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ORIGINAL ARTICLE / ÖZGÜN MAKALE

STRUCTURAL INSIGHTS AND ANTICANCER POTENTIAL OF MELITTIN IN CD147 INTERACTION

MELİTTİN'İN CD147 İLE ETKİLEŞİMİNE YAPISAL BAKIŞ VE ANTİKANSER POTANSİYELİ

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ABSTRACT

Objective: *This study investigates the interaction between melittin (PDB ID: 2MLT), a bioactive peptide from honeybee venom, and CD147 (PDB ID: 5XF0), a glycosylated transmembrane protein implicated in tumor progression.*
Material and Method: *Employing molecular docking and bioinformatics tools, our structural analysis reveals diverse binding features, including hydrogen bonds, salt bridges, and non-bonded contacts, between the CD147 complex and melittin.*
Result and Discussion: *Non-bonded interactions between 2MLT and specific amino acids (Gly181 and Arg201) of CD147 are highlighted, resembling aspects of the CypA/CD147 binding mechanism (Pro180-Gly181 and Arg201). The elevated anticancer potential of 2MLT was substantiated by utilizing the AntiCP 2.0 server and the ENNAACT server, employing machine learning and artificial neural network algorithms. Additionally, hydrophobicity analysis aligns with characteristics associated with anticancer peptides. Notably, thermodynamic stability variations with temperature underscore the robust binding affinity of 2MLT to the 5XF0 receptor. While our study comprehensively explores molecular interactions and predictive analyses, further in vitro and in vivo investigations are crucial to validate these findings for potential therapeutic applications.*
Keywords: *Anticancer peptides, CD147, cyclophilins, melittin, molecular docking*

ÖZ

Amaç: *Bu çalışma, arı zehrinden elde edilen biyoaktif bir peptit olan melittin (PDB ID: 2MLT) ile tümör ilerlemesinde rol oynayan bir glikozile transmembran protein olan CD147 (PDB ID: 5XF0) arasındaki etkileşimi incelemektedir.*
Gereç ve Yöntem: *Moleküler bağlanma ve biyoinformatik araçlar kullanılarak yürütülen yapısal analizimiz, CD147 kompleksi ile melittin arasında hidrojen bağları, tuz köprüleri ve bağlanmamış temaslar dahil olmak üzere çeşitli bağlanma özelliklerini ortaya çıkarmaktadır.*
Sonuç ve Tartışma: *2MLT ile CD147'nin belirli amino asitleri (Gly181 ve Arg201) arasındaki etkileşimlerin, CypA/CD147 bağlanma mekanizmasına (Pro180-Gly181 ve Arg201) benzer şekilde oluştuğu gözlemlenmiştir. 2MLT'nin yüksek antikanser potansiyeli, AntiCP 2.0 server ve ENNAACT server kullanılarak, makine öğrenimi ve yapay sinir ağı algoritmalarını içeren yöntemlerle desteklenmiştir. Ayrıca, hidrofobiklik analizi, antikanser peptitlerle ilişkilendirilen özelliklerle uyumludur. Özellikle, sıcaklıkla olan termodinamik stabilite değişiklikleri, 2MLT'nin 5XF0 reseptörüne güçlü bağlanma eğilimini vurgulamaktadır. Çalışmamız, moleküler etkileşimlerin*

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kapsamlı bir keşfini ve analizlerini sunarken, bu bulguların potansiyel terapötik uygulamalar için geçerliliğini doğrulamak için ileri in vitro ve in vivo çalışmalarının zorunlu olduğunu belirtmektedir.
Anahtar Kelimeler: *Antikanser peptidler, CD147, melittin, moleküler bağlanma, siklofilinler*

INTRODUCTION

Cancer poses a significant threat to the survival of living organisms, with high morbidity and mortality rates. Conventional cancer therapies demonstrate effectiveness primarily against malignant tumors. Yet, their efficacy diminishes in advanced stages due to metastasis, recurrence, heterogeneity, resistance to chemotherapy and radiation therapy, and immune evasion. Additionally, the presence of cancer stem cells has been identified as a contributing factor to treatment failures. In pursuing more successful cancer treatments, numerous studies have identified biomarkers and signaling pathways crucial in promoting malignant features like proliferation, anti-apoptosis, invasion, angiogenesis, therapeutic resistance, and stemness [1,2]. To overcome the limitations of conventional medications and their associated adverse effects, there is a concerted effort to explore alternative treatment strategies. Among these, the utilization of biotoxins, including those derived from animal venom, has gained prominence. These biotoxins, developed by living organisms as a defense mechanism against predators, exhibit toxicological and pharmacological effects [2].

