

# **Effect of Marine Metabolites on Neurodegenerative Disease-**

## ***An In silico Study***

Submitted in partial fulfillment of the requirements under Bachelor of technology  
degree in Biotechnology

By

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**DEPARTMENT OF BIOTECHNOLOGY**

**SCHOOL OF BIO AND CHEMICAL ENGINEERING**

# **SATHYABAMA**

**Institute of Science and Technology**

**(Deemed to be University)**

Accredited with Grade "A" by NAAC

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APRIL-2021



# **SATHYABAMA**

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### **BONAFIDE CERTIFICATE**

This is to certify that this project report is the bonafide work of **S.Mohana Priya (37230046)** who carried out the project entitled "**Effect Of Marine Metabolites On Neurodegenerative Disease - An Insilico Study**" under the supervision of Dr.K.Renugadevi , Assistant Professor ,Sathyabama Institute of Science and Technology, Chennai 119.

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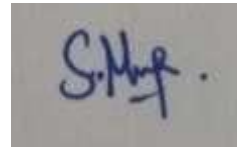
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## **DECLARATION**

I **S.Mohana Priya (37230046)** hereby declare that the Project Report entitled **“Effects Of Marine Metabolites On Neurodegenerative Disease – An Insilico Study”** done by me under the guidance of **Dr.K.Renugadevi, Assistant Professor, Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai-119** is submitted in partial fulfillment of the requirements for the award of **Bachelor Of Technology** degree in **Biotechnology**.

**Date :16.04.21**

**Place: Chennai**

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**Signature of the Candidate**

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## ABSTRACT

Neurodegenerative diseases are most commonly characterized by the neuronal cell death that are occurred most commonly by neuroinflammation, oxidative stress, aging, life style factors and in some cases due to mutation or variants in genes. The most common neurodegenerative disease are Alzheimer's and Parkinson's disorder. No treatment or medication is available for complete cure of this disease but certain medication may reduce the risk and symptoms which may delay the onset of the disease. Limited drugs are available on this disease since it is highly complex to understand the brain pathway. Thus, this study focuses on bringing out ideas of utilizing Saturated fatty acid from blue green algae(cyanobacteria) as a novel therapeutic lead compounds to reduce the risks of neurodegenerative disease. In silico analysis has showed that palmitic acid has very good and effective binding activity against maximum of possible targets. Comparison of palmitic acid with the available drug compound showed relatively similar binding energy with better biological activity and ADMET properties. Virtual screening on the analogs of lead compound palmitic acid potentially showed that 10 oxo hexanoic acid had better binding when compared with the first, which could be analyzed for in vivo potency that may be a lead compounds in future. From in silico study, the saturated fatty acid such as Palmitic acid may be a promising therapeutic lead molecule in future which may possibly reduce the risk associated with neurodegenerative disease.

**Keywords:** Neurodegenerative, Marine metabolite, Alzheimer's, Parkinson's, Computational analysis.

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## ABBREVIATIONS

|      |                                                 |
|------|-------------------------------------------------|
| AD   | Alzheimer's Disease                             |
| PD   | Parkinson's Disease                             |
| ADME | Absorption, Distribution, Metabolism, Excretion |
| NMR  | Nuclear Magnetic Resonance                      |
| MAO  | Mono Amine Oxidase                              |
| mCHR | Muscarinic Acetylcholine Receptor               |
| APP  | Amyloid Precursor Protein                       |
| NMDA | N-methyl-D-aspartate                            |
| PCA  | Posterior cortical atrophy                      |
| PPA  | Primary progressive aphasia                     |
| NCBI | National Centre for Biotechnology Information   |
| BTIS | Bio-Technology Information System               |
| BBB  | Blood Brain Barrier                             |
| HIA  | Human Intestinal Absorption                     |
| MDCK | Madin-Darby Canine Kidney                       |
| CYP  | Cytochrome P                                    |
| hERG | Human Ether-a-go-go Related Gene                |
| TA   | Therapeutic Area                                |
| CMC  | Chemistry Manufacturing and Control             |
| MDDR | Molecular Detection of Drug Resistance          |
| WDI  | World Drug Index                                |
| PASS | Prediction of Activity Spectra for Substances   |

# CHAPTER 1

## INTRODUCTION

### 1.1. AN OVERVIEW

Neurodegenerative diseases represent a major threat to human health. These age-dependent disorders are becoming increasingly prevalent, in part because the elderly population has increased in recent years (Heemels MT, 2016). The most common neurodegenerative diseases are Alzheimer's disease and Parkinson's disease. All neurodegenerative diseases would affect different regions of the brain, which may exhibit distinctive and evident characteristics at the phenotypic level, i.e., progressive loss of sensory-motor may occur and cognitive functions. (Woolley JD, *et al* 2011). But on overall analysis they share similar etiology at the molecular and cellular level (Vadakkan KI 2016) (Hervas R, *et al* 2012) (Gitler AD, *et al* 2017). A common cause of these conditions is a long run effect of period until which sufficient protein accumulates, followed by a cascade of symptoms over 2-20 years, with increasing disability leading to death of cells. This provides a broader therapeutic ideas, especially early diagnostic of this groups at risk are identified earlier and preclinical diagnosis becomes feasible. The increasing incidence with age can be seen as a threat especially for this disorder. (Williams A. 2002).

### 1.2. ALZHEIMER'S DISEASE:

Alzheimer's are the progressive disease that may cause the brain and its functions to shrink including the death of the brain cells. The most common cause of this disorder is not fully known, but certain study shows that it is age related along with certain genetic, environmental and most probably the lifestyle factors. Alzheimer's basically destroys the memory and thinking skills in slow progress. Alzheimer's disease (AD) is a neurodegenerative and prominent protein-conformational change disease (PCD) (Adav SS, *et al* 2016) primarily caused by the aberrant processing and multiple polymerization of normally soluble proteins (Leandro P, *et al* 2008). Major cause of the condition is said to be the formation of tangles and plaques in the brain due to certain abnormalities. Plaques usually develops between the dying brain cells. They are made from beta-amyloid which is protein. The tangles occur within the nerve cells. They are made from tau, an another type of protein in

brain. When misfolding of soluble neuronal proteins attain altered conformations at certain point due to mutation, external factors, or aging, and aggregate, leads to abnormal neuronal functions and loss (Horwich A 2002). The loss of connection between the nerve cells or neurons in the brain also serves as a main reason. Alzheimer's disease can range from mild, moderate to severe. The field of research toward understanding AD pathogenesis and designing efficient therapies is vast. AD is a highly complex and progressive neurodegenerative disease (Henry W.*et al* 2010) It had drastically become one of the leading cause of dementia cases worldwide.

### 1.3. PARKINSON'S DISEASE:

Parkinson's disease is the neurodegenerative disease characterized by a progressive loss of dopaminergic neurons in the brain region called substantia nigra pars compacta. In PD, there is a long dormancy between the first damage to cells in nuclei of the nervous system, and for the onset and progression of clinical symptoms. The symptoms of PD does not develop until 70–80% of dopaminergic neurons have already been lost which makes this disorder more incurable. (El-Agnaf OMA, *et al* 2006). Thus, identifying patients in the interval between the first onset of dopaminergic cell loss and the advent of clinical parkinsonism may be more difficult and important for the development of effective neuroprotective treatment strategies (Berendse HW,*et al* 2001). Decrease in dopamine levels leads to abnormal brain activity that causes impaired movement and other symptoms of Parkinson's disease. Specific genetic mutations may cause Parkinson's disease, but it is uncommon and rare in many cases. Clumps of specific substances in brain cells are microscopic markers of Parkinson's disease called Lewy bodies and researchers believe that Lewy bodies has an important hint to cause Parkinson's disease. Although many substances are found within Lewy bodies, scientists believe an important one is the widespread protein called alpha-synuclein. Alpha synuclein is found in all Lewy bodies in a form of clumps which cannot be break down by the normal cells. This is presently an important focus among scientist on Parkinson's disease research. There is no record or proof of exact medication on this disease since the cause of this disease is still a mystery.

#### 1.4. MARINE SOURCE:

More than seventy percent of the earth's surface is overall covered by water but research on exploring pharmacological aspect is limited since many are unexplored. Several drugs from marine species are still serving an efficient potential on diseases like cancer. Many aquatic organisms are screened on basis of anti inflammatory, antioxidant, antibacterial etc. (Harshad Malve 2016). Marine diversity is nowadays depended majorly for new drug discovery for several diseases. Blue green algae also known as cyanobacteria are generally more abundant and are found in every habitat from oceans to lakes which belongs to either types of macroalgae present. Certain species of cyanobacteria has enormous amount of health benefits on human life. Some types of blue green algae are used and taken by the human kind for its high protein content. There are so many bioactive compounds present in cyanobacteria. One among which is saturated fatty acid group present in them. Certain study and researches has come out with certain health benefits of these fatty acids on human life and in relevance with neurodegenerative disease. .Blue green algae has higher amount of saturated fatty acids compared to other monounsaturated and unsaturated fatty acids.

#### 1.5. IN SILICO ANALYSIS:

In Silico approach provides a fastest way of finding an effective lead compounds against the target of any particular disease. In silico approach narrow downs the search of potential and efficient lead compound.

##### **1.5.1. RCBS -PDB:**

RCBS-PDB(Research collaboratory for structural Bioinformatics Protein Data Bank),which is an online tool to obtain 3D structures of the target.The data are typically obtained by, NMR spectroscopy, X-ray crystallography, cryo-electron. These are submitted by biologists and biochemists for reference .(Helen Berman ,et al 2000).The PDB was originally established with 7 structure by Brookhaven National Laboratory under the leadership of Walter Hamilton in 1971.

##### **1.5.2. Pubchem:**

PubChem is a database of all chemical molecules present in all species and their activities against biological assays. The lead compound from any source can be

obtained from Pubchem online database. PubChem contains one of the largest corpus of publicly available chemical information which enables researchers worldwide to obtain information about any chemical compound that is useful in many fields like biotechnological, Pharmaceutical, therapeutical chemistry and majorly for drug discovery.

#### **1.5.3. Drug Bank:**

The Drug Bank database is an online tool which is free, accessible and comprehensive database that contains information on drugs and drug target. Certain targets can be obtained through this platform by giving respective drug name whose interaction, half life, toxicity information are detailed whose further interaction can be carried out. Drug Bank database is frequently updated with the approved and emerged drugs. (Wishart DS *et al* 2007).

#### **1.5.4. Pharmacokinetics:**

Pharmacokinetics studies enables biologist to study even more about the lead compound. Pharmacokinetics prediction gives detailed study on possibilities of drug likeliness, ADME and toxicity. (Meibohm B *et al* 2002). By using the range given in the tool, the toxicity, drug like compounds can be predicted from the lead compound. Thus, this data helps in rapid identification of lead compound.

#### **1.5.5. Pharmacodynamics:**

Pharmacodynamics studies predicts the possible biological activity of the lead compound. The mechanism of action on human body can be predicted through this study. The selected targets and the lead compound can be studied for interaction through docking. (Meibohm B *et al* 2002).

#### **1.5.6. Molecular interaction:**

Molecular interaction designed to predict an interactive environment for viewing and editing molecular structures, X-ray reflection data sequences, scripts, and other data. It also provides viewers for displaying plots and other graphical representations of interactions and data. Important steps considered in molecular interaction is the binding of receptor to the ligand compound and poses of interaction (orientations). Molecular interaction enables to achieve better binding information based on the research expectations. Major challenge in drug

development is predicting better binding which can be easily predicted through softwares.( Pagadala NS *et al* 2017) .The application runs on Window and is a fully integrated desktop environment. Therefore, computational approach on drug discovery is fastest and cost efficient which helps researcher's to find the effective lead compound without any delayed or failure process.

#### ***1.5.7. Virtual screening:***

Virtual screening is the screening of the small molecules to bind to its particular receptor of a disease. The similar structure, substructures can be obtained through virtual screening tools. One among them is Zinc Database (ZINC 15).Zinc database provides details on all possible similar structure ,available compounds in the market and details about its manufacturers.

## CHAPTER 2

### REVIEW OF LITERATURE

*Armand Schachter AS et al.,(2000)* suggests that Alzheimer's has become most common type of dementia from past few decades. The current therapeutic approaches on dementia has an incomplete understanding towards the basic pathology of AD. Cholinergic deficits have been well described to make cholinesterase inhibitors (donepezil, tacrine, rivastigmine and galantamine) which has become the recommended treatment of cognitive disturbance in patients with AD. Treatments with antioxidants, anti-inflammatory agents are still controversial though they are showing effective results.

*George DM et al.,(2015)* suggested that Parkinson's is a chronic and progressive disorder related to both motor and non motor features. The ultimate goal in the management of PD is to treat the symptomatic motor and nonmotor features of the disorder, with the objective of improving the patient's overall quality of life. Therapies that slow the progression of the disease or provide a neuroprotective effect have not been identified yet.

*Helen Berman et al.,(2000)* describes that Protein Data Bank (PDB) was established at Brookhaven National Laboratories (BNL) in 1971 as an archive for crystal structure of biological macromolecules

*Kim S et al.,(2016)* confirms that PubChem is committed to continue serving as a key chemical information resource not only to the biomedical research community but also to the scientific community as a whole. Report of September 2015 suggests that PubChem has more than 157 million depositor-provided data's from biologist and scientist on chemical substance descriptions. In addition 60 million unique chemical structures and its biological assay descriptions majorly covered about 10 thousand unique protein target sequences are been deposited. These data's are updated regularly.

*Mridula S et al.,(2019)* stated that acetylcholinesterase is the actual key enzyme which hydrolyses the neurotransmitter acetylcholine. Acetylcholinesterase plays an important role in the treatment of AD as a target. Inhibition of acetylcholinesterase

is mainly concentrated which evolved to formulate certain drug to reduce the symptoms of AD. The drug loses their efficacy at certain period of time. Thus, acetylcholinesterase enzyme plays as an important target for Alzheimer's.

*RangelBarajas C et al., (2015)* suggests that the Dopamine receptors play an important role in neurotoxicity and neurodegeneration. DA receptors are prone to change under certain pathological conditions. The rearrangements of intracellular signal pathways may occur that hardens the symptoms in aging diseases or neurodegenerative process. Thus dopamine receptor plays a crucial role as a target for Alzheimer's and Parkinson's disease.

*Petzer et al., (2009)* suggests dual-target-directed target for drugs and also may exhibit enhanced neuroprotective properties. MAO-B inhibition may enhance the increase of dopamine levels after levodopa treatment. This is particularly used in early stages of the disease when dopamine production may not be so severely effective or damaged. It provides neuroprotective properties in part by reducing the damaged effect caused by the dopamine in the brain.

*Scarffe LA et al., (2014)* reports that Parkin is a unique multifunctional ubiquitin ligase plays vital role in maintaining neuronal survival. It is underscored by our current recognition that Parkin dysfunction represents not only important cause of family related Parkinsonism but also a formal risk factor for the more common sporadic form of Parkinson's disease.

*Nguyen L et al., (2017)* brings out that Sigma receptors are now acknowledged as targets for novel therapeutic intervention in neurodegenerative conditions. These proteins are incriminated in diverse neural mechanisms, which includes the modulation of cell survival and function, calcium signalling, neurotransmitter release, synaptogenesis and inflammation. Sigma-1 receptors has multiple effect on signal pathways which appears to be promising, druggable targets to help combat the complex pathophysiology of neurodegenerative disorders.

*Jiang S Li Y et al., (2014)* reports M1 mAChR plays a crucial role in cognitive functions like learning and memory. Downregulation of M1 mAChR attributes to AD

and the specific activation and focus on M1 subtype is considered to be a promising strategy and therapeutic target for AD treatment.

*Bonifacio et al., (2007)* reports that COMT has also been suggested to play a role in modulating prefrontal dopamine neurotransmission. Levodopa is particularly useful when given with decarboxylase inhibitors, but its subsequent metabolism by catechol-O-methyltransferase (COMT) markedly limits its availability to the brain. The last decade showed that the use of catechol-O-methyltransferase inhibitors as adjuvants to the levodopa as a treatment, significantly improves the clinical benefits of this therapy.

*Brundin et al., (2017)* demonstrates Alpha-synuclein as a therapeutic target for PD has been growing recently not only due to the genetic link, but also because of the pathology of alpha-synuclein spreading through the nervous system and the cell-to-cell propagation of aggregated alpha-synuclein which makes them to focus as a major target.

