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Brief Communication

Pharmacoinformatics study of Piperolactam A from *Piper betle* root as new lead for non steroidal anti fertility drug development

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ABSTRACT

Fertility control is a burning problem all over the world to regulate population overflow and maintain ecological balance. This study is an *in-silico* approach to explore a non-steroidal lead as contraceptive agent in order to avoid several contraindications generated by steroidal analogues. Piperolactam A, an aristolactam isolated from *Piper betle* Linn. showed binding affinity towards estrogen and progesterone receptor as −8.9 and −9.0 Kcal/mol (inhibition constant K_i = 0.294 μM and 0.249 μM) respectively which is even larger than that of reported antagonists such as Rohitukine and OrgC (binding affinity −8.7 and −8.4 Kcal/mol; K_i 0.443 μM and 0.685 μM respectively). The binding site exploration displayed more hydrogen bonding of Piperolactam A (His 524, Leu 346, Thr 347) than Rohitukine and OrgC (Leu 718) with associated receptors which was further confirmed by molecular dynamics simulations. The drug-likeliness of the compound has been proved from its tally with Lipinsky's Rule of Five and lowered toxicity such as cardiac toxicity, liver toxicity, mutagenicity and ecological toxicity. Endocrine disruptome and later docking guided molecular simulations revealed that Piperolactam A has weaker binding affinity and/or lower probability of binding with nuclear receptors especially hERG and cytochrome P450. The high Caco-2 permeability suggested more bioavailability hence more therapeutic efficacy of the drug.

1. Introduction

In recent days, fertility control is an issue of global public health concern. Gradually increasing growth rate of the world's population gives broad impact on environment, economic growth, that's why family planning now turns in global need (Whitehead and Nussey, 2001; Azmeera et al., 2012). To find out new non-steroidal contraceptive compound, the World Health Organization (WHO) has planned for fertility regulation (Kar, 2014). As hormonal contraceptives have been found to be more hazardous to health, long term use of the same may increase the chance of venous thromboembolism (including deep vein thrombosis [DVT] and pulmonary embolism) (Angeles and Manuel, 2010; Rang Humphrey et al., 2012; Kanis et al., 2013) especially in case of women elder than 35. Furthermore, estrogen can decrease levels of biological amines-serotonin, thereby causing depression (Kulkarni and Jayashri, 2005). In fact, steroidal contraceptives deplete vitamin B_6 level in the body which is a cofactor for enzymes which metabolize tryptophan into serotonin. Serotonin is a potential mediator in neurotransmission, thus reduction of neuronal serotonin level especially within brain, causes depression (Saarawy et al., 1982; Brogan 2013). Furthermore, withdrawal of such contraceptives causes idiopathic increase of serotonin level inside body often leading to serotonin syndrome characterized by involuntary movement, restlessness, diarrhoea, fever, muscle rigidity and so others. In addition, aminopeptidase P (AP-P), an enzyme which breaks Bradykinin, is increased due to use of Hormonal Contraceptive finally leading to hypertension (La Corte et al., 2008).

To minimize the side effects of hormonal contraceptives, various measures have been taken but there is little success (Kar, 2014). Due to serious adverse effects produced by synthetic steroidal contraceptives, attention has now been focused on non_steroidial synthetic contraceptives. As the synthetic medicines have many drawbacks, attempts have been undertaken to derive or isolate contraceptive molecules from natural origin. The anti-fertility effect of tradition herbal medicine was approved in many literatures (Bhavya et al., 2013; Abu et al., 2012).

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Earlier reports demonstrate that the leaves of *Piper betle* Linn. (belonging to the family of Piperaceae) (Cheng et al., 2012) has multitude of pharmacological effets including carminative, aphrodisiac, laxative and appetite improving effect (Periyanayagam et al., 2012; Chandra et al., 2011) Not only the leaves, but the roots also showed contraceptive action. Ethanolic extract of *Piper betle* displayed anti-fertility activity after systemic study on albino rats (Sharma et al., 2007). Thus, this plant derived compounds can be a covetable target for screening and isolation of newer generation contraceptives.

Nowadays, in biochemical research computational methods to predict protein structure and ligand-protein interaction have been successfully applied. Being a part of rational drug design, docking is used not only in the prediction of the binding orientation of ligands or drug candidates, but also in the prediction of the affinity and activity of the molecules (Shen et al., 2013). Due to binding interaction with receptor and ligands, activation or inhibition of the particular enzyme may result. By computational methods of new drug design, many ligands such as agonist and antagonist may be developed. The scope of molecular modelling (Kitchen et al., 2004; Morris et al., 2009; Trott and Olson, 2010) has allowed the search for novel non-steroidal contraceptive which has inhibitory action on estrogen receptor (Anonymous, 2011).

