RESEARCH PAPER

Exploring the Therapeutic Landscape of N9-Substituted Purines from Synthesis to Targeted Drug Design

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ABSTRACT

Purines represent a fundamental class of heterocyclic compounds that play a central role in biological systems and medicinal chemistry. The N9-substituted purine derivatives have attracted significant attention due to their structural similarity to natural nucleosides and their broad spectrum of biological activities, therefore, advancement in the synthesis, regioselective functionalization, molecular docking, structure–activity relationships and therapeutic applications of N9-substituted purine derivatives are summarized over the period from 2010 to 2026. The synthetic strategies, including regioselective N9-alkylation, N-arylation, solid-phase synthesis, microwave-assisted methods, and green chemistry approaches, are discussed with emphasis on selectivity and sustainability. The pharmacological potential of these compounds is highlighted across multiple therapeutic areas, including anticancer, antiviral, antibacterial, antifungal, anti-tuberculosis, and metabolic disorders, supported by molecular docking and computational studies targeting kinases, viral polymerases, bromodomains, and purine biosynthesis enzymes. SAR analyses reveal that substitutions at the N9, C2, and C6 positions critically influence potency, selectivity, and cellular uptake. Furthermore, emerging concepts such as purinosome targeting, nano-pharmaceutical delivery, PROTAC development, and AI-assisted drug design underscore the expanding role of the 9-purine scaffold in modern drug discovery to establish N9-substituted purines as versatile and privileged scaffolds with strong potential for future therapeutic development.

Keywords: Purines, N9-substituted purines derivatives, SAR, Molecular docking, Green synthesis, Biological activities.

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1. Introduction

The nitrogen containing heterocyclic scaffolds are an important class of compounds in medicinal and organic chemistry. Pyrrolidine, pyrazoline and triazole, which are five membered nitrogen heterocycles, have garnered much interest. Interestingly, the pyrrolidine ring exhibits a number of biological activities, such as anticancer, antimalarial, antioxidant, antileishmanial, antidepressant, antidiabetic, and antifungal as well as antibacterial activity. The pyrrolidine nucleus, in particular, is highly favoured in the field of pharmaceutical science and drug design, thanks to its versatility. This adaptability makes an indispensable scaffold for the development of novel bioactive molecules and establishing novel therapeutic agents. Furthermore, it has potential applications other than medicinal chemistry, such as optical probes and biomarkers in material science [1]. Beyond medicinal uses, nitrogen-containing heterocycle is also known to have remarkable photophysical and electronic properties, which makes them attractive for materials science, bio imaging, molecular sensing and drug discovery. Heteroatoms (e.g., N) affect the π-electron delocalization, intramolecular charge transfer, fluorescence emission, absorption properties and stability of molecules. The ultraviolet-visible (UV–Vis) absorption spectra, fluorescence quantum yield, HOMO–LUMO energy gaps, and solvatochromic behavior are strongly affected by substituent effects and the conjugation patterns. Recent studies showed that as a component of a heterocyclic system N atoms showed

increased photostability, tunable fluorescence properties, and increased molecular interactions with the biological targets. The photophysical properties are now being exploited in theranostic applications, as well as in the field of drug delivery, biosensing and molecular imaging [2]. The purines are privileged scaffolds in medicine due to their biological and physicochemical importance, and derivatives of purines are used in many therapeutic applications. Structural changes at various sites on the purine ring, especially N9, have significant impact on receptor binding, target selectivity, PK properties, and electronic properties. The use of the molecular docking, computational chemistry, photophysical analysis, and structure–activity relationship (SAR) studies has boosted N9-substituted purines as potential candidates for anticancer, antiviral, antimicrobial, and enzyme-targeted therapies [3]. The purine (imidazo[4,5-d]pyrimidine) nucleus serves as a basic and flexible heterocyclic framework in many physiologically important molecules. In the present review, we examine the chemical, biological, synthetic and therapeutic studies of N9-substituted purines published since 2010 until the present. Two different nitrogen-containing rings—a five-membered imidazole ring and a six-membered pyrimidine ring—combine to produce this bicyclic framework. The rings are joined in such a way that atoms C and N are joined in two places that are close together. It is due to the presence of many nitrogen atoms in the purine ring system, which can engage in proton transfer, that the ring exists in a number of tautomeric forms. Four N–H tautomeric forms are thought to be the most stable and frequently seen of these. The aromatic character that the fused ring system retains, has a significant effect on the relative stability of these tautomers because more aromatic stabilization means lower energy of such molecule and the stabilization of certain proton configurations [4]. Purine's chemical identification and laboratory synthesis were established as early as the mid-1800s, although its biological significance was not readily apparent at that time. Purine's function in biological systems was not well understood in the early research, (Figure 1) which mostly concentrated on it as a separate heterocyclic molecule produced by chemical synthesis [5]. The discovery that purine is a basic structural element of genetic material came about just a few decades after purine bases were effectively separated from naturally occurring biomolecules, especially nucleic acids. The restricted analytical methods available in the early stages of biochemical study made it incredibly difficult to separate and identify the elements of nucleic acids, which is why this discovery was delayed [6]. Differences in aromatic character within the fused heterocyclic ring system significantly influence the relative stability of various N–H tautomeric forms of purine. The energetic favourability of the tautomer rises with the degree of aromatic stabilization [7].

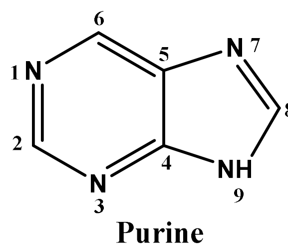


Figure 1. Parent core of Purine moiety

Theoretically and experimentally, purine protonation sites are stable in the following order: 9-H > 7-H > 3-H > 1-H. Based on this, protonation at N1 is the less thermodynamically stable configuration, while protonation at N9 is the most thermodynamically favorable configuration. Thorough aromaticity investigations reveal that the pyrimidine ring is more aromatic when the proton is removed from the pyrimidine N atoms (particularly the H9 and H7 tautomers). The

imidazole ring, on the other hand, is less aromatic in the H1 and H3 tautomers, where it is the imidazole unit that is more populated with protons. This results in a reduction in the purine framework's overall stability. These results have been objectively confirmed using the Harmonic Oscillator Model of Aromaticity (HOMA) in which the extent of bond length equalization was used as an indicator of aromatic character. In the pyrimidine part of purine, HOMA calculations are always larger for the tautomers with the H9 and H7 position than the ones with the H9 and H3 or H7 and H3 position, which hinders the delocalization of the electrons. The distribution of aromaticity in the fused system [9-11] thus determines the preferred tautomer of purine. The atoms in the purine ring are numbered in the usual IUPAC fashion to ensure consistent assignment of substitution sites and structure. Numbering begins at the N1 of the six membered pyrimidine ring [8]. Then rotates clockwise around this ring C2, N3, C4, C5, and C6. When the pyrimidine part is complete it is numbered into the fused five-membered imidazole ring. The imidazole ring is numbered clockwise starting with N7, then C8, then N9, as opposed to the pyrimidine ring [9]

Table 1: Atoms and its position

ATOMS	POSITIONS	SOURCE
Nitrogen	1	Amino group of aspartates
Carbon	2	Formyl THFA
Nitrogen Carbon	3 4	amide *N* of glutamine glycine
Carbon	5	glycine
Carbon	6	Respiratory CO_2
Nitrogen	7	glycine
Carbon	8	Methylene THFA
Nitrogen	9	Amide *N* of glutamine
Adenine	-	6-amino purine
Guanine	-	2-amino, 6-oxypurine
Hypoxanthine	-	6-oxy purine
Xanthine	-	2,6-dioxy purine
Uric acid	-	2,6,8-tri-oxopurine

2. Geometry of N9 position of purine

Depending on the particular tautomeric form of the molecule and the type of substitution at that location, the nitrogen atom at the N9 position of the imidazole ring in the purine framework may display pyramidal or almost planar geometry. The partial sp^3 character of the lone pair on N in the 9H tautomer of purine makes it protonated and has a hydrogen atom attached, preferring a slightly pyramidal structure. However, in the process of purine making nucleosides, the hydrogen at N9 is replaced by a sugar, and the sugar is connected to the N9 atom of the purine by an N-glycosidic bond to the anomeric carbon of the sugar, whether it be ribose or deoxyribose. These nucleoside compounds typically adopt a more planar conformation of the N9 atom and it is suggested that this N9 is more sialic, associated with conjugation and glycosidic linkage stability. Crystallographic and spectroscopic studies of purine nucleosides indicate that the length of the C–N9 glycosidic bond is typically between 1.45 and 1.50 Å and is consistent with it having a partial double-bond character due to electron delocalization between the purine base and the sugar ring. All these geometric

features are important for base stacking interactions and enzyme recognition processes, and for maintaining correct relative orientation of bases within DNA and RNA helices [10]. Stable optimized structures for the N9H and N7H purine tautomers have been verified by quantum chemical simulations using MP2-level geometry optimization under Cs symmetry restrictions. Effective electron delocalization in the fused ring system is supported by the bond angle at the N9 location, namely the C4–N9–C8 angle of around 115°. In these tautomeric forms, this geometric arrangement greatly enhances aromatic stability [6]. In order to verify the stable optimized structures of the N9H and N7H tautomers of purine, quantum chemical simulations with geometry optimization at the MP2 level under Cs symmetry have been performed. The bond angle C4–N9–C8 is approximately 115° which is conducive to effective electron delocalization in the fused ring system. The X-ray crystallographic studies of 9-substituted purine derivatives indicate that this arrangement is basically planar with the root-mean-square deviations typically under 0.05Å, and places a large degree of aromatic stability. Conversely, substituents attached at N9 tend to assume a twisted conformation and can be measured with respect to the dihedral angle of the purine ring plane. This conformational behavior shows the steric and electronic effects caused by N9 substitution and maintains the aromaticity of the system [11].

3. Metabolic Pathway of Purine

3.1. De-novo pathways

The process of building the purine ring directly on a phosphoribosyl (PRPP) scaffold utilizing amino acids, carbon dioxide, and one-carbon units from folate as basic precursors is known as de novo purine nucleotide biosynthesis. Instead of reusing preexisting bases, this route builds the nucleotide step by step. It is the main pathway by which cells produce purine nucleotides [12]. De novo purine biosynthesis is a highly conserved and energy-intensive process that uses ATP, glutamine, glycine, aspartate, CO_2, and one-carbon units in ten enzyme-catalyzed stages to construct the purine ring on PRPP, with IMP serving as the primary intermediary. To satisfy cellular needs for growth and metabolism, IMP is later transformed into AMP or GMP via distinct enzyme pathways. Concurrently, the salvage route via HGPRT and APRT can recycle free purine bases, preserving cellular energy [13]. The coordinated action of several enzymes that can form transient multi-enzyme complexes tightly regulates the DNPB pathway, increasing pathway flux by almost seven times and preferentially directing IMP toward AMP synthesis, resulting in AMP levels that are three to four times higher than GMP (Figure 2) [14]. During periods of high nucleotide demand, this metabolic channelling reduces diffusion losses and improves catalytic performance. According to recent research, these dynamic assemblies are known as purinosomes, or purine corpuscles, which are found close to mitochondria and link the supply of ATP with biosynthetic activity. Purine synthesis can quickly respond to cellular proliferation and metabolic stress because to its spatial and functional arrangement [15].

