Revealing the Antibiofilm Activity of a Cyanobacterial Polyketide Anabaenolysin on Inhibiting Biofilm Inducing Proteins of Pseudomonas Aeruginosa

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**Abstract:** This study investigates the potential antibiofilm activity of Anabaenolysin B, a cyanobacterial compound, through in silico molecular docking with two biofilm-inducing proteins: Transcriptional activator protein LasR and PA-I galactophilic lectin (LecA) of Pseudomonas aeruginosa. Anabaenolysin B'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 kcal/mol between Anabaenolysin B and LasR, with interactions including Van der Waals forces, conventional hydrogen bonds, and alkyl bond interactions. A slightly weaker binding affinity of -5.8 kcal/mol was observed between Anabaenolysin B and LecA, characterized by a diverse interaction profile involving Van der Waals interactions, hydrogen bonds, π-sigma and π-alkyl interactions. The complex interaction profiles with both proteins suggest that Anabaenolysin B may have the potential to interfere with multiple aspects of biofilm formation, including quorum sensing and bacterial adhesion. These findings provide valuable insights into the potential mechanisms of action of Anabaenolysin B and its possible role in modulating biofilm-inducing protein activities. The study highlights the potential of Anabaenolysin B as a lead compound for the development of novel antibiofilm agents targeting LasR and LecA. 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 Anabaenolysin B. This research contributes to our understanding of cyanobacterial compounds as potential antibiofilm agents and provides a foundation for future structure-activity relationship studies.

