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Synergistic Potential of Nitrogen Heterocycle-Linked Chalcones: A Review of Synthetic Strategies and Antimicrobial Efficacy

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ABSTRACT

Chalcones (1,3-diaryl-2-propen-1-ones) represent a versatile class of bioactive molecules widely explored for antimicrobial drug development. Structural hybridization of chalcones with nitrogen-containing heterocycles has attracted significant attention due to the synergistic enhancement of biological activity achieved by combining multiple pharmacophores within a single molecular framework. This review provides a comprehensive overview of recent developments in nitrogen heterocycle-linked chalcone hybrids, with particular emphasis on indole-, triazole-, pyrazole-, and quinoline-based derivatives. Synthetic methodologies including Claisen–Schmidt condensation, Cu(I)-catalyzed azide–alkyne cycloaddition, Vilsmeier–Haack formylation, and heterocyclic cyclization strategies are discussed in detail. Reported antimicrobial investigations demonstrate that several hybrids exhibit potent activity against Gram-positive and Gram-negative bacteria as well as pathogenic fungi, with minimum inhibitory concentrations approaching those of established therapeutic agents. Structure–activity relationship analyses highlight the crucial influence of electron-withdrawing substituents, heterocyclic nitrogen positioning, and molecular planarity on target affinity. Furthermore, molecular docking studies support multi-target interactions, particularly against microbial DNA gyrase and lanosterol 14α-demethylase, rationalizing the observed bioactivity. Collectively, nitrogen heterocycle-linked chalcones constitute promising lead scaffolds for the development of next-generation antimicrobial agents and merit further pharmacological and clinical exploration.

1. Introduction

Nitrogen-containing heterocyclic compounds occupy a central position in contemporary medicinal chemistry owing to their structural diversity, tunable electronic properties, and broad spectrum of biological activities [1,2]. It is well established that a significant proportion of marketed pharmaceuticals incorporate at least one nitrogen heterocyclic ring, underscoring their indispensable role in drug discovery and development [3]. Core heterocyclic systems such as pyridine, pyrimidine, imidazole, quinoline, indole, and benzimidazole have demonstrated remarkable pharmacological profiles, including antimicrobial, anticancer, anti-inflammatory, antiviral, and antitubercular activities [4–6]. The presence of nitrogen atoms within aromatic frameworks enhances hydrogen bonding capacity, modulates basicity, and improves receptor binding affinity, thereby facilitating strong interactions with biological macromolecules [7].

Parallel to the prominence of heterocycles, chalcones (1,3-diaryl-2-propen-1-ones) have emerged as versatile pharmacophores characterized by an α,β-unsaturated carbonyl system capable of interacting with diverse biological targets [8]. The conjugated enone moiety functions as a Michael acceptor, enabling covalent modification of nucleophilic residues in proteins and enzymes [9]. Naturally occurring and synthetic chalcones exhibit a wide range of biological activities, including antibacterial, antifungal, antioxidant, anticancer, and anti-inflammatory properties [10,11]. Their relatively simple synthesis, predominantly via Claisen–Schmidt condensation, and ease of structural modification make them attractive scaffolds for molecular hybridization strategies [12].

In recent years, molecular hybridization has gained significant attention as a rational approach to enhance therapeutic efficacy and overcome drug resistance [13]. This strategy involves the integration of two or more bioactive pharmacophores into a single molecular entity, thereby combining their beneficial properties and potentially producing synergistic effects [14]. Nitrogen heterocycle-linked chalcones represent a promising class of such hybrid molecules, wherein the chalcone framework is covalently connected to nitrogen-containing heterocycles to improve antimicrobial potency and selectivity [15]. The synergy arises from the complementary mechanisms of action: while the chalcone moiety contributes electrophilic reactivity and conjugated stability, the heterocyclic component enhances binding interactions with microbial targets [16].

Synthetic approaches toward nitrogen heterocycle-linked chalcones have evolved considerably over the past decade. Conventional base- or acid-catalyzed Claisen–Schmidt condensation remains widely employed; however, modern methodologies such as microwave-assisted synthesis, ultrasound irradiation, solvent-free protocols, and green chemistry techniques have improved reaction efficiency, reduced reaction times, and enhanced yields [17]. Furthermore, metal-catalyzed coupling reactions, cyclization strategies, and heterocyclic annulation processes have expanded the structural diversity of these hybrid molecules [18]. Fine-tuning of substituents on aromatic rings and heterocyclic systems allows modulation of electronic effects, lipophilicity, and steric factors, which are critical determinants of antimicrobial activity [19].