In our recent study, we identified a number of phytochemicals obtained from *Piper betle* root through literature survey. Then the phytochemicals were screened through *in-silico* docking analysis to find potential lead compounds for anti-fertility activity. The molecular docking tools such as AutoDock tools and AutoDockVina (The Scripps Research Institute, La Jolla, CA, USA) were used to dock all phytochemicals against 1ERE (human estrogen receptor binding with 17-β estradiol) and 1E3K (human progesterone receptor). The Piperolactams were also analyzed for their absorption, distribution, metabolism, and excretion-toxicity (ADMET) profile by using admetSAR software (http://lmmd.ecust.edu.cn:8000/) (Cheng et al., 2012). We also studied the Drug-likeness attributes by using the molinspiration softwares and the Osiris Property Explorer (http://www.organic-chemistry.org/prog/peo/) (Mirza et al., 2014).

2. Materials and methods

2.1. Materials

For computation, Chem 3D Ultra 8.0, AutoDock Tools-1.5.6 (MGLTools-1.5.6) [18], Discovery Studio 3.5 Visualizer (Accelrys, Biovia, USA), AutoDock Vina [19], admetSAR, Osiris Property Explorer(20), PyMOL (PyMOL molecular graphics system, Schrodinger, LLC) are used.

2.2. Computational methods

The Docking studies, to analyze the inhibitory action of the ligand onto the active site of the estrogenic and progesterone receptors, were divided in three steps i. Preparation of the receptor and ligand ii. Defining the binding site of the ligand inside the receptor and iii. Generating the docking score in form of binding affinity having rohitukine and OrgC as the standard antagonists against estrogen and progesterone receptors respectively.

