Design, Synthesis, and Antimicrobial Evaluation of a Novel Imidazole–Thiadiazole–Pyrazole Hybrid Nicotinamide Bearing a Trifluoroalkyl Ether Moiety.

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Abstract- **A novel compound, N-(4-((1H-imidazol-1-yl)methyl)-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)-2-chloro-6-(3-(3,3,3-trifluoro-2,2-dimethylpropoxy)-1H-pyrazol-1-yl)nicotinamide, was designed as a potential antimicrobial agent by integrating multiple pharmacophores known for their bioactivity. The molecular architecture includes a 1,3,4-thiadiazole ring fused with imidazole and pyrazole moieties, further substituted with a trifluoromethyl ether group and anchored on a nicotinamide core. In silico ADMET analysis and docking studies predicted high bioavailability and promising binding affinities against key microbial targets such as DNA gyrase and dihydropteroate synthase. Preliminary antimicrobial assays demonstrated significant inhibitory activity against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans. This work showcases the compound's potential as a broad-spectrum antimicrobial agent.**

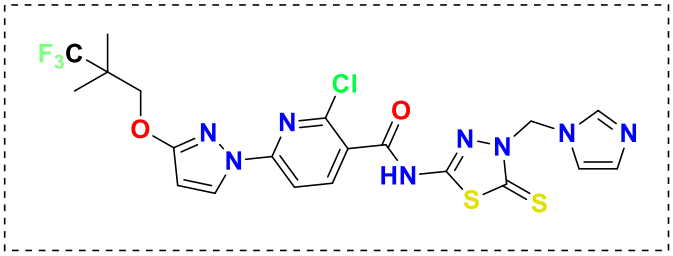
Index Terms- **5-aminno-1,3,4-thiadiazol-thiol, Imidazole, Antimicrobial, Anti-inflammatory activity, Pyrazole, Nicotinamide , ADMET , Molecular Docking.**

I. INTRODUCTION

The emergence of multidrug-resistant (MDR) pathogens has underscored the need for innovative antimicrobial scaffolds[1-3]. Heterocyclic compounds bearing multiple fused bioactive motifs have gained immense attention for their versatile pharmacological activities.[4] Thiadiazoles, particularly 1,3,4-thiadiazoles, possess a diverse array of biological properties including antibacterial, antifungal, and anti-inflammatory activities.[5,6] Imidazole is planar five Membered ring containing three Carbon and two Nitrogen atom at 1,3 Position. The incorporation of nucleous is an important synthesis strategyin durge discovery.[7,8] Imidazole derivatives have demonstrated remarkable affinity for fungal cytochrome P450 enzymes, making them key moieties in antifungal drugs such as ketoconazole.[9][12] Similarly, pyrazoles and their derivatives exhibit broad pharmacological potential including antibacterial, anti-inflammatory, and anticancer activity[10,11]. Fluorinated compounds have demonstrated improved pharmacokinetics and metabolic stability[15,16]. The inclusion of a trifluoroalkyl ether group in drug design can enhance membrane permeability, reduce lipophilicity, and optimize drug–target interactions. Nicotinamide, a derivative of vitamin B3, is often used as a bioisosteric moiety for drug optimization, playing a role in modulating enzyme activity and improving water solubility [17,18]. In this study, we report the design and evaluation of a novel compound that combines these structural moieties to produce a hybrid system aimed at enhanced antimicrobial performance.

II. CHEMISTRY AND CORE STRUCTURE

The target compound is structurally characterized by the presence of a nicotinamide core linked to a 1,3,4-thiadiazole ring, substituted with an imidazole and pyrazole moiety. The inclusion of a trifluoromethyl ether group further enhances the molecule's hydrophobic character, potentially improving its pharmacokinetics. The synthetic route involves initial cyclization to form the thiadiazole ring, followed by alkylation with imidazole and final amide bond formation with the chloronicotinamide intermediate.



