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Review Article

A Focused Review and Comparative Analysis of Molecular Docking Strategies and Software Applications in Piperazine-Based Antimicrobial Drug Development

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ABSTRACT

The rise of resistant bacterial and fungal strains remains a major public health issue. To address this challenge, new antimicrobial agents are being developed by modifying the structures of existing compounds. Computational techniques, including molecular docking and in silico modeling, assist researchers in predicting drug-target interactions and help identify and optimize effective pharmaceutical derivatives. Piperazine serves as a valuable scaffold for developing new antimicrobial agents due to its favorable physicochemical and pharmacological properties. Structural modifications of piperazine can improve target affinity and selectivity, thereby increasing therapeutic effectiveness and overcoming bacterial resistance mechanisms. Popular molecular docking software, such as Surflex-Dock, Molecular Operating Environment (MOE), and AutoDock, enable the simulation and prediction of compound binding properties, facilitating the development of models for in vivo drug activity. Comparative analyses show that MOE provides fast and reliable performance with energy-minimization algorithms, Surflex-Dock is particularly good at predicting binding flexibility and affinity, and AutoDock uses rigorous energy-based conformational sampling to clarify ligand binding interactions. Together, these advances support rational design of piperazine-based antimicrobials and aid in creating next-generation agents that are effective against resistant pathogens, thereby improving clinical outcomes.

INTRODUCTION

Bacterial and fungal infections present significant global health threats, primarily due to the increasing resistance towards pathogens to

existing antimicrobial drugs. The continued rise in antimicrobial resistance has prompted medicinal chemists to design and develop new drugs to overcome this challenge.[1] The effectiveness of

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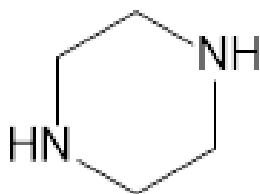
conventional therapies is diminishing as pathogenic microorganisms evolve mechanisms to evade antibiotic action, necessitating the development of new anti-infective agents that can overcome resistant strains. Scaffold modification of existing antibiotics, both natural and synthetic, has proven essential for enhancing pharmacological activity, improving target selectivity, restoring potency against resistant pathogens, and reducing toxicity. Strategies such as functional group substitution, heterocycle incorporation, bioisosteric replacement, and hybridization facilitate the creation of novel derivatives that retain the advantages of established drugs while addressing their limitations. These approaches have yielded promising antimicrobial candidates with improved efficacy, pharmacokinetic profiles, and resistance-breaking capabilities. Rational redesign of known antibacterial pharmacophores remains a critical strategy in combating microbial resistance and advancing next-generation therapeutics.[2] The reduction in effective drugs due to antimicrobial resistance underscores the urgency of discovering new, potent agents. Heterocyclic compounds, particularly piperazine derivatives, have attracted attention for their structural diversity and broad biological activities, including antibacterial, antifungal, antitubercular, anticancer, antiviral, and antioxidant effects. This versatility positions the piperazine scaffold as a valuable foundation for developing next-generation antimicrobial agents to address current resistance challenges.[3]

Piperazine

Piperazine is an organic heterocycle composed of a six-membered ring containing two nitrogen atoms positioned opposite each other. This structural framework is highly valuable in modern medicinal chemistry due to its versatility and ability to interact with a wide range of biological

