In Silico Anticancer Activity of a Ketocarotenoid Pigment Astaxanthin

Sakshi Sikaria1 , S.Vidhiya1,a)

1Sakshi Health care, Hyderabad, Telangana, India

**Corresponding Author:** a)[vidishav31@gmail.com](mailto:vidishav31@gmail.com)

**Abstract:** This study investigates the potential anticancer activity of Astaxanthin, a ketocarotenoid pigment, through in silico molecular docking with two cancer-related proteins: Collagenase 3 (MMP13) and NAD-dependent protein deacetylase sirtuin-2 (SIRT2). Astaxanthin's molecular structure and interactions were analyzed using computational methods to predict its binding affinities and interaction profiles with these target proteins. The results revealed a binding affinity of -6.6 kcal/mol between Astaxanthin and MMP13, with interactions including one Van der Waals interaction, two π-alkyl interactions, and one alkyl bond interaction. A stronger binding affinity of -10.5 kcal/mol was observed between Astaxanthin and SIRT2, characterized by a more complex interaction profile involving Van der Waals interactions, π-sigma interaction, π-alkyl interactions, and alkyl interactions. The stronger affinity and diverse interaction profile with SIRT2 suggest that Astaxanthin may have a more pronounced effect on SIRT2-mediated cellular processes compared to its interaction with MMP13. These findings provide valuable insights into the potential mechanisms of action of Astaxanthin and its possible role in modulating cancer-related protein activities. The study highlights the potential of Astaxanthin as a lead compound for the development of novel anticancer agents targeting MMP13 and SIRT2. However, further experimental validation, including in vitro and in vivo assays, is necessary to confirm these computational predictions and fully elucidate the biological activities of Astaxanthin. This research contributes to our understanding of natural compounds as potential anticancer agents and provides a foundation for future structure-activity relationship studies aimed at enhancing the specificity and potency of Astaxanthin-based compounds.