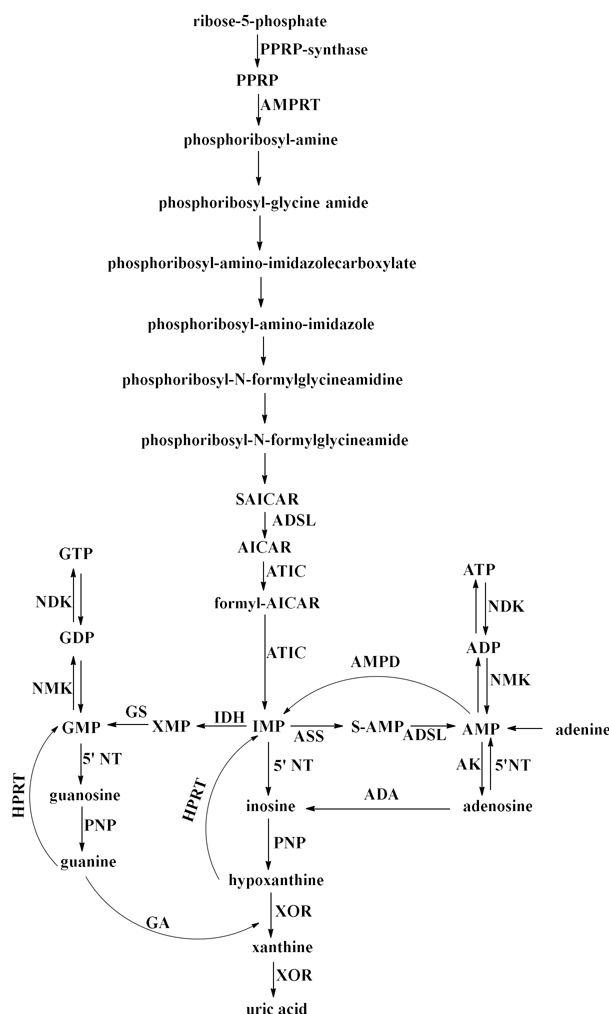


Figure 2. Purine metabolic pathways. Abbreviations: 5′NT, 5′-nucleotidase; AICAR, aminoimidazole carboxamide riboside; ADA, adenosine deaminase; AK, adenosine kinase; ADP, adenosine diphosphate; ADSL, adenyl succinate lyase; AMP, adenosine monophosphate or adenylic acid; AMPD, adenylate deaminase; AMPRT, amidophosphoribosyltransferase; APRT, adenine phosphoribosyl transferase; ASS, adenyl succinate synthetase; ATIC, AICAR-transformists/IMP-cyclopyrrolone; ATP, adenosine triphosphate; GA, guanase; GDP, guanosine diphosphate; GMP, guanosine monophosphate or guanylic acid; GS, GMP-synthase; GTP, guanosine triphosphate; HPRT, hypoxanthine-guanine phosphoribosyl transferase; IDH, IMP-dehydrogenase; IMP, inosine monophosphate or inosine acid; NDK, nucleoside diphosphate kinase; NMK, nucleoside monophosphate kinase; PRPP, phosphoribosyl pyrophosphate; SAI-CAR, succinyl-aminoimidazole carboxamide riboside; S-AMP, succinyl-AMP or adenyl succinate; XMP, xanthine monophosphate or xanthylic acid; XOR, xanthine oxidoreductase.

3.2. Salvage Pathway

The purine salvage route eliminates the requirement for energy-intensive de novo synthesis by recovering free purine bases and nucleosides produced by nucleic acid turnover and converting them back into nucleotides [1]. In organs like the brain and bone marrow that have limited biosynthetic capability or fast cell division, this recycling process is very important. The salvage mechanism sustains ongoing DNA and RNA production as well as general metabolic balance by preserving sufficient nucleotide pools. By moving ribose phosphate from PRPP to purine bases, important enzymes including HGPRT and APRT play crucial roles in this process [16]. By repurposing purine bases from food or intracellular nucleotide breakdown, the salvage mechanism works in tandem with de novo synthesis to produce nucleotides with low energy consumption, usually needing just one ATP equivalent per purine. The primary mediators of this system are APRT, which changes adenine into AMP, and HPRT1, which uses PRPP as the ribose phosphate donor to change hypoxanthine and guanine into IMP and GMP, respectively [17]. The resulting IMP can then be transformed into AMP or GMP, which undergo further phosphorylation to produce diphosphate and triphosphate nucleotides. Therefore, under different metabolic settings, the salvage method offers an effective and adaptable mechanism to maintain nucleotide pools (Figure 3) [8,18].

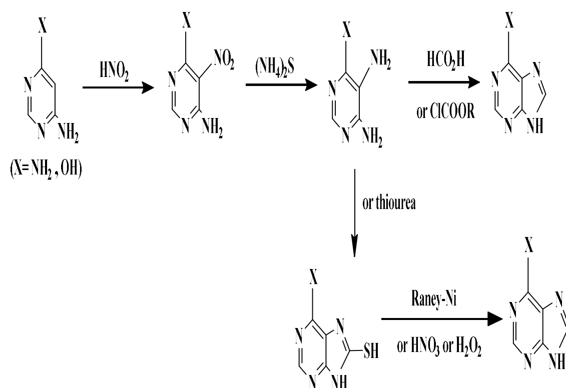


Figure 3. Synthetic pathway for substituted purine derivatives involving diazotization, thiolation, reduction, and oxidative transformation steps

4. Significance of N-9 substitution

Rapid synthesis of a wide range of purine derivatives substituted at both the C-8 and N-9 positions has been made possible by a unique and creative method, greatly expanding the structural variety accessible for biochemical and therapeutic applications [19]. The nitrogen atom in the imidazole ring of the purine scaffold, which is essential to the chemical reactivity and biological recognition of purines, is especially targeted by N9 substitution, which is accomplished by alkylation or arylation. Because it resembles the natural glycosidic bond in DNA and RNA, where the sugar moiety binds to the N9 of adenine and guanine, this alteration is very significant. N9 substitution improves chemical and thermal stability by locking the purine in a particular tautomeric state, which is essential for creating stable nucleoside analogs in drug development [20]. Additionally, these substituted purines are useful resources for researching base pairing interactions, enzyme selectivity, and potentially medicinal nucleic acid analogues [21]. Chemists may selectively alter the imidazole nitrogen while maintaining control over the molecule's general structure thanks to N9-substitution, which is essential in guiding the regioselective synthesis of purine analogs. Alkylation of purine anions with alkyl halides is a common strategy that predictably adds a range of substituents at the N9 position. As an alternative, the purine scaffold can be built using annulation techniques that begin with imidazole precursors and already include N9 substitution [22]. These techniques greatly simplify access to libraries of structurally diverse molecules by efficiently synthesizing a variety of 8,9-disubstituted purine derivatives in one or two synthetic steps. In medicinal chemistry, these regioselective techniques are extremely useful for quickly producing nucleoside analogs and other bioactive purines with specific characteristics [23].

The ability of a number of 9-substituted purine derivatives to replicate protein A binding to human IgG antibodies was produced and methodically assessed. The purine core was essential for biological activity, according to structure–activity relationship (SAR) investigations, underscoring its crucial function in molecular recognition [15]. Among the substances examined, Purine 14 showed a strong binding affinity that was similar to that of natural protein A, indicating that these analogs may serve as protein mimetics. These results imply that N9-substituted purines may be viable options for therapeutic intervention in autoimmune diseases, where it is desirable to modify antibody interactions. The findings highlight how rational purine alteration may be used to create physiologically active compounds with target engagement [11].

5. Synthesis of 9-Purine derivatives

5.1. Alkylation Method

2,6-dichloropurine was the primary precursor used in the synthesis of 9-substituted purines. This process produced a mixture of N9- and N7-alkylated regioisomers (Figure 4) by alkylation with different alkyl halides under basic conditions in DMF at room temperature. Because of the intrinsic regioselectivity at

the imidazole nitrogen, the reaction mostly favored the synthesis of N9-substituted products [24]. The selective character of the reaction was confirmed by using high-resolution HMBC assays to clearly differentiate between N9 and N7 substitution patterns. In order to ensure structural confirmation and purity, the resultant compounds were subsequently thoroughly characterized using conventional spectroscopic methods, such as IR, mass spectrometry (MS), and ^{1}H- and ^{13}C-NMR. Together, these techniques confirm the effectiveness and dependability of this strategy for obtaining N9-functionalized purines for further biological assessment [10,25,26].

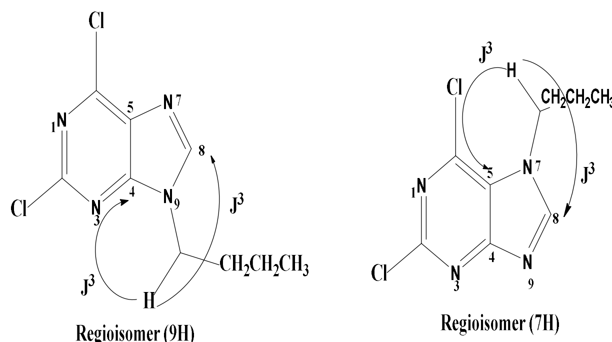
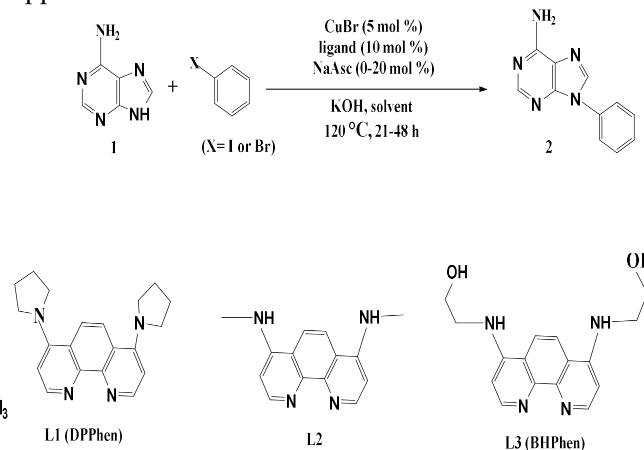


Figure 4. N7/N9 regioselectivity observed in purine alkylation leading to 7H and 9H Regio isomers.

5.2. Arylation Methods

By coupling adenine with aryl iodides and bromides that have a range of polar and non-polar electron-donating and electron-withdrawing substituents, N-arylation of purine derivatives was investigated. Alcohols, phenols, amines, carboxylic acids, cyano, trifluoromethyl, nitro, ketone, and fluoro were among the functional groups that produced high to exceptional yields. CuBr catalyzed (Scheme 1) the reactions in a DMF/H_2O solvent combination at high temperatures, using BHPhen as a ligand, KOH as the base, and sodium ascorbate as an additive [26]. In these circumstances, the arylation mostly took place at the purine ring's N9 position, giving regioselective access to N9-arylated products with moderate to high efficiency and illustrating how spatial limitations affect regioselectivity. This methodology provides a versatile and practical approach for synthesizing structurally diverse purine derivatives for potential biological applications [23,27].

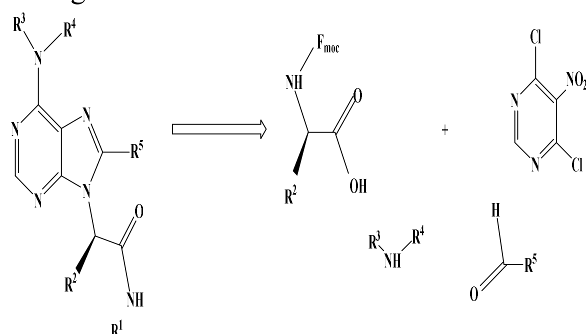


Scheme 1. Copper-catalysed Ullmann-type N-arylation of purine derivatives using CuBr and ligand under basic conditions.

6. Solid phase synthesis

One effective method for creating N9-substituted purine derivatives is solid-phase synthesis, which allows for the quick construction of structurally varied libraries. Fmoc-protected α-amino acids are initially used to functionalize a polymer-bound amine resin at the start of the procedure. Free amine groups are produced by subsequent Fmoc-deprotection [28]. These groups thereafter undergo stepwise nucleophilic aromatic substitution with dichloro-nitro pyrimidines. While still affixed to the solid support, other changes such as intramolecular cyclization and nitro group reduction aid in the creation of the purine scaffold (Scheme 2). Ultimately, the completely formed N9-substituted purine derivatives are released in high yield by cleavage from the resin. This strategy highlights the potential of solid-phase techniques for the quick, effective, and adaptable synthesis of complex purine-

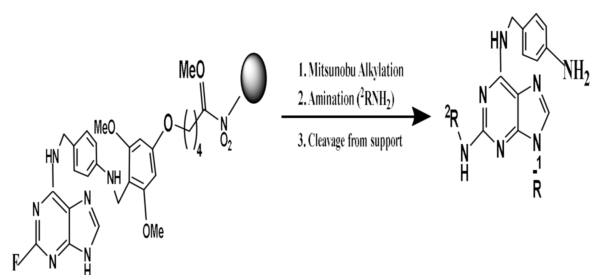
based compounds appropriate for biological assessment [29,30].



