Molecular Docking Simulations to Identify Ubiquitin Carboxyl-Terminal Hydrolase Isozyme L1 (UCLH1) Biomarker and Therapeutic Target for Aggressive Neuroendocrine Carcinomas

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Published Jun, 2025

Abstract: Neuroendocrine carcinoma is a rare and aggressive form of cancer that affects the endocrine (hormones) and nervous systems, with a 5-year survival rate of only 39%. Detecting and treating neuroendocrine carcinoma is difficult due to late diagnosis, tumor heterogeneity, and limited effective therapies. UCHL1 is a novel protein found to be elevated in the tissue and plasma of affected NEC patients and can be used as a potential biomarker and therapeutic target. In our work, we have utilized molecular docking simulations to predict inhibitors that can bind to the UCHL1 protein and be used in the diagnosis and treatment of neuroendocrine carcinomas. We hypothesized that ligands will effectively bind to the binding site of the UCHL1 protein. We then used various software to create the UCHL1 protein structure, identify potential ligands, and perform molecular docking simulations. The Alphafold 3 software was used to represent the UCHL1 protein structure visually using its amino acid sequence. The ScanNet software identified the potential binding site. 1092 chemical compounds were scanned and then docked on the UCHL1 binding site using the Autodock Vina software. The results of the molecular docking simulations identified five ligands with the highest binding potential with the UCHL1 binding site. The pharmaceutical properties and binding energy calculations indicate high gastrointestinal absorption, binding potential, and drug likeness. This current work will help develop a novel diagnostic agent for Neuroendocrine carcinoma and pave the way for many potential new treatment methods against the disease.

1. INTRODUCTION

Making up only 0.5% of all cancers, Neuroendocrine carcinomas are cancers that affect the endocrine (hormones) and nervous systems. [1] Neuroendocrine cells regulate the body's hormone levels by producing hormones such as adrenaline, gastrin, insulin, and serotonin. Neuroendocrine tumors originate from these cells and can occur in various locations affecting hormones, including the digestive tract, skin, lungs, endocrine glands, pancreas, and reproductive system. [2] These tumors disrupt the body's state of homeostasis, causing issues with energy levels, mood, and digestion. [2] They can lead to serious health issues such as anemia, heart damage, liver damage, bowel obstruction, seizures, etc. Symptoms of neuroendocrine cancer vary based on the location of the tumor and which hormone it affects. [3] General symptoms include anxiety due to hormone imbalance, coughing up blood, blood stools, facial flushing, dizziness, fatigue, loss of appetite, seizures, etc. Current treatment for neuroendocrine carcinomas varies based on what stage the cancer is in, its location, and what hormone it affects. Typically, treatment involves chemotherapy and radiation therapy, which often harm healthy cells in the process. There is also existing medicine that blocks the production of a specific hormone to prevent the spread of the tumor. Surgical options such as hepatic embolization can be used to kill tumors. These methods often come with dangerous side effects and aren't reliable. [4]

Scientists at the UCLA Cancer Research Center have identified a protein, UCHL1. [5] UCHL1, or ubiquitin carboxy-terminal hydrolase L1, is a deubiquitinating enzyme that may contribute to the poor prognosis of neuroendocrine cancer. [5] The UCHL1 protein was found in high amounts in patients with aggressive neuroendocrine carcinomas. It promotes the progression of cancer by regulating nucleoporin, POM121, and p53 (proteins involved in cell function). It can, therefore, be used as a marker for diagnosing neuroendocrine cancer. Additionally, inhibiting the function of UCHL1 can be utilized to slow the growth of these tumors. [5]

Molecular Docking can be used to determine potential in-

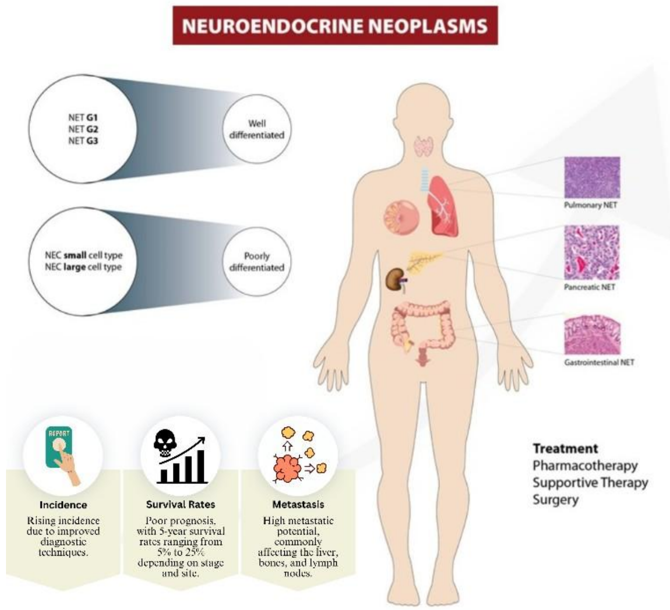

Fig. 1. Schematic representation of the aggressive neuroendocrine carcinomas.

