CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Exploration of *Ranunculus hirtellus* Metabolites Inhibitory Role in Polycystic Ovarian Syndrome - An Insilico Study

by

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CERTIFICATE OF APPROVAL

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(Sara Parveen)

Abstract

Polycystic Ovary Syndrome (PCOS) is an intricate endocrine disorder that is influenced by hormonal dysregulation, metabolic dysfunction, and reproductive issues. The underlying mechanisms for its pathogenesis are multifaceted and include genetic, epigenetic, and environmental elements, which include insulin resistance and inflammation. Given the exponential rise in the prevalence of PCOS worldwide, reliable treatment modalities are required urgently. This work explores the therapeutic potential of the metabolic compounds of *Ranunculus hirtellus* that has been shown to possess abundant amounts of metabolic compounds. Selected metabolic compounds such as the methyl ester, 2,4-di-tert-butylphenol, turmerone, succinic acid, and phytyl were analyzed for their interaction capabilities with identified proteins that have been shown to be of prime importance in the pathogenesis of PCOS, which include Gamma-Aminobutyric Acid B receptor subunit 2, Apolipoprotein A-1, C-Reactive Protein, Follicle-Stimulating Hormone Receptor, and Kisspeptin. Three-dimensional structures of identified proteins and metabolic compounds were employed for molecular docking. Moreover, phytyl, which is an important metabolite of the *Ranunculus hirtellus* plant, showed promising ADME/T characteristics, docking score of -7.6 against kisspeptin protein, and good protein interaction profile, confirming it as a drug of choice. To test the additional efficiency of phytyl, it was evaluated against the commercial anti-PCOS drug clomiphene citrate. Comparing phytyl with clomiphene citrate with respect to ADME/T analysis, which was comparable, though the docking score and interaction profile of phytyl are better than clomiphene citrate. Thus, it is concluded at this stage that phytyl possesses the ability to be an anti-PCOS drug of the future.

Keywords: polycystic ovary syndrome, phytyl, ADMET profile, bioactive metabolites, therapeutics

Contents

List of Figures

List of Tables

Abbreviations

ADMET	absorption, distribution, metabolism, excretion, toxicity
AR	Androgen receptor
ApoA-1	Apolipoprotein A-1
CAH	Congenital Adrenal Hyperplasia
CRP	C-reactive protein
CRP	C-reactive protein
DHEAS	Dehydroepiandrosterone sulfate
FSH	Follicle-Stimulating Hormone
FSHR	Follicle-Stimulating Hormone Receptor
FSHR	Follicle-stimulating hormone receptor
GBR2	Gamma-aminobutyric acid type B, receptor subunit 2
GnRH	gonadotropin-releasing hormone
HDL	High-density lipoprotein
INSR	Insulin receptor
IVF	in vitro fertilization
LH	Luteinizing Hormone
NAFLD	Non-Alcoholic Fatty Liver Disease
OSA	Obstructive sleep apnea
PCOS	Polycystic Ovary Syndrome
PI3K	Phosphoinositide 3-kinase
WHO	World Health Organization

Chapter 1

Introduction

Polycystic Ovary Syndrome is one of the most complex, common endocrine disorders that many women face in their reproductive period. Studies have estimated that it globally affects approximately 5-18% of this population. PCOS is a syndrome with a myriad of presentations, encompassing menstrual irregularities, acne, hirsutism, and infertility, thus posing a complex challenge to women's health. The syndrome is not only a reproductive issue but also has strong links with metabolic disorders as well as mental health, thereby being an extremely important public health issue requiring integrated management approaches [1].

PCOS has a multifactorial etiology and is shaped by genetic tendencies, environmental conditions, and individual lifestyle choices. At the core of the pathophysiology of PCOS is the presence of insulin resistance, which is present in approximately 70% of affected women. This insulin resistance accelerates hyperandrogenism, which is a major cause of the clinical manifestations of PCOS. This nexus of hormonal disturbances and metabolic disorders makes the management of PCOS a challenge because a multifactorial treatment strategy is required along with addressing the cause of the syndrome [2].

Another important feature of PCOS is endocrine dysfunction, specifically hormonal imbalance. The principal defect in the pathophysiology of PCOS can be attributed to the impairment of the hypothalamic-pituitary-ovarian axis. In patients with PCOS, there may be an abnormal episodic secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus, culminating in an abnormal secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland. This translates to an excess of LH over FSH, thereby impairing ovarian function. The excess secretion from the ovaries is characterized by the overproduction of male hormones like testosterone, thereby manifesting as excess hair growth, acne, and irregular menstruation [3].

In addition, the pathophysiology of PCOS is also affected by various proteins and insulin resistance. Insulin resistance, which is associated with reduced responsiveness of the body cells to insulin and an increased level of insulin in the bloodstream, is prevalent in women who suffer from this condition. Besides exacerbating the hormonal overproduction of androgens in the ovaries, this hyperinsulinemia impacts the metabolic processes of the patient, leading to increased risks of type 2 diabetes, cardiovascular diseases, and weight gain. The presence of PCOS in women is also exacerbated by the vicious cycle that is developed between the impacts of insulin resistance and hormonal imbalance [4]. Moreover, the proteins involved in insulin receptor activity, androgen receptor, and follicle-stimulating hormone receptor include: Gamma-aminobutyric acid type B, receptor subunit 2 (GBR2), Apolipoprotein A-1 (ApoP-1), C-reactive protein (CRP), FSHR, kisspeptin also have major contributions to PCOS development with their critical roles in the regulation of cellular processes, follicular development, and ovarian functions [5].

In fact, some of the drugs that have been traditionally utilized in the treatment and management of PCOS include hormonal contraceptives, anti-androgen medications, and the use of insulin-sensitizing drugs such as metformin. Although these drugs have been very effective, they have also been known to produce several side effects that have made it difficult for some women to follow the traditional treatment approaches. This has made alternative treatments using natural drugs derived from plants a common trend [6]. Among these natural alternatives, one plant, *Ranunculus hirtellus*, has gained popularity because of its richness in bioactive compounds that are believed to be useful for women with PCOS [7].

Traditionally, *Ranunculus hirtellus* also known as softly hairy buttercup or piryali has been used in several traditional medicinal practices due to its diverse medicinal values, such as relieving inflammatory, painful, and digestive disorders [8]. Modern scientific research has finally identified that a range of phytochemicals can be obtained from this plant, such as flavonoids, saponins, and phenolic compounds, that have long been recognized due to their strong antioxidant as well as anti-inflammatory properties, which are critical for the treatment of several symptoms shown by patients suffering from PCOS [9].

Further, the antioxidant properties of *Ranunculus hirtellus* are useful for combating oxidative stress, which plays a significant role in the pathophysiology of PCOS. This disorder's symptoms could be exacerbated by oxidative stress, leading to inflammation and damage to cells. The active compounds of *Ranunculus hirtellus* may help to support ovarian function, boosting overall reproductive well-being by eliminating free radicals, which could serve as an effective complement to traditional management strategies [10].

The objective of this research work is to comprehensively examine the bioactive compounds in *Ranunculus hirtellus* and their prospective roles in the treatment of PCOS. Through the analysis of literature and understanding how these compounds achieve their beneficial outcomes, it is proposed that this research work shall provide important information regarding how these compounds could be effectively combined with conventional treatment for PCOS sufferers. The long-term goal of this proposed approach is to ensure that affected women experience an improvement in their quality of life.

1.1 Problem Statement

Polycystic ovary syndrome is an emerging health issue globally as its prevalence continuously rises, especially in developing countries. The traditional medication for this condition comes with many side effects, resulting in the need for the development of safer and more effective alternatives.

1.2 Hypothesis

The *Ranunculus hirtellus* bioactive metabolites might have an active role in the context of polycystic ovarian syndrome.

1.3 Aim and Objectives

This research aims to explore potential bioactive metabolites from *Ranunculus hirtellus* showing desirable properties to combat polycystic ovarian syndrome.

i. To screen *Ranunculus hirtellus* metabolites with PCOS-treatment properties.

ii. To analyze the interaction between specific *Ranunculus hirtellus* metabolites and the desired target proteins.

iii. To identify the best docked metabolite as an inhibitory compound against polycystic ovarian syndrome.

Chapter 2

Literature Review

Polycystic Ovary Syndrome is a common endocrine system disorder that has become prevalent in most women of reproductive age. This medical issue is associated with hormonal distortions, metabolic disorders, and reproductive system problems. Women normally suffer from the developed presence of numerous cysts in their ovaries, in addition to irregular menstrual cycle, excessive hair growth, and obesity. The World Health Organization has termed Polycystic Ovary Syndrome one of the major public health concerns owing to its high prevalence and association with the development of major health complications like infertility, type 2 diabetes, and cardiac issues [11].

2.1 Polycystic Ovary Syndrome

Polycystic Ovary Syndrome is a very common complex endocrine disorder that affects a large proportion of women of reproductive age worldwide. This condition presents a set of diverse symptoms, such as infrequent menstrual cycles, excess male hormones, and the presence of polycystic ovaries, thus giving rise to a variety of health issues connected to the reproductive system. Women suffering from this condition undergo menstruation irregularities, either in the form of oligomenorrhea, or a condition known as amenorrhea, whereby there are no menstruation cycles at all. Moreover, hirsutism, acne, and alopecia or thinning hair, for instance, are common characteristics of the overproduction of male hormones [1]. The causes of this condition can be attributed to a combination of genetic, familial, and reproductive factors, as a large percentage of first-degree relatives of patients suffering from this condition exhibit similar symptoms. The core of the illness can be attributed to the relative overproduction of main hormones, specifically the overproduction of male hormones, as a large percentage of patients suffer from excess male hormones, thus providing a principal reason for a considerable percentage of the diverse health issues shown by patients using figure 2.1 below.

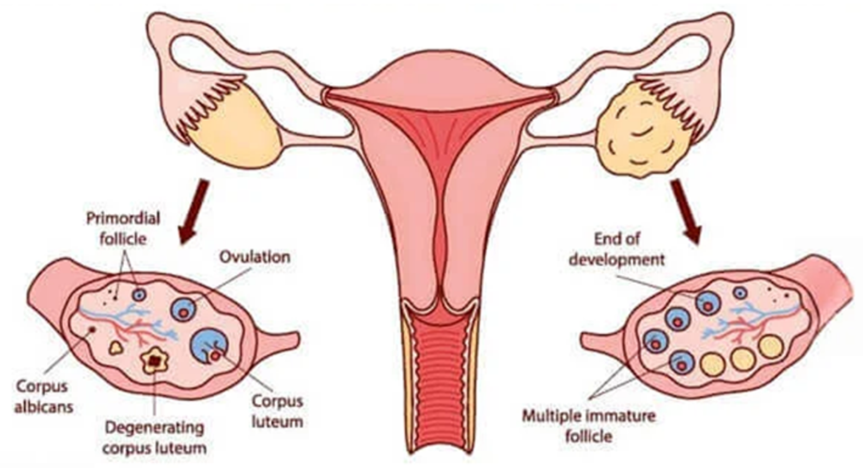


FIGURE 2.1: Polycystic ovary syndrome. This image outlines the female reproductive system, focusing on the ovaries, uterus, stages of ovarian follicles (from primordial follicles to corpus luteum), ovulation process, and development of an immature follicle resulting in PCOS [12].

2.2 Types of Polycystic Ovary Syndrome

Polycystic Ovary Syndrome is a heterogeneous disorder that presents in several different ways. Knowledge about the different presentations is key in designing treatment programs that take into account of the differing needs of the patients, as illustrated in figure 2.2. Below are the main types of PCOS.

2.2.1 Insulin-Resistant PCOS

This is the most common form of PCOS and insulin-resistance, wherein the body's cells become less responsive to insulin, as a result of which high insulin levels are also generated. Women with insulin-resistant PCOS are also predisposed to an increased risk of developing metabolic syndrome and type 2 diabetes. Diet and exercise regimens, apart from medications such as metformin, are usually advised to treat insulin-resistant PCOS. In addition, ovarian enlargement, also known as ovarian cysts, is another common occurrence in women affected with PCOS [13].

2.2.2 Inflammatory PCOS

The presence of low-grade inflammation within the body indicates that the inflammatory PCOS is characterized by low-grade inflammation. This particular condition can exacerbate the insulin sensitivity associated with the disorder, as well as the hormonal imbalances that are typical of the health problem. In addition, women who suffer from inflammatory PCOS tend to suffer from symptoms including fatigue, weight, and issues with their skin in the form of acne or eczema [14].

2.2.3 Adrenal PCOS

Adrenal PCOS involves excessive production of androgens in the adrenal glands and not the ovaries. This form of PCOS is less frequent but can manifest with symptoms such as excessive hair growth, acne, and menstrual irregularities. Women with adrenal PCOS can have excessive production of dehydroepiandrosterone sulfate (DHEAS), which is produced in the adrenal glands. Management of adrenal PCOS can be achieved through regulating androgen production with corticosteroids and/or hormonal contraceptives [15].

2.2.4 Post-Pill PCOS

In some women, symptoms of PCOS can be triggered by the stopping of hormonal contraceptives, making symptoms of hormonal imbalance less noticeable. This type of PCOS is also called post-pill PCOS, and symptoms like irregular menses and acne can return to women who take the pill. This condition can be treated with lifestyle modifications and hormonal therapies to regulate the menstrual cycle [16].

2.2.5 Non-Classic Congenital Adrenal Hyperplasia PCOS

This form is linked to congenital adrenal hyperplasia, which impacts the production of hormones in the adrenal glands. In the case of non-classic CAH, females often have symptoms of hyperandrogenism like excessive hairiness and irregular menses, but they are less severe than in the classical form.

Testing for hormones will confirm the diagnosis, and it can be treated with glucocorticoids, preventing the surplus production of hormones [17].

2.2.6 Lean PCOS

While many women with PCOS are overweight or obese, lean PCOS refers to those of normal body weight yet still with symptoms of the syndrome. This variant can also present with insulin resistance and hormonal abnormalities similar to other types. Management may include lifestyle modifications regarding diet and exercise, and medications that treat symptoms [18].

Figure 2.2: Types of PCOS. This image categorizes different types of PCOS, including insulin-resistant, post-pill, adrenal, and inflammatory PCOS, along with dietary considerations [13].

2.3 Prevalence of PCOS

PCOS is projected to affect between 5-10% of women of childbearing age worldwide; this makes PCOS one of the most prevalent endocrine disorders. However, the prevalence of PCOS may differ depending on the population being measured. There has been a concerning increase in the prevalence of PCOS, especially in the urban population, where changes in dietary habits and physical activity levels have been observed [19]. However, the lack of awareness among doctors as well as patients has led to the undertreatment of PCOS. PCOS has been observed to affect around 15% to 22% of women of childbearing age in Pakistan [20].

2.4 Normal Mechanism of the Ovary

The ovaries play a very integral role in the female reproductive system. They play a key part in hormone secretion. The regular working of the ovaries is very important in the regulation of the menstrual cycle. The detailed processes involved in the ovaries help a person gain information regarding the changes that occur in the body during conditions like PCOS.

2.4.1 Follicular Development

Follicular development is an intrinsic part of ovarian functions, which is basically the maturation of ovarian follicles that are fluid-filled structures that harbor immature eggs. In the onset of every menstrual cycle, some of the follicles develop in size due to the release of some hormones, especially the Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH), secreted from the pituitary glands. With the development of these follicles comes the process whereby usually only one dominant follicle fully develops and is secreted through ovulation, while most of the follicles die through a process called atresia. However, in women who suffer from PCOS, this carefully regulated process is abnormal in that, instead of only one dominant follicle developing, multiple immature follicles build up, giving rise to the polycystic ovarian structure depicted through ultrasound images. This is not only infertility but also leads to the hormonal imbalance exhibited in the syndrome [21].

2.4.2 Hormonal Regulation

The regulation of the ovarian cycle is a complex phenomenon involving a balance of hormones that promote the development of follicles and ovulation. FSH is important in the stimulation of the growth of follicles in the ovary, while LH is important in the process of ovulation, which is the secretion of a mature egg from the dominant follicle.

However, in the case of PCOS patients, this delicate balance of hormones is often affected. Androgen secretion is often high in PCOS patients. This can affect the sensitivity of the ovaries to FSH. This often leads to ovulation. This is the absence of ovulation and leads to ovulatory disturbances of the menstrual cycle, a key cause of infertility among women [22].

2.4.3 Insulin Sensitivity

An important consideration within the regulation of ovulation is the role of insulin. This is because insulin has been known to increase the sensitivity of the ovulation cells of a healthy individual to FSH; hence the development and fulfillment of the full maturation of the follicle.

On the other hand, insulin resistance, which is the reduced response of the body's cells to insulin, is typical among PCOS women. Such insulin resistance may result in hyperinsulinemia, which is the production of high insulin levels within the blood.

This stimulates the ovaries to produce large amounts of androgen. Beyond disrupting the balance of the hormonal environment required for ovulation, the production of this hormone is behind the clinical manifestations of PCOS, such as the occurrence of acne and hirsutism [23].

2.4.4 Ovulation

Ovulation represents an important aspect in the menstrual cycle as it denotes the discharge of the mature ovum from the ovary as shown in figure 2.3 below, to allow the possibility of conception to take place through the process of fertilization, as it reoccurs on a monthly basis in the ordinary cycle of a woman's life.

In PCOS, the resultant imbalance in the body's hormones usually leads to an anovulatory cycle whereby the egg does not get discharged as it would in the ordinary cycle, and this represents a key factor in the infertility exhibited in women suffering from PCOS since it may be accompanied by other factors such as the irregular menses and the possibility of the patient suffering from the problem of endometrial hyperplasia as a consequence of prolonged exposure to the hormone estrogen [24].

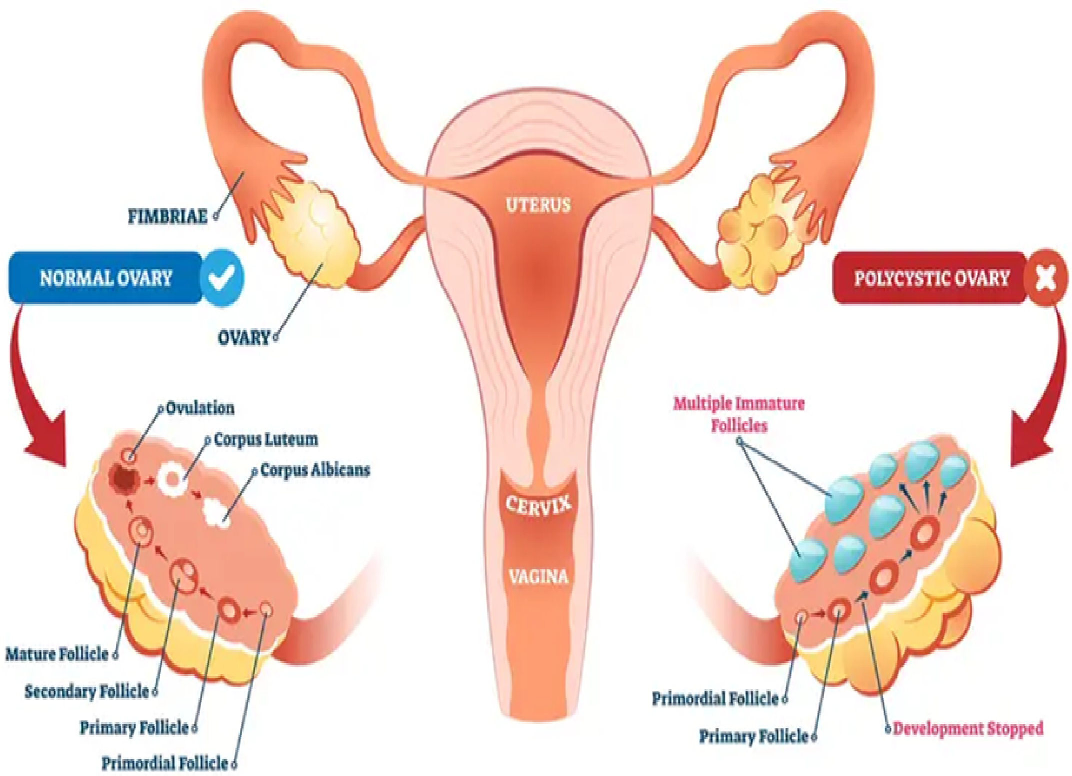


FIGURE 2.3: Difference between normal ovary and polycystic ovary. This picture portrays the contrast between the normal ovary and the polycystic ovary, showing the vast differences in the development stages of the follicles. In the normal ovary, the stages range from mature to the ovulation stage, while the polycystic ovary has several immature follicles whose development has been halted [21].

2.5 Formation of Polycystic Ovary Syndrome

Polycystic Ovary Syndrome is a complex endocrine dysfunction that occurs due to interaction of multiple genetic, hormonal, and environmental factors. Ovarian dysfunction, hyperandrogenism, and insulin resistance in PCOS are created through multiple biological mechanisms. Knowledge of molecular mechanisms of PCOS is necessary for the treatment of this disorder. An imbalance of growth factors plays an important role in the pathogenesis of PCOS.

2.5.1 Genetic Factors

Genetic predisposition is a significant contributing factor in the manifestation of PCOS. Family and twin studies indicate that there is a heritable component, while many candidate genes have been implicated for the disorder. It is believed that genes that regulate insulin signaling, androgen biosynthesis, and ovarian function have some contributions to the risk of developing PCOS. Gamma-aminobutyric acid type B, receptor subunit 2, Apolipoprotein A-1, C-reactive protein, follicle-Stimulating Hormone Receptor, and kisspeptin also have significant contributions in the development of PCOS regarding their critical roles in regulating cellular processes, follicle development and ovarian function [25]. Furthermore, genetic polymorphisms affecting inflammatory pathways and metabolic processes are found to be associated with the development of PCOS, suggesting genetic complexity in the underlying etiology of the disorder [26].

2.5.2 Hormonal Imbalances

PCOS is characterized by hormonal abnormalities, especially those of androgens, LH, and FSH. In cases of PCOS, women tend to have high production of androgens like testosterone, resulting in physical symptoms like hirsutism and acne. The excess production of androgens is mainly caused by excessive stimulation of the ovaries by LH, which tends to be high in cases of PCOS. This sharp variation between high and low production of hormones causes irregular growth of follicles, resulting in the maturation of many immature follicles, which form the cysts seen in PCOS [3].

In the regular menstrual cycle, the FSH stimulates the growth and development of the ovarian follicles, and the sudden surge in the LH value triggers the ovulation process. However, it has been perceived that there is less responsiveness of the ovaries to FSH in PCOS, which causes the ovaries not to develop properly, thus resulting in the condition of anovulation, as depicted in Figure 2.4. This augments the production of androgens and thus increases the cycle of the symptoms of the PCOS [27].

2.5.3 Insulin Resistance

Insulin resistance is a critical feature of PCOS, occurring in up to 70% of women with the syndrome. This condition decreases the ability of the body to recognize insulin, leading to compensatory hyperinsulinemia. Insulin resistance is interrelated with PCOS in several ways because high levels of insulin stimulate ovarian theca cells to overproduce androgens, further contributing to hyperandrogenism. It is related to obesity, which often comes with this kind of disorder and can be disruptive in metabolic dysfunction outright [28].

One of the biochemical pathways through which insulin resistance has been linked is alterations in the insulin signaling cascade. Impaired activation of the insulin receptor results in reduced absorption of glucose by target tissues, thus increasing blood glucose levels. Moreover, this sets off a response toward the pro-inflammatory pathway that leads to the low-grade chronic inflammation often reported among individuals with PCOS [29].

2.5.4 Inflammation

The other important component of the pathophysiology of PCOS is the presence of low-grade chronic inflammation. The level of pro-inflammatory cytokines like interleukins and C-reactive protein has been found to be higher in women with PCOS. Chronic low-grade inflammation may exacerbate the abnormalities of insulin resistance and hormonal imbalance, giving rise to a vicious cycle that perpetuates the disease. The activity of the ovaries may also be affected by the pro-inflammatory cytokines, which may interfere with the normal growth of the follicles, resulting in anovulatory cycles, which is the characteristic feature of PCOS [30].

2.5.5 Ovarian Dysfunction

An imbalance of the hormonal environment regulating ovulation and follicular growth is the core characteristic of the ovarian dysfunction found in PCOS. The accumulated number of immature developing follicles, which do not progress into ovulation, is the result of the constant stimulation of the ovaries by the excessive secretion of LH. As a result, irregular menstrual cycles, anovulation, and even infertility may be prevalent in women with PCOS. Growth factors and signaling pathways, such as the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway, contribute to the molecular events of follicular growth. The presence of cysts in the ovaries may be further complicated by the disturbances in the aforementioned signaling pathways, causing atresia of the follicles and reduced activity of the granulocytes [31].

2.5.6 Metabolic Pathways

The biochemical pathways in the case of PCOS are complicated and include dysregulation in lipid metabolism, glucose regulatory pathways, and energy. This is because the insulin resistance found in the condition leads to excess lipids in the body, particularly in the adipose tissues. This, in turn, leads to the development of obesity, thereby increasing the chances of various other disorders in the body. The condition also includes the risks associated with disorders like “cardiovascular diseases,” because the dyslipidemia linked with the condition leads to low levels of HDL cholesterol, thereby increasing the triglycerides. Moreover, the effects and responses related to the androgens in the body might affect the body composition and fat distribution. This is because the excess levels of androgens in the body lead to the accumulation of excess fat in the visceral area [32].

