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In Silico Elucidation of the Binding Mechanisms and Molecular Dynamics of Oroxylin A -2,3-Dioxygenase Interaction: An Insight into Therapeutic Potentiation of Quercetin's Cardioprotection

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ABSTRACT

Objective: Elucidating the intricate interplay between enzymes and natural compounds is essential for designing therapeutic strategies.

Methods: This study employs advanced computational techniques to explore the binding mechanisms between quercetin 2,3-dioxygenase (QDO) and oroxylin A, revealing specific interaction patterns and key residues crucial to the formation of the QDO-oroxylin A complex.

Results: Molecular docking simulations revealed a favorable binding affinity (docking score: -5.6 kcal/mol) between Oroxylin A and the active site cavity of QDO, which was supported by Oroxylin A's specific orientation (Pose 3). Despite an observed root-mean-square deviation (RMSD) value of 2.776 indicating a moderate deviation between the docked pose and the reference structure, the formation of two hydrogen bonds with GLN 93 chain D underscores specific molecular interactions driving the binding process. This hydrogen bond formation suggested the presence of a stable and specific binding mode between Oroxylin A and QDO, likely influencing the functional dynamics of the enzyme, necessitating further refinement and validation of the docking model.

Conclusion: The ensuing deliberation on the implications of Oroxylin A include its potential as a modulator of QDO activity, emphasizing the importance of molecular-level insights in comprehending enzyme-compound interactions. Oroxylin A, a quercetin 2,3-dioxygenase inhibitor, was used in combination with other agents to prolong the biological impacts of quercetin, thereby amplifying its antioxidant and anti-inflammatory effects. This strategic approach exhibits promise in augmenting cardioprotective benefits, immune system support, and protection against diverse pathological conditions. Subsequent considerations of dosage, bioavailability, and healthcare professional consultation are imperative for judicious supplementation, particularly in individuals with prevailing health conditions or medications. This ongoing in silico study is dedicated to revealing the potential synergistic interactions of Oroxylin A, potentiating the long-term effects of quercetin and advancing our understanding of these intricacies.

Keywords: Quercetin, Quercetin 2,3-dioxygenase (QDO), Oroxylin A, Molecular docking, Antioxidant, Cardioprotection

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Introduction

Cardiovascular diseases (CVDs) stand as prominent contributors to global mortality rates and necessitate the development of novel therapeutic interventions to combat this global health burden (Liu et al., 2021). Quercetin, a flavonoid abundantly found in various dietary sources, has garnered increased amounts of attention for its potential cardioprotective effects (Oboh et al., 2016). Quercetin, a polyphenolic compound abundant in various vegetables, fruits, and beverages such as red wine and tea, it exhibits a diverse array of pharmacological activities (Batiha et al., 2020). As a potent antioxidant, quercetin scavenges reactive oxygen species (ROS) furthermore, it provides protection against oxidative stress-induced damage in vascular endothelial cells and cardiomyocytes (Chang et al., 2021). Additionally, quercetin demonstrates anti-inflammatory properties by inhibiting proinflammatory cytokines and regulating intracellular signaling pathways implicated in the development of CVD (Hu et al., 2019). Additionally, quercetin has been shown to inhibit platelet aggregation, endothelial dysfunction, and smooth muscle cell proliferation, thus exerting antiatherogenic effects (Li and Zhang 2023). The pathophysiology of CVD is multifactorial and involves complex interactions between genetic, environmental, and lifestyle factors (Aggarwal et al., 2023). Quercetin targets several key mechanisms implicated in the progression and development of CVDs, comprising oxidative stress, endothelial dysfunction, dyslipidemia, inflammation, and thrombosis (Zhang et al., 2023). By enhancing nitric oxide synthase produced by endothelial cells (eNOS) activity and promoting the molecule known as nitric oxide (NO) production, quercetin improves endothelial function and vascular tone, thereby reducing the risk of hypertension and atherosclerosis (Yamagata 2023). Furthermore, quercetin attenuates lipid peroxidation, inhibits the expression of adhesion molecules, and suppresses The pathway of nuclear factor-kappa B (NF-κB), resulting in diminished vascular inflammatory response and plaque formation (Yan et al., 2023). Moreover, quercetin modulates lipid metabolism by regulating the activation of genes involved in lipid synthesis and breakdown, resulting in improved lipid profiles and a reduced risk of dyslipidemia-related CVDs (Papakyriakopoulou et al., 2022). Experimental studies using cell culture and animal models have provided compelling evidence supporting the cardioprotective effects of quercetin. These studies demonstrated that quercetin supplementation attenuates myocardial ischemia–reperfusion injury, preserves heart function, and decreases the size of tissue damage in animal models of heart attack (Papakyriakopoulou et al., 2022; Bartekova et al., 2010). Moreover, research has demonstrated that administering quercetin improves hypertension, improve endothelial function, and inhibit atherosclerotic lesion formation in various animal models of CVD (Patel et al., 2018). In clinical trials and meta-analyses involving human subjects, quercetin supplementation has been linked to enhancements in endothelial function, blood pressure, lipid profiles, and glycemic control, suggesting its potential therapeutic utility in CVD prevention and management (Yamagata and Yamori 2020). However,the effectiveness of quercetin therapy is constrained by its poor bioavailability and rapid metabolism, which are primarily mediated by enzymes such as quercetin 2,3-dioxygenase (Guo et al., 2022). Recent studies have identified oroxylin A, a natural flavone extracted from Scutellaria baicalensis Georgi, as a potential modulator of quercetin metabolism through interaction with quercetin 2,3-dioxygenase (Wang et al., 2014). Recent research has explored strategies to modulate quercetin metabolism and enhance its therapeutic potential. One such approach involves the use of oroxylin A, a natural flavone found in the plant Scutellaria baicalensis Georgi. Oroxylin A has been shown to interact with QDO, potentially influencing the degradation pathways of quercetin and altering its biological effects.