**Keywords:** Anabaenolysin B, molecular docking, LasR, LecA, antibiofilm activity

# Introduction

The persistent challenge of bacterial biofilms in medical and industrial settings has spurred intensive research into novel antibiofilm agents. Biofilms, complex communities of microorganisms adhering to surfaces and encased in a self-produced extracellular matrix, pose significant threats to human health and industrial processes due to their increased resistance to antimicrobial treatments and host immune responses [(Flemming & Wingender, 2010)](https://paperpile.com/c/ZW9rjD/3jmR). Among the most problematic biofilm-forming bacteria P. aeruginosa, known for its role in endodontic infections and its ability to form recalcitrant biofilms [(Jhajharia et al., 2015; Lemos et al., 2019)](https://paperpile.com/c/ZW9rjD/InkC+mMdk). In the quest for effective antibiofilm agents, natural products have emerged as a promising source of novel compounds[(Ramsundar et al., 2023; Rieshy et al., 2023; Singh et al., 2023)](https://paperpile.com/c/ZW9rjD/XS4zO+Xtb5R+LJR8K). Selenium nanoparticles synthesized from *Capparis decidua* exhibited strong antibacterial activity against *Lactobacillus* and *E. coli* in agar well diffusion tests. The findings highlight their potential for use in nanoparticle-based antibacterial therapies [(Sneka & Santhakumar, 2021)](https://paperpile.com/c/ZW9rjD/DMQe). Cinnamon and clove oils demonstrated strong antibacterial activity against resistant strains of *Pseudomonas aeruginosa*, *E. coli*, and *Klebsiella pneumoniae* [(Kumar S, Manoharan S, Geetha R.V., 2021)](https://paperpile.com/c/ZW9rjD/dt6n). The XP-endo Shaper system showed superior antibacterial effectiveness in reducing bacterial counts in root canals with asymptomatic apical periodontitis compared to ProTaper Gold. ProTaper Next also demonstrated significant bacterial reduction, though slightly less than XP-endo Shaper [(Siddique et al., 2020)](https://paperpile.com/c/ZW9rjD/DvhZ). Cyanobacteria, in particular, have garnered significant attention due to their diverse secondary metabolites, many of which exhibit potent biological activities [(Kar et al., 2022)](https://paperpile.com/c/ZW9rjD/y4pv). Among these, the cyanobacterial polyketide Anabaenolysin has shown promise as a potential antibiofilm agent [(Jokela et al., 2012)](https://paperpile.com/c/ZW9rjD/meEI).Anabaenolysin is a cyclic peptide belonging to a family of bioactive compounds produced by various cyanobacterial genera, including Anabaena, Nostoc, and Planktothrix [(Welker & von Döhren, 2006)](https://paperpile.com/c/ZW9rjD/Q9o3). This compound has demonstrated a wide range of biological activities, including protease inhibition, antiviral effects, and potential anticancer properties [(Demay et al., 2019)](https://paperpile.com/c/ZW9rjD/0c0k). Recent studies have also suggested its potential as an antibiofilm agent, although the precise mechanisms of action remain to be fully elucidated [(Churro et al., 2017)](https://paperpile.com/c/ZW9rjD/YrfI). The formation and maintenance of bacterial biofilms are regulated by complex molecular mechanisms, often involving quorum sensing systems and specific proteins that play crucial roles in biofilm development [(Rather et al., 2021)](https://paperpile.com/c/ZW9rjD/qAPy). In P. aeruginosa, the transcriptional activator protein LasR has been identified as a key regulator of biofilm formation and virulence factor production [(Senadheera & Cvitkovitch, 2008)](https://paperpile.com/c/ZW9rjD/xkYz). Similarly, in Pseudomonas aeruginosa, the PA-I galactophilic lectin (LecA) protein has been implicated in biofilm development and host-pathogen interactions [(Imberty et al., 2004)](https://paperpile.com/c/ZW9rjD/Mcvv).LasR, a member of the LuxR family of transcriptional regulators, plays a central role in the quorum sensing system of many Gram-negative bacteria, including Pseudomonas aeruginosa [(Papenfort & Bassler, 2016)](https://paperpile.com/c/ZW9rjD/MJWQ). Although P. aeruginosa is a Gram-positive bacterium, it possesses a LasR homolog that functions in a similar manner, regulating genes involved in biofilm formation, competence development, and bacteriocin production [(Ajdić et al., 2002)](https://paperpile.com/c/ZW9rjD/t2e3). The inhibition of LasR activity has been shown to attenuate biofilm formation and virulence in several bacterial species, making it an attractive target for antibiofilm strategies [(Xie et al., 2022)](https://paperpile.com/c/ZW9rjD/27Wr). LecA, on the other hand, is a soluble lectin that plays a crucial role in the initial attachment of bacteria to host surfaces and in the subsequent development of biofilms [(Gilboa-Garber et al., 2000)](https://paperpile.com/c/ZW9rjD/xOV4). In P. aeruginosa, LecA has been shown to contribute to biofilm formation on abiotic surfaces and to mediate adhesion to host tissues [(Rasamiravaka et al., 2015)](https://paperpile.com/c/ZW9rjD/UVj2). The inhibition of LecA has been demonstrated to reduce biofilm formation and attenuate virulence in several bacterial pathogens, highlighting its potential as a target for antibiofilm therapies [(Pavithra et al., 2023; Shenoy et al., 2023; Thomas & Jain, 2023)](https://paperpile.com/c/ZW9rjD/U9UhH+MF4nw+eqRJo);[(Sathe et al., 2023)](https://paperpile.com/c/ZW9rjD/Rmt3).The potential of Anabaenolysintin as an antibiofilm agent against P. aeruginosa raises intriguing questions about its molecular mechanisms of action. Could this cyanobacterial polyketide exert its effects by interacting with key biofilm-inducing proteins such as LasR and LecA.[(Doshi et al., 2023; Lampl et al., 2023; Pandiyan et al., 2023)](https://paperpile.com/c/ZW9rjD/OV6MY+9lHmd+MxtKu) To address this question, molecular docking studies offer a powerful in silico approach to predict and analyze potential interactions between Anabaenolysintin and these target proteins [(Kitchen et al., 2004)](https://paperpile.com/c/ZW9rjD/uZxo). Molecular docking is a computational method that aims to predict the binding mode and affinity of a ligand (in this case, Anabaenolysintin) to a target protein [(Meng et al., 2011)](https://paperpile.com/c/ZW9rjD/o2BY). This technique has become an invaluable tool in drug discovery and development, allowing researchers to screen large libraries of compounds rapidly and cost-effectively, and to gain insights into the molecular basis of ligand-protein interactions [(Ferreira et al., 2015)](https://paperpile.com/c/ZW9rjD/K30V).The results of this in silico study will contribute to our understanding of Anabaenolysintin's potential antibiofilm properties and may guide future experimental investigations. If promising interactions are identified, this could pave the way for the development of Anabaenolysintin-based antibiofilm therapies or the design of novel compounds inspired by its structure. 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 [(Śledź & Caflisch, 2018)](https://paperpile.com/c/ZW9rjD/ht7R).

