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Effectiveness of Temporins for the Treatment of HPV Infections

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Abstract

Objectives: *Human papillomavirus (HPV), especially high-risk variants like type-16 have been linked to the formation of malignant tumors. HPV-16 has been implicated in most cases of cervical cancer in women. This study aims to assess the effectiveness of three types of temporin molecules, i.e., antiviral peptides secreted by certain frog species, for the inhibition of the viral L1 protein.* ***Methods:*** *In the present study, various computational tools were used to find the temporin molecule that was most effective in inhibiting the attachment of the L1 viral major capsid protein of HPV type-16 to the heparan sulfate proteoglycan residues present on the basement membrane of the host cell. HPEPDOCK 2.0 and pyDockWEB servers were used to carry out protein-peptide and protein–protein docking, respectively. Binding affinity scores revealed the efficiencies of the different temporin molecules in inhibiting the action of the L1 protein.* ***Results:*** *According to the results obtained from this docking study, the temporin-L peptide, which was isolated from Rana temporaria was found to be the most efficient in preventing the attachment of the viral L1 major capsid protein to the host cell HSPG residues present on the basement membrane, thereby hindering the mechanism of viral entry.* ***Conclusion:*** *Further studies can be conducted on the mechanism of action of the temporin-L antiviral peptide. It can be a potential new therapeutic option for the treatment and management of high-risk HPV infections. Further research can be conducted on the temporin-L molecule for the formulation of new antiviral therapeutics for the treatment of HPV-16 infections. Temporins can be formulated into topical creams or gels, which could be applied directly to genital warts caused by HPV. These formulations might help reduce the size and discomfort of warts or even promote their clearance.*

Keywords: Human papillomavirus, temporins, antiviral peptides, L1 protein

INTRODUCTION

Human papillomavirus (HPV), belonging to the family Papillomaviridae, is a double-stranded DNA (dsDNA) virus approximately 8 kb [1]. Basal cells of the stratified epithelium are the only cells in which HPV can replicate; in the process of viral entry into the host cell, the L1 major capsid protein of the virus must first bind to heparan sulfate proteoglycan (HSPGs) residues present on the basement membrane [2]. In a study that used virus-like particles (VLPs) of HPV, it was observed that during the initial attachment of the particles to the host cell, the L1 protein played a more prominent role than the L2 protein [3]. In another study, the importance of the heparan sulfate proteoglycan residue was demonstrated using heparinase; it was found that the binding of the virus and subsequent infection was prevented [4, 5]. As the major capsid protein L1 seems to play an important role in mediating viral entry, inhibitors of this protein could act as potential anti-HPV drugs.

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Human papillomavirus (HPV) infection is associated with cervical cancer [6]. Although most HPV infections do not cause oncogenesis, certain

strains like HPV type-16 and HPV type-18 are known to cause cancers of the cervix in affected individuals [7]. Based on a report published on March 2023 by the Catalan Institute of Oncology (ICO)/International Agency for Research on Cancer (IARC), cervical cancer is the leading form of cancer among Indian women; it is estimated that approximately 123,907 new cases of cervical cancer are recorded in India (as per estimates for 2020) [8]. These results show that the development of new therapeutic options for the treatment of HPV infections is of utmost importance.

Temporins are antimicrobial peptides that are naturally produced by certain species of frogs. These compounds are secreted specifically by the skin of frogs and their granular glands at a significantly high concentration. These peptides are part of the innate immune system and defend animals against various pathogenic microorganisms [9, 10]. Temporins have several properties that make them promising candidates for the formulation of new antiviral drugs: their small size and therefore more efficient chemical synthesis, amphipathic properties, non-toxicity to mammalian cells, and in vivo studies, they are effective as topical preventive agents against infections [11, 12]. They are short peptides that typically consist of 8–17 amino acid residues. Amidation at the C-terminal end was observed in isolated peptides, and biological assays conducted using synthetic peptides demonstrated antibacterial activity in all of them, except for temporin-D and -H. Over 150 peptides categorized within the temporin family have been extracted from various genera of Ranid frogs, including *Amolops, Hylarana, Lithobates, Odorrana, Pelophylax, Rana,* and *Hylarana* [13].

Conventional techniques for the discovery and production of new antiviral drugs are known to be a very long and tedious process, with many potential candidate drugs failing the ADMET screening process towards the end after years of formulation [14]. Molecular docking and in silico techniques have greatly improved drug discovery outcomes. This study aimed to make use of in silico techniques such as protein–protein molecular docking to reveal the effectiveness of temporins A, B, and L for treating high-risk type-16 HPV infections.