Despite the promising findings from preclinical and clinical studies, several challenges must be addressed to realize the full therapeutic potential of quercetin in CVD treatment. These challenges include issues related to bioavailability, formulation optimization, dose selection, and standardization of quercetin-containing products (Alizadeh and Ebrahimzadeh 2022; Khan et al., 2021). Moreover, the heterogeneity of study designs, patient populations, and outcome measures in clinical trials necessitates careful interpretation of the existing evidence and emphasizes the necessity for randomized controlled trials and well-designed with longer follow-up periods and larger sample sizes. Additionally, future investigation efforts should focus on elucidating the underlying mechanisms of quercetin's actions on specific CVD subtypes and identifying potential synergistic interactions with other cardiovascular medications.

In addition to performing a computer-based investigation, this study aimed to closely examine the intricate relationship between quercetin 2,3-dioxygenase and oroxylin A. The goal was to uncover crucial insights that can improve the effectiveness of quercetin and open the door to innovative therapeutic strategies for CVD treatment. By using computational methods, we aimed to thoroughly explore this interaction, shedding light on its

potential as a target for CVD drug intervention. This research not only investigated the molecular dynamics (MD) of the interaction between quercetin 2,3-dioxygenase and oroxylin A but also emphasized its broader implications for cardiovascular health, offering a hopeful avenue for the advancement of novel medications for cardiovascular disease therapy.

Materials and Methods

Ligand and protein preparation

The preparation of ligands and proteins for molecular docking commenced with the creation of PDBQT files, facilitated by UCSF Chimera software version 1.17.3 UCSF Chimera serves as a versatile tool for molecular visualization and analysis platform renowned for its advanced features, including structure visualization, trajectory analysis, and sequence alignment. While UCSF Chimera remains freely available for academic purposes, its successor, UCSF ChimeraX, provides expanded capabilities and is recommended for activities including structure preparation, acquiring 3D structures and docking (Pettersen et al., 2004). For docking a strategy that involved a flexible ligand and a rigid protein was implemented (Totrov and Abagyan 2008), wherein the receptor molecule maintained a rigid conformation while the ligand exhibited flexibility. Preparing the protein receptor included eliminating water molecules, consolidating nonpolar atoms, and assigning Kollman and Gasteiger charges (Mustafa et al., 2023).

The 3D structure of Oroxylin A (CID: 5320315) was retrieved from the PubChem database, which contains a comprehensive platform interconnecting substance, compound, and bioAssay database) (Kim et al., 2023). Subsequently, optimization and minimization of Oroxylin A were performed using the ChemBioDraw Ultra 14 suite, which is renowned for analyzing and drawing chemical structures (PerkinElmer 2023).

To visualize Oroxylin A in 3D, PyMOL software was used; PyMOL is known for its ability to generate high-quality molecular structures and facilitate molecular analysis, including structure visualization and simulation trajectory visualization (Figure 1A).