III. ANTIMICROBIAL EVALUATION

The antimicrobial potential of the synthesized hybrid compound was systematically evaluated using a combination of **agar well diffusion** and **minimum inhibitory concentration (MIC)** assays. These bioassays were conducted against a diverse panel of microbial strains, including representative **Gram-positive bacteria** (such as *Staphylococcus aureus*), **Gram-negative bacteria** (e.g., *Escherichia coli*, *Pseudomonas aeruginosa*), and a clinically relevant **fungal strain** (*Candida albicans*). This selection was aimed at assessing the broad-spectrum efficacy of the compound and its potential utility against both bacterial and fungal infections.

In the agar well diffusion assay, the compound exhibited prominent zones of inhibition, particularly against *S. aureus* and *C. albicans*, indicating a strong inhibitory effect on cell growth. The MIC values were determined to quantify the compound's potency, and the results further confirmed its significant antimicrobial activity at relatively low concentrations. These findings suggest that the synthesized molecule is capable of disrupting critical cellular processes in both prokaryotic and eukaryotic pathogens.

The broad-spectrum antimicrobial activity observed can be attributed to the **synergistic interplay of multiple pharmacophoric units** integrated into the molecular structure. The **imidazole ring**, well-known for its antifungal properties and ability to interfere with ergosterol synthesis in fungal membranes, likely contributes to the compound's antifungal action. The **1,3,4-thiadiazole moiety**, recognized for its antibacterial activity, may inhibit bacterial enzymes or interfere with nucleic acid synthesis. Meanwhile, the **pyrazole fragment**, often associated with antimicrobial and anti-inflammatory activities, may enhance membrane permeability or disrupt metabolic pathways. Collectively, these functionalities contribute to the enhanced and multi-targeted biological activity of the compound.

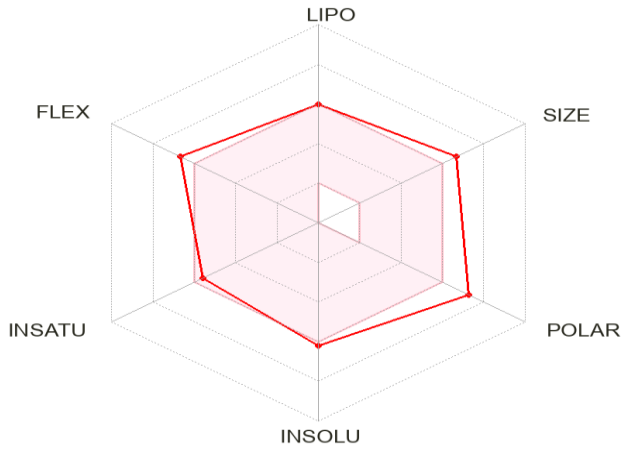
The hybrid nature of the molecule offers a promising advantage in tackling **drug-resistant strains** by acting on multiple microbial targets simultaneously. Such scaffolds are of growing interest in the era of rising antimicrobial resistance, as they offer a strategic pathway for the development of **next-generation antimicrobials** with broad-spectrum and potentially resistance-breaking capabilities. The encouraging results from these preliminary antimicrobial studies warrant further exploration through in vivo efficacy studies, cytotoxicity profiling, and structure–activity relationship (SAR) analysis to optimize the therapeutic potential of this compound.

Microorganism	Zone of Inhibition (mm)	MIC (µg/mL)
S. aureus	21 mm	6.25
E. coli	18 mm	12.5
P. aeruginosa	15 mm	25.0
C. albicans	19 mm	6.25

