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Design, Molecular Docking, and ADMET Evaluation of Pyrazoline-Pyridine Derivatives as Dual-Target Ligands for Glucokinase (1V4S) and CDK5 (1UNL)

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Abstract

The development of dual-target ligands is an emerging strategy in medicinal chemistry to address complex diseases. This study aimed to evaluate six pyrazoline-pyridine derivatives (**5A-5F**) as potential dual-target ligands for Glucokinase (1V4S) and Cyclin-Dependent Kinase 5 (CDK5, 1UNL). Molecular docking was performed using AutoDock4 to assess binding affinity and interactions profiles, following validation of the docking protocol (RMSD < 2 Å). The results showed that compound **5F** exhibited the strongest binding affinity toward Glucokinase (-10.96 kcal/mol) and high affinity toward CDK5 (-8.34 kcal/mol), forming key interactions with residues Thr65 and Arg63 (Glucokinase) as well as Asn144, and Lys33 (CDK5). ADMET prediction indicated that all compounds comply with Lipinski's rule and exhibit high gastrointestinal absorption. However, most compounds were predicted to inhibit multiple CYP enzymes and show potential hepatotoxicity, highlighting the need for further optimization. Structure-activity relationship (SAR) analysis revealed that the pyrazoline scaffold contributes to molecular flexibility, while the pyridine ring enhances hydrogen bonding and hydrophobic interactions. Overall, compound **5F** is identified as the most promising candidate for further investigation as a dual-target ligand.

Keywords: *Pyrazoline-pyridine derivative, Glucokinase, CDK5, molecular docking, ADMET*

INTRODUCTION

Alzheimer's disease (AD) and type 2 diabetes mellitus (T2DM) are two global health conditions that place a significant burden on healthcare systems and on individuals' quality of life. AD, the most common form of neurodegenerative disease in the geriatric population, is projected to affect 150 million people worldwide by 2050, with the majority of dementia cases caused by AD (Kerwin et al., 2022; Li et al., 2022). The pathology of AD is characterized by abnormal hyperphosphorylation of tau protein and the accumulation of amyloid-β (Aβ) plaques, which progressively leads to neuronal dysfunction, loss of hippocampal neurons, and neurotransmitter dysregulation (Scheltens et al., 2021).

At the same time, T2DM is classified as a chronic metabolic disease characterized by elevated blood glucose levels resulting from insulin resistance or impaired insulin secretion. Its prevalence is showing a significant upward trend, from approximately 537 million cases globally, and is projected to reach 643 million by 2030 and 783 million by 2045 (Hossain et al., 2024; Saeedi et al., 2019). The main causes are obesity, low physical activity, unhealthy eating habits, and reduced insulin sensitivity (Kruczkowska et al., 2024).

The link between T2DM and AD is increasingly recognized, with T2DM significantly increasing the risk of dementia and AD by 45-90% (Hossain et al., 2024; Kruczkowska et al., 2024; Saeed et al., 2024; Xue et al., 2019). A recent meta-analysis from 2024 confirmed a 59% increased risk of dementia in patients with diabetes compared to those without diabetes (Cao et al., 2024). The pathophysiological convergence between these two diseases, often referred to as "type 3 diabetes" in the case of AD, involves common mechanisms such as oxidative stress, impaired insulin signaling, mitochondrial dysfunction, neuroinflammation, the formation of advanced glycation end products (AGEs), and metabolic syndrome (Kadohara, et al., 2017).

At the molecular level, glucokinase and Cyclin-Dependent Kinase 5 (CDK5) have emerged as key targets linking T2DM and AD (Paul et al., 2024a; Sharma et al., 2022). Glucokinase, which acts as a glucose sensor in the pancreas and neurons, exhibits reduced activity in conditions of insulin resistance, contributing to the dysregulation of systemic glucose homeostasis and neuronal energy deficiency (Sharma et al., 2022). Meanwhile, hyperglycemia triggers the activation of calpain, which cleaves p35 into p25, thereby forming a hyperactive CDK5/p25 complex. This hyperactive CDK5 plays a central role in AD pathology by inducing tau hyperphosphorylation, the formation of neurofibrillary tangles (NFTs), neuroinflammation, and neuronal death (Paul et al., 2024). In addition, CDK5 also affects insulin secretion and sensitivity, creating a pathological feedback loop between the two diseases.

In the context of modern drug design, the "one drug multiple targets" strategy or multi-target agents has become highly relevant for treating complex, interrelated diseases such as T2DM and AD (Artasensi et al., 2020; Ramsay et al., 2018). This approach reduces polypharmacy, improves treatment adherence, and simultaneously targets common pathological pathways. Pyrazoline-based heterocyclic compounds are known to have a broad spectrum of biological activity, including antidiabetic activity (Abdelgawad et al., 2025; Mortada et al., 2024), anti-Alzheimer's (Alkahtani et al., 2023; Machado et al., 2022), and anti-inflammatory (Mawla & Omar, 2025). Several pyrazole and pyrazoline derivatives have previously shown potential as potent inhibitors of α-glucosidase and α-amylase, as well as multi-target activity against AD targets (Abdelgawad et al., 2025).

