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Research Article

In Silico Targeting and Structural Stabilization of LRRC66 by 3′,5,7-Trihydroxy-4′- methoxy flavanone A Computational Strategy for Therapeutic Modulation of Cardiac Remodeling

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Abstract: Cardiovascular diseases (CVDs) remain the leading cause of global mortality, responsible for approximately 20.5 million deaths in 2021. The impact of genetic alterations on protein stability was assessed using MUpro and I-Mutant 2.0. GalaxyRefine was used to refine the tertiary structure modeled by SWISS-MODEL, and validation was performed using Errat (overall quality factor 89.87) and Ramachandran plot analysis (93.4% residues in favored regions). Binding affinity was determined by molecular docking with AutoDock, and pharmacokinetic profiles were predicted by SwissADME. Finally, structural stability and B-factor flexibility were evaluated by molecular dynamics simulations using the iMODS approach. A docking binding affinity of −7.8 kcal/mol and a spontaneous binding free energy of −35.4 kcal/mol indicated strong interaction. 3',5,7-Trihydroxy-4'-methoxyflavanone optimized the structural dynamics of the solenoid domain by. We conclude that 3',5,7-Trihydroxy-4'-methoxyflavanone is a promising pharmacological approach for preventing heart failure and cardiac metabolic dysfunction by efficiently targeting and stabilizing the LRRC66 membrane sensor.

Keywords: cardiovascular disease; drug design; LRRC66 gene; cardiac hypertrophy; molecular docking

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1. Introduction

Cardiovascular diseases (CVDs) remain the biggest challenge to global public health, responsible for approximately 20.5 million deaths worldwide in 2021[1-3]. Heart failure (HF) is one of these illnesses that has reached epidemic proportions, with a steady and concerning increase in rate across all demographics[4,5]. According to current epidemiological data, there are about 6.7 million 8.7 million by 2030 and an unbelievable 11.4 million by 2050[6,7]. One in four people will be diagnosed with HF at some point in their lives due to the increased lifetime risk of 24%[8,9]. HF was a contributing factor in 425,147 fatalities in the US. In 2022, More than 45% of deaths were linked to the cardiovascular disease[10,11]. The financial impact is also alarming; in 2020, direct medical expenditures for heart failure were predicted to be $32 billion, and by 2050, total costs, including indirect costs from lost productivity, might reach $858 billion[12,13]. Less than 25% of eligible patients receive optimal multiple therapy, despite advancements in guideline-directed medical therapies (GDMT)[14]. This global shortage is predicted to cost 1.19 million lives annually. There is an urgent need for new molecular targets that control cardiac structural resilience and stop irreversible remodeling because the transition from adaptive hypertrophy to terminal heart failure includes a complex interaction of genetic variables and mechanical stress[15-17]. Leucine-Rich Repeat-Containing Protein 66 (LRRC66) is a crucial "hub gene" in cardiac developmental networks and cardiomyocyte differentiation, according to recent transcriptomic and genomic discoveries. LRRC66 is a member of the LRR superfamily, a collection of proteins with adaptable solenoid-shaped domains that promote signal transduction, cell adhesion, and intricate protein-protein interactions. It is found on chromosome 4q12. LRRC66 is an 880 amino acid multi-pass transmembrane protein with two helical transmembrane segments at residues 4–24 and 376–396, as well as a large extracellular domain with six conserved LRR motifs. It is hypothesized that LRRC66 serves as a key membrane sensor in the cardiac environment, converting physical heart-wall strain into signals for cellular growth,

serving as a molecular link between the exterior matrix and the internal cytoskeleton of the cardiomyocyte. The protein is at a crucial junction of vascular resistance and cardiac structural integrity because variations in the LRRC66 locus have been closely associated with systolic blood pressure regulation. Its function and structure are similar to those of the cardiac-specific protein LRRC10, whose mutations are directly associated with human idiopathic dilated cardiomyopathy (DCM)[18,19]. When these membrane sensors are disrupted, homeostasis is compromised, resulting in apoptosis, suppression of antioxidant systems, and reduced mitochondrial bioenergetics, which ultimately lead to irreversible hypertrophy or fibrosis[20,21]. The evaluation of natural polyphenols as safe and efficient medicinal substitutes has grown as a result of the hunt for pharmacological stabilizers of such proteins. Citrus fruits like oranges and lemons contain a bioactive flavanone called 3',5,7-Trihydroxy-4'-methoxyflavanone, which has strong anti-inflammatory, cardioprotective, and antioxidant qualities[22]. This substance helps shield heart cells from harm by scavenging free radicals and lowering oxidative stress[23]. To prevent fatal arrhythmias, 3',5,7-Trihydroxy-4'-methoxyflavanone is known to modify vital ion channels like Nav1.5 and hERG and block pro-hypertrophic signaling cascades like the PI3K/AKT and MAPK pathways[16]. It increases the inactivation rate of human mutants linked to long QT syndrome type 3 by interacting with the voltage-gated Na+ channel's local anesthetic binding region[24,25]. Additionally, it has been demonstrated that 3',5,7-trihydroxy-4'-methoxyflavanone reduces the systemic inflammatory conditions that cause cardiac fibrosis by lowering the expression of pro-inflammatory cytokines such as TNF-alpha, IL-6, and IL-beta. 3',5,7-Trihydroxy-4'-methoxyflavanone is a great option for targeting the LRRC66 sensor to preserve cardiac membrane architecture under long-term mechanical stress because of its good drug-likeness and safe toxicological profile (LD50 > 2000 mg/kg)[26]. The LRRC66 mRNA (NM_001024611.3) and protein (NP_001019782.1) sequences were retrieved from the NCBI database to start the computational pipeline. The EXPASY program was then used to find the right open reading frames[27]. Using a multi-tool approach that included BLASTx, MUpro, PolyPhen-2, and I-Mutant 2.0 to assess the consequent changes in Gibbs free energy, genetic alterations impacting LRRC66 stability were confirmed[28,29]. PsiPred secondary structure prediction was used to determine the structural organization[30,31]. SWISS-MODEL was used to model 3D homology, and the GalaxyRefine web service was used to refine the structure for the best side-chain packing[32,33]. High-affinity molecular docking of the 3',5,7-Trihydroxy-4'-methoxyflavanone ligand utilizing the PyRx and AutoDock platforms was made possible by the refined model's stereochemical validation using Errat and Ramachandran plots. SwissADME and the "boiled egg" model were used for thorough pharmacokinetic profiling in order to verify complete adherence to the Lipinski rule of five and high human intestinal absorption[34,35]. Finally, molecular dynamics simulations using the IMODS methodology were used to evaluate thermodynamic and structural stability. The ligand-protein interaction was quantified using eigenvalue and B-factor calculations[36,37].

2. Results

2.1 Nucleotide sequence of LRRC66

The complete sequence of the altered LRRC66 quality is determined by an investigation using the NCBI database and the accession number NM_001024611.3. This research is especially crucial for estimating the protein's fundamental structure.

Table 1: Displays the gene sequence that was obtained from the NCBI.