Scheme 2. Synthesis of purine–amino acid conjugates via amide bond formation using Fmoc-protected amino acid derivatives.

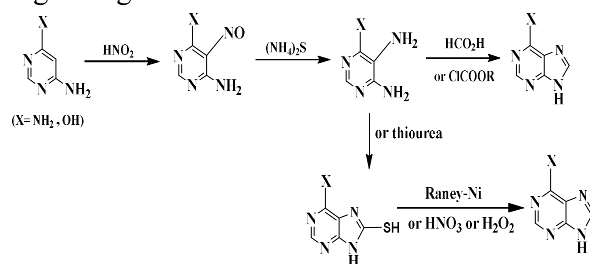
6.1. Classical Approaches

For the combinatorial synthesis of 2,9-disubstituted purine derivatives, a flexible technique has been established, providing quick access to structurally varied purine libraries. This method offers a simple and regioselective way to introduce different alkyl or aryl groups by selectively alkylating the purine scaffold's N9 nitrogen atom via a Mitsunobu reaction [31]. After N9 functionalization, an amination process modifies the purine ring's C2 position, enabling the insertion of a variety of amine substituents. The effective production of 2,9-disubstituted purines in a modular form is made possible by the sequential coupling of these two processes, which allows for the independent variation of both substituents. For medicinal chemistry applications, this combinatorial technique is very useful since it allows for quick investigation of structure–activity correlations and the identification of bioactive purine analogs (Scheme 3) [32].



Scheme 3. Solid-phase combinatorial synthesis of 2,9-disubstituted purine derivatives via Mitsunobu alkylation, C2 amination, and resin cleavage.

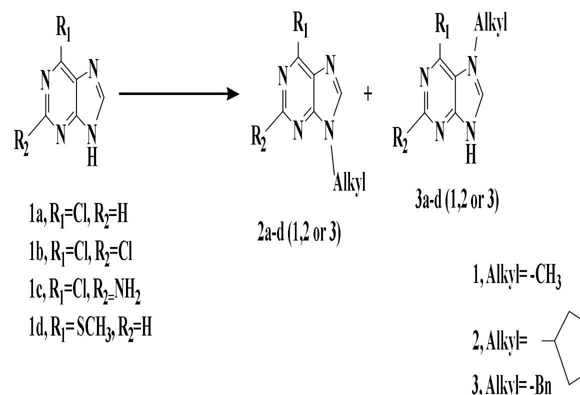
A traditional method that builds the purine ring system from pyrimidine precursors is the Traube purine synthesis. Purines are characterized by the production of a fused imidazole-pyrimidine scaffold, which is made possible via a series of transformations that include nitrosation, reduction, and formylation [33]. One of the main benefits of this approach is that N9 substituents may be added early on, before the purine ring is finished, giving control over regioselectivity and making the synthesis of N9-functionalized derivatives easier (Scheme 4) [31]. This method has been widely used to produce structurally varied purine analogs in both medicinal chemistry and natural product synthesis. The Traube synthesis is still a useful method for obtaining purines with specific chemical and biological characteristics by pre-installing substituents and closely regulating reaction conditions [33].



Scheme 4. Traube purine synthesis reaction

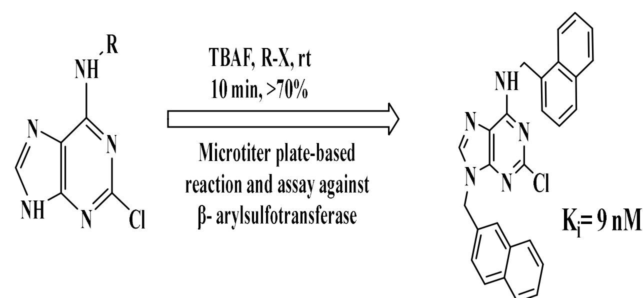
7. Regioselectivity of 9-purine

Purine alkylation at the N9 position is very interesting for the synthesis of several molecules with possible medical uses. The effective production of N9-alkylated purines was made possible in this work by the development of a reliable and regioselective technique under moderate circumstances [34]. When compared to traditional heating techniques, microwave-assisted reactions greatly shorten reaction durations, improve yields, improve regioselectivity, and raise crude product purity. Furthermore, employing tetrabutylammonium hydroxide ($(Bu)_4NOH$) as a base enhances purine solubility and further boosts yields, overcoming typical problems with conventional N9-alkylation that frequently result in combinations of N-alkylated Regio isomers. Alkylation at either the N7 or N9 sites can yield purine derivatives, and regioselectivity is an important factor [26]. Tetrabutylammonium hydroxide $(Bu)_4NOH$ produced the best results in this study, which examined a variety of bases and improved reaction conditions to promote selective N9-alkylation. The choice of base and solvent has a significant impact on the reaction's efficiency. Additionally, microwave irradiation improves N9 selectivity, shortens reaction durations, and lowers the production of unwanted secondary products. This process makes it possible to synthesize N9-functionalized purines with greater control and yield for possible medicinal uses (Scheme 5) [23,33].



Scheme 5. TBAF-Mediated Regioselective N- Alkylation of Purine Derivatives

Purine scaffolds react with organic halides in the presence of tetrabutylammonium fluoride (TBAF) to produce N-alkylation quickly and reliably. This method is a useful technique for synthesizing N9- or N7-substituted purine derivatives because it provides great yields and high selectivity toward the target product (Table 2). The technique is very useful for producing various purine analogs in medicinal chemistry applications because of its effectiveness and repeatability (Scheme 6) [35].



Scheme 6. TBAF- Promoted Rapid N9-Selective Alkylation of Purine Scaffold

Table 2. Comparative summary of N9-selective functionalization strategies for purine derivatives, highlighting selectivity, reaction conditions, and substrate scope.

Method	Selectivity	Conditions	Scope

TBAOH + Microwave	Highest (>95% N9)	Microwave, alkyl halides	Alkyl groups
TBAF	Very high (>90% N9)	RT, Alkyl halides	Combinatorial
Cu/BHPhen Arylation	Exclusive N9	120˚C, aryl halides	Aryls, heteroaryl
Transition-metal-free amidoalkylation	Selective N9	Sp3 C-H activation	Amido alkyl

8. Molecular docking studies

The binding affinities of N9-substituted purine derivatives to protein targets implicated in cancer-related signalling cascades, including kinases like MAPKAPK2 and eIF4E, are often evaluated by molecular docking studies. These derivatives participate in important hydrophobic and hydrogen bonding interactions inside the enzyme active sites; they frequently have alkyl or cyclopropyl methyl substituents at N9 [36]. Their potential as powerful inhibitors have been confirmed by computational assessments utilizing docking tools such as Auto Dock and CDOCKER. The therapeutic importance of N9-substituted GMP analogs is highlighted by their ability to prevent Ser209 phosphorylation when docked to eIF4E, with binding energies of up to –54 kcal/mol through hydrogen bonds and π–π interactions [37]. 2,6,9-trisubstituted purine derivatives, which were computationally docked into MAPKAPK2 (PDB:2JBP) and MAPK-1 (PDB:1PME), have been employed in recent investigations to target anticancer kinases. These compounds have substantial binding affinities, similar to the co-crystallized ligands, with estimated energies ranging from –65 to –79 kcal/mol. The stability of the binding is achieved via hydrophobic contacts with Ile74 and hydrogen bonds with residues like Leu141, indicating the potential of these purine derivatives as strong kinase inhibitors [36,38]. The binding affinities of the 2-amine-9H-purine scaffold and similar purine derivatives toward enzymes such bromodomains, kinases, and mutant versions of EGFR are often studied. Specifically, an induced-fit process between the 2-amine-9H-purine scaffold and BRD9 results in the conformational reconfiguration of the acetyllysine recognition pocket. The scaffold's usefulness in creating strong enzyme inhibitors and epigenetic modulators is highlighted by its adaptive binding, which permits high-affinity interactions that frequently exceed nanomolar potency (Table 3) [39]. In order to maximize binding predictions, Glide SP mode molecular docking investigations on BRD9 structures, including PDB entries containing Tyr106, provide variable selection of ligand poses with scaled van der Waals interactions. It has been demonstrated that N9-arenethenyl purine derivatives target Src and Abl kinases in both their DFG-in and DFG-out conformations, allowing for conformational flexibility. The significance of residues like Ala403 and Phe405, whose binding is mostly driven by van der Waals interactions, is highlighted by docking in conjunction with molecular dynamics simulations. Furthermore, structure–activity connection investigations have been supported by the use of CoMFA (Comparative Molecular Field Analysis) models to forecast the biological activity of these derivatives [38]. L858R/T790M/C797S EGFR mutants have been shown to be strongly inhibited by 9-heterocyclyl 9H-purine derivatives, with lead drug D9 showing an IC50[1] of around 18 nM. According to docking experiments, the N9-linked cyclopropylsulfonamide and Ser797 establish important hydrogen bonds that improve the triple mutant's selectivity.

Similar to this, N9-cyclopentyl purine-hydrazones dock efficiently into the active sites of EGFR and HER2, often outperforming reference compounds in predicted binding scores. Similar computational methods have been used to confirm ligand poses and interactions for various targets, such as HSV-1 thymidine kinase, Aurora kinases, katanin, and CDK9, utilizing programs like Glide, Auto Dock, and molecular dynamics simulations [36–38].

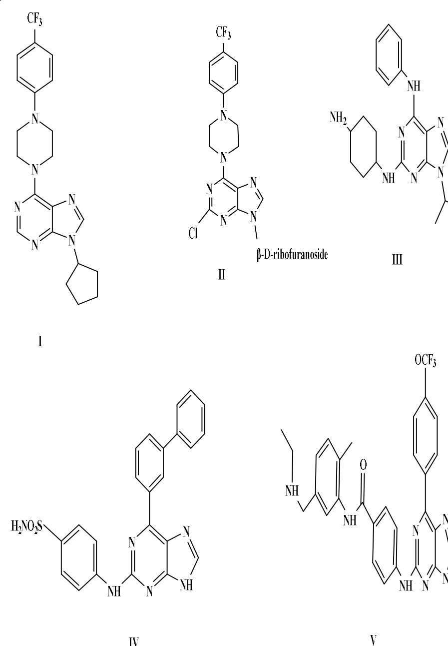
Table 3. Summary of molecular docking results for substituted purine derivatives against selected protein targets, highlighting key interactions and predicted binding energies.

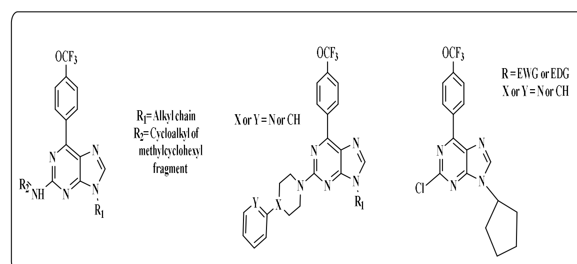
Target	Derivative Example	Key Bonds	Binding Energy (kcal/mol)
MAPKAPK2(2JBP)	9a (n-hexyl at N9)	N7-Leu141 H-bond, alkyl-Va178 hydrophobic	-69.07
ElF4E	4b (benzyl at N7, phosphonamidite)	Carbonyl-Ser209 H-bond, pi-Lys159	-54.02
TLR9(5ZLN)	Purine-pyrimidine hybrids	Multiple H-bonds to Ala/Asn	-8.1

9. Biological activities of 9-substituted purine derivatives

9.1. Anti-Cancer activity

It was discovered that a key factor influencing cytotoxic action was substitution at the purine core's C-2 location [1]. Specifically, antiproliferative effects were significantly enhanced when nitrogen-containing substituents (Series I and II) were replaced with a chlorine atom (Series III), suggesting that a less bulky and electron-withdrawing group at C-2 is more advantageous for biological activity. Additionally, only when linked at the C-6 position (Series III) did aryl-piperidinyl and aryl-piperazinyl moieties significantly contribute to cytotoxicity; their attachment at the C-2 position (Series II) resulted in decreased activity, underscoring the strong influence of substitution pattern on structure–activity relationships (Scheme 7) [40].





