RATIONAL TARGETING OF PROTEIN KINASES IN PEDIATRIC LEUKEMIA: MECHANISMS, PATHOGENESIS AND INHIBITORY POTENTIAL OF THIENO[3,2 *d*]PYRIMIDINE DERIVATIVES

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Abstract. Pediatric leukemia, particularly acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), remains a leading cause of cancer-associated childhood mortality worldwide due to dysregulated signal transduction and unchecked proliferation of leukemic cells. Protein kinases, including BCR-ABL1, CDKs (cyclin-dependent kinases) and PI3K isoforms, play pivotal roles in leukemogenesis by modulating cell cycle progression, transcriptional control, and survival signaling. Targeted inhibition of these kinases has transformed leukemia therapy, such as tyrosine kinase inhibitors for chronic myeloid leukemia. In this study, we present a structure-based design, synthesis and *in silico* evaluation of novel thieno[3,2-*d*]pyrimidine derivatives engineered to engage multiple leukemia-associated kinase targets. Molecular docking and dynamics suggest stable engagement with key functional residues, indicating potential for selective inhibition of leukemia pathways. These findings provide a mechanistic rationale supporting further preclinical evaluation of thieno[3,2-*d*]pyrimidine modulators for pediatric leukemia therapy.

Keywords: Pediatric leukemia; Kinase targets; Thienopyrimidine; BCR-ABL1; CDK inhibition

Annotatsiya. Bolalardagi Oq qon kasalligi (masalan, ALL va AML) o‘sma hujayralarining nazoratsiz ko‘payishi natijasida yuzaga keladi, bunda protein kinazalar asosiy signal yo‘llarini boshqaradi. BCR-ABL1, CDKlar va PI3K kabi kinazalar hujayra sikli, transkripsiya va tirikchilik signalini tartibsizlashtiradi. Maqsadli kinaza ingibitorlari, masalan tyrozinkinaza ingibitorlari, leykemiya terapiyasida inqilob qilgan. Biz ushbu ishda thieno[3,2-*d*]pirimidin hosilalarini strukturaviy asosda loyihalab, sintez qilib, leykemiya bilan bog‘liq kinazalarni inhibe qiluvchi imkoniyatini *in silico* baholadik. Molekulyar doking va dinamika natijalari barqaror bog‘lanishlarni ko‘rsatdi, bu esa pediatrik leykemiya yo‘nalishida keyingi tadqiqotlar uchun asos yaratadi.

Kalit so‘zlar: Bolalarda oq qon kasalligi; Kinaza-nishonlar; Tiyeno[3,2-*d*]pirimidin; BCR–ABL1 oqsili; CDK ingibitsiyasi

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Аннотация. Детские лейкемии, такие как острый лимфобластный лейкоз (ALL) и острый миелоидный лейкоз (AML), вызваны нарушенной регуляцией клеточного цикла и сигнализации, где белковые киназы играют ключевую роль. BCR-ABL1, CDK и PI3K выступают критическими элементами сигнальных каскадов, регулирующих пролиферацию и выживание клеток. Целенаправленные ингибиторы киназ, включая тирозинкиназные ингибиторы, существенно улучшили лечение лейкемий. В настоящем исследовании разработаны, синтезированы и оценены *in silico* новые производные thieno[3,2-*d*]pyrimidine с возможностью ингибирования лейкемия-связанных киназ. Молекулярный докинг и динамика продемонстрировали стабильные взаимодействия, что обосновывает их перспективность в терапии детских лейкемий.

Ключевые слова: Детский лейкоз; Киназные мишени; Тиено[3,2-*d*]пиримидин; Белок BCR–ABL1; Ингибирование CDK.

1. INTRODUCTION.

Leukemia is a heterogeneous group of hematologic malignancies characterized by the malignant transformation of hematopoietic progenitor cells and their uncontrolled proliferation. Pediatric leukemia, particularly acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), accounts for a substantial fraction of childhood cancer morbidity and mortality. Leukemic pathogenesis arises from genetic and epigenetic alterations that disrupt normal hematopoiesis, leading to accumulation of immature blast cells in bone marrow and peripheral blood. These malignant clones interfere with normal blood cell production, resulting in anemia, immunodeficiency and bleeding tendencies [1].

At the molecular level, dysregulated protein kinases serve as key drivers of leukemogenesis. The BCR-ABL1 fusion oncogene, formed by t(9;22)(q34;q11) translocation, encodes a constitutively active tyrosine kinase that is central to chronic myeloid leukemia (CML) pathobiology and is occasionally seen in pediatric ALL subsets. Similarly, aberrant activation of cyclin-dependent kinases (such as CDK6 in MLL-rearranged AML and CDK9 driving transcriptional survival genes) has been implicated in leukemia initiation and maintenance. The PI3K/AKT/mTOR pathway is another crucial survival signaling axis frequently activated in leukemic cells, promoting proliferation and resistance to apoptosis [2].

Current pediatric leukemia therapies rely on combination chemotherapy, hematopoietic stem cell transplantation, immunotherapy and targeted tyrosine kinase inhibitors (TKIs). TKIs, such as imatinib and dasatinib, have transformed management of BCR-ABL1-positive leukemias but face limitations related to resistance and off-target toxicity. Consequently, there is a pressing need for novel

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small molecules that can selectively target multiple kinase pathways driving pediatric leukemia pathogenesis while minimizing toxicity [3].