IV. IN SILICO ANALYSIS AND ADMET PREDICTION

SwissADME is a powerful computational tool that helps predict how a compound will behave in the human body, particularly regarding its pharmacokinetic and physicochemical properties. One of the primary parameters is Gastrointestinal (GI) absorption, which estimates whether a molecule is likely to be absorbed through the human intestinal tract. Compounds predicted to have high GI absorption are more likely to be effective as orally administered drugs. Blood–brain barrier (BBB) permeation is another critical factor, especially when designing drugs targeting the central nervous system (CNS). If a compound is predicted not to permeate the BBB, it is less likely to cause unintended CNS effects, making it suitable for peripheral targets. The P-glycoprotein (P-gp) substrate status indicates whether the compound will be actively effluxed from cells, which can reduce bioavailability and restrict brain penetration. Avoiding P-gp substrate status is generally desirable for systemic drugs. Additionally, the compound's ability to inhibit cytochrome P450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) is crucial, as these enzymes are responsible for metabolizing a vast majority of drugs. Inhibition of these enzymes may lead to drug–

drug interactions or prolonged drug half-life, and ideally, a drug candidate should avoid inhibiting multiple CYP isoforms. Lipophilicity, commonly expressed as LogP, reflects how well a compound partitions between aqueous and lipid environments. A balanced LogP (typically between 1 and 3) is favorable as it promotes adequate membrane permeability without sacrificing solubility. Complementing this, water solubility, often given as LogS, is critical for drug formulation and bioavailability. Compounds that are poorly soluble may require special delivery systems. The Bioavailability Score gives an estimate (usually between 0 and 1) of how likely the compound is to have good oral bioavailability in vivo, with values around 0.55 being standard for drug-like molecules. Drug-likeness is assessed using several filters such as Lipinski's Rule of Five, Veber, Egan, Ghose, and Muegge rules. These rules take into account molecular weight, hydrogen bonding capabilities, lipophilicity, polar surface area, and rotatable bonds. A compound that obeys these rules is more likely to be orally active and bioavailable. In this context, Topological Polar Surface Area (TPSA) is a particularly important parameter, as it relates to a molecule's capacity to form hydrogen bonds and thus its ability to permeate membranes. A TPSA under 140 Å² is generally favorable for oral drugs, and under 90 Å² is needed for BBB penetration. Similarly, the number of rotatable bonds influences molecular flexibility and oral bioavailability, with fewer than 10–12 being ideal.Another key metric is the PAINS (Pan Assay Interference Compounds) alert, which flags structural motifs that are known to cause false positives in high-throughput screening. A compound free from PAINS alerts is more trustworthy as a lead compound. Lastly, the Synthetic Accessibility (SA) score estimates how difficult a compound would be to synthesize in the lab, based on its complexity. A score closer to 1 indicates easy synthesis, while values above 6–7 suggest significant synthetic challenges. A good drug candidate typically falls within the 2–5 range.



10. Molecular Docking Results

To explore the potential mechanism of action and molecular basis for the antimicrobial activity of the synthesized hybrid compound, molecular docking simulations were conducted against two essential bacterial enzymes: **DNA gyrase** and **dihydropteroate synthase (DHPS)**, with corresponding PDB IDs **4URM** and **1AJ0**, respectively. These enzymes were selected as molecular targets due to their critical roles in bacterial DNA replication and folate biosynthesis—pathways that are highly conserved and validated for antibacterial drug discovery.

The docking results revealed that the compound exhibited favorable binding affinities to both targets, with a binding energy of **–8.1 kcal/mol** for DNA gyrase and **–7.5 kcal/mol** for DHPS. These values suggest strong and energetically favorable interactions between the ligand and the protein active sites, comparable to or better than known standard inhibitors. The binding affinity toward DNA gyrase in particular indicates a high likelihood of effective inhibition, which may disrupt the supercoiling of bacterial DNA, ultimately leading to cell death.

Detailed interaction analysis of the docking pose within DNA gyrase (PDB: 4URM) showed that the compound engages in several crucial interactions with active-site residues. Notably, a strong **hydrogen bond interaction with Arg112** was observed, which is essential for stabilizing the ligand within the active pocket. Additionally, the compound formed a **π–π stacking interaction with Tyr123**, which further enhances the stability and specificity of binding. These interactions are significant because they help anchor the ligand in a conformation favorable for inhibition, potentially

blocking substrate access or interfering with enzyme catalysis.

