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4-(2-Phenylhydrazinyl)-7H-pyrrolo[2,3-d]pyrimidine Analogues: A Comprehensive Review

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Abstract

Pyrrolo[2,3-d]pyrimidine derivatives represent a versatile and extensively studied class of fused heterocyclic compounds, known for their diverse biological, medicinal, and physicochemical properties. Owing to their structural similarity to purine frameworks, these molecules exhibit excellent bioisosteric compatibility with nucleic acid bases and enzyme cofactors, enabling their involvement in various molecular recognition processes. Over the past decade, the introduction of hydrazinyl, aryl, alkyl, and heteroatom-containing substituents has significantly expanded their chemical diversity, resulting in analogues with enhanced potency, selectivity, and improved drug-like characteristics.

Among these, 4-(2-phenylhydrazinyl)-7H-pyrrolo[2,3-d]pyrimidine derivatives have emerged as a particularly significant subgroup. Their importance stems from structural flexibility, ease of functionalization, and the ability to form multiple non-covalent interactions, including hydrogen bonding, π–π stacking, and metal coordination. These properties contribute to strong binding affinities with biological targets, positioning them as promising lead compounds in therapeutic areas such as oncology, infectious diseases, inflammatory disorders, and enzyme inhibition.

Advancements in modern synthetic methodologies have further accelerated research in this field. Techniques such as cyclization, cross-coupling, annulation, metal-catalyzed C–N and C–C bond formation, and multicomponent reactions now allow efficient and scalable synthesis of substituted pyrrolo[2,3-d]pyrimidine cores. These strategies not only improve yields and reaction efficiency but also enable precise modulation of steric, electronic, and lipophilic properties, facilitating the design of biologically active hydrazinyl derivatives. Simultaneously, progress in analytical and computational approaches has deepened the understanding of these compounds. Spectroscopic techniques, including NMR, HRMS, and FTIR, along with crystallographic studies, provide detailed structural insights. Computational studies and structure–activity relationship (SAR) analyses have revealed that substitutions on the phenyl ring, variations in hydrazone linkages, and the introduction of electron-donating or electron-withdrawing groups significantly influence biological activity, bioavailability, and pharmacokinetics.

This review presents a comprehensive overview of 4-(2-phenylhydrazinyl)-7H-pyrrolo[2,3-d]pyrimidine derivatives, focusing on their synthesis, structural features, and biological applications. Emphasis is placed on SAR trends associated with antibacterial, anticancer, antiviral, anti-inflammatory, and enzyme-inhibitory activities, supported by recent literature. Furthermore, mechanistic insights, computational predictions, and structure-based design strategies are discussed to highlight their role in the development of high-affinity ligands. Finally, existing challenges, research gaps, and future prospects are outlined, emphasizing the potential of these compounds as promising scaffolds for next-generation drug discovery and medicinal chemistry research.

Keywords: 4-(2-phenylhydrazinyl)-7H-pyrrolo[2,3-d]pyrimidine, 2-Phenylhydrazinyl, Anticancer Activity, structure–activity relationships , Modern synthetic route

1. Introduction

Heterocyclic compounds containing fused nitrogen-rich systems have always remained central to medicinal chemistry due to their broad interactions with biological macromolecules. These privileged scaffolds often participate in diverse biochemical processes because their multiple nitrogen atoms enable them to act as hydrogen-bond donors and acceptors, and as electron-rich aromatic units capable of engaging in π-stacking and dipolar interactions with proteins, nucleic acids, and enzymatic cofactors. Within this category, frameworks composed of annulated pyridine, pyrazine, and imidazole analogues have been intensively investigated; however, nitrogen-dense fused systems such as pyrrolo-pyrimidines continue to stand out owing to their structural resemblance to naturally occurring nucleobases. The pyrrolo[2,3-d]pyrimidine framework, structurally related to purine bases, has shown high affinity toward enzymes involved in nucleic-acid processing, kinase regulation, and folate metabolism [1–3]. This purine-mimicking behavior allows the scaffold to interact with ATP-binding pockets, nucleoside-recognition domains, and allosteric regulatory sites with notable selectivity. Over the past decade, advancements in synthetic methodologies and structure-based design have further highlighted the value of this heterocycle, positioning it among the most promising fused systems in modern medicinal chemistry.

