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Molecular Docking Study of Pyrazoline Derivatives against Topoisomerase II and DNA Gyrase Subunit B for Antimicrobial Evaluation

Alka Yadav, Amit Modi, Neeraj Sharma, Udai Bhan Singh Rathore, Pratibha S. Kurup
Faculty of Pharmacy, Bhagwant University, Ajmer, Rajasthan
Indore Mahavidyalaya, Indore, M.P.
Department of Pharmacy, JRNRV(Deemed To Be)University, Udaipur, Rajasthan
IPS Academy College of Pharmacy, Indore, M.P.
Department of Chemistry, Bharti Vishwavidyalaya, Durg, Chhattisgarh
Corresponding author's mail ID: neerajsharma236@gmail.com

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Abstract:
Molecular docking has emerged as a crucial *in-silico* tool to predict the binding affinity and interaction profile of compounds with target proteins. This study evaluates the docking efficiency of synthesized pyrazoline derivatives (4a–4h) against two vital microbial enzymes—Topoisomerase II (PDB ID: 1JIJ) and DNA Gyrase Subunit B (PDB ID: 1KZN)—involved in DNA replication. PyRx 0.8 and AutoDock Vina were employed for docking, while binding interactions were visualized using Maestro and Discovery Studio Visualizer. Compounds 4h and 4e exhibited the highest affinity against 1JIJ (-10.0 and -9.8 kcal/mol respectively), while 4a showed the highest binding score (-9.1 kcal/mol) against 1KZN. The interactions revealed significant hydrogen bonding, polar, hydrophobic, and π–π stacking interactions, suggesting potential antimicrobial efficacy.
Keywords: Antimicrobial Evaluation; Topoisomerase II; DNA Gyrase subunit B; AutoDock Vina; *In-silico*

1. Introduction

Antimicrobial resistance (AMR) has emerged as one of the most critical global health challenges of the 21st century. The excessive and often irrational use of conventional antibiotics has led to the evolution of resistant microbial strains, rendering several once-effective drugs obsolete. According to the World Health Organization (WHO), antimicrobial resistance threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses, and fungi. Consequently, there is an urgent and unmet need for the discovery and development of new therapeutic agents with novel mechanisms of action to combat resistant microorganisms [1].

A key approach to addressing antimicrobial resistance involves the identification and inhibition of essential microbial enzymes that are crucial for their survival and replication. Among these, topoisomerases and DNA gyrases are particularly attractive targets. These enzymes are involved in maintaining the topology of DNA during vital cellular processes such as replication, transcription, and recombination. Topoisomerase II, for instance, introduces

transient double-stranded breaks into DNA to relieve torsional stress, while DNA gyrase (a type II topoisomerase found exclusively in prokaryotes) introduces negative supercoils into DNA, which is critical for bacterial chromosome compaction and gene regulation [2]. Inhibiting these enzymes can disrupt DNA processes and lead to microbial cell death, making them validated targets in the development of antibiotics like fluoroquinolones [3].

Molecular docking has emerged as a powerful in silico technique in drug discovery, allowing researchers to predict the binding affinity and interaction profile of small molecules with target macromolecules. It offers valuable insights into how a drug candidate may fit into the active site of a protein and what kind of interactions stabilize the ligand-protein complex. Docking studies not only help in understanding the potential of a molecule to act as an inhibitor but also facilitate the optimization of lead compounds through structure-based drug design approaches [4,5].

Pyrazoline derivatives represent a diverse and pharmacologically important class of heterocyclic compounds known for their wide range of biological activities, including anti-inflammatory, antitumor, antidiabetic, antioxidant, and notably, antimicrobial properties [6]. These five-membered heterocycles contain two nitrogen atoms and one double bond, offering a versatile scaffold for chemical modifications that can enhance bioactivity. The structural flexibility and favorable physicochemical properties of pyrazolines have inspired the synthesis of numerous analogs aimed at targeting microbial enzymes. Recent studies have suggested that certain substituted pyrazolines exhibit potent activity against Gram-positive and Gram-negative bacteria, which further strengthens their potential as antimicrobial drug candidates [7,8].

In the present study, a series of eight synthesized pyrazoline derivatives, labeled 4a through 4h, were evaluated for their binding affinity and interaction behavior with two key microbial enzymes: topoisomerase II (PDB ID: 1JIJ) and DNA gyrase subunit B (PDB ID: 1KZN) using molecular docking techniques. The protein targets were chosen due to their indispensable role in microbial proliferation and their proven track record as antibiotic targets. AutoDock Vina integrated within the PyRx 0.8 platform was utilized for the docking simulations, while ligand preparation and energy minimization were carried out using Open Babel. The docking results were further analyzed and visualized to interpret the binding conformations, interaction types, and possible mechanistic inhibition pathways.

