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Spectroscopic (FT-IR and UV-Vis), Electronic and Docking Studies on the Red Clover Isoflavone Irilone as a Progesterone Receptor (PR) Effect Supporter in Endometrial and Ovarian Cancer Cell Lines

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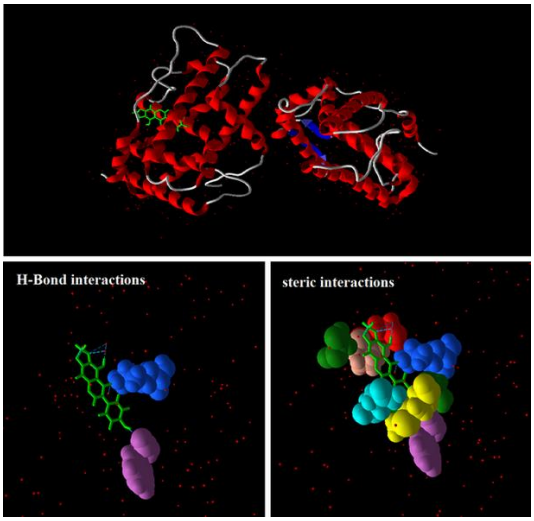
ABSTRACT

This work tries to present theoretical studies and docking analysis on the small novel molecule irilone as a progesterone receptor (PR) effect supporter in endometrial and ovarian cancer cell lines. The quantum mechanical computations are done using B3LYP/6-31+G(d,p) level of theory on the molecule under study at room temperature. The theoretical calculations showed that irilone is a stable small molecule with high electrophilicity property. The density of states (DOS) graph indicated that the virtual orbitals of the said compound have more density than that of the occupied orbitals. The studies indicated that the title compound can make a complex with progesterone receptor (PR) using steric and hydrogen bond (HB) interactions. The docking analysis showed that the receptor (PR-B isoform) residues Pro-696, Gln-725, Met-759, Arg-766, Glu-695, Asp-697, Leu-758, Lys-822, Ile-699, Val-698 and Trp-755 play the main role in receptor-ligand complex formation.

GRAPHICAL ABSTRACT



1. Introduction

Estrogen and Progesterone receptors (ER, PR) are ligand-induced transcription factor members of the steroid hormone receptor (SR) as the subfamily of nuclear receptors. Those receptors and their corresponding steroid hormones (estradiol and progesterone) play their roles through complex mechanisms to adjust biological processes critical for women's health.[1-2] Two common isoforms (A and B) are formed from the same gene through alternate translational start sites; PR-B relates to the full-length receptor, while PR-A is an N-terminally shorten version (additional amino acid at position 164 in PR-B).[3-4] PR-A is mostly localized in the nucleus, whereas PR-B continuously transports between nuclear and cytoplasmic compartments. Progesterone is recognized to its vital role in regulation of the normal physiology of the ovary, uterus, mammary gland, as well as brain progress during childhood. Progesterone also acts via the maintenance of the cardiovascular, central nervous and skeletal systems.[5-6] Despite the fact that many drugs act through PR-mediated mechanisms, few educations have identified the natural product components detected in herbal supplements that act via the progesterone receptor.[7] Red clover which is used to alleviate menopausal symptoms contains phytoestrogens. These are phenolic non-steroidal compounds with a comparable steric structure as steroidal

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estrogens. This similarity donates them the chance to bind to steroid receptors and exert estrogenic or anti-estrogenic effects.[8] Previously, reports suggested that the red clover constituent kaempferol showed progestogenic effects in ovariectomized rats.[9] Irilone, identified to be present in red clover plants, has been recently recognized in red clover based compounds for the first time, accounting for about 10 % of the total isoflavone content.[10-11] This extract supported the effect of progesterone in both endometrial and ovarian cancer cell lines. In these cancers, progesterone acts were associated with positive outcomes and reduced hazard of disease occurrence .[12, 13]

From the literature survey, it was found that the identification of the structural properties of the molecule under study had not been performed previously in the light of computational chemistry[14-16] and hence the study was undertaken. The main aim of the present study is to give a comprehensive description of the electronic properties andspectroscopic (FT-IR and UV-Vis) profiles of the novel antagonist irilone as a progesterone receptor (PR) effect supporter in endometrial and ovarian cancer cell lines by quantum-mechanical (QM) and molecular docking methods.It is believed that the outputs of this study will provide a deep and accurate understanding of the possible biological activities of the title compound.

