

L-Proline promoted green and regioselective synthesis of novel pyrazole based trifluoromethylated fused thiazolopyran scaffold and their biological evaluation

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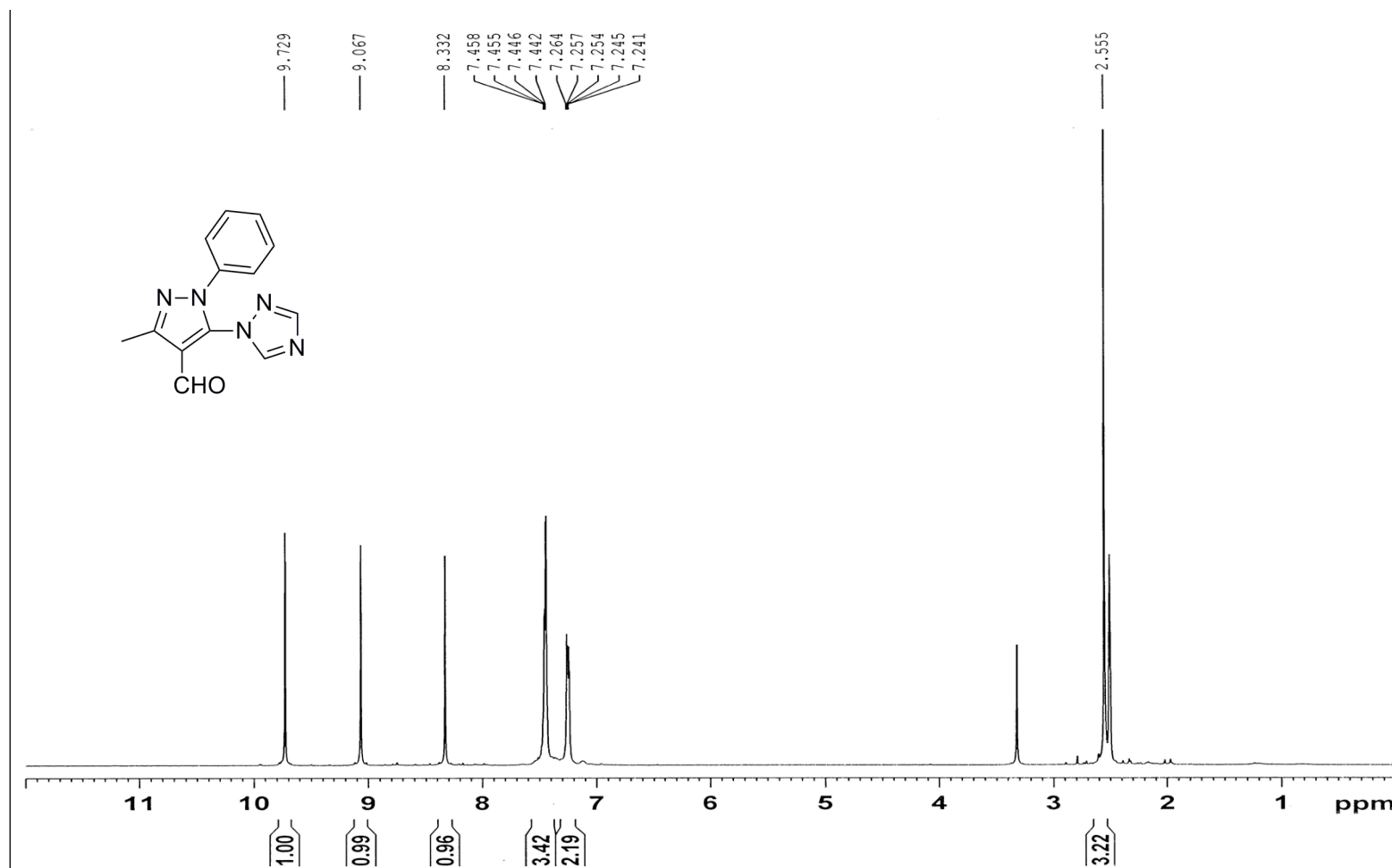
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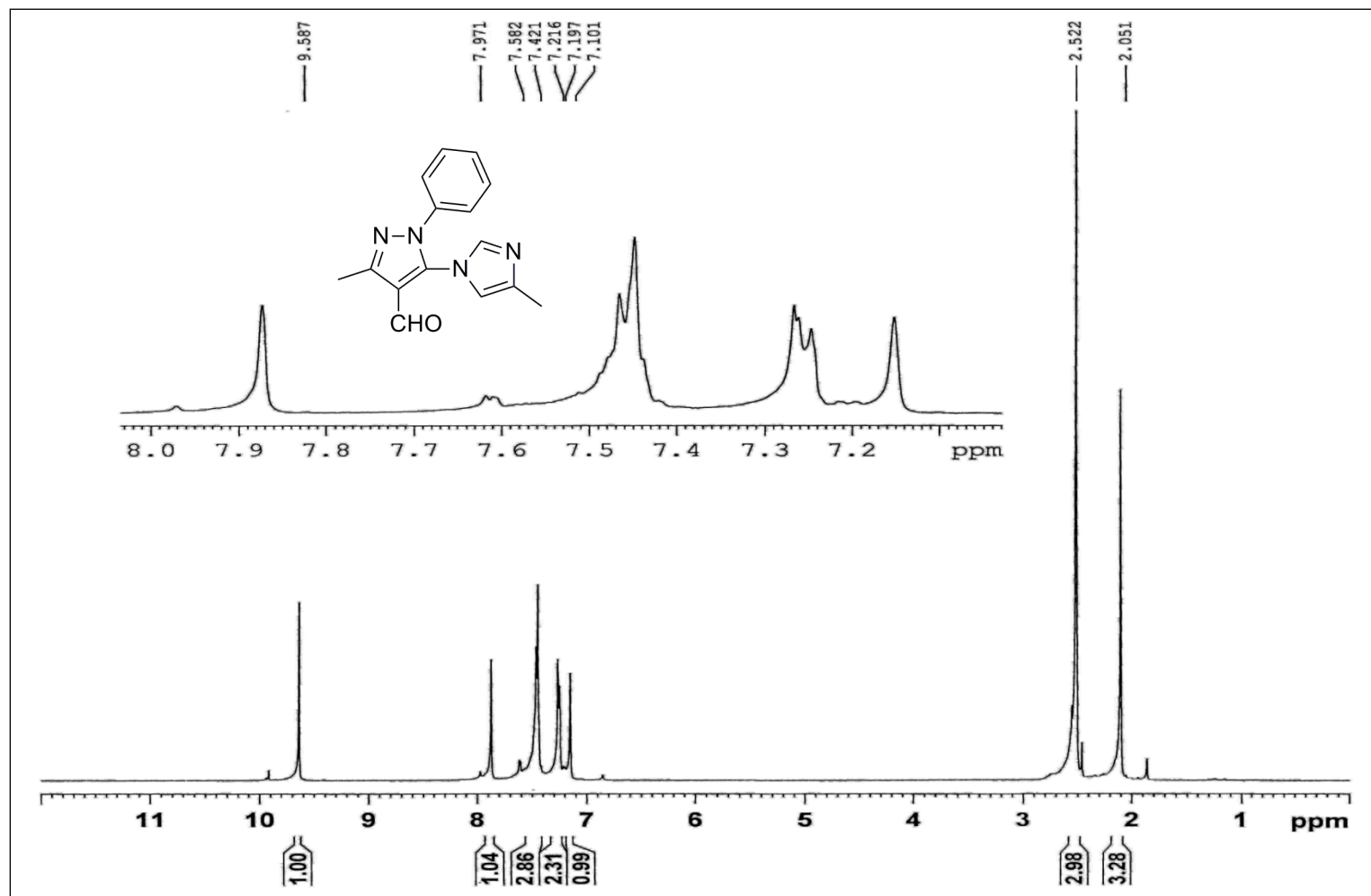
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Supporting information

