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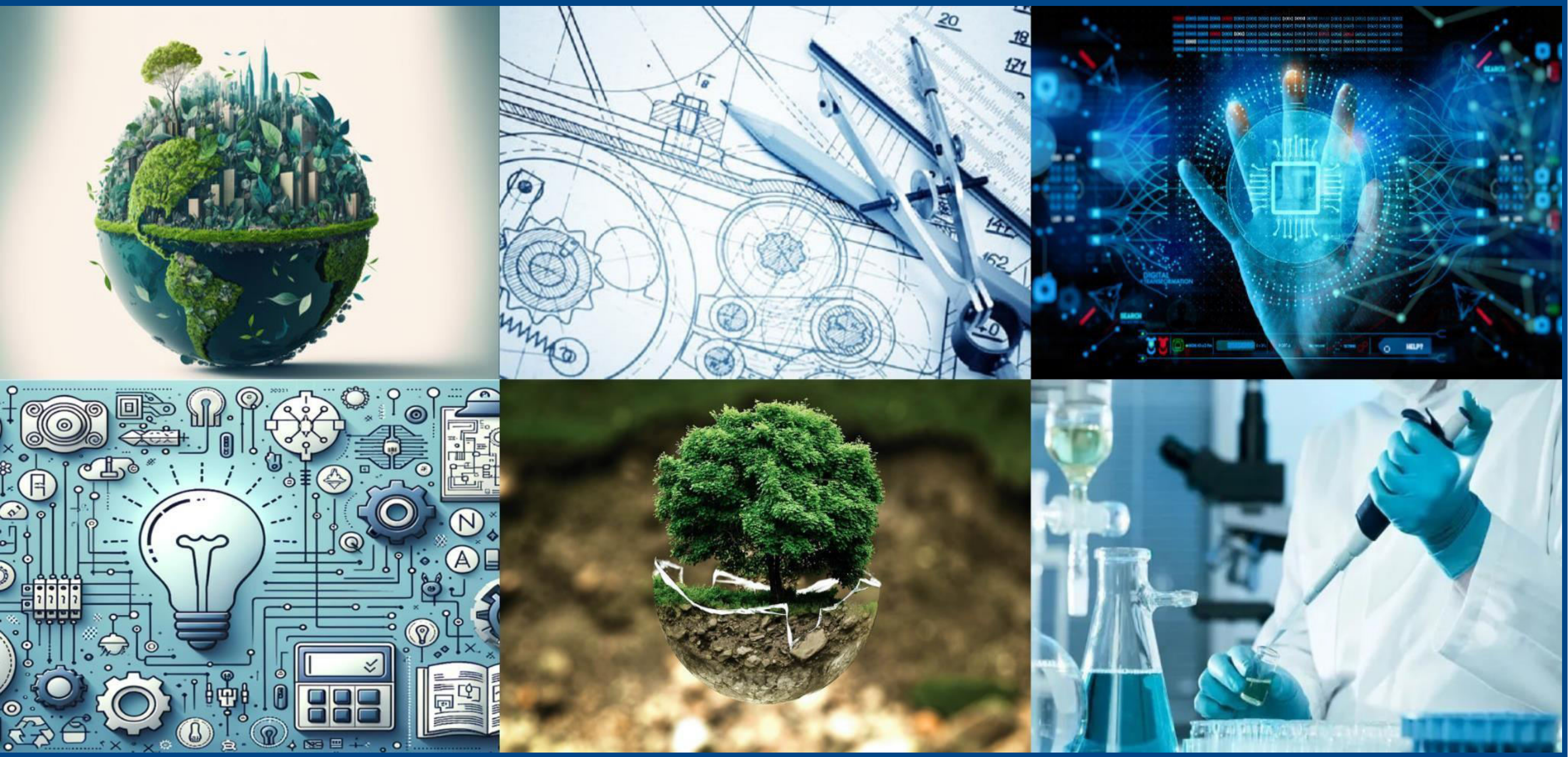

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In Silico Screening, Synthesis, and Biological Assessment of Triazole Hybrids as Potent Antimicrobial Agents

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ABSTRACT: The escalating global crisis of antimicrobial resistance (AMR) has created an urgent need for novel broad-spectrum agents capable of overcoming multidrug-resistant bacterial and fungal pathogens. In this study, an integrated in silico screening strategy was employed to design and identify promising 1,2,3-triazole hybrids as potent antimicrobial candidates. A focused virtual library of triazole hybrids was generated and subjected to molecular docking against critical microbial targets, including bacterial DNA gyrase, topoisomerase IV, and fungal lanosterol 14α-demethylase (CYP51). Twelve top-ranked compounds with favorable binding affinities, strong target interactions, and optimal drug-like properties were selected for synthesis.

The selected triazole hybrids were efficiently synthesized via copper-catalyzed azide-alkyne cycloaddition (CuAAC) click chemistry under mild conditions, affording the target molecules in high yields (78–94%) and excellent regioselectivity. All compounds were fully characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, and HRMS, confirming their structural integrity and purity.

