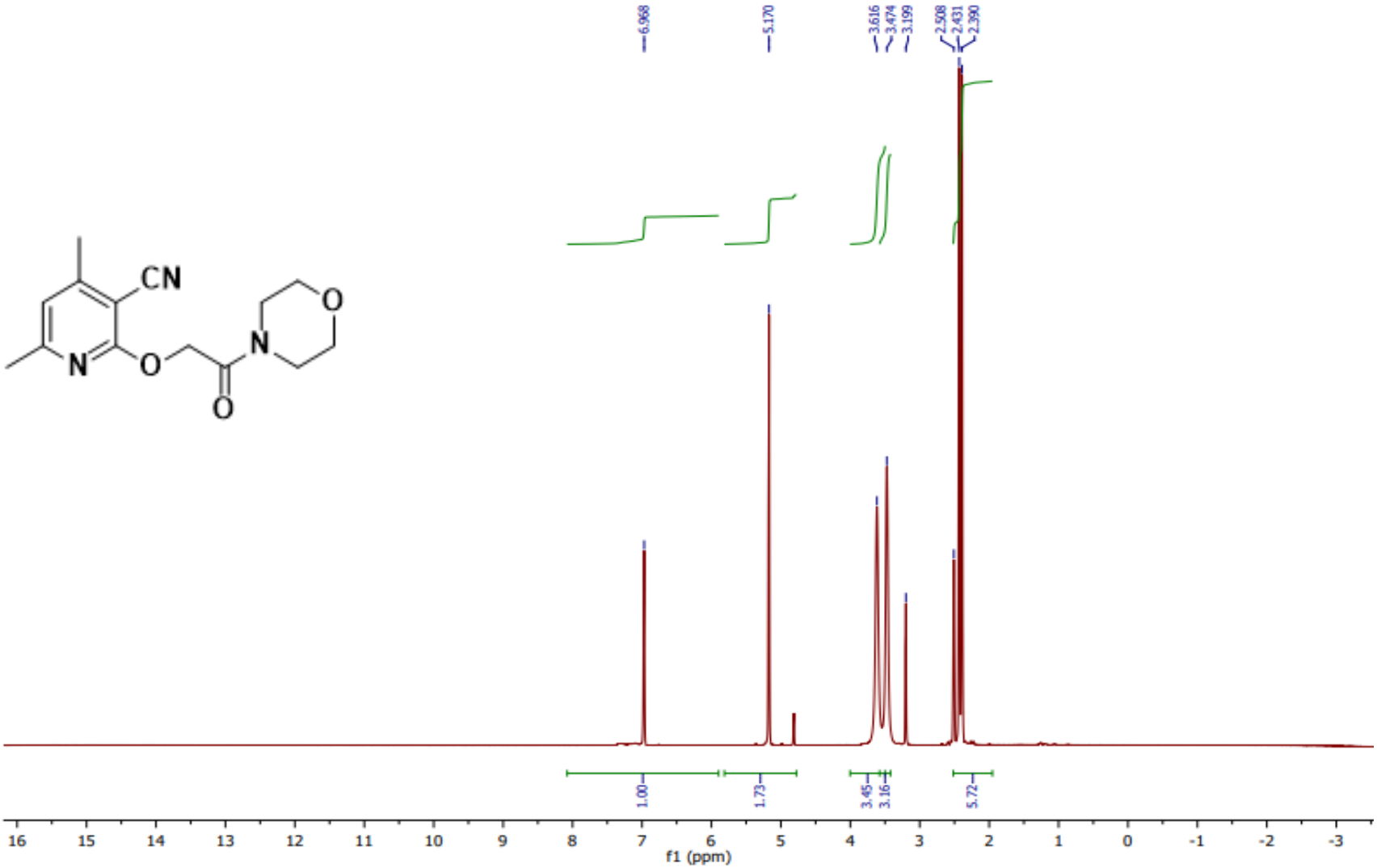


Fig.S20 : ^{1}H-NMR (DMSO-d$_6$)spectrum of compound 12