Previous research by Mawla and Omar (2025) successfully synthesized and evaluated, both experimentally and computationally, a series of new Pyridine-ring-based pyrazoline compounds (compounds 5A-5F), as shown in Table 1. This prior study indicates that heterocyclic-based pyrazoline derivatives exhibit favorable molecular interactions with biological targets and have the potential to be further developed as multitarget therapeutic agents.

Although the biological potential of pyrazoline derivatives with a pyridine ring scaffold has been reported to exhibit both antidiabetic and neuroprotective activities, most previous studies have focused only on a single target and have not evaluated the potential of the pyrazoline-pyridine scaffold as a dual-target agent that simultaneously targets interrelated metabolic and neurodegenerative pathways. The novelty of this study lies in the evaluation of pyrazoline-pyridine derivatives (5A–F) as dual-target ligands against glucokinase and CDK5, which simultaneously target these two key enzymes that play a crucial role in the pathophysiology of T2DM and AD.

Based on this gap, the present study focuses on analyzing the molecular interactions between pyrazoline-pyridine derivative ligands (5A-5F) and amino acid residues at the active sites of glucokinase and CDK5 through molecular docking studies, structure-activity relationship (SAR) analysis, and predictions of pharmacokinetic and toxicity properties (ADMET) to assess the compounds' potential for safety and efficacy as drug candidates.

Through this computational approach, a deeper understanding of ligand-receptor mechanisms of action is obtained, accelerating the drug discovery process and identifying innovative, more efficient, and safer multi-target drug candidates for managing the rising prevalence of T2DM and AD comorbidities.

METHODOLOGY

Hardware and Software

The hardware used in this study was one HP Pavilion x360 14-dhxxx laptop with the specifications: Intel Core i5 CPU 1007U @ 1.50 GHz; 8 GB of RAM; and the Windows® 10 operating system.

The software programs used were ChemDraw Professional 15.0, GaussView 5.0 (Gaussian, Inc.), Chimera 1.13 from the Resource for Biocomputing, Visualization, and Informatics (RBVI), AutoDockTools 1.5.6, and MGL Tools 1.5.6, which includes AutoDock4 (ver. 4.2.6) from The Scripps Research Institute. Discovery Studio Visualizer 2019 (DSV) from BIOVIA.

Procedure

The Protein Preparation

The structures of the target proteins glucokinase and CDK5 were downloaded from the RCSB Protein Data Bank at https://www.rcsb.org/. The human glucokinase crystal structure used has the PDB ID 1V4S with a resolution of 2.30 Å and contains the native ligand 2-amino-4-fluoro-5-[(1-methyl-1H-imidazol-2-yl)sulfanyl]-N-(1,3-thiazol-2-yl)benzamide (MRK). Meanwhile, the CDK5 enzyme is a crystallographic structure of Cyclin-Dependent Kinase 5 (PDB ID: 1UNL; resolution 2.20 Å) that forms a complex with the ligand roscovitine (RRC). The target protein structure was accessed and saved in Protein Data Bank (.pdb) format.

The receptor was prepared using Chimera 1.13 by removing all nonstandard residues and the native ligand. The receptor was then saved in .pdb format. Polar hydrogen atoms were added to the entire receptor structure using AutoDockTools 1.5.6.

The Ligand Preparation

All the ligands for docking, namely, pyrazoline derivatives containing a pyridine ring, specifically compounds 5A, 5B, 5C, 5D, 5E, and 5F were drawn in GaussView 5.0 and optimized using the PM6 semi-empirical method with Gaussian(R) 09W software. The optimization and energy minimization were performed to determine the most stable molecular conformation with the lowest energy level (Azkiyah et al., 2025). The optimized structures of ligands 5A-5F were then saved in SybylMOL2 format.

