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Original Article

In silico prediction of cinnamaldehyde on the PI3K/AKT pathway activator of anti-apoptotic potential

[Predicción *in silico* del potencial antiapoptótico del cinamaldehído sobre el activador de la vía PI3K/AKT]

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Abstract

Context: The dysregulation of the phosphoinositide 3-kinase protein kinase B (PI3K/AKT) signaling pathway has been associated with diabetes and its complications. Cinnamaldehyde (CA) can potentially inhibit diabetes complications through its pleiotropic effects, including anti-inflammatory, anti-oxidative, and anti-apoptosis. However, whether these effects are related to PI3K/AKT pathway modulation is unknown.

Aims: To evaluate the interaction of cinnamaldehyde with proteins involved in the PI3K/AKT pathway.

Methods: This study involved pharmacokinetic analysis, molecular docking, and molecular dynamics. The ligand used was cinnamaldehyde, and the proteins were eight crystal structures of proteins related to the PI3K/AKT pathway. For comparison purposes, we selected compound UCL-TRO-1938 (PI3K activator), baicalin (AKT activator), and bisperoxovanadium (PTEN inhibitor) as control ligands.

Results: The results of this study indicated that CA has the potential to be an oral-drug candidate as it fulfills all Lipinski criteria and shows good cell membrane permeability. Molecular docking showed that CA exhibited good binding affinities toward target proteins. CA showed stronger binding affinity to the p110α subunit of PI3K (-6.4 vs. -6.3 kcal/mol) and PTEN (-5.8 vs. -5.0 kcal/mol) compared to the control ligand. CA also interacts with similar amino acid residues compared to control ligands. A molecular dynamic study revealed that CA and control ligands-protein complexes have similar stability with few conformational alterations in several amino acid residues.

Conclusions: CA has the potential to be a drug candidate to ameliorate diabetes complications by up-regulating the PI3K/AKT pathway. Further experimental study is required to ascertain its efficacy.

Keywords: AKT; cinnamaldehyde; *in silico*; molecular docking; molecular dynamics; PI3K.

Resumen

Contexto: La desregulación de la vía de señalización de la proteína quinasa B de la fosfoinosítido 3-quinasa (PI3K/AKT) se ha asociado con la diabetes y sus complicaciones. El cinamaldehído (CA) puede inhibir potencialmente las complicaciones de la diabetes a través de sus efectos pleiotrópicos, que incluyen efectos antiinflamatorios, antioxidantes y antiapoptósicos. Sin embargo, se desconoce si estos efectos están relacionados con la modulación de la vía PI3K/AKT.

Objetivos: Evaluar la interacción del cinamaldehído con las proteínas involucradas en la vía PI3K/AKT.

Métodos: Este estudio implicó análisis farmacocinético, acoplamiento molecular y dinámica molecular. El ligando utilizado fue el cinamaldehído y las proteínas fueron ocho estructuras cristalinas de proteínas relacionadas con la vía PI3K/AKT. Para fines de comparación, seleccionamos el compuesto UCL-TRO-1938 (activador de PI3K), baicalina (activador de AKT) y bisperoxovanadio (inhibidor de PTEN) como ligandos de control.

Resultados: Los resultados de este estudio indicaron que el CA tiene el potencial de ser un candidato a fármaco oral, ya que cumple con todos los criterios de Lipinski y muestra una buena permeabilidad de la membrana celular. El acoplamiento molecular mostró que el CA exhibió buenas afinidades de unión hacia las proteínas objetivo. El CA mostró una afinidad de unión más fuerte a la subunidad p110α de PI3K (-6,4 frente a -6,3 kcal/mol) y PTEN (-5,8 frente a -5,0 kcal/mol) en comparación con el ligando de control. El CA también interactúa con residuos de aminoácidos similares en comparación con los ligandos de control. Un estudio de dinámica molecular reveló que tanto el CA como los complejos de proteína-ligando de control tienen una estabilidad similar con pocas alteraciones conformacionales en varios residuos de aminoácidos.

Conclusiones: El CA tiene el potencial de ser un candidato a fármaco para mejorar las complicaciones de la diabetes mediante la regulación positiva de la vía PI3K/AKT. Se requieren más estudios experimentales para determinar su eficacia.

Palabras Clave: acoplamiento molecular; AKT; cinamaldehído; dinámica molecular; *in silico*; PI3K.

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Abbreviation: AKT: protein kinase B; CA: cinnamaldehyde; CYP: cytochrome P450; P-gp: P-glycoprotein; PI3K: phosphoinositide 3-kinase; PIP2: phosphatidylinositol (4,5)-bisphosphate; PIP3: phosphatidylinositol (3,4,5)-triphosphate; PTEN: phosphatase and tensin homolog on chromosome 10; RMSD: root mean square deviation; RMSF: root mean square fluctuation; T2D: type 2 diabetes mellitus.

INTRODUCTION

Type 2 diabetes mellitus (T2D) is a metabolic disorder that has become a global pandemic in recent decades. The global prevalence of diabetes is around 536 million in 2021 and is estimated to continue to increase to 783 million in 2045 (International Diabetes Federation, 2021). At least 6 million deaths per year are caused by diabetes and its complications, making it the ninth leading cause of death worldwide (Khan et al., 2020). Many diabetes complications are associated with cellular injury caused by hyperglycemia. Apoptosis is known as physiological programmed cell death. However, if apoptosis occurs excessively, it potentially leads to organ failure (Volpe et al., 2018). Many *in vitro* studies consistently reported that high glucose exposure induced cellular apoptosis (Bai et al., 2022; Dong et al., 2020; E and Jiang, 2020; Varela et al., 2021)

The phosphoinositide 3-kinase protein kinase B (PI3K/AKT) signaling pathway is essential in various cell signaling processes associated with insulin signaling, cell survival, metabolism, growth, and apoptosis (Kumar et al., 2023). PI3K is classified into three classes: I, II, and III. Class IA PI3K is a ubiquitous heterodimeric protein consisting of two different subunits: a p85 regulatory subunit and a p110 catalytic subunit (p110α, β, or δ) (Saltiel, 2021). Class IA PI3K catalyzes the phosphorylation of phosphatidylinositol (4,5)-bisphosphate (PIP2) into phosphatidylinositol (3,4,5)-triphosphate (PIP3), which then interacts with AKT and initiates its translocation to the plasma membrane. Activated AKT proceeds to phosphorylate several downstream effectors related to the apoptosis pathway, thus resulting in the inhibition of apoptosis cascades (Vidal et al., 2022).

