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EVALUATION OF THE BINDING AFFINITY OF 3,5-DI-O-CAFFEOYLQUINIC ACID AND CYNARIN TO CAV1.2 CHANNELS USING VIRTUAL MODELING

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Abstract. L-type calcium channels (CaV1.2) play a central role in regulating calcium influx and vascular tone. In this study, the interactions of 3,5-di-O-caffeoylquinic acid and cynarin with the L-type calcium channel were investigated using molecular docking analysis. Both compounds exhibited significant binding affinities, forming multiple hydrogen bonds, hydrophobic interactions, and electrostatic contacts with key residues within the channel's binding pocket. The binding energies and inhibition constants indicated strong inhibitory potential, with 3,5-di-O-caffeoylquinic acid demonstrating a higher affinity than cynarin. These results provide a molecular basis for the potential modulatory effects of these natural compounds on L-type calcium channel activity and highlight their promise as candidates for further exploration in cardiovascular therapeutics.

Keywords: L-type calcium channel, 3,5-di-O-caffeoylquinic acid, cynarin, molecular docking, channel inhibition, flavonoids, binding interactions

Introduction

Voltage-gated calcium channels play a central role in the regulation of cellular excitability, muscle contraction, neurotransmitter release, and gene expression. Among these channels, the L-type calcium channel CaV1.2 is of particular importance in cardiovascular physiology, as it mediates calcium influx in cardiomyocytes and vascular smooth muscle cells, thereby regulating cardiac contractility and vascular tone [1]. Abnormal activity or overactivation of CaV1.2 channels has been closely linked to the development of hypertension, cardiac hypertrophy, and other cardiovascular pathologies, making this channel a key pharmacological target in cardiovascular therapy. Currently available CaV1.2 channel blockers, including dihydropyridines, phenylalkylamines, and benzothiazepines, are widely used in clinical practice. However, their long-term use may be associated with undesirable side effects such as peripheral edema, reflex tachycardia, and negative inotropic effects. These limitations have driven increasing interest in the discovery of alternative calcium channel modulators, particularly those derived from natural sources, which may offer improved safety profiles and additional biological benefits [2]. Plant-derived phenolic compounds have gained considerable attention due to their antioxidant, anti-inflammatory, and vasoprotective properties. Caffeoylquinic acid derivatives, including 3,5-di-O-caffeoylquinic acid and cynarin (1,3-di-O-caffeoylquinic acid), are abundant in medicinal plants such as Cynara scolymus and are known for their cardioprotective and antihypertensive potential. Previous pharmacological studies have suggested that these compounds can induce vascular relaxation and improve endothelial function; however, the precise molecular mechanisms underlying their effects, particularly their direct interactions with voltage-gated

calcium channels, remain insufficiently understood. Recent advances in computational biology and molecular modeling have provided powerful tools for exploring ligand–protein interactions at the molecular level. In silico approaches such as molecular docking and binding affinity prediction allow for the characterization of binding modes, interaction networks, and potential inhibitory mechanisms of bioactive compounds toward ion channels [3]. These techniques are especially valuable for studying CaV1.2 channels, whose experimental structural analysis is complex and limited. In this context, the present study aims to evaluate the binding affinity and interaction patterns of 3,5-di-O-caffeoylquinic acid and cynarin with the CaV1.2 calcium channel using virtual modeling methods. The findings of this work are expected to provide mechanistic insight into the potential calcium channel–modulating properties of these natural compounds and to support their further development as promising candidates for cardiovascular drug discovery [4].

Materials and methods

Datasets and software

This study employed publicly available structural and computational resources for in silico analysis. The three-dimensional structure of the L-type voltage-gated calcium channel CaV1.2 (α1C subunit) was obtained from the Protein Data Bank (PDB; PDB ID: 7K0M). Ligand structures of 3,5-di-O-caffeoylquinic acid and cynarin (1,3-di-O-caffeoylquinic acid) were retrieved from the PubChem database in SDF format and subsequently converted to PDB format using Open Babel software. Molecular visualization, structural inspection, and preparation of protein–ligand complexes were performed using PyMOL (version 2.5). Molecular docking simulations were carried out using AutoDock 4.2, with AutoDock Tools (ADT) employed for ligand and receptor preparation, grid box definition, and docking parameter setup [5].