hibition strategies. [6] Molecular Docking is a computational simulation used to predict interactions between two structures, particularly proteins and drugs. To perform molecular docking successfully, it is crucial to identify a strong potential binding site. After the site is identified, this method considers electrostatic interactions, Van der Waals forces, Coulombic forces, and hydrogen bonds to assess ligand-protein compatibility. The sum of the interactions between these two structures results in a docking score that indicates the bonding potential. This is done through a two-step algorithm. First, the simulation examines the conformational space in which the molecule can bind to the target. Then, it calculates the energy value required for coupling at each site. The lowest energy values outputted are considered the most promising sites.

2. METHOD

First, the amino acid sequence was obtained from Uniprot. [7] Then, the amino acid sequence was uploaded onto the AlphaFold 3 server to produce the protein's 3D structure. [8] The protein's 3D structure was also uploaded to P2Rank to create an additional representation of the protein, highlighting various surface residues. [9] ScanNet was then used to identify the potential binding site of UCHL1. [10] To determine which ligands would best inhibit UCHL1, 1092 ligands were downloaded from the ZINC database through simulation. In preparation for the molecular docking process, we created a folder containing the following files: 1.pl, 2.pl, 3.pl, conf_vs.txt, UCHL1.pdbqt, vina, and model files. The various ligands were then analyzed to determine if they could bind to the UCHL1 active site. Finally, we performed molecular docking simulations. AutodockVina is a flexible molecular docking program that accurately explores the binding potential between proteins and ligands. [11] To perform molecular docking, we first prepared the necessary files within each folder, including scripts named 1.pl, 2.pl, and 3.pl, a configuration file (conf_vs.txt), the protein structure file (UCHL1.pdbqt), all required Vina files (vina.exe, vina_license.rtf, vina_split.exe, Vina_windows.pl), and model files containing 1,000 ligand structures each. For the docking step, we executed the Perl scripts sequentially—Perl 1.pl, Perl 2.pl, Perl 3.pl, and Perl vina_windows.pl—and entered Ligand.txt to initiate the docking of ligands to the UCHL1 protein. In the final analysis phase, we ran Python analysis_1.pl > 1.txt, followed by Python analysis_2.pl, to evaluate binding scores and identify the top ligand candidates for further study.

3. RESULTS

In our current research, we conducted molecular docking simulations to identify potential ligands that can bind to the UCHL1 protein, serving as biomarkers and therapeutic targets for aggressive neuroendocrine carcinomas.

Surface properties of UCHL1: Figures 2a and 2b depict the predicted binding site of UCHL1. A binding site is a region on a macromolecule, such as a protein, where a specific molecule, known as a ligand, can bind. Figure 2a was determined using a deep learning-based model through the ScanNet software. Figure 2b was determined using a machine learning- based method through the P2Rank software. Figure 2c shows the electrostatic surface potential map of UCHL1. This displays the three-dimensional charge distributions of molecules. The ChimeraX software was used to create the ESP model.

Molecular Docking and Analysis: We used Autodock Vina for molecular docking simulations. The results of the molecular docking simulations identified five ligands that are most likely to bind to the UCHL1 binding site. These may be utilized in the development of drugs for neuroendocrine carcinoma. The 2D interactions formed between the protein and ligands are shown in Figure 3.

