

A Structural Blueprint for Antibacterial Discovery: Microwave- and Ultrasound-Assisted Synthesis of Pyrrolidine-Fused Quinoxalines as Novel Inhibitors of DNA Gyrase and Biofilm

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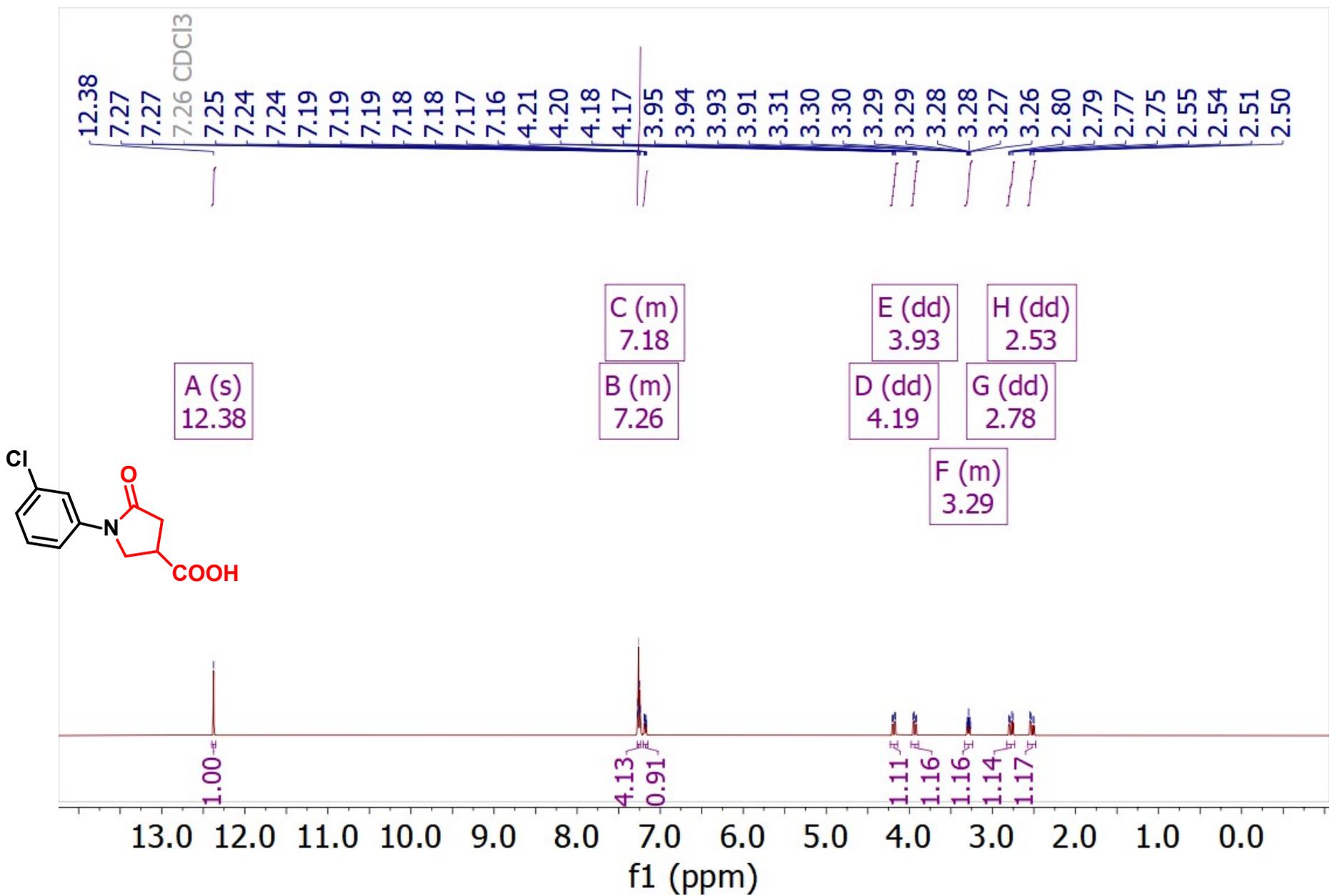
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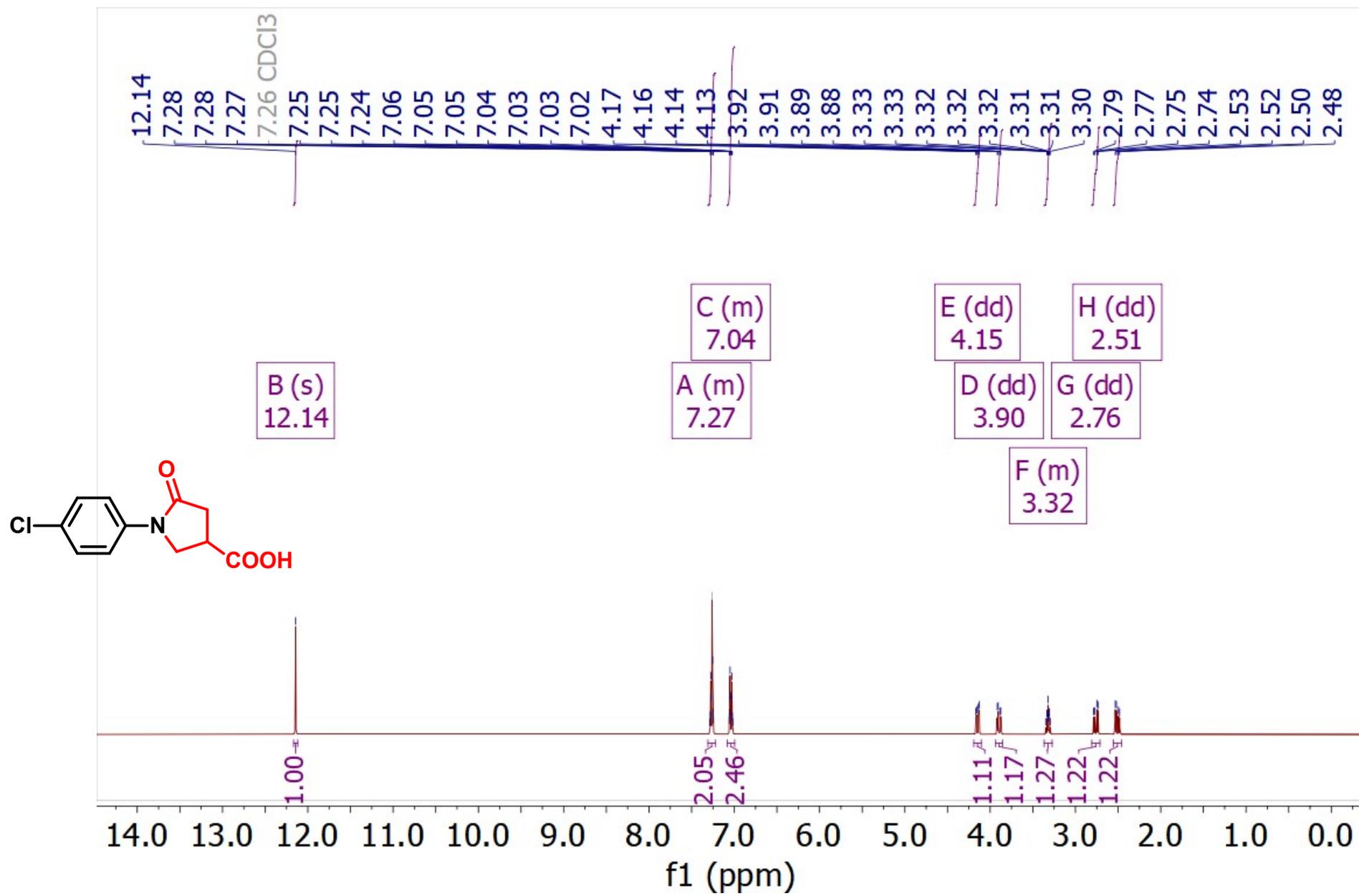
Chemistry

Spectral Data

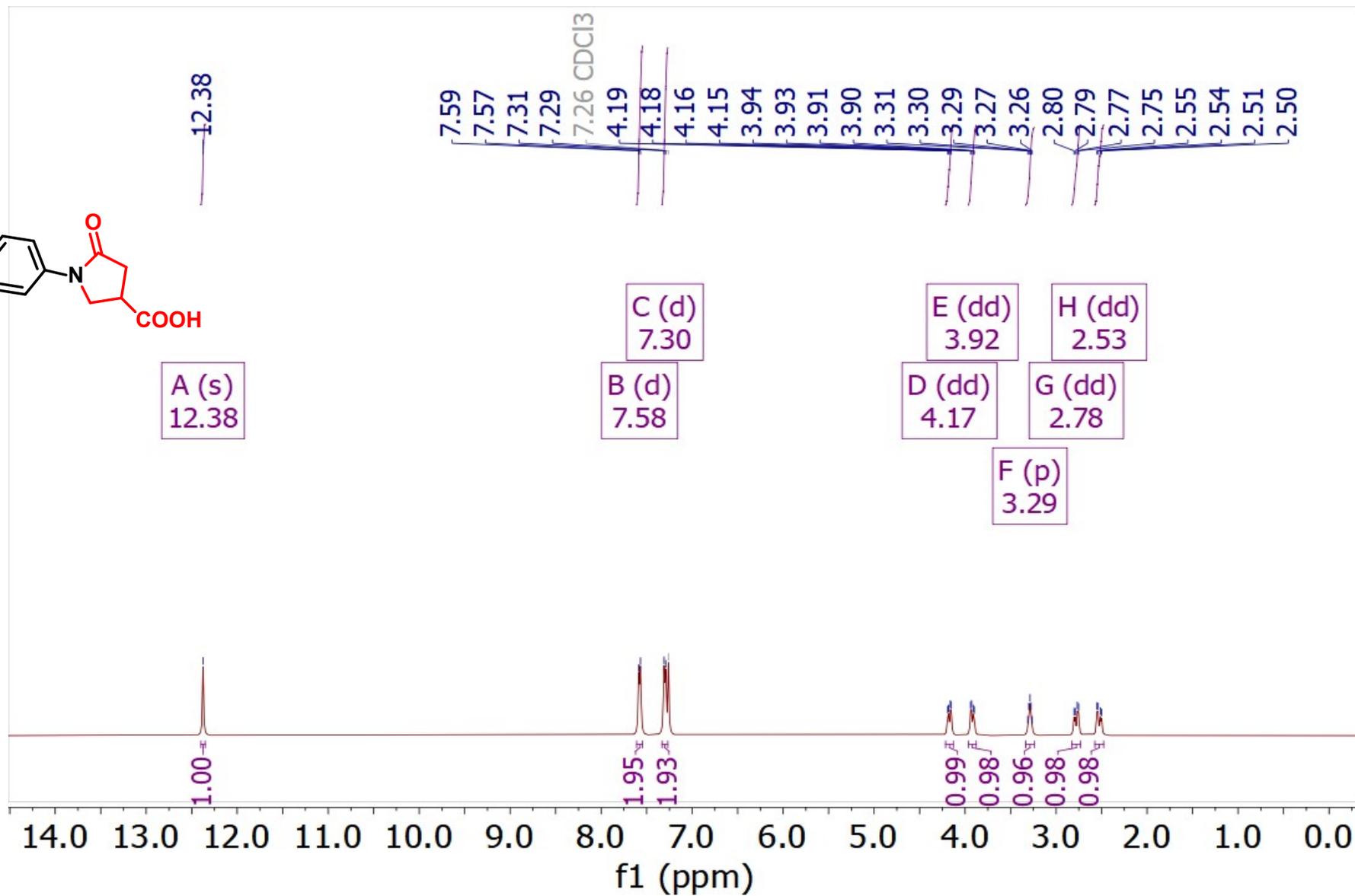
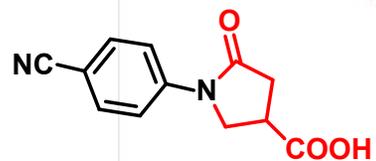
Compound 16