Ligand and receptor preparation

The receptor proteins were prepared by removing all water molecules, co-crystallized ligands, and heteroatoms from the PDB structures. Polar hydrogen atoms were added, and Kollman partial charges were assigned using AutoDock Tools [6]. Ligand structures were energy-minimized to achieve the most stable conformations and then saved in the .pdbqt format compatible with AutoDock [7].

Grid parameter setup

A three-dimensional grid box was generated around the active site of each protein to define the docking search area. The grid box dimensions were adjusted to completely cover the binding pocket and allow ligand flexibility. All parameters were stored in a .gpf (Grid Parameter File) [8].

AutoGrid operation

The AutoGrid4 module was used to create atomic affinity maps for each ligand atom type. These maps represent the potential energy landscape around the protein and were later used by AutoDock to estimate interaction energies between the ligand and receptor [9].

Docking protocol and binding energy calculation

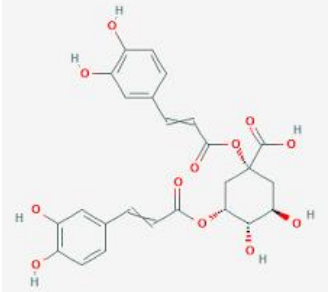
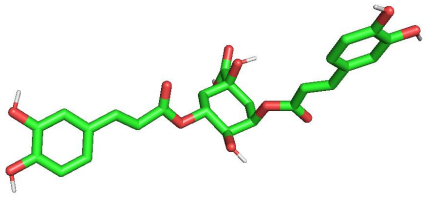
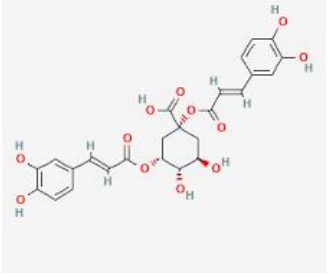
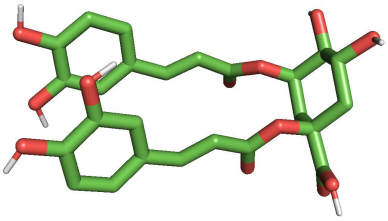
Docking simulations were carried out using AutoDock 4.2 with the Lamarckian Genetic Algorithm (LGA) as the search method. Default parameters were used for population size, energy evaluations, and mutation rates to ensure reproducibility. Each ligand was docked with all five target proteins to predict the most favorable binding conformation.

Binding free energy (ΔG, kcal/mol) values were automatically calculated by AutoDock based on the scoring function. The inhibition constant (Ki, μM) was determined from the binding energy using the following equation:

$$Ki = e^{\wedge}\frac{\Delta G x 1000}{RxT}$$

where ΔG is the binding energy in kcal/mol, R is the universal gas constant (1.987 cal·mol^{-1}·K^{-1}), and T is the absolute temperature (298.15 K) [10].

Table 1. 2D and 3D Structures of Ligands Selected for Molecular Docking Analysis.

Ligands	2D	3D
3,5-di-o-caffeoylquinic acid		
Cynarin		

Visualization and interaction analysis

The resulting protein–ligand complexes obtained from molecular docking were examined using PyMOL and Discovery Studio Visualizer to identify key intermolecular interactions, including hydrogen bonds, hydrophobic contacts, electrostatic interactions, and π–π stacking between the ligands and amino acid residues of the CaV1.2 channel. Both two-dimensional and three-dimensional interaction maps were generated to visualize the residues involved in ligand recognition and stabilization within the channel structure, thereby providing insights into the potential modulatory mechanisms of 3,5-di-O-caffeoylquinic acid and cynarin on L-type calcium

channel function [11]. Furthermore, Discovery Studio Visualizer was employed for automated detection and characterization of ligand-binding pockets within the CaV1.2 channel. This analysis included mapping of hydrophobic regions, identification of aromatic residue clusters, and determination of the spatial coordinates of the binding cavity. Such analysis enabled precise localization of the functional binding site and supported a detailed understanding of the structural basis of ligand–channel interactions [12].