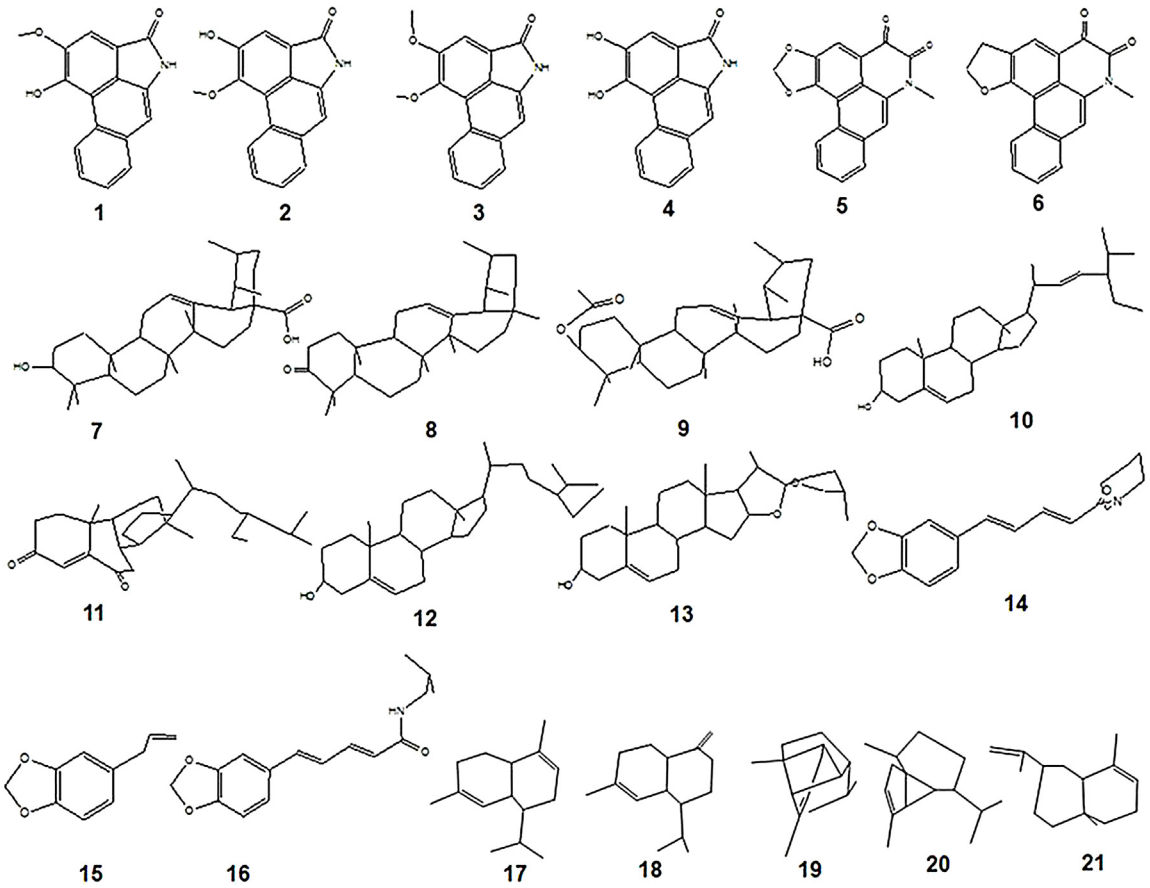

Fig 1. Chemical structure of phytochemicals having various biological activities isolated from the root of *Piper bettle*.

2.3. Optimization of the receptor and ligand

The compounds structures were drawn by Chem Draw Ultra (Fig. 1), followed by energy minimization by Chem 3D guided molecular dynamics. The compounds were saved in PDB format, reopened in AutoDock Tools-1.5.6 and saved in PDBQT format setting all the torsions and bonds freely rotable during docking.

Both the oestrogenic and progesteronic receptors were downloaded from PDB repository having PDB ID namely 1ERE and 1E3K respectively. The estrogen receptor (PDB id: 1ERE) was ligated with 17-β estradiol (Resolution 3.10 Å, R-value: 0.218) whereas the inbound ligand for progesterone receptor (PDB id: 1E3K) was found as metribolone (Resolution: 2.80 Å, R-value: 0.240). The receptors were freed from water molecules and inbound ligand by Discovery Studio 3.5 Visualizer. The ligand and water free receptor molecules were converted to PDBQT format by AutoDock Tools-1.5.6 after adding polar hydrogens to the receptors and consequently merging the non-polar hydrogens. The protein structures were energy minimized before docking and all bonds and Gasteiger charges within ligands were allowed to rotate freely during docking. Since use of Gasteiger charges may create incompleteness during docking, later kollman charges were added onto the compounds (http://sebastianraschka.com/Articles/2014_autodock_energycomps.html) and docked again obeying the same algorithm.

2.4. Defining the binding site of the ligand inside the receptor

To define the binding site inside the corresponding receptor, first the ligand bound crystalline protein structure was opened in Discovery Studio Visualizer 3.5 and the binding site was mapped by labelling with amino acids. The binding site was then covered with grid box in AutoDock Tools 1.5.6. In order to reconfirm the binding site, the optimized ligand was subjected to whole receptor docking by AutoDock Vina that uses a local search for a global optimizer as a part its optimization algorithm. The best possible conformer having maximum binding affinity was chosen as the global optimum and fitted within the receptor through script mediated ligand-fit interactions algorithm in Discovery Studio 3.5 Visualizer. The binding site atoms (in conjunction with the results within PDB derived protein crystal) were defined consequently, saved in PDB format and the corresponding grid box was assigned in AutoDock Tools-1.5.6.

2.5. Generating the docking score in form of binding affinity

The docking scores in form of binding affinity were generated by AutoDock vina. For oestrogenic receptor (1ERE), the search grid was identified as center_x = 21.6, center_y = 57.09, center_z = 88.7, and the dimension was size_x = 40, size_y = 40, size_z = 40. For progesteronic receptor (1E3 K), the search grid was set as center_x = 13.9, center_y = 14.7, center_z = 28.1, and the dimensions were size_x = 22, size_y = 20, size_z = 20. The AutoDock Vina uses machine learning approach to its scoring function, hence finds the global optimum within the search space.

2.6. Evaluating binding affinity towards other nuclear receptors

In order to evaluate any potential side effect of Piperolactam A (PLA), binding profile of the same with other nuclear receptors has been studied. For screening of nuclear receptor library, an open source software "Endocrine disruptome" has been studied (http://endocrinedisruptome.ki.si/) (Kolsek et al., 2014; Plosnik et al., 2015). Again, to reconfirm the results, two significant nuclear receptors hERG (human Ether-à-go-go Related Gene or KCNH2) and different variants of CYP450 (human cytochrome P450) have been chosen. The two receptors were subjected to docking with PLA in order to estimate the binding affinity together with binding profile with the aforementioned ligand.

