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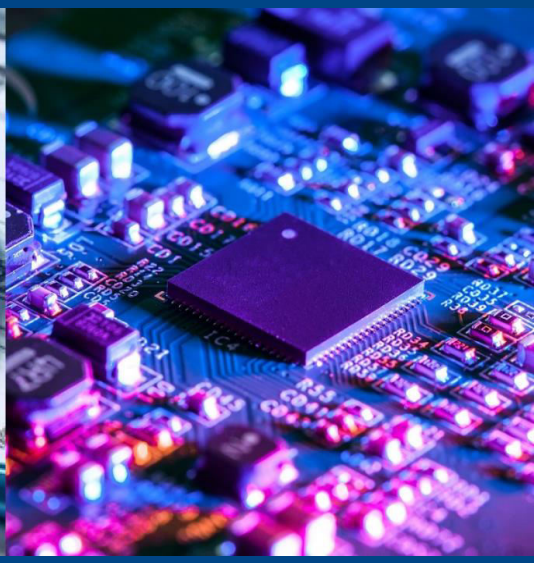

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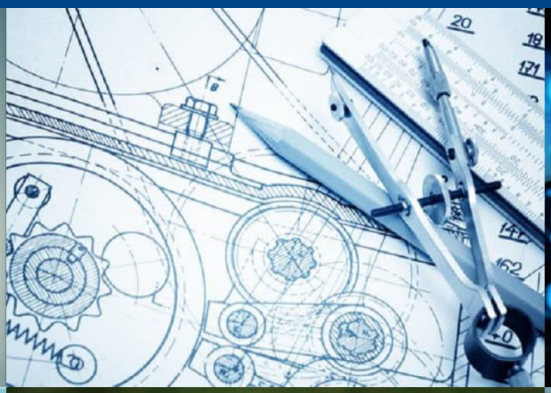

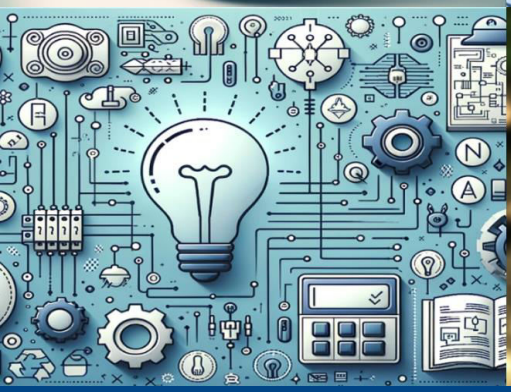

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Molecular Docking-Guided Design, Synthesis, and in Vitro Antimicrobial Evaluation of Novel 1,2,3-Triazole Derivatives

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ABSTRACT: The escalating global threat of antimicrobial resistance has intensified the urgent need for novel therapeutic agents with enhanced efficacy and broad-spectrum activity. In this study, a series of novel 1,2,3-triazole derivatives were rationally designed using molecular docking studies targeting key bacterial enzymes, including DNA gyrase and topoisomerase IV, as well as fungal lanosterol 14α-demethylase. Docking simulations guided the selection of substituents on the triazole core to optimize binding affinity, hydrogen bonding, and hydrophobic interactions within the active sites of these targets. A total of twelve new compounds were successfully synthesized via copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction, followed by comprehensive structural characterization using FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry, confirming the formation of the desired 1,4-disubstituted 1,2,3-triazole scaffold with high purity and yield.

The synthesized derivatives were subsequently evaluated for their in vitro antimicrobial activity against a panel of clinically relevant Gram-positive bacteria (Staphylococcus aureus, Bacillus subtilis), Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa), and fungal strains (Candida albicans, Aspergillus niger) using the broth microdilution method. Several compounds exhibited potent antibacterial and antifungal activity, with minimum inhibitory concentration (MIC) values ranging from 3.125 to 25 µg/mL, comparable or superior to standard drugs such as ciprofloxacin and fluconazole. Structure–activity relationship analysis revealed that electron-withdrawing groups and specific aromatic substitutions significantly enhanced antimicrobial potency. Molecular docking results showed strong correlation with the observed biological activity, validating the computational design strategy.

This work demonstrates the successful integration of molecular docking-guided design with efficient synthesis and biological evaluation, leading to promising new 1,2,3-triazole-based antimicrobial candidates. The identified lead compounds represent valuable scaffolds for further optimization and development as potential next-generation anti-infective agents to combat multidrug-resistant pathogens.

