"IN SILICO ANALYSIS OF ANTIMICROBIAL ACTIVITY IN NOVEL PYRIMIDINOL DERIVATIVES"

Meenakshi Tyagi[1], Mukesh Maithani[2], Mayank Yadav*

1*Department of Pharmaceutical Sciences, Adarsh Vijendra Institute of Pharmaceutical Sciences, Shobhit University, Gangoh, Saharanpur, UP
2Department of Pharmaceutical Sciences, HNB Garhwal University, Srinagar Garhwal, U.K.

***Corresponding Author:** Dr. Mayank Yadav
Email: **mayankmp@gmail.com**

Abstract: The burgeoning of resistance to antimicrobial drugs is a key the ample defence behind continued research into new compounds. Ciprofloxacin (CPFX) was chosen as standard compound, a virtual screening refinement was appliance to examine the interaction between the compound and its potential targets. Compounds that showed stronger interactions with the target than Ciprofloxacin (CPFX) were selected for further development. The in - silico analysis of these compounds suggested they had drug-like properties and demonstrated good bioactivity and pharmacokinetic properties. Fifteen derivatives of Pyrazol- pyrimidine were synthesized and confirmed by using molecular docking techniques.[1]The process of molecular docking, which involves the arrangement of new Pyrazol- pyrimidine derivatives drawn by Chem Draw 3D (version 16.0) was constitute at the subatomic level by using conventional bond lengths and angles. This was achieved through the use of the Auto Dock Vina software, which was detected by the Discovery Studio Client (version 1.5.7). The structure of protein was also analyzed i.e Escherichia coli (PDB.ID: 1AJ6).All these structures(Table:3) were generated using Auto Dock Vina and were reviewed by the Discovery Studio Client (version 4.2) to confirm the structures and thei specific arrangement of atoms within the binding pocket of each protein. Additionally, The antibacterial activity of some derivatives were examined against a range of bacteria, including both Gram-positive and Gram-negative strains.[2,3] Among the synthesized derivatives, 2,3,7,9,12 showed most potent and 1,5,6,8,10,11,13,14,15more potent and 4 derivative was showed less antibacterial profile than ciprofloxacin. Docking studies showed that these substances were potent candidates for antimicrobial use, with docking scores that were superior to those of the standard ciprofloxacin.

Keywords: Ciprofloxacin, Antibacterial, molecular docking, Pyrazole- Pyrimidine

Introduction:

Antimicrobial resistance (AMR) is a growing global health crisis characterized by the ability of microorganisms—such as bacteria, fungi, viruses, and parasites—to resist the effects of medications that once effectively controlled them. This resistance results in infections that are harder to treat, resulting in extended sickness, higher medical expenses, and higher mortality rates. The creation of innovative antibacterial agents is crucial to addressing the limitations of existing treatments. [4] The effectiveness of conventional antibiotics is declining against resistant strains, highlighting the necessity for novel medications with distinct mechanisms of action. These new agents should be capable of targeting bacterial or fungal pathways with a lower likelihood of developing resistance. [5]

In this context, the Pyrazol bearing Pyrimidine derivatives represent a promising class of compounds. [6] These derivatives are being investigated due to their potential to interact with key microbial targets, such as lyase enzymes, which are essential to microbial metabolism and pathogenicity. By focusing on these compounds, the study aims to identify new leads that might provide practical remedies for resistant strains as compared to Ciprofloxacin drug as standard. [7]

The research into the Pyrazol bearing Pyrimidine derivatives involves evaluating their potential as antimicrobial agents using computer techniques such as molecular docking. This approach helps predict how well these compounds can bind to and inhibit target enzymes, which is crucial for creating medications that are highly effective and minimal resistance development.[8]The identification of such potent inhibitors could result in the creation of novel therapies, contributing significantly to fight against antimicrobial resistance and improving treatment options for resistant infections.[9]

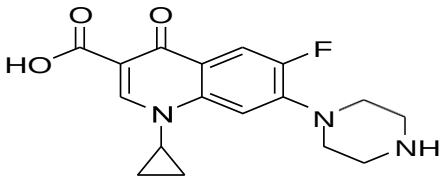

Figure 1: Structure of ciprofloxacin

Overview & Importance of Lyase Enzymes: The synthesis and breakdown of biomolecules are two of the biochemical processes in which a general classes of enzymes identified as lyases Plays a central role roles. [10] In constructiveness to hydrolysis or oxidation, they bolster the cleavage of chemical bonds, often forming new rings or double bonds in the process. Because of their vital functions in microbial metabolism and pathogenesis, lyases are desirable targets for medications development. [11]

1. Key Role in Microbial Metabolism:

Lyase enzymes are integral to several metabolic pathways in microorganisms. For instance, in bacteria and fungi, they participate in processes such as metabolism of amino acids and the production of cell wall and detoxification. Disruption of these pathways can impair microbial growth and survival, making lyases valuable targets for antimicrobial therapy. [12, 13]

2. Novel Target for Drug Development:

Targeting lyase enzymes offers a strategy for discovering novel antibacterial agents with unique mechanisms of action. Unlike traditional antibiotics that often target ribosomes or cell wall synthesis, inhibiting lyase enzymes can interfere with less common but crucial biological processes. [14]This can help overcome existing resistance mechanisms and provide alternative treatment options.

3. Reduction of Cross-Resistance:

Many existing antibiotics face challenges with cross-resistance, where bacteria develop resistance to multiple drugs. [15] By targeting lyase enzymes, which more unlikely to be targeted by conventional antibiotics, it is possible to lessen the likelihood of cross-resistance and address infections that are resistant to current treatments.

4. Selective Inhibition:

Lyase enzymes often have unique structures and functions specific to particular microorganisms. This specificity allows for the design of selective inhibitors that can target pathogenic microbes while minimizing effects on human cells. Such selective targeting enhances the efficacy of antimicrobial agents and reduces potential side effects. [16]

5. Potential for Combination Therapies:

Inhibitors of lyase enzymes can be used in combination with existing antibiotics to enhance therapeutic efficacy. By disrupting multiple aspects of microbial metabolism, combination therapies can provide a synergistic effect, leading to more effective treatment outcomes and reducing the likelihood of resistance development. [17, 18]

Relevance to 2-(2-(5-Chloro-3-Methyl-1-Phenyl-1H-Pyrazol-4-Yl) Vinyl)-6-Methylpyrimidin-4-Ol Derivatives:

The2-(2-(5-Chloro-3-Methyl-1-Phenyl-1H-Pyrazol-4-Yl)Vinyl)-6-Methylpyrimidin-4-Ol derivatives are being explored as potential antimicrobial agents targeting lyase enzymes. These derivatives are designed to bind specifically to the active sites of lyase enzymes, inhibiting their function and thereby disrupting essential microbial processes. [19] The specificity and novel new way in which these derivatives make them attractive prospects for developing effective treatments against resistant microbial infections.