2.7. Molecular dynamics (MD) simulation

The molecular dynamics (MD) provides some information of active residues which are mostly responsible for binding interaction between ligand and protein in simulated condition. The MD study together with the docking analyses is a strong scientific approach for reliable prediction of protein drug interaction (Chuang et al., 2015). So, in the present study the MD study has been performed after docking the ligand, Piperolactam A (PLA) with a human progesterone receptor (PDB: 1E3 K) and an estrogen receptor (PDB: 1ERE) to ensure the degree of reproducibility of docking results with the simulation study. The MD study has been performed in TIP3P water model (i.e, transferable inter molecular potential 3P) (Mark and Nilsson, 2001) in Desmond/Maestro using OPLS molecular mechanics force field. An orthorombic simulation cell was established with periodic boundary conditions. Here, the MD simulation each of the trajectory frames has been set up to capture the events at every 4.8 ps of time interval. The total system was neutralized by Na^+ counter ions. It was also controlled under NPT to maintain simulation volume, constant with pressure and density conditions for 10 ns at 300 K. Nose-Hoover chain thermostat and Martyna-Tobias-Klein barostat were captured at 300 K temperature and at 1.01325 bar pressure for equilibration of temperature and pressure (Pradhan et al., 2014). Finally, the stability of the protein-ligand complex was evaluated after checking the root-mean-square deviation (RMSD) and root-mean-square fluctuation (RMSF) of the protein-ligand complex (Elengoe et al., 2014).

2.8. ADMET analysis and drug-likeliness

In-silico analysis of drug likeness was performed in order to check the potential 1ERE and 1E3K ligands for their ability to follow Lipinski's rule of five (Lipinski, 2004). Online programme Molinspiration (http://www.molinspiration.com/cgi-bin/properties) was used to elucidate the drug likeliness by calculating the ligand molecular properties. The chemical structures of potential ligands were uploaded to admetSAR (http://lmmd.ecust.edu.cn:8000/) server for *in-silico* prediction of ADME-Tox (absorption, distribution, metabolism, excretion and toxicity) properties. The SMILE format of the Chemical structure of phytochemicals isolated from the root of *Piper bettle* was given as input to the admetSAR software and the data predicted from software were noted. Furthermore, online server Osiris Property Explorer (http://www.organic-chemistry.org/prog/peo/) was used to predict the tumorigenic, reproductive, and mutagenic risks.

2.9. De novo *ligand design*

The *de novo* designed molecules are generated by online *de novo* design tool, e-LEA3D (Wang et al., 2002).

3. Results and discussion

3.1. Docking analysis

Docking analysis is performed to screen a set of test compounds undergoing activity trial to select the best candidate compound employing drug-receptor interaction. Candidate compound is identified by selecting the compound with strongest binding affinity with the specific ligand. In this study, compounds reported from the extract of *Piper betle* have been docked to reveal the

Table 1
Binding affinity as calculated from AutoDock Vina along with estrogen and progesterone receptor inhibition constant for compounds isolated from *Piper betel* root.

No[a]	Estrogen receptor (PDB: 1ERE)		Progesterone receptor (PDB: 1E3K)	
	Binding Affinity, ΔG (Kcal/mol)	Inhibition constant, K_i (μM)	Binding Affinity, ΔG (Kcal/mol)	Inhibition constant, K_i (μM)
1	−8.9	0.294	−9.0	0.249
2 (Aristolactam AII)	−8.4	0.685	−8.5	0.579
3 (Aristolactam BII	−8.3	0.812	−8.3	0.812
4 (*o*-dihydoxy Piperolactam A)	−9.2	0.177	−8.5	0.579
5	−7.8	1.889	−5.9	46.824
6	Not binding	–	−6.2	28.205
7	Not binding	–	−0.4	508712.6
8	Not binding	–	−0.5	429626.1
9	Not binding	–	10.8	84186504.34 M
10	Not binding	–	−5.7	65.650
11	Not binding	–	−5.6	77.735
12	Not binding	–	−7.2	5.206
13	Not binding	–	1.3	8.994 M
14	Not binding	–	6.3	41981.515 M
15	−6.2	28.209	−6.0	39.545
16	Not binding	–	−5.6	77.735
17	−7.7	2.237	−4.9	253.681
18	−8.5	0.579	−7.9	1.595
19	−8.5	0.579	−7.6	2.648
20	−7.9	1.595	−7.8	1.889
21	−8.3	0.812	−7.7	2.237
Rohitukine	−8.7	0.413	–	–
OrgC	–	–	−8.4	0.685

No[a] = Compound number; K_i is calculated from the formula, $K_i = \exp(\Delta G^{*}1000/RT)$, where, R = 1.986 cal/mol; T = 298 K.

