In Silico Drug Designing and Molecular Docking Analysis of Modified Drug Analogues Against SLC18A2 Protein Target Expressed in Huntington's Disease

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Abstract: Huntington's disease (HD) is a progressive, genetic neurodegenerative disorder characterised by motor, cognitive, and psychiatric symptoms. The disease is caused by the expansion of CAG repeats in the HTT gene; however, other genetic factors, including the *SLC18A2* gene, which encodes vesicular monoamine transporter 2 (VMAT2), also play a role in disease pathology. Current treatments primarily manage symptoms without addressing the underlying neurodegeneration. This study explores the potential of targeting VMAT2 in drug design for HD. Using computational tools such as Open Targets, Swiss-Model, PrankWeb, DrugBank, ProTox, and PyMol, we identified active sites on VMAT2, screened potential drug candidates, and performed molecular docking. Our research suggests that targeting VMAT2 could be a promising strategy for treating Huntington's disease. We identified several potential drug analogues, which show strong potential to bind effectively to VMAT2 and could lead to improved treatments for HD. This approach has the potential to significantly enhance patient outcomes and quality of life by addressing the root causes of the disease. Further laboratory testing will help confirm these findings and advance the development of new therapies.

Keywords: Active sites, drug analogue, drug designing, molecular docking, Protein target.

I. INTRODUCTION

Huntington's disease (HD) is a genetic neurodegenerative disorder that progressively impairs motor, cognitive, and psychiatric functions. First described by George Huntington in 1872, HD is an autosomal dominant condition caused by an expansion of cytosine-adenine-guanine (CAG) repeats within the *Huntingtin* (*HTT*) gene located on chromosome 4p16.3 [1]. This abnormal trinucleotide expansion leads to the production of mutant huntingtin protein, which aggregates and disrupts normal cellular processes, ultimately resulting in neuronal degeneration. The number of CAG repeats correlates inversely with the age of onset and disease severity, although additional genetic factors also influence disease progression. Among these, mutations in the *SLC18A2* gene play a crucial role in regulating neurotransmitter balance, thereby contributing to HD pathophysiology [2].

The *SLC18A2* gene encodes vesicular monoamine transporter 2 (VMAT2), a protein essential for packaging and releasing monoamine neurotransmitters such as dopamine. In HD, the striatum, a brain region rich in dopamine, undergoes progressive degeneration, leading to an imbalance of dopamine and other neurotransmitters [3]. This imbalance contributes to the motor, cognitive, and psychiatric symptoms characteristic of HD. Targeting VMAT2 presents a potential therapeutic strategy for restoring neurotransmitter homeostasis. Modulating VMAT2 activity may help reduce symptom severity and improve patients' quality of life. Recent studies suggest that selectively enhancing or inhibiting VMAT2 function could offer therapeutic benefits with fewer side effects compared to broader monoamine depletion strategies. This more targeted modulation could potentially address both motor and psychiatric symptoms more effectively[4].

Currently available treatments for HD, such as tetrabenazine for chorea and various psychiatric medications, primarily address symptoms and do not halt or reverse neurodegeneration. These treatments often cause significant side effects, underlining the need for new therapies that directly target the underlying disease mechanisms [5]. While several

therapeutic approaches are under exploration, drug design strategies targeting root causes hold particular promise [6]. By slowing disease progression and alleviating core symptoms, such strategies can significantly enhance both lifespan and quality of life for HD patients. In this context, targeting *SLC18A2* (VMAT2) emerges as a promising approach for developing more effective and comprehensive treatment options, thereby expanding the scope of therapeutic research in HD [7].

This study addresses the urgent need for such treatments by focusing on novel drug design strategies aimed at targeting the *SLC18A2* protein. Our approach integrates computational modelling, virtual screening of chemical libraries, and structure- based drug design to identify lead compounds that selectively bind to *SLC18A2* [8]. By combining these methods, we aim to advance existing drug design frameworks and contribute to the development of new therapeutic options. The following sections detail the methodology, present our findings, discuss their implications, and outline directions for future research. Specifically, we explore the feasibility of targeting VMAT2 for HD therapy. By investigating the molecular mechanisms of VMAT2 and its role in neurotransmitter dynamics [9], we aim to identify drug candidates capable of modulating its activity. Through this comprehensive approach, we hope to provide valuable insights into the potential of VMAT2-targeted therapies to improve outcomes for HD patients.