Melittin is a biologically active peptide found in the venom of the honeybee (*Apis mellifera*), constituting approximately half of the venom's composition. This peptide exhibits an amphiphilic structure comprising 26 amino acids [2,3]. The therapeutic potential of melittin has been reported to encompass anti-inflammatory, anti-cancer, and anti-microbial effects [4]. Although the therapeutic potential of melittin is constrained by its hemolytic activity, molecular biology techniques offer avenues for N- and C-terminal adjustments, residue substitutions with both natural and unnatural amino acids, hybridization, cyclization, truncation, arginine enrichment, and the addition of C-terminal cysteine for optimizing the peptides' characteristics [5].

Melittin exhibits a specific binding affinity to phosphatidylcholine membranes [6] and is more attractive to negatively charged membranes within cancer microenvironments. Consequently, its affinity for the membranes of cancer cells, rich in anionic phospholipids, surpasses that of healthy cells [7]. Melittin demonstrates heightened activity in disrupting cancer cells, as evidenced by its targeted and redox-responsive conjugates exhibiting anticancer efficacy against MCF-7, C33A, and HeLa cancer cell lines in a study by Sahsuvar (2023) [8]. The extensive investigation of melittin for the treatment of various cancer types reveals recent advancements, including discussions on its synergistic combination with standard anticancer drugs and the recent progress in formulating a nano-version of melittin to enhance targeted delivery [9].

Further exploration of melittin's direct cytotoxic effects on cancer cells and its diverse immunomodulatory functions is highlighted. Due to its unique dual mechanism of action, involving cell cycle arrest, apoptosis, regulation of cancer cell pathways such as metastasis, angiogenesis, and inflammation through interactions with various signaling molecules, melittin is considered a broad-spectrum antitumor agent [9]. Its impact extends to significant molecular targets associated with growth inhibition and apoptotic induction, including Bax, Bcl-2, caspases, Akt, HIF-α, NF-kB, Wnt, STAT3, matrix metalloproteinases (MMPs), VEGF, and TNF-α [2]. A noteworthy study asserts that melittin-loaded niosomes exhibit more excellent anticancer effects compared to free melittin, underscoring the suitability of niosomes as vesicle carriers for melittin in comparison to its free form [10].

CD147, also known as basigin or EMMPRIN, is a glycosylated transmembrane protein abundant on tumor and stromal cell surfaces, functioning as an inducer of matrix metalloproteinases and a promoter of tumor progression. CD147 is implicated in various mechanisms related to tumor cell invasion, metastasis, and angiogenesis [11]. Recent studies highlight CD147's potential as a biomarker and therapeutic target for various diseases, including cancer, due to its involvement in oncogenic signaling pathways [1].

A study focused on the role of CD147 in the development and diagnosis of hepatocellular carcinoma elucidates its molecular structure and regulatory role in cancer progression [11]. Another

investigation into the expression and functional roles of CD147 in breast cancer cells reveals its involvement in crucial protein modulation associated with functions such as cell migration, invasion, drug resistance, and cancer progression [12].

Initially identified as a regulator of MMP, CD147 emerges as a promising target for cancer therapy due to its engagement in cell-matrix and cell-cell interactions. Beyond MMP regulation, CD147 is overexpressed in cancer cells and plays a regulatory role in cell proliferation, drug resistance, and cell stromal adhesion properties. Additionally, it possesses diverse functions, interacting with various molecular partners to modulate multiple signaling pathways. CD147's role extends to angiogenesis by regulating the production of vascular endothelial growth factor (VEGF) in tumor and stromal cells. It also impacts cancer-associated fibroblasts, promoting tumorigenesis and development. For instance, CD147 expression on melanoma cells induces tumor cell invasion by stimulating fibroblast production of matrix metalloproteinases [11].

Furthermore, the interaction between Cyclophilin A (CypA) and CD147 is pivotal in signal transduction. This interaction, facilitated by binding Pro180 amino acid and subsequent interaction through Pro211, induces signaling. The Glu218 amino acid is crucial for signal response. The CypA/CD147 interaction generates signals outside the cell through proline isomerization, leading to signal transmission inside the cell [1]. Significantly, the CypA/CD147 interaction induces the expression of MMP-2 and MMP-9, essential for the invasion and metastasis of cancer cells [13].