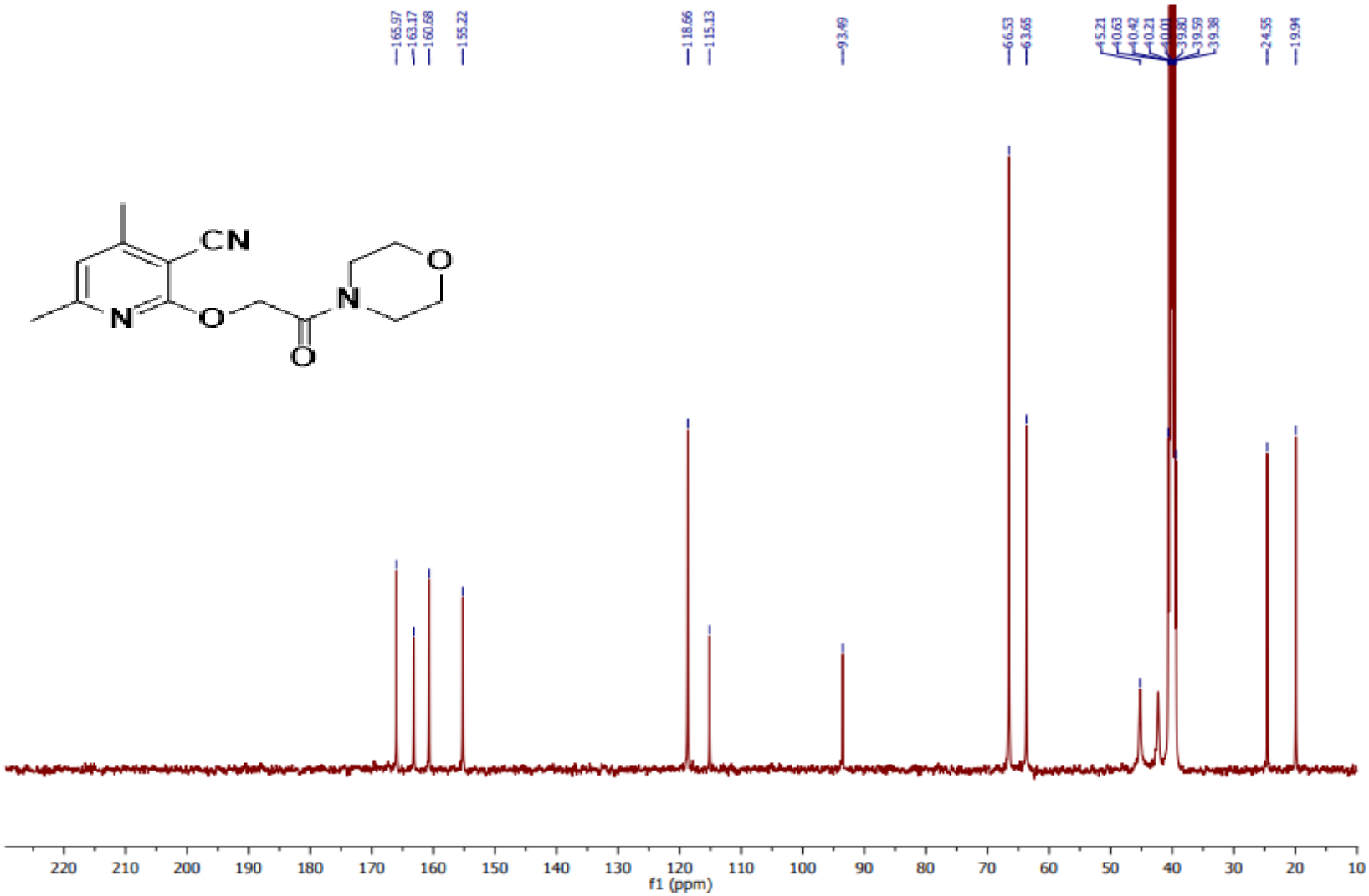


Fig.S21 : ^{13}C-NMR (DMSO-d$_6$)spectrum of compound 12