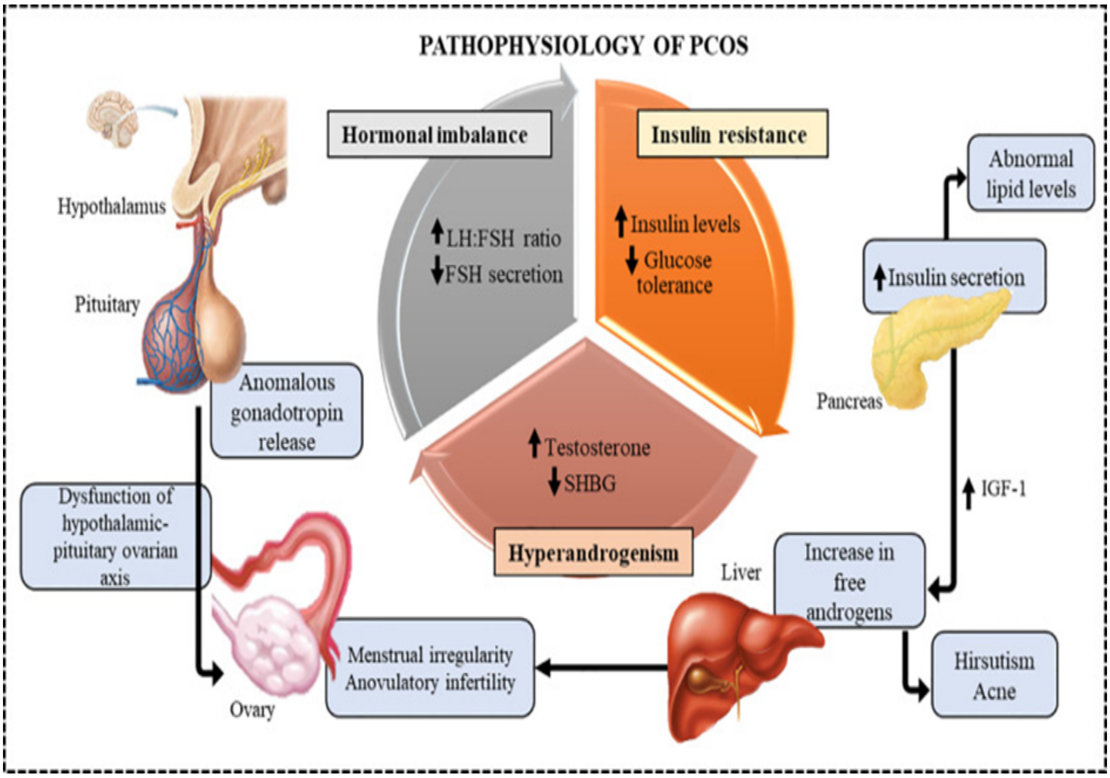


FIGURE 2.4: Pathophysiology of PCOS. This image indicates the pathophysiology of PCOS. Excess insulin secretion leads to elevated testosterone levels. This causes several conditions like irregular menses and hirsutism. Another important point taken into consideration in the image is the involvement of the hypothalamus, pituitary gland, and liver [27].

2.6 Symptoms of Polycystic Ovary Syndrome

The symptoms of polycystic ovary syndrome are diverse and might differ greatly from person to person. Hormonal abnormalities, especially those involving androgens and insulin, are frequently the cause of these symptoms and can affect various aspects of a woman's health, as shown in figure 2.5s. Understanding these symptoms is crucial for early diagnosis and effective management of the condition. One of the most prevalent symptoms is irregular menstrual cycles, manifested as oligomenorrhea, where cycles occur at intervals longer than 35 days, or amenorrhea, characterized by the absence of menstruation for several months. This irregularity is primarily due to anovulation, where the ovaries do not release eggs, thus disrupting the hormonal regulation crucial for regular menstruation [33].

Hyperandrogenism is another key feature of PCOS, resulting in elevated androgen levels that lead to physical symptoms such as hirsutism, excessive hair growth in typical male distribution areas and acne, particularly on the face, chest, and back. Alopecia, or thinning hair, may also occur due to increased sensitivity of hair follicles to these heightened androgen levels, significantly impacting self-esteem and psychological well-being [34].

Additionally, many women with PCOS experience challenges with weight gain or find it difficult to lose weight, often linked to insulin resistance, which contributes to increased abdominal adiposity. Obesity can further complicate PCOS symptoms and elevate the risk of developing other serious health conditions, including type 2 diabetes and cardiovascular disease [35].

Insulin resistance is frequently reported among individuals with PCOS, while many may not exhibit overt symptoms, it can manifest as fatigue or intense cravings for sugary foods, complicating dietary adherence [36].

Furthermore, skin changes such as acanthosis nigricans, which include dark, velvety patches of skin associated with insulin resistance and the development of skin tags, can also be prevalent [37].

Infertility remains a significant concern for women with PCOS due to ovulatory dysfunctions, emphasizing the necessity for various management strategies, including pharmacologic treatments and assisted reproductive technologies [38].

In addition, psychological ramifications manifested as anxiety and depression are common, influenced by factors like weight gain and infertility, indicating the need for a multidisciplinary approach to treatment that addresses both physical and mental health [39, 40].

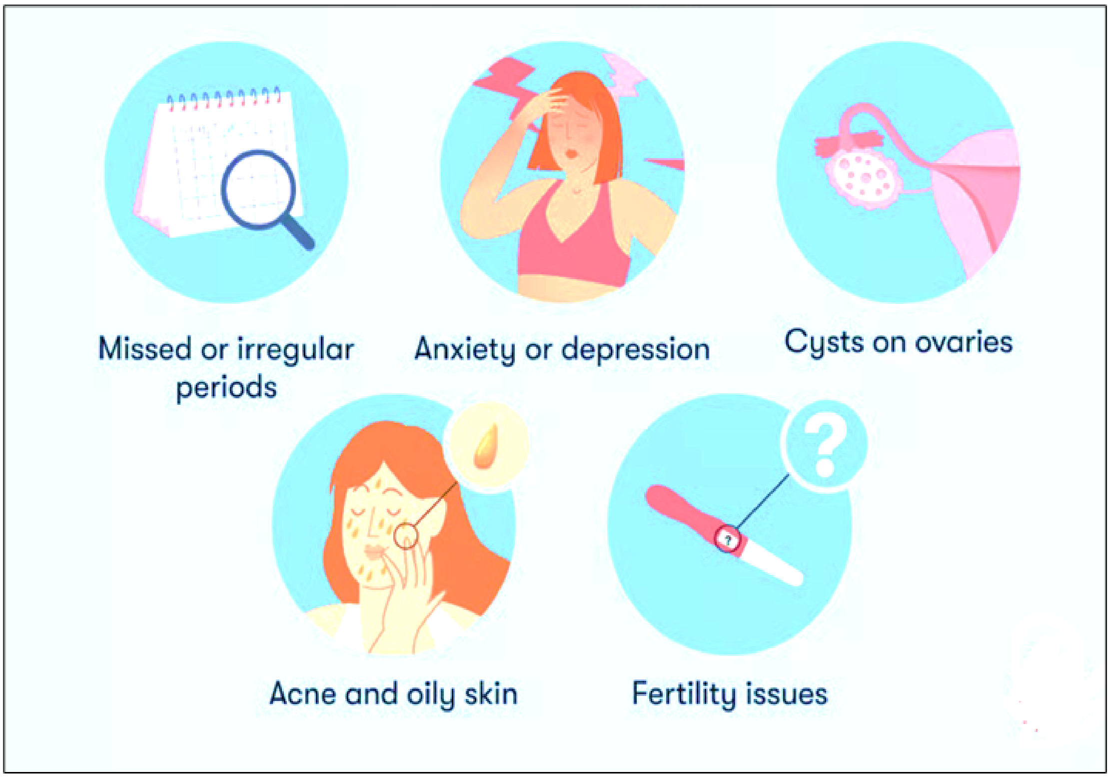


FIGURE 2.5: Symptoms of PCOS. The image depicts common symptoms and issues associated with PCOS, including missed periods, anxiety, and ovarian cysts [33].

2.7 Associated Disorders

Apart from the reproductive issues, Polycystic Ovary Syndrome has several comorbidities, which have the potential to greatly impact the health and well-being of women. These health problems pose grave threats to the well-being of the woman and make the medical treatment of PCOS even more challenging, as depicted in figure 2.6.

Insulin resistance is seen in women with PCOS, which increases the chances of developing metabolic syndrome and type 2 diabetes.

Osteoporosis, endometrial hyperplasia, and obesity are also evident, in addition to hormonal imbalance and infertility problems, along with cardiovascular and depression-related problems, as well as the potential development of non-alcoholic fatty liver disease and acne, all of which can be treated with proper management plans [41].

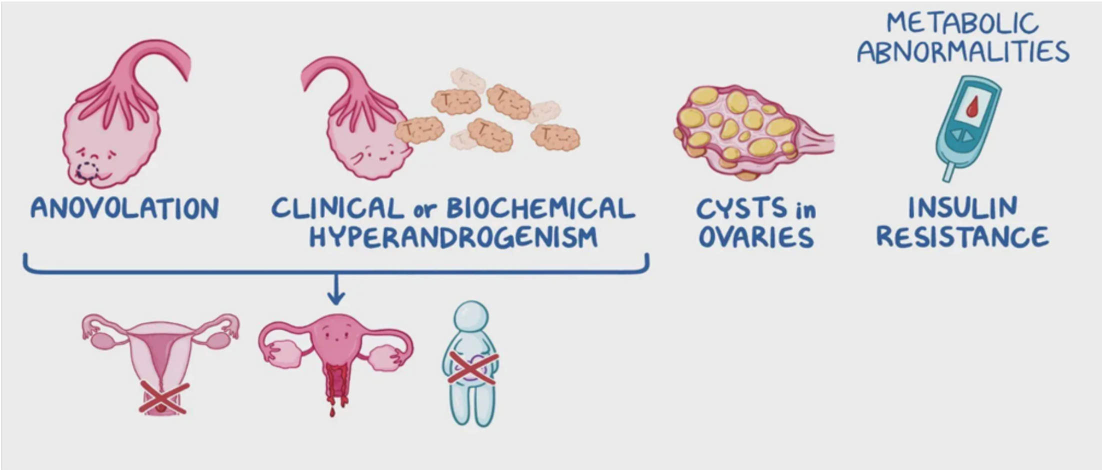


FIGURE 2.6: PCOS-associated disorders. This image illustrates key features of PCOS, including anovulation, hyperandrogenism, and the presence of ovarian cysts and insulin resistance [41].

2.8 Treatments for Polycystic Ovary Syndrome

The management of Polycystic Ovary Syndrome requires a tailored approach that considers individual symptoms, health status, and personal preferences. Some treatment options for PCOS are shown in figure 2.7. Weight loss through healthy eating and regular exercise is essential, as even modest weight reductions can improve insulin sensitivity and promote regular menstrual cycles. A balanced diet focusing on whole foods, along with physical activity, significantly alleviates symptoms and reduces the risk of metabolic complications [42]. Hormonal therapies, such as combined oral contraceptives containing progestin and estrogen, are commonly prescribed to regulate menstrual patterns and manage excessive androgen-related symptoms like hirsutism and acne. Anti-androgen medications, such as spironolactone, further address these issues, while insulin-sensitizing agents like metformin can improve insulin sensitivity and aid in reproductive health [43, 44].

For women desiring to conceive, ovulation induction is often indicated, with medications like clomiphene citrate being effective. If unsuccessful, alternatives such as letrozole or gonadotropins may be explored, along with assisted reproductive technologies when needed [45]. Furthermore, anti-inflammatory approaches, including supplements like omega-3 fatty acids and vitamin D, can enhance overall metabolic function and counteract inflammation [46]. Mental health management is essential, as PCOS is associated with psychological issues like anxiety and depression, making therapies and support networks valuable [47]. Given the multifaceted nature of PCOS, a multidisciplinary approach involving endocrinologists, gynecologists, dietitians, and mental health professionals ensures comprehensive care and optimizes the quality of life for affected individuals [48].

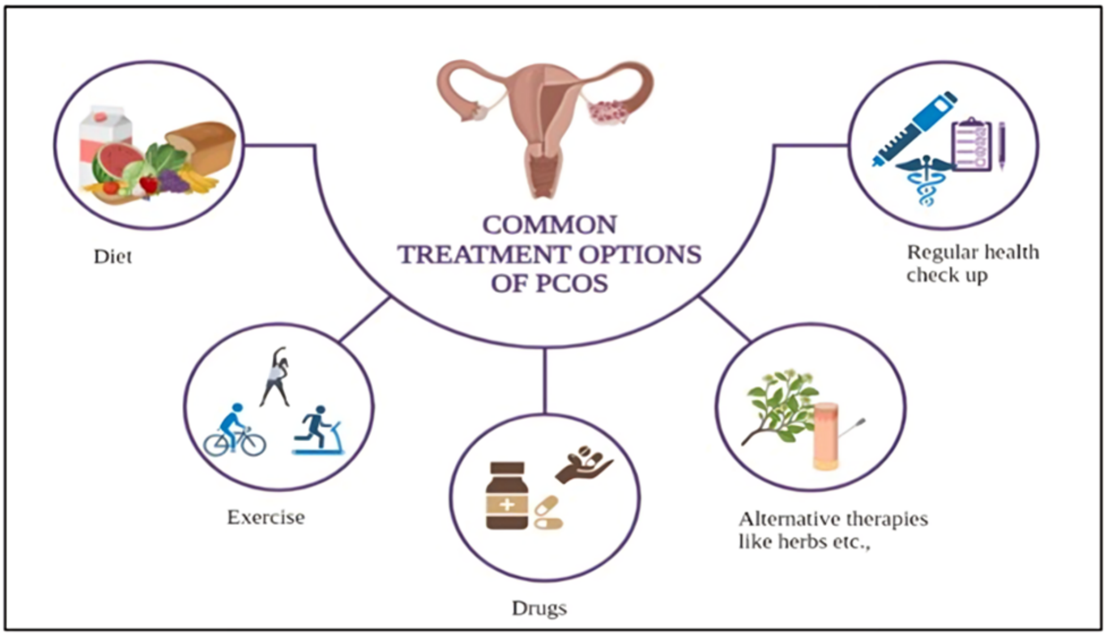


Figure 2.7: Treatment options for PCOS. This picture illustrates the treatment of PCOS, stressing the need to have a good diet, health check-ups, and exercise. It also incorporates the role of medications and alternative treatments, including herbs, in managing the condition [43].

2.9 Side Effects of Available Treatments for PCOS

Although there exist several methods for the treatment of Polycystic Ovary Syndrome that can work effectively in order to control the symptoms and maintain the quality of life, the treatments can have several side effects, as can be seen in figure 2.8. Knowledge of the side effects is imperative in the effective management of the condition.

Hormonal medications, such as combined oral contraceptives, are commonly prescribed to regulate menstrual cycles and mitigate symptoms; however, they may lead to hormonal imbalances that can result in mood swings, weight gain, decreased libido, and potential breakthrough bleeding in the initial months of use [49].

Similarly, insulin sensitizers like metformin, while effective in lowering diabetes risk, often cause gastrointestinal issues, including diarrhea, nausea, and stomach cramps, which may compel some patients to discontinue their use.

Gradual dosage increases and regular monitoring can help manage these side effects. Furthermore, weight fluctuations can occur, as certain hormonal treatments can lead to fluid retention and increased appetite, while others like metformin, may facilitate weight loss [50].

In addition to physical side effects, psychological effects are significant in the treatment of PCOS. Hormonal therapies can exacerbate emotional disturbances, including depression and anxiety, necessitating ongoing emotional support from healthcare professionals. Skin reactions may also arise from anti-androgen medications like spironolactone, causing dryness, rashes, or peeling, which may require additional skincare management [51].

Patients may experience menstrual irregularities such as amenorrhea or heavy flow despite treatment intentions, and variability in insulin sensitivity can lead to hypoglycemia if dietary controls are not carefully managed [52].

For women undergoing fertility treatments, side effects such as ovarian hyperstimulation syndrome (OHSS) can occur, necessitating close supervision [53].

Overall, the experience of managing PCOS can be emotionally taxing and may lead to body image issues and increased anxiety, highlighting the need for comprehensive emotional support from medical professionals [54].

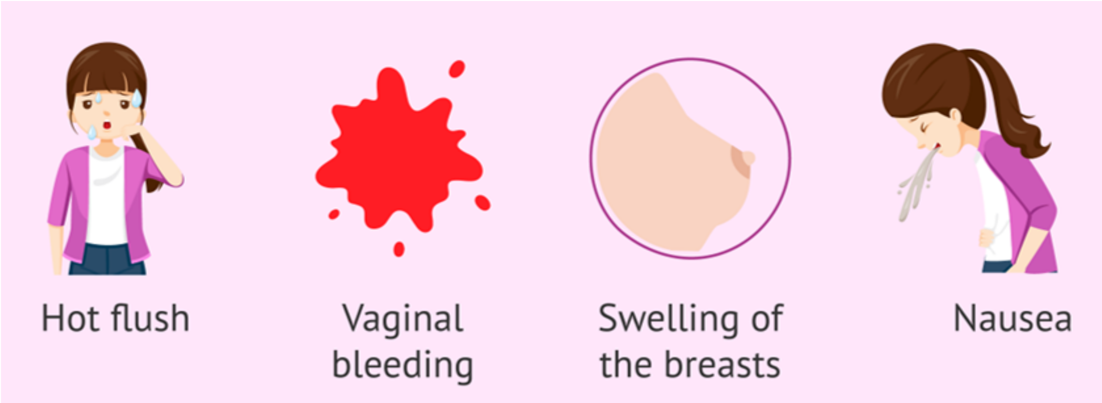


FIGURE 2.8: Side effects of available treatments. The image displays common symptoms associated with hormonal changes, including hot flashes, vaginal bleeding, breast swelling, and nausea [49].

2.10 Herbal Treatments for PCOS : Potential Benefits and Bioactive Metabolites

A common endocrine condition that affects many women worldwide, polycystic ovary syndrome is characterized by hormonal abnormalities, insulin resistance, and a number of reproductive and metabolic problems. While conventional treatments such as hormonal therapies, lifestyle modifications, and medications are commonly used to manage PCOS, there is an increasing interest in complementary therapies, particularly those derived from herbal sources. Many plants contain bioactive metabolites that may provide protective or therapeutic effects against the symptoms of PCOS [55]. Several herbs and their active compounds have been investigated for their potential benefits in alleviating this condition, as shown in figure 2.9.

Due to the active component, curcumin, *Curcuma longa*, or turmeric, has been among the plants tested extensively for any possible connections to PCOS. Due to the powerful anti-inflammatory and antioxidant properties, curcumin could potentially reduce the chronic inflammation that can be a common symptom of, as well as be associated with, PCOS. Curcumin can improve sensitivity to insulin levels, as well as regulate menstruation, by interfering with diverse hormonal pathways, as has been shown by diverse studies. This herb works well as a dietary supplement for treating symptoms of PCOS, as curcumin has been proven to inhibit the growth of ovarian cells as well as possibly reduce the risk of metabolic syndromes, besides functioning as a complementary agent that can improve cognitive health due to the ability to boost such functions [56].

Mentha spicata, or spearmint, is another natural remedy that has been noted for its use as a means of dealing with androgenic effects. The use of spearmint tea has been considered effective for decreasing hirsutism among females suffering from PCOS due to the low levels of testosterone induced by spearmint consumption. The active ingredient, rosmarinic acid, has been observed to be a potential source of antioxidants within spearmint, thus helping deal with endocrine imbalances that may cause issues like excess hair growth among females suffering from PCOS [57].

Cinnamomum verum, also known as cinnamon, has shown potential in dealing with symptoms related to PCOS. Researchers have revealed that cinnamon has the capacity to increase the sensitivity of the body to insulin and even help in regulating the woman's monthly cycle. The active elements of cinnamon, including cinnamaldehyde, help increase the ovulation process in females. Consume cinnamon daily, promoting the body's hormonal balance, which has numerous benefits in terms of boosting the body's metabolism, thereby being a simple but efficient way to manage the symptoms related to PCOS [58].

Vitex agnus-castus, vitex, and chaste tree are some common names for it, one of the oldest herbs traditionally known for the treatment of irregular menses and symptoms of hormonal imbalance. It is assumed that compounds found in vitex help regulate the pituitary gland by assisting in the regulation of the secretion of luteinizing hormone and thereby help decrease an elevated level of prolactin in blood. It helps in regulating menses and symptoms caused by PCOS, thereby making vitex an excellent herbal treatment for women looking for an alternative to modern medicine [59].

Another indian plant with a root that can be used as a vegetable, *Lepidium meyenii* or 'maca,' has adaptogenic functions. Maca has been proposed to improve fertility and hormonal control in women with PCOS, based on studies. Maca's bioactive compounds, such as glucosinolates and other amino acids, have been suggested to improve sexual desire and ovarian functions, according to modern research. A holistic approach to managing the condition and improving overall reproductive health can possibly be offered through the use of maca in the diet [60].

Fenugreek seeds, also known as *Trigonella foenum-graecum*, have also been studied for potential benefits in the management of PCOS. Fenugreek is rich in soluble fibers and has been shown to improve the sensitivity of the tissues to insulin and manage blood sugar levels. In addition, its high phytoestrogen content makes fenugreek an appropriate supplement in the diet of women diagnosed with PCOS, offering relief from excess hair growth and irregular menstrual cycles [61].

Urtica dioica, also known as nettle, is yet another herb that can potentially provide benefit to women with PCOS. The anti-inflammatory properties of nettle leaves, rich in vitamins as well as minerals, can work towards reducing the inflammation associated with this disease.

This herb can work very well as a supplement to various treatments available for PCOS because it can aid in achieving balance in hormones as well as improve reproductive health [62].

FIGURE 2.9: Herbal treatments for PCOS. The image highlights the role of herbal treatments for PCOS. Herbal teas and extracts are examples of natural therapies that may help reduce symptoms and maintain hormonal balance [55].

2.11 *Ranunculus hirtellus*

It is also known as softly hairy buttercup or piryali is a perennial herb that grows mainly in the Himalayas at altitudes between 3000 to 5000 meters. It grows to about 4.5 to 27 cm high with softly hairy stems and leaves. The basal leaves are 3 parted or deeply lobed, the flowers are bright yellow, about 1-1.5 cm diameter, with hairy sepals underneath. It flowers from May to July, thriving in subalpine habitats across areas such as Kashmir, Tibet, and western China. Its characteristics include having fibrous roots, with puberulent stems, and its habitat in high-altitude mountainous terrain as indicated in figure 2.10 [4].

FIGURE 2.10: *Ranunculus hirtellus.* The image showcases *Ranunculus hirtellus*, a flowering plant characterized by its delicate petals and vibrant yellow centers [4].

2.11.1 Morphology and Taxonomy

Ranunculus hirtellus can be described as a perennial herb, growing between 30 to 50 centimeters tall, with a strong upright growth habit. The plant has a unique structure whereby the leaves are deeply lobed, divided, and palmately arranged, have a dark green color, and can grow between 5 to 15 centimeters long, attached to the stem by a short petiole that ensures a large surface area, thereby facilitating photosynthesis, hence effective growth of the plant.

The flowering stage of *Ranunculus hirtellus* occurs in late spring to early summer, when the plant grows cup-shaped flowers that attract many people not only by their appearance but also because they attract many pollinators. Every cup-shaped flower in the plant has five to seven petal-like sepals that range in color from yellow, but in some cases, the colors may vary depending on the environmental conditions. The petals are also slightly cup-shaped, creating a bowl that holds nectar, thereby attracting many pollinators. Following fertilization, the blooms grow into a cluster of tiny, dry fruits called achenes that hold individual seeds. The achenes are usually scattered by water and wind, which helps the plant spread in its native environment. The plant grows well in soils that drain properly and is often found in meadows, grasslands, and other open areas, showcasing its adaptability to various environmental conditions.

Taxonomically, *Ranunculus hirtellus* falls into the genus Ranunculus; this genus consists of a diverse group of flowering plants known for their ornamental and medicinal values. It is placed under the family Ranunculaceae, which is widely famous for its herbaceous plants and some shrubs. The scientific classification of *Ranunculus hirtellus* is given below:

i. Kingdom: Plantae
ii. Phylum: Tracheophyta
iii. Class: Magnoliopsida
iv. Order: Ranunculales
v. Family: Ranunculaceae
vi. Genus: Ranunculus
vii. Species: *Ranunculus hirtellus*

The genus Ranunculus includes many species, some of which are economically important and used in traditional medicine and horticulture. The genus is commonly divided into sections based on morphological, ecological, and geographical features of the plants. *Ranunculus hirtellus* belongs to a section that includes several other species based on similar floral structures and growth habits [63].

2.11.2 Traditional and Ethnomedical Uses of *Ranunculus hirtellus*

However, there are only a few scientific studies on *R. hirtellus* and learning from the Ranunculus family, there are important morphological and phytochemical properties associated with this plant, including potential applications for the treatment of Polycystic Ovary Syndrome. Phytochemically, Ranunculus contains abundant bioactive compounds with great therapeutic uses, including strong anti-inflammatory, hormonal, and antioxidant properties, including bioactive components like flavonoids, saponins, triterpenoids, and polyphenols. In addition, several plants from the Ranunculus family have long been explored and utilized in folk medicine for years to treat inflammatory, pulmonary, and intestinal disorders, establishing their significance and usefulness to human health [64]. That being said, the therapeutic uses of these plants, their ability to treat symptoms associated with inflammation and an imbalance in the metabolic system, imply their potentially useful role for *R. hirtellus*, aiming to treat symptoms associated with the metabolic and endocrine disorder, Polycystic Ovary Syndrome. With the existing collective bioactivity of the existing bio-phytocompounds in the Ranunculus family, *R. hirtellus* represents an important and novel therapeutic target for improving health in women with PCOS.

Ranunculus hirtellus has been used in traditional medicine for a very long period of time in diverse cultures, especially in countries where the species is native. This is because its medicinal values have been acknowledged for many centuries, and thus its practices have been an essential part of alternative medicine. This herb has been in extensive use for its potential health benefits, most especially in treating cases of the respiratory, digestive, and inflammatory systems. Its analgesic and anti-inflammatory properties have been put to use in traditional European medicine. In order to treat inflammation, the herb has been given topically in the form of poultices for the relief of pain associated with arthritis and rheumatoid conditions. Leaves and flowers of the herb have been crushed together with some other natural ingredients for the determination of remedies for the reduction of inflammation and thus for promotion of healing in particular areas. *Ranunculus hirtellus* has been used in traditional medicine for a very long period of time in diverse cultures, especially in countries where the species is native. This is because its medicinal values have been [65].