The objective of this study is to elucidate the interaction between melittin and CD147 through the utilization of molecular docking and bioinformatics tools.

MATERIAL AND METHOD

The molecule with Protein Data Bank (PDB) ID 5XF0 represents the three-dimensional structure of the solute CD147's Ig1 domain (residues 99–205 at the C-terminal Ig domain of CD147). This structure was obtained using the nuclear magnetic resonance (NMR) method [14]. The molecule with PDB ID 2MLT corresponds to the three-dimensional structure of melittin, a significant toxin from *Apis mellifera*. This structure, comprising 26 amino acid residues, was determined using X-ray diffraction at a resolution of 2.00 Å [15].

These molecules have been saved in PDB format. It is noted that the structure of 2MLT is a homotetramer composed of chains A and B [15], with chain A selected as a ligand for the docking procedures.

The amino acid sequence of the selected chain (GIGAVLKVLTTGLPALISWIKRKRQQ) was obtained from the PDB. Subsequently, using default settings (Model 1, SVM threshold: 0.45) on the AntiCP 2.0 server [16], sequence-specific parameters (hydrophobicity, hydropathicity, amphipathicity, hydrophilicity, charge, isoelectric point, and molecular weight) were predicted. Hydrophobicity analysis was additionally validated using the ProtScale program (The SIB Swiss Institute of Bioinformatics, Swiss) employing the Kyte-Doolittle method (scoring window size: 3, the relative weight of the window edges compared to the window center: 100%).

The anti-cancer activity of the ligand was investigated using the ENNAACT web server, and the anti-cancer activity scores were predicted to vary within the range of 0 to 1 as normalized sigmoid scores [17].

The amino acid sequence of the ligand was submitted to the PEP-FOLD4 server to predict its secondary structure. The files corresponding to the best model were converted to PDB format and subsequently saved for later use. The sequences and chain IDs of the receptor, 5XF0, and the ligand were verified in PyMOL.

Subsequently, the A chain of 5XF0 was retrieved from the PDB database, and the single-chain form of the ligand was uploaded from the local computer memory to perform docking using the ClusPro 2.0 server [18–21] The docking process was conducted with default settings.

The PDB file of the receptor-ligand complex was uploaded to the Protein Binding Energy Prediction (PRODIGY) server to compare the strength of protein-protein interactions and their thermodynamic stabilities under specific conditions at temperatures of 25°C and 40°C. The free energy change (ΔG, kcal.mol^{-1}) and dissociation constant Kd (M) were predicted from this.

The PDB file of the receptor-ligand complex was analyzed using the Uniprot PDBsum tool. This tool facilitated the visualization of interactions between polypeptide chains, residues, and atoms, including hydrogen bonds, non-bonded contacts, and salt bridges. The interaction region designates the receptor protein chain with ID A and the ligands' protein chain with ID B.

RESULT AND DISCUSSION

The PDB file representing the receptor-ligand interaction was visualized in PyMOL using the molecular surface method. The visualizations included the structural examination of the 5XF0 and 2MLT molecules, revealing their chain structures and interactions (Figure 1).

