Synthesis and Molecular Docking Studies of Pyrazolo-Oxazole Derivatives as Potential Inhibitors of *P. gingivalis* Heme-Binding Protein

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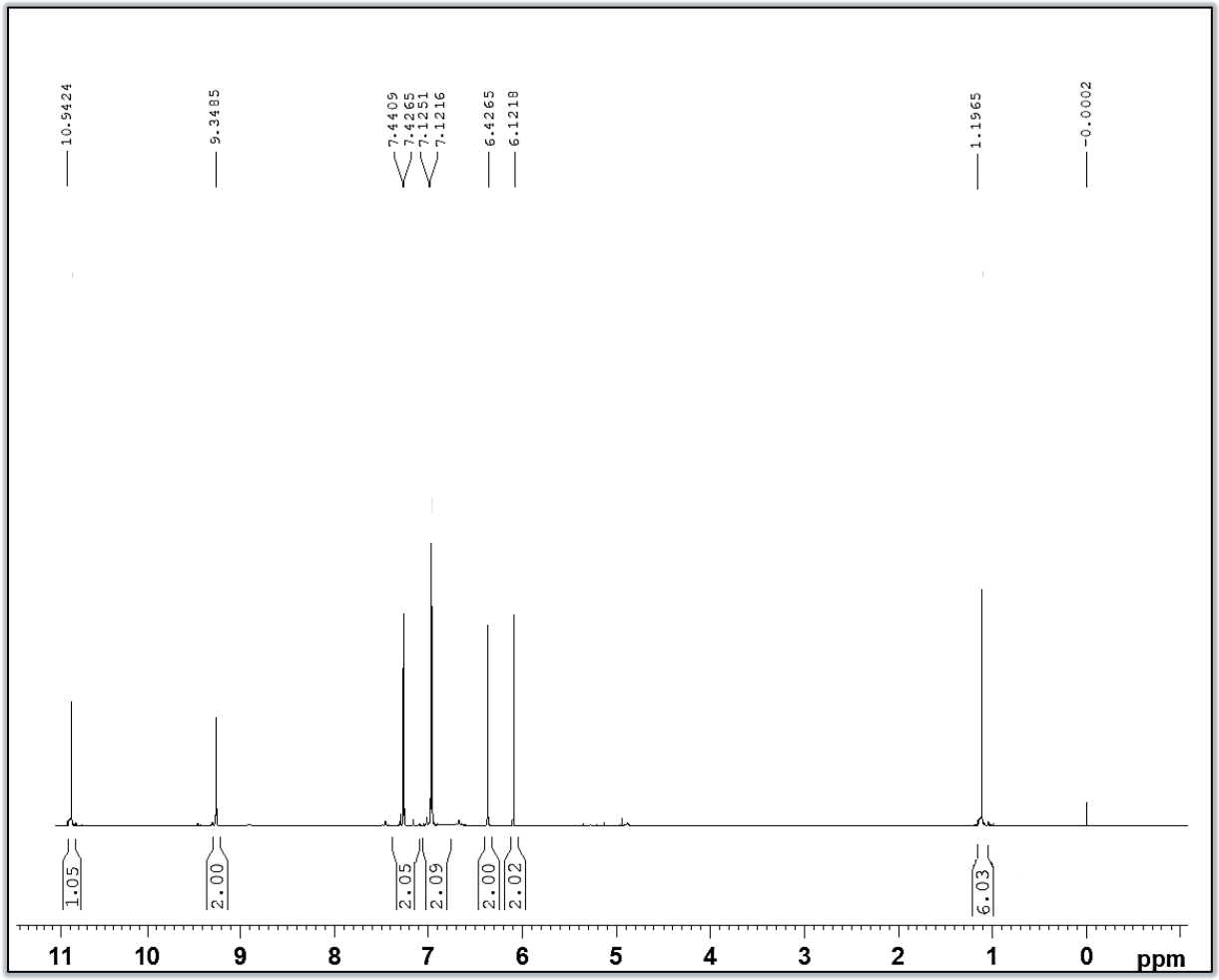

Figure 1: ^{1}H NMR Spectra of compound 2-(4-Aminoanilino)-5,5-dimethyl-5,6-dihydro-1,3-benzoxazol-7(4*H*)-one (4b)

