

DISCOVERY OF NOVEL INHIBITORS FOR DRUG TARGETS IN OBESITY

Submitted in partial fulfillment of the requirements for the award
Master of Science degree in bioinformatics.

by

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SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

**Accredited with Grade “A” by NAAC | 12B Status by UGC | Approved by
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APRIL- 2023**



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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **G. Radha Priya(41739006)** who carried out the project entitled “ **Discovery of novel inhibitors for drug targets in Obesity**” under the supervision from January 2023 to May 2023

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DECLARATION

I, G. Radha Priya(41739006), hereby declare that the Project Reported entitled **“Discovery of novel inhibitors for drug targets in Obesity”** done by me under the guidance of **Dr. Swetha Sunkar** at Sathyabama Institute of Science and Technology is submitted in partial fulfillment of the requirements for the award of Master of Science in Bioinformatics and Data Science.

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ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to the **Board of Management of SATHYABAMA** for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr. JEMMY CHRISTY, Head of the Bioinformatics Department**, for providing me with the necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide **Dr. SWETHA SUNKAR** for her valuable guidance, suggestions, and constant encouragement that paved the way for the successful completion of my project work.

I would like to express my gratitude to **Athista.M** from the Department of Bioinformatics for their invaluable assistance throughout the project, which was crucial to its successful completion.

ABSTRACT

This study focuses on the Virtual screening and identification of novel inhibitors to ACC1 and SCD. The acetyl-CoA carboxylase-1 (ACC1) and stearyl-CoA desaturase-1 (SCD1) are the enzymes in lipogenesis and the upregulation of these enzymes contributes to the aggressive nature of cancer cells and the development of certain metabolic diseases. Therefore, the inhibition of ACC1 and SCD1 has emerged as a potential therapeutic strategy for the treatment of these diseases. The study aims to identify potential inhibitors to both targets and their effectiveness in inhibiting multiple diseases. Ligands were obtained from virtual screening and then subjected to pharmacokinetic analysis using SwissADME to assess their drug-like properties. Toxicity analysis was performed using the Osiris toxicity predictor to evaluate the safety of the compounds. Finally, the identified compounds were subjected to docking using Lib dock in Discovery Studio to determine their binding affinity and potential inhibition of protein functions. The present study identified 23 potential compounds for SCD1 and 10 compounds for ACC1 that may inhibit the functions of the target. The study provides insights into the development of new therapeutics for the treatment of diseases associated with lipid metabolism pathways, such as cancer, diabetes, and obesity. Further studies may be needed to evaluate the effectiveness and safety of these compounds. This research may be useful for further analysis and development of treatments for these diseases.

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ABBREVIATION

ACC1- Acetyl Co A carboxylase, SCD-1 – Stearoyl Co A Desaturase, TG- Tri glycerol, TCA – Tricarboxylic acid cycle, ACYL- ATP citrate lyase, CIC- Citrate/ isocitrate carrier, OAA – Oxaloacetate, FASN – Fatty acid synthase, CHREBP- carbohydrate response element binding protein, SREBP - stearoyl regulatory element binding protein, BMI – Body Mass Index, NICE - National Institute of Health and Care Excellence, DNL – De novo lipogenesis, BT- Biotin carboxylase, CT- Carboxyl Transferase, GEWAS – genome-wide association studies, TOFA - 5-tetradecyl oxy-2-furoic acid, PDB – Protein Data bank, EBI- European bioinformatics institute, SIB- Swiss Institute of Bioinformatics, BLAST- Basic Local Alignment Search Tool, NCBI- National Center Of Biotechnology Information, NIH- National Institute Of Health, ADME- Absorption, metabolism, distribution, and excretion, SVM – Support vector Machine, QSPR - Quantitative structure-property relationship, BCCP- biotin containing carboxylase carrier protein.

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CHAPTER 1

INTRODUCTION

Obesity is an extensive, persistent sickness that begins with fat accumulation and leads to being overweight. Globally every 1 in 5 females and 1 in 7 males may reside with obesity according to the World Health Obesity Atlas by 2030. Obese people in the community are at risk for a long-term illness. It is a challenging disease for doctors to treat in today's world and leads to several illnesses such as diabetes mellitus, thyroid, cancer, high blood pressure, etc. It is an inherited disease that also happens due to overconsumption of carbohydrates, hormonal imbalances, and an inactive way of life. Fatty acids are produced by lipogenesis which occurs in adipose tissue and the liver. Fat breakdown takes place through lipolysis and it is a catabolic process. Elevation in obesity is a prime contributor to fat aggregation. For that reason, the inhibition of certain enzymes accelerates the depletion of obesity. (Batchuluun *et al.*, 2022).

Bioenergetic substances, signaling molecules, and structural components of fatty acids are essential for survival. In the presence of excessive carbohydrate intake fat aggregation takes place and is utilized as energy later in time and unused fat is stored in the form of triglycerides also called tri-glycerol (TAG). At the time of fasting fats are revealed by subcutaneous fat and aggregation takes place in the form of triglycerides (TG) in non-adipose tissues like the heart, muscle, etc. so the accumulation of triglycerides in the liver accelerates diseases such as NASH (non-alcoholic steatohepatitis), cancers, heart diseases, reduced insulin secretion, cell dysfunction, apoptosis, etc. As the imbalance in lipid metabolism is the initial root of lipotoxicity, changes in lipolysis and lipogenesis lead to insulin resistance. (Saponaro *et al.*, 2015).

1.1 Lipogenesis pathway

De novo lipogenesis is a strictly monitored process that basically occurs in fatty tissue and other organs like the pancreas, fatty tissues, liver, etc. Various hormones manage lipogenesis and one of them is insulin, which stimulates adipogenesis and lipid synthesis. A sequence of enzymatic reactions occurs in lipogenesis. Initially, pyruvate, which is the final product of glycolysis, is transported to the powerhouse of the cell and

then transforms into acetyl-CoA. Acetyl-CoA enters into the TCA (tricarboxylic acid cycle) to synthesize citrate. In an overabundance of carbohydrates, citrate moves to the cytosol by CIC (citrate/isocitrate carrier) and is shattered into acetyl-CoA and oxaloacetate (OAA) by using ATP-citrate lyase (ACLY). Acetyl-CoA is then translated to Malonyl-CoA by acetyl-CoA carboxylase. The first metabolic intermediate is Malonyl-CoA in lipogenesis. Utilizing Malonyl-CoA and SCD1 (stearoyl-CoA Desaturase) along with FASN (fatty acid synthase) triglycerides are generated.

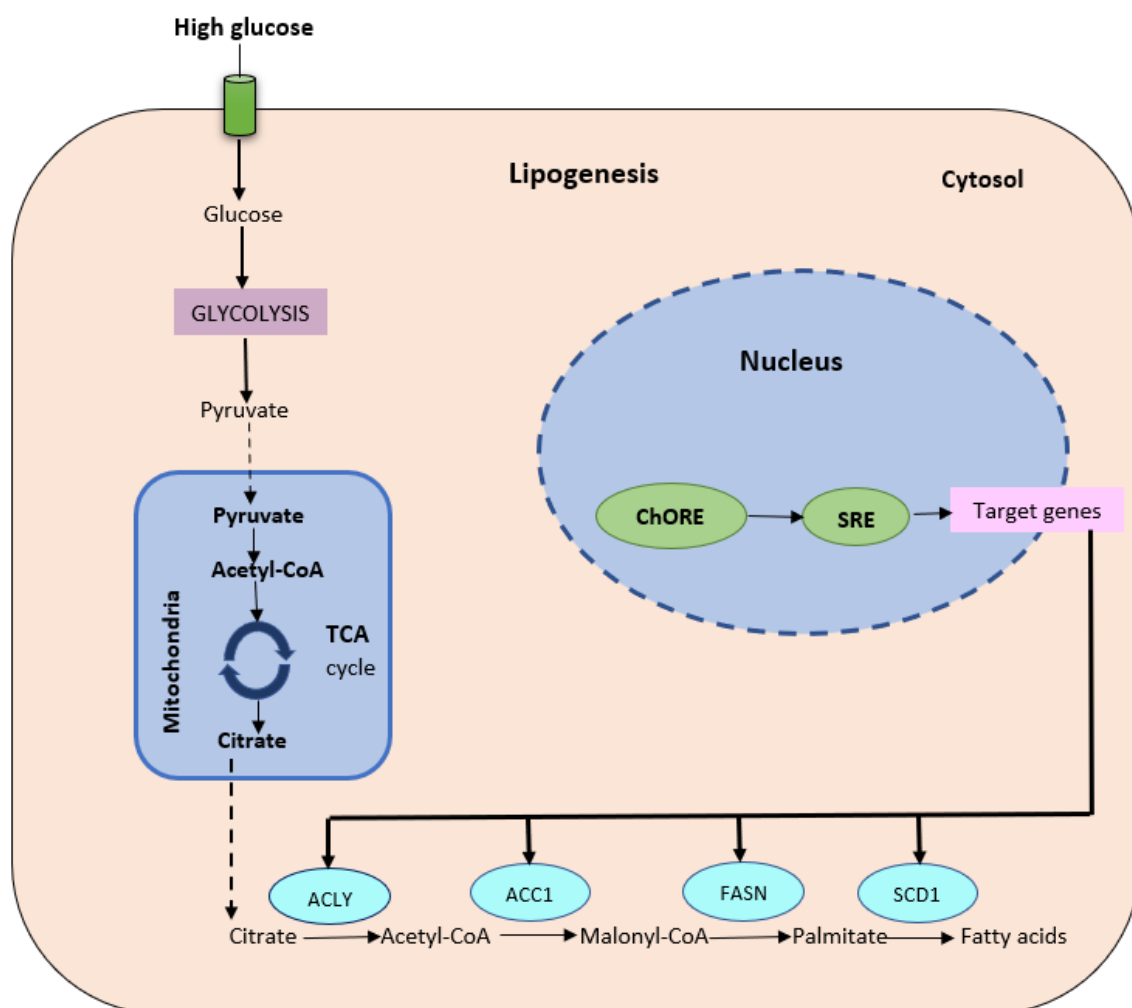


Figure 1.1 De novo lipogenesis pathway.

ChREBP (carbohydrate response element binding protein) takes part a vital role in lipogenesis and it is activated by the glucose metabolites generated by glycolysis then along with MLX (Max-like protein) binds to carbohydrate response element to the promoter region of the target genes that encode stearoyl-CoA desaturase (SCD-1),

fatty acid synthase (FASN), Acetyl-CoA Carboxylase (ACC1), ATP-citrate lyase (ACLY) and ChREBP-beta which activates target gene expression and leads to the synthesis of triglycerides. Other than ChREBP, SREBP-1 (sterol regulatory element binding protein) is a transcription regulatory factor that plays a major role in lipogenesis. (Song *et al.*, 2018).

1.2 Consequences of obesity

Because of the rapid changes in the modern lifestyle such as poor physical activity, processed food, and a poor nutrition diet leads to metabolic diseases such as obesity. (Petridou, *et al* 2019). In most of countries, the high consumption of carbohydrates is the major reason. Diabetes, hypertension, thyroid, coronary artery disease, cancer, and non-alcoholic fatty acid liver are some of the diseases that obesity makes us more likely to develop. In Western nations, obesity is one of the major reasons for sudden deaths and sickness. This leads to the belief that obesity is a disease in the twenty-first century. Understanding the mechanism of the disease at the molecular level will be helpful to develop effective therapies and prevention, research is still going on. (Endalifer and Diress., 2020).