METHODOLOGY

Preparation of Viral Protein Structure

The structure of the L1 protein of human papillomavirus (type 16) (HPV16) with the PDB identifier 1DZL was retrieved from the RCSB PDB database (*https://www.rcsb.org/)* [15]. Protein structure purification involves the removal of heteroatoms, water molecules, and other bound ligands. This step was performed using the BIOVIA Discovery Studio tool ((https://discover.3ds.com/discovery-studio-visualizer-download) [16]. The protein structure was further prepared for docking by adding hydrogen atoms to the UCSF Chimera software (*https://www.cgl.ucsf.edu/chimera/*) [17].

Preparation of Antiviral Peptides

Temporin-L, temporin-A, and temporin-B are antiviral peptides (produced by *Rana temporaria*) chosen for this docking study. Temporins are short peptides secreted by frogs that are known to have antimicrobial properties. The sequences of these peptides were obtained from the NCBI for Biotechnology Information protein database (*https://www.ncbi.nlm.nih.gov/protein/*) [18] in the FASTA format. AlphaFold2 (*https://neurosnap.ai/login?path=/service/AlphaFold2*) [19] is a tool used to model the 3D structures of input protein sequences using AI and deep learning methods. The FASTA sequences of the antiviral peptides were used as the input for the AlphaFold2 tool, and the 3D structures of the peptides were obtained. These results were further used as inputs for protein-peptide docking simulations.

Protein-peptide Docking

This step was carried out on the HPEPDOCK 2.0 web server (http://huanglab.phys.hust.edu.cn/hpepdock/) [20–24]. It is a tool that performs flexible protein-peptide docking, achieved by rapid modeling of peptide conformations. The de novo method was used to add polar hydrogen atoms to peptide molecules. This algorithm predicted more than 100 binding models; however, the top ten

models were the most probable. The results included binding affinity values and model ranks. Docking simulations were visualized using the PyMol tool (*https://pymol.org/2/*) [25], and interaction analysis was performed using BIOVIA Discovery Studio.

Preparation of Receptor Structure

Viral entry into the host cell is mediated by the attachment of the L1 protein of HPV16 to the heparan sulfate proteoglycan (HSPG) present on the basement membrane of the host cell. Therefore, inhibition of the L1 protein by antiviral peptides to prevent their attachment to HSPGs can be a potential solution. The structure of the HSPG receptor was retrieved from the RCSB PDB database (PDB ID: 1GL4). Purification was a two-step process: heteroatoms, water molecules, and the bound ligand nidogen-1 were removed using the Discovery Studio tool, and polar hydrogen atoms and surface charges were added using UCSF Chimera. This purified protein structure was used as the input for the protein–protein docking step. Another ligand, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid was also found to bind to the HSPG receptor structure. However, the removal of this ligand led to the fragmentation of the protein complex when visualized. Therefore, this ligand was retained in the protein–protein docking process.

Protein–Protein Docking

The pyDockWEB server (*https://life.bsc.es/pid/pydockweb*) [26] was used to perform protein–protein docking simulations. The inputs for this tool were protein-antiviral peptide complexes obtained after protein-peptide docking simulations and the purified HSPG receptor structure.

This tool uses the FTDock algorithm and returns the best rigid-body docking orientations between the input protein structures. The pyDock scoring function was used to predict the most probable binding models, with the top 10 being the most significant. The results included values for electrostatics, desolvation, total energy, and rank for each configuration.

These results were further analyzed using the DimPlot plugin available in LigPlus software (*https://www.ebi.ac.uk/thornton-srv/software/LigPlus/*) [27]. The DimPlot plugin was specifically used for the analysis of protein–protein interactions. The diagrams generated by DimPlot show the residues interacting with their chains and their names. It also provides an option for choosing the chains of the two protein structures whose interaction diagrams need to be generated.

