



Predictive Data Modeling of CISD2 Activation for Neuroprotection: Insights from In Silico and Machine Learning Approaches

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ABSTRACT

The optimal operation of mitochondria and the endoplasmic reticulum (ER) relies on CISD2, also known as CDGSH iron-sulfur domain 2. Changes in CISD2 relate to neurodegenerative diseases like Parkinson's disease (PD) & Alzheimer's disease (AD). Found primarily in the endoplasmic reticulum including mitochondria, CISD2 is a component within the CDGSH protein family as well as alters redox balance, mitochondrial integrity, including calcium (Ca²⁺) homeostasis within these organelles. Often marked by mitochondrial malfunction, oxidative stress, and cell death, neurodegenerative diseases cover regions where CISD2 is absolutely important. Amyloid-beta (A β) accumulates in Alzheimer's disease to cause mitochondrial calcium excess, oxidative stress, and mitochondrial damage. This stimulates the mitochondrial permeability transition pore (MPTP), releasing cytochrome c (CytC) and so activating caspases (CASP3 and CASP9), hence causing neuronal death. By means of the IRE1 α and PERK pathways, A β disturbs Ca²⁺ homeostasis, induces the unfolded protein response (UPR), and promotes caspase 12 (CASP12)-mediated death. CISD2 mitigates oxidative damage and contributes to the stabilization of the mitochondrial membrane, thereby functioning as a protective agent against these phenomena. In neurodegenerative illnesses, diminished CISD2 levels exacerbate oxidative stress and mitochondrial injury, hence leading to neuronal death. Activated microglia and astrocytes, responding to A β toxicity, induce neuroinflammation, which accelerates dementia. Enhancing CISD2 expression has been shown to safeguard mitochondria, reduce reactive oxygen species (ROS), and prevent cell death. Furthermore, by maintaining Ca²⁺ homeostasis, CISD2 mitigates ER stress and pro-apoptotic signaling; hence, elevated CISD2 expression indicates a protective effect against neurodegeneration by diminishing oxidative stress, mitochondrial dysfunction, and endoplasmic reticulum stress. The natural flavonoid Liquiritigenin, found in licorice root, significantly enhances the expression of CISD2, hence augmenting mitochondrial integrity and stabilizing the endoplasmic reticulum in neurodegenerative models. Molecular docking and in silico research indicate that liquiritigenin and other small molecules bind to CISD2, hence activating its protective functions. Liquiritigenin reduces oxidative stress, mitochondrial damage, and endoplasmic reticulum stress by increasing CISD2 expression, hence decelerating neurodegeneration. Targeting CISD2 with Liquiritigenin or analogous chemicals may reduce neuroinflammation and improve mitochondrial health, thereby offering a viable treatment strategy for Alzheimer's disease and other neurodegenerative illnesses..

INTRODUCTION

Neurodegenerative illnesses such as Parkinson's disease (PD) and Alzheimer's disease (AD) are characterized by the progressive degradation of neurons, oxidative stress, mitochondrial dysfunction, and cellular death.[1] These diseases substantially undermine healthcare systems, impacting millions of individuals globally. Numerous diseases remain with their fundamental origins ambiguous, and now, no treatments are available for them.[2] This has garnered increased attention towards utilizing regulatory proteins and mitochondrial health as

potential therapeutic strategies for many disorders.[3] CISD2 (CDGSH iron-sulfur domain 2), associated with neuronal degeneration, is essential for maintaining mitochondrial and endoplasmic reticulum (ER) functionality.[4] CISD2 plays a crucial role in regulating cellular homeostasis, as it is a member of the CDGSH protein family and encompasses CISD1 and CISD3. CISD2 is primarily located in the endoplasmic reticulum and the outer mitochondrial membrane, where it regulates oxidative phosphorylation, redox balance, and calcium (Ca²⁺) homeostasis.[5] Disruptions in these systems lead



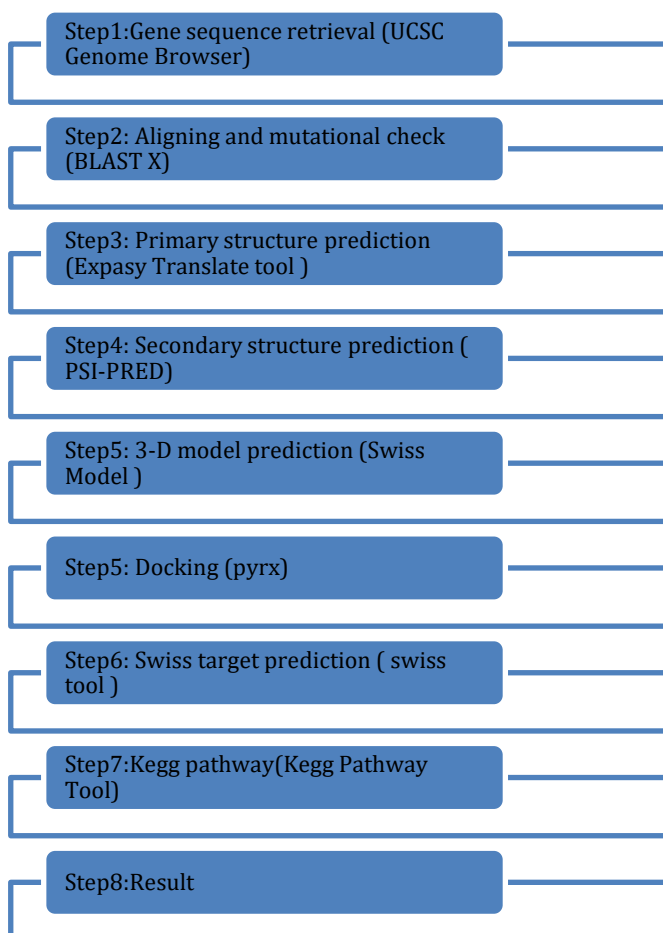
to mitochondrial dysfunction, resulting in the generation of reactive oxygen species (ROS), energy depletion, and ultimately, cellular demise.[6] The etiology of neurodegenerative disorders fundamentally relies on these pathways. Cisd2 is crucial for mitochondrial function and the optimal functioning of the endoplasmic reticulum, which is responsible for Ca²⁺ storage, lipid synthesis, and protein folding—each of which is vital for neuronal survival. Endoplasmic reticulum (ER) stress, arising from disturbances in ER functions, can trigger the unfolded protein response (UPR) and ultimately lead to cellular apoptosis if left unaddressed.[7] Accelerated neurodegeneration has been linked to reduced Cisd2 expression, hence highlighting the protective role of Cisd2 in both organelles. This research investigates Cisd2's ability to reduce oxidative stress, prevent cell death, and regulate mitochondrial and endoplasmic reticulum functions, positioning it as a potential therapeutic target for neurodegenerative diseases.[8] Our research indicates that enhancing Cisd2 expression will rectify cellular dysfunctions associated with neurodegenerative diseases.[9] Mitigating mitochondrial fragmentation, enhancing Ca²⁺ buffering, and restricting ROS accumulation essential for averting neuronal degeneration may facilitate Cisd2 expression to save neurons.[10] Liquiritigenin, a natural flavonoid derived from *Glycyrrhiza uralensis*, was identified as a promising small-molecule activator of Cisd2. Our research demonstrates that Liquiritigenin enhances the production of Cisd2, hence augmenting mitochondrial function and reducing oxidative stress.[10] This small molecule was identified through in silico approaches and subsequently evaluated for binding efficacy and therapeutic potential by molecular docking experiments.[11] Computational tools such as molecular docking facilitated the rapid identification of medicines with high affinity for binding to the CDGSH domain of Cisd2, hence supporting their essential role in mitochondrial and endoplasmic reticulum regulation.[12] In addition to docking analysis, in silico pharmacokinetic profiling was employed to predict the drug-like characteristics of these compounds.[13] We evaluated factors such as absorption, distribution, metabolism, excretion, and toxicity (ADMET) to identify candidates with robust binding potential and pharmacological efficacy.[14] Liquiritigenin is particularly notable for its capacity to enhance Cisd2 expression and exhibit good ADMET properties, making it an ideal option for further research.[15] The role of Cisd2 in critical cellular pathways associated with neurodegeneration was further examined. The mitophagy-lysosome pathway is a crucial mechanism for the elimination of damaged mitochondria. Neurodegenerative illnesses such as Parkinson's and Alzheimer's impair mitophagy; therefore, the ability of Cisd2 to regulate mitochondrial health suggests that activating Cisd2 may promote mitophagy.[16] Facilitating the efficient removal of impaired mitochondria may reduce ROS production and the secretion of pro-apoptotic proteins, thereby protecting neurons against apoptosis. Moreover, Cisd2 may play a role in inhibiting the opening of the mitochondrial permeability transition pore (mPTP) within the mitochondrial membrane. The mPTP facilitates the release of cytochrome c, the dissipation of

mitochondrial membrane potential, and the initiation of apoptotic pathways.[17] Our research suggests that the activation of Cisd2 may inhibit the opening of the mPTP under stress conditions, hence offering neuroprotection against cell death.[18] This research emphasizes the significance of Cisd2 in maintaining mitochondrial and endoplasmic reticulum homeostasis, as well as its potential as a therapeutic target for neurodegenerative diseases.[19] We have identified a novel small-molecule activator of Cisd2 through the integration of computational and experimental approaches.[20] Utilizing these activators enhances Cisd2 activity, offering a promising approach to mitigate mitochondrial and ER dysfunction associated with dementia.[21] The findings of this study suggest that Cisd2-targeted therapies warrant further investigation for the treatment of additional age-related conditions, such as diabetes and cardiovascular diseases, thereby broadening the therapeutic scope of Cisd2 activation.

