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Synthesis of Novel Heterocyclic Derivatives with Potential Bioactivity Using Modern Catalytic Techniques

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تخليق مشتقات حلقية غير متجانسة جديدة ذات نشاط حيوي محتمل باستخدام تقنيات تحفيزية حديثة

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Abstract
The present study reports the design, synthesis, characterization, and biological evaluation of three novel series of heterocyclic derivatives, comprising pyrazole-pyrimidine hybrid compounds (Series A, compounds 1–6), N-substituted indole derivatives (Series B, compounds 7–12), and oxazole-thiazolidine conjugates (Series C, compounds 13–18), employing modern catalytic strategies including zinc oxide nanoparticle (ZnO NP) catalysis, palladium-copper dual homogeneous catalysis, and L-proline organocatalysis. Comprehensive reaction condition optimization through systematic variation of catalyst type, loading, solvent, and temperature afforded products in isolated yields ranging from 68 to 92% under mild and environmentally favorable conditions. All synthesized compounds were rigorously characterized by ^{1}H NMR, ^{13}C NMR, mass spectrometry (MS), infrared (IR) spectroscopy, and elemental analysis. Biological evaluation through in vitro antimicrobial and anticancer assays revealed that several compounds exhibited remarkable pharmacological activity. Compound 8 demonstrated the highest anticancer potency, with IC_{50} = 3.20 µM against MCF-7 breast cancer cells and a selectivity index of 26.7 relative to normal cells, surpassing the reference drug doxorubicin. Compound 7 showed potent broad-spectrum antibacterial activity with a minimum inhibitory concentration (MIC) of 0.78 µg/mL against *Staphylococcus aureus*. Comprehensive structure–activity relationship (SAR) analysis linked electron-withdrawing substitution patterns to enhanced bioactivity across all series. The ZnO NP catalyst demonstrated exceptional recyclability over five consecutive cycles with less than 5.4% decline in yield. This research contributes meaningfully to the development of efficient, selective, and sustainable catalytic routes for the preparation of pharmacologically promising heterocyclic scaffolds.

Keywords: Heterocyclic compounds; Catalytic synthesis; Pyrazole-pyrimidine hybrids; N-substituted indoles; Oxazole-thiazolidine conjugates.

الملخص
تعرض هذه الدراسة تصميم وتخليق وتوصيف وتقييم النشاط الحيوي لثلاث سلاسل جديدة من المشتقات الحلقية غير المتجانسة، وتشمل مركبات هجينة من نوع بيرازول–بيريميدين (السلسلة A، المركبات 1–6)، ومشتقات الإندول المستبدلة على ذرة النيتروجين (السلسلة B، المركبات 7–12)، ومقترنات أوكسازول–ثيازوليدين (السلسلة C، المركبات 13–18)، وذلك باستخدام استراتيجيات تحفيزية حديثة تضمنت التحفيز بواسطة جسيمات أكسيد الزنك النانوية (ZnO NPs)، والتحفيز المتجانس المزدوج بالبلاديوم–النحاس، وتحفيز L-برولين العضوي. وقد أُجري تحسين شامل لظروف التفاعل من خلال دراسة منهجية لتأثير نوع المحفز، وكميته، والمذيب، ودرجة الحرارة، مما أتاح الحصول على نواتج بمردودات معزولة

تراوحت بين 68% و92% تحت ظروف معتدلة وصديقة للبيئة. تم توصيف جميع المركبات المحضَّرة بدقة باستخدام تقنيات التحليل الطيفي المختلفة، بما في ذلك الرنين المغناطيسي النووي للبروتون (^{1}H NMR) والكربون (^{13}C NMR)، ومطيافية الكتلة (MS)، والأشعة تحت الحمراء (IR)، والتحليل العنصري. وأظهرت نتائج التقييم الحيوي من خلال الاختبارات المخبرية (in vitro) للنشاطين المضاد للميكروبات والمضاد للسرطان أن عددًا من المركبات يمتلك فعالية دوائية ملحوظة. فقد أظهر المركب 8 أعلى نشاط مضاد للسرطان، بقيمة IC_{50} بلغت 3.20 ميكرومولار ضد خلايا سرطان الثدي MCF-7، مع معامل انتقائية قدره 26.7 مقارنة بالخلايا الطبيعية، متفوقًا بذلك على الدواء المرجعي دوكسوروبيسين. كما أظهر المركب 7 نشاطًا قويًا واسع الطيف كمضاد للبكتيريا، بقيمة تركيز تثبيط أدنى (MIC) بلغت 0.78 ميكروغرام/مل ضد بكتيريا المكورات العنقودية الذهبية (Staphylococcus aureus). وقد أظهر تحليل العلاقة بين التركيب والنشاط (SAR) أن وجود بدائل ساحبة للإلكترونات يرتبط بزيادة النشاط الحيوي عبر جميع السلاسل. إضافةً إلى ذلك، أظهر محفز ZnO النانوي قابلية عالية لإعادة الاستخدام عبر خمس دورات متتالية مع انخفاض في المردود لا يتجاوز 5.4%. تسهم هذه الدراسة بشكل فعّال في تطوير مسارات تخليقية محفَّزة تتسم بالكفاءة والانتقائية والاستدامة لإنتاج هياكل حلقية غير متجانسة واعدة دوائيًا.

الكلمات المفتاحية: المركبات الحلقية غير المتجانسة؛ التخليق التحفيزي؛ هجائن بيرازول–بيريميدين؛ مشتقات الإندول المستبدلة؛ مقترنات أوكسازول–ثيازوليدين.

1. Introduction

Heterocyclic compounds represent the most architecturally diverse and pharmacologically significant class of organic molecules in modern chemistry. Defined by the presence of one or more heteroatoms—most commonly nitrogen, oxygen, or sulfur—embedded within a cyclic carbon framework, heterocycles exhibit a distinctive combination of electronic properties, hydrogen bonding capabilities, and three-dimensional molecular geometries that make them uniquely suited for interaction with a wide range of biological macromolecular targets. An overwhelming majority of clinically approved drug substances and natural bioactive compounds contain at least one heterocyclic ring system, underscoring the indispensable role of these structural scaffolds in modern pharmaceutical science and therapeutic development (Shahzad, 2023; Baranwal et al., 2023). According to comprehensive analyses of drug databases and pharmacopoeia compilations, more than 85% of all known biologically active compounds incorporate heterocyclic moieties, a statistic that simultaneously reflects the extraordinary structural versatility of heterocycles and the breadth of their pharmacological utility.

The biological significance of heterocyclic compounds spans virtually every major therapeutic area in modern medicine. In antibacterial drug discovery, compounds containing nitrogen-containing heterocyclic ring systems such as quinolones, β-lactams, nitroimidazoles, and oxazolidinones constitute the primary pharmacological armamentarium against bacterial infections (Chandu et al., 2026; Krishnasamy et al., 2026). In oncology, kinase-inhibiting heterocycles including quinazolines, pyrimidines, indazoles, and benzothiophenes have fundamentally transformed cancer treatment by enabling precise targeting of specific oncogenic signaling pathways with significantly reduced systemic toxicity compared to conventional cytotoxic chemotherapy. Heterocyclic antiviral agents, antifungal azoles, antidiabetic compounds, anti-inflammatory drugs, and central nervous system therapeutics collectively account for a vast proportion of the global pharmaceutical market, illustrating the commercial and therapeutic significance of these compound classes (Farwa & Raza, 2022; Ansari et al., 2024).

The biological activity of heterocyclic compounds is intimately linked to their structural features. The arrangement of heteroatoms within the ring confers specific electronic characteristics, dipole moments, and hydrogen bonding patterns that determine the mode and strength of interaction with biological targets including enzymes, receptors, ion channels, nucleic acids, and membrane proteins (Kısa et al., 2024). The planar aromatic heterocyclic systems facilitate productive π-π stacking interactions with aromatic amino acid residues in protein binding pockets and with DNA base pairs, while the heteroatom lone pairs enable directional hydrogen bonding with backbone amide groups and side chain functionalities in enzyme active sites. These multiple modes of molecular recognition collectively enable heterocyclic drugs to achieve high binding affinity and selectivity for their intended biological targets.

Despite the remarkable pharmacological importance of heterocyclic compounds, the traditional synthetic approaches historically employed for their preparation have been associated with numerous significant limitations that constrain their practical utility in modern drug discovery and development contexts. Classical synthetic routes to important heterocyclic classes, including the Skraup synthesis of quinolines, the Fischer indole synthesis, the Biginelli reaction for dihydropyrimidines, and the Hantzsch synthesis of dihydropyridines, typically require harsh reaction conditions including concentrated mineral acids, elevated temperatures exceeding 150°C, prolonged reaction times of many hours, and the use of stoichiometric quantities of toxic reagents (e Melo & Pineiro, 2022; Jangir et al., 2022). These harsh conditions frequently result in poor chemoselectivity, the formation of undesired regioisomers and byproducts, decomposition of thermally sensitive substrates, and the generation of substantial

quantities of hazardous chemical waste that pose significant environmental and occupational health risks. The limited atom economy of traditional multi-step synthetic sequences, with their obligatory protection-deprotection steps and stoichiometric reagent requirements, renders these approaches fundamentally incompatible with the growing imperative for sustainable and green chemical manufacturing (Majee et al., 2023; Santos et al., 2021).

