Journal of
Food and Pharmaceutical Sciences

Original Article

Potential Activity of Caryophyllene Derivatives as Xanthine Oxidase Inhibitor: An in silico Quantitative Structure-Activity Relationship Analysis

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Received: 1 September 2022; Revised: 3 November 2022; Accepted: 10 November 2022; Published: 31 December 2022

Abstract: As the enzyme responsible for uric acid formation, Xanthine oxidase was considered to be a therapeutic target for hyperuricemic treatment. This study was carried out to assess the potential of caryophyllene, and its derivates usually present in the natural product to inhibit Xanthine oxidase. The molecular docking using Autodock Tool and Biovia Discovery Studio was conducted to visualize the molecular interaction and to reveal the structure-activity relationship of those compounds. The results showed that the derivates of caryophyllene showed a higher affinity to Xanthine Oxidase than Allopurinol. Among those all, caryophyllene oxide has the most stable bonding to xanthine oxidase. Structure-activity relationship analysis showed that the compound's chemical properties affected the affinity and molecular interaction to the enzyme as the target site in this study. The number of double bonds, substituents position, conformational structure related to steric hindrance, and the presence of lactone ring was assumed to influence the xanthine oxidase inhibitory activity of caryophyllene derivates. It can be concluded that the high affinity of caryophyllene oxide is directly linked to its chemical properties allowing it to interact with essential amino acid residues of xanthine oxidase.

Keywords: Xanthine oxidase; α-caryophyllene; β-caryophyllene; γ-caryophyllene; caryophyllene oxide; QSAR

1. INTRODUCTION

Xanthine oxidase (XO) is an enzyme promoting hyperuricemia by catalyzing the biosynthesis of purines, xanthine, and other biochemicals to uric acid [1,2]. Within the biosynthesis of uric acid, two reactions of purine catabolism are catalyzed by the XO, particularly the oxidation of hypoxanthine to xanthine and xanthine to uric acid with the reduction of NAD^+ or O_2, simultaneously [3,4]. Overproduction of XO will increase the oxidation of hypoxanthine and xanthine, leading to the elevation of uric acid production [5]. Therefore, the XO has been considered as the therapeutic target to obstruct the biosynthesis of uric acid.

Many XO inhibitors are currently available and have been used to lower uric acid serum levels. However, conventional XO inhibitors like allopurinol and febuxostat have excessive side effects and toxicity [6]. Steven-Johnson syndrome, liver function abnormality and dysfunction, agranulocytosis, and toxic epidermal necrolysis syndrome have been observed in patients treated

with these drugs [7–9]. As a consequence, new drug candidates acting as XO inhibitors with low side effect necessary to be discovered.

Natural product-derived compounds (NPCs) and their structural analogs have made a vital contribution to drug discovery [10]. NPCs provide unique characteristics with numerous structural complexities and diversity, which possess high molecular rigidity that can be used to tackle protein-protein interactions [11,12]. Caryophyllene and its derivatives are highly rigid molecules that have spacious biological activities. They are usually present in mixed forms and contribute to almost all pharmacological activity of clove and other essential oils [13,14]. Several studies reported that caryophyllene and its isomeric compounds possess anti-cancer and antiproliferative activity [15,16], antioxidant and antioxidative stress [17,18], and anti-inflammatory activity [19]. Even though they have broad pharmacological activities, there has been no report regarding the xanthine oxidase inhibitor potential of caryophyllene and its derivatives.

Therefore, this study was an initial effort to assess their potential for XO inhibitors by analyzing their molecular interactions and quantifying the structure-activity relationship of caryophyllene derivatives.

2. MATERIALS AND METHODS

2.1. Protein and ligand preparation

The crystallographic structure of Xanthine oxidase (XO) was obtained from Protein Data Bank (www.rcsb.org) with PDB ID: 3NVY. The XO was prepared in Biovia Discovery Studio by removing all unnecessary components. In particular, waters, ligands, and heteroatoms were removed from the XO structure and saved in PDB format. On the other hand, the 3D structure of caryophyllene derivatives α-caryophyllene, β-caryophyllene, γ-caryophyllene, and caryophyllene oxide was downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). The 3D structure of all ligands was saved in PDB format.

