CAPITAL UNIVERSITY OF SCIENCE AND
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# Active Drug Targeting of Cannabis Constituents against Mutated SMN1 Protein to Cure Spinal Muscular Atrophy

by

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A thesis submitted in partial fulfillment for the
degree of Master of Science

in the

Faculty of Health and Life Sciences
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*This thesis dedicate to my dear and supportive family and friends who have Fully helped me in achieving my life goals.*

# CERTIFICATE OF APPROVAL

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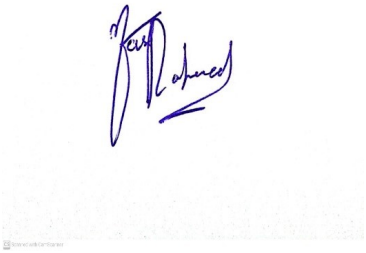

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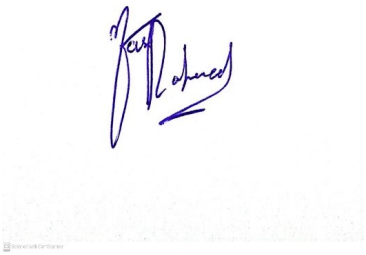

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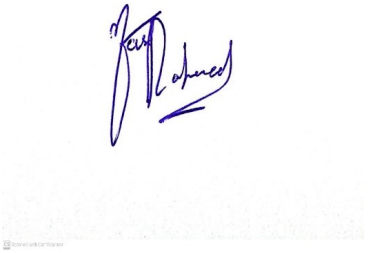

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# *Abstract*

Spinal muscular atrophy (SMA), a neurological disorder, is caused by autosomal recessive genes. The survival motor neuron (SMN1) gene is impacted, which reduces the amounts of the SMN protein, which is the source of the disease. In order to stop SMA from getting worse, promote independence, and enhance the patient's quality of life, manage the condition that focuses on disease-modifying treatments. For this purpose, many plants have been exploited to find natural compounds to work against this disease. The detailed study of the mutated SMN1 gene shows that mutations in the SMN1 gene cause a lack of the SMN protein in people with SMA. The active compound in cannabis (marijuana) were selected to be docked against the mutated SMN1 protein. 20 ligands from different classes were selected for this purpose. These ligands were then screened out based on Lipinski Rule and through studying the ADMET properties of the ligands. After the docking of the selected ligands with the receptor protein through the CB dock, the lead compound lysine was selected against the standard drug Evrysdi (Risdiplam). The docking results of both compounds were visualized via PyMol and were analyzed by the use of LigPlot. The result showed that lysine can be more effective against SMN1 protein rather than Evrysdi (Risdiplam). However further research has to be carried for investigating lysine for potential medicinal use.

**Keywords:** SMA, Cannabis (marijuana), CB-dock, ADMET, Lysine, Evrysdi

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# Abbreviations

| | |
|---|---|
| ADME | Absorption, Distribution, Metabolism and Excretion |
| BBB | Blood Brain Barrier |
| FDA | Food Drug Authority |
| MRTD | Maximum Rate Tolerated Dose |
| PDB | Protein Data Bank |
| PKcsm | Pharmacokinetic Properties |
| SMA | Spinal Muscular Atrophy |
| SMN | Survival Motor Neurons |
| T. Pyriformis | Tetrahymena pyriformis |
| VDss | Volume Distribution |

# Chapter 1

# Introduction

## 1.1 Background

One of the main genetic conditions linked to infant mortality is spinal muscular atrophy (SMA). The Survival Motor Neuron 1 (SMN1) gene is source of mutations or deletions in over 90% of SMA cases. Since exon 7 is primarily skipped, SMN1 is lost and its nearly similar duplicate, SMN2, cannot make up for it [1]. Estimated to affect 1 in 6,000 to 1 in 10,000 live births, SMA is the second most frequent disorder after cystic fibrosis, with a carrier frequency of 1/401/60 [2].

Motor neurons, the specialized nerve cells in charge of regulating voluntary muscle movement, degenerate in Spinal Muscular Atrophy a hereditary condition. The survival motor neuron gene which found on the chromosome, is where the majority of the mutations that cause it occur. The survival motor neuron protein which is essential for the survival and operation of motor neurons, is produced according to instructions from the SMN1 gene. Mutations in the SMN1 gene cause a lack of the SMN protein in people with SMA. Motor neurons' stability and health are impacted by this deficit which ultimately causes their degeneration [3]. Deletion of the survival motor neuron gene, which is supposed to be found in the telomeric region of chromosome 5q13, is the cause of this disease. A gene called SMN2, which is found in the centromeric region and is related to SMN1, is the primary factor that determines severity [4]. The survival motor neuron protein has

decreased as a result of this genetic modification to the SMN1 gene. Because the SMD2 gene only generates 25% of the SMN protein, it cannot fully compensate for the lack of SMN1 expression.

The absence of SMN protein causes the alpha () motor neurons in the spinal cord's ventral horn to degenerate, resulting in symmetrical muscle weakness that progresses over time. Muscle weakness, atrophy (wasting) and other muscle-related problems result from the motor neurons' decline because they can no longer communicate with the muscles. While the SMN1 gene is the primary gene linked to SMA, other genes can affect the severity a course of the condition. The survival motor neuron 2 (SMN2) gene, which closely resembles SMN1 is one of these genes. The SMN2 gene, however, generates a less stable and shorter form of the SMN protein as a result of a minor genetic difference. Due to the increased production of the SMN protein, those who have more copies of the SMN2 gene typically have milder types of SMA. The number of functioning SMN2 gene copies, along with other genetic and environmental factors, can affect how severe SMA is [5]. Multiple drugs have been given the by the Food and Drug Administration (FDA) to treat spinal muscular atrophy. These medicines Spinraza is marketed as Nusinersen. One of the first therapies for SMA to receive FDA approval was nusinersen. The survival motor neuron protein can be produced more often thanks to an antisense oligonucleotide that alters the SMN2 gene. The drug onasemnogene abeparvovec, also known as Gene therapy called Zolgensma has been licensed for the treatment of SMA in young individuals [6]. Multiple natural substances with a history of displaying significant anti- inflammatory and therapeutic characteristics have come under study in the drive to further medical research for spinal muscular atrophy. Through the use of computer- assisted drug discovery and design techniques the area of medical research has considerably advanced the creation of prospective therapeutic interventions over the past three decades.

One such priceless technology is molecular docking, a computational strategy that significantly outperforms conventional manual procedures in terms of speed and cost-effectiveness in identifying prospective drug candidates. This strategy has the potential to hasten the development of SMA therapies [7]. Medicinal plants have been previously used to combat several diseases. Cannabis (marijuana) is showing remarkable outcomes for many

patients with neuromuscular conditions like muscular dystrophy and spinal muscular atrophy. Attempts have been made to identify small molecules extracted from the plants that exhibits inhibition activity against the SMA. Marijuana is a plant that contains over 500 different chemical compounds. Just a small portion of them have been investigated. It is widely accepted that delta-9-tetrahydrocannabinol (THC). Cannabinoids, or other substances related to THC chemically, have been found in more than 100 different types of marijuana. The mechanisms by which cannabinoids work are mediated by the relatively new [8].

## 1.2 Problem Statement

Spinal muscular atrophy a neurological disorder, is caused by autosomal recessive genetics. The survival motor neuron gene is impacted, leading to reduced accumulation of SMN protein, which is the reason behind disease. Mutations in the SMN1 gene, which lead to the loss of the functional Survival Motor Neuron protein, are the main cause of the debilitating neuromuscular condition known as spinal muscular atrophy. There is a vivid gap of study that could target mutated SMN1 protein of SMA with the active compounds having antiseptic properties present in Cannabis (marijuana) for the conduction of extensive computational studies through molecular docking.

## 1.3 Aim and Objectives

To predict potential compounds of Cannabis (marijuana) showing inhibitory properties against mutated SMN1 protein to control Spinal Muscular Atrophy.

1. To identify the probable inhibitory compounds present in Cannabis (marijuana) against SMN1 protein of SMA.

2. To analyze the interaction between ligand and protein complex by performing molecular docking.

3. To find the best of the interacting molecules that show inhibitory effects against the Spinal muscular atrophy SMN protein

## 1.4 Scope

The study of Spinal Muscular Atrophy covers a range of topics including disease comprehension, patient diagnosis, patient management, and the development of efficient therapies. It's important to understand the genetic roots and molecular processes of SMA. This entails looking at how SMN1 protein mutations result in motor neuron degeneration and looking into potential modifiers that affect disease severity. The scope of SMA research includes investigating and creating novel therapeutics such gene therapies, small molecule medications, and targeted interventions that are intended to address the underlying molecular abnormalities and enhance patients' quality of life. Recent research has shown the potential of particular substances as spinal muscular atrophy inhibitors, highlighting the necessity of future research into natural substances having inhibitory characteristics against the underlying mechanisms of the disease. In order to delay or stop the advancement of the disease, Spinal muscular atrophy research looks for innovative substances that can target and reduce the molecular mechanisms of motor neuron degeneration. An in-silico method is used in this research, this study takes an in silico approach, using molecular docking to forecast how active compound of Cannabis (marijuana) would interact with the mutated SMN1 gene and other biological structures .

# Chapter 2

# Literature Review

The term "spinal muscular atrophy," or "SMA," refers to a group of inherited diseases that are all caused by anterior horn cell degeneration, which weakens the muscles. The homozygous deletion or mutation of the 5q13 survival of motor neuron (SMN1) gene, an autosomal recessive disorder, accounts for about 95% of instances of SMA. Overall carrier frequency was one in 54 with an incidence of one in 11,000 in a thorough multiethnic study to evaluate the feasibility of high throughput genetic testing for SMA carriers [9].

## 2.1 Type I

This type is noticeable before the age of six months. The most severe instances have decreased mobility, muscle/tendon shortening (contractures), low muscle tone, no tendon reflexes, problems, and challenges with feeding and swallowing. Many affected youngsters don't live past age 2 without treatment.

## 2.2 Type II

Between the ages of 6 and 18 months, parents usually discover that their kid can sit but needs help standing or walking. Some people have trouble breathing. Although many people live into adolescence or young adulthood, life expectancy is shorter.

## 2.3 Type III

Seen around 18 months, these kids can walk on their own but may have trouble running, getting out of a chair, or climbing stairs. There's a chance of respiratory infections, contractures, and spinal curvature. The average lifespan may increase with treatment.

## 2.4 Type IV

This form first shows symptoms after the age of 21, including mild to moderate leg muscular weakness. Mobility, and life expectancy vary between SMA type's early diagnosis, care, and management have a big impact on outcomes [10].

## 2.5 Origin

The hereditary cause of spinal muscular atrophy a condition is well known. The SMN1 gene which is found on chromosome 5 has mutations that lead to the condition. The precise time when the genetic cause of SMA was discovered can be dated to the late 20th century, when developments molecular genetics made it possible for researchers to comprehend the genetic basis of the condition. In particular, it was in the 1990s that the SMN1 gene and its connection to SMA were discovered. Drs. Arthur Burghes and Adrian Krainer's research team made a ground breaking discovery in 1995 when they determined that the SMN1 gene is the main genetic component causing SMA.

A rare neuromuscular condition with variable symptoms and severity is called spinal muscular atrophy. In the US, it affects between 10,000 and 25,000 people. Depending on the kind of SMA, symptoms may start to manifest as early as birth [11].

## 2.6 Classification of SMA

Based on the age at illness onset and the maximal motor function attained, there are four SMA classes as shown in Table 2.1 [12].

Table 2.1: Classification of SMA Disease [12]

| **SMA type** | **Other Names** | **Age of Onset** | **Life Span** | **Highest Motor Activity** |
|---|---|---|---|---|
| Type-I years (Severe) | Werdnig-Hoffmann disease | 0-6 months | 2-5 | Never sit |
| Type-II (Intermediate | SMA, Dubowitz type | 7-18 months | 2 years | Sit, Never stand |
| Type-III (Mild) | Kugelberg-Welander disease | 18 months | Adult | Stand and walk (may require assistance) |
| Type-IV (Adult) | —— | Adulthood | Normal | Walk during Adulthoodunassisted (some muscle weakness) |

### 2.6.1 Type I SMA

Also known as severe SMA, Werdnig Hoffmann disease, or acute SMA, is distinguished (between 0 and 6 months of age) failure to develop capacity to sit up and an extremely low life expectancy (less than 2 years). When a child is diagnosed with this kind they cough wail and have very little control over their heads. Before they turn a year old they lose the capacity to swallow and feed. It is uncommon for a regular respiratory cycle to form when intercostal muscles begin to weaken along with the trunk and limbs.

### 2.6.2 Type II SMA

Also known as chronic SMA, typically appear between the ages of 6 and 18 months, however they might appear sooner. Some patients with type II SMA are capable of

sitting up unassisted, whereas others can sit still if they are properly positioned but are unable to do so. Better developed patients can maintain their standing position with support, but they will not be able to walk on their own. In some kids, bulbar weakness and trouble swallowing can prevent them from gaining as much weight. Furthermore, these patients face issues like coughing and clearing tracheal secretions may experience fine trembling (sometimes referred to as fasciculation) develop scoliosis and contractures. Average lifespan is between 10 and 40 years.

### 2.6.3 Type III SMA

The onset is after 18 months, however the precise age varies widely. The disease is categorized as Type IIIa SMA if it first manifests before the age of three and Type IIIb SMA if it does so beyond that age. The capacity to walk is preserved in one case, but not the other.

### 2.6.4 Type IV SMA

There is disagreement on the disease's initial age.Russman claims that it appears after the age of 10, although Wang et al. claim that it typically appears in the second or third decade of life or around the age of 30. There are no issues with deglutition or respiration and there is just a minor involvement of the motor functions. These patients may walk normally and are expected to live regular lives [12].

## 2.7 Mutation Causes in SMA

Full-length mRNA from the SMN1 gene results in a useful protein. In contrast, the SMN2 gene skips exon 7 during the synthesis of mRNA, resulting in the unstable and shortened SMNA protein. SMNA is less effective and quickly broken down by cells (in Figure 2.1). This molecular process assisted in the creation of SMN-increasing medications like nusinersen. Because it is essential for the generation of the SMN protein in SMA patients,

SMN2 is crucial. About 10% of the SMN protein from SMN2 is functional, helping to make up for the loss of SMN production in some small way. SMA patients possess one or more copies of SMN2, the quantity varies depending on the kind of SMA. In general, and more copies of SMN2 tend to reduce the severity of the condition, although this isn't always the case because of other influencing factors [13].

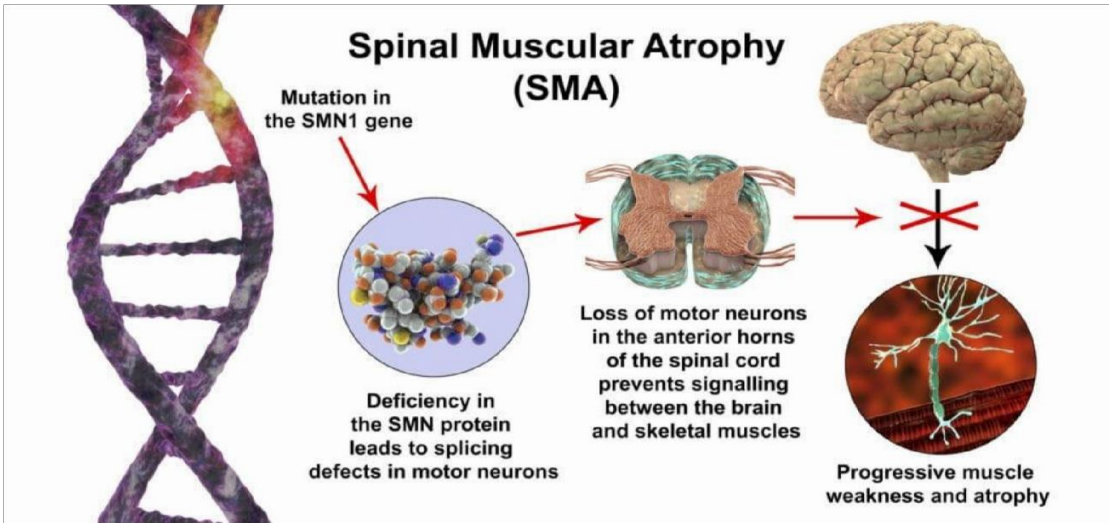



FIGURE 2.1: Spinal Muscular Atrophy [13]

## 2.8 Symptoms

Spinal muscular atrophy is a neuromuscular illness characterized by tongue mobility difficulties, muscle weakness and floppiness in the limbs, and tremors. It can result in anomalies of the bones and joints such scoliosis. There may also be issues with swallowing, eating, and breathing. SMA has no negative effects on intelligence or causes learning problems [14].

### 2.8.1 Muscle Weakness

Muscle weakness is a typical SMA symptom. It occurs when there is insufficient of the necessary SMN protein, which prevents muscles from receiving signals from motor neurons. Muscles weaken and eventually atrophy as a result of motor neurons' inability to deliver messages [15].

## 2.9 Statistics

More than half of all deaths worldwide and over half of all recorded instances of motor neuron disorders occur in high-income regions like North America, Western Europe, and Australasia. In other regions of the world, motor neuron disorders were significantly less common and more seldom occurred. Specifically because of the aging population, the total prevalence increased more between 1990 and 2016 than the age-standardized prevalence. Cousin marriages are the most frequently cited cause of SMA in Pakistan, despite the paucity of evidence needed to estimate its incidence mentioned in Table 2.2 [16].

TABLE 2.2: Statistics of SMA in Pakistan and other Countries [16]

| **Author Name & Study Year** | **Reported Proportion** | **Country** |
|---|---|---|
| Shawky(2011) | 46% | Egypt |
| Koul (2007) | 49% | Oman |
| Salahshourifar (2007) | 65% | Iran |
| Al Rajeh (1998) | 81% | Saudi Arabia |
| Ibrahin et al(our study) | 68% | Pakistan |

## 2.10 Treatment for SMA

In order to stop SMA from getting worse, promote and enhance the patient's quality of life, treatment for the condition focuses on disease-modifying treatments.

With the approval of three drugs, SMA disease-modifying therapy has advanced significantly.

1. Spinraza (nusinersen)
2. Zolgensma (onasemnogene abeparovec-xioi)
3. Evrysdi (Risdiplam) [17]

With these medications, the prognosis and course of SMA may be improved. The long-term result not yet known because these treatments are still very new. Other crucial elements of controlling SMA are in addition to these disease-modifying treatments.

- Physical Medicine

Using assistive technology, including feeding tubes, ventilators, and wheelchairs. (SMA can need a wide variety of assistive devices. It is advisable to talk about this with your medical team).

- Spinal Deformity Surgery

Families should collaborate with a healthcare team using a multidisciplinary approach, according to doctors. Over the course of a patient's lifespan, the SMA patient should have several medical evaluations. Family members need to receive genetic counseling, which is crucial. Researchers who have received funding from the NIH have also gathered information on children who have SMA types I, II, or III and their unaffected siblings. This study's objective is to counsel and inform parents about potential clinical trial options.

However, there has been a sizable advancement in the creation of medications that can help control the condition and enhance the standard of living for SMA patients. The following are some possible SMA treatments.

### 2.10.1 Zolgensma (Onasemnogene Abeparvovec)

An FDA-approved gene treatment for SMA designed exclusively for newborns and young children is called Zolgensma (Onasemnogene abeparvovec). It entails the one-time replacement of a healthy SMN1 gene with a damaged gene hence enhancing the synthesis of the SMN protein. However, the discovery of the genetic cause of SMA has led to the development of several treatment options that affect the genes involved in SMA.

### 2.10.2 Nusinersen (Spinraza)

Spinraza is an FDA-approved medication for SMA It modifies the splicing of the SMN2 gene to produce functional SMN protein. It's normally treated continuously and is provided by intrathecal injection (into the spinal fluid).

### 2.10.3 Risdiplam (Evrysdi)

This FDA-approved medication boosts the production of SMN protein by altering SMN2 gene splicing. It's consumed orally [18].

## 2.11 Medicinal Plants

Early people were able to recognize and use particular plants with proven curative characteristics through trial and error and their innate instincts. They would have been fully aware of which plant to utilize for specific maladies, injuries, or diseases. The systematic examination of numerous plants' therapeutic properties as a result of this careful use of medicinal plants ultimately helped greatly to the advancement of modern medicine and the identification of key drugs.

Cannabis (often known as marijuana) and hemp, two plants that are very closely related, both contain the active ingredient CBD. In contrast to marijuana's psychotropic properties, CBD does not cause a "high." A CBD-based medication called EpidiolexR has received FDA approval to treat seizures caused by three different illnesses. While clinical research examines CBD's potential to reduce pain and anxiety, there isn't yet solid clinical proof that it can effectively treat SMA-related discomfort. The FDA classifies CBD as a dietary supplement because it is exempt from strict safety and purity requirements. Although there are many different rules governing CBD's legal status, it is still available in most of the United States, and experts predict that it will soon be made more widely accessible and legal [19]. However, its legal status varies greatly around the world, with some countries and states legalizing its recreational and/or medicinal use, while others maintain strict prohibition.

## 2.12 Cannabis (Marijuana)

The cannabis (Figure 2.2) and the cannabinoids found in it to cure illness or relieve discomfort. It has been used in a variety of medical settings, including: Vomiting and Nausea: Medical marijuana is used to treat nausea and vomiting, especially in people receiving chemotherapy. Also, it is used to increase appetite, especially in those suffering from diseases like HIV/AIS, when losing weight might be a major concern. Pain management: Medical cannabis is used to treat muscular spasms and chronic pain, providing relief to people who suffer from a variety of severe diseases Study on Stroke.

