IN SILICO APPROACH, TO STUDY THE PHYTOCHEMICALS FROM NERIUM OLEANDER AS POTENTIAL MEDICAMENT FOR CARDIAC ARRHYTMIA

A Dissertation Submitted
in Partial Fulfillment of the Requirement for the Degree of

MASTER OF SCIENCE

in

BIOTECHNOLOGY

by

Nisha Saini
(Roll No. 2K22/MSCBIO/61)

Under the supervision of
Prof. YASHA HASIJA
(Professor)
Head of Department
Delhi Technological University

To the
Department of Biotechnology

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly called as Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-110042,India

June, 2024

Title:

IN SILICO APPROACH, TO STUDY THE PHYTOCHEMICALS FROM *NERIUM OLEANDER* AS POTENTIAL MEDICAMENT FOR CARDIAC ARRHYTHMIA

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-42

ACKNOWLEDEMENT

It is my privilege to express my profound sense of gratitude and indebtedness to my mentor Professor YASHA HASIJA, HOD of the Department of Biotechnology, Delhi Technological University for her valuable guidance and consistent encouragement during the progress of the project work. This dissertation would not be completed in short duration of time without her insightful suggestion and support.

I am equally grateful and wish to express my wholehearted thanks to respected lab seniors Ms. Neha, Ms. Khushi for their kind support and help in the course of my research work.

Nisha Saini
(2K22/MSCBIO/61)

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-42

CANDIDATE'S DECLARATION

I NISHA SAINI hereby certify that the work which is being presented in the thesis entitled In silico approach, to study the phytochemicals from *Nerium oleander* as potential medicament for cardiac arrhythmia in partial fulfilment of the requirements for the award of the Degree of Master in Biotechnology, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from January to May under the supervision of

Professor Yasha Hasija

The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

NISHA SAINI
2K22/MSCBIO/61

Candidate's Signature

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-42

CERTIFICATE BY THE SUPERVISOR

Certified that Nisha Saini (2k22/MSCBIO/61) has carried out their search work presented in this thesis entitled In silico approach, to study the phytochemicals from Nerium oleander as potential medicament for cardiac arrhythmia for the award of Master in Biotechnology from Department of Biotechnology, Delhi Technological University, Delhi, under my supervision.

The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/ Institution.

Supervisor Signature

Prof. Yasha Hasija
(Professor)
Head of Department
Delhi Technological University
Delhi

Date-

Abstract:

Cardiac arrhythmias pose a significant burden on global health, contributing to morbidity and mortality worldwide. Ion channels play a crucial role in cardiac physiology, and dysfunction in these channels is implicated in the pathogenesis of various arrhythmia syndromes. Conventional drugs, which are often prescribed to treat cardiac arrhythmias, are costly and have negative effects. Therefore, a more reliable, affordable, and powerful alternative is needed. Medicinal herbs are, thus, the most important kind of treatment for cardiac arrhythmia.

This dissertation explores the computational discovery of phytochemical for targeting protein which in turn target ion channels implicated in cardiac arrhythmias, focusing on molecular docking simulations and understanding therapeutic approach by using herbal plant. Key findings include the identification of potential phytochemical with the ability to modulate ion channel function and restore normal cardiac rhythm. Insights into the mechanism of action of cardiac glycoside reveal their ability to regulate cardiac rhythm by primarily increasing vagal tone, which slows AV node conduction and reduces the ventricular rate. The implications for drug discovery and therapeutic development are discussed, highlighting the potential of phytochemical as a precision medicine approach for personalized treatment strategies tailored to the underlying molecular mechanisms of arrhythmia subtypes. Recommendations for future research include experimental validation of lead compounds, structural biology studies to elucidate ligand-protein interactions, clinical translation of computational predictions, and the investigation of personalized medicine approaches based on patient-specific genetic mutations. By addressing these recommendations, the field can further advance our understanding of cardiac arrhythmias and facilitate the development of innovative therapeutic interventions for improving patient care and outcomes.

Keywords:

cardiac arrhythmias, ion channels, molecular docking, structure-based drug design, precision medicine, computational modeling, drug discovery, therapeutic development.

Table of Contents

List of Figures

CHAPTER 1

INTRODUCTION

Cardiac arrhythmias pose a significant challenge in contemporary healthcare, contributing to morbidity and mortality worldwide. These irregularities in heart rhythm are often rooted in the malfunction of ion channels, crucial components orchestrating the cardiac electrical signaling cascade. The intricate interplay of these channels, regulating the influx and efflux of ions across the cellular membrane, is paramount for maintaining cardiac rhythmicity. However, dysregulation of ion channels can lead to arrhythmogenic conditions, ranging from benign palpitations to life-threatening ventricular fibrillation.

According to Robbins and Cotran (2021), "Cardiac arrhythmias encompass a broad spectrum of rhythm disturbances, from benign premature contractions to life-threatening ventricular fibrillation, posing significant challenges in both diagnosis and management" (p. 178). This underscores the clinical urgency and complexity associated with arrhythmia management.

To address this challenge, researchers have explored novel therapeutic avenues, among which phytochemicals use emerges as a promising strategy. Plants create bioactive substances called phytochemicals to defend themselves. More than a thousand phytochemicals have been identified to far, and they may be obtained from a variety of foods, including whole grains, fruits, vegetables, nuts, and herbs. Significant phytochemicals include dietary fibers, certain polysaccharides, carotenoids, polyphenols, isoprenoids, phytosterols, and saponins. These phytochemicals have

antibacterial, antidiarrheal, anthelmintic, antiallergic, antispasmodic, and antiviral properties in addition to their potent antioxidant activity(1)

Leveraging computational techniques such as molecular docking, researchers can systematically screen vast chemical libraries to identify potential phytochemical with therapeutic efficacy.

In this dissertation, we delve into the realm of computational study of phytochemical, focusing on the identification of phytochemical targeting ion channels implicated in cardiac arrhythmias. In addition, we explore the potential of different compounds derived from *Nerium oleander* to modulate ion channel activity. Through a molecular docking simulations, we investigate its binding affinity and potential effects, contributing to the comprehensive understanding of arrhythmia pathophysiology and paving the way for innovative therapeutic interventions.

1.2 Background and Significance of Cardiac Arrhythmias

Cardiac arrhythmias represent a diverse array of rhythm disturbances, ranging from benign palpitations to life-threatening conditions such as ventricular fibrillation. These irregularities in the heart's electrical activity disrupt its ability to pump blood effectively, leading to symptoms such as dizziness, chest pain, and in severe cases, sudden cardiac arrest (Robbins & Cotran, 2021).

The prevalence of cardiac arrhythmias is substantial, with millions of individuals affected globally. Age, genetics, structural heart disease, electrolyte imbalances, and medication side effects are among the multifactorial contributors to arrhythmia development (Ziaeian & Fonarow, 2016). Importantly, cardiac arrhythmias pose significant challenges in both

diagnosis and management, necessitating a comprehensive understanding of their underlying mechanisms.

Robbins and Cotran (2021) underscore the clinical significance of cardiac arrhythmias, stating that they "encompass a broad spectrum of rhythm disturbances, posing significant challenges in both diagnosis and management" (p. 178). Indeed, the unpredictable nature of arrhythmias and their potential for adverse outcomes necessitate ongoing research efforts to improve risk stratification, diagnostic modalities, and therapeutic interventions.

From a pathophysiological standpoint, cardiac arrhythmias often arise from abnormalities in the generation or propagation of electrical impulses within the heart. Dysregulation of ion channels, specialized proteins embedded in the cardiac cell membrane, plays a pivotal role in disrupting the finely orchestrated sequence of electrical events. Alterations in ion channel function can lead to aberrant depolarization or repolarization, culminating in arrhythmogenic substrates predisposing individuals to various rhythm disturbances (Nattel et al., 2017).

Understanding the underlying molecular mechanisms driving cardiac arrhythmias is essential for the development of targeted therapies aimed at restoring normal electrical activity and reducing morbidity and mortality associated with these conditions. Moreover, advancements in computational techniques, such as molecular docking, offer promising avenues for the discovery of novel therapeutic agents capable of modulating ion channel function with precision and efficacy.

1.3 Role of Ion Channels in Cardiac Physiology

Ion channels are integral membrane proteins responsible for regulating the flow of ions across the cell membrane, thereby governing the electrical excitability of cardiac myocytes. These specialized channels play a fundamental role in orchestrating the complex sequence of events underlying cardiac physiology, including action potential generation, propagation, and contraction (Bers, 2002).

In the context of cardiac electrophysiology, several types of ion channels contribute to the dynamic changes in membrane potential during each cardiac cycle. Sodium (Na^+), potassium (K^+), calcium (Ca^2+), and chloride (Cl^-) channels are among the key players involved in mediating the influx and efflux of ions, thereby modulating the electrical properties of cardiac cells (Noble et al., 2010).

During the resting membrane potential phase, ion channels maintain a polarized state by selectively allowing specific ions to permeate the membrane. Upon depolarization, voltage-gated sodium channels rapidly open, allowing a transient influx of sodium ions, which initiates the action potential. Subsequent activation of voltage-gated potassium channels facilitates repolarization by promoting the efflux of potassium ions, restoring the resting membrane potential (Bers, 2002).

In addition to sodium and potassium channels, calcium channels play a crucial role in excitation-contraction coupling, where calcium influx triggers calcium release from the sarcoplasmic reticulum, leading to myocardial contraction. These intricate processes are tightly regulated to ensure proper cardiac function and maintain hemodynamic stability (Bers, 2002).

The role of ion channels extends beyond normal cardiac physiology, as their dysregulation can give rise to arrhythmogenic substrates predisposing individuals to cardiac arrhythmias. Abnormalities in ion channel function,

whether due to genetic mutations, acquired disorders, or pharmacological interventions, can disrupt the delicate balance of ion fluxes, leading to conduction abnormalities and rhythm disturbances (Noble et al., 2010).

Understanding the intricate interplay between ion channels and their contribution to cardiac electrophysiology is paramount for elucidating the pathophysiological mechanisms underlying cardiac arrhythmias.

1.4 Phytochemical as a Therapeutic Approach

Conventional drugs, which are often used to treat cardiovascular diseases, are costly and have negative effects. Therefore, a more reliable, affordable, and powerful alternative is needed. Medicinal herbs are therefore the most important kind of treatment for cardiovascular disease. Treatment of CVDs is more likely to involve medicinal herbs. Due to increased understanding of how herbs enhance health and quality of life, herbal medicine has been widely accepted in medicine. A different and promising approach to CVD prevention is the use of phytochemicals and plant-based whole meals, according to mounting data. Arrhythmia, congestive heart failure, cerebral and venous insufficiency, atherosclerosis, angina pectoris, and systolic hypertension have all been treated with herbal remedies. In addition to therapeutic herbs, exercise is believed to cardioprotective in nature.

Natural goods contain a wide variety of chemical entities that are included in the phytochemical category, including vitamins, flavonoids, steroidal saponins, polyphenols, and organosulfur compounds. Numerous bioactive substances that are often derived from terrestrial plants, including diosgenin, resveratrol, quercetin, sulforaphane, tocotrienols, and carotenoids, have been shown to lower the risk of cardiovascular illnesses and support cardioprotection, which is important because cardiovascular disease is the world's leading cause of death. These include isoflavones,

diosgenin, resveratrol, quercetin, catechin, sulforaphane, tocotrienols, and carotenoids. The antioxidative, antihypercholesteroemic, antiangiogenic, anti-ischemic, suppression of platelet aggregation, and anti-inflammatory properties of the different phytochemicals may be responsible for their cardioprotective benefits.(2)

Despite the immense potential of phytochemicals, several challenges remain, including identifying standardization, bioavailability, efficacy and safety while using phytochemical in treatments. Nonetheless, advances in computational techniques, such as molecular docking, have facilitated the identification and screening of phytochemicals, accelerating drug discovery efforts in this field.

1.5 Molecular Docking as a Computational Tool

Molecular docking serves as a powerful computational tool for predicting the binding interactions between small molecules (ligands) and target proteins, such as ion channels, with high accuracy and efficiency. This technique plays a pivotal role in drug discovery and design by facilitating the identification of potential lead compounds that selectively bind to specific protein targets, thereby modulating their activity (Morris et al., 2009).

At its core, molecular docking involves the computational simulation of ligand-protein interactions within the three-dimensional space of the protein's binding site. By employing algorithms that account for factors such as ligand flexibility, protein conformational changes, and electrostatic complementarity, docking programs can predict the most energetically favorable binding pose of a ligand within the protein's binding pocket (Kitchen et al., 2004).

The accuracy and reliability of molecular docking simulations depend on several factors, including the quality of the protein structure, the choice of scoring function, and the incorporation of solvent effects and protein flexibility (Leach et al., 2006). Additionally, validation against experimental data, such as crystallographic structures or binding affinity measurements, is essential for assessing the predictive performance of docking algorithms.

Despite its computational nature, molecular docking has proven to be a valuable tool in virtual screening campaigns aimed at identifying potential drug candidates from large chemical libraries. By screening millions of compounds in silico, researchers can prioritize promising leads for further experimental validation, thereby accelerating the drug discovery process and reducing the time and cost associated with traditional high-throughput screening methods (Kitchen et al., 2004).

In the context of ion channels implicated in cardiac arrhythmias, molecular docking holds immense promise for identifying allosteric modulators with therapeutic potential. By virtually screening chemical libraries against ion channel structures, researchers can pinpoint compounds that interact with specific allosteric sites, thereby modulating channel function and restoring normal cardiac rhythm (Kolb et al., 2011).

1.6 Research Objectives and Scope

The primary objective of this research is to leverage molecular docking techniques to identify potential phytochemical targeting protein implicated in cardiac arrhythmias. Specifically, the study aims to:

1. Select a protein which is known to play critical roles in cardiac electrophysiology and arrhythmogenesis, based on a comprehensive review of the literature and expert consultation.