Gastrointestinal Absorption: All the ligands showed a high

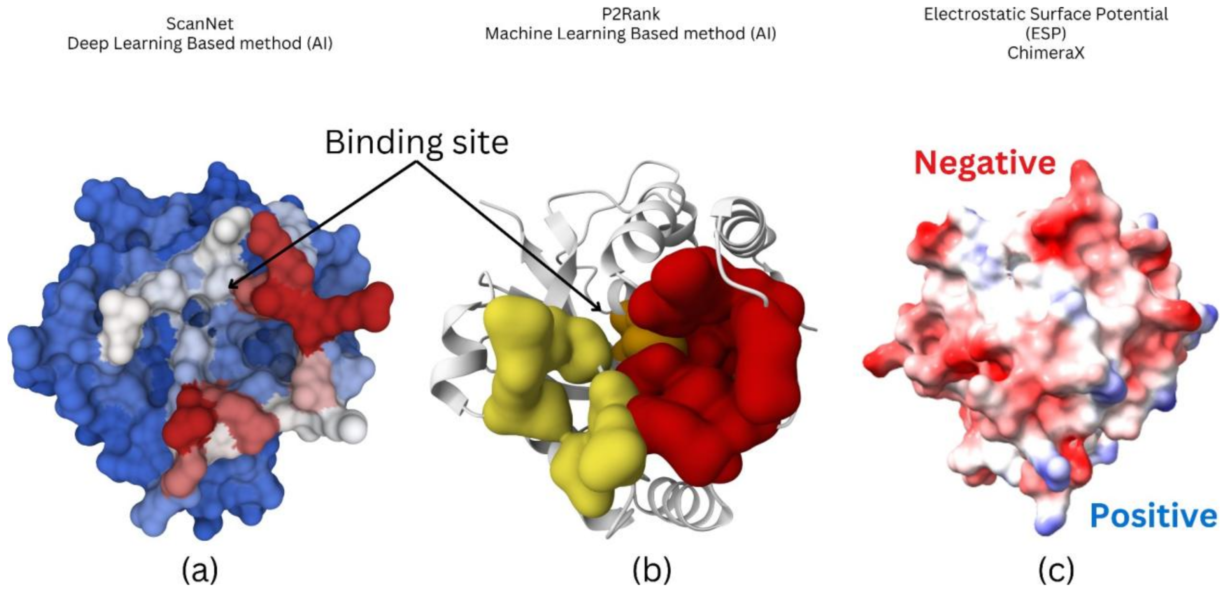

Fig. 2. Surface properties of the UCHL1 protein:(a) The red and white regions indicate the binding site on the protein surface, while the blue region represents the non-binding region. This calculation was performed using deep learning; (b) The red and yellow areas indicate the binding site on the protein's surface. The gray region is the nonbinding region. This model was calculated using a machine learning method; (b) The red region indicates the negatively charged areas of the protein, while the blue region indicates the positively charged areas. The white areas are neutral and have no charge. This model was calculated using the ChimeraX software.

GI absorption. GI absorption tests whether the drug can be absorbed through the gastrointestinal tract and ingested by the stomach. A high GI Absorption indicates that the drug can be successfully injected and is a positive sign when considering drug likelihood. Blood-brain barrier permeation: All the ligands tested negative for blood barrier permeation. The blood-brain barrier is a highly selective membrane that protects the brain by regulating which molecules can pass into the brain. Blood-brain barrier permeability is a measure used to determine whether a drug will pass through barrier and reach the brain. (Lipinski): All the ligands tested positive for drug likeness. Drug likeness is an important factor in determining whether a chemical substance can be developed into a drug. Lipinski's rule of 5 is useful in determining whether a compound is drug-like. It tests for a Molecular Weight < 500 Da, the number of H bond acceptors < 10, number of hydrogen bond donors < 5, Rotatable bonds < 10, and Lipophilicity < 5. If the compound adheres to these rules, it has a good chance of being developed into a drug.

4. DISCUSSION

The biophysical and compositional properties of a protein play a crucial role in guiding rational drug discovery. In the first panel, deviations from population averages in amino acid composition and structural properties help identify features that distinguish the target protein. For example, an unusual enrichment in aromatic residues or disordered regions suggests the protein may be involved in dynamic interactions or signaling pathways, which are often implicated in disease. These unique characteristics can be leveraged to design drugs with higher specificity, reducing the likelihood of off-target interactions and adverse effects.

The second panel shows the windowed charge distribution across the protein, highlighting regions of positive and negative electrostatic potential. These charged zones are critical in identifying druggable pockets, as many drug molecules form ionic or polar interactions with their targets. Positively charged areas may attract negatively charged ligands such as nucleic acids or phosphate-based compounds, while negatively charged regions are favorable for binding to cationic drugs or peptides. Charge mapping is particularly valuable when designing inhibitors for DNA-binding proteins, ion channels, or surface receptors involved in cancer or neurological diseases.

The third panel illustrates the folding propensity of different regions in the protein sequence, which provides insight into structural dynamics. Folded regions are typically well-defined and can harbor enzymatic active sites or ligand-binding pockets, making them prime candidates for structure-based drug design. Conversely, intrinsically disordered regions (IDRs) often mediate protein-protein interactions and can be targeted with small molecules or peptides that induce conformational changes. These insights are particularly useful for targeting challenging proteins involved in signaling pathways, transcription regulation, or phase separation, expanding the range of therapeutic interventions beyond traditional active-site inhibition.