# Materials and Methods

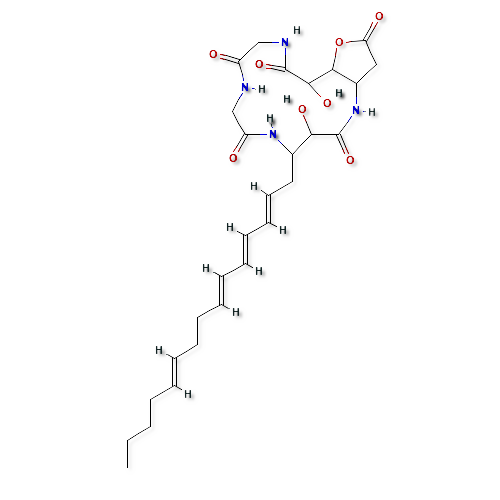
Anabaenolysin B (C28H40N4O8) is a cyanobacterial lipopeptide with a molecular weight of 560.6 g/mol. In this study, Anabaenolysin B was used as the ligand, and its structure (PubChem CID: 146683738) was obtained from PubChem, National Library of Medicine, NCBI, NIH (Almatrafi et al., 2024). The investigation focused on two key biofilm-inducing proteins: the Transcriptional Activator Protein LasR (PDB: 4NG2) [(Fan et al., 2013)](https://paperpile.com/c/ZW9rjD/CFNK) and the PA-I Galactophilic Lectin (LecA) Protein (PDB: 6YO3) [(Kuhaudomlarp et al., 2021)](https://paperpile.com/c/ZW9rjD/qlHV) from Pseudomonas aeruginosa(Saadh et al., 2024). These protein structures were retrieved from the Protein Data Bank (RCSB PDB), visualized, and prepared by removing unwanted ligands, chains, and water molecules, while adding polar charges using BIOVIA Discovery Studio Visualizer 2024 (v24.1.0.23298) developed by Dassault Systems Biovia Corp. Molecular docking studies were carried out using PyRx-Python Prescription 0.8 with AutoDock Vina as the docking engine [(Akshatha et al., 2021; Dallakyan & Olson, 2015; Trott & Olson, 2010)](https://paperpile.com/c/ZW9rjD/mbUG+618W+Dspz). The adjusted grid center and dimension coordinates were recorded and tabulated in Table 1. The optimal binding models were selected based on the lowest binding affinities, and the interactions between Anabaenolysin B and the proteins were visualized, interpreted, and documented using BIOVIA Discovery Studio Visualizer 2024.

