

COMPUTATIONAL DRUG DISCOVERY FOR RAYNAUD'S DISEASE

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Science in Bioinformatics and Data Science

by

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**DEPARTMENT OF BIOINFORMATICS
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY(DEEMED TO BE UNIVERSITY)

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APRIL 2024



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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **JERUSHA.R (41738005)**, who carried out the project entitled "**COMPUTATIONAL DRUG DISCOVERY FOR RAYNAUD'S DISEASE**" under my supervision from **January 2024 to April 2024**.

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Submitted for Viva voce Examination held on 27/04/24

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DECLARATION

I, **JERUSHA.R (41738005)** hereby declare that the Project Report entitled **COMPUTATIONAL DRUG DISCOVERY FOR RAYNAUD'S DISEASE** done by me under the guidance of **Dr.SWETHA SUNKAR** , Jeppiaar Nagar, Rajiv Gandhi Road, Chennai-600119, Tamil Nadu, India, is submitted in partial fulfillment of the requirements for the award of **Bachelor of Science degree in Bioinformatics and Data Science**.

DATE : 27/04/2024

PLACE: CHENNAI



SIGNATURE OF THE CANDIDATE

ACKNOWLEDGEMENT

I am delighted to express my heartfelt acknowledgement to the Board of Management of **SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY** for their kind encouragement and support throughout the duration of my project. Their guidance has been invaluable in ensuring the successful completion of this project, and I am sincerely grateful for their contribution.

I convey my thanks to **Dr. Rekha Chakravarthy, Dean of the School of Science and Humanities**, for providing me necessary support and details at the right time during the progressive reviews.

I sincerely thank **Dr. H. Jemmy Christy, Head of the Department, Department of Bioinformatics, Sathyabama Institute of Science and Technology**, for her unwavering support and timely provision of necessary details during the progressive reviews. Her support has been instrumental in helping me to overcome obstacles and achieve success in this project.

I am deeply indebted to my **Project Guide, Dr. Swetha Sunkar**, for her invaluable guidance, suggestions, and constant encouragement throughout the project. Her expertise and insights have been instrumental in shaping my work and ensuring its successful completion.

I also wish to extend my gratitude to my family for their support and assistance in various ways, which have contributed to the successful completion of this project.

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CERTIFICATE

This is to certify that the dissertation entitled "**Computational Drug Discovery for Raynaud's Disease**" was carried out by **Ms. JERUSHA.R** (Reg. No. **41738005**), Department of Bioinformatics and Data Science, Sathyabama Institute of Science and Technology, Chennai in partial fulfillment of the requirements of the award of the **Degree of Bachelor of Science in Bioinformatics and Data Science** 2021-2024, is a bonafide record of the project work done under my guidance and supervision. This thesis or part, therefore, has not been submitted elsewhere for any other degree or diploma.

Date: 27/04/24

Place: CHENNAI

A handwritten signature in blue ink, appearing to read 'Swetha'.

Dr. SWETHA SUNKAR

ABSTRACT

Raynaud's disease is a common but sometimes undiagnosed vascular condition that is characterized by a temporary and periodic, but reversible constriction of the peripheral blood vessels. It is induced by excessive vasoconstriction of the peripheral microcirculation in response to environmental factors like cold, and also stress or emotions. Through this study, it was attempted to computationally discover drugs that assist in managing the symptoms of Raynaud's disease. Computational drug discovery (CDD) refers to the use of computational techniques, algorithms, and modelling approaches to quicken the process of discovering and developing new drugs. Advancements in CDD have increased the speed and decreased the costs of the drug discovery process, by eliminating the need to physically test several compounds in the lab, while also increasing the efficiency by applying high throughput screening methods to screen a large number of compounds in a short period of time. Several data were analysed to identify the target as the voltage-gated calcium channel and more specifically, the CaV1.2 present in the smooth muscles. The UNIPROT database was used to identify the drug binding sites as the regions from 1109 to 1198 and from 1478 to 1546. After the target was identified, several plants were found to have calcium channel blocking properties. From the list of plants, 5 of them that were not studied extensively were chosen. A library of ligands (phytochemicals from the selected plants) was obtained from PUBCHEM and were then filtered using its properties like absorption, distribution, metabolism, excretion, toxicity, etc. Molecular docking was done using the ligands and the target protein voltage- gated calcium channel using Discovery Studio. Through the study, it was understood how the improvements in computational methods in drug discovery have impacted the overall drug discovery process and reduced the cost as well as the time required to search for new potential drug candidates. CDD has definitely become a very useful tool for pharmaceutical companies and academic research groups to make their work more efficient.

Keywords: Computational drug discovery, Raynaud's disease, target, ligands, ADME/T properties, molecular docking

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LIST OF ABBREVIATIONS

RP	Raynaud's phenomenon
RD	Raynaud's disease
AR	Adrenoreceptors
ROS	Reactive oxygen species
MLCK	Myosin light-chain kinase
CCB	Calcium channel blockers
LTCC	L-type calcium channels
SR	Sarcoplasmic reticulum
RYP	Ryanodine receptors
VGCC	Voltage gated calcium channels
DHP	Dihydropyridines
IMPPAT	Indian Medicinal Plants, Phytochemistry and Therapeutics
OSIRIS	Organized Structure of Interfaces from Reduced Information Sets
NCBI	National Centre for Biotechnology Information
NIH	National Institutes of Health
PDB	Protein Data Bank
ADME	Absorption, Distribution, Metabolism, Excretion
DS	Discovery studio
SDF	Structure Data File
MW	Molecular weight
Rot. bonds	Rotatable bonds
HBA	Hydrogen bond acceptors
HBD	Hydrogen bond donors
TPSA	Topological Polar Surface Area

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CHAPTER 1

INTRODUCTION

1.1 RAYNAUD'S DISEASE

Raynaud's disease, sometimes referred to as Raynaud's phenomenon or Raynaud's syndrome, is characterised by phases of decreased blood flow to certain extremities, usually the fingers and toes. It is generally triggered by exposure to cold weather or due to psychological stress. Initially reported by the French physician Maurice Raynaud in 1862 (*Raynaud, 1888*), this condition causes the small arteries supplying blood to the skin to constrict or spasm momentarily. The affected areas typically become pale or bluish-white due to this constriction, and as blood flow recovers, they take on a reddish hue.

A person suffering from Raynaud's disease may feel a numb, tingling, or cool feeling in their fingers, toes, or occasionally other extremities like the nose or ears. This disease affects up to 10% of the population (*Gerbracht et al., 2015*) and the intensity of these symptoms can differ across individuals; some may feel minor discomfort while others may experience more severe pain. There are two commonly recognised classifications of Raynaud's disease: primary, which occurs when there is no underlying medical condition, and secondary, which is associated with other health issues such as vascular diseases, autoimmune diseases like lupus or scleroderma, or exposure to particular drugs or chemicals (*Block and Sequeira, 2011*).

The symptoms of Raynaud's illness can greatly affect a person's quality of life and may point to an underlying medical problem that needs to be addressed, even though the disease itself is not life-threatening.

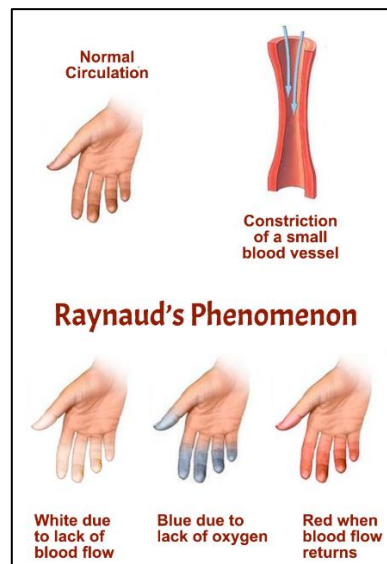


Fig 1.1 Raynaud's disease

1.1.1 Pathophysiology

The pathophysiology of this disease is mainly contributed to neural as well as vascular effects. There are slight differences in the pathophysiology for both the primary and secondary causes of RP, but for primary RP, it is known to be generally due to functional abnormalities since it is an isolated vasospastic condition. (Herrick, 2012)

1.1.1.1 Vascular Abnormalities

Endothelium is a very sensitive layer and even the slightest defect in it can cause abnormal functioning and obstruct it from performing its regular functions of reacting to and producing a huge variety of vasodilators, such as nitric oxide (NO) and prostacyclin. (Haque and Hughes, 2020). This decreased production of vasodilators NO and prostacyclin may cause the pathogenesis of RP.

Another function of the Endothelium is to produce vasoconstrictive substances like endothelin-1 (Herrick, 2012). Since endothelin-1 is a vasoconstrictive substance, production of it in large amounts can lead to RP. (Zamora et al., 1990). Similar to endothelin-1, angiotensin is another strong vasoconstrictor that can have the same effects.

1.1.1.2 *Neural Abnormalities*

Generally, when the human body is subjected to cold, α_1 - and α_2 -adrenoreceptors respond to norepinephrine and decrease the flow of blood to the skin to reduce the loss of heat. This is a natural reflex of the sympathetic nervous system and if this reflex is exaggerated due to some abnormality, it can lead to RP (*Flavahan et al., 2003*)

1.1.2 *Management and Treatment*

Management of Raynaud's disease focuses on minimizing vasospastic episodes, relieving symptoms, and preventing complications. Non-pharmacological interventions such as lifestyle modifications (e.g., avoiding cold exposure, smoking cessation) and thermal biofeedback techniques (*Karavidas et al., 2006*) are recommended as initial measures. Pharmacological therapy includes the use of calcium channel blockers, vasodilators, and agents that improve peripheral circulation. In refractory cases, surgical interventions such as sympathectomy or botulinum toxin injection may be considered to alleviate symptoms and improve digital perfusion

1.2 COMPUTATIONAL DRUG DISCOVERY

Computational drug discovery is a novel method to drug development that uses computational approaches and algorithms to speed up the identification and optimisation of potential therapeutic compounds. The pharmaceutical industry has found this field to be an invaluable asset in recent years, with the potential to speed up the discovery process, cut expenses, and eventually provide safer and more effective therapies for a range of illnesses.

Computational drug discovery is fundamentally based on an interdisciplinary method combining concepts from statistics, chemistry, biology, and computer science. Researchers can simulate molecular interactions, predict the behaviour of potential drugs, and screen enormous libraries of chemical compounds with previously unattainable speed and precision by utilising computational models. By using these techniques, scientists can find promising drug candidates more quickly, which reduces the amount of time it takes from early discovery to clinical trials.

1.2.1 Challenges in traditional drug discovery

The traditional drug discovery process, while effective, has a number of flaws that can significantly hinder advancement and the development of novel therapeutic agents. The considerable amount of time and money needed to find and improve possible drug candidates is one of the most significant drawbacks. Conventional approaches mostly depend on labour-intensive experimental procedures that can be expensive and time-consuming, like combinatorial chemistry and high-throughput screening. This lengthy schedule may cause new medications to be introduced later than they would otherwise, especially for ailments that are fast advancing or for diseases for which there are few therapeutic options.

