

Structure-Based Drug Discovery of New Compounds Targeting Exon 1 N-terminus Region of Mutant Huntingtin

Yash Jaju¹ and Divya Ramamoorthy^{1*}

¹ Moxie Scientist, Georgetown, Texas, USA.

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ABSTRACT

Affecting hundreds of thousands of people around the world, Huntington's Disease is a neurodegenerative disorder causing involuntary movements, poor-decision making, nervous system shutdown, and ultimately death. The aim of this project was to identify molecules as inhibitors to limit the disease-causing mutation, known as mHTT. We used the PDB 4FEB in this study. Three groups of compounds, CNS compounds, mHTT inhibitors, and peptidomimetics that we screened in the study. In addition, we analyzed the binding site and created a docking grid to prepare the mHTT protein for SP and XP docking. We also performed Wikprop analysis, which gave us the log BB, molMW, and PercentHumanOralAbsorption of the ligand. Finally, we created ligand interaction diagrams between the top-performing ligands and the 4FEB chain to demonstrate the key interactions. While the top-scoring ligands are likely not directly suitable for clinical use in treating or preventing Huntington's Disease, our goal is to continue researching ligands that can drive the design of new compounds for effectively targeting and defeating Huntington's Disease.

INTRODUCTION

Huntington's disease (HD) is a hereditary neurodegenerative disorder characterized by progressive loss of motor function, cognitive decline, psychiatric disturbances, and dementia¹. The disease affects medium spiny neurons in the striatum of the basal ganglia, along with cortical neurons, which leads to the clinical symptoms of neurodegeneration of the striatum and cortex and loss of control over voluntary movement and cognitive ability². In the early stages of the disease, the primary symptom is involuntary movement, and people are able to perform their daily tasks as usual. However, the disease gradually worsens over a period of 10-20 years following its onset, and many of the observable clinical symptoms are present³.

In Western countries and Oceania, Huntington's disease affects approximately 5-10 individuals per 100,000 population, which is far greater than observed in Asian and most African

populations, where estimates range from 0.1-0.5 per 100,000⁴. An example of this is that a person of Japanese descent has a one-tenth less chance of developing Huntington's Disease⁵. Geographic differences between the two regions of the world are thought to influence the disease's risk across their populations. In European populations, chromosomes are grouped into 3 main haplogroups: A, B, and C, with haplogroups A1 and A2 being the highest risk of HD. The chromosomes of East Asian populations are more closely associated with those of haplogroup C, the least risk haplogroup for Huntington's Disease⁶.

The disease is caused by an expansion of the CAG codon on chromosome 4p16.3's short arm, which is the mutation that leads to HD. Normally, there are 10-35 repetitions of the codon cytosine-adenine-guanine, but the expansion coding for Huntington's Disease contains 36 or more of these repetitions⁷. The normal, non-mutated HD gene codes for the HTT protein when translated, a large protein with 3,144 amino acids⁸. However, when the mutation is present, the gene codes for the mutated protein mHTT, which is the toxic form of the HTT protein. mHTT includes a polyglutamine expansion of the exon-1 N-terminus (EX1) of the HTT protein, affecting the way it is folded and hence its function⁹. This mutated protein damages neurons and their functions, ultimately leading to the degradation of the neurons in the basal ganglia, and hence the disease¹⁰.

The average age of onset for Huntington's disease is 35 years of age, but additional mutations of the CAG expansion can lead to earlier onset. 40-50 repetitions of the CAG codon lead to adult-onset HD, while 50-120 repetitions lead to juvenile HD, where symptoms can begin to show as early as the teenage years¹¹.