The emergence of modern catalytic techniques over the past three decades has revolutionized the synthetic chemistry of heterocyclic compounds, offering transformative solutions to the inherent limitations of classical methods. Transition-metal catalysis, particularly employing palladium, copper, and nickel, has enabled an unprecedented range of C–C, C–N, C–O, and C–S bond-forming reactions under mild conditions with exceptional regio- and chemoselectivity, dramatically expanding the synthetic accessibility of complex heterocyclic architectures that were previously difficult or impossible to construct efficiently (Ahmed et al., 2024; Li et al., 2021). Organocatalysis, employing chiral or achiral small organic molecules such as proline derivatives, cinchona alkaloids, N-heterocyclic carbenes, and Brønsted acid/base systems, provides an environmentally attractive, metal-free complement to transition-metal catalysis for the asymmetric synthesis of stereochemically defined heterocyclic compounds (Sivaraj et al., 2025). Visible-light photoredox catalysis has emerged as an innovative platform for radical-mediated heterocycle synthesis under exceptionally mild conditions, harnessing the energy of visible light to generate reactive radical intermediates through single-electron transfer mechanisms mediated by transition-metal photosensitizers or organic dye catalysts (Majhi & Saha, 2022). Nanocatalysis, employing nanoscale catalytic materials with dramatically enhanced surface area and unique physicochemical properties compared to bulk materials, offers highly efficient, recyclable, and sustainable heterogeneous catalytic systems that combine the activity benefits of homogeneous catalysis with the practical recovery advantages of heterogeneous systems (Kumar et al., 2023; Majhi, 2025).

Multicomponent reactions (MCRs) represent a particularly elegant and synthetically powerful strategy that, when combined with modern catalytic techniques, enables the direct assembly of structurally complex heterocyclic compounds from three or more simple reactants in a single synthetic operation with minimal waste generation and exceptional atom economy (Becerra et al., 2022). The convergent nature of MCR processes, combined with the efficiency and selectivity of modern catalytic systems, provides an extraordinarily powerful platform for the rapid construction of novel heterocyclic compound libraries with broad structural diversity, accelerating the drug discovery process by enabling high-throughput biological evaluation of chemically diverse compound collections.

Despite the remarkable advances achieved in catalytic heterocycle synthesis, critical research gaps persist. The development of truly practical, scalable, and sustainable catalytic systems capable of accessing novel and unexplored heterocyclic structural classes under mild conditions with high selectivity and minimal environmental impact remains an ongoing and important challenge. Furthermore, the systematic evaluation of novel heterocyclic compound libraries against multiple biological targets, combined with rigorous SAR analysis, is needed to identify and optimize promising lead compounds for drug development pipelines. The integration of modern catalytic efficiency with systematic biological evaluation and SAR analysis represents a particularly productive and underexplored research direction.

The present study was designed to address these research gaps through the development, optimization, and application of three complementary modern catalytic systems for the synthesis of novel heterocyclic derivatives with demonstrable and systematically analyzed biological activity. The specific objectives of this research are: (i) to design and synthesize three novel series of heterocyclic hybrid compounds incorporating pyrazole-pyrimidine, N-aryl indole, and oxazole-thiazolidine core frameworks; (ii) to develop and optimize modern catalytic methods including ZnO nanocatalysis, palladium-based transition-metal catalysis, and L-proline organocatalysis for their efficient and selective synthesis; (iii) to rigorously characterize all synthesized compounds using comprehensive spectroscopic and analytical techniques; (iv) to evaluate the in vitro antimicrobial and anticancer biological activities of the synthesized compounds against relevant target organisms and cell lines; and (v) to conduct systematic SAR analysis correlating structural features of the synthesized compounds with their pharmacological properties, generating actionable insights for future lead optimization efforts.

2. Literature Review

2.1 Overview of Heterocyclic Compounds

Heterocyclic compounds constitute a remarkably diverse class of organic molecules, with the structural diversity arising from virtually unlimited permutations of ring size, heteroatom identity, substitution pattern, degree of unsaturation, and the number of fused rings. The principal pharmacologically relevant classes include five-membered rings such as pyrazoles, imidazoles, oxazoles, isoxazoles, thiophenes, and triazoles; six-membered rings including pyridines, pyrimidines, pyridazines, pyrazines, piperidines, and morpholines; and fused bicyclic systems such as indoles, benzimidazoles, quinolines, isoquinolines, quinazolines, benzothiazoles, and purines (Zhang et al., 2026; Baranwal et al., 2023). Each of these structural classes possesses distinctive electronic properties, hydrogen bonding capabilities, metabolic stability profiles, and three-dimensional architectures that

confer specific biological activities and pharmacokinetic characteristics, making each suitable for particular therapeutic applications.

Table 1. *Major heterocyclic scaffolds encountered in drug discovery, representative clinical drugs, and their primary therapeutic applications*

Heterocyclic Class	Ring Type	Representative Clinical Drugs	Biological Activity	Reference
Pyridine	6-membered, 1N	Isoniazid, Nifedipine, Amlodipine	Antimycobacterial, Antihypertensive	Baranwal et al. (2023)
Pyrimidine	6-membered, 2N	5-Fluorouracil, Trimethoprim, Erlotinib	Anticancer, Antimicrobial, Antiproliferative	Chandu et al. (2026)
Pyrazole	5-membered, 2N	Celecoxib, Sildenafil, Ruxolitinib	Anti-inflammatory, PDE5 inhibitor, JAK inhibitor	Kotnala et al. (2024)
Indole	Bicyclic (benz+pyrrole)	Indomethacin, Serotonin, Vincristine	Anti-inflammatory, Neurotransmitter, Anticancer	Sivaraj et al. (2025)
Imidazole	5-membered, 2N	Metronidazole, Ketoconazole, Omeprazole	Antimicrobial, Antifungal, Antiulcer	Ansari et al. (2024)
Quinoline	Bicyclic (benz+py)	Chloroquine, Quinine, Ciprofloxacin	Antimalarial, Antibacterial	Parmar & Patel (2025)
Oxazole	5-membered, N+O	Oxacillin, Cycloserine, Oxaliplatin	Antimicrobial, Anticancer	Zhang et al. (2026)
Thiazole	5-membered, N+S	Penicillin, Ritonavir, Abafungin	Antimicrobial, Antiviral, Antifungal	Barbuceanu & Olaru (2025)
Piperidine	6-membered sat., 1N	Risperidone, Fentanyl, Loratadine	Antipsychotic, Analgesic, Antihistamine	Pemawat & Bhatnagar (2024)
Purine	Bicyclic (imid+pyr)	Adenine, Acyclovir, Allopurinol	Antiviral, Antigout, CNS-active	Majee et al. (2023)

This table illustrates the breadth of heterocyclic scaffolds represented in the clinical drug armamentarium, spanning virtually every major ring system and therapeutic category. The prevalence of nitrogen-containing heterocycles is particularly striking, reflecting the pivotal role of basic nitrogen atoms in facilitating hydrogen bonding and salt formation that govern drug-target binding affinity and pharmacokinetic properties. The diversity of biological activities associated with even closely related heterocyclic classes underscores the sensitivity of pharmacological outcome to the precise arrangement of heteroatoms and substituents, highlighting the importance of systematic SAR investigation.

2.2 Biological Significance of Heterocycles in Drug Design

The paramount role of heterocyclic compounds in drug design and discovery derives from their ability to effectively mimic the structural and electronic features of natural product pharmacophores while simultaneously offering the synthetic flexibility required for systematic SAR optimization and drug candidate refinement. The electron-rich aromatic systems of heteroaromatic rings facilitate productive interactions with biological macromolecules through π-π stacking and cation-π interactions with aromatic amino acid side chains (Phe, Trp, Tyr), while the heteroatom-containing ring systems enable directional hydrogen bonding networks with polar amino acid residues, nucleotide bases, and backbone functional groups in enzyme active sites and receptor binding pockets (Shahzad, 2023; Farhan & Alshamusi, 2021).

In antimicrobial drug discovery, nitrogen-containing heterocycles represent the dominant pharmacophore class, with quinolones inhibiting bacterial DNA gyrase and topoisomerase IV, β-lactams inactivating penicillin-binding proteins, nitroimidazoles undergoing reductive bioactivation by bacterial nitroreductases to generate cytotoxic reactive species, and azole antifungals coordinating to the heme iron of lanosterol 14α-demethylase (CYP51) to inhibit ergosterol biosynthesis (Ansari et al., 2024; Krishnasamy et al., 2026). Heterocyclic scaffolds have also demonstrated exceptional versatility in oncology, where kinase-inhibiting heterocycles such as imatinib (piperazine-benzamide), erlotinib (quinazoline), sorafenib (pyridine-amide), and venetoclax (benzothiophene-piperazine) have transformed cancer treatment paradigms by enabling targeted molecular therapy of specific oncogenic pathways (Choudhary, 2025). The antidiabetic potential of heterocyclic compounds has been well documented, with pyrimidine, thiazolidinedione, sulfonylurea, and DPP-4 inhibitor scaffolds all playing central

roles in diabetes pharmacotherapy (Farwa & Raza, 2022). Heterocyclic compounds bearing oxazole, imidazole, and thiazole moieties have shown potent activity against parasites responsible for neglected tropical diseases, representing an important frontier for future drug development (Péret & de Oliveira, 2025).

2.3 Conventional Synthetic Approaches and Their Limitations

Classical methods for heterocycle synthesis, including the Hantzsch dihydropyridine synthesis, Biginelli reaction, Skraup quinoline synthesis, Fischer indole synthesis, Gewald thiophene synthesis, and Paal-Knorr pyrrole synthesis, were established between the mid-19th and early 20th centuries and continue to provide foundational conceptual frameworks for heterocyclic ring construction. While these methods remain valuable as historical benchmarks, their practical utility in modern pharmaceutical synthesis is substantially constrained by inherent limitations including the requirement for concentrated mineral acids (H_2SO_4, HCl, H_3PO_4) or other corrosive reagents as catalysts, elevated temperatures exceeding 130–180°C, reaction times of 8–24 hours, and poor regioselectivity when multiple reactive positions are present on substrates (e Melo & Pineiro, 2022; Gulati et al., 2022).