By comparing the binding energies and interaction profiles of the tested ligands with those of the standard antimicrobial drug streptomycin, this study aims to identify promising candidates for further biological evaluation. Ultimately, the insights derived from this docking study could contribute to the development of new antimicrobial agents based on the pyrazoline scaffold, offering a potential solution to the growing problem of drug-resistant infections.

2. Materials and Methods

2.1 Hardware and Software

Windows 10 (64-bit) operating systems with 4 GB RAM and 2.50 GHz Intel(R) Core(TM) i5-

7200U processor was used for executing the docking process. PyRx version 0.8, available at https://pyrx.sourceforge.io/ was used to perform the docking in Auto Dock Vina Wizard. Autodock Tools 4.2.6 which is made accessible by the Scripps Research Institute at https://autodock.scripps.edu/, was used for preparing the proteins and for grid generation, Ligands were processed using Open babel and PyRx 0.8 and interaction poses of ligands were visualized and analysed using Discovery Studio Visualizer.

2.2 Protein Selection and Preparation

Crystal structures of microbial targets—topoisomerase II (PDB ID: 1JIJ) and DNA gyrase subunit B (PDB ID: 1KZN)—were obtained from the RCSB Protein Data Bank [9]. The structures were prepared for docking by removing water molecules and heteroatoms, adding polar hydrogens and Kollman charges using AutoDock Tools, and converting them to PDBQT format for compatibility with docking software [10–12].

2.3 Ligand Preparation

The crystal structures of target proteins (PDB ID: 1JIJ – topoisomerase II, PDB ID: 1KZN – DNA gyrase subunit B) were downloaded from the RCSB Protein Data Bank [15], and the proteins were prepared using AutoDock Tools 4.2.6 [13-15]. In this step, attached water molecules and bound heteroatoms/ligands were removed, polar hydrogens and Kollman charges were added, the charge was distributed equally over all atoms, and residues were checked for any missing atoms. The prepared PDB files were then converted to PDBQT format for docking.

Ligands in SMILES format were converted to SDF files, and 3D coordinates for all ligands were generated using Open Babel via command-line execution [16-17]. The 3D structure files were processed in PyRx using UFF energy minimization and then converted to PDBQT format (AutoDock-compatible format) [18].

2.4 Grid Generation

The grid box was initially set over the attached ligand using AutoDock Tools and then manually adjusted to the desired dimensions using PyRx. The grid dimensions were set as −11.857 × 13.512 × 87.379 Å³ with 25 grid points in X, Y, Z directions for PDB ID: 1KZN and 20.562 × 30.804 × 35.946 Å³ with 25 grid points in each direction for PDB ID: 5D6P [19].

2.5 Docking Parameters

Docking was carried out using AutoDock Vina with the exhaustiveness set to 8. The best docking pose, based on the lowest binding energy, was selected for further interaction analysis [20].