In this work, we applied structure-based drug design techniques to aid in the identification of novel classes of compounds. In contrast to traditional drug screening, structure-based drug design integrates structural insights with computational docking and scoring functions to identify molecules with optimized binding capabilities with the target structure. We selected the mHtt36Q-EX1 region as the target structure because it effectively demonstrates the expanded polyglutamine characteristic of the Huntington's Disease gene. Among the four available structures for this region, we selected the mHtt36Q-EX1-X1-C2(Beta) crystal structure PDB 4FEB due to its high atomic resolution at 2.8Å, providing near-atomic detail of the conformational structure and ligand binding site¹². Previous studies have shown that the EX1 N-terminal sequence before the polyglutamine tract influences the progression of Huntington's Disease⁹, so the structure chosen in our research was carefully selected before proceeding.

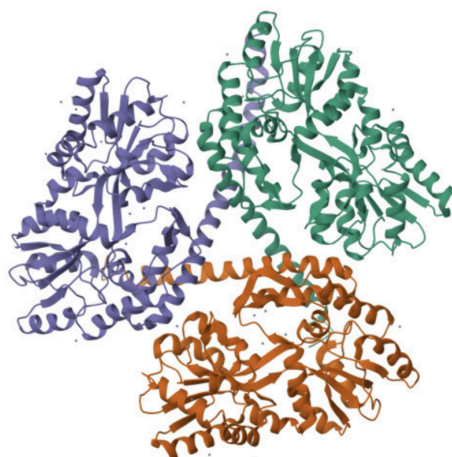


Figure 1. Crystal Structure of Htt 36Q3H-EX1-X1-C2(Beta) (PDB: 4FEB).

We attempted to develop peptidomimetics and cyclopeptides to target the pathological consequences of CAG expansion. As the initial step towards the design of a peptidomimetic or a cyclopeptide, we have screened the peptidomimetics library. As compared to traditional drugs, which are small molecules and protein therapeutics, peptidomimetics are promising for targeting intracellular proteins that are traditionally considered undruggable, complementing treatment solutions with small molecules¹³.

MATERIALS AND METHODS

Computational Drug Discovery Workflow

All computational studies, including protein preparation, ligand optimization, molecular docking, and binding affinity calculations, were performed using the Schrödinger Suite 2024-3 (Schrödinger, LLC, New York, NY).

Protein Preparation

The crystal structure(s) of the target proteins were retrieved from the Protein Data Bank (PDB). Before docking studies, the protein was prepared using the Protein Preparation Wizard (Schrödinger Suite 2024-3) to ensure a chemically and sterically sound structure. This process involved several steps:

1. Bond Order Assignment: Covalent bonds, bond orders, and formal charges were assigned.
2. Hydrogen Addition: Hydrogen atoms were added to the protein structure, and their positions were optimized.
3. Metal Ion State Correction: The protonation and oxidation states of metal cofactors (e.g., zinc, magnesium) were corrected.

4. Water Removal: All water molecules outside a 5 Å radius of the active site were removed. The remaining water molecules were retained.
5. Side Chain Correction: The protonation states of histidine residues were determined based on the local hydrogen-bonding network.
6. Energy Minimization: A final energy minimization was performed using the Optimized Potentials for Liquid Simulations (OPLS4) force field to relieve steric clashes and optimize the geometry of the protein, while maintaining the positions of heavy atoms.

Ligand Preparation

The ligands used in this study were obtained from Selleck, PubChem search, and NCI libraries. Each ligand was prepared using the LigPrep tool (Schrödinger Suite 2024-3) to generate three-dimensional (3D) conformers and a range of ionization and tautomeric states. This process included:

1. Protonation State Assignment: The pH was set to 7.0 ± 2.0 to generate appropriate protonation states for each ligand.
2. Stereoisomer Generation: All possible stereoisomers for each ligand were generated and retained.
3. Energy Minimization: The structures were minimized using the OPLS4 force field.

Molecular Docking

Molecular docking was performed using Glide (Schrödinger Suite 2024-3) to predict the most favorable binding pose and affinity of each ligand to the prepared protein active site.