potential compound having best contraceptive activity. Rohitukine (Keshri et al., 2007), a potential reported estrogen antagonist has been taken as standard for estrogen receptor docking and for progesterone receptor, the standard compound has been selected as Org C (Svensson et al., 2001). Since AutoDock Vina works on Monte Carlo algorithm, the scoring function ΔG signifies the binding energy of the ligand with the receptor. Furthermore, ΔG being the Gibb's free energy for the reaction, negative ΔG suggests spontaneity of the reaction while large ΔG indicates higher binding constant for the reaction (ΔG = −RT ln K). PLA showed docking affinity as −8.9 Kcal/mol (inhibition constant, K_i = 0.294 μM) and −9.0 Kcal/Mol (K_i 0.249 μM) for estrogenic and progesterone receptor respectively whereas Aristolactam AII showed docking affinity −8.4 Kcal/mol and −8.5 Kcal/mol for the respective receptors (Table 1). Again, Aristolactam BII revealed lesser binding affinity than PLA towards both the receptors whereas *o*-dihydroxy Aristolactam AII exhibited higher binding affinity (−9.2 Kcal/mol, K_i 0.685 μM) compared to PLA towards estrogen receptor only. Interestingly, aporphine group of alkaloids showed either poor binding (compound 5) or no binding towards receptors (compound 6). Furthermore, the steroidal derivative of compounds (compound 7–13) did not reveal any binding affinity towards estrogen receptor. In contrast, volatile oils derived from *Piper betle* (compound 17–21) showed substantial binding affinity (−7.7 to −8.5 Kcal/mol) towards both the receptors, albeit lower compared to PLA. Noteworthily, PLA demonstrated higher binding affinity compared to reported estrogen and progesterone antagonists such as Rohitukine (−8.7 Kcal/mol, K_i 0.443 μM) and Org C (−8.4 Kcal/mol, K_i 0.685 μM). The 3D coordinates of PLA-1ERE and PLA-1E3 K complexes have been provided in Supplementary information.

As a proof of the concept approach and for validatation of the docking methodology, we have re-docked native inbound ligands such 17β-estradiol, metribolone with their mother receptors and subsequently aligned with original binding poses of the same. The two poses nearly coincided onto themselves suggesting accuracy of our methodology of docking (supplementary Fig. S1).

3.2. Binding residues analysis

The binding site analysis was performed by PyMOL. The study revealed that the compounds bind in the same binding pocket as the standard antagonists undertaken for the study. For example, PLA binds within estrogen receptor pocket surrounded by residues such as L525, H524, M343, I424, L346, F404, L391, R394, E353, L387, A350 (Fig. 2A) which in turn is proved to be the same binding pocket of compound 15 (Fig. 2B), 18 (Fig. 2C) and rohitukine (Fig. 2D). Similarly, PLA binding groove inside progesterone receptor is engulfed by F778, M801, L718, L715, Y890, C891, N719, M909, M756, M759, L721, Q725, L763 and R766 (Fig. 3A) which are the same residues that encompasses the binding site of Org C (Fig. 3D) together with compound 15 (Fig. 3B) and 18 (Fig. 3C). This suggests the potential of PLA to act as a contraceptive agent by inhibiting the same receptor in the same way that a reported antagonist has been revealed to.

The binding affinity of the compounds towards receptor can be explained by hydrogen bonding interaction between the compounds and the receptor. For example, rohitukine undergoes hydrogen bonding between the double bonded hydrogen of His524 and the carbonyl group of the compound (Fig. 2D) exhibiting only single hydrogen bonding for the receptor interaction. On the other hand, carbonyl group of PLA showed three hydrogen bonding interactions with His524 and Leu346 and Thr347 (Fig. 2A) suggesting stronger interaction with the receptor than rohitukine. Interestingly, compound 15 and 18 showed no hydrogen bonding with the estrogen receptor indicating lower affinity towards it (Fig. 2B and C). Even in case of progesterone receptor, the carbonyl group of PLA exploits H-bonding with Leu 718 (Fig. 3A) which is comparable with the hydrogen bonding of Org C with the Gly834 of the progesterone receptor (Fig. 3D). Interestingly again, compound 15 and 18 revealed no hydrogen bonding interaction with the progesterone receptor (Fig. 3B, 3C). Compound 18 showed only vander Waals binding with either estrogen or progesterone receptor (Fig. 4A and B). In our docking experiments we get nanomolar range binding affinities for both estrogen receptor α