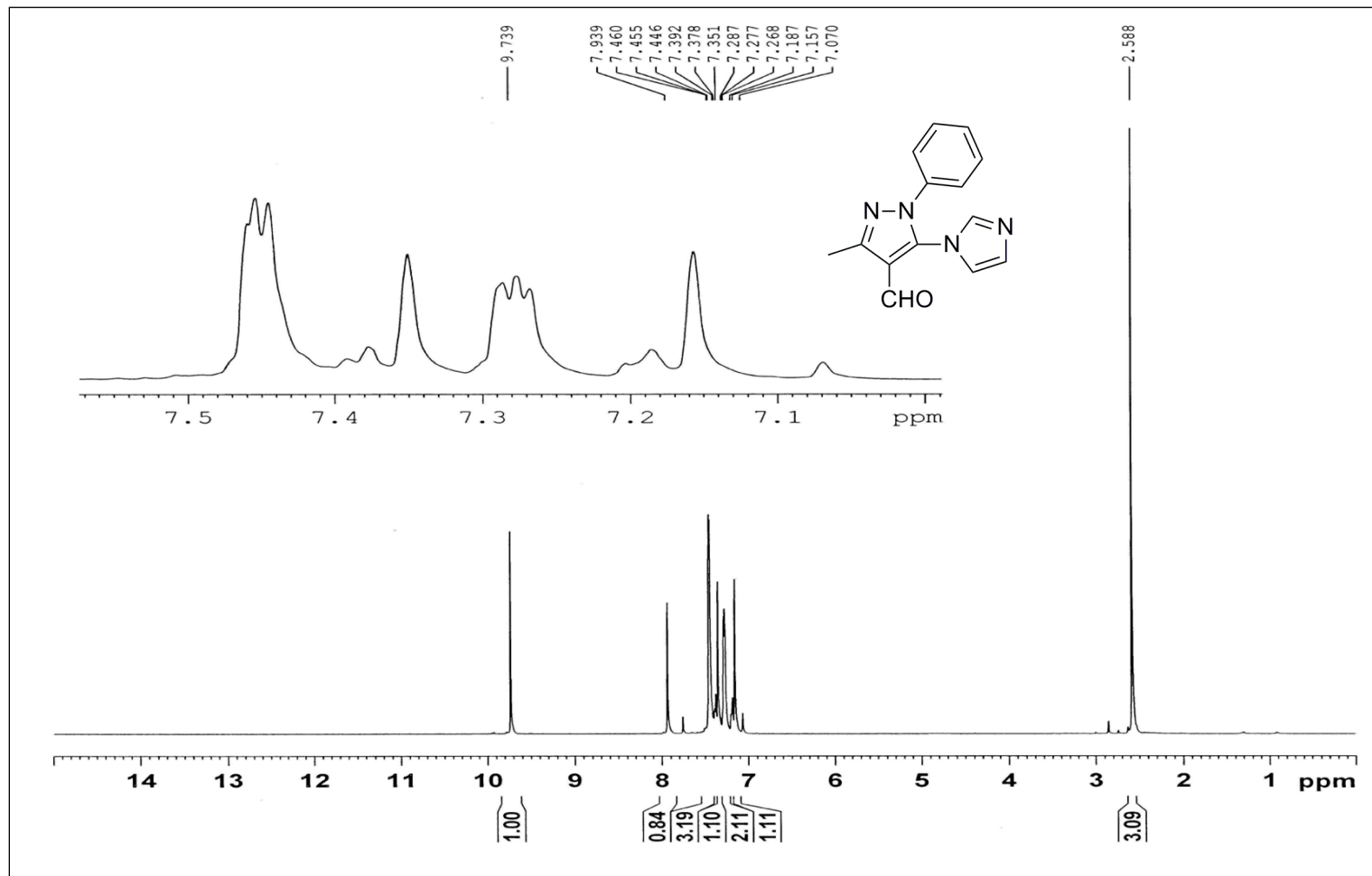
¹H NMR spectra of compound **4a**



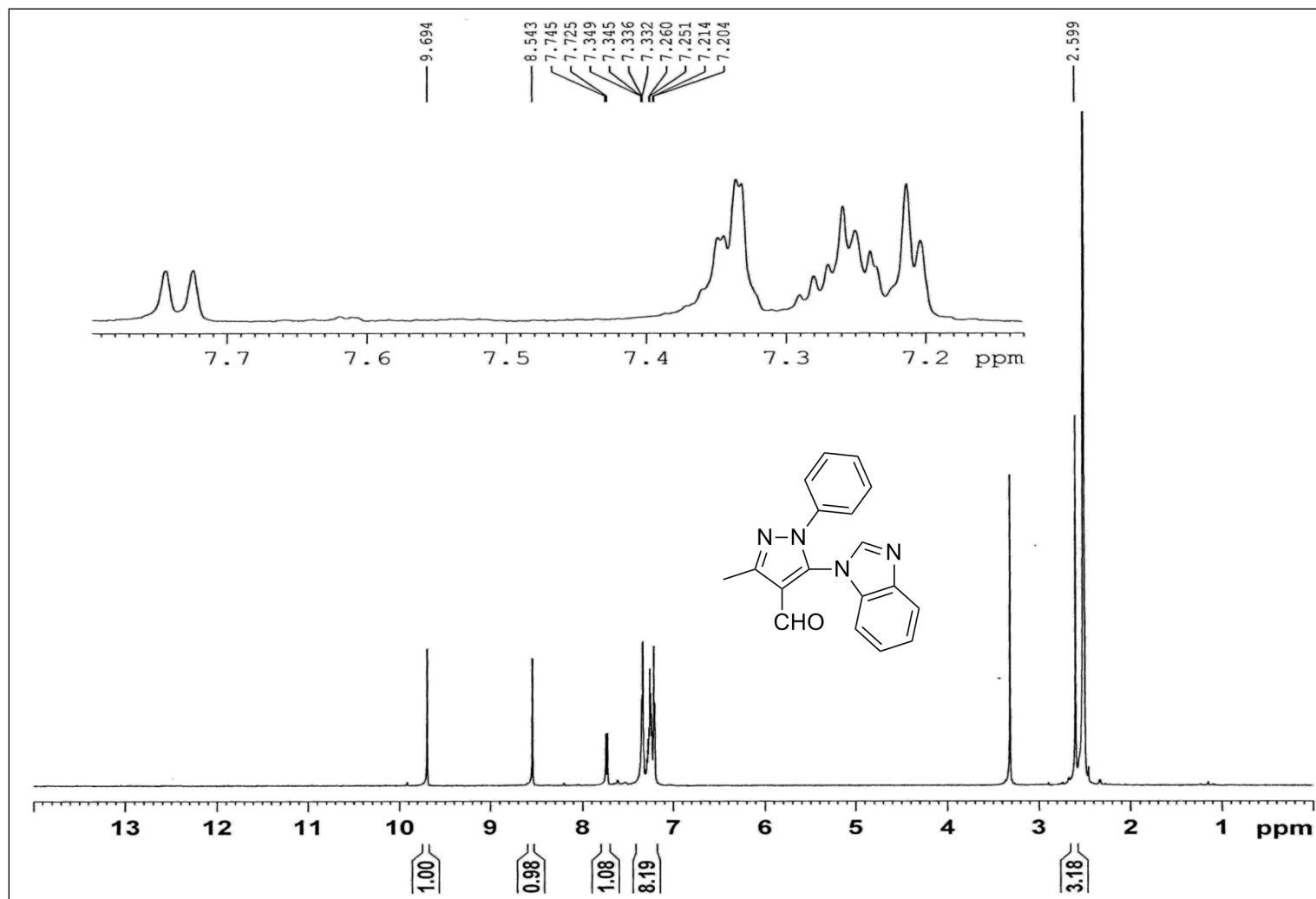
^1H NMR spectra of compound **4b**