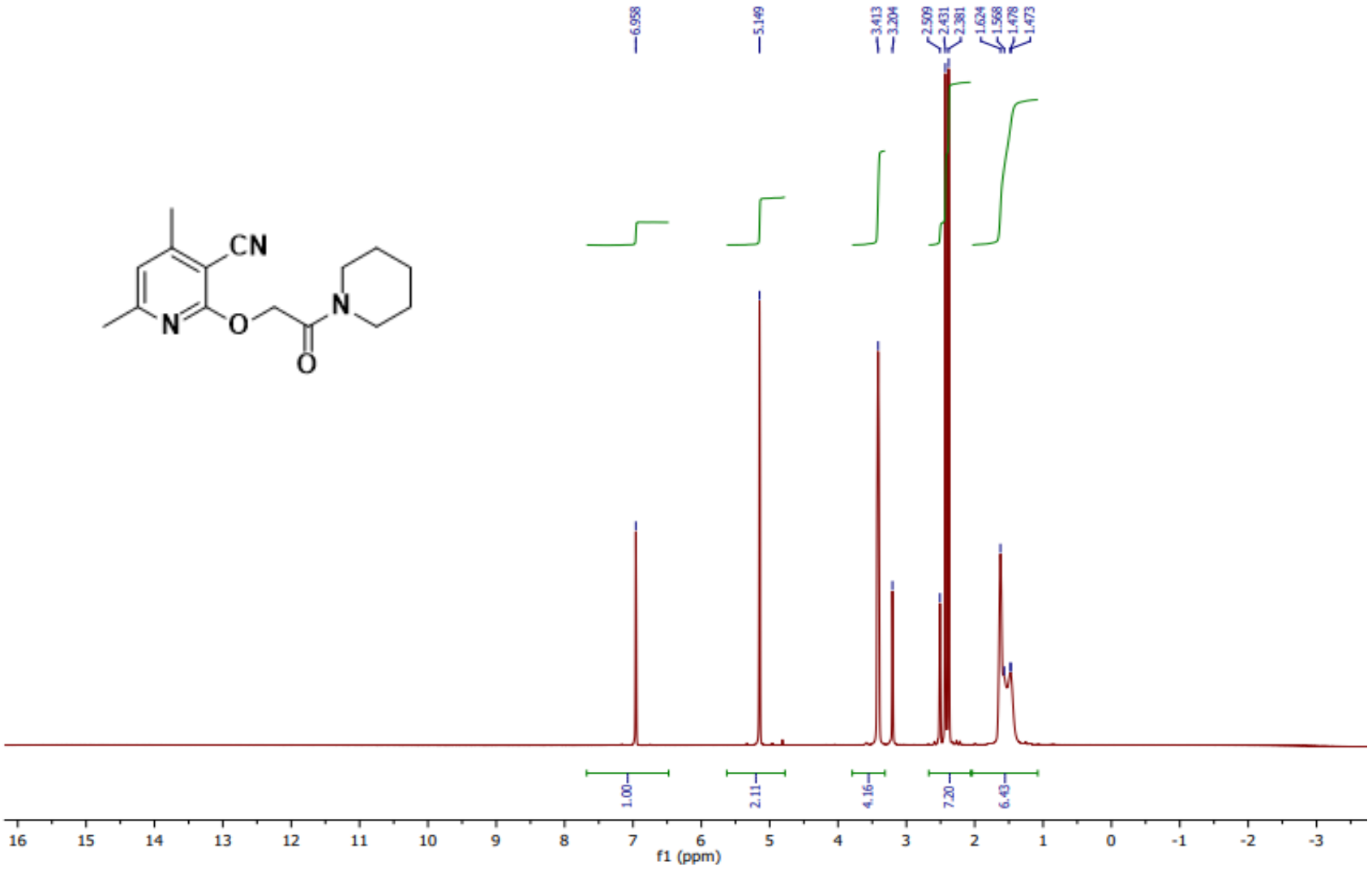


Fig. S22: ^{1}H-NMR (DMSO-d$_6$)spectrum of compound 13