RESULTS

Preparation of Antiviral Peptides

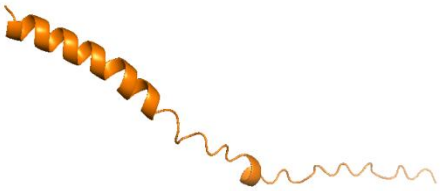
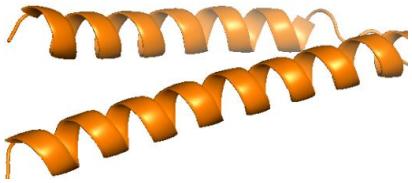
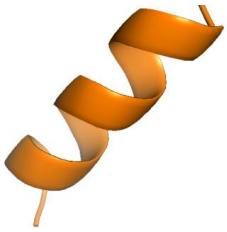
The antiviral peptides selected for this study were temporin-A (Identifier: QIU80529.1), temporin-B (Identifier: P79874.1), and temporin-L (Identifier: P57104). The sequences for these proteins retrieved from the NCBI Protein database were used as input for the AlphaFold2 web server to model the 3D structures. The number of recycles corresponding to the number of steps taken by the tool to construct the model was set to five. In this study, the PDB files were downloaded for visualization and docking. The structures generated using AlphaFold2 are presented in Table 1. These structures were visualized using the PyMol software.

Protein-Peptide Docking

Three antiviral peptides belonging to the temporin class (temporin-A, -B, and -L) secreted by frogs of the species *Rana temporaria* (common frog) were chosen for this docking study. The sequences were used as the input for the AlphaFold2 web server, and the resulting 3D structures were assessed for their ability to bind to the L1 protein of human papillomavirus type 16. L1 protein is known to play an important role in viral entry into the host cell. The HPEPDOCK 2.0, web server, was used to simulate protein-peptide docking. The results are presented in Table 2. From these results, it is evident that temporin-L, with a binding affinity of -215.248 kJ/mol, binds strongly to the L1 protein of HPV16. This is followed by temporin-A with a binding affinity of -188.815 kJ/mol, and finally temporin-B with a

binding affinity of -177.8 kJ/mol. The binding positions of the peptides with the L1 protein of HPV16 are shown in Figure 1.

Table 1. 3D structures generated by AlphaFold2.

