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Research Article

Structure Based Virtual Docking and Molecular Dynamics Guided Identification of Potential Phytoconstituents from Traditionally Used Female Antifertility Plant

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Abstract

Background: Oral contraceptives are very widely used agents to check unwanted pregnancies. They contain synthetic analogues of estrogen and progesterone hormones. Estrogen is an important hormone that plays a significant role in menstrual cycle, ovulation, fertilization and implantation. Estrogen receptor α (ERα) can modulate the ovulation, fertilization or receptivity of the uterus. Oral contraceptives pose mild to severe adverse effects such as menstrual cycle disorders, metabolic alterations and increased risk of cancers. It is essential to identify and screen alternative contraceptives that are safer to use. The present study was aimed at identifying the compounds from *Cissampelos pareira* that is traditionally used for antifertility activity.

Methods: The compounds reported from the plant were collected and prepared using the LigPrep wizard. The protein, ERα was selected from protein data bank (1G5O) and prepared. The ligands were docked with the protein and the hits were selected for further screening of free energy calculation, induced fit docking and molecular dynamics simulations based on the respective scores and various interactions.

Results: Among various compounds, Coclaurine and Norruffscine have been identified to interact with ERα and possess similar interactions as that of the endogenous ligand, estradiol. The compounds also showed drug-like properties in Qikprop analysis and promising result in the molecular dynamics simulation studies.

Conclusion: Considering the dock scores, molecular interactions with the ERα receptor and energy calculations, the compounds Coclaurine and Norruffscine were found to have good binding properties. Further *in vitro* and *in vivo* evaluations are warranted for confirmation.

Introduction

With an increase in population at an alarming rate, the effects can be seen in various sectors like healthcare, employment, sanitation, environment, housing, etc. Use of contraceptives as a measure to check unnecessary pregnancies is also increasing as couples prefer nuclear families. With growing knowledge about the intricate pathways involved in reproductive physiology and better understanding about the role of various hormones in the process of fertilization, newer antifertility agents are being developed. Although there are many interventions for contraception like use of male and female condoms, injectable contraceptives, intrauterine devices etc, one of the most widely used birth control methods is the use of oral contraceptive pills.[1] In females, they inhibit ovulation or fertilization process. Combined oral contraceptive pills mainly contain synthetic estrogen and progesterone hormonal analogues at varying ratios.[2] Although they are considered as one of the most effective ways of birth control, there are many adverse effects relating to cardiovascular system, menstrual cycle irregularities, metabolism, increasing the risk of cancers, etc.[3] Hence, there is a need for search of safer and effective antifertility agents with reversible effects.[4] During the last few decades, value of Asian traditional medicinal system has been credited and there is an increase in the research on traditional medicine. India has a wide practice of herbalism since ages and there is an extensive research now being performed to scientifically evaluate the medicinal activity of the plants that have been a part of traditional medicine. In the field of antifertility research, several animal studies have shown anti-zygotic, blastocystotoxic, anti-implantation and

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abortifacient activities with some plant extracts.[5] They were also found to have a reversible activity prompting their safe use in female reproductive cycle.[6] A folklore plant that is being traditionally used to check pregnancy is *Cissampelos pareira*.[7,8] To validate the use of this plant as an antifertility agent, a study was performed on methanolic extract of the plant. The extracts have shown potent antifertility activity.[9] Therefore, the main objective of the present study is to identify the compounds of the plant that are responsible for the activity. As a part of this, an attempt has been made to screen a dataset of the reported compounds of the plant for their agonistic activity with estrogen receptor α (ERα). ERα is mainly present in the theca cells of ovaries and in the uterus. ERα is considered one of the most important receptors that play a pivotal role in menstrual cycle, fertility and pregnancy in females.[10]

An extensive literature search has been carried out to create a dataset of all the reported compounds from the plant *Cissampelos pareira*. Ligands and the estrogen receptor α protein (PDB ID: 1G50) were prepared with necessary corrections for carrying out docking. Molecular mechanics energies generalized Born and surface area continuum Solvation, Molecular Mechanics-Generalized Born Surface Area (MM/GBSA) and induced fit docking (IFD) were performed and the hits were selected based on the scores and pivotal interactions with the receptor protein. ADME parameters were analysed and the hits were taken forward for molecular dynamics (MD) study. Best compounds, based on the interactions are reported.

Methods

All the *in silico* studies have been performed in Maestro version 11.4, a commercial software of Schrodinger Inc.[11,12] Grid-based Ligand Docking with Energetics (GLIDE), MM-GBSA, IFD, molecular dynamics simulation by Desmond and ADME analysis by Qikprop were executed on a HP computer supported by Linux Ubuntu 18.04.1 LTS platform, Intel Haswell graphics, 8 GB RAM and Intel Core i3-4160 processor.

Protein preparation and receptor grid generation

Use of appropriate protein structure is an important step in docking. X-ray crystal structure of human ERα co-crystallized with the agonist 17β-estradiol ($C_{18}H_{24}O_2$), 1G50 was retrieved from RCSB Protein Data Bank (PDB) into Maestro.[13] 17β-estradiol is a naturally occurring estrogen and has a very high affinity for the estrogen receptors α and β. The crystal structure 1G50 is of 2.9 Å consisting of trimer with 3 chains, A, B and C each bound to the ligand. The chains were separated, and only chain A was used for the study. Protein was prepared using Protein Preparation Wizard (PPW) tool involving the steps i. Import and refine ii. Review and modify iii. Minimization.[14,15] Missing residues and side chains were filled, water molecules beyond 5 Å were deleted, hydrogens were added in order to define correct tautomeric states and ionization of amino acid residues. Minimization was done with the default constraint of 0.3 Å of root mean square deviation (RMSD). Receptor grid was generated by grid generation panel around the active site of the protein where the ligand is bound using Glide.