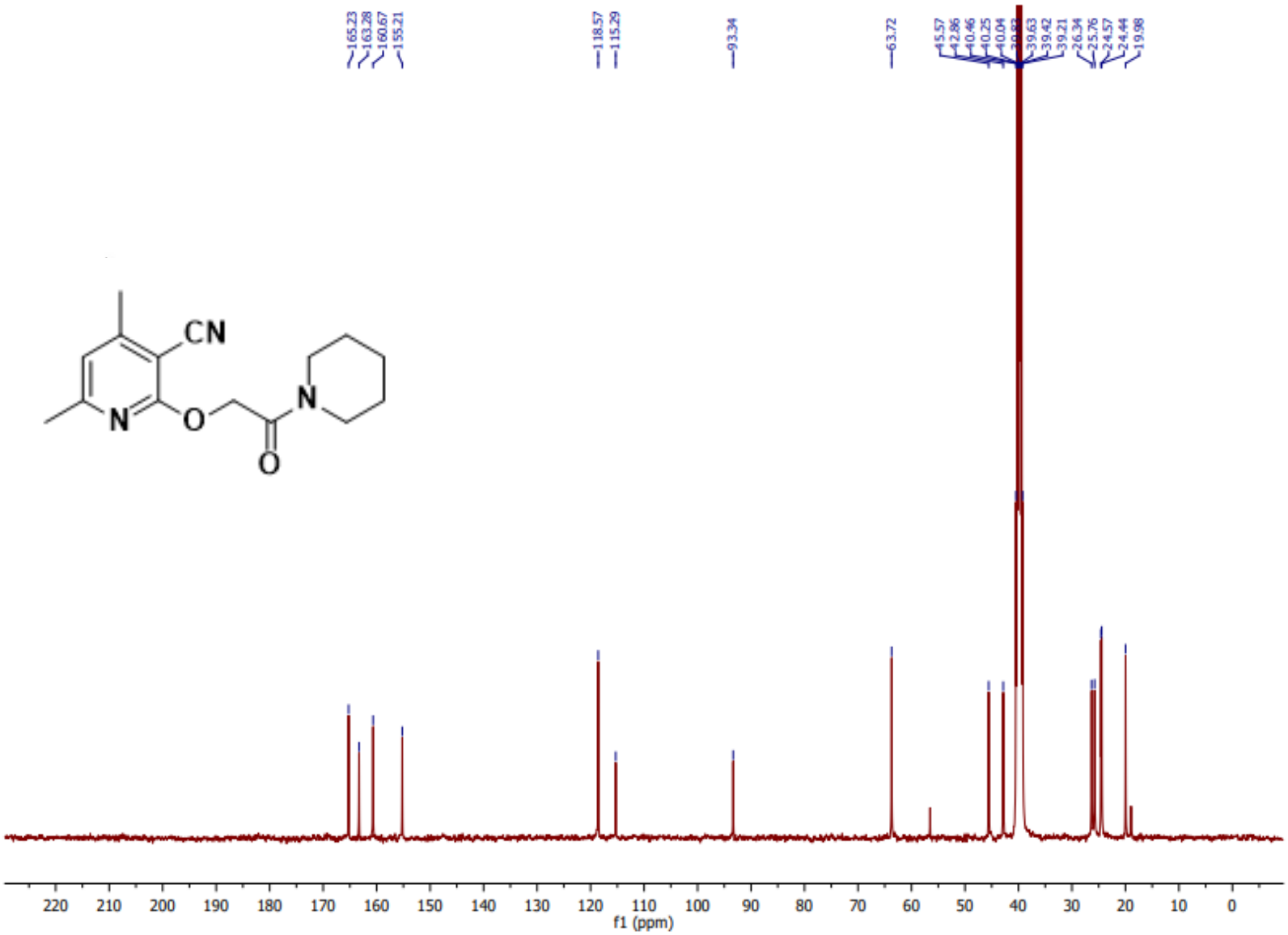


Fig.S23 : ^{13}C-NMR (DMSO-d$_6$)spectrum of compound 13

