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# Base and additive free click chemistry strategy to accomplish the synthesis of amalgamated pyrazolo-triazole heterocyclic scaffolds and their molecular docking study

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**C H R O N I C L E**

*Article history*:
Received February 25, 2024
Received in revised form
March 25, 2024
Accepted July 3, 2024
Available online
July 3, 2024

*Keywords*:
*Click Chemistry*
*Copper Catalyst*
*1, 2, 3-Triazole linked*
*Pyrazolone*
*Molecular Docking*

**A B S T R A C T**

The current work involves the synthesis of amalgamated heterocyclic scaffolds embracing pyrazolone and triazole nuclei. The suggested methodology leads to streaming in the targeted synthesis in a multicomponent reaction manner resulting in 91% yield of the structural motifs. This strategy makes use of the click reaction mechanism of copper-catalyzed azide-alkyne (CuAAC) cycloaddition. The structures of all the synthesized compounds were ascertained considering spectro-analytical data from $^{1}$H NMR, $^{13}$C NMR, and FTIR and Mass studies. Subsequently, molecular docking studies were performed taking into account the *P. gingivalis* as the heme binding targeted protein.



## Supporting Information

### Experimental Section

All chemicals were purchased from Sigma Aldrich, India, and used without further purification. The reactions were performed in an aerobic atmosphere without any specific precautions. Melting points were determined with open capillary tubes on a Veego melting-point apparatus and were uncorrected. FT-IR spectra were recorded in the range of4400–400cm$^{-1}$ using Perkin-Elmer spectrum FTIR SP 10 STD. The $^{1}$H NMR and $^{13}$C NMR spectra of the synthesized compounds were recorded at 400 and 400 MHz, respectively, using Bruker Advance 400-MHz NMR spectrometer in DMSO-$d_6$ solvent. The chemical shifts were expressed in parts per million relative to tetramethylsilane (TMS) as an internal standard and coupling constants (*J*) are given in hertz (Hz). Splitting patterns are indicated as s (singlet), d (doublet), t (triplet), dd (doublet of doublet), q (quartet), and m (multiplet). Thin-layer chromatography (TLC) was performed using percolated aluminum sheets with silica gel 60 F254.

### General procedure for the preparation of pyrazolone (3a-l)

The mixture of an appropriate(0.38mmol), ethyl 2-((4-nitrophenyl)diazenyl)-3-oxobutanoate**(1a-l)** and hydrazine (0.37 mmol) was taken in evaporating dish. It was heated in a boiling water bath for about 2 hours and stirred from time to time with a glass rod. To this 100 ml of ether was added vigorous stirring of the mixture. The solid, which is insoluble in ether, solidified within 15 minutes and settled down. The solid was filtered and washed thoroughly with ether to remove coloured impurities. The filtered solid was recrystallized from hot water to get the desired product.

### Synthesis of alkyne-substitutedpyrazolone(4a-l)

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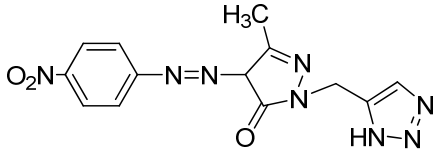
doi: 10.5267/j.ccl.2024.7.002

The mixture of pyrazolone (1mmol) (3a-l), and propargyl bromide (1.2mmol) in DMF(5ml) was stirred with potassium carbonate (1.5 mmol) at room temperature for 20 hours. The progress of the reaction was monitored by TLC (ethyl acetate/petroleum ether). After completion of the reaction, the mixture was poured into cold water and the solid product was separated, filtered out and dried. It was used directly in the next preparation without any further purification.

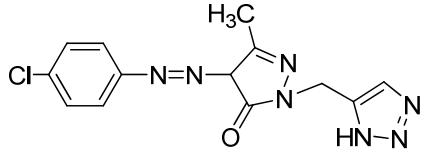
**Synthesis of 1, 2, 3-triazole (5a-l)**

The mixture of alkyne substituted pyrazolone **(4a-l)** (1mmol), sodium azide (1mmol) and copper iodide (5mol %) in DMF (5ml) was stirred for 20 minutes at room temperature. After completion of the reaction (monitored by TLC), water was added, and the desired compound was separated with the help of DCM in a separatory funnel (10x2). The solvent was removed by a rotary evaporator and triazole-linked pyrazolone was obtained in pure form.