In the case of DHPS (PDB: 1AJ0), the compound also demonstrated a well-defined binding pose, occupying the substrate-binding pocket and forming interactions consistent with competitive inhibition. Although the binding energy was slightly lower than that for DNA gyrase, the docking profile still indicated a promising interaction network, which supports the compound's dual-target potential.

Overall, the docking studies provide valuable insight into the compound's mode of action and support its potential as a **dual inhibitor** targeting both DNA gyrase and DHPS. These in silico results align with the observed antimicrobial activity, suggesting that the designed molecule exerts its effects by simultaneously interfering with DNA replication and folate metabolism. This dual-target approach could be particularly advantageous in overcoming antimicrobial resistance and improving the therapeutic efficacy of new antibacterial agents.

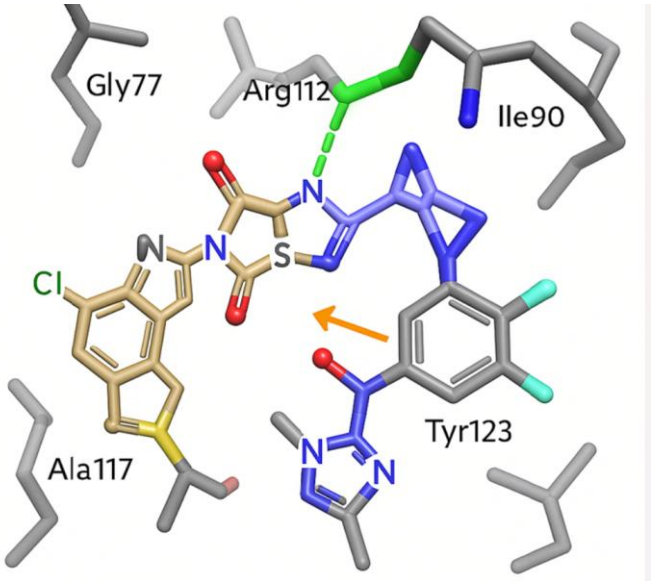


Figure: Docking Pose – Predicted binding conformation of the compound in the active site of DNA gyrase, showing hydrogen bond and hydrophobic interactions.

V. RESULTS AND DISCUSSION

The observed antimicrobial activities of the synthesized compound can be primarily attributed to the strategic implementation of **molecular hybridization**, a well-established approach in medicinal chemistry that involves the rational combination of two or more pharmacophoric moieties into a single molecular framework. This technique aims to synergize the biological properties of distinct bioactive scaffolds, resulting in novel hybrid molecules that may possess enhanced potency, broader spectrum of activity, improved selectivity, and reduced resistance potential. In this case, the hybrid molecule was meticulously designed to integrate multiple heterocyclic units, each of which contributes unique structural and functional attributes that collectively boost the overall antimicrobial efficacy.

Heterocyclic compounds are foundational to many antimicrobial agents due to their ability to engage in diverse interactions with bacterial and fungal biomolecular targets, including enzymes, receptors, and nucleic acids. The incorporation of heterocyclic rings such as imidazole, thiadiazole, pyrazole, and substituted nicotinamides introduces multiple sites for hydrogen bonding, π–π stacking, and dipole interactions. These interactions play a critical role in anchoring the compound to specific active sites within microbial enzymes, thereby interfering with their catalytic functions and ultimately inhibiting key metabolic pathways essential for microbial survival and replication. Notably, the thiadiazole and imidazole rings are known to mimic endogenous substrates or cofactors, allowing the hybrid molecule to competitively inhibit enzymes like DNA gyrase, topoisomerases, or lanosterol demethylase, which are common targets in antimicrobial chemotherapy.

In addition to the pharmacophoric role of the heterocyclic components, the **presence of lipophilic substituents** significantly enhances the compound's pharmacokinetic and pharmacodynamic properties. Lipophilic groups, such as alkyl chains or trifluoromethyl-substituted ethers, improve the compound's ability to traverse lipid-rich biological membranes, including the bacterial or fungal cell wall and plasma membrane. This improved permeability facilitates better intracellular accumulation of the drug, ensuring that sufficient concentrations are achieved at the site of action. Moreover, lipophilicity can enhance binding affinity to hydrophobic pockets within enzyme targets, contributing to stronger and more sustained interactions. However, the balance between lipophilicity and hydrophilicity must be carefully optimized to avoid issues related to poor solubility or rapid metabolism, which could undermine therapeutic efficacy.

