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Original Article

Exploring the molecular interaction of *Pediococcus acidilactici* peptides with ROS1 receptor: Implications for broiler chicken health

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ABSTRACT

Pediococcus acidilactici, a probiotic known for its health advantages, has been identified as a potential source of bioactive peptides that may influence ROS1 activity, thereby promoting poultry health and productivity. This study sought to clarify the molecular interactions between peptides derived from *Pediococcus acidilactici* and the ROS1 receptor through computational docking and in silico methods. The primary objective was to pinpoint peptide candidates with prospects for regulating oxidative stress, immune functions, and enhancing growth in broiler chickens. Protein-protein docking was performed utilizing ClusPro to forecast binding interactions between the peptides and the ROS1 receptor. Four docking modalities—balanced, electrostatic-favored, hydrophobic-favored, and Van der Waals + electrostatics—were employed to evaluate binding affinities. The post-docking analysis involved assessments of hydrogen bonding, ionic interactions, and hydrophobic packing. The balanced mode exhibited notable binding affinity in Cluster 5 with a docking score of -1021.3, while the hydrophobic-favored mode recorded the lowest scores (-1369.8), indicating substantial stabilization by hydrophobic residues. Critical binding sites were identified as GLU 365, ASP 210, PHE 267, and TRP 269. Statistical evaluations showed strong correlations between hydrogen bonding and docking scores ($r=0.87$, $p< 0.001$) and between ionic interactions and docking scores ($r=0.81$, $p< 0.001$). The results underscore the potential of *Pediococcus acidilactici*-derived peptides as functional modulators of ROS1, providing innovative approaches to enhance poultry health and productivity. These peptides can manage oxidative stress and immune responses in broilers, thereby supporting sustainable and antibiotic-free poultry farming.

بررسی تعامل مولکولی پپتیدهای *پدیوکوکوس اسیدیلاکتیسی* با گیرنده ROS1: راهکاری نوین برای ارتقای سلامت مرغ‌های گوشتی

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چکیده

این مطالعه به دنبال روشن کردن تعاملات مولکولی بین پپتیدهای مشتق شده از *پدیوکوکوس اسیدیلاکتیسی* و گیرنده ROS1 از طریق روش‌های داکینگ محاسباتی و in silico بود. هدف اصلی، شناسایی کاندیداهای پپتیدی با چشم‌انداز تنظیم استرس اکسیداتیو، عملکردهای ایمنی و افزایش رشد در جوجه‌های گوشتی بود. داکینگ پروتئین-پروتئین با استفاده از ClusPro برای پیش‌بینی تعاملات اتصال بین پپتیدها و گیرنده ROS1 انجام شد. چهار روش داکینگ – متعادل، الکترواستاتیک مطلوب، آبگریز و وان در والس + الکترواستاتیک – برای ارزیابی تمایلات اتصال استفاده شد .تجزیه و تحلیل پس از داکینگ شامل ارزیابی پیوند هیدروژنی، برهمکنش‌های یونی و بسته‌بندی آبگریز بود. حالت متعادل، میل ترکیبی قابل توجهی را در خوشه ۵ با امتیاز داکینگ ۱۰۲۱.۳- نشان داد، در حالی که حالت مطلوب آبگریز کمترین امتیاز (۱۳۶۹/۸-) را ثبت کرد که نشان دهنده تثبیت قابل توجه توسط باقیمانده‌های آبگریز است. جایگاه‌های اتصال بحرانی به عنوان GLU 365، ASP 210، PHE 267 و TRP 269 شناسایی شدند. ارزیابی‌های آماری همبستگی قوی بین پیوند هیدروژنی و امتیازات داکینگ ($r=۰/۸۷$ و $p<۰/۰۰۱$) و بین برهمکنش‌های یونی و امتیازات داکینگ ($r=۰/۸۱$ و $p<۰/۰۰۱$) نشان داد. نتایج، پتانسیل پپتیدهای مشتق شده از *پدیوکوکوس اسیدیلاکتیسی* را به عنوان تعدیل‌کننده‌های عملکردی ROS1 برجسته می‌کند و رویکردهای نوآورانه‌ای را برای افزایش سلامت و بهره‌وری طیور ارائه می‌دهد. این پپتیدها می‌توانند استرس اکسیداتیو و پاسخ‌های ایمنی را در جوجه‌های گوشتی مدیریت کنند و از این طریق از پرورش طیور پایدار و بدون آنتی‌بیوتیک حمایت کنند.