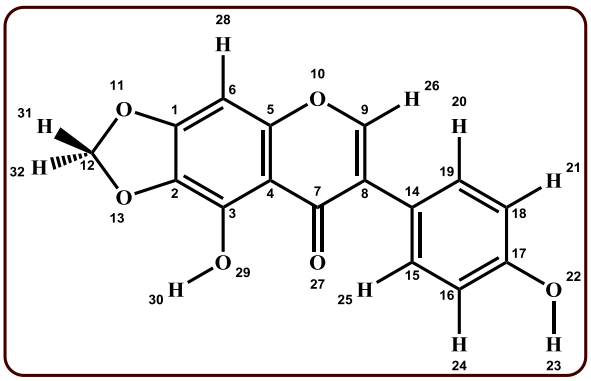
2. Computational Methods

Quantum mechanics (QM) has revolutionized our understanding of the reactivity and structure of the small molecular systems. All quantum mechanical simulations and structural properties calculations were done with the Gaussian 03 software.[17] Most quantum mechanical studies on the small molecules employ density functional theory (DFT), [18] also in the present study, the novel antagonist irilone was optimized at the B3LYP/6-31+G(d,p) level of theory at room temperature. After optimizing the molecular structure of the compound irilone, the global reactivity and moleculecular electrostatic potential (MEP) calculations were performed at the mentioned basis set of theory to describe the stability and reactivity of the molecule under study. On the other hand, the molecule-protein interactions were investigated using Molecular Virtual Docker (MVD) program.[19]

3. Results and Discussion

3.1. Irilone Structural Properties Study

Jung-Ho Lee and his colleagues found that the compound irilone (Scheme 1) potentiated the progesterone effect in both endometrial and ovarian cancer cell lines.[20] The molecular structure of this natural compound was characterized and identified using proton and carbon-13 nuclear magnetic resonance (NMR) technique.[20] Unfortunately, this article doesn't give us more data about the molecular geometry, reactivity and stability of the molecule under study. So, we computed the mentioned properties theoretically using DFT method. Firstly, the molecular structure of the title compound was optimized by B3LYP/6-31+G(d,p) level of theory. Figure 1 shows the optimized molecular structure of this small molecule. As can be shown in Figure 1, the B and C rings are planar and aromatic. Due to the aromaticity property, the pi electrons and oxygen-10 nonbonding electrons are in resonance with together. So, the bond length of the C-O10 bond (1.36 Å) is shorter than the normal singlet C-O bond (1.43 Å). On the other hand, the D ring has been twisted to the B and C rings due to the steric strain of the atoms of the title rings (H20, H25, H26 and O27). The C9-C8-C14-C19 dihedral angle is 41.13 Å. Figure 2 indicates the dependence between the theoretical and experimental bond lengths of the molecule under study. This dependence is shown by the equation y=0.931x+0.0751. The higher correlation coefficient (R^2=0.9725) for this equation shows a great convergence. So, the B3LYP/6-31+G(d,p) basis set of theory is a good method to compute the electronic properties of the said compound.



Scheme 1. Irilone molecular structure.

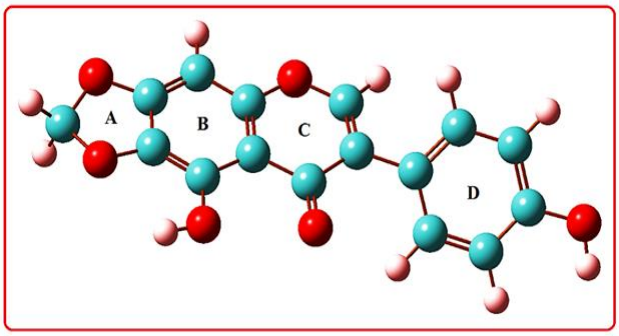


Fig 1. The theoretical geometric structure of Irilone.

