Advances in Pharmacology and Pharmacy 10(4): 281-308, 2022 http://www.hrpub.org
DOI: 10.13189/app.2022.100407

In Silico Analysis of Plant-Based Bioactive Compounds Targeting Progesterone Receptors for the Treatment of Uterine Leiomyoma

M. Rajamiriyam[1], C. Anchana Devi[1,*], Farnaz Nabiya[1], N. Pushpa[2]

[1]PG and Research Department of Biotechnology, Women's Christian College, an Autonomous Institution, Affiliated to University of Madras, Chennai, Tamil Nadu, India
[2]PG and Research Department of Microbiology, Cauvery College for Women, Tiruchirapalli, Tamil Nadu, India

Received January 17, 2022; Revised June 27, 2022; Accepted August 23, 2022

Cite This Paper in the Following Citation Styles

(a): *[1] M. Rajamiriyam, C. Anchana Devi, Farnaz Nabiya, N. Pushpa , "In Silico Analysis of Plant-Based Bioactive Compounds Targeting Progesterone Receptors for the Treatment of Uterine Leiomyoma," Advances in Pharmacology and Pharmacy, Vol. 10, No. 4, pp. 281 - 308, 2022. DOI: 10.13189/app.2022.100407.*

(b): *M. Rajamiriyam, C. Anchana Devi, Farnaz Nabiya, N. Pushpa (2022). In Silico Analysis of Plant-Based Bioactive Compounds Targeting Progesterone Receptors for the Treatment of Uterine Leiomyoma. Advances in Pharmacology and Pharmacy, 10(4), 281 - 308. DOI: 10.13189/app.2022.100407.*

Abstract Uterine leiomyomas are the most common benign gynaecological tumours that affect 50% - 60% of females belonging to reproductive age. The role of progesterone in the growth and development of leiomyomas is evident. Progesterone is regulated by progesterone receptors and it acts by activating PI3K/AKT pathway, by increasing proliferating cell nuclear antigen (PCNA) levels and Bcl-2 expression. Thus, modulating the receptor can help in the treatment of leiomyomas. The current study involves testing the efficiency of the various plant-based bioactive compounds to bind to the progesterone receptors, which may possess inhibitory or modulatory effects on or against progesterone receptors. These compounds can be further experimented with using other in vivo and in vitro methods to produce phytomedicine based on drugs for the treatment of uterine leiomyomas. This study is an in silico analysis performed using various bioinformatics databases and software. The receptor and ligand structures were retrieved from PDB and PubChem databases. Their pharmacokinetics and pharmacodynamics properties were analyzed using various databases. AutoDock4.2.6 was used for molecular docking, and the results were visualized using LigPlot+. Among 15 compounds selected, 8 compounds had higher binding energies than the control drug Ulipristal acetate i.e., -8.21Kcal/mol. Of which Ursolic acid, Vitamin-D2, and Betulinic acid had binding energies above -9.0 Kcal/mol. This concludes that these compounds can modulate the progesterone receptors, thus decreasing the proliferation and growth of uterine leiomyomas. Further studies can bring out the potential therapeutic effects of these bioactive compounds and they can act as a potential lead compound in the design of novel drug targets for the treatment of uterine leiomyoma.

Keywords Uterine Leiomyoma, Selective Progesterone Receptor Modulator (SPRM), *In Silico* Analysis, AutoDock 4.2.6 and LigPlot

1. Introduction

Uterine leiomyomas, also known as uterine fibroids, are benign smooth muscle tumours located in numerous layers of the uterus. A leiomyoma rarely becomes cancerous. Uterine leiomyomas are the most frequent tumours of the female reproductive system, affecting 50 percent to 70 percent of women of reproductive age by the age of 50 [1]. Also, epidemiological studies have reported that 40% to 60% of hysterectomies are performed due to the presence of myomas [2]. There are no specific symptoms to diagnose the disease, but it is commonly associated with symptoms such as excessive menstrual bleeding,

dysmenorrhea, intermenstrual bleeding, chronic pelvic pain, and pressure symptoms such as bloating, increased urinary frequency (due to the larger size of the fibroid squeezing the bladder behind), and bowel disturbances. These leiomyomas also interfere with the reproductive functions of females causing infertility, early pregnancy loss, and later pregnancy complications [3]. Most women with leiomyomas are asymptomatic, thus most of them are undiagnosed and consequently get less medical attention.

1.1. Pathophysiology of Uterine Leiomyoma

The pathophysiology of uterine leiomyomas remains unclear because of the diverse nature of lesions. This disease is said to be heterogeneous i.e., different leiomyoma within the same uterus have different etiologies. Pathogenesis of leiomyomas depends on the type of risk factor to which the women are exposed. The various physiological, genetic and environmental factors that cause leiomyomas include age, race, reproductive factors, endogenous hormones, exogenous hormones, epigenetic factors, and lifestyle factors [4, 5]. Though there are several elements that play a role in the development of uterine leiomyomas, ovarian steroid hormones are thought to be one of the most essential. Oestrogen is considered to be a major factor in the formation of leiomyoma, and also it is a highly studied molecule when compared to progesterone. However, current research has accumulated a large body of evidence suggesting that progesterone and its receptor may play a role in the formation and development of leiomyomas [6]. Many clinical, pharmacological, biochemical and histological studies have reported the effects of progesterone in leiomyoma [7, 8]. Also, studies have demonstrated that the mitotic activity of the uterine leiomyomas is higher in the secretory phase (progesterone is predominant) when compared to the proliferative phase (oestrogen is dominant) [9].

Only a few research have been provided that show a direct link between progesterone-regulated genes in leiomyoma, but other supportive studies look at gene expression in different phases of the menstrual cycle, giving a clear picture of which genes are controlled by ovarian hormones. The higher level of PCNA and lower levels of (phosphatase and tensin homolog) PTEN can be correlated to increased mitogenesis and decreased apoptosis in leiomyoma [10] which can be regulated through progesterone and its receptor. Increased expression of the anti-apoptotic B-cell lymphoma 2 (Bcl-2) genes is seen in leiomyoma. Also, progesterone was seen to up-regulate Bcl-2 mRNA by binding to the Bcl-2 promoter thus enhancing their transcription [11].

There are non-genomic effects of progesterone that are regulated through its receptors. The mechanism remains unclear/ unknown by which the progesterone receptor is said to activate the pathway. The amino terminal of progesterone receptor (PR) has unrecognized polyproline-rich SRC Homology 3 (SH3) recognition motifs which interact with the SH3 domain of c-Src tyrosine kinase. This interaction further mediates the activation of the Mitogen-activated protein kinases (MAPK) pathway, which leads to cell division and proliferation in leiomyoma [12]. Recent studies have shown progesterone-mediated activation of the PI3K/AKT pathway in rat endometrial stromal cells. Progesterone can rapidly phosphorylate AKT (Protein kinase B) in the leiomyoma cells. The AKT pathway increases proliferation and decreases apoptosis in leiomyoma cells. The functional role and pathways activated by progesterone and its receptor in uterine leiomyoma are mentioned in Figure 1. Thus, these genes, growth factors, and pathways that are up-regulated and down-regulated by progesterone and its receptors provide an important role of progesterone in the growth and development of uterine leiomyoma [13]. All the physiological actions of progesterone are regulated by the progesterone receptors. Thus, bloating or modulating those receptors can ameliorate the effects produced by the progesterone hormone.

1.2. Treatment for Uterine Leiomyoma

Only a few research have been provided that show a direct link between progesterone-regulated genes in leiomyoma, but other supportive studies look at gene expression in different phases of the menstrual cycle, giving a clear picture of which genes are controlled by ovarian hormones. Surgical removal of fibroid or the whole uterus has been the most effective treatment till now, but it comes with some drawbacks, surgical procedures may affect the uterus in case of future pregnancies, recurrence of fibroids after surgery, surgical related scar tissue can lead to adhesion in the abdominal cavity over a long period. Scars and adhesion can cause pain in the lower abdomen. Thus relieving all these disadvantages, other non-invasive, simpler medical techniques are required for the treatment of leiomyomas. One such treatment modality is Selective Progesterone Receptor Modulators (SPRM). Ulipristal acetate is now the only SPRM approved to treat uterine leiomyoma and is also a clinically approved medicine for the treatment of mild to the severe uterine fibroid. This chemical drug is used to treat the bleeding symptoms and also to reduce the size of the leiomyoma [15]. It is sold under the brand name Esmya ® and EllaOne®.

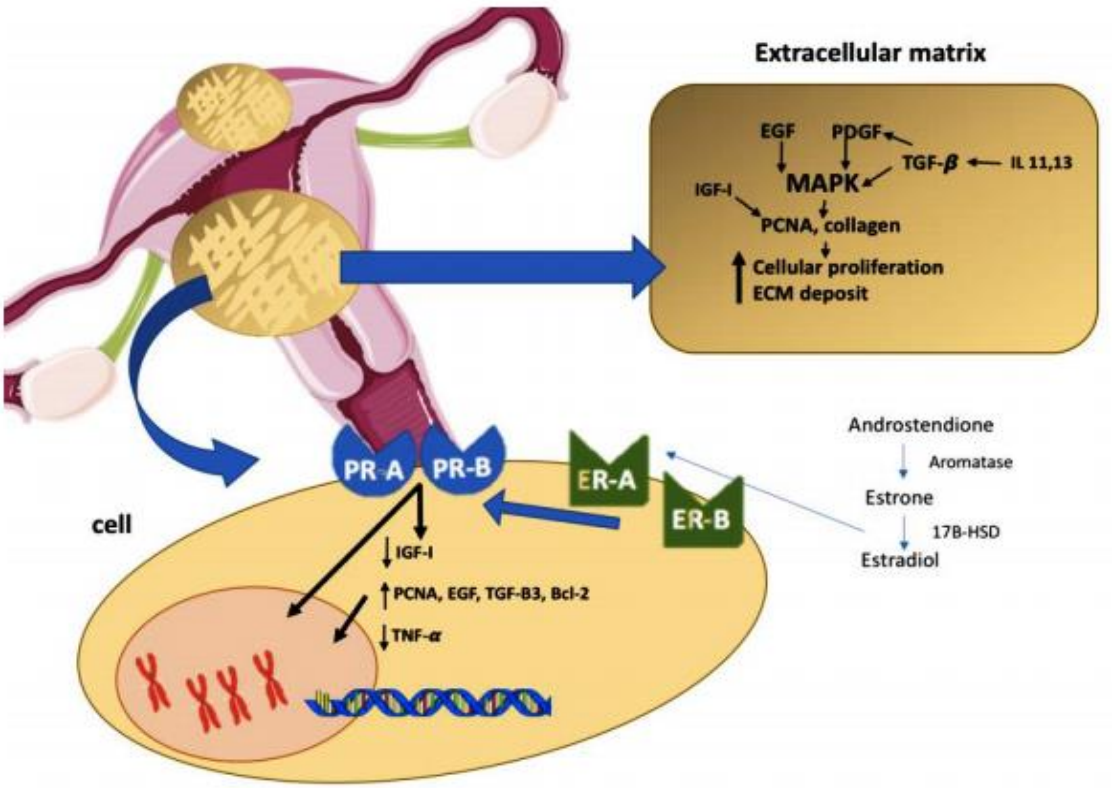

Figure 1. Functional role of progesterone and its receptor in uterine leiomyoma [14]

1.3. Current Approaches Using *In Silico* Methods

The effectiveness of plant-based phytochemicals against uterine leiomyoma has been researched by a number of studies, and it has been determined that these plant-based bioactive substances could be a viable option for the prevention and treatment of uterine leiomyoma [16]. The current study involves finding the potency of 15 plant bioactive compounds to act as SPRM. This study is an in-silico model which utilizes various software and databases to analyze the bioactive compounds. Also, docking is performed between the receptor and ligand to find the affinity between them. The results might help in finding the potential lead compounds that can act against the progesterone receptor for the treatment of uterine leiomyoma and further by in vitro and in vivo research, a potent SPRM can be formulated. Thus the main aim of the current study is to find suitable plant-based bioactive compounds that can effectively target the progesterone receptors to be used as SPRM. The in silico work can then be translated to in vitro and in vivo experiments to create an efficient SPRM for the treatment of uterine leiomyomas.

2. Materials and Methods

2.1. Databases and Software Used

- Databases - Uniprot, PDB, PubChem, PreADMET, SwissADME, pkCSM, CASTp.
- Softwares - OpenBabel, AutoDock 4.2.6, LigPlot+.

2.2. Receptor Selection, Structure, and Active Site Retrieval

Progesterone (PR) receptor were selected as a key receptor to be used for the study. The structure of the progesterone receptor was retrieved from the Uniprot [17]. The PR receptor structure with PDB ID **1SQN** was downloaded in the PDB format (Protein data bank) [18]. The active site sequence of the receptor was retrieved using the Computed Atlas of Surface Topography of proteins (CASTp) database [19].