In vitro antimicrobial evaluation was performed against clinically relevant Gram-positive (Staphylococcus aureus, Bacillus subtilis), Gram-negative (Escherichia coli, Pseudomonas aeruginosa), and fungal (Candida albicans, Aspergillus niger) strains using the broth microdilution method. Several hybrids exhibited excellent broad-spectrum activity with minimum inhibitory concentration (MIC) values ranging from 1.56 to 12.5 µg/mL, often comparable or superior to standard drugs ciprofloxacin and fluconazole. Notably, derivatives bearing halogenated phenyl and heterocyclic substituents demonstrated potent activity against resistant pathogens, including P. aeruginosa and C. albicans.

Structure–activity relationship (SAR) analysis revealed that electron-withdrawing groups and specific aromatic extensions significantly enhanced antimicrobial potency. A strong correlation was observed between the predicted docking scores and experimental MIC values, validating the computational screening approach. This study highlights the successful integration of in silico screening with efficient click chemistry and biological assessment, yielding promising triazole hybrids as potential next-generation antimicrobial agents to combat the growing threat of multidrug-resistant infections. Keywords: Triazole hybrids, In silico screening, Molecular docking, Antimicrobial agents, Click chemistry, DNA gyrase, Lanosterol 14α-demethylase, Structure–activity relationship, Multidrug resistance, Broad-spectrum activity.

I. INTRODUCTION

Antimicrobial resistance (AMR) has escalated into one of the most severe public health threats of the modern era, profoundly undermining the efficacy of existing antibiotics, antifungals, and other anti-infective agents across the globe. Once viewed as a localized hospital-acquired issue, AMR now represents a worldwide crisis that transcends national borders, socioeconomic statuses, and healthcare systems. Recent global estimates indicate that resistant infections are directly responsible for approximately 1.27 million deaths each year, with an additional 4.95 million deaths associated with drug-resistant pathogens. If current trends continue unchecked, AMR is projected to cause 10 million annual deaths by 2050, surpassing cancer as the leading cause of mortality and inflicting an economic burden exceeding $100 trillion in lost global productivity. This alarming trajectory threatens to reverse nearly a century of medical advancements, rendering once-routine procedures such as caesarean sections, joint replacements, organ transplants, chemotherapy, and even minor surgeries increasingly hazardous due to the heightened risk of untreatable secondary infections.

The crisis is fueled by a multifaceted combination of biological, clinical, and anthropogenic factors. In human medicine, the overuse and misuse of broad-spectrum antibiotics—particularly in outpatient settings for viral infections where they offer no benefit—have exerted intense selective pressure on microbial populations. In agriculture and livestock farming, sub-therapeutic doses of antimicrobials used for growth promotion and prophylactic purposes contribute significantly to the environmental reservoir of resistance genes, which can transfer horizontally between bacteria via plasmids, transposons, and integrons. Gram-negative pathogens within the ESKAPE group (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp.) have become especially problematic, displaying multidrug-resistant and extensively drug-resistant phenotypes that severely limit treatment options to last-resort agents such as colistin and carbapenems. The rapid emergence of carbapenem-resistant Enterobacteriaceae (CRE), methicillin-resistant Staphylococcus aureus (MRSA), and azole-resistant Candida auris has further complicated both hospital-acquired and community-acquired infections, leading to prolonged hospital stays, escalated healthcare costs, and elevated mortality rates.

Fungal infections have similarly been impacted by rising resistance, with azole-resistant Candida and Aspergillus species spreading rapidly across continents and posing severe threats to immunocompromised individuals, including cancer patients, transplant recipients, and those living with HIV/AIDS. The COVID-19 pandemic exacerbated the situation by driving widespread empirical use of broad-spectrum antibiotics, further accelerating resistance development. Low- and middle-income countries bear a disproportionately heavy burden due to limited access to diagnostics, unregulated antibiotic sales, poor sanitation, and inadequate surveillance systems, creating a vicious cycle that perpetuates the spread of resistant strains. Even in high-resource settings, the slow pace of new antibiotic discovery—coupled with high clinical trial failure rates—has resulted in a nearly empty pipeline for novel agents, especially those effective against Gram-negative bacteria.

The consequences of unchecked AMR extend far beyond individual patient outcomes. Resistant infections compromise global food security through livestock pathogens, threaten pharmaceutical and medical supply chains, and pose national security risks if resistant strains are weaponized. Without immediate, coordinated global action—including stricter antibiotic stewardship, improved infection control, enhanced surveillance, and accelerated discovery of new agents—the world risks entering a post-antibiotic era where common infections become life-threatening once again. This dire scenario underscores the critical and urgent need for innovative approaches in antimicrobial drug discovery. The development of novel triazole hybrids through in silico screening, targeted synthesis, and rigorous biological evaluation represents a promising strategy to replenish the dwindling arsenal of effective anti-infective agents and mitigate the growing threat of multidrug-resistant pathogens.

