ROLE OF SORL1 GENE AND ITS PHARMACOLOGICAL INTERACTIONS IN ALZHEIMER'S DISEASE

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CANDIDATE'S DECLARATION

I Akanksha Sahu, Roll Number: 2K22/MSCBIO/05 hereby certify that the work which is being presented the thesis entitled- **"Role of SORL1 gene and its Pharmacological Interactions in Alzheimer's Disease"** in partial fulfilment of the requirements for the award of Master of Science, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from May 2023 to May 2024 under the supervision of Prof. Pravir Kumar.

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.

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ROLE OF SORL1 GENE AND ITS PHARMACOLOGICAL INTERACTIONS IN ALZHEIMER'S DISEASE

AKANKSHA SAHU

ABSTRACT

Alzheimer's Disease is considered to be a remarkable global health challenge, with a crucial need for highly effective treatments. The main reason behind AD is abnormal amassing of neurofibrillary tangles and amyloid beta plaques in neurons. Among the several genetic factors implicated in AD, the SORL1 gene (Sorting Protein-Related Receptor1) or SORLA or LR11 appeared as a key player, influencing amyloid precursor protein metabolism eventually contributing to disease pathology. According to the researchers, several independent methods, including clinical pathology, human genetics, and exploratory studies in animal models, all point towards SORL1 receptor as a key player in the progression of Alzheimer's disease. This abstract focus on elucidating an effective role of gene variants of SORL1 in AD which can reduce the likelihoods of AD by synthesizing SORL1 protein. Since lipids often affect lipoprotein receptors, it has been assumed that Docosahexaenoic acid (DHA), an important ω-3 fatty acid correlated to a lower risk of Alzheimer's disease (AD) and degraded accumulation of Aβ, might influence the expression of SORL1 or LR11 protein. According to the studies, DHA vividly elevate the level of SORL1 in a variety of biological systems such as mice and human neuronal cells. The interaction of DHA containing drug called Vascepa and other dementia related drugs with SORL1 protein has also been suggested to provide insights into therapeutic potential of these drugs in treatment of AD by affecting the activity of SORL1 protein. Furthermore, a comprehensive comparison of binding energies of drugs with SORL1 protein receptor has been made to determine the efficacy of each drug in increasing the protein expression, eventually reducing the progression of neurodegenerative disorder. Additional research and studies need to be done on SORL1 protein to discover the possible participation in this neurodegenerative disorder.

Keywords— Alzheimer's disease, binding energy, molecular docking, DHA, amyloid beta, neurofibrillary tangles, Vascepa, SORL1

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LIST OF ABBREVIATIONS

AD: Alzheimer's Disease

SORL1: Sorting Protein-Related Receptor 1

DHA: Docosahexaenoic acid

EPA: Eicosapentaenoic Acid

APP: Amyloid Precursor Protein

APOE: Apolipoprotein E

PSEN 1: Presenilin 1

EOAD: Early-onset Alzheimer disease

LOAD: late-onset Alzheimer disease

SNP: Single nucleotide polymorphisms

CSF: Cerebrospinal fluid

LDLR: Low-density lipoprotein receptor

TGN: Trans-Golgi network

AAO: Age-at-Onset

PUFA: Polyunsaturated fatty acids

LR11: Lipoprotein Receptor

CR: Clustered Repeats

FN: Fibronectin

ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicity

CHAPTER 1

INTRODUCTION

1.1 Background

Alzheimer's disease stands as the most prevalent form of progressive neurodegenerative disorder across the globe. The molecular principles which govern the proteolytic processing of amyloid precursor protein (APP) into amyloid-β peptides (Aβ) and the rapid accumulation of neurotoxic Aβ proteins in the brain cells eventually lead to the progression of neurodegeneration of neurons in both uncommon and common forms of Alzheimer's disease. AD have been the most widely studied from of dementia among all the other forms of neurodegenerative disorders. In the clinical analysis, aiming on the accumulation of Aβ in AD affected patients shows a lot of promise to the scientific community. Presently, this approach is quite limited to a very small number of targets, which include all the enzymes, β- and γ-secretases, involved in degradation of APP into Aβ (Schmidt V et al., 2012). This neurodegenerative disease has no permanent treatment till date and is considered to be matter of global concern. Developing helpful cures for this incapacitating neurodegenerative condition has proved to be one of the largest catastrophes to aged populations in all civilizations (Scherzer et al., n.d.).

Alzheimer's disease (AD) has a diverse genetic makeup which means that several genes are involved in the pathogenesis. AD pathogenesis involves tremendous rare form of mutations in genes such as PSEN2, APP, PSEN1, or are seen in certain families (Yin et al., 2015). These pathogenic mutations in AD related genes have a specific autosomal dominant transmission pattern. This eventually results in early and late onset of AD. Regardless of when the AD initiates, the difficulty of determinism is more sophisticated in the majority cases of AD. A majority of genetic risk factors have been discovered in a variety of AD cases which differ widely in terms of degree of impact and frequency in pathogenesis.

AD can be divided into two main categories as follows - late-onset AD (LOAD) and early-onset AD (EOAD). Genome-wide association studies (GWAS) have widely altered the research in AD genetics (Borges et al., 2018). GWAS have discovered over 20 genes which are directly linked with LOAD. Studies suggests that 95% of AD cases belong to the LOAD category which primarily evolves sporadically in a population.

LOAD is highly manipulated by some combination of various genetic and environmental risk factors as in Apolipoprotein E4 allele. Experiment suggests that LOAD is less caused by single mutation (Vardarajan et al., 2014). On the other hand, autosomal dominant variants such as presenilin 2(PSEN1) and presenilin 1(PSEN 2) show major contribution in EOAD cases. But modern studies have implicated greater than hundred genes responsible for AD pathogenesis, among which mutation in SORL gene has proved to be a key player ("2015 Alzheimer's Disease Facts and Figures," 2015).

1.2 SORL1 Mutations and Alzheimer's Disease

Sortilin-related receptor 1 (also known as LR11) is a type-1 membrane protein of molecular weight of 250-kDa and it fall under the category of Vacuolar protein sorting 10 (VPS10) domain family and low-density lipoprotein receptor (LDLR) family (Andersen et al., 2016), that is found to be expressed in nerve cells of peripheral and central nervous system. SORL1 is found to be intensely associated with both LOAD and EOAD in 2004. The level of SORL1 protein is decreased in case of AD which shows positive correlation of amyloid beta accumulation with the protein. SORL1 primarily interacts with certain cytosolic adaptors for both retrograde and anterograde transportation of APP between the trans-Golgi network (TGN) and early endosomes. The interaction between SORL1 and adaptors potently coordinate the delivery of the precursor to endocytic compartments, which are conducive to deterioration of amyloid proteins. SORL1 gene is positioned on chromosomal locus of 11q23.2-q24.2. SORL1 is responsible for the regulation of synthesis of amyloid beta protein and sorting of the amyloid precursor protein (APP) in neurons. Whole-exome sequencing has recognized SORL1 mutations in cases with both EOAD and LOAD. Few clusters of common variants of SORL1 are linked to AD while rare variants of SORL1 gene is highly responsible for early onset of Alzheimer's disease (Verheijen et al., 2016).

Studies unveiled that multiple single nucleotide polymorphisms (SNP) of SORL1 gene have been discovered as risk factors for the occurrence of sporadic AD in diverse populations. Further, experimental studies have demonstrated that significant corelations were situated in 2 distinct regions: the 30 end and the 50 end of the SORL1 gene [(Mehmedbasic et al., 2015). So far, only few studies provided evidence of interconnection of SORL1 genetic variants in AD pathogenesis.

1.3 Pharmacological Interactions with SORL1 Protein

The term pharmacological interactions are defined as the physiological effects that results from a specific drug interacting with food, or a drug interacting with some one or more drugs, supplements or herbs. These interactions usually modify the safety, efficiency, or toxic effects and help in analysing how both of the interacting compounds affect each other's function. It is very essential to Comprehend the pharmacological interactions in clinical studies to ensure the possible therapeutic outcome thereby reducing the side effects.

Pharmacodynamic and Pharmacokinetic interactions are the two categories of mechanisms that is fundamental to pharmacological interactions: Pharmacodynamic Interactions: these interactions occur when two or more drugs interact with each other on the identical physiological target or system. The nature of pharmacodynamic interactions can be synergistic, additive or antagonistic in nature. For example, mixing a and a beta-blocker and beta-agonist may neutralise the effects of each other. Pharmacokinetic Interactions: These interactions define the alterations in the methods drugs are being absorbed, distributed, metabolized, or excreted (ADMET). Pharmacokinetic interactions involve modification of pH of stomach impacting the competition for metabolic enzymes or interference with hepatic clearance mechanisms or some other renal mechanisms.

Some Dietary supplements Containing DHA such as krill oil and Fish oil and might increase LR11 gene expression level to diminish AD risk. Omega-3 fatty acids have been the subject of interest among researchers in recent years. But when it comes to nutraceuticals, Docosahexaenoic acid (DHA), which is an indispensable polyunsaturated fatty acid (PUFA) is found to play a significant role in AD.DHA supplements significantly protect synaptic protein loss, improve cognitive deficits and help in lowering insoluble amyloid beta. DHA might increase the level of LR11 protein which could contribute to the reduction of amyloid beta witnessed in nerve cells, thus reducing the risk for AD in late and early stage of life (Guo et al., 2012). Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) are two major types of omega-3 fatty acids exclusively found in krill oil, which is a DHA supplement obtained from small Antarctic crustaceans. According to 2015-2020 Dietary Guidelines for Americans, approximately 250 mg of EPA and DHA has been suggested for a general population. EFSA stated that intakes of 4-5 g/day DHA and EPA as a supplement do not cause any harmful impacts on a general population. In an experimental study on a mice model, mice were injected with Aβ25–35 followed by krill oil treatment (Decandia et al., 2023). Studies concluded that krill oil enhanced memory functions while simultaneously causing neurons death and reducing neuronal oxidative stress. Moreover, this 12-week krill oil treatment was performed to enhance the cognitive functioning but currently no direct relation of krill oil's effects for AD patients available yet (Calon F et al., 2005).

In this thesis we made attempts to understanding the complex web of the potential synergy between DHA-containing drugs like Vascepa and dementia medications with SORL1 protein to identify a promising strategy for alleviating Alzheimer's disease, highlighting the significance of exploring innovative therapeutic approaches to combat neurodegenerative disorders.

OBJECTIVES OF THE STUDY

a) To explore the role and potentials of SORL1 gene in AD pathogenesis
b) Analyse the pharmacological interactions of SORL1 receptor protein with existing drugs to understand the potential impact of drug-protein interactions within AD progression.
c) To unravel the potential of DHA in upregulating the expression level of SORL1 gene.

CHAPTER 2

LITERATURE REVIEW

2.1 SORL1 gene as a late-onset AD risk factor

Alzheimer's disease (AD) is found to be the most widespread form of progressive neurodegenerative disease which is marked by disturbances of memory and cognitive functions. The assembly of amyloid plaques, neurofibrillary tangles, and dystrophic neurites which possess hyperphosphorylated tau proteins in nerve cells are the major hallmark of AD (Schneider et al., 2009). The key genes in AD are found to be presenilin 2, presenilin 1, amyloid precursor protein (APP). Apart from PSEN1, APP, and PSEN2 as the key genes, SORL1 has proved to be a substantial vulnerability factor for late-onset Alzheimer's disease (LOAD) in various cases. Additionally, there is still room to investigate many other potential genes linked to LOAD susceptibility, as the APOE genotype carries approximately half of the risk for late-onset sporadic Alzheimer disease (Campion et al., 2019). In order to clarify the condition's wider genetic landscape, scientists have conducted extensive genome-wide association studies (GWASs) and candidate gene analyses. SORL1 also interact with ApoE gene both of which act on a similar metabolic pathway associated with LOAD. Interactions of SORL1 gene with other AD related genes such as PSEN1, PSEN2 also play an active role in Late onset of Alzheimer's disease (LOAD) (Rovelet-Lecrux et al., 2021). SORL1 is a family of SORCS 1, SORCS2, and SORCS3 members. SORL1 acts as a sorting receptor such that it regulates its trafficking and proteolytic processing into Aβ by binding with APP. It has been confirmed that the five Single nucleotide polymorphisms (SNPs) of SORL1 -rs560573, rs668387, rs689021, rs641120, rs1614735(SNP 6, 8, 9, 10, and 27) showed significant associations with LOAD. SNP (rs985421) also exhibited a resilient association with LOAD including other SNPs such as rs3781834, SNP 7 and rs3781836 (Cuccaro et al., 2016).