Correspondingly, 4-(2-phenylhydrazinyl)-7H-pyrrolo[2,3-d]pyrimidine derivatives have emerged as potent and strategically modifiable scaffolds, largely due to the synergistic contributions of the pyrrolopyrimidine core and phenylhydrazinyl substituent, offering rich possibilities for hydrogen bonding, π–π stacking, and fine-tuning of physicochemical properties [4]. The phenylhydrazinyl moiety provides an additional flexible pharmacophoric element, enabling conformational adaptability and stronger intermolecular interactions with charged or hydrophobic residues inside enzyme cavities. The presence of the hydrazine nitrogen atoms further broadens the scope for derivatization through acylation, condensation, Schiff-base formation, and metal-complexation reactions, allowing medicinal chemists to rapidly expand the structural diversity of this class. Such structural versatility makes these molecules ideal templates for targeting multi-domain proteins, facilitating dual-site binding, and enhancing both potency and selectivity.

These compounds have been explored for anticancer, antibacterial, antifungal, anti-inflammatory, and antioxidant activities, establishing them as valuable candidates for further therapeutic optimization [5–7]. Their anticancer potential has been supported by studies demonstrating inhibition of kinases, folate-dependent pathways, and cell-cycle regulatory enzymes, while antibacterial and antifungal evaluations have revealed promising activity against resistant microbial strains. Moreover, the inherent redox properties of the hydrazinyl group contribute to notable antioxidant effects, further broadening their pharmacological spectrum. The combined evidence from these biological domains underscores the importance of 4-(2-phenylhydrazinyl)-substituted pyrrolo[2,3-d]pyrimidines as adaptable, multi-target agents with significant potential in next-generation drug-discovery programs.

2. Structural Features and Chemical Significance

2.1.The Pyrrolo[2,3-d]pyrimidine Core

The rigid bicyclic architecture of pyrrolo[2,3-d]pyrimidine results from angular fusion of pyrrole and pyrimidine rings, creating an electron-rich system capable of interacting strongly with ATP-binding domains and DNA-interactive proteins [8]. The nitrogen atoms of the pyrimidine ring provide sites for protonation and tautomerism, influencing binding affinity and target selectivity.

2.2. Role of 2-Phenylhydrazinyl Substitution

The C-4 substitution with a 2-phenylhydrazine moiety contributes significantly to biological activity by introducing several key structural and electronic advantages. First, the presence of the –NH–NH– functionality provides an additional pair of hydrogen-bond donors and acceptors, enabling stronger and more versatile interactions with receptor binding pockets, enzyme active sites, and nucleic-acid grooves. This dual hydrogen-bonding capability often enhances molecular anchoring and recognition, thereby improving target specificity and reducing off-target interactions. Second, the appended phenyl ring enhances π-stacking interactions, allowing the molecule to engage more effectively with aromatic residues such as Phe, Tyr, Trp, and nucleotide bases. Such stabilizing interactions frequently contribute to higher binding affinities, especially within hydrophobic or planar biological environments.

Furthermore, the incorporation of this hydrazinyl-phenyl system has been shown to improve membrane permeability by increasing the overall lipophilic surface area while preserving a balanced polar profile that facilitates passive and carrier-mediated transport. This balance is critical for achieving optimal cellular uptake and favorable pharmacokinetic behavior. In addition, the hydrazine unit provides multiple synthetically accessible sites, enabling facile derivatization at both nitrogen atoms and at the aromatic positions of the phenyl ring. This chemical flexibility allows for the fine-tuning of steric, electronic, and lipophilic parameters, enabling rapid generation of analogues with optimized biochemical properties.

Collectively, these features enhance biological performance, strengthen receptor binding affinity, and contribute positively to metabolic stability and overall pharmacokinetics [9,10].

3. Synthetic Approaches

3.1. Classical Synthetic Route

The most common method involves nucleophilic substitution of 4-chloro-7H-pyrrolo[2,3-d]pyrimidine with 2-phenylhydrazine in refluxing polar solvents such as ethanol, DMF, or n-butanol. This route is efficient, providing moderate to excellent yields with minimal purification requirements [11].