3. Results and Discussion

3.1 Docking with Topoisomerase II (1JIJ)

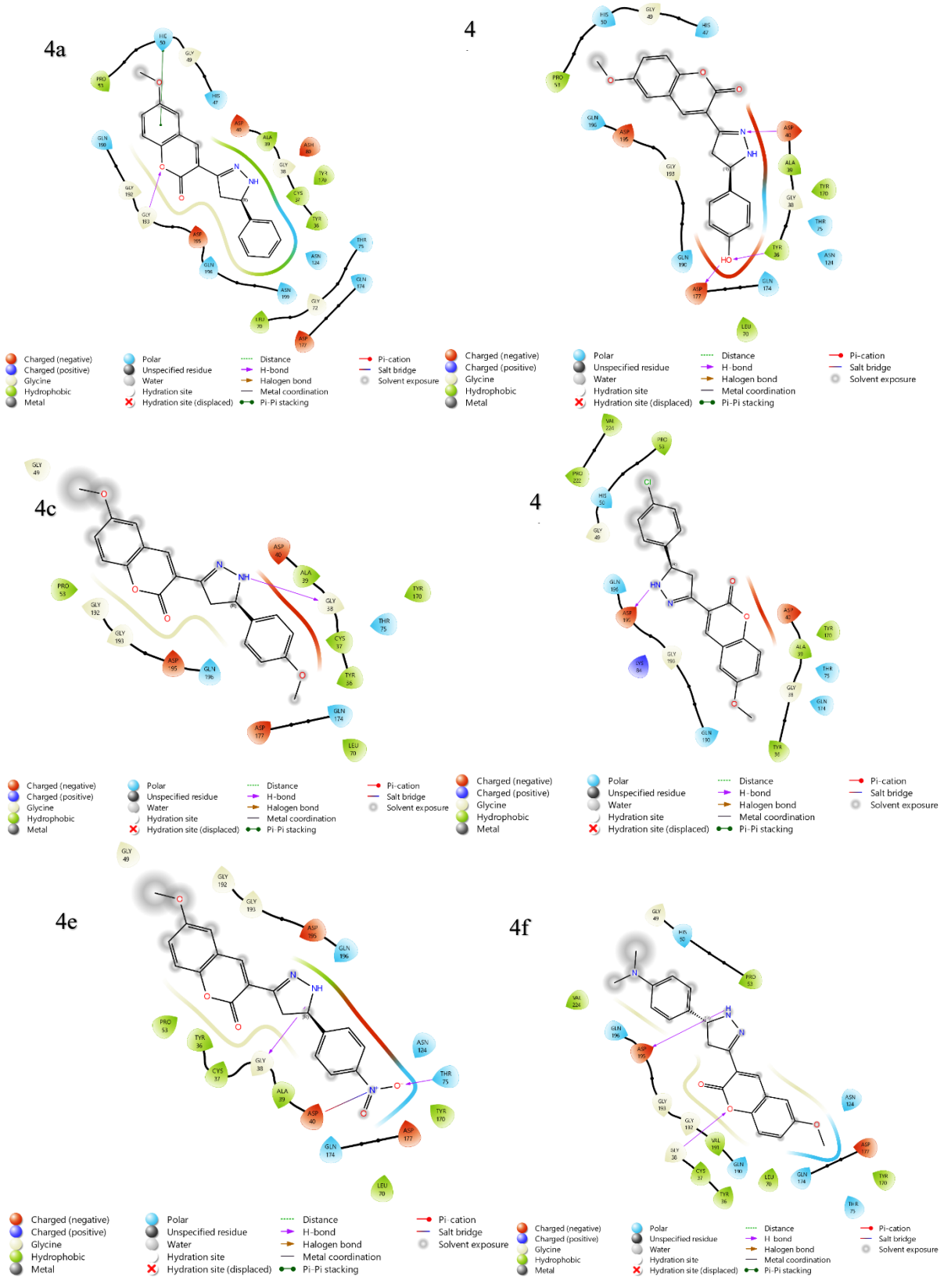
All compounds exhibited strong binding with 1JIJ, with binding energies ranging from -8.8 to -10.0 kcal/mol. Compound 4h showed the best binding affinity (-10.0 kcal/mol), forming hydrogen bonds with residues ASP40, GLN174, GLY49, and TYR170, along with π–π

stacking interactions with HIS50 and TYR36 (Table 1).

Table 1: Molecular Docking Study of Compounds 4a-4h with PDB id 1JIJ

Ligands	Affinity (Kcal/mol)	Charged (Negative)	Charged (Positive)	Glycine	Hydrophobic	Polar	H-Bond	Pi-Pi Stacking
4a	-9.5	ASP177, ASP195, ASP40	HIS47, HIS50	GLY192, GLY193, GLY38, GLY49	ALA39, LEU70, PRO53	ASN124, CYS37, GLN174, GLN196, HIS47, HIS50, THR75, TYR170, TYR36	GLY193, GLY38	HIS50, TYR36
4b	-9.6	ASP177, ASP195, ASP40	HIS47, HIS50	GLY193, GLY38, GLY49	ALA39, LEU70, PRO53	ASN124, GLN174, GLN190, GLN196, HIS47, HIS50, THR75, TYR170, TYR36	ASP177, ASP40, GLY193, HIS50, TYR36	HIS50
4c	-9	ASP177, ASP195, ASP40		GLY192, GLY193, GLY38, GLY49	ALA39, LEU70, PRO53	CYS37, GLN174, GLN196, THR75, TYR170, TYR36	GLY193, GLY38	
4d	-9	ASP195, ASP40	HIS50, LYS84	GLY193, GLY38, GLY49	ALA39, PRO222, PRO53, VAL224	GLN174, GLN190, GLN196, HIS50, THR75, TYR170, TYR36	ASP195, ASP40, GLN174, GLN190	HIS50, TYR36
4e	-9.8	ASP177, ASP		GLY192, GLY1	ALA39, LEU70, PRO53	ASN124, CYS37, GLN174,	ASN124, ASP177, GLY193, GLY38, THR75	