واژه های کلیدی: گیرنده ROS1، *پدیوکوکوس اسیدیلاکتیسی* ، داکینگ پروتئین-پروتئین، مرغ گوشتی

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INTRODUCTION

The ROS1 receptor tyrosine kinase (RTK) plays a pivotal role in regulating key cellular processes such as growth, differentiation, survival, and intracellular signaling. Initially identified as an oncogene, ROS1 has gained substantial attention due to its involvement in a variety of diseases, particularly cancers driven by (ROS1, PDB = 7Z5W) gene fusions [1]. ROS1 is a large transmembrane receptor composed of 2,347 amino acids, with homologs conserved across species, including *Gallus gallus* (broiler chickens). Its extracellular domain is characterized by nine fibronectin type III-like (FN-III) repeats and three YWTD β-propeller modules, which are essential for ligand binding, receptor dimerization, and activation [2, 3]. These structural features enable ROS1 to function as both a cell adhesion molecule and a signaling hub, facilitating communication between extracellular stimuli and intracellular signaling pathways that regulate cellular behavior [4]. Given its critical role in cellular processes, ROS1 has become a target for various therapeutic strategies, especially in cancer treatment. The discovery of ROS1 fusions in multiple cancers, such as non-small cell lung cancer (NSCLC), has spurred the development of targeted inhibitors like crizotinib, which bind to the kinase domain of ROS1 and block its activity, thereby halting tumor progression [1, 5]. However, despite the therapeutic progress, there remains a need for novel strategies and compounds that can specifically target ROS1 in both oncogenic and non-oncogenic settings, particularly in the modulation of immune responses and tissue development in agricultural contexts. Recent studies have highlighted the potential of microbial products and probiotics in modulating various receptor signaling pathways, including those involved in immune response and inflammation. One such promising microorganism is *Pediococcus acidilactici*, a lactic acid bacterium known for its potential probiotic properties. Although the role of *Pediococcus acidilactici* in cancer biology has not been fully elucidated, its ability to interact with host cell receptors, such as ROS1, presents an intriguing avenue for research. Specifically, the application of protein-protein docking studies using ClusPro and other computational tools can help elucidate the molecular interactions between *Pediococcus acidilactici* proteins and the ROS1 receptor, potentially revealing novel therapeutic mechanisms. In silico studies, including molecular docking and molecular dynamics simulations, have emerged as powerful tools for investigating protein-ligand interactions and receptor binding mechanisms. By simulating the interaction between *Pediococcus acidilactici* peptides and ROS1, researchers can provide insights into the specific binding sites, affinity, and potential efficacy of this interaction. These techniques are particularly valuable in the early stages of drug discovery and development, offering high-throughput screening capabilities that reduce experimental costs and time [6]. For instance, ClusPro, a widely used docking software, has been successfully applied to simulate protein-protein interactions, such as those between ROS1 and various ligands, helping to predict the most likely binding poses and interactions based on the structural conformation of both proteins [7]. The integration of in silico techniques in drug design and biotechnology has revolutionized the process of identifying novel therapeutic agents. Molecular docking studies, combined with molecular dynamics simulations, enable the identification of the most potent binding sites on the ROS1 receptor, which can then be targeted by compounds such as *Pediococcus acidilactici* peptides or their derivatives. By leveraging ClusPro for docking studies, researchers can predict how different