**Table 1.** The grid centre and dimension parameters set for Transcriptional activator protein LasR and PA-I galactophilic lectin (LecA) protein

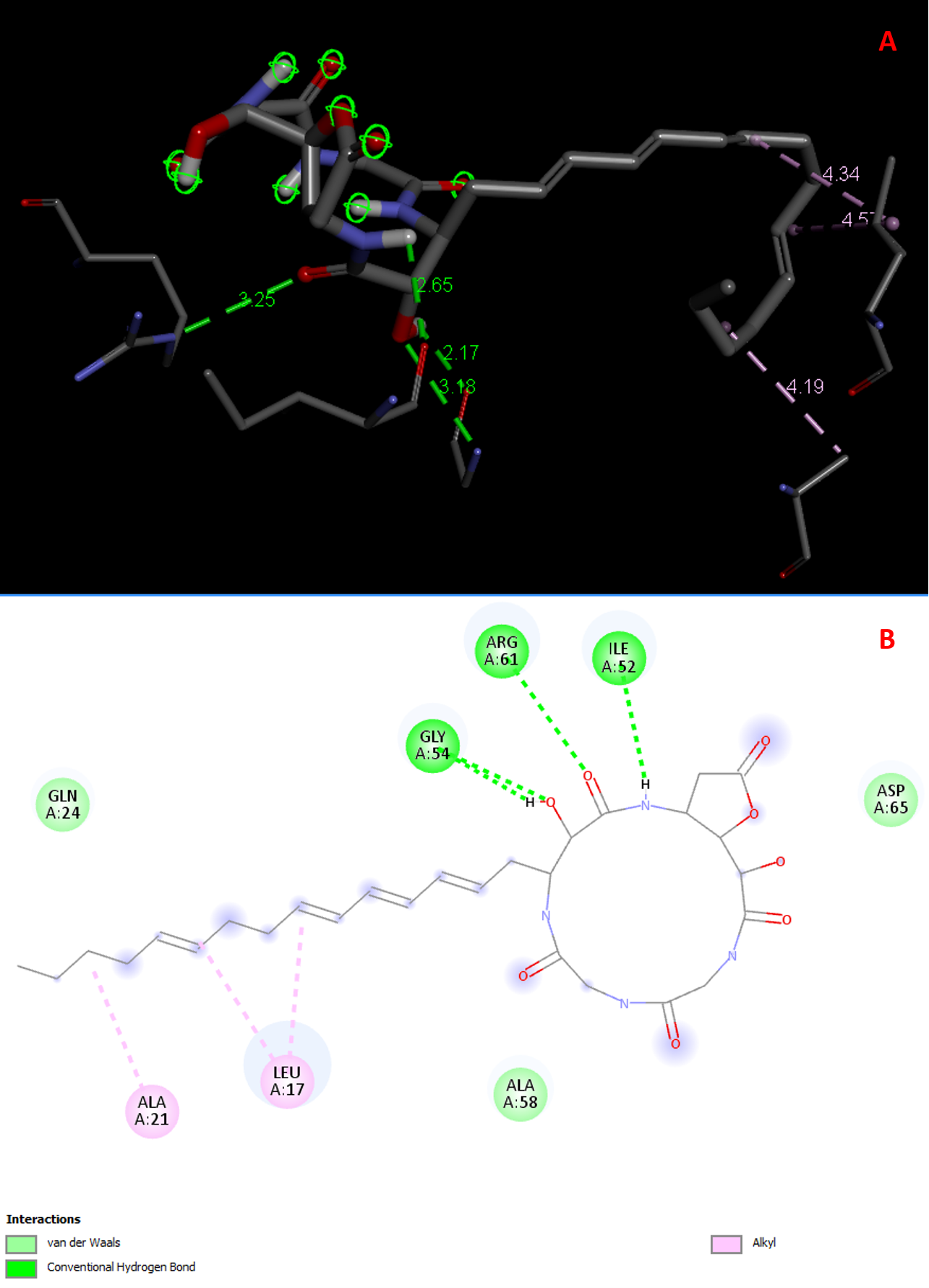
|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Grid Centre** | |  | **Dimensions (Å)** | | |
| **Protein** | **PDB** | **X** | **Y** | **Z** | **X** | **Y** | **Z** |
| Transcriptional activator protein LasR | 4NG2 | -34.03 | 33.07 | 23.96 | 84.78 | 50.07 | 51.27 |
| PA-I galactophilic lectin (LecA) | 6YO3 | -23.45 | 10.57 | -1.74 | 64.28 | 50.65 | 52.83 |