Coding region of LRRC66 gene (NM_001024611.3)
AAATCCCTACCTGGCTTGATCCAGATATCCACCCATTTGATAACCACTTAGGATAC-
GAAGGCATTATGAAAAACCTCTATTTCAGAGTCATTACCATAGTTATAGGTCTTTATTTTACTGGAATAATGACAAATGCATC
AAGAAAAAGCAATATTTTATTCAATTCTGAATGCCAATGGAATGAATATATTCTGACAAATT-
GTTCTTTTACCGGAAAGTGTGATATACCTGTGGACATATCACAGACAGCAGCCACTGTGGATGTAAGTTTCAATTTCTTTAGA
GTTCTCTTACAGTCTCACACGAAAAAAGAAGAGTGGAAAATAAAACATCTGGACCTCAG-
TAACAATCTCATATCAAAAATAACCTTAAGCCCTTTTGCATATTTACATGCTTTGGAAGTGTTAAACCTCAGCAACAATGCCAT
CCACTCCCTCTCATTGGATCTACTCAGTCCTAAGTCCTCATGGGTGAAACGCCACAGAA-
GCAGCTTCAGAAACAGGTTTCCATTGCTGAAGGTGCTCATTCTTCAAAGAAATAAACTCAGTGACACTCCCAAGGGACTGTGG
AAACTGAAGTCATTGCAGAGTTTGGATCTGTCATTCAATGGGATATTGCAAA-
TAGGGTGGTCTGATTTTCACAACTGCCTGCAACTGGAGAATCTCTGTTTAAAGAGCAACAAGATATTCAAAATTCCCCCACAA
GCCTTCAAGGACCTCAAAAAATTACAGGTCATAGACCTTAGCAACAATGCTCTGATTAC-
CATCCTACCAATGATGATCATAGCTCTAGAATTTCCCCATCTAGTGGTTGACTTGGCTGATAATAACTGGCAGTGTGATGATA
GTGTGGCAGTCTTTCAAAATTTTATTTCTGAATCCTGGAGGAAAAAGTGGAATGTCATTT-
GCAACAGGTCTATAGGGAGTGAGGAGGCCAACGGGGGCACTCCCCAGAGCAGGATTTCCAGGGAAACCCGCCTTCCTCCCAT
TCATCTGCATCGCATGAAAAGCCTCATAAGGAGCAAAGCAGAGAGGCCCCAGGGAG-
GAAGGCACACGGGCATTTCTACTCTGGGGAAGAAGGCAAAGGCCGGCTCTGGTCTCAGGAAGAAGCAGAGACGGCTGCCAA
GGAGTGTTAGAAGCACCCGCGATGTGCAGGCTGCCGGCAAAAAAAGAGGACGCTCCCCAGGAC-
CTGGCTCTGGCGGTGTGCCTGTCAGTGTTCATCACATTCCTTGTCGCCTTCAGCCTGGGGGCTTTCACAAGGCCTTATGTTGAC
AGACTGTGGCAAAAAAAGTGCCAGAGCAAAAGCCCTGGCCTGGACAACGCGTATTCAAAC-
GAGGGCTTCTACGATGACATGGAAGCTGCGGGGCACACACCACACCCAGAGACCCATCTGCGCCAAGTATTTCCTCATCTAA
GCCTCTACGAGAACCAGACCCCTTTCTGGGTGACACAGCCACACCCACACGCCACCG-
TAATTCCTGATAGAACTCTGGGAAGGAGCAGAAAGGATCCTGGCAGTTCGCAGAGCCCAGGACAGTGCGGGGACAACACCG

GGGCAGGAAGTGGAAATGATGGTGCAGTCTATTCCATTCTCCAGAGACATCCACATGCCGG-
TAACCGTGAACTAATGTCAGCAGCGCAGGACCACATCCATAGGAATGATATTCTCGGAGAATGGACTTATGAAACTGTGGCC
CAGGAAGAGCCTCTCAGTGCACATTCAGTGGGCGTCTCTTCTGTAGCTGGCACGTCTCAC-
GCTGTCTCTGGCTCAAGCCGTTATGATTCCAATGAATTAGACCCTTCCCTCTCCGGAGAAATAACAGCTTCCCTCTGTAAAATG
CTAACACATGCAGAAGCACAGAGGACTGGAGATAGTAAGGAAAGAGGGGGCACTGAACAG-
TCACTTTGGGACTCGCAGATGGAATTTTCTAAGGAAAGGCAAGTGAGTTCATCCATTGATTTGCTGAGCATACAGCAGCCAAG
GCTGTCCGGGGCAAGGGCTGAGGAAGCGCTTTCAGCCCAC-
TACAGCGAGGTTCCATACGGTGACCCAAGAGACACAGGCCCATCAGTCTTTCCTCCAAGATGGGACAGTGGCCTGGATGTCA
CTCCTGCTAACAAGGAACCAGTGCAGAAATCCACTCCTTCTGACACTTGCTGTGAGTTGGA-
GAGTGACTGTGACTCTGATGAGGGGTCTCTGTTCACTCTGAGCTCCATAAGTTCAGAGAGTGCAAGGAGCAAGACTGAAGAG
GCAGTGCCTGATGAGGAGTCCCTGCAGGACGAGAGCTCAGGGGCAAGCAAGGACAATGTGAC-
GGCTGTAGACAGTCTTGAGGAAAATGTTACCTTCCAAACAATTCCAGGGAAATGCAAGAATCAAGAAGATCCCTTTGAAAAA
CCTCTCATTTCTGCTCCAGACTCTGGCATGTACAAGACTCATCTGGAAAATGCCTCTGACAC-
TGATAGATCTGAGGGCCTGTCACCCTGGCCCAGGTCACCAGGGA

2.2 Translation EXPASY: Interpreting Modified DNA Sequences to Examine Fundamental Protein Structure

The EXPASY method can be used to anticipate the fundamental structure of an LRRC66 protein that is causing issues.

Table 2: Primary structure of the LRRC66 protein (NP_001019782.1) as predicted by the EXPASY translate tool.

Primary protein sequence
MKNLYFRVITIVIGLYFTGIMTNASRKSNILFNSECQWNEYILTNCSFTGKCDIPVDIS- QTAATVDVSFNFFRVLLQSHTKKEEWKIKHLDLSNNLISKITLSPFAYLHALEVLNLSNNAIHSLSLDLLSPKSSWVKRHRSSFRNRF PLLKVLILQRNKLSDTPKGLWKLKSLQSLDLSFNGILQIGWSDFHNCLQLENLCLK- SNKIFKIPPQAFKDLKKLQVIDLSNNALITILPMMIIALEFPHLVVDLADNNWQCDDSVAVFQNFSESWRKKWNVICNRSIGSEEANG GTPQSRISRETRLPPIHLHRMKSLIRSKAERPQGGRHTGISTLGKKAKAGSGLRKKQRRL- PRSVRSTRDVQAAGKKEDAPQDLALAVCLSVFITFLVAFSLGAFTRPYVDRLWQKKCQSKSPGLDNAYSNEGFYDDMEAAGHTPH PETHLRQVFPHLSLYENQTPFWVTQPHPHATVIPDRTLGRSRKDPGSS- QSPGQCGDNTGAGSGNDGAVYSILQRHPHAGNRELMSAAQDHIHRNDILGEWTYETVAQEEPLSAHSVGVSSVAGTSHAVSGSSR YDSNELDPSLSGEITASLCKMLTHAEAQRTGDSKERGGTEQSLWDSQMEFSKERQVSSSI- DLLSIQQPRLSGARAEEALSAHYSEVPYGDPRDTGPSVFPPRWDSGLDVTPANKEPVQKSTPSDTCCELESDCDSDEGSLFTLSSISSE SARSKTEEAVPDEESLQDESSGASKDNVTAVDSLEENVTFQTIPGKCKNQEDPFEKPLISAP- DSGMYKTHLENASDTDRSEGLSPWPRSPGNSPLGDEFPGMFTYDYDTALQSKAAEWHCSLRDLEFSNVDVLQQTPPCSAEVPSDPD KAAFHERDSDILK

2.3 Identification and Verification of LRRC66 Gene Sequence Mutations: Utilising BLASTX and Specialised Tools

The decrease in the expression of the LRRC66 protein is largely caused by changes to the protein's sequence. It is a significant characteristic that these mutations are located inside the protein binding pocket. Table 3 lists the alterations that were attached utilizing a range of analytical techniques.

Table 3: Mutations in LRRC66 verified using MUpro, I-Mutant, and PolyPhen-2, showing the predicted effect on protein stability.

