

# *In vitro* and molecular docking studies of an anti-inflammatory scaffold with human peroxiredoxin 5 and tyrosine kinase receptor

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#### Abstract

A new series of 4-(3-(2-amino-3,5-dibromophenyl)-1-(4-substitutedbenzoyl)-4,5-dihydro-1H-pyrazol-5-yl)benzotrile (4a-h) compounds were synthesized and evaluated for in-vitro anti-inflammatory activities. The spectral (IR, NMR) and elemental analyses data of the product indicated the formation of new pyrazoles 4a-h. Compound 4e exhibited potent anti-inflammatory property with 85.45 % inhibitions. This value was compared with standard diclofenac sodium. This data is explained using molecular docking analysis of receptor-ligand binding. These results demonstrated that pyrazole derivatives are potential inhibitors of Human Peroxiredoxin 5 and Tyrosine kinase receptor in the treatment of inflammation related illness.

Keywords: *in vitro* anti-inflammatory, pyrazoline, docking, autodock

#### Background:

Inflammation is the natural defense mechanism of the body to deal with the infection and tissue damage [1]. However uncontrolled in diseases like chronic asthma, rheumatoid and osteo-arthritis, flammatory cascades is answerable for various multiple sclerosis, inflammatory bowel diseases and psoriasis, diabetic nephropathy [2] tumor initiation, and malignant progression [3]. Pain is the most common inflammatory indication needing medical attention and

increase financial burden annually [4]. It is widely believed that deaths related to sepsis and sepsis will continue to rise. Research efforts in the field of sepsis have largely focused on the innate immune system and have conceptually viewed sepsis as a syndrome of hyper-inflammation [5,6]. Under this paradigm, overzealous activation of the host inflammatory response, ostensibly intended for pathogen eradication, becomes deregulated and consequently causes auto injury to the host which leads to

multiple organ failure and death [7]. Peroxiredoxin 5 (PRDX5), also known as PrxV/ AOEB166/PMP20/ACR1, is a novel thioredoxin peroxidase widely expressed in mammalian tissues. z,4-6 PRDX5 may be addressed intracellularly to mitochondria, peroxisomes and the cytosol, suggesting that this peroxiredoxin may have an important role as antioxidant in organelles that are major sources of ROS, namely mitochondria and peroxisomes, and in the control of signal transduction due to its localization in the cytosol [8-10]. Moreover, the physiological importance of PRDX5 has recently been emphasized by its ability to prevent p53-induced apoptosis and to inhibit intracellular hydrogen peroxide accumulation by TNF $\alpha$  [11-12]. It is of interest to report the synthesis, biological evaluation and docking studies of a anti-inflammatory scaffold with binding features with protein target for further consideration.

## Materials and Methods:

Without pre-cleaning, all chemicals were purchased commercially. In the open capillary tube, melting points were identified and uncorrected. On PERKIN ELMER 240 CHN analyzer, elemental testing was carried out. On a Shimadzu FTIR spectrophotometer in the 400-4000 cm<sup>-1</sup> range with KBr pellets, an FT-IR spectrum of title compounds was recorded. The <sup>1</sup>H NMR spectrometer was recorded with the solvents of DMSO and CDC13 on a 400 MHz NMR BRUKER AVANCE. The ppm was reported to have chemical shifts. As an internal reference to every NMR spectrum, tetramethylsilane (TMS) was used, with chemical shifts reported as standard in the case of units (parts per million).

## Synthesis of 4-(3-(2-amino-3,5-dibromophenyl)-1-(4-substitutedbenzoyl)-4,5-dihydro-1H-pyrazol-5-yl)benzonitrile:

Chalcones (3) were obtained by condensation in the first step 2-amino-3, 5-dibromobenzaldehyde (2) 4-acetylbenzonitrile (1). Then, a mixture in 25 mL of acetic acid was refluxed with 3 (0.01 mol) of aryl hydrazide (0.01 mol), 8 hours. Filtration and purification of the precipitate were performed by ethanol recrystallation. Compound synthetic pathway is shown in Scheme 1 4a-h.