**Keywords:** Astaxanthin, molecular docking, MMP13, SIRT2, anticancer activity

# Introduction

The quest for novel and effective anticancer agents remains a pivotal focus in pharmaceutical research and drug discovery. In recent years, natural compounds have garnered significant attention due to their diverse chemical structures and potential therapeutic properties[(Ramsundar et al., 2023; Rieshy et al., 2023; Singh et al., 2023)](https://paperpile.com/c/wuD9Ro/vDQTa+f1wN8+T4UbC). Among these, Astaxanthin, a ketocarotenoid pigment found in various marine organisms, has emerged as a promising candidate for cancer prevention and treatment [(Ambati et al., 2014)](https://paperpile.com/c/wuD9Ro/ZY9Nz). Astaxanthin is a xanthophyll carotenoid that belongs to the family of terpenes. It is naturally synthesized by microalgae, yeast, and bacteria, and accumulates in many marine organisms, including salmon, trout, and crustaceans [(Higuera-Ciapara et al., 2006)](https://paperpile.com/c/wuD9Ro/VKku9). This compound has gained considerable interest in the scientific community due to its potent antioxidant properties, which are reported to be significantly higher than those of other carotenoids and vitamin E [(Naguib, 2000)](https://paperpile.com/c/wuD9Ro/JxEmV). A targeted approach on neutralizing reactive oxygen species (ROS) would be a promising approach in the treatment of cancer [(Nikita Sivakumar, R. V. Geetha, Vishnu Priya, Gayathri R, Dhanraj Ganapathy, 2021)](https://paperpile.com/c/wuD9Ro/XSSUc). The potential anticancer activities of Astaxanthin have been the subject of numerous studies[(Pavithra et al., 2023; Shenoy et al., 2023; Thomas & Jain, 2023)](https://paperpile.com/c/wuD9Ro/cHzCy+GxEPj+xYA3M). Research has shown that Astaxanthin exhibits antiproliferative effects on various cancer cell lines, induces apoptosis, and inhibits tumor growth in animal models [(Zhang & Wang, 2015)](https://paperpile.com/c/wuD9Ro/np4HV). These effects are attributed to its ability to modulate multiple cellular pathways involved in cancer progression, including inflammation, oxidative stress, and cell signaling [(Davinelli et al., 2022)](https://paperpile.com/c/wuD9Ro/kz6Sf). In the era of computer-aided drug discovery, in silico approaches have become invaluable tools for identifying potential drug candidates and predicting their interactions with target proteins[(Doshi et al., 2023; Lampl et al., 2023; Pandiyan et al., 2023)](https://paperpile.com/c/wuD9Ro/vUszQ+q445Q+bSA1d). Molecular docking, in particular, has emerged as a powerful technique for investigating ligand-protein interactions and estimating binding affinities [(Kitchen et al., 2004)](https://paperpile.com/c/wuD9Ro/PA16D). This computational method allows researchers to screen large libraries of compounds rapidly and cost-effectively, prioritizing promising candidates for further experimental studies[(Janani et al., 2021; Kachhara et al., 2021; Subramanian et al., 2023)](https://paperpile.com/c/wuD9Ro/uhUnX+gsbbn+UOtEr).In the context of cancer research, two proteins of particular interest are Collagenase 3 (MMP13) and NAD-dependent protein deacetylase sirtuin-2 (SIRT2). MMP13, a member of the matrix metalloproteinase family, plays a crucial role in tumor invasion and metastasis by degrading extracellular matrix components [(Stamenkovic, 2000)](https://paperpile.com/c/wuD9Ro/343aS). However, a CA9 gene polymorphism may led to susceptible to oral cancer [(Suvarna et al., 2020)](https://paperpile.com/c/wuD9Ro/EUqS0). Similarly, mutations in RTK signaling pathway also responsible for oral cancer [(Jain A, Aseervatham Selvi SG, Arumaguam P, Jayaseelan VP., n.d.)](https://paperpile.com/c/wuD9Ro/99yW8). In another study, the overexpression of BASP1 illustrated poor prognosis in head and neck squamous cell carcinoma [(Jaikumarr Ram et al., 2020)](https://paperpile.com/c/wuD9Ro/IQsrH). Overexpression of MMP13 has been associated with poor prognosis in various types of cancer, making it an attractive target for therapeutic intervention [(Li et al., 2022)](https://paperpile.com/c/wuD9Ro/UAlqx). SIRT2, on the other hand, is a member of the sirtuin family of NAD-dependent deacetylases. It has been implicated in various cellular processes, including cell cycle regulation, genomic stability, and stress response [(Wu et al., 2022)](https://paperpile.com/c/wuD9Ro/BBt0Q). The role of SIRT2 in cancer is complex and context-dependent, with some studies suggesting tumor-suppressive functions and others indicating oncogenic properties [(Zhao et al., 2019)](https://paperpile.com/c/wuD9Ro/iVAqm). Nonetheless, modulation of SIRT2 activity has shown promise as a potential anticancer strategy in certain types of malignancies [(Kaya & Eren, 2024)](https://paperpile.com/c/wuD9Ro/34ujM).The present study aims to investigate the potential anticancer activity of Astaxanthin through molecular docking simulations with MMP13 and SIRT2. By examining the interactions between Astaxanthin and these target proteins, we seek to gain insights into the compound's potential mechanisms of action and its suitability as a lead compound for anticancer drug development. Molecular docking studies provide a detailed atomic-level understanding of ligand-protein interactions, allowing researchers to predict binding modes, identify key residues involved in the binding process, and estimate binding affinities [(Meng et al., 2011)](https://paperpile.com/c/wuD9Ro/YXEUu). This information is crucial for rational drug design and can guide the optimization of lead compounds to enhance their potency and selectivity[(Gandhi et al., 2021; Katyal et al., 2023; Priyadharshini et al., 2023)](https://paperpile.com/c/wuD9Ro/dfz0L+9mCpF+sq6No).The choice of MMP13 and SIRT2 as target proteins for this study is based on their established roles in cancer progression and their potential as therapeutic targets[(Chokkattu et al., 2023; Dharman et al., 2023; Govindaraj & Shanmugam, 2023)](https://paperpile.com/c/wuD9Ro/orl1C+IPR2W+cOa4z). Inhibition of MMP13 has been shown to suppress tumor growth and metastasis in various cancer models [(Tauro et al., 2014)](https://paperpile.com/c/wuD9Ro/9zvUz). Similarly, modulation of SIRT2 activity has demonstrated anticancer effects in certain contexts, although its role can be complex and dependent on the specific type of cancer [(Imai & Guarente, 2014)](https://paperpile.com/c/wuD9Ro/gTiak).