II. MATERIALS AND METHODS

The overall *in silico* workflow from target identification through molecular docking and toxicity analysis is summarised in the Appendix (Fig. A1).

Target Protein Selection

The target protein for this study is vesicular monoamine transporter 2 (VMAT2), encoded by the *SLC18A2* gene. VMAT2 plays a crucial role in transporting monoamines such as dopamine, serotonin, and norepinephrine into synaptic vesicles for release into the synaptic cleft. The Open Targets Platform was utilised to identify and validate VMAT2 as a potential drug target for Huntington's disease. Open Targets is an integrative bioinformatics resource that connects genomic, transcriptomic, and clinical data to support systematic drug target discovery [10].

Prediction of Active Sites

The three-dimensional structure of the target protein (VMAT2) was retrieved from the Protein Data Bank (PDB ID: 8JSW). To ensure its reliability, the protein structure was analysed using the Swiss-Model web server. Upon uploading the structure, a Ramachandran plot was generated to evaluate the quality of the model, as shown in Fig.1. The plot showed that the majority of residues fell within the allowed regions, indicating that the model was of high quality and suitable for further analysis. This validation step is crucial as it confirms the accuracy of the protein model, making it reliable for subsequent studies[11].
Following the validation, the high-quality structure was uploaded to PrankWeb, an online tool designed to predict protein-ligand binding sites, as highlighted in Fig.2. PrankWeb analyses the protein structure and generates a list of potential active sites, each assigned a score reflecting its likelihood to bind a drug molecule effectively. This scoring system is particularly useful for prioritising which sites to target in drug development efforts, as it helps researchers focus on the most promising areas of the protein for binding interactions[12].

Screening of Drug Candidates

Screening of drug candidates is a systematic approach to identify and test potential new drugs from a large collection of chemicals. The objective is to discover compounds that interact effectively with a protein target implicated in Huntington's Disease. For this study, the protein sequence of *SLC18A2* was obtained from UniProt and downloaded in FASTA format. This sequence was then submitted to the DrugBank Target Search Tool, which provided a list of drug candidates predicted to interact with the target protein[13]. Each candidate was accompanied by data detailing binding affinity, mechanism of action, and therapeutic indications. Following the identification of potential drug candidates from DrugBank, it is essential to assess their toxicity to ensure safety for further development. This assessment involves uploading a structure file, such as the SMILES (Simplified Molecular Input Line Entry System) format, to the ProTox 3.0 tool. ProTox
3.0 analyses the chemical structure and predicts

various types of toxicity, including acute toxicity, carcinogenicity, and mutagenicity, as observed in Figs. 4 and 5. The tool provides a comprehensive report on the predicted toxicity of each drug candidate, which is crucial for determining their viability for further research and development[14].

Structural Modifications of Drug Candidates

Selected drug candidates were downloaded in Structure Data File (SDF) format from PubChem, a public chemical compound repository. These files were imported into MarvinSketch, a chemical drawing software used for editing and modifying molecular structures as presented in Fig.3. Functional groups were added or replaced to improve binding affinity and reduce toxicity. The modified molecules were then converted to SMILES format using ChemSketch (by ACD/Labs), a widely used chemical structure drawing tool [15].

The modified SMILES strings were re-evaluated using ProTox 3.0 to assess whether the structural changes reduced predicted toxicity while maintaining drug-likeness.

Molecular Docking

Molecular docking studies were conducted to analyse how modified drug candidates interact with VMAT2. The protein structure was cleaned and prepared using AutoDock Tools by removing water molecules, adding polar hydrogen atoms. Docking simulations were carried out using AutoDock Vina, an open-source program that predicts binding affinities and orientations by performing a grid-based search and scoring function optimisation [16].