targets. Since its introduction in the early 20th century, initially as an anthelmintic agent for the treatment of intestinal worm infections, piperazine has become a core scaffold in numerous therapeutic agents. Today, the piperazine ring is incorporated into many clinically important drugs, spanning diverse pharmacological classes such as antipsychotics, antihistamines, antianginal agents, antidepressants, anticancer agents, antivirals, cardioprotective drugs, anti-inflammatory agents, and diagnostic imaging compounds. Even minor structural modifications at different positions on the piperazine ring can significantly alter the biological profile of the resulting derivatives, enabling the development of molecules with enhanced potency, selectivity, or improved pharmacokinetic properties. This adaptability makes piperazine a privileged and widely used scaffold in rational drug design.[4] Piperazine, originally identified as a component derived from the black pepper plant (*Piper nigrum*), contains two reactive secondary amine groups located at the 1 and 4 positions of the ring. Statistical analyses of drug substructures indicate that piperazine is one of the most commonly used nitrogen-containing heterocycles in pharmaceutical compounds, ranking third after piperidine and pyridine. This six-membered ring system, featuring two nitrogen atoms positioned opposite each other, contributes important physicochemical and pharmacokinetic advantages. Its structural rigidity, significant polar surface area, and ability to participate in hydrogen bonding make it an attractive scaffold in drug design. These characteristics often translate into improved target binding affinity, enhanced selectivity, better aqueous solubility, and favorable oral bioavailability. Additionally, piperazine-containing molecules frequently demonstrate improved ADME profiles, including efficient absorption, distribution, metabolism, and excretion, which further supports their wide use in modern medicinal chemistry.[5]





Drug Discovery and Development

Drug discovery refers to the comprehensive process through which new therapeutic agents are identified and developed. Within this broader framework, drug design plays a crucial role. It is a systematic and rational approach aimed at discovering, selecting, and optimizing molecules that have the potential to become effective drugs. This strategy relies on understanding the physicochemical properties of compounds and analyzing how these molecules interact at the molecular level with specific biological targets. The primary objective of drug design is to identify compounds that can bind selectively and effectively to targets, such as proteins, enzymes, receptors, or nucleic acids, which play crucial roles in disease mechanisms. By studying these interactions, researchers can modify and enhance molecular structures to improve binding affinity, specificity, stability, and safety. Ultimately, drug design helps guide the development of molecules that can modulate biological pathways with precision, laying the foundation for innovative and effective therapeutic agents.[6] Drug discovery is an extremely resource-intensive process, often requiring more than a decade to move a potential therapeutic from the research stage to regulatory approval. The total time and financial investment can vary widely depending on the type of drug being developed and the complexity of the disease it targets. On average, bringing a single new medicine to market can take 10–15 years and cost over \$2 billion, reflecting the extensive research, testing, and regulatory evaluations required before a drug becomes available to patients.[7]

Steps Involved in Drug Discovery and Development

1. Target identification
2. Target validation
3. Lead identification
4. Lead optimization
5. Product characterization
6. Formulation and development
7. Preclinical
8. Investigating a new drug
9. Clinical
10. New drug application
11. Approval

Drug Design

Drug design typically begins by identifying a biological target, usually a macromolecule such as a protein, enzyme, or receptor, that plays a key role in the disease process. Once the target is defined, the next step is to discover a “hit” compound, meaning a ligand capable of interacting with that target. Through iterative optimization, this initial hit is gradually refined to improve its affinity, selectivity, safety, and overall drug-like properties, ultimately leading to a viable therapeutic candidate. Drug design strategies are generally categorized into two main approaches: ligand-based drug design and structure-based drug design. A major advancement in this field has been the evolution of *in silico* (computational) technologies, which support virtual screening, rational compound design, energy calculations, SAR and QSAR studies, ADME prediction, and modeling of drug–target interactions. Molecular docking is one of the most important tools in this area, as it predicts how two molecules, typically a ligand and its biological target, interact. Docking simulations estimate the orientation and conformation of the ligand within the binding site and calculate the binding energy of the resulting complex. Lower binding energy generally

indicates a more stable and favorable interaction. Another widely used virtual screening technique is pharmacophore-based screening, which identifies essential structural features required for biological activity and searches for molecules that match these features.[8]