Moreover, there is a high failure rate in the traditional drug discovery process at different phases of development (*Shekhar, 2008*). Preclinical testing yields many drug candidates that do not meet safety or efficacy specifications, resulting in major setbacks and resource loss. Additionally, depending too much on empirical screening techniques may lead to the discovery of compounds with unexpected toxicities, off-target effects, or poor bioavailability, which will only complicate the development process and raise the risk of clinical trial failures.

1.2.2 Advantages

On the other hand, there are a number of benefits associated with computational drug discovery that counter these drawbacks and transform the field of drug development. Through the use of computational modelling, scientists can evaluate thousands or even millions of compounds quickly and effectively, greatly accelerating the discovery process in its early phases. Scientists can discover viable drug leads faster by prioritising the most promising candidates for additional experimental validation thanks to this accelerated screening process. This saves time, money and resources.

1.2.3 Emerging trends and future directions

Looking ahead, the convergence of computational drug discovery with advances in artificial intelligence, machine learning, and related fields holds immense promise for accelerating drug development in Raynaud's disease and beyond. Deep learning algorithms can analyse large-scale omics datasets and identify subtle patterns associated with disease progression and treatment response. Furthermore, the integration of patient-specific data, such as electronic health records and wearable sensor data, enables the tailoring of therapeutic strategies to individual needs, maximizing efficacy and minimizing adverse effects. As computational drug discovery continues to evolve, it heralds a new era of precision medicine where innovative therapies for Raynaud's disease and other complex disorders are within reach. (*Paul et al., 2021*)

CHAPTER 2

REVIEW OF LITERATURE

Raynaud's disease (RD) was discovered by Maurice Raynaud in 1862 but was only labelled as "Raynaud's disease" in 1883 (*Susol, 1999*). The complex mechanisms causing this disease are explored by *MacDonald, et al. 1997*. Blood vessel spasms that cause insufficient blood flow are the indicators of Raynaud's disease; these usually occur in the extremities, such as the fingers and toes.

According to previous studies, it was shown that the α_2 -adrenoreceptors (ARs) actually consist of three subtypes: α_2A , α_2B , and α_2C . Through the search to find out which subtype was responsible for the vasoconstriction, α_2A -ARs did not seem to play any role, while some reports showed possible use of α_2B -AR antagonists as a treatment option for vasospasms in RP, but no strong experimental or clinical data was found to support the role of α_2B -AR antagonists in the treatment of Raynaud's Disease.

Since hormones, particularly oestrogen, intensify the vasoconstriction induced by alpha adrenoreceptors, they are thought to be linked to an increased incidence of vascular diseases (*Fraenkel and Liana, 2002*). Premenopausal females have a high prevalence of RP, which can be linked to the fact that oestrogen activates the α_2C -adrenoreceptor gene promoter, increasing the expression of α_2C -adrenoreceptors.

Bailey et al., 2004 has said that Reactive oxygen species (ROS) produced by the mitochondria cause cold-induced vasoconstriction. This organelle functions as a thermos-sensor in the smooth muscles of the blood vessels, detecting changes in temperature. Low temperatures stimulate it, which results in the generation of reactive oxygen species (ROS), a redox signal, and stimulation of the Rho/Rho-kinase pathway. This leads to the translocation and activation of α_2C -adrenoreceptors, which in turn promotes vasoconstriction.

Wigley, 2002 discusses the physiological processes involved, including irregular vasoconstriction, endothelial failure, and the sympathetic nervous system's function. They also discuss the different elements that can cause Raynaud's disease, such as extreme cold, stress, etc.

Hinze et al., 2018 has noted that, in the pharmacologic treatment of RP, phosphodiesterase 5 inhibitors have also been demonstrated to be helpful in symptom reduction, even if calcium channel blockers are still the main choice. Although when digital ischemia is present, intravenous prostanooids (They are active lipid mediators that regulate inflammatory response) are the usual course of treatment. Patients with scleroderma who take bosentan (It is an endothelin receptor antagonist derived from sulfonamide) have demonstrated benefits in preventing further ulcers. A clinical investigation involving scleroderma patients found that botulinum toxin therapy was ineffective; further controlled research in different patient subgroups is required.

Amberg et al., 2013 has explained the dynamics of calcium in vascular smooth muscles. It is said that the cells of the muscles usually contract when the level of calcium ions increases. This contraction occurs when the calcium forms a complex with the calcium binding protein called calmodulin, which then in turn increases the myosin light-chain kinase (MLCK) activity. When the vascular smooth muscle is either contracted or dilated, it causes a change in the vascular diameter. These changes in diameter directly influences the amount of blood flowing through the vessels, and thereby the tissue the vessels are present in. Therefore, any event that influences calcium is very important in the regulation of vascular function.

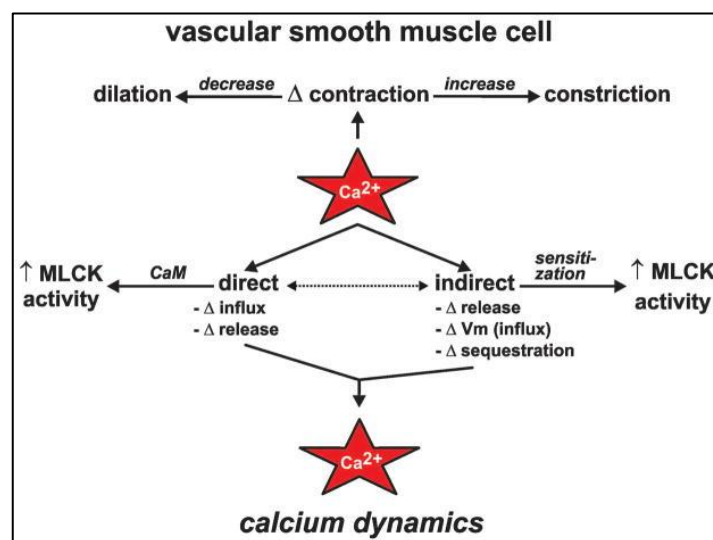


Fig 2.1 Ca²⁺ dynamics in vascular smooth muscle (*Amberg et al., 2013*)

Ennis et al., 2016, conducted a study with seven trials that were randomised with 269 participants. For the study, they had used 2 commercially available calcium blockers, Nifedipine and Nicardipine. The 6 trials were compared with placebos while one trial was with dazoxiben as well as placebo. Out of the 7 trials, 4 were using nifedipine and the rest were using nicardipine.

Their results show that oral calcium channel blockers (CCBs) were useful in reducing the frequency of attacks, but the difference between the duration of attacks was not very significant when comparing the results of nicardipine and placebo.

In total, the risk of bias within these trials were either low or not clear, but for number of attacks, the quality of the evidence provided was moderate and for duration of attacks, the quality of evidence given was very low.

Striessnig et al., 2014, says that, one of the three main classes of voltage-gated calcium channels (Cav1-3) is represented by L-type calcium channels (Cav1). They were shown to be the target of calcium channel blockers (CCBs, also known as calcium antagonists).

The pore of L-type calcium channels (LTCCs) is formed by four of the ten known $\alpha 1$ subunits (Cav1.1–Cav1.4), which have high-affinity drug-binding sites for dihydropyridines and other chemical families of organic CCBs.

Cav1.1 channels are only present in skeletal muscle, where they cause the sarcoplasmic reticulum's (SR) ryanodine receptors (RYRs) to release Ca^{2+} when depolarization occurs. Cav1.4 channel expression is likewise highly confined, mostly manifesting in the retina. Conversely, Cav1.2 and Cav1.3 are found in the same cells and have a substantially overlapping pattern of expression across a wide range of tissues. Their distinct gating characteristics and protein interactions, however, enable them to perform various physiological roles. The most important distinction is the approximately 9–15 mV greater negative activation range of Cav1.3 channels. They contribute to pacemaking and plateau potential stabilisation by opening at threshold potentials in neurons and sinoatrial node (SAN) cells. They also vary in terms of how C-terminal alternative splicing modifies them.

When membrane depolarizations occur, VGCCs open, letting Ca^{2+} ions enter cells. All VGCC types have voltage-sensitive pores that are generated by $\alpha 1$ subunits. The majority of the gating and pharmacological characteristics are dictated by $\alpha 1$ yet the channel function is fine-tuned and supported by auxiliary β and $\alpha 2\delta$ subunits that help the channel target the plasma membrane.

When the voltage sensor depolarizes, the positive charges of the S4 helices appear to flow outward through a scaffold formed by the S1–S3 helices. The cytoplasmic terminals of the S5 and S6 helices receive their movement through the S4–S5 linkers. As a result, the activation gate that the S6 helices on the channel's inner side formed opens.

All LTCC $\alpha 1$ subunits exhibit sensitivity to the primary types of CCBs, namely benzothiazepines, phenylalkylamines, and dihydropyridines (DHPs), however they vary in terms of tissue expression and gating properties.

Elliott et al., 2011 has noted that Calcium channel blockers, which dilate arteries by lowering calcium flow into cells, are approved to treat angina or cardiac dysrhythmias and effectively lower blood pressure, especially when used in combination with other medications.

Some other notable observations are:

- Calcium channel blockers lower blood pressure in all patient groups, irrespective of age, sex, race/ethnicity, or amount of sodium consumed in the diet.
- Non-dihydropyridine calcium channel blockers are more inotropic (The ability of a drug or any compound to change the force or energy of the contraction of muscles) and negatively chronotropic (The ability to decrease heart rate) than the dihydropyridine subclass, which is significant for patients who require β -blockers or have cardiac dysrhythmias.
- With the exception of heart failure, which is better treated with a diuretic, extensive experience in comparative randomised trials suggests that an early calcium antagonist can avoid all main forms of cardiovascular disease.

- Although members of the non-dihydropyridine subclass can lower albuminuria, initial dihydropyridine calcium channel blockers have not been able to slow down the progression of renal disease as much as renin-angiotensin system inhibitors.

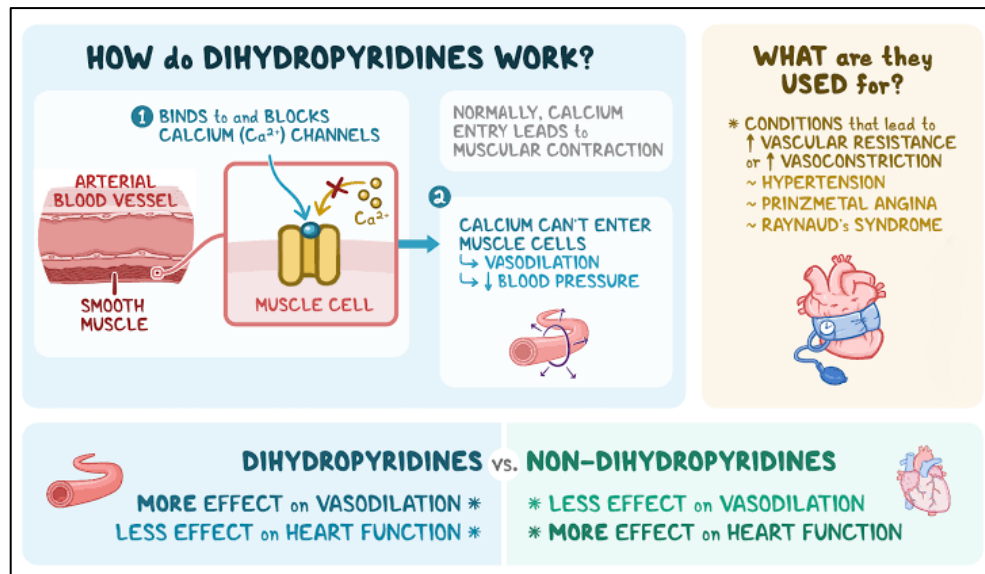


Fig 2.2 Dihydropyridines (Hernández et al., 2020)

CHAPTER 3

AIM AND OBJECTIVE

3.1 AIM

The aim of this project is to identify possible drug candidates for the treatment of Raynaud's disease by utilising computational methods. Through the use of computational techniques including ADME studies, toxicity prediction, and molecular docking, the research seeks to identify molecules that may be useful in targeting the underlying mechanisms of Raynaud's disease by sorting through large libraries of chemical compounds.