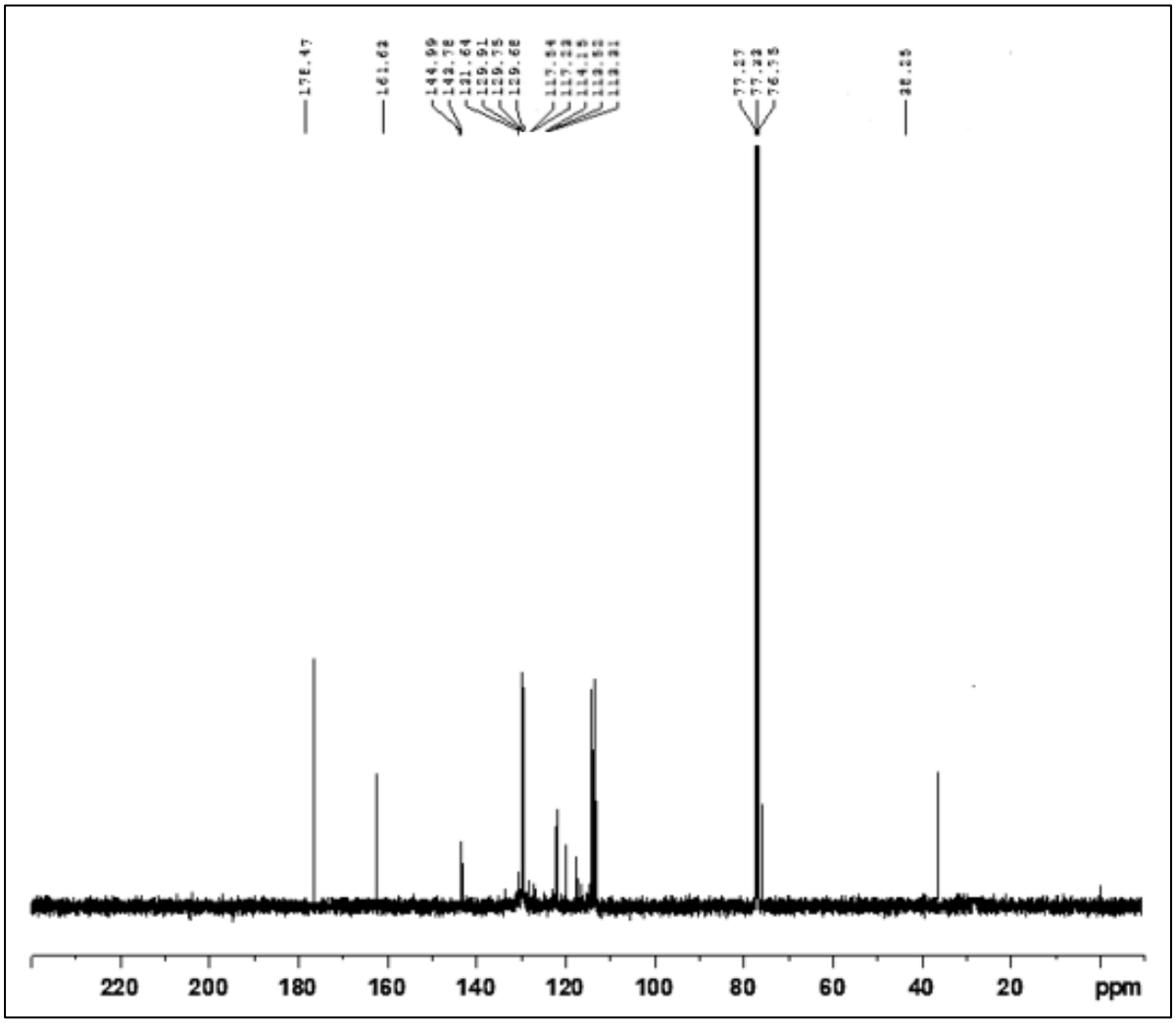

Figure 2: ^{13}C NMR Spectra of compound 2-(4-Aminoanilino)-5,5-dimethyl-5,6-dihydro-1,3-benzoxazol-7(4*H*)-one (4b)