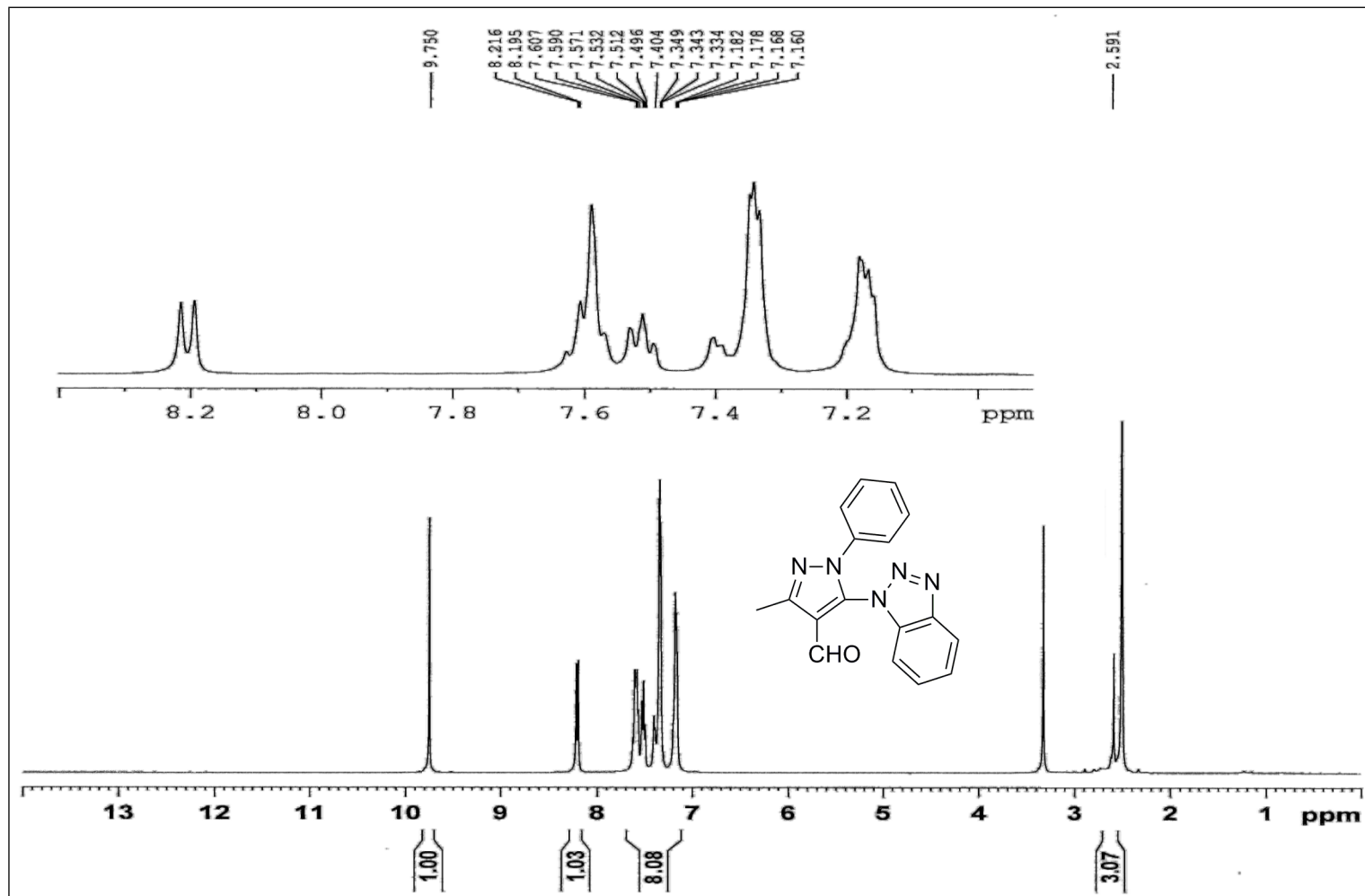
¹H NMR spectra of compound **4c**



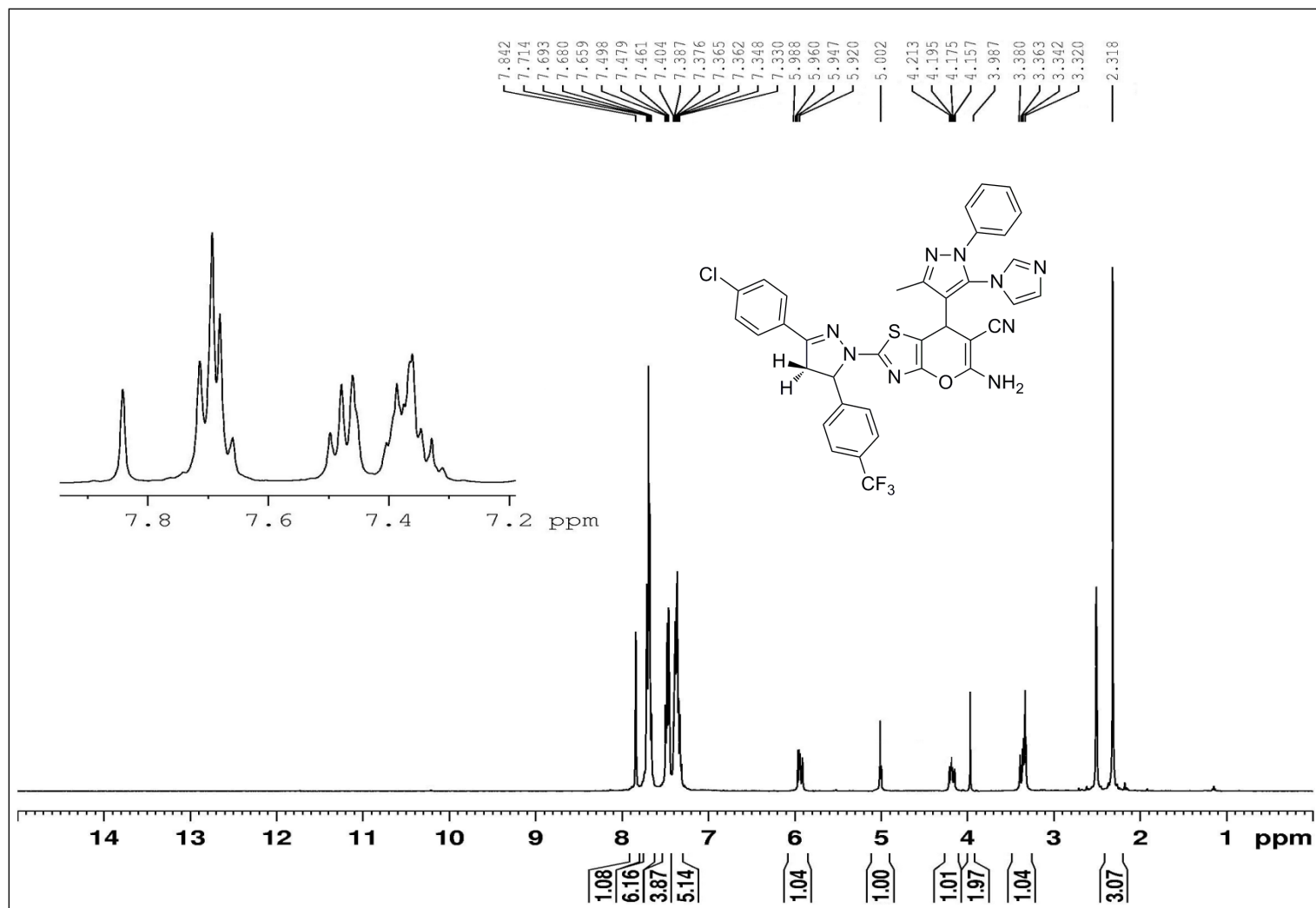
^1H NMR spectra of compound **4d**



