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Identification of Potential Inhibitors of Thrombocytopenia from *Carica papaya*

by

Fizza Jamil

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

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I dedicate this project to Allah Almighty, my creator, my strong pillar, and my source of inspiration, wisdom, knowledge, and understanding. He has been the source of my strength throughout this program.Every challenging work needs self-efforts as well as the guidance of elders especially those who are very close to our hearts. My humble efforts I dedicated to my loving and caring Father and Mother whose affection, love, encouragement and prays of day and night make me able to get such success and honor.

CERTIFICATE OF APPROVAL

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Acknowledgement

"Trust in the LORD with all your heart and lean not on your understanding; in all your ways acknowledge him and he shall direct your paths". (Proverbs 3:5, 6)

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(Fizza Jamil)

Abstract

Dengue virus infection is progressively known as the world's evolving contagious disease. It is transmitted through *A. aegypti*, a flaviviridae virus. In recent years, there has been a significant increase in the severity and incidence of dengue fever as well as deaths caused by its complications. Although there have been significant efforts made in the development of antivirals and vaccines for dengue, there is currently just supportive care available for patients. In addition to fever, patients face a major decline in the thrombocytes causing thrombocytopenia which eventually causes complications and may be mortal. It is said that *C. papaya* leaves extracts have a positive effect on thrombocyte count and patients go for a speedy recovery of platelets. The fast spread of the dengue virus has been impacting over a million people and it is now necessary to explore the potent antiviral drug for the dengue virus. This study identifies the active constituents of *C. papaya* that could counteract thrombocytopenia. *C. papaya* is a rich source of many phytochemicals, such as flavonoids, alkaloids, saponins, phenolics, glycosides, tannins, phytosterols, and terpenoids. Two proteins of dengue such as NS5 and NS2B-NS3 were reported to have a significant role in the replication of the virus, thus proposing a potential antiviral drug target. So, 20 active ingredients of *C. papaya* were chosen and docked against receptor proteins with the potential to inhibit the target protein. The most effective possible inhibitor against the dengue virus was found after physiochemical research and identification of the active domains of NS5 and NS2B-NS3 in these compounds. The docking data was evaluated and represented using PyMol and LigPlot. The Lipinski rule of five and ADMET characteristics was applied to these 20 compounds to determine their potential as drugs. The substance with the highest binding affinity and the most favorable pharmacological characteristics was revealed, is Caffeic acid. S-adenosylmethionine (SAM) for NS5 and Hydroxychloroquine (HCQ) for NS2B-NS3 was selected as their reported inhibitor compounds. The results imply that Caffeic acid may be a more effective potential inhibitor of dengue proteins. However, more investigation is required to determine their potential medical applications.

Contents

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Abbreviations

A.aegypti: *Aedes aegypti*

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

BBB: Blood-brain barrier

BITC: Benzyl isothiocyanate

C. papaya: *Carica papaya*

CADD: Combined Annotation Dependent Depletion

CB Dock: Cavity-detection guided Blind Docking

CNS: Central Nervous System

CYP: Cytochromes P450

DENV: Dengue Virus

DHF: Dengue Hemorrhagic Fever

GCMS: Gas chromatography–mass spectrometry

GRAVY: Grand average of hydropathicity

HCQ: Hydroxychloroquine

hERG: Human Ether-a-go-go-Related Gene

ICM: Internal Coordinate Mechanics

IGC_{50}: Inhibited the Growth of Cells by 50%

II: Instability index

kDa: Kilodalton

LD_{50}: Lethal Dose 50

LOAEL: Lowest-observed-adverse-effect level

MRTD: Maximum rate tolerated dose

MTase: Methyltransferase

NS: Non-structural

OCT: Organic cation transporter

ORF: Open Reading Frame

PDB: Protein Data Bank

RdRp: RNA-dependent RNA polymerase

RNA: Ribonucleic acid

SAM: S-Adenosyl methionine

VDss: Volume distribution at steady state

Chapter 1

Introduction

Indigenous knowledge about natural products is as ancient as human civilizations. Knowledge has been transferred to people from their ancestors in verbal form. Plants have been in the use of people for different purposes since old ages [1]. The significance of ethnomedicinal knowledge in this era exists in the evolution of novel drugs, however, to make certain the therapeutic value of medicinal plants, standardization of traditional medications is obvious. Much of the modern medications are the result of ancient and folklore remedies [2]. Indigenous knowledge is general knowledge of the overall ecosystem and about specific plants used as food, medicine, and building material [3]. These precious bits of knowledge have not been transferred completely as less than 1% of the knowledge of medicinal plants or other natural products has been surveyed globally [4]. In primordial eras, natural products were efficaciously used to administer various illnesses due to their improved susceptibility in the human community, better affinity with the body, and their natural influence to medicate disease by symbiotic effects and nullifying blends to minimize antagonistic effects [5]. Therapeutic plants have no or fewer adverse reactions as compared to synthetic medication [6], [7]. Medicinal herbs and plants can be used in a variety of forms such as, in fresh or powdered form, extracts, seeds, and their fruits or mixtures sometimes [8], [9]. Almost 265,000 species of plants exist on earth, however, only half of them are so far explored for their curative importance and chemical composition. According to an estimation

of about a decade, [10], [11] around 80% population depends on medicinal plants for the cure of different diseases in developing countries while 60 % population of the developed country uses these plants for various health issues [6]. Approximately 42% population in the USA, 40-50% in Germany, 48% in Australia, and about 49% population of France relies on plants for various health problems [12].

In Pakistan, around 6000 species of higher plants exist, of which 600 – 700 are used for therapeutic purposes [13]. 3000 species are reported from northern areas of Pakistan among those, 124 species have pharmaceutical significance [14], [15]. Unfortunately, Pakistan has reported only 10% remedial plants out of the total species [16]. *C papaya* is a tropical tree and its different parts have been used medicinally for the cure of a very large range of diseases such as diabetes, intestinal worms, wound restoration, dengue fever, and an abortion agent. Most commonly papaya is consumed as ripe fruit, however, plant tissues are used for therapeutic purposes including young leaves, seeds, latex, or immature green fruit. The main agent that is accountable for action has not been known but there is some evidence of activity of Benzyl isothiocyanate (BITC) in different curative perspectives. Young leaves, seeds, and latex are good sources of BITC. For speedy recovery of the burn wound, protease is used as topical use and unripe green fruit is a good source of protease. Papaya tissues give some active constituents in ailments and some activities have been exhibited by papaya extracts including hypertension, diabetes, dengue fever, etc. Still, active compounds that are responsible remain unidentified [17].

The fruit is enriched in proteins, vitamin C, fiber, amino acids, carbohydrates, and other nutrients. All tissues of papaya consist of white latex, which is rich in an enzyme known as papain that plays a crucial role in the cure of many diseases, for industrial use and as well as for drug designing for instance pharmaceutical provisions and meat tenderizers [19]. In different aspects papaya extract plays a role that may include vascular muscle relaxation, increasing blood cells count in dengue fever, lowering blood glucose levels, boosting cell proliferation in wound repair, for abortion purposes by contracting uterine muscles, etc. Even though there has been vast research in the past decade on the active site of extracts of

C. papaya, more advancement in studies may increase the knowledge of active constituents liable for therapeutic purposes. It will assist to pass the traditional knowledge of remedies to conventional medicinal usage [17].

It has been reported, that as an appetizer, the flavor-active compounds in papaya are commonly linalool and benzaldehyde. The presence of linalool could be the reason for diverse sweet flowery flavors in foods. Whereas, benzaldehyde is another appetite inducer that might lead to a range of flavors in papaya fruit with its variety of benzaldehyde products [18]. In the last 50 years, the prevalence of dengue fever is increased by 30 times worldwide, in more than 100 countries. South Western America and South East Asia are two main hotspots where the dengue virus has influenced considerable populations [23], [24]. Climate change effects are somehow responsible for making temperatures in the specific area more appropriate for breeding mosquito-borne ailments, like dengue fever [25], [26]. The statistics in more recent years have proposed that the concentration of dengue fever has transferred from mild to severe, as dengue fever in Pakistan has transformed in terms of survival rate in contradiction of several aspects [29].

At present, the unlicensed but traditional use of *C. papaya* plant extract is for dengue infection as a herbal remedy in South East Asian countries such as Pakistan, India, Sri Lanka, and Malaysia [20]. Studies suggest that *C. papaya* leaves have the capacity for antiviral action in contradiction to DENV. The dengue virus is transmitted by the bite of a female mosquito from the family Aedes aegypti and spread into the bodies of mammals including humans [21]. The initial signs of dengue disease are just like common cold and fever with the headache that increases over time. Four types of dengue viruses have been known so far named DENV1, DENV2, DENV3, and DENV4 [22]. Until now there is no antiviral drug or vaccine for the dengue virus. In addition to fever, thrombocytes face a major decline in the human body causing thrombocytopenia. The decrease in thrombocyte count results in dengue hemorrhagic fever (DHF) which ultimately causes complications and may be mortal. It is said that *C. papaya* leaves extracts have a positive effect on thrombocyte count and patients go for a speedy recovery of platelets. The fast spread of the dengue virus has been impacting over a million

people and it is now necessary to explore the potent antiviral drug for the dengue virus.

1.1 Problem Statement

Dengue infection is typically a self-limited sickness in which only supportive care is given. No vaccine is existing for the inhibition of dengue infection. Vector mosquito bite avoidance is the only way to avert dengue disease attainment. Numerous reports have proposed that *C. papaya* leaves can repair damaging effects on platelets by the dengue virus. As papaya leaves have therapeutic efficacy against dengue infection, so this study has been conducted to explore the curative agent in leaf extracts as anti-thrombocytopenia. In this study, two proteins of dengue (NS32B and NS5) has been targeted by computational study through molecular docking with active compounds of *C. papaya* leaves having antiviral properties.

1.2 Aims and Objectives

This study aims to find the potential inhibitors of thrombocytopenia caused by the dengue virus in natural anti-viral compounds of *C. papaya*. So by using computational tools we focus on protein-ligand interactions for the structural drug design against thrombocytopenia.

The objectives of this study are as follows:

1. To determine various bioactive compounds of *C. papaya* as potential inhibitors of dengue proteins.
2. To scrutinize the interaction between targeted dengue proteins and bioactive compounds of *C. papaya*.
3. To recognize the best interacting molecule that exhibits inhibitory effects against the disease.

1.3 Scope

Currently, there is no specific treatment available, the management is entirely supportive like keeping body temperature below 39°C, and giving the patient paracetamol. Advice to drink large amounts of fluids along with the patient's normal diet. According to many reports, *C. papaya* leaves extracts show positive effects on thrombocytopenia. So there is a need for exploration of certain natural potential compounds in *C. papaya* having a restraining effect against the dengue virus. Identification of inhibitory compounds against dengue disease could be done by *In-silico* molecular docking that will ultimately help in drug design to encounter an increasing death rate due to dengue fever.

Chapter 2

Review of Literature

2.1 Significance of Medicinal Plants

From prehistoric times plants have been used for therapeutic purposes. Remedies with natural products are considered to be very safe as there are no or fewer adverse reactions. Medicinal plant therapeutics are synchronized with nature and this is the prime benefit. The primordial researchers only considered that medicinal plants are merely a way to treat many problems and diseases of well-being. Many studies have been done to account for accurate conclusions in terms of the usefulness of various medicinal herbs. These drugs are free of side effects and due to this, herbal remediation is popular across the globe. The medicinal quality of these plants gives a rational method for the cure of many internal ailments, that are else complicated to cure.

According to the well-documented scientific literature, plants have been used for therapeutic purposes for about the previous 60,000 years [30]. Millions of people are dependent on medicinal herbs for their welfare across the world [31]. In rural communities, natural remedies are being used regularly as the synthetic medication is difficult to avail of or even inaccessible. This is in contradiction to westernized communities where curative floras are normally used as an unconventional or complement to recommended medication [31]. These medicinal herbs

are the important primary source of medication however, for the development of new drugs they also play a role as phytochemical building blocks [32]. As per an estimation, around 67% of drugs employed in chemotherapy are derivatives of natural products [33]. In addition to this, medicinal plants are cost-productive as they offer chances for rural inhabitants to attain cash income [34].

The traditional knowledge of herbal remedies has been transferred over the centuries among human populations. Secondary metabolites of active components are generally the cause of the biological activity of plant species used all over the globe for diverse reasons comprising cures for infectious diseases [35].

Traditional methods of medication are widely practiced for various problems. Insufficient resources of medications, unwanted effects of numerous synthetic medicines, population intensification, an unaffordable budget of cures, and improvement of resistance to presently utilized drugs for contagious ailments have led to amplified prominence of the consumption of plant materials as a source of remedies for an extensive range of human disease. The best advantage to the use of herbal medicines is independent of any age group and the sexes. In the previous few years, several new compounds have been sequestered from marine creatures from which many of the constituents have been explored to possess intriguing biological activities [36]. Presently the major focus of many researchers is on the isolation of pharmacologically active constituents obtained from natural sources in the region of such diseases where presently accessible medicines are not so beneficial. Herbal medicines are nowadays experiencing a greater revival as people are returning to the base for their well-being because nature is the solution to every problem. Herbal remedies are now known as alternative medicines [37].

2.2 *Carica papaya*

Papaya (*Carica papaya L.*) is an important fruit crop nurtured in tropical and subtropical zones. Papaya originated from Costa Rica or Southern Mexico afterward it was familiarized as a plantation crop in Hawaii, Sri Lanka, Australia, South Africa,

India, and in all tropical and sub-tropical regions. It is grown both commercially and domestically [38]. Papaya is a common man fruit available cheaply but has enriched in nutritive value. It is rich in natural vitamins and minerals. Papaya is low in calories and carotene in comparison to other fruits like plantains, apple, or guava that aid in the prevention of damage by free radicals. Immature green papaya is used as a vegetable that contains all nutrients except carotene [39]. Each component of the papaya tree has significant economic worth due to which it is now grown on a commercial scale. In last recent decades, there is substantial advancement regarding the natural and biological activity as well as remedial application of papaya due to which it is now considered a beneficial nutraceutical fruit plant.

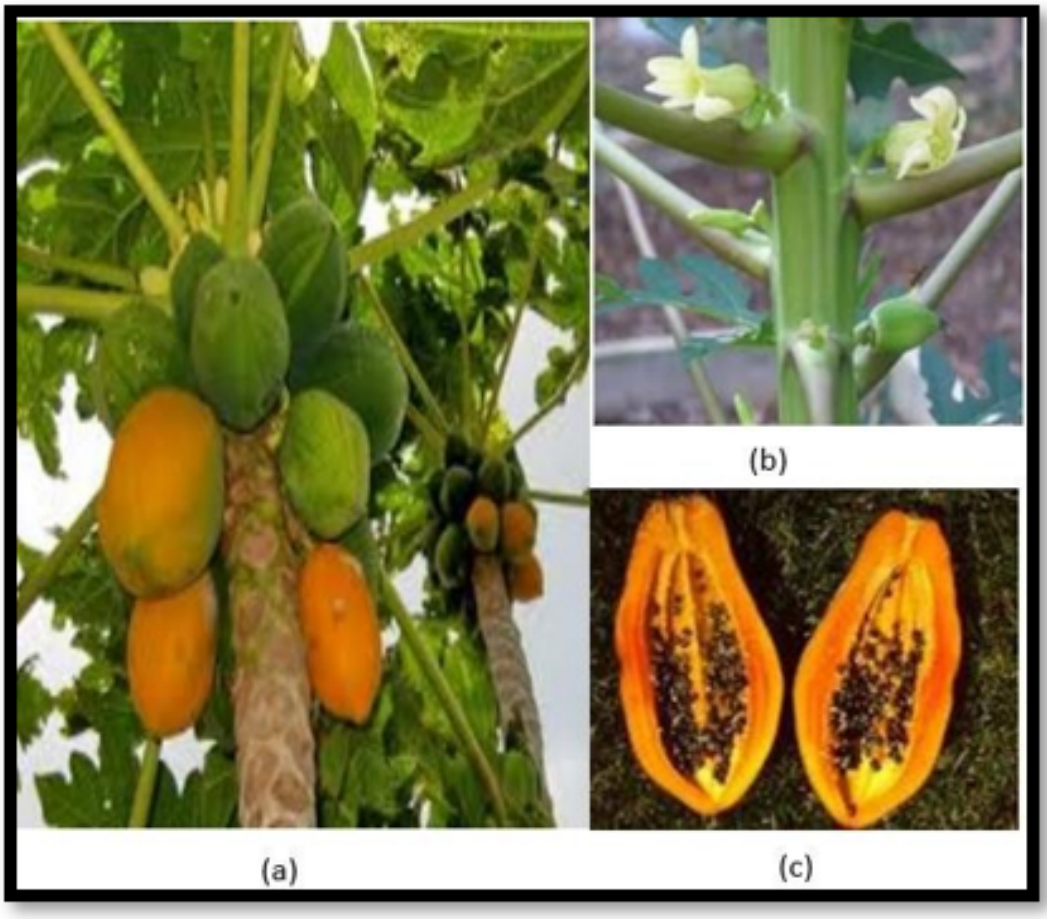


FIGURE 2.1: *C. papaya* plant with fruits (a), *C. papaya* flower (b), C. papaya fruit with its seeds (c) [93].

Some of the uses of every part of *C. papaya* are as follows as shown in table 2.1, [38], [39]:

TABLE 2.1: Pharmacological & Medicinal properties of different parts of the C. papaya plant

Parts of plant	**Uses**
Leaves	Inhibition of cancer cells, Anti-malarial & Anti-plasmodial, Facilitates digestion, Enhances thrombocytes in dengue fever, Meat tenderizer.
Fruit	Regularize bowel movement, 'Papain' is used for indigestion, Void the stroke or heart attack.
Seeds	Antibacterial, Used as a skin irritant, Improves digestion.
Peel	Skin lightening agent, Pain reliever, Muscle relaxant.
Roots	Ease urinary trouble, Uses in cure of dyspepsia.
Latex	Cures diarrhea, bleeding hemorrhoids, whooping cough.

2.3 Morphology of *Carica papaya*

The papaya plant is an evergreen tree-like herb with a single stem and thin branches. Its average height is almost 5-10m. The plant's leaves are arranged in an umbrella-like canopy to the top of the stem. Leaves are about 20-18 inches in diameter and are palmately lobed leaves. It contains white latex in all parts of

the plant [40]. Its fruit is melon-like. Its shape varies from round to oblong with the size of 3 to 5 inches in diameter. The peel is thin and smooth, and its color change from green (immature fruit) to deep orange-yellow (ripe fruit). The inner flesh is 1 to 2 inches in thickness, mildly sweet in taste and color varies from pale yellow to deep salmon-pink. Fruit also contains numerous black round seeds [42].

The flowers of *C. papaya* are 5-parted yellowish-white petals and are very dimorphic. Petals are a fusion of male and female flowers. The ovary is present in the female flower and five petals are connected with the base [40]. Papaya is a polygamous species with the three classifications of primary sex type. It includes,

1. Female (pistillate)
2. Hermaphroditic (bisexual)
3. Male (staminate)

Moreover, some plants produce more than one type of flower at the same time. Some species of papaya exhibit a variety of degrees of femaleness and maleness. This affinity to alter sexual expression is triggered by climate influences like flexible temperature and drought [41].

2.4 Taxonomy of *Carica papaya*

The taxonomy of the *C. papaya* as shown in table 2.2 below .

TABLE 2.2: Taxonomy of *C. papaya* [93]

Domain	**Flowering Plant**
Kingdom	Plantae
Sub Kingdom	Tracheobionta
Class	Magnoliopsida
Subclass	Dilleniidae
Subdivision	Spermatophyta

TABLE 2.2: Taxonomy of *C. papaya* [93]

Domain	**Flowering Plant**
Phylum	Steptophyta
Order	Brassicales
Family	Caricaceae
Genus	*Carica*
Botanical Name	*C. papaya*

2.5 Phytochemical Composition of *Carica papaya*

Leaves of *C. papaya* are comprised of flavonoids, alkaloids, tannin, saponin, and glycosides. Ca, Mg, Mn, Zn, K, Fe, and various other minerals are present in its shoots. Papain and chymopapain enzymes are present in unripe or immature fruit. The chemical composition of mature ripen fruit shows the presence of carotenoids such as cryptoxanthin and Beta carotene. Roots contain glucosinolates carposide and benzyl isothiocyanate. The fruit of papaya contains mono-terpenoids, 4-terpinol and Linalool however seed's oil fruit comprises flavonoids, myricetin, and kaemferol [43].

According to studies enzymes found in latex are papain, chymopapain, Protease omega, and caricain [44], [45], [46]. Some other reports show that Latex of *C. papaya* also contains enzymes named chitinase, glutaminyl cyclase, and cysteine endopeptidases [47].

2.6 *Dengue Fever*

Dengue fever is a flu-like sickness that influences people in every age group i.e infants, young children, and adults. Its symptoms may vary regarding the age of

the patient. Slight feverish sickness with a little bit of rash is the symptom of disease in infants. While older children and adults may face a mild fever or the usual harsh disease with sudden onset and high febrile condition, pain behind the eyes, headache, muscle, and joint pains, and rash [48]. Transmission of dengue fever virus is through a female mosquito *A. aegypti*, which transfers the disease by feeding on the blood of an infected person to the non-infected. The habitation of this species of mosquito is indoors, in closets, and in other dark places around human dwellings, however, it rests in a cool and shady area, outside. Stored and exposed water are the favored breeding places. One of the four different serotypes of the dengue virus is responsible for infection (DENV1, DENV2, DENV3 and DENV4).

FIGURE 2.2: Dengue spread by *A. aegypti* [94].

Dengue virus infection is progressively known as the world's evolving contagious disease. Almost 500,000 victims of Dengue Hemorrhagic Fever (DHF) and 50-100 million dengue fever infectants are reported every year with around 24,000 deaths cases [49]. In Pakistan, the first case was testified in 1994; though, it properly gained consideration in the mid-2000s when the infectants count has been elevated

in the coastal areas of Karachi [54]. The dengue virus exists in the tropical and subtropical areas of Pakistan as it is a favorable environment for the virus [50], [51], [52]. Heavy rainfalls in the monsoon season may intensify the dengue cases in Pakistan at that time [53].

Dengue virus is an enveloped single-stranded RNA virus. The virion has a diameter of 30nm and possesses a spherical nucleocapsid core and icosahedral envelope organization [55], [56]. DENV genome is 11kb long with a 5'cap at the 5'end. This genome lacks a poly-A tail [57]. The genome of this virus comprises mRNA ORF (Open Reading Frame) for translation, which gives two peptides (2k and ER), three structural proteins (envelope (E), capsid (C), membrane (M)), and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [58]. There is no particular cure for dengue fever. Victims of this virus should seek medical assistance, take rest and stay hydrated. Supportive medication could be taken to control the fever and some pain killer for body aches [54].

2.7 Molecular Docking

From small molecule databases, it is now possible to discover novel potential inhibitors against the target of concern. Molecular docking is the computer modeling that envisages shaping a receptor-ligand complex. To predict the feasible conformations of a binary complex, every docking program makes use of one or more precise search algorithms [59].

Molecular docking is a technique that estimates the possible orientation, affinity, and association of a ligand in the binding site of a protein. Information on orientation could be used to determine the accurate structure of the ligand molecule within the target binding site through a special scoring function. The input of docking is the 3D structures of the target proteins and the ligands which represents a frequently used approach in structure-based drug design. The strength of the binding affinity among ligand molecule and drug target is determined in docking through the scoring function [60]. Moreover, it also aids in revealing the

essential properties, such as high binding affinity, and the reasonable mechanism of absorption, excretion, distribution, and metabolism help in the selection of lead for the target [61]. Ligand and protein docking is one of the main key areas of molecular docking, as structure- based drug designing plays a significant role in its acceptance and appreciation. Molecular docking could be done by various software such as CB Dock, ICM, Auto Dock, Auto Dock vina, etc. The most used algorithms in molecular docking are the distance geometry method, molecular dynamics and genetics algorithm, etc [62].

2.8 Targeted Protein

2.8.1 NS5

The largest and most conserved protein among all viral proteins is NS5 (103 kDa) [71]. NS5 protein is almost 900 amino acids long and contains an RNA- dependent RNA polymerase domain at its C-terminal end and a methyl-transferase domain at its N terminus. The enzymatic activities from both termini provide an attractive target for antiviral drug development [72]. These enzymes codified by NS5 demonstrate that they have a significant role in the replication of the virus, thus proposing NS5 as a potential antiviral drug target. 320-368 residues are precisely conserved among the dengue viruses and are implicated to interact with NS3 [73]. NS5 protein is confined within the nucleus in DENV infections, however not all flavivirus RNA-dependent RNA polymerase is localized to the nucleus. Its definite enzymatic functions in the virus life cycle are vital in the cytoplasm and are presently unidentified but are dynamically being investigated [74].

