European Review for Medical and Pharmacological Sciences 2023; 27: 6831-6842

Side chain inset of neurogenerative amino acids to metalloproteins: a therapeutic signature for huntingtin protein in Huntington's disease

M. NAVEED[1], N. UL AIN[1], T. AZIZ[2], M.A. SHABBIR[1], A. SALEEM[1], A. ZAFAR[1], N. GHULAM[3], M. ALHARBI[4], A. ALSHAMMARI[4], A.F. ALASMARI[4]

[1]Department of Biotechnology, Faculty of Life Sciences, University of Central Punjab, Lahore, Pakistan
[2]Department of Agriculture, University of Ioannina, Arta, Greece
[3]Institute of Nature Conservation, Polish Academy of Sciences, Krakow, Poland
[4]Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

Abstract. – OBJECTIVE: Huntington's disease is a dominant autosomal inherited neurodegenerative disease that results in progressive impairment, characterized by dementia, chorea, and behavioral and cognitive decline. The objective of this study was to investigate the potential activity of metalloproteins against the huntingtin protein using various insertion-based engineering computational methods. In this study, metalloproteins, metal protein complexes involved in important biochemical and physiological processes, were explored as potential drug candidates for Huntington's disease.

MATERIALS AND METHODS: A total of 18 metalloproteins were selected as drug candidates and studied to assess their potential inhibitory effects on the huntingtin protein. The screening was based on the lowest binding energy resulting from docking analysis. The metalloprotein with the lowest docking score was chosen for side chain insertion of neurogenerative amino acids. The engineered metalloprotein was then evaluated based on physiochemical properties, allergenicity, toxicity, and surface accessibility. Cloning and expression analysis was performed to further investigate its potential as a therapeutic agent.

RESULTS: The metalloprotein chosen for side chain insertion, cytochrome C oxidase, showed promising results. It was computed as a probable non-allergen and exhibited non-toxic domains, indicating its non-toxic nature. Additionally, it demonstrated a strong binding affinity with the huntingtin protein, with a binding energy of -1,253.3 Kcal/mol.

CONCLUSIONS: Metal-based proteins, when engineered with additional neurogenerative amino acids, hold potential as drug candidates for treating neurodegenerative diseases such as Huntington's disease. The successful development of these engineered metalloproteins could offer therapeutic advantages. Further testing, both *in vitro* and *in vivo*, is necessary to evaluate their efficacy and validate their potential activity as novel drugs for the treatment of neurodegenerative diseases.

Key Words:
Huntingtin disease, *HTT* gene, Metalloproteins, Cytochrome C oxidase, Side chains.

Introduction

The neurodegenerative disorder Huntington's disease (HD) is becoming more prevalent in the aging population worldwide[1]. Huntington's disease is a fatal autosomal dominant inherited disease associated with neurodegeneration of the central nervous system[2]. In the US, the total mortality rate due to Huntington's disease was 2.27 per million people annually. Currently, there are no treatments that can stop, slow down, or reverse the progression of HD[3]. People with HD typically die between 10 to 20 years after the onset of symptoms, mostly due to pneumonia infection and injuries incurred in falls[4]. It is characterized by progressive dementia, behavioral, and cognitive dysfunction, chorea, and uncontrolled extreme movement of motor neurons[5]. The expansion of a dominant polyglutamine (poly-Q) in the N-terminal of huntingtin protein results in protein misfolding and intracellular aggregation in inclusions is the root cause of Huntington's disease (HD)[6]. Degeneration predominates and is more prevalent in the striatum and cerebral cortex. Huntingtin is the major protein associated with HD, despite not being a metalloprotein, is an iron-responsive brain-developmental protein that

Corresponding Author: M. Naveed, MD; e-mail: dr.naveed@ucp.edu.pk; iwockd@gmail.com

regulates the transferrin receptors, one of the essential iron metabolism proteins. Huntingtin has been considered a potential biomarker for brain huntingtin in biofluids. So, biomarkers are required as they could predict the onset and progression of HD more accurately. It has also been shown[7] that cerebrospinal fluid (CFS) huntingtin levels correspond to brain levels, and elevate with the disease stage.

In the human body, metal ions play a key role in maintaining cell structure, including protein stability, function, and structure. The binding of metal ions or clusters to proteins increases the structural and functional diversity of proteins in nature. In these proteins, metal ions are present in about one-third of the whole composition. Metal ion-binding proteins, also known as metalloproteins, are involved in several biological and chemical processes in nature. Metalloproteins are essential for numerous cellular processes that support life[9]. A subclass of metalloproteins known as metalloenzymes is a metal ion-containing enzyme primarily responsible for catalyzing the majority of complex transformation chemical reactions. Metalloenzymes act on a molecule known as the substrate that undergoes a net chemical change and performs particular catalytic activities. They are necessary for biological pathways to maintain life. Metalloproteins are relatively common metal-based proteins involved in a wide range of functions as enzymes, storage and transport proteins, regulators of gene expression, and signal transduction cascades proteins. For many years, biological research[10] has mostly been concerned with metalloproteins.

Movement anomalies, cognitive impairment, and psychiatric symptoms are the clinical hallmarks of the disease. In Huntington's disease (HD), significant and persistent abnormalities in iron homeostasis have been periodically reported[11] and serum ferritin inadequacy has been considered the reason for the higher brain iron levels seen in HD patients. However, metallomics studies on HD patient biofluids are unusual and sparse. This disease shows multiple symptoms in humans and directly affects the Central Nervous System through the progressive degeneration of neurons in the brain. Currently, there are no known disease-modifying drugs or curative treatments for Huntington's disease[12]. However, symptomatic medications, including tetrabenazine[13], neuroleptics[14,15], etc., have been licensed to treat the symptoms of chorea in HD and are used to suppress the effect of involuntary jerking and writhing movements. Huntington's disease is not particularly treated by licensed medications because long-term exposure to these drugs may cause drowsiness somnolence, blurred vision, insomnia, depression, panic attacks, akathisia, and paranoia[16]. Therefore, there is an urgent need to design an effective drug specifically for Huntington's disease, as no particular drug has been developed and licensed for this disease.

The way to reduce the burden of HD globally is to cure this disease by different means. In this case, the *in-silico* approach is an efficient method to check metalloproteins' efficiency and potential activity. These tools, provide a framework for screening the potential activity of metalloproteins towards specific targets/receptors, which helps to select the ones with the greatest potential activity for subsequent future *in vitro* and *in vivo* study. The purpose of this study is to determine and assess the efficacy of side chain amino acid-inserted metalloproteins against Huntingtin proteins to design a drug candidate for Huntington's disease. The design of metalloprotein-based drugs has an enormous impact on neurodegenerative diseases such as HD, and they have been widely used to treat diverse effects of neurodegenerative disorders.