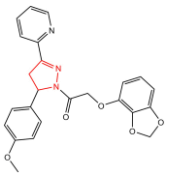
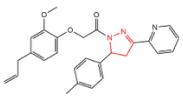
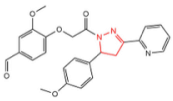
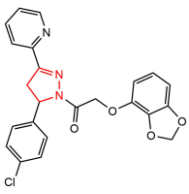
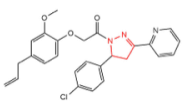
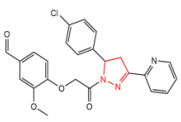
The docking and Visualization

Docking simulations were performed using AutoDock 4 1.5.6, targeting the active sites of glucokinase and CDK-5. The geometry of the glucokinase binding site was prepared using AutoGrid, with grid points defined by the coordinates (x, y, z) = (40, 40, 40) and a grid spacing of 0.375 Å. Meanwhile, the coordinate points for the CDK-5 enzyme were (x, y, z) = (40, 40, 50), with the same grid spacing of 0.375 Å. Protein-ligand complex interactions were analyzed using BIOVIA Discovery Studio Visualizer 2019 and PyMOL. Method validation was performed by redocking the native ligands (RRC and MRK). The validity of the redocking method is considered good if the Root Mean Square Deviation (RMSD) value is less than 2.00 Å.

ADMET Profile Evaluation

The pharmacokinetic parameters of drug-likeness and DME for ligands 5A-5F were analyzed using the SwissADME server (http://www.swissadme.ch/index.php). Drug-likeness evaluation was performed based on Lipinski's Rules of Five, namely: molecular weight ≤500 g/mol, hydrogen bond donors ≤5, hydrogen bond acceptors ≤10, and LogP ≤5, to predict the compounds' potential for oral bioavailability. Toxicity evaluation was performed using ProTox II (https://www.tox.charite.de/protox3/index.php?site=compound_input) by entering the ligand structure in SMILES format. The toxicity parameters examined included hepatotoxicity, carcinogenicity, immunogenicity, and mutagenicity.

Table 1. The chemical structure of the ligand 5A-5F

Compound	IUPAC Name	Structure
5A	2-(benzo[d][1,3]dioxol-4-yloxy)-1-(5-(4 methoxyphenyl)-3-(pyridin-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one	
5B	2-(4-allyl-2-methoxyphenoxy)-1-(3-(pyridin-2yl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one	
5C	3-methoxy-4-(2-(5-(4-methoxyphenyl)-3(pyridin-2-yl)-4,5-dihydro-1H-pyrazol-1-yl) 2oxoethoxy)benzaldehyde	
5D	2-(benzo[d][1,3]dioxol-4-yloxy)-1-(5-(4chlorophenyl)-3-(pyridin-2-yl)-4,5-dihydro-1H-pyrazol-1yl)ethan-1-one	
5E	2-(4-allyl-2-methoxyphenoxy)-1-(5-(4chlorophenyl)-3-(pyridin-2-yl)-4,5-dihydro-1H-pyrazol-1yl)ethan-1-one	
5F	4-(2-(5-(4 chlorophenyl)-3-(pyridin-2-yl)-4,5dihydro-1H-	

	pyrazol-1-yl)-2-oxoethoxy)-3 methoxybenzaldehyde

RESULT AND DISCUSSION

The dual-target ligand approach in medicinal chemistry is becoming an increasingly relevant strategy for treating complex diseases, particularly those involving metabolic and neurodegenerative dysregulation. In this study, pyridine-ring-substituted pyrazoline derivatives were evaluated in silico against two protein targets: glucokinase (1V4S) as an activator and CDK5 (1UNL) as an inhibitor.

ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) Pharmacokinetic Profile

Evaluation of the ADMET profile is a crucial step in drug development for predicting the pharmacokinetic behavior and safety of compounds. The chemical structure characteristics of pyrazoline-pyridine derivatives also influence the compounds' pharmacokinetic profiles. Based on the data presented, all six pyrazoline compounds (5A, 5B, 5C, 5D, 5E, 5F) exhibit promising characteristics as drug candidates. The ADMET pharmacokinetic analysis data are presented in Table 2.

Absorption. All compounds meet Lipinski's Rule of Five, indicating good potential for oral bioavailability. LogP values range from 3.60 to 4.42, indicating moderate lipophilicity, which supports absorption through biological membranes. All compounds are predicted to have high gastrointestinal (GI) absorption and varying blood-brain barrier (BBB) permeability; compounds **5B, 5D,** and **5E** are predicted to cross the BBB, while **5A, 5C**, and **5F** are not**.** This suggests that compounds 5B, 5D, and 5E have the potential to reach the central nervous system.

Distribution. Good BBB permeability for most compounds (**5B, 5D, 5E**) is a positive indicator for Alzheimer's therapy.

Metabolism. In terms of metabolism, there is variation in interactions with cytochrome P450 (CYP) enzymes. Compounds **5A** and **5D** are predicted to be CYP1A2 inhibitors, while compounds **5A, 5C**, and **5D** are CYP2D6 inhibitors. In addition, all compounds are inhibitors of CYP2C19, CYP2C9, and CYP3A. This broad CYP inhibition profile warrants further attention as it may lead to undesirable drug interactions.