MATERIALS AND METHODS

Within the field of drug development, several different bioinformatics technologies have been utilized. A selection of the instruments that we have utilized in our endeavors within this field are described in the following:

Chart



Utilising Verification Procedures and Annotated Data for Gene Sequencing: An Investigation of Genomic Landscapes Utilising the UCSC Genome Browser

Often used tool with many visual representations of genetic data it offers is the UCSC Genome

Browser(<https://genome.ucsc.edu/>). Together with other partners, the Genome Bioinformatics Group at the University of California, Santa Cruz created this application that effectively shows annotated regions of genomes including genes, mRNA, and DNA variants. This book covers in great detail issues including gene control, phenotypic expression, DNA variations, and interspecies genomic comparisons. Combining thorough data into a single easily accessible interface helps the UCSC Genome Browser to facilitate the study and comprehension of complicated biological events. Consequently, our knowledge of the natural world gets better (**accessed on October 1, 2023**). After acquiring the nucleotide sequence of the CISD2 gene by means of the UCSC Genome Browser, we next subjected it to further investigation and validation using complementary techniques.

Tools for modern bioinformatics that can anticipate the function of proteins and analyse genetic characteristics

One of the three BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) algorithms—along with tBLASTn and tBLASTx—the BLASTx method is designed especially to search databases for sequences like DNA and protein sequences. It converts DNA sequences into protein sequences so that possible amino acid matches across DNA sequence nucleotide content may be found by means of comparison with known proteins. Usually taking more time than other techniques, BLASTx demands looking at all six possible reading frames against protein databases. According to the findings, open reading frames relate to like sequences. Effective in gene identification and prediction, BLASTx helps to find protein-coding genes in DNA and clarifies the proteins expressed by particular DNA segments. It also helps ascertain whether a given new DNA sequence codes for a protein.

A strong tool meant to forecast the possible consequences of genetic changes on protein function within the framework of human biology is the PolyPhen-2 computer software. To reach this, it takes into account the physical properties of the changed amino acid as well as the evolutionary preservation of the relevant residue across like proteins.

A clever computer tool called MuPro forecasts how variations in amino acids affect protein stability. Examining many facets of amino acids, including their interactions with water, lengths, and charges, it seeks to elucidate how gene modifications affect proteins. By means of these elements, MuPro clarifies illness mechanisms, protein production, and changes in protein stability, thereby enabling researchers to grasp how mutations might affect these aspects. For biologists studying how gene mutations could influence protein stability as well as for protein researchers, this is an invaluable tool. MuPro can produce accurate forecasts and is easy to use.

Computed with a back vector machine, the I-Mutant 2.0 algorithm evaluates how a single modification influences protein stability. By means of additional attributes or more generally, protein grouping, this program may continuously forecast changes in proteins.

For pathogenicity of genetic variations, PhD-SNP integrates several data sources using machine learning approaches. Based on investigations of both the DNA sequence alteration and its surrounding context, it uses Support Vector Machines (SVMs) to separate benign from disease-associated Single Nucleotide Polymorphisms (SNPs).

SIFT is a computer tool meant to examine how differences in amino acids affect protein performance. Through analysis of the genetic code, SIFT aids in the identification of mutations most likely to influence protein function, so enabling the prioritising of more study on particular genetic variants. (**accessed on October 1, 2023**).

Proteoprotein Sequences Derived from DNA and RNA Expressed in Translation

One of the tools that can be **accessed on October 2, 2024** is called Expasy(<https://www.expasy.org/>) Translate. Its purpose is to convert sequences of DNA or RNA into sequences of proteins.

Advanced Protein Structure Analysis and PSI-BLAST Integration are the two components of PSIPRED. Utilisation of Neural Networks

PSIPRED(<https://bioinf.cs.ucl.ac.uk/psipred/>) provides a comprehensive method for the investigation of protein structures by employing PSI-BLAST for predictive modelling. In order to integrate information and provide assistance with decision-making, it makes use of artificial neural networks. Users are able to project protein structures based on their fundamental ingredients by using this tool, which operates as a server-based application and features an online interface that is simple to navigate. The PSIPRED software can be downloaded as a piece of software or it can be accessed online. We **accessed it on October 2, 2024**.

In the context of investigatory work, the Swiss-Model is an improvement in the modelling of protein structure.

Swiss-Model(<https://swissmodel.expasy.org/>) is a sophisticated online tool that is designed to assist researchers in the process of creating protein structures that are exact and efficient. It does this by using the structural data that is already available as a blueprint. Consequently, it makes it easier for users to construct protein models with varying degrees of complexity by enabling them to develop models that closely resemble the proteins that are now in existence (**retrieved on October 2, 2024**).

Contributing to the Advancement of Scientific Research: Recent Developments in Protein Exploration and Drug Development The Discovery Studio of BIOVIA

The objective of the BIOVIA Discovery Studio is to enhance the understanding of molecular structures and to make it possible to develop novel medications by combining three decades of research with cutting-edge computational technologies. Utilising this cutting-edge technology, which was **accessed on October 3, 2024**, researchers are able to investigate proteins, which ultimately leads to the advancement of the discovery of innovative drugs.

Enhancing the Effectiveness of Docking: The versatility of Autodock Vina

Autodock Vina is a versatile tool that is extensively utilised in a variety of docking applications, including protein-ligand docking, site-specific docking, and blind docking, among other applications. In addition to docking, Autodock Vina (Autodock Tools) assists in the structural modification of ligands and proteins. Additionally, it furnishes a method for evaluating molecular groupings of both a little and a large size. Docking efficiency and accuracy are significantly improved by Autodock Vina, which does so by utilising innovative scoring strategies, simplified optimisation methods, and multithreading capabilities (as of October 3, 2024).

Disclosing Molecular Impacts: Swiss Target Projection

This online Swiss Target prediction (<https://www.swisstargetprediction.ch/>) is designed to anticipate the physiological responses of humans and animals to small chemical compounds. Available on October 3, 2024, this predictive instrument elucidates intricate molecular systems and anticipates various impacts induced by specific compounds, hence emphasising potential undesirable outcomes.

Swiss ADME: Enhancing Drug Discovery with Computational Tools

The Swiss ADME (<https://www.swissadme.ch/>) website provides a valuable platform for calculating chemical properties and predicting their behaviour within the human body. This information, accessed on October 3, 2024, is beneficial for researching potential pharmaceutical compounds, facilitating the identification of novel treatments.

Making Protein Interaction Analysis More Comprehensive by Investigating the Functional Insights and Comprehensive Database provided by STRING

Designed to enable scientists better grasp and forecast the interactions between proteins in biological systems, STRING (<https://string-db.org/>) is a sophisticated database and web tool. The STRING database is a great resource for the scientific community by means of a thorough range of experimental data, predictive analytics, and publically available literature. Users all over can quickly access the platform, which always changes with fresh ideas. STRING not only compiles large amounts of data but also lets users investigate the functional links between their given protein lists using accepted classification systems as GO, Pfam, and KEGG. Especially the most recent edition, Version 11b, has a large collection of around 24.5 million proteins from more than 5,000 distinct species (accessible on October 4, 2024).

Unlocking Discoveries at the Molecular Level: Navigating the KEGG PATHWAY Database

A comprehensive understanding of molecular interactions in biological systems is provided by the KEGG PATHWAY (<https://www.genome.jp/kegg/pathway.html%20>) database, which also offers comprehensive visual representations of these interacting molecules. In addition to a condensed three- or four-letter name that indicates the organism that is the subject of the investigation, each

pathway is uniquely identifiable by a five-digit number that is associated with a specific code such as map, ko, ec, or rn (accessed on October 4, 2024).