Mutation	MUpro	I-Mutant	PolyPhen-2
I42V	Decrease	Decrease	Benign
C52H	Decrease	Decrease	Benign
I58T	Decrease	Decrease	Benign
N70G	Decrease	Decrease	Benign
L75F	Decrease	Decrease	Benign
S78C	Decrease	Decrease	Benign
N145K	Decrease	Decrease	Benign
HI92Q	Decrease	Decrease	Benign
P211H	Decrease	Decrease	Benign
I224V	Decrease	Decrease	Benign
I240M	Decrease	Decrease	Benign
N255H	Decrease	Decrease	Benign
R304H	Decrease	Decrease	Benign

R312S	Decrease	Decrease	Benign
M313V	Decrease	Decrease	Benign
I332V	Decrease	Decrease	Benign
K340R	Decrease	Decrease	Benign
G342S	Decrease	Decrease	Benign
S392C	Decrease	Decrease	Benign
V401I	Decrease	Decrease	Benign
A419G	Decrease	Decrease	Benign
M429I	Decrease	Decrease	Benign
V445A	Decrease	Decrease	Benign

2.4 Secondary Structure

In structural bioinformatics, the PSIPRED tool is frequently used to predict the secondary structure of proteins, particularly those having unstable conformations. This predictive model produces sequence plots that illuminate the structural structure of the protein by displaying strand, helix, and coil forms. According to the results in Figs. 1, the following experimental measures.

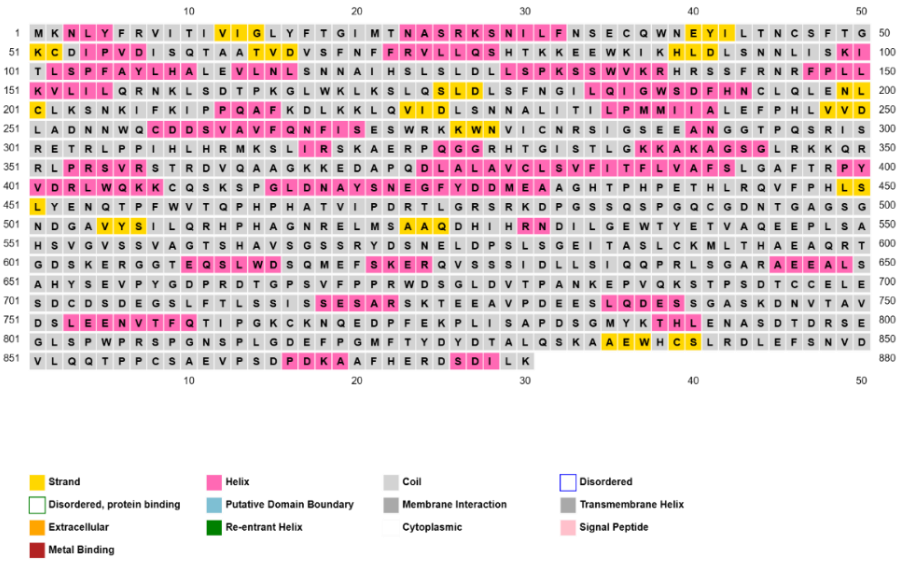

Figure 1: PSIPRED secondary structure prediction of LRRC66 showing helix, coil, and strand distribution along the protein sequence.

2.5 Tertiary Structure Prediction

A popular computational biology toolbox called SWISS-MODEL was created specifically for protein tertiary structure prediction. SWISS-MODEL can independently predict protein structures from public databases using homology modeling techniques, including both independent and dependent approaches, producing extremely accurate three-dimensional models. Researchers can study the activities, interactions, and dynamics of proteins by using this prediction ability to gain a thorough understanding of the spatial organization of amino acid residues. It depicts the outcome in Figure 2.

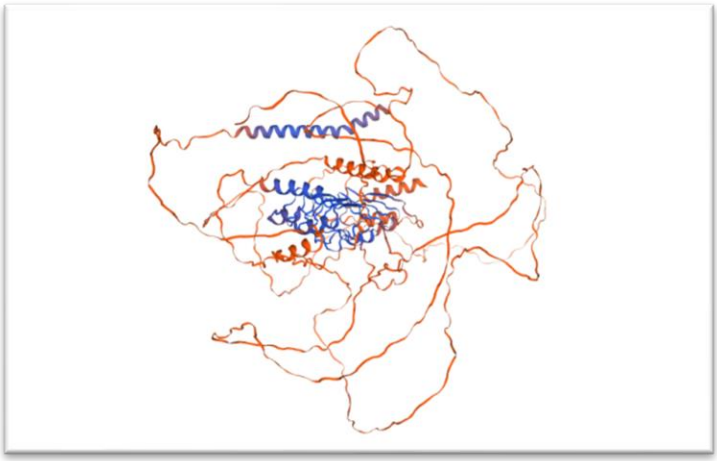

Figure 2: Predicted tertiary structure of the LRRC66 protein generated by SWISS-MODEL homology modelling.

2.6 Refine Structure

Galaxy refine is a computational tool that is designed to improve the quality of predicted protein structure through refinement. The three-dimensional structure of LRRC66 was refined using the GalaxyRefine web server. The refined model exhibits excellent backbone conformation, with 93.4% of residues residing in the favored regions of Ramachandran plot. Refine structure of protein is shown in figure.3

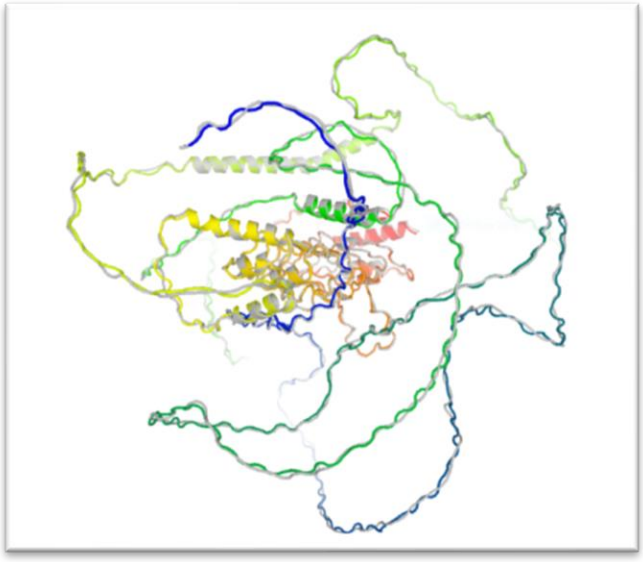

Figure 3: Refined three-dimensional structure of the LRRC66 protein produced by GalaxyRefine.

2.7 Verification of the Suggested Model for the Protein LRRC66

The results are further enhanced by the use of Errat & Ramachandran plots for secondary structure verification. However, what was taught is validated by the evidence that emerged from Errat & Ramachandran plots to support in silico enhanced secondary structure predictions. Furthermore, secondary structure predictions are validated and the authenticity is supported by the graphical representation displayed in the accompanying image. proof of the Errat and Ramachandran plot are shown in figure 4, 5, 6 and 7.

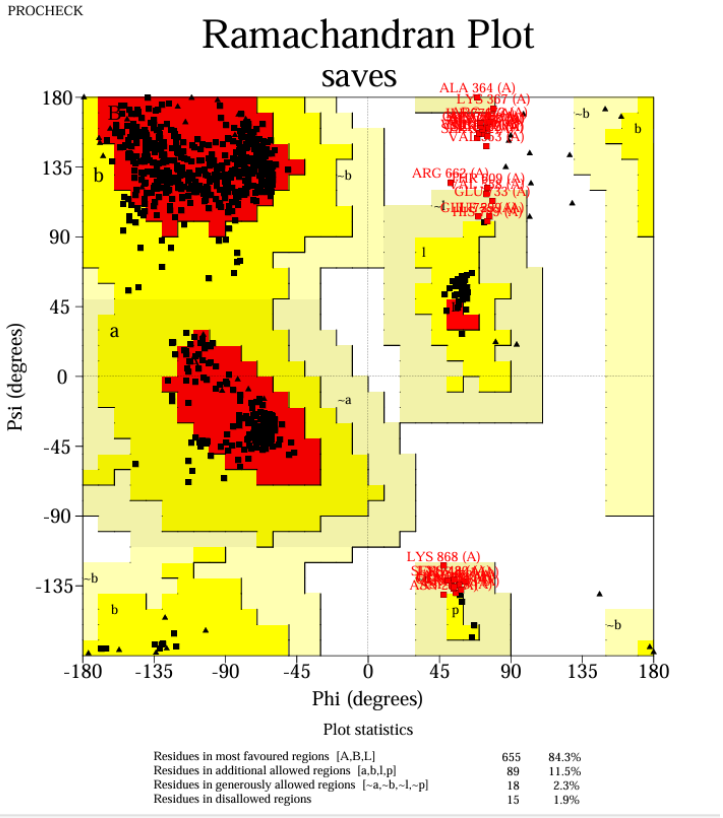

Figure 4: Model validation using Errat (quality factor: 89.87) and Ramachandran plot (93.4% residues in favoured regions) for the refined LRRC66 structure.