The dysregulation of the PI3K/AKT signaling pathway has been associated with diabetes and its complications (Ramasubbu and Devi Rajeswari, 2023). On the other hand, accumulating studies suggest that activation of the PI3K/AKT pathway could offer a beneficial effect on diabetics, providing tissue protection and enhancing cell regeneration. Increased PI3K/AKT pathway activation by various pharmacological agents has been proven to successfully ameliorate cellular injury due to high glucose (Bathina et al., 2020). Therefore, there is growing interest in the development of potential PI3K/AKTagonists.

Several natural-based molecules have been shown to activate AKT, like baicalin (Yang et al., 2019), chlorogenic acid (Gao et al., 2018), kaempferol-3-glucuronide, and quercetin-3-glucuronide (Fang et al., 2021). Besides, Kumar et al. (2023) identified two lead molecules that may act as AKT activators through ligand and structure-based computational approaches. A recent study also reported the discovery of UCL-TRO-1938, a small molecule activator of the PI3Kα (Gong et al., 2023).

Cinnamon (*Cinnamomum* sp.) has been widely utilized as both a flavoring agent and a medicinal remedy for centuries in many regions, including the Middle, East, and Southeast Asia (Shang et al., 2021). Previous research has revealed cinnamon's antimicrobial, antidiabetic, and antilipidemic properties. Much of this research has focused on cinnamon bark powder, which is rich in essential oils, antioxidants, flavonoids, and carotenoids (Stevens and Allred, 2022). However, the volatile essential oil of cinnamon may also possess significant medicinal properties. Cinnamaldehyde (CA), which constitutes approximately 50–90% of the chemical composition of cinnamon essential oil, is the primary contributor to the sweet, spicy aroma typically associated with cinnamon essential oil (Doyle and Stephens, 2019). Besides providing a direct antidiabetic effect by increasing insulin secretion and sensitivity, recent evidence indicates that CA also potentially inhibits diabetes complications through its pleiotropic effects, including anti-inflammatory, anti-oxidative, and anti-apoptotic (Shang et al., 2021). Several studies have proven that both the insulin-sensitizing and anti-apoptosis effects of CA are mediated, at least in part, by the up-regulation of the PI3K/AKT pathway (Abdelmageed et al., 2019; Endharti et al., 2024; Urasaki and Le, 2022). However, it is still unclear whether these effects are mediated by the direct activation of the PI3K/AKT pathway.

Despite the fact that CA's antidiabetic and anti-apoptotic effects have been empirically proven, the potency of CA as a direct PI3K or AKT activator is relatively understudied. Additionally, PI3K and AKT are valuable therapeutic targets for developing new therapeutic strategies against various diseases, including diabetes mellitus and its complications. Therefore, the present study aims to predict the potency of CA as a candidate for PI3K/AKT activator using *in silico* virtual screening, molecular docking, and molecular dynamics simulations.

MATERIAL AND METHODS

Preparation of ligands and targets

Crystal structures of several proteins involved in the PI3K/AKT pathway were obtained from Protein Data Bank (PDB) (https://www.rcsb.org/) databases. The protein targets used in this study are listed in Table 1. Subsequently, PyMol (version 2.5.5) was employed to prepare the protein targets, including adding hydrogen atoms, elimination of water molecules, and metal ions. On the other hand, the 3D structure of CA (Compound ID: 637511) was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Cinnamaldehyde underwent energy minimization by using OpenBabel and then saved as .pdbqt format before molecular docking was performed. Several control ligands were selected for comparison, including UCL-TRO-1938 (compound ID: 166451134) for 1st-4th proteins, baicalin (compound ID: 64982) for the 5th-7th proteins, and bisperoxovanadium (compound ID: 54692867) for the 8th protein (Gong et al., 2023; Walker et al., 2019; Yang et al., 2019).

Table 1. Target proteins used in molecular docking.

Protein No.	Protein	PDB ID
1	p110α subunit	8OW2
2	apo p110α/p85α	7PG6
3	apo p110β/p85β	2Y3A
4	apo p110δ/p85α	5DXU
5	AKT1	2UZT
6	PH domain AKT1	1UNR
7	AKT2	1O6L
8	PTEN	1D5R

Molecular docking

The binding affinities of the CA and targeted proteins were determined by using PyRx AutoDock Vina. The active binding site information was obtained from either previous experimental studies or the web-server active site prediction http://www.scfbio-iitd.res.in/dock/ActiveSite.jsp. The center of the grid box for molecular docking was chosen based on the location of the predicted active site region, and its volume was chosen based on the coverage of the entire active site pocket. Amongst them, the conformation with the lowest root mean square deviation (RMSD) values was selected and documented for their binding affinity. Lastly, 2D and 3D visualization of CA and targeted protein interaction was performed using BIOVIA Discovery Studio Software (BIOVIA, San Diego, USA).