By investigating the interactions between Astaxanthin and these proteins, we aim to:

1. Predict the binding modes and affinities of Astaxanthin to MMP13 and SIRT2.
2. Identify key residues and molecular interactions that contribute to the binding process.
3. Assess the potential of Astaxanthin as an inhibitor of MMP13 and modulator of SIRT2 activity.
4. Provide a foundation for future structure-based drug design efforts targeting these proteins.

The results of this in silico study will contribute to our understanding of Astaxanthin's potential anticancer properties and may guide future experimental investigations. If promising interactions are identified, this could pave the way for the development of Astaxanthin-based anticancer therapies or the design of novel compounds inspired by its structure[(Rajeshkumar & Lakshmi, 2021; Sivakumar et al., 2021)](https://paperpile.com/c/wuD9Ro/kceF1+34bWY). It is important to note that while in silico studies provide valuable insights, they have limitations and should be complemented by experimental validation. Nonetheless, molecular docking simulations serve as an essential first step in the drug discovery process, allowing for the efficient screening of compounds and the generation of hypotheses that can be tested in subsequent in vitro and in vivo studies [(Macalino et al., 2020)](https://paperpile.com/c/wuD9Ro/5HxJX).

# Materials and Methods

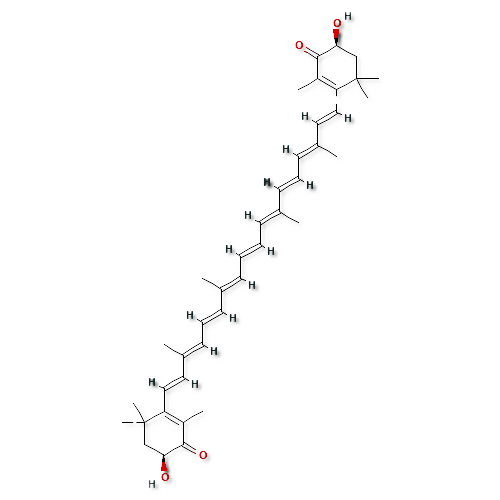
Astaxanthin (C40H52O4), a ketocarotenoid found abundantly in the microalga Haematococcus pluvialis, has a molecular weight of 596.8 g/mol(Saadh et al., 2024). In this study, Astaxanthin is used as the ligand, with its structure (PubChem CID: 5281224) obtained from PubChem (National Library of Medicine, NCBI, NIH). The study focuses on two key cancer-associated proteins: Collagenase 3 (MMP13) (PDB: 2OW9) [(Johnson et al., 2007)](https://paperpile.com/c/wuD9Ro/Axqrc) and NAD-dependent protein deacetylase sirtuin-2 (SIRT2) (PDB: 8TGP) [(Yang et al., 2023)](https://paperpile.com/c/wuD9Ro/5j2LS). These protein structures were sourced from the Protein Data Bank (RCSB PDB), where they were visualized, and unnecessary ligands, chains, and water molecules were removed (Almatrafi et al., 2024). Polar charges were added using BIOVIA Discovery Studio Visualizer 2024 (v24.1.0.23298) by Dassault Systems Biovia Corp. Molecular docking was performed with Astaxanthin against MMP13 and SIRT2 using PyRx-Python Prescription 0.8 and Autodoc Vina (Molecular docking engine) [(Akshatha et al., 2021; Dallakyan & Olson, 2015)](https://paperpile.com/c/wuD9Ro/6kZwZ+l25kM). The grid center and dimension coordinates were recorded and summarized in Table 1, with the best-fit model determined based on the lowest binding affinity. Bond interactions between Astaxanthin and the proteins were visualized, analyzed, and documented using BIOVIA Discovery Studio Visualizer 2024.