2.8.2 NS2B-NS3

NS2B-NS3 protease complex is a serine protease that belongs to the family of chymotrypsin with a standard Ser-His-Asp catalytic triad [63]. NS3 protease

(NS3pro185; amino acids1476 to 1660) requires a cofactor for its proper functioning and participation in substrate recognition, NS2B (amino acids 1394 to 1440) is referred to as that cofactor [64], [65]. The cleavage at 8 of the 13 polyprotein cleavage positions occurred due to the dengue virus protease complex [66]. For the maturation of the viral particle, these cleavages are required. However, these cleavages make the dengue virus NS2B-NS3 protease complex a perfect target for drug development. The dengue virus proteases are important for viral replication and infectivity, including NS2B-NS3. In yellow fever virus is genetically altered and contains an inactive NS2B-NS3 protease so it is unable to infect the target cells [67]. Likewise, a report shows that the inhibition of NS2B-NS3 protease reduces dengue virus infections by up to 80 % through the treatment of cells with a peptide [68]. Trypsin identifies sites that contain a single cationic residue, however, NS2B-NS3 protease distinguishes sites that consist of two cationic residues. So it has been a requirement for the development of novel classes of inhibitors for directing the active sites [69]. The structure of NS3 suggests that its domain encoded the functional protease however, further studies show that NS2B cofactor is crucial for NS3 protease activation [65].

2.9 Inhibitors of Compounds in *Carica papaya*

Natural compounds are a significant source for the development of new antiviral drugs. The aqueous leaf extracts of *C. papaya* show potential anti-dengue activity, especially in the case of thrombocytopenia. A report shows that the administration of leaf extracts increased platelet counts from 55x103/microliter to 168x103/microliter in a dengue-infected patient [76]. Phytochemical analysis of leaves of *C. papaya* reveals the occurrence of carbohydrates, flavonoids, alkaloids, saponins, phenolics, glycosides, tannins, phytosterols, and terpenoids [77]. An analysis states that the *C. papaya* leaves extract comprises phenolic compounds, such as caffeic acid, kaempferol, quercetin, chlorogenic acid, protocatechuic acid, p-coumeric acid, and 5,7-dimethoxycoumarin [78]. A study shows the presence of 21 compounds in the aqueous leaf extracts of *C. papaya*. They are named: Ascorbic

acid, kaempferol, deoxykaempferol, quercetin, deoxyquercetin, coumaroylquinic acid, carpaine, tocopherol, dicoumarol, p-coumaroyl alcohol, cysteine, homocysteine, folic acid, cysteine sulphoxide, dimethoxy phenol, coumarin, glutamic acid, phenylalanine, caffeoyl alcohol, umbelliferone and methyl nonyl ketone [92].

Chapter 3

Research Methodology

3.1 Methodology Flowchart

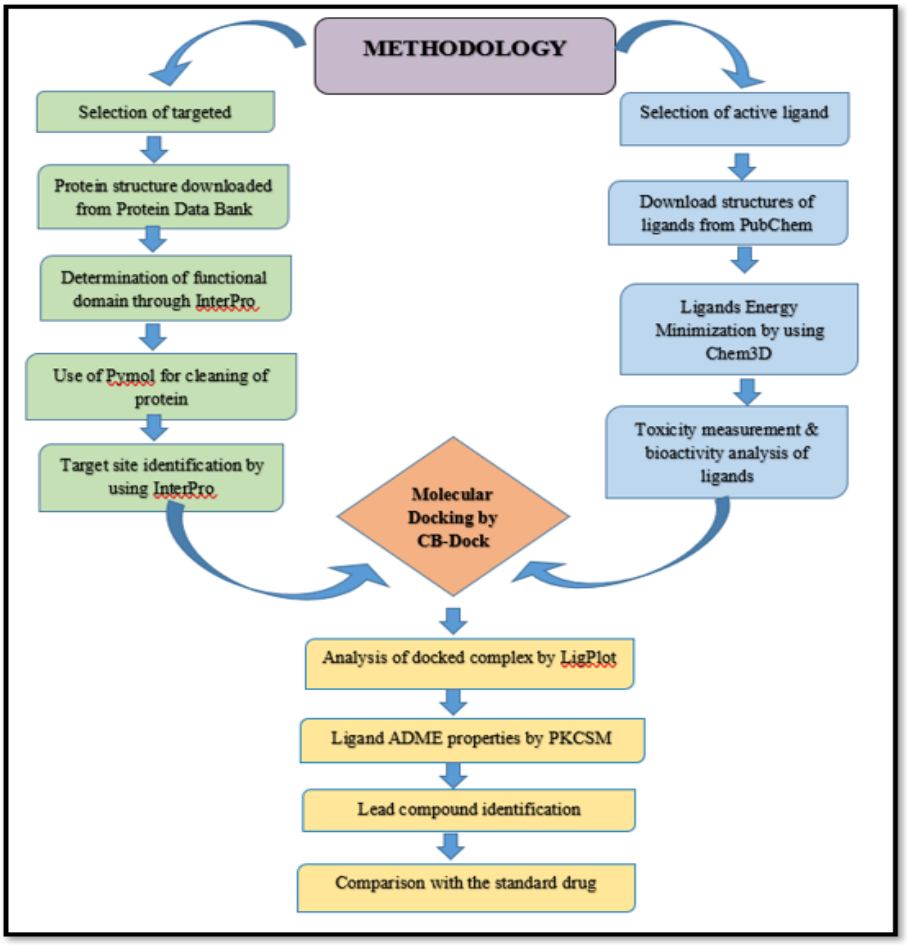


FIGURE 3.1: The flowchart of research methodology.

3.2 Selection of Disease

Dengue virus (DENV) is transmitted to the human body by *A. aegypti*. In tropical and subtropical areas, around 2.5 billion people are at risk of infection [79]. It exists in four distinguished serotypes (DENV-1, DENV-2, DENV-3 and DENV-4). Among four serotypes, infection with any of them may result in a wide range of clinical symptoms [80]., such as from mild-flu-like syndrome to the most severe form of disease i.e., thrombocytopenia, which may be fatal. The formation of a potent vaccine remains global health precedence. A tetravalent vaccine is required that should be effective against all four serotypes of the dengue virus.

3.3 Selection of Protein

As there are four serotypes of DENV, those four serotypes have a cosmopolitan genotype DENV-2 [81]. NS3 is a multifunctional protein with serine protease, that binds with the NS2B cofactor which involves the cleavage of DENV polyprotein [82]. The NS2B-NS3 protease complex shows the major involvement of processes of the viral polyprotein and virus replication [83]. Another protein NS5 also plays a vital role in virus replication [84]. Thus, both proteins are an attractive target for the development of antiviral therapeutics [82], [83]. The 3D structures of both the proteins have been downloaded from the Protein Data Bank (PDB) in .pdb format (PDB ID of NS2B-NS3: 5GXJ and NS5: 5ZQK).

3.4 Analysis of Physiochemical Properties

The study of chemical and physical properties plays a significant role in determining the function of proteins. A tool of Expasy, i.e ProtParam, is used to predict these properties.

Properties include no. of amino acids, molecular weight, instability index, isoelectric point, No. of positively charged residues (Arg + Lys), No. of negatively

charged residues (Asp + Glu), grand average of hydropathicity, and atomic composition will be evaluated by using ProtParam [85].

3.5 Distinguishing the Functional Domains of the Targeted Proteins

For analysis of protein, its functional sites, family classification, and prediction of domains, a database has been used named InterPro. This database uses predictive models, termed signatures, through a variety of databases and forms an Interprofessional Consortium [86]. We will get the polypeptide binding sites and homodimer interfaces by adding the FASTA sequence of the main protease in InterPro.

3.6 Identification of Active Site

With the protein, the ligand shows the maximum interaction on the active site of the target protein. Amino acids play a vital role in the formation of a ligand-protein complex. These protein-binding pockets are identified by CASTp [87].

3.7 Preparation of Ligands

The world's largest assortment of freely available chemical information is the PubChem. We will get a 3-dimensional (3D) structure of ligands from PubChem. Chemical information consists of chemical names, simple and 3D structures, molecular formulas, their isomers, canonical similes, and other information about molecular activities [88].

From the PubChem, the structure of ligands will be downloaded and the MM2 energy of the ligands will be minimized by using Chem3D ultra. If in case the

targeted structures are not available on PubChem, we can draw them on software ChemDraw by inserting canonical similies taken from data available on PubChem. After minimizing the MM2 energy ligand's structure will be downloaded in .sdf format.

3.8 Bioactivity Analysis of Ligands and Toxicity Measurements

Chemical compounds that were used as ligands should follow the Lipinski rule of five which is a rule to evaluate the effectiveness of an orally active drug. The potential success of a compound relies on its ADMET properties. PkCSM (https://omictools.com/pkcsm-tool) is an online tool that aids in finding the ADMET properties of compounds [89].

The Lipinski rules are as follows:

- The value of logP of most "drug-like" molecules should be confined to 5.
- Molecular weight should be below 500 g/mol.
- The maximum No. of hydrogen bond acceptors should be 10.
- The Maximum No. of hydrogen bond donors should be 5.

3.9 Molecular Docking of Ligands and Proteins

Molecular docking helps to find the best conformational interaction among ligands and targeted proteins. It will be done by Cavity-detection guided Blind Docking (CB-Dock) which recognizes the binding sites and performs molecular docking with AutoDock Vina [90]. We will get docked results of interactive 3D visualization in 5 different poses by uploading the 3D structure of proteins (pdb format) and ligands (sdf format). Selection of best pose is done based on minimum vina score.

3.10 Visualization of Docked Molecule by PyMol

An open-source molecular visualization is available, named as PyMol. It can give high- quality 3D images of proteins, nucleic acids, small molecules and electron densities, etc. Docked complex of protein and ligand will be visualized by PyMol [91]. Docked poses produced by CB- Dock will be visualized by PyMol.

3.11 Analysis of Docked Complex by LigPlot

LigPlot is used for the analysis of docked complex, that automatically gives schematic diagrams of ligand-protein interactions (.pdb format). These interactions are altered by hydrogen bonds and through hydrophobic contacts. LigPlot gives 2D schematic depictions of the ligand-protein complex which enables the prompt assessment of many enzyme complexes [92].

3.12 Ligand ADME Properties

The study of pharmacokinetics and toxicity properties is done after the analysis of docked complex. The weak drug candidates would be eliminated in the early stages of preclinical ADME, leaving behind the potential drug candidates selected against the disease. Optimization of ADME properties of drug molecules will be done by PkCSM [87], [93].

3.13 Identification of Lead Compounds

The most active inhibitor will be determined after a comprehensive analysis of protein and ligand interactions, docking scores, and toxicity studies. The nominated compound is our lead compound [89], [94].

Chapter 4

Results and Discussions

4.1 Sequence Retrieval of Protein

4.1.1 NS5

The FASTA sequence of NS5 protein obtained from PDB is as under: 5ZQK-1—Chains A, B—Non Structural Protein 5—Dengue virus 2 (11060)

>5ZQK_1|Chains A, B|Non Structural Protein 5|Dengue virus 2 (11060)

MHHHHHHSSGVDLGTENLYFQSMGTGNTGETLGEKWKNRLNALGKSEFQIYKKSGIQEV
DRTLAKEGIKRGETDHHAVSRGSAKLRWFVERNLVTPEGKVVDLGCGRGGWSYYCGGL
KNVKEVKGLTKGGPGHEEPIPMSTYGWNLVRLQSGVDVFFTPPEKCDTLLCDIGESSPNPT
VEAGRTLRVLNLVENWLNNNTQFCIKVLNPYMPSVIEKMEALQRKYGGALVRNPLSRNS
THEMYWVSNASGNIVSSVNMISRMLINRFTMRHKKATYEPDVDLGSGTRNIGIESETPNL
DIIGKRIEKIKQEHETSWHYDQDHPYKTWAYHGSYETKQTGSASSMVNGVVRLLTKPWD
VIPMVTQMAMTDTTPFGQQRVFKEKVDTRTQEPKEGTKKLMKITAEWLWKELGKKKTP
RMCTREEFTRKVRSNAALGAIFTDENKWKSAREAVEDSGFWELVDKERNLHLEGKCETC
VYNMMGKREKKLGEFGKAKGSRAIWYMWLGARFLEFEALGFLNEDHWFSRENSLSGVE
GEGLHKLGYILRDVSKKEGGAMYADDTAGWDTRITLEDLKNEEMVTNHMEGEHKKLAE
AIFKLTYQNKVVRVQRPTPRGTVMDIISRRDQRGSGQVVTYGLNTFTNMEAQLIRQMEGE
GVFKSIQQLTATEEIAVKNWLVRVGRERLSRMAISGDDCVVKPLDDRFASALTALNDMG
KVRKDIQQWEPSRGWNDWTQVPFCSHHFHELIMKDGRVLVVPCRNQDELIGRARISQGA
GWSLRETACLGKSYAQMWSLMYFHRRDLRLAANAICSAVPSHWVPTSRTTWSIHATHE
WMTTEDMLTVWNRVWIQENPWMEDKTPVESWEEIPYLGKREDQWCGSLIGLTSRATWA
KNIQTAINQVRSLIGNEEYTDYMPSMKRFRREEEEAGVLW

FIGURE 4.1: Sequence Retrieval

The structure of NS5 that is available on PDB is shown in Figure 4.2

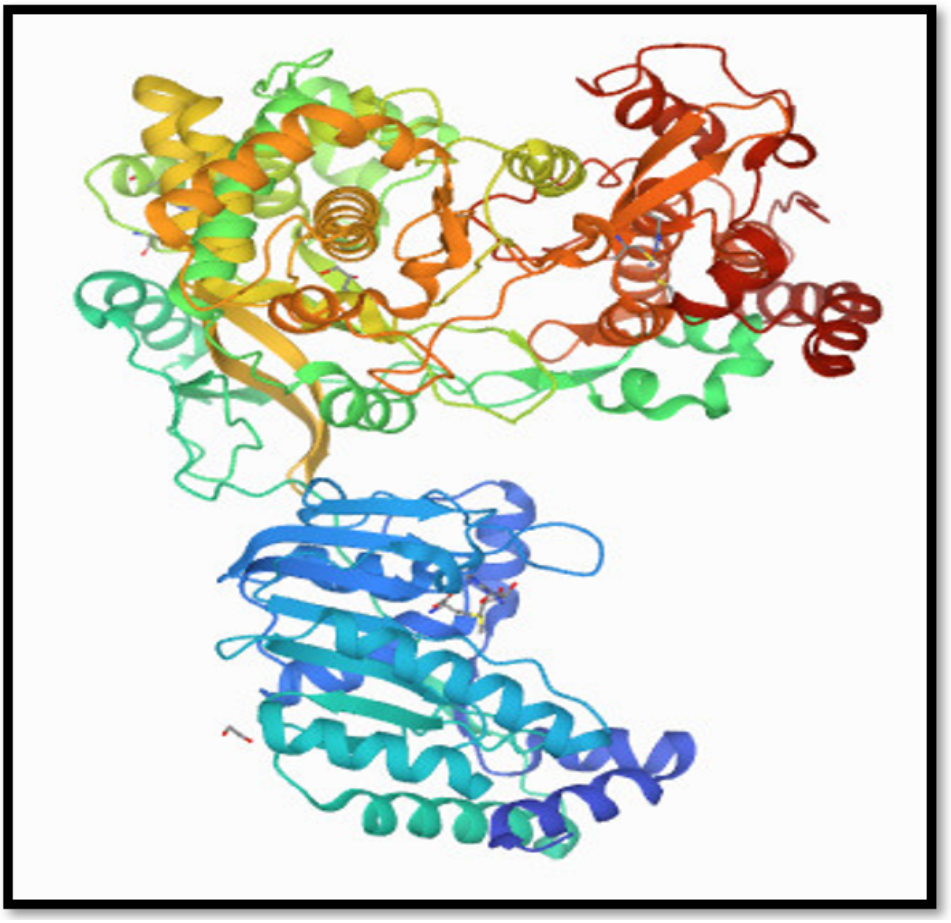


FIGURE 4.2: Structure of NS5 Protein

Non-structural protein 5 (NS5) of Flavivirus comprises a C-terminal polymerase (RNA-dependent RNA polymerase [RdRp]) domain and an N-terminal methyltransferase (MTase) domain bonded by a 9-amino-acid linker. The NS5 domains are structurally conserved in the full-length protein. Targeting the NS5 protein is a likely approach for generating attenuated flavivirus strains for vaccine proposal [95].

4.1.2 NS2B-NS3

The FASTA sequence of NS2B-NS3 protein obtained from PDB is as under: 5GXJ-1—Chains A, B—FLAVIVIRUS-NS2B, LINKER, Peptidase S7—Zika virus (64320)

>5GXJ_1|Chains A, B|FLAVIVIRUS_NS2B, LINKER, Peptidase S7|Zika virus (64320)

SVDMYIERAGDITWEKDAEVTGNSPRLDVALDESGDFSLVEDDGP
GGGGSGGGGSGALWDVPAPKEVKKGETTDGVYRVMTRRLLGST
QVGVGVMQEGVFHTMWHVTKGSALRSGEGRLDPYWGDVKQDL
VSYCGPWKLDAAWDGHSEVQLLAVPPGERARNIQTLPGIFKTKD
GDIGAVALDYPAGTSGSPILDKCGRVIGLYGNGVVIKNGSYVSAIT
QGRR

FIGURE 4.3: Sequence Retrieval

The structure of NS2B-NS3 that is available on PDB is shown in Figure 4.4

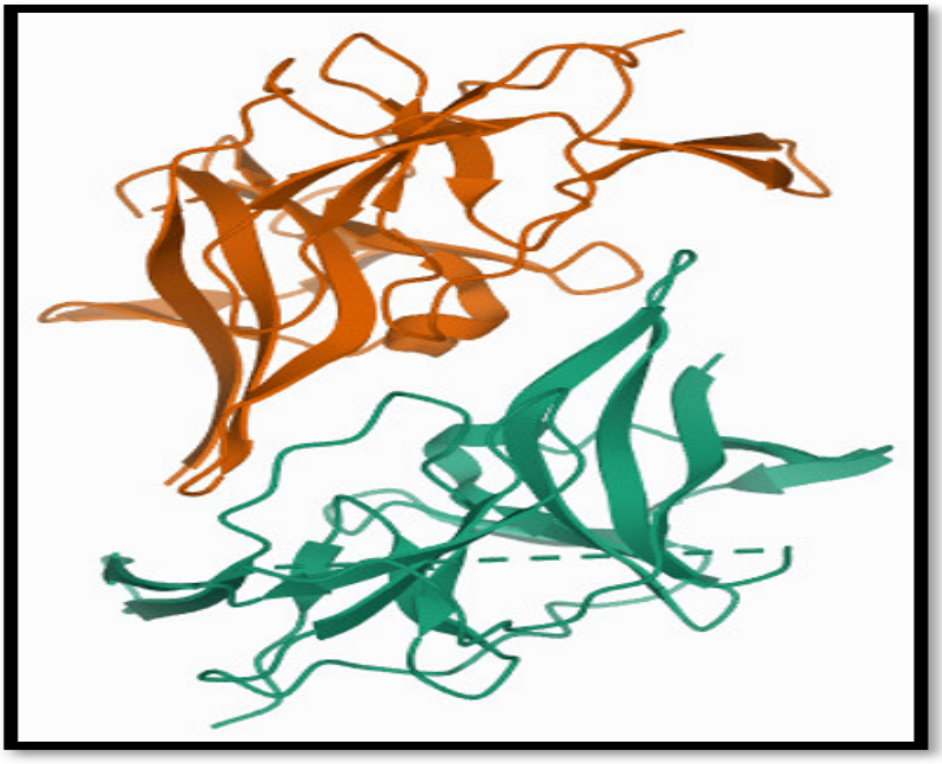


FIGURE 4.4: Structure of NS2B-NS3 Protein

NS2B-NS3 protease, a non-structural protein complex, is a good and attractive antiviral target as it plays a significant role in the maturation of viral non-structural

proteins [96]. This protease is a two-component serine protease. The N terminal part of NS3 and the cofactor of NS2B fused to become a membrane protein vital for the membrane site of NS3 [97].

4.2 Analysis of Physicochemical Properties of Targeted Proteins

Various chemical and physical parameters of nominated proteins can be found by a tool, ProtParam [98]. Analysis of physicochemical parameters reveals that the length of NS5 and NS2B-NS3 polypeptides are 923 and 224 amino acids long respectively. Whereas, NS5 and NS2B-NS3 contain a molecular weight of 105748.25 da and 23681.49 da. It shows a stable protein. Table 4.1 shows the physicochemical properties of NS5 and NS2B-NS3.

TABLE 4.1: Physiochemical properties of NS5 & NS2B-NS3

Parameters	**NS5**	**NS2B-NS3**
Molecular weight	105748.25 Dalton	23681.49 Dalton
No. of amino acids	923	224
Theoretical pI	8.58	5.01
Instability index (II)	36.44 (stable)	29.85 (stable)
No. of negatively charged residues (Asp+ Glu)	119	30
No. of positively charged residues (Arg+Lys)	127	23
Aliphatic index	70.85	78.26
Grand average of hydropathicity (GRAVY)	-0.620	-0.0330

TABLE 4.1: Physiochemical properties of NS5 & NS2B-NS3

Parameters	**NS5**	**NS2B-NS3**
Atomic composition	Carbon-4664	Carbon-1039
	Hydrogen-7296	Hydrogen-1629
	Nitrogen-1338	Nitrogen-293
	Oxygen-1381	Oxygen-329
	Sulphur-48	Sulfur-6
Total no. of atoms	14727	3296
Amino acid composition	Ala-47 (5.1%)	Ala-14 (6.2%)
	Arg-63 (6.8%)	Arg-12 (5.4%)
	Asn-44 (4.8%)	Asn-4 (1.8%)
	Asp-40 (4.3%)	Asp-19 (8.5%)
	Cys-14 (1.5%)	Cys-2 (0.9%)
	Gln-31 (3.4%)	Gln-6 (2.7%)
	Glu-79 (8.6%)	Glu-11(4.9%)
	Gly-76 (8.2)	Gly-37 (16.5)
	His-27 (2.9%)	His-3 (1.3%)
	Ile-41 (4.4%)	Ile-9 (4.0%)
	Leu-70 (7.6%)	Leu-16(7.1%)
	Lys-64 (6.9%)	Lys-11(4.9%)
	Met-34 (3.7%)	Met-4 (1.8%)
	Phe-25 (2.7%)	Phe-3 (1.3%)
	Pro-31 (3.4%)	Pro-11 (4.9%)
	Ser-56 (6.1%)	Ser-15(6.7%)
	Thr-64 (6.9%)	Thr-12(5.4%)
	Trp-33 (3.6%)	Trp-6 (2.7%)
	Tyr-24 (2.6%)	Tyr-7 (3.1%)
	Val-60 (6.5%)	Val-22 (9.8%)
	Pyl-0 (0.0%)	Pyl-0 (0.0%)
	Sec-0 (0.0%)	Sec-0 (0.0%)

4.3 Identification of Functional Domains

Numerous proteins have active regions called functional domains that participate in interactions between proteins and other substances [114]. NS5 has six functional domains and their residues i.e., Pyrv/Fd/Flavodoxin Oxrdtase N (residues 6 to 243) , PFOR II (residues 265 to 347), Pyrv/ketoisovalerate Oxred cat (residues 426 to 610), 4Fe4S Fe-S-bd (residues 680 to 709) and 4Fe4S Fe-S-bd (residues 736 to765). Figure 4.5 shows the functional domains of protein NS5.

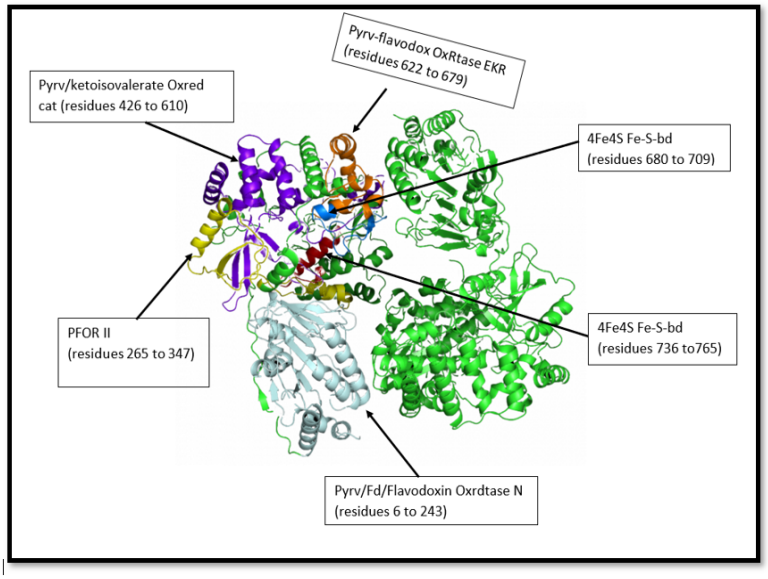


FIGURE 4.5: Functional Domain of Selected Protein NS5

NS2B-NS3 has two functional domains and their residues i.e., Flavi-NS2B (residues 1-83) and Flavivirus-NS3-S7 (residues 55-224). Figure 4.6 shows the functional domains of protein NS2B-NS3.