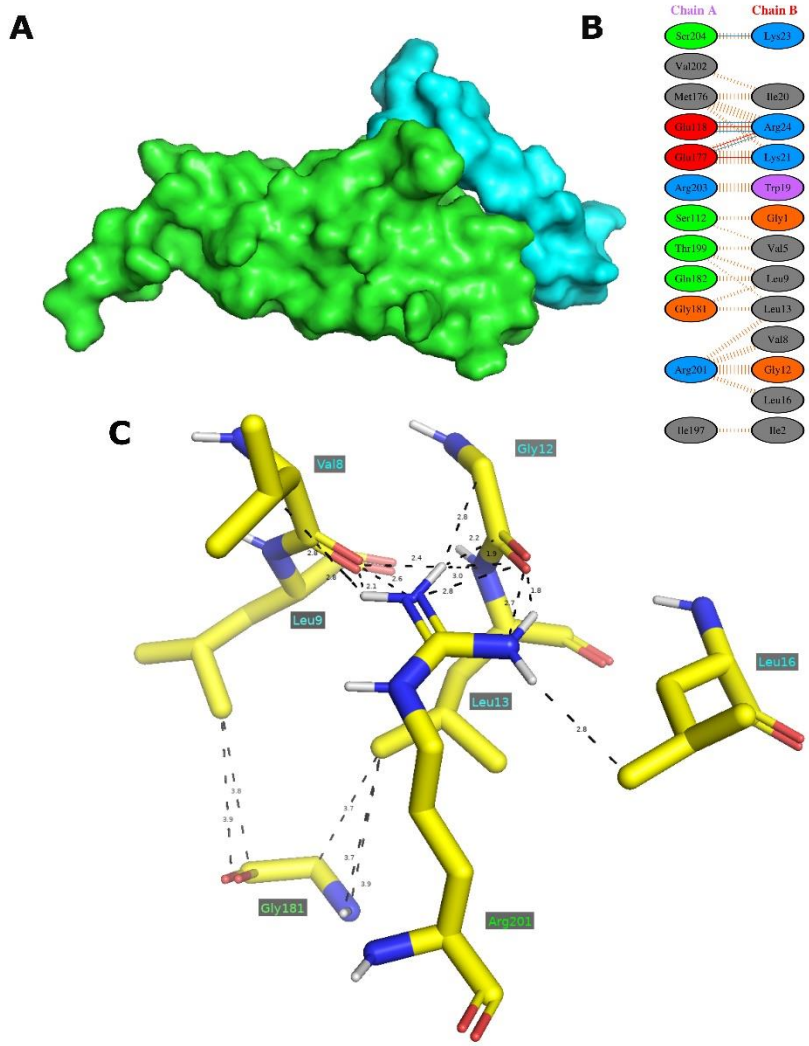

Figure 1. Illustration and molecular interactions of the CD147 Ig1 domain with melittin ligand: docking model and PDBsum analysis

The interaction between the Ig1 domain of CD147 protein and the melittin ligand was investigated. (A) The docking model illustrates the interaction between the IgI domain of the CD147 protein (depicted in green) and the 2MLT ligand (depicted in cyan). (B) The molecular interactions between CD147 (chain A) and the 2MLT ligand (chain B) residues were elucidated using PDBsum. The names of amino acid residues are inscribed within oval shapes of various colors. Key interactions between residues are represented by lines, with color codes indicating salt bridges (in red), hydrogen bonds (in blue), and non-bonded contacts (in orange). (C) The interaction between 5XF0 and 2MLT demonstrates non-bonded contacts involving Gly181 with Leu9 and Leu13, and Arg201 with Leu13, Val8, Gly12, and Leu16. A set of labels represented by green rectangles corresponds to the CD147 protein, while labels depicted in cyan rectangles indicate the amino acids of the 2MLT ligand. The black dashed lines represent non-bonded contacts, and the accompanying numerical values indicate the distances of these interactions in angstroms

The percentage of participating amino acids in the receptor-ligand interaction was delineated. Valine (15%), serine (15%), arginine (15%), glycine (15%), and lysine (10%) collectively constituted 70% of the interaction, with the remaining amino acids (isoleucine, threonine, methionine, lysine, glutamine, tryptophan) contributing to the rest.

The structural analysis revealed four hydrogen bonds between Ser204, Glu118, and Glu17 residues of 5XF0 and Lys23 and Arg24 residues of 2MLT, along with three salt bridges between Glu118 and Glu177 residues of 5XF0 and Arg24 and Lys21 residues of 2MLT. Additionally, 79 non-bonded contacts were identified between various amino acids.

The designed peptide and prediction results obtained from the AntiCP 2.0 server are presented in Table 1, providing the ligand's SVM score, terms describing its interaction with water, and chemical properties. The ligand's high hydrophobicity was observed based on the Kyte–Doolittle method obtained from ProtScale (Figure 2).

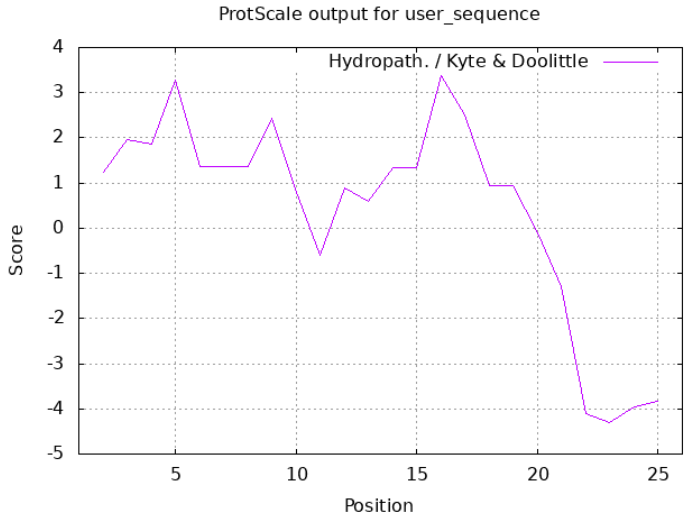

Figure 2. Hydrophobicity analysis of peptide sequence using ProtScale
The peptide sequence (GIGAVLKVLTTGLPALISWIKRKRQQ) underwent hydrophobicity analysis using the ProtScale tool. The horizontal axis represents the amino acid positions, while the vertical axis depicts the hydrophobicity levels. Values above zero correspond to the presence of hydrophobic amino acids within the peptide