Peptide	Structure generated by AlphaFold2
Temporin-A	
Temporin-B	
Temporin-L	

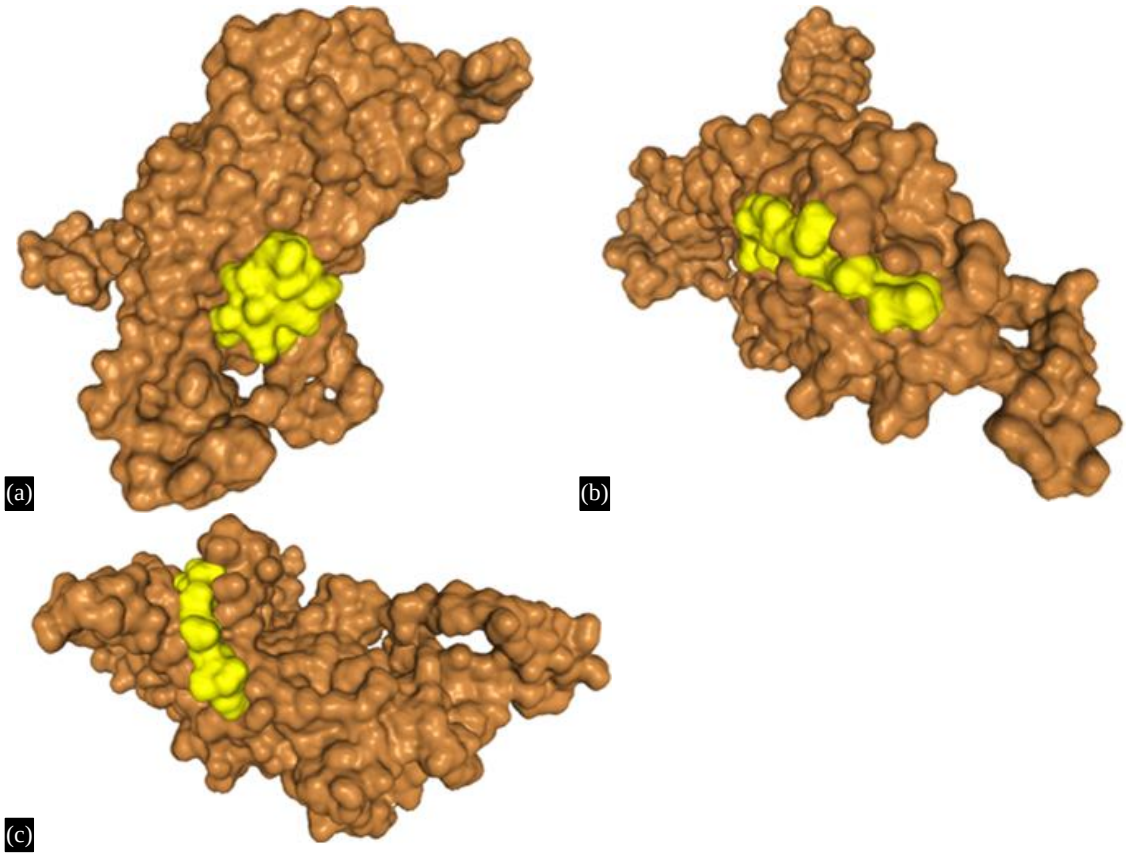


Figure 1. Binding poses of peptides (in yellow) with L1 protein (in brown): (a) temporin-L, (b) temporin-A, (c**)** temporin-B.

Table 2. Results of protein-peptide docking.

Name of peptide	Binding affinity (kJ/mol)
Temporin-L	–215.248
Temporin-A	–188.815
Temporin-B	–177.800

Table 3. Results of protein–protein docking simulations.

Protein-peptide complex	Electrostatics (kJ/mol)	Desolvation (kJ/mol)	van der Waals interactions (kJ/mol)	Total energy (kJ/mol)
Temporin-L plus -L1 protein of HPV16	-23.179	-10.985	19.517	-32.212
Temporin-A plus -L1 protein of HPV16	-16.383	-17.649	-2.818	-34.314
Temporin-B plus -L1 protein of HPV16	-15.540	-17.075	-9.416	-33.557

Protein–Protein Docking

Protein–protein docking was performed to assess the ability of the protein-antiviral peptide complex to prevent attachment to the heparan sulfate proteoglycan receptor (HSPG) present on the basement membrane of host cells. The pyDockWEB tool was used to simulate protein–protein docking. The viral protein-antiviral peptide complex generated in the previous step and the HSPG receptor were used as inputs for this tool. The results of the protein–protein docking simulation are shown in Table 3. All total energy values are negative for the temporin-A complex, which has the most negative total energy.

From these results, the temporin-L, -L1 protein complex is the most effective in preventing attachment to the HSPG receptor. This is indicated by its (comparatively lesser) binding energy.

The binding energy value represents the energy released due to bond formation; therefore, a lower binding energy value indicates a greater degree of binding. However, since the main goal of this study was to identify peptide complexes that *prevent* the attachment of viral proteins to the HSPG receptor, a greater value of binding energy is considered favorable, as this indicates that the viral protein-antiviral peptide complex binds weakly to the receptor structure, thereby hindering the viral entry process.

The most probable models for protein–protein docking generated by the pyDockWEB server, as visualized by PyMol, are shown in Figure 2.

DimPlot Interaction Analysis

The DimPlot plugin, available in the LigPlus software, was used to analyze the interactions between the docked protein structures. The input for the DimPlot was the docked protein complex generated by pyDockWEB. The results of the DimPlot analysis are shown in Figure 3.

The greater binding affinity of temporin-A and -B is evidenced by the greater number of interacting residues when docked with the HSPG receptor structure. In the interaction plot for temporin-L, we observed that the only interactions at play were hydrophobic. However, in the case of temporins A and B, hydrogen bonding interactions were also present. This, along with a greater number of interacting residues overall, contributes to a greater binding affinity for temporins A and B. Table 4 shows the interacting residues participating in the protein–protein docking simulation.

The EPE ligand (Figure 4), attached to the HSPG receptor structure, was retained for the protein–protein docking process. Removal of this ligand led to fragmentation of the protein complexes when visualized using PyMol.