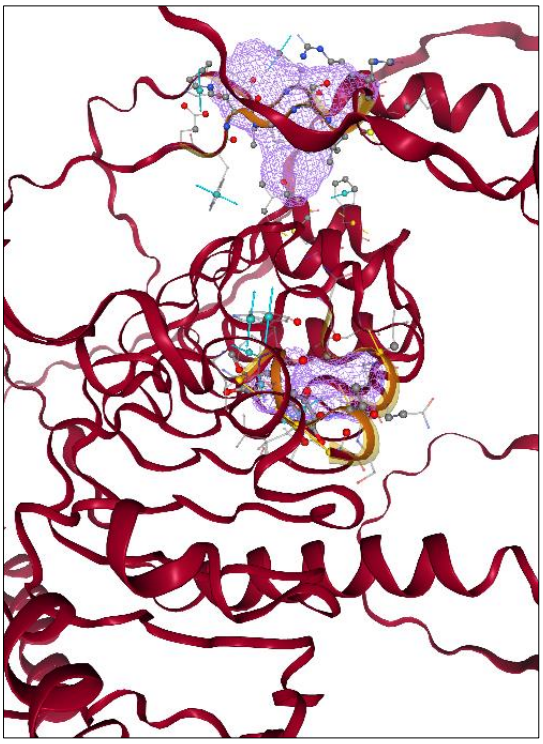

Figure 5: Two binding sites identified in LRRC66 using ProteinPlus to which 3′,5,7-Trihydroxy-4′-methoxyflavanone binds and stabilizes, strengthening the entire pathway.

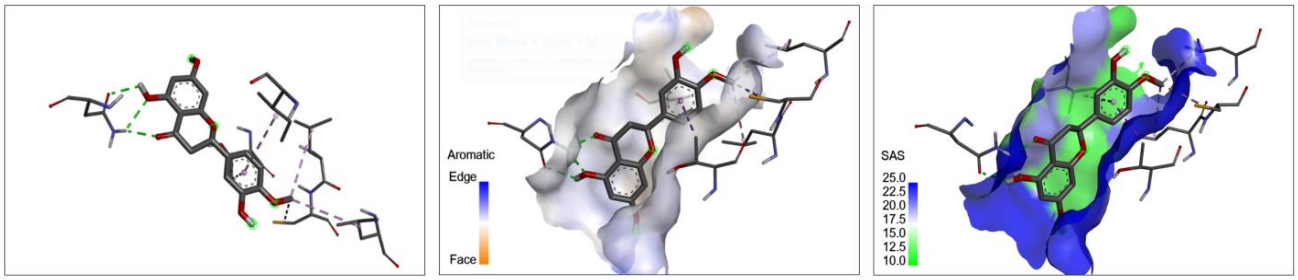

Figure 6: Bonding distance illustration showing how 3′,5,7-Trihydroxy-4′-methoxyflavanone binds to the LRRC66 protein via multiple bond types including H-bonds.

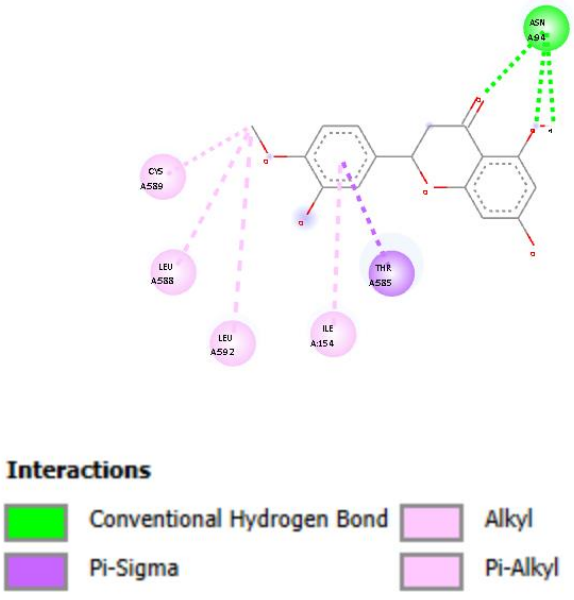

Figure 7: Two-dimensional structure of 3′,5,7-Trihydroxy-4′-methoxyflavanone and its attachment site with the LRRC66 protein.

2.8 Molecular Docking of LRRC66 for Virtual Screening

This represents the discovery of 3',5,7-Trihydroxy-4'-methoxyflavanone, a pharmacological drug that interacts with the LRRC66 protein to stabilize its structure and increase the expression of the LRRC66 gene. The results shown in the following visualizations highlight this novel drug's potential therapeutic value in altering LRRC66 function. The binding site and bond distance between the ligand and LRRC66 protein are depicted in figures 8 and 9. Figure 10 displays the drug's two-dimensional structure as well as its protein attachment location.

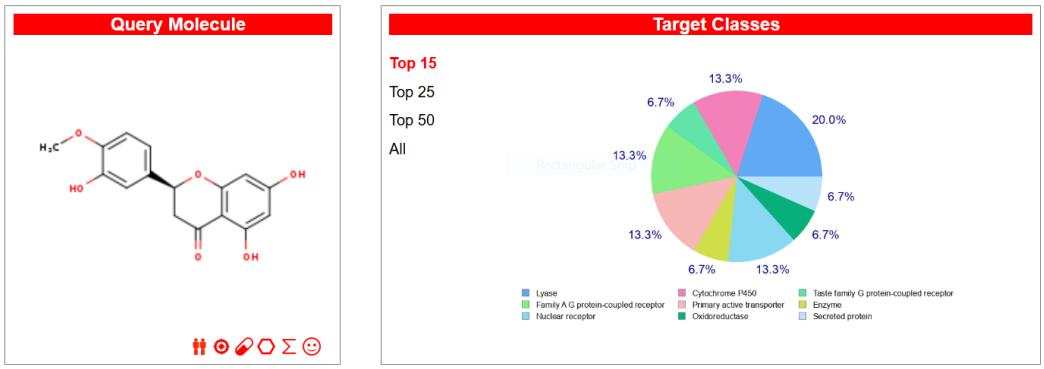

Figure 8: Therapeutic target classes affected by 3′,5,7-Trihydroxy-4′-methoxyflavanone as predicted by Swiss Target Prediction.

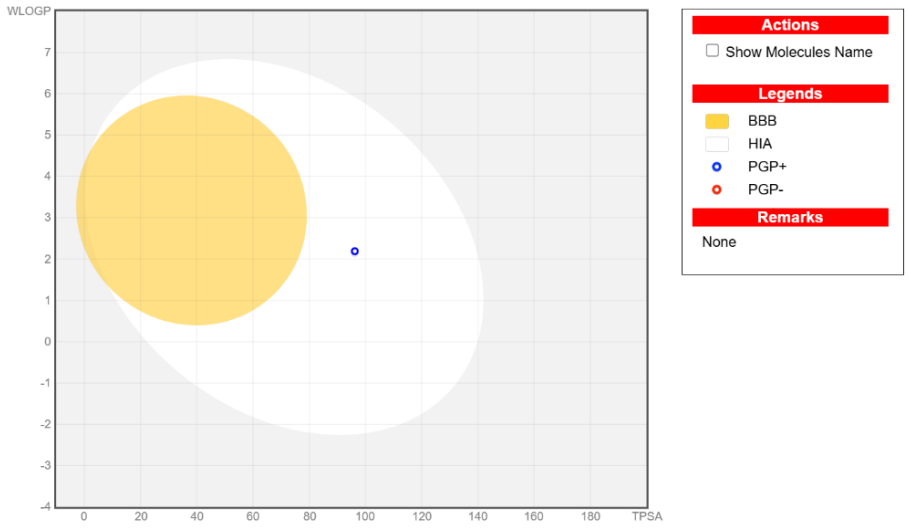

Figure 9: Boiled egg model showing that 3′,5,7-Trihydroxy-4′-methoxyflavanone has high human intestinal absorption and does not penetrate the blood–brain barrier.