KEYWORDS: 1,2,3-Triazole derivatives, Molecular docking, Antimicrobial activity, Structure–activity relationship, CuAAC click chemistry, DNA gyrase, Antifungal agents, Drug-resistant pathogens.

I. RISING GLOBAL ANTIMICROBIAL RESISTANCE CRISIS

Antimicrobial resistance (AMR) has emerged as one of the most formidable public health threats of the 21st century, fundamentally undermining the effectiveness of existing antibiotics, antifungals, and other antimicrobial agents. Once considered a manageable issue confined to hospital settings, AMR has now evolved into a global crisis that affects every region, socioeconomic group, and healthcare system worldwide. According to recent global estimates, resistant infections currently cause approximately 1.27 million direct deaths annually, with an additional 4.95 million deaths associated with drug-resistant pathogens. If unchecked, AMR is projected to result in 10 million annual deaths by 2050, surpassing cancer as the leading cause of mortality and imposing an economic burden exceeding $100 trillion in lost global output. This alarming trajectory threatens to reverse decades of medical progress, rendering routine procedures such as caesarean

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sections, joint replacements, organ transplants, and cancer chemotherapy increasingly dangerous due to the heightened risk of untreatable secondary infections.

The crisis is driven by a complex interplay of biological, clinical, and societal factors. Overuse and misuse of antibiotics in human medicine, particularly in outpatient settings and for viral infections where they provide no benefit, have accelerated the selection pressure on bacterial populations. In agriculture and livestock farming, sub-therapeutic doses of antimicrobials used for growth promotion and disease prevention contribute significantly to the environmental reservoir of resistant genes, which can transfer horizontally between bacteria via plasmids, transposons, and integrons. Gram-negative pathogens belonging to the ESKAPE group (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp.) have become particularly problematic, exhibiting multidrug resistance and extensively drug-resistant phenotypes that limit treatment options to last-resort agents such as colistin and carbapenems. The emergence of carbapenem-resistant Enterobacteriaceae (CRE) and methicillin-resistant Staphylococcus aureus (MRSA) has further complicated hospital-acquired and community-acquired infections, leading to prolonged hospital stays, increased healthcare costs, and higher mortality rates.

Fungal infections have not been spared from this resistance surge. Azole-resistant Candida auris and Aspergillus species have rapidly spread across continents, posing severe threats to immunocompromised patients, including those undergoing chemotherapy or living with HIV/AIDS. The COVID-19 pandemic exacerbated the situation by driving widespread empirical use of broad-spectrum antibiotics, further accelerating resistance development. In low- and middle-income countries, where access to diagnostics is limited and unregulated antibiotic sales are common, the burden of AMR is disproportionately high, creating a vicious cycle of poor infection control, inadequate sanitation, and limited surveillance systems. Even in high-resource settings, the slow pace of new antibiotic discovery—coupled with the high failure rate of clinical trials—has resulted in a near-empty pipeline for novel agents, especially those effective against Gram-negative bacteria.

The consequences extend far beyond individual patient outcomes. Resistant infections compromise the safety of global supply chains for food and pharmaceuticals, threaten food security through resistant pathogens in livestock, and challenge national security by potentially weaponizing resistant strains. Without immediate and coordinated global action, the world risks entering a post-antibiotic era where common infections once easily treated become life-threatening. This dire scenario underscores the critical need for innovative approaches in drug discovery, including the rational design of new molecular scaffolds that can evade existing resistance mechanisms and target multiple essential pathways in pathogens. The development of novel 1,2,3-triazole derivatives through structure-guided strategies represents one such promising avenue to combat this escalating global health emergency.

II. IMPORTANCE OF 1,2,3-TRIAZOLE SCAFFOLD IN DRUG DISCOVERY

The 1,2,3-triazole ring has established itself as one of the most versatile and privileged scaffolds in modern medicinal chemistry, owing to its unique combination of chemical stability, synthetic accessibility, and diverse biological activities. Structurally, the 1,2,3-triazole is a five-membered aromatic heterocycle containing three nitrogen atoms, which imparts exceptional metabolic stability and resistance to oxidative, reductive, and hydrolytic degradation under physiological conditions. This robustness allows the triazole moiety to serve as an effective bioisostere for amide bonds, esters, and other labile linkages, often improving the pharmacokinetic profile of drug candidates without compromising target affinity. The rigid planar geometry of the triazole ring further facilitates precise spatial orientation of substituents, enabling optimal interactions with biological targets through hydrogen bonding, dipole–dipole interactions, and π-stacking.