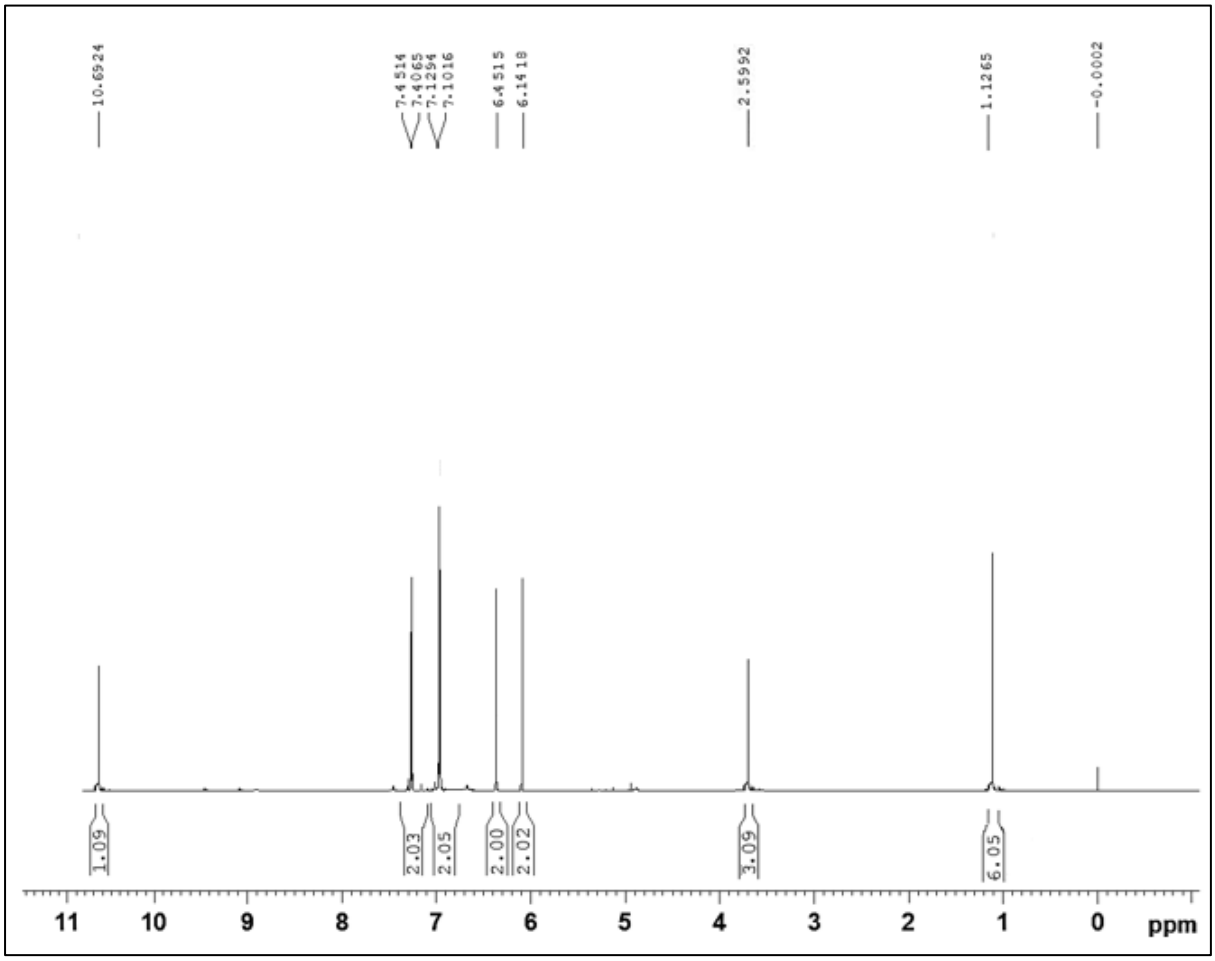

Figure 3: ^{1}H NMR Spectra of compound 2-(3-Methoxyanilino)-5,5-dimethyl-5,6-dihydro-1,3-benzoxazol-7(4*H*)-one (4e)