By targeting lyase enzymes with these derivatives, the study aims to harness their potential to offer new solutions in the fight against antimicrobial resistance, potentially leading to the development of next-generation antimicrobial agents with improved efficacy and safety profiles. [20]

Objective:

The primary objective of this study is to evaluate the potential of 2-(2-(5-Chloro-3-Methyl-1-Phenyl-1H-Pyrazol-4-Yl)Vinyl)-6-Methylpyrimidin-4-Ol derivatives as antimicrobial agents. This evaluation is conducted through molecular docking simulations to assess their ability to interact with and inhibit key lyase enzymes implicated in microbial patho- genicity.

3. Materials and Methods:

Pyrazol- pyrimidine derivatives drawn by Chem Draw Ultra 12.0 was utilised to draw the 2D formula of the compounds. The 2- dimensional structures (2D) of the analogues were retrieved from the database CAS Scifinder^{n} in format accessed on November 12, 2022, 8:29AM. [21]For preparing the protein and ligands and determining the Grid map, Autodock 1.5.6 was used. Chain A was selected. Then, the water was deleted, and polar hydrogens and Gasteiger charges were added. A grid map was determined **(Table1).** Energy minimisation was applied with Avogadro software. The visualisation of interactions was made by Discovery Studio 3.5.Docking studies were also conducted to investigate potential interactions between the strong compounds. [22]

To optimise the docking process, crystal structure of Ciprofloxacin (CPFX) was compared with the predicted conformations of docking results. **2,3,7,9,12** showed most potent and **1,5,6,8,10,11,13,14,15** shows best ciprofloxacin considerable lyase enzyme inhibitory effect. Molecular Docking was employed to ascertain the potential optimum binding pose of the compounds **(1-15)** by which they may be sorted for discovering promising leads. The primary steps in molecular docking research are choosing and preparing the right protein, creating the grid and the ligand, and then analysing the results of the docking and how they interact. The BIOVIA Discovery Studio perceiver (Discovery Studio perceiver 2023-12-01T4;30:32z) was prepare the 1AJ6 protein structure for docking.[23] The orientation of the different functional groups, the expunction of ligands and water molecules, and the hydrogen deficiency were all observed adaptations. lyase enzymes it was discovered to be efficient as an antibiotic against Escherichia coli is PDB.ID:1AJ6.

Grid box dimension		Pdb Id
X	60.334	1AJ6
Y	5.741	

Z	40.532	

Table 1: Grid box dimension

Evaluation of predicted protein structure:

The Ramachandran plot is used to assess how accurate the predicted protein structure is. PDBSUM SERVER, MOLPROBITY, STAN SERVER, and other servers and programs were used in Ramachandran plots. Several protein structure stereochemical properties were predicted using five tools in the extensive toolbox saves v6.0 (https://saves.mbi.ucla.edu/): ERRAT, VERIFY3D, PROVE, PROCHECK, AND WHAT CHECK. The Ramachandran plot is used to examine the structural stereochemical property.[24] By contrasting the overall model geometry with the geometry of a specific residue, the procheck assesses the stereochemical quality of a predicted model. The Procheck tool, which generates the Ramachandran plot, needs a modelled protein file as input in order to confirm the structural soundness. [25]

Procheck, a tool that uses Ramachandran plots for structural verification, was employed to evaluate the
stereochemical quality of the 3D model of protein 1Aj6 (figure 2). A high-quality model for research is indicated by the generated protein's Ramachandran plot, which shows the amino acids of the total residues in the most favoured region and the amino acids in the additionally permitted zone.

