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Original Article

Natural Carotenoids Targeting Matrix Metalloproteinase-3: Implications for Caries Prevention

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Abstract

Background: Dental caries, a pervasive chronic disease, arises from a complex interplay of cariogenic bacteria, dietary sugars, and host factors. Matrix metalloproteinase-3 (MMP-3), an enzyme detected in demineralized human dentin and bovine predentine, contributes to caries progression through its proteoglycan-degrading activity. This study aimed to evaluate a series of carotenoids for their potential to inhibit the MMP-3 active site.

Methods: A molecular docking analysis was performed using AutoDock 4.0 to estimate the binding affinity of 19 carotenoid compounds to the catalytic cleft of MMP-3. The specific binding modes and molecular interactions were subsequently analyzed and visualized using the Discovery Studio Visualizer. The results were compared with those of a positive control inhibitor, MBS.

Results: Three carotenoids, including astaxanthin, canthaxanthin, and capsanthin, displayed notably strong binding affinities, with free energy values ($\Delta G_{binding}$) lower than -10 kcal/mol. Among these, astaxanthin was the most potent inhibitor. Interaction profiling indicated that capsanthin established the most extensive hydrophobic network, engaging five key residues, while canthaxanthin formed two stabilizing hydrogen bonds.

Conclusion: The findings suggest that astaxanthin, canthaxanthin, and capsanthin are promising natural compounds for inhibiting MMP-3. This positions them as potential candidates for novel therapeutic strategies to prevent dental caries progression.

Keywords: Carotenoids, Dental caries, Drug, Inhibitor, Matrix metalloproteinase-3

Introduction

Dental caries is a global chronic health issue stemming from the complex, long-term interactions among acid-producing bacteria, fermentable carbohydrates, and individual patient factors [1]. According to the World Health Organization, oral diseases affect approximately 3.5 billion people, and untreated tooth decay alone is the most common health problem on a global scale [2]. This condition is highly prevalent in children, impacting 43% worldwide [3, 4]. Despite modern advances in dentistry, the burden of caries remains substantial, particularly in developing countries and among at-risk populations [5]. Therefore, early detection of cavities and preventive strategies are vital for controlling disease progression and preserving natural teeth [6].

The pathogenesis of dental caries involves a shift in microbial communities within oral biofilms. The primary mechanism involves cariogenic bacteria, notably *Streptococcus mutans* (*S. mutans*), metabolizing fermentable carbohydrates in plaque

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biofilms to generate acids [7]. This leads to dysbiosis, reinforcing *S. mutans* dominance and creating persistent acidic microenvironments that demineralize enamel and dentin, thereby initiating caries [8, 9]. In addition to bacterial acids, the degradation of the dentinal matrix is facilitated by host-derived enzymes, specifically matrix metalloproteinases (MMPs), which degrade collagen fibrils. The clinical relevance of this process is evidenced by the use of MMP inhibitors to pretreat dentin before restorative bonding [10]. Among these enzymes, MMP-3 has been identified in demineralized human dentin, where it acts as a proteoglycanase. Its specific proteolytic activity in this context is well-documented, and its presence has been further confirmed in the predentine layer and dentinal tubules of bovine molars [11-14].

Carotenoids are widely distributed pigments responsible for yellow, orange, and red hues. Plants, bacteria, and fungi biosynthesize these C40 tetraterpenoids, but animals do not produce them and must obtain them through nutrition. Their medicinal appeal stems from a range of biological functions, including scavenging free radicals, exerting anti-inflammatory and provitamin A activities, and displaying anti-aging properties [15-20].

Carotenoids have shown inhibitory effects against MMPs. For example, a previous study by Sajjad et al. [21] demonstrated through molecular docking that astaxanthin exhibits a high binding affinity for MMP-3, interacting with its hydrophobic S1' active site. Building on this finding, the present study aimed to evaluate the inhibitory potential of a diverse set of carotenoids against MMP-3 using AutoDock 4.0 and to critically compare their binding modes and affinities with those of the potent MMP-3 inhibitor (MBS; PubChem ID: 446165). This comparative investigation identified novel, plant-derived MMP-3 inhibitors, providing a foundational rationale for developing herbal-based interventions to combat collagen degradation in dentin and help prevent dental caries.

Materials and Methods

Re-docking Analysis

Prior to carotenoid screening, the docking protocol was validated by re-docking MBS (the co-crystallized ligand from MMP-3, PDB ID: 1HY7) using the same AutoDock 4.0 settings. Root-mean-square deviation (RMSD) between the re-docked and crystal poses was calculated using LigRMSD [22] to confirm binding mode reproducibility.