## Analysis of the anti-inflammatory activities using HRBC membrane stabilization Method:

The antiinflammatory activity of compounds 4a-h was assessed by *in vitro* HRBC membrane stabilization method. Blood was collected from healthy volunteers. Th collected blood was mixed with equal volume of Alsever solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and distilled water 100 mL) and centrifuged with isosaline. To 1 mL of HRBC suspension, equal volume of test drug in three different concentrations, 100, 250, and 500  $\mu$ g/mL, was added. All the assay mixtures were incubated at 37  $^{\circ}$ C for 30 minutes and centrifuged. The haemoglobin content in

the supernatant solution was estimated by using spectrophotometer at 560 nm [22]. Here, the negative control used was Alsever's solution with blood in it and it contained no Aspirin [15].

## Molecular docking:

Crystal structures of the protein complex used in this study were obtained from the the protein data bank ([www.rcsb.org/pdb](http://www.rcsb.org/pdb)) [16]. Docking calculation was carried out using autodock 4.2 [17,18]. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out on DNA gyrase protein model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of auto-Dock tools [16]. Affinity (grid) maps of  $\times \times$   $\text{Å}$  grid points and 0.375 $\text{Å}$  spacing were generated using the autogrid program [16]. Auto-Dock parameter set- and distance-dependent dielectric functions were used in the calculation of the vander Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method [19]. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 1 50. During the search, a translational step of 0.2  $\text{Å}$ , and quaternion and torsion steps of 5 were applied.

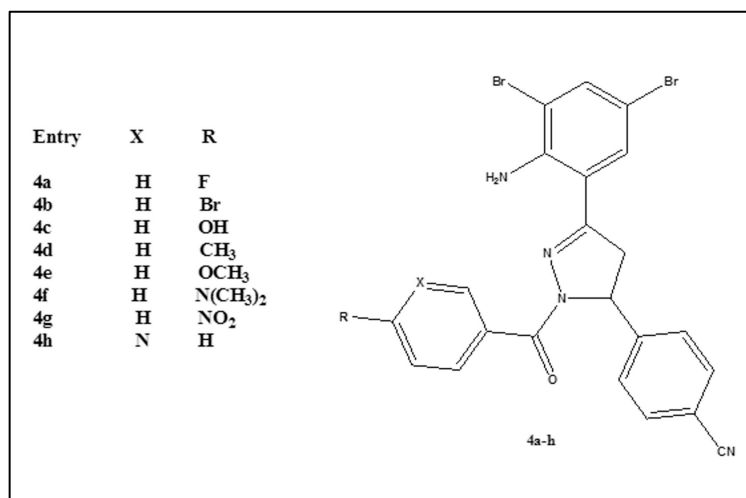
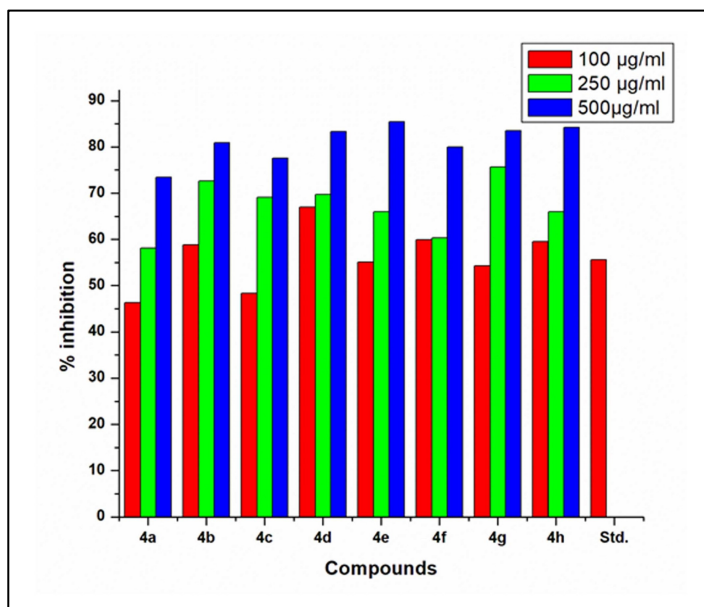
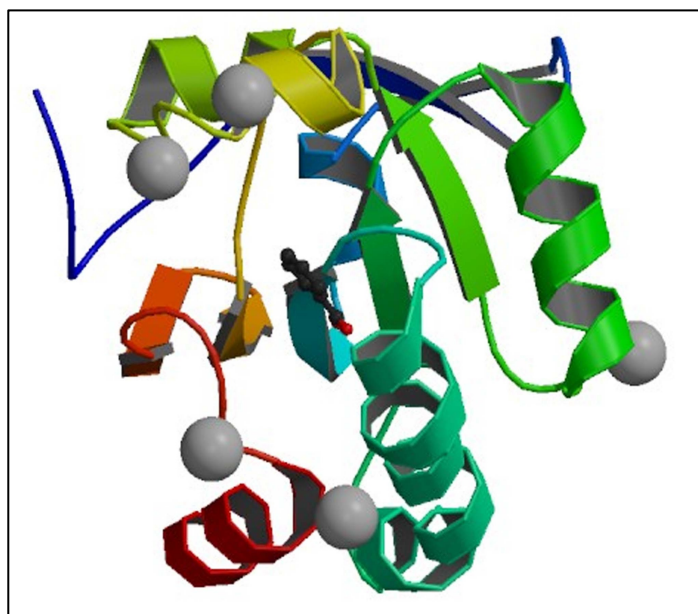


