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Characterization of Plumbagin by implying various *in silico* studies

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***Abstract*---**Naphthoquinone derivative was established to be an important chemical versatile scaffold belongs to *Plumbago indica (Plumbaginaceae family). Its* 5-hydroxy-2-methyl-1,4-naphthoquinone substituent, Plumbagin belongs to one of the diverse largest groups of plant metabolites and is obtained from the plant roots. Although the interactions of ligand receptor have not yet been confirmed, but it is found that it possess excellent antagonist properties against the multiple signaling proteins of cancer. *The biggest problem like overflow of population is to be monitored to establish a balance ecologically. To resolve this problem,* plumbagin is found to be a potential lead to overcome several side effects and contraindications resulted from other synthetic contraceptive analogues. The present investigation provides detailed information for understanding the various structural insights into progesterone receptors and estrogen receptors using different binding modes. It displayed excellent binding affinity against the progesteronereceptor (7.2 Kcal/mol (Ki 0.249 mM) and estrogen receptors (10.5 Kcal/mol (Ki = 0.294 mM)).

***Keywords*---**Estrogen, *in-silico*, plumbagin, progesterone receptors.

Introduction

The control of fertility is serious issue of concern related to health concern. Worldwide, the accelerated population produces the detrimental effects on the

environment ultimately leading to confer the various fertility consequences.[1,2] In this regard, WHO (World Health Organization) has developed certain fertility regulations for investigating novel non-steroidal contraceptive compounds.[3]The studies of post-market of hormonal contraceptives have revealed their hazardous nature as its long term use might improve the venous thromboembolism chances in older women like pulmonary embolism [PE] and deep vein thrombosis [DVT].[4,5] In addition, estrogen decreases biological amines-serotonin levels, resulting in depression .[6] Moving further, the literature revealed that contraceptives especially steroidal structure decrease the levels of vitamin B6 level throughout the body, it is believed to be an essential cofactor for enzymes metabolizing the conversion of tryptophan into its starting reactant serotonin .[7] Furthermore, the levels of vitamin B6 is decreased in the body by steroidal contraceptives that metabolize the conversion of tryptophan (trp) into serotonin (ser). Serotonin, a potential neurotransmission mediator, reduces the level of neuronal serotonin in the brain, thus causes depression.[8] Although extreme exertions have been implied to minimize hormonal contraceptive side effects, but efficient realization has not yet been achieved .[9]

Since synthetic steroidal contraceptives produces serious side effects, the use of non-steroidal synthetic contraceptives has attained importance nowadays. Due to multiple side-effects caused by synthetic medicines, the investigation has been engrossed on developing novel contraceptive compounds by utilizing naturally occurring substances. The tradition herbal medicine was endorsed for its anti-fertility effect in numerous literary works.[10,11] In our new review, we distinguished various phytochemicals acquired from Plumbagin through writing overview.

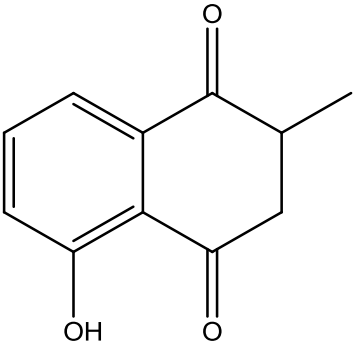


Figure 1. Structure of Plumbagin.

After that, molecular modeling docking was performed using *in silico* docking simulation tools such as AutoDock softwares from The Scripps Research Institute, CA, USA (AutoDock Vina and AutoDock tools) to screen the phytochemicals for understanding for anti-fertility activity of the lead compounds. Isolated Plumbagin from the phytochemicals was screened against human progesterone receptor (1E3K) and human estrogen receptor (1ERE) binding with 17-b estradiol. Moving further, it was accessed for ADMET profile (absorption, distribution, metabolism, and excretion-toxicity) using ADMET SAR software (http://lmmd.ecust.edu.cn:8000/) .[12] The Drug-likeness attributes were analyzed by using the SwissADME webserver and Osiris Property Explorer (http://www.organic chemistry.org/prog/peo/) .[13]

Materials and Methods

Chem 3D Ultra version 8.0, AutoDock Tools (MGLTools)-1.5.7, AutoDock Vina,, ArgusLab and PyMOL are utilized for computation studies.[14]

Computational methods

The *in silico* investigation studies were found to be very useful to hypothesize the mechanism of accomplishment of plumbagin phytochemical within the both receptors (progesterone and estrogenic receptors). The inhibition of the ligands within the focused active site of these receptors was assessed using *in silico* tools of molecular docking to understand it in detail. The docking technique of computer aided drug design is used to hypothesize the favored orientation within the target receptors. The molecular docking forecast the inhibitory activity of ligand into the dynamic active binding site and it was performed in three stages as mentioned below:

- Receptor Preparation using optimization
- Ligand Preparation
- Characterizing the limiting docking site of the ligand
- Prediction of binding affinity in terms of docking score, where the standard antagonists were used against progesterone and estrogen receptors separately.