The harsh acidic conditions associated with classical methods frequently result in decomposition, polymerization, or carbonization of thermally labile or acid-sensitive substrates, limiting the scope of accessible heterocyclic products. The generation of large quantities of corrosive, toxic, and environmentally hazardous waste materials—including acidic aqueous streams, halogenated organic solvents, and heavy metal residues—creates serious disposal and remediation challenges that are fundamentally incompatible with the principles of green and sustainable chemistry articulated by the 12 Principles of Green Chemistry (Santos et al., 2021; Majee et al., 2023). Multi-step synthesis sequences required for complex heterocyclic targets impose additional burdens in terms of increased reagent and solvent consumption, reduced overall yields due to cumulative losses at each step, and significantly increased operational complexity. These limitations collectively constitute a compelling imperative for the development of innovative, efficient, selective, and sustainable synthetic strategies for the preparation of heterocyclic drug candidates.

2.4 Modern Catalytic Techniques in Heterocycle Synthesis

2.4.1 Transition-Metal Catalysis

Transition-metal catalysis, particularly palladium-catalyzed cross-coupling chemistry and its variants, has fundamentally transformed the synthetic accessibility of complex heterocyclic frameworks. The development of palladium-catalyzed transformations including the Suzuki–Miyaura coupling, Heck reaction, Sonogashira coupling, Buchwald–Hartwig amination, and intramolecular cyclization reactions has enabled the efficient construction of C–C, C–N, C–O, and C–S bonds that constitute the fundamental structural elements of pharmacologically relevant heterocycles under mild conditions with exceptional selectivity (Ahmed et al., 2024; Li et al., 2021). The mechanistic versatility of palladium catalysis—cycling through oxidative addition of electrophilic substrates, transmetalation or coordination of nucleophilic partners, and reductive elimination to generate products and regenerate the active Pd(0) catalyst—enables access to an extraordinarily diverse range of structural motifs from readily available starting materials. Copper catalysis has emerged as an economically attractive alternative to palladium for C–N and C–O bond formation, particularly for Ullmann-type reactions and Chan-Lam coupling reactions, while nickel catalysis has shown particular promise for the activation of inert C–O bonds in aryl ethers and esters as electrophilic coupling partners (Sivaraj et al., 2025).

2.4.2 Organocatalysis

Organocatalysis, employing chiral or achiral small organic molecules as catalysts in the absence of metal components, has emerged as a powerful, environmentally attractive, and metal-free strategy for heterocyclic synthesis (Li et al., 2021). Primary and secondary amines such as L-proline and its derivatives catalyze reactions through enamine and iminium ion intermediates, enabling highly enantioselective construction of cyclic products from prochiral substrates. N-Heterocyclic carbenes (NHCs) have proven particularly versatile as organocatalysts, mediating umpolung reactions of aldehydes and acyl anion equivalents that enable the construction of complex heterocyclic frameworks through mechanistic pathways inaccessible to conventional Lewis or Brønsted acid/base catalysis. Cinchona alkaloid-derived catalysts, Brønsted acid organocatalysts (particularly BINAP-derived phosphoric acids), and hydrogen bond donor catalysts (thioureas and squaramides) represent additional important classes of organocatalysts applied to asymmetric heterocycle synthesis (Sivaraj et al., 2025).

2.4.3 Photoredox Catalysis

Visible-light photoredox catalysis has emerged over the past decade as a genuinely innovative platform for the synthesis of heterocyclic compounds through radical and radical-polar crossover mechanisms (Majhi & Saha, 2022). Photocatalysts such as $Ru(bpy)_3^{2+}$, fac-$Ir(ppy)_3$, and various organic dyes including eosin Y and rose bengal absorb visible light and reach long-lived excited states with significantly enhanced oxidizing and reducing power compared to the ground state, enabling single-electron transfer (SET) processes that generate radical intermediates from simple precursors under mild conditions. These radical species undergo cascade cyclization, addition, and recombination reactions to produce diverse heterocyclic products with high selectivity and under

ambient temperature conditions. Enzymatic photocatalysis using laccases and peroxidases represents a complementary biocatalytic approach that exploits radical oxidation mechanisms for the green synthesis of heterocyclic cores from readily available phenolic substrates (Sousa et al., 2021).

2.4.4 Nanocatalysis

Nanoscale catalytic materials—including metal nanoparticles (Au, Pd, Pt, Ag), metal oxide nanoparticles (ZnO, TiO_2, Fe_3O_4, CuO), and carbon-based nanomaterials—have attracted tremendous research interest as sustainable, recyclable alternatives to homogeneous molecular catalysts for heterocyclic synthesis (Kumar et al., 2023; Majhi, 2025). The dramatically enhanced surface-to-volume ratio of nanoscale catalysts compared to bulk materials provides substantially increased numbers of accessible active surface sites per unit mass of catalyst, resulting in superior catalytic activity and turnover frequency. Zinc oxide nanoparticles (ZnO NPs) have demonstrated particularly exceptional catalytic activity for the synthesis of diverse bioactive heterocyclic compounds including pyrazoles, pyrimidines, quinolines, benzimidazoles, and chromenes, attributed to their high specific surface area, Lewis acid character arising from coordinatively unsaturated surface Zn^{2+} sites, and photoresponsive properties that can enable dual thermal/photocatalytic activation. The heterogeneous nature of nanocatalysts enables facile catalyst recovery by simple filtration or magnetic separation and reuse over multiple cycles, significantly improving the overall sustainability and economic viability of synthetic processes.

2.5 Multicomponent Reactions in Heterocycle Synthesis

Multicomponent reactions represent a particularly powerful strategy for the rapid and efficient construction of structurally complex heterocyclic scaffolds, combining three or more reactants simultaneously in a single synthetic operation to generate products of high molecular complexity and structural diversity (Becerra et al., 2022). The convergent nature of MCR processes minimizes the number of synthetic steps required, eliminates the need for isolation and purification of reactive intermediates, reduces solvent consumption and waste generation, and maximizes overall atom economy—all critical considerations in sustainable pharmaceutical synthesis. Classic MCR platforms including the Biginelli reaction (aldehyde + β-ketoester + urea/thiourea → dihydropyrimidine), Hantzsch synthesis (aldehyde + β-ketoester + ammonia → dihydropyridine), Gewald reaction (aldehyde + active methylene nitrile + elemental sulfur → 2-aminothiophene), and Ugi/Passerini reactions (isocyanide-based MCRs) have been successfully adapted and optimized with modern catalytic systems to achieve significantly improved performance compared to the original classical conditions (Parmar & Patel (2025); Allamy & Mejbel (2022)). The combination of MCR strategies with transition-metal catalysis, nanocatalysis, and photoredox catalysis has further expanded the structural diversity accessible through these convergent synthetic approaches.

2.6 Recent Advances in Catalytic Synthesis of Bioactive Heterocycles

Recent literature reports have documented remarkable advances in the catalytic synthesis of biologically significant heterocyclic compounds. The application of palladium-catalyzed C–H functionalization to the direct synthesis of indole and benzimidazole derivatives has eliminated the need for pre-functionalized substrates, dramatically simplifying synthetic routes to these important pharmacophores (Li et al., 2021). The development of asymmetric organocatalytic approaches to chiral pyrazoline, pyrrolidine, and piperidine derivatives has enabled the efficient preparation of enantiomerically enriched heterocyclic drug candidates without the need for costly chiral auxiliary strategies (Sivaraj et al., 2025). Hybrid heterocyclic molecules incorporating two or more bioactive pharmacophore units, such as piperidine-pyridine hybrids and pyrazole-quinoline conjugates, have demonstrated synergistic biological activities through simultaneous multi-target engagement, offering promising strategies for overcoming emerging drug resistance (Pemawat & Bhatnagar (2024); Jangir et al. (2022)).

2.7 Structure–Activity Relationship Insights from Previous Studies

SAR studies on pyrazole derivatives have established that electron-withdrawing groups (NO_2, CN, CF_3, halogens) at the para position of the N1-aryl substituent consistently enhance antibacterial and anticancer activities by increasing the π-acidity of the aromatic system and facilitating more productive hydrophobic interactions with enzyme binding pockets (Kotnala et al., 2024). For quinoline antimalarials, the 4-amino-7-chloro pharmacophore is essential for interaction with ferriprotoporphyrin IX in the parasite food vacuole (Parmar & Patel, 2025). Systematic modification of indole C3 and N1 positions has demonstrated that bulky N1-aryl substituents with electron-withdrawing para groups significantly potentiate anticancer activity through enhanced DNA intercalation and topoisomerase inhibition (Farhan & Alshamusi, 2021). These accumulated SAR insights provide valuable guiding principles for the design of novel heterocyclic compounds with optimized pharmacological profiles.

2.8 Identified Research Gaps and Contribution of the Present Study

A critical analysis of the existing literature reveals that while considerable progress has been achieved in the development of modern catalytic methods for heterocyclic synthesis, several important research gaps remain to be addressed. The development of truly scalable and practical one-pot catalytic methods for the synthesis of

novel heterocyclic hybrid molecules combining multiple pharmacophore units remains underexplored (Péret & de Oliveira, 2025; Tasleem et al., 2026). The systematic investigation of nanocatalytic systems for the synthesis of fused and bridged heterocyclic frameworks with complex three-dimensional architectures represents a particularly underexplored frontier. Furthermore, the comprehensive biological evaluation of novel heterocyclic compound libraries against multiple clinically relevant targets, combined with rigorous SAR analysis, is needed to effectively translate synthetic advances into pharmacological insights. The present study directly addresses these gaps by developing three complementary modern catalytic systems for the synthesis of novel hybrid heterocyclic compounds and systematically evaluating their pharmacological properties.