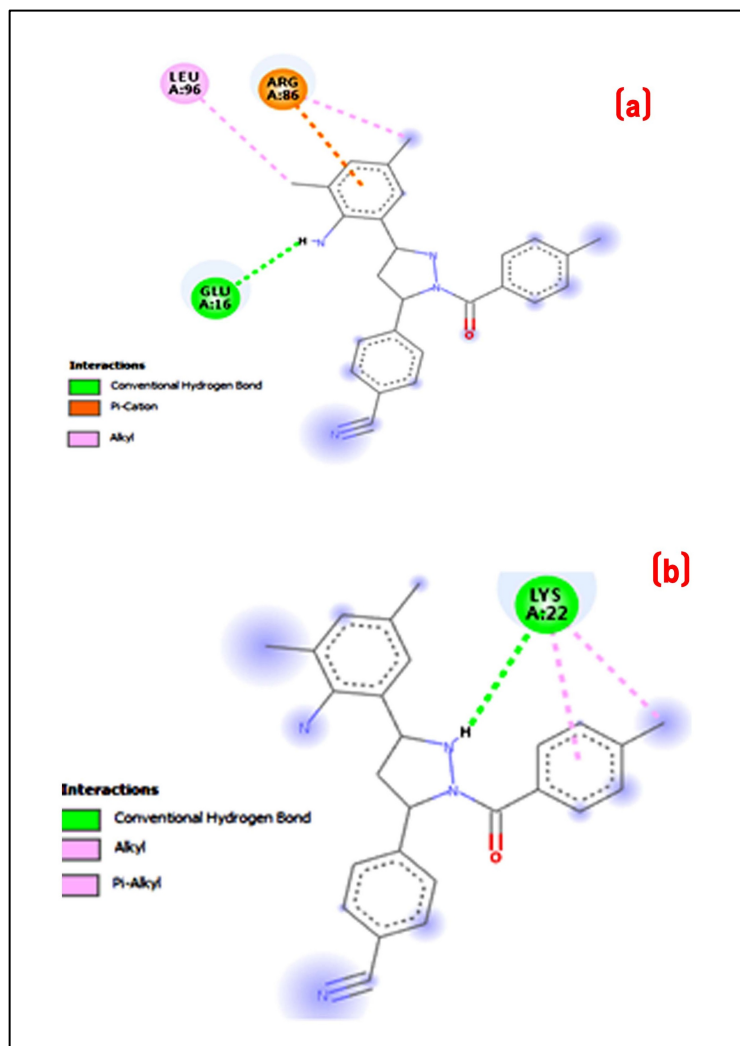
Figure 1: Molecular structure of 1,3,5-trisubstituted pyrazoline derivatives 4a-h