2.2 Molecular docking study

The Molecular docking study was conducted using Autodock 4.2 (https://autodock.scripps.edu), assisted by Autodock Tools (https://ccsb.scripps.edu/mgltools). The XO macromolecule prepared from Biovia Discovery Studio (https://discover.3ds.com) was input into Autodock and was further processed by adding the hydrogen atoms (polar only). The ligand was prepared by setting the number of rotatable bonds and the center node with the output in the PDBQT format. In the grid box preparation, the coordinate of the grid box was set to x: 39.291, y: 21.87, and z: 20.22, following the native ligand binding site. The adjustment of the grid box was made by setting the grid box dimensional size of x, y, and z at 40 x 40 x 40 with a spacing of 0.375Å. The molecular docking used the Genetic Algorithm (GA) and was set at 100 runs with 250.000 evaluations for docking parameters and the Lamarckian Genetic Algorithm as an output. The molecular docking was run as many as ten repetitions and used allopurinol as the positive control.

2.3 QSAR analysis

The quantitative structure-activity relationship study was carried out by comparing the docking score of the ligands and the molecular interactions. The docking score was obtained from the docking study by observing the free binding energy of each ligand. The best conformation of each ligand obtained from the docking study was saved in PDB format and processed in Biovia Discovery Studio for molecular interactions analysis

3. RESULTS AND DISCUSSION

The plain crystallographic structure of XO (Figure 1) was successfully obtained from the protein preparation by separating waters, heteroatoms, and ligands so that the unnecessary interactions due to the intervention of those components can be avoided.

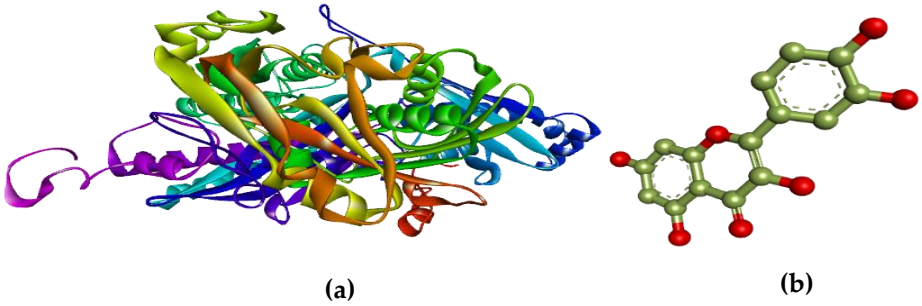

Figure 1. Protein preparation result. **(a)** plain crystallographic xanthine oxidase structure, **(b)** native ligand 3D structure.

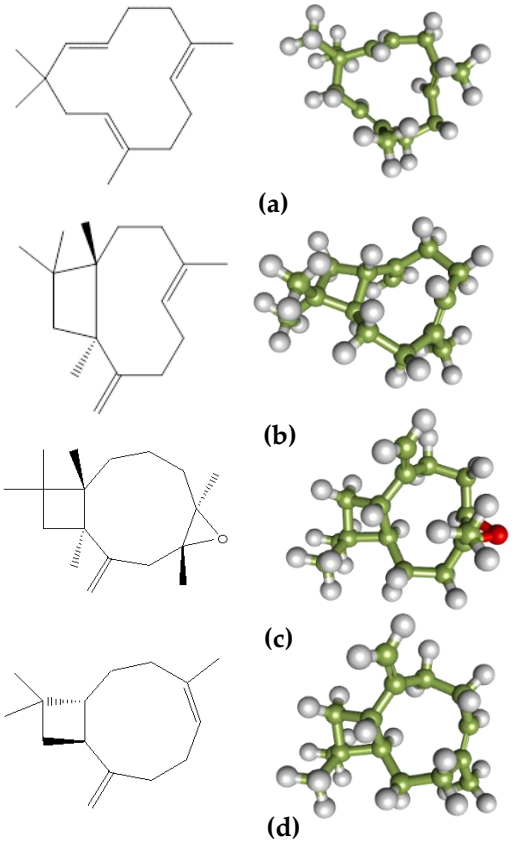

Figure 2. The 2D and 3D structure of caryophyllene derivatives. (**a**) α-caryophyllene, (**b**) β-caryophyllene, (**c**) caryophyllene oxide, and (**d**) γ-caryophyllene.