Matrix Metalloproteinase-3 (MMP-3) and Carotenoids Structural Preparation

To establish a high-resolution structural framework for this investigation, the crystal structure of MMP-3 (PDB ID: 1HY7) was retrieved from the RCSB Protein Data Bank [23, 24]. This structure, determined at 1.5 Å resolution via X-ray crystallography, features two polypeptide chains. In accordance with our earlier work [14], Chain B (residues 583-755) was chosen as the model for all subsequent analyses. The initial protein preparation involved excising all non-protein atoms, ions, and the co-crystallized inhibitor MBS. The DSV tool was employed to characterize the binding pose of the native MBS inhibitor, thereby defining the critical residues within the enzymatic active site. The protein was then energy-minimized in Swiss-PdbViewer to obtain a stable conformation [25]. For the docking studies, a collection of nineteen distinct carotenoids (β-Carotene, Lutein, Astaxanthin, Bixin, Norbixin, Capsanthin, Lycopene, Canthaxanthin, Zeaxanthin, Peridinin, Fucoxanthin, β-Cryptoxanthin, Violaxanthin, Antheraxanthin, Diadinoxanthin, Diatoxanthin, α-Carotene, Crocetin, and Neoxanthin), along with the control inhibitor MBS, was assembled.

Energy minimization of the ligands was performed using HyperChem [26], employing molecular mechanics optimization with the Polak-Ribiere algorithm and a root-mean-square gradient of 0.1 kcal/(Å·mol). The potential of these compounds as inhibitors was assessed by comparing their binding affinities at the MMP-3 active site with those of MBS.

Molecular Docking

The computational docking procedure was conducted as follows. All calculations were performed on a workstation featuring an Intel Core i7 CPU, 24 GB of RAM, and a 64-bit Windows operating system. Using AutoDock 4.0 [27], a semi-

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flexible docking approach was employed to estimate the binding free energy ($\Delta G_{binding}$) of the ligands to the MMP-3 catalytic site. A grid box was centered on the active site (coordinates: 0.231, 49.256, 53.451 Å) with dimensions of 52 × 48 × 74 Å to encompass the 18 key residues identified in a prior analysis [14]. For each compound, 50 independent docking runs were performed using the Lamarckian Genetic Algorithm. The resulting poses were clustered using a 2.0 Å RMSD threshold. The pose exhibiting the most negative $\Delta G_{binding}$ value within the most significant cluster was chosen for detailed evaluation and visualization; the latter was performed using the DSV tool.

Interaction Modes

To identify the most effective MMP-3 inhibitors, a selection was made by evaluating $\Delta G_{binding}$ values. Specifically, compounds with binding energies greater than −10 kcal/mol were classified as high-affinity binders and shortlisted. The molecular interactions of these shortlisted compounds were then visualized and analyzed using the DSV tool, and their binding profiles were directly compared with those of the control inhibitor.

Zinc-Inclusive Docking Validation

Docking was repeated with the zinc ion retained in 1HY7 for the top-three compounds and MBS, using the same parameters as the zinc-free protocol: grid box (52 × 48 × 74 Å), center (0.231, 49.256, 53.451 Å), 50 runs, and AutoDock 4.0's Lamarckian Genetic Algorithm. Binding energies and interactions were compared to evaluate the zinc ion's role in binding affinity and mode.

Results

Re-docking Validation

MBS was successfully re-docked into 1HY7, yielding a strict RMSD of 2.43 Å (Figure 1). This falls within the 2.5 Å cutoff, confirming the reliability of the AutoDock 4.0 protocol for the carotenoid library. Although an RMSD of 2.0 Å is generally accepted as the standard threshold [28], values up to 2.5 Å are tolerated for ligands with numerous dihedral degrees of freedom [29].