		195, ASP 40		93, GLY3 8, GLY4 9		GLN196, THR75, TYR170, TYR36		
4f	-9.7	ASP 177, ASP 195	HIS5 0	GLY1 92, GLY1 93, GLY3 8, GLY4 9	LEU70, PRO53, VAL191, VAL224	ASN124, CYS37, GLN174, GLN190, GLN196, HIS50, THR75, TYR170, TYR36	ASP195, CYS37, GLN196, GLY38, THR75	TYR 36
4 g	-8.8	ASP 195, ASP 40, ASP 80	HIS4 7, HIS5 0	GLY3 8, GLY4 9	ALA39, LEU223, LEU52, PRO222, VAL224	ASN124, GLN174, GLN196, HIS47, HIS50, THR75, TYR170	ASP40, GLN174, GLY38, HIS50, PRO222	HIS5 0
4 h	-10	ASP 195, ASP 40, ASP 80	ARG 88, HIS4 7, HIS5 0, LYS 84	GLY3 8, GLY4 9	ALA39, LEU70	ASN124, GLN174, GLN190, GLN196, HIS47, HIS50, THR75, TYR170, TYR36	ASP40, GLN174, GLY49, LYS84, THR75, TYR170	HIS5 0, TYR 36
strep tomy cin	-7.8	ASP 195, ASP 40, ASP 80	ARG 88, HIS4 7, HIS5 0, LYS 84	GLY1 92, GLY1 93, GLY3 8, GLY4 9	ALA39, ILE48, LEU223, LEU52, PHE232, PRO222, PRO53, VAL224	GLN174, GLN196, HIS47, HIS50, SER194, THR42	ARG88, ASP195, ASP80, GLN196, GLY193, GLY38, GLY49, HIS47, HIS50, LYS84, PRO222, VAL224	HIS4 7


4a
4
4c
4
4e
4f
Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Metal
Polar
Unspecified residue
Water
Hydration site
Hydration site (displaced)
Distance
H-bond
Halogen bond
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

