DESIGN AND EVALUATION OF THIENO[3,2-*d*]PYRIMIDINONE-BASED CDK INHIBITORS

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Abstract. The integration of *in silico* and experimental approaches is a key strategy in modern drug discovery. In this study, novel tricyclic thieno[3,2-*d*]pyrimidinone derivatives were rationally designed, synthesized, and evaluated as potential cyclin-dependent kinase inhibitors. Molecular docking against several CDK-related kinases revealed favorable binding energies and conserved hinge-region interactions. Compound **6e** showed strong affinity toward the CDK2 ATP-binding pocket. Molecular dynamics simulations confirmed complex stability, supporting the observed antiproliferative effects and CDK-targeted mechanism of action.

Keyword: Anticancer, CDKs, Docking, In silico, Thienopyrimidinone

Annotatsiya. Zamonaviy dori vositalarini yaratishda *in silico* va eksperimental yondashuvlarning integratsiyasi muhim strategiya hisoblanadi. Ushbu tadqiqotda yangi trisiklik tiyeno[3,2-*d*]pirimidinon hosilalari siklinlarga bog‘liq kinazalarning potentsial ingibitorlari sifatida oqilona loyihalandi, sintez qilindi va baholandi. Bir qator CDK bilan bog‘liq kinazalarga nisbatan o‘tkazilgan molekulyar doking tadqiqotlari qulay bog‘lanish energiyalari hamda hinge-mintaqaga xos saqlanib qolgan o‘zaro ta’sirlarni ko‘rsatdi. **6e** birikmasi CDK2 ning ATP-bog‘lanish cho‘ntagiga yuqori affinitet namoyon etdi. Molekulyar dinamika simulyatsiyalari kompleksning barqarorligini tasdiqlab, kuzatilgan antiproliferativ ta’sirlar va CDKga yo‘naltirilgan ta’sir mexanizmini qo‘llab-quvvatladi.

Kalit so‘zlar: CDKlar, Doking, *In silico*, Saratonga qarshi, Tiyenopirimidinon.

Аннотация. Интеграция *in silico* и экспериментальных подходов является ключевой стратегией в современной разработке лекарственных средств. В настоящем исследовании новые трициклические производные тиено[3,2-*d*]пиримидинона были рационально спроектированы, синтезированы и оценены как потенциальные ингибиторы циклинзависимых киназ. Молекулярный докинг в отношении ряда CDK-ассоциированных киназ выявил благоприятные энергии связывания и консервативные взаимодействия в области шарнирного участка. Соединение 6e продемонстрировало высокое сродство к АТФ-связывающему карману CDK2. Молекулярно-динамические симуляции подтвердили стабильность

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комплекса, что согласуется с наблюдаемыми антипролиферативными эффектами и CDK-опосредованным механизмом действия.

Ключевые слова: CDK, Докинг, *In silico*, Противоопухолевый, Тиенопиримидинон

INTRODUCTION

Modern drug discovery increasingly relies on the integration of *in silico* and experimental methodologies to overcome the limitations of conventional trial-and-error approaches in medicinal chemistry. The exponential growth of computational power and algorithmic sophistication has enabled in silico techniques to play a central role in early-stage drug discovery by allowing rapid screening of large chemical libraries and prioritization of promising lead compounds. In this context, computational tools such as molecular docking, molecular dynamics simulations, and in silico ADMET (absorption, distribution, metabolism, excretion, and toxicity) profiling have become indispensable for predicting ligand–target interactions, conformational stability, binding energetics, and pharmacokinetic behavior prior to chemical synthesis.

The implementation of such rational design strategies significantly reduces time consumption, experimental costs, and material resources while simultaneously increasing the probability of identifying biologically relevant compounds with favorable drug-like properties. Moreover, *in silico* evaluation provides mechanistic insights into structure–activity relationships (SAR), enabling systematic structural optimization and informed modification of molecular scaffolds at an early stage of development.

Among diverse heterocyclic systems explored in medicinal chemistry, the thieno[3,2-*d*]pyrimidine framework has emerged as a privileged scaffold due to its remarkable structural versatility and broad spectrum of biological activities. Numerous studies have demonstrated that thieno[3,2-*d*]pyrimidine derivatives exhibit significant anticancer, antimicrobial, antiviral, anti-inflammatory, and enzyme inhibitory properties, often through interaction with key biological targets such as kinases, receptors, and nucleotide-binding enzymes. The fused bicyclic architecture of this scaffold provides a rigid and planar conjugated system, which facilitates strong π–π stacking, hydrogen bonding, and hydrophobic interactions within biological binding sites.

Furthermore, the presence of multiple functionalizable positions within the thieno[3,2-*d*]pyrimidine core enables fine-tuning of electronic, steric, and lipophilic properties, making it particularly attractive for multi-target drug design. The pyrimidinone moiety, in particular, contributes to enhanced binding affinity through its ability to act as both hydrogen bond donor and acceptor, thereby

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improving molecular recognition and biological efficacy.

In light of these considerations, the present study is dedicated to the rational design, chemical synthesis, and comprehensive in silico evaluation of a series of novel thieno[3,2-*d*]pyrimidinone derivatives. The computational assessment aims to predict their binding behavior, stability, and pharmacokinetic profiles, thereby identifying promising candidates for further biological investigation as potential pharmacologically active agents.