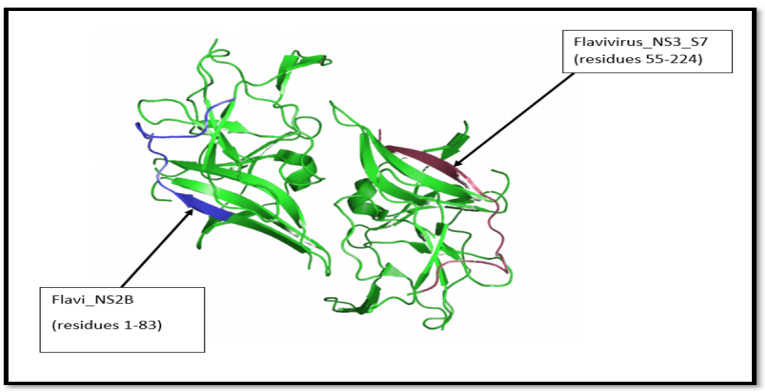


FIGURE 4.6: Functional Domain of Selected Protein NS2B-NS3

4.4 Structure of Protein Cleaned for Docking

The targeted proteins are cleaned in PyMol which will be used for molecular docking. Inhibitors were removed from the molecule and now the refined structures (Figures 4.7 and 4.8) of both proteins are all set for docking.

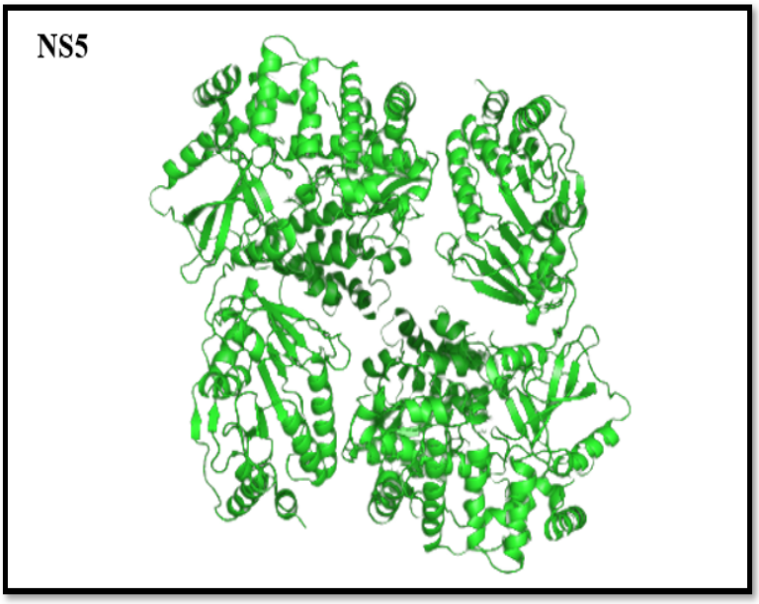


FIGURE 4.7: Refined structure of NS5

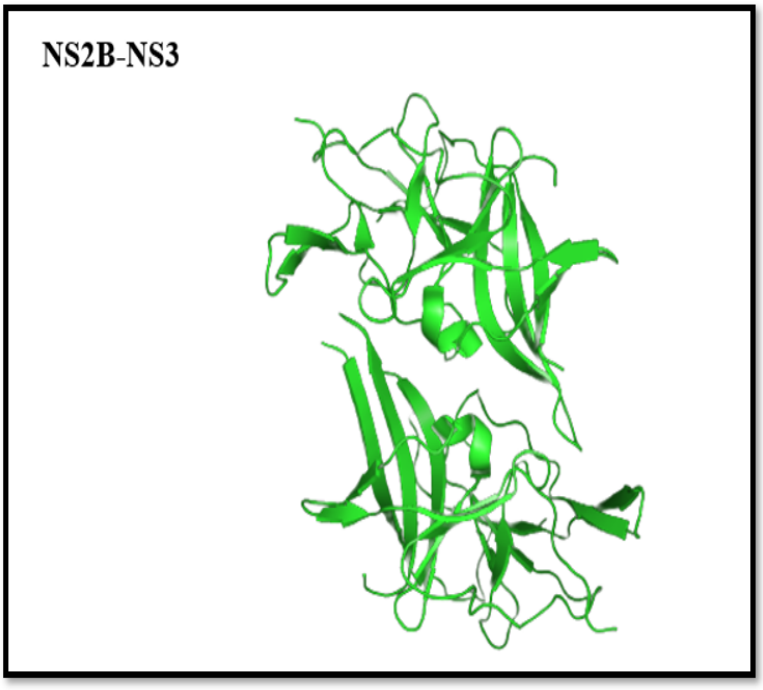


FIGURE 4.8: Refined structure of NS2B-NS3

4.5 Ligand Selection

Leaves of *C. papaya* contain many bioactive compounds which show a potential to target the dengue proteins. Selected proteins are involved in the process of replication and thus proven as a potential target for the discovery of drugs [72]. The discovery of structures of proteins i.e NS5 and NS2B-NS3 gives an inordinate prospect to find potential drugs as a cure for thrombocytopenia.

Table 4.2 illustrates the names and structures of selected ligands along with their molecular weight, molecular formula and minimized energies. Structures were obtained from PubChem and their energies have been minimized through Chem3D.

TABLE 4.2: Selected ligands from *C. papaya*

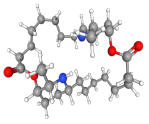
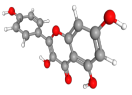
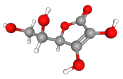
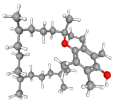
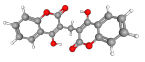
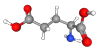
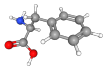
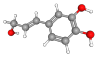
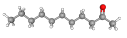
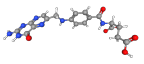
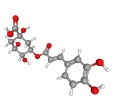
Compounds names	**Molecular weight**	**Molecular formula**	**Minimized Energy kcal/mol**	**Structure**
Carpaine	478.7	$C_{28}H_5ON_2O_4$	55.7125	
Kaempferol	286.24	$C_{15}H_{10}O_6$	21.7794	
Ascorbic acid	176.12	$C_6H_8O_6$	12.7338	
Tocopherol	430.7	$C_{29}H_{50}O_2$	22.4002	
Dicoumarol	336.3	$C_{19}H_{12}O_6$	7.7598	
Cysteine	121.16	$C_3H_7NO_{23}S$	-0.7090	
Homo-cysteine	135.19	$C_4H_9NO_2S$	-0.1615	
Dimethoxy phenol	154.16	$C_8H_{10}O_3$	4.1876	

TABLE 4.2: Selected ligands from *C. papaya*

Compounds names	**Molecular weight**	**Molecular formula**	**Minimized Energy kcal/mol**	**Structure**
Coumarin	146.14	$C_9H_6O_2$	9.5426 kcal/mol	
Glutamic acid	147.13	$C_5H_9NO_4$	-0.6497	
Pheny-lalanine	165.19	$C_9H_{11}NO_2$	-3.5146	
Caffeoyl alcohol	166.17	$C_9H_{10}O_3$	-7.4674	
Umbelli-feron	162.14	$C_9H_6O_3$	8.5749	
Methyl nonyl ketone	170.29	$C_{11}H_{22}O$	13.0070	
Folic acid	441.4	$C_{19}H_{19}N_7O_6$	18.5989	
Caffeic acid	180.16	$C_9H_8O_4$	-7.2745	
p-coumaric acid	164.16	$C_9H_8O_3$	-0.5365	
5,7-dimethoxy coumarin	206.19	$C_{11}H_{10}O_4$	24.9949	
Chlorogenic acid	354.31	$C_{16}H_{18}O_9$	13.9129	
Protocatechuic acid	154.12	$C_7H_6O_4$	-4.5060	

4.6 Virtual Screening

An online tool (PkCSM) has been used to find the Lipinski rule of five. According to that rule, the logP value of the molecule should be limited to 5, molecular weight should be below 500, the maximum number of Hydrogen bond acceptors should be 10 and the maximum number of Hydrogen bond donors should be 5 [89]. The Lipinski Rule of Five has been applied to selected ligands and the results are given in Table 4.3.

TABLE 4.3: Applicability of Lipinski Rule on Selected Ligands

Ligands	**LogP Value**	**Molecular Weight (g/mol)**	**Rotatable Bonds**	**H-bond Acceptor**	**H-bond Donor**
Carpaine	5.566	478.718	0	6	2
Kaempferol	2.282	286.239	1	6	4
Ascorbic acid	-1.407	176.124	2	6	4
Tocopherol	8.840	430.717	12	2	1
Dicoumarol	2.901	336.299	2	6	2
Cysteine	-0.671	121.161	2	3	3
Homocysteine	-0.281	135.188	3	3	3
Dimethoxy phenol	1.409	154.165	2	3	1
Coumarin	1.793	146.145	0	2	0
Glutamic acid	-0.736	147.13	4	3	3
Phenylalanine	0.641	165.192	3	2	2
Caffeoyl alcohol	1.103	166.176	2	3	3
Umbelliferon	1.498	162.144	0	3	1
Methylnonyl ketone	3.716	170.296	8	1	0
Folic acid	-0.0448	441.404	9	9	6
Caffeic acid	1.195	180.159	2	3	3

TABLE 4.3: Applicability of Lipinski Rule on Selected Ligands

Ligands	**LogP Value**	**Molecular Weight (g/mol)**	**Rotatable Bonds**	**H-bond Acceptor**	**H-bond Donor**
p-coumaric acid	1.49	164.16	2	2	2
5,7-dimethoxy coumarin	1.810	206.197	2	4	0
Chlorogenic acid	-0.645	354.311	4	8	6
Protocatechuic acid	0.796	154.121	1	3	3

Table 4.3 shows that out of 20 selected ligands, 4 ligands do not follow one rule of Lipinski i.e., Carpaine and Tocopherol have logP values greater than 5, whereas, Folic acid and Chlorogenic acid consist of 6 hydrogen bond donors.

4.7 ADMET Properties of Ligands

pkCSM, an online tool, is used to identify ADMET properties by placing input of ligands as SMILES. These properties give the kinetic and pharmacological activity of a compound and also the effect on drug level [102].

4.7.1 Absorption

A compound reaches the target cells after passing through the bloodstream, most frequently through mucous surfaces, for instance, the digestive tract. The digestive tract works on absorption, termed intestinal absorption [103]. It includes Water Solubility, $CaCO_2$ Permeability, Intestinal Absorption (human), Skin Permeability, P-glycoprotein Substrate, and P-glycoprotein I and II Inhibitors.

Water solubility shows the solubility of the compound in water, however, $CaCO_2$ Permeability and Skin Permeability are the permeability coefficients. Intestinal Absorption is the absorbing value in the small intestine. P-glycoprotein Substrate is the biological barrier for chemicals and other toxins and the P-glycoprotein I and II give the prediction of the presence or absence of inhibitors [104]. Some properties of absorption of nominated ligands are given in Table 4.4 and 4.5.

TABLE 4.4: Absorption Properties of Selected Ligands.

Ligands	**Water Solubility**	**$CaCO_2$ Permeability**	**Intestinal Absorption (Human)**	**Skin Permeability**
Carpaine	-4.724	0.849	91.891	-2.782
Kaempferol	-3.04	0.032	74.29	-2.735
Ascorbic acid	-1.556	-0.255	39.154	-2.955
Tocopherol	-6.901	1.345	89.782	-2.683
Dicoumarol	-3.983	0.203	93.074	-2.736
Cysteine	-2.888	0.386	74.807	-2.737
Homocysteine	-2.889	0.519	73.687	-2.735
Dimethoxy phenol	-1.4	1.734	93.789	-2.745
Coumarin	-1.517	1.649	97.344	-1.921
Glutamic acid	-2.892	-0.487	28.979	-2.735
Phenylalanine	-2.89	0.62	76.21	-2.734
Caffeoyl alcohol	-0.939	-0.939	74.333	-2.949
Umbelliferon	-2.131	1.206	94.551	-2.6
Methylnonyl ketone	-4.684	1.486	94.005	-1.333
Folic acid	-2.88	-0.877	1.108	-2.735
Caffeic acid	-2.33	0.634	69.407	-2.722
p-coumaric acid	-2.378	1.21	93.494	-2.715

TABLE 4.4: Absorption Properties of Selected Ligands.

Ligands	**Water Solubility**	**$CaCO_2$ Permeability**	**Intestinal Absorption (Human)**	**Skin Permeability**
5,7-dimethoxy -coumarin	-2.12	1.279	98.027	-2.41
Chlorogenic acid	-2.449	-0.84	36.377	-2.735
Protocatechuic acid	-2.069	0.49	71.174	-2.727

Each compound's water solubility shows solubility at 25. The results of absorption in Table 4.4 demonstrate the good $CaCO_2$ solubility of tocopherol, dimethoxyphenol, coumarin, umbelliferon, methyl nonyl ketone, and 5,7-dimethoxycoumarin. Only folic and glutamic acids exhibit low intestinal absorption among all the ligands. All of the ligands are expected to be skin permeable based on the skin permeability data.

TABLE 4.5: Absorption Properties of Selected Ligands.

Ligands	**P-glycoprotein Substrate**	**P-glycoprotein I Inhibitor**	**P-glycoprotein II Inhibitor**
Carpaine	Yes	No	No
Kaempferol	Yes	No	No
Ascorbic acid	No	No	No
Tocopherol	No	No	Yes
Dicoumarol	Yes	No	No
Cysteine	No	No	No
Homocysteine	No	No	No
Dimethoxy phenol	No	No	No
Coumarin	No	No	No
Glutamic acid	No	No	No
Phenylalanine	No	No	No

TABLE 4.5: Absorption Properties of Selected Ligands.

Ligands	**P-glycoprotein Substrate**	**P-glycoprotein I Inhibitor**	**P-glycoprotein II Inhibitor**
Caffeoyl alcohol	No	No	No
Umbelliferon	No	No	No
Methylnonyl ketone	No	No	No
Folic acid	Yes	No	No
Caffeic acid	No	No	No
p-coumaric acid	No	No	No
5,7-dimethoxy coumarin	No	No	No
Chlorogenic acid	Yes	No	No
Protocatechuic acid	No	No	No

Table 4.5 shows that Carpaine, Kaempferol, Dicoumarol, Folic acid, and the chlorogenic acid act as the P-glycoprotein substrate. P-glycoprotein-I is not inhibited by any ligand. However, just Tocopherol is the only inhibitor of P-glycoprotein II among all the ligands.

4.7.2 Distribution

According to pharmacology, distribution is the transmission of a drug inside the body from one spot to another. The drug enters the systemic circulation through absorption and is distributed into the intracellular and interstitial fluid.

The volume of Distribution (VDss) is the estimation of how much medication will be needed overall to spread evenly throughout the blood plasma. The Blood-Brain

Barrier, which shields the brain from external substances, is a crucial factor. It thus aids in anticipating CNS targets, adverse effects, and non-CNS treatments alike [105]. Distributive properties of ligands are shown in Table 4.6.

TABLE 4.6: Distributive Properties of Selected Ligands

Ligands	**VDss (Human)**	**Fraction Unbound (Human)**	**BBB Permeability**	**CNS Permeability**
Carpaine	0.812	0.378	-0.351	-2.948
Kaempferol	1.274	0.178	-0.939	-2.228
Ascorbic acid	0.218	0.825	-0.985	-3.217
Tocopherol	0.709	0	0.876	-1.669
Dicoumarol	-0.379	0.025	-0.393	-2.051
Cysteine	-0.486	0.49	-0.398	-3.476
Homocysteine	-0.51	0.464	-0.365	-3.464
Dimethoxy phenol	-0.129	0.435	-0.204	-2.226
Coumarin	-0.143	0.367	-0.007	-1.926
Glutamic acid	-0.291	0.414	-0.692	-3.554
Phenylalanine	-0.326	0.492	-0.271	-2.675
Caffeoyl alcohol	-0.047	0.503	-0.344	-2.566
Umbelliferon	0.032	0.432	-0.278	-2.741
Methylnonyl ketone	0.324	0.317	0.694	-1.937
Folic acid	0.046	0.37	1.615	-4.262
Caffeic acid	-1.098	0.529	-0.647	-2.608
p-coumaric acid	-1.151	0.428	-0.225	-2.418
5,7-dimethoxy coumarin	-0.349	0.318	0.154	-2.37
Chlorogenic acid	0.581	0.658	-1.407	-3.856

TABLE 4.6: Distributive Properties of Selected Ligands

Ligands	**VDss (Human)**	**Fraction Unbound (Human)**	**BBB Permeability**	**CNS Permeability**
Protocatechuic acid	-1.298	0.648	-0.683	-3.305

The parameters used to establish the distribution properties (Table 4.6) includes the VDss, which demonstrates the levels of folic acid, ascorbic acid, dimethoxyphenol, coumarin, caffeine acid, umbelliferon, and methyl nonyl ketone within the normal range. The fraction of these ligands that are unbound indicates that, of the total dose, this proportion will not bind to the protein. Only methyl nonyl ketone and folic acid, according to BBB permeability values, can easily pass the brain barrier. Only three ligands—tocopherol, coumarin, and methyl nonyl ketone—can penetrate the central nervous system.

4.7.3 Metabolism

Metabolism is the catabolic and anabolic enzymatic reactions of compounds in the body. The cytochrome P450 (CYP) enzyme is thought to be the most important enzyme in drug metabolism and is used to anticipate how compounds will be metabolized by the CYP enzyme [105]. The metabolic properties of nominated ligand compounds are given in Table 4.7 and 4.8.

TABLE 4.7: Metabolic Properties of Selected Ligands.

Ligands	**CYP2D6 Substrate**	**CYP3A4 Substrate**	**CYP1A2 Inhibitor**	**CYP2C19 Inhibitor**
Carpaine	No	Yes	No	No
Kaempferol	No	No	Yes	No
Ascorbic acid	No	No	No	No
Tocopherol	No	Yes	No	Yes
Dicoumarol	No	Yes	Yes	Yes

TABLE 4.7: Metabolic Properties of Selected Ligands.

Ligands	**CYP2D6 Substrate**	**CYP3A4 Substrate**	**CYP1A2 Inhibitor**	**CYP2C19 Inhibitor**
Cysteine	No	No	No	No
Homocysteine	No	No	No	No
Dimethoxy phenol	No	No	No	No
Coumarin	No	No	Yes	No
Glutamic acid	No	No	No	No
Phenylalanine	No	No	No	No
Caffeoyl alcohol	No	No	No	No
Umbelliferon	No	No	Yes	No
Methylnonyl ketone	No	No	No	No
Folic acid	No	No	No	No
Caffeic acid	No	No	No	No
p-coumaric acid	No	No	No	No
5,7-dimethoxy coumarin	No	No	Yes	No
Chlorogenic acid	No	No	No	No
Protocatechuic acid	No	No	No	No

According to Table 4.7, no ligand is a CYP2D6 substrate. Substrates for CYP3A4 include carpaine, tocopherol, and dicoumarol.

TABLE 4.8: Metabolic Properties of Selected Ligands.

Ligands	**CYP2C9 Inhibitor**	**CYP2D6 Inhibitor**	**CYP3A4 Inhibitor**
Carpaine	No	No	No
Kaempferol	No	No	No
Ascorbic acid	No	No	No

TABLE 4.8: Metabolic Properties of Selected Ligands.

Ligands	**CYP2C9 Inhibitor**	**CYP2D6 Inhibitor**	**CYP3A4 Inhibitor**
Tocopherol	No	No	No
Dicoumarol	Yes	No	No
Cysteine	No	No	No
Homocysteine	No	No	No
Dimethoxy phenol	No	No	No
Coumarin	No	No	No
Glutamic acid	No	No	No
Phenylalanine	No	No	No
Caffeoyl alcohol	No	No	No
Umbelliferon	No	No	No
Methylnonyl ketone	No	No	No
Folic acid	No	No	No
Caffeic acid	No	No	No
p-coumaric acid	No	No	No
5,7-dimethoxy coumarin	No	No	No
Chlorogenic acid	No	No	No
Protocatechuic acid	No	No	No

CYP2C19 is inhibited by tocopherol and dicoumarol. The sole CYP2C9 inhibitor is dicoumarol. All of the ligands do not act as CYP2D6 and CYP3A4 inhibitors (Table 4.7 and 4.8).

4.7.4 Excretion

The process in which compounds and their metabolites are removed through kidneys or feces is termed excretion. The kidney or the liver, where medications are removed in the form of urine or bile, respectively, can carry out the drug excretion process [105]. Many medications are excreted by the body without being metabolized. Although the idea of "Renal Clearance" of medications had long been widely acknowledged, it has only lately been obvious that unmodified pharmaceuticals can also be "Totally Cleared" by the liver [106]. Properties of excretion of compounds are shown in Table 4.9.

TABLE 4.9: Excretory Properties of Selected Ligands.

Ligands	**Total Clearance**	**Renal OCT2 Substrate**
Carpaine	0.856	No
Kaempferol	0.477	No
Ascorbic acid	0.631	No
Tocopherol	0.794	No
Dicoumarol	0.698	No
Cysteine	0.53	No
Homocysteine	0.503	No
Dimethoxy phenol	0.213	No
Coumarin	0.97	No
Glutamic acid	0.205	No
Phenylalanine	0.452	No
Caffeoyl alcohol	0.157	No
Umbelliferon	0.706	No
Methyl nonyl ketone	1.574	No
Folic acid	0.527	No
Caffeic acid	0.508	No
p-coumaric acid	0.662	No

TABLE 4.9: Excretory Properties of Selected Ligands.

Ligands	**Total Clearance**	**Renal OCT2 Substrate**
5,7-dimethoxy coumarin	0.832	No
Chlorogenic acid	0.307	No
Protocatechuic acid	0.551	No

Since none of these ligands are renal OCT2 substrates, as shown in Table 4.9, they won't be excreted from the body, hence the overall clearance values are presented in accordance.

4.7.5 Toxicity

It is the specific amount to which an organism cannot be damaged by a particular chemical substance.

Individual hazardous chemical limits are measured by the maximum tolerable dose (MRTD). The ability of any substance to result in the blockage of potassium channels triggered by the hERG is determined using the hERG I and II inhibitors model. A chemical's lethal dose (LD_{50}) is the concentration that results in the death of 50% of test animals (mice). While LOAEL seeks to determine the lowest dosage of a molecule with a significant adverse effect, it forecasts the toxicity of a likely compound.

Hepatotoxicity is a significant safety concern during drug development since it indicates a medicine's potential to harm the liver. A possible side effect of skincare and applied products is skin sensitivity. A protozoan bacteria called *T. pyriformis* produces a toxin that limits growth by 50% and is frequently used as a toxic endpoint (IGC_{50}) [107]. The toxicity values of ligands are shown in Tables 4.10 and 4.11.

TABLE 4.10: Toxicity values of Selected Ligands

Ligands	**Max. Tolerated Dose (Human)**	**hERG I inhibitor**	**hERG II inhibitor**	**Oral Rat Acute Toxicity (LD_{50})**	**Oral Rat Chronic Toxicity (LOAEL)**
Carpaine	-0.685	No	No	2.968	-1.167
Kaempferol	0.531	No	No	2.449	2.505
Ascorbic acid	1.598	No	No	1.063	3.186
Tocopherol	0.775	No	Yes	2.072	1.987
Dicoumarol	0.395	No	Yes	2.396	2.167
Cysteine	1.133	No	No	1.982	2.6
Homocysteine	1.234	No	No	2.062	2.626
Dimethoxy phenol	1.218	No	No	2.047	1.923
Coumarin	0.435	No	No	2.112	1.903
Glutamic acid	0.898	No	No	2.443	2.444
Phenylalanine	0.935	No	No	2.193	1.954
Caffeoyl alcohol	1.282	No	No	2.019	1.936
Umbelliferon	0.689	No	No	2.047	1.751
Methylnonyl ketone	0.379	No	No	1.602	2.33
Folic acid	-0.586	No	No	2.67	3.153
Caffeic acid	1.145	No	No	2.383	2.092
p-coumaric acid	1.111	No	No	2.155	2.534
5,7-dimethoxy coumarin	0.447	No	No	2.106	2.328

TABLE 4.10: Toxicity values of Selected Ligands

Ligands	**Max. Tolerated Dose (Human)**	**hERG I inhibitor**	**hERG II inhibitor**	**Oral Rat Acute Toxicity (LD_{50})**	**Oral Rat Chronic Toxicity (LOAEL)**
Chlorogenic acid	-0.134	No	No	1.973	2.982
Protocatechuic acid	0.814	No	No	2.423	2.021

According to Table 4.10, Tocopherol and Dicoumarol are hERG II inhibitors that may cause potassium channel inhibition and ultimately lead to QT syndrome. Chlorogenic acid, p-coumaric acid, Folic acid, homocysteine, and ascorbic acid have more values of Oral Rat Chronic Toxicity (LOAEL) than its normal range i.e. it should be less than 2.5 Log/mg, which means these ligands are toxic.

TABLE 4.11: Toxicity values of Selected Ligands

Ligands	**Hepato-toxicity**	**Skin Sensitization**	***T.Pyriformis* toxicity**	**Minnow toxicity**
Carpaine	No	No	0.285	1.127
Kaempferol	No	No	0.312	2.885
Ascorbic acid	No	No	0.285	4.386
Tocopherol	No	No	1.017	-3.324
Dicoumarol	Yes	No	0.33	0.762
Cysteine	No	No	0.149	2.992
Homocysteine	No	No	0.284	2.662
Dimethoxy phenol	No	No	-0.129	2.101

TABLE 4.11: Toxicity values of Selected Ligands

Ligands	**Hepato-toxicity**	**Skin Sensitization**	***T.Pyriformis* toxicity**	**Minnow toxicity**
Coumarin	No	No	0.365	1.555
Glutamic acid	No	No	0.285	3.182
Phenylalanine	Yes	No	0.269	2.247
Caffeoyl alcohol	No	Yes	0.05	2.487
Umbelliferon	Yes	No	0.546	1.714
Methylnonyl ketone	No	Yes	1.465	0.248
Folic acid	Yes	No	0.285	4.009
Caffeic acid	No	No	0.293	2.246
p-coumaric acid	No	No	0.319	1.607
5,7-dimethoxy coumarin	No	No	0.565	1.593
Chlorogenic acid	No	No	0.285	5.741
Protocatechuic acid	No	No	0.273	2.451

Dicoumarol, Phenylalanine, and Folic Acid are identified in Table 4.11 as Hepatotoxic, indicating that they are toxic to the liver and may cause liver damage. Caffeoyl alcohol and Methyl nonyl ketone are marked positive for skin sensitization. Tocopherol and Methyl nonyl ketone are minnow toxic.