Dynamics in Molecular Simulation

Molecular dynamics simulations run on the iMODS platform (<https://imods.iqf.csic.es/>) predicted the main structural and functional aspects of the C1SD3 protein. These characteristics comprise the B-factor, which denotes the flexibility of different protein domains; deformability, which emphasises areas prone to structural changes; and the eigenvalue, which gauges the rigidity of the protein and the energy required for conformational changes. Moreover, variance was computed to ascertain the general mobility of the protein; hence, residue and atom indices were investigated to find particular areas and atoms engaged in these dynamics. These simulations, especially in connection with ligands like "(2~{r})2-[3,4-Bis(Oxidaryl)phenyl]-6-Oxidanyl-2,3-Dihydrochromen-4-One," improve our knowledge of protein stability and its possible influence on C1SD3's function in tackling metabolic malfunction and age-related illnesses. (Accessed September 20, 2024.)

RESULTS

Acquiring the sequence of the nucleotides:

For almost twenty years, the scientific community has benefited much from the UCSC Genome Browser database, which provides a thorough forum for investigating gene chromosomal localisation and acquiring genetic data and annotations linked to the genome. Innovative approaches that can efficiently show this enormous amount of data are in increasing demand as the field of gene research keeps developing and new technologies produce a continuous flow of data (Gene Browser database: 2021 update - Oxford Academic). These elements help to explain the need of fresh approaches. Access and extraction of the nucleotide sequence of the C1SD2 gene as shown in [fig 01](#) depends on this priceless tool from the UCSC Genome Browser database, which we use in our work. Blastx uses this sequence also for aligning and finding mutations; the ExPASy translation tool forecasts protein basic structure. This therefore makes it possible to investigate C1SD2's functions more fully.

Figure 01

The nucleotide sequence that was extracted from the UCSC Genome Browser is shown this Figure

```
>hg38_knownGene_ENST00000273986.10 range=chr4:182869885-182887430 5'pad=0 3'pad=0 strand=+ repeatMasking=none
ATGTTCTCTGAGAGCGTGGCCCGTATCGTGAAGTGCAGCTCCCTGCTCA
TCTGAAGCGCTCCCACTCCCTGGAAGCATTACCGGTTCCCTAGGCTCA
CAGTTTCAAGATGGCTTCGGTTATTGCTTTCCTTGGTGTACTCGCACTT
CTTGGTCACTTGCAGTTCTCGTCACTTCCCTCCGGAAGAAACACAGAA
GGATAGCTTGAATTAATCTTAAATACAAAGGAAATCCGAAAGTAGTGA
ATGAATAAATCAATGAAGATTGTGTCTTCTAAGACAGCTTATTGTAGG
TGTGGCGTTCTAAAGCTTTCCTGCTGCGATGGTTCACATAATAAACA
CAATGAATTGCAGGAGATAATGTGGTCCACTAATACTGAAGAAGAAAG
AAGTATAA
```

For the purpose of full sequence analysis and mutation detection, BLAST X and other specialist tools were utilised.

After we had obtained the genetic code from the United States Genome Browser, we utilised BLAST X in order to evaluate and match the code with other sequences, which made it possible for us to identify mutations as well. After that, we utilised Polyphen2, Mupro, I.mutant, PHDSnp, and

Sift in order to analyse the arranged codes which had mutations as shown in [Table 1](#). The most important thing is to be aware of the internal changes that have occurred inside the collection, and those tools offer invaluable records that are almost capable of undergoing mutations. The desk is where the developments are shown.

Table 1

This table 1 contains comprehensive information regarding mutations, along with validated results obtained through the application of a variety of mutation analysis methods.

Mutation	Polyphen 2	Mupro	I-mutant	PHDamp	Sift
T28V	Benign	Decrease stability	Increase	Neutral	Predict tolerated
T28L	Benign	Decrease stability	Increase	Neutral	Predict tolerated
T28S	Benign	Decrease stability	Decrease	Neutral	Predict tolerated
P24L	Benign	Decrease stability	Decrease	Neutral	Predict tolerated

Translation from Expasy: Deciphering Changing DNA Sequences for Protein Structure Analysis

In our field of work, searching for DNA sequences and researching mutations depends critically on the UCSC Genome Browser database. Once changes inside the DNA collection have been found, the crucial next action is to change the statistics using ExPASy Translate in [Fig 2](#). The well-known bioinformatics tool ExPASy Translate helps us to identify the main building parts of proteins. One could say: This level is essential since it helps one to understand the amino acid composition of the protein. - Working together, ExPASy Translate and the USA Genome Browser helps one to understand how genetic variations affect protein characteristic and structure.

Figure 02

PSIpred forecasts the protein's configuration by means of the acquired records from the interpretation assessment accomplished with ExPASy Translate and the projection of a protein's number one motif.



Protein 2D Structure: Predictive Modelling and Analysis

In this work, we investigate how Swiss Model and contemporary PC software like PSI-Pred understand protein basic sequences. For protein visualisation in a flat shape, PSI-Pred is indispensable. These techniques provide useful information regarding the helical and coiled architectures of the protein by using predictive modelling to identify the sites of particular amino acids inside it as shown in [Fig 3](#), [Fig 4](#) and [Fig 5](#). This method helps us to

better appreciate the complex form and shape of the protein. Our evaluation of protein structure is much enhanced by the combination of PSI-Pred with Swiss-Model, therefore guaranteeing total accuracy. This helps one to grasp the special qualities and links of the protein more precisely. To completely understand the form of the protein, one must take into account the observations given by these predictive models.

Figure 03

PSIpred-Derived Two-Dimensional Protein Structure Visualisation Showing Diverse Structural Elements in Varied Colour Representations



Figure 04

Visualisation of Protein Structural Regions Using PSIPred: Distinct Colour Coding for Polar, Aromatic, Hydrophobic, Small Nonpolar Regions

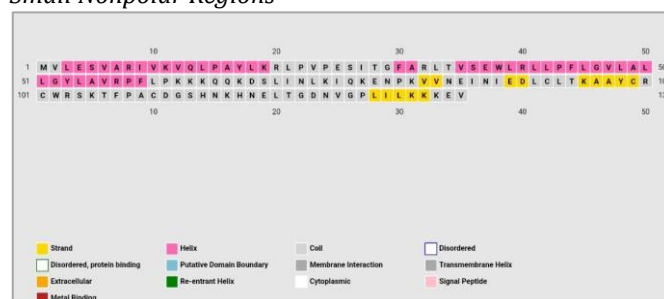
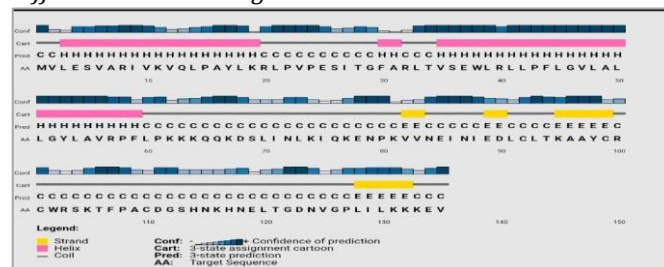


Figure 05

PSIpred-Derived Visualisation of Target Sequence, Confidence Score, and Three-State Prediction Employing Different Colour Coding: Two-Dimensional Protein Structure



An All-Inclusive Method for Validating the Three-Dimensional Structure of Proteins

Through the use of cutting-edge computer hardware, we make a guess as to what the three-dimensional form of the protein looks like and then correctly identify it. Knowing this is vitally necessary in order to have a complete understanding of its extensive preparation and operation. Through the utilisation of specific validation techniques such as the Ramachandran plot and Errat, we assess the precision of this three-dimensional version. We investigate the structure of the protein and the manner in which its components are assembled there. We are able to make the

prediction of three-dimensional forms more accurate and straightforward by first breaking it down into smaller bits and then doing detailed analysis on it. With the use of these methodologies, we are able to execute a more effective approach to shape prediction. We are able to identify the protein more accurately because to this meticulous method of inspection, which also ensures that the three-dimensional form of the protein is accurate. Therefore, this contributes to the definition of the degree of destiny research on their capabilities and how they interact with other things. By conducting rigorous inspections, we ensure that the findings of our investigations are accurate and trustworthy. Researchers and scientists working on drugs are able to come up with certain fairly certain results as a result of this.