Materials and methods. Cyclin-dependent kinases (CDKs) are a highly conserved family of serine/threonine kinases that play a central role in the regulation of transcription, cell cycle progression, and cellular homeostasis. Among them, CDK7 and CDK9 function as key transcriptional kinases by regulating RNA polymerase II activity through phosphorylation of its C-terminal domain. Aberrant activation or overexpression of these kinases has been strongly associated with uncontrolled cell proliferation, impaired apoptosis, and tumor progression in various cancer types, thereby establishing CDK7 and CDK9 as attractive molecular targets for anticancer drug development (Figure 1).

In this study, a series of structurally diverse tricyclic thieno[3,2-*d*]pyrimidine derivatives were rationally designed and synthesized with the aim of achieving optimal complementarity to the ATP-binding sites of CDKs. The rigid and conjugated tricyclic framework was selected to enhance planarity and facilitate π–π stacking interactions with aromatic residues located in the kinase hinge and gatekeeper regions. In addition, the presence of heteroatoms within the pyrimidinone moiety was intended to promote the formation of key hydrogen bonds with conserved hinge residues, which are crucial for potent ATP-competitive kinase inhibition.

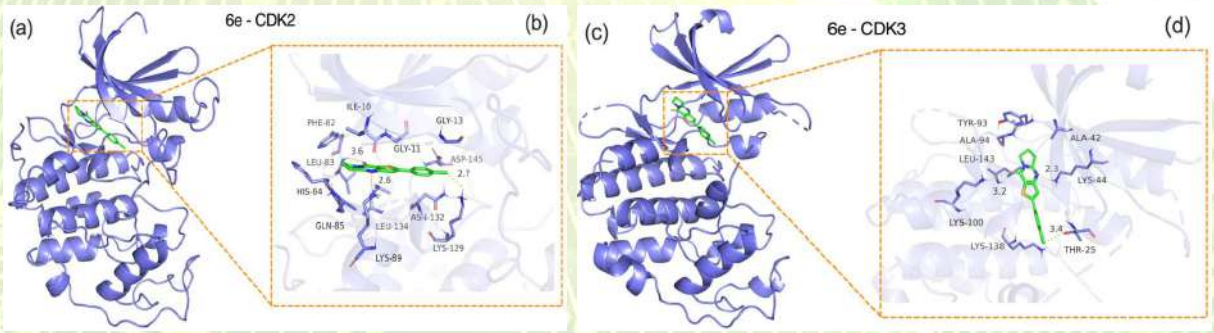


Figure 1. illustrates the representative docking poses of compound 6e within the active sites of CDK2, CDK3, and CDK5–p25 complexes. Panels (a, c) depict the overall binding conformation of compound **6e** (green) inside the kinase active sites (blue), whereas panels (b, d) highlight the key amino acid residues involved in ligand stabilization. Hydrogen bonds are indicated by dashed lines along with corresponding bond distances (Å), providing insight into binding orientation,

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interaction geometry, and predicted interaction strength.

Table-1

Structure	Target	PDB ID	Cdocker Energy (kcal/mol)	Combination Type
	CDK2	2C5Y	−9.4959	Hydrogen bond, Hydrophobic interaction, Electrostatic interaction
	CDK3	3GC0	0.1148	Hydrogen bond, Hydrophobic interaction, Electrostatic interaction, π-interaction
	CDK5–p25	1UNG	−5.7945	Hydrogen bond, Hydrophobic interaction, Electrostatic interaction, π-interaction
	CDK5–p35	1UNH	−8.3051	Hydrogen bond, Hydrophobic interaction, Electrostatic interaction, π-interaction
	CK1γ1	6F1W	−7.2382	Hydrogen bond, Hydrophobic interaction, van der Waals forces, Electrostatic interaction
	CSK	1BYG	−2.2890	Hydrogen bond, Hydrophobic interaction, Electrostatic interaction, π-interaction

To evaluate the binding feasibility and inhibitory potential of the synthesized compounds, molecular docking studies were performed against several CDK-related kinases using experimentally resolved crystal structures obtained from the Protein Data Bank. Docking simulations were conducted to predict plausible binding orientations, interaction patterns, and relative binding affinities of the ligands within the ATP-binding pocket. The calculated Cdocker energy values were used as an indicator of binding strength and complex stability, while detailed interaction analyses allowed identification of hydrogen bonds, hydrophobic contacts, electrostatic interactions, van der Waals forces, and π-interactions contributing to ligand recognition (Table 1).

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As summarized in Table 1, compound **6e** exhibited favorable docking scores and interaction profiles across multiple CDK-related kinases, including CDK2, CDK3, CDK5–p25, and CDK5–p35, as well as other signaling kinases such as CK1γ1 and CSK. The observed interaction patterns suggest a multi-kinase targeting potential, characterized by a combination of hydrogen bonding with conserved hinge residues, hydrophobic interactions within the ATP-binding cleft, electrostatic contacts, and stabilizing π-interactions.

Overall, the docking and molecular simulation results indicate that compound **6e** is capable of engaging multiple CDK-associated signaling pathways, thereby providing a plausible structural basis for its experimentally observed dose-dependent antiproliferative and pro-apoptotic effects in HeLa cells. Flow cytometry analysis revealed pronounced G1/S and G2/M phase cell cycle arrest, which is consistent with functional CDK inhibition. Notably, docking studies demonstrated strong binding of compound **6e** within the CDK2 ATP-binding pocket, characterized by stable hydrogen bonds with Leu83 and Glu81, as well as hydrophobic interactions involving Asp145. Furthermore, molecular dynamics simulations over a 100 ns timescale confirmed the stability of the ligand–protein complex, supporting a CDK-targeted mechanism of action that warrants further validation through enzymatic kinase inhibition assays and biochemical studies.

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