Ligand library and preparation

Literature was thoroughly explored to extract all the reported compounds from the plant *Cissampelos pareira*.[16–21] A library of 37 identified compounds was made and used for screening. The standard agonist, 17β-estradiol was taken as standard and prepared in the same way as ligands by Ligprep tool. For good representation of the ligands during protein-ligand interactions, the ligands were prepared with all the necessary conditions like modification of torsions, assigning an appropriate protonation state using the 'Ligprep' tool.[12] Optimized potentials for liquid simulations (OPLS 3e) force field was used for producing lowest energy state 3D conformation of compounds with all the required corrections. Some of these compounds satisfy the conditions of ER agonists and can therefore be probable ER agonists.[10]

Molecular docking

To identify the binding affinity of the ligands, molecular docking was performed by using GLIDE tool. The library of compounds was docked against ERα (PDB id, 1G50). All the compounds were docked using extra precision (XP) which uses descriptor and explicit water technology.[22,23] As the ligands were prepared using Ligprep, Epik score penalties were added to avoid any binding mode discrimination. All the ligands exceeding 300 atoms and 50 rotatable bonds were excluded from scoring. All other default settings including scaling factor of 0.8 Van der Waal's radii were followed. XP scoring is more reliable as it uses explicit water technology and descriptors.

Free binding energy calculation

Although poses were generated using the extra precision docking, to understand how stable the ligand-protein interaction is, MM-GBSA was run in triplicates. This panel calculates a number of properties including the ligand binding energies and strain energies of the selected ligands with the desired protein.[24] Default harmonic potential of 1.0 kcal mol^{-1} $Å^{-2}$ was used for constraining the flexibility of the residues. Solvation model VSGB and OPLS3e forcefield were used with water as solvent.[25] The binding free energy

$$ÄG\ bind = G\ complex - (G\ protein + G\ ligand) \quad \textbf{Eq. (1)}$$

of ERα-ligands' complexes were calculated by the formula: where, G= MME (molecular mechanics energy) + GSGB (SGB salvation model for polar solvation) + GNP (nonpolar solvation).

Induced fit docking

IFD was performed in order to take into account the protein flexibility when ligand binds to it. IFD functions

with a combination of GLIDE rigid docking and prime.[26] This 3-step tool was followed to predict the cluster of ligands binding with flexible receptor based on scoring. Limit for the number of ligands poses was set to 20. Residue refinement was performed within a distance of 5Å from the ligand conformation. Scores were calculated after docking with the induced-fit protein structure and scores were used to predict the best ligand molecules. The IFD score was calculated as following:

$$IFD\ Score = 1.0 \times Prime\_Energy + 9.057 \times GLIDE\ Score + 1.428 \times GLIDE\_Ecoul \quad \text{Eq. (2)}$$

Validation of docking protocol

Validation was performed by redocking 17β-estradiol, the co-crystallized ligand into the protein 1G50 to obtain the RMSD. Docking was performed on the grid generated in the protein by superimposing and aligning the ligand with the co-crystallized ligand.

ADME analysis

After identifying the top hits by molecular docking studies, *in silico* ADME properties were predicted using QikProp tool, version 5.4 of Maestro.[27] Various kinetic properties helped us predict if the molecules could act as drugs and if they posed any toxicity. Pharmacokinetic descriptors like molecular weight, hydrogen bond donor (HB^d), hydrogen bond acceptor (HB^a) along with predicted properties like octanol/water partition coefficient (QPlogPo/w), IC_{50} value for blockage of HERG K^+ channels (QPlogHERG), aqueous solubility (QlogS), apparent Caco-2 cell permeability (QPPCaco), human oral absorption (HOA), predicted permeability through blood-brain barrier (QPlog BB), Van der Waals surface area of polar nitrogen and oxygen atoms and carbonyl carbon atoms (PSA) and number of violations of Lipinski's rule of five (RoF) were calculated using multi-regression model.

Molecular dynamics simulation studies

To predict the interactions and binding stability accurately within the physiological conditions, MD simulation studies were carried out. Hits based on docking score, MM-GBSA values, IFD score and important interactions with the receptor were selected for molecular dynamics simulation using Desmond software that uses OPLS3e forcefield.[28,29] This involves three-steps: (a) system builder, (b) minimization (c) molecular dynamics. The system used for MD simulation comprises of the ERα protein molecule complexed with selected hit ligands, explicit SPC solvent, and counter ions to neutralize system. The input files for MD simulations were the protein-ligand complex structures obtained after IFD docking. In the system builder, in order to reduce the excessive solvent volume, an orthorhombic box shape was selected as boundary with a buffer distance of 10 Å. Sodium and chloride ions were used to neutralize the charges. Minimization was carried out to in order to comfortably align the structure of protein within the simulation boundaries. Loading the model system (.cms file) into the molecular dynamics panel, the simulation was performed for 20ns with the ensemble class NPT at 300 K temperature and 1 bar pressure. The stability is determined by RMSD which is measured as an average change in the displacement of selected atoms of a defined frame with respect to the reference frame and calculated by the formula:

$$RMSD_X = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(r'_i(t_x)) - r_i(t_{ref}))^2} \quad \text{Eq. (3)}$$