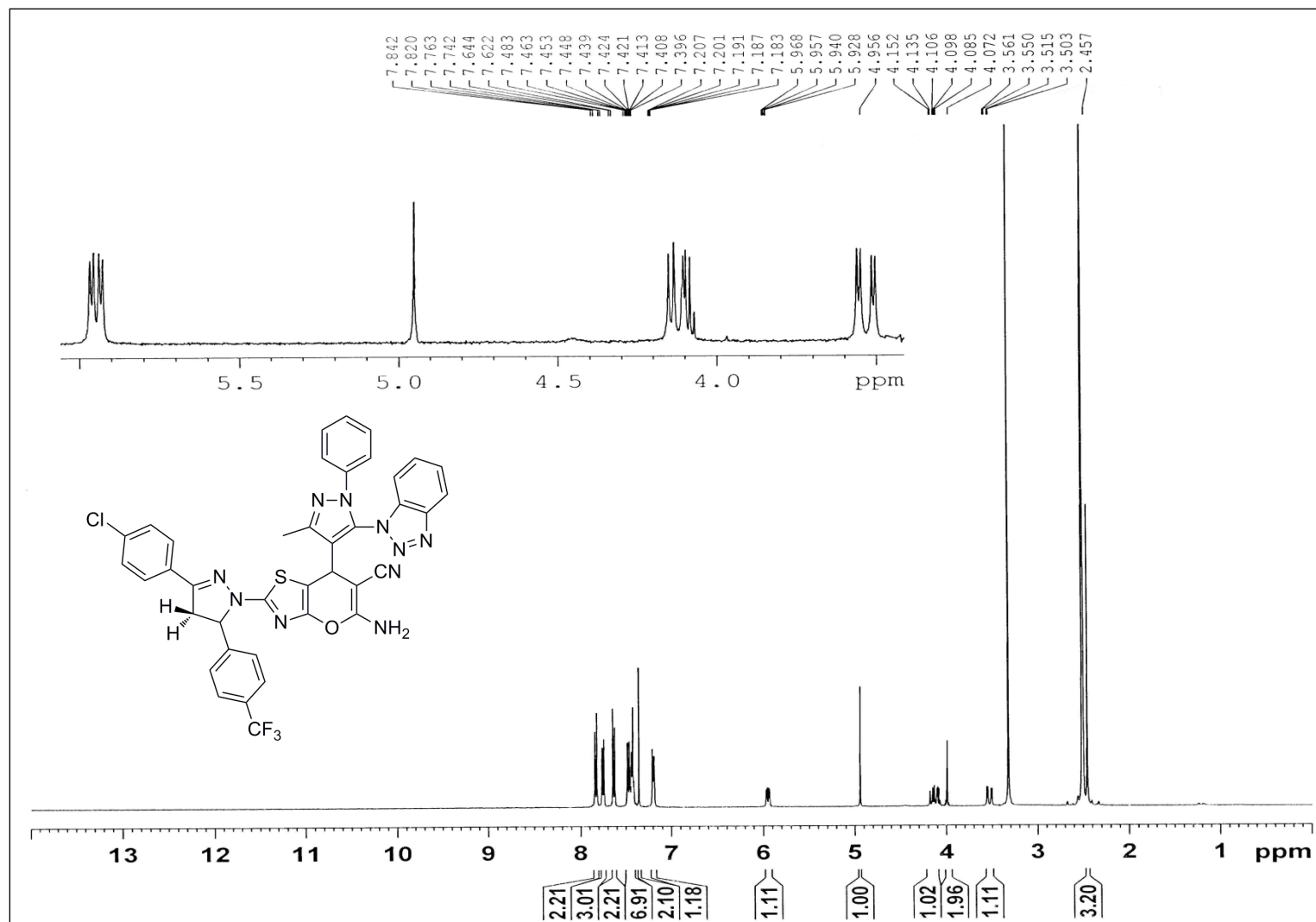
^1H NMR spectra of compound **4e**



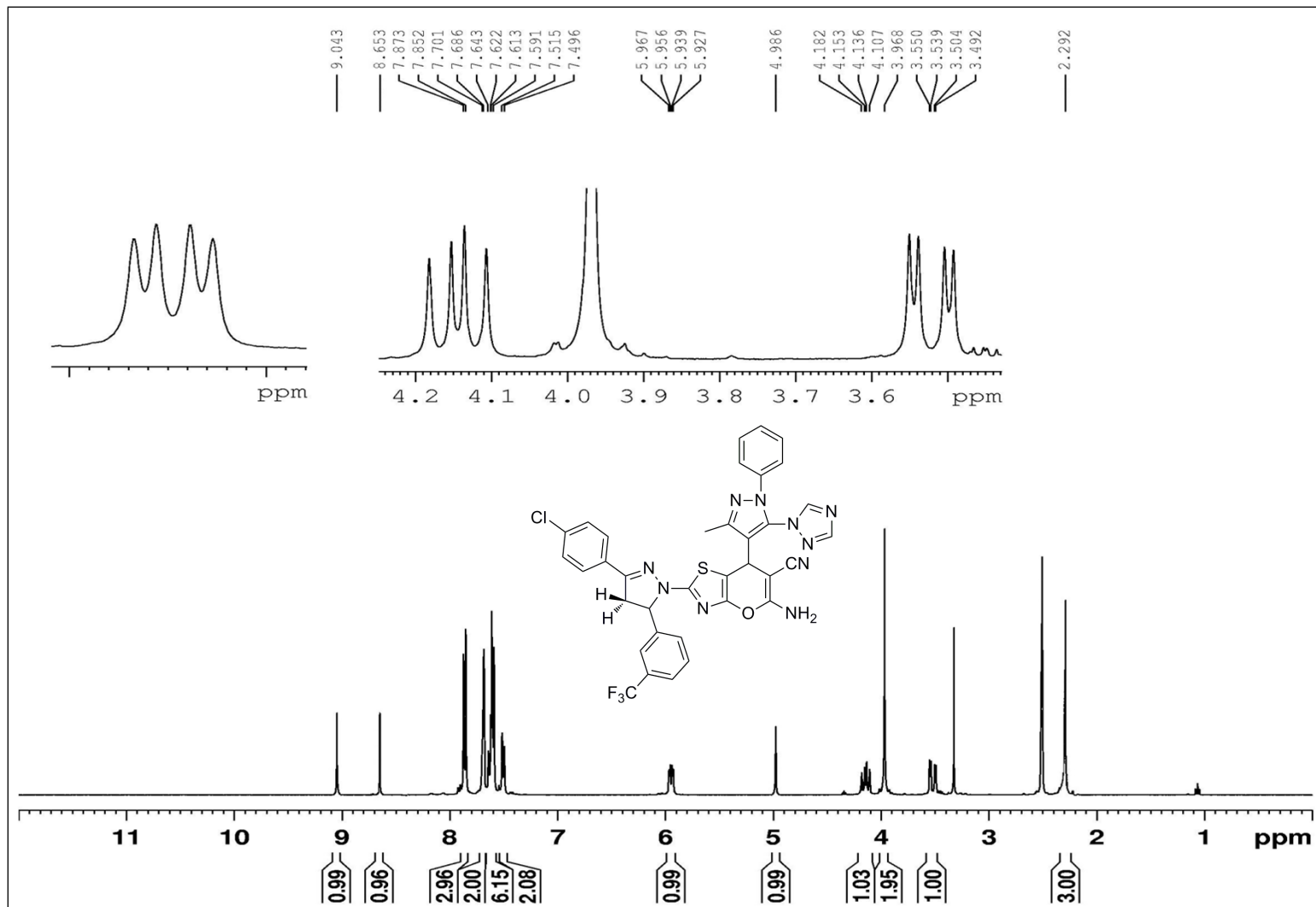
¹H NMR spectra of compound **11c**