For various medical diseases, however, medical cannabis has varying degrees of efficacy. Medical cannabis use for a brief period of time might have both modest and serious negative effects. Dizziness, exhaustion, nausea, and hallucinations are typical adverse reactions. Concerns about cannabis usage include issues with memory and cognition the possibility for addiction a rise in the incidence of schizophrenia in young individuals and unintentional consumption by children. In conclusion, medicinal cannabis is used to treat a variety of medical diseases and symptoms, but its efficacy varies and its use has potential negative health implications, especially with chronic use [20].

FIGURE 2.2: Cannabis (Marijuana) [20]

## 2.13 Active Constituents of Cannabis (Marijuana)

Over 550 different chemical components have been found in cannabis, and over 100 of them are phytocannabinoids, which include cannabidiol (CBD) and 9- tetrahydrocannabinol (THC).

Scientifically speaking, cannabis, also referred to as hemp or Cannabis sativa. Cannabis, a plant used for thousands of years for a variety of applications such as medical, recreational, industrial fiber, and seed oil, contains physiologically active and psychoactive substances known as THC have an impact on behavior and mental states. Furthermore, psychoactive canna-binoids with several medical advantages include cannabichromene (CBC), cannabigerol (CBG), and cannabidiol (CBD). This review concentrates on the phytochemistry of cannabis, emphasizing the compounds that make it up, especially the cannabinoids. Monoterpenes are secondary metabolites that could be linear or present in a rin-ged structure, shows antifungal, anti-inflammatory, antitumor, antibacterial, and insecticidal properties [22]. Tetrahydrocannabinol shows anticancer properties. Canabinol have anti –inflammatory and anti-microbial effects.

Cannabichromene ant inflammatory and anti-microbial properties. Ocimene has a pleasant, woodsy aroma and anti-tumor, anti-fungal, and anti-convulsant effects. Sesquiterpenes are present in the plant. Some of these which is an antiviral, antiparasitic, antimalarial, ant fibrotic, and anti-inflammatory [23]. Methionine increases glutathione intracellular concentration, supporting redox control and antioxidant-mediated cell defense. Lysine for healthy growth, a vitamin that helps decrease cholesterol and convert fatty acids into energy. Phenolic compounds in the classes of cyclitol, phenolic acids, and flavonoids are also present. Cannabichromenic acid have anti-analgesic and anti-inflammatory activity. Pinene have qualities that are antibiotic, apoptotic, antimicrobial, and antimetastatic. Limonene have anti-stress, anti-inflammatory, antioxidant, & perhaps disease-preventive qualities. Terpineol shows antihypertensive and cardiovascular consequences. Humulene and myrcene shows an anti-inflammatory and an anabolic agent [24].

## 2.14 Taxonomic Hierarchy

Table 2.3 shows the taxonomic hierarchy of Cannabis (marijuana). There is proof that prehistoric tribes in Africa and Eurasia used cannabis as a mind altering drug through archeological finds. The first known description of cannabis use is found in the Greek historian Herodotus, who describes the Scythians of central Eurasia taking steam baths with cannabis. From the Islamic dominion in the Middle East to North Africa, around 1545 cannabis made its way into the western hemisphere [25].

TABLE 2.3: Taxonomic Hierarchy of Cannabis [25]

| **S.No.** | **Domain** | **Scientific Name and Common Name** |
|---|---|---|
| 1 | Kingdom | *Plantae* - Plants |
| 2 | Subkingdom | *Tracheobionta* - Vascular plants |
| 3 | Division | *Magnoliophyta* - Flowering plants |
| 4 | Class | *Magnoliopsida* - Dicotyledons |
| 5 | Subclass | *Hamamelididae* |
| 6 | Order | *Urticales* |
| 7 | Family | *Cannabaceae* Martinov - Hemp family |
| 8 | Genus | *Cannabis* L. - hemp |
| 9 | Specie | *Cannabis sativa* L. - marijuana |

## 2.15 Molecular Docking

For more than three decades, computer assisted drug design and molecular biology have used the potent technique of molecular docking. It is essential for the virtual screening of chemicals in enormous databases, enabling an effective understanding of their functionalities. For the purpose of optimizing lead compounds for drug development, docking is favored since it can foretell how a ligand will interact with a protein. To forecast the results of receptor ligand interactions, many docking programmers use one or more search algorithms [26]. This adaptability is a key factor in why molecular docking has established itself as a crucial tool in drug discovery and molecular modelling. A scoring system

that quantifies the interaction is provided by the docking data, and the precision of this scoring function improves the accuracy of forecasting the ligand's binding pose. Additionally, it helps in locating the ligand's binding site, which in turn helps in the search for prospective lead medications connected to the target protein. In essence, molecular docking is a crucial technique for applications in structural biology and rational drug design, greatly aiding the creation of novel pharmaceuticals [27].

## 2.16 SMN Protein

The 38 kDa polypeptide known as the SMN protein which expressed in the cytoplasm and nucleus in vertebrates. It's composed of 2-294 amino acids. It is crucial for preserving the wellbeing of motor neurons, specialized nerve cells found in the spinal cord. The brain sends messages to the muscles, which in turn govern how the body's muscles move. This is done by motor neurons. Small nuclear ribonucleoproteins (snRNPs), necessary parts of the spliceosome, can be assembled and functioned more easily thanks to the SMN protein. The splicing of pre mRNA (messenger RNA precursors) into mature mRNA is carried out by the spliceosome. Splicing determines how many genes are expressed correctly, including those necessary for motor neurons to survive and function. The primary cause of spinal muscle atrophy is a deficiency of SMN protein (SMA). The SMN protein is produced by the survival motor neuron 1 (SMN1) gene, and mutations in this gene cause the deficiency.

Low levels of functional SMN protein result in motor neuron degeneration, weakness, and muscle atrophy. SMA manifests in varying degrees of severity, with the more severe forms causing significant difficulties with breathing and movement [28]. The survival motor neuron (SMN) protein has been reported in a number of different structures each of which sheds light on the protein's three dimensional arrangement and intermolecular interactions. Crystal graphic details of the SMN complexed with Gemins 2 and 8 (Gemin2 and Gemin8) are among the noteworthy structures that have been published. Splicing and the assembly of snRNPs both depend on this complex. There have also been numerous other structures and models investigated, including those involving the Tudor domain of SMN [29].

## 2.17 Natural Compounds as Inhibitors of Mutated SMN1 Gene

In contrast to enzymes or receptors, the SMN protein itself directly bind with small molecules and substances in many metabolic processes. The SMN protein instead functions as a structural and regulatory component of the cell.

Since the SMN protein's function is not dependent on enzymatic activity or direct binding to small molecules, the term "active compound" is not frequently used in relation to SMN proteins. Instead, its "activity" relates to how it participates in cellular functions and the construction of macromolecular complexes. These substances are not "active compounds" of the SMN protein are made to target particular elements of the cellular machinery that affect the creation of SMN protein. For instance, two such drugs, Nusinersen (Spinraza) and Risdiplam (Evrysdi) work to boost SMN protein levels by altering the splicing of the SMN2 gene, which, as a result of a genetic variant, produces less function al SMN protein. Treatment for SMA involves the use of these substances [30].

## 2.18 Inhibitors against Mutated SMN1 Gene of SMA in Cannabis(Marijuana)

There are large number of naturally occurring compounds that can serve as antiseptic to inhibit the activity of mutated SMN1 gene of SMA. The natural compounds have shown minimal side effects with low toxicity and the important thing is they are easily available to a large mass. The plant Cannabis (Marijuana) have been used from the earlier times. Different metabolic compounds are obtained from the roots, oil and leaves of the plant which includes terpenes, monoterpenes, polyphenols, flavonoids, coumarins and sesquiterpenoids. Tetrahydrocannabinol, also known as 9-THC.

Plants of the Cannabis sativa L. genus are widely distributed and grow well in temperate and tropical regions. With alcohol, tobacco, and caffeine, it is one of the most commonly used substances worldwide. Since ancient times, it has been used as a fiber source and

narcotic. The dried flowering tops and leaves of cannabis are used in herbal remedies. Hash oil, also known as cannabis resin, is a solvent-based extract of cannabis. Almost always, cannabis is smoked, frequently combined with tobacco [31]. The majority of resin and herbal while dronabinol medication in some countries for the treatment of nausea associated with cancer chemotherapy, cannabis has also been shown to have some therapeutic effect as an analgesic. THC and cannabis products are regulated internationally. While cannabis imported as an herbal medicine may come from South- East Asia or West Africa cannabis resin is mostly a product of either North Africa or Afghanistan Local producers frequently use solvent extraction to manufacture cannabis oil (hash oil) from a cannabis or cannabis resin. In Europe and elsewhere, intensive indoor cultivation is now very common.

# Chapter 3

# MATERIALS AND METHODS

## 3.1 Selection of Disease

Specialized nerve cells responsible for controlling voluntary muscle action will deteriorate. Most of the mutations happen in the (SMN1) gene. The SMN1 gene provide instructions for the production of the survival motor neuron protein which is necessary for the survival and functionality of motor neurons. Mutations in the (SMN1) gene had the genetic etiology of SMA. Due to its genetic foundation it is good candidate for studies on the mechanisms behind genetic disorders, gene treatments, and other genetic interventions [32].

## 3.2 Selection of Protein

The main role of SMN protein is to promote the spliceosome's crucial subunits small nuclear ribonucleoproteins (snRNPs) their synthesis and operation. Pre mRNA (messenger RNA precursors) are spliced into mature mRNA by a structure called a spliceosome.

The correct expression of genes, particularly those essential for the survival and function of motor neurons, depends on this splicing process [33]. The structure of SMA mutated SMN1 gene had been downloaded from the available resource of protein data bank (PDB).

The DOI and the PDB ID 4QQ6 the Crystal Structure of Tudor domain of SMN1 in complex with a small organic molecule had been downloaded [34].

## 3.3 Determination of Physiochemical Properties of Genes

To understand how a gene will function in a pathway. To investigate and determine its physical and chemical characteristics. ProtParam and an ExPAsy tool were used for this purpose. The molecular weight, isoelectric point, quantity of amino acids present, and instability index had only a few examples of the physiochemical parameters that were researched [35].

## 3.4 Cleaning of the Downloaded Protein

After downloading the protein structure, the extra constituents attached to the protein needs to be removed which was done by the use of an open source system Pymol. The linear chain consisting of range 1-294 amino acids was kept referring as the A chain and remaining all the constituents of the protein were eliminated so that further process is done effectively [36].

## 3.5 Determination of Functional Domains of Target Proteins

For determining the domains of the target protein was analyzed using InterPro, a comprehensive database renowned for its ability to scrutinize protein sequences. InterPro furnishes its structural and functional characteristics, including details about protein families, functional sites and the domains of the protein under study [37]. By inserting the FASTA sequence of the SMN1 gene we obtained the polypeptide binding sites and homodimer interfaces.

## 3.6 Selection of Active Metabolic Ligands

Those ligands were selected that had previously shown some antiviral and antimalarial properties. These includes the terpenes, monoterpenes, sesquiterpenes, phenolic compounds, flavonoids, coumarins and sterols [38].

## 3.7 Ligand Preparation

By using the database PubChem, 3-dimensional structure of the above selected ligands was downloaded. PubChem is under the National Center of Biotechnology Information (NCBI) and information regarding the chemical molecules. The information stored is related to the chemical names, molecular formulas. 3 dimensional or simple structures, their isomers, canonical similies and information regarding the activities of the molecules against the biological assays [38]. The structure of the ligands which are obtained from PubChem were downloaded and then the ligands MM2 energy was minimized by using Chem3D ultra. If in case the selected ligand structure was not available aim was to download the canonical similies from PubChem and then insert them in the software Chem Draw and after obtaining the 3D structure repeated the energy minimization step using Chem3D ultra. At the end SDF format was selected to save the energy minimized structure of the ligands.

## 3.8 Molecular Docking

A computational method called molecular docking was frequently employed in drug discovery and the investigation of protein-ligand interactions. Although it may not be the main method utilized in the study of spinal muscular atrophy itself, it may be used in adjacent research fields to comprehend the molecular mechanisms behind the illness and perhaps uncover therapeutic targets [38]. Docking is used to calculate the binding affinities of different small molecules or chemicals to particular proteins or enzymes involved in the

pathophysiology of SMA. This method was known as "target-identification" by analyzing the ways in which these substances interact with the intended proteins. Virtual-screening is a technique that uses computer screening to find possible therapeutic candidates for a specific target from massive databases of chemical compounds. Repurposing existing drugs can be used to analyze already-approved medications for other illnesses to find possible candidates for repurposing as SMA treatments. In the process of developing new drugs, this method could save time and money. Researchers create small molecules or other substances that specifically target the pathways implicated in the pathogenesis of SMA. This could be a step in the process of rational drug design [39].

## 3.9 Visualization of Docking Result via PyMol

Over the past few years the PyMol emerged as an efficient molecular tool of visualization. The graphics and its ability to view 3D structures were extraordinary. PyMol provides a plugin which can access the results and make their visualization clearer so that the docking results could be easily studied. The pictures of the docking result could be captured also. For all the process the docking result was saved in the pdb format and after visualization in the PyMol, was also be saved in the pdb file format [40].

## 3.10 Analysis of Docked Complex via LigPlot

Once we get the docked complex with the lowest vina score the next step was the analysis of the complex. The complex was in the pdb format. This analysis was done by using the software LigPlot. For the given pdb file format the schematic diagrams of the protein and ligand interactions were generated automatically. These connections are modified by hydrogen bonds and through hydrophobic contacts. LigPlot is a powerful tool utilized for analyzing the hydrophobic and hydrogen bonding interactions within protein-ligand complexes. Through its sophisticated algorithms, LigPlot generates detailed 2D representations of these complexes, offering a visually intuitive depiction of the molecular interactions between the protein and its ligand [41].

## 3.11 Ligand ADMET Properties

After the analysis the next step was study of pharmacokinetic and toxicity properties. The weak candidates of the drug were eliminated during preclinical ADMET screening. The remaining applicants might be chosen to develop medications to treat the illness. By using the PkCSM optimization of the ADMET which is Absorption, Distribution, Metabolism, Excretion and Toxicity related to human body was done [42].

## 3.12 Lead Compound Identification

After all the work was performed the next step was to find the lead compound. The lead compound was identified after applying the rule of 5 which includes.

1. The log value of the drug-like compound had to limited to 5.
2. The molecular weight should had to be lesser than 500.
3. Hydrogen bond acceptors maximum number should be 10.
4. Hydrogen bond donor's maximum number should be 5.

Once the compound fulfills these rule it was selected as the lead compound [43].

## 3.13 Comparison with the Standard Drug

Two such drugs, Nusinersen (Spinraza) and Risdiplam (Evrysdi) work to boost SMN protein levels by altering the splicing of the SMN2 gene, which, as a result of a genetic variant produced less functional SMN protein. Treatment for SMA involved the use of these substances [44].

## 3.14 Drug-Proposed against SMA

Even though much work had been done on developing and using medications to treat SMA there is still a gap in the care and cure for this condition. The active cannabis

(marijuana) components would be chosen as the chemical and will be compared to the existing medication to find its efficacy [45].

## 3.15 Overview of Methodology

Overview of methodology opted for this study is shown in Figure 3.1.

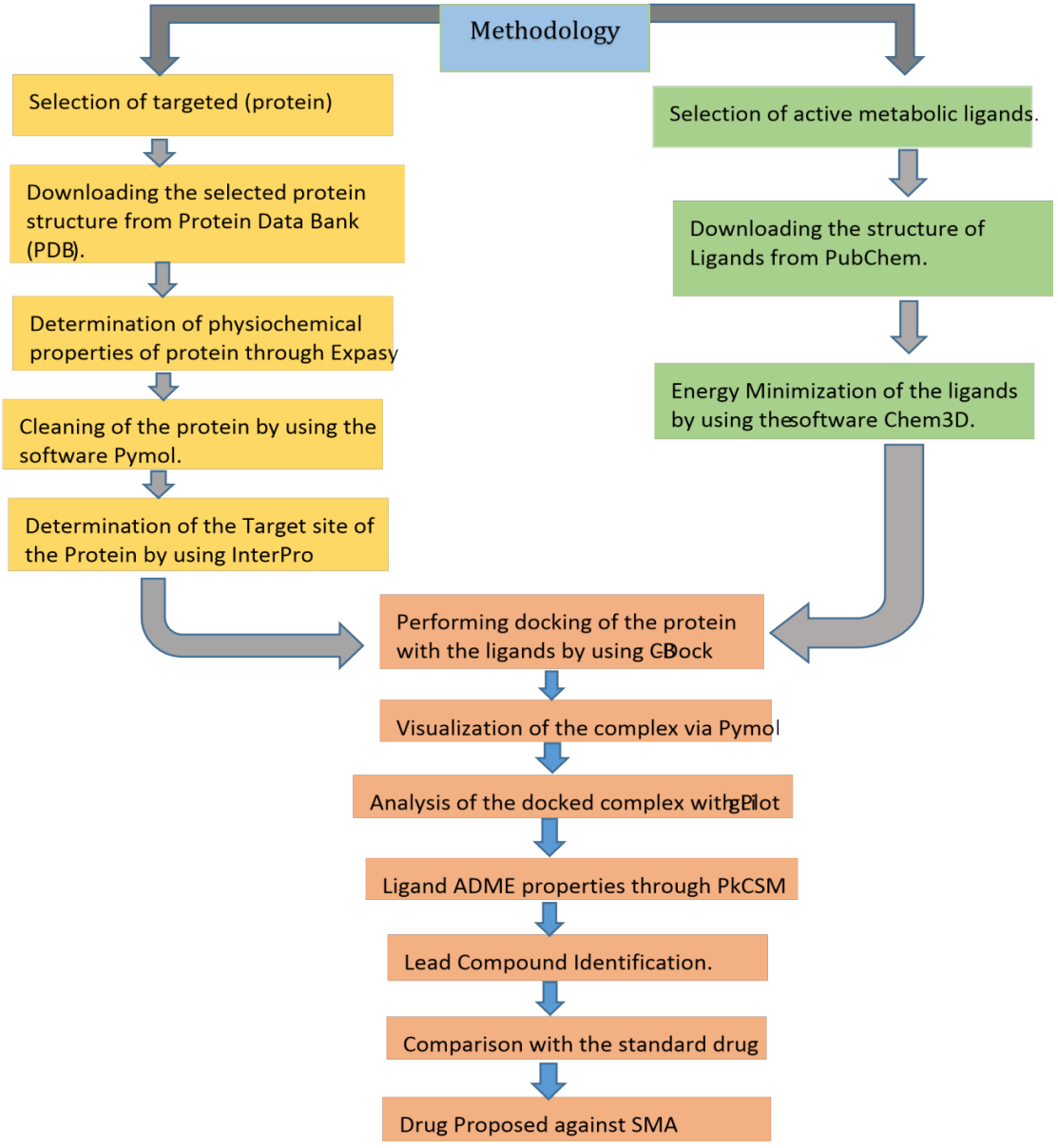



FIGURE 3.1: Methodology Opted for this Study

# Chapter 4

# Result and Discussion

## 4.1 Structure Modelling

Mutated SMN1 gene was selected as the target protein to act against the essential components present in cannabis (marijuana). Anterior horn cell degeneration, which results in muscle atrophy and weakening is the cause of several genetic disorder in which mutated SMN1 gene is involved [46].

### 4.1.1 3D Structure of the Protein

The protein selected is mutated SMN1 which plays an important role to provide instructions for the production of the survival motor neuron protein which is necessary for the survival and functionality of motor neurons. The SMN complex is crucial to the splicing of cellular pre mRNAs because it catalyzes the formation of small nuclear ribonucleoproteins (snRNPs), which are the spliceosome's building blocks. Mutated SMN1 protein is a 7.6 kDa protein which is making it an efficient drug target. The PDB (Protein Data bank) contains a large amount of data regarding the protein-ligand complexes. The structure of SMA mutated SMN1 gene was downloaded from the available resource of protein data bank (PDB). With the DOI and the PDB ID 4QQ6 the Crystal Structure of Tudor domain of SMN1 in complex with a small organic molecule was downloaded [47], which is given in Figure 4.1.

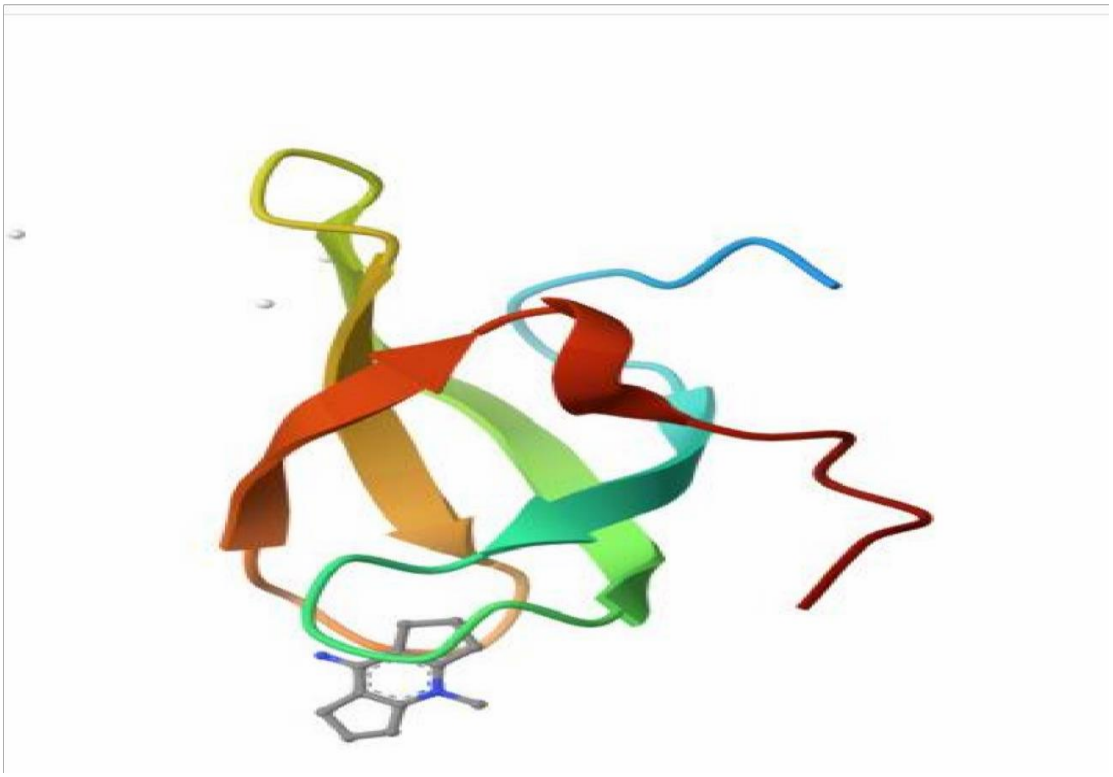


FIGURE 4.1: The Tudor Domain of SMN1 Crystals Structure [47]

### 4.1.2 Physical Properties of Protein

For studying the properties of mutated SMN1 protein a tool of ExPASy named as ProtParam was used. It is an online tool that was used for computing the physical and chemical properties of proteins that are entered in the Swiss-prot or TrEMBL or for the proteins entered by the users. The parameters which were studied include the molecular weight, protein's amino acid composition, atomic composition, theoretical pI, estimated half-life, extinction co- efficient, instability index, aliphatic index, and the last is the grand average of hydropathicity [48].