2.2 Genetic Variants of SORL1 in AD

Rogaeva et al found out that late onset AD is connected to genetic kinds of the neuronal Sortilin-related receptor, i.e. SORL1 (Lee et al., 2007). While studying AD among which there were 296 patients in general, Lee et al stated that different SNPs and haplotypes in the SORL1 gene were related to it and studied how seven genes that can be passed onto offspring through SORL1 are associated with the AAO (age-at-onset), he found that two of these genes, rs536360and rs556349are linked to AAO (Cellini et al., 2009). The respondents' outcomes showed that there was an extensive allelic

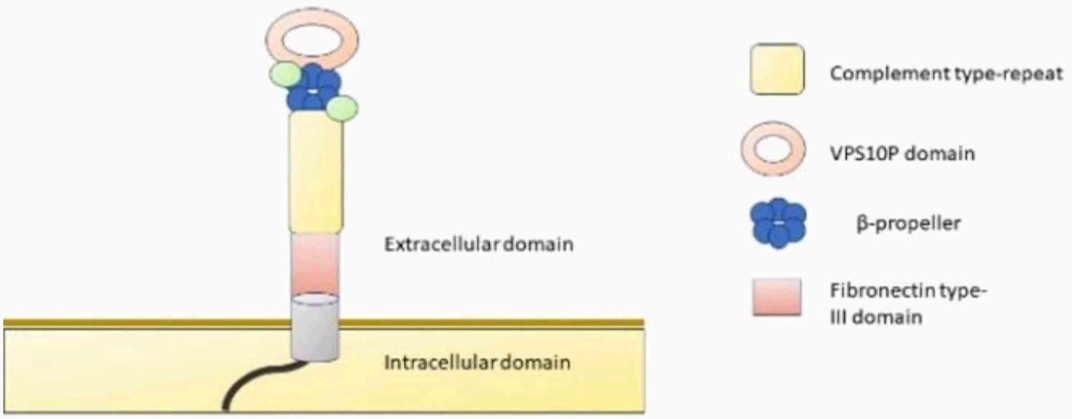
heterogeneity noted in SORL1, which meant that certain single nucleotide polymorphisms (SNPs) were just limited to some groups. In one of their articles Cellini et al. stated that correlation between SORL1 and late-onset AD was finally proved by their research study (Reitz et al. 2003) attribute their affirmation to the acquired knowledge that there exist numerous SORL1 polymorphisms associating the Alzheimer's disease susceptibility (Wang et al., 2014). The linear regression model, Kaplan-Meier survival analysis and the Cox proportional hazard model were used in this study to investigate the relationship between 43 SNPs on the gene of SORL1 and AAO of AD. In the male sample, five SNPs were found to be significantly associated with the AAO of AD using the Cox proportional hazard model and Kaplan-Meier survival analysis. On the other hand, only one SNP was found to show relation with the AAO of AD in the female sample of $P < 0.05$. Moreover, two SNPs (rs2282649 and rs726601) were proved to show association with AAO in the total sample according to linear regression model. Furthermore, logrank test showed that SNP rs11218313was related to AAO in males, which is similar to the results obtained from the Cox model for the same males' AAO (Zhang et al., 2017).

The process of protein truncation and rare pathogenic missense variants of SORL1 gene show association with AD. Low CSF-SORLA levels are found in carriers of pathogenic missense or truncating mutations, and this could be a biomarker for compromised SORL1 (Holstege et al., 2017). There were pathogenic mutations across the whole SORL1 gene, independent of a particular functional domain. Only four cases and no older controls had moderately harmful mutations in the VPS10 domain found in our discovery study (Holstege, 2023). On the other hand, only control participants had somewhat harmful mutations in the LDL-receptor A and fibronectin domains. The large number of young reference subjects in the replication sample who might yet develop the AD condition at a later age, however, may have contributed to our inability to confirm these findings in the combined or replication samples. Larger samples will be required in the future to elucidate whether moderately harmful mutations in the VPS10 domain that binds Aβ could potentially impair SORL1 function dangerously (Berman et al., 2000).

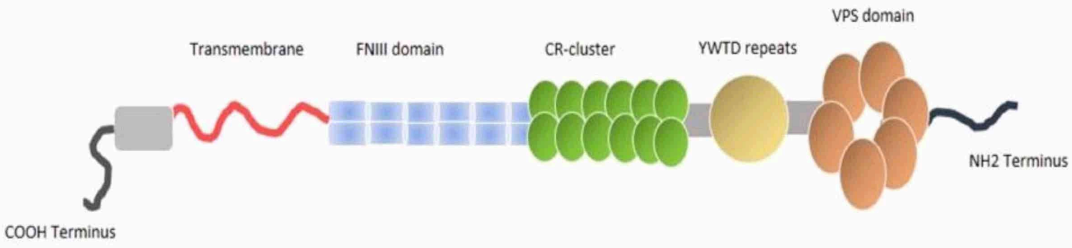
2.3 Biochemical Properties of SORL1

The human SORL1 or LR11 gene which consists of 121452203-121633762 base pairs is found to be located on chromosome 11q23.2-q24.2 of a human genome. SORL1, a 250 kDa membrane protein includes seven different domains, which can be explained as follows: domain II which is identical to VPS10, domain III which has five tandem LDLR "YWTD" repeats (Zhang et al., 2017), domain IV forms the amino-terminus of the SORL1 receptor includes a sequence of 350 amino acids, domain VI is a membrane-spanning region , domain V forms an assembly of 11 complement-type repeats (CR) , six motifs linked to the fibronectin-type (FN) III repeat (Wang et al., 2023) which can also appear in some neural adhesion proteins and domain VII which is a cytoplasmic region of SORL1 receptor present at the COOH terminus. The SORL1 protein is denoted as a hybrid receptor because it holds structural units from several other proteins and is capable of performing diverse physiological functions, such as signalling, intracellular sorting, transportation of cargoes and chaperone-like activities.

SORL1 expression is not only distributed throughout the brain, it is particularly noticeable in the neurons of the hippocampus, Purkinje cells, some brain stem nuclei, and at the subcellular level, where it is mostly located in the trans-Golgi network (TGN) and early endosomes. Regarding its functions, SORL1 binds the ApoE-rich lipoprotein as a member of the LDLR family (Cencini et al., 2022).



(a)



(b)

Fig 2.1: Schematic Representation of SORL1 receptor in Cell Membrane

Through its modulation of Aβ aggregation and clearance as well as its involvement in inflammation, ApoE plays a vital role in the progression of AD. Therefore, through its interaction with ApoE, SORL1—an ApoE receptor—is a significant biomarker for AD. The extracellular portion of the VPS10 family, which includes yeast sorting receptors, is where the VPS10 domain originates. The yeast receptor ligand-binding site it forms guides lysosomal hydrolases from the TGN to the vacuoles. These

receptors are located in addition to SORL1. The cytosolic adaptors SORL1 interacts with specific to the retrograde and anterograde transit of APP between the early endosomes and TGN. This restriction of APP delivery to the endocytic compartments favours amyloidogenic degradation leading to the production of Aβ.

APP follows the secretory pathway where it is transported from the endoplasmic reticulum (ER) to the membrane via Golgi apparatus, where it can be fragmented by the α-secretase present inside the Aβ coding sequence of APP, which ultimately leads to the synthesis of non-amyloidogenic fragments. On the other hand, amyloid precursor protein (APP) can go through clathrin-dependant endocytosis, so that APP can be easily directed to endosomes. In late endosomes, successive cleavage of APP by the β- and γ- secretases results in the fabrication of the Aβ which can be either directed to the lysosome or can be secreted in the extracellular compartment. SorLA protein has the ability to bind with both Aβ and APP where it acts as a repressor of Aβ secretion in three distinct pathways: (1) it can delay the release of APP protein molecules from the Golgi apparatus (2) it can readdress APP to the cell membrane or to the Golgi apparatus and (3) it can target formative amyloid beta proteins to the lysosome in order to limit Amyloid beta secretion on the cell membrane.

2.4 SORL1 protein in APP processing

SORL1 is responsible for breaking down of amyloid precursor beta protein which further limit the production of amyloid beta protein. This 250 kDa transmembrane protein called previously been identified while looking for a lipoprotein receptor which were found to be expressed in the mammalian brain cells. Scherzer and his colleagues, used global gene expression profiling method in their study to identify genes which were differently expressed in AD pathology. Their study provided the first proof which showed the correlation between SORL1 gene to AD pathogenesis. This study demonstrated that in some sporadic cases of AD, there is a 2.5-fall in SORLA levels in nerve cells but in such affected individuals, absence of SORL1 protein expression has been observed in the hippocampus and cortex but not in the cerebellum of the brain of affected individuals. In another experiment performed on a group of people suffering from early-onset Alzheimer's disease (EOAD) and late-onset Alzheimer's disease (LOAD) were the participants of whole-exome sequencing method, which was followed by detailed functional examinations of particular variations. In order to identify the effects of SORL1 mutations, an extensive clinical review of medical records was conducted. Moreover, functional analyses were performed to gauge the consequences of SORL1 mutations on the formation of Aβ and the trafficking of amyloid precursor protein (APP).

SORL1 effectively binds with amyloid precursor protein and synchronizes the sorting process into endocytic recycling or secretory pathways (Lee et al., 2008). It was found that SORLA directly interact with APP in neurons of brain cells. It was discovered that a specific domain of SORLA, known as complement-type repeat (CR) cluster, interact with carbohydrate domain of APP. In an experiment where SH-SY5Y cells were used to understand the outcome of mutation in CR domain, it was concluded that mutations in residues in the SorLA CR-domains modified the O-linked glycosylation of APP. This experimental demonstration provides novel insights into various processes

involved in the regulation of post-translational glycosylation in the Golgi apparatus by activity of SORLA protein receptor, suggesting novel strategies to eliminate the process of amyloidogenesis in AD. This interaction holds APP within the cell's 'Golgi' network. This prevents APP from moving to the cell's surface where it would typically undergo processing that can lead to the production of amyloid beta. SORLA prevents the formation of homodimer in APP which is also responsible for amyloid beta accumulation (Mishra et al., 2022).

SorLA resides primarily in endosomes and Golgi compartments at the cellular level, with a tiny quantity of the protein also found on the extracellular surface of the cell. In an attempt to prevent ligand interaction, the 53 amino acid residues pro peptide which makes up SorLA folds back on the VPS10P domain during production in the neurons. The mature protein receptor found in the trans-Golgi network (TGN) is generated by cleavage of the pro peptide, and it can later interact with its target proteins. Constitutive secretory vesicles transfer the mature receptor protein from the TGN to the cell membrane. A significant number of SorLA molecules present on the cell surface are endocytosed in a clathrin-mediated mechanism (LM et al., 2005) Internally localised receptors migrate to the early endosomes and are eventually sorted to the trans-Golgi, where they repeatedly leave their homes between the endosomes and TGN.

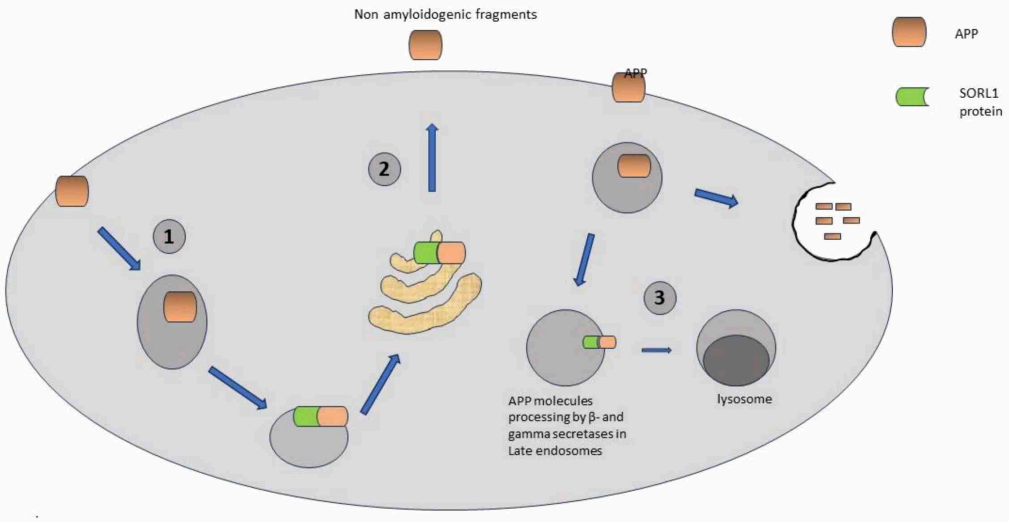


Fig 2.2: The primary Protection against Aβ secretion can be provided SorLA. SORL1 protein reduces the expression level of amyloid precursor protein by three different pathways: (1) binding of APP with SORL1 protein can guide APP protein towards membrane or Golgi apparatus;(2) APP exit from the cell can be slowed down by Golgi apparatus, and (3) SORL1 protein can target nascent amyloid beta protein to lysosome where it can be digested by the hydrolytic enzymes.

2.5 DHA containing drug and SORL1 protein in AD

DHA (Docosahexaenoic Acid), a lengthy chain of polyunsaturated omega-3-fatty acid which plays an important role in simplifying cognitive functions. Neuroprotectin D-1(NPD-1) is the derivative of DHA such that it has neuroprotective properties in case of Alzheimers disease. DHA is highly responsible for upregulating the expression of SORL1 gene. Studies suggest that regular consumption of DHA can reduce the underlying effect of AD. Experimental studies in humans and mice cells suggest that docosahexaenoic acid increases the SORL1 protein level. many DHA containing drugs have been discovered which interact with SORL1 protein. One of them is vascepa which can interact with SORL1 protein (Oksman et al., 2006). Complete interaction of dha and SORL1 is still unknown. Sea algae, fish oil and krill oil are the natural source of DHA.DHA indirectly increases the level of SORL1 protein by DHA methylation and histone modifications. Transthyretin (TTR) is a protein responsible of amyloid beta clearance. DHA can increase the level of TTR to reduce the risk of amyloid beta accumulation. DHA also regulate the activity of insulin degrading enzyme which destroy amyloid beta (Xin et al., 2018)