Where, N is the number of atoms in the atom selection; t_{ref} is the reference time and r' is the position of the selected atoms in frame x

Results and Discussion

Grid-based molecular docking

The library of all compounds was initially docked using XP mode of GLIDE with the prepared protein 1G50 in which the grid was generated based on the coordinates of the crystallized ligand. Screening of the ligand library resulted in the generation of docking score for 07 compounds. Rest of the compounds did not show any binding with the protein and therefore no score was assigned. Table 1 shows dock score of the compounds along with the co-crystallized standard ligand 17β-estradiol. Docking generates ligand poses which is a combination of position along with the orientation and different conformations. Only the poses that pass this stage enter the latter stage after which the final scoring was assigned to the energy minimized poses. Docking score of the co-crystallized ligand, 17β-estradiol was -11.964 kcal/mol. Ligand interaction diagram of 17β-estradiol in XP docking has shown π-π stacking interaction at PHE404, H-bond interactions at ARG394, GLU353, HIE524 and hydrophobic interactions at LEU391, MET388, LEU387, LEU384, TRP383, ALA350, LEU349, THR347, LEU346, MET343, LEU428, PHE425, ILE424, MET421, GLY521, LEU525, MET528. The XP dock scores for the ligands ranged between -10.576 and -4.845Kcal/mol. Coclaurine showed the highest XP docking score. The hydrophobic interactions, MET343, LEU346, LEU349, ALA350, LEU387, MET388, MET421, ILE424, LEU525 were found to be common in the ligands and 17β-estradiol. π-π stacking interaction, which is an important interaction with 17β-estradiol was found in Bulbocapnine, Corytuberine, Magnocurarine, Norruffscine, Coclaurine and Pareirine.

Validation of docking protocol

The docking protocol was validated by docking 17β-estradiol, the co-crystallised ligand with ERα and an RMS deviation of 0.1665 was observed, optimizing the docking accuracy. The redocked ligand and co-crystallized ligands were superimposed as shown in Figure 1.

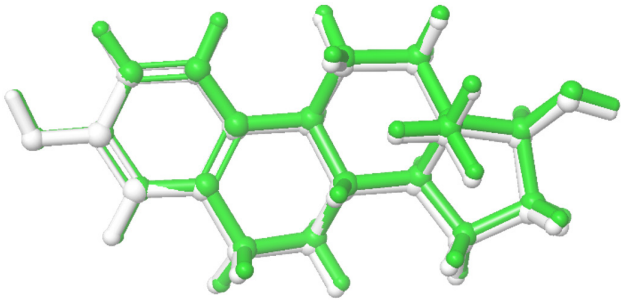


Figure 1. Superimposition of docked 17β-estradiol and co-crystallized ligand of ERα for validating the of docking protocol. Observed RMSD was 0.1665.

Free ligand binding energy calculation

Top 8 compounds obtained from XP docking were subjected to free ligand energy calculation (ΔG) using Prime MM-GBSA. The co-crystallized drug, 17β-estradiol showed ΔG binding energy of -78.41 Kcal/mol and Norruffscine showed a highest ΔG of -43.45 Kcal/mol among the ligands. The energy of the uncomplexed ERα was observed to be -9969.729 kcal/mol when calculated in triplicate in MMGBSA. Similarly, the energy of the selected free ligands and the protein-ligand complexes have been tabulated in the Table 2. The compounds Pareirubrine B, Grandirubrine, Oblongine and Bulbocapnine did not show stability in the docked poses and their binding energies were found to be positive.

Induced fit docking

Induced fit docking was performed on top 3 compounds based on docking score, MM-GBSA score and important interactions. IFD was performed to obtain accurate ligand structures at the binding pocket which cannot be generated in the XP docking where they are docked with a rigid protein. This is because many target proteins are not rigid in the biological system and undergo conformational changes to accommodate the ligand molecule in the binding pocket. This conformational change in protein structure is otherwise not considered during the XP docking. As a number of conformers are generated in IFD, it is possible to obtain most accurate binding structure. In case of virtual docking, since only one conformation of a compound is screened, the result pertaining to the compounds other than the hits could be false negative and might result in losing a potential compound. Therefore, to avoid this, IFD was used to generate different conformers for a single ligand and screened. IFD score and the interactions were further analysed. The IFD scores with 2D interaction diagrams are shown in Table 3 and the 3D IFD interactions are shown in Figure 2.

Table 1. Docking score (kcal/mol) of the hit ligands along with the standard 17β estradiol.

No.	Compound	Docking Score
1	17β estradiol	-11.964
2	Coclaurine	-10.576
3	Corytuberine	-9.714
4	Norruffscine	-8.313
5	Pareirubrine B	-7.78
6	Grandirubrine	-7.49
7	Pareitropone	-7.11
8	Lineolic acid	-7.011

ADME analysis

ADME analysis was performed for three compounds, Coclaurine, Norruffscine and Pareitropone using Qikprop tool. Properties analysed for three ligands are summarized in Table 4. The recommended values are also given in the table for comparison. All the descriptors of three compounds fall well within the recommended values. Caco-2 cells are a model for the gut-blood barrier and the compounds Norruffscine and Pareitropone showed great Caco cell permeability. All the three compounds showed good oral absorption. Also, all the compounds obey Lipinski's rule of five and therefore can be considered 'drug-like'.