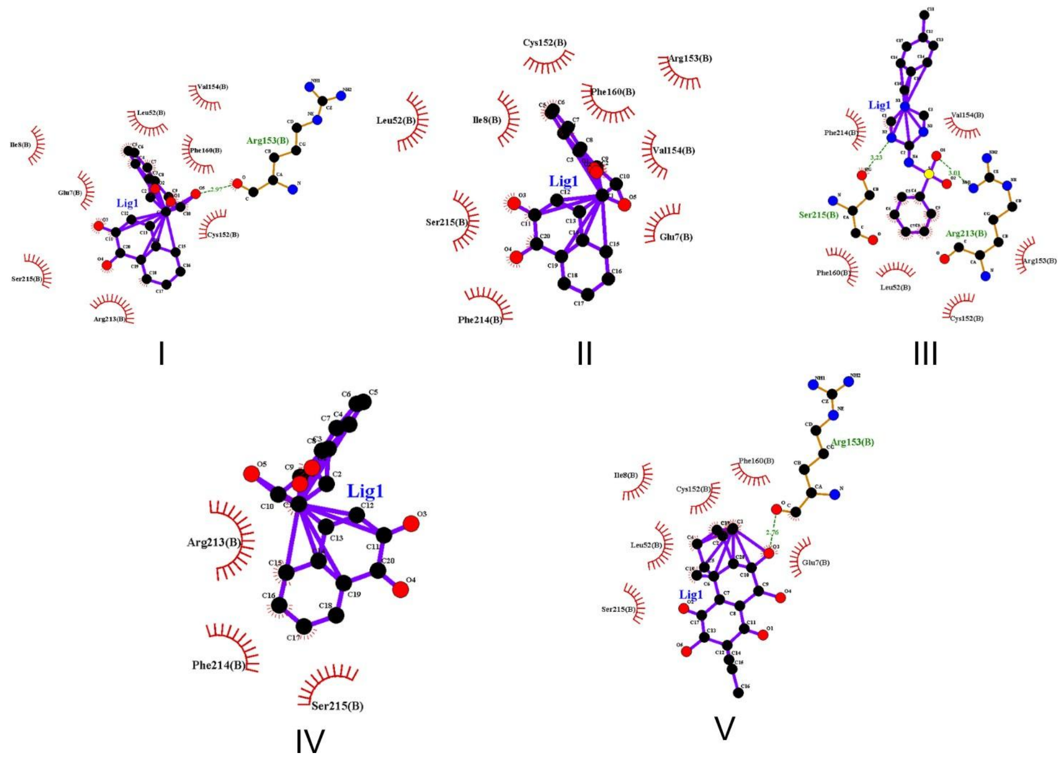

Fig. 3. Protein-ligand 2D interactions: The purple-colored structures represent the ligands, and the surrounding amino acids are depicted in blue. The hydrogen bonds are highlighted in green, and the red eyelash structures are hydrophobic interactions.

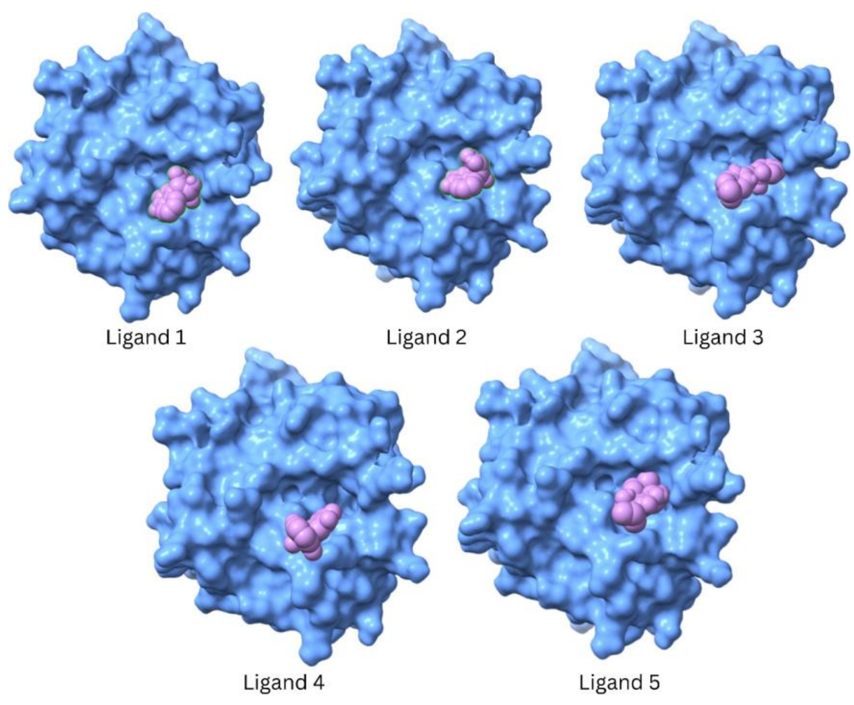

Fig. 4. Ligands at UCHL1 binding site: The pink structures are the ligands, and the blue structure is the UCHL1 protein. The figure shows how the ligands bind to the site.