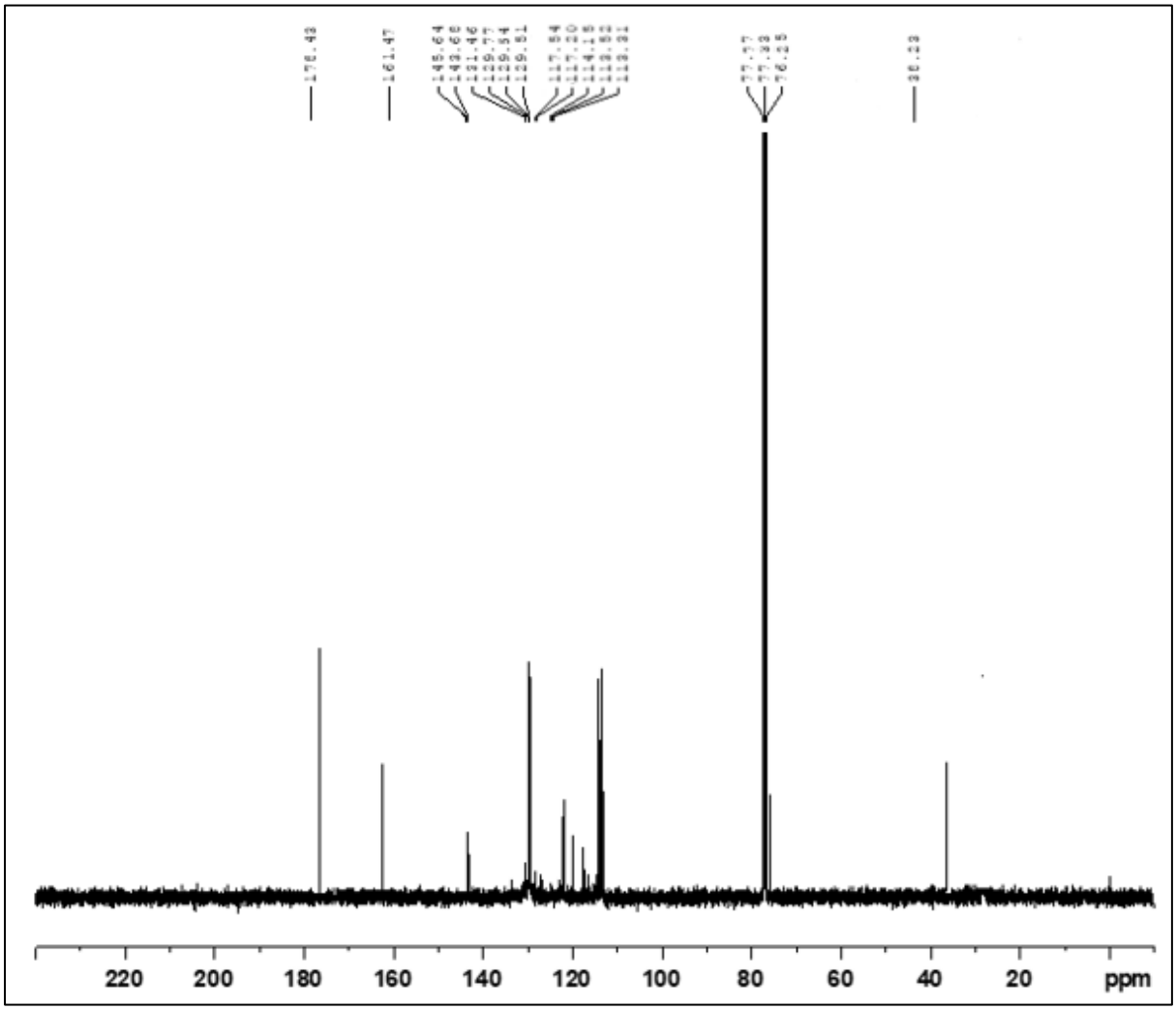

Figure-4: ^{13}C NMR Spectra of compound 2-(3-Methoxyanilino)-5,5-dimethyl-5,6-dihydro-1,3-benzoxazol-7(4*H*)-one (4e)

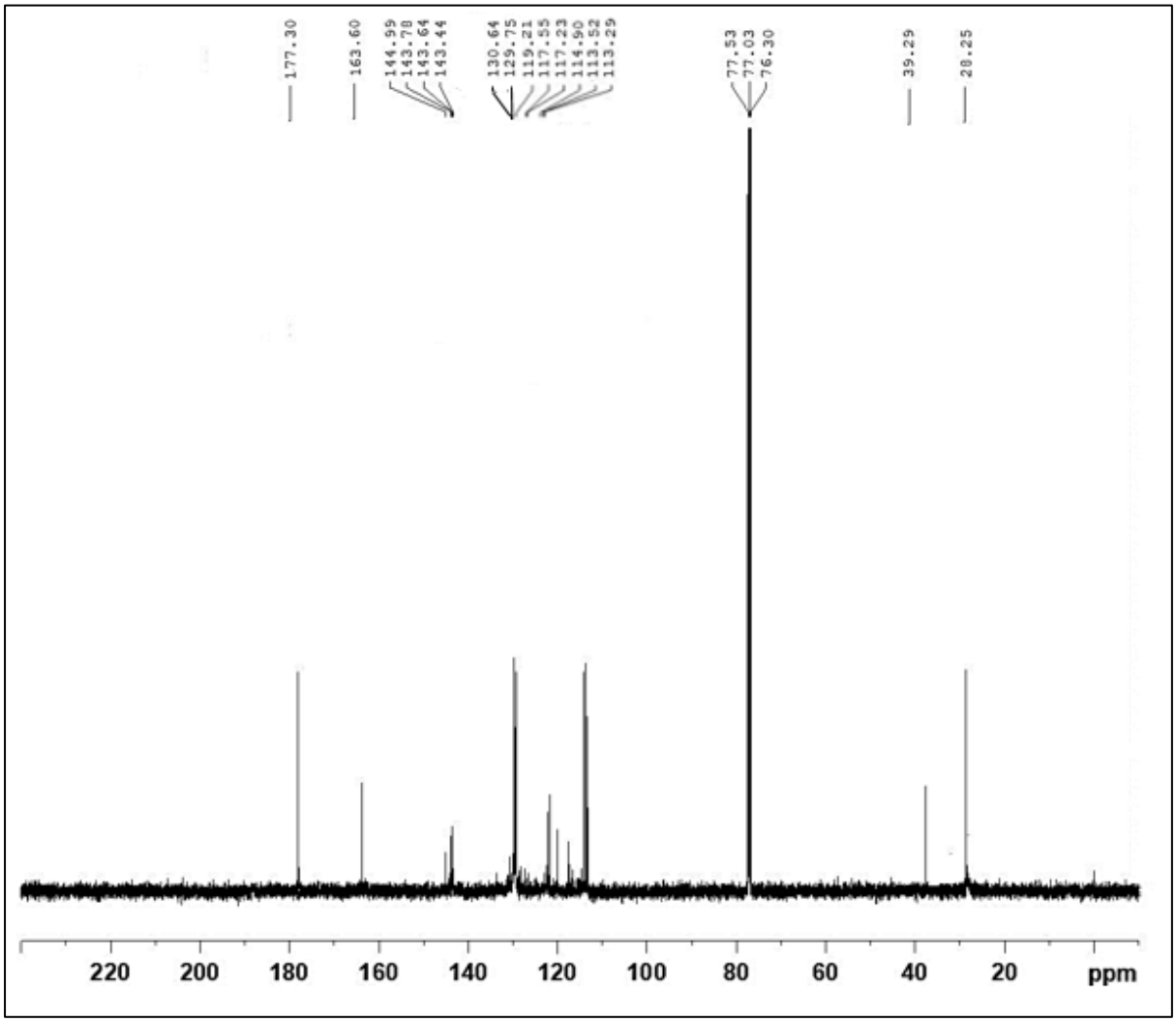

Figure 5: ^{13}C Spectra of compound 22-[(1E)-1-(4-Hydroxyphenyl)-5-hydroxy-3-methyl-1H-pyrazol-4-ylazo]-5,5-dimethyl-1-oxa-3-aza-5,6-dihydroinden-7(4H)-one **(8c)**