Molecular dynamics simulation studies

Although induced fit docking was performed, it does not provide an in detail intrinsic flexibility of the receptor and explicit water treatment. These can be well understood in molecular dynamics studies wherein the exact biological conditions are simulated. This enables us to study the protein-ligand interactions in detail. MD simulation was carried out on three top molecules based on docking scores, binding energies, IFD scores and interactions.

Throughout the simulation, RMSD of the protein and the ligand was monitored. 1000 frames, each pertaining to

Table 2. Table representing the binding free energy which was calculated from the receptor free energy, ligand free energy and the free energy of protein-ligand complex.

No.	Compound	MMGBSA dG Bind±SD (kcal/mol)	Receptor Energy±SD (kcal/mol)	Complex Energy±SD (kcal/mol)	Ligand Energy±SD (kcal/mol)
1	17β estradiol	-78.43±0.023	-9969.729±0.00	-10048±0.002	0.199±0.020
2	Coclaurine	-9.67±1.100	-9969.729±0.00	-9934.62±0.008	44.779±1.105
3	Corytuberine	-38.1±0.078	-9969.729±0.00	-10021.8±0.047	-13.976±0.030
4	Norruffscine	-43.42±0.037	-9969.729±0.00	-9973.59±0.000	39.560±0.040
5	Pareirubrine B	1.39±0.121	-9969.729±0.00	-9942.13±0.003	26.212±0.120
6	Grandirubrine	29.75±0.127	-9969.729±0.00	-9883.32±0.001	56.651±0.131
7	Pareitropone	-29.99±0.058	-9969.729±0.00	-9970.12±0.003	29.600±0.060
8	Lineolic acid	-11.70±0.724	-9969.729±0.00	-10015.3±0.052	-33.894±0.673

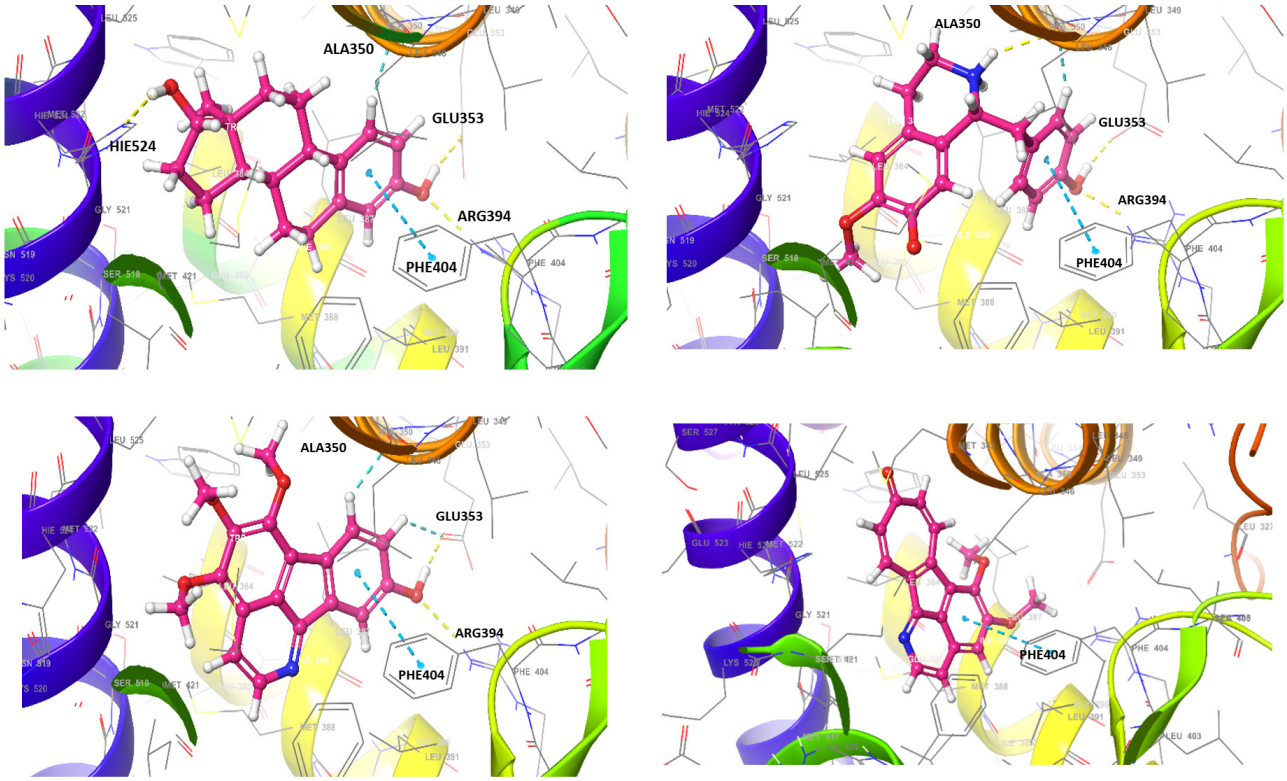


Figure 2. 3D illustration of interactions by Induced fit docking (IFD) captured from the IFD tool of Maestro module. Top left: 17β-estradiol, top right: Coclaurine, Bottom left: Norruffscine and bottom right: Pareitropone.