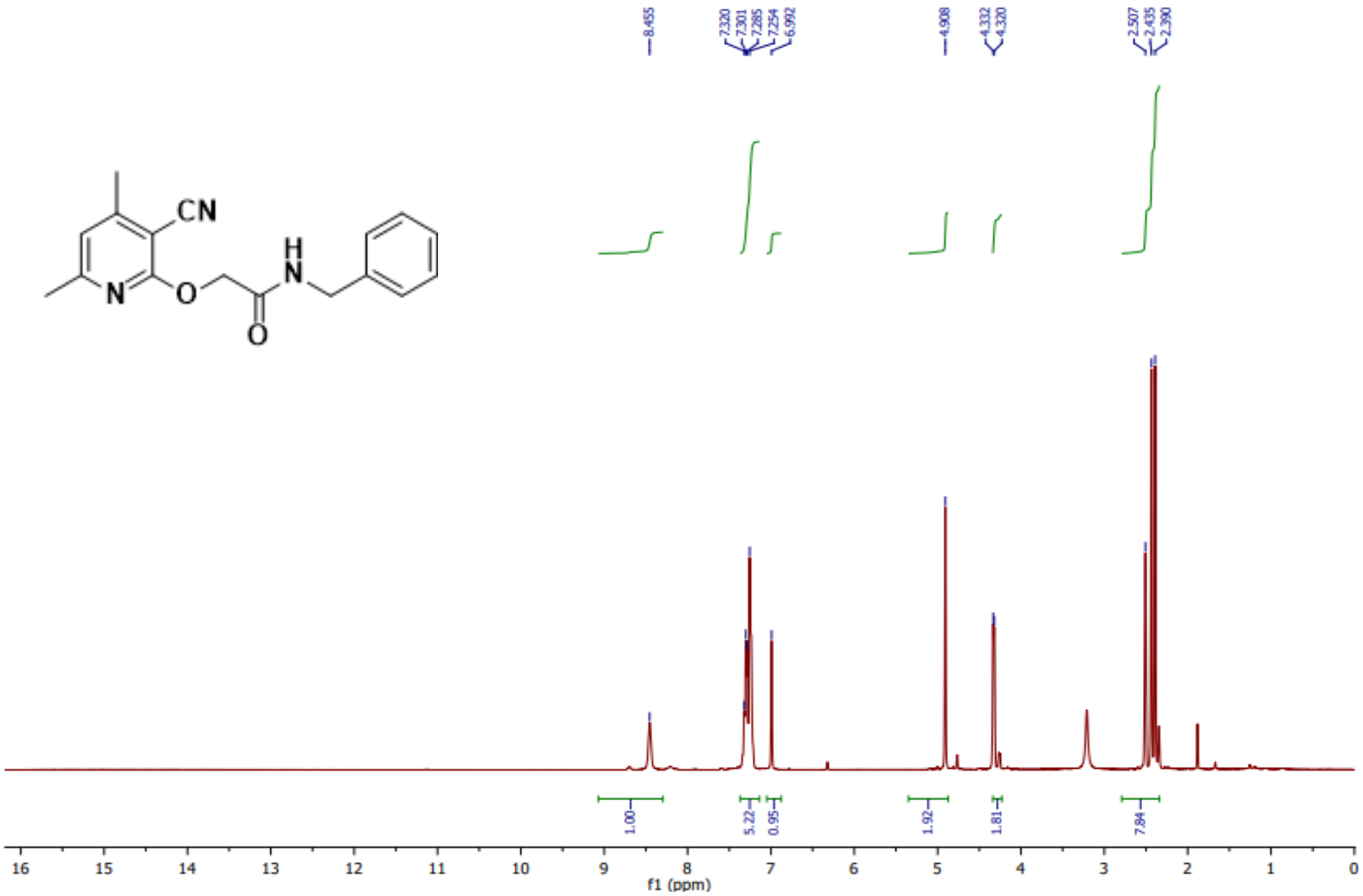


Fig. S24: ^{1}H-NMR (DMSO-d$_6$)spectrum of compound 14

