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A COMPREHENSIVE REVIEW OF PYRAZOLO[3,4-*d*]PYRIMIDINE SCAFFOLD BIOLOGICAL ACTIVITIES

Ibrahim M. Salem[1], Sally A. El Zohny[1], Mai Alaa Hayallah[2] and Alaa M. Hayallah[1,3*]

[1]*Department Pharmaceutical Chemistry, Faculty of Pharmacy, Sphinx University, New Assiut 10, Egypt*
[2]*Department of Pharmacy Practice, Faculty of Pharmacy, Sphinx University, New Assiut 10, Egypt*
[3]*Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt*

Pyrazolo[3,4-*d*]pyrimidine represents a fused bicyclic scaffold formed by the condensation of pyrazole and pyrimidine rings. Initially synthesized as an analog of purine nucleosides for anticancer therapy, this structural motif has since demonstrated a wide spectrum of biological properties, including antimicrobial, antiviral, antimycobacterial, anti-inflammatory, antimalarial, antidiabetic, anti-sterility effects, as well as xanthine oxidase inhibition for the treatment of gout. In recent years, the incorporation of the pyrazolo[3,4-*d*]pyrimidine pharmacophore into drug design has garnered significant interest among medicinal chemists and pharmacologists, prompting extensive exploration of its biological potential. This review provides a comprehensive survey of the literature on this nucleus derivatives published between 2012 and 2024, highlighting recent advancements and emerging trends in their therapeutic applications. This review may open the door for medicinal chemists and pharmacologists to emphasis the importance of this nucleus as probable future drug candidates for different diseases. Also, may encourage the researchers to improve physical and chemical letterings and modify their pharmacological properties.

Keywords: Pyrazolo[3,4-*d*]pyrimidines, Synthetic methodologies, Pyrazole derivatives, Pyrimidine frameworks, Pharmacological properties.

1. INTRODUCTION

This heterocyclic structure has a bicyclic framework that garnered significant scientific interest due to its potential role as a purine isostere. Structurally, it comprises a pyrazole ring fused with a pyrimidine nucleus, replacing the imidazole ring typically found in purine systems. Owing to this structural analogy, compounds based on this scaffold were initially characterized as adenosine receptor antagonists, primarily explored for their anticancer and antiviral properties. In recent years, derivatives of this nucleus have attracted increasing attention within the field of medicinal chemistry, owing to their diverse pharmacological activities. These include antimicrobial, antiviral, anticancer, antimycobacterial, anti-inflammatory, antimalarial, antidiabetic, anti-sterility effects, and their application as xanthine oxidase inhibitors in the management of gout. This review provides a comprehensive summary of the wide range of biological investigations carried out on the privilege scaffold and highlights recent advances in the pharmaceutical formulations of compounds derived from this chemical class (Fig. 1).

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*Corresponding author: Alaa M. Hayallah, E-mail: alaa_hayalah@yahoo.com

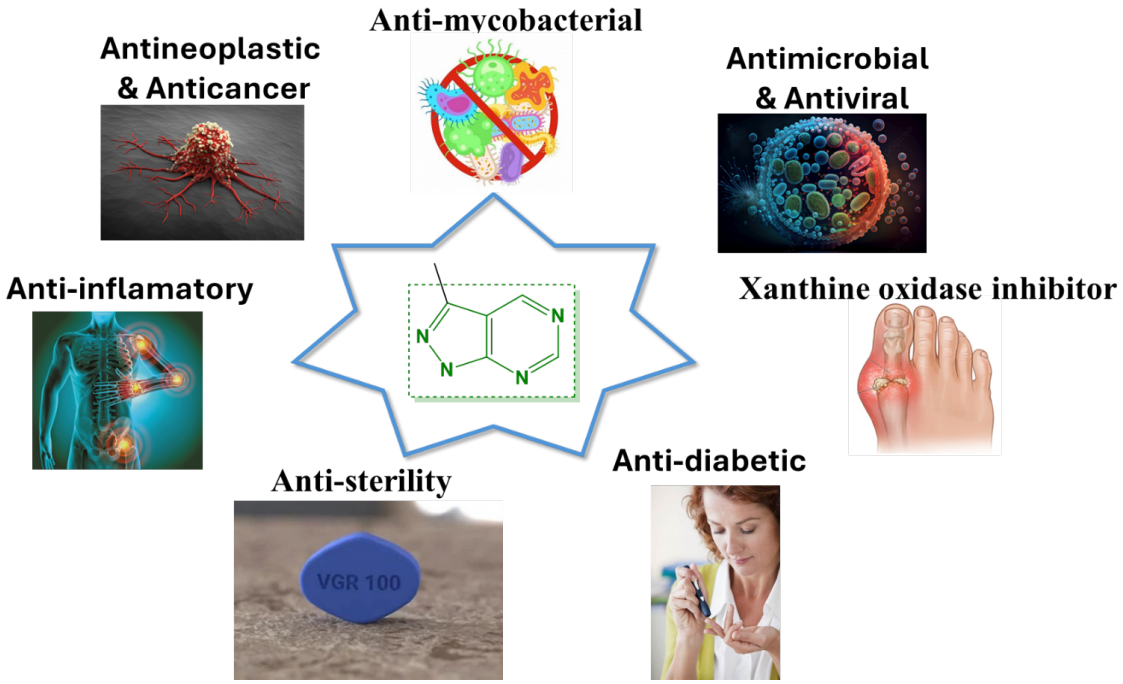


Fig. 1: The therapeutic potential of pyrazolo[3,4-*d*]pyrimidine as antineoplastic, anti-inflammatory, antimicrobial, antiviral, antimycobacterial, xanthine oxidase inhibitor, anti-diabetic and anti-sterility.