Table 3. 2D interactions of the top four compounds with IFD scores.

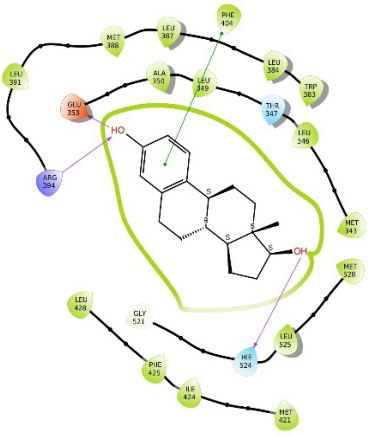
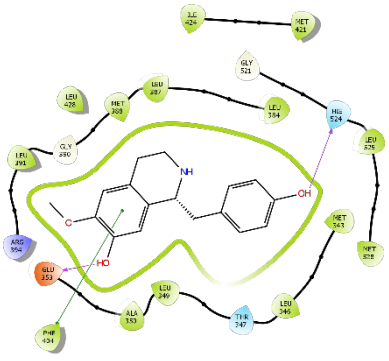
Compound	2D Interaction	Non-bonding interactions		IFD score
17β estradiol		H-bond ARG394, GLU353, HIE524	Hydrophobic bonding MET343, LEU346, THR347, LEU349, ALA350, GLU353, TRP383, LEU384, LEU387, MET388, ARG394, MET421, ILE424, PHE425, LEU428, GLY521, LEU525, MET528. Polar interaction THR347, HIE524 Charged Positive ARG394 Charged Negative GLU353 π- π stacking PHE404	-517.8
Coclaurine		H-bond GLU353, HIE524	Hydrophobic bonding MET343, LEU346, LEU349, ALA350, LEU384, LEU387, MET388, LEU391, MET421, ILE424, LEU525, MET528 Polar interaction THR347, HIE524 Charged Positive ARG394 Charged Negative GLU353 π- π stacking PHE404	-517.1

Table 3. Continued.

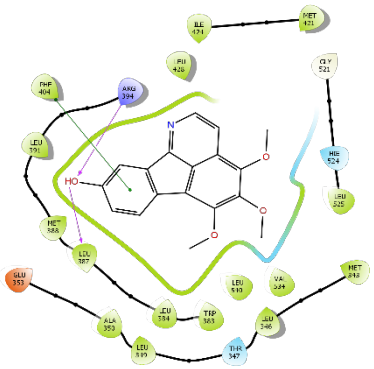
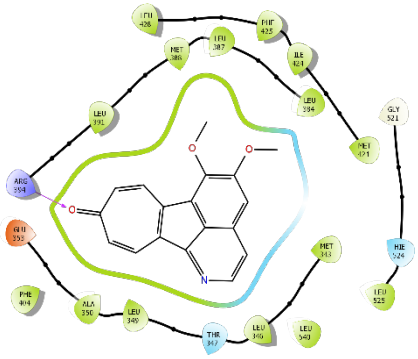
Norruffscine		H-bond ARG394, LEU387	Hydrophobic bonding MET343, LEU346, LEU349, ALA350, TRP383, LEU384, LEU387, MET388, LEU391, MET421, ILE424, LEU428, LEU525, VAL534, LEU540 Polar interaction THR347, HIE524 Charged Positive ARG394 Charged Negative GLU353 π- π stacking PHE404	-514.3
Pareitropone		H-bond ARG394	Hydrophobic bonding MET343, LEU346, LEU349, ALA350, LEU387, MET388, LEU391, PHE404, MET421, ILE424, PHE425, LEU428, LEU525, LEU540 Polar interaction THR347, HIE524 Charged Negative GLU353	-512.8

Table 4. ADME properties by Qikprop for top 3 compounds with recommended values in parenthesis.

Compound	MW (130.0–725.0)	HBD (0.0–6.0)	HBA 2.0–20.0	QPlog Po/w (-2.0–6.5)	QPlog HERG (Concern below -5)	QPlogS (-6.5–0.5)	QPP Caco <25 poor, >500 great	QPlog BB (-3.0 –1.2)	HOA (1-low, 2-medium, 3-high)	PSA (7.0–200.0)	RoF (Max. 4)
Coclaurine	285.3	3	3.75	2.046	-5.362	-3.05	159.98	-0.454	3	65.4	0
Norruffscine	309.3	1	4	3.22	-4.73	-4.857	1940.983	-0.339	3	57.6	0
Pareitropone	291.3	0	4.5	2.661	-4.451	-3.891	1893.071	-0.213	3	55.6	0

MW: Molecular weight; HBD: hydrogen bond donor; HBA: hydrogen bond acceptor; QlogPo/w: Predicted octanol/water partition coefficient; QPlogHERG: Predicted IC_{50} value for blockage of HERG Kþ channels; QPlogS: Predicted aqueous solubility, QPPCaco: Predicted apparent Caco-2 cell permeability in nm/sec; QPlog BB: Predicted permeability through blood-brain barrier: %HOA: Human oral absorption. Predicted human oral absorption on a 0–100% scale. The prediction is based on a quantitative multiple linear regression model; PSA: Van der Waals surface area of polar nitrogen and oxygen atoms and carbonyl carbon atoms; RoF: Number of violations of Lipinski's rule rule of five.

every 20 ps were captured during the 20 ns simulation. All the frames, saved as a trajectory gives us insights into the structural conformation during the interaction. This also indicates the stability of the interaction along with the information as of whether the simulation has been equilibrated. Coclaurine, Norruffscine and Pareitropone were taken forward for MD simulation as they have shown better docking scores, found to be stable in the MM-GBSA and did not violate the recommended ADME values. Further, no toxicity was found with the compounds. Figure 3 shows the MD trajectories and RMSD of both receptor and ligand for both the compounds.