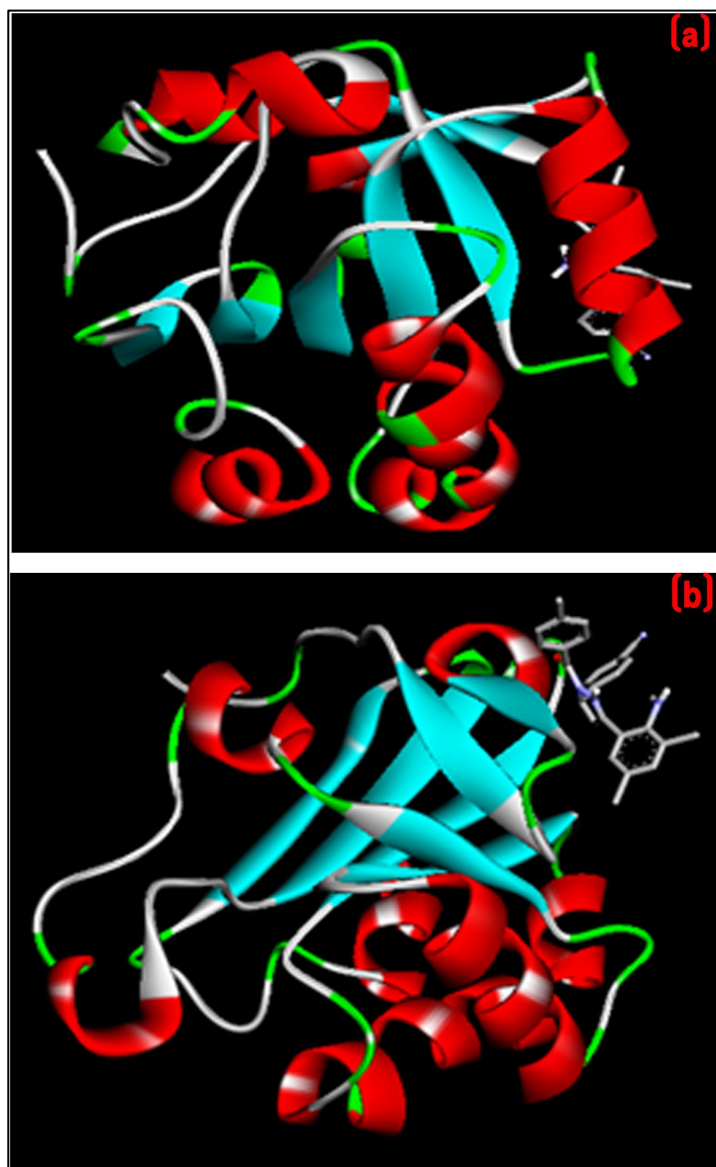
**Figure 2:** Antioxidant inhibition profile of tested compounds at 100,250 and 500 µg/ml concentrations



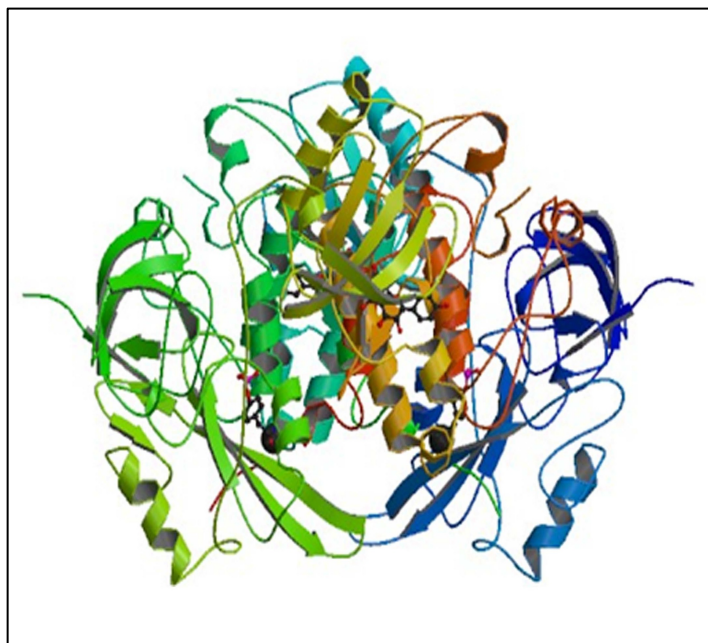
**Figure 3** 3D view of human peroxiredoxin 5 receptor (PDB ID: 1HD2)