Toxicity. In terms of toxicity, all compounds are predicted to be hepatotoxic and immunogenic. Compounds 5A-5C are predicted to be carcinogenic, while compounds 5D-5F are not. However, none of the compounds exhibited mutagenicity, which is a good indicator of safety. The LD50 (50% lethal dose) values indicate that compound 5A has the lowest toxicity (6000 mg/kg), followed by 5C (4540 mg/kg), and the other compounds (5B, 5D, 5E, 5F) have an LD50 of 1000 mg/kg. Based on toxicity classification, compound 5A falls into Class 6 (practically non-toxic), 5C into Class 5 (low toxicity), and 5B, 5D, 5E, and 5F into Class 4 (moderate toxicity). This toxicity profile requires further confirmation through in vitro and in vivo studies.

Table 2. ADMET Profiles of Pyrazoline-Pyridine Compounds Using SwissADME and Protox II

Drug Profile	**Compound**					
	5A	**5B**	**5C**	**5D**	**5E**	**5F**
Lipinski	✓	✓	✓	✓	✓	✓
MW (g/mol)	431	441	445	435	462	449
Hydrogen Donor	-	-	-	-	-	-
Hydrogen Acceptor	7	5	7	6	5	6
LogP	3.96	4.33	3.69	3.48	4.42	3.60
ADME						
GI Absorption	High	High	High	High	High	High
BBB	×	✓	×	✓	✓	×
Substrate P-gp	×	×	×	×	×	×
Inhibitor CYP1A2	✓	×	×	✓	×	×
Inhibitor CYP2C19	✓	✓	✓	✓	✓	✓
Inhibitor CYP2C9	✓	✓	✓	✓	✓	✓
Inhibitor CYP2D6	✓	×	✓	✓	×	×
Inhibitor CYP3A4	✓	✓	✓	✓	✓	✓
Skin Permeation (LogKp)	-6.48	-5.39	-6.84	-6.05	-5.33	-6.40
Toxicity						
Hepatotoxicity	Active	Active	Active	Active	Active	Active
Carcinogenicity	Active	Active	Active	Inactive	Inactive	Inactive
Immunogenicity	Active	Inactive	Inactive	Active	Active	Active
Mutagenicity	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
LD50 (mg/kg)	6000	1000	4540	1000	1000	1000
Toxicity Class	6	4	5	4	4	4

Overall, the ADMET profile indicates that these pyrazoline compounds have potential as drug candidates with good oral absorption and the ability to cross the blood-brain barrier for most compounds.

However, their broad CYP inhibitory potential and the presence of hepatotoxicity and immunogenicity require further investigation to optimize the safety profile and minimize drug interactions.

Ligand binding to the enzyme glucokinase

Method validation for the docking protocol was performed by redocking the native Mrk ligand from the glucokinase enzyme (1V4S) to the enzyme's active site to validate the geometric conformation. The 3D conformation (superimposition) of the native ligand resulting from redocking, which matches the pose of the native ligand in the co-crystal structure, is indicated by an RMSD value < 2.00 Å. The redocking results for the native MRK ligand yielded an RMSD value of 0.35 Å with a binding affinity of −10.01 kcal/mol. An RMSD value ≤ 2.00 Å indicates that the orientation of the redocked ligand shows a high degree of alignment with the position of the native ligand in the co-crystal structure (Kurniawan, et al., 2025). This alignment is also confirmed through superimposition visualization using Discovery Studio Visualizer (DSV) in Figure 1.

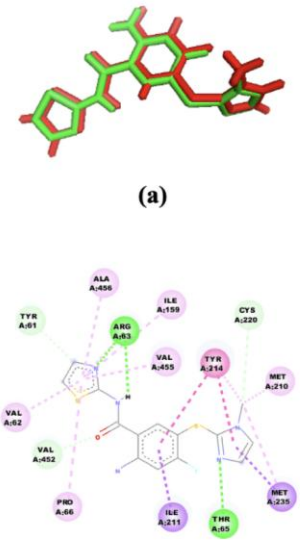
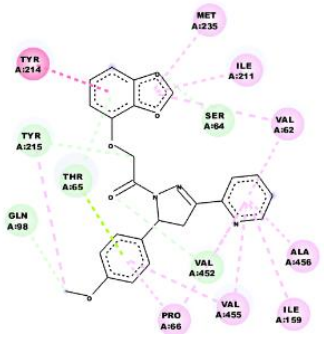


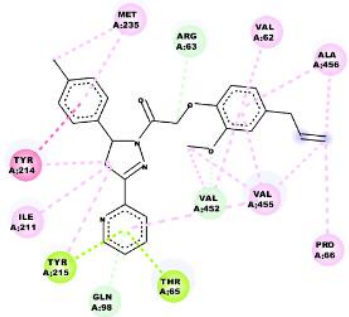
Figure 1. Visualization of the redocking of the native MRK ligand on glucokinase (PDB ID: 1V4S): (a) Superimposition of the redocking pose (green) with the co-crystallographic ligand (red); (b) Ligand interactions with amino acid residues in the active site

Interactions at the active site of the enzyme are indicated by the involvement of amino acid residues that form hydrogen bonds with Arg63 and Thr65; carbon-hydrogen interactions with Cys220, Ser64, Val452, and Tyr61; and hydrophobic interactions with amino acid residues such as Tyr214, Ala456, Ile159, Val455, Ile221, Met235, Met210, Pro66, and Val62.

