Interaction analysis of quinolone alkaloids derived from Euodia fruit with human pancreatic lipase through docking simulation

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Abstract. Obesity is associated with various metabolic disorders, and pancreatic lipase inhibitors are important therapeutic agents that suppress fat absorption. Quinolone alkaloids isolated from Euodia fruit have been reported to exhibit inhibitory activity against pancreatic lipase. In this study, docking simulations were performed using 33 ligands, including 14 isolated quinolone alkaloids and 19 virtual stereoisomers, against human pancreatic lipase. The ligands were classified into three structural groups based on carbon chain saturation and the presence of hydroxyl groups, and their docking results were compared with experimental activity. Overall, the docking results were consistent with the experimental data, showing positive correlations between structural groups 1 and 3. Notably, Mol 10, which interacted with His151, exhibited a high docking score despite its low experimental activity, whereas Mol 14-1, which interacted with His263, demonstrated strong inhibitory activity and a positive correlation with the docking scores. These findings suggest that the docking score may not always strongly correlate with experimental potency, as it can depend on the docking pose. This study highlights the potential of quinolone alkaloids as pancreatic lipase inhibitors and emphasizes the importance of analyzing ligand–residue interactions.

1 Introduction

Recent reports have indicated an increasing global trend in the prevalence of obesity [1]. Obesity directly induces cardiovascular risk factors, which in turn lead to various disorders, such as dyslipidemia, type 2 diabetes, hypertension, and sleep disorders [2]. In Japan, cardiovascular diseases, including heart and cerebrovascular diseases, account for a substantial proportion of mortality [3]. Generally, the primary treatment for obesity consists of behavioral interventions, including dietary and exercise therapy. However, in cases where these approaches are challenging to implement, yield insufficient effects, or are associated with markedly severe symptoms, pharmacotherapy may be considered as

an adjunct treatment [4]. Currently, the anti-obesity drugs approved in Japan include mazindol, semaglutide, and orlistat.

Mazindol and semaglutide promote weight loss by suppressing appetite, thereby improving obesity. Orlistat and cetilistat act as inhibitors of human pancreatic lipase. Orlistat forms a covalent bond with serine, which is its active site residue [5]. Cetilistat interacts with serine and histidine via hydrophobic interactions [6]. Both agents improve obesity by inhibiting lipid hydrolysis and reducing the intestinal absorption of fatty acids. However, the primary adverse effects associated with these agents include gastrointestinal disturbances, such as loose stools, increased defecation frequency, and potential interference with the absorption of fat-soluble vitamins [7].

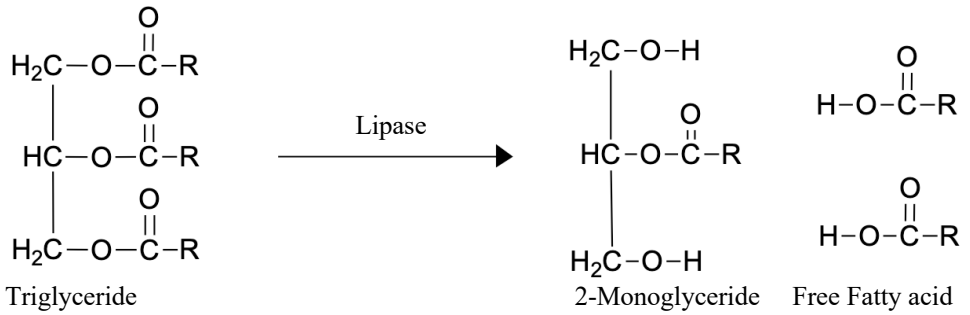

Fig. 1. Triglyceride decomposition reaction.

Dietary fats are composed of triglycerides that cannot be absorbed in their native form by humans (Figure 1). For intestinal absorption to occur, triglycerides must first be hydrolyzed, and this reaction is catalyzed by lipases. In humans, multiple types of lipases exist, including gastric, hepatic, endothelial, and pancreatic lipases, with pancreatic lipase accounting for 50–70% of triglyceride hydrolysis [8]. Therefore, the inhibition of pancreatic lipase is considered the most effective strategy for reducing excessive fat absorption. Human pancreatic lipase consists of A and B chains, with a catalytic pocket located in the B chain. The most critical residues in the active site of human pancreatic lipase are Asp176, His263, and Ser152 [9, 10]. Asp176 donates hydrogen to the imidazole ring of His263, which increases the pKa of the histidine nitrogen, enabling histidine to function as a stronger base and deprotonate the hydroxyl group of Ser152. The deprotonated Ser152 acts as a nucleophile, attacking the ester bond of the lipid substrate. This nucleophilic attack results in the formation of a tetrahedral intermediate between triglycerides and Ser152. Phe77 and Leu153 contribute to the stabilization of this tetrahedral intermediate.