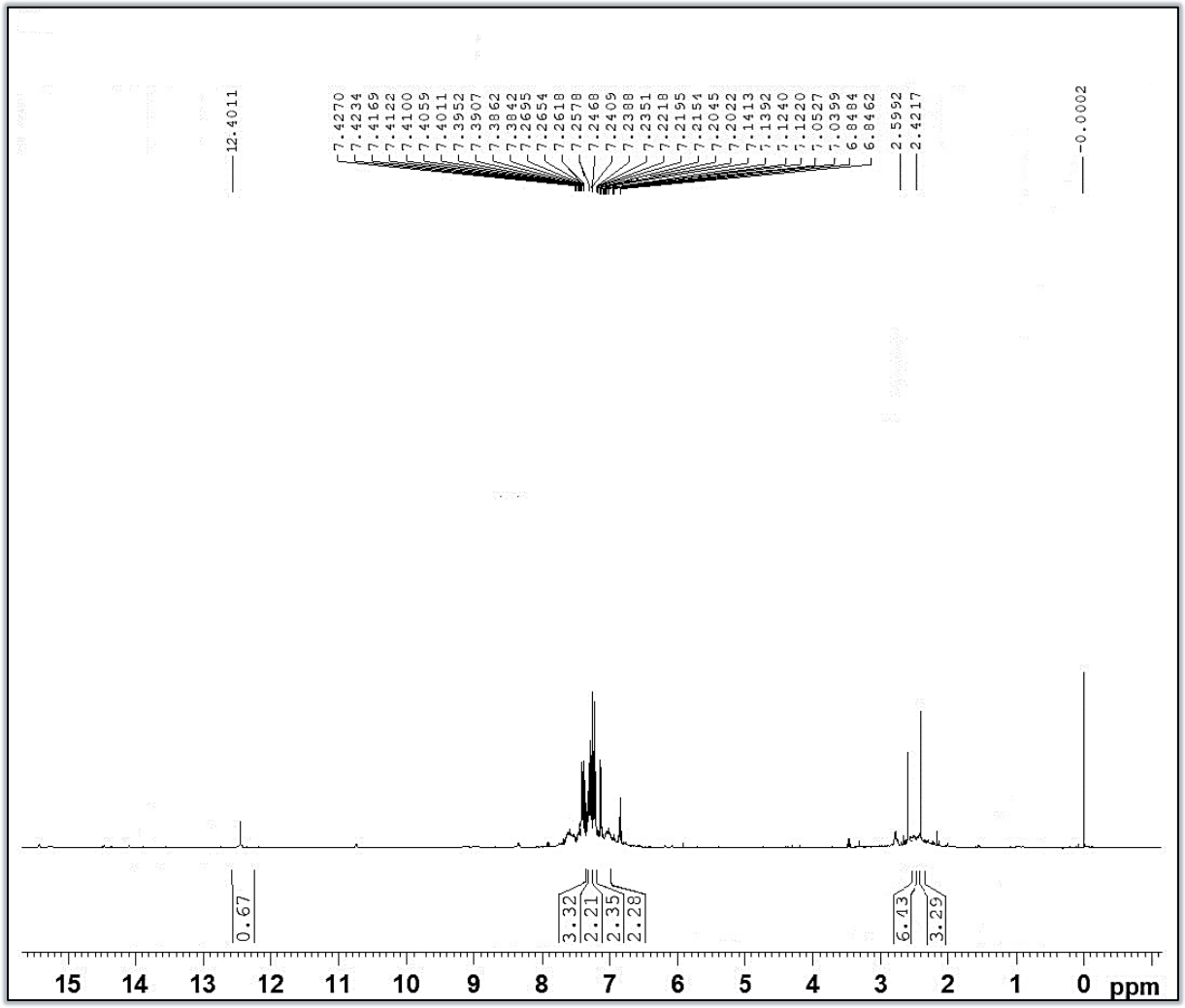

Figure 6: ^{1}H NMR Spectra of compound 2-[(1E)-5-Hydroxy-3-methyl-1-(phenyl)-1H-pyrazol-4-ylazo]-5,5-dimethyl-1-oxa-3-aza-5,6-dihydroinden-7(4H)-one **8d**