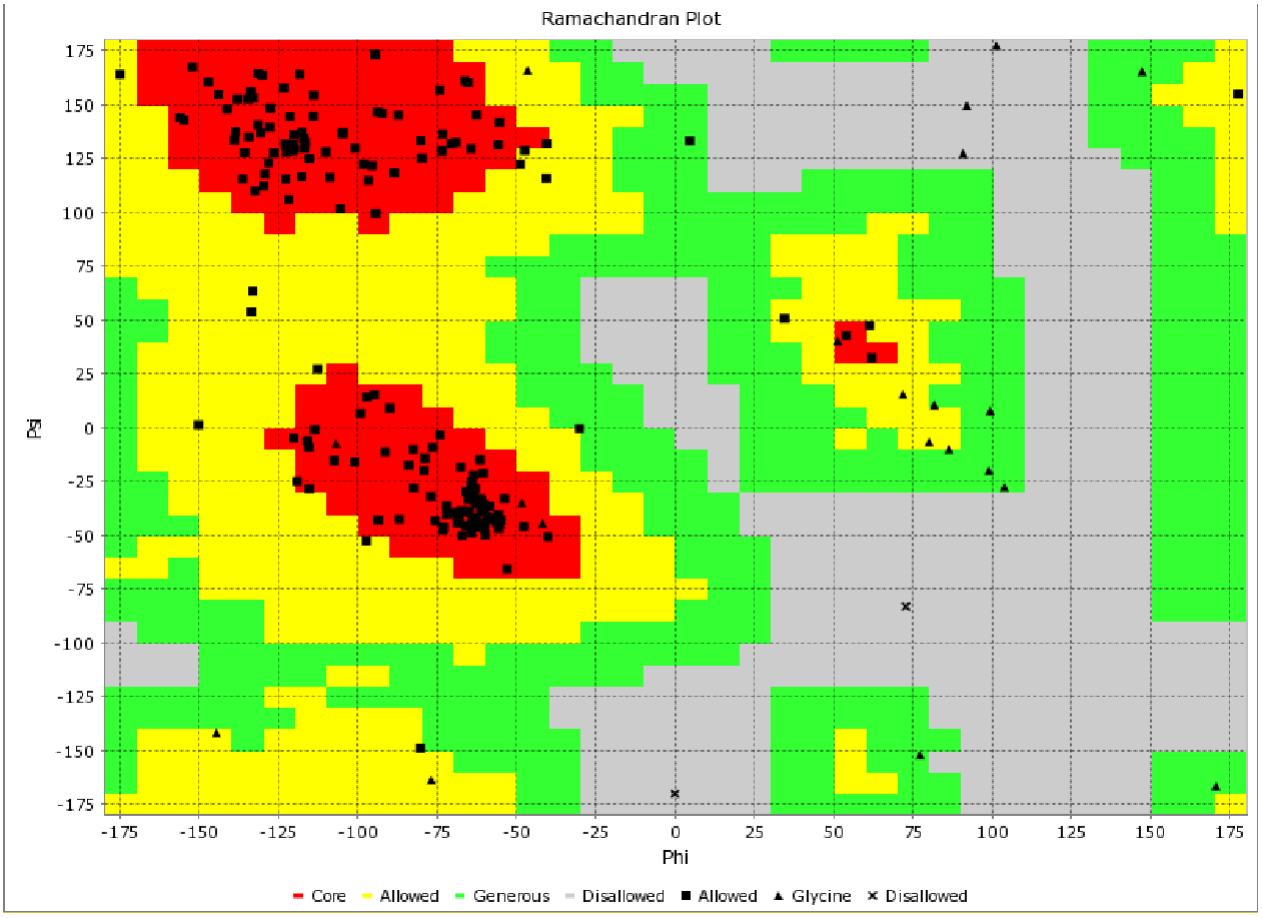

Figure 2: Stereochemical analysis of 1AJ6.

Materials and Methods:

AutoDock Vina is a widely used open-source tool for molecular docking that provides flexible docking and high-quality results.[26] It is renowned for its speed and accuracy in predicting ligand binding affinities.For evaluating the binding potential of 2-(2-(5-Chloro-3-Methyl-1-Phenyl-1H-Pyrazol-4-Yl)Vinyl)-6-Methylpyrimidin-4-Ol derivatives, the following software and tools are commonly used in molecular docking studies:

Target Enzyme Selection: Asparaginase (PDB ID: 1AJ6):

- **Biological Relevance:** Asparaginase is crucial in the metabolism of the amino acid asparagine and is used as Antimicrobial agent to target various microorganisms. **PDB ID: 1AJ6** (Asparaginase from *Escherichia coli*).
- **Structures of Derivatives:** The chemical structures created by molecular drawing software (e.g., ChemDraw, Marvin Sketch) of the 2-(2-(5-Chloro-3-Methyl-1-Phenyl-1H-Pyrazol-4-Yl)Vinyl)-6-Methylpyrimidin-4-Ol derivatives.

Compounds	R^1	R^2	molecular formula	M. w.(g/mol)
1.	**H**	**H**	$C_{10}H_9ClN_4O$	236.66
2.	**H**	**Cl**	$C_{10}H_8Cl_2N_4O$	271.10
3.	**H**	**NO_2**	$C_{10}H_8ClN_5O_3$	281.66
4.	**H**	**CH_3**	$C_{11}H_{11}ClN_4O$	250.69
5.	**Cl**	**H**	$C_{10}H_8Cl_2N_4O$	271.10
6.	**OCH_3**	**H**	$C_{11}H_{11}ClN_4O_2$	266.69
7.	**F**	**CH_3**	$C_{11}H_{10}ClFN_4O$	268.68
8.	**NO_2**	**H**	$C_{10}H_8ClN_5O_3$	281.66
9.	**NH_2**	**H**	$C_{10}H_{10}ClN_5O$	251.67
10.	**F**	**H**	$C_{10}H_8ClFN_4O$	254.65
11.	**Br**	**H**	$C_{10}H_8BrClN_4O$	315.56
12.	**OCH_3**	**Cl**	$C_{11}H_{10}Cl_2N_4O_2$	301.13
13.	**Br**	**NO_2**	$C_{10}H_7BrClN_5O_3$	360.55
14.	**NO_2**	**OH**	$C_{10}H_8ClN_5O_4$	297.66
15.	**CH_3**	**F**	$C_{11}H_{10}ClFN_4O$	268.68

Table 2: The formula of ligands tested in molecular dockin

Table3: 3D and 2D interaction of best conformer with Lyase receptor with 1AJ6 Protein