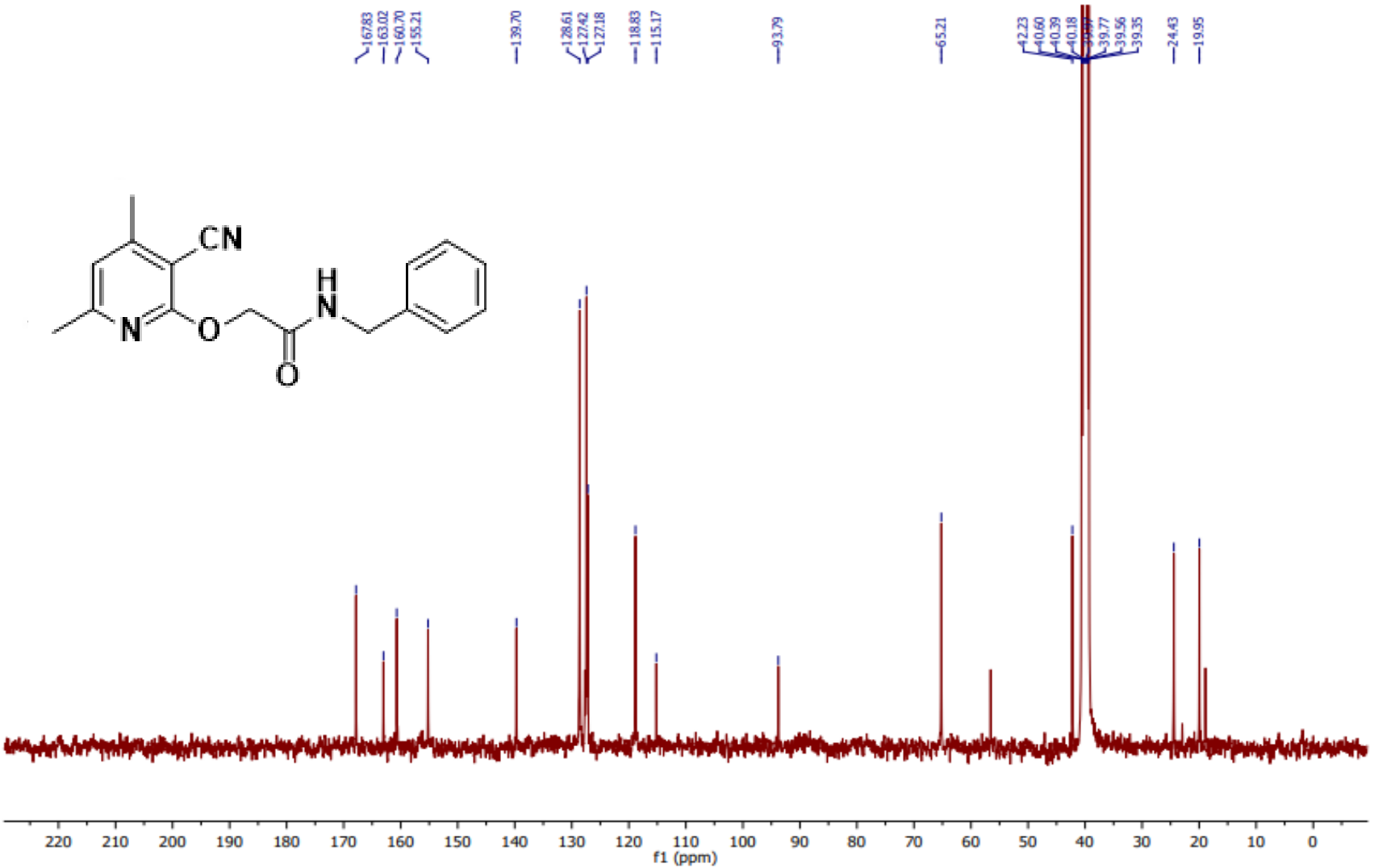


Fig.S25 : ^{13}C-NMR (DMSO-d$_6$)spectrum of compound 14

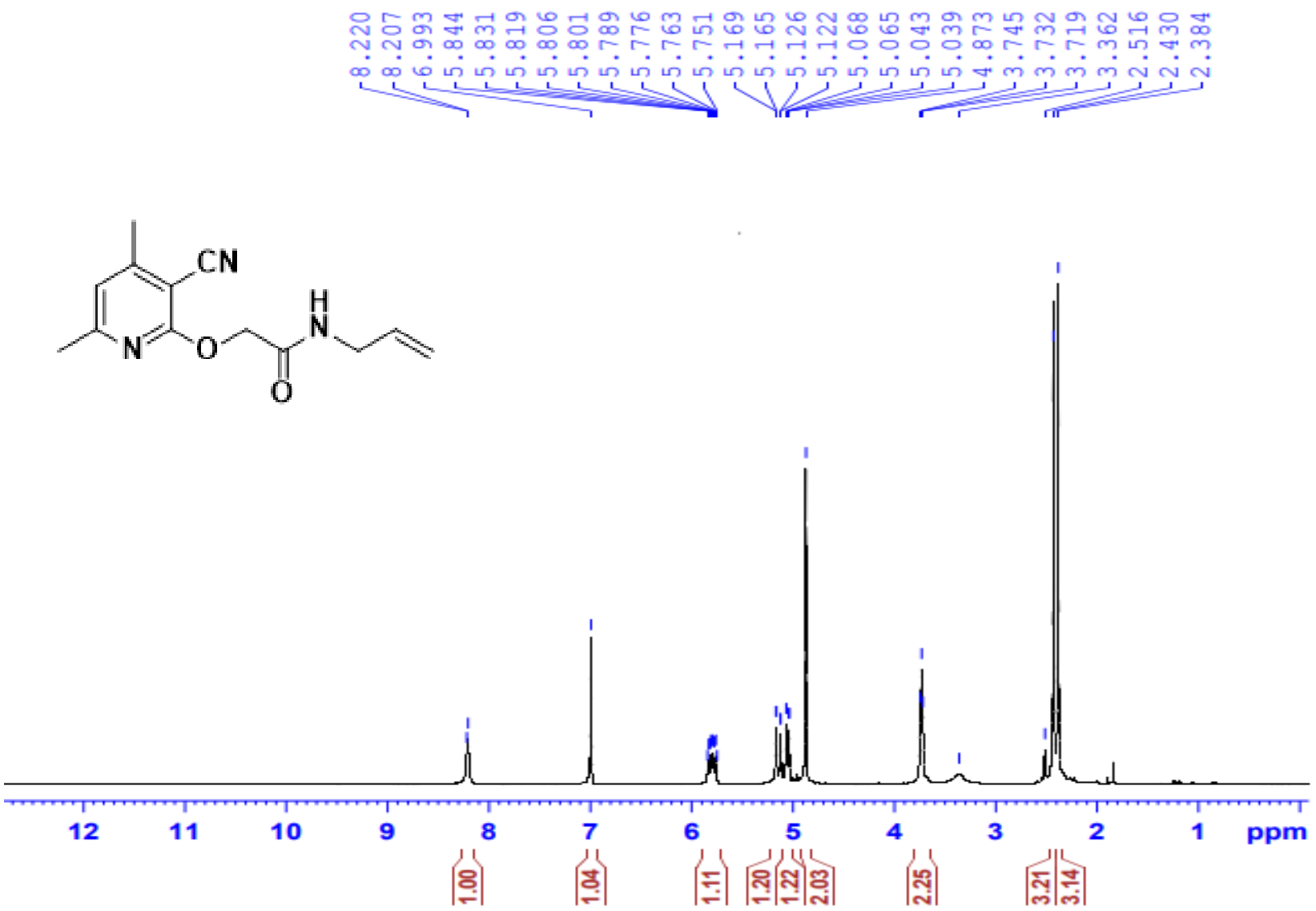
