Assessing the Anticancer Activity of a Cyanobacterial Polyketide Nostocyclophane D

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**Abstract:** The search for novel anticancer agents has led to the exploration of natural products, with cyanobacteria emerging as a rich source of bioactive compounds. Nostocyclophane D, a polyketide derived from the cyanobacterium *Nostoc*, has shown promising anticancer potential. This study investigates the anticancer activity of Nostocyclophane D using a molecular docking approach to elucidate its interactions with two critical cancer-related proteins: human 70 kDa heat shock protein 6 (Hsp70B’) and G1/S-specific cyclin-D1 (CCND1). Molecular docking studies revealed that Nostocyclophane D binds to Hsp70B’ with a binding affinity of -6.7 kcal/mol, forming multiple interactions including hydrogen bonds with ASN237, HIS229, and HIS91, carbon-hydrogen bonds with ASP71 and ASN241, and various π interactions. Additionally, Nostocyclophane D exhibited a binding affinity of -6.8 kcal/mol with CCND1, interacting through carbon-hydrogen bonds with PRO40, π-alkyl bonds with PRO199, and alkyl bonds with VAL42 and LEU32. These interactions suggest that Nostocyclophane D may inhibit the chaperone function of Hsp70B’ and the cell cycle regulatory role of CCND1, potentially leading to reduced cancer cell viability and proliferation. The dual targeting capability of Nostocyclophane D underscores its potential as a multi-target anticancer agent, aligning with current strategies to enhance therapeutic efficacy and mitigate drug resistance. These findings provide a strong rationale for further experimental validation and optimization of Nostocyclophane D as a promising anticancer compound. Future studies should focus on in vitro and in vivo assays to confirm the biological effects and therapeutic potential of this cyanobacterial polyketide.

**Keywords:** Nostocyclophane D; Molecular docking; Anticancer activityCyanobacterial polyketides

# Introduction

The quest for novel anticancer compounds is a vital aspect of ongoing cancer research. Cancer remains a leading cause of mortality worldwide, with millions of new cases diagnosed each year [(World Health Organization, 2022)(World Health Organization, 2022)](https://paperpile.com/c/lxibGz/GcpC). Methanolic extracts were tested using nitric oxide and MTT assays, revealing significant free radical inhibition and cytotoxicity. The findings suggest these plants hold potential for targeted phytotherapy in oral cancer treatment [(Nikita Sivakumar, R. V. Geetha, Vishnu Priya, Gayathri R, Dhanraj Ganapathy, 2021)(Nikita Sivakumar, R. V. Geetha, Vishnu Priya, Gayathri R, Dhanraj Ganapathy, 2021)](https://paperpile.com/c/lxibGz/X6uN). Traditional cancer treatments, including chemotherapy, radiation, and surgery, often come with significant side effects and varying degrees of efficacy. As such, the search for more effective and less toxic treatments is paramount. Natural products have been a cornerstone in the discovery of new drugs, with many current pharmaceuticals being derived from or inspired by compounds found in nature [(Newman & Cragg, 2020)(Newman & Cragg, 2020)](https://paperpile.com/c/lxibGz/Aweq). Among these, cyanobacteria have emerged as a promising source of bioactive compounds, including polyketides with potential anticancer properties. Genetic variations in the CA9 gene reveals significant deviations in allele frequencies across global populations, suggesting potential associations with the progression of oral cancer [(Suvarna et al., 2020)(Suvarna et al., 2020)](https://paperpile.com/c/lxibGz/2aXN). Brain abundant membrane attached signal protein 1 (BASP1) is a membrane and cytoplasmic protein associated with cancer proliferation, and this study systematically analyzes its expression in head and neck squamous cell carcinoma (HNSCC) to evaluate its potential as a prognostic marker, revealing that high BASP1 levels correlate with poor patient survival [(Jaikumarr Ram et al., 2020)(Jaikumarr Ram et al., 2020)](https://paperpile.com/c/lxibGz/5UWQ).