Quinolone alkaloids have also been isolated from Euodia fruit [11]. Matsuo et al. [12] reported that several of the 14 quinolone alkaloids isolated from Euodia fruit exhibited inhibitory activity against porcine pancreatic lipase. Quinolone alkaloids with shorter carbon chains demonstrated stronger inhibitory activity. These findings suggest that the chemical space surrounding the quinolone alkaloid scaffold contains structural motifs that are useful for pancreatic lipase inhibition. Therefore, further exploration of this chemical space, using quinolone alkaloids as lead compounds, is required. The objective of the present study is to elucidate the structure–activity relations of the isolated alkaloids.

To this end, we performed molecular docking simulations of human pancreatic lipase with 33 ligands, comprising both isolated alkaloids and their hypothetical stereoisomers that were not naturally isolated.

2 Method

Docking simulations were performed using AutoDock Vina version 1.2.5 [13]. The protein structure was obtained from the X-ray crystallographic data of human pancreatic lipase available in the Protein Data Bank (PDB ID: 1LPB) [10]. The retrieved structure contained bound ions and ligands in addition to the protein. Therefore, these non-protein components were removed using PyMOL [14], and the resulting structure was used as the receptor for docking (Figure 2).

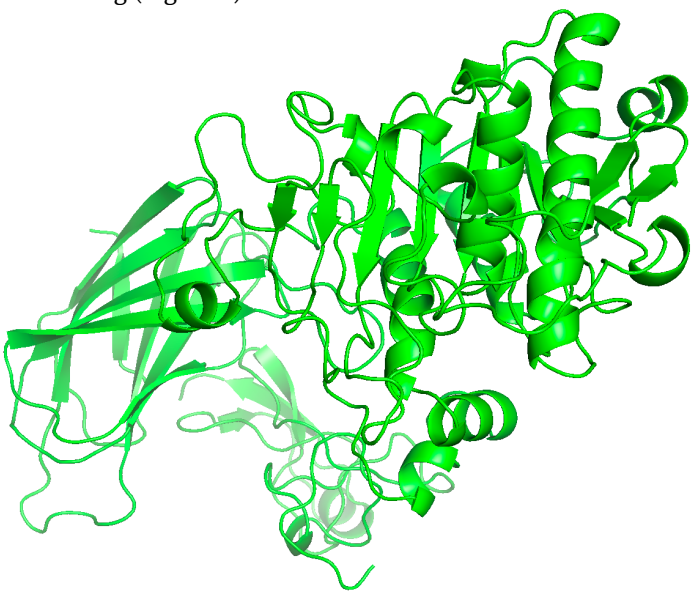

Fig. 2. Structure of human pancreatic lipase (PDB ID: 1LPB).

Ligand structures were drawn using ChemDraw version 23.1.2 and exported in SMILES format. For quinolone alkaloids 6–14 in the study by Matsuo et al. [12], all possible stereoisomers were generated using RDKit, resulting in 33 SMILES strings. These SMILES strings were then converted into three-dimensional structures using the Gen3D [15] module of OpenBabel [16], and the resulting structures were used as ligands (Figure 3a). For the clinically approved drugs orlistat and cetilistat, the 3D structures obtained from PubChem were used as ligands (Figure 3b and c).