As a result of covid -19 global health problems have increased a lot. Fat accumulation is the key indicator of metabolic diseases. Chronic exposure to unusual metabolic conditions may leads to decreased innate and adaptive immunity, abnormalities in lung and cranial physiology, and an increase in infectivity and virulence ultimately all these factors may lead to increased risk for covid -19 (Zhou, *et al* 2021). During pregnancy, obesity may cause negative neonatal outcomes such as respiratory distress syndrome, injury to the peripheral nervous system, hyperglycemia, and skeletal muscle injury, it also leads to cesarean section and women's death. (Endalifer and Diress., 2020).

1.3 Obtainable Treatments for Obesity

The three major macronutrients available in food are fatty acids, carbohydrates, and proteins. The desirable target for weight loss is fats as it is the most calorie-dense and satiating. Instead, consuming food with high protein content will be helpful for weight loss.

Along with a calorie-reduced diet and the best possible exercises, NICE(National Institute of Health and Care Excellence) advises pharmaceutical treatments for weight loss. Treatment is based on the BMI(Body Mass Index). If a BMI of > 27 kg/m generally gets a description up to 3 months. Orlistat- It irreversibly inhibits pancreatic lipases that convert fats to absorb freely fatty acids which are then expelled in the stool. 32% of absorbed lipids are stopped from being absorbed by orlistat. Liraglutide (Saxena)- It is given subcutaneously once a day to treat obesity and GLP-1 agonists. It affects both centrally and peripherally. (Ruban *et al.*,2019).

It is recommended when dietary therapy or pharmacotherapy fails to show progress in treatment. On weight reduction and co-morbidities, bariatric surgery showed better outcomes when compared to non-surgical therapies. There are four major categories in bariatric surgery: Gastric bypass, sleeve gastrectomy, gastric band, and duodenal switch.

In this method, a much larger bottom and smaller upper part and then a small intestine are connected to both the upper and lower parts. This method also showed a significant decrease in the stomach's functional volume. The stomach is removed to roughly 15% by the surgical removal of the major part of the stomach. This surgery is made laparoscopically and is everlasting. Around the stomach, an inflatable band is wrapped at the top portion and that leads to a tiny chamber above the band. Hence this is also called adjustable gastric banding. A significant portion of the stomach is removed which directs to the small intestine and this results in the decreased absorption of calories and nutrients. (Ruban *et al.*,2019).

1.4 Side effects of drugs

Orlistat and bupropion/zonisamide are gastrointestinal lipase inhibitor and it inhibits the breakdown of triglycerides into fatty acids reducing the calorie intake and fat absorption of the intestine finally leading to a 30% rise in bowel fat excretion. The most adverse are nausea, dizziness, numbness in hands or feet, etc.(Francisco Bonamichi *et al.*, 2018).

Fatty acids are responsible for cell growth and play a major part in metabolism. But upregulation of lipogenesis leads to several harmful diseases so inhibition of certain

enzymes associated with lipogenesis will be helpful for treating diseases. The attractive targets in lipogenesis are acetyl co A carboxylase (ACC), ATP-citrate lyase, Fatty acid synthase (FASN), and Stearoyl CoA desaturase (SCD). The identification of different responses of specific cells across rodents and humans has proven to be a substantial obstacle to the therapeutic development of DNL inhibitors.

ACC1 and SCD1 are the two major enzymes associated with lipogenesis and their inhibition will be helpful for inhibiting certain diseases such as cancer, vulgaris, obesity, etc. (Batchuluun *et al.*, 2022). ACC1 and ACC2 are the two isoforms of ACC in humans. ACC2 is mainly seen in the breast, skeletal muscles, liver, and adipose whereas ACC1 is omnipresent. Amino acid sequence similarity of ACC isoforms exhibit 76% and 82% in the carboxyl transferase (CT) and biotin carboxylase (BT) domains. ACC inhibitors are still under clinical trials as the binding specificity towards the ACC isomers is low. Stearoyl co-A desaturase plays a major part in lipogenesis and is the restricting factor in fatty acid synthesis. In metabolic syndrome and obesity SCD,1 is a potential target for treatment. (Chen, *et al* 2018) SCD1 inhibitors showed an adverse effect on the animal models hence they are still under preclinical trials. (Ravaut, *et al.* ,2020).

CHAPTER 2

LITERATURE SURVEY

Obesity is an extensive disease generally occurring among all age groups, lipogenesis is the major reason for obesity. Fats play a crucial part in cellular functions and act like structural components, signal stimulants, etc. High expression of lipogenesis generates various metabolic diseases like diabetes mellitus, cancer, NASH (non-alcoholic steatohepatitis), etc. (Batchuluun, *et al.*, 2022).

Lipogenesis is a complex and well-monitored pathway where triglycerides are produced by utilizing freely circulating cellulose and then converted to fats. It mainly takes place in cellular organelles like the liver and adipose. Recent studies are paying major insight into lipogenesis passage and genes corresponding to fat production, and regulation.(Ziyi, *et al.*, 2018). The presence of excess sugars lipogenesis is upregulated then leads to excess fat production. The lipogenesis pathway involves a series of enzyme responses. De novo lipogenesis is regulated through various hormones like insulin that stimulate adipogenesis. At first pyruvate, the end product of glycolysis is carried to mitochondria and converted into Acetyl CoA, then that AcetylCoA enters into the tricarboxylic acid cycle (TGC) to produce citrate. In the abundance of carbohydrates, citrate is carried to the cytosol by Isocitrate then breaks into Acetyl CoA and Oxaloacetate (OAA) by ATP Citrate lyase. Acetyl CoA carboxylase converts the Acetyl CoA into Malonyl CoA. It is the first metabolic intermediate in De novo lipogenesis. Utilizing Malonyl-CoA and stearoyl-CoA Desaturase and FASN (fatty acid synthase) fatty acids are generated. (Svensson *et al.*, 2020).

There are some genes that upregulate lipogenesis and it leads to metabolic diseases. ChREBP (carbohydrate response element binding protein) takes part a vital part in fatty acid synthesis and it is switched on by the metabolites generated by glycolysis then along with MLX (Max-like protein) binds to carbohydrate response element to the promoter region of the responsible genes that encode stearoyl-CoA desaturase (SCD-1), Acetyl-CoA Carboxylase (ACC1), ATP-citrate lyase (ACLY) and ChREBP-beta which activates target gene expression and leads to the synthesis of triglycerides (Iizuka *et al.*, 2020).

Other than ChREBP, SREBP-1 (stearoyl regulatory element binding protein) is a transcription regulatory element that plays a major part in lipogenesis. It is activated by the hormone insulin and initiates the gene expression connected with Lipogenesis, its inhibition may reduce the growth and migration of cancer cells. It maintains lipid metabolism, and transcription factors in the regulation of homeostasis. (Sun *et al.*, 2020).

In this modern era, a lot of changes take place in the lifestyle with poor physical activities. Obesity is common in both children and adults. There are several factors that may cause obesity such as food habits, poor physical activities, hormonal imbalance, genetics, etc. GEWAS (genome-wide association studies) showed that more than 250 genes are corresponding to obesity. Obesity leads to multiple metabolic diseases like diabetes, cancers, heart diseases, high blood pressure, premature death, etc (Endalifer *et al.*, 2020). There are multiple treatments available to treat obesity based on the stages. Drugs such as Liraglutide, orlistat, etc are available to treat obesity at its early stages. Bariatric surgery is also available to treat in case of need. Changing lifestyle by including healthy food and avoiding fast food and increasing physical activity is the best way to stop all diseases. (Ruban *et al.*, 2019).

The drugs available in the market may cause several side effects such as dizziness, vomiting, constipation, numbness in hands or feet, etc (Francisco Bonamichi *et al.*, 2018). Inhibition of certain enzymes in lipogenesis may inactivate the lipogenesis and they are Acetyl CoA, Isocitrate, Stearoyl CoA desaturase, ATP Citrate, etc. Lipogenesis inhibitors are in preclinical studies and may become a starting point for therapeutic targets. (Simeone *et al.*, 2021). Acetyl CoA is one of the therapeutic targets in lipogenesis and ACC undergoes carboxylation and forms malonyl CoA. ACC has isoforms ACC1 as well as ACC2 and is located in the cytoplasm. It plays a major role in causing diseases like cancer, diabetes, etc hence it is one of the attractive targets in lipogenesis. ACC inhibition leads to changes in the post-translational modifications and shows different effects on biological pathways. There are some inhibitors in the preclinical studies they are 5-tetradecyl oxy-2-furoic acid (TOFA), Soraphen A, and some small molecules which target BC or CT domain and they are ND-630, ND-646, ND-654, TOFA, CP-640186, piperidiny derived analogs. (Wang *et al.*, 2022). BC and

CT domains take a major role in lipogenesis. The functions of the three regions take place on three levels. At first, ATP is utilized by the BC region and promotes the carboxylation of the BC region, then bicarbonates the carbonyl portion then the BCCP region carries the bicarbonate to the CT region. Finally, carboxylation takes place at the CT domain and converts Acetyl CoA into Malonyl CoA.(Wang, Yu, *et al*, 2022) The BC domain consists of two chains A and B and the sequence length of the BC domain is 540. (Wang *et al.*, 2022). The active site in the ACC1 is CT regions where carboxylation takes place and its functions are inhibited when the ligand binds to Ser80, Ser1201, Ser1216, Ser1263, Ser1259, and Ser1157 (Wang *et al.*, 2022).

SCD1 plays a key role in lipogenesis and utilizing Malonyl-CoA and SCD1 (stearoyl-CoA Desaturase) and FASN (fatty acid synthase) fatty acids are generated. Multiple studies showed that SCD1 is responsible for cancer cell growth, migration, etc. It is taking part in multiple physiological processes such as lipid homeostasis, insulin sensitivity inflammation, and lipogenesis. It is expressed in adipose tissues, mammary glands, liver, etc, and is regulated by signaling pathways and hormones.(Tracz-Gaszewska and Dobrzyn., 2019).

SCD1 consists of multiple domains and they are the N-terminal cytoplasmic region, a transmembrane portion, and a C-terminal catalytic portion. The Catalytic region of SCD1 has an iron core that is needed for enzymatic functions and it consists of a hydrophobic pocket.(Petroff *et al.*, 2021).The Molecular Weight of SCD1 is nearly 41kDa and its length is 359 with a single chain.

The Human SCD1 is folded in Mushroom like architecture with a cytoplasmic region and four alpha transmembrane helices. These transmembranes are connected to each other through ER-lumen loops and polypeptide linkages. SCD1 of the cytoplasmic domain consists of two metal ion binding regions and it has a Hydrophobic tunnel near acyl tail residues associated are ILE115, TRP153, TRP262, AND ALA260, ASN148, ASN265, TRP262, HIS157. SCD1 consists of hydrophobic binding pockets near the metal ion binding region, the structure consists of a dimetal center which is present proximal to a prominent kink in the hydrophobic tunnel (Wang *et al.*, 2015).

CHAPTER 3

AIM AND SCOPE

The aim of the present study is to find novel inhibitors for drug targets in obesity as obese people in the community are at risk for long-term illness. It is a challenging disease for doctors to treat in today's world and leads to several illnesses such as diabetes mellitus, thyroid, cancer, high blood pressure, etc. Obesity is an extensive disease generally occurring among all age groups, lipogenesis is the major reason for obesity. Fats play a crucial part in cellular functions and act like structural components, signal stimulants, etc. High expression of lipogenesis generates various metabolic diseases like diabetes mellitus, cancer, NASH (non-alcoholic steatohepatitis), etc. ACC1 and SCD1 are the two major enzymes associated with lipogenesis and their inhibition will be helpful for inhibiting certain diseases such as cancer, vulgaris, and obesity. SCD1 is a potential approved target and its upregulation leads to various diseases such as diabetes, obesity, and cancer. Hence the present study may be useful for further analysis to find potential inhibitors to both targets which are responsible for multiple diseases. Inhibition of ACC1 will not only help to treat obesity but also be useful to treat diabetes, cancer, cardiovascular disease, and neurogenerative diseases. ACC1 is a potential target to treat multiple diseases. It doesn't have approved specific drugs as it is in preclinical studies because of efficacy and safety concerns.