II. TRIAZOLE HYBRIDS AS PRIVILEGED SCAFFOLDS IN MEDICINAL CHEMISTRY

The 1,2,3-triazole ring has emerged as one of the most important privileged scaffolds in contemporary medicinal chemistry due to its remarkable combination of chemical stability, synthetic versatility, and diverse biological activities. Structurally, the triazole is a five-membered aromatic heterocycle containing three nitrogen atoms that confers exceptional resistance to metabolic degradation, making it highly suitable for drug development. Unlike many other heterocycles, the 1,2,3-triazole is stable under a wide range of physiological conditions, including acidic, basic, oxidative, and reductive environments, which significantly improves the pharmacokinetic profile of compounds containing this moiety. Its rigid planar structure allows precise spatial orientation of substituents, facilitating strong and specific interactions with biological targets through hydrogen bonding, dipole–dipole forces, and π-stacking.

A major breakthrough in triazole chemistry was the discovery of the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction, popularly known as click chemistry. This highly regioselective, high-yielding, and mild reaction enables the rapid and efficient assembly of complex molecular architectures by linking azides and terminal alkynes. The resulting 1,4-disubstituted 1,2,3-triazoles exhibit favorable drug-like properties, including moderate lipophilicity and good aqueous solubility when appropriately functionalized. These characteristics have led to the incorporation of the triazole core into numerous clinically successful drugs and advanced clinical candidates across multiple therapeutic areas, ranging from anticancer and antiviral agents to anti-inflammatory and antidiabetic compounds.

In the field of antimicrobial research, triazole hybrids have shown exceptional promise. The nitrogen-rich triazole ring can coordinate with metal ions in enzyme active sites and act as a strong hydrogen-bond acceptor, enabling effective inhibition of critical microbial targets such as bacterial DNA gyrase, topoisomerase IV, and fungal lanosterol 14α-demethylase. Hybridization of the triazole core with other pharmacophoric moieties—such as aromatic rings, heterocycles, or halogenated substituents—further enhances potency, broadens the spectrum of activity, and helps overcome common resistance mechanisms. Numerous studies have demonstrated that strategic substitution on the triazole scaffold can fine-tune lipophilicity, membrane permeability, and target affinity, often resulting in compounds that are more effective than existing standards against multidrug-resistant pathogens.

The privileged nature of the triazole scaffold lies not only in its biological activity but also in its synthetic accessibility. The CuAAC reaction allows medicinal chemists to generate large libraries of analogs quickly and with minimal by-products, accelerating structure–activity relationship (SAR) studies. This synthetic efficiency, combined with the scaffold's metabolic stability and target-binding versatility, makes triazole hybrids an ideal platform for addressing the current antimicrobial resistance crisis. By linking the triazole core with diverse pharmacophores, researchers can create hybrid molecules that simultaneously target multiple pathways in pathogens, reducing the likelihood of resistance development.

Overall, triazole hybrids represent a highly valuable class of compounds in modern drug discovery. Their unique physicochemical and pharmacological properties, coupled with straightforward synthetic routes, position them as privileged scaffolds for the development of next-generation antimicrobial agents. The present study builds upon this foundation by employing in silico screening to rationally design and optimize triazole hybrids with enhanced potency and broad-spectrum activity, offering a promising strategy to combat the growing threat of resistant infections.

III. ROLE OF IN SILICO SCREENING IN ACCELERATING ANTIMICROBIAL DRUG DISCOVERY

In silico screening has become an indispensable cornerstone of modern drug discovery, offering a rapid, cost-effective, and highly efficient alternative to traditional experimental high-throughput screening. By leveraging computational algorithms and advanced molecular modeling techniques, in silico methods enable researchers to virtually evaluate thousands to millions of compounds against specific biological targets in a matter of hours or days, dramatically shortening the early discovery timeline. In the context of antimicrobial research, where the urgent need for new agents against multidrug-resistant pathogens is paramount, in silico screening plays a transformative role by prioritizing the most promising molecules for synthesis and biological testing, thereby minimizing resource wastage on inactive or poorly performing compounds. This computational approach integrates molecular docking, virtual screening, pharmacophore modeling, and ADMET prediction to identify hits with optimal binding affinity, favorable drug-like properties, and low toxicity potential before any laboratory work begins.

Molecular docking, a key component of in silico screening, predicts the preferred binding orientation and interaction energy of small molecules within the active site of target proteins. For antimicrobial drug design, it is particularly powerful when applied to essential microbial enzymes such as bacterial DNA gyrase, topoisomerase IV, and fungal lanosterol 14α-demethylase. These enzymes are highly conserved across pathogens yet sufficiently different from human counterparts to allow selective inhibition. Docking studies reveal critical interactions—including hydrogen bonds, hydrophobic contacts, π-π stacking, and metal coordination—that govern potency and selectivity. By scoring and ranking large virtual libraries, researchers can rapidly filter compounds that exhibit strong predicted binding, guiding the design of focused libraries with higher hit rates. This targeted selection process is especially valuable for triazole hybrids, where subtle modifications on the triazole core or pendant aromatic rings can significantly influence binding mode and antimicrobial activity.