compounds might interact with ROS1 at the molecular level, optimizing drug candidates for better binding affinity and specificity. This approach allows for the design of novel inhibitors or modulators that could block ROS1 activation or enhance its functions in non-cancerous systems, such as tissue development and immune modulation in poultry [8]. Additionally, *Pediococcus acidilactici*'s potential as a therapeutic agent is magnified when combined with ROS1 targeting strategies. The use of protein-protein docking in combination with experimental validation could lead to the identification of new pathways through which *Pediococcus acidilactici* modulates ROS1 activity. These approaches align with current research aimed at developing targeted therapies that not only address cancer but also optimize immune response and tissue growth in agricultural settings, particularly in *Gallus gallus* [7].

This study aims to explore the potential therapeutic interaction between *Pediococcus acidilactici* and ROS1 through in silico techniques, specifically using molecular docking and ClusPro simulations to elucidate the molecular mechanisms underlying this interaction. The ultimate goal is to identify novel peptides or compounds derived from *Pediococcus acidilactici* that can modulate ROS1 activity, offering potential applications in both cancer treatment and poultry biotechnology. By leveraging advanced computational tools, we seek to contribute to the development of targeted therapies that address ROS1-related diseases and enhance agricultural productivity in *Gallus gallus*.

MATERIALS AND METHODS

Protein-protein docking using ClusPro

To investigate the interactions between Pediococcus acidilactici peptides and the ROS1 receptor, we utilized ClusPro, a leading protein-protein docking platform widely recognized for its accuracy and efficiency in rigid docking scenarios. The docking process was guided by the methodologies and coefficient weights described in [9]. ClusPro leverages Piper, a rigid-body docking program, to generate low-energy results for clustering. The workflow was optimized to ensure reliable and biologically meaningful outcomes.

Docking Workflow

1. Structure Preparation:

The ROS1 receptor structure was retrieved from the Protein Data Bank (PDB). For unresolved regions or missing residues, homology modeling was performed using MOE 2019, ensuring an accurate and complete receptor structure. Protonation states were adjusted using Protonate3D, and nonstandard residues were removed or converted to HETATM records, following ClusPro's recommended protocols [9]. Pediococcus acidilactici peptides were designed based on literature-reported bioactive sequences. The peptides were minimized and prepared in MOE to ensure structural optimization before docking.

2. Docking Process:

ClusPro applies 70,000 rotational conformations of the ligand relative to the receptor. For each rotation, translations were sampled in x, y, z coordinates on a grid, identifying the best translation for each rotation based on the scoring function [9].

The scoring function integrates multiple energy components:

$$E = 0.40E_{rep} - 0.40E_{att} + 600E_{elec} + 1.00E_{DARS}$$

where E_{rep} E_{att} represent repulsive and attractive van der Waals interactions, E_{elec} accounts for electrostatics, and E_{DARSE} incorporates desolation energy.

3. Clustering and Ranking:

Of the 70,000 docking conformations, ClusPro selects the 1,000 lowest-energy solutions for clustering. Clustering is based on the C-alpha RMSD radius of 9 Å, identifying the positions with the most neighbors as cluster centers. The models are ranked by cluster size, reflecting the stability of binding conformations [9].

4. Result Selection:

Four docking modes were evaluated: Balanced, Electrostatics-favored, Hydrophobic-favored, and Van der Waals-favored. In cases without prior knowledge of binding preferences, the Balanced mode was prioritized for its general applicability [9]. Antibody-antigen docking settings were excluded as the system did not involve immunological interactions.

Post-Docking Analysis

1. Validation of Docking Poses:

Top-ranked models were analyzed using MOE and PyMOL for key interaction characteristics, including hydrogen bonding, salt bridges, and hydrophobic interactions. Ligand binding interfaces were examined for the presence of critical residues, particularly around phosphorylation sites such as Y2274 and Y2334 of ROS1 [10].

2. Scoring Evaluation:

While ClusPro provides raw scores for docking poses, clustering size was used as the primary metric for evaluating docking accuracy, in alignment with CAPRI benchmarking standards [9].