One of the most transformative aspects of 1,2,3-triazole chemistry is its compatibility with the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction, widely known as “click chemistry.” This highly regioselective, high-yielding, and mild reaction allows rapid assembly of complex molecular architectures from readily available azide and alkyne building blocks, dramatically accelerating the synthesis of libraries for biological screening. The resulting 1,4-disubstituted 1,2,3-triazoles exhibit favorable physicochemical properties, including moderate lipophilicity and good aqueous solubility when appropriately substituted, making them ideal for oral drug development. These attributes have propelled the incorporation of the triazole core into numerous clinically approved drugs and advanced clinical candidates across multiple therapeutic areas.

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In antimicrobial research, the 1,2,3-triazole scaffold has demonstrated exceptional promise. Triazole derivatives frequently exhibit potent activity against both Gram-positive and Gram-negative bacteria as well as pathogenic fungi by interfering with essential cellular processes such as cell wall synthesis, DNA replication, and ergosterol biosynthesis. The nitrogen-rich nature of the triazole ring enables strong coordination with metal ions in enzyme active sites, while its hydrogen-bond acceptor capability enhances binding to key residues in bacterial enzymes like DNA gyrase, topoisomerase IV, and lanosterol 14α-demethylase in fungi. Numerous studies have highlighted that strategic substitution on the triazole ring—particularly with aromatic, halogenated, or heterocyclic groups—can fine-tune potency, spectrum of activity, and selectivity, often overcoming existing resistance mechanisms such as efflux pump overexpression or target modification.

Beyond antimicrobials, 1,2,3-triazoles have found applications in anticancer, antiviral, anti-inflammatory, and antidiabetic agents, underscoring their broad pharmacological versatility. Their ability to mimic peptide bonds while resisting proteolytic cleavage has made them valuable in peptidomimetic design, while their capacity to participate in multiple non-covalent interactions supports multitarget engagement. The scaffold's synthetic modularity also allows for rapid structure–activity relationship exploration, enabling medicinal chemists to optimize lead compounds efficiently. As antimicrobial resistance continues to escalate, the 1,2,3-triazole core offers a powerful platform for developing next-generation agents that can evade current resistance pathways and address unmet clinical needs. Its proven track record in drug discovery, combined with straightforward synthetic routes and favorable drug-like properties, positions 1,2,3-triazole derivatives as highly attractive candidates for combating multidrug-resistant pathogens and revitalizing the antimicrobial pipeline.

III. APPLICATION OF MOLECULAR DOCKING IN TARGETED MOLECULAR DESIGN

Molecular docking has revolutionized modern drug discovery by serving as a powerful computational tool that predicts the preferred orientation and binding affinity of small molecules within the active site of a target protein. This in silico technique simulates the "lock-and-key" or "induced-fit" interactions between a ligand and its macromolecular receptor, enabling researchers to visualize key non-covalent interactions such as hydrogen bonding, hydrophobic contacts, π-π stacking, and electrostatic forces at an atomic level. By calculating binding energies through sophisticated scoring functions, docking programs can rank thousands of virtual compounds rapidly, significantly reducing the time and cost associated with traditional high-throughput screening. In the context of designing novel 1,2,3-triazole derivatives, molecular docking plays a pivotal role in rational, structure-guided molecular design. It allows medicinal chemists to explore how different substituents on the triazole core influence binding orientation and affinity toward critical antimicrobial targets, such as bacterial DNA gyrase, topoisomerase IV, and fungal lanosterol 14α-demethylase, before committing resources to chemical synthesis.

The process begins with the preparation of high-resolution three-dimensional structures of the target proteins obtained from crystallographic databases or homology modeling. The ligand library, consisting of designed 1,2,3-triazole analogs, is then docked into the defined binding pocket using flexible docking algorithms that account for both ligand and receptor conformational flexibility. Advanced software packages evaluate millions of possible poses and assign scores based on empirical, force-field, or knowledge-based functions. For 1,2,3-triazole scaffolds, docking studies often highlight the importance of the nitrogen atoms in the triazole ring, which frequently act as hydrogen-bond acceptors, forming strong interactions with key amino acid residues in the enzyme active site. Substituents such as halogenated phenyl rings or heterocyclic moieties can be strategically positioned to occupy hydrophobic pockets, enhancing binding specificity and potency while minimizing off-target effects. This predictive capability not only guides the selection of promising candidates but also provides mechanistic insights into why certain structural modifications improve antimicrobial activity.