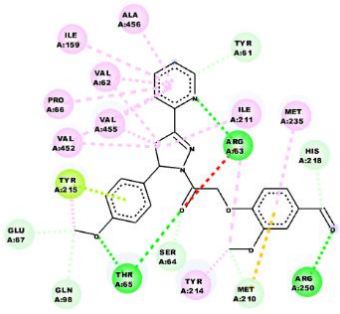
The docking results show that most of the test compounds have an affinity comparable to or higher than that of the native ligand, which served as the positive control (-10.10 kcal/mol). The highest binding affinities for each test compound, in descending order, were 5F < 5C < 5A < 5B < 5E < 5D, specifically: -10.96, -10.72, -10.42, -9.70, -9.13, and -8.96 kcal/mol. Furthermore, the results of the interaction profile analysis showed that the test compounds successfully occupied the binding site of the glucokinase enzyme and were able to interact with key amino acid residues through conventional hydrogen bonds, carbon-hydrogen bonds, and hydrophobic interactions characterized by the presence of alkyl, pi-alkyl, pi-pi stacking, and pi-sulfur bonds. The visual docking results are presented in Figure 2, while a detailed evaluation is provided in Table 1.



5A



5B



5C

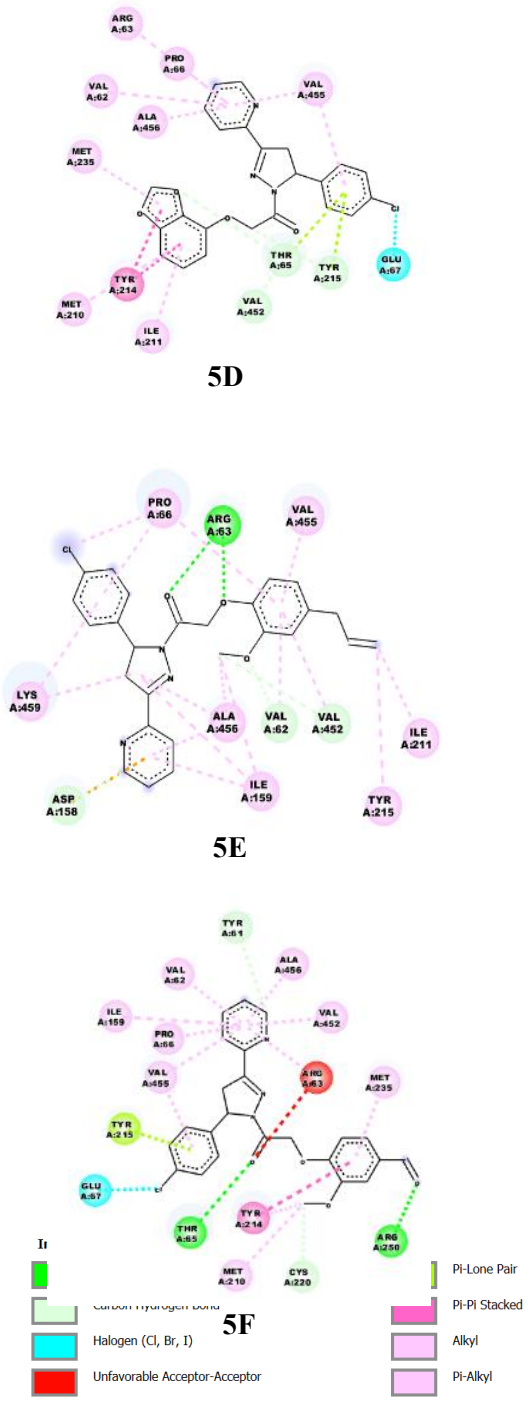


Figure 2. 2D conformational visualization of the test ligands (5A-5F) with the active amino acid residues of the glucokinase enzyme

Table 3. Binding Energy and Ligand Interaction Data for the Active Site of Glucokinase (PDB ID: 1V4S)