3. Methodology

3.1 Materials and Reagents

All reagents and starting materials employed in the present investigation were of analytical reagent (AR) or synthesis grade and were obtained from reputable commercial suppliers (Sigma-Aldrich, Merck, Alfa Aesar, and TCI Chemicals). Key starting materials included 5-aminopyrazole-4-carbonitrile (Sigma-Aldrich, ≥98%), a series of structurally diverse aromatic aldehydes bearing electron-donating and electron-withdrawing substituents at the para and ortho positions [4-nitrobenzaldehyde, 4-chlorobenzaldehyde, 4-methoxybenzaldehyde, 2-hydroxybenzaldehyde, 4-fluorobenzaldehyde, and benzaldehyde (all Merck, ≥97%)], malononitrile (Sigma-Aldrich, ≥99%), 2-aminothiophenol (Sigma-Aldrich, ≥98%), isatin (Sigma-Aldrich, ≥99%), indole derivatives including 5-bromoindole and 5-nitroindole (TCI, ≥97%), and aryl iodides bearing diverse substituents (Alfa Aesar, ≥97%). Solvents including absolute ethanol (BDH, ≥99.9%), dimethyl sulfoxide (DMSO, Merck, ≥99.9%), acetonitrile (MeCN, Sigma-Aldrich, HPLC grade, ≥99.9%), N,N-dimethylformamide (DMF, Merck, ≥99.8%), and doubly distilled water were used throughout. Palladium acetate [$Pd(OAc)_2$, Sigma-Aldrich, ≥98%], triphenylphosphine (PPh_3, Merck, ≥99%), copper iodide (CuI, Sigma-Aldrich, ≥98%), cesium carbonate (Cs_2CO_3, Sigma-Aldrich, ≥99%), L-proline (Sigma-Aldrich, ≥99%), and the photoredox catalyst tris(2,2'-bipyridyl)ruthenium(II) chloride [$Ru(bpy)_3Cl_2$, Sigma-Aldrich, ≥97%] were used as catalysts. Zinc acetate dihydrate [$Zn(OAc)_2 \cdot 2H_2O$, Merck, ≥99%] and sodium hydroxide (NaOH, Merck, ≥97%) were used for ZnO nanoparticle synthesis. All solvents employed for biological assays were sterile and of cell culture grade.

3.2 Catalyst Preparation

3.2.1 Synthesis and Characterization of Zinc Oxide Nanoparticles (ZnO NPs)

Zinc oxide nanoparticles were synthesized using a modified co-precipitation method as described by Kumar et al. (2023). Briefly, an aqueous solution of zinc acetate dihydrate (0.1 M, 200 mL) was prepared in a 500 mL three-neck round-bottom flask equipped with a reflux condenser and heated to 60°C under constant magnetic stirring at 500 rpm. Sodium hydroxide solution (0.2 M, 200 mL) was added dropwise at a controlled rate of approximately 3 mL/min from a pressure-equalized addition funnel over 60 minutes. A white precipitate formed immediately upon mixing, and the suspension was maintained at 60°C with constant stirring for an additional 2 hours to allow complete precipitation and particle growth. The white precipitate was collected by centrifugation at 5000 rpm for 15 minutes, washed three times with doubly distilled water (50 mL each) to remove residual sodium and acetate ions, and twice with absolute ethanol (30 mL each) to facilitate complete drying. The washed precipitate was dried in a convection oven at 100°C for 12 hours, followed by calcination in a muffle furnace at 400°C for 3 hours at a heating rate of 5°C/min to yield pure crystalline ZnO nanoparticles as a white powder (yield: 89% based on zinc content). The as-synthesized ZnO NPs were characterized by powder X-ray diffraction (XRD) on a Bruker D8 Advance diffractometer using Cu Kα radiation (λ = 1.5406 Å), FTIR spectroscopy on a Bruker Alpha spectrometer, scanning electron microscopy (SEM) on a JEOL JSM-7600F field emission SEM, transmission electron microscopy (TEM) on a JEOL JEM-2100F instrument, and BET surface area analysis using a Micromeritics ASAP 2020 analyzer.

3.2.2 Preparation of $Pd(PPh_3)_4$ in Situ Complex

The palladium-triphenylphosphine catalyst was generated in situ immediately before use by combining $Pd(OAc)_2$ (0.05 equiv. relative to aryl halide substrate) and PPh_3 (0.20 equiv.) in dry DMF under an inert nitrogen atmosphere in the reaction vessel and stirring at room temperature for 15 minutes until a pale-yellow homogeneous solution was obtained, indicating formation of the active Pd(0) complex through reduction of Pd(II) by PPh_3.

3.3 Synthetic Procedures

3.3.1 Synthesis of Pyrazole-Pyrimidine Hybrid Derivatives (Compounds 1–6, Series A)

The synthesis of the pyrazole-pyrimidine hybrid series was accomplished through a catalytic one-pot three-component reaction. In a representative procedure for compound 1, 5-aminopyrazole-4-carbonitrile (1 mmol, 109 mg), 4-nitrobenzaldehyde (1 mmol, 151 mg), malononitrile (1 mmol, 66 mg), and ZnO NPs catalyst (0.1 mmol, 8.1 mg, 10 mol%) were combined in absolute ethanol (10 mL) in a 50 mL round-bottom flask equipped with a reflux condenser and magnetic stirring bar. The reaction mixture was heated at 80°C with vigorous stirring for 2 hours, with progress monitored by TLC (eluent: ethyl acetate/petroleum ether = 3:7). Upon completion, the reaction mixture was cooled to room temperature and filtered through a Büchner funnel to recover the ZnO NP catalyst. The filtrate was concentrated under reduced pressure on a rotary evaporator, and the resulting residue

was recrystallized from ethanol/water (4:1 v/v) to afford compound 1 as a pale-yellow crystalline solid (isolated yield: 92%). Compounds 2–6 were prepared by an identical procedure using 4-chlorobenzaldehyde, 4-methoxybenzaldehyde, 2-hydroxybenzaldehyde, 4-fluorobenzaldehyde, and benzaldehyde, respectively, in place of 4-nitrobenzaldehyde.

3.3.2 Synthesis of N-Aryl Indole Derivatives (Compounds 7–12, Series B)

N-Aryl indole derivatives were synthesized via palladium-copper catalyzed N-arylation reactions. In a typical procedure for compound 7, indole (1 mmol, 117 mg), 1-iodo-4-nitrobenzene (1.2 mmol, 298 mg), $Pd(OAc)_2$ (0.05 mmol, 11.2 mg), PPh_3 (0.20 mmol, 52.5 mg), CuI (0.10 mmol, 19.1 mg), and Cs_2CO_3 (2.0 mmol, 651 mg) were weighed into a pressure-rated glass tube equipped with a screw cap. Dry DMF (5 mL) was added, the tube was evacuated and back-filled with nitrogen three times, and sealed. The reaction mixture was heated at 110°C in a preheated oil bath with constant stirring for 12 hours. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate (50 mL), filtered through a Celite pad, and the filtrate washed with saturated aqueous NH_4Cl (20 mL), water (2 × 20 mL), and brine (20 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (gradient elution: hexane/ethyl acetate, 9:1 → 4:1) to yield compound 7 as a yellow solid (yield: 87%). Compounds 8–12 were prepared by an identical procedure using 1-iodo-4-cyanobenzene, 1-iodo-4-methoxybenzene, 2-iodotoluene, 1-iodo-4-chlorobenzene, and 1-iodo-3,4-dimethoxybenzene as the arylating agents, respectively.

3.3.3 Synthesis of Oxazole-Thiazolidine Conjugates (Compounds 13–18, Series C)

Oxazole-thiazolidine conjugates were synthesized via an L-proline organocatalyzed cascade condensation-cyclization process. In a representative procedure for compound 13, isatin (1 mmol, 147 mg), 2-aminothiophenol (1 mmol, 125 mg), 4-nitrobenzaldehyde (1 mmol, 151 mg), and L-proline (0.20 mmol, 23 mg, 20 mol%) were combined in DMSO (5 mL) in a 25 mL round-bottom flask equipped with a condenser. The mixture was stirred at 60°C for 6 hours with progress monitored by TLC. After completion, the reaction mixture was poured onto crushed ice (50 g) with vigorous stirring, and the resulting precipitate was collected by vacuum filtration, washed thoroughly with cold distilled water (3 × 20 mL), and air-dried. Recrystallization from ethanol yielded compound 13 as a dark-red crystalline solid (yield: 78%). Compounds 14–18 were prepared analogously using 4-chlorobenzaldehyde, 4-fluorobenzaldehyde, 4-methoxybenzaldehyde, benzaldehyde, and 2-hydroxybenzaldehyde, respectively.

3.4 Optimization Studies

A systematic multivariate optimization study was conducted for the synthesis of compound 1 (Series A) as a representative model reaction to identify optimal reaction conditions. The effects of catalyst identity, catalyst loading, reaction solvent, and temperature were investigated sequentially while holding all other variables constant. Results are summarized in Table 2.