This herb can be applied to the treatment and management of gastrointestinal issues owing to its anti-inflammatory components. Certain cultures prepare various infusions from the leaves of this herb to alleviate swelling, indigestion, and pain associated with the abdomen. Its high concentration of bioactive components such as flavonoids and saponins, contributes to this by potentially increasing the health and efficiency of the digestive systems. Traditional Chinese medicine would see the utilization of the seeds and roots of *Ranunculus hirtellus*. This herb has been traditionally used owing to its supposed health merits regarding the respiratory system. Its seeds, commonly known by the name "bitter almonds," contain the supposed health benefit attributes of being expectorants to alleviate the symptoms of coughs and other respiratory tract diseases. This herb is commonly applied to the preparation of various herbal drugs aimed at the treatment and management of bronchitis and colds due to its superior health advantages regarding the increase and promotion of the health of the lungs [10].

Most recently, the putative anticancer activity of *Ranunculus hirtellus* is highly promising; for example, the extracts of the plant suppress the proliferation of some cancerous cell lines, but the exact details of the mechanism are yet to be deciphered. Such findings align with the traditional use of the plant in promoting general health and well-being and point toward its importance in holistic healthcare. In addition to its uses for medicine, it has been part of many folk remedies and cultural traditions. It is considered a plant with cooling properties in some regions, which is helpful in addressing inflammatory diseases and fevers. This is evident through the use of the plant in tea form. *Ranunculus hirtellus* has several uses in traditional medicine; apart from being a nutritional plant, the fruit of the plant has been used in the treatment of diverse diseases. The use of *Ranunculus hirtellus* in traditional medicine as a means of improving the health of the skin and the eyes is attributed to the high vitamin and antioxidant levels in the plant. Additionally, the use of the plant in the treatment of conditions such as constipation and indigestion showed the many benefits of the plant [66].

2.11.3 Bioactive Compounds in *Ranunculus hirtellus*

Apart from the conventional medicinal uses, *Ranunculus hirtellus* also gains importance because of the high presence of bioactive compounds, as shown in Figure 2.11, that could serve as an additional therapeutic component, particularly in managing symptoms of Polycystic Ovary Syndrome. The bioactive compounds demonstrate multiple bioactivities, including antioxidant, anti-inflammatory, and hormonal regulation, and these are imperative in managing the multiple symptoms associated with PCOS.

2.11.3.1 Flavonoids

Flavonoids constitute one of the most important classes of bioactive compounds in *Ranunculus hirtellus*, with strong antioxidant and anti-inflammatory properties. Of particular interest in the flavonoids is the role of quercetin in suppressing the proliferation and promoting the apoptosis of cancer cells, and thus its importance in cancer prevention activities can never be overstated. In additional benefits, quercetin has the ability to relieve oxidative stress by preventing the formation of free radicals, thus offering protection from damage. This particular benefit will be valuable for women with PCOS, considering that oxidative stress is one of the causative agents in the development of the condition. According to studies, quercetin can be effective in regulating associated metabolic problems such as insulin resistance and weight gain that occur in PCOS [67].

2.11.3.2 Saponins

Another class of active compounds present in *Ranunculus hirtellus* are saponins. These possess immune-stimulant and anti-inflammatory actions, thus being very useful in patients with PCOS. Saponins can regulate blood sugar levels, improve insulin sensitivity and reduce the risk of type 2 diabetes, a frequent complication of PCOS. Moreover, saponins have been reported to be used for digestive health, further promoting overall well-being [68].

2.11.3.3 Triterpenes

The triterpenes present in *Ranunculus hirtellus* are responsible for anti - inflammatory and anticancer properties. These agents might act as cancer prevention agents because it was shown that this agent modifies a series of signaling pathways which are implicated in cell division and death. Triterpenes form an important part of herbal medication due to their anti-inflammatory role in reducing pain and inflammation associated with ovarian dysfunction in women with PCOS [9].

2.11.3.4 Polyphenols

Ranunculus hirtellus contains high amounts of polyphenols, which are widely recognized for their ability to counteract oxidative stress, an important contributing factor in the pathophysiology of PCOS. Polyphenols are believed to work towards improving metabolic function and protect various cellular components from damage. They also help to regulate hormonal imbalances and thereby work towards improving fertility and managing menstruation, as a result of their ability to reduce oxidative stress. Moreover, polyphenols also contribute towards improving the health of the cardiovascular system, thereby helping women who are predisposed to cardiovascular diseases because of PCOS [69].

2.11.3.5 Essential Oils

Many bioactive compounds possess medicinal properties, some of which could be extracted from the essential oils obtained from the *Ranunculus hirtellus*. The antibacterial and anti-inflammatory properties in the oils might help alleviate the presence of PCOS symptoms, which could include infections and irritations in the skin. The relaxation effects associated with the essential oils might help alleviate the general well-being, perhaps reducing stress and anxiety that impacts many women who have PCOS [9].

2.11.3.6 Vitamins and Minerals

Several key vitamins and minerals that play an important role in general health can be derived from *Ranunculus hirtellus*. For example, vitamin C aids in skin care and works well as an antioxidant in the body by improving its antioxidant properties. It is vital in the synthesis of collagen and assists in treating hormonal conditions like hirsutism and acne, which can be associated with PCOS. The herb is also rich in minerals that include magnesium, which is critical in maintaining good cell function, considering that it participates significantly in DNA synthesis, which is vital for maintaining hormonal balance [10].

2.11.3.7 Dietary Fiber

Apart from the bioactive compounds found in *Ranunculus hirtellus*, this herb also contains fiber, which plays a crucial role in supporting digestion. This fiber helps stimulate the evacuation of bowels, thereby helping with weight issues, an issue that affects most women with PCOS. This fiber also helps with the clearance of toxins and possible carcinogenic compounds from the body, thereby reducing the development of cancer within the colon, an aspect that affects most women with PCOS.

2.11.3.8 Alkaloids

The potential uses in medicine of *R. hirtellus* might be linked to its alkaloids. These are biological compounds that contain nitrogen and display different biological activities, including analgesic and anti-inflammatory effects. They can contribute to the suppression of pain and discomfort symptoms, benefiting women with PCOS, as they would be able to relieve their symptoms to some extent [64].

2.11.3.9 Phenolic Acids

Phenolic acids, another class of bioactive compounds present in *Ranunculus hirtellus*, are known to possess very high antioxidant activity and could be useful in combating oxidative stress. These substances have been proven to inhibit cancer cell growth and ensure proper cell function. They are very useful in PCOS patients because of their anti-inflammatory components, which ensure general health and well-being [64].

2.11.3.10 Fatty Acids

Ranunculus hirtellus health benefits can also be attributed to the fatty acids present in it. Due to the known anti-inflammatory properties, Omega-3 and Omega-6 fatty acids can be beneficial in reducing the inflammatory symptoms associated with PCOS. These two fatty acids are known to improve the hormonal and cardiovascular systems, which are essential in managing PCOS symptoms [70].

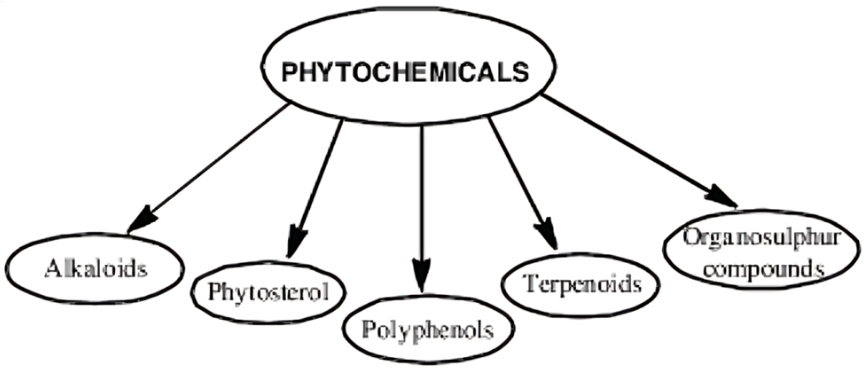


FIGURE 2.11: Types of phytochemicals. The image shows the classification of phytochemicals, which focuses on the important classes of phytochemicals like alkaloids, phytosterols, polyphenols, terpenoids, and organosulfurs. Phytochemicals are known for their numerous health benefits and for their role as plant protectants [67].

2.11.4 Medical Uses of *Ranunculus hirtellus*

Ranunculus hirtellus has a great history of traditional medicinal use, and research into its pharmacology is ongoing. This plant is valued for its broad profile of bioactive components, which substantiate its multiple health benefits, particularly regarding digestive issues, inflammatory diseases, and other disorders.

2.11.4.1 Digestive Health

Ranunculus hirtellus is highly beneficial in terms of digestive health. The bioactive constituents present within the plant can help relieve gastrointestinal distress and move the bowels smoothly.

Its anti-inflammatory properties soothe the digestive tract and make it beneficial for people with bloating, constipation, and indigestion. Infusions derived from the leaves themselves form part of traditional remedies, which allegedly enhance gut function and support the process of digestion. [9].

2.11.4.2 Anti-inflammatory Properties

Ranunculus hirtellus contains active anti-inflammatory compounds that can be used for the treatment of numerous inflammatory conditions. The active compounds of the plant have been shown to be able to decrease inflammation in tissues associated with inflammatory conditions like arthritis and muscle strain.

The species has been appropriately documented in traditional medicine for its ability to relieve inflammation and pain when used in creams and pastes [10].

2.11.4.3 Respiratory Health

In conventional medicine, extracts of *R. hirtellus* are used to promote respiratory health. It is thought to be an expectorant, helping to relieve coughs and facilitate the ejection of mucus from the respiratory tract.

This makes it a potential remedy for those with respiratory ailments like bronchitis and cough. It also enhances respiratory functions and, therefore, could ensure respiratory health [71].

2.11.4.4 Antioxidant Effects

Rich in antioxidants, *Ranunculus hirtellus* is useful in fighting oxidation within the body. The antioxidants work by neutralizing the effects of free radicals, which protect the cells within the body and thus deter chronic diseases like some cancers. It is worth noting that the antioxidant properties in *Ranunculus hirtellus* also have significant applications in the prevention of PCOS, which is spurred by oxidation within the body [72].

2.11.4.5 Immune Support

The bioactive compounds found in *R. hirtellus* have the capacity to increase the potency of the immune system. The herbal plant helps in fighting diseases by increasing the powers of the immune system. The plant has been traditionally used to increase the immune and natural defenses of the body by consuming teas or extracts, which helps in improving overall health, especially during flu seasons [9].

2.11.4.6 Skin Health

Because of its anti-inflammatory and healing properties, *Ranunculus hirtellus* is traditionally used to treat different skin conditions. To soothe the irritation, promote the healing process, and reduce inflammation, the leaves and flowers can be applied directly to the area. These methods can be considered the natural answer to the currently available drugs when applied to small skin injuries, rashes, or irritation of the skin [73].

2.11.4.7 Potential Anticancer Properties

Preliminary reports suggest that some bioactive compounds in the *Ranunculus hirtellus* species could possess anti-cancer properties. It has been indicated that the use of plant extracts results in the induction of apoptosis and the prevention of the progression of certain cancer cells. There is great potential in the use of *Ranunculus hirtellus* as an adjuvant for the treatment of cancers, regardless of the need for further experimentation to confirm the findings [74].

2.11.4.8 Antimicrobial Activity

The plant can possess antimicrobial properties, which can be very useful in the prevention of infection. In traditional medicine, the different parts of the plant *R. hirtellus* can be utilized to treat wounds and dermatological infections. This is due to the natural capacity to resist the pathogenic agents.

This shows that the plant can be utilized to prepare drugs to aid in the promotion of healing and the inhibition of infection [64].

2.11.4.9 Hormonal Balance

Ranunculus hirtellus can be used as a means of regulating hormonal issues, making it very useful for females experiencing issues related to conditions like PCOS.

The ability of the plant to regulate hormones can be a natural means by which females can control issues like irregular menstruation, as well as issues like hirsutism and mood swings, among others, associated with hormonal disorders [75].

2.11.4.10 Pain Relief

Ranunculus hirtellus is also known as buttercup weed. This is employed in the treatment of various forms of pain owing to its analgesic properties. Its anti-inflammatory component is also useful in the alleviation of any form of discomfort that comes along with joint pain, headaches, and sore muscles. This is especially useful for people who experience relief without the side effects of prescribed medications [76].

2.11.4.11 Cardiovascular Health

Recent research shows that bioactive compounds found in *Ranunculus hirtellus* plant extracts may boost heart health. The plant may be used to improve blood flow and prevent any cardiac-related issues by decreasing any signs of stress on blood cells due to oxidation. The plant may be embraced as part of a healthy cardiac diet due to its ability to boost overall metabolism [77].

2.11.4.12 Neuroprotective Effects

It is proposed by some studies that *Ranunculus hirtellus* bioactive agents could possess neuroprotective abilities to promote the well-being of the brain cells. Because these agents can decrease the level of neuronal cells' inflammation and oxidative stress, they could also help to reduce the prevalence of neurodegenerative diseases. This potential attribute underlines the importance of the *Ranunculus hirtellus* to promote overall cognitive well-being [78].

Although *Ranunculus hirtellus* has some encouraging potential in treating health issues, however, in order to explicitly establish the link between its potential and the specific proteins associated with polycystic ovarian disease, further research is needed. The previous studies have been more inclined towards overall health aspects rather than pointing towards any interaction between those specified proteins and the plant. The goal of the current research work is to explore potential PCOS-fighting phytocompounds in the *Ranunculus hirtellus* herb that can help in smooth ovary functioning and overall wellness of women affected by polycystic ovarian disease.

Chapter 3

Materials and Methods

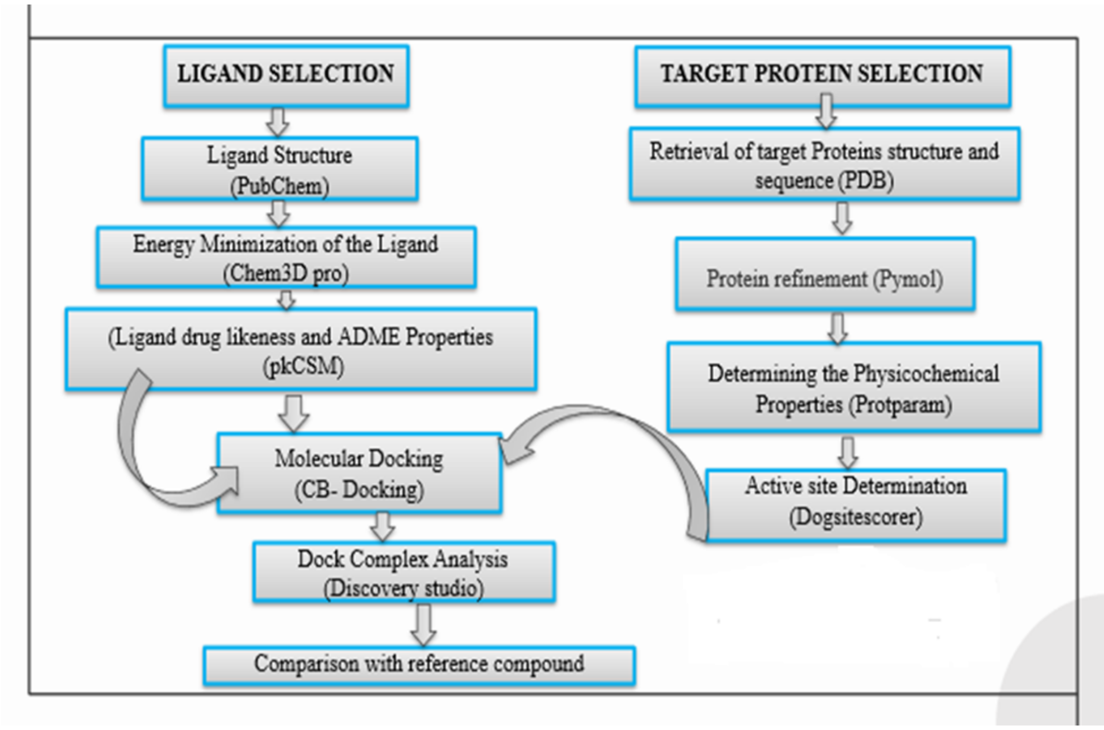


FIGURE 3.1: Flow chart of methodology

3.1 Selection of Target Proteins

The choice of specific proteins for polycystic ovary syndrome study centers on their important function for the regulation of cell processes, development, as well as ovulation functions. Gamma-aminobutyric acid type B, receptor subunit 2 (GBR2)

[79], Apolipoprotein A-1 (ApoP-1) [80], C-reactive protein (CRP) [81], Follicle-Stimulating Hormone Receptor (FSHR) [82], and kisspeptin [83] were selected for their significant contributions in the development of PCOS.

GBR2 abnormal levels can affect the release of gonadotropin-releasing hormone (GnRH). Apolipoprotein A-1, when differentially expressed in follicular fluid, impacts cellular processes. Elevated levels of CRP indicate chronic low-grade inflammation. FSHR abnormalities affect follicle development and ovarian function.

By interfering with the hypothalamic-pituitary-gonadal axis, kisspeptin contributes significantly to PCOS by causing aberrant GnRH pulses, increased LH/FSH ratios, hyperandrogenism, and anovulation, which ultimately result in problems with fertility. When taken as a whole, these proteins are crucial targets for comprehending and possibly treating PCOS.

3.2 Proteins 3D Structure Prediction and Refinement

The 3D structures of target proteins were predicted through PDB (https://www.rcsb.org/). The FASTA sequences were also retrieved using the same database [84].

PyMOL (https://pymol.org/) is an open-source molecular graphics application widely used worldwide to examine and portray various proteins, small molecules, nucleic acids, electron densities, and varying surfaces, as well as trajectories.

It is also used for editing the molecules, tracing the ray, and also to make animations and movies. This is software that is based on Python and also contains many plugin tools to enhance its use and facilitate drug targeting and designing by the use of PyMol software.

The excess components linked to the protein must be deleted after downloading the protein's structure which was done by the use of an open-source system PyMol [85].

3.3 Analysis of Physicochemical Properties of Target Proteins

The function of the proteins is significantly influenced by their physicochemical characteristics. ProtParam expasy tool (https://web.expasy.org/protparam/) was used for the prediction of these features of target proteins.

ProtParam was used to calculate the number of negatively charged residues (Asp+ Glu) and positively charged residues (Arg+ Lys), theoretical pI, molecular weight, aliphatic index, grand average of hydrophobicity, instability index, Ext coefficient (with Cys) and Ext coefficient (excluding Cys) [86].

3.4 Active Site Identification

The area in which the target protein's active site is located is where the ligand exhibits the greatest or maximal interaction with the protein. Amino acids have a major role in the ligand-protein complex building process.

Dogsitescorer (https://proteins.plus/help/dogsite) software was used for the detection of protein binding pockets [87].

3.5 Selection of Active Metabolic Ligands

After an extensive literature review, approximately among fifty identified compounds, those phytocompounds from *Ranunculus hirtellus* were selected that have previously shown some potential medicinal properties. These include methyl ester, 2,4-di-tert-butylphenol, turmerone, succinic acid and phytyl.

These compounds exhibit strong antioxidant, antidiabetic, anti-inflammatory, and lipid-regulating activities, which are crucial in mitigating PCOS pathophysiology [4].

3.6 Retrieval of Chemical Structure of Ligands and Energy Minimization

PubChem (https://pubchem.ncbi.nlm.nih.gov/) is the world's largest repository of easily accessible chemical information databases. So the chemical compounds that were selected as potential ligands were taken from the PubChem database in SDF format.

If the selected ligand structure is not available, then our next attempt would be to download the canonical smiles from PubChem and then insert them in the software ChemDraw to obtain the 3D structure [88].

Ligand's energy was minimized by using Chem3D Pro. It is a necessary step to refine the ligands before performing docking; otherwise, there will be unreliable docking scores [89].

3.7 Virtual Screening and ADMET Analysis of Ligands

An essential criterion for determining whether ligands are likely to be drugs is the Lipinski rule. Certain chemicals are likely to be utilized as active pharmaceuticals in humans if they adhere to the lipinski rule of five. pkCSM (https://omictools.com /pkcsm-tool) is an online tool that helps to check whether ligands obey lipinski rule or not.

According to these rules, the log P value, rotatable bond and hydrogen bond donors should be in the range of five. While the hydrogen bond acceptors should be limited to ten, and the molecular weight should be below five hundred grams [90].

After filtering the ligands by applying the lipinski rule, the next step of the study was the prediction of pharmacokinetic and toxicity properties. The weak candidates of the drug would be eliminated during ADMET analysis [91]. The remaining candidates can be selected as potential drugs against the disease.

3.8 Molecular Docking and Analysis of Docked Complexes

For performing the molecular docking between the protein and the ligand, CB-dock (Cavity detection guided blind docking) was used. CB-dock (http://clab.labshare.cn/cbdock/blinddock) finds the sites of docking automatically. CB-Dock is a method of protein and ligand docking that indicates the sites of bonding, the size, and the center calculated [92].

The box size is adjusted according to the ligand and then docking is performed. The docking is performed through AutoDock Vina. Its accuracy ratio is greater because the docking process is more focused on cavity binding. We uploaded the proteins to do docking, using the 3D structure in pdb format and the ligand's 3D structure in SDF format.

After this, docking is performed, the result would be 5 different poses of interaction. To select the best pose, we would look at the lowest docking score which is given in KJ/m-1 CB- Dock will provide an interactive 3D visualization of results in 5 different poses. Based on the lowest vina score expressed in (kJ/m1), the optimal position was chosen [93].

To interpret docking findings, the interaction between the ligand and the protein's active pockets was calculated. Different types of interactions were examined including hydrophobic and hydrogen bonding. Discovery studio 2025 Client was used to analyze the protein-ligand interactions. The protein-ligand interactions for the designated ligands in the PDB file are automatically schematically diagrammed by this application [94].

3.9 Lead Compound Identification

The most active inhibitor was found after a thorough examination of docking scores, pharmacokinetic studies, toxicity features, and protein and ligand interactions. Our lead compound was the one that followed all these parameters.

3.10 Reference Drug Selection

The purpose of this step is to identify the commercial drug that are already in use for PCOS therapy. The Drug Bank (https://go.drugbank.com/) database was used for this purpose because it provides details about drugs and their pathways [95].

3.11 Comparison between Lead Compound and Reference Drug

Docking values, molecular interactions, and pharmacokinetic features were compared between the reference drug and the suggested lead molecule.

Chapter 4

Results

4.1 3D Structure Prediction and Refinement of Selected Proteins

3D structure of target proteins i.e. GRB2, apolipoprotein A-1, CRP, FSHR and kisspeptin was taken from PDB under PDB IDs 4F11, 3K2S, 1GNH, 8I2H and 8XGS respectively. Using PyMol, the protein structures were refined by eliminating any ligands and water molecules. To obtain stable conformation the absent polar hydrogens were added, and other atoms were removed to prevent overlaps and the modified file was saved in PDB format. The refined structures of target proteins are shown in figure 4.1 below.