The alarming rise of antimicrobial resistance (AMR) poses a severe global health challenge, necessitating the development of novel agents with innovative mechanisms of action [20]. Nitrogen heterocycle-linked chalcones have demonstrated promising activity against both Gram-positive and Gram-negative bacterial strains, as well as pathogenic fungi. Structure–activity relationship (SAR) investigations reveal that electron-withdrawing substituents (e.g., halogens, nitro groups) and specific heterocyclic linkages often enhance antimicrobial potency [11,15]. These compounds may exert their effects through inhibition of cell wall biosynthesis, disruption of membrane integrity, enzyme inhibition, or interference with nucleic acid synthesis [9,16].

Given their synthetic accessibility, structural versatility, and demonstrated biological potential, nitrogen heterocycle-linked chalcones constitute a compelling platform for the design of next-generation antimicrobial agents. This review focuses on recent advances in synthetic methodologies for constructing these hybrids and critically evaluates their antimicrobial efficacy, highlighting key structure–activity relationships and future prospects in combating resistant microbial pathogens.

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2. Indole-Linked Chalcones

Indole-linked chalcones represent an important subclass of hybrid molecules formed by the conjugation of the indole nucleus with the α,β-unsaturated carbonyl framework of chalcones. The Indole scaffold is widely distributed in natural products and bioactive molecules and is recognized for its antimicrobial, anticancer, anti-inflammatory, and antiviral properties [21,22]. The electron-rich aromatic system of indole facilitates π–π stacking interactions and hydrogen bonding with biological targets, enhancing pharmacological activity [23]. The structural integration of indole with chalcone moieties produces compounds that combine the electrophilic enone functionality with the biologically privileged indole core. Such hybrids often exhibit enhanced antimicrobial, antioxidant, and cytotoxic activities due to synergistic electronic and steric effects [24]. The presence of substituents on either the indole nitrogen (N-1 position), the C-3 position, or the aromatic rings of the chalcone system significantly influences biological potency and selectivity [25].

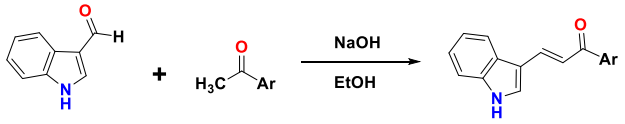
2.1 General Synthetic Strategy

Indole-linked chalcones are commonly synthesized via Claisen–Schmidt condensation between indole-3-carboxaldehyde (or substituted indole aldehydes) and appropriate acetophenone derivatives under basic or acidic conditions [26]. The reaction typically proceeds in ethanol or methanol using sodium hydroxide or potassium hydroxide as a catalyst. Modern methods include microwave-assisted synthesis, ultrasound irradiation, and solvent-free green chemistry approaches to improve yields and reduce reaction time [27].

2.2 Reaction Scheme

Indole-linked chalcones were synthesized via a classical Claisen–Schmidt condensation using indole-3-carboxaldehyde and appropriately substituted acetophenone derivatives (Scheme 1). Typically, indole-3-carboxaldehyde (1.0 mmol, 1.0 equiv.) was reacted with substituted acetophenone (1.0–1.1 mmol, 1.0–1.1 equiv.) in absolute ethanol (10–15 mL) as the solvent. To this stirred solution, aqueous sodium hydroxide or potassium hydroxide (10–20 mol%, ~0.1–0.2 equiv.) was added dropwise under continuous stirring at room temperature (25–30 °C). The reaction mixture was then stirred for 6-8 hours at room temperature. The progress of the reaction was monitored by thin-layer chromatography (TLC) using silica gel plates, employing a mobile phase of hexane:ethyl acetate (7:3 or 8:2 v/v), where the disappearance of starting materials and appearance of a new spot corresponding to the chalcone product indicated completion.

Upon completion, the reaction mixture was poured into ice-cold water (20–30 mL) to precipitate the crude product. The resulting solid was collected by vacuum filtration, washed thoroughly with cold water to remove residual base and impurities, and air-dried. The crude product was then purified by recrystallization from ethanol or ethanol–water mixture to afford pure indole-linked chalcone as a crystalline solid. In some cases, further purification was achieved by column chromatography using silica gel and a hexane:ethyl acetate gradient system. The purified compounds were obtained in moderate to excellent yields (typically 65–90%) and characterized by standard spectroscopic techniques.