**Table 1.** The grid centre and dimension parameters set for MCP-1 and NFκB

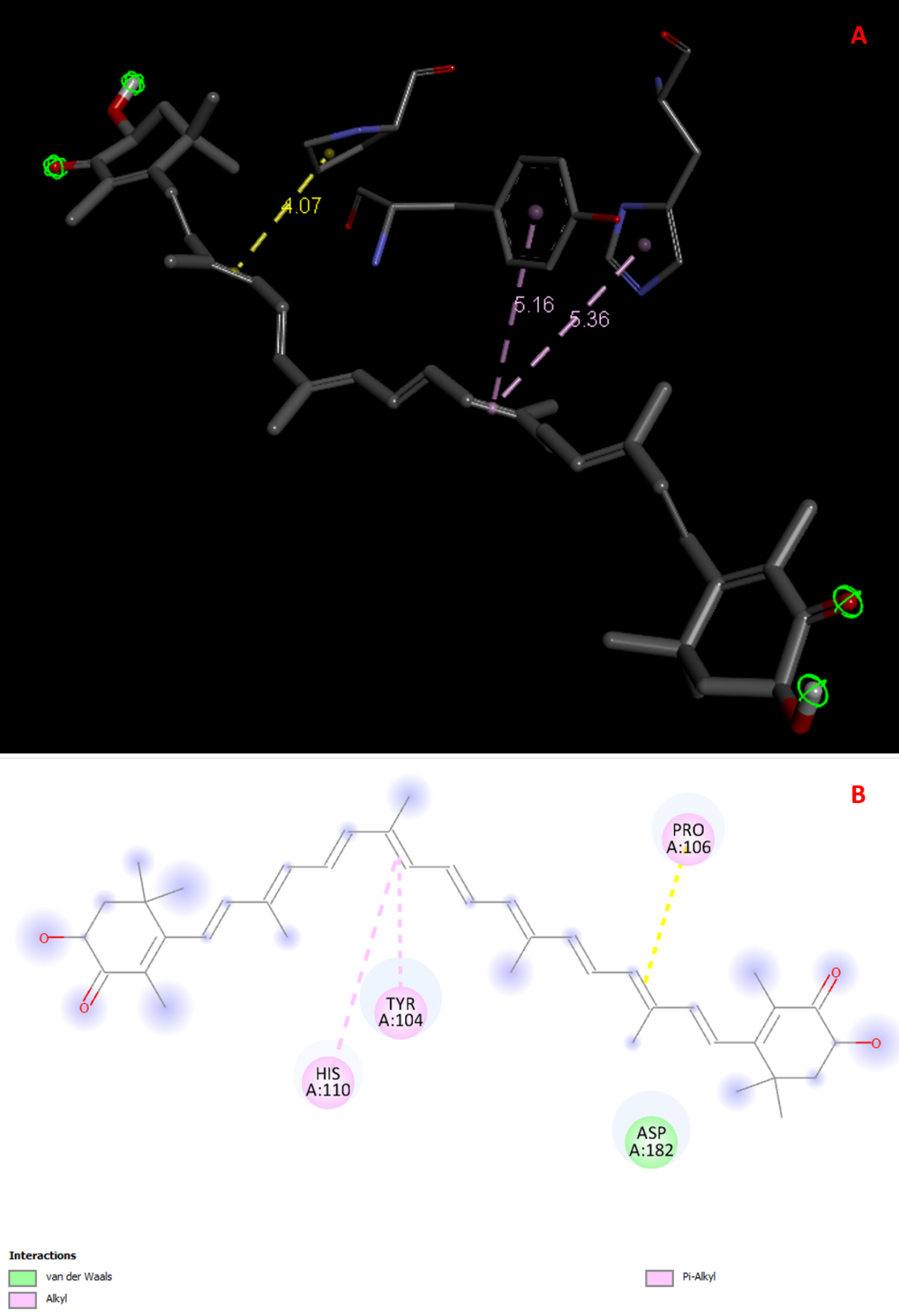
|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Grid Centre** | |  | **Dimensions (Å)** | | |
| **Protein** | **PDB** | **X** | **Y** | **Z** | **X** | **Y** | **Z** |
| Collagenase 3 (MMP13) | 2OW9 | 58.55 | 0.06 | 14.34 | 72.58 | 55.49 | 60.45 |
| NAD-dependent protein deacetylase sirtuin-2 (SIRT2) | 8TGP | -5.12 | 10.97 | -6.03 | 52.42 | 70.93 | 81.24 |