3.2 OBJECTIVES

This project seeks to address several key objectives. They are:

- First and foremost, the goal is to identify particular molecular targets or pathways that are connected to the pathophysiology of Raynaud's disease and understand how they contribute to the irregular vascular response that is seen in those who are suffering from it. By using experimental validation and computational studies, we hope to validate these targets and prioritise them for subsequent drug discovery efforts.
- To find lead compounds with promising therapeutic benefits against Raynaud's disease, a virtual screening of chemical libraries will be carried out. These compounds will then undergo iterative filtration using computational methods.
- In order shed light on the underlying mechanisms of action and possible therapeutic outcomes, the research intends to predict and analyse the interactions between potential drug candidates and their molecular targets.
- This project also attempts to evaluate the risk and predict the toxicity profiles of such compounds.

CHAPTER 4

METHODOLOGY

4.1 IDENTIFICATION OF THE TARGET AND RETRIEVAL OF INFORMATION

Raynaud's disease is a disease in which the blood flow to the fingers and toes decreases due to the vasoconstriction of the blood vessels.

In order to relieve the symptoms of the disease, calcium channel blockers (CCBs) were used. CCBs block the calcium channels, preventing calcium ions from entering the channel. This helps to increase the blood flow thereby relieving the symptoms of Raynaud's disease.

An example of a calcium channel blocker used is Nifedipine. It is from the dihydropyridine class and they are the first line of treatment after the non-drug therapy. Nifedipine stops the influx of extracellular calcium ions from passing the cell membrane of myocardial and vascular smooth muscles without changing the concentration of calcium ions in the serum. This stops the muscles from contracting and causes dilation of the arteries which decreases the peripheral resistance and increases heart rate.

Thus, the target for the drug was determined to be the calcium channel. More specifically, the L-type voltage gated calcium channels.

After the target was identified as the calcium channel, it was studied using the UNIPROT database (<https://www.uniprot.org/>)

UNIPROT: UniProt is a comprehensive and freely accessible database of protein sequences and functional information. It provides an extensive amount of information about proteins derived from numerous organisms, making it a crucial resource for academics and researchers across the globe. To give precise annotations and descriptions of proteins, UniProt combines data from multiple sources, such as computer predictions, experimental data, and human curation. Protein sequences, structures, functions, pathways, and interactions can all be explored by users, supporting a variety of study fields including drug discovery, proteomics, and genomics.

4.2 SELECTION OF PLANTS

Several articles and papers based on plants with calcium channel blocking properties were studied and analysed. Based on these past studies, the plants were chosen. These plants were assumed to have calcium channel blocking properties but were not worked on extensively using in-silico methods. In their study for hypertension, *Joshi et al., 2021*, described the application of a variety of medicinal plants that include phytoconstituents that inhibit calcium channels to treat hypertension.

4.3 RETRIEVAL OF PLANT COMPOUNDS

All the compounds from each of the plants were collected from the Indian Medicinal Plants, Phytochemistry and Therapeutics 2.0 (IMPPAT 2.0) database (<https://cb.imsc.res.in/imppat/>) and listed in an excel sheet along with their SMILES notations.

The **IMPPAT** database 2.0 is an extensive repository of information related to medicinal plants found in India. It serves as a valuable resource for researchers, healthcare professionals, and practitioners in traditional medicine.

The database contains comprehensive data on a wide range of topics, including the botanical characteristics of medicinal plants, their traditional uses in Indian medicine systems like Ayurveda, Siddha, and Unani, as well as modern scientific research on their phytochemistry and therapeutic properties.

4.4 PHARMACOKINETIC PROPERTIES ANALYSIS

To analyse the pharmacokinetic properties of the compounds, their SMILES notations were submitted to SWISSADME (<http://www.swissadme.ch/index.php>)

SWISSADME is a tool used in drug discovery and development to predict various pharmacokinetic properties of a molecule. These properties include absorption, distribution within the body, metabolism, and excretion, which are crucial factors in determining a drug's efficacy and safety. SWISSADME utilizes computational models and algorithms to estimate these properties based on the molecular

structure of a compound. It's valuable for screening and prioritizing compounds in the early stages of drug development, helping researchers make informed decisions about which molecules have the most potential for further investigation.

The compounds were then screened and filtered manually using the Lipinski's rules. Christopher A. Lipinski, a medicinal chemist, developed a series of criteria known as Lipinski's rules, or the Rule of Five, in 1997 to evaluate whether small molecule compounds are drug-like or not. These rules suggest that the compounds that follow them are more likely to have good pharmacokinetic profiles.

The rules are as follows:

- **Molecular Weight (MW):** The compound should have a molecular weight less than 500 daltons. Higher molecular weight compounds might find it more difficult to pass through biological membranes, which would in turn reduce their bioavailability.
- **Lipophilicity (LogP):** The logP value, or the octanol-water partition coefficient has to be lower than 5. Overly lipophilic compounds may not dissolve well in water and may bind to proteins in an unspecific manner.
- **Hydrogen Bond Donors (HBD):** The number of hydrogen bond donors should be less than or equal to 5. Too many hydrogen bond donors in a compound can make it difficult to pass through cell membranes.
- **Hydrogen Bond Acceptors (HBA):** The number of hydrogen bond acceptors should be less than or equal to 10. Compounds with too many hydrogen bond acceptors might have poor oral absorption because of their increased polarity.

4.5 TOXICITY ANALYSIS

Toxicity analysis was performed using the OSIRIS Property explorer tool which stands for “Organized Structure of Interfaces from Reduced Information Sets Property Explorer”. It was downloaded from the openmolecules.org website (<https://openmolecules.org/propertyexplorer/>).

OSIRIS Property Explorer is a software tool frequently used in toxicity studies within the field of chemistry and pharmacology. It makes it easier to predict a range of chemical compound features, including those related to toxicity. Using OSIRIS, scientists and toxicologists can evaluate a compound's possible toxicity based on its physicochemical and structural qualities. Molecular structures and property data are analysed by OSIRIS, which helps guide decision-making in chemical safety evaluations and drug development by pointing out possible risks.

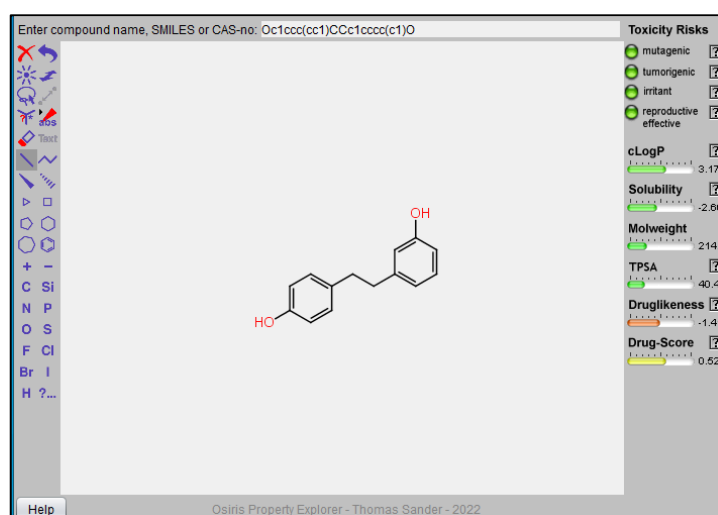


Fig 4.1 OSIRIS Property Explorer

4.6 DOCKING

The currently filtered compounds after pharmacokinetic studies and toxicity prediction were docked along with the target protein, the L-type calcium channel (LTCC). This was done using the BIOVIA Discovery Studio software suite (<https://www.3ds.com/products/biovia/discovery-studio>).

BIOVIA Discovery Studio is a comprehensive software suite designed for modelling and simulation in the life sciences and materials research fields. It was created by Dassault Systèmes and offers a large set of molecular modelling, simulation, and visualisation tools to support materials science, bioinformatics, and drug development research.

The 3D structures of the ligand compounds were downloaded from the PubChem repository.

The National Centre for Biotechnology Information (NCBI), a branch of the National Institutes of Health (NIH) in the United States, is responsible for maintaining **PubChem**, a comprehensive chemical database. It is a publicly available resource for anyone looking for chemical knowledge, including researchers, students, and the general public. PubChem provides details on the chemical structures, biological targets, and biological activity of small compounds.

Apart from the ligand compounds, the commercially available CCB, Nifedipine (a dihydropyridine drug) was also docked with the target protein, as a reference point. The structure of this compound was also obtained from PubChem.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 IDENTIFICATION OF THE TARGET AND RETRIEVAL OF INFORMATION

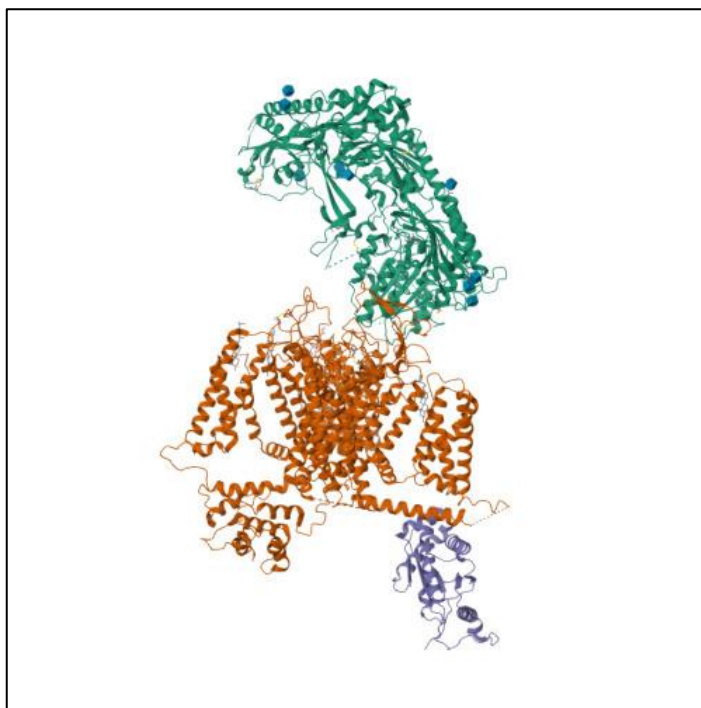


Fig 5.1: Structure of the human L-type voltage-gated calcium channel Cav1.2 obtained from PDB with the accession ID 8FD7

From PDB, it was recognized that the method used to obtain the structure was Electron Microscopy, and through UniProt, it was identified that the drugs were binding to a binding site in the alpha-1C subunit of the calcium channel called the Dihydropyridine binding site

Target name: Voltage-dependent L-type calcium channel subunit alpha-1C

Gene name: CACNA1C

Organism name: Homo sapiens (Human)

Accession ID: Q13936

Amino acids: 2221

In electrically excitable mammalian cells, voltage-gated calcium channels come in at least five distinct forms. L-type calcium channels (Cav1) is one of the major classes, and the only one in the family of calcium channels to have high-affinity binding regions contained within their alpha 1-subunits for various chemical groups of drugs (Ca²⁺ channel antagonists; examples like isradipine, verapamil and diltiazem). (Striessnig *et al.*, 1998).

