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In Silico Investigation of Two Benzoxanthone-Flavonoids: ADMET Analysis and Xanthine Oxidase Binding †

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Abstract: Natural products, particularly flavonoids, which possess medicinal properties such as anticancer, anti-inflammatory, and antioxidant effects are known to inhibit the xanthine oxidase (XO) enzyme, which plays a key role in purine metabolism and generates reactive oxygen species (ROS). Inhibiting XO may help manage diseases associated with uric acid accumulation and ROS production. Molecular docking was performed to analyze the interactions of two benzoxanthone-flavonoid compounds, Artonin E and (+)-Artobiloxanthone, with the XO enzyme. These compounds demonstrated excellent stability within the site active of the XO enzyme, with estimated docking scores of −9.64 and −7.99 kcal/mol, respectively, and formed significant interactions, similar to those observed in the quercetin-XO complex. Additionally, ADMET analyses suggest that these compounds have promising therapeutic potential.

Keywords: XO enzyme; benzoxanthone-flavonoids; molecular docking; ADMET analysis

1. Introduction

Natural products have long been recognized as a valuable source of bioactive compounds [1], playing a vital role in traditional medicine and modern drug discovery. These compounds, including polyphenols, flavonoids, benzoxanthones, and other phytochemicals, have garnered significant attention due to their diverse biological activities and therapeutic potential [2]. Among them, flavonoids stand out as a prominent group of polyphenols, widely distributed in fruits, vegetables, tea, and other plant-based foods. As secondary metabolites, flavonoids serve various ecological functions in plants, such as defense against pathogens and ultraviolet radiation [3]. In humans, they exhibit a broad spectrum of pharmacological effects, making them a subject of increasing interest in medicinal chemistry [4].

The health benefits of flavonoids are multifaceted. Their anticancer properties have been attributed to their ability to modulate various signaling pathways involved in cell proliferation, apoptosis, and angiogenesis [5]. In addition, flavonoids possess anti-inflammatory effects, acting through the inhibition of pro-inflammatory mediators such as cytokines and enzymes like cyclooxygenase [6]. Another critical role of flavonoids is their antioxidant activity, which enables them to neutralize free radicals and reduce oxidative stress, a contributing factor in the pathogenesis of chronic diseases such as cardiovascular disorders, neurodegenerative conditions, and diabetes [7].

One of the key mechanisms by which flavonoids exert their therapeutic effects is through the inhibition of the XO enzyme. XO is a molybdenum-containing enzyme responsible for catalyzing the oxidation of hypoxanthine to xanthine and subsequently to

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uric acid, a process that generates ROS as byproducts. While ROS play essential roles in cellular signaling and immune response, their excessive production can lead to oxidative damage, contributing to the development of conditions such as hyperuricemia, and cardiovascular diseases [8].

The inhibition of XO, therefore, emerges as a promising therapeutic approach for managing diseases characterized by abnormal purine metabolism and oxidative stress. Flavonoids, with their potent XO inhibitory activity, have the potential to serve as natural alternatives or complementary agents to conventional drugs such as allopurinol, a well-known XO inhibitor.

Recent studies have further explored the molecular interactions between flavonoids and XO, utilizing advanced techniques like molecular docking and structure-activity relationship (SAR) analysis [9]. These investigations provide deeper insights into how specific flavonoids bind to the active site of XO, stabilize within the enzyme, and inhibit its activity.

In this context, we studied the interaction mode of two known benzoxanthone-flavanoids (Artonin E and (+)-Artobiloxanthone) to verify their ability to inhibit the XO enzyme and to show its capacity as an antioxidant agent; the studies were carried out by molecular docking and ADMET analysis.

2. Materials and Methods

2.1. Molecular Docking

The X-ray crystal structure of the Quercetin-XO complex (PDB: 3NVY) was obtained from the RSC protein data bank [10]. The structure was prepared using the Protein preparation wizard in the Schrödinger suites software package. The three-dimensional structures were generated using Maestro software and further optimized with Ligprep using the OPLS3e force field [11]. The final prepared PDB files for both the protein and benzoxanthone-flavanoids compounds were submitted for the docking process. Docking studies were conducted using the Glide software with extra precision settings. The output files containing the docked compounds in complex with the XO enzyme were visualized using Chimera X [12].