The protein showing pI greater than 7 means the basic nature of the protein whereas a pI value lesser than 7 indicates the acidic nature of the protein. Extinction coefficient indicates light absorption whereas instability index represents stability level of protein if it is lesser than 40 then that means the protein is stable and value greater than 40 shows that protein is unstable [49]. The aliphatic index shows thermo-stability of a protein. The molecular weight (MW) of protein shows both positive and the negative amino acid

residues. NR indicates the negative residues (Asp+Glu) and PR represents the positive charge residues (Arg+Lys). The low GRAVY value shows the interaction with water molecules. All the above- mentioned parameters were shown in Table 4.1 taken into consideration [50].

TABLE 4.1: The Physical Properties of the Selected Mutated SMN1 Protein

| **MW** | **pI** | **NR** | **PR** | |
|---|---|---|---|---|
| 31848.72 | 6.13 | 33 | 31 | |
| **Ext. Co 1** | **Ext. Co 2** | **Instability Index** | **Aliphatic Index** | **GRAVY** |
| 45420 | 44920 | 62.07 | 55.78 | -0.770 |

The Table 4.1 shows the molecular weight of mutated SMN1 protein as 31848.72 which is a collective weight of negative and positive amino acids residues. The pI is 6.13 which indicates that the selected protein is acidic in nature. The values of light absorption in terms of extinction coefficient is 45420 and 44920. The instability index value of 62.07 shows that selected protein mutated SMN1 quite an unstable protein. Aliphatic index also shows that selected protein is thermo-stable. Low value of GRAVY -0.770 shows that mutated SMN1 protein has good interactions with water molecules.

### 4.1.3 Identification of Functional Domains of the Protein

For identifying the functional domains InterPro consortium is used. InterPro helps in finding the functional analysis of proteins and classifies them into families which is done by finding functional domains and other important sites. Functional domains are the active part of the protein that is used by the protein for interacting with other proteins or other substances. The job ID for finding the functional domain of 4QQ6. The protein known as Survival of Motor Neuron in humans is made up of 294 amino acids and is divided into different domains. Figure 4.2 shows functional domains include the proline rich and YG domain at the C-terminal, a central Tudor domain and the N- terminal Gemni2 and nucleic acid-binding domains. The helical Gemin2 binding domain, the

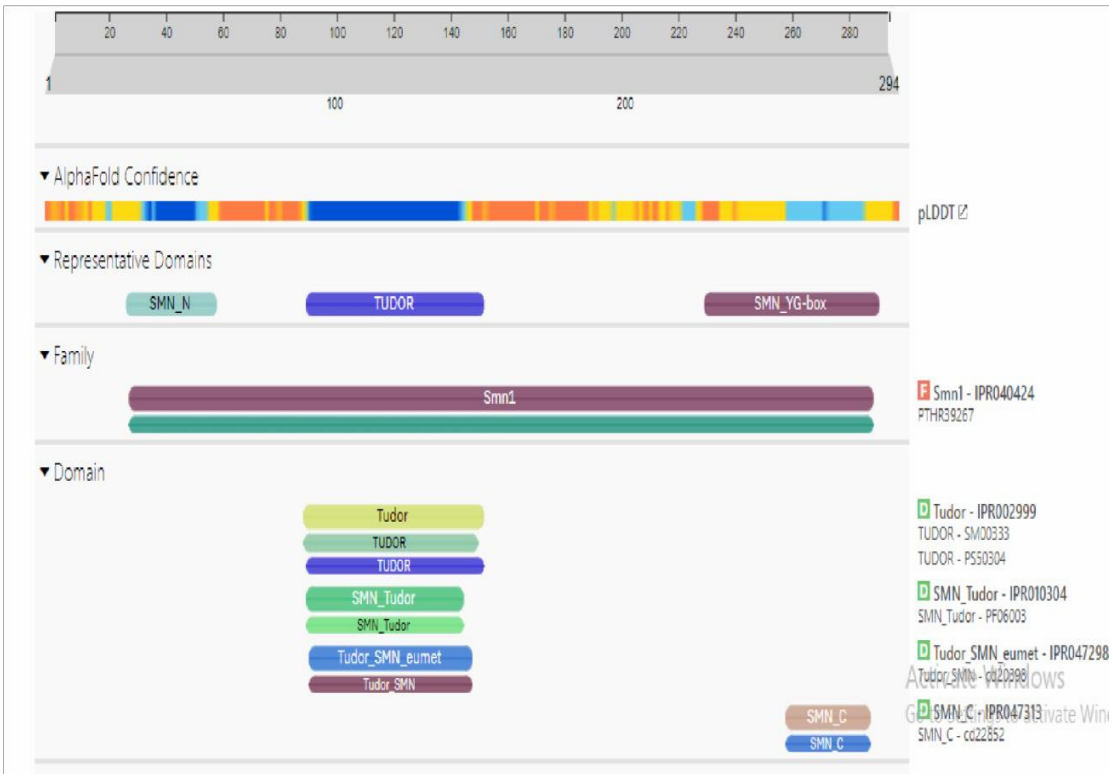



FIGURE 4.2: Functional Domains of Targeted Protein

barrel Tudor domain and the YG box domain including a glycine zipper motif from a dimeric SMN molecules are rendered visible in structure models. The N and C termini of these domain are displayed in the structural representation in lower panel [51].

### 4.1.4 Structure of Protein Refined for Docking

Structure of the protein was refined by the use of PyMol. The hydrogen bond was removed from the protein structure as shown in Figure 4.3.

## 4.2 Ligand Selection

The ligands which are the active constituents of the selected plant were searched from the world's largest chemical databank- PubChem. The 3D structures of these ligands were downloaded from PubChem in the SDF format. Table 4.2 shows all the selected ligands with the information regarding their structure [52-56].

FIGURE 4.3: Cleaned Protein of SMA (Mutated SMN Protein)

After downloading the structures of the ligands that were selected the next step that was performed was minimizing the energy of these ligands. This step is an important one as we can't use simply the downloaded structure as the ligands are unstable and it can directly affect the docking vina scores.

TABLE 4.2: Selected Ligand with Structure Information

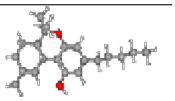
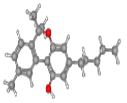
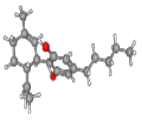
| **Sr. No.** | **Ligand Name** | **Molecular Formula** | **Molecular Weight** | **Structure** |
|---|---|---|---|---|
| 1. | Tetrahydrocannabinol | C21H30O2 | 314.5 g/mol |  |
| 2. | Cannabinol | C21H26O2 | 310.4 g/mol |  |
| 3. | Cannabidiol | C21H30O2 | 314.5 g/mol |  |

Table 4.2 - Continued from Previous Page

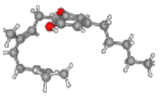
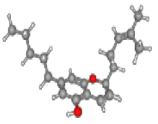
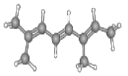
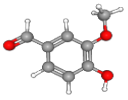
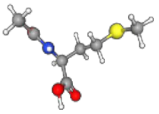
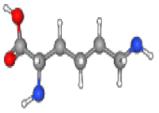
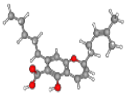
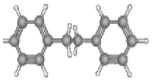
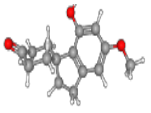
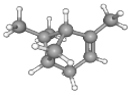
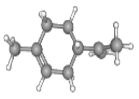
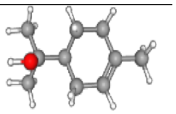
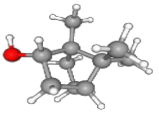
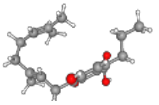
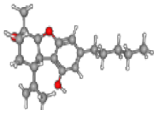
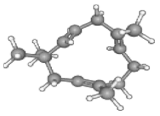
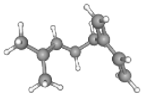
| **Sr. No.** | **Ligand Name** | **Molecular Formula** | **Molecular Weight** | **Structure** |
|---|---|---|---|---|
| 4. | Cannabigerol | C21H32O2 | 316.5g/mol |  |
| 5. | Cannabi-chromene | C21H30O2 | 314.5g/mol |  |
| 6. | Ocimene | C10H16 | 136.23g/mol |  |
| 7. | Resins | C8H8O3 | 152.15g/mol |  |
| 8. | Methionin | C7H13NO3S | 191.25g/mol |  |
| 9. | Lysine | C6H14N2O2 | 146.19g/mol |  |
| 10. | Cannabich-romenic Acid | C22H30O4 | 358.5 g/mol |  |
| 11 | Dihydrosti-lbene | C14H14 | 182.26g/mol |  |
| 12. | Cannabispiran | C15H18O3 | 246.3g/mol |  |
| 13. | Pinene | C10H16 | 136.23g/mol |  |
| 14. | limonene | C10H16 | 136.23g/mol |  |

Table 4.2 - Continued from Previous Page

| Sr. No. | Ligand Name | Molecular Formula | Molecular Weight | Structure |
|---|---|---|---|---|
| 15. | Terpineol | C10H18O | 154.25g/mol |  |
| 16. | Borneol | C10H18O | 154.25g/mol |  |
| 17. | Cannabiger-ovarinic acid | C20H28O4 | 332.4g/mol |  |
| 18. | Cannabielsoin | C21H30O3 | 330.5g/mol |  |
| 19. | Humulene | C15H24 | 204.35g/mol |  |
| 20. | Myrcene | C10H16 | 136.23g/mol |  |

## 4.3 Toxicity Prediction through Lipinski Rule

The compounds underwent screening to classify them as either drug-like or non-drug-like, adhering to the Lipinski rule of five and ADME (Absorption, Distribution, Metabolism, Excretion) properties, as outlined in references [57] and [58]. The Lipinski rule of five encompasses specific parameters, including a molecular weight not exceeding 500, a log P (octanol-water partition coefficient) of 5 or less, no more than 5 hydrogen bond donors, and no more than 10 hydrogen bond acceptors [59]. A compound is considered a potential drug candidate if it satisfies three or more of these rules. Conversely, if a compound violates two or more rules, it is deemed poorly absorbed, diminishing its potential as a drug candidate. This rigorous screening process ensures that only compounds with favorable pharmacokinetic properties and a high likelihood of oral bioavailability are considered for further evaluation in drug development efforts.

Table 4.3: Value of Lipinski Rule for the Selected Ligands

| **Sr. No.** | **Ligand** | **Log-P Value** | **Molecular Weight** | **H-Bond Accepter** | **H-bond Donor** |
|---|---|---|---|---|---|
| 1. | Tetrahydrocannabinol | 5.7358 | 314.5 g/mol | 1 | 2 |
| 2. | Cannabinol | 5.72782 | 310.4 g/mol | 2 | 1 |
| 3. | Cannabidiol | 12.19222 | 314.5 g/mol | 2 | 2 |
| 4. | Cannabigerol | 6.0657 | 316.5g/mol | 2 | 2 |
| 5. | Cannabichromene | 6.0356 | 314.5g/mol | 2 | 1 |
| 6. | Ocimene | 3.475 | 136.23g/mol | 0 | 0 |
| 7. | Methionin | 0.3288 | 191.25g/mol | 4 | 2 |
| 8. | Resins | 1.2133 | 152.15g/mol | 3 | 1 |
| 9. | Lysine | -0.4727 | 146.19g/mol | 4 | 3 |
| 10. | Cannabichromenic Acid | 5.7338 | 358.5 g/mol | 4 | 2 |
| 11. | Dihydrostilbene | 3.4718 | 182.26g/mol | 0 | 0 |
| 12. | Cannabispiran | 2.7279 | 246.3g/mol | 3 | 1 |
| 13. | Pinene | 2.9987 | 136.23g/mol | 0 | 0 |
| 14 | Limonene | 3.3089 | 136.23g/mol | 0 | 0 |
| 15. | Terpineol | 2.5037 | 154.25g/mol | 1 | 1 |
| 16 | Borneol | 2.1935 | 154.25g/mol | 1 | 1 |
| 17 | Cannabigerovarinic acid | 4.9837 | 332.4g/mol | 4 | 3 |
| 18 | Cannabielsoin | 4.7066 | 330.5g/mol | 3 | 2 |
| 19 | Humulene | 5.0354 | 204.35g/mol | 0 | 0 |
| 20 | Myrcene | 3.475 | 136.23g/mol | 0 | 0 |

### 4.3.1 Toxicity Prediction

PkCSM was an online tool that was used to predict the values of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) of the bioactive compounds and drugs. This tool will allow us to assess the toxicity of the ligands selected, for this different methods are used to test whether a given ligand is toxic or not. AMES toxicity test used to test the mutagenic potential of the compound by using bacteria. If it shows a positive response, then the ligand is mutagenic which can also act as a carcinogen [60]. T. Pyriformis toxicity method uses T. Pyriformis (protozoa bacteria) toxicity as a toxic end

point. Any value >-0.5 log ug/L is considered toxic. The values predicted in the Minnow toxicity test are used to represent the concentration at which the compound could cause the death of 50% of the minnows. The value below 0.5 mM is regarded as acute toxic.

The MRTD (maximum recommended tolerable dose) is an essential parameter used to determine the first dosage of a medicine in clinical phase I trials. A value of 0.477 log mg/kg/day is classified as low, indicating the need for a cautious first dose. Values over this threshold are considered high, requiring close observation for potential negative consequences.

In the context of oral rat chronic toxicity testing, the anticipated logarithmic value of the lowest observed adverse impact in logarithmic milligrams per kilogram body weight per day offers information about the concentration of the substance needed for therapy over a specific period.

A hepatotoxicity assessment is conducted to ascertain the hepatotoxic potential of a chemical, namely its ability to affect liver function. Similarly, a skin test assesses the probability of a substance causing negative skin responses. The hERG I and II inhibitor test assesses a compound's ability to hinder potassium channels linked to the human ether-a-go-go-related gene (hERG). Blocking these channels can result in QT syndrome, which increases the likelihood of ventricular arrhythmia when exposed for an extended period of time. This extensive battery of tests aids in evaluating the safety profile of pharmaceutical candidates, providing information for regulatory choices and reducing potential dangers to human health.

Table 4.4: Toxicity Predicted Values of the Selected Ligands

| **Sr. No.** | **Name** | **AMES** | **Max Dose** | **GI** | **GII** | **Oral** | **Chroinc** | **Hepa** | **Skin** | **T.pyrif** | **Minnow** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Tetrahydro | No | 0.121 | No | No | 2.543 | 1.95 | No | No | 2.419 | -0.854 |
| 2 | Cannabinol | No | 0.43 | No | yes | 2.066 | 1.731 | No | No | 1.036 | -0.681 |
| 3 | Cannabidiol | No | 0.308 | No | yes | 2.383 | 0.915 | No | No | 0.285 | -4397 |
| 4 | Cannabigerol | No | 0.532 | No | Yes | 2.542 | 2.217 | No | yes | 1.669 | -1.282 |
| 5 | Cannabichromene | No | 0.591 | No | yes | 2.406 | 1.977 | No | No | 2.338 | -1.09 |
| 6 | Ocimene | No | 0.636 | No | No | 1.636 | 2.427 | No | No | 0.792 | 0.784 |
| 7 | Resins | No | 1.285 | No | No | 1.937 | 2.007 | No | No | -0.014 | 1.899 |
| 8 | Methionin | No | 0.882 | No | No | 1.879 | 1.564 | No | No | 0.18 | 2.434 |
| 9 | Lysine | No | 1.227 | No | No | 2.046 | 3.083 | No | No | 0.274 | 2.542 |
| 10 | Dihydrostilbene | No | 0.988 | No | No | 1.877 | 1.266 | yes | yes | 1.439 | 0.646 |

Table 4.4 - Continued from Previous Page

| Sr. No. | Name | AM-ES | Max Dose | GI | GII | Oral | Chroinc | Hepa | Skin | T.pyrif | Minnow |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 11 | Canna-bichr | No | 0.447 | No | No | 2.637 | 1.925 | No | No | 0.294 | -0912 |
| 12 | Cannabispiran | No | -0.265 | No | No | 2.318 | 1.8 | No | No | 1.318 | 1.126 |
| 13 | Pinene | No | 0.48 | No | No | 1.77 | 2.262 | No | No | 0.45 | 0.579 |
| 14 | Limonene | No | 0.77 | No | No | 1.88 | 2.336 | No | yes | 1.159 | 1.203 |
| 15 | Terpineol | No | 0.886 | No | No | 1.923 | 1.945 | No | yes | 0.008 | 1.8 |
| 16 | Borneol | No | 0.577 | No | No | 1.707 | 1.877 | No | yes | 0.175 | 1.727 |
| 17 | Cannabigerovarinic acid | No | 0.306 | No | No | 2.595 | 1.971 | No | No | 0.289 | -0.35 |
| 18 | Cannabielsoin | No | -0.009 | No | No | 2.657 | 1.771 | No | No | 1.558 | -0.24 |
| 19 | Humulene | yes | 0.551 | No | No | 1.766 | 1.336 | No | Yes | 1.451 | 0.716 |
| 20 | Myrcene | No | 0.617 | No | No | 1.643 | 2.406 | No | No | 0.894 | 0.736 |

The toxicity values of all ligands are given in Table 4.4. The toxicity values of tetrahydrocannabinol and cannabinol shows that both have a low MRTD value. All other test values are in the safe range that shows that both are tetrahydrocannabinol and cannabinol not the cause for AMES Toxicity. They both are the hERG I. They both have a safe toxic rate with respect to test on rat and on T. pyriformis with that they are toxic to liver and does not provide any sensitivity to skin. The toxicity values of cannabidiol and cannabigerol indicates that cannabigerol has a high MRTD value that it is also sensitive to skin. Cannabidiol and cannabigerol can inhibit the hERG II inhibitor. Cannabigerol are sensitive to skin all other parameters of toxicity and hepatoxicity in the positive range. The toxicity values of cannabichromene, ocimene and resins indicate that all these three ligands have high MRTD values.

Among these three parameters cannabichromene, hERG II inhibitor can inhibit and others are in safe range. T.pyriformis toxicity of resins are not in safe range other two are positive range. The toxicity values through pkcsm of methionin and lysine have high MRTD value indicate that all other parameters of toxicity and hepatotoxicity and that of hERG1 and II inhibitors and T.pyroformis and skin sensitivity are all in the positive range. Dihydrostilbene, cannabispiran and pinene all have the value in range of the AMES toxicity, hERG I and II inhibitors, and T.pyriformis. Among these three, dihydrostilbene and pinene have high MRTD value. Hepatoxicity and skin sensitization of cannabispiran and pinene are in positive range. Limonene, terpineol both have AMES safe range, high MRTD value and indicates skin sensitization. All the three ligands have shown values in the range that is determined by pkcsm. Among all of these, humulene show AMES toxicity and skin sensitization and also indicates high MRTD in humulene and myrcene.

## 4.4 Molecular Docking

Molecular docking a technique that was used for estimation of the strength between a ligand bonded to a receptor protein through the vina score function and for determining the correct structure of the ligand that binds to the binding site. The 3D structure of the ligands and the protein are taken to perform docking. For this purpose, CB dock an online blind auto docking tool is used [62].

CB Dock computes the cavity sizes and predicts the protein binding locations. CB Dock provides us with the top five possess and receptor models upon docking. Based on the cavity size and the vina score, the optimal position was chosen from these five [63].

Molecular docking was performed by using SMN1 protein as the receptor protein and the 20 ligands selected above. The protein was in the PDB format and the ligands were in the SDF format [64]. Among five best conformations best one was selected based on high-affinity score and interaction between protein and the ligand. Ligands showing the best binding score between the selected ligands and the protein SMN1 are shown in Tables 4.5.

The docking result of ligands that is of tetrahydrocannabinol, cannabinol, cannabidiol, cannabigerol, and cannabichromene. It shows that tetrahydrocannabinol has a binding score of -6.6, with accepting one and donating two hydrogen. The logP value of this docked result is 5.7358. Cannabinol shows the docking score of -5.5 with accepting two or donating one hydrogen, and gives a logP value of 5.72782 which is similar to tetrahydrocannabinol as these both are isomers of each other. Both tetrahydrocannabinol and cannabigerol have shown same binding score of -6.6. Cannabidiol and cannabichromene shows a low binding score as -6.3 and -6.9 which is less than the binding score of cannabinol which gives the binding score as -5.5. Cannabidiol, cannabigerol, and cannabichromene gives a logP value of 12.19222, 6.0657 and 6.0356.