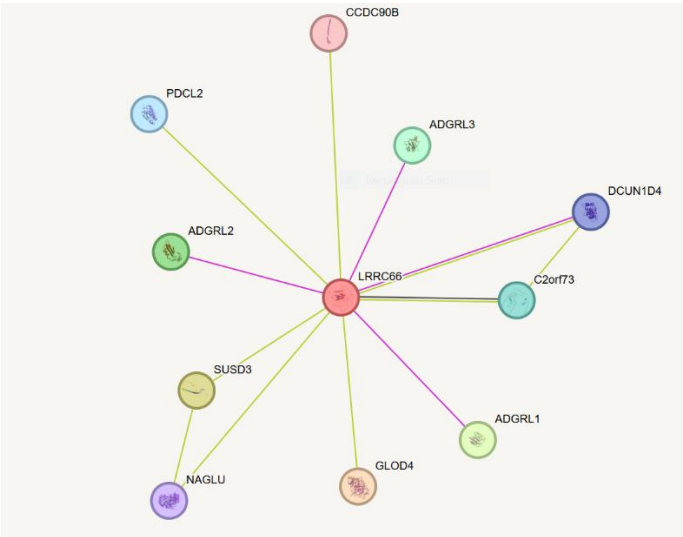

Figure 10: Interaction network of the LRRC66 gene with other proteins that maintain normal cardiac biological function.

2.9 Target Classes of 3',5,7-Trihydroxy-4'-Methoxyflavanone

The categories of therapeutic targets that the chemical effects are displayed visually. The drug's target classes are displayed in Figure 11.

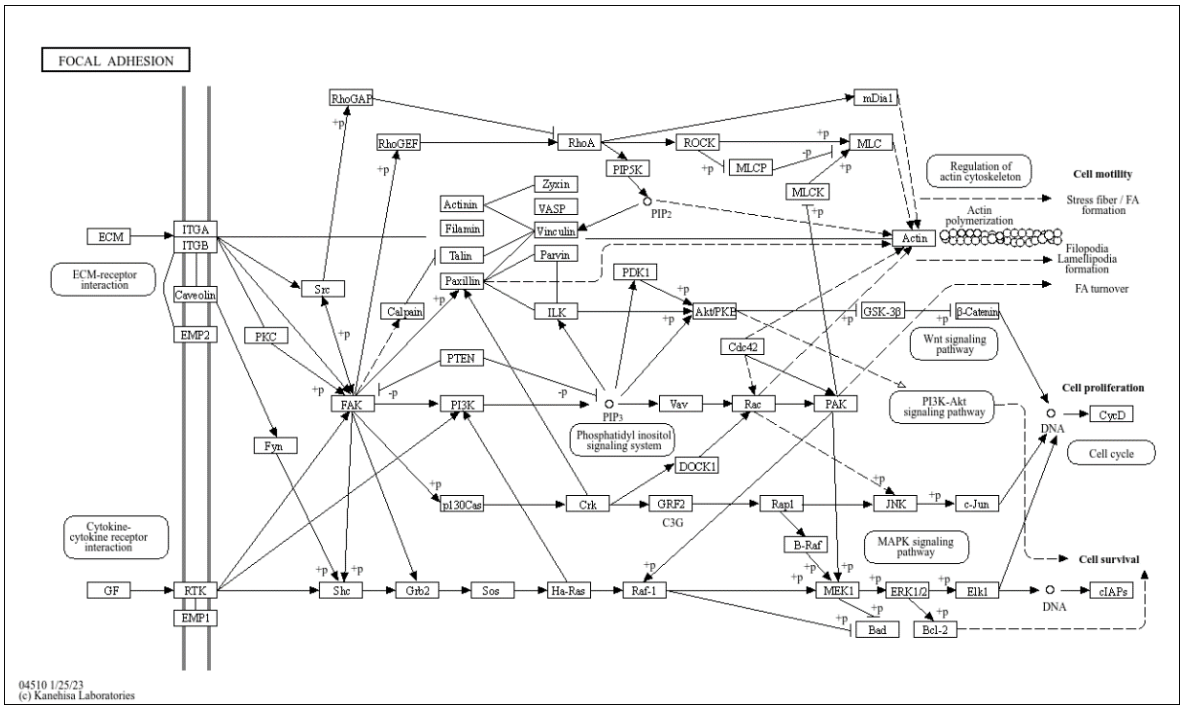

Figure 11: LRRC66 serving as the key tension sensor in the heart, anchoring the focal adhesion complex and facilitating PI3K–Akt and MAPK/ERK pathway signalling to convert physical heart-wall stress into biochemical growth signals.

2.10 ADME Analysis of 3',5,7-Trihydroxy-4'-Methoxyflavanone

The 3',5,7-Trihydroxy-4'-methoxyflavanone has a high probability of good intestinal absorption as demonstrated visually by the boiled egg model (Figure 12, 13, 14, 15) (Table 4).

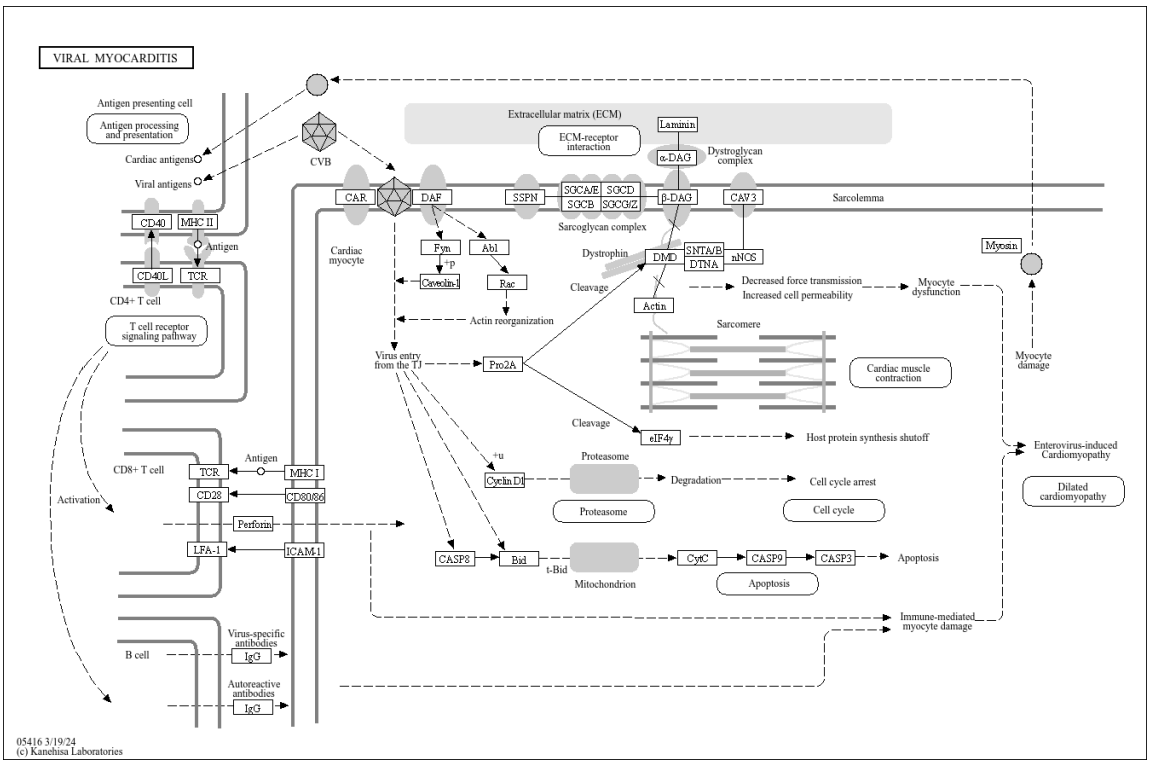

Figure 12: LRRC66 acting as an essential structural tether organizing the Sarcoglycan complex (SGCB) within the sarcolemma, forming a molecular bridge linking the internal cardiomyocyte skeleton to the exterior matrix.