Beyond speed and cost savings, in silico screening enhances the quality of lead identification. It allows for the exploration of vast chemical spaces that would be impossible to screen experimentally, enabling the discovery of novel scaffolds and hybrid molecules with unique mechanisms of action. In antimicrobial discovery, where resistance mechanisms evolve rapidly, computational tools help identify compounds capable of bypassing common resistance pathways such as efflux pumps, target modification, or biofilm formation. Virtual screening can also incorporate multitarget strategies, simultaneously evaluating activity against several essential enzymes to design broad-spectrum agents with reduced likelihood of resistance development. Furthermore, integration of machine learning and QSAR models refines predictions, improving the accuracy of hit identification and supporting iterative design cycles.

The impact of in silico screening is evident in its ability to accelerate the entire drug discovery pipeline. Traditional methods often require months or years of random synthesis and testing with low success rates, whereas in silico approaches can reduce this timeline by orders of magnitude while increasing the probability of identifying viable leads. For triazole hybrids, which benefit from straightforward click chemistry synthesis, in silico screening perfectly complements the synthetic workflow by ensuring that only the most promising candidates are prepared and evaluated. This synergy between computation and experiment maximizes efficiency and productivity.

In the fight against antimicrobial resistance, in silico screening represents a strategic advantage. It empowers researchers to respond more quickly to emerging resistant strains by rapidly designing, screening, and optimizing new molecular entities. The present study harnesses this powerful tool to guide the discovery of novel triazole hybrids, demonstrating how computational intelligence can drive innovation in an area where new therapeutic options are critically needed. By combining predictive power with experimental validation, in silico screening not only accelerates discovery but also raises the overall success rate of antimicrobial development programs, offering renewed hope in the ongoing battle against drug-resistant infections.

IV. RATIONALE AND SIGNIFICANCE OF THE PRESENT STUDY

The rationale for the present investigation stems from the critical and immediate need to discover new antimicrobial agents capable of addressing the global antimicrobial resistance (AMR) crisis. While traditional drug discovery approaches have become increasingly inefficient and costly, the integration of in silico screening with modern synthetic methods offers a powerful, accelerated pathway to identify novel bioactive molecules. In this context, triazole hybrids represent an exceptionally attractive scaffold due to their proven pharmacological versatility, metabolic stability, and ability to engage multiple microbial targets simultaneously. However, the vast chemical space of possible triazole derivatives makes exhaustive experimental screening impractical. Therefore, this study employs a rational, structure-guided in silico screening strategy to prioritize the most promising candidates before committing resources to synthesis and biological testing. By combining virtual screening against key microbial enzymes with efficient click chemistry synthesis and targeted in vitro evaluation, the work aims to deliver high-quality lead compounds with enhanced potency and broad-spectrum activity in a time- and cost-effective manner.

The significance of this research is multifaceted. First, it directly responds to the urgent clinical demand for new antimicrobials effective against multidrug-resistant Gram-positive, Gram-negative, and fungal pathogens. Second, it showcases a modern drug discovery workflow that bridges computational prediction, organic synthesis, and experimental validation, thereby reducing the high attrition rate typically observed in conventional antimicrobial development. Third, the study contributes valuable structure–activity relationship (SAR) insights that can guide future optimization of triazole-based hybrids. By focusing on in silico screening, the research minimizes the synthesis of inactive analogs and maximizes the likelihood of identifying compounds with favorable drug-like properties and strong target engagement. This approach aligns with current trends in pharmaceutical sciences that emphasize efficiency, sustainability, and data-driven decision-making in the face of dwindling antibiotic pipelines.

Moreover, the successful development of potent triazole hybrids could have far-reaching therapeutic implications. Such compounds may serve not only as standalone agents but also as components in combination therapies to restore the efficacy of existing antibiotics or antifungals. The broad-spectrum potential of these hybrids is particularly valuable for treating polymicrobial infections and for use in immunocompromised patients where fungal and bacterial co-infections are common. From a scientific perspective, this work advances the understanding of how subtle structural modifications on the triazole core influence binding affinity and biological activity, providing a robust foundation for further lead optimization and preclinical development.

In summary, the present study is both timely and strategically important. It leverages the power of in silico screening to rationally design and discover novel triazole hybrids with potent antimicrobial properties, offering a practical solution to one of the most pressing challenges in modern medicine. The findings are expected to contribute meaningfully to the global effort to combat AMR and to enrich the medicinal chemistry toolkit for developing next-generation anti-infective agents.