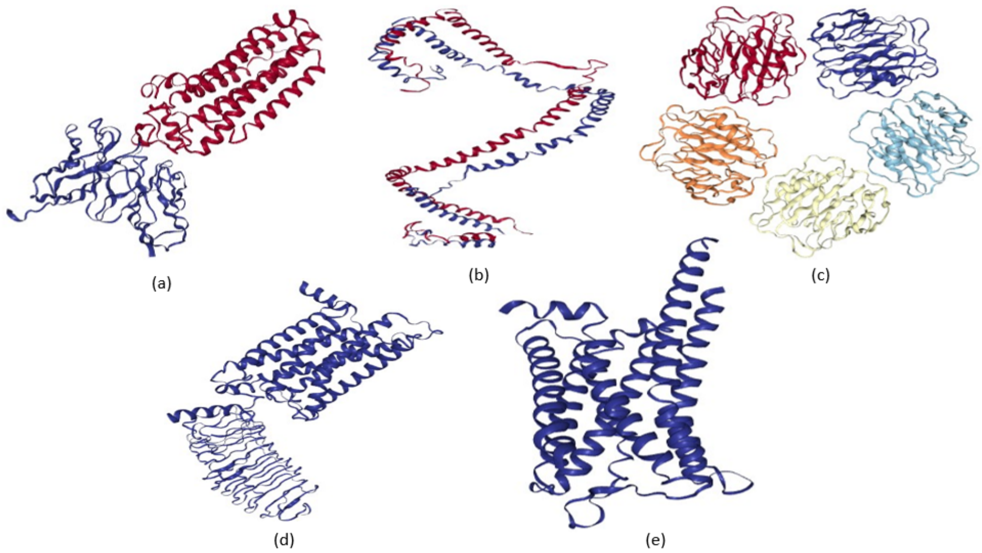


FIGURE 4.1: Structures of refined target proteins (a) GRB2 (b) Apolipoprotein A-1 (c) CRP (d) FSHR (e) Kisspeptin

FASTA sequences were also obtained by using PDB under the same IDs given above and shown in figure 4.2.

```
>4F11_1|Chain A|Gamma-aminobutyric acid type B receptor subunit 2|Homo sapiens (9606)
WARGAPRPPPSSPPLSIMGLMPLTKEVAKGSIGRGVLPAVELAIEQIRNESLLRPYFLDLRLYDTECDNAKGLKAFYDAIKYGPNHLMVFGGVCPSVT
SIIAESLQGWNLVQLSFAATTPVLADKKKYPYFFRTVPSDNAVNPAILKLLKHYQWKRVGTLTQDVQRFSEVRNDLTGVLYGEDIEISDTESFSNDPC
TSVKKLKGNDVRIILGQFDQNMAAKVFCCAYEENMYGSKYQWIIPGWYEPSWWEQVHTEANSSRCLRKNLLAAMEGYIGVDFEPLSSKQIKTISG
KTPQQYEREYNNKRSGVGPSKFHGYAYDGIWVIAKTLQRAMETLHASSRHQRIQDFNYTDHTLGRIILNAMNETNFFGVTGQVVFRNGERMGTIK
FTQFQDSREVKVGEYNAVADTLEIINDTIRFQGSEPPKDDYKDDDDK
>3K2S_1|Chains A, B|Apolipoprotein A-I|Homo sapiens (9606)
DEPPQSPWDRVKDLATVYVDVLKDSGRDYVSQFEGSALGKQLNLKLLDNWDSVTSTFSKLREQLGPVTQEFWDNLEKETEGLRQEMSKDLEEVK
AKVQPYLDDFQKKWQEEMELYRQKVEPLRAELQEGARQKLHELQEKLSPLGEEMRDRARAHVDALRTHLAPYSDELRQRLAARLEALKENGGA
RLAEYHAKATEHLSTLSEKAKPALEDLRQGLLPVLESFKVSFLSALEEYTKKLNTQ
>1GNH_1|Chains A, B, C, D, E|C-REACTIVE PROTEIN|Homo sapiens (9606)
QTDMSRKAFVFPKESDTSYVSLKAPLTKPLKAFTVCLHFYTELSSTRGYSIFSYATKRQDNEILIFWSKDIGYSFTVGGSEILFEVPEVTVAPVHICTS
WESASGIVEFWVDGKPRVRKSLKKGYTVGAEASIILGQEQDSFGGNFEGSQSLVGDIGNVNMWDFVLSPDEINTIYLGGPFSPNVLNWRALKYEV
QGEVFTKPQLWP
>8I2H_1|Chain A|Follicle-stimulating hormone receptor|Homo sapiens (9606)
LGSGCHHRICHCSNRVFLCQESKVTEIPSDLPRNAIELRFVLTKLRVIQKGAFSGFGDLEKIEISQNDVLEVIEADVFSNLPKLHEIRIEKANNLLYINPE
AFQNLPNLQYLLISNTGIKHLPDVHKIHSLQKVLLDIQDNINIHTIERNSFVGLSFESVILWLNKNGIQEIHNCAFNGTQLDELNLSDNNNLEELPNDV
FHGASGPVILDISRTRIHSLPSYGLENLKKLRARSTYNLKKLPTLEKLVALMEASLTYPSHCCAFANWRRQISELHPICNKSILRQEVDYMTQARGQR
SSLAEDNESSYSRGFDMTYTEFDYDLCNEVVDVTCSPKPDAFNPCEDIMGYNILRVLIWFISILAITGNIIVLVILTTSQYKLTVPRFLMCNLAFADLCI
GIYLLLIASVDIHTKSQYHNYAIDWQTGAGCDAAGFFTVFASELSVYTLTAITLERWHTITHAMQLDCKVQLRHAASVMVMGWIFAFAAALFPIFGI
SSYMKVSICLPMDIDSPLSQLYVMSLLVLNVLAFVVICGCYIHIYLTVRNPNIVSSSSDTRIAKRMAMLIFTDFLCMAPISFFAISASLKVPLITVSKAK
ILLVLFHPINSCANPFLYAIFTKNFRRDFFILLSKCGCYEMQAQIYRTETSSTVHNTHPRNGHCSSAPRVTNGSTYILVPLSHLAQN
>8XGS_1|Chain A|KiSS-1 receptor|Homo sapiens (9606)
MHTVATSGPNASWGAPANASGCPGCGANASDGPVPSPRAVDAWLVPLFFAALMLLGLVGNSLVIYVICRHKPMRTVTNFYIANLAATDVTFLLCCV
PFTALLYPLPGWVLGDFMCKFVNYIQQVSVQATCATLTAMSVDRWYVTVFPLRALHRRTPRLALAVSLSIWVGSAAVSAPVLALHRLSPGPRAYCS
EAFPSRALERAFALYNLLALYLLPLLATCACYAAMLRHLGRVAVRPAPADSALQGQVLAERAGAVRAKVSRLVAAVVLLFAACWGPIQLFLVLQAL
GPAGSWHPRSYAAYALKTWAHCMSYSNSALNPLLYAFLGSHFRQAFRRVCPCAPRRPRRPRRPGPSDPAAPHAELLRLGSHPAPARAQKPGSSGLA
ARGLCVLGEDNAPL
```

FIGURE 4.2: FASTA sequence of target proteins

4.2 Physicochemical Characterization of Proteins

We utilized an online tool called ProtParam to forecast a variety of parameters, including the molecular and structural properties of specific proteins. Table 4.1 lists the physicochemical characteristics of target proteins.

TABLE 4.1: The target proteins' physicochemical characteristics

S. No	Target Proteins	MW	PI	NR	PR	Ext Co1.	Ext Co2.	Instability Index	Aliphatic Index	GRAVY
1	GRB2	449069.66	6.75	52	51	74175	73800	39.16	77.46	-0.453
2	ApoP-I	28078.62	5.27	46	37	32430	31278	41.48	82.72	-0.84
3	CRP	23047.14	5.28	23	19	45045	44920	39.94	80.83	-0.14
4	FSHR	76892.23	6.8	59	56	68645	67270	43.87	106.66	-0.182
5	Kisspeptin	42586.04	9.93	13	36	64245	63370	48.25	99.67	-0.383

All proteins exhibit molecular weights ranging from approximately 23 kDa to 76 kDa, with isoelectric points varying from 5.27 to 9.93, suggesting they are likely soluble under physiological conditions.

The instability index values are relatively within range except for FSHR and kisspeptin, indicating that these proteins are likely stable in physiological environments.

Moreover, the proportionate volume of the aliphatic side chains is represented by the aliphatic index, and this index is high for all proteins, indicating the tendency to be thermostable.

Additionally, the GRAVY value is negative, indicating that the proteins are hydrophilic and help in interactions with the aqueous environment. Overall, these properties suggest that the proteins are well-suited for their biological roles.

4.3 Active Site Identification

The dogsitescorer software, which determines the number of pockets that can be bound and gives details on their surface area and volume, was utilized to determine the active sites of the protein.

Figure 4.3 below illustrates the areas and volumes of target proteins. The coloured areas depict the active sites available for a particular protein.

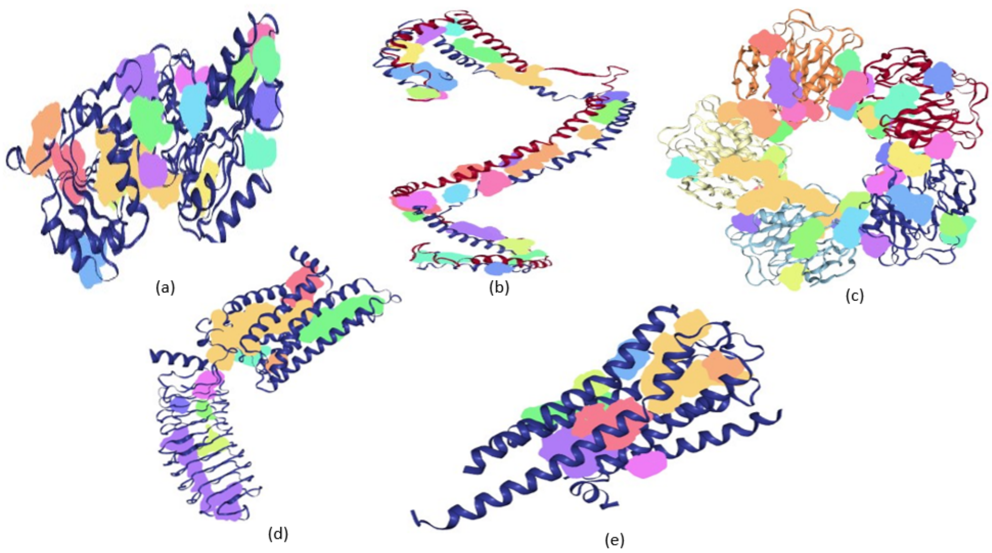


FIGURE 4.3: Active sites of refined target proteins (a) GRB2 (b) Apolipoprotein A-1 (c) CRP (d) FSHR (e) Kisspeptin

Dogsitescorer data depicts different numbers of pockets for each protein. According to this data, the GRB2 consists of fifteen pockets, ApoP-1 consists of 24 pockets, CRP consists of 38 pockets, FSHR consists of ten pockets, and kisspeptin consists of nine pockets.

4.4 Retrieval of Chemical Structure of the Ligands

The ligand to be selected should be on the best resolution structure based on crystal-chemical class and their binding affinities. With that, what matter is the conformational selection of the ligand. A ligand preferentially binds to one of the conformers in this selection process, boosting its numbers in comparison to the overall population and fortifying it of that protein. The largest chemical databank in the world, PubChem, was searched for ligands. These ligands' 3D structures were extracted in SDF format from the PubChem database. After downloading the structures, the next step 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. The refined structures of ligand obtained after energy minimization along with other information are given in table 4.2.

TABLE 4.2: Chemical structure of ligands

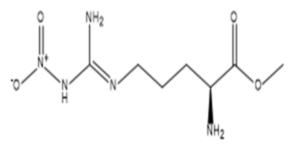
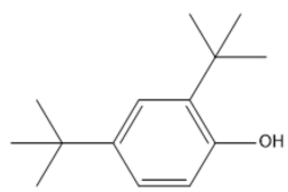
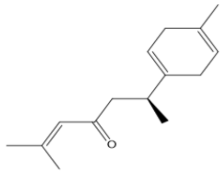
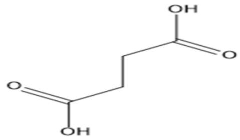
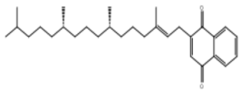
S.No	**Ligands Name**	**Molecular Formula**	**Molecular Weight**	**Structure**
1	Methyl ester	$C_7H_{15}N_5O_4$	233.23	
2	2,4-di-tert-butylphenol	$C_{14}H_{22}O$	206.32	

Table 4.2 continued from previous page

S.No	Ligands Name	Molecular Formula	Molecular Weight	Structure
3	Turmerone	$C_{15}H_{22}O$	218.33	
4	Succinic acid	$C_4H_6O_4$	118	
5	Phytyl	$C_{30}H_{44}O_2$	436.7	

4.5 Virtual Screening of Ligands

For compounds to be separated as both drug-like and nondrug-like virtual screening and pharmacokinetic properties are followed. The Lipinski rule addresses specific parameters, including molecular weight ($\leq$ 500), log P ($\leq$ 5), H-bond donors ($\leq$ 5), H-bond acceptors ($\leq$ 10), and rotatable bonds ($\leq$ 10). Orally active chemicals must adhere to these guidelines. The manner of administration affects the drug-like behaviour. When a chemical satisfies three or more requirements, it is classified as a drug; if it violates more than two, its absorption is poor [96]. LC-MS analysis depicted various compounds in *Ranunculus hirtellus* but only those that exhibited strong antioxidant, antidiabetic, anti-inflammatory, and lipid-regulating activities, which are crucial in mitigating PCOS pathophysiology. Virtual screening of selected ligands is shown is table 4.3.

TABLE 4.3: Virtual screening of ligands

S. No	Ligand Name	Molecular Weight	Log P Value	Rotatable Bonds	H-bond Acceptors	H-bond Donors
1	Methyl ester	233	-1.63	6	6	3
2	2,4-di-tert-butylphenol	206	3.98	1	1	1
3	Turmerone	218	4	4	1	1
4	Succinic acid	118	-0.064	3	2	2

Table 4.3 continued from previous page

S. No	Ligand Name	Molecular Weight	Log P Value	Rotatable Bonds	H-bond Acceptors	H-bond Donors
5	Phytyl	436	5	10	2	1

All ligands adhere to the Lipinski rule, as table 4.4 demonstrates. All ligands have log P values less than 5, and the molecular weight of all is also below 500. The hydrogen bond donor, acceptor, and rotatable bonds are also in range for all ligands.

4.6 ADMET Analysis of Ligands

A second investigation was conducted utilizing the online program pkCSM to ascertain ligands' ADMET characteristics as a pharmacokinetic metric following the lipinski rule. There are two general words in pharmacology: pharmacodynamics and pharmacokinetics. Within the field of pharmacology, pharmacodynamics examines how medications affect the body. In pharmacokinetics, we investigate how medications are absorbed, distributed, metabolized, and excreted [97].

4.6.1 Absorption Properties of Ligands

The absorption parameters reveal important criteria for evaluating the bioavailability of compounds. For water solubility, the higher the number, the better the solubility, helping with absorption. In the case of $Caco_2$ permeability, a desirable number should be above 0, and when above 1, permeability and absorption are high. For intestinal absorption, desirable values should be above 50%, representing good absorption in human metabolism. For skin permeation, desirable values should be above -2, helping with absorption. Finally, being a P-Glycoprotein substrate is desirable when "Yes" as it can be preferably transported by P-glycoprotein, depending on the case. Ideally, for P-glycoprotein inhibitors, a "No" status is preferred to avoid potential drug interactions that could compromise therapeutic efficacy [98]. The absorption properties of ligands are given in table 4.4.

TABLE 4.4: Absorption properties of ligands

	ADMET Properties	**Methyl ester**	**2,4-di-tert-butylphenol**	**Turmerone**	**Succinic acid**	**Phytyl**
Absorption	Water solubility	-2.716	-4.972	-4.693	-2.66	-7.593
	Caco2 Permeability	0.176	1.442	1.378	0.603	1.396
	Intestinal absorption (human)	53	94	97	71	92
	Skin permeability	-2.735	-2.734	-2.677	-2.735	-2.689
	P-glycoprotein substrate	Yes	Yes	Yes	No	Yes
	P-glycoprotein I inhibitor	No	No	No	No	No
	P-glycoprotein II inhibitor	No	No	No	No	No

All ligands have good water solubility. Caco2 permeability is in range for all ligands, intestinal absorption for all ligands is above 50. Skin permeability is also in range. No ligand is inhibitor of P-glycoprotein while some ligands are identified as P-glycoprotein substrates, which could affect their systemic availability

4.6.2 Distribution Properties of Ligands

The theoretical volume or VDss indicates the entire dosage of the medication that must be dispersed evenly to produce a concentration similar to that of blood plasma. For VDss a good value is typically greater than 0.5 L/kg, indicating favorable distribution in body tissues. The fraction unbound (Fu) should ideally be above 0.1, which signifies a significant proportion of the drug is available for therapeutic action. The blood-brain barrier reduces the amount of exogenous substances that can reach the brain directly while protecting it. Regarding BBB permeability (log BB), values greater than 0 suggest the ability to cross the blood-brain barrier. For CNS permeability (log PS), values closer to 0, ideally above -2 are preferred, as they indicate potential for penetration into the central nervous system [99]. Table 4.5 shows the distribution properties of ligands. The table indicates all ligands have safe range which is given below.

TABLE 4.5: Distribution properties of ligands

	ADMET Properties	Methyl ester	2,4-di-tert-butylphenol	Turmerone	Succinic acid	Phytyl
Distribution	VDss (human)	-0.466	0.047	0.536	-1.013	0.442
	Fraction unbound (human) Fu	0.679	0.13	0.303	0.638	0.2
	BBB permeability log BB	-1.006	-0.297	-0.593	-0.163	-0.143
	CNS permeability log PS	-3.63	-0.43	-1.967	-3.06	-0.997

VDSS and fraction unbound values are in range for all ligands. The BBB and CNS value are negative indicating they cannot cross the blood brain barriers and the CNS.

4.6.3 Metabolism Properties of Ligands

The enzyme cytochrome P450 is in charge of the liver's detoxification process. Many drugs get deactivated by this enzyme but certain drugs are capable of activating. This enzyme's inhibitors can directly affect the metabolism of the drug hence should not be used. Similarly, CYP2D6 and CYP3A4 are responsible for the drugs' metabolism. Inhibition of these affects the pharmacokinetics of the drug in use [100]. The ligand metabolism prediction is shown below. The metabolic characteristics of ligands are displayed in Table 4.6.

TABLE 4.6: Metabolism properties of ligands

	ADMET Properties	Methyl ester	2,4-di-tert-butylphenol	Turmerone	Succinic acid	Phytyl
Meta	CYP2D6 substrate	No	No	No	No	No
	CYP3A4 substrate	No	No	No	No	No
	CYP1A2 inhibitor	No	Yes	No	No	No

Table 4.6 continued from previous page

ADMET Properties	Methyl ester	2,4-di-tert-butylphenol	Turmerone	Succinic acid	Phytyl
CYP2C19 inhibitor	No	No	No	No	Yes
CYP2C9 inhibitor	No	No	No	No	Yes
CYP2D6 inhibitor	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No

The metabolic properties of the ligands indicate that none of the compounds are substrates for the CYP2D6 or CYP3A4 enzymes, which suggests they are not metabolized by these pathways. This is beneficial as it reduces potential drug interactions that could arise from competition for these important metabolic enzymes. 2,4-di-tert-butylphenol and phytyl show inhibition of the CYP1A2 enzyme, which can lead to increased levels of drugs metabolized by this enzyme.

4.6.4 Excretion Properties of Ligands

Two organs are involved in drug excretion, the liver, which is engaged in biliary excretion, and the kidneys, which are involved in renal excretion. Excretion may also include other organs, such as the lungs in the case of volatile or gaseous substances. Moreover, drugs can be expelled through tears, saliva, and perspiration [101]. The excretion values of the ligands are given in table 4.7.

TABLE 4.7: Excretion properties of ligands

	ADMET Properties	Methyl ester	2,4-di-tert-butylphenol	Turmerone	Succinic acid	Phytyl
Excretion	Total Clearance	0.022	0.71	1.237	0.722	0.907
	Renal OCT2 substrate	No	No	No	No	No

The total clearance values for the compounds are in range. Notably, all compounds are classified as non-substrates for renal OCT2, which implies that they do not rely on this renal transport mechanism for excretion. This is beneficial as it minimizes the risk of interactions with other drugs that may utilize the same pathway.

4.6.5 Toxicity Properties of Ligands

By using pkCSM we determined the toxicity of the ligands. AMES toxicity test is 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. The toxicity of *T. pyriformis* (protozoa bacterium) is used as a toxic endpoint in the *T. pyriformis* toxicity method. Any value >-0.5 log ug/L is considered toxic [102]. 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 expected log value of the oral rat chronic toxicity test's lowest recorded adverse effect is correlated with the drug concentration that requires a specific duration of treatment, expressed in log mg/kg bw/day. A hepatotoxicity test predicts that if a compound could affect liver functioning or not. Higher maximum tolerated dose (MRTD) values indicate better safety [103]. The toxicity values of all ligands are given in table 4.8.

TABLE 4.8: Toxicity properties of ligands

	ADMET Properties	**Methyl ester**	**2,4-di-tert-butylphenol**	**Turmerone**	**Succinic acid**	**Phytyl**
Toxicity	AMES toxicity	No	No	No	No	No
	Max tolerated dose (human)	0.771	1.756	1.078	0.641	0.329
	hERG I inhibitor	No	No	No	No	No
	hERG II inhibitor	No	No	No	No	No
	Oral rat acute toxicity (LD50)	2.19	1.821	2.157	1.618	1.91
	Oral rat chronic toxicity (LOAEL)	2.244	1.764	1.139	3.052	3.204
	Hepatotoxicity	No	No	No	No	No
	Skin sensitization	No	No	No	No	No
	T.Pyriformis toxicity	0.284	2.268	1.65	0.065	0.478
	Minnow toxicity	2.136	-0.222	0.248	2.829	2.697

No inhibition of hERG I or hERG II was seen in any ligand. None of the ligands demonstrated hepatotoxicity, AMES toxicity and skin sensitivity. Every ligand's MRTD value is within the range. *T. pyriformis* activity was seen in all ligands at least 0.5 log μg/L. The tolerable threshold of 0.5 mM was exceeded by the minnow toxicity levels of all ligands.

4.7 Molecular Docking

To carry out docking, the three-dimensional structures of the protein and ligands are used. An online blind auto docking program called CB dock is utilized for this. 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 among these five [104].

Target proteins and ligands were used in molecular docking. Ligands are in SDF format, while the proteins are in PDB format. After verifying the input files, CB Dock uses Open Babel and MGL tools to transform them into files in the pdbqt format. Next, CB dock determines the receptor's cavities as well as the diameters and centers of the top five cavities. The protein-ligand interaction's high-affinity score determines which of the five optimal conformations is the best [105]. The scores obtained after the docking of proteins and ligands are shown in tables 4.9.

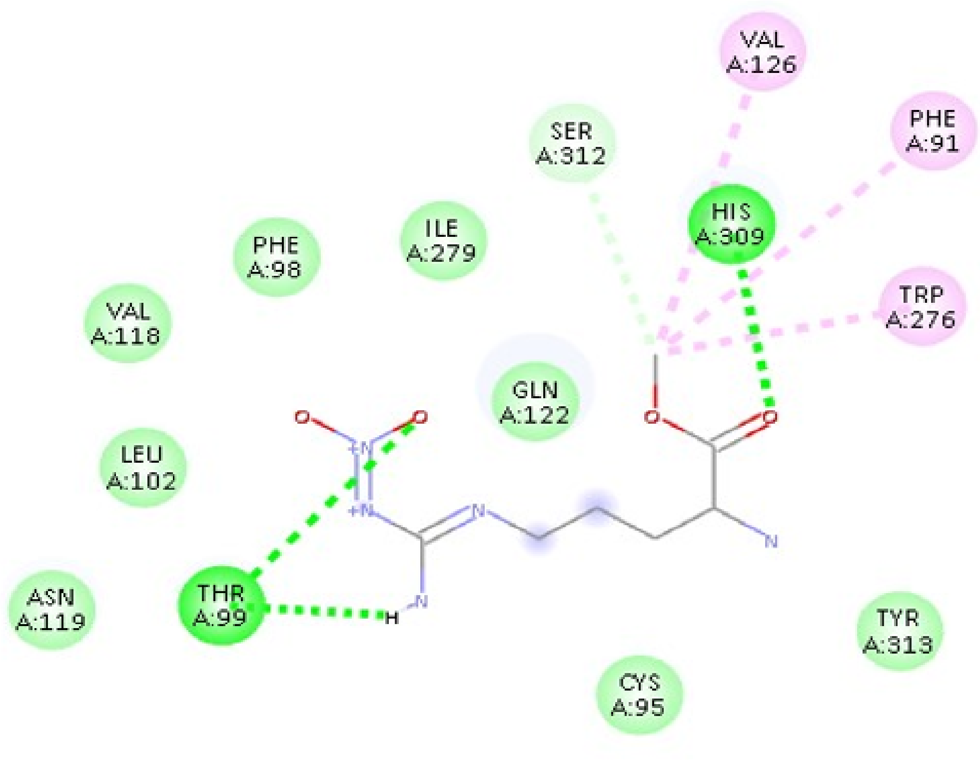
TABLE 4.9: Docking score of ligand-protein complexes

Ligands	**Proteins**				
	GRB2	**ApoP-1**	**CRP**	**FSHR**	**Kisspeptin**
2,4-di-tert-butylphenol	-5.3	-5.3	-5.7	-5.4	-6.6
Turmerone	-6	-6.4	-7	-6.3	-5.8
Methyl ester	-5.7	-4	-6	-4.6	-5.4
Succinic acid	-4.7	-4	-4.7	-3.9	-4.2
Phytyl	-6.7	-5.2	-5.6	-6.6	-7.6

Table 4.10 shows the docking result of receptors with ligands. It shows that phytyl has the highest binding score of -7.6 with kisspeptin, followed by -6.7 with GRB2.

4.8 Analysis of Docked Complexes via Discovery Studio

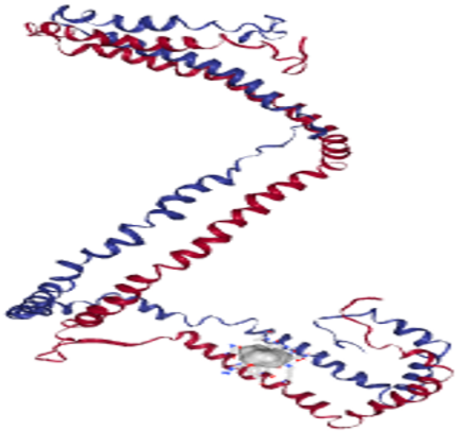
To understand docking data, ligand and protein interactions were estimated. Hydrogen bonding, alkyl and van der waals interactions are the main types of interactions that were investigated. Discovery Studio 2025 Client was used to analyze these interactions between proteins and ligands. The saved conformations for the ligand-receptor complex of each molecule were analyzed in detail. This program creates schematic representations of the protein-ligand interactions between the specified ligands in the PDB file automatically [106]. Numerous interactions between the ten ligands and the three target proteins were seen in the docked data, which were submitted in PDB format. The docked complexes and ligand-receptor interactions are depicted in the following diagrams. The (a) part of the figure represents the docking complex, while (b) part of the figure provides information about the interaction between protein and ligand.



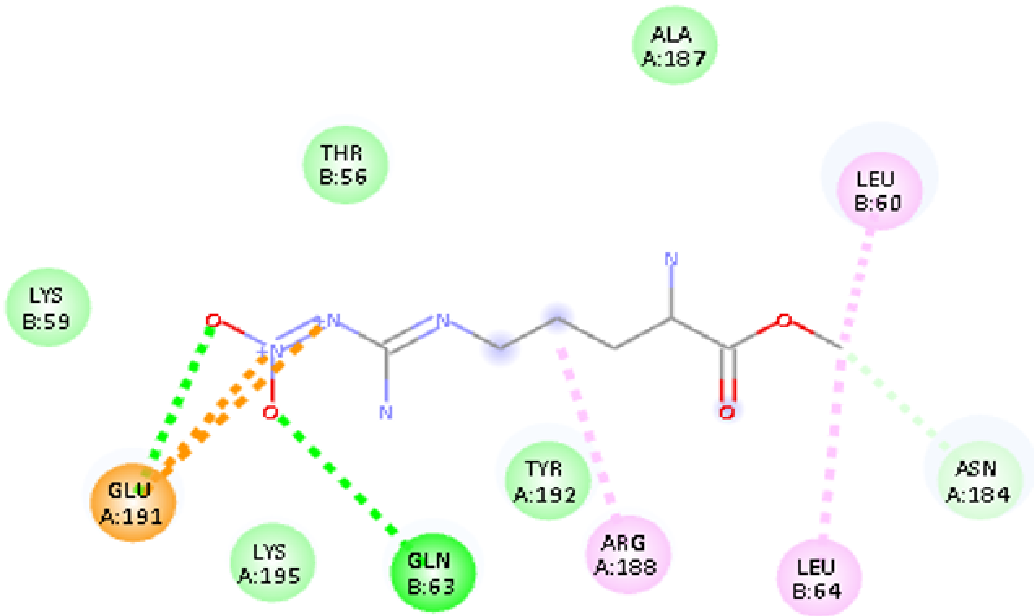
(b)

FIGURE 4.4: Analysis of dock complexes of methyl ester with kisspeptin

Figure 4.4 shows the interaction of methyl ester with kisspeptin. It shows there are three conventional hydrogen bonds, one carbon hydrogen bond, three alkyl bonds and eight van der waals interactions.