3.2. Alternative Cyclization Strategies

Cyclization of β-ketoesters, acylated amino esters, or substituted aminopyrimidines can yield the pyrrolopyrimidine core, followed by hydrazinylation at C-4. Such approaches allow multi-point substitution and structural variation, essential for SAR studies [12,13].

3.3. Modern Synthetic Trends

Recent advancements emphasize several innovative synthetic strategies that have significantly improved the efficiency, sustainability, and structural diversity of pyrrolo[2,3-d]pyrimidine analogues. Microwave-assisted synthesis has received substantial attention for its ability to drastically reduce reaction times while simultaneously boosting product yields and purity, largely due to uniform heating, enhanced molecular collisions, and minimized side-product formation [14]. This technique has proven especially valuable for multi-step heterocyclic transformations, where conventional heating often results in prolonged reaction periods and lower efficiency.

In parallel, solvent-free and green-chemistry protocols utilizing ionic liquids or deep eutectic solvents have emerged as environmentally responsible alternatives to traditional organic solvents [15]. These media provide unique physicochemical properties such as high polarity, tunable viscosity, and recyclability, which collectively promote cleaner reactions, improved atom economy, and lower environmental impact. Their ability to dissolve diverse substrates and stabilize reactive intermediates has made them particularly suitable for hydrazine incorporation, cyclization reactions, and heteroatom-functionalization steps required for generating phenylhydrazinyl derivatives.

Another major development is the application of palladium-catalyzed C–N cross-coupling reactions, such as the Buchwald–Hartwig amination, which enables efficient late-stage functionalization of the pyrrolopyrimidine core [16]. This transformation allows for precise installation of amine-containing substituents under mild conditions, opening avenues for rapid analogue diversification and structure–activity relationship (SAR) exploration. The mildness and compatibility of these catalytic systems with various functional groups further enhance their utility in medicinal chemistry workflows. Collectively, these modern synthetic approaches align with sustainable chemistry principles while offering substantial versatility in analogue generation.

4. Biological Activities

4.1. Anticancer Activity

Numerous pyrrolo[2,3-d]pyrimidines show potent anticancer properties, especially as kinase inhibitors and antifolate agents [17,18]. Their structural similarity to purine nucleotides allows them to effectively compete with endogenous substrates and bind to regulatory enzymatic pockets with high precision. This purine-mimicking behavior, combined with the rigid fused-heterocycle geometry, enables these molecules to modulate a wide range of signaling pathways involved in uncontrolled cell proliferation, angiogenesis, and survival. Within this broad class, hydrazinyl-substituted analogues have attracted particular attention due to their favorable interaction profiles and superior binding adaptability. The hydrazinyl-substituted analogues demonstrate strong affinity toward ATP-binding pockets, enabling them to interfere with phosphorylation cascades central to cancer pathogenesis. They also exhibit marked inhibition of EGFR, JAK, and CDK-family kinases, which play essential roles in tumor growth, inflammation-driven malignancy, and cell-cycle progression. In addition to kinase inhibition, the extended planar π systems present in these molecules promote DNA intercalation, allowing them to disrupt replication and transcription processes at the genomic level.

Studies reveal activity against breast, lung, and leukemia cancer cell lines, underscoring their broad therapeutic potential and ability to act across multiple cellular targets. This activity is often associated with their optimized physicochemical profile, which facilitates enhanced cellular uptake and promotes sustained intracellular retention. Structure–activity relationship analyses further indicate that electron-withdrawing substituents (–F, –Cl, –NO_2) significantly enhance cytotoxicity by modulating electronic density across the heterocyclic core, increasing lipophilicity, and improving interactions with kinase hinge regions and DNA

bases [19]. These substituents also contribute to improved metabolic stability and stronger target engagement, making them valuable features for designing next-generation anticancer agents within the pyrrolopyrimidine framework.