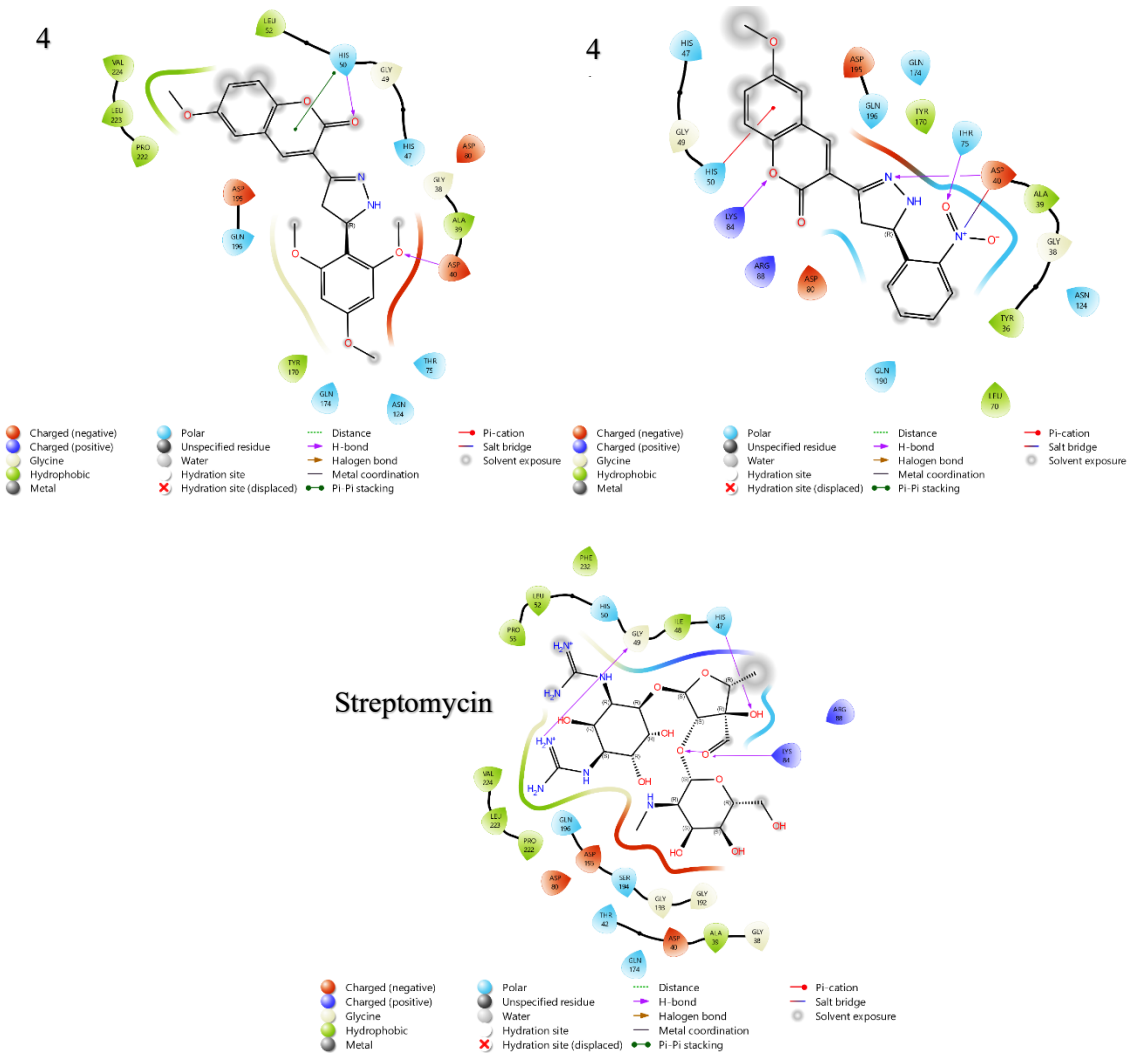


Figure 1: Binding Interactions of Compounds 4a-4h with PDB id 1JIJ

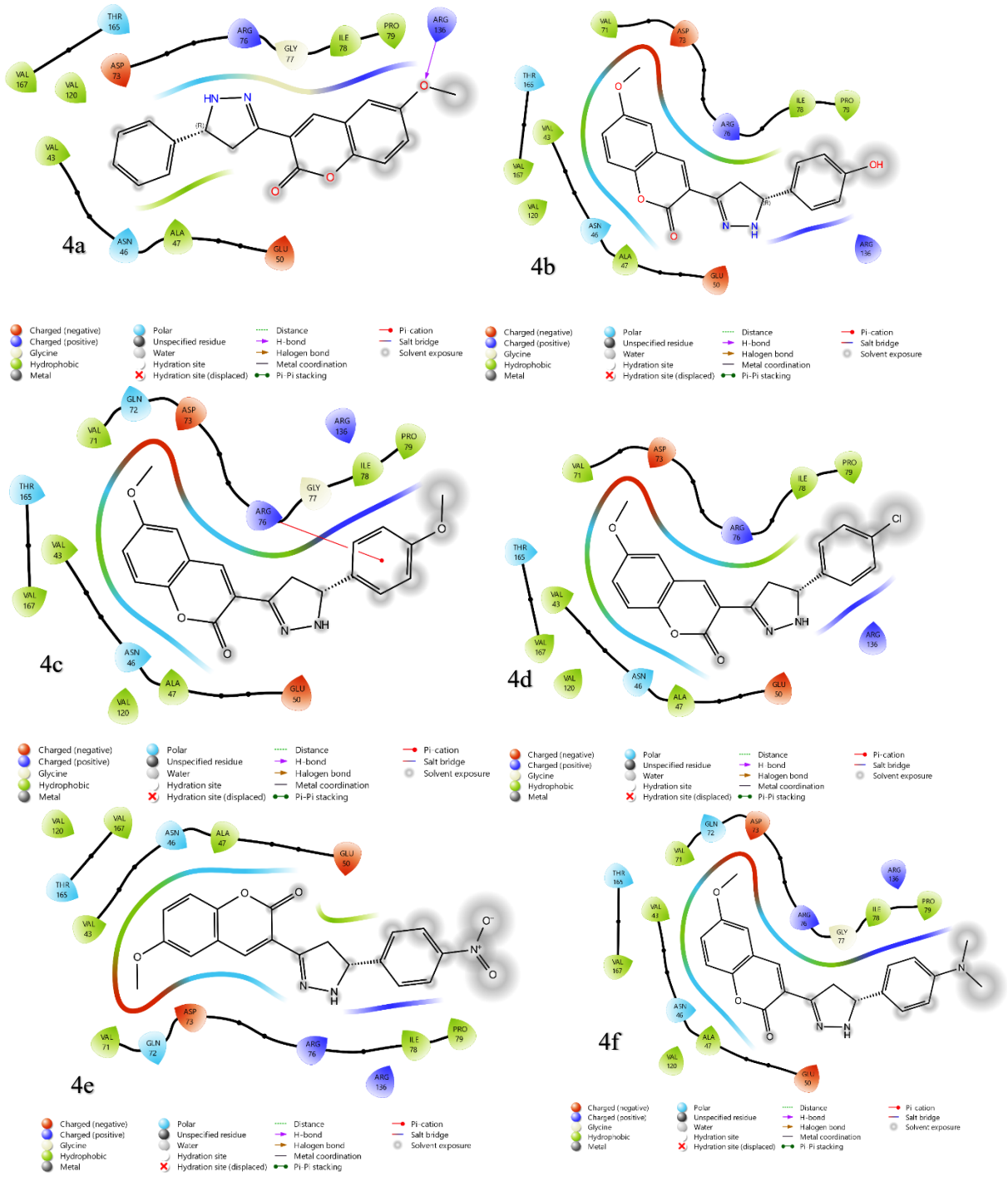
3.2 Docking with DNA Gyrase Subunit B (1KZN)