*Elena Anahi Bignante et al.,(2013)* discusses about the A $\beta$  deposition in the brain region is an early event that occurs in the AD patients. This may precede to neuronal degeneration and cognitive decline by several years or even decades. Aggregated Ab-induced multimerization of APP at the cell surface represents a primary pathogenic event that triggers dying-back degeneration of neurons.

*Singh et al., (2006)* On examining Late-onset sporadic AD, It showed a significant genetic component, estimated at 50%–70%. The *APOE* gene has three variants which encode proteins such as ApoE2, ApoE3, and ApoE4 that differ at two amino acids. The *APOE E4* allele, which shares 10%–20% of various populations drastically increases the risk for AD Thus it plays effective role as a therapeutic target for Alzheimer's disease.

*Parameshwaran et al., (2008)* suggests that the impairment which occurs in AD also has a relation with synaptic plasticity. NMDA receptor plays a critical role in this neurodegeneration as a part. (*Parameshwaran et al., 2008*). Glutamatergic neurotransmission via NMDAR is crucial for synaptic plasticity and neurons survival. However, excessive NMDAR activity can lead to excitotoxicity and promotes cell death, which has an important potential mechanism of neurodegeneration occurred in AD.

*Demetrius M Maraganore et al., (2004)* reported that UCHL1 (Ubiquitin carboxy-terminal hydrolase L1) plays an important role in Parkinson's disorder which is a putative cysteine protease found abundantly in neuron and specifically expressed in neurons. He reported an adverse relation between the ubiquitin carboxy-terminal hydrolase L1 (UCHL1) gene S18Y variant type and Parkinson's disease (PD) has strong biological plausibility. Though it is not fully confirmed of being a promising therapeutic drug, it may act as a cause at some point. Mutations in UCHL1 has been showed in Parkinson diseased patients. A point mutation near the active site might change Ile93 to Met (I93M) which is linked to an increased risk of developing an autosomal dominant form of PD.

*Ripamonti E et al.,(2016)* illustrated a case study that there is no specific pattern related or reported as a strong diagnostic reason of a specific degenerative disease. Overall, AD patients were significantly more impaired in reading nonwords than words. Lexical decision impairment in the visual modality was seen to be related to PCA, while a similar deficit in the auditory modality is more suggestive of AD. A multiple examination and observations were carried out on the reading performance of the patients to identify the distribution of different kinds of dyslexia: phonological dyslexia occurred in 50% of patients affected by PCA: it fortunately occurred less in patients affected by Alzheimer's (15.8%) and PPA (16.7%). Surface dyslexia was observed only in one case of AD.

*Shingo Nakajima et al.,(2020)* demonstrated that lauric acid which is a natural saturated fatty acid has a potential to enhance mRNA expression of growth factor. These development was mediated by the ERK signalling. Lauric acid induced neuronal maturation required the presence of astrocytes. Astrocyte-conditioned medium also increase the presynaptic protein levels which acts in giving better neuroprotection. Therefore lauric acid could be one of the lipids activator for the neuron-glia cell communication for development of neurons.

## CHAPTER 3

### AIM & SCOPE

#### 3.1. AIM:

- To study the effective lead compound from marine metabolite for neurodegenerative disease such as Alzheimer's and Parkinson's through Insilico study.

#### 3.2. SCOPE:

- Insilico analysis on ultimately finding the lead drug compound for neurodegenerative disease such as Alzheimer's and Parkinson's disease will reduce the time and cost effectively in future research.
- The detailed prediction through pharmacokinetics and pharmacodynamics for the respective lead compounds enabled maximum determination for further studies. Thus this in silico approach has wide application and makes invitro and in vivo analysis easier for design and development of drug.
- Drugs can be formulated if they get qualified in both in vitro and in vivo studies.

## CHAPTER 4

### MATERIALS AND METHODS

#### 4.1. SELECTION OF TARGETS:

Targets are selected based on literature review for both Alzheimer's and Parkinson's which are the most common neurodegenerative disease. In this study, 24 target on both Alzheimer's and Parkinson's disorder has been analyzed and studied. The target structure are retrieved from PDB (Protein Data Bank), which is a database to obtain three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. The target structure for this study entirely depends on various literature survey. The targets selected not only focuses on the major targets of neurodegenerative disease such as Acetylcholinesterase, Dopamine receptor, NMDA receptor, Monoamine oxidase, Parkin, DJ 1 Protein etc, but also on the emerging target which may act as a promising therapeutic drug in future.

**TABLE 4.1. Target selected for the study on neurodegenerative disease**

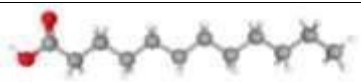
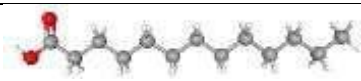
| <b>TARGET SELECTED</b>                                      | <b>PDB ID</b> |
|-------------------------------------------------------------|---------------|
| Human acetylcholinesterase                                  | 5HFA          |
| Structure of the D2 Dopamine Receptor                       | 6CM4          |
| The C-JUN-N-Terminal Kinase (JNK3S)                         | 1JNK          |
| Glucocerebrosidase                                          | 6Q1N          |
| Structure of human Parkin G319A                             | 5C9V          |
| Human sigma-1 receptor                                      | 5HK1          |
| Human Monoamine Oxidase B                                   | 1GOS          |
| Structure of the human M1 muscarinic acetylcholine receptor | 5CXV          |
| Crystal Structure of Human Catechol O-Methyltransferase     | 3BWM          |
| Human alpha-synuclein                                       | 3Q28          |
| Apolipoprotein E4, 22k domain                               | 1GS9          |
| Human DJ-1                                                  | 1PDW          |
| Human prion protein                                         | 1QLX          |

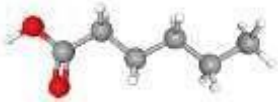
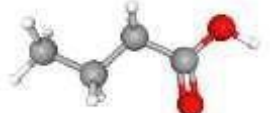
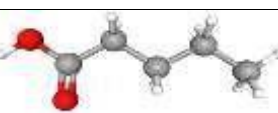
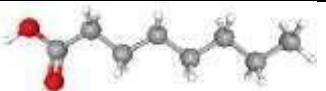
|                                                                                 |      |
|---------------------------------------------------------------------------------|------|
| KEAP1 Kelch domain(Nrf2)                                                        | 6TYP |
| TNF alpha converting enzyme                                                     | 2FV9 |
| Adaptor Protein 2 Associated Kinase (AAK1                                       | 4WSQ |
| Glycogen synthase kinase 3 beta                                                 | 1H8F |
| NMDA Receptor                                                                   | 1PBQ |
| Human GABA(B) receptor                                                          | 4MRM |
| ROC domain from the Parkinson's disease-associated leucine-rich repeat kinase 2 | 2ZEJ |
| Human A2A Adenosine Receptor                                                    | 3EML |
| I93M Mutant of Ubiquitin Carboxy-terminal Hydrolase L1                          | 3IRT |
| Human Beta Secretase                                                            | 4D8C |
| E2 domain of Human Amyloid Precursor-Like Protein                               | 3Q7G |

#### 4.2. SELECTION OF LIGANDS:

Ligands are selected based on the GC-MS analysis on fatty acid composition of blue green algae through literature review. In this study Saturated fatty acids are focussed for their positive activity on neurodegenerative disease. The ligand compounds are retrieved from pubchem.3D conformer structure is been obtained for interaction studies. The selected saturated fatty acids are most generally present in all maximum species of blue green alga

**TABLE: 4.2. List of Ligands**

| LIGANDS            | PUBCHEM ID | STRUCTURE                                                                             |
|--------------------|------------|---------------------------------------------------------------------------------------|
| Lauric acid        | 3893       |  |
| Palmitic acid      | 985        |  |
| Tetradecanoic acid | 11005      |  |
| Tridecanoic acid   | 12530      |  |

|                    |       |                                                                                     |
|--------------------|-------|-------------------------------------------------------------------------------------|
| Hexanoic acid      | 8892  |  |
| Butanoic acid      | 264   |  |
| Pentanoic acid     | 7991  |  |
| Pentadecanoic acid | 13849 |  |
| Undecanoic acid    | 8180  |  |
| Octanoic acid      | 379   |  |
| Decanoic acid      | 2969  |  |

#### 4.3. DRUG BANK:

Drug bank was used to verify the selected targets using the known drug available for neurodegenerative disease. Certain well known and trusted drugs in treatment and medication are donepezil, levodopa. The targets of these drugs and mechanism of action can be clearly studies through drug bank database. The comparative studies on drug with the target was done by retrieving 3D structure of the drug from drug bank.

#### 4.4. ONLINE SMILES TRANSLATOR:

The next procedure after the selection of ligands is to carry out pharmacodynamics studies. To carry out this study, canonical smiles notation was retrieved from PubChem for the respective ligands. Since pharmacodynamics study using PreADMET tool needed mol format of the lead compound ,Online smiles translator was used for conversion. Online smiles translator can be used to obtain any form of format by either uploading the smiles notation or by uploading the PDB file format. The structure for certain lead compound which is not available in PubChem can also be obtained using online smiles translator. In this study all mol format of the lead

compounds were obtained from online smiles translator using canonical smiles notation.

#### 4.5. PHARMACOKINETICS STUDIES:

Pharmacodynamic studies was carried out using PreADMET studies ,an online tool for approximate prediction on the lead compound overall.

Pharmacodynamics studies included toxicity , drug likeliness and ADME study. All selected saturated, fatty acids were analysed. Based on the results the further procedures were carried out.

##### 4.5.1. ADME Prediction:

###### 4.5.1.1. Blood Brain Barrier Penetration:

Blood-Brain Barrier (BBB) penetration is represented as  $BB = [Brain]/[Blood]$ , where [Brain] and [Blood] are the steady-state concentration of radiolabelled compounds in brain and peripheral blood. Predicting BBB penetration means predicting whether compounds pass across the blood-brain barrier. (Ajay GW. *et al* 1999) and (Lobell M *et al* 2003).

**Table.4.3. Parameter for prediction of BBB Penetration**

|                       |                             |
|-----------------------|-----------------------------|
| CNS active compound   | More than 0.4/More than 1.0 |
| CNS inactive compound | Less than 0.4/Less than 1.0 |

**Table.4.4. Actual Parameter for BBB used by PreADMET [ Ma X. *et al* 2005]**

|                          |               |
|--------------------------|---------------|
| High absorption to CNS   | More than 2.0 |
| Middle absorption to CNS | 2.0 – 0.1     |
| Low absorption to CNS    | Less than 0.1 |

###### 4.5.1.2. Human Intestine absorption:

Predicting human intestinal absorption of drugs is very important for identify potential drug candidate. In PreADMET we can predict the percentage of human intestinal absorption (%HIA) of the particular lead compound.

**Table.4.5. Parameter for HIA Prediction**

|                              |         |
|------------------------------|---------|
| Well absorbed compound       | 70-100% |
| Moderately absorbed compound | 20-70%  |
| Low absorbed compound        | 0-20%   |

**4.5.1.3. Caco2 cell and MDCK Permeability:**

Numerous *in vitro* methods have been used in the drug selection process for assessing the intestinal absorption of drug candidates. Among them, Caco-2 cell model and MDCK cell model has been recommended as a reliable *in vitro* model for the prediction of oral drug absorption.

**Table.4.6. Parameter for Caco2 cell and MDCK Permeability**

|                     |              |
|---------------------|--------------|
| High Permeability   | More than 70 |
| Medium Permeability | 4-70         |
| Low Permeability    | Less than 4  |

**4.5.2. Toxicity Prediction:**

Toxicity Prediction gives detailed information about the toxicity of the lead compound. Generally toxicity is predicted using mouse or rat in *in vivo* studies. InPreADME tool below is the parameter followed.

**Table.4.7. Parameter for Toxicity Prediction**

|          |                                         |
|----------|-----------------------------------------|
| Negative | Clear evidence of carcinogenic activity |
| Positive | No evidence of carcinogenic activity    |

**4.5.3. Druglikeness:**

Drug likeness prediction included the following parameters to be considered to design and develop the drug compound.

#### 4.5.3.1. *Lipinski's Rule:*

Lipinski's Rule, so called "Rule of Five", is published by Christopher A. (Lipinski CA 2004). in Pfizer Central Research (Groton, NJ, USA). A subset of 2245 compounds from WDI (World Drug Index) database was selected and drug-likeness was designed.

- No. of hydrogen bond donors  $\leq 5$
- No. hydrogen bond acceptor  $\leq 10$
- Molecular weight  $\leq 500$

#### 4.5.3.2. *CMC Rule:*

CMC rule is similar to that of Rule of Five. The full form of CMC is Chemistry, Manufacturing and Control. The indication of qualified means it is CMC clean lead molecule. If it shows not qualified, then it has certain parameters which on further study can be analysed and taken up for drug discovery.

#### 4.5.3.3. *MDDR:*

Rule of five cannot be used to discriminate whether the lead compound is drug or non drug like directly. Thus MDDR (Molecular Detection of Drug Resistance) like rule is used to predict whether a lead compound may or may not be a drug molecule. MDDR-like rule are the number of rings, the number of rigid bonds and the number of rotatable bonds.

#### 4.6. PHARMACODYNAMICS:

Pass analysis was used in this study to predict the pharmacodynamics of the selected lead compounds. It is simply known as way to drug. The lead compounds after their pharmacokinetics predictions, the lead compounds are further analysed for their biological activities on human body. The mechanism of action and adverse effects are analysed using Pass server tool. This study mainly focused on the activity related to neurological disorder which may be effective enough for future drug design.

#### 4.7. MOLECULAR INTERACTION:

The targets and ligands after all analysis on particular are docked to predict the binding affinity and their interaction. In this study discovery studio 2020 (a latest and updated version has been used) from Madras Veterinary College, BTIS centre. This updated version was useful in exact interaction study on the selected targets and ligands.

#### 4.8. INTERACTION STUDY:

Discovery studio visualizer latest version was used to obtain the interacting residue, number of hydrogen bonds interacted. software was also used to measure the bond length on accordance with the interacted target and the ligand. These detailed studies may be useful to find drug even more effectively with no or low failure chances in in vivo and invitro studies.

#### 4.9. VIRTUAL SCREENING:

Virtual Screening was performed using Zinc Database. In this study, virtual screening is performed. Zinc database is an online database tool which has a collection of commercially available chemical compounds. Zinc database is different from other chemical database because it gives related chemical compound and three dimensional structure. Stock availability details and details on their manufacturers are also available. Virtual screening is a computational technique used to obtain small molecules to interact with the receptors, to obtain similarity structure, substructures etc .Zinc database for virtual screening is highly effective which gives detailed information on similar structure, chemical availability, FDA approved compounds etc.

## CHAPTER 5

### RESULTS AND DISCUSSION

The ligands compounds were obtained from a detailed literature study and its ADME (Absorption, Distribution, metabolism and excretion) of that particular metabolites was evaluated using PreADMET online tool.

Toxicity and drug likeliness was also predicted using PreADMET online tool and the below are the following results.

#### 5.1. ADME PREDICTION:

**Table.5.1. ADME Prediction on Lauric acid, Pentanoic acid, Palmitic acid:**

| ID                         | Pentanoic acid | Lauric acid | Palmitic acid |
|----------------------------|----------------|-------------|---------------|
| BBB                        | 1.22           | 2.30        | 8.21          |
| Buffer_solubility_mg_L     | 1322.53        | 1814.12     | 136.24        |
| Caco2                      | 20.53          | 22.27       | 26.07         |
| CYP_2D6_inhibition         | Non            | Non         | Non           |
| CYP_2D6_substrate          | Non            | Non         | Non           |
| CYP_3A4_inhibition         | Inhibitor      | Inhibitor   | Inhibitor     |
| CYP_3A4_substrate          | Non            | Non         | Non           |
| HIA                        | 89.08          | 97.00       | 98.29         |
| MDCK                       | 61.75          | 55.65       | 70.50         |
| Pgp_inhibition             | Non            | Non         | Inhibitor     |
| Plasma_Protein_Binding     | 76.37          | 100.00      | 100.00        |
| Pure_water_solubility_mg_L | 12442.40       | 47.22       | 1.70          |
| Skin_Permeability          | -2.38          | -0.79       | -0.58         |
| SKlogD_value               | 0.27           | 3.45        | 5.26          |
| SKlogP_value               | 1.52           | 4.70        | 6.51          |
| SKlogS_buffer              | -0.11          | -2.04       | -3.27         |
| SKlogS_pure                | -0.91          | -3.62       | -5.17         |

The result of ADME studies on Lauric acid Pentanoic acid and Palmitic acid has been represented in table 5.1. The blood brain barrier of all three acids presented above comes under the criteria according to the parameters of Pre ADME studies. Human intestinal absorption was more than 70% for all the acids in the table which means that they are suitable for oral intake. MDCK prediction suggests that pentanoic acid and lauric acid has medium permeability and palmitic acid has higher permeability. Pgp which means P-glycoprotein inhibition has been calculated through this study which suggest that inhibition of Pgp can result in increased bioavailability. Palmitic acid is inhibitor of Pgp. Lauric acid and pentanoic acid are not inhibitor of Pgp but may be effect in invitro study.