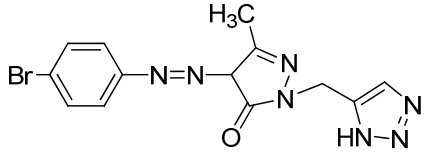
**1-((1H-1,2,3-triazol-5-yl)methyl)-3-methyl-4-((4-nitrophenyl)diazenyl)-1H-pyrazol-5(4H)-one: (5a)**

Dark Red Brown solid: mp 385-387 $^0$C; IR(cm$^{-1}$): 3346, 3071, 2950, 2927, 2195, 1728, 1651, 1606, 1547, 1163, 1102, cm$^{-1}$; $^1$H NMR (400 MHz, DMSO):δ 8.14 (d, *J*= 7.2Hz, 1H), 7.96 (d, *J*=7.2Hz, 1H), 7.09 (d, *J*=7.5Hz, 1H), 7.08(d, *J*=7.5Hz, 1H), 5.80(s, 1H), 4.86(s, 2H), 2.50 (s1H), 1.79(s, 3H) ppm; $^{13}$C NMR(400 MHz, DMSO): 173.0, 156.6, 145.1, 142.4, 127.86, 126.78, 113.16, 64.30, 40.38, 29.65 ppm.



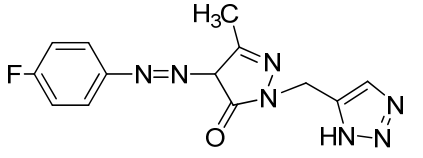
**1-((1H-1,2,3-triazol-5-yl)methyl)-4-((4-chlorophenyl)diazenyl)-3-methyl-1H-pyrazol-5(4H)-one (5b):**

Light Brown solid: mp: 374-375 $^0$C. IR(cm$^{-1}$): 3342, 3065, 2946, 2922, 2191, 1722, 1655, 1595, 1580, 1160, 1190, cm$^{-1}$; $^1$H NMR (400 MHz, DMSO):δ8.04 (d, *J*= 7.2Hz, 1H), 7.89(d, *J*=7.2Hz, 1H), 6.89 (d, *J*=7.5Hz, 1H), 6.88(d, *J*=7.5Hz, 1H), 5.70(s, 1H), 4.76(s, 2H), 2.49 (s1H), 1.78(s, 3H) ppm; $^{13}$C NMR(400 MHz, DMSO): 172.02, 155.65, 144.12, 137.44, 127.86, 126.78, 112.16, 62.30, 40.01, 25.65 ppm.



**1-((1H-1,2,3-triazol-5-yl)methyl)-4-((4-bromophenyl)diazenyl)-3-methyl-1H-pyrazol-5(4H)-one**: **(5c):**

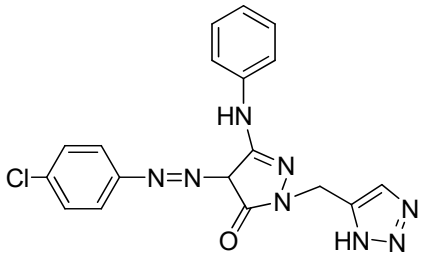
Red Brown solid: mp: 373-374 $^0$C. IR(cm$^{-1}$): 3344, 3070, 2952, 2927, 2194, 1725, 1653, 1598, 1579, 1152, 1105, 1191, 600 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO):δ8.024(d, *J*= 7.2Hz, 1H), 7.94(d, *J*=7.2Hz, 1H), 6.99 (d, *J*=7.5Hz, 1H), 7.09(d, *J*=7.5Hz, 1H), 5.82(s, 1H), 4.87(s, 2H), 2.52 (s1H), 1.76(s, 3H)ppm; $^{13}$C NMR(400 MHz, DMSO): 171.02, 154.65, 147.12, 139.44, 128.86, 125.78, 111.16, 61.30, 42.01, 27.65 ppm.