Prediction of pharmacokinetic properties

The drug-likeness properties are invaluable in predicting the potential of a compound to be used as a drug candidate. The SwissADME (Swiss Institute of Bioinformatics) database (http://www.swissadme.ch/) and TCM System Pharmacology (TCMSP) Database (https://tcmsp-e.com/) were used to predict the absorption, distribution, metabolism, and excretion (ADME) characteristics of CA. To assess the drug-likeness of CA, several properties were considered, including but not limited to molecular weight, oral bioavailability, predicted central nervous system activity, predicted aqueous solubility, Lipinski rule of 5, and predicted human oral absorption. The analysis was carried out by submitting the CA canonical smile obtained from the PubChem database to the respective website. A compound is said to be a good drug candidate if it does not violate the Lipinski rule, has five or fewer hydrogen bond donors, ten or fewer hydrogen bond acceptors, and a molecular mass of 500 kg/mol or less (Hollingsworth and Dror, 2018).

Molecular dynamics analysis

Molecular dynamics were carried out to estimate the stability of CA interaction with target proteins, which were simulated as if the process was taking part in the cell and compared to the control ligands. Cinnamaldehyde was previously docked with target proteins using the PyRx software and saved in PDB format. The data was then submitted to the web-based protein structure modeling − CABS-flex 2.0 (http://biocomp.chem.uw.edu.pl/CABSflex2). The simulation was performed through 50 cycles, and the selection chain chosen in this case is the A chain. Other settings used in this simulation follow the default web server settings. The root mean-square fluctuation (RMSF) value was evaluated. A higher RMSF value indicates more flexibility (Hollingsworth and Dror, 2018). The result is presented as residual fluctuation of plot data stored in CSV and graphic formats.

Data analysis

The data obtained were analyzed at each step of the experiment. Firstly, the internal validation of the docking method was confirmed by redocking the native ligand in each crystal structure of the target protein using PyRx software. The validity was assessed based on the Root Mean Square Deviation (RMSD) value (<2Å), which reflects the accuracy of the docking method by re-docking the native ligand to the target protein as similar as possible to the original structure. The pharmacokinetics analysis, including the predicted value of the Lipinski rule, the prediction of absorption and distribution, and the physi-

cochemical properties of CA, was averaged from two different databases (SwissADME and TCMSP) if possible. The chemical interaction, the binding affinity, and molecular dynamics interaction of each ligand and target protein were compared to those of control ligands obtained from literature, including UCL-TRO-1938, baicalin, and bisperoxovanadium (Gong et al., 2023; Walker et al., 2019; Yang et al., 2019).

RESULTS

Molecular docking simulation of cinnamaldehyde with PI3K/AKT pathway proteins

To further evaluate the potential effect of CA on the eight key hypothetical target proteins related to the PI3K/AKT pathway, we performed a molecular docking analysis *in silico*. The binding affinity, the interacting residues, their respective type of bond, and the grid box coordinates are shown in Table 2. Molecular docking analysis of CA with targeted proteins was presented in Table 3. The binding affinity values of ligands and proteins can be interpreted as the average free energy of the conformations of all interacting molecules. The lower the binding affinity, the higher the possibility of forming a bond. Overall, CA binding affinity was less than −5.0 kcal/mol, which indicated a good interaction between CA and the targeted proteins (Xue et al., 2023). However, only its binding with p110α subunit (8OW2) and PTEN (1D5R) was stronger than the control ligands. CA exhibited a binding affinity of -6.4 Kcal/mol to the p110α catalytic subunit, whereas compound 1938 (control) had a binding affinity of -6.3 Kcal/mol (data not presented). CA also has a more negative binding affinity to PTEN (-5.8 Kcal/mol) than bisperoxovanadium (-5.0 Kcal/mol), a known PTEN inhibitor. On the other hand, CA showed comparable binding affinity towards other target proteins, including class IA PI3K (apo p110α/p85α, apo p110β/p85β, apo p110δ/p85α) AKT1, PH domain AKT1, and AKT2. These results imply that treatment with CA may affect PI3K/AKT/PTEN in diabetic patients.

Table 2. The results of molecular docking between CA and target proteins.

Target protein (PDB ID)	Binding affinity (kcal/mol)	Interacting residues	Type of bond	Grid box coordinate
p110α subunit (8OW2)	-6.4*	**Glu259**	Hydrogen	x: 5.99
		Tyr260	Hydrogen	y: -13.60
		Arg662	Hydrophobic	z: -36.43
		Pro757	Hydrophobic	
apo p110α/p85α (7PG6)	-6.0	**Val166**	Hydrogen	x: -0.44
		Tyr167	Hydrogen	y: -20.77
		Pro168	Hydrogen	z: 31.76
		Asp258	Hydrogen	
		Glu259	Hydrogen	
		Tyr260	Hydrogen	
		Gln661	Hydrogen	
		Arg662	Hydrogen	
		His665	Hydrogen	
		Tyr698	Hydrogen	
		Leu752	Hydrogen	
		Pro757	Hydrophobic	
		Ala758	Hydrogen	
apo p110β/p85β (2Y3A)	-6.7	**Ile329**	Hydrogen	x: 32.01
		Val347	Hydrogen	y: 46.29
		Arg348	Hydrogen	z: 2.79
		Ala349	Hydrogen	
		Trp375	Hydrogen	

Table 2. The results of molecular docking between CA and target proteins (continued...)