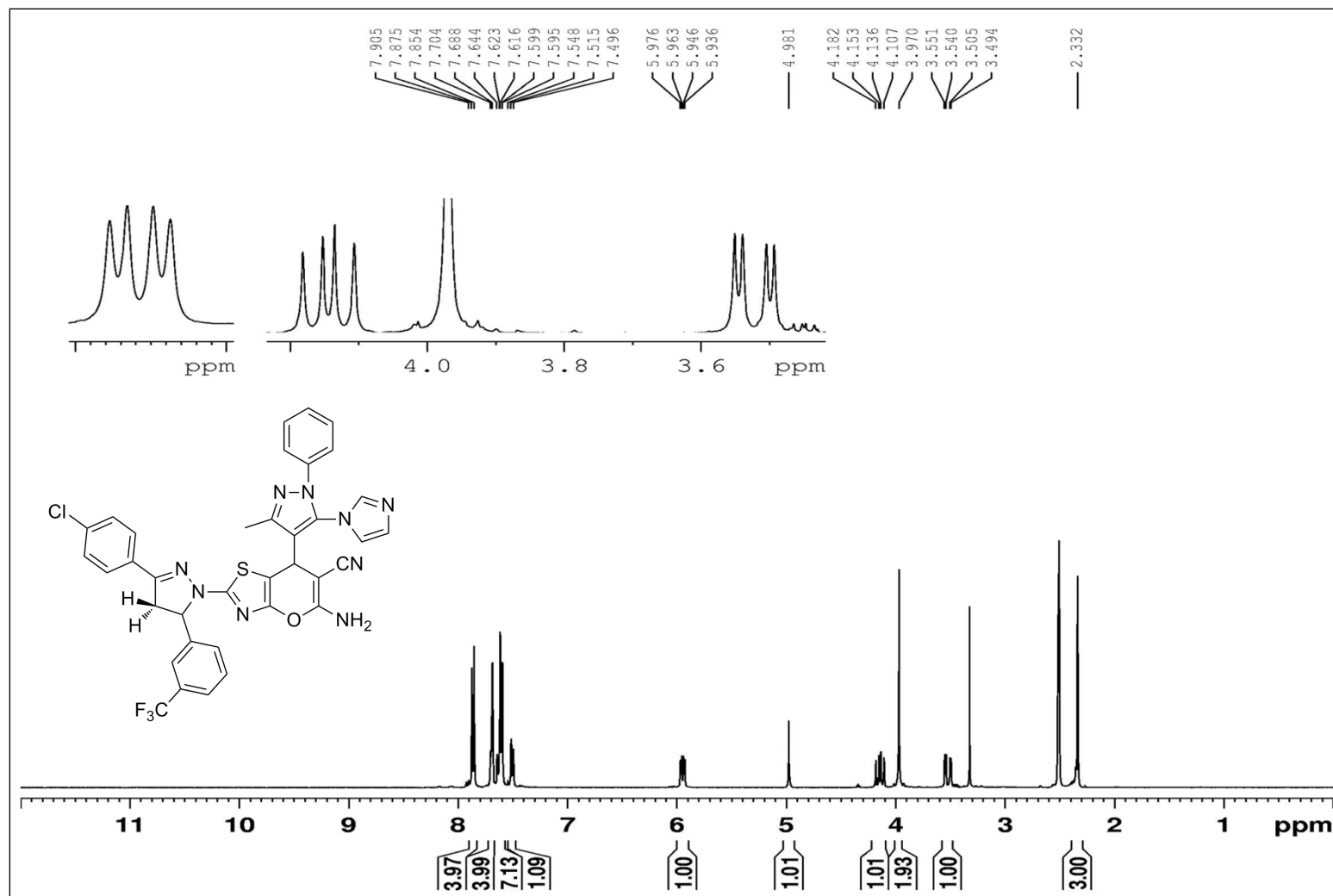
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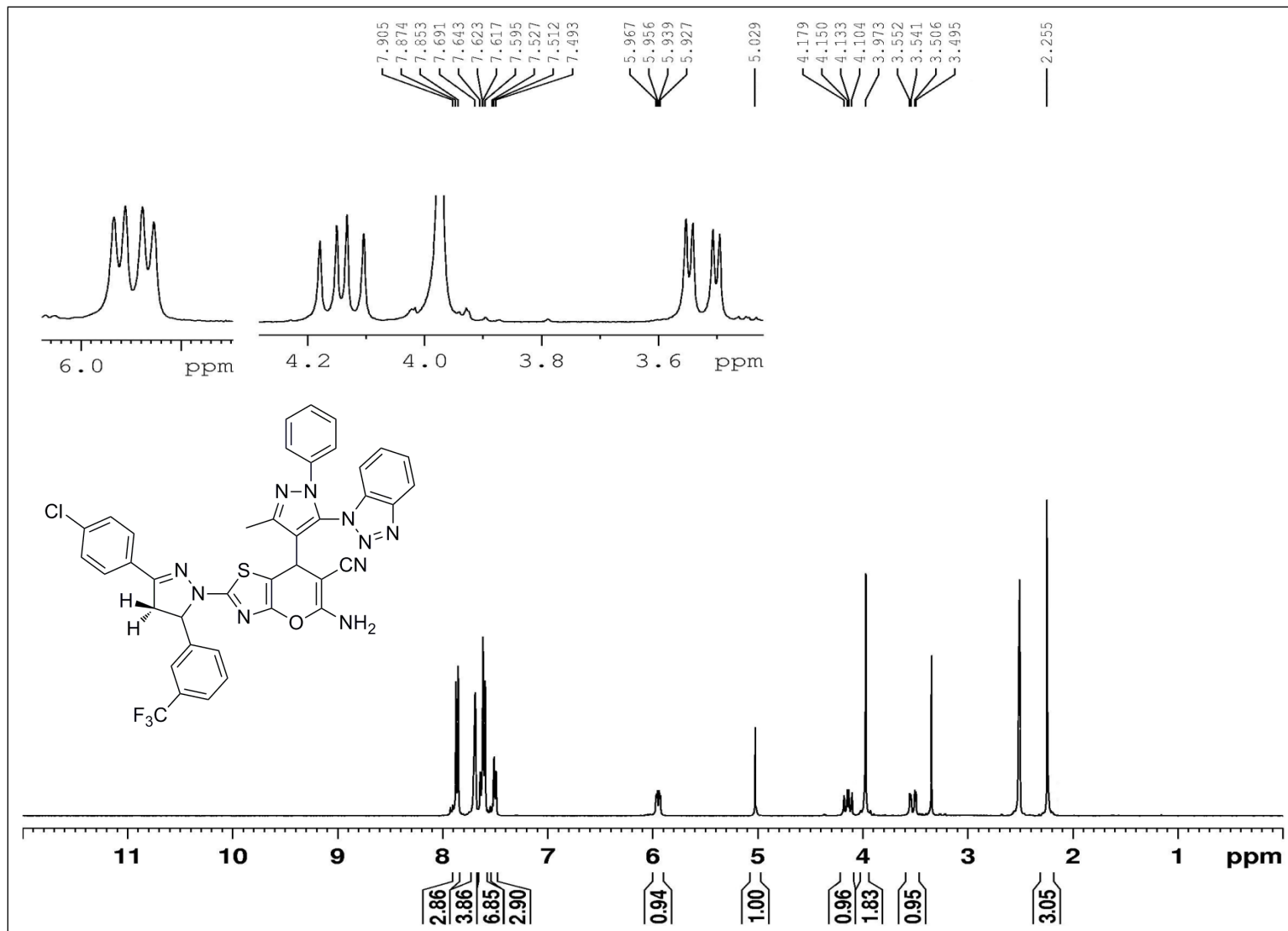
¹H NMR spectra of compound **11f**