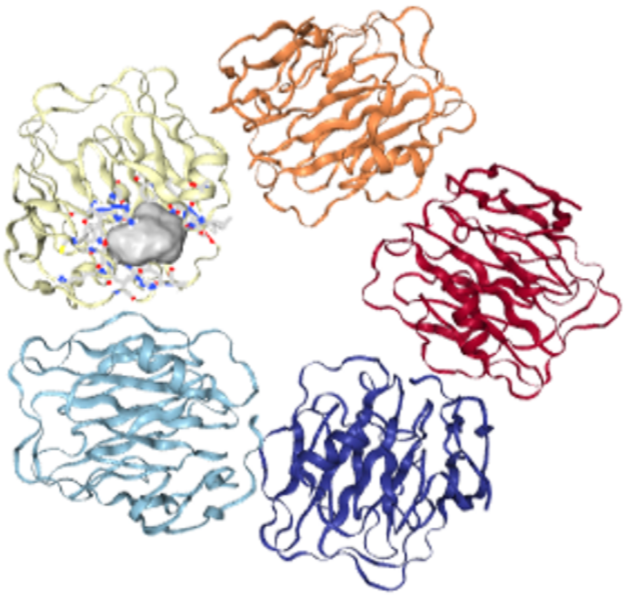
(a)



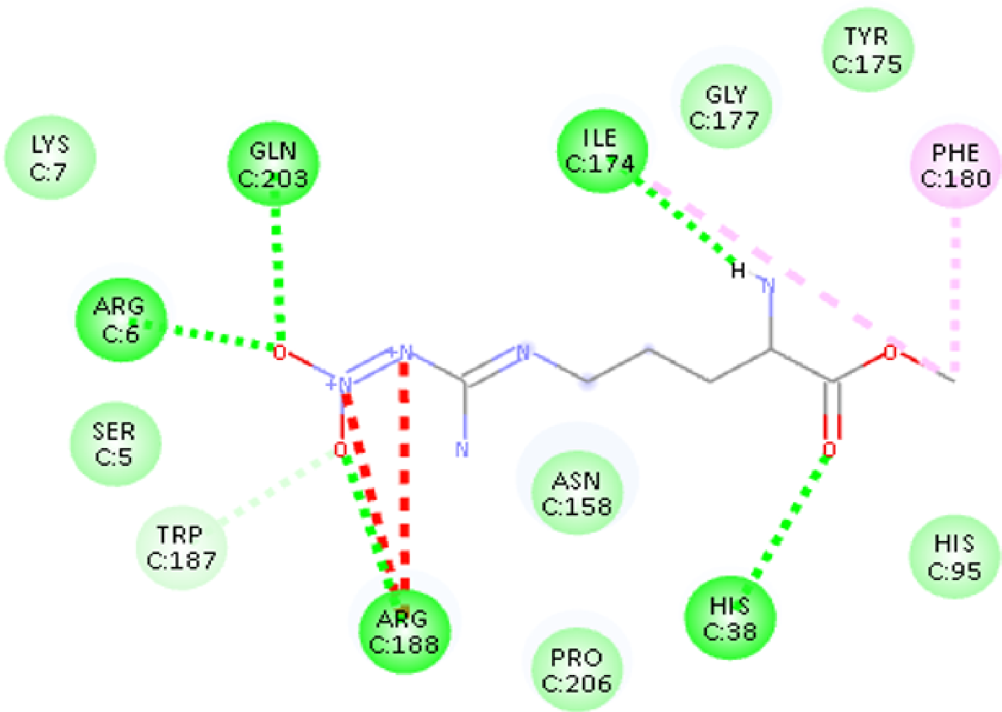
(b)

FIGURE 4.5: Analysis of dock complexes of methyl ester with ApoA-1

Figure 4.5 shows the interaction of methyl ester with ApoA-1. It shows there is one attractive charge, one conventional and one carbon hydrogen bond, three alkyl bonds and five van der waals interactions.



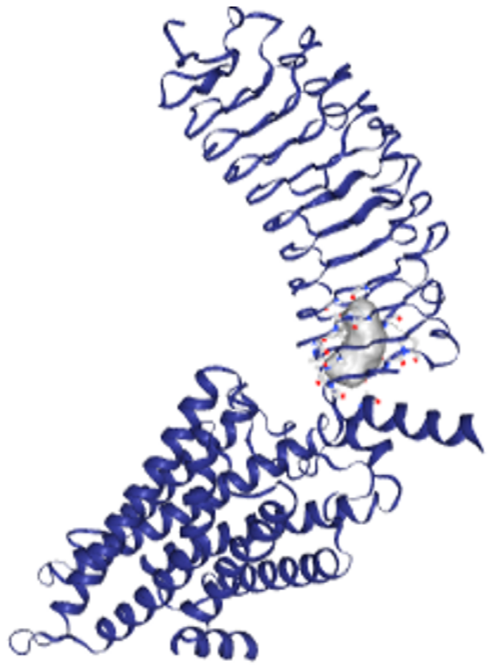
(a)



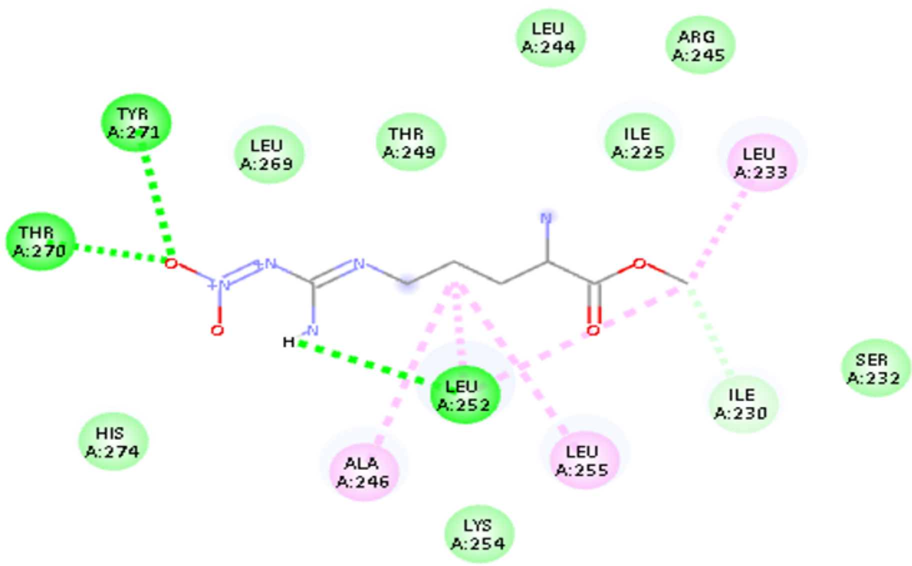
(b)

FIGURE 4.6: Analysis of dock complexes of methyl ester with CRP

Figure 4.6 shows the interaction of methyl ester with CRP. It shows there is one alkyl bond, five conventional and one carbon hydrogen bond and seven van der waals interactions.



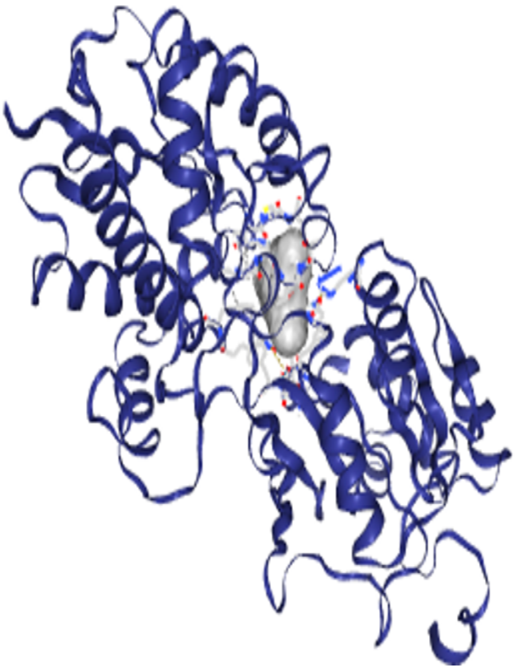
(a)



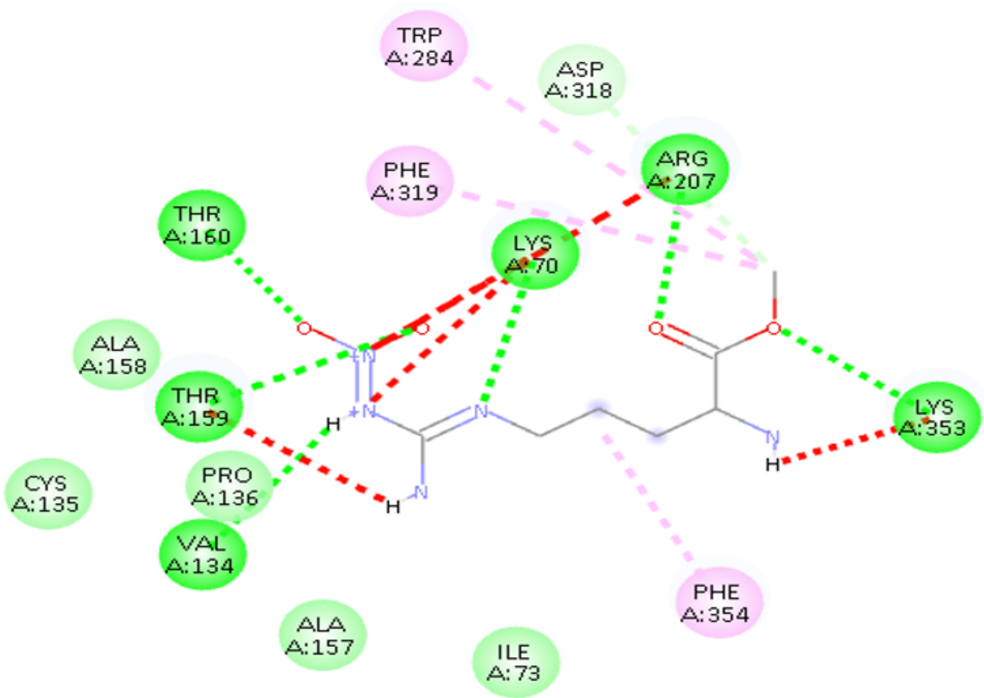
(b)

FIGURE 4.7: Analysis of dock complexes of methyl ester with FSHR

Figure 4.7 shows the interaction of methyl ester with FSHR. It shows there are three conventional hydrogen bonds, one carbon hydrogen bond, three alkyl bonds and eight van der waals interactions.



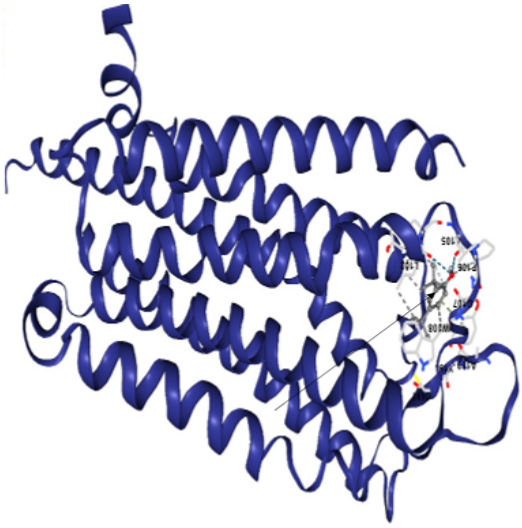
(a)



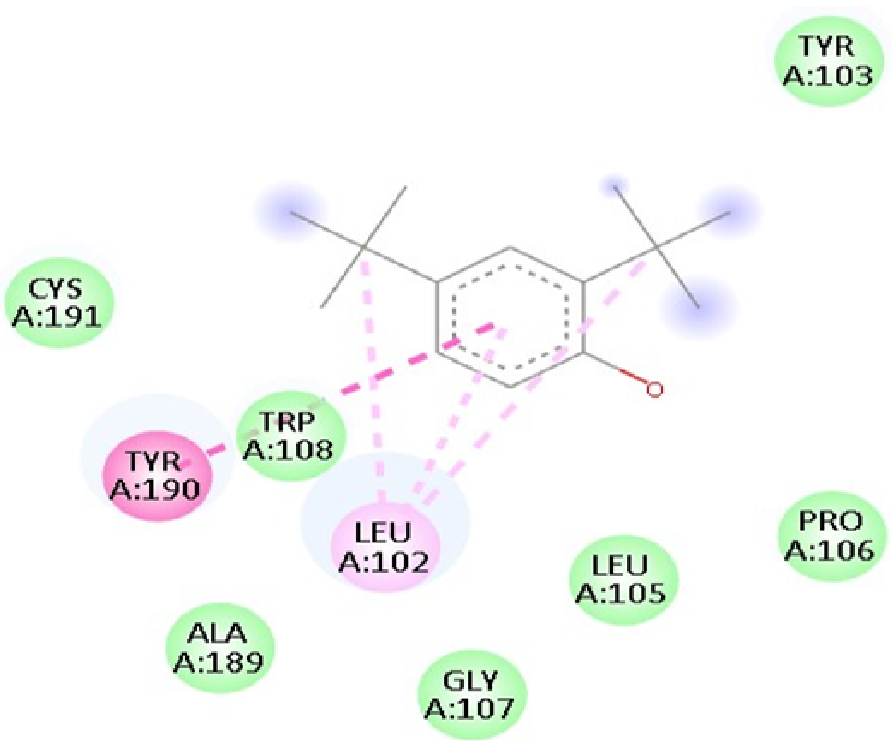
(b)

FIGURE 4.8: Analysis of dock complexes of methyl ester with GRB2

Figure 4.8 shows the interaction of methyl ester with GRB2. It shows there are six conventional hydrogen bonds, one carbon hydrogen bond, three alkyl bonds and five van der waals interactions.



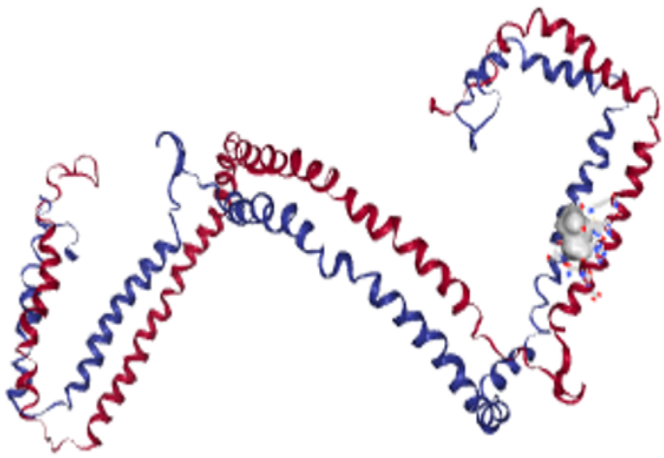
(a)



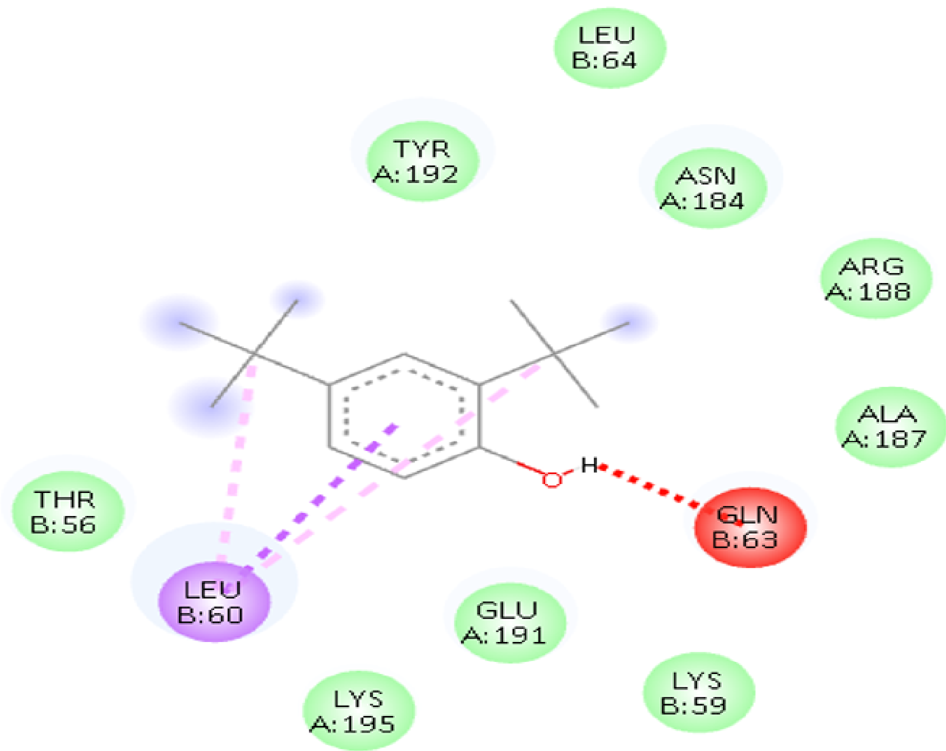
(b)

FIGURE 4.9: Analysis of dock complexes of 2,4-di-tert-butylphenol with kisspeptin

Figure 4.9 shows the interaction of 2,4-di-tert-butylphenol with kisspeptin. It shows there is one pi-bond, three alkyl bonds and seven van der waals interactions.



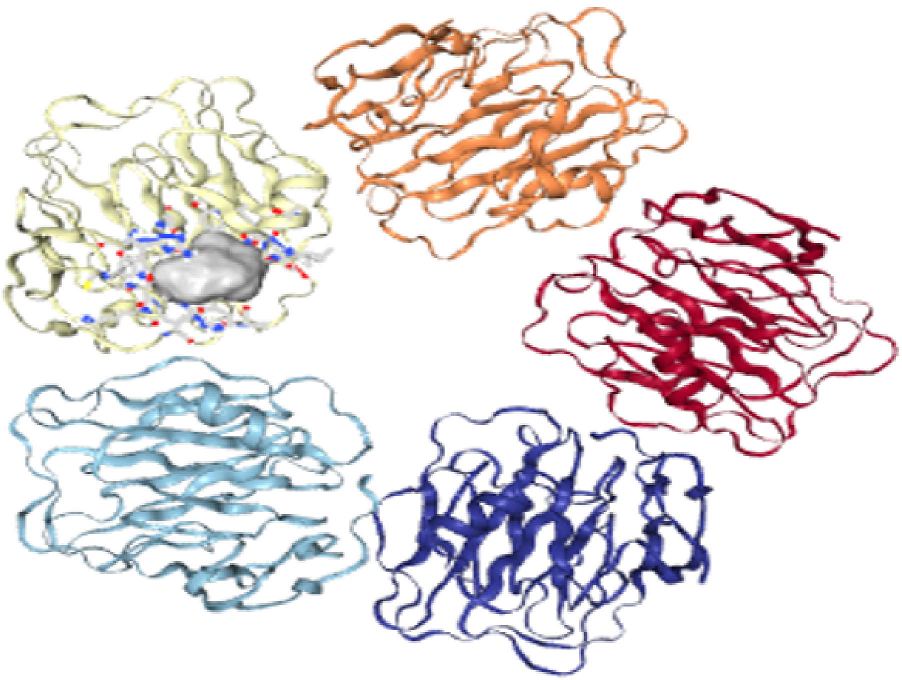
(a)



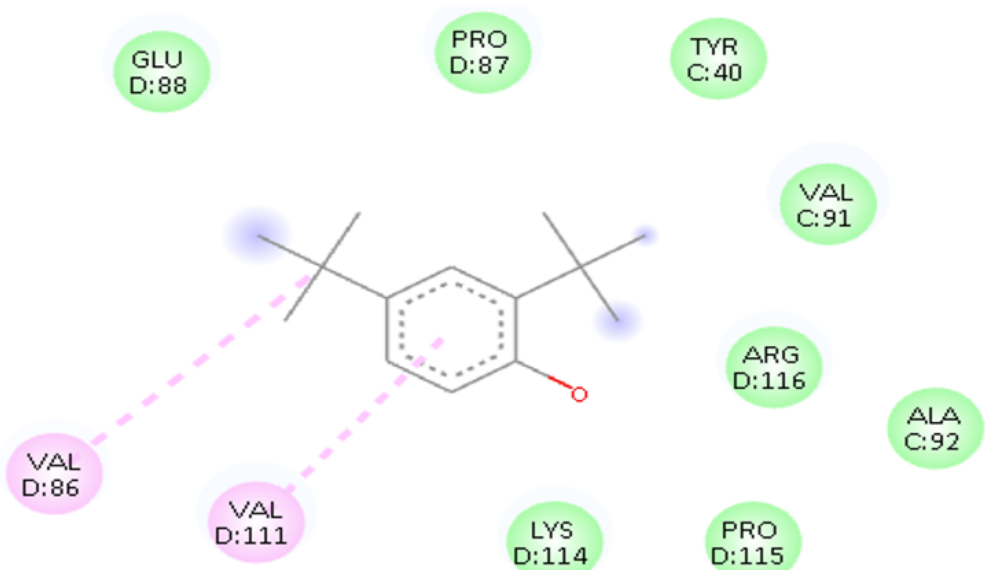
(b)

FIGURE 4.10: Analysis of dock complexes of 2,4-di-tert-butylphenol with ApoA-1

Figure 4.10 shows the interaction of 2,4-di-tert-butylphenol with ApoA-1. It shows there is one sigma bond, one unfavorable interaction and nine van der waals interactions.



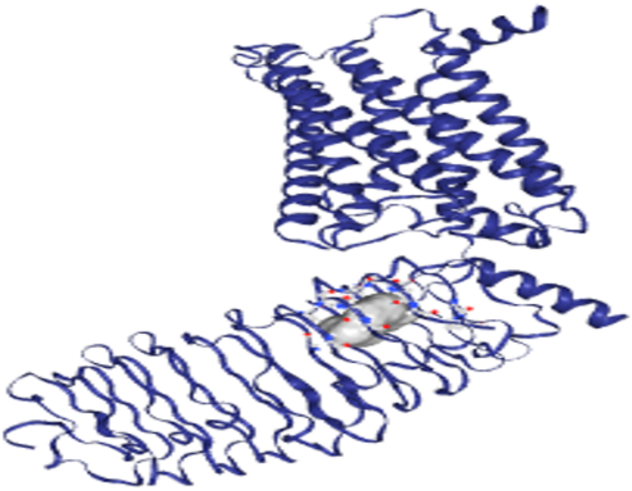
(a)



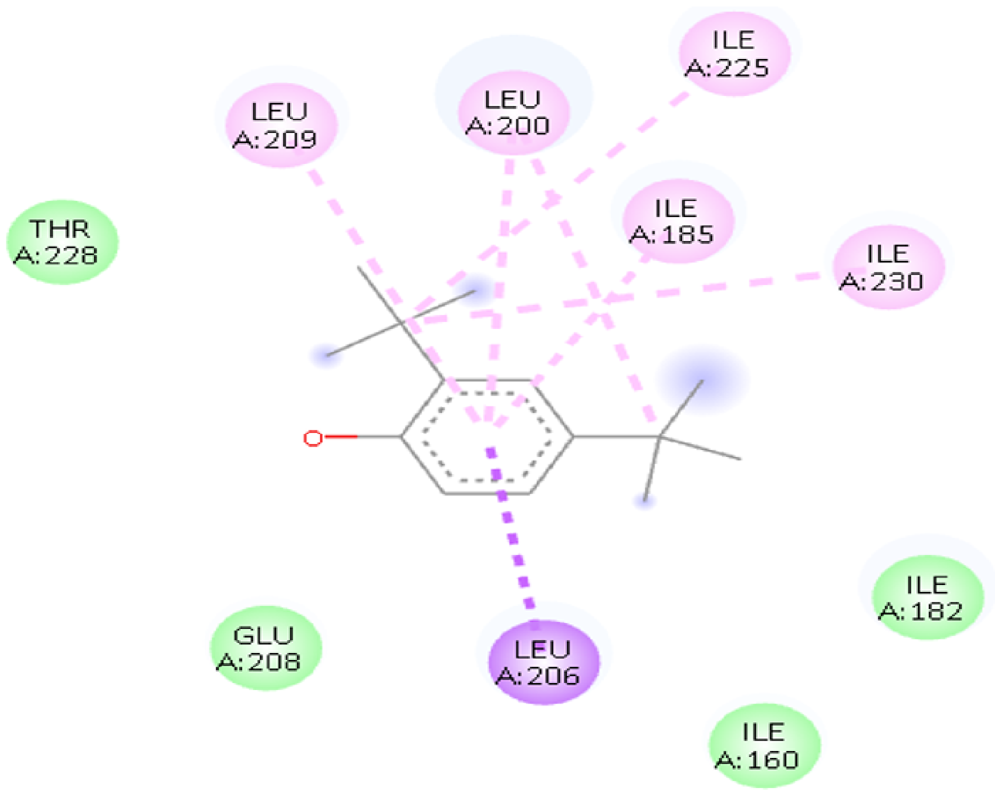
(b)

FIGURE 4.11: Analysis of dock complexes of 2,4-di-tert-butylphenol with CRP

Figure 4.11 shows the interaction of 2,4-di-tert-butylphenol with CRP. It shows there are two alkyl bonds and eight van der waals interactions.