2.3. Ligand Selection Bioactive Compound and Structure Retrieval

Bioactive compounds from plant sources were selected for the study due to the easy sourcing out of the compound from plants. From various plant-based bioactive compounds, 15 bioactive compounds were selected using Lipinski's rule. Lipinski's thumb rule of 5 was formulated to evaluate the drug-likeness of any compound [20]. It was developed to set druggability guidelines for new molecular entities [21]. 15 plant-based bioactive compounds selected are Curcumin, Vitamin-D2, Resveratrol, Indole-3-Carbinol, Isoliquiritigenin, Quercetin, Genistein, Allicin, Ellagic acid, Fisetin, Kaempferol, Betulinic acid, Butein, Ursolic acid, 2,3,5,4′ - tetrahydroxystilbene - 2 - O - β - glucoside (THSG). The Structure data files (SDF)

structure of all these bioactive compounds were retrieved from the PubChem database of the National Center for Biotechnology Information (NCBI) [22]. All the files that are required for docking must be in PDB format. Hence SDF structures were converted to PDB format using the Open Babel software [23].

2.4. Prediction of Pharmacokinetics, ADMET Properties and Biological Activities of Bioactive Compounds

Table 1 lists the various databases that were used to predict pharmacokinetics, absorption, metabolism, excretion, and toxicity properties as well as various biological activities of the ligand.

Table 1. List of Databases used for predictions

Sl. No	Databases	Parameters analyzed
1	Pubchem	Molecular formula, molecular weight, X log P, Hydrogen Bond Donor, Hydrogen Bond Acceptor, Rotatable Bond Count, Topological Polar Surface Area.
2	PreADMET	Carcinogenicity, AMES test
3	Swiss ADME	Bioavailability score
4	pkCSM	ADMET predictions

2.5. Molecular Docking of Receptor and Ligand

Molecular docking can be used to analyze whether ligands bind effectively to receptors and to determine their binding efficiency. This was done using the software Auto Dock 4.2.6 [24]. All the selected 15 bioactive compounds were docked with progesterone receptors using AutoDock 4.2.6. The number of bonds formed, binding energy, ligand efficiency, intermolecular energy, and other energy levels was noted from the docking and the results were interpreted.

2.6. Visualization of Docking Results

The LigPlot^{+} software helps in the 2D visualisation of the interactions between the receptor and ligand [25]. The final result pdbqt file from Auto Dock was converted to PDB format and pasted at the end of the 1SQN PDB file and was saved as the final result file for visualization, which then was opened in LigPlot to view the interaction between the ligand and receptor in 2D form.

3. Results and Discussion

3.1. Receptor Structure Retrieval

PDB structure of human Progesterone receptor with PDB ID: 1SQN was retrieved from the Uniprot (available at:https://www.uniprot.org/) and PDB (available at: https://www.rcsb.org) database. Figure 2 represents the 3D structure of the progesterone receptor. The CASTp database (available at: http://sts.bioe.uic.edu/castp/index.html?2cpk) was used to retrieve the active site sequence of the particular progesterone receptor.

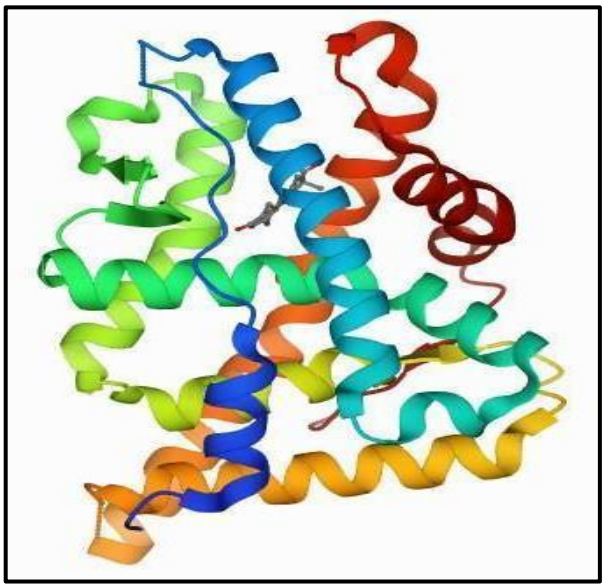

Figure 2. 3D structure of Progesterone receptor (1SQN)

3.2. Bioactive Compound Selection

15 Bioactive compounds of different plant sources were selected based on Lipinski's rule to be used as ligands for the progesterone receptors. Lipinski's rule helps in testing the drug-likeness of the bioactive compound with medicinal properties. The physical and chemical properties like molecular formula, molecular weight, XlogP, hydrogen bond donor and acceptor count, rotatable bond count, Topological polar surface area (TPSA), and bioavailability scores were obtained using PubChem (available at: https://pubchem.ncbi.nlm.nih.gov/), SwissAdme (available at: http://www.swissadme.ch/), PreADMET (available at: https://preadmet.bmdrc.kr/toxicity/) databases. Table 2 depicts the list of bioactive compounds selected through Lipinski's rule.

Table 2. Selection of bioactive compounds using Lipinski's rule of five

Sl.No	Compound	Molecular Weight (g/mol)	X log p	Hydrogen bond donor	Hydrogen bond acceptor	Rotatable bond count	Topological Polar Surface Area (Å)	Bioavailability Score	Lipinski's rule violation
1	Ulipristal acetate (C)	475.6	3.5	0	5	5	63.7	0.55	0
2	Allicin	162.3	1.3	0	3	5	61.6	0.55	0
3	Indole-3- carbinol	147.17	1.1	2	1	1	36	0.55	0
4	Resveratrol	228.24	3.1	3	3	2	60.7	0.55	0
5	THSG	406.4	1	7	9	5	160	0.55	0
6	Ellagic acid	302.19	1.1	4	8	0	134	0.55	0
7	Betulinic acid	456.7	8.2	2	3	2	57.5	0.85	1
8	Butein	272.25	2.8	4	5	3	98	0.55	0
9	Curcumin	368.4	3.2	2	6	8	93.1	0.55	0
10	Vitamin-D2	396.6	7.4	1	1	5	20.2	0.55	1
11	Genistein	270.24	2.7	3	5	1	87	0.55	0
12	Isoliquiritigenin	256.25	3.2	3	4	3	77.8	0.55	0
13	Fisetin	286.24	2	4	6	1	107	0.55	0
14	Ursolic acid	456.7	7.3	2	3	1	57.5	0.85	1
15	Kaempferol	286.24	1.9	4	6	1	107	0.55	0
16	Quercetin	302.23	1.5	5	7	1	127	0.55	0

The molecular weight of the compound determines the bioavailability, hence it should not exceed 500g/mol. Relatively all the compounds considered for the study had molecular weight lesser than 500 g/mol. Molecules like betulinic acid and ursolic acid had a molecular weight of more than 450 g /mol. According to the literature, the molecular weight and permeability of the compounds are inversely proportional. X log P is the partition coefficient which is a common measure of lipophilicity and these values determine the solubility, membrane adsorption, and penetration. And the values should not exceed 5. Most compounds had an X Log P value of less than 5, but compounds like betulinic acid, vitamin-D2, and ursolic acid had values of more than 5. But according to Lipinski's rule violation of anyone the rule is permitted. The number of hydrogen bond donors and acceptors plays an important role in expressing the specificity of the ligand towards the receptor. An ideal compound should not have more than 5 hydrogen bond donors and should not have more than 10 hydrogen bond acceptors. All the compounds had values as specified and no violation was observed.

TPSA represents the drug's ability to penetrate the cells and the values should not be more than 140 $Å^2$. Compounds with values more than 140 $Å^2$ are poor in penetrating the membrane. All the compounds had values less than 140 $Å^2$ indicating all compounds have better membrane penetrating ability. The bioavailability score is a semi-quantitative score that relies on the total charge, TPSA, and Lipinski's rule violation. Most of the compounds have a bioavailability score of 0.55 which are considered to be drugs with good bioavailability. But betulinic acid and ursolic acid had a bioavailability score of 0.85, which indicates an In-silico model that these compounds had better bioavailability than other compounds. Bioavailability is one of the most important factors in drug selection as the score determines the availability of the drug into circulation to reach the target organ or target site. Compared to the control - ulipristal acetate, the plant-based bioactive compounds have similar physicochemical properties. In fact, certain bioactive compounds have better properties

3.3. Ligand Structure Retrieval

The 15 plant-based bioactive compounds were selected according to Lipinski's rule. The chemical structure of all 15 compounds was retrieved from PubChem. The PubChem ID, Molecular formula, Canonical SMILES, and Chemical structure of the respective bioactive compounds and control ulipristal acetate are tabulated in the following table 3. Simplified Molecular-Input Line-Entry System (SMILES) is a form of line notation that uses ASCII strings, which helps in describing the structure of the chemical species. These SMILES structures of the compounds are required for further database searches for knowing the ADME properties using the pkCSM database (available at: http://biosig.unimelb.edu.au/pkcsm/)

3.4. ADMET Properties of Bioactive Compounds

ADMET is Absorption, Distribution, Metabolism, Excretion, and Toxicology. The ADMET properties of the compound are very important in determining the efficacy of the compound to be used as a drug. The ADMET properties of all compounds were analyzed using the pkCSM database. This database helps to analyze the drug-like properties of the bioactive compound. Thus, the bioactive compound can be turned into a drug that produces desired effect in the body with low or nil toxicity. The following table 4 below describes the ADMET properties of each compound.

3.4.1. Absorption

The absorbing property of the compound depends on the water solubility, intestinal adsorption values, and skin permeability. Any drug to be adsorbed should be present in soluble form hence solubility of the compound in water helps with better absorption and also water-soluble compounds are easier for formulation into drugs. Compounds like allicin and indole-3-carbinol have log S values between 0 to -2 log mol/L which indicates them to be good water-soluble drugs. While other compounds except vitamin-D2 had values between -2 to -4 log mol/L and are considered to be slightly soluble compounds. But the control ulipristal acetate had the lowest value of -5.998 log mol/L and values less than -4 log mol/L are considered to be an insoluble molecule. Molecules with less than 30% of intestinal adsorption are considered to be poorly absorbed drugs. The compound ursolic acid had 100% absorption and betulinic acid had a 99.76 % absorption range, which makes them be the best intestinally absorbed bioactive compound than the control ulipristal acetate which had 98.90%.

3.4.2. Distribution

The distribution of the adsorbed drug depends on the VDss and fraction unbound values. VDss is a steady-state volume of distribution. It is a theoretical volume of compounds needed for uniform distribution in various tissues. The VDss value should range between 0.71 to 2.81 L/Kg. Most compounds had better VDss values. While compounds like allicin, betulinic acid, curcumin, and ursolic acid that had distribution value is considered to be relatively lower. Fraction unbound is the amount of drug present in an unbound state in plasma. The efficacy of the drug is affected by the degree to which the compound is to the blood proteins. The more they are bound, the less they get distributed. Data retrieved showed that ulipristal acetate had better distribution than the bioactive compounds.

3.4.3. Metabolism

The metabolism of the compound depends on the liver enzymes. The bioavailability of the compound decreases when they are metabolized by the liver enzymes. Cytochrome P450 is the most important enzyme of metabolism in the liver.

Table 3. PubChem CID, Molecular formula, Canonical SMILES, and chemical structures of the bioactive compounds

Sl. No	Compound name	PubChem CID	Molecular formula	Canonical SMILES
1	Ulipristal acetate (Control)	130904	$C_30H_37NO_4$	CC(=O)C1(CCC2C1(CC(C3=C4CCC(=O)C=C4CCC23)C5=CC=C(C=C5)N(C)C)C)OC(=O)C
2	Allicin	65036	$C_6H_{10}OS_2$	C=CCSS(=O)CC=C
3	Indole-3- carbinol	3712	C_9H_9NO	C1=CC=C2C(=C1)C(=CN2)CO
4	Resveratrol	445154	$C_{14}H_{12}O_3$	C1=CC(=CC=C1C=CC2=CC(=CC(=C2)O)O)O
5	THSG	5321884	$C_{20}H_{22}O_9$	C1=CC(=CC=C1C=CC2=C(C(=CC(=C2)O)O)OC3C(C(C(C(O3)CO)O)O)O)O
6	Ellagic acid	5281855	$C_{14}H_6O_8$	C1=C2C3=C(C(=C1O)O)OC(=O)C4=CC(=C(C(=C43)OC2=O)O)O
7	Betulinic acid	64971	$C_{30}H_{48}O_3$	CC(=C)C1CCC2(C1C3CCC4C5(CCC(C(C5CCC4(C3(CC2)C)C)(C)C)O)C)C(=O)O
8	Butein	5281222	$C_{15}H_{12}O_5$	C1=CC(=C(C=C1C=CC(=O)C2=C(C=C(C=C2)O)O)O)O
9	curcumin	969516	$C_{21}H_{20}O_6$	COC1=C(C=CC(=C1)C=CC(=O)CC(=O)C=CC2=CC(=C(C=C2)O)OC)O
10	Vitamin-D2	5280793	$C_{28}H_{44}O$	CC(C)C(C)C=CC(C)C1CCC2C1(CCCC2=CC=C3CC(CCC3=C)O)C
11	Genistein	5280961	$C_{15}H_{10}O_5$	C1=CC(=CC=C1C2=COC3=CC(=CC(=C3C2=O)O)O)O
12	Isoliquiritigenin	638278	$C_{15}H_{12}O_4$	C1=CC(=CC=C1C=CC(=O)C2=C(C=C(C=C2)O)O)O
13	Fisetin	5281614	$C_{15}H_{10}O_6$	C1=CC(=C(C=C1C2=C(C(=O)C3=C(O2)C=C(C=C3)O)O)O)O
14	Ursolic acid	64945	$C_{30}H_{48}O_3$	CC1CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)O)C)C)C2C1C)C)C(=O)O
15	Kaempferol	5280863	$C_{15}H_{10}O_6$	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O
16	Quercetin	5280343	$C_{15}H_{10}O_7$	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O