The X-ray crystallographic coordinates of QDO (PDB: 1JUH) were obtained from the RCSB database, which serves as a comprehensive archive of three-dimensional structural data for biological molecules (Figure 1.B). Finally, the preparation of docking and minimization structures for both ligands and receptors were accomplished using software UCSF Chimera version 1.17.3, enabling comprehensive preparation for subsequent molecular docking simulations.

Docking Protocol for Molecular Interactions

At the first,the CASTp online server was used for detecting active site of protein (Tian et al., 2016). Subsequently, the grid box dimensions were established as (20 × 20 × 20 Å), with the grid center set at coordinates (51.823, 12.609, 32.233) relative to the QDO using the software's configuration parameters. Molecular docking simulations of Oroxylin A and QDO were conducted utilizing the UCSF Chimera+Autodockvina32 software platform (Trott and Olson 2010). AutoDock Vina, a widely used open-source software designed specifically for molecular docking, was used for the computational prediction of the preferred orientation of Oroxylin A in relation to QDO to form a stable complex. This analysis encompassed evaluating hydrogen bond interactions and binding affinities, providing crucial insights into the molecular interactions between Oroxylin A and QDO within the active site.

Interaction between Quercetin 2,3-dioxygenase and the Oroxylin A complex

For analyzing proteins and ligand interactions, the Protein Ligand Interaction Profiler (PLIP) online server was performed (Adasme et al., 2021). This online server offers a free platform for examining protein–ligand interactions, offering valuable insights into binding energies, binding modes and interaction types. Widely employed in structural biology and drug discovery, the PLIP server facilitates the exploration of protein–ligand interactions crucial for designing novel drugs and therapies. Additionally, a 2D representation of the QDO and Oroxylin A complex was obtained from Discovery Studio Visualizer v21.1.0.20298 software ((BIOVIA, Dassault Systèmes, San Diego, CA, USA). The BIOVIA Discovery Studio Visualizer software represents a robust molecular visualization tool equipped with advanced features tailored for small molecule and protein data analysis. Its functionalities include creating high-fidelity 3D structures, facilitating comprehensive structure analysis, visualizing simulation trajectories and molecular docking. Users can leverage features such as distance measurements, angle determination, dihedral analysis, neighbor identification, and visualization of electrostatic surface potentials. In this study, we employed the BIOVIA Discovery Studio Visualizer to generate both 2D interaction visualizations and 3D representations of the QDO and

Oroxylin A complex, allowing for detailed examination and analysis of their molecular interactions.

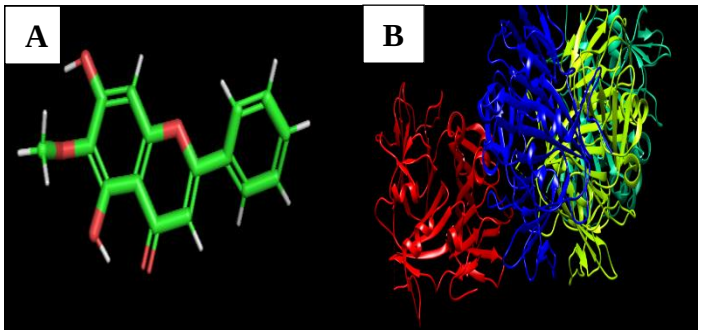

Figure 1. 3D structure of Oroxylin (A); Crystal structure of QDO (PDB: 1JUH) (B)

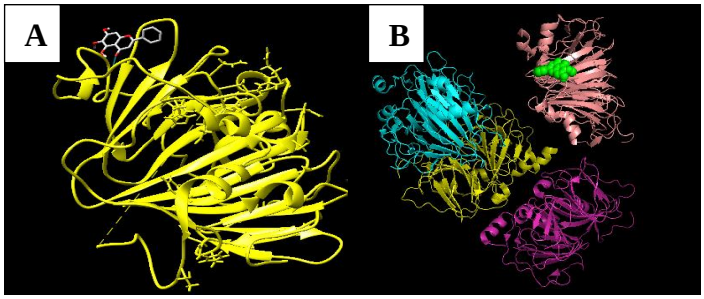

Figure 2. Docking of QDO and Oroxylin A (A); QDO and Oroxylin A complex (B)