**TABLE 5.2. ADME Prediction on Butanoic acid, Hexanoic acid, Octanoic acid**

| ID                         | Butanoic acid | Hexanoic acid | Octanoic acid |
|----------------------------|---------------|---------------|---------------|
| BBB                        | 1.12          | 1.19          | 0.91          |
| Buffer_solubility_mg_L     | 231817        | 74027         | 22261         |
| Caco2                      | 20.17         | 20.73         | 1.21          |
| CYP_2D6_inhibition         | Non           | Non           | Non           |
| CYP_2D6_substrate          | Non           | Non           | Non           |
| CYP_3A4_inhibition         | Inhibitor     | Inhibitor     | Inhibitor     |
| CYP_3A4_substrate          | Non           | Non           | Non           |
| HIA                        | 86.76         | 90.92         | 93.71         |
| MDCK                       | 74.40         | 66.59         | 133.78        |
| Pgp_inhibition             | Non           | Non           | Non           |
| Plasma_Protein_Binding     | 76.52         | 84.63         | 100.00        |
| Pure_water_solubility_mg_L | 26203.30      | 5796.76       | 1207.58       |
| Skin_Permability           | -2.63         | -2.10088      | -1.52308      |
| SKlogD_value               | 0.17          | 0.72          | 1.63          |
| SKlogP_value               | 1.06          | 1.97          | 2.88          |
| SKlogS_buffer              | -0.42         | -0.19         | -0.81         |
| SKlogS_pure                | -0.52         | -1.30         | -2.07         |

From the table 5.2. the ADME studies of butanoic acid hexanoic acid and octanoic acid has been predicted. BBB permeant of all acids above was suitable according to the parameters. Human intestine absorption was good in all the acids as it had best high absorption .MDCK prediction suggested that hexanoic acid had middle

absorption. Butanoic acid and octanoic acid had high absorption in accordance with the parameters .Pgp inhibition showed non for all the three acids in table 5.2.

**TABLE 5.3.Adme Prediction on Decanoic acid, Pentadecanoic acid**

| <b>ID</b>                  | <b>Decanoic acid</b> | <b>Pentadecanoic acid</b> |
|----------------------------|----------------------|---------------------------|
| BBB                        | 0.72                 | 6.64                      |
| Buffer_solubility_mg_L     | 6440.83              | 261.68                    |
| Caco2                      | 19.74                | 25.04                     |
| CYP_2D6_inhibition         | Non                  | Non                       |
| CYP_2D6_substrate          | Non                  | Non                       |
| CYP_3A4_inhibition         | Inhibitor            | Inhibitor                 |
| CYP_3A4_substrate          | Non                  | Non                       |
| HIA                        | 95.67                | 98.11                     |
| MDCK                       | 165.36               | 70.79                     |
| Pgp_inhibition             | Non                  | Inhibitor                 |
| Plasma_Protein_Binding     | 100.00               | 100.00                    |
| Pure_water_solubility_mg_L | 242.04               | 3.92                      |
| Skin_Permeability          | -1.06                | -0.61                     |
| SKlogD_value               | 2.54                 | 4.81                      |
| SKlogP_value               | 3.79                 | 6.06                      |
| SKlogS_buffer              | -1.42                | -2.96                     |
| SKlogS_pure                | -2.85                | -4.79                     |

Table 5.3. represents the details of ADME study on decanoic acid, pentadecanoic acid. According to parameter the BBB permeant was suitable for all the above acids. Pentadecanoic acid acted as a Pgp inhibitor which can result in increased bioavailability. Human intestinal absorption was high for all three acids and MDCK prediction suggested that pentadecanoic acid had middle permeability and decanoic acid had high permeability.

**Table.5.4. ADME Prediction on Undecanoic acid, Tetradecanoic acid, Tridecanoic acid:**

| ID                         | Undecanoic acid | Tridecanoic acid | Tetradecanoic acid |
|----------------------------|-----------------|------------------|--------------------|
| BBB                        | 1.24            | 3.54             | 5.03               |
| Buffer_solubility_mg_L     | 3427.98         | 955.34           | 500.94             |
| Caco2                      | 21.29           | 23.16            | 24.07              |
| CYP_2D6_inhibition         | Non             | Non              | Non                |
| CYP_2D6_substrate          | Non             | Non              | Non                |
| CYP_3A4_inhibition         | Inhibitor       | Inhibitor        | Inhibitor          |
| CYP_3A4_substrate          | Non             | Non              | Non                |
| HIA                        | 96.41           | 97.48            | 97.84              |
| MDCK                       | 37.98           | 65.82            | 69.88              |
| Pgp_inhibition             | Non             | Inhibitor        | Inhibitor          |
| Plasma_Protein_Binding     | 100.00          | 100.00           | 100.00             |
| Pure_water_solubility_mg_L | 107.22          | 20.70            | 9.03               |
| Skin_Permability           | -0.90           | -0.71            | -0.65              |
| SKlogD_value               | 2.99            | 3.90             | 4.36               |
| SKlogP_value               | 4.24            | 5.15             | 5.60               |
| SKlogS_buffer              | -1.73           | -2.35            | -2.65              |
| SKlogS_pure                | -3.23           | -4.01            | -4.40              |

ADME studies on undecanoic acid, tetradecanoic acid and tridecanoic acid are represented in table 5.4. BBB permeant was suitable for all the above acids. Tetradecanoic acid and tridecanoic acid had Pgp inhibition which may result in increased bioavailability. All three acids had high human intestine absorption. MDCK prediction showed middle permeability for all three acids mentioned in table 5.4.

## 5.2. TOXICITY PREDICTION:

Toxicity was predicted using PreADMET Online tool and the result are given below.

**TABLE.5.5. Toxicity Prediction on Lauric acid , Pentanoic acid, Palmitic acid, Tetradecanoic acid :**

| ID            | Lauric acid | Pentanoic acid | Palmitic acid | Tetradecanoic acid |
|---------------|-------------|----------------|---------------|--------------------|
| Carcino_Mouse | Positive    | Negative       | Negative      | Negative           |
| Carcino_Rat   | Positive    | Positive       | Positive      | Positive           |

Toxicity prediction was carried out on ligand compounds. Table 5.5.represents the toxicity study on lauric acid, pentanoic acid, palmitic acid and tetradecanoic acid. Carcinogenicity on mouse and rat was predicted through this tool. Rodent carcinogenicity was positive for all the ligands in the table which means it has no carcinogenicity or toxicity. Lauric acid had positive on both mouse and rat. All these details are predictions and further study must be carried out for drug design.

**TABLE.5.6. Toxicity Prediction on Butanoic acid, Hexanoic acid, Octanoic acid, Decanoic acid:**

| ID            | Butanoic acid | Hexanoic acid | Octanoic acid | Decanoic Acid |
|---------------|---------------|---------------|---------------|---------------|
| Carcino_Mouse | Negative      | Negative      | Negative      | Positive      |
| Carcino_Rat   | Positive      | Positive      | Positive      | Positive      |

Toxicity prediction on Butanoic acid , hexanoic acid ,octanoic acid and decanoic acid is presented in table 5.6.Carcinogenicity on rat showed positive for all ligands in the table. Decanoic acid showed positive for both mouse and rat means it has no toxicity according to prediction.

**TABLE.5.7. Toxicity Prediction on Pentadecanoic acid, undecanoic acid, tridecanoic acid, Tetradecanoic acid:**

| <b>ID</b>     | <b>Pentadecanoic acid</b> | <b>Undecanoic acid</b> | <b>Tridecanoic Acid</b> |
|---------------|---------------------------|------------------------|-------------------------|
| Carcino_Mouse | Negative                  | Positive               | Negative                |
| Carcino_Rat   | Positive                  | Positive               | Positive                |

Toxicity prediction on pentadecanoic acid, undecanoic acid and tridecanoic acid is represented in table 5.7. All ligands showed positive in rodent carcinogenicity. undecanoic acid showed positive indication in both mouse and rat carcinogenicity prediction

The overall toxicity prediction of all the lead compounds suggests that Lauric acid, undecanoic acid and decanoic acid showed positive in both mouse and rat which means that it completely has no toxicity. Palmitic acid, Pentanoic acid, Pentadecanoic acid, Tetradecanoic acid, Tridecanoic acid, hexanoic acid, Butanoic acid, octanoic acid showed negative in mouse and positive in rat which means that it showed a little toxicity against mouse. All the details presented in the table are prediction. Further drug development must undergo invitro studies.

### 5.3. PREDICTION OF DRUGLIKELINESS:

**TABLE:5.8. Druglikeliness study on all the lead compounds**

| LIGAND COMPOUNDS   | CMC RULE      | MDDR LIKE RULE | RULE OF FIVE |
|--------------------|---------------|----------------|--------------|
| Lauric acid        | Qualified     | Mid structure  | Suitable     |
| Undecanoic acid    | Qualified     | Mid structure  | Suitable     |
| Palmitic acid      | Not Qualified | Mid structure  | Suitable     |
| Tetradecanoic acid | Qualified     | Mid structure  | Suitable     |
| Tridecanoic acid   | Qualified     | Mid structure  | Suitable     |
| Pentanoic acid     | Not Qualified | Non drug like  | Suitable     |
| Pentadecanoic acid | Not Qualified | Mid structure  | Suitable     |
| Hexanoic acid      | Not Qualified | Non drug like  | Suitable     |
| Decanoic acid      | Qualified     | Mid structure  | Suitable     |
| Butanoic acid      | Not Qualified | Non drug like  | Suitable     |
| Octanoic acid      | Not Qualified | Mid structure  | Suitable     |

The above table 5.8 suggests the study on drug likeliness of the lead compound. The prediction explains that the criteria like CMC, MDDR, and Rule of five are considered in this study. CMC is same as rule of five/Lipinski's rule. MDDR is a prediction which shows whether the lead compound would be a drug or non drug. All lead compounds are analysed on this aspect and the results showed that all compounds are suitable in accordance with the rule of five. This prediction would be further analysed in the process of drug design.

## 5.4. PHARMACODYNAMIC PREDICTION OF LIGANDS:

Pass online server was used in this study to bring out all possible biological activity, Pharmacological effects, mechanism of action, side effects, interaction with metabolic enzymes etc. It was in particularly attempted to bring out its biological activity which would be used as aid in neurodegenerative disease such as Alzheimer's disease and Parkinson's disease.

### 5.4.1. Lauric acid:

The smiles notation for lauric acid is CCCCCCCCCCCC(=O)O. It was retrieved from PubChem and given in the pass online server to obtain the prediction. The possible activities was retrieved from this analysis.

**TABLE:5.9. Pharmacodynamic Prediction on Lauric acid:**

| Pa    | Pi    | Activity                                     |
|-------|-------|----------------------------------------------|
| 0,942 | 0,002 | Sarcosine oxidase inhibitor                  |
| 0,902 | 0,002 | Aminoacylase inhibitor                       |
| 0,891 | 0,002 | Macrophage colony stimulating factor agonist |
| 0,852 | 0,002 | GABA aminotransferase inhibitor              |
| 0,835 | 0,002 | Guanidinoacetase inhibitor                   |
| 0,798 | 0,004 | Antihypoxic                                  |
| 0,783 | 0,004 | Antimutagenic                                |
| 0,781 | 0,003 | Oxygen scavenger                             |
| 0,686 | 0,004 | GABA C receptor agonist                      |
| 0,646 | 0,009 | TNF expression inhibitor                     |
| 0,515 | 0,052 | Antiinflammatory                             |
| 0,432 | 0,014 | Antiparkinsonian, rigidity relieving         |

### 5.4.2. Palmitic acid:

The smiles notation for palmitic acid is CCCCCCCCCCCCCCCC(=O)O. The below are the list of activities predicted. All these activities are predicted and further invitro and invivo analysis should be carried out.

**TABLE:5.10. Pharmacodynamic Prediction on Palmitic acid**

| Pa    | Pi    | Activity                                     |
|-------|-------|----------------------------------------------|
| 0,973 | 0,001 | Acylcarnitine hydrolase inhibitor            |
| 0,942 | 0,002 | Sarcosine oxidase inhibitor                  |
| 0,906 | 0,002 | Acetylcarnitine Inhibitor                    |
| 0,902 | 0,002 | Aminoacylase inhibitor                       |
| 0,891 | 0,002 | Macrophage colony stimulating factor agonist |
| 0,852 | 0,002 | GABA aminotransferase inhibitor              |
| 0,836 | 0,006 | Membrane Permeability Inhibitor              |
| 0,835 | 0,002 | Guanidinoacetase inhibitor                   |
| 0,798 | 0,004 | Antihypoxic                                  |
| 0,785 | 0,016 | 5Hydroxytryptamine release stimulant         |
| 0,783 | 0,004 | Antimutagenic                                |
| 0,781 | 0,003 | Oxygen scavenger                             |
| 0,686 | 0,004 | GABA C receptor agonist                      |
| 0,646 | 0,009 | TNF expression inhibitor                     |
| 0,515 | 0,052 | Antiinflammatory                             |
| 0,432 | 0,014 | Antiparkinsonian, rigidity relieving         |

**5.4.3. Tetradecanoic acid:**

The smiles notation for tetradecanoic acid is CCCCCCCCCCCCCCCC(=O)O. The biological activity predicted are listed below.

**TABLE:5.11. Pharmacodynamic Prediction on Tetradecanoic acid**

| Pa    | Pi    | Activity                                     |
|-------|-------|----------------------------------------------|
| 0,942 | 0,002 | Sarcosine oxidase inhibitor                  |
| 0,907 | 0,005 | Ubiquinol-cytochrome-c reductase inhibitor   |
| 0,902 | 0,002 | Aminoacylase inhibitor                       |
| 0,894 | 0,005 | G-protein-coupled receptor kinase inhibitor  |
| 0,894 | 0,005 | Beta-adrenergic receptor kinase inhibitor    |
| 0,891 | 0,002 | Macrophage colony stimulating factor agonist |
| 0,852 | 0,002 | GABA aminotransferase inhibitor              |

#### 5.4.4. Tridecanoic acid:

The smiles notation for tridecanoic acid is CCCCCCCCCCCCC(=O)O. Activities Predicted are given below.

**TABLE:5.12. Pharmacodynamic Prediction on Tridecanoic acid**

| Pa    | Pi    | Activity         |
|-------|-------|------------------|
| 0,798 | 0,004 | Antihypoxic      |
| 0,783 | 0,004 | Antimutagenic    |
| 0,781 | 0,003 | Oxygen scavenger |

#### 5.4.5. Pentadecanoic acid:

The smiles notation of pentadecanoic acid is CCCCCCCCCCCCCCC(=O)O. The possible activities are listed below.

**TABLE:5.13. Pharmacodynamic Prediction on Pentadecanoic acid**

| Pa    | Pi    | Activity                                   |
|-------|-------|--------------------------------------------|
| 0,798 | 0,004 | Antihypoxic                                |
| 0,794 | 0,003 | Gluconolactonase inhibitor                 |
| 0,795 | 0,005 | Nitrate reductase (cytochrome) inhibitor   |
| 0,797 | 0,008 | Glucan endo-1,6-beta-glucosidase inhibitor |
| 0,783 | 0,004 | Antimutagenic                              |
| 0,781 | 0,003 | Oxygen scavenger                           |
| 0,779 | 0,005 | Hydrogen dehydrogenase inhibitor           |

The biological activities predicted through pharmacodynamics study reveals that the lead compounds mentioned above had relatively most relatable activities on neurodegenerative disease. The major activity trusted to be effective on all target is antihypoxic which is the prevention against the decrease of dopamine. Palmitic acid showed direct anti parkinsonian , rigidity relieving. Certain ligand compounds shows acetylerase inhibitor which would a promising biological activity for treating neurodegenerative disease. Thus the ligand compounds can definitely be a promising therapeutic drug in future for neurodegenerative disease. Most of the ligands had similar activity at the respective percentages.

## 5.5. DOCKING RESULTS:

Molecular interaction was carried out for all possible target against the lead compounds. Complex structure obtained from PDB was minimized in Discovery Studio. Protein preparation, loop minimization, protein cleaning was achieved by Discovery studio software. Prepared proteins were docked against the lead compounds.