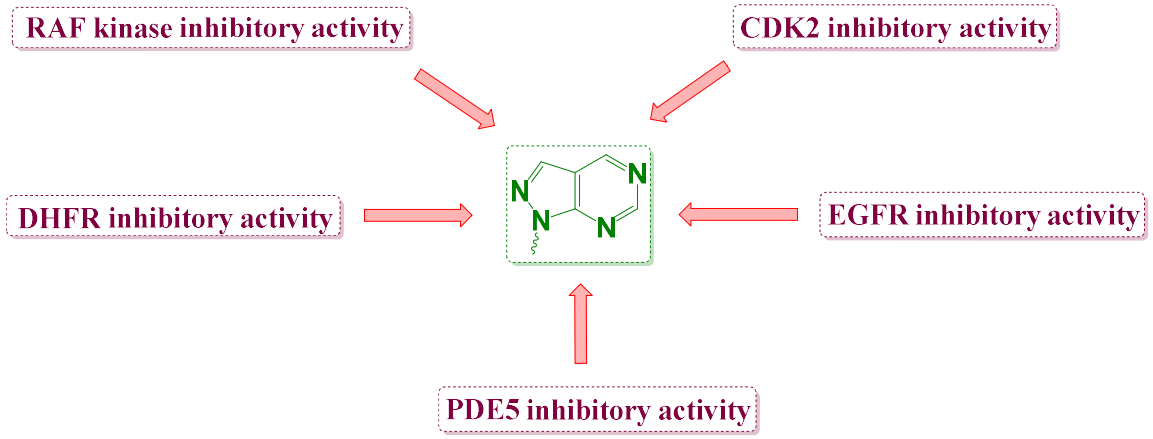


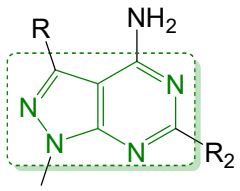
Fig. 2: Diverse molecular targets associated with pyrazolo[3,4-*d*]pyrimidine derivatives under investigation as prospective antineoplastic agents.

2. Biological inspection

2.1. Antineoplastic activity

The pyrazolo[3,4-*d*]pyrimidine scaffold exhibits significant antineoplastic potential through multiple well-characterized mechanistic pathways, as extensively documented in figure 2.

The chemistry core present in compound **1** represents a prominent fused heterocyclic framework that has attracted considerable interest within the field of medicinal chemistry, largely owing to its diverse biological properties and pronounced anticancer potential. Structurally analogous to purines, this scaffold exhibits isosteric characteristics that enable it to engage with a range of enzymatic and receptor targets implicated in oncogenic signalling pathways[1-3].



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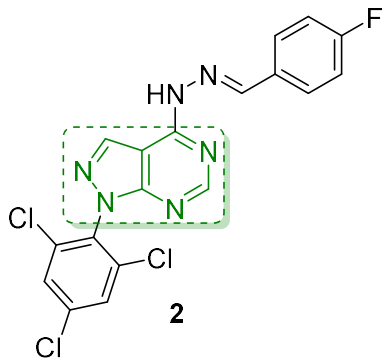
2.1.1. Mechanisms of antineoplastic activity

These chemical scaffold derivatives exert potent anticancer effects primarily through the targeted inhibition of several key kinases integral to oncogenic processes. A prominent class of these targets includes:

2.1.1.1. Cyclin-dependent kinases (CDKs) inhibition

CDKs are essential regulators of cell cycle progression. Pharmacological inhibition of CDKs disrupts the orderly transition between cell cycle phases, leading to cell cycle arrest. This disruption is often followed by the induction of apoptosis – an outcome particularly desirable in oncological therapeutics due to its role in eliminating malignant cells.

Targeting CDKs thus represents a strategic approach to impede tumor cell proliferation. Notably, multiple fused rings analogs have demonstrated significant efficacy as CDK inhibitors. Compounds **2** and **3** have exhibited pronounced inhibitory activity against CDK2 and related isoforms. Mechanistically, these derivatives interact with the CDK active site in a manner that closely resembles the binding of endogenous ligands or classical inhibitors, thereby competitively obstructing kinase function. This mode of action underscores the therapeutic promise of these scaffolds in the design of next-generation anticancer agents[4].



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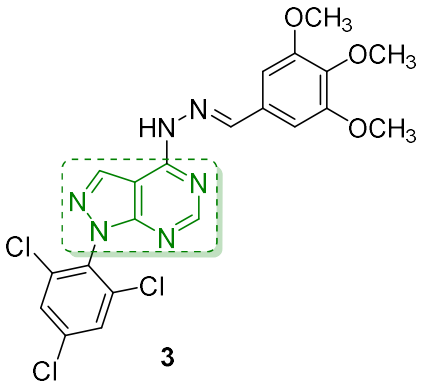

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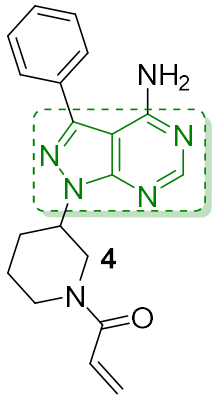
2.1.1.2. Epidermal growth factor receptor (EGFR) inhibition

EGFR represents pivotal molecular target in oncological research, owing to its integral involvement in key signaling cascades that govern cellular proliferation, survival, and metastatic progression. Aberrant expressions or activating mutations of EGFR are frequently correlated with enhanced tumor aggressiveness and unfavorable clinical outcomes. Consequently, EGFR has emerged as a prominent focus in the development of targeted cancer therapeutics.

The inhibition of epidermal growth factor receptor (EGFR) has emerged as a critical therapeutic strategy for attenuating tumor progression and metastatic dissemination by interfering with key oncogenic signaling pathways. Clinically, multiple classes of EGFR-targeted agents, including monoclonal antibodies and small-molecule tyrosine kinase inhibitors, have been developed and implemented. Among these, compounds incorporating the pyrazolopyrimidine core – such as compound **4**, have demonstrated notable efficacy in preclinical and clinical models, significantly suppressing tumor cell proliferation and hindering metastatic potential. These agents exert their therapeutic effect by either obstructing ligand-EGFR interactions or by directly inhibiting the receptor's intrinsic kinase activity, thereby prominent contacting elucidation such as, hydrogen bonding donor effect with Gln791 residue, along with two significant hydrogen bond acceptor interactions with the critical Met793 and Cys797 amino acid. The robust preclinical and clinical data supporting these outcomes underscore the therapeutic relevance of EGFR as a molecular target in the design of next-generation anticancer interventions[5].