Target protein (PDB ID)	Binding affinity (kcal/mol)	Interacting residues	Type of bond	Grid box coordinate
		Leu379	Hydrogen	
		Phe396	Hydrogen	
		Ala397	Hydrogen	
		Val398	Hydrogen	
		Leu485	Hydrophobic	
		Ile487	Hydrogen	
apo p110δ/p85α (5DXU)	-6.1	**Ile777**	Hydrogen	x: 33.02
		Asp787	Hydrogen	y: 12.77
		Tyr813	Hydrogen	z: 32.44
		Ile825	Hydrogen	
		Glu826	Hydrogen	
		Val827	Hydrogen	
		Val828	Hydrophobic	
		Ser831	Hydrogen	
		Met900	Hydrophobic	
		Phe908	Hydrogen	
		Ile910	Hydrophobic	
		Asp911	Hydrogen	
AKT1 (2UZT)	-6.4	**Val15**	Hydrogen	x: 5.46
		Phe18	Hydrophobic	y: 18.86
		Leu19	Hydrogen	z: 21.58
		Phe100	Hydrogen	
		Leu152	Hydrogen	
		Glu155	Hydrogen	
		Tyr156	Hydrogen	
		Ile303	Hydrogen	
		Tyr306	Hydrophobic	
PH domain AKT1 (1UNR)	-4.8	**Tyr38**	Hydrogen	x: 24.65
		Lys39	Hydrogen	y: -4.13
		Glu40	Hydrogen	z:14.56
		Arg41	Hydrogen	
		Pro42	Hydrogen	
		Glu49	Hydrogen	
		Ala50	Hydrogen	
		Pro51	Hydrogen	
		Leu52	Hydrogen	

Table 2. The results of molecular docking between CA and target proteins (continued...)

Target protein (PDB ID)	Binding affinity (kcal/mol)	Interacting residues	Type of bond	Grid box coordinate
AKT2 (1O6L)	-6.1	Leu158	Hydrogen	x: 33.78
		Gly159	Hydrogen	y: 24.54
		Val166	Hydrophobic	z: 105.51
		Thr213	Hydrogen	
		Met229	Hydrogen	
		Glu230	Hydrogen	
		Ala232	Hydrogen	
		Met282	Hydrophobic	
		Lys290	Hydrogen	
		Thr292	Hydrogen	
		Phe439	Hydrogen	
PTEN (1D5R)	-5.8*	**Phe206**	Hydrogen	x: 31.76
		Met270	Hydrogen	y: 76
		Lys327	Hydrophobic	z: 23.7
		Ala328	Hydrogen	
		Asp331	Hydrogen	
		Tyr336	Hydrophobic	
		Phe337	Hydrogen	
		Asp268	Hydrogen	

Bold indicates identical interacting residues between CA and control ligand to target protein. *Asterisk indicates lower binding affinity value than control ligand.

The target proteins and CA interaction were further visualized by BIOVIA Discovery Studio software, as presented in Fig. 1. The type of interaction between the ligand and the target protein is a critical parameter in predicting the quality of interactions (Daisy et al., 2011). Docking results show that the binding between CA and the target proteins is mainly mediated by hydrogen bonds and, to a lower extent, by hydrophobic bonds (Fig. 2). Both hydrogen bonding and optimized hydrophobic interactions are critical in stabilizing the ligands at the target site, and helping alter binding affinity and drug efficacy (Patil et al., 2010). CA forms hydrogen bonds with Glu259 and Tyr260, while it shows hydrophobic bonds with Arg662 and Pro757 of the p110α subunit. Three of those interactions are also observed in compound 1938 (control ligand) interaction with the p110α subunit. Meanwhile, CA binds to PTEN through six hydrogen bonds with Phe206, Met270, Ala328, Asp331, Phe337, and Asp268 and two hydrophobic bonds with Lys327 and Tyr336. Similar interactions are also observed in the bisperoxovanadium interaction with PTEN.

Predictor ADME properties of cinnamaldehyde

CA was subjected to ADME analysis using the Swiss ADME and TCMSP databases. ADME properties of CA and control ligands used in this study were tabulated in Table 3. CA suitability for oral delivery was assessed using Lipinski's 'Rule of Five' criterion. An orally active drug-like compound should not have more than one violation of these criteria: hydrogen bond donors not greater than 5, hydrogen bond acceptors not greater than 10, molecular weight not greater than 500 Da, and octanol-water partition coefficient (log P) not greater than 5. CA has molecular weights of 132.17 Da, AlogP o/w value of 1.97, zero hydrogen bond donors, and one hydrogen bond acceptor. CA obeys all Lipinski's rules, which indicate its drug-like properties. Interestingly, the predicted aqueous solubility (logS) of CA was within the acceptable range of –2 to –4, meaning that it is slightly water soluble.