Computer-Aided Drug Design

Computer-aided drug design (CADD) is a modern and powerful tool in pharmaceutical research that applies computational techniques to streamline and enhance the drug discovery process. The primary objective of CADD is to screen, refine, and evaluate potential molecules based on their predicted interactions with a biological target. By integrating structural biology, chemistry, and computational algorithms, CADD enables researchers to identify promising drug candidates more efficiently and with greater precision. CADD is now widely used in both academic research and the pharmaceutical industry as part of a multidisciplinary strategy aimed at increasing therapeutic effectiveness while minimizing adverse effects. This approach is particularly valuable because traditional drug discovery is often lengthy, complex, costly, and associated with a high failure rate. Challenges such as identifying suitable targets, achieving selectivity, predicting toxicity, and ensuring good pharmacokinetic properties often complicate the development pipeline. CADD helps address many of these obstacles by providing early insights into molecular behavior, predicting biological activity, reducing experimental workload, and assisting in rational optimization of lead compounds. As a result, the integration of computational methods has become essential for improving success rates and reducing the overall time and financial investment required in drug development.[9]

Depending on whether 3D protein structures or

ligands are available, CADD employs two distinct approaches. They are referred to as:

1. Structure-based drug design (SBDD)
2. Ligand-based drug design (LBDD)
3. In certain instances, combining the two methods has demonstrated high precision in identifying the lead compounds.^[10]

1. Structure-Based Drug Design (SBDD)

Structure-based drug design (SBDD) utilizes the three-dimensional structural information of a biological target to guide the development of potential inhibitors. Typically, the receptor structure is obtained through experimental methods such as X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy. When an experimentally determined structure is not available, computational techniques such as threading and homology modeling can be used to predict the protein's 3D conformation. Among these methods, homology modeling has become the most widely adopted approach for generating reliable structural models in situations where no crystal structure exists.[11]

2. Ligand-Based Drug Design (LBDD)

Ligand-based drug design is applied when the three-dimensional structure of the receptor is not available. Instead of relying on the target protein, this approach uses information from known active molecules to identify or design new compounds that are likely to interact with the same biological target.[11] Three-dimensional quantitative structure-activity relationship (3D-QSAR) methods and pharmacophore modeling are among the most important and commonly used ligand-based drug design tools. These approaches help identify the key structural features required for biological activity and provide predictive models that support the optimization of existing



compounds as well as the discovery of new lead molecules.[6]

Theory Of Docking

The primary goal of molecular docking is to computationally predict the most plausible structure of a ligand-receptor complex. The docking process generally involves two key stages: first, generating a range of possible ligand conformations within the protein's active site, and second, evaluating and ranking these conformations using a scoring function to identify the most favorable binding pose.[12] Molecular docking is one of the most widely applied techniques in structure-based drug design because it can predict how small molecules fit and interact within the binding site of a target protein. The objective of docking is to identify the most favorable conformation and orientation of both the ligand and the protein during their interaction, such that the overall binding free energy of the system is minimized.[13]

Types Of Docking

There are 2 types of Docking

1. Rigid docking
2. Flexible docking

Rigid Docking

In this docking approach, both the ligand and the receptor are treated as rigid structures. Programs such as DOCK use this principle by first determining the optimal orientation of a ligand within the binding pocket and then evaluating how well it fits based on a scoring function. Because the molecules are assumed to be inflexible, the process involves finding the best spatial arrangement of the ligand relative to the receptor that maximizes their geometric and chemical complementarity. In this model, the ligand's

conformation is fixed, and its ability to bind does not depend on any structural adjustments of either molecule during the interaction. [14]

Flexible docking

In this type of docking, the receptor is typically considered rigid, meaning its structure remains fixed during the simulation. However, the ligand is allowed to explore different conformations. By assessing ligand flexibility during the transformation steps, the method aims to identify the most favorable conformations of both the ligand and its interaction with the receptor as they would appear in the final bound complex.[15]

Molecular docking

Molecular docking refers to the computational process of positioning a ligand within the binding site of a receptor in an orientation that optimizes its interaction. It has become an essential tool in modern drug discovery, particularly for the virtual screening of phytochemicals, nutraceuticals, and other small molecules as potential therapeutic candidates. The first docking program was developed in the mid-1980s by Irwin Kuntz at the University of California, and since then, continuous advancements have been made to improve the accuracy and efficiency of docking algorithms. [16]