**1-((1H-1,2,3-triazol-5-yl)methyl)-4-((4-fluorophenyl)diazenyl)-3-methyl-1H-pyrazol-5(4H)-one**: **(5d):**

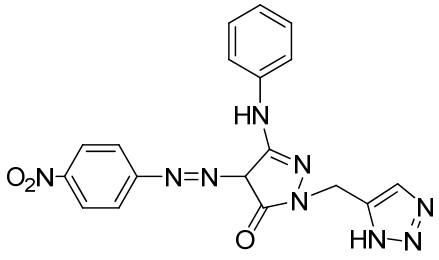
Light red Brown solid: mp: 371-372 $^0$C. IR(cm$^{-1}$): 3340, 3069, 2965, 2932, 2198, 1729, 1658, 1610, 1595, 1166, 1120, 1198, 790 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO):δ7.91 (d, *J*= 7.2Hz, 1H), 7.95(d, *J*=7.2Hz, 1H), 7.19 (d, *J*=7.5Hz, 1H), 7.18(d,

*J*=7.5Hz, 1H), 5.88(s, 1H), 4.89(s, 2H), 2.58 (s1H), 1.80(s, 3H) ppm; $^{13}$C NMR(400 MHz, DMSO): 175.02, 156.65, 148.12, 141.44, 129.86, 126.78, 113.16, 64.30, 45.01, 29.65 ppm.



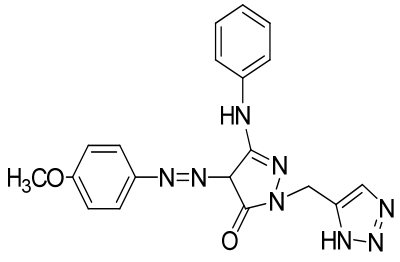

**1-((1H-1,2,3-triazol-5-yl)methyl)-4-((4-chlorophenyl)diazenyl)-3-(phenylamino)-1H-pyrazol-5(4H)-one: (5e):**

Dark Brown solid: mp: 375-377 $^{0}$C. IR(cm$^{-1}$): 3405, 3370, 3075, 3070, 2959, 2948, 2935, 2190, 1728, 1690, 1620, 1585, 1164, 1109, 1190, 759 cm$^{-1}$; $^{1}$H NMR (400 MHz, DMSO):δ 7.98(d, *J*=7.5Hz, 1H), 7.86(d, *J*=7.5Hz, 1H), 7.02(s, 1H), 7.06(d, *J*=7.2Hz, 2H), 7.21(t, *J*=7.2Hz, 2H), 7.14(t, 1H), 6.99(d, *J*=7.5Hz, 1H), 6.45(d, *J*=7.5Hz, 1H),4.40(s, 2H), 2.80(s, 1H) ppm; $^{13}$C NMR(400 MHz, DMSO): 169.30, 158.56, 154.40, 146.41, 142.14, 128.21, 120.98, 118.36, 115.25, 60.10, 43.11, 40.98, ppm.



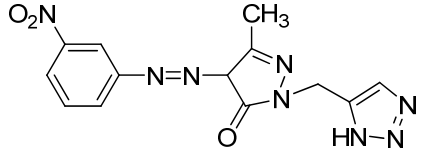

**1-((1H-1,2,3-triazol-5-yl)methyl)-4-((4-nitrophenyl)diazenyl)-3-(phenylamino)-1H-pyrazol-5(4H)-one(5f):**

Dark Brown solid: mp: 385-386 $^{0}$C. IR(cm$^{-1}$): 3395, 3354, 3075, 3065, 2955, 2947, 29230, 2197, 1725, 1655, 1610, 1595, 1555, 1169, 1115, 1200 cm$^{-1}$; $^{1}$H NMR (400 MHz, DMSO):δ 8.04(d, *J*=7.5Hz, 1H), 8.01(d, *J*=7.5Hz, 1H), 7.54(s, 1H), 7.45(d, *J*=7.2Hz, 2H), 7.20(t, *J*=7.2Hz, 2H), 6.98(t, 1H), 6.75(d, *J*=7.5Hz, 1H), 6.69(d, *J*=7.5Hz, 1H),4.47(s, 2H), 2.99(s, 1H) ppm; $^{13}$C NMR(400 MHz, DMSO): 174.30, 159.57, 155.42, 145.42, 141.15, 127.22, 120.98, 119.3, 114.25, 59.10, 42.12, 39.98, ppm.