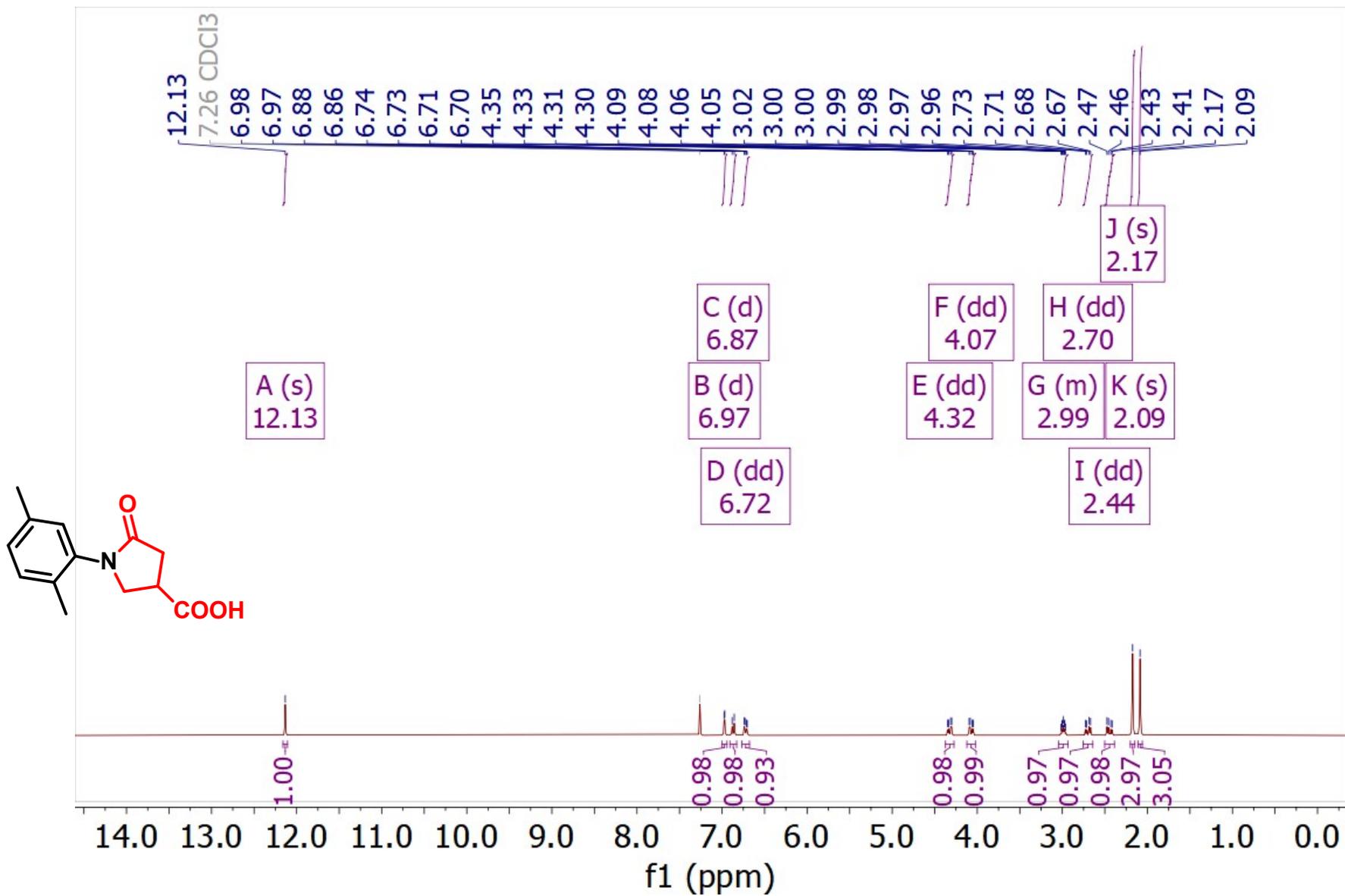
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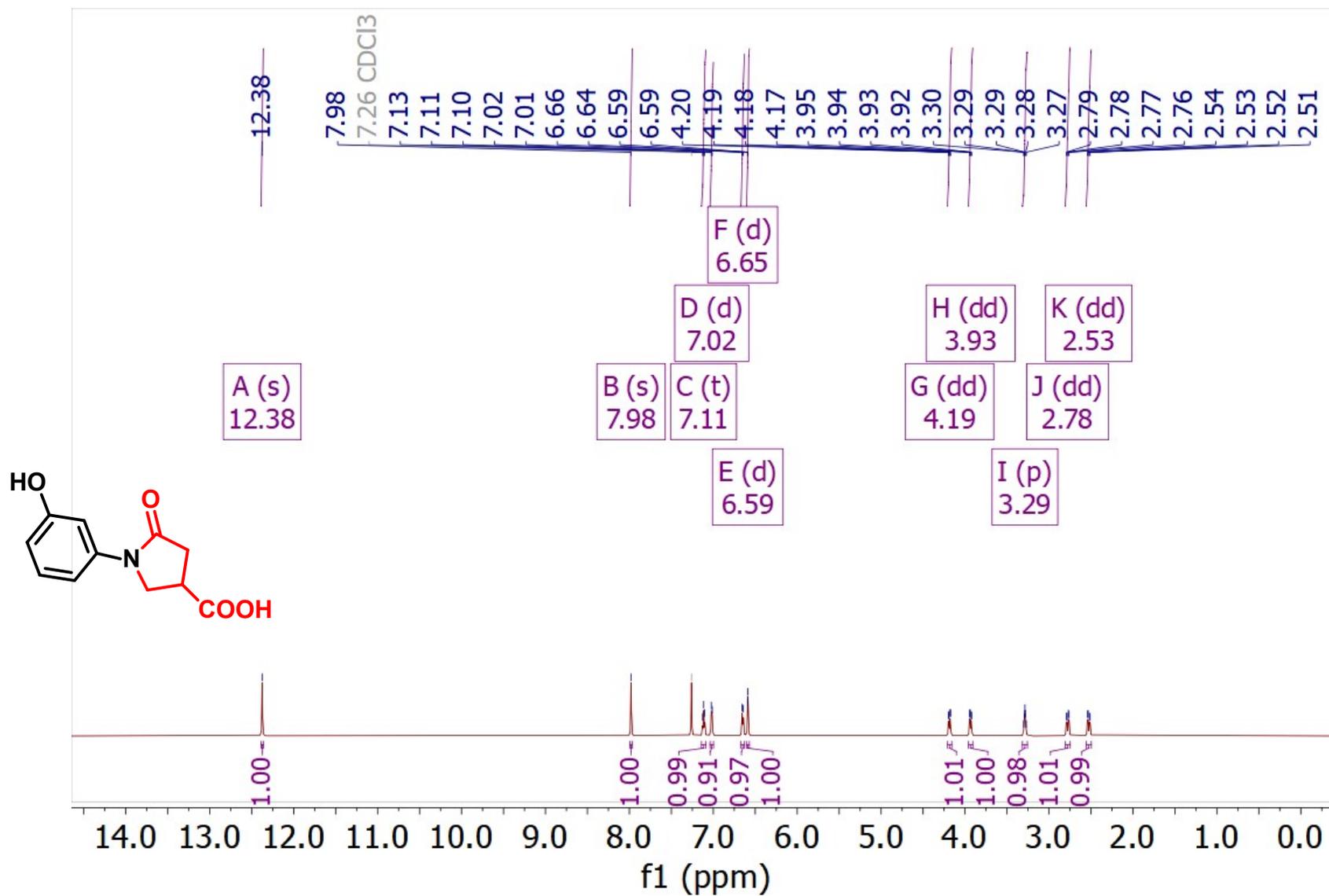
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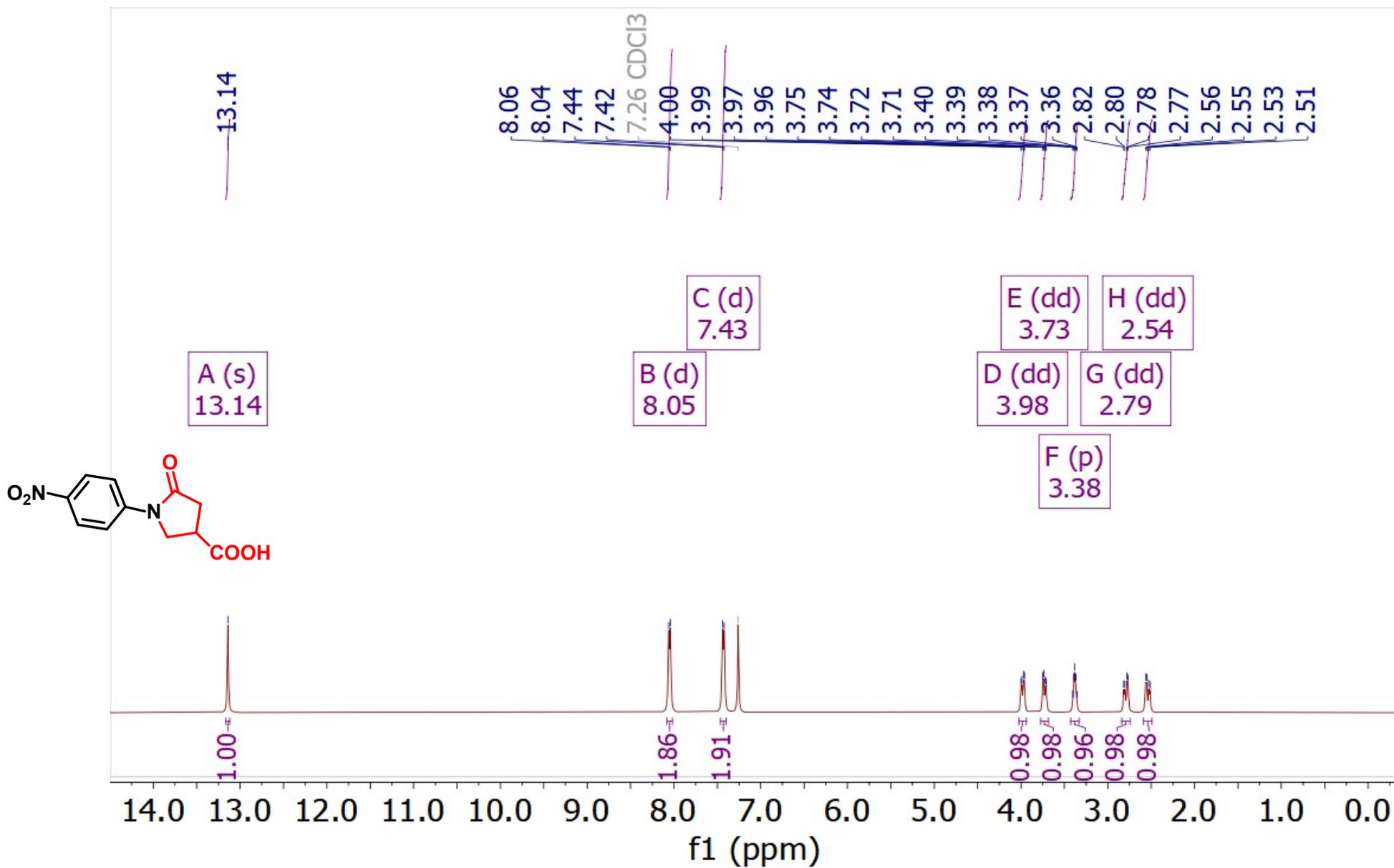
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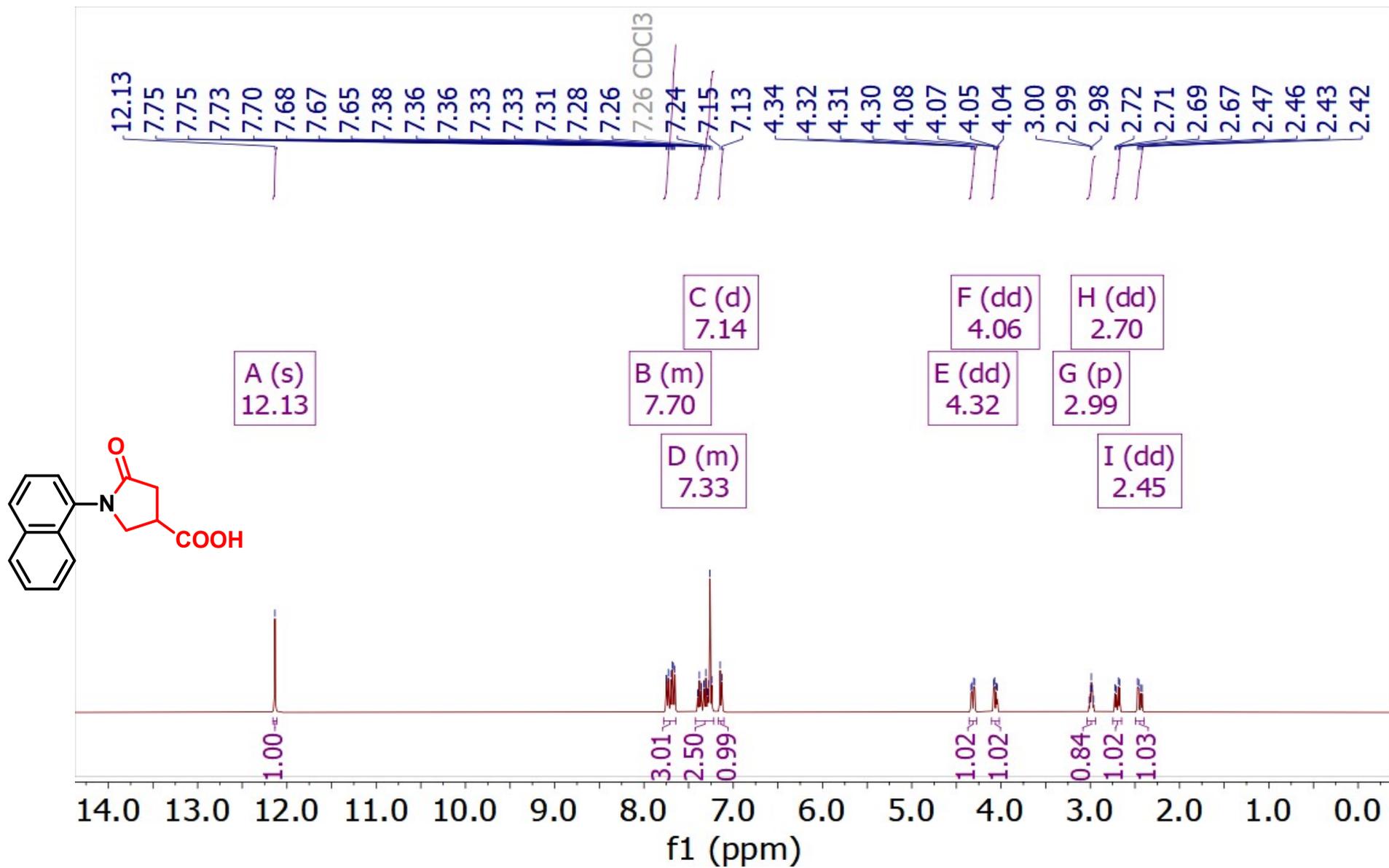
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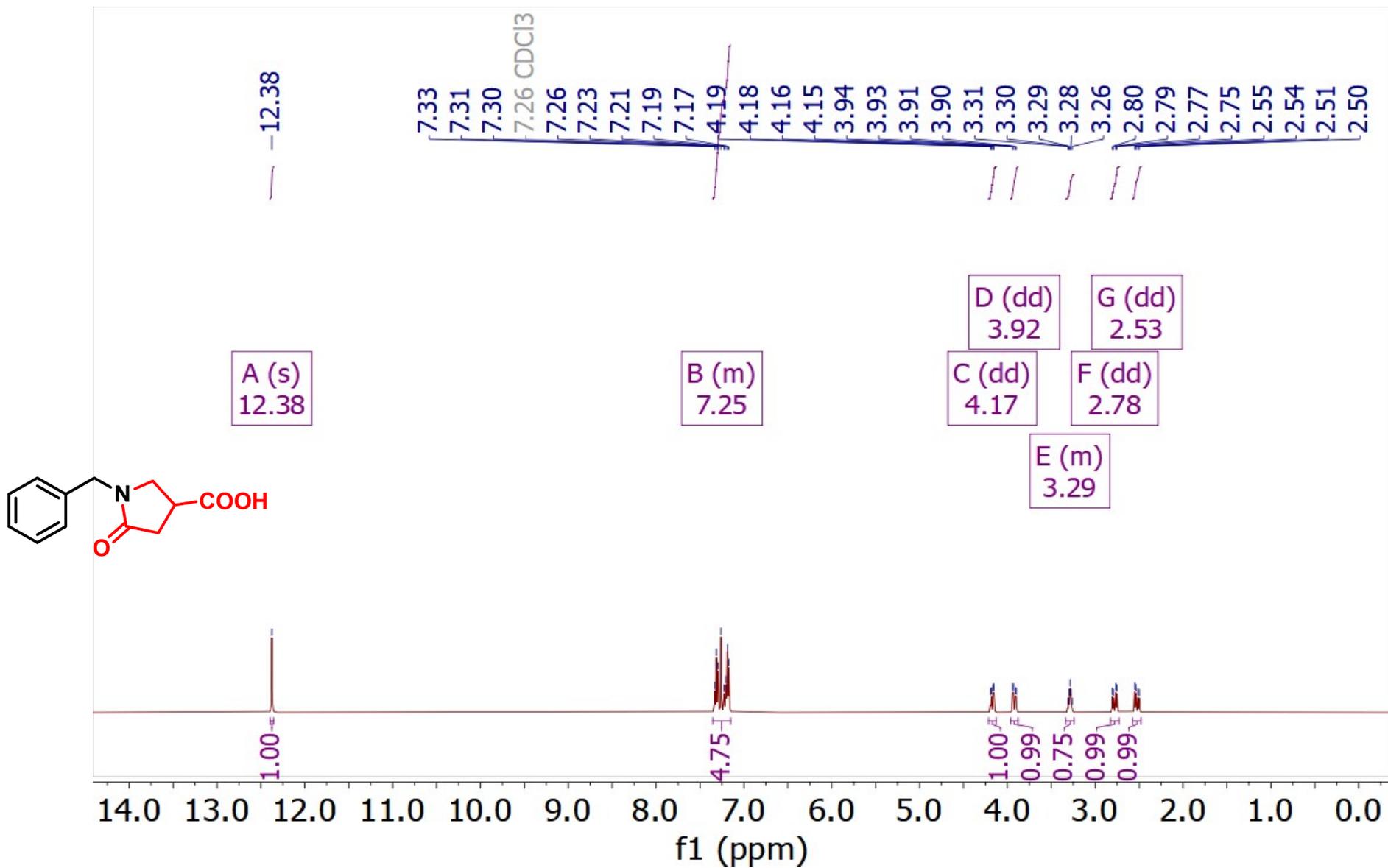
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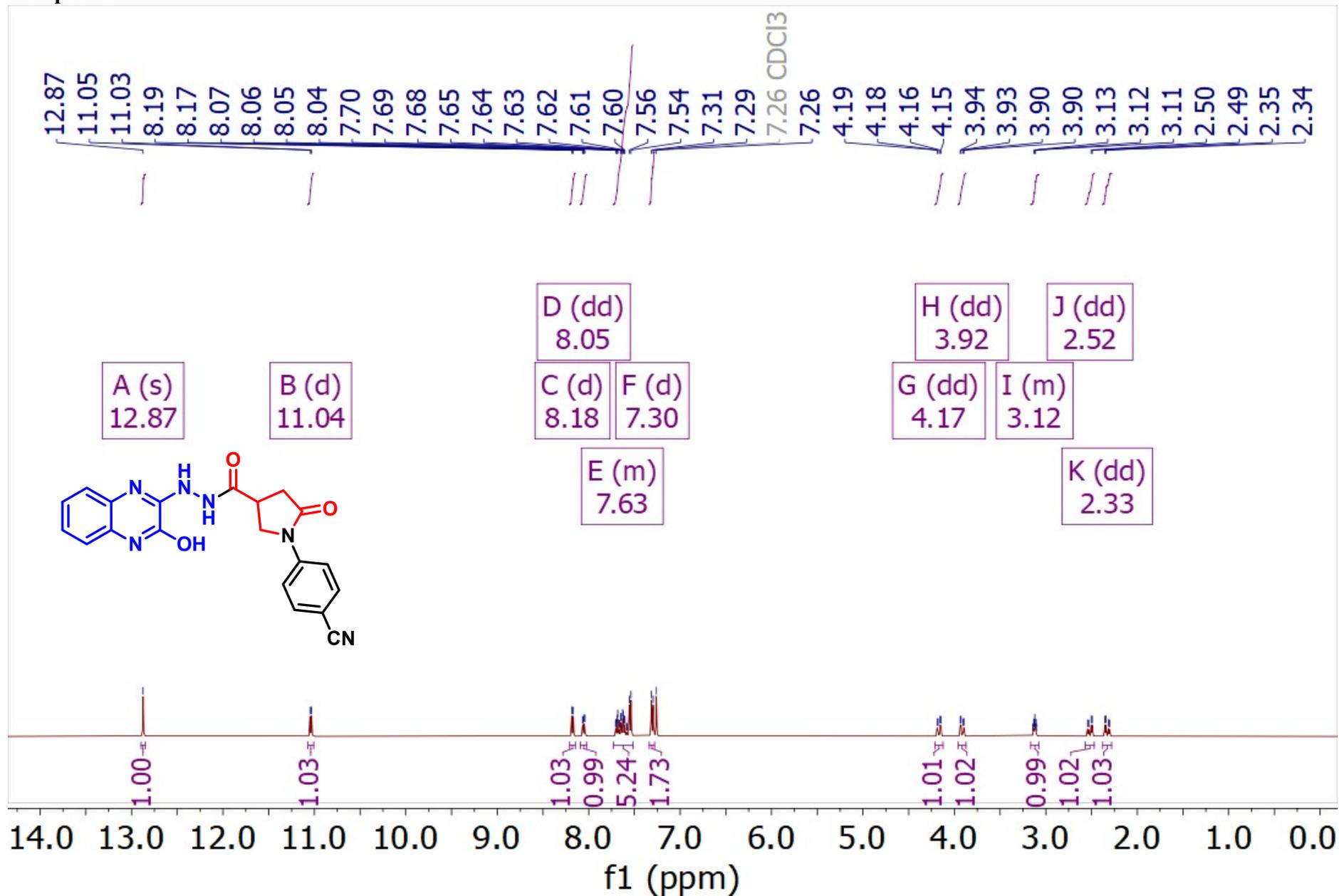
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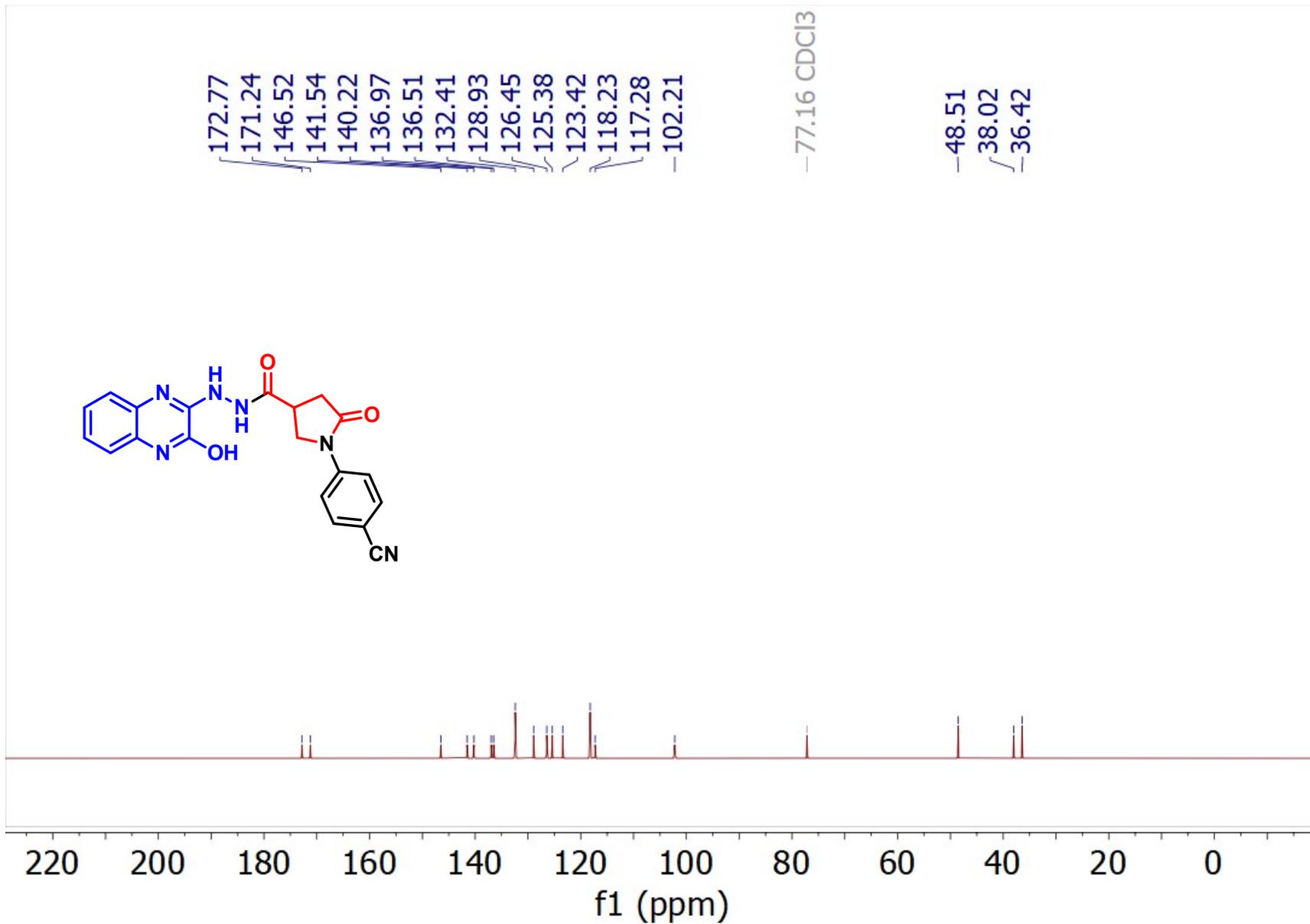
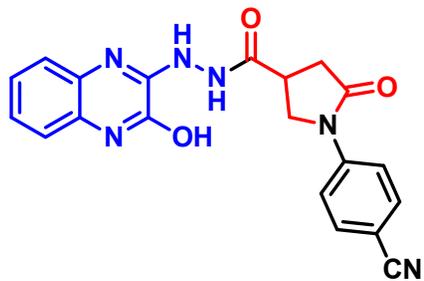


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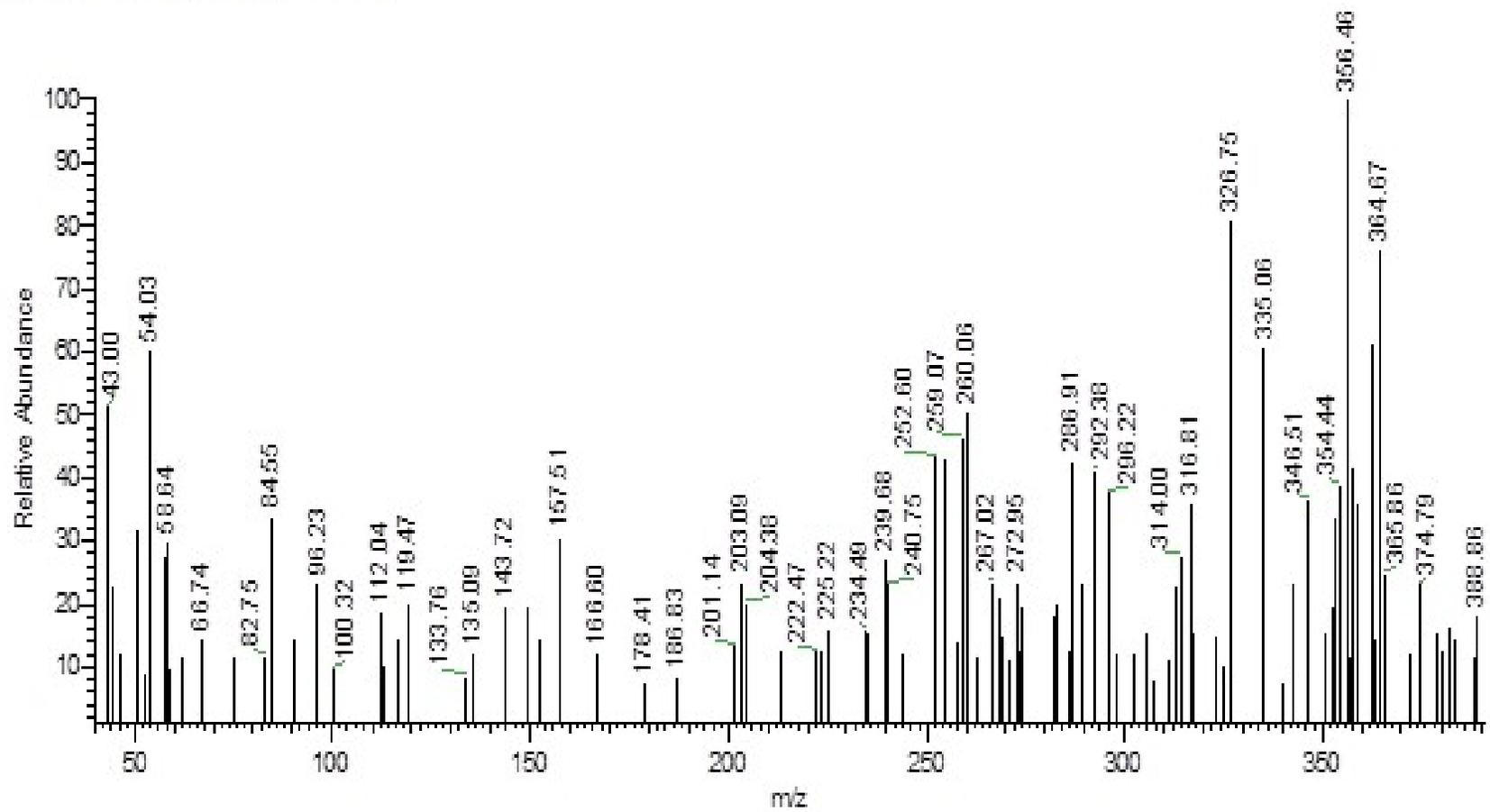


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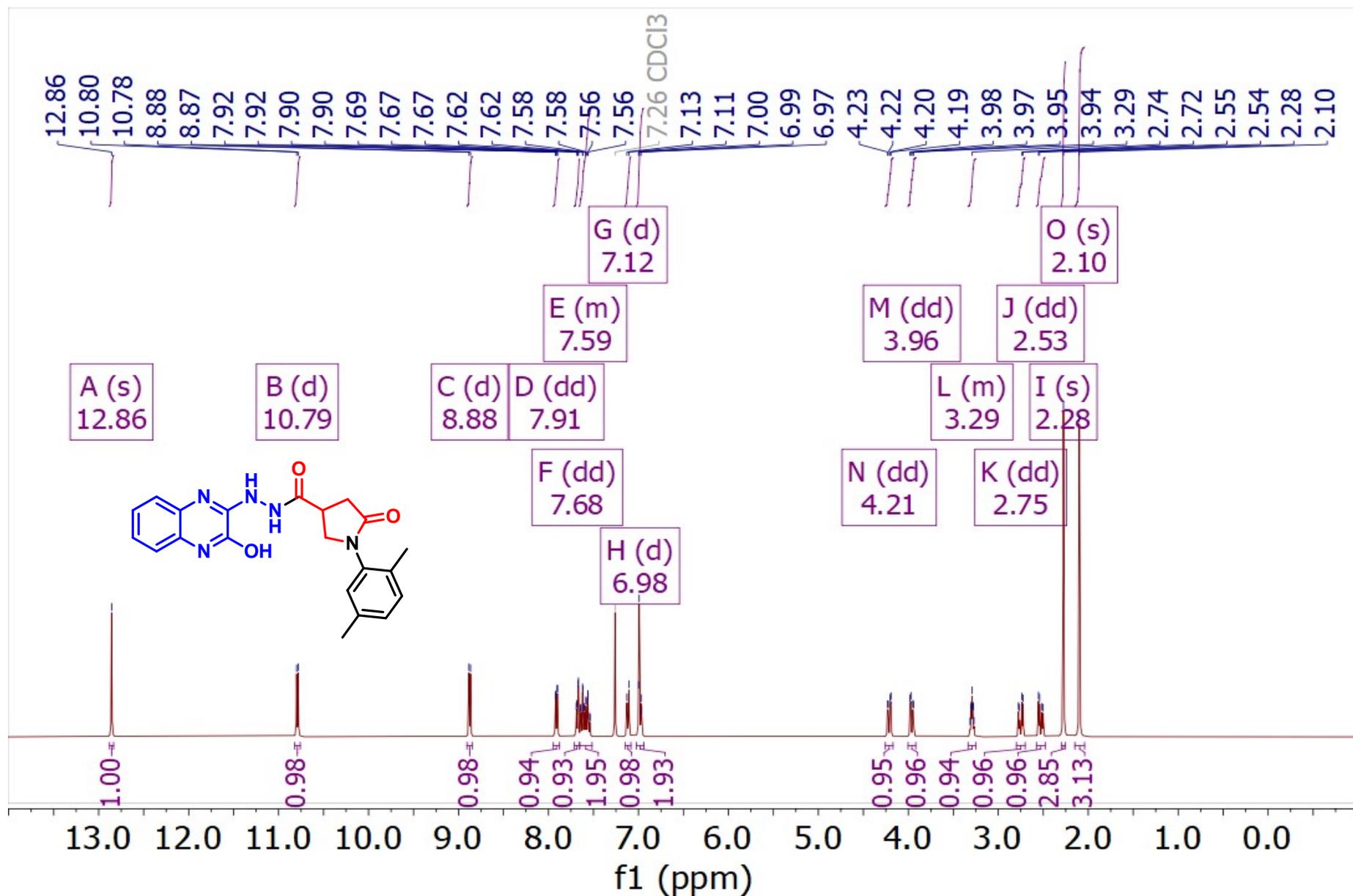