Materials and Methods

Retrieval of Hungtintin Protein

Huntington's disease targeted protein addressed in this study is huntingtin protein with ID mapping O00291·HIP1_HUMAN obtained from Uniprot (Universal Protein Resource, available at: https://www.uniprot.org/). Uniprot is an online database of protein sequences that gives structural and functional information about proteins. It helps to predict biological processes, molecular functions, and cellular components of the cell. The tertiary structures of selected proteins were visualized using Discovery Studio Visualizer (3D Boston Campus USA, available at: https://discover.3ds.com/discovery-studio-visualizer-download). It is a protein modeling software used to visualize, analyze, and simulate protein structures[17,18].

Prediction of Binding Pockets

For better and more effective interaction analysis, it is important to predict or identify the binding sites present in the protein molecule. To identify and detect active sites of protein, a meta server method COACH (available at: https://zhanggroup.

org/COACH/) was used. It works based on two different algorithms TM-SITE and S-SITE. The BioLiP protein function database identifies the sites in the ligand binding templates with structural and sequence analysis. This step identified the potential binding pockets in the protein molecule. The COACH analysis was performed using the Protein Data Bank (PDB) structure of the protein as input.

Retrieval of 3D Structures of Metalloproteins

Protein Data Bank (PDB) (available at: https://www.rcsb.org/) is an online server, used to retrieve the 3D structures of metalloproteins, including aconitase (1L5J), iron-responsive element-binding protein (IRE-BP) (3SN2), hydrogenase (1H2A), catalase (3RGP), beta-amyloid (5TPT), aminopeptidase (4QPE), carboxypeptidase (2PJ7), alcohol dehydrogenase (1HSO), nitrite reductase (2DWT), nitrous-oxide reductase (5I5J), laccase (4JHV), cytochrome c oxidase (CcO) (7CP5), Calmodulin (2RO8), Ceruloplasmin (5N0K), ferritin (2FHA), cytochromes (1W0E), rubredoxin (3KYX), and tyrosinase (3AWU). It can provide complete access to structural biology and structural genomics information[19].

Docking Analysis of Metalloproteins

ClusPro 2.0 (available at: https://cluspro.bu.edu/) was used for protein-protein docking purpose[20]. ClusPro is an online automated, web-based program used the check the interaction and binding energies between two protein molecules[21]. Docking was carried out between Huntington protein and cytochrome C oxidase. In the docking process, steric and physicochemical compatibility is evaluated by predicting the structure of the protein-protein complex, and proteins recognize one another, usually in a tight-packed manner, and bind in a very specific way. It is used to understand cellular functions, and docking X-ray or nuclear magnetic resonance (NMR) structures of proteins.

Side-chain Insertion of Amino Acids into Metalloproteins

The side-chain insertion of neurogenerative amino acids to metalloproteins was done by the SwissSidechain software (available at: https://www.swisssidechain.ch/). The SwissSidechain database contains several hundred natural and unnatural amino acid sidechains, which can be inserted into natural proteins or peptides computationally to enhance the effectiveness of proteins[22]. The cytochrome C oxidase structure was opened, and four amino acids sequence of tryptophan, tyrosine, histidine, and asparaginen were inserted on both sides of the chain of cytochrome C oxidase. Tyrosine and tryptophan were inserted into the N-terminal of it, whereas histidine and asparagine were inserted into the C-terminal of metalloproteins. This step was aided by adding the sequence of these amino acids to the FASTA sequence of cytochrome C oxidase at specific terminals[23]. The output PDB structure of this side chain amino acid constructive metalloproteins was again docked through Cluspro to predict its enhanced activity.

Prediction of Physiochemical Properties

The physicochemical properties of the compound can be determined by using Expasy ProtParam (available at: https://web.expasy.org/protparam/). AA-Prop is a tool for predicting the physicochemical properties of proteins. The AA-Prop accurately predicts many properties like Molecular weight, Instability Index, GRAVY-Grand Average of Hydropathicity, aliphatic index, extinction coefficient, isoelectric point, molecular weight, and the net charge on protein. It also predicts amino acid composition and a protein molecule's atomic composition[24].

Prediction of Disulfide Bonds

Cyspred (available at: http://gpcr.biocomp.unibo.it) is an online software used to predict the bonding state of cysteine or disulfide bonds in the specified metalloprotein. It can be used to separate and differentiate reactive and nonreactive cysteine, by analyzing the H-bond network and using profiling techniques. It is used to characterize the structural and functional characteristics of proteins by predicting disulfide bridges from protein sequences. The stability and folding of many proteins depend on the formation of disulfide bonds between the appropriate pairs of cysteine residues[25].

Prediction of Surface Accessibility

To predict the surface accessibility of metalloproteins, NetSurfP (available at: http://www.cbs.dtu.dk/services/NetSurfP-1.1/) software was used. The simultaneous prediction of local structural topography is easily simplified by this prediction. NetSurfP is an online tool used to predict secondary structure, structural disorder, solvent accessibility, and surface accessibility. Surface

prediction accessibility is mainly based on the Z score. The Z score is used to predict the validity of surface accessibility and surface prediction[26].

Prediction of Allergenicity

By identifying the allergic compounds in the protein molecules, the allergenicity of metalloproteins was determined using AllerTOP v.20 (available at: https://www.ddg-pharmfac.net/AllerTOP/). It is an online service used to predict allergenicity based on the physicochemical properties of proteins. By comparing the protein sequences in databases, this technique evaluates the metalloprotein's ability to cause symptoms based on a cross-reactivity[27].

Prediction of Toxicity

ToxDL is an online tool (available at: http://www.csbio.sjtu.edu.cn/bioinf/ToxDL/) that can be used to classify toxic and non-toxic chains in a protein molecule. It works based on the multimodal deep learning-based approach. It was used to categorize the toxicity of metalloproteins according to the presence of toxic domains and moieties in the secondary structure of ligands[28].

Prediction of Secondary Structure

PsiPred (available at: http://bioinf.cs.ucl.ac.uk/psipred/) is an online web server used to predict the secondary structure of proteins based on position-specific scoring matrices. It is a highly accurate prediction method that works on the principle of artificial neural networks and machine learning methods in its algorithm[29]. The secondary structure of proteins contains alpha helix, beta-pleated sheets, coils, extracellular regions, cytoplasmic region, protein bindings, and transmembrane helix. PSIPRED took the output of the Position-Specific Iterated BLAST (PSI-BLAST) and analyzed it by two feed-forward neural networks to predict the 2D structure of the protein[30].

Prediction of Tertiary Structure

The 3D structure of metalloprotein was predicted using TrRosetta (available at: https://yanglab.nankai.edu.cn/trRosetta/help/). The transform-restrained Rosetta (TrRosetta) algorithm predicts protein structures rapidly and effectively. It is an online web server that builds the structure of a protein using direct energy minimizations and restrained/constrained Rosetta. The inter-residue geometries, including orientations and distance, are initially predicted using a deep neural network, and then predicted geometries are subsequently converted into protein models to forecast the structure[31].