The anticancer activity prediction results from the Employing Neural Networks for Anticancer Activity Classification for Therapeutic Peptides (ENNAACT) server are also presented in Table 1, including the ligand's amino acid sequence and a normalized sigmoid score (PROB score) within the range of 0-1.

Table 1. Integrated results of AntiCP 2.0 server for designed peptide and predictions, alongside anticancer activity predictions from the ENNAACT server

ID	Sequence	SVM	Hydro-phobicity	Hydro-pathicity	Amphi-pathicity	Hydro-philicity	Charge	pI	Mol wt	PROB
2MLT	GIGAVLKVLTTGLP ALISWIKRKRQQ	1.0	-0.08	0.27	0.71	-0.20	5.00	12.03	2847.91	0.997

The table presents details regarding the input query sequence. The PROB score from the ENNAACT server is a normalized sigmoid score ranging from 0 to 1. A score of 0 signifies a high probability of being a non-anticancer, whereas a score of 1 denotes a high probability of being an anticancer. Abbreviations: pI, isoelectric point; SVM, support vector machine score

The receptor-ligand complex resulting from docking, with the highest cluster members (model 0), was saved in PDB format. Scores and coefficients for this model are presented in Table 2, indicating that the VdW+Elec mode of the 2MLT model had the highest cluster members.

Table 2. Docking model rankings in various modes and corresponding free energy changes and dissociation constant values at two different temperatures

Receptor-Ligand Complex	Balanced	Electrostatic-favored	Hydrophobic-favored	VdW+Elec	ΔG (kcal.mol^{-1})	Kd (M) at 25.0°C/40.0°C
5XF0-2MLT	112	126	179	297	-9.6	8.4e-08/1.8e-07

The table presents cluster rank values based on the docking model (Model 0) across different modes: Balanced, Electrostatic-favored, Hydrophobic-favored, and VdW+Elec. Moreover, in the VdW+Elec mode, the table includes measurements of free energy changes (ΔG, kcal.mol^{-1}) and dissociation constant Kd (M) values, recorded at two distinct temperatures (25.0°C and 40.0°C)

PRODIGY server outputs for receptor-ligand docking, also presented in Table 2, provided ΔG and Kd values to compare the strength and thermodynamic stability of protein-protein interactions under different temperature conditions. Notably, the 5XF0-2MLT complex exhibited strong ΔG and binding affinity (-9.6 kcal.mol^{-1}; 8.4e-08 M). Additionally, as temperature increased, a consistent decrease in binding affinity between the receptor and ligand was observed, aligning with the patterns of ΔG and binding affinity.

The investigation of the 5XF0 complex and its interaction with the 2MLT molecule, as determined by the PDBsum server, reveals several noteworthy binding characteristics. A thorough analysis of the 5XF0-2MLT interactions unveiled a diverse array of interaction types, including hydrogen bonds (4 occurrences, ranging from a minimum of 2.73 Å to a maximum of 2.85 Å), salt bridges (3 occurrences; ranging from a minimum of 2.59 Å to a maximum of 2.76 Å), and non-bonded contacts (a total of 79 contacts among 22 amino acids; ranging from a minimum of 2.56 Å to a maximum of 3.89 Å). These interactions imply that the specific arrangement of distinct amino acid residues can bolster the stability of the complex and influence binding affinities. Establishing weak interactions originating from hydrogen bonds can guide recognition and binding processes. The potential for hydrogen bonds among the amino acids of the receptor and ligand relies on specific groups in their side chains capable of forming hydrogen bonds, enabling these amino acids to engage in various hydrogen bond formations with each other and other molecules. Salt bridges represent ionic interactions between the receptor and ligand, contributing to increased stability and specific molecular recognition. This aspect is critical in determining the nature of interactions between the receptor and ligand. The distinctive binding characteristics among amino acids may hold mechanistic significance for subsequent investigations.