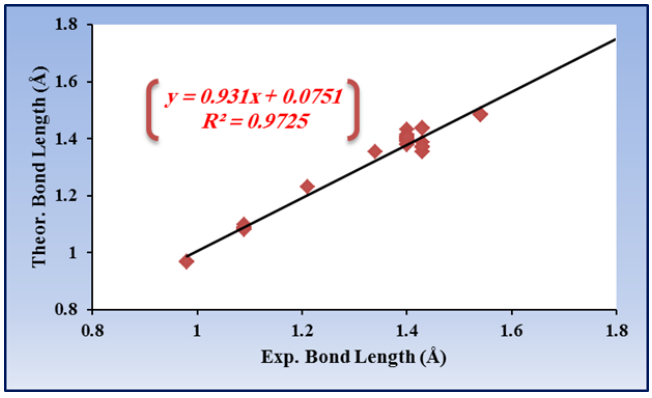


Fig 2. The experimental and theoretical bond lengths relationship of Irilone.

3.2. Stability and Reactivity Study of the Compoundirilone

Frontier molecular orbital (FMO) theory is an application of the molecular orbital (MO) theory describing the highest

occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) interactions.[21]The frontier molecular orbitals (HOMO and LUMO) of the molecule irilone have been indicated in Figure 3. We can see that the LUMO is mainly on the A, B and C rings, while the HOMO has been made by the atoms of the B, C and D rings. It can be deduced that the B and C rings participate in the formation of both HOMO and LUMO. It happens due to the electron current of these planar rings.The stability and global reactivity indices of an organic compound can be gained using FMO theory.[22] The global reactivity descriptors like energy gap (Eg), ionization potential (IP), electron affinity (EA), chemical hardness (η), chemical softness (S), electronegativity (χ), electronic chemical potential (μ) and electrophilicity index (ω) can be obtained from the energies of the frontier orbitals. These reactivity indices are achieved by following formulas[23]:

$$E_g = E_{LUMO} - E_{HOMO}$$

$$IP = -E_{HOMO}$$

$$EA = -E_{LUMO}$$

$$\eta = \frac{(\varepsilon_{LUMO} - \varepsilon_{HOMO})}{2}$$

$$\chi = \frac{-(\varepsilon_{LUMO} + \varepsilon_{HOMO})}{2}$$

$$\mu = \frac{(\varepsilon_{LUMO} + \varepsilon_{HOMO})}{2}$$

$$\omega = \frac{\mu^2}{2\eta}$$

$$S = \frac{1}{\eta}$$

Table 1 which has been listed the global reactivity indices and frontier molecular orbitals energies of irilone. As can be seen from the data, the energies of HOMO and LUMO are -5.870 eV and -1.565 eV, respectively. The HOMO/LUMO energies gap (Eg) is 4.305 eV. This energy gap shows the high stability of the said compound. On the other hand, the high amount of ionization potential (5.870 eV) indicates the low affinity of the title compound in reaction to the oxidizing agents. So, this natural compound is really stable under biological conditions of cells. Also, the low amounts of electron affinity and chemical hardness and high amounts of the chemical softness and electrophilicity index show the high tendency of the molecule under study to interaction with residues of the proteins like receptors.Figure 4 shows the density of states (DOS) graph of the title compound. This graph indicates that the virtual orbitals have more density than the occupied orbitals. So, the molecule irilone likes more to react with the electron rich agents such as receptors. The molecular electrostatic potential (MEP) graph of irilone is shown in Figure 5. In this graph, the regions with blue, green and red colors are related to the molecular segments with positive, zero and negative electrostatic potentials, respectively. We can see that all atoms, except atoms H31, H32, H30, O29, O27, O22 and H23, are nearly zero from the electrostatic potential point of view. It can be deduced that these hydrogen atoms have acidic property. On the other hand, the atoms O22, O27, O29, H23 and H30 can be participated in the hydrogen bond formation with hydrogen bond acceptor (HBA) agents or hydrogen bond donor (HBD) agents.