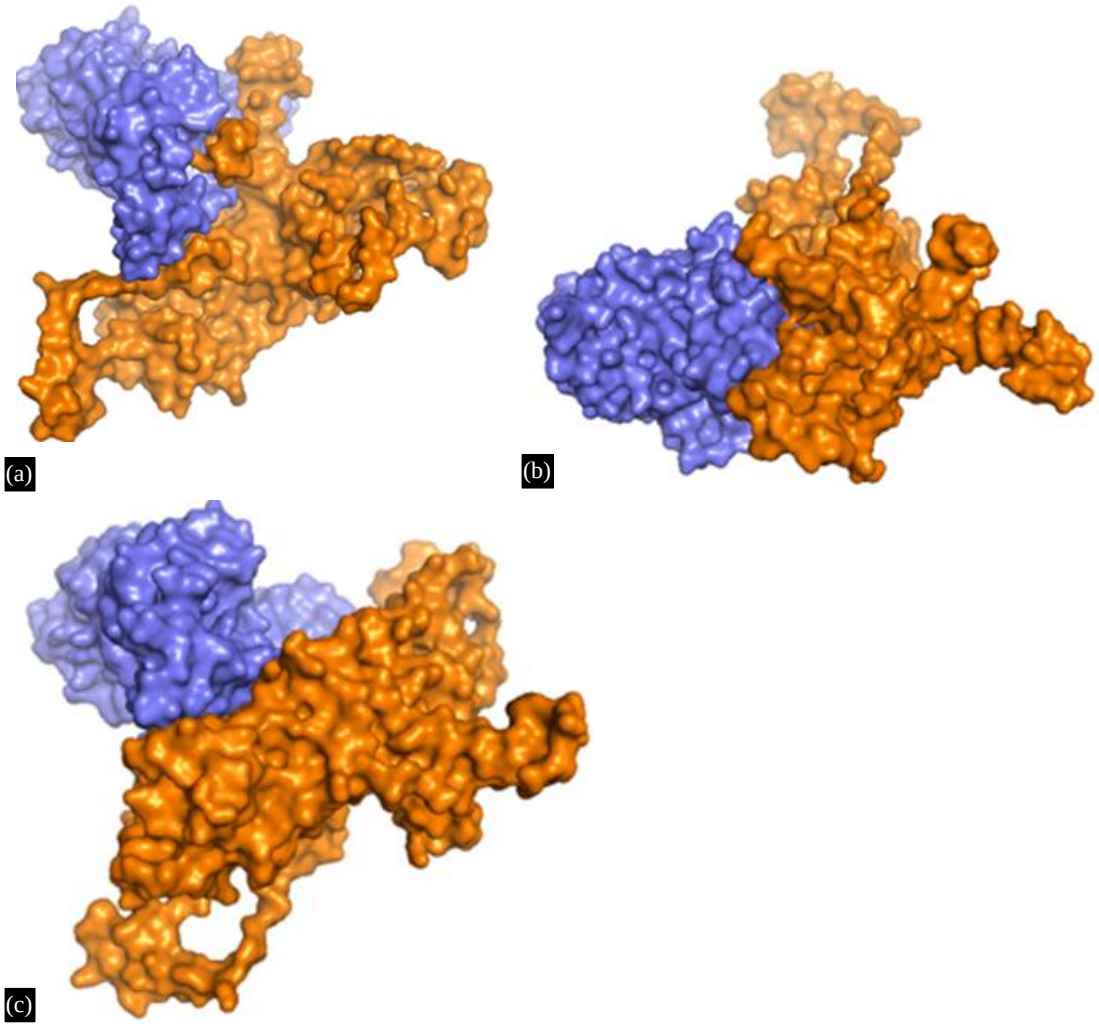
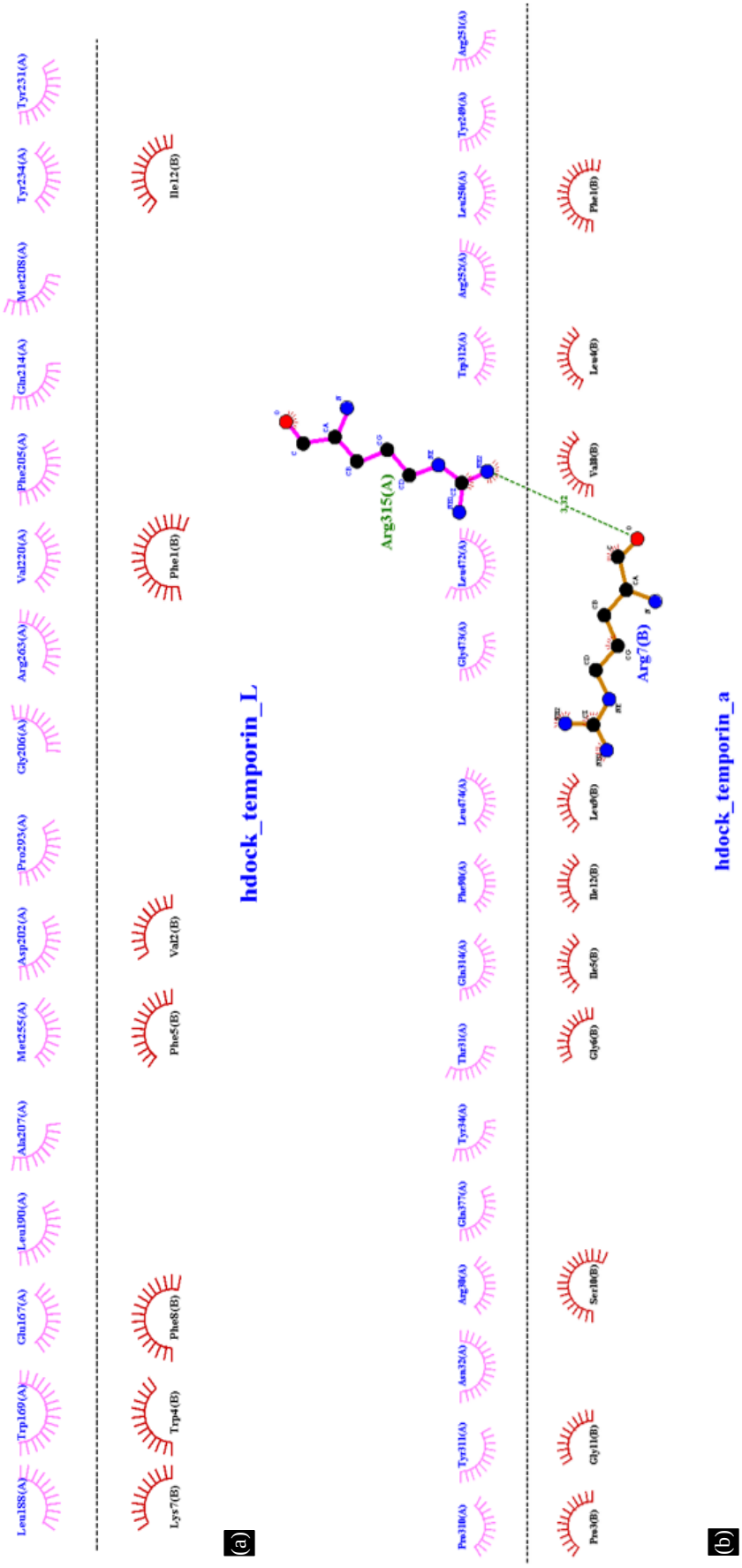