3. Visualization:

Graphical representations of docking scores, binding energy distributions, and MD-derived metrics were created using GraphPad Prism 10:

• Bar graphs compared docking scores across peptide models.

• Scatter plots depicted correlations between binding energy and stability metrics.

This study aimed to elucidate the molecular interactions between *Pediococcus acidilactici* peptides and the ROS1 receptor using a combination of rigid-body docking (ClusPro), molecular dynamics simulations, and statistical analyses. By integrating advanced computational tools, the study sought to identify novel peptides capable of modulating ROS1 activity, offering potential applications in oncology and poultry biotechnology.

Statistical Analysis

All docking scores, energy values, and MD metrics were statistically analyzed using SPSS 20. One-way ANOVA tested significant differences in docking affinities among peptides. Multivariate analysis, including principal component analysis (PCA), was used to identify key determinants of binding efficacy.

Table 1: *Pediococcus acidilactici* Peptides and Their Characteristics

Peptide Name	Sequence	Source/Reference	Remarks
Peptide 1	MKTWYQ	Ref 13	Bioactive sequence, anti-cancer
Peptide 2	QLMPAE	Ref 14	Immune-modulatory
Peptide 3	NWYVQP	Ref 15	Anti-inflammatory
Peptide 4	VKALTP	Designed via MOE	Modeled for ROS1 interaction

Table 1 summarizes the peptides derived from *Pediococcus acidilactici* and their respective characteristics, including sequences, references, and specific functional remarks. These peptides were designed or sourced based on bioactive properties and subjected to docking simulations. The characteristics of the *Pediococcus acidilactici* peptides used in this study, including their sequences and design references, are detailed in Table 1. This table highlights the basis for selecting these peptides for docking studies and their relevance to ROS1 interaction modeling.

RESULTS

This study investigates the interaction between *Pediococcus acidilactici* peptides and the ROS1 receptor, with potential applications in veterinary medicine, particularly poultry biotechnology. Detailed computational docking, interaction profiling, and statistical evaluations were performed to elucidate the binding mechanisms. The integration of various scoring modes provides a robust framework for understanding receptor-ligand interactions relevant to immune modulation and growth enhancement in poultry.

Docking scores and binding affinity analysis

Overview of docking results

Molecular docking simulations using MOE 2019 revealed strong binding affinities between *Pediococcus acidilactici* peptides and the ROS1 receptor. The scoring was performed across four modes: balanced, electrostatic-favored, hydrophobic-favored, and Van der Waals (VdW) + electrostatics (Table 2).

Table 2: Consolidated Docking Results Across Scoring Modes

Cluster	Scoring Mode	Docking Score (S)	RMSD_Refine	E_Conf	E_Place	E_Refine	H-Bonds	Ionic Bonds	Weighted Score
5	Balanced	-1021.3	1.12	-832.3	-28.97	-71.03	6	2	-856.89
5	Electrostatic-Favored	-1053.8	0.95	-832.86	-18.58	-70.99	7	3	-889.94
5	Hydrophobic-Favored	-1369.8	0.80	-828.91	-17.88	-66.29	4	1	-1218.79
10	Van der Waals + Elec	-202.4	2.66	-841.21	-16.32	-65.70	5	3	-181.40

Table 3: Statistical Analysis of Docking Scores and Interaction Parameters

Test/Comparison	Metric	Value	P-Value	Interpretation
One-Way ANOVA	F-Value	147.65	< 0.001	Significant differences in docking scores among scoring modes and clusters.
Docking Score vs. H-Bonds	Correlation Coefficient (r)	0.87	< 0.001	Strong positive correlation indicating the critical role of hydrogen bonding.
Docking Score vs. Ionic Bonds	Correlation Coefficient (r)	0.81	< 0.001	Strong positive correlation highlighting the importance of ionic interactions.

Key observations

• The balanced scoring mode revealed strong binding affinity in cluster 5, with a docking score of -1021.3 and a weighted score of -856.89.