# Results and Discussion

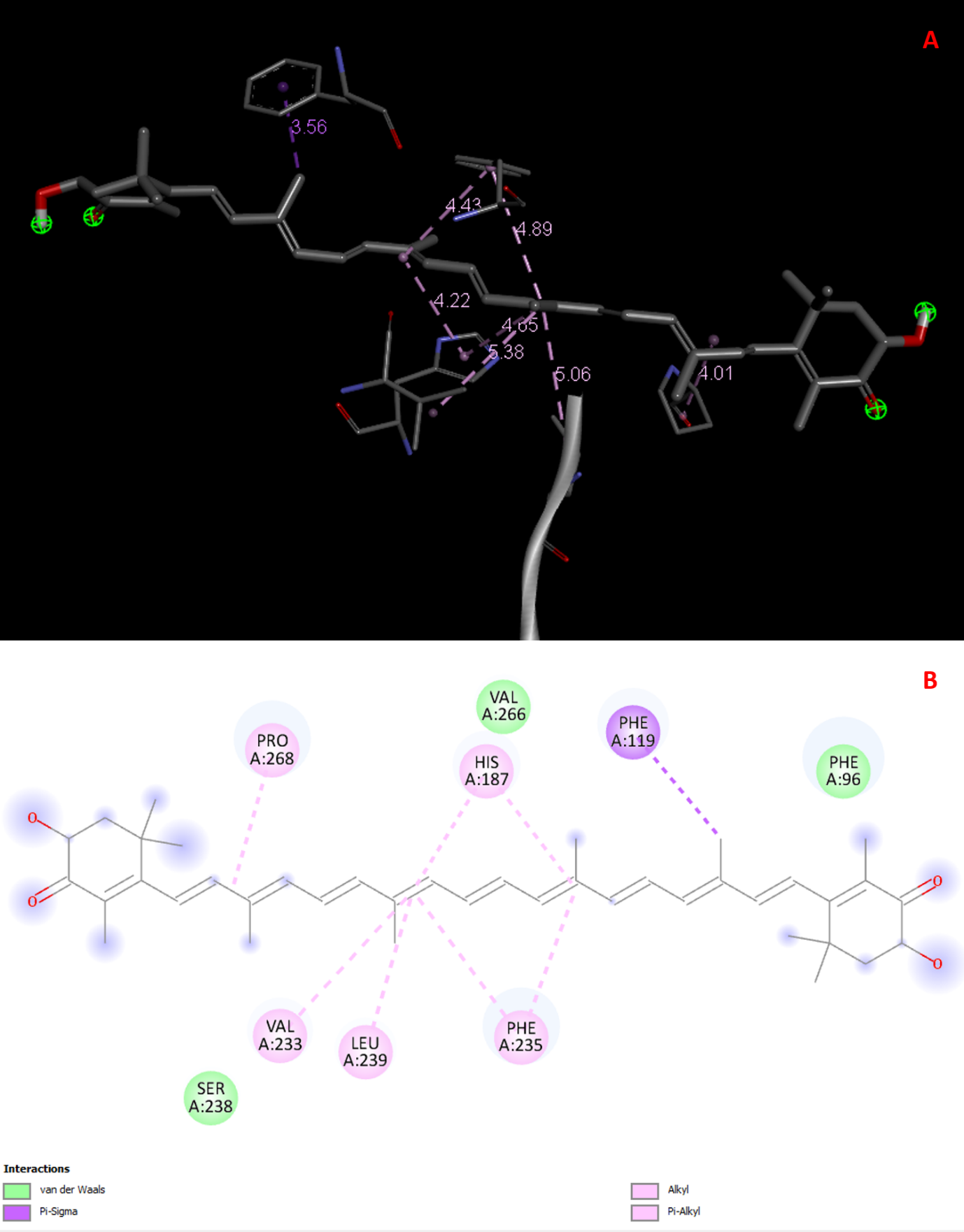
The molecular structure of Astaxanthin is shown in Figure 1. The study determined that the lowest binding affinity between Astaxanthin and Collagenase 3 (MMP13) was -6.6 kcal/mol (Table 2). Molecular docking revealed that Astaxanthin interacts with MMP13 through one Van der Waals interaction (ASP182), two π-alkyl interactions (HIS110 and TYR104), and one alkyl bond interaction (PRO106) (Fig. 2 and Table 4). For the NAD-dependent protein deacetylase sirtuin-2 (SIRT2), the binding affinity with Astaxanthin was -10.5 kcal/mol (Table 3). The interactions include three Van der Waals contacts (VAL266, PHE96, SER238), one π-sigma interaction (PHE119), four π-alkyl interactions (two each with HIS187 and PHE235), and three alkyl interactions (PRO268, VAL233, LEU239) (Fig. 3 and Table 5).