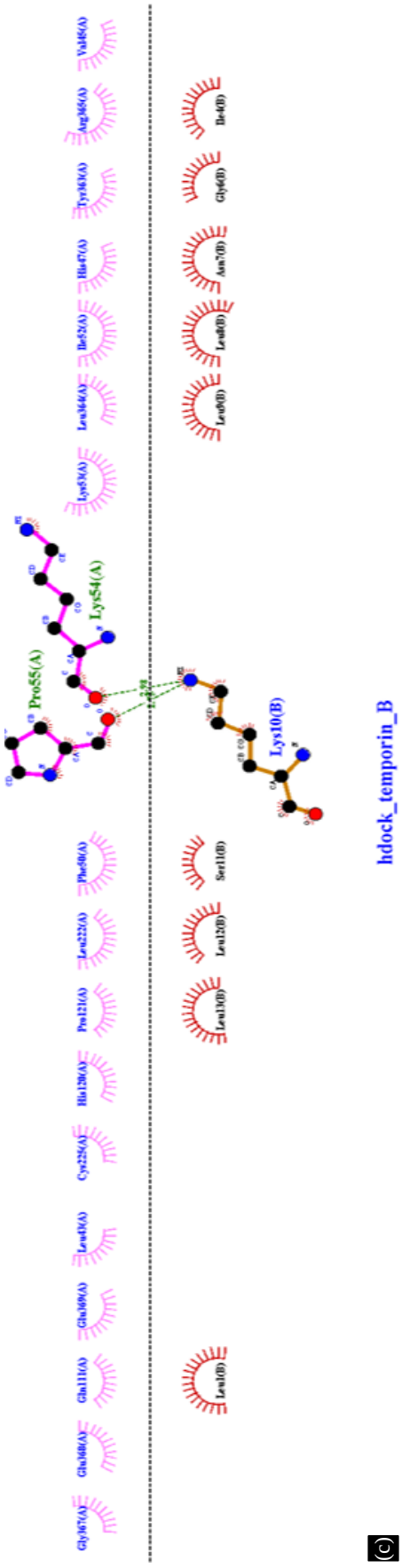