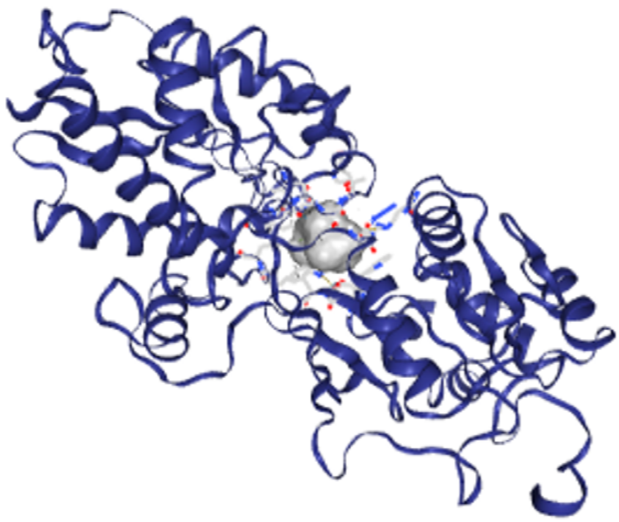
(a)



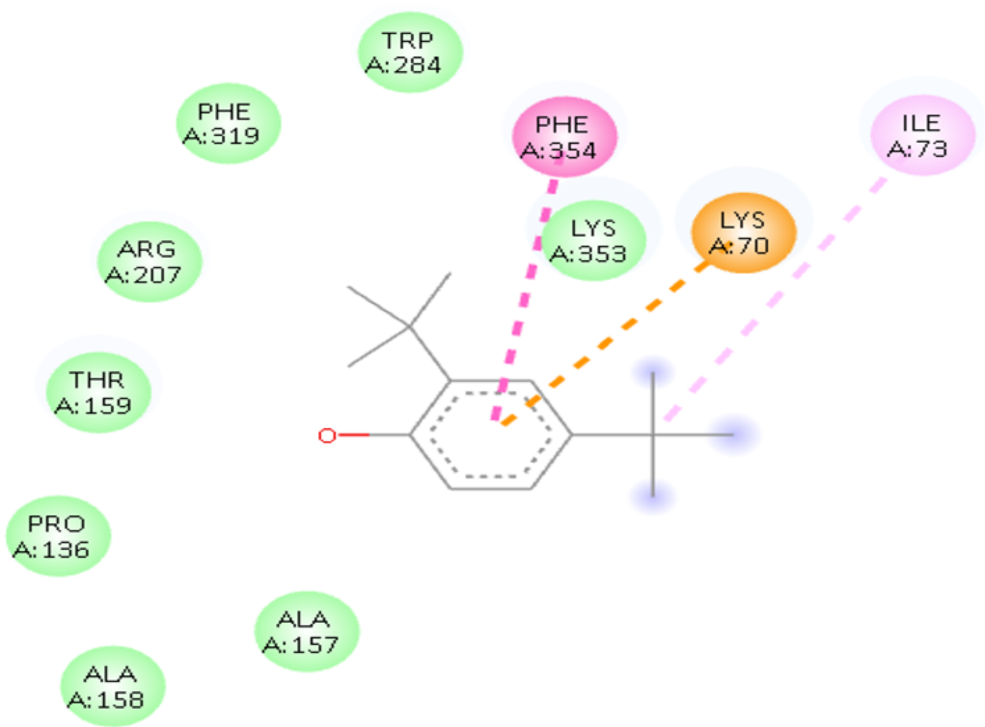
(b)

FIGURE 4.12: Analysis of dock complexes of 2,4-di-tert-butylphenol with FSHR

Figure 4.12 shows the interaction of 2,4-di-tert-butylphenol with FSHR. It shows there is one sigma bond, five alkyl bonds and four van der waals interactions.



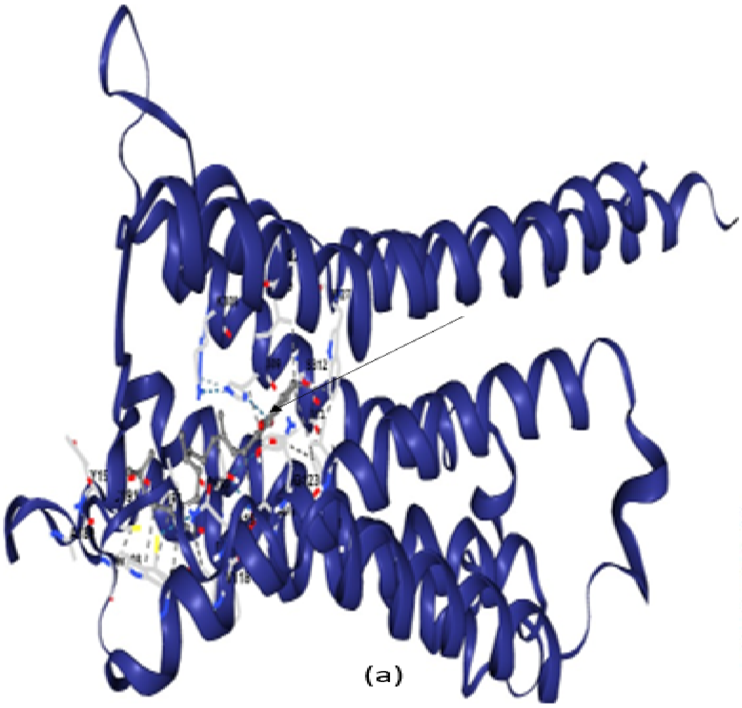
(a)



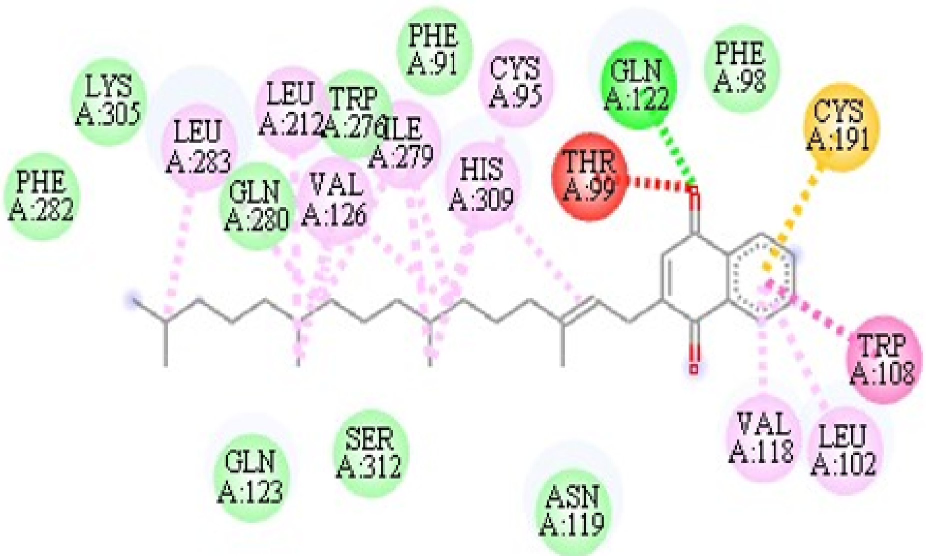
(b)

FIGURE 4.13: Analysis of dock complexes of 2,4-di-tert-butylphenol with GRB2

Figure 4.13 shows the interaction of 2,4-di-tert-butylphenol with GRB2. It shows there is one pi-bond, one alkyl bond, one pi-cation and eight van der waals interactions.



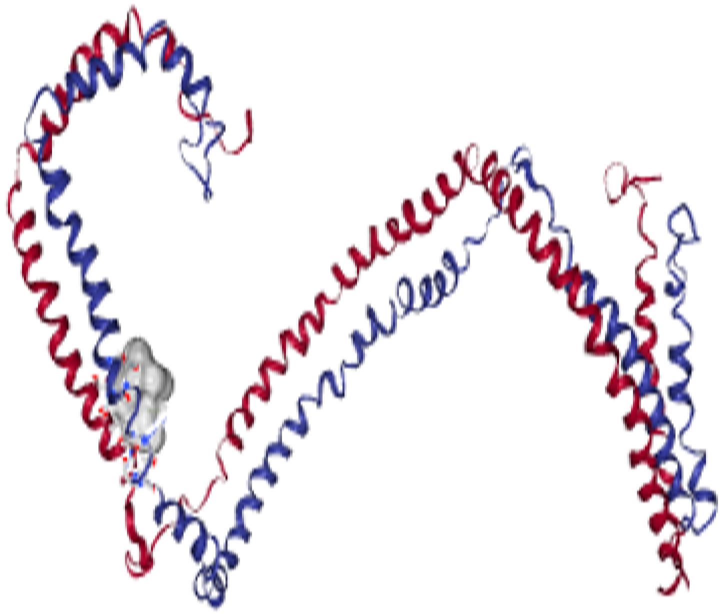
(a)



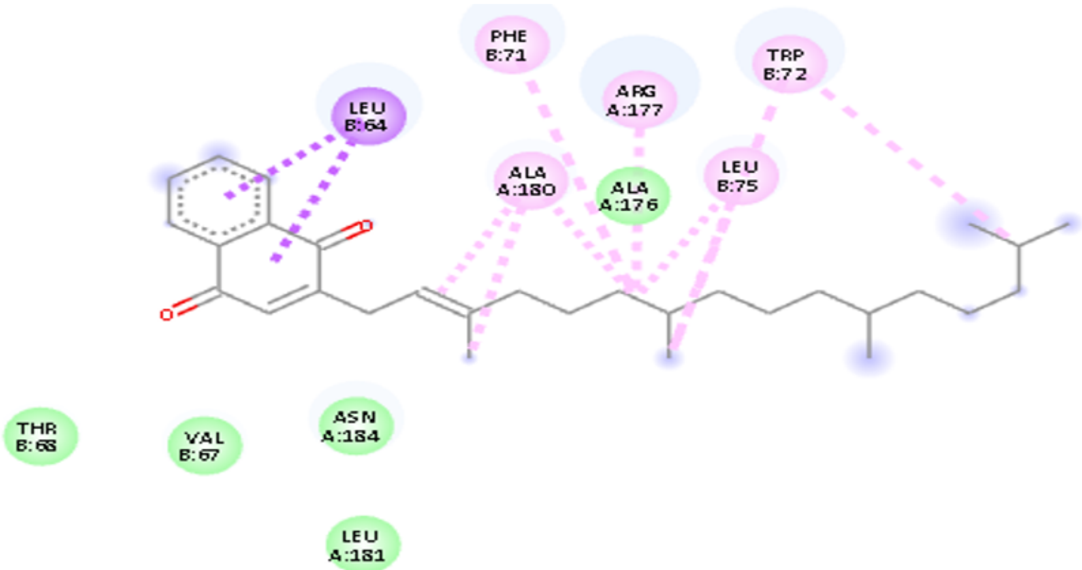
(b)

FIGURE 4.14: Analysis of dock complexes of phytyl with kisspeptin

Figure 4.14 shows the interaction of phytyl with kisspeptin. It shows there is one pi-bond, one pi-sulfur, twelve alkyl bonds, one conventional hydrogen bond, nine van der waals and one unfavorable interaction.



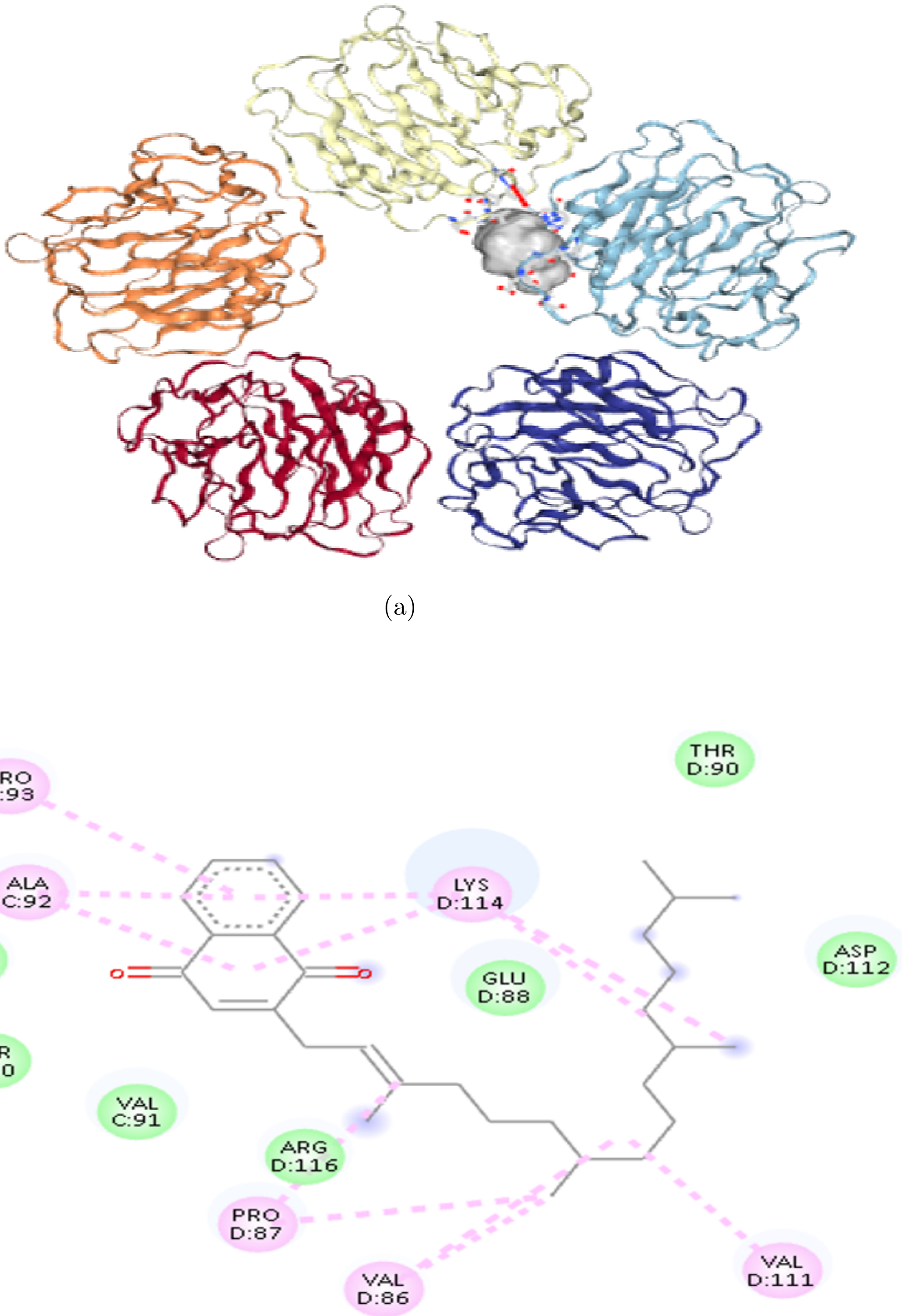
(a)



(b)

FIGURE 4.15: Analysis of dock complexes of phytyl with ApoA-1

Figure 4.15 shows the interaction of phytyl with ApoA-1. It shows there are three is one sigma bond, nine alkyl bonds and five van der waals interactions.

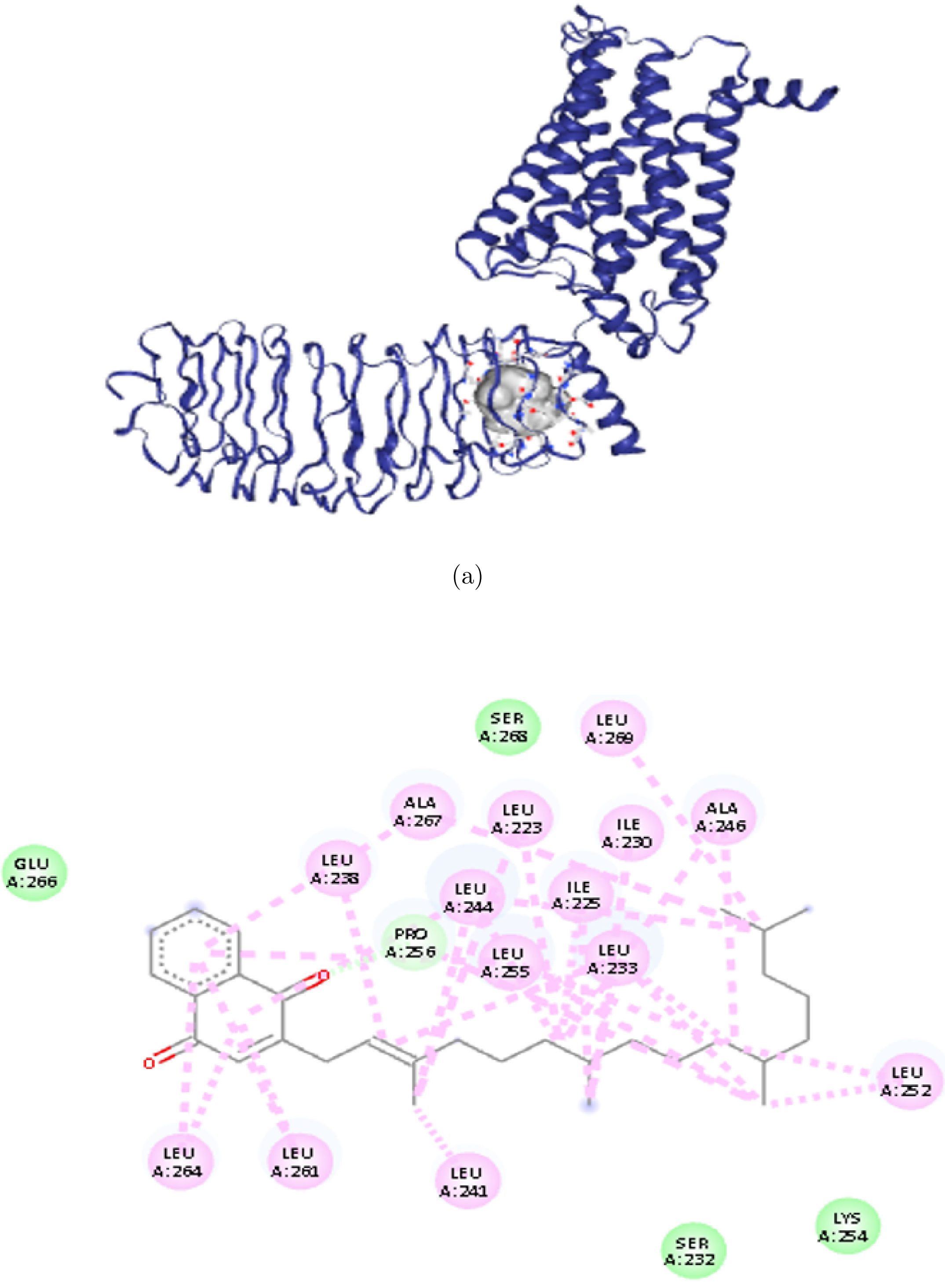


(a)

(b)

FIGURE 4.16: Analysis of dock complexes of phytyl with CRP

Figure 4.16 shows the interaction of phytyl with CRP. It shows there are eleven alkyl bonds and seven van der waals interactions.

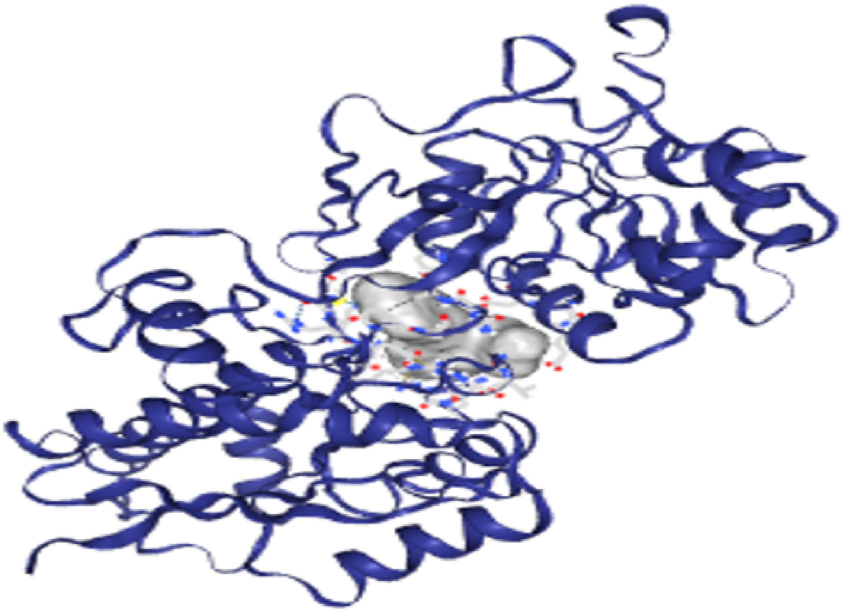


(a)

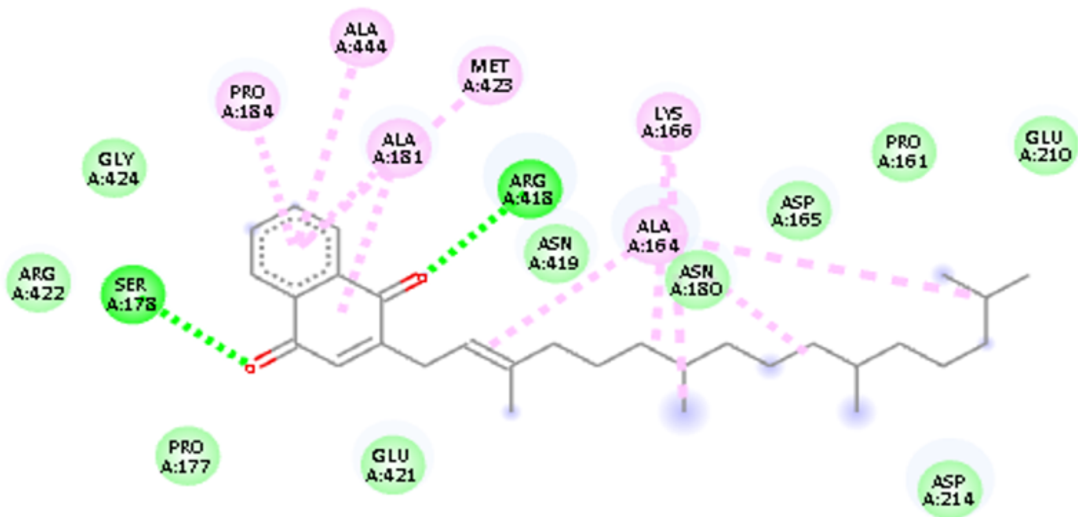
(b)

FIGURE 4.17: Analysis of dock complexes of phytyl with FSHR

Figure 4.17 shows the interaction of phytyl with FSHR. It shows there is one carbon hydrogen bond, twenty two alkyl bonds and four van der waals interactions.



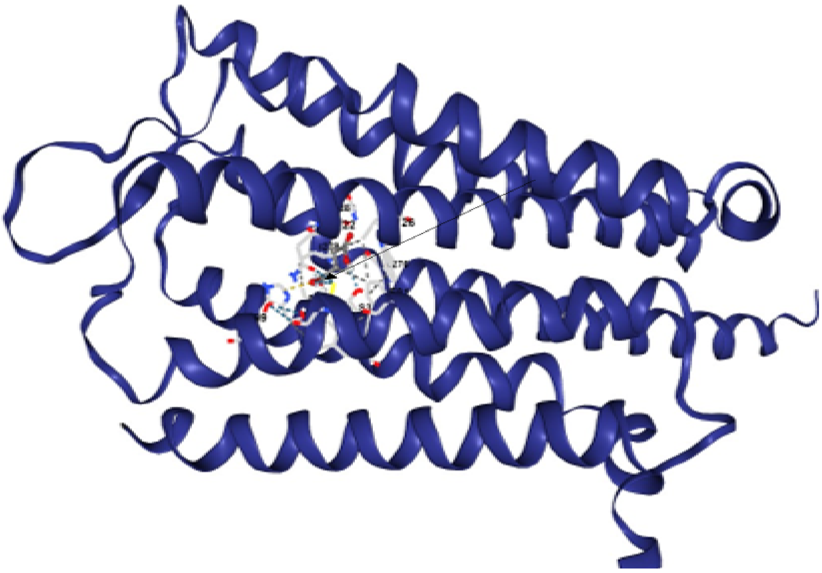
(a)



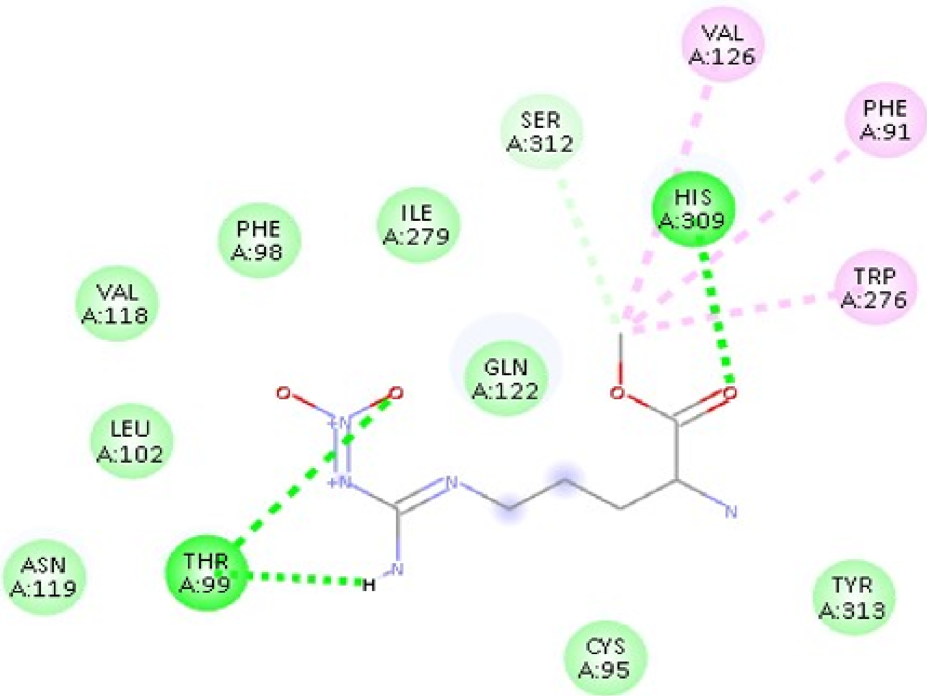
(b)

FIGURE 4.18: Analysis of dock complexes of phytyl with GRB2

Figure 4.18 shows the interaction of phytyl with GRB2. It shows there are two conventional hydrogen bonds, eleven alkyl bonds and ten van der waals interactions.