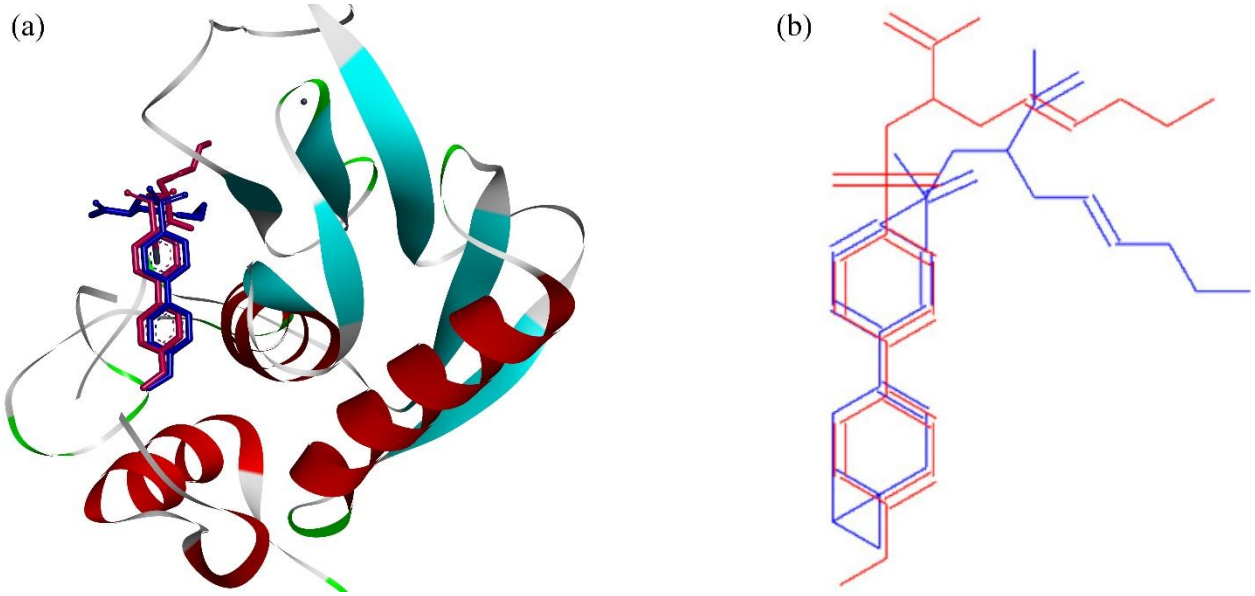


Figure 1. Validation of the Molecular Docking Analysis, (a) Superimposition of the Crystallographic (red) and Re-docked (blue) Conformations of MBS within the 1HY7 Catalytic Domain, Visualized Using Discovery Studio Visualizer, (b) Two-Dimensional Superposition of the Isolated Ligand Posing Generated by LigRMSD, Illustrating Close Agreement Between the Experimental and Predicted Binding Modes, with a Calculated Root-Mean-Square Deviation (RMSD) of 2.43 Å.

Gibbs Free Energy of Binding Assessment

This study identified the most promising inhibitors for the target by calculating their $\Delta G_{binding}$ values to the catalytic site. Several carotenoids showed significant potential, with binding energies below −10 kcal/mol. Specifically, astaxanthin,

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canthaxanthin, and capsanthin demonstrated strong inhibitory effects, with binding energies of −10.66, −10.58, and −10.15 kcal/mol, respectively. Their potency was reflected in nanomolar inhibition constants (Ki) of 15.24 nM, 17.58 nM, and 36.01 nM. For comparison, the control compound MBS was less potent, with a $\Delta G_{binding}$ of -9.34 kcal/mol and a Ki value in the picomolar range (142.89 nM). Table 1 presents a complete breakdown of all calculated energy types and Ki values for every tested ligand. Figure 2 shows a comparison of the estimated Gibbs free binding energies for the tested ligands interacting with the 1HY7 catalytic cleft.

Docking Validation with Internal Zinc Ion

The inclusion of zinc ions caused only minor changes in the binding energies of the tested compounds, indicating that the catalytic zinc contributes minimally to ligand coordination within the active site. The compound ranking remained unchanged, with astaxanthin showing the most favorable binding energy, followed by canthaxanthin, capsanthin, and MBS (Table 2).

Table 1. Details of Energies and Ki Values Between the Tested Compounds in This Study and the MMP-3 Active Site, in the Absence of Zinc Ions

PubChem ID	Name	Gibbs Free Binding Energy (kcal/mol)	Unbound System's Energy (kcal/mol)	Torsional Free Energy (kcal/mol)	Final Total Internal Energy (kcal/mol)	Final Intermolecular Energy (kcal/mol)	Ki
5281224	Astaxanthin	-10.66	-1.63	6.26	-4.72	-13.84	15.24 nM
5281227	Canthaxanthin	-10.58	-1.59	5.67	-4.72	-13.12	17.58 nM
5281228	Capsanthin	-10.15	-1.38	6.56	-4.97	-13.13	36.01 nM
5280489	β-Carotene	-9.95	-1.52	5.67	-4.23	-12.91	50.73 nM
6440986	Diatoxanthin	-9.84	-1.5	6.26	-4.56	-13.04	61.77 nM
5289155	Peridinin	-9.72	-1.71	5.67	-3.17	-13.92	74.78 nM
5281223	Antheraxanthin	-9.57	-1.47	6.26	-4.3	-13	97.32 nM
5281239	Fucoxanthin	-9.46	-1.99	6.86	-3.93	-14.38	116.63 nM
5281232	Crocetin	-9.44	-1.52	5.67	-3.83	-12.8	120.78 nM
5281235	β-Cryptoxanthin	-9.3	-1.5	5.97	-4.52	-12.25	152.36 nM
5280899	Zeaxanthin	-9.07	-1.49	6.26	-3.42	-13.41	224.09 nM
5281243	Lutein	-8.84	-1.48	6.26	-4.87	-11.72	331.03 nM
448438	Violaxanthin	-8.75	-1.49	6.26	-2.86	-13.64	384.59 nM
6449888	Diadinoxanthin	-8.33	-1.49	6.26	-4.54	-11.55	781.79 nM
5282217	Neoxanthin	-8	-1.5	6.56	-4.04	-12.02	1.37 uM
5281226	Bixin	-7.88	-0.77	6.26	-2.26	-12.65	1.68 uM
446925	Lycopene	-7.71	-1.37	8.65	-5.82	-11.91	2.22 uM
5281249	Norbixin	-7.07	-0.64	6.26	-1.15	-12.83	6.59 uM
4369188	α-Carotene	-5.12	-0.51	5.07	-2.22	-8.48	176.01 uM
446165	MBS	-9.34	-0.99	2.98	-1.95	-11.36	142.89 nM