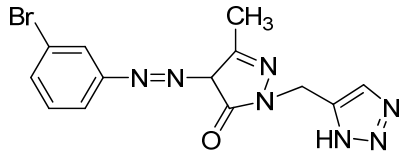

**1-((1H-1,2,3-triazol-5-yl)methyl)-4-((4-methoxyphenyl)diazenyl)-3-(phenylamino)-1H-pyrazol-5(4H)-one (5g):**

Dark Brown solid: mp: 385-386 $^{0}$C. IR(cm$^{-1}$): 3395, 3354, 3075, 3065, 2955, 2947, 29230, 2197, 1725, 1655, 1610, 1595, 1555, 1169, 1200 cm$^{-1}$; $^{1}$H NMR (400 MHz, DMSO):δ8.14(d, *J*=7.5Hz, 1H), 8.06(d, *J*=7.5Hz, 1H), 7.54(s, 1H), 7.45(d, *J*=7.2Hz, 2H), 7.22(t, *J*=7.2Hz, 2H), 6.94(t, 1H), 6.71(d, *J*=7.5Hz, 1H), 6.65(d, *J*=7.5Hz, 1H),4.48(s, 2H), 3.19(s, 3H) ppm; $^{13}$C NMR(400 MHz, DMSO): 176.33, 159.98, 156.45, 147.44, 142.14, 128.23,121.98, 118.4, 115.26, 58.12, 43.12, 40.98, ppm.



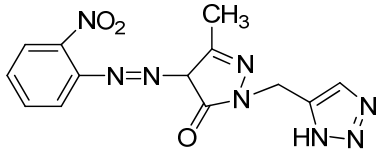
**1-((1H-1, 2, 3-triazol-5-yl)methyl)-3-methyl-4-((3-nitrophenyl)diazenyl)-1H-pyrazol-5(4H)-one(5h):**

Dark Red Brown solid: mp: 385-387 $^0$C. IR(cm$^{-1}$): 3360, 3075, 2955, 2935, 2202, 1729, 1659, 1625, 1598, 1557, 1163, 1116, 1205, cm$^{-1}$; $^1$H NMR (400 MHz, DMSO):δ8.14(s, 1H), 8.11(d, *J*=7.2Hz, 1H), 7.98(t, 1H), 7.65(d, 1H), 6.95(s, 1H), 4.82(s, 2H), 2.98(s, 1H), 1.61(s, 3H) ppm; $^{13}$C NMR(400 MHz, DMSO): 173.10, 155.60, 144.12, 140.40, 127.88, 127.77, 114.18, 65.35, 40.31, 29.69 ppm.



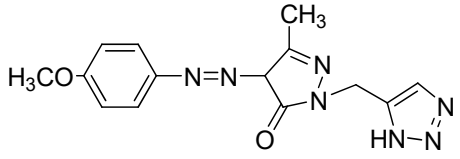

**1-((1H-1,2,3-triazol-5-yl)methyl)-4-((3-bromophenyl)diazenyl)-3-methyl-1H-pyrazol-5(4H)-one(5i):**

Red Brown solid: mp: 375-377 $^0$C. IR(cm$^{-1}$): 3348, 3077, 2959, 2930, 2192, 1723, 1647, 1601, 1583, 1195, 1161, 1111, 610 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO):δ7.97 (d, *J*= 7.2Hz, 1H), 7.95(d, *J*=7.2Hz, 1H), 7.20 (s, 1H), 7.19(t, *J*=7.5Hz, 1H), 5.90(s, 1H), 4.86(s, 2H), 2.56 (s, 1H), 1.89(s, 3H) ppm; $^{13}$C NMR(400 MHz, DMSO): 171.02, 154.65, 146.11, 138.45, 126.86, 124.78, 113.18, 61.38, 41.01, 26.60 ppm.