Figure 2. Binding poses of the two-protein involved in the protein–protein docking simulation: HSPG receptor (in orange) and the L1 protein-antiviral peptide complexes (in blue), (a) temporin-L complex, (b) temporin-A complex, (c) temporin-B complex.

Table 4. Interacting residue names with chain identifiers.

Protein–protein complex	Interacting residues	Hydrogen bonding residues
Temporin-L complex	Leu188(A), Trp169(A), Glu167(A), Leu190(A), Ala207(A), Met255(A), Asp202(A), Pro293(A), Gly206(A), Arg263(A), Val220(A), Phe205(A), Gln214(A), Met208(A), Tyr234(A), Tyr231(A), Lys7(B), Trp4(B), Phe8(B), Phe5(B), Val2(B), Phe1(B), Ile12(B)	NIL
Temporin-A complex	Pro310(A), Tyr311(A), Asn32(A), Arg30(A), Gln377(A), Tyr34(A), Thr31(A), Gln314(A), Phe90(A), Leu474(A), Gly473(A), Leu472(A), Trp312(A), Arg252(A), Leu258(A), Tyr249(A), Arg251(A), Pro3(B), Gly11(B), Ser10(B), Gly6(B), Ile5(B), Ile12(B), Leu9(B), Val8(B), Leu4(B), Phe1(B)	Arg315(A), Arg7(B)
Temporin-B complex	Gly367(A), Glu368(A), Gln111(A), Glu369(A), Leu43(A), Cys225(A), His120(A), Pro121(A), Leu222(A), Phe50(A), Lys53(A), Leu364(A), Ile52(A), His47(A), Tyr363(A), Arg365(A), Val49(A), Leu1(B), Leu13(B), Leu12(B), Ser11(B), Leu9(B), Asn7(B), Gly6(B), Ile4(B)	Pro55(A), Lys54(A), Lys10(B)





(c)

Figure 3. Protein–protein interaction diagrams generated by DimPlot: (a) temporin-L complex, (b) temporin-A complex, (c) temporin-B complex.

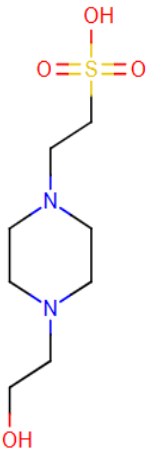


Figure 4. The 2D representation of the EPE ligand (Data Bank, 2024).

DISCUSSIONS

Human papillomaviruses (HPV) are pathogenic organisms mainly associated with conditions such as benign warts and malignancy, usually in the cervix in women as well as several other anatomical sites in both men and women [28]. They are known to be the causative agents of most sexually transmitted diseases [29]. 80 subtypes of the virus have been categorized so far, with HPV-16 being the "high-risk" variant as it has been observed to be the cause of most cervical cancer cases along with HPV type 18; it has been estimated that 84.1% of all invasive cervical cancer cases has been attributed to the subtypes of the human papillomavirus [30, 31]. Therefore, finding better and more efficient treatment options for these subtypes could save numerous people from developing cancers in which the causative agent is human papillomavirus.

The viral L1 protein (55 kDa), also known as the major capsid protein or major late protein, can self-assemble to form VLPs [32]. It has been observed that the L1 protein plays a major role in the initial attachment of the virus to the heparan sulfate residues. Several studies have supported this observation using cultured cell lines and murine vaginal challenge models. One study found that the binding of the virion was prevented by using heparin, a highly sulfated form of heparan sulfate, and another study observed the same results after enzymatically removing heparan sulfate residues using heparinase [33–35]. Therefore, targeting the L1 major capsid protein to develop new therapeutic options for the treatment of high-risk HPV infections would be beneficial.

Temporins A, B, and L, isolated from frogs belonging to the genus *Rana* are the most investigated temporins; they have been studied for their antimicrobial and antibacterial properties [11]. Therefore, these three classes of temporins were chosen for this study owing to their effectiveness as antiviral peptides. Temporin-A is effective against a broad spectrum of pathogens, including bacteria, fungi, and protozoa. It is also known to exhibit anticancer properties [13]. According to some studies, temporin-A is the most effective against gram-positive bacteria [36]. In an experiment conducted using a subcutaneous rat pouch model, this peptide showed synergistic effects in combination with imipenem, ceftazidime, polymyxin E, and linezolid [37, 38]. These antimicrobial properties that have been attributed to temporin-A make it a promising candidate and a potential treatment option for high-risk HPV infections.