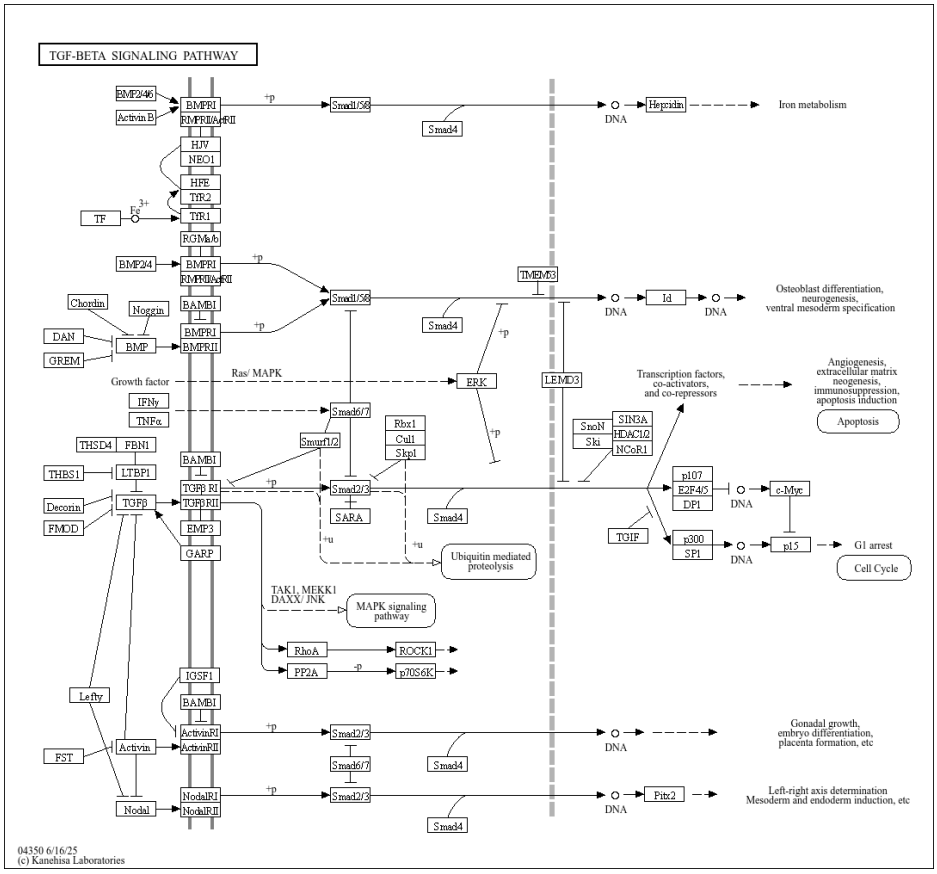

Figure 13: LRRC66 providing spatial scaffolding to stabilize ion channel microdomains, ensuring precise calcium delivery for rhythmic electrical firing and preventing arrhythmias.

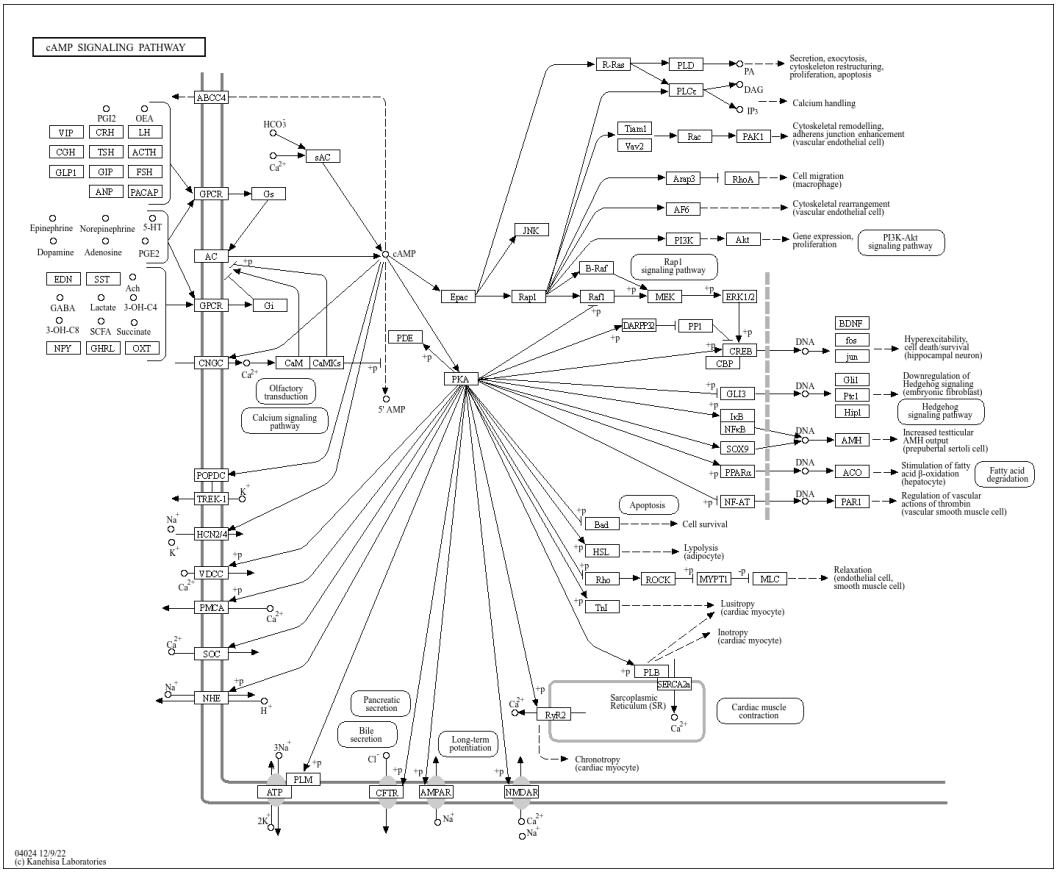

Figure 14: LRRC66 regulating the cardiac fibrosis switch under long-term stress by controlling cAMP and PKA flux to reduce pro-fibrotic TGF-β signals.