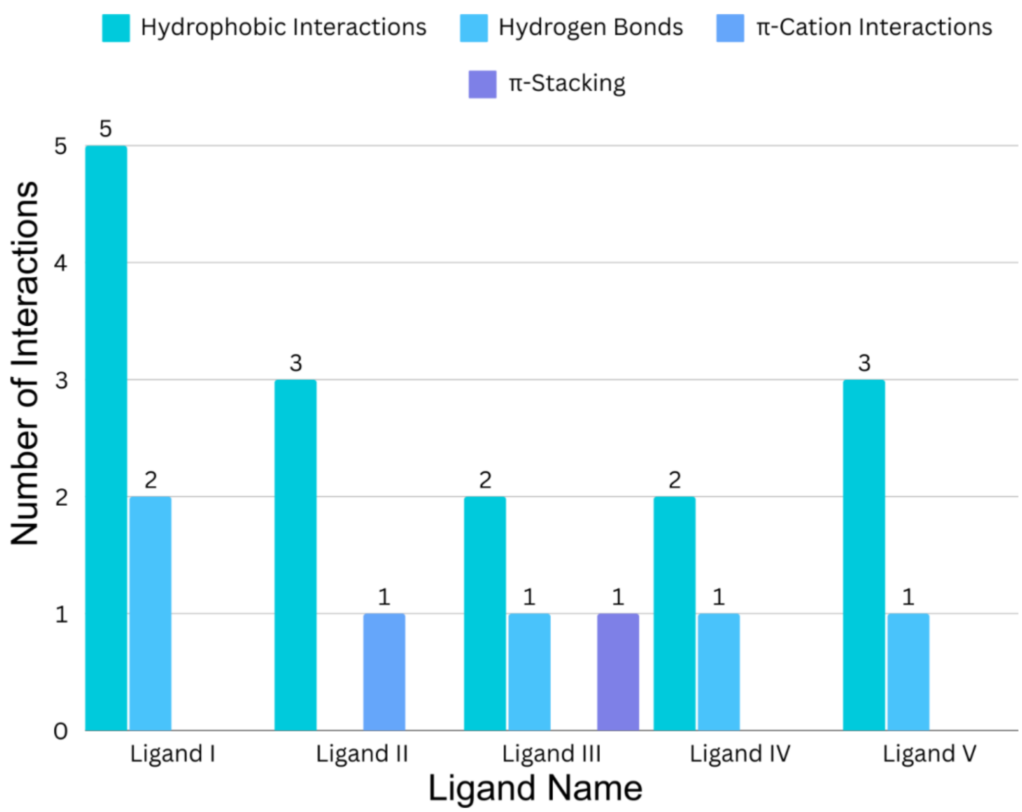

Fig. 5. Protein Ligand interactions.

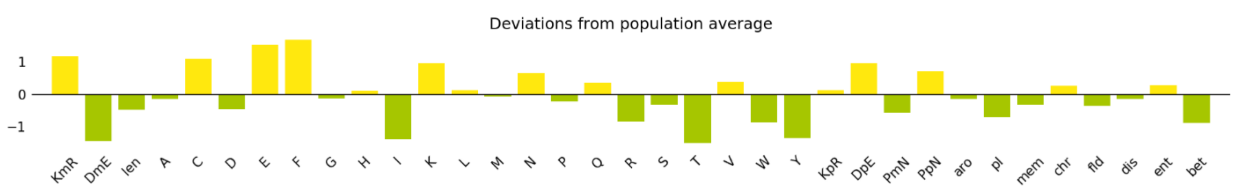

Fig. 6. Deviation of Protein Features from Population Averages. This chart compares the target protein's amino acid composition and structural/functional properties with those of a reference population of proteins. Positive deviations (yellow bars) indicate enrichment, while negative deviations (green bars) suggest depletion, highlighting unique characteristics relevant for targetability and drug design.

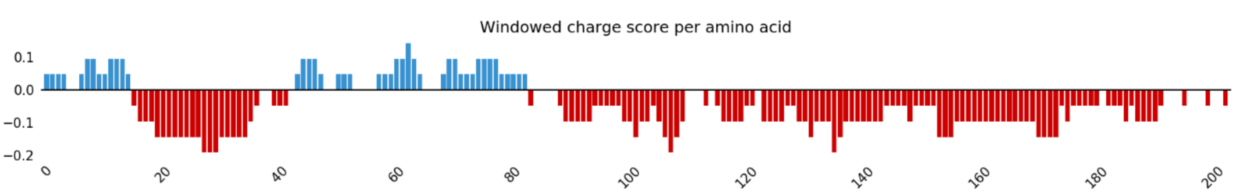

Fig. 7. Windowed Charge Distribution Along the Protein Sequence. The graph displays localized charge scores calculated using a sliding window across the amino acid sequence. Blue bars represent regions with net positive charge, and red bars indicate negatively charged segments—key indicators for identifying electrostatically active binding or interaction sites.