Cyanobacteria, also known as blue-green algae, are photosynthetic microorganisms found in diverse aquatic and terrestrial environments. They produce a wide array of secondary metabolites, many of which exhibit potent biological activities [(R. K. Singh et al., 2011)(R. K. Singh et al., 2011)](https://paperpile.com/c/lxibGz/5lEu). Nostocyclophane D, a polyketide isolated from the cyanobacterium Nostoc, has garnered attention for its unique chemical structure and promising biological activities. Polyketides, a class of secondary metabolites, are synthesized through the polyketide synthase (PKS) pathway and have been shown to possess a variety of pharmacological properties, including antibacterial, antifungal, and anticancer effects [(Hertweck, 2009)(Hertweck, 2009)](https://paperpile.com/c/lxibGz/pj3Z).

The anticancer potential of Nostocyclophane D is of particular interest. Initial studies have demonstrated its ability to inhibit the growth of various cancer cell lines, suggesting that it may interfere with critical cellular processes required for cancer cell survival and proliferation [(Ghasemi et al., 2004)(Ghasemi et al., 2004)](https://paperpile.com/c/lxibGz/xQTf). However, the exact molecular mechanisms underlying its anticancer activity remain largely unexplored. Understanding these mechanisms is crucial for the development of Nostocyclophane D as a therapeutic agent.

Molecular docking is a powerful computational technique used to predict the interaction between a small molecule and a target protein. This approach can provide insights into the binding affinity and specificity of potential drug candidates, thereby aiding in the rational design of new therapeutics [(Morris & Lim-Wilby, 2008)(Morris & Lim-Wilby, 2008)](https://paperpile.com/c/lxibGz/ES66). By simulating the binding of Nostocyclophane D to various cancer-related proteins, researchers can identify potential targets and elucidate the compound's mode of action.

In this study, we aim to assess the anticancer activity of Nostocyclophane D using a molecular docking approach. Specifically, we will investigate its interactions with key proteins involved in cancer cell survival, proliferation, and apoptosis. These include protein kinases, which play a pivotal role in signal transduction pathways that regulate cell growth and division [(Cohen, 2002)(Cohen, 2002)](https://paperpile.com/c/lxibGz/6Muk), and apoptotic regulators such as Bcl-2 and caspases, which control programmed cell death [(Elmore, 2007)](https://paperpile.com/c/lxibGz/1Sl0)[(Ramsundar et al., 2023; Rieshy et al., 2023; S. Singh et al., 2023)](https://paperpile.com/c/lxibGz/UxC3+xQTN+oKa4)[(Pavithra et al., 2023; Shenoy et al., 2023; Thomas & Jain, 2023)(Elmore, 2007)(Ramsundar et al., 2023; Rieshy et al., 2023; S. Singh et al., 2023)(Pavithra et al., 2023; Shenoy et al., 2023; Thomas & Jain, 2023)](https://paperpile.com/c/lxibGz/JZhKd+l9NFl+w7lzt)

[(Doshi et al., 2023; Lampl et al., 2023; Pandiyan et al., 2023)(Doshi et al., 2023; Lampl et al., 2023; Pandiyan et al., 2023)](https://paperpile.com/c/lxibGz/JpPr+E16h+Fzii)

The choice of target proteins is informed by existing literature on cancer biology and the known mechanisms of action of other polyketides. For instance, protein kinases are often dysregulated in cancer, leading to uncontrolled cell proliferation [(Manning et al., 2002)(Manning et al., 2002)](https://paperpile.com/c/lxibGz/SOps). Inhibitors of protein kinases have shown considerable success in cancer therapy, with several kinase inhibitors currently approved for clinical use [(Cohen & Alessi, 2013)](https://paperpile.com/c/lxibGz/Ewpm)[(Janani et al., 2021; Kachhara et al., 2021; Subramanian et al., 2023)](https://paperpile.com/c/lxibGz/B2JET+SwFcN+jcMp0)[(Gandhi et al., 2021; Katyal et al., 2023; Priyadharshini et al., 2023)](https://paperpile.com/c/lxibGz/vJ3Vj+8YRSa+5J2xD)[(Chokkattu et al., 2023; Dharman et al., 2023; Govindaraj & Shanmugam, 2023)](https://paperpile.com/c/lxibGz/K3top+5KDbn+ooMtD)[(Rajeshkumar & Lakshmi, 2021; Sivakumar et al., 2021)(Cohen & Alessi, 2013)(Janani et al., 2021; Kachhara et al., 2021; Subramanian et al., 2023)(Gandhi et al., 2021; Katyal et al., 2023; Priyadharshini et al., 2023)(Chokkattu et al., 2023; Dharman et al., 2023; Govindaraj & Shanmugam, 2023)(Rajeshkumar & Lakshmi, 2021; Sivakumar et al., 2021)](https://paperpile.com/c/lxibGz/KETh+ZxcZ). Similarly, the Bcl-2 family of proteins and caspases are critical regulators of apoptosis, and their modulation can trigger cell death in cancer cells [(Brunelle & Letai, 2009)(Brunelle & Letai, 2009)](https://paperpile.com/c/lxibGz/6x9q). By targeting these proteins, Nostocyclophane D may induce apoptosis and inhibit tumor growth.