2. Compile a database of phytochemical with the potential to act as ligand, drawing from diverse chemical libraries and bioactivity databases.

3. Utilize molecular docking simulations to predict the binding interactions between selected protein and phytochemical which as a ligand, considering ligand flexibility and receptor conformational changes.

4. Validate the docking results through comparison with experimental data, where available, to assess the reliability and predictive performance of the computational approach.

5. Analyse the top-ranking docking poses and scoring metrics to prioritize lead compounds for further experimental evaluation, considering factors such as binding affinity, specificity, and drug-likeness.

6. Discuss the implications of the findings for the development of novel therapeutic strategies targeting different ion channels in cardiac arrhythmias.

The scope of the research encompasses computational and theoretical investigations aimed at elucidating the molecular mechanisms underlying ion channel modulation and arrhythmia pathophysiology. While the study primarily focuses on molecular docking as a predictive tool, it integrates principles from structural biology, pharmacology, and bioinformatics to provide a comprehensive understanding of the phytochemicals targeting ion channels.

CHAPTER 2

Literature Review

Cardiac arrhythmias represent a diverse spectrum of rhythm disturbances that pose significant challenges in both diagnosis and management (Robbins & Cotran, 2021). These abnormalities in cardiac rhythm can range from benign premature contractions to life-threatening ventricular fibrillation, underscoring the clinical urgency and complexity associated with arrhythmia management. The prevalence of cardiac arrhythmias is substantial, affecting millions of individuals globally, and is influenced by various factors including age, genetics, structural heart disease, electrolyte imbalances, and medication side effects (Ziaeian & Fonarow, 2016). Consequently, there is a pressing need for comprehensive research to enhance our understanding of arrhythmia pathophysiology and develop effective therapeutic interventions.

Ion channels play a pivotal role in cardiac electrophysiology, regulating the influx and efflux of ions across the cellular membrane to maintain normal cardiac rhythm (Nattel et al., 2017). Abnormalities in ion channel function can disrupt the delicate balance of ion fluxes, leading to conduction abnormalities and arrhythmogenic substrates predisposing individuals to various rhythm disturbances. Understanding the molecular basis of ion channel dysfunction is essential for elucidating the underlying mechanisms of cardiac arrhythmias and identifying potential therapeutic targets.

Traditional plant-based remedies have been used extensively in health care from ancient times to the present. Natural bioactive substances called phytochemicals are present in fruits, vegetables, medicinal plants, fragrant

plants, leaves, flowers, and roots. They function as the body's defensive mechanism against illness. Their presence of anti-inflammatory, anti-arrhythmic, and antioxidant substances contributes to the preservation of cardiovascular health in general. Thus, we can target the ion channels linked to cardiac arrhythmias by using phytochemicals to target a specific heart protein, which leads to the development of a therapeutic method.(2)

Molecular docking serves as a powerful computational tool for predicting the binding interactions between small molecules (ligands) and target proteins, such as ion channels, with high accuracy and efficiency (Morris et al., 2009). By simulating ligand-protein interactions within the three-dimensional space of the protein's binding site, docking programs can predict the most energetically favorable binding pose of a ligand, facilitating the identification of potential lead compounds for further experimental validation. Despite its computational nature, molecular docking has proven to be a valuable tool in virtual screening campaigns aimed at identifying potential drug candidates from large chemical libraries.

2.1 Overview of Cardiac Arrhythmias and Their Impact

Cardiac arrhythmias encompass a spectrum of rhythm disturbances that can significantly impact cardiovascular health and patient outcomes (Robbins & Cotran, 2021). These disturbances in the heart's electrical activity can manifest as irregular heartbeats, palpitations, and in severe cases, life-threatening arrhythmias such as ventricular fibrillation. The prevalence of cardiac arrhythmias is substantial, affecting millions of individuals worldwide and representing a leading cause of morbidity and mortality in both developed and developing countries (Ziaeian & Fonarow, 2016).

The impact of cardiac arrhythmias on patient health and quality of life is multifaceted. Arrhythmias can lead to symptoms such as dizziness,

lightheadedness, chest pain, and shortness of breath, which can significantly impair daily functioning and diminish overall well-being. Moreover, certain arrhythmias, particularly those involving ventricular tachycardia or fibrillation, can precipitate sudden cardiac arrest, a life-threatening event requiring immediate medical intervention to restore normal heart rhythm and prevent irreversible damage or death.

Beyond the acute consequences, cardiac arrhythmias are associated with an increased risk of adverse cardiovascular outcomes, including heart failure, stroke, and myocardial infarction (Nattel et al., 2017). The hemodynamic instability induced by arrhythmias can compromise cardiac function and exacerbate underlying cardiovascular conditions, leading to further complications and a higher likelihood of hospitalization and mortality.

The economic burden of cardiac arrhythmias is also substantial, encompassing healthcare expenditures related to hospital admissions, diagnostic testing, pharmacological management, and invasive procedures such as catheter ablation or implantable device placement. Moreover, the indirect costs associated with arrhythmia-related disability, loss of productivity, and decreased quality of life further contribute to the socioeconomic impact of these conditions.

2.2 Molecular Basis of Ion Channel Function and Dysfunction in Cardiac Arrhythmias

Ion channels play a fundamental role in regulating the electrical activity of cardiac myocytes, thereby governing the initiation and propagation of action potentials essential for cardiac contraction (Nattel et al., 2017). These specialized transmembrane proteins facilitate the selective passage of ions across the cell membrane, generating the membrane potential changes that underlie the cardiac action potential.

The cardiac action potential is orchestrated by the sequential activation and inactivation of various ion channels, each contributing to distinct phases of depolarization and repolarization. Voltage-gated sodium (Na^+) channels initiate the rapid upstroke of the action potential by allowing the influx of sodium ions into the cell, leading to depolarization of the membrane. This phase is followed by the activation of voltage-gated potassium (K^+) channels, which promote the efflux of potassium ions, facilitating repolarization and restoration of the resting membrane potential.

In addition to sodium and potassium channels, calcium (Ca^2+) channels also play a crucial role in cardiac electrophysiology, particularly in excitation-contraction coupling. L-type calcium channels facilitate the influx of calcium ions during the action potential, triggering calcium release from the sarcoplasmic reticulum and initiating myocardial contraction. Calcium influx also contributes to the plateau phase of the action potential, prolonging depolarization and modulating contractility.

Dysfunction of ion channels can disrupt the delicate balance of ion fluxes, leading to arrhythmogenic substrates predisposing individuals to cardiac arrhythmias (Nattel et al., 2017). Genetic mutations, acquired disorders, and pharmacological interventions can alter ion channel function, resulting in conduction abnormalities, repolarization disturbances, and susceptibility to arrhythmia development.

For example, mutations in genes encoding cardiac ion channels, such as SCN5A (sodium channel), KCNQ1/KCNE1 (potassium channel), and CACNA1C (calcium channel), have been implicated in various arrhythmia syndromes, including long QT syndrome, Brugada syndrome, and catecholaminergic polymorphic ventricular tachycardia. These mutations can disrupt channel gating, alter ion selectivity, or impair channel trafficking, leading to abnormal electrical activity and arrhythmia susceptibility.

Moreover, acquired disorders such as ischemia, heart failure, and electrolyte imbalances can further exacerbate ion channel dysfunction, creating a substrate conducive to arrhythmia initiation and perpetuation. Pharmacological agents, including antiarrhythmic drugs and medications used to treat other cardiovascular conditions, can also modulate ion channel function, sometimes with unintended proarrhythmic effects.

2.3 Molecular Docking Methods and Applications in Drug Discovery

Molecular docking is a computational technique widely used in drug discovery to predict the binding interactions between small molecules (ligands) and target proteins, with applications ranging from lead identification and optimization to virtual screening and structure-based drug design (Kitchen et al., 2004). The method involves simulating the geometric complementarity and non-covalent interactions between the ligand and receptor within the binding site, thereby predicting the most energetically favorable binding pose and affinity.

Several molecular docking algorithms have been developed, each with its unique features and strengths. Among the most widely used are AutoDock, AutoDock Vina, and DOCK, which employ different scoring functions and search algorithms to explore ligand conformational space and optimize ligand-receptor interactions. These programs incorporate various parameters such as ligand flexibility, protein flexibility, and solvent effects to improve the accuracy and reliability of docking predictions.

Molecular docking has numerous applications in drug discovery, including lead identification, where it can efficiently screen large chemical libraries to identify potential ligands with high binding affinity and specificity for the target protein (Kitchen et al., 2004). Virtual screening, another key

application, involves docking a database of compounds against a target protein to prioritize promising candidates for experimental testing based on their predicted binding energy and fit within the binding site.

Structure-based drug design relies heavily on molecular docking to guide the rational optimization of lead compounds by systematically exploring the chemical space around the binding site and identifying key interactions driving ligand binding and potency (Kitchen et al., 2004). Docking simulations can also aid in understanding structure-activity relationships and predicting the impact of chemical modifications on ligand binding affinity and selectivity.

Moreover, molecular docking serves as a valuable tool for studying protein-ligand interactions, elucidating the molecular mechanisms underlying drug action and resistance, and guiding the design of novel therapeutic agents targeting various disease pathways. By integrating computational and experimental approaches, docking simulations can accelerate the drug discovery process, reduce costs, and increase the success rate of lead optimization and development.

2.4 Phytochemicals

Nerium oleander, a member of the Apocynaceae family, is referred to as Kaner in several Asian and Mediterranean nations. Because of the numerous bioactive chemicals that have been identified from this plant, such as cardiac glycosides, oleandrin, α-tocopherol, digitoxingenin, urosolic acid, quercetin, odorosides, and adigoside, it has been well-known to have substantial medicinal potential. One of N. oleander's most powerful and pharmacologically active phytochemicals is oleandrin, a saponin glycoside. Its extraordinary pharmacotherapeutic potential has been

described as antioxidant, antibacterial, neuroprotective, anti-HIV, anti-inflammatory, and anticancer. (3)

2.4.1 Mechanism of action

In cardiac muscle cells, cardiac glycosides modify the activity of the sodium-potassium ATPase pump. These sodium-potassium pumps normally transfer sodium ions out and potassium ions in. Nevertheless, cardiac glycosides block this pump by keeping it stable in the E2-P transition state, which prevents sodium from being extruded and raises intracellular sodium concentration. Changes in extracellular potassium concentration may have an impact on therapeutic efficacy because potassium and cardiac glycosides compete with one another for binding to the ATPase pump, which affects potassium ion transport. Nevertheless, such negative effects can be prevented by closely monitoring the dosage. To reiterate the mechanism, elevated intracellular sodium levels block the action of NCX, a second membrane ion exchanger that is in charge of expelling calcium ions from the cell and sodium ions, which are present in a 3Na+/Ca2+ ratio. As a result, calcium ions are likewise not ejected and will instead start to accumulate within the cell. Increased calcium uptake via the SERCA2 transporter into the sarcoplasmic reticulum (SR) results from disturbed calcium homeostasis and elevated cytoplasmic calcium concentrations. Increased calcium release upon stimulation is made possible by higher calcium reserves in the SR, which enables the myocyte to contract more quickly and forcefully through cross-bridge cycling. Because cardiac glycosides lengthen the AV node's refractory time, they also lower heart rate. Digoxin, for instance, increases cardiac output and lowers heart rate without significantly altering blood pressure; this property enables it to be used medicinally in the treatment of cardiac arrhythmia.(4)

For many years, it has been known that N. oleander is harmful. Cardiac glycosides are present in every part of the plant, although they are particularly abundant in the seeds and roots. Cardiac glycosides and digitoxin, a substance found in foxglove plants, share a similar structure. According to a number of research, N. oleander may have antibacterial, pesticidal, rodenticidal, and insecticidal properties. Five Nerium oleander leaves can be consumed and result in fatal poisoning. One N. oleander leaf was said to have extremely harmful effects on kids, though. It is controversial to report that a 7-year-old child who consumed three N. oleander leaves experienced mild poisoning but no further complications. After consuming five leaves of N. oleander, a woman in her adulthood experienced mild poisoning without experiencing severe symptoms. Consequently, figuring out the N. oleander toxicity lethal dose has not been fully determined, and additional research is needed to determine the plant's fatal dosages.(3)

2.4.2Human Angiotensin Receptor

Renin-angiotensin system (RAS) proteases methodically break down angiotensinogen, a serpin family protein that is mostly generated by the liver, to create hormone peptides. The systemic physiological response to renin-angiotensin syndrome (RAS) is regulated by specific cellular signals produced by cell surface receptors for at least three different angiotensin peptides. Angiotensin type 1 receptor (AT1 receptor) and type 2 receptor (AT2 receptor) are two well-characterized receptors. They react to the hormone angiotensin II, an octapeptide. A potential Ang receptor is the oncogene product MAS (1–7). Despite being G-protein coupled receptors (GPCRs), type 2 transmembrane proteins may be the binding sites for angiotensin IV in vivo. In addition to controlling inflammation, matrix-cell interactions, cell proliferation, survival, neurological, renal, and cardiovascular processes, these four receptors work together to control hemodynamic, neural, and endothelial activities. Angiotensin receptors are significant targets for treatment in a number of illnesses.(5) So in this dissertation we target Human angiotensin receptor with phytochemicals of *Nerium oleander.*

CHAPTER 3

METHODOLOGY

The methodology employed in this study integrates computational techniques with molecular docking simulations to identify potential phytochemical of *Nerium oleander* targeting 4YAY protein for treatment of cardiac arrhythmias.

Here, we used PyRx for virtual screening of multiple ligands(phytochemicals) targeting a protein 4YAY. The following paragraphs detail the approach utilized for ligand selection, protein preparation, molecular docking, and validation procedures.

1.Downloading the protein

- Use PDB (www.rcsb.org) to download the 4YAY structure.
- If you experience any issues loading the structure, you can download the PDBx/mmCIF format in addition to the PDB format as normal.
- Put this file into a folder.