Mechanism of docking

This method uses molecular docking to predict how a small molecule interacts with a protein at the atomic level. Docking plays a key role in drug discovery by helping identify potential inhibitors, refining lead compounds, and understanding the principles of molecular recognition. The interacting molecule, known as a ligand, may act as an inhibitor by binding to the active or allosteric site of the protein. Accurate docking requires a

high-resolution 3D structure of the target protein, typically obtained through techniques such as X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy. [17]

Applications of molecular docking

Although molecular docking is a computational technique, it offers insights that closely resemble those obtained from experimental biochemical methods. Docking simulations allow researchers to virtually examine how a small molecule fits into the binding site of a protein and how the two interact at the atomic level. By analyzing these predicted interactions, it becomes possible to infer whether a compound may function as an activator, inhibitor, or simply a weak binder toward a specific biological target. One of the major strengths of molecular docking is its ability to map out the types of non-covalent interactions, such as hydrogen bonding, hydrophobic contacts, π - π stacking, electrostatic interactions, and metal coordination that contribute to the stability of a ligand protein complex. Understanding these interactions helps explain why certain molecules exhibit strong biological activity, while others do not. Docking studies are especially valuable in characterizing protein–ligand relationships where experimental structural data may be limited or expensive to obtain. For example, docking can help elucidate how potential drug candidates interact with active sites involved in essential cellular pathways, such as enzyme catalytic pockets or receptor-binding domains. Furthermore, docking can predict binding behavior in systems involving nucleic acids such as DNA gyrase, topoisomerases, or transcriptional regulators by showing how ligands interact with DNA bases or backbone components.

Beyond predicting binding affinity, molecular docking also helps in:

- **Lead optimization:** suggesting chemical modifications that could strengthen binding.
- **Virtual screening:** rapidly filtering large libraries of compounds to identify promising hits.
- **Mechanistic understanding:** providing clues about how a molecule may inhibit or disrupt a biological process.
- **Guiding experimental work:** helping prioritize which compounds should be synthesized and tested first.

Overall, molecular docking bridges the gap between theoretical modeling and practical drug discovery, reducing experimental workload and providing mechanistic insights that support rational drug design. [18]

Comparison Study of Docking using Different Software

1. Using Molecular Operating Environment

Molecular docking was performed using MOE software (version 2014). The most active compounds 2b, 2c, 5a, 5c, 5e, 5h, and 5i were docked against *S. aureus* DNA gyrase to explore their potential interactions within the enzyme's active site. The crystal structure of the gyrase–DNA–ciprofloxacin complex (PDB ID: 2XCT) was obtained from the RCSB Protein Data Bank for this analysis. Molecular modeling of the co-crystallized ligand, ciprofloxacin, used as the reference drug, demonstrated various interactions inside the gyrase active site. These included metal chelation with Mn^{2+} , hydrogen bonding with nucleotide DC X13, hydrophobic contacts, and a π -cation interaction with nucleotide DG Y9. Docking results for the ciprofloxacin-based hybrids (2b, 2c, 5b, 5c, 5e, 5h, and 5i) containing the piperazine moiety indicated that these derivatives also fit well within the active site. They formed notable hydrophobic interactions along



with additional hydrogen bonds with key amino acid residues and DNA bases, consistent with their enhanced antibacterial activity against *S. aureus*. All tested molecules exhibited strong binding affinity toward gyrase, with docking scores (ΔG values ranging from -12.8884 to -10.5550 kcal/mol) significantly better than that of ciprofloxacin ($\Delta G = -8.7725$ kcal/mol). The structures of all ligands were drawn using ChemDraw Professional and prepared for docking by adding hydrogens, followed by energy minimization using the MMF94FX force field with an RMS gradient of 0.001 kcal/mol. The enzyme structure was optimized using MOE's QuickPrep protocol. Docking was performed using the Triangle Matcher placement algorithm, generating ten poses per compound under default settings. Refinement employed the Forcefield method, and scoring was based on the Affinity ΔG scoring function. All docking poses were

inspected visually, and the pose with the lowest free energy and the most favorable hydrophobic, hydrogen-bonding, and electrostatic interactions within the active site was selected for each compound.[19]

2. Using Surflex docking software

In this study, molecular docking was carried out using the X-ray crystal structure of the *E. coli* 24 kDa gyrase B domain complexed with clorobiocin (PDB ID: 1KZN, 2.30 \AA resolution). The Surflex-Dock module of the Sybyl-X software package was used to explore the potential interactions of the compounds within the active site of the gyrase enzyme. All seventeen synthesized inhibitors were docked into the binding pocket, and their predicted binding energies were evaluated to assess their affinity toward the target.