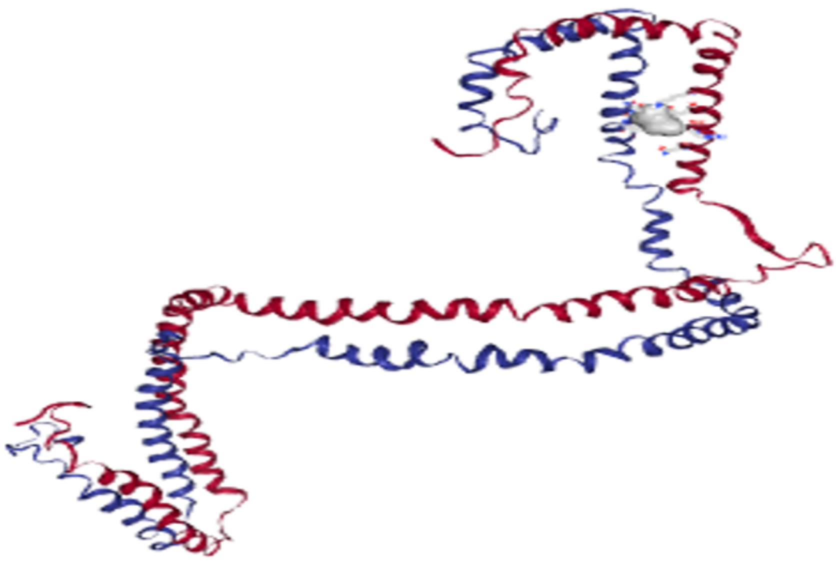
(a)



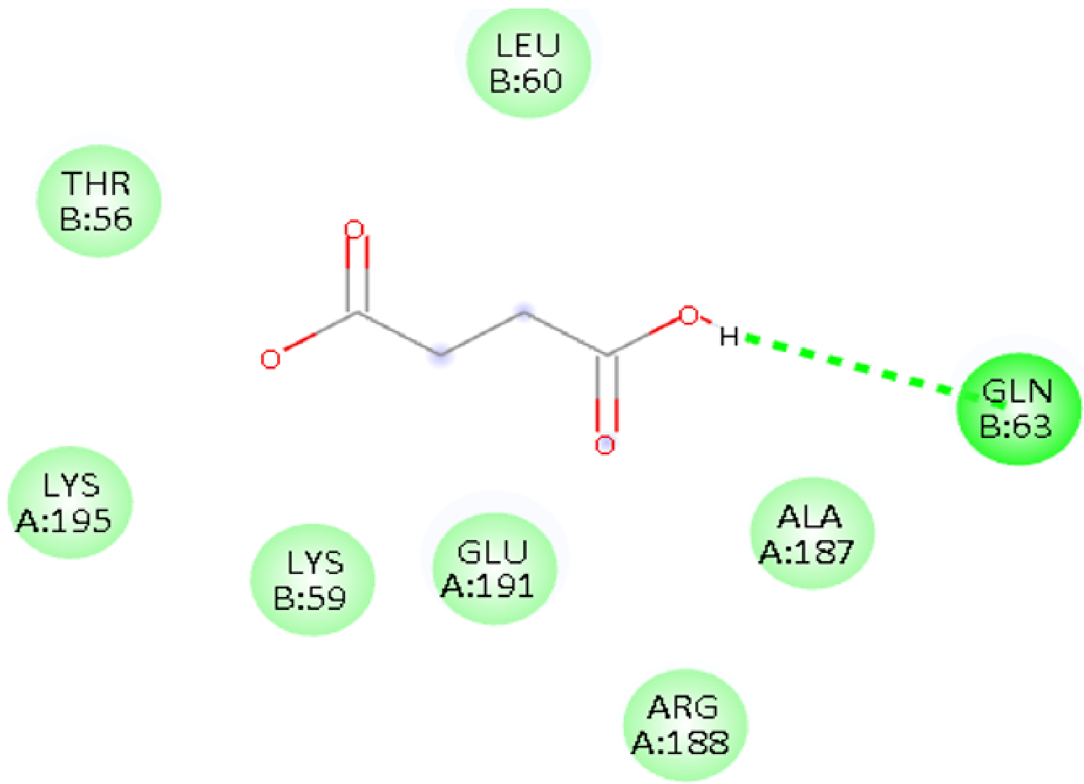
(b)

FIGURE 4.19: Analysis of dock complexes of succinic acid with kisspeptin

Figure 4.19 shows the interaction of succinic acid with kisspeptin. It shows there are two conventional hydrogen bonds and eight van der waals interactions.



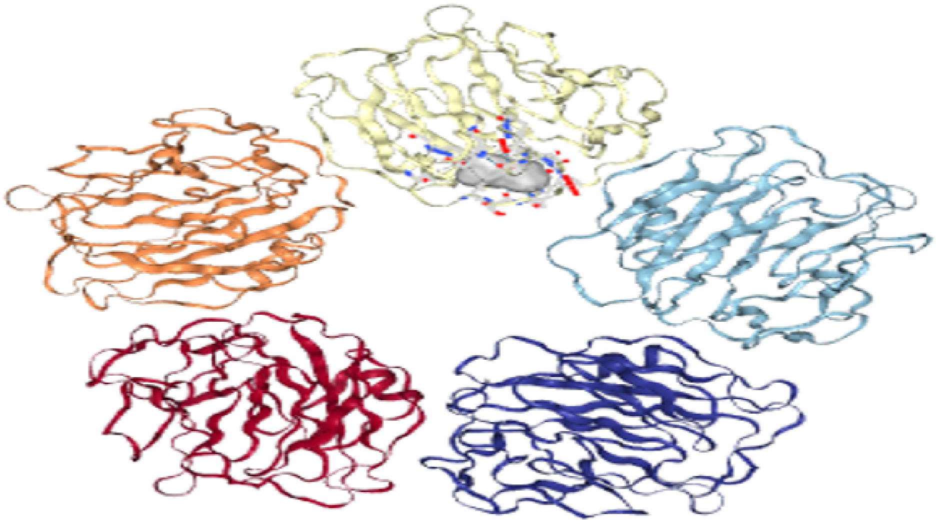
(a)



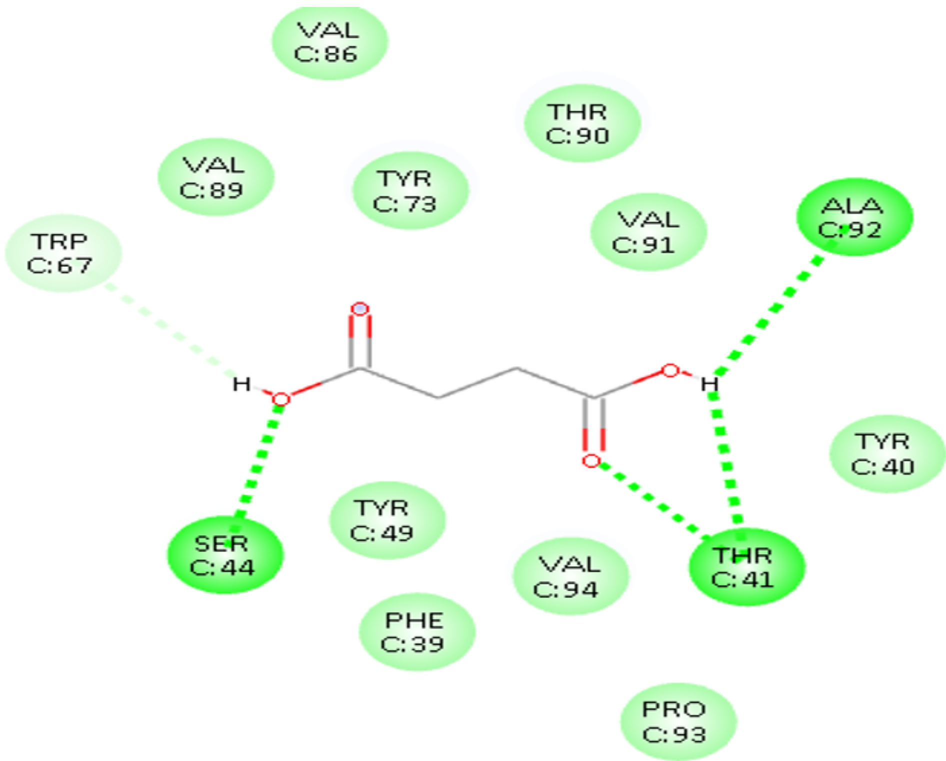
(b)

FIGURE 4.20: Analysis of dock complexes of succinic acid with ApoA-1

Figure 4.20 shows the interaction of succinic acid with ApoA-1. It shows there is one conventional hydrogen bond and seven van der waals interactions.



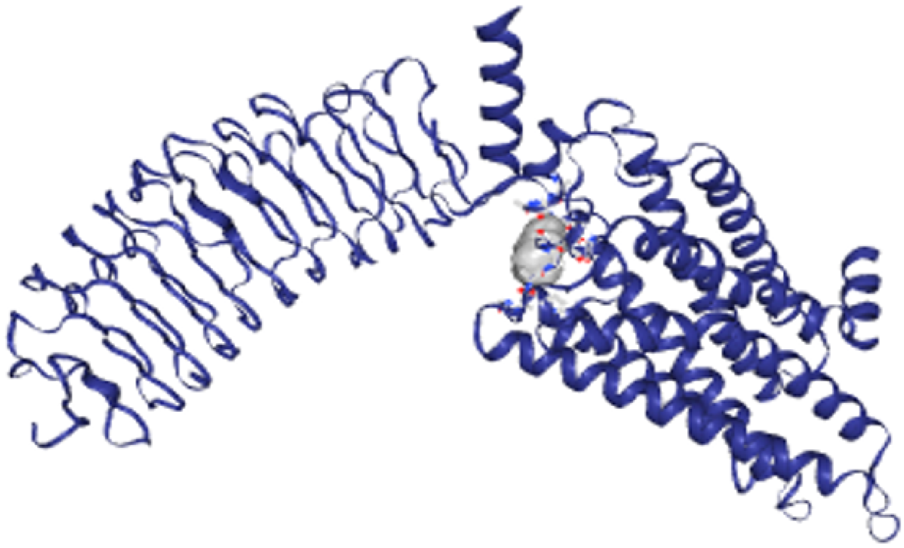
(a)



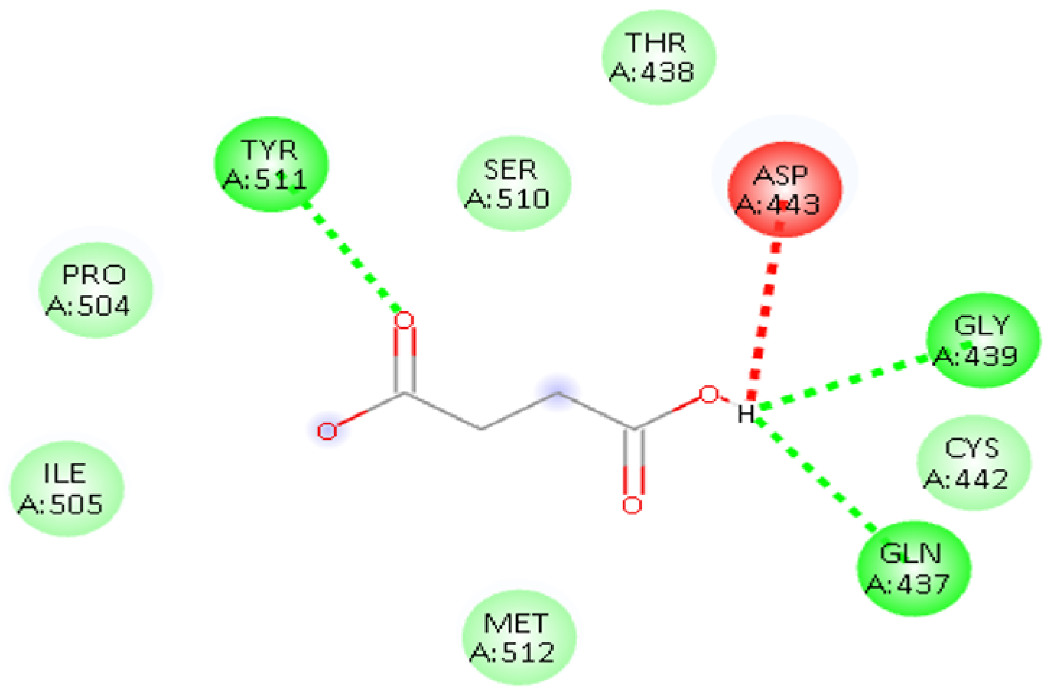
(b)

FIGURE 4.21: Analysis of dock complexes of succinic acid with CRP

Figure 4.21 shows the interaction of succinic acid with CRP. It shows there are four conventional hydrogen bonds, one pi-donor hydrogen bond and ten van der waals interactions.



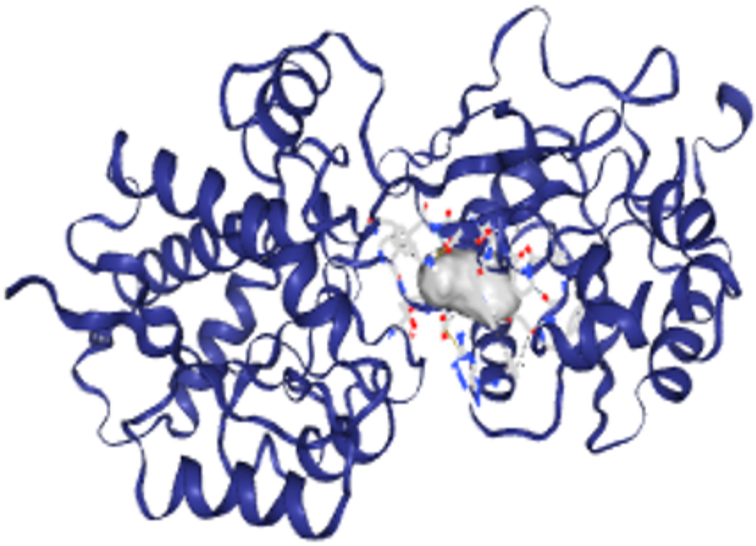
(a)



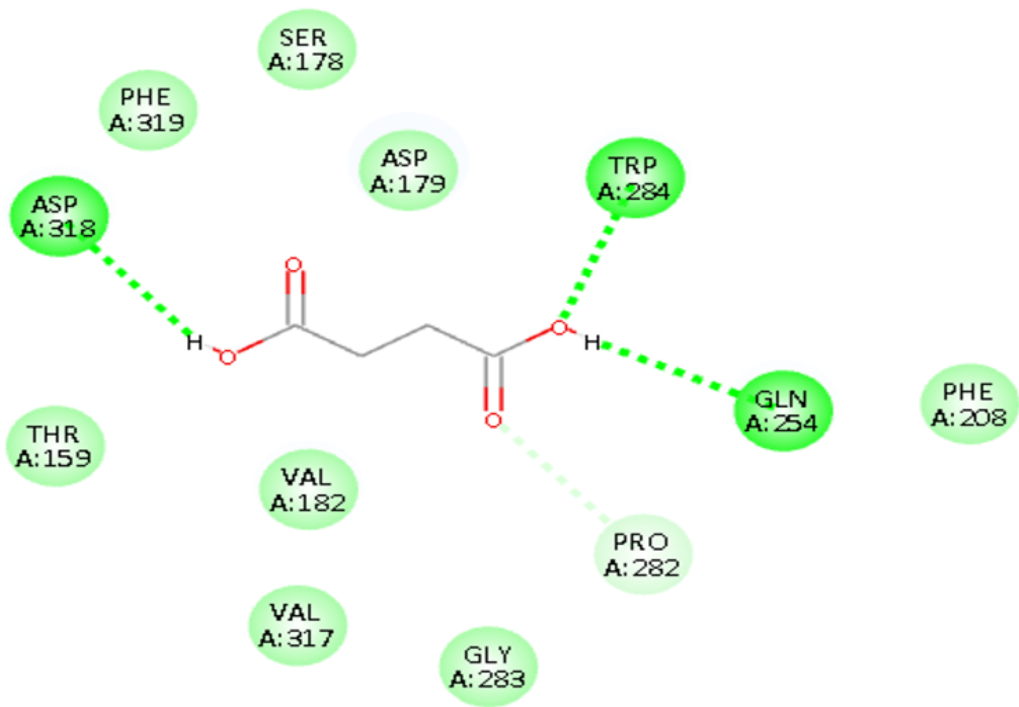
(b)

FIGURE 4.22: Analysis of dock complexes of succinic acid with FSHR

Figure 4.22 shows the interaction of succinic acid with FSHR. It shows there are three conventional hydrogen bonds, six van der waals and one unfavorable interaction.



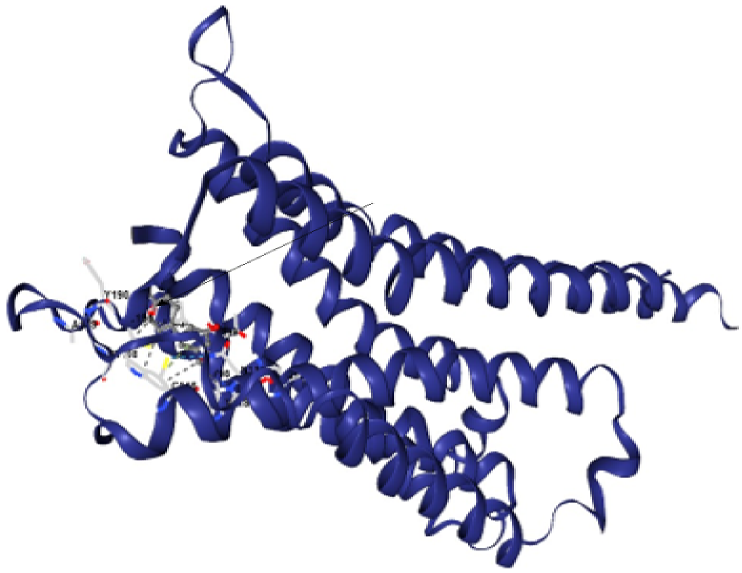
(a)



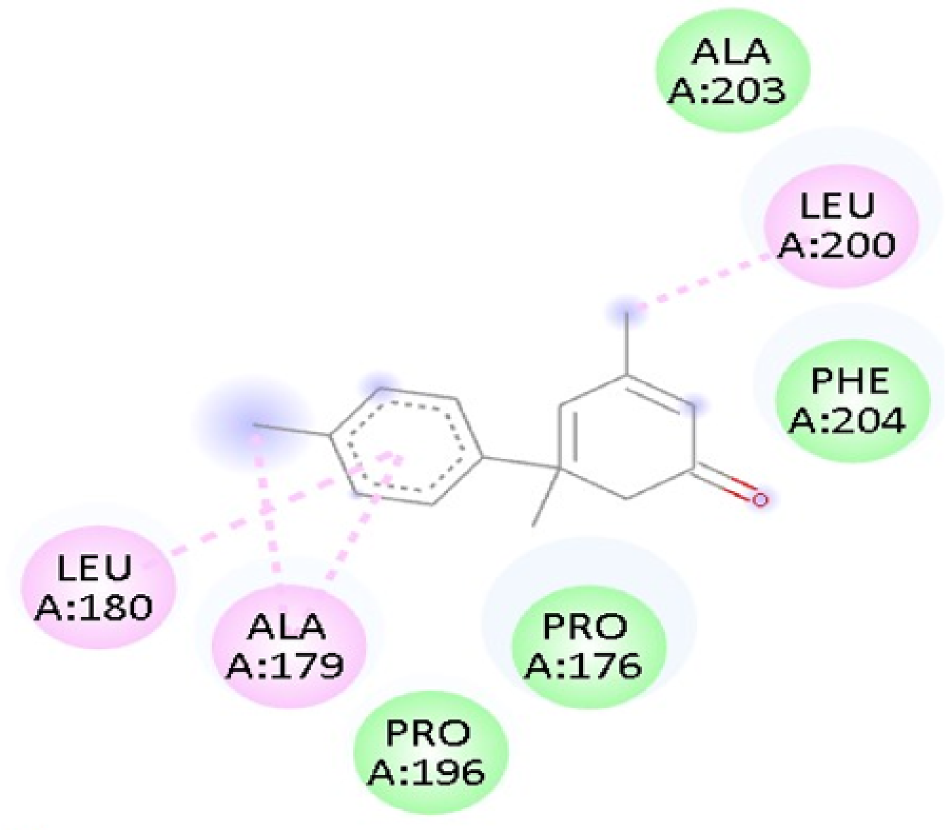
(b)

FIGURE 4.23: Analysis of dock complexes of succinic acid with GRB2

Figure 4.23 shows the interaction of succinic acid with GRB2. It shows there are three conventional hydrogen bonds, one carbon hydrogen bond and eight van der waals interactions.



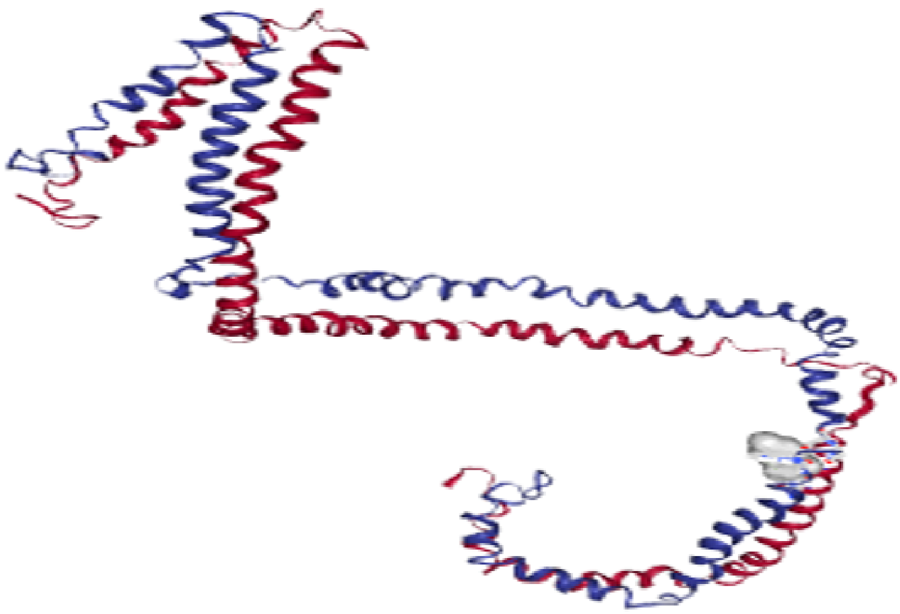
(a)



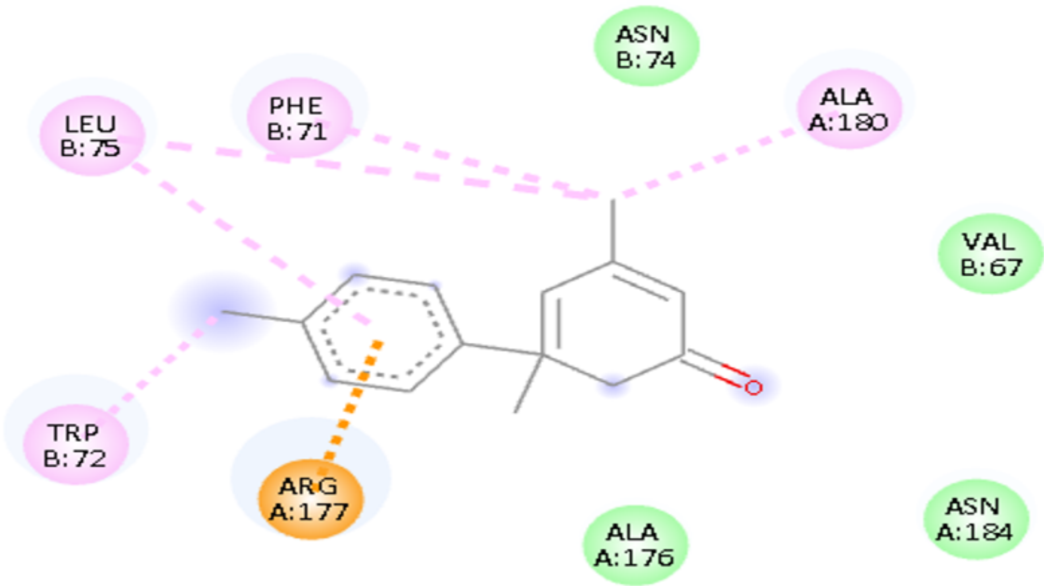
(b)

FIGURE 4.24: Analysis of dock complexes of turmerone with kisspeptin

Figure 4.24 shows the interaction of turmerone with kisspeptin. It shows there are four alkyl bonds and four van der waals interactions.