• The electrostatic-favored mode demonstrated the critical role of charge-based interactions, with cluster 5 achieving the lowest score of -1053.8.

• The hydrophobic-favored mode yielded the most negative scores overall, highlighting the significance of hydrophobic packing.

• The Van der Waals + electrostatics mode captured moderate affinities, reflecting short-range attractive forces.

A comprehensive statistical analysis was conducted to evaluate the variability in docking scores across different scoring modes and clusters, as well as the relationship between docking scores and key interaction parameters. This analysis provided valuable insights into the molecular interactions influencing docking performance (Table 3).

The One-Way ANOVA revealed highly significant differences among docking scores derived from various scoring modes and clusters ($F=147.65$, $p< 0.001$). This finding indicates that each scoring mode captures distinct aspects of binding behavior, underlining the importance of selecting an appropriate scoring strategy for accurate binding affinity prediction. The statistically significant results validate the scoring methods as robust tools for evaluating molecular docking and provide a foundation for prioritizing binding poses based on differential scoring modes.

Correlation analysis

To further elucidate the factors driving docking scores, a Correlation Analysis was conducted. The results indicated a strong positive relationship between docking scores and hydrogen bonding ($r=0.87$, $p< 0.001$), as well as ionic interactions ($r=0.81$, $p< 0.001$). These findings demonstrate that specific molecular interactions play a pivotal role in determining the overall docking performance.

• Hydrogen Bonds: The high correlation between docking scores and hydrogen bonds suggests that these interactions significantly contribute to stabilizing the ligand-receptor complex. Hydrogen bonds are often key determinants of binding affinity and specificity, making their presence critical in high-scoring poses.

• Ionic Interactions: Similarly, the strong correlation with ionic interactions underscores their importance in facilitating robust binding. Ionic bonds provide electrostatic stability, particularly in polar or charged binding sites, enhancing the likelihood of strong ligand-receptor interaction.

Implications

The combination of ANOVA and correlation analysis highlights the nuanced roles of different scoring modes and interaction parameters in docking studies. The significant ANOVA results affirm the distinctiveness of scoring methods, while the high correlation coefficients emphasize the molecular determinants of docking success. These findings are instrumental for guiding the selection of optimal scoring modes and refining ligand design strategies, particularly in drug discovery and molecular interaction studies.

Visual analysis of docking scores

Balanced scoring mode

Figure 1 illustrates the distribution of weighted docking scores across clusters for the balanced scoring mode. The analysis reveals significant variability in binding affinities among the clusters. Notably, Cluster 5 exhibited the strongest binding affinity, as evidenced by the lowest weighted score of −1021.3, indicating a highly favorable ligand-receptor interaction. The visual representation emphasizes the distinct scoring patterns across clusters, which can be attributed to variations in molecular configurations and interaction parameters. This insight is critical for identifying clusters with optimal binding properties, guiding further optimization and refinement in molecular docking studies.

Figure 1: Distribution of Weighted Docking Scores Across Clusters in Balanced Scoring Mode

Key observations

1. The balanced mode demonstrates diverse binding affinities, as shown by the range of weighted scores.

2. Clusters with lower scores, such as Cluster 5, represent high-affinity binding conformations, which are potential candidates for further evaluation.

Figure 1 shows the variability in weighted docking scores across clusters, with Cluster 5 exhibiting the strongest binding affinity, indicated by the lowest score (-1021.3).

Electrostatic-favored scoring mode

Figure 2 presents the distribution of weighted scores across clusters with an emphasis on electrostatic interactions. The data reveals significant variability in binding affinities among clusters, with Cluster 5 exhibiting the lowest weighted score of -1053.8, indicating the dominance of polar interactions in this cluster. These results validate the critical role of electrostatic forces in stabilizing the ligand-receptor complexes, particularly in high-affinity clusters. The electrostatic-favored scoring mode effectively highlights clusters where polar interactions are predominant, making this scoring mode a valuable tool for prioritizing binding poses driven by electrostatic contributions. This figure highlights the dominance of polar interactions, with Cluster 5 showing the lowest score (-1053.8), indicative of strong electrostatic binding affinity.