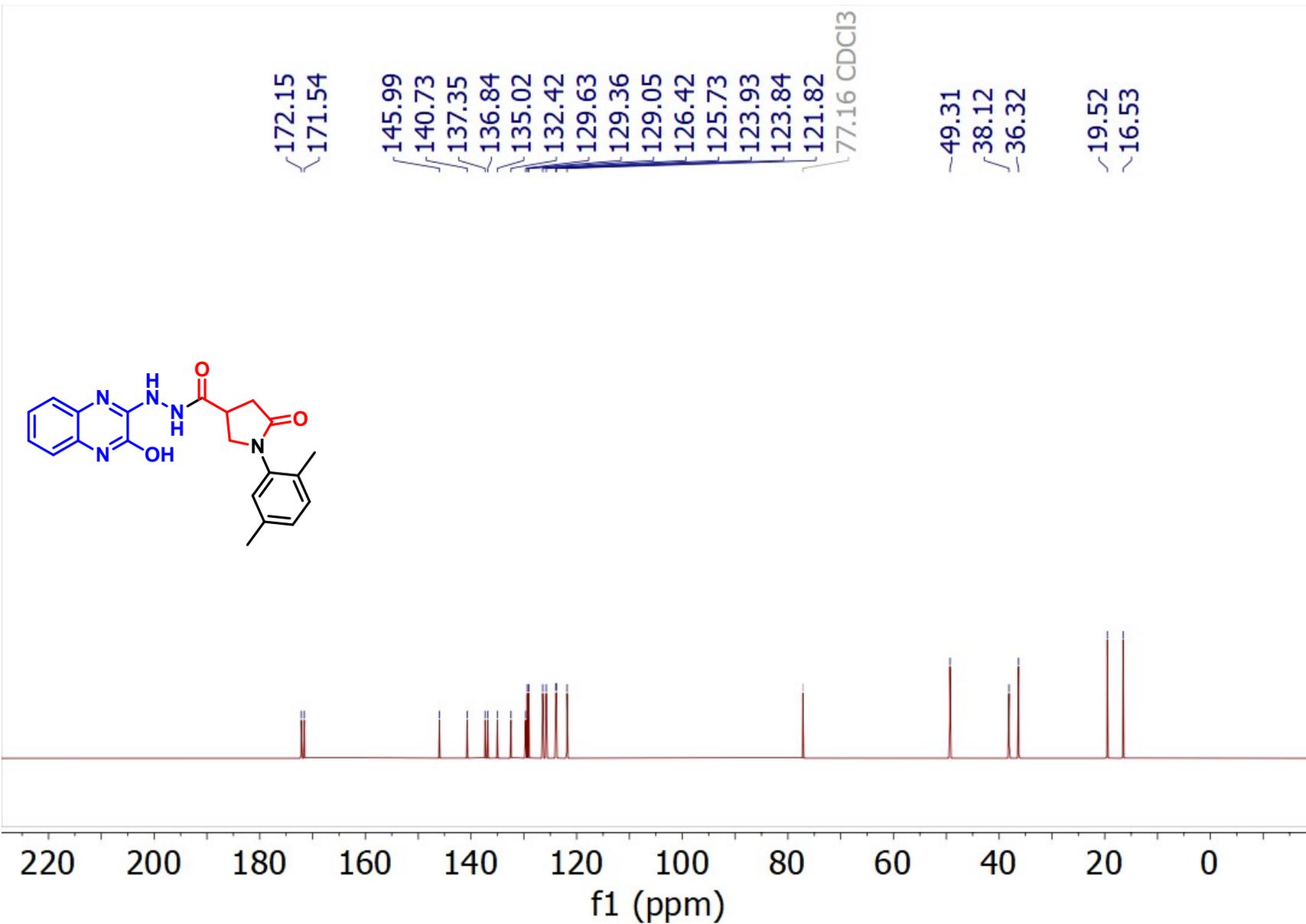
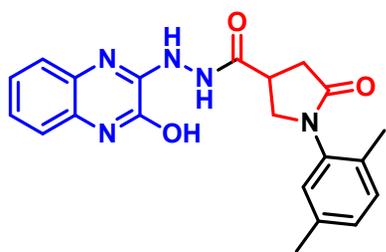


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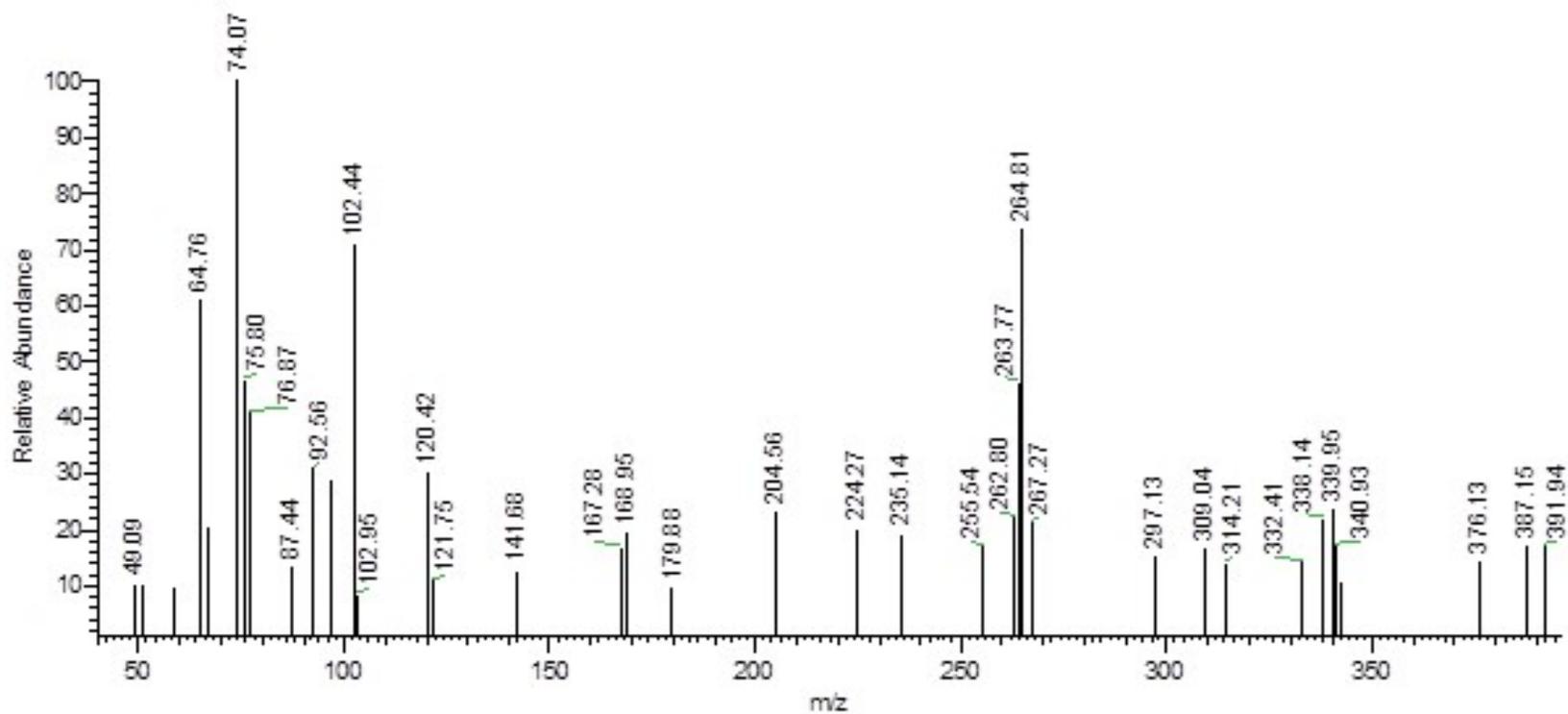


Compound 25

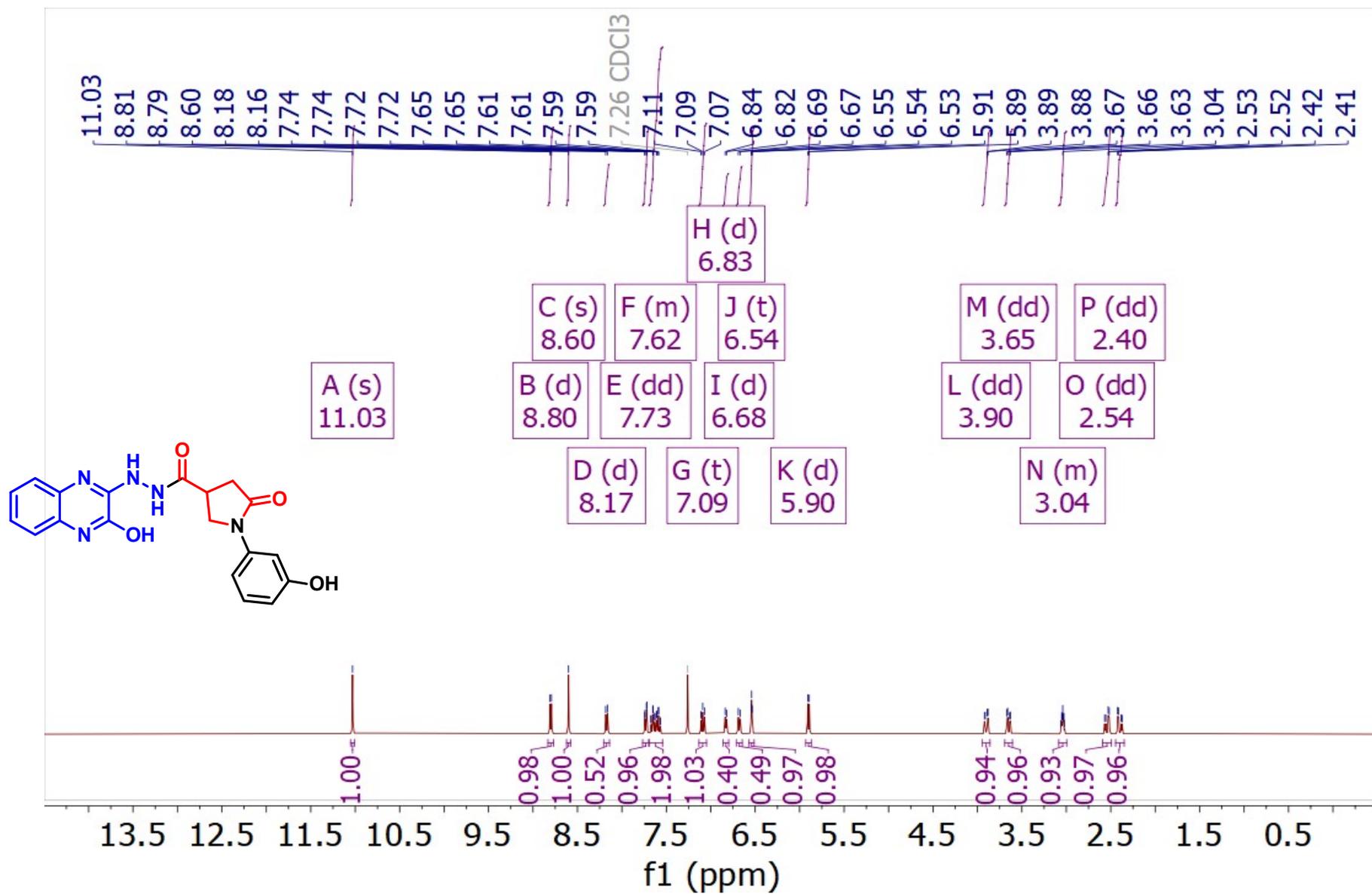


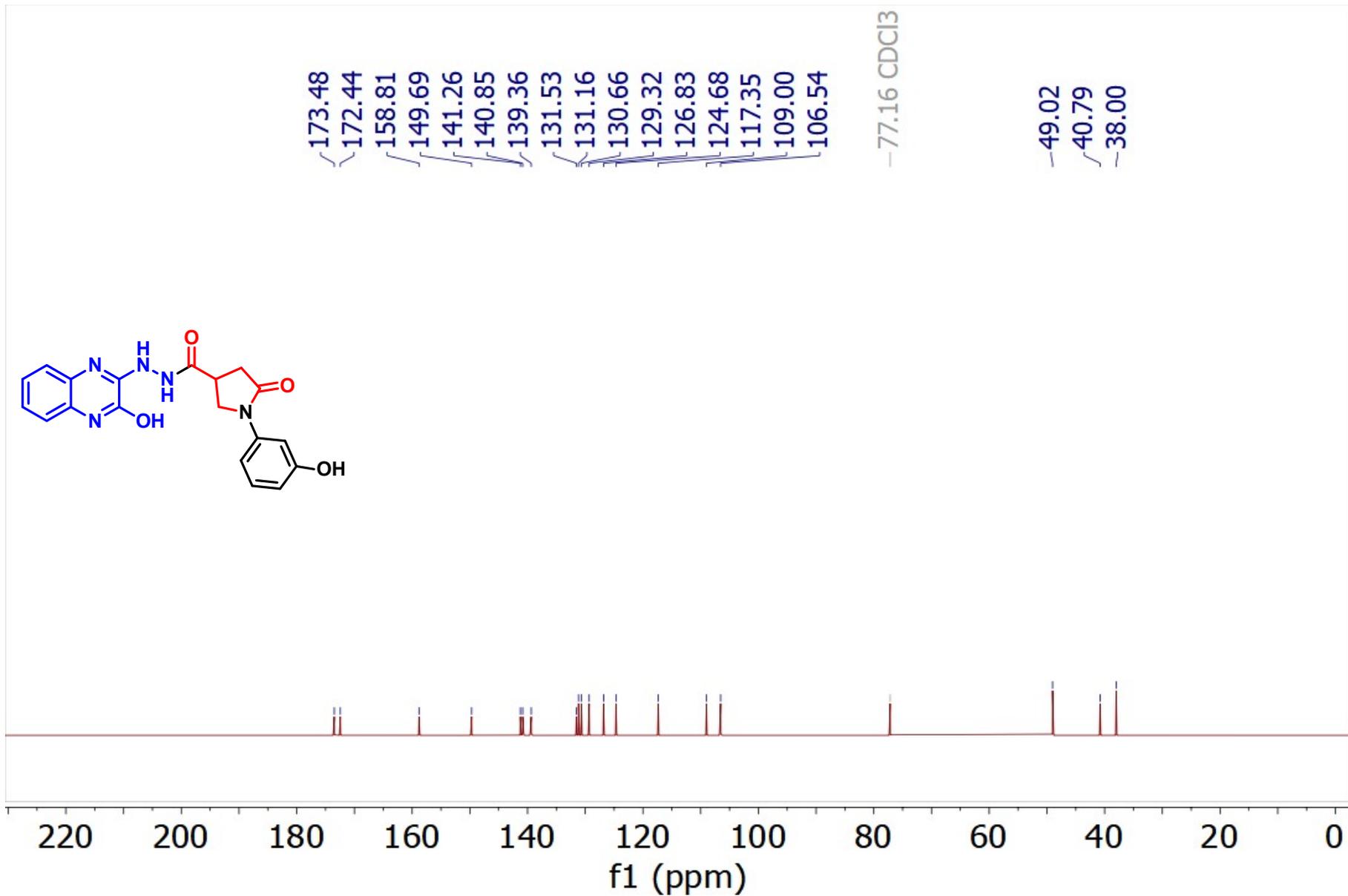
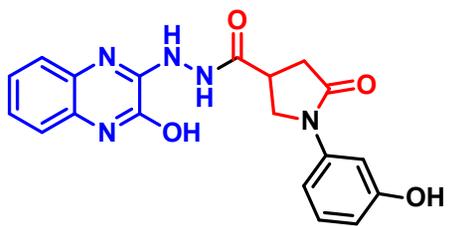


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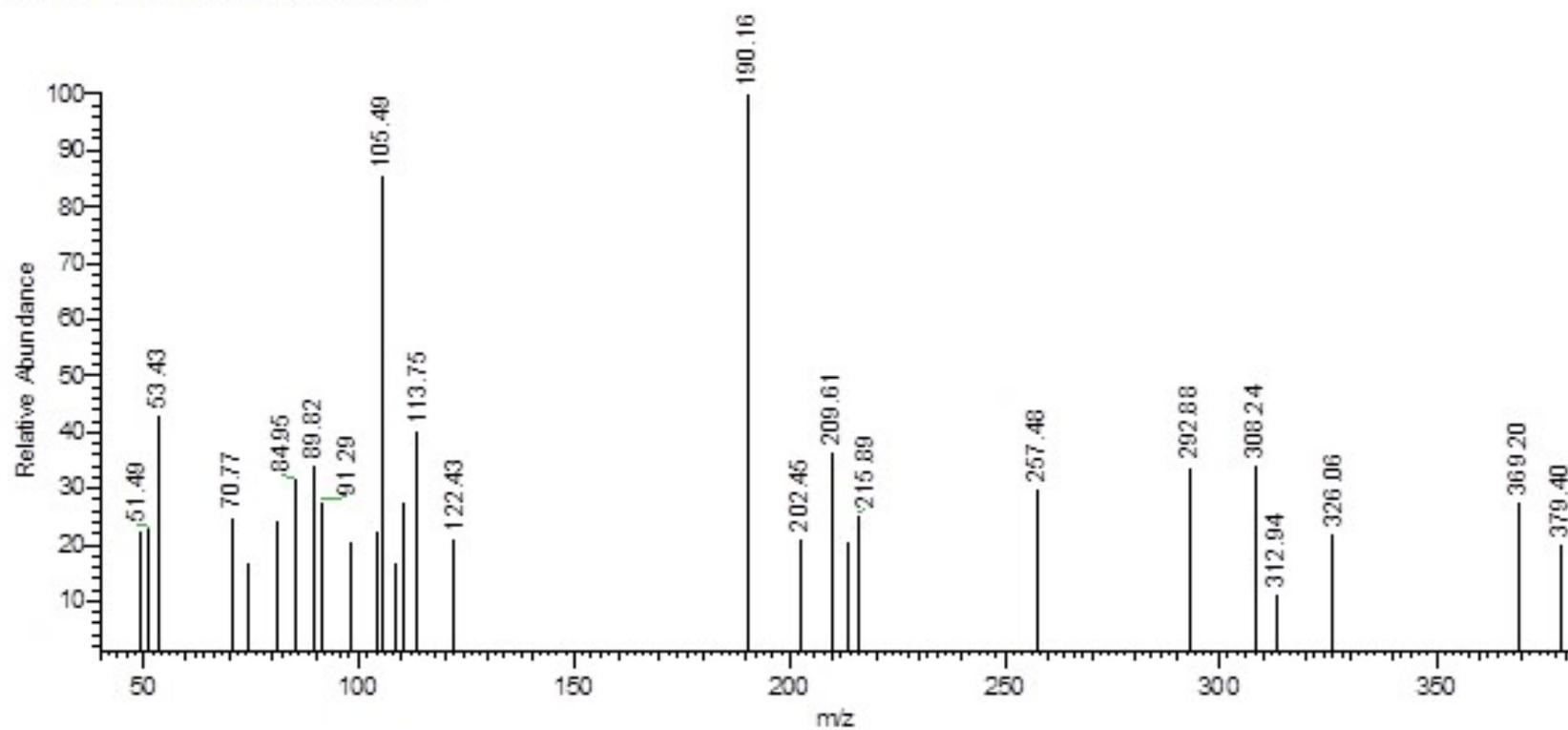


Compound 26

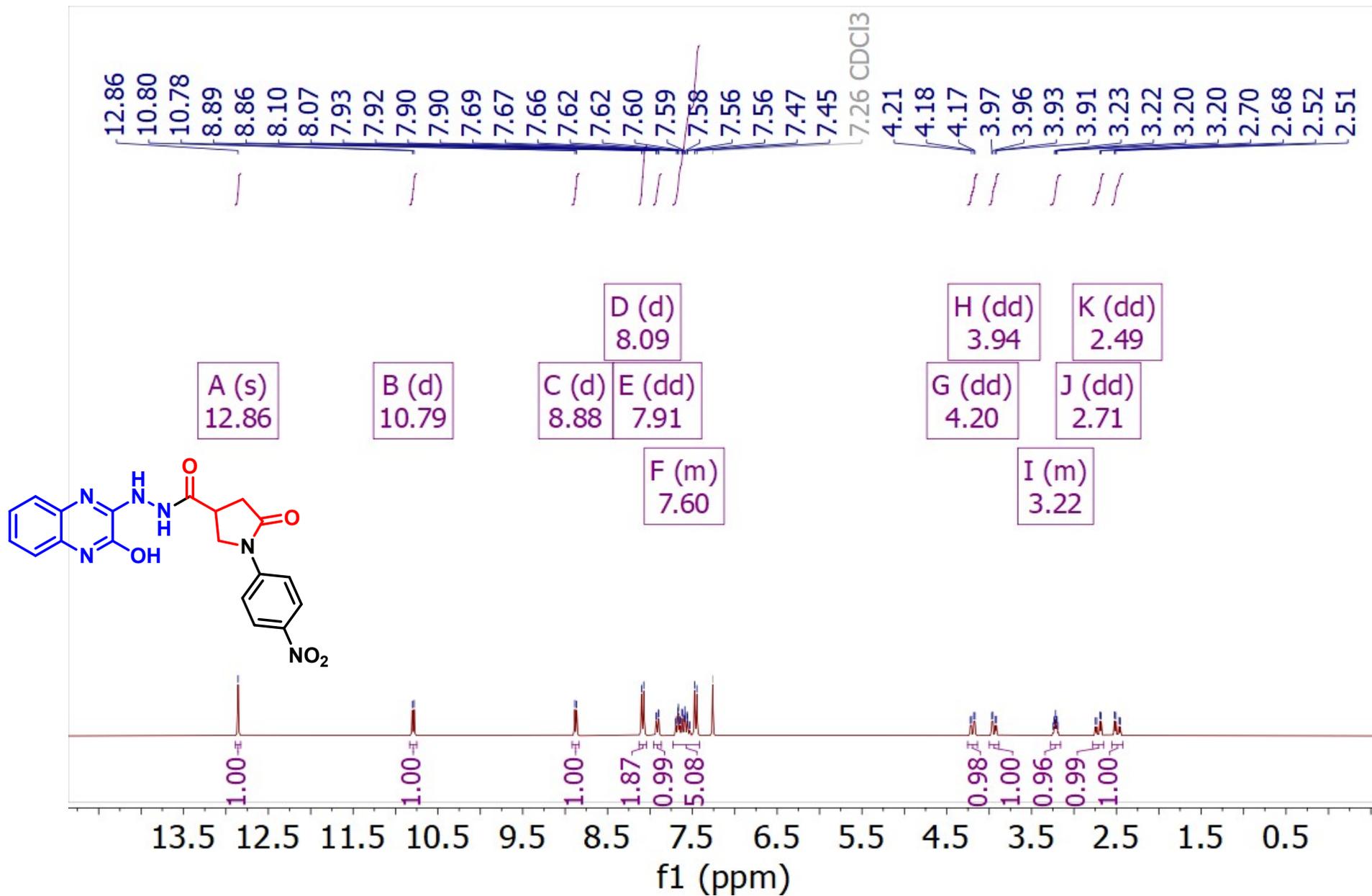


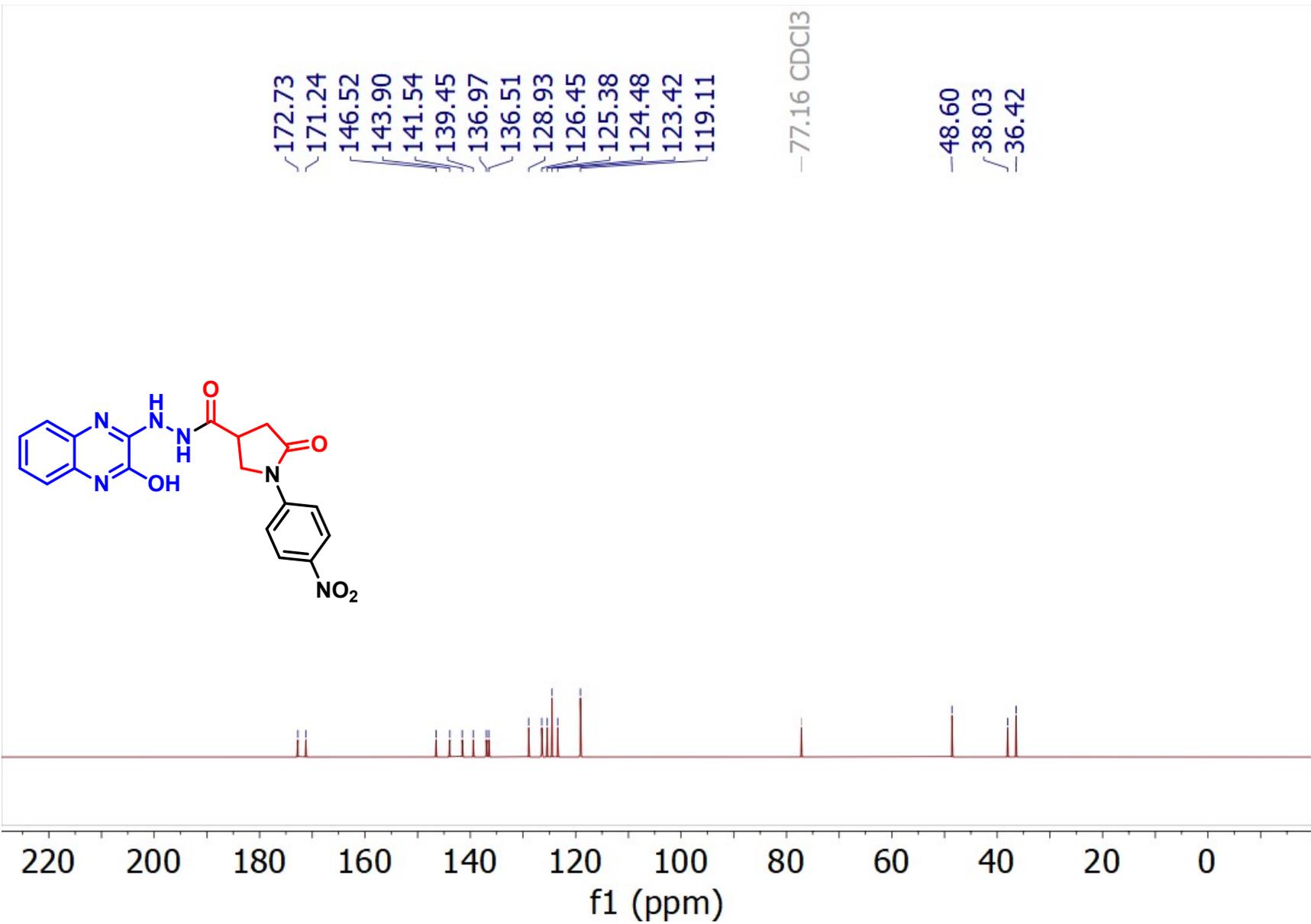
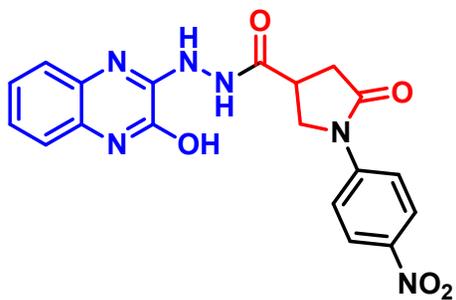