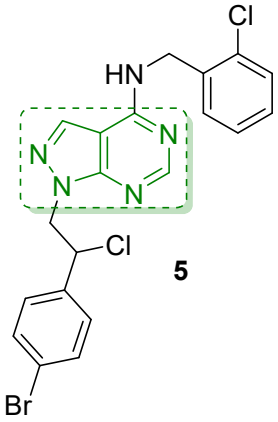
Due to its significant role in tumorigenesis, EGFR has become a primary target for cancer therapy. Several strategies have been hired to inhibit EGFR activity, including:

1. Monoclonal antibodies specifically target the extracellular domain of the epidermal growth factor receptor (EGFR), thereby inhibiting the binding of endogenous ligands and subsequent receptor activation. Clinically approved agents in this category include cetuximab and panitumumab, both of which have demonstrated efficacy in various EGFR-expressing malignancies.
2. Tyrosine Kinase Inhibitors (TKIs) are low-molecular-weight compounds designed to interfere with the tyrosine kinase activity within the intracellular domain of EGFR. By obstructing this enzymatic function, these agents effectively disrupt downstream signalling cascades critical for tumor cell proliferation and survival. Prominent examples include gefitinib, erlotinib, and afatinib, all of which are employed in the targeted therapy of EGFR-mutant cancers[6].

2.1.1.3. Src family kinases (SFKs) inhibition

The Src family kinases (SFKs) constitute a class of non-receptor tyrosine kinases that are critically involved in the regulation of diverse cellular functions such as proliferation, survival, differentiation, migration, and programmed cell death. This kinase family comprises several structurally related members, with Src serving as the archetype. Prominent additional members include Yes, Fyn, Lyn, Lck, and Hck. SFKs participate in a wide array of intracellular signaling cascades and frequently associate with various membrane-bound receptors, including growth factor receptors and integrins, thereby modulating key physiological and pathological processes[7].

In compound **5**, they disclosed substantial inhibition of SRC kinase family (SFKs), leading to a significant reduction in cell viability and induction of apoptosis, while sparing non-tumor cells such as primary human skin fibroblasts and differentiated C2C12 myoblasts. In addition, compound **5** impaired *in-vitro* cell migration and invasion, and suppressed tumor growth in a rhabdomyosarcoma (RMS) xenograft model. SFK inhibition also promoted muscle differentiation in RMS cells by modulating the NOTCH3 receptor–p38 MAPK signalling axis, which governs the balance between cellular proliferation and differentiation[8].



1. **Cell proliferation and survival:** Src family kinases (SFKs) are critical regulators of cellular proliferation and survival, primarily through the activation of key downstream signalling cascades, including the RAS–RAF–MEK–ERK and PI3K–AKT pathways, which collectively promote cell cycle progression and suppress apoptotic mechanisms[2].
2. **Cell migration and invasion:** SFKs are critical in processes such as cell motility, which is essential for normal physiological functions and the metastatic spread of cancer cells. They modulate cytoskeletal dynamics and integrin signaling to facilitate these processes[9].
3. **Signal transduction:** SFKs act as critical nodes in transducing signals from various extracellular stimuli, including growth factors and adhesive signals from the extracellular matrix[10].

Src family kinases (SFKs) are frequently overexpressed or aberrantly activated in a broad spectrum of malignancies, where they play a critical role in promoting oncogenic processes. Dysregulated SFK signalling has been associated with enhanced tumor proliferation, metastatic potential, and evasion of apoptosis, underscoring their relevance as promising therapeutic targets in cancer treatment. Among the SFKs, c-Src is particularly well-characterized and has been extensively implicated in the pathogenesis and progression of several major cancers, including those of the breast, prostate, and colon[11].

Due to their significant roles in cancer progression, SFKs are considered promising targets for therapeutic intervention. Several small-molecule inhibitors targeting SFKs have been developed, showing promise in preclinical and clinical settings. This strategy aims to inhibit SFK activity to reverse or prevent the malignant behaviors influenced by these kinases[12].

In summary, SFKs are essential regulatory proteins involved in various cellular mechanisms and disease processes, particularly in cancer, and represent a viable target for novel therapeutic strategies.

2.1.1.4. m-TOR and JAK kinases inhibition

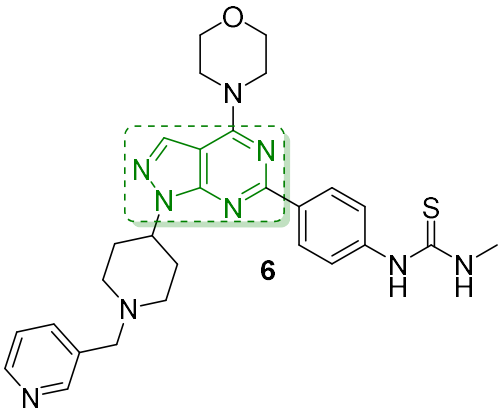
They are critical signaling pathways involved in cell growth, proliferation, and survival, and they play significant roles in cancer biology[13].

mTOR is a serine/threonine kinase that functions as a central regulator of cellular metabolism, growth, proliferation, and survival. It integrates signals from nutrients, growth factors, and cellular energy status to control processes such as:

- **Protein synthesis:** mTOR promotes the translation of mRNA into proteins, which is important for cell growth and propagation.
- **Autophagy:** It inhibits autophagy under nutrient-rich conditions, which is a process that degrades and recycles cellular components[7].
- **Cell cycle progression:** mTOR signaling influences cell cycle progression, particularly the transition from the G1 phase to the S phase[14].

mTOR was identified as a therapeutic target in various cancers, leading to the development of mTOR inhibitors, such as rapamycin and its analogs, which aim to halt cancer growth and proliferation[15].

Verheijen *et al.*[16] combined 4-morpholino-*1H* pyrazolopyrimidines with alkylureido-phenyl groups at the C-3 position of the pyrazolo[3,4-*d*]pyrimidine ring to produce target compound **6**.



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2.1.1.5. JAK Kinases inhibition

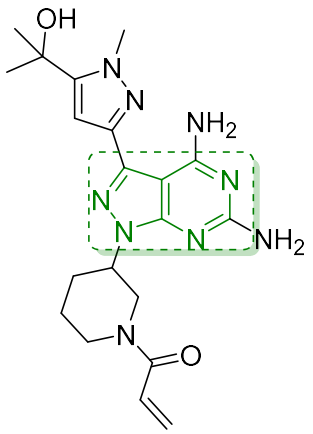
JAKs are a family of non-receptor tyrosine kinases that associate with cytokine receptors and are critical for the signaling pathways of many growth factors and hematopoietic cytokines. They are involved in mediating the effects of cytokines and growth factors through the JAK/STAT (Signal Transducer and Activator of Transcription) signaling pathway, which regulates various biological processes, including[17]:

- **Immune response:** JAKs play a key role in mediating responses to cytokines involved in the immune system.
- **Cell proliferation and differentiation:** They are involved in the proliferation and differentiation of hematopoietic cells.
- **Inflammation:** JAK signaling is implicated in inflammatory responses.