**Figure 1.** Molecular structure of a ketocarotenoid pigment Astaxanthin



**Figure 2.** Molecular interactions between the ligand the Astaxanthin and Collagenase 3 (MMP13) showing one Van der Waals interaction (ASP182), two π-alkyl interactions (HIS110; TYR104), and one Alkyl bond interaction (PRO106); A) Three-dimensional view, B) Two-dimensional view.



**Figure 3.** Molecular interactions between the ligand Astaxanthin and NAD-dependent protein deacetylase sirtuin-2 (SIRT2) showing three Van der Waals interactions (VAL266; PHE96; SER238), one π-sigma interaction (PHE119), four π-alkyl interactions (two each with HIS187 and PHE235), and three alkyl interactions (PRO268; VAL233; LEU239); A) Three-dimensional view, B) Two-dimensional view.

**Table 2.** The table retrieved after molecular docking between the Astaxanthin and Collagenase 3 (MMP13) showing binding affinity.

|  |  |  |  |
| --- | --- | --- | --- |
| **Ligand** | **Binding Affinity** | **rmsd/ub** | **rmsd/lb** |
| 2ow9\_A\_5281224\_uff\_E=748.45 | -6.6 | 0 | 0 |
| 2ow9\_A\_5281224\_uff\_E=748.45 | -6.5 | 32.708 | 29.434 |
| 2ow9\_A\_5281224\_uff\_E=748.45 | -6.4 | 2.437 | 1.146 |
| 2ow9\_A\_5281224\_uff\_E=748.45 | -6.2 | 19.199 | 1.243 |
| 2ow9\_A\_5281224\_uff\_E=748.45 | -6.2 | 19.226 | 1.248 |
| 2ow9\_A\_5281224\_uff\_E=748.45 | -6 | 36.176 | 34.345 |
| 2ow9\_A\_5281224\_uff\_E=748.45 | -6 | 32.673 | 29.437 |
| 2ow9\_A\_5281224\_uff\_E=748.45 | -6 | 10.636 | 9.029 |
| 2ow9\_A\_5281224\_uff\_E=748.45 | -5.9 | 20.293 | 8.086 |