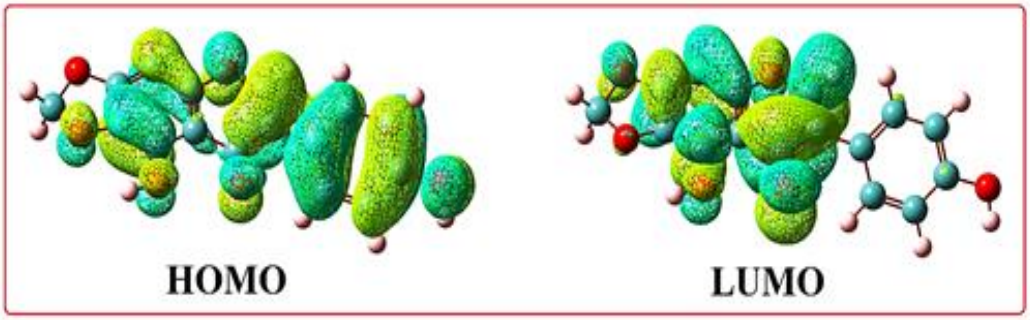


Fig 3. The frontier molecular orbitals of irilone.

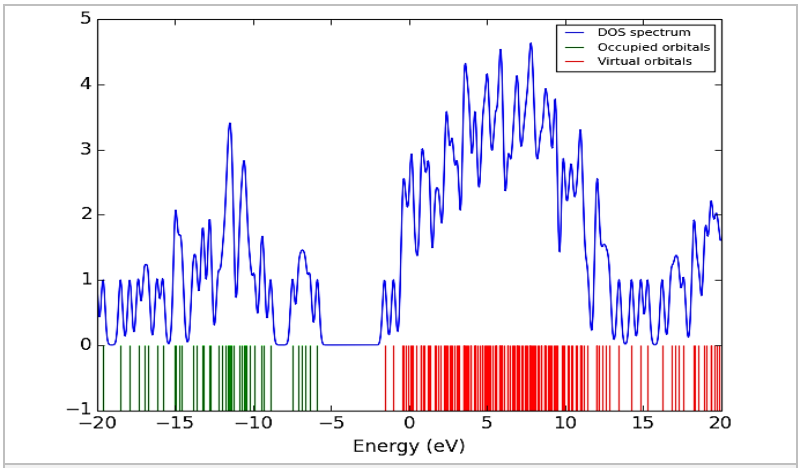


Fig 4. The density of states (DOS) graph of irilone.

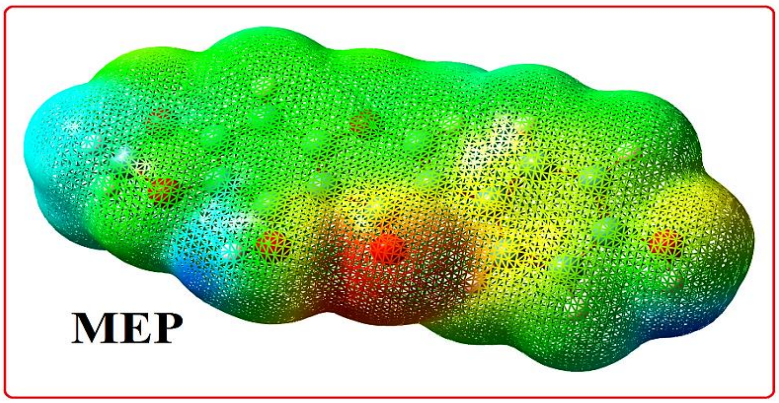


Fig 5. The molecular electrostatic potential (MEP) graph of irilone.

Table 1. Global reactivity indices of irilone.

Parameter	Energy value (eV)
HOMO	-5.870
LUMO	-1.565
Ionization Potential (IP)	5.870
Electron Affinity (EA)	1.565
Energy Gap (Eg)	4.305
Electronegativity (χ)	3.718
Chemical Potential (μ)	-3.718
Chemical Hardness (η)	2.153
Chemical Softness (S)	0.464
Electrophilicity index (ω)	3.210