Temporin-B is also known for its antimicrobial properties, specifically against gram-positive bacteria, similar to those of temporin-A. It was found to be significantly effective against a bacterial species, *Acinetobacter junii*, which is responsible for causing urinary tract infections [11]. This molecule has also been shown to inhibit the activity of herpes simplex virus type-1 in human epithelial cells [39].

Another promising candidate is the 13 amino acid temporin-L peptide isolated from the skin region *Rana temporaria* [13]. This peptide is most effective against gram-positive bacterial infections as well as fungal infections caused by *Candida* [40]. Temporin-L exhibits cytotoxic properties in various cancer cell lines, including Hut-78, U-937, and K562 [41]. These anticancer properties of temporin-L are beneficial, as high-risk HPV-16 infections have been strongly linked to cervical cancer cases, as mentioned previously.

Protein-peptide docking was performed using the HPEPDOCK 2.0 web server for all three temporin molecules: temporin-A, temporin-B, and temporin-L. As shown in Table 2, the results indicated that the temporin-L and viral L1 major capsid protein complex showed the strongest binding affinity with a value of -215.248 kJ/mol, followed closely by the temporin-A and viral L1 protein complex with a binding affinity score of -188.815 kJ/mol. The complexes with the lowest binding affinity were temporin-B and viral L1 protein complexes. These findings indicate that temporin-L is more efficient in terms of binding to the L1 major capsid protein. Strong binding to the L1 protein could indicate that the temporin molecule can inhibit it and prevent subsequent attachment to the host cell heparan sulfate proteoglycan residues present on the basement membrane. This can be tested by conducting a protein–protein docking experiment, with the two input molecules being the temporin molecule-viral L1 protein complex and the HSPG receptor.

The pyDockWEB server was used to perform protein–protein docking. The results of docking, as indicated in Table 3, show that the temporin-L-L1 complex and the HSPG receptor have the lowest binding affinity values (-32.212 kJ/mol). This value of total energy indicates that the temporin-L molecule is more successful in inhibiting the L1 protein, as the binding affinity values with the HSPG receptor structure have decreased. The temporin-B-L1 complex showed the highest binding affinity with the HSPG residue, which is consistent with the previous observation in protein-peptide docking, where the temporin-B-L1 complex had the lowest binding affinity; therefore, it was unable to prevent the attachment of the L1 protein to the HSPG residue as efficiently as temporin-L.

A DimPlot was used to assess the binding interactions between the two protein complexes. As shown in Figure 4, the interaction diagram for the temporin-L complex showed only hydrophobic interactions, with no hydrogen bonding. This result was consistent with the binding affinity scores obtained on the pyDockWEB server. Temporin-A and temporin-B complexes show one and two hydrogen bonds, respectively, in the interaction diagram. Hydrogen bond formation indicates greater binding; therefore, the temporin-B complex with the highest binding affinity has two hydrogen bonds.

The EPE ligand (Figure 3), also known as 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid, was found to bind to the 3D structure of the HSPG receptor retrieved from the PDB database. The removal of this ligand caused fragmentation of the protein structure when visualized using PyMol software. The EPE ligand is used as a buffer in various experiments; it is mainly used to maintain the stability of the protein structure and to balance the pH of the solution [42].

Therefore, the role played by EPE in maintaining the structural integrity of the protein could be the reason for the fragmentation of the protein structure upon removal of the ligand.

Although vaccines for the prevention of HPV infections exist [43, 44], there is no evidence to indicate that these vaccines can clear the virus in individuals with established infections [45, 46]. Therefore, there is a need to develop new therapeutic options to combat HPV infection.

CONCLUSION

The three temporin molecules (temporin-A, temporin-B, and temporin-L) were assessed for their effectiveness in inhibiting the viral L1 major capsid protein of the “high-risk” human papillomavirus (type-16). From the results obtained, it was observed that the temporin-L molecule showed the highest

potential as an inhibitor of the viral protein L1, as evidenced by the high binding affinity scores obtained after the protein-peptide docking step. This result was also consistent with the binding affinity scores obtained after the protein–protein docking simulations.

The development of an effective delivery method for temporins will play a crucial role in their clinical application. This might involve techniques such as nanoparticle-based delivery or other such drug delivery systems to ensure targeted and sustained release at the site of infection. Considerations about production costs and accessibility of temporins should be prioritized, as affordable and widely available treatments are essential for widespread use, especially for conditions such as HPV, which can affect a large demographic.

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Conflict of Interest

The author declares that there is no conflict of interest.

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