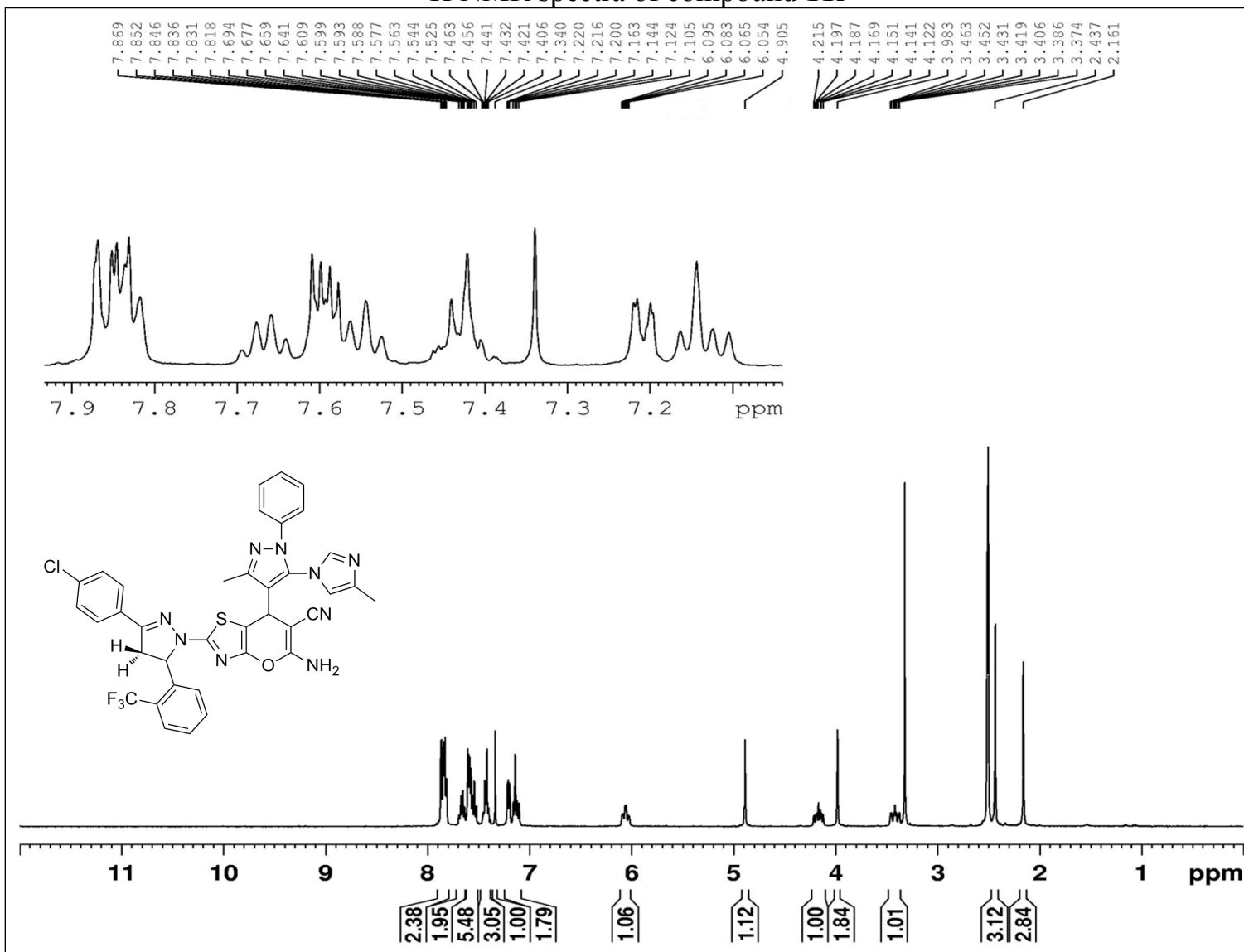
¹H NMR spectra of compound **11h**



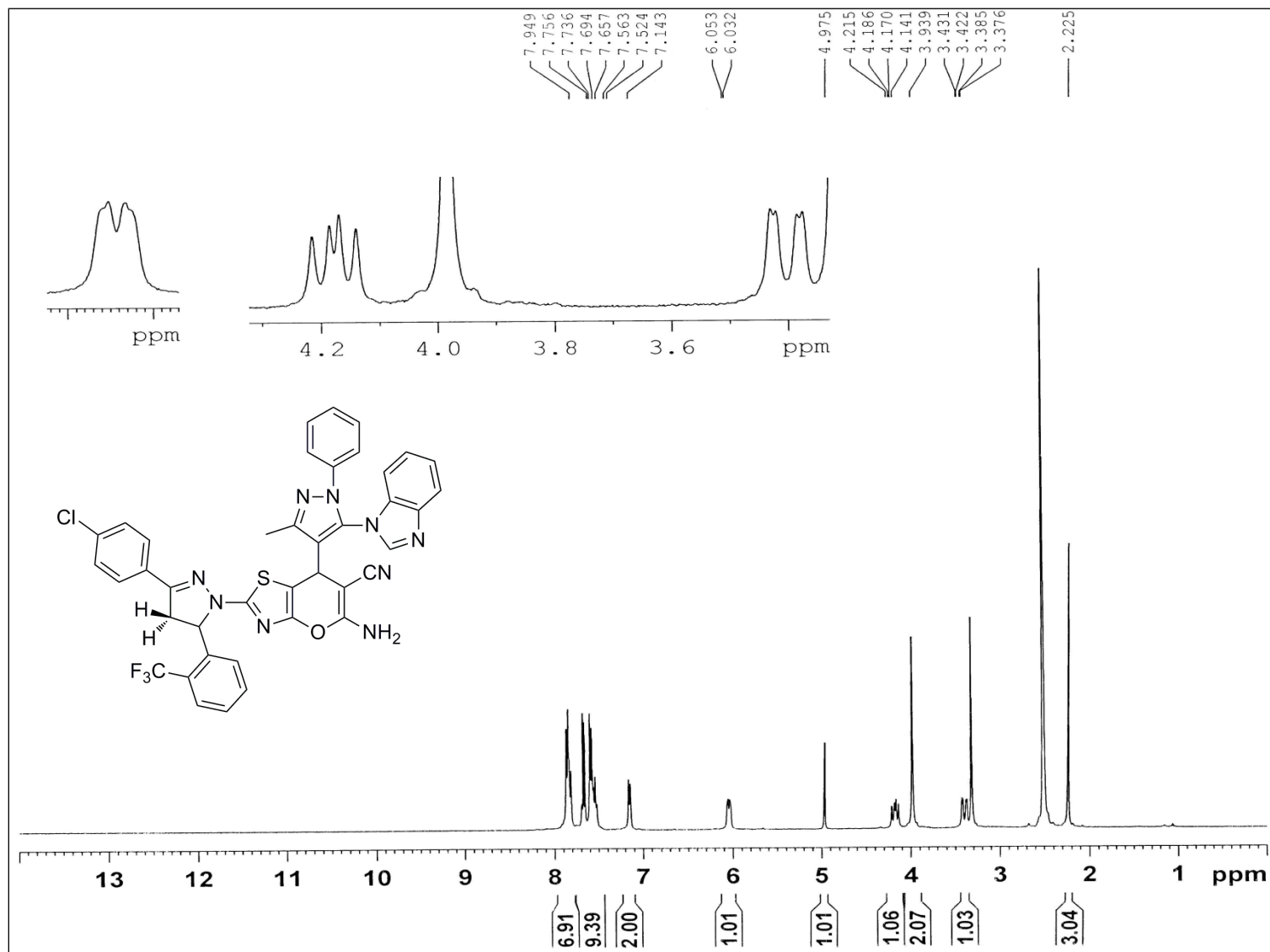
¹H NMR spectra of compound **11j**



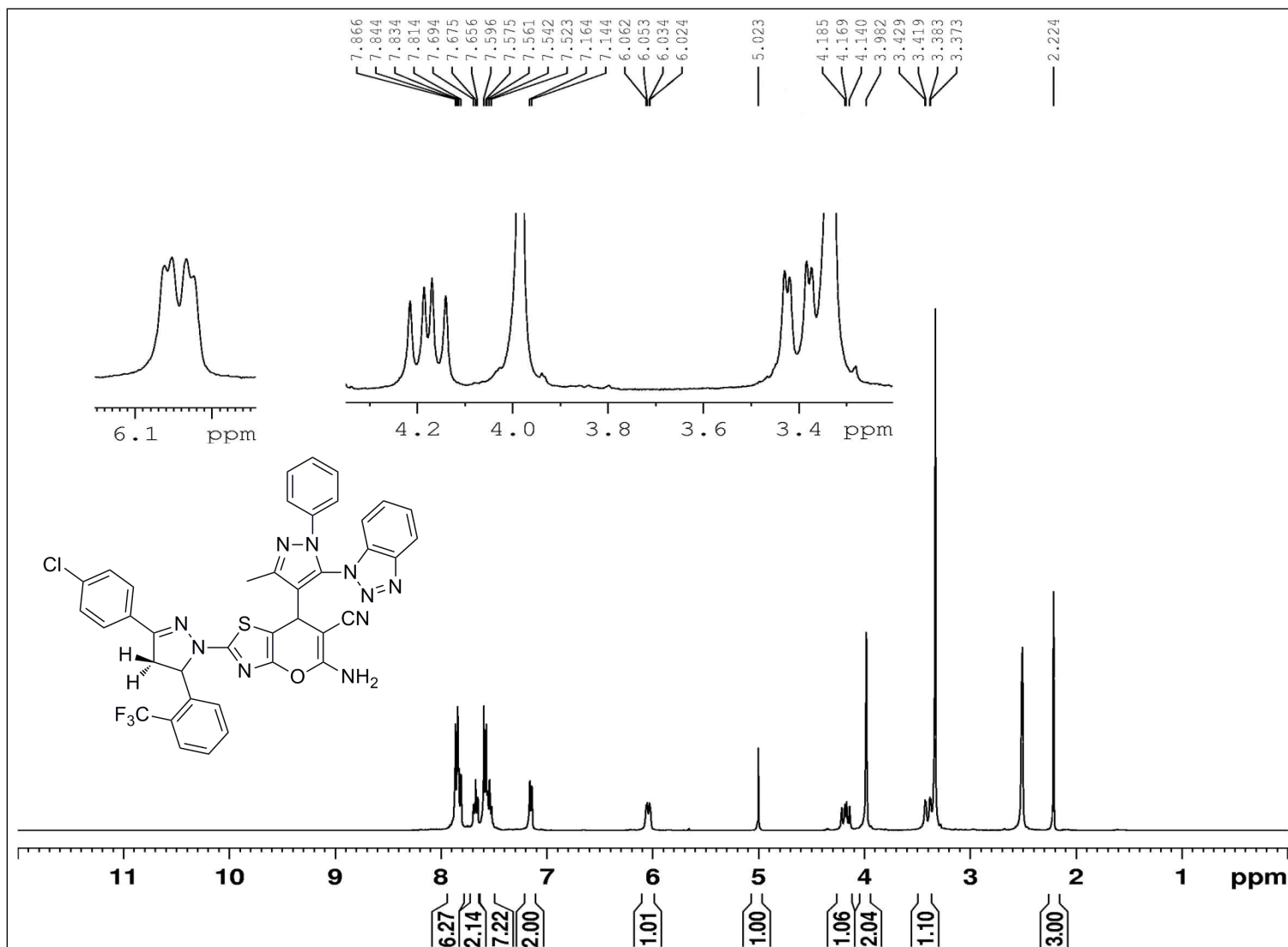
¹H NMR spectra of compound **11l**



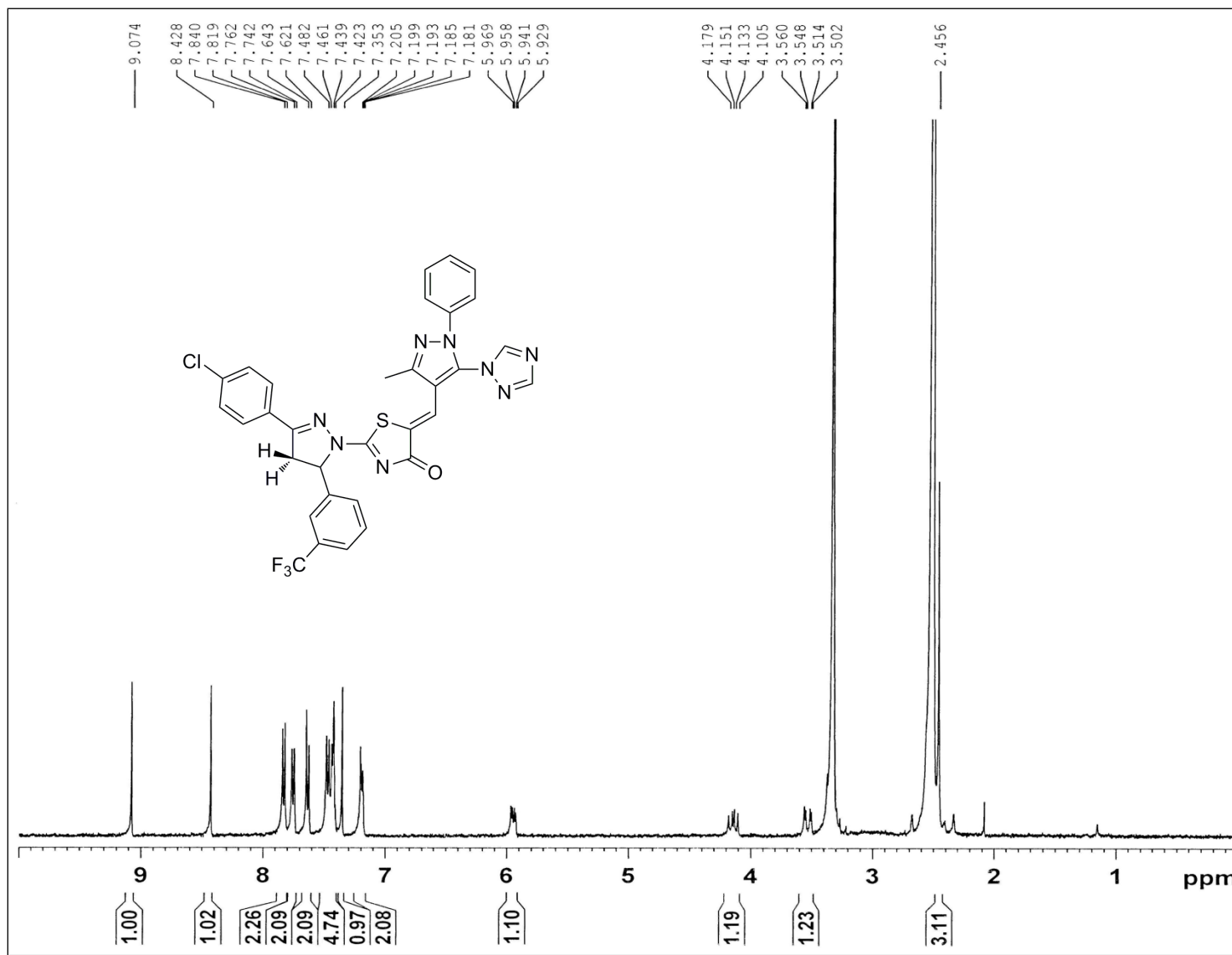
¹H NMR spectra of compound **11n**



¹H NMR spectra of compound **11o**



¹H NMR spectra of compound **12a**



1. Biological evaluation

1.1. *In vitro* antimicrobial assay

The *in vitro* antimicrobial activity of newly synthesized compounds (**11a-o**) was carried out by broth microdilution method. Mueller – Hinton broth was used as nutrient medium to grow and dilute the compound suspension for the test bacteria. 2% DMSO in water was used as the diluent to get desired concentration of compounds to test upon standard bacterial strains. Sabouraud Dextrose broth was used for fungal nutrition. Inoculum size for test strain was adjusted to 10^8 CFU mL⁻¹ by comparing the turbidity. Serial dilutions were prepared in primary and secondary screening. Each synthesized compound and the standard drugs were diluted obtaining 2000 µg/mL concentration as a stock solution. The drugs which