Figure 06

Swiss-model-based three-dimensional protein structure modelling is shown in Figure

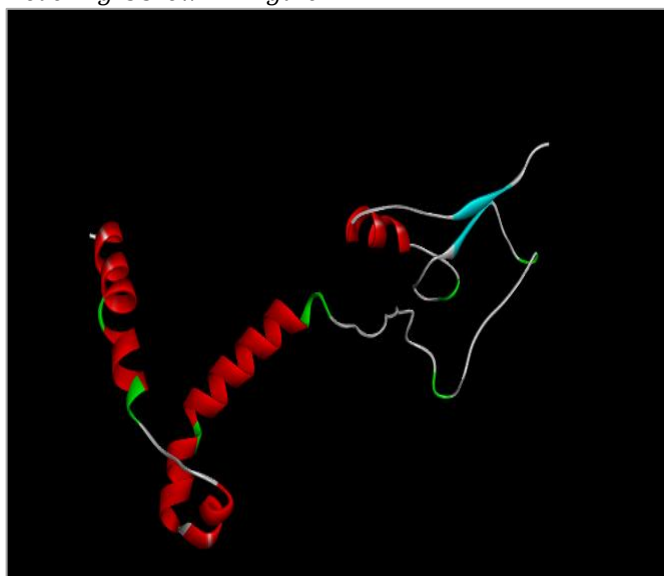


Figure 07

The evaluation of the shape and structure check of the Errat Ray is shown in Figure

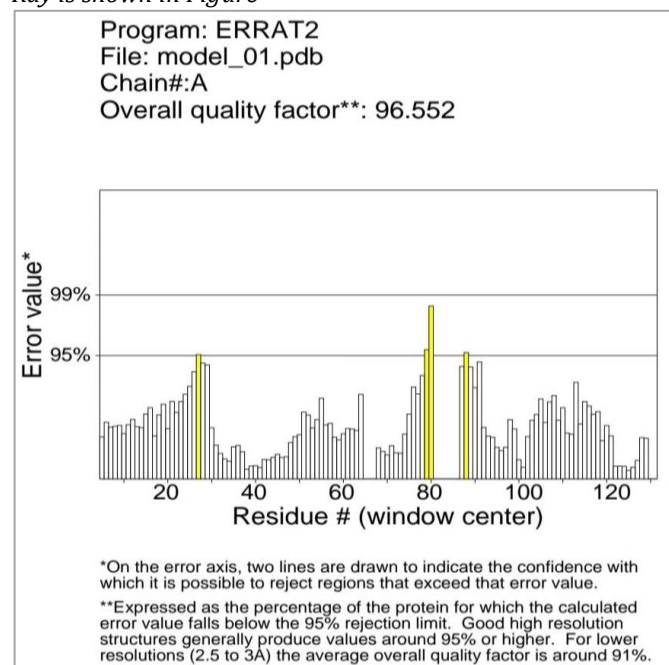
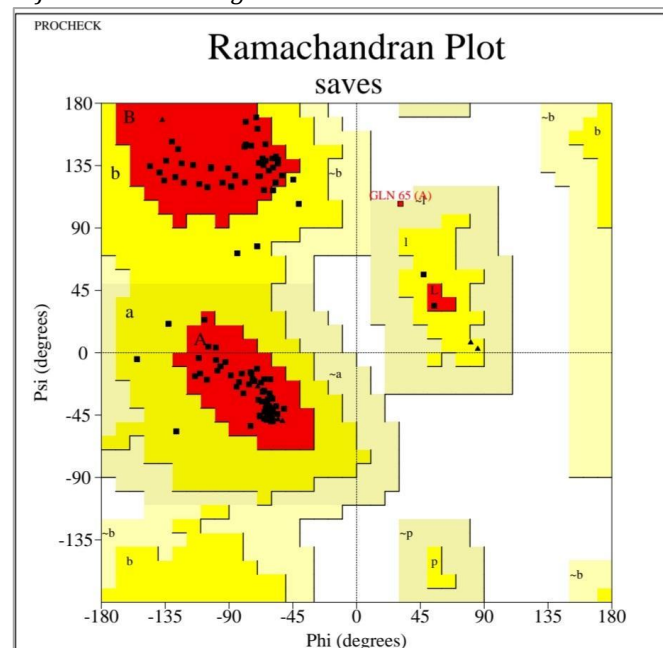


Figure 08

Using a completely unique chart known as a Ramachandran Plot, a determination of the definition and relationship of an object is shown in Figure



Investigate the relationship between the shape of the Cisd2 protein and the sparkling molecular connections.

Our increasing understanding of the structure of the Cisd2 protein has surely driven the creation of new medications. This result emphasises the need of having a strong awareness of protein structures in the design of new drugs. Currently under research for possible interactions with several drugs, including Liquiritigenin and CDGSH modulators, is the Cisd2 protein. The hunt for novel medications gains a major step forward when Liquiritigenin and CDGSH modulators are found as possible binders to the Cisd2 protein. This observation underlines the important need of knowing protein structures in the development of drugs and shows how such expertise can help to produce more successful treatments. This surprising result generally fits our earlier studies and creates interesting directions for creating treatments for particular disorders as well as for clarifying the function of the Cisd2 protein in the body.

Information Obtained from Autodock Vina Analyses Regarding the Ligand Binding Analysis of the Cisd2 Protein

Within the scope of our investigation, we discovered that the Cisd2 protein exhibits robust interactions with the ligand by the utilisation of Autodock Vina. To be more specific, the results of our study indicate that Liquiritigenin may have an impact on the mode of operation of the device. The identification of a specific region in which the protein binds to various molecules is an essential step in the process of comprehending the functioning of the protein. In addition to this, it provides us with essential information on the functions that the protein performs within the body as well as the ways in which it might be utilised to treat ailments. The results of this study demonstrate that knowledge of the ways in which

molecules and proteins change their forms can be extremely useful in the development of novel treatments. By making this new discovery, we are able to understand how the Cisd2 protein functions, which will lead to the development of novel treatments and improved medications. In general, those findings are consistent with what we are now investigating, and they represent a significant advancement in our understanding of how the Cisd2 protein functions and the potential therapeutic applications of this protein.

Substance-Cisd2 Protein Contact Patterns: Visual Verification of the Interaction Patterns

The findings of our research shed light on some fascinating new views about the interplay between Cisd2 and Liquiritigenin, as well as neurodegenerative illnesses. Through the utilization of computer imaging, researchers are able to investigate the chemical interactions that occur between Liquiritigenin and Cisd2 as shown [Fig 09](#). The results of this study shed light on the mechanism by which Liquiritigenin may activate Cisd2 within the body, hence enhancing our understanding of the regulating function of this protein. According to the findings, Liquiritigenin may be able to assist in the regulation of Cisd2 levels, hence reducing the potential risk of neurological illnesses that are associated with the aging process. Furthermore, it is becoming clear that these models are valuable tools for figuring out the complex biochemical pathways that are responsible for managing neuronal state and the aging process. Because of this study, therapeutic techniques that are intended to provide support for healthy brain function and to combat neurodegenerative diseases associated with aging have found their way forth.

Figure 09

Figure depicts the introduction of Liquiritigenin, a medication that binds with the Cisd2 protein in order to stabilise it and increase the expression of the Cisd2 gene.