Table 4. ADMET properties of all bioactive compounds analyzed using pkCSM database

Compound name	Absorption		Distribution		Metabolism		Excretion	Toxicity		
	Water solubility (log mol/ L)	Intestinal absorption (%)	VDss (log L/ kg)	Fraction unbound (Fu)	CYP2D6 substrate	CYP3CA4 substrate	Total clearance (log ml/ min/ Kg)	LD 50 (mol/ Kg)	LC 50 (log mM)	Ames test
Ulipristal acetate (Control)	-5.998	98.90	0.227	0	no	yes	0.249	2.71	-0.38	no
Allicin	-1.72	96.22	-0.045	0.577	no	no	0.714	2.37	1.41	no
Indole-3- carbinol	-1.874	92.09	0.212	0.446	no	no	0.515	2.39	1.77	no
Resveratrol	-3.178	90.94	0.296	0.166	no	yes	0.076	2.53	1.52	yes
THSG	-2.699	40.92	0.609	0.383	no	no	0.061	2.58	5.26	no
Ellagic acid	-3.181	86.68	0.375	0.083	no	no	0.537	2.40	2.11	no
Betulinic acid	-3.122	99.763	-1.18	0.018	no	yes	0.116	2.26	-1.17	no
Butein	-3.777	72.57	0.741	0.232	no	no	0.015	2.46	2.82	no
Curcumin	-4.01	82.19	-0.215	0	no	yes	-0.002	1.83	-0.08	no
Vitamin-D2	-6.969	94.75	0.156	0	no	yes	0.811	2.30	-2.05	no
Genistein	-3.595	93.38	0.094	0.087	no	no	0.151	2.27	1.94	no
Isoliquiritigenin	-3.06	91.10	0.485	0.17	no	no	0.087	2.43	2.08	no
Fisetin	-3.181	83.75	0.718	0.16	no	no	0.421	2.47	2.27	no
Ursolic acid	-3.072	100	-1.088	0	no	yes	0.083	2.35	-0.77	no
Kaempferol	-3.04	74.29	1.274	0.178	no	no	0.477	2.45	2.89	no
Quercetin	-2.925	77.20	1.559	0.206	no	no	0.407	2.47	3.72	no

CYP2D6 and CYP3CA4 are the two main subtypes of cytochrome P450. Compounds that do not form the substrate of these enzymes have better bioavailability. From the data retrieved from the pkCSM database, it is evident that none of the compounds is a substrate of CYP2D6. But some molecules like resveratrol, Bbetulinic acid, curcumin, vitamin-D2, and ursolic acid are substrates of the CYP3CA4 enzyme. The control was metabolized in a similar way to that of betulinic acid and ursolic acid i.e., ulipristal acetate was a substrate of CYP3CA4 but it was not a substrate to CYP2D6 enzyme.

3.4.4. Excretion

Total clearance is the combination of hepatic and renal clearance. These levels are important for determining the bioavailability and the dosage levels of the drug. Compounds like Vitamin-D2, allicin, ellagic acid, indole-3-carbinol, fisetin, kaempferol, and quercetin had the highest total clearance rate respectively, while other compounds had relatively lower values. Compared to the control bioactive compounds had a better total clearance rate.

3.4.5. Toxicity

LD50 represents the amount of compound that causes death in 50% of the test animals. LC50 represents the concentration of molecules required to cause death in 50% of the test. From data obtained it was said that compounds with a log LC50 value less than -0.3 are considered to be compounds with high acute toxicity. Data retrieved reported that none of the compounds exceeds the LC 50 values. AMES test helps in determining the mutagenicity of the compound with the help of bacteria. Except for resveratrol none of the compounds were mutagenic. The cumulative data acquired from LD50, LC50, and Ame's test exposed that most of the bioactive compounds chosen through Lipinski's rule did not pose any toxicity. But the control drug had a high acute toxicity level which had an LC50 level of -0.377, which is higher than the prescribed value.

From the overall comparison of the ADMET properties between plant-based bioactive compounds and Control ulipristal acetate, it was that the plant-based compounds had better ADMET properties than the control specifically in terms of absorption, excretion, and toxicity. Among the 15 plant-based bioactive compounds, ursolic acid, and betulinic acid had better absorbing properties, 7 compounds had better excreting properties and most plant-based bioactive compounds were non-toxic.

3.5. Molecular Docking Results

Docking of the receptor and ligands were performed using the open-source software AutoDock version 4.2.6 (available at: http://autodock.scripps.edu/downloads/autodock-registration/autodock-4-2-download-page/, http://mgltools.scripps.edu/downloads). The PDB structure of the progesterone receptor and the PDB structure of the ligand (15 bioactive compounds + 1 control) were loaded onto the software. The docking results from each compound were tabulated in table 5. The docking results provided the following information about the receptor-ligand interaction. The number of hydrogen bonds infers the strong interaction that the ligand has made with the receptor. Binding energy is the sum of all interactions formed between the ligand and receptor. It helps in determining the ability of the ligand to make a complex interaction with the receptor. Ligand efficiency is a measure of binding energy per atom of the ligand to the receptor. This explains the ligand`s potency not only to optimally fit the target but also the ability of the ligand to establish a good interaction. Intermolecular energy is the energy between the non-bound atoms in the molecules. vdW _ hb_ desolv_energy is the electrostatic or Vander Waal`s energy loss of interaction between ligand and protein upon binding. Electrostatic energy is the non-bound energy of ligand and protein upon binding. Torsional energy is another form of internal energy 15 plant-based bioactive compounds were docked with the progesterone receptor and the results obtained from docking were compared to the control ulipristal acetate. The binding energies of the bioactive compounds were compared to that of the control. Based on the docking results, the binding energy of ulipristal acetate was found to be -8.21 Kcal/mol. Seven out of 15 compounds had higher binding energy than that of control, thus those compounds can effectively act against and modulate the progesterone receptor.

From the results of interaction between bioactive compounds and progesterone receptors, it was found that ursolic acid had the highest binding energy of -9.77 Kcal/mol with 13 interactions; followed by vitamin-D2 and betulinic acid with binding energies -9.63 and -9.13 Kcal/mol respectively. Thus, these compounds can act as potential lead compounds for designing a therapeutic drug for the treatment of uterine leiomyoma. Also compared to the binding energy of the control compound (i.e., -8.21 Kcal/mol), kaempferol, quercetin, isoliquiritigenin, and butein had higher binding energies of -8.59, -8.53, -8.34, and -8.21 Kcal/mol respectively. Thus, these compounds can also act as a potent progesterone receptor modulators. Compounds resveratrol, genistein, fisetin, and ellagic acid had binding energies of -8.15, -8.13, -8.02, -8.01 Kcal/mol respectively. These binding energies are near to that of control binding energy. The binding energies value concludes that these compounds may possess some progesterone receptor modulating properties nearer to that of ulipristal acetate. Binding energy is not the only factor for determining the specificity of the ligand towards the receptor. It is considered to be one of the key factors to test the efficacy of the ligand. Other factors include the physical, chemical, biological properties, ADMET properties, and also bioavailability. These all factors must be taken into consideration upon selecting the compound for further drug formulation and development.

Table 5. Summary of docking results obtained from the binding of progesterone receptor and 15 bioactive compounds

Sl.no	Molecular Docking of Progesterone Receptor with	No. of H bonds	Binding Energy (Kcal/ mol)	Ligand Efficiency	Inter molecular energy (Kcal/ mol)	vdW _ hb_ desolv_ energy	Electrostatic energy (Kcal/ mol)	Torsional energy (Kcal/ mol)	Total internal unbound energy (Kcal/ mol)
1	Ulipristal acetate (Control)	1	-8.21	-0.23	-9.7	-9.24	-0.46	1.49	-1.81
2	Ursolic acid	2	-9.77	-0.3	-10.67	-10.3	-0.36	0.89	0.0
3	Vitamin-D2	2	-9.63	-0.33	-11.42	-11.32	-0/1	1.79	-1.33
4	Betulinic acid	2	-9.13	-0.28	-10.33	-9.05	-1.28	1.19	-0.15
5	Kaempferol	4	-8.59	-0.41	-10.8	-9.83	-0.25	1.49	-1.32
6	Quercetin	6	-8.53	-0.39	-10.32	-10.1	-0.22	1.79	-2.35
7	Isoliquiritigenin	3	-8.34	-0.44	-10.13	-9.92	-0.22	1.79	-0.91
8	Butein	4	-8.21	-0.41	-10.3	-10.11	-0.19	2.09	-1.69
9	Resveratrol	5	-8.15	-0.48	-9.65	-9.38	-0.27	1.49	-0.26
10	Genistein	4	-8.13	-0.41	-9.32	-9.13	-0.19	1.19	-0.95
11	Fisetin	5	-8.02	-0.38	-9.51	-9.3	-0.21	1.49	-1.81
12	Ellagic acid	4	-8.01	-0.36	-9.2	-8.98	-0.23	1.19	-2.13
13	Curcumin	3	-7.54	-0.28	-10.52	-10.17	-0.35	2.98	-1.89
14	THSG	5	-7.18	-0.25	-10.76	-10.39	-0.37	3.58	-4.28
15	Indole-3-carbinol	4	-6.0	-0.55	-6.6	-6.45	-0.14	0.6	0.0
16	Allicin	2	-5.36	-0.6	-6.85	-6.68	-0.16	1.49	-0.27

3.6. Visualization of Receptor-Ligand Interaction

Using LigPlot+, the interactions formed by hydrogen bonds between the receptor and the compound are visualized in a 2D manner (available at: https://www.ebi.ac.uk/thornton-srv/software/LigPlus/download.html). This software also tells about the amino acid residues that are having interactions and also the distance of hydrogen bonds formed. The interaction between the ligand and the receptor is depicted in Figures 3 to 18.

LigPlot analysis shows the details of all the interacting amino acid residues involved in the formation of that particular bond and it also shows other non-interacting key amino residues involved in the interaction. The violet-colored line depicts the bond in the ligand, the orange-colored line depicts the non-ligand bonds, and the green-colored dotted lines depict the hydrogen bond and the length of the bond formed between the ligand and the receptor. The non-interacting amino acids are involved in the formation of hydrophobic bonds, which are stronger than hydrogen bonds.