The ligand structures were also prepared in PDBQT format using AutoDock Tools. A configuration file was created to define the centre and dimensions of the docking grid box. After running the docking simulations, binding affinities and interaction poses were visualized using PyMOL, a molecular visualization software used to inspect the docked conformations and hydrogen bonding interactions, as visualized in Fig. 6 [17].

Analysis of Binding Interactions

The final docking poses were analysed using PyMOL to visualize how the modified drug analogues interacted with the active site of VMAT2. Key binding interactions, such as hydrogen bonds, hydrophobic contacts, were evaluated to determine the binding mode and orientation of the ligand within the binding site [18].

III. RESULTS

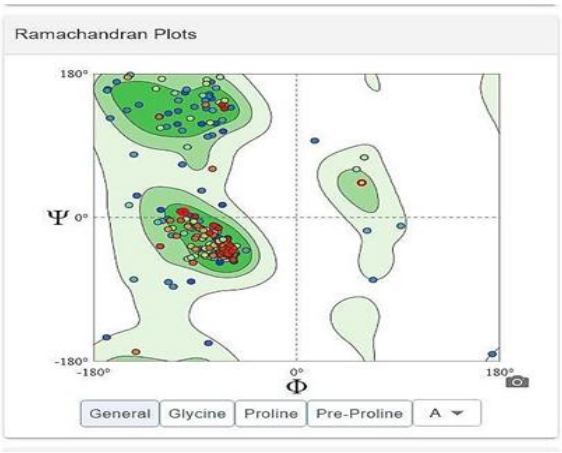

Fig. 1. Ramachandran plot for Protein *SLC18A2*. The majority of residues fall into most favoured regions, indicating a well-folded structure.

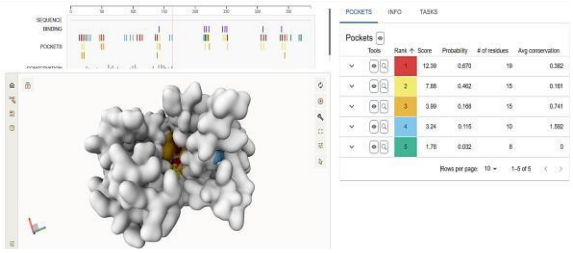

Fig. 2. 3D structure of Protein *SLC18A2* with predicted active sites highlighted in red, yellow and blue. The red-highlighted areas are high-confidence active sites. These regions are important for ligand binding.

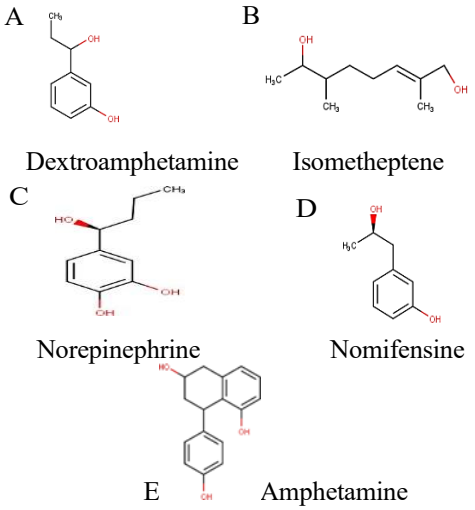

Fig. 3. Structures of drug analogues after structural modifications. Structural changes in compounds like

dextroamphetamine, isomethepteneе, norepinephrine, nomifensine, and amphetamine were made to enhance binding affinity, reduce toxicity, and improve pharmacological properties for potential therapeutic use.