The docking result of ligands that are ocimene, resins, methionin, lysine, and cannabichromenic acid. All ligands have different binding score and highest binding score of ligand lysine -3.8. Lysine have -4.2, resins have -5.3, ocimene have -6.1, and cannabichromenic acid have 6.5. Among the docking result of ligands dihydrostillbene, cannabispirin, pinene, limonene, terpineol, the highest binding score of pinene is -4.1 then terpeniol -4.5.limonene have -5.6, cnabispirin have -5.8 and dihydrostilbene have score -7.3.

Table 4.5: Docking Result of all Ligands

| Sr. No. | Name | Score | Size | HBD | HBA | Log p | Weight | Bonds | Grid Map |
|---|---|---|---|---|---|---|---|---|---|
| 1. | Tetrahy drocann abinol | -6.6 | 47 | 2 | 1 | 5.7358 | 314.5 g/mole | 4 | 22 |
| 2. | Cannabinol | -5.5 | 47 | 1 | 2 | 5.72782 | 310.4g/mole | 4 | 22 |
| 3. | Cannabi diol | -6.3 | 47 | 2 | 2 | 12.19222 | 314.5 g/mole | 6 | 22 |
| 4. | Cannabige rol | -6.6 | 47 | 2 | 2 | 6.0657 | 316.5 g/mole | 9 | 23 |
| 5. | Cannabi chromene | -6.9 | 47 | 1 | 2 | 6.0356 | 314.5 g/mole | 7 | 24 |
| 6. | Ocimene | -6.1 | 47 | 0 | 0 | 3.475 | 136.23g/mole | 3 | 19 |
| 7. | Resins | -5.3 | 47 | 1 | 3 | 1.2133 | 152.15g/mole | 2 | 17 |
| 8. | Methion in | -4.2 | 78 | 2 | 4 | 0.3288 | 191.25 g/-mole | 5 | 18 |
| 9. | Lysine | -3.8 | 78 | 3 | 4 | -0.4727 | 146.19g/mole | 5 | 18 |
| 10. | Canabic hromeni c Acid | -6.5 | 47 | 2 | 4 | 5.7338 | 358.5 g/mole | 8 | 24 |

Table 4.5 - Continued from Previous Page

| Sr. No. | Name | Score | Size | HBD | HBA | Log p | Weight | Bonds | Grid Map |
|---|---|---|---|---|---|---|---|---|---|
| 11. | Dihydro stilbene | -7.3 | 47 | 0 | 0 | 3.4718 | 182.26g/mole | 3 | 20 |
| 12. | Canabispir in | -5.8 | 69 | 1 | 3 | 2.7279 | 246.3g/mole | 1 | 19 |
| 13. | pinene | -4.1 | 69 | 0 | 0 | 2.9987 | 136.23 g/mole | 0 | 16 |
| 14. | Limonene | -5.6 | 47 | 0 | 0 | 3.3089 | 136.23 g/mole | 1 | 17 |
| 15. | Terpineol | -4.5 | 33 | 1 | 1 | 2.5037 | 154.25 g/mole | 1 | 15 |
| 16. | Borneol | -3.6 | 33 | 1 | 1 | 2.1935 | 154.25g/mole | 0 | 16 |
| 17. | Cannabi gerovar nic Acid | -6.8 | 47 | 3 | 4 | 4.9837 | 332.4g/mole | 8 | 23 |
| 18. | Cannabi elsoin | -5.6 | 47 | 2 | 3 | 4.7066 | 330.5 g/mole | 5 | 22 |

Table 4.5 - Continued from Previous Page

| **Sr. No.** | **Name** | **Score** | **Size** | **HBD** | **HBA** | **Log p** | **Weight** | | **Bonds** | **Grid Map** |
|---|---|---|---|---|---|---|---|---|---|---|
| 19. | Humulene | -5.5 | 69 | 0 | 0 | 5.0354 | 204.35 mole | g/- | 0 | 18 |
| 20 | Myrcene | -5.7 | 47 | 0 | 0 | 3.475 | 136.23 mole | g/- | 4 | 18 |

## 4.5 Interaction of Ligands and the Targeted Protein

The result deducted from docking is analyzed through LigPlot and PyMol. The interaction between the Ligands and the receptor protein is predicted through LigPlot. Based on the 3D coordinates, the LigPlot graphical system automatically creates the 2D images of interaction. Dimensional images show the hydrophobic contacts and hydrogen bond interactions between the ligand and the side chain or main chain of the receptor protein [65]. The 2D diagrams of the interaction of ligands and protein are shown in Figure 4.4 to 4.23. Figure 4.4 shows the interaction of tetrahydrocannabinol with receptor SMN protein. It shows that tetrahydrocannabinol has formed five hydrophobic interactions and two hydrogen bond.

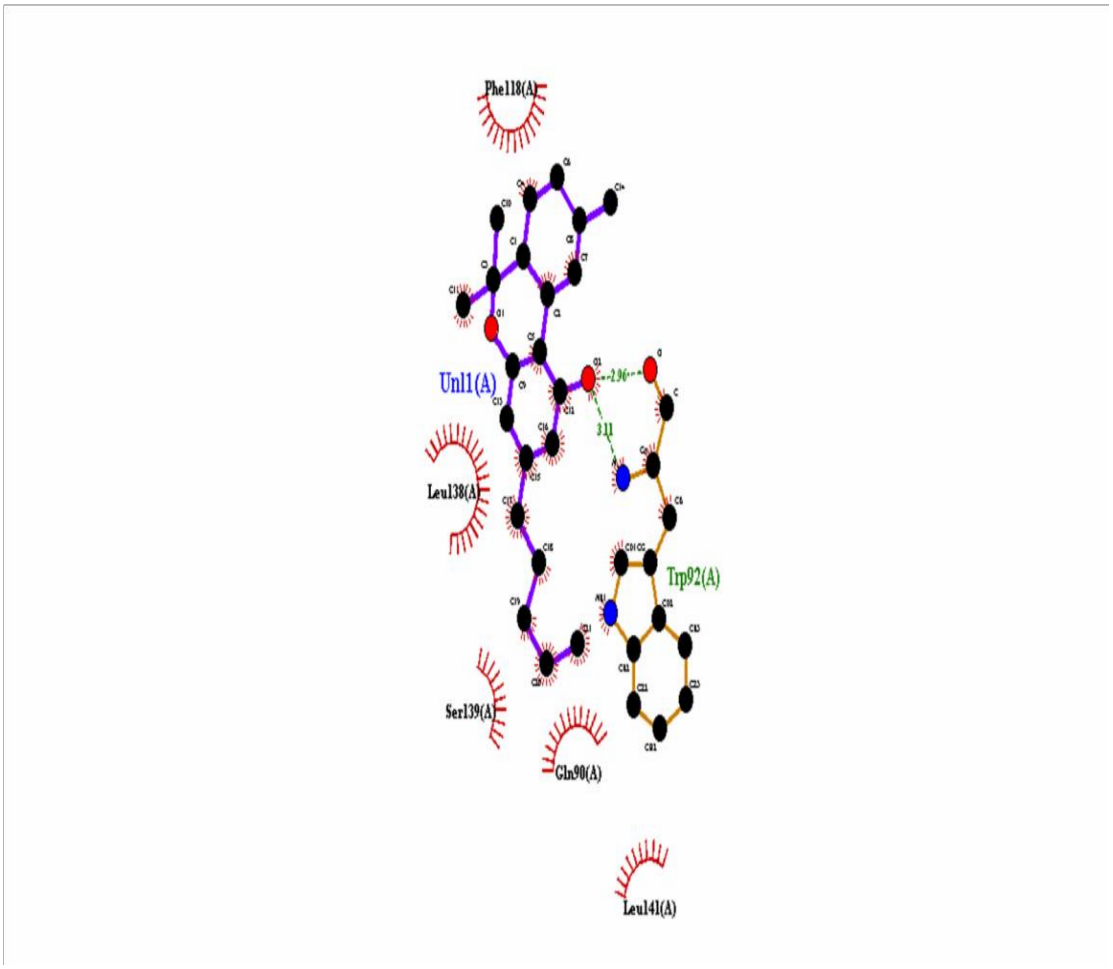



FIGURE 4.4: Interaction of Tetrahydrocannabinol with the Receptor Protein)

Figure 4.5 shows the interaction of cannabinol with receptor SMN protein. It shows that cannabinol has formed five hydrophobic interactions.

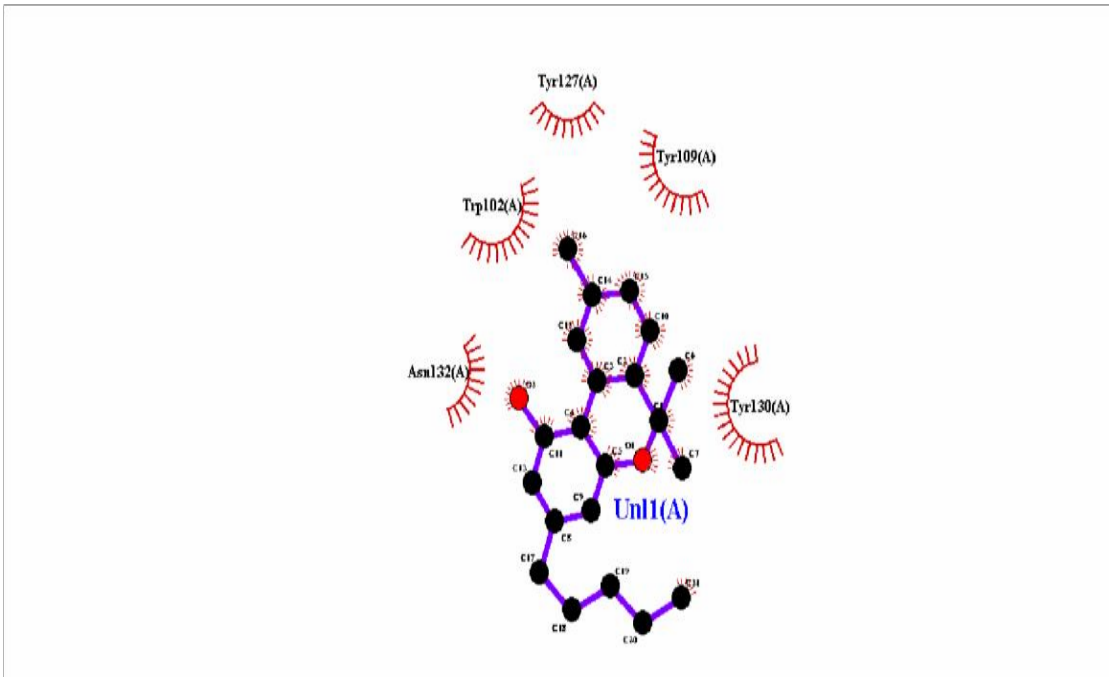



FIGURE 4.5: Interaction of Cannabinol with the Receptor Protein

Figure 4.6 shows the interaction of cannabidiol with receptor SMN protein. It shows that cannabidiol has formed six hydrophobic interactions.

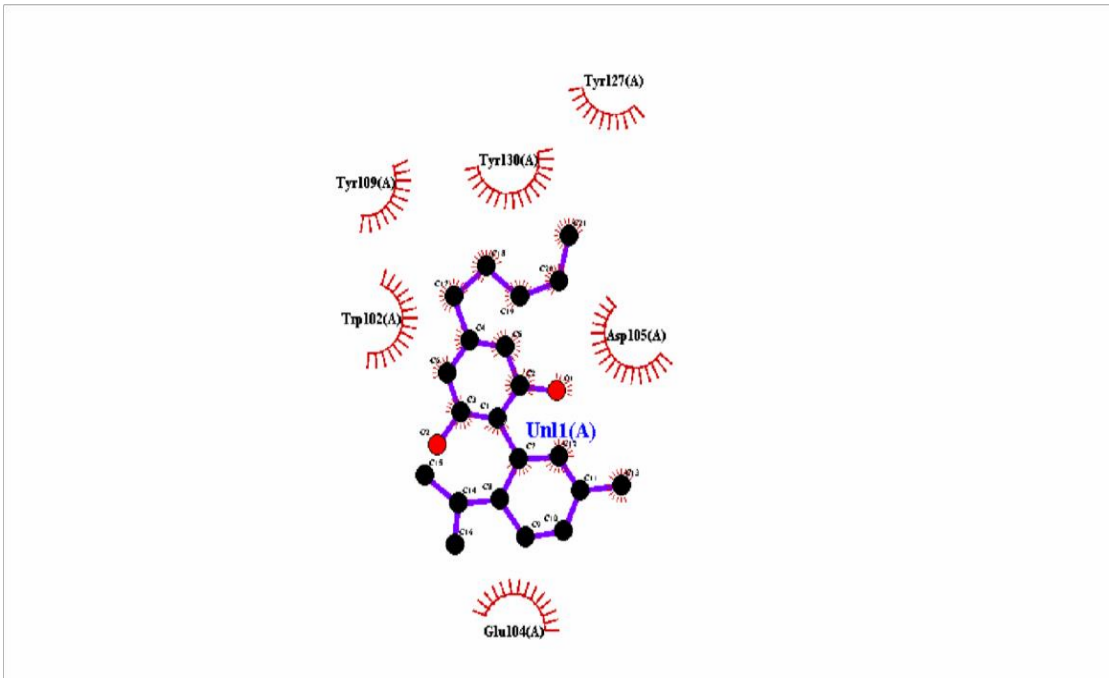



FIGURE 4.6: Interaction of Cannabidiol with Receptor Protein

Figure 4.7 shows the interaction of cannabigerol with receptor SMN protein.It shows that cannabigerol has formed seven hydrophobic interactions and one hydrogen bond.

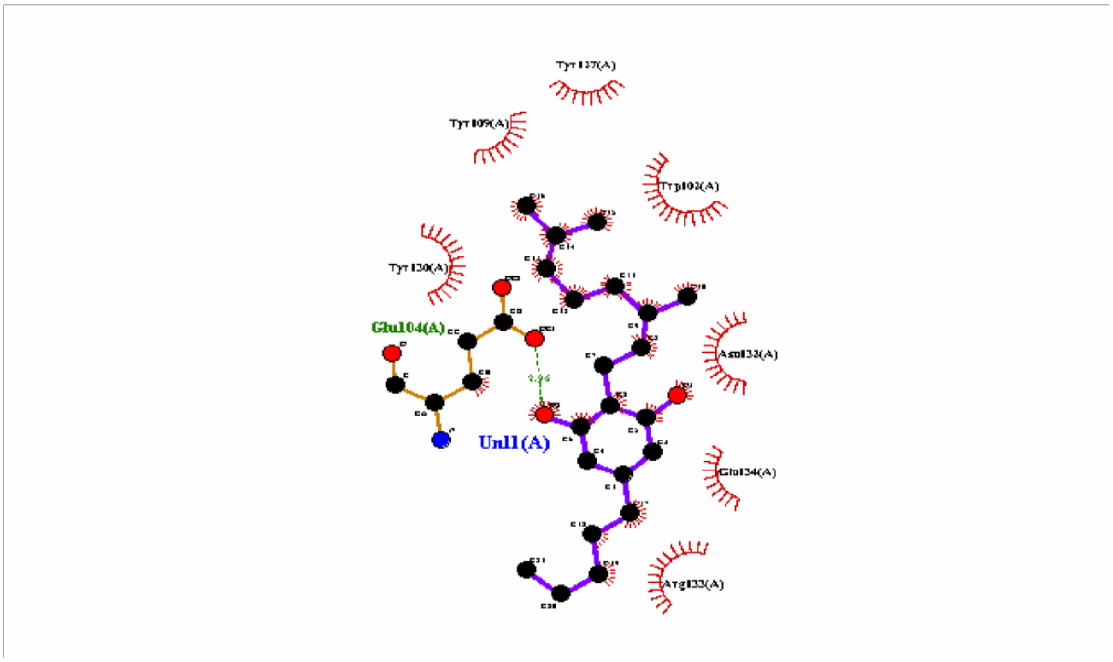



FIGURE 4.7: Interaction of Cannabigerol with Receptor Protein

Figure 4.8 shows the interaction of cannabichromene with receptor SMN protein. It shows that cannabichromene has formed nine hydrophobic interactions.

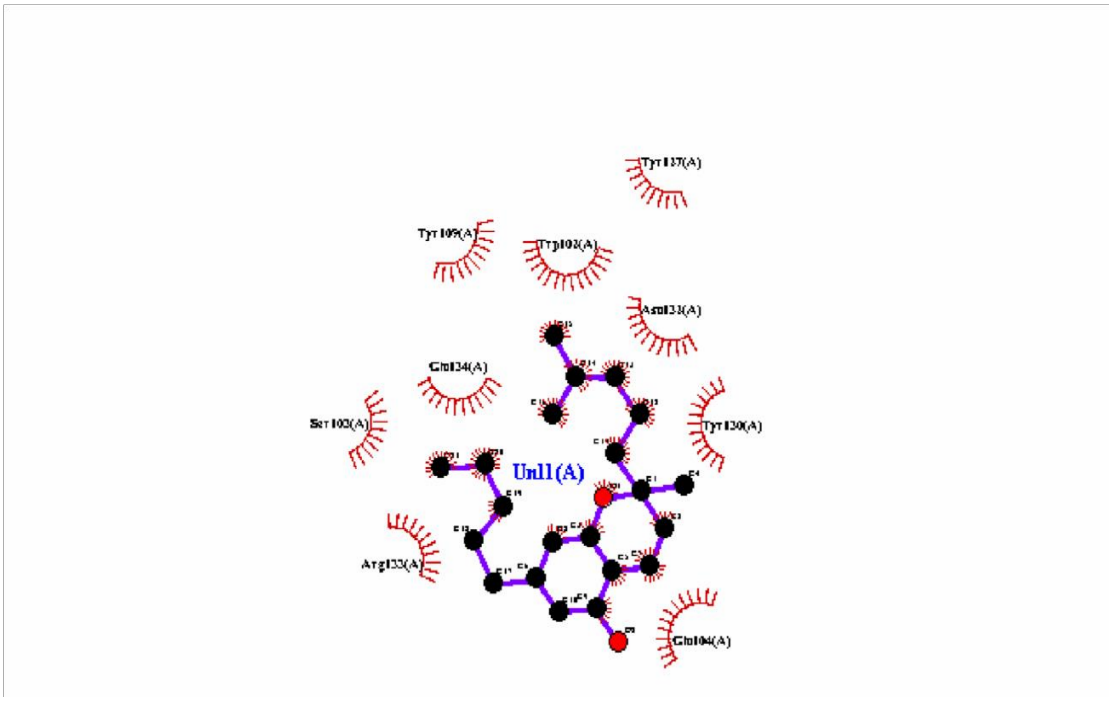



FIGURE 4.8: Interaction of Cannabichromene with Receptor Protein

Figure 4.9 shows the interaction of ocimene with receptor SMN protein. It shows that ocimene has formed five hydrophobic interactions.

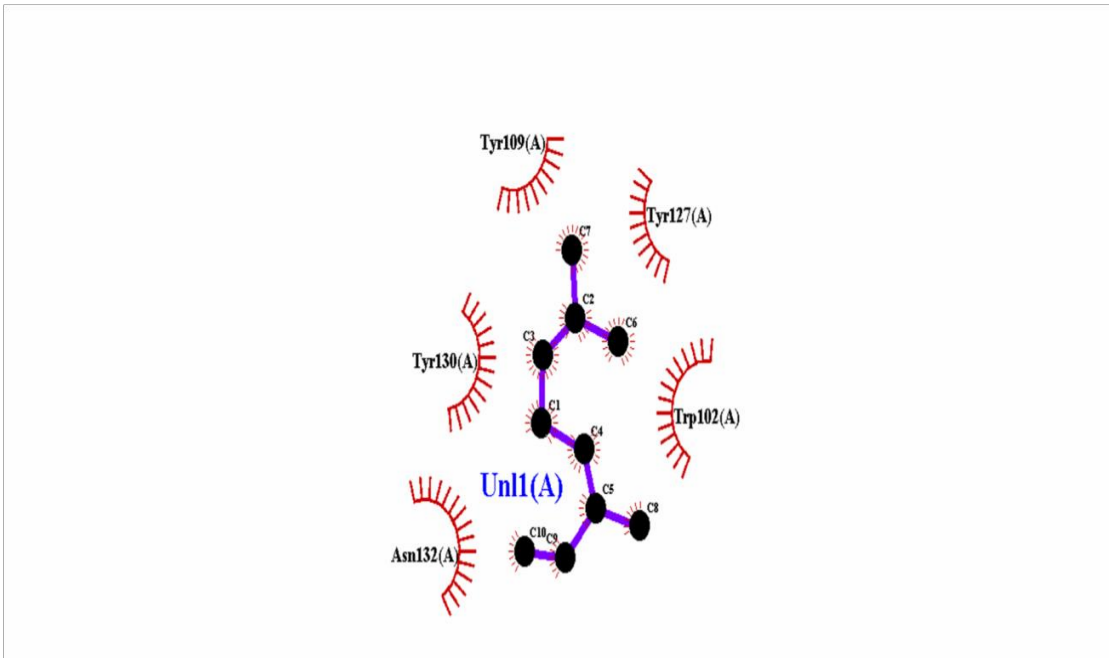



FIGURE 4.9: Interaction of Ccimene with Receptor Protein

Figure 4.10 shows the interaction of resins with receptor SMN protein. It shows that resins has formed four hydrophobic interactions and two hydrogen bonds.