2.Download the ligand

Visit PubChem at www.pubchem.ncbi.nlm.nih.gov.

- Look up "oleanolic acid" as a compound. Download the corresponding 3D conformer in SDF format. Put it in a separate folder.
- Download the 3D structures of Oleandrin, Alpha tocopherol, Adigoside, Scopolin and Ouabain the same way as mentioned in previous steps and save it.

3.Preparing the protein

- The binding location and binding residues must be known in order to prepare the protein for docking. They may involve two chains of proteins, or they may be found in just one. Examine the related paper that was released for that structure.
- Here we know the binding site of 4YAY protein.
- Open pdb file and delete hetatoms. Since the structure we're utilizing is a crystal structure complexed with ligand(s), we remove the bound ligand by deleting hetatoms from the PDB file in order to dock the desired ligand with the protein at that specific spot. We won't obtain the right results if we dock our ligand without first eliminating the complexed ligand. Moreover, we can quickly eliminate ligands by using BIOVIA discovery studio to see the protein.
- Extra chains were removed from the protein structure to avoid the complexity.
- Here we kept chain A and rest were removed. Now save the file as “protein.pdb”.
- We have prepared our protein structure to proceed further for docking.

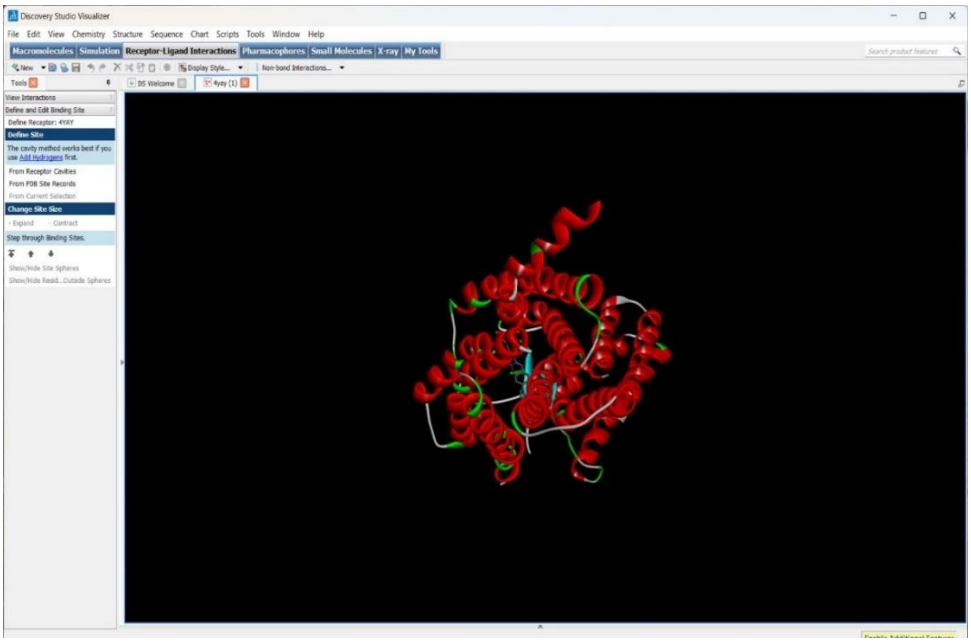


Figure 3.1- Screenshot showing loading of protein in discovery studio

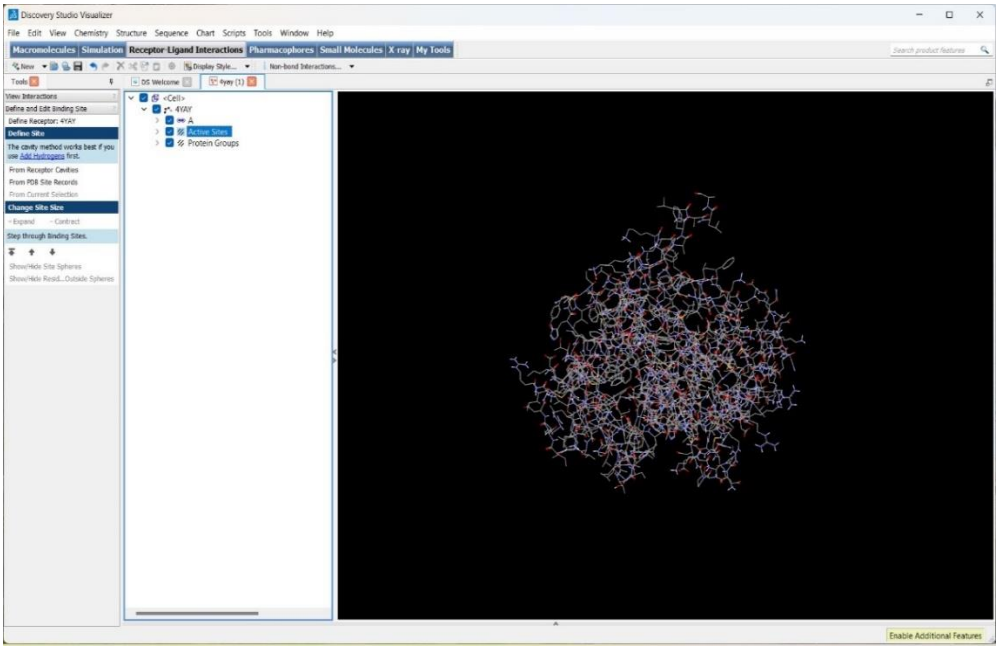


Figure 3.2- Screenshot showing protein after modification

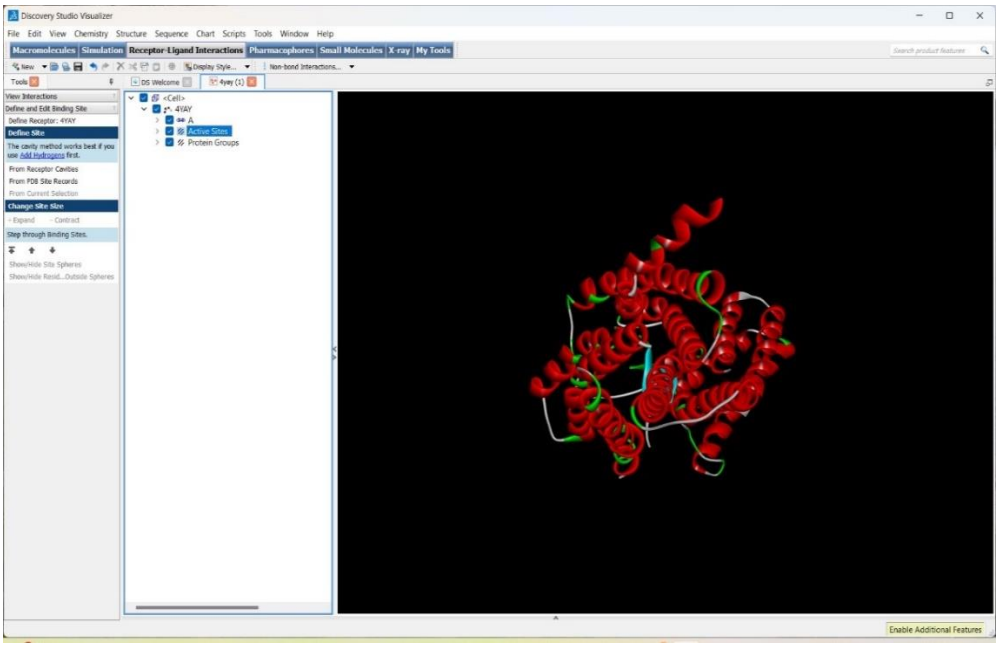


Figure 3.3- Screenshot showing protein after modification

4.Performing docking using pyrx

Autodock Vina will be the docking tool that we use. We use the Vina algorithm to dock it in Pyrx. Launch Pyrx GUI and followed the steps given below:

4.1Protein Loading

- Select "File" -> "Load Molecule" or simply click the first icon in the upper left corner. Choose the protein structure that you downloaded. referred to here as "4YAY.pdb."
- convert pdb format of protein to pdbqt by right clicking on 4YAY then on display and now select macromolecule.

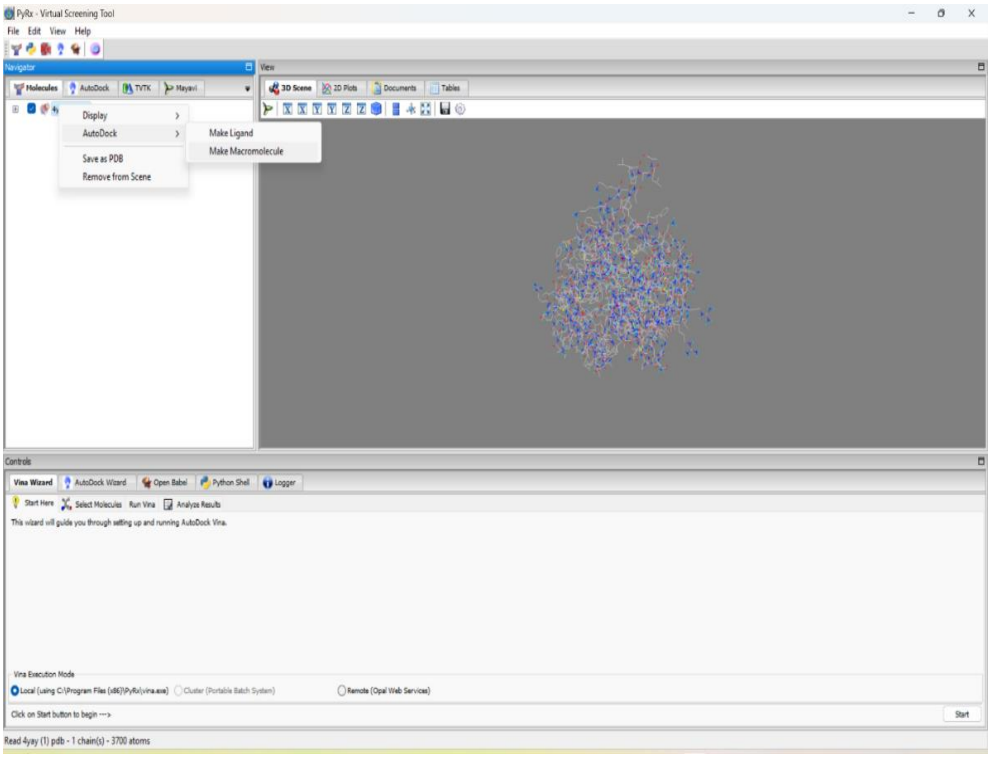


Figure 3.4- Screenshot showing conversion of protein file pdb to pdbqt

4.2 Ligand loading

- In PyRx , click on OpenBabel and select on insert new item present on bottom right corner.
- Now select each ligand from folder one by one and upload it.

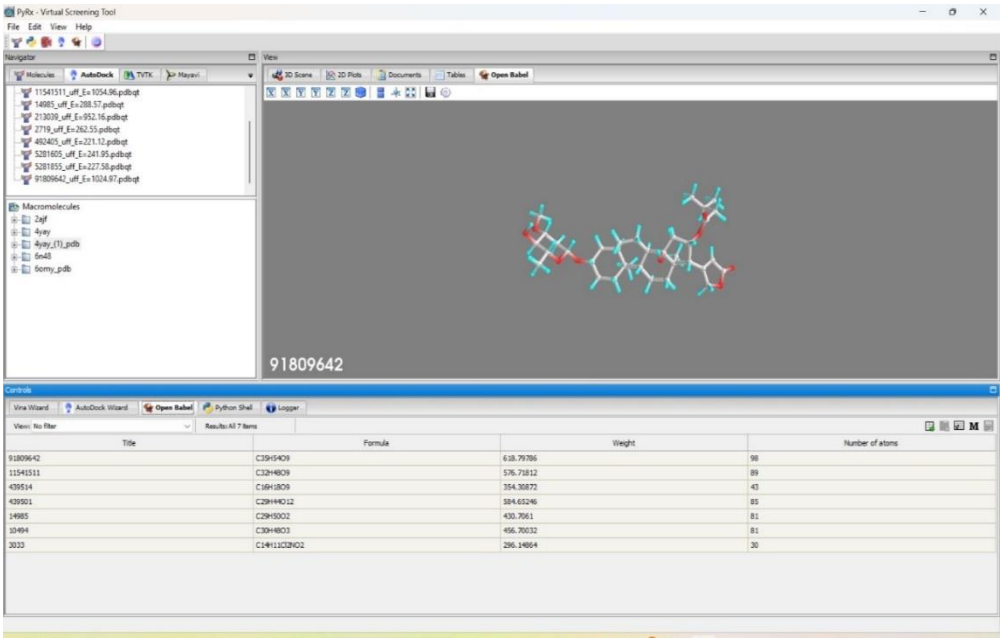


Figure 3.5- Screenshot showing loading of ligands

- After uploading all ligands , right click on ligand and select minimise all to decrease the energy.