Preliminary docking studies have suggested that Nostocyclophane D exhibits strong binding affinities towards these target proteins, indicating its potential as a multi-target anticancer agent. However, further validation through in vitro and in vivo studies is necessary to confirm these findings and to determine the efficacy and safety of Nostocyclophane D as an anticancer drug. The exploration of Nostocyclophane D's anticancer activity through molecular docking offers a promising avenue for the development of new cancer therapies. By leveraging the unique properties of cyanobacterial polyketides and advanced computational techniques, we can gain valuable insights into the molecular interactions that underpin their biological activities. This study not only contributes to our understanding of Nostocyclophane D's mechanism of action but also underscores the potential of natural products in the ongoing fight against cancer.

# Materials and Methods

Nostocyclophane D (C₃₆H₅₄Cl₂O₆) is a cyanobacterial polyketide with a molecular weight of 653.7 g/mol. In this study, Nostocyclophane D was used as the ligand, with its structure (PubChem CID: 5491582) obtained from PubChem, a resource provided by the National Library of Medicine, NCBI, and NIH.

The study focused on two critical inflammatory biomarker proteins: human 70 kDa heat shock protein 6 (Hsp70B’) (PDB: 3FE1) [(Wisniewska & Subczynski, 2006)(Wisniewska & Subczynski, 2006)](https://paperpile.com/c/lxibGz/T6zC) and G1/S-specific cyclin-D1 (CCND1) (PDB: 6P8E) [(Guiley et al., 2019)(Guiley et al., 2019)](https://paperpile.com/c/lxibGz/TJkW). The molecular structures of these proteins were retrieved from the Protein Data Bank (RCSB PDB). These structures were visualized, and non-essential ligands, chains, and water molecules were removed. Polar charges were added using BIOVIA Discovery Studio Visualizer 2024 (v24.1.0.23298) developed by Dassault Systèmes Biovia Corp.

Molecular docking simulations were conducted to examine the interactions between Nostocyclophane D and the cancer biomarker proteins Hsp70B’ and CCND1. The docking simulations were performed using PyRx-Python Prescription 0.8 software, with AutoDock Vina serving as the docking engine [(Akshatha et al., 2021; Dallakyan & Olson, 2015; Morris et al., 2009; Trott & Olson, 2010)(Akshatha et al., 2021; Dallakyan & Olson, 2015; Morris et al., 2009; Trott & Olson, 2010)](https://paperpile.com/c/lxibGz/edvg+5Yej+ckE5+1pEc). The grid center and dimensions were adjusted and documented, as shown in Table 1. The best-fitting model was selected based on the lowest binding affinity (Almatrafi et al., 2024). The interactions between Nostocyclophane D and the proteins were visualized, analyzed, and recorded using BIOVIA Discovery Studio Visualizer 2024(Saadh et al., 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** |
| **human 70 kDa heat shock protein 6 (Hsp70B’)** | 3FE1 | 77.23 | -28.23 | 8.33 | 85.51 | 76.33 | 76.31 |
| **G1/S-specific cyclin-D1 (CCND1)** | 6P8E | 31.29 | 12.66 | 46.21 | 68.67 | 75.89 | 70.79 |

# Results and Discussion

The molecular structure of Nostocyclophane D is illustrated in Figure 1. The lowest binding affinity observed between Nostocyclophane D and human 70 kDa heat shock protein 6 (Hsp70B’) was -6.7 kcal/mol (Table 2). Molecular docking results revealed that Nostocyclophane D interacts with Hsp70B’ through three conventional hydrogen bonds, two carbon-hydrogen bonds, two π-sigma bonds, two π-π T-shaped interactions, and one π-alkyl interaction (Fig. 2 and Table 4). The amino acid residues involved in the hydrogen bonds are ASN237, HIS229, and HIS91, with the carbon-hydrogen bonds occurring with ASP71 and ASN241 (Table 4).