Table I. Toxicity Analysis of Drug Analogues Before and After Modification

Drug Analogue	*LD50 Value (mg/kg)*	*Toxicity class*	*Toxic Effects Before Modifications*	*Improvements After Modifications*
Dextroamphet amine (A)	160	Class 3	Neurotoxicity, Respiratory toxicity, BBB barrier, Ecotoxicity, Nutritional toxicity	LD50 increased to 435, Reduced to Class 4, no toxic effects predicted
Isometheptene (B)	134	Class 3	BBB barrier, Neurotoxicity, Ecotoxicity	LD50 increased to 5000, reduced to Class 5, eliminated the BBB barrier, and Ecotoxicity
Norepinephrin e (C)	20	Class 2	Highly toxic, Respiratory toxicity	LD50 increased to 2000, Reduced to Class 4, eliminated Respiratory toxicity
Nomifensine (D)	260	Class 3	Hepatotoxicity, Neurotoxicity, Respiratory toxicity, Carcinogenicity, BBB barrier, Ecotoxicity, Clinical toxicity, CYP2D6, Acetylcholinesterase (AChE)	LD50 increased to 5000, reduced to Class 5, eliminated Hepatotoxicity, Neurotoxicity, Carcinogenicity, BBB barrier, Ecotoxicity, Clinical toxicity. CYP2D6, Acetylcholinesterase (AChE)
Amphetamine (E)	160	Class 3	Neurotoxicity, Respiratory toxicity, BBB barrier, Ecotoxicity, Nutritional toxicity	LD50 increased to 2000, reduced to Class 4, and eliminated all toxic effects.

Fig. 4(a)

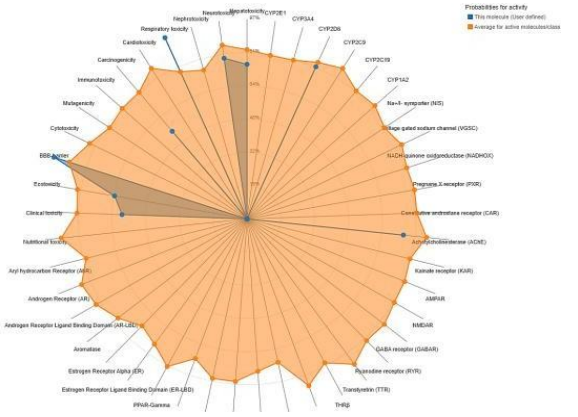

Fig. 4(b)

Fig. 4(a)(b). Drug Toxicity Before Modification and Toxicity Radar Chart for Nomifensine(D).

This figure shows the toxicity prediction results for the Nomifensine drug analogue(D) before modification. The top panel displays the predicted LD50(Lethal Dose) of 260 mg/kg, classifying the drug in Toxicity Class 3 with 100% prediction accuracy. The middle panel presents a detailed toxicity model report, highlighting active and inactive toxicity targets across various biological systems. The bottom panel features a toxicity radar chart, illustrating the compound's predicted toxicity across different

pathways and providing a visual overview of potential risks. This approach was applied to all drugs in the study.

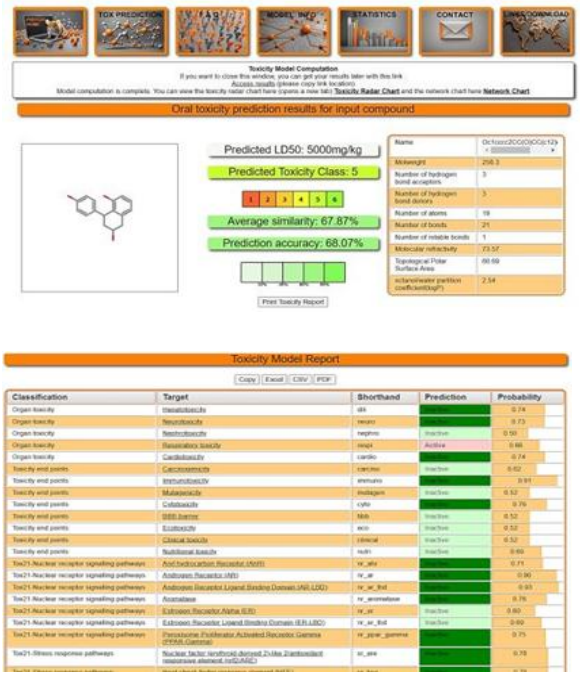

Fig. 5(a)