In general, for a ligand to interact well with an ER, it should possess two OH groups linked by a lipophilic central scaffold placing them at a distance of about 11 Å. Among the two OH groups, at least one must be a phenol or a phenol-bioisostere.[10]

MD Simulation report also gives us various protein interactions with ligand throughout the simulation time. Pareitropone did not show prominent interactions with the receptor. It could not retain the bond at PHE404 but only had a H-bond and a water bridge at HIS524 and therefore not considered a good ligand for the receptor. Norruffscine showed H-bonding with LEU349, GLU353, ARG394 and water bridges with LEU349, ALA350, GLU353, LEU387, ARG394, LEU403, PHE404. Coclaurine showed hydrogen bonding with GLU353, HIS524 and water bridges with LEU349, GLU353, LEU387, ARG394, PHE404. In both the compounds, protein-ligand contact with PHE404, which was found to be an essential interaction for the

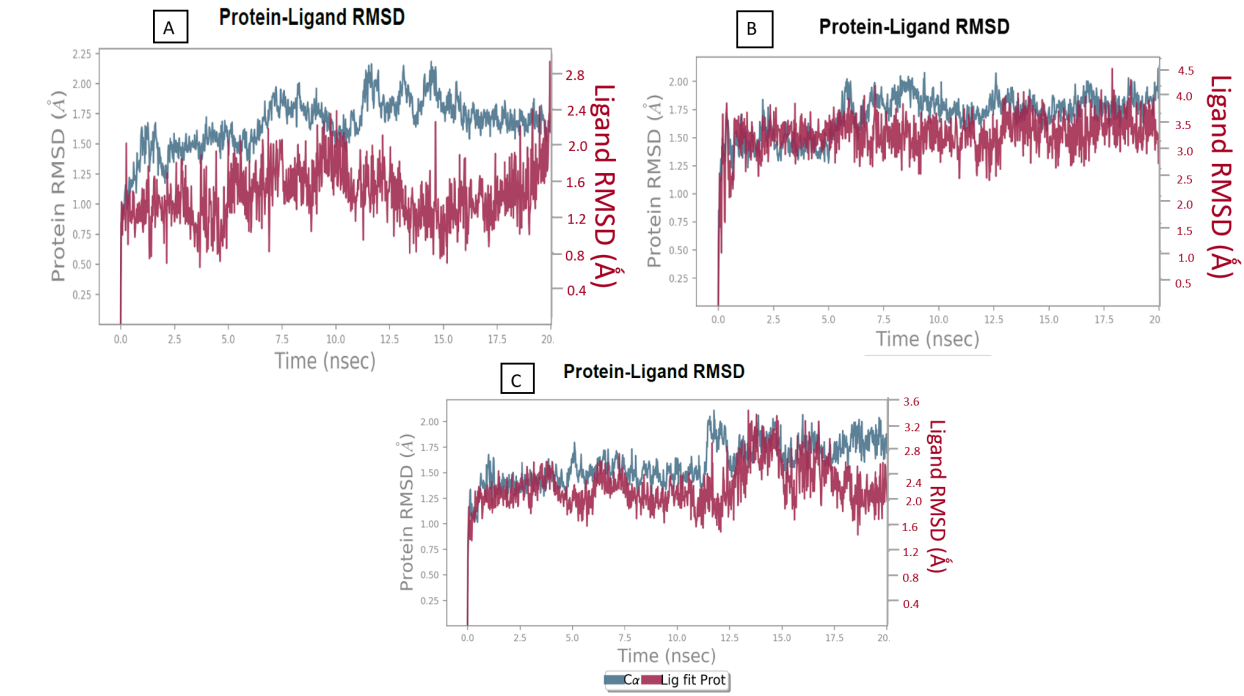


Figure 3. Plots showing RMSD of ERα with the ligands obtained from the Desmond tool for performing molecular dynamics simulation. (A) Coclaurine, (B) Norruffscine and (C) Pareitropone from top to bottom respectively.