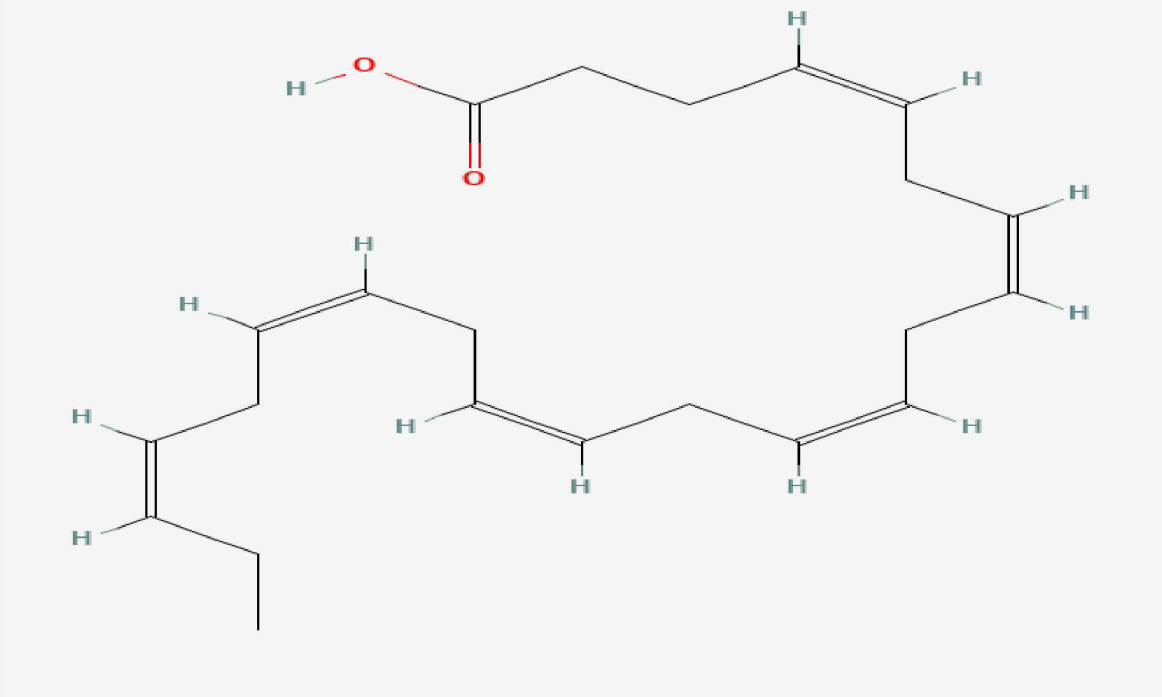


Fig 2.3: 2D-Structure of Docosahexaenoic Acid

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 TOOLS AND SOFTWARE

3.1.1 BIOVIA

BIOVIA Discovery Studio is an open-source simulation software which gather highly efficient in silico techniques such as free energy calculations, molecular mechanics, biotherapeutics developability to aid drug development, analysing protein-protein interactions and molecular modeling. This unique software earlier known as Accelrys Discovery Studio, is developed and designed by **Dassault Systèmes BIOVIA.**

Visualisation -A feature-rich, free molecular modeling program called BIOVIA is available for the easy viewing, sharing, and analysis of protein and small molecule data. It makes it simple for specialists and associates to share findings, guaranteeing that scientific data and time are kept completely intact. Through Discovery Studio, a plethora of tools for data analysis and visualization are accessible. Users can see and analyse chemical properties, protein-ligand interactions, molecular structures, and other relevant information. Along with features like scatter plots, histograms, and 2D and 3D visualization, it can be used for data mining.

Pharmacophore modeling-BIOVIA is also used for the purpose of pharmacophore modeling which is the leading-edge technology to obtain and identify the interactions between receptor-ligand complex. These interactions help scientists recognise novel therapeutic candidates conveniently.

Simulations- BIOVIA Discovery Studio is widely used by the researchers to analyse the dynamic behaviour protein-ligand complexes, solvents, lipid bilayers, ions and biomolecules using molecular dynamics simulations programmes supported by the software. Molecular simulations usually provide insights into the properties and mechanism of these biomolecular process. Simulation programmes such as NAMD and CHARMm are the best simulation programmes used by BIOVIA (Sharma et al., 2020).

3.1.2 PyRx

PyRx is an open-source software which can be used on all major operating systems such as Mac OS, Windows and Linux devised for virtual screening for computational drug discovery purposes. It proves highly beneficial for virtual screening of compound libraries against known targets for drugs. It also assists in job submission, data preparation, and result analysis. PyRx is a valuable tool for computer-aided drug design because it has an intuitive user interface and a docking wizard. Additionally, PyRx has a strong visualization engine which is very vital for structure-based drug creation.

PyRx 1.1 version has more advanced features than old version. This version is capable of simplifying data analysis,2-D structure visualisation of Open Babel molecules, identifying the complex protein structures using Ramachandran plot and automatic centering of grid box eventually enhancing molecular docking studies and accurately analysing docked compounds using machine learning scoring function (Kamble et al., 2023).

3.1.3 STRING Database

STRING-DB is an online bioinformatic tool and database to analyse and retrieve protein-protein interactions (PPIs), functional interactions, and networks. This database collects data from diverse resources to produce a broad network which range from identifiable to presumed protein interactions. Usually, STRING database collects and merges data about thousands of protein interactions from the experimental studies, literature and computational approaches. It designs functional relationships from various sources such as co-occurrence, co-expression, and common domains with physical interaction.

STRING database allows users to analyse networks of protein-protein interactions. The visual representation of nodes and edges present in the network is used to summarise the interconnections of thousands of important proteins where nodes represent proteins and edges represent inter-connections. Visualization can be differentiated by various parameters such as types of interaction and confidence scores to obtain the pure results. This database has 25 tools that perform functional enriching analysis in order to identify biological pathways and processes which are found to be overrepresented by a given group of proteins. This information may help in understanding the genera related to protein family and their role in a specific biological pathway.

STRING database stores detailed information on protein properties which include domain structures, functional annotations, other information related with protein families. It is also linked to other databases to obtain detailed information about the functions and features of some unknown proteins. Another way for STRING-DB to obtain more details concerning proteins, pathways, and functional annotations is through collaborating with various external databases and resources of information like UniProt, KEGG, or GO (Gene Ontology). Researchers of molecular biology or systems biology often resort to STRING DB when they want to understand protein-

protein interactions or find out the molecular mechanism behind diverse biological processes and diseases (Szklarczyk et al., 2020).

3.1.4 Molecular Docking

Molecular docking is a type of computational modeling and a key tool in computer-assisted drug design and structural molecular biology with a goal to predict the predominant binding orientation and affinity of a ligand with a protein of known three-dimensional structure. Molecular docking is a valuable tool used to determine how well small molecules, like potential drugs, fit together with specific targets in the body, such as DNA or proteins. It is found to be very crucial for designing new drugs that perform better and are more precise. This computer tool helps scientists figure out how these molecules might work together in a biological entity. Researchers usually employ a molecular docking score system, which are mathematical functions used to identify the binding affinity between two molecules. Scoring function helps understand how interacting molecules bind together in a certain orientation and also identify the stability and affinity towards each other. The primary aim of molecular docking is to identify the best binding orientation between two interacting molecules such that the resulting complex is a stable structure with minimum energy (Agarwal & Mehrotra, 2016).

Limitations of Molecular Docking

Rigidity

Ligand can take on a variety of conformations when bound to a receptor. But rigidity of receptors is found to one of the main obstacles in molecular docking which correlate to a single receptor conformation, thus results in frequent false negatives output.

Uncertainty in scoring function

A significant drawback of molecular docking is the absence of trust regarding scoring functions which produce precise binding energies of interacting molecules. This uncertainty originates from entropy change and the solvation effect during docking. Further the accuracy of scoring function reduces because of its lack of ability to identify some intermolecular interactions precisely.

Presence of water molecules

Presence of water molecules may interfere with binding of ligand with active site of receptor. Presently there is no solid theoretical framework for estimating the impact of ligands on water molecules. Precisely controlling the water molecules in the binding pocket during the docking process is still a major challenge for the scientist. It becomes more difficult to locate water molecules in a receptor due to inability to locate hydrogen atoms that could serve as a bridge between the ligand and the receptor [30].

3.1.5 Protein Data Bank (PDB)

The Protein Data Bank (https://www.rcsb.org/?ref=nav_home) is an archive of 3D crystalline structures of large biological macromolecules such as DNA, RNA and proteins and was established at Brookhaven National Laboratories (BNL) in 1971.This database is very essential for a diverse group of research scientists in the field of biotechnology, drug development and biochemistry. Rapid advancement in technology as in crystallographic process and NMR spectroscopy methodologies attributed to widespread use of this database across the globe.

The primary goal of PDB is to enhance accessibility to 3D structures of biological molecules stored in the Protein Data Bank (PDB) repository, with the goal of expanding the limitations of biomedicine, fundamental biology, and biotechnology. This priceless database will encourage sustainability while provoking research and innovation in healthcare industry. This goal was attained through a combination of information from external sources and artificial intelligence/machine learning approaches (Pires et al., 2015).

3.1.6 PubChem

PubChem is a freely accessible public chemistry database established by National Institutes of Health (NIH) in 2004. Its open accessibility encourages students and researchers to give their scientific contributions. PubChem primarily focus on small molecules, but also covers larger biological compounds like lipids, carbohydrates, nucleotides, peptides, and other chemically-altered macromolecules. This invaluable repository is a treasure of information covering physical and biological properties, chemical structures, toxicity profiles, identifiers, health and safety data. PubChem manages the massive amount of data into three inter-linked databases: compound, substance and BioAssay databases (Kim et al., 2023).

3.2 METHODOLOGY

3.2.1 Protein preparation using BIOVIA

SORL1 receptor protein has been prepared by using BIOVIA Discovery Studio 2021 which is an open-source software widely used by the research community for protein preparation, visualisation and analysing receptor-ligand complex. The three-dimensional structure of SORL1 protein containing VPS10 domain in a ligand free form has been downloaded from freely accessible protein data bank database in PDB format. The desired protein has been prepared by removing ligand groups, deletion of water molecules and heteroatoms from the macromolecule to prevent undesirable interaction of receptor protein with the ligand. Additionally, polar hydrogens were added to the crystalline structure of protein for efficient docking. A specific ligand configuration was selected from a wide range of ligand configurations found in the

protein crystal structure, and the protein's attributes X, Y, and Z were found to assess its binding affinity.

3.2.2 Ligand preparation

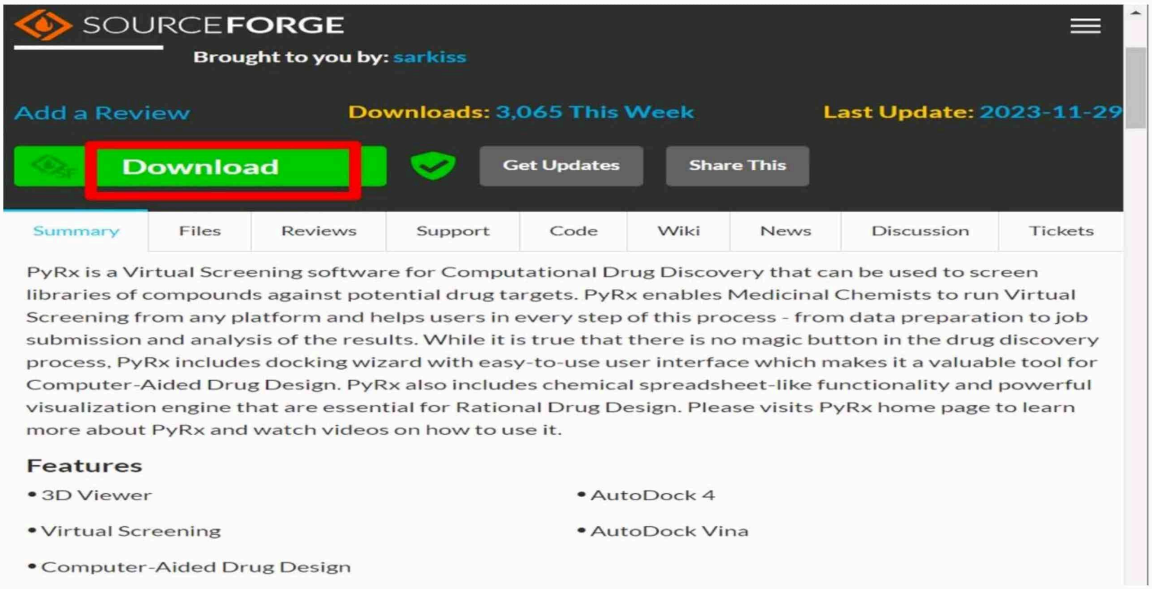
The three-dimensional structures of DHA (Docosahexaenoic Acid) containing drug called vascepa and other dementia related drugs (Doxorubicin, Memantine, Galantamine etc) were downloaded from PubChem database in .sdf format. Vascepa is a long-chain fatty acid ethyl ester synthesised from condensation of hydroxy group of ethanol and carboxy group of eicosapentaenoic acid. It is a highly purified omega-3 fatty acid that has anticholesteremic properties and antidepressant properties. PubChem is a public database which is a repository of biological macromolecules like peptides, lipids, carbohydrates, nucleotides, and other chemically compounds such as drugs. This database also contains the two-dimensional structures, toxicity profile, chemical nature, biochemistry, physical properties molecular formula and literature citations of vast number of compounds.

3.2.3 Ligand-protein docking using PyRx

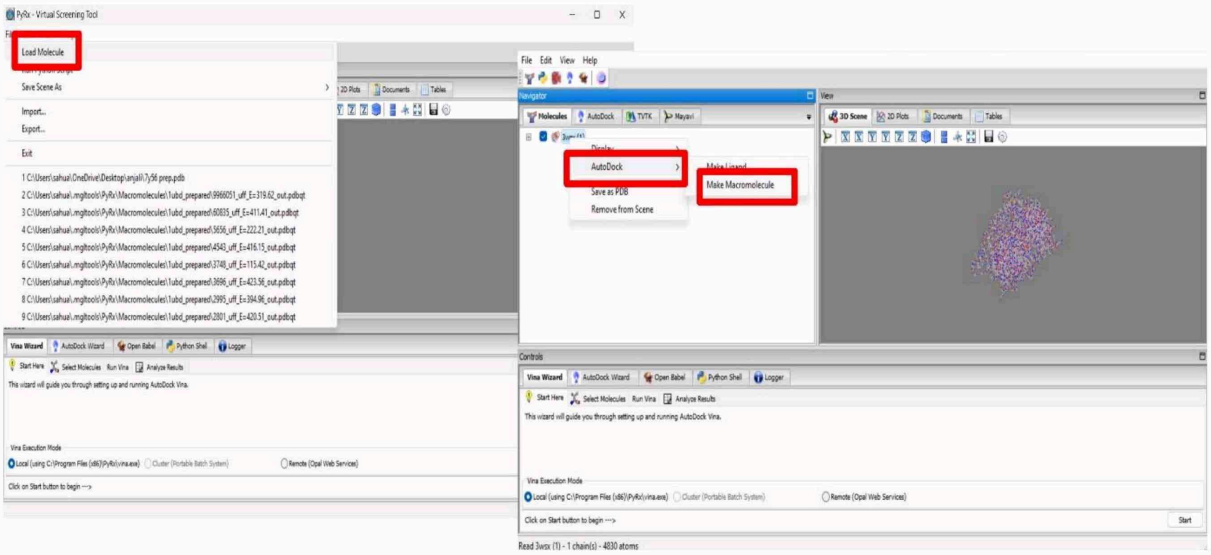
The receptor protein and ligands are prepared as mentioned above. Protein-ligand docking is a vital tool in understanding protein-ligand interaction which can be used to predict protein-ligand binding mode and affinity. Pyrx (https://pyrx.sourceforge.io/) is an open access virtual screening software which is helpful in analysing receptor-ligand interactions for computational drug discovery against potential drug targets. For this experiment, SORL1 receptor protein and ligands are successfully uploaded on the software and converted into. pdbqt file in Pyrx and the blind docking of protein-ligand was initiated. Blind docking is generally used to investigate the reproducibility of the receptor-ligand complex. The dimensions of the grid box in Angstrom(Å) are: X: -93.88 Y- 80.44 and Z- 25 with an exhaustiveness of 8.