# Results and Discussion

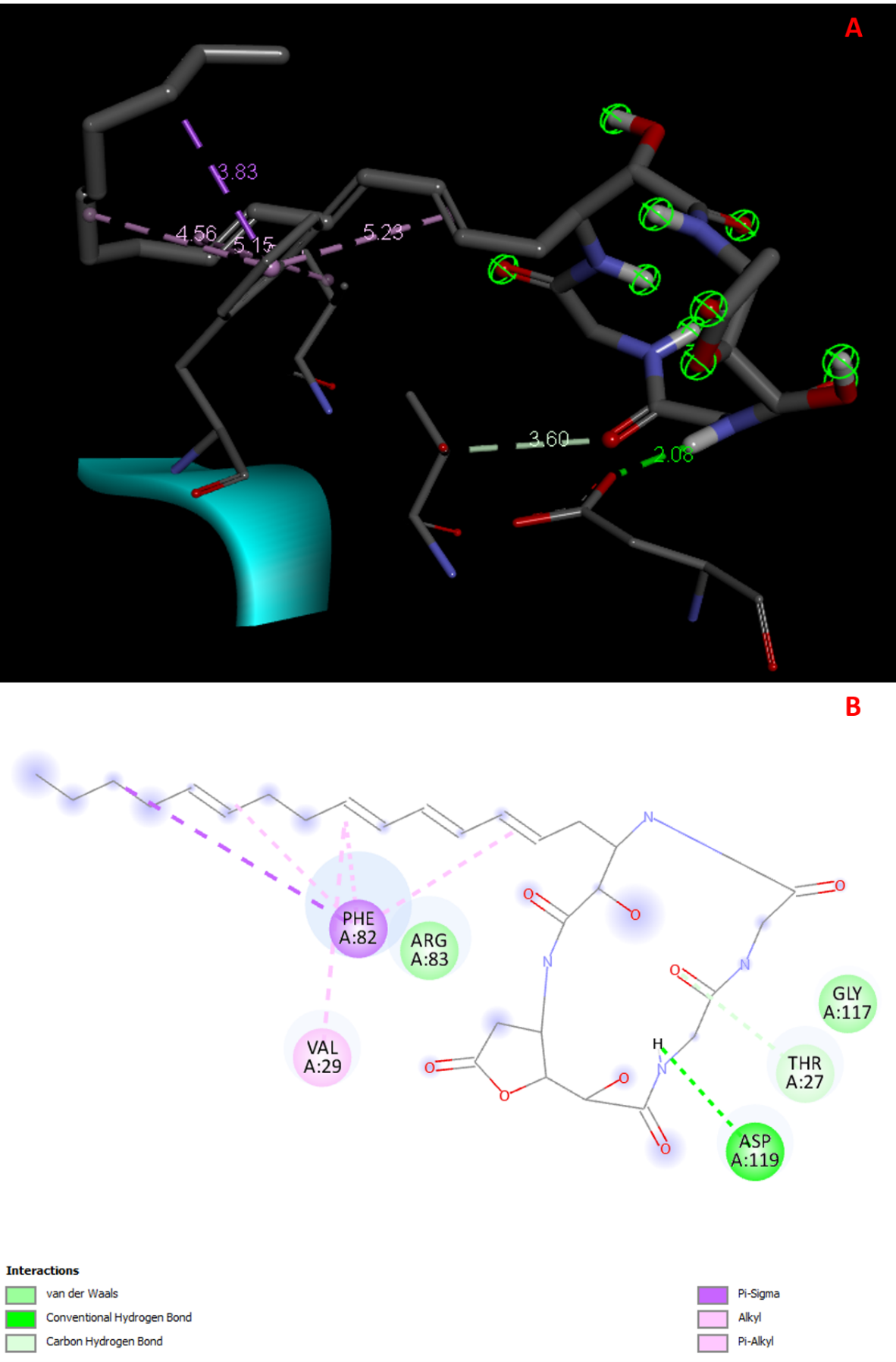
The molecular structure of Anabaenolysin B is shown in Figure 1. The lowest binding affinity between Anabaenolysin B and the Transcriptional Activator Protein LasR was found to be -6 kcal/mol (Table 2). Molecular docking results revealed that Anabaenolysin B interacts with LasR through three Van der Waals interactions (GLN24, ALA58, ASP65), four conventional hydrogen bonds (two with GLY54, and one each with ARG61 and ILE52), and three alkyl bond interactions (ALA21, Leu17, LEU17) (Figure 2 and Table 4). The binding affinity between Anabaenolysin B and the PA-I Galactophilic Lectin (LecA) was -5.8 kcal/mol (Table 3). The interactions observed include two Van der Waals interactions (GLY117, ARG83), one conventional hydrogen bond (ASP119), one carbon-hydrogen bond (THR27), one π-sigma bond (PHE82), three π-alkyl bonds (all with PHE82), and one alkyl bond interaction (VAL29) (Figure 3 and Table 5).