**Figure 4:** 2D Docked conformation of most active compound 4d with protein (a) human peroxiredoxin 5 receptor; (b) tyrosine kinase HCK receptor



**Figure 5:** Ribbon binding model compound **4d** (a) human peroxiredoxin 5 receptor (b) tyrosine kinase HCK receptor



**Figure 6:** 3D view of tyrosine kinase HCK receptor (PDB ID: 2HCK)

#### Results and Discussion:

Our research work started with the preparation of 4-(3-(2-amino-3,5-dibromophenyl)-1-(4-substitutedbenzoyl)-4,5-dihydro-1H-pyrazol-5-yl) benzonitrile (**4a-h**). Pyrazole analogues (**4a-h**) were synthesized by condensation of (E)-4-(3-(2-amino-3,5-dibromophenyl)acryloyl)benzonitrile and aryl hydrazide in the presence of acetic acid using conventional heating (**Figure 1**). The spectral characterization (IR, NMR) and elemental analysis data of the product indicated the formation of new pyrazoles **4a-h**. The absorption bands in the region  $1598-1620\text{ cm}^{-1}$  for C=N stretching group [20] in pyrazoles **4a-h**. A strong absorption bands in the region  $2351-2360\text{ cm}^{-1}$  are ascribed to CN stretching. The carbonyl stretching vibrations are observed in the around  $1660\text{ cm}^{-1}$ . In title compounds, N-H [21,22] stretching vibration was observed *ca.*  $3456\text{ cm}^{-1}$ , which supports the formation of pyrazole ring. The  $^1\text{H}$  NMR spectra revealed the presence of a two doublet of doublet signals around 3.74 and 3.09 ppm are easily assigned to proton  $\text{CH}_\text{B}$  and  $\text{CH}_\text{A}$  protons, respectively in pyrazole molecule. The benzylic proton in pyrazole moiety appeared as a doublet of doublet in the region 4.86-3.95 ppm. The  $-\text{NH}_2$  protons are noticed by proton NMR spectra as a sharp singlet around 5.42-5.90 ppm. Additionally, aromatic protons are resonating as multiplets in the

range between 6.90-8.69 ppm. These signals confirm the formation of pyrazoles **4a-h**.

Table 1: Anti-inflammatory activity of pyrazoles 4a-h and diclofenac sodium

Compound	Concentration ( $\mu\text{g/ml}$ )	% inhibition	ic50
4a	100	27.54	207.9
	250	56.67	
	500	78.09	
4b	100	32.1	180.3
	250	60.43	
	500	75.66	
4c	100	19.11	352.1
	250	38.91	
	500	66.62	
4d	100	30.99	192
	250	58.09	
	500	76.77	
4e	100	18.8	271.9
	250	46.32	
	500	80.84	
4f	100	36.27	159.1
	250	62.76	
	500	86.01	
4g	100	32.31	185
	250	59.01	
	500	83.88	
4h	100	15.76	370.6
	250	34.34	
	500	69.57	
DFS	100	42.66	127.3
	250	66.01	
	500	86.01	