4.8 Molecular Docking

Ligand-protein is a potent tool for Computer-Aided Drug Discovery (CADD). Cavity-detection-guided Blind Docking (CB-Dock) is used to execute molecular docking of proteins and ligands [108]. CB-Dock displays result in five distinct poses and with an interactive 3D visualization. The lowest vina score (in kJ/m) is selected as the best pose. After getting proteins and ligands ready for docking,

CB Dock, an established online tool for blind auto docking, does the docking. The architecture of the protein, ligands, refinements, and net speed all affect the docking process' outcomes and time requirements. Five potential possess and receptor models were provided by CB Dock, and the best possess model was chosen by looking at many characteristics including vina score and cavity size, among others. CB Dock, a user-friendly blind docking web server, predicts and estimates a binding site for a given protein, calculates centers and sizes using a novel rotation cavity detection method, and docks with the well-known docking program Auto dock Vina. This allows for molecular docking without knowledge of binding sites [109]. CB-Dock examines the input files and converts them to pdb. formatted files after receiving the input files (Protein file in pdb. format and ligand file in sdf. format).The best binding/vina scores of all ligands with NS5 and NS2B-NS3 are given in Table 4.12, table 4.13 and table 4.14 and table 4.15.

TABLE 4.12: Ligands with best binding score values with NS5

Compounds	**Binding score (kJ/mol)**	**Cavity size**	**H-Bond Donor**	**H-Bond Acceptor**
Carpaine	-9.6	2919	2	6
Kaempferol	-8.1	2919	4	6
Ascorbic acid	-5.9	1660	4	6
Tocopherol	-6.5	2919	1	2
Dicoumarol	-8.7	2919	2	6
Cysteine	-4.0	1660	3	3
Homocysteine	-4.2	2919	3	3
Dimethoxy phenol	-5.3	1660	1	3
Coumarin	-6.6	2919	0	2
Glutamic acid	-5.2	1660	3	3
Phenylalanine	-6.5	2919	2	2
Caffeoyl alcohol	-6.1	2919	3	3
Umbelliferon	-6.5	1660	1	3
Methyl nonyl ketone	-5.0	470	0	1

TABLE 4.12: Ligands with best binding score values with NS5

Compounds	**Binding score (kJ/mol)**	**Cavity size**	**H-Bond Donor**	**H-Bond Acceptor**
Folic acid	-9.0	2919	6	9
Caffeic acid	-6.5	1660	3	3
p-coumaric acid	-6.3	2919	2	2
5,7-dimethoxycoumarin	-6.1	1660	0	4
Chlorogenic acid	-8.4	2919	6	8
Protocatechuic acid	-6.3	2919	3	3

TABLE 4.13: Ligands with best binding score values with NS5

Compounds	**LogP value**	**Mol. Weight**	**Rotatable Bonds**
Carpaine	5.566	478.71	0
Kaempferol	2.282	286.23	1
Ascorbic acid	-1.407	176.12	2
Tocopherol	8.840	430.71	12
Dicoumarol	2.901	336.29	2
Cysteine	-0.671	121.16	2
Homocysteine	-0.281	135.18	3
Dimethoxy phenol	1.409	154.16	2
Coumarin	1.793	146.14	0
Glutamic acid	-0.736	147.13	4
Phenylalanine	0.641	165.19	3
Caffeoyl alcohol	1.103	166.17	2
Umbelliferon	1.498	162.14	0
Methyl nonyl ketone	3.716	170.29	8
Folic acid	-0.044	441.40	9
Caffeic acid	1.195	180.15	2
p-coumaric acid	1.49	164.16	2

TABLE 4.13: Ligands with best binding score values with NS5

Compounds	**LogP value**	**Mol. Weight**	**Rotatable Bonds**
5,7-dimethoxycoumarin	1.810	206.19	2
Chlorogenic acid	-0.645	354.31	4
Protocatechuic acid	0.796	154.12	1

Table 4.12 and 4.13 shows the docking score of ligands and NS5. It also clarifies the logP value of the selected ligands. Carpaine indicates the highest binding score and Cysteine has the lowest one among all ligands. Chlorogenic acid and Folic acid have 6 hydrogen bonds.

TABLE 4.14: Ligands with best binding score values with NS2B-NS3

Compounds	**Binding Score (kJ/mol)**	**Cavity Size**	**H-Bond Donor**	**H-Bond Accepter**
Carpaine	-8.5	5928	2	6
Kaempferol	-8.4	5928	4	6
Ascorbic acid	-6.6	5928	4	6
Tocopherol	-7.8	211	1	2
Dicoumarol	-8.9	5928	2	6
Cysteine	-4.0	5928	3	3
Homocysteine	-4.5	5928	3	3
Dimethoxy phenol	-5.7	5928	1	3
Coumarin	-6.1	5928	0	2
Glutamic acid	-5.7	5928	3	3
Phenylalanine	-5.5	5928	2	2
Caffeoyl alcohol	-6.6	5928	3	3
Umbelliferon	-6.5	5928	1	3
Methyl nonyl ketone	-5.5	5928	0	1
Folic acid	-9.8	5928	6	9

TABLE 4.14: Ligands with best binding score values with NS2B-NS3

Compounds	**Binding Score (kJ/mol)**	**Cavity Size**	**H-Bond Donor**	**H-Bond Accepter**
Caffeic acid	-7.1	5928	3	3
p-coumaric acid	-6.4	5928	2	2
5,7-dimethoxy coumarin	-6.2	5928	0	4
Chlorogenic acid	-8.1	5928	6	8
Protocatechuic acid	-6.7	5928	3	3

TABLE 4.15: Ligands with best binding score values with NS2B-NS3

Compounds	**LogP value**	**Mol. Weight**	**Rotatable Bonds**
Carpaine	5.566	478.71	0
Kaempferol	2.282	286.23	1
Ascorbic acid	-1.407	176.12	2
Tocopherol	8.840	430.71	12
Dicoumarol	2.901	336.29	2
Cysteine	-0.671	121.16	2
Homocysteine	-0.281	135.18	3
Dimethoxy phenol	1.409	154.16	2
Coumarin	1.793	146.14	0
Glutamic acid	-0.736	147.13	4
Phenylalanine	0.641	165.19	3
Caffeoyl alcohol	1.103	166.17	2
Umbelliferon	1.498	162.14	0
Methyl nonyl ketone	3.716	170.29	8
Folic acid	-0.044	441.40	9
Caffeic acid	1.195	180.15	2
p-coumaric acid	1.49	164.16	2

TABLE 4.15: Ligands with best binding score values with NS2B-NS3

Compounds	**LogP value**	**Mol. Weight**	**Rotatable Bonds**
5,7-dimethoxycoumarin	1.810	206.19	2
Chlorogenic acid	-0.645	354.31	4
Protocatechuic acid	0.796	154.12	1

Table 4.14 and 4.15 shows the docking score of ligands and NS2B-NS3. It also illustrates the logP value of the selected ligands. Folic acid indicates the highest binding score and Cysteine has the lowest one among all ligands. Chlorogenic acid and Folic acid have 6 hydrogen bonds.

4.9 Interaction of Ligands and Nominated Protein

In Computational Biology, LigPlot produces a schematic 2D depiction of the protein-ligand complex, which eases the prompt assessment of various enzyme complexes and exhibits a simple and explanatory illustration of the intermolecular interactions and their strengths, these comprise hydrophobic interactions, hydrogen bonds, and atom approachability [100]. Investigation of docked complex (.pdb) through LigPlot creates automatically schematic illustrations of protein-ligand interactions for specified PDB files [101]. 2D demonstrations of 20 designated docked complexes with NS5 are revealed in figures from Figure 4.9 to Figure 4.28 and NS2B-NS3 are shown from Figure 4.29 to Figure 4.48. Hydrophobic interactions and hydrogen bonding amongst proteins and particular twenty ligands were shown in Appendix as Tables 5.1 and 5.2.

2D representations of docked complexes of NS5

Figure 4.9 shows the Carpaine-NS5 docked complex, which has a binding energy

of -9.6. It has no Hydrogen bond and consists of nine Hydrophobic interactions (Ser796, Ile797, Cys709, Glu459, Lys461, Arg472, Asp539, Asn610, Asp663).

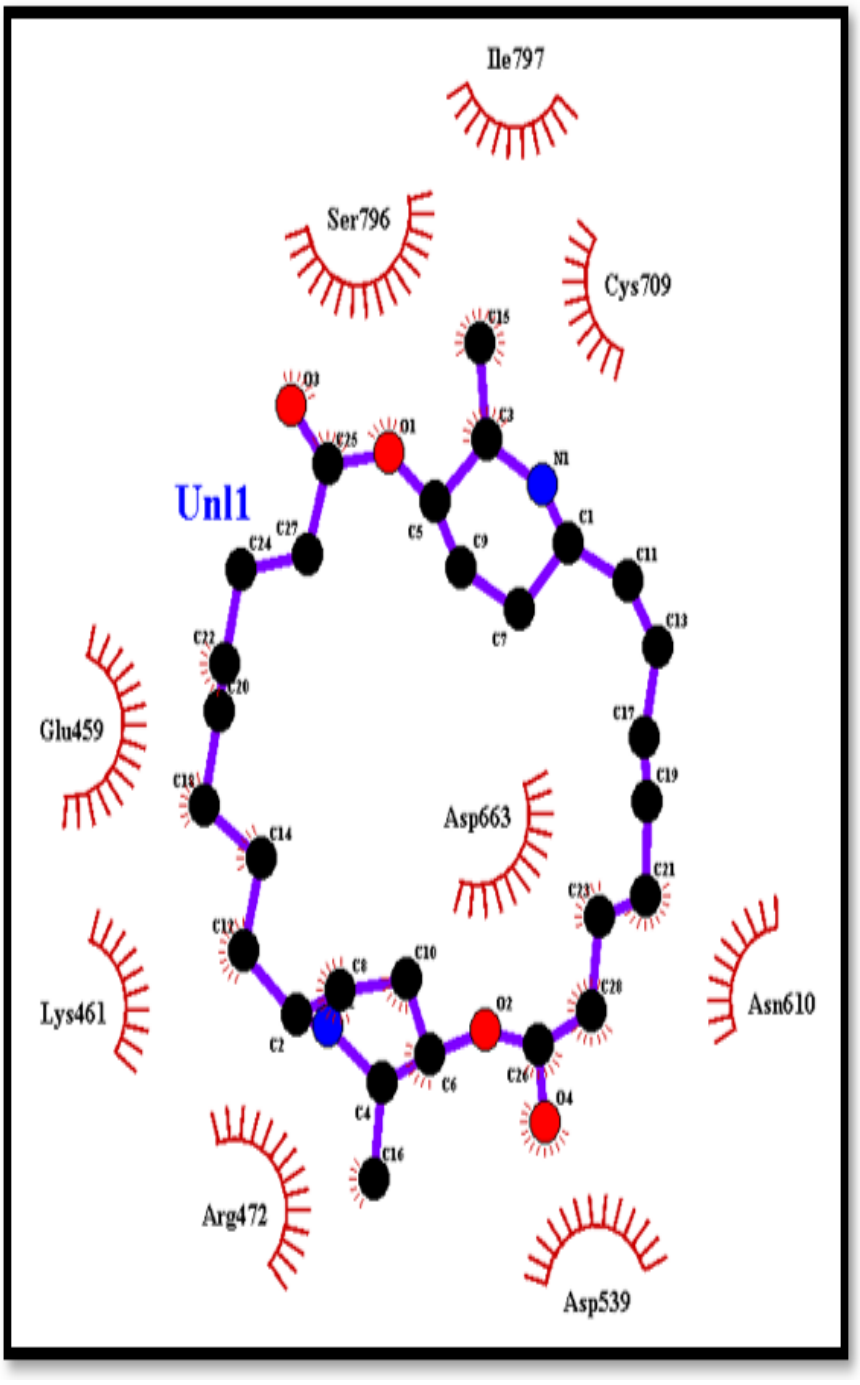


FIGURE 4.9: 2D depiction of docked complex Carpaine-NS5

Figure 4.10 shows the Kaempferol-NS5 docked complex, which has a binding energy of -8.1. It has no Hydrogen bond and consists of three Hydrophobic interactions (Thr794, Trp795, Arg737).

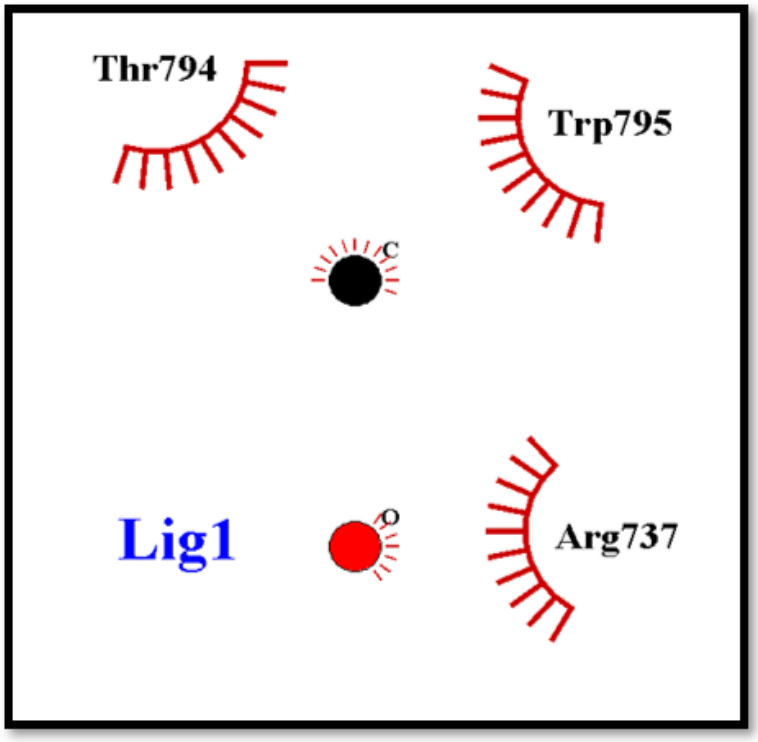


FIGURE 4.10: 2D depiction of docked complex Kaempferol-NS5

Figure 4.11 shows the Ascorbic acid-NS5 docked complex, which has a binding energy of -5.9. It has one Hydrogen bond (Amino acid: Thr794) and consists of one Hydrophobic interaction (Trp795).

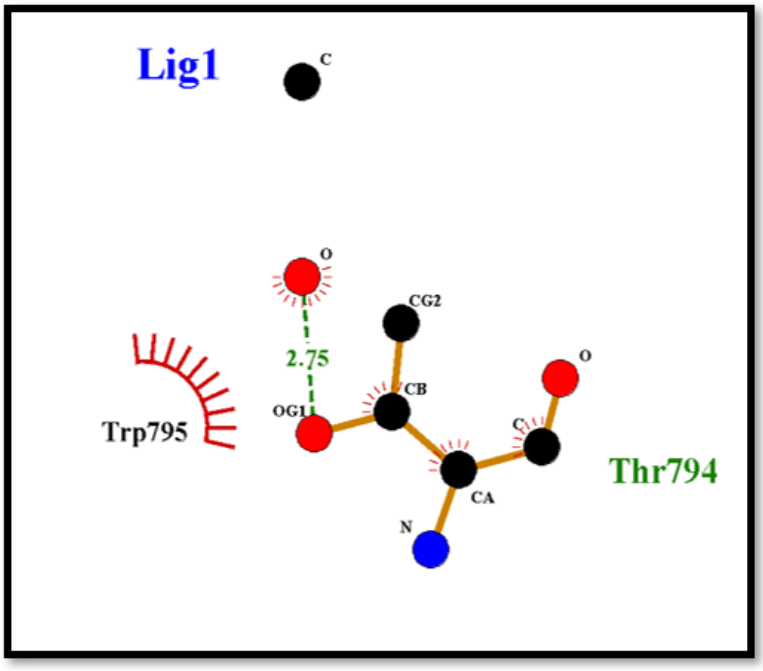


FIGURE 4.11: 2D depiction of docked complex Ascorbic acid-NS5

Figure 4.12 shows the Tocopherol-NS5 docked complex, which has a binding energy of -6.5. It has no Hydrogen bond and consists of three Hydrophobic interactions (Arg472, Asp539, Ala473).

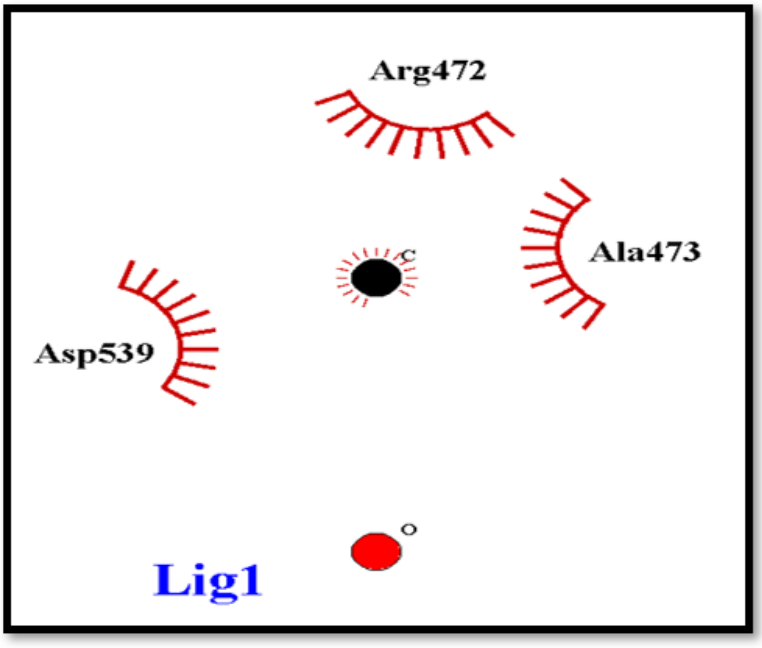


FIGURE 4.12: 2D depiction of docked complex Tocopherol-NS5

Figure 4.13 shows the Dicoumarol-NS5 docked complex, which has a binding energy of -8.7. It has no Hydrogen bond and consists of three Hydrophobic interactions (Arg737, Thr793, Thr794).

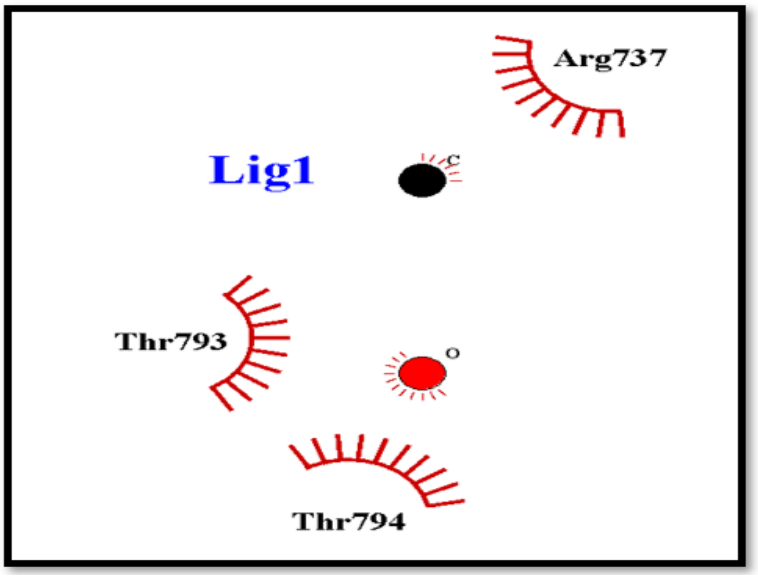


FIGURE 4.13: 2D depiction of docked complex Dicoumarol-NS5

Figure 4.14 shows the Cysteine-NS5 docked complex, which has a binding energy of -4.0. It has three Hydrogen bonds (Amino acid: Thr793, Tyr758, Lys460) and consists of five Hydrophobic interactions (Gln742, Thr794, Arg792, Glu459, Trp795).

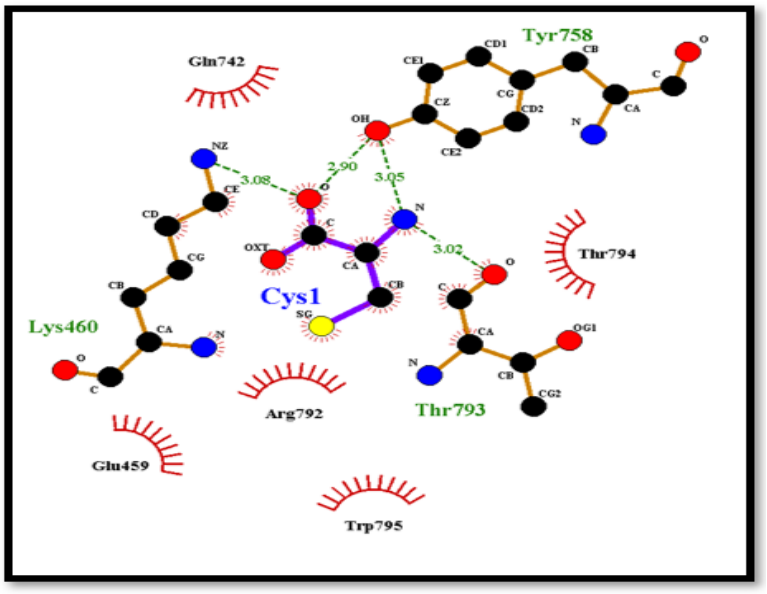


FIGURE 4.14: 2D depiction of docked complex Cysteine-NS5

Figure 4.15 shows the Homocysteine-NS5 docked complex, which has a binding energy of -4.2. It has two Hydrogen bonds (Amino acid: Tyr758, Thr793) and consists of two Hydrophobic interactions (Lys460, Arg792).

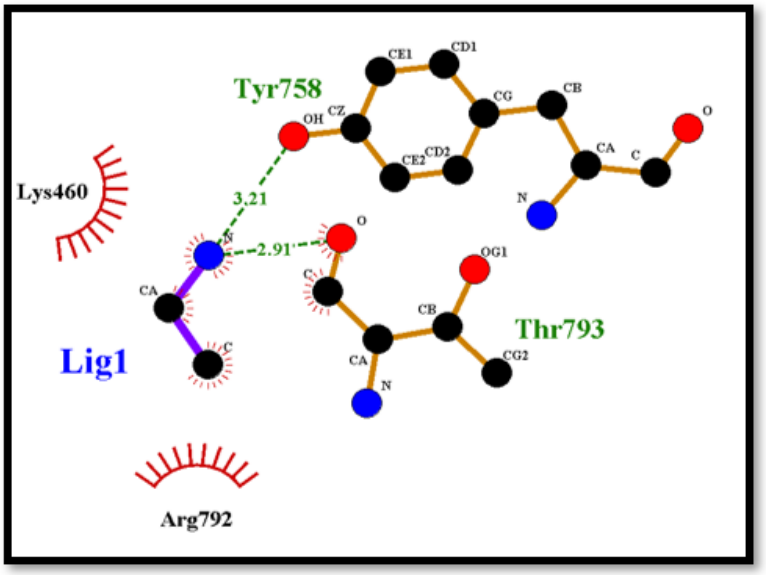


FIGURE 4.15: 2D depiction of docked complex Homocysteine-NS5

Figure 4.16 shows the Dimethoxyphenol-NS5 docked complex, which has a binding energy of -5.3. It has four Hydrogen bonds (Amino acid: Thr793, Arg737, Lys460, Tyr758) and consists of four Hydrophobic interactions (Gln742, Arg792, Thr794, Glu459).

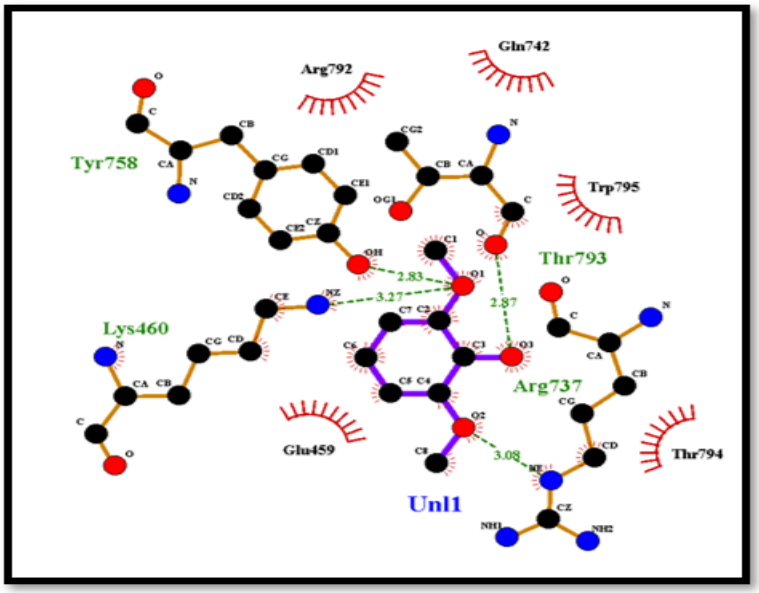


FIGURE 4.16: 2D depiction of docked complex Dimethoxyphenol-NS5

Figure 4.17 shows the Coumarin-NS5 docked complex, which has a binding energy of -6.6. It has no Hydrogen bonds and consists of eight Hydrophobic interactions (Arg458, Lys460, Glu459, Gln742, Thr794, Thr793, Trp795, Arg792).