Validation of the Tertiary Structure of the Protein

The 3D structure of the huntingtin protein was evaluated and verified by the Ramachandran plot. The Ramachandran plot was used to verify the 3D model by using PROCHECK (available at: https://saves.mbi.ucla.edu/). PROCHECK determines the stereochemical properties and the geometry of the residues that are found in the protein structure. The plot shows the phi (Φ) and psi (ψ) angles of the residues present in the amino acid sequence.

Expression Analysis

Snap Gene (available at: https://www.snapgene.com) is an online molecular biology software used for expression analysis. It is used for DNA visualization and molecular cloning. For expression analysis, PuC18 vector was selected and restriction sites were identified and marked. JCAT (Java Codon Optimization Tool) (available at: http://www.jcat.de/) was utilized for accurate and efficient expression analysis. The fragment was subsequently selected for metalloprotein; cytochrome C oxidase was subjected to adequate expression scrutiny[32].

Results

Retrieval of Protein Sequence

Huntington protein was retrieved from UniProt with ID mapping O00291.HIP1_HUMAN. This protein contains 4 chains consisting of 107 amino acids starting with Glycine (G) and ending at Histidine (H), as presented in Figure 1A.

Binding Site Identification

The structure of the huntingtin protein having PDB Id 2aq7 was given to COACH. It is a meta-server online tool used for the identification of active sites in a protein molecule. The BioLiP protein function database with structural and sequence analysis was used to identify the sites and detect the ligand binding templates by using two comparative approaches TM-SITE and S-SITE. The possible predicted binding sites in a huntingtin protein are shown in Figure 1B.

The TM-SITE results showed a C-score of 0.25, a cluster size of 19, and the ligands were NUC, DPV and III with the residues 20, 23, 24, 27, 28, 31, 35, 38, and 39, respectively. The S-SITE results have a C-score of 0.16, the cluster size is 3,

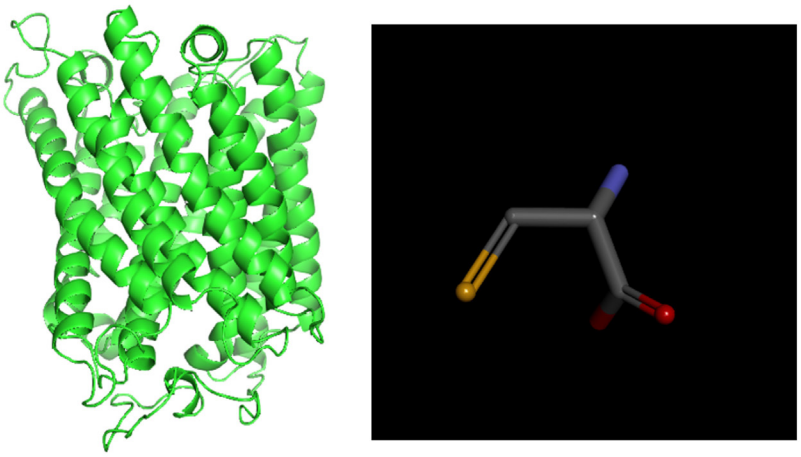


Figure 1. **A**, 3D structure of Huntingtin protein; a major player in the prognosis of Huntingtin diseases. **B**, Binding sites of huntingtin protein by COACH.

and the ligands are EYK, CA, and SE with the predicted binding site residues 58, 63, 66, 67, 68, and 70, respectively.

Protein-Protein Docking

The protein-protein docking of metalloproteins and huntingtin protein receptors was simulated by ClusPro 2.0. To evaluate the outcomes of docking results, the lowest energies of VdW Electric were selected. The docking score of the cytochrome C oxidase was -1,253.3, which is high among other drug candidates. The docking energies of drug candidates metalloproteins and metalloenzymes with huntingtin protein are shown in Table I.

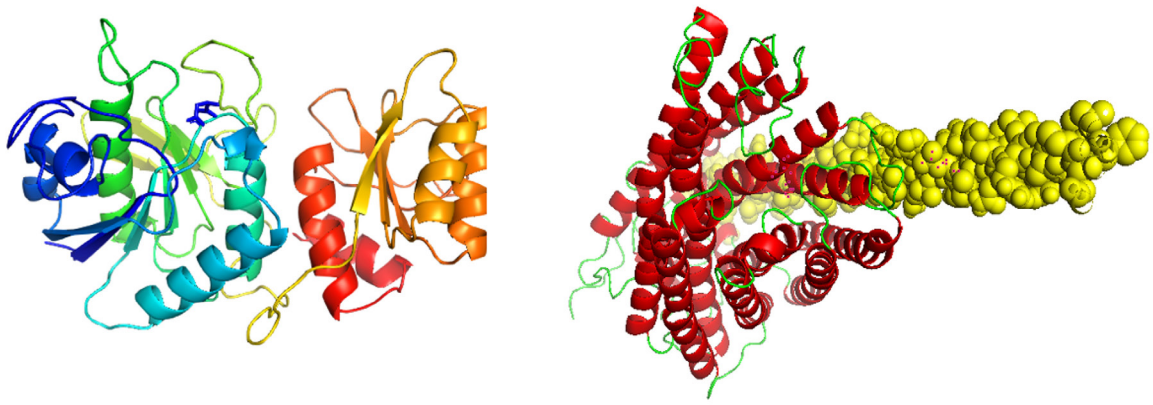
Side-Chain Insertion of Neurogenerative Amino Acids to Metalloproteins

The addition of four amino acids in the metalloprotein structure of cytochrome C oxidase resulted in the increase of efficacy of targeted metalloprotein as all of these amino acids play important roles in the neuroregeneration as proved by the docking analysis and this was further validated by physiochemical analysis. Figure 2A shows the side chain insertion of

Table I. Binding energies of different metalloproteins and huntingtin protein by ClusPro.

Sr. No.	Metalloproteins	PDB ID	Cluster Members	Binding Affinities (kcal/mol)	
				Centres	Highest
1	Aconitase	1L5J	90	-779.2	-874.3
2	Iron-responsive element-binding protein	3SN2	35	-685.0	-906.4
3	Hydrogenase	1H2A	66	-815.6	-823.8
4	Catalase	3RGP	55	-878.4	-1,175.3
5	Beta amyloid	5TPT	57	-691.9	-840.4
6	Aminopeptidase	4QPE	102	-614.7	-673.8
7	Carboxypeptidase	2PJ7	111	-735.1	-772.4
8	Alcohol dehydrogenase	1HSO	70	-594.7	-807.2
9	Nitrite reductase	2DWT	73	-649.2	-745.8
10	Nitrous-oxide reductase	5I5J	49	-869.3	-946.2
11	Laccase	4JHV	81	-647.5	-660.5
12	Cytochrome C oxidase	7CP5	45	-1091.1	-1,253.3
13	Calmodulin	2RO8	135	-474.8	-612.0
14	Ceruloplasmin	5N0K	48	-796.2	-804.5
15	Ferritin	2FHA	71	-816.3	-1,077.8
16	Cytochromes	1W0E	145	-542.2	-636.2
17	Rubredoxin	3KYX	202	-463.6	-484.7
18	Tyrosinase	3AWU	97	-630.7	-711.7

Adjusted for age, infertility duration, BMI, number of MII oocytes, number, and quality of embryos transferred.