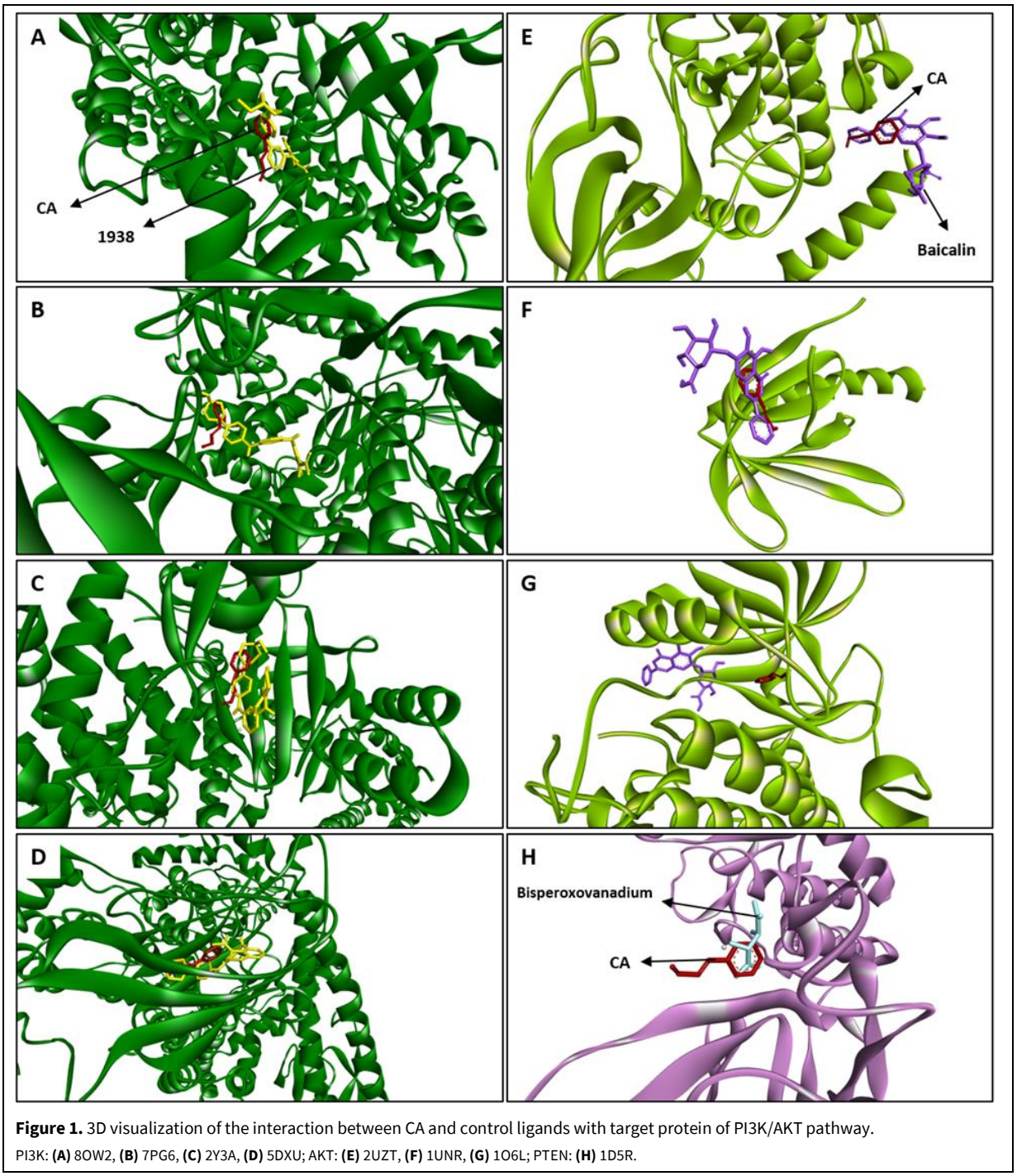

Figure 1. 3D visualization of the interaction between CA and control ligands with target protein of PI3K/AKT pathway. PI3K: **(A)** 8OW2, **(B)** 7PG6, **(C)** 2Y3A, **(D)** 5DXU; AKT: **(E)** 2UZT, **(F)** 1UNR, **(G)** 1O6L; PTEN: **(H)** 1D5R.

The pharmacokinetics properties analyzed were oral bioavailability, drug half-life, Caco-2 permeability, predicted interaction with P-glycoprotein (P-gp) and cytochrome P450 (CYP) proteins, and BBB permeability. CA had a predicted oral bioavailability value of 32% with a half-life of 4.73 hours, which is classified as the mid-elimination group. The Caco-2 permeability value of CA was 1.35 nm/s, representing moderate intestinal epithelial permeability. CA was also predicted to not act as a P-gp substrate and not inhibit CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 proteins. In addition, CA has a TPSA value of less than 60 angstroms, indicating that it is cell membrane permeable; however, it is also considered blood-brain barrier penetrative (BBB value of >0.3).

Molecular dynamics analysis of ligand-protein complexes

Molecular dynamics simulations were performed to investigate the dynamic features of the ligand-receptor complex in order to mimic the behavior in the actual environment.