NE #63 RT: 0.90 P: + NL: 5.14E2
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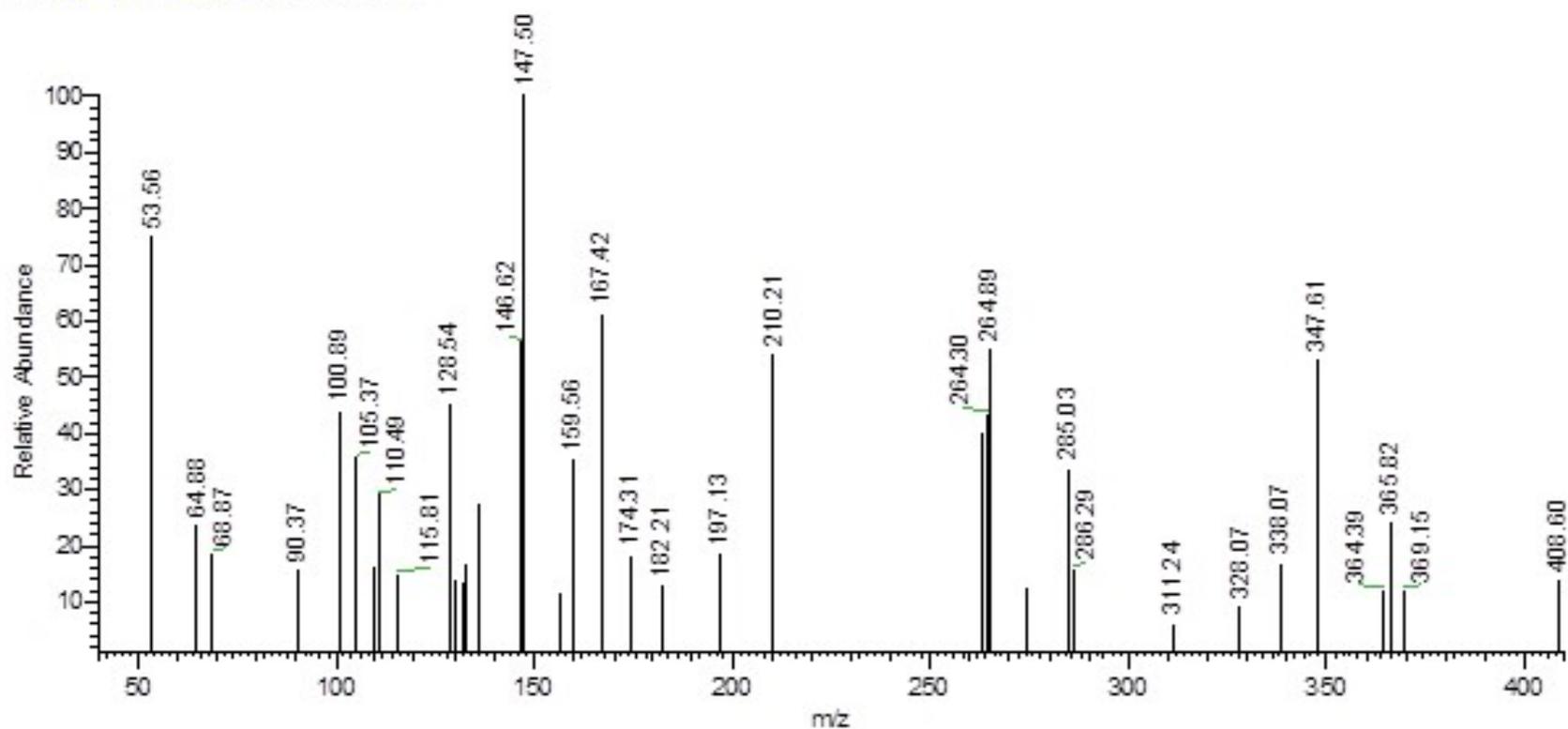


Compound 27

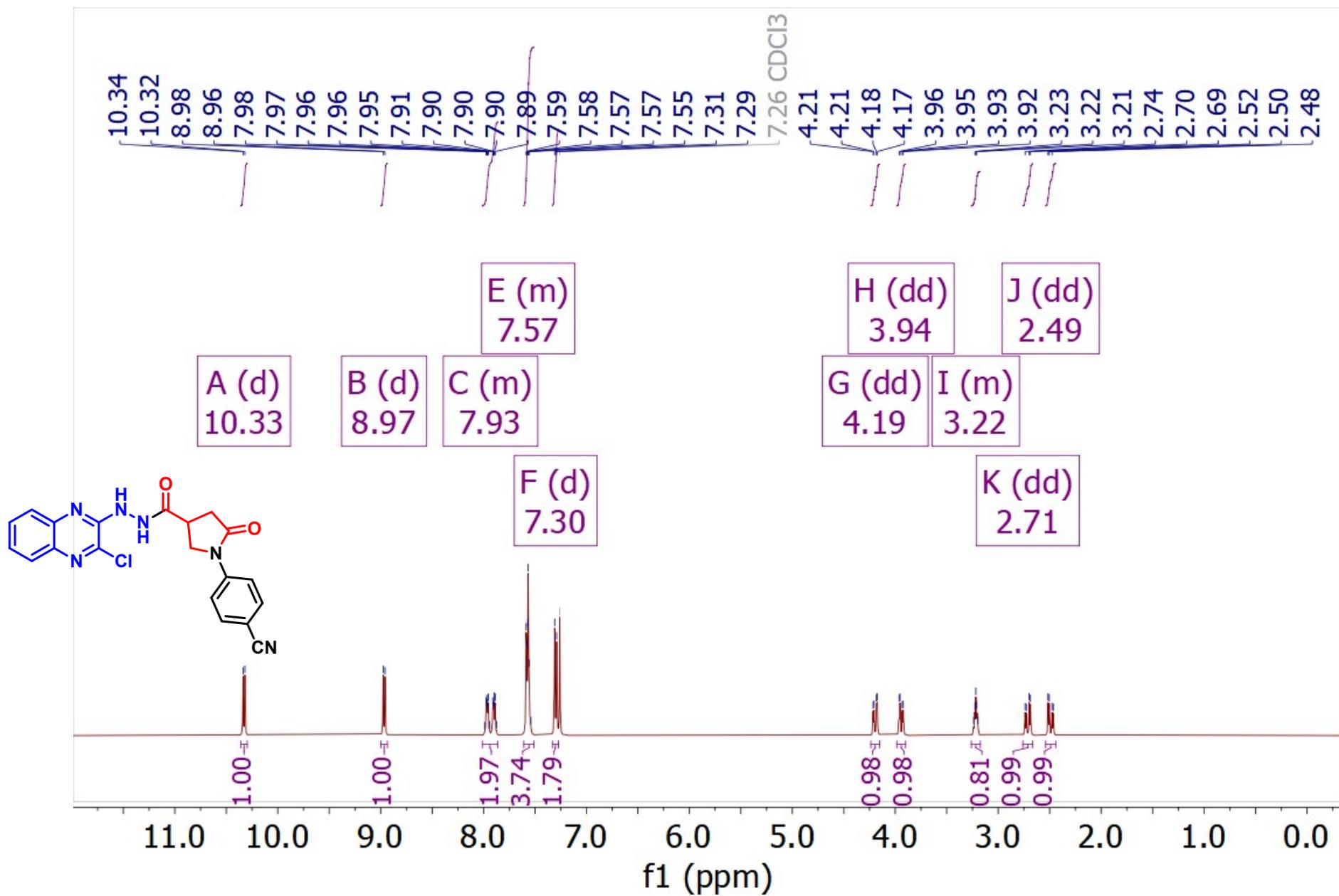


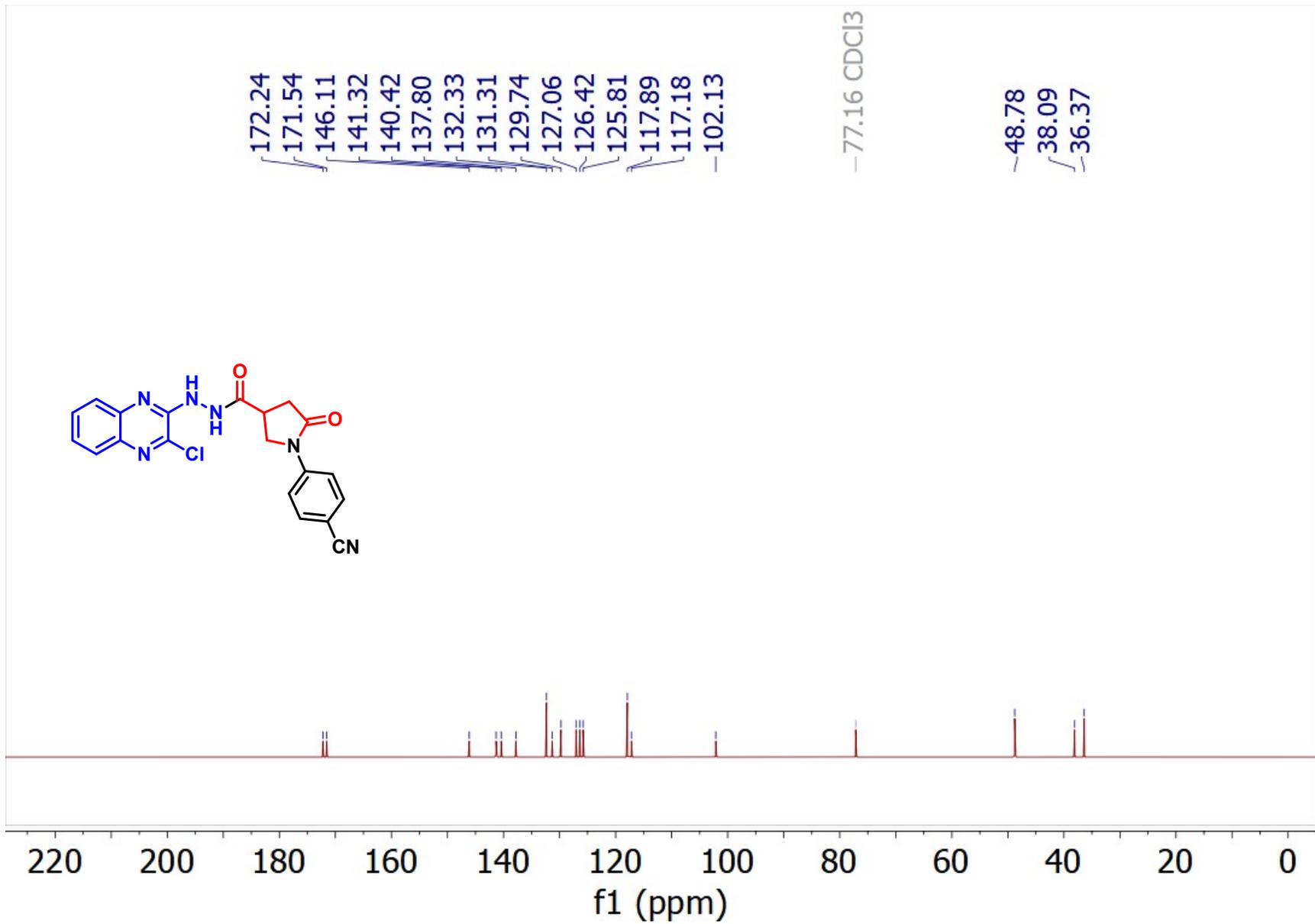
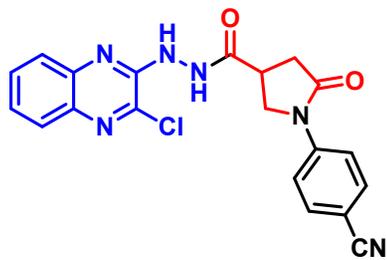


NG#263 RT: 4.42 P: + NL: 5.30E2
T: {0,0} + cEI Full ms [40.00-1000.00]

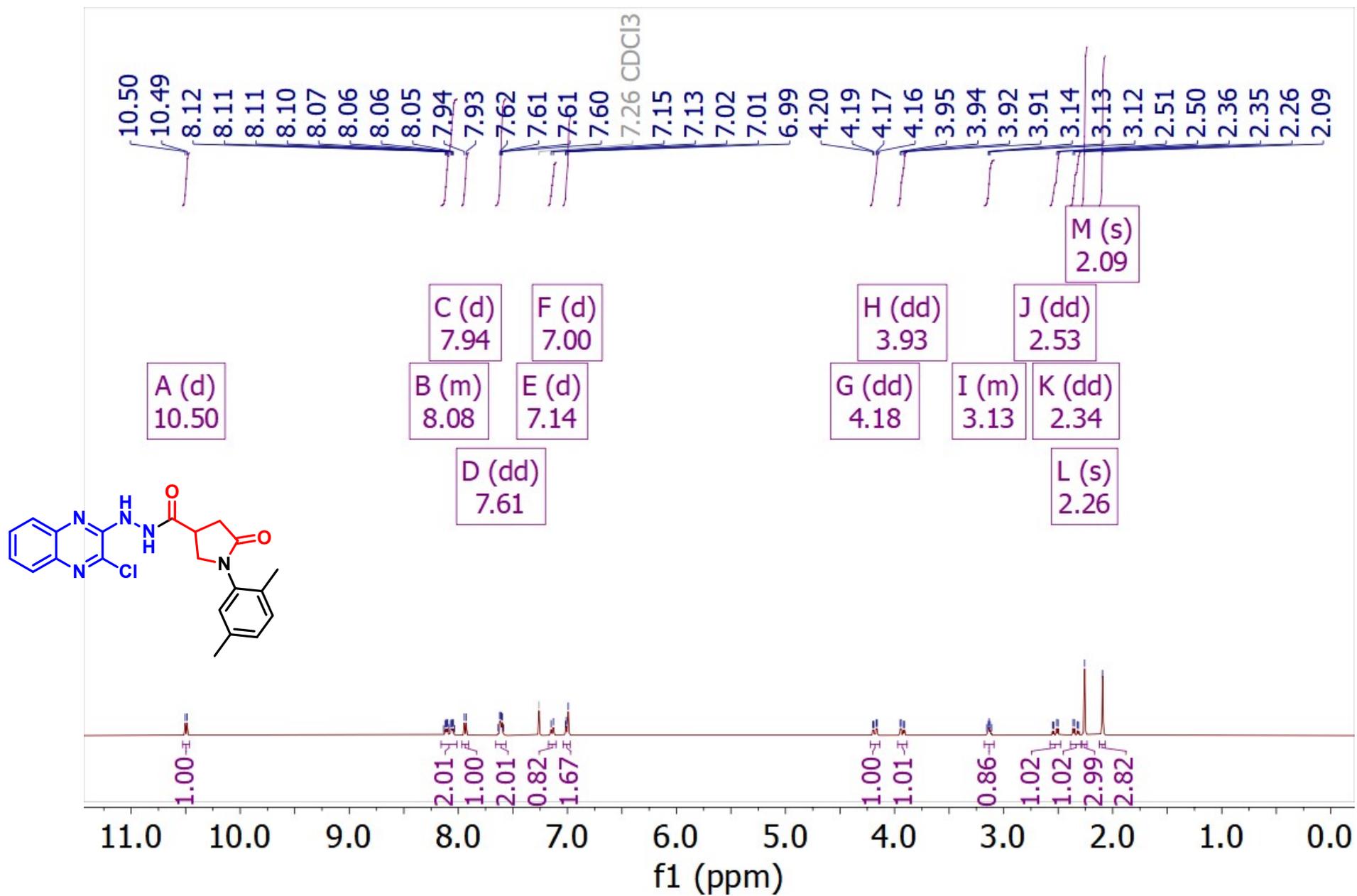


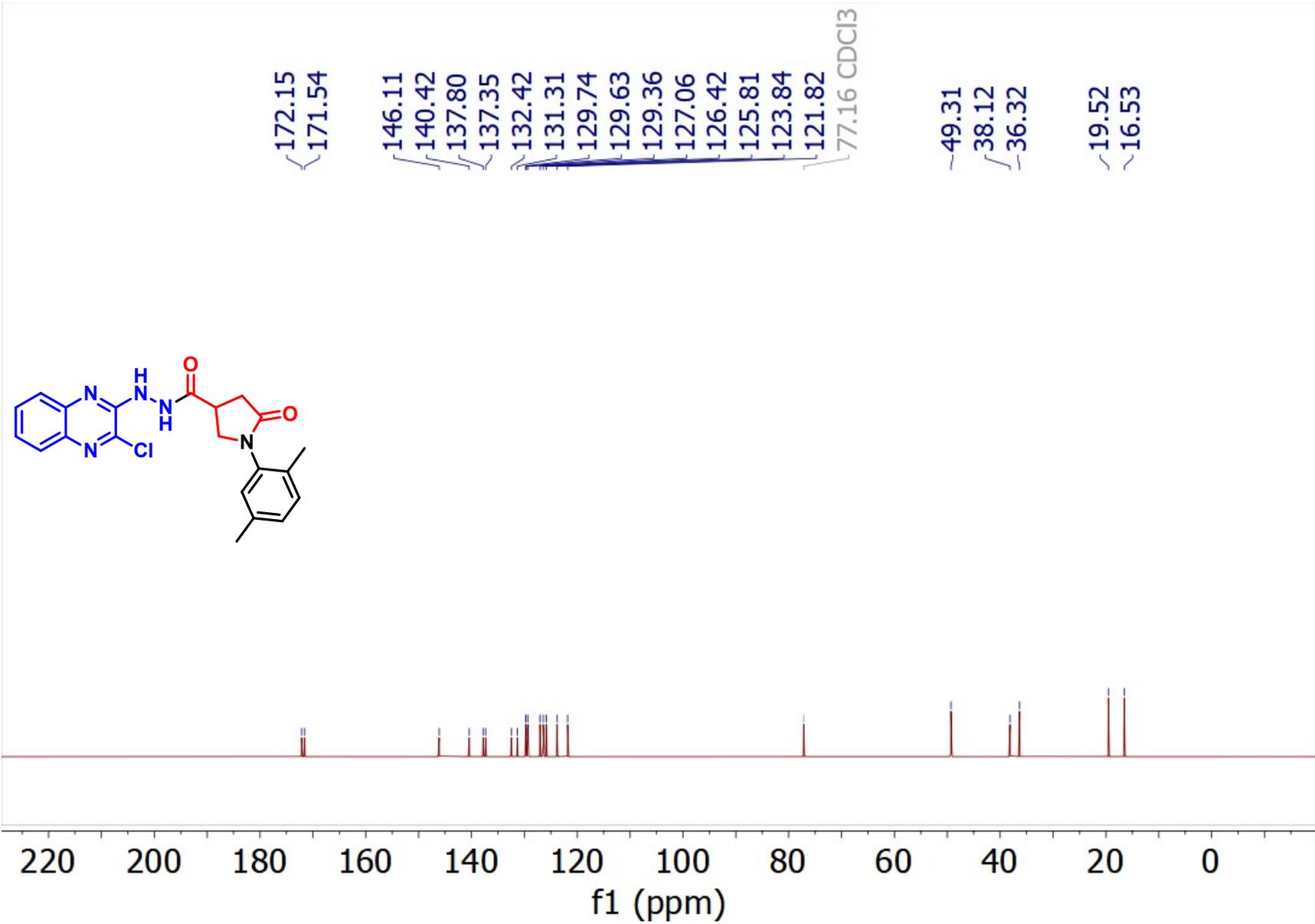
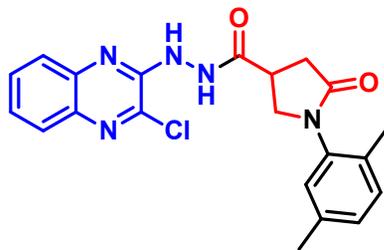
Compound 28