CHAPTER 4

MATERIALS AND METHODS

4.1 Target identification

The target will be retrieved from PDB and heteroatoms will be removed from the target. Protein Data Bank (PDB) consists of solid 3D structures of biomolecules namely DNA, RNA, and polypeptides. It is a precious resource for scientists and students to get data on biological molecules. PDB provides the 3D structure of proteins that are present in the living world. Identification of the structure of macromolecules helps to find the mechanism of diseases. It helps to determine how structure and biomolecules interact. Uniport is used to get information on proteins and their associated functions. In Uniport the protein sequence data is annotated in a detailed manner such as localization, post-translational modifications, and interactions. The Swiss Institute of Bioinformatics(SIB), European bioinformatics institute (EBI), and protein information resources work together in developing Uniprot. Uniprot provides access to some other protein sequence databases such as UniProt KB/Swiss-prot, UniProtKB/TrEMBL, and UniRef. The UniProtKB/TrEMBL has not yet experimentally determined. UniProtKB/Swiss-Prot is a database that has experimental characterization. Protein sequences are grouped to avoid redundancy. Uniprot provides tools that are useful for sequence analysis and one of the tools is BLAST which allows finding sequences that are identical and PROSITE will help to find protein motifs and domains.

4.2 Screening of Ligands

MIT open screen tool is utilized to find the possible drug compounds that bind to respective target molecules. The selection of specific target molecules is the first step in screening. The target may be already identified as a potential target or it should play a key role in a particular disease.

After identification of the target, the MTi open screen tool will search through a vast library of small molecules to find molecules that are more likely to bind with high affinity to the target protein. The prediction of small molecules is done by utilizing computational techniques like machine learning and molecular docking. MTi open screening tool can be utilized to predict novel drug candidates and it is the best

resource for drug discovery. The complete information of the ligand can be retrieved from databases such as PubChem, zinc database, etc.

PubChem is an online database of chemicals and their attributes, and biological functions. PubChem is managed by the National Center of Biotechnology Information (NCBI) and is a part of the National Institute of Health (NIH). It consists of documented millions of chemical compounds and consists of information on their characteristics, name, chemical formulae, and safety information. PubChem is connected to other databases like GenBank, and PubMed. The zinc database online repository of easily obtainable compounds for virtual screening and drug discovery. The zinc database consists of information on almost 230 million compounds along with detailed information. It has various chemical compounds such as natural, and synthetic which have drug-like properties. It is utilized in various computational techniques like structure-based drug designing and molecular docking.

4.3 Analysis of Pharmacokinetic Properties of Ligand

Swiss ADME is an online tool that is useful to learn the pharmacokinetic properties of drugs and it is introduced by The Swiss Institute of Bioinformatics (SIB). The major pharmacokinetic factors that affect a drug's effectiveness are Absorption, metabolism, distribution, and excretion. Swiss ADME is developed to assist chemists and researchers to study quickly and evaluate the pharmacokinetic properties of respective compounds and can locate issues that can limit their drug-likeness. Swiss ADME predicts a huge range of ADME-related properties such as permeability, blood-brain barrier penetration, solubility, and more. It uses various computational algorithms such as ML, and SVM to predict the properties of the compounds. Based on Lipinski's rule of five the compounds will be filtered and these are a set of guidelines used in drug discovery and development. Lipinski's rules include, molecular weight should be less than or equal to 500 Daltons, lipophilicity is also known as Log P, should be less than 5, hydrogen bonds should be less than five, and H-bond acceptors should be less than ten.

4.4 Toxicity Analysis

Osiris property explorer is an online tool to predict the toxicity of compounds. It is developed at the University of Minnesota by the organic chemistry group. It is used to predict the physicochemical and toxicological properties of compounds. The tool uses Quantitative structure-property relationship(QSPR) models which are trained on large datasets, these properties are used to predict the properties of compounds such as solubility, mutagenicity, tumorigenicity, drug-likeness, etc. The goal of toxicity analysis is to find the proper range of dose of a compound beyond its toxic effects. It is a free web-based application that runs in a Java environment. Using canonical smiles as an input can generate predictions of respective compounds. If any compound having toxic effects such as tumorigenicity, mutagenicity, reproductive effectiveness, or irritant is filtered and compounds with toxic effects are shown in red, moderate effects in orange, and no toxic effects in green.

4.5 Docking

Docking is the prediction of binding fashion and affinity of the ligand towards the target and the purpose of docking is to find the best confirmation and orientation of ligand to the target site along with the calculation of binding energy of the protein-ligand complex.

Discovery Studio is a powerful and versatile software for molecular modeling, computer-aided drug designing, and simulations. It is developed by BIOVIA and consists of various applications and tools for bioinformatics, drug designing, and data analysis. There are four major steps in docking: preparation of the target consists of the target protein being downloaded in 3D format and unwanted atoms such as water being removed. Ligand is being downloaded in SDF format. Then define and edit the binding site. Lib dock, a module in discovery studio is used for docking and it uses rigid body docking algorithms. The analysis of the results is based on the binding site and affinity of the ligand toward the target protein.

CHAPTER 5

RESULT AND DISCUSSION

5.1 Target Identification

Based on the literature survey two potential targets have been recognized which are associated with lipogenesis. The biotin-dependent enzymes Acetyl CoA Carboxylase and Stearoyl CoA Desaturase are the two targets that play a major part in lipogenesis.

Acetyl CoA Carboxylase is an enzyme that plays a major part in lipogenesis and comprises two homologs ACC1, and ACC2. ACC1 is generally manifest in the hepatic system(liver), and fatty tissue, and it is regulated by hormones like insulin. For treating various metabolic diseases such as cancer, obesity, and diabetes Acetyl CoA carboxylase 1 is one of the potential targets. The function of ACC1 is the Carboxylation for the production of Malonyl CoA which is a restricting step in the fatty acid synthesis. Inhibition of ACC1 will not only help to treat obesity but also cancers. In most tumors, ACC1 is upregulated and makes the tumor aggressive. So ACC1 is an attractive target for treating several metabolic diseases (Chen *et al.*, 2019).

Eukaryotic ACC1 consists of several domains and is encoded by the ACACA gene and its molecular weight is 265 kDa with 2356 amino acids. Biotin Carboxylase (BC) domain, biotin-containing carboxylase carrier protein(BCCP), and Carboxyl transferase (CT) domain are the three major domains in ACC1. Carboxylation is carried out by the BC domain and is considered an active site in ACC1. BC and CT domain plays a major role in fatty acid synthesis. The functions of the three domains take place in three steps. At first, ATP is utilized by the BC domain and promotes the carboxylation of biotin, then bicarbonates the carbonyl moiety then the BCCP domain carries the bicarbonate to the CT domain. Finally, carboxylation takes place at the CT domain and converts Acetyl CoA into Malonyl CoA. (Wang, *et al*, 2022) The structure of ACC1 has been obtained from the Protein Data bank (PDB- 6G2D). The BC domain consists of two chains A and B and the sequence length of the BC domain is 540 (Wang *et al.*, 2022).

The active site in the ACC1 is the CT domain where carboxylation takes place and its functions are inhibited when a ligand binds to Ser80, Ser1201, Ser1216, Ser1263, Ser1259, and Ser1157 (Wang *et al.*, 2022).

The enzyme stearoyl CoA Desaturase has a major role in lipogenesis and it introduces a double bond at delta-nine positions to fatty acyl CoA which leads to the production of Monounsaturated fatty acids. This is called desaturation and is responsible for the production of several other Monounsaturated fatty acids such as palmitoleic acid, vaccenic acid, and oleic acid. These monounsaturated fatty acids are important parts of signaling molecules, storage lipids, and membrane phospholipids.

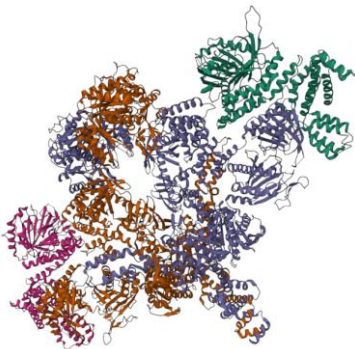
It is taking part in multiple physiological processes such as lipid homeostasis, insulin sensitivity inflammation, and lipogenesis. It is expressed in adipose tissues, mammary glands, liver, etc, and is regulated by signaling pathways and hormones (Tracz-Gaszewska and Dobrzynl, 2019). Hence it is an attractive target to treat several diseases such as obesity, cancer, diabetes, etc. so SCD1 inhibition may inhibit lipid collection and promote insulin sensitivity and it showed desired results in preclinical studies. Reduced metastasis and poor cancer cell proliferation have been shown by inhibiting SCD1. Yet it is still in preclinical studies as it showed adverse effects on animal models.(Ravaut *et al.*, 2020).

SCD1 consists of multiple domains and they are the N-terminal cytoplasmic domain, a transmembrane domain, and a C-terminal catalytic domain. The Catalytic domain of SCD1 has an iron core which is needed for enzyme functions and it consists of a hydrophobic pocket.(Petroff *et al.*, 2021). The PDB Id of SCD1 is 4ZYO. The Molecular Weight of SCD1 is nearly 41kDa and its length is 359 with a single chain.

The Human SCD1 is folded in Mushroom like architecture with a cytoplasmic domain and four alpha transmembrane helices. These transmembranes are connected to each other through ER-lumen loops and polypeptide linkages. SCD1 of the cytoplasmic domain consists of two metal ion binding regions and it has a Hydrophobic tunnel near acyl tail residues associated are ILE115, TRP153, TRP262, AND ALA260, ASN148, ASN265, TRP262, HIS157(Wang *et al.*, 2015).

SCD1 consists of hydrophobic binding pockets near the metal ion binding region, the structure consists of a dimetal center which is present proximal to a prominent kink in the hydrophobic tunnel(Wang *et al.*, 2015).

Table - 5.1 Structures and properties of SCD1 and ACC1

Name	PDB ID	Protein structure	Resolution	Sequence length	method
SCD1	4ZYO		3.25 Å	359	X-RAY DIFFRACTION
ACC1	6G2D		5.40 Å	2407	Electron Microscopy

5.2 Identification of Ligand

The intention of ligand identification is to find a proper legend that can bind to the active site of a protein and to find the binding affinity of protein and ligand. (Stanzione *et al.*, 2021). There are various techniques to screen ligands among them Structure-based in silicon screening is one of the potential methods to find a novel bioactive compound that can inactivate a protein by binding at specific amino acids in the pockets. (Sun *et al et al.*,2017). In order to obtain the best possible inhibitors for the two targets obtained from PDB in silico screening has been performed. Structure-based in silico screening has been done using MTi open screening to obtain possible bioactive ligands for target inhibition. Totally 320 Zinc compounds were acquired from the MTi open screening and they are obtained from the Zinc repository in 3D SDF format. The zinc repository is a curated collection of chemical compounds that are freely available. It has 3.7 billion specified compounds in 2D format.