**Table 5.14. Docking Results – Palmitic acid, Tetradecanoic acid, Tridecanoic acid**

| PDB ID<br>OF<br>TARGET | PALMITIC ACID |        | TETRADECANOIC<br>ACID |        | TRIDECANOIC ACID |        |
|------------------------|---------------|--------|-----------------------|--------|------------------|--------|
|                        | DOCK<br>SCORE | ENERGY | DOCK<br>SCORE         | ENERGY | DOCK<br>SCORE    | ENERGY |
| 5HFA                   | 127.11        | -11.33 | 108.43                | -3.96  | 102.38           | -10.04 |
| 1JNK                   | 119           | -5.97  | 112                   | -3.42  | 110              | -9.83  |
| 3BWM                   | 0             | -      | 0                     | -      | 77.25            | -7.25  |
| 5CXV                   | 122.07        | -12.47 | 110.3                 | -6.77  | 103.4            | -9.21  |
| 1QLX                   | 96.09         | -7.88  | 87.85                 | -16.48 | 74.54            | -9.93  |
| 6TYP                   | 103.89        | -16.83 | 93.14                 | -11.09 | 88.91            | -16.65 |
| 6Q1N                   | 122.72        | -2.44  | 109.55                | -1.42  | 99.78            | 0      |
| 4MRM                   | 103.27        | -9.28  | 104.96                | -3.3   | 96.36            | -3.86  |
| 4WSQ                   | 97.21         | -7.13  | 88.52                 | -0.3   | 86.1             | -14.76 |
| 3Q28                   | 108           | -11.52 | 104.29                | -7.04  | 103.37           | -10    |
| 1GS9                   | 0             | -      | 0                     | -      | 60.93            | -8.65  |
| 1PDW                   | 94.84         | -15    | 93.78                 | -7.52  | 79.84            | -13    |
| 2FV9                   | 89.04         | -2.8   | 83.9                  | -5.3   | 81.49            | -3.86  |
| 1GOS                   | 107           | -12.12 | 102.10                | 8.7    | 98.20            | -9.36  |
| 6CM4                   | 119.81        | -11.98 | 111.151               | -11.23 | 106.37           | -11.62 |
| 5HK1                   | 104.87        | -12.34 | 95.8                  | -4.57  | 92.22            | -14.85 |
| 1H8F                   | 100.7         | -7.13  | 91.81                 | -9.6   | 92               | -5.04  |
| 31RT                   | 102.41        | -4     | 92.86                 | -4.5   | 90.47            | -5.04  |
| 6HUE                   | 107.57        | -17.94 | 95.1                  | -6.77  | 92.35            | -17    |
| 1PBQ                   | 97.59         | -9.22  | 97.49                 | -1.23  | 89.05            | -13.78 |
| 4D8C                   | 113.39        | -4.66  | 100.61                | -13.31 | 108.46           | -15.92 |
| 5C9V                   | 96.23         | -11.2  | 88.2                  | -8.94  | 90.15            | -5.2   |
| 3EML                   | 101.66        | -16.03 | 92.05                 | -6.72  | 93.22            | -12.95 |
| 2ZEJ                   | 96.5          | -15.3  | 95.59                 | -9.7   | 92.13            | -6.9   |

**Table 5.15. Docking results – Pentadecanoic acid, Lauric acid, Undecanoic acid**

| PDB ID<br>OF<br>TARGET | PENTADECANOIC<br>ACID |        | LAURIC ACID   |        | UNDECANOIC ACID |        |
|------------------------|-----------------------|--------|---------------|--------|-----------------|--------|
|                        | DOCK<br>SCORE         | ENERGY | DOCK<br>SCORE | ENERGY | DOCK<br>SCORE   | ENERGY |
| 5HFA                   | 108.39                | -8.54  | 109.61        | -3.15  | 90.63           | -3.43  |
| 1JNK                   | 114                   | -7.42  | 109           | -9.76  | 94              | -6.8   |
| 3BWM                   | 0                     | -      | 81.62         | -4.88  | 78.34           | -6.12  |
| 5CXV                   | 115                   | -6.37  | 100.23        | -10.3  | 94.18           | -3.43  |
| 1QLX                   | 84.45                 | -5.88  | 80.85         | -4.34  | 89.71           | -3.91  |
| 6TYP                   | 96.95                 | -8.53  | 83.14         | -4.5   | 79.67           | -4.33  |
| 6Q1N                   | 117                   | -6     | 93.69         | 0      | 89              | -5     |
| 4MRM                   | 103.36                | -13.54 | 95.5          | -9.83  | 86.82           | -4.94  |
| 4WSQ                   | 94.19                 | -4.13  | 81.29         | -3.72  | 82.12           | -4.72  |
| 3Q28                   | 104.57                | -4.51  | 98.86         | -4.34  | 86.99           | -4.33  |
| 1GS9                   | 57.97                 | -7.98  | 62.70         | -9.12  | 68.16           | -14    |
| 1PDW                   | 86.05                 | -10    | 75.88         | -15.08 | 71.89           | -15    |
| 2FV9                   | 86.62                 | -3.62  | 78.8          | -3.51  | 71.98           | -4.33  |
| 1GOS                   | 100                   | -3     | 99            | -1.3   | 88              | -5     |
| 6CM4                   | 118.23                | -7.6   | 100.86        | -5.34  | 98.2            | -8.29  |
| 5HK1                   | 98.69                 | -13.26 | 87.74         | -4.27  | 86.59           | -9.11  |
| 1H8F                   | 96.3                  | -12.03 | 86.7          | -3.15  | 74.98           | -13.8  |
| 31RT                   | 97.11                 | -5.48  | 84.48         | -9.76  | 77.93           | 0      |
| 6HUE                   | 96.07                 | -11.7  | 85.81         | -5.34  | 83.61           | -15.37 |
| 1PBQ                   | 96.61                 | -13.26 | 85.86         | -6.36  | 80.3            | -5.9   |
| 4D8C                   | 101.4                 | -6.6   | 97.33         | -13.71 | 93.98           | -4.91  |
| 5C9V                   | 95.97                 | -9.1   | 84.21         | -9.83  | 78.38           | -5.48  |
| 3EML                   | 101.82                | -16.19 | 85.14         | -3.72  | 83              | -15.95 |
| 2ZEJ                   | 99.5                  | -7.9   | 88.08         | -18.05 | 82.73           | -4.45  |

**Table 5.16. Docking results – Hexanoic acid, Pentanoic acid, Octanoic acid**

| PDB ID<br>OF<br>TARGET | HEXANOIC ACID |        | PENTANOIC ACID |        | OCTANOIC ACID |        |
|------------------------|---------------|--------|----------------|--------|---------------|--------|
|                        | DOCK<br>SCORE | ENERGY | DOCK<br>SCORE  | ENERGY | DOCK<br>SCORE | ENERGY |
| 5HFA                   | 62.77         | -1.87  | 56.29          | -0.64  | 74.14         | -0.82  |
| 1JNK                   | 58            | -1.11  | 54             | -1.47  | 81.28         | -7.67  |
| 3BWM                   | 64.67         | -3.04  | 0              | -      | 74.48         | -5.64  |
| 5CXV                   | 57.95         | -3.04  | 54.12          | 0.01   | 75.27         | -2.56  |
| 1QLX                   | 67.25         | -1.8   | 58.76          | -1.47  | 76.23         | -5.35  |
| 6TYP                   | 53.3          | -2.29  | 50.51          | -1.11  | 63.72         | -2.56  |
| 6Q1N                   | 69            | -9     | 60             | -1     | 76            | -14    |
| 4MRM                   | 62.07         | -2.21  | 56.88          | 0      | 75.83         | -3.16  |
| 4WSQ                   | 66.7          | 0.13   | 59.77          | -0.06  | 69.34         | -9.45  |
| 3Q28                   | 59.06         | -1.18  | 55.06          | -1.15  | 66.97         | 0      |
| 1GS9                   | 62            | 0      | 58             | 0      | 64            | -5     |
| 1PDW                   | 58.21         | -1.56  | 52.83          | -1.52  | 66.42         | -14    |
| 2FV9                   | 47.09         | -1.13  | 41.06          | -0.37  | 57.15         | -5.52  |
| 1GOS                   | 64            | 0      | 58             | 0      | 70            | 0      |
| 6CM4                   | 66.03         | -0.01  | 89.41          | -0.01  | 77.84         | -7.67  |
| 5HK1                   | 65            | -0.73  | 59             | -1.05  | 69            | -4.32  |
| 1H8F                   | 54.16         | -0.03  | 45.23          | -0.67  | 58.72         | -2.56  |
| 31RT                   | 53.66         | -0.01  | 48.31          | -0.6   | 60.54         | -9.4   |
| 6HUE                   | 65.35         | -4.7   | 58.54          | -0.6   | 71.14         | -5.6   |
| 1PBQ                   | 56.47         | -0.73  | 47.71          | -0.01  | 69.3          | 0      |
| 4D8C                   | 63.2          | -3.09  | 57.32          | -4.08  | 76.6          | -3.16  |
| 5C9V                   | 53.01         | -2.62  | 44.92          | -1.47  | 65.64         | -4.32  |
| 3EML                   | 69.07         | -0.55  | 57.53          | 0      | 71.92         | -3.16  |
| 2ZEJ                   | 57.4          | -1.5   | 53.1           | -0.6   | 67            | -0.8   |

**Table 5.17. Docking results – Butanoic acid, Decanoic acid**

| PDB ID<br>OF<br>TARGET | BUTANOIC ACID |        | DECANOIC ACID |        |
|------------------------|---------------|--------|---------------|--------|
|                        | DOCK<br>SCORE | ENERGY | DOCK<br>SCORE | ENERGY |
| 5HFA                   | 50.68         | -0.72  | 86.27         | -2.12  |
| 1JNK                   | 44.6          | -0.49  | 83.64         | 0      |
| 3BWM                   | 56.05         | -0.42  | 76.73         | -5.76  |
| 5CXV                   | 47.44         | -0.04  | 77.46         | -2.12  |
| 1QLX                   | 50.47         | -0.42  | 62.25         | -6.34  |
| 6TYP                   | 48.39         | -0.42  | 72.47         | -6.7   |
| 6Q1N                   | 53            | 0      | 87            | -1     |
| 4MRM                   | 45.77         | -0.42  | 77.38         | -9.83  |
| 4WSQ                   | 55.15         | -0.81  | 76.24         | -5.27  |
| 3Q28                   | 52.17         | -0.49  | 81.12         | -2.12  |
| 1GS9                   | 53            | 0      | 62            | -5     |
| 1PDW                   | 36.33         | -0.81  | 72.38         | -6.52  |
| 2FV9                   | 36.19         | -0.81  | 67.16         | -4.15  |
| 1GOS                   | 57            | 0      | 82            | -9     |
| 6CM4                   | 57            | -0.7   | 88.54         | -6.71  |
| 5HK1                   | 53.01         | 0      | 91.41         | -6.52  |
| 1H8F                   | 45.96         | -0.7   | 70.37         | -6.35  |
| 31RT                   | 39.83         | -0.04  | 72.71         | -1.5   |
| 6HUE                   | 48.79         | -0.7   | 77.57         | -16.56 |
| 1PBQ                   | 42            | -0.42  | 75.05         | -5.7   |
| 4D8C                   | 49.37         | -0.72  | 89.27         | -1.75  |
| 5C9V                   | 40.87         | -0.72  | 75.56         | -5.12  |
| 3EML                   | 50.56         | -0.08  | 77.07         | -6.52  |
| 2ZEJ                   | 47.3          | 0      | 79.49         | -6.52  |

**Table .5.18. Best Docking Results**

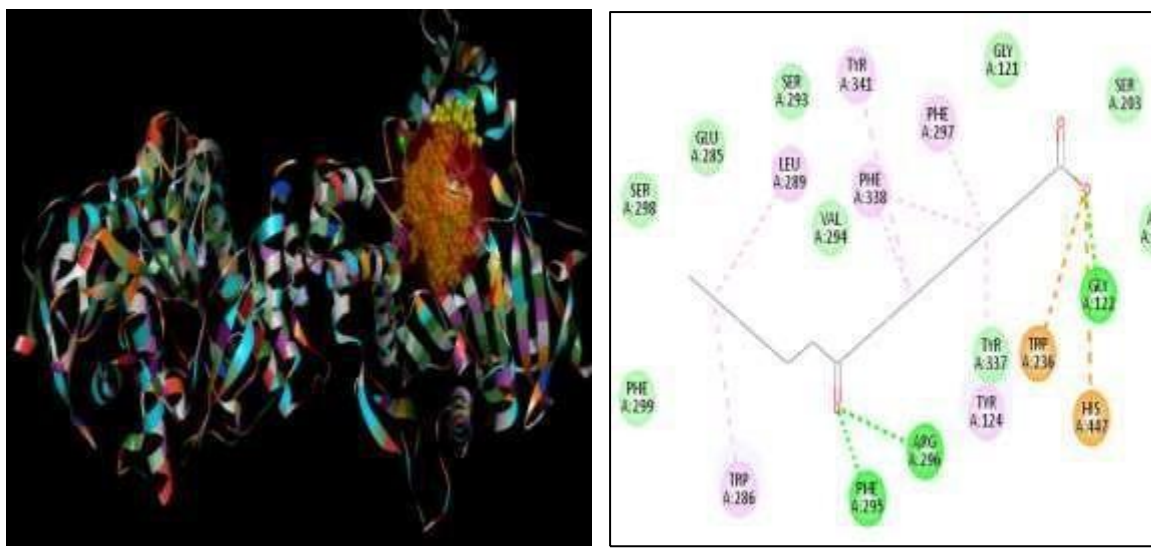
| <b>TARGET</b>                        | <b>LEAD COMPOUND</b> | <b>DOCK SCORE</b> | <b>RELATIVE ENERGY</b> |
|--------------------------------------|----------------------|-------------------|------------------------|
| Acetylcholinesterase                 | Palmitic acid        | 127.11            | -11.33                 |
| DopamineReceptorD2                   | Palmitic Acid        | 119.81            | -11.98                 |
| C-Jun N terminal kinase              | Palmitic Acid        | 119.12            | -5.97                  |
| Glucocerebrosidase                   | Palmitic Acid        | 122.72            | -2.44                  |
| Parkin                               | Palmitic Acid        | 112.89            | -4.18                  |
| Sigma 1 Receptor                     | Palmitic acid        | 104.87            | -12.34                 |
| Monoamine Oxidase B                  | Palmitic acid        | 107.77            | -12.12                 |
| Muscarinic Acetylcholine Receptor M1 | Pentadecanoic acid   | 115.35            | -6.37                  |
| Catechol o methyltransferase         | Lauric acid          | 81.62             | -4.88                  |
| Alpha Synuclein                      | Palmitic acid        | 108               | -11.52                 |
| APOE4                                | Undecanoic acid      | 68.16             | -14.85                 |
| DJ1 Protein                          | Palmitic acid        | 94.84             | -15                    |
| Prion Protein                        | Palmitic acid        | 96.09             | -7.88                  |
| Nrf2                                 | Palmitic acid        | 103.89            | -16.83                 |
| TNF alpha converting enzyme          | Palmitic acid        | 89.04             | -2.80                  |
| Adaptor protein 2 associated kinase  | Palmitic acid        | 97.21             | -7.13                  |
| Glycogen synthase kinase 3 beta      | Palmitic acid        | 100.7             | -7.13                  |
| NMDA Receptor                        | Palmitic acid        | 97.59             | -9.22                  |
| GABA Receptor                        | Tetradecanoic acid   | 104.96            | -3.3                   |
| Leucine rich repeat kinase           | Pentadecanoic acid   | 99.5              | -7.9                   |

|                                    |                    |        |        |
|------------------------------------|--------------------|--------|--------|
| Adenosine receptor A2A             | Pentadecanoic acid | 101.82 | -16.19 |
| Ubiquitin carboxyl terminal kinase | Palmitic acid      | 102.41 | -4     |
| Beta Secretase                     | Palmitic acid      | 113.39 | -4.66  |
| Amyloid Precursor protein          | Palmitic acid      | 93.69  | -5.7   |

Acetylcholinesterase(127.11),Glucocerebrosidase(122.72),Dopamine D2 Receptor,(119.81),C-Jun N terminal kinase(119.12) , Muscarinic Acetylcholine Receptor M1(115.35) are five highest docked score target with the ligands

### 5.5.2. Interaction of acetylcholinesterase with palmitic acid:

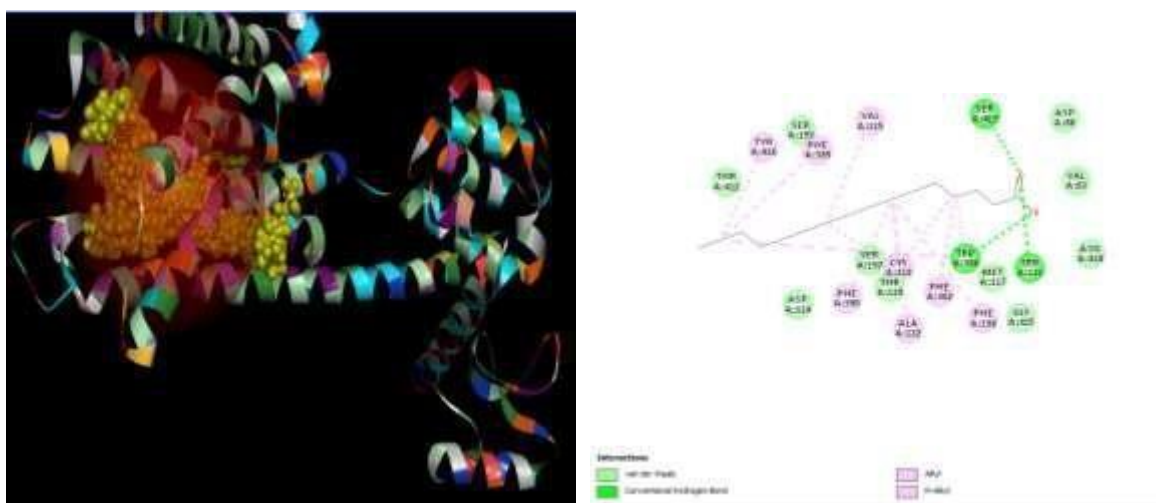
On performing molecular interaction of palmitic acid (lead compound) with acetylcholinesterase,the Lib dock score obtained was 127.11 with a relative energy of -11.33.The estimated bond length is 2.91 Å.