2.2. ADMET Prediction

To evaluate the possibility of our benzoxanthone-flavanoids compounds successfully advancing through clinical trials, we conducted an analysis based on several key parameters, including Lipinski's Rule of Five, Veber's Rule, Egan's Rule, polar surface area (TPSA), the number of rotatable bonds, as well as ADME/T properties and bioactivity scores. These calculations were carried out using online tools SwissADME, ProTox, and Molsoft server [13].

3. Results and Discussion

3.1. Molecular Docking

To investigate the interactions between the two selected benzoxanthone-flavonoids, Artonin E and (+)-Artobiloxanthone, and the XO enzyme, molecular docking was performed using the quercetin-XO complex.

The molecular docking protocol was validated by re-docking quercetin into the active pocket of the XO enzyme, where the docked quercetin nearly overlapped with the crystallized form (RMSD = 0.24 Å). Quercetin demonstrated significant stability in the active pocket (docking score: −9.33 kcal/mol) due to the formation of several hydrogen bonds between the hydroxyl and carbonyl groups of its flavone fragment and the residues Thr 1010, Val 1011, Arg 880, Glu 1261, and Ala 1079. Additionally, the flavone fragment engaged in π-π stacking interactions with the aromatic rings of residues Phe 1009 and Phe 914 (Figure 1).

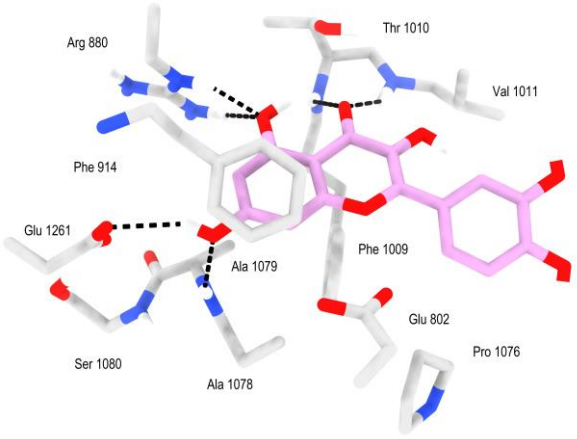


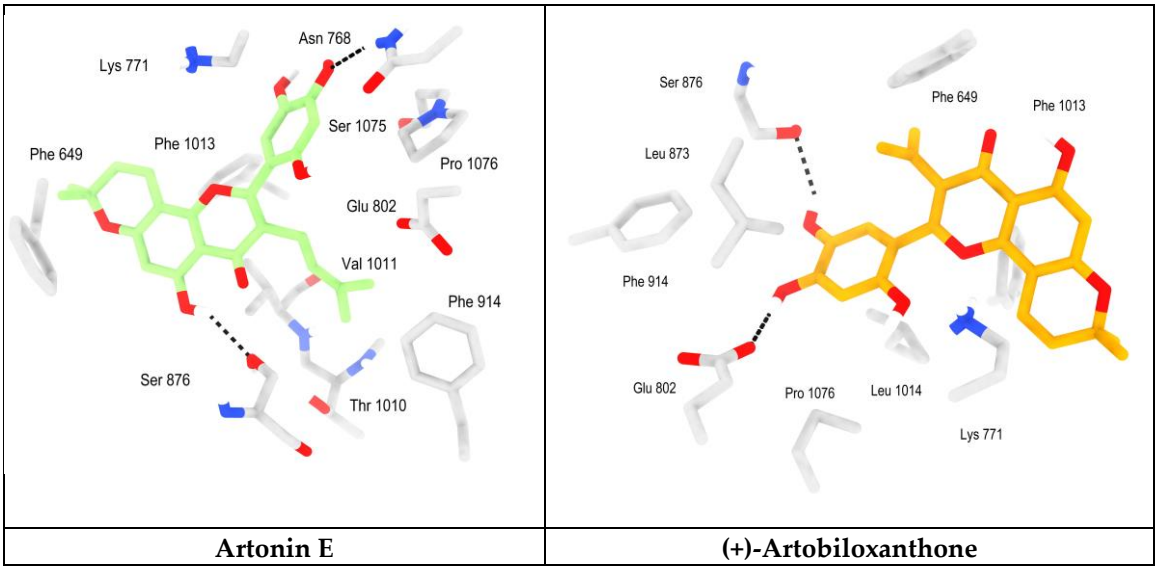
Figure 1. 3D binding interactions of quercetin after docking calculations in the active site of XO enzyme. The amino acid residues were shown as grey stick model and H-bonds were shown as black lines.