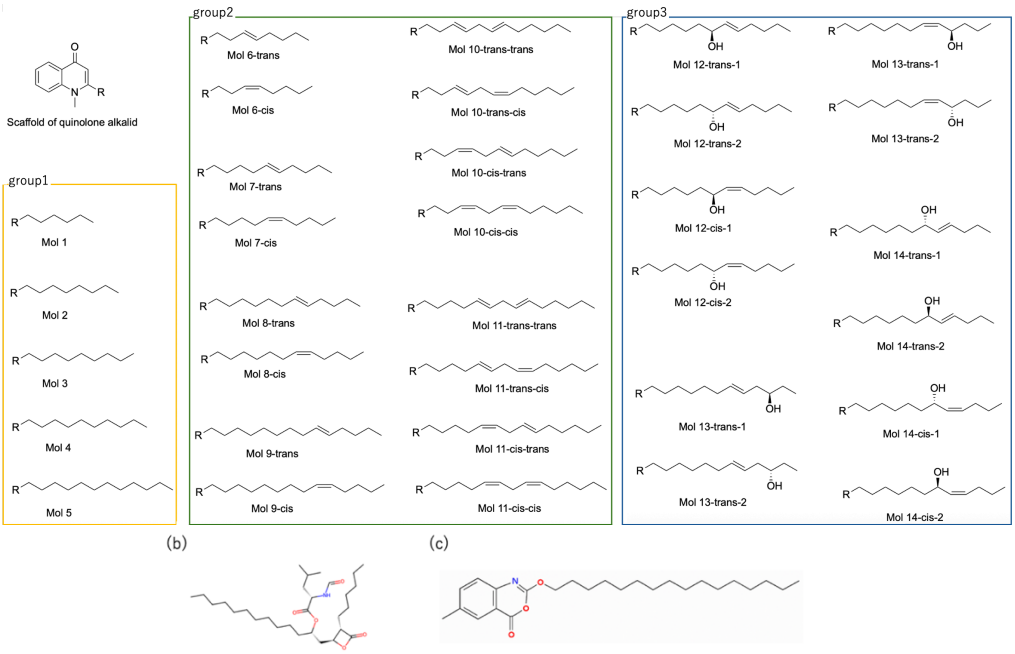

Fig. 3. Structure of ligands. (a) 33 quinolone alkaloids, including virtual stereoisomeric forms; (b) Orlistat; (c) Cetilistat.

In addition, quinolone alkaloids were classified into three structural groups based on their characteristic differences for further analysis. Structural Group 1 consists of quinolone alkaloids with carbon chains composed exclusively of single bonds, corresponding to Mol 1–5. Structural Group 2 includes compounds with carbon chains containing double bonds, corresponding to Mol 6–11. Structural Group 3 consists of compounds with carbon chains containing double bonds and hydroxyl groups, corresponding to Mol 12–14. These groups were defined based on the previously isolated compounds. As shown in Figure 3, among the stereoisomers, the isolated structure corresponds to the cis form. Furthermore, Mols 12, 13, and 14 were recently identified and isolated by Matsuo et al. [12].

Docking simulations using AutoDock Vina can be performed in two modes: basic docking, in which the protein is treated as a rigid body, and flexible docking, in which selected protein residues are allowed to move. In any docking method, ligands are treated as flexible. For each ligand, 50 independent runs of basic docking and five independent runs of flexible docking were conducted. For flexible docking, rotational freedom of 14 residues (Phe77, Ile78, Asp79, Tyr114, His151, Ser152, Leu153, Asp176, Ile209, Leu213, Phe215, Arg256, His263, and Leu264) was permitted. These residues were selected based on the findings of Nguyen et al. [17], who reported that they interact as auxiliary residues surrounding the catalytic triad. The docking simulation parameters used in this study are summarized in Table 1. The scoring function was evaluated using Vina.

Table 1. Parameters of docking simulation.

	Basic docking	Flexible docking
Grid center	X -2.7 Y 29.9 Z 38.5	X 9.82 Y 23.50 Z 50.87
Box size(Å)	X 85.0 Y 65.0 Z 75.0	X 35.0 Y 35.0 Z 35.0
Grid space	0.375	0.375
Exhaustiveness	32	32

For basic docking, the box size was set to cover the entire protein; therefore, ligands were not necessarily positioned within the binding pocket. However, because more than 90% of the ligands were located in the binding pocket based on the basic docking results, the box size for flexible docking was restricted to the vicinity of the binding pocket. Docking simulations were performed under the aforementioned conditions, and the correlation with the strength of inhibitory activity observed in the experiments was examined. In addition, the least-squares method and R^2 values were used for analysis.

3 Results

For both basic and flexible docking, the mean and standard deviation of the best scores obtained in each simulation were calculated (Figure 4). The docking scores obtained from basic and flexible docking showed qualitative agreement. Furthermore, many of the results were consistent with those reported by Matsuo et al. [12]. However, for Mol 10 and Mol 11, although strong inhibitory activity was not observed experimentally, docking simulations predicted favorable scores, that is, low binding energies.