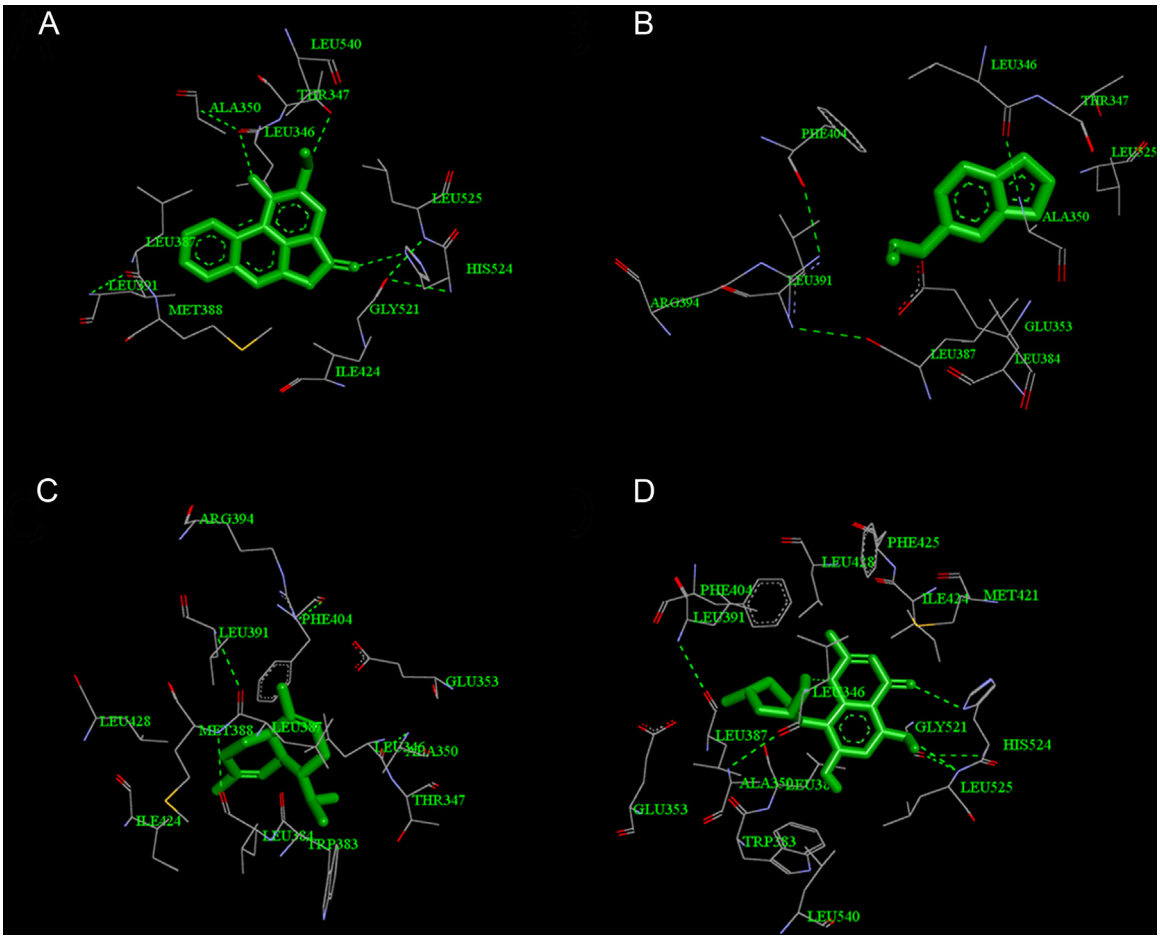

Fig. 2. Estrogen Receptor (Pdb id 1ERE) and ligand interactions: The binding residues and hydrogen bondings are represented as constructed in Discovery Studio Visualizer 3.5 (Accelrys, Biovia, USA) (A) Compound 1 (Piperolactam A), (B) Compound 15, (C) Compound 18 and (D) Rohitukine.

and β subtypes (29.4 nm) and also with the progesterone receptor (-9.0). For estrogen receptor, in our docking pose the hydroxyl group is engaged in hydrogen bonding interaction with Leu 346 residue and there is no steric hindrance found. In case of progesterone receptor we found hydrogen bonding interaction of the hydroxyl group with Leu 718 residue as well.

Why water molecules have been deleted from the protein structures prior to docking? The binding cavities of both estrogen and progesterone receptors are devoid of water molecules. If water molecules are not deeply buried into the hydrophobic pocket, they are not likely to be considered as structural water molecules. Thus, we have deleted the water molecules from the proteins otherwise they could distort the pose search. However as a confirmatory approach to investigate any such role of water molecules, we have performed docking in presence of water molecules within both the receptors. Interestingly, we have founds almost no change in the binding affinity or docking scores in this context (supplementary Fig.S2).