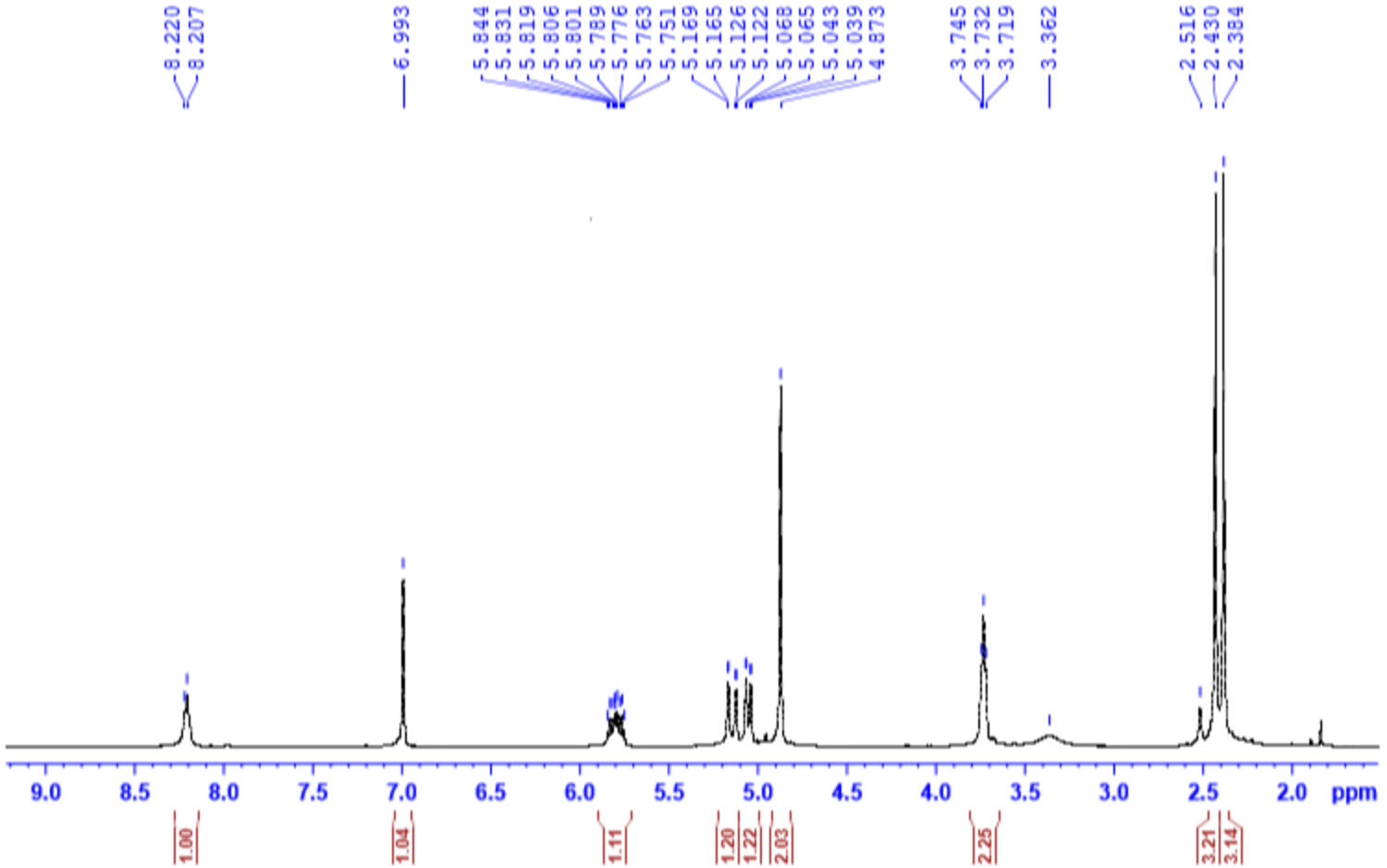
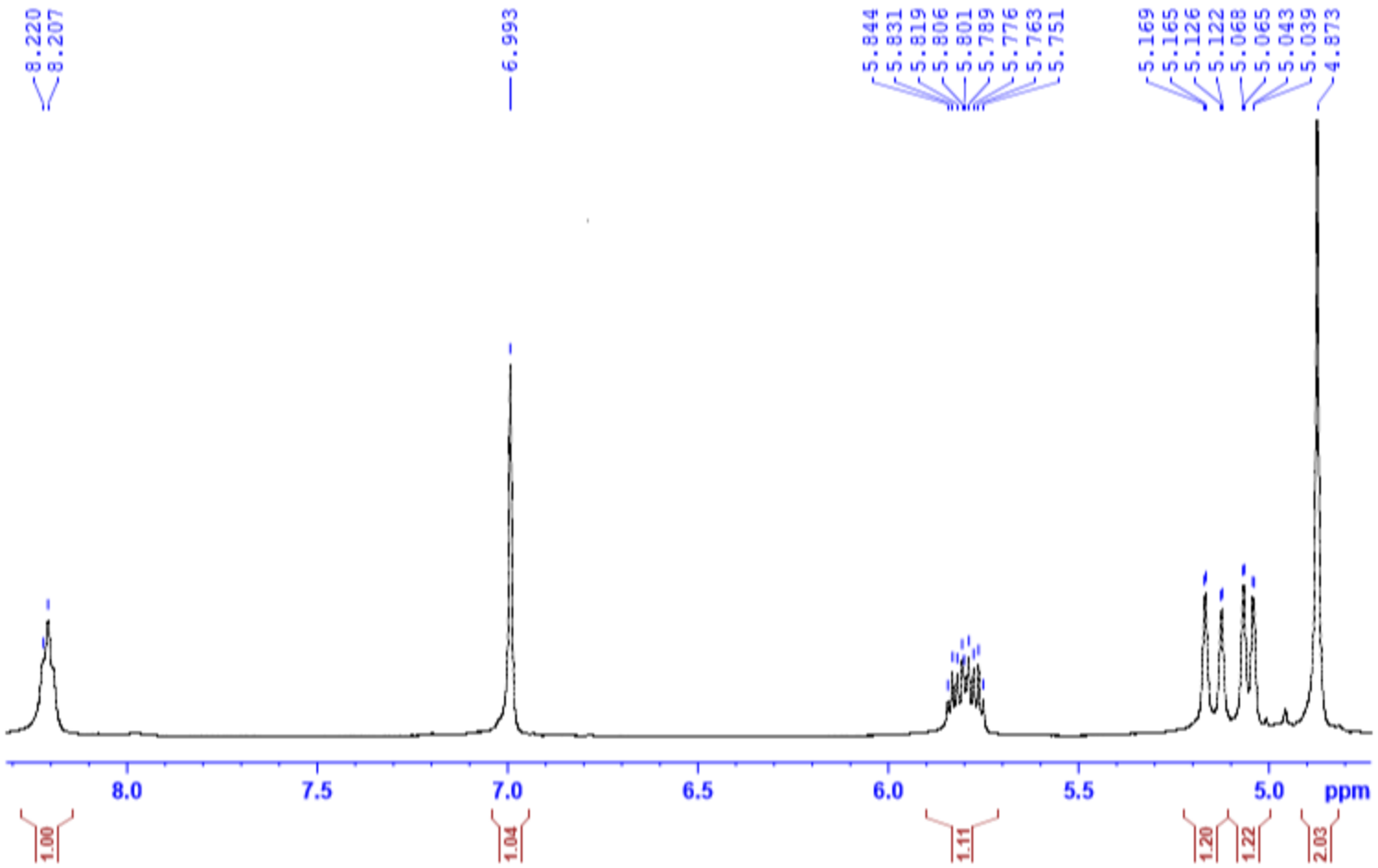