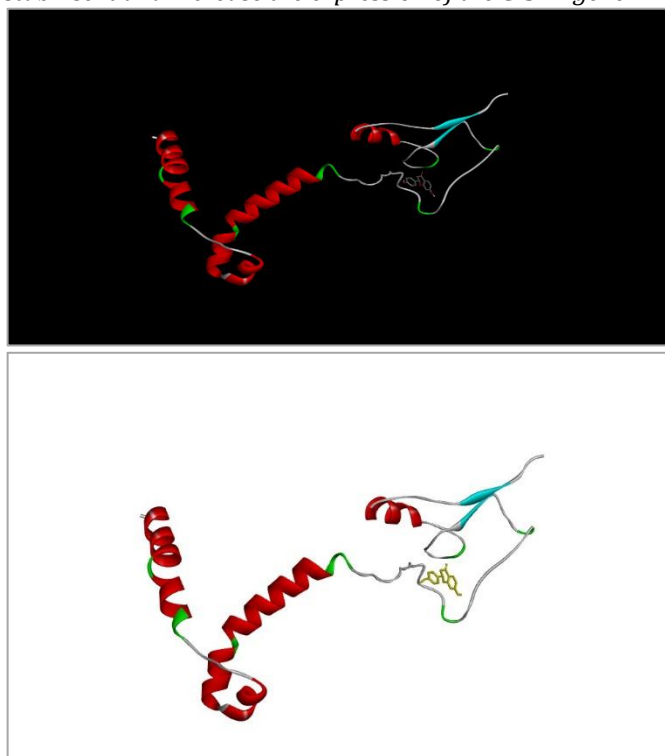


Figure 10

The binding point of Liquiritigenin with the Cisd2 protein is depicted in Figure

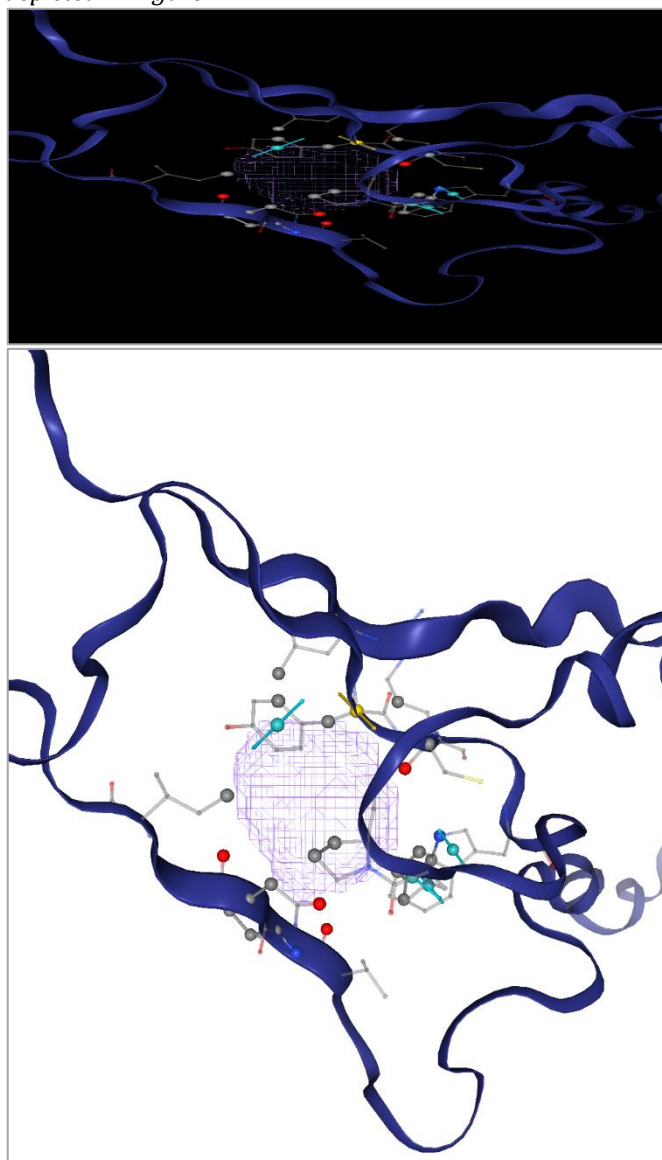


Figure 11

The bond distance is depicted in this figure.

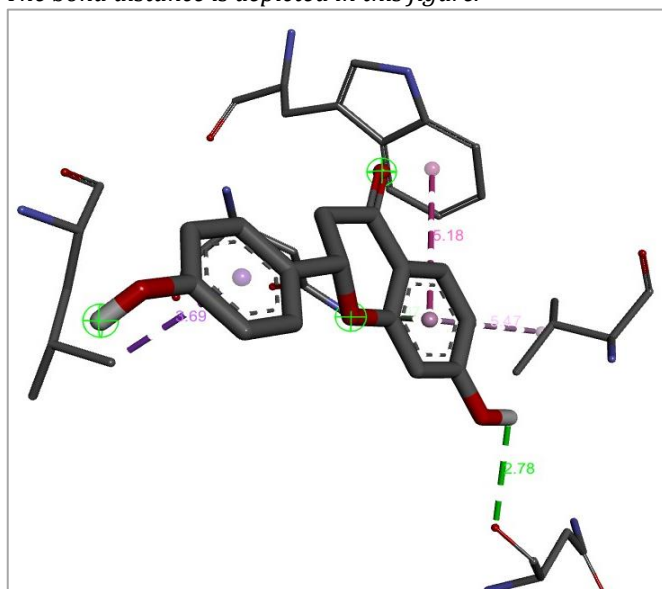
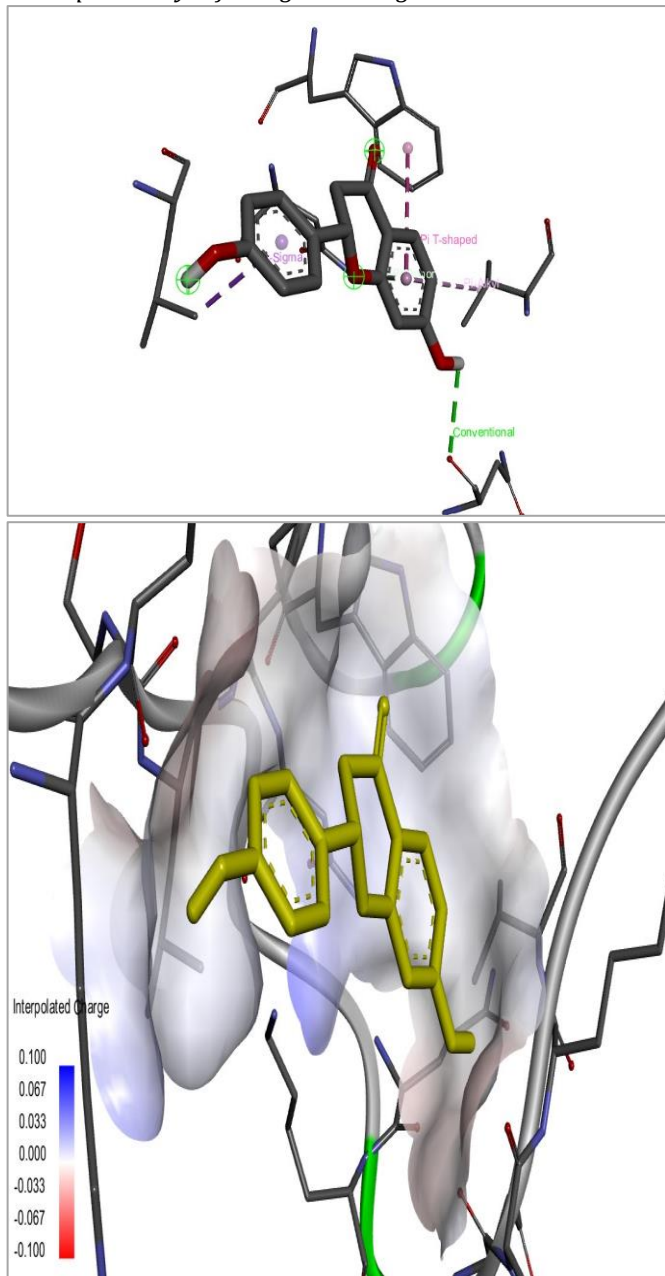
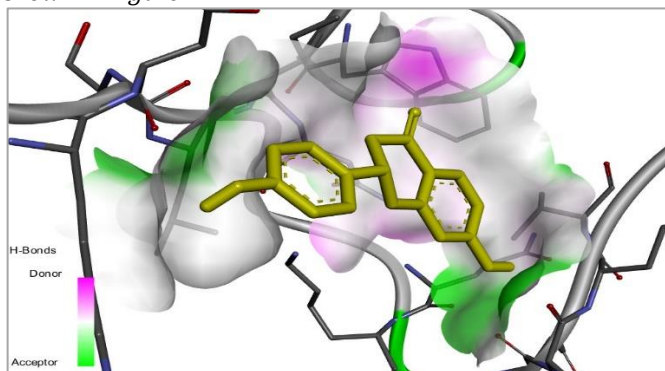


Figure 12

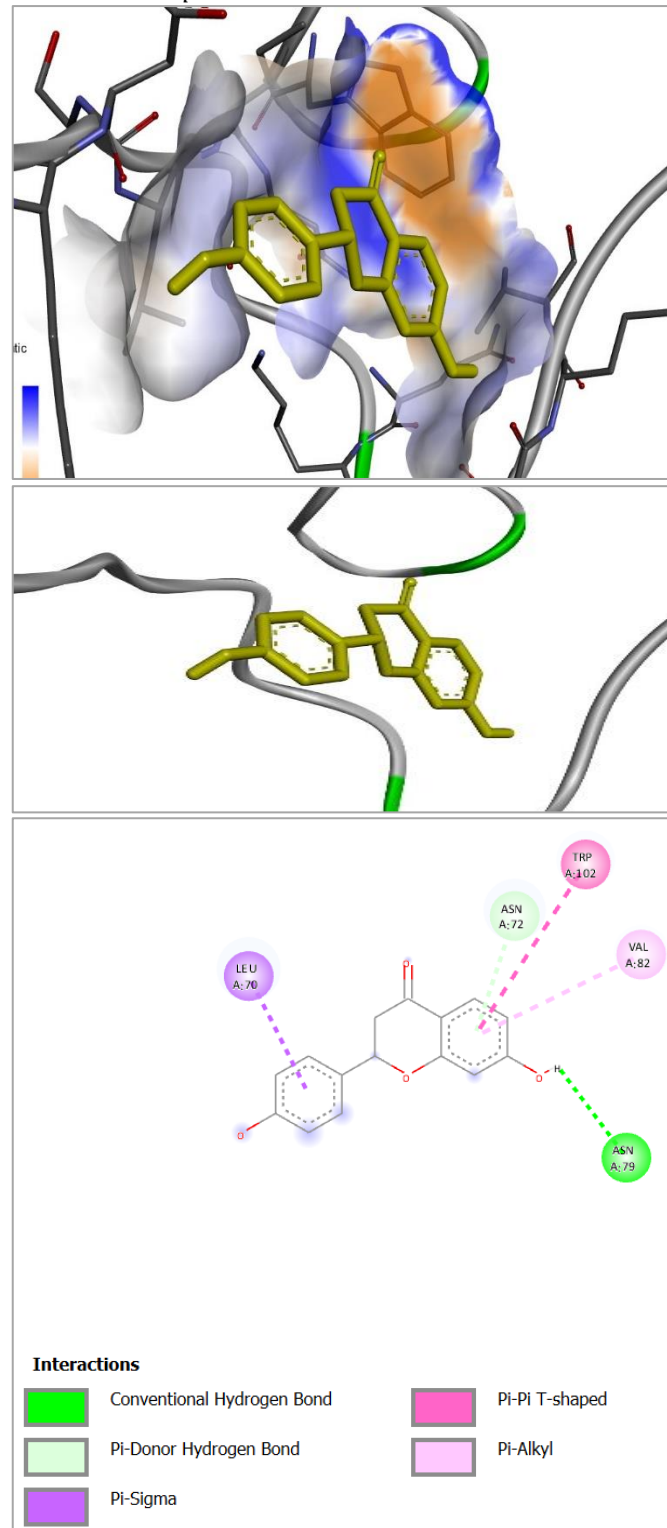
It is possible to gain an understanding of the many bonds that are involved in the binding of Liquiritigenin to the C1SD2 protein by referring to this Figure