(A) (B)

Figure 2. A, 3D structure of side chain amino acids constructive cytochrome C oxidase (red, light blue and green chains); tryptophan (dark blue chain), tyrosine (green chain), histidine (orange chain) and asparagine (yellow chain). **B**, The docking complex of Huntingtin protein and side chain amino acids constructive Cytochrome C oxidase. Chains of huntingtin protein receptor (red helix and green loops) and inserted side chains of amino acids in cytochrome C oxidase (yellow).

4 amino acids (tryptophan in dark blue chain, tyrosine in green chain, histidine in orange chain and asparagine in yellow chain) to cytochrome C oxidase. The docked complex of hungtintin protein with side chain amino aicds constructive cytochrome C oxidase is depicted in Figure 2B.

Prediction of Physiochemical Prosperities

Expasy ProtParam was used to specify the physiochemical properties of side chain-engineered cytochrome C oxidase metalloprotein. All characteristic physical and chemical parameters, including the total number of negatively and positively charged amino acids, were 25 and 17, respectively. The instability index of side chain-engineered cytochrome C oxidase was 25.81. The physicochemical properties of side chain-engineered cytochrome C oxidase metalloprotein are presented in Table II.

Prediction of Disulfide Bonds

The disulfide bonds calculated by CYSPred showed that the side chain amino acids constructive cytochrome C oxidase have all cysteines and disulfide bonds were present in the bonding states with a reliability factor of 2 to 9, respectively.

Prediction of Surface Accessibility

NetSurfP predicted the surface accessibility of side chain amino acids constructive cytochrome C oxidase. In the results, the most exposed amino acid number, its relative surface accessibility, and absolute surface accessibility are 197, 0.931, and 162.576, respectively.

Prediction of Allergenicity

The allergenicity of side chain amino acids constructive cytochrome C oxidase was predicted as a probably non-allergen. The metalloprotein-based drug candidate was considered to be safe enough to use as a drug candidate due to its low allergenicity and non-allergen activity.

Prediction of Toxicity

Based on toxicity domains in the secondary structure of metalloproteins, the toxicity of side chain-engineered cytochrome C oxidase was predicted by the ToxDL tool. The toxicity score is 0.000000005. No toxic domains were found in side chain amino acids constructive cytochrome C oxidase claiming that it is safe to use as a drug in the future.

Table II. Physiochemical properties of side chain amino acids constructive cytochrome C oxidase.

Name	Extinction Coefficient	Estimated Half Life	Instability Index	Aliphatic Index	Grand average of hydropathicity (GRAVY)
Side chain amino acids constructive Cytochrome C oxidase	2.136	30 hours	25.81	102.06	0.685

Prediction of Secondary Structure

Psipred was used the generate the secondary structure of the side chain-engineered cytochrome C oxidase metalloprotein. The 2D structure obtained was a long chain of amino acids, including coils, helix and strands, as shown in Figure 3.

Validation of 3D Structure

The 3D structure of side chain amino acids constructive cytochrome C oxidase was verified and validated by PROCHECK based on quality factors. The Ramachandran plot shows that 91.9% of residues are in the most favored regions, and 7.4% are in additional allowed regions, as presented in Figure 4.

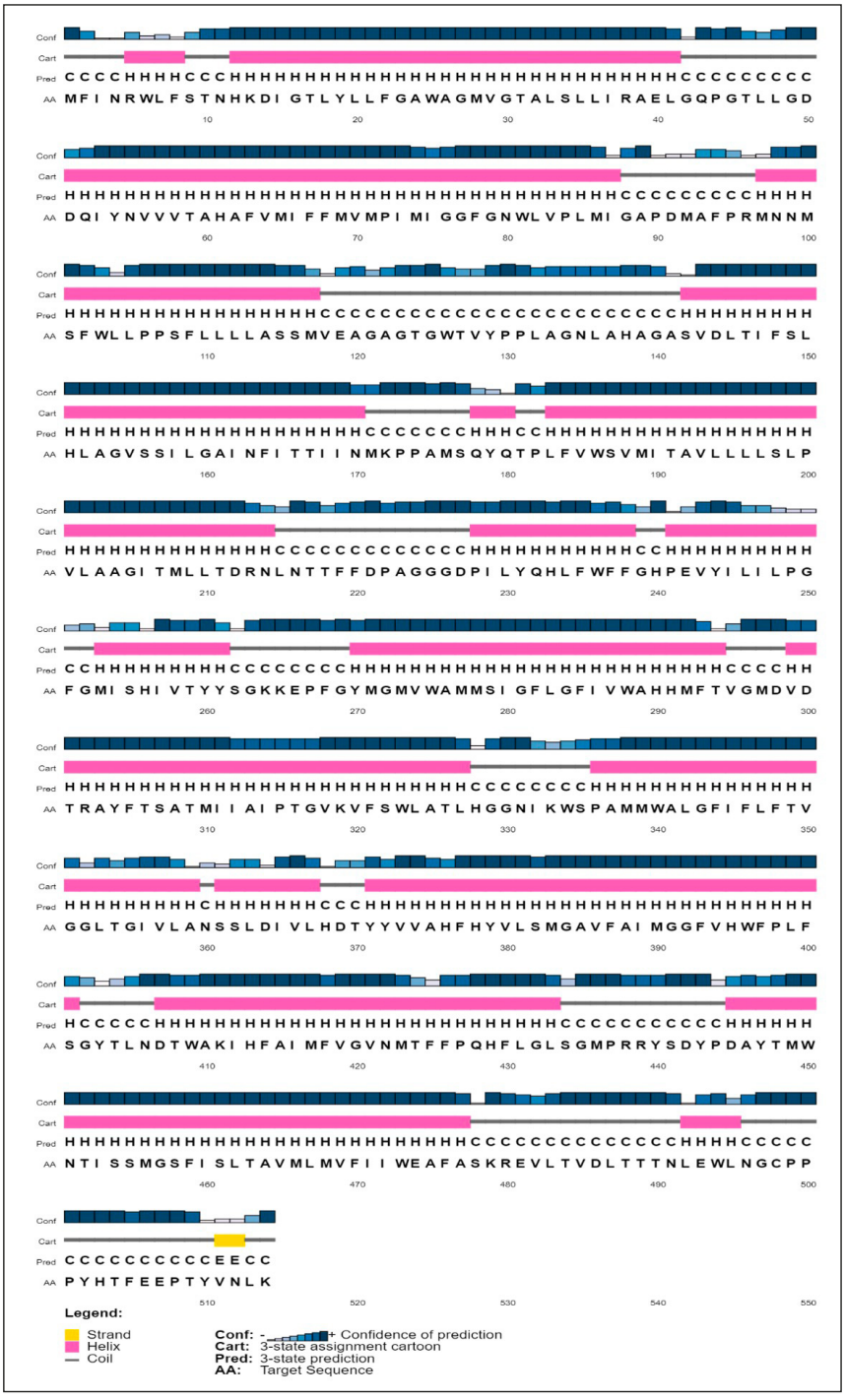


Figure 3. Predicted secondary structure of side chain amino acids constructive cytochrome C oxidase metalloprotein by Psipred.