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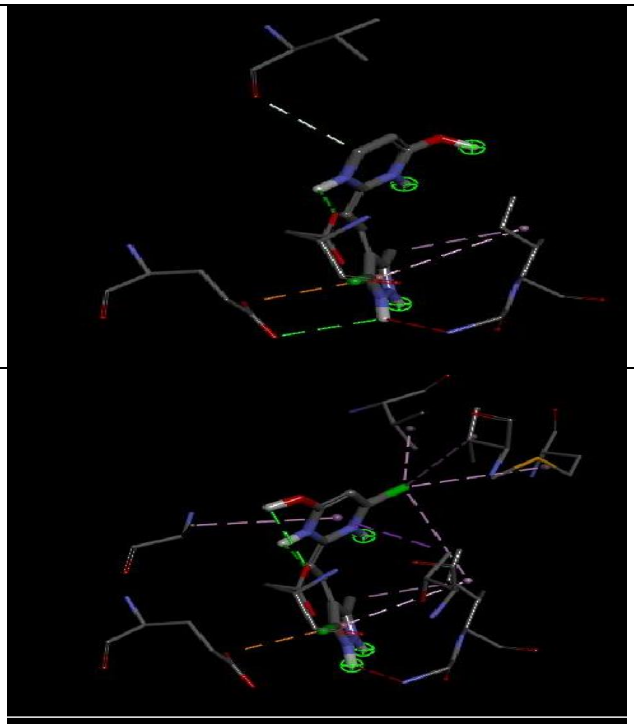
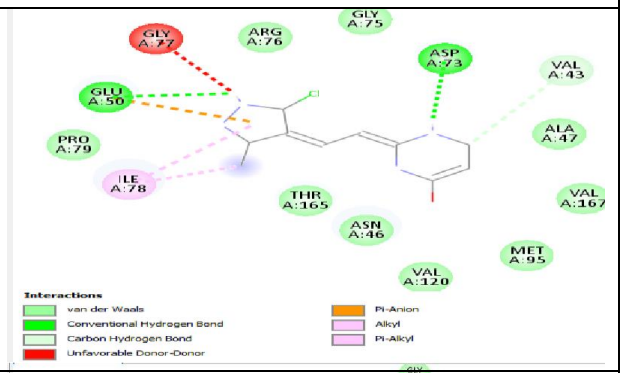
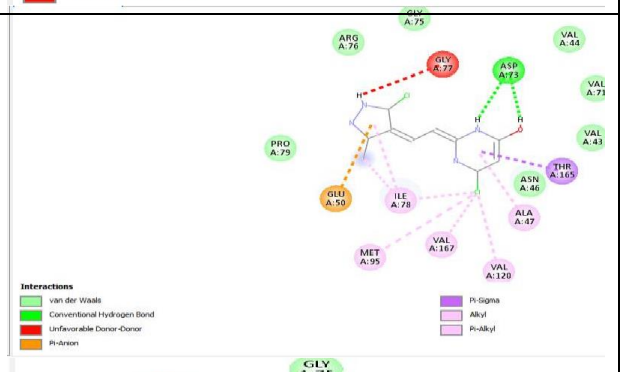
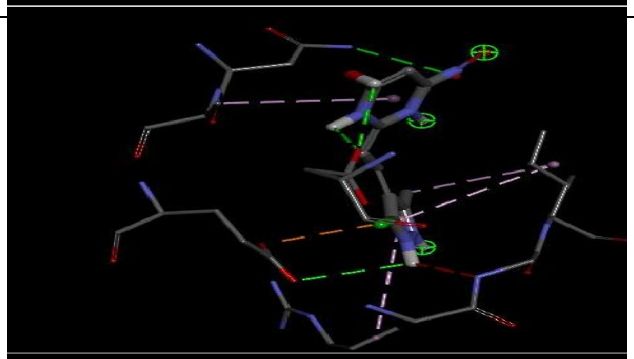
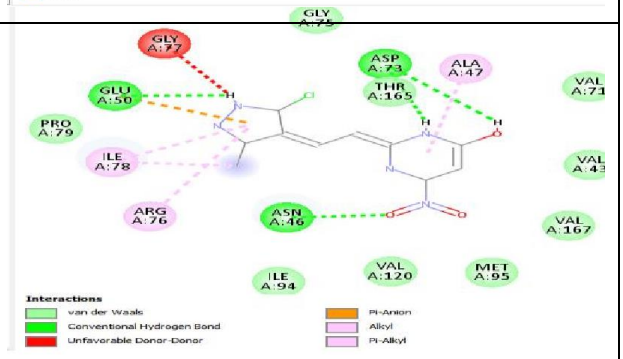
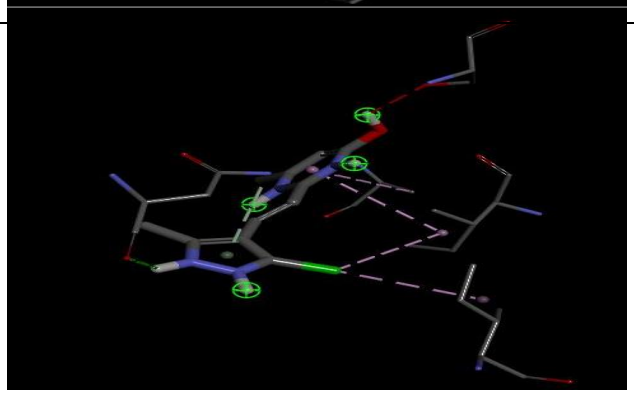
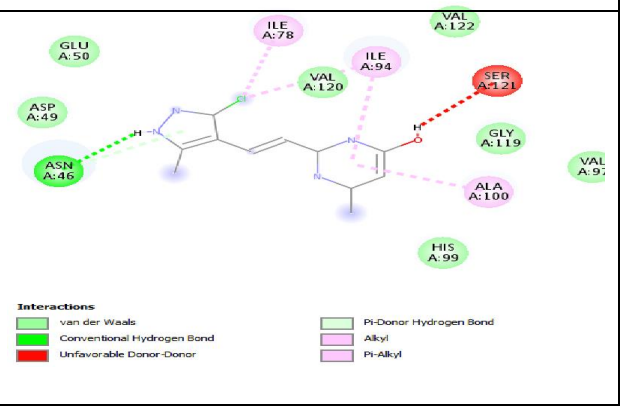
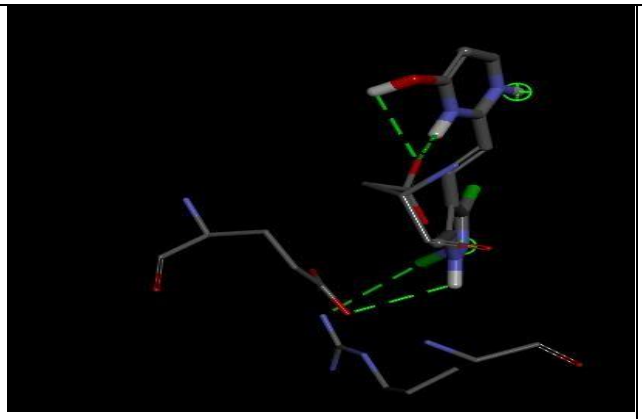
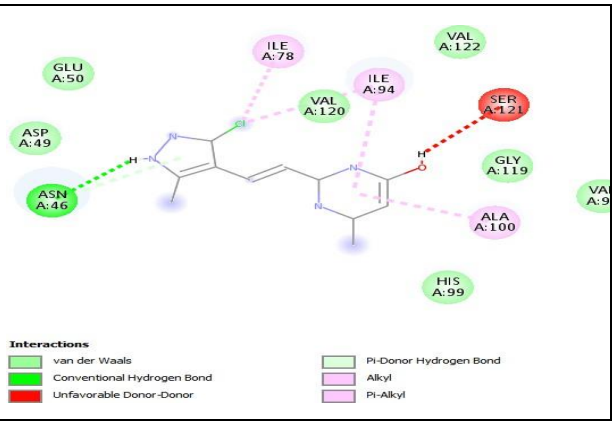
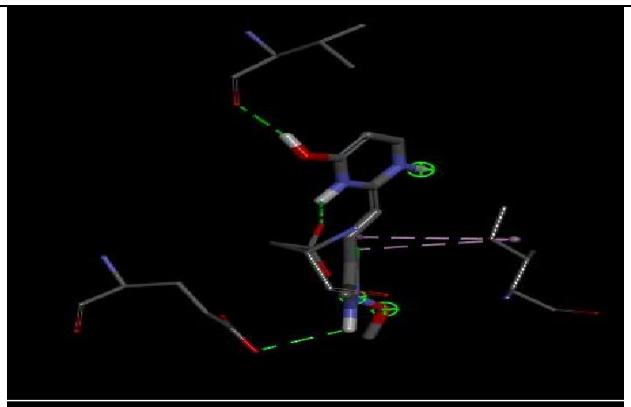
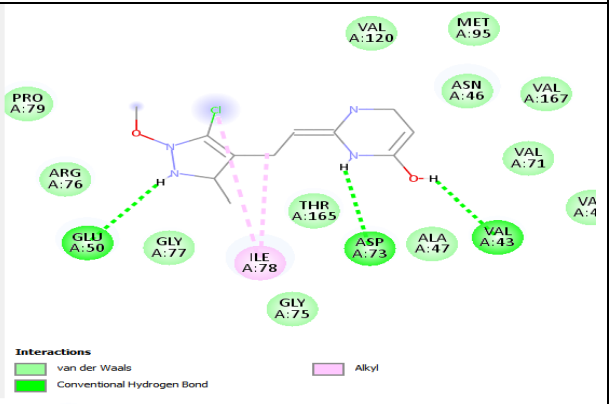
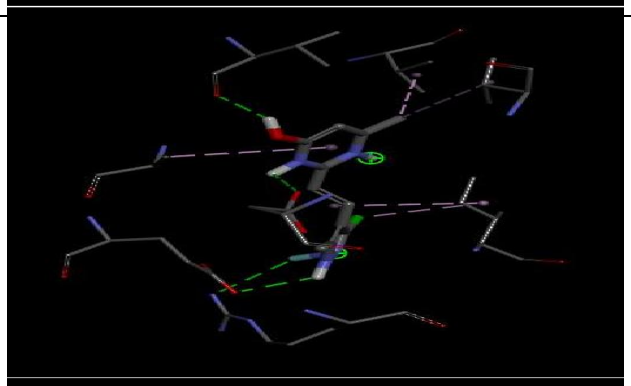
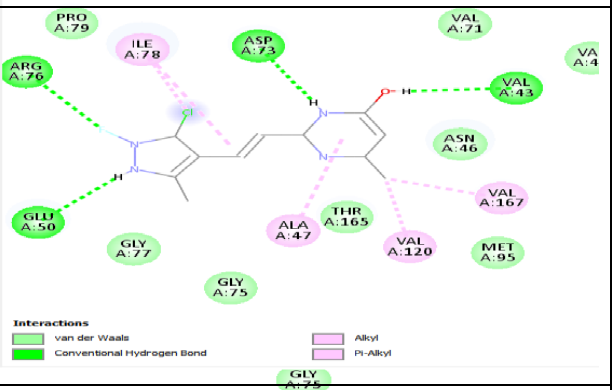
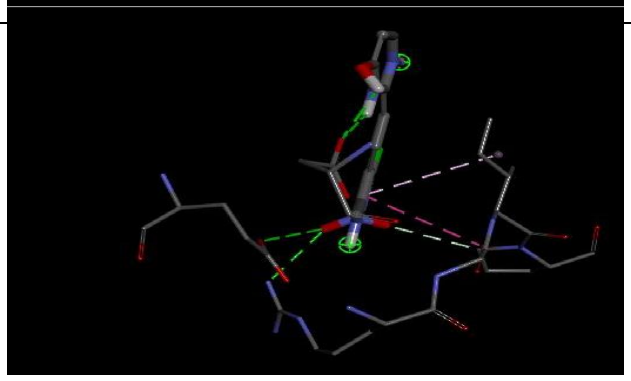