**Figure 13**

Several distinct kinds of hydrogen bonds are involved in the process of Liquiritigenin attachment to the C1SD2 protein, as shown in Figure

**Figure 14**

The depiction in Figure 14 depicts the two-dimensional structure of Liquiritigenin as well as the binding site that it shares with the protein.



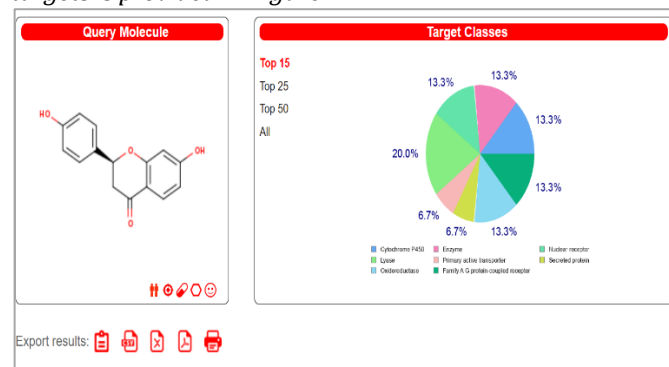
Swiss-Target Prediction Tool for Drug Target Analysis and Analysis of Drug Targets

Employed with great care, the Swiss-Target prediction tool is used to perform a thorough investigation of the several targets of the medicine across several physiological systems in the human body. This methodical technique helps us to emphasise the several pharmacological effects of the medicine that go beyond its main target, therefore

clarifying its several uses. We obtain important understanding of the wide spectrum of physiological processes influenced by the medicine by dissecting the intricate network of molecular interactions between the drug and its designated protein targets. This knowledge helps scientists to decide how best to improve the efficacy of the treatment for a variety of diagnosis and diseases. Moreover, it provides a basis for knowing how the medication works in the body over time.

Figure 15

A representation of the many categories of pharmacological targets is provided in Figure



Evaluation of Liquiritigenin Absorption and Properties for the Purpose of Enhancing Clinical Prospects: A Swiss-ADME Analysis

Our investigation using Swiss-Adme shows that Liquiritigenin is quickly absorbed in the stomach, which is a crucial determinant of the pharmacological efficacy of a medicine. These results suggest that the chemical has possible therapeutic use and is efficient within the body. The Lipinski rule supports Liquiritigenin's fit as a potential medication option for additional research. Its rapid absorption also implies that it could be a good way for drug delivery, so facilitating patient oral medicine intake and following their recommended treatment plan. All things considered, these discoveries improve our knowledge of the pharmacological profile of Liquiritigenin and offer a solid foundation for more investigation and clinical uses meant to maximise its therapeutic possibilities for metabolic malfunction and age-related disorders.

Figure 16

Using a boiled egg model, figure 16 shows the noteworthy absorption characteristics of Liquiritigenin, especially its high gastrointestinal absorption in humans (HIA).

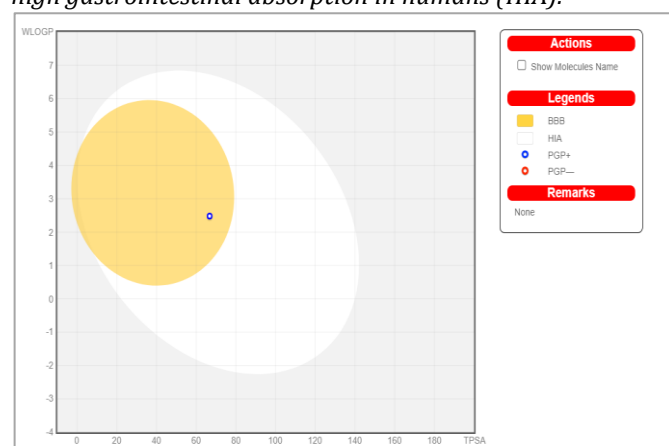
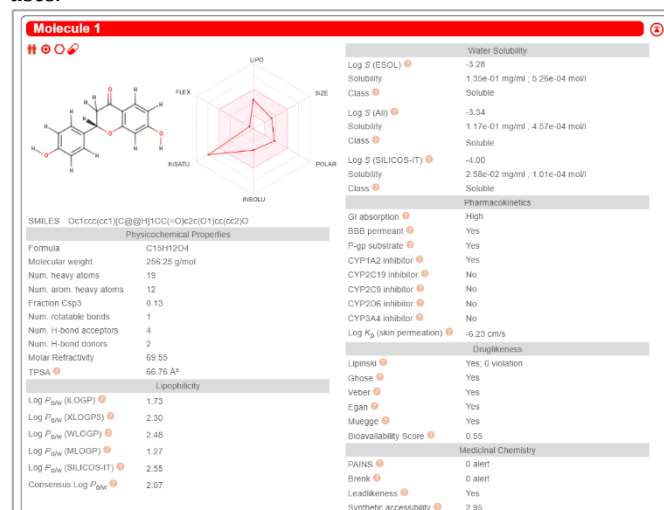


Figure 17

Figure 17 shows that Liquiritigenin complies with the Lipinski rule, has great gastrointestinal absorption, and is water-soluble—all of which suggest its possible medical uses.



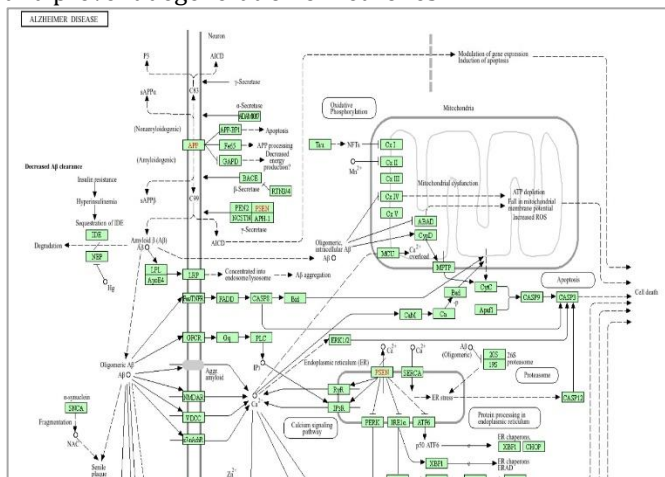
The Analysis of the Mechanism of Action Employing the KEGG Pathway Tool

Alzheimer's disease (AD) is characterized by the presence of complex mechanisms that revolve around amyloid-beta (Aβ) peptides. These peptides manifest themselves as a result of the breakdown of amyloid precursor protein (APP) via β-secretase (BACE) and γ-secretase (PSEN). These peptides combine to form oligomers, which disrupt the homeostasis of the cell and result in the formation of amyloid plaques. Aβ accumulation leads to mitochondrial Ca²⁺ excess, oxidative stress, and ATP depletion, which ultimately leads to caspase activation-mediated death. This occurs as a result of calcium (Ca²⁺) dysregulation. Furthermore, Aβ is responsible for creating stress in the endoplasmic reticulum (ER), which in turn leads to a reduction in protein folding and additional neuronal death through prolonged ER stress. Despite the fact that neuroinflammation makes the damage worse, neuronal degeneration is caused by mitochondrial malfunction and endoplasmic reticulum stress both working together. The C1SD2 protein found in mitochondria is responsible for both the reduction of oxidative stress and the maintenance of cellular integrity. One treatment method that shows promise for protecting neurons from Alzheimer's disease is the expression of C1SD2, which is being increased as shown in Fig18. A compound called liquiritigenin, which is produced from licorice, has demonstrated potential in enhancing the function of C1SD2, particularly in neurodegenerative illnesses that are associated with natural aging. Through the protection of mitochondrial integrity and the reduction of oxidative stress, liquiritigenin has the potential to assist in the delaying of the onset of neurodegenerative diseases such as Alzheimer's. In addition, computational investigations have demonstrated that liquiritigenin metabolites have the ability to boost C1SD2 activity even further, hence presenting novel therapeutic avenues for the treatment of neurodegenerative illnesses associated with aging. These systems highlight the significant role that C1SD2 plays in maintaining neuronal health and the potential therapeutic

benefit of Liquiritigenin in the treatment of Alzheimer's disease.