Scheme 7. Design and Structural Optimization of Substituted Purine Derivative (Series I-V)

Comparing Series I and Series II further revealed that, in comparison to bulkier arylpiperazine or arylpiperidine fragments, smaller substituents like cycloalkyl or methylcyclohexyl groups at C-2 increased cytotoxicity against HL-60 leukemia cells. This suggests that steric hindrance at this position adversely affects receptor binding or cellular uptake. The idea that reducing substituent volume at C-2 enhances molecular fit inside the biological target site is supported by this data [18,41].

Sufficient biological data from many cancer cell lines (NCI-H460, HL-60, K-562, and MCF7) enabled more trustworthy SAR findings for Series III compounds, where a chlorine atom occupies the C-2 position. Across all studied cell lines, a similar pattern showed that para-substitution of the arylpiperidine moiety with electron-withdrawing groups like nitro, trifluoromethyl, or chloro groups greatly improved cytotoxic efficacy. Electron-donating or unsubstituted phenyl analogues, on the other hand, showed somewhat less activity, indicating that electronic effects are crucial in controlling target interactions [1,15].

Furthermore, phenylpiperidine substitution at the C-6 position was often more effective than phenylpiperazine, especially against NCI-H460 lung cancer and HL-60 leukemia cells, suggesting that basicity and ring type may affect cellular permeability and binding affinity. Overall, our results show that the kind and location of substituents on the purine scaffold significantly control antitumor efficacy, offering helpful recommendations for future purine-based anticancer drug improvement (Table 4) [23,30].

Table 4. Structure -activity relationship of substituted purine derivatives highlighting the influence of substitution position, electronic effects, and steric factors on cytotoxic activity

Structural Position	Substitution	Effect on cytotoxicity Activity	SAR Results
C-2 position	Chlorine (Series III)	Increased cytotoxicity vs Series I& II	Cl at C-2 is favourable
C-2 position	Nitrogenated fragments (Series I &II)	Lower cytotoxicity	Less effective than halogen
Position of aryl piperidinyl/aryl piperazinyl group	Attached at C-6 (Series III)	Higher cytotoxicity	C-6 is optimal attachment site
Position of aryl piperidinyl/aryl piperazinyl group	Attached at C-2 (Series II)	Reduced activity	C-2 attachment unfavourable
N-9 position	Long alkyl chain (general)	No significant effect	N-9 not a key determinant
N-9 position	Pentyl group	Enhanced activity in selected cell lines	Hydrophobicity contributes to potency
C-2 substituent size	Cycloalkyl/methyl	Increased cytoto	Smaller substituents

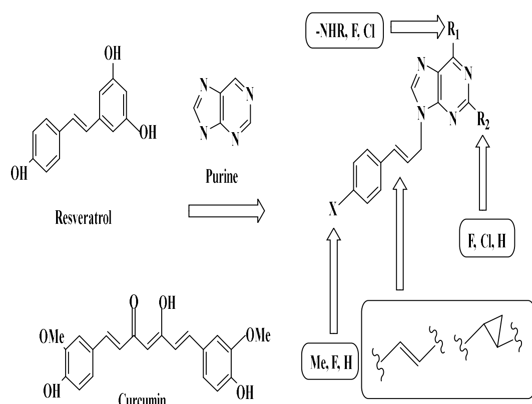
	cyclohexyl	xicity (HL-60)	preferred
C-2 substituent size	Aryl-piperidine / aryl-piperazine	Decreased activity	Large substituents
C-6 arylpiperidine substitution	Para-EWS (NO_2, CF_3, Cl)	Increased activity in all tested cell lines	EWGs enhance antitumor activity
C-6 arylpiperidine substitution	EDG or no substitution	Reduced cytotoxicity	EDGs unfavourable
Aryl ring type (HL-60)	Benzene ring	Required for high cytotoxicity	Aromatic benzene essential
Aryl ring type (HL-60)	Nitrogenated heterocycle	Reduced activity	Heterocycles less favourable
C-6 moiety type	Phenylpiperidine	Higher cytotoxicity	Piperidine prefferred
C-6 moiety type	Phenylpiperazine	Lower cytotoxicity	Less potent analogue

9.2. Anti-viral activity

A similar sequence of 9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphate derivatives was produced in this work by consistently substituting a β-D-ribophorins moiety at the N-9 position of all synthesized molecules [42]. Since the ribophorins group is essential for imitating natural nucleosides and being recognized by cellular transporters and metabolic enzymes, no structural changes were made at N-9. The cellular absorption and subsequent phosphorylation processes, which are crucial for antiviral action, are facilitated by this sugar moiety [43]. Therefore, N-9 substitution cannot be blamed for differences in biological response among the compounds. Rather, variations in antiviral effectiveness were mostly determined by the type of substituents added at the purine ring's C-6 position, highlighting C-6 as a crucial location for structure–activity optimization [44].

9.3. Anti-inflammatory

The SAR study of N9-substituted purine derivatives demonstrated that functionalization at the N-9 position is a significant determinant of anti-inflammatory activity, providing the fundamental pharmacophoric scaffold necessary for interaction with the TLR4/MyD88/NF-κB signalling cascade [45]. The insertion of a cinnamyl moiety at N-9, in particular, greatly increased biological response, while substituents on both the cinnamyl phenyl ring and the purine core further modulated the reaction. The maximum inhibitory action was obtained by para-methyl substitution on the cinnamyl phenyl ring, demonstrating that moderately hydrophobic electron-donating groups enhance receptor binding, whereas unsubstituted or fluoro counterparts had decreased effects (Scheme 8) [46,47].



Scheme 8. SAR-Guided Design of Resveratrol/ Curcumin- Inspired Purine Derivatives

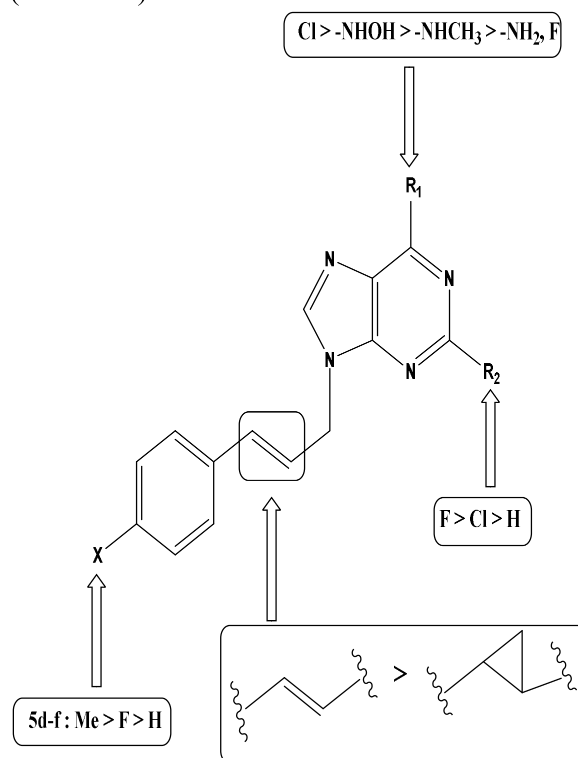
Halogen substitution at the purine nucleus, particularly chlorine at the C-2 and C-6 locations, significantly boosted activity, most likely due to increased lipophilicity and favorable electrical interactions inside the binding pocket. In contrast, replacing chloro groups with polar functions such as amino or hydroxylamine resulted in lower efficacy, suggesting that excessive polarity at these positions is deleterious (Table 5). Overall, the SAR reveals that an appropriate balance of hydrophobic N9 substitution paired with electron-withdrawing halogens on the purine ring is crucial for maximising anti-inflammatory activity in this chemical series [47].

Table 5. Structure–Activity Relationship (SAR) Trends of N9-Substituted Purine Derivatives with Anti-Inflammatory Activity

Modification	Trend in Activity
N9-cinnamyl core	Required for activity
Para-Me on cinnamyl phenyl	Increased activity
Cl at purine positions (X, Y)	Increased potency
Amino substitutions on purine	Decreased activity
Fluoro substitutions on purine	Typically, decreased or neutral

9.3.1. Mechanism of N9- purine derivatives

N9-substituted purine derivatives have antiviral efficacy primarily via functioning as structural mimics of natural nucleosides, allowing them to access viral replication pathways. After intracellular activation, these analogues interfere with viral DNA or RNA polymerases, resulting in chain termination or incorrect genome synthesis [46,47]. This disruption effectively prevents viral reproduction and dissemination within host cells. Many therapeutically employed antiviral purine analogues exhibit well-known nucleoside-mimicking activity (Scheme 9) [47].



Scheme 9. Structure–Activity Relationship (SAR) of N9-Substituted Purine Analogues

Following intracellular phosphorylation, several N9-substituted purine analogues

function as chain terminators or competitive inhibitors of viral polymerases. For example, 9-[2-(phosphonomethoxy)alkoxy] purines (PMEA and similar derivatives) are transformed into active diphosphate forms that substantially block herpesvirus and retrovirus DNA polymerases, including visna virus. Their selectivity is mostly owing to preferential activation by viral thymidine kinase in infected cells, which minimizes damage to uninfected host tissues, a process similar to acyclovir. This tailored activation leads to the excellent antiviral effectiveness and enhanced safety profiles [48,49].

Some purine nucleoside derivatives with sulfonamide groups improve plant resistance to viral infections like tobacco mosaic virus (TMV) and potato virus Y (PVY) by boosting host defense systems rather than having direct virucidal effects. These chemicals increase the expression of antioxidant enzymes such as peroxidase (POD) and superoxide dismutase (SOD), as well as defence-related genes like PR-1. They also enhance the production of photosynthesis-associated proteins. This stimulation of plant immunological and physiological processes improves viral tolerance while generating no phytotoxicity, even at relatively high treatment doses. Host-directed antiviral techniques provide an ecologically friendlier alternative to traditional antivirals [47]. Some purine nucleoside derivatives with sulfonamide groups improve plant resistance to viral infections like tobacco mosaic virus (TMV) and potato virus Y (PVY) by boosting host defence systems rather than displaying direct virucidal activity. These chemicals increase the production of antioxidant enzymes such as peroxidase (POD) and superoxide dismutase (SOD), as well as defence-related genes like PR-1 and photosynthesis-associated proteins. Even at relatively high treatment concentrations, activation of plant immunological and physiological mechanisms improves viral tolerance while avoiding phytotoxicity. These host-directed antiviral techniques provide a safer alternative to traditional antivirals [47,50].

9.3.2. Structure Activity Relationship

The antiviral activity of N9-substituted purine derivatives is greatly impacted by both the substituent at the N-9 position and other purine ring modifications. Lipophilic groups at N-9 consistently improve membrane permeability and target binding, resulting in increased effectiveness against viruses such as herpesviruses, enteroviruses, and rhinoviruses, according to SAR data. Furthermore, particular ring modifications, such as halogens or electron-drawing groups at C-2 and C-6, improve potency by stabilising interactions with viral enzymes. These combined structural properties are thus crucial for establishing broad-spectrum antiviral effects [51]. The nature of the N-9 substituent is important in defining the antiviral spectrum of purine derivatives. Alkoxy side chains, such as 3-hydroxypropoxy, promote viral kinase activation, resulting in increased anti-HSV activity and, in some studies, greater effectiveness than acyclovir. In contrast, aryl substituents like 2-fluorobenzyl suppress enteroviruses, particularly when coupled with C-6 halogenation, resulting in EC_{50} values in the 5-8 μM range. Furthermore, acyclic phosphonomethoxyalkyl chains give significant selectivity for viral DNA polymerases in retroviruses, creating active diphosphate metabolites [45,52]. Additionally, sulfonamide groups at C-6 can stimulate plant defense gene expression against TMV and PVY, while trifluoromethyl substitution at C-2 has been linked to inhibition of rhinovirus uncoating processes. These observations confirm that both hydrophobicity and electronic effects at ring positions critically govern antiviral and host-response activities. Halogen substitution, such as chlorine or bromine at

the purine ring's C-2, C-6, or C-8, while polar groups, such as methoxy significantly reduce antiviral potency (Table 6) [53].