Ligands used in this study were caryophyllene derivatives such as α-caryophyllene, β-caryophyllene, γ-caryophyllene, and caryophyllene oxide, which have similar structural conformation as illustrated in Figure 2.

The validation of docking methods was previously conducted prior to the docking of the test ligands by re-docking the XO native ligands. In the validation process, the docking method was stated valid if the root means square deviation (RMSD) obtained from the re-docking of the native ligand was less than 2 Å [20,21]. The similarity of structural conformation between the re-docking native ligand with the original native ligand is represented by the RMSD value. The low RMSD value (< 2 Å) indicates that the structural conformation of the re-docking ligand is similar to that of the original native ligand. The re-docking result revealed that the RMSD value of the re-docking of the native ligand was 1.53 Å indicating that the docking parameters were valid.

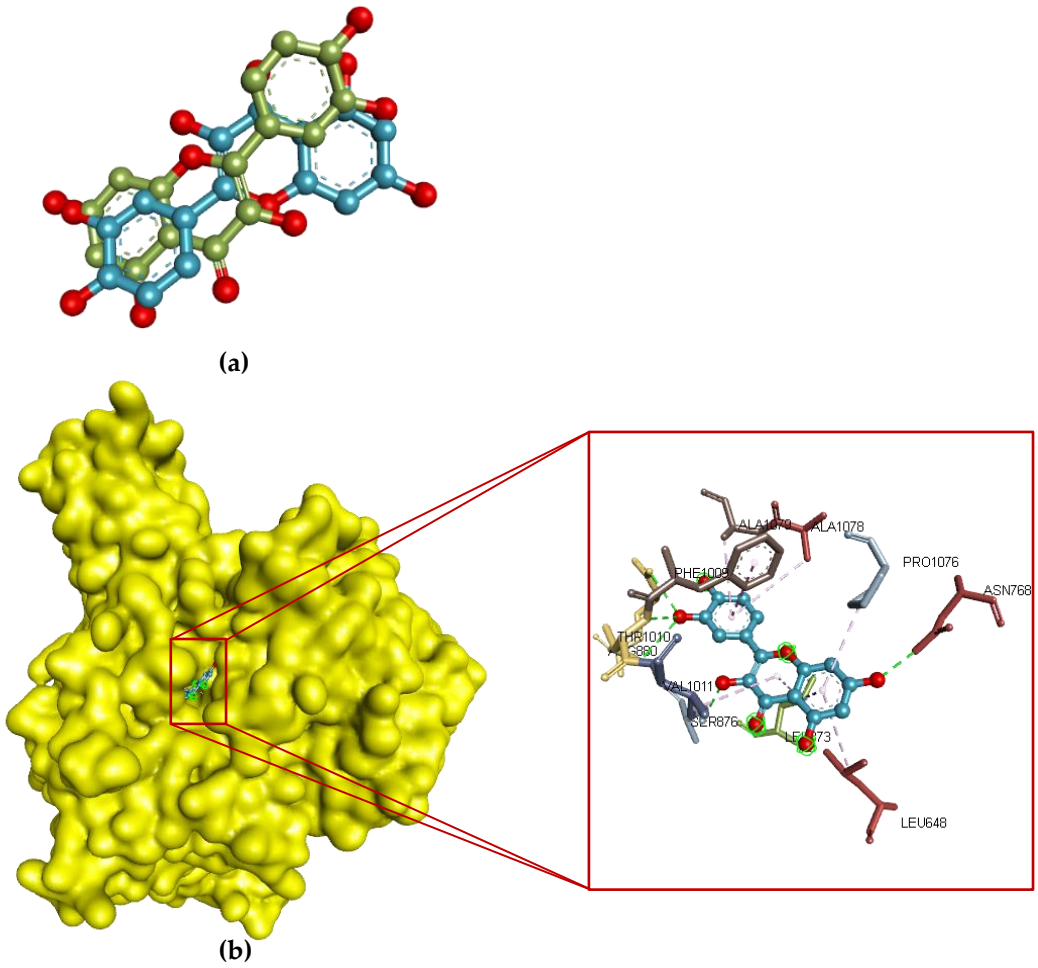

Figure 3. Result of docking method validation. (**a**) re-docking of the native ligand (blue assigned to re-docking ligand; green assigned to original native ligand), (**b**) 3D molecular interactions of the native ligand with XO.