3.3. Vibrational and Electronic Spectral Analyses of the Compound Irilone

In the current section, a detailed analysis of the compound irilone has been done through FT-IR and UV-Vis spectroscopy techniques. The FT-IR spectrum of the molecule is indicated in Figure 6. It can be seen from the title spectrum that the vibrational frequencies in ranges 3780-3820 cm^{-1}, 3000-3250 cm^{-1}, 1630-1710 cm^{-1}, 1300-1550 cm^{-1} and1020-1290 cm^{-1} are related to the O-H, C-H, C=O, C-H (bending) and C-O vibrations, respectively. The detailed vibrational modes of the compound under study are: 26.6338, 50.2406, 58.9206, 75.8149, 105.9890, 137.1457, 158.6545, 186.6165, 206.2506, 217.7725, 251.9591, 286.4582, 298.2487, 328.7449, 336.1726, 346.6072, 376.8986, 399.9449, 423.3983, 426.3956, 437.1135, 448.5555, 474.1609, 519.8643, 536.7466, 584.1813, 596.8732, 605.3844, 610.9425, 647.9093, 655.0521, 667.1823, 700.4325, 723.7782, 742.3640, 773.3349, 791.2200, 817.1994, 821.4719, 822.0385, 839.4746, 845.8451, 907.4906, 928.6943, 950.9707, 958.9455, 966.4519, 1026.9986, 1044.7690, 1065.0055, 1081.0078, 1112.1124, 1131.1020, 1145.9609, 1180.1595, 1186.6270, 1202.2470, 1215.8891, 1234.1445, 1264.2484, 1283.5154, 1289.7597, 1325.5239, 1332.6743, 1368.7811, 1384.1202, 1392.2250, 1419.8745, 1433.8671, 1465.4275, 1499.1627, 1511.0375, 1541.0728, 1549.6011, 1628.5458, 1647.7739, 1660.1175, 1667.1292, 1686.1361, 1712.4694, 3026.5686, 3150.5752, 3167.2075, 3185.0297, 3211.5671, 3235.7555, 3237.5988, 3247.2491, 3786.8225 and 3826.6346 cm^{-1}.

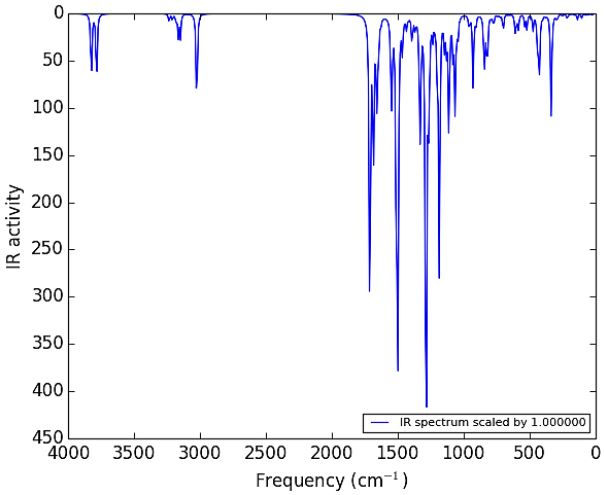


Fig 6. The FT-IR spectrum of irilone.

The structures of medicinal compounds can absorb visible (Vis) or ultraviolet (UV) lights. The most important point in this method is that the different molecular structures absorb radiation of different wavelengths. An absorption spectrum will show the absorption bands corresponding to the structural

groups within the molecule [24]. The UV-Vis spectrum of the compoundirilone is shown in Figure 7. The electronic transitions of irilone molecular structure in UV-Vis region have been tabulated in Table 2. The electronic transitions of the said compound are seen in wavelengths 333 nm, 326 nm and 297 nm with energies 29990.32 cm^{-1}, 30677.51 cm^{-1} and 33662.59 cm^{-1}, respectively. The most importantelectronic transition is related to the HOMO to LUMO transition. This electronic transition is seen in wavelength 325.972 nm.

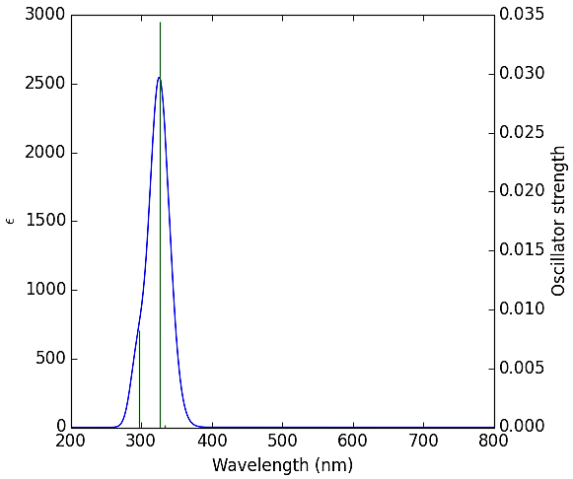


Fig 7. The UV-Vis spectrum of irilone.