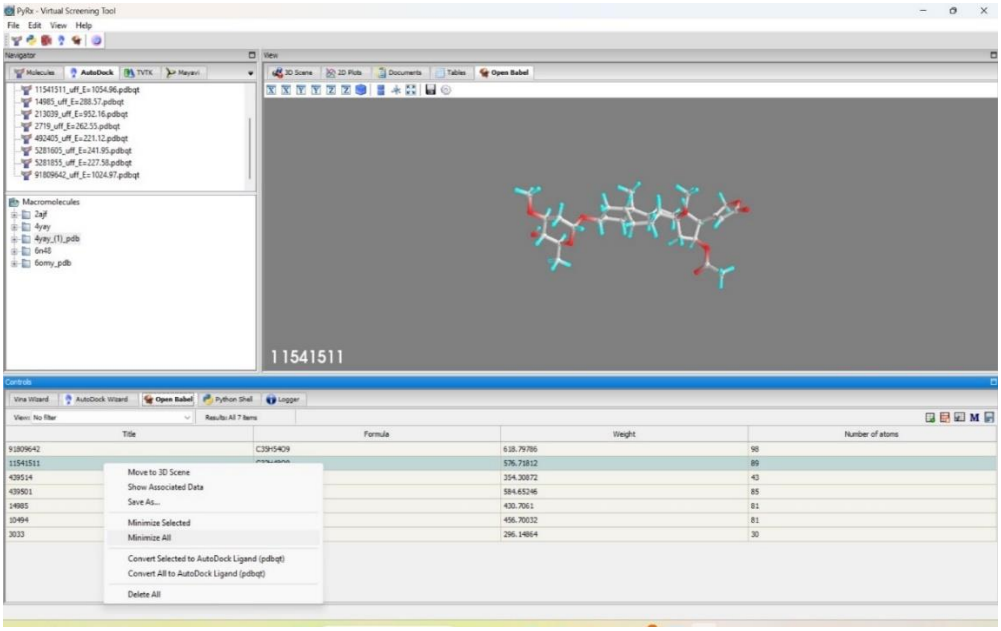


Figure 3.6- Screenshot showing minimisation of energy of ligand

- Again right click and select convert all to AutoDock ligand (pdbqt) to convert all ligands to pdbqt format.

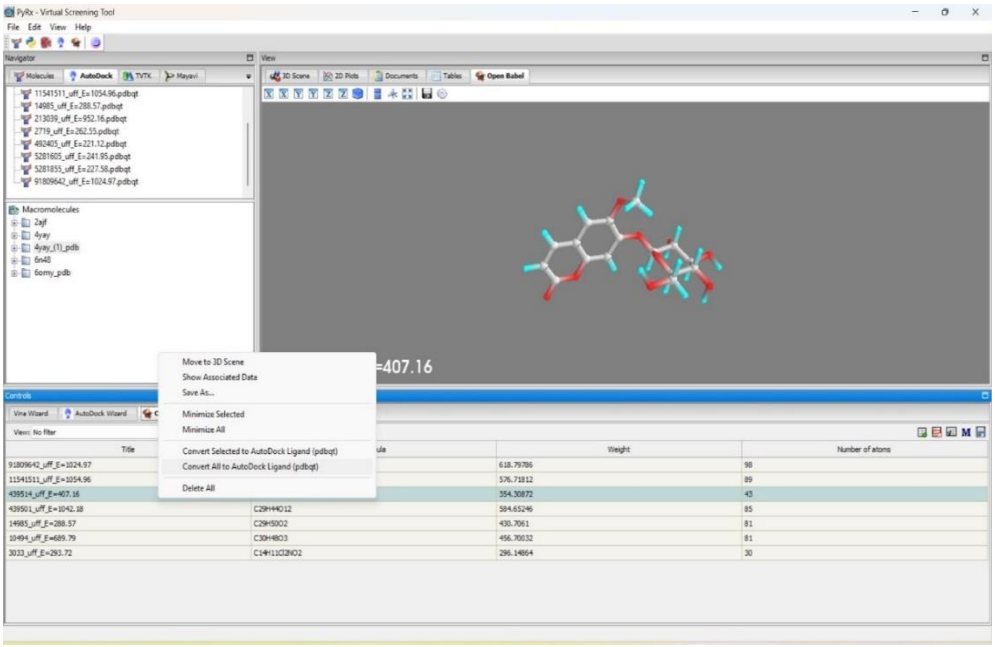


Figure 3.7- Screenshot showing conversion of all ligands to pdbqt

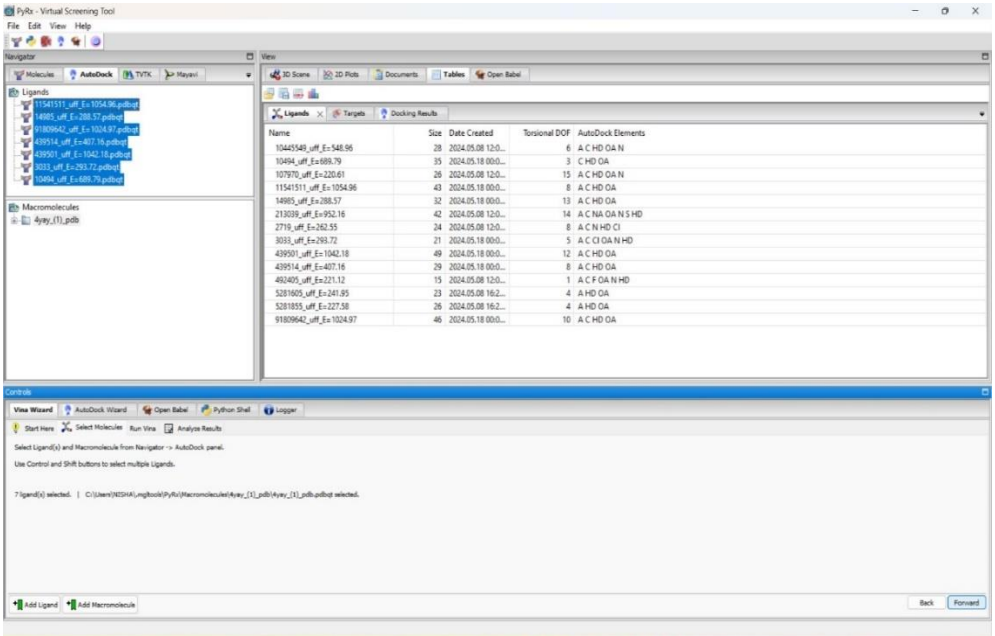


Figure 3.8- Screenshot showing display of all ligands

5.Defining ligands and proteins

The loaded protein and ligand are shown under the "Molecules" tab. It is now necessary to identify which is a ligand and which is a protein. • Right-click on the protein –> “Autodock” –> “Make Macromolecule” to accomplish that.
Perform a right-click on the ligand, select "Autodock," then "Make Ligand."After that, you'll see that it has automatically prepared their PDBQT files under the 'Autodock' page.

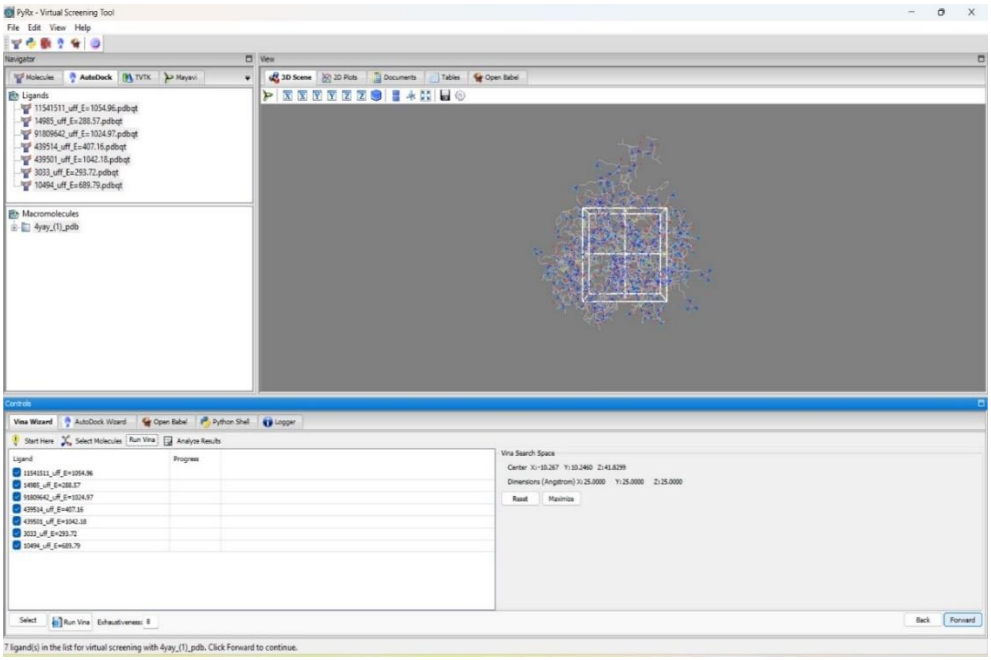


Figure 3.9- Screenshot showing grid box

6.Defining grid box

- Now click on Vina Wizard and select start option on bottom right corner. Start selecting protein and ligand one by one by pressing shift and control button.
- Click on forward. Grid box appears. Return to the 'Molecules' tab located on the right-hand side. Click the loaded protein's "+" symbol.

- All of the residues in the chain will be visible to you. To choose the binding residues, right-click on the residue and choose Atoms, Display, Label, and Atoms. The atoms will start to show up on the protein.
  Now make the appropriate adjustments to the grid box so that it contains all of the selected residues. The ligand does not need to be enclosed within the grid box.

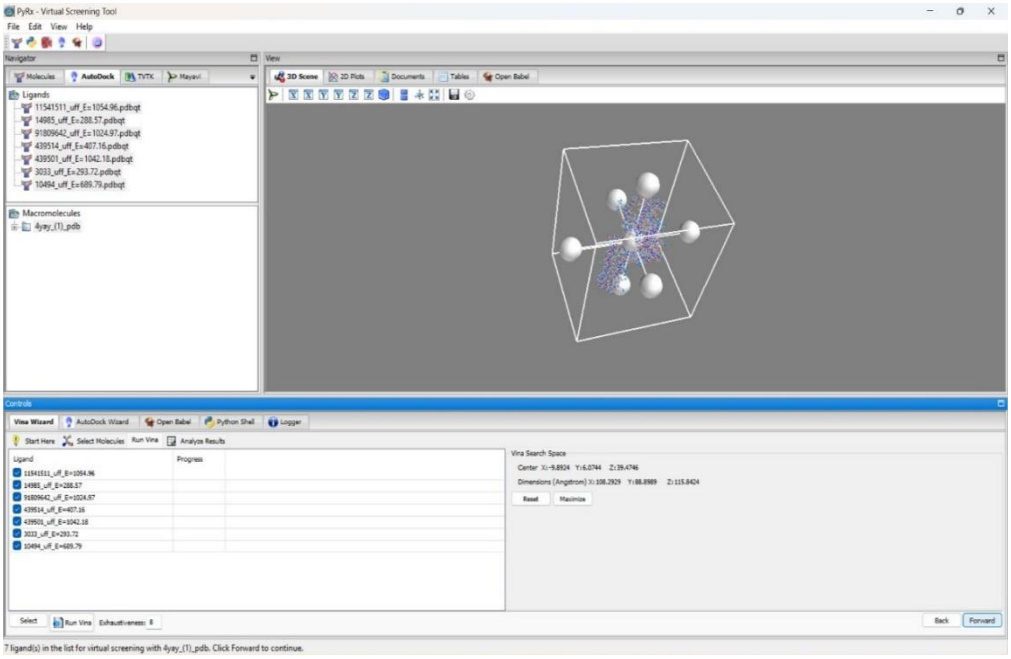


Figure 3.10- Screenshot showing defining of grid box

7.Running vina for docking

- To adjust the exhaustiveness, simply enter the desired number in the box located in the left-bottom corner. Once everything has been adjusted, press the "Forward" button.
- Docking will begin, and the processing will be shown. The bottom panel will display the poses and their binding affinities after the docking process is complete. It will show all poses along with RMSD values. Save your file in excel sheet.

- Now analyse the result and the one ligand which has the highest energy with negative sign is selected. Again open pyrx tab and click on AutoDock and select macromolecule and select the ligand with highest binding energy. Now right click and select display then all models of the ligands gets displayed and select your desired model and save it in pdb format.