Receptor Preparation

Chem 3D Draw Ultra was used to draw the structures of compounds, followed by energy minimization by dynamics module of molecular modeling installed in Chem 3D. Moving further, PDB format was used to save the compounds and opened again with AutoDock software. The defined protocols were used to prepare the receptor such as defining torsions and making bonds freely rotatable. After that, it was saved in the format of PDBQT with during docking. PDB repository containing PDB IDs 1E3K and 1ERE was chosen and was downloaded to obtain the progesteronic and oestrogenic receptors for evaluation of the molecular modeling studies.

PDB id: 1ERE was found to be the estrogen receptor with 17-b estradiol co-crystal (resolution 3.10 Å; R-value: 0.218), whereas PDB id: 1E3K has the inbound ligand metribolone was established to be progesterone receptor (resolution: 2.80 Å; R-value: 0.240). The water, co-crystal and cofactor molecules were removed to isolate the receptors using Miscellaneous module. The non-polar hydrogens were merged consequently after addition of polar types of hydrogen to the receptors. The inbound ligand and water was extracted to obtain the receptor molecules and then said molecule was converted from PDB into the format of PDBQT by AutoDock software version 1.5.7.

Prior to docking, protein structures were energy minimized followed by addition of charges of Gasteiger within ligands to make it rotatable freely. During docking, it was displayed that the same charges may generate incompleteness that will mislead the wrong results. To remove this error, incorporation of kollman charges

was retrieved on the compounds, and re-docking was performed into the same cavity conforming the same algorithm.

Preparation of Ligand

After receptor preparation, ligand is prepared by optimization using force field by the above mentioned settings.

Characterizing the limiting docking site

Firstly the docking software was used to open the ligand bound (co-crystal) crystalline protein structure and active limiting binding site was drawn using active amino acids labelling. The focused active amino acid was defined to dock the ligands within the corresponding receptors. The box of grid was prepared to cover the binding site for performing the docking studies. AutoDock Vina was employed for alignment of the co-crystal optimized ligand into whole receptor to insure the presence of active binding site, which uses a global optimizer in terms of local search as a part of its algorithm of optimization. The optimum ligand was confirmed to be the best possible conformer having maximum binding affinity and was fitted within the receptor in visualization module such as MGL visualizer through ligand-fit interactions using script mediated algorithm. The binding site atoms were defined consequently in relationship with the results obtained from PDB derived protein crystal, and its PDB format was saved. The resultant corresponding grid box was aligned in AutoDock module.

Prediction of binding affinity in terms of docking score, where the standard antagonists were used against progesterone and estrogen receptors separately. The affinity of binding was determined in terms of docking scores against the progesteronic receptor (1E3K) using vina module of AutoDock software. The search grid was identified as mentioned below:
Center of x = 17.510,
Center of y = 4.408,
Center of z = 19.866,
and the dimension of size was
size of x = 52,
size of y = 76,
size of z =78.
For Estrogen gesteronic receptor (1ERR). The search grid was set as
Center of x = 68.201,
Center of y = 33.808,
Center of z = 75.063,
and the dimension of size was
size of x = 30,
size of y = 36 and
size of z =44

The machine learning approach was achieved to the scoring function using AutoDock Vina, therefore the optimum of global was found within the search space.

Evaluation of binding affinity within the other nuclear receptors

The molecular docking of Plumbagin (PLN) with other nuclear receptors was done to determine the binding activity in terms of restricting affinity as the standard antagonists against progesterone and estrogen receptors separately.[13]

Analysis of Binding residues

Analysis of binding site was performed using both protein structure of target receptors such as progesterone and estrogen receptors by MGL tools. The respective co-crystals are extracted for creating the docking grid for accommodating the new compounds into the cavity. It was retrieved from the crucial investigation that the herbal ligand binds in the same binding pocket as the standard inhibitors undertaken for the study. For example, PLN binds within progesterone receptor pocket surrounded by residues such as Phe778, Met801, Leu718, Leu715, Tyr890, Cys891, Trp719, Met909, Met756, Met759, Leu721, Gln725, Leu763 and Arg766 which in turn is proved to be the same binding pocket of PLN compound.