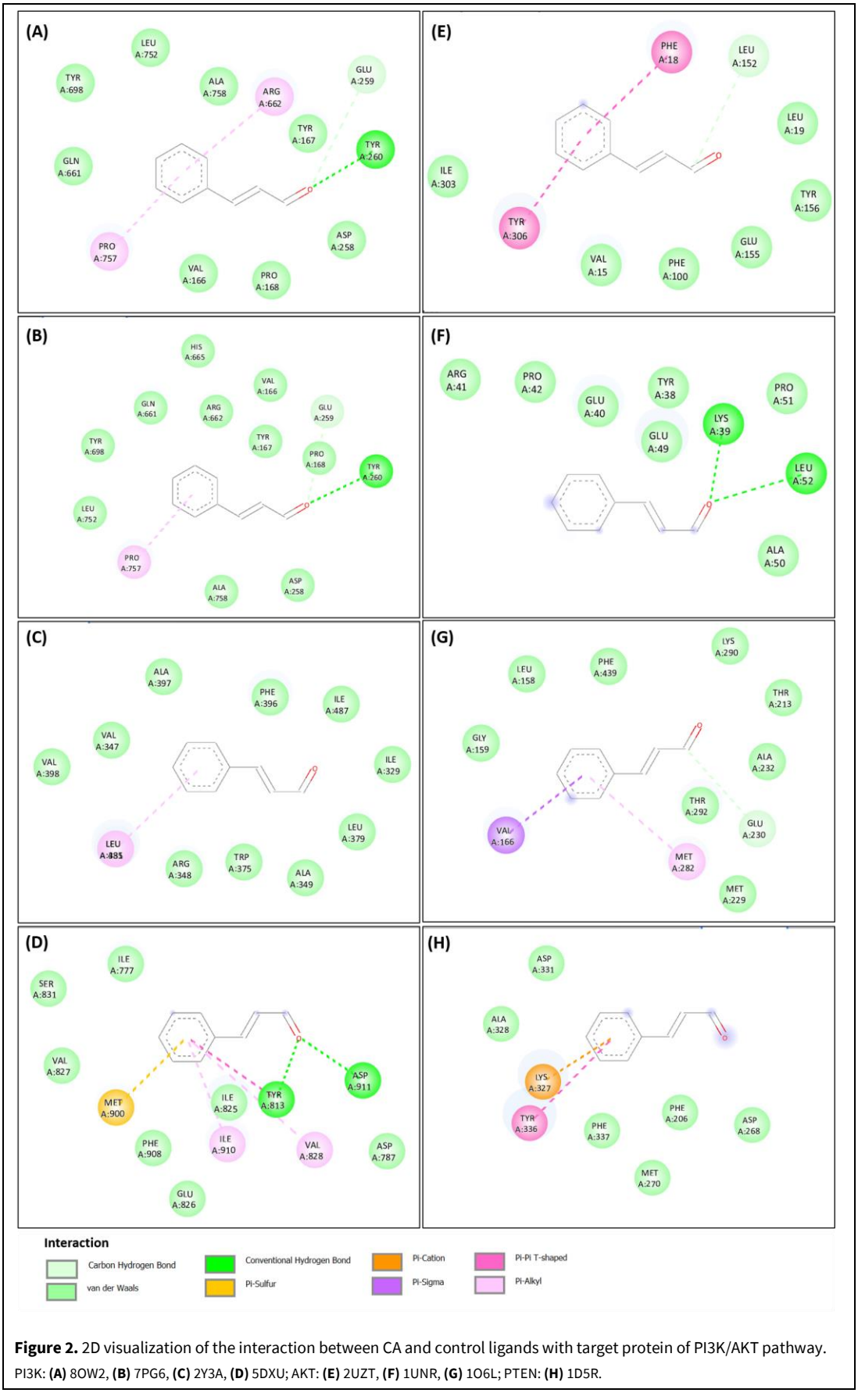

Figure 2. 2D visualization of the interaction between CA and control ligands with target protein of PI3K/AKT pathway. PI3K: **(A)** 8OW2, **(B)** 7PG6, **(C)** 2Y3A, **(D)** 5DXU; AKT: **(E)** 2UZT, **(F)** 1UNR, **(G)** 1O6L; PTEN: **(H)** 1D5R.

Table 3. Pharmacokinetics of CA and control ligands.

Compound	Physicochemical properties						Pharmacokinetics						
	MW (g/mol)	LogS	LogP o/w	Hdon	Hacc	TPSA (Å²)	OB (%)	HL (h)	Caco-2 (nm/s)	P-gp substrate	CYP inhibitor	BBB	Lipinski violation
Cinnamaldehyde	132.17	-2.17	1.97	0	1	17.07	32	4.73	1.35	No	No	1.48	0
UCL-TRO-1938	456.58	-5.1	3.43	2	3	63.74	n/a	n/a	n/a	Yes	No	n/a	0
Baicalin	446.39	-3.41	0.22	6	11	187.12	40	17.36	-0.85	Yes	No	-1.74	2
Bisperoxovanadium	351.27	-0.88	-1.03	5	10	n/a	n/a	n/a	n/a	No	No	n/a	0

MW: molecular weight; LogS: water solubility; LogP o/w: octanol-water partition coefficient; Hdon: hydrogen bond donor; Hacc: hydrogen bond acceptor; TPSA: topological polar surface area; OB: oral bioavailability; HL: half-life; Caco-2: human intestinal cell line Caco-2 permeability; P-gp: P-glycoprotein; CYP: cytochrome P450; BBB: blood-brain barrier.

Table 4. Average RMSF value of MD simulations.

No	Protein	Average RMSF (Å)	
		Cinnamaldehyde	Control ligand
1	p110α subunit	0.85	0.88
2	apo p110α/p85α	0.86	0.85
3	apo p110β/p85β	0.83	0.82
4	apo p110δ/p85α	0.76	0.80
5	AKT2	0.97	0.81
6	AKT1	1.12	0.91
7	PTEN	0.95	1.04
8	PH domain AKT1	0.83	1.14

These calculations were performed using CABSflex 2.0). Simulation results are explained based on statistical parameters such as root mean square fluctuation (RMSF). RMSF is an important parameter that provides information about the structural flexibility of each residue in the corresponding system (Ul Haq et al., 2017). The molecular dynamic results indicate that the ligands used in this study form stable bonds to the target proteins. In the current system, the average α-carbon atom fluctuations between CA and target proteins compared to control ligands are tabulated in Table 4. Overall, the average RMSF value of CA and target proteins was similar compared to control ligands, indicating that CA binding to target proteins is as stable as control ligands.

Based on the plot presented in Fig. 3, it can be observed that several residues underwent fluctuations. The structure is considered stable if the fluctuation of the RMSF value is in the range of 1-3 Å. However, higher peaks indicate high fluctuation rates, and significant conformational changes are anticipated (Blaszczyk et al., 2016). The RMSF of each ligand and target protein was performed to understand the stability of each residue in the simulation trajectory. Fig. 3 depicts the comparison between CA and control ligands. The trajectories of molecular dynamics simulations for all complexes presented mostly mild oscillations despite several higher peaks (>3 Å) being noticed in a few specific amino acid residues.

DISCUSSION

The defective PI3K/AKT insulin signaling pathway has been associated with diabetes and its complications. Deterioration of the PI3K/AKT pathway induces apoptosis, autophagy, protein misfolding, and insulin resistance in many cells, including cardiomyocytes, neurons, pancreatic cells, hepatocytes, and nephrons (Ramasubbu and Devi Rajeswari, 2023). Therefore, activation of the PI3K/AKT pathway could benefit diabetics by providing tissue protection and enhancing cell regeneration. Activation of the PI3K/AKT pathway by means of natural-based molecules has been investigated (Fang et al., 2021; Gong et al., 2023; Kumar et al., 2023).