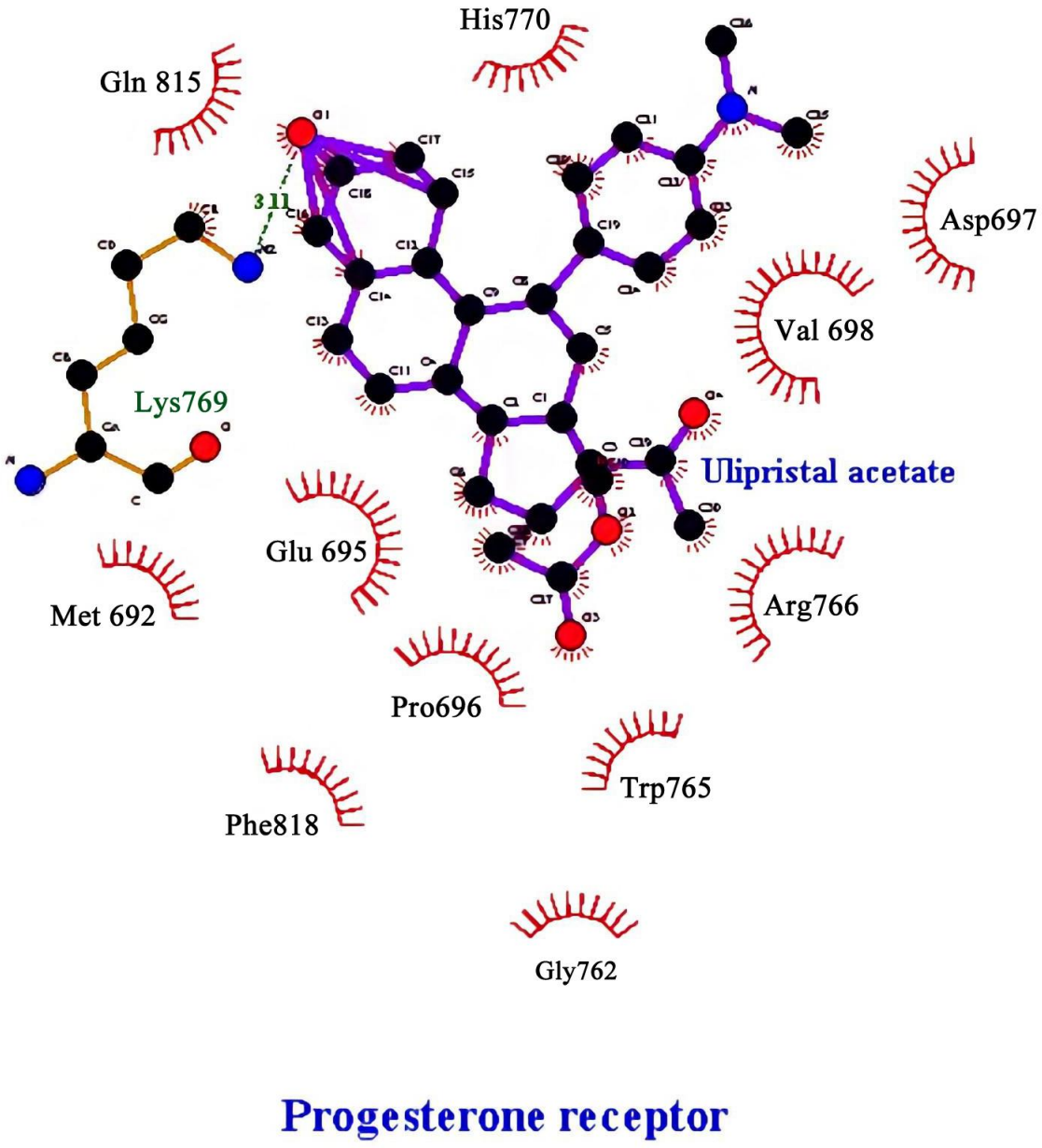

Figure 3. Docking of Progesterone receptor with Ulipristal acetate

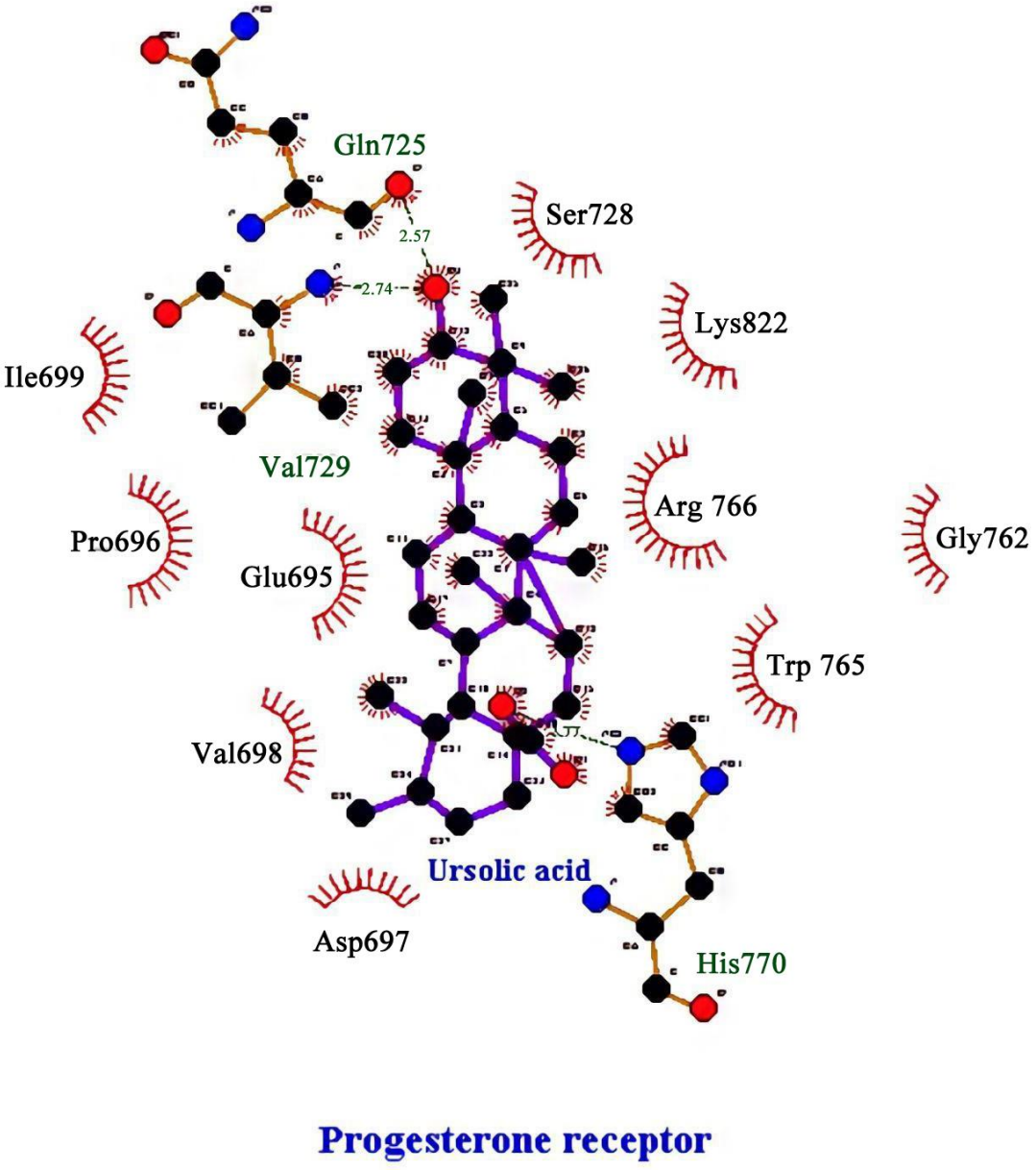

Figure 4. Docking of Progesterone receptor with Ursolic acid

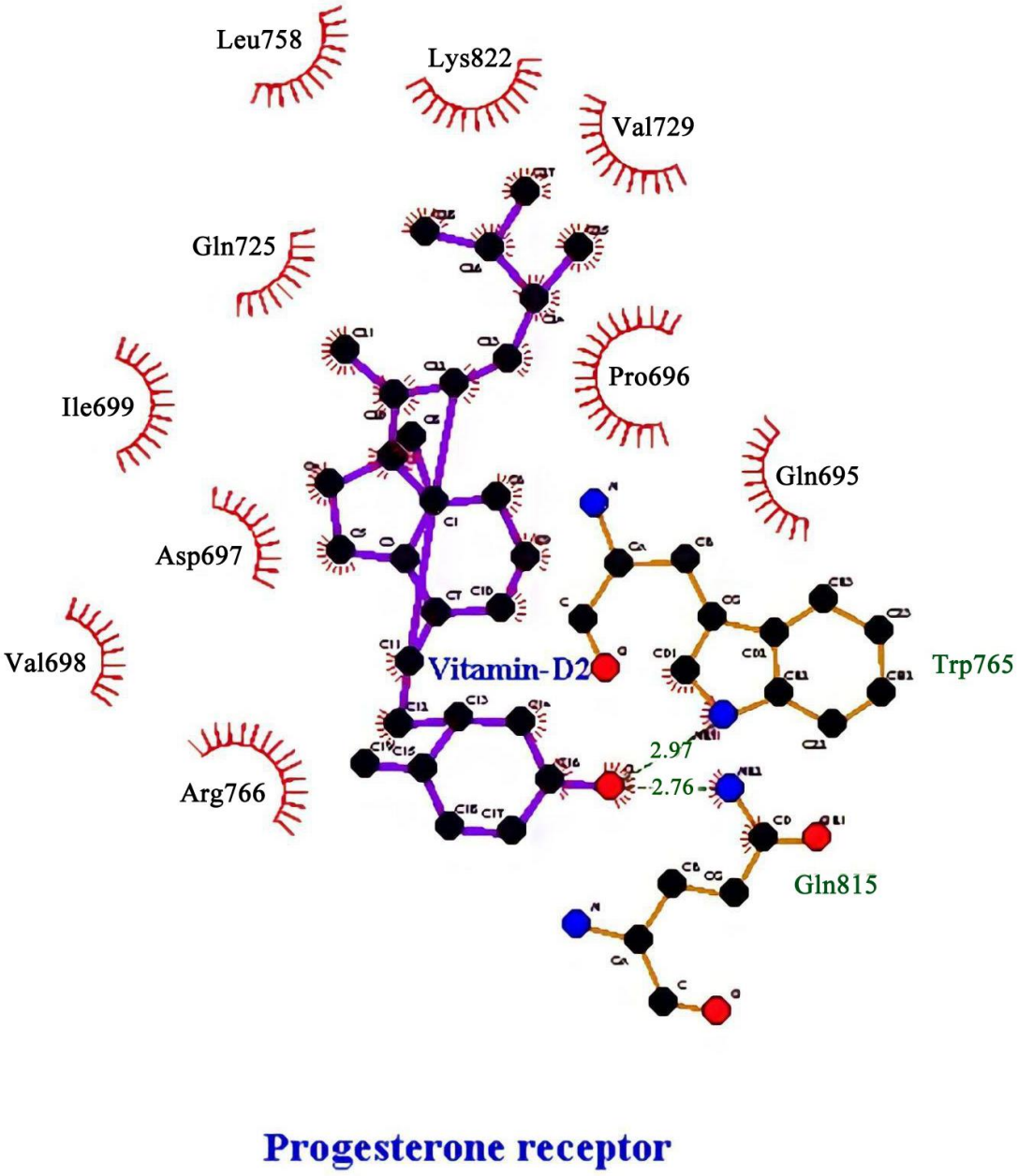

Figure 5. Docking of Progesterone receptor with Vitamin-D2

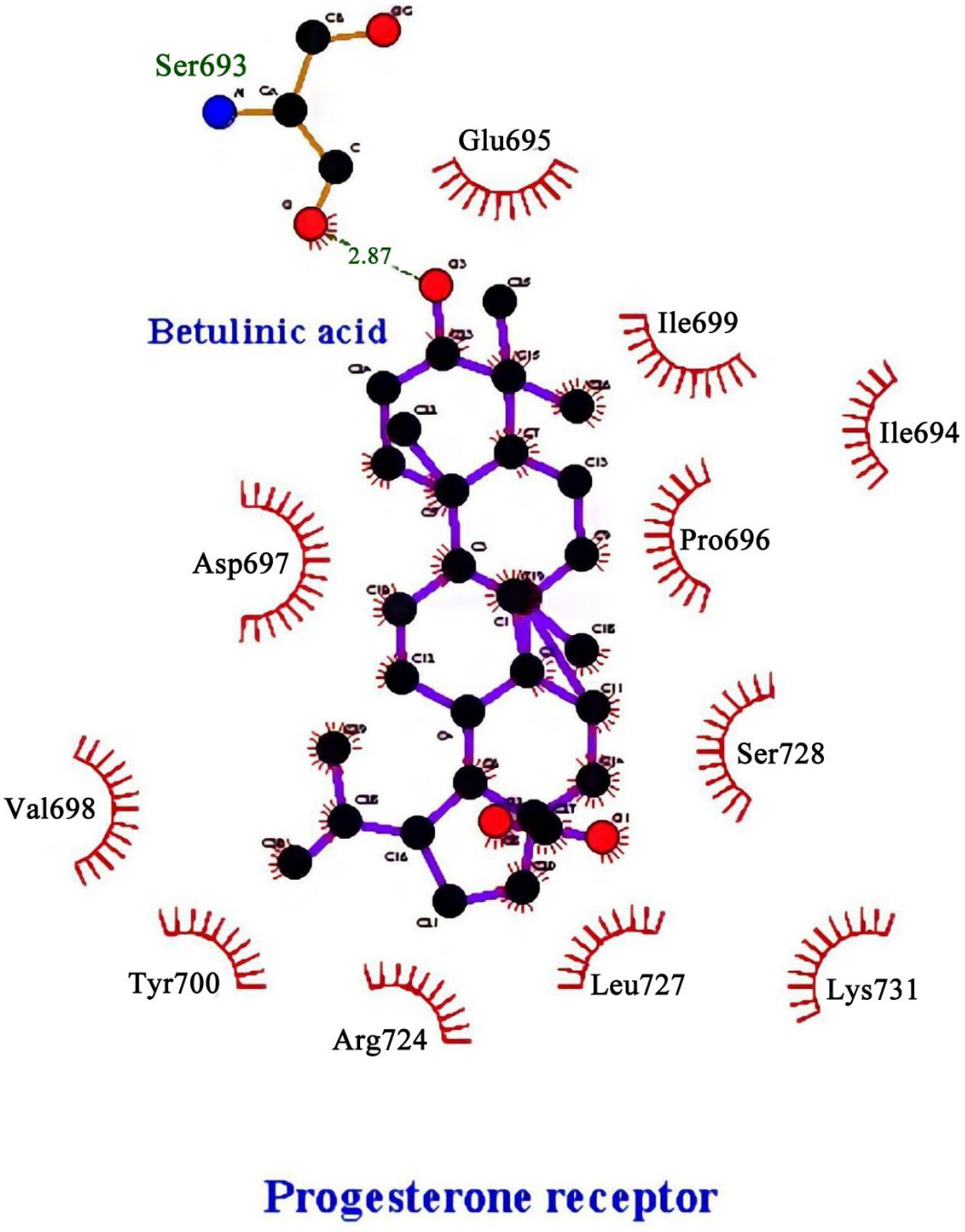

Figure 6. Docking of Progesterone receptor with Betulinic Acid

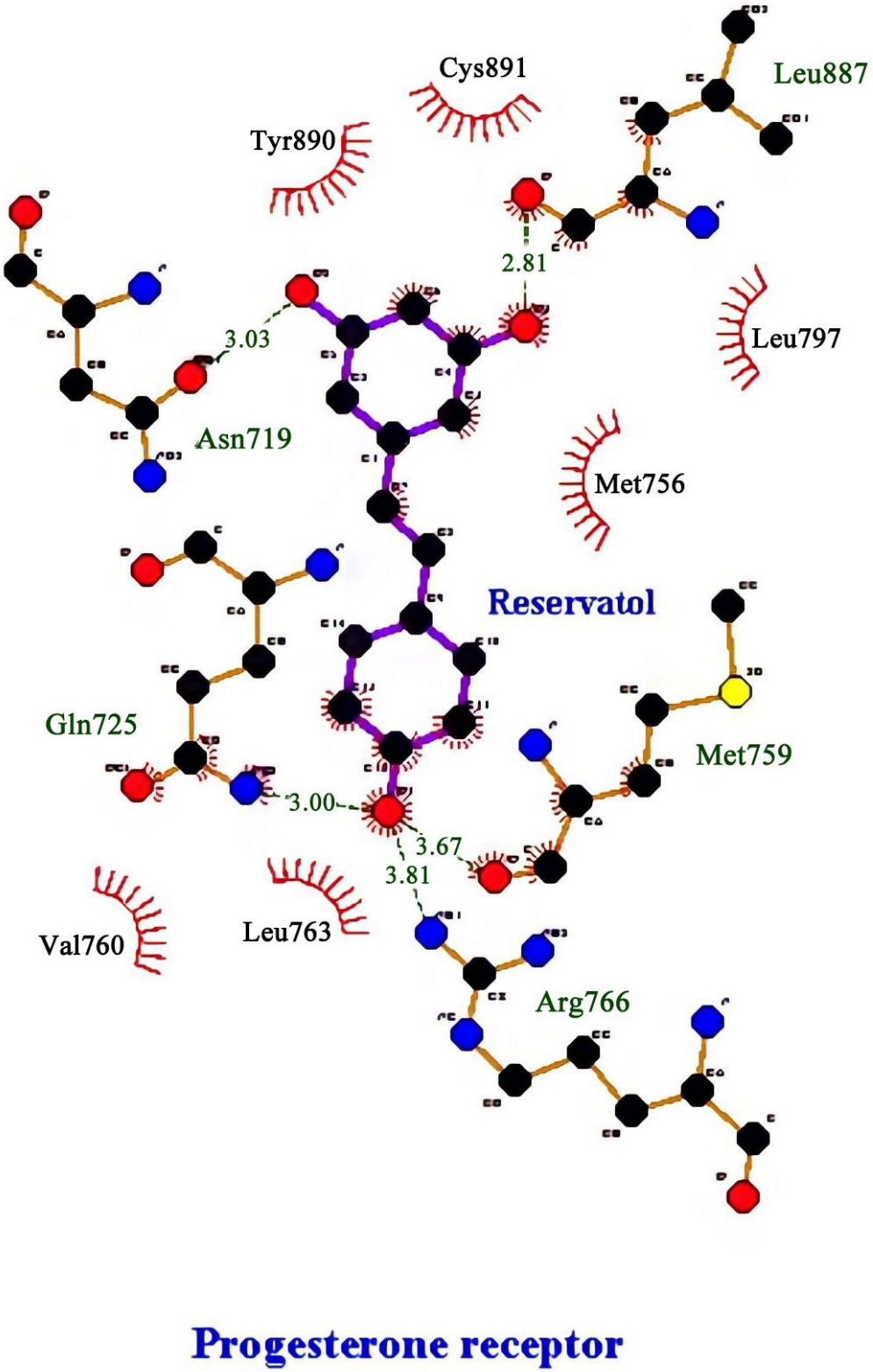

Figure 7. Docking of Progesterone receptor with Resveratrol

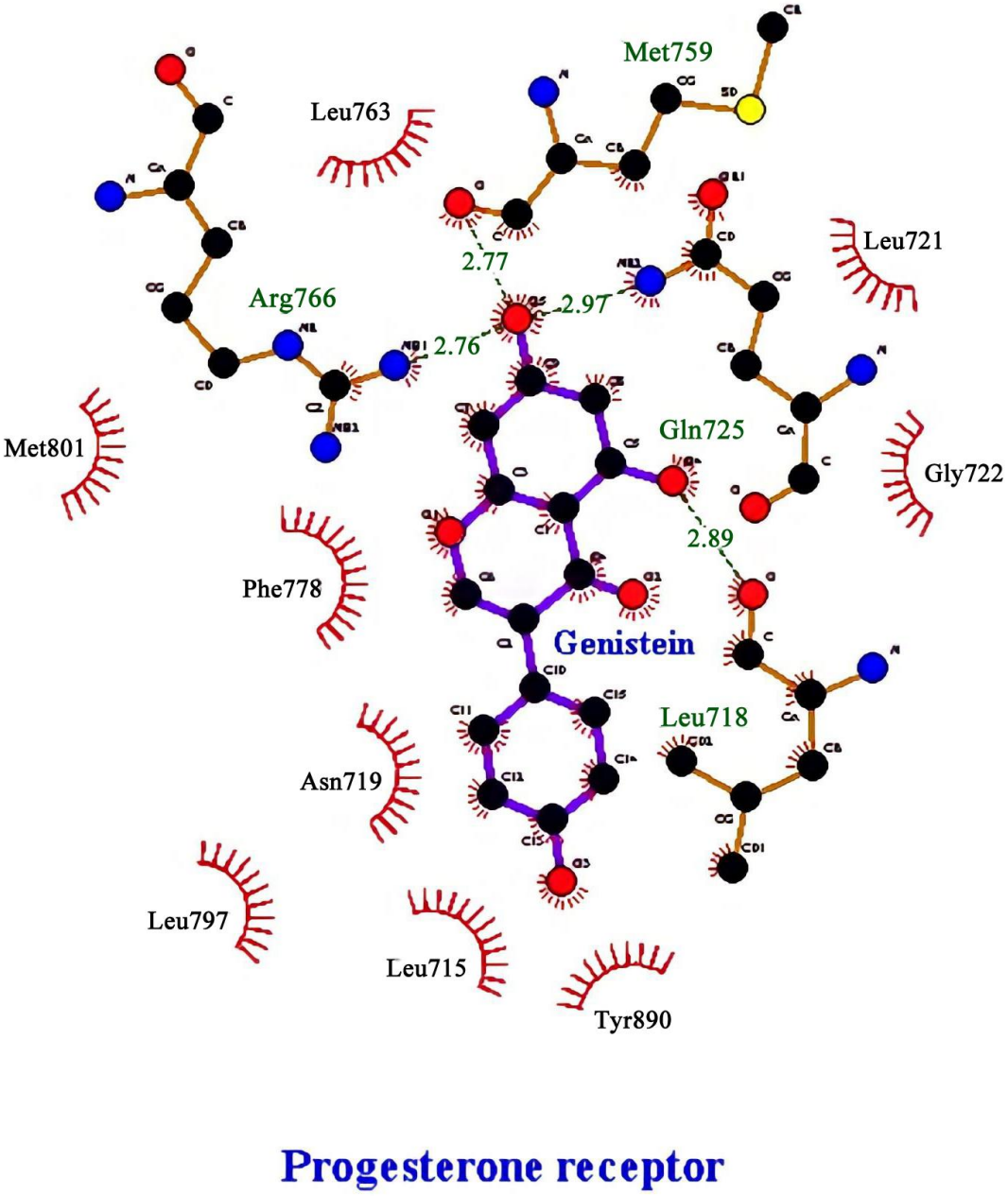

Figure 8. Docking of Progesterone receptor with Genistein

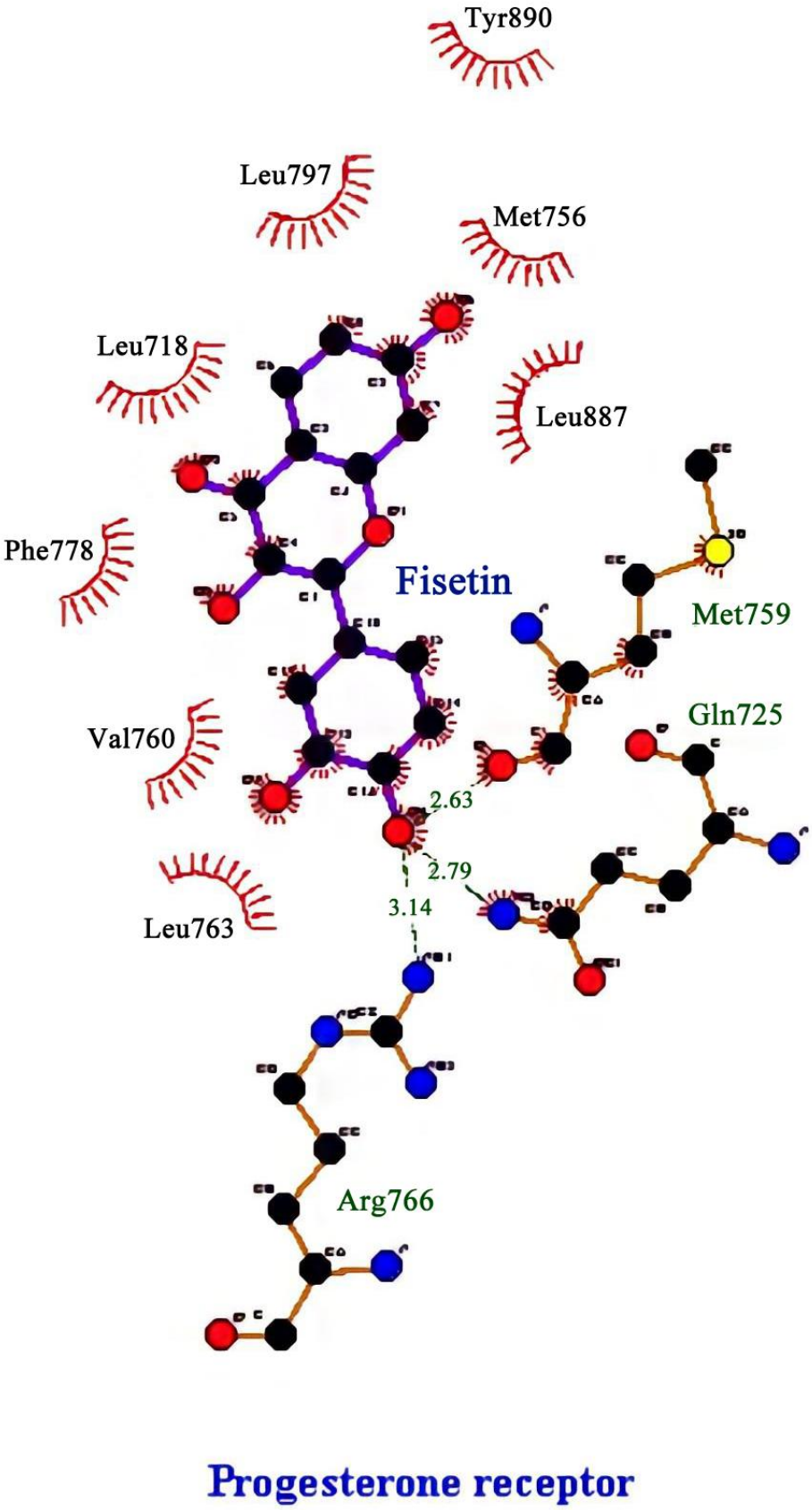

Figure 9. Docking of Progesterone receptor with Fisetin

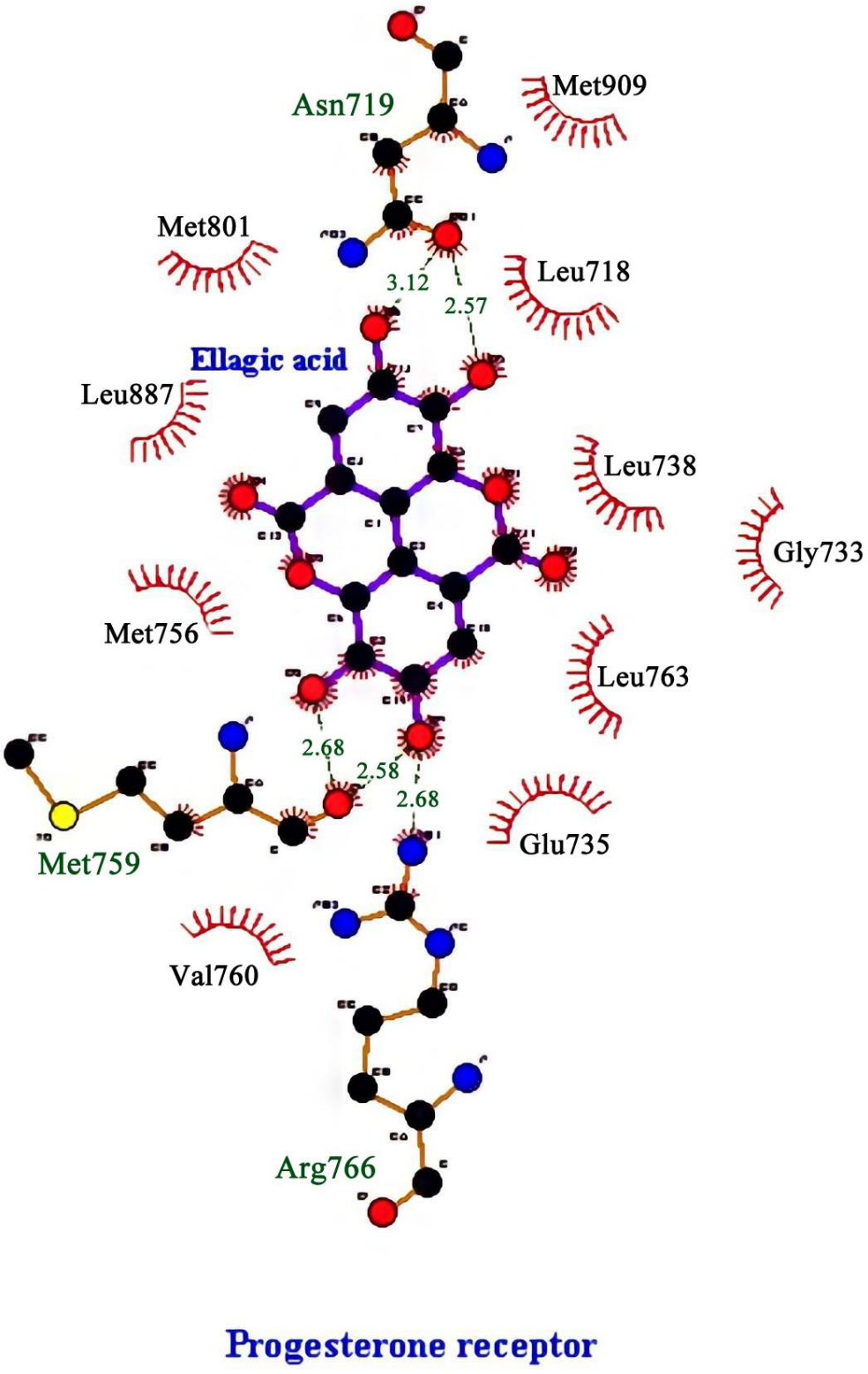

Figure 10. Docking of Progesterone receptor with Ellagic acid

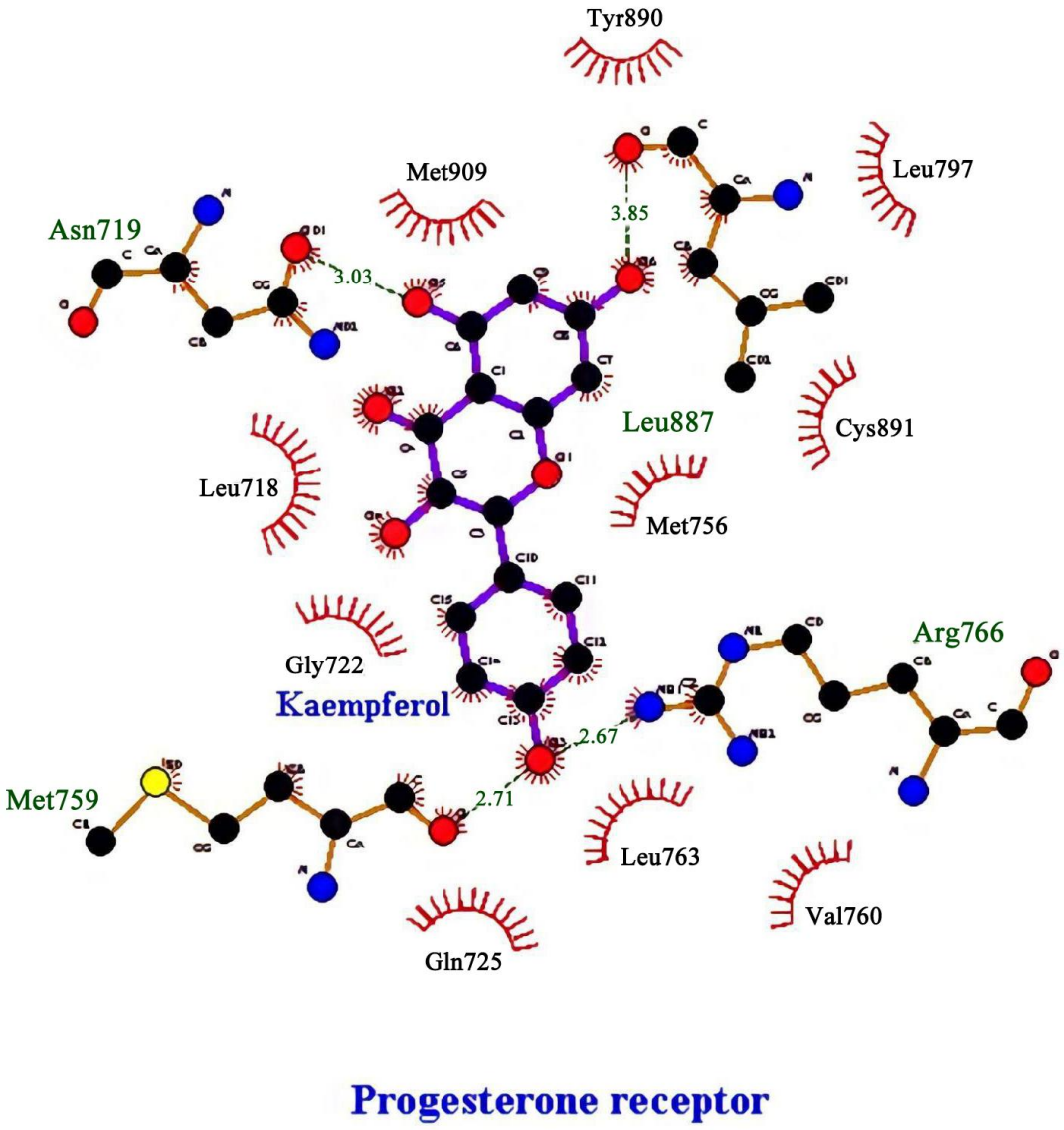

Figure 11. Docking of Progesterone receptor with Kaempferol

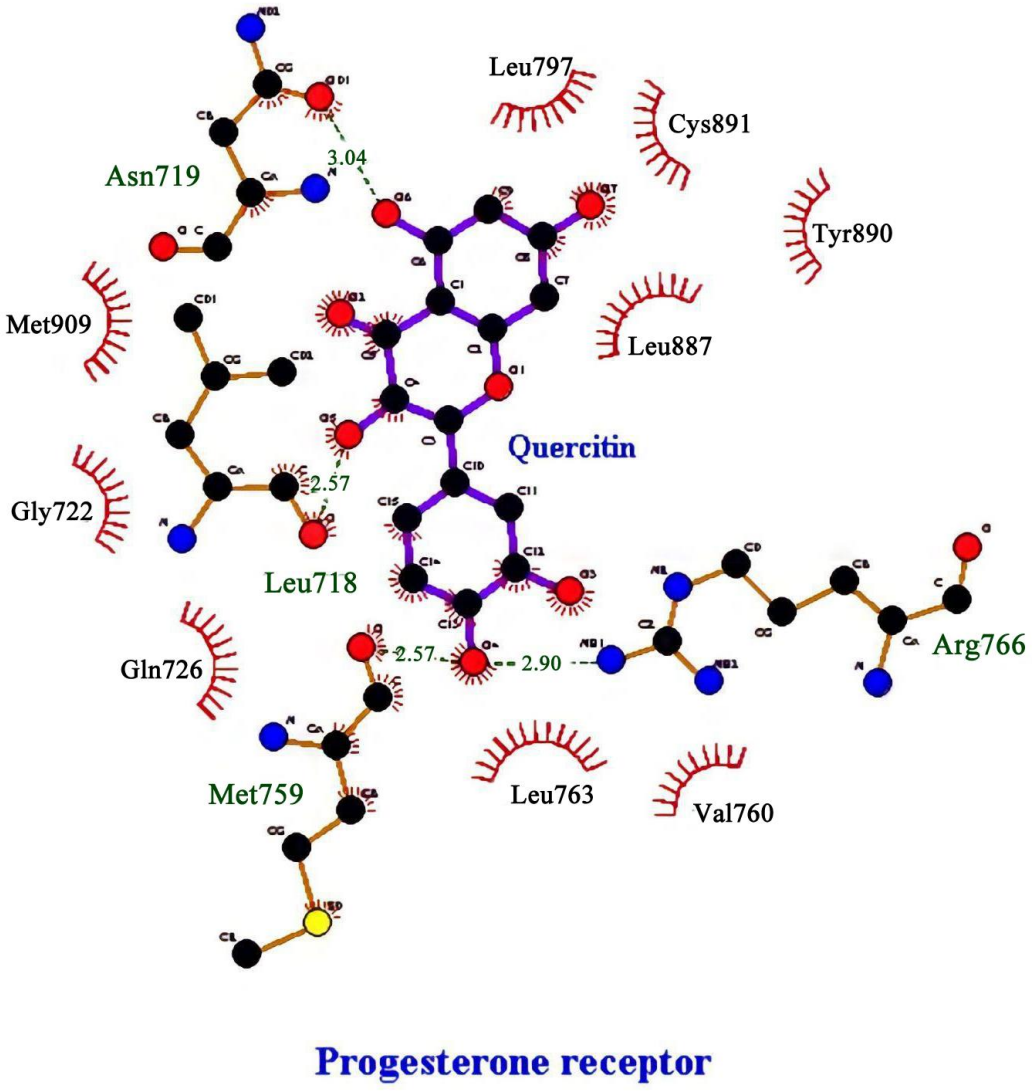

Figure 12. Docking of Progesterone receptor with Quercetin

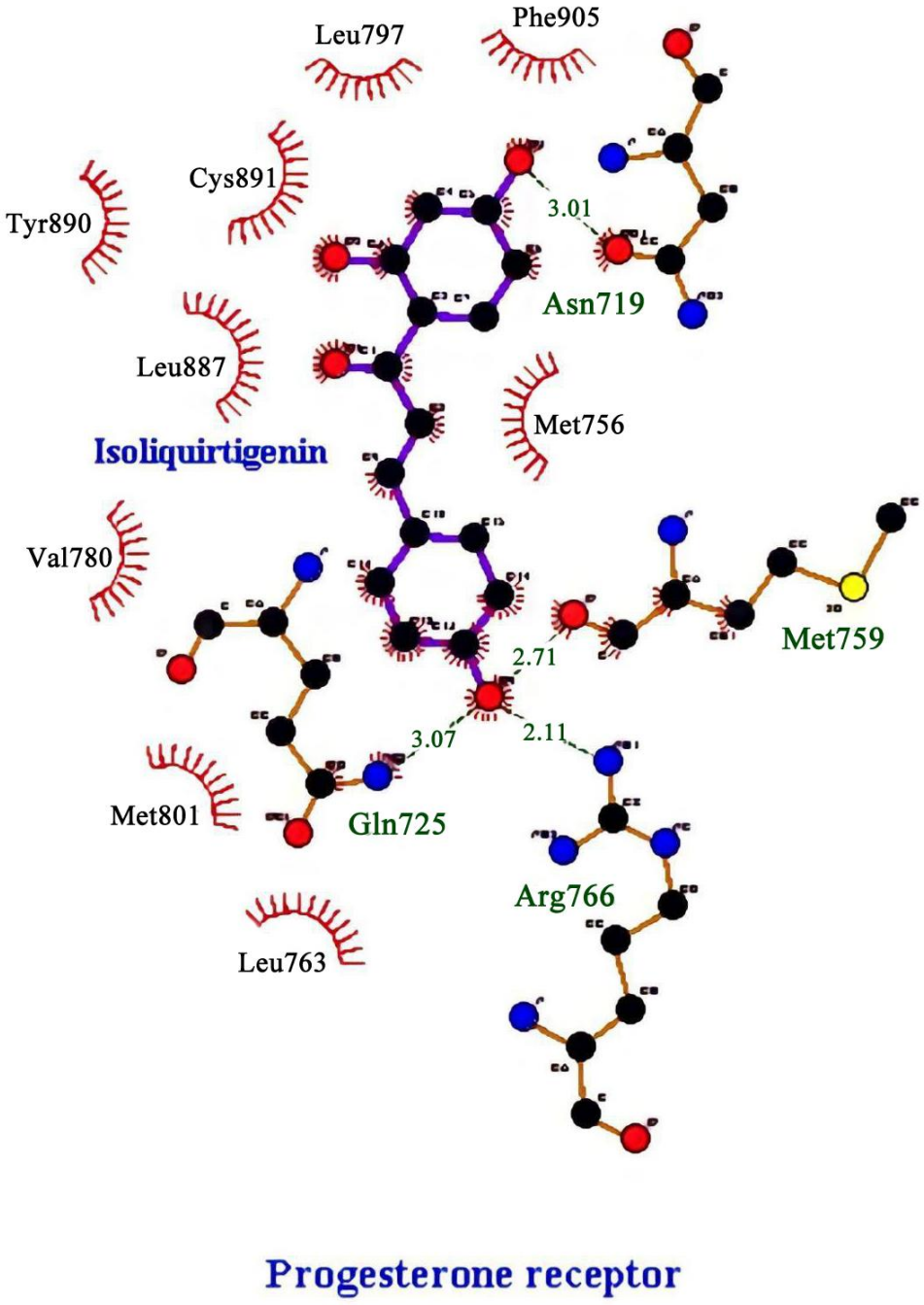

Figure 13. Docking of Progesterone receptor with Isoliquiritigenin

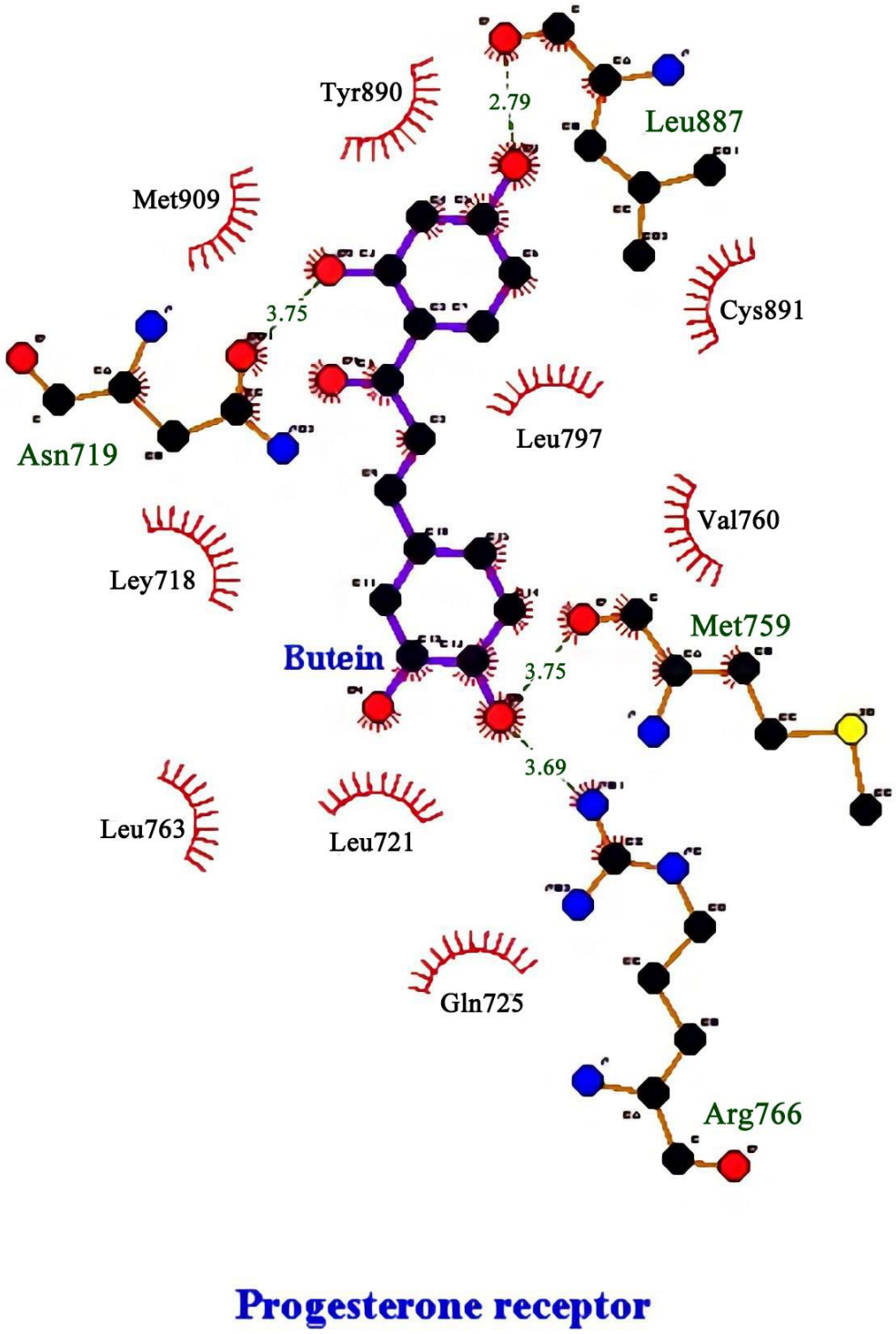

Figure 14. Docking of Progesterone receptor with Butein

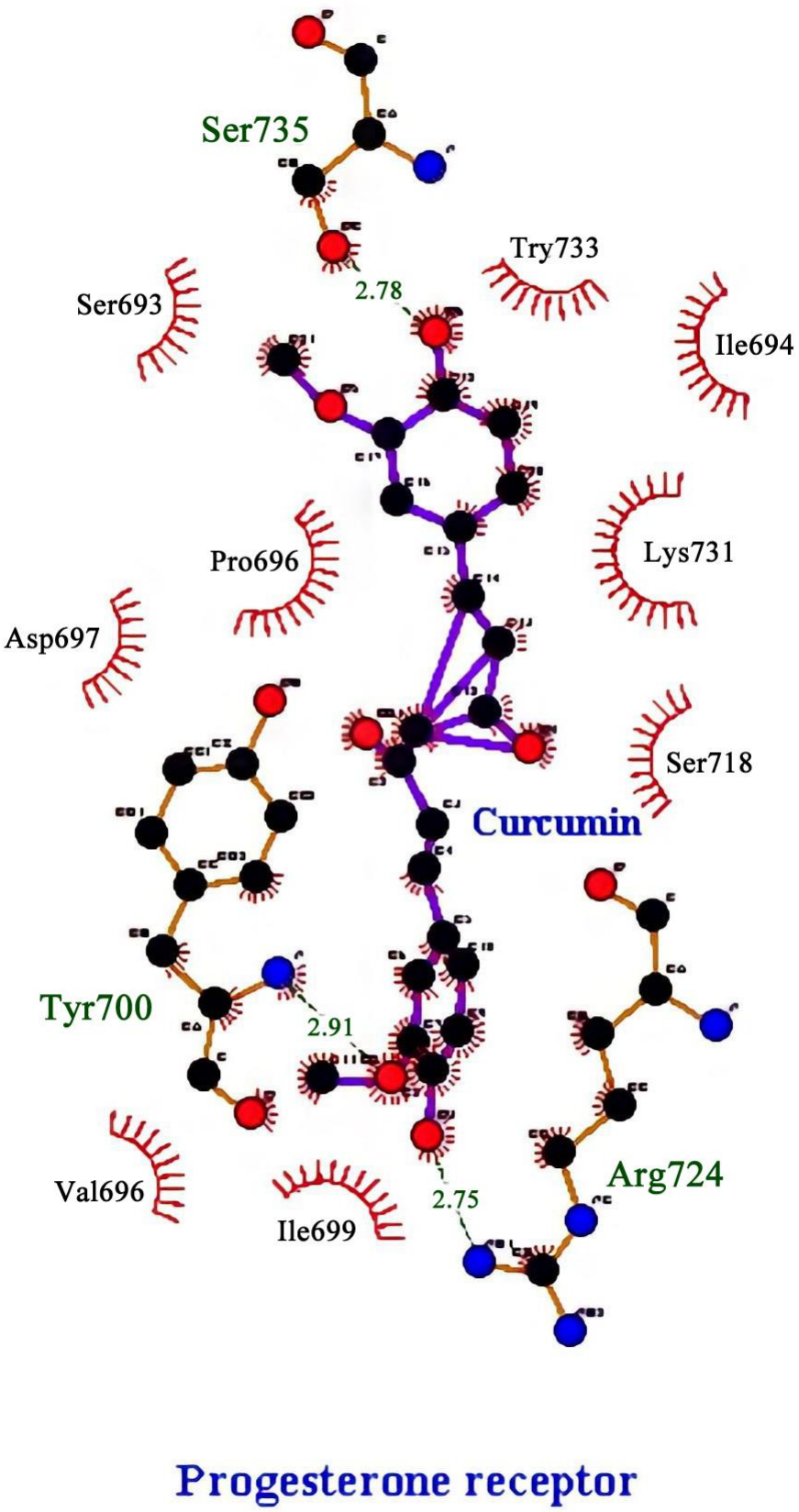

Figure 15. Docking of Progesterone receptor with Curcumin

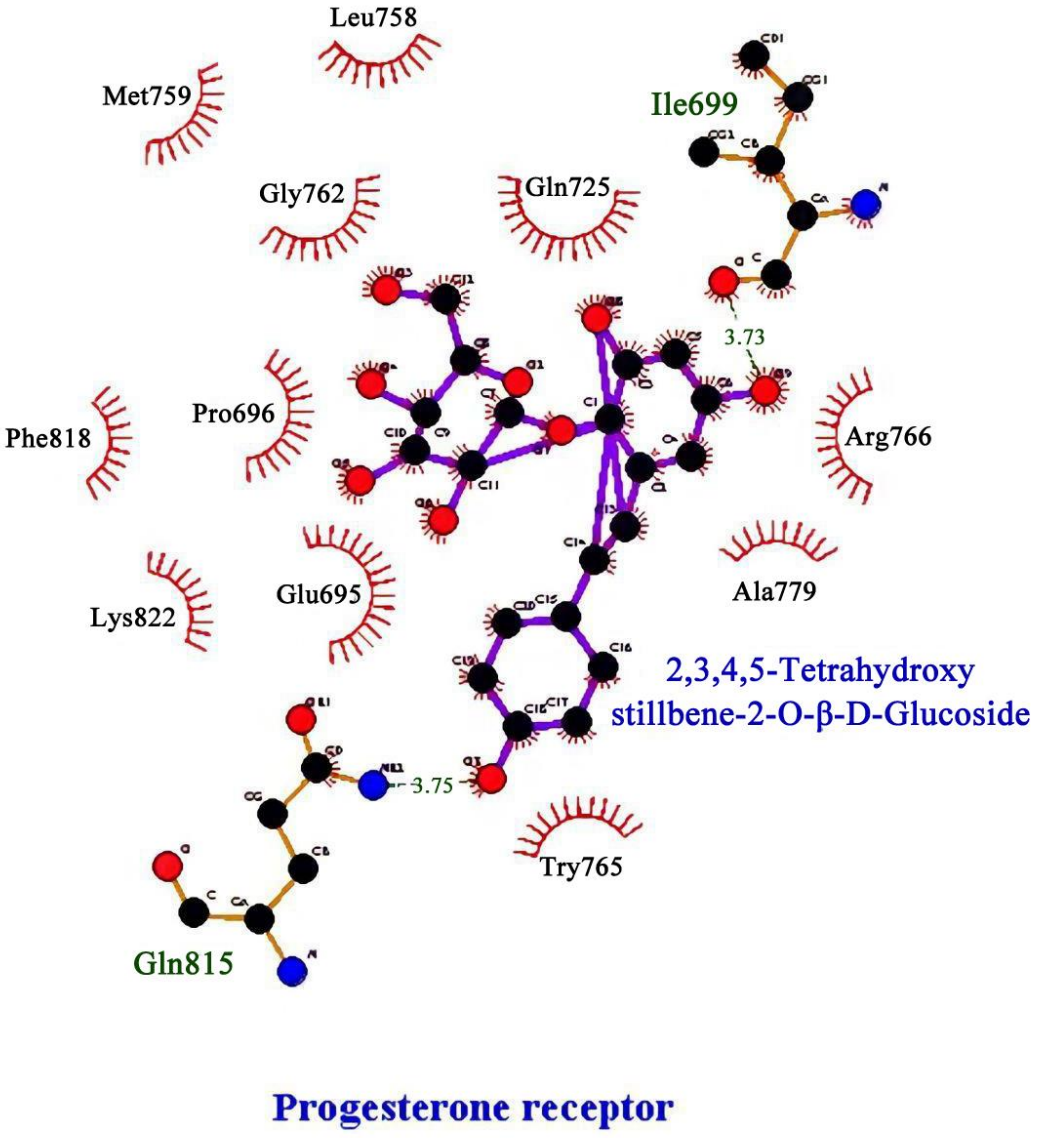

Figure 16: Docking of Progesterone receptor with THSG

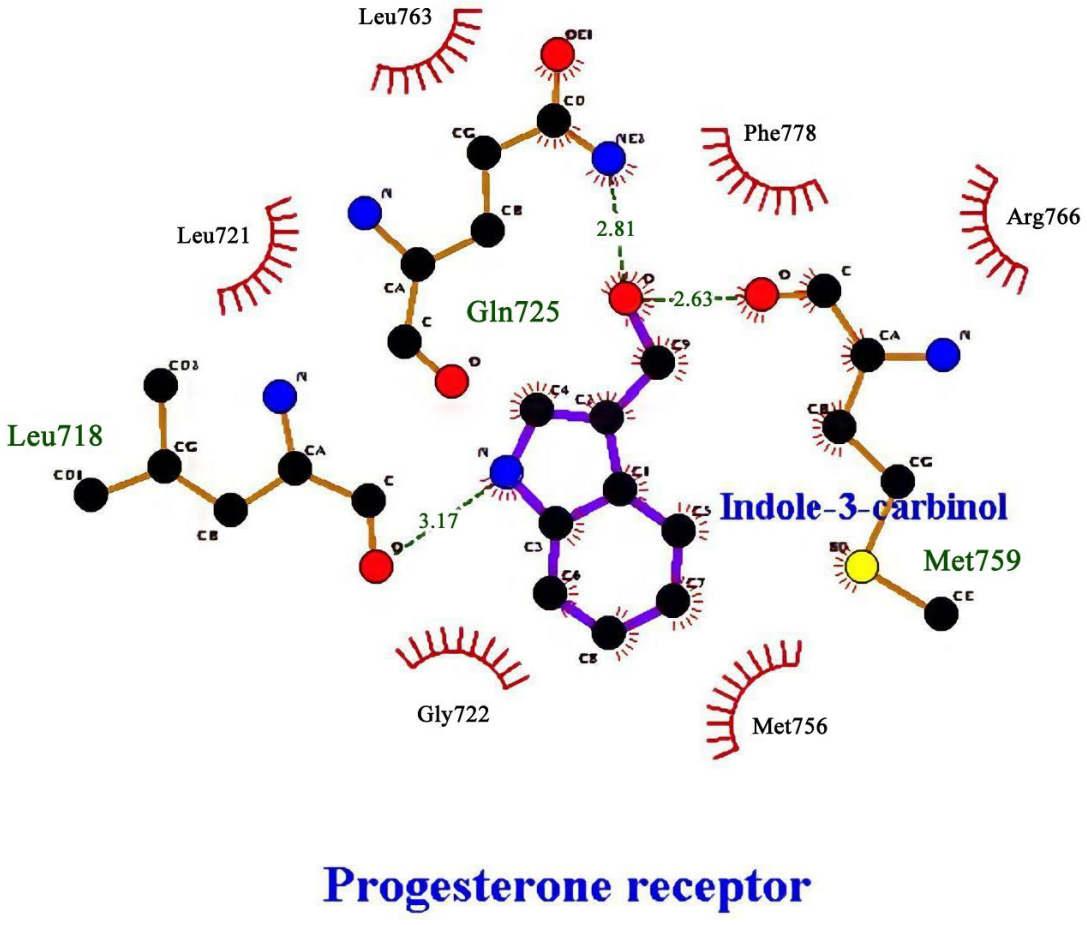

Figure 17. Docking of Progesterone receptor with Indole-3-carbinol

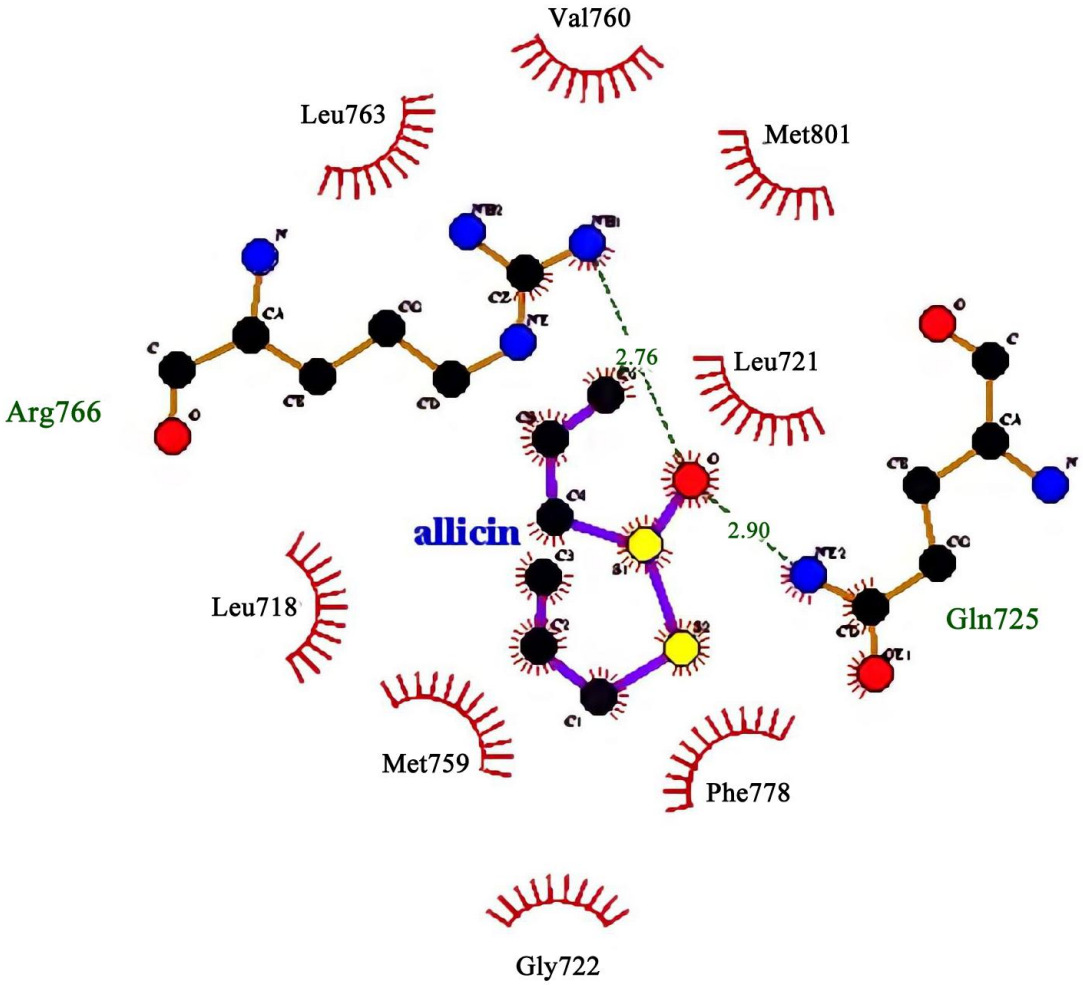

Figure 18. Docking of Progesterone receptor with Allicin

4. Conclusions

Uterine leiomyomas are the most common benign gynaecological tumours. However, a considerable amount of evidences have proved the role of progesterone and its receptors in the growth and development of uterine leiomyomas. Many researchers have reviewed the efficacy of various herbal formulations against uterine leiomyomas. In silico methods are time-saving methods, where multiple compounds can be analyzed in less time and compounds with high efficiency can be identified and can be further used in other in vivo and in vitro techniques.