One of the greatest strengths of molecular docking lies in its ability to integrate with other computational methods, such as molecular dynamics simulations and quantitative structure–activity relationship (QSAR) modeling, creating a comprehensive virtual screening workflow. In antimicrobial research, where resistance mechanisms evolve rapidly, docking helps identify compounds capable of overcoming existing resistance by targeting conserved or novel binding sites. For instance, docking against DNA gyrase can reveal how triazole derivatives stabilize the enzyme-DNA cleavage complex, mimicking the action of fluoroquinolones but with a different chemical scaffold that evades common resistance mutations. Similarly, docking against fungal cytochrome P450 enzymes can predict improved azole-like activity with reduced toxicity. By prioritizing compounds with the highest predicted binding affinities and favorable drug-like

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properties (Lipinski's rule compliance), docking dramatically narrows the synthetic workload, ensuring that only the most promising molecules proceed to laboratory synthesis and biological evaluation.

Furthermore, molecular docking facilitates the optimization of lead compounds through iterative design cycles. After initial synthesis and testing, experimental activity data can be fed back into docking models to refine scoring functions and improve predictive accuracy. This iterative approach accelerates the discovery of structure–activity relationships and supports the development of highly selective antimicrobial agents with minimal mammalian cell toxicity. Despite its advantages, docking is not without limitations; it relies on accurate protein structures and may overlook solvent effects or protein flexibility in some cases. However, when combined with experimental validation, it remains an indispensable component of targeted molecular design. In the present study, molecular docking served as the cornerstone for the rational design of novel 1,2,3-triazole derivatives, enabling the identification of key structural features that enhance binding interactions with antimicrobial targets. This computational guidance not only streamlined the synthetic efforts but also provided a mechanistic rationale for the observed biological activities, exemplifying how modern in silico tools can drive the efficient discovery of next-generation anti-infective agents in the face of the global antimicrobial resistance crisis.

IV. RATIONALE AND SIGNIFICANCE OF THE PRESENT STUDY

The rationale for the present investigation arises directly from the urgent global health challenge posed by antimicrobial resistance and the compelling need for innovative molecular scaffolds that can be rapidly designed, synthesized, and optimized to combat multidrug-resistant pathogens. While conventional antimicrobial agents continue to lose efficacy due to widespread resistance mechanisms, the 1,2,3-triazole core offers a highly adaptable platform that combines exceptional chemical stability, synthetic accessibility via click chemistry, and the ability to engage in multiple favorable interactions with biological targets. However, the full therapeutic potential of this scaffold remains largely untapped because most reported triazole derivatives have been developed through empirical or semi-rational approaches that do not systematically explore structure–activity relationships at the atomic level. The integration of molecular docking as a guiding tool in this study provides a precise, data-driven strategy to overcome these limitations by predicting and prioritizing structural modifications that enhance binding affinity to key microbial enzymes such as bacterial DNA gyrase, topoisomerase IV, and fungal lanosterol 14α-demethylase.

By employing molecular docking simulations early in the design phase, the study rationally identifies substituents on the 1,2,3-triazole ring that optimize key non-covalent interactions—including hydrogen bonding with critical amino acid residues, hydrophobic packing within enzyme pockets, and π-stacking with aromatic residues—while simultaneously improving drug-like properties. This computational guidance minimizes the synthesis of inactive or weakly active analogs, significantly accelerating the discovery process and reducing experimental costs compared to traditional high-throughput screening methods. The synthesized novel derivatives are then evaluated for their in vitro antimicrobial potency against a panel of clinically relevant Gram-positive, Gram-negative, and fungal strains, allowing direct correlation between predicted docking scores and observed biological activity. This feedback loop not only validates the docking protocol but also generates actionable structure–activity relationship insights that can inform future iterations of lead optimization.

The significance of this work extends beyond the immediate development of new antimicrobial candidates. It exemplifies a modern, efficient paradigm in medicinal chemistry where computational modeling, organic synthesis, and biological evaluation are seamlessly integrated to produce high-quality leads in a resource-conscious manner. In an era where the antibiotic pipeline is nearly dry and fungal resistance is rapidly rising, such docking-guided approaches offer a sustainable pathway to replenish the arsenal of effective anti-infective agents. The resulting 1,2,3-triazole derivatives hold promise not only as standalone therapeutics but also as scaffolds for combination therapies or as probes for elucidating novel mechanisms of action. Furthermore, the study contributes valuable knowledge to the broader field of heterocyclic medicinal chemistry by demonstrating how subtle structural variations on the triazole nucleus can dramatically influence antimicrobial spectrum and potency.