5.2.1 Database compounds

To inhibit the target protein small molecules like substrates are also used and these are acquired from the Phyto Hub Repository. It is a freely accessible repository and it has complete knowledge of the metabolites and phytochemicals which are available from food sources and natural products. Phyto Hub came up with physical and chemical properties and the phytocompounds along with canonical smiles. There are 1200 phytochemicals and plant metabolites are there and human metabolites are less than 560. In Ayurveda, natural foods products such as lemon, green tea, cinnamon, amla, tomato, and various herbal compounds are used in treating weight loss for people who are facing obesity.(Conforti and Pan, 2016). So, from 44 natural sources around 268 compounds are collected. PubChem is used to find their chemical structures in 2D and 3D formats. PubChem is also an open-source repository and it contains detailed information on synthetic chemicals and also small molecules. It also contains details on the chemically modified macromolecules such as nucleotides, lipids, etc. (Wang *et al.*,2009).

5.3 Pharmacological Property Analysis

Pharmacokinetic property analysis has been performed to evaluate the ADME (absorption, metabolism, distribution, and excretion) properties of ligands along with the lethality, efficacy, bioavailability, and safety of a drug.(Guleria *et al.*, 2021). The Swiss ADME web application is used for pharmacological property analysis. Swiss ADME is a free online tool to evaluate pharmacological properties such as absorption, metabolism, toxicity, etc. To make sure they do not violate the properties of drug-likeness and Lipinski's rule(Dania *et al.*,2017). In this property, analysis is based on Lipinski's rule of five the compounds obtained by screening are filtered. Lipinski's rule of five is a set of guidelines to determine the drug-likeness and bioavailability of compounds and the guidelines contain (a) the H-bonds should be less than or equal to 5 (b) H- bond acceptors should be 10(c) weight should be less than 500 Da (d) clog p-value should be less than 5. After pharmacological analysis, 247 zinc compounds and 50 small molecules have been filtered.

5.4 Toxicity Analysis

To find the adverse effects of potential drug toxicity analysis made in drug discovery. The reason behind the drug is identified in toxicity analysis and it takes place in animal models in preclinical studies. Toxicity analysis is a major step in drug discovery and development in order to identify safe and potential drugs for human use. Osiris property explorer is being used in toxicity prediction It predicts toxicological and physicochemical characteristics and its application runs in a Java environment. Users are allowed to draw chemical structures along with the prediction of toxicity. Based on some harmful endpoints such as mutagenicity, tumorigenicity, carcinogenicity, reproductive effectiveness, etc are the parameters used to predict toxicity. Just by providing the canonical smile of the chemical compound, it provides the toxicity levels of the compound along with drug-likeness, clog P value, solubility, etc. After toxicity analysis of the compounds obtained from MTi open screening and small molecules from natural resources, there are 149 zinc compounds, and 26 small molecules are filtered.

5.5 Docking

The library of compounds is constructed in Biovia Discovery studio and their 3D confirmations are downloaded in SDF format from ZINC and PubChem databases. Discovery Studio is a powerful software for Molecular docking and finding the interaction between proteins and small molecules. An in-silico technique called docking analyses how ligands bind to the active sites of proteins, enzymes, etc. It is a major tool in structure-based drug designing, biochemical pathway analysis, and optimization (Saikia and Bordoloi,2019). It is commercial software for modeling macromolecules and their interaction with drugs. Docking is used in various fields such as protein engineering, drug development, discovery, protein engineering, etc.(Raval *et al.* , 2022). The Lib Dock program is being used in the discovery studio for docking, and it predicts the ligand orientation and affinity of molecules in the pockets of proteins. The systematic conformational search algorithm is used by the lib dock module to find the possible binding sites. The energy minimization in docking is the Smart minimizer algorithm and CHARMM force field. To identify potential targets, lib dock is being used to predict the ligands with a better binding affinity towards the target. After performing docking 9 small molecules and 28 zinc compounds got docked to SCD1 and for ACC1 17 compounds got docked.

Table 5.1 List of compounds docked with SCD1

SCD1- Small Molecules										
sl.no	Name/source	Relative energy	Absolute energy	Libdock Score	H-bond total	Residue of H bond	Unfavorable bonds	Carbon - Hydrogen Bonds and Residues	No of non-covalent interactions	Non-Covalent bond number, residues
1	FALCARINOL / Fennel/parsley	17.6867	31.805	110.449	1	ASN148	0	2- TYR251,PHE146	3	HIS171,HIS157, HIS160
2	Falcarindiol / Fennel/parsley	8.00813	23.7326	103.67	1	ASN148	0	0	6	LEU78,MET79,LEU82,HIS157,TRP153,HIS171
3	FARNESOL / Fennel	10.7705	35.1033	102.086	1	ASN148	0	0	9	TRP153,HIS157, HIS171,HIS160, VAL264,HIS120
4	PHLORETIN/ Apple	56.1529	36.3881	119.112	2	ASN148,ASP156	0	0	2	TRP153,VAL264
5	Hexacosanol / diospyros	13.9498	16.5777	137.13	1	ASN148	0	1-ASP156	2	PHE146,VAL246

6	Cedrelopsis / oldenlandia	7.69772	37.6446	72.8761	2	ASN148, TYR254	0	0	6	PHE146, MET79, TRP184, TRP262, LEU185
7	Pheophorbide A methyl ester / oldenlandia	17.623	162.813	94.2213	1	ASN148	0	0	14	LYS219, LEU223, LEU78, PHE251, PHE227, LEU82, PHE146, MET79, TYR254, LEU185, LEU258
8	Scan – doside/ oldenlandia	16.5487	46.7202	72.8761	3	GLN214, TYR217, PRO220	0	0	1	MET225
9	Beta sitosterol/ oldenlandia	11.0557	15.1876	136.449	0	0	0	0	22	ILE115, HIS120, HIS171, HIS157, TRP153, TRP184, LEU185, TRP262, VAL264, HIS298, ALA298
SCD1- MTi open screening										
10	dimethyl,2,4,5,6,7,8,9,11,12,14,15-dodecahydrocyclopenta[a]phenanthren-3-one	22.1918	40.1798	67.3681	1	TRP153	0	0	7	MET79, LEU185, TRP184, TRP262

11	5alpha-Androst-16-en-3beta-ol	12.4302	31.764	100.287	0	0	0	0	9	VAL264,1-HIS171,3-TRP153,1-HIS120,HIS157
12	Cyclo(D-Leu-L-Pro)	3.68026	19.8154	87.2218	2	ASN265,HIS157	0	2-HIS171,HIS160	5	TRP153,1-ILE115,TRP184,HIS171
13	[2-[(2-Chloropyridin-3-yl)amino]-2-oxoethyl] 2,3-dichlorobenzoate	10.8939	75.9463	58.0912	0	0	0	0	0	0
14	N-hydroxy-L-tryptophan	9.38691	91.3172	65.3765	0	0	0	1-LEU78	6	PHE251,PHE227,LEU82
15	5-Epi-alpha-selinene	11.8648	21.6252	89.6192	0	0	0	0	11	TRP153,HIS171,TRP184,LEU258,LEU185
16	(1R,2R,5R)-N-ethyl-5-methyl-2-propane-2-ylcyclohexane-1-carboxamide	12.6946	22.2095	84.2757	1	TRP153	0	0	8	TRP153,HIS157,HIS171,HIS160,TRP184
17	alpha-Eudesmol	5.03261	16.9943	93.6942	1	TRP262	0	0	6	TRP153,TRP184,HIS171

18	(2R)-2-methyl-3-[(1R,2R,5S)-5-methyl-2-propan-2-ylcyclohexyl]oxopropane-1,2-diol	11.3939	25.2318	88.0948	0	0	0	1-ASN148	4	HIS171,TRP153
19	N-(3-Indolyl Acetyl)-L-isoleucine	13.0144	102.854	87.8513	3	ASN148, TYR254, PHE146	0	0	3	LEU185, 2-PHE146
20	7,8-Dihydro Yangonin	3.88071	42.46	117.85	1	ASN148	0	2-PHE146, ASN265	2	TRP153, LEU185
21	Tyrosol 4-sulfate	8.11629	23.3411	82.7173	3	HIS157, ASN265, TRP153	0	2-HIS160, ASN148	2	HIS171, TRP153
22	Liriodenine	0	91.9463	131.206	1	THR261	0	4-GLU152, LYS189, ASN265, TRP262	8	TRP153, TRP184, HIS171, LEU185
23	1,3-Propanedione, 1-(4-methoxy-5-benzofuranyl)-3-phenyl-	11.1765	133.017	129.798	2	ASN265, HIS157	0	5-ASN148, TRP153, HIS171, HIS157, HIS160	4	TRP153, TRP184, ILE 115

24	5-[(2R,3S)-7-methoxy-3-methyl-5-prop-2-enyl-2,3-dihydro-1-benzofuran-2-yl]-1,3-benzodioxole	6.13642	110.241	135.865	0	0	0	2-ASN265,THR261,1- THR116	6	HIS157,HIS120,HIS171,VAL264,TRP153
25	[(1S,2R,4S,7S,8R,9S,12S,13R,16S)-7,9,13-trimethyl-6-oxo-5-oxapentacyclo[10.8.0.02,9.04,8.013,18]icos-18-en-16-yl] acetate	7.18942	50.9881	85.9611	0	0	0	1-PRO191	4	ARG188,PRO191,TYR59
26	Nodakenetin	0	48.7944	106.218	2	ASN148	0	1-PHE146	5	TRP184,TRP153,LEU185
27	Methyl 4-[(4-methoxy benzoyl)amino]benzoate	0.879218	54.8153	114.237	1	ASN148	0	1-TYR256	4	LEU258,LEU185,TRP153,TRP184
28	[(2S,3R,4R)-2-(3,4-dimethoxyphenyl)-4-[(3,4-dimethoxyphenyl)methyl]oxolan-3-yl]methanol	6.73033	72.1154	106.501	1	ASN148	0	1-THR261,ASN265,ASN148	3	TPR153,LEU185,PHE146

29	[(1R,4aS,5R,8aR)-5-(hydroxymethyl)-2,5,8a-trimethyl-1,4,4a,6,7,8-hexahydronaphthalene-1-yl]methanol	14.9466	40.1022	91.2717	1	ASN148	0	1-ASP156	7	TRP153,HIS171,TRP184
30	methyl (1S,15S,18R,19S,20S)-18-hydroxy-1,3,11,12,14,15,16,17,18,19,20,21-dodecahydroyohimban-19-carboxylate	9.40377	98.4019	94.312	2	ASN148,ASN75	0	1- TRP262	4	TRP184,LEU185
31	(2R,3S)-2,3-bis(1,3-benzodioxol-5-ylmethyl)butane-1,4-diol	6.36035	127.327	118.895	2	THR261	1	2-GLN147,TYR254,PHE146	4	MET79,PHE146,TRP184,LEU185
32	Ubenimex	5.27038	32.3092	112.863	1	ASN148	0	1- TRP153	4	LEU185,PHE146,HIS157,TRP153
33	(3R,6R)-3-[(4-hydroxyphenyl)methyl]-6-(2-methylpropyl)piperazine-2,5-dione	11.7199	34.0096	119.656	2	THR261,THR116	0	1-HIS157	6	HIS171,TRP153,VAL264,ILE115,ALA292

34	5-[(2R,3S)-7-methoxy-3-methyl-5-prop-2-enyl-2,3-dihydro-1-benzofuran-2-yl]-1,3-benzodioxole	6.13642	110.241	135.865	0	0	0	1-ASN148	5	TRP153,LEU258,TRP264,2-LEU185
35	(3S)-3-hydroxy-5-methoxy-2,2-dimethyl-7-(2-phenylethyl)-3,4-dihydrochloride-8-carboxylic acid	6.29948	64.4004	71.6433	1	HIS361	0	1- GLU284,TYR276	5	LEU345,ILE348, GLU312,TYR313 ,
36	Guibourtinidol-4 alpha-ol	9.27832	39.4904	108.349	1	HIS157	0	1-ASN265	2	ILE115,VAL264
37	4-hydroxy-3-[(2E,4R,6E)-4-hydroxy-3,7,11-trimethyldodecane-2,6,10-trienyl]benzoic acid	6.39516	49.4555	121.836	2	ASN148, TRP262	0	0	5	LEU185,MET79, LEU82

5.5.1 Docking result of SCD1

The electrostatic attractiveness of a negatively charged group and H-atom is called a Hydrogen bond. The ligand forms a hydrogen bond to the target protein at the active site showing specificity as well as directionality to interaction. Hydrogen bonds are omnipresent and play a crucial role in molecular interaction, protein folding, catalysis, and some biological processes. H- bonds are an important factor in ligand-protein stability and affinity. Proteins have functional sites called active sites, when a ligand forms an H-bond to the target site it may lead to conformational alterations and interrupts its functions. (Chen *et al.*, 2016) .