Lead compound selection not only depends on the binding energies, but it also depends on the ligand efficiency, and the pharmacokinetic properties of the compound. All these parameters along with the good binding energy of the compound are taken into consideration while inventing a new Selective Progesterone Receptor Modulator (SPRM). The data obtained via various bioinformatics software and databases have provided in-depth knowledge about all 15 bioactive compounds and the control ulipristal acetate. 15 plant-based bioactive compounds were docked with the progesterone receptor and the results obtained from docking were compared to the control ulipristal acetate. Ulipristal acetate is a clinically approved drug for the treatment of uterine fibroid. The binding energies of the bioactive compounds were compared to that of the control. Based on the docking results, the binding energy of ulipristal acetate is -8.21 Kcal/mol. Seven out of 15 compounds had higher binding energy than that of control, thus those compounds can effectively act against and modulate the progesterone receptor. Ursolic acid had the highest binding energy of -9.77 Kcal/mol with 13 interactions. Followed by vitamin-D2 and betulinic acid with binding energies of -9.63 and 9.13 Kcal/mol

respectively. Also comparing to the binding energy of the control compound i.e., -8.21 Kcal/mol Kaempferol, Quercetin, Isoliquiritigenin, and Butein had higher binding energies of -8.59, -8.53, -8.34, and -8.21 Kcal/mol respectively. Thus, these compounds can also act as a potent progesterone receptor modulator.

Thus the study concludes that ursolic acid and betulinic acid are the best bioactive compounds among the 15 compounds in terms of binding energies and all other properties than the control ulipristal acetate. Hence further in vitro and in vivo studies can be performed on these two compounds and they can be used as a lead compound for the discovery of novel SPRM drugs and can be used to treat uterine leiomyomas by targeting progesterone receptors.