Deregulation of JAK activity is associated with several cancers, including leukemia and lymphomas. JAK inhibitors, such as ruxolitinib and tofacitinib, are being utilized to treat certain hematological malignancies and autoimmune disorders[18].

Yin *et al.*[19] designed new pyrazole-based derivatives using a structure-based drug design strategy. This study resulted in compound **7**. In summary, JAK kinase is critical for cell signaling and contributes to cancer progression. Compound **7** exhibited an exceptional binding

inhibition of the JAK Kinase of 6.5 nM. Targeting this kinase represents a promising therapeutic strategy in oncology, with ongoing research aimed at optimizing their inhibition and overcoming resistance mechanisms.



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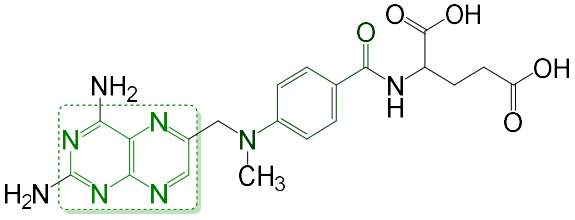
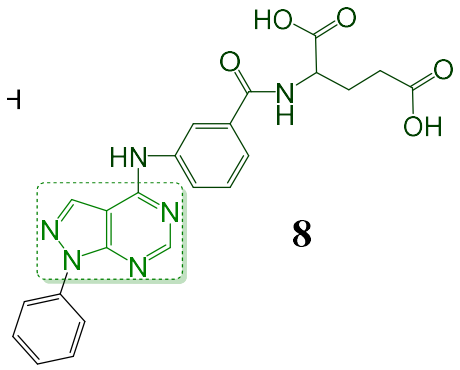
2.1.1.6. DHFR inhibition

In 2022, Salem *et al.*[20] reported the design and synthesis of a novel glutamic acid-conjugated pyrazolo[3,4-*d*]pyrimidine derivative (Compound **8**), structurally analogous to methotrexate. This structural mimicry was achieved by substituting the pteridine moiety of methotrexate with the isosteric pyrazolo[3,4-*d*]pyrimidine scaffold. The biological evaluation of compound **8** revealed potent anti-proliferative activity across various human cancer cell lines, primarily attributed to its inhibition of dihydrofolate reductase (DHFR). Notably, compound **8** demonstrated a significantly enhanced DHFR inhibitory effect, with an IC_{50} value of 0.24 µM, markedly surpassing that of the reference drug methotrexate (IC_{50} = 5.57 µM).



Methotrexate



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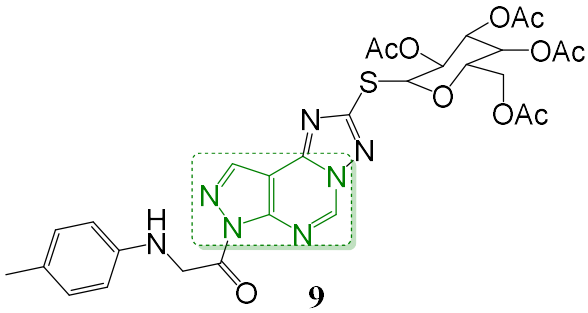
Besides, Nassar *et al.*[21] carried out a novel glutamic acid-conjugated of this heterocyclic scaffold (designated as compound **9**) was designed and synthesized, exhibiting structural similarity to methotrexate through the strategic replacement of the pteridine core with an isosteric pyrazolo[3,4-*d*]pyrimidine scaffold. This rational design approach aimed to preserve key pharmacophoric features while potentially enhancing biological activity. Comprehensive *in-vitro* evaluations demonstrated that compound **9** possesses robust anti-proliferative efficacy against a spectrum of human cancer cell lines, predominantly mediated through potent inhibition of dihydrofolate reductase (DHFR).



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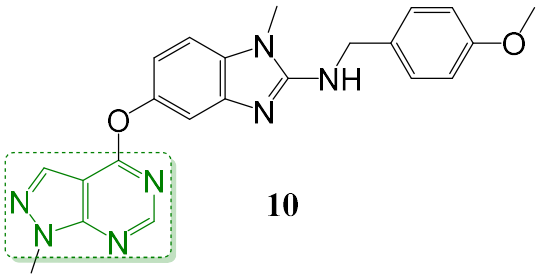
Furthermore, Wang *et al.*[22] developed a series of pyrazolo[3,4-*d*]pyrimidine derivatives incorporating a benzimidazole moiety (Compound **10**) and assessed their anti-proliferative efficacy against A375 and H-29 cell lines, both of which express the oncogenic BRAF^V600E mutation known to drive aberrant cellular signaling. Among the synthesized compounds, compound **10** exhibited significant cytotoxic activity, demonstrating half-maximal inhibitory concentration (IC_{50}) values of 1.74 µM and 6.92 µM against A375 and H-29 cells, respectively.



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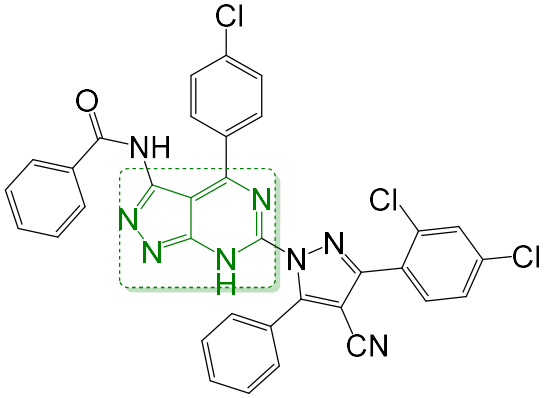
In a recent 2024 study, Hassaballah *et al.*[23], reported the synthesis of a novel pyrazolo[3,4-*d*]pyrimidine/pyrazole hybrid compound, designated as compound **11**, and evaluated its cytotoxic properties across the National Cancer Institute's panel of 60 human cancer cell lines. The compound demonstrated potent and broad-spectrum antiproliferative activity, as evidenced by its performance in the NCI 5-log dose screening assay. Notably, the growth inhibition (GI_{50}) values ranged from 0.018 to 9.98 μM, suggesting a strong correlation between its cytotoxic effects and epidermal growth factor receptor (EGFR) inhibitory activity.