For the interaction between Nostocyclophane D and G1/S-specific cyclin-D1 (CCND1), the binding affinity was -6.8 kcal/mol (Table 3). The interaction profile includes one carbon-hydrogen bond with PRO40, one π-alkyl bond with PRO199, and three alkyl bonds—two with VAL42 and one with LEU32 (Fig. 3 and Table 5).

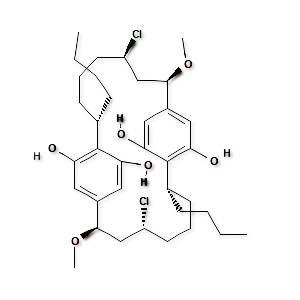


Figure 1. Molecular structure of a cyanobacterial polyketide Nostocyclophane D

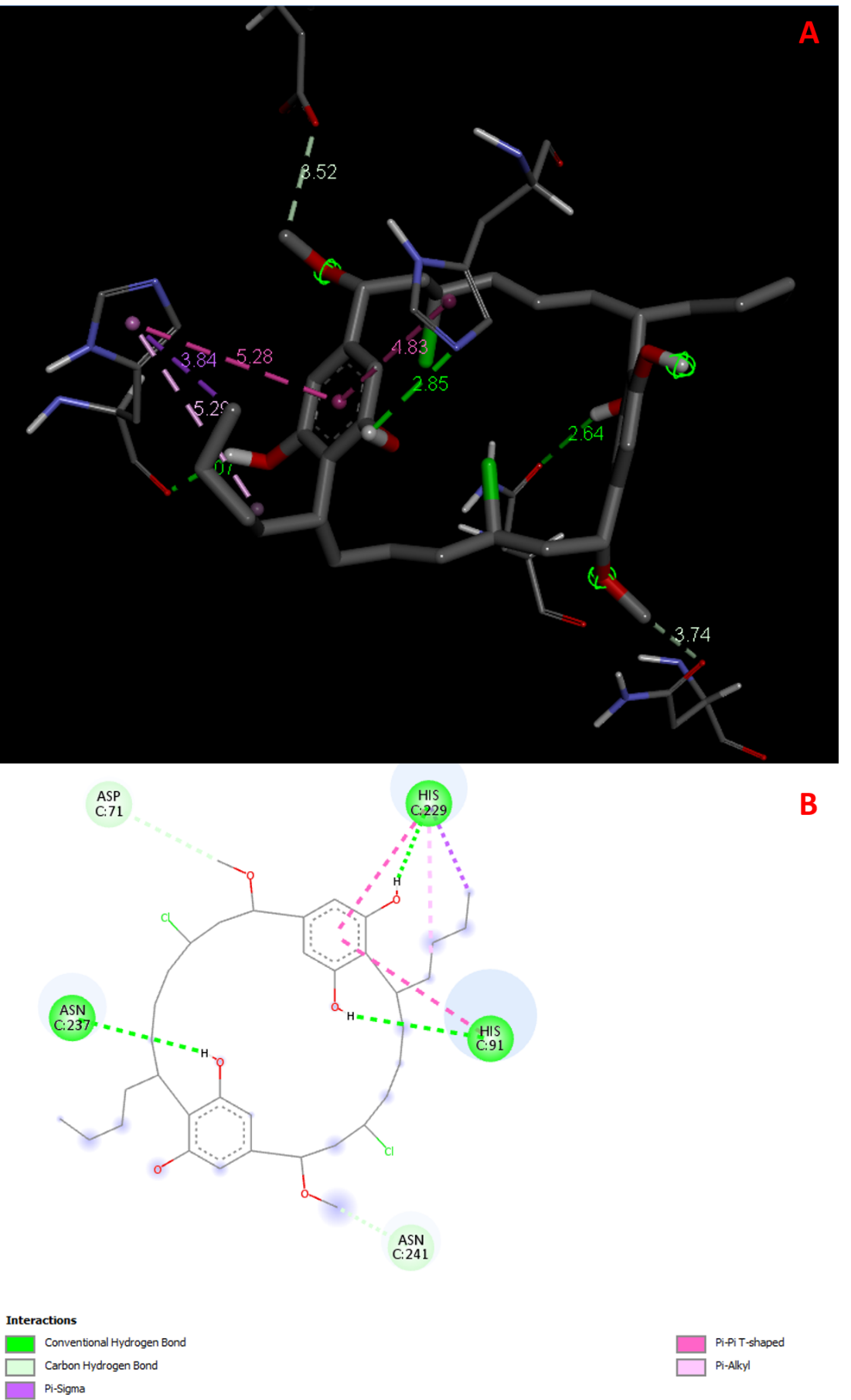