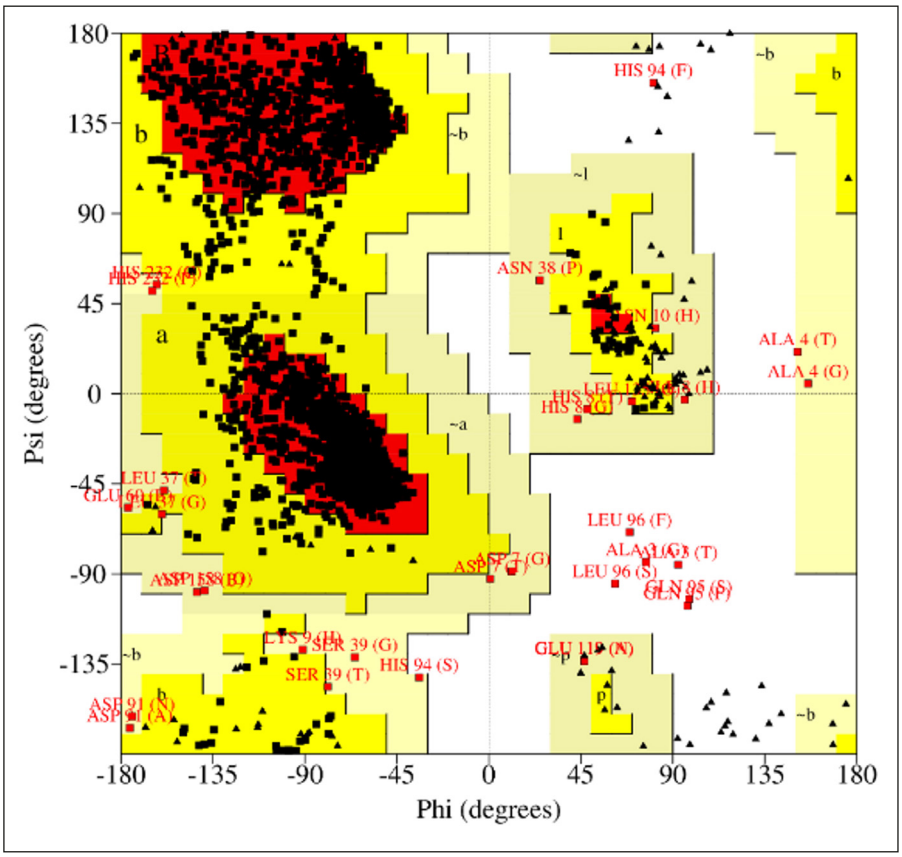


Figure 4. Ramachandran plot of 3D structure validation of side chain amino acids constructive cytochrome C oxidase.

Expression Analysis

Snap gene software (available at: https://www.snapgene.com/) was employed for the expression analysis of side chain amino acids constructive cytochrome C oxidase metalloprotein with the PuC-18 vector. The complete expressional accuracy was achieved through codon optimization. The metalloprotein-based drug candidate used as an insert in the vector will be further modified to produce a drug on a commercial scale. The side chain amino acids constructive cytochrome C oxidase metalloproteins based drug construct and generated using the PUC-18 vector using Snap Gene, is shown in Figure 5.

Discussion

In Huntingtin disease (HD), the huntingtin protein contains a polyglutamine expansion known as poly-Q, which leads to the misfolding and aggregation of the protein[33]. Metallothionein, specifically the MT-III isoform, has been shown[34] to significantly reduce poly-Q aggregation and toxicity when overexpressed in mammalian cells. This metalloprotein has therefore been proposed as a potential target for therapeutic intervention in HD. However, there has been little investigation into the levels of metalloproteins, including MT-III, in biofluids such as cerebrospinal fluid (CSF) in HD patients[35]. A study conducted by Zakiyanov et al[36] expected that MT-III levels in CSF may be elevated in HD due to the increased brain iron levels observed in the patients.

Matrix metalloproteinases (MMPs) are a family of zinc-containing proteolytic enzymes involved in various pathological processes. They are known[37] to degrade extracellular matrix proteins and have been linked to diseases such as rheumatoid arthritis, kidney disease, cardiovascular disease, and cancer. An imbalance of MMP and tissue inhibitor metalloproteinase (TIMP) activity is thought to contribute to these diseases. MMP inhibition has been shown[38] to reduce neuronal damage after transient cerebral ischemia, and mice lacking MMP-9 have a significantly decreased level of striatal neuronal cell death after intracerebral hemorrhage. The MMP-3/MMP-9 inhibitor SB-3CT (selective, mechanism-based inhibitor of the gelatinases) has also been found to decrease