**Fig: 5.1. Representation of 3D and 2D Interaction of acetylcholinesterase with palmitic acid**

It is been visualized that GLY A:122 ARG A:296 PHE A:295 interacts better with the lead compound (palmitic acid).Total hydrogen bond is 261.

### 5.5.3. Interaction of dopamine receptor D2 with Palmitic Acid:

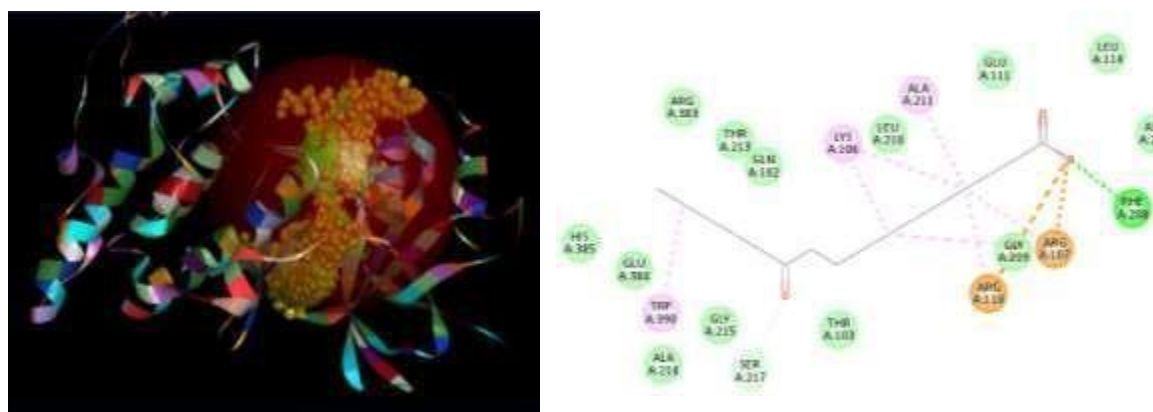


**Fig: 5.2. Representation of 3D and 2D Interaction of dopamine receptor D2 with Palmitic Acid**

The estimated bond distance is 2.76Å and total hydrogen bond is 161

The interacting residues are SER A:419, SER A:121, TRP A:386.

### 5.5.4. Interaction of C-Jun N terminal kinase with palmitic acid:

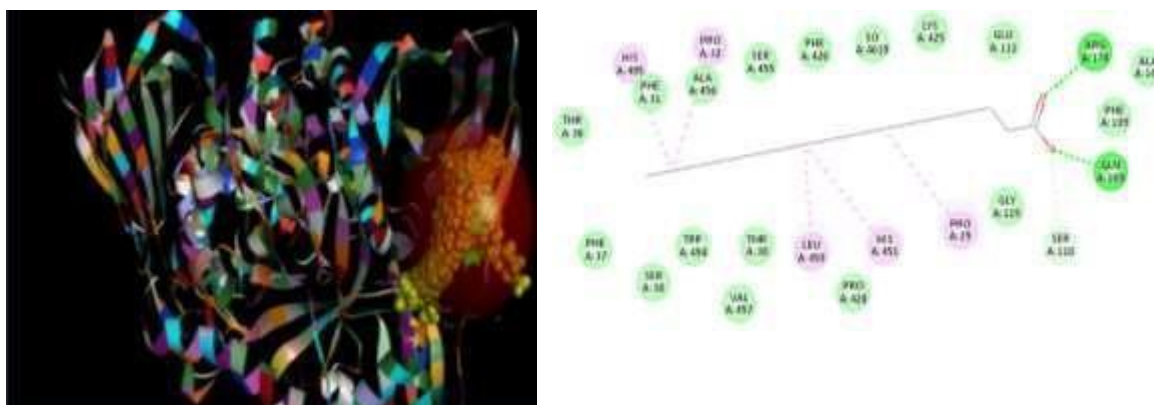


**Fig.5.3. Representation of 3D and 2D Interaction of C-Jun N terminal kinase with palmitic acid**

The estimated bond distance is 2.77Å and total hydrogen bond is 55

The interacting residue is PHE A:208

### 5.5.5. Interaction of glucocerebrosidase with palmitic acid:

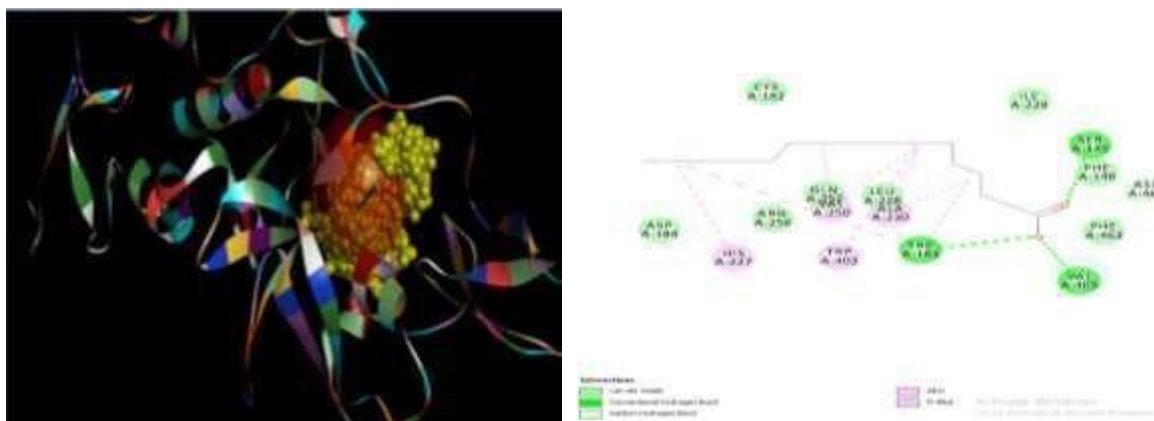


**Fig: 5.4. Representation of 3D and 2D Interaction of glucocerebrosidase with palmitic acid**

The estimated bond length is 2.16 Å and total number of hydrogen bond is 286

The interacting residues are ARG A:170 GLN A:169

### 5.5.6. Interaction of parkin with palmitic acid:

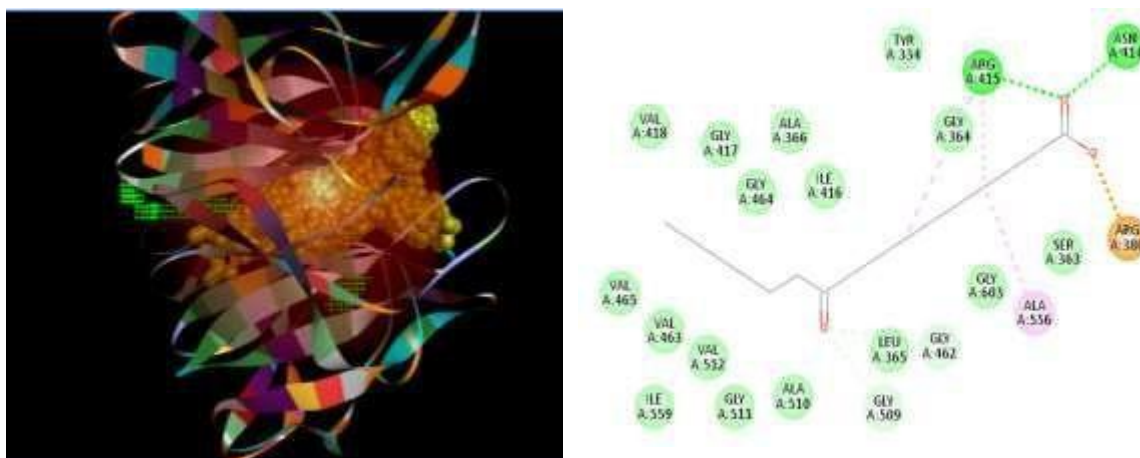


**Fig:5.5. Representation of 3D and 2D Interaction of parkin with palmitic acid**

The estimated bond length is 2.71Å.

The interacting residues are SER A:145,TRP A:183 , VAL A:465.

### 5.5.7. Interaction of NrF2 with palmitic acid:

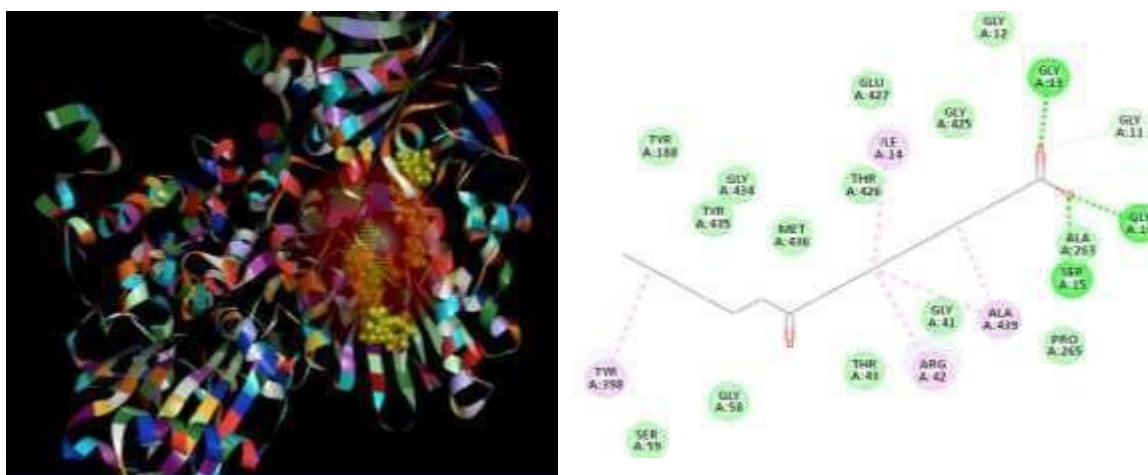


**FIG.5.6. Representation of 3D and 2D Interaction of NrF2 with palmitic acid**

The bond length calculated is 2.09Å and the total number of hydrogen bonds are 362

The interacting residues are ARG A:415 ASN A:414.

### 5.5.8. Interaction of Monoamine Oxidase B with Palmitic acid:

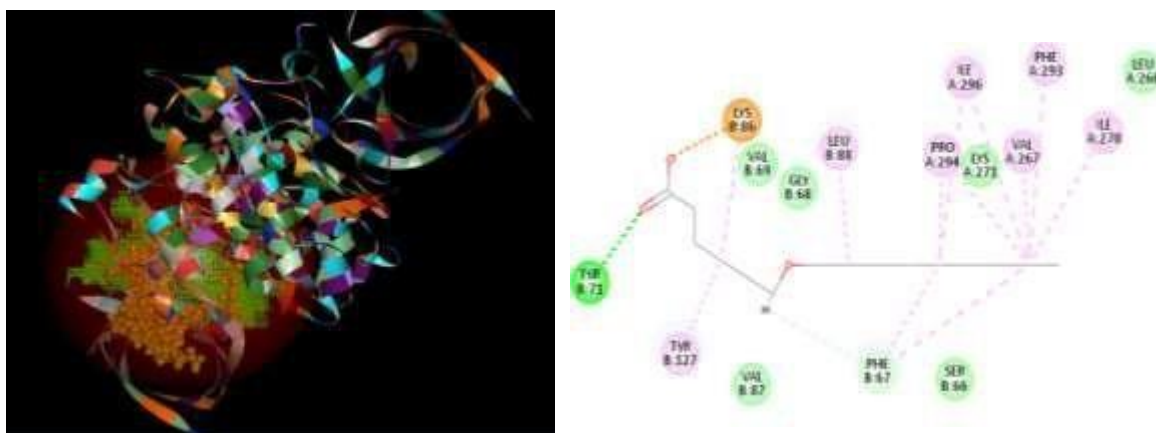


**FIG.5.7. Representation of 3D and 2D Interaction of Monoamine Oxidase B with Palmitic acid**

The bond length distance is 2.74 Å and total hydrogen bond is 22.

The interacting residues are GLY A:13 GLY A:16 SER A:15.

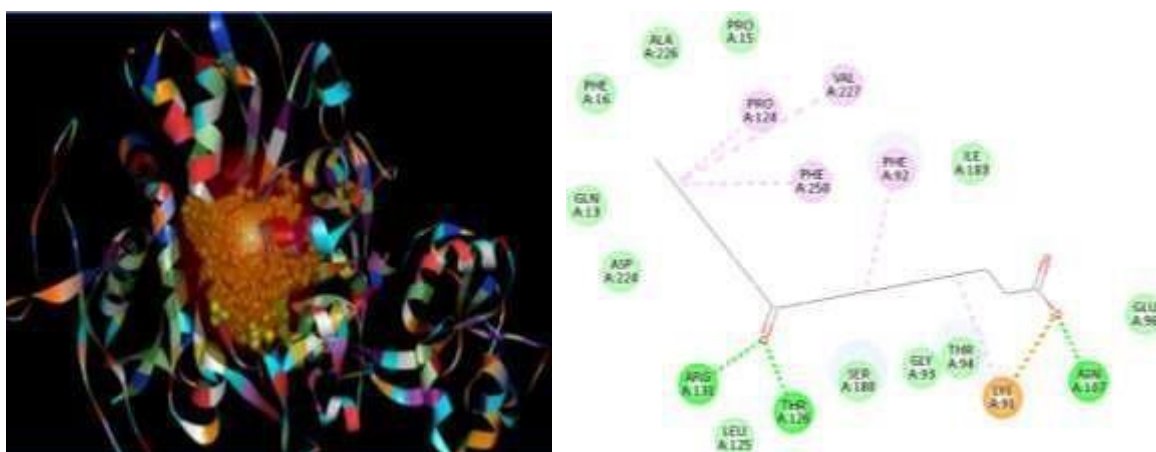
### 5.5.9. Interaction of glycogen synthase kinase 3 beta with Palmitic acid:



**FIG.5.8. Representation of 3D and 2D Interaction of glycogen synthase kinase 3 beta with Palmitic acid**

The bond distance calculated is 2.46 Å and total number of hydrogen bond is 59. The interacting residues are TYR B:71.