Table 6. Structure–Activity Relationship (SAR) of Substituents on Antiviral Activity

Position	Favorable	Unfavorable	Target Viruses
N9	Aryl-halogen, alkoxy	Bulky polar	HSV, Enterovirus
C6/C8	Cl/Br	CN/OH	Rhinovirus, Grp94-related
C2	CF_3, amino	Methyl	Rhinovirus

9.4. Anti-diabetic activity

Dipeptidyl peptidase-4 (DPP-4) has been shown to be selectively inhibited by certain 8-substituted purine derivatives that are structurally similar to N9-modified purines. This prolongs the action of incretin hormones like GLP-1 and GIP and increases insulin production. Oral treatment of these substances dramatically decreased fasting blood glucose and HbA1c levels by around 1% in Zucker diabetic fatty (ZDF) rat models, demonstrating successful glycemic management [54]. There was no discernible toxicity during therapy, and their pharmacological profile and effectiveness were similar to those of the clinically utilized DPP-4 inhibitor vildagliptin. These results imply that, in addition to their antiviral uses, substituted purine scaffolds may potentially be effective antidiabetic medicines [53]. By inhibiting gluconeogenesis and increasing glycogen synthesis, purine nucleosides like xanthosine have been demonstrated to regulate hepatic glucose metabolism and support blood glucose homeostasis in diabetic animals. Furthermore, the incidence of type 2 diabetes is inversely correlated with purine catabolites such as hypoxanthine, suggesting possible preventive benefits through the control of cellular energy metabolism and nucleotide turnover. These findings imply that purine nucleosides and their metabolic products may be crucial for metabolic health and glycemic regulation (Table 7) [51].

Table 7. Mechanisms and Effects of Purine-Based Anti-Diabetic Compounds

Compound	Mechanism	Model/Effect
8-Purine DPP-4 inhibitor	Incretin stabilization	ZDF rats: ↓HbA1c 1%
Xanthopsin	↓Gluconeogenesis, ↑glycogenesis	Hepatic cells
Purine catabolites	Energy pathway modulation	T2D risk reduction

9.5. Anti-fungal

9-Purine derivatives exhibit encouraging antifungal potential, especially when fungal inositol polyphosphate kinases such as Arg1 and Kcs are specifically inhibited. According to recent research, DT-23, a substituted purine analogue, is a strong inhibitor of Kcs1 (MIC50 = 15 µg/mL) and Cryptococcus neoformans Arg1 (IC50 = 0.68 µM). At modest dosages (1.5 µg/mL), it works in concert with amphotericin B to improve survival in insect infection models and increase effectiveness against C. neoformans in vitro [51]. These purines interfere with the production of inositol phosphate, which is necessary for fungal virulence. DT-23 reduces IP7 in a dose-dependent manner and alters gene expression in a way that resembles IPK deletions. DT-23 has greater solubility and dual-targeting for more extensive antifungal effects than earlier equivalents like TNP [50].

9.5.1. Structure activity relationship

Incorporating a trifluoromethyl ($–CF_3$) group at the meta-position of the N2-benzylamino substituent significantly

improves cell permeability and inhibitory potency against Cryptococcus neoformans Arg1 (IC • ≈ 0.6 μM), enabling effective growth suppression (MIC • ≈ 15 μg/mL) without combination therapy [50]. The significance of this lipophilic substituent is shown by the low antifungal activity of analogues like DT-12 that lack the $–CF_3$ group. Additionally, dual inhibition of Arg1 and Kcs1 is maintained by substituting an improved nitrobenzylamino group for TNP's simple benzylpiperazine at the N6 position, with Kcs1 IC·$_0$ values of around 0.68 μM. These changes show how purine substituents may be specifically tuned electronically and lipophilic ally to greatly increase antifungal activity [47].

9.6. Anti-bacterial

The primary mechanisms by which N9-substituted purine derivatives exhibit antibacterial action are the inhibition of vital bacterial enzymes and the disruption of important metabolic pathways. Several analogues have potency that is on par with conventional antibiotics like ciprofloxacin, even when used against resistant strains [46]. Interestingly, compound, which has a 4-chlorobenzylamino substituent at C-6 and N9 alteration, showed strong action against clinical and standard MRSA isolates, much like ciprofloxacin. Furthermore, the unsubstituted N9 analogue and 9-cyclopentyl-6-[(4-fluorobenzyl) amino]-9H-purine were notably successful against Gram-positive bacteria, particularly Staphylococcus aureus [44]. These findings demonstrate the potential of N9-functionalized purines as starting points for the creation of novel antimicrobial compounds (Table 8) [42].

9.6.1. Structure activity relationship

Numerous N9-substituted purine derivatives have antibacterial properties through the inhibition of important bacterial enzymes, including glutamate racemase, which is necessary for the manufacture of peptidoglycan; 9-benzyl substitutions provide micromolar-level inhibition [31]. Furthermore, newly created 9-alkyl purine–thiazole hybrid compounds, including compound 3A7, have potent action against Xanthomonas species and other plant harmful bacteria by rupturing cell membranes and generating intracellular reactive oxygen species (ROS). These hybrids have EC_{20} values of around 25.5 μg/mL, suggesting that multi-target mechanisms effectively restrict growth [26]. These results show that purine structural hybridization can expand the antimicrobial range and improve antibacterial efficacy [33].

Table 8. Antibacterial Activity of Selected Compounds Against Target Strains

Compound	Target Bacteria	Activity (MIC/EC50)
22	MRSA (std/clinical)	Comparable ciprofloxacin
24	S. aureus, B. subtilis	3.12 μg/mL (re antifungal)
3A7	Xanthomonas oryzae	25.5 μg/mL
9-Benzyl purines	Glutamate racemase	Micromolar inhibition

9.7. Anti-HIV

HIV reverse transcriptase (RT) is the main mechanism via which purine derivatives, particularly nucleoside and nucleotide analogs, have strong anti-HIV activity. These analogs compete with natural deoxynucleotides for inclusion into the expanding viral DNA strand following intracellular phosphorylation to their active triphosphate forms [36]. The inclusion of these chemicals causes premature chain termination, which stops proviral DNA synthesis since many of them lack a functional 3′-hydroxyl group. Certain purine analogs reduce viral replication even further by acting as competitive inhibitors of reverse transcriptase in addition to direct chain termination [35,55]. Purine-based nucleoside analogs are a key component of antiretroviral therapy due to this dual

mechanism, which also explains their continued therapeutic significance in HIV treatment [18]. With IC•^{1} values ranging from 2 to 60 μM, 2-Amino-9-(3-azido-2,3-dideoxy-β-D-erythro-pentofuranosyl)-6-substituted-9H-purine derivatives with 6-alkoxy, 6-alkylamino, or 6-arylamino groups demonstrate significant anti-HIV activity by shielding MT-4 lymphocyte cells from HIV-1 (IIIB)-induced cytopathic effects. Their antiviral activity is explained by intracellular phosphorylation, which is followed by HIV reverse transcriptase inhibition, which stops the manufacture of viral DNA [32]. Furthermore, adenine and 2,6-diaminopurine are the sources of the acyclic nucleoside phosphonates (RS)-FPMPA and (RS)-FPMPDAP, which have selective efficacy against both HIV-1 and HIV-2. These substances have better antiviral selectivity with less host-cell toxicity than PMEA and PMEDAP, as evidenced by their higher therapeutic indices 2-Amino-9-(3-azido-2,3-dideoxy-β-D-erythro-pentofuranosyl)-6-substituted-9H-purine derivatives with 6-alkoxy, 6-alkylamino, or 6-arylamino groups show significant anti-HIV activity by shielding MT-4 lymphocyte cells from the cytopathic effects of HIV-1 (IIIB), with IC_{50} values between 2 and 60 μM [38]. Their intracellular phosphorylation and subsequent inhibition of HIV reverse transcriptase, which results in the cessation of viral DNA synthesis, are responsible for their antiviral effect. Furthermore, the acyclic nucleoside phosphonates (RS)-FPMPA and (RS)-FPMPDAP, which are synthesized from 2,6-diaminopurine and adenine, have specific action against HIV-1 and HIV-2. Compared to PMEA and PMEDAP, these substances had greater therapeutic indices, suggesting enhanced antiviral selectivity with less host-cell toxicity (Table 9) [56].

Table 9. Anti-HIV Activity of Purine-Based Nucleoside and Nucleotide Analogues

Compound Class	Substitution	HIV IC50/ EC50	Cell Protection
6-Alkoxy/ amino-purines	N9-azido-dideoxyribosyl	2-60 μM	MT-4 vs HIV-1IIIB
(RS)-FPMPA	Adenine-FPMP	Selective (low μM)	HIV-1/2 > DNA viruses
(RS)-FPMPDAP	2,6-Diaminopurine-FPMP	High therapeutic index	Broad retroviruses
9-Alkoxy purines	Di/polyhydroxypropoxy	Moderate	HIV replication

9.7.1. Structure activity relationship

With IC_{50} values ranging from 2 to 60 μM, N9-azido-dideoxyribosyl purine derivatives with C6-alkoxy or C6-arylamino substituents—especially those with electron-donating groups—show notable anti-HIV action. In order to successfully stop the production of viral DNA, their technique entails intracellular activation followed by reverse transcriptase (RT) chain termination. Retroviral selectivity is also significantly improved by adding a fluorophosphonylpropyl side chain to adenine or 2,6-diaminopurine cores. This change enhances target specificity toward viral polymerases while decreasing host-cell toxicity, leading to improved therapeutic profiles [56,57].

9.8. Anti-tuberculosis

By specifically targeting Mycobacterium tuberculosis's de novo purine biosynthesis pathway, purine derivatives show encouraging anti-tuberculosis potential. Key enzymes like PurF (amidophosphoribosyltransferase), which is necessary for the synthesis of purine nucleotides and bacterial viability, are

inhibited by a number of analogs. Mtb's growth and replication are hampered when this pathway is inhibited because it interferes with the production of DNA and RNA. These inhibitors provide a targeted and therapeutically appealing approach to treating tuberculosis since humans depend less on de novo purine production [58]. The 9-Benzylpurine derivatives containing phenyl ethynyl, trans-styryl, or aryl substituents at C6, as well as chlorine at the C2 position, have strong inhibitory action against Mycobacterium TB. Furthermore, 6-oxo and 6-thio analogs with appropriate N9 substitutions increase effectiveness against both H37Rv and H37Ra strains, demonstrating positive structure-activity correlations. JNJ-6640, a specific PurF inhibitor, lowers lung bacterial burden by 1.5-2 log CFU in both acute and chronic rat tuberculosis models [59]. Its synergistic results with linezolid and bedaquiline highlight the therapeutic value of inhibiting purine biosynthesis in combination TB regimens. These drugs work against mycobacteria by inhibiting PurF (amidophosphoribosyltransferase), a crucial enzyme involved in de novo purine production in Mycobacterium TB. Importantly, they demonstrate strong selectivity for the mycobacterial enzymes and negligible action against human orthologs, as indicated by IC_{50} values surpassing 100 μM in mammalian cancer cells. Structural optimization studies show that 2-chloro substitution on the purine ring and suitable N9 alterations dramatically increase antimycobacterial efficacy, most likely by increasing target binding and cellular absorption [60].

9.8.1. Structure activity relationship

The addition of a chloro substituent at the C2 position significantly improves antimycobacterial activity across 9-benzyl-6-substituted purines, consistently outperforming the corresponding unsubstituted analogues against M. tuberculosis H37Rv. Lipophilic N9 substituents, particularly benzyl or phenethyl groups, are ideal because they improve membrane penetration and intracellular accumulation [59]. In comparison to alkylamino substituents, phenylethynyl and trans-styryl moieties at the C6 position have higher efficacy. Furthermore, to attain modest MIC values, 6-oxo and 6-thio purine analogues require suitable N9 substitutions, with thio-derivatives often exhibiting stronger activity than oxo counterparts [58]. Bulky and hydrophobic aryl substituents at the C6 position, as shown in precursor scaffolds linked to JNJ-6640, greatly improve binding to PurF, but the inclusion of polar functional groups reduces antimycobacterial activity. The reduction of the purine ring to 2,3-dihydropurine analogues does not destroy biological activity, as long as the critical C2 and C6 substituents are intact. This suggests that hydrophobic interactions at C6 and appropriate substitution at C2 are important factors of PurF inhibition (Table 10) [58].