REFERENCES

[1] Borahay, M. A., Al-Hendy, A., Kilic, G. S., & Boehning, D. Signalling Pathways in Leiomyoma: Understanding Pathobiology and Implications for Therapy. Molecular medicine (Cambridge, Mass.), Vol.21, No.1, 242–256, 2015.

[2] Sparic, R., Mirkovic, L., Malvasi, A., & Tinelli, A. Epidemiology of Uterine Myomas: A Review. International journal of fertility & sterility, Vol.9, No.4, 424–435, 2016.

[3] Ciavattini A, Di Giuseppe J, Stortoni P, Montik N, Giannubilo SR, Litta P, Islam MS, Tranquilli AL, Reis FM, Ciarmela P. Uterine fibroids: pathogenesis and interactions with endometrium and endomyometrial junction. ObstetGynecol Int. 2013;2013:173184. doi: 10.1155/2013/173184, PMID: 24163697; PMCID: PMC3791844

[4] Kim, J. J., & Sefton, E. C. The role of progesterone signalling in the pathogenesis of uterine leiomyoma. Molecular and cellular endocrinology, Vol.358, No.2, 223–231, 2012.

[5] Othman, E. E., & Al-Hendy, A. Molecular genetics and racial disparities of uterine leiomyomas. Best practice & research. Clinical obstetrics &gynaecology, Vol.22No.4, 589–601, 2008.

[6] He, Y., Zeng, Q., Dong, S., Qin, L., Li, G., & Wang, P. Associations between uterine fibroids and lifestyles including diet, physical activity, and stress: a case-control study in China. Asia Pacific Journal of clinical nutrition, Vol.22, No.1, 109–117, 2013.

[7] Cermik, D., Arici, A., & Taylor, H. S. Coordinated regulation of HOX gene expression in myometrium and uterine leiomyoma. Fertility and sterility, Vol.78,No.5, 979–984, 2002.

[8] Cermik, D., Karaca, M., & Taylor, H. S. HOXA10 expression is repressed by progesterone in the myometrium: differential tissue-specific regulation of HOX gene expression in the reproductive tract. The Journal of clinical endocrinology and metabolism, Vol.86, No.7, 3387–3392, 2001.