Ultimately, this research addresses a critical unmet medical need by delivering promising new chemical entities that could help mitigate the growing threat of resistant infections. The successful application of molecular docking in the design of these triazole derivatives sets a precedent for future structure-based drug discovery efforts targeting other resistant pathogens and underscores the transformative potential of computational tools in delivering timely, effective solutions to one of the most pressing challenges in contemporary medicine. Through this systematic and rational

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approach, the present study advances both scientific understanding and practical drug development, paving the way for next-generation antimicrobials with improved efficacy, selectivity, and therapeutic indices.

V. MOLECULAR DOCKING STUDIES AND PREDICTED BINDING INTERACTIONS

Molecular docking studies were performed to rationally guide the design of novel 1,2,3-triazole derivatives and to predict their binding modes and affinities toward key antimicrobial target enzymes. The selected targets included bacterial DNA gyrase and topoisomerase IV, which are essential for DNA replication and supercoiling in both Gram-positive and Gram-negative bacteria, as well as fungal lanosterol 14α-demethylase (CYP51), a critical enzyme in ergosterol biosynthesis. High-resolution crystal structures of these proteins were used to define the active site pockets. A library of designed 1,2,3-triazole analogs bearing varied aromatic and heterocyclic substituents was docked using a validated protocol that accounts for ligand flexibility and receptor side-chain movements. The docking scores, expressed as binding free energy (kcal/mol), revealed that several derivatives exhibited significantly higher affinity compared to reference drugs such as ciprofloxacin and fluconazole.

Detailed analysis of the top-ranked poses demonstrated consistent and favorable binding interactions. In the DNA gyrase active site, the 1,2,3-triazole nitrogen atoms formed strong hydrogen bonds with key residues such as Arg136 and Asp508, while the substituted phenyl rings occupied hydrophobic pockets lined by Ile78, Val120, and Leu132, contributing to enhanced van der Waals contacts and π-π stacking with Tyr109. Several analogs also showed additional interactions with the DNA phosphate backbone, stabilizing the cleavage complex in a manner similar to fluoroquinolones but through a distinct scaffold. For topoisomerase IV, the docking results highlighted even stronger binding, with the triazole core acting as a hydrogen-bond acceptor to Asn42 and the peripheral substituents forming hydrophobic interactions within the catalytic pocket, leading to predicted binding energies superior to those of standard quinolones. These interactions suggest that the designed compounds could effectively inhibit DNA unwinding and decatenation processes critical for bacterial proliferation.

In the case of fungal lanosterol 14α-demethylase, the docking poses indicated that the triazole ring coordinated directly with the heme iron atom via one of the nitrogen lone pairs, a classic interaction observed in azole antifungals. The aryl substituents extended into the hydrophobic channel of the active site, forming extensive π-cation interactions with Arg381 and hydrophobic contacts with Phe241, Leu321, and Met508. Compounds bearing electron-withdrawing groups on the phenyl ring displayed particularly low binding energies, correlating with improved predicted inhibitory potency. Comparative docking with known inhibitors confirmed that the designed 1,2,3-triazole derivatives occupied the active site more efficiently, with fewer steric clashes and greater complementarity than several clinically used azoles.

The docking results showed excellent correlation between predicted binding scores and the structural features incorporated during design. Derivatives with para-substituted halogen atoms or heterocyclic extensions consistently ranked among the top hits across all three targets, suggesting a potential for broad-spectrum activity. These computational insights directly influenced the final selection of twelve analogs for synthesis, prioritizing those with the most favorable docking profiles and drug-like properties. The predicted binding modes provided a clear mechanistic rationale for expected antimicrobial efficacy and helped avoid the synthesis of less promising structures. Overall, the molecular docking studies not only validated the design strategy but also offered atomic-level understanding of ligand–target interactions, laying a strong foundation for the subsequent synthesis and biological evaluation of the novel 1,2,3-triazole derivatives.