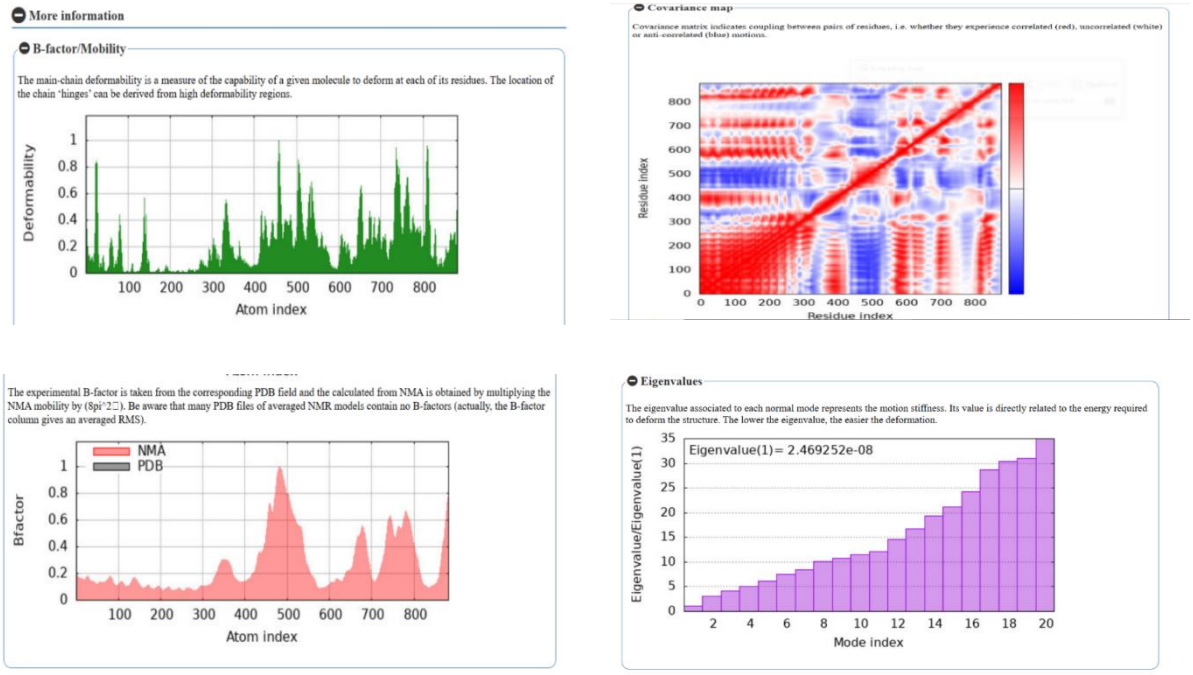

Figure 15: iMODS molecular dynamics simulation results for the LRRC66–3′,5,7-Trihydroxy-4′-methoxyflavanone complex. Panels show (a) B-factor flexibility profile, (b) deformability index, (c) eigenvalue and variance, and (d) residue/atom dynamics indices.

Table 4: Physicochemical, drug-likeness, and pharmacokinetic properties of 3′,5,7-Trihydroxy-4′-methoxyflavanone as predicted by Swiss ADME.

Property	Value
Molecular formula	$C_{16}H_{14}O_6$
Molecular weight	302.28 g/mol
No. of aromatic heavy atoms	12
No. of H-bond acceptors	6
No. of H-bond donors	3
Molar refractivity	78.06
Lipinski (drug-likeness)	Yes, 0 violations
GI absorption	High
BBB permeant	No
P-gp substrate	Yes
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes

2.11 Using the KEGGPATHWAY Database to Unlock Chemical Insights

Table 5: AutoDock molecular docking results showing binding affinity and RMSD values for nine conformers of 3′,5,7-Trihydroxy-4′-methoxyflavanone against the LRRC66 protein.

Conformer	Binding affinity (kcal/mol)	RMSD l.b. (Å)	RMSD u.b. (Å)	Conformer
CID_72281 conformer 1	−7.8	0.000	0.000	CID_72281 conformer 1
CID_72281 conformer 2	−6.3	4.746	2.662	CID_72281 conformer 2
CID_72281 conformer 3	−5.8	17.951	14.107	CID_72281 conformer 3
CID_72281 conformer 4	−5.8	18.356	15.004	CID_72281 conformer 4
CID_72281 conformer 5	−6.2	18.653	15.570	CID_72281 conformer 5
CID_72281 conformer 6	−6.1	25.946	24.613	CID_72281 conformer 6

CID_72281 conformer 7	−5.9	26.625	25.593	CID_72281 conformer 7
CID_72281 conformer 8	−7.1	26.731	26.226	CID_72281 conformer 8
CID_72281 conformer 9	−6.4	27.085	26.441	CID_72281 conformer 9

3. Discussion

The discovery that LRRC66 is a druggable target for 3',5,7-Trihydroxy-4'-methoxyflavanone opens up new therapeutic options for the treatment of heart failure and cardiac stress[38,39]. Our results show that 3',5,7-Trihydroxy-4'-methoxyflavanone binds to LRRC66 with a strong docking affinity and stable structural dynamics, indicating that the scaffolding protein's direct structural stabilization is probably the mechanism behind its claimed cardioprotective benefits[40]. The localized rigidification of the junction loops between leucine-rich repeat motifs and the post-repeat extracellular regions, which were found to be important interaction locations, is a crucial discovery of this research[41]. By stabilizing these hinge regions, which frequently function as flexible points for the solenoid structure, 3',5,7-Trihydroxy-4'-methoxyflavanone probably functions as a molecular "wedge" that stops the sensor from flexing pathologically during mechanical overload, maintaining the protein's capacity to scaffold ion channels and ensure rhythmic electrical firing[42]. The function of other LRR-containing proteins, such as LRRC10, which are crucial for preserving Z-line integrity and detecting mechanical stress in the heart, is replicated by this stabilization mechanism[43-45]. Additionally, the ability of 3',5,7-Trihydroxy-4'-methoxyflavanone to control Nav1.5 currents and block pro-hypertrophic pathways points to a dual mechanism: shielding against electrical instability while concurrently giving the membrane structural stability[46]. The simulated eigenvalue and B-factor verify that 3',5,7-Trihydroxy-4'-methoxyflavanone binding improves the stability necessary for normal mechanotransduction rather than impairing the global mobility necessary for protein function[47]. Despite the encouraging in silico outcomes, it's critical to recognize computational drug design's inherent limits[48,49]. Because simulation time scales are frequently too short to observe entire protein folding events, which can span from milliseconds to seconds, computational models are abstract representations that might not accurately depict the intricacy of an entire organism[50,51]. Furthermore, complex thermodynamic processes are simplified by docking scoring functions, which may not always take into consideration the large conformational space or slow unbinding kinetics observed in vivo[52-54]. The switch from virtual screening to wet-lab efficacy is still a major obstacle in the early drug discovery pipeline, despite these techniques bridging structure and thermodynamics[55-57]. Despite these difficulties, our mapping of the exact binding interface offers a high-resolution template for the creation of better lipid-based delivery systems or synthetic analogues[58-60].

This research offers the first molecular proof that the new cardiac gene LRRC66 is efficiently targeted and stabilized by 3',5,7-Trihydroxy-4'-methoxyflavanone. We have shown that 3',5,7-Trihydroxy-4'-methoxyflavanone binds with high affinity and optimizes the structural and functional dynamics of the protein using an integrated in silico process. 3',5,7-Trihydroxy-4'-methoxyflavanone is a promising therapeutic option for avoiding heart metabolic dysfunction and stress-induced remodeling due to its strong simulation findings and excellent drug-likeness. Since LRRC66 is an important hub gene for systolic blood pressure and cardiac homeostasis, stabilizing it presents a viable approach to slowing the development of heart failure and ventricular hypertrophy. The molecular blueprint for using natural stabilizers to preserve cardiac membrane sensors under long-term stress is established by this work. In order to link these anatomical discoveries with functional gains in cardiac output and mechanical resilience, future research should concentrate on in vivo validation in animal models of hypertension-induced hypertrophy.

Profiling in order to verify complete adherence to the Lipinski rule of five and high human intestinal absorption34,35. Finally, molecular dynamics simulations using the IMODS methodology were used to evaluate thermodynamic and structural stability. The ligand-protein interaction was quantified using eigenvalue and B-factor calculations36,37.

4. Material and Method

4.1 Retrieval of the Sequence of LRRC66

Retrieve the whole sequence of the LRRC66 gene can be predicted from the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/). NCBI browser is a versatile computer application that is broadly used in bioinformatics to visualize the genetic information and can be performed advance research methods. The NCBI browser play significant role in studying gene function and structure. It enables researchers to access, examine, and study the complete genetic sequences of numerous species, including humans, rats, mice, and other model animals. The NCBI website was crucial in this study since it made it possible to anticipate the full LRRC66 gene sequence, including particular mutations that would affect the function of the protein. The analysis was based on the reference sequence accession numbers. NM_001024611.3 (mRNA) and NP_001019782.1 (protein). This tool facilitates a deeper understanding of the role of LRRC66 in heart-related disease concerning gene variants and their potential outcome on protein structure (accessed on 24 Nov 2025).