After performing docking for SCD1 only 37 compounds got docked. Among them, 9 compounds do not form H- bonds to the target protein, and compound 2 compounds made H-bond at the other region of the protein rather than its active sites, so protein activity may be partially inhibited as H-bond leads to conformational changes. Then 2 compounds bind in the same hydrophobic pocket but with different amino acids. Hence these two compounds which bind to the same pocket may inhibit the protein function Here one compound has 1 unfavorable bond. In docking Scoring functions are used to estimate the binding affinity of the protein-ligand complexes. In order to determine the most favorable conformation of ligands, factors like conventional hydrogen bonds, van der Waals and hydrophobic interactions are considered. Improper geometry, confirmations with high energy, and steric clashes and repulsions are represented in docking. Unfavorable bonds are not seen in the biological systems and inaccuracies in the confirmations are represented in unfavorable bonds, so in docking conformations with unfavorable bonds are not considered as they are not reliable.(Dhorajiwala *et al.*, 2019). So, the one compound with one unfavorable bond may not show the desired inhibitory activity.

Around 23 compounds bind with H- bond to the amino acids in the hydrophobic pocket which plays a major role in the function of the proteins. The H-bond may lead to conformational changes and leads to the inactivation of protein functions. SCD1 of cytoplasmic domain consists of two metal ion binding regions and it has a Hydrophobic tunnel near the acyl tail residues associated are ILE115, TRP153, TRP262, AND ALA260, ASN148, ASN265, TRP262, HIS157. (Wang, *et al*, 2015) There are 7 small

molecules that bind to the active site and 16 zinc compounds bind to the desired active sites. In docking, the interaction of protein and ligand takes place by the covalent and noncovalent interactions such as van der Waals, pi-alkyl interactions, H-bond interactions, etc. Covalent interactions are stronger than noncovalent interactions, yet noncovalent interactions show major effects on the stability of the complex. (Landeros *et al.*, 2023). Overexpression of the enzyme SCD1 has been implicated in the development of metabolic diseases. Sterculic acid is a natural product that has been shown to inhibit SCD1 activity. Studies have demonstrated that sterculic acid binds to the hydrophobic pocket of SCD1, which is a critical region responsible for the enzyme's function in lipid metabolism. By binding to this pocket, sterculic acid can effectively inhibit the activity of SCD1, leading to decreased levels of monounsaturated fatty acids and triglycerides, which are associated with metabolic diseases such as obesity and diabetes. (Pelaz *et al.*, 2020). So, all the compounds bind at the active site of SCD1 may inhibit the protein functions.

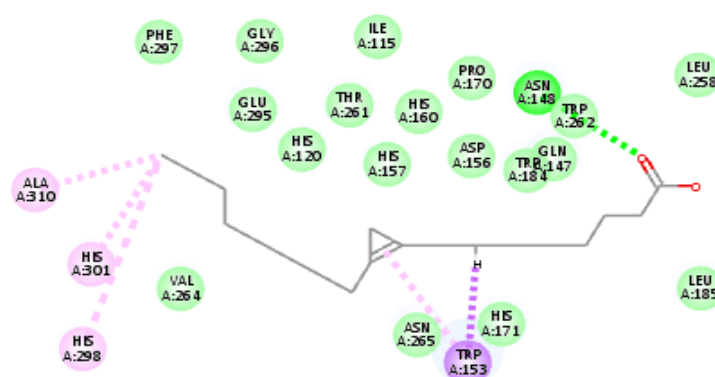
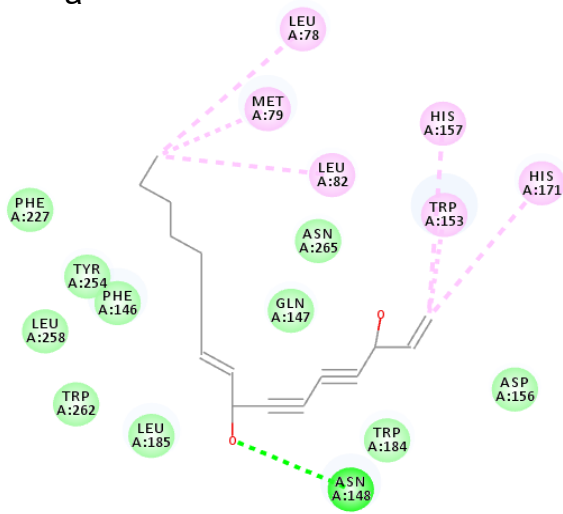
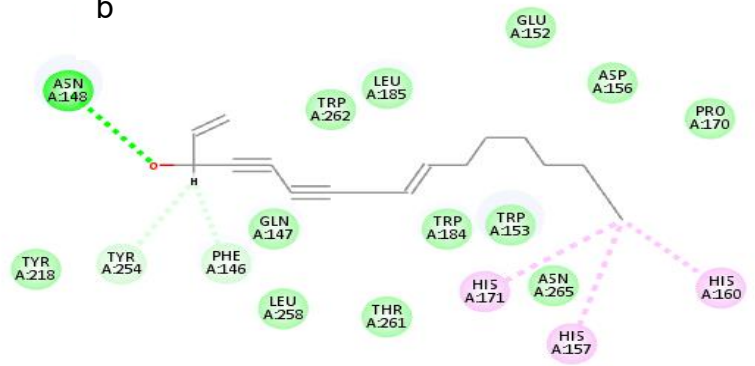