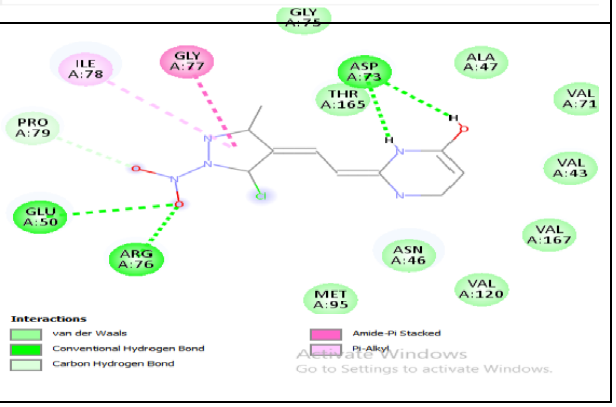
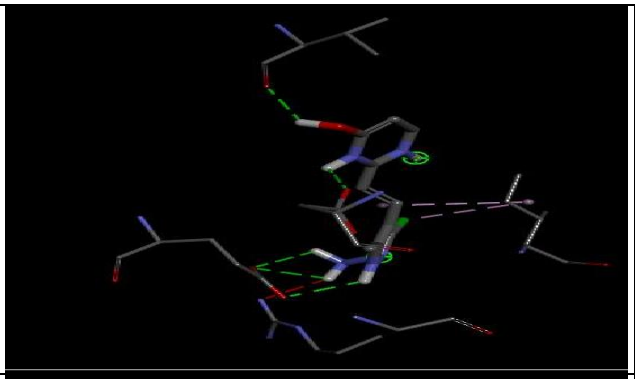
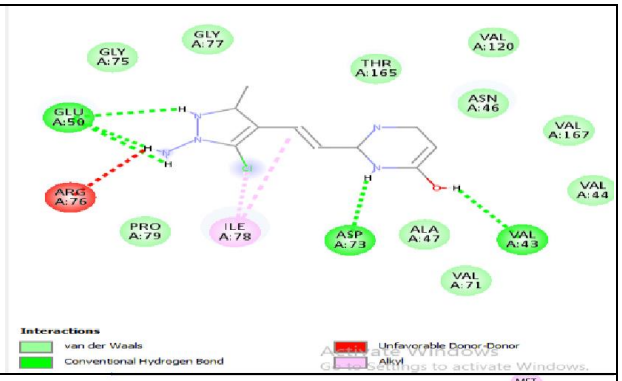
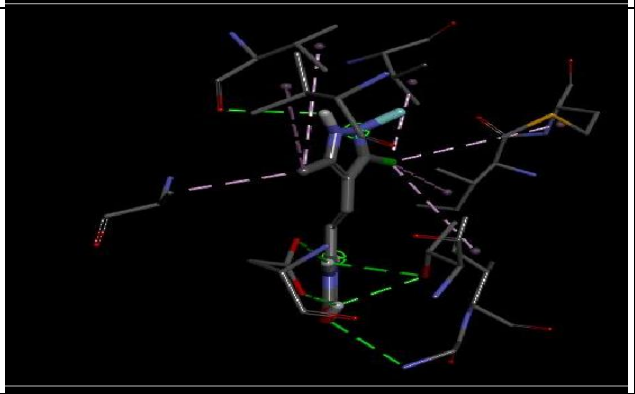
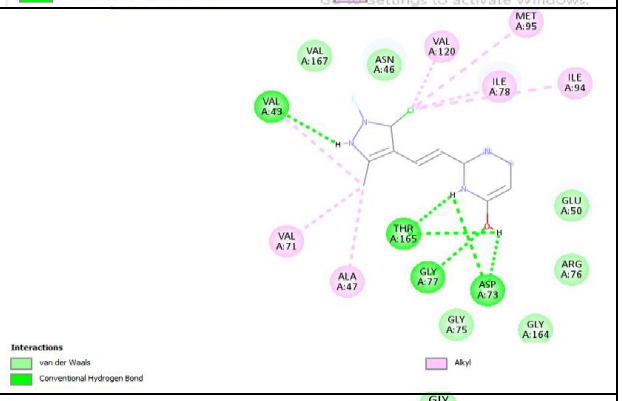
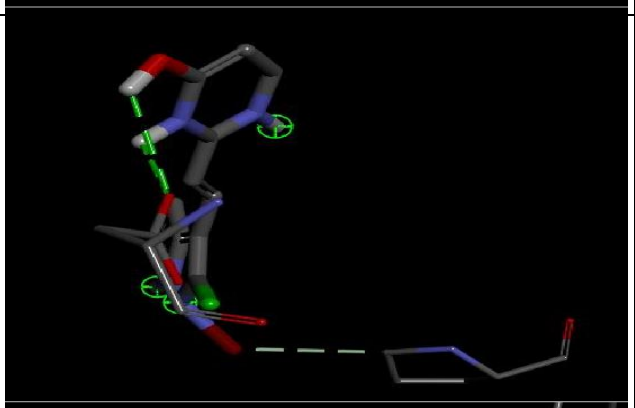
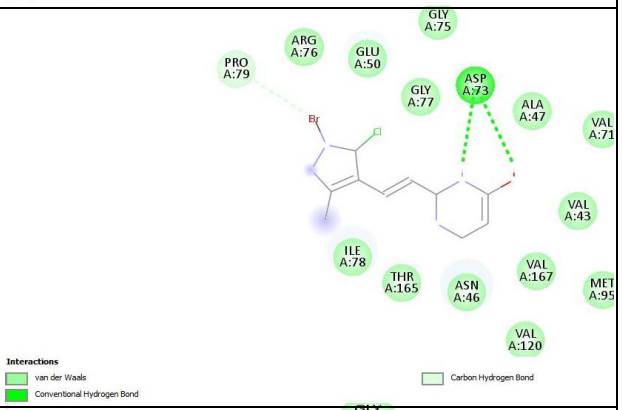
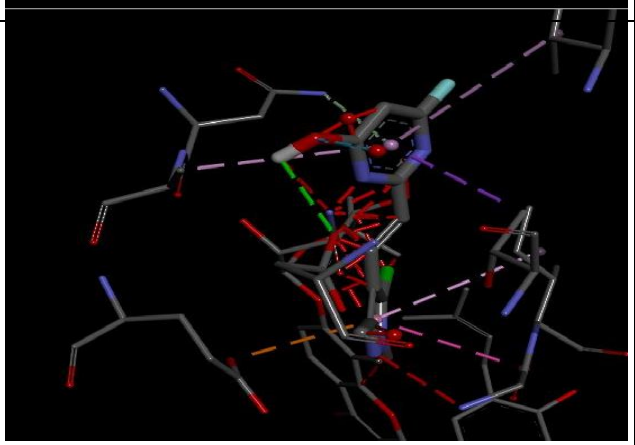
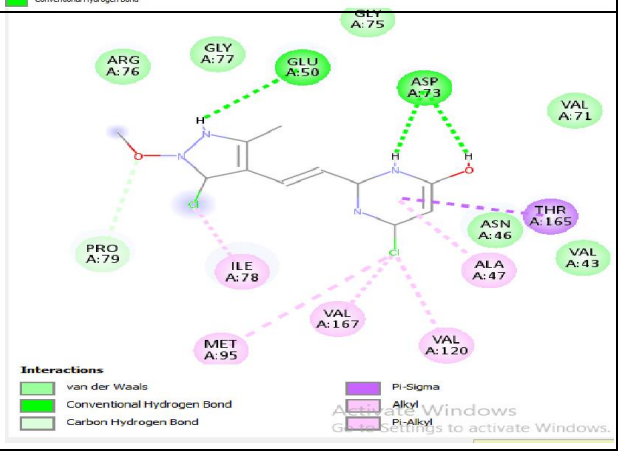
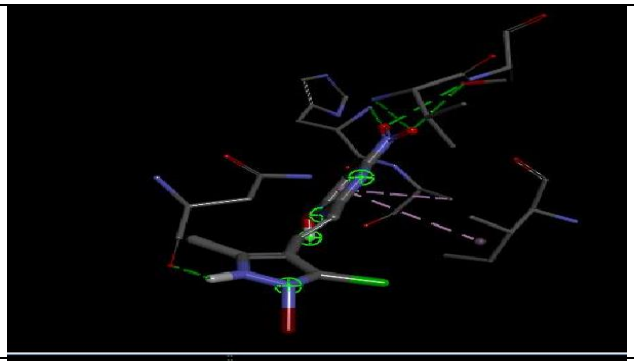
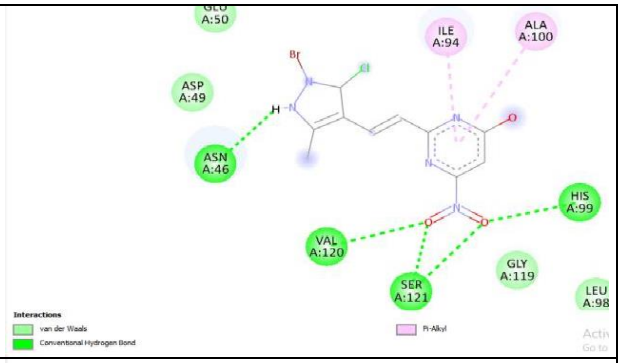
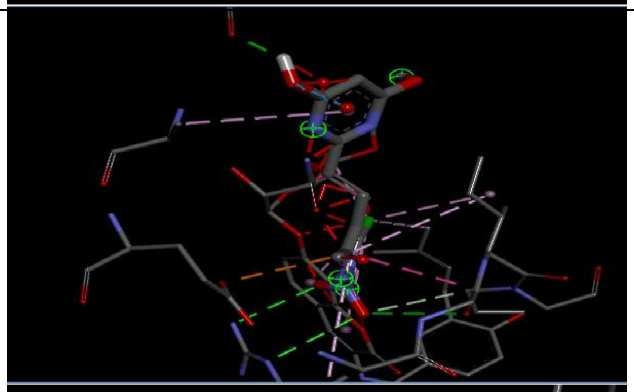
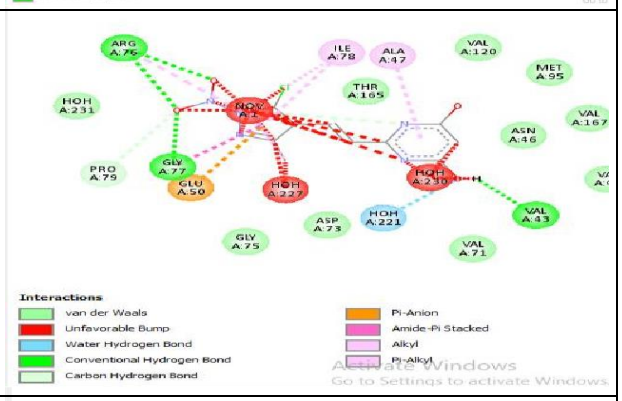
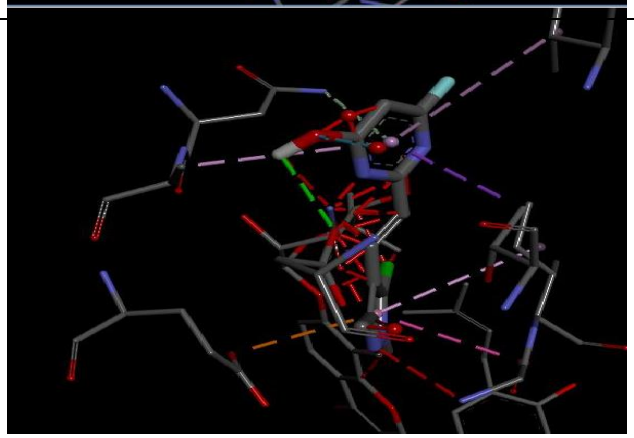
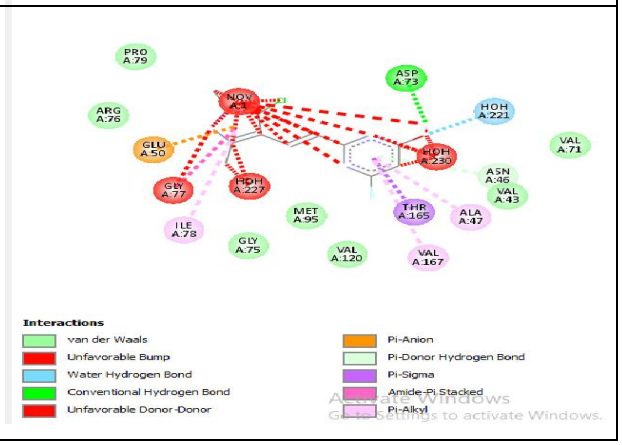
1.		
2.		
3.		
4.		