Steps for docking

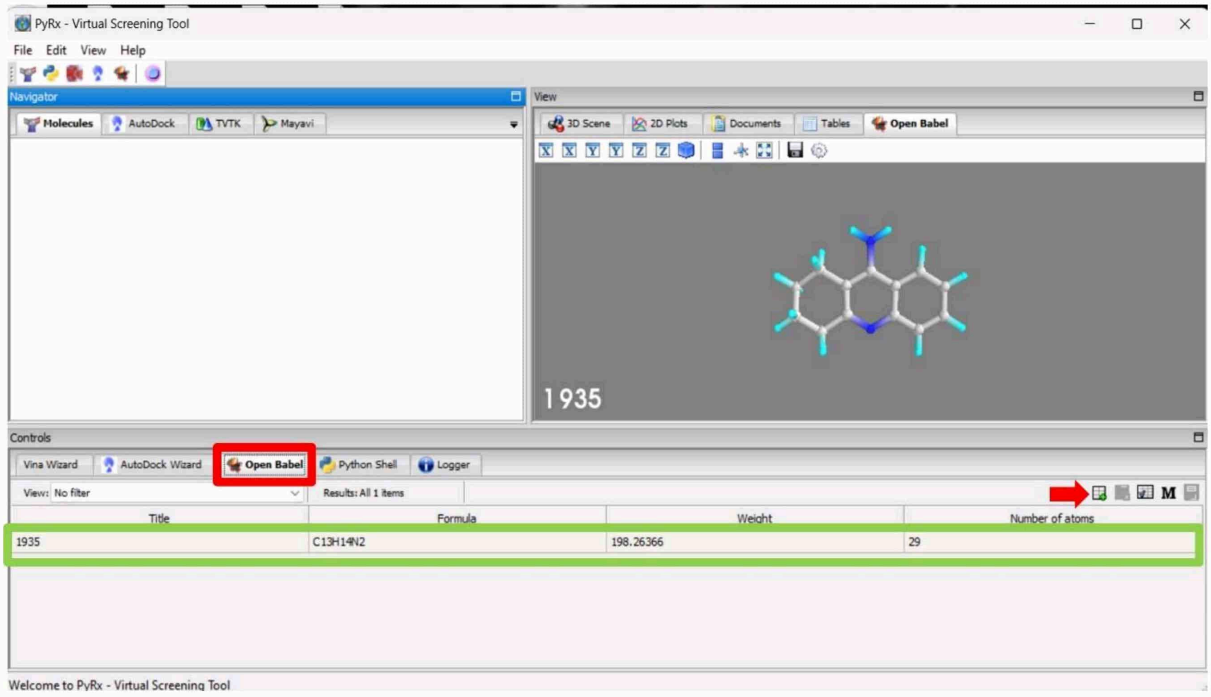
STEP 1- Download PyRx from https://sourceforge.net/projects/pyrx/ on operating system (Windows, LINUX, MacOS), run the set up and open the software.



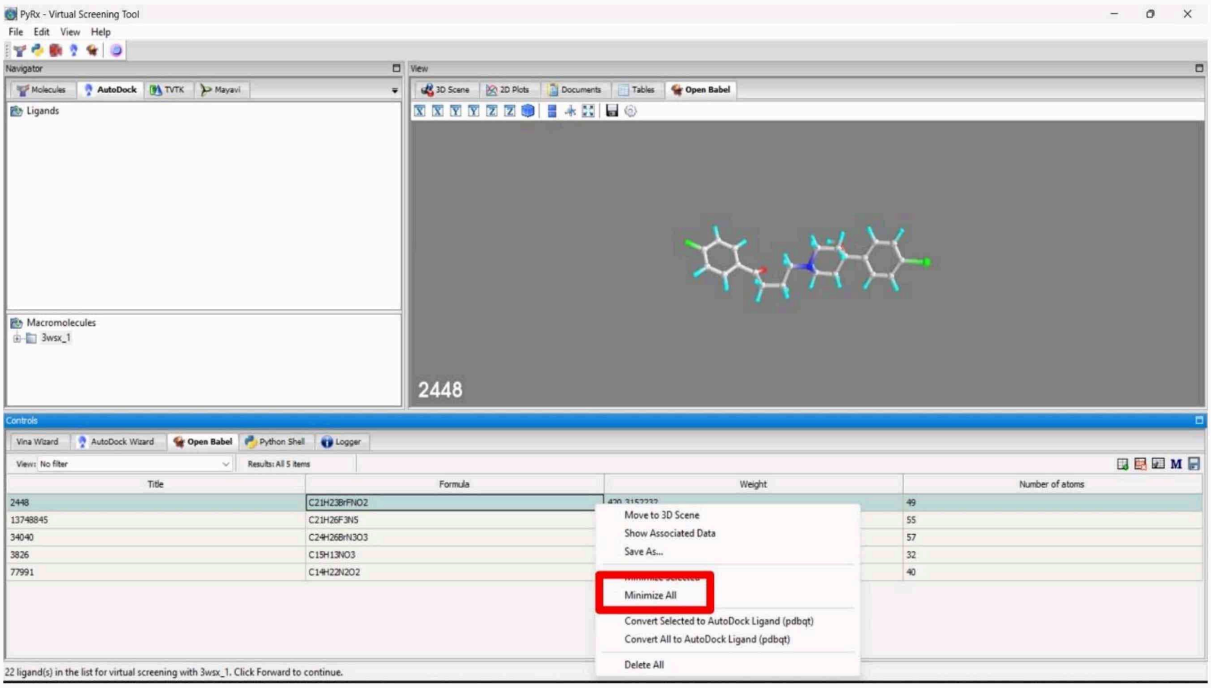
STEP 2- Click on *File* and then select load molecule. Now right click on the receptor protein loaded on the screen, select Autodock and click on make Macromolecule.



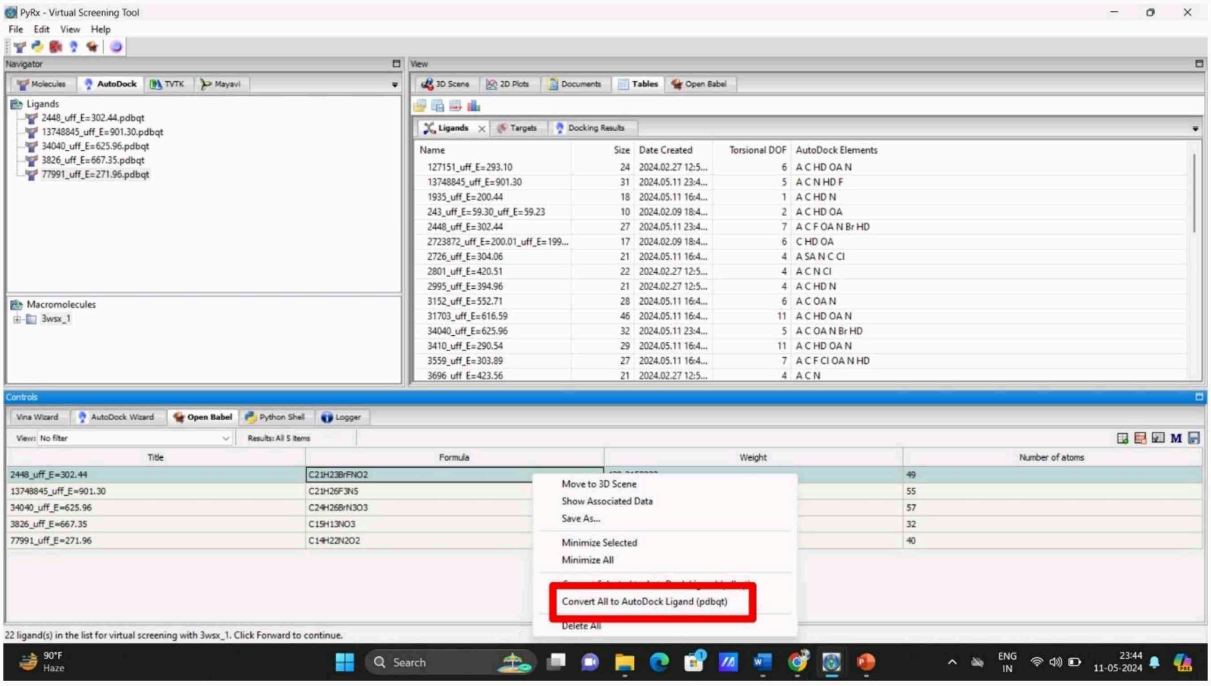
STEP 3- Import the ligand molecules from your computer which will be displayed in Open Babel present below 3D view with column header.



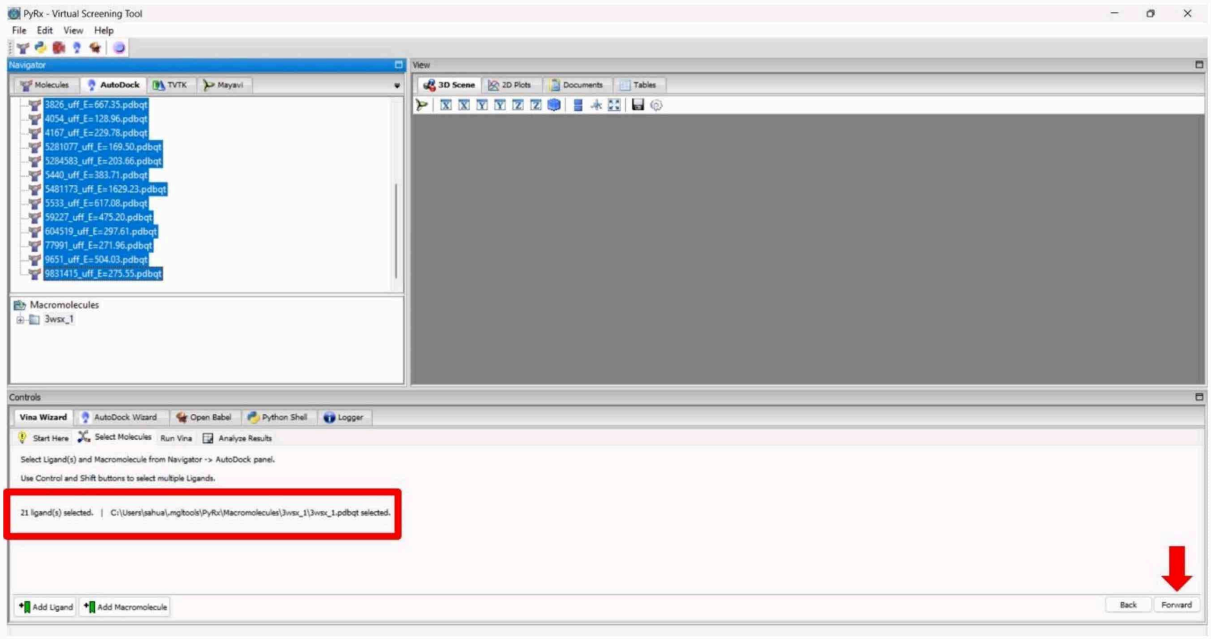
STEP 4- Right-click on all the ligand molecules and select *minimize all* to minimize the energy.



STEP 5- Right click on all minimised ligands and select convert all to Autodock ligand to convert selected compounds from sdf to pdbqt.