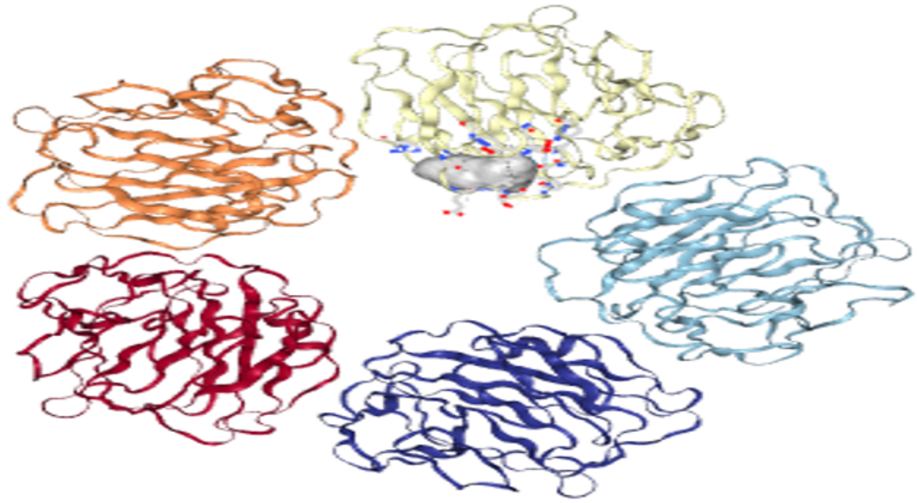
(a)



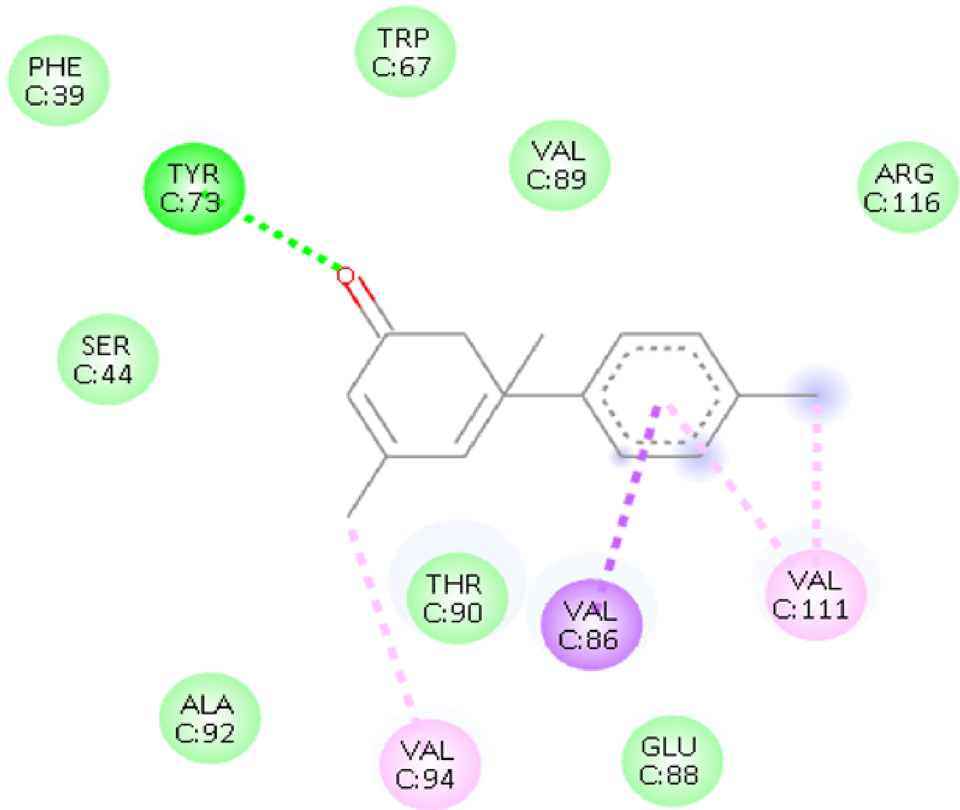
(b)

FIGURE 4.25: Analysis of dock complexes of turmerone with kisspeptin

Figure 4.25 shows the interaction of turmerone with ApoA-1. It shows there is one pi-cation, five alkyl bonds and four van der waals interactions.



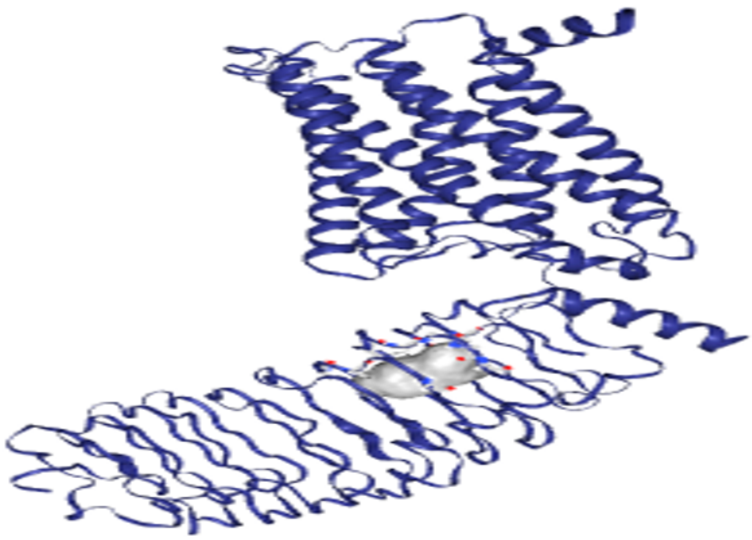
(a)



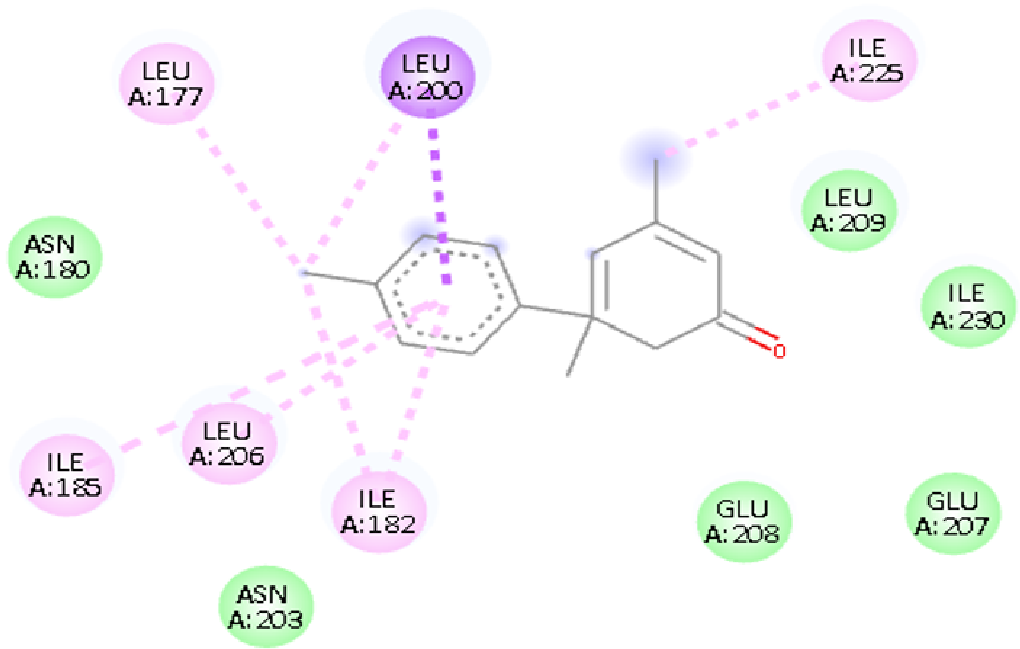
(b)

FIGURE 4.26: Analysis of dock complexes of turmerone with CRP

Figure 4.26 shows the interaction of turmerone with CRP. It shows there is one conventional hydrogen bond, one sigma bond, three alkyl bonds and eight van der waals interactions.



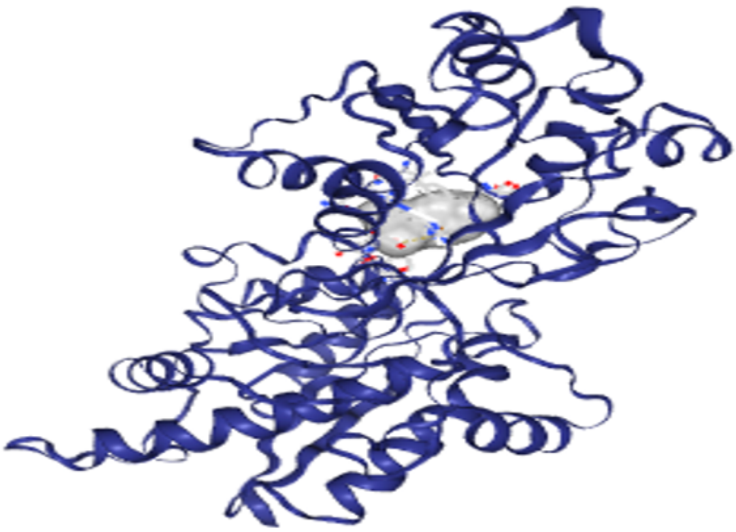
(a)



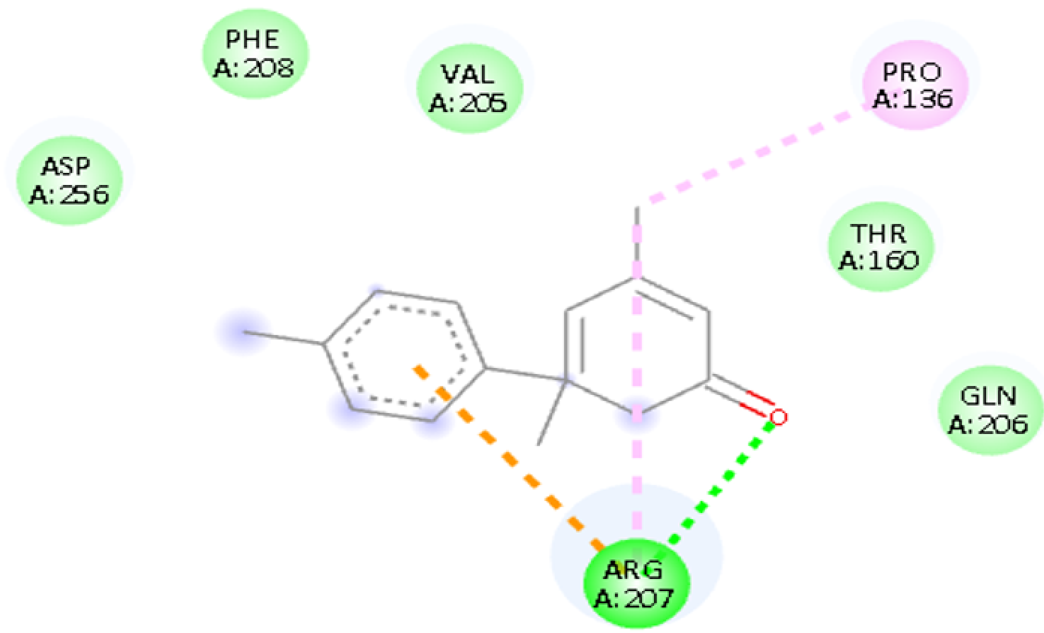
(b)

FIGURE 4.27: Analysis of dock complexes of turmerone with FSHR

Figure 4.27 shows the interaction of turmerone with FSHR. It shows there is one sigma bond, six alkyl bonds and six van der waals interactions.



(a)



(b)

FIGURE 4.28: Analysis of dock complexes of turmerone with GRB2

Figure 4.28 shows the interaction of turmerone with GRB2. It shows there is one conventional hydrogen bond, one alkyl bond and five van der waals interactions.

4.9 Lead Compound Identification

The ligands' pharmacokinetic and physicochemical properties determine their fate as either drug or non-drug compounds. Lipinski's rule is the first filter and pharmacokinetics is the second filter for this identification. All ligands were seen obeying the lipinski rule of five, so they all got selected for docking. The next knockout stage is pharmacokinetic screening and docking score. In this screening, phytyl was selected as it showed the best ADMET values concerning high water solubility, good intestinal absorption, and minimal toxicity. Docking score of phytyl is also good against all receptors and highest i.e. -7.6 against kisspeptin. Additionally, phytyl has a significant quantity of residues having van der waals interactions, alkyl bonds and hydrogen bonds, so phytyl was selected as the lead compound.

4.10 Reference Anti-PCOS Drug Identification

Clomiphene citrate is a commonly used medication for PCOS, particularly for women struggling with ovulation and fertility. It works by blocking estrogen receptors in the brain, which increases the release of hormones that stimulate the ovaries. This helps promote the growth and release of eggs, making it easier for women to conceive. Clomiphene citrate is essential for those looking to regulate their menstrual cycles and improve their chances of pregnancy [107].

4.11 Clomiphene citrate and Lead Compound Comparison

To identify the better treatment for PCOS and the best bioactive metabolite for controlling PCOS mechanisms, the lead compound phytyl and the standard drug clomiphene citrate were compared. The comparison was being performed through parameters like ADMET properties, lipinski rule and docking complexes analysis.

4.12 Clomiphene citrate Structure Prediction

First of all clomiphene citrate structure was downloaded in SDF format from PubChem. Then its energy was minimized by using chem3D pro to get the refined structure. The chemical formula of clomiphene citrate is $C_{32}H_{36}ClNO_8$ and its refined structure is given in figure 4.29.

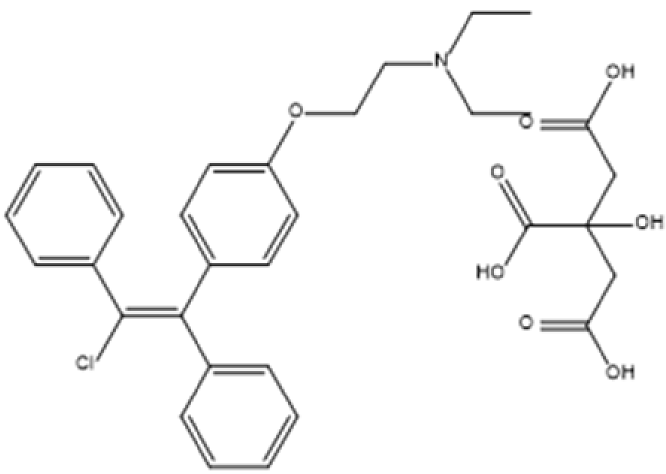


FIGURE 4.29: Structure of Clomiphene citrate

4.13 Lipinski Rule Comparison

The clomiphene citrate and phytyl were compared to observe their response to the lipinski rule and to evaluate their pharmacokinetic properties, to assess their bioavailability, safety, efficacy, and drug-likeness. The comparison is given in table 4.10.

TABLE 4.10: Virtual screening of ligands

S. No	**Ligand Name**	**Molecular Weight**	**Log P Value**	**Rotatable Bonds**	**H-bond Acceptors**	**H-bond Donors**
1	Clomiphene citrate	5.31	598	4	6	14
2	Phytyl	5	436	1	2	10

Table 4.11 shows that clomiphene citrate molecular weight and rotatable bonds are above range defined by lipinski rule.

4.14 ADMET Properties Comparison

The ADMET qualities were used to evaluate the drug's and the lead chemical's absorption, distribution, metabolism, excretion, and toxicity to identify a better drug candidate.

4.14.1 The Absorption Properties Comparison

A comparison between clomiphene citrate and phytyl for checking absorbance models is given in table 4.11.

TABLE 4.11: Absorption properties of ligands

	ADMET Properties	**Phytyl**	**Clomiphene citrate**
Absorption	Water solubility	-7.593	-3.596
	Caco2 Permeability	1.396	-0.277
	Intestinal absorption (human)	92	15.48
	Skin permeability	-2.689	-2.735
	P-glycoprotein substrate	No	Yes
	P-glycoprotein I inhibitor	No	Yes
	P-glycoprotein II inhibitor	No	Yes

Table 4.11 shows that both phytyl and clomiphene citrate show comparable absorption properties except intestinal absorption which is too low for clomiphene citrate.

4.14.2 Distribution Properties Comparison

The comparison between the distribution properties of clomiphene citrate and phytyl is given in table 4.12.

TABLE 4.12: Distribution properties of ligands

	ADMET Properties	**Phytyl**	**Clomiphene citrate**
Distribution	VDss (human)	0.442	-0.998

Table 4.12 continued from previous page

	ADMET Properties	Phytyl	Clomiphene citrate
	Fraction unbound (human) Fu	0.2	0.168
	BBB permeability log BB	-0.143	-1.649
	CNS permeability log PS	-0.997	-3.156

The above table 4.12 shows the comparative distribution properties of clomiphene citrate and phytyl. Both drugs share comparable distribution properties except VDSS, which is too low for clomiphene citrate.

4.14.3 Metabolism Properties Comparison

The comparison between the metabolism properties of clomiphene citrate and phytyl is given in table 4.13.

TABLE 4.13: Metabolism properties of ligands

	ADMET Properties	Phytyl	Clomiphene citrate
Metabolism	CYP2D6 substrate	No	No
	CYP3A4 substrate	No	Yes
	CYP1A2 inhibitor	No	No
	CYP2C19 inhibitor	Yes	No
	CYP2C9 inhibitor	Yes	No
	CYP2D6 inhibitor	No	No
	CYP3A4 inhibitor	No	Yes

Table 4.13 shows that both phytyl and clomiphene citrate share similar metabolism profiles, except that clomiphene citrate is a substrate of CYP3A4 and an inhibitor of CYP3A4.

4.15 Excretion Properties Comparison

The comparison between the excretion properties of clomiphene citrate and phytyl is given in table 4.14.

TABLE 4.14: Excretion properties of ligands

	ADMET Properties	Phytyl	Clomiphene citrate
Excretion	Total Clearance	0.907	-0.314
	Renal OCT2 substrate	No	No

Table 4.14 shows that phytyl has a higher total clearance rate compared to clomiphene citrate, indicating that phytyl is eliminated from the body more efficiently. Neither phytyl nor clomiphene citrate are substrates for the renal OCT2 transporter, which suggests they do not rely on this pathway for renal excretion.

4.15.1 Toxicity Properties Comparison

The comparison between the toxicity properties of clomiphene citrate & phytyl is given in table 4.15.

TABLE 4.15: Toxicity properties of ligands

	ADMET Properties	Phytyl	Clomiphene citrate
Toxicity	AMES toxicity	No	No
	Max tolerated dose (human)	0.329	0.273
	hERG I inhibitor	No	No
	hERG II inhibitor	No	Yes
	Oral rat acute toxicity (LD50)	1.91	1.865
	Oral rat chronic toxicity (LOAEL)	3.204	2.561
	Hepatotoxicity	No	Yes
	Skin sensitization	No	No
	T.Pyriformis toxicity	0.478	0.286
	Minnow toxicity	2.697	-0.898

Table 4.15 shows that both phytyl and clomiphene citrate have comparable toxicity profiles, as neither drug shows AMES toxicity and hERG I inhibition, but clomiphene citrate shows hepatotoxicity and hERG II inhibition. The minnow toxicity value of the standard drug is also below the predicted range.

4.16 Docking Score Comparison

We docked the lead and standard compounds with the target proteins, and then we obtained the binding value from the docking results. The docking score comparison between the lead compound phytyl and the conventional medication clomiphene citrate is displayed in table 4.16.

Table 4.16: Docking comparison of clomiphene citrate and phytyl

Ligands	**Proteins**				
	GRB2	**ApoP-1**	**CRP**	**FSHR**	**Kisspeptin**
Phytyl	-6.7	-5.2	-5.6	-6.6	-7.6
Clomiphene citrate	-4.5	-4.4	-5	-3.9	-5

As can be shown in table 4.16, the vina scores of the lead compound phytyl are significantly greater than those of the generic medication clomiphene citrate. The docking scores of clomiphene citrate the against target proteins GRB2, ApoP-1, CRP, FSHR and kisspeptin are -4.5, -4.4, -5, -3.9 and -5 respectively, while for phytyl these scores are -6.7, -5.2, -5.6, -6.6 and -7.6, respectively. These results show that the lead compound phytyl can bind with target proteins more efficiently than the standard drug clomiphene citrate.

4.17 Docking Analysis Comparison

Discovery studio evaluated the docking results depending upon the number of interactions between ligand and protein. The following figure shows the docking analysis of standard drug clomiphene citrate with highest docking score -5 against target protein kisspeptin.

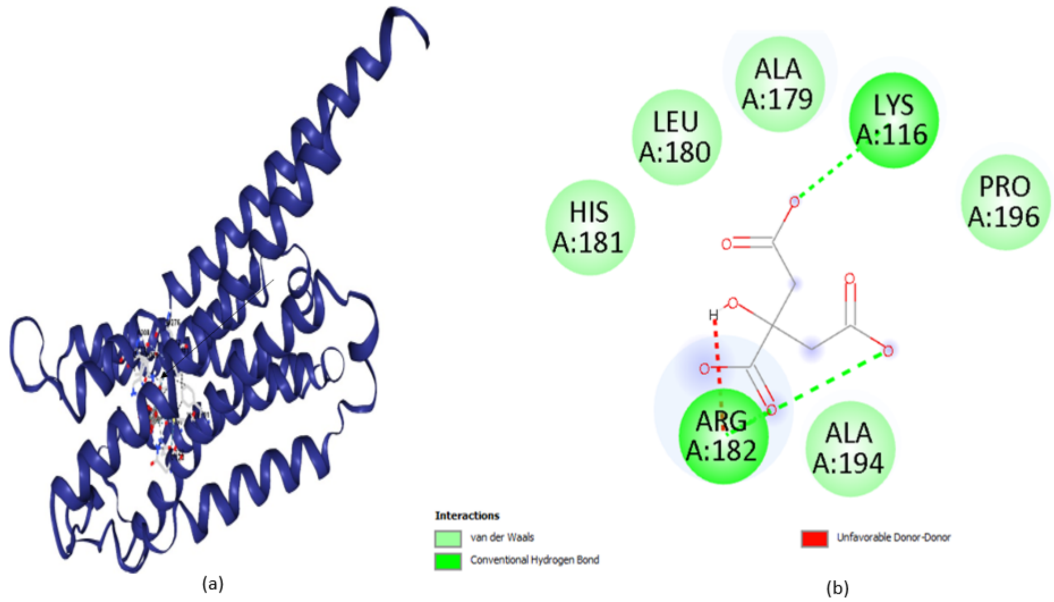


FIGURE 4.30: Docking interaction of clomiphene citrate with kisspeptin

Figure 4.30 shows the interaction of clomiphene citrate with kisspeptin. It shows there are two conventional hydrogen bonds and five van der waals interactions. Whereas in the case of interaction of phytyl with kisspeptin, figure 4.14 above, there is one pi-bond, one pi-sulfur, twelve alkyl bonds, one conventional hydrogen bond, and nine van der waals interactions. The data demonstrate that in comparison to clomiphene citrate, the phytyl shows a robust interaction profile.

Overall, the comparison of clomiphene citrate with phytyl shows that both share comparable lipinski rules and ADMET analysis. But the docking scores and receptor-ligand interaction profile of the lead compound phytyl are better than those of the standard drug clomiphene citrate. It shows that phytyl can act as a promising anti-PCOS therapeutic candidate in the future.

Chapter 5

Discussion

The exploration of *Ranunculus hirtellus* and its metabolites for their potential therapeutic effects on PCOS is an emerging area of research that combines traditional knowledge with modern scientific inquiry. PCOS is considered to be an endocrine disorder that affects a large number of women around the world. This disorder occurs due to several factors, including symptoms of menstrual problems, hyperandrogenism, and insulin resistance [108]. As it is clear that PCOS is a complex disorder, treatment is essential. The available treatment options, although effective, but result in several side effects.

Ranunculus hirtellus, a herbal plant used by different cultures for its medicinal values, has remained vastly unexplored for its potential in the management of PCOS. Various previous studies have identified bioactive compounds in the *Ranunculus* species, which have included flavonoids and phenolic compounds like quercetin and phenolic acid, that have both anti-inflammatory, anti-diabetic, and antioxidant properties [10]. As an illustration, quercetin, a type of flavonoid found in *Ranunculus hirtellus*, has proven its potential in increasing insulin sensitivity and decreasing the levels of inflammation in animal models of PCOS. This correlates with the Nielsen and Rose findings that the anti-inflammatory properties of quercetin can have the potential to greatly impact the metabolic disturbances in the pathophysiology of PCOS [109].

Additionally, the action mechanism of the aforementioned metabolites, particularly their role in the interaction with relevant proteins such as the insulin receptor and the androgen receptor, holds importance in terms of therapeutic application. The present study validates the fact that the bioactive molecules present in the *Ranunculus* species are indeed capable of modulating the hormonal imbalances associated with PCOS [110]. Various research studies indicate that though herbs like *Ranunculus* are beneficial, it needs to be considered as an element in an overall treatment strategy that includes diet and exercise for effective results [111]. Furthermore, this strategy has been in accordance with the findings in other studies and holds the fact that by making use of pharmacological and lifestyle management together, it leads to improved results in the treatment of PCOS symptoms [112]. The present study is based on the insilico analysis by molecular docking and predicting the interactions of the bioactive molecules with target proteins suggesting phytyl as the lead compound based on drug-like properties, docking score, and the nature of interactions.

Chapter 6

Conclusion and Future Prospects

The current research makes it clear that the promising therapeutic use of the metabolites found in *Ranunculus hirtellus* offers immense hope in managing the complexities of Polycystic Ovary Syndrome. With the rising cases of this disease worldwide, it has become imperative to look for efficient management techniques that not only help manage these complications but also work to reduce the side effects associated with existing treatments. The phytyl derived from *Ranunculus hirtellus*, which houses the bioactive molecules, displays excellent ADMET characteristics, docking score, and ligand interaction, making it an excellent alternative plant medicine that can be used by women with Polycystic Ovary Syndrome. However, it is important to conduct more studies to confirm these discoveries through randomized controlled trials that test their efficiency and toxicity in different populations. Moreover, research focusing on the mechanism by which it works will help in comprehending how these drugs can be used to manage Polycystic Ovary Syndrome, which revolves around their impact on metabolic pathways.

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