5.		 GLU A:50 ILE A:78 VAL A:122 ILE A:94 VAL A:120 SER A:121 ASP A:49 GLY A:119 ASN A:46 ALA A:100 HIS A:99 Interactions van der Waals Conventional Hydrogen Bond Unfavorable Donor-Donor Pi-Donor Hydrogen Bond Alkyl Pi-Alkyl
6.		 VAL A:120 MET A:95 PRO A:79 ASN A:46 VAL A:167 VAL A:71 ARG A:76 THR A:165 GLU A:50 GLY A:77 ASP A:73 ALA A:47 VAL A:43 ILE A:78 GLY A:75 Interactions van der Waals Conventional Hydrogen Bond Alkyl
7.		 PRO A:79 VAL A:71 ILE A:78 ASP A:73 ARG A:76 VAL A:43 ASN A:46 VAL A:167 GLU A:50 ALA A:47 THR A:165 VAL A:120 MET A:95 GLY A:77 GLY A:75 Interactions van der Waals Conventional Hydrogen Bond Alkyl Pi-Alkyl
8.		 GLY A:75 ILE A:78 GLY A:77 ASP A:73 ALA A:47 THR A:165 VAL A:71 PRO A:79 VAL A:43 GLU A:50 VAL A:167 ARG A:76 ASN A:46 MET A:95 VAL A:120 Interactions van der Waals Conventional Hydrogen Bond Carbon Hydrogen Bond Amide-Pi Stacked Pi-Alkyl Activate Windows Go to Settings to activate Windows.