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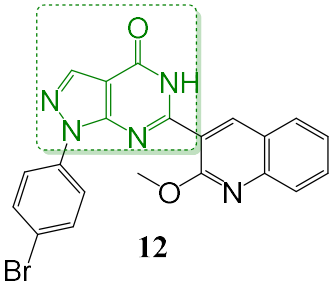
Furthermore, in 2020, Ibrahim *et al.*[24] developed a new compound linked to a quinoline moiety (Compound **12**), which was subsequently evaluated for its potential as a phosphodiesterase type 5 (PDE-5) inhibitor and pro-apoptotic agent. Compound **12** exhibited marked PDE-5 inhibitory potency, with an IC_{50} value of 1.57 nM, indicating strong enzymatic suppression. Mechanistic investigations revealed that the compound triggered the intrinsic apoptotic pathway, as evidenced by a significant downregulation of the anti-apoptotic Bcl-2 protein, thereby affirming its role in promoting programmed cell death.



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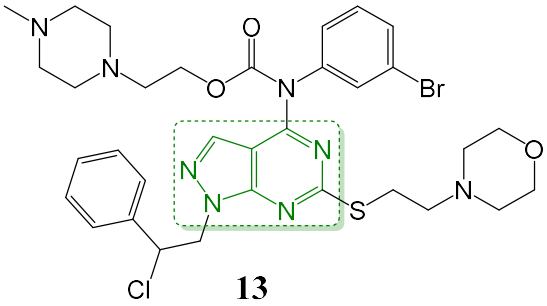
2.1.1.7. Targeting multidrug resistance (MDR)

A major impediment to the efficacy of chemotherapy is the development of multidrug resistance (MDR), frequently mediated by ATP-binding cassette (ABC) transporters such as P-glycoprotein (P-gp). Recent studies have demonstrated that pyrazolo[3,4-*d*]pyrimidine-based tyrosine kinase inhibitors (TKIs), particularly compound **13**, exhibit significant potential in circumventing MDR mechanisms. This compound effectively inhibits the ATPase activity of P-gp, thereby impeding drug efflux processes. As a result, it enhances the intracellular accumulation of chemotherapeutic agents, contributing to the restoration of drug sensitivity to agents such as doxorubicin and paclitaxel[25].



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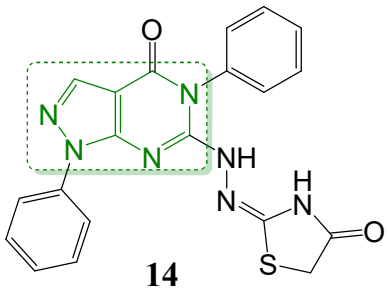
2.2. Anti-inflammatory activity

Numerous scientific investigations have highlighted the significant anti-inflammatory potential of the pyrazolo-pyrimidine scaffold[26]. In a study by Tageldin *et al.*[27], novel thiazolidinone-substituted pyrazolo[3,4-*d*]-pyrimidine derivatives, specifically compounds **14** and **15**, were synthesized and evaluated for their *in-vitro* cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) inhibitory activities. The findings demonstrated that both compounds exhibited superior anti-inflammatory efficacy compared to the standard drug diclofenac, as assessed using the formalin-induced paw edema model.



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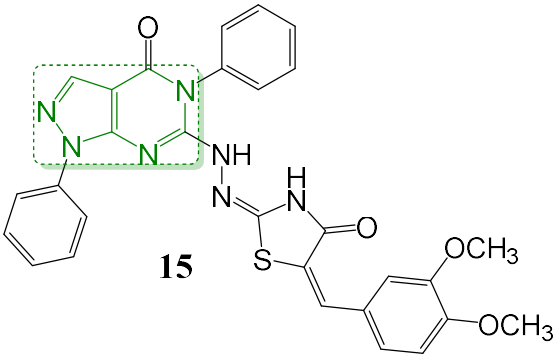

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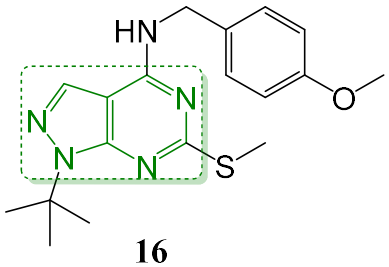
Similarly, in a study conducted by Lee *et al.* in 2019[10], compound **16**, was reported. The experimental findings demonstrated that compound **16** significantly upregulated intracellular Nrf2 levels and exhibited high binding affinity toward Keap1, a negative key regulator of Nrf2. By effectively disrupting the Keap1–Nrf2 interaction, this compound showed potential neuroprotective properties. These results suggest that compound **16** may serve as a promising therapeutic candidate for the management of oxidative stress and inflammation-associated neurodegenerative diseases, including Parkinson's disease.



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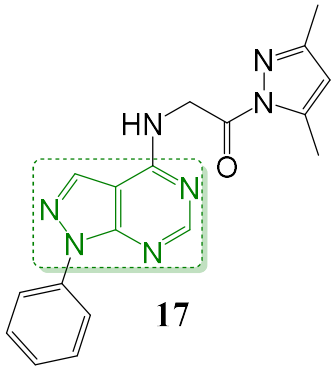
2.3. Antimicrobial activity

The pyrazolo[3,4-*d*]pyrimidine scaffold has been extensively documented for its significant antibacterial and antifungal properties[28]. In a study conducted by El-Sayed *et al.*[29] novel derivatives bearing a 1-phenyl-*1H*-pyrazolo[3,4-*d*]pyrimidine core were synthesized, incorporating both pyrazole **17** and 1,3,4-oxadiazole **18** moieties. The resulting compounds exhibited a broad-spectrum antimicrobial profile, demonstrating potent activity against Gram-positive bacteria (*Streptococcus pneumoniae*), Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*), as well as fungal strains including *Aspergillus fumigatus* and *Candida albicans*.



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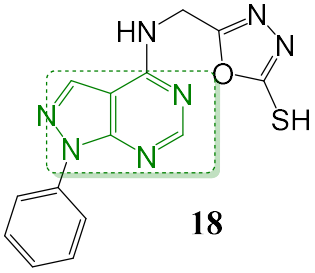

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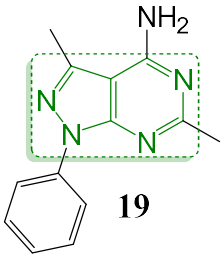
Furthermore, Beyzaei *et al.*[30] synthesized the 4-amino-1-phenylpyrazolo[3,4-*d*]pyrimidine derivative, designated as compound **19**, which demonstrated notable antibacterial activity against *Streptococcus pyogenes* and *Pseudomonas aeruginosa*, exhibiting a minimum inhibitory concentration (MIC) of 32 µg/mL.