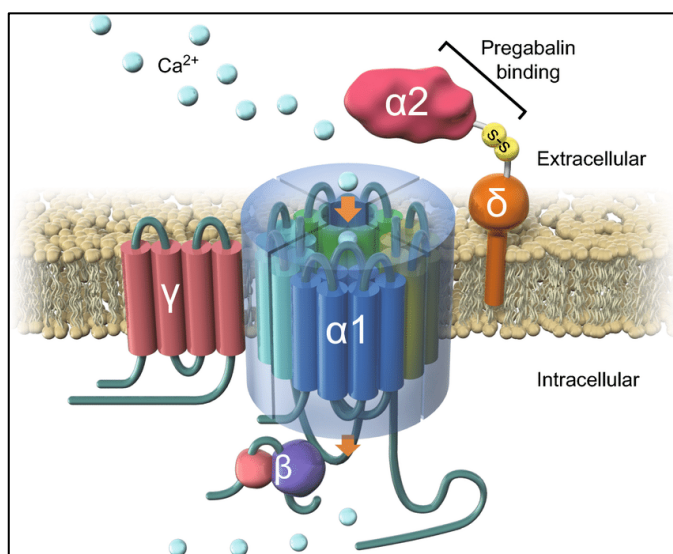


Fig 5.2: Structure of L-type calcium channel (Ninomiya, 2020)

Calcium channels of the Cav1 L-type are hetero-oligomeric proteins made up of alpha1 ($\alpha1$ -) main subunit with alpha2delta ($\alpha2\delta$ -), beta ($\beta1$ –4-), and gamma (γ -) auxiliary subunits.

The biggest subunit, alpha1, is responsible for forming the gating mechanism, voltage sensor, ion-conducting pore, and binding sites for calcium channel blockers. Its molecular weight ranges from 190 to 250 kDa.

The four homologous repeats (I–IV) that make up the alpha1 subunit each have six transmembrane segments (S1–S6). The loop connecting transmembrane segments S5 to S6 and the S6 segment in each domain make up the pore structure.

The loops also contain the selectivity filter of the channel. The S4 segments serve as voltage sensor. The auxiliary beta subunits are intracellularly located proteins ranging from 50 to 72 kDa (*Priori et al., 2018*).

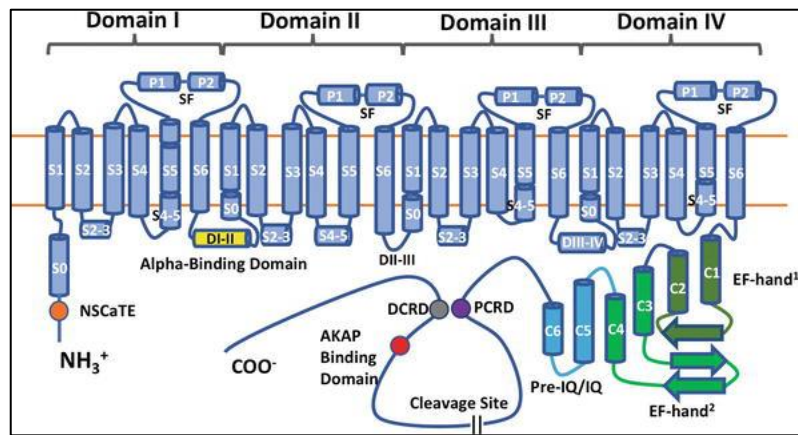


Fig 5.3: Structure of the $\alpha 1$ -subunit

CACNA1c encodes the $\alpha 1$ -subunit, CaV1.2. The gene spans >500 kb, and it undergoes extensive alternative splicing with at least 42 known splice variants. Some of these splice variants are expressed in the heart and have been associated with a diversity of biophysical properties, including inactivation kinetic, drug sensitivity, and phosphorylation sensitivity.

The accessory subunits modulate gating, trafficking, and responses to neurohormonal stimuli. (*Moosmang and Hofmann, 2009*)

At least five different types of voltage-gated Ca^{2+} channels exist in electrically excitable mammalian cells. Only one type, the family of L-type Ca^{2+} channels (L channels), contains high-affinity binding domains within their alpha 1-subunits for different chemical classes of drugs (Ca^{2+} channel antagonists; examples like isradipine, verapamil and diltiazem). Their stereoselective, high-affinity binding induces block of channel-mediated Ca^{2+} inward currents in heart and smooth muscle, resulting in antihypertensive, cardiodepressive and antiarrhythmic effects (*Striessnig et al., 1998*).

5.2 SELECTION OF PLANTS

The plants were:

1. *Apium graveolens* (Celery)



Fig 5.4 *Apium graveolens* (Celery)

Celery (*Apium graveolens*) is a low-calorie vegetable that belongs to the Apiaceae family, which also includes carrots, parsley, and fennel. It's known for its long, fibrous stalks and distinctive flavour.

This plant was chosen due to the available evidence of its calcium channel blocking and vasodilatory characteristics (*Alobaidi et al., 2024*).

2. *Ammi visnaga* (Khella Baldi or toothpick weed)



Fig 5.5 *Ammi visnaga* (Toothpick weed)

Some of the other names for *Ammi visnaga* are *Ammi daucoides* and *Daucus visnaga*. It is also of the family Apiaceae/Umbelliferae (Bhagavathula et al., 2014). These plants consist of large groups of small white flowers as seen in **Fig 5.5**. It is an herbaceous, annual or occasionally biennial plant that can reach heights of up to 1.3 metres for its green aerial portion. It is native to Europe, Asia, and North Africa's Mediterranean region. In addition, it is also found in Argentina, Mexico, Chile, the Atlantic islands, and North America. In Asia, it is found in Iraq, Iran, and some other nations in the west and south.

3. *Lavandula stoechas* (Spanish lavender)



Fig 5.6 *Lavandula stoechas* (Spanish lavender)

Lavandula stoechas, of the family Lamiaceae, is a woody evergreen subshrub that has flowers on spikes that sprout in the spring and early summer on leafless stems. While it can withstand a variety of environments, it is typically found in dry, alkaline, low-organic matter soil that is in full sun on hot, sunny days. It is dispersed throughout three continents, Asia, Africa and Europe, and grows in the Mediterranean basin, covering Morocco, Spain, Greece, France, Italy, and some other places. It can also be found in Iran and Saudi Arabia. This plant contains analgesic, sedative, and antibacterial qualities. It also helps with wounds and UTIs. (Zubi, 2020).

4. *Marrubium vulgare* (White Horehound)



Fig 5.7 *Marrubium vulgare* (White Horehound)

White horehound, or *Marrubium vulgare*, is a perennial shrub of the Lamiaceae family. This plant is widely distributed throughout California's grasslands, scrub, and riparian zones. For the most part, it has no effect on native species. It originated in Europe, Central Asia, and North Africa, but is regarded as an invasive species in some areas of Australia and New Zealand. However, it has become naturalised over most of North and South America. Herbal medicines for coughs and mild pulmonary disorders occasionally use horehound infusions or extracts in the form of syrups, teas, or lozenges. The leaves and flowering tops are used as flavouring for drinks and candies.

5. *Valeriana jatamansi* (Indian Valerian)



Fig 5.8 *Valeriana jatamansi* (Indian Valerian)

This highly valued medicinal herb, *Valeriana jatamansi*, of the family Caprifoliaceae, is native to several Asian countries. It is widely acknowledged that the species is used in both conventional and alternative medicine. For example, *V. jatamansi* is highly significant due to its antihelmethic and insect-repelling qualities. Similar to this, reports of the species' sedative, neurotoxic, cytotoxic, antidepressant, antioxidant, and antibacterial properties in a variety of illnesses in the traditional medical system, especially in Asia, have been made (*Jugran et al., 2019*).

5.3 RETRIEVAL OF PLANT COMPOUNDS

Table 5.1 Number of phytochemicals from each plant:

Sl.No.	Name of plants		No. of phytochemicals
	Scientific name	Common name	
1	<i>Apium graveolens</i>	Celery	170
2	<i>Ammi visnaga</i>	Khella Baldi or toothpick weed	94
3	<i>Lavandula stoechas</i>	Spanish lavender	201
4	<i>Marrubium vulgare</i>	White Horehound	130
5	<i>Valeriana jatamansi</i>	Indian Valerian	201
Total			796

Total unique compounds: 567 (After removing duplicates between each plant)

5.4 PHARMACOKINETIC PROPERTIES ANALYSIS

Information about the Molecular weight (MW), Rotatable bonds (Rot bonds), H-bond acceptors (HBA), H-bond donors (HBD), Topological Polar Surface Area (TPSA), and the LogP value for all the compounds were analysed using SWISSADME and there was a total of 122 compounds filtered out.

These 122 compounds are predicted to have good pharmacokinetic properties that show how these compounds might be absorbed, distributed, metabolised, and eliminated by the body. and may also show positive levels of oral bioavailability.

5.5 TOXICITY ANALYSIS

After the toxicity analysis, a total of 62 compounds were further filtered out and were selected out of the previous 122 compounds. The drug likeness score and the drug score of the selected compounds were also noted to be within acceptable ranges.

This shows that these compounds have passed the assessment of the potential harmful effects of substances on living organisms, and a number of toxicity-related issues including irritation, carcinogenicity, acute and chronic toxicity, and reproductive toxicity, have been eliminated.