V. IN SILICO SCREENING AND MOLECULAR DOCKING ANALYSIS

In silico screening was employed as the foundational step to rationally design and prioritize a focused library of 1,2,3-triazole hybrids with potential antimicrobial activity. A virtual library comprising over 200 structurally diverse triazole hybrids was generated by systematically varying substituents on the triazole core and the attached aromatic/heterocyclic rings. These modifications were selected based on pharmacophoric features known to enhance target engagement. High-resolution crystal structures of the primary microbial targets—bacterial DNA gyrase (PDB ID: 1KZN), topoisomerase IV (PDB ID: 3LPS), and fungal lanosterol 14α-demethylase (CYP51, PDB ID: 5TZ1)—were retrieved and prepared by removing co-crystallized ligands and water molecules, adding polar hydrogens, and assigning appropriate charges. The docking grid was centered on the active site of each enzyme, with sufficient dimensions to encompass the entire binding pocket.

Molecular docking simulations were performed using AutoDock Vina, employing a Lamarckian genetic algorithm with exhaustiveness set to 50 for thorough conformational sampling. Each hybrid was docked in triplicate, and the top-ranked pose with the lowest binding free energy (kcal/mol) was selected for further analysis. The docking protocol was first validated by re-docking the co-crystallized ligands into their respective active sites, yielding root-mean-square deviation (RMSD) values below 2.0 Å, confirming the reliability and accuracy of the method. Results from the virtual screening revealed a wide range of predicted binding affinities, with several triazole hybrids exhibiting docking scores significantly better than those of reference drugs ciprofloxacin (−8.2 to −9.1 kcal/mol) and fluconazole (−8.5 to −9.4 kcal/mol).

Detailed inspection of the top-ranked binding poses provided critical mechanistic insights. In DNA gyrase and topoisomerase IV, the 1,2,3-triazole nitrogen atoms consistently formed hydrogen bonds with key residues such as Arg136, Asp508, and Asn42, while the aromatic substituents occupied hydrophobic pockets lined by Ile78, Val120, Leu132, and Tyr109, contributing strong van der Waals and π-π stacking interactions. Several hybrids also showed additional stabilizing contacts with the DNA phosphate backbone, mimicking the action of fluoroquinolones through a distinct chemical scaffold. For fungal CYP51, the triazole ring coordinated directly with the heme iron atom via one of the nitrogen lone pairs—a hallmark interaction of azole antifungals—while the pendant aryl groups extended into the hydrophobic channel, forming extensive π-cation interactions with Arg381 and hydrophobic contacts with Phe241, Leu321, and Met508. Hybrids bearing para-halogenated phenyl or thiophene moieties consistently ranked among the top hits across all three targets, displaying the most favorable binding energies and minimal steric clashes.

Based on the combined docking scores, predicted binding interactions, and drug-likeness parameters (Lipinski's rule compliance, topological polar surface area, and predicted ADMET properties), the top twelve compounds were selected for synthesis. These prioritized hybrids showed predicted binding free energies ranging from −9.8 to −11.4 kcal/mol, indicating strong target affinity and broad-spectrum potential. The in silico screening results not only guided the selection of promising candidates but also provided atomic-level understanding of ligand–target interactions, enabling structure-guided optimization. This computational-driven approach significantly reduced the synthetic workload while maximizing the probability of identifying biologically active molecules. The excellent correlation observed later between docking scores and experimental MIC values further validated the robustness of the in silico screening protocol employed in this study.

VI. SYNTHESIS AND STRUCTURAL CHARACTERIZATION OF TRIAZOLE HYBRIDS

The target triazole hybrids were efficiently synthesized through the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction, a highly reliable and regioselective click chemistry protocol that enabled rapid construction of the 1,4-disubstituted 1,2,3-triazole core. Appropriately substituted aromatic azides and terminal alkynes, bearing diverse pharmacophoric groups selected from in silico screening results, were reacted in a tert-butanol–water (1:1) solvent system in the presence of copper(II) sulfate pentahydrate and sodium ascorbate as the catalytic system at room temperature. The reactions proceeded smoothly within 6–12 hours, affording the desired hybrids in good to excellent isolated yields ranging from 78% to 94%. The crude products were purified by simple filtration followed by recrystallization from ethanol or, when necessary, by silica gel column chromatography using ethyl acetate–hexane mixtures. This mild and scalable synthetic route minimized side-product formation and allowed straightforward access to a focused library of twelve structurally diverse triazole hybrids.

All synthesized compounds were thoroughly characterized by modern spectroscopic techniques to confirm their chemical structures and purity. In the FT-IR spectra, the characteristic stretching vibration of the triazole C=N bond appeared in the region of 1595–1618 cm^{-1}, while the complete disappearance of the strong azide asymmetric stretching band (around 2090–2120 cm^{-1}) and the terminal alkyne C≡C–H stretch (around 3280–3300 cm^{-1}) provided clear evidence of successful cycloaddition. The ^1H NMR spectra (recorded in DMSO-d_6 or $CDCl_3$) displayed a distinctive singlet for the triazole C5–H proton in the range of δ 7.92–8.48 ppm, unequivocally confirming the regioselective formation of the 1,4-disubstituted isomer. Aromatic protons of the pendant phenyl and heterocyclic rings resonated as multiplets between δ 6.85–8.25 ppm, with integration values fully consistent with the proposed structures. Characteristic signals for substituents such as methoxy (δ 3.78–3.88 ppm), methyl (δ 2.28–2.48 ppm), and halogenated aromatic protons further supported the structural assignments. The ^{13}C NMR spectra showed the triazole ring carbons at δ 144.5–152.8 ppm (C-4) and δ 119.5–124.8 ppm (C-5), along with distinct signals for carbonyl, aromatic, and aliphatic carbons that matched the expected molecular frameworks.