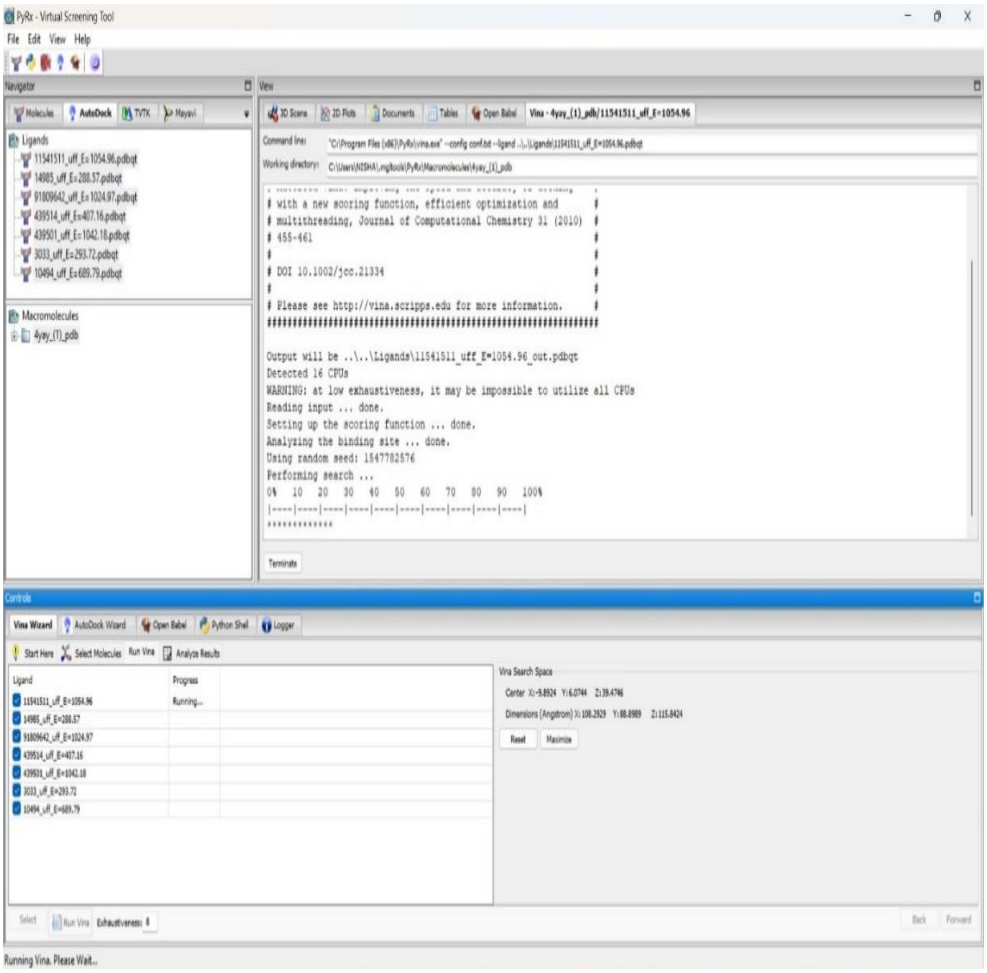


Figure 3.11- Screenshot showing running of vina wizard

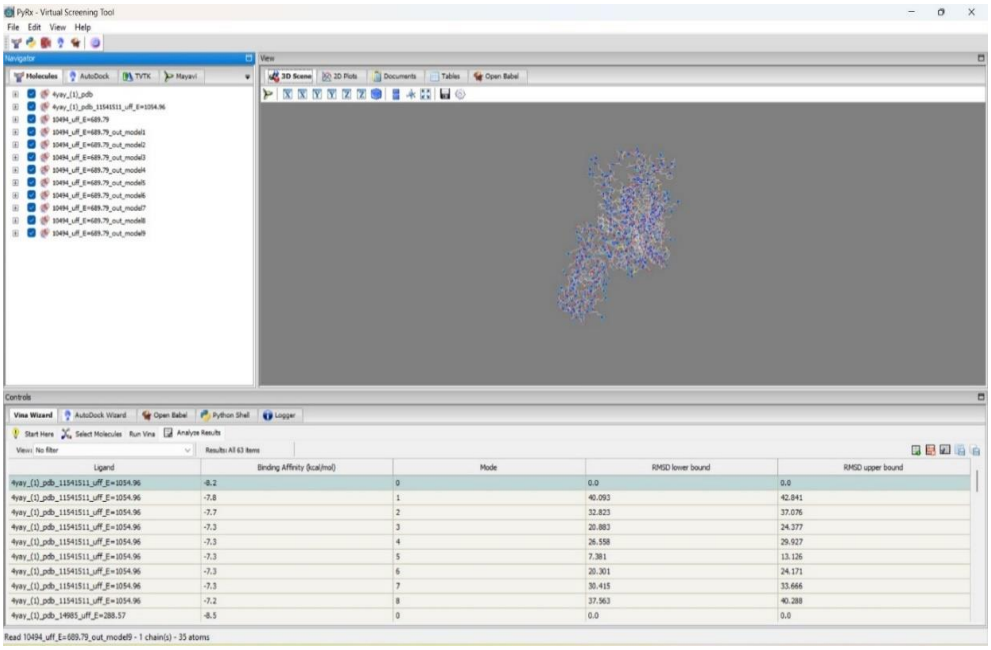


Figure 3.12- Screenshot showing binding affinity of ligands with protein

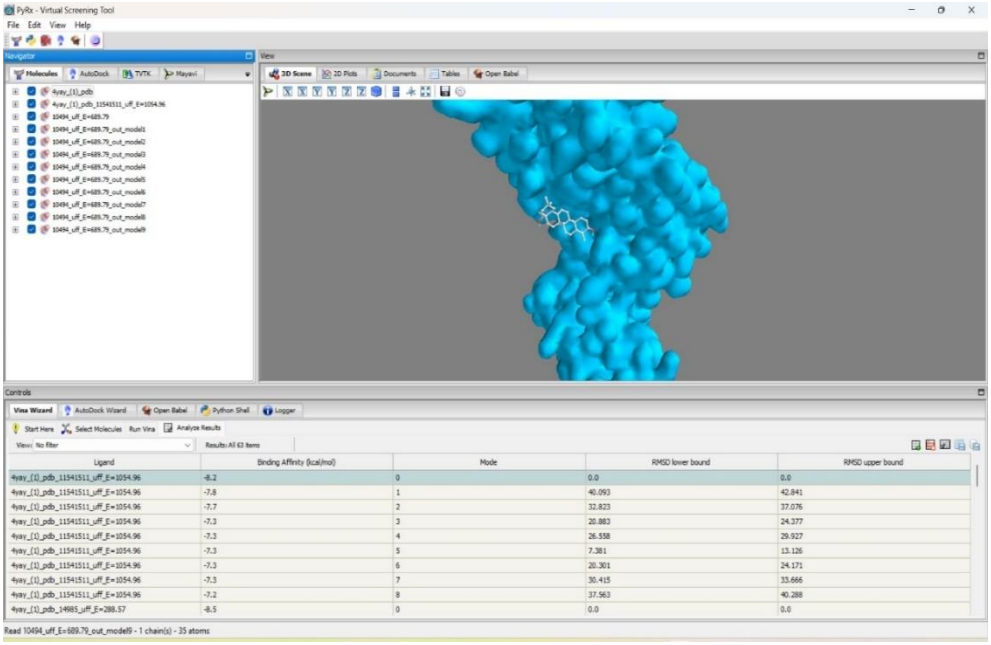


Figure 3.13– Screenshot showing macromolecular structure of protein with different ligands

8.Open Discovery Studio: - Start a new project in Discovery Studio.

9.Protein Structure Import:

- Open Discovery Studio and import the structure of the target protein.
- Choose your protein structure file (such as *.pdb) by using File > Open.

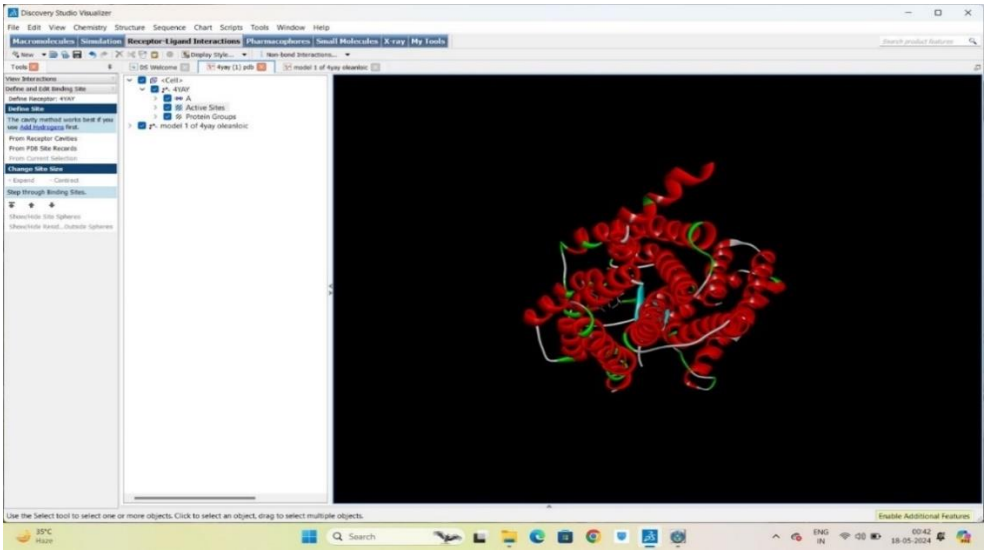


Figure 3.14- Screenshot showing visualisation of protein structure in discovery studio

10. Import Docked Ligand Conformations:

- Import the docked ligand conformations.
- Use File > Open and select the converted ligand file (e.g., *.pdb or *.mol2).

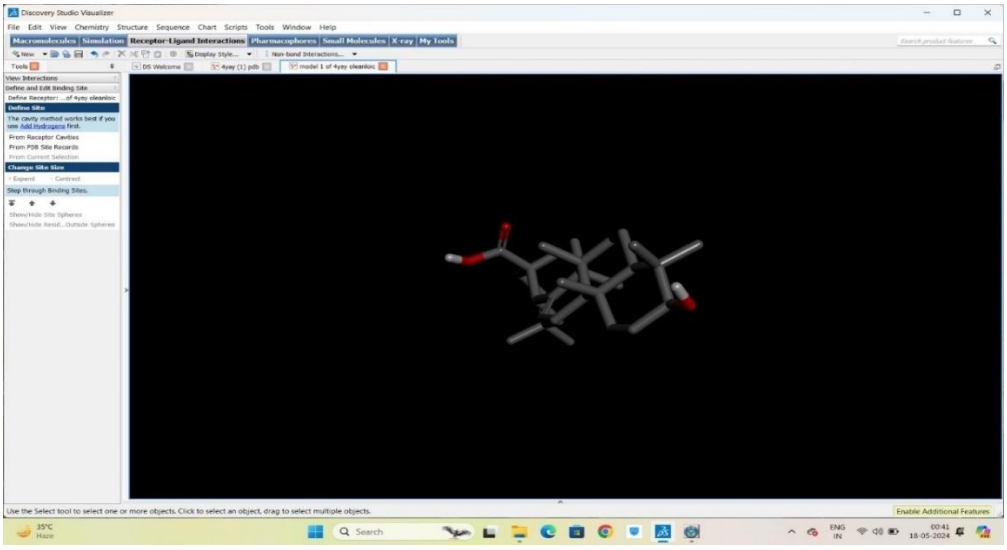


Figure 3.15- Screenshot showing structure of Oleanolic acid

11.Visualize Docked Poses:

- Display the protein and ligand together in the 3D workspace.
- Use the View > Sequence panel to ensure the correct structures are loaded.
- Adjust the display settings to show interactions clearly (e.g., stick or surface representations).

12.Analyse Binding Affinities:

- Check the binding affinities associated with each docked pose.
- This information can be found in the PyRx log files or the output summary from PyRx..
- Record the binding affinity scores (typically in kcal/mol) for reference.

13. Examine Binding Interactions:

- Use the Analyze > Receptor-Ligand Interactions tool to identify and visualize key interactions between the ligand and the protein.
- Highlight hydrogen bonds, hydrophobic interactions, salt bridges, and π-π stacking interactions. Check for consistency with known binding sites or important residues in the binding pocket.

14.Evaluate Docked Poses:

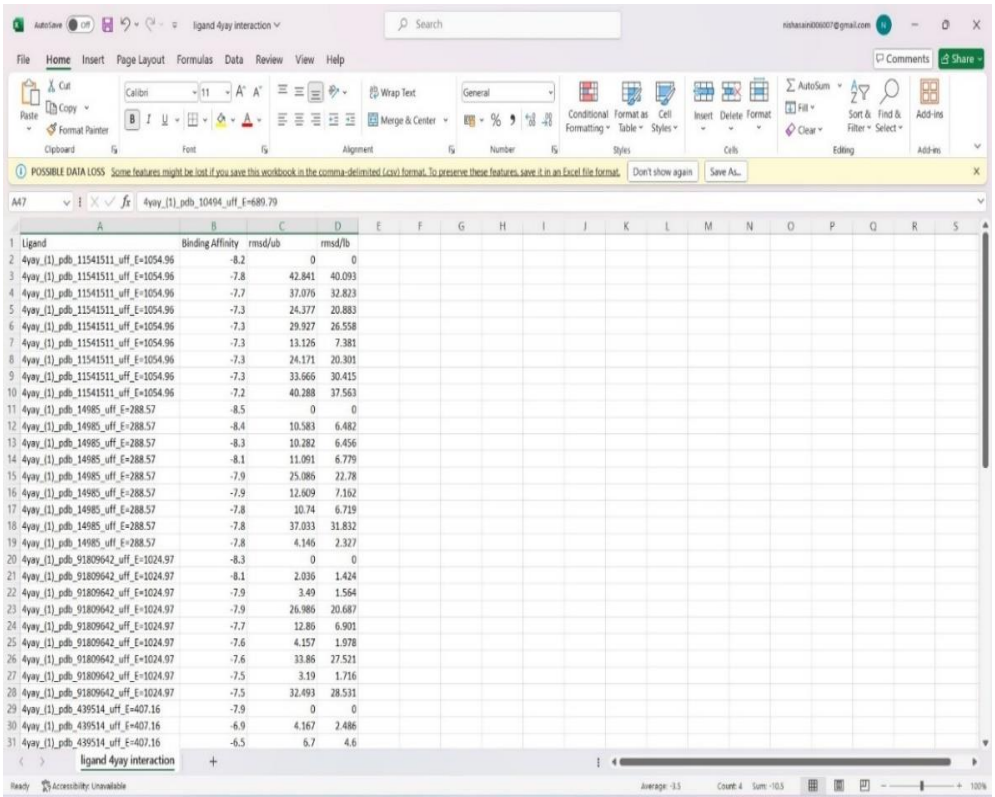
- Compare multiple docked poses to determine if there is a consensus binding mode.
- Use the View > Compare tool to overlay different conformations and evaluate their similarity.
- Consider the Root Mean Square Deviation (RMSD) values to understand the variance among poses.