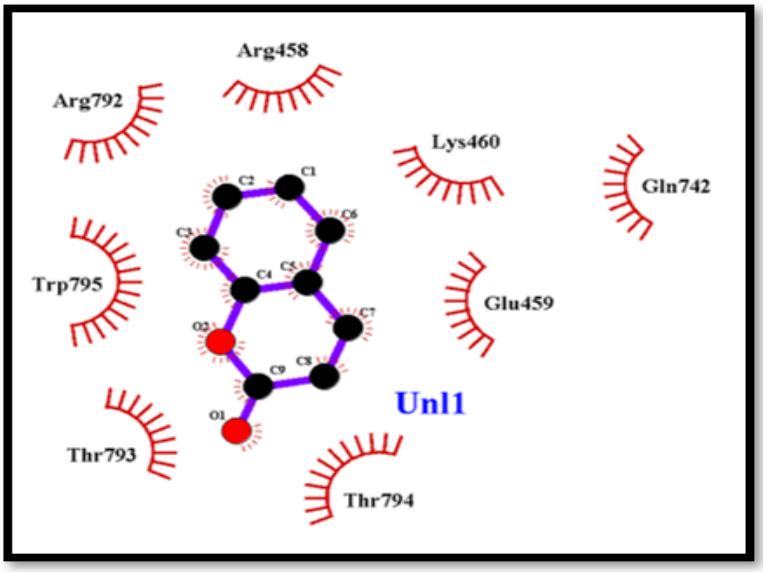


FIGURE 4.17: 2D depiction of docked complex Coumarin-NS5

Figure 4.18 shows the Glutamic acid-NS5 docked complex, which has a binding energy of -5.2. It has four Hydrogen bonds (Amino acid: Thr793, Arg737, Lys460, Tyr758) and consists of five Hydrophobic interactions (Gln742, Arg792, Thr794, Trp795, Glu459).

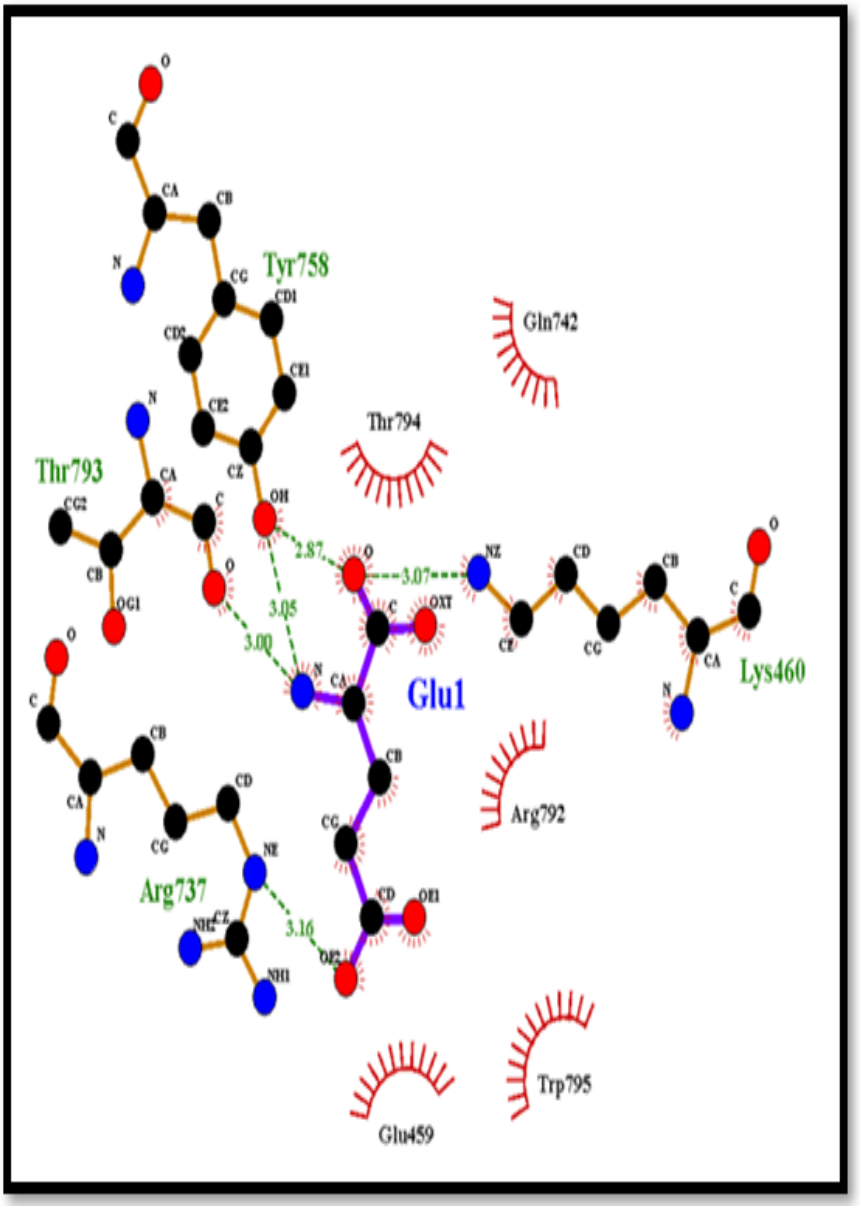


FIGURE 4.18: 2D depiction of docked complex Glutamic acid-NS5

Figure 4.19 shows the Phenylalanine-NS5 docked complex, which has a binding energy of -6.5. It has two Hydrogen bonds (Amino acid: Arg737, Lys460) and consists of six Hydrophobic interactions (Arg458, Gln742, Arg792, Thr794, Trp795, Thr793).

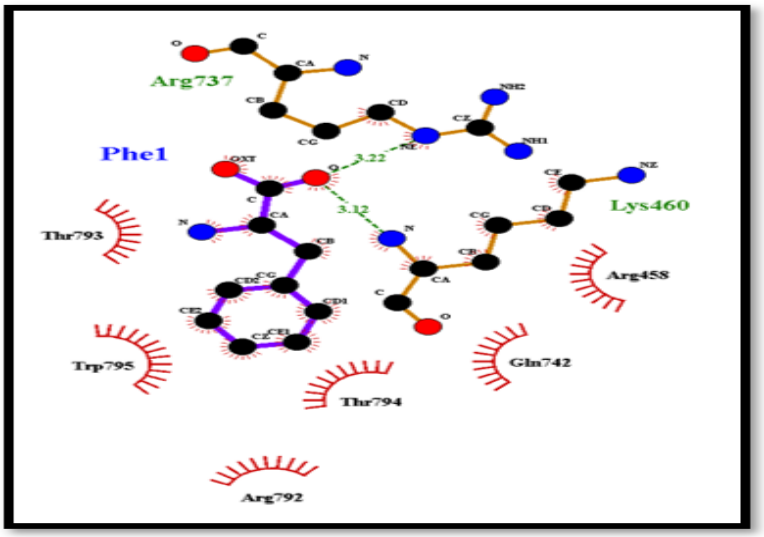


FIGURE 4.19: 2D depiction of docked complex Phenylalanine-NS5

Figure 4.20 shows the Caffeoyl alcohol-NS5 docked complex, which has a binding energy of -6.1. It has two Hydrogen bonds (Amino acid: Arg737, Lys460) and consists of three Hydrophobic interactions (Trp795, Lys461, Glu459).

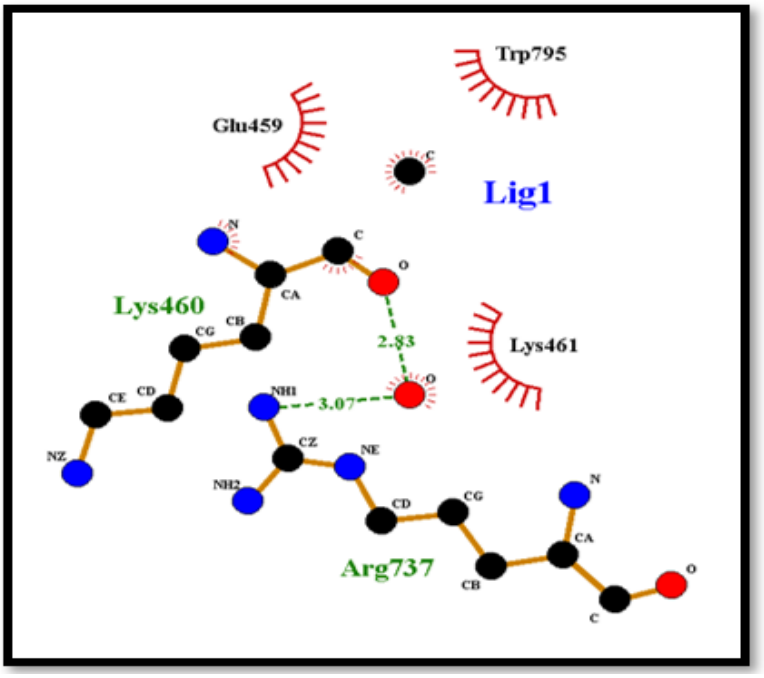


FIGURE 4.20: 2D depiction of docked complex Caffeoyl alcohol-NS5

Figure 4.21 shows the Umbelliferon-NS5 docked complex, which has a binding energy of -6.5. It has three Hydrogen bonds (Amino acid: Arg737, Lys460, Arg458)

and consists of seven Hydrophobic interactions (Met343, Gln742, Trp795, Thr793, Tyr758Arg792, Thr794, Glu459).

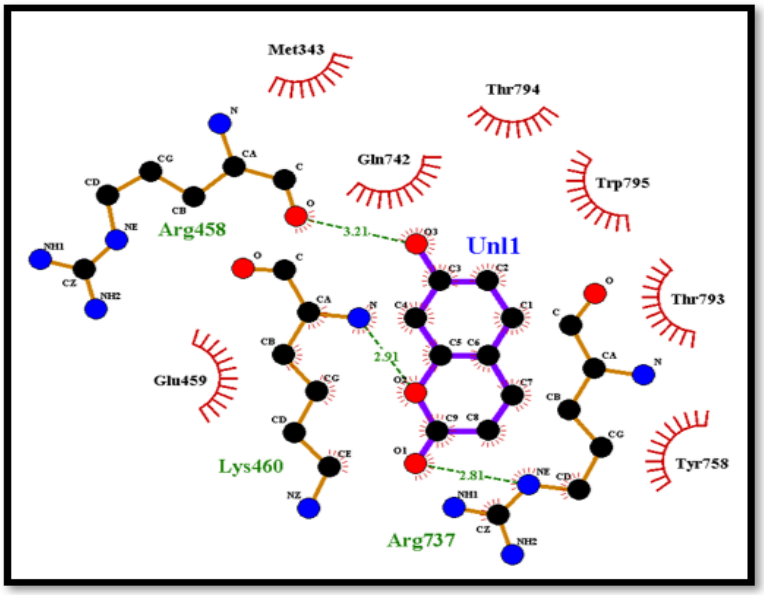


FIGURE 4.21: 2D depiction of docked complex Umbelliferon-NS5

Figure 4.22 shows the Methyl nonyl ketone-NS5 docked complex, which has a binding energy of -5.0. It has two Hydrogen bonds (Amino acid: Arg579, Glu287) and consists of three Hydrophobic interactions (Trp478, Thr449, Lys283).

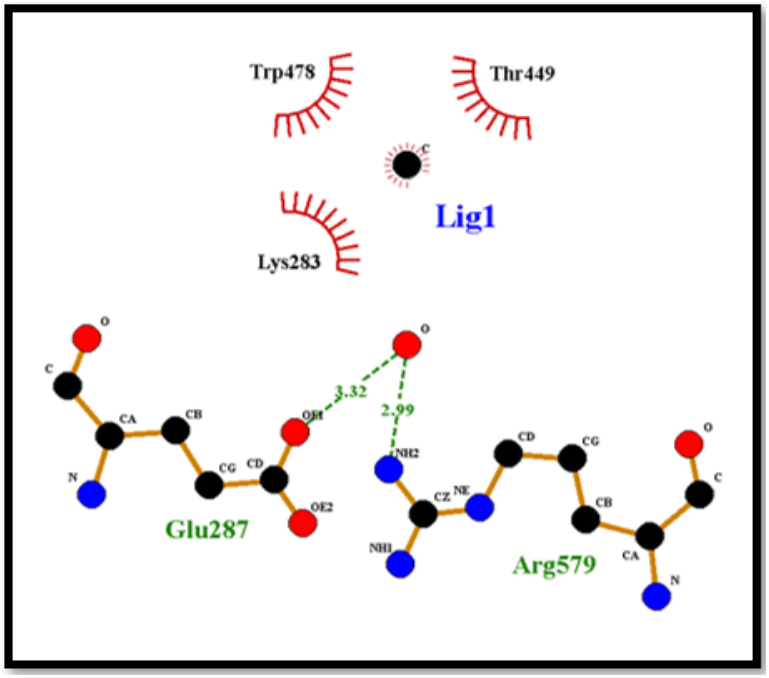


FIGURE 4.22: 2D depiction of docked complex Methyl nonyl ketone-NS5

Figure 4.23 shows the Folic acid-NS5 docked complex, which has a binding energy of -9.0. It has five Hydrogen bonds (Amino acid: Ile797, Ser796, Gln603, Thr606, Asp663) and consists of nine Hydrophobic interactions (Trp475, Tyr607, Ser661, Ser710, Gly662, Cys709, His798, Ile 474, Asp 539).

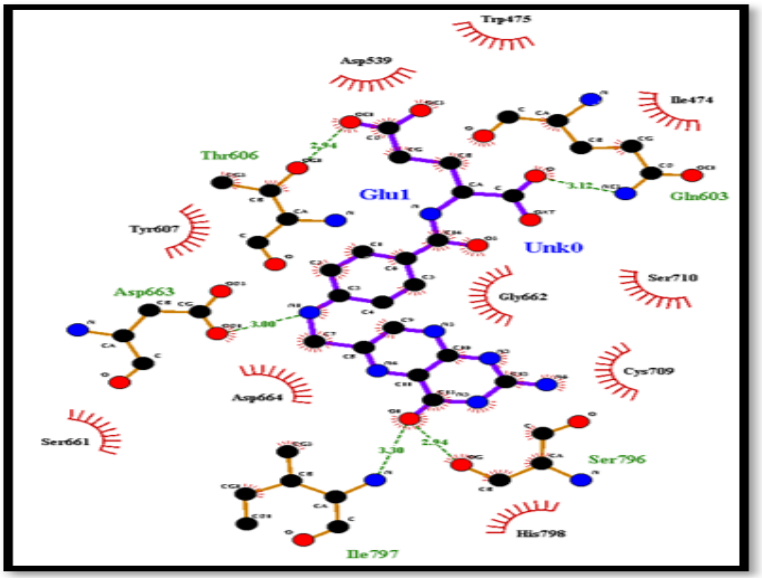


FIGURE 4.23: 2D depiction of docked complex Folic acid-NS5

Figure 4.24 shows the Caffeic acid-NS5 docked complex, which has a binding energy of -6.5. It has four Hydrogen bonds (Amino acid: Arg729, Thr794, Arg729, Trp795) and consists of seven Hydrophobic interactions (Gln742, Met343, Arg458, Arg737, Thr793, Lys460, Tyr758).

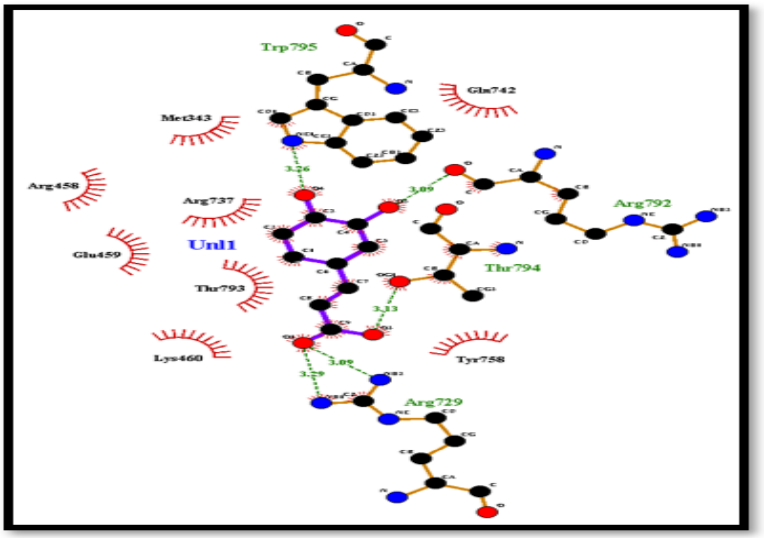


FIGURE 4.24: 2D depiction of docked complex Caffeic acid-NS5

Figure 4.25 shows the p-coumaric acid-NS5 docked complex, which has a binding energy of -6.3. It has three Hydrogen bonds (Amino acid: Arg792, Lys460, Arg737) and consists of seven Hydrophobic interactions (Met343, Glu742, Arg458, Glu459, Thr793, Trp795, Thr794).

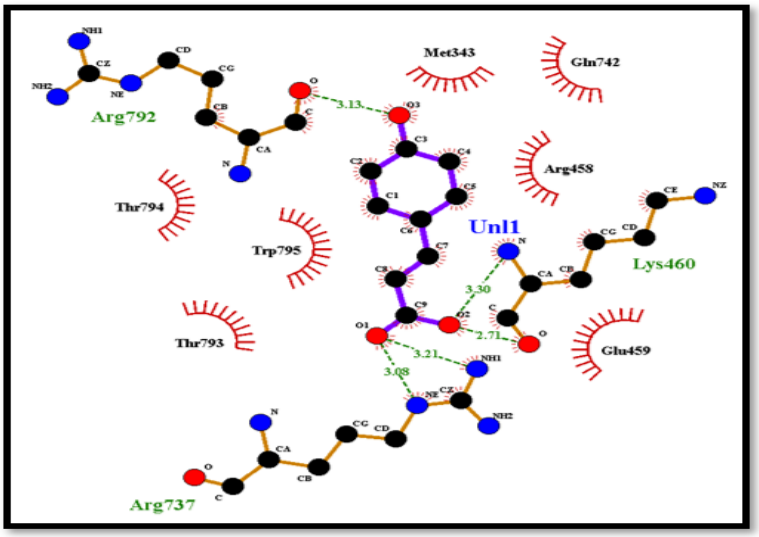


FIGURE 4.25: 2D depiction of docked complex p-coumaric acid-NS5

Figure 4.26 shows the 5,7-dimethoxycoumarin-NS5 docked complex, which has a binding energy of -6.1. It has two Hydrogen bonds (Amino acid: Thr793, Tyr758) and consists of two Hydrophobic interactions (Arg737, Lys460).

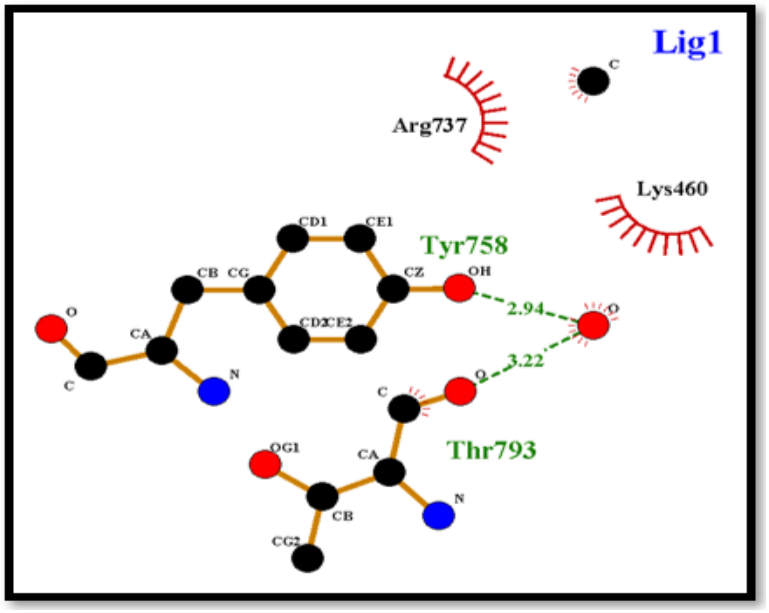


FIGURE 4.26: 2D depiction of docked complex 5,7-dimethoxycoumarin-NS5

Figure 4.27 shows the Chlorogenic acid-NS5 docked complex, which has a binding energy of -8.4. It has no Hydrogen bonds and consists of two Hydrophobic interactions (Thr794, Trp795).

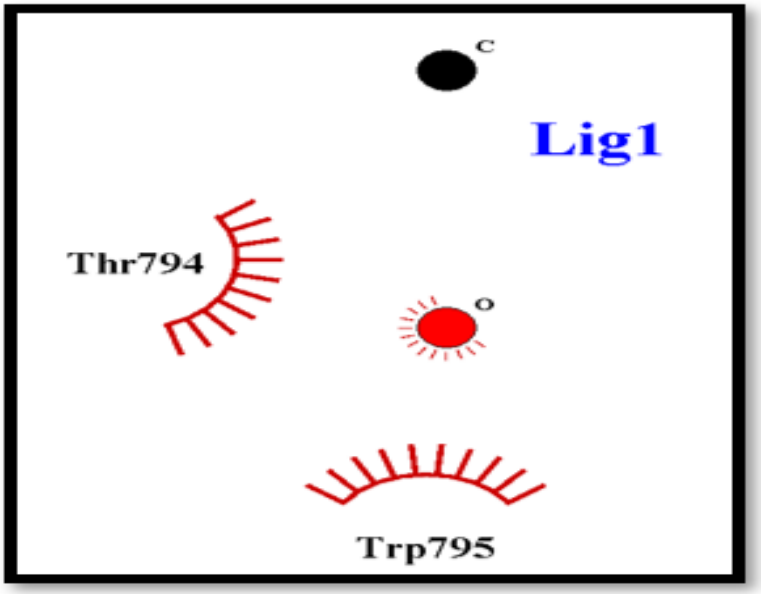


FIGURE 4.27: 2D depiction of docked complex Chlorogenic acid-NS5

Figure 4.28 shows the Protocatechuic acid-NS5 docked complex, which has a binding energy of -6.3. It has three Hydrogen bonds (Amino acid: Thr606, Ser601, Trp475) and consists of six Hydrophobic interactions (Asp539, Ala473, Gln603, Gly600, Gly602, Arg599).

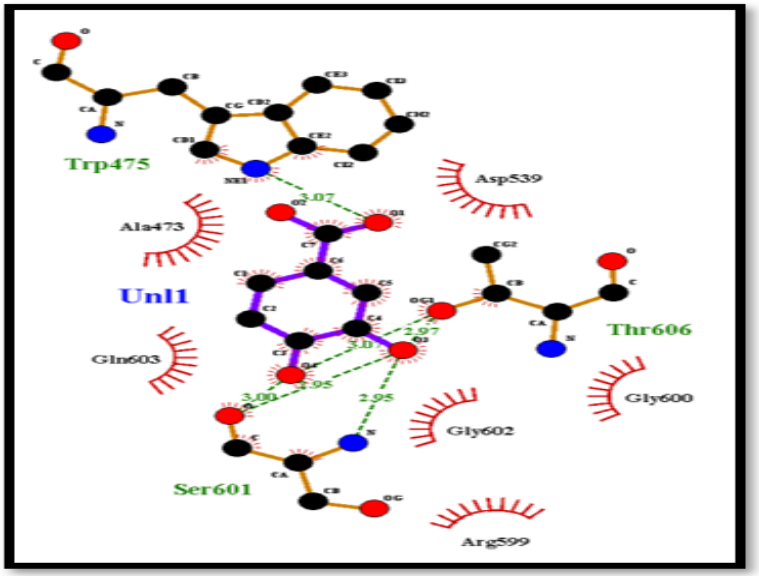


FIGURE 4.28: 2D depiction of docked complex Protocatechuic acid-NS5

The information about the hydrogen bond and hydrophobic interactions between the chosen ligand and receptor protein NS5 is displayed in Appendix as Table 5.1.

2D representations of docked complexes of NS2B-NS3

Figure 4.29 shows the Carpaine-NS2B-NS3 docked complex, which has a binding energy of -8.5. It has one Hydrogen bond (Amino acid: Ser48) and consists of eight Hydrophobic interactions (Arg1059, Arg1064, Met51, Ser1056, Lys1073, Val1072, Val49, Asp50).