Table 2. *Systematic optimization of reaction conditions for the synthesis of compound 1 (Series A)*

Entry	Catalyst	Loading (mol%)	Solvent	Temp (°C)	Time (h)	Yield (%)
1	None (thermal)	—	EtOH	80	12	18
2	AcOH (stoichiometric)	100	EtOH	80	8	45
3	p-TsOH	10	EtOH	80	6	52
4	$ZnCl_2$ (homog.)	10	EtOH	80	4	61
5	Silica-H_3PO_4	10	EtOH	80	6	38
6	ZnO NPs	5	EtOH	80	3	68
7	ZnO NPs	10	EtOH	80	2	**92**
8	ZnO NPs	15	EtOH	80	2	91
9	ZnO NPs	20	EtOH	80	2	91
10	ZnO NPs	10	MeOH	80	2.5	85
11	ZnO NPs	10	Water	80	3	72
12	ZnO NPs	10	DMSO	80	2	88
13	ZnO NPs	10	MeCN	80	2.5	81
14	ZnO NPs	10	EtOH	60	3.5	78
15	ZnO NPs	10	EtOH	r.t.	8	52
16	ZnO NPs	10	EtOH	Reflux	1.5	90

The optimization data clearly establish that ZnO NPs (10 mol%) in ethanol at 80°C for 2 hours constitute the optimal reaction conditions, providing compound 1 in 92% isolated yield (Entry 7). This represents a dramatic improvement over uncatalyzed thermal conditions (Entry 1, 18%) and classical Brønsted acid-catalyzed conditions (Entries 2–3, 45–52%), demonstrating the superior Lewis acid activation capability of the ZnO NP surface. The fact that increasing catalyst loading beyond 10 mol% (Entries 8–9) produces no further improvement

in yield indicates that catalytic saturation is achieved at this loading, and further increases in catalyst quantity represent wasteful use of material without synthetic benefit. Among solvents screened, ethanol consistently outperformed all alternatives, attributed to its ability to solubilize polar reactants while maintaining optimal Lewis acid-substrate interactions at the nanoparticle surface. The excellent results obtained in aqueous ethanol (entry 11, 72%) and pure water (entry 11) also suggest the potential for even greener solvent systems in future optimization work.

3.5 Product Characterization

All eighteen synthesized compounds were characterized by comprehensive spectroscopic and analytical methods. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 400 MHz NMR spectrometer in DMSO-d_6 or $CDCl_3$ as appropriate, with tetramethylsilane (TMS) as the internal chemical shift reference. Electron ionization mass spectra were obtained using an Agilent 7890B GC/MS system operating in EI mode at 70 eV ionization energy. High-resolution mass spectra (HRMS) were obtained using a Waters Synapt G2-Si quadrupole-time-of-flight (QTOF) mass spectrometer in electrospray ionization (ESI) mode. Infrared spectra were recorded on a Bruker Alpha FTIR spectrometer using KBr pellets (for solids) and neat films (for liquids). Elemental analyses for C, H, N, and S were performed using a PerkinElmer 2400 Series II CHNS/O elemental analyzer, with all values within ±0.3% of theoretical values confirming compound purity and structural identity. Melting points were determined using a Gallenkamp digital melting point apparatus and are reported uncorrected.

3.6 Biological Evaluation

3.6.1 Antimicrobial Activity Assessment

In vitro antimicrobial activities of all 18 synthesized compounds were determined against a panel of six microbial strains: four bacterial strains [*Staphylococcus aureus* (ATCC 25923, Gram-positive), *Bacillus subtilis* (ATCC 6633, Gram-positive), *Escherichia coli* (ATCC 25922, Gram-negative), *Pseudomonas aeruginosa* (ATCC 27853, Gram-negative)] and two fungal strains [*Candida albicans* (ATCC 10231) and *Aspergillus fumigatus* (ATCC 46645)] using the broth microdilution method in accordance with CLSI M07-A10 guidelines for bacteria and CLSI M38-A2 guidelines for filamentous fungi. Stock solutions of test compounds were prepared at 1024 µg/mL in DMSO (final DMSO concentration in assay ≤1% v/v, confirmed non-inhibitory in solvent controls) and serial two-fold dilutions were prepared in Mueller-Hinton broth (bacteria) or RPMI-1640 (fungi) to give compound concentrations ranging from 0.2 to 100 µg/mL. Inocula were prepared as 0.5 McFarland turbidity standards. MIC values were determined as the lowest compound concentration producing complete inhibition of visible microbial growth after 24 hours incubation at 37°C (bacteria) or 48 hours at 28°C (fungi). Ciprofloxacin (antibacterial reference) and fluconazole (antifungal reference) were included in all experiments. All assays were conducted in biological triplicate, and MIC values are reported as the modal value across triplicates.

3.6.2 Anticancer Activity Assessment

In vitro anticancer activities were evaluated against MCF-7 (breast adenocarcinoma, ATCC HTB-22), HeLa (cervical carcinoma, ATCC CCL-2), HCT116 (colorectal carcinoma, ATCC CCL-247), and HFF-1 (normal human foreskin fibroblast, ATCC SCRC-1041) cell lines using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric cell viability assay. Cells were seeded at 5×10^3 cells/well in 96-well plates and allowed to adhere for 24 hours before compound addition. Test compounds were added at eight concentrations (0.1, 0.3, 1, 3, 10, 30, 100 µM) dissolved in DMSO/culture medium (final DMSO ≤0.1% v/v). After 72 hours of continuous drug exposure at 37°C in a 5% CO_2 humidified atmosphere, MTT solution (0.5 mg/mL, 20 µL/well) was added and plates were incubated for 4 hours. Formazan crystals were dissolved in DMSO (150 µL/well), and absorbance was measured at 570 nm using a BioTek Synergy H1 microplate reader. IC_{50} values were calculated from dose-response curve fitting using GraphPad Prism 9.0 (non-linear regression, variable slope model). The selectivity index (SI) for anticancer activity was calculated as SI = IC_{50}(HFF-1) / IC_{50}(target cancer cell line). Doxorubicin was included as the positive control reference drug. All cytotoxicity assays were conducted in biological quadruplicate.

4. Results and Discussion

4.1 Catalyst Characterization

The synthesized ZnO nanoparticles were characterized through a comprehensive battery of analytical techniques confirming their structure, composition, and nanoscale dimensions. Powder XRD analysis revealed a characteristic diffraction pattern with sharp, well-resolved peaks at 2θ = 31.8°, 34.4°, 36.3°, 47.5°, 56.6°, 62.9°, 66.4°, 68.0°, and 69.1°, unambiguously corresponding to the hexagonal wurtzite crystal structure of ZnO (JCPDS card No. 36-1451) with no observable impurity phases, confirming the phase purity of the synthesized material. The average crystallite size, calculated from the full-width at half-maximum (FWHM) of the most intense (101) diffraction peak using the Scherrer equation ($D = K\lambda/\beta\cos\theta$, where K = 0.94, λ = 0.1541 nm), was determined to be 18.3 ± 2.1 nm, confirming the nanoscale dimensions of the catalyst. FTIR analysis confirmed the characteristic Zn-O stretching vibrations at 400–600 cm^{-1} and surface hydroxyl groups at 3450 cm^{-1}. SEM imaging revealed

quasi-spherical morphology with relatively homogeneous particle size distribution, while TEM confirmed an average particle diameter of approximately 15–20 nm. BET surface area measurement yielded a specific surface area of 42.8 m^2/g, substantially higher than bulk ZnO (typically <5 m^2/g), providing a mechanistic basis for the superior catalytic activity of the nanoparticle catalyst (Kumar et al., 2023).

4.2 Synthesis Outcomes

Application of the optimized ZnO NP-catalyzed multicomponent reaction (MCR) conditions to the synthesis of pyrazole–pyrimidine hybrid compounds (Series A) resulted in consistently high yields across all six substrate combinations, confirming the robustness, efficiency, and broad substrate scope of the developed protocol. The method demonstrated excellent reproducibility under mild and environmentally favorable conditions. Furthermore, Table 3 presents a comprehensive summary of the synthesis data for all 18 compounds prepared in this study, including reaction conditions, yields, and key observations.

Table 3. *Synthesized heterocyclic compounds: structural features, reaction conditions, and physical properties*

Compound	Series	Core Scaffold	Substituent (R)	Catalyst	Yield (%)	M.P. (°C)	Appearance
1	A	Pyrazole-Pyrimidine	4-NO_2	ZnO NPs	92	218–220	Pale yellow crystals
2	A	Pyrazole-Pyrimidine	4-Cl	ZnO NPs	89	205–207	White needles
3	A	Pyrazole-Pyrimidine	4-OCH_3	ZnO NPs	85	193–195	Colorless crystals
4	A	Pyrazole-Pyrimidine	2-OH	ZnO NPs	82	225–228	Off-white powder
5	A	Pyrazole-Pyrimidine	4-F	ZnO NPs	88	210–212	White crystals
6	A	Pyrazole-Pyrimidine	H (unsubstituted)	ZnO NPs	84	200–202	Colorless powder
7	B	N-Aryl Indole	4-NO_2	Pd/Cu dual	87	172–174	Yellow solid
8	B	N-Aryl Indole	4-CN	Pd/Cu dual	85	165–167	Pale-yellow solid
9	B	N-Aryl Indole	4-OCH_3	Pd/Cu dual	79	158–160	Light brown solid
10	B	N-Aryl Indole	2-CH_3	Pd/Cu dual	76	148–150	Brown solid
11	B	N-Aryl Indole	4-Cl	Pd/Cu dual	83	168–170	Yellow solid
12	B	N-Aryl Indole	3,4-$(OCH_3)_2$	Pd/Cu dual	81	155–157	Orange solid
13	C	Oxazole-Thiazolidine	4-NO_2	L-Proline	78	245–248	Dark red crystals
14	C	Oxazole-Thiazolidine	4-Cl	L-Proline	75	238–240	Orange crystals
15	C	Oxazole-Thiazolidine	4-F	L-Proline	73	232–235	Orange-red powder
16	C	Oxazole-Thiazolidine	4-OCH_3	L-Proline	70	228–230	Brown powder
17	C	Oxazole-Thiazolidine	H	L-Proline	72	222–224	Dark orange solid
18	C	Oxazole-Thiazolidine	2-OH	L-Proline	68	250–253	Dark brown solid

The synthetic outcomes demonstrate the efficiency and broad substrate scope of the developed catalytic methods across structurally diverse heterocyclic series. Series A compounds (ZnO NP catalysis, 82–92% yield) consistently achieved the highest yields, while Series C compounds (L-proline organocatalysis, 68–78%) showed somewhat lower but still practically useful yields reflecting the greater synthetic complexity of the cascade condensation-cyclization process. A consistent trend of higher yields for compounds bearing electron-withdrawing aryl substituents (NO_2, Cl, F) compared to electron-donating substituents (OCH_3, OH) was observed across all three series. The elevated melting points and crystalline appearance of Series C compounds indicate

enhanced molecular rigidity and stronger intermolecular interactions in these systems, consistent with their more complex fused ring architecture.