Fig.S26 : ^{1}H-NMR (DMSO-d$_6$)spectrum of compound 15



Zoomed image 1



Zoomed image 2

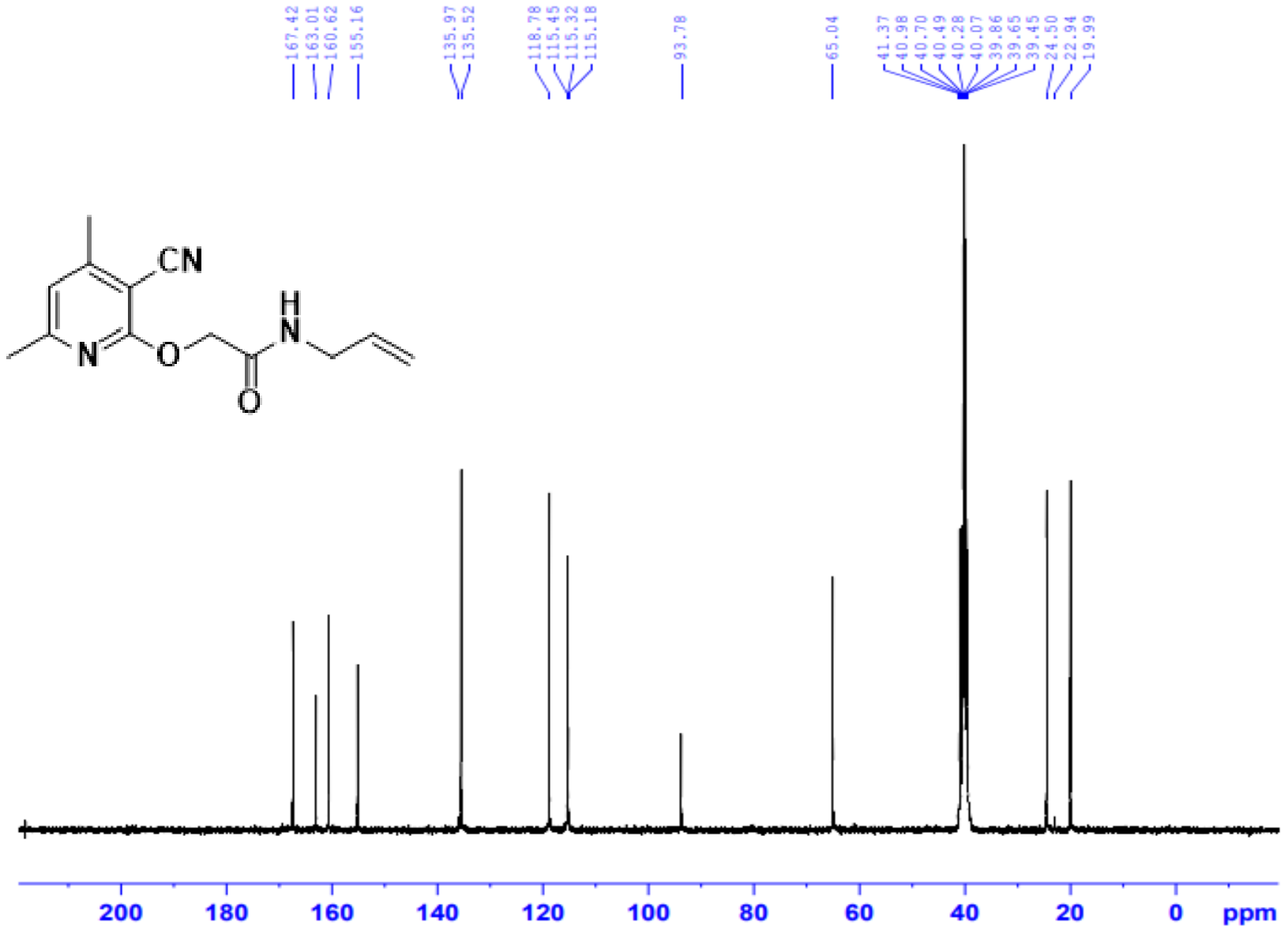


Fig.S27 : ^{13}C-NMR (DMSO-d$_6$)spectrum of compound 15

In Vivo Assay

Animals and tumor cell line

Adult female Swiss albino mice purchased from Theodor Bilharzia Research Institute, Giza, Egypt, with an average body weight of (18-23) g was used. Mice were housed under constant conditions of 12 h light/dark cycle in a temperature under conditions of controlled humidity (22 ± 2 °C), with free access to standard laboratory mice food and water .All procedures related to care and maintenance of the animals were performed according to the international guiding principles for animal research and approved by Faculty of Science, Suez Canal University bioethics and animal ethics committee (Approval number REC220/2023).

Solid Ehrlich carcinoma (SEC) were got from the National Cancer Institute (Cairo University, Egypt). The tumor cell line was proliferated in mice through serial intraperitoneal (I.P.) transplantation of a volume of 0.2 mL physiological saline contains 1×10^6 viable cells for 24 h. SEC cells were collected 7 days after I.P. implantation. The harvested cells were diluted with saline to obtain a concentration of 5×10^6 viable SEC cells/mL. A volume of 0.2 mL saline contains 1×10^6 SEC cells that were I.P. implanted into each normal mouse. SEC cells (1×10^6 tumor cells/mouse) were implanted subcutaneously into the right thigh of the hind limb.

The experimental animals were randomly divided into four groups. Group 1 served as the normal saline control (5 mL/kg B.Wt., I.P.). Group 2 served as the SEC control (1×10^6 cells/mouse). Group 3 served as the compound-treated group (5 mg/kg B.Wt., I.P.). Group 4 received the standard anticancer drug of Doxorubicin (DOX) (5 mg/kg BW, I.P.) and is considered as a reference control. Body weight and survival were recorded daily until the 24^{th} day in both treated and control groups. At the end of experiment, the blood of each group was collected under light anesthesia for estimation of hematological and biochemical assays. The anesthetized animals were then sacrificed for evaluation of the antitumor activity and histopathological examination.

Antitumor potentiality

It includes tumor volume, weight, and tumor inhibition ration (TIR%). Time interval measurements of tumor volume using digital Vernier caliper (Tricle Brand, Shanghai, China). Measure tumor length and width using clipper and then calculate tumor volume using formulations V = (L x W x W)/2, where V is tumor volume, W is tumor width, L is tumor length. While TIR% was calculated according to the following equation $\frac{Tumor\ volume\ (Control) - Tumor\ volume\ (treated)}{Tumor\ volume\ (control)} x\ 100$.

Blood assays

At the end of the experiment, animals from different groups were sacrificed, and blood samples were collected for determination of liver enzymes ALT, AST levels. Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were evaluated using commercial kits (ELITech clinical systems, France). Serum albumin level was determined by kit purchased from STANBIO Company (USA).

Histopathological study

Specimens of liver-sacrificed mice were fixed in 10% saline formalin. The fixed liver specimens were dehydratedin ascending series of ethyl alcohol and embedded in paraffin. Sections at 5 mm thicknesses were stained with hematoxylin and eosin and examined under light microscopy.

References

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[2] M. Dicato, L. Plawny, M. Diederich, Anemia in cancer, Annals of Oncology. 21 (2010) vii167–vii172. https://doi.org/10.1093/annonc/mdq284.

[3] H.S.A. ElZahabi, M.S. Nafie, D. Osman, N.H. Elghazawy, D.H. Soliman, A.A.H. EL-Helby, R.K. Arafa, Design, synthesis and evaluation of new quinazolin-4-one derivatives as apoptotic enhancers and autophagy inhibitors with potent antitumor activity, European Journal of Medicinal Chemistry. 222 (2021) 113609. https://doi.org/10.1016/j.ejmech.2021.113609.

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