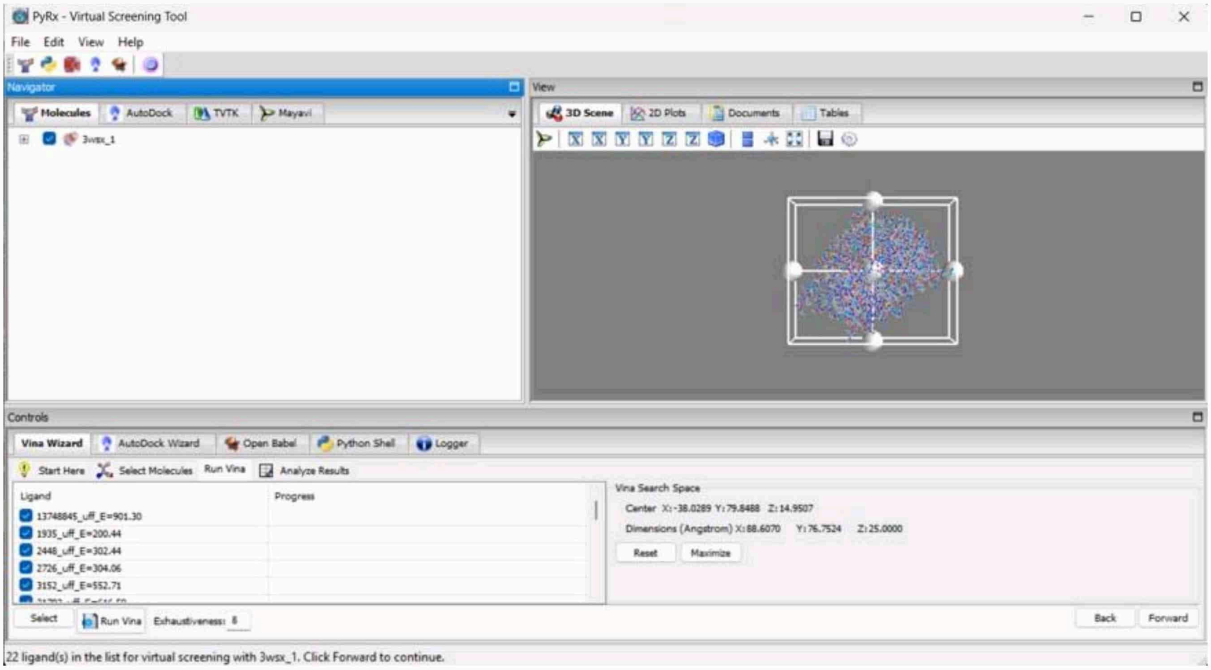
STEP 6- Click Vina wizard in control view and then click on start button.



STEP 7- Select the macromolecule and 3D structures of the compounds appear on the screen.

STEP 8-Select all the ligands by pressing shift button.

STEP 9-Click forward and set box size to cover the active sites of the receptor protein. The docking process is initiated which can take hours to complete.



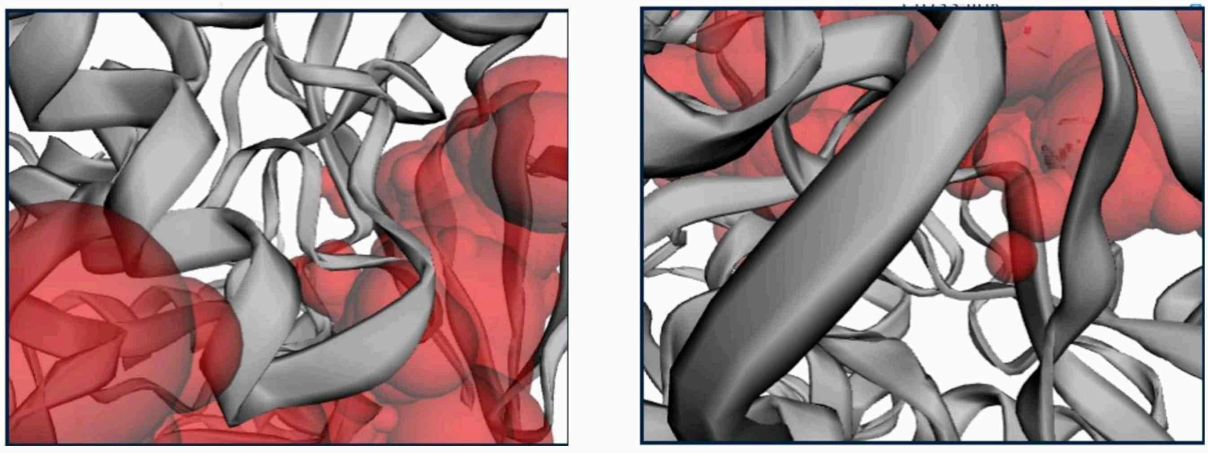
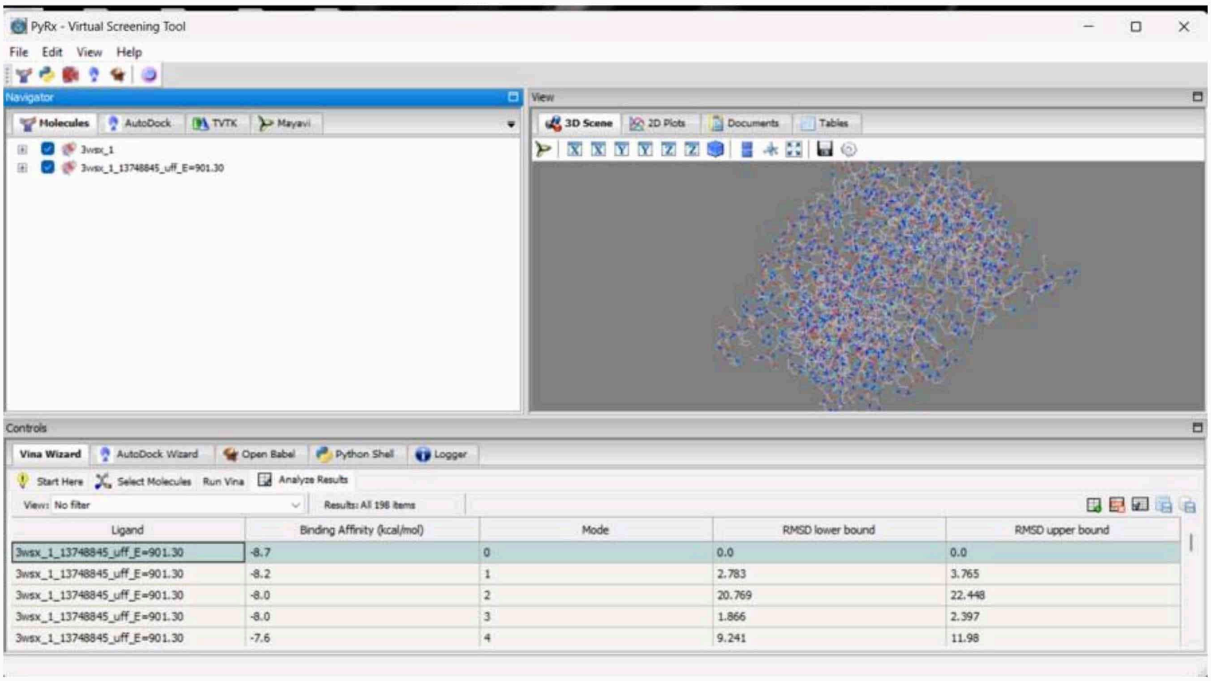
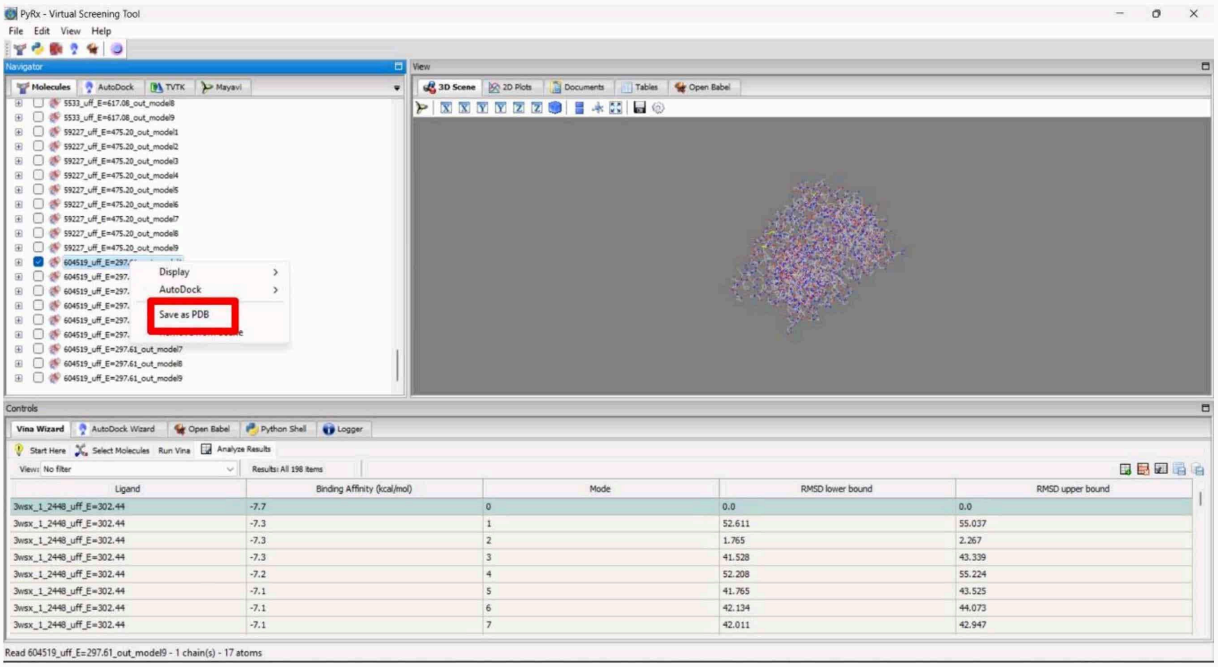


Fig 3.1: (a) Chain A active site (b) Chain C chain active site

STEP 10- Binding energies will be displayed in an excel sheet. Lower the binding energies higher will be the affinities of ligand towards the receptor protein.



STEP 11- Now save all the docked compounds by selecting the desired model and right-click on it. A dialog box appears. Now click on *save as PDB*.



3.2.4 Structural Evaluation

After saving the docked receptor-ligands, these interacting compounds are exported to BIOVIA Discovery studio to understand and analyse the interactions between receptor and ligands. The hierarchy of the docked compounds are copied in the hierarchy of the receptor to retrieve the 2-D and 3-D configurations of receptor-ligand interactions as shown in Fig. These configurations can be used to identify the amino acids and functional groups present involved in the interactions.

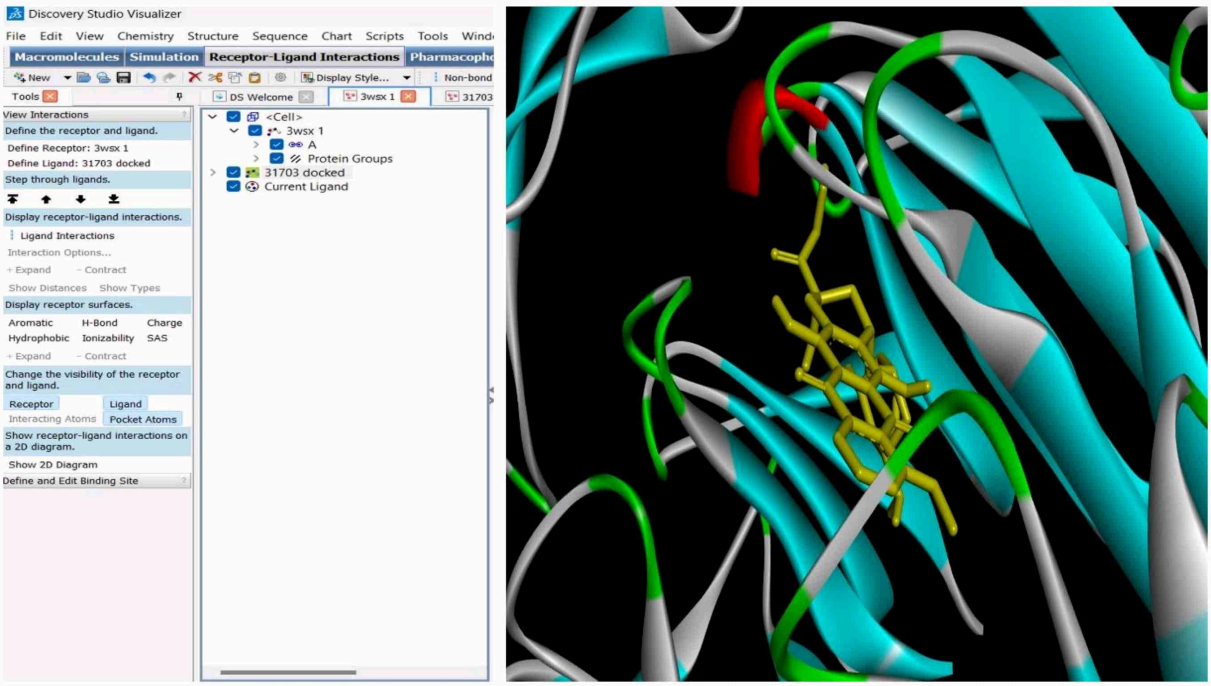


Fig 3.2: Structural Evaluation of docked receptor-ligand in BIOVIA

3.3 PPI Analysis

Protein-protein interactions (PPI) can be studied using the most widely applicable web-based visualization tool STRING (https://string-db.org/). Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) is an extensive database which for offers information on the ability of thousands of proteins to interact with one another. These interactions might be functional which means that how the proteins are involved in the biological process or pathway. The complicated web of connections between proteins may be seen and analysed by scientists using STRING-DB, which helps in their understanding the cooperation of proteins within a cell or organism. This tool is especially useful in identifying important regulatory proteins and pathways that may be involved in disorders or some particular biological responses.

3.4 ADMET Analysis

pkCSM which is a freely accessible web-based tool (http://structure. bioc.cam.ac.uk/pkCSM), which offers a platform to quickly analyse the pharmacokinetic and toxicity properties of drugs. This tool allows us to speculate ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) parameters essential for drug discovery. The Canonical SMILES of the drugs were retrieved from PubChem database and the pharmacokinetic properties of all twenty-one ligands are evaluated pkCSM tool. This tool provides insights into water-solubility, BBB (Blood Brain Barrier) permeability, total clearance, intestinal absorption and various other pharmacokinetics parameters (Ma et al., 2007).