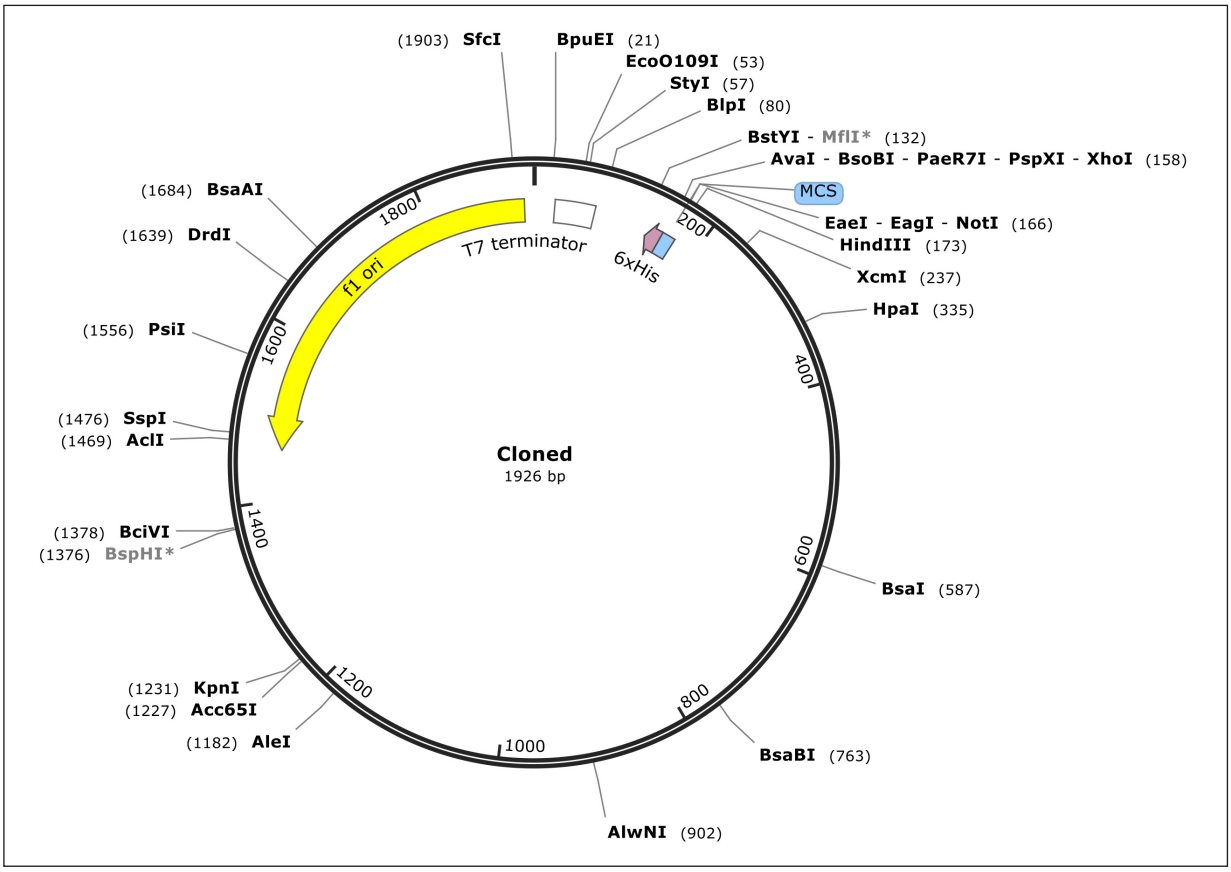


Figure 5. The side chain amino acids constructive cytochrome C oxidase cloning in PuC-18 by SnapGene; vector containing restriction sites for mentioned restriction enzymes.

MMP activity and infarct size in a mouse stroke model[39]. While MMPs are considered important in the development of certain diseases, more research is needed to understand their role in normal cell function and disease. Several studies[40] have utilized neuroimaging techniques to better understand the progression of Huntingtin disease (HD) and to evaluate the effectiveness of potential therapies. Some of the therapies that have been tested in clinical trials for HD include phosphodiesterase 10a inhibitor, resveratrol, tetrahydrocannabinol, cannabidiol, coenzyme Q10, riluzole, minocycline, cystamine, minocycline, essential fatty acids, sodium butyrate and creatine inhibitors[41]. However, these therapeutic agents have not successfully inhibited or modified the progression of HD. Currently, different drugs such as laquinimod, semaphorin-4D and pridopidine-neutralizing antibodies are in the experimental and evaluation process for their potential to treat HD[42].

The current study is based on the screening of metalloproteins, leading to the side-chain insertion of neurogenerative amino acids to metalloproteins acting as as a drug candidate against the huntingtin protein (HTT). This chain of computational analysis shows that cytochrome C oxidase has the highest binding affinity with the Huntingtin protein (-1,091 kcal/mol energy), and it was predicted to be non-allergen and safe for drug administration further for clinical trials[43,44]. The side-chain addition of amino acids to the cytochrome C oxidase shows increased efficacy and binding affinity. All these four amino acids tyrosine, tryptophan, histidine and asparagine play an important role in neurogeneration. A study by Suzuki et al[45] shows the role of seven essential amino acids, namely leucine, phenylalanine and lysine supplemented with histidine isoleucine, tryptophan and valine to delay the onset of dementia. These amino acids were given orally to the participants in the form of granular powder, and results showed that the 6 g intervention group (6gIG) significantly improved cognitive functions and psychosocial function, which was expected to reduce cognitive decline[45].

Since delivering nucleic acids to the brain is difficult, this strategy faces many challenges. Furthermore, once the nervous system is cured, many

other tissues and organs got affected. Therefore, developing conventional drugs is important to cure HD without side effects[46-53]. Drugs that have been used for Hungtinton disease, such as antipsychotics, tranquilizers, cludemonoamine depleters and antidepressants do not prevent cognitive, behavioral and psychotic dysfunction linked to HD. Moreover, their long-term use causes chronic side effects. Several drugs have shown significant therapeutic effects against HD that provide an alternative to this, such as protein-based drugs. Furthermore, herbal drugs are also used as an alternative therapeutic method, but their permeability and drug solubility to reach the target site have not been able to reach the clinical exploration stage[54]. In the current study, side chain amino acids constructive cytochrome C oxidase shows good ADME (absorption, distribution, metabolism and excretion) Aproperties, high solubility and permeability, making it drug-permeable and showing no toxicity as its toxicity score is 6.0772003e-05. The validation of the 3D structure of cytochrome C oxidase shows 91.9% of residues are in the most favored regions, and 7.4% of residues are present in additional allowed regions. Conclusively, this designed study contains many computational facts and information through which a specifically targeted drug against Huntingtin diseases could be propagated. The targeted drug complex shows no allergenicity and toxicity that proves it to be safe for administration as a drug and could be implicated for further investigation for *in-vivo* studies.

Conclusions

Huntington's disease is a fatal genetic disorder that affects the central nervous system. It is caused by a mutation in the *HTT* gene, which leads to the abnormal expansion of a poly-Q tract in the HTT protein. This expansion confers toxic functions on the protein and causes it to accumulate and aggregate within cells. One potential treatment for Huntington's disease involves targeting the HTT protein with metalloprotein-based drugs. A molecular docking study was conducted to identify suitable drug candidates, and it was found that cytochrome C oxidase had the highest binding energy. Finally, side chain amino acids constructive cytochrome C oxidase was subjected for physiochemical properties, toxicity and allergenicity predictions, suggesting that this side chain constructive metalloproteins are safe for oral administration and could be further tested in *in vitro* and *in vivo* studies. Overall, this research provides computational pharmacological data that could support the development of HTT-targeted drugs for Huntington's disease.

Conflict of Interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' Contributions

Conceptualization, M.N, N.A, M.A, A.S, A.Z and T.A.; methodology, T.A., M.G, and A.S; software, M.A.; validation, M.G and A.S.; formal analysis, M.N, N.A, M.A, A.Z, A.S and T.A; investigation, M.N, N.A, M.A, A.S and A.Z. resources, M.A, and N.A.; data curation, N.A.; writing and original draft preparation, T.A and M.A; writing, review and editing, A.F and A.S.A; visualization, G.N and M.N.; supervision, M.N and T.A.; project administration, A.S.A.; funding acquisition, T.A.