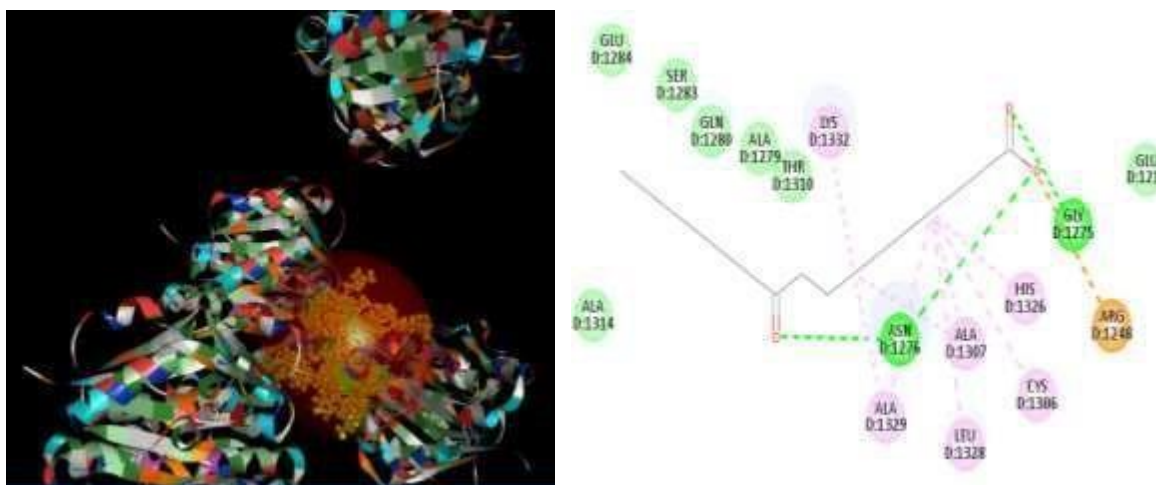
### 5.5.10. Interaction of NMDA Receptor with Palmitic acid:



**FIG.5.9. Representation of 3D and 2D Interaction of NMDA Receptor with Palmitic acid**

The bond distance is 2.15 Å and total number of hydrogen bond calculated is 300. The interacting residues are ARG A:131 THR A:126 ASN A:107

#### 5.5.11. Interaction of DJ 1 Protein with Palmitic acid:

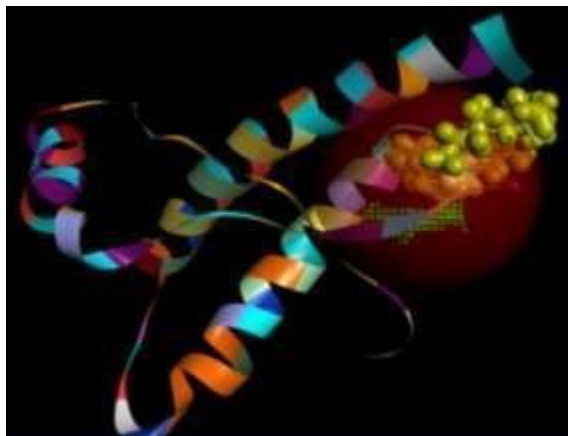


**Fig.5.10. Representation of 3D and 2D Interaction of DJ 1 Protein with Palmitic acid**

The bond distance is 2.89 Å and total number of hydrogen bond is 84.

The interacting residues are ASN D:1276 GLY D:1275.

#### 5.5.12. Interaction of Prion protein with Palmitic acid:

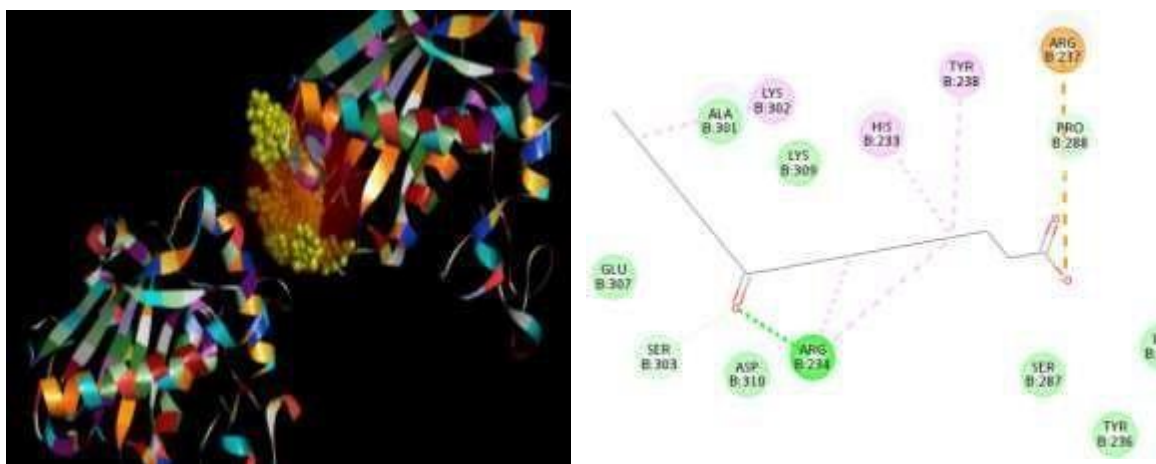


**FIG.5.11. 3D Interaction of Prion protein with Palmitic acid**

The bond distance is 2.73 Å and the total hydrogen bond is 16.

The 2D interaction of this interaction was not obtained.

### 5.5.13. Interaction of TNF alpha Converting enzyme with Palmitic acid:

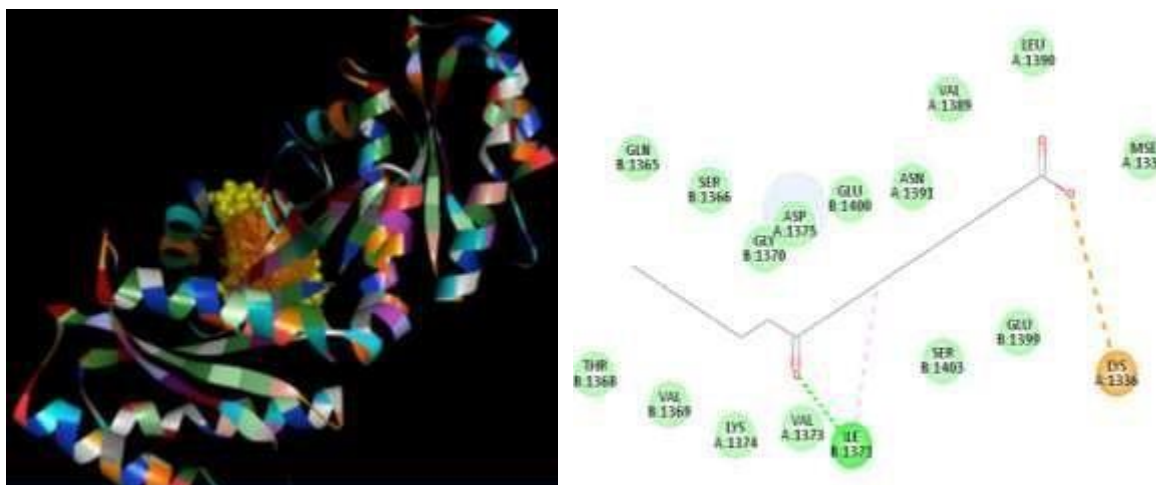


**FIG.5.12: Representation of 3D and 2D Interaction of TNF alpha Converting enzyme with Palmitic acid**

The bond distance is 2.88 Å and the total number of hydrogen bond is 238.

The interacting residues are ARG B:234.

### 5.5.14. Interaction of LRRK2 with pentadecanoic acid:

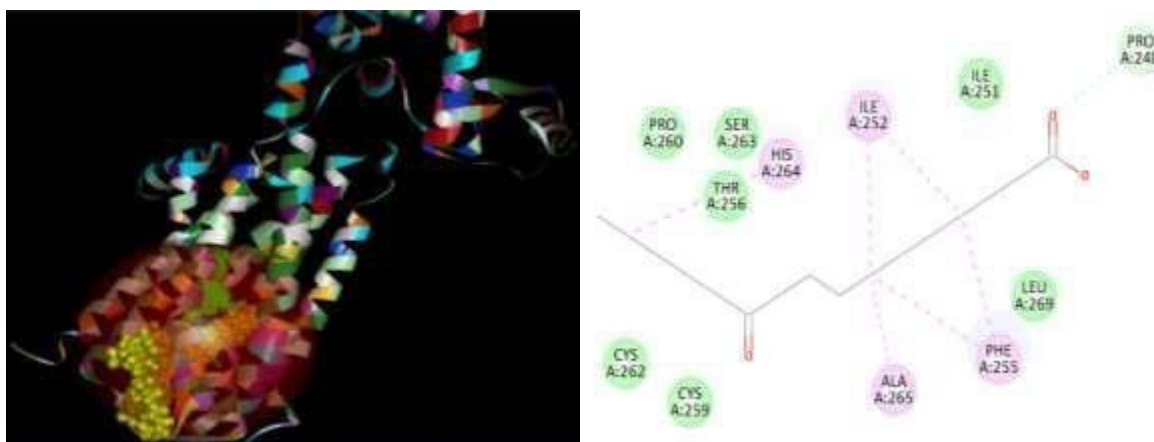


**FIG.5.13. Representation of 3D and 2D Interaction of LRRK2 with pentadecanoic acid**

The bond distance is 2.69 Å and the total number of hydrogen bond is 230.

The interacting residues are ILE B:1371.

#### 5.5.15. Interaction of Adenosine A2a Receptor with pentadecanoic acid:

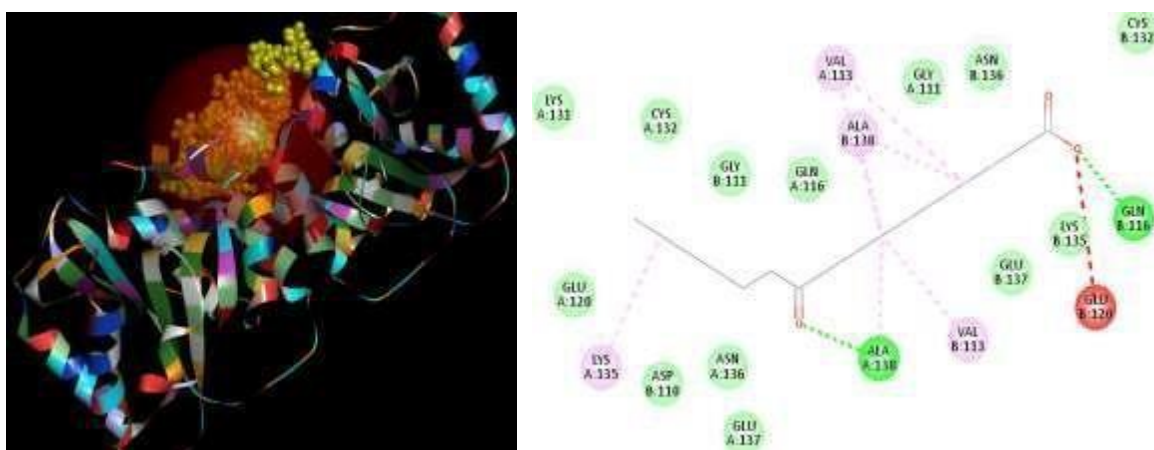


**FIG.5.14. Representation of 3D and 2D Interaction of Adenosine A2a Receptor with pentadecanoic acid**

The bond distance is 2.86 Å and total number of hydrogen bond is 145.

It has not interacted with the conventional hydrogen bond which may not be considered as an effective binding.

#### 5.5.16. Interaction of Ubiquitin carboxyl terminal kinase with palmitic acid:

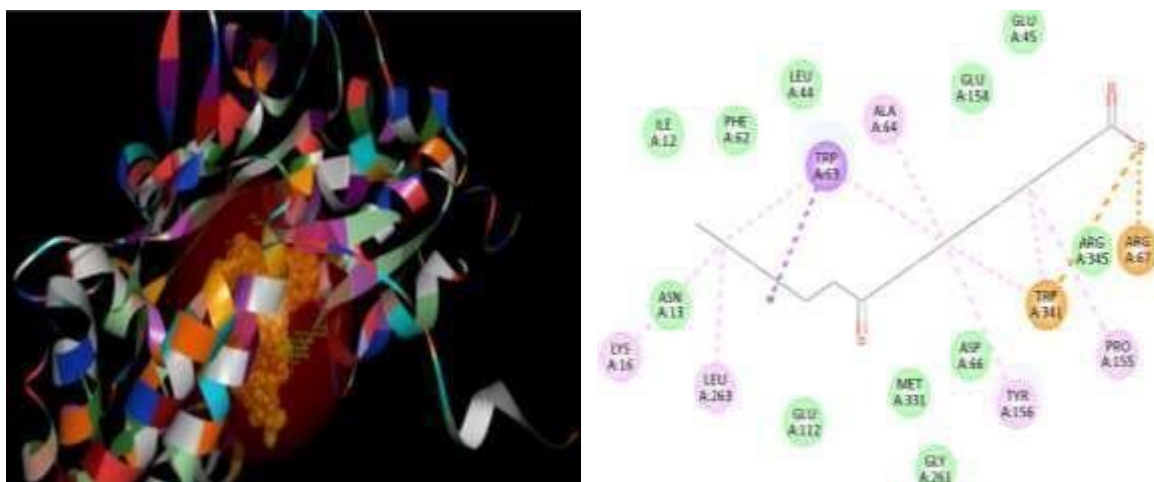


**FIG.5.15. Representation of 3D and 2D Interaction of Ubiquitin carboxyl terminal kinase with palmitic acid**

The bond length distance is 2.26 Å and the total number of hydrogen bond is 275.

The interacting residues are ALA A:138 GLN B:116.

### 5.5.17. Interaction of Alpha Synuclein with Palmitic acid:

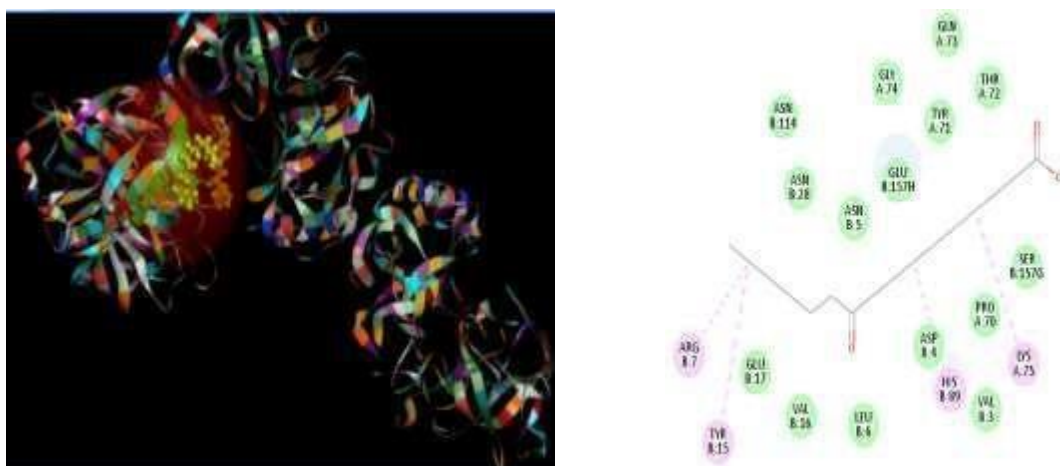


**FIG.5.16. Representation of 3D and 2D Interaction of Alpha Synuclein with Palmitic acid**

The bond distance is 2.83 Å and the total number of hydrogen bond is 239.

It has not interacted with the conventional hydrogen bond which may not be considered as an effective binding

### 5.5.18. Interaction of Beta Secretase with palmitic acid:

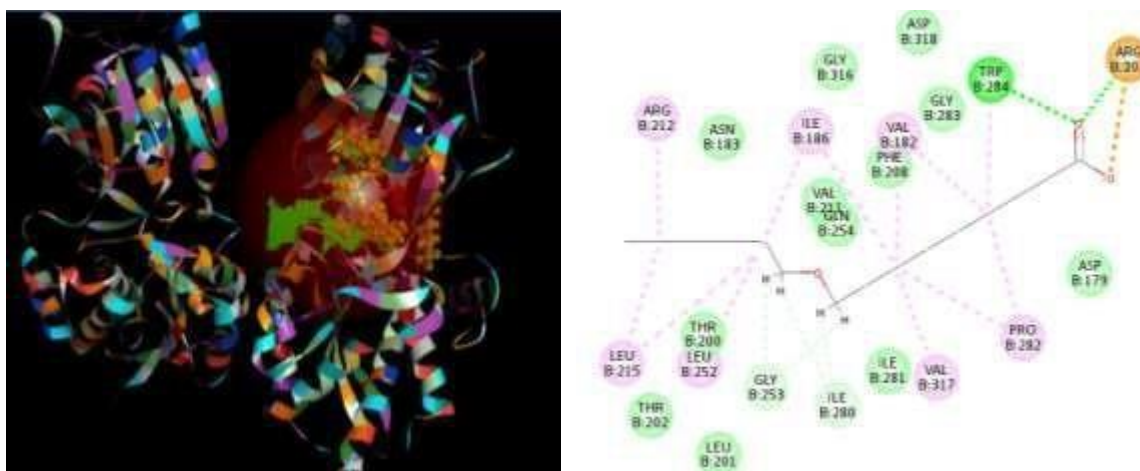


**FIG.5.17. Representation of 3D and 2D Interaction of Beta Secretase active site with palmitic acid**

The bond distance is 2.77 Å and the total hydrogen bond is 6

It has not interacted with the conventional hydrogen bond which may not be considered as an effective binding.