Table 5.2: Toxicity analysis results

Compound	MW	Rot. bonds	HBA	HBD	TPSA	Con. Log P	Drug like ness	Drug score
6-acetyl-2,2-dimethyl-2H-1-benzopyran	202.25	1	2	0	26.3	2.77	-1.01	0.54
Norseychellanon	206.32	0	1	0	17.07	3.3	-1.79	0.48
Sedanonic acid	210.27	5	3	1	54.37	2.17	-7.01	0.46
Lunularin	214.26	3	2	2	40.46	2.89	-1.41	0.52
epi-Cubebol	220.35	1	1	1	20.23	3.22	1.39	0.77
Eudesma-4(15),7-dien-1-b-ol	220.35	1	1	1	20.23	3.37	-4.53	0.42
Jatamol A	220.35	1	1	1	20.23	3.57	19.82	0.4
(4E,7E)-1,5,9,9-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-4,7-diene	220.35	0	1	0	12.53	3.72	-3.15	0.37
Humulene epoxide II	220.35	0	1	0	12.53	3.72	-3.15	0.37
Isofraxidin	222.19	2	5	1	68.9	1.48	-3.06	0.5
Bulnesol	222.37	1	1	1	20.23	3.41	-2.13	0.45
alpha-Muurolol	222.37	1	1	1	20.23	3.42	-4.24	0.42
Guaiol	222.37	1	1	1	20.23	3.42	-2.02	0.45
(-)-alpha-Cadinol	222.37	1	1	1	20.23	3.43	-4.24	0.42
tau-Cadinol	222.37	1	1	1	20.23	3.43	-4.24	0.42
Cedrelanol	222.37	1	1	1	20.23	3.44	-4.24	0.42
epi-Cubenol	222.37	1	1	1	20.23	3.5	-4.36	0.42
6-Valerene-11-ol	222.37	1	1	1	20.23	3.5	-1.55	0.48

alpha-Eudesmol	222.37	1	1	1	20.23	3.51	-2.86	0.43
Nardol	222.37	1	1	1	20.23	3.51	-4.31	0.41
Patchouli alcohol	222.37	0	1	1	20.23	3.56	-1.38	0.5
Drimenol	222.37	1	1	1	20.23	3.58	-4.53	0.42
Carotol	222.37	1	1	1	20.23	3.59	-4.69	0.41
beta-Eudesmol	222.37	1	1	1	20.23	3.6	-7	0.4
Pogostol	222.37	1	1	1	20.23	3.63	-	0.39
Juniperol	222.37	0	1	1	20.23	3.64	-4.29	0.42
(1S,5S,9R)-9-Isopropyl-1-methyl-6-methylenespiro[4.5]decan-1-ol	222.37	1	1	1	20.23	3.66	-6.29	0.4
(1R,4aR,8aR)-1,4a-dimethyl-7-propan-2-ylidene-3,4,5,6,8,8a-hexahydro-2H-naphthalen-1-ol	222.37	0	1	1	20.23	3.69	-1.15	0.49
Selin-11-en-4alpha-ol	222.37	1	1	1	20.23	3.76	-	0.39
Elemol	222.37	3	1	1	20.23	3.77	-	0.38
alpha-Elemol	222.37	3	1	1	20.23	3.77	-	0.38
Valeranone	222.37	1	1	0	17.07	3.89	-8.89	0.39
(-)-Valeranone	222.37	1	1	0	17.07	3.89	-8.89	0.39
Farnesol	222.37	7	1	1	20.23	4.46	-3.38	0.32
Eudesmol	224.38	1	1	1	20.23	3.79	-4.2	0.4
Visnagin	230.22	1	4	0	52.58	2.32	-6.84	0.41

(1R,5R)-4-(furan-2-yl)-6,8,9-trimethyl-3-oxabicyclo[3.3.1]non-6-ene	232.32	1	2	0	22.37	2.94	-0.02	0.65
Jatamansic acid	234.33	2	2	1	37.3	3.2	-1.96	0.49
Occidenol	236.35	1	2	1	29.46	2.92	-1.9	0.49
Proximadiol	240.38	1	2	2	40.46	2.94	-2.58	0.46
Oxacyclohexadecan-2-one	240.38	0	2	0	26.3	4.22	-	0.3
Khellol	246.22	2	5	1	72.81	1.53	-0.16	0.63
Isopimpinellin	246.22	2	5	0	61.81	2.16	-3.4	0.45
Nodakenetin	246.26	1	4	1	59.67	2.09	-2.53	0.48
Lunularic acid	258.27	4	4	3	77.76	2.52	-3.23	0.46
Celerin	260.29	3	4	1	59.67	2.92	-8.05	0.42
Apigravin	260.29	3	4	1	59.67	2.99	-3.21	0.44
Rutaretin	262.26	1	5	2	79.9	1.69	-2.83	0.48
Celereoin	262.26	1	5	2	79.9	1.7	-2.83	0.48
Vulgarin	264.32	0	4	1	63.6	1.64	2.8	0.9
Patchoulyl acetate	264.4	2	2	0	26.3	3.99	-2.01	0.42
Isoimperatorin	270.28	3	4	0	52.58	3.3	-3.7	0.38
2,3-Dihydro-4-hydroxy-2-(1-hydroxy-1-methylethyl)-7-methyl-5H-furo(3,2-g)(1)benzopyran-5-one	276.28	1	5	2	79.9	2	-6.41	0.43
alpha-Kessyl acetate	280.4	2	3	0	35.53	3.2	-2.23	0.45

6-Methylapigenin	284.26	1	5	3	90.9	2.46	0.98	0.75
Acacetin	284.26	2	5	2	79.9	2.52	-1.47	0.51
Jatamol B	320.47	5	3	1	46.53	4.04	21.78	0.32
Marrubiin	332.43	3	4	1	59.67	3.47	0.04	0.57
6-ethyl-3-[(Z)-naphthalen-2-ylmethylideneamino]thieno[2,3-d]pyrimidin-4-one	333.41	3	3	0	75.49	4.23	3.74	0.47
Marrubenol	336.47	4	4	3	73.83	2.94	-7.48	0.4
(1S,4aS,5R,6R,8R,8aR)-5-[2-(furan-3-yl)ethyl]-5,8-dihydroxy-1,4a,6-trimethyl-3,4,6,7,8,8a-hexahydro-2H-naphthalene-1-carboxylic acid	350.45	4	5	3	90.9	2.72	-1.57	0.47
Virolin	358.43	8	5	1	57.15	3.76	1.84	0.68

5.6 DOCKING

Biovia Discovery Studio (DS) was used for molecular docking investigations. The target structure, sourced from the RCSB Protein Data Bank (PDB) with the identifier 8FD7, underwent preparation using DS. This preparation involved the removal of hetero-molecules and water molecules, resulting in the retention of the K-chain, which contained the binding site relevant for calcium channel blockage.

Furthermore, ligand compounds were obtained from PubChem and assembled into a comprehensive library within DS. These efforts led to successful docking simulations, where the compiled library of ligands was docked against the prepared target protein structure.

Through this computational approach, insights into potential ligand-target interactions and binding affinities were obtained. This comprehensive analysis provides valuable information for understanding the molecular mechanisms underlying the interactions between ligands and the target protein, offering potential avenues for further research and therapeutic development in the field of drug discovery and design.

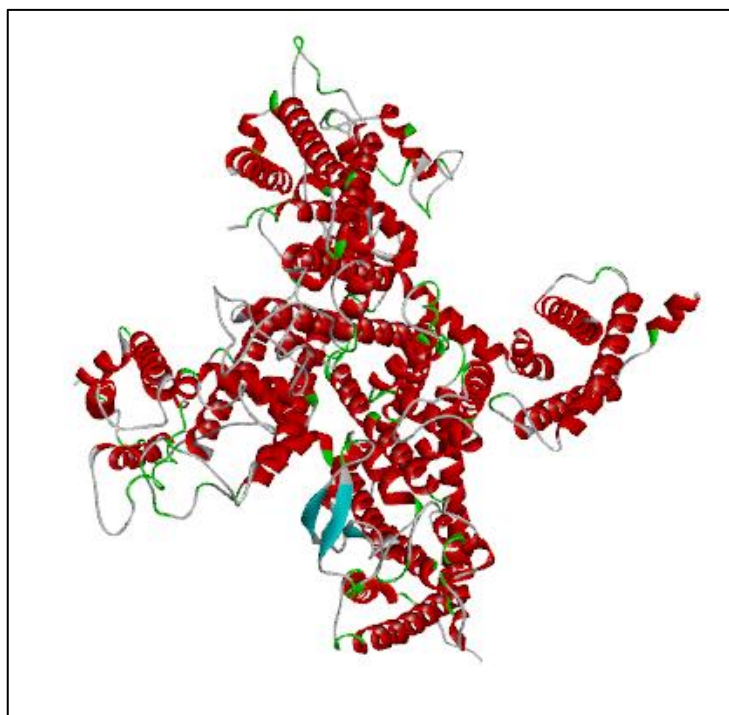


Fig 5.9 K chain of the alpha-1c subunit

The LibDock algorithm was used for this docking, and it predicts the ligand orientation and the binding affinity of the compounds to the binding sites of the target protein. The scoring function used by LibDock is based on a pharmacophore model, which illustrates the spatial arrangement of chemical elements needed for ligand binding to the target protein, including aromatic rings, hydrophobic areas, and donors and acceptors of hydrogen bonds. It samples the ligand's conformational space, assessing possible binding poses according to how well they complement the pharmacophore model and how well they can build advantageous interactions with the binding site of the protein.

After performing the docking procedure, 23 compounds were docked to the binding site of the target protein. Each of the different poses were visualised to determine the best pose.

Table 5.3 Compounds that were docked

CID	Molecule name	Absolute energy	Libdock score	No. of H-bonds	Residue of H-bonds	Unfavourable bonds
6716	Visnagin	103.806	72.1629	3	TYR583, LYS629, ARG626	0
91753440	alpha-Muurolol	13.0194	70.1535	1	TYR583	0
101286216	Nardol	29.0697	71.3364	1	TYR583	0
101618010	Jatamol A	12.7345	76.5714	1	TYR583	0
101635455	alpha-Kessyl acetate	30.832	72.032	1	TYR583	0
154496810	(1R,3aS,8aR)-1-methyl-7-propan-2-yl-1,2,3,3a,8,8a-hexahydroazulene-4-carboxylic acid	27.6392	75.3223	1	TYR583	0
26305	Nodakenetin	49.0032	70.3856	1	TYR583	0
68079	Isopimpinellin	113.397	84.6598	2	TYR583, ARG626	0
68081	Isoimperatorin	120.327	69.9211	2	TYR583, LYS629	0
73401	Marrubiin	53.9443	99.2649	2	TYR583, ARG626	0
90785	Bulnesol	35.805	67.2219	1	TYR583	0
91457	beta-Eudesmol	10.7806	72.979	2	TYR583, ASP592	0
92762	alpha-Eudesmol	13.6791	66.2065	1	TYR583	0
94253	Vulgarin	25.7635	85.5671	2	TYR583, ASP592	0
156431	Celerin	67.5281	69.8291	2	TYR583, ASP592	0
161413	Lunularic acid	35.3425	83.7337	2	TYR583, LYS629	0

177040	1-(2,2-Dimethylchromen-6-yl)ethanone	28.4161	65.0549	1	TYR583	0
181511	Lunularin	24.0784	83.5298	1	TYR583	0
227829	Guaiol	35.1847	71.409	1	TYR583	0
445070	Farnesol	33.6277	86.4049	1	TYR583	0
609798	Norseychellanon	23.98	68.4444	1	TYR583	0
5318734	(+)-Juniper Camphor	18.9342	70.9577	1	TYR583	0
5320651	Pogostol	23.0481	77.9623	1	TYR583	0