Funding

This research work received no external funding.

Acknowledgments

The authors greatly acknowledge and express their gratitude to the Researchers Supporting Project number (RSP2023R335), King Saud University, Riyadh, Saudi Arabia.

Ethics Approval

Not applicable.

Informed Consent

Not applicable.

Availability of Data and Materials

All the data generated during this research study has been included in the manuscript.

ORCID ID

M. Naveed. 0000-0002-4333-8226
Tariq A: 0000-0003-0905-8076
Aqib MS: 0000-0001-7912-3983
Abdulrahman A: 0000-0002-9888-9826
Abdullah FA: 0000-0001-7569-4287

References

1) Azam S, Haque ME, Balakrishnan R, Kim IS, Choi DK. The ageing brain: molecular and cellular basis of neurodegeneration. Fron Cell Deve Biol 2021; 9: 459-563.

2) Albin RL, Tagle DA. Genetics and molecular biology of Huntington's disease. Trends Neurosci 1995; 18: 11-14.
3) Coppen EM, Roos RA. Current pharmacological approaches to reduce chorea in Huntington's disease. Drug Inf J 2017; 77: 29-46.
4) Folstein SE, Leigh RJ, Parhad IM, Folstein MF. The diagnosis of Huntington's disease. Neurol Int 2021; 36: 1279-1281.
5) MacDonald ME, Ambrose CM, Duyao MP, Myers RH, Lin C, Srinidhi L, Barnes G, Taylor SA, James M, Groot N. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Int J Cell Biol 1993; 72: 971-983.
6) Burke KA, Kauffman KJ, Umbaugh CS, Frey SL, Legleiter J. The interaction of polyglutamine peptides with lipid membranes is regulated by flanking sequences associated with huntingtin. J Biol Chem 2013; 288: 14993-15005.
7) Ricci C, Marzocchi C, Battistini S. MicroRNAs as biomarkers in amyotrophic lateral sclerosis. Int J Cell Biol 2018; 7: 219.
8) Fuss JO, Tsai CL, Ishida JP, Tainer JA. Emerging critical roles of Fe–S clusters in DNA replication and repair. Biochim Biophys Acta Mol Cell Res 2015; 34: 1253-1271.
9) Akcapinar GB, Sezerman OU. Computational approaches for de novo design and redesign of metal-binding sites on proteins. Biosci Rep 2017; 37: 123-134.
10) Mittler R. ROS are good. Trends Plant Sci 2017; 22: 11-19.
11) Sturrock A. The assessment of proton MRS as a biomarker for Huntington disease. J Plant Sci 2015; 45: 455-566.
12) Jimenez-Sanchez M, Licitra F, Underwood BR, Rubinsztein DC. Huntington's disease: mechanisms of pathogenesis and therapeutic strategies. Cold Spring Harb Perspect Biol 2019; 7: 242-246.
13) Group HS. Tetrabenazine as antichorea therapy in Huntington disease: a randomized controlled trial. Trends Neurosci 2019; 66: 366-372.
14) Deroover J, Baro F, Bourguignon R, Smets P. Tiapride versus placebo: a double-blind comparative study in the management of Huntington's chorea. Curr Med Res Opin 2021; 9: 329-338.
15) Quinn N, Marsden CD. A double-blind trial of sulpiride in Huntington's disease and tardive dyskinesia. J Psychiatry Neurosci 2020; 47: 844-847.
16) Jankovic J, Beach J. Long-term effects of tetrabenazine in hyperkinetic movement disorders. Neurol Int 2020; 48: 358-362.
17) Bairoch A, Apweiler R, Wu CH, Barker WC, Boeckmann B, Ferro S, Gasteiger E, Huang H, Lopez R, Magrane M. The universal protein resource for hungtinton disease. Curr Med Res Opin 2020; 33: 154-159.
18) Nainan OV, Xia G, Vaughan G, Margolis HS. Diagnosis of hungtiton disease: a molecular approach. Clin Microbiol Rev 2018; 19: 63-79.
19) Kouranov A, Xie L, de la Cruz J, Chen L, Westbrook J, Bourne PE, Berman HM. Combination high-dose omega-3 fatty acids and high-dose cholecalciferol in hungtinton disease: a potential role in preservation of beta-cell mass. Eur Rev Med Pharmacol Sci 2018; 20: 3313-3318.
20) Kozakov D, Hall DR, Xia B, Porter KA, Padhorny D, Yueh C, Beglov D, Vajda S. The ClusPro web server for protein protein docking. Nat Protoc 2017; 12: 255-278.
21) Comeau SR, Gatchell DW, Vajda S, Camacho CJ. ClusPro: a fully automated algorithm for protein protein docking. Nat Protoc 2019; 32: 96-99.
22) Wang X, Kleerekoper Q, Revtovich AV, Kang D, Kirienko NV. Identification and validation of a novel anti-virulent that binds to pyoverdine and inhibits its function. Virulence 2020; 11: 1293-1309.
23) Naveed M, Shabbir MA, Ain Nu, Javed K, Mahmood S, Aziz T, Khan AA, Nabi G, Shahzad M, Alharbi ME. Chain-Engineering-Based De Novo Drug Design against MPXVgp169 Virulent Protein of Monkeypox Virus: A Molecular Modification Approach. Bioengineering 2023; 10: 11-12.
24) Gasteiger E, Hoogland C, Gattiker A, Wilkins MR, Appel RD, Bairoch A. Protein identification and analysis tools on the ExPASy server. Clin Microbiol Rev 2006; 45: 571-607.
25) Muskal SM, Holbrook SR, Kim SH. Prediction of the disulfide-bonding state of cysteine in proteins. Protein Eng Des Sel 1990; 3: 667-672.
26) Høie MH, Kiehl EN, Petersen B, Nielsen, M, Winther O, Nielsen H, Hallgren J, Marcatili P. NetSurfP-3.0: accurate and fast prediction of protein structural features by protein language models and deep learning. Nat Protoc 2022; 34: 340-345
27) Dimitrov I, Bangov I, Flower DR, Doytchinova I. AllerTOP 2a server for in silico prediction of allergens. J Mol Model 2014; 20: 1-6.
28) Pan X, Zuallaert J, Wang X, Shen HB, Campos EP, Marushchak DO, De Neve W. ToxDL: deep learning using primary structure and domain embeddings for assessing protein toxicity. Bioinformatics 2021; 36: 5159-5168.
29) Jones DT. Protein secondary structure prediction based on position-specific scoring matrices. J Mol Biol 1999; 29: 195-202.
30) McGuffin LJ, Bryson K, Jones DT. The psipred protein structure prediction server. Bioinformatics 2000; 16: 404-405.
31) Du Z, Su H, Wang W, Ye L, Wei H, Peng Z, Anishchenko I, Baker D, Yang J. The trRosetta server for fast and accurate protein structure prediction. Nat Protoc 2021; 16: 5634-5651.
32) Choi SY, Ro H, Yi H. DNA cloning: a hands-on approach. Springer 2019; 56: 567-589.
33) Daldin M, Fodale V, Cariulo, C, Azzollini L, Verani M, Martufi P, Spiezia MC, Deguire SM, Cherubini M, Macdonald D. Polyglutamine expansion affects huntingtin conformation in multiple Huntington's disease models. Sci Rep 2017; 7: 11-15.
34) Sussulini A, Hauser-Davis R. A. Metallomics applied to the study of neurodegenerative and mental diseases. Metallomics 2018; 45: 21-37.