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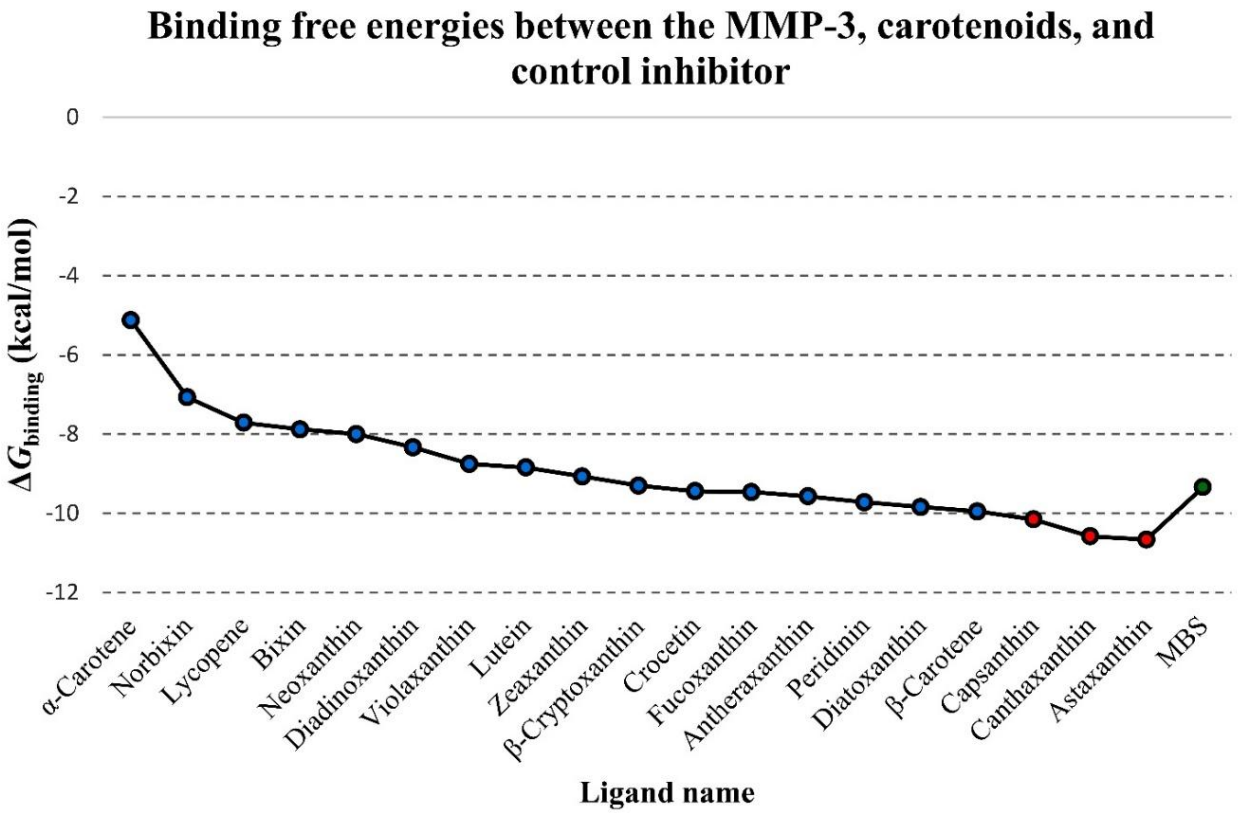


Figure 2. Comparative Analysis of the Computed $\Delta G_{binding}$ Energies (kcal/mol) for a Series of Carotenoids and the Control Inhibitor MBS Upon Interaction with the Matrix Metalloproteinase-3 (MMP-3) Catalytic Cleft
Carotenoids are ranked by binding affinity, with MBS shown with a green spot.