Fig 5.5.1 Sterculic Acid binding at the active site

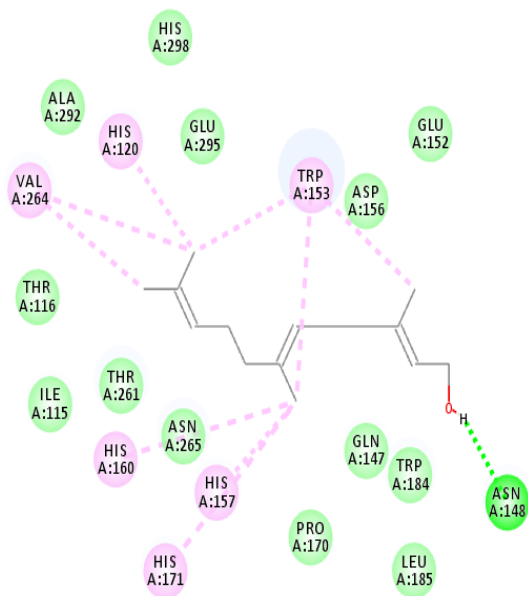
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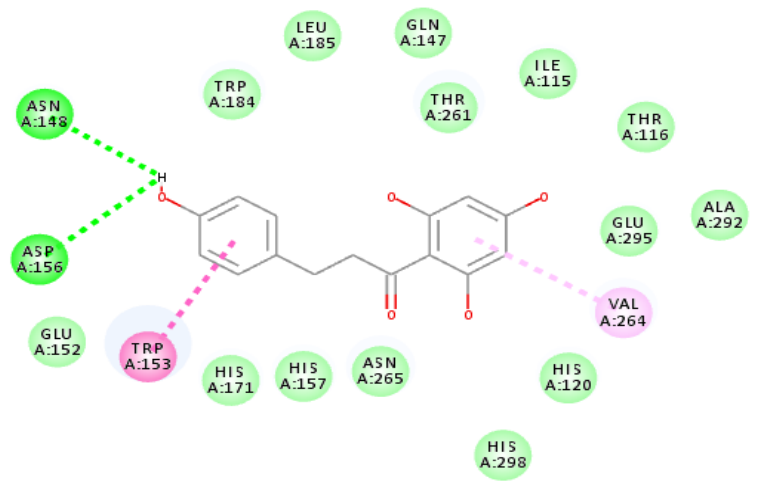
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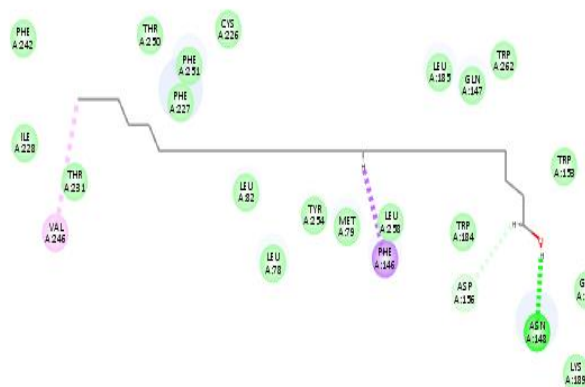
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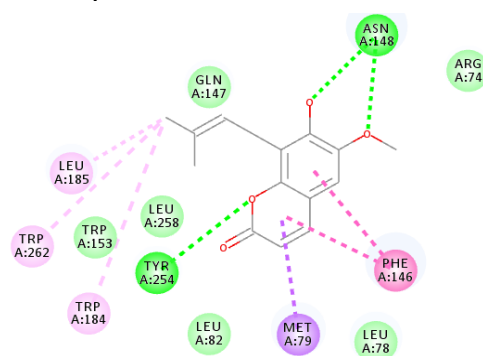
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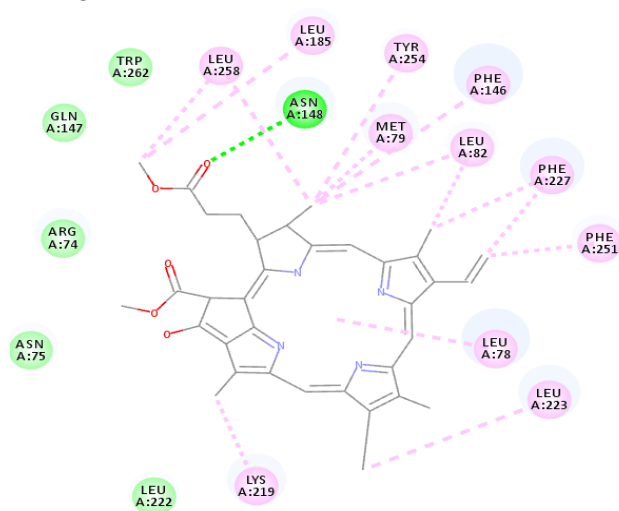
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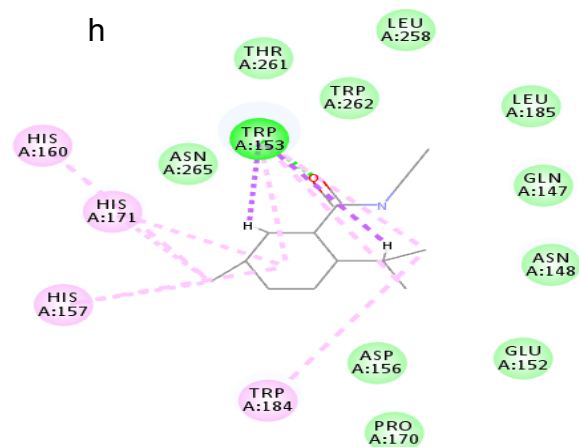
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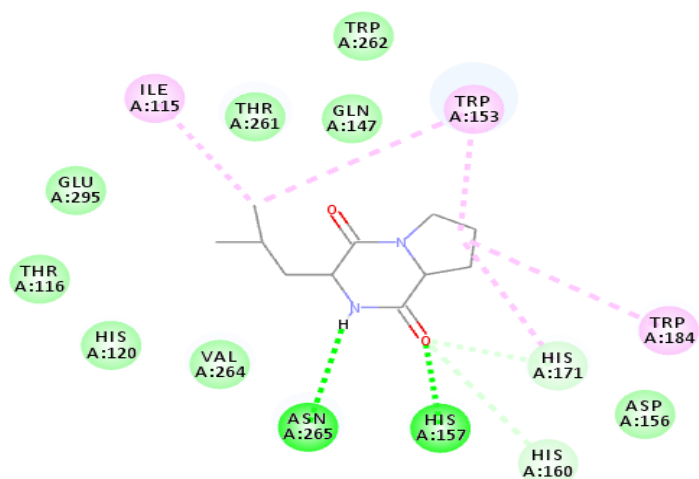
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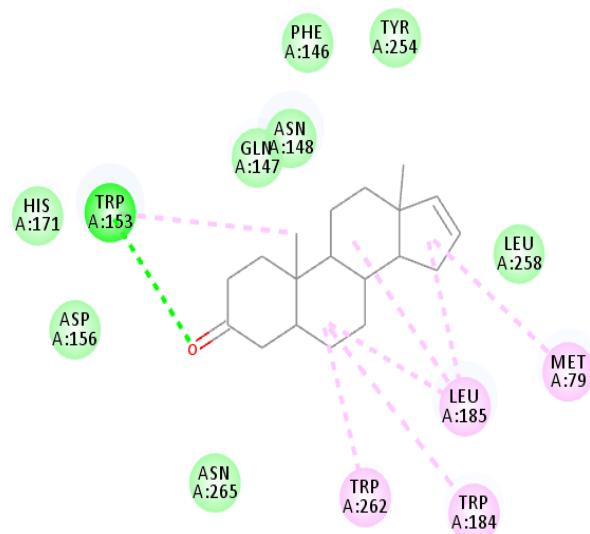
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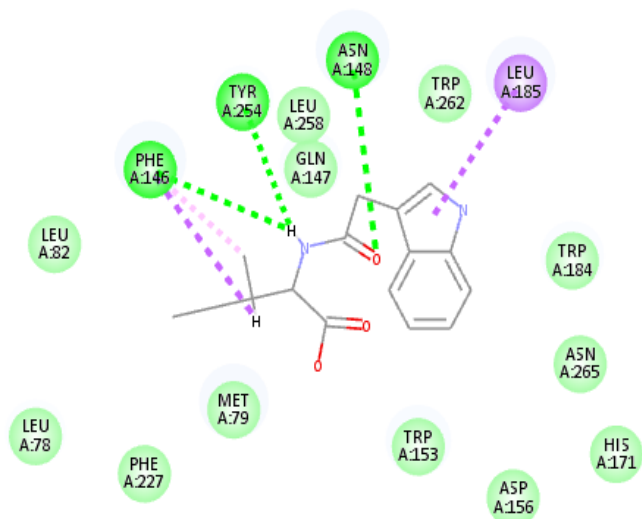
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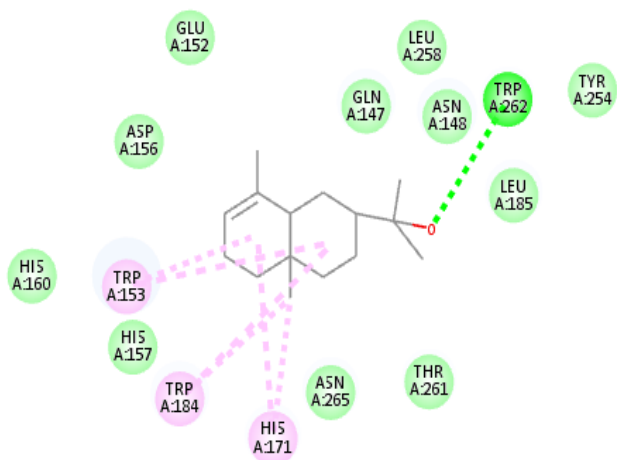
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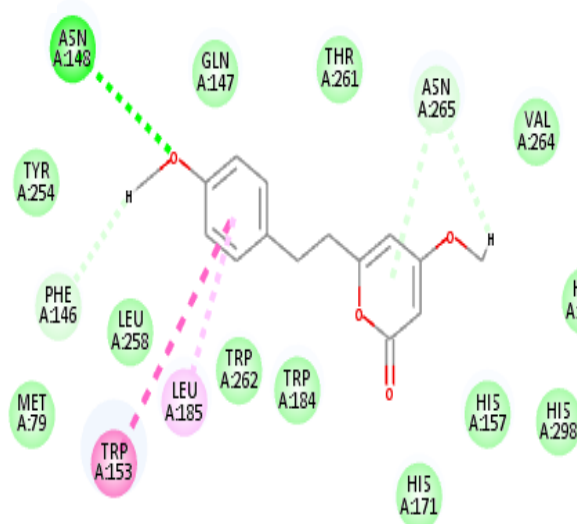
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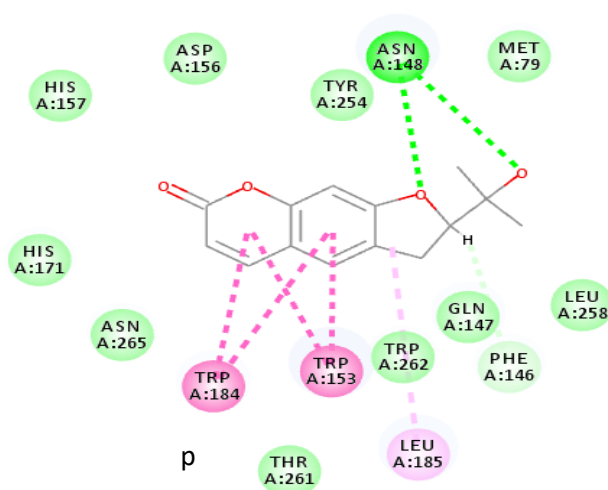
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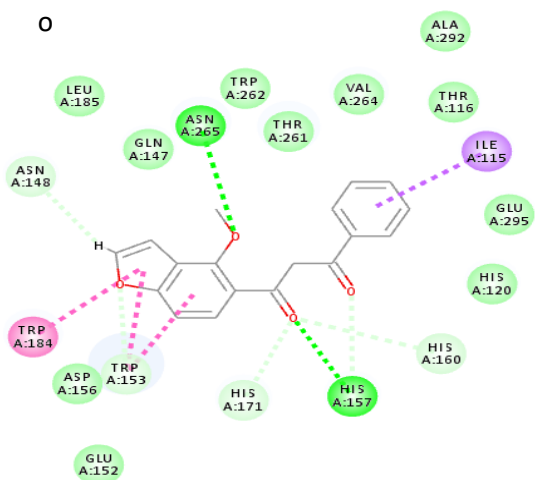
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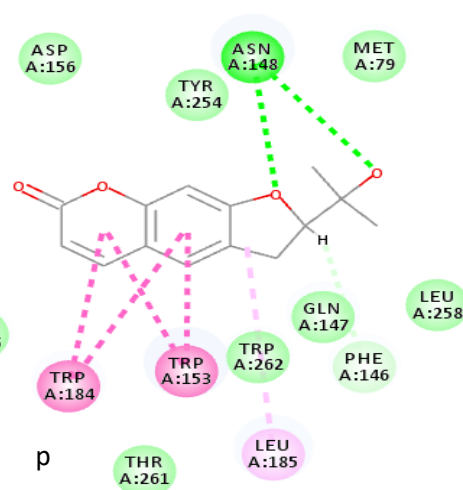
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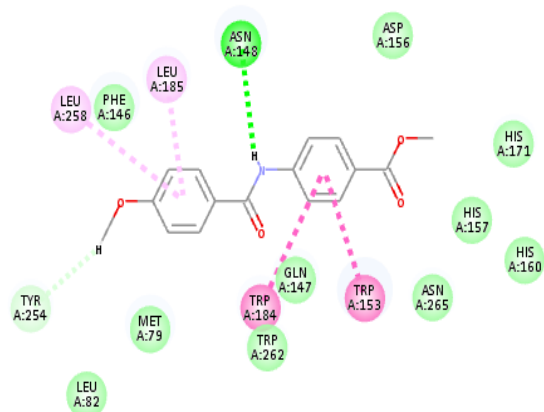
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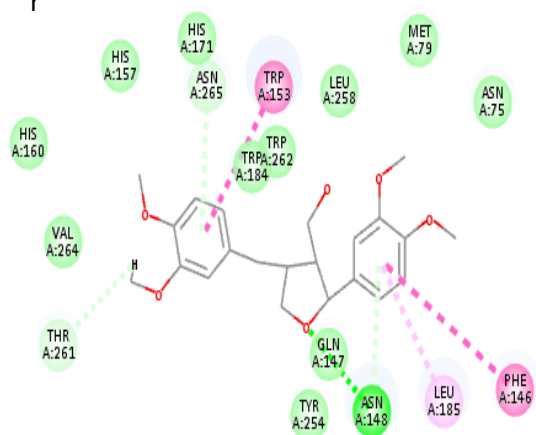
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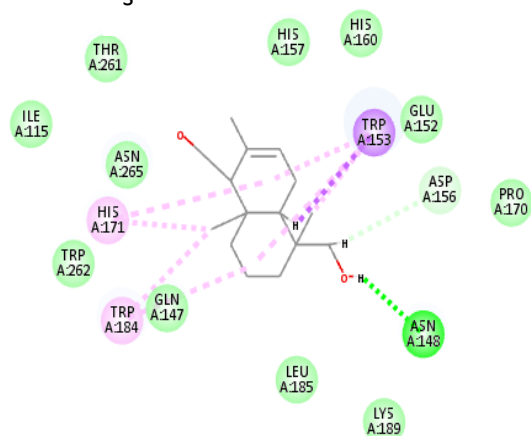
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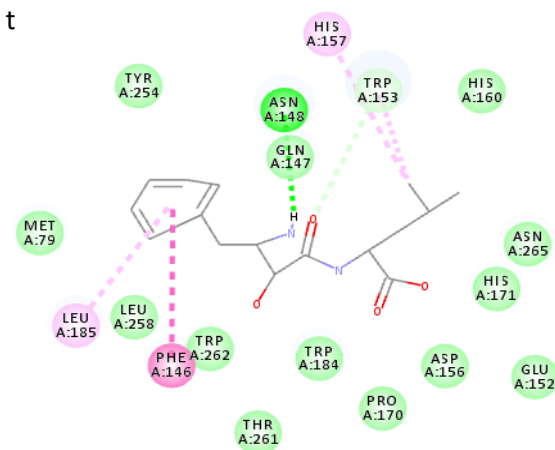
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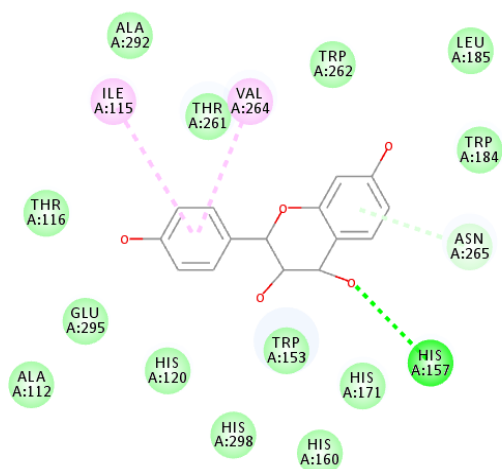
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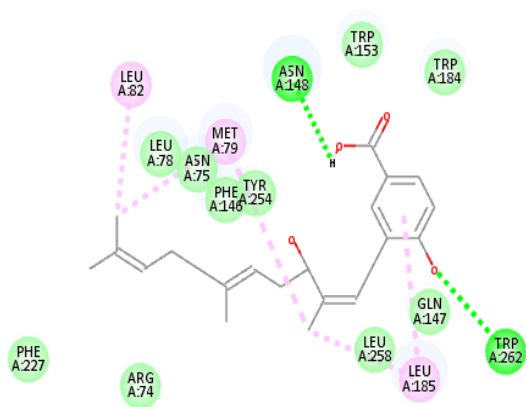
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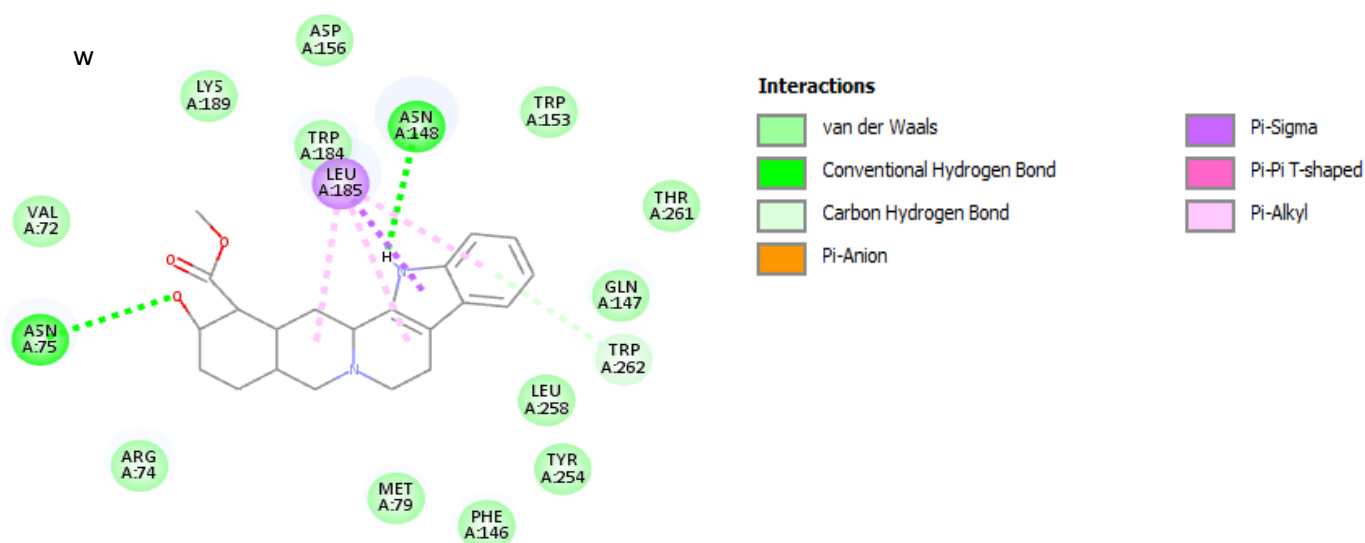