Table 2. Electronic transitions of irilone in UV-Vis region.

Energy (cm^{-1})	Wavelength (nm)	Osc. strength	Electronic transition (possibility)
29990.320	333.441	0.0002	HOMO-2→LUMO (88%), HOMO-4→LUMO (3%)
30677.510	325.972	0.0344	HOMO→LUMO (89%)
33662.588	297.066	0.0082	HOMO-1→LUMO (72%), HOMO→LUMO+1 (15%), HOMO-3→LUMO+1 (3%)

3.4. Charge Distribution and Molecular Docking

The Mulliken charge distribution on atoms of the title small molecule is shown in Figure 8. In this graph, the negative, zero and positive charges are seen with red, black and green colors, respectively. The atoms with negative and positive charges prefer to react with electron-poor and electron-rich atoms of a protein structure, respectively. It can be seen from the Figure 8 that the carbon atoms of B, C and D rings alternately have positive and negative charges. This charge distribution on carbon atoms of the title rings is due to the electron current of pi orbitals. On the other hand, oxygen and hydrogen atoms of the molecular structure show negative and positive charge distributions, respectively. So, hydrogen and oxygen atoms of the compound irilone can play HBD and HBA roles, respectively. Figure 9 indicates the two-dimensional electron localization graph of irilone. This graph clearlyshows the electron current on all rings of the title compound. So, all rings can participate in the steric interactions with the residues of the progesterone receptor (PR).

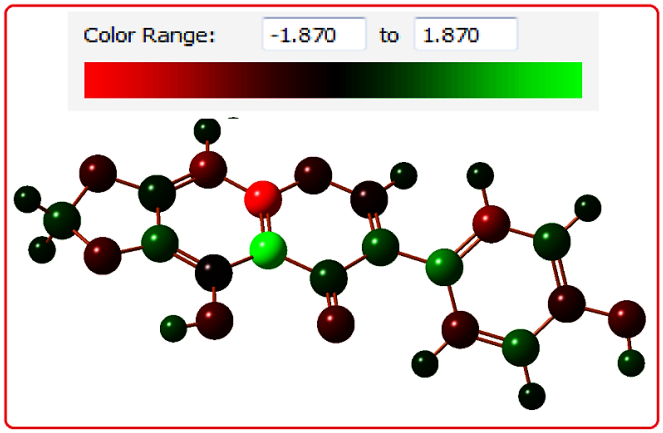


Fig 8. The charge distribution of irilone.

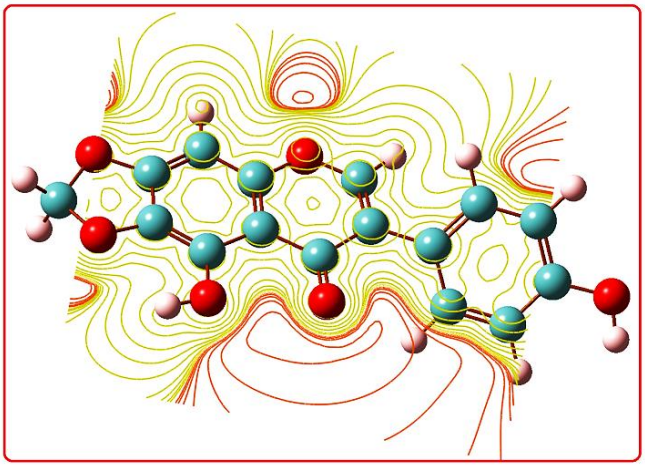


Fig 9. The two-dimensional electron localization graph of irilone.