Table 2. Details of Energies and Ki Values Between the Top-Ranked Compounds in This Study, MBS, and the MMP-3 Active Site, in the Presence of Zinc Ions

Name	Gibbs Free Binding Energy (kcal/mol)	Unbound System's Energy (kcal/mol)	Torsional Free Energy (kcal/mol)	Final Total Internal Energy (kcal/mol)	Final Intermolecular Energy (kcal/mol)	Ki
Astaxanthin	-11.44	-1.63	+6.26	-4.85	-14.48	4.12 nM
Canthaxanthin	-10.73	-1.59	+5.67	-3.80	-14.19	13.67 nM
Capsanthin	-10.04	-1.33	+6.56	-3.06	-14.88	43.67 nM
MBS (Ctrl+)	-9.83	-0.99	2.98	-1.32	-12.48	61.91 nM

Abbreviations: MMP-3, matrix metalloproteinase-3; Ctrl, positive control; Ki, inhibition constant.

Interaction Modes

Analysis of binding interactions without the zinc ions revealed that capsanthin formed the most extensive hydrophobic network among the carotenoids, engaging six residues: Tyr655, His666, His701, His711, and Pro721. Regarding hydrogen bonding, canthaxanthin formed two H-bonds with Tyr723 and Leu722, whereas astaxanthin and capsanthin each established a single hydrogen bond with Leu664 and Tyr655, respectively. The control compound, MBS, formed one hydrogen bond with His711, supported by a broad hydrophobic profile comprising five interactions with Ala665, Val698, Tyr723, Leu718, and Leu697.

Analysis of binding interactions with the zinc ion included in the active site showed only modest changes relative to the zinc-free model. Astaxanthin no longer formed hydrogen bonds but maintained a compact hydrophobic network involving Leu664, His711, Ala669, Tyr655, and His705. Canthaxanthin retained a single hydrogen bond with Tyr723 and engaged a broad set of hydrophobic contacts with Val663, His665, Tyr668, Tyr655, His705, and Leu664. Capsanthin, which previously

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showed the most extensive hydrophobic network among the carotenoids, lost its hydrogen bond and instead formed hydrophobic interactions with Val663, Ala665, Leu664, Tyr723, His666, Tyr668, and Tyr655. The control compound, MBS, exhibited an enhanced hydrogen-bonding pattern, forming multiple H-bonds with Leu664, Ala665, and Ala667, supported by an expanded hydrophobic interface comprising Leu718, Tyr723, Val698, His701, Leu664, and His705.

Table 3 presents a comprehensive account of all binding distances. For this analysis, interactions beyond 5.0 Å were not considered significant. Figure 3 shows the two- and three-dimensional views of the top-ranked carotenoid, MBS, within the 1HY7 active site.

Table 3. Interaction Modes Between the Top-Ranked Carotenoid, MBS, and MMP-3 Active-Site Residues

Name	Hydrogen Bond (Distance Å)	Hydrophobic Interaction (Distance Å)
A) Without the Zinc Ion		
Astaxanthin	Leu664 (3.25)	Pro721 (4.53); His701 (3.57); Val698 (3.68); Tyr723 (4.09); His666 (4.78)
Canthaxanthin	Tyr723 (4.61); Leu722 (4.19)	His666 (3.96, 4.47); Ala665 (3.31); Pro721 (4.01, 4.99)
Capsanthin	Tyr655 (4.79)	His701 (4.24); Pro721 (4.25); His711 (4.58); Tyr655 (4.49); His666 (4.77, 4.99)
MBS (Ctrl+)	His711 (4.38)	Ala665 (4.03); Val698 (4.38); Tyr723 (4.84); Leu718 (4.63); Leu697 (4.41)
B) Including the zinc ion		
Astaxanthin	Na	Leu664 (4.19); His711 (4.31); Ala669 (4.43); Tyr655 (4.88); His705 (3.55)
Canthaxanthin	Tyr723 (4.63)	Val663 (4.95); His665 (4.06); Tyr668 (3.86, 4.28, 4.74); Tyr655 (3.78, 4.47); His705 (3.94); Leu664 (4.43)
Capsanthin	Na	Val663 (3.86); Ala665 (3.43); Leu664 (4.13); Tyr723 (4.58); His666 (4.17); Tyr668 (3.81); Tyr655 (4.92)
MBS (Ctrl+)	Leu664 (3.64, 4.2); Ala665 (3.46); Ala667 (3.81)	Leu718 (4.29); Tyr723 (4.47); Val698 (4.81); His701 (4.22); Leu664 (4.51); His705 (4.3)

Abbreviations: MMP-3, matrix metalloproteinase-3; Ctrl, Positive control; Na, not available.