were found to be active in primary screening (i.e. 500, 250 and 200 µg/mL concentrations) were further screened in their second set of dilution at 100, 50, 25 and 12.5 µg/mL concentration against all microorganisms. 10 µL suspensions were further inoculated on appropriate media and growth was noted after 24 and 48 hrs. The control tube containing no antibiotic was instantaneously subcultured by spreading a consistently over an area of plate of medium fitting for the growth of the test organism. The tubes were then put for incubation at 37°C overnight. The maximum dilution preventing appearance of turbidity after spot subculture was considered as minimal inhibitory concentration (MIC, µ/L). All the tubes showing no visible growth (same as control tube) were subcultured and incubated overnight at 37 °C. The amount of growth from the control tube before incubation was compared. In this study Ampicillin, Norfloxacin and Ciprofloxacin were used as the standard antibacterial drugs while Nystatin and Griseofulvin were used as standard antifungal drugs.

2.2. *In vitro* antituberculosis assay

The antitubercular activity of all the synthesized compounds against *Mycobacterium tuberculosis* H37Rv was performed by Lowenstein-Jensen method¹ with minor modification where 250 µg/mL and 100 µg/mL dilution of each compound was added to Lowenstein-Jensen medium and then media was uncontaminated by inspissations method. A culture of *Mycobacterium tuberculosis* H37Rv growing on

Lowenstein-Jensen medium was harvested in 0.85% saline in bijou bottle. The stock solutions of the title compounds were prepared in DMSO i.e. 100 µg/mL. These tubes were then incubated at 37 °C for 1 day followed by streaking of *Mycobacterium tuberculosis* H37Rv (5 _ 10⁴ bacilli per tube). The growth of bacilli was seen after two weeks, three weeks and finally after four weeks of incubation. The tubes having the compounds were compared with control tubes where medium alone was incubated with *Mycobacterium tuberculosis* H37Rv. The concentration at which complete inhibition of colonies occurred was taken as active concentration of the tested compound. The standard drugs isoniazid and rifampicin were used for comparison purpose.

References

1. A. Rattan, *Antimicrobials in Laboratory Medicine*. Churchill B. I., Livingstone, New Delhi,, 2000, 85.