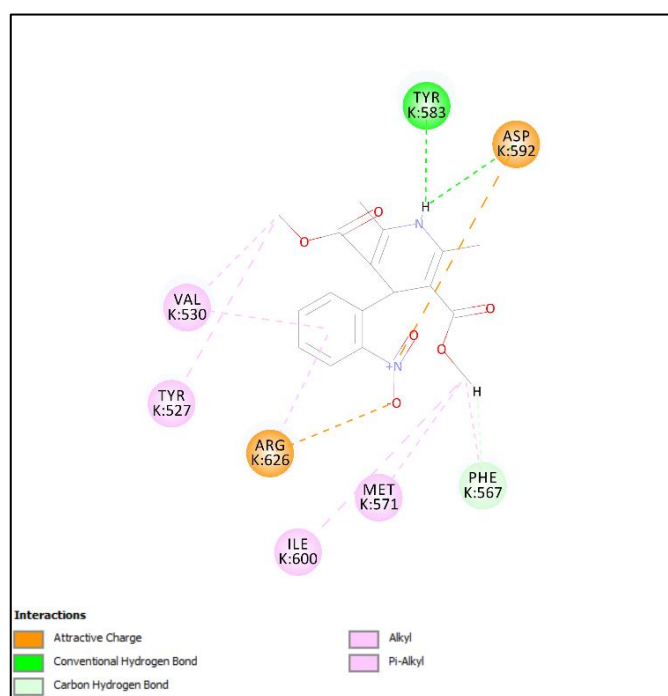
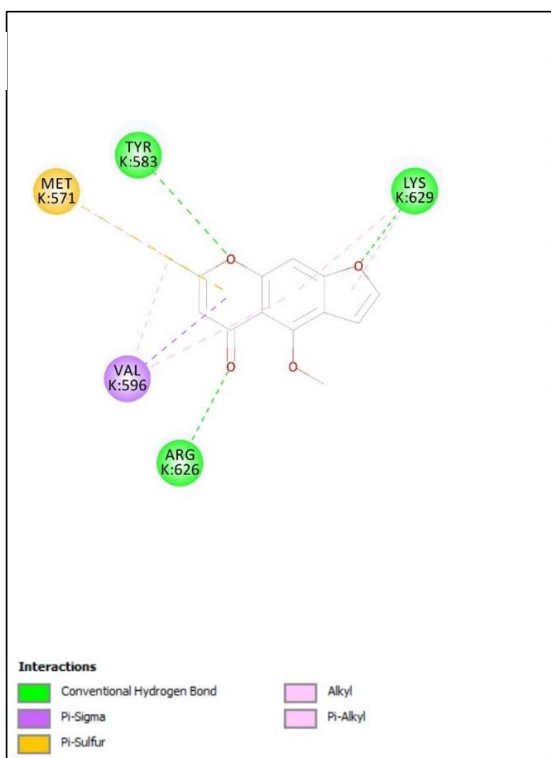
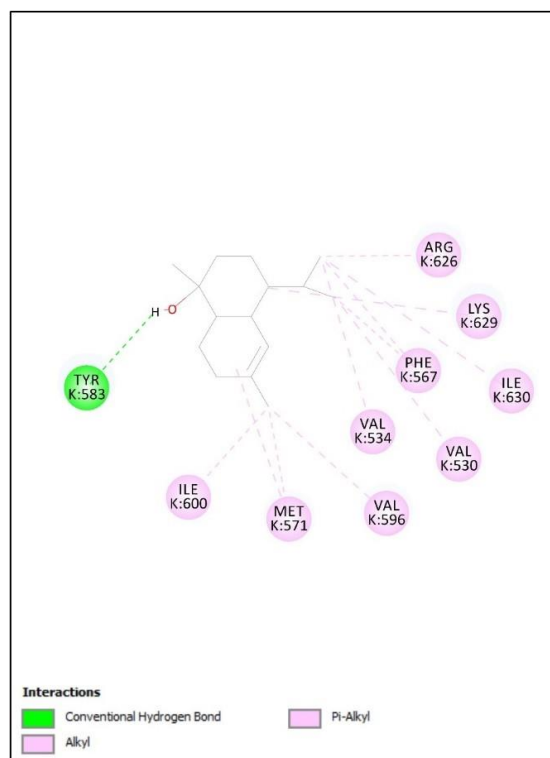