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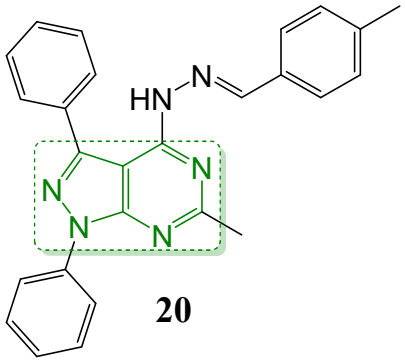
Additionally, Hassaneen *et al.*[31], synthesized a novel pyrazolo[3,4-*d*]pyrimidine derivative conjugated with a hydrazone moiety

(Compound **20**). The antimicrobial properties of this compound were systematically evaluated against *Bacillus subtilis* and *Pseudomonas aeruginosa*. Notably, compound **20** exhibited significant inhibitory activity, with minimum inhibitory concentration (MIC) values recorded at 40 µg/mL and 60 µg/mL for *B. subtilis* and *P. aeruginosa*, respectively.



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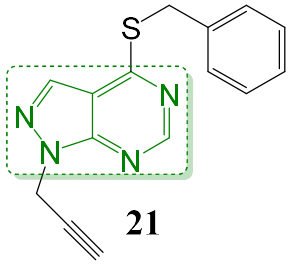
2.4. Anti-mycobacterial activity

Moukha-Chafiq *et al.*[32], reported the synthesis of a benzylthiopyrazolo[3,4-*d*]pyrimidine derivative, designated as compound **21**. Subsequent biological evaluation demonstrated that this compound **21** exhibited notable anti-tubercular activity.



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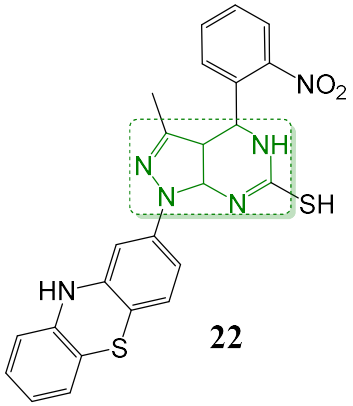
Too, in 2010 Trivedi *et al.*[33], synthesized a novel phenothiazine-conjugated pyrazolo[3,4-*d*]pyrimidine derivative, designated as compound **22**. The *in-vitro* evaluation of its antimycobacterial potential demonstrated significant inhibitory activity against *Mycobacterium tuberculosis*, with a minimum inhibitory concentration (MIC) of less than 6.25 µg/mL, highlighting its promise as a potent anti-tubercular agent.



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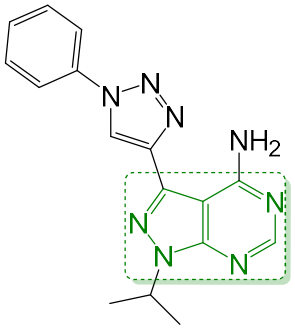
2.5. Anti-malarial activity

The pyrazolo[3,4-*d*]pyrimidine scaffold has been validated as a promising pharmacophore exhibiting notable antimalarial activity. Klein *et al.*[34], synthesized a series incorporating 1,2,3-triazole moieties to generate compounds **23** and **24**. This analog was subsequently evaluated for their antimalarial efficacy against *Plasmodium falciparum*, with a specific focus on their inhibitory effect on Plasmodium falciparum protein kinase 7 (PfPK7). Both compounds demonstrated selective inhibitory activity against PfPK7, exhibiting IC_{50} values in the range of 10–20 µM, thereby indicating their potential as lead candidates for further optimization in antimalarial drug development.



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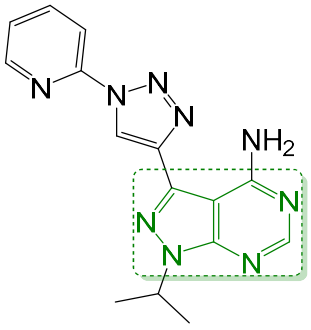

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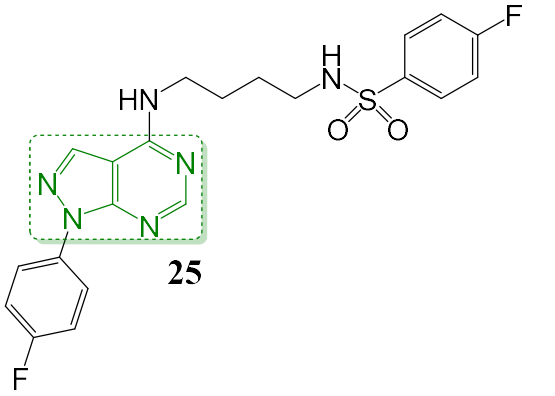
Similarly, Silveira *et al.*[35] synthesized pyrazolo[3,4-*d*]pyrimidine derivatives, specifically compounds **25** and **26**, incorporating a benzene sulfonamide moiety via a flexible butyl linker. These analogs were subjected to *in-vitro* evaluation for antimalarial efficacy against *Plasmodium falciparum*. Notably, both compounds demonstrated inhibitory activity against chloroquine-resistant *P. falciparum* strains, with half-maximal inhibitory concentration (IC_{50}) values ranging from 5.13 to 43.40 µM.