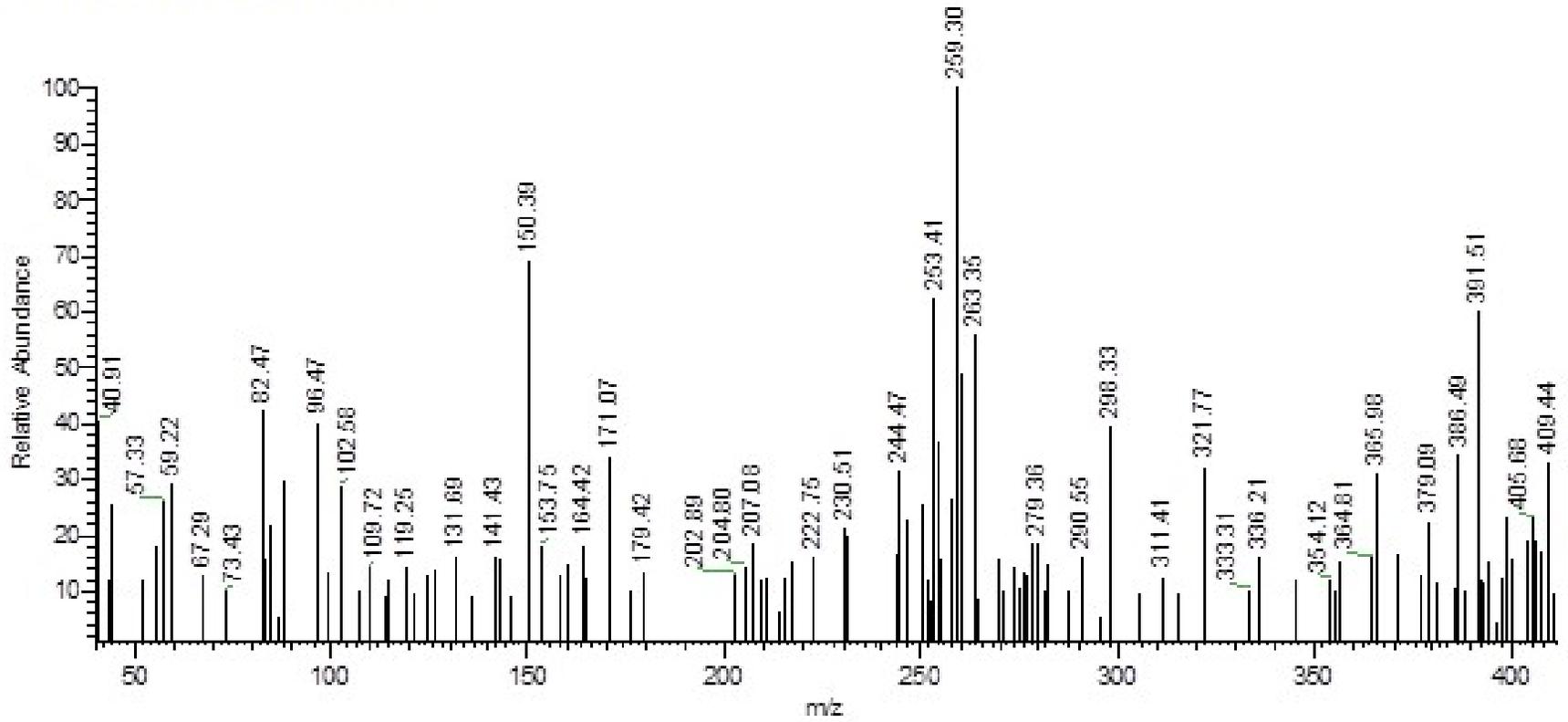


Compound 29

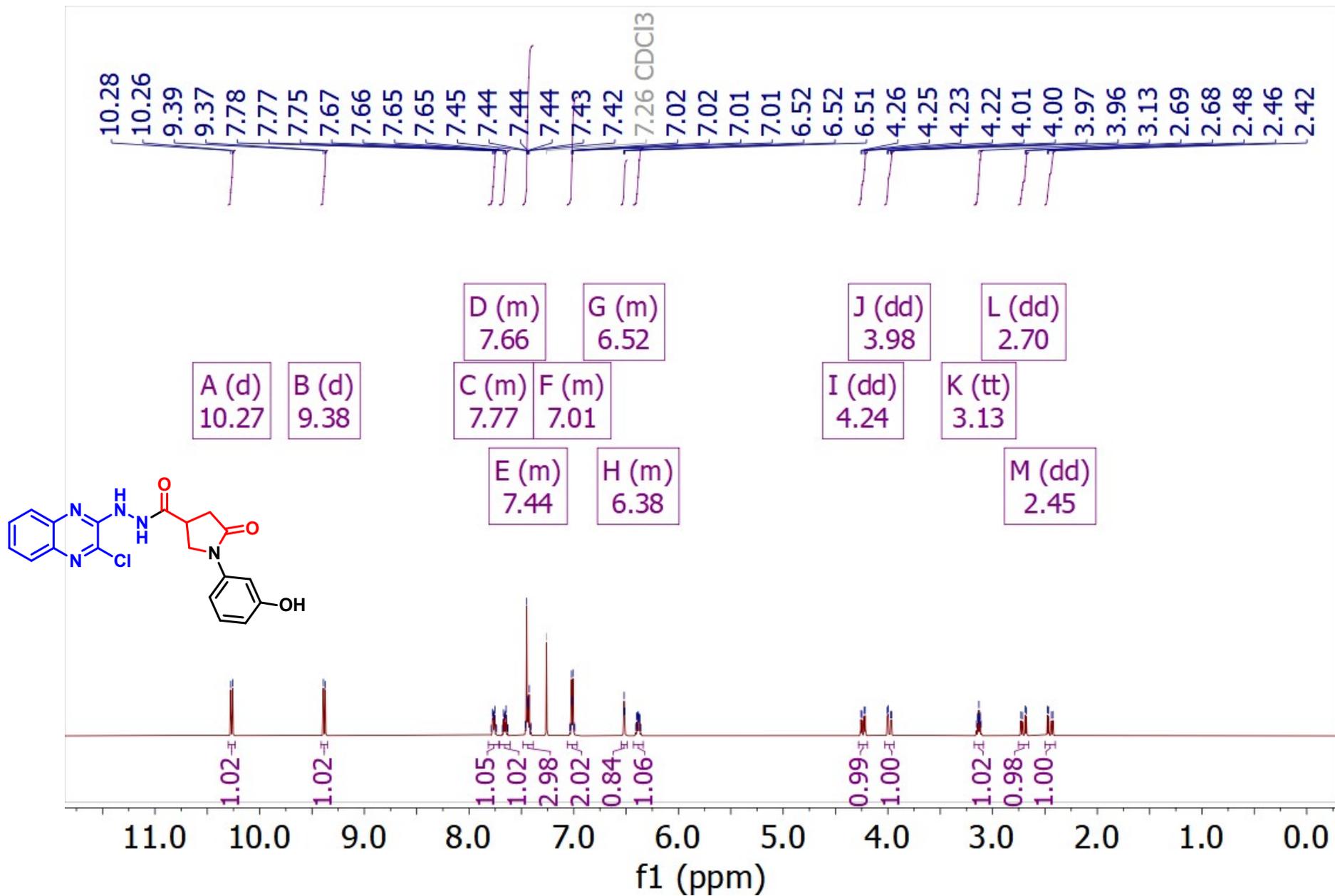


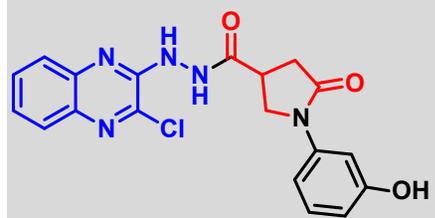


ND #2 RT: 0.05 P: + NL: 7.20E2
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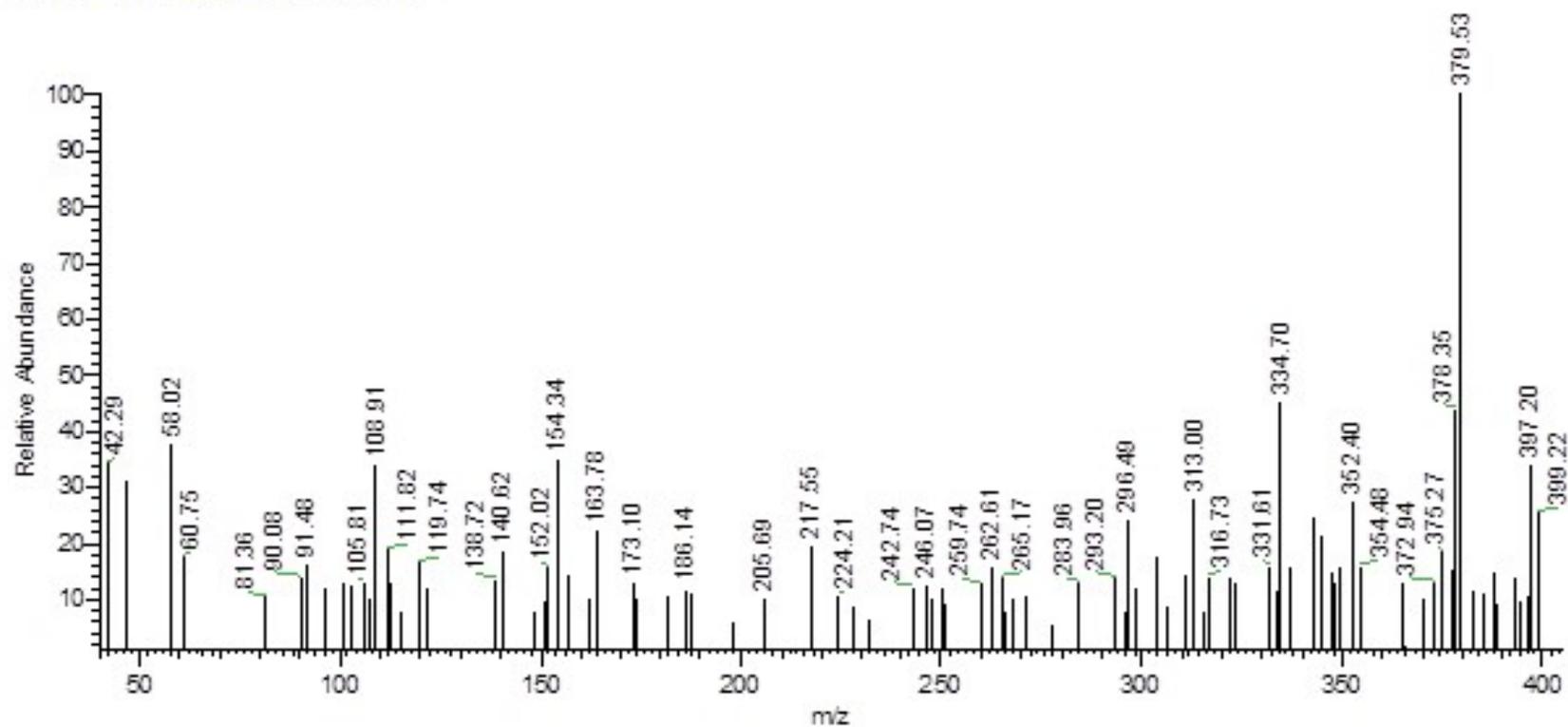


Compound 30

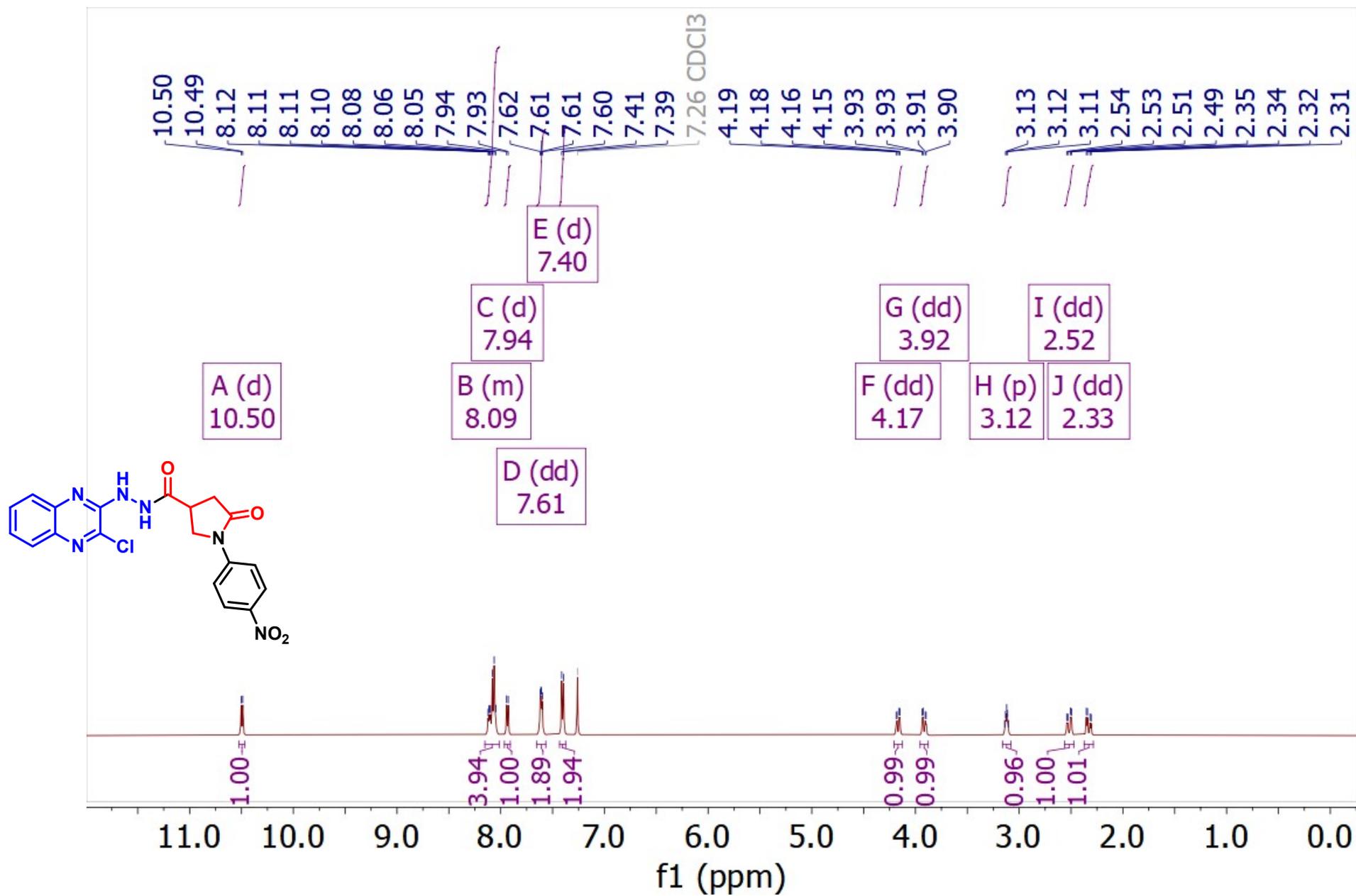


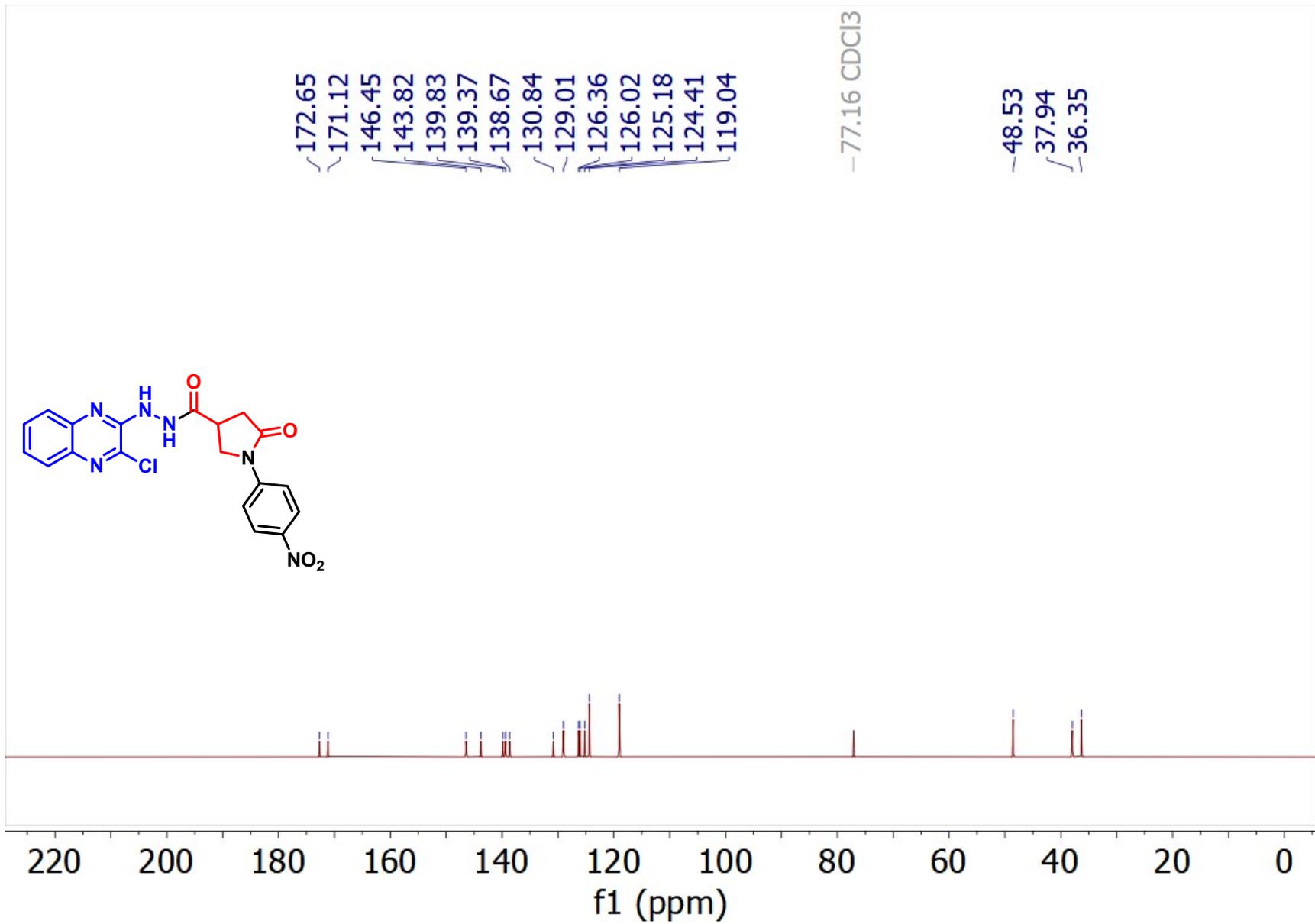
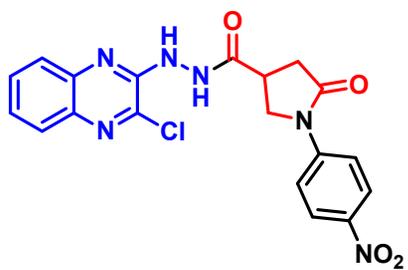


NF #23 RT: 0.40 P: + NL: 7.94E2
T: {0,0} + cEI Full ms [40.00-1000.00]

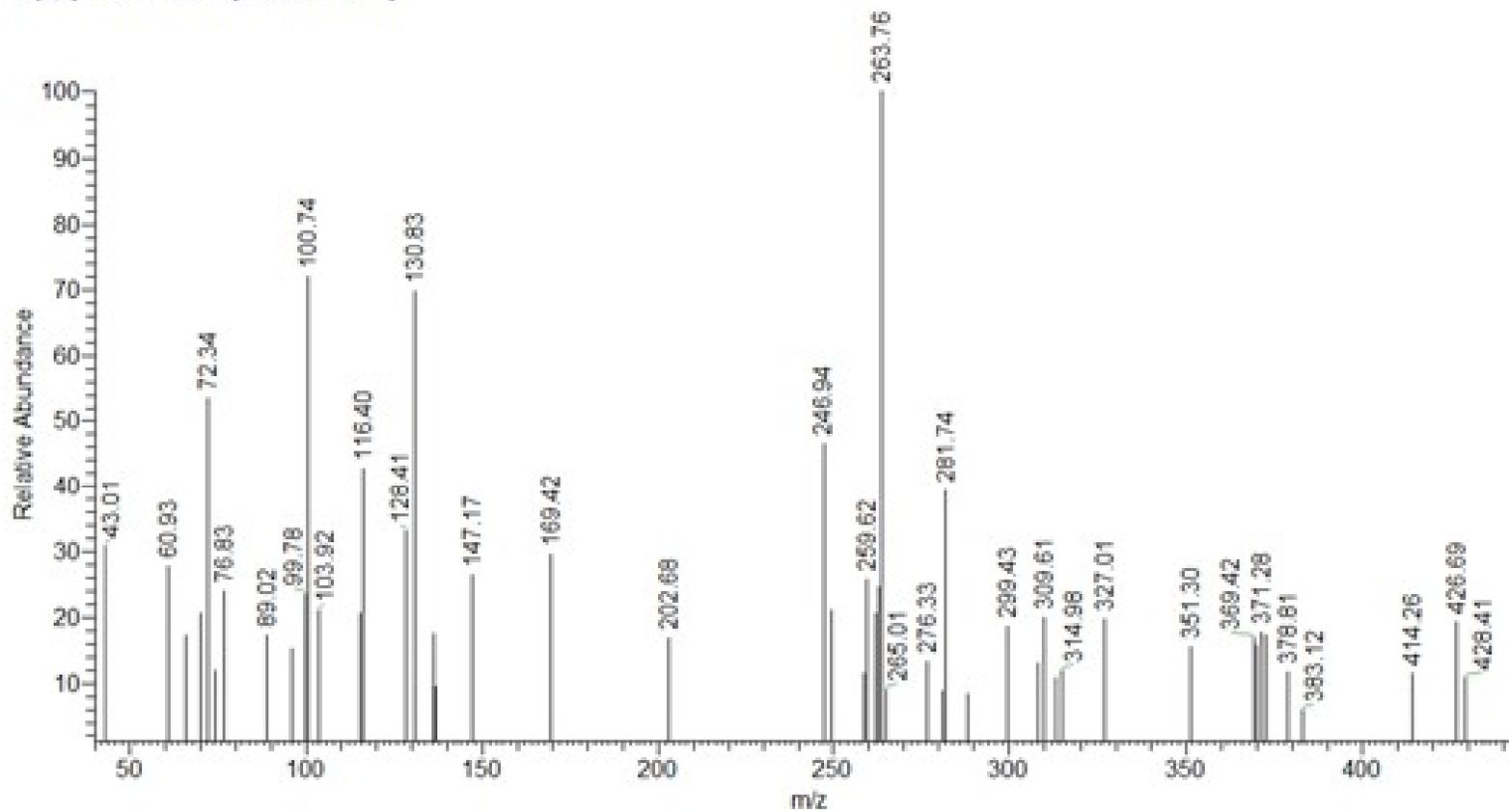


Compound 31





NH #262 RT: 4.40 P: + NL: 7.41E2
T: (0,0) + c EI Full ms [40.00-1000.00]



Biology

DNA Gyrase Inhibition Assay

The DNA Gyrase (Topoisomerase II) (ProFoldin, Worcester, MA) Assay Kit Plus was utilized to assess the activity of DNA gyrase *in vitro* in accordance with the vendor's instructions. Each reaction had a total volume of 40 μL and was conducted on a 96-well plate with a black, transparent bottom. DNA gyrase (20 nM), ATP (1 mM), relaxed plasmid DNA (25 $\mu\text{g}/\text{mL}$), and manufactured hybrids (2.5-200 $\mu\text{g}/\text{mL}$) were all dissolved in 1 \times buffer with 5% DMSO for 1 hour at room temperature. The final composition of the 1 \times buffer (supplied by ProFoldin) was 20 mM Tris-HCl, 35 mM NH_4OAc , 4.6% glycerol, 1 mM DTT, 0.005% Brij35 and 8 mM MgCl_2 . After applying 250 μL of the freshly made H19 dye solution (supplied by ProFoldin) to each well for five minutes, fluorescence at 535 nm was detected using an $\lambda_{\text{ex}} = 485 \text{ nm}$ using a Spectra Max MS-Plate Reader (Molecular Devices). The DNA gyrase activity was standardized against the antibiotic-free reaction. In the test, ciprofloxacin-using positive controls and one without ATP were employed. Every experiment was carried out in triplicate [104-109].