Compound	ΔG (kcal/mol)	Interaction	
		Hidrogen Bond	Hidrophobic Interaction
MRK	-10.01	Arg63, Thr65, Cys220, Ser64, Val452, Tyr61	Tyr214, Ala456, Ile159, Val455, Ile221, Met235, Met210, Pro66, Val62
5A	-10.42	Tyr65, Val452, Ser64, Gln98	Tyr214, Ala456, Ile159, Val455, Ile211, Met235, Pro66, Val62, Tyr215
5B	-9.70	Arg63, Thr65, Val452, Tyr215, Gln98	Tyr214, Ala456, Val455, Ile211, Val452, Pro66, Met235, Val62, Gln98 Tyr215
5C	-10.72	Arg63, Thr65, Cys220, Glu67, Arg250	Tyr214, Ala456, Val455, Met210, Met235, Ile159, Val62, Pro66, Ile211, Tyr215
5D	-8.96	Thr65, Tyr215, Val452	Tyr214, Ala456, Val455, Met210, Ile211, Met235, Val62, Pro66, Arg63, Glu67
5E	-9.13	Arg63, Val62, Val452, Asp158	Val62, Ala456, Val455, Val452, Ile159, Ile211, Pro66, Lys459
5F	-10.96	Thr65, Cys220, Arg250, Tyr215, Tyr61	Tyr214, Val455, Ala456, Met210, Met235, Pro66, Ile159, Val62, Val452, Arg63, Glu 67

Compounds 5F, 5C, and 5A have the highest binding affinities compared to the other test compounds. This indicates that these three test compounds have greater potential for activating glucokinase than the native ligand. In contrast, compounds with lower energy, such as 5D and 5E, exhibit a limited number of hydrogen bonds and a less-than-optimal distribution of hydrophobic interactions, making them unable to effectively stabilize the allosteric site of glucokinase.

Compound 5F interacts with the glucokinase enzyme through the formation of two conventional hydrogen bonds. These bonds form between the oxygen atom of the carbonyl group (C=O) and residues Arg250 and Thr65. Additionally, compound 5F also forms carbon-hydrogen bonds with Tyr61, Cis220, and Tyr215. The presence of a pyridine ring and aromatic groups enhances hydrophobic interactions, which also play a significant role in the binding of compound 5F via pi-alkyl bonds with residues Val455, Pro66, Ile159, Val62, Ala456, Val452, Met235, and Arg63; alkyl bonds from the carbon atom in the methoxy group with Met210 and Tyr214; the presence of a stacked pi-pi bond with Tyr214 further underscores the hydrophobic and aromatic nature of these interactions, which contribute to the stabilization of the glucokinase allosteric pocket. This combination of hydrogen-bonding and hydrophobic interactions is a common characteristic of glucokinase activators (Frimayanti et al., 2021).

Ligand binding to the CDK5 enzyme

A molecular docking study was also conducted on the Cyclin-Dependent Kinase 5 (CDK5) enzyme (PDB ID: 1UNL), specifically the crystallographic structure of the human CDK5 enzyme complex with the activator protein p25 and the inhibitor roscovitine (RRC). The selection of the 1UNL receptor was based on the presence of the native RRC ligand, which contains pyrazoline and pyridine groups, structurally relevant to the test compounds, which are pyrazoline derivatives substituted with a pyridine skeleton.

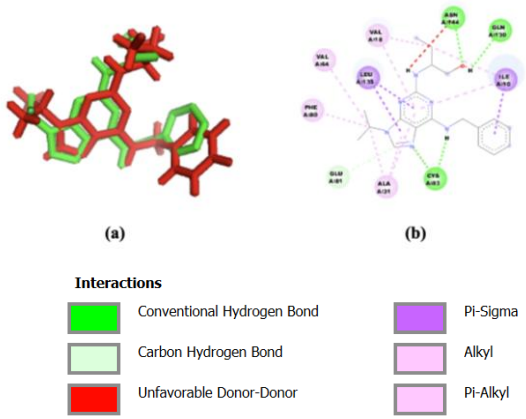
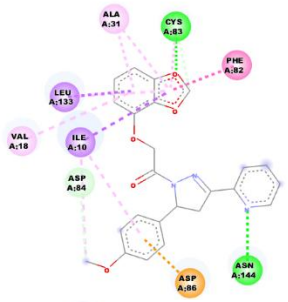


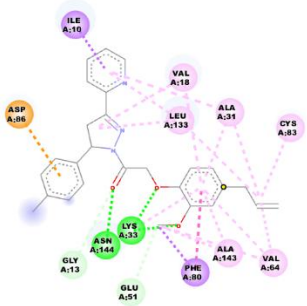
Figure 3. Visualization of the redocking of the native RRC ligand with the CDK5 enzyme (PDB ID: 1UNL): (a) Overlay of the redocked native ligand (green) with the CDK5 enzyme co-crystal ligand (red); (b) Interactions between the redocked RRC ligand and the active amino acid residues of the CDK5 enzyme.