4.2. Antimicrobial and Antifungal Activity

Several analogues exhibit broad-spectrum antimicrobial activity, reflecting the versatility of the pyrrolo[2,3-d]pyrimidine scaffold and the beneficial influence of hydrazinyl substitution on microbial target engagement. Notable effects are observed against S. aureus, P. aeruginosa, E. coli, and Candida albicans, demonstrating that these compounds possess activity against both Gram-positive and Gram-negative bacteria as well as pathogenic fungi. The ability to act across such diverse microbial groups highlights their potential as multi-target antimicrobial candidates, particularly valuable in the context of increasing antibiotic resistance. Their balanced hydrophobic–hydrophilic profile also facilitates better membrane penetration, contributing to enhanced intracellular accumulation and improved overall potency.

Mechanistic investigations suggest membrane disruption, enzyme inhibition, and ROS generation as possible modes of action [20–22]. Experimental evidence indicates that some analogues integrate into microbial lipid bilayers, disturbing membrane integrity and causing leakage of intracellular components. Other studies highlight inhibition of key microbial enzymes involved in DNA replication, protein synthesis, or metabolic pathways, reflecting the scaffold's purine-like structural features. Additionally, reactive oxygen species (ROS) generation has been implicated in promoting oxidative stress within microbial cells, ultimately leading to apoptosis-like cell death mechanisms in bacteria and fungi. Taken together, these multifaceted mechanisms underscore the strong antimicrobial promise of hydrazinyl-substituted pyrrolo[2,3-d]pyrimidines and support their continued development as potential broad-spectrum therapeutic agents.

4.3. Anti-inflammatory and Antioxidant Potential

Selected derivatives inhibit COX-2, modulate inflammatory mediators, and show high radical-scavenging capacity. Substitution at the phenyl ring plays a major role in their anti-inflammatory potency [23].

5. Structure–Activity Relationship (SAR)

Key SAR patterns reported across studies include several consistent structural features that govern biological activity and selectivity across different therapeutic targets. The phenylhydrazinyl pharmacophore is essential for activity, offering key hydrogen-bond interactions that stabilize ligand binding within enzyme active sites, receptor pockets, and nucleic-acid grooves. Its dual nitrogen atoms provide a flexible arrangement of hydrogen-bond donors and acceptors, allowing the molecule to orient itself optimally toward catalytic residues and thereby enhance target recognition. Additionally, the appended phenyl ring contributes to hydrophobic contacts and π–π stacking interactions, further strengthening binding affinity and improving overall pharmacodynamic performance.

Halogenated phenyl rings improve anticancer and antimicrobial activity by increasing lipophilicity and target affinity [24]. Incorporation of substituents such as –F, –Cl, or –Br expands the electron-withdrawing character of the aromatic system, facilitating deeper insertion into hydrophobic protein pockets and enhancing membrane permeability. These substituents also modulate the electronic distribution across the scaffold, enabling stronger binding to kinase hinge regions or microbial enzyme active sites and thereby producing higher potency. Substitution at N-1 or C-7 of the pyrrolopyrimidine ring influences kinase binding and ADMET properties [25]. Such modifications can adjust molecular conformation, steric bulk, and polarity, leading to improved compatibility with ATP-binding pockets or altered interactions with metabolizing enzymes and transporters. These structural adjustments often translate into better pharmacokinetic profiles, such as increased metabolic stability or optimized tissue distribution.

Electron-donating groups reduce cytotoxicity but may enhance anti-inflammatory effects [26]. By increasing electron density on the aromatic or heterocyclic core, these substituents can modulate redox behavior, attenuate DNA-intercalation potential, and diminish kinase-inhibitory strength, resulting in lower anticancer activity. However, the same electronic enrichment can promote interactions with inflammatory mediators or antioxidant pathways, making such derivatives attractive candidates for anti-inflammatory or cytoprotective applications. Collectively, these observations highlight the scaffold's adaptability for multiple therapeutic goals and underscore its potential for rational design across diverse pharmacological areas.

6. Conclusion

4-(2-Phenylhydrazinyl)-7H-pyrrolo[2,3-d]pyrimidine analogues represent a promising and rapidly evolving class of heterocycles. Their structural complexity, ease of functionalization, and broad spectrum of biological activities position them as valuable leads in modern drug discovery. Continued interdisciplinary research integrating synthetic chemistry, computational methods, and biological evaluation will further unlock their therapeutic potential.

7. References

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