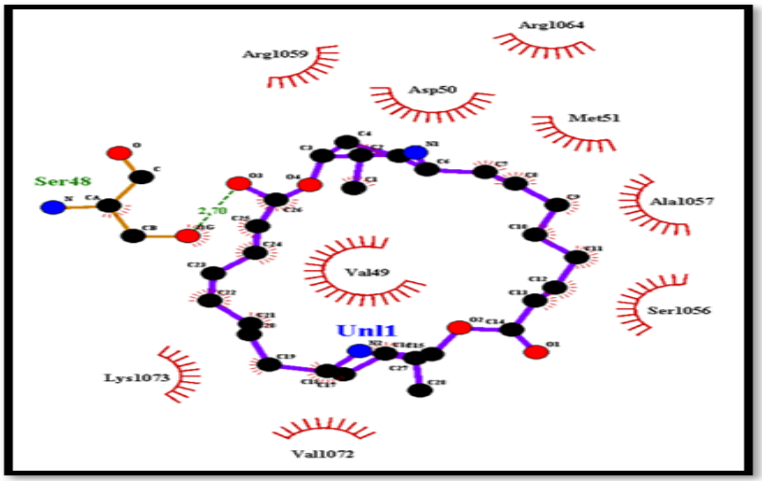


FIGURE 4.29: 2D depiction of docked compound Carpaine-NS2B-NS3

Figure 4.30 shows the Kaempferol- NS2B-NS3 docked complex, which has a binding energy of -8.4. It has two Hydrogen bonds and no Hydrophobic interactions.

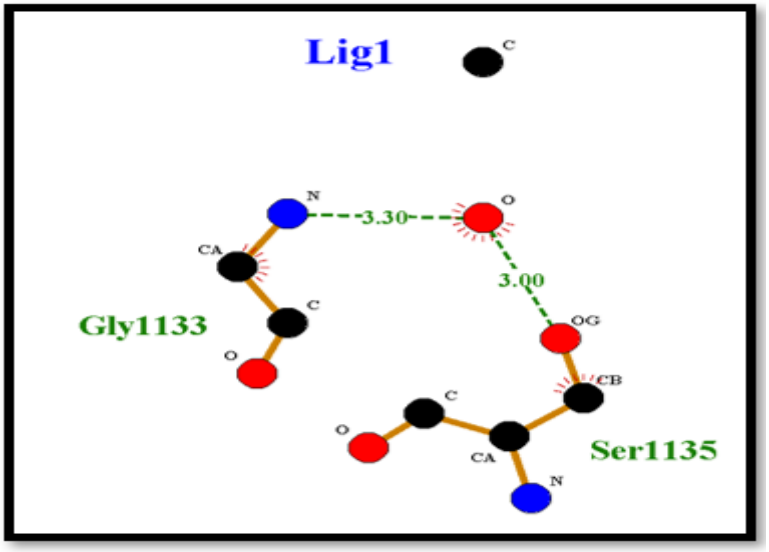


FIGURE 4.30: 2D depiction of docked compound Kaempferol-NS2B-NS3

Figure 4.31 shows the Ascorbic acid- NS2B-NS3 docked complex, which has a binding energy of -6.6. It has no Hydrogen bond and consists of four Hydrophobic interactions (Ser1135, Tyr1150, Arg1029, Thr1027).

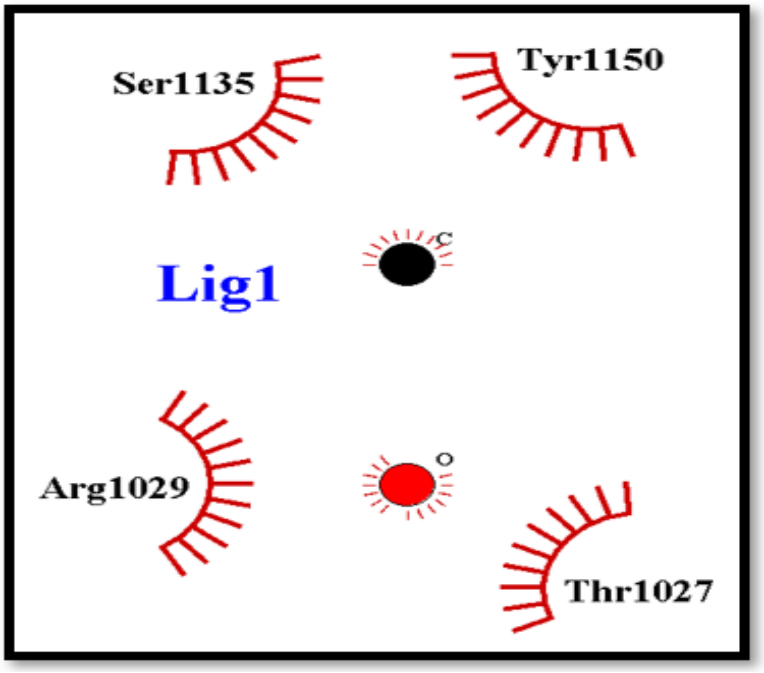


FIGURE 4.31: 2D depiction of docked compound Ascorbic acid-NS2B-NS3

Figure 4.32 shows the Tocopherol-NS2B-NS3 docked complex, which has a binding energy of -7.8. It has no Hydrogen bond and consists of five Hydrophobic interactions (Leu1085, Val1146, Leu1076, Trp1083, Gly1148).

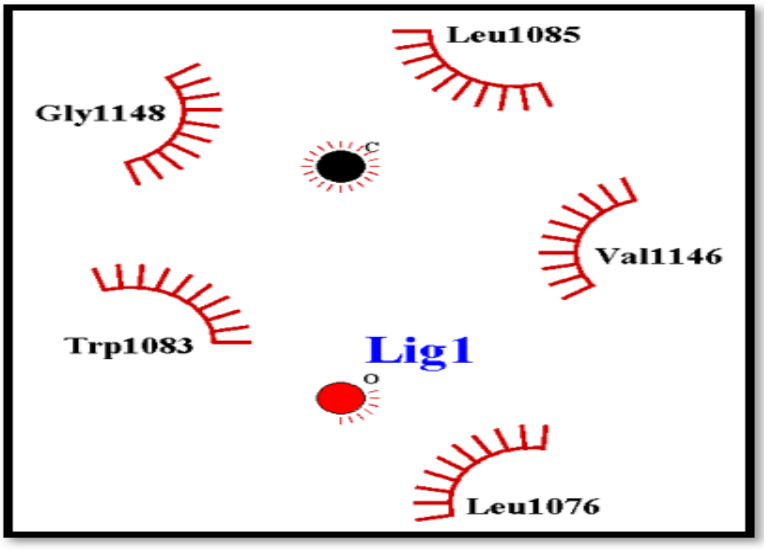


FIGURE 4.32: 2D depiction of docked compound Tocopherol-NS2B-NS3

Figure 4.33 shows the Dicoumarol-NS2B-NS3 docked complex, which has a binding energy of -8.9. It has one Hydrogen bond (Amino acid: Arg1029) and consists of three Hydrophobic interactions (Pro1131, Tyr1130, Gly1151).

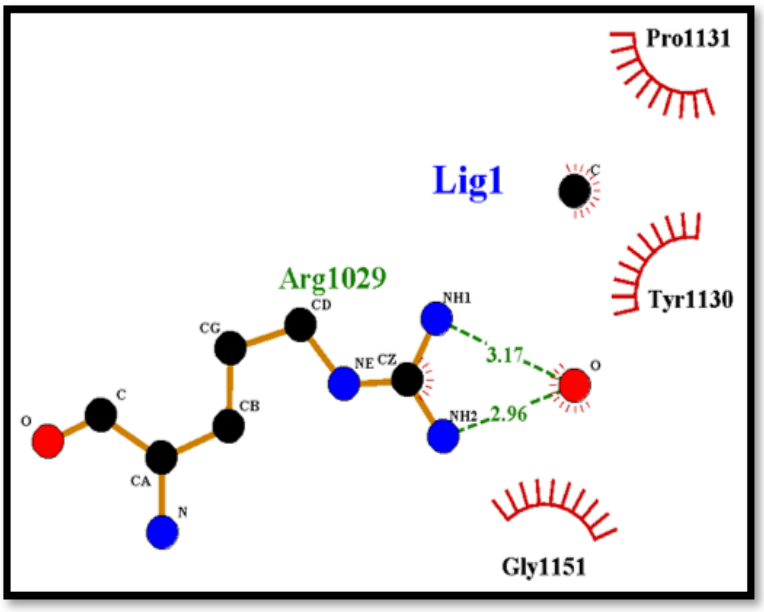


FIGURE 4.33: 2D depiction of docked compound Dicoumarol-NS2B-NS3

Figure 4.34 shows the Cysteine-NS2B-NS3 docked complex, which has a binding energy of -4.0. It has four Hydrogen bonds (Amino acid: Ser1135, Thr1134, Gly1133, Thr1034) and consists of four Hydrophobic interactions (Val1036, Ala1132, Pro1131, Arg1029).

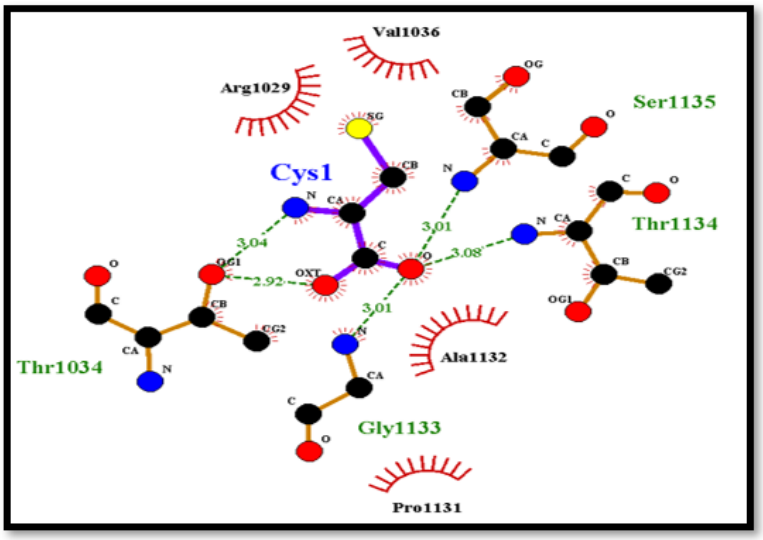


FIGURE 4.34: 2D depiction of docked complex Cysteine-NS2B-NS3

Figure 4.35 shows the Homocysteine-NS2B-NS3 docked complex, which has a binding energy of -4.5. It has one Hydrogen bond (Amino acid: Tyr1130) and consists of three Hydrophobic interactions (Tyr1150, Thr1034, Ser1135).

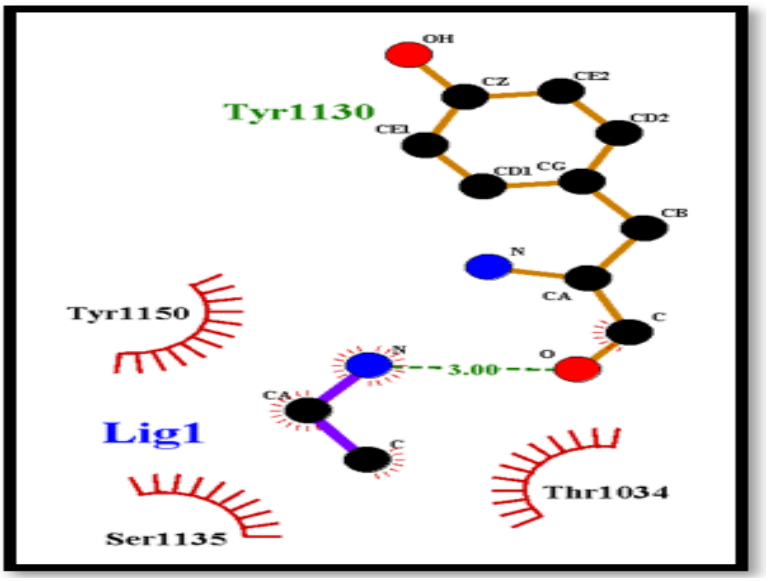


FIGURE 4.35: 2D depiction of docked complex Homocysteine-NS2B-NS3

Figure 4.36 shows the Dimethoxyphenol-NS2B-NS3 docked complex, which has a binding energy of -5.7. It has two Hydrogen bonds (Amino acid: Tyr 1130, Tyr1150) and consists of ten Hydrophobic interactions (Asp1129, Ala1132, Pro1131, Thr1034, Gly1133, Ser1135, Gly1151, Arg1029, Leu1128, Gly1032).

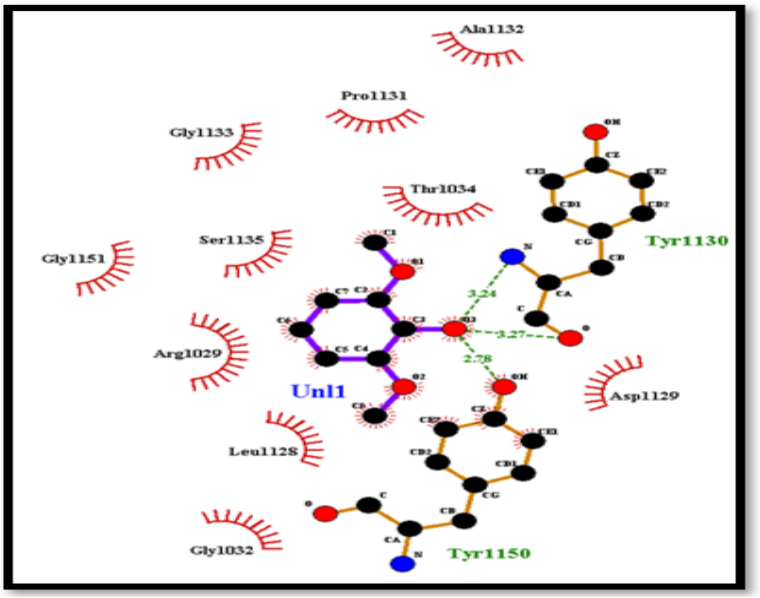


FIGURE 4.36: 2D depiction of docked complex Dimethoxy phenol-NS2B-NS3

Figure 4.37 shows the Coumarin-NS2B-NS3 docked complex, which has a binding energy of -6.1. It has two Hydrogen bonds (Amino acid: Thr1034, Thr1027) and consists of seven Hydrophobic interactions (Met51, Lys1054, His1051, Val1036, Ala1132, Gly1133, Arg1029).

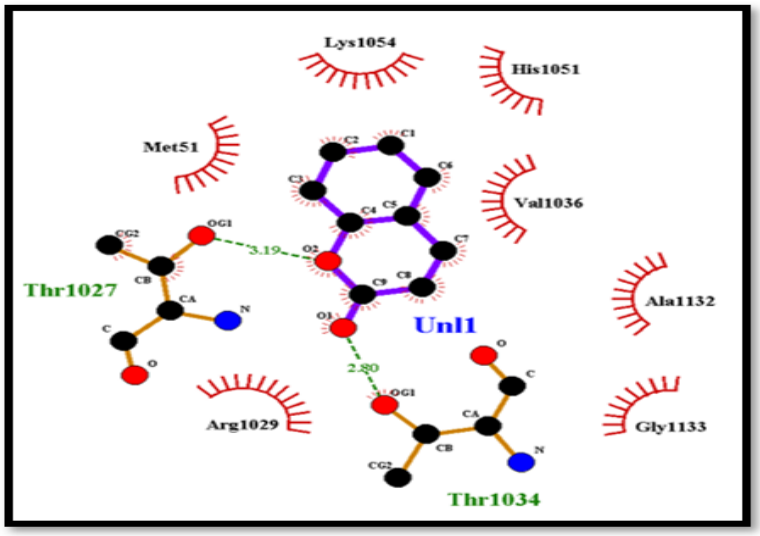


FIGURE 4.37: 2D depiction of docked complex Coumarin-NS2B-NS3

Figure 4.38 shows the Glutamic acid-NS2B-NS3 docked complex, which has a binding energy of -5.7. It has four Hydrogen bonds (Amino acid: Ser1135, Thr1134, Gly1133, Thr1034) and consists of nine Hydrophobic interactions (Asp1129, Gly1151, Tyr1150, Pro1131, Tyr1130, Ala1132, Gly1032, Arg1029, Ser1033).

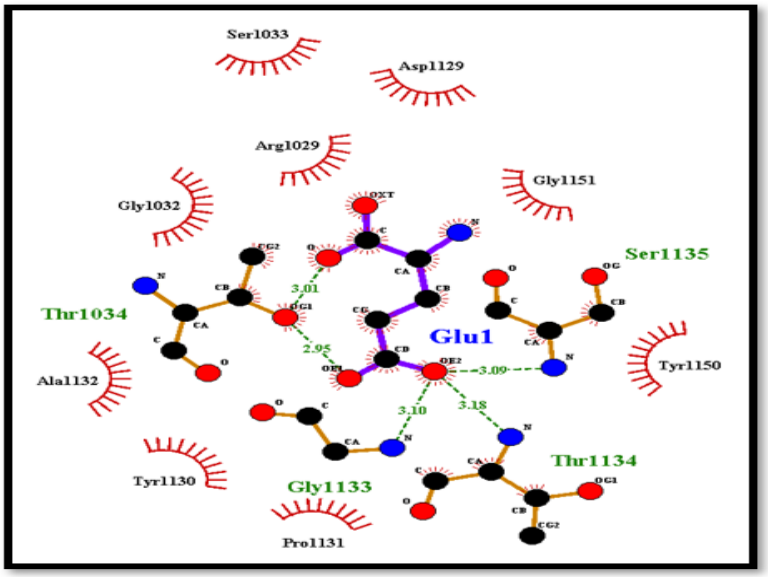


FIGURE 4.38: 2D depiction of docked complex Glutamic acid-NS2B-NS3

Figure 4.39 shows the Phenylalanine-NS2B-NS3 docked complex, which has a binding energy of -5.5. It has one Hydrogen bond (Amino acid: Val1036) and consists of five Hydrophobic interactions (Gln1035, Thr1034, Val1036, Gly1133, His1051).

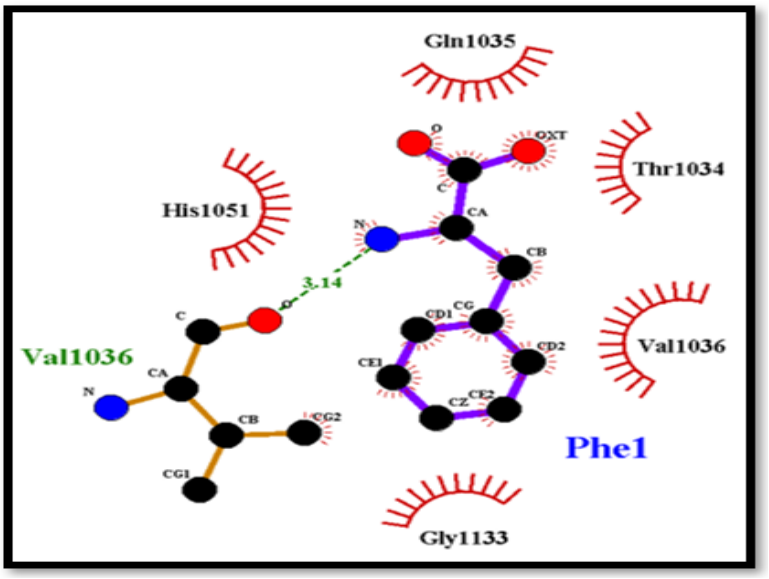


FIGURE 4.39: 2D depiction of docked complex Phenylalanine-NS2B-NS3

Figure 4.40 shows the Caffeoyl alcohol-NS2B-NS3 docked complex, which has a binding energy of -6.6. It has one Hydrogen bond (Amino acid: Val1126) and consists of two Hydrophobic interactions (Tyr1150, Gly1151).

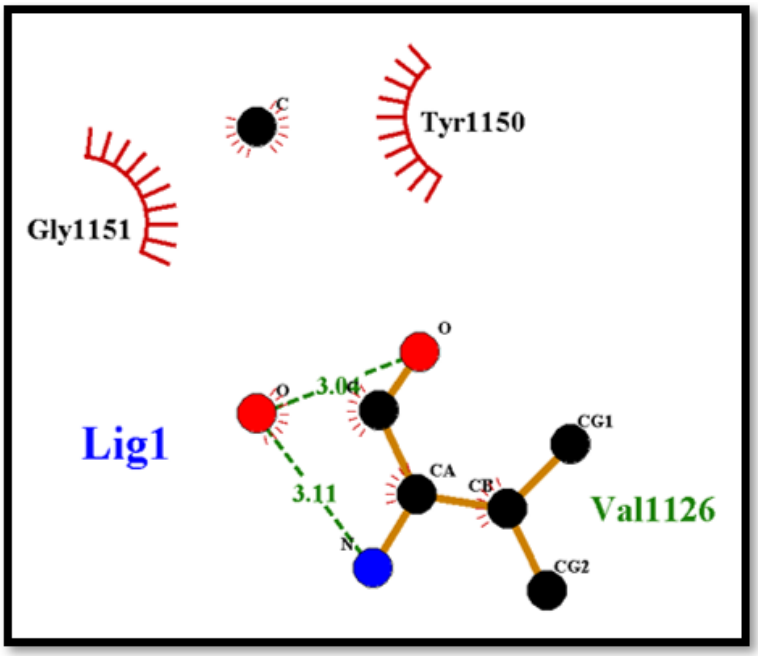


FIGURE 4.40: 2D depiction of docked complex Caffeoyl alcohol-NS2B-NS3

Figure 4.41 shows the Umbelliferon-NS2B-NS3 docked complex, which has a binding energy of -6.5. It has two Hydrogen bonds (Amino acid: Ala1087, Val1146) and consists of eight Hydrophobic interactions (Leu1085, Ile1147, Val1155, Gly1153, Leu1076, Asn1152, Gly1148, Trp1083).

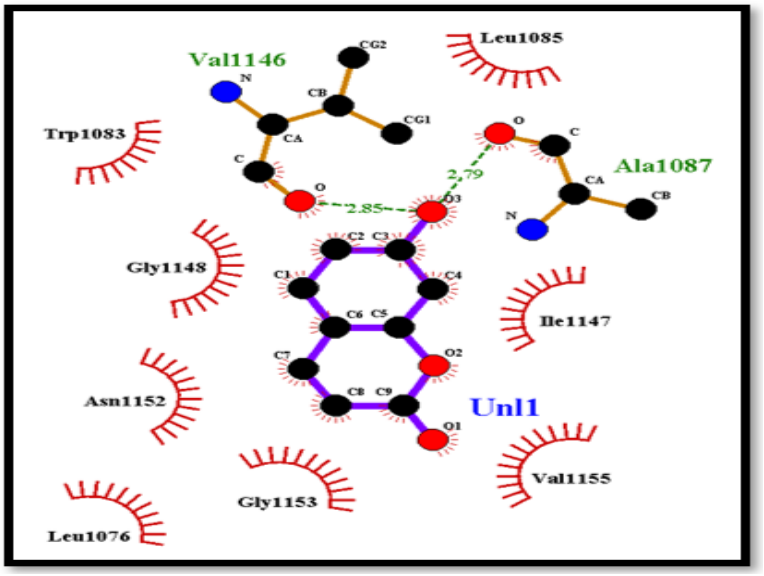


FIGURE 4.41: 2D depiction of docked complex Umbelliferon-NS2B-NS3

Figure 4.42 shows the Methyl nonyl ketone-NS2B-NS3 docked complex, which has a binding energy of -5.5. It has four Hydrogen bonds (Amino acid: Ser1135, Thr1134, Gly1133, Pro1131) and consists of two Hydrophobic interactions (Arg1029, Ala1132).

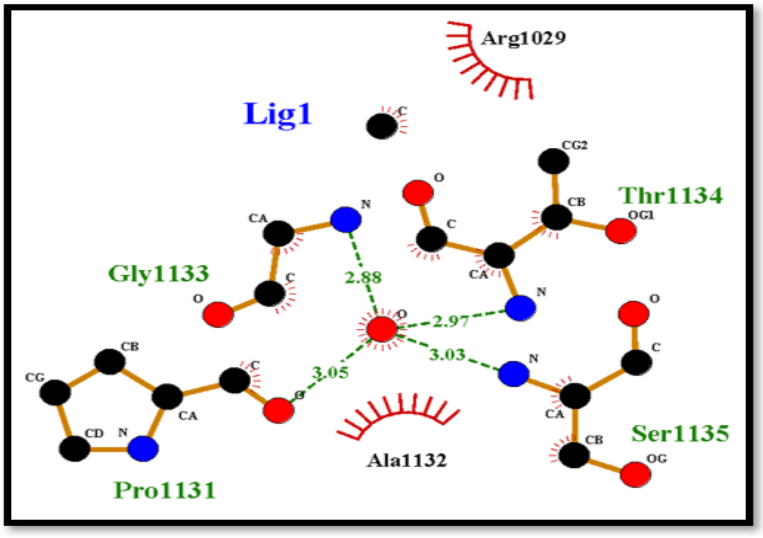


FIGURE 4.42: 2D depiction of docked complex Methyl nonyl ketone-NS2B-NS3

Figure 4.43 shows the Folic acid-NS2B-NS3 docked complex, which has a binding energy of -9.8. It has six Hydrogen bonds (Amino acid: Tyr1130, Thr1027, Ser1135, Arg1029, Ser1056, Lys1054) and consists of fifteen Hydrophobic interactions (Tyr1150, Gly1133, Gln1035, Val1036, Val1036, Val1052, Met51, Thr1053, His1051, Lys1054, Gly1133, Ala1132, His1051, Pro1131, Thr1034).