All of the results showed that PLA binds with the female sex hormonal receptors even firmer than reported standard antagonists. Thus, it is a plausible assumption that PLA can be used as a contraceptive agent for human physiological cycles.

In order to find out both agonistic and antagonistic binding profile of PLA with associated receptors, we took Estrogen receptor α (ERα) and Estrogen receptor β (ERβ) as two model receptors and performed subsequent analyses. The receptor conformations in the complexes of estrogen (ER) bound with the agonist and antagonists are different. Thus we have compared the docking of piperolactam A in complex with estrogen receptor in bound to agonist (pdb 1ERE, 3OLS) and antagonist (1SJ0, 1QKN). Piperolactam binds with the agonist and antagonist conformations of ERα and ERβ with variable affinities. For example, ERα agonistic and antagonistic binding affinities were noted as −8.9 and −7.9 Kcal/mol whereas ERβ agonistic and antagonistic binding affinities were noted as −8.9 and −9.2 Kcal/mol respectively. Thus, PLA can be docked to both agonist and antagonist conformation of ERα and ERβ, such that no type determinations can be made. The agonistic binding interactions with ER α and ERβ have been provided in supplementary Figs. S3 and S5 respectively, whereas antagonistic interactions with the same receptors have been provided in supplementary Fig. S4 and S6 respectively.

3.3. Molecular dynamics (MD) simulation analysis

The docked protein (PDB: 1ERE) and (PDB: 1E3K) complexes of the ligand were subjected to 10 ns MD simulation. The RMSD value of ligand with progesterone and estrogen protein complexes is depicted in Fig. 5A and B (PLA-estrogen receptor, PLA-progesterone receptor respectively), which indicates that binding interactions pattern of both complexes is similar, and up to 6 ns they show lower RMSD value. However, ligand with progesterone protein shows low RMSD value than ligand-estrogen receptor complex. For getting suggestion about the local changes along the protein chain, the RMSF values of the residues for the both complexes are shown in Fig. 6A and B (PLA-estrogen receptor, PLA-progesterone receptor respectively). The fluctuations between the side chain and