The present study reported the potential effect of cinnamaldehyde on the modulation of the PI3K/AKT pathway by *in silico* methods. CA was subjected to molecular docking against three crystal structures of PI3K, three of which were PI3K crystal structures of PI3K class A (apo p110α/p85α, apo p110β/p85β, and

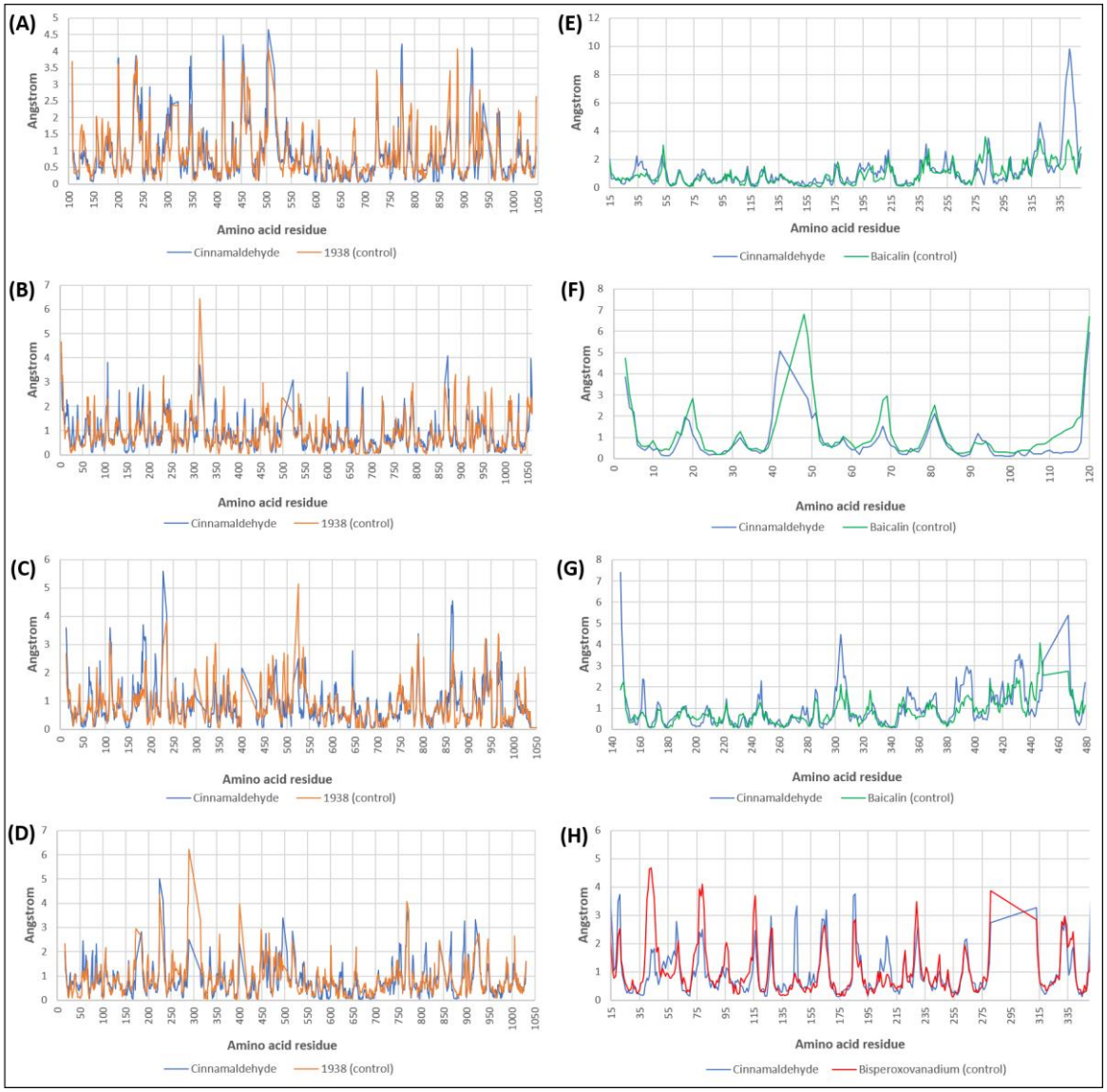

Figure 3. RMSF plot of molecular dynamics simulation between CA with target protein of PI3K/AKT pathway compared to control ligand.

PI3K: **(A)** 8OW2, **(B)** 7PG6, **(C)** 2Y3A, **(D)** 5DXU; AKT: **(E)** 2UZT, **(F)** 1UNR, **(G)** 1O6L; PTEN: **(H)** 1D5R.

apo p110δ/p85α), while one was only the p110α subunit of PI3K. We found that CA showed better binding affinity against the p110α subunit of PI3K compared to UCL-TRO-1938, a small molecule activator of the PI3Kα. CA also bound to apo p110α/p85α, apo p110β/p85β, and apo p110δ/p85α, despite the binding affinities were not as strong as UCL-TRO-1938. However, it seems that CA interacting residues with proteins above are relatively similar to UCL-TRO-1938, which allosterically activates PI3Kα by enhancing multiple steps of the PI3Kα catalytic cycle and causing conformational changes in the PI3Kα structure (Gong et al., 2023).

We also performed molecular docking simulation of CA towards several available AKT crystal structures. AKT is the central effector protein of the PI3K/AKT pathway, whose phosphorylation controls many downstream pathways (Kumar et al., 2023). The structure and activation mechanism of the three AKT isoforms (AKT1, AKT2, AKT3) are highly conserved. Each AKT kinase comprises three conserved domains: an N-terminal pleckstrin homology (PH) domain, a central catalytic (CAT) domain, and a C-terminal extension (EXT) domain. The binding of small molecules to the PH domain of AKT has been reported to facilitate its phosphorylation in the cytoplasm and thus up-regulates the AKT pathway (Kumar et al., 2023). We found that CA showed acceptable binding affinity towards AKT1, PH domain AKT1, and AKT2 (>5 Kcal/mol), although weaker than the control ligand, baicalin. In line with our findings, CA has been reported to increase the phosphorylation of AKT2 at

T450 and Y475, and prime AKT2 for insulin-stimulated phosphorylation at S474 (Urasaki and Le, 2022).