Molecular Docking

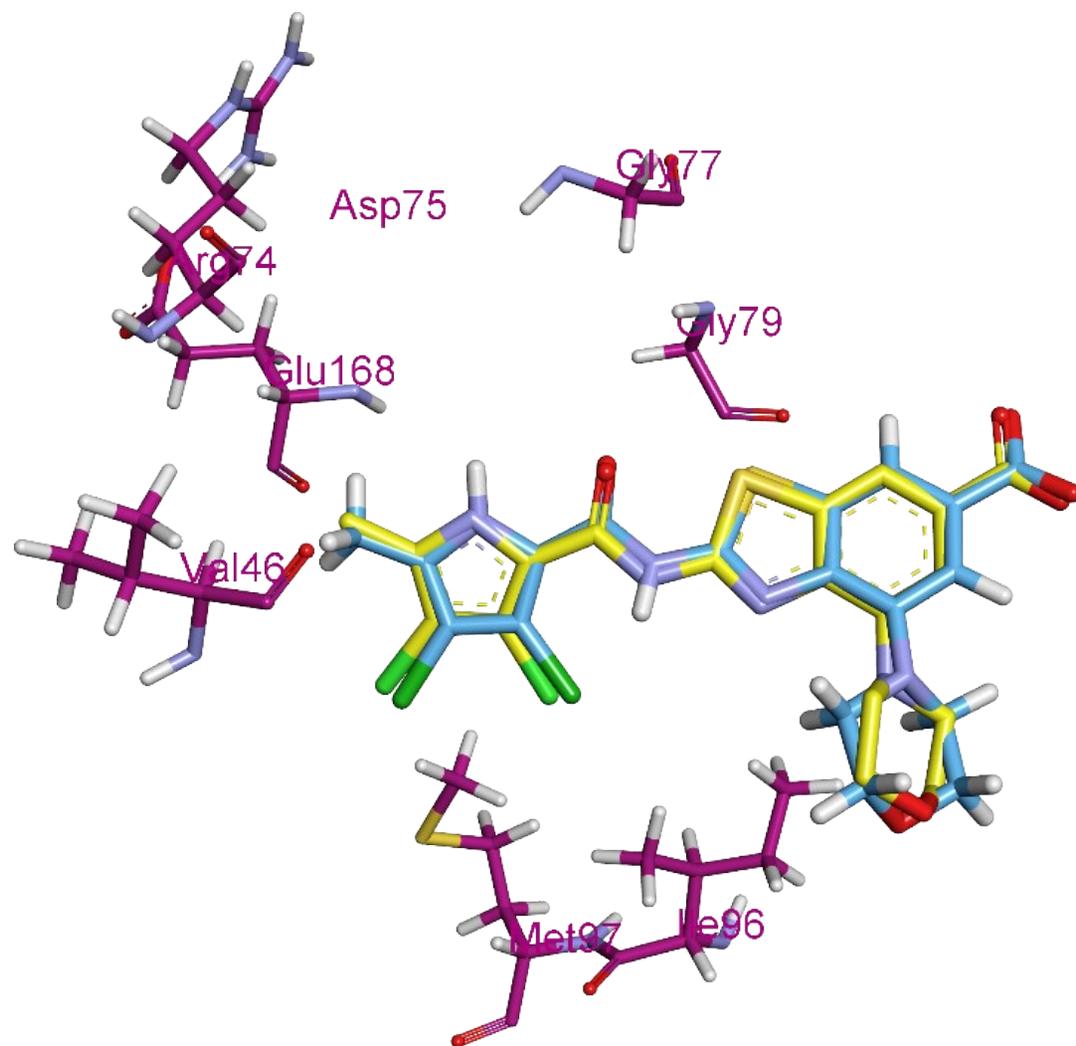


Figure S1: Overlay of bound ligand (cyan) and redocked ligand (yellow)

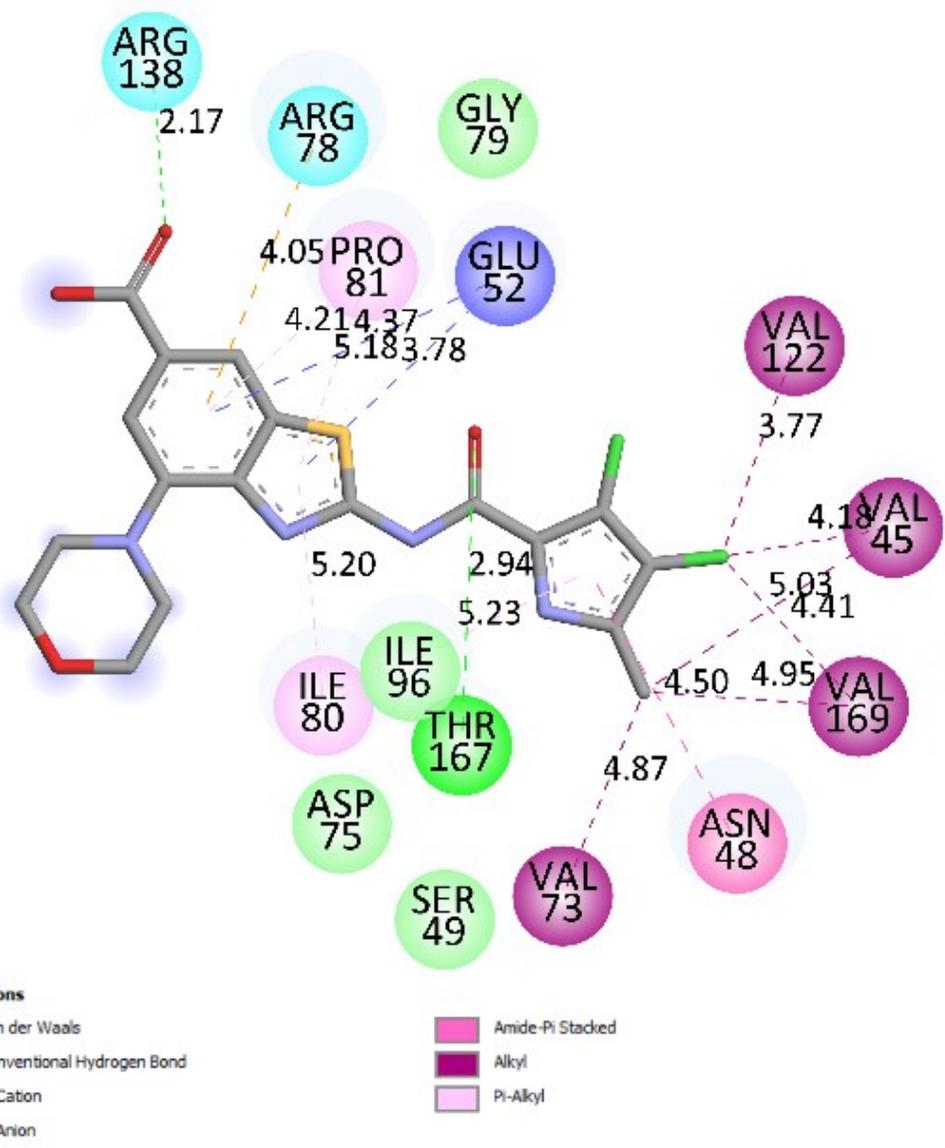


Figure S2: bound ligand interactions

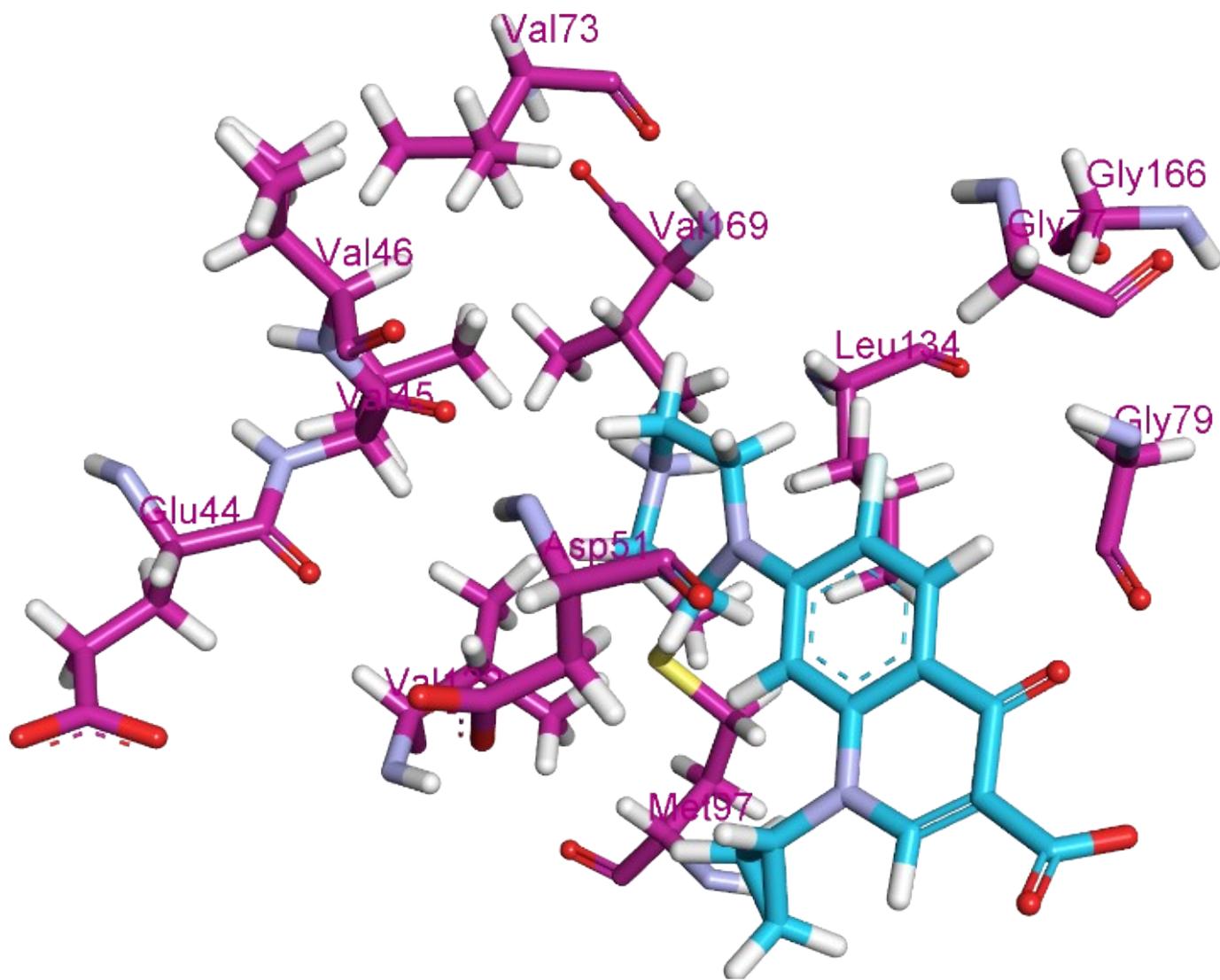


Figure S3: Binding mode of Ciprofloxacin.

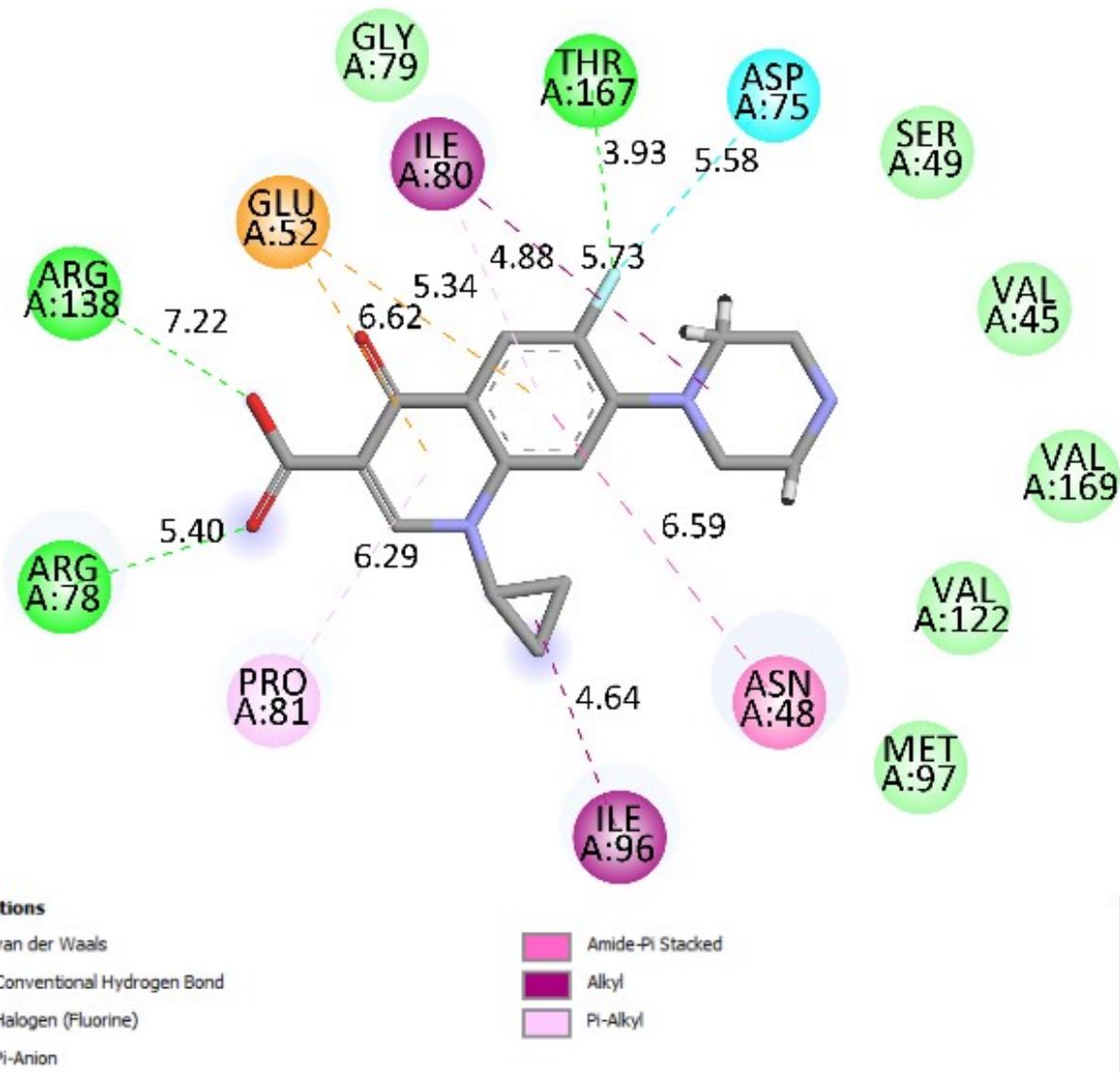


Figure S4: ligand interactions of Ciprofloxacin.