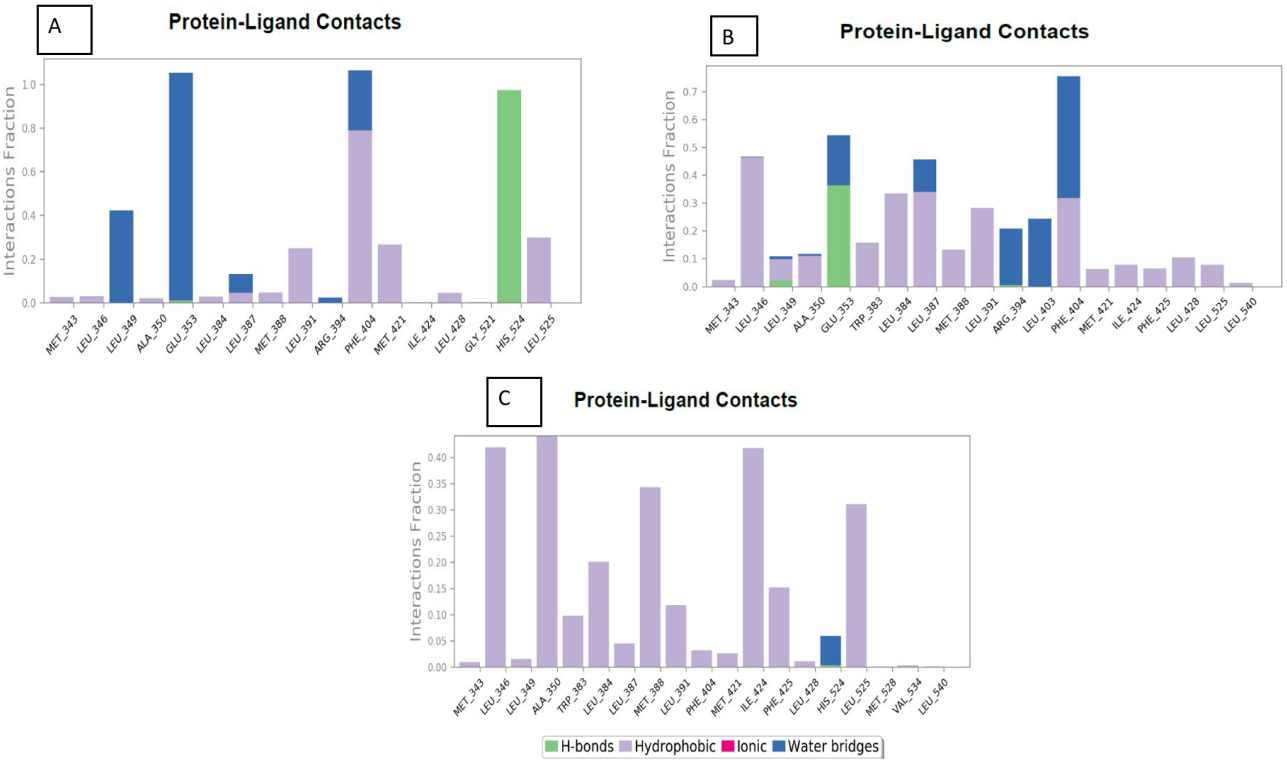


Figure 4. Protein-ligand interaction diagrams obtained after performing molecular dynamics simulations using Desmond tool. Interaction of (A) Coclaurine (B) Norruffscine and (C) Pareitropone with estrogen receptor α at different residues with estrogen receptor α at different residues. Stacked bar chart indicates interactions at various residues with respect to the % of simulation time. Values over 1.0 are due to multiple interactions of same subtype of the residues with the ligand. Interactions with the pivotal residues of estrogen receptor α ligand binding pocket, LEU384 and MET421 were found with both Coclaurine and Norruffscine.

ERα-ligand was found to be present for more than 70% of the simulation time. It was observed that overall, the protein-ligand interactions remained common in both XP docking and MD simulation. Exceptions were that with Norruffscine, an extra hydrogen bond at LEU349 and additional water bridges were observed. In case of Coclaurine, hydrogen bond at LEU346 was lost in the MD simulation. All the MD receptor-ligand contacts are shown