9.		 GLY A:77 GLY A:75 VAL A:120 THR A:165 ASN A:46 GLU A:50 VAL A:167 ARG A:76 VAL A:44 PRO A:79 ILE A:78 ASP A:73 ALA A:47 VAL A:43 VAL A:71 Interactions van der Waals Conventional Hydrogen Bond Unfavorable Donor-Donor Alkyl
10.		 MET A:95 VAL A:167 VAL A:120 ASN A:46 ILE A:78 ILE A:94 VAL A:43 GLU A:50 THR A:165 VAL A:71 ALA A:47 GLY A:77 ASP A:73 ARG A:76 GLY A:75 GLY A:164 Interactions van der Waals Conventional Hydrogen Bond Alkyl
11.		 GLY A:75 ARG A:76 PRO A:79 GLU A:50 ASP A:73 GLY A:77 ALA A:47 VAL A:71 VAL A:43 ILE A:78 THR A:165 ASN A:46 VAL A:167 MET A:95 VAL A:120 Interactions van der Waals Conventional Hydrogen Bond Carbon Hydrogen Bond
12.		 GLY A:75 ARG A:76 GLY A:77 GLU A:50 ASP A:73 VAL A:71 THR A:165 ASN A:46 PRO A:79 VAL A:43 ILE A:78 ALA A:47 VAL A:167 MET A:95 VAL A:120 Interactions van der Waals Conventional Hydrogen Bond Carbon Hydrogen Bond Pi-Sigma Alkyl Pi-Alkyl