Molecular docking simulation between CA and Phosphatase and tensin homolog on chromosome 10 (PTEN) was carried out to investigate whether CA can up-regulate the AKT pathway by inhibiting PTEN. PTEN is a phosphatase that directly opposes the activation of the PI3K/AKT signaling pathway (Álvarez-Garcia et al., 2019). Moreover, overexpression of PTEN has been associated with defective signaling pathways and glucose uptake, causing insulin resistance (Khokhar et al., 2020). Therefore, it is plausible that PTEN inhibition may restore PI3K signaling, leading to ameliorated cellular injury and improved cell survival. Based on a molecular docking study, CA interaction with PTEN exhibited stronger binding affinity than control ligand bisperoxovanadium. Bisperoxovanadium is a potent PTEN inhibitor, which has been proven to up-regulate PI3K/AKT activity and reduce cellular injury (Walker et al., 2019). Therefore, it is plausible that CA might possess the potential to be a PTEN inhibitor.

ADME analysis using the Swiss ADME and TCMSP database was carried out to determine the pharmacokinetics properties of CA and control ligands**.** Based on the data obtained, CA is suitable for oral delivery because it has hydrogen bond donors not greater than 5, hydrogen bond acceptors not greater than 10, molecular weight not greater than 500 Da, and log P not greater than 5. CA has molecular weights of 132.17 Da, AlogP o/w value of 1.97, zero hydrogen bond donors, and one hydrogen bond acceptor. The aqueous solubility of drug candidates is an important factor in drug development, especially for drugs intended for oral route administration. Drug candidates with high aqueous solubility tend to have good absorption rates and distribution in the body. Interestingly, the predicted aqueous solubility (logS) of CA was considered slightly water soluble. Whereas previous studies have reported that CA was non-polar and hydrophobic, meaning that it has low water solubility (Muhoza et al., 2023). Other pharmacokinetics parameters, namely Caco-2 permeability, oral bioavailability, and TPSA value, are all within desirable ranges, proving the drug-like properties of CA. In terms of absorption parameters, CA has a molecular weight of less than 500 Da (132.17 Da); therefore, it is predicted to have the ability to be easily absorbed by the intestine. Regarding drug metabolism, the predicted interactions of CA with P-gp and CYP were evaluated. P-glycoprotein is an efflux transporter that limits the systemic exposure of its substrates. Therefore, the coadministration of a drug that inhibits or induces P-gp may alter the bioavailability of P-gp substrates (Elmeliegy et al., 2020). Here, we found that CA is predicted as a non-P-gp substrate. The prediction of CYP inhibition is important because this class of liver enzymes metabolizes 75% of drugs. Understanding the interaction between drugs and CYP is required to predict potential drug-drug interactions that may alter its pharmacological response (Espiritu et al., 2020). Results obtained from this study showed that the enzymes belonging to the CYP subtype, including the CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4, were predicted not to be inhibited by cinnamaldehyde. Therefore, it could be interpreted that cinnamaldehyde administration would unlikely affect the metabolism of other drugs by CYP.

We further performed molecular dynamics simulations to study the dynamic features of the ligand-protein complex in a biological environment. The fluctuations of the mean structure of the ligand-protein complex, expressed in terms of RMSF value, were calculated. The average RMSF value of CA and target proteins was similar compared to control ligands, indicating that CA binding to target proteins is as stable as control ligands. However, several higher peaks (>3 Å) were noticed in specific amino acid residues. Thus, it can be concluded that both CA and control ligands-protein complexes have similar stability. However, there were few conformational alterations in several amino acid residues, which may reflect the location of flexible key residues around the binding site of the target protein (Li et al., 2020).

In this study, we have applied virtual screening, molecular docking, and molecular dynamics simulation through data mining and analysis to predict the pharmacological activity of CA in the context of PI3K/AKT pathway modulation. To our knowledge, this is the first *in silico* study of this set of multiple proteins along the PI3K/AKT pathway and their theoretical interactions with CA. Our results provide molecular insights into the mechanism of CA in the treatment of diabetes mellitus and will inform further development of CA as a direct PI3K activator and PTEN inhibitor.

CONCLUSION

Cinnamaldehyde is proven to have a good pharmacokinetics profile and is suitable for oral route administration. Molecular docking results revealed that cinnamaldehyde can bind to the p110α catalytic subunit of PI3K class IA and PTEN with stronger affinity than control ligands. This is further supported by the similarity of the interacting amino acid residues, predominated by hydrogen bonds, between CA and target protein compared to control ligands. The molecular dynamic parameter of the RMSF of CA

shows bond stability and acceptable fluctuation. Therefore, CA shows potential as a drug candidate, both as a PI3K activator and PTEN inhibitor. Further validation through *in vitro* and *in vivo* experiments is necessary to confirm its activity.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Fukata E	Aulanni'am	Rohman MS	Fakurazi S	Malek NANN	Kawamoto Y	Endharti AT
Concepts or ideas	x	x	x	x	x	x	x
Design	x			x	x	x	x
Definition of intellectual content	x	x	x	x	x	x	x
Literature search	x	x	x	x	x	x	x
Experimental studies	x	x	x	x	x	x	x
Data acquisition	x	x	x	x	x	x	x
Data analysis	x	x	x				x
Statistical analysis							
Manuscript preparation	x	x	x	x	x	x	x
Manuscript editing	x	x	x	x	x	x	x
Manuscript review	x	x	x	x	x	x	x

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