Against 1KZN, compound 4a demonstrated the highest affinity (-9.1 kcal/mol), interacting with key residues such as ARG76, THR165, and ASP73. Streptomycin, used as a standard, exhibited significantly lower affinity (-6.5 kcal/mol), supporting the potential of the pyrazoline series as antimicrobial leads (Table 2 and figure 2).

Table 2: Molecular Docking Study of Compounds 4a-4h with PDB id 1KZN

Ligands	Affinity (Kcal/mol)	Charged (Negative)	Charged (Positive)	Glycine	Hydrophobic	Polar	H-Bond	Pi-Pi Stacking
4a	-9.1	ASP73, GLU50	ARG136, ARG	GLY77	ALA47, ILE78, PRO79, VAL120, VAL167, VAL43	ASN46, THR165	ARG136, ARG76, ASP73, THR165	

			G76					
4b	-8.4	ASP73, GLU50	ARG136, ARG76		ALA47, ILE78, PRO79, VAL120, VAL167, VAL43, VAL71	ASN46, THR165	ASN46, GLU50, VAL71	
4c	-8.6	ASP73, GLU50	ARG136, ARG76	GLY77	ALA47, ILE78, PRO79, VAL120, VAL167, VAL43, VAL71	ASN46, GLN72, THR165	ASN46, GLU50, THR165, VAL71	
4d	-8.7	ASP73, GLU50	ARG136, ARG76		ALA47, ILE78, PRO79, VAL120, VAL167, VAL43, VAL71	ASN46, THR165	ASN46, GLU50, VAL71	
4e	-8.6	ASP73, GLU50	ARG136, ARG76		ALA47, ILE78, PRO79, VAL120, VAL167, VAL43, VAL71	ASN46, GLN72, THR165	ASN46, GLU50, VAL71	
4f	-8.9	ASP73, GLU50	ARG136, ARG76	GLY77	ALA47, ILE78, PRO79, VAL120, VAL167, VAL43, VAL71	ASN46, GLN72, THR165	ASN46, GLU50, THR165, VAL71	
4g	-8.1	ASP73, GLU50	ARG136, ARG76	GLY77	ALA47, ILE78, ILE90, PRO79, VAL120, VAL167, VAL43, VAL71	ASN46, THR165	ARG76, ASN46, ASP73, GLU50, THR165	
4h	-8.5	ASP73, GLU50	ARG136, ARG76		ALA47, ILE78, PRO79, VAL120, VAL167, VAL43, VAL71	ASN46, THR165	ASN46, GLU50, VAL71	
streptomycin	-6.5	ASP49, ASP73, GLU42, GLU50	ARG76, HIS95	GLY117, GLY119	ALA47, ALA96, ILE78, ILE90, PRO79, VAL118, VAL120	ASN46, HIS95, SER121, THR165	ALA96, ASN46, ASP49, GLY117, VAL118, VAL120	HIS95


4a
4b
4c
4d
4e
4f
Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Metal
Polar
Unspecified residue
Water
Hydration site
Hydration site (displaced)
Distance
H-bond
Halogen bond
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