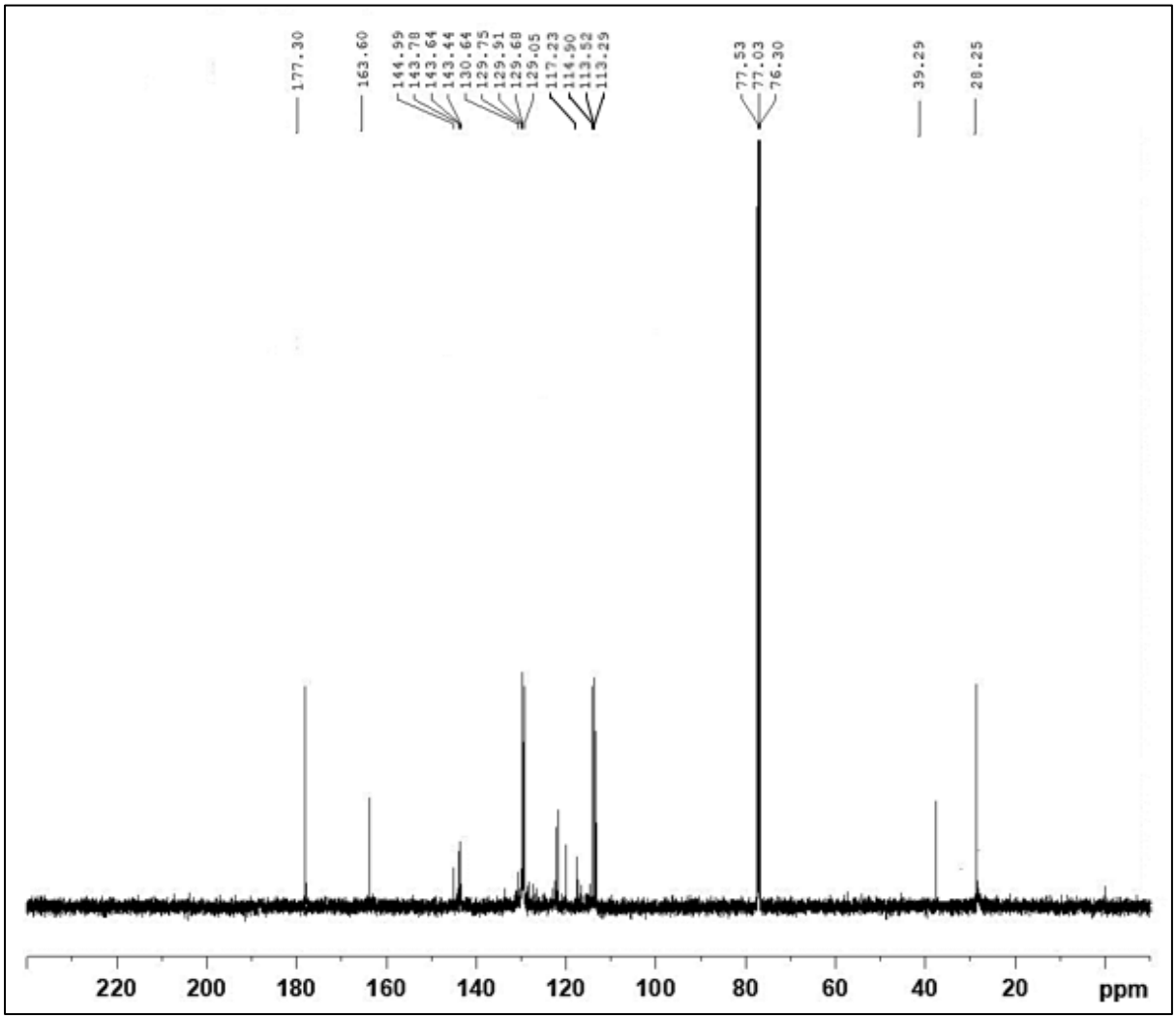

Figure 7: ^{13}C NMR Spectra of compound 2-[(1E)-5-Hydroxy-3-methyl-1-(phenyl)-1H-pyrazol-4-ylazo]-5,5-dimethyl-1-oxa-3-aza-5,6-dihydroinden-7(4H)-one **8d**