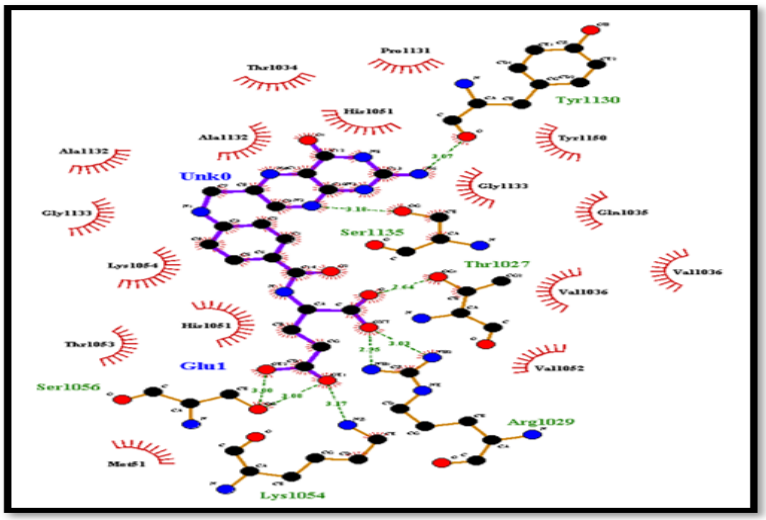


FIGURE 4.43: 2D depiction of docked complex Folic acid-NS2B-NS3

Figure 4.44 shows the Caffeic acid-NS2B-NS3 docked complex, which has a binding energy of -7.1. It has two Hydrogen bonds (Amino acid: Val1126, Thr1034) and consists of nine Hydrophobic interactions (Ser1135, Tyr1150, Gly1151, Ala1125, Leu1031, Leu1030, Arg1029, Gly1032, Ser1033).

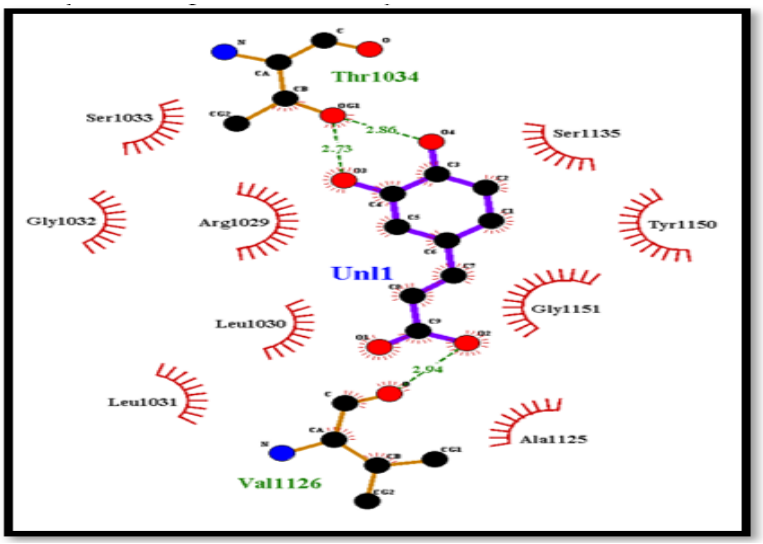


FIGURE 4.44: 2D depiction of docked complex Caffeic acid-NS2B-NS3

Figure 4.45 shows the p-coumaric acid-NS2B-NS3 docked complex, which has a binding energy of -6.4. It has five Hydrogen bonds (Amino acid: Ser1135, Gly1133, Thr1134, Pro1131, Thr1034) and consists of seven Hydrophobic interactions (Ala1132, His1051, Val1036, Met51, Arg1029, Thr1027, Lys1054).

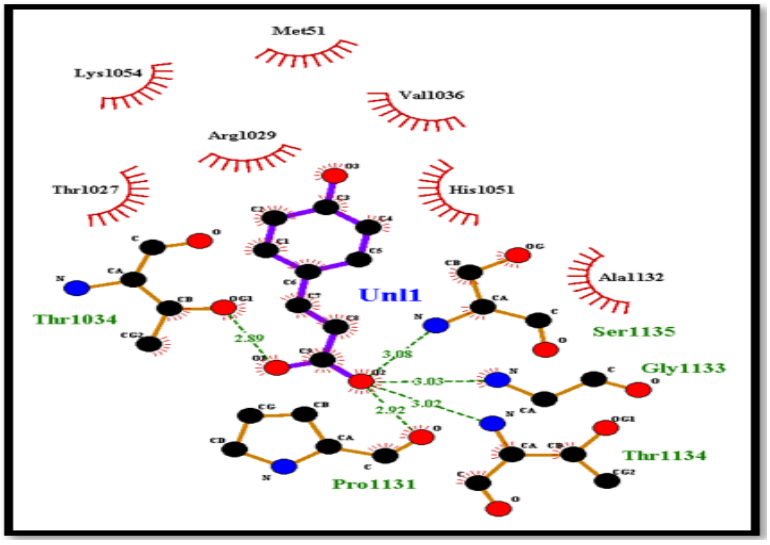


FIGURE 4.45: 2D depiction of docked complex p-coumaric acid-NS2B-NS3

Figure 4.46 shows the 5,7-dimethoxycoumarin-NS2B-NS3 docked complex, which has a binding energy of -6.2. It has four Hydrogen bonds (Amino acid: Ser1135, Val1036, His1051, Asp1075) and no Hydrophobic interactions.

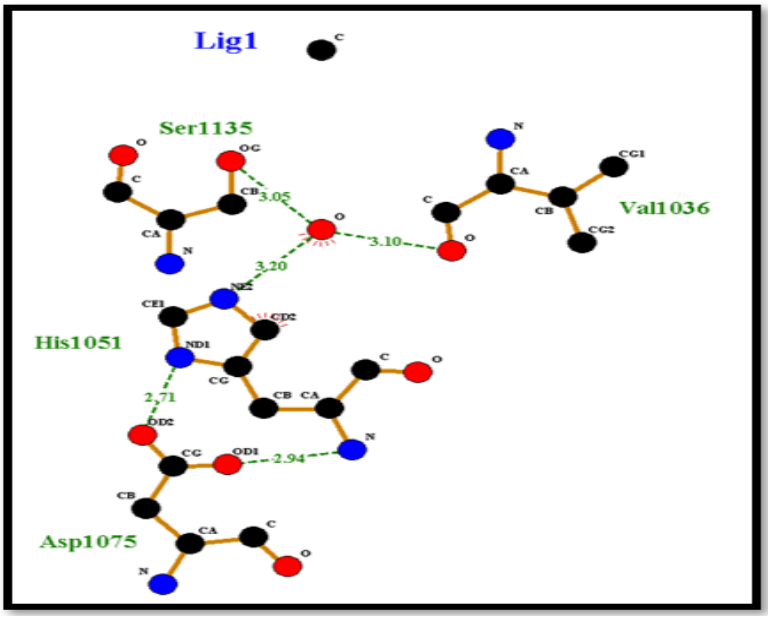


FIGURE 4.46: 2D depiction of docked complex 5,7-dimethoxycoumarin-NS2B-NS3

Figure 4.47 shows the Chlorogenic acid-NS2B-NS3 docked complex, which has a binding energy of -8.1. It has no Hydrogen bonds and consists of two Hydrophobic interactions (Thr1034, Ala1132).

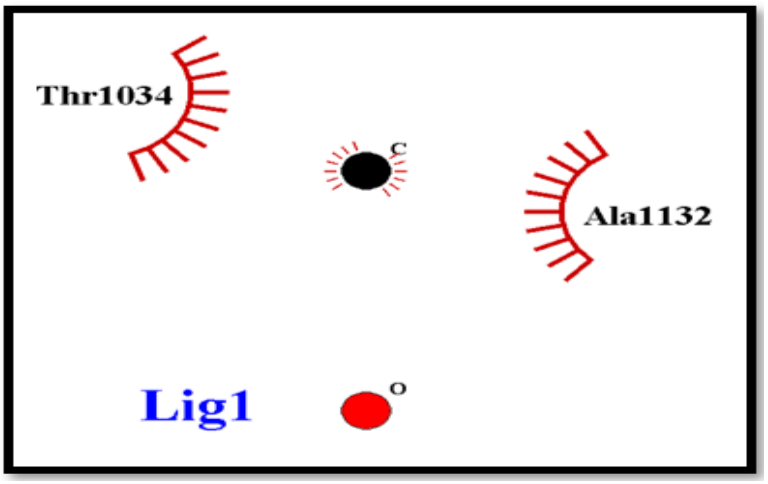


FIGURE 4.47: 2D depiction of docked complex Chlorogenic acid-NS2B-NS3

Figure 4.48 shows the Protocatechuic acid-NS2B-NS3 docked complex, which has a binding energy of -6.7. It has four Hydrogen bonds (Amino acid: Tyr1150, Arg1029, Thr1034, Leu1128) and consists of five Hydrophobic interactions (Gly1032, Gly1151, Ser1033, Thr1027, Ser1135).

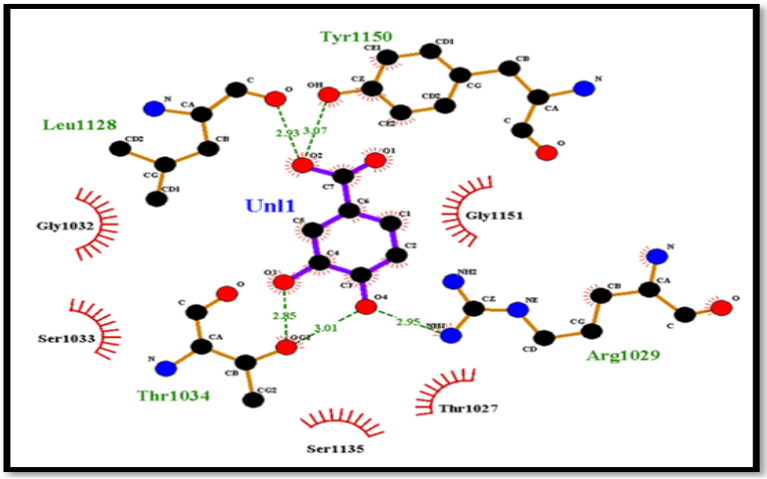


FIGURE 4.48: 2D depiction of docked complex Protocatechuic acid-NS2B-NS3

The information about the hydrogen bond and hydrophobic interactions between the chosen ligand and receptor protein NS2B-NS3 is displayed in Appendix as Table 5.2. The table explain all about the lingands.

4.10 Lead Compound Identification

The ligands' physiochemical and pharmacokinetic characteristics decide whether a molecule will become a drug or not. The Lipinski Rule of Five serves as the first filter for this identification, and pharmacokinetic properties serve as the second. Compounds that don't adhere to more than two rules are not regarded as drug-like. Folic acid and Chlorogenic acid, both, have 6 Hydrogen-bond donors but follow other properties, hence it is acceptable. Even though Carpaine and Tocopherol have Log P values of more than 5, they are however allowed to move on to the next phase. Therefore, there are no eliminations in the initial evaluation, and all compounds proceeded to the next phase. After this step, screening is based on good Interaction and Binding scores. Then, the succeeding step is pharmacokinetic screening. In this screening, Phenylalanine and Umbelliferon are omitted as both are hepatotoxic and may damage the liver, whereas due to the status as hERG II inhibitors, Tocopherol is eliminated. Cysteine, Homocysteine, p-coumaric acid, and Chlorogenic acid are eradicated because they have properties of chronic toxicity. Glutamic acid is knockout due to its poor Intestinal Absorption. After this screening, there are four compounds left i.e., Carpaine, Dimethoxyphenol, Caffeic acid, and Protocatechuic acid. Among all these compounds, Caffeic Acid shows the best binding energy as well as more hydrogen bonds with both targeted proteins (NS5 and NS2B-NS3).

4.11 Inhibitor Identification Against Targeted Proteins

Although there have been significant efforts made in the development of antivirals and vaccines for dengue, there is currently just supportive care available for patients [23], [73], [75], [84]. After this screening, there are four compounds left i.e., Carpaine, Dimethoxyphenol, Caffeic acid, and Protocatechuic acid. Among all these compounds, Caffeic Acid shows the best binding energy. Numerous antiviral

medications and other host immune modulators are still undergoing clinical trials and won't be ready for usage in patients for a while. Several dengue vaccines are being developed and others have previously received approval for use, but neither of these has overwhelmingly demonstrated benefits for all age groups or immunity against all serotypes. Careful fluid management, along with regular monitoring and supportive care, is the cornerstone of treatment for dengue [20]. The inhibition of viral enzymes is the most successful strategy among those employed in the quest for and development of a dengue antiviral [73]. So for this research inhibitors of Selected Proteins have been chosen. SAM and HCQ are selected as inhibitors of NS5 and NS2B-NS3, respectively.

4.11.1 Inhibitor of NS5: S-Adenosyl-L-methionine (SAM)

All living things contain the significant chemical S-Adenosyl-L-methionine (SAM). Although SAM is produced in the cytosol of every cell, the liver plays a crucial role in maintaining its homeostasis as the primary site of both its production and breakdown [110]. In Europe, SAM has been widely utilized as a prescription-only dietary supplement, and lactic acid bacteria with high SAM synthesizing capacity are anticipated to be employed as probiotics that increase SAM levels. SAM is regarded as a crucial metabolite in living organisms, just like ATP, and it is now more widely known that it plays important role in the treatment of many disorders, including liver disease, osteoarthritis, neurologic syndrome, depression, Alzheimer's dementia, and others [111].

4.11.2 Inhibitor of NS2B-NS3:Hydroxychloroquine (HCQ)

Hydroxychloroquine (HCQ) is a medication that has been used for many years to treat skin conditions and autoimmune diseases. It is also becoming more popular as a treatment for cancer and pediatric inflammatory disorders [112]. Using a drug library that has been approved by the Food and Drug Administration, molecular docking, and molecular dynamics simulations, the drug HCQ which has already

been approved for use in pregnancy has been discovered as a potential inhibitor of NS2B-NS3 protease. Enzyme kinetic experiments further demonstrated that hydroxychloroquine suppresses the activity of the NS2B-NS3 protease, providing further insight into its potential inhibitory effects. Furthermore, hydroxychloroquine greatly lowers placental cell infection with the Zika virus [113].

4.12 Inhibitor's ADMET Properties

Inhibitors are tested for drug score, drug similarity and toxicity. The ADMET properties of the choosen inhibitors are discovered by using the web tool pkCSM. In this the absorption, distribution, metabolism, excretion and toxicity was noticed.

4.12.1 Absorption

Table 4.16 shows the absorptive properties of Selected Inhibitors. These properties revealed that both compounds are less soluble in water. The Intestinal Absorption of HCQ is more as compared to SAM. Skin permeability is low and both show positive results for P-glycoprotein substrate and negative for P-glycoprotein I and II inhibitors.

TABLE 4.16: Absorption properties of SAM & HCQ

		Inhibitor of NS5	**Inhibitor of NS2B-NS3**
Sr.No.	**Absorption Properties**	**S-adenosyl methionine (SAM)**	**Hydroxy chloroquine (HCQ)**
1	Water Solubility	-2.892	-3.627
2	$CaCO_2$ Permeability	-0.844	1.543
3	Intestinal Absorption (Human)	17.436	90.217
4	Skin Permeability	-2.735	-2.849

TABLE 4.16: Absorption properties of SAM & HCQ

		Inhibitor of NS5	**Inhibitor of NS2B-NS3**
5	P-glycoprotein Substrate	Yes	Yes
6	P-glycoprotein I Inhibitor	No	No
7	P-glycoprotein II Inhibitor	No	No

4.12.2 Distribution

The distribution properties of selected inhibitors are given below in Table 4.17. The table illustrates that HCQ has a higher VDss value than SAM, indicating that it is dispersed more in tissue and less in plasma. The permeability to the brain barrier is indicated by BBB Permeability. Weak results are displayed by both inhibitors, indicating that their BBB permeability is poor.

TABLE 4.17: Distribution properties of SAM & HCQ

		Inhibitor of NS5	**Inhibitor of NS2B-NS3**
Sr.No.	**Distribution Properties**	**S-adenosyl-methionine (SAM)**	**Hydroxy chloroquine (HCQ)**
1	VDss (Human)	0.027	1.076
2	Fraction Unbound	0.437	0.247
3	BBB Permeability	-1.02	0.074
4	CNS Permeability	-3.559	-2.511

4.12.3 Metabolism

Table 4.18 elaborates on the metabolic properties of inhibitory compounds.

TABLE 4.18: Metabolic properties of SAM & HCQ

		Inhibitor of NS5	**Inhibitor of NS2B-NS3**
Sr.No.	**Metabolism Properties**	**S-adenosyl-methionine (SAM)**	**Hydroxy-chloroquine (HCQ)**
1	CYP2D6 Substrate	No	Yes
2	CYP3A4 Substrate	No	Yes
3	CYP1A2 Inhibitor	No	Yes
4	CYP2C19 Inhibitor	No	No
5	CYP2C9 Inhibitor	No	No
6	CYP2D6 Inhibitor	No	Yes
7	CYP3A4 Inhibitor	No	No

Table 4.18 illustrates that HCQ is metabolized by CYP2D6 and CYP3A4 while SAM cannot. CYP2D6 and CYP3A4 are the two main isoforms of cytochrome P450 (detoxification enzyme of the liver) that plays role in the excretion of exogenous compounds by oxidizing them. SAM cannot inhibit CYP2D6 as HCQ does.

4.12.4 Excretion

Table 4.19 lists the anticipated values for the inhibitors' excretion. The hepatic and renal clearance of SAM and HCQ is represented by the total clearance reported as a log (CL tot) value. SAM and HCQ, both, predict a "No" for the presence of the renal OCT2 substrate, indicating that it does not affect how OCT2 functions in cells.

TABLE 4.19: Excretory properties of SAM & HCQ

		Inhibitor of NS5	**Inhibitor of NS2B-NS3**
Sr.No.	**Excretion Properties**	**S-adenosyl-methionine (SAM)**	**Hydroxy-chloroquine (HCQ)**
1	Total Clearance	0.446	1.152
2	Renal OCT2 Substrate	No	No

4.12.5 Toxicity

The table 4.20 shows that the maximum tolerated dose value of SAM and HCQ is 0.463 and -0.091 respectively. However, HCQ predicts itself as an hERG II inhibitor which means it inhibits potassium channels. Drug toxicity is predicted by the LD_{50}, and the LOAEL identifies the lowest dose at which side effects are likely to occur.

TABLE 4.20: Toxicity measurements of SAM & HCQ

		Inhibitor of NS5	**Inhibitor of NS2B-NS3**
Sr.No.	**Toxicity Properties**	**S-adenosyl-methionine (SAM)**	**Hydroxy-chloroquine (HCQ)**
1	Max. Tolerated Dose (Human)	0.463	-0.091
2	hERG I inhibitor	No	No
3	hERG II inhibitor	No	Yes
4	Oral Rat Acute Toxicity (LD_{50})	2.482	2.656
5	Oral Rat Chronic Toxicity (LOAEL)	2.019	1.407
6	Hepatotoxicity	Yes	Yes

TABLE 4.20: Toxicity measurements of SAM & HCQ

		Inhibitor of NS5	**Inhibitor of NS2B-NS3**
7	Skin Sensitization	No	No
8	*T.Pyriformis* toxicity	0.285	1.061
9	Minnow toxicity	3.178	1.325

4.13 Inhibitor's Docking with Targeted Proteins

SAM and HCQ (as a ligand) with NS5 and NS2B-NS3 were docked using the web program CB Dock. Five conformational poses were produced through docking, and the best one was chosen. Table 4.21 displays the docking outcomes for the chosen protein-ligand complex.

TABLE 4.21: Docking Scores of Inhibitors with Targeted Protein Via CB Dock

		Docked with NS5	**Docked with NS2B-NS3**
Sr.No.	**Properties**	**S-adenosyl-methionine (SAM)**	**Hydroxy-chloroquine (HCQ)**
1	Binding Score	-7.8	-7.6
2	Cavity size	1660	5928
3	H-Bond Donor	4	2
4	H-Bond Acceptor	11	4
5	Log P value	-3.2569	3.783
6	Molecular Weight	398.4	335.9
7	Rotatable Bonds	7	9

4.14 Comparison of Lead Compound with Inhibitors

To determine the bioavailability, drug-likeness, efficacy, and safety of the Inhibitors and lead compound, their physicochemical and pharmacokinetic properties are compared.

4.14.1 Comparison of Lipinski Rule of Five

All compounds pass the Lipinski rule criteria for drug-likeness. However, Caffeic acid indicates less molecular weight than both inhibitors. Table 4.22 shows that Caffeic acid has less no. of hydrogen bond donor and acceptor as compared to SAM and HCQ.

TABLE 4.22: Comparison of Lipinski Rule of Five of Inhibitors and Lead Compounds

	Inhibitor of NS5	**Inhibitor of NS2B-NS3**	**Lead Compound**
Parameters	**S-adenosyl-methionine (SAM)**	**Hydroxy-chloroquine (HCQ)**	**Caffeic Acid**
LogP value	-3.2569	3.783	1.195
Molecular weight (g/mol)	398.4	335.9	180.15
Rotatable Bonds	7	9	2
H-Bond Acceptor	11	4	3
H-Bond Donor	4	2	3

4.14.2 Comparison of ADMET Properties

When choosing chemicals as therapeutic candidates, pharmacokinetic qualities are a key consideration. To compare the ADMET characteristics, adsorption, distribution, metabolism, excretion, and toxicity are examined. Tables contain a list of these attributes.

4.14.2.1 Absorption

According to absorptive comparison, it is determined that intestinal absorption of SAM is lower and HCQ is higher than the standard range i.e. 30-70%. None of them is the inhibitor of P-glycoprotein I and II, Caffeic acid, however, is not a P-glycoprotein substrate (Table 4.23).

TABLE 4.23: Comparison of Absorptive properties of Inhibitors and Lead Compounds

	Inhibitor of NS5	**Inhibitor of NS2B-NS3**	**Lead Compound**
Absorption Properties	**S-adenosyl-methionine (SAM)**	**Hydroxy-chloroquine (HCQ)**	**Caffeic Acid**
Water Solubility	-2.892	-3.627	-2.33
$CaCO_2$ Permeability	-0.844	1.543	0.634
Intestinal Absorption	17.436	90.217	69.407
Skin Permeability	-2.735	-2.849	-2.722
P-glycoprotein Substrate	Yes	Yes	No
P-glycoprotein I Inhibitor	No	No	No
P-glycoprotein II Inhibitor	No	No	No

4.14.2.2 Distribution

Table 4.24 shows the comparison of distribution properties. Inhibitors and lead compounds have reasonable value for VDss, as if it exceeded 2.8 L/kg then the drug is more distributed in the tissue rather than blood plasma. The fraction unbound value of Lead Compound is more than both inhibitors which shows Caffeic acid is more effective than reference inhibitors in case of unbounded friction present in plasma.

In contrast to SAM, which has poor BBB and CNS permeability values, caffeine acid and HCQ can pass across the brain barrier and into the CNS.

TABLE 4.24: Comparison of Distribution properties of Inhibitors and Lead Compounds

	Inhibitor of NS5	**Inhibitor of NS2B-NS3**	**Lead Compound**
Distributive Properties	**S-adenosyl-methionine (SAM)**	**Hydroxy-chloroquine (HCQ)**	**Caffeic Acid**
VDss (Human)	0.027	1.076	-1.098
Fraction Unbound (Human)	0.437	0.247	0.529
BBB Permeability	-1.02	0.074	-0.647
CNS Permeability	-3.559	-2.511	-2.608

4.14.2.3 Metabolism

Table 4.25 elaborates on the compared metabolic properties of Inhibitors and lead.

TABLE 4.25: Comparison of Metabolic properties of Inhibitors and Lead Compounds

	Inhibitor of NS5	**Inhibitor of NS2B-NS3**	**Lead Compound**
Metabolism Properties	**S-adenosyl methionine (SAM)**	**Hydroxy-chloroquine (HCQ)**	**Caffeic Acid**
CYP2D6 Substrate	No	Yes	No
CYP3A4 Substrate	No	Yes	No
CYP1A2 Inhibitor	No	Yes	No
CYP2C19 Inhibitor	No	No	No
CYP2C9 Inhibitor	No	No	No
CYP2D6 Inhibitor	No	Yes	No
CYP3A4 Inhibitor	No	No	No

The cytochrome P450 isoforms CYP2D6, CYP3A4, CYP1A2, CYP2C19, and CYP2C9 are used to predict the metabolic characteristics of substances. In contrast to caffeic acid, which is not projected to be a substrate of these isoforms, Hydroxychloroquine (HCQ) is listed in the table as a CYP3A4 isoform substrate.

4.14.2.4 Excretion

Excretion attributes are represented in Table 4.26 as two models with projected values.