Increased expression of CypA and CD147 in the signaling cascade of tumor cells triggers cancer pathogenesis [1,22]. CypA facilitates signal transduction by binding to Pro180 and Pro211 of CD147. The crucial role of Glu218 in this mechanism has also been reported [1]. According to a recent study, the CypA/CD147 binding process is regulated by Pro180-Gly181, and Arg201 is identified as an essential residue for binding [23]. Similarly, in our study, the interaction between 5XF0 and 2MLT reveals non-bonded contacts between Gly181 and Leu9, Leu13 and Arg201 with Leu13, Val8, Gly12, and Leu16, indicating specific interactions among amino acids without chemical bonding. The binding of CypA to CD147 induces conformational changes acting as a molecular chaperone, while the non-bonded interactions of 2MLT are a subject for further investigations in subsequent studies.

The SVM score of the ligand is at its maximum level (1.0), indicating a potentially high anticancer capacity for this peptide. Trained explicitly on a dataset of 861 anticancer peptides and 861 non-anticancer peptides using machine learning, the AntiCP 2.0 server [16] performed anticancer scoring for 2MLT. The potential anticancer peptide feature of 2MLT may be explained by its interaction with CD147, yet further *in vitro* and *in vivo* investigations are necessary.

According to the Kyte-Doolittle method, the hydrophobicity values for 2MLT are notably high. This observation suggests the presence of hydrophobic features that tend to bind to the target cell membrane, a characteristic often associated with anticancer peptides. However, it can be argued that the effects of the hydrophobicity feature of 2MLT on its interaction with CD147 cannot be predicted based on the results of this study. Nevertheless, evaluating interaction forms of amino acid residues through hydrogen bonds and salt bridges may provide insights for future studies.

Artificial neural networks that classify anticancer peptides take specific peptide features (e.g., amino acid sequence, hydrophobicity, electric charge) as input. They are trained to classify the anticancer activity based on these features. Due to their ability to learn patterns in large and complex datasets, artificial neural networks can assist in understanding and classifying the properties and activities of anticancer peptides [17]. In our study, the ENNAACT server, trained with neural network algorithms, indicates the high potential anticancer activity of 2MLT due to its high PROB score (0.997), which the AntiCP 2.0 server has corroborated. However, further *in vitro* and *in vivo* investigations are required.

Since 2MLT exhibits the highest cluster members in the VdW+Elec mode, it can be inferred that this mode most strongly supports the interaction of 2MLT. According to the PRODIGY server's results for receptor-ligand docking, lower (negative) ΔG values indicate stronger protein-protein interactions. Lower Kd values signify strong binding (high-affinity interaction). Lower ΔG and Kd values indicate a more robust and more stable interaction. This suggests that 2MLT has a preferred binding affinity to the 5XF0 receptor. The binding energy score of the complex varied with temperature. With the elevation in temperature, a discernible reduction in the binding affinity between the receptor and the ligand was observed. Nevertheless, it can be asserted that the 5XF0-2MLT complex exhibits a diminished sensitivity to the temperature increment. This observation implies a decline in thermodynamic stability concomitant with the temperature rise.

In conclusion, this study delved into the structural aspects and molecular interactions between the 5XF0 complex and the 2MLT molecule, revealing intricate binding features such as hydrogen bonds, salt bridges, and non-bonded contacts. Exploring the interaction between 5XF0 and 2MLT elucidated specific amino acid assemblies influencing complex stability and binding affinities. The study also shed light on the role of the non-bonded interactions of 2MLT, which remains a subject for future investigations. The anticancer peptide (2MLT) demonstrated a high SVM score, suggesting a potential anticancer capacity supported by its interaction with CD147. The study leveraged machine learning and neural networks to classify anticancer peptides, providing insights into 2MLT's possible activity. Analyzing hydrophobicity values and thermodynamic stability variations with temperature also offered valuable perspectives. While the study comprehensively explores molecular interactions and predictive analyses, further *in vitro* and *in vivo* investigations are imperative to validate the findings and translate them into potential therapeutic applications.

AUTHOR CONTRIBUTIONS

Concept: B.D.; Design: B.D.; Control: B.D.; Sources: B.D.; Materials: B.D.; Data Collection and/or Processing: B.D.; Analysis and/or Interpretation: B.D.; Literature Review: B.D.; Manuscript Writing: B.D.; Critical Review: B.D.; Other: -

CONFLICT OF INTEREST

The author declares that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The author declares that the ethics committee approval is not required for this study.

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