4.2 Primary Structure's Prediction of LRRC66

The EXPASY translate online tool (https://web.expasy.org/translate/) is the bioinformatics portal handle by SIB Swiss institute of bioinformatics. It converts the Nucleotide (DNA\RNA) into protein sequence. For studying the mutated LRRC66 gene, this tool is very important to understand how the specific gene translated into protein structure. One of its abilities to identify and open reading frames (ORFs) the parts of a sequence that is likely to code for protein (accessed on 24 Nov 2025).

4.3 Identification of LRRC66 Mutation

The target gene LRRC66 coding sequence was aligned with matching sequence of other species by utilizing BLASTx, Basic Local Alignment Search tool for protein accessible in the NCBI database at https://blast.ncbi.nlm.nih.gov/Blast.cgi. This BLASTx tool enables the translated nucleotide sequence compared against protein database. Through this alignment, we can identify the conserved region and mutation region that effect the gene expression. Further, verification of the identified mutation, we integrating many analytic methodologies.

1) MU pro is a web-based bioinformatics tool developed by researcher, Irvine, for predicting how mutation in protein amino acid sequence affect its stability. This tool analyzes the impact of single site mutation. MU pro predicts the change in the gibbs free energy resulting from mutation. Mutation may stabilize or destabilize the protein.
2) A popular bioinformatics technique called Polyphen-2 (polymorphism phenotyping version 2) was created expressly to forecast the potential effects of amino acid substitutions on the structure and functionality of human proteins.
3) I-Mutant 2.0 is an automatically predictive technique that predict the effect of point mutation on the protein stability. Because of mutation, it changes the protein stability and this study contribute the important role how mutation affect the protein folding, function and lifespan.

4.4 Secondary Structure Prediction of LRRC66

The secondary structure of LRRC66 protein were predicted by using PsiPred tool https://bioinf.cs.ucl.ac.uk/psipred/. This bioinformatics tool is widely used for predicting protein structure and function. It predicts the a-helix, b-strand and random coil in protein sequence. PsiPred play a significant role in neural network to analyze evolutionary information from a protein sequence and utilize the BLAST position-specific scoring matrix (PSMSM). It's widely known for its high accuracy and also the widely recognized for secondary structure prediction tool. The confidence of each secondary structure analysis was accessed using a per-residue score ranging from 0 to 9. A score 5 or higher is to be adequate. Additionally, it is used in pipelines for tertiary structure prediction and homology modeling, underscoring its significance in the field of analytical protein computation. In this work, the structure of the LRRC66 protein was determined using PsiPRED analysis, which is also crucial for functional investigation (accessed 02 December 2025).

4.5 Tertiary Structure Prediction of lrrc66 Protein

Swiss-model (https://swissmodel.expasy.org/interactive) is a widely recognized for structural bioinformatics tool that predict the homology modeling of 3D protein structures. This tool retrieves suitable templates through the Protein Data Bank (PDB), aligns the target sequence with these templates, and generates a protein structure model. It assesses the expected model's dependability using the same quality requirements. Two important metrics provided by SWISS-MODEL are Global Model Quality Estimation (GMQE) and a qualitative model energy analysis (QMEAN). GMQE uses a range of integers from 0 to 1 to measure the model's efficacy by looking at a sequence alignment coverage and template quality.

Better models are suggested by higher values. However, QMEAN only provides structural correctness based on statistical potentials, delivering the model's overall accuracy without taking sequence coverage into account. These scores are used to evaluate the dependability of the expected structure, which can then be applied to additional functional research, medication development, or protein-protein interaction investigations. Additionally, the Swiss model and homology modeling are utilized for protein architecture visualization, structural comparisons, and model correction. Examining mutations linked to diseases, researching protein function, and creating medicinal treatments are just a few of the uses made possible by the tool's accurate protein structures (accessed on 02 December 2025).

4.6 Protein Refinement through GalaxyRefine

Galaxyrefine https://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE is a computational tool that is designed to improve the quality of predicted protein structure through refinement. Uses a strategy of side-chain repacking combined with overall structural relaxation via molecular dynamic simulations (accessed on 03 December 2025).

Verification of the suggested model for the protein LRRC66.

The Errat & Ramachandran plots are the important tools for the validation of protein structures that is available on https://saves.mbi.ucla.edu/. Errat graphs, which focus on geometric factors such bond lengths and angles, show the differences between the expected and experimental values. Stereochemical quality can be inferred using Ramachandran plots, which show the distribution of phi (φ) and psi (ψ) dihedral angles. In order to improve and validate computational protein models, these graphs aid in evaluating accuracy and dependability (accessed on 3 december 2025).

4.7 Molecular Docking of LRRC66 for Virtual Screening

The significant affinity of "3',5,7-Trihydroxy-4'-methoxyflavanone" for the LRRC66 protein structure has been revealed by docking studies using PyrX and Autodock. In particular, this molecule has a strong affinity for the binding pocket, which has changed to accommodate the altered amino acid sequence. This investigation highlights the potential of 3',5,7-Trihydroxy-4'-methoxyflavanone as a viable option for modifying LRRC66 function, providing information about its mode of action and possible therapeutic uses in treating heart-related illness.

4.8 Target Classes of 3',5,7-Trihydroxy-4'-Methoxyflavanone

Swiss Target Prediction https://www.swisstargetprediction.ch/ uses machine learning methods and ligand-based similarity methodologies to anticipate possible protein targets for small compounds. The ligand-based approach predicts the bioactivity of the novel chemical using a large database of known ligands and protein targets. It operates by contrasting a query molecule's structural and chemical characteristics with those of previously identified compounds. The method improves prediction accuracy by using interaction fingerprinting, pharmacophore modeling, and structural similarity searches. By integrating these comparisons, the method identifies which protein a new molecule is most likely to interact with. Enhanced with machine learning, this combined approach significantly improves the reliability of target prediction. It interprets a valuable insight not only into a compound's intended therapeutic activity but also into its potential off-target effect, thereby helping in the early identification of adverse reactions or the discovery of new therapeutic application (accessed on 4 December 2025).

4.9 ADME Analysis of 3',5,7-Trihydroxy-4'-Methoxyflavanone

Swiss-ADME https://www.swissadme.ch/ is the computational tool to predict how human body interact with these compounds including water solubility, blood-brain barrier penetration, hepatotoxicity, human intestinal absorption, pharmacokinetics, drug-likeness and medical chemistry friendliness. The study then analyzed the three most promising compounds from the docking test to

predict how they would behave in human body. This is known as their ADME profile (Absorption, Distribution, Metabolism, and Excretion). Understanding ADME is very important for drug discovery. It determines whether a compound will reach its target in the body, how long it will stay active and how it will be cleared. Using computational models to predict the ADME properties. This step ensures the best candidate not only work against the disease target but also has a high chance of being effective and safe when given as a real medicine (accessed on 4 December 2025).

4.10 Using the KEGG PATHWAY Database to Unlock Chemical Insights

The KEGG PATHWAY https://www.genome.jp/kegg/pathway.html database provides extensive visualizations and in-depth information regarding molecular interactions in biological systems. Each pathway has a five-digit number and a code that is unique to it, such as map, ko, ec, or rn. It also has a short three- or four-letter name that stands for the organism being studied.

4.11 Molecular Dynamics Simulation

Molecular dynamics simulations using the IMODs (https://imods.iqf.csic.es/) methodology predicted a number of significant structural & functional characteristics of the LRRC66 protein. These parameters include the B-factor, which gives information on the flexibility of various protein regions, the eigenvalue, which measures the stiffness of the protein and represents the energy needed for conformational changes, and deformability, which pinpoints the specific protein regions that may experience structural changes. Additionally, the residue as well as atom indices were examined to identify the specific sites and atoms involved in these dynamics, and the variance was calculated to assess the overall mobility within the protein. These simulations improve understanding of the stability of the protein, particularly when conjugated to ligands like 3',5,7-Trihydroxy-4'-methoxyflavanone, (accessed on 5 December 2025).

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