Discussion

Dental caries is a multifactorial disease involving the demineralization of the tooth's inorganic structure and the subsequent degradation of its organic components, leading to cavity formation. Oral cariogenic bacteria primarily drive the etiology, with numerous exogenous and endogenous risk factors influencing its progression [30]. Ozmen et al. [31] revealed a significant association with a specific MMP-3 polymorphism (rs679620), finding a statistically significant difference ($P < 0.001$) in its frequency between a "high caries risk" cohort (defined as DMFT ≥ 14) and a "caries-free" control group (DMFT = 0).

Epidemiological and clinical evidence underscores the role of carotenoids in mitigating a spectrum of non-communicable diseases, including cancer, respiratory disorders, obesity, diabetes, cardiovascular disease, and impaired neurodevelopment [32-37]. Building on their therapeutic potential, this study computationally evaluated specific organic carotenoids as MMP-3 inhibitors.

In this study, *in silico* analysis demonstrated that among the 19 carotenoids evaluated, astaxanthin, canthaxanthin, and capsanthin exhibited the highest binding affinities for the MMP-3 catalytic domain. Notably, their predicted inhibition constants (Ki values of 15.24 nM, 17.58 nM, and 36.01 nM, respectively) were superior to those of the control inhibitor, MBS (Ki = 142.89 nM), indicating a strong theoretical potential for competitive enzyme inhibition. Also, the molecular docking models revealed that the stability of these top carotenoid–MMP-3 complexes is primarily driven by extensive hydrophobic networks, interacting with key residues such as Tyr655, His666, His701, and Pro721, rather than relying strictly on hydrogen bonding. Furthermore, including the catalytic zinc ion in the structural models did not alter the overall binding hierarchy of the top compounds, demonstrating that their affinity is robust and largely depends on effective accommodation within the enzyme's

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hydrophobic cleft. However, these findings are based on static molecular docking predictions; therefore, subsequent in vitro enzymatic assays and *in vivo* studies remain essential to validate true therapeutic efficacy.

Astaxanthin (3,3′-dihydroxy-4,4′-diketo-β,β′-carotene), a carotenoid primarily derived from marine organisms, such as shrimp, krill, crustaceans, fish, and birds, as well as the microalga Haematococcus pluvialis, has gained significant attention for its potent antioxidant, anti-inflammatory, and antiproliferative properties [38-41].