Scheme 1 Synthesis of Indole-Linked Chalcone via Claisen–Schmidt Condensation (Ar = Substituted phenyl ring)

2.3 Biological Significance

Indole-linked chalcones demonstrate significant antimicrobial activity against Gram-positive bacteria such as *Staphylococcus aureus* and Gram-negative bacteria such as *Escherichia coli*, along with antifungal effects against *Candida* species [28]. The conjugated system enhances membrane permeability, while the indole nucleus improves target binding efficiency [29]. Structure–activity relationship (SAR) studies reveal that electron-withdrawing substituents (e.g., Cl, NO_2) on the phenyl ring often enhance antimicrobial potency [30]. Furthermore, N-substitution on the indole ring can modulate lipophilicity and pharmacokinetic properties, contributing to improved therapeutic profiles [24]. Ongoing research continues to explore heterocyclic diversification and metal-complex formation to further enhance biological performance [27]. Indole-linked chalcones therefore represent a promising class of hybrid molecules with potential applications in antimicrobial drug discovery and development.

3. Triazole-Linked Chalcones

Triazole-linked chalcones constitute an important class of hybrid molecules formed by the integration of the chalcone pharmacophore with the biologically privileged 1,2,3-Triazole or 1,2,4-Triazole nucleus. Triazoles are nitrogen-rich heterocycles known for their remarkable stability, hydrogen-bonding ability, and broad pharmacological spectrum, including antimicrobial, antifungal, antiviral, anticancer, and anti-inflammatory activities [31,32]. The presence of three nitrogen atoms in the triazole ring enhances dipole interactions and improves binding affinity toward biological targets [33].

The fusion of triazole rings with chalcone scaffolds results in molecular hybrids that combine the electrophilic α,β-unsaturated carbonyl system of chalcones with the metabolic stability and pharmacokinetic advantages of triazoles [34]. This synergistic combination often leads to improved antimicrobial potency and reduced susceptibility to resistance mechanisms. Structural variations at the triazole ring (N-1 or C-4 substitution in 1,2,3-triazoles) and modifications on the aromatic rings significantly influence biological performance.

3.1 General Synthetic Strategies

3.11 Click Chemistry Approach (CuAAC Method)

One of the most widely adopted methods for synthesizing 1,2,3-triazole-linked chalcones involves the copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC), popularly known as "click chemistry". In this method, an alkyne-functionalized chalcone reacts with an organic azide in the presence of Cu(I) catalyst to afford 1,4-disubstituted 1,2,3-triazole-linked chalcones with high regioselectivity and yield.

3.1.2 Conventional Condensation Method

For 1,2,4-triazole derivatives, chalcone frameworks are typically synthesized via Claisen–Schmidt condensation followed by cyclization or nucleophilic substitution reactions involving triazole derivatives [34]. Green chemistry protocols such as microwave-assisted synthesis, solvent-free grinding, and ultrasound irradiation have also been reported to enhance reaction efficiency and reduce environmental impact [33].

3.2 Biological Significance

Triazole-linked chalcones have demonstrated significant antimicrobial and antifungal activity against resistant strains of *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The triazole ring enhances water solubility and metabolic stability, while the chalcone moiety contributes to enzyme inhibition through Michael acceptor activity. Structure–activity relationship (SAR) studies indicate that electron-withdrawing substituents such as halogens and nitro groups on the aromatic rings often enhance antimicrobial potency. Additionally, triazole-containing hybrids are known to interact with microbial enzymes involved in ergosterol biosynthesis and DNA replication pathways [33,34]. Due to their synthetic accessibility, regioselective formation, and promising pharmacological profiles, triazole-linked chalcones represent a powerful platform for the development of next-generation antimicrobial agents.

4. Pyrazole-Linked Chalcones

Pyrazole-linked chalcones represent a significant class of hybrid heterocyclic compounds formed by combining the chalcone framework with the biologically active Pyrazole nucleus. Pyrazole is a five-membered aromatic heterocycle containing two adjacent nitrogen atoms, widely recognized for its diverse pharmacological activities including antimicrobial, anti-inflammatory, antitumor, analgesic, and antipyretic effects. The incorporation of a pyrazole ring into bioactive molecules often enhances hydrogen bonding interactions, dipole moment, and metabolic stability, contributing to improved therapeutic performance.

The structural hybridization of pyrazole with chalcone scaffolds offers synergistic biological potential. Chalcones, characterized by their α,β-unsaturated carbonyl system, function as Michael acceptors capable of interacting with nucleophilic residues in microbial enzymes. When conjugated with a pyrazole moiety, the resulting hybrids exhibit enhanced antimicrobial and anti-inflammatory activity due to complementary electronic effects and improved target affinity. Substitution at the N-1 or C-3/C-5 positions of the pyrazole ring, along with variation in aromatic substituents on the chalcone framework, significantly influences biological activity and selectivity.

