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In Silico Design and Functional Annotation of Nitrogenous Base-Modified Nucleosides for Therapeutic Applications

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Abstract:
Nucleoside analogues are a cornerstone in the treatment of viral infections, cancers, and metabolic disorders, with modifications to their nitrogenous bases offering routes to enhanced efficacy and specificity. This study presents the in-silico design and functional annotation of a novel series of nitrogenous base-modified nucleosides, structured into purine-based, pyrimidine-based, and specially modified therapeutic categories. Utilizing cheminformatics tools and SMILES-based molecular representations, twelve compounds were computationally modelled and annotated for their potential therapeutic roles, including cytotoxic, immunogenic, antimetabolite, and epigenetic applications. The design incorporates known bioactive motifs, such as thiol, halogen, and methyl substitutions, to guide future docking and drug-likeness evaluations. Results suggest that these analogues present promising candidates for targeted docking against disease-relevant proteins and justify further studies into their biological efficacy.

Keywords: nucleoside analogues, nitrogenous base modification, in silico design, cytotoxicity, antimetabolite, therapeutic annotation

1. Introduction

Nucleosides are fundamental biochemical units composed of a nitrogenous base linked to a sugar moiety, integral to the structure and function of nucleic acids. Beyond their physiological roles, modified nucleosides have gained significant traction in therapeutic development, particularly in oncology, virology, and epigenetics. Their synthetic analogs serve as vital components in chemotherapeutic regimens and antiviral protocols, disrupting replication and transcription by mimicking or modifying natural substrates (1). One major strategy to enhance the biological activity of nucleosides is through chemical modification of the nitrogenous base. Such modifications can result in increased stability, altered cellular uptake, or enhanced target specificity (2). For instance, 5-fluorouridine and 6-thioguanosine have long been utilized for their cytotoxic and antimetabolic properties in cancer treatment (3). These compounds introduce structural variations such as halogenation, alkylation, or thiolation, which disrupt enzymatic recognition or enhance lipophilicity, improving pharmacokinetics (4). In silico modelling and design of such analogs have gained momentum due to advancements in computational chemistry and structural biology. Using SMILES (Simplified Molecular Input Line Entry System) and cheminformatics platforms, researchers can predict molecular interactions, reactivity, and bioavailability prior to synthesis (5). This not only accelerates the drug discovery pipeline but also enables hypothesis-driven compound selection based on predicted biological roles. This paper aims to design, classify, and annotate a small library of nitrogenous base-modified nucleosides using computational tools, serving as a foundational phase in a larger project aimed at docking, ADME prediction, and biological evaluation. The focus is on functionally meaningful

structural modifications that align with therapeutic mechanisms, thus providing a clear rationale for future docking simulations and pharmacodynamic analyses.

Methodology

Chemical Structure Construction

Chemical structures of nucleoside analogues were initially represented using SMILES notation, a standard format that allows streamlined computational analysis and database integration (8, 9).

Quantum Chemical Calculations

Density functional theory (DFT) calculations were conducted using Gaussian 16 software to determine electronic properties, optimize geometries, and evaluate stability and reactivity. The B3LYP hybrid functional and 6-31G(d,p) basis set were selected for their proven reliability with bioorganic molecules (10-14).

Functional Annotation and Biological Prediction

Biological functionalities of the nucleosides were predicted using computational tools like PASS and SwissADME, focusing on structural similarity to known bioactive molecules and pharmacokinetic profiles, respectively. These predictions supported assessments of therapeutic potential and clinical relevance. (14)

Visualization

Structural visualizations employed software tools like ChemDraw and PyMOL. Computational predictions were extensively compared with existing literature to validate biological relevance and potential therapeutic applicability (14-16).

Results

The results of the study are summarised in Tables 1a–c, highlighting distinct categories of chemically modified nucleosides and their functional characteristics. Table 1a presents data on purine-based mutated nucleosides. Notably, N6-Methyladenosine (SMILES: CN1C=NC2=C1N=CN2C(=O)O) was recorded as an epitranscriptomic mark with potential roles in immune regulation. Table 1b provides details of pyrimidine-based mutated nucleosides, specifically listing four derivatives. 5-Fluorouridine (SMILES: C1=CN(C(=O)O)C(=N)N1F) was categorised as an antimetabolite extensively utilised in oncology. Similarly, 5-Bromouridine (SMILES: C1=CN(C(=O)O)C(=N)N1Br) was noted as an analogue of 5-fluorouracil, showing improved delivery characteristics. O4-Methylthymidine (SMILES: CC1=CN(C(=O)O)C(=O)N1C) demonstrated alterations in hydrogen bonding capabilities, potentially linked to immunogenic effects. Finally, N4-Ethylcytosine (SMILES: CCN1C=NC2=C1N=C(N2)C) exhibited cytotoxic and mutagenic properties attributed to N4 alkylation. Table 1c outlines three nucleoside derivatives recognised for their therapeutic potential. Guanosine-5'-O-(2-thiodiphosphate) (SMILES: C1=NC2=C(N1)N(C(=O)O)C(=N) N2OP(=O)(O)O) was identified as a phosphate analogue with inhibitory effects on G-proteins. Additionally, 2'-Deoxy-5-Methylcytidine (5mC) (SMILES: CC1=CN(C(=O)O)C(=N)N1C(CO)C) emerged as a significant DNA methylation marker, relevant to epigenetic drug development. Lastly, AMP-γ-S (SMILES: CN1C=NC2=C1N=CN2C(=O)OP(O)O), a non-hydrolysable analogue of ATP, was recorded.