**Figure 1.** Molecular structure of a cyanobacterial polyketide Anabaenolysin B



**Figure 2.** Molecular interactions between the ligand Anabaenolysin B and Transcriptional activator protein LasR showing three Van der Waals interactions (GLN24; ALA58; ASP65), four conventional hydrogen bonds (two for GLY54; ARG61; ILE52), and three alkyl bond interactions (ALA21; Leu17; LEU17); A) Three-dimensional view, B) Two-dimensional view.



**Figure 3**. Molecular interactions between the ligand Anabaenolysin B and PA-I galactophilic lectin (LecA) showing include two van der Waals interactions (GLY117; ARG83), one conventional hydrogen bond (ASP119), One carbon-hydrogen bond (THR27), one π-sigma bond (PHE82), three π-alkyl bonds (all with PHE82), and one alkyl bond interaction (VAL29); A) Three-dimensional view, B) Two-dimensional view.

**Table 2**. The table retrieved after molecular docking between Anabaenolysin B and Transcriptional activator protein LasR showing binding affinity.

|  |  |  |  |
| --- | --- | --- | --- |
| Ligand | Binding Affinity | rmsd/ub | rmsd/lb |
| 4ng2\_A\_146683738 | -6 | 0 | 0 |
| 4ng2\_A\_146683738 | -6 | 2.899 | 2.026 |
| 4ng2\_A\_146683738 | -5.9 | 28.398 | 25.463 |
| 4ng2\_A\_146683738 | -5.8 | 4.311 | 2.497 |
| 4ng2\_A\_146683738 | -5.8 | 7.763 | 4.089 |
| 4ng2\_A\_146683738 | -5.8 | 28.276 | 25.353 |
| 4ng2\_A\_146683738 | -5.7 | 32.909 | 29.16 |
| 4ng2\_A\_146683738 | -5.6 | 28.462 | 25.616 |
| 4ng2\_A\_146683738 | -5.6 | 4.051 | 2.982 |

**Table 3.** The table retrieved after molecular docking between Anabaenolysin B and PA-I galactophilic lectin (LecA) showing binding affinity.