Fig 5.10 Nifedipine binding to the binding site

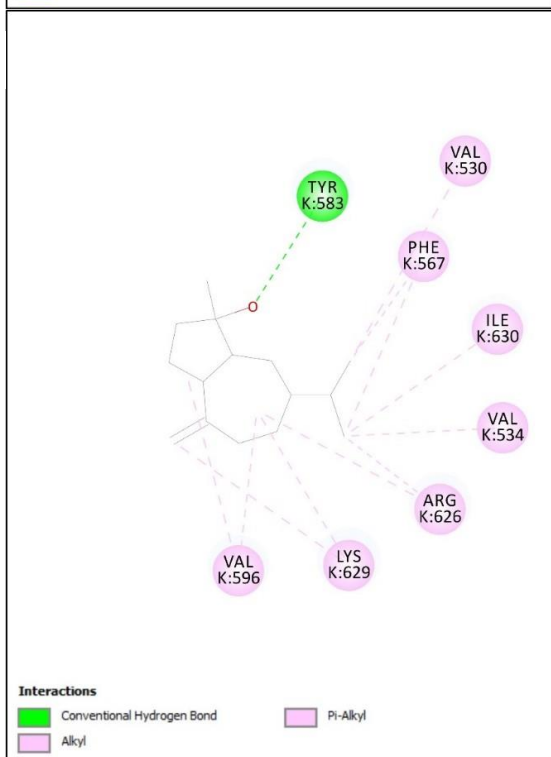
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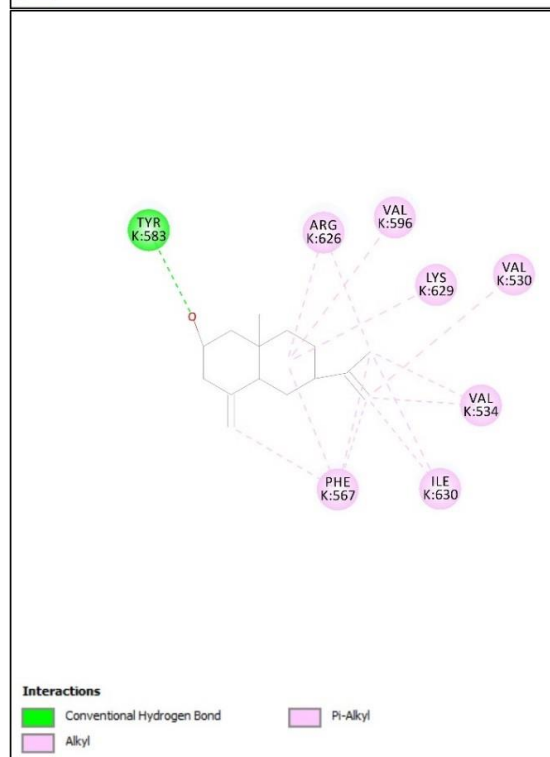
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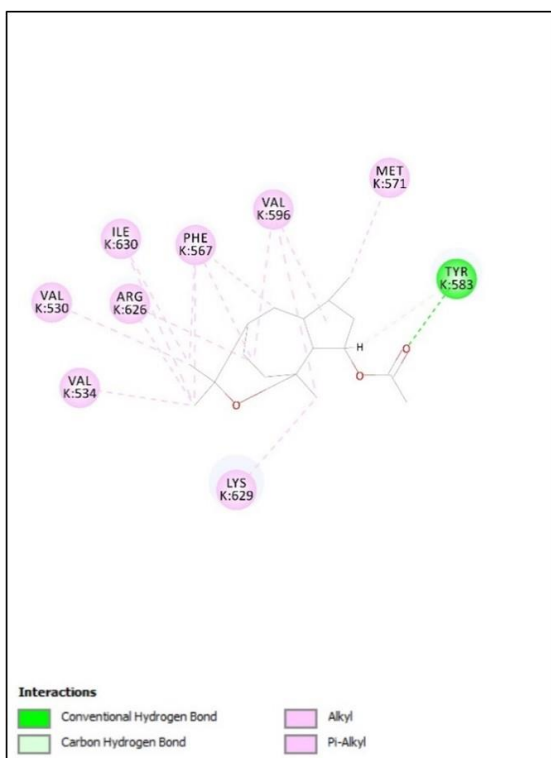
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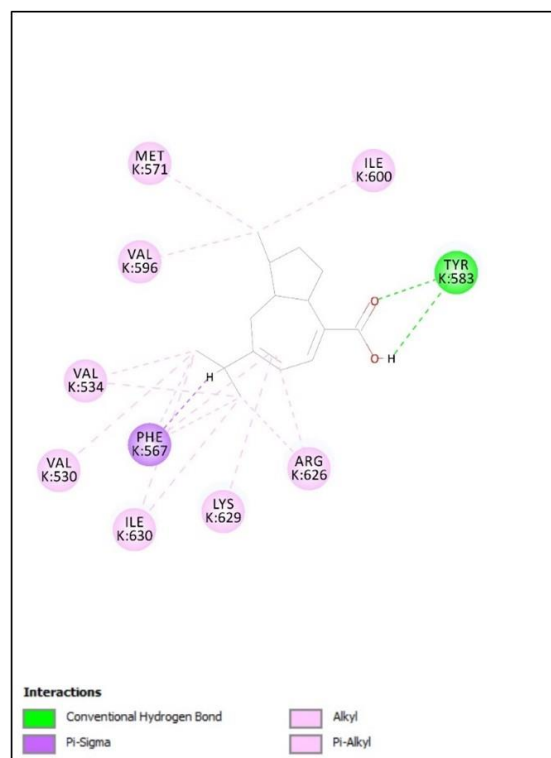
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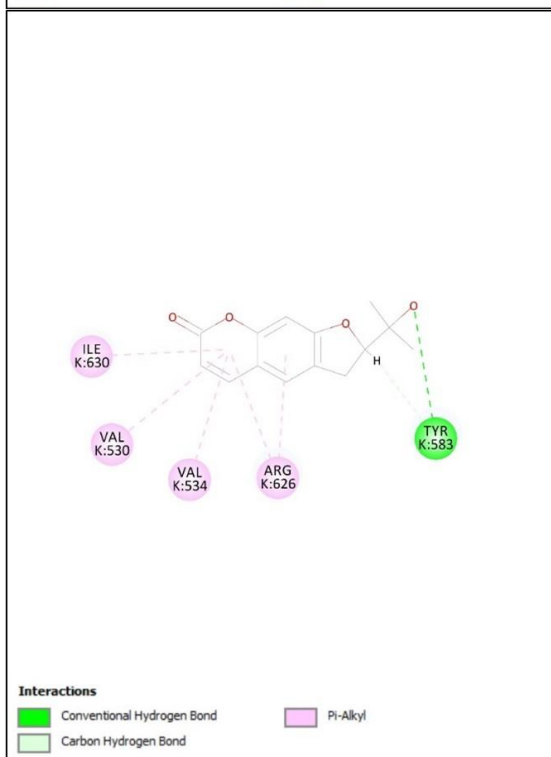
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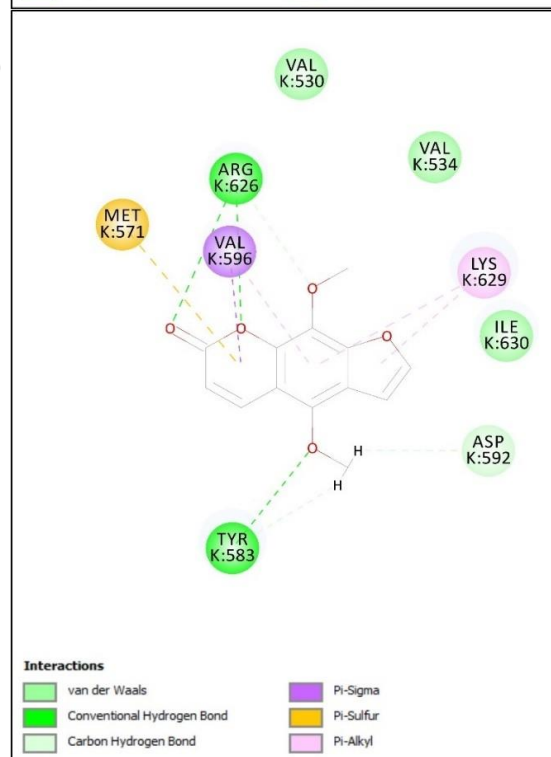
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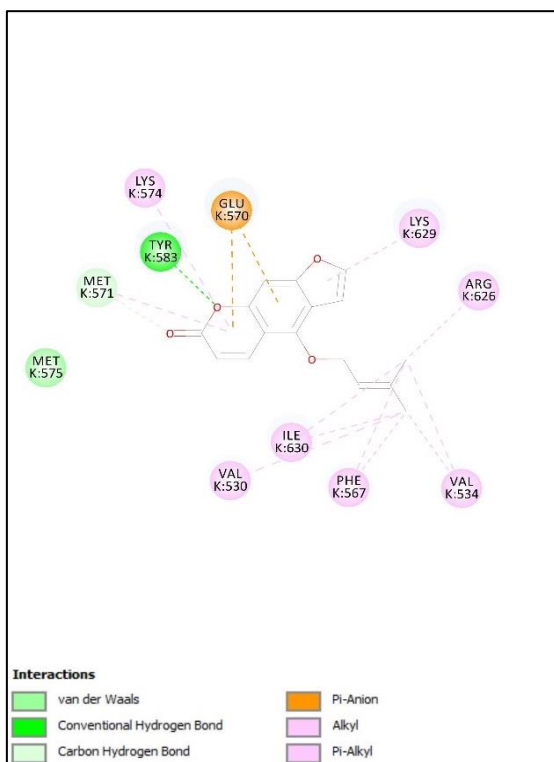
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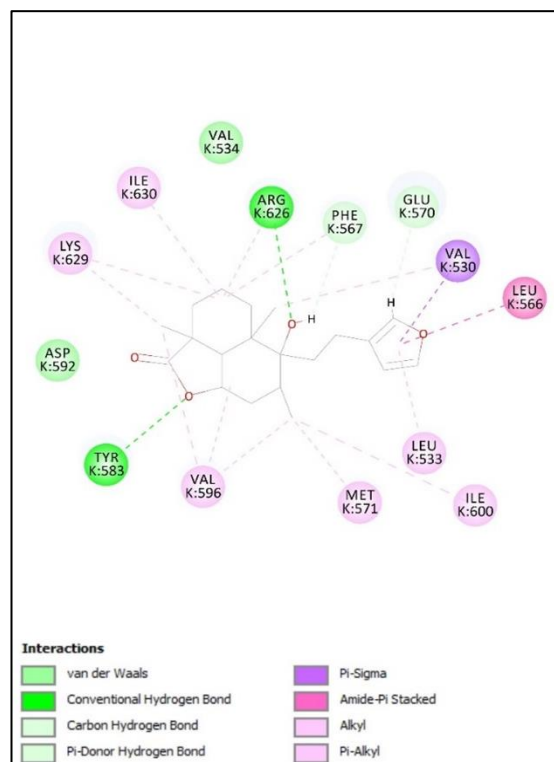
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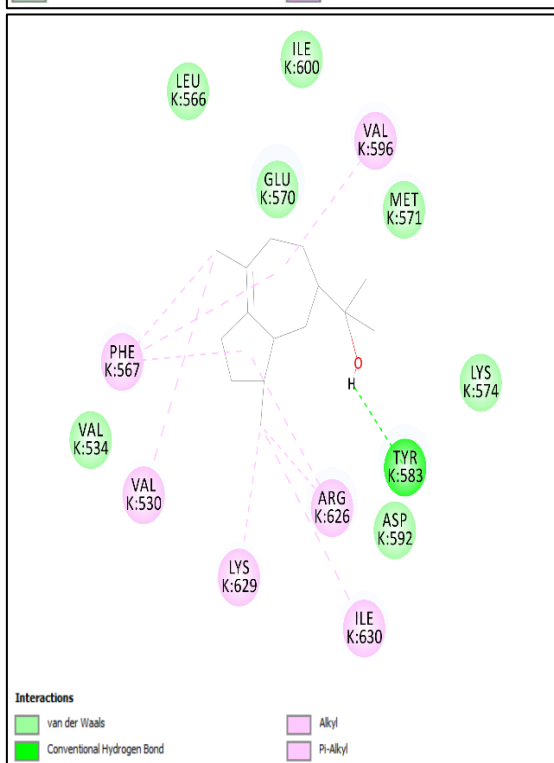
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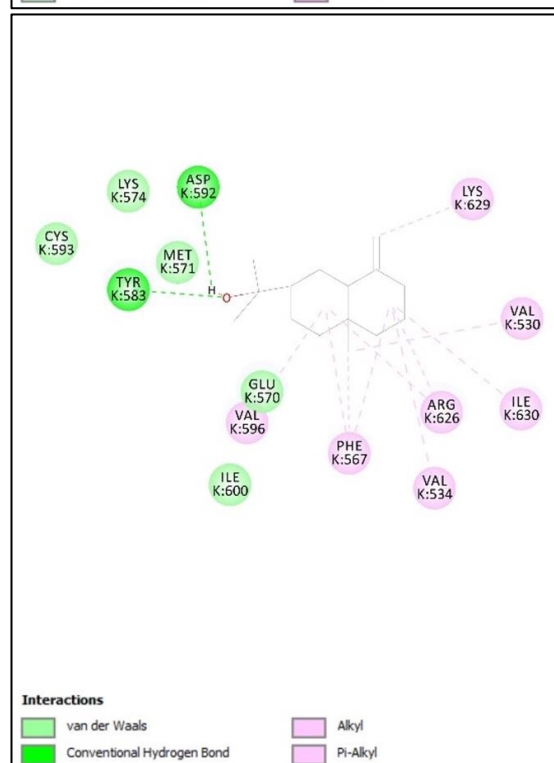
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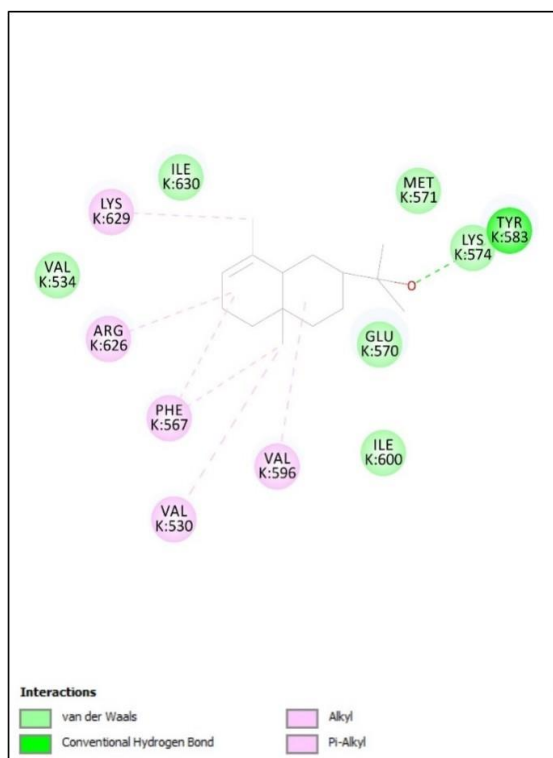
k)



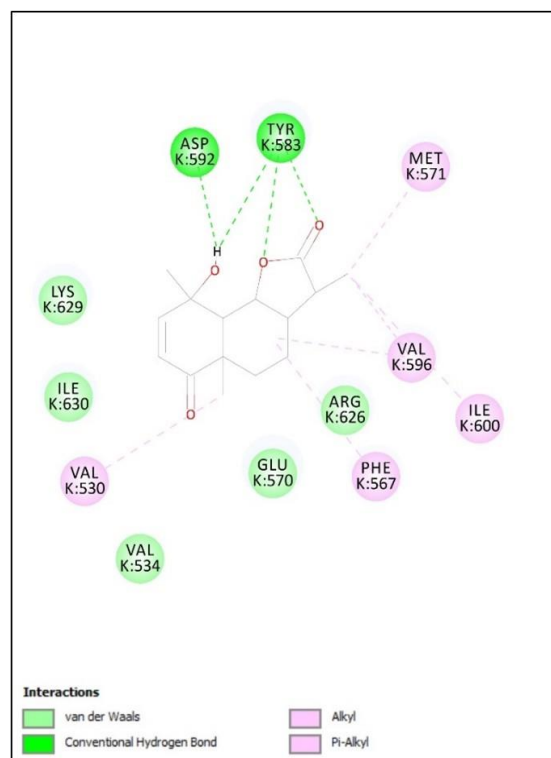
l)



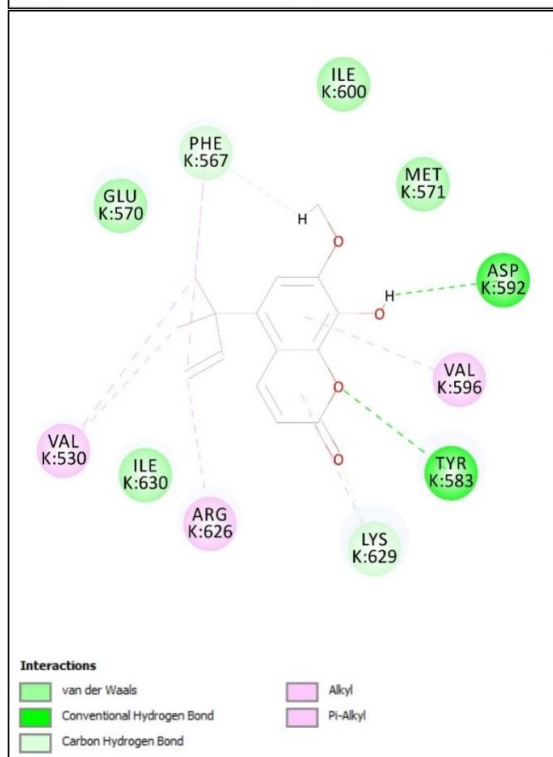
m)