Similarly, PLN binding groove inside esterogen receptor is engulfed by Leu525, His524, Met343, Ile424, Leu346, Phe404, Leu391, Arg394, Glu353, Leu387, Ala350 which are found to be the same residues that encompasses the binding site. This conclusion suggests that PLN is assumed to act as a contraceptive agent by inhibition of the same receptor in the similar manner that a reported antagonist has been discovered.

ArgusLab: Calculation of binding energy

ArgusLab is found to be the classical method by which the binding energy is correlated with binding affinity, which is expressed in the negative order. It is the software used for computing the ligand-receptor binding energies of the ligands within the receptors i.e. proteins or enzymes. The unit of binding energy is kcal/mol expressed in the terms of Dock Score, that is performed in two steps.

- Preparation of ligand
- Docking

The compounds were firstly drawn in the chem 3D Ultra and then its geometry was optimized. The ligand molecules were energy minimized after addition of hydrogen atoms, using UFF (Universal Force Field) executed in Arguslab. There are two types of docking engines; GA Dock and ArgusDock. During trials, it was decided to choose Ascore scoring function and ArgusDock search engine. Docking was performed using Ascore scoring function with type of search engine Argusdock with 0.4 Å grid resolution. Furthermore, size of the binding site bounding box (22.351×15.765×13.957 Å) was determined automatically using Arguslab. Docked poses with energy were displayed in the molecule tree view window.

Results and Discussion

Molecular Docking analysis

A bunch of test compounds was docked to screen for docking examination through action preliminary to choose the best up-and-comer compound utilizing drug-receptor interactions. Up-and-comer compound is recognized by choosing the compound with most grounded restricting liking with the particular ligand. In this communication, compounds detailed from the concentrate of Plumbagin have been docked *in-silico* to uncover potential compound having best preventative movement. Atomic docking has been performed utilizing both programming Autodock vena and ArgusLab to discover the docking score and restricting energy separately.

A potential uncovered pogestrogen ligand, Organization C has been considered as a standard for progesterone receptor docking and for estrogen receptor, the standard compound has been picked as Rohitukine. Monte Carlo estimation phenomenon was employed using AutoDock Vina, which works on the scoring limit DG to infer the restricting energy of the ligand with respect to the receptor. Moving forward, DG being the Gibb's free energy for the reaction, negative DG suggests instantaneousness of the reaction although huge DG shows higher confining consistent for the reaction (DG = RT ln K). PLN showed docking prejudice as 10.5 Kcal/mol (obstruction consistent, Ki = 0.294 mM) and 7.2 Kcal/mol (Ki 0.249 mM) for progesterone and estrogenic receptor exclusively.

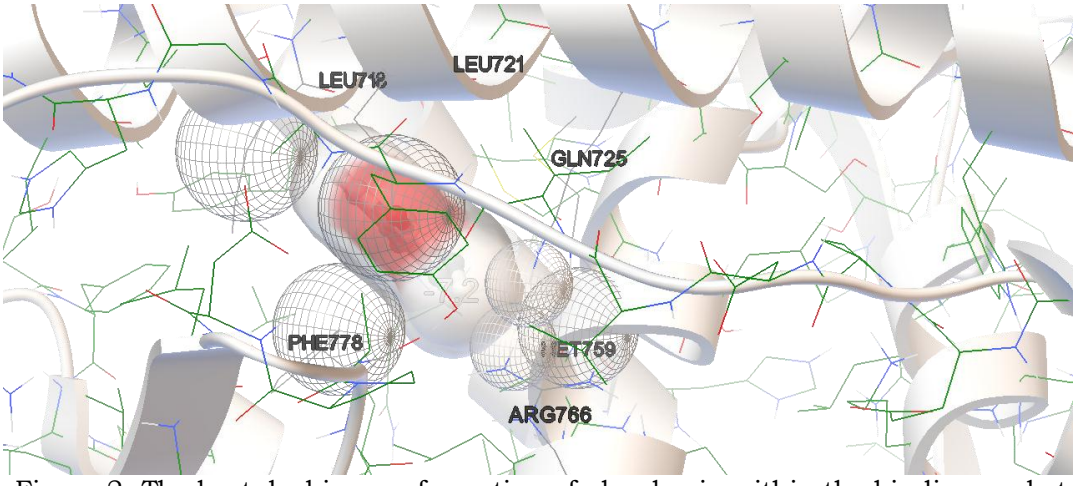


Figure 2. The best docking conformation of plumbagin within the binding pocket of enzyme covered by electrostatic potential cloud (PDB ID: 1E3K).