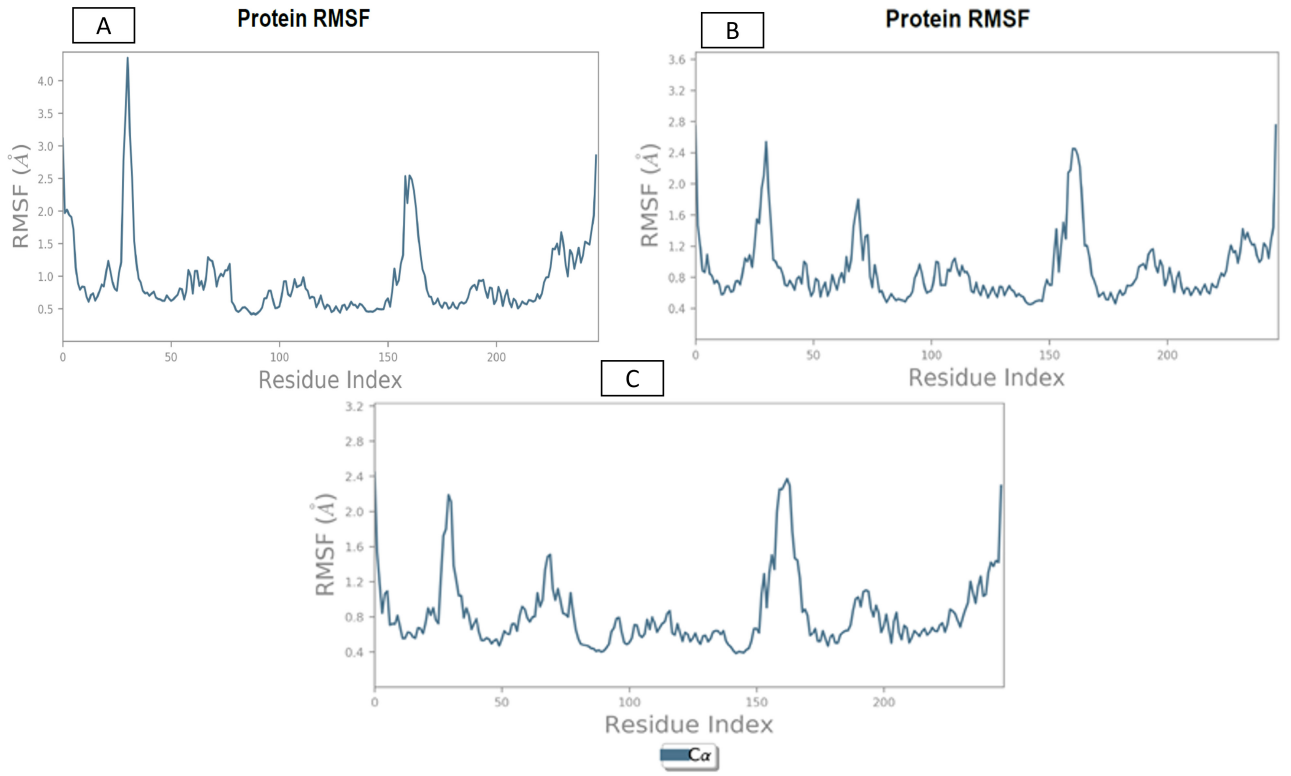


Figure 5. RMSF plots depicting fluctuations in the secondary structure of protein after different ligand binding obtained from Desmond tool after performing molecular dynamics simulations. a. RMSF Plot of protein during binding with Coclaurine b. RMSF Plot of protein during binding with Norruffscine c. RMSF Plot of protein during binding with Pareitropone.

in Figure 4. Also, as stated earlier about the presence of OH groups as an ideal structural requirement for a compound to act as ER ligand, one of these OH groups is supposed to form a strong attractive interaction with H-bond network comprising the residues Glu353/305, Arg394/346 and a water molecule, where the phenolic OH group of the A-ring of estradiol binds. In the MD simulation, H-bond and a water bridge at both GLU353 and ARG394 were observed with Norruffscine and an H-bond at GLU353 and a water bridge at ARG394 were found with Coclaurine. In addition, hydrophobic interactions were found at important positions in ligand binding cavities, LEU384 and MET421 with both Norruffscine and Coclaurine.

In addition, after 12.5 ns, the bond at LEU349 with Coclaurine has been lost which was evident in the RMSD plot but it was re-established by the end of 19 ns after which the RMSD has been stabilised. In case of Norruffscine, the RMSD was almost stable throughout the simulation except for around 1 to 1.5 ns after 10.5 and 17.5 ns. The RMSD was stable and did not show any significant deviations in case of Pareitropone. However, the number of interactions for Pareitropone were not many.

The Root Mean Square Fluctuation (RMSF) plots of the protein bound to all the selected hit ligands showed stable protein structure with slight fluctuations during entire molecular simulation study. The Estrogen receptor α protein bound to Coclaurine showed 66% of alpha helices and 3.22% of beta strand structure. Protein bound to Norruffscine comprised of 65.40% of alpha helices and 2.78% of beta strand. Similarly, protein with Pareitropone contained 65.09% of alpha helices and 3.04% of beta stand. ERα protein bound to Coclaurine molecule showed a maximum RMSF up to 4Å majorly due to the changes in the amino acid residues ranging from 25-35. However, the amino acid residues from 25-35 showed least fluctuations in the ERα protein bound to Norruffscine with fluctuation of 2.6 Å and Pareitropone with an RMSF 2.2 Å. Similarly, amino acid residues ranging from 60-80 of ERα protein showed fluctuation of 1.2 Å in protein bound to Coclaurine and around 1.6 Å in protein bound to both Norruffscine and Pareitropone as observed in Figure 5.

Conclusion

Estrogen receptor α plays a significant role in menstrual cycle, fertilization and pregnancy in females. Estrogen can modulate the ovulation process and hence estrogen receptor is found to be an important target for the agents that alter the ovulation process. One of the targets that all the combined oral contraceptives present in the market have are the estrogen receptors. Although oral contraceptive use becomes inevitable at some point of fertile age in a female's lifespan, they pose mild to severe adverse effects such as menstrual cycle disorders, metabolic alterations and increased risk of cancers. It is necessary to find safer alternatives that are effective and elicit reversible actions. The present study was aimed at identifying the compounds from *Cissampelos pareira* that is being traditionally used for antifertility activity. Considering the dock scores,

molecular interactions and energy calculations, the compounds Coclaurine and Norruffscine were found to possess good binding properties and interactions with the Estrogen receptor α. Further *in vitro* and *in vivo* studies are required to validate their activity and screen for any possible interactions with other proteins.

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Author Contributions

KP: Ideation of the concept, Literature search, designing the study, data analysis and interpretation, manuscript drafting. SM: Designing the study, data analysis and interpretation and manuscript editing. RS: Data analysis and manuscript editing. MMS & UVB: Review of manuscript. KSRP: Data analysis and review of manuscript.

Conflict of Interest

The authors report no conflicts of interest.

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