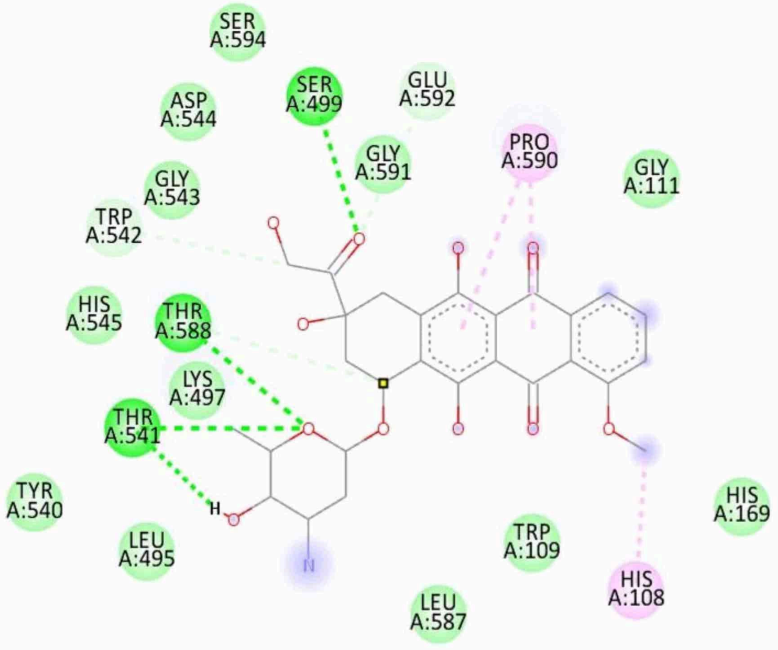
CHAPTER 4

RESULTS

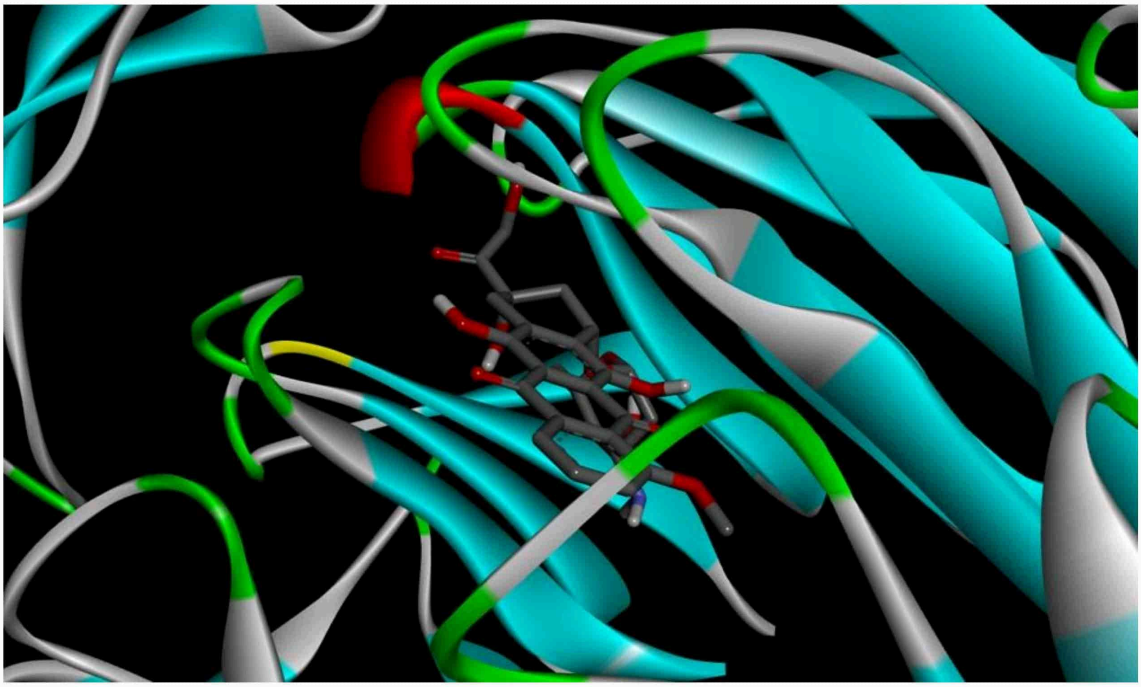
4.1 Interaction between SORL1 and Doxorubicin

The docking results of SORL1 gene and Doxorubicin, inferred the successful interaction between the drug and the gene. After the result analysis, the binding energy of Doxorubicin was found to be -9 kcal/mol which signifies the most efficient interaction between the drug and protein in comparison to all other drugs. The 2-D and 3-D configuration of Doxorubicin interacting with SORL1 is given in Fig 4.1.
The interactions of SOLR1 with other dementia related medications is also carried out using PyRx and their binding energies are evaluated using the given formula (1):

$$\Delta Gbind = \Delta Gbound\ polar - \Delta Gfree\ polar + \Delta\Delta Gbind\ non{-}polar \quad (1)$$



(a)



(b)

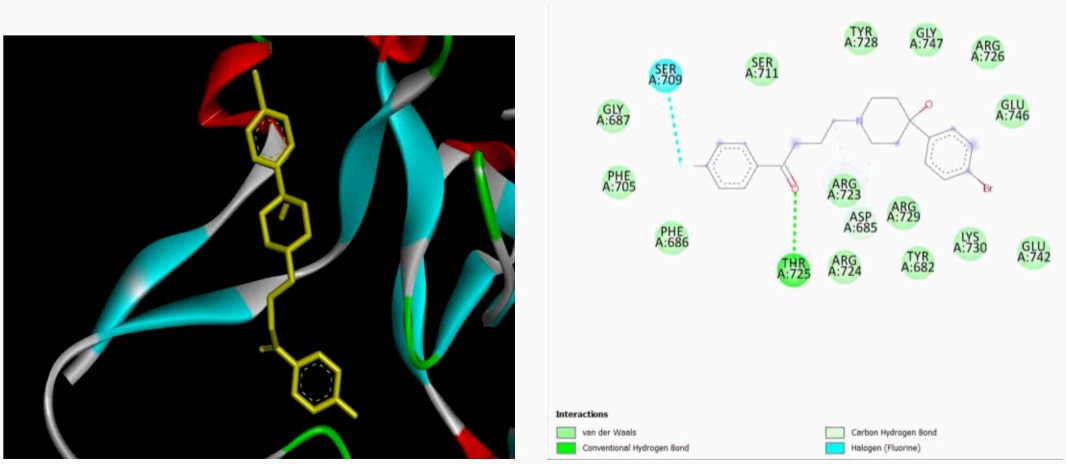
Fig 4.1: (a) 2D Configuration (b) 3D Configuration of Doxorubicin

Table I. Binding Energies of Drugs Interacting with SORL1

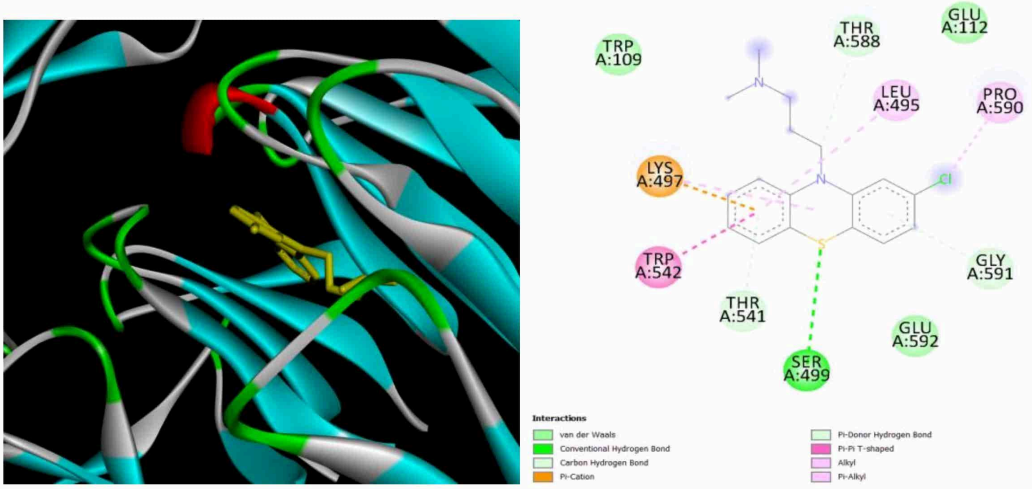
Drugs	PUBCHEM ID	Molecular Formula	Binding Energy
Vascepa	9831415	$C_{22}H_{34}O_2$	-4.9
Thorazine	2726	$C_{17}H_{19}ClN_2S$	-6.3
Rivastigmine	77991	$C_{14}H_{22}N_2O_2$	-5.9
Nicergoline	34040	$C_{24}H_{26}BrN_3O_3$	-8

Memantine	**4054**	**$C_{12}H_{21}N$**	**-6**
Levetiracetam	**5284583**	**$C_8N_{14}N_2O_2$**	**-5.5**
Ketorolac	**3826**	**$C_{15}H_{13}NO_3$**	**-6.9**
Galantamine	**9651**	**$C_{17}H_{21}NO_3$**	**-7.3**
Formoterol	**3410**	**$C_{19}H_{24}N_2O_4$**	**-6.9**
Doxorubicin	**31703**	**$C_{27}H_{29}NO_{11}$**	**-9**
Donepezil	**3152**	**$C_{24}H_{29}NO_3$**	**-8.2**
Lorpiprazole	**13748845**	**$C_{21}H_{26}F_3N_5$**	**-8.7**
Ipidacrine	**604519**	**$C_{12}H_{16}N_2$**	**-6.3**

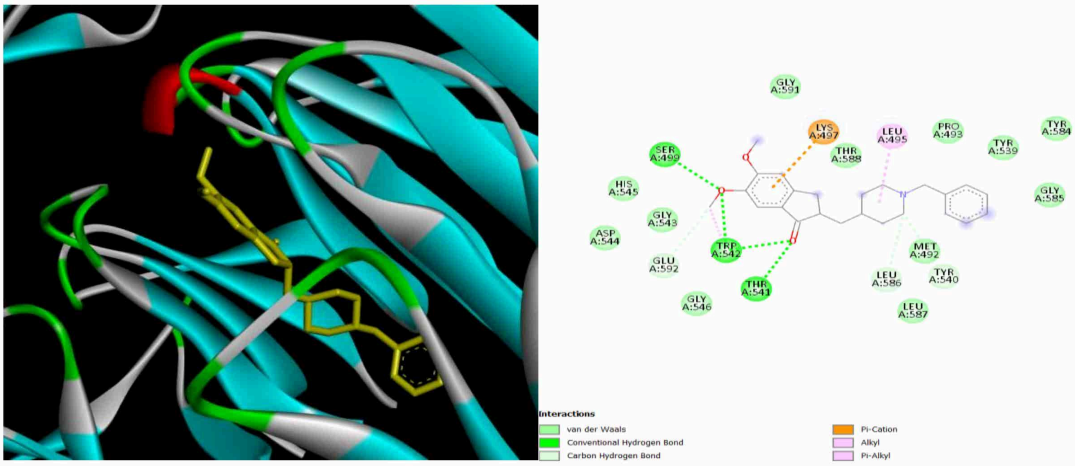
Rotigotine	**59227**	**$C_{19}H_{25}NOS$**	**-6.8**
Trazodone	**5533**	**$C_{19}H_{22}ClN_5O$**	**-7.3**
Thiethylperazine	**5440**	**$C_{22}H_{29}N_3S_2$**	**-6.9**
Metixene	**4167**	**$C_{20}H_{23}NS$**	**-7.2**
Haloperidol	**3559**	**$C_{21}H_{23}ClFNO_2$**	**-7.5**
Tacrine	**1935**	**$C_{13}H_{14}N_2$**	**-6.7**
Ceftazidime	**5481173**	**$C_{22}H_{22}N_6O_7S_2$**	**-7.4**
Bromperidol	**2448**	**$C_{21}H_{23}BrFNO_2$**	**-7.7**
Citalopram	**2771**	**$C_{20}H_{21}FN_2O$**	**-6.3**