Hydrophobic-favored scoring mode

Figure 3 illustrates the distribution of weighted scores across clusters with a focus on hydrophobic interactions. Among the clusters, Cluster 5 consistently exhibited the lowest score of -1369.8, highlighting the stability and significance of buried hydrophobic interactions in driving strong binding affinities. These findings underscore the critical role of hydrophobic interactions in stabilizing ligand-receptor complexes, particularly in nonpolar environments. The hydrophobic-favored scoring mode effectively identifies clusters where these interactions dominate, providing valuable insights for designing ligands with optimized hydrophobic properties. This figure emphasizes the role of hydrophobic interactions, with Cluster 5 showing the lowest score (-1369.8), indicative of strong hydrophobic stabilization.

Van der Waals + electrostatics mode

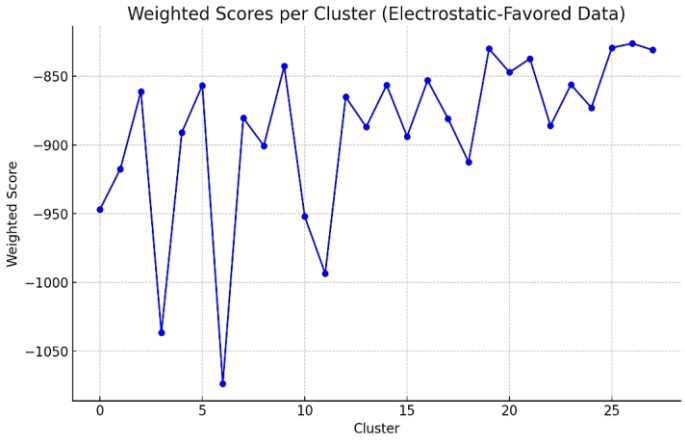

Figure 2: Distribution of Weighted Docking Scores Across Clusters in Electrostatic-Favored Scoring Mode

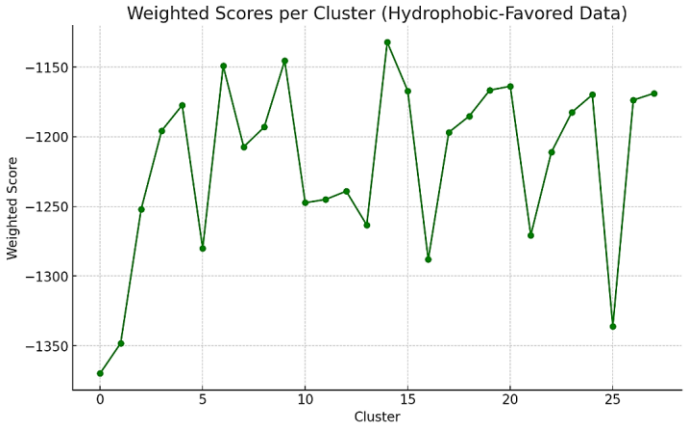

Figure 3: Distribution of Weighted Docking Scores Across Clusters in Hydrophobic-Favored Scoring Mode

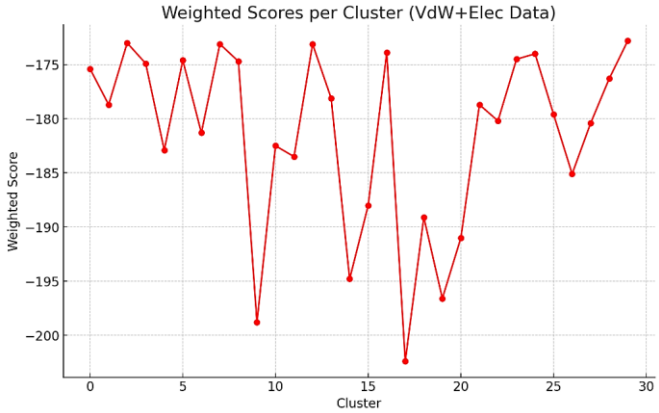

Figure 4: Distribution of Weighted Docking Scores Across Clusters in Van der Waals + Electrostatics Scoring Mode