Results,

3,5-di-o-caffeoylquinic acid

As a result of molecular docking analysis, 3,5-di-O-caffeoylquinic acid was found to interact with the L-type calcium channel at the active binding site identified using Discovery Studio, with the coordinates center_x = 168.279698, center_y = 176.439484, and center_z = 180.397000. The calculated binding energy for this interaction was −9.6 kcal/mol (Table 2), indicating a highly stable and strong association between the compound and the calcium channel. Based on the obtained binding energy value, the inhibitory potential of the extract was estimated using the corresponding thermodynamic relationship, yielding an inhibition constant of 0.085 μM. This low inhibition constant clearly suggests that 3,5-di-O-caffeoylquinic acid exhibits a very strong inhibitory potential toward the L-type calcium channel [13.14].

Table 2. Molecular docking results of 3,5-di-O-caffeoylquinic acid with the L-type calcium channel

№	Affinitet (kcal/mol)	RMSD l.b. (Å)	RMSD u.b. (Å)
1	-9.6	0.000	0.000
2	-9.0	1.772	3.668
3	-8.9	2.958	8.314
4	–8.9	1.626	3.762
5	–8.6	2.969	8.102
6	–8.4	3.329	7.720
7	–8.4	3.334	8.308
8	–8.0	28.923	32.122
9	-7.9	13.097	17.179

When 3,5-di-O-caffeoylquinic acid interacts with the L-type CaV1.2 calcium channel, the compound forms multiple stabilizing interactions with several key amino acid residues within the channel structure. Conventional hydrogen bonds were observed with ASP A:598, TYR A:585, ASP A:586, and SER F:267, indicating strong polar interactions that play a crucial role in anchoring the ligand within the binding pocket. These residues are predominantly located in functionally important regions of the channel, where polar side chains contribute to ligand recognition and stabilization through hydrogen bonding networks. A π–alkyl interaction was identified with ARG A:593, suggesting additional hydrophobic and electrostatic stabilization of the ligand. Arginine residues within CaV1.2 channels are known to participate in electrostatic interactions and contribute to maintaining the structural integrity of the binding cavity, thereby

facilitating effective ligand accommodation. An unfavorable acceptor–acceptor interaction was detected with GLU A:591, which may reflect local steric or electrostatic constraints within the binding site; however, such interactions can also indicate tight spatial proximity and may not significantly compromise overall binding stability when compensated by multiple favorable contacts. In addition, a π-donor hydrogen bond was observed with LEU F:269, highlighting the involvement of hydrophobic residues in reinforcing ligand binding. Leucine residues are commonly found within transmembrane regions of L-type calcium channels and contribute to shaping the hydrophobic environment of the channel pore or adjacent regulatory domains. The interaction of 3,5-di-O-caffeoylquinic acid with both polar and hydrophobic residues suggests that the ligand is well accommodated within a structurally and functionally relevant binding cavity of the CaV1.2 channel (Figure 1). Overall, the observed interaction profile indicates that 3,5-di-O-caffeoylquinic acid preferentially binds to residues that are critical for channel gating and ion conduction, which may underlie its strong binding affinity and potential inhibitory effect on L-type calcium channel activity [15].