CHAPTER 4

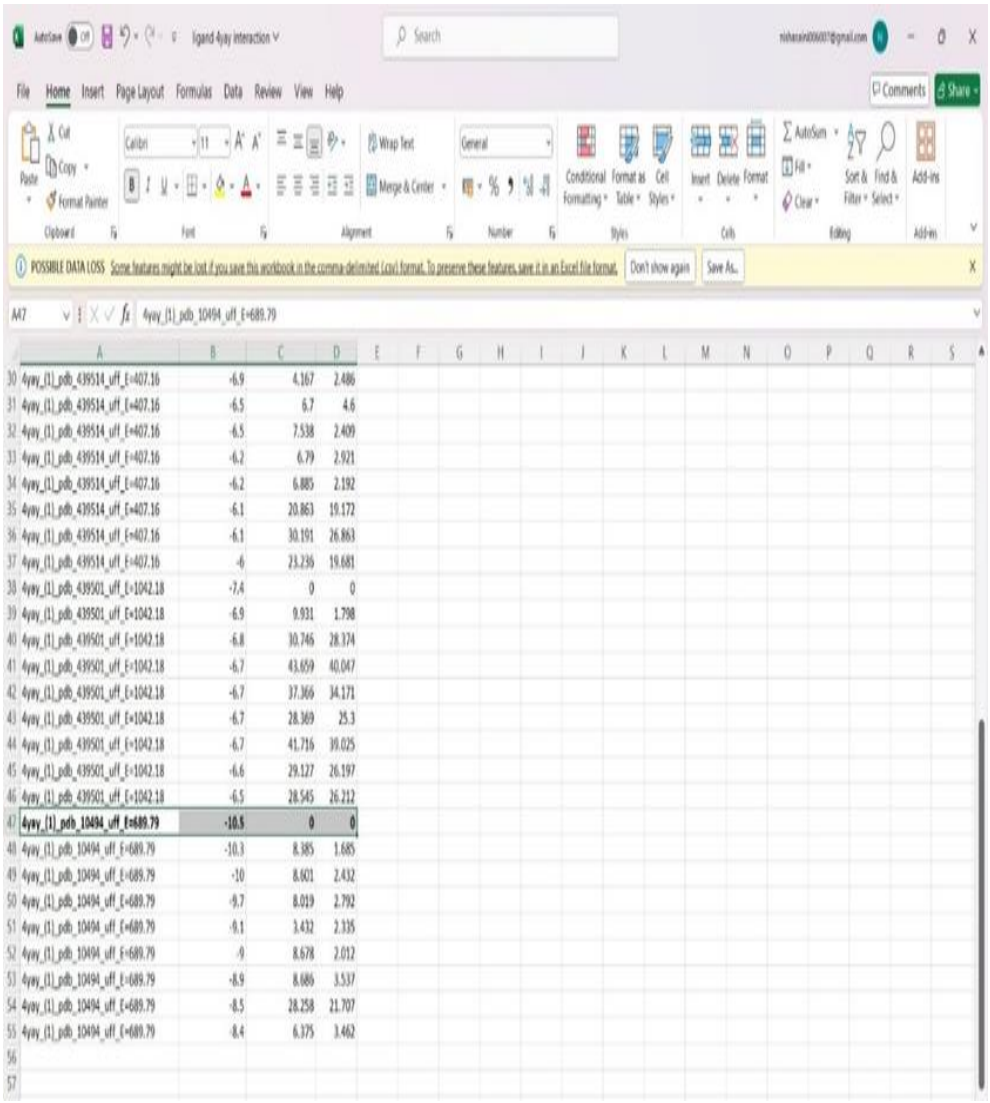
RESULT

The results of the molecular docking simulations revealed promising candidate for treatment in cardiac arrhythmias. Through virtual screening of a diverse compound from Nerium oleander against protein 4yay, several compounds were identified with favourable binding affinities and potential therapeutic effects. The phytochemical which has highest binding score is selected and its model is studied using discovery studio for visualisation of interaction. We also check the binding score of Ouabain which is already in use for the treatment of cardiac arrythmia with our target protein.

The following images show the docking result of different phytochemicals with 4yay



Ligand	Binding Affinity	rmsd/ub	rmsd/lb
4yay_(1)_pdb_11541511_uff_E=1054.96	-8.2	0	0
4yay_(1)_pdb_11541511_uff_E=1054.96	-7.8	42.841	40.093
4yay_(1)_pdb_11541511_uff_E=1054.96	-7.7	37.076	32.823
4yay_(1)_pdb_11541511_uff_E=1054.96	-7.3	24.377	20.883
4yay_(1)_pdb_11541511_uff_E=1054.96	-7.3	29.927	26.558
4yay_(1)_pdb_11541511_uff_E=1054.96	-7.3	13.126	7.381
4yay_(1)_pdb_11541511_uff_E=1054.96	-7.3	24.171	20.301
4yay_(1)_pdb_11541511_uff_E=1054.96	-7.3	33.666	30.415
4yay_(1)_pdb_11541511_uff_E=1054.96	-7.2	40.288	37.563
4yay_(1)_pdb_14985_uff_E=288.57	-8.5	0	0
4yay_(1)_pdb_14985_uff_E=288.57	-8.4	10.583	6.482
4yay_(1)_pdb_14985_uff_E=288.57	-8.3	10.282	6.456
4yay_(1)_pdb_14985_uff_E=288.57	-8.1	11.091	6.779
4yay_(1)_pdb_14985_uff_E=288.57	-7.9	25.086	22.78
4yay_(1)_pdb_14985_uff_E=288.57	-7.9	12.609	7.162
4yay_(1)_pdb_14985_uff_E=288.57	-7.8	10.74	6.719
4yay_(1)_pdb_14985_uff_E=288.57	-7.8	37.033	31.832
4yay_(1)_pdb_14985_uff_E=288.57	-7.8	4.146	2.327
4yay_(1)_pdb_91809642_uff_E=1024.97	-8.3	0	0
4yay_(1)_pdb_91809642_uff_E=1024.97	-8.1	2.036	1.424
4yay_(1)_pdb_91809642_uff_E=1024.97	-7.9	3.49	1.564
4yay_(1)_pdb_91809642_uff_E=1024.97	-7.9	26.986	20.687
4yay_(1)_pdb_91809642_uff_E=1024.97	-7.7	12.86	6.901
4yay_(1)_pdb_91809642_uff_E=1024.97	-7.6	4.157	1.978
4yay_(1)_pdb_91809642_uff_E=1024.97	-7.6	33.86	27.521
4yay_(1)_pdb_91809642_uff_E=1024.97	-7.5	3.19	1.716
4yay_(1)_pdb_91809642_uff_E=1024.97	-7.5	32.493	28.531
4yay_(1)_pdb_439514_uff_E=407.16	-7.9	0	0
4yay_(1)_pdb_439514_uff_E=407.16	-6.9	4.167	2.486
4yay_(1)_pdb_439514_uff_E=407.16	-6.5	6.7	4.6



	A	B	C	D
30	4yay_(1)_pdb_439514_uff_E=407.16	-6.9	4.167	2.486
31	4yay_(1)_pdb_439514_uff_E=407.16	-6.5	6.7	4.6
32	4yay_(1)_pdb_439514_uff_E=407.16	-6.5	7.538	2.409
33	4yay_(1)_pdb_439514_uff_E=407.16	-6.2	6.79	2.921
34	4yay_(1)_pdb_439514_uff_E=407.16	-6.2	6.885	2.192
35	4yay_(1)_pdb_439514_uff_E=407.16	-6.1	20.863	19.172
36	4yay_(1)_pdb_439514_uff_E=407.16	-6.1	30.191	26.863
37	4yay_(1)_pdb_439514_uff_E=407.16	-6	23.236	19.681
38	4yay_(1)_pdb_439501_uff_E=1042.18	-7.4	0	0
39	4yay_(1)_pdb_439501_uff_E=1042.18	-6.9	9.931	1.798
40	4yay_(1)_pdb_439501_uff_E=1042.18	-6.8	30.746	28.374
41	4yay_(1)_pdb_439501_uff_E=1042.18	-6.7	43.659	40.047
42	4yay_(1)_pdb_439501_uff_E=1042.18	-6.7	37.366	34.171
43	4yay_(1)_pdb_439501_uff_E=1042.18	-6.7	28.369	25.3
44	4yay_(1)_pdb_439501_uff_E=1042.18	-6.7	41.716	39.025
45	4yay_(1)_pdb_439501_uff_E=1042.18	-6.6	29.127	26.197
46	4yay_(1)_pdb_439501_uff_E=1042.18	-6.5	28.545	26.212
47	**4yay_(1)_pdb_10494_uff_E=689.79**	**-10.5**	**0**	**0**
48	4yay_(1)_pdb_10494_uff_E=689.79	-10.3	8.385	1.685
49	4yay_(1)_pdb_10494_uff_E=689.79	-10	8.601	2.432
50	4yay_(1)_pdb_10494_uff_E=689.79	-9.7	8.019	2.792
51	4yay_(1)_pdb_10494_uff_E=689.79	-9.1	3.432	2.335
52	4yay_(1)_pdb_10494_uff_E=689.79	-9	8.678	2.012
53	4yay_(1)_pdb_10494_uff_E=689.79	-8.9	8.686	3.537
54	4yay_(1)_pdb_10494_uff_E=689.79	-8.5	28.258	21.707
55	4yay_(1)_pdb_10494_uff_E=689.79	-8.4	6.375	3.462
56				
57				

By comparing our docking result we identified that the Oleanolic acid have highest binding score of -10.5 with Energy E=689.79. Then we studied its model further and check its interactions with our target protein.