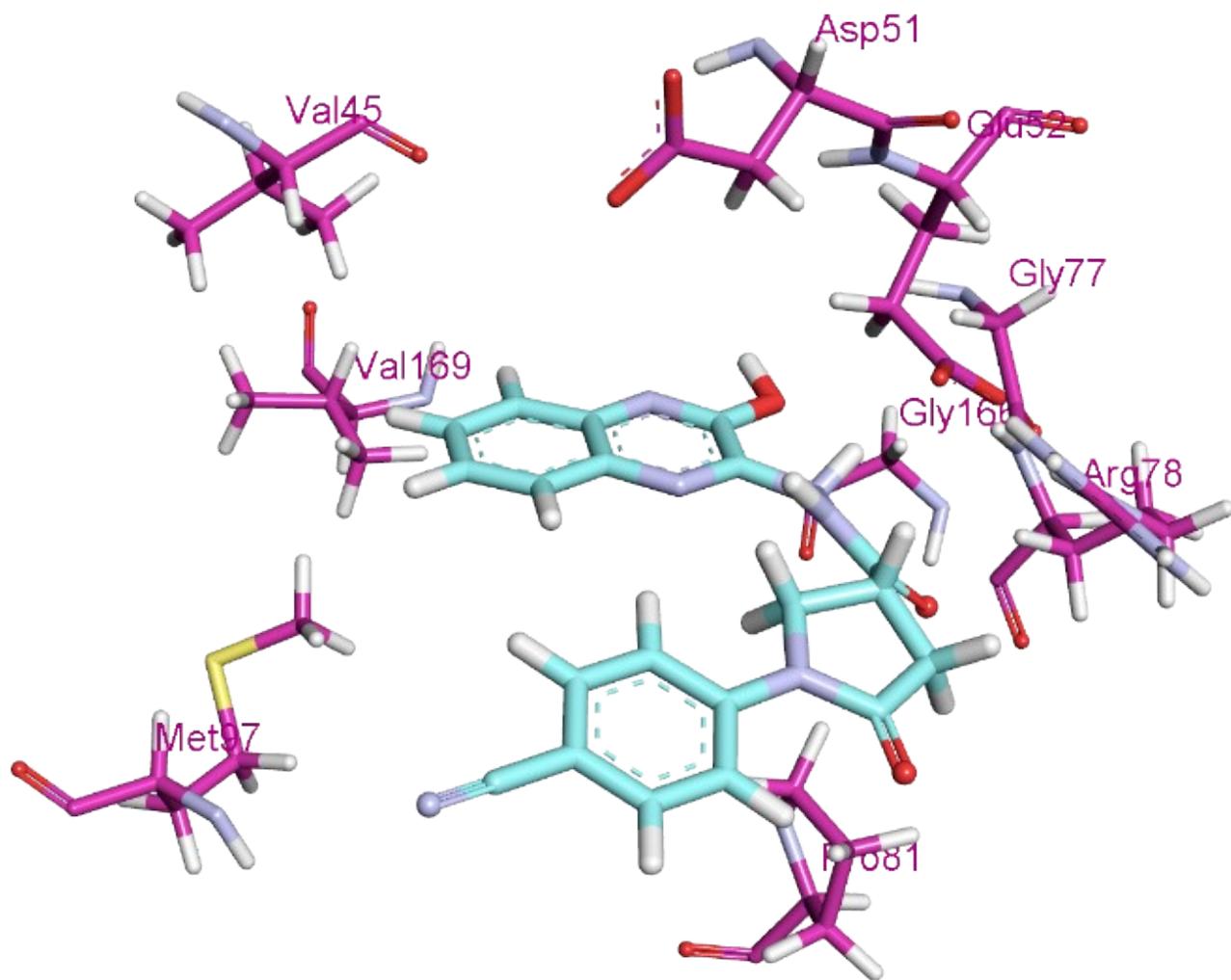


Figure S5: Binding mode of 24

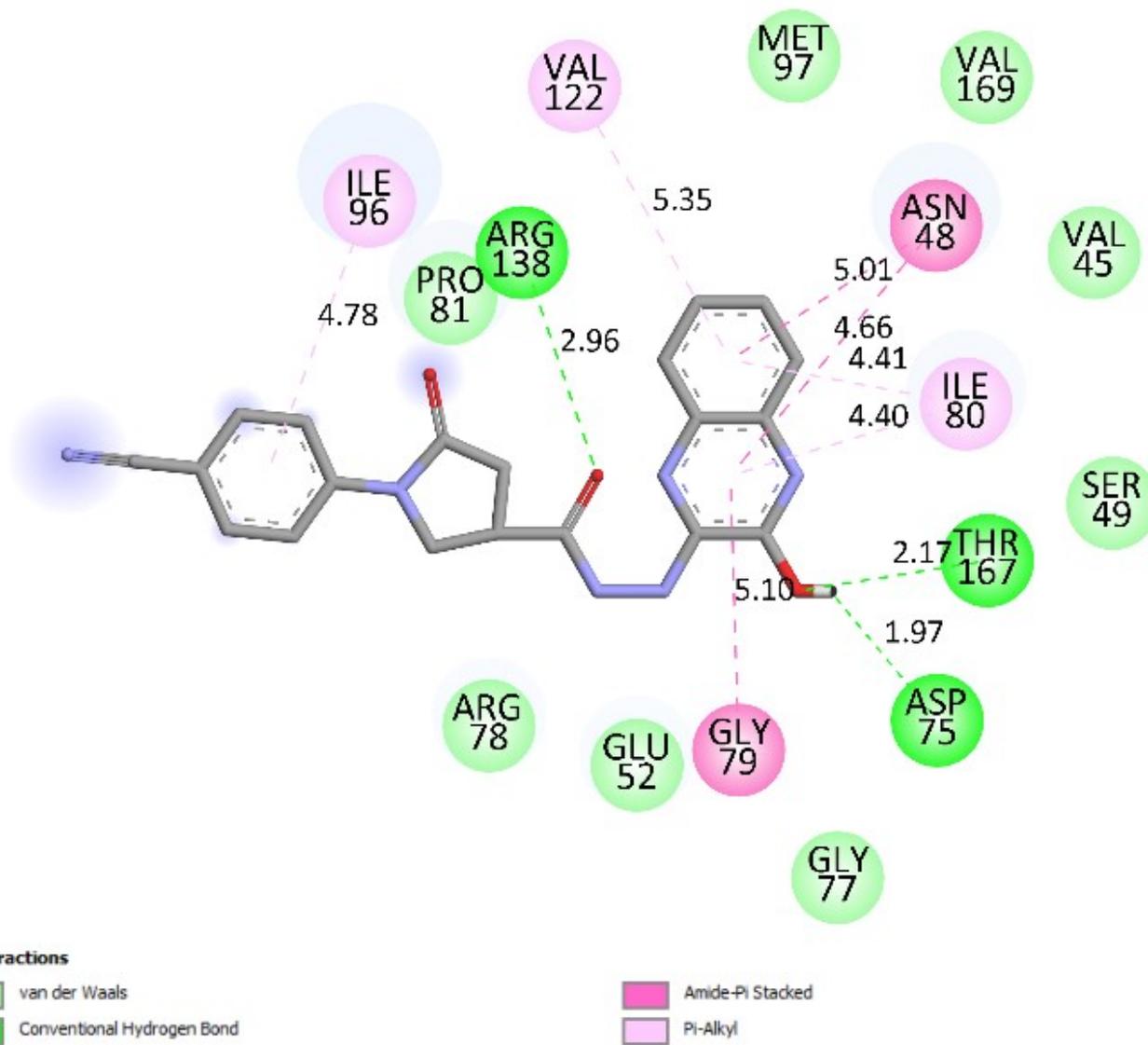


Figure S6: ligand interactions of 24

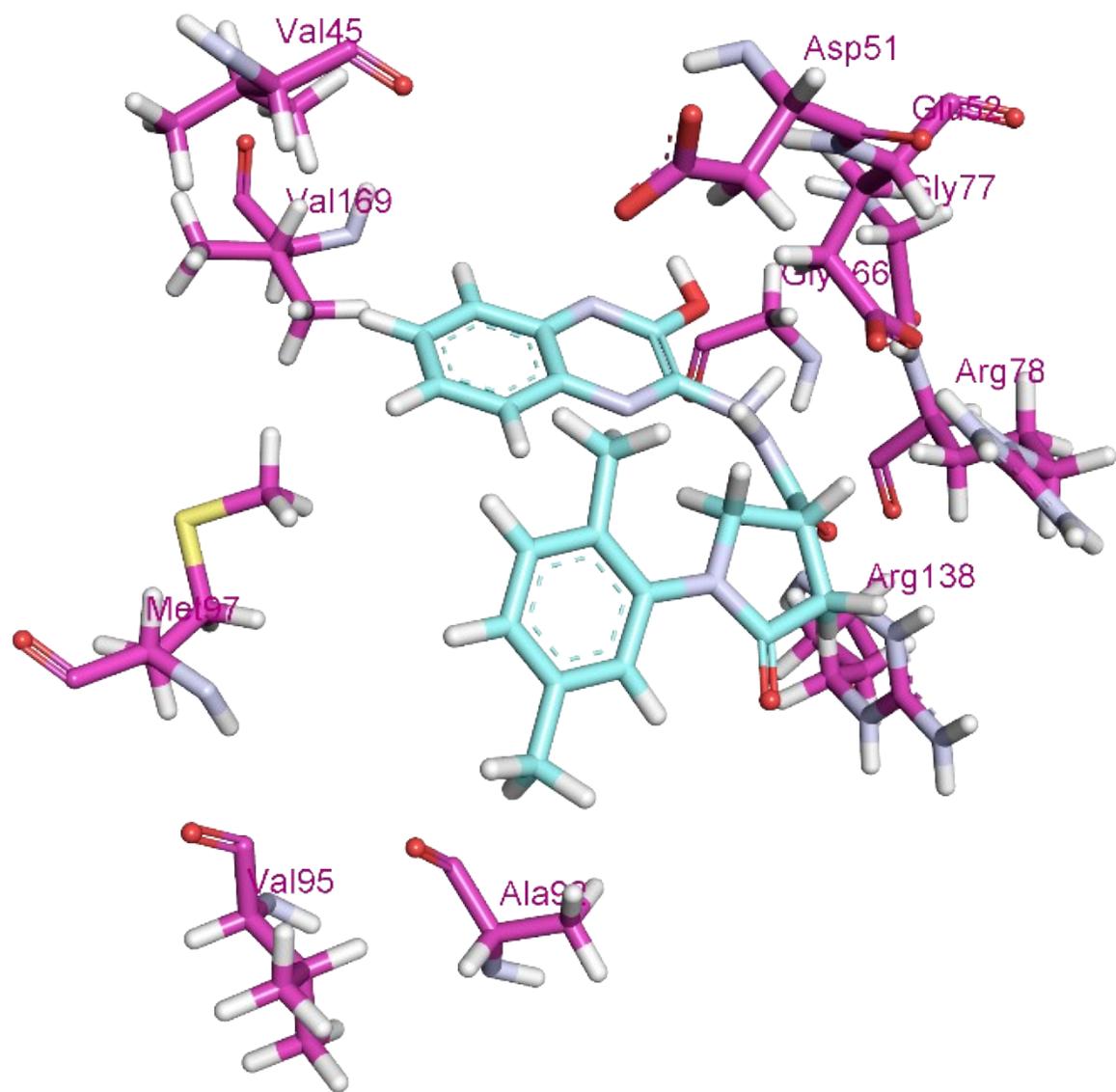
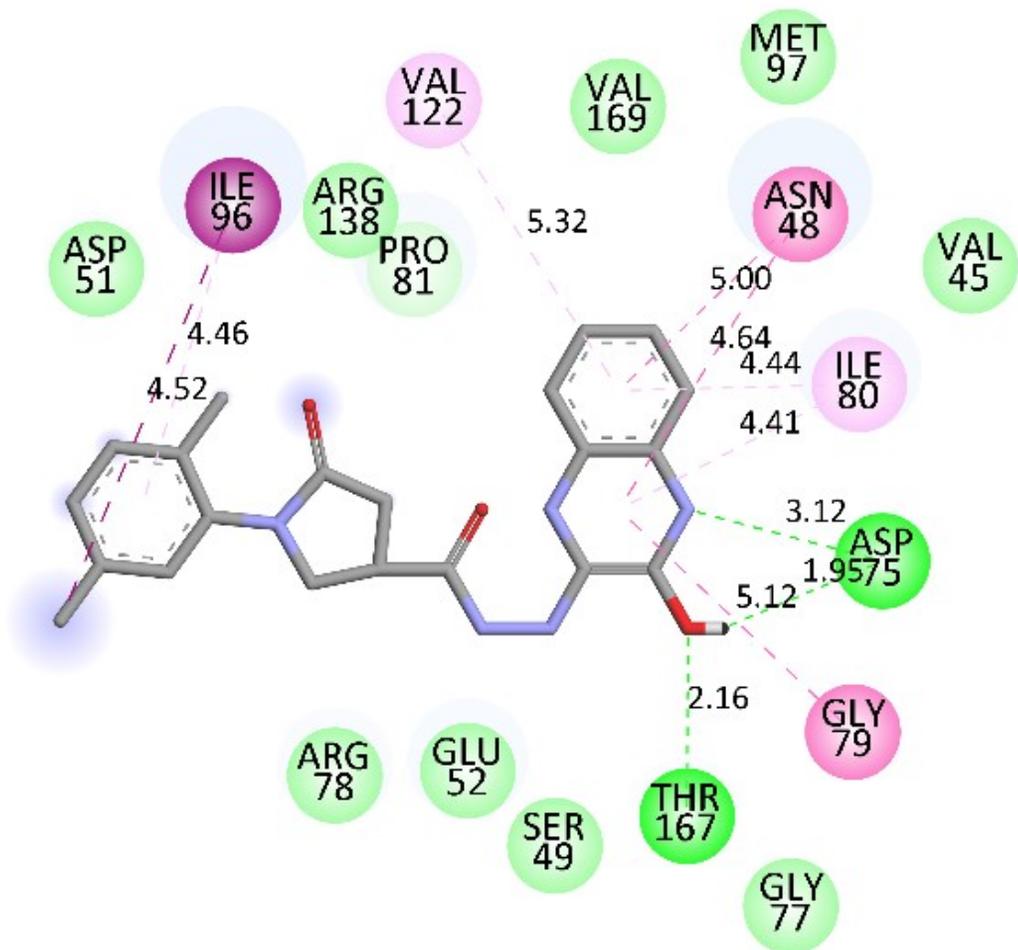