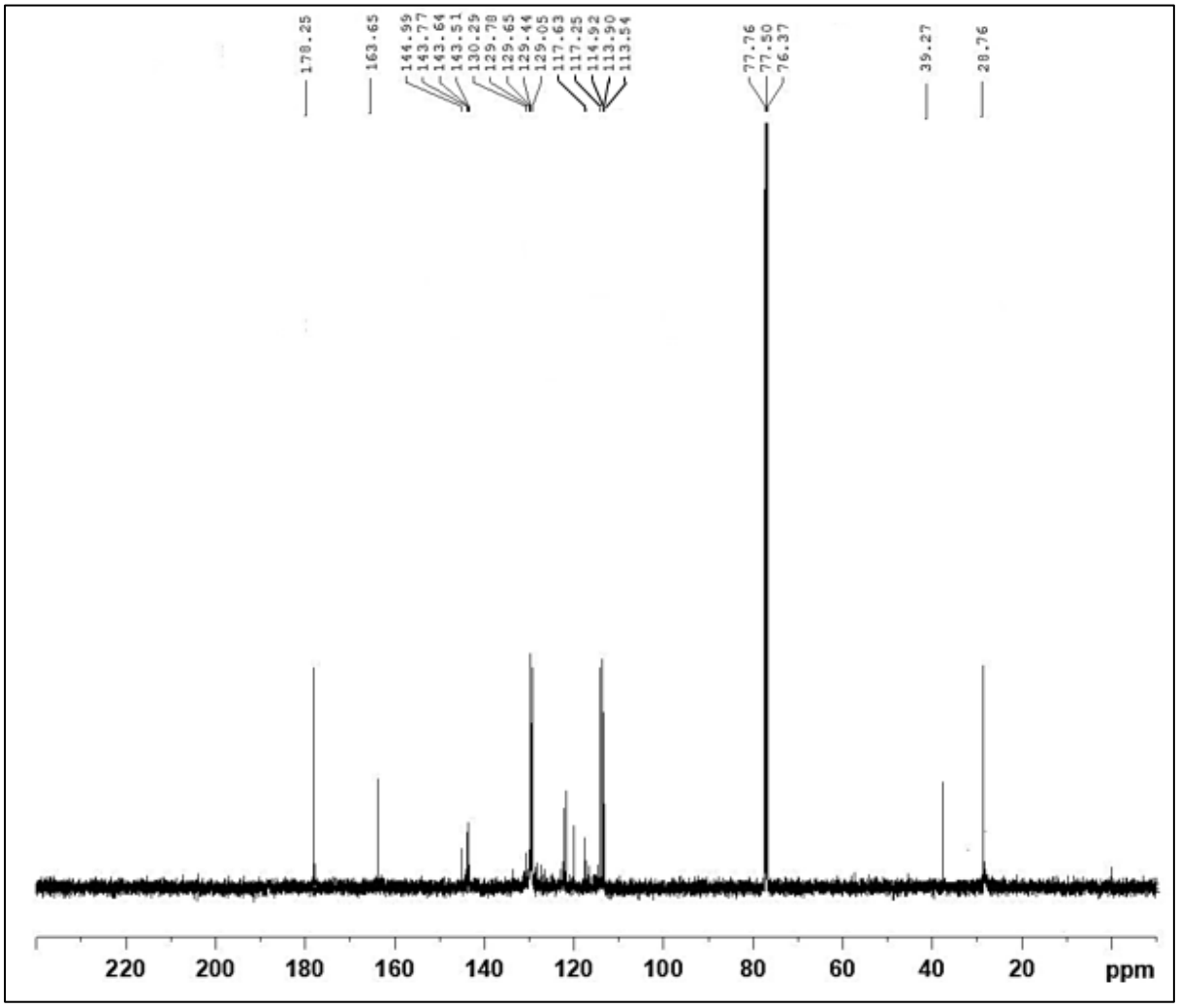

Figure 8: ^{1}H NMR Spectra of compound 2-[(1E)- 5-Hydroxy-3-methyl-1-(2,4-dinitrophenyl)-1H-pyrazol-4-ylazo]-5,5-dimethyl-1-oxa-3-aza-5,6-dihydroinden-7(4H)-one **8e**