The validity of the docking method was assessed through a redocking process of the native RRC ligand. The three-dimensional (3D) conformations generated from this redocking were then compared to the inhibitor ligand's pose in the enzyme's crystallographic structure, and the degree of similarity was measured using the Root Mean Square Deviation (RMSD) parameter (Figure 4). The redocking results showed an RMSD value of 0.91 Å, indicating a high degree of pose alignment with the native ligand found in the co-crystallographic data. Therefore, the docking method used is considered valid and applicable for the docking of test ligands.

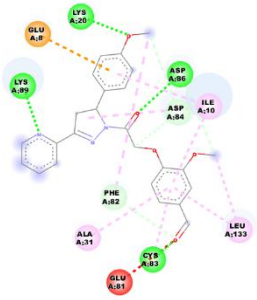
5A



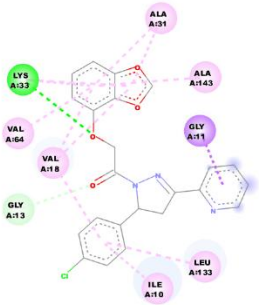
5B



5C



5D



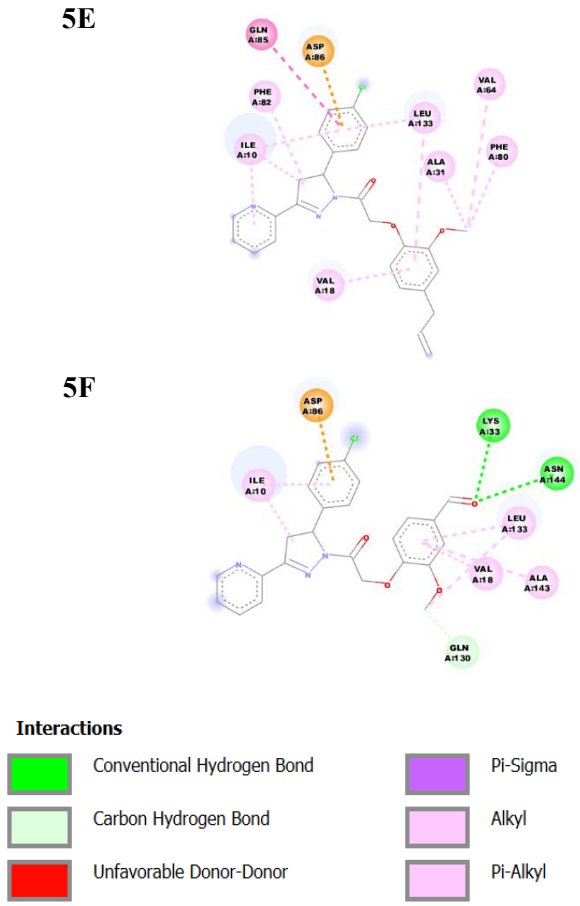


Figure 4. 3D visualization of the test ligands (5A-5F) and the active site residues of the CDK5 enzyme

At the CDK5 target (1UNL), the native ligand has an affinity energy of -7.73 kcal/mol and forms three hydrogen bonds: between the nitrogen of the pyrazoline group and the Cys83 residue; between the carbonyl group and the Asn144 and Gln130 residues; and a carbon-hydrogen bond with the Glu81 residue. Hydrophobic interactions are also formed via pi-sigma bonds between the benzene ring and Ile10 and between the pyridine and pyrazoline rings and Leu33; alkyl bonds with Val64 and Phe80; and pi-alkyl bonds with Val18, Ile10, and Ala31.

Analysis of the docking results for the test compounds shows that, in general, the pyrazoline-pyridine derivative test compounds exhibit inhibitory activity against the CDK5 enzyme, as evidenced by high binding affinity values ranging from -7.47 to -8.35 kcal/mol, as well as non-covalent interactions with key residues at the enzyme's active site. The order of binding affinities from highest to lowest is 5B < 5F < 5D < 5A < 5C < 5E, with values of -8.35, -8.34, -8.11, -7.93, -7.65, and -7.47 kcal/mol, respectively. Compounds 5B, 5F, 5D, and 5A have higher binding affinities than the native ligand (-7.73 kcal/mol) and other test compounds.