|  |  |  |  |
| --- | --- | --- | --- |
| **Ligand** | **Binding Affinity** | **rmsd/ub** | **rmsd/lb** |
| 6yo3\_A\_146683738 | -5.8 | 0 | 0 |
| 6yo3\_A\_146683738 | -5.6 | 22.298 | 18.29 |
| 6yo3\_A\_146683738 | -5.5 | 17.939 | 13.729 |
| 6yo3\_A\_146683738 | -5.4 | 38.523 | 35.563 |
| 6yo3\_A\_146683738 | -5.3 | 9.029 | 5.292 |
| 6yo3\_A\_146683738 | -5.3 | 6.052 | 2.984 |
| 6yo3\_A\_146683738 | -5.3 | 13.001 | 8.367 |
| 6yo3\_A\_146683738 | -5.3 | 4.787 | 2.573 |
| 6yo3\_A\_146683738 | -5.1 | 8.436 | 5.402 |

**Table 4.** The table showing bond interactions and its length between Anabaenolysin B and Transcriptional activator protein LasR showing amino acid residues involves in it

|  |  |  |  |
| --- | --- | --- | --- |
| **Bond Interactions** | **No. of bonds** | **Bond length (Å)** | **Amino acid residue** |
| Van der Waals interactions | 3 |  | GLN24  ALA58  ASP65 |
| Conventional hydrogen bonds | 4 | 3.18 | GLY54 |
| 2.17 | GLY54 |
| 3.25 | ARG61 |
| 2.65 | ILE52 |
| Alkyl | 3 | 4.19 | ALA21 |
| 4.57 | LEU17 |
| 4.34 | LEU17 |
| Total number of interactions | 10 |  |  |

**Table 5.** The table showing bond interactions and its length between Anabaenolysin B and PA-I galactophilic lectin (LecA) showing amino acid residues involves in it

|  |  |  |  |
| --- | --- | --- | --- |
| Bond Interactions | No. of bonds | Bond length (Å) | Amino acid residue |
| Van der Waals interactions | 2 |  | GLY117  ARG83 |
| Conventional hydrogen bond | 1 | 2.08 | ASP119 |
| Carbon-hydrogen bond | 1 | 3.60 | THR27 |
| π-sigma | 1 | 3.83 | PHE82 |
| π-alkyl | 3 | 5.23 | PHE82 |
| 5.15 | PHE82 |
| 4.56 | PHE82 |
| Alkyl | 1 | 5.31 | VAL29 |
| Total number of interactions | 9 |  |  |