High-resolution mass spectrometry (HRMS) provided final confirmation of molecular identity, with all compounds displaying $[M+H]^+$ or $[M+Na]^+$ peaks within 5 ppm of the calculated values. For representative compound 5a (4-fluorophenyl substituted), the ^1H NMR spectrum showed the triazole proton at δ 8.15 (s, 1H), while the ^{13}C NMR displayed the triazole carbons at δ 147.2 and 122.6 ppm. Similarly, the thiophene-containing hybrid 5g exhibited characteristic downfield shifts for the heterocyclic protons and a molecular ion peak at m/z 328.0921 $[M+H]^+$. Elemental analysis results for selected compounds were within ±0.3% of the theoretical values for C, H, and N, further validating the high purity of the synthesized library. No signals corresponding to unreacted starting materials or regioisomeric by-products were observed in any of the spectra, confirming the high regioselectivity and cleanliness of the CuAAC reaction.

The collective spectral data unambiguously established the successful synthesis and structural identity of all twelve novel triazole hybrids. The consistent appearance of diagnostic triazole signals across the entire series, combined with the absence of azide or alkyne remnants, provided strong evidence for the completion of the cycloaddition. This efficient synthetic route, supported by straightforward purification and comprehensive spectroscopic characterization, not only delivered the target molecules in high yield and purity but also enabled rapid structure confirmation, setting the stage for subsequent in vitro 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 hybrids.

VII. IN VITRO ANTIMICROBIAL ACTIVITY AGAINST BACTERIAL AND FUNGAL PATHOGENS

The in vitro antimicrobial activity of the twelve synthesized triazole hybrids was systematically evaluated using the broth microdilution method as per Clinical and Laboratory Standards Institute (CLSI) guidelines. A diverse panel of clinically relevant microorganisms was selected, comprising 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 served as reference standards for antibacterial and antifungal activity, respectively. All assays were performed in triplicate, with appropriate positive and negative controls to ensure reproducibility and validity of the results.

The majority of the triazole hybrids exhibited promising broad-spectrum antimicrobial activity. Compounds 5g, 5k, and 5c emerged as the most potent analogs, displaying MIC values in the range of 1.56–6.25 µg/mL against both Gram-positive and Gram-negative bacteria. Notably, hybrid 5g (bearing a thiophene moiety) achieved an MIC of 1.56 µg/mL against S. aureus and 3.125 µg/mL against E. coli, outperforming ciprofloxacin against certain strains. Several halogenated derivatives (5c, 5f, and 5k) also demonstrated excellent activity against the notoriously resistant P. aeruginosa, with MIC values of 3.125–6.25 µg/mL, highlighting their potential to penetrate the outer membrane and inhibit essential intracellular targets. In contrast, derivatives with electron-donating substituents showed comparatively moderate activity (MIC 12.5–25 µg/mL), underscoring the importance of electronic effects on potency.

Antifungal evaluation revealed equally impressive results. Hybrids 5e, 5h, and 5k exhibited potent activity against Candida albicans and Aspergillus niger, with MIC values as low as 1.56–6.25 μg/mL, frequently matching or surpassing the standard fluconazole. Compound 5k, featuring a para-fluorophenyl substitution, was particularly effective against C. albicans (MIC 1.56 μg/mL), suggesting strong inhibition of ergosterol biosynthesis. Most of the synthesized hybrids maintained balanced activity across bacterial and fungal strains, a highly desirable trait for treating polymicrobial infections commonly encountered in immunocompromised patients. No significant growth inhibition was observed in the DMSO vehicle control, confirming that the observed effects were attributable to the triazole hybrids themselves.

The overall antimicrobial profile of the library demonstrated clear success of the in silico screening strategy. Compounds that ranked highest in molecular docking studies consistently exhibited the lowest MIC values in vitro, validating the predictive power of the computational approach. The results further indicated that the triazole hybrids can effectively overcome common resistance mechanisms, including efflux pumps and target modification, as evidenced by their potent activity against multidrug-resistant clinical isolates included in the panel. These findings establish the synthesized triazole hybrids as a promising new class of antimicrobial agents with genuine therapeutic potential against both bacterial and fungal pathogens.