### 5.5.19. Interaction of GABA Receptor with tetradecanoic acid:

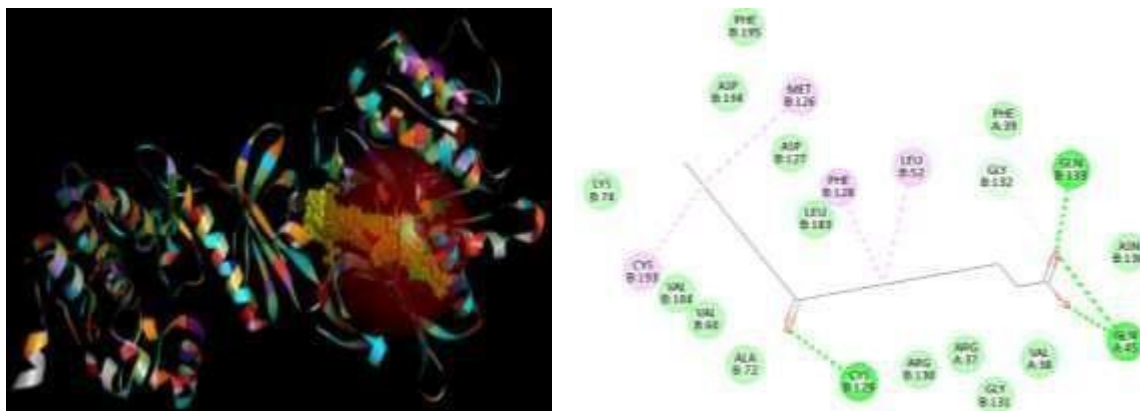


**FIG.5.18: Representation of 3D and 2D Interaction of GABA Receptor with tetradecanoic acid**

The bond distance is 2.42 Å and total hydrogen bond calculated is 28

The interacting residues are TRP B:284.

### 5.5.20. Interaction of Adaptor 2 associated protein kinase with palmitic acid:

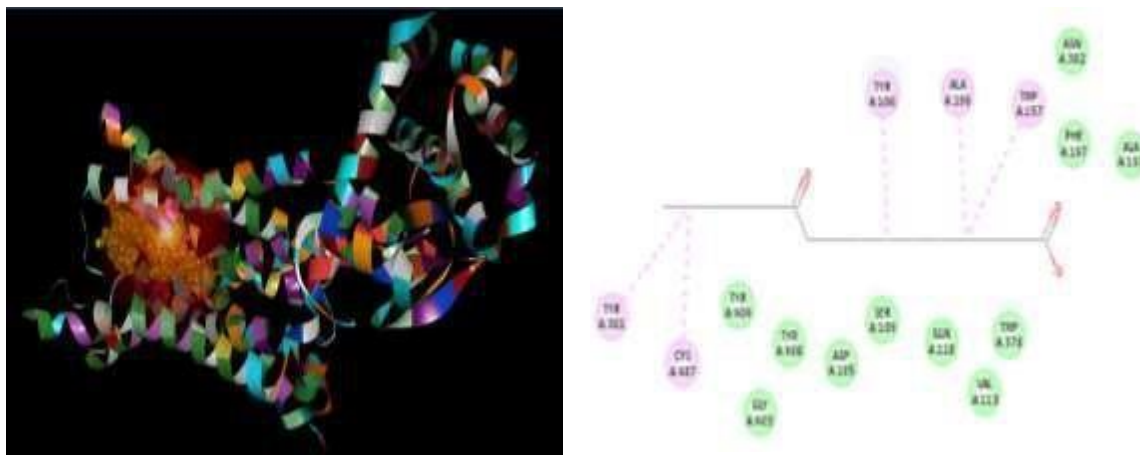


**FIG.5.19. Representation of 3D and 2D Interaction of Adaptor 2 associated protein kinase with palmitic acid**

The bond distance is 2.72 Å and the total hydrogen bond is 202.

The interacting residues are CYS B:129 GLN B:133 GLN A:45.

#### 5.5.21. Interaction of Muscarinic Acetylcholine Receptor M1 with Pentadecanoic acid:

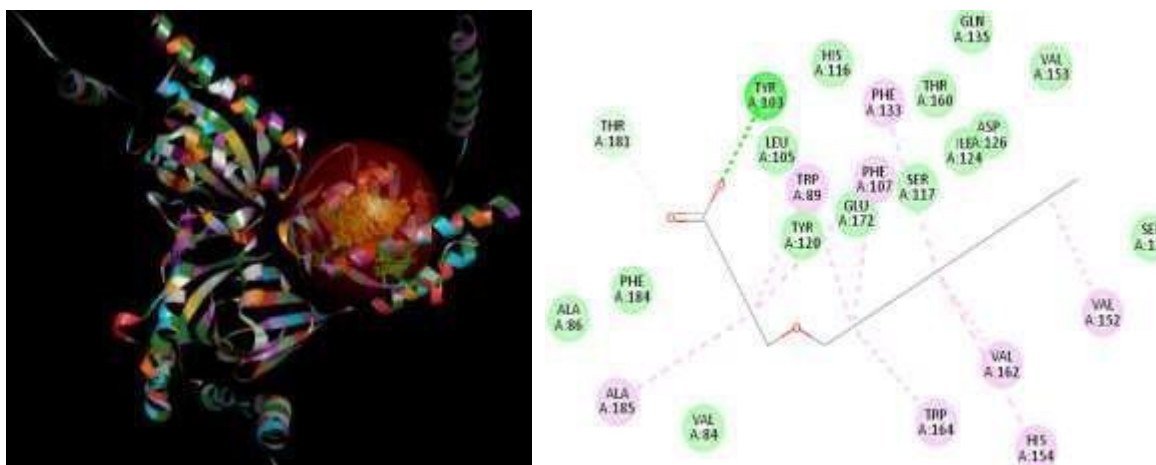


**Fig.5.20. Representation of 3D and 2D Interaction of Muscarinic Acetylcholine Receptor M1 with Pentadecanoic acid**

The bond distance is 2.20 Å and the total number of hydrogen bond is 140.

It has not interacted with the conventional hydrogen bond which may not be considered as an effective binding

#### 5.5.22. Interaction of Sigma 1 Receptor with palmitic acid:

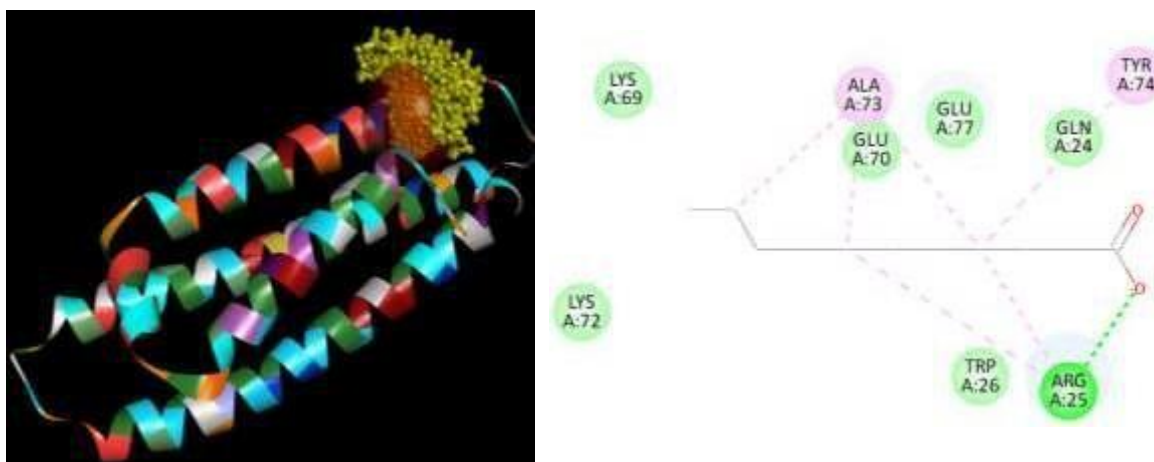


**FIG.5.21. Representation of 3D and 2D Interaction of Sigma 1 Receptor with palmitic acid**

The bond distance is 1.92 Å and the total hydrogen bond is 605

The interacting residues are TYR A:103.

### 5.5.23. Interaction of APOE4 with Undecanoic acid:

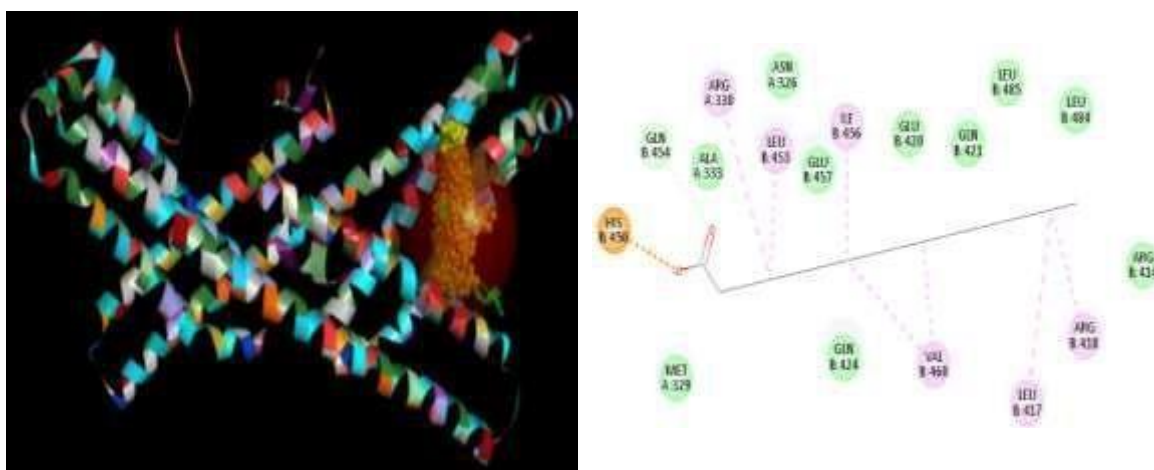


**FIG.5.22. Representation of 3D and 2D Interaction of APOE4 with Undecanoic acid**

The bond distance is 2.67 Å and the total number of hydrogen bond is 106

The interacting residues are ARG A:25.

### 5.5.24. Interaction of Amyloid Precursor protein with palmitic acid:

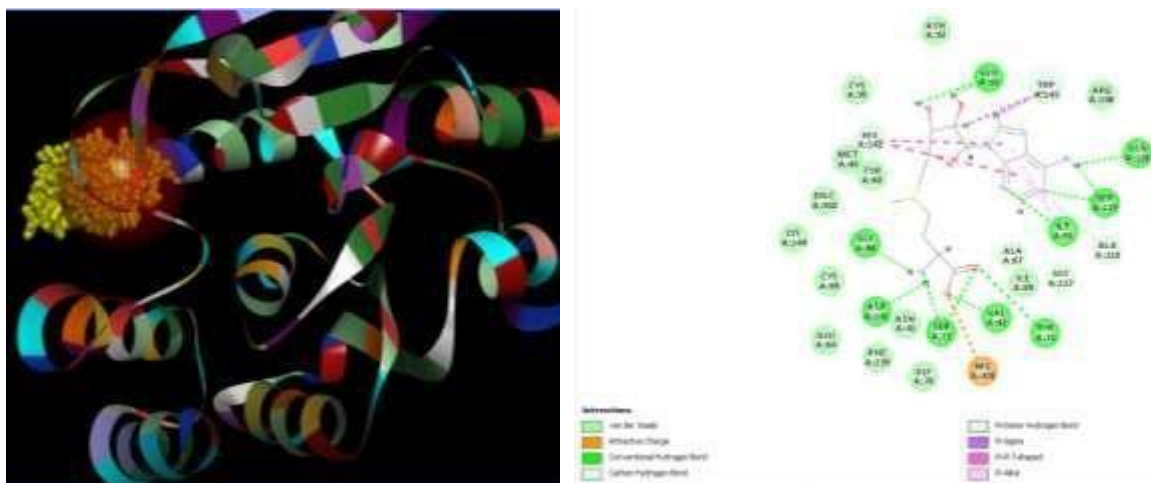


**FIG.5.23. Representation of 3D and 2D Interaction of Amyloid Precursor protein with palmitic acid**

The bond distance is 2.48 Å and the total number of hydrogen bond is 115

It has not interacted with the conventional hydrogen bond which may not be considered as an effective binding.

### 5.5.25. Interaction of Catechol o methyltransferase with Lauric acid:



**FIG.5.24. Representation of 3D and 2D Interaction of Catechol o methyltransferase with Lauric acid**

The estimated bond distance is 3.25Å and the total hydrogen bond calculated is 72. The bond distance between the receptor and the ligand should be less than 3. Bond distance below 3 suggest that the binding distance is not too far and has good binding. Catechol o methyltransferase shows bond distance more than 3. Thus further analysis is needed on this which may be effective.

The interacting residues are GLU A:90 GLU A:120 SER A:119 ILE A:91 TYR A:71 VAL A:42 SER A:72 ASP A:142 GLY A:66.

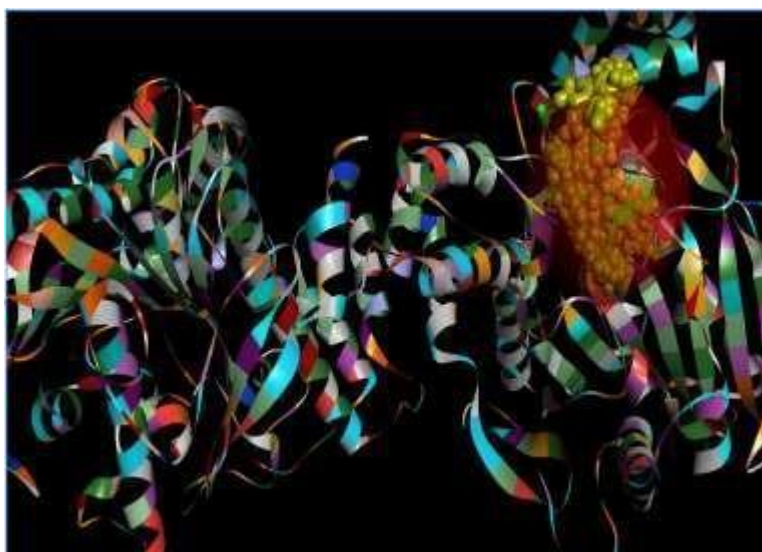
Relative Estimated bond length should be less than 3Å. Bond distance is the binding of ligand with the receptor. If the distance is more than 3Å, it may become a very weak interaction. Thus bond distance is an important parameter.

### 5.6. COMPARATIVE STUDY WITH THE DRUGS:

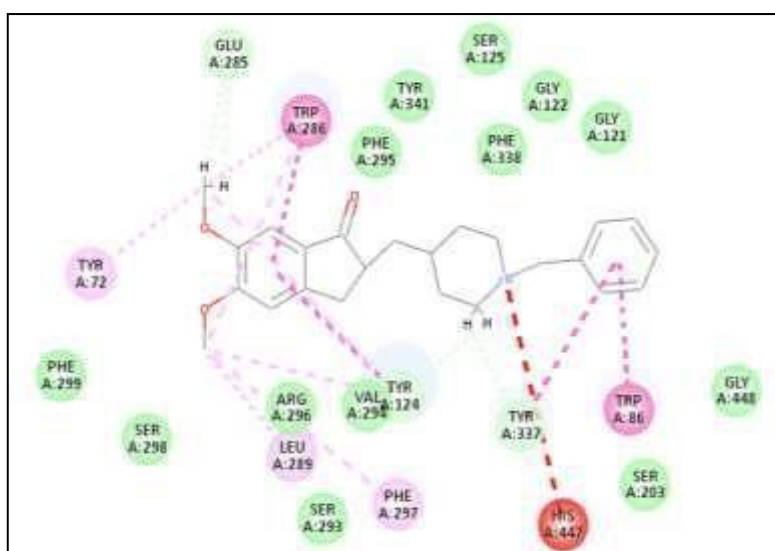
Comparative study was carried out by docking the drug which is in common use for Alzheimer's and Parkinson with its respective target to study the best binding activity between the selected lead compound and the drug.