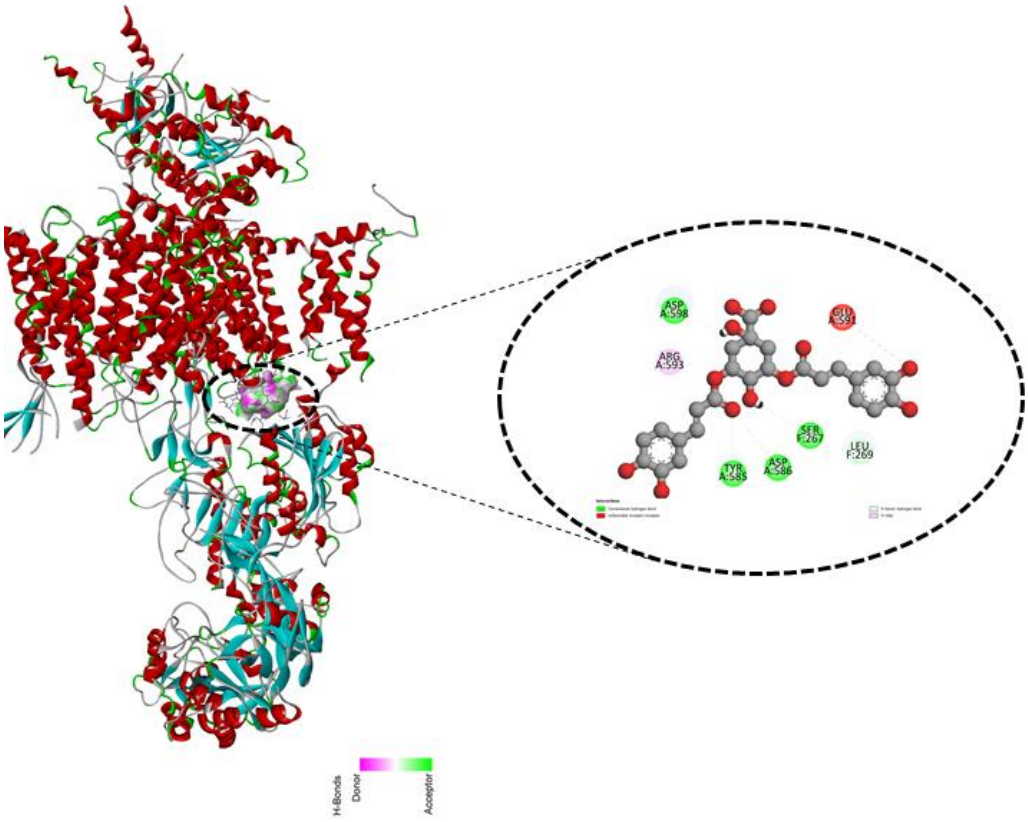


Figure 1. Molecular docking visualization of 3,5-di-O-caffeoylquinic acid interacting with the CaV1.2 L-type calcium channel

Cynarin

In subsequent analyses, the interaction between the cynarin extract and the L-type calcium channel at the active binding site defined by the coordinates center x = 168.279698, center y = 176.439484, and center z = 180.397000 resulted in a binding energy of −8.1 kcal/mol. Based on this binding energy, the inhibition constant was calculated to be 1.09 µM, indicating that cynarin also exhibits a high inhibitory potential toward the L-type calcium channel.

Table 3. Molecular docking results of Cynarin with the L-type calcium channel

№	Affinitet (kcal/mol)	RMSD l.b. (Å)	RMSD u.b. (Å)
1	-8.1	0.000	0.000
2	-8.0	13.965	19.614
3	-7.8	1.911	8.344
4	–7.7	1.862	8.154
5	–7.5	1.784	4.660
6	–7.4	2.874	8.130
7	–7.4	15.197	20.858
8	–7.4	9.582	12.745
9	-7.3	15.505	18.543