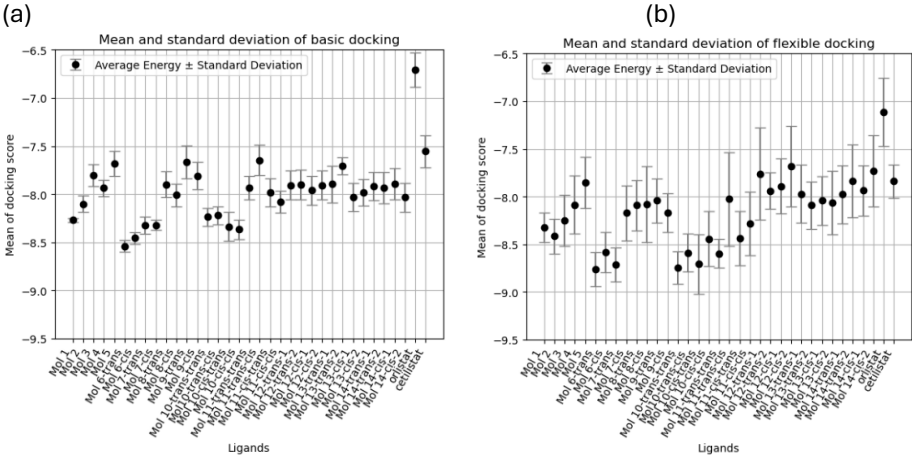

Fig. 4. Mean and standard deviation of docking simulations. (a) Basic docking; (b) Flexible docking.

Figure 5 shows a graph illustrating the correlation between the experimental values reported by Matsuo et al. [12] and flexible docking scores for the 14 structures.

Experimental values represent the activity of porcine pancreatic lipase, with the control set at 100%. Values closer to zero indicate stronger inhibitory activity. The docking scores represent the binding affinities calculated by the scoring function, with lower values indicating more stable docking poses. For comparison with the experimental values reported by Matsuo et al. [12], only the results for the cis isomers were used for structural Groups 2 and 3. Positive correlations were observed between structural Groups 1 and 3. In addition, the R^2 values were 0.88 for Group 1 and 0.75 for Group 3, indicating that a correlation with the experimental values was obtained for most structures.

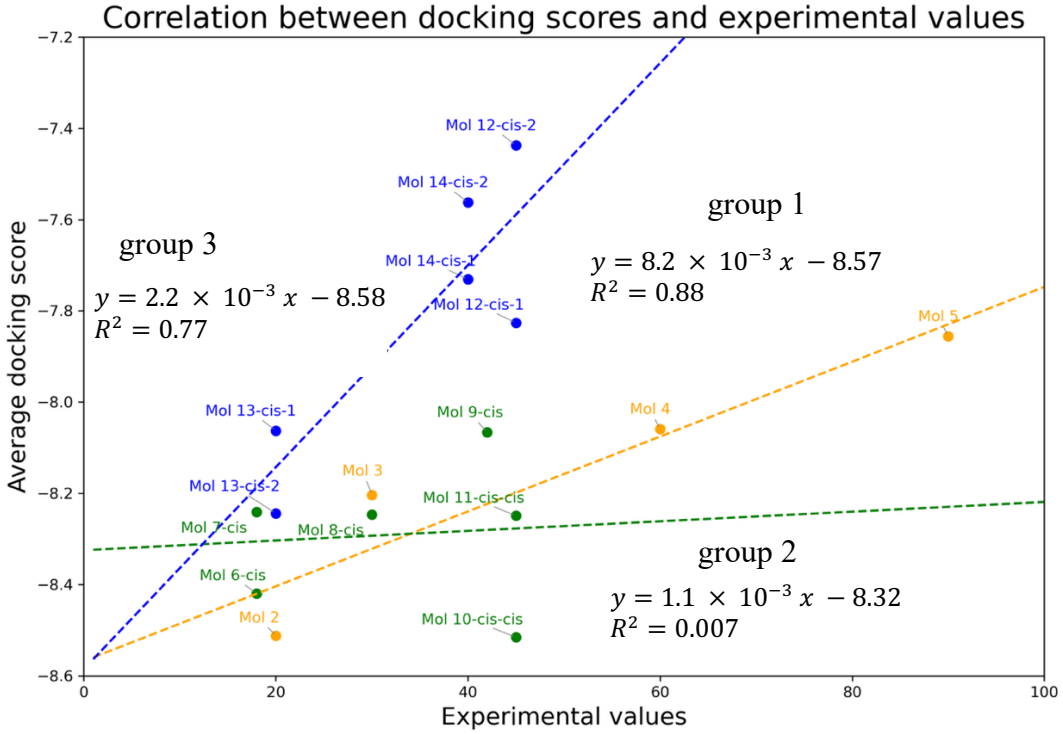

Fig. 5. Correlation of experimental data and docking scores within each structural group. The points represent the experimental values and docking score data points, and the lines indicate the regression lines for each group.