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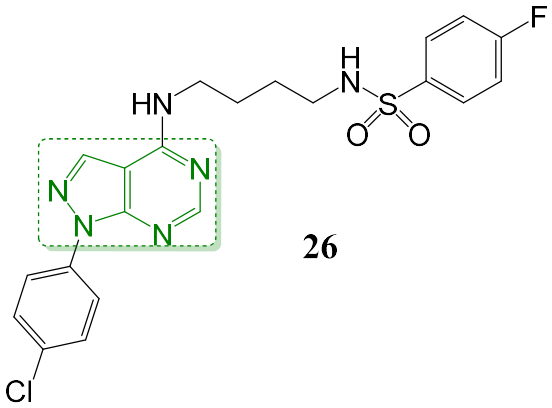
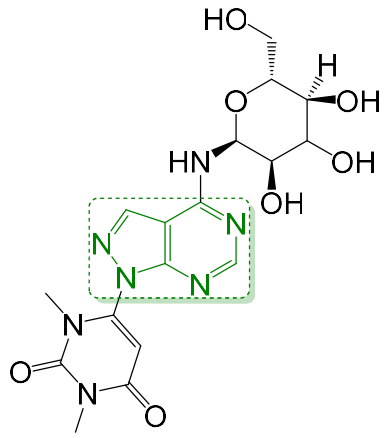


26

2.6. Anti-viral activity

Due to its structural resemblance to purine bases, the pyrazolo[3,4-*d*]pyrimidine scaffold has garnered considerable attention for its potential as an antiviral pharmacophore. El-Sayed *et al.*[36] synthesized N4-β-D-glycosidic derivatives (Compounds **27** and **28**) and assessed their efficacy against the hepatitis B virus. The findings demonstrated that both compounds exhibited pronounced antiviral activity, with a notably low minimum inhibitory concentration (MIC) of 0.2 µM, indicating strong therapeutic potential.



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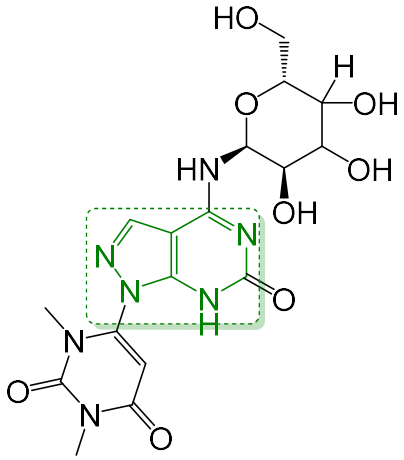
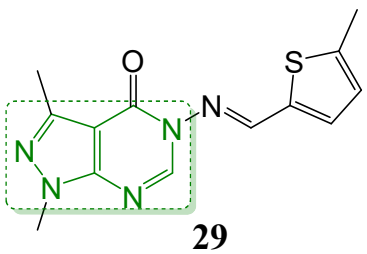


28

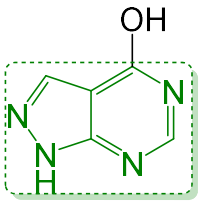
In a study conducted by Wang *et al.*[37] a novel pyrazolo[3,4-*d*]pyrimidine derivative incorporating a Schiff base moiety (Compound **29**) was successfully synthesized and subsequently evaluated for its antiviral efficacy against the tobacco mosaic virus (TMV). The findings revealed that compound **29** exhibited remarkable antiviral activity, demonstrating an effective concentration (EC_{50}) of 53.65 µg/mL, significantly surpassing the performance of the standard antiviral agent ribavirin, which showed an EC_{50} of 150.45 µg/mL.



29

2.7. Xanthine oxidase inhibitory activity

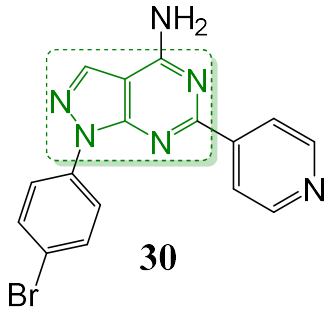
The pyrazolo[3,4-*d*]pyrimidine scaffold constitutes the fundamental structural motif of allopurinol (Fig. 3), which was the first xanthine oxidase inhibitor to receive approval from the U.S. Food and Drug Administration (FDA) for the sustained treatment of chronic gout-related conditions[38].



(Allopurinol)

Fig. 3: The structure of the first FDA approved pyrazolo[3,4-*d*]pyrimidine containing drug Allopurinol as xanthine oxidase inhibitor.

Oliveira-Campos *et al.*[39], 1-phenyl-pyrazolo[3,4-*d*]pyrimidine derivatives 30 and 31 were synthesized and subsequently assessed for their inhibitory activity against xanthine oxidase, with allopurinol employed as the reference standard.



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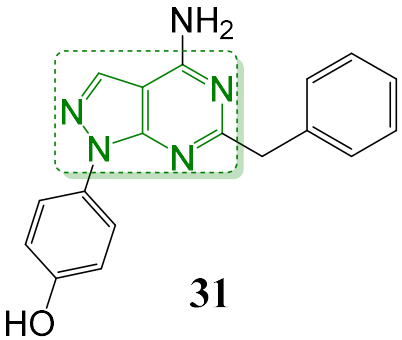
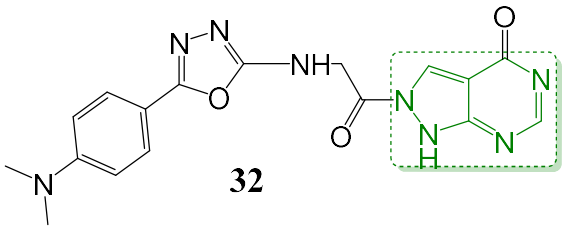


31

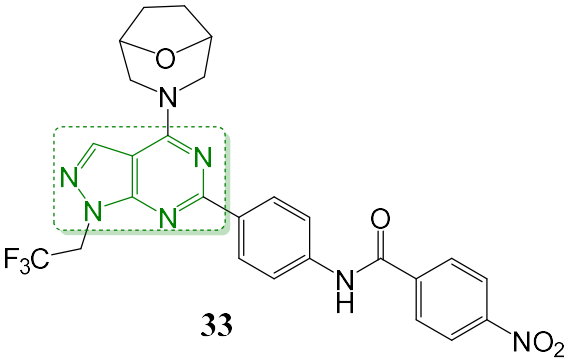
In a study conducted by Khammas *et al.*[40], the xanthine oxidase inhibitory potential of a derivative incorporating a 1,3,4-oxadiazole moiety (designated as compound 32) was systematically evaluated. The compound demonstrated significant inhibitory activity against xanthine oxidase, with an IC_{50} value of 1.32±0.05 µM. This potency is notably comparable to that of the standard inhibitor allopurinol, which exhibited an IC_{50} of 2.61±0.07 µM under the same experimental conditions.



32

2.8. Anti-diabetic activity

Reddy and colleagues synthesized a novel compound, designated as compound 33, which incorporates a pyrazolo[3,4-*d*]pyrimidine core connected with a bicyclic moiety and a benzanilide functional group. This compound demonstrated superior anti-diabetic activity, exhibiting an IC_{50} value of 1.60±0.48 µM, thereby outperforming the standard reference drug acarbose, which showed an IC_{50} of 1.73±0.05 µM.