35) Sapna G, Gokul S, Bagri-Manjrekar K. Matrix metalloproteinases and periodontal diseases. Oral Dis 2014; 20: 538-550.
36) Zakiyanov O, Kalousová M, Zima T, Tesař V. Matrix metalloproteinases in renal diseases: a critical appraisal. Kidney Blood Press Res 2019; 44: 298-330.
37) Lee H, Park JW, Kim SP, Lo EH, Lee SR. Doxycycline inhibits matrix metalloproteinase-9 and laminin degradation after transient global cerebral ischemia. Neurobiol Dis 2009; 34: 189-198.
38) Xue M, Fan Y, Liu S, Zygun DA, Demchuk A, Yong VW. Contributions of multiple proteases to neurotoxicity in a mouse model of intracerebral haemorrhage. Brain Res J 2019; 132: 26-36.
39) Gu Z, Cui J, Brown S, Fridman R, Mobashery S, Strongin AY, Lipton SA. A highly specific inhibitor of matrix metalloproteinase-9 rescues laminin from proteolysis and neurons from apoptosis in transient focal cerebral ischemia. J Neurosci 2015; 25: 6401-6408.
40) Tabrizi SJ, Scahill RI, Owen G, Durr A, Leavitt BR, Roos RA, Borowsky B, Landwehrmeyer B, Frost C, Johnson H. Predictors of phenotypic progression and disease onset in premanifest and early-stage Huntington's disease in the TRACK-HD study: analysis of 36-month observational data. Lancet 2013; 12: 637-649.
41) Hersch SM, Schifitto G, Oakes D, Bredlau AL, Meyers CM, Nahin R, Rosas HD. The CREST-E study of creatine for Huntington disease: A randomized controlled trial. J Psychiatry Neurosci 2017; 89: 594-601.
42) McGarry A, McDermott M, Kieburtz K, de Blieck EA, Beal F, Marder K, Ross C, Shoulson I, Gilbert P, Mallonee WM. A randomized, double-blind, placebo-controlled trial of coenzyme Q10 in Huntington disease. J Psychiatry Neurosci 2017; 88: 152-159.
43) Kim YC, Miller A, Lins LC, Han SW, Keiser MS, Boudreau RL, Davidson BL, Narayanan NS. RNA interference of human α-synuclein in mouse. Front Neurol 2017; 8: 15.
44) Keiser MS, Kordasiewicz HB, McBride JL. Gene suppression strategies for dominantly inherited neurodegenerative diseases: lessons from Huntington's disease and spinocerebellar ataxia. Hum Mol Genet 2016; 25: 53-64.
45) Suzuki H, Yamashiro D, Ogawa S, Kobayashi M, Cho D, Iizuka A, Tsukamoto-Yasui M, Takada M, Isokawa M, Nagao K. Intake of seven essential amino acids improves cognitive function and psychological and social function in middle-aged and older adults: a double-blind, randomized, placebo-controlled trial. Front Nutr 2020; 7: 586-596.
46) Trujillo Del Río C, Tortajada Pérez J, Gómez Escribano A, Casterá F, Peiró C, Millán J, Herrero M, Vázquez-Manrique R. Metformin to treat Huntington disease: A pleiotropic drug against a multi-system disorder. Mech Ageing Dev 2022; 45: 111-670.
47) Naveed M, Ain N, Aziz T, Ali I, Aqib Shabbir M, Javed K, Alharbi M, Alshammari A, Alasmari AF. Revolutionizing treatment for toxic shock syndrome with engineered super chromones to combat antibiotic-resistant Staphylococcus aureus. Eur Rev Med Pharmacol Sci 2023; 27: 5301-5309.
48) Muhammad N, Waseem M, Tariq A, Hassan JU, Makhdoom SI, Ali U, Alharbi M, Alsahammari A. Identification of Bacterial Strains and Development of anmRNA-Based Vaccine to Combat Antibiotic Resistance in Staphylococcus aureus via In Vitro and In Silico Approaches. Biomedicines 2023; 11: 1039.
49) Naveed M, Makhdoom SI, Ali U, Jabeen K, Aziz T, Khan AA, Jamil S, Shahzad M, Alharbi M, Alshammari A. Immunoinformatics Approach to Design Multi-Epitope-Based Vaccine against Machupo Virus Taking Viral Nucleocapsid as a Potential Candidate. Vaccines 2022; 10: 1732.
50) Muhammad N, Sheraz M, Amin A, Waseem M, Aziz T, Khan AA, Ghani M, Shahzad M, Alruways MW, Dablool A.S. Designing a Novel Peptide-Based Multi-Epitope Vaccine to Evoke a Robust Immune Response against Pathogenic Multidrug-Resistant Providencia heimbachae. Vaccines 2022; 10: 1300.
51) Naveed M, Ain NU, Aziz T, Javed K, Shabbir MA, Alharbi M, Alsahammari A, Alasmari AF. Artificial Intelligence Assisted Pharmacophore Design for Philadelphia Chromosome-Positive Leukemia with Gamma-Tocotrienol: A Toxicity Comparison Approach with Asciminib. Biomedicines 2023; 11: 1041.
52) Muhammad N, Urooj A, Tariq A, Rasool MJ, Ijaz A, Alharbi M, Alharbi ME, Alshammari A, Alasmari AF. A reverse vaccinology approach to design an mRNA-based vaccine to provoke a robust immune response against HIV-1. Acta Biochim Pol 2023; 17:407-418.
53) Naveed M, Mughal MS, Jabeen K, Aziz T, Naz S, Nazir N, Shahzad M, Alharbi M, Alshammari A, Sadhu SS. Evaluation of the whole proteome to design a novel mRNA-based vaccine against multidrug-resistant Serratia marcescens. Front Microbiol. 2022; 18:960285.
54) Vishwas S, Gulati M, Kapoor B, Gupta S, Singh SK, Awasthi A, Khan A, Goyal A, Bansal A, Baishnab S. Expanding the arsenal against Huntington's disease-Herbal drugs and their nanoformulations. Curr Neuropharmacol 2021; 19: 957-989.