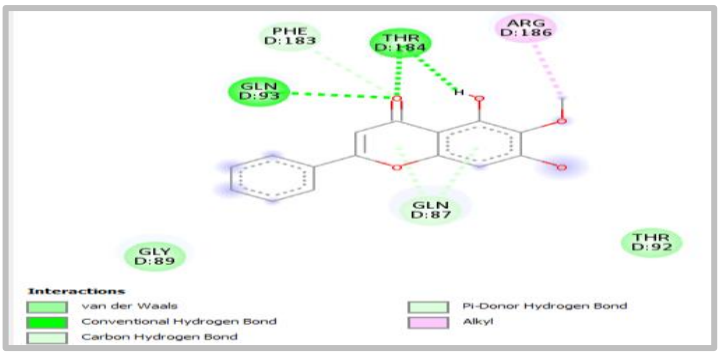

Figure 3. 2D diagram of the interaction between QDO and Oroxylin A

Table 1. Interaction between QDO and Oroxylin A according to the PLIP server

Hydrogen Bond									
Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein Donor	Side Chain	Donor Atoms	Acceptor Atoms
1	93D	GLN	2,57	3,14	110.07	V	V	8498 (Nam)	10355 (O3)
2	184D	THR	1.89	2.81	154.36	V	X	9079 (Nam)	10339 (O2)

MD Study

We utilized the GROMACS 2023 software (Rao et al., 2023) to conduct the simulations. To begin with, we prepared topology and coordinate files, employing the CHARMM36 force field for the intermolecular potential which was represented as a summation of Lennard-Jones (LJ) force and pairwise Coulomb interaction. The long-range electrostatic force was managed using the particle mesh Ewald (PME) method, and the numerical integration was executed through the velocity Verlet algorithm (Gharaghani et al., 2013). The system, under the condition of periodic boundary, was submerged in a water box shaped like a cube that contained water molecules with extended simple point-charge (SPC) atoms, positioned at a distance of 1 nm from each wall side. The neutrality of the system was confirmed, and 15 sodium ions were introduced in order to neutralize it, resulting in a system with 15 NA+ ions and 15756 solvent atoms. The energy minimization process, comprising of 50,000 steps for 2 fs, was followed by the equilibration stage at a constant temperature (NVT) of 300 K, utilizing the Berendsen thermostat, with a cutoff radius set at 1.2 nm. Subsequently, the system underwent equilibration at constant pressure (NVT) of 1 bar to optimize solvent molecules' arrangement around the solute. Finally, a 100 ns main simulation run at 300 K and 1 bar pressure was conducted.

To simulate the QDO-ligand complex, we utilized the SWISSPARAM server for the CHARMM36 force field to generate the topology file for the ligand. Subsequently, we integrated the topology parameters and ligand coordinates with those of QDO. The MD simulation of the protein-ligand complex mirrored the protein simulation and lasted for a duration of 100 ns. For all subsequent analyses associated with the simulation, such as the evaluation of intermolecular hydrogen bonds, RMSD, Rg, Root mean square fluctuation (RMSF) and solvent surface tension parameter (SASA) methods, we employed the GROMACS 2023 software. The MD simulation was carried out on an Ubuntu 22.04 Linux computer.

Results

The data provided here offer valuable insights into the molecular docking outcomes of Oroxylin A within the active site cavity TYDQAGSNCLEIPFVWH of QDO. A negative docking score of -5.6 kcal/mol indicated favorable binding affinity between Oroxylin A and QDO, while Pose 3 elucidated a specific orientation within the active site. Furthermore, the RMSD value of 2.776 revealed the deviation between the docked pose and the reference structure. Notably, Oroxylin A formed a hydrogen bond with GLN 93 chain D, suggesting its potential as a modulator of QDO activity. These findings underscore the promising pharmacological properties of Oroxylin A and its potential as a therapeutic agent in ongoing drug development endeavors (Figure 2A, B; Figure 3) (Table 1).

The initial pivotal stage in MD simulations involves assessing the RMSD, which signifies the stability and structural alterations occurring throughout the simulation period. RMSD represents the deviation of particle positions from the original structure at any given time (Sargsyan et al., 2017). A lower RMSD value indicates minimal fluctuation during the simulation, implying high stability of the protein (Elangovan et al., 2021). The mean average RMSD values for QDO and its complex with oryoxacilin were 0.325 and 0.239, respectively (Fig 4. D).

The RMSF algorithm evaluates regions of the structure with varying degrees of fluctuations compared to their reference structure. This parameter elucidates how ligand binding induces conformational changes at the residue level (Saravanan et al., 2019) (Fig. 4C).

The gyration radius is a measure used to assess compression changes during MD simulations. Protein compression during interactions with the ligand is influenced by protein chains and relies on the ligand's flexibility (Fig. 4E).