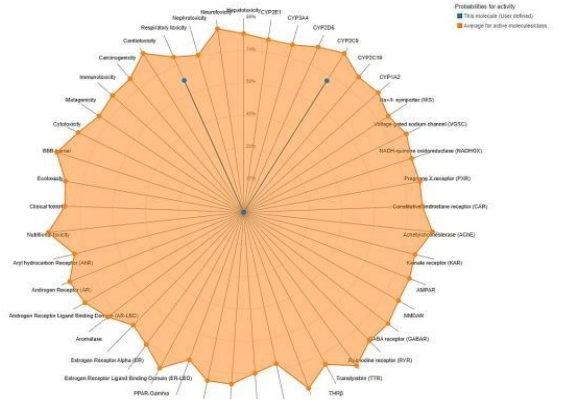

Fig. 5(b)

Fig. 5(a)(b). Drug Toxicity Analysis After Modification and Toxicity Radar Chart for Nomifensine(D).

This figure shows the toxicity analysis for the modified drug analogue. The top panel presents the updated LD50 value of 5000 mg/kg, classifying the drug in Toxicity Class 5 (low toxicity) with 100% prediction accuracy. The middle panel displays the updated toxicity model report, indicating that the modifications have successfully eliminated Hepatotoxicity, Neurotoxicity, and Carcinogenicity, BBB barrier, Ecotoxicity, Clinical toxicity, CYP2D6, and Acetylcholinesterase (AChE). The bottom panel illustrates the updated toxicity radar chart, reflecting the changes in the toxicity profile and highlighting the reduced risk across various biological pathways after modification. This approach was applied to all drugs in the study.

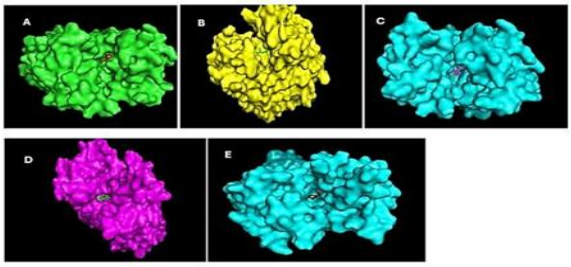

Fig. 6. Molecular Docking of Protein-Ligand Interactions

The figure shows five distinct molecular docking simulations, labelled A, B, C, D, and E. Each panel showcases a different protein structure with a ligand bound to it. The ligand colours are green (A), yellow (B), cyan (C and E), and magenta (D). This colouring highlights the binding sites within the protein structures.

Table II. Binding Affinities of Modified Drug Analogues with *SLC18A2*

Drug Analogue	*Binding Affinity (Kcal/mol)*	*Mode*	*Observations*
Dextroamphetamine (A)	-6.1	Mode 1	Moderate binding, ligand fits well in the active site.
Isomethheptene (B)	-6.2	Mode 1	Strong interaction with the active site
Norepinephrine (C)	-5.9	Mode 1	Moderate interaction, favourable binding pose.
Nomifensine (D)	-8.1	Mode 1	Strongest binding affinity observed, highly favourable interaction.
Amphetamine (E)	-5.5	Mode 5	Moderate binding, effective interaction with protein.

IV. DISCUSSION

This study explored the therapeutic potential of modified drug analogues targeting the *SLC18A2* protein (VMAT2) for Huntington's disease using an *in silico* drug design approach. Among the five

analogues designed Dextroamphetamine (A), Isometheptene (B), Norepinephrine (C), Nomifensine (D), and Amphetamine (E) the Nomifensine analogue (D) demonstrated the strongest binding affinity (-8.1 kcal/mol, Table II), suggesting a high likelihood of stable interaction with VMAT2. Molecular docking simulations (Figure 6) confirmed effective interactions with predicted active regions (Figure 2), supporting the structural relevance of the chosen binding sites. Toxicity analysis (Table I, Figures 4–5) showed that rational structural modifications led to improved safety profiles, including higher LD50 values and reductions in key toxic effects such as neurotoxicity, hepatotoxicity, and blood-brain barrier penetration.