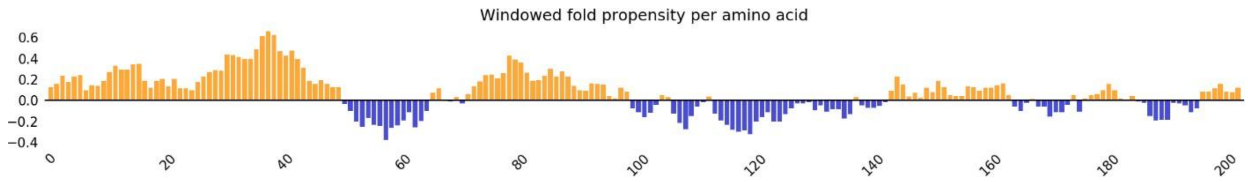

Fig. 8. Windowed Fold Propensity of the Protein Sequence. This chart shows the predicted folding potential of different sequence segments. Orange bars indicate regions with high propensity to form stable structures, while blue bars suggest disordered or flexible regions. These data are crucial for identifying structured domains versus dynamic regions, each offering different drug-targeting strategies.

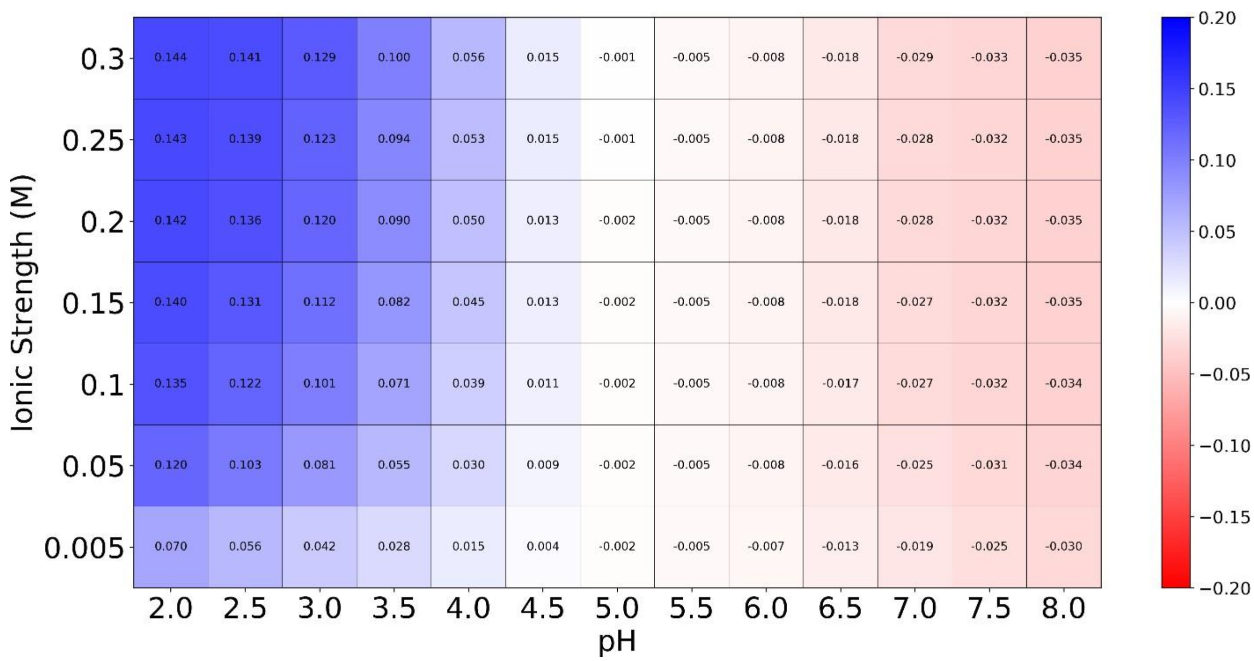

Fig. 9. This heatmap illustrates the variation in electrostatic interaction energy (or a related physicochemical parameter) as a function of pH and ionic strength, providing valuable insights for drug discovery and formulation. In the context of therapeutic development, such data can be instrumental in identifying optimal conditions for drug-target binding. For example, the shift from positive to negative values across the pH gradient suggests a change in electrostatic behavior, which can directly influence ligand binding affinity or nanoparticle stability. This is particularly relevant when designing pH-sensitive drug delivery systems, such as aptamer-based therapeutics or nanoparticle formulations, that require effective function in environments like the acidic tumor microenvironment or intracellular compartments. Moreover, the ionic strength-dependent behavior helps in understanding solubility and aggregation tendencies, which are crucial for ensuring drug stability and bioavailability. Overall, the heatmap serves as a powerful tool in rational drug design, helping researchers tailor therapeutic agents and delivery platforms to specific physiological or pathological conditions.

Limitations: One of the primary limitations of this study is the reliance on virtual experiments, which precludes direct experimental validation. The molecular docking simulations conducted with 1,092 ligands restrict the generalizability of the findings, as they do not encompass the full range of potential ligand interactions. This limitation is further exacerbated by the low permeability of the tested ligands across the blood-brain barrier, which diminishes the practical applicability of the results. Enhancing the validity of these findings necessitates physical experimentation and an expansion of the ligand repertoire to include a broader diversity, which may yield more comprehensive insights. Consequently, future research should prioritize these areas to bolster confidence in the study's conclusions and foster more robust applications in targeted neuroendocrine carcinoma therapies.