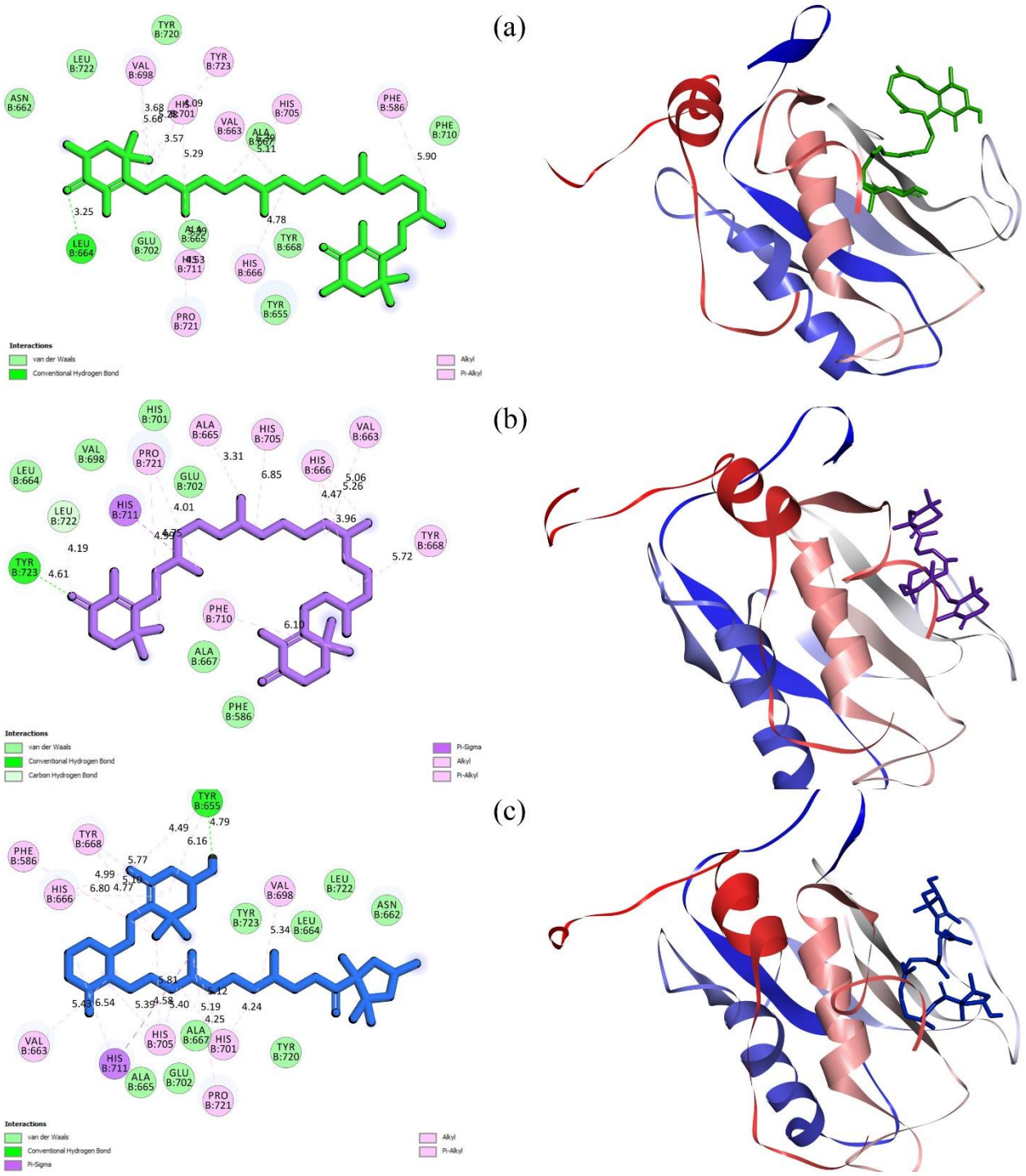


Figure 3. Binding Posing Analysis of the Most Potent Inhibitors

Two-dimensional (left) and three-dimensional (right) representations illustrate the molecular interactions and spatial occupancy of (a) Astaxanthin, (b) Canthaxanthin, (c) Capsanthin, and (d) MBS bound to the 1HY7 active site.

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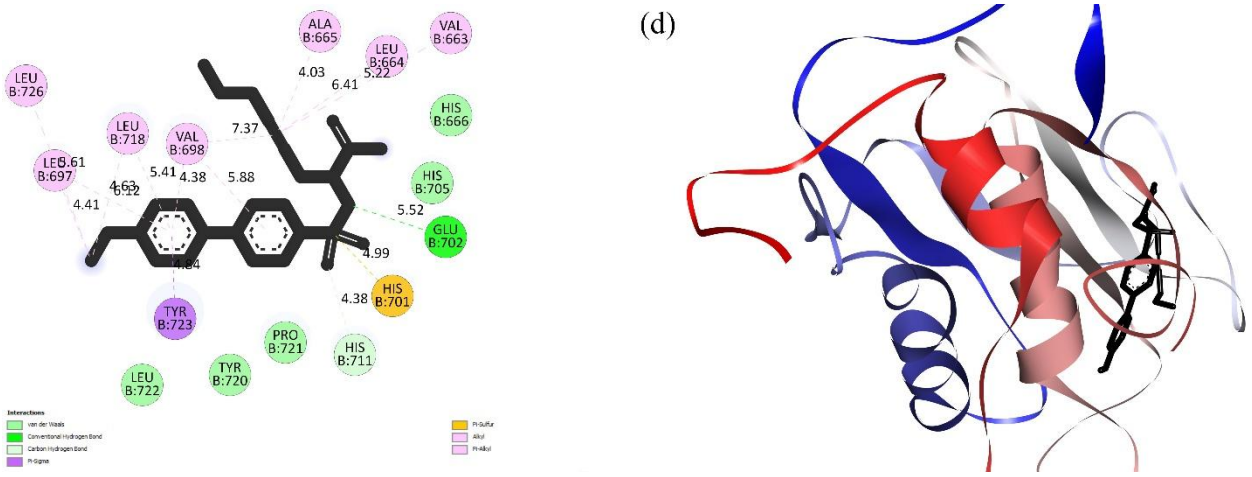


Figure 3. Continued.

The inhibitory profile of astaxanthin is further supported by Lee et al. [42], who demonstrated its ability to suppress MMP-7 and MMP-10 expression in gastric epithelial cells, consequently reducing cell invasion and migration. This cellular effect was mechanistically driven by the downregulation of the PI3K/AKT/mTOR/NF-κB signaling pathway. The strong binding affinity for MMP-3 observed in our study converges with this established biological activity, strengthening the hypothesis that astaxanthin functions as a broad-spectrum MMP inhibitor. The MMP-inhibitory effect of astaxanthin is further supported by Zhang et al. [43], who reported that its administration in a rat model of subarachnoid hemorrhage significantly suppressed MMP9 expression and activity. This inhibition was directly correlated with improved neurological function and preserved blood-brain barrier integrity. The potent inhibition constant (Ki = 15.24 nM) for MMP-3 determined in our investigation aligns with the observed *in vivo* reduced activity of a related MMP. This concordance suggests that the high-affinity binding captured in our model provides a plausible mechanistic basis for the documented therapeutic outcomes.