In Group 2, no positive correlation was observed. Moreover, the R^2 value was as low as 0.007, which is close to zero, indicating that the docking scores varied considerably among structures. Therefore, the docking pose of Mol 10, which was identified as having the largest discrepancy between its experimental inhibitory activity and docking score, was examined using PyMOL. For comparison, Mol 14-1 was selected because it possesses the same number of carbon atoms in its carbon chain and demonstrated nearly identical experimental inhibitory activity. Despite the negative charge of the binding pocket, both ligands bound strongly to the receptor via the oxygen atom of the quinolone alkaloid scaffold. Figure 6 illustrates the best-scoring docking pose from each of the five flexible docking runs using human pancreatic lipase. The ligands are represented in pink, yellow, beige, white, and purple, corresponding to the first through fifth runs, respectively. In

the five docking simulations, the best scores were –8.492 for Mol 10-cis-cis in the fifth run and –8.110 for Mol 14-cis-1 in the fourth run.

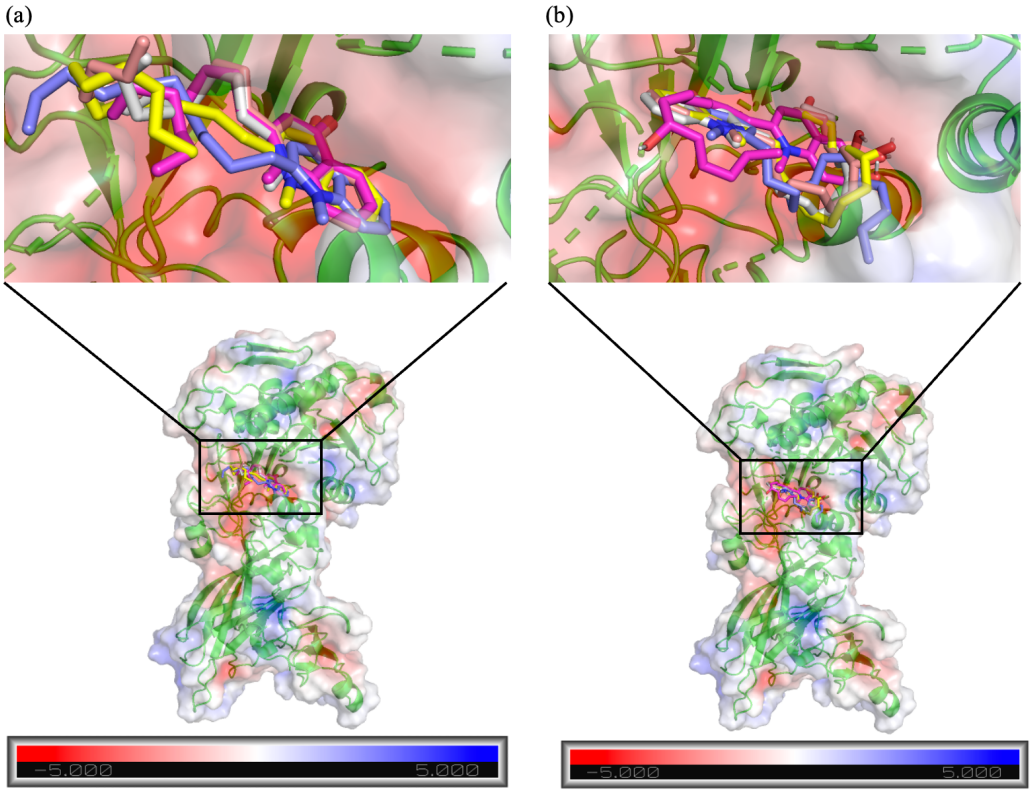

Fig. 6. Docking poses with pancreatic lipase (a) Mol 10 (b) Mol 14-1.

In addition, interaction analysis was performed using LigPlot [18]. Figure 7 illustrates the interactions observed in the docking poses that yielded the best docking scores.