Table 10. Structure–Activity Relationship (SAR) of Purine Derivatives Against Bacterial Targets

Position	Favourable	Unfavourable	Impact on MIC
C2	Cl, F	H, alkyl	2-5x potency boost
N9	Benzyl, phenethyl	H, small alkyl	Enhances uptake
C6	Phenylethynyl, styryl	Amino, alkoxy	Strongest inhibition
6-Oxo/Thio	+N9-alkyl	Unsubstituted	Moderate activity

10. Green synthesis of 9-purine

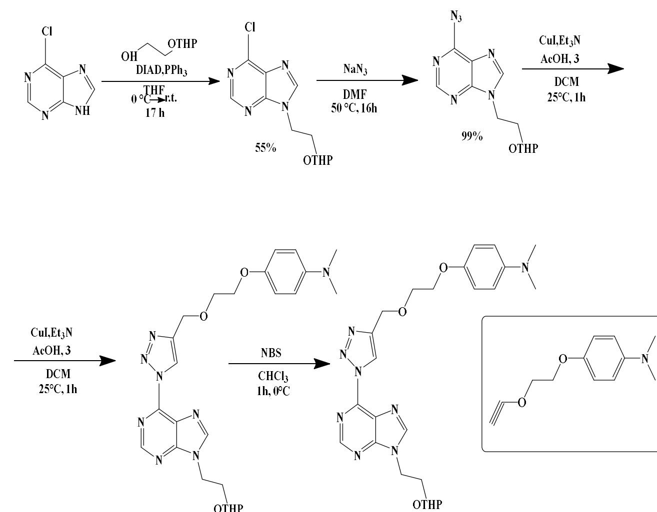
10.1. Green N-9 alkylation/arylation

Green synthesis techniques for 9-substituted purines focus on using

environmentally friendly strategies to functionalize the purine nucleus's N9 position [61]. These technologies frequently use natural or biodegradable catalysts, alternative energy sources, and green solvents like water or ethanol, reducing toxic waste [59]. Enzymatic or solvent-free methods limit the use of hazardous chemicals and transition metals, making the process more sustainable. Overall, these environmentally friendly techniques provide quick access to N9-substituted purines while adhering to green chemistry principles [60].

10.1.1. Mitsunobu-type green synthesis

This process is a crucial green and efficient way for the synthesis of N9-substituted purine derivatives, using alcohols such as cinnamyl or iodoethyl alcohols as alkylating agents and purine bases such as 6-chloro-9H-purine or THP-protected purines. The reaction follows the Mitsunobu technique, which uses DIAD and triphenylphosphine in mild solvents such as THF or water at room temperature, and normally takes 2-4 hours to complete [62]. High isolated yields (70-90%) are usual following routine workup, which includes basification, organic extraction, and silica gel chromatography. The moderate conditions, short reaction periods, and superior regioselectivity make this approach ideal for long-term N9 functionalization of purines (Scheme 10) [63].

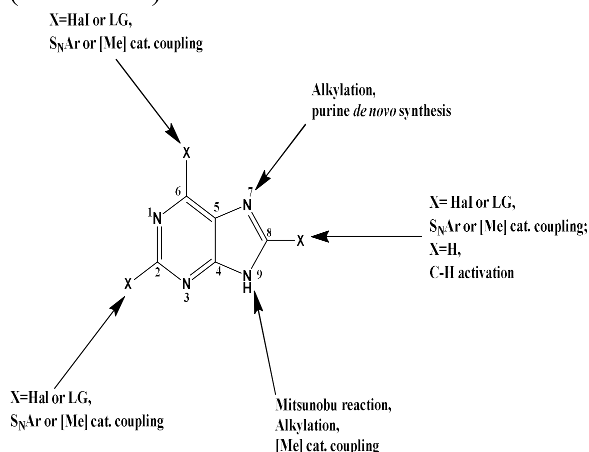


Scheme 10. Stepwise Synthesis of Functionalized N9-Substituted Purine Derivatives

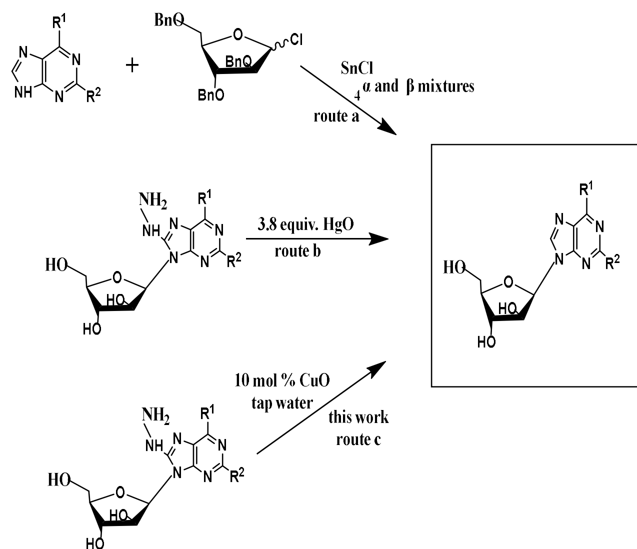
10.2. Green N-9 alkylation / arylation

Recent literature studies emphasize effective methods for N9-alkylation of purine scaffolds using mild bases, phase-transfer catalysts, or microwave-assisted conditions [64]. These reactions are typically performed in green solvent systems such as ethanol-water combinations, which allow for the insertion of alkyl or aryl substituents at the N9 position with high to outstanding yields. Microwave irradiation considerably reduces reaction times, enhances regioselectivity, and reduces by-product generation. Overall, these methods decrease solvent waste and energy consumption, resulting in a lower E-factor and more sustainability for purine functionalization [65]. Several synthetic methods use reusable solid catalysts, such as supported copper complexes or basic metal oxides, to aid in the coupling of purine substrates with alkyl or aryl chlorides. These reactions are frequently carried out in aqueous or solvent-free settings, increasing their environmental friendliness. Heterogeneous catalysts can be easily recovered and reused with little loss of activity [66]. Such methods provide

effective N9-functionalization while minimizing metal contamination, solvent use, and overall environmental impact (Scheme 11) [67].



Scheme 11. Synthetic Strategies for Site-Selective Functionalization of the Purine Core



Scheme12. Synthesis of Nucleoside Analogues via Vorbrüggen Glycosylation and Green CuO-Catalyzed Hydrolysis

10.3. Green synthesis of purine nucleosides (9-glycosylpurines)

A green and effective CuO-catalyzed dehydrazination process has been established for the synthesis of purine arabinosides, giving a realistic pathway to 9-glycosylpurines. The process is carried out in water, avoiding hazardous organic solvents, and uses a modest loading of heterogeneous CuO catalyst (≈10 mol%). This approach has a broad substrate breadth and high yields, showing its operational simplicity and potential scalability. The catalyst's recyclability contributes to its applicability for sustainable large-scale synthesis (Scheme 12) [68,69].

11. Future perspective

The future of 9-purine research is evolving from a basic chemical scaffold to a highly functioning "privileged" core in multidisciplinary science. Current study by 2026 shows many distinct trajectories [70]. The invention of Targeted Protein Degraders (PROTACs) marks the most important shift in the medicinal use of the 9-purine scaffold. Rather than just blocking an enzyme, researchers are employing 9-purine derivatives as ligands to attract E3 ligases, resulting in total destruction of disease-causing proteins. Future research will focus on "next-generation" inhibitors of EGFR and HER2 kinases, which will employ 9-purine-hydrazone hybrids to reduce off-target damage in cancer treatment. Scientists are shifting their focus from individual enzymes to the Purinosome, a multi-enzyme complex that develops near mitochondria with high purine demand [71].

Targeting the creation of these clusters, rather than the enzymes themselves,

provides a unique approach to "starve" cancer cells that rely on de novo purine synthesis. Because many purine-based medications have limited solubility and severe toxicity, the future is highly reliant on nano pharmaceuticals. Using PLGA or lipid nanoparticles to transport 9-purine analogs (such as acyclovir or 6-mercaptopurine) to specific organs (for example, the liver or across the blood-brain barrier). Stimuli-responsive coatings are being developed to release purine derivatives exclusively in the presence of particular tumor settings [72].

Future clinical applications will most likely employ levels of certain purine metabolites as early diagnostic indicators for hypoxia, Alzheimer's disease, and multiple sclerosis. Enzymatic extraction and synthetic biology (for example, modified yeast or bacteria) are increasingly being used to manufacture 9-purine derivatives, lowering pharmaceutical manufacturing's environmental footprint. The application of artificial intelligence (AI) to forecast the structure-activity relationship (SAR) of 2,6,9-trisubstituted purines, considerably reducing the drug development schedule.

The rational design of N9-substituted purines is expected to be revolutionized by artificial intelligence (AI) and machine learning. AI-driven prediction of structure–activity relationships (SAR), molecular docking, pharmacokinetics (PK) and toxicity data can dramatically reduce the time and costs associated with traditional drug development. The creation of effective and targeted treatment candidates will be accelerated by the quick identification of ideal replacements at the N9, C2, and C6 locations made possible by computational methods. Artificial Intelligence (AI), Machine Learning (ML), Molecular Dynamics Simulations and Predictive QSAR Platforms are expected to revolutionize purine-based Medicinal Chemistry. Predict optimal substitution patterns at N9 and other positions, reduce trial and error synthesis, speed up lead optimization, boost ADMET prediction accuracy, discover new bioactive scaffolds via virtual screening, to name a few. Overall, the 9-purine scaffold has the potential to serve as a vital link between chemistry, biology, and medicine, translating basic research into clinically relevant and patient-friendly treatments.

12. Conclusion

The family of N9 substituted purine derivatives is a large and important class of heterocyclic compounds that exhibit a broad spectrum of biological activity, and excellent structural flexibility. The chemistry, regioselective synthesis, molecular interactions, structure – activity correlations (SAR) and medicinal aspects of these compounds have been well described in this study. The results show that biological activity, target selectivity, lipophilicity, and pharmacokinetic behaviour are all greatly impacted by changes at the N9 position as well as alterations at the C2 and C6 locations. In particular, electron withdrawing groups such as trifluoromethyl and halogens can enhance activity in many therapeutic areas. A variety of synthetic methods have enabled the efficient synthesis of purine derivatives with diverse structures, including regioselective N9-alkylation and arylation, microwave assisted synthesis, solid-phase synthesis, Traube purine synthesis and green chemistry strategies. Selective N9 functionalization was successfully achieved using TBAF, TBAOH or copper-catalyzed approaches. The molecular docking and computational studies revealed that N9-substituted purines bind with high affinity to a variety of biologically important targets including enzymes for purine biosynthesis, TLR pathways, viral polymerases, BRD9 bromodomains, and EGFR and MAPK kinases, making purine scaffolds promising candidates for rational drug design.

Based on other biological studies, N9 substituted purines possess significant anticancer, antiviral, anti-inflammatory, antibacterial, antifungal, anti-HIV, antitubercular and antidiabetic activities. The therapeutic relevance of many derivatives was highlighted by their strong activity against metabolic targets, cancer cell lines and resistant microorganisms. The incorporation of SAR-guided optimization, molecular modelling, green synthetic strategies, nanotechnology delivery systems, and advancements in AI-driven drug discovery represents a significant leap forward in the field, positioning N9-substituted purines as highly promising scaffolds for future medicinal chemistry and pharmaceutical research.

Overall, these findings underscore that purine derivatives are not only pharmacologically versatile but also synthetically accessible through sustainable approaches, making them promising candidates for future therapeutic applications in antiviral, antibacterial, and antidiabetic domains. The integration of SAR insights with green chemistry strategies paves the way for the rational design of safer, more efficient purine-based drugs.

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14. Conflict of interest

The authors declare that there is no conflict of interest.