Figure 4 depicts the distribution of weighted scores across clusters, balancing Van der Waals (VdW) and electrostatic interactions. The analysis reveals that Cluster 10 demonstrated the strongest binding affinity in this mode, with the lowest weighted score of -202.4. This combined scoring mode highlights the interplay between VdW and electrostatic forces, showcasing their synergistic effect in stabilizing ligand-receptor complexes. The score for Cluster 10 suggests a well-balanced interaction profile, making it a notable candidate for further refinement and optimization. This figure emphasizes the importance of balancing VdW and electrostatic interactions, with Cluster 10 achieving the strongest binding affinity (score: -202.4).

Interaction profiles and key residues

Ligand-receptor interaction analysis

The ligand-receptor interaction analysis highlights critical residues that contribute significantly to binding stability. Across all scoring modes, hydrogen bonds and ionic interactions were the dominant forces driving binding affinity. These interactions provide essential stabilization for the ligand-receptor complex, enhancing the likelihood of a strong and specific binding conformation.

Key observations

1. Hydrogen bonds with residues such as GLU 365 and ASP 210 were consistently observed, demonstrating their pivotal role in stabilizing the ligand-receptor complex.

2. Ionic interactions, particularly with PHE 267, further reinforced binding stability, contributing substantially to the overall binding energy.

3. The detailed interaction profiles for key residues are summarized in Table 4.

The dominance of hydrogen bonds and ionic interactions across the binding interface underscores their importance in determining binding specificity and stability. This detailed analysis provides a foundation for further exploration of ligand optimization strategies and can inform the development of targeted therapeutic interventions.

Table 4: Detailed Ligand-Receptor Interactions

Residue	Interaction Type	Distance (Å)	Energy Contribution (kcal/mol)
GLU 365	Hydrogen Bond	2.76	-9.4
GLY 258	Hydrogen Bond	3.23	-1.0
ASP 210	Hydrogen Bond	3.20	-5.1
TRP 269	Hydrogen Bond	2.90	-3.6
PHE 267	Ionic Bond	2.76	-6.3

DISCUSSION

The binding mechanisms underlying the observed ligand-receptor interactions provide critical insights into the molecular forces driving binding stability and efficiency within the ROS1 binding pocket. This section explores the role of key residues, the contributions of different scoring modes, and their relevance in designing high-affinity ligands for therapeutic and veterinary applications. The analysis of ligand-receptor interactions identified several critical residues that consistently contributed to binding stability. These residues act as molecular hotspots, mediating specific interactions crucial for ligand retention and efficacy. Their contributions are supported by previous findings on ROS1 and other receptor families [11, 12]. GLU 365 and ASP 210 (Hydrogen Bonding Hotspots): These residues formed stable and consistent hydrogen bonds across all scoring modes. Hydrogen bonding plays a pivotal role in stabilizing the ligand-receptor complex, particularly in polar environments such as the ROS1 binding pocket. For instance, GLU 365 demonstrated a significant energy contribution (−9.4 kcal/mol), emphasizing its importance as a primary binding site. Similarly, ASP 210 (−5.1 kcal/mol) provided stabilization by anchoring the ligand through electrostatic interactions. These findings align with studies highlighting the critical role of glutamic and aspartic residues in ligand-receptor interactions [13]. PHE 267 and GLY 258 (Electrostatic and Hydrophobic Stability): PHE 267 contributed through ionic interactions (−6.3 kcal/mol), reinforcing the electrostatic stability of the complex, particularly under electrostatic-favored scoring modes. Ionic bonds are essential for mediating high-affinity interactions in charged binding pockets, as shown in similar veterinary receptor studies [14]. On the other hand, GLY 258 formed weaker hydrogen bonds (−1.0 kcal/mol), which, while modest, supported the structural orientation of the ligand. TRP 269 (Versatile Hydrophobic Stabilizer): The interaction of TRP 269 with the ligand highlights the significance of hydrophobic residues in nonpolar environments. With an energy contribution of −3.6 kcal/mol, TRP 269 aids in burying the ligand within the receptor's hydrophobic core, a