Fig 5.5.2 Ligands binding at the active site of the SCD1

a. Falcarinol, b. Falcarindiol, c. Farnesol, d. Phloretin, e. Hexacosanol, f. Cedrelopsin, g. Pheophorbide A methyl ester, h. dimethyl-1,2,4,5,6,7,8,9,11,12,14,15-dodecahydro cyclopenta[a]phenanthren-3-one, i. Cyclo(D-Leu-L-Pro), j. (1R,2R,5R)-N-ethyl-5-methyl-2-propan-2-ylcyclohexane-1-carboxamide, k. alpha-Eudesmol, l. N-(3-Indolylacetyl)-L-isoleucine, m. 7,8-Dihydroyangonin, n. Tyrosol 4-sulfate, o. 1,3-Propanedione, 1-(4-methoxy-5-benzofuranyl)-3-phenyl, p. Nodakenetin, q. Methyl 4-[(4-methoxybenzoyl)amino]benzoate, r. [(2S,3R,4R)-2-(3,4-dimethoxyphenyl)-4-[(3,4-dimethoxyphenyl)methyl]oxolan-3-yl]methanol, s. [(1R,4aS,5R,8aR)-5-(hydroxymethyl)-2,5,8a-trimethyl-1,4,4a,6,7,8-hexahydronaphthalen-1-yl]methano, t. Ubenimex, u. Guibourtinidol-4 alpha-ol, v. 4-hydroxy-3-[(2E,4R,6E)-4-hydroxy-3,7,11-trimethyldodeca-2,6,10-trienyl]benzoic acid, w. (1S,15S,18R,19S,20S)-18-hydroxy-1,3,11,12,14,15,16,17,18,19,20,21-dodecahydroyohimban-19-carboxylate

5.5.2 Docking Results of ACC1

For ACC1 there are 17 compounds docked, among them 4 compounds don't form H-bonds, and 4 consist of unfavorable bonds, only 10 compounds formed H-bonds at the N-terminal of the CT domain of ACC1. Biotin Carboxylase (BC) domain, biotin-containing carboxylase carrier protein(BCCP), and Carboxyl transferase (CT) domain are the three major domains in ACC1. Carboxylation is carried out by the BC domain and is considered an active site in ACC1. BC and CT domain plays a major role in fatty acid synthesis. The functions of the three domains take place in three steps. At first, ATP is utilized by the BC domain and promotes the carboxylation of biotin, their bicarbonates the carbonyl moiety then the BCCP domain carries the bicarbonate to the CT domain. Finally, carboxylation takes place at the CT domain and converts Acetyl CoA into Malonyl CoA. (Wang *et al.*, 2022).

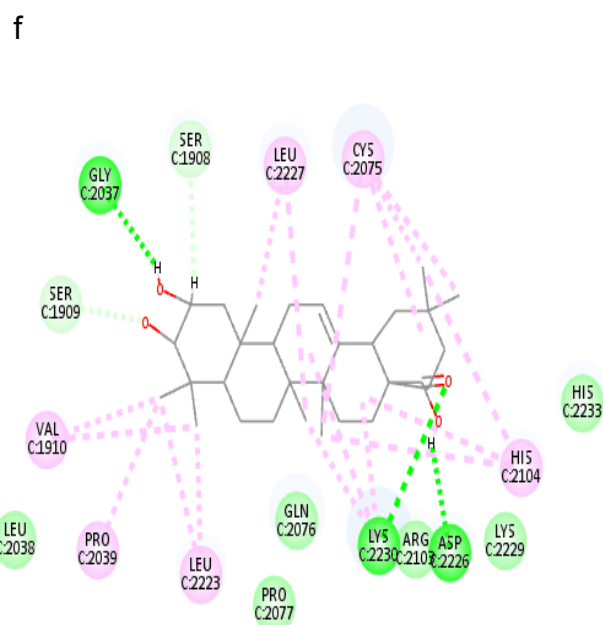
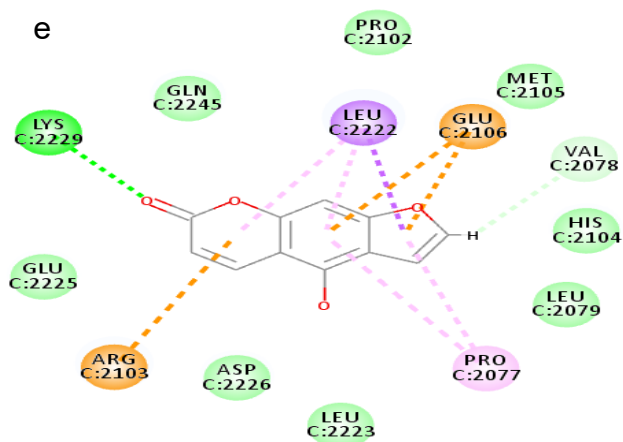
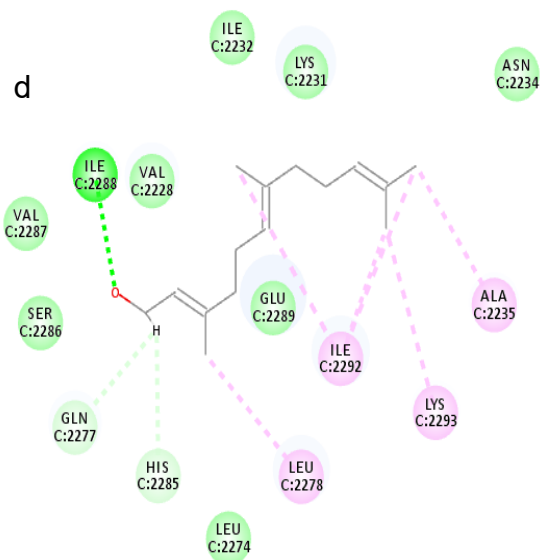
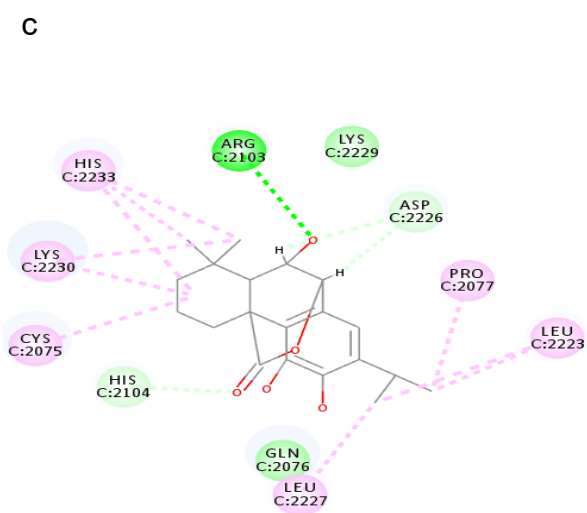
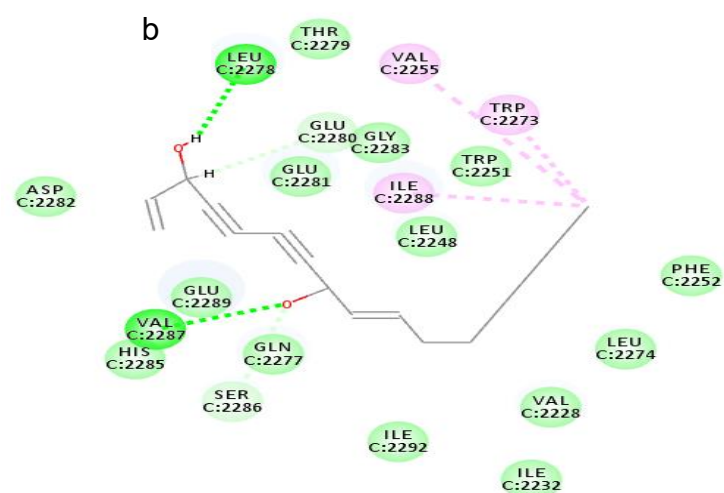
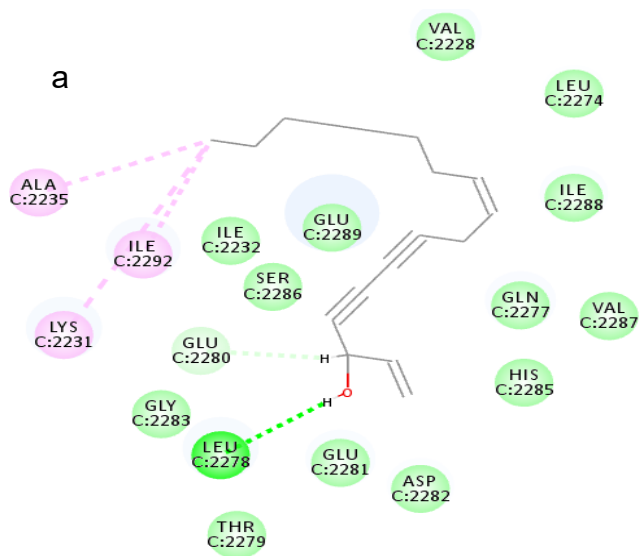
Inhibition of the CT (carboxyltransferase) domain of ACC1 (acetyl-CoA carboxylase 1) may have significant effects on fatty acid synthesis and energy metabolism in the body. Since the CT domain is responsible for carboxylation, inhibiting this domain can lead to a decrease in malonyl-CoA production, which is a key precursor for fatty acid synthesis. Several drugs have been developed that target the CT domain of ACC1, such as soraphen A and ND-630. These drugs have been shown to be effective in reducing the fatty acid synthesis and promoting fatty acid oxidation in preclinical studies. (Madauss *et al.* , 2009) There are 10 compounds that bind at the CT domain with h-bonds and may inhibit the protein functions.