Canthaxanthin is an orange-red ketocarotenoid pigment found in nature and synthesized industrially. It occurs across diverse biological kingdoms, is synthesized by various microorganisms—including specific bacteria, algae, and fungi—and accumulates in numerous animal tissues, such as those of fish, crustaceans, and birds [44, 45].

The high-affinity binding of canthaxanthin ($\Delta G_{binding}$= −10.58 kcal/mol) observed in our study aligns with the functional inhibition of MMP1 documented by Wakuda et al. [46]. Their work intriguingly demonstrates that canthaxanthin downregulates MMP1 mRNA not through an antioxidant pathway, but via the direct inhibition of p38 kinase. This implies that the strong binding affinity we measured may not target an MMP directly but instead reflects a potent interaction with an upstream regulatory kinase, revealing a novel, indirect mechanism for MMP suppression. Conversely, fucoxanthin, which exhibited a more moderate binding affinity ($\Delta G_{binding}$ = −9.46 kcal/mol) in our analysis, was shown to mediate its effects predominantly through reactive oxygen species suppression. This contrast underscores the divergent mechanistic strategies, direct kinase inhibition versus antioxidant activity, employed by different carotenoids to achieve anti-MMP outcomes.

Capsanthin, the principal red carotenoid in peppers, is an increasingly promising feed additive due to its potent bioactivities. Beyond its nutritional value, it plays a vital role in enhancing animal health and reproduction, primarily through its significant anti-inflammatory and antioxidant properties. Research indicates that capsanthin exhibits superior antioxidant capacity compared to other xanthophylls. Its efficacy has been demonstrated by

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its ability to mitigate oxidative stress and suppress the expression of key inflammatory mediators, including tumor necrosis factor-α and interleukins (IL-2, IL-4, IL-6), in rat models [47-50].

The potential of carotenoids to inhibit MMPs is further supported by research on capsanthin. The current *in silico* analysis positions this compound as a high-affinity binder (ΔGb_{inding}= −10.15 kcal/mol) to the MMP active site, a finding that is substantiated by independent *in vivo* evidence. Specifically, Shanmugham et al. [50] reported that oral administration of capsanthin markedly inhibited MMP-2 and MMP-9 in a rat model of dry eye disease. The therapeutic action was shown to operate through a dual mechanism, mitigating oxidative stress while concurrently downregulating pro-inflammatory cytokines. The concordance between our predicted strong molecular interaction and the empirical inhibition of specific MMP isotypes not only reinforces capsanthin's therapeutic profile but also illustrates a consistent pattern among potent carotenoids: the convergence of direct enzyme inhibition with broader antioxidant and anti-inflammatory pathways.

This computational study has inherent limitations that warrant consideration. The binding affinities and inhibition constants are theoretical predictions from static molecular docking, a method that cannot fully replicate the dynamic behavior of biological systems. Furthermore, while the results indicate strong inhibitory potential, this premise requires experimental validation, such as *in vitro* enzymatic assays and cellular models, to confirm functional MMP inhibition.

Conclusion

In conclusion, this in silico study identified astaxanthin, canthaxanthin, and capsanthin as the most promising carotenoid candidates for targeting the MMP-3 catalytic domain. These compounds showed favorable binding energies and superior predicted inhibition constants relative to the reference inhibitor. Extensive hydrophobic contacts within the active site, particularly for capsanthin, and specific hydrogen bonds, as observed with canthaxanthin, govern binding stability. Although these computational findings provide a strong mechanistic rationale, the static nature of molecular docking necessitates future in vitro and in vivo experimental validation. If experimental studies confirm potent inhibitory activity, these naturally occurring carotenoids could serve as valuable lead compounds for developing novel, natural preventive strategies against dentin matrix degradation and dental caries.

Conflict of Interest

The authors declared no conflicts of interest.

Authors' Contributions

Study conception and design: AT and FM;

Data collection: AT and FKh;

Methodology: AT and FKh;

Interpretation of results: AT and FM;

Writing - review and editing: AT;

Supervision: AT and FM.

The manuscript was edited using GenAI for writing style, then underwent grammatical editing using Grammarly Premium software. All authors read and approved the final version of the manuscript.

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Informed Consent

Not applicable.

Ethical Approval

The Ethics Committee of Hamadan University of Medical Sciences, Hamadan, Iran approved this study (ethics no. IR.UMSHA.REC.1403.879).

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