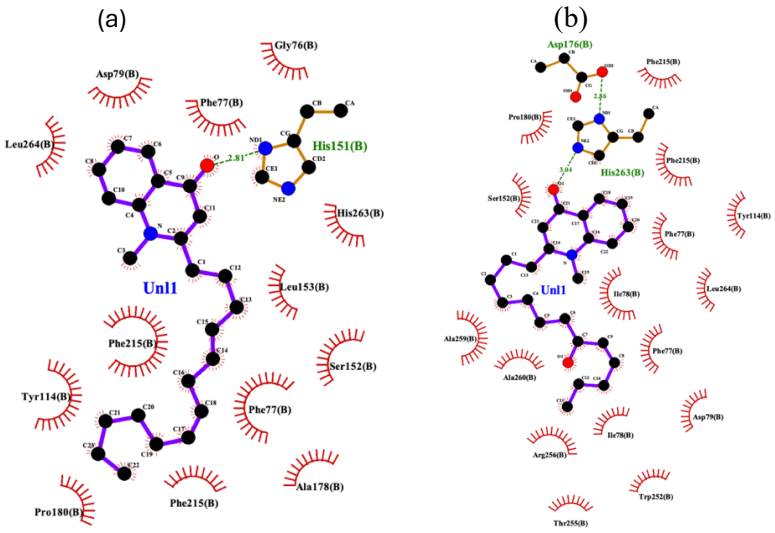

Fig. 7. Interactions with pancreatic lipase (a) Mol 10 (b) Mol 14-1.

Mol 10-cis-cis contacted His151 and showed high docking scores but weak and inconsistent experimental inhibition, indicating no correlation. By contrast, Mol 14-cis-1 contacted His263, with additional contact with Asp176, and exhibited favorable docking scores together with strong experimental inhibition, indicating a positive correlation.

4 Discussion

Docking simulations indicated that the quinolone scaffold oxygen frequently engaged histidine residues near the lipase pocket. Poses biased toward His151—proximal but outside the catalytic triad—often achieved favorable docking scores yet aligned poorly with experimental inhibition, whereas interactions involving catalytic His263 correlated more closely with measured activities. A plausible explanation is that His263 engagement positions the ligand oxygen for hydrogen bonding and possible proton exchange with imidazole, preventing the increase in pKa required for triad activation. The deeper location of His151 may drive over-penetration during pose search and inflate scores, accounting for the weak score–activity correlation. Overall, inhibition strength did not uniformly follow docking scores; therefore, pose- and residue-level inspection should be prioritized. Future studies will employ QM/MM approaches.

5 Conclusion

Matsuo et al. [12] isolated 14 quinolone alkaloids from Euodia fruit, three of which were identified as novel compounds. Several of the isolated alkaloids exhibited strong inhibitory activity against porcine pancreatic lipase. These findings suggest that useful compounds with potential as human pancreatic lipase inhibitors may exist within the chemical space surrounding quinolone alkaloids and that exploring this class of compounds could be an effective strategy for discovering novel inhibitors. In this study, docking simulations were conducted for 33 quinolone alkaloids, including hypothetical stereoisomers, with human pancreatic lipase, and their docking poses and interactions were analyzed. The results demonstrated that compounds that interacted with the catalytic residue His263 exhibited a strong correlation with experimental inhibitory activity. By contrast, compounds that did not show such a correlation tended to interact with His151, which is located deeper in the binding pocket than the catalytic residues. This phenomenon is thought to result from the docking algorithm preferentially searching for lower-energy poses. Therefore, when performing docking simulations on chemical structures lacking experimental data, analyzing the docking poses and interactions is useful. Moreover, because charge transfer is not accounted for in docking simulations, quantum chemical calculations are required to further elucidate the mechanisms underlying this inhibitory activity.

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methodology, and M.N., C.Y., Y.N., and K.W. performed the validation. C.Y., Y.N., and K.W. performed formal analysis and investigation. Resources were provided by M.T., R.M., and Y.N., whereas data curation was performed by C.Y. and Y.N. The original draft was prepared by K.W., Y.N., and M.T. All authors contributed to the review and editing of the manuscript. Figures and visualizations were generated by K.W. and C.Y. Supervision was provided by Y.M., Y.N., R.M., and M.T. Project administration and funding acquisition were managed by Y.N., R.M., and M.T. All authors have read and approved the final manuscript.

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