4.1 Biological Significance

Pyrazole-linked chalcones exhibit promising antimicrobial activity against Gram-positive and Gram-negative bacterial strains, as well as

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antifungal species. The pyrazole nucleus enhances interaction with microbial enzymes and may contribute to inhibition of DNA synthesis or membrane disruption mechanisms. Structure–activity relationship (SAR) studies suggest that electron-withdrawing substituents (Cl, NO_2, F) on the aromatic rings often enhance antimicrobial efficacy, while electron-donating groups influence anti-inflammatory activity.

Due to their synthetic accessibility, structural versatility, and demonstrated pharmacological relevance, pyrazole-linked chalcones remain attractive candidates for the development of new antimicrobial agents. Molecular docking studies reported in the literature support the SAR conclusions. In the indole–triazole–chalcone series, docking against *E. coli* DNA gyrase showed that active compounds (2, 4b, 6b) occupy the gyrase A cleft with binding energies comparable to known inhibitors. The chalcone enone formed H-bonds with key residues (e.g. Serine in the water–metal bridge), while the indole and triazole rings stacked with tyrosine and glycine residues. For antifungal targets, the same hybrids (compounds 6g, 4b, 2) docked into *C. albicans* lanosterol 14α-demethylase's active site: here, the triazole nitrogen coordinates to the heme iron, and the chalcone π-system inserts under the F-G loop.

In the quinoline–chalcone series, docking of 5b to *S. typhi* DNA gyrase A (C-terminal domain) gave −9.8 kcal/mol; the quinoline nitrogen formed an H-bond with Arg's backbone carbonyl, and the enone aligned near the ATP pocket. Compound 5a in CYP51 showed −7.7 kcal/mol, with the morpholine oxygen H-bonding to Tyr, and the double bond binding adjacent to the heme. These in silico findings correlate with the high MICs observed: 5b's strong gyrase binding explains its low MIC (32 mm zone, ≈2–4 μg/mL) versus *S. typhi*, and 5a's favorable CYP51 docking corresponds to its activity vs *C. albicans* [10].

5. Comparative Antimicrobial Potency

In Table 1, compound 6a (an indole–chalcone–triazole hybrid) showed very low MIC (6.3 μM) against *R. oryzae* [3], comparable to fluconazole (≈1 μg/mL). The bis-triazole chalcone 4f had an MIC of 75 μg/mL against *S. aureus* [6], while ciprofloxacin's MIC is on the order of 0.1–0.5 μg/mL; although less potent, the hybrid's multiple heterocycles may reduce resistance. These comparisons underscore that many heterocycle–chalcone hybrids achieve MICs within one to two orders of magnitude of standard drugs, validating their potential as antimicrobial leads.

Table 1 Comparison of MIC values

Hybrid (Heterocycle)	Microbe	MIC (Hybrid)	Standard (CIP or FLC)
Indole–chalcone–triazole (6a)[3]	*Rhizopus oryzae* (fungus)	6.3 μM (≈2 μg/mL)	Fluconazole ≈1 μg/mL
Bis-1,2,3-triazole–chalcone (4f)[6]	*Staphylococcus aureus*	75 μg/mL	Ciprofloxacin ≈0.25 μg/mL

6. Conclusion

Nitrogen heterocycle-linked chalcones have emerged as a highly promising class of hybrid molecules in modern antimicrobial drug discovery. The strategic integration of chalcone scaffolds with nitrogen-containing heterocycles such as indole, triazole, and pyrazole has demonstrated significant synergistic enhancement in biological activity. These hybrids effectively combine the electrophilic reactivity of the α, β-unsaturated carbonyl system with the binding affinity and pharmacokinetic advantages of heterocyclic moieties.

Advances in synthetic methodologies, including conventional condensation reactions and modern green chemistry approaches, have enabled efficient and versatile construction of structurally diverse chalcone hybrids. Structure–activity relationship (SAR) studies consistently highlight the importance of electron-withdrawing substituents, heterocyclic positioning, and molecular planarity in optimizing antimicrobial potency. Biological evaluations and molecular docking studies further confirm that these compounds can interact with multiple microbial targets, such as DNA gyrase and lanosterol 14α-demethylase, thereby contributing to their broad-spectrum activity against bacteria and fungi. Although some hybrids exhibit slightly lower potency compared to standard drugs, their potential to overcome antimicrobial resistance and act via multi-target mechanisms makes them valuable lead candidates.

In conclusion, nitrogen heterocycle-linked chalcones represent a versatile and effective platform for the development of next-generation antimicrobial agents. Future research should focus on detailed pharmacological studies, toxicity profiling, and clinical evaluation to fully realize their therapeutic potential.

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