A molecular docking study of the test ligands was carried out following the parameters that have been validated. The docking result was analyzed by observing the free binding energy of each ligand. The more negative free binding energy, the stronger the affinity of the ligand with the protein [22]. It means that ligands with smaller free binding energy than native ligands indicated that their affinity to the protein was better than the native ligand. The docking result is shown in Table 1.

Table 1. Molecular docking results of caryophyllene derivatives against xanthin oxidase

Ligands	Free binding energy (kcal/mol)	Inhibition constant Ki (μM)
α-caryophyllene	-5.44 ± 0.008	101.06 ± 0.600
β-caryophyllene	-5.80 ± 0.005	55.52 ± 0.074
γ-caryophyllene	-6.39 ± 0.005	20.76 ± 0.040
Caryophyllene oxide	-6.66 ± 0.003	13.09 ± 0.022
Allopurinol	-5.96 ± 0.029	41.27 ± 0.167
Native ligand	-10.32 ± 0.004	0.027 ± 0.184

As illustrated in Table 1, γ-caryophyllene and caryophyllene oxide have more negative free binding energy than the positive control, allopurinol indicating that they are well-bound and stable to XO compared to allopurinol. It can be predicted that γ-caryophyllene and caryophyllene oxide possess a better affinity with XO than allopurinol. In addition, the inhibition constant is also one of the parameters in assessing the binding affinity between the ligand and the target protein. The smaller the value of the inhibition constant, the higher the affinity of the ligand to the protein [23,24]. The inhibition constant is directly proportional to the free binding energy. The more negative the free binding energy, the smaller the inhibition constant. The inhibition constant of γ-caryophyllene and caryophyllene oxide was significantly different from the inhibition constant of allopurinol. It is obvious that the affinity of γ-caryophyllene and caryophyllene oxide to XO was better than allopurinol. However, between these two compounds, caryophyllene oxide is the best and the most stable compound to bind with XO.