SASA values are employed to analyze the extent and significance of ligand binding to the receptor, as well as the alterations in protein conformation resulting from ligand binding (Shukla et al., 2019) (Fig. 4B).

Various interactions, including hydrogen bonds, hydrophobic interactions, and ionic interactions, stabilize the protein–ligand complex. Hydrogen bonds are particularly crucial and specific transient interactions for protein–ligand stabilization (Shukla et al., 2019; Eskandarzadeh et al., 2021) (Fig. 4A).

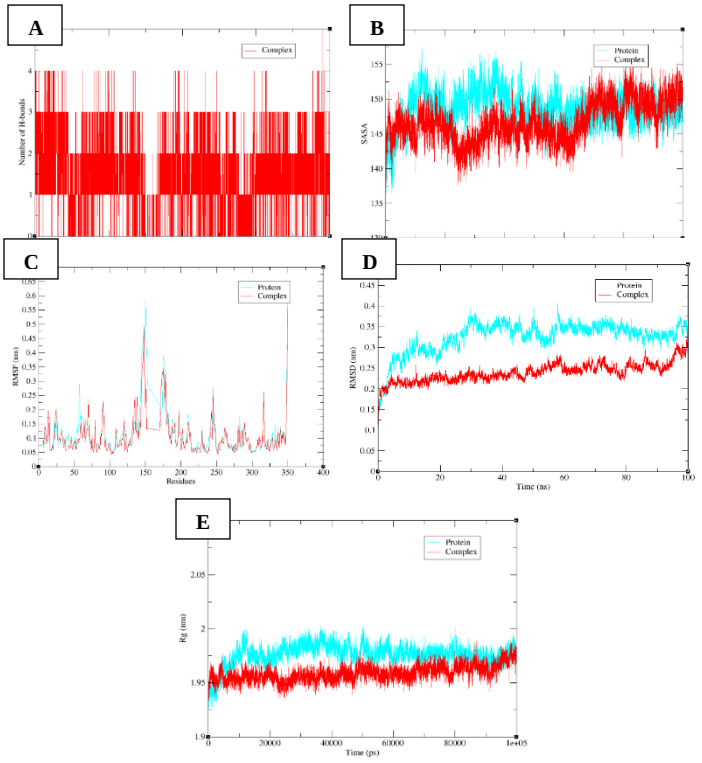

Figure 4. Molecular dynamics results generated by Gromacs software; number of hydrogen bonds ; SASA ; RMSF ; RMSD ; and Rg

Discussion

Quercetin, an extensively distributed natural compound found in diverse dietary sources, has attracted considerable attention owing to its purported therapeutic efficacy in the management of CVDs (Guillermo Gormaz et al., 2015). A growing body of research has elucidated its multifaceted properties, characterizing it as a promising candidate for promoting cardiovascular health. The documented antioxidant, anti-inflammatory, and antiatherogenic attributes of quercetin underscore its potential significance in mitigating the intricate pathophysiological processes associated with CVDs.

The antioxidant activity of quercetin is manifested through its ability to scavenge ROS, thereby providing a protective shield against oxidative stress, a pivotal player in the initiation and progression of cardiovascular malignancies. Moreover, the cardioprotective effects of quercetin are attributed to its anti -inflammatory properties by dampening the inflammatory cascade implicated in the development and exacerbation of cardiovascular pathologies. Its ability to modulate lipid metabolism adds another dimension to its therapeutic potential, as dysregulated lipid homeostasis is intricately

linked to atherosclerotic processes underlying CVDs. As scientific understanding advances, the exploration of the intricate molecular interactions of quercetin and its translation into targeted therapeutic interventions holds promise for addressing the complex landscape of cardiovascular health with precision and efficacy.

However, the therapeutic efficacy of quercetin is limited by its susceptibility to degradation by enzymes such as QDO, which can compromise its bioavailability and effectiveness (Siegbahn, 2004). This limitation underscores the importance of identifying inhibitors of QDO, such as oroxylin A, to preserve the beneficial effects of quercetin in managing CVDs.

Oroxylin A, a natural flavone compound, has shown promise as an inhibitor of QDO, thereby potentially enhancing the bioavailability and efficacy of quercetin in combating CVDs (Ren et al., 2020). By inhibiting the degradation of quercetin, Oroxylin A may prolong its therapeutic effects and improve its potency in cardiovascular health management.