Table no. 1 Surflex Docking Scores of Piperazine Derivatives for *E. coli* (PDB: 1KZN)

Mol. #	C Score	Crash Score	Polar Score	D Score	PMF Score	G Score	Chem Score
Clorobiocin (Ligand)	12.14	-1.21	5.33	-189.513	-76.407	-135.711	-34.514
3a	2.93	-0.87	0.00	-66.065	-20.007	-115.193	-16.162
3b	5.48	-1.69	1.46	-114.895	-23.772	-236.789	-25.490
3c	3.60	-4.44	1.32	-122.691	-18.028	-243.061	-24.762
3d	5.38	-0.62	0.96	-103.551	-31.084	-173.693	-27.155
3e	5.41	-1.77	0.64	-128.063	-12.168	-225.387	-24.369
3f	5.54	-1.99	0.92	-131.171	-12.123	-213.098	-24.211
3g	3.64	-0.82	0.05	-95.843	-8.012	-131.015	-19.207
3h	4.99	-0.61	1.04	-109.661	-35.048	-170.947	-27.589
3i	5.55	-2.28	1.33	-131.189	1.405	-231.666	-30.644
3j	2.72	-1.19	0.00	-93.647	8.001	-149.443	-15.933
3k	5.20	-1.39	0.00	-122.741	-1.386	-230.423	-21.916
3l	5.30	-0.76	1.11	-102.217	-28.486	-168.898	-26.264
3m	3.94	-3.38	0.04	-142.042	-6.368	-245.636	-26.355
3n	3.97	-1.02	1.46	-95.522	-7.291	-154.593	-21.745
3o	2.77	-2.56	0.00	-108.918	3.070	-145.135	-20.229
3p	2.41	-0.92	1.82	-77.155	-18.425	-101.856	-15.749
3q	5.24	-1.74	1.81	-123.589	-36.455	-205.531	-30.026

C-Score (Consensus Score)

A combined score that averages multiple scoring functions to give one overall value. It provides the



final ranking of how strongly the ligand binds to the receptor.

Crash Score

Indicates whether the ligand is penetrating incorrectly or clashing with the binding site. Values close to 0 = good fit. Negative values = steric clashes or wrong penetration.

Polar Score

Shows how much polar interactions (mainly hydrogen bonds) contribute to the binding. Useful to identify ligands that form meaningful hydrogen bonds.

D-Score

Represents electrostatic (charge) and van der Waals interactions between ligand and protein. Higher magnitude means better overall physical interactions.

PMF-Score (Potential of Mean Force Score)

Estimates the free energy of interaction between protein and ligand based on statistical data. Helps in evaluating overall binding strength and stability.

G-Score

Measures the combined contribution of:

- Hydrogen bonding
- Protein–ligand interactions
- Internal stability of ligand
- Lower values generally indicate better binding energy.

Chem-Score

Evaluates:

- Hydrogen bonds

- Hydrophobic contacts
- Rotational freedom of the ligand
It gives a chemical interaction-based estimate of binding affinity.