Furthermore, the transition from virtual to in-person applications is essential for reinforcing the current results and addressing the limitations imposed by the computational nature of the study. Conducting physical experiments would allow direct observation and validation of ligand interactions, thereby heightening the confidence in the study's findings. Additionally, expanding the library of ligands beyond the 1,092 used in this research would enhance the robustness and applicability of the results, offering a broader understanding of potential interactions and therapeutic targets. This expansion could reveal novel ligand conformations and improve their permeability across the blood-brain barrier, an identified limitation needing resolution to advance drug delivery strategies effectively. In summary, both in-person validation and an inclusive ligand range are central to the evo-

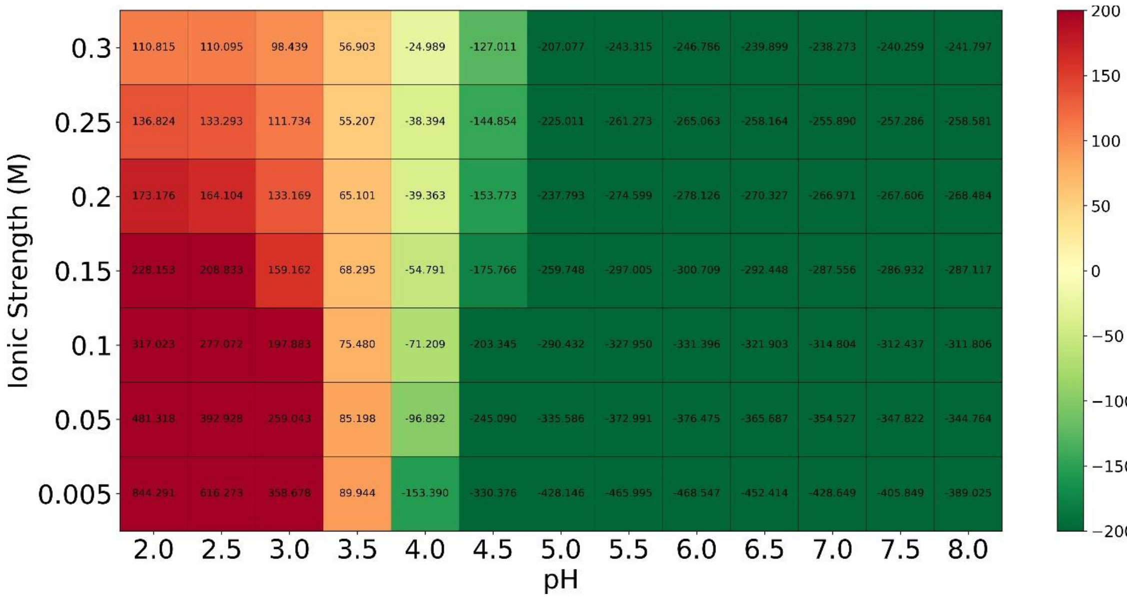

Fig. 10. The energy heatmap provides critical insights into optimizing drug-target interactions under varying physiological conditions. It reveals that binding is most energetically favorable at mildly acidic pH values (around 5.5–6.0) and low ionic strength (0.005 M), with the minimum energy reaching –468.547 J/aa, indicating highly stable interactions. In contrast, high energy values at low pH and high ionic strength suggest destabilized or unfavorable binding. This trend highlights the importance of electrostatic interactions in drug design, as increasing salt concentrations likely shield key charge-based interactions. Such data can guide drug formulation strategies by identifying optimal pH and salt conditions that maximize drug efficacy, particularly for biologics, peptides, or aptamer-based therapeutics that are sensitive to their biochemical environment.

lution of the research and its contributions to neuroendocrine carcinoma therapies.

Moreover, addressing the low permeation of ligands across the blood-brain barrier is crucial for enhancing the therapeutic efficacy of the investigated compounds. The current study reveals a gap in ligand permeability that hinders the potential application of the results in practical drug development. To overcome this, strategies such as employing advanced drug delivery systems, optimizing the physicochemical properties of the ligands to improve their lipid solubility, and utilizing specific transporter mechanisms can be considered. These approaches may significantly enhance the capability of therapeutic molecules to traverse the barrier effectively, thereby increasing their bioavailability in target brain regions. Future work in this area could focus on integrating these permeability enhancements with ligand binding studies to develop more effective and targeted treatments for neuroendocrine carcinomas.