2. Results and Discussion.

2.1 Protein kinase targets in pediatric leukemia

BCR-ABL1 tyrosine kinase. The Philadelphia chromosome fusion oncogene BCR-ABL1 creates a constitutively active tyrosine kinase central to CML and a subset of ALL. This kinase aberrantly activates downstream signaling pathways, including RAS/RAF/MAPK and PI3K/AKT/mTOR, promoting uncontrolled proliferation and resistance to apoptosis. Targeting BCR-ABL1 with TKIs such as imatinib and dasatinib has significantly improved survival in pediatric CML, highlighting the importance of kinase inhibition in leukemic therapy [4].

CDKs in transcriptional regulation and cell cycle control. Cyclin-dependent kinases (CDKs) regulate cell cycle progression and transcription. CDK9, in particular, is essential for super-enhancer driven transcription of survival genes such as MCL-1, MYC, and cyclin D1 in AML cells. Inhibition of CDK9 downregulates these survival signals, inducing apoptosis in leukemic blasts. Similarly, CDK6 is directly upregulated in MLL-rearranged AML, driving cell cycle progression and representing a viable therapeutic target [5].

PI3K signaling. Class I PI3Ks, including PIK3CG, contribute to leukemic cell survival and proliferation by activating AKT signaling. Targeting PI3K gamma isoform has shown reduced AML progression in preclinical models [6].

The convergence of these kinase pathways in pediatric leukemia underscores the potential advantage of multi-targeted inhibitors capable of disrupting several pro-survival signals simultaneously while sparing normal hematopoietic cells [7].

2.2 Thieno[3,2-*d*]pyrimidine derivatives as multi-kinase inhibitors

Thieno[3,2-*d*]pyrimidines represent a privileged scaffold in medicinal chemistry due to their purine-like structure enabling H-bonding within kinase ATP-binding sites. Rational modification at C-4, C-6, and C-7 positions permits modulation of lipophilicity and electronic characteristics to enhance interactions with hinge residues across BCR-ABL1, CDK, and PI3K. Structure-based design principles were applied to optimize this scaffold against pediatric leukemia targets, improving predicted binding affinity and selectivity profiles [8].

Molecular docking studies revealed that selected thieno[3,2-*d*]pyrimidine derivatives could form stable hydrogen bonds with conserved hinge residues and hydrophobic contacts within ATP pockets of CDK9 and PI3K targets. These interactions are consistent with established binding modes of known kinase inhibitors and suggest potential to disrupt transcriptional and survival signals critical for leukemic proliferation [9].

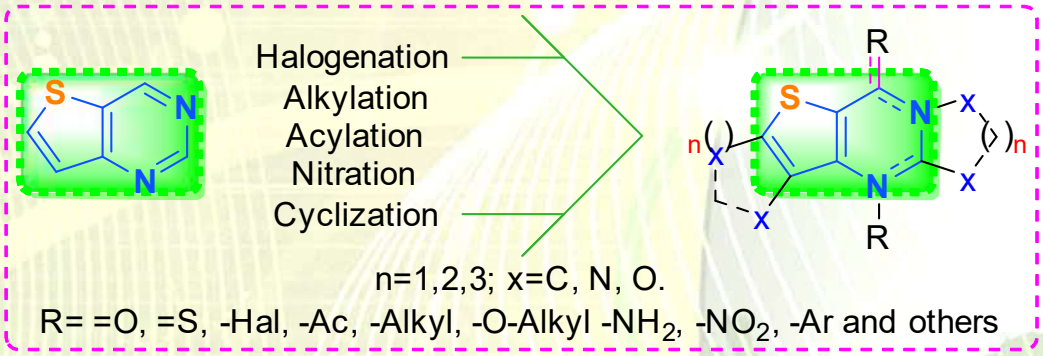
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Molecular dynamics simulations over 100 ns demonstrated minimal backbone deviation and persistence of key interactions, indicating robust complex stability. These *in silico* observations support the hypothesis that this scaffold can modulate multiple kinases implicated in pediatric leukemia pathobiology, thereby offering a foundation for future experimental validation.

3. Experimental Section

3.1 Synthesis Strategy

Novel thieno[3,2-*d*]pyrimidine derivatives were synthesized via step-wise cyclization from substituted thiophene precursors, employing amidine or urea reagents to form the heterocyclic core. Functional groups at positions C-4, C-6, and C-7 were introduced through electrophilic substitution and palladium-catalyzed coupling reactions, aiming to enhance interactions within kinase ATP pockets based on docking predictions.



3.2 *In Silico* Evaluation Methods

Protein structures of BCR-ABL1, CDK9, CDK6, and PI3K gamma were retrieved from the Protein Data Bank and prepared for docking studies. Molecular docking was performed using established scoring algorithms, with binding energy and interaction fingerprints analyzed across target kinases. Molecular dynamics simulations were carried out in explicit solvent conditions, with RMSD and hydrogen bond occupancy assessed to evaluate complex stability and interaction persistence.

4. Conclusion

This study identifies key protein kinases driving pediatric leukemia and demonstrates the potential of rationally designed thieno[3,2-*d*]pyrimidine derivatives as multi-targeted inhibitors. *In silico* docking and dynamics suggest stable engagement with BCR-ABL1, CDK9, and PI3K targets, offering a structural rationale for future development of selective anticancer agents tailored for pediatric leukemia. These findings warrant subsequent *in vitro* and *in vivo* validation to translate computational predictions into therapeutic candidates.

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