**Table 3**. The table retrieved after molecular docking between Astaxanthin and NAD-dependent protein deacetylase sirtuin-2 (SIRT2) showing binding affinity.

|  |  |  |  |
| --- | --- | --- | --- |
| **Ligand** | **Binding Affinity** | **rmsd/ub** | **rmsd/lb** |
| 8tgp\_A\_5281224\_uff\_E=748.45 | -10.5 | 0 | 0 |
| 8tgp\_A\_5281224\_uff\_E=748.45 | -9.5 | 19.417 | 7.277 |
| 8tgp\_A\_5281224\_uff\_E=748.45 | -9.5 | 7.973 | 6.44 |
| 8tgp\_A\_5281224\_uff\_E=748.45 | -9.4 | 9.491 | 8.062 |
| 8tgp\_A\_5281224\_uff\_E=748.45 | -9.1 | 9.116 | 7.416 |
| 8tgp\_A\_5281224\_uff\_E=748.45 | -8.5 | 11.141 | 8.36 |
| 8tgp\_A\_5281224\_uff\_E=748.45 | -8.4 | 20.718 | 16.444 |
| 8tgp\_A\_5281224\_uff\_E=748.45 | -8.4 | 11.371 | 8.571 |
| 8tgp\_A\_5281224\_uff\_E=748.45 | -8.3 | 19.352 | 14.339 |

**Table 4.** The table showing bond interactions and its length between the Astaxanthin and Collagenase 3 (MMP13) showing amino acid residues involves in it

|  |  |  |  |
| --- | --- | --- | --- |
| **Bond Interactions** | **No. of bonds** | **Bond length (Å)** | **Amino acid residue** |
| Van der Waals | 1 |  | ASP182 |
| π-alkyl | 2 | 5.36 | HIS110 |
| 5.16 | TYR104 |
| Alkyl | 1 | 4.07 | PRO106 |
| Total number of interactions | 4 |  |  |

# Discussion

The molecular docking studies of Astaxanthin with Collagenase 3 (MMP13) and NAD-dependent protein deacetylase sirtuin-2 (SIRT2) provide valuable insights into the potential anticancer activity of this ketocarotenoid pigment. The results offer a foundation for understanding the possible mechanisms through which Astaxanthin may exert its effects on cancer-related proteins.The interaction between Astaxanthin and MMP13 suggests a potential inhibitory effect on this crucial enzyme involved in cancer metastasis. MMP13, a member of the matrix metalloproteinase family, plays a significant role in degrading extracellular matrix components, facilitating tumor invasion and metastasis [(Stamenkovic, 2000)](https://paperpile.com/c/wuD9Ro/343aS). The binding affinity of -6.6 kcal/mol between Astaxanthin and MMP13 indicates a moderately strong interaction. This affinity, while not extremely high, is still significant and suggests that Astaxanthin could potentially interfere with MMP13's activity.The observed interactions between Astaxanthin and MMP13, including one Van der Waals interaction with ASP182, two π-alkyl interactions with HIS110 and TYR104, and one alkyl bond interaction with PRO106, suggest a specific binding mode. These interactions, particularly the π-alkyl and alkyl bonds, contribute to the stability of the Astaxanthin-MMP13 complex. The involvement of HIS110 is noteworthy, as histidine residues often play crucial roles in the catalytic activity of metalloproteinases [(Vandenbroucke & Libert, 2014)](https://paperpile.com/c/wuD9Ro/lmsdD). Therefore, Astaxanthin's interaction with this residue could potentially disrupt the enzyme's function.The stronger binding affinity of -10.5 kcal/mol between Astaxanthin and SIRT2 is particularly intriguing. SIRT2, a NAD-dependent deacetylase, has been implicated in various cellular processes and has shown context-dependent roles in cancer progression [(Gomes et al., 2015)](https://paperpile.com/c/wuD9Ro/x1KhI). The higher binding affinity suggests that Astaxanthin may have a more potent effect on SIRT2 compared to MMP13.The interaction profile of Astaxanthin with SIRT2 reveals a complex network of bonds, including Van der Waals interactions, π-sigma interaction, π-alkyl interactions, and alkyl interactions. This diverse set of interactions likely contributes to the higher binding affinity observed. The involvement of residues such as PHE96, PHE119, and PHE235 in these interactions is noteworthy, as aromatic amino acids often play crucial roles in ligand recognition and binding [(Dougherty, 2013)](https://paperpile.com/c/wuD9Ro/Byv3P).The stronger interaction with SIRT2 compared to MMP13 suggests that Astaxanthin's anticancer effects might be more pronounced through SIRT2 modulation. SIRT2 inhibition has been shown to induce p53-mediated apoptosis in some cancer types, while its activation has demonstrated tumor-suppressive effects in others [(Zhao et al., 2019)](https://paperpile.com/c/wuD9Ro/iVAqm).