In this comprehensive investigation, molecular docking outcomes were examined, and subsequent MD simulations were subsequently conducted to elucidate the intricate interaction between Oroxylin A and QDO. This molecular docking study provides insights into the underlying binding mechanisms involved, unveiling a favorable binding affinity with a computed docking score of -5.6 kcal/mol, indicating a thermodynamically favorable interaction at the active site cavity of QDO. This interaction is further supported by the specific orientation of Oroxylin A, as exemplified by Pose 3. Despite a moderate deviation with an observed RMSD value of 2.776 between the docked pose and the reference structure, the formation of two hydrogen bonds with the GLN 93 chain D underscores the specificity and stability of the QDO-Oroxylin A complex (Shadidizaji et al., 2024).

The results of the molecular docking analysis revealed a substantial negative docking score of -5.6 kcal/mol, indicating a robust and favorable binding affinity between Oroxylin A and QDO (Shadidizaji et al., 2023). The significance of this interaction is underscored by the specific orientation observed within the active site, emphasizing Oroxylin A's potential efficacy in modulating QDO activity (Shadidizaji et al., 2023). The establishment of a hydrogen bond with GLN 93 chain D further substantiates the specificity of Oroxylin A in its interaction with QDO, suggesting its potential role as a therapeutic agent (Shadidizaji et al., 2024; Rad et al., 2023).

Subsequent MD simulations were employed to determine the stability and structural dynamics of the QDO-Oroxylin A complex. Despite the moderate deviation revealed by the RMSD analysis between the docked pose and the reference structure, the mean average RMSD values indicate an enhanced stability of the complex compared to the unbound QDO (Matta 2003). Further analyses encompassing the RMSF, gyration radius, and SASA provided nuanced insights into conformational changes and protein–ligand interactions at the molecular level (Bayan et al., 2023; Rezaei et al., 2022).

The exploration of interactions stabilizing protein–ligand complexes has placed particular emphasis on hydrogen bonds as specific and transient interactions crucial for stabilization. This nuanced understanding of molecular interactions at the atomic level is pivotal for rational drug design and optimization, offering potential therapeutic avenues for oroxylin A in modulating QDO activity and addressing CVDs (Shadidizaji et al., 2023; Rad et al., 2023). These findings contribute to the broader landscape of structure-based drug discovery, providing a foundation for the development of targeted therapeutics with oroxylin A as a potential candidate for CVD intervention.

The results unveiled the pharmacological properties of Oroxylin A and its potential applications in therapeutic interventions, with a specific focus on CVD treatment. The study illuminates encouraging findings that underscore the inherent potential of Oroxylin A, positioning it as a compelling candidate meriting in-depth scrutiny within the realm of drug development initiatives directed toward ameliorating the complexities associated with CVDs and allied conditions. The identified outcomes not only elucidate the ability of Oroxylin A to interact with molecular targets implicated in cardiovascular pathophysiology but also emphasize its promising attributes, thereby suggesting its inclusion in the cohort of compounds earmarked for further exploration.

It is essential to acknowledge certain limitations in the current study. While computational approaches provide valuable insights into Oroxylin A and QDO interactions, rigorous experimental validation through in vivo studies and functional assays is necessary to confirm the modulatory effects of Oroxylin A on QDO activity and its impact on quercetin stability and functionality. Additionally, thorough exploration of the pharmacokinetics, bioavailability, and potential off-target effects of Oroxylin A is crucial for assessing its suitability as a therapeutic agent for CVD treatment. These

considerations emphasize the need for a comprehensive and multidisciplinary approach to clarify the potential of Oroxylin A as a QDO inhibitor, optimizing the effectiveness of quercetin for cardiovascular health.

Conclusion

In summary, the potential of quercetin for managing CVDs is hindered by enzymatic degradation, necessitating QDO inhibitors such as oroxylin A. Molecular docking and simulations reveal a strong binding affinity, suggesting that oroxylin A is a potential CVD intervention. Despite a moderate deviation, the QDO-Oroxylin A complex exhibited enhanced stability. Despite these limitations, further experimental validation and comprehensive exploration of the pharmacokinetics of Oroxylin A are crucial. This multidisciplinary approach is essential for optimizing the potential of Oroxylin A as a QDO inhibitor, as it enhances the effectiveness of quercetin for improving cardiovascular health.

Ethics Committee Approval: Ethical approval isn't necessary.
Peer-review: Externally peer-reviewed.
Author Contributions: KTC and AS designed the study. ÖA, MW collected the data. SM and FG drafted and wrote the paper. FG, AM, and DA discussed the results. MW supervised the study, interpreted the data and finalized the paper.
Conflict of Interest: The authors have no conflicts of interest to declare.
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