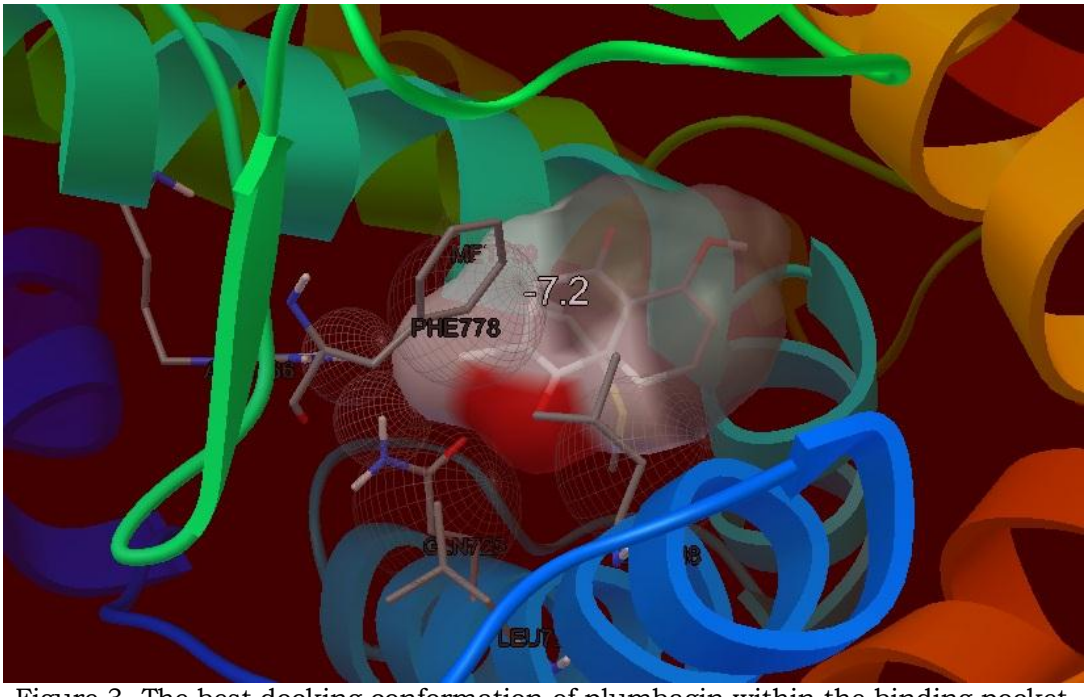


Figure 3. The best docking conformation of plumbagin within the binding pocket of enzyme (PDB ID: 1E3K) surrounding by the protein.

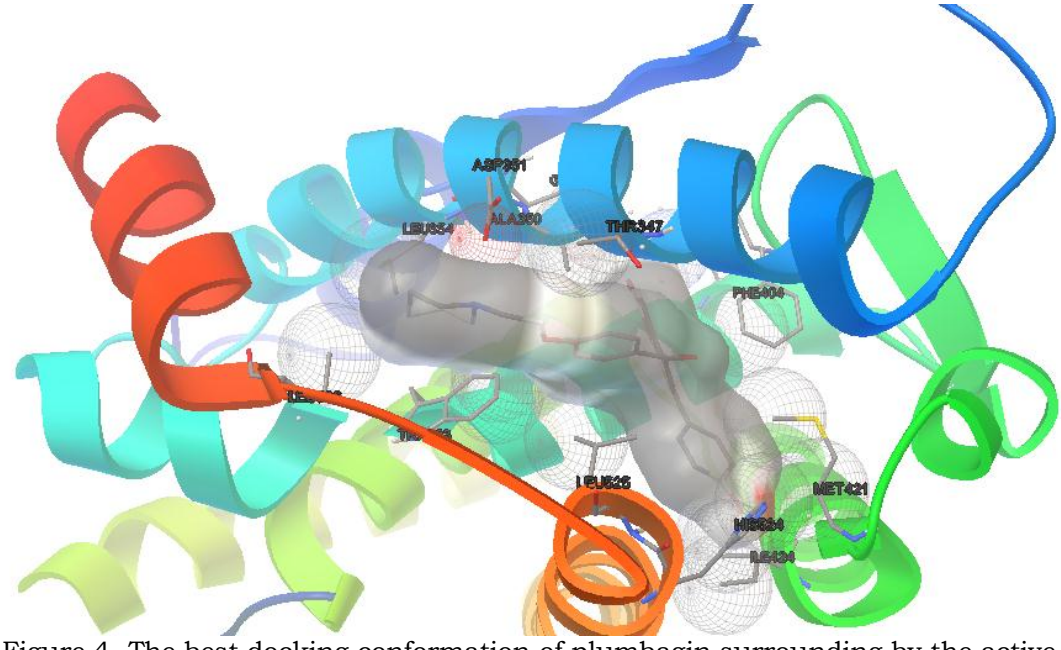


Figure 4. The best docking conformation of plumbagin surrounding by the active amino acids of enzyme (PDB ID: 1ERR)