The two studied compounds, Artonin E and (+)-Artobiloxanthone, exhibited excellent stability within the active site of the XO enzyme, with docking scores of −9.64 and −7.99 kcal/mol, respectively.

Artonin E, which had a better docking score than quercetin, formed two hydrogen bonds with the Asn 768 and Ser 876 residues. It also engaged in π-π stacking interactions with Phe 649 and a π-cation interaction with the Lys 771 residue. Hydrogen bonding is crucial in maintaining the compound's position and orientation, facilitating more effective inhibition. Additionally, Artonin E formed significant hydrophobic interactions, similar to those observed with quercetin, involving residues such as Phe 1009, Phe 914, Phe 1013, and Met 770.

Regarding the compound (+)-Artobiloxanthone, it is equally significant as Artonin E. This compound formed two hydrogen bonds with the residues Ser 876 and Glu 802. Additionally, it engaged in π-π stacking interactions with Phe 1013 and a π-cation interaction with Lys 771 (Figure 2).

All of these interactions contribute to a lower energy state, reinforcing the stability of the ligand-enzyme complex. The presence of these interactions suggests that Artonin E (+)-and Artobiloxanthone are not only able to fit well into the binding site but also can form multiple stabilizing interactions that enhance its inhibitory potential.



Artonin E	**(+)-Artobiloxanthone**

Figure 2. 3D binding interactions of Artonin E and (+)-Artobiloxanthone after docking calculations in the active site of XO enzyme. The amino acid residues were shown as grey stick model and H-bonds were shown as black lines.

3.2. ADMET Prediction

A potential drug candidate must undergo various tests to evaluate its ability to penetrate the organism, including assessments of pharmacokinetic properties and toxicity levels. A comprehensive prediction of the ADMET properties for the two studied compounds was conducted, and the results are presented in the table below.

The ADME study revealed that none of the compounds violate the Lipinski's rule of five, which is a set of criteria used to assess the drug-likeness of a chemical compound based on its physicochemical properties, including molecular weight, lipophilicity, hydrogen bonding capacity, and solubility. Moreover, according to Tox-Prediction, both compounds exhibited a toxicity level of 2500 mg/kg, placing them in Class V. This indicates that the compounds may be harmful if swallowed". (Table 1).

Table 1. Pharmacokinetic parameters and drug likeness score (DLS) Artonin E and (+)-Artobiloxanthone.

Proprieties / Compounds	Molecular Weight (g/mole)	Rotatable Bonds	H-Bond Donor	H-Bond Acceptor	Violations	Log Po/W iLogP	Log S ESOL
Artonin E	436.45	3	4	7	0	3.72	−5.98
(+)-Artobiloxanthone	408.40	2	4	7	0	3.16	−5.56
Proprieties / Compounds	**Bioavailability Score**	**BBB**	**Log Kp (cm/s)**	**GI**	**TPSA (°A²)**	**DLS**	**LD50 (mg/kg)**
Artonin E	0.55	2.36	−5.29	Low	120.36	−0.36	2500
(+)-Artobiloxanthone	0.55	2.45	−5.50	High	120.36	−0.77	2500

4. Conclusions

In summary, the in silico study of two benzoxanthone-flavonoids, Artonin E and (+)-Artobiloxanthone, demonstrated their potential as effective xanthine oxidase inhibitors through molecular docking and ADMET analysis. Both compounds exhibited strong binding affinities and stability within the active site of XO enzyme, surpassing the reference compound quercetin in terms of docking scores and interaction strength. The ADMET analysis confirmed favorable pharmacokinetic properties and low toxicity levels, making these flavonoids promising candidates for further drug development, especially in managing diseases related to oxidative stress and uric acid accumulation. These findings highlight the therapeutic potential of natural products in drug discovery, especially for conditions involving XO inhibition.

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

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