1. Receptor Grid Generation: A grid box of dimensions [e.g., 20 x 20 x 20 Å] was centered on the native ligand (present in the crystal structure) to define the active site. The grid was generated with default settings. In the absence of a ligand in the crystal structure, the grid was centered over the residues around the binding site, as identified by SiteMap analysis, as described above.
2. Docking Calculations: Both Standard Precision (SP) and Extra Precision (XP) docking modes were employed. SP docking was used for an initial, large-scale virtual screening to filter out non-binding compounds based on a preliminary scoring function. XP docking was then used for a more rigorous and accurate assessment of the top-ranked ligands from the SP screen. XP docking uses a more extensive sampling and a more refined scoring function to better account for ligand-protein interactions, which is crucial for identifying high-affinity binders.

Physiochemical Property Prediction (QikProp)

The drug-likeness and ADME (absorption, distribution, metabolism, and excretion) properties of the top-ranked ligands were predicted using QikProp (Schrödinger Suite 2024-3). QikProp calculates over 30 relevant molecular descriptors, including molecular weight, logP, solubility, and blood-brain barrier permeability. These predictions were used to filter out compounds with unfavorable pharmacokinetic profiles, ensuring the selected

candidates were more likely to be orally bioavailable and safe.

Ligand Interaction Analysis

The nature of the interactions between the ligands and the target protein was analyzed to identify key binding motifs and residues. Visual analysis of the docked poses was performed using Maestro to identify crucial hydrogen bonds, π - π stacking, cation- π , and hydrophobic interactions between the ligand and the active site residues.

RESULTS AND DISCUSSION

As Huntington's Disease directly impacts the brain, any potential treatment must be able to cross the blood-brain barrier to effectively target intracellular processes and DNA structures. This proves a challenge for drug discovery for Huntington's Disease and emphasizes the importance of carefully selected compounds.

To address this challenge, we screened compounds for the central nervous system (CNS) database provided by SELLECK. These were selected because they were already optimized for activity within brain cells and have high log BB values, allowing for more effective penetration into the brain. In addition to these CNS compounds, we also screened peptidomimetics from a specialized peptidomimetics database. Peptidomimetics are designed to have many of the same behaviors as regular peptides while retaining a smaller size and greater stability, making them more efficient in targeting unwanted intracellular proteins compared to traditional approaches.

The ligands we screened were taken from three main groups: central nervous system compounds, known mHTT inhibitors, and peptidomimetics. Each group was included in the study for a specific reason. CNS compounds have a known ability to function in the brain, mHTT inhibitors play a direct role in mitigating the disease's mechanism, and peptidomimetics demonstrate potential to bind effectively to mutant proteins while ensuring stability is maintained in the cell.

Using molecular docking methods, we analyzed how well each ligand interacted with the target mHTT protein. Docking scores were used to measure binding affinity, as lower scores indicate stronger and more effective protein-ligand interactions. Firstly, we identified the binding site of the mHTT protein, and then prepared the ligands for docking. Glide then explored the ligand's conformations and positions through different precision levels, SP and XP, to test for the binding's speed and accuracy. After applying this method to the ligands in the three different groups, we identified the compounds that produced the best docking scores.

Table 1: Results from Qikprop

Title	mol MW	QPlogPw	QPlogPo/ w	QPlogS	HumanOralAb sorption	PercentHumanOral Absorption
16875867	409.422	16.288	1.803	-4.382	3	74.998