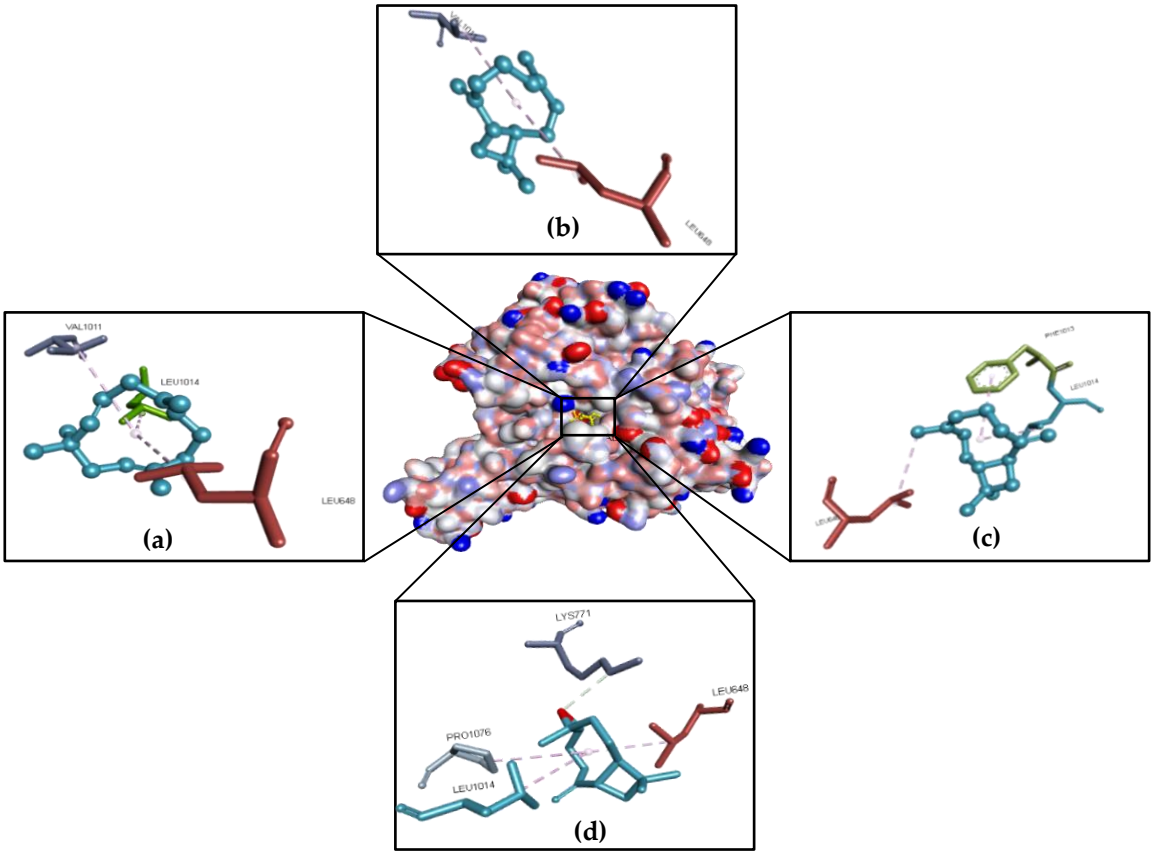

Figure 4. 3D molecular interaction of ligands with XO. (**a**) α-caryophyllene, (**b**) β-caryophyllene, (**c**) γ-caryophyllene, and (**d**) caryophyllene oxide.

In Table 1, even though they have a similar structure, every ligand provides different free binding energy and inhibition constant, indicating that they possess a diverse affinity with XO. The diversity of the affinity of each ligand might be caused by the differences in their conformational structure related to the structure-activity relationship. Conformational structure heterogeneous of caryophyllene derivatives directly influences the differences of occurred molecular interactions.