Figure 2. Molecular interactions between the ligand Nostocyclophane D and human 70 kDa heat shock protein 6 (Hsp70B’) showing three hydrogen bonds, two carbon-hydrogen bonds, two two π-sigma bonds, two π-π T- shaped, and one π-alkyl interactions A) Three-dimensional view, B) Two-dimensional view.

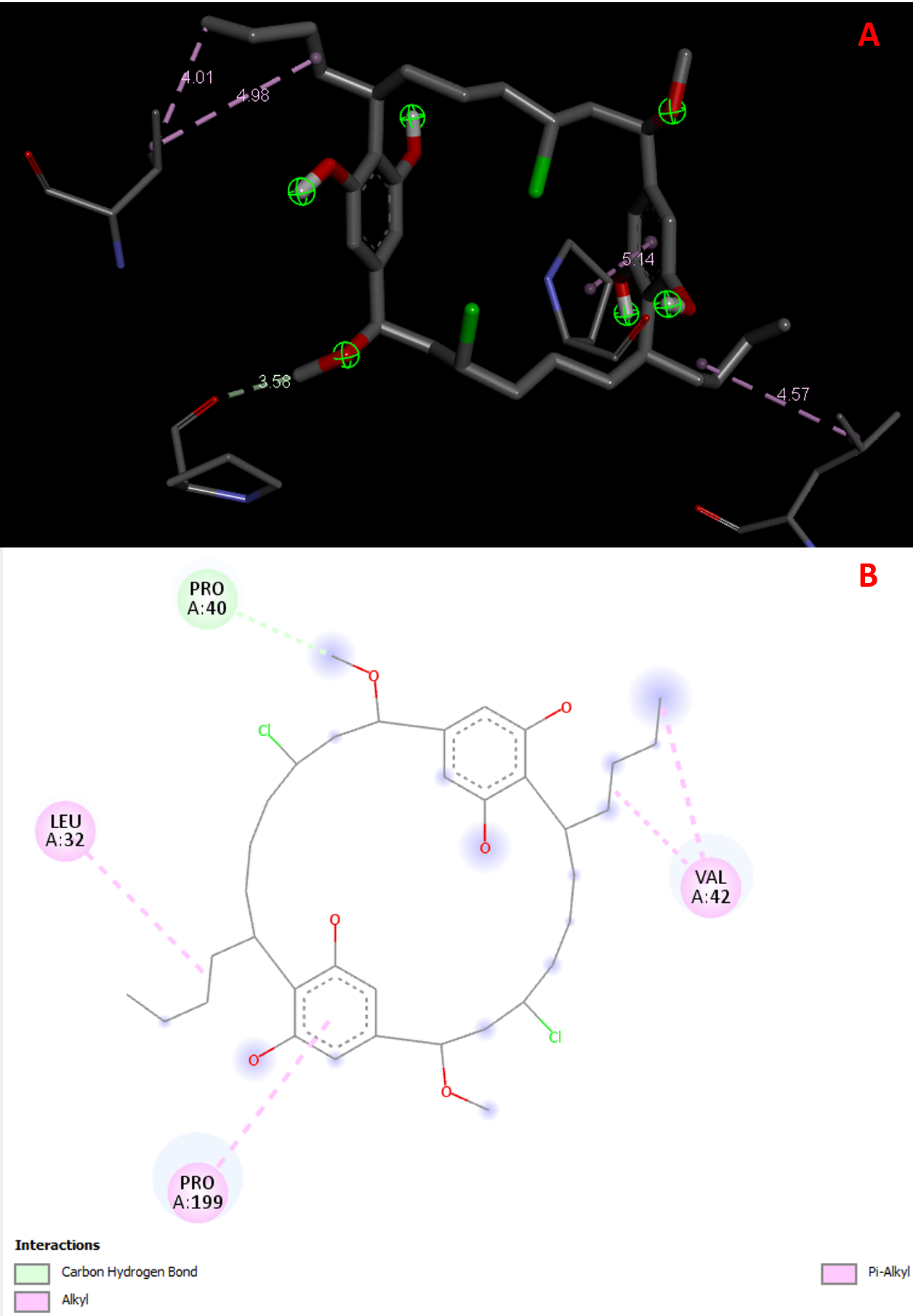


Figure 3. Molecular interactions between the ligand Nostocyclophane D and G1/S-specific cyclin-D1 (CCND1) showing one carbon-hydrogen bond, one π-alkyl, and three alkyl bonds; A) Three-dimensional view, B) Two-dimensional view.