The designed compounds were studied for in vitro anti-inflammatory activity by HRBC membrane stabilization method. The anti-inflammatory activity data (Table 1) indicated that all the test compounds exhibited significant activity when compared to standard diclofenac sodium. The data obtained were presented in Table 1. All tested compounds offered adequate protection in a dose-dependent manner. The activity was increased with increasing concentration. The result of the in vitro membrane stabilization activity of synthesized pyrazoline (4a-h) is presented in Table 1 and Fig.2. According to these results all the compounds showed dose dependent inhibition of hemolysis. Compound 4f (IC<sub>50</sub> = 159.1  $\mu\text{g/ml}$ ) and 4b (IC<sub>50</sub> = 180.3  $\mu\text{g/ml}$ ) displayed very good activity among the series as compared to standard Diclofenac sodium (IC<sub>50</sub> = 127.3  $\mu\text{g/ml}$ ). Other compound 4g (IC<sub>50</sub> = 185.0  $\mu\text{g/ml}$ ) and 4d (IC<sub>50</sub> = 192  $\mu\text{g/ml}$ ) showed moderate activity and 4c (IC<sub>50</sub> = 352.1  $\mu\text{g/ml}$ ) and 4h (IC<sub>50</sub> = 370.6  $\mu\text{g/ml}$ ) had exhibited lower anti-inflammatory activity as compared to standard DCS.

In an attempt to explain how our designed compounds interact with active site of the Human Peroxiredoxin 5 (Figure 3) flexible docking simulations were carried out to predict the receptor-ligand interactions by using autodock 4.2. The protein crystal structure Human Peroxiredoxin 5 (PDB ID: 1HD2) was obtained from the PDB (www.rcsb.org/pdb). The graphical depiction of protein is mentioned in Fig. 2. Compounds **4a-h** have scores ranged from -5.01 to -6.06 Kca/mol. According to the docked structure of compound **4a** forms one hydrogen bond with THR147. Further interactions, including hydrophobic and Alkyl/Pi-Alkyl were observed giving a total binding energy value of -5.01 kcal/mol with protein. Replacing of fluoro group by bromo (compound **4b**) leads to increasing binding energy (-5.60 kcal/mol). Compound **4b** also showed hydrophobic interactions with PHE128, ILE119, PRO45 and LEU116 amino acids. The hydroxyl substituted compound **4c** showed binding energy value of -5.95 kcal/mol and makes one hydrogen bond with THR147. It was noticeable that compounds with methyl group substitution (**4d**) at phenyl group had a significant impact on the activity. Compound **4e** has highest binding scores in this experiment as shown Table 2. Figures. 4a and 5a shows three-dimensional binding pose of two active compound **4d** with Human Peroxiredoxin 5. A hydrophobic interaction was observed between the ligand and protein. Compound **4e** also showed  $\pi$ -alkyl interactions with THR44 and PRO40 amino acids. The compounds **4f** and **4g** showed similar binding energy. The replacing of phenyl group by pyridine ring (compound **4h**) showed binding energy about -5.61 kcal/mol.

Figures 4b and 5b shows three-dimensional binding pose of two active compound **4d** with tyrosine kinase HCK. The X-ray crystal structure of protein tyrosine kinase Hck (PDB: 2HCK) is shown in Figure 6. The newly designed compounds **4a-h** was docked into tyrosine kinase Hck protein to understand the binding interactions. Compound **4a** has binding energy -5.41 kcal/mol with three hydrogen bonds *viz.* THR179, GLN529 and GLN528, when introducing bromo substitution (**4b**) in phenyl group. It is pertinent to note that the ligand **4b** exhibit nice binding energy -5.84 kcal/mol.

As depicted in Table 3 compound **4b** showed hydrogen interactions with GLN528, GLN526 and GLN529. In addition it has  $\pi$ -alkyl interaction with LYS203, THR523 residues. The compound **4c** show nice binding energy -6.63 kcal/mol and makes hydrogen bond with GLN526 and GLN525 amino acid residues. Further, it makes  $\pi$ -alkyl interaction with GLU524, THR523, LYS203, THR179 and ARG155 residues. The binding pattern of ligand **4d** with protein clearly revealed that the ligand polar interactions with ARG205, ARG175 and SER185. In addition, it showed  $\pi$ -alkyl

interaction with LYS203 and THR523. As seen from **Table 3**, ligand **4e** showed higher (-7.4 kcal/mol) binding affinity within the protein and it forms hydrogen bond interaction with THR179 amino acid. A polar interaction was observed between the ligand and protein. Compound **4e** also showed  $\pi$ -alkyl interactions with GLN529, HIS201, THR523, ARG155 and ARG175 amino acids. The compounds **4f** and **4g** showed binding energies -5.99 and -5.23 kcal/mol. In this series, compound **4g** shows the least binding energy. The pyridine substituted compound **4h** gives binding energy -5.94 kcal/mol and makes one hydrogen bond with GLN526 amino acid residue. Further, it makes  $\pi$ -alkyl interaction with ARG155, ARG205, ARG175, SER185 and GLN529 residues.