In this study, ligand docking and binding affinity estimation were carried out using the Surflex-Dock tool integrated within the Sybyl X-2.0 software package, which provides an automated platform for protein–ligand interaction analysis. Errors in atom typing within the PDB structure were corrected, and the proline Φ angles were adjusted accordingly. Side-chain amide orientations were refined to enhance possible hydrogen-bond interactions, and van der Waals clashes in the side chains were inspected and resolved. Necessary hydrogen atoms were then added to complete the structure. The final protein model was examined for any conformational issues using the ProTable module in the Sybyl software suite. The quality of the protein structure was evaluated through a Ramachandran plot to inspect the ϕ and ψ backbone torsion angles, as well as by checking the local geometry and verifying that polar residues were properly buried and non-polar residues appropriately exposed. After this assessment, the protein structure underwent energy minimization using the Powell algorithm for 3000 cycles, applying the Kollman united atom force field with a non-bonded cut-off distance of 9.0 and a dielectric constant of 4.0.

The synthesized ligands, including clorobiocin, were also energy-minimized under similar conditions using the Tripos force field and Gasteiger charges. Docking studies were then performed by positioning the compounds into the active site of DNA gyrase subunit A (PDB ID: 1KZN) through the Surflex-Dock module in the Sybyl environment, which uses an incremental build-up strategy to optimize ligand placement within the binding pocket. The resulting docked

poses were finally evaluated and ranked using a combined scoring system, reflected in the consensus score (C-score).^[1]

3. Using Auto Dock Vina 4.2 Software

The newly prepared compounds demonstrated moderate to strong antitubercular activity against the Mtb H37Rv strain. To understand their binding behavior, molecular docking was carried out to determine the key amino acid residues in the target protein that interact with these molecules. It is worth mentioning that the newly developed compounds are structural derivatives of Rifampicin. Rifampicin functions by blocking bacterial DNA-dependent RNA polymerase activity, forming a stable complex with the enzyme's β -subunit in *Mycobacterium tuberculosis*. Hence, the docking simulation is done between the new molecules and Mtb RNAP (PDB ID: 5UHC). The binding energies of the synthesized compounds docked with the 5UHC protein. For every compound, the pose showing the lowest binding energy among the ten generated conformations was taken as the representative result. To evaluate whether the docking scores relate to biological activity, the corresponding MIC values for each molecule have also been

added. Docking results indicate that all synthesized compounds display reasonably strong binding affinity toward the target protein. Although the difference in binding energies between the most active compound (7a) and the least active one (6b) is only about 1.25 kcal/mol, their biological responses differ significantly. Compound 7a shows nearly a twenty-fold lower MIC compared to 6b. This highlights that binding energy alone does not fully account for the overall antimicrobial potency of a molecule. This observation is reinforced by the fact that compound 7a has a lower MIC than the reference drug Rifampicin, even though its binding affinity is approximately half that of Rifampicin. Therefore, understanding the specific interactions within the binding pocket becomes essential. Detailed docking analysis reveals that compounds forming stronger hydrogen bonds with residue ASN-493 (and, to a lesser extent, GLN-438) tend to display higher inhibitory activity. For instance, compound 7a forms a hydrogen bond of 2.2 Å with ASN-493, which is notably stronger than Rifampicin's corresponding interaction at 3.5 Å., which reinforces the higher potency of the former compound. The strongest indication of this trend is observed when comparing the activities of the closely related derivatives 6a, 6b, 7a, and 7b.^[20]

Table no. 2

Entry	MIC ($\mu\text{g/mL}$)	BindingEnergy (kcal/mol)	H-Bond Distance with ASN-493 (Å)	Interacting Residues
6a	1.56	-6.95	2.4	ARG-454, ASN-493
6b	12.5	-6.05	NI	ARG-454, SER-456
6c	3.12	-7.23	NI	SER-437
6d	6.25	-6.30	NI	ARG-454, ARG-613
7a	0.65	-7.30	2.2	ASN-493
7b	6.25	-5.98	NI	GLN-438, ARG-454, SER-456
7c	1.56	-7.15	4.2	ASN-493, ARG-454
7d	1.56	-6.60	4.2	GLN-438, SER-456, ASN-493
Rifampicin	0.75	-13.69	3.5	GLN-438, HIS-451, ARG-454, SER-456, ASN-493

Comparative Conclusions

Although all three software platforms accurately predict ligand binding modes, each excels in different scenarios:

Table no. 3

Software	Best Use Case	Key Strength
MOE	Drug-like ligands, flexible scaffolds	Reliable ΔG scoring and refinement
Surflex-Dock	Detailed interaction mapping	Strong consensus scoring & protomol accuracy
AutoDock/Vina	Large-scale screening, energy-based ranking	Fast and consistent binding energy predictions

DISCUSSION

Docking studies on piperazine-based antimicrobials have been consistently performed on experimentally validated targets such as bacterial DNA gyrase and Mtb RNAP. Patil et al. docked a series of 1,4-di(hetero)aryl piperazines into the clorobiocin-bound *E. coli* DNA gyrase A domain (PDB 1KZN) using Surflex-Dock and showed that the most active analogues 3e and 3c established strong hydrogen bonds with ASP73, THR165, and GLY77, along with high consensus scores, suggesting a gyrase-mediated mechanism for the observed antibacterial activity. Similarly, ciprofloxacin-chalcone and ciprofloxacin-pyrimidine hybrids were docked into *S. aureus* DNA gyrase (PDB 2XCT) with MOE, where the best inhibitors (2b, 2c, 5b, 5c, 5e, 5h and 5i) retained the canonical ciprofloxacin metal chelation and DNA contacts but gained additional hydrogen bonds and hydrophobic interactions; these features translated into more favorable binding energies (-12.9 to -10.6 kcal/mol) and superior DNA gyrase IC_{50} /MIC values compared to ciprofloxacin. In the case of antitubercular piperazines, Sanka et al. employed AutoDock 4.2

against Mtb RNAP (PDB 5UHC) and identified ASN-493 as a key hotspot residue: compounds 6a and particularly 7a formed short H-bonds with ASN-493 (2.4 and 2.2 Å, respectively) and showed the best MIC values, whereas nitro-substituted analogues 6b/7b lacked this interaction and were markedly less active. Across these studies, docking consistently supports the experimental SAR and highlights specific amino acid residues ASP73/THR165/GLY77 in DNA gyrase and ASN-493 in RNAP, as critical for the activity of piperazine-containing scaffolds. However, all three rely on rigid-receptor single-pose docking without extensive validation (RMSD, MD), so the docking results should be viewed as qualitative mechanistic support rather than definitive proof of binding mode.

CONCLUSION

The molecular docking analyses from all three published studies collectively highlight the significance of structural modification on piperazine-based antimicrobial agents and their interaction with key bacterial targets. The first paper on ciprofloxacin hybrids demonstrated that derivatives such as 2b, 2c, 5a, 5c, 5e, 5h, and 5i consistently showed stronger binding affinities toward *S. aureus* DNA gyrase compared to the parent drug, ciprofloxacin. Their improved ΔG values and enhanced hydrogen-bonding and hydrophobic interactions directly aligned with their superior in vitro antibacterial activity, confirming their potential as next-generation DNA gyrase inhibitors. The second published study, employing Surflex-Dock on the *E. coli* DNA gyrase B domain (1KZN), validated through rigorous protein refinement, revealed that several derivatives, particularly 3b, 3d, 3e, 3f, 3h, 3i, 3k, 3l, and 3q, exhibited strong consensus scores and deeper stabilization within the ATP-binding pocket. This consistency across multiple scoring

functions emphasizes their promising inhibitory potential, with compound 3i emerging as a notable lead. The third published work on Mtb RNA polymerase established that specific residue interactions, especially hydrogen bonding with ASN-493, govern antitubercular activity. Compound 7a, despite a moderate binding energy, achieved superior potency due to optimal residue-level interactions. Overall, these three studies collectively show that binding affinity alone is not the sole determinant of biological potency; rather, precise interactions with signature amino acid residues, metal-chelating sites in DNA gyrase, and ASN-493 in RNAP play a decisive role. Together, these findings provide a strong scientific foundation for the continued development of piperazine-based antibacterial and antitubercular agents, confirming that all three published papers meaningfully contribute to advancing structure-guided antimicrobial drug design.

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