[9] Kawaguchi, K., Fujii, S., Konishi, I., Nanbu, Y., Nonogaki, H., & Mori, T. Mitotic activity in uterine leiomyomas during the menstrual cycle. American journal of obstetrics and gynaecology, Vol.160, No.3, 637–641, 1989.

[10] Shimomura, Y., Matsuo, H., Samoto, T., & Maruo, T. Up-regulation by progesterone of proliferating cell nuclear antigen and epidermal growth factor expression in human uterine leiomyoma. The Journal of clinical endocrinology and metabolism, Vol.83, No.6, 2192–2198, 1998.

[11] Yin, P., Lin, Z., Cheng, Y. H., Marsh, E. E., Utsunomiya, H., Ishikawa, H., Xue, Q., Reierstad, S., Innes, J., Thung, S., Kim, J. J., Xu, E., &Bulun, S. E. Progesterone receptor regulates Bcl-2 gene expression through direct binding to its promoter region in uterine leiomyoma cells. The Journal of clinical endocrinology and metabolism, Vol.92,No.11, 4459–4466, 2007.

[12] Boonyaratanakornkit, V., Scott, M. P., Ribon, V., Sherman, L., Anderson, S. M., Maller, J. L., Miller, W. T., & Edwards, D. P. Progesterone receptor contains a proline-rich motif that directly interacts with SH3 domains and activates c-Src family tyrosine kinases. Molecular cell, Vol.8, No.2, 269–280, 2001.

[13] Hoekstra, A. V., Sefton, E. C., Berry, E., Lu, Z., Hardt, J., Marsh, E., Yin, P., Clardy, J., Chakravarti, D., Bulun, S., & Kim, J. J. Progestins activate the AKT pathway in leiomyoma cells and promote survival. The Journal of clinical endocrinology and metabolism, Vol.94,No.5, 1768–1774, 2009.

[14] Bot á, C.P, Camarasa, S.C, Baixauli, F.R, & Sanchez, A.C. Uterine Fibroids: Understanding their Origins to Better Understand their Future Treatments. Journal of Tumour Research Vol.3,130, 2017.

[15] Murji, A., Whitaker, L., Chow, T. L., & Sobel, M. L. Selective progesterone receptor modulators (SPRMs) for uterine fibroids. The Cochrane database of systematic reviews, Vol.4,No.4, CD010770, 2017.