Figure 18

Alzheimer's disease (AD) results from the complicated series of events started by β -secretase (BACE) & γ -secretase (PSEN) breaking down amyloid precursor protein (APP). Amyloid-beta ($A\beta$) peptides produced by this pathway combine to form oligomers that upset cellular homeostasis and produce amyloid plaques. By raising Ca^{2+} influx into neurones via NMDAR and IP3R receptors, $A\beta$ induces calcium (Ca^{2+}) dysregulation as it accumulates. By means of the mitochondrial calcium uniporter (MCU2), this excess Ca^{2+} passes into the mitochondria and causes oxidative stress, ATP depletion, mitochondrial malfunction, and opening of the permeability transition pore in the mitochondria (MPTP). These events cause caspases (CASP9 and CASP3) to activate and cytochrome c (CytC) to be released, therefore inducing neural death. $A\beta$ buildup also causes endoplasmic reticulum (ER) stress, which disturbs protein folding and throws further off intracellular calcium equilibrium. Pathways include PERK and IRE1 α set off the unfolded protein response (UPR), while extended ER stress causes cell death via caspase 12 (CASP12) and CHOP. Along with neuroinflammation brought on by astrocytes and activated microglia in response to $A\beta$, mitochondrial malfunction increases oxidative stress and neurodegeneration. Particularly by means of drugs as Liquiritigenin, the figure highlights the possibility of improving Cisd2 expression to control mitochondrial function, reduce oxidative stress, and prevent degeneration of neurones.



Liquiritigenin, Cisd2, and CDGSH Modulators have been shown to have numerous therapeutic benefits for neurodegenerative diseases, and their mechanisms of action are being investigated

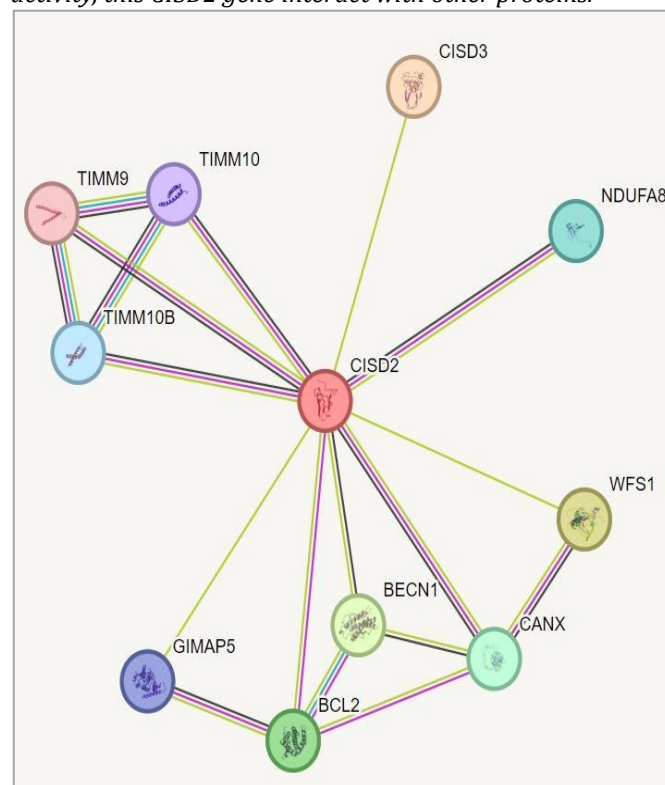
In the treatment of neurodegenerative illnesses, liquiritigenin and Cisd2 can be of tremendous assistance due to their distinct mechanisms of action. The antioxidant known as liquiritigenin protects neurons from the damaging effects of oxidative stress, hence enhancing the cells' structural integrity. Because of its anti-inflammatory properties, it helps to reduce the inflammation that is associated with neurodegenerative illnesses and contributes to the fight against neurodegeneration itself. The power of antioxidant

proteins to fight off hazardous substances is enhanced by this molecule, which in turn strengthens the antioxidant defense mechanisms. Through the inhibition of the production of pro-inflammatory mediators, liquiritigenin is able to maintain neuronal activity for an extended period of time. The mitochondrial energy generation and function are both preserved in a significant way by Cisd2, which is essential for the health of the neural network. Through the maintenance of mitochondrial activity, Cisd2 protects neurons from damage and stress, hence lowering the generation of reactive oxygen species (ROS). The presence of this protein protects cells against the effects of stress and increases the lifetime of cells, so ensuring that neuronal health is maintained continuously. Furthermore, by modifying processes that are associated with death and endoplasmic reticulum (ER) stress, it enhances its protective effects against neurodegenerative illnesses. This is particularly beneficial in its reaction to the accumulation of amyloid-beta ($A\beta$) and endoplasmic reticulum (ER) stress, which are the primary factors that lead to neurodegeneration. CDGSH modulators provide therapeutic benefits, but these benefits are contingent on the existence of Cisd2, which is identified by its CDGSH iron-sulfur domain. Through intricate molecular pathways, this protein acts as a critical mediator, which enables the numerous therapeutic applications of CDGSH modulators in the treatment of a variety of ailments, such as neurodegenerative diseases, diabetes, and metabolic dysfunctions that are associated with aging. When these pathways are targeted, Liquiritigenin and Cisd2 will be able to play a more significant role in the therapy and prevention of neurodegenerative illnesses.

String Analysis Results

Figure 19

This suggests that to guarantee appropriate physiological activity, this Cisd2 gene interact with other proteins.



Summary of Docking Results

Table 2

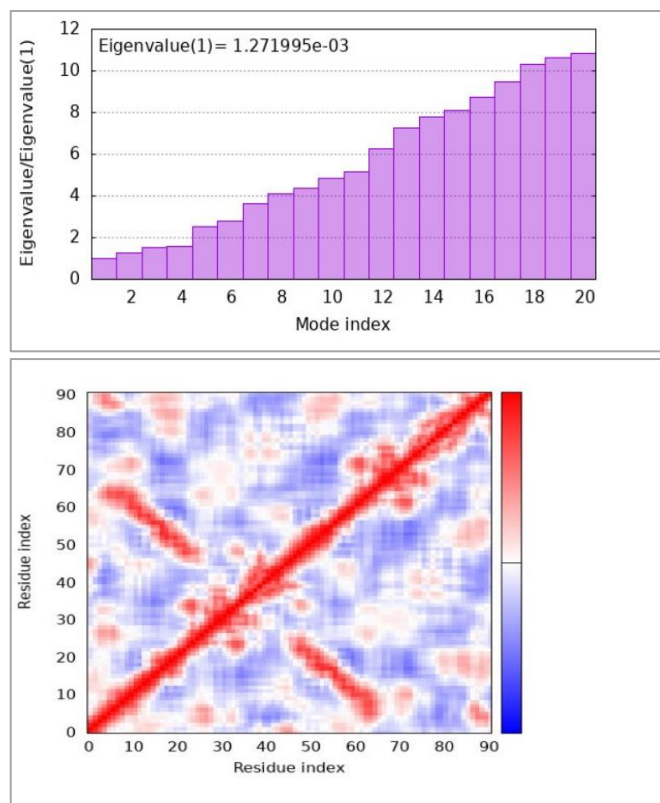
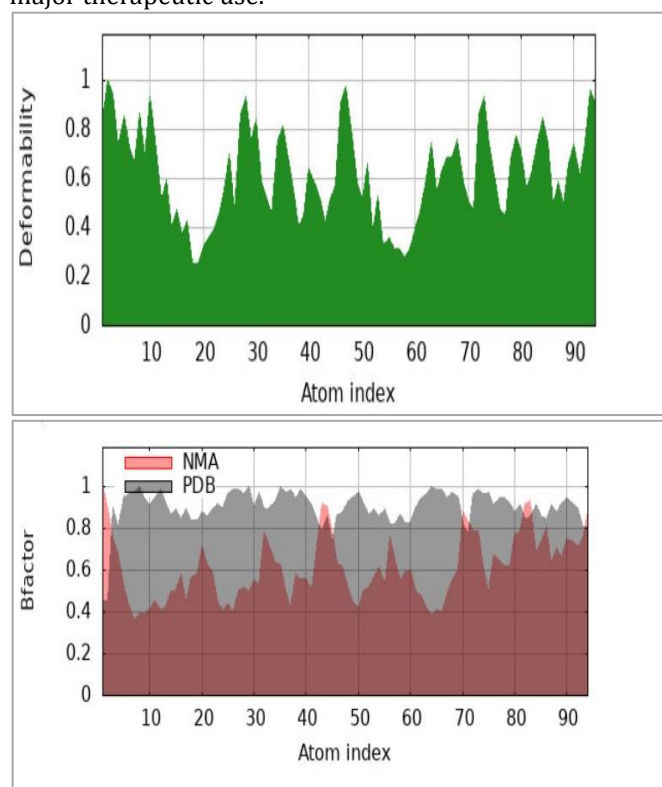
Reflectively with a value of (-6.2), the docking results show a significant binding affinity of Liquiritigenin with the CISD2 protein.