Docking results of cynarin with L-type calcium channel show that the ligand forms multiple interactions with specific amino acid residues within the channel. Conventional hydrogen bonds are formed with GLY 1015, GLU 1014, GLU 614, ASN 239, GLN 1299, ARG 1318, and ASP 615, which stabilize the ligand within the binding pocket. A Pi-alkyl interaction occurs with ALA 1324, providing additional hydrophobic stabilization. An unfavorable acceptor–acceptor interaction is observed with ASP 615, indicating slight electronic or steric repulsion. ASP 296 participates in a Pi-anion interaction, contributing further electrostatic stabilization. These amino acids play important roles in the channel's function. GLU 1014 and GLU 614 are negatively charged residues in the pore region that coordinate calcium ions during conduction. ASP 615 and ASP 296 are acidic residues contributing to ion selectivity and electrostatic interactions. ASN 239 and GLN 1299 are polar residues that stabilize both the channel structure and ligand binding through hydrogen bonds. ARG 1318 is a positively charged residue that enhances ligand affinity. GLY 1015 provides backbone flexibility for optimal ligand accommodation. ALA 1324 is a small hydrophobic residue that interacts with the aromatic rings of cynarin (Figure 2). Cynarin binds to these residues through a combination of hydrogen bonding, hydrophobic interactions, and electrostatic complementarity. Polar residues form stable contacts with hydroxyl and carboxyl groups of the ligand, while hydrophobic residues interact with the ligand's aromatic moieties. This binding pattern suggests that cynarin fits well within the L-type calcium channel binding pocket and may modulate its activity [16.17].

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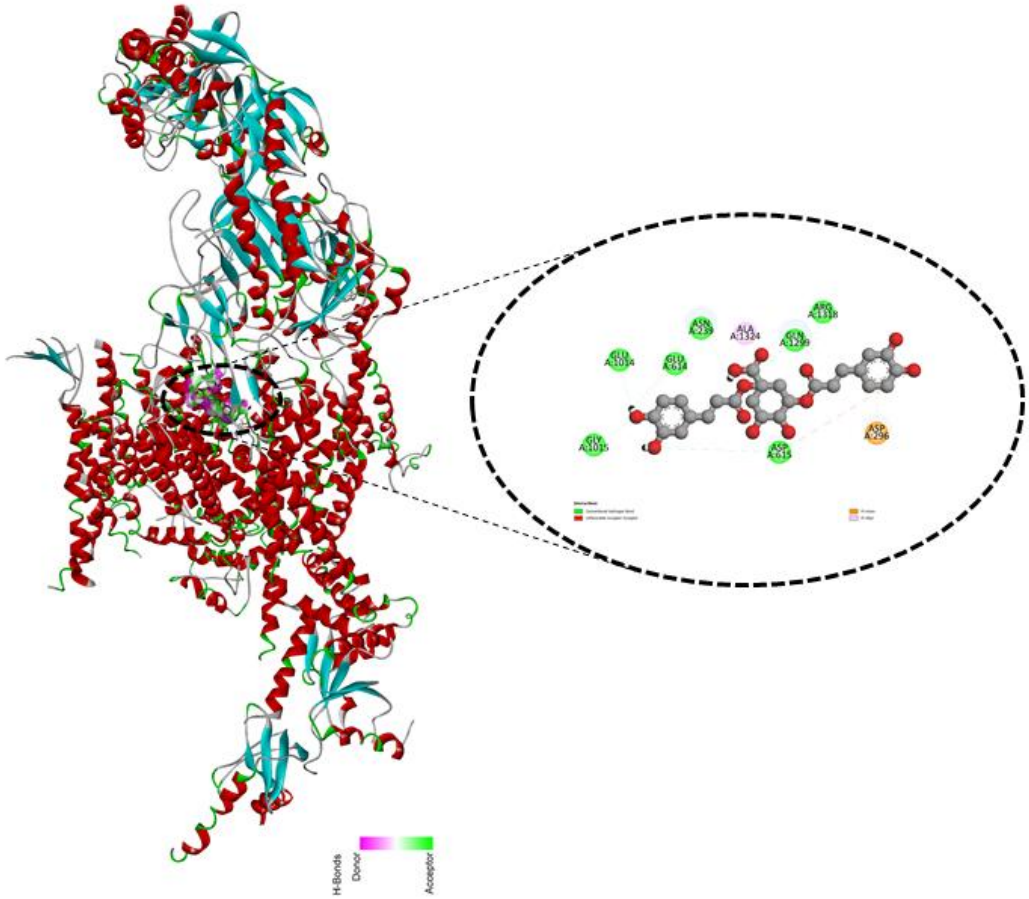