[16] Islam, M. S., Akhtar, M. M., Ciavattini, A., Giannubilo, S. R., Protic, O., Janjusevic, M., Procopio, A. D., Segars, J. H., Castellucci, M., & Ciarmela, P. Use of dietary phytochemicals to target inflammation, fibrosis, proliferation, and angiogenesis in uterine tissues: promising options for prevention and treatment of uterine fibroids?. Molecular nutrition & food research, Vol.58,No.8, 1667–1684, 2014.

[17] The UniProt Consortium, UniProt: the universal protein knowledgebase in2021, Nucleic Acids Research, Volume 49, Issue D1, 8 January 2021, Pages D480–D489.

[18] Williams, S.P., Madauss, K.P., Deng, J.-S., Austin, R.J.H., Lambert, M.H., McLay, I., Pritchard, J., Short, S.A., Stewart, E.LUings, I.J. Progesterone Receptor Ligand Binding Domain with bound Norethindrone J Med Chem Vol 47, 3381-3387, 2004.

[19] Tian, W., Chen, C., Lei, X., Zhao, J., & Liang, J. (2018). CASTp 3.0: computed atlas of surface topography of proteins. Nucleic acids research, Vol.46(W1), W363–W367.

[20] Lipinski C. A. Drug-like properties and the causes of poor solubility and poor permeability. Journal of pharmacological and toxicological methods, Vol.44No.1, 235–249, 2000.

[21] Benet, L. Z., Hosey, C. M., Ursu, O., & Oprea, T. I. BDDCS, the Rule of 5, and druggability. Advanced drug delivery reviews, Vol.101, 89–98, 2016.

[22] Kim, S., Thiessen, P. A., Bolton, E. E., Chen, J., Fu, G., Gindulyte, A., Han, L., He, J., He, S., Shoemaker, B. A., Wang, J., Yu, B., Zhang, J., & Bryant, S. H. PubChem Substance and Compound databases. Nucleic acids research, 44(D1), D1202–D1213, 2016.

[23] O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. Open Babel: An open chemical toolbox. Journal of Cheminformatics, Vol.3, 33, 2011.

[24] Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., and Olson, A. J. Autodock4 and AutoDockTools4: automated docking with selective receptor flexibility. J. Computational Chemistry, Vol.16, 2785-2791, 2009.

[25] Wallace, A. C., Laskowski, R. A., & Thornton, J. M. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. Protein engineering, Vol.8,No.2, 127–134, 1995.