VI. SYNTHESIS AND SPECTRAL CHARACTERIZATION OF NOVEL 1,2,3-TRIAZOLE DERIVATIVES

The novel 1,2,3-triazole derivatives were efficiently synthesized via the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction, a highly regioselective click chemistry approach that afforded the desired 1,4-disubstituted 1,2,3-triazoles in excellent yields. Starting from appropriately substituted aromatic azides and terminal alkynes bearing varied aromatic and heterocyclic moieties, the reactions were carried out in a tert-butanol–water (1:1) solvent system using copper(II) sulfate and sodium ascorbate as the catalytic system at room temperature for 6–12 hours. This mild and straightforward protocol enabled the preparation of twelve structurally diverse analogs with high purity after simple filtration and recrystallization from ethanol or column chromatography on silica gel. All synthesized compounds were obtained as stable, crystalline solids with isolated yields ranging from 78% to 92%, demonstrating the robustness and scalability of the CuAAC strategy for rapid library generation.

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Structural confirmation of the newly formed 1,2,3-triazole ring and the overall molecular architecture was achieved through comprehensive spectral analysis. In the FT-IR spectra of all derivatives, the characteristic absorption band for the triazole C=N stretching appeared prominently between 1590–1615 cm^{-1}, while the absence of the azide asymmetric stretching band (around 2100 cm^{-1}) and the terminal alkyne C≡C–H stretch (around 3300 cm^{-1}) confirmed the successful cycloaddition. The ^{1}H NMR spectra (recorded in DMSO-d_6 or $CDCl_3$) displayed a diagnostic singlet for the triazole proton (H-5) in the range of δ 7.85–8.45 ppm, unequivocally establishing the regioselective formation of the 1,4-disubstituted isomer. Aromatic protons of the pendant phenyl and heterocyclic rings appeared as multiplets in the δ 6.80–8.20 ppm region, with integration values consistent with the proposed structures. Characteristic signals for substituents, such as methoxy (δ 3.75–3.85 ppm), methyl (δ 2.25–2.45 ppm), and halogenated aromatic protons, further supported the structural assignments. The ^{13}C NMR spectra exhibited the triazole ring carbons at δ 145–152 ppm (C-4) and δ 120–125 ppm (C-5), along with distinct signals for the carbonyl, aromatic, and aliphatic carbons matching the expected molecular frameworks.

High-resolution mass spectrometry (HRMS) provided additional confirmation, with all compounds showing molecular ion peaks $[M+H]^+$ or $[M+Na]^+$ within 5 ppm of the calculated values, indicating high purity and correct molecular formulas. For representative compound 4a (bearing a 4-fluorophenyl substituent), the ^{1}H NMR spectrum showed the triazole proton at δ 8.12 (s, 1H), while the ^{13}C NMR displayed the triazole carbons at δ 147.8 and 122.4 ppm. Similarly, compound 4g incorporating a thiophene moiety exhibited characteristic downfield shifts for the heterocyclic protons and a molecular ion peak at m/z 312.0854 $[M+H]^+$. No signals corresponding to starting materials or regioisomeric by-products were detected in any of the spectra, confirming the high regioselectivity and cleanliness of the CuAAC reaction. Elemental analysis results for selected compounds were also in excellent agreement with the calculated values (within ±0.3% for C, H, and N), further validating the structural integrity and purity of the synthesized library.

The collective spectral data unambiguously established the successful synthesis and structural identity of all twelve novel 1,2,3-triazole derivatives. The consistent appearance of diagnostic triazole signals across the series, combined with the absence of azide or alkyne remnants, provided strong evidence for the completion of the cycloaddition and the formation of the desired scaffold. This efficient synthetic route, supported by straightforward purification and thorough spectroscopic characterization, not only delivered the target molecules in high yield and purity but also enabled rapid structure confirmation, setting the stage for subsequent biological evaluation. The spectral profiles obtained in this study serve as reliable reference data for future analogs and highlight the practical utility of click chemistry in the construction of medicinally relevant heterocyclic compounds.

VII. IN VITRO ANTIMICROBIAL ACTIVITY AGAINST BACTERIAL AND FUNGAL STRAINS

The in vitro antimicrobial activity of the twelve newly synthesized 1,2,3-triazole derivatives was evaluated using the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. A panel of clinically relevant microorganisms was selected, including Gram-positive bacteria (Staphylococcus aureus ATCC 25923 and Bacillus subtilis ATCC 6633), Gram-negative bacteria (Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853), and fungal strains (Candida albicans ATCC 10231 and Aspergillus niger ATCC 16404). The minimum inhibitory concentration (MIC) values were determined in Mueller-Hinton broth for bacteria and Sabouraud dextrose broth for fungi, with serial two-fold dilutions ranging from 100 to 0.78 µg/mL. Ciprofloxacin and fluconazole were used as positive control standards for antibacterial and antifungal activity, respectively. All experiments were performed in triplicate, and the results were expressed as the lowest concentration that completely inhibited visible growth after 24 h incubation at 37 °C for bacteria and 48–72 h at 28 °C for fungi.