STRUCTURE OF STUDIED NATURAL COMPOUNDS

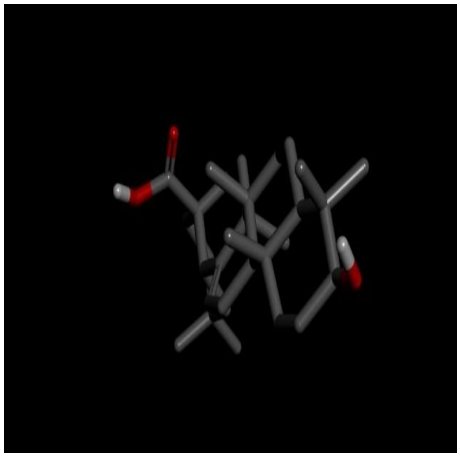


Figure 4.1- Oleanolic acid

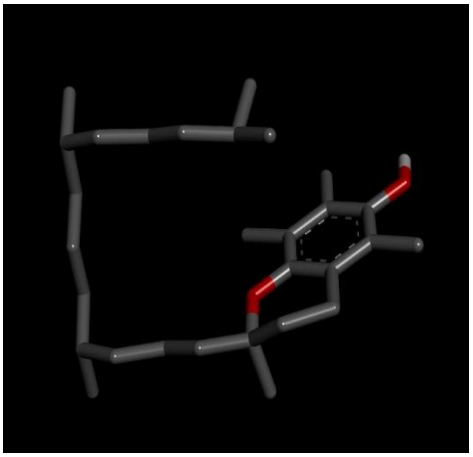


Figure 4.2- Alpha-tocopherol

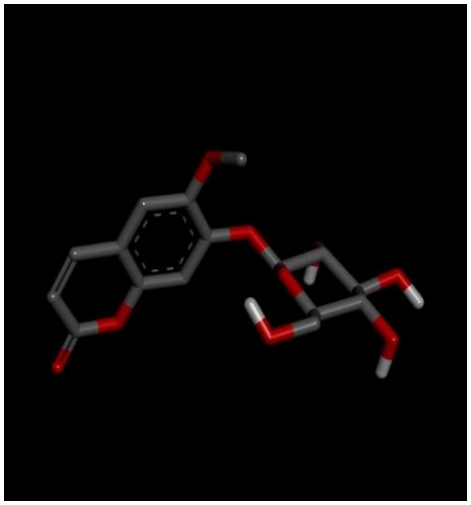


Figure 4.3- Scopolin

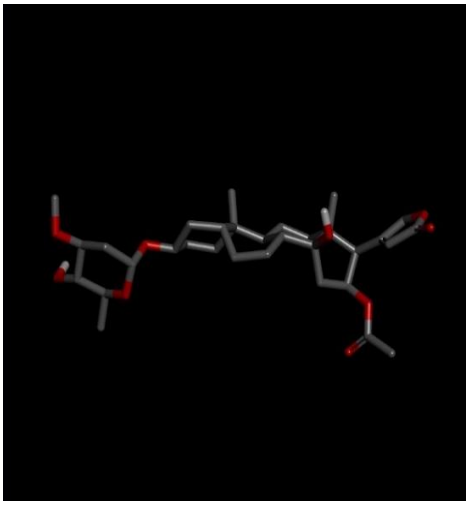


Figure 4.4- Oleandrin

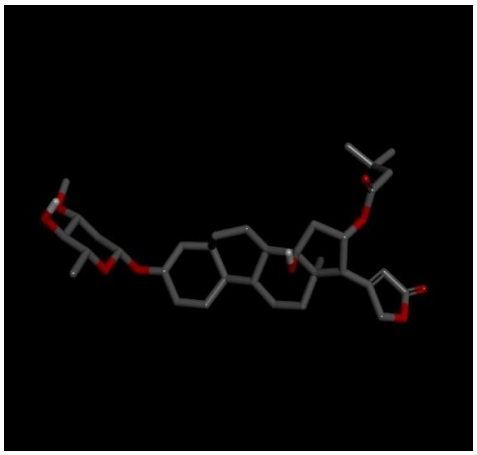


Figure 4.5- Adigoside

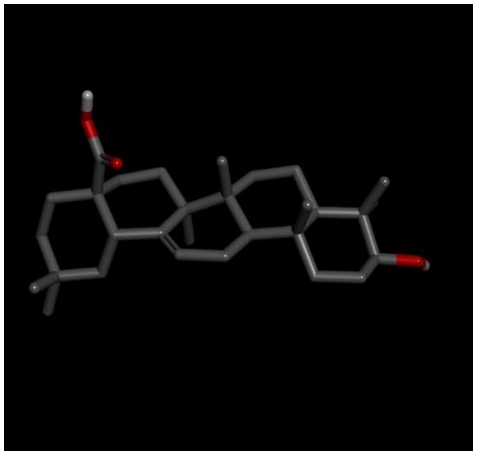


Figure 4.6- Ouabain

Docking of different phytochemical with 4yay

Docking of Adigoside with 4yay

Docking results in binding between adigoside and 4yay shows

E score=1024.97, binding affinity -8.3 kcal/mol and rmsd value =0

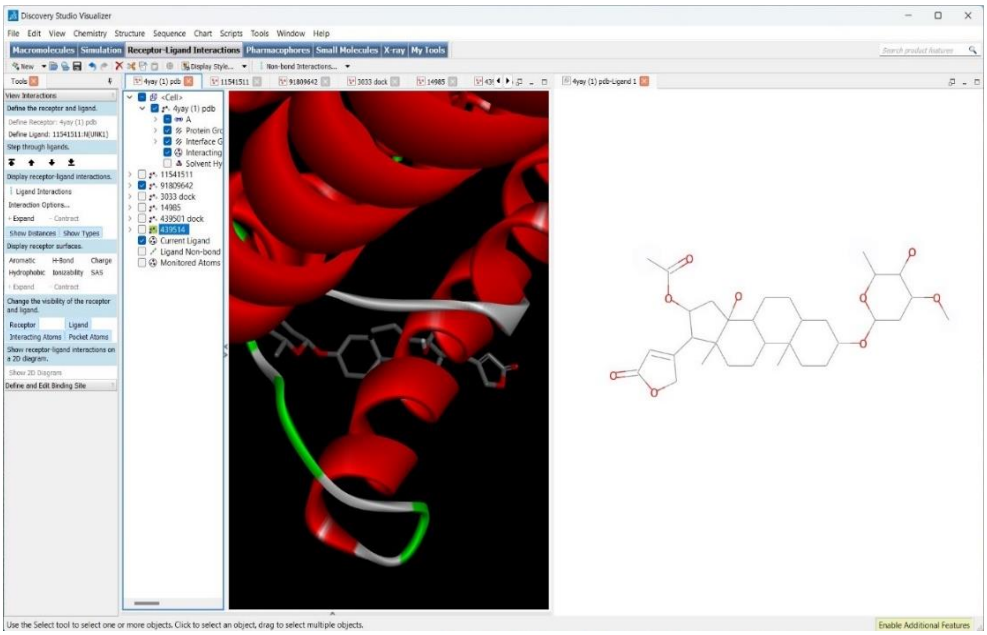


Figure 4.7- Docking of Adigoside with 4YAY

Docking of Alpha -tocopherol with 4YAY

Docking results in binding between alpha-tocopherol with 4yay shows

E score= 288.57, binding affinity -8.5kcal/mol and rmsd value 0

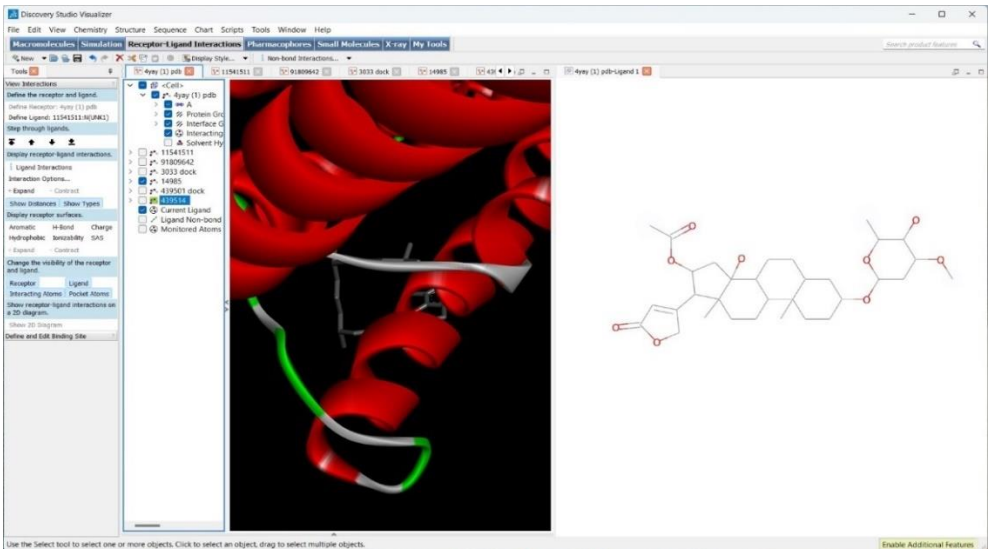


Figure 4.8 – screenshot showing docking of alpha-tocopherol with 4YAY

Docking of Ouabain with 4YAY

Docking results in binding between ouabain with 4yay shows

E score =1042.18, binding affinity -7.4 kcal/mol and rmsd 0

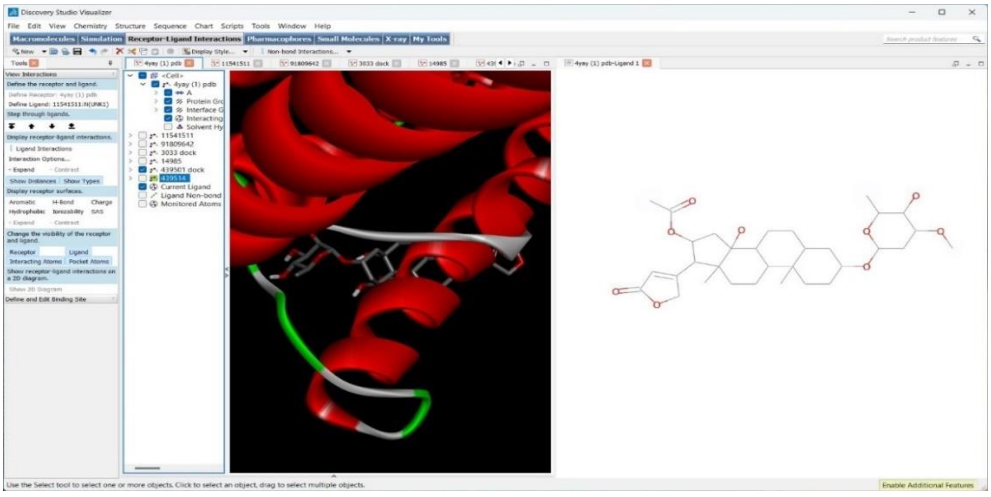


Figure 4.9-Screenshot showing docking of Ouabain with 4YAY

Docking of Scopolin with 4yay

Docking results in binding between scopolin with 4yay shows

E score=407.16, binding affinity -7.9kcal/mol and rmsd value 0

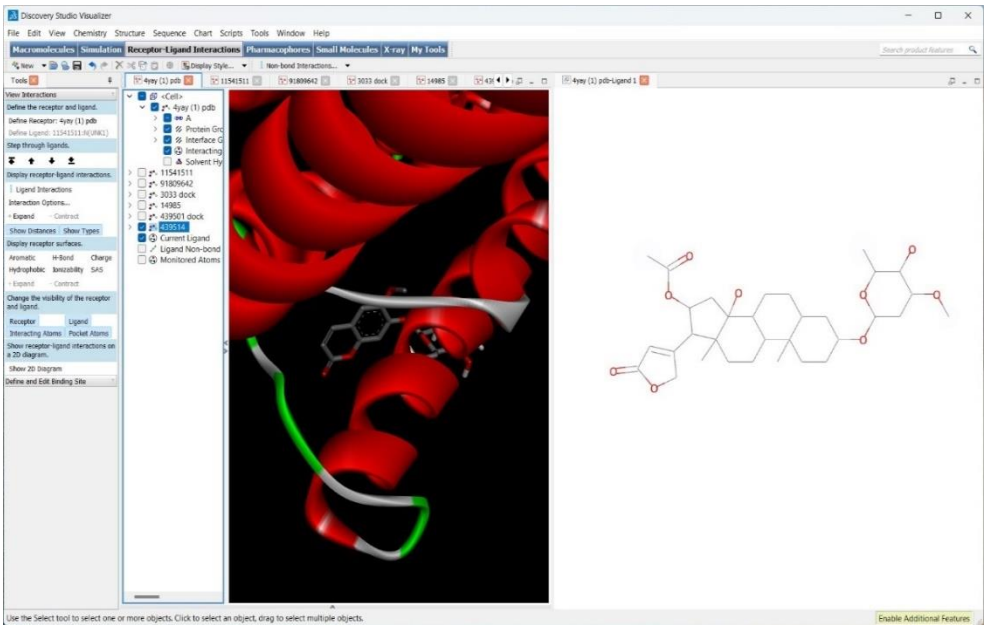


Figure 4.10- Screenshot of docking of scopolin with 4YAY

Docking of Oleandrin with 4YAY

Docking results in binding between oleandrin with 4yay shows

E score=1054.96, binding affinity -8.2kcal/mol and rmsd value 0

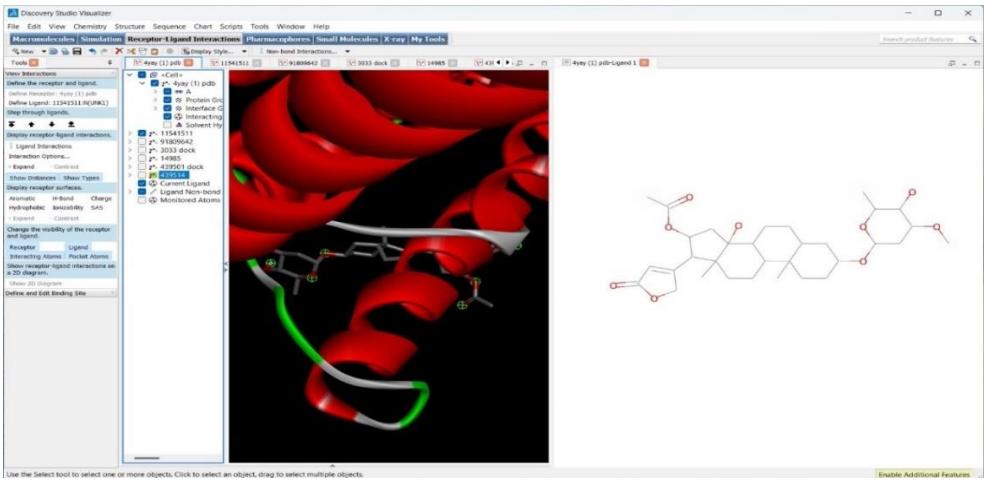


Figure 4.11- Screenshot showing docking of Oleandrin with 4YAY

Docking of Oleanolic acid with 4YAY

Docking results in binding between oleanolic acid with 4yay shows

E score=689.79, binding affinity -10.5kcal/mol and rmsd 0

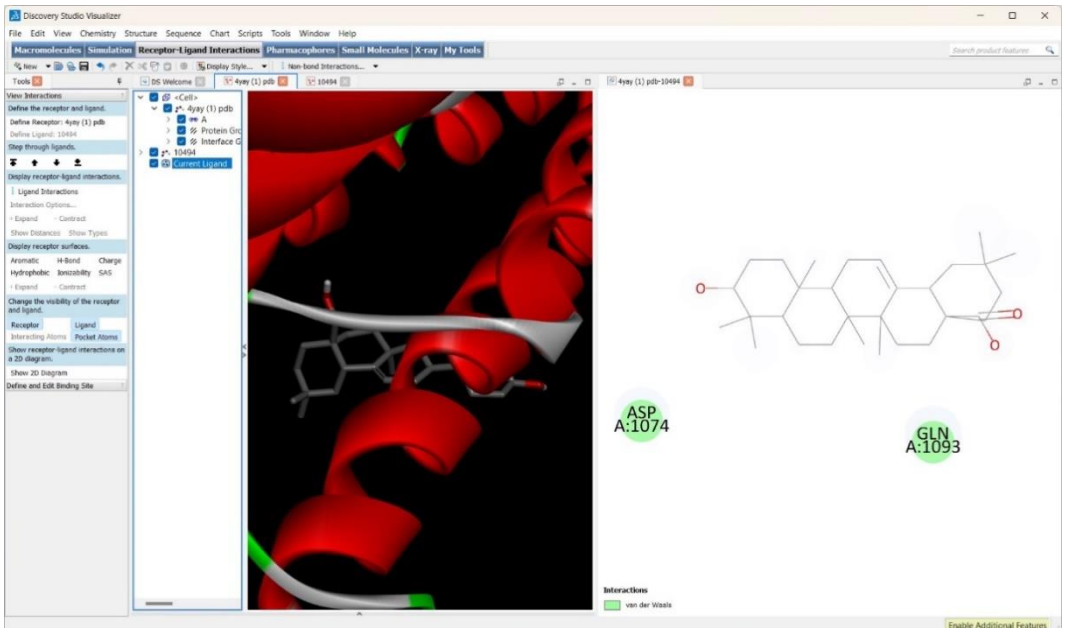


Figure 4.12- Screenshot showing docking of Oleanolic acid with 4YAY

From the above result we identify that Oleanolic acid has high binding affinity with our target protein so we studied its 2-D and 3-D structure which is shown below.