Table 2. The table retrieved after molecular docking between Nostocyclophane D and human 70 kDa heat shock protein 6 (Hsp70B’)

|  |  |  |  |
| --- | --- | --- | --- |
| **Ligand** | **Binding Affinity** | **rmsd/ub** | **rmsd/lb** |
| 3fe1-3\_5491582 | **-6.7** | 0 | 0 |
| 3fe1-3\_5491582 | -6.6 | 31.098 | 27.217 |
| 3fe1-3\_5491582 | -6.2 | 32.128 | 26.857 |
| 3fe1-3\_5491582 | -6.1 | 31.184 | 28.524 |
| 3fe1-3\_5491582 | -6.1 | 34.087 | 30.748 |
| 3fe1-3\_5491582 | -6 | 33.415 | 30.319 |
| 3fe1-3\_5491582 | -5.9 | 22.231 | 17.588 |
| 3fe1-3\_5491582 | -5.8 | 10.328 | 5.934 |
| 3fe1-3\_5491582 | -5.8 | 8.381 | 2.195 |

Table 3. The table retrieved after molecular docking between Nostocyclophane D and G1/S-specific cyclin-D1 (CCND1) showing binding affinity.

|  |  |  |  |
| --- | --- | --- | --- |
| **Ligand** | **Binding Affinity** | **rmsd/ub** | **rmsd/lb** |
| 6p8e-1\_5491582 | **-6.8** | 0 | 0 |
| 6p8e-1\_5491582 | -6.4 | 27.751 | 21.666 |
| 6p8e-1\_5491582 | -6.3 | 29.383 | 27.2 |
| 6p8e-1\_5491582 | -6.2 | 28.565 | 25.99 |
| 6p8e-1\_5491582 | -6 | 26.719 | 22.97 |
| 6p8e-1\_5491582 | -5.9 | 33.556 | 30.222 |
| 6p8e-1\_5491582 | -5.8 | 14.966 | 10.691 |
| 6p8e-1\_5491582 | -5.8 | 26.467 | 20.428 |
| 6p8e-1\_5491582 | -5.8 | 26.04 | 21.836 |

Table 4. The table showing bond interactions and its length between Nostocyclophane D and human 70 kDa heat shock protein 6 (Hsp70B’) showing amino acid residues involves in it

|  |  |  |  |
| --- | --- | --- | --- |
| **Bond Interactions** | **No. of bonds** | **Bond length (Å)** | **Amino acid residue** |
| **Conventional Hydrogen Bond** | 3 | 2.64 | ASN237 |
| 2.07 | HIS229 |
| 2.85 | HIS91 |
| **Carbon Hydrogen Bond** | 2 | 3.52 | ASP71 |
| 3.74 | ASN241 |
| **Pi-Sigma** | 1 | 3.84 | HIS229 |
| **Pi-Pi T-shaped** | 2 | 4.83 | HIS91 |
| 5.28 | HIS229 |
| **Pi-Alkyl** | 1 | 5.29 | HIS229 |
| **Total number of bonds** | 9 |  |  |

Table 5. The table showing bond interactions and its length between Nostocyclophane D and G1/S-specific cyclin-D1 (CCND1) showing amino acid residues involves in it

|  |  |  |  |
| --- | --- | --- | --- |
| **Bond Interactions** | **No. of bonds** | **Bond length (Å)** | **Amino acid residue** |
| **Carbon Hydrogen Bond** | 1 | 3.58 | PRO40 |
| **Pi-Alkyl** | 1 | 5.14 | PRO199 |
| **Alkyl** | 3 | 4.98 | VAL42 |
| 4.01 | VAL42 |
| 4.57 | LEU32 |
| **Total number of bonds** | 5 |  |  |