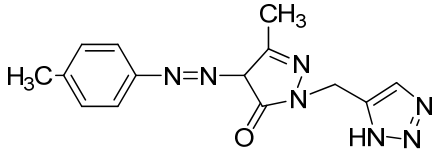

**1-((1H-1,2,3-triazol-5-yl)methyl)-3-methyl-4-((2-nitrophenyl)diazenyl)-1H-pyrazol-5(4H)-one(5j):**

Dark Red Brown solid: mp: 390-391 $^0$C. IR(cm$^{-1}$): 3365, 3085, 2961, 2938, 2206, 1732, 1654, 1616, 1596, 1554, 1200, 1173, 1112, cm$^{-1}$; $^1$H NMR (400 MHz, DMSO): δ8.12(d, *J*=7.5Hz, 1H), 7.78(t, *J*=7.2, 1H), 7.72(t, *J*=7.2, 1H), 7.45(d, 1H), 5.98(s, 1H),4.69(s, 2H), 2.98(s, 1H), 1.90(s, 3H) ppm; $^{13}$C NMR(400 MHz, DMSO): 173.10, 156.60, 145.10, 142.40, 128.86, 127.79, 113.14, 64.31, 40.33, 28.64 ppm.




**1-((1H-1,2,3-triazol-5-yl)methyl)-4-((4-methoxyphenyl)diazenyl)-3-methyl-1H-pyrazol-5(4H)-one(5k):**

Brown Black solid: mp: 375-377 $^0$C. IR(cm$^{-1}$): 3350, 3065, 2955, 2935, 2190, 1720, 1645, 1610, 1585, 1090, 1150, 1093, 1195, cm$^{-1}$; $^1$H NMR (400 MHz, DMSO):δ8.05 (d, *J*= 7.2Hz, 1H), 7.99(d, *J*=7.2Hz, 1H), 7.29 (d, *J*=7.5Hz, 1H), 7.18(d, *J*=7.5Hz, 1H), 5.84(s, 1H), 4.81(s, 2H), 3.45 (s, 3H), 1.79(s, 3H) ppm; $^{13}$C NMR(400 MHz, DMSO): 176.15, 158.63, 147.10, 143.42, 129.86, 126.79, 112.14, 63.33, 41.38, 29.69 ppm.




**1-((1H-1,2,3-triazol-5-yl)methyl)-3-methyl-4-(p-tolyldiazenyl)-1H-pyrazol-5(4H)-one(5l):**

Dark Brown solid: mp: 371-372 $^0$C. IR(cm$^{-1}$): 3341, 3065, 2945, 2929, 2188, 1717, 1660, 1615, 1587, 1164, 1108, 1181, cm$^{-1}$; $^1$H NMR (400 MHz, DMSO):δ 7.79 (d, *J*=7.2Hz, 1H), 7.59 (d, *J*= 7.2Hz, 1H), 7.56 (s, 1H), 6.93 (d, *J*=7.5Hz, 1H), 6.79(d, *J*=7.5Hz, 1H), 5.77 (s, 1H), 3.92 (s, 2H), 2.40 (s, 3H), 1.56(s, 3H) ppm; $^{13}$C NMR(400 MHz, DMSO): 169.19, 154.51, 147.16, 140.40, 126.10, 124.46, 110.12, 61.34, 40.38, 26.69 ppm.