15. Declarations

15.1. Ethical Approval:

Not applicable. This article does not contain any studies involving human participants or animal studies that need ethical approval.

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REFERENCES-

1. Manna, T., Maji, S., Maity, M., Debnath, B., Panda, S., Khan, S. A., & Akhtar, M. J. (2025). Anticancer potential and structure activity studies of purine and pyrimidine derivatives: an updated review. *Molecular Diversity*, *29*(1), 817-848. doi:10.1007/s11030-024-10870-4
2. Jezuita A, Wieczorkiewicz PA, Krygowski TM, Szatylowicz H. Influence of the Solvent on the Stability of Aminopurine Tautomers and Properties of the Amino Group. Molecules. 2023 Mar 27;28(7):2993. doi:10.3390/molecules28072993
3. Wei M, Zuo J, Tian G, Hua W. Temperature and Tautomeric Effects in High-Resolution Oxygen 1s X-ray Photoelectron Spectroscopy of Purines and Pyrimidines [Internet]. 2024 Mar 14. doi:10.1063/5.0224090
4. Parmar MB, Vara MK, Pandya JH. A brief review on imidazole, triazine, and isatin derivatives: synthesis approaches and their applications. Discover Chemistry. 2024 Nov 26;1(1):56. doi:10.1007/s44371-024-00057-z
5. Harutyunyan, A. A Benzo[4’,5’]imidazo[2’,1’:6,1]pyrido[2,3-d]pyrimidines: Past and Present. IgMin Research. 2023 Nov 16;1(1):025–31. doi:10.61927/igmin113
6. Agranat I. Aromaticity – a theoretical notion. Structural Chemistry. Springer; 2024. p. 715–

20. doi:10.1007/s11224-024-02328-y

7. Sh. Sabirov D. Information entropy of mixing molecules and its application to molecular ensembles and chemical reactions. Comput Theor Chem. 2020 Oct;1187:112933. doi:10.1016/j.comptc.2020.112933
8. Jezuita A, Szatylowicz H, Marek PH, Krygowski TM. Aromaticity of the most stable adenine and purine tautomers in terms of Hückel's 4N+2 principle. Tetrahedron. 2019 Aug;75(35):130474. doi:10.1016/j.tet.2019.130474
9. Cao H, He J, Li T, Huang SY. Deciphering Protein Secondary Structures and Nucleic Acids in Cryo-EM Maps Using Deep Learning. J Chem Inf Model. 2025 Feb 10;65(3):1641–52. doi:10.1021/acs.jcim.4c01971
10. Escayola Gordils, S. (2024). Exploring the boundaries of aromaticity through computational analysis of excited states and complex molecular topologies.
11. Ahunovych, Volodymyr, Anton A. Klipkov, Maksym Bugera, Karen Tarasenko, Serhii Trofymchuk, Oleh Stanko, Andrii Boretskyi, Mykola Zheludenko, Iryna V. Sadkova, and Pavel K. Mykhailiuk. "General and scalable approach to trifluoromethyl-substituted cyclopropanes." *The Journal of Organic Chemistry* 88, no. 6 (2023): 3859-3870. doi:10.1021/acs.joc.3c00123
12. Oziminski WP, Bycul A. Thermodynamic and Kinetic Characteristics of Molnupiravir Tautomers and Its Complexes with RNA Purine Bases as an Explanation of the Possible Mechanism of Action of This Novel Antiviral Medicine: A Quantum-Chemical Study. J Org Chem. 2023 Oct 6;88(19):14048–64. doi:10.1021/acs.joc.3c01580
13. Sabirov, D. S. Information entropy of mixing molecules and its application to molecular ensembles and chemical reactions. Comput Theor Chem. 2020 Oct;1187:112933. doi:10.1016/j.comptc.2020.112933
14. Farooqui F, Khan AR, Khan MA, Nasibullah M, Ansari JA. Purine derivatives as potent anticancer agents: a comprehensive review. Future Med Chem. 2026 Jan 2;18(1):103–12. doi:10.1080/17568919.2025.2594966
15. Rana N, Grover P, Singh H. Recent Developments and Future Perspectives of Purine Derivatives as a Promising Scaffold in Drug Discovery. Curr Top Med Chem. 2024 Mar;24(6):541–79. doi:10.2174/0115680266290152240110074034
16. Kasahara, Kazuyuki, Robert L. Kerby, Qijun Zhang, Meenakshi Pradhan, Margarete Mehrabian, Aldons J. Lusis, Göran Bergström, Fredrik Bäckhed, and Federico E. Rey. "Gut bacterial metabolism contributes to host global purine homeostasis." *Cell Host & Microbe* 31, no. 6 (2023): 1038-1053. doi:10.1016/j.chom.2023.05.011
17. Singh S, Anand R. Diverse strategies adopted by nature for regulating purine biosynthesis via fine-tuning of purine metabolic enzymes. Curr Opin Chem Biol. 2023 Apr;73:102261. doi:10.1016/j.cbpa.2022.102261
18. Fedeles BI, Li D, Singh V. Structural Insights into Tautomeric Dynamics in Nucleic Acids and in Antiviral Nucleoside Analogs. Front Mol

Biosci. 2022 Jan 25;8. doi:10.3389/fmolb.2021.823253

19. Das G, Harikrishna S, Gore KR. Influence of Sugar Modifications on the Nucleoside Conformation and Oligonucleotide Stability: A Critical Review. The Chemical Record. 2022 Dec;22(12). doi:10.1002/tcr.202200174
20. Rothkegel J, Kaufmann S, Wilsch-Braeuninger M, Mateus R. The Role of Purine Interactions in Biogenic Crystal Shape Determination. 2024. doi:10.1101/2024.09.18.613275
21. Jena S, Dutta J, Tulsiyan KD, Sahu AK, Choudhury SS, Biswal HS. Noncovalent interactions in proteins and nucleic acids: beyond hydrogen bonding and π-stacking. Chem Soc Rev. 2022;51(11):4261–86. doi:10.1039/D2CS00133K
22. Jezuita A, Szatylowicz H, Krygowski TM. Impact of the Substituents on the Electronic Structure of the Four Most Stable Tautomers of Purine and Their Adenine Analogues. ACS Omega. 2020 May 26;5(20):11570–7. doi:10.1021/acsomega.0c00820
23. Raczyńska, E. D., Gal, J. F., Maria, P. C., Kamińska, B., Igielska, M., Kurpiewski, J., & Juras, W. (2020). Purine tautomeric preferences and bond-length alternation in relation with protonation-deprotonation and alkali metal cationization. *Journal of Molecular Modeling*, *26*(5), 93. doi:10.1007/s00894-020-4343-6 PubMed PMID: 32248379.
24. Moghimi P, Bolourian S, Shiri A, Eshghi H, Hosseini F, Sabet-Sarvestani H. The origin of experimental regioselectivity in ring-closing reaction of pyrido[1,2- *e*]purine systems and comparison of the aromaticity character of probable products: a mechanistic study based on DFT insights. New Journal of Chemistry. 2023;47(23):11123–33. doi:10.1039/D3NJ01276J
25. Iacob, Simona, Claudia-Simona Stefan, Aurel Nechita, Madalina-Nicoleta Matei, Elena-Lacramioara Lisa, Dana Tutunaru, Iuliu Fulga, Ana Fulga, Alina-Georgiana Cristea, and Oana-Maria Dragostin. "Hybrid molecules with purine and pyrimidine derivatives for antitumor therapy: News, Perspectives, and future directions." *Molecules* 30, no. 13 (2025): 2707.doi:10.3390/molecules30132707
26. Rosales-Martínez C, Matilla-Hernádez A, Choquesillo-Lazarte D, Frontera A, Castiñeiras A, Niclós-Gutiérrez J. The Copper(II)-Thiodiacetate (tda) Chelate as Efficient Receptor of N9-(2-Hydroxyethyl)Adenine (9heade): Synthesis, Molecular and Crystal Structures, Physical Properties and DFT Calculations of [Cu(tda)(9heade)(H2O)]·2H2O. Molecules. 2023 Aug 2;28(15):5830. doi:10.3390/molecules28155830
27. Tber, Zahira, Nicolas G. Biteau, Luigi Agrofoglio, Julien Cros, Stéphane Goffinont, Bertrand Castaing, Cyril Nicolas, and Vincent Roy. "Microwave-Assisted Suzuki–Miyaura and Sonogashira Coupling of 4-Chloro-2-(trifluoromethyl) pyrido [1, 2-e] purine Derivatives." *European Journal of Organic Chemistry* 2019, no. 33 (2019): 5756-5767. doi:10.1002/ejoc.201900921
28. Tashchilova, A., Podoplelova, N., Sulimov, A., Kutov, D., Ilin, I., Panteleev, M., Shikhaliev, K., Medvedeva, S., Novichikhina, N., Potapov, A. and Sulimov, V., 2022. New blood coagulation factor XIIa inhibitors: molecular modeling, synthesis, and experimental

confirmation. *Molecules*, *27*(4), p.1234. doi:10.3390/molecules27041234

29. Doganc F, Aydin AS, Şahin E, Göker H. Regioselective N-alkylation of some 2 or 6-chlorinated purine analogues. J Mol Struct. 2023 Jan;1272:134200. doi:10.1016/j.molstruc.2022.134200
30. Shaik, Khaja Mohiddin, Komala Pandurangan, Tejeswara Rao Allaka, Seshadri Nalla, Srinivas Ganta, Mohammad Z. Ahmed, Srinivasadesikan Venkatesan, and Pilli Veera Venkata Nanda Kishore. "Novel purine-linked 1, 2, 3-triazole derivatives as effective anticancer agents: design, synthesis, docking, DFT, and ADME-T investigations." *Scientific Reports* 15, no. 1 (2025): 26853. doi:10.1038/s41598-025-95669-5
31. Abad, N., Buhlak, S., Hajji, M., Saffour, S., Akachar, J., Kesgun, Y., & Mardinoglu, A. (2024). Unveiling structural features, chemical reactivity, and bioactivity of a newly synthesized purine derivative through crystallography and computational approaches. *Journal of Molecular Structure*, *1311*, 138400. doi:10.1016/j.molstruc.2024.138400
32. Ali Z, Goyal A, Jhunjhunwala A, Mitra A, Trant JF, Sharma P. Structural and Energetic Features of Base–Base Stacking Contacts in RNA. J Chem Inf Model. 2023 Jan 23;63(2):655–69. doi:10.1021/acs.jcim.2c01116
33. Sharma M, Sharma A, Nuthakki VK, Bhatt S, Nandi U, Bharate SB. Design, synthesis, and structure–activity relationship of caffeine-based triazoles as dual AChE and BACE-1 inhibitors. Drug Dev Res. 2022 Dec 26;83(8):1803–21. doi:10.1002/ddr.21998
34. Mohamed AR, El Kerdawy AM, George RF, Georgey HH, Abdel Gawad NM. Design, synthesis and in silico insights of new 7,8-disubstituted-1,3-dimethyl-1H-purine-2,6(3H,7H)-dione derivatives with potent anticancer and multi-kinase inhibitory activities. Bioorg Chem. 2021 Feb;107:104569. doi:10.1016/j.bioorg.2020.104569
35. Sharma S, Mehndiratta S, Kumar S, Singh J, Bedi P, Nepali K. Purine Analogues as Kinase Inhibitors: A Review. Recent Pat Anticancer Drug Discov. 2015 Sep 16;10(3):308–41. doi:10.2174/1574892810666150617112230
36. Silva CHTP, Silva M, Iulek J, Thiemann OH. Structural Complexes of Human Adenine Phosphoribosyltransferase Reveal Novel Features of the APRT Catalytic Mechanism. J Biomol Struct Dyn. 2008 Jun;25(6):589–97. doi:10.1080/07391102.2008.10507205
37. Sathya U, Nirmalram JS, Gomathi S, Dhivya D, Jegan Jennifer S, Abdul Razak I. A study of the crystal structures, supramolecular patterns and Hirshfeld surfaces of bromide salts of hypoxanthine and xanthine. Acta Crystallogr E Crystallogr Commun. 2022 Jun 1;78(6):652–9. doi:10.1107/S2056989022005278
38. Pastor-Anglada M, Pérez-Torras S. Who Is Who in Adenosine Transport. Front Pharmacol. 2018 Jun 14;9. doi:10.3389/fphar.2018.00627
39. Pareek V, Pedley AM, Benkovic SJ. Human de novo purine biosynthesis. Crit Rev Biochem Mol Biol. 2021 Jan 2;56(1):1–16. doi:10.1080/10409238.2020.1832438
40. Cicero AFG, Fogacci F, Di Micoli V, Angeloni C, Giovannini M, Borghi C. Purine Metabolism Dysfunctions:

Experimental Methods of Detection and Diagnostic Potential. Int J Mol Sci. 2023 Apr 10;24(8):7027. doi:10.3390/ijms24087027

41. Bhat MA, Tüzün B, Alsaif NA, Ali Khan A, Naglah AM. Synthesis, characterization, molecular modeling against EGFR target and ADME/T analysis of novel purine derivatives of sulfonamides. J Mol Struct. 2022 Jun;1257:132600. doi:10.1016/j.molstruc.2022.132600
42. Tian, Y., Cui, F., Bian, Z., Tao, X., Wang, H., Zhang, N., & Zhu, G. (2024). Construction of porous aromatic frameworks with specifically designed motifs for charge storage and transport. *Accounts of Chemical Research*, *57*(15), 2130-2143. doi:10.1021/acs.accounts.4c00258
43. Abushaheen, M. A., Fatani, A. J., Alosaimi, M., Mansy, W., George, M., Acharya, S., & Jhugroo, P. (2020). Antimicrobial resistance, mechanisms and its clinical significance. *Disease-a-month*, *66*(6), 100971. doi:10.1016/j.disamonth.2020.100971
44. Buchy, P., Ascioglu, S., Buisson, Y., Datta, S., Nissen, M., Tambyah, P. A., & Vong, S. (2020). Impact of vaccines on antimicrobial resistance. *International Journal of Infectious Diseases*, *90*, 188-196. doi:10.1016/j.ijid.2019.10.005
45. Ali ES, Ben-Sahra I. Regulation of nucleotide metabolism in cancers and immune disorders. Trends Cell Biol. 2023 Nov;33(11):950–66. doi:10.1016/j.tcb.2023.03.003
46. Frenguelli BG. The Purine Salvage Pathway and the Restoration of Cerebral ATP: Implications for Brain Slice Physiology and Brain Injury. Neurochem Res. 2019 Mar 24;44(3):661–75. doi:10.1007/s11064-017-2386-6
47. Ragab A, Abusaif MS, Aboul-Magd DS, Wassel MMS, Elhagali GAM, Ammar YA. A new exploration toward adamantane derivatives as potential anti-MDR agents: Design, synthesis, antimicrobial, and radiosterilization activity as potential topoisomerase IV and DNA gyrase inhibitors. Drug Dev Res. 2022 Sep 18;83(6):1305–30. doi:10.1002/ddr.21960
48. Valdes F, Brown N, Morales-Bayuelo A, Prent-Peñaloza L, Gutierrez M. Adenosine Derivates as Antioxidant Agents: Synthesis, Characterization, in Vitro Activity, and Theoretical Insights. Antioxidants. 2019 Oct 9;8(10):468. doi:10.3390/antiox8100468
49. Yang M, Xu X. Important roles of transporters in the pharmacokinetics of anti-viral nucleoside/nucleotide analogs. Expert Opin Drug Metab Toxicol. 2022 Aug 3;18(7–8):483–505. doi:10.1080/17425255.2022.2112175
50. Tber, Z., Biteau, N. G., Agrofoglio, L., Cros, J., Goffinont, S., Castaing, B., ... & Roy, V. (2019). Microwave-Assisted Suzuki–Miyaura and Sonogashira Coupling of 4-Chloro-2-(trifluoromethyl) pyrido [1, 2-e] purine Derivatives. *European Journal of Organic Chemistry*, *2019*(33), 5756-5767. doi:10.1002/ejoc.201900921
51. Nadaf, A. Q. A., Najare, M. S., Garbhagudi, M., Mantur, S., Sunagar, M. G., Gaonkar, S., ... & Khazi, I. A. M. (2020). Synthesis of 6-[4-(4-propoxyphenyl) piperazin-1-yl]-9h-purine derivatives as antimycobacterial and antifungal agents: In vitro evaluation and in silico study. *Chemistry &*

Biodiversity, *17*(5), e2000053. doi:10.1002/cbdv.202000053

52. Ohler A, Taylor PE, Bledsoe JA, Iavarone AT, Gilbert NC, Offenbacher AR. Identification of the Thermal Activation Network in Human 15-Lipoxygenase-2: Divergence from Plant Orthologs and Its Relationship to Hydrogen Tunneling Activation Barriers. ACS Catal. 2024 Apr 5;14(7):5444–57. doi:10.1021/acscatal.4c00439
53. Ragab A. Recent advances in the synthesis, reaction, and bio-evaluation potential of purines as precursor pharmacophores in chemical reactions: a review. RSC Adv. 2025;15(5):3607–45. doi:10.1039/D4RA08271K
54. Xiang Y, Luo P, Hao T, Xiong W, Song X, Ding Q. Copper-mediated formal [5+1] annulation of 2-vinylanilines and glyoxylic acid: A facile approach for the synthesis of 4-arylated quinolines. Tetrahedron. 2021 Jan;79:131832. doi:10.1016/j.tet.2020.131832
55. Chen, Y., Lv, M., Zhang, Y., Wu, Y., Ying, L., Tang, J., ... & Song, Z. (2022). C–H diselenation and monoselenation of electron-deficient alkenes via radical coupling at room temperature. *The Journal of Organic Chemistry*, *87*(24), 16175-16187. doi:10.1021/acs.joc.2c01567
56. Tsujihara, T., Sasaki, R., Fukkoshi, M., Hatakeyama, S., Takehara, T., Suzuki, T., & Kawano, T. (2022). Synthesis of 6, 7-benzene-fused tropane derivatives from isoindoline-aminal hybrid compound. *Tetrahedron Letters*, *95*, 153724.doi:10.1016/j.tetlet.2022.153724
57. Jayasingha JACC, Lee KT, Jin C, Choi YH, Lee CS, Kim G. Sakuranin Is a Novel Anti-Inflammatory Agent Targeting TLR4–NF-κB Signaling Pathways. Chem Biodivers. 2025 Nov 25;22(11). doi:10.1002/cbdv.202501167
58. Yang S, Peng Y, Wu M, Yang J, Chen X, Rong L. An efficient and green synthesis of multi-heteroatom heterocycles via a three components reaction in acetic acid/aqueous condition. J Heterocycl Chem. 2023 Apr 20;60(4):670–80. doi:10.1002/jhet.4621
59. Tber, Z., Biteau, N. G., Agrofoglio, L., Cros, J., Goffinont, S., Castaing, B., Roy, V. (2019). Microwave-Assisted Suzuki–Miyaura and Sonogashira Coupling of 4-Chloro-2-(trifluoromethyl) pyrido [1, 2-e] purine Derivatives. *European Journal of Organic Chemistry*, *2019*(33), 5756-5767. doi:10.1002/ejoc.201900921
60. Verma, A. K., Ahmed, S. F., Hossain, M. S., Bhojiya, A. A., Mathur, A., Upadhyay, S. K., Bahadur, N. M. (2022). Molecular docking and simulation studies of flavonoid compounds against PBP-2a of methicillin-resistant Staphylococcus aureus. *Journal of Biomolecular Structure and Dynamics*, *40*(21), 10561-10577. doi:10.1080/07391102.2021.1944911
61. Saha SK, Ahmed CM, Haque T, Al Mamun MA, Hussain Mohd Z. Assessment of atrial septal defects using 3-dimensional transthoracic echocardiography prior to percutaneous device closure: first report from Bangladesh. Ther Adv Cardiovasc Dis. 2023 Jan 11;17. doi:10.1177/17539447231193290
62. Eissa, I. H., Yousef, R. G., Elkaeed, E. B., Alsfouk, A. A., Husein, D. Z., Ibrahim, I. M., ... & Metwaly, A. M. (2023). Anticancer derivative of the natural alkaloid, theobromine, inhibiting EGFR protein: Computer-

aided drug discovery approach. *Plos one*, *18*(3), e0282586. doi:10.1371/journal.pone.0282586

63. Yang Y, Song R, Wang S, Zu G, Song B. Antiviral activity and mechanism of purine morpholine nucleoside analogues incorporating a sulfonamide fragment. J Adv Res. 2026 Mar;81:43–56. doi:10.1016/j.jare.2025.05.062
64. Abu-Hashem, A. A., Hakami, O., El-Shazly, M., El-Nashar, H. A., & Yousif, M. N. (2024). Caffeine and purine derivatives: A comprehensive review on the chemistry, biosynthetic pathways, synthesis-related reactions, biomedical prospectives and clinical applications. *Chemistry & Biodiversity*, *21*(7), e202400050. doi:10.1002/cbdv.202400050
65. Shah, A., Teraiya, N., Kamdar, J. H., Juneja, T., Sangani, C. B., Ahmed, S., & Kapadiya, K. (2024). Novel purine derivatives as selective CDK2 inhibitors with potential anticancer activities: Design, synthesis and biological evaluation. *Bioorganic Chemistry*, *153*, 107841. doi:10.1016/j.bioorg.2024.107841
66. Ayman R, Abusaif MS, Radwan AM, Elmetwally AM, Ragab A. Development of novel pyrazole, imidazo[1,2-b]pyrazole, and pyrazolo[1,5-a]pyrimidine derivatives as a new class of COX-2 inhibitors with immunomodulatory potential. Eur J Med Chem. 2023 Mar;249:115138. doi:10.1016/j.ejmech.2023.115138
67. Dhiman A, Sharma R, Singh RK. Target-based anticancer indole derivatives and insight into structure–activity relationship: A mechanistic review update (2018–2021). Acta Pharm Sin B. 2022 Jul;12(7):3006–27. doi:10.1016/j.apsb.2022.03.021
68. Yadav M, Kumar R, Krishnamurthy R. Chemistry of Abiotic Nucleotide Synthesis. Chem Rev. 2020 Jun 10;120(11):4766–805. doi:10.1021/acs.chemrev.9b00546
69. Perkins JJ, Shurtleff VW, Johnson AM, El Marrouni A. Synthesis of C6-Substituted Purine Nucleoside Analogues via Late-Stage Photoredox/Nickel Dual Catalytic Cross-Coupling. ACS Med Chem Lett. 2021 Apr 8;12(4):662–6. doi:10.1021/acsmedchemlett.0c00673
70. Park, Y. J., Jeon, M. S., Lee, S., Kim, J. K., Jang, T. S., Chung, K. H., & Kim, K. H. (2021). Anti-fibrotic effects of brevilin A in hepatic fibrosis via inhibiting the STAT3 signaling pathway. *Bioorganic & Medicinal Chemistry Letters*, *41*, 127989. doi:10.1016/j.bmcl.2021.127989
71. Sharma, S., Gangal, S., & Rauf, A. (2008). Green chemistry approach to the sustainable advancement to the synthesis of heterocyclic chemistry. *Rasayan J. Chem*, *1*(4), 693-717.
72. Zeleke D, Damena T. Advance in green synthesis of pharmacological important heterocycles using multicomponent reactions and magnetic nanocatalysts (MNCs). Results Chem. 2024 Jan;7:101283. doi:10.1016/j.rechem.2023.101283
73. Langer P. Synthesis of Purines and Related Molecules by Cyclization - Reactions of Heterocyclic Enamines. Synlett. 2022 Mar 22;33(05):440–57. doi:10.1055/s-0040-1719845
74. Ahmed SA, Sarma P, Barge SR, Swargiary D, Devi GS, Borah JC. Xanthosine, a purine glycoside mediates hepatic glucose homeostasis through inhibition of gluconeogenesis and activation of glycogenesis via regulating the

AMPK/ FoxO1/AKT/GSK3β signaling cascade. Chem Biol Interact. 2023 Feb;371:110347. doi:10.1016/j.cbi.2023.110347