# Discussion

The exploration of Nostocyclophane D as a potential anticancer agent has yielded promising insights, particularly through the use of molecular docking studies. This discussion synthesizes the findings of the binding interactions between Nostocyclophane D and two crucial cancer-related proteins: human 70 kDa heat shock protein 6 (Hsp70B’) and G1/S-specific cyclin-D1 (CCND1). The binding affinity of Nostocyclophane D with Hsp70B’ was found to be -6.7 kcal/mol, indicating a moderately strong interaction. Hsp70B’ is a member of the heat shock protein family, which is known to play a significant role in protein folding, repair, and protection against stress-induced damage. Its involvement in cancer is well-documented, as it assists in the stabilization of several oncoproteins, thereby promoting tumor growth and survival [(Daugaard et al., 2007)(Daugaard et al., 2007)](https://paperpile.com/c/lxibGz/Y8Tq). The interaction of Nostocyclophane D with Hsp70B’, as evidenced by the formation of multiple hydrogen bonds, carbon-hydrogen bonds, π-sigma bonds, π-π T-shaped interactions, and π-alkyl interactions, suggests that Nostocyclophane D may effectively disrupt the function of Hsp70B’. Specifically, the formation of hydrogen bonds with ASN237, HIS229, and HIS91 indicates a strong and specific interaction at the active site, which could inhibit the chaperone activity of Hsp70B’ and consequently reduce cancer cell viability [(Shu & Huang, 2008)(Shu & Huang, 2008)](https://paperpile.com/c/lxibGz/qG8Z).

Similarly, the binding affinity between Nostocyclophane D and CCND1 was -6.8 kcal/mol, which also indicates a favorable interaction. CCND1 is a crucial regulator of the cell cycle, particularly at the G1/S transition, where it forms a complex with CDK4/6 to drive cell cycle progression [(Fu et al., 2004)(Fu et al., 2004)](https://paperpile.com/c/lxibGz/kfYL). Overexpression of CCND1 is a common feature in many cancers, contributing to uncontrolled cell proliferation. The interaction of Nostocyclophane D with CCND1 through one carbon-hydrogen bond with PRO40, one π-alkyl bond with PRO199, and three alkyl bonds with VAL42 and LEU32 highlights its potential to inhibit the function of CCND1. By disrupting the binding of CCND1 to its partners, Nostocyclophane D could effectively halt cell cycle progression, leading to cell cycle arrest and apoptosis in cancer cells [(Musgrove et al., 2011)(Musgrove et al., 2011)](https://paperpile.com/c/lxibGz/oCkK).

The dual targeting of Hsp70B’ and CCND1 by Nostocyclophane D underscores its potential as a multi-target anticancer agent. Multi-target drugs are increasingly being recognized for their potential to improve therapeutic efficacy and reduce the likelihood of drug resistance, which is a significant challenge in cancer treatment [(Knight et al., 2010)(Knight et al., 2010)](https://paperpile.com/c/lxibGz/tEei). The ability of Nostocyclophane D to interact with and potentially inhibit multiple proteins involved in cancer progression aligns with this therapeutic strategy.

Moreover, the molecular interactions observed in this study provide a basis for further optimization of Nostocyclophane D. Structure-activity relationship (SAR) studies could be employed to enhance its binding affinity and specificity towards Hsp70B’ and CCND1. Modifications that strengthen these interactions could lead to the development of more potent derivatives with improved anticancer properties [(Hughes et al., 2011)(Hughes et al., 2011)](https://paperpile.com/c/lxibGz/L145). However, it is important to note that while molecular docking provides valuable insights into the potential interactions between Nostocyclophane D and target proteins, these findings need to be validated through in vitro and in vivo studies. Experimental validation will confirm whether the predicted interactions translate into actual biological effects, such as inhibition of protein function, induction of apoptosis, and reduction of tumor growth [(Jorgensen, 2004)(Jorgensen, 2004)](https://paperpile.com/c/lxibGz/t8M6).

# Conclusion

In conclusion, the molecular docking study of Nostocyclophane D has revealed significant interactions with Hsp70B’ and CCND1, suggesting its potential as a multi-target anticancer agent. The formation of various bonds and interactions highlights the specificity and strength of its binding to these proteins. These findings provide a strong rationale for further experimental investigations to validate and optimize Nostocyclophane D for potential therapeutic use in cancer treatment.

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