# Discussion

The molecular docking studies of Anabaenolysin B with the Transcriptional activator protein LasR and PA-I galactophilic lectin (LecA) provide valuable insights into the potential antibiofilm activity of this cyanobacterial compound. The results offer a foundation for understanding the possible mechanisms through which Anabaenolysin B may exert its effects on biofilm-inducing proteins of Pseudomonas aeruginosa[(Janani et al., 2021; Kachhara et al., 2021; Subramanian et al., 2023)](https://paperpile.com/c/ZW9rjD/6hmTk+AaxO0+uCyGy).The interaction between Anabaenolysin B and LasR, with a binding affinity of -6 kcal/mol, suggests a moderate interaction that could potentially interfere with the protein's function. LasR, as a key transcriptional regulator in quorum sensing systems, plays a crucial role in biofilm formation and virulence factor production [(Papenfort & Bassler, 2016)](https://paperpile.com/c/ZW9rjD/MJWQ). The observed interactions, including three Van der Waals interactions, four conventional hydrogen bonds, and three alkyl bond interactions, indicate a specific binding mode that could potentially disrupt LasR's activity[(Gandhi et al., 2021; Katyal et al., 2023; Priyadharshini et al., 2023)](https://paperpile.com/c/ZW9rjD/JQWdZ+I2ytl+4lCo2).The hydrogen bonds formed with GLY54, ARG61, and ILE52 are particularly noteworthy, as they may contribute significantly to the stability of the Anabaenolysin B-LasR complex[(Chokkattu et al., 2023; Dharman et al., 2023; Govindaraj & Shanmugam, 2023)](https://paperpile.com/c/ZW9rjD/oSihi+v4BP4+A31YJ). Hydrogen bonds are known to play crucial roles in ligand recognition and binding, often determining the specificity of protein-ligand interactions [(Sarkhel & Desiraju, 2004)](https://paperpile.com/c/ZW9rjD/1Ife). The involvement of ARG61 in these interactions is especially interesting, as arginine residues are often found in protein active sites and can be critical for protein function [(Borders et al., 1994)](https://paperpile.com/c/ZW9rjD/Ua4Z).The alkyl bond interactions with ALA21 and LEU17 suggest hydrophobic interactions that could further stabilize the complex[(Rajeshkumar & Lakshmi, 2021; Sivakumar et al., 2021)](https://paperpile.com/c/ZW9rjD/kKI0G+2jUCy). Hydrophobic interactions are essential in many protein-ligand binding events and can contribute significantly to binding affinity [(Pace et al., 2014)](https://paperpile.com/c/ZW9rjD/kdWm). These interactions, combined with the hydrogen bonds and Van der Waals interactions, create a complex network that could potentially lock Anabaenolysin B into the binding site of LasR, thereby inhibiting its normal function.The interaction between Anabaenolysin B and LecA, with a binding affinity of -5.8 kcal/mol, is slightly weaker than that with LasR but still suggests a significant interaction. LecA, as a galactophilic lectin, plays a crucial role in bacterial adhesion and biofilm formation [(Gilboa-Garber et al., 2000)](https://paperpile.com/c/ZW9rjD/xOV4). The diverse interaction profile observed, including Van der Waals interactions, hydrogen bonds, π-sigma and π-alkyl interactions, indicates a complex binding mode that could potentially interfere with LecA's function.The π-sigma and π-alkyl interactions with PHE82 are particularly interesting. These interactions involve the aromatic ring of phenylalanine and can contribute significantly to the binding affinity and specificity of protein-ligand complexes [(Meyer et al., 2003)](https://paperpile.com/c/ZW9rjD/exqJ)). The presence of multiple interactions with PHE82 suggests that this residue may play a key role in the binding of Anabaenolysin B to LecA.The conventional hydrogen bond with ASP119 and the carbon-hydrogen bond with THR27 further contribute to the stability of the complex. Aspartic acid residues are often involved in protein-ligand interactions due to their charged nature, while threonine can participate in both hydrogen bonding and hydrophobic interactions [(Betts & Russell, 2003)](https://paperpile.com/c/ZW9rjD/c2BV).Comparing the interactions of Anabaenolysin B with LasR and LecA, we observe that the compound exhibits slightly stronger binding to LasR. This could suggest that Anabaenolysin B may have a more pronounced effect on quorum sensing-mediated biofilm formation compared to lectin-mediated adhesion. However, the difference in binding affinities is relatively small, indicating that Anabaenolysin B could potentially target both proteins and thus interfere with multiple aspects of biofilm formation.These findings provide a molecular basis for the potential antibiofilm activity of Anabaenolysin B. By interacting with key proteins involved in biofilm formation, Anabaenolysin B could disrupt the complex processes that lead to biofilm development in P. aeruginosa. This dual-targeting approach, if confirmed experimentally, could make Anabaenolysin B a particularly effective antibiofilm agent.However, 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. 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 LasR and LecA in the presence of Anabaenolysin B would provide crucial data on the compound's actual biological effects. Additionally, biofilm formation assays with P. aeruginosa in the presence of Anabaenolysin B would offer insights into its overall antibiofilm potential.

# Conclusion

In conclusion, this molecular docking study provides valuable insights into the potential antibiofilm mechanisms of Anabaenolysin B through its interactions with LasR and LecA. The results suggest that Anabaenolysin B may exert its effects through multiple pathways, potentially interfering with both quorum sensing and adhesion processes in biofilm formation. These findings lay the groundwork for future experimental studies and may contribute to the development of Anabaenolysin B-based antibiofilm therapies or the design of novel compounds inspired by its structure.

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