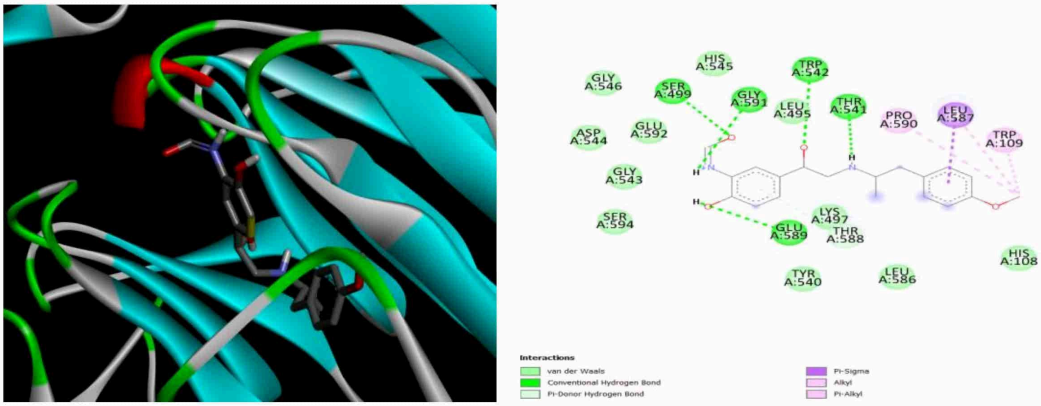
Bromperidol



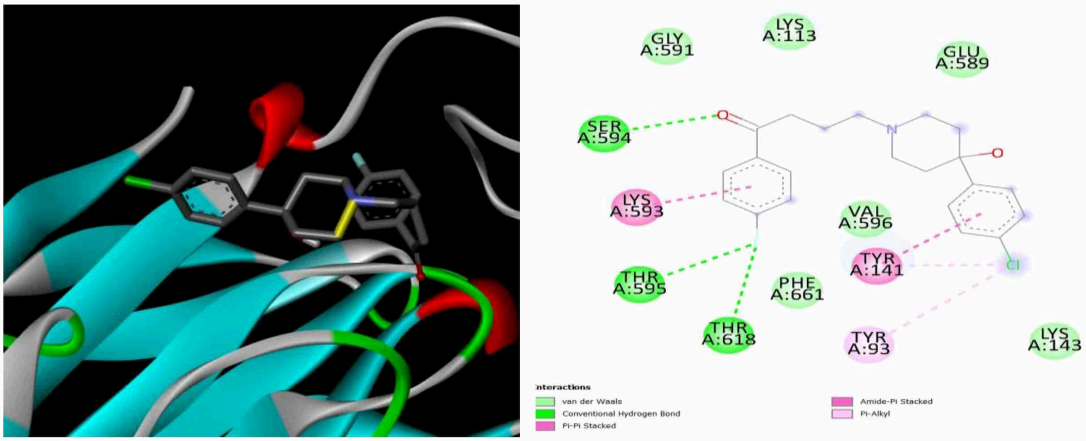
Thorazine



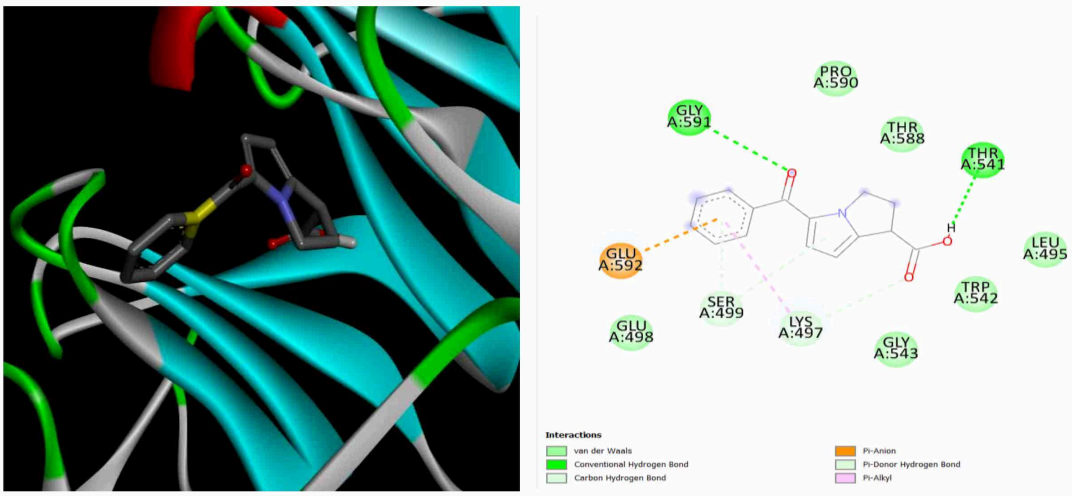
Donepezil



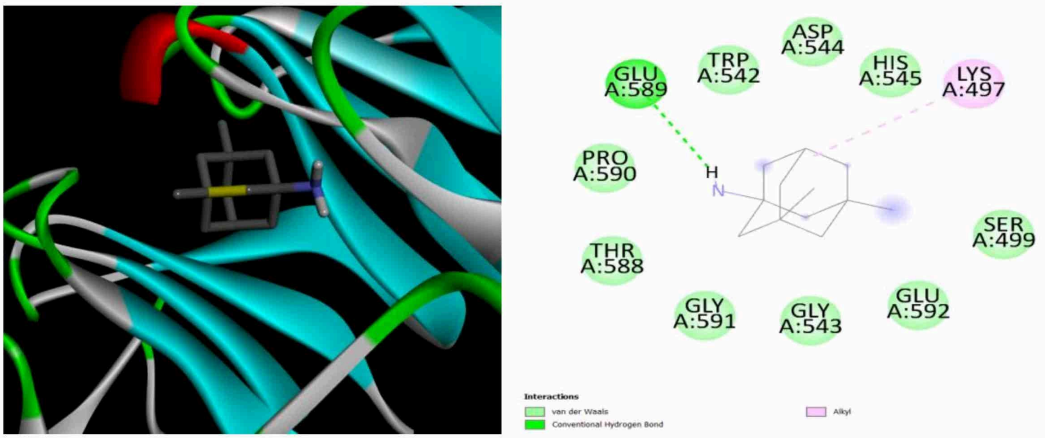
Formoterol



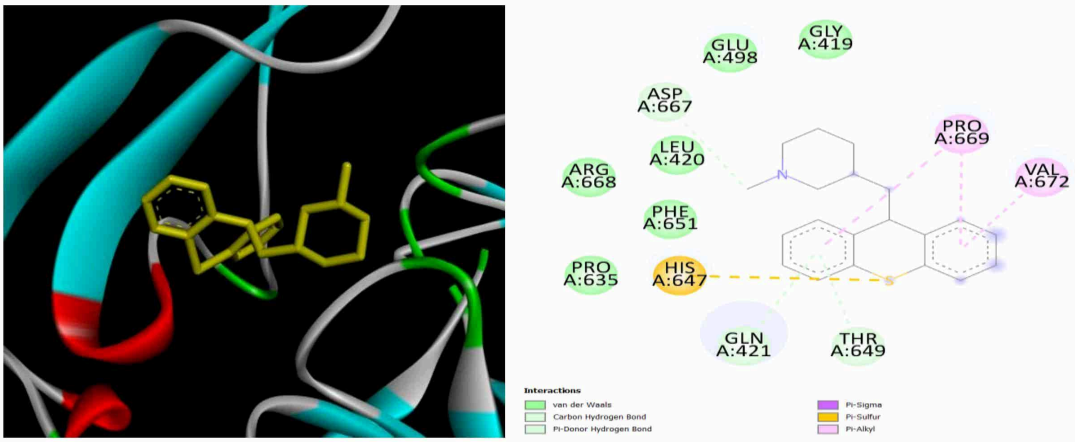
Haloperidol



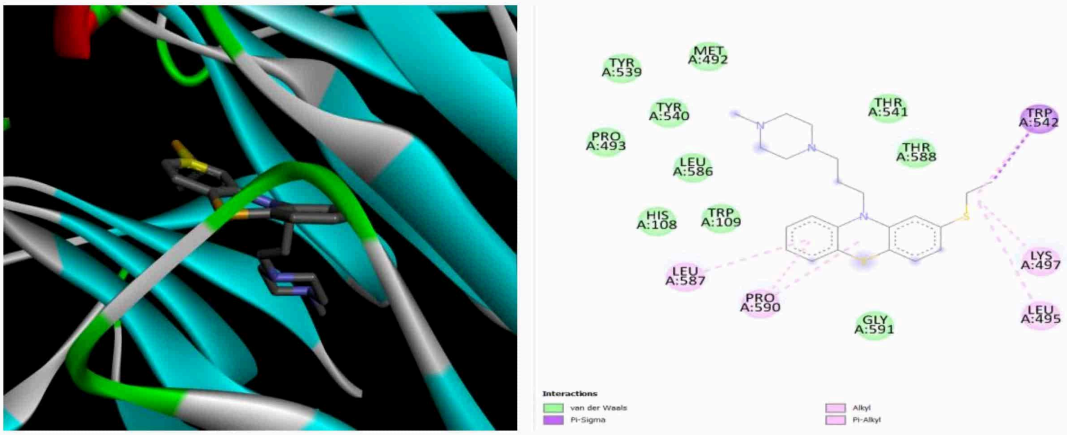
Ketorolac



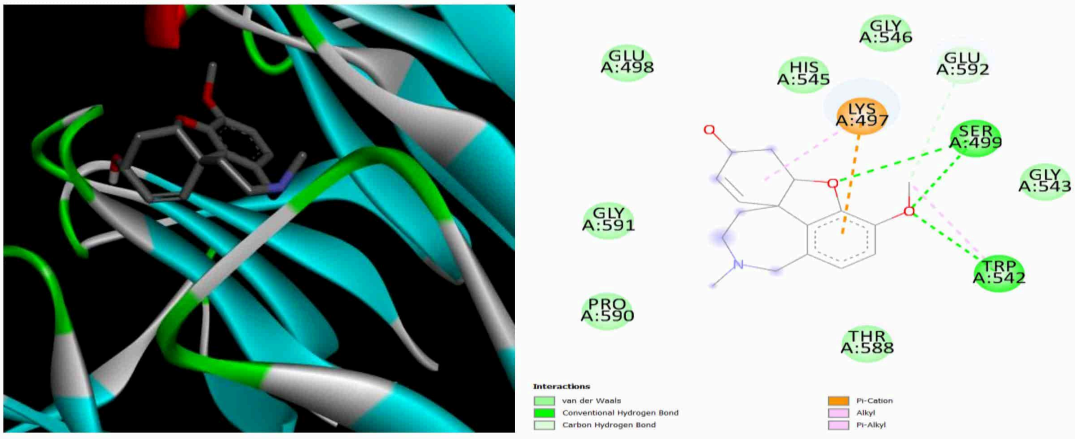
Memantine



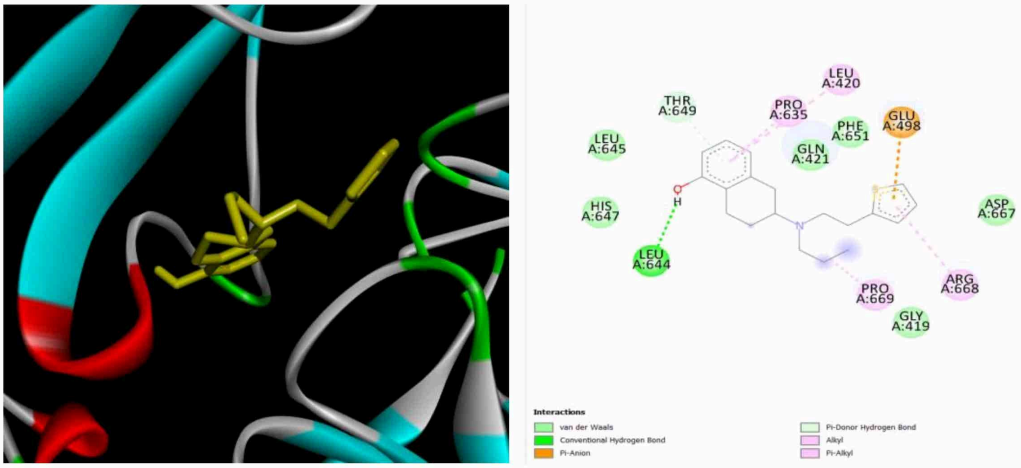
Metixene



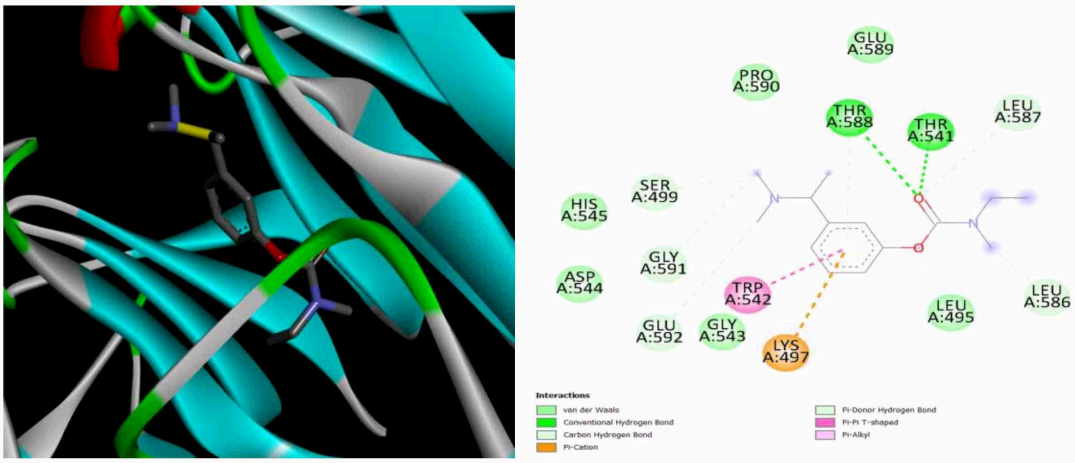
Thiethylperazine



Galantamine

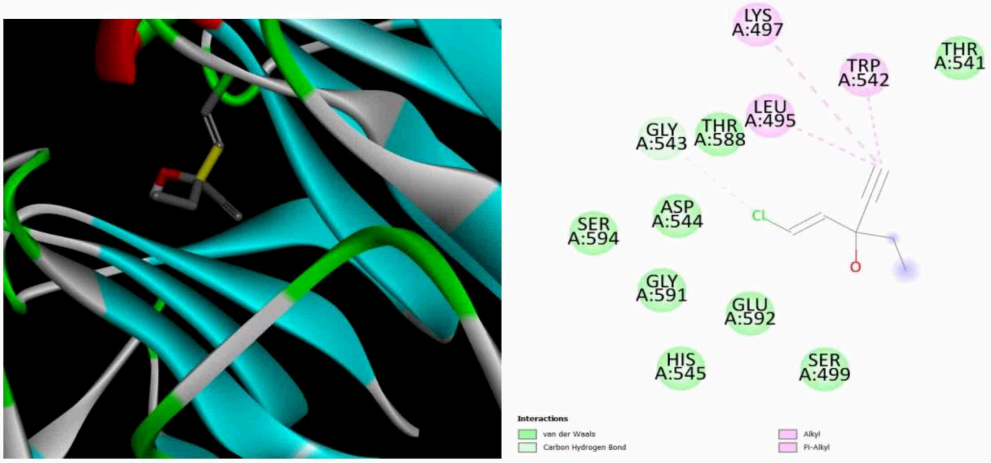


Rotigotine

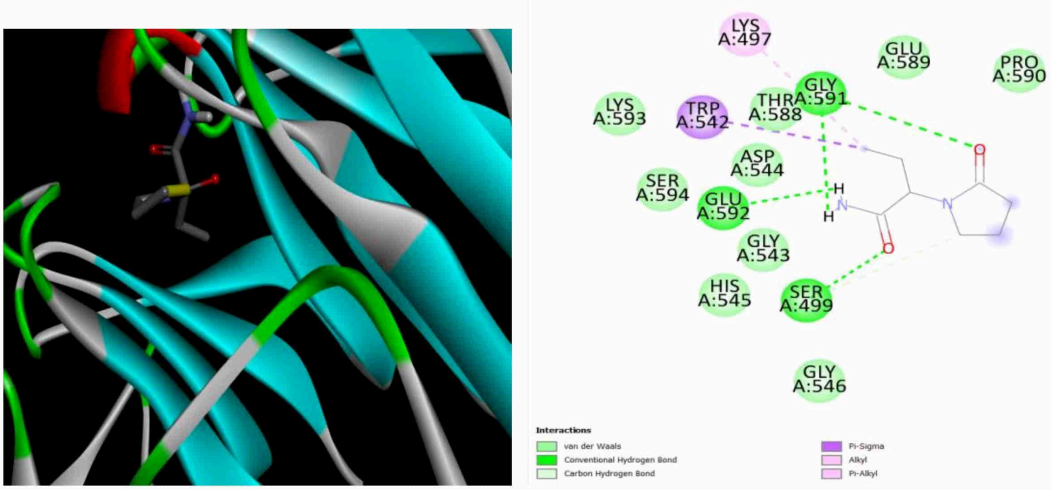


Rivastigmine

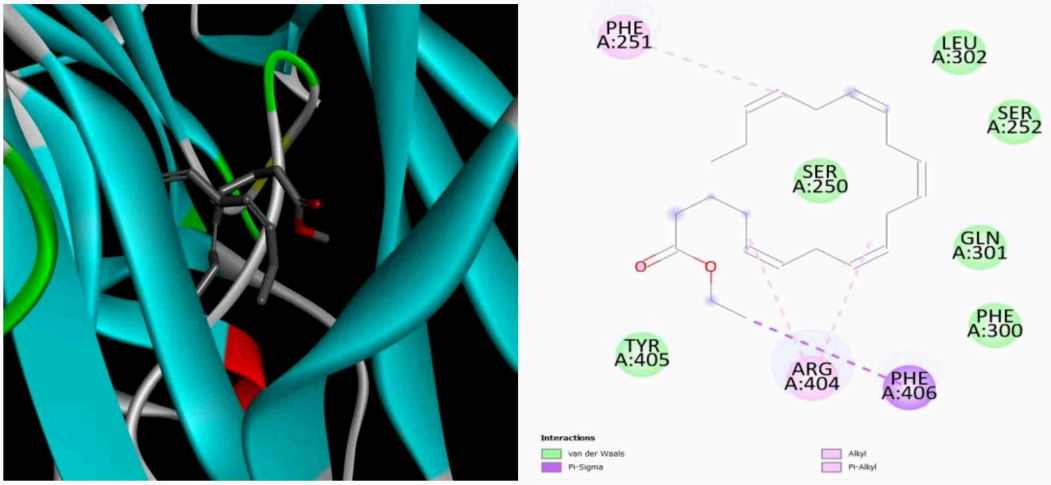
(i)



Ethchlorvynol



Levetiracetam



Vascepa

Fig 4.2. 3D and 2D Docking results of Drugs with SORL1

4.2 PPI Analysis

After the PPI analysis of SOLR1 at highest confidence of 0.900, it was discovered that the protein has 7 nodes and 8 edges as shown in Fig 4.3. Highest confidence in STRING-DB gives pure results of protein-protein interactions. In this network, nodes represent the distinct proteins while edges represent the interactions between them. The network stats have been mentioned in Table II.

Average node degree of SORL1 is found to be 2.29 which is defined as the number of interactions that a specific protein has on an average in the network stats. The degree of connectivity between the nodes is defined by the clustering coefficient which is found to be 0.876. There are several networks with connections having high values of clustering coefficient. If the nodes were chosen randomly, the predicted number of edges indicates how many edges would be expected in a network stat. The observed number of edges is quite considerable and the nodes shown are not random in nature, which is well indicated by a tiny PPI enrichment p-value that is found to be 0.262.

Table II. Network Stats of SORL1 using STRING-DB

Number of nodes	7
Number of Edges	8
Average node degrees	2.29
Avg. Local clustering Coefficient	0.876
PPI enrichment p-value	0.262
Expected number of edges	6

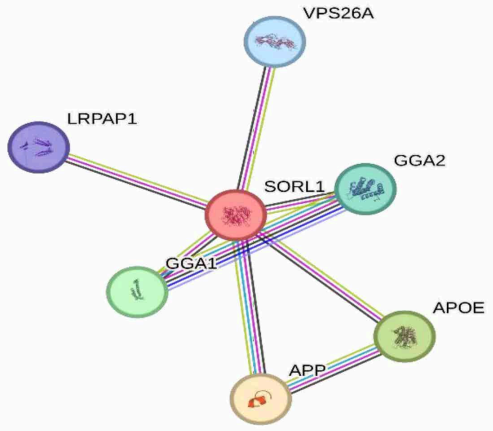


Fig 4.3. PPI analysis of SORL1 using STRING-DB

4.3 ADMET Analysis

An ADMET analysis of 21 drugs was performed by using the pkCSM software in order to identify their Absorption, Distribution, Metabolism, Excretion, and Toxicity properties. This analysis is very essential for assessing the safety profiles and pharmacokinetic properties of the drugs. ADMET analysis of a drug has a significant role in drug development for their potential therapeutic application. The results of this comprehensive assessment are given in Table, which provides information of the performance of each drug across several ADMET parameters. These parameters include blood-brain barrier permeability, oral bioavailability, Intestinal absorption, cytochrome P450 enzyme inhibition, total clearance, and potential toxic effects. The data mentioned Table III. highlight the limitations and strengths of each drug, thus helps in further optimization.

Table III. ADMET analysis of the Interacting Drugs

Drugs	Intestinal Absorption (%)	BBB permeability (log BB)	CNS permeability (log PS)	Total clearance (log ml/min/kg)	Max. Tolerated dose (log mg/kg/day)
Doxorubicin	62.37	-1.379	-4.307	0.987	0.081
Vascepa	93	0.784	-1.293	2.296	-0.257
Bromperidol	89	0.283	-1.394	1.009	-0.071
Thorazine	92	0.788	-1.412	0.6	0.387
Rivastigmine	88.45	0.508	-2.225	0.557	0.382
Memantine	91.23	0.603	-2.478	0.548	0.322
Galantamine	94	-0.081	-2.511	0.991	-0.423
Donepezil	93	0.157	-1.464	0.987	-0.217

Formoterol	89.71	-0.832	-2.977	1.111	0.144
Levetiracetam	84.22	-0.279	-3.427	0.447	1.049
Tacrine	82.44	-0.285	-1.296	0.463	0.342

DISCUSSION

The abnormal accumulation of neurofibrillary tangles and amyloid beta in nerve cells are considered to be the major hallmark of Alzheimer's disease. Besides Apolipoprotein allele, PSEN1 and PSEN2 gene which act as a major contributor in AD, SORL1 gene emerged as a foremost breakthrough in AD. Increased expression level of SORL1 gene reduces the probability of Alzheimer's disease and any mutation in SORL1 can trigger the chances of EOAD. Dietary supplements containing DHA such as fish oil has been found to enhance the expression level of SORL1 gene. DHA has been found to be the biomarker in the expression of SORL1 gene.