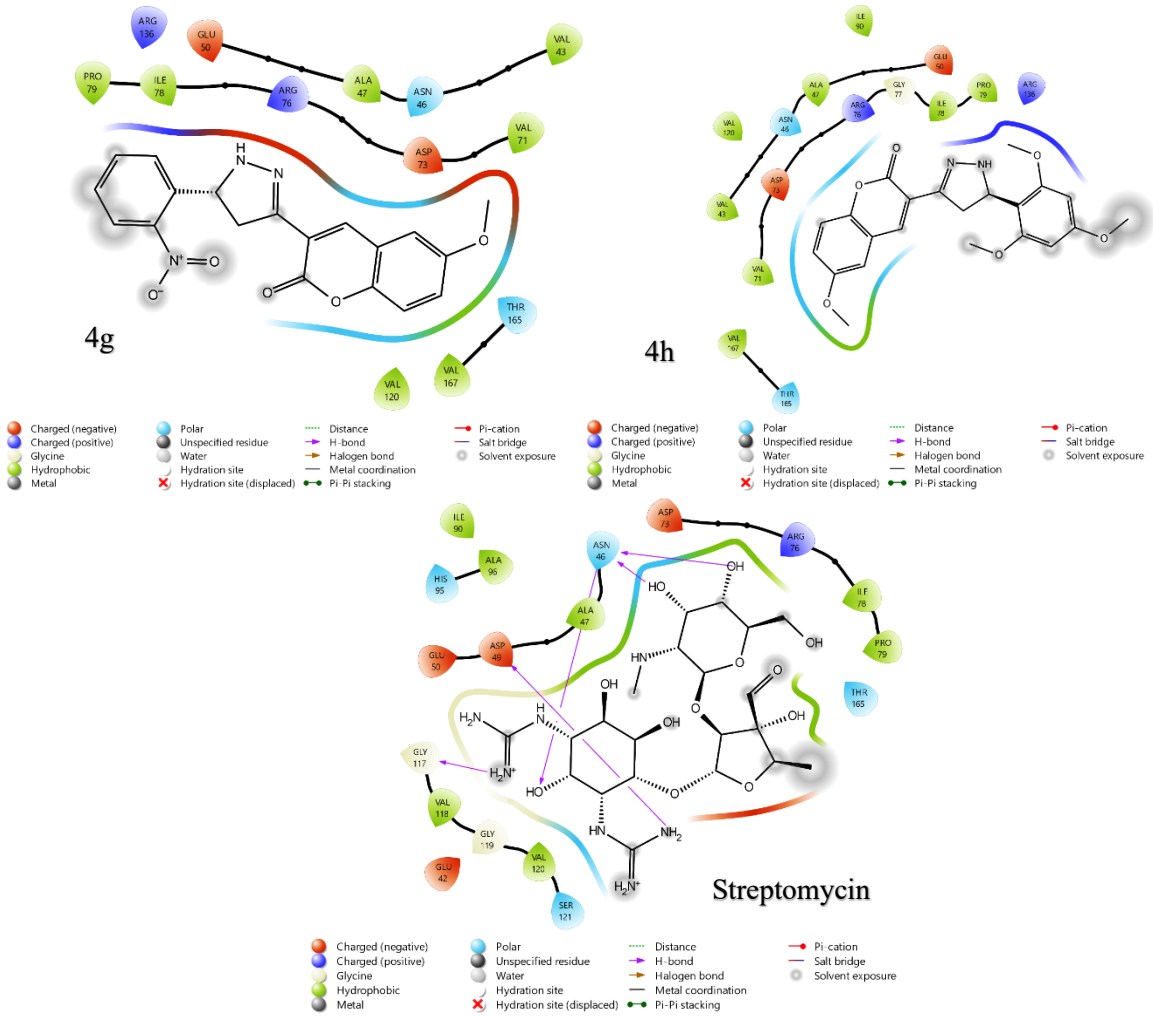


Figure 2: Binding Interactions of Compounds 4a-4h with PDB id 1KJN

3.3 3.3 Interaction Analysis

Molecular docking results revealed that the binding affinities of the pyrazoline derivatives (4a–4h) toward the microbial targets—topoisomerase II (PDB ID: 1JIJ) and DNA gyrase subunit B (PDB ID: 1KZN)—were governed by a variety of non-covalent interactions. These included hydrogen bonds, π–π stacking, polar interactions, and hydrophobic contacts, which collectively contributed to the stability and specificity of the ligand-protein complexes.

For topoisomerase II (1JIJ), compounds such as 4h, 4e, and 4f demonstrated strong binding affinities, ranging from –9.7 to –10.0 kcal/mol. The binding pocket interactions commonly involved the residues GLY38, HIS50, and TYR36, which appeared recurrently across multiple ligand complexes. These residues played critical roles in forming hydrogen bonds and π–π stacking interactions, particularly with the aromatic rings of the pyrazoline core and its substituents. For example, HIS50 engaged in π–π stacking with several ligands (notably 4h and 4b), stabilizing the ligand in the active site. Additionally, ASP40, GLN174, and GLY193 were observed to participate in polar and hydrogen bonding interactions, enhancing the orientation and binding depth of the ligands.