#### Conclusion:

We describe the synthesis, biological evaluation (85.45% inhibition) and docking studies of a anti-inflammatory scaffold [74-(3-(2-amino-3, 5-dibromophenyl)-1-(4-substitutedbenzoyl)-4,5-dihydro-1H-pyrazol-5-yl) benzonitrile] with molecular binding features with the protein targets such as the human peroxiredoxin 5 and tyrosine kinase Hck for further consideration.

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**Table 2:** Docking results of pyrazoles 4a-h with human peroxiredoxin 5

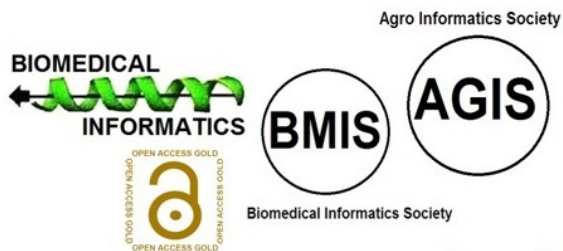
S. No	Substituent	Binding energy Kca/mol-1	Hydrogen	Halogen	Cation-pi	Polar	Hydophobic	Other bonds Alkyl/Pi-Alkyl
4a	Fluoro	-5.01	THR147	ASP145	-	LYS49, ARG127	PRO45, LEU116, PHE120, LEU149	ILE119
4b	Bromo	-5.6	-	THR147	-	ARG205	PHE128, ILE119, PRO45, LEU116	THR44, ARG127, LEU149, PRO40
4d	Methyl	-5.33	THR147, GLY46	-	-	ARG127, LYS49	PRO45, PHE120, LEU149, LEU116, ILE119	ASP145
4e	Methoxy	-6.06	-	-	-	ARG124	PHE120, PRO45, ILE119	THR44, PRO40
4f	N-dimethyl	-5.76	THR147	THR44	PHE120	-	LEU116, LEU112	LEU149, ARG127, SER115, PRO40, ASP145
4g	Nitro	-5.76	ILE119	PHE43	-	ARG127	PRO45	THR44, THR147, PRO40
4h	pyridine	-5.61	GLY46, THR147	-	-	ARG127, THY44, LYS49	PRO45, PRO40,	-

**Table 3:** Docking results of pyrazoles 4a-h with Tyrosine kinase Hck

S. No	Substituent	Binding energy Kca/mol-1	Hydrogen	Halogen	Polar	Hydophobic	Other bonds Alkyl/Pi-Alkyl
4a	Fluoro	-5.41	THR179, GLN529, GLN528	SER185	LYS203, ARG205	-	ARG155, GLN526, THR180, GLU178, ARG175
4b	Bromo	-5.84	GLN528, GLN526, GLN529	HIS201, TYR202,	ARG205	-	LYS203, THR523
4c	Hydroxy	-6.63	GLN526, GLN525	SER185	GLN528, GLN529	-	GLU524, THR523, LYS203, THR179, ARG155
4d	Methoxy	-6.89	GLN529	-	ARG205, ARG175,	HIS201	LYS203, THR523
4e	Methoxy	-7.4	THR179	TYR202, GLU178,	GLN526,	-	GLN529, HIS201,
4f	N-dimethyl	-5.99	THR523, GLU524	-	ARG155, ARG205, ARG175, GLN526, SER185	HIS201	LYS203, THR179, GLU178, GLN529, SER177
4g	Nitro	-5.23	ARG175, GLN5, SER 185	-	LYS203, ARG155, THR179, SER177	-	GLN529, GLU178
4h	Pryridine	-5.94	GLN526	-	LYS203	HIS201	ARG155, SRG205, ARG175, SER185, GLN529

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