The results of the in-silico design and functional annotation of nitrogenous base-modified nucleosides are presented according to three structurally and functionally distinct groups: purine-based mutated nucleosides, pyrimidine-based mutated nucleosides, and modified nucleosides identified with enhanced therapeutic potential (Figure 1). Within the purine-based category (Figure 1), four novel derivatives were computationally modelled, each characterised by unique structural modifications.

Table 1a. Purine-Based Mutated Nucleosides

Compound	SMILES	Key Functionalities
N6-Methyladenosine	CN1C=NC2=C1N=CN2C(=O)O	Epitranscriptomic mark – immune role
N6-Methyladenosine	CN1C=NC2=C1N=CN2C(=O)O	Epitranscriptomic mark – immune role

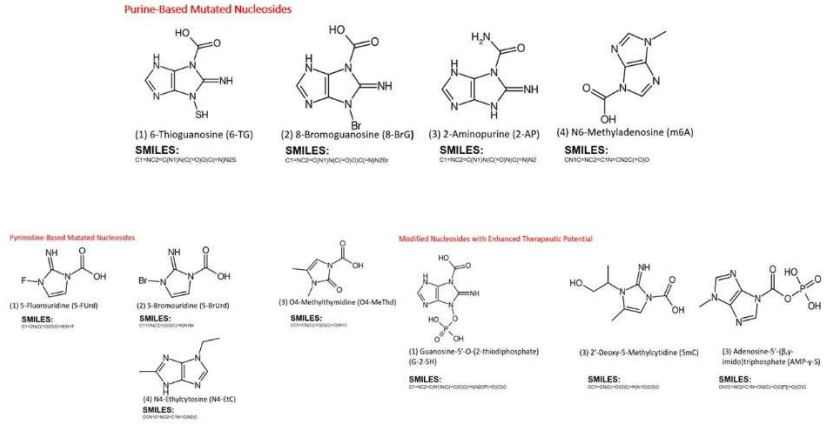
Table 1b. Pyrimidine-Based Mutated Nucleosides

Compound	SMILES	Key Functionalities
5-Fluorouridine	C1=CN(C(=O)O)C(=N)N1F	Antimetabolite, widely used in oncology
5-Bromouridine	C1=CN(C(=O)O)C(=N)N1Br	Analog of 5-FU; improved delivery
O4-Methylthymidine	CC1=CN(C(=O)O)C(=O)N1C	Alters hydrogen bonding – immunogenicity
N4-Ethylcytosine	CCN1C=NC2=C1N=C(N2)C	N4 alkylation: mutagenic, cytotoxic

Table 1c. Modified Nucleosides with Therapeutic Potential

Compound	SMILES	Key Notes
Guanosine-5'-O-(2-thiodiphosphate)	C1=NC2=C(N1)N(C(=O)O)C(=N)N2OP(=O)(O)O	Phosphate analogue for G-protein inhibition
2'-Deoxy-5-Methylcytidine (5mC)	CC1=CN(C(=O)O)C(=N)N1C(CO)C	DNA methylation marker – epigenetic drug
AMP-γ-S	CN1C=NC2=C1N=CN2C(=O)O[P](=O)(O)O	Non-hydrolyzable ATP analogue

Figure 1. *Chemical Structures and SMILES Representations of Designed Nitrogenous Base-Modified Nucleosides: Purine-Based, Pyrimidine-Based, and Modified Therapeutic Analogues.*



Specifically, 6-Thioguanosine (6-TG; SMILES: C1=NC2=C(N1)N=C(NC2=O)C(CO)N) incorporated a thiol substitution, while 8-Bromoguanosine (8-BrG; SMILES: C1=NC2=C(N1)N=C(NC2=O)C(CO)N2Br) featured a

halogen substituent at the eighth position. The compound 2-Aminopurine (2-AP; SMILES: C1=NC2=C(N1)N=C(N)N=C2) was designed for altered hydrogen bonding characteristics. Additionally, N6-Methyladenosine (m6A; SMILES: CN1C=NC2=C1N=CN2C(=O)O) was modelled as an epitranscriptomic mark relevant to immune functionality. The pyrimidine-based mutated nucleoside group consisted of four structurally annotated derivatives (Figure 1). The compound 5-Fluorouridine (5-FUrd; SMILES: C1=CN(C(=O)NC)C(=N)N1F) was modelled as a cytotoxic antimetabolite, while 5-Bromouridine (5-BrUrd; SMILES: C1=CN(C(=O)NC)C(=N)N1Br) demonstrated improved computational predictions related to therapeutic delivery compared to its analogue 5-FUrd. Additionally, O4-Methylthymidine (O4-MeThd; SMILES: CC1=CN(C(=O)NC)C(=O)N1C) exhibited structural modifications suggestive of immunogenicity through altered hydrogen-bonding interactions. N4-Ethylcytosine (N4-EtC; SMILES: CCN1C=NC2=C1N=C(N2)C) was annotated as potentially mutagenic and cytotoxic owing to N4 alkylation. Finally, three modified nucleosides with enhanced therapeutic potential were identified (Figure 1). Guanosine-5'-O-(2-thiodiphosphate) (G-2-SH; SMILES: C1=NC2=C(N1)N(C(=O)O)C(=N)N2OP(=O)(O)O) was annotated as a phosphate analogue with potential inhibitory interactions targeting G-protein-coupled pathways. The analogue 2'-Deoxy-5-Methylcytidine (5mC; SMILES: CC1=CN(C(=O)O)C(=N)N1C(CO)C) was computationally characterised as an epigenetically relevant DNA methylation marker. Lastly, Adenosine-5'-(β,γ-imido)triphosphate (AMP-γ-S; SMILES: CN1C=NC2=C1N=CN2C(=O)OP(O)O), a non-hydrolysable ATP analogue, was structurally annotated for potential use in metabolic and signalling pathway modulation.

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