mechanism critical in the stability of many veterinary drug molecules targeting similar receptors [15]. The scoring modes used in the analysis provided complementary insights into the diverse forces governing ligand-receptor interactions. Each mode emphasizes unique aspects of binding stability, offering a multifaceted view of molecular docking. In Balanced Scoring Mode (Comprehensive Binding Profile): This mode integrates all major forces, including hydrogen bonds, ionic interactions, and hydrophobic effects. It serves as a holistic approach to evaluate overall binding efficiency, capturing both polar and nonpolar contributions. The comprehensive nature of the balanced mode has been validated in other docking studies targeting veterinary enzymes [16].

In Electrostatic-Favored Scoring Mode (Charge-Driven Binding): Charge-based interactions, such as ionic bonds and polar hydrogen bonds, dominated in this mode. Residues like GLU 365 and PHE 267 demonstrated high contributions, reflecting the charged nature of the ROS1 binding pocket. Electrostatic interactions are particularly important in veterinary medicine for targeting polar regions of enzymes and receptors, especially in inflammatory and infectious diseases [17].

In Hydrophobic-Favored Scoring Mode (Buried Residue Stabilization): This mode highlights the importance of nonpolar interactions in ligand binding. Cluster 5, with the strongest hydrophobic stabilization (−1369.8 kcal/mol), underscores the role of buried hydrophobic residues like TRP 269 and PHE 267. Hydrophobic forces are crucial for drug molecules designed to penetrate lipophilic environments, such as cellular membranes or hydrophobic binding pockets [18].

In Van der Waals + Electrostatics Scoring Mode (Synergistic Interactions): This mode balances short-range Van der Waals forces and long-range electrostatic interactions, capturing the intermediate effects of these forces. Cluster 10, with a score of −202.4 kcal/mol, represents a cluster with strong combined interactions. Such scoring modes are frequently employed in veterinary docking studies for designing drugs that require multi-force stability.

Understanding the binding mechanisms of the ROS1 binding pocket is critical for advancing veterinary therapeutics. The identified residues and scoring modes provide a blueprint for designing ligands that optimize binding stability and specificity. For instance, veterinary drugs targeting inflammatory or cancer pathways could leverage the insights on hydrogen bonding and hydrophobic stabilization to enhance therapeutic efficacy and reduce off-target effects. Moreover, the analysis of different scoring modes highlights the importance of considering diverse interaction forces during drug design. Future directions could include experimental validation of the computational findings using techniques such as crystallography or NMR spectroscopy, along with in vivo efficacy studies in veterinary models. Such approaches would provide a robust framework for developing next-generation veterinary drugs.

CONCLUSION

This study provides a comprehensive understanding of the binding mechanisms of Pediococcus acidilactici-derived peptides with ROS1, utilizing advanced computational docking, statistical analyses, and detailed interaction profiling. The key findings of this study are as follows:

1. Key Residues and Binding Stability: Residues such as GLU 365, ASP 210, PHE 267, and TRP

269 were identified as critical contributors to binding stability, with each playing a unique role in mediating hydrogen bonding, electrostatic interactions, and hydrophobic stabilization.

2. Scoring Modes and Binding Efficiency: The use of multiple scoring modes provided a multifaceted view of ligand-receptor interactions, highlighting the importance of balancing polar and nonpolar forces in drug design.

3. Therapeutic and Agricultural Applications: The findings have significant implications for designing peptide-based inhibitors targeting ROS1 in veterinary medicine and improving animal health and productivity through microbiome-based strategies in agriculture.

ETHICS

Approved.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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