The present study used the computational approach to predict the future prospects and therapeutic potential of 21 different dementia medications by showing the interaction with SORL1 receptor protein. Molecular docking using PyRx is used to analyse the protein-drug interaction by analysing their binding energies. Docking results demonstrated that, Doxorubicin is found to possess the most negative binding energy of -9 kcal/mol among all, thereby showing the most efficient interaction with SORL1.It shows the highest binding affinity with the receptor followed by Lorpiprazole and Nicergoline. DHA containing drug, Vascepa, which also showed the binding affinity of -4.9 kcal/mol with SORL1 suggesting it to be a significant key player in SORL1 gene expression. Docking results expected to show Vascepa as the most interactive drug with SORL1 in comparison with other dementia medications but still some laboratory investigations must be performed to furnish robust evidence about the interaction of vascepa with SORL1 receptor.

PPI network analysis of SOLR1 resulted that SORL1 show interactions with APOE, APP, CGA1, GGA2, LRPAP1 and VPS26A using STRING-DB. According to this information, these interactions suggested the involvement of SOLR1 in numerous neurological pathways specifically in neurodegenerative disorders. The pharmacokinetic properties of the drugs have been identified by pkCSM software. This ADMET analysis gave valuable insights of intestinal absorption permeability, minimum toxicity dose and bioavailability. However, further research and studies needs to be done on SORL1 gene to identify the most appropriate therapeutic potentials in treatment of AD.

CHAPTER 5

CONCLUSION

The computational approach conducted in this study in order to understand the role and pharmacological interactions of SORL1 in treating Alzheimer's disease (AD) offers a promising opportunity to identify the advancing therapeutic potentials in future. Bioinformatics tools such as molecular docking elucidated the complex interactions between SORL1 and various pharmacological drugs, which successfully provides insights role of SORl1 in amyloid precursor protein (APP) processing and trafficking. These computational approaches help in identifying the therapeutic potentials and the optimization of existing therapies. Experimental validations have further catalysed the discovery of novel compounds that has the capability to modulate SORL1 function which is to reduce amyloid-beta accumulation and tau-neurofibrillary tangles in neurons. Additionally, computational methods assisted in drug repurposing in order to use existing drugs for treating AD. This approach can help in personalizing treatment by assessing genetic profiles of each patient, leading to increased effectiveness of a drug. Nevertheless, this computational approach may hinder the accuracy of the quality of the data and models. But persistent advancements and innovations in computational algorithms and high-throughput biological data collection will further enhance the accuracy in these models. In conclusion, computational approaches are found to be integral to the future of treatment of AD to propose a deeper understanding of role of SORL1 and its pharmacological interactions, thereby directing towards innovative therapies.

Future Perspectives

Based on the present study, the following multifaceted and promising future perspectives can be drawn:

Combination Therapies: Synergistic approaches of using anti-amyloid, anti-tau or cholinesterase therapies in combination with SORL1-targeted therapies can provide a multidimensional approach to treat AD. Further investigations must be done to cure AD using synergistic approach.

Experimental Validations: In the light of present study, further in vivo and in vitro experiments must be implemented by the scientists to understand pharmacological interactions of SORL1 with DHA containing drug Vascepa which could result in upregulation of SORL1 gene expression.

Clinical Studies: Appropriate selection of patient populations is essential to understand the effectiveness of drugs in upregulating the SORL1 gene expression. Clinical Studies must choose patients with different stages of Alzheimer's disease to examine how SORL1-targeted therapies outperforms other therapies across different severity levels. Genetic screening for SORL1 variants could also be helpful identifying the subvariants that might benefit from these therapies.

Drug Delivery Systems: The pharmacodynamic effectiveness of the drugs in body can be enhanced by using efficient drug delivery systems and drug formulations. Nanoparticles mediated drug delivery systems such as carbon tube, liposome mediated, dendrimers, quantum dots etc. can be used to boost the effectiveness of a drug against target protein.

Gene Editing: Future efforts must be made to utilise gene editing tools such as CRISPR/CAS system can be used to replace mutated SORL1 gene with a corrected gene as a permanent cure to early onset of AD.

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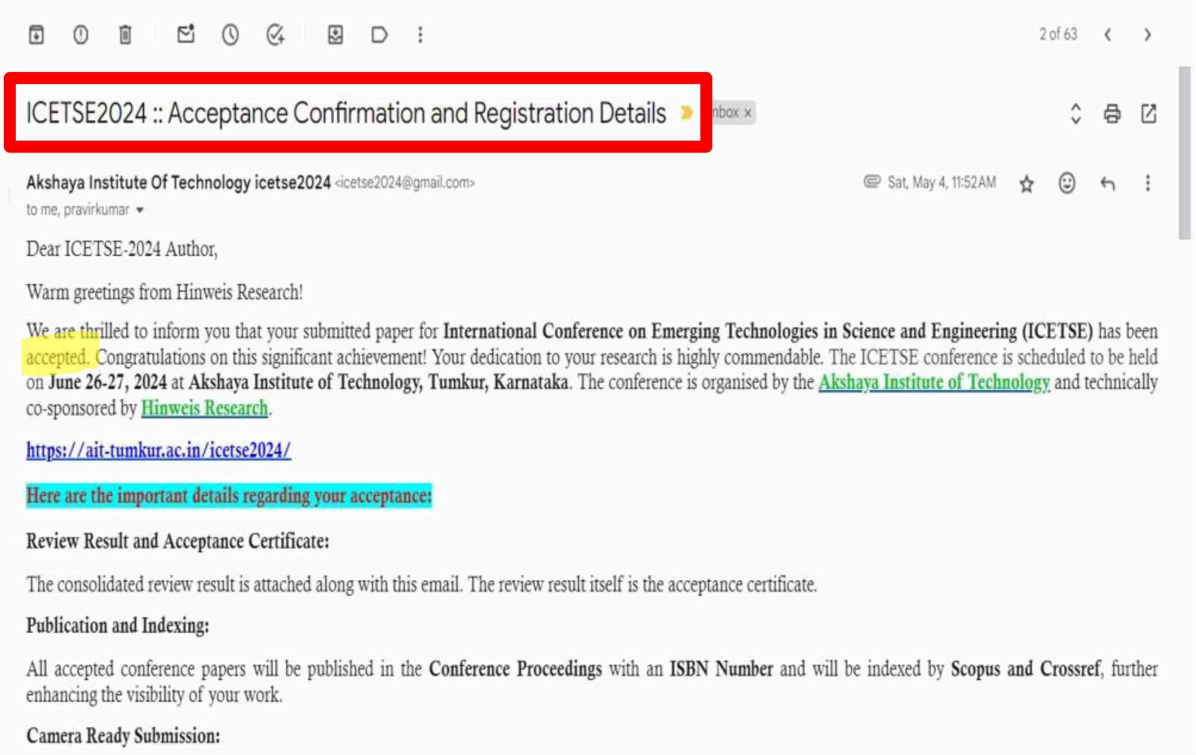

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