Future Studies: Research should progress toward experimental validation of ligand binding to the UCHL1 protein, a critical area for future exploration in the context of neuroendocrine carcinomas. According to Grethe et al., this validation can illuminate specific interactions at the molecular level, offering insights into therapeutic targeting strategies. Additionally, enhancing drug permeability through the blood-brain barrier remains a significant focus, as improving ligand delivery systems can increase therapeutic efficacy. Integrating advanced drug delivery approaches with measured permeability outcomes could effectively boost ligand concentrations at key brain sites, further advancing treatment potential. Future studies may also consider genetic modifications or the use of external stimuli, such as ultrasound, to disrupt the blood-brain barrier transiently, thereby facilitating more effective drug passage. Additionally, future studies aiming to overcome the current limitations could employ a multifaceted approach integrating both technological and methodological advancements. One promising methodology involves the use of high-throughput screening technologies to evaluate a broader spectrum of ligands, extending beyond the initial set of 1,092, which could provide a more comprehensive assessment of ligand efficacy and specificity. Enhancements in computational modeling, such as integrating artificial intelligence and machine learning algorithms, could refine the accuracy of molecular docking simulations, thereby reducing reliance on in-person experiments. Meanwhile, methodologies that enhance ligand permeability across the blood-brain barrier, such as the development of novel nanoparticle-based delivery systems, promise to augment drug bioavailability and efficacy in target brain regions. These advancements, coupled with the experimental validation of ligand-UCHL1 interactions, could significantly advance the development of targeted therapies for neuroendocrine carcinomas.

Pharmaceutical Properties of the Ligands: This table indicates that all ligands exhibit high GI absorption and drug likeness but low BBB permeation. These properties were computed using the SwissADME and is shown in Table 1.

Applications: The results of this study have practical applications in the development of targeted therapies for neuroendocrine carcinomas, mainly through the regulation of the

Table 1. Pharmaceutical Properties of the Ligands: This table shows that all the ligands exhibit high GI absorption and drug likeness but low BBB permeation. These properties were computed using the SwissADME.

	I	II	III	IV	V
Gastrointestinal Absorption	High	High	High	High	High
Blood-brain barrier permeation	No	No	No	No	No
Drug likeliness (Lipinski)	Yes	Yes	Yes	Yes	Yes
Binding energy	-5.34	-5.33	-5.36	-5.26	-6.20

Table 2. Types of UCHL1 Protein: Characteristics and Functions.

	Characteristics	Function
Native Monomeric UCHL1	223 amino acids; ~25 kDa; soluble cytosolic protein; contains active site cysteine	Deubiquitinating enzyme (DUB); hydrolyzes ubiquitin from ubiquitinylated proteins
Dimeric UCHL1	Formed under oxidative or pathological conditions	Acts as an E3 ligase-like enzyme promoting ubiquitin ligation under some conditions
Oxidized UCHL1	Formed in oxidative stress; cysteine oxidized or S-nitrosylated	Loses DUB activity; involved in neurodegeneration (e.g., Parkinson's, Alzheimer's)
UCHL1 with I93M Mutation	Ile93 → Met mutation; linked to Parkinson's disease	Impaired ubiquitin recycling; increased aggregation propensity; mitochondrial dysfunction
UCHL1 with S18Y Polymorphism	Ser18 → Tyr variant; neuroprotective allele found in some populations	Enhances antioxidant response and stability; linked to reduced risk of Parkinson's disease
Membrane-associated UCHL1	UCHL1 is lipidated or associates with membranes	Possible role in axonal transport, synaptic function, and exosome-mediated signaling
Nuclear UCHL1	Translocates to nucleus under stress/pathology	May regulate transcription factors, chromatin remodeling, or nuclear protein degradation
Extracellular UCHL1	Secreted via exosomes or passively released	Emerging role as a biomarker for neurodegeneration and certain cancers (e.g., pancreatic, lung)

UCHL1 protein. By inhibiting the UCHL1 binding site, drug candidates could potentially prevent tumor formation, fundamentally altering the progression of these carcinomas. This inhibitory mechanism would not only target tumor growth but also enable the use of UCHL1 protein levels as a biomarker for early diagnosis, offering a dual approach in both therapeutic and diagnostic contexts [5]. Furthermore, the identification of UCHL1 as a specific target enables a more focused drug design, facilitating the creation of molecules that selectively disrupt the protein's activity, thereby enhancing treatment efficacy. As a result, these innovative strategies underscore the relevance of the study's findings in advancing therapeutic options for patients with neuroendocrine tumors.

Furthermore, utilizing UCHL1 protein levels as a biomarker for early diagnosis in neuroendocrine carcinomas holds great promise for advancing diagnostic precision in clinical settings. UCHL1's expression patterns provide a reliable indicator of tumor presence, offering a proactive measure in identifying malignancies before they progress significantly [5]. This biomarker application aligns with the dual therapeutic and diagnostic approach mentioned earlier, as it facilitates both timely intervention and guides treatment strategies targeting the UCHL1 binding site. Additionally, early detection through UCHL1 levels could enable more personalized treatment regimens, enhancing patient outcomes by tailoring therapies to the specific molecular profile of the tumor. Thus, future research should focus on optimizing techniques for reliably measuring UCHL1 in biological samples, ensuring its effective integration into routine diagnostic protocols for neuroendocrine carcinomas.

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