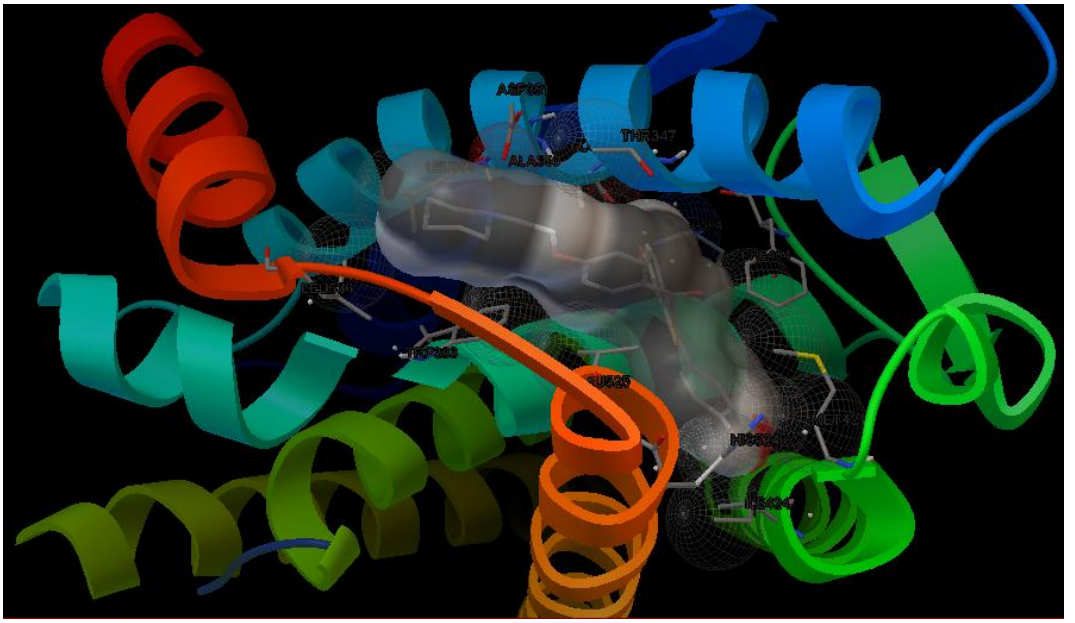


Figure 5. The best docking conformation of plumbagin within the binding pocket of enzyme (PDB ID: 1ERR)

The limiting partiality of the phytochemical PLN towards the both receptors can be clarified by hydrogen holding collaboration between the ligands and the receptor. For instance, carbonyl gathering of PLN showed three hydrogen holding associations with H524 and L346 and T347 recommending solid communication with the receptor. Strangely, showed just vander Waals restricting with one or the other estrogen or progesterone receptor.

ArgusLab

For performing the docking with ArgusLab software, the active amino acids have been identified using Ramachadran Plot which shows all the active amino acids present in the allowed and disallowed regions. Active amino acids are Leu715, Leu718, Asn719, Leu721, Gln725, Met756, Leu763, Phe778, Met759, Arg766, Phe778, Met801, Tyr890, Cys891 and Met 909 from the crucial studies of the protein active binding site.