The literature review clearly shows that the medicinal compound irilone can be used as a progesterone receptor (PR) effect supporter in endometrial and ovarian cancer cell lines. The present section of our investigationis the irilone-PR docking analysis to clear the desired protein-ligand binding site and its binding affinity. The three dimensional crystal structure of the progesterone receptor (PR) was obtained from protein data bank (PDB) and the docking analysis was carried out using Molegro Virtual Docker (MVD) program. As can be seen from the Figure 10, the said natural compound formed a stable complex with the PR-B isoform ofprogesterone receptor (PR) with Steric and hydrogen bond (HB) interactions. From the data of the Table 3, the MolDock scores of the Steric (by PLP), Steric (by LJ12-6) and HB interactions in the receptor-ligand complex formation are 106, 13 and 11, respectively. So, the steric interactions play main role in the formation ofirilone-PRB complex. On the other hand, the title ligand can interact with water molecules.It can be seen from the data of the Table 3, the water-ligand interactions value is about 22. Figure 11 indicates the most important hydrogen bond (HB) and steric interactions in irilone-PRB complex. From the Figure 11 and the data of the

Table 4, the most important interactions are related to the PR-B isoform residues Pro-696, Gln-725, Met-759, Arg-766, Glu-695, Asp-697, Leu-758, Lys-822, Ile-699, Val-698 and respectively.

Trp-755. Also, the residues Trp-755 and Lys-822 form hydrogen bonds with oxygen atoms O-22 and O27,