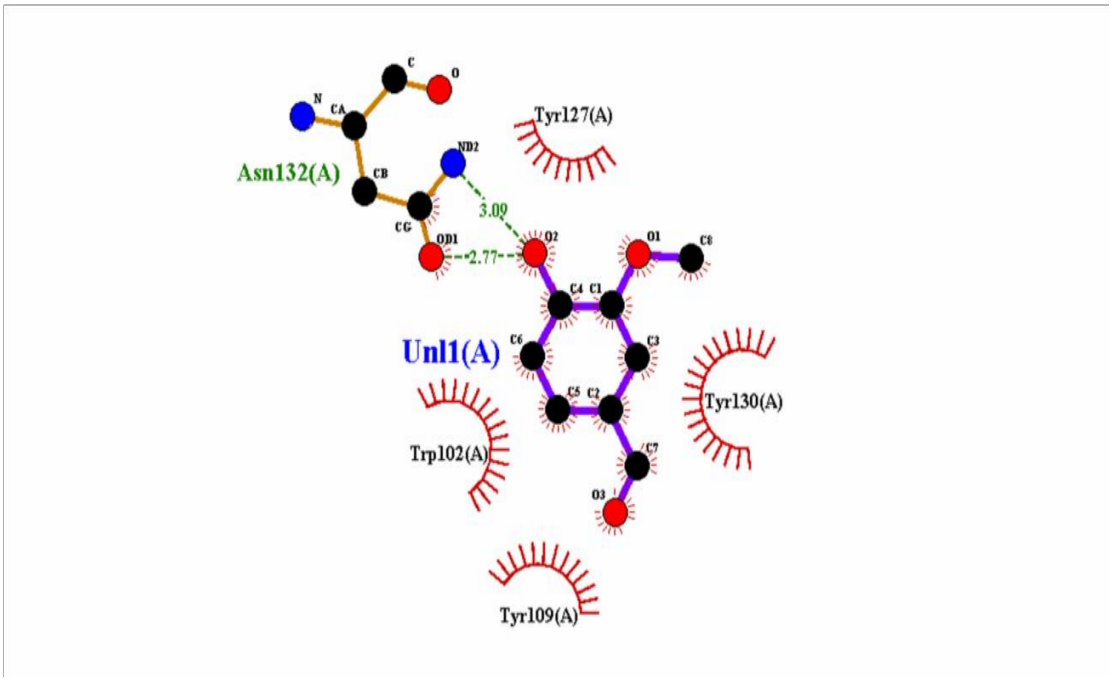



FIGURE 4.10: Interaction of Resins with Receptor Protein

Figure 4.11 shows the methionin with receptor SMN protein. It shows that methionin has formed five hydrophobic interactions and two hydrogen bonds.

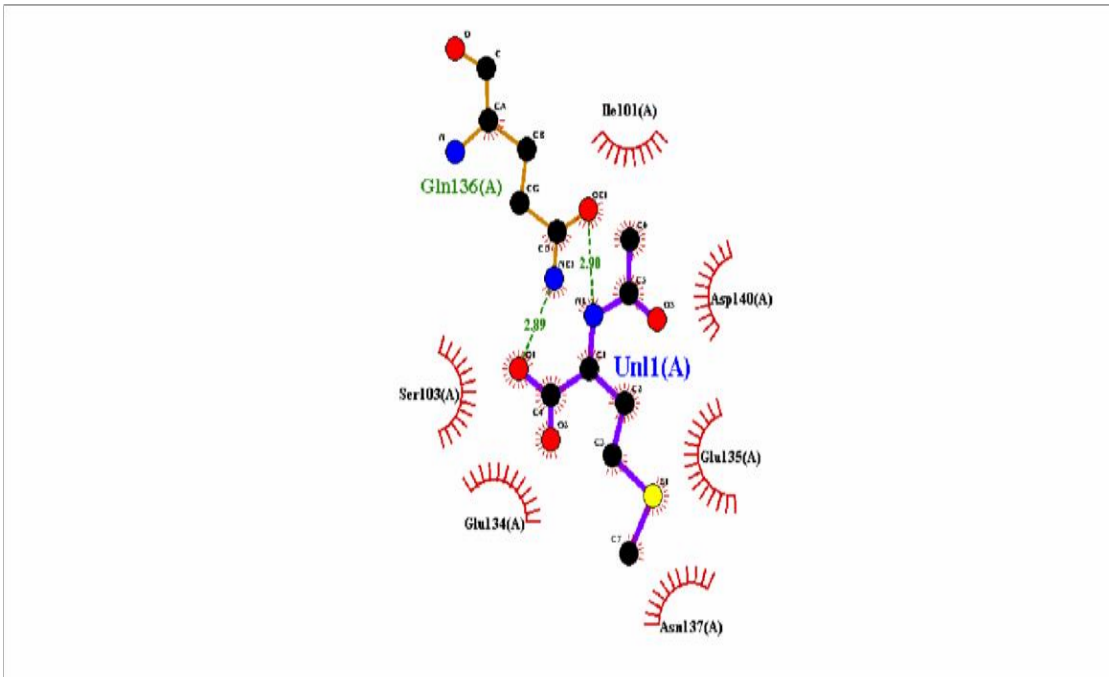



FIGURE 4.11: Interaction of Resins with Receptor Protein

Figure 4.12 shows the interaction of lysine with receptor SMN protein. It shows that lysine has formed two hydrophobic interactions and three hydrogen bonds.

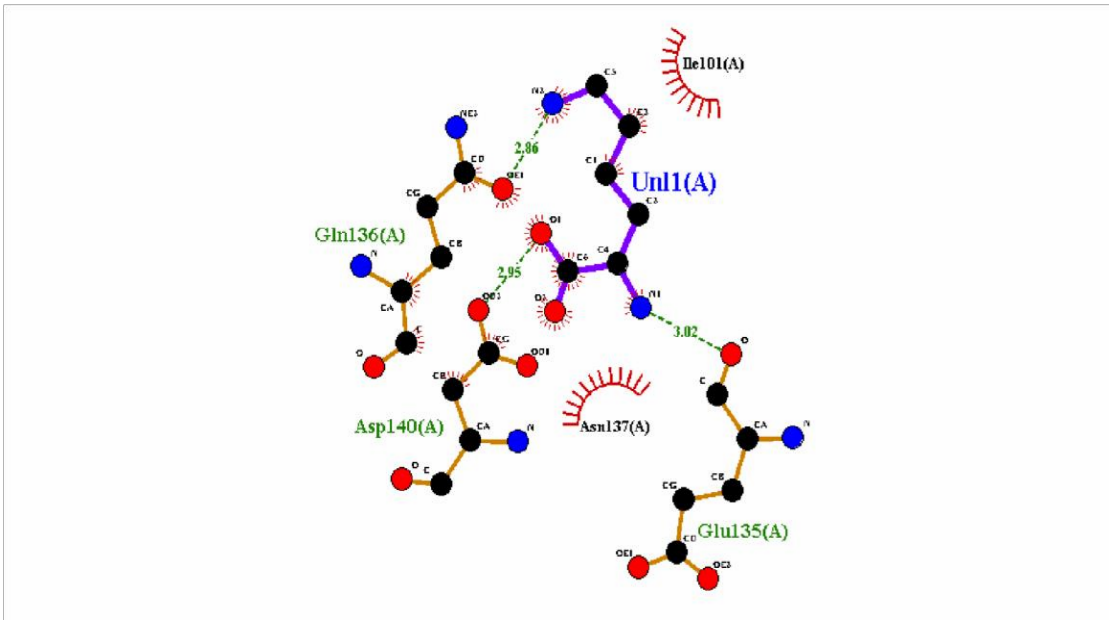



FIGURE 4.12: Interaction of Lysine with Receptor Protein

Figure 4.13 shows the interaction of canabichromenic acid with receptor SMN protein. It shows that canabichromenic acid has formed seven hydrophobic interactions and one hydrogen bonds.

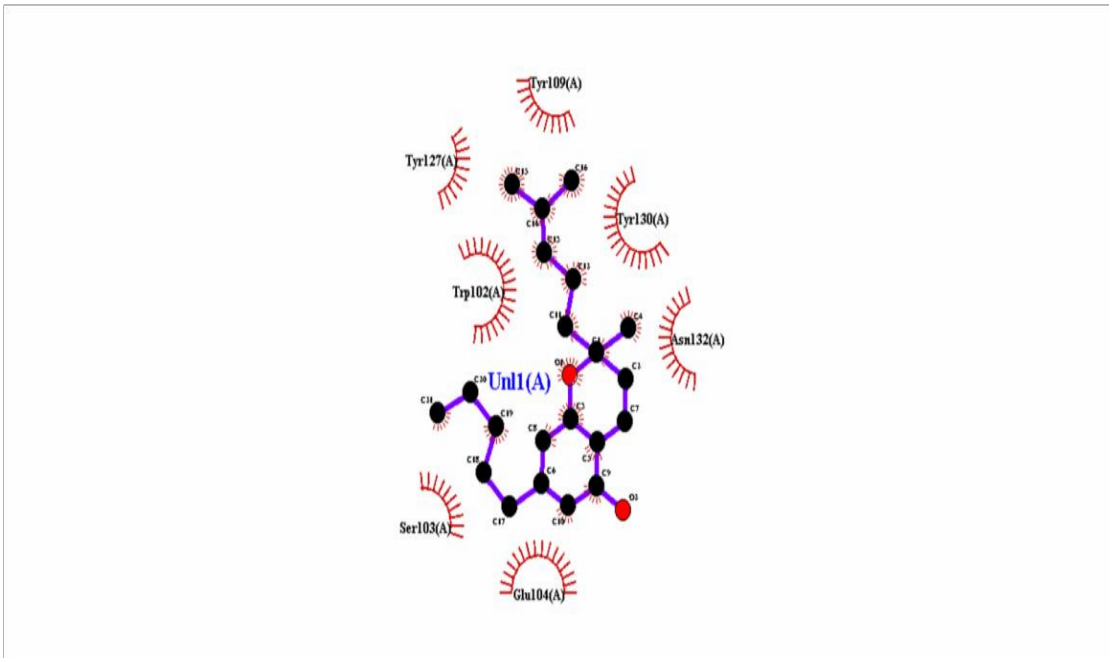



FIGURE 4.13: Interaction of Canabichromenic Acid with Receptor Protein

Figure 4.14 shows the interaction of dihydrostilbene with receptor SMN protein. It shows that dihydrostilbene has formed five hydrophobic interactions.

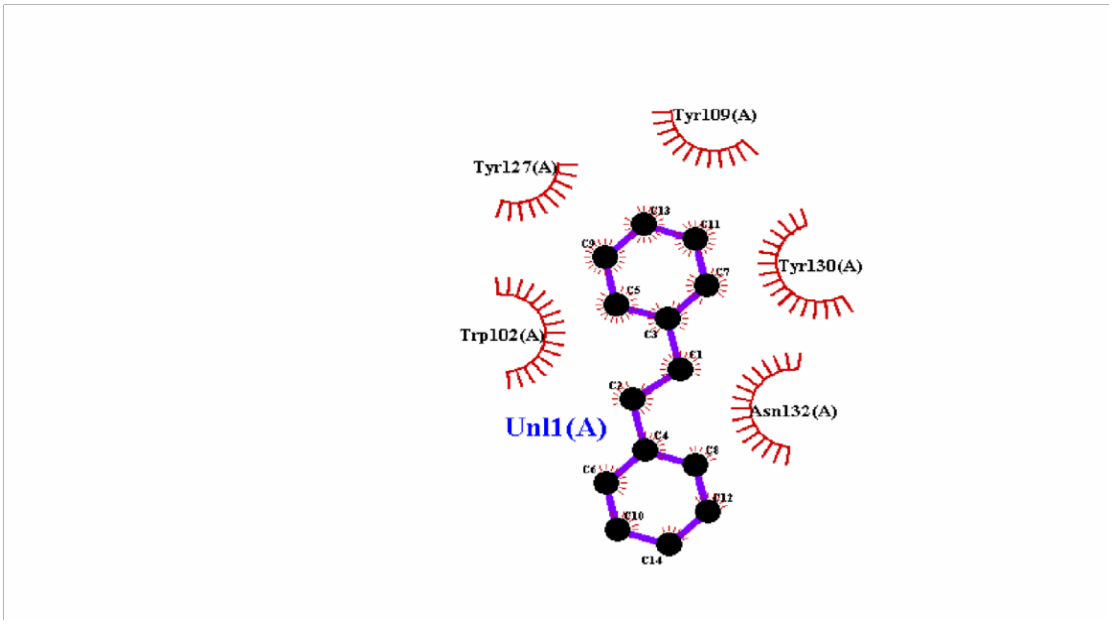



FIGURE 4.14: Interaction of Dihydrostilbene with Receptor Protein

Figure 4.15 shows the cannabispiran with receptor SMN protein. It shows that cannabispiran has formed five hydrophobic interactions and one hydrogen bond.

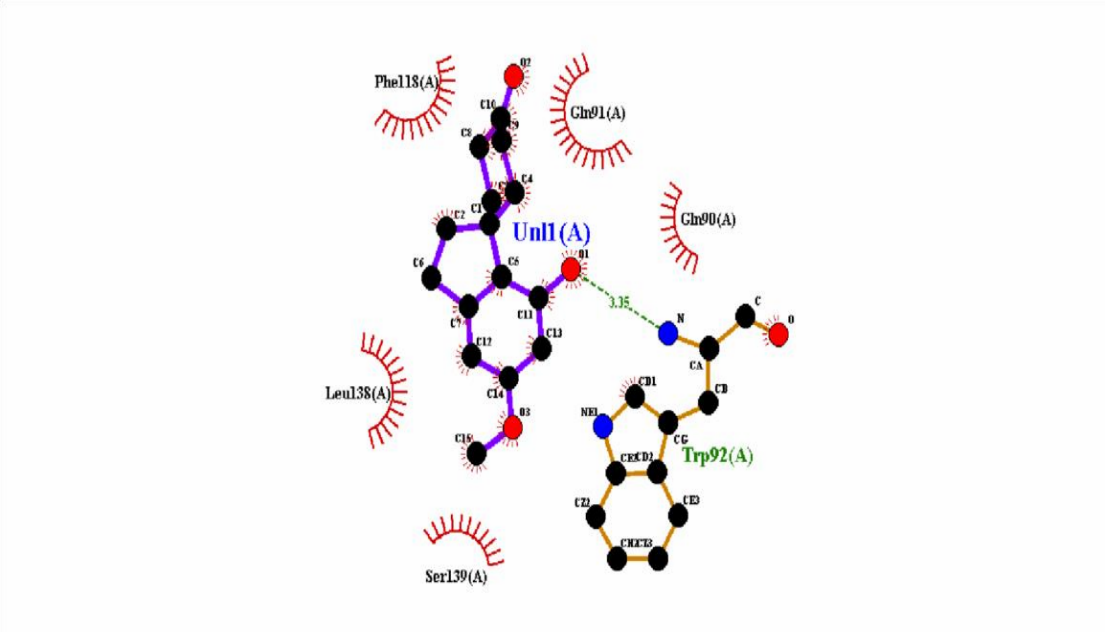



FIGURE 4.15: Interaction of Cannabispiran with Receptor Protein

Figure 4.16 shows the interaction of pinene with receptor SMN protein. It shows that pinene has formed five hydrophobic interactions.

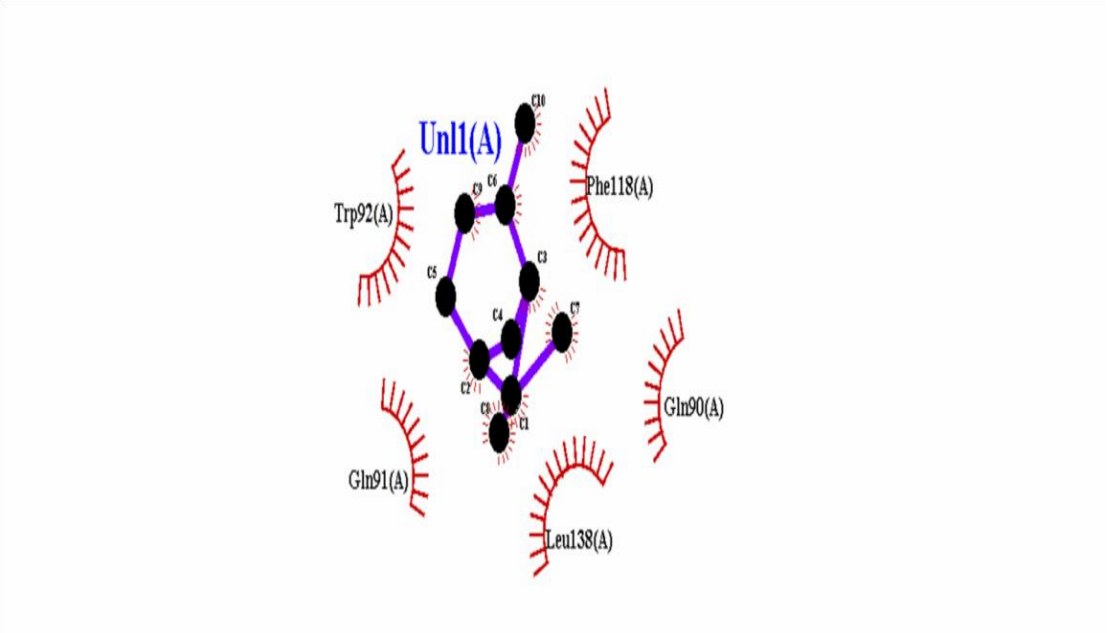



FIGURE 4.16: Interaction of Pinene with Receptor Protein

Figure 4.17 shows the interaction of limonene with receptor SMN protein. It shows that limonene has formed five hydrophobic interactions.

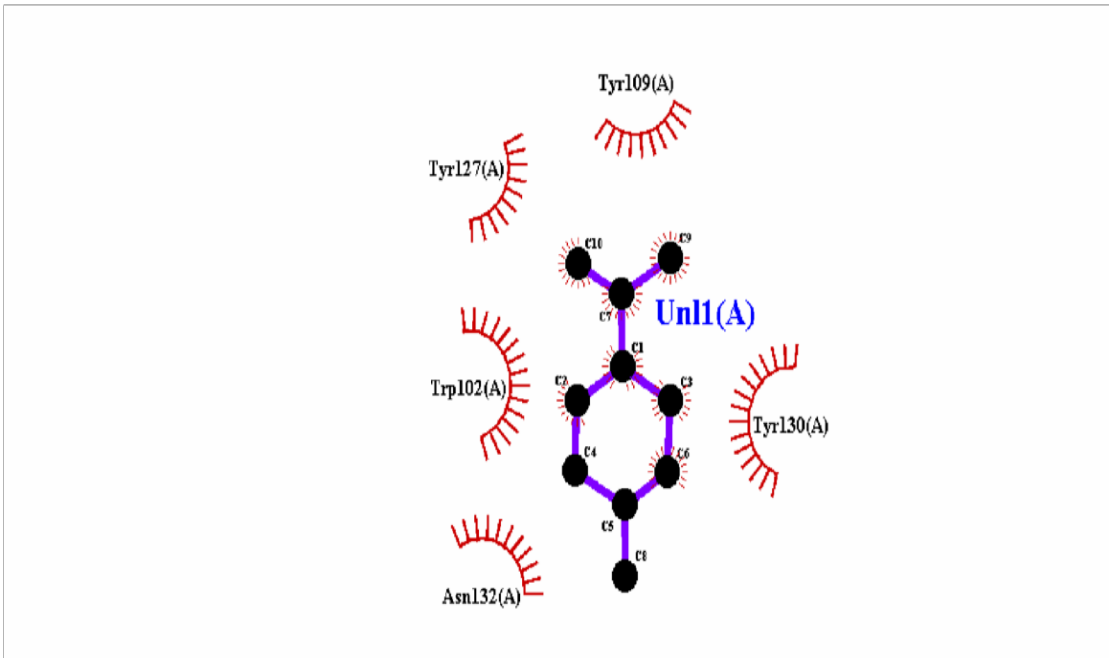



FIGURE 4.17: Interaction of Limonene with Receptor Protein

Figure 4.18 shows the interaction of terpineol with receptor SMN protein. It shows that terpineol has formed seven hydrophobic interactions and one hydrogen bonds.

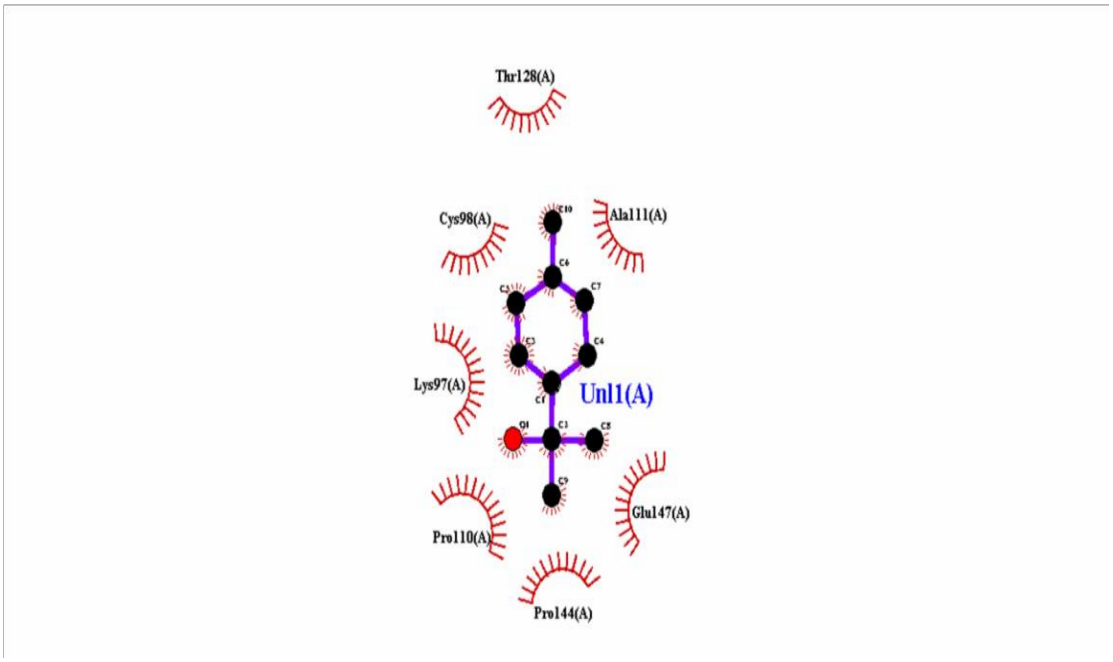



FIGURE 4.18: Interaction of Terpineol with Receptor Protein

Figure 4.19 show the borneol with receptor SMN protein. It shows that borneol has formed five hydrophobic interactions.