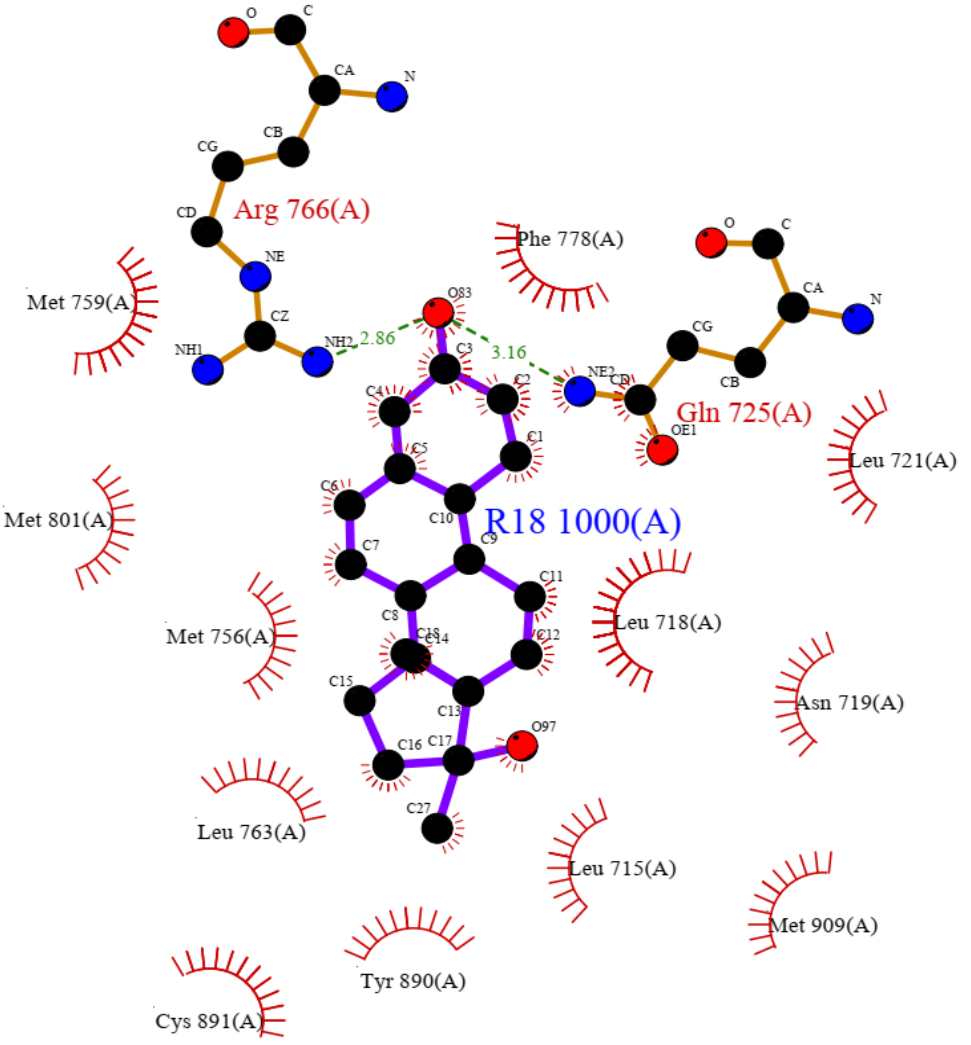


Figure 6. Ramachadran Plot showing active amino acids

The Ramachandran plot is found to be a fundamental tool in the analysis of protein structures and identified the specific interactions that affect the backbone. Considering the basic four types, Ramachandran plots are classified depending on the amino acid stereo-chemistry, which is composed of 18 non-glycine non-proline amino acids (generic amino acids), pre-proline, glycine, and proline. The distribution of torsion angles was assessed using Ramachandran plot, that provides the information about the amino acids in a protein structure and shows that the torsion angles corresponding to the two major types of secondary structure elements such as β-sheets and α-helices, which are evidently assembled within the specified separate regions.

The active amino acids present in the favourable regions are found to be Leu715, Leu718, Leu721, Gln725, Met756, Met759, Leu763, Arg766, Phe778, Tyr890, Met801, Cys891 and Met909. In addition to it, this plot gives the detailed

information about the active amino acids present in the allowed and disallowed regions. Grid has been made by knowledge of this plot using biased method. The details of it are given below:

Grid min (x,y,z) : 18.9049, -21.6146, -4.47904
Grid max (x,y,z) : 38.5049, 1.98542, 17.921
Grid dimensions: 50 x 60 x 57
Total number of grid points: 171000
Total memory required for grids = 17784000 bytes

GA Dock is used to target the active site using 2000 random revolutions at different angles and out of all, 149 conformations have been chosen for analyzing the different ligand poses. Binding energy of the compound PLN is calculated using GA Dock method is given below in the table 1.

Table 1
Binding Energy of Plumbagin in the terms of docking score

S.No	Ligand	PDB ID	Binding Energy
1.	PLN	1E3K	-8.50927
2.	PLN	1ERR	-9.65402

Binding residues analysis

PyMOL and MGL software tool kits were utilized to understand the crucial binding site analysis. The crucial study of the poses demonstrated that the interactions possessed by the ligand depicts the same pattern in the similar binding pocket as it is presented by the standard antagonists for the study. For example, PLN binds within progesterone receptor pocket surrounded by residues such as Phe778, Met801, Leu718, Leu715, Tyr890, Cys891, Asn719, Met909, Met756, Met759, Leu721, Gln725 and Leu763 that is found to be the same binding pocket of compound. On the other front, PLN binding groove inside the binding cavity of estrogen receptor is engulfed by Leu525, His524, Met343, Ile424, Leu346, Phe404, Leu391, Arg394, Glu353, Leu387 and Ala350.