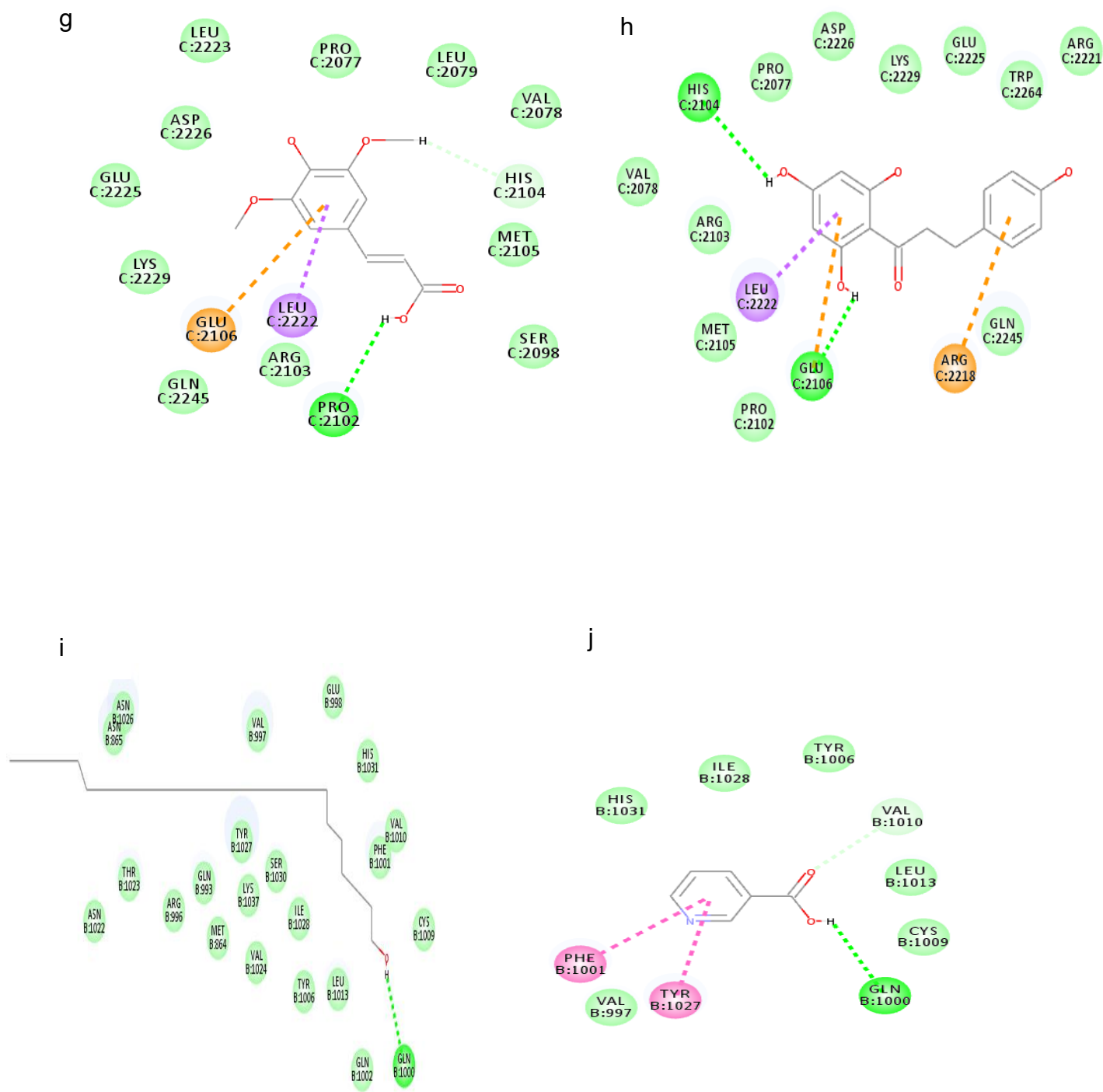
Table 5.2 List of compounds docked with ACC1

ACC1 inhibitors- small molecules										
Sl. no	Name	Relative energy	Absolute energy	Libdock Score	H-bond total	The residue of the H bond	Unfavorable bonds	Carbon - Hydrogen Bonds and Residues	No of non-covalent interactions	Non-Covalent bond number, residues
1	Rotundone	4.67654	23.7265	54.322	0	0	0	0	11	VAL2228, LYS2231, ILE2229, ILE2228, ILE2232, LEU2274
2	FALCARINOL/fennel, parsley	5.96624	20.0845	100.173	1	LEU2278	0	0	3	ALA2235, ILE2292, LYS2231
3	FALCARINDIOL / fennel, parsley	7.42986	23.1544	105.54	1	LEU2278	0	0	3	VAL2255, TRP2273, ILW2288
4	Ursolic acid	0	65.7475	102.822	2	ASP2226, ARG2103	1	2	12	LEU2227, LEU2223, VAL1910, HIS2104
5	Carnosic acid	1.69204	47.2636	72.9495	0	0	0	0	11	GLU22889, LEU2278, ILE2288, ILE2292,

6	Nuatagenin	0.78225	46.02778	98.5754	0	0	0	0	19	ALA2235,ILE2292,TRP2273,LEU2274,ILE2288,VAL2228
7	Rasmaquinone A	8.39593	39.142	80.7091	1	ARG2218	1	1	8	ARG2249,TRP2264,LYS2229,GLU2106,LEU2222
8	Rasmanol	0	45.2192	63.272	1	GLU2225	2	2	4	LYS2229,LEU2222,GLU2106
9	Epiisorasmanol / rosemary	3.4444	72.885	111.16	1	ARG2103	0	3	10	HIS2233,LYS2230,CYS2075,LEU2227,LEU2223,PRO2077
10	Nootkatone	0	17.6966	43.4074	0	0	0	0	14	LEU2278,LEU2274,TRP2273ILE2288,VAL2228,LYS2231,
11	Farnesol	8.08867	32.4215	87.6167	1	ILE2288	2	2	6	ALA2235,LYS2293,ILE2292,LEU2278
12	Bergaptol / grapefruit	0	97.6736	80.0579	1	LYS2229	0	1	8	LEU2222,GLU2106,PRO2077,ARG2103
13	Maslinic acid/apple, olive	15.0504	77.5074	96.295	3	GLY2037,LYS2230,ASP2226	0	2	17	LEU2227,CYS2075,HIS2104,LYS2230,LEU2223,PRO2039,VAL1910

14	Sinapic acid/orange, olive	0.49927	42.8059	86.5118	1	PRO2102	0	1	2	GLU2106, LEU2222
15	Phloretin/apple	7.03373	42.5175	95.9174	2	HIS2104, GLU2106	0	0	3	LEU2222, ARG2218, GLU2106
16	Hexacosanol/Diospyros	11.8462	14.474	122.933	1	GLN1000	0	0	0	0
17	Nicotinic acid / oldenlandia	0.0266112	14.9731	66.5992	1	GLN1000	0	1	2	PHE1001, TYR1027





5.5.3 Ligands binding at the CT domain of ACC1

a. Falcarinol, b. Falcarindiol, c. Epi-isorasmanol, d. Farnesol, e. Bergaptol, f. Maslinic acid, g. Sinapic acid, h. Pholorectin, i. Hexacosanol, j. Nicotinic acid.

DISCUSSION

Obesity, cancer, and diabetes are some of the major metabolic diseases that take place globally through the lipid metabolism pathway. Acetyl-CoA carboxylase-1 (ACC1) and stearoyl-CoA desaturase-1 (SCD1) are enzymes that play a crucial role in this pathway by catalyzing the conversion of acetyl-CoA to malonyl-CoA and stearoyl-CoA to oleoyl-CoA, respectively. These reactions are key steps in the synthesis of fatty acids, which are important for energy storage and cell membrane formation. However, it has been shown that the overexpression of ACC1 and SCD1 is associated with various metabolic disorders, including obesity and cancer. In particular, in many tumors, ACC1 and SCD1 are upregulated and contribute to the aggressive nature of cancer cells. Therefore, inhibition of these enzymes has emerged as a potential therapeutic strategy for the treatment of these diseases. When a ligand binds to the active site of a protein and forms hydrogen bonds with specific residues, it may inhibit conformational changes that disrupt the protein's function. This study has identified compounds that can bind to the active sites of ACC1 and SCD1 and inhibit their functions through hydrogen bonding interactions. Specifically, there are 23 compounds that have been shown to bind to the active site of SCD1 with good Lipinski's rule of five scores and without any unfavorable interactions. Furthermore, 10 compounds have been identified that can bind to the CT domain of ACC1 and may inhibit ACC1. Taken together, these findings suggest that the inhibition of ACC1 and SCD1 through hydrogen bonding interactions may be a promising therapeutic strategy for the treatment of metabolic disorders and cancer. Further research is needed to optimize these inhibitors and assess their safety and efficacy in preclinical and clinical settings. Zinc compounds are commercially available and the small molecules are obtained from natural resources which are edible. For ACC1 the 10 compounds which bind at the active site are the compounds obtained from edible sources such as fennel, grapes, olives, almonds, etc. For SCD1 7 small molecules and 16 zinc compounds are binding at the active site.

CONCLUSION

This study concludes that there are 10 potential inactivators for ACC1 and 23 compounds that may inhibit SCD1 activity. All of these compounds have been shown to bind to the active site of the target protein and may have potential inhibitory activity. These compounds may hold promise as potential therapeutics for the treatment of metabolic disorders and cancer. The commercially available chemical compounds that are obtained from the zinc database by virtual screening and these compounds are being used to treat cancer, asthma, obesity, neurogenic diseases and the ability to obtain small molecules from natural sources using laboratory techniques make these potential inhibitors more attractive from a drug discovery perspective. However, further research is needed to optimize these inactivators and assess their safety and efficacy in preclinical and clinical studies. It is also important to note that the inhibition of these enzymes may have unintended consequences, and careful consideration should be given to potential off-target effects. Overall, the findings of this study provide valuable insights into the potential use of small molecule inhibitors for the treatment of metabolic disorders and cancer, and future research in this area could have important clinical implications.

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