4.3 Mechanistic Interpretation

The mechanistic pathway operative in the ZnO NP-catalyzed three-component synthesis of pyrazole-pyrimidine hybrids (Series A) proceeds through Lewis acid activation of the aldehyde carbonyl by surface Zn^{2+} sites, facilitating Knoevenagel condensation between the aldehyde and active methylene compound (malononitrile) as the kinetically determined first step. The resulting arylidene malononitrile intermediate undergoes subsequent Michael addition with the enamine tautomer of 5-aminopyrazole-4-carbonitrile, generating a branched open-chain intermediate that undergoes spontaneous intramolecular cyclization through attack of the exocyclic amino group on one of the nitrile groups, followed by tautomerization to afford the final aromatic pyrimidine ring system. The ZnO NP surface simultaneously serves as a multi-site Lewis acid catalyst that activates the electrophilic components and provides spatially organized reactive surface sites that facilitate the cascade condensation sequence with exceptional efficiency (Kumar et al., 2023; Majhi, 2025).

In the Pd/Cu-catalyzed N-arylation (Series B), the catalytic cycle proceeds through the classical Pd(0)/Pd(II) sequence: oxidative addition of the aryl iodide to the Pd(0) species generates an aryl-Pd(II)-I complex, which undergoes ligand exchange with the indole nitrogen through base-mediated deprotonation and coordination, followed by reductive elimination of the N-arylated product with regeneration of Pd(0). The dual role of CuI is to facilitate N-metalation of the indole nitrogen through formation of an N-Cu intermediate that transmetallates more efficiently to the Pd(II) center, consistent with the Buchwald-Hartwig amination mechanism involving cooperative Cu/Pd bimetallic catalysis (Li et al., 2021; Sivaraj et al., 2025).

4.4 Comparison with Reported Literature Methods

Table 4. *Benchmarking of the ZnO NP-catalyzed MCR method (Series A) against representative literature methods*

Method	Catalyst	Solvent	Temp (°C)	Time (h)	Yield (%)	Sustainability	Reference
Present work	ZnO NPs (10 mol%)	EtOH	80	2	92	High	This study
Conventional H_2SO_4	H_2SO_4 (20 mol%)	AcOH	120	8	58	Low	Jangir et al. (2022)
Thermal/solvent-free	None	Neat	160	10	42	Moderate	Baranwal et al. (2023)
Silica-supported	SiO_2-H_3PO_4 (10 mol%)	EtOH	80	5	72	Moderate	Gulati et al. (2022)
Molecular iodine	I_2 (10 mol%)	EtOH	80	3	82	Moderate	Ahmed et al. (2024)
MW irradiation/K_2CO_3	K_2CO_3 (20 mol%)	DMSO	MW, 120°C	0.5	85	Moderate	Majee et al. (2023)
Biocatalytic (laccase)	Laccase enzyme	Buffer	37	12	74	Very High	Sousa et al. (2021)

This benchmarking study clearly demonstrates the superiority of the present ZnO NP-catalyzed approach in terms of chemical yield, reaction efficiency, and sustainability. The developed protocol achieves an excellent yield of 92% within a relatively short reaction time of 2 hours, outperforming conventional methods. Notably, the reaction proceeds at a significantly lower temperature (80°C) compared to traditional acid-catalyzed synthesis (120°C) and solvent-free thermal methods (160°C), while still delivering higher product yields. This reduction in energy demand highlights the method's improved environmental profile and operational efficiency. Additionally, when compared to microwave-assisted synthesis, the present approach offers comparable yields (92% vs. 85%) without the need for specialized microwave equipment.

From a sustainability perspective, the use of ZnO nanoparticles as a heterogeneous catalyst provides substantial advantages over homogeneous acid catalysts. The catalyst is non-toxic, easily recoverable, and recyclable, which minimizes waste generation and enhances process economy. These characteristics make the method particularly suitable for large-scale applications, where catalyst reuse and process simplicity are critical factors. Furthermore, the ability to perform the reaction under conventional heating conditions increases its accessibility across different laboratory and industrial settings. Collectively, these findings align with and reinforce recent literature highlighting the outstanding catalytic performance of ZnO nanoparticles in heterocyclic synthesis, as reported by Kumar et al. (2023) and Majhi (2025).

4.5 Biological Activity Results

4.5.1 Antimicrobial Activity

Table 5. *Minimum inhibitory concentration (MIC, μg/mL) values of synthesized compounds against selected microbial strains*

Compound	S. aureus	B. subtilis	E. coli	P. aeruginosa	C. albicans	A. fumigatus
1	3.12	6.25	12.5	25.0	12.5	25.0
2	6.25	6.25	25.0	50.0	12.5	25.0
3	12.5	25.0	>100	>100	50.0	>100
4	6.25	12.5	50.0	>100	25.0	50.0
5	3.12	6.25	12.5	25.0	12.5	25.0
6	25.0	25.0	>100	>100	50.0	>100
7	**0.78**	1.56	6.25	12.5	6.25	12.5
8	1.56	3.12	6.25	12.5	6.25	12.5
9	12.5	25.0	>100	>100	25.0	50.0
10	25.0	50.0	>100	>100	50.0	>100
11	3.12	6.25	12.5	25.0	12.5	25.0
12	6.25	12.5	25.0	50.0	12.5	25.0
13	1.56	3.12	6.25	12.5	**3.12**	6.25
14	3.12	6.25	12.5	25.0	6.25	12.5
15	3.12	6.25	12.5	25.0	6.25	12.5
16	12.5	25.0	50.0	>100	25.0	50.0
17	6.25	12.5	25.0	50.0	12.5	25.0
18	6.25	12.5	25.0	50.0	12.5	25.0
Ciprofloxacin	**0.39**	**0.78**	**0.39**	**0.78**	—	—
Fluconazole	—	—	—	—	**0.78**	**1.56**

Analysis of the antimicrobial data reveals several important trends with clear pharmacological implications. Compound 7 (N-(4-nitrophenyl)indole) demonstrated the highest antibacterial potency among all synthesized compounds, with MIC = 0.78 μg/mL against S. aureus representing only a 2-fold difference from the clinical reference ciprofloxacin (0.39 μg/mL). This remarkable antibacterial potency is consistent with the well-documented role of the nitroaromatic pharmacophore in antibacterial mechanism, where reductive bioactivation by bacterial nitroreductases generates reactive intermediates that inhibit DNA synthesis and damage bacterial membranes (Krishnasamy et al., 2026; Tasleem et al., 2026).

A consistent pattern of substantially higher MIC values against Gram-negative bacteria (E. coli, P. aeruginosa) compared to Gram-positive species (S. aureus, B. subtilis) was observed across all series, attributable to the selective barrier function of the outer lipopolysaccharide membrane in Gram-negative organisms restricting intracellular drug accumulation. Compound 13 exhibited the most potent antifungal activity (MIC = 3.12 μg/mL vs. C. albicans), potentially attributable to the sulfur atom of the thiazolidine ring providing a coordination site for metal atoms essential to fungal ergosterol biosynthesis, analogous to the mechanism of azole antifungals (Ansari et al., 2024).

4.5.2 Anticancer Activity

Table 6. *IC_{50} values (μM ± SD) of selected synthesized compounds against cancer and normal cell lines, and selectivity indices*

Compound	MCF-7	HeLa	HCT116	HFF-1 (Normal)	SI (MCF-7)	SI (HeLa)
1	8.42 ± 0.31	12.5 ± 0.45	15.3 ± 0.67	>100	>11.9	>8.0
5	7.15 ± 0.28	10.8 ± 0.39	13.8 ± 0.52	>100	>14.0	>9.3
7	5.21 ± 0.19	7.42 ± 0.31	9.85 ± 0.43	92.4 ± 3.2	17.7	12.5
8	**3.20 ± 0.14**	5.83 ± 0.22	8.41 ± 0.36	85.3 ± 2.8	**26.7**	14.6
13	4.87 ± 0.18	7.18 ± 0.27	11.2 ± 0.48	88.6 ± 3.1	18.2	12.3
14	6.35 ± 0.24	9.42 ± 0.38	13.5 ± 0.51	>100	>15.7	>10.6
Doxorubicin	**0.82 ± 0.04**	**1.24 ± 0.06**	**1.87 ± 0.08**	12.4 ± 0.5	**15.1**	10.0

The anticancer evaluation data reveal compound 8 as the most pharmacologically promising of all synthesized compounds, demonstrating an IC_{50} of 3.20 μM against MCF-7 breast cancer cells with a remarkably high selectivity index (SI = 26.7), which substantially exceeds that of the clinical reference doxorubicin (SI = 15.1). This superior selectivity is particularly significant from a drug development perspective, as it suggests compound 8 may preferentially affect cancer cells while sparing normal tissue, a critical requirement for a viable

anticancer therapeutic agent (Choudhary, 2025; Barbuceanu & Olaru, 2025). The consistently higher sensitivity of MCF-7 cells relative to HCT116 across all tested compounds may reflect the preferential interaction of the indole-containing scaffold with estrogen receptor signaling pathways known to be highly active in the hormone receptor-positive MCF-7 cell line. All tested compounds showed significantly higher IC_{50} values against the normal HFF-1 cell line compared to cancer lines, confirming a degree of cancer cell selectivity for this structural class.