Acetylcholinesterase was docked with the well known drug Donepezil. Donepezil binds to acetylcholinesterase. The dock score obtained was 153.37 and the estimated relative energy was -10.67.

### 5.6.1. Interaction of acetylcholinesterase with donepezil drug:



**FIG: 5.25. Interaction of acetylcholinesterase with donepezil drug**

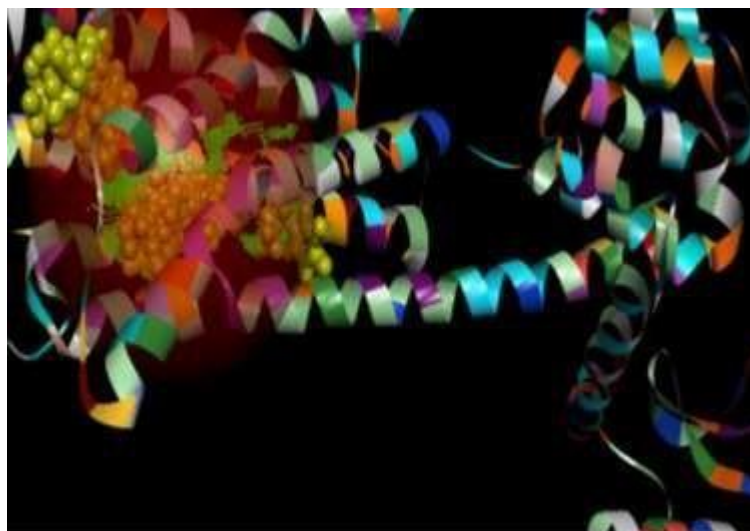


**FIG:5.26. Interaction of donepezil with the active site of acetylcholinesterase**

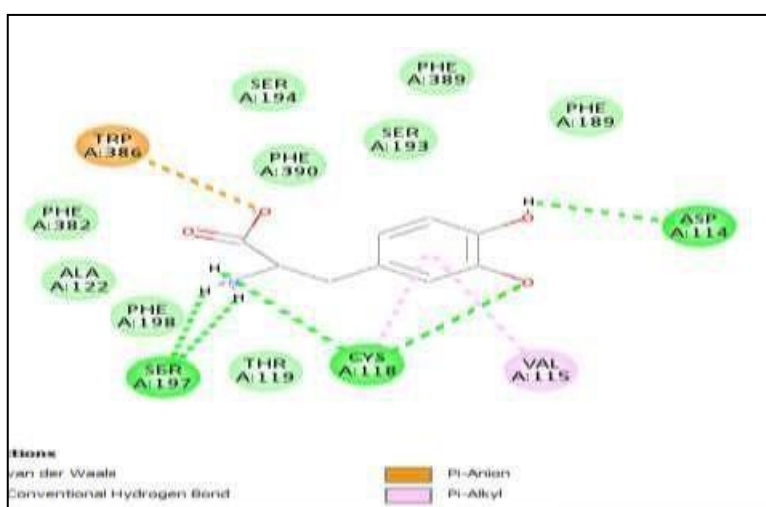
The interacting residues are GLU A:285.

The distance bond length is 2.83Å and total number of hydrogen bond is 95.

### 5.6.2. Interaction of Dopamine Receptor D2 with Levadopa:



**FIG:5.27. Interaction of Dopamine Receptor D2 with Levadopa**



**FIG: 5.28. Interaction of levodopa with the binding site of dopamine receptor D2**

The interacting residues are ASP A:114, SER A:197, CYS A:118.

The bond distance length is 2.92Å and the total number of hydrogen bonds are 278.

**Table.5.19. Comparison of drug with the highest docked lead compound palmitic acid**

| TARGET WITH DRUGS    | 5HFA                 |                 | 6CM4                 |                 |
|----------------------|----------------------|-----------------|----------------------|-----------------|
|                      | Acetylcholinesterase |                 | Dopamine Receptor D2 |                 |
|                      | Dock score           | Relative Energy | Dock Score           | Relative Energy |
| Donepezil            | 153.37               | -10.67          | -                    | -               |
| Levodopa             | -                    | -               | 88.67                | -2.37           |
| <b>Palmitic Acid</b> | <b>127.11</b>        | <b>-11.33</b>   | <b>119.81</b>        | <b>-11.98</b>   |

On comparing the available drug with palmitic acid, the result were relatively good. Donepezil had higher dock score than palmitic acid but was not that low. Palmitic acid had higher dock score compared to Dopamine D2 receptor. Thus palmitic acid may act as a lead compound.

**Table.5.20. Comparison of drugs and lead compound palmitic acid with its pharmacokinetics and pharmacodynamics Properties.**

| Properties                            | DONEPEZIL             | LEVADOPA        | PALMITIC ACID             |
|---------------------------------------|-----------------------|-----------------|---------------------------|
| BBB Permeant                          | 0.18                  | 0.43            | 8.21                      |
| Human intestinal absorption           | 97.95                 | 59.66           | 98.29                     |
| Rodent Carcinogenicity                | <b>Negative Toxic</b> | <b>Positive</b> | <b>Positive Non toxic</b> |
| CMC Rule                              | Qualified             | Qualified       | Not qualified             |
| MDDR rule                             | Drug like             | Mid structure   | Mid structure             |
| Rule of Five                          | Suitable              | Suitable        | Suitable                  |
| Antihypoxic                           | -                     | 75%             | 79%                       |
| Oxygen Scavenger                      | 37%                   | 66%             | 78%                       |
| Membrane Permeability inhibitor       | 65%                   | 59%             | 83%                       |
| Antiparkinsonian, rigidity relieving  | -                     | -               | 43%                       |
| Antiparkinsonian, tremor relieving    | 19%                   | -               | 34%                       |
| 5 Hydroxytryptamine release stimulant | 67%                   | 58%             | 78%                       |

The drug donepezil showed carcinogenicity in both mouse and rat whereas palmitic acid had no carcinogenicity on rat. The predicted pharmacodynamics activity range was lesser for drug when compared to the selected palmitic acid. Therefore the obtained ligand compound palmitic acid can definitely be a promising drug for all possible target since it has good activity and effective binding score which was analysed through pass analysis and interaction.

#### 5.7. VIRTUAL SCREENING:

Virtual screening was carried out on palmitic acid since it was not qualified on CMC rule i.e. Chemistry Manufacturing and control rule. The main aim for performing virtual screening is to bring out even more effective ligand compounds which has the similar structure with palmitic acid. Zinc Database (ZINC 15) online tool was used to perform virtual screening. Similar structures were obtained for palmitic acid. Smiles notation was given to obtain the similar structure. 45 similar compounds were obtained in accordance with palmitic acid.

The 45 similar compounds are listed with the Zinc accession number. Similar compounds denoted the similar structure.

#### 5.21. List of similar compounds for palmitic acid from Zinc database

|                         |              |                             |              |
|-------------------------|--------------|-----------------------------|--------------|
| Margarate               | ZINC24805047 | Heptadec-6-ynoic Acid       | ZINC64393100 |
| 10 oxohexadecanoic acid | ZINC4798470  | 7-Hexadecynoic acid         | ZINC79754211 |
| 11 oxopalmitic acid     | ZINC14619628 | Sapienic acid               | ZINC85617707 |
| 6oxopalmitic acid       | ZINC71418182 | 8E-heptadecenoic acid       | ZINC85832106 |
| 9oxopalmitic acid       | ZINC79244776 | 10zHeptadecenoic Acid       | ZINC95626152 |
| 5keto palmitic acid     | ZINC86037074 | TRANS-10-HEPTADECENOIC ACID | ZINC96023470 |
| 7 keto palmitic acid    | ZINC86037082 | cis-8-heptadecenoic acid    | ZINC97654295 |

|                               |               |                               |               |
|-------------------------------|---------------|-------------------------------|---------------|
| 8oxo palmitic acid            | ZINC86037089  | cis-9-heptadecenoic acid      | ZINC102181517 |
| 5-decoxypentanoic acid        | ZINC96907384  | 7Z-heptadecenoic acid         | ZINC102181818 |
| 6-nonoxyhexanoic acid         | ZINC96907385  | 7-Heptadecenoic acid          | ZINC102182203 |
| 9-hexoxynonanoic acid         | ZINC96907386  | 7-Heptadecynoic acid          | ZINC102182208 |
| 10-pentoxydecanoic acid       | ZINC96907387  | 8-Heptadecynoic acid          | ZINC102182212 |
| 6-octoxyhexanoic acid         | ZINC112715832 | 9-Heptadecynoic acid          | ZINC102182216 |
| trans-9-hexadecenoic acid     | ZINC14881154  | (E)-heptadec-6-enoic acid     | ZINC115798226 |
| cis-10-hexadecenoic acid      | ZINC15274080  | cis-6-pentadecenoic acid      | ZINC115801202 |
| 9E-heptadecenoic acid         | ZINC30731064  | 11Z-heptadecenoic acid        | ZINC118550555 |
| 10-hexadecenoic acid          | ZINC33822115  | 6-(decylamino) hexanoic acid  | ZINC124855399 |
| (E)-heptadec-11-enoic acid    | ZINC34211738  | 7-(nonylamino) heptanoic acid | ZINC147304910 |
| 9-Hexadecynoic acid           | ZINC59825433  | 6-(octylamino) hexanoic acid  | ZINC160919742 |
| 5-(decylamino) pentanoic acid | ZINC232943961 | 5 (nonylamino) pentanoic acid | ZINC310463921 |

All similar structure were studied for their pharmacokinetics properties. ADME studies, Toxicity and druglikeness was predicted.

Certain similar compounds was not suitable on their BBB permeant which was below 0.4 and certain compounds showed negative on both rodent and mouse carcinogenicity. Those compounds was eliminated from this study.

After pharmacokinetics, overall of 24 similar structure were obtained. For analysing the work even more better, the compounds which was highly reliable with all the parameters of PreADME prediction was considered for further screening to study the molecular interaction.

However from 24 similar structures, only 7 lead similar structure had highly efficient characteristics and was qualified in CMC rule for them to be a lead effectively in future. Those structures were interacted with all the targets and was checked whether it was reliably effective than palmitic acid.

**Table 5.22. Best similar compounds selected after pharmacokinetics study**

|                          |               |
|--------------------------|---------------|
| cis-6-pentadecenoic acid | ZINC115801202 |
| 9-hexoxynonanoic acid    | ZINC96907386  |
| 10-pentoxydecanoic acid  | ZINC96907387  |
| 6-nonoxyhexanoic acid    | ZINC96907385  |
| 5-decoxypentanoic acid   | ZINC96907384  |
| 11 oxopalmitic acid      | ZINC14619628  |
| 10 oxohexadecanoic acid  | ZINC4798470   |

Docking of these seven compounds against all targets showed excellent binding affinity than palmitic acid. Highest binding affinity was obtained for 10 oxo hexadecenoic acid for the following targets. Acetylcholinesterase(136) ,dopamine receptorD2(120),MonoamineoxidaseB(113),alphasynuclein(112),DJ1protein(101), Nrf2(108),TACE(93),AAK1(105),NMDAreceptor(109),UCHL1(108),Betasecretase( 107),LRRK2 (107)showed better binding than palmitic acid.

11 oxo palmitic acid had higher binding affinity for M1 Muscarinic receptor(123),AdenosineA2areceptor(112),AmyloidPrecursorProtein(118),Parkin(123),Glucocerebrosidase (131)and C-Jun-N terminal kinase(132).

Thus both 10 oxo hexanoic acid and 11 palmitic acid had highest binding affinities with maximum of the neurodegenerative targets.

Certain other compounds showed higher affinity for other targets. 5-decoypentanoic acid had higher binding activity on sigma 1 receptor (109). 9-hexoxynonanoic acid had higher binding towards GABA receptor (123). 10-pentoxydecanoic acid had higher binding on Prion protein (96) and finally 6-nonoxyhexanoic acid had higher binding towards Glycogen synthase kinase 3 beta (104).

However all the compounds had better molecular interaction with the targets which are different compositions of fatty acids. Though it had higher binding than palmitic acid, the biological activity predicted through pharmacodynamics study was nearly similar. Anyway to further proceed this lead compounds, in vivo and in vitro analysis is highly needed.

## DISCUSSION

Neurodegenerative disease is a progressive disease which has no possible complete cure. Drugs like Donepezil, carbidopa- levadopa, rivastigmine, memantine are in common use to reduce the symptoms of Alzheimer's and Parkinson's disease. The study was carried out to find effective compounds amongst the saturated fatty acid from most abundant marine source blue green algae against the most possible targets for Alzheimer's and Parkinson's disease.

Pharmacokinetics study using PreADMET online tool showed various parameters such as solubility, BBB permeant, human intestinal absorption, Pgp inhibition, MDCK on the ligand compounds. According to the study the ligand compounds such as Lauric acid, butanoic acid, Hexanoic acid, Octanoic acid, Decanoic acid, Undecanoic acid had BBB range of 0.4 to 2. Pentanoic acid, Palmitic acid, Tetradecanoic acid, Pentadecanoic acid, Tridecanoic acid has range of more than 2. The actual prediction followed by PreADMET studies suggests that the compound which has range more than 2 are highly absorbed by the central nervous system and the compounds that are less than 2 are active compounds in CNS. Palmitic acid, butanoic acid, octanoic acid, decanoic acid, pentadecanoic acid had high permeability. Other acids had medium permeability. All ligand molecules has relatively good permeability on human body. Therefore the ligand compounds selected are equally effective according to this prediction and may be an effective drug compound through further studies.

Lauric acid, Tetradecanoic acid, Tridecanoic acid, decanoic acid, undecanoic acid has good drug likeliness property with the qualified result on CMC rule. Palmitic acid, pentadecanoic acid and octanoic acid can definitely be a drug with analysing certain parameters. Butanoic and pentanoic showed non drug like property. Since all the above parameters are prediction on the ligand compounds, certain detailed parameter would definitely pave way to formulate drug from those ligands since it has some activity.

Pharmacodynamics Prediction on all ligand compounds showed biological activities related with neurodegenerative disease. The most trusted activity of these ligand compounds would be Acetyl esterase inhibition, anti hypoxic activity, GABA

aminotransferase inhibition, GABA agonist, anti-inflammatory, antimutagenic, Oxygen scavengers, Membrane permeability inhibition etc.

The molecular interaction study on neurodegenerative disorder target against the saturated fatty acids has shown very effective results. Palmitic acid had maximum highest binding affinity towards major targets. Pentadecanoic acid, Tetradecanoic acid and tridecanoic acid has shown good binding affinity on interacting with the targets and had very good pharmacokinetics and biological activity. In addition, Lauric acid which has no toxicity also contributed more or less equal binding effect with the selected targets. Though Palmitic acid had highest binding affinity with all targets, Lauric acid equally had better binding with all targets as well with similar biological activity and with no toxicity.

Virtual screening on palmitic acid has shown certainly that 10 oxo palmitic acid showed highest binding affinity than palmitic acid. However the biological effects are not that much satisfying when comparing with palmitic acid. Further study on 10 oxo palmitic acid is mandatory for it to act as a lead compound.

From this study, it would be evident that palmitic acid has high and may have possible effects to reduce the symptoms of neurodegenerative disease. In vitro and In vivo studies would definitely be required to develop an effective therapeutic drug having the present study as an initiative approach towards the development of drug on neurodegenerative disease.

## CHAPTER 6

### SUMMARY AND CONCLUSION

The study on effect of marine metabolite on neurodegenerative disease through in silico approach has revealed certain effective activity and new approach towards the use of saturated from marine blue green algae which is very abundant in terrestrial, freshwater, ocean. Computational study was done to obtain detailed information on the lead compounds. All possible and comparative prediction tools and study was carried out to know better about the ligands. Toxicity, drug likeliness and ADME studies on PreADMET tool exhibited more details on ligands related to drug design which had certain limitation as well. During the process on drug design these data's will be efficient for easy development. Pharmacodynamics study revealed the biological activity of the lead compound which may be effective to reduce neurodegenerative disease. Molecular docking of the target with the ligand showed interaction, binding affinity, residues interacted, relative energy. bond length, 2D prediction. The comparison of drug with the highest dock scored lead compound palmitic acid showed more effective biological activity. Virtual screening paved way for analysing even more ligand compounds similar to palmitic acid. From the study, it is evident that palmitic acid may have some potential activity to reduce the risk of neurodegenerative disease. However, in vivo and invitro analysis should be carried out in future to exhibit detailed potency of the lead compound.

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