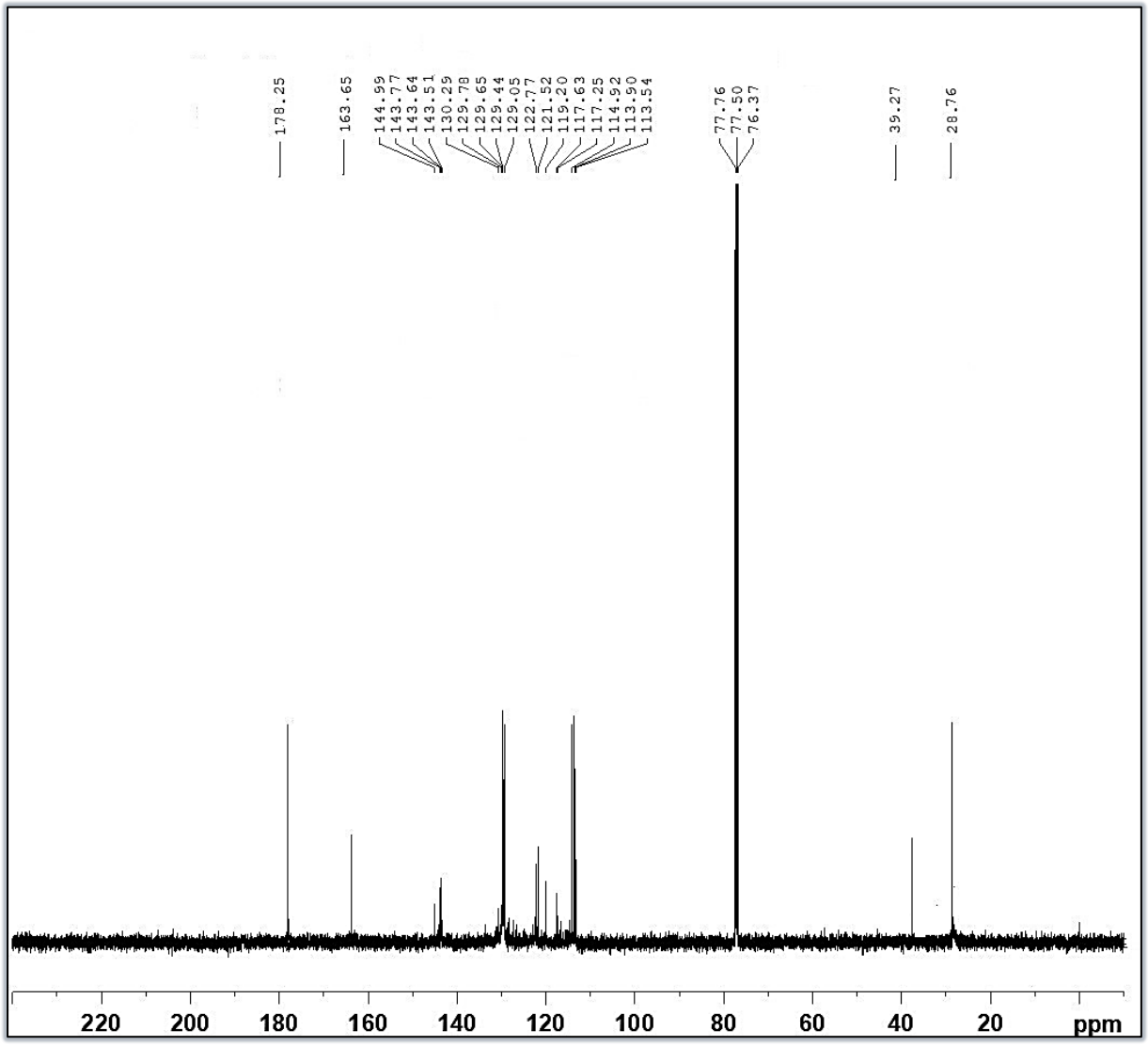

Figure 9: ^{13}C Spectra of compound 2-[(1E)- 5-Hydroxy-3-methyl-1-(2,4-dinitrophenyl)-1H-pyrazol-4-ylazo]-5,5-dimethyl-1-oxa-3-aza-5,6-dihydroinden-7(4H)-one **8e**

Preparation of Macromolecule

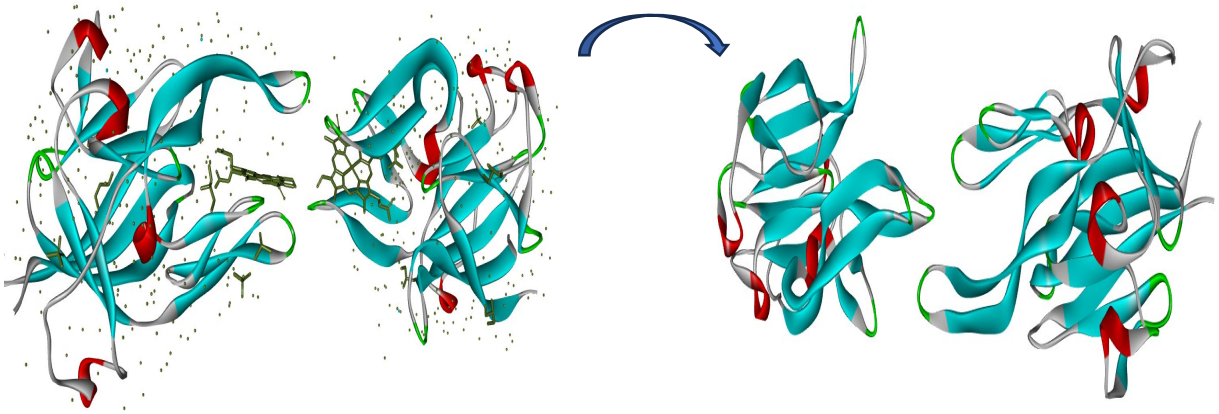

Figure-11: 3D structure of unprepared protein and prepared protein of *P. gingivalis* (PDB ID: 3H8T)

The 3D structure of P. gingivalis heme binding was obtained from the protein data bank, PDB ID: 3H8T. After stripping out co-crystallized ligands, water molecules, and other heteroatoms, it prepared the protein by adding polar hydrogens and kollman charges as the standard protocol was conducted with MGL tools and Biovia Discovery Studio software.

Molecular docking

It also comprised the use of a virtual screening tool, AutoDock Vina PyRx. The autoDock Vina employs a scoring function that is highly optimized to suit multi-threading ability. PyMOL is an open-source version of the virtual screening tool, hence it was installed in a PC that came fitted with an 11th generation Intel(R) Core (TM) i5-1135G7 @ 2.40GHz - 2.42GHz processor, as well as having 8.00 GB of RAM. In the process of drawing the ligand molecules, the ChemSketch was the software used. The molecules were saved in MDL mole (.mol) format, then converted to PDB files using PyMOL. The crystal structure of the HeLa cell line protein caspase-3 (PDB ID: 3H8T) was obtained from the protein data bank and used to explore interactions between the target receptor and newly synthesized ligands. To prepare the proteins, the Biovia Discovery Studio software tool available at https://discover.3ds.com/discovery-studiovisualizer-download was used to remove the water molecules and introduce polar hydrogens. The target protein was loaded onto PyMOL using Autodock commands, which were then saved as a PDBQT. Ligands were then loaded utilizing the input wizard of PyMOL.

The ligand energy in reduced forms was converted to PDBQT format afterward. Simulations of docking were performed by Auto Dock Vina, which located a grid box within the target molecule's active site pocket.

Graphical user interface, Auto Dock Vina38, was applied in ligand-protein docking interactions while the molecular docking research was made possible by free visual user interface, for Auto Dock Vina termed as Auto Dock Tools (ADT). The docking of synthetic compounds against the protein active site with the predefined spacing and dimensions of grid point centre was carried out by Auto Dock Vina. Nine conformations of each ligand were generated and ranked according to their binding energies. The compounds synthesized were then docked to the active site of the protein with Auto Dock Vina. The spacings applied to the center of the grid point were -1.507, 47.298, and 9.104 along the x, y, and z axes, respectively.

The dimensions of the grid box in Angstroms are 58, 92, and 80 along the x, y, and z directions, respectively. Post-docking evaluations were done using the Biovia Discovery Studio Visualiser and PyMOL. The lowest binding energy conformation is used as the best docking score. They were ranked according to their binding energies.