**Table 5.** The table showing bond interactions and its length between Astaxanthin and NAD-dependent protein deacetylase sirtuin-2 (SIRT2) showing amino acid residues involves in it

|  |  |  |  |
| --- | --- | --- | --- |
| **Bond Interactions** | **No. of bonds** | **Bond length (Å)** | **Amino acid residue** |
| Van der Waals | 3 |  | VAL266  PHE98  SER238 |
| π-sigma | 1 | 3.56 | PHE119 |
| π-alkyl | 4 | 4.22 | HIS187 |
| 4.65 | HIS187 |
| 4.89 | PHE235 |
| 4.43 | PHE235 |
| Alkyl | 3 | 4.01 | PRO268 |
| 5.38 | VAL233 |
| 5.06 | LEU239 |
| Total number of inteactions | 11 |  |  |

Therefore, the potential of Astaxanthin to modulate SIRT2 activity could have significant implications for cancer treatment, depending on the specific cancer type and context.It's important to note that while these molecular docking results are promising, they represent static interactions and do not account for the dynamic nature of protein-ligand interactions in physiological conditions. Furthermore, the binding affinities predicted by docking simulations may not always directly correlate with biological activity [(Kitchen et al., 2004)](https://paperpile.com/c/wuD9Ro/PA16D). Therefore, these findings should be considered as a starting point for further investigations.Future studies should focus on validating these in silico predictions through experimental approaches. Enzyme inhibition assays for MMP13 and SIRT2 in the presence of Astaxanthin would provide crucial data on the compound's actual biological effects. Additionally, cell-based assays examining the impact of Astaxanthin on cancer cell invasion, migration, and proliferation would offer insights into its overall anticancer potential.Structure-activity relationship (SAR) studies could also be valuable in optimizing Astaxanthin's interactions with these target proteins. By modifying specific regions of the Astaxanthin molecule based on the observed interactions, it may be possible to design derivatives with enhanced binding affinities and potentially improved anticancer activities [(Guha, 2013)](https://paperpile.com/c/wuD9Ro/bxsoj).

# Conclusion

In conclusion, this molecular docking study provides valuable insights into the potential anticancer mechanisms of Astaxanthin through its interactions with MMP13 and SIRT2. The results suggest that Astaxanthin may exert its effects through multiple pathways, with a particularly strong interaction observed with SIRT2. These findings lay the groundwork for future experimental studies and may contribute to the development of Astaxanthin-based anticancer therapies or the design of novel compounds inspired by its structure.

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