For DNA gyrase subunit B (1KZN), compound 4a displayed the best binding affinity at –9.1

kcal/mol, followed by 4f and 4d. Key interacting residues included ASN46, GLU50, and VAL71, which facilitated hydrogen bonding and van der Waals interactions with functional groups of the ligands. Hydrophobic residues such as PRO79, ILE78, and VAL120 contributed to the stability of the complex via hydrophobic interactions, while ARG76 and ARG136 formed electrostatic interactions with negatively charged or polar ligand moieties. The presence of glycine residues, such as GLY77, in proximity to the binding pocket indicated flexible loop regions that might accommodate various structural features of the ligands.

Overall, the detailed interaction mapping across both protein targets indicates that the pyrazoline derivatives were capable of occupying the active site effectively, forming a network of interactions that mimic or exceed those of known antimicrobial agents.

3.4 Comparison with Standard Drug

In order to benchmark the docking results, streptomycin, a well-known aminoglycoside antibiotic, was used as a standard for comparative docking against both protein targets. The binding affinity of streptomycin was –7.8 kcal/mol for topoisomerase II and –6.5 kcal/mol for DNA gyrase subunit B, which were significantly lower than those observed for the synthesized pyrazoline derivatives.

In terms of interaction profiles, streptomycin showed fewer and less diverse interactions. While it formed some hydrogen bonds with residues such as ASP195, GLY193, and HIS47, and a few polar interactions with ARG88 and LYS84, its overall engagement with the active site was less extensive. It lacked strong π–π stacking interactions and had limited hydrophobic contact with the pocket compared to pyrazoline analogs. Additionally, the binding orientation of streptomycin appeared more superficial within the active site in comparison to the deeply embedded poses observed with 4h, 4e, and 4a.

This disparity in binding energies and interaction patterns suggests that the tested pyrazoline compounds have a stronger and more stable interaction with both microbial targets, likely due to their structural rigidity, electron-rich aromatic rings, and functional groups capable of forming multiple types of bonds.

4. Conclusion

The present molecular docking investigation was conducted to evaluate the binding potential of a series of synthesized pyrazoline derivatives (4a–4h) against two critical bacterial enzymes: topoisomerase II (PDB ID: 1JIJ) and DNA gyrase subunit B (PDB ID: 1KZN), both of which are well-established antimicrobial targets involved in DNA replication and supercoiling processes. Using AutoDock Vina through the PyRx platform, the ligands were analyzed for their interaction profiles and binding energies in comparison with the standard antibiotic streptomycin.

The docking results revealed that all pyrazoline derivatives demonstrated notable binding affinities, with compounds 4h, 4e, and 4a emerging as the most potent candidates. Compound 4h exhibited the highest binding affinity towards topoisomerase II (–10.0 kcal/mol), while 4a showed the strongest interaction with DNA gyrase subunit B (–9.1 kcal/mol). These

compounds engaged in diverse molecular interactions including hydrogen bonds, π–π stacking, polar contacts, and hydrophobic interactions, particularly involving critical amino acid residues such as GLY38, HIS50, TYR36 in topoisomerase II and ASN46, GLU50, VAL71 in DNA gyrase subunit B. Such interactions suggest strong and specific ligand accommodation within the active site of both targets.

In contrast, streptomycin, though widely used clinically, displayed lower binding affinities (–7.8 kcal/mol and –6.5 kcal/mol for 1JIJ and 1KZN, respectively) and formed fewer stabilizing interactions with the active site residues. This observation underscores the promising binding performance of the pyrazoline scaffold and its potential as a viable pharmacophore for further antimicrobial development.

Overall, the molecular docking data support the hypothesis that structural modifications on the pyrazoline ring can lead to enhanced interaction with microbial targets. The superior binding profiles of selected derivatives highlight their potential as novel antimicrobial agents that may offer advantages over current therapies in the fight against drug-resistant bacterial infections. However, while these in silico findings provide a valuable preliminary framework, further in vitro antimicrobial screening, MIC determination, and in vivo efficacy and toxicity evaluations are essential to confirm their pharmacological potential and safety. These follow-up studies could ultimately lead to the development of effective new-generation antibiotics derived from the pyrazoline core.

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