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
model_01_114829_uff_E=226.64_uff_E=226.54	-6.2	0	0
model_01_114829_uff_E=226.64_uff_E=226.54	-5.9	3.053	2.422
model_01_114829_uff_E=226.64_uff_E=226.54	-5.9	2.305	1.655
model_01_114829_uff_E=226.64_uff_E=226.54	-5.8	2.243	1.645
model_01_114829_uff_E=226.64_uff_E=226.54	-5.7	5.603	3.092
model_01_114829_uff_E=226.64_uff_E=226.54	-5.7	5.572	3.34
model_01_114829_uff_E=226.64_uff_E=226.54	-5.6	12.707	9.865
model_01_114829_uff_E=226.64_uff_E=226.54	-5.6	5.213	3.083
model_01_114829_uff_E=226.64_uff_E=226.54	-5.6	6.449	1.839

Dynamics in Molecular Simulation

Figure 20

From the B-factor, which shows the flexibility of different protein regions; deformability, which points out areas prone to structural changes; and the eigenvalue, which assesses the rigidity of the protein and the energy required for conformational adjustments, one gains important understanding of protein behaviour. Our results reveal that these values are within ideal ranges, therefore suggesting a good balance between stability and adaptability. This implies that functional needs call for little structural changes. These findings substantially increase our trust in the suggested protein model and verify that our method of investigation is sound and has considerable possibility for major therapeutic use.



DISCUSSION

The significance of CISD2 in neurodegenerative illnesses including Parkinson's disease (PD) and Alzheimer's disease (AD) is highlighted by the findings of these investigations.[22] Systems that are affected by problems related with endoplasmic reticulum (ER) stress, oxidative stress, and mitochondrial dysfunction cannot function properly without the presence of CISD2, which is an essential regulator.[23] Our research has demonstrated that increasing the activity of CISD2 can lessen the effects of oxidative stress, slow down the process of cell death, and make it easier for mitochondria and the endoplasmic reticulum to operate properly.[24] These are all important variables that have been identified as contributing to neurodegeneration. In line with the findings of previous research that has demonstrated the role of CISD2 in maintaining redox equilibrium and mitochondrial integrity, these findings are consistent.[25] An important finding that emerged from this investigation was the realization that the flavonoid compound known as Liquiritigenin has the ability to boost the expression of CISD2.[26] Increasing the levels of CISD2 makes it easier for Liquiritigenin to reduce the effects of oxidative stress and mitochondrial malfunction, both of which are frequently seen in neurodegenerative illnesses.[27] Liquiritigenin's status as a CISD2 activator was further supported by the computational drug discovery techniques that we developed.[28] Through the use of molecular docking simulations, we were able to determine that Liquiritigenin possesses a substantial potential to bind and activate CISD2, hence providing a unique therapeutic approach for the treatment of neurodegenerative illnesses. Immediate evaluation of a number of compounds was carried out using in silico techniques, and the compounds that exhibited the highest

binding affinity for C1SD2 were identified.[29] These computational tools not only helped us gain a better knowledge of how C1SD2 is activated, but they also sped up the process of finding new drugs. The idea that the overexpression of C1SD2 maintains mitochondrial and endoplasmic reticulum function, hence shielding neurons against degeneration, is supported by the observations that we have obtained. Liquiritigenin-induced activation of C1SD2 resulted in a considerable reduction of important factors that contribute to neuronal death in neurodegenerative illnesses.[30] These factors include oxidative stress, increased mitochondrial Ca^{2+} , and the release of pro-apoptotic proteins, including cytochrome c. Through the maintenance of mitochondrial membrane potential and the regulation of the formation of reactive oxygen species (ROS), C1SD2 reduces the risk of cellular death. Moreover, C1SD2 is responsible for the regulation of Ca^{2+} concentrations in the endoplasmic reticulum (ER) and the reduction of ER stress, which is linked to the activation of pathogenic pathways such as CHop, IRE1 α , and PERK.[31] During the progression of Alzheimer's disease, the reduction in C1SD2 expression that occurs as a result of the accumulation of amyloid-beta ($\text{A}\beta$) peptides exacerbates the effects of oxidative stress and mitochondrial dysfunction.[32] According to our findings, the enhancement of C1SD2 expression with the use of Liquiritigenin has the potential to reduce the mitochondrial damage caused by $\text{A}\beta$, hence proposing a potential therapeutic approach for Alzheimer's disease and its associated symptoms.[33] It is possible that the activation of C1SD2 in Parkinson's disease protects dopaminergic neurons by preserving the integrity of mitochondria. Taking into consideration that the progression of Parkinson's disease is predominantly driven by oxidative stress and mitochondrial complex I malfunction, the capacity of Liquiritigenin to boost C1SD2 expression may result in beneficial benefits.[34] When astrocytes and activated microglia are exposed to $\text{A}\beta$ toxicity and other neurodegenerative stimuli, C1SD2 is responsible for regulating the neuroinflammation that occurs as a result. Liquiritigenin has the ability to activate C1SD2, which indicates that it has the potential to reduce neuroinflammation.[35] Neuroinflammation is a significant role in the course of many neurodegenerative illnesses, including dementia. In spite of the fact that our computational analysis produced encouraging results, a fundamental drawback of our research is that we did not conduct any in vitro or in vivo experiments to validate the therapeutic efficacy of Liquiritigenin as a C1SD2 activator. Despite the fact that our in silico approaches have yielded substantial insights, it is essential to do additional biological validation.[36] Liquiritigenin should be evaluated for its neuroprotective properties in cellular and animal models of dementia, and its efficacy in upregulating C1SD2 should be experimentally determined. This research should be conducted in the future. Ultimately, the results of our research offer a solid basis for the development of treatments that are aimed at C1SD2. In its capacity as a C1SD2 activator, liquiritigenin presents a potentially fruitful treatment approach that has the ability to slow

down or even reverse the progression of neurodegenerative diseases including Parkinson's disease and Alzheimer's disease.[37] Through the modulation of mitochondrial and endoplasmic reticulum function, the reduction of oxidative stress, and the prevention of neuronal death, liquiritigenin offers a novel therapeutic path. It is vital to conduct additional study, which should include both in vitro and in vivo investigations, in order to validate these discoveries and develop viable treatments for neurodegenerative illnesses. Although silico studies have revealed substantial insights, further research is currently required.

CONCLUSION

This research highlights the crucial function of C1SD2 in maintaining the integrity of mitochondria and the endoplasmic reticulum (ER), essential for averting neuronal damage associated with Alzheimer's disease (AD), Parkinson's disease (PD), and other neurodegenerative disorders. Our findings suggest that activating C1SD2 may provide a viable treatment strategy by addressing mitochondrial malfunction, oxidative stress, and endoplasmic reticulum stress, which are critical contributors to the advancement of neurodegenerative disorders. We identified small-molecule activators of C1SD2 through computational drug discovery methods that demonstrate significant potential to enhance C1SD2 activity. The naturally occurring flavonoid liquiritigenin was found to significantly enhance C1SD2 expression, hence promoting beneficial changes in the cell. By stabilizing mitochondrial and endoplasmic reticulum function, it diminishes the generation of reactive oxygen species (ROS) and prevents cell death, hence activating C1SD2 caused by liquiritigenin. Liquiritigenin not only safeguards brain cells from mitochondrial injury and apoptosis but also mitigates neuroinflammation, hence augmenting its neuroprotective properties. The findings validate the notion that a novel category of therapies aimed at maintaining cellular homeostasis in the mitochondria and endoplasmic reticulum may be developed by targeting C1SD2 with Liquiritigenin or other compounds, thereby decelerating the progression of neurodegenerative diseases. Further laboratory (in vitro) and animal (in vivo) research are necessary to establish the efficacy and safety of these C1SD2 activators despite these promising results. Additional study is essential to enhance therapeutic strategies, as the molecular mechanisms by which C1SD2 sustains mitochondrial and endoplasmic reticulum function require scrutiny. Future study should investigate the timing and context in which C1SD2 activation, particularly with compounds such as Liquiritigenin, would be most advantageous during disease progression. Employing small-molecule activators to target C1SD2 offers a novel strategy for the treatment of neurodegenerative illnesses. C1SD2 activation may delay or halt neuronal degeneration by maintaining cellular equilibrium in mitochondria and the endoplasmic reticulum, therefore presenting possibilities for enhanced therapies for Alzheimer's disease, Parkinson's disease, and other neurodegenerative illnesses.

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