TABLE 4.26: Comparison of Excretory Properties of Inhibitors and Lead Compounds

	Inhibitor of NS5	**Inhibitor of NS2B-NS3**	**Lead Compound**
Excretion Properties	**S-adenosyl methionine (SAM)**	**Hydroxy-chloroquine (HCQ)**	**Caffeic Acid**
Total Clearance	0.446	1.152	0.508
Renal OCT2 Substrate	No	No	No

When compared to Caffeic Acid, HCQ has a higher projected value and SAM has a lower one, for drug clearance as measured by total clearance. All substances fall under the "No" category for the "Renal OCT2 Substrate Model," which means they do not disrupt the normal operation of the Organic Cation Transporter 2 (OCT2), which is involved in the renal clearance of medicines.

4.14.2.5 Toxicity

Of all the pharmacokinetic (ADMET) qualities, toxicity is the most crucial one. Maximum tolerated dosage aids in determining the maximum suggested tolerated dose; if the value is equal to or less than 0.477 log mg/kg/day, it is regarded as low. Table 4.27 shows that Caffeic acid has a more tolerated value than both inhibitors (SAM and HCQ). The hERG I and II inhibitor models indicate whether or not the drugs under study will inhibit potassium channels. If "yes," the chemical might not be suitable for the medicine. Table 4.25 makes it clear that HCQ acts as an hERG II inhibitor. The lowest dose of a medicine that can cause harmful effects over a long period (chronic use) is determined by oral rat chronic toxicity (LOAEL). LOAEL value of SAM (inhibitor) and Caffeic acid (Lead Compound) is almost equal. However, HCQ predicts lower values as compared to other ones. The term

"hepatotoxicity" refers to a liver injury that can be classified as either positive or negative. While both inhibitors are hepatotoxic substances, the predicted outcome for Caffeic Acid demonstrates that it is not. (Table 4.27).

TABLE 4.27: Comparison of Toxicity of Lead compound & Inhibitors

	Inhibitor of NS5	**Inhibitor of NS2B-NS3**	**Lead Co -mpound**
Toxicity Properties	**S-adenosyl-methionine (SAM)**	**Hydroxy-chloroquine (HCQ)**	**Caffeic Acid**
Max. Tolerated Dose (Human)	0.463	-0.091	1.145
hERG I inhibitor	No	No	No
hERG II inhibitor	No	Yes	No
Oral Rat Acute Toxicity (LD_{50})	2.482	2.656	2.383
Oral Rat Chronic Toxicity (LOAEL)	2.019	1.407	2.092
Hepatotoxicity	Yes	Yes	No
Skin Sensitization	No	No	No
T.Pyriformis toxicity	0.285	1.061	0.293
Minnow toxicity	3.178	1.325	2.246

Both Inhibitors and the Lead Compound does not cause any allergic skin reaction. *T. pyriformis* toxicity value i.e., greater than - 0.5 is considered toxic according to which all compounds show toxicity against *T. pyriformis*. Toxicity levels for minnows are deemed toxic if they are less than 0.5mM.

4.14.3 Comparison of Physiochemical Properties and Docking Results

The essential characteristics of a compound are described by its physiochemical properties, which also serve as the main filters to separate compounds with desirable characteristics. According to Molecular Formula, SAM and HCQ consist of 49 and 50 atoms of Carbon, Hydrogen, Oxygen, Sulphur, Iodine, and Nitrogen, whereas, Caffeic Acid consists of 21 atoms of Carbon, Hydrogen, and Oxygen which shows its simplicity as a bio-compound. Compared to both inhibitors, Caffeic Acid has a lower molecular weight. Caffeic Acid can donate 3 Hydrogen bonds while inhibitors have values of 2 and 4. If there are more than 10, the oral bioavailability of rotatable bonds decreases.

In contrast to inhibitors, Caffeic Acid contains two rotatable bonds. SAM consists of 7 rotatable bonds whereas HCQ has 9 as shown in Table 4.28. When compared to the inhibitors, Caffeic Acid has a lower Binding score and shares the same cavity size as SAM; however, HCQ has a higher value for the cavity size.

TABLE 4.28: Comparison of Physiochemical Properties and Docking Scores of Inhibitors and Lead Compounds

	Inhibitor of NS5	**Inhibitor of NS2B-NS3**	**Lead Compound**
Properties	**S-adenosyl-methionine (SAM)**	**Hydroxy-chloroquine (HCQ)**	**Caffeic Acid**
Binding Score	-7.8	-7.6	-6.5
Cavity size	1660	5928	1660
H-Bond Donor	4	2	3
H-Bond Acceptor	11	4	3

TABLE 4.28: Comparison of Physiochemical Properties and Docking Scores of Inhibitors and Lead Compounds

	Inhibitor of NS5	**Inhibitor of NS2B-NS3**	**Lead Compound**
Log P value	-3.2569	3.783	1.195
Molecular Formula	$C_{15}H_{22}N_6O_5S$	$C_{18}H_{26}ClN_3O$	$C_9H_8O_4$
Molecular Weight	398.4	335.9	180.1
Rotatable Bonds	7	9	2

4.14.4 Comparison of Docking Analysis

LigPlot is used to show the docking results, and the number of hydrogen bonds, hydrophobic contacts, steric interactions, and interacting amino acids is evaluated.

NS5:with Lead Compound and Inhibitor (SAM)

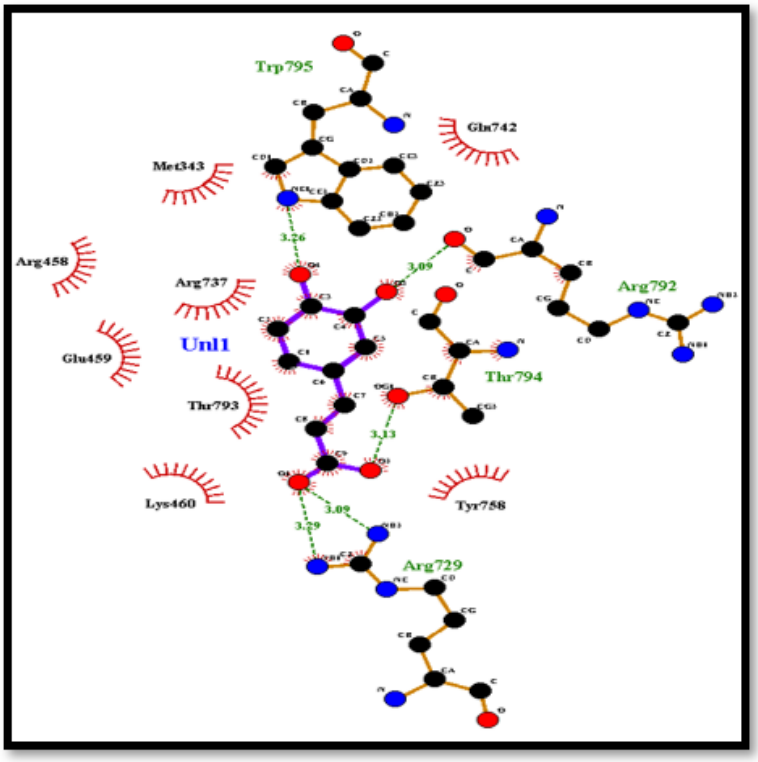


FIGURE 4.49: 2D depiction of docked complex Caffeic Acid-NS5

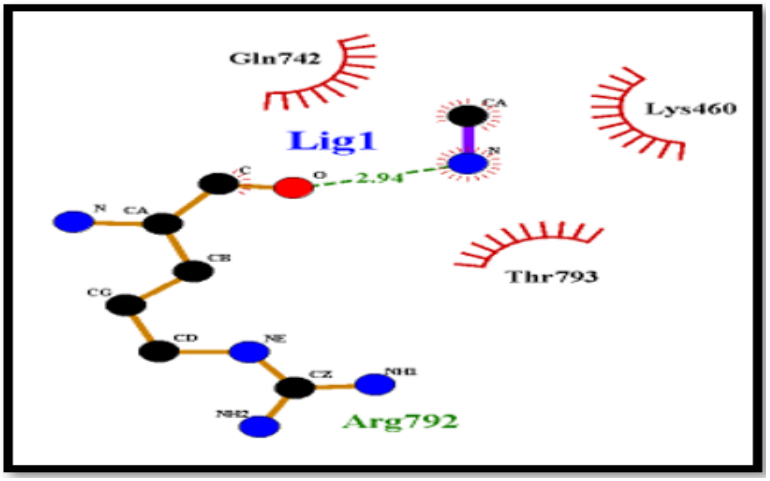


FIGURE 4.50: 2D depiction of docked complex S-adenosylmethionine-NS5

TABLE 4.29: Docking analysis Comparison with Protein NS5

Compounds	**Binding Energy**	**No.of Hydrogen Bonds**	**Hydrogen Bonding**		**Hydrophobic Interactions**
			Amino Acids	**Distance**	
Caffeic acid	-6.5	4	Arg729 Thr794 Arg729 Trp795	3.29 3.13 3.09 3.26	Gln742 Met343 Arg458 Arg737 Thr793 Lys460 Tyr758

TABLE 4.29: Docking analysis Comparison with Protein NS5

Compounds	**Binding Energy**	**No.of Hydrogen Bonds**	**Hydrogen Bonding**		**Hydrophobic Interactions**
			Amino Acids	**Distance**	
S-adenosyl-methionine (SAM)	-7.8	1	Arg792	2.94	Gln742 Lys460 Thr793

Table 4.29 displays the specifics of the hydrogen bond and hydrophobic interaction. The presence of oxygen atoms is significant since it is necessary for the establishment of an H-bond with the target protein. While S-adenosylmethionine only creates one hydrogen bond with Arg, Caffeic Acid forms four with Arg, Thr, and Trp residues. Additionally, S-adenosylmethionine has fewer hydrophobic interactions than Caffeic Acid.

NS2B-NS:with Lead Compound and Inhibitor (HCQ)

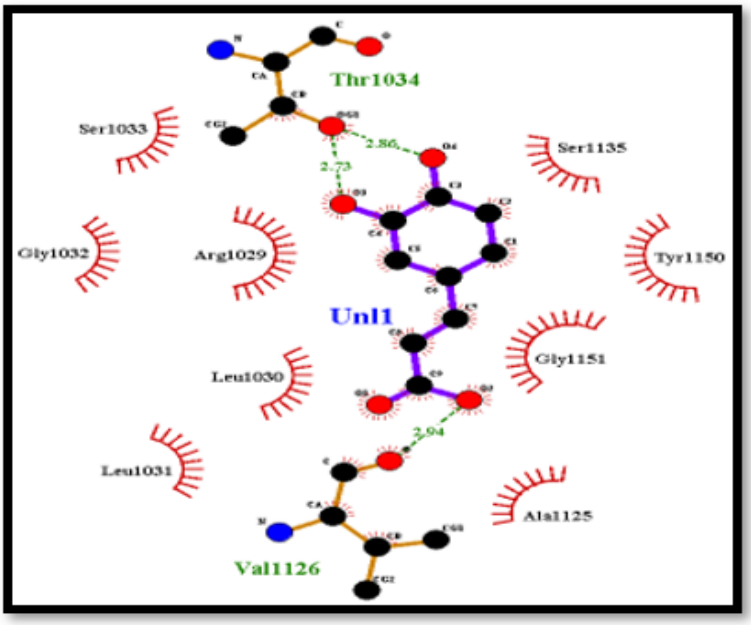


FIGURE 4.51: 2D depiction of docked complex Caffeic acid-NS2B-NS3

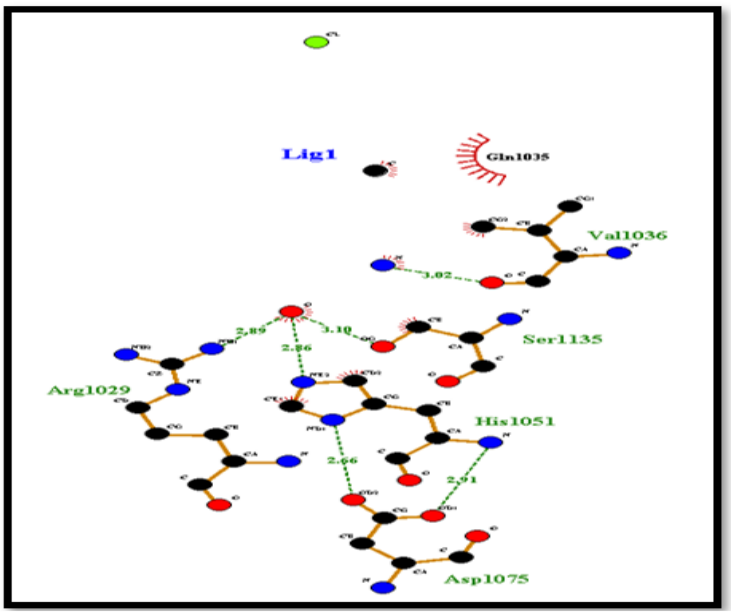


FIGURE 4.52: 2D depiction of docked complex Hydroxychloroquine-NS2B-NS3

Caffeic Acid forms four hydrogen bonds having residues of Arg, Thr, and Trp, whereas, Hydroxychloroquine contains 5 hydrogen bonds with residues Val, Ser, Arg, Asp and His. But Hydroxychloroquine has not proper interaction with protein. In addition, Table 4.30 demonstrates that Caffeic Acid has a greater number of hydrophobic interactions than Hydroxychloroquine (HCQ).

TABLE 4.30: Docking analysis Comparison with Protein NS2B-NS3

Compounds	**Binding Energy**	**No.of Hydrogen Bonds**	**Hydrogen Bonding**		**Hydrophobic Interactions**
			Amino Acids	**Distance**	
Caffeic acid	-6.5	4	Arg729 Thr794 Arg729 Trp795	3.29 3.13 3.09 3.26	Gln742 Met343 Arg458 Arg737 Thr793

TABLE 4.30: Docking analysis Comparison with Protein NS2B-NS3

Compounds	**Binding Energy**	**No.of Hydrogen Bonds**	**Hydrogen Bonding**		**Hydrophobic Interactions**
			Amino Acids	**Distance**	
Hydroxy-chloroquine	-7.6	5	Arg1029	2.89	Gln1035
			Asp1075	2.66	
			His1051	2.91	

Chapter 5

Conclusions and Recommendations

Undeniably, papaya is a traditional herbal remedy. Its therapeutic and nutritive benefits are well known all over the world. It has been utilized as a nutraceutical in ethnomedicine to treat or prevent a variety of illnesses, including cancer. According to several investigations, papaya leaves can boost platelet counts and prevents the damaging effects on platelets by dengue virus. The goal of this study was to identify active ingredients of *C. papaya* that could counteract thrombocytopenia. 20 ligands that may inhibit the target protein were chosen and docked against receptor proteins. PyMol and LigPlot were used to evaluate and visualize the docking data. Caffeic acid was chosen as the lead drug against both proteins, NS5 and NS2B-NS3, after a thorough investigation of their binding score, physicochemical qualities, and ADMET properties.

The possible inhibitors, based on earlier research on the targeted proteins, S-adenosylmethionine (SAM) for NS5 and hydroxychloroquine (HCQ) for NS2B-NS3, were compared with the virtual screening findings, physiochemical properties, and pharmacokinetics features of these compounds. These results imply that caffeic acid is regarded as a positive indicator for the creation of antiviral drugs to treat thrombocytopenia caused by dengue fever. Overall, it has been determined

that additional research into the phenolic molecule "caffeic acid" could lead to its potential development as a potent anti-dengue drug. To develop therapeutic uses for thrombocytopenia in vivo, more research is required to pinpoint the precise pharmacological activity of caffeic acid.

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An Appendix

TABLE 5.1: Active Ligand showing Hydrogen and Hydrophobic Interactions with NS5

Compounds	**Binding Energy**	**No. of Hydrogen Bonds**	**Hydrogen Bonding**		**Hydrophobic Interactions**
			Amino Acids	**Distance**	
Carpaine	-9.6	0	-	-	Ser796 Ile797 Cys709 Glu459 Lys461 Arg472 Asp539 Asn610

TABLE 5.1: Active Ligand showing Hydrogen and Hydrophobic Interactions with NS5

Compounds	**Binding Energy**	**No. of Hydrogen Bonds**	**Hydrogen Bonding**		**Hydrophobic Interactions**
			Amino Acids	**Distance**	
Kaempferol	-8.1	0	-	-	Thr794 Trp795
Ascorbic acid	-5.9	1	Thr794	2.75	Arg737 Trp795 Arg472
Tocopherol	-6.5	0	-	-	Asp539 Ala473 Arg737
Dicoumarol	-8.7	0	-	-	Thr793
Cysteine	-4.0	3	Thr793 Tyr758 Lys460	3.02 3.05 3.08	Thr794 Thr794 Arg792 Glu459 Trp795

TABLE 5.1: Active Ligand showing Hydrogen and Hydrophobic Interactions with NS5

Compounds	**Binding Energy**	**No. of Hydrogen Bonds**	**Hydrogen Bonding**		**Hydrophobic Interactions**
			Amino Acids	**Distance**	
Homocysteine	-4.2	2	Tyr758	3.21	Lys460
			Thr793	2.91	Arg792
			Thr793	3.08	Gln742
Dimethoxy phenol	-5.3	4	Arg737	2.87	Arg792
			Lys460	3.27	Thr794
			Tyr758	2.83	Glu459
					Arg458
					Lys460
					Glu459
					Gln742
Coumarin	-6.6	0	-	-	Thr794
					Thr793
					Trp795
					Arg792

TABLE 5.1: Active Ligand showing Hydrogen and Hydrophobic Interactions with NS5

Compounds	**Binding Energy**	**No. of Hydrogen Bonds**	**Hydrogen Bonding**		**Hydrophobic Interactions**
			Amino Acids	**Distance**	
Glutamic acid	-5.2	4	Lys460	3.07	Gln742
			Arg737	3.16	Arg792
			Thr793	3.05	Trp795
			Tyr758	2.87	Glu459
					Arg458
					Gln742
					Thr794
Phenyl-alanine	-6.5	2	Arg737	3.22	Arg792
			Lys460	3.12	Trp795
					Thr793
					Trp795

TABLE 5.1: Active Ligand showing Hydrogen and Hydrophobic Interactions with NS5

Compounds	**Binding Energy**	**No. of Hydrogen Bonds**	**Hydrogen Bonding**		**Hydrophobic Interactions**
			Amino Acids	**Distance**	
Caffeoyl alcohol	-6.1	2	Arg737	3.07	Trp795
			Lys460	2.83	Glu459
					Lys461
					Met343
					Gln742
Umbelliferon	-6.5	3	Arg737	2.81	Thr794
			Lys460	2.91	Trp795
			Arg458	3.21	Thr793
					Tyr758
					Glu459
					Trp478
Methyl nonyl ketone	-5.0	2	Arg579	2.99	Thr449
			Glu287	3.32	Lys283

TABLE 5.1: Active Ligand showing Hydrogen and Hydrophobic Interactions with NS5

Compounds	**Binding Energy**	**No. of Hydrogen Bonds**	**Hydrogen Bonding**		**Hydrophobic Interactions**
			Amino Acids	**Distance**	
Folic acid	-9.0	5	Ile797	3.30	His798
					Cys709
			Ser796	2.94	Gly662
					Ser710
			Gln603	3.12	Ile474
					Trp475
			Thr606	2.94	Asp539
					Tyr607
			Asp663	3.00	Ser661
					Gln742
Caffeic acid	-6.5	4	Arg729	3.29	Met343
			Thr794	3.13	Arg458
					Arg737
			Arg729	3.09	Thr793
			Trp795	3.26	Lys460
					Tyr758

TABLE 5.1: Active Ligand showing Hydrogen and Hydrophobic Interactions with NS5

Compounds	**Binding Energy**	**No. of Hydrogen Bonds**	**Hydrogen Bonding**		**Hydrophobic Interactions**
			Amino Acids	**Distance**	
p-coumaric acid	-6.3	3	Arg792	3.13	Met343 Glu742
			Lys460	3.30	Arg458 Glu459
			Arg737	3.21	Thr793 Trp795
5,7-dimeth- -oxy coumarin	-6.1	2	Tyr758	2.94	Thr794 Arg737
			Thr793	3.22	Lys460
Chlorogenic acid	-8.4	0	-	-	Thr794 Trp795
Protocatechuic acid	-6.3	3	Trp475	3.07	Asp539 Ala473 Gln603
			Thr606	2.97	Gly600 Gly602
			Ser601	3.00	Arg599

TABLE 5.2: Active Ligand showing Hydrogen & Hydrophobic Interactions with NS2B-NS3

Compounds	**Binding Energy**	**No. of Hydrogen Bonds**	**Hydrogen Bonding**		**Hydrophobic Interactions**
			Amino Acid	**Distance**	
Carpaine	-8.5	1	Ser48	2.70	Arg1059 Arg1064 Met51 Ser1056 Lys1073 Val1072 Val49 Asp50
Kaempferol	-8.4	2	Gly1133 Ser1135	3.30 3.00	-
Ascorbic acid	-6.6	0	-	-	Ser1135 Tyr1150 Arg1029 Thr1027 Leu1085
Tocopherol	-7.8	0	-	-	Val1146 Leu1076 Trp1083 Gly1148 Pro1131
Dicoumarol	-8.9	1	Arg1029	3.17	Tyr1130 Gly1151

TABLE 5.2: Active Ligand showing Hydrogen & Hydrophobic Interactions with NS2B-NS3

Compounds	**Binding Energy**	**No. of Hydrogen Bonds**	**Hydrogen Bonding**		**Hydrophobic Interactions**
			Amino Acid	**Distance**	
Cysteine	-4.0	4	Ser1135 Thr1134 Gly1133 Thr1034	3.01 3.08 3.01 3.04	Val1036 Ala1132 Pro1131 Arg1029
Homocysteine	-4.5	1	Tyr1130	3.00	Tyr1150 Thr1034 Ser1135 Asp1129 Ala1132 Pro1131
Dimethoxy phenol	-5.7	2	Tyr1130 Tyr1150	3.27 2.78	Thr1034 Gly1133 Ser1135 Gly1151 Arg1029
Coumarin	-6.1	2	Thr1034 Thr1027	2.80 3.19	Met51 Lys1054 His1051 Val1036 Ala1132 Gly1133 Arg1029

TABLE 5.2: Active Ligand showing Hydrogen & Hydrophobic Interactions with NS2B-NS3

Compounds	**Binding Energy**	**No. of Hydrogen Bonds**	**Hydrogen Bonding**		**Hydrophobic Interactions**
			Amino Acid	**Distance**	
Glutamic acid	-5.7	4	Ser1135 Thr1134 Gly1133 Thr1034	3.09 3.18 3.10 3.01	Asp1129 Gly1151 Tyr1150 Pro1131 Tyr1130 Ala1132 Gly1032 Arg1029 Ser1033 Gln1035 Thr1034
Phenyl-alanine	-5.5	1	Val1036	3.14	Val1036 Gly1133 His1051
Caffeoyl alcohol	-6.6	1	Val1126	3.11 3.04	Tyr1150 Gly1151 Val1155 Gly1153
Umbelliferon	-6.5	2	Ala1087 Val1146	2.79 2.85	Leu1076 Asn1152 Gly1148 Trp1083

TABLE 5.2: Active Ligand showing Hydrogen & Hydrophobic Interactions with NS2B-NS3

Compounds	**Binding Energy**	**No. of Hydrogen Bonds**	**Hydrogen Bonding**		**Hydrophobic Interactions**
			Amino Acid	**Distance**	
Methyl nonyl ketone	-5.5	4	Ser1135	3.03	Arg1029
			Thr1134	2.97	Ala1132
			Gly1133	2.88	Tyr1150
			Pro1131	3.05	Gly1133
					Gln1035
					Val1036
					Val1036
					Val1052
					Met51
Folic acid	-9.8	6	Tyr1130	3.07	Thr1053
			Thr1027	2.64	His1051
			Ser1135	3.18	Lys1054
			Arg1029	3.03	Gly1133
			Ser1056	3.00	Ala1132
			Lys1054	3.17	His1051
					Pro1131
					Thr1034

TABLE 5.2: Active Ligand showing Hydrogen & Hydrophobic Interactions with NS2B-NS3

Compounds	**Binding Energy**	**No. of Hydrogen Bonds**	**Hydrogen Bonding**		**Hydrophobic Interactions**
			Amino Acid	**Distance**	
Caffeic acid	-7.1	2			Ala1125
					Leu1031
					Leu1030
			Val1126	2.94	
			Thr1034	2.86	Arg1029
					Gly1032
					Ser1033
					Ala1132
p-coumaric acid	-6.4	5	Ser1135	3.08	His1051
			Gly1133	3.03	Val1036
			Thr1134	3.02	Met51
			Pro1131	2.92	Arg1029
			Thr1034	2.89	Thr1027
					Lys1054

TABLE 5.2: Active Ligand showing Hydrogen & Hydrophobic Interactions with NS2B-NS3

Compounds	**Binding Energy**	**No. of Hydrogen Bonds**	**Hydrogen Bonding**		**Hydrophobic Interactions**
			Amino Acid	**Distance**	
5,7-dimethoxy coumarin	-6.2	4	Ser1135	3.05	-
			Val1036	3.10	
			His1051	2.71	
Chlorogenic acid	-8.1	0	Asp1075	2.94	Thr1034
			-	-	Ala1132
Protocatechuic acid	-6.7	4	Tyr1150	3.07	Gly1032
			Arg1029	2.95	Gly1151
			Thr1034	3.01	Ser1033
			Leu1128	2.93	Thr1027
					Ser1135