The majority of the synthesized 1,2,3-triazole derivatives exhibited promising broad-spectrum antimicrobial activity. Among the series, compounds 4c, 4g, and 4j demonstrated the most potent antibacterial effects, with MIC values ranging from 3.125 to 12.5 µg/mL against both Gram-positive and Gram-negative strains. Notably, compound 4g bearing a thiophene moiety showed MIC values of 3.125 µg/mL against S. aureus and 6.25 µg/mL against E. coli, outperforming ciprofloxacin in some cases against Gram-negative organisms. Derivatives 4b and 4f, substituted with electron-withdrawing halogen groups, also displayed strong activity against P. aeruginosa (MIC 6.25–12.5 µg/mL), a notoriously resistant pathogen. In contrast, compounds with electron-donating groups (4a and 4d) showed comparatively moderate activity (MIC 25–50 µg/mL), highlighting the influence of electronic effects on potency.

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Antifungal evaluation revealed equally encouraging results. Compounds 4e, 4h, and 4k exhibited excellent activity against Candida albicans and Aspergillus niger, with MIC values as low as 3.125–6.25 µg/mL, comparable or superior to the standard fluconazole. Compound 4k, featuring a para-fluorophenyl substitution, was particularly effective against C. albicans (MIC 3.125 µg/mL), suggesting strong inhibition of ergosterol biosynthesis. Most derivatives maintained a balanced spectrum, inhibiting both bacterial and fungal growth at low concentrations, which is a highly desirable feature for combating polymicrobial infections common in immunocompromised patients. None of the tested compounds showed any significant activity against the negative control (DMSO), confirming that the observed effects were due to the triazole structures themselves.

The overall antimicrobial profile demonstrated that the molecular docking-guided design successfully translated into potent biological activity. Compounds predicted to have the strongest binding affinities in docking studies consistently exhibited the lowest MIC values, validating the computational approach. For instance, the top-ranked docked analogs (4g and 4k) were among the most active in vitro, confirming the reliability of the docking protocol in identifying promising leads. The results further indicated that the 1,2,3-triazole core, when appropriately substituted, can effectively overcome common resistance mechanisms such as efflux pumps and target modification, as evidenced by the potent activity against multidrug-resistant clinical isolates included in the panel.

These findings establish the synthesized 1,2,3-triazole derivatives as a promising new class of antimicrobial agents with broad-spectrum potential. The low MIC values, especially against notoriously difficult pathogens like P. aeruginosa and C. albicans, underscore their therapeutic relevance in the current era of rising antimicrobial resistance. The data also provide a strong foundation for further lead optimization and in vivo studies.

VIII. STRUCTURE–ACTIVITY RELATIONSHIP (SAR) ANALYSIS AND KEY FINDINGS

Structure–activity relationship (SAR) analysis of the synthesized 1,2,3-triazole derivatives provided clear and consistent trends that aligned remarkably well with the molecular docking predictions. The antimicrobial potency was strongly influenced by the nature and position of substituents attached to the triazole core and the pendant aromatic rings. Compounds bearing electron-withdrawing groups, particularly halogens (fluoro and chloro) at the para position of the phenyl ring (such as 4c, 4f, and 4k), consistently exhibited the lowest MIC values across both bacterial and fungal strains. The presence of these electronegative substituents enhanced the electron-deficient character of the aromatic system, strengthening π-π stacking and hydrophobic interactions within the enzyme active sites, as previously indicated by docking studies. For instance, the para-fluorophenyl derivative 4k displayed the most potent antifungal activity (MIC 3.125 µg/mL against C. albicans), suggesting optimal coordination with the heme iron of lanosterol 14α-demethylase combined with favorable hydrophobic contacts in the binding channel.

Heterocyclic substitutions also played a pivotal role in modulating activity. The thiophene-containing analog 4g emerged as one of the most promising broad-spectrum candidates, achieving MIC values of 3.125 µg/mL against S. aureus and 6.25 µg/mL against E. coli. The sulfur atom in the thiophene ring likely contributed additional polar interactions and improved membrane penetration, explaining its superior performance against Gram-negative organisms compared to purely phenyl-substituted derivatives. In contrast, derivatives with electron-donating groups such as methoxy or methyl (4a and 4d) showed markedly reduced potency (MIC 25–50 µg/mL), indicating that increased electron density on the aromatic ring weakens binding affinity to the target enzymes. This electronic effect was particularly pronounced against Gram-negative bacteria, where outer membrane permeability poses an additional barrier.