33

2.9. Anti-sterility activity

Sildenafil, the first FDA-approved agent targeting infertility through the inhibition of cyclic guanosine monophosphate-specific phosphodiesterase type 5 (PDE5), has been extensively utilized in the clinical management of erectile dysfunction. Structurally, sildenafil is characterized by a pyrazolo[4,3-*d*]pyrimidine core conjugated to a benzene sulfonamide moiety (Fig. 4). Notably, structural modifications – specifically the substitution of the N4-methyl group on the piperazine ring

with an ethyl group, while preserving both the pyrazolo[4,3-*d*]pyrimidine and benzene sulfonamide motifs – have led to the development of a more potent analog, Vardenafil, representing a novel chemical entity with enhanced pharmacological efficacy (Fig. 4)[41].

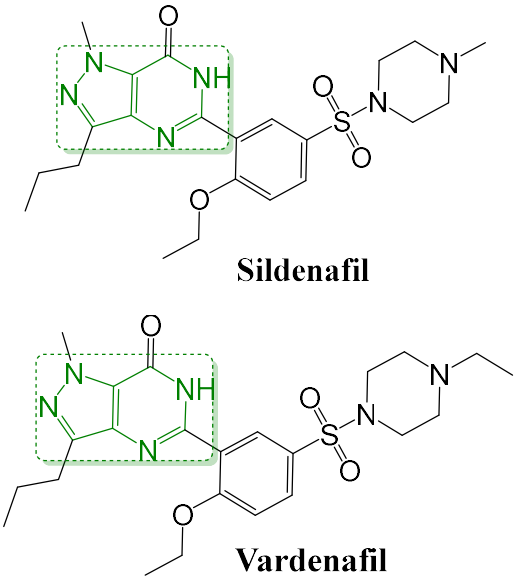
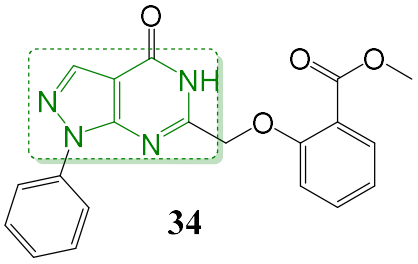
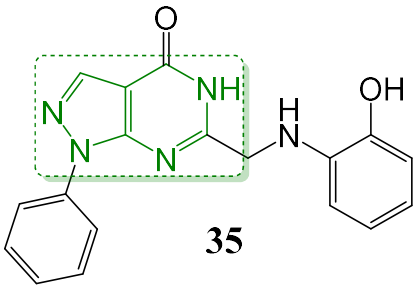


Fig. 4: The chemical structure pyrazolo[4,3-*d*]-pyrimidine dependant anti-sterility drug Sildenafil and Vardenafil.

In a related study, Shaaban *et al.*[42] employed a combination of virtual screening and pharmacophore modeling to design and synthesize a series including compounds 34 and 35. These molecules demonstrated significant relaxant activity on isolated rat corpus cavernosum tissue, exhibiting pEC_{50} values between 8.31 and 5.16 µM. Notably, their pharmacological efficacy was comparable to that of the reference drug sildenafil, achieving a response equivalent to 100%.





3. Conclusion

The pyrazolo[3,4-*d*]pyrimidine scaffold has emerged as a prominent structural motif in medicinal chemistry, owing to its extensive range of pharmacological properties, which encompass anti-inflammatory, antimycobacterial, antimicrobial, anticancer, antiviral, antidiabetic, antimalarial, xanthine oxidase inhibitory, and antifertility activities. This review critically examines the therapeutic relevance of this heterocyclic framework, emphasizing lead compounds that integrate this nucleus. The objective is to support the rational design and synthesis of innovative bioactive molecules derived from this core structure, thereby promoting systematic biological assessments aimed at optimizing pharmacological efficacy and specificity. Furthermore, these interesting findings this review may open the door towards this privilege skeleton to develop new pharmacological leads of potential therapeutic applications.

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بحث مرجعى شامل للأنشطة البيولوجية لهيكل بيريزولو[4،3-د]بيريميدين

ابراهيم سالم[1] – سالى الذهنى[1] – مى علاء حياالله[2] – علاء حياالله[1،3]

[1]قسم الكيمياء الصيدلية ، كلية الصيدلة ، جامعة سفنكس ، أسيوط الجديدة 10 ، مصر
[2]قسم الممارسة الصيدلية ، كلية الصيدلة ، جامعة سفنكس ، أسيوط الجديدة 10 ، مصر
[3]قسم الكيمياء العضوية الصيدلية ، كلية الصيدلة ، جامعة أسيوط ، أسيوط 71526 ، مصر

يُمثل بيرازولو[4،3-د]بيريميدين هيكلًا ثنائي الحلقة مُلتحمًا ، مُكوّنًا من تكثف حلقات البيرازول والبيريميدين. صُنع هذا الهيكل في البداية كنظير لنوكليوسيدات البيورين لعلاج السرطان ، وقد أظهر منذ ذلك الحين طيفًا واسعًا من الخصائص البيولوجية ، بما في ذلك تأثيرات مضادة للميكروبات ، ومضادة للفيروسات ، ومضادة للفطريات ، ومضادة للالتهابات ، ومضادة للملاريا ، ومضادة لمرض السكري ، ومضادة للعقم ، بالإضافة إلى تثبيط أوكسيديز الزانثين لعلاج النقرس. في السنوات الأخيرة ، حظي دمج فارماكوفور بيرازولو[4،3-د]بيريميدين في تصميم الأدوية باهتمام كبير بين الكيميائيين وعلماء الصيدلة ، مما دفع إلى استكشاف واسع لإمكانياته البيولوجية. يقدم هذا البحث المرجعى مسحًا شاملاً للأبحاث المنشورة حول مشتقات هذه النواة بين عامي 2012 و2024 ، مُسلّطةً الضوء على التطورات الحديثة والاتجاهات الناشئة في تطبيقاتها العلاجية. قد يفتح هذا البحث الباب أمام الكيميائيين الطبيين وعلماء الأدوية لتأكيد أهمية هذه النواة كمرشحات محتملة للأدوية في المستقبل لعلاج أمراض مختلفة. كما قد تشجع الباحثين على تحسين الخواص الفيزيائية والكيميائية وتعديل خصائصها الدوائية.