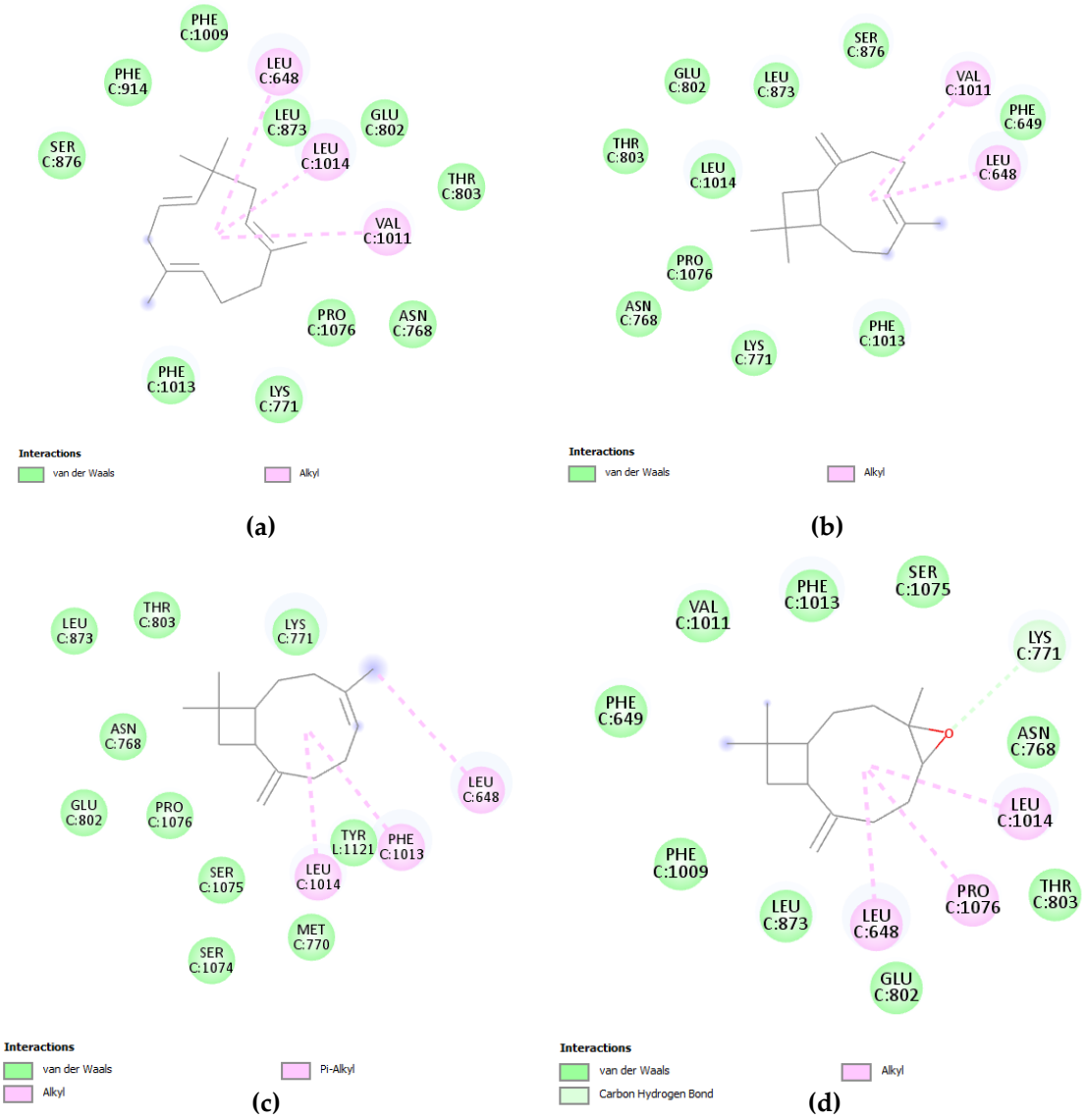

Figure 5. 2D molecular interactions of ligands with XO. (**a**) α-caryophyllene, (**b**) β-caryophyllene, (**c**) γ-caryophyllene, and (**d**) caryophyllene oxide.

As illustrated in Figure 5, every ligand has slightly different molecular interactions with XO. The number of double bonds, the location of the substituents, the conformational model, and the presence of a lactone ring within the molecular structure is the factors that influence the interactions. α-caryophyllene in Figure 5a showed that it has three double bounds that are supposed to contribute to π interactions. However, these π bonds do not assist the π-interactions due to the unfavorable position of the double bond in the structure of α-caryophyllene. It is evident that the position of the double bond greatly influences molecular interaction [25]. The conformational structure of α-

caryophyllene provides many steric hindrances that prevent molecular interaction. This is the primary cause of the affinity of α-caryophyllene with XO being diminished.

β-caryophyllene and γ-caryophyllene possess equal double bond (Figure 5b and 5c). However, the affinity of γ-caryophyllene was better than β-caryophyllene due to the position of the double bond. The structure of the γ-caryophyllene provides less steric hindrance than that of β-caryophyllene. This increases the possibility for γ-caryophyllene to form π-interaction with XO, leading to increased γ-caryophyllene affinity. Different from the three other caryophyllene derivatives, caryophyllene oxide (Figure 5d) possesses a lactone ring within its structure. This lactone ring makes a significant contribution to the XO inhibitory activity of caryophyllene oxide. The oxygen atom in the lactone ring interacts with XO by forming a hydrogen bond with Lysine 771, increasing its affinity. Additionally, the lactone ring in the structure of caryophyllene oxide will interact with the protein by nucleophilic addition to sulfhydryl or amino group through the Michael reaction [26].

Therefore, it can be assumed that the XO inhibitory activity of caryophyllene derivatives is influenced by their chemical properties, such as alkylating center reactivity, side chain, and molecular conformation.

4. CONCLUSION

A total of 4 Caryophyllene derivatives were subjected to molecular docking-based virtual screening against Xanthin Oxidase. Due to their conformational structure, those compounds have different binding affinities on XO, as was shown by each free binding energy and its inhibition constant. Among those compounds, Caryophyllene oxide was the most promising compound to inhibit the XO compared to Allopurinol. The chemical properties such as the number of double bonds, substituents position, steric hindrance, and presence of lactone ring in this study were revealed to contribute to the compound structure-activity relationship.

Funding: This research received no external funding

Acknowledgments: The authors would like to thank Ms. Nurul Hidayati, Department of Pharmaceutics, School of Pharmacy, Bandung Institute of Technology for conducting the statistical analysis.

Conflicts of interest: The authors declare no conflict of interest.

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