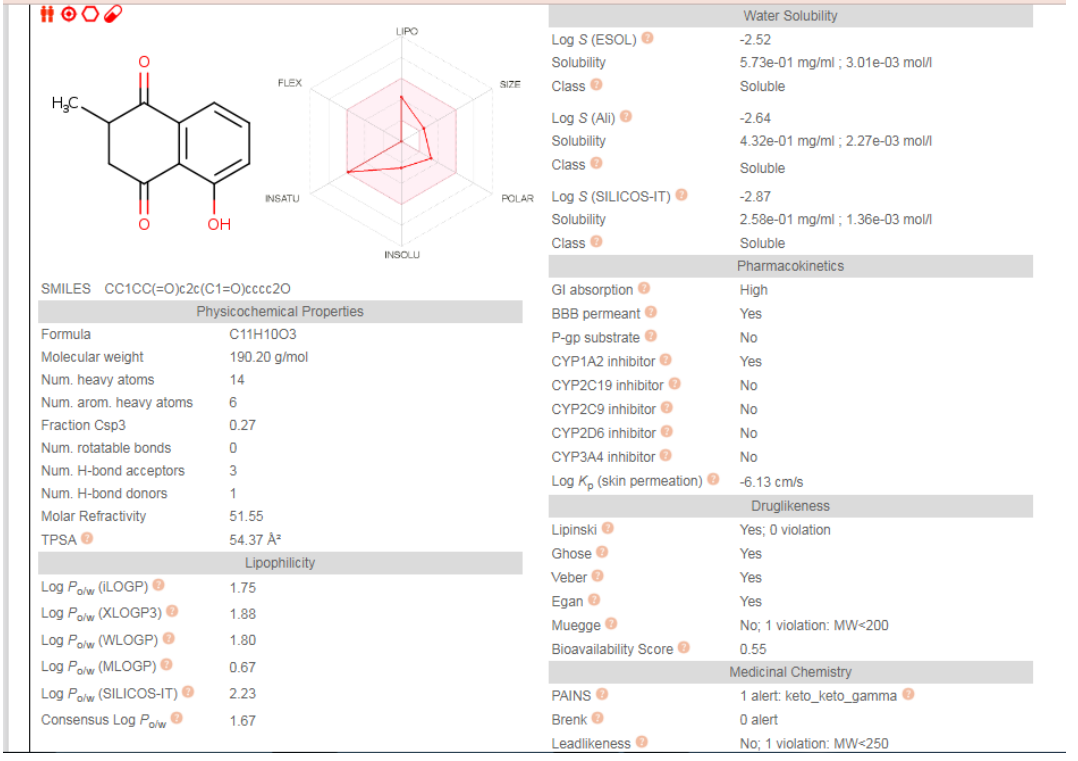
This detailed investigation suggests PLN to possess the activity against contraception by modulating the same receptor in the same way that a reported antagonist has been revealed. Hydrogen bond formed between the compound and the receptor was found to be important for explaining and determining the binding affinity towards receptor. For example, hydrogen bond between carbonyl group of the compound and His524 is formed via double bonded. On the other hand, it exhibits only single hydrogen bonding for understanding the ligand-receptor interaction.

It was found from the crucial interaction studies that “carbonyl group of PLN showed three hydrogen bonding interactions with His524 and Leu346 and Thr347 suggesting strong interaction with the receptor than rohitukine. Even in case of progesterone receptor, the carbonyl group of PLA exploits H-bonding with Leu 718 which is comparable with the hydrogen bonding of Org C with the Gly834 of the progesterone receptor.” Besides that, it showed only vander Waals binding with

either progesterone or estrogen receptor. It was recapitulated from the above study that PLN is found to be an excellent candidate as contraceptive agent.

Physicochemical and ADME properties

Physicochemical properties are found to amalgamation of chemical and physical properties of the compounds and that governs the ADME profile of the ligand. ADME properties have been computed using SwissADME webserver, ADMETSAR and OSIRIS calculator. For calculation purposes, the atomic design of the promising ligand was transferred to the respective calculator to compute different properties, for example, drug likeliness and ADMET properties. This multitude of potential compounds kept Lipinski's guideline of five with no infringement ADMET investigations more than three mixtures with promising exercises have been performed to assess on the off chance that they could make any unfriendly impact human. Curiously, every one of the mixtures showed some of both harmful and non-poisonous impacts.