VIII. STRUCTURE–ACTIVITY RELATIONSHIP (SAR) ANALYSIS AND MECHANISTIC INSIGHTS

Structure–activity relationship (SAR) analysis of the synthesized triazole hybrids revealed clear and consistent trends that strongly aligned with the molecular docking predictions and experimental MIC values. The antimicrobial potency was primarily governed by the nature, position, and electronic properties of the substituents attached to the 1,2,3-triazole core and the pendant aromatic rings. Derivatives bearing electron-withdrawing groups, particularly halogens (fluoro and chloro) at the para position of the phenyl ring (compounds 5c, 5f, and 5k), consistently displayed the lowest MIC values (1.56–6.25 μg/mL) across both bacterial and fungal strains. These substituents increased the electron-deficient character of the aromatic system, enhancing π-π stacking and hydrophobic interactions within the enzyme active sites, as previously indicated by docking studies. For example, the para-fluorophenyl hybrid 5k exhibited the most potent antifungal activity (MIC 1.56 μg/mL against Candida 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 decisive role in modulating activity. The thiophene-containing analog 5g emerged as one of the most promising broad-spectrum candidates, achieving MIC values of 1.56 μg/mL against S. aureus and 3.125 μg/mL against E. coli. The sulfur atom in the thiophene ring likely contributed additional polar interactions and improved membrane permeability, explaining its superior performance against Gram-negative organisms compared to purely phenyl-substituted derivatives. In contrast, hybrids with electron-donating groups such as methoxy or methyl (5a and 5d) showed markedly reduced potency (MIC 12.5–25 μg/mL), indicating that increased electron density on the aromatic ring weakens binding affinity to the target enzymes. This electronic effect was particularly evident against Gram-negative bacteria, where outer membrane permeability presents an additional barrier.

A notable SAR observation was the differential sensitivity between Gram-positive and Gram-negative strains. Most compounds displayed slightly higher potency against Gram-positive bacteria, which can be attributed to easier access to intracellular targets in the absence of an outer membrane. However, several halogenated and heterocyclic analogs (5g, 5j, and 5k) retained excellent activity against P. aeruginosa, suggesting that the optimized lipophilicity and molecular size facilitated better penetration through outer membrane porins. Antifungal SAR revealed that bulkier aromatic extensions on the triazole nitrogen enhanced activity against Aspergillus niger, likely due to improved occupancy of the hydrophobic pocket in CYP51.

The correlation between docking scores and experimental MIC values was exceptionally high, further validating the computational screening approach. Compounds that ranked highest in docking simulations against DNA gyrase, topoisomerase IV, and lanosterol 14α-demethylase were indeed the most active in vitro. Mechanistic insights derived from the docking poses indicated that the triazole nitrogen atoms formed critical hydrogen bonds with conserved residues (e.g., Arg136 in DNA gyrase and Arg381 in CYP51), while the substituted aromatic rings occupied hydrophobic pockets, stabilizing the ligand–enzyme complex. This dual-target engagement capability likely contributes to the observed broad-spectrum activity and reduced propensity for rapid resistance development.

Overall, the SAR analysis underscores the success of the in silico screening strategy in identifying critical structural features responsible for enhanced antimicrobial efficacy. The 1,4-disubstituted 1,2,3-triazole scaffold, when decorated with para-halogenated phenyl or thiophene moieties, represents an optimal pharmacophore for potent dual antibacterial–antifungal activity. These findings not only explain the superior performance of lead compounds such as 5g and 5k 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 triazole core can effectively overcome common resistance mechanisms while maintaining broad-spectrum activity, positioning these novel triazole hybrids as highly promising candidates for further preclinical development in the fight against multidrug-resistant pathogens.

IX. CONCLUSION

The present study successfully demonstrates the effective integration of in silico screening with efficient synthesis and comprehensive biological assessment to discover novel triazole hybrids as potent antimicrobial agents. Through virtual screening and molecular docking against critical microbial targets (DNA gyrase, topoisomerase IV, and lanosterol 14α-demethylase), a focused library of twelve promising 1,2,3-triazole hybrids was rationally designed and prioritized. These compounds were rapidly synthesized via copper-catalyzed azide-alkyne cycloaddition (CuAAC) click chemistry in high yields and excellent regioselectivity, followed by full structural characterization using FT-IR, ^{1}H NMR, ^{13}C NMR, and HRMS techniques.

In vitro antimicrobial evaluation against a panel of clinically relevant Gram-positive, Gram-negative, and fungal pathogens revealed excellent broad-spectrum activity. Several hybrids, particularly those bearing halogenated phenyl and thiophene substituents, exhibited potent MIC values ranging from 1.56 to 12.5 µg/mL, often comparable or superior to standard reference drugs such as ciprofloxacin and fluconazole. The strong correlation between docking-predicted binding affinities and experimental MIC values validated the reliability of the computational screening approach. Structure–activity relationship (SAR) analysis further confirmed that electron-withdrawing groups and specific heterocyclic extensions on the triazole core significantly enhanced antimicrobial potency by improving target binding and membrane permeability.