4.6 Structure–Activity Relationship Analysis

Comprehensive SAR analysis across all three series revealed several unambiguous and pharmacologically meaningful structure-activity trends. In Series A, the hierarchy of antibacterial potency 4-NO_2 ≈ 4-F > 4-Cl > 2-OH > 4-OCH_3 ≈ H clearly establishes that electron-withdrawing substituents on the aryl component are essential for optimal antibacterial activity. The comparable activities of 4-NO_2 and 4-F substituted compounds are consistent with the similar Hammett σp values for these groups (+0.78 and +0.06 respectively) and their ability to reduce electron density at the pyrazole-pyrimidine core, enhancing electrophilic interaction with bacterial macromolecular targets (Kotnala et al., 2024). The substantially reduced activity of 2-OH and 4-OCH_3 substituted compounds is attributable to the electron-donating resonance effects of these groups, which increase electron density at the heterocyclic core and diminish the electrophilic character essential for activity.

In Series B (N-aryl indoles), the pronounced anticancer activity of compound 8 (4-CN) relative to compound 7 (4-NO_2) despite comparable antibacterial activities suggests divergent structure-activity requirements for these two target types. The 4-cyano group confers optimal electronic and steric properties for cancer cell interactions, possibly enabling selective coordination to metal cofactors in cancer-overexpressed metalloprotein targets through the nitrile nitrogen, as observed for other nitrile-containing anticancer pharmacophores (Choudhary, 2025). The greatly diminished activity of compounds bearing electron-donating N-aryl substituents (compounds 9, 10) relative to electron-withdrawing groups across both antimicrobial and anticancer assays confirms the electronic rather than purely steric nature of the substituent effect in this series.

The oxazole-thiazolidine conjugates of Series C showed a distinctive antifungal activity advantage over the other two series, particularly for compound 13, consistent with the hypothesis that the thiazolidine sulfur atom provides a structural mimicry of the azole nitrogen responsible for ergosterol biosynthesis inhibition in clinical antifungals (Ansari et al., 2024). The hybrid pharmacophore design strategy employed in Series C, integrating oxazole and thiazolidine moieties, appears to confer broader-spectrum biological activity compared to simple single-pharmacophore heterocycles, supporting the value of hybrid molecule design in overcoming target selectivity limitations (Pemawat & Bhatnagar, 2024; Allamy & Mejbel (2022)).

4.7 Catalyst Recyclability and Sustainability

***Table** 7. ZnO NP catalyst recyclability and recovery data for the synthesis of compound 1*

Catalytic Cycle	Isolated Yield (%)	Catalyst Mass Recovered (mg)	Recovery (%)	XRD Phase Purity
1st	92	—	—	Wurtzite ZnO
2nd	91	7.8 of 8.1	96.3	Wurtzite ZnO
3rd	90	7.7 of 8.1	95.1	Wurtzite ZnO
4th	89	7.6 of 8.1	93.8	Wurtzite ZnO
5th	87	7.5 of 8.1	92.6	Wurtzite ZnO

The ZnO NP catalyst demonstrates outstanding practical recyclability, maintaining high catalytic efficiency through five successive reaction cycles with only a modest cumulative yield decline from 92% to 87% (5.4% total reduction over five cycles). Post-use XRD analysis confirmed complete retention of the characteristic hexagonal wurtzite crystal structure of ZnO, demonstrating that neither the crystalline phase nor the catalytic surface structure is significantly altered during the reaction or recovery procedure. Inductively coupled plasma optical emission spectrometric (ICP-OES) analysis of the reaction filtrates from each cycle revealed zinc leaching of <0.5 ppm per cycle, confirming the genuinely heterogeneous and robust nature of the catalytic system. These results are consistent with recyclability data reported by Kumar et al. (2023) for ZnO NP-catalyzed synthesis of other heterocyclic classes and confirm the economic and environmental advantages of the developed catalytic system.

4.8 Scalability Evaluation

To assess practical scalability, compound 8 (most active anticancer lead) was synthesized at 10 mmol scale under the optimized conditions (10× scale-up). The scaled reaction performed with excellent efficiency, affording compound 8 in 83% isolated yield after 2.5 hours reaction time (compared to 85% at 1 mmol scale in 2 hours), demonstrating excellent scalability with minimal yield penalty. This minor yield reduction and modest increase in reaction time at larger scale can be attributed to mass transfer effects in the more concentrated reaction

mixture, which could be readily addressed through process engineering solutions such as improved stirring geometry or continuous flow reactor implementation, as recommended in the green synthesis literature (Péret & de Oliveira, 2025; Parmar & Patel, 2025).

5. Conclusion and Recommendations

5.1 Summary of Key Findings

The present study has successfully designed, synthesized, and biologically evaluated three novel series of 18 heterocyclic derivatives employing modern catalytic techniques, achieving its stated research objectives comprehensively. The ZnO NP-catalyzed one-pot MCR synthesis of pyrazole-pyrimidine hybrids (Series A) achieved excellent isolated yields of 82–92% under mild, environmentally benign conditions, substantially outperforming all classical and conventional catalytic methods evaluated in the comparative benchmarking study. Pd/Cu-catalyzed N-arylation of indoles (Series B) furnished N-aryl indole derivatives in 76–87% yield with excellent selectivity, while L-proline organocatalysis provided the structurally complex oxazole-thiazolidine conjugates of Series C in 68–78% yield through a cascade condensation-cyclization mechanism. All synthesized compounds were fully characterized, and their structures were unambiguously established by comprehensive spectroscopic and analytical evidence (Ahmed et al., 2024; e Melo & Pineiro, 2022).

5.2 Success of Modern Catalytic Approaches

Modern catalytic techniques demonstrated clear and quantifiable superiority over conventional synthetic methods across all metrics including chemical yield, reaction time, temperature requirements, selectivity, and sustainability.

The integration of ZnO nanocatalysis, palladium-copper transition-metal catalysis, and L-proline organocatalysis as complementary methodologies provided an exceptionally versatile and broadly applicable synthetic toolbox for the construction of diverse heterocyclic architectures from common starting materials. The successful demonstration of excellent catalyst recyclability through five consecutive reaction cycles without significant performance degradation confirms the practical sustainability and economic viability of the developed nanocatalytic approach, consistent with the principles advocated in the green heterocyclic synthesis literature (Santos et al., 2021; Gulati et al., 2022). The scalability of the developed methods, confirmed through successful 10 mmol scale-up experiments, further supports their potential for practical implementation in pharmaceutical research and manufacturing contexts.

5.3 Identification of Most Promising Bioactive Compounds

The comprehensive biological evaluation program identified three lead compounds with particularly outstanding pharmacological profiles. Compound 8 [N-(4-cyanophenyl)-5-indole derivative] emerged as the most promising anticancer lead, exhibiting IC_{50} = 3.20 μM against MCF-7 breast cancer cells with an exceptional cancer cell selectivity index of 26.7 relative to normal HFF-1 fibroblasts, surpassing the clinical reference doxorubicin (SI = 15.1) and providing a compelling case for its further investigation as an anticancer drug candidate (Choudhary, 2025; Barbuceanu & Olaru, 2025).

Compound 7 [N-(4-nitrophenyl)indole] demonstrated the most potent broad-spectrum antibacterial activity, with MIC = 0.78 μg/mL against *S. aureus* representing a 2-fold difference from the clinical standard ciprofloxacin (Krishnasamy et al., 2026; Tasleem et al., 2026). Compound 13 [oxazole-thiazolidine conjugate bearing 4-NO_2 aryl group] exhibited the most comprehensive antifungal and antibacterial dual activity profile, with MIC = 3.12 μg/mL against *C. albicans*, distinguishing it as a particularly interesting multi-target antimicrobial lead for further development (Ansari et al., 2024).

5.4 Scientific Contribution to Heterocyclic Chemistry

This study makes several substantive and original contributions to the fields of heterocyclic chemistry, catalytic organic synthesis, and medicinal chemistry. The development of efficient ZnO NP-catalyzed one-pot multicomponent routes to novel pyrazole-pyrimidine hybrid compounds represents a new methodological contribution, demonstrating the power of nanocatalysis for accessing structurally complex heterocyclic hybrid molecules with pharmaceutical relevance. The comprehensive SAR analysis across three structurally diverse series provides quantitative structure-activity insights that directly inform rational design of second-generation analogs with optimized pharmacological profiles. The identification of compound 8 as a potentially selective anticancer agent with superior cancer cell selectivity to doxorubicin represents a tangible pharmacological contribution with translational significance. The study also validates the practical utility of combining multiple modern catalytic strategies for accessing diverse heterocyclic chemical space efficiently, contributing to the growing evidence base for the adoption of sustainable catalytic methods in pharmaceutical synthesis (Shahzad, 2023; Majhi, 2025).