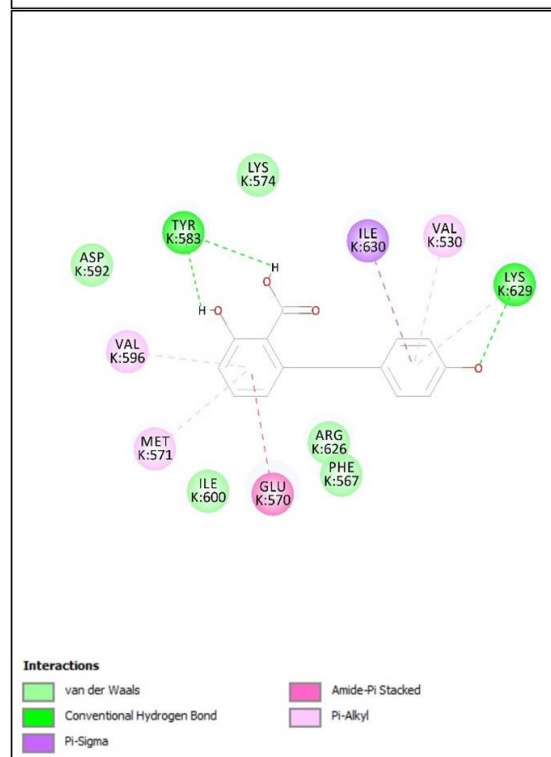
n)



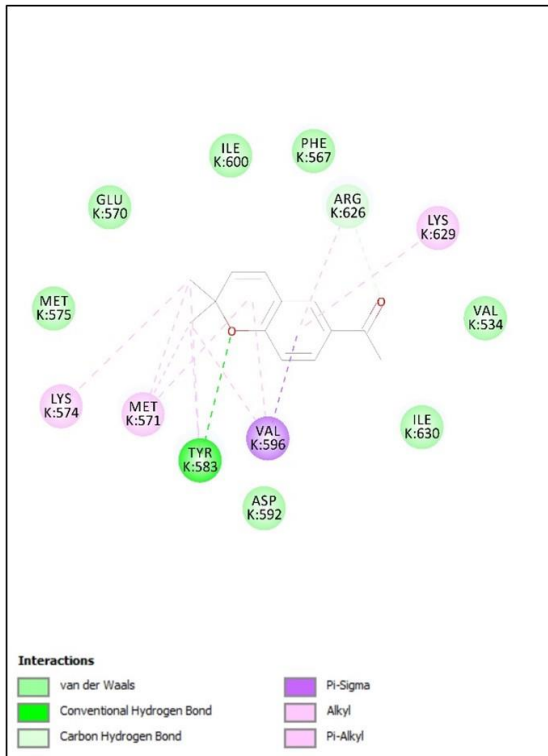
o)



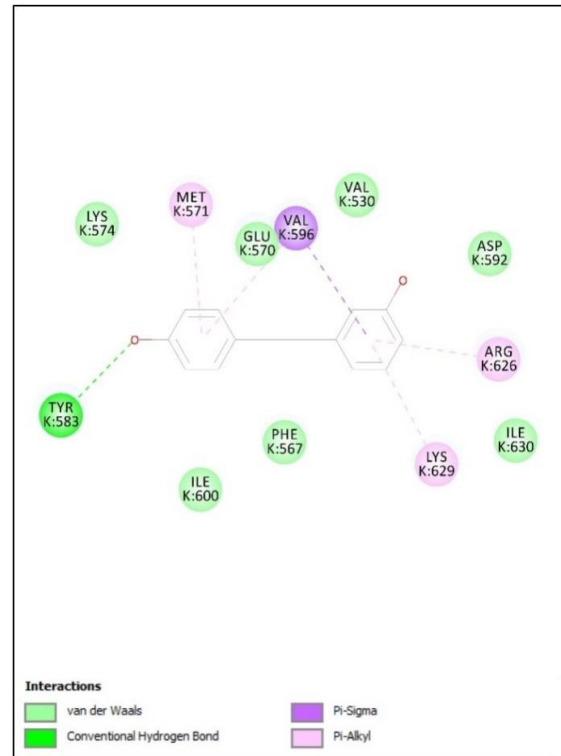
p)



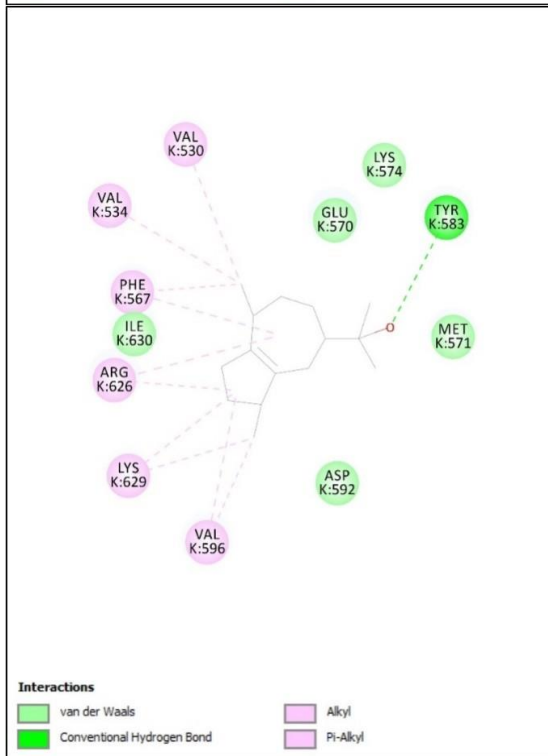
q)



r)



s)



t)

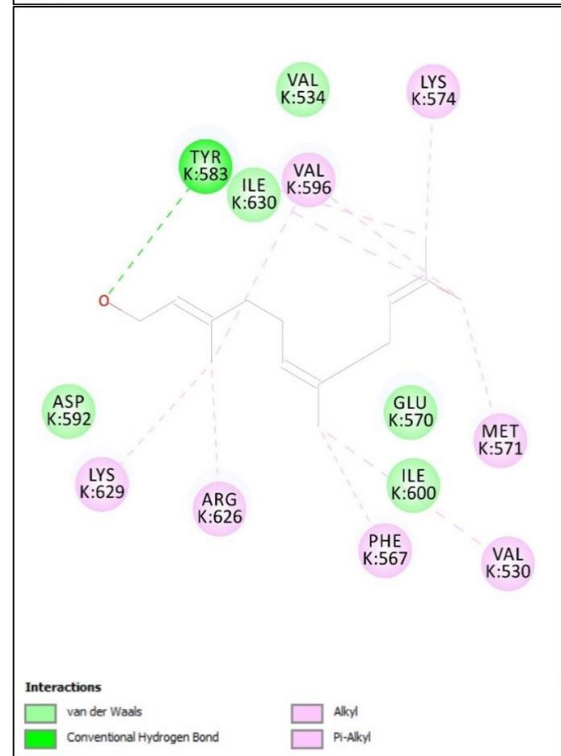




Fig 5.11: 2D diagrams of the interactions of docked compounds

- a) Visnagin b) alpha-Muurolol c) Nardol d) Jatamol A e) alpha-Kessyl acetate
 f) (1R,3aS,8aR)-1-methyl-7-propan-2-yl-1,2,3,3a,8,8a-hexahydroazulene-4-carboxylic acid g) Nodakenetin h) Isopimpinellin i) Isoimperatorin
 j) Marrubiin k) Bulnesol l) beta-Eudesmol m) alpha-Eudesmol

n) Vulgarin o) Celerin p) Lunularic acid q) 1-(2,2-Dimethylchromen-6-yl)ethenone
 r) Lunularin s) Guaiol t) Farnesol u) Norseychellanon
 v) (+)-Juniper Camphor w) Pogostol

CCBs prevent the influx of calcium ions into the cells, thereby dilating the blood vessels and relieving the symptoms of Raynaud's disease (RD). These CCBs work by binding to the active site of the calcium channel and inhibiting its function perhaps by changing its conformation.

This study has identified compounds that can bind to the active site of the L-type calcium channel (LTCC) through hydrogen bonds and possibly inhibit its function.

The active site was identified by docking a commercially available drug, Nifedipine, to the LTCC. It was found to form hydrogen bonds with the TYR583 residue as seen in **Fig 5.10**. Following this, the rest of the 62 compounds were docked in the same site and 23 compounds were found to form hydrogen bonds with the same TYR583 residue. The name of the compounds, their binding energy, LibDock score, number of hydrogen bonds, residues of each bond, and the number of unfavourable bonds are all represented in Table 5.3.

The conformation of the compounds while binding to the active sites can be seen in **Fig 5.11** along with their covalent and non-covalent bonds. Out of the several poses provided by DS for each compound, these poses were chosen due to its satisfactory LibDock score, energy levels, hydrogen bonded interactions as well as several other factors that aid in the binding of the ligand to the active site.

These 23 compounds that have been shown to bind to the active site of LTCC with good scores in Lipinski's rule of five and without any unfavourable interactions.

These findings suggest that the inhibition of LTCC may be a promising therapeutic strategy for the treatment of Raynaud's disease. Further research is needed to optimize these inhibitors and assess their safety and efficacy in preclinical and clinical settings.

CHAPTER 6

SUMMARY AND CONCLUSION

6.1 SUMMARY

In this project, the aim was to identify, filter, and optimise possible drug candidates for the treatment of Raynaud's disease by utilising computational methods. Raynaud's disease is a common vascular condition that is can be usually noticed by a change in colour of the fingers or toes from white to blue to red. It is generally temporary and periodic, and is caused by the constriction of the peripheral blood vessels. To relieve the symptoms of this disease, drugs that have vasorelaxant properties are used. These are most commonly calcium channel blockers like Nifedipine. The calcium ions moving within the cell causes the constriction of blood vessels, therefore, if the calcium ions are prevented from entering, it reduces the constriction of blood vessels and promotes relaxation, thereby relieving the symptoms of Raynaud's disease. This theory was followed to search for plants that contained calcium channel blocking properties. The plants *Apium graveolens*, *Lavandula stoechas*, *Marrubium vulgare*, and *Valeriana jatamansi* were found to have these calcium channel blocking properties. The phytochemicals from these plants were taken from IMPPAT database and passed through various screening steps like pharmacokinetic analysis and toxicity analysis to obtain a total of 62 compounds. These compounds were then docked using Discovery Studio and a total of 23 compounds were docked in the active site of the target. The drug Nifedipine was also docked with the target in order to identify the active site and to be used as a reference.

Overall, the aim was achieved, and 23 possible calcium channel inhibiting compounds were identified. These compounds have to be further tested for its efficiency and risk factors before moving on to preclinical or clinical trials.

It has to be noted that these compounds do not completely cure Raynaud's disease but rather relieves the symptoms and reduces the frequency of attacks.

6.2 CONCLUSION

This study concludes that there are 23 potential compounds that may inhibit the activity of L-type VGCCs. All of these compounds have been shown to bind to the active site of the target protein and may have potential inhibitory activity. These compounds may hold promise as potential therapeutics for the treatment of Raynaud's disease. These chemical compounds that are obtained from the PubChem database by virtual screening open up new possibilities in the field of drug discovery by displaying possible inhibitory actions. The ability to obtain small molecules from natural sources using laboratory techniques make these potential inhibitors more attractive from a drug discovery perspective. However, further research is needed to optimize these inactivators and assess their safety and efficacy in preclinical and clinical studies. It is also important to note that the inhibition of these proteins may have unintended consequences, and careful consideration should be given to potential off-target effects. Overall, the findings of this study provide valuable insights into the potential use of small molecule inhibitors for the treatment of Raynaud's disease and future research in this area could have important clinical implications.

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