Figure S7: Binding mode 25



Interactions

- van der Waals
- Conventional Hydrogen Bond
- Amide-Pi Stacked

- Alkyl
- Pi-Alkyl

Figure S8: ligand interactions of 25

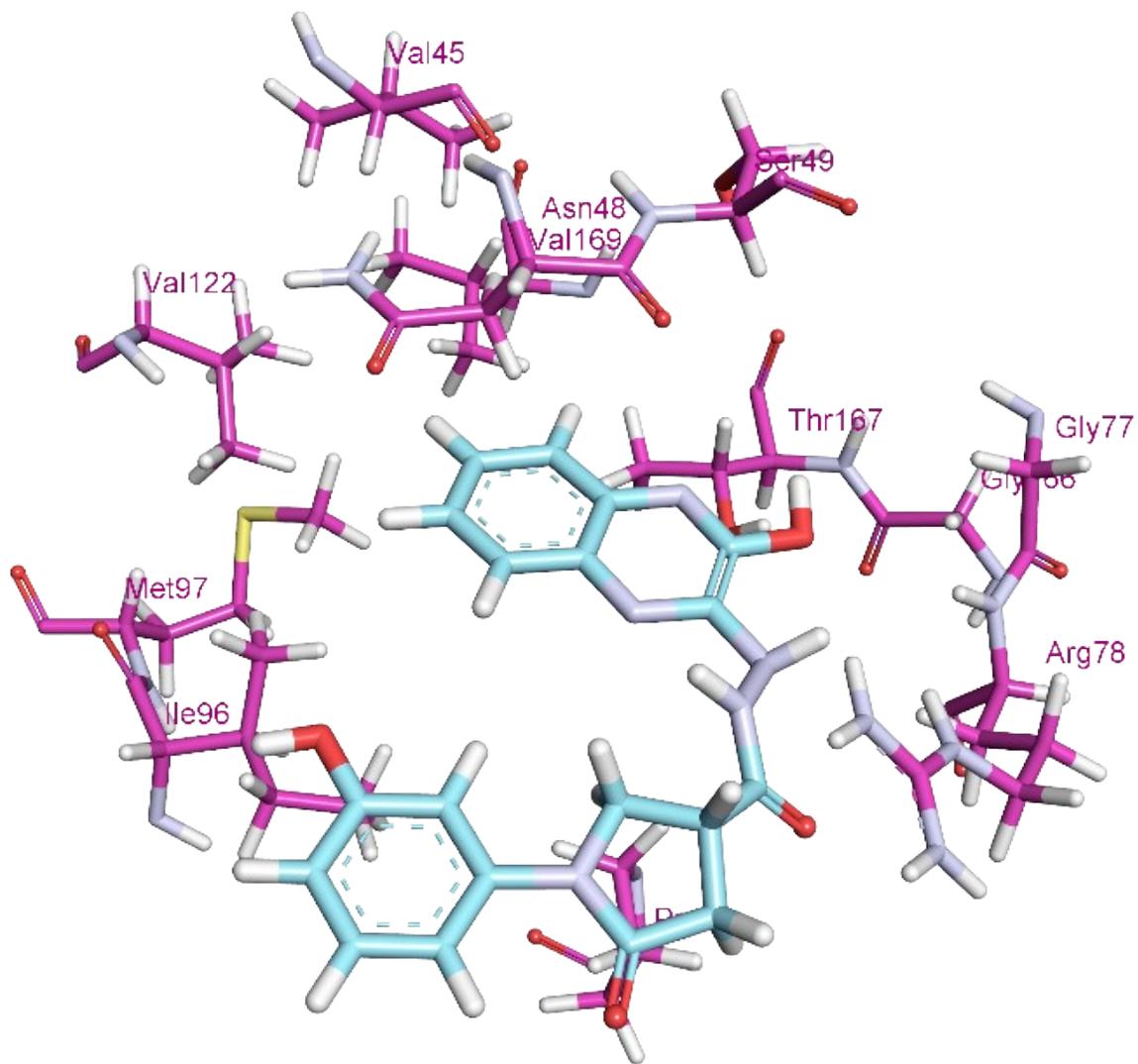


Figure S9: Binding mode of 26

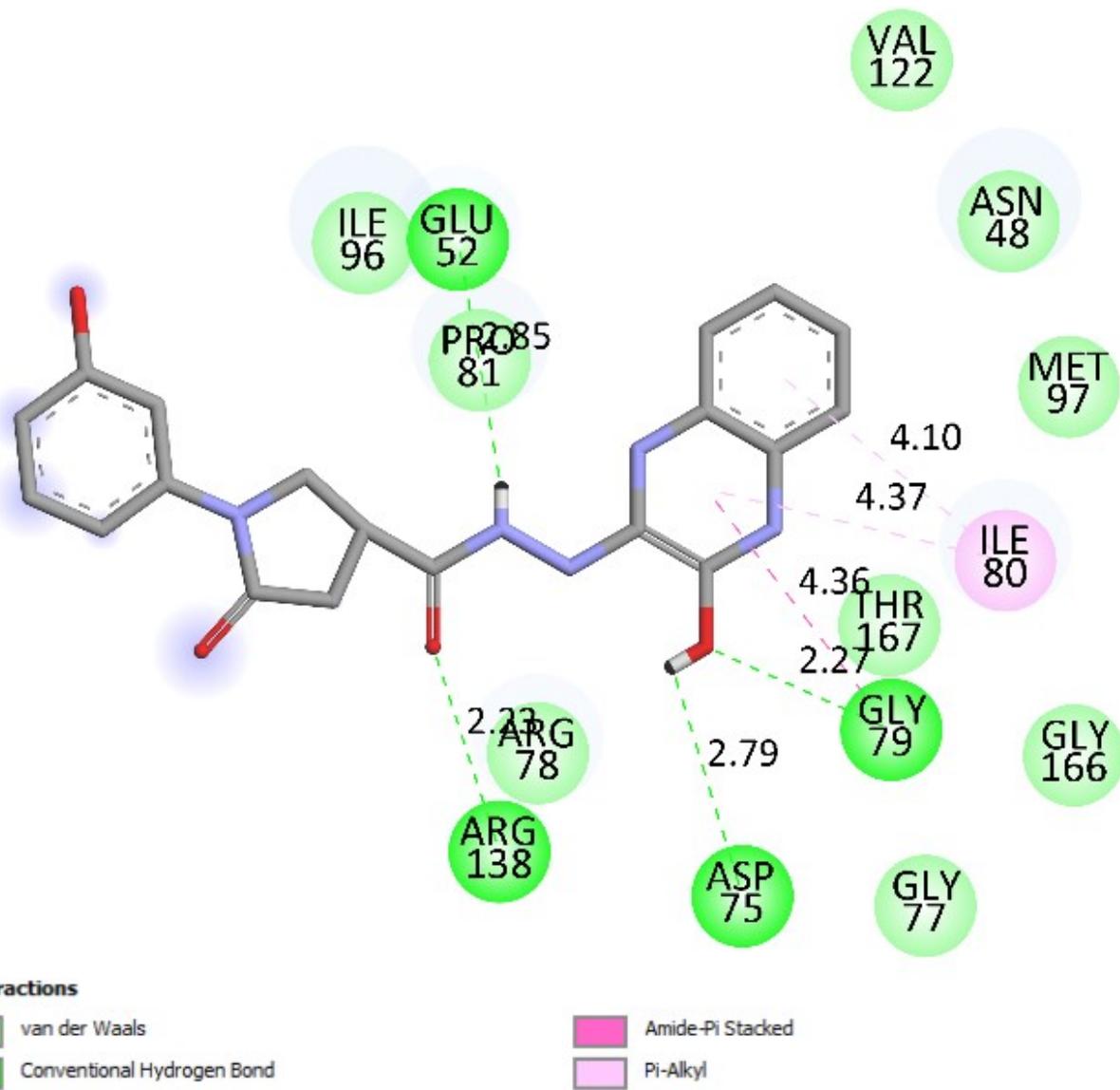


Figure S10: ligand interactions of 26

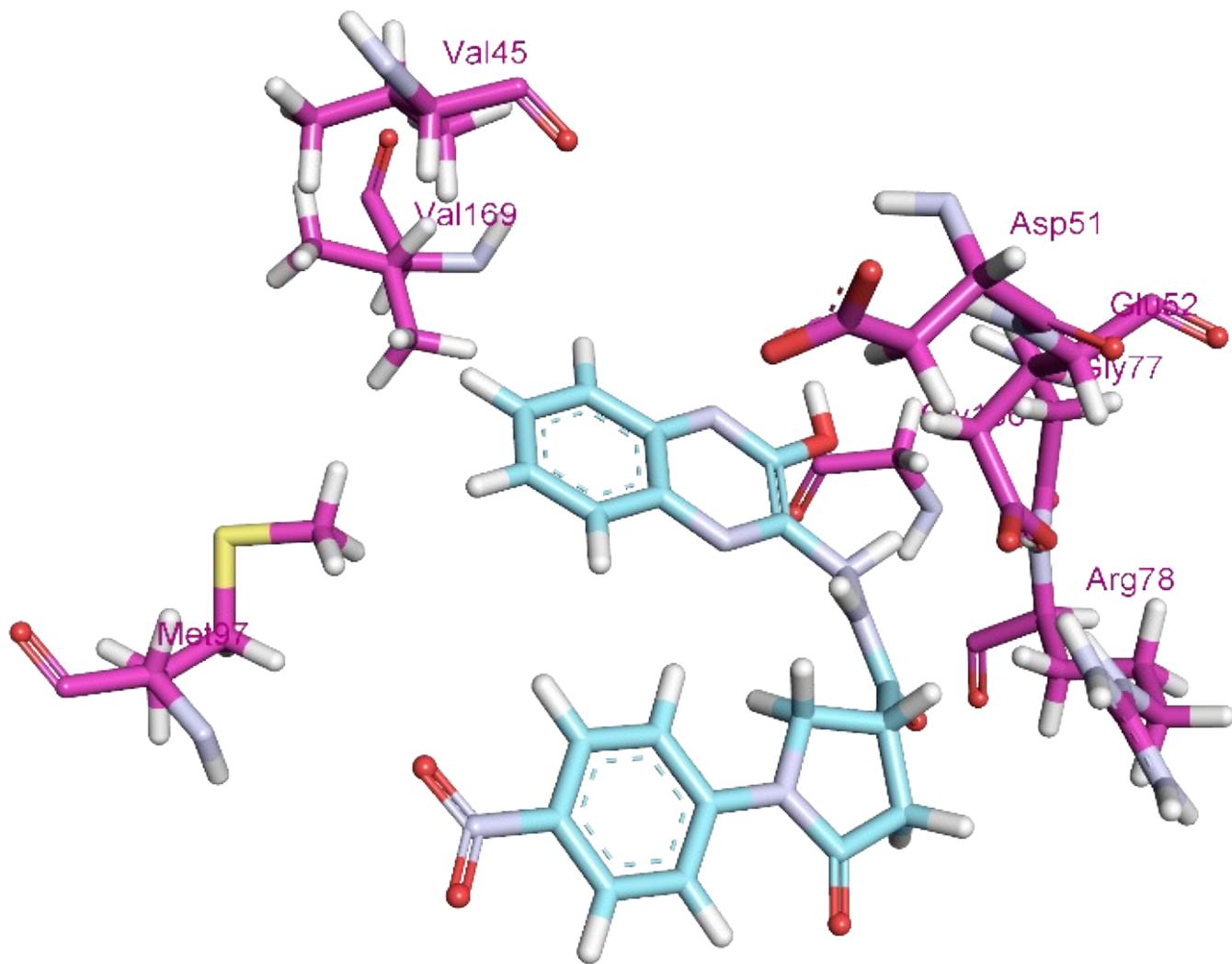


Figure S11: Binding mode of 27

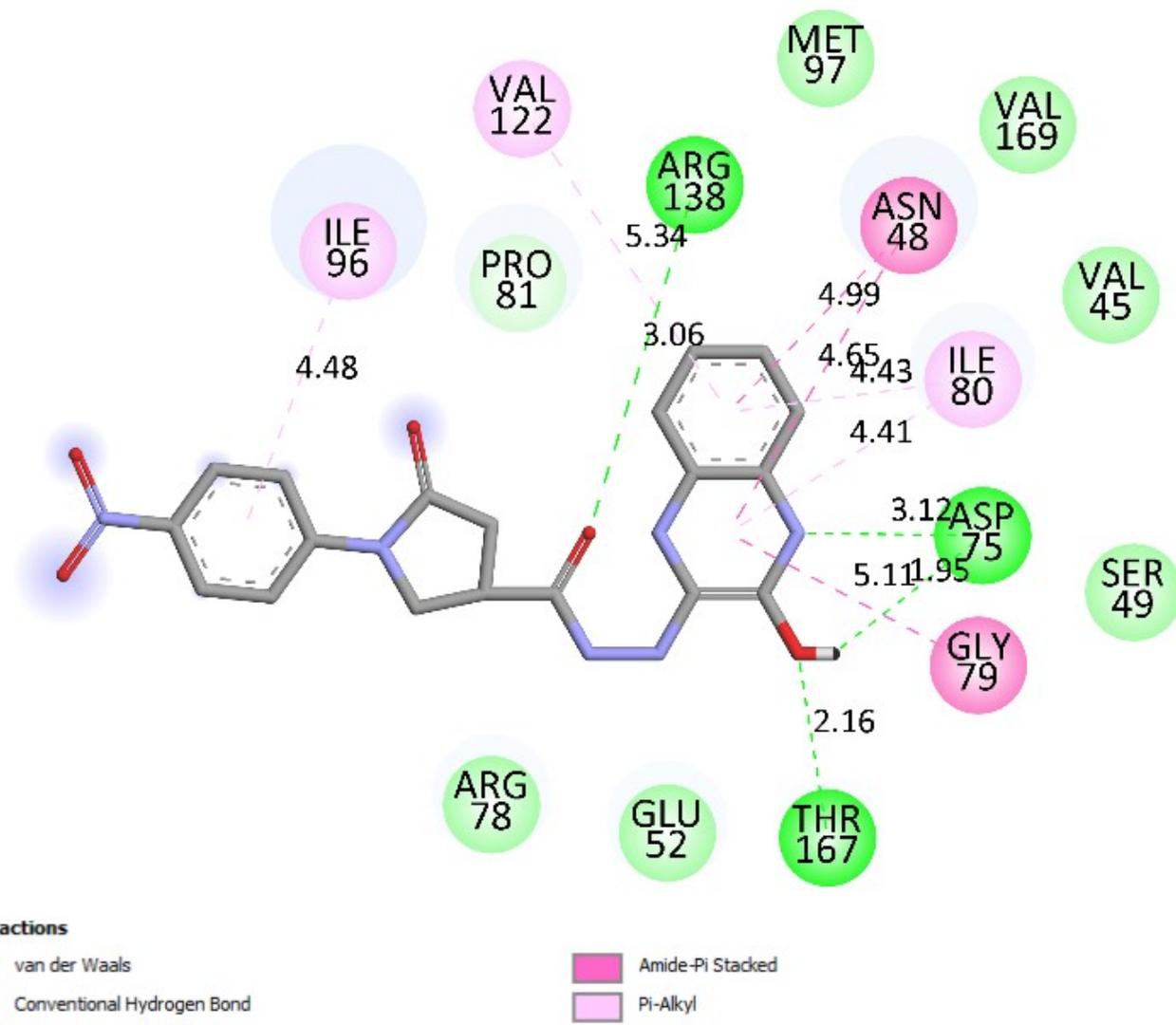


Figure S12: ligand interactions of 27

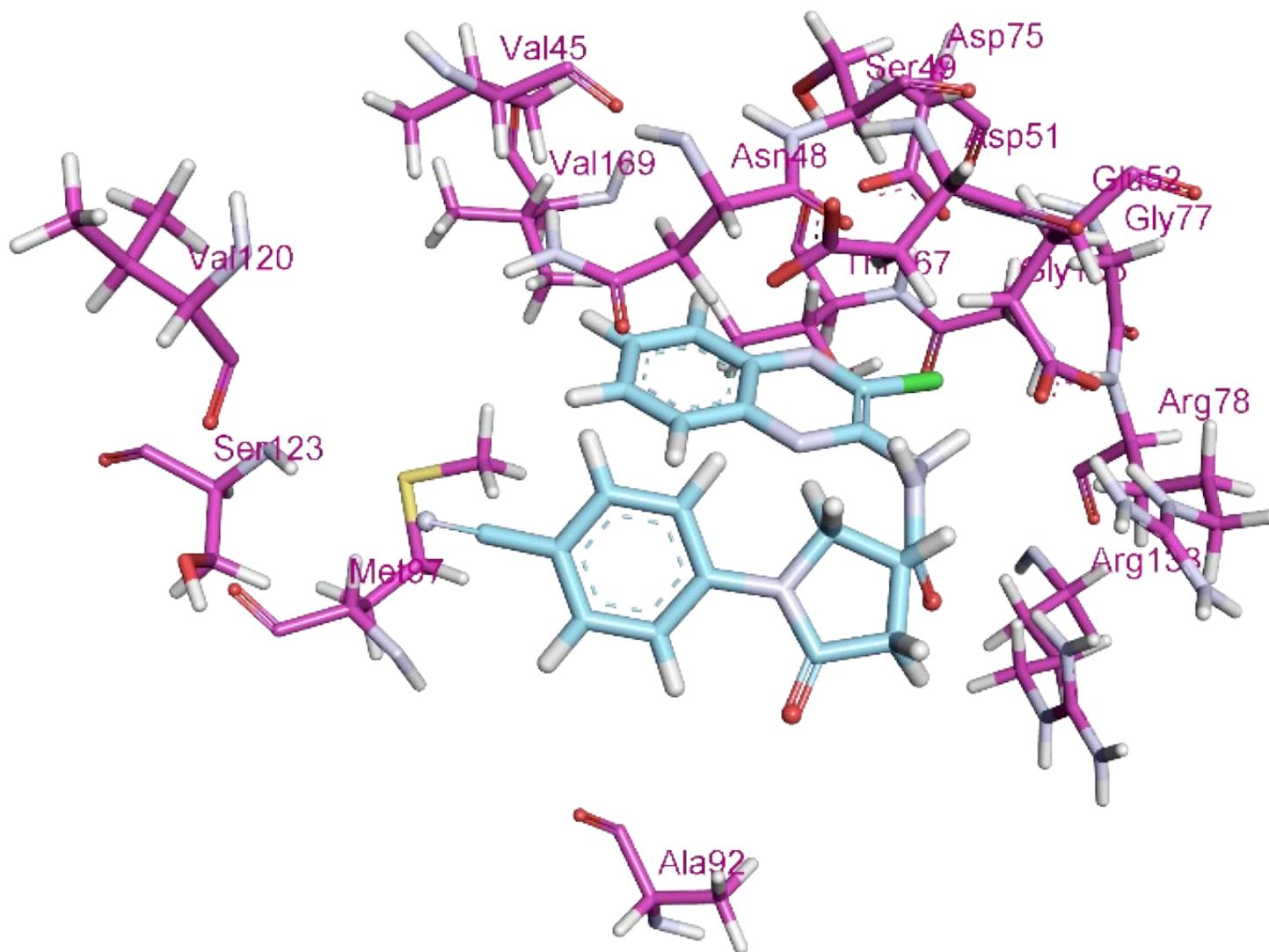


Figure S13: Binding mode of 28

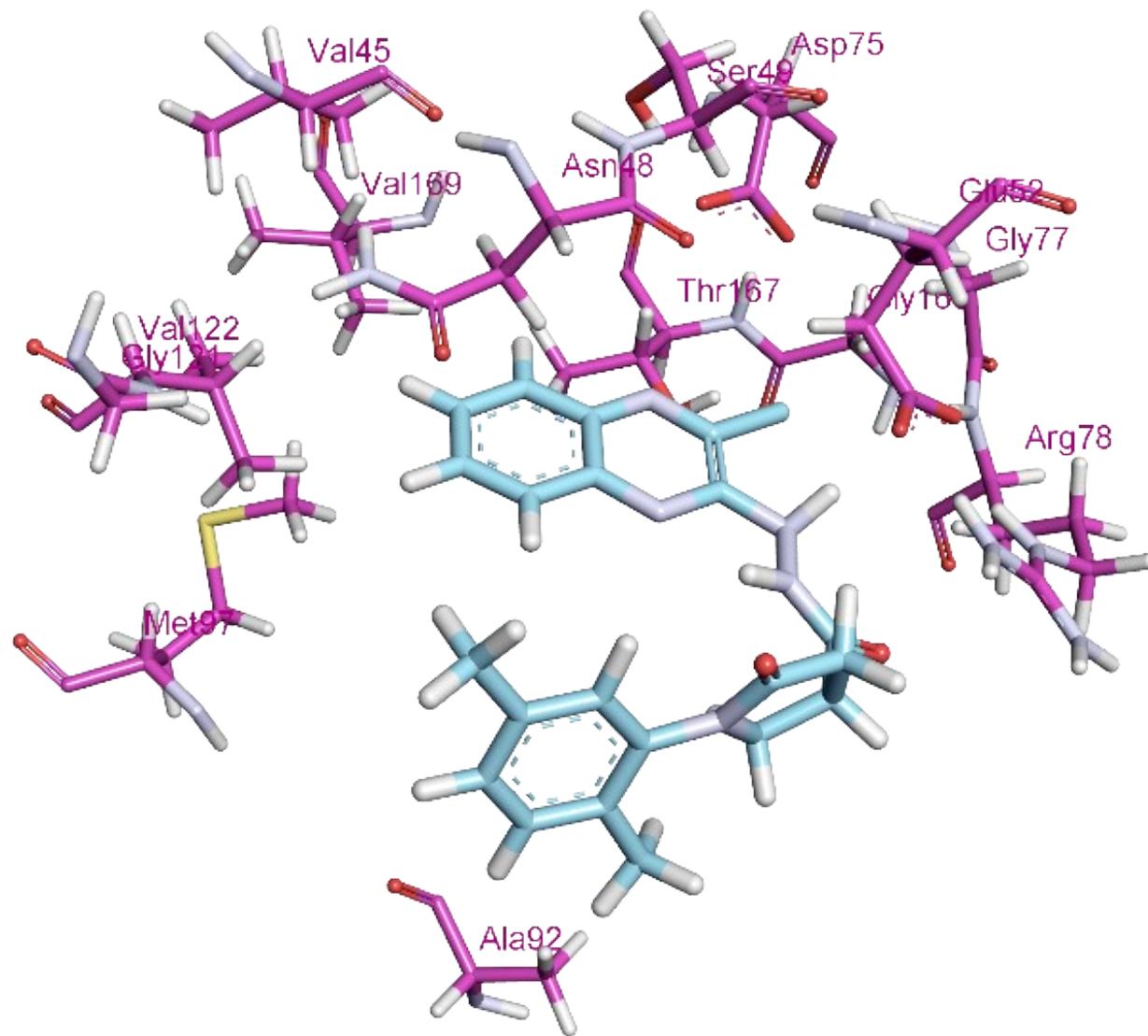


Figure S15: Binding mode of 29

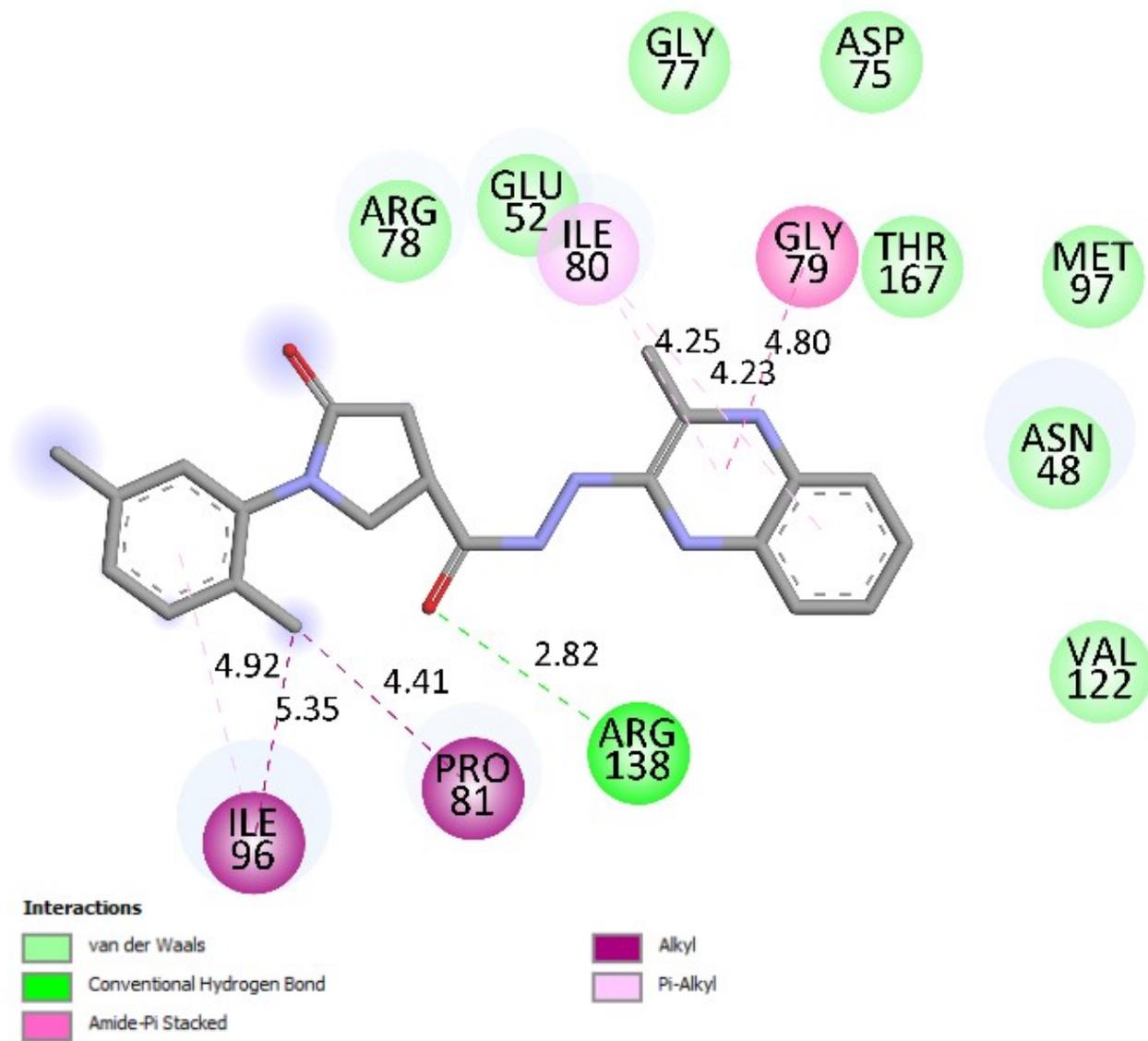


Figure S16: ligand interactions of 29

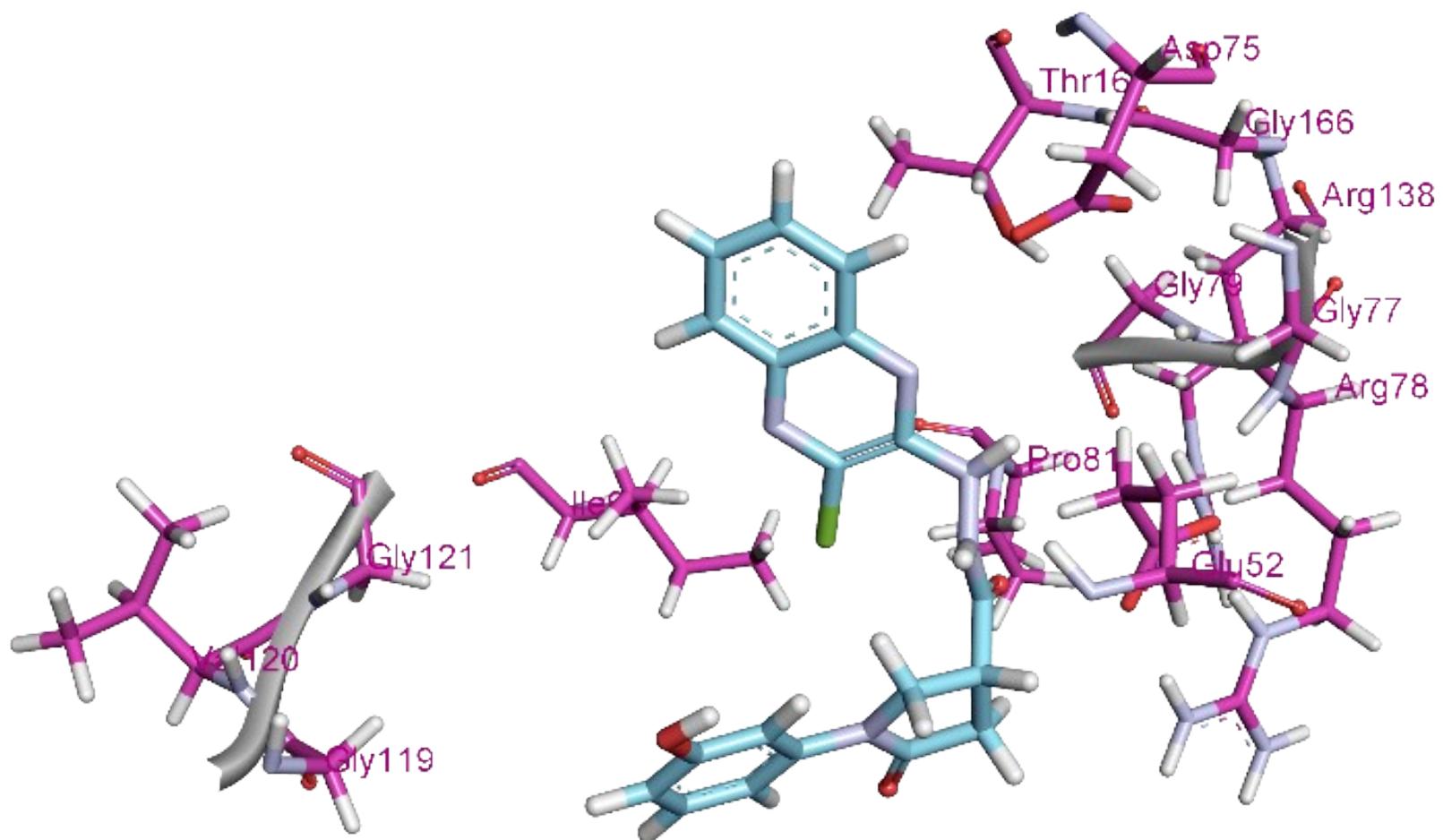


Figure S17: Binding mode 30

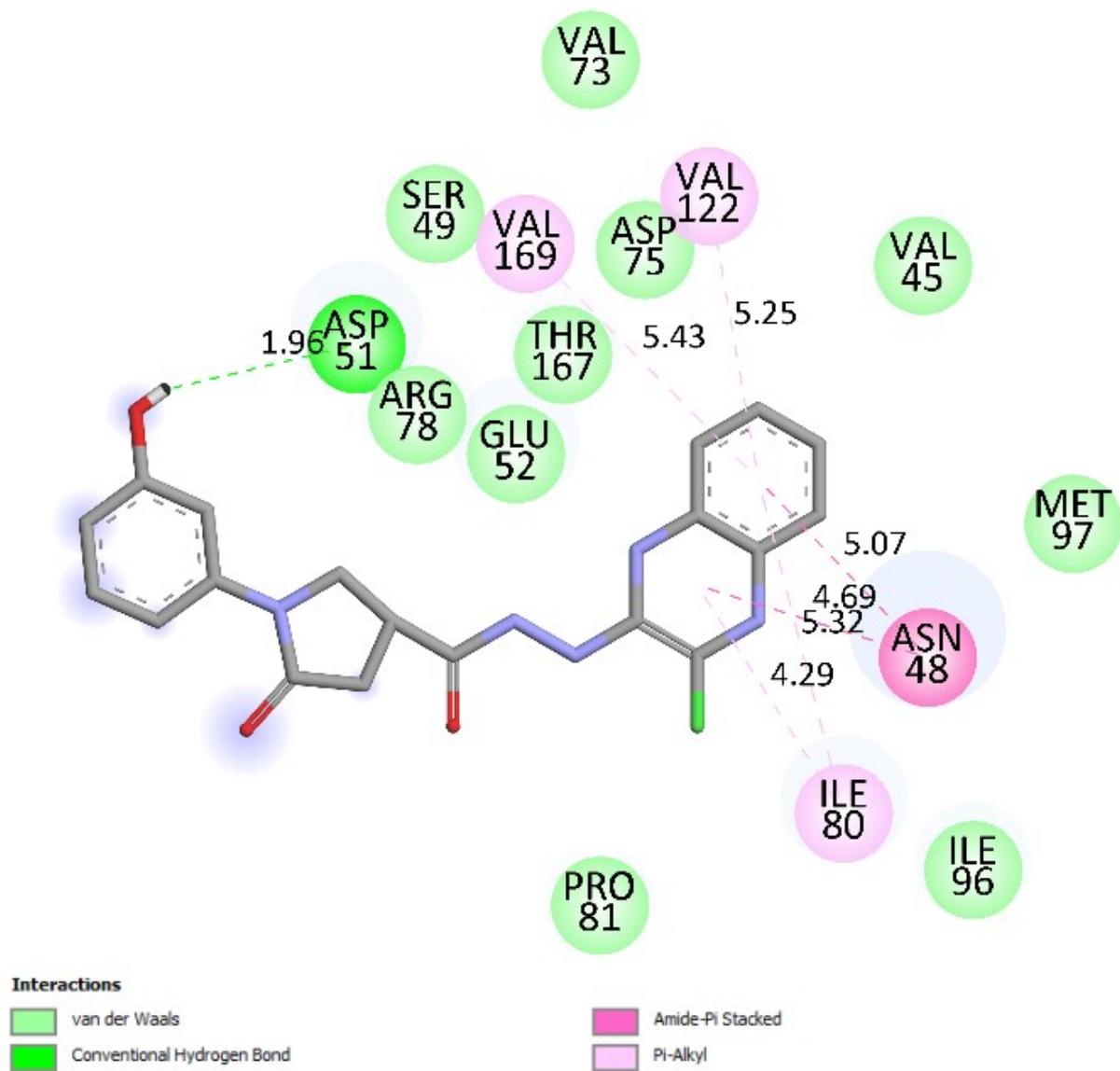


Figure S18: ligand interactions of 30

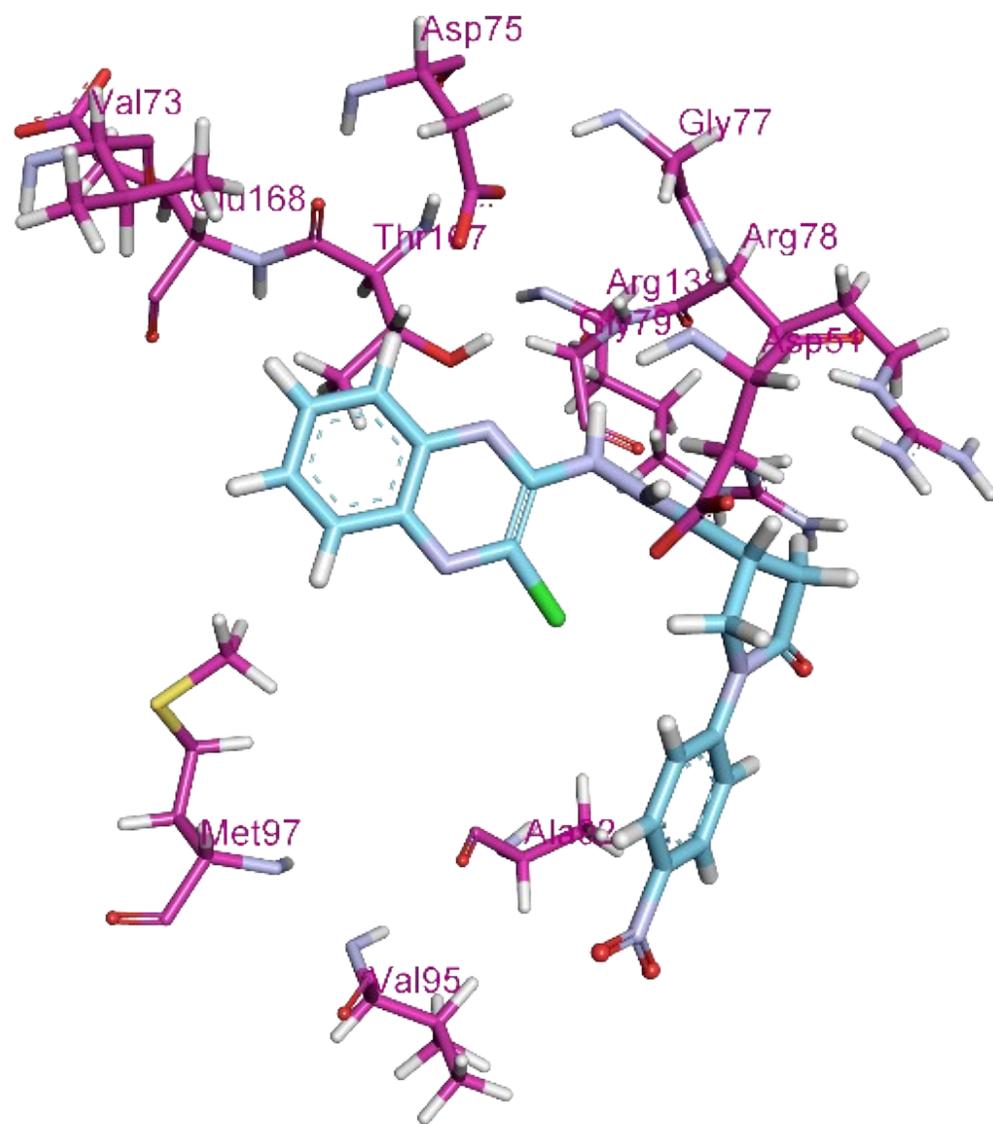


Figure S19: Binding mode of 31

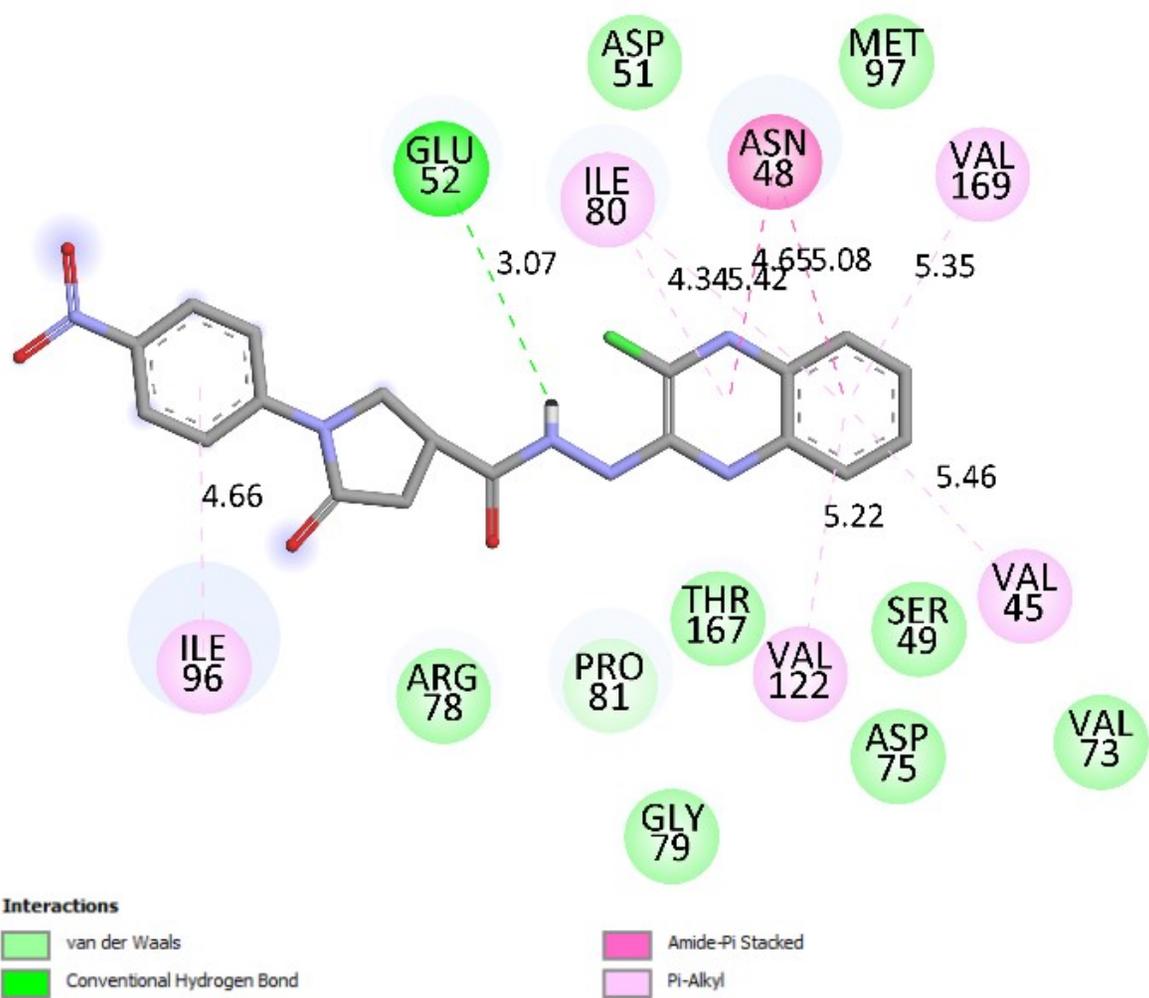


Figure S20: ligand interactions 31