7						
mHTT-IN-2	395.438	13.658	2.911	-5.188	3	84.521

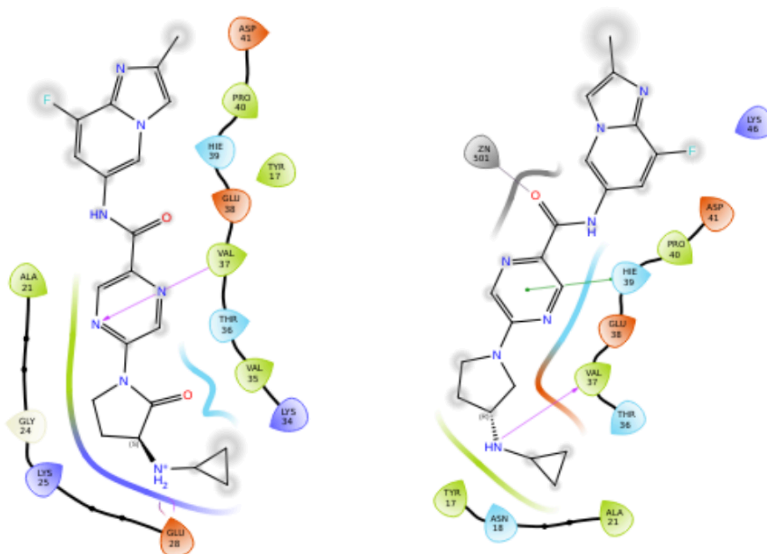


Figure 2. Ligand Interaction between 4FEB chain and the ligand 168758677 (left), Ligand Interaction between 4FEB chain and mHTT-IN2 inhibitor (right). The green arrows represent π - π stacking (a hydrophobic interaction between two aromatic rings). The pink arrows represent hydrogen bonds, with the arrow pointing from the donor to the acceptor.

QikProp showed similar results for Figure 2 and Figure 3 compounds, showing it can be used in future work. The lower docking scores mean that the ligand fits better to holding the mHTT protein together, as this is vital in preventing Huntington's Disease. Thus, for the ligands we tried, 168758677 and the mHTT-IN2 inhibitor each worked about as well.

When analyzing the first ligand interaction diagram, we observed several key amino acid residues within the binding region that contributed to ligand stabilization. The ligand interacted with residues ASP41, PRO40, HIE39, GLU38, VAL37, THR36, and VAL35, and these are clustered around the active site and known to play important roles in interactions between proteins and ligands. Additionally, interactions were observed with LYS34, TYR17, ALA21, GLY24, LYS25, and GLU28, meaning that hydrogen bonding and hydrophobic contacts are observed.

The second ligand interaction diagram showed a similar interaction pattern, meaning there is an observable consistency in ligand binding. The ligand formed interactions with ASP41, PRO40, HIE39, GLU38, VAL37, and THR36, and additional contacts with TYR17, ASN18, ALA21, and

LYS46. In this interaction, one key feature of importance is the presence of a zinc ion (ZN502) in proximity to the ligand. This may play a role in stabilization or coordination with the binding site. The similar residues between both complexes highlight an important feature in the binding region that is key for designing inhibitors in the future.

CONCLUSION

The toxicology study found that the compounds 168758677 and mHTT-IN-2 had the best docking scores and they both have similar interactions with the binding site of mHTT. Additionally, in both ligand-protein complexes, hydrogen bonds, π - π stacking, and hydrophobic interactions were formed, along with the amino acid residues ASP41, PRO40, HIE39, GLU38, VAL37, and THR36. The observation of a zinc ion in the interaction between mHTT-IN-2 and the mHTT binding site may serve as a feature of additional importance when it comes to the stabilization and ligand binding. These findings strongly support the potential of both peptidomimetics and optimized CNS compounds for further investigation.

Even though this study is limited to computational modeling and docking analyses, it demonstrates that peptidomimetics and CNS compounds have a strong potential in treating and preventing Huntington's Disease. More work is needed to discover molecular dynamics, stability, and specificity of identified compounds. Nevertheless, this research contributes to the development of novel yet effective therapeutic strategies designed to target mHTT.

AUTHOR INFORMATION

Corresponding Author

*Email: divya@moxiescientist.com

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

CAG, Cytosine–Adenine–Guanine trinucleotide repeat; CNS, Central Nervous System; EX1, Exon-1 N-terminus region; HD, Huntington's Disease; HTT, Huntingtin protein; mHTT, Mutant huntingtin protein.

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