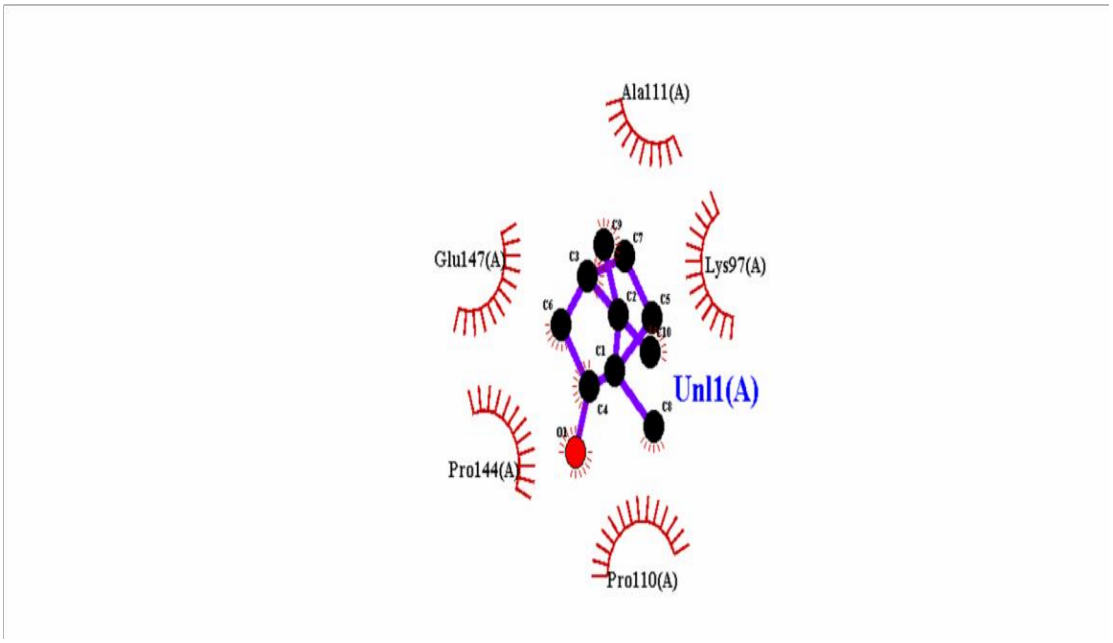



FIGURE 4.19: Interaction of Borneol with Receptor Protein

Figure 4.20 shows the interaction of cannabigerovarinic acid with receptor SMN protein. It shows that Cannabigerovarinic acid has formed six hydrophobic interactions and 4 hydrogen bonds.

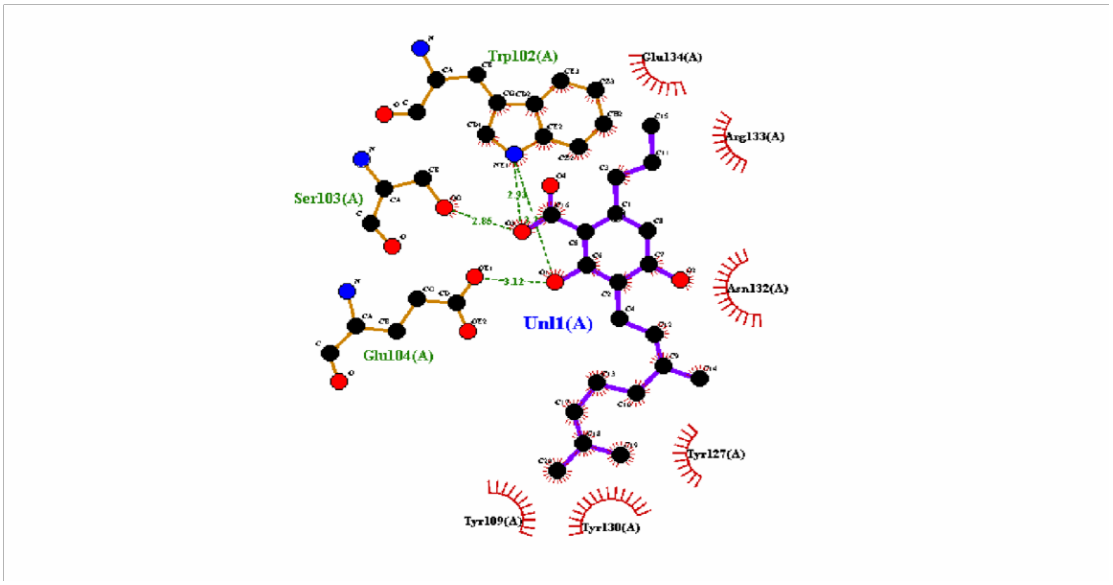



FIGURE 4.20: Interaction of Cannabigerovarinic Acid with Receptor Protein

Figure 4.21 cannabielsoin with receptor SMN protein. It shows that cannabielsoin has formed six hydrophobic interactions.

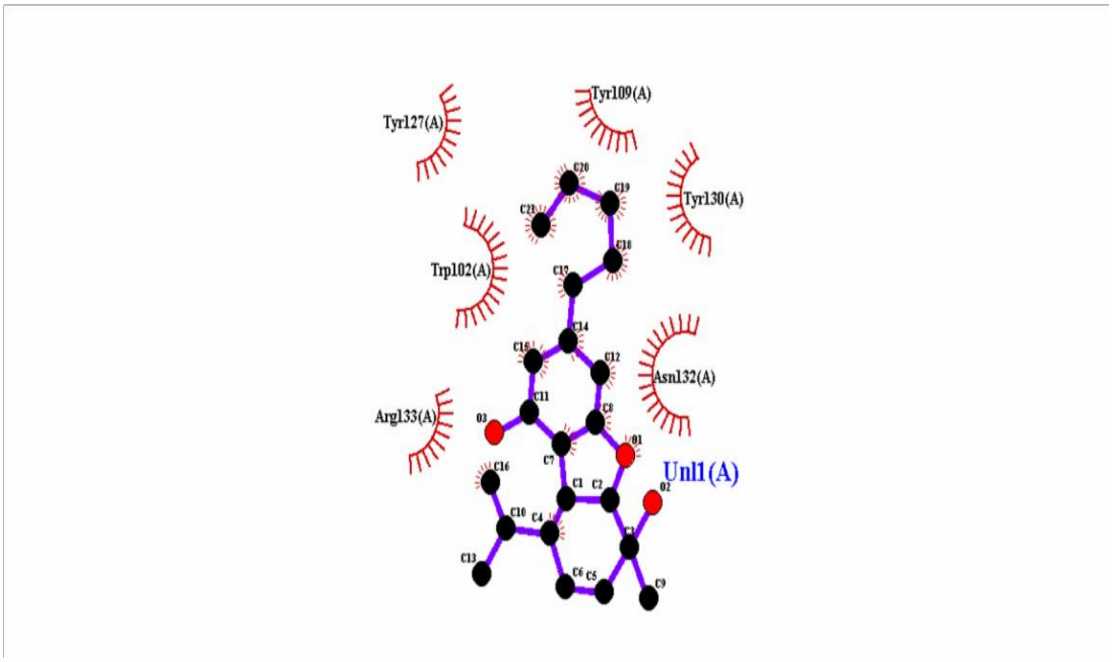



FIGURE 4.21: Interaction of Cannabielsoin with Receptor Protein

Figure 4.22 shows the interaction of humulene with receptor SMN protein. It shows that humulene has formed seven hydrophobic interactions.

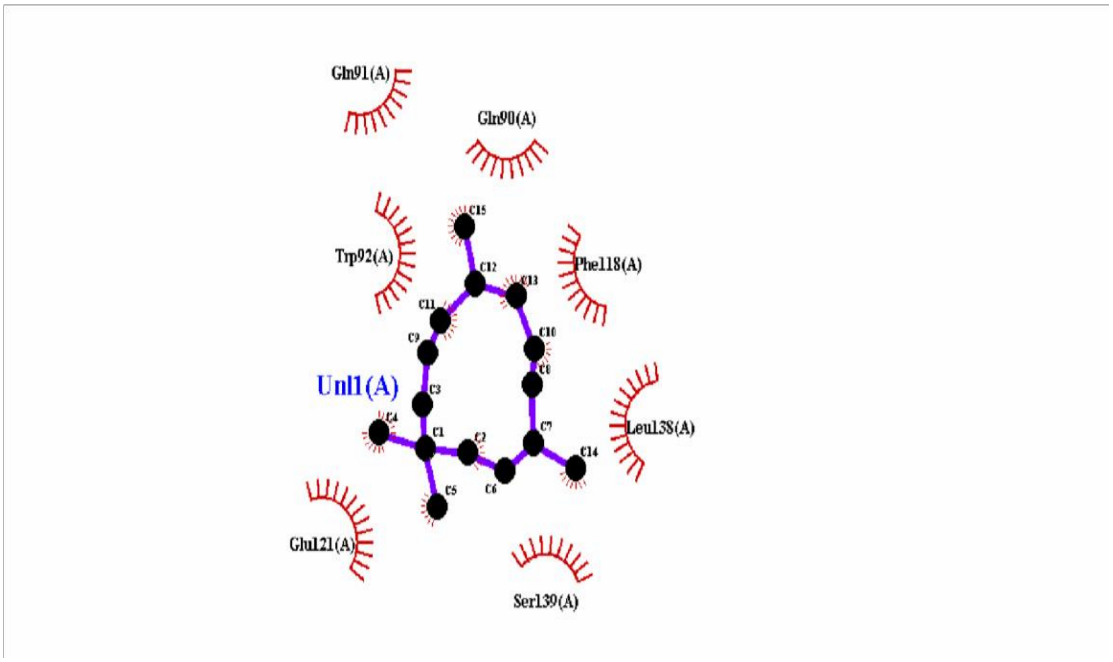



FIGURE 4.22: Interaction of Humulene with Receptor Protein

Figure 4.23 myrcene with receptor SMN protein. It shows that myrcene has formed five hydrophobic interactions.

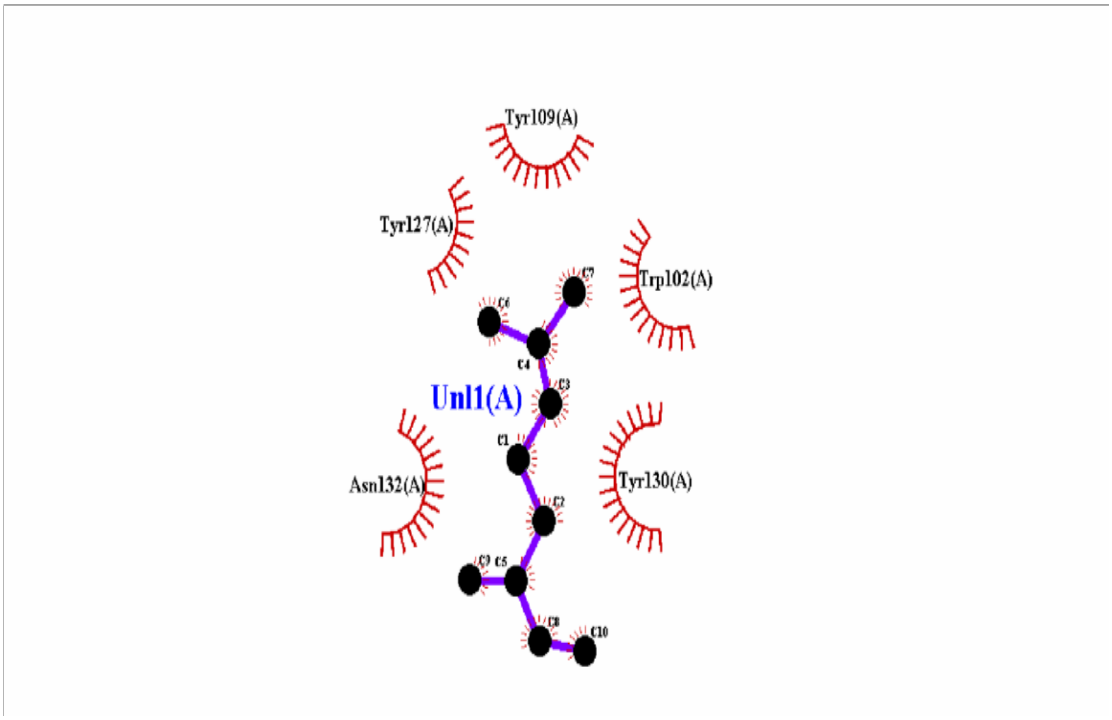



FIGURE 4.23: Interaction of Myrcene with Receptor Protein

The Table 4.6 below shows the details of hydrogen and hydrophobic interactions of the selected ligands with the receptor protein. The values show that cannabichromene forms the highest hydrophobic interactions in number which is 9 next is canabigerol, cannabichromenic acid, terpeniol, humulene with 7 hydrophobic bonds. Methionine, cannabispirin, canabigerovarnic acid and canabielsoin with hydrophobic bonds 6. Tetrahydrocannabinol, canabinol, ocimene, dihyrostilbene, pinene, borneol and mycrene have 5 hydrophobic interactions. Resin have 4 hydrophobic interactions. Lysine have 2 hydrophobic interactions.

## 4.6 ADME Properties of Ligands

In the first step of the evaluation process, Lipinski's five drug law is utilized to determine whether the availability of the substance is verbal or artificial [66]. With regard to the evaluation of ADME qualities, the second instrument that is utilized is known as PkCSM [67].

Table 4.6: Active Ligand Showing Hydrogen and Hydrophobic Interactions

| **Sr. No.** | **Ligand Name** | **Binding Energy** | **HBs No.** | **Amino Acids** | **Distance** | **Hydrophobic Bonding** |
|---|---|---|---|---|---|---|
| 1 | Tetrahydrocannabinol | -6.6 | 2 | 0-Trp92-01 N-Trp92-01 | 2.96 3.11 | Leu141 Glu90 Ser139 Leu138 Phe118 |
| 2 | Cannabinol | -5.5 | 0 | | | Tyr102 Tyr 109 Tyr127 Tyr130 Asn132 |
| 3 | Cannabidiol | -6.3 | 0 | | | Tyr109 Tyr127 Tyr130 Trp102 Asp105 Glu104 |
| 4 | Cannabiger ol | -6.6 | 1 | OLI-Glu104-01 | 2.69 | Tyr102 Tyr109 Tyr127 Tyr130 Asn132 Glu134 Arg133 |
| 5 | Cannabichromene | -6.9 | 0 | | | Trp102 Tyr109 Tyr127 Tyr130 Asn132 Glu134 Ser103 Arg133 Glu104 |
| 6 | Ocimene | -6.1 | 0 | | | Tyr109 Tyr127 Tyr130 Trp102 Asn132 |

Table 4.6 - Continued from Previous Page

| Sr. No. | Ligand Name | Binding Energy | HBs No. | Amino Acids | Distance | Hydrophobic Bonding |
|---|---|---|---|---|---|---|
| 7 | Resins | -5.3 | 2 | ND2-Asn132-02 OD1-Asn132-02 | 3.09 2.77 | Tyr109 Tyr127 Tyr30 Trp102 |
| 8 | Methionin | -4.2 | 2 | DE-Gln136-N1 NE3-Gln136-01 | 2.90 2.89 | Lle101 Asp140 Glu135 Asn137 Glu134 Ser103 |
| 9 | Lysine | -3.8 | 3 | OE1-Gln136-N2 OD2-Asp140-O1 O-Glu135-N1 | 2.86 2.95 3.02 | Lle101 Asn137 |
| 10 | Cannabichromenic Acid | -6.5 | 0 | | | Tyr109 Tyr127 Tyr130 Trp102 Asn132 Glu104 Ser103 |
| 11 | Dihydrostilbene | -7.3 | 0 | | | Tyr109 Tyr127 Tyur130 Trp102 Asn132 |
| 12 | Cannabispiran | -5.8 | 1 | N-Trp92-01 | 3.35 | Gln90 Gln91 Phe118 Leu138 Ser139 |

Table 4.6 - Continued from Previous Page

| Sr. No. | Ligand Name | Binding Energy | HBs No. | Amino Acids | Distance | Hydrophobic Bonding |
|---|---|---|---|---|---|---|
| 13 | Pinene | -4.1 | 0 | | | Phe118 Gln90 Gln91 Leu138 Trp92 |
| 14 | limonene | -5.6 | 0 | | | Tyr109 Tyr127 Tyr130 Trp102 Asn132 |
| 15 | Terpineol | -4.5 | 0 | | | Thr128 Ala111 Glu147 Pro110 Pro144 Lys97 Cys98 |
| 16 | Borneol | -3.6 | 0 | | | Ala111 Lys97 Pro110 Prp144 Glu147 |
| 17 | Cannabiger ovarinic acid | -6.8 | 4 | OE1-Glu1040- ON<br>OC-Ser103-O3 NE1-<br>Trp102- ON NE1-<br>Trp102- O3 | 3.12<br>2.85<br>3.2<br>2.93 | Glu134 Arg133 Asn132 Tyr109 Tyr127 Tyr130 |

Table 4.6 - Continued from Previous Page

| Sr. No. | Ligand Name | Binding Energy | HBs No. | Amino Acids | Distance | Hydrophobic Bonding |
|---|---|---|---|---|---|---|
| 18 | Cannabielsoin | -5.6 | 0 | | | Tyr109 Tyr127 Tyr130 Asn132 Trp102 Arg133 |
| 19 | Humulene | -5.5 | 0 | | | Gln90 Phe118 Leu138 Ser139 Glu121 Trp92 Gln91 |
| 20 | Myrcene | -5.7 | 0 | | | Tyr109 Tyr127 Tyr130 Trp102 Asn132 |

### 4.6.1 Pharmacodynamics

One of the broader terms used in pharmacology is pharmacodynamics which deals with the study of drug effects on the body [68].

### 4.6.2 Pharmacokinetics

The other term used in pharmacology is pharmacokinetics which deals with the study of the reaction of the body to the drug, that how the body reacts after the drug enter the body. The absorption, distribution, metabolism, and excretion of drugs are also studied [69].

### 4.6.3 Absorption

The CaCO2 solubility helps in predicting the absorption of the drugs which are administered orally. Value >0.90 (log Papp in 10-6 cm/s) is considered as high CaCO2 permeability [58]. The water solubility of the ligands is given as log mol/L. This indicates the compound solubility in water at 25o C. Hence the lipid-soluble drugs will be less soluble than the water-soluble drugs . Intestinal absorption indicates the value or proportion of the compound that will absorb into the intestines. A value less than 30% is considered poorly absorbed [58]. P-glycoprotein is an ABC transporter that functions to extrude toxins or other xenobiotics from the cells by acting as a biological barrier [59]. P-glycoprotein inhibition can be a therapeutic target or it can act in contradiction. Skin permeability is important for developing transdermal drugs. Any compound with a value ¿ -2.5 has a low skin permeability [59]. Absorption properties of all ligands are given in Table 4.7. Among five ligands tetrahydrocannabinol, cannabinol, canabidiol, canabigerol, ocimene, cannabinol, canabidiol, canabigerol all have low skin permeability except cannabichromene, with that these five tetrahydrocannabinols and cannabichromene is not the glycoprotein substrates. All have high CaCO2 solubility. Apart from all these the values of other parameters are in the range. Ligands like ocimene, resins, methionin, lysine, cannabichromenic acid shows that methionin, lysine, cannabichromenic acid have

low CaCO2 solubility. All have good intestinal absorption. Whereas cannabichromenic acid is a glycoprotein substrate and P-glycoprotein II inhibitor.

Ligands limonene and terpineol are P-glycoprotein substrates, with that cannabispiran also has low skin permeability. Other than that water solubility, CaCO2 solubility, intestinal absorption values are all in the pkcsm range. All these ligands are not the P-glycoprotein I and II inhibitor. Cannabigerovarinic acid has low CaCO2 solubility. Cannabigerovarinic acid, cannabielsoin, humulene is a P-glycoprotein and not have I and II inhibitor. Whereas cannabigerovarinic acid, cannabielsoin, humulene is not a P-glycoprotein. The remaining ligands in Table 4.7 are the values of absorption parameters which are water solubility, CaCO2 solubility, intestinal absorption, skin permeability, P-glycoprotein substarte and its inhibitors, all have indicated the values in pkcsm range. Some of the parameters of absorption properties of tetrahydrocannabinol has already been studied Hanasono GK, Sullivan HR, – 1987. Some parameters of absorption of have been studied methionin, cannabichromenic acid and by Webb J-1997. Pkcsm absorption properties of limonene, terpineol reported by Carpena M. Nunez-Estevez B-2021 [71]. Pkcsm absorption properties of cannabigerovarinic acid, cannabielsoin,have already been reported by Izzo AA, Borrelli F in 2009. Some parameters of absorption properties of canabinol and canabidiol have been studied Kauert GF, Ramaekers JG in 2007 [72]. Pkcsm absorption properties of cannabichromene has already been reported by Izzo AA, in 2009 [74].