Figure 1. Molecular docking visualization of Cynarin interacting with the CaV1.2 L-type calcium channel

Discussion

The molecular docking results provide insight into the potential mechanisms by which 3,5-di-O-caffeoylquinic acid and cynarin modulate L-type calcium channel activity. Both compounds demonstrated significant binding affinities toward the CaV1.2 channel, as indicated by the calculated binding energies and low inhibition constants, suggesting that they can effectively interact with functionally important regions of the channel. For 3,5-di-O-caffeoylquinic acid, the formation of multiple conventional hydrogen bonds with residues such as ASP 598, TYR 585, ASP 586, and SER 267 underscores the importance of polar interactions in stabilizing the ligand within the binding pocket. These residues are located in regions critical for channel gating and ion conduction, implying that ligand binding at these sites may influence the conformational states required for calcium ion passage. The observed π–alkyl interaction with ARG 593 and the π-donor hydrogen bond with LEU 269 further highlight the contribution of hydrophobic interactions to binding stability, suggesting that a combination of polar and hydrophobic contacts is essential for effective ligand accommodation. Although an unfavorable acceptor–acceptor interaction with GLU 591 was detected, it is likely compensated by multiple stabilizing interactions, maintaining overall high affinity. Similarly, cynarin interacts with a network of polar and hydrophobic residues, including GLY 1015, GLU 1014, GLU 614, ASN 239, GLN 1299, ARG 1318, and ASP 615. Hydrogen bonding with these residues ensures precise

positioning of the ligand, while hydrophobic interactions with ALA 1324 and electrostatic stabilization via ASP 296 support strong binding. The involvement of both negatively and positively charged residues suggests that cynarin binding may influence ion selectivity and channel conductance by occupying key functional sites within the channel pore. Collectively, these findings suggest that both 3,5-di-O-caffeoylquinic acid and cynarin are capable of modulating L-type calcium channel activity through a combination of hydrogen bonding, hydrophobic interactions, and electrostatic complementarity. The preference of these ligands for residues critical for gating and ion coordination indicates a potential mechanism by which they exert inhibitory effects, which could be relevant for their vasorelaxant and cardioprotective properties. Furthermore, the differential binding energies and inhibition constants reflect variations in ligand potency, with 3,5-di-O-caffeoylquinic acid showing a stronger inhibitory potential than cynarin, likely due to a more extensive network of stabilizing interactions. Overall, these results provide a molecular basis for understanding how naturally occurring flavonoids and related compounds interact with L-type calcium channels and support their potential as modulators of calcium-dependent physiological processes. Future experimental validation, including electrophysiological studies, would further clarify the functional implications of these interactions and guide the development of novel channel-targeting therapeutics.

Conclusion

Molecular docking analysis demonstrated that both 3,5-di-O-caffeoylquinic acid and cynarin effectively bind to the L-type calcium channel, forming multiple stabilizing interactions with key amino acid residues. These interactions involve hydrogen bonding, hydrophobic contacts, and electrostatic complementarity, targeting residues crucial for channel gating and ion conduction. Among the two ligands, 3,5-di-O-caffeoylquinic acid exhibited a stronger binding affinity and lower inhibition constant, suggesting a higher inhibitory potential compared to cynarin. The findings provide a molecular basis for the potential modulatory effects of these compounds on L-type calcium channels and support their role as promising candidates for further studies aimed at developing natural modulators of calcium-dependent physiological processes.

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