13.		
14.		
15.		

Docking Scores: Present the docking scores (binding affinities) of the derivatives against the target lyase enzyme(s). **(Table4)**

Compound	Binding energy (K.Cal/Mol)
1	**-7.7**
2	**-8.4**
3	**-8.5**
4	**-6.4**
5	**-7.1**
6	**-7.3**
7	**-7.9**
8	**-7.4**
9	**-7.8**
10	**-7.6**
11	**-7.0**
12	**-7.9**

13	**-7.2**
14	**-7.7**
15	**-7.7**
Ciprofloxacin (standard)	**-6.5**

Binding Interactions: The Important interactions between the derivatives and the site that is currently active enzyme like as hydrophobic interactions & hydrogen bonding& other non-covalent interactions. **(Table5)**

Compound	Hydrophobic interaction	No. of hydrogen bond
1.	ARG 76, GLY 76, PRO 79, THR165	2
2.	ARG 76, GLY 75, VAL44, VAL 43, VAL 71	1
3.	GLY 75, VAL 71, VAL 167,I LE 94, MET 95	3
4.	GLY 119, VAL 97, HIS 99	1
5.	ARG 76, GLY 77,PRO:79, GLY 75, VAL 71	3
6.	PRO 79, ALA 47, THR 165, VAL 71, VAL 44	3
7.	PRO 79 GLY 119, ASN46 VAL 71, MET 95 THR 165	4
8.	GLY 119, ALA 47, PRO:79 ASN46, GLY75, VAL 97	3
9.	GLY 75, GLY 77, THR 165, ASN46, VAL 167	3
10.	VAL 167, VAL 46 GLY 75, GLY 164, ARG 76	4
11.	ARG 76, GLU 50, GLY 75 THR 165, ASN46	1
12.	ARG 76, GLU 50, GLY 75, ASN46, PRO 79	2
13.	GLU 50, ASP 49 GLY 119	4
14.	VAL 120, THR 165, MET 95, ASN 46, PRO79	3
15.	VAL 43, MET95, PRO 79	1

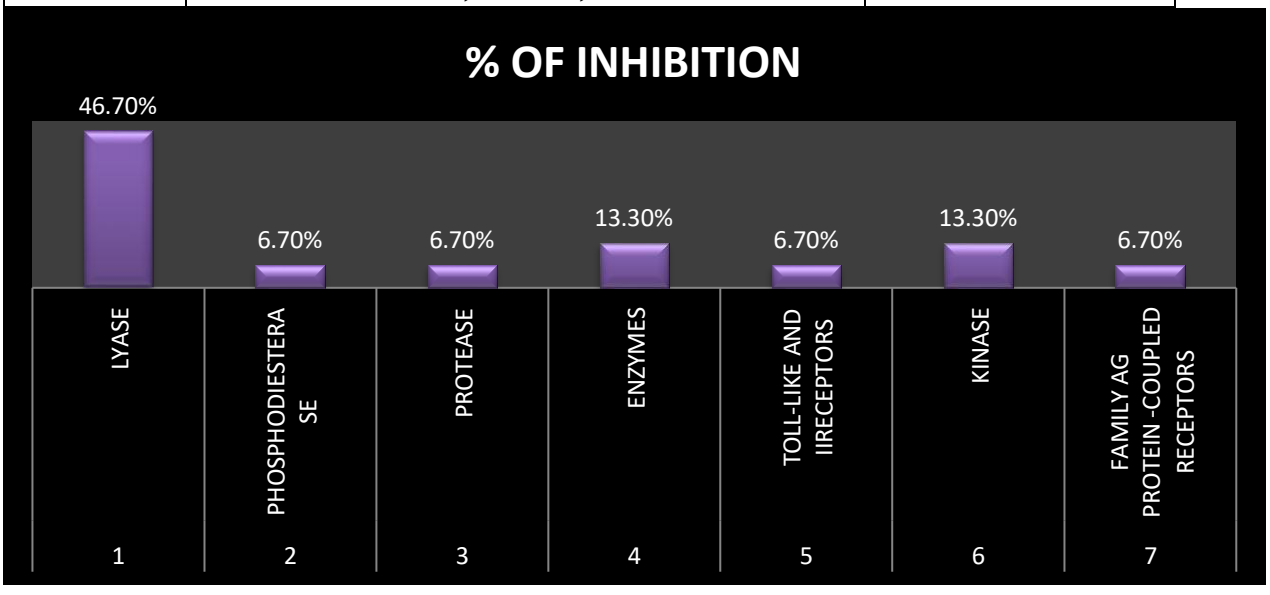


Figure3: Result of Interaction in percentage of Ligands with different Enzymes and Receptors

Discussion:

The molecular docking technique is used to predict their possible targets.The protein enzyme Lyase (PDB ID:1AJ6) was used and the docking score values of the synthesized compounds are given in the Table-4.The compound **2,3,7 and 9,12** shows Excellent binding score **-8.4,-8.5,-7.9** and **-7.8,-7.9** for an enzyme Lyase(PDB ID:1AJ6) with grid box dimensions **X=60.334, Y=5.741,Z=40.532.**

In the Table 4the compound **1** has the binding energy(-7.7) andhydrophobic interactions**ARG:76, GLY76:201, PRO:79, THR:165.**The compound **2** has the binding energy(-8.4)and hydrophobic interactions **ARG :76, GLY:75, VAL:44, VAL:43, VAL:71 .**The compound **3** has the binding energy(-8.5)and hydrophobic interactions **GLY:75, VAL:71, VAL:167,ILE:94, MET:95 .**The compound **4** has the binding energy(-6.4)and hydrophobic interactions **GLY:119, VAL:97, HIS:99 .**The compound **5** has binding energy(-7.1)and hydrophobic interactions **ARG:76, GLY:77,PRO:79, GLY:75, VAL:71 .**The compound **6** has the binding energy(-7.3)and hydrophobic interactions **PRO:79, ALA:47, THR:165, VAL:71, VAL:44 .**The compound **7** has the binding energy(-7.9)and hydrophobic interactions **PRO:79 GLY:119, ASN:46 VAL:71, MET:95 THR:165 .**The compound **8** has the binding energy(-7.4)and hydrophobic interactions **GLY:119, ALA:47, PRO:79 ASN:46, GLY:75, VAL:97 .**The compound **9** has the binding energy(-7.8)and hydrophobic interactions **GLY:75, GLY:77, THR:165, ASN:46, VAL :167.**The compound **10** has the binding energy(-7.6)and hydrophobic interactions **VAL:167, VAL:46 GLY:75, GLY:164, ARG:76**. The compound **11** has the binding energy (-7.0) and hydrophobic interactions **ARG: 76, GLU: 50, GLY: 75 THR: 165, ASN: 46.** The compound **12** has the binding energy (-7.9) and hydrophobic interactions **ARG: 76, GLU: 50, GLY: 75, ASN:46, PRO:79 .** The compound **13** has the binding energy (-7.2) and hydrophobic interactions **GLU:50, ASP:49 GLY:119 .** The compound **14** has the binding energy(-7.7)and hydrophobic interactions **VAL:120, THR:165, MET:95, ASN:46, PRO:79 .** The compound **15** has the binding energy (-7.7) and hydrophobic interactions **VAL:43, MET:95, PRO:79.**

Conclusion:

In order to fully understand the drug-target interactions, fifteen pyrazole-bearing pyrimidine derivatives were investigated for docking against Ciprofloxacin in the current studies. Auto-DockVina applications was used to model and dock the derivatives' structures. According to our findings, all derivatives have the maximum affinity for binding to 1AJ6 because of electron-withdrawing groups such NO2, Cl, Br, F, OCH3, and NH2, but **2, 3, 7, 9 and 12** exhibit the most activity compared to the conventional drug ciprofloxacin. As a result, all derivatives seem to work better than ciprofloxacin.In summary, the study highlights the possibility of application of pyrazole derivatives containing pyrimidine, which may have a promising antimicrobial effect in treating a range of infections.

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