Based on the information, we get through pkcsm absorption running we can screen several ligands which could be a step behind other ligands. Based on low CaCO2 cannabigerovarinic acid has low CaCO2 solubility stays a back in the selection of lead compound whereas the values of absorption parameters which are water solubility, CaCO2 solubility, intestinal absorption, skin permeability, P-glycoprotein substarte and its inhibitors, all have indicated the values in pkcsm range.

Table 4.7: Absorption Properties Ligands

| Sr.No. | Ligands Name | Water Sol. | CaCo2 Sol. | Intestinal Abs. | Skin Permea | Pglycoprotein | Pglycoprote in 1 Inhibitor | P-glycoprote in II Inhibitor |
|---|---|---|---|---|---|---|---|---|
| 1. | Tetrahy drocann | -6.275 | 1.519 | 93.091 | -2.538 | No | Yes | No |
| 2. | Cannabinol | -5.78 | 1.633 | 92.487 | -2.723 | yes | No | yes |
| 3. | Cannabidiol | -4.901 | 1.79 | 90.657 | -2.795 | yes | yes | No |
| 4. | Cannabigero l | -5.266 | 1.572 | 87.846 | -2.717 | yes | yes | yes |
| 5. | Cannabichro mene | -6.155 | 1.128 | 91.138 | -2.485 | No | yes | No |
| 6. | Ocimene | -4.446 | 1.406 | 94.726 | -1.065 | No | No | No |
| 7. | Resins | -1.308 | 1.219 | 84.976 | -2.832 | No | No | No |
| 8. | Methionin | -0.81 | 0.647 | 84.272 | -2.275 | No | No | No |
| 9. | Lysine | -2.888 | 0.737 | 62.673 | -2.735 | No | No | No |
| 10. | Canabic hromenic Acid | -3.342 | 0.626 | 95.921 | -2.73 | Yes | No | yes |

Table 4.7 - Continued from Previous Page

| Sr.No. | Ligands Name | Water Sol. | CaCo2 Sol. | Intestinal Abs. | Skin Permea | Pglycoprotein | Pglycoprote in 1 Inhibitor | P-glycoprote in II Inhibitor |
|---|---|---|---|---|---|---|---|---|
| 11. | Dihydro stillbene | -4.63 | 1.572 | 95.675 | -1.922 | No | No | No |
| 12. | Canabis pirin | -2.73 | 1.32 | 94.555 | -3.251 | No | No | No |
| 13. | pinene | -3.733 | 1.38 | 96.041 | -1.827 | No | No | No |
| 14. | limonene | -3.568 | 1.401 | 95.898 | -1.721 | yes | No | No |
| 15. | Terpineol | -2.039 | 1.489 | 94.183 | -2.418 | yes | No | No |
| 16. | Borneol | -2.462 | 1.484 | 93.439 | -2.174 | No | No | No |
| 18. | Cannabielsoin | -5.392 | 1.37 | 91.97 | -3.298 | yes | yes | No |
| 19. | Humulene | -5.191 | 1.421 | 94.682 | -1.739 | yes | No | No |
| 20. | Myrcene | -4.497 | 1.4 | 94.696 | -1.043 | No | No | No |

### 4.6.4 Distribution

The VDss is the theoretical volume which tells about the total dose of the drug which will be needed to be distributed uniformly to give the same concentration as it is in the blood plasma. If the VDss value exceeds 2.81 L/kg, then the drug is more distributed in the tissues than in the plasma. The VDss will be low if the value is below 0.71 L/kg [58]. Many drugs in the plasma exist in an equilibrium between a bounded and an unbounded state to the serum proteins. As a drug binds more to the serum proteins it will have less efficiency of diffusion to cellular membranes. The blood brain barrier protects the brain and reduces the exogenous compounds to enter directly into the brain. If a compound has a value of logBB ¿0.3 then it will easily cross the BBB barrier hence been effective and if it is logBB¡-1 then it is poorly distributed. Compounds with a value of logPS¿-2 penetrate the CNS whereas value logPS¡-3 does not penetrate the CNS [59].

The values of the distribution of ligands tetrahydrocannabinol, cannabinol, canabierol, canabichromene are given below in Table 4.8. The range of VDss values provides crucial insights into the extent of distribution throughout the body, encompassing both vascular and extravascular compartments. The values of the fraction unbound of these ligands shows that out of the total dose this fraction will not be bounded to the protein. All these ligands mentioned in Table 4.8 can cross the blood brain barriers of all these had pass the CNS. The distribution properties of ocimene, resins, methionin, lysine, cannabichromenic acid indicates all had cross the blood brain barrier and with that methionin, lysine are not permeable to central nervous system.

Other parameters gives the distribution of ligands and gives the amount of the unbounded ligand. The distribution properties of dihydrostilbene, cannabispiran, pinene, limonene, terpineol indicates that all these ligands are permeable to the central nervous system and that they can easily cross the blood brain barrier. Their distribution and fraction unbounded values are also given. The distribution properties of borneol, cannabigerovarinic acid, cannabielsoin, humulene, myrcene indicates that borneol, cannabigerovarinic acid, cannabielsoin, humulene, myrcene ligands as drugs can pass through the central nervous system. With that cannabielsoin can poorly pass the blood brain barrier.

Table 4.8: Distribution Properties Ligands

| **Sr. No.** | **Name of Ligands** | **VDss (human)** | **Fraction Unbound** | **BBB** | **CNS** |
|---|---|---|---|---|---|
| 1. | Tetrahydrocannabinol | 0.977 | 0 | 0.448 | -2.104 |
| 2. | Cannabinol | 0.758 | 0 | 0.771 | -1.606 |
| 3. | Cannabidiol | 0.939 | 0.012 | -0.113 | -1.886 |
| 4. | Cannabigerol | 0.717 | 0 | -0.225 | -1.268 |
| 5. | Cannabichromene | 0.717 | 0 | 0.491 | -1.998 |
| 6. | Ocimene | 0.336 | 0.387 | 0.761 | -1.848 |
| 7. | Resins | -0.152 | 0.43 | -0.243 | -2.236 |
| 8. | Methionin | -1.1 | 0.716 | -0.276 | -3.059 |
| 9. | Lysine | -0.511 | 0.47 | -0.518 | -3.497 |
| 10. | Canabichromenic Acid | -1.386 | 0.076 | -0.218 | -2.102 |
| 11. | Dihydrostillbene | 0.76 | 0.015 | 0.68 | -1.226 |
| 13. | pinene | 0.667 | 0.425 | 0.791 | -2.201 |
| 14. | limonene | 0.396 | 0.48 | 0.732 | -2.37 |
| 15. | Terpineol | 0.207 | 0.565 | 0.305 | -2.807 |
| 16. | Borneol | 0.337 | 0.486 | 0.646 | -2.331 |
| 17. | Cannabigerovarnic Acid | -1.661 | 0.056 | -0.921 | -2.18 |
| 18. | Cannabielsoin | 0.631 | 0.006 | -0.131 | -1.962 |
| 19. | Humulene | 0.505 | 0.347 | 0.663 | -2.555 |
| 20. | Myrcene | 0.363 | 0.39 | 0.781 | -1.902 |

Some of the parameters of distribution properties of tetrahydrocannabinol has already been studied Hanasono GK, Sullivan HR, – 1987. Some parameters of distribution of have been studied, methionin cannabichromenic acid by Webb J-1997 [73]. Some parameters of distribution properties of canabinol and canabidiol have been studied by Kauert GF, Ramaekers JG in 2007. Pkcsm distribution properties of limonene, terpineol reported by Carpena M. Nunez-Estevez B's 2021 study [74] explored cannabichromene dispersion, based on Izzo AA's 2009 research. Izzo AA and Borrelli F's 2009 study also examined cannabigerovarinic acid, borneol, and cannabielsoin distribution. various investigations illuminate the pharmacokinetic characteristics and biological distribution patterns of various drugs. Researchers can better comprehend the chemicals' therapeutic and pharmacological effects by elucidating their distribution features. This allows for further medication development and clinical study.

### 4.6.5 Metabolism

Cytochrome P450 is an enzyme held responsible for detoxification in the liver. Many drugs get deactivated by this enzyme but certain drugs can be activated. Inhibitors of this enzyme can directly affect the metabolism of drug hence should not be used [58]. Similarly, CYP2D6 and CYP3A4 are responsible for the metabolism of the drugs. Inhibition to these affects the pharmacokinetics of the drug in use [59]. Table 4.9 shows the prediction of the metabolism of all ligands. Among tetrahydrocannabinols, cannabinol, canabidiol, canabigerol, cannabichromene, all the five ligands mentioned are neither the CYP2D6 substrates nor CYP2D6 inhibitor. Substrates nor they are CYP1A2, CYP2C19 not inhibitors. The metabolic properties of ocimene, resins, methionin, lysine, cannabichromenic acid indicates that all the five ligands mentioned are not substrates. Ocimene, resins, methionin, lysine, cannabichromenic acid are not the CYP1A2 inhibitors.

All five ligands are not CYP2C9 and CYP2D6 inhibitors. Among dihydrostilbene, cannabispiran, pinene, limonene, terpineol, all the five ligands are not CYP2D6 substrates dihydrostilbene, cannabispiran are CYP3A4 substrates. Ligands dihydrostilbene, cannabispiran are inhibitors to CYP2C19, CYP1A2.and CYP3A substrate. Dihydrostilbene, cannabispiran are CYP3A4 substrates. Ligands borneol, cannab-igerovarinic acid, are inhibitors to CYP2C19, CYP1A2.and CYP3A substrate. The five ligands borneol, cannabigerovarinic acid, cannabielsoin, humulene, and myrcene are not CYP2D6 substrates while dihydrostilbene, cannabispiran are CYP3A4 substrates. Ligands borneol, cannabigerovarinic acid, are inhibitors to CYP2C19, CYP1A2.and CYP3A substrate.

Some of the parameters of metabolism properties of canabinol, tetrahydrocannabinol has already been studied Hanasono GK, Sullivan HR, – 1987. Some parameters of metabolism of have been studied methionin cannabichromenic acid by Webb J-1997 [76]. Some parameters of metabolism properties of canabinol and canabidiol have been studied Kauert GF, Ramaekers JG in 2007. Pkcsm absorption properties of limonene, cannabispiran reported by Carpena M. NunezEstevez B-2021 [78]. Pkcsm metabolism properties Cannabichromene of has already been reported by Izzo AA, in 2009 [79].

Table 4.9: Metabolism Properties Ligands

| Sr.No. | Ligands Name | CYP2D6 Substrate | CYP3A4 Substrate | CYP1A2 Inhibitor | CYP2C19 Inhibitor | CYP2C9 Inhibitor | CYP2D6 Inhibitor | CYP3A4 Inhibitor |
|---|---|---|---|---|---|---|---|---|
| 1. | Tetrahydrocannabinol | No | yes | yes | yes | No | No | No |
| 2. | Cannabinol | No | yes | yes | yes | yes | No | yes |
| 3. | Cannabidiol | No | yes | yes | yes | No | No | No |
| 4. | Cannabigerol | No | yes | yes | yes | yes | No | Yes |
| 5. | Cannabichromene | No | yes | yes | yes | No | No | No |
| 6. | Ocimene | No | No | No | No | No | No | No |
| 7. | Resins | No | No | No | No | No | No | No |
| 8. | Methionin | No | No | No | No | No | No | No |
| 9. | Lysine | No | No | No | No | No | No | No |
| 10. | Canabichromenic Acid | No | No | No | No | No | No | No |
| 11. | Dihydrostillbene | No | yes | yes | yes | yes | No | No |
| 12. | Canabispirin | No | yes | yes | yes | yes | No | No |
| 13. | pinene | No | No | No | No | No | No | No |

Table 4.9 - Continued from Previous Page

| Sr.No. | Ligands Name | CYP2D6 Substrate | CYP3A4 Substrate | CYP1A2 Inhibitor | CYP2C19 Inhibitor | CYP2C9 Inhibitor | CYP2D6 Inhibitor | CYP3A4 Inhibitor |
|---|---|---|---|---|---|---|---|---|
| 14. | limonene | No | No | No | No | No | No | No |
| 15. | Terpineol | No | No | No | No | No | No | No |
| 16. | Borneol | No | yes | yes | yes | yes | No | No |
| 17. | Cannabigerovarnic Acid | No | yes | yes | yes | No | No | No |
| 18. | Cannabielsoin | No | yes | No | yes | No | No | No |
| 19. | Humulene | No | No | No | No | No | No | No |
| 20. | Myrcene | No | No | No | No | No | No | No |

### 4.6.6 Excretion

The Renal OCT2 substrate acts as a transporter that helps in clearing the drugs and other compounds. Total clearance indicates hepatic clearance which means the drug is metabolized and renal clearance indicates the drug is excreted [59]. The excretion values of the ligands are given below. Table 4.10 shows the excretory properties of all ligands.

Among tetrahydrocanabinol, canabinol, canabidiol, canabigerol and canabichromene, it indicates that all these ligands are not renal OCT2 substrates which means the ligands would not be cleared out of the body and hence the total clearance values are given accordingly. Borneol, cannabigerovarinic acid, cannabielsoin, humulene, and myrcene exhibit excretion, suggesting they are not renal organic cation transporter 2 substrates. Thus, renal excretion routes cannot efficiently clear them. Thus, total clearance values for these ligands are based on alternative clearance processes to show their main elimination routes. These data help us understand these compounds' pharmacokinetic behavior, directing their therapeutic use and drug development techniques to optimize their pharmacological effects and safety. The excretory properties of ocimene, resins, methionin, lysine, cannabichromenic acid indicates that all these ligands are not renal OCT2 substrates which means the ligands would not be cleared out of the body and hence the total clearance values are given accordingly. The excretory properties of dihydrostilbene, cannabispiran, pinene, limonene, terpineol indicates that all these ligands are not renal OCT2 substrates which means the ligands would not be cleared out of the body and hence the total clearance values are given accordingly.

Some of the parameters of excretory properties of canabidiol, tetrahydrocannabinol has already been studied Hanasono GK, Sullivan HR, – 1987 [80]. Some parameters of excretory properties of canabigerol and canabidiol have been studied Kauert GF, Ramaekers JG in 2007 [81]. Pkcsm excretory properties of Cannabichromene has already been reported by Izzo AA, in 2009 [82]. Some parameters of excretory of have been studied ocimene, cannabichromenic acid and by Webb J1997 [83]. Pkcsm excretory properties of cannabispiran, terpineol reported by Carpena M. Nunez-Estevez B-2021 [47]. Pkcsm excretory properties of humulene, cannabielsoin have already been reported by Izzo AA, Borrelli F in 2009 [84].

TABLE 4.10: Excretion Properties Ligands

| Sr. No. | Name of Ligands | Total Clearance | Renal OCT2 Substrate |
|---|---|---|---|
| 1. | Tetrahydrocannabinol | 0.883 | No |
| 2. | Cannabinol | 0.777 | No |
| 3. | Cannabidiol | 1.092 | No |
| 4. | Cannabigerol | 1.31 | No |
| 5. | Cannabichromene | 1.093 | No |
| 6. | Ocimene | 0.441 | No |
| 7. | Resins | 0.601 | No |
| 8. | Methionin | 0.304 | No |
| 9. | Lysine | 0.5 | No |
| 10. | Canabichromenic Acid | 0.954 | No |
| 11. | Dihydrostillbene | 0.204 | No |
| 12. | Canabispirin | 0.948 | No |
| 13. | pinene | 0.043 | No |
| 14. | limonene | 0.213 | No |
| 15. | Terpineol | 1.219 | No |
| 16. | Borneol | 1.035 | No |
| 17. | Cannabigerovarnic Acid | 1.099 | No |
| 18. | Cannabielsoin | 0..887 | No |
| 19. | Humulene | 1.282 | No |
| 20. | Myrcene | 0.438 | No |

## 4.7 Lead Compound Identification

The physiochemical and the pharmacokinetics properties of the ligands determine their fate as for being drug or non-drug compounds. Lipinski's rule is the first filter and pharmacokinetics is the second filter for this identification. All of the follow the Lipinski Rule as the molecular weight, H bond acceptors, and hydrogen bond donor Lipinski rule, canabidiol, canabigerol, canabichromene does not follow the Lipinski rule as the logP value exceed the Lipinski rule but as it falls from one it is acceptable. The next knockout stage is pharmacokinetic screening. In this screening humulene because of being carcinogenic have been knocked out. At the end of this, the compound left are tetrahydrcanbinol, canabinol, ocimene, resins, methionin, lysine, canabichromenic acid, dihydrostillbene, canabispirin, pinene, limonene, terpeineol, bormeol, canabigerovarnic acids, canabielsoin,

mycrene. Among all of these lysine and canabielsoin taken and I have selected lysine as a lead compound [85].

## 4.8 Drug Identification against SMA

Drug Identification against SMA Evrysdi is one of the medications that has been used in several nations, including the UK, India, Pakistan, and many more [86].

### 4.8.1 Evrysdi

Evrysdi Drug to treat spinal muscular atrophy in children and adults. It is a FDA approved drug. The current treatments for SMA aim to boost the expression of survival motor neuron proteins (SMN), which are produced at a reduced rate due to the disease. The first and only oral drug authorized for the treatment of SMA is risdiplam. With evrysdi, it may prove beneficial against Spinal Muscular Atrophy [87].

## 4.9 Drug ADMET Properties

The drug ADMET properties are studied by using the same software as above which is pkCSM [88].

### 4.9.1 Toxicity Prediction of Reference Drug

Table 4.11 shows that even though Evrysdi's toxicity characteristics are within the positive range, the medicine may still be harmful to the liver. Evrysdi's lack of skin sensitivity induction and hERG I inhibition suggests safety. However, it inhibits hERG II channels. The detection of AMES toxicity suggests Evrysdi may cause cancer. These findings emphasize the necessity of complete safety studies in assessing pharmaceutical intervention risks and benefits for regulatory and clinical decision-making.

Table 4.11: Toxicity Properties of Evrysdi

| **Sr. No.** | **Reference Drug** | **Predicted Value** |
|---|---|---|
| 1. | AMES Toxicity | Yes |
| 2. | Max. tolerated dose (human) | 0.652 |
| 3. | hERG I inhibitor | No |
| 4. | hERG II inhibitor | yes |
| 5. | Oral rat acute toxicity | 2.406 |
| 6. | Oral rat chronic toxicity | 1.043 |
| 7. | Hepatoxicity | Yes |
| 8. | Skin sensitization | No |
| 9. | *T.pyriformis* toxicity | 0.285 |
| 10. | Minnow toxicity | 2.461 |

### 4.9.2 Absorption Properties

Table 4.12 shows the absorption properties of Evrysdi. The values show that Evrysdi have high CaCO2 solubility. Though the intestinal absorption is high but it still is in the safe range. Evrysdi also has a lower value of skin permeability. Evrysd is also a P-glycoprotein substrate and an inhibitor to P-glycoprotein I and P-glycoprotein II inhibitor [90].

Table 4.12: Absorption Properties of Evrysdi

| **Sr. No.** | **Reference Drug** | **Evrysdi** |
|---|---|---|
| 1 | Water solubility | -2.81 |
| 2 | CaCo2 Solubility | 1.436 |
| 3 | Intestinal absorption | 100 |
| 4 | Skin Permeability | -2.735 |
| 5 | p-glycoprotein substrate | Yes |
| 6 | p-glycoprotein 1 inhibitor | No |
| 7 | P- glycoprotein II inhibitor | No |

### 4.9.3 Distribution Properties

Table 4.13 shows the distribution properties of Evrysdi. The distribution parameters value shows that the value of VDss is low which means the drug would not be distributed properly [91].

Table 4.13: Distribution Properties of Evrysdi

| **Sr. No.** | **Reference Drug** | **Evrysdi** |
|---|---|---|
| 1 | VDSS | 0.34 |
| 2 | Fraction unbound (human) | 0.401 |
| 3 | BBB permeability | -1.198 |
| 4 | CNS Permeability | -3.073 |

### 4.9.4 Metabolic Properties

Table 4.14 shows the metabolic properties of Evrysdi. It indicates that Evrysdi is CYP1A2 substrate and CYP3A4 substrate. It also shows that Evrysdi is not a CYP2C19, CYP2C9. CYP2D6, CYP2D6 and CYP3A4 inhibitor [92].

Table 4.14: Metabolic Properties of Evrysdi

| **Sr. No.** | **Reference Drug** | **Evrysdi** |
|---|---|---|
| 1 | CYP2D6 Substrate | No |
| 2 | CYP3A4 Substrate | NO |
| 3 | CYP1A2 Inhibitor | Yes |
| 4 | CYP2C19 Inhibitor | No |
| 5 | CYP2C9 Inhibitor | No |
| 6 | CYP2D6 Inhibitor | No |
| 7 | CYP3A4 Inhibitor | Yes |

### 4.9.5 Excretion Properties

Table 4.15 shows the excretion properties of Evrysdi. The above table gives the values of excretory properties of Evrysdi [57]. It shows that Evrysdi is not a renal OCT2 Substrate which means it will not help in clearing of the drug. With that the value of total clearance as 0.768 is also given with respect to its liver and renal clearance [93].

TABLE 4.15: Excretion Properties of Evrysdi

| **Sr. No.** | **Reference Drug** | **Evrysdi** |
|---|---|---|
| 1 | Total clearance | 0.768 |
| 2 | Renol OCT2 Substrate | No |

## 4.10 Evrysdi Mechanism of Action

People who have spinal muscular atrophy (SMA), which is caused by chromosome 5q mutations that cause an insufficiency of SMN protein, are treated with Evrysdi (risdiplam), a survival of motor neuron (SMN2) splicing modifier.