SMILES CC1CC(=O)c2c(C1=O)cccc2O

Physicochemical Properties	
Formula	C11H10O3
Molecular weight	190.20 g/mol
Num. heavy atoms	14
Num. arom. heavy atoms	6
Fraction Csp3	0.27
Num. rotatable bonds	0
Num. H-bond acceptors	3
Num. H-bond donors	1
Molar Refractivity	51.55
TPSA	54.37 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	1.75
Log $P_{o/w}$ (XLOGP3)	1.88
Log $P_{o/w}$ (WLOGP)	1.80
Log $P_{o/w}$ (MLOGP)	0.67
Log $P_{o/w}$ (SILICOS-IT)	2.23
Consensus Log $P_{o/w}$	1.67

Water Solubility	
Log *S* (ESOL)	-2.52
Solubility	5.73e-01 mg/ml ; 3.01e-03 mol/l
Class	Soluble
Log *S* (Ali)	-2.64
Solubility	4.32e-01 mg/ml ; 2.27e-03 mol/l
Class	Soluble
Log *S* (SILICOS-IT)	-2.87
Solubility	2.58e-01 mg/ml ; 1.36e-03 mol/l
Class	Soluble
Pharmacokinetics	
GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-6.13 cm/s
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	No; 1 violation: MW<200
Bioavailability Score	0.55
Medicinal Chemistry	
PAINS	1 alert: keto_keto_gamma
Brenk	0 alert
Leadlikeness	No; 1 violation: MW<250

For instance, PLN uncovered low hydrophilic solubility recommending less bioavailability in blood circulation and inclination to cross BBB (blood brain barrier), that can actuate contraindication on the CNS (central nervous system). Moreover, the particles likewise showed AMES toxicity recommending they may be mutagenic on use. Notwithstanding, then again, all the compounds showed great gastrointestinal retention and cell porousness). Most remarkably, a bunch of isoforms of CYP450, for example, 2C9, 2D6, 2C19, and 3A4 have been viewed as non-inhibited by the toxicological forecast; just 1A2 is repressed by the

expectation. The cytochrome P450 superfamily shows a huge role in processing the medication and its clearance in the liver. In this manner, the restraint of cytochrome P450 isoforms may influence the medication digestion and lift the toxicity level.[14,15]

The SMILE pattern of the Chemical structure was used as an input to the admetSAR software to predict and the data. By virtue of negative retention, circulation, digestion and end (ADME) boundaries, the vast majority of the potential helpful specialists neglect to arrive at the center. ADMET examination and medication likeliness *In-silico* investigation of medication similarity was acted to check the potential 1E3K and 1ERE ligands for their capacity to keep Lipinski's guideline of five [16-17]. [16,17]Web-based program Molinspiration was utilized to explain the medication likeliness by computing the ligand sub-atomic properties. The synthetic designs of potential ligands were transferred to admetSAR server for *in-silico* forecast of ingestion, dispersion, digestion, discharge and poisonousness (ADME-Tox) properties.

In addition to it, online server Osiris Property Explorer was used to predict the tumorigenic, reproductive, and mutagenic risks. From all these parameters, it was found that compound PLN exhibits significant ADME properties in acceptable range.For evaluating the drug likeness, Molinspiration property engine v2018.10 was used to calculate it and parameters have been given below:

miLogP	1.75
TPSA	54.37
natoms	14
MW	190.20
nON	3
nOHNH	1
nviolations	0
nrotb	0
volume	169.38

Conclusions

The recent manuscript has been found to be an *in-silico* way to deal with explore PLN capability as a fertile prophylactic specialist. The docking scores of PLN uncovered more restricting liking towards applicable receptors as compared to the standard adversaries. The limiting binding site of the ligand was reconfirmed by definition of the sub-atomic elements reproduction of the PLN-receptor docked complex while all over again ligand configuration recommended expected subordinates of the ligand applying antifertility potential. The compound displayed more hydrogen holding towards progesterone and estrogen receptor than the norms. Besides, the ADMET investigation uncovered that PLN effectively cleared the filter of Lipinsky's Standard of Five and showed unimportant poisonousness like cardiovascular harmfulness, atomic receptor harmfulness and natural harmfulness. Hence this research paper proposes that PLN envelops sufficient potential to be uncovered as an antifertility specialist and be effectively utilized alone or in blend with other helpful specialists.

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Conflicts of Interest
"The authors declare no conflict of interest."

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