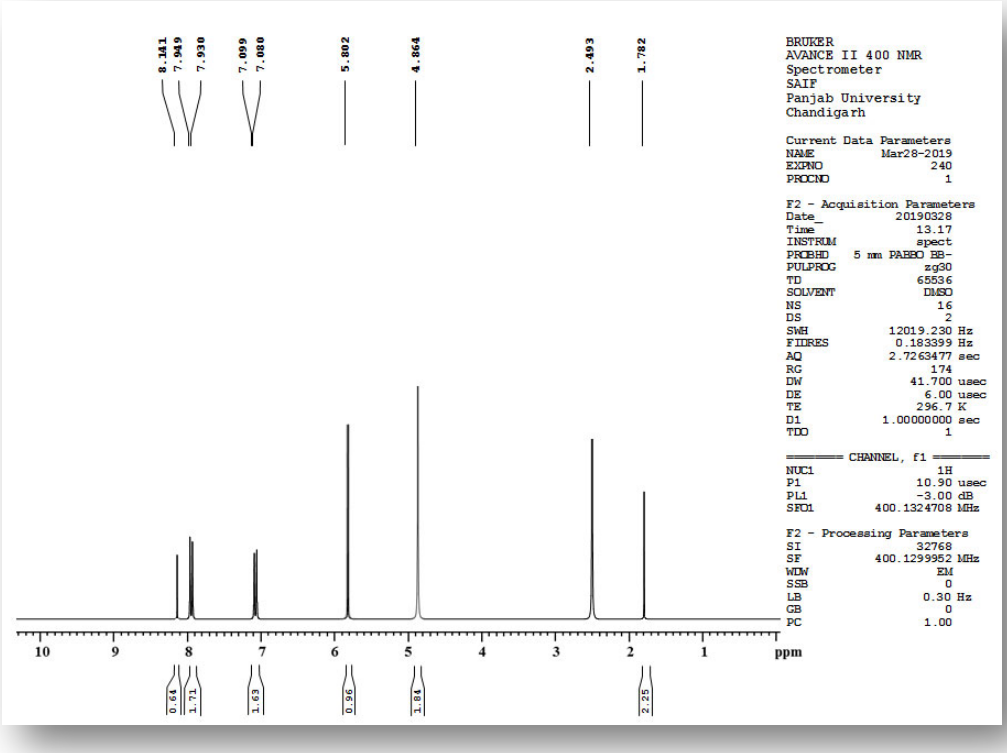



**Figure 1:[1]H NMR Spectra of compound 5a**

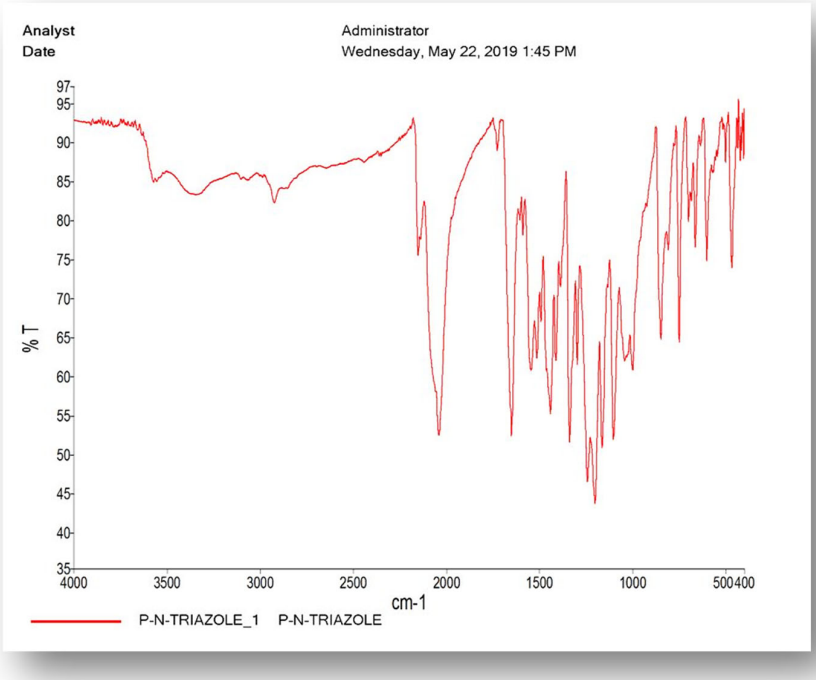



**Figure 2: IR Spectra of compound 5a**

**Preparation of Macromolecule**

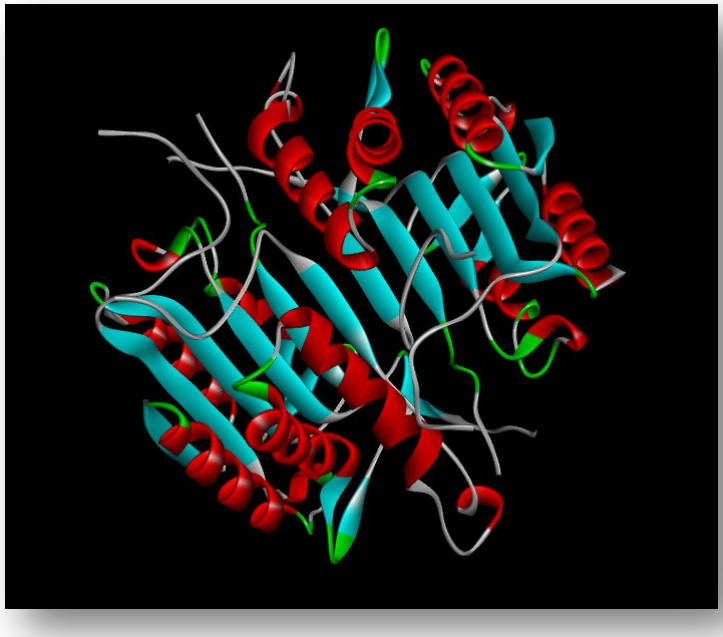


**Figure 3. P. Gingivalis 3D Structure (PDB ID: 5IAE)**

The 3D structure (**Figure 3**) of the heme binding of P. gingivalis was retrieved from the protein data bank (PDB ID: 5IAE). Water molecules, other hetero atoms, co-crystallized ligands were removed, and the protein was prepared by adding polar hydrogens and kollman charges in accordance with the standard protocol employing the software Biovia Discovery Studio and MGL tools.

## Molecular docking

In this study, the virtual screening tool AutoDock Vina PyRx was utilized, providing enhanced docking efficiency and accuracy. AutoDock Vina employs a scoring function with efficient optimization and multithreading capabilities. The PyMOL virtual screening tool, an open-source software (https://pymol.org/2/), was downloaded and installed on a computer configured with an Intel(R) Core (TM) i5-8250U CPU @ 1.60 GHz 1.80 GHz processor and 8.00 GB RAM capacity. Ligand molecules were drawn using the ChemSketch software tool (https://www.acdlabs.com) and saved in MDL mole (.mol) format, subsequently converted to a PDB file using PyMOL.

To investigate interactions between the newly synthesized ligands and the target receptor, the crystal structure of the HeLa cell line protein caspase-3 (PDB id: 5IAE) was obtained from the Protein Data Bank (https://www.rcsb.org). Protein