It was discovered that risdiplam enhanced the production of full-length SMN protein in the brain and the inclusion of exon 7 in SMN2 messenger ribonucleic acid (mRNA) transcripts through the use of in vitro tests and studies carried out on transgenic animal models of SMA. Results from studies conducted in vivo and in vitro indicate that risdiplam may cause alternative splicing of additional genes, including MADD and FOXM1. It has been discovered that FOXM1 and MADD may have a role in the detrimental consequences seen in animals [95].

Studies conducted on risdiplam analogues have demonstrated that the drug acts through two distinct mechanisms (Figure 4.24) that target distinct regions of SMN2 exon 7. The initial target in the premRNA generated from SMN2 is the 5-splice site (TSL2) of exon-7. Here, risdiplam stabilizes the duplex formed by the 5-splice site RNA sequence and the U1 snRNP RNA sequence to encourage splicing initiation. The second field of study focuses on the internal structure of the exonic splicing enhancer 2 (ESE2) of SMN2 exon-7. The

TABLE 4.16: Docking Results of Evrysdi

| Com. | Score | Size | HBD | HBA | logP | Weight | Bond | Grid |
|---|---|---|---|---|---|---|---|---|
| Evrysdi | -7.4 | 47 | 1 | 8 | 1.96324 | 401.474 | 2 | 25 |

stem-loop structure (TSL1) of the first half of SMN2 exon 7 is changed risdiplam binds to the ESE2 region [96].

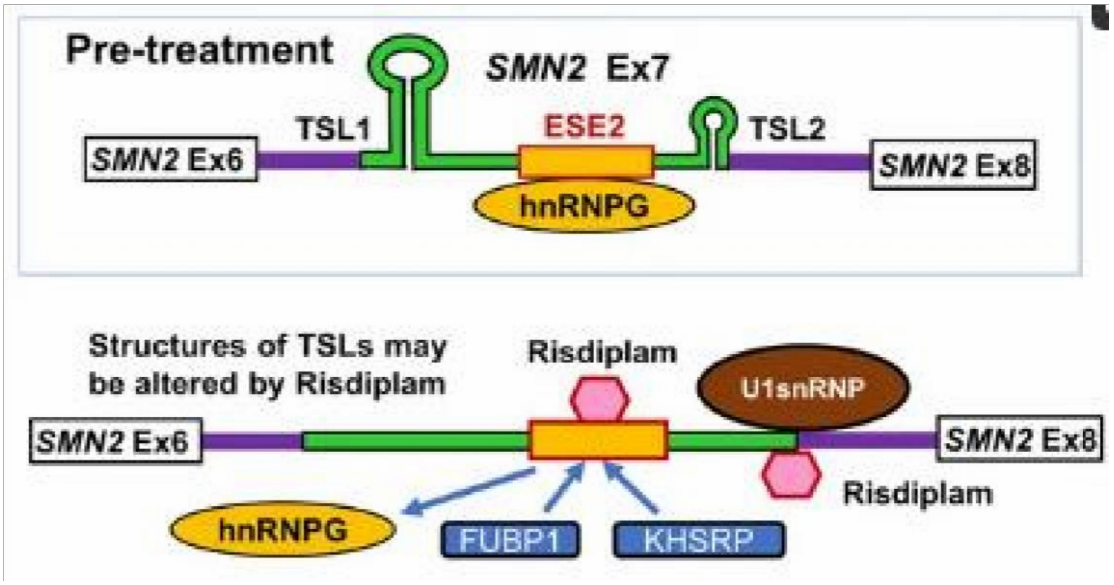



FIGURE 4.24: Evrysdi (Risdiplam) Mechanism of Action

## 4.11 Evrysdi (Risdiplam) Effect on Body

After taking the medicine, headache, diarrhea, fever, and rash are the most typical side effects. For upper respiratory infections, runny nose, sneezing, and sore throat in infants with SMA lower respiratory tract infection (lung infection) [96].

## 4.12 Evrysdi (Risdiplam) Docking

Table 4.16 shows the docking result of Evrysdi (Risdiplam). The table indicates that Evrysdi (Risdiplam) has a binding score of -7.4.

The docking results of Evrysdi (Risdiplam) with SMN protein shows that it has quite a good binding score. And has one hydrogen bond donars, and eight hydrogen bond acceptors that breaks one of the Lipinski rule, Evrysdi (Risdiplam) has two numbers of Rotatable bonds.

### 4.12.1 Evrysdi (Risdiplam) Comparison with Lead Compound

The standard drug Evrysdi (Risdiplam) is compared with the lead compound lysine and their physicochemical and pharmacokinetic properties are compared for the assessment of bioavailability, efficiency, safety, and drug-likeness. The Table 4.17 shows lead compound lysine not breaks of Lipinski's rules.

Table 4.17: Lipinski Rule Comparison

| S. No. | Compound | logP | Weight g/mole | HBA | HBD |
|---|---|---|---|---|---|
| 1 | Evrysdi | 1.96324 | 401.474 | 8 | 1 |
| 2 | Lysine | -0.4727 | 146.19g/mol | 4 | 3 |

## 4.13 ADMET Properties Comparison

The ADMET properties comparison is done to check the absorption, distribution, metabolic excretion, and toxicity properties of the drug and the lead compound for finding a better drug candidate [97].

### 4.13.1 Toxicity Comparison

Nine models are used to assess the toxicity of the lead chemical and the conventional medication. According to Model 1 of AMES toxicity, lead and standard chemicals do not cause mutations. According to Model 2 of the Maximum Tolerated Dosage, a number is deemed low if it is equal to or less than 0.477 log mg/kg/day, whereas a higher value

TABLE 4.18: Toxicity Properties Comparison

| S.No. | Reference Drug | Evrysdi Predicted Value | Lysine Predicted Value |
|---|---|---|---|
| 1. | AMES Toxicity | Yes | No |
| 2. | Max. tolerated dose (human) | 0.652 | 1.227 |
| 3. | hERG I inhibitor | No | No |
| 4. | hERG II inhibitor | Yes | No |
| 5. | Oral rat acute toxicity | 2.406 | 2.046 |
| 6. | Oral rat chronic toxicity | 1.043 | 3.083 |
| 7. | Hepatoxicity | Yes | No |
| 8. | Skin sensitization | No | No |
| 9. | *T.pyriformis* toxicity | 0.285 | 0.274 |
| 10. | Minnow toxicity | 2.461 | 2.542 |

is deemed excessive. The table below demonstrates the high tolerated dose value of lysine. The third model is hERG. Relative toxicity is evaluated using the fourth oral rat acute toxicity model. Model 5 of oral rat chronic toxicity provides the lowest dose values that could have a negative outcome. The hepatic Model 6 indicates that a medicine may harm the liver. As can be seen from the table, Evrysdi is hepatotoxic [98]. The number seven is used to verify the dermal goods model's sensitivity to the skin. The lead chemical-based and the standard are not skin-sensitive. Models 8 and 9 employ T. Pyriformis and minnows, respectively to assess toxicity. Both lysine and Evrysdi are fairly poisonous for T. Pyriformis levels ¿-0.5, and both chemicals pass this toxicity test for minnows,which has toxicity values ¡ 0.5mM. The relative toxicity ratings of lysine and Evrysdi are displayed in Table 4.18. Toxicity Properties Comparison

### 4.13.2 Absorption Properties Comparison

Six models form the basis of the absorption parameter. The compound's solubility in water at 25o is indicated by the water solubility model. The oral medication absorption is predicted using the CaCO2 solubility model. High drug absorption is defined as values higher than 0.90. Less than 30% on the intestinal absorption model is regarded as inadequate absorption. Lysine has a high intestinal absorption rate, as indicated by the standard and lead compound values provided. As per the skin permeability model, transdermal medicines with a value less than log Kp ¿ -2.5 are deemed poor; hence, neither of

the compounds can pass the skin permeability test. Because P glycoprotein is an ABC transporter and a biological barrier, the P-glycoprotein substrate model is crucial. The substrates are Evrydsi. The final P glycoprotein inhibitor model illustrates whether a given substance functions as an inhibitor or not.

Table 4.19: Absorption Properties Comparison

| **S. No.** | **Reference Drug** | **Evrysdi** | **Lysine** |
|---|---|---|---|
| 1 | Water solubility | -2.81 | -2.888 |
| 2 | CaCo2 Solubility | 1.436 | 0.737 |
| 3 | Intestinal Absorption | 100 | 62.673 |
| 4 | Skin Permeability | -2.735 | -2.735 |
| 5 | p-glycoprotein substrate | yes | No |
| 6 | p-glycoprotein 1 inhibitor | No | No |
| 7 | P- glycoprotein II inhibitor | No | No |

Table 4.19 demonstrates that neither of the drugs inhibits P-glycoprotein I or II [99].

### 4.13.3 Metabolic Properties Comparison

P450 is mostly located in the liver and is considered a detoxification enzyme since it oxidizes foreign substances to make them easier for the body to eliminate. It either deactivates or activates some medicines. Therefore, determining whether a chemical is a P450 substrate or not, as well as if it is a P450 inhibitor, is crucial [65]. Table 4.20 indicates that lysine is not an inhibitor of CYP3A4 or CYP1A2, although Evrysdi is.

### 4.13.4 Distribution Properties Comparison

Table 4.21 shows the relative distribution characteristics of lysine and Evrysdi. Based on four models, the distribution parameter is determined. When the drug's volume of

TABLE 4.20: Metabolic Properties Comparison

| S. No. | Reference Drug | Evrysdi | Lysine |
|---|---|---|---|
| 1 | CYP2D6 Substrate | No | No |
| 2 | CYP3A4 Substrate | No | No |
| 3 | CYP1A2 Inhibitor | Yes | No |
| 4 | CYP2C19 Inhibitor | No | No |
| 5 | CYP2C9 Inhibitor | No | No |
| 6 | CYP2D6 Inhibitor | No | No |
| 7 | CYP3A4 Inhibitor | Yes | No |

distribution (VDss) exceeds 2.81 L/kg, it indicates that the medication is more evenly distributed in the tissues than in the blood plasma. The VDss measures the drug's uniform distribution in blood plasma. Lysine and Evrysdi have similar low VDss values. The second model is predicated on the proportion of medicines in plasma that are unbound, since medications that are bounded have an impact on drug efficiency. The amount of medicine that is still unbounded is indicated by the given value. It is evident from these readings that lysine has a high value, indicating easy distribution, and Erysdi has a low value, suggesting inadequate distribution to the brain. The CNS model operates on the premise that drugs possessing a logPS value exceeding 2 exhibit facile penetration into the central nervous system (CNS), while those with a logPS value below -3 face significant barriers to CNS entry. Due to its notably low logPS value, Erysdi is unlikely to traverse the blood-brain barrier and access the CNS. This characteristic has implications for its pharmacological activity, suggesting a limited impact on CNS-related functions and potential side effects associated with central nervous system interactions.

TABLE 4.21: Distribution Properties Comparison

| S. No. | Reference Drug | Evrysdi | Lysine |
|---|---|---|---|
| 1 | VDSS | 0.34 | -0.511 |
| 2 | Fraction unbound (human) | 0.401 | 0.47 |
| 3 | BBB permeability | -1.198 | -0.518 |
| 4 | CNS Permeability | -3.073 | -3.497 |

### 4.13.5 Excretion Properties Comparison

The evaluation of the medication dose rates is dependent on the total clearance value, which is a sum of the hepatic and renal clearance. Compared to lysine, Evrysdi has a higher overall clearance. The second model is of the Renal OCT2 (Organic Cation Transporter 2), a transporter that aids in the renal clearance of various substances, including medications. In respect to inhibitors, one may experience negative effects from being an OCT2 substrate [99]. Thus, lysine and Evrysdi are not renal OCT2 substrates. The excretory characteristics of Evrysdi and lysine are displayed in Table 4.22.

Table 4.22: Interaction of Resins with Receptor Protein

| S. No. | Reference Drug | Evrysdi | Lysine |
|---|---|---|---|
| 1 | Total clearance | 0.768 | 0.5 |
| 2 | Renal OCT2 Substrate | No | No |

## 4.14 Physiochemical Properties Comparison

The physiochemical properties of the compounds are investigated in order to ascertain their basic characteristics. Using this screening, it is possible to determine that Evrysdi contains 22 carbon atoms, 23 hydrogen atoms, and 70 nitrogen atoms, while lysine contains 6 carbon atoms, 14 hydrogen atoms, 2 nitrogen atoms, and 2 oxygen atoms. This demonstrates that, in relation to lysine, Evrysdi is a basic bio-compound. As a sign of the oxidation state, lysine can only transfer three hydrogen atoms, whereas Erysdi can only donate one. Lysine and Evrysdi, which are subject to the Lipinski rule. Even though Evrysdi's Log P value is higher than lysine's, its molecular weight is significantly higher and it does not follow the Lipinski rule. When comparing the number of rotatable bonds, lysine has just five, while Evrysdi has two. The comparison of the physiochemical characteristics of lysine and Evrysdi is presented in Table 4.23.

TABLE 4.23: Physiochemical Properties Comparison

| **Sr. No.** | **Drug** | **Formula** | **H Donor** | **H Accept.** | **logP** | **Weight** | **Bond** |
|---|---|---|---|---|---|---|---|
| 1 | Evrysdi | C22H23N7O | 1 | 8 | 1.96324 | 401.474 | 2 |
| 2 | Lysine | C6H14N2O2 | 3 | 3 | 0.4727 | 146.19 | 5 |

## 4.15 Docking Score Comparison

Standard drug and lead compound docked against the SMN protein, and the best binding score was obtained from the docking result. Table 4.24 demonstrates that lysine, the lead chemical, has a significantly higher vine score than Evrysdi, the conventional medication. Evrysdi's binding score is -7.4, while lysine's is -3.8, both of which are higher than those of the conventional medication. This finding indicates that lysine is able to either inhibit or bind with the mutant SMN1 gene more effectively than Evrysdi.

TABLE 4.24: Docking Score Comparison

| **Sr. No.** | **Compound** | **Binding Score** |
|---|---|---|
| 1 | Evrysdi | -7.4 |
| 2 | lysine | -3.8 |

## 4.16 Docking Analysis Comparison

The docking results were analyzed by LigPlot based on the number of hydrogen bonds, number of hydrophobic interactions, number of interacting amino acids, and that of steric interactions.

Figure 4.25 and 4.26 shows the docking results of Evrysdi and lysine. Figure 4.25 shows that Evrysdi has formed five hydrophobic interactions and lysine formed two hydrophobic interaction and three hydrogen bond.

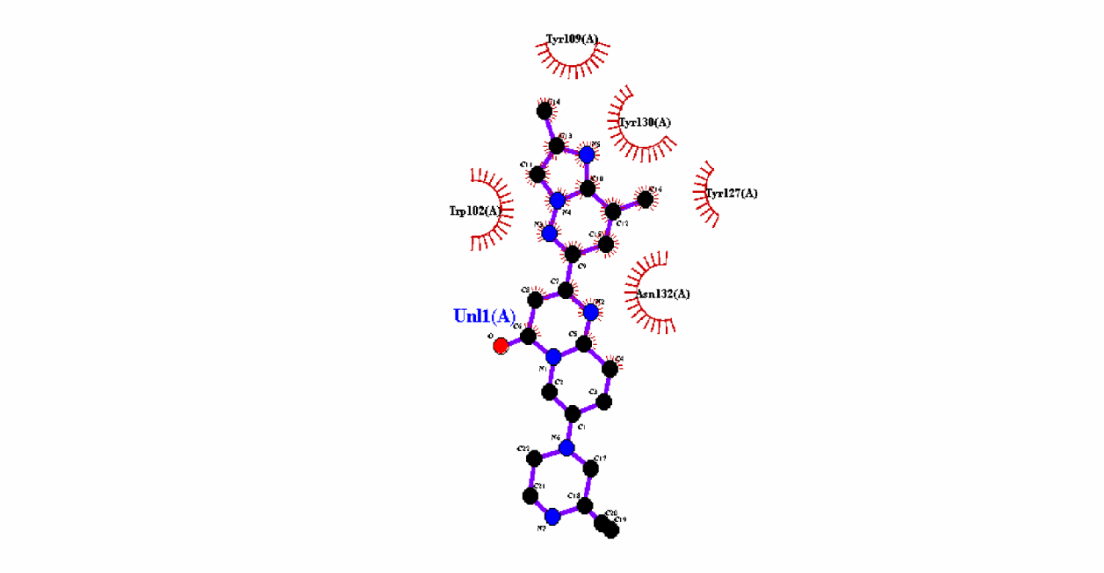



FIGURE 4.25: Interaction of Evrysdi with the Receptor

Figure 4.26 shows that lysine has formed six hydrophobic interactions and three hydrogen bonds.

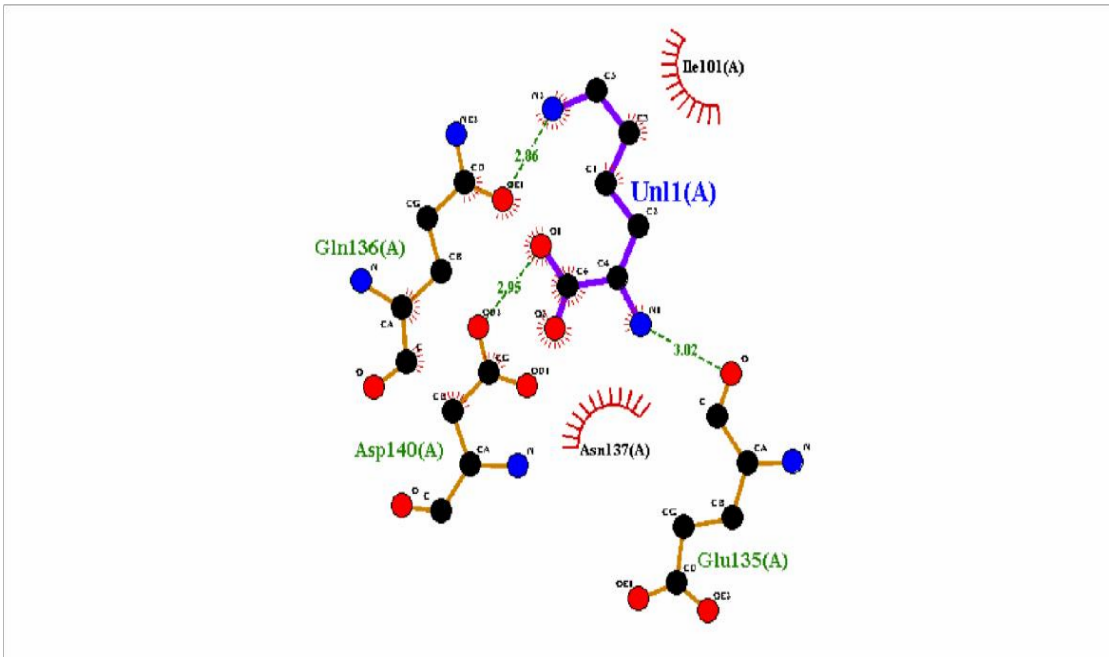



FIGURE 4.26: Interaction of lysine with Receptor

The details of hydrogen and hydrophobic interactions are mentioned in the Table 4.25. Lysine forms three hydrogen bonds whereas Evrysdi form no hydrogen bond, this is mainly because lysine N1, N2 and O1 has made interactions with the receptor. Evrysdi makes 5 hydrophobic interactions whereas lysine makes 2 of them. With all this information lysine succeeds to be much better than Evrydsi.

TABLE 4.25: Docking Analysis Comparison

| **Sr. No.** | **Ligand** | **Binding** | **HBs** | **Amino** | **Distance** | **Hydrophobic** |
|---|---|---|---|---|---|---|
| 1 | Evrysdi | -7.4 | 0 | – | – | Tyr109 |
| | | | | | | Tyr127 |
| | | | | | | Tyr130 |
| | | | | | | Asn132 |
| | | | | | | Trp102 |
| 2 | Lysine | -3.8 | 3 | OE1-Gln136-N2 | 2.86 | Lle101 |
| | | | | OD2-Asp140-O1 | 2.95 | Asn137 |
| | | | | O-Glu135-N1 | 3.02 | |

The 4.25 shows Tyr109, Tyr127, Tyr130, Asn132, Trp102 participates forming hydrophobic interaction between protein and Evrysdi. Whereas Lle101, Asn137 participates forming hydrophobic interaction between protein and lysine. The oxygen atom of Gln136 termed as OE1 forms a hydrogen atom with N2 of lysine forming an OE1-Gln136-N2 bond. The oxygen of Asp140 named as OD2 bonds to first oxygen of lysine forming an OD2-Asp140-O1 bond, similarly the oxygen of Glu135 forms a bond with first oxygen of lysine forming an O-Glu135-N.

# Chapter 5

# CONCLUSION AND FUTURE PROSPECTS

The study aimed to determine active constituents in the plant Cannabis (Marijuana) which is also known as grass, hashish, joint in common language. For this purpose, 20 ligands were selected to be dock against the SMN protein of SMA. All the ligands were docked against the receptor protein via CB Dock. The results were visualized using PyMol and were analyzed through LigPlot. Out of those 20 ligands, canabinol, canabidiol and canabigerol disobeyed one Lipinski rule that are of LogP value. After these 17 ligands were left and out of those lysine and canabielsoin were the two best active ligands selected. Based on the hydrophobic and hydrogen bonding, lysine was selected as a lead compound against the standard drug Evrysdi, which is in use for the treatment of spinal muscular atrophy SMA. With the final results, it was cleared that lysine can bind better to mutated SMN protein than that of Evrysdi.

## 5.1 Recommendation

As per the findings of this research lysine should be exploited more against SMA. With this other active constituent like canabielsoin, resins. Tetrahyrocanabinol and canabisprin have also shown a positive result in response to SMN protein. Previously Cannabis

(Marijuana) has been used as anti-viral, anti-inflammatory and anti-oxidants for this reason Cannabis (Marijuana) should be explored more for its effectiveness against SMA.

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