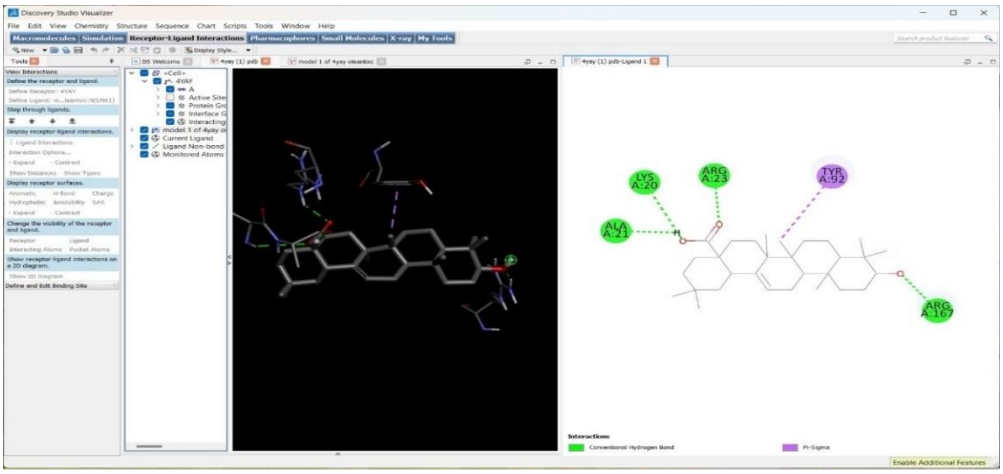


Figure 4.13- Screenshot showing 3-D and 2-D structure of Oleanolic acid

Discussion

In this study, the docking simulation technique was used to identify the potential natural compound used in the treatment of cardiac arrythmia. Analysis of docking scores and binding poses indicated that compounds from the Nerium oleander exhibited varying degrees of interaction with the active sites of target. Docking simulations predicted specific binding interactions, including hydrogen bonds, π-π stacking, and hydrophobic interactions, contributing to ligand binding and stabilization within the binding site (Kumar et al., 2019).The docking results obtained from molecular simulations provide valuable insights into the binding interactions between potential phytochemical and target protein which in turn targets ion channels implicated in cardiac arrhythmias. Analysis of docking scores and binding poses offers crucial information regarding ligand binding affinity, specificity, and mode of interaction within the target proteins. The docking scores, often represented as binding energies or scoring functions, quantify the predicted strength of ligand binding to the target protein. Lower docking scores correspond to more favourable binding interactions, indicating higher affinity and stability of the ligand-protein complex. The oleanolic acid found in Nerium oleander and also present in many plants have high binding affinity with the target protein. The binding interaction energy of oleanolic acid with target 4yay is E=689.79 and highest binding score of -10.5kcal/mol. It is a triterpenoid and have antioxidant property which helps in treatment of cardiac arrythmia. Although some research have indicated that Nerium oleander is hazardous in nature, this plant has the potential to treat cardiac arrhythmia because to the presence of several cardiac glycosides which have as much as potential like Ouabain which is extracted from plant strophantus seeds and already have been used. So we can work on the dosage of the compounds obtained from Nerium oleander and maybe used their compounds as therapeutic approach in cardiac arrythmia while keeping in mind its toxicity.

Recommendations for Future Research

Even while antiarrhythmic medications (AADs) continue to be among the most significant therapeutic alternatives, there are still a number of issues with their administration that need to be resolved. These issues include their limited therapeutic window and adverse drug reactions, which should be taken into account in future drug research. It is therefore necessary to look into cutting-edge, secure, and efficient treatment alternatives with antiarrhythmic action.

Experimental Validation: Future research efforts should focus on experimental validation and optimization of ligand compounds identified through computational modelling, including assessment of binding affinities, pharmacokinetic properties, safety profiles, and efficacy in relevant disease models.

Clinical Translation: Further research is needed to translate computational predictions into clinically viable therapeutics, including preclinical validation studies, formulation development, and clinical trials to evaluate safety and efficacy in human patients.

Personalized Medicine Approaches: Investigating the potential of personalized medicine approaches based on patient-specific genetic mutations or ion channel subtypes can improve treatment outcomes and inform the development of targeted therapeutic strategies for individual patients.

By addressing these recommendations for future research, the field can further advance our understanding of cardiac arrhythmias and facilitate the development of innovative therapeutic interventions for improving patient care and outcomes.

Reference:

1 Kumar A, Nirmal P, Kumar M, Jose A, Tomer V, Oz E, et al. Major Phytochemicals: Recent Advances in Health Benefits and Extraction Method. Vol. 28, Molecules. 2023.

2 Bachheti RK, Worku LA, Gonfa YH, Zebeaman M, Deepti, Pandey DP, et al. Prevention and Treatment of Cardiovascular Diseases with Plant Phytochemicals: A Review. Vol. 2022, Evidence-based Complementary and Alternative Medicine. 2022.

3 NERIUM OLEANDER: PHYTOCHEMISTRY, POISONING AND CASE STUDIES. Int J Biol Pharm Allied Sci. 2022 Jul 1;11(7).

4 Soltani D, Azizi B, Rahimi R, Talasaz AH, Rezaeizadeh H, Vasheghani-Farahani A. Mechanism-based targeting of cardiac arrhythmias by phytochemicals and medicinal herbs: A comprehensive review of preclinical and clinical evidence. Vol. 9, Frontiers in Cardiovascular Medicine. 2022.

5 Dhanachandra Singh K, Karnik SS. Angiotensin Receptors: Structure, Function, Signaling and Clinical Applications. J Cell Signal. 2017;01(02).

6 1.Zhang, Y., Wang, K., & Yu, Z. (2020). Drug development in channelopathies: Allosteric modulation of ligand-gated and voltage-gated ion channels. *Journal of Medicinal Chemistry*, *63*(24), 15258-15278.

7 2.Wacker, S., Noskov, S. Y., & Perissinotti, L. L. (2017). Computational models for understanding of structure, function and pharmacology of the cardiac potassium channel Kv11. 1 (hERG). *Current Topics in Medicinal Chemistry*, *17*(23), 2681-2702.

8 Villoutreix, B. O., & Taboureau, O. (2015). Computational investigations of hERG channel blockers: New insights and current predictive models. *Advanced drug delivery reviews*, *86*, 72-82.

9 3.Tikhonov, D. B., & Zhorov, B. S. (2020). Molecular Modeling in Studies of Ion Channels and their Modulation by Ligands. *Neuroscience and Behavioral Physiology*, *50*(7), 928-937.

10 4.Pliushcheuskaya, P., & Künze, G. (2023). Recent advances in computer-aided structure-based drug design on ion channels. *International Journal of Molecular Sciences*, *24*(11), 9226.

11 5.Lagoutte-Renosi, J., Allemand, F., Ramseyer, C., Yesylevskyy, S., & Davani, S. (2022). Molecular modeling in cardiovascular pharmacology: current state of the art and perspectives. *Drug Discovery Today*, *27*(4), 985-1007.

12 6.Zhang, Q., Gao, Z., & Yang, H. (2016). Computer-aided drug discovery and design targeting ion channels. *Current Topics in Medicinal Chemistry*, *16*(16), 1819-1829.

13 Xu, L., Ding, X., Wang, T., Mou, S., Sun, H., & Hou, T. (2019). Voltage-gated sodium channels: structures, functions, and molecular modeling. *Drug discovery today*, *24*(7), 1389-1397.

14 Vagos, M., van Herck, I. G., Sundnes, J., Arevalo, H. J., Edwards, A. G., & Koivumäki, J. T. (2018). Computational modeling of electrophysiology and pharmacotherapy of atrial fibrillation: recent advances and future challenges. *Frontiers in physiology*, *9*, 402624.

15 Tikhonov, D. B., & Zhorov, B. S. (2019). Molecular Modeling in Studies of Ion Channels and their Modulation by Ligands. *Российский физиологический журнал им. ИМ Сеченова*, *105*(11), 1333-1348.

16 Cavalluzzi, M. M., Imbrici, P., Gualdani, R., Stefanachi, A., Mangiatordi, G. F., Lentini, G., & Nicolotti, O. (2020). Human ether-à-go-go-related potassium channel: Exploring SAR to improve drug design. *Drug Discovery Today*, *25*(2), 344-366.

17 Taboureau, O., & Steen Jorgensen, F. (2011). In silico predictions of hERG channel blockers in drug discovery: from ligand-based and target-based approaches to systems chemical biology. *Combinatorial Chemistry & High Throughput Screening*, *14*(5), 375-387.

18 Zhorov, B. S., & Tikhonov, D. B. (2016). Computational structural pharmacology and toxicology of voltage-gated sodium channels. In *Current topics in membranes* (Vol. 78, pp. 117-144). Academic Press.

19 Yu, Z., Liu, J., van Veldhoven, J. P., IJzerman, A. P., Schalij, M. J., Pijnappels, D. A., ... & de Vries, A. A. (2016). Allosteric modulation of Kv11. 1 (hERG) channels protects against drug-induced ventricular arrhythmias. *Circulation: Arrhythmia and Electrophysiology*, *9*(4), e003439.

20 Pliushcheuskaya, P., & Künze, G. (2023). Recent advances in computer-aided structure-based drug design on ion channels. *International Journal of Molecular Sciences*, *24*(11), 9226.

21 Eldstrom, J., Wang, Z., Xu, H., Pourrier, M., Ezrin, A., Gibson, K., & Fedida, D. (2007). The molecular basis of high-affinity binding of the antiarrhythmic compound vernakalant (RSD1235) to Kv1. 5 channels. *Molecular pharmacology*, *72*(6), 1522-1534.

22 Hanck, D. A., Nikitina, E., McNulty, M. M., Fozzard, H. A., Lipkind, G. M., & Sheets, M. F. (2009). Using lidocaine and benzocaine to link sodium channel molecular conformations to state-dependent antiarrhythmic drug affinity. *Circulation research*, *105*(5), 492-499.

23 Stansfeld, P. J., Sutcliffe, M. J., & Mitcheson, J. S. (2006). Molecular mechanisms for drug interactions with hERG that cause long QT syndrome. *Expert opinion on drug metabolism & toxicology*, *2*(1), 81-94.

24 Roden, D. M., Balser, J. R., George Jr, A. L., & Anderson, M. E. (2002). Cardiac ion channels. *Annual review of physiology*, *64*(1), 431-475

25 Tikhonov, D. B., & Zhorov, B. S. (2017). Mechanism of sodium channel block by local anesthetics, antiarrhythmics, and anticonvulsants. *Journal of General Physiology*, *149*(4), 465-481.

26 Bottino, D., Penland, R. C., Stamps, A., Traebert, M., Dumotier, B., Georgieva, A., ... & Lett, G. S. (2006). Preclinical cardiac safety assessment of pharmaceutical compounds using an integrated systems-based computer model of the heart. *Progress in biophysics and molecular biology*, *90*(1-3), 414-443.

27 Hoffmann, P., & Warner, B. (2006). Are hERG channel inhibition and QT interval prolongation all there is in drug-induced torsadogenesis? A review of emerging trends. *Journal of pharmacological and toxicological methods*, *53*(2), 87-105.

28 Clancy, C. E., Kurokawa, J., Tateyama, M., Wehrens, X. H., & Kass, R. S. (2003). K+ channel structure-activity relationships and mechanisms of drug-induced QT prolongation. *Annual review of pharmacology and toxicology*, *43*(1), 441-461.

29 Clancy, C. E., Tateyama, M., & Kass, R. S. (2002). Insights into the molecular mechanisms of bradycardia-triggered arrhythmias in long QT-3 syndrome. *The Journal of clinical investigation*, *110*(9), 1251-1262.

30 Jiang, D., Xiao, B., Zhang, L., & Chen, S. W. (2002). Enhanced basal activity of a cardiac Ca2+ release channel (ryanodine receptor) mutant associated with ventricular tachycardia and sudden death. *Circulation research*, *91*(3), 218-225.

31 Abriel, H., & Kass, R. S. (2005). Regulation of the voltage-gated cardiac sodium channel Nav1. 5 by interacting proteins. *Trends in cardiovascular medicine*, *15*(1), 35-40.

32 Karawya M, Balbaa S, Khayyal S. Estimation of cardenolides in *Nerium oleander*. *Plant Med* 1973;

Similarity Report

PAPER NAME

study of phytochemical from Nerium ole ander for treatment of cardiac arrhythmi aa.docx

WORD COUNT	CHARACTER COUNT
7052 Words	**44199 Characters**
PAGE COUNT	FILE SIZE
42 Pages	**5.3MB**
SUBMISSION DATE	REPORT DATE
May 31, 2024 3:04 PM GMT+5:30	**May 31, 2024 3:05 PM GMT+5:30**

8% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

- 5% Internet database
- 3% Publications database
- Crossref database
- Crossref Posted Content database
- 5% Submitted Works database

Excluded from Similarity Report

- Bibliographic material
- Cited material
- Small Matches (Less then 10 words)

8% Overall Similarity

Top sources found in the following databases:

- 5% Internet database
- Crossref database
- 5% Submitted Works database
- 3% Publications database
- Crossref Posted Content database

TOP SOURCES

The sources with the highest number of matches within the submission. Overlapping sources will not be displayed.

1	researchgate.net Internet	<1%
2	bioinformaticsreview.com Internet	<1%
3	fastercapital.com Internet	<1%
4	Universiti Teknologi Malaysia on 2021-09-19 Submitted works	<1%
5	mdpi.com Internet	<1%
6	Shyam Tripathi, Kusum Rani, V. Samuel Raj, Rashmi K. Ambasta. "Drug... Crossref	<1%
7	National University of Singapore on 2015-01-21 Submitted works	<1%
8	ijmcmed.org Internet	<1%

9	"Voltage-gated Sodium Channels: Structure, Function and Channelopat... Crossref	<1%
10	University of Sydney on 2023-04-28 Submitted works	<1%
11	universititeknologimara on 2024-02-18 Submitted works	<1%
12	pediatrix.com Internet	<1%
13	Jagdish S. Bankar, Kajal N. Bondre, Pratiksha P. Wagh, Surbhi S. Bhop... Crossref	<1%
14	Sir George Monoux College on 2024-04-29 Submitted works	<1%
15	University of Wales Institute, Cardiff on 2022-05-07 Submitted works	<1%
16	lra.le.ac.uk Internet	<1%
17	ncbi.nlm.nih.gov Internet	<1%
18	science.gov Internet	<1%
19	Anglia Ruskin University on 2013-09-17 Submitted works	<1%
20	The University of Texas at Arlington on 2023-11-27 Submitted works	<1%

21	University of South Florida on 2023-05-01 Submitted works	<1%
22	University of Strathclyde on 2023-11-27 Submitted works	<1%
23	School of Accounting & Management on 2010-02-07 Submitted works	<1%
24	University of Leeds on 2017-12-04 Submitted works	<1%
25	University of Newcastle on 2013-10-01 Submitted works	<1%
26	University of Witwatersrand on 2013-11-14 Submitted works	<1%
27	link.springer.com Internet	<1%
28	news.samsung.com Internet	<1%
29	fedoa.unina.it Internet	<1%