These findings align with existing literature that emphasises the importance of reducing systemic toxicity while maintaining target-specific binding for CNS-targeted drugs [6]. Unlike conventional VMAT2 inhibitors like tetrabenazine, which are associated with significant neurological side effects, the analogues in this study may allow for more selective modulation of VMAT2 activity. Given VMAT2's role in regulating dopamine and serotonin levels, both of which are disrupted in HD [3], its targeted modulation could alleviate motor, cognitive, and psychiatric symptoms without inducing global monoamine depletion [12]. While these computational results are promising, further experimental validation through in vitro and in vivo studies is necessary to confirm their therapeutic efficacy, pharmacokinetics, and safety in a biological system.

V. CONCLUSION

This study highlights the therapeutic potential of targeting the *SLC18A2* protein (VMAT2) in Huntington's disease through a comprehensive *in silico* approach involving structural modelling, toxicity prediction, and molecular docking. Among the five modified drug analogues evaluated, the Nomifensine analogue (D) demonstrated the strongest binding affinity (–8.1 kcal/mol) and the most favourable safety profile. Structural modifications led to significant improvements in LD50 values and reduced key toxicities, including neurotoxicity and blood-brain barrier penetration, ecotoxicity, and respiratory toxicity, enhancing the overall drug-likeness of the compounds. Stable interactions observed at VMAT2 active sites support their potential to restore neurotransmitter balance in HD. These computational findings provide a strong foundation for further in vitro and in vivo validation and underscore the importance of rational drug design in accelerating the identification of promising lead candidates. Overall, this work contributes to the growing evidence that in silico strategies can effectively support early-stage drug discovery for complex neurodegenerative disorders like Huntington's disease.

VI. FUTURE PROSPECTS

The future prospects of this study involve advancing drug discovery for Huntington's Disease by refining computational techniques like molecular dynamics simulations to improve drug-protein interaction predictions and optimise drug analogues. Experimental validation through in vitro assays using *SLC18A2*-expressing cell lines will assess binding affinity and efficacy, while in vivo studies on animal models will provide insights into pharmacokinetics, pharmacodynamics, and therapeutic potential. Detailed toxicological evaluations, along with pharmacokinetic studies, will ensure the safety and feasibility of the drug candidates. Further, the integration of artificial intelligence and machine learning can enhance drug discovery efficiency, while personalised medicine and combination therapies offer promising approaches for tailored and more effective treatments. Preclinical and clinical trials will ultimately validate the therapeutic impact on disease progression and patient outcomes.

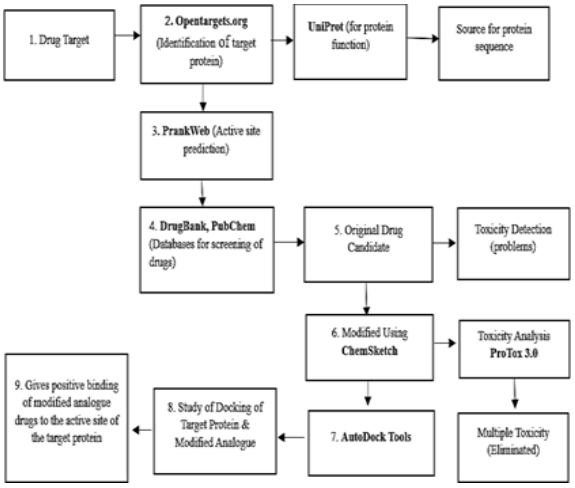

Fig. A1. Bioinformatics pipeline

This systematic workflow illustrates the application of bioinformatics tools and software to identify, design, and validate drugs targeting specific proteins, ensuring their binding efficacy and safety through detailed computational analysis.

ACKNOWLEDGMENT

F.A. expresses sincere gratitude to Mrs. Ruchita Dalvi for her support and for providing essential resources that enabled the completion of this research. Appreciation is also extended to parents, friends, and batchmates for their continued encouragement and cooperation throughout this journey.

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