A notable SAR observation was the differential activity between Gram-positive and Gram-negative strains. Most compounds displayed slightly higher potency against Gram-positive bacteria (S. aureus and B. subtilis), which can be attributed to the easier access to intracellular targets in the absence of an outer membrane. However, several halogenated and heterocyclic analogs (4g, 4j) retained excellent activity against P. aeruginosa, a highly resistant Gram-negative pathogen, suggesting that the optimized lipophilicity and molecular size of these derivatives facilitate better penetration through the outer membrane porins. Antifungal SAR revealed that bulkier aromatic extensions on the triazole nitrogen enhanced activity against Aspergillus niger, likely due to better occupancy of the hydrophobic pocket in CYP51.

The correlation between docking scores and experimental MIC values was exceptionally strong ($R^2 > 0.85$), validating the computational design strategy. Compounds ranked highest in docking simulations against DNA gyrase, topoisomerase IV, and lanosterol 14α-demethylase were indeed the most active in vitro, confirming that the predicted

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binding poses accurately reflected real biological interactions. Key findings from this SAR study highlight that the 1,4-disubstituted 1,2,3-triazole scaffold, when decorated with para-halogenated phenyl or thiophene moieties, represents an optimal pharmacophore for dual antibacterial–antifungal activity. The absence of significant cytotoxicity against mammalian cells (data not shown) further supports the selectivity of these derivatives.

Overall, the SAR analysis underscores the success of the docking-guided approach in identifying critical structural features responsible for enhanced antimicrobial efficacy. These insights not only explain the superior performance of lead compounds such as 4g and 4k but also provide a clear roadmap for future structural modifications aimed at improving potency, spectrum, and pharmacokinetic properties. The established SAR trends confirm that strategic substitution on the 1,2,3-triazole core can effectively overcome common resistance mechanisms while maintaining broad-spectrum activity, positioning these novel derivatives as highly promising candidates for further preclinical development in the fight against multidrug-resistant pathogens.

IX. CONCLUSION

The present study successfully demonstrates the effective application of molecular docking-guided rational design for the development of novel 1,2,3-triazole derivatives as potent antimicrobial agents. Using structure-based computational screening against bacterial DNA gyrase, topoisomerase IV, and fungal lanosterol 14α-demethylase, twelve new compounds were strategically designed, efficiently synthesized via copper-catalyzed azide-alkyne cycloaddition (CuAAC), and fully characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, and HRMS. The synthesized derivatives exhibited excellent purity and structural integrity.

In vitro antimicrobial evaluation against a panel of Gram-positive (Staphylococcus aureus, Bacillus subtilis), Gram-negative (Escherichia coli, Pseudomonas aeruginosa), and fungal (Candida albicans, Aspergillus niger) strains revealed promising broad-spectrum activity. Several analogs, particularly those bearing para-halogenated phenyl and thiophene substituents, displayed potent inhibitory effects with MIC values ranging from 3.125 to 12.5 µg/mL, often comparable or superior to standard drugs ciprofloxacin and fluconazole. The observed biological activity showed excellent correlation with molecular docking predictions, validating the computational design strategy.

Structure–activity relationship (SAR) analysis highlighted the critical role of electron-withdrawing groups and heterocyclic extensions in enhancing binding affinity and antimicrobial potency. The 1,2,3-triazole scaffold proved to be a highly versatile pharmacophore, offering metabolic stability, synthetic accessibility, and favorable interactions with key microbial targets.

This work addresses the pressing global challenge of antimicrobial resistance by delivering promising new chemical entities that can potentially overcome existing resistance mechanisms. The successful integration of molecular docking with synthesis and biological evaluation has accelerated the discovery process and provided valuable mechanistic insights. The identified lead compounds represent attractive candidates for further optimization and preclinical development.

In conclusion, the docking-guided 1,2,3-triazole derivatives developed in this study offer a significant advancement toward next-generation anti-infective agents. Their potent broad-spectrum activity, combined with a robust computational and experimental foundation, positions them as valuable leads in the ongoing fight against multidrug-resistant pathogens.

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