Table 4. Data on Binding Energy and Ligand-Enzyme Interactions with CDK5 (1UNL)

Compound	ΔG (kcal/mol)	Interaction	
		Hidrogen Bond	Hidrophobic Interaction
RRC	-7.73	Asn144, Gln130, Cys83	Ile80, Ala31, Val18, Val64, Phe80, Glu81
5A	-7.93	Asn144, Cys83	Ala31, Ile10, Val18, Leu133, Phe82, Asp84, Asp86
5B	-8.35	Asn144, Lys33, Gly133, Glu51	Ala31, Il10, Val18, Leu133, Val64, Phe80, Cys83, Ala143, Asp64
5C	-7.65	Cys83, Asp86, Lys20, Lys89	Ala31, Il10, Leu133, Phe82, Asp84, Glu81
5D	-8.11	Lys33	Ala31, Ile10, Leu133, Val18, Val64, Ala143, Gly11
5E	-7.47		Ala31, Ile10, Leu133, Val18, Val64, Phe80, Phe82, Gln85, Asp86
5F	-8.34	Asn144, Lys33	Leu133, Ile10, Val18, Ala143, Asp86, Gln130

The main interactions occur at Cys83, which serves as the hinge region (the flexible hinge region of the protein), as well as at Asn144 and Lys33. The Cys83 residue is a key component of the kinase's hinge region, acting as an anchor point for the inhibitor through the formation of hydrogen bonds. Compounds 5A, 5B, 5D, and 5F exhibit interactions with these residues, indicating appropriate ligand orientation within the ATP-binding pocket.

Specifically, compounds 5B and 5F form simultaneous interactions with Asn144 and Lys33, thereby enhancing the stability of the ligand-protein complex, while hydrophobic interactions with residues such as Val18, Leu133, and Ile10 contribute to the filling of hydrophobic spaces. In contrast, compound 5E does not show hydrogen bonding, indicating weak specific interactions with the active site, although

nonspecific hydrophobic interactions are still present. The visual docking results are presented in Figure 5, while a detailed evaluation is provided in Table 3.

Structure-Activity Relationships

The structure-activity relationship with respect to the target molecule indicates that the biological activity of pyrazoline derivatives is highly dependent on the type and position of substituents attached to various positions on the pyrazoline ring. Electronegative and steric factors at the N-1, C-3, and C-5 positions play a crucial role in determining the binding affinity, molecular orientation, and pharmacokinetic characteristics of these compounds (Mehmood et al., 2022). Functional groups such as methoxy (-OCH_3) as electron donors and halogens such as chlorine (Cl) as electron-withdrawing groups tend to enhance the compounds' potential as inhibitors of biological targets, as previously reported (Mawla & Omar, 2025). Meanwhile, pyridine and benzyl groups also contribute significantly to the overall binding energy through strong hydrophobic-aromatic interactions, as evidenced by the presence of pi-sigma, pi-pi-stacked, alkyl, and pi-alkyl bonds with amino acid residues at the active site of the protein.

Identification of Dual-Target Compounds Based on Molecular Docking, ADMET, and SAR Results

The relationship between molecular docking results, structure-activity relationship (SAR) analysis, and ADMET profiles indicates that the characteristics of the substituents on the pyrazoline-pyridine scaffold play a crucial role in determining the biological activity and pharmacokinetic properties of the compounds. Based on the docking results and SAR analysis, compound 5F emerged as the most promising dual-target ligand candidate, exhibiting the highest binding affinity for Glucokinase and CDK5, supported by specific interactions at key residues and the presence of pyridine and aromatic groups that contribute to strong hydrophobic interactions, consistent with SAR principles. Although it has a promising ADMET profile in terms of oral absorption and is not carcinogenic, its broad CYP inhibition potential and hepatotoxicity require further evaluation. Compounds 5B and 5D, although selective as CDK5 inhibitors with the ability to cross the BBB, also exhibit similar toxicity profiles. In contrast, compound 5A, which also demonstrates good dual-target potential, has the lowest toxicity (LD50 6000 mg/kg) among all compounds, making it an attractive candidate for further optimization. Overall, although some compounds exhibited less-than-ideal ADMET profiles, the relationships between structure, activity, and pharmacokinetic properties identified through this analysis provide an important foundation for structural modifications aimed at improving efficacy and safety in the development of dual-target drugs.

CONCLUSION

This study confirms the potential of pyrazoline-pyridine derivatives as promising multitarget ligands against glucokinase (1V4S) and CDK5 (1UNL). Based on the integration of molecular docking results, structure-activity relationship (SAR) analysis, and ADMET predictions, compound 5F was identified as the most promising candidate because it exhibited the highest binding affinity for both targets and was able to form interactions with key residues involved in stabilizing the ligand-protein complex. The presence of a pyrazoline scaffold and a pyridine ring is known to contribute to the formation of hydrogen and hydrophobic interactions that support the compound's dual-target activity. All test compounds exhibited good drug-likeness characteristics with high gastrointestinal absorption and low to moderate toxicity. However, predictions of inhibition of several CYP isoenzymes and potential hepatotoxicity indicate the need for further structural optimization. Overall, these results support the development of pyrazoline-pyridine derivatives as multi-target candidate agents for type 2 diabetes mellitus and Alzheimer's disease, which still need to be validated through molecular dynamics studies as well as in vitro and in vivo assays.

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