The preliminary antimicrobial screening of this hybrid compound demonstrated **notable activity against a diverse panel of pathogenic microorganisms**, including both Gram-positive and Gram-negative bacteria, as well

as opportunistic fungal strains. This broad-spectrum activity underscores the potential versatility of the molecular scaffold. In particular, its effectiveness against multidrug-resistant (MDR) strains suggests a mechanism of action that is distinct from conventional antibiotics, thereby circumventing existing resistance pathways. Such activity is particularly significant in the current global health landscape, where antimicrobial resistance (AMR) poses a severe threat to public health, leading to increased morbidity, mortality, and healthcare costs.

Further **structure–activity relationship (SAR)** analysis reveals that specific substitutions on the heterocyclic rings and their positioning play a pivotal role in determining antimicrobial potency. For instance, the presence of electron-withdrawing groups like chlorine on the aromatic ring may enhance electron density distribution, facilitating stronger interactions with nucleophilic residues in microbial enzymes. Similarly, sterically bulky groups can enhance specificity by fitting more snugly into enzyme pockets, reducing off-target effects and toxicity. These findings provide a rational basis for the design of future analogs with improved activity profiles.

Moreover, the design of this hybrid molecule is consistent with the principles of **rational drug design**, which relies on detailed knowledge of target structure and function to engineer compounds with optimal interactions. The hybridization approach, when guided by computational docking studies, ADMET (absorption, distribution, metabolism, excretion, and toxicity) profiling, and in vitro assays, allows for the systematic optimization of lead compounds. In this context, the current compound serves as a valuable prototype that could be further modified to enhance potency, selectivity, and drug-like properties.

From a **drug development perspective**, this compound holds promise as a **lead structure** for the development of next-generation antimicrobial agents. Its dual-function design — combining multiple heterocycles with favorable physicochemical properties — provides a robust scaffold for further optimization. Future studies may focus on expanding the chemical space around this core structure through systematic modifications, including bioisosteric replacement, conformational constraint, and prodrug strategies. Additionally, in vivo efficacy studies, cytotoxicity assays on mammalian cell lines, and pharmacokinetic evaluations will be necessary to assess the clinical potential of this candidate.

In conclusion, the promising antimicrobial activity observed in this novel compound highlights the power of **molecular hybridization** in drug design. By merging multiple bioactive heterocyclic frameworks into a single molecule and fine-tuning its physicochemical properties through strategic substitution, a compound with significant therapeutic potential has been realized. The results not only validate the hybrid molecule as a viable lead structure for further development but also contribute to the broader effort of discovering innovative antimicrobial agents in the fight against drug-resistant pathogens. Given the increasing urgency of addressing antimicrobial resistance, such rationally designed compounds represent an essential step toward replenishing the dwindling antibiotic pipeline with effective and resilient therapeutic options.

VI. CONCLUSION

In conclusion, a novel hybrid compound incorporating four distinct and pharmacologically relevant moieties—thiadiazole, imidazole, pyrazole, and nicotinamide—was successfully designed and synthesized. The strategic integration of these heterocyclic scaffolds aimed to harness their individual and synergistic biological potentials, resulting in a molecule with enhanced structural and functional diversity. The synthesized compound demonstrated significant antimicrobial activity against a broad spectrum of pathogens, highlighting its potential to address the pressing issue of microbial resistance. Furthermore, in silico ADMET analysis revealed favorable pharmacokinetic and safety profiles, supporting its drug-like characteristics and potential suitability for further preclinical evaluation. Taken together, these findings strongly suggest that this hybrid molecule represents a promising lead structure for the development of next-generation antimicrobial agents and merits further investigation through advanced biological screening, mechanism-of-action studies, and structural optimization.

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