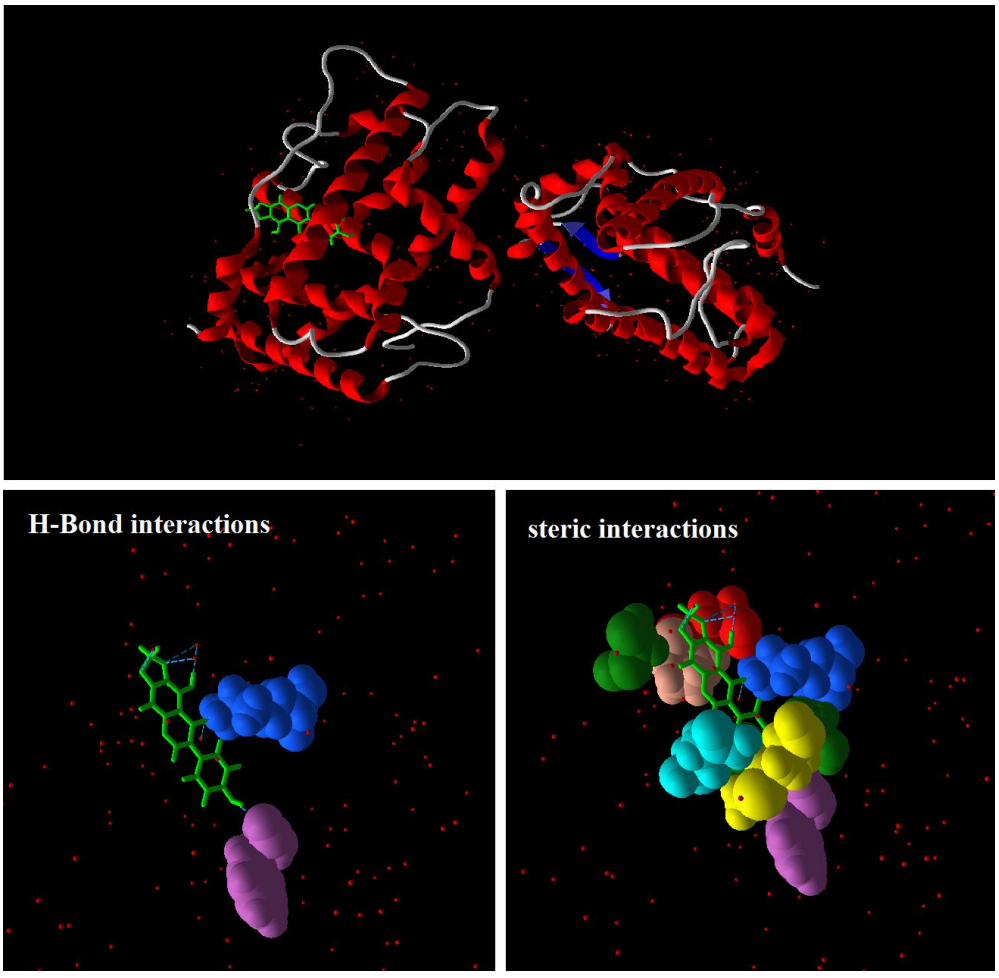


Fig 10. Ligand irilone embedded in the active site of progesterone receptor

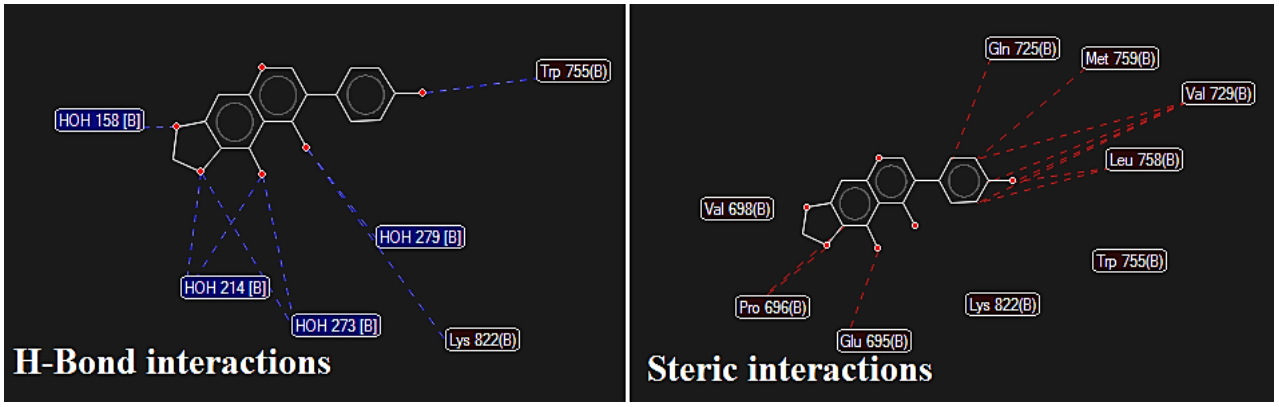


Fig 11. H-bond and steric interactions of ligand irilone embedded in the active site of progesterone receptor.

Table 3. The ligand-receptor interactions.

Interactions		MolDock Score
Protein-Ligand Interactions	Steric (by PLP)	-105.726
	Steric (by LJ12-6)	-12.936
	Hydrogen bonds	-3.905
	Hydrogen bonds (no directionality)	-7.168
Water-Ligand Interactions		-21.743
Internal Ligand Interactions	Steric (by PLP)	22.226
	Steric (by LJ12-6)	79.332

Table 4. The participated residues of progesterone receptorin ligand-receptor interactions.

Residue/HOH	Total energy score
Pro 696 [B]	-20.205
Gln 725 [B]	-15.534
Met 759 [B]	-11.283
Arg 766 [B]	-10.511
Glu 695 [B]	-8.479
Asp 697 [B]	-8.393
Leu 758 [B]	-6.873
Lys 822 [B]	-6.550
Water HOH 125	-6.232
Ile 699 [B]	-5.952
Val 698 [B]	-5.482
Trp 755 [B]	-5.206
Water HOH 190	-4.869
Water HOH 246	-3.590
Water HOH 64	-2.949
Water HOH 184	-2.859
Ser 728 [B]	-1.531
Leu 726 [B]	-1.450
Gly 762 [B]	-1.250
Water HOH 12	-0.927
Water HOH 99	-0.883
Phe 818 [B]	-0.828
Water HOH 86	0.567
Val 729 [B]	2.007

4. Conclusions

The present article is related to study the structural and electronic properties and docking analysis of the novel compound irilone as a progesterone receptor (PR) effect supporter in endometrial and ovarian cancer cell lines. The mentioned investigations are theoretically carried out using density functional theory (DFT) method (B3LYP/6-31+G(d,p)) at room temperature. Optimization process of the molecular structure showed electron current on the sextet rings of the molecule. Thus, the rings are aromatic and obey from Huckel's rule (4n+2). The frontier molecular orbitals (FMOs) theory calculations indicated the stability of the said compound and its high tendency to react with electron rich

agents. On the other hand, the electronic properties study showed that the title molecule likes to make complex with progesterone receptor (PR) by steric and hydrogen bond interactions. The ligand-receptor docking analysis indicated that the receptor (PR-B isoform) residues Pro-696, Gln-725, Met-759, Arg-766, Glu-695, Asp-697, Leu-758, Lys-822, Ile-699, Val-698 and Trp-755 play the main role in irilone-PRB complex formation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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