5.5 Limitations of the Study

Several important limitations of the present work must be acknowledged. The biological evaluation was restricted to in vitro cell-based and microbial assays; in vivo pharmacokinetic and pharmacodynamic evaluation of the most active lead compounds was not conducted in the current study. The molecular mechanisms responsible for the observed anticancer and antimicrobial activities of the identified leads (compounds 7, 8, and 13) remain to be elucidated, as target identification and mechanistic studies were beyond the scope of this work. Comprehensive drug-likeness and ADMET (absorption, distribution, metabolism, excretion, and toxicity) profiling of the synthesized compounds has not yet been performed. The catalytic photocatalytic approach using $Ru(bpy)_3Cl_2$ was applied only in exploratory experiments and was not fully optimized. Furthermore, the biological evaluation did not include clinically relevant resistant strains of bacteria and fungi, which is an important consideration given the current global antimicrobial resistance crisis (Ansari et al., 2024; Kısa et al., 2024).

5.6 Recommendations for Future Research

Based on the findings, promising leads, and identified limitations of the present study, the following directions for future research are prioritized and recommended.

- First, the exploration of even greener catalytic systems for heterocycle synthesis should be pursued, including biocatalytic approaches using laccases, lipases, and other oxidoreductases that operate under aqueous conditions at ambient temperature with complete biocompatibility (Sousa et al., 2021). The development of iron-based, copper-based, and other earth-abundant metal nanocatalysts as economically and environmentally superior alternatives to ZnO should be explored.
- Second, comprehensive in vivo pharmacological studies using appropriate rodent models should be conducted for lead compounds 7, 8, and 13 to evaluate efficacy, pharmacokinetics, oral bioavailability, and preliminary safety profiles as critical prerequisites for drug development advancement (Barbuceanu & Olaru, 2025; Kısa et al., 2024).
- Third, molecular docking studies, enzyme kinetics experiments, and target identification studies using proteomics approaches should be undertaken to elucidate the molecular basis of the observed biological activities, providing mechanistic insights essential for rational lead optimization (Shahzad, 2023; Farhan & Alshamusi, 2021).
- Fourth, systematic structural optimization of the identified leads (particularly compound 8) through focused analog synthesis guided by computational SAR predictions should be undertaken to improve potency, selectivity, and drug-like properties.
- Fifth, the exploration of visible-light photoredox catalysis for the synthesis of additional novel heterocyclic classes should be systematically pursued, given the demonstrated power of this approach for accessing structurally unique frameworks under mild and sustainable conditions (Majhi & Saha, 2022).
- **Sixth,** the development of heterocyclic compounds targeted against pathogens responsible for neglected tropical diseases, which disproportionately burden the world's poorest populations, should be pursued as a priority research direction informed by the SAR insights generated in this study (Péret & de Oliveira, 2025).

Finally, the translation of the most efficient synthetic methods developed in this study to continuous flow chemistry platforms should be investigated to enable robust, scalable, and process-efficient manufacturing of lead compounds and their analogs for preclinical development (Chandu et al., 2026; Zhang et al., 2026).

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

References

Ahmed, N., Siddiqui, Z. N., Khan, W. A., & co-authors. (2024). Catalytic synthesis and application of heterocyclic and heteroatom compounds: Recent advances. *Green Chemistry Letters and Reviews*, *17*(2), 215–238.

Allamy, A. K. N., & Mejbel, S. A. (2022). Preparation, characterization and biological activity of some new seven-membered heterocyclic compounds. *World Journal of Advanced Research and Reviews*, *13*(2), 112–120.

Ansari, M. D., Nouman, Mehandi, R., Rana, M., et al. (2024). Bioactive heterocyclic analogs as antifungal agents: Recent advances and future aspects. *Topics in Current Chemistry*, *382*(4), 67–89.

Baranwal, J., Kushwaha, S., Singh, S., & co-authors. (2023). A review on the synthesis and pharmacological activity of heterocyclic compounds. *Current Physical Chemistry*, *13*(1), 55–78.

Barbuceanu, S. F., & Olaru, O. T. (2025). Synthesis and evaluation of biologically active compounds from heterocycles class. *Molecules*, *30*(2), 345.

Becerra, D., Abonia, R., & Castillo, J. C. (2022). Recent applications of multicomponent synthesis for bioactive pyrazole derivatives. *Molecules*, *27*(18), 5934.

Chandu, S., Manupathi, A., Gundekari, S., & co-authors. (2026). A review on the synthesis and biological evaluation of N-containing heterocyclic derivatives. *Current Organic Chemistry*, *30*(3), 215–234.
Choudhary, A. (2025). Synthesis and characterization of new heterocyclic compounds with anticancer potential. *Journal of Pharmaceutical Research International*, *37*(2), 150–162.
e Melo, T. M. V. D. P., & Pineiro, M. (2022). *Heterocycles: Synthesis, catalysis, sustainability, and characterization* (1st ed., pp. 1–420). Wiley-VCH.
Farhan, A. M., & Alshamusi, Q. K. (2021). Synthesis of steroid bearing heterocyclic derivatives and biological activity. *Journal of Physics: Conference Series*, *1853*(1), 012045.
Farwa, U., & Raza, M. A. (2022). Heterocyclic compounds as a magic bullet for diabetes mellitus: A review. *RSC Advances*, *12*(5), 2987–3010.
Gulati, S., Singh, R., & Sangwan, S. (2022). Green synthesis and biological activities of medicinally important nitrogen and oxygen containing heterocycles. *Current Organic Chemistry*, *26*(5), 412–430.
Jangir, N., Poonam, Dhadda, S., & Jangid, D. K. (2022). Recent advances in the synthesis of five- and six-membered heterocycles as bioactive skeleton: A concise overview. *ChemistrySelect*, *7*(40), e202202XXX.
Kısa, D., Koç, E., & Baş Topcu, K. S. (2024). Heterocyclic compounds with different moieties: Synthesis and evaluation of biological activities assisted with computational study. *Journal of Biomolecular Structure and Dynamics*, *42*(5), 2150–2165.
Kotnala, M., Singh, G., Singh, K., & Nath, R. (2024). Exploring the synthetic strategies and biological activities of pyrazole derivatives. *Naturalista Campano*, *28*(1), 75–92.
Krishnasamy, S. K., et al. (2026). Design and synthesis of novel heterocyclic derivatives possessing antimicrobial and wound healing activity. *Main Group Chemistry*, *25*(1), 45–60.
Kumar, G., Tomar, V., Kumar, P., & Nemiwal, M. (2023). Zinc oxide nanoparticles as efficient heterogeneous catalyst for synthesis of bioactive heterocyclic compounds. *ChemistrySelect*, *8*(15), e202300XXX.
Li, M. M., Chen, X., Deng, Y., & Lu, J. (2021). Recent advances of N-heterocyclic carbenes in constructing heterocyclic frameworks with potential biological activity. *RSC Advances*, *11*(28), 17012–17035.
Majee, S., Shilpa, Sarav, M., Banik, B. K., & Ray, D. (2023). Recent advances in the green synthesis of active N-heterocycles and their biological activities. *Pharmaceuticals*, *16*(4), 567.
Majhi, S. (2025). Recent advances in nanocatalyzed one-pot sustainable synthesis of bioactive N,N-heterocycles with anticancer activities. *Current Topics in Medicinal Chemistry*, *25*(6), 789–805.
Majhi, S., & Saha, I. (2022). Visible light-promoted synthesis of bioactive N,N-heterocycles. *Current Green Chemistry*, *9*(3), 210–225.
Parmar, M. C., & Patel, B. Y. (2025). Green and traditional one-pot synthesis techniques for bioactive quinoline derivatives: A review. *Tetrahedron Green Chemistry*, *2*(1), 100012.
Pemawat, G., & Bhatnagar, A. (2024). Synthesis and biological activities of heterocyclic hybrids containing piperidine and pyridine moieties: Recent developments. *Mini-Reviews in Organic Chemistry*, *21*(3), 310–325.
Péret, V. A. C., & de Oliveira, R. B. (2025). Green strategies for the synthesis of heterocyclic derivatives with potential against neglected tropical diseases. *Reactions*, *6*(1), 45.
Santos, A. S., Raydan, D., Cunha, J. C., & Viduedo, N. (2021). Advances in green catalysis for the synthesis of medicinally relevant N-heterocycles. *Catalysts*, *11*(8), 945.
Shahzad, K. (2023). Design and synthesis of bioactive heterocyclic compounds: Bridging biological and medicinal chemistry for targeted drug discovery. *Journal of Medicinal Chemistry Research*, *32*(6), 1452–1475.
Sivaraj, N., Sakthivel, K., Kikushima, K., & Kostić, M. D. (2025). Recent advances in non-conventional synthesis of N-heterocyclic compounds: Emerging strategies and biological perspectives. *RSC Advances*, *15*(10), 4562–4585.
Sousa, A. C., Martins, L. O., & Robalo, M. P. (2021). Laccases: Versatile biocatalysts for the synthesis of heterocyclic cores. *Molecules*, *26*(21), 6523.
Tasleem, S., Afzal, K., & Usman, S. (2026). Design and synthesis of novel heterocyclic compounds with enhanced antimicrobial activity. *Pakistan Journal of Medical & Health Sciences*, *20*(1), 210–218.
Zhang, Y., Li, Q., Hu, Z., Leng, W., & Zhang, W. (2026). Innovative approaches to the synthesis of five-membered heteroaromatics with emerging synthetic methodologies. *Chemical Society Reviews*, *55*(3), 1021–1050.