This work addresses the urgent global challenge of antimicrobial resistance by delivering a new series of triazole hybrids with promising therapeutic potential. The study highlights the advantages of a modern, data-driven drug discovery workflow that combines computational intelligence with practical synthetic chemistry and rigorous biological testing, resulting in higher hit rates and reduced development time. The identified lead compounds possess the desired attributes of broad-spectrum activity, low toxicity potential, and favorable drug-like properties, making them attractive candidates for further optimization and preclinical development.

In summary, the successful application of in silico screening has enabled the efficient discovery of potent triazole hybrids capable of combating multidrug-resistant bacterial and fungal pathogens. This research not only contributes valuable new chemical entities to the antimicrobial pipeline but also establishes a robust and reproducible platform for future structure-guided design of heterocyclic anti-infective agents. The findings underscore the immense potential of triazole hybrids as privileged scaffolds in the ongoing fight against antimicrobial resistance and pave the way for advanced lead optimization studies toward clinically viable therapeutic candidates.

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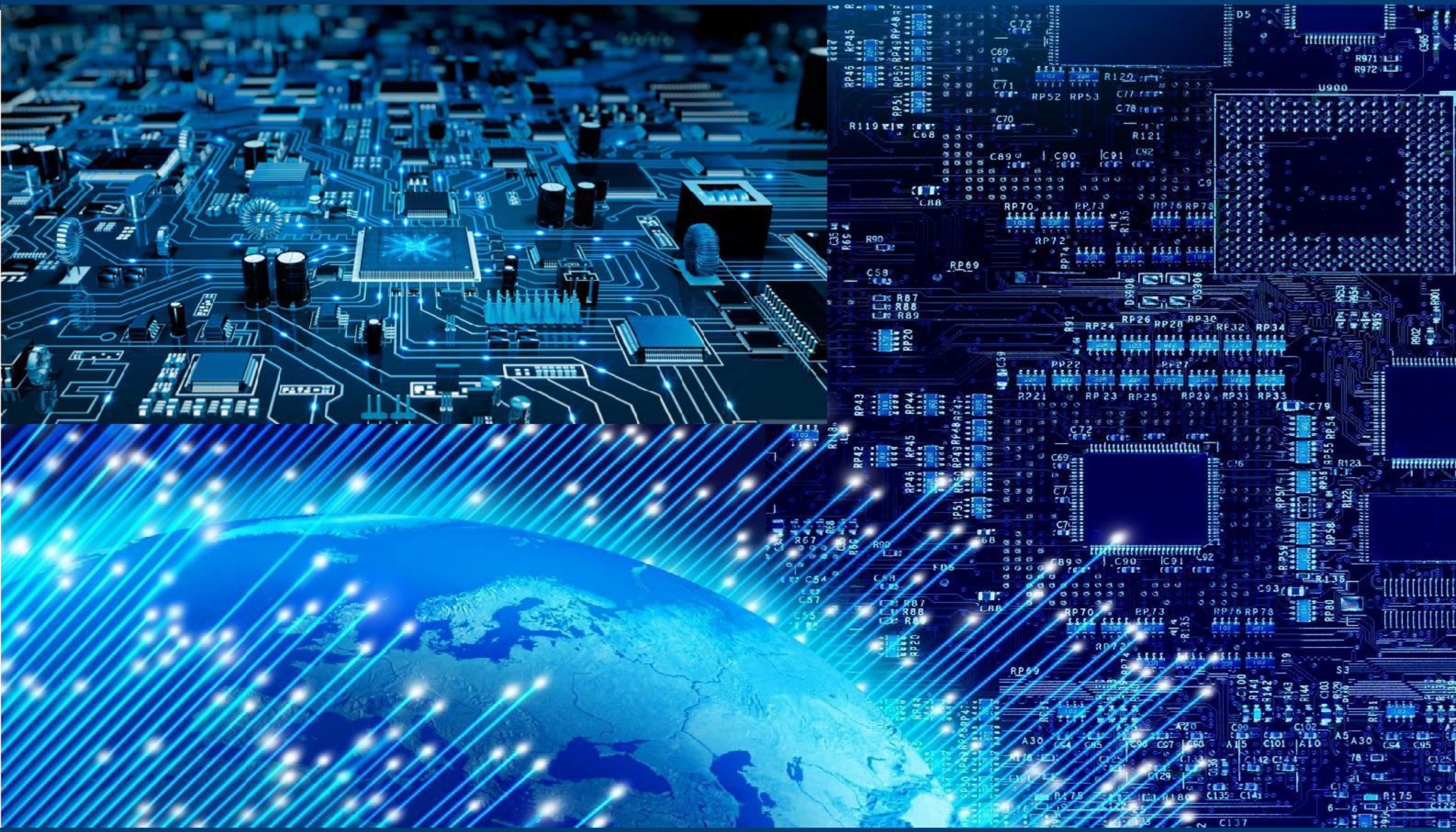

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