preparation involved the removal of water molecules and addition of polar hydrogens using the Biovia Discovery Studio software tool (https://discover.3ds.com/discovery-studiovisualizer-download). The target protein was loaded into PyMOL, and saved as a PDBQT file using Autodock commands, and ligands were loaded using the PyMOL input wizard. Ligand energies were minimized and converted to PDBQT file format. Docking simulations were performed using AutoDock Vina with a grid box set up in the active site pocket of the target molecule.

The graphical user interface Auto Dock Vina[42] was employed for Ligand-Protein docking interactions, with Auto Dock Tools (ADT), a free visual user interface (GUI) for AutoDock Vina, facilitating molecular docking research. Synthesized compounds were docked against the protein's active site, employing Auto Dock Vina with specific grid point center spacing and dimensions. Nine alternative conformations were created for each ligand and ranked based on their binding energies. The compounds Synthesized were docked against the protein's active site, Auto Dock Vina was employed with a grid point center spacing of 0.957, 6.134, and 144.599 along the x, y and z axis respectively. The dimensions (Angstrom) of the grid box are 40, 40, and 39.555 points in the x, y, and z directions respectively. The post-docking evaluations were conducted using PyMOL and Biovia Discovery Studio Visualizer. Conformations were ranked according to their binding energy, with the conformation displaying the lowest binding energy considered as the best docking score.