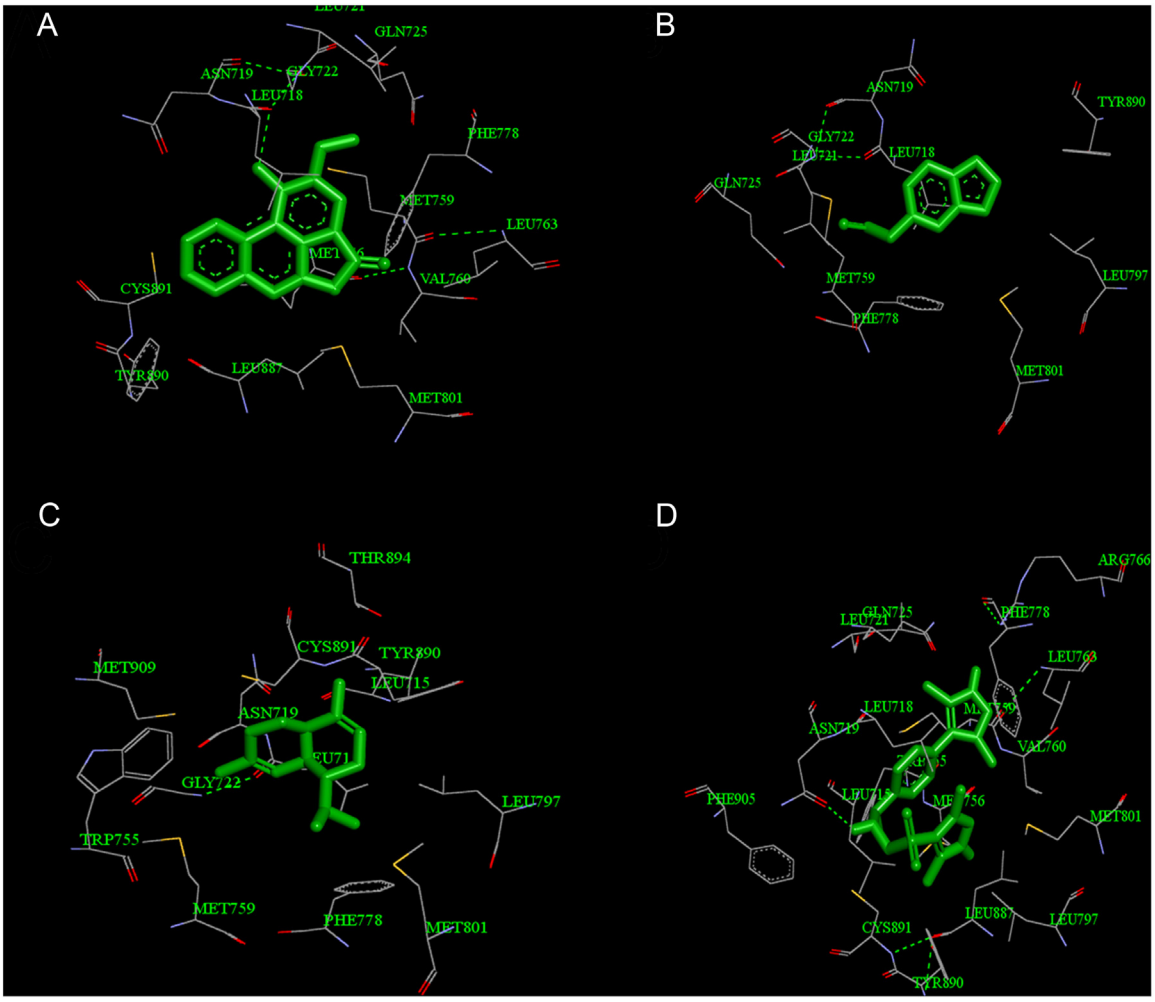

Fig. 3. Progesterone Receptor (Pdb id 1E3K) and ligand interactions: The binding residues and hydrogen bondings are represented as constructed in Discovery Studio Visualizer 3.5 (Accelrys, Biovia, USA) (A) Compound 1 (Piperolactam A), (B) Compound 15, (C) Compound 18 and (D) Org C.

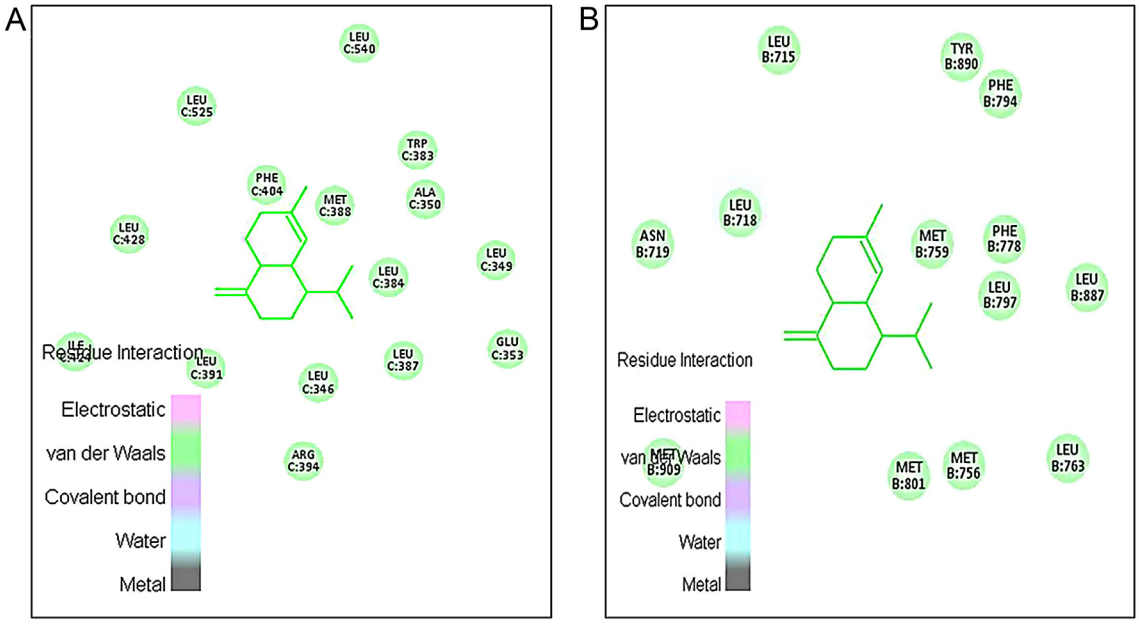

Fig. 4. 2D diagram depicting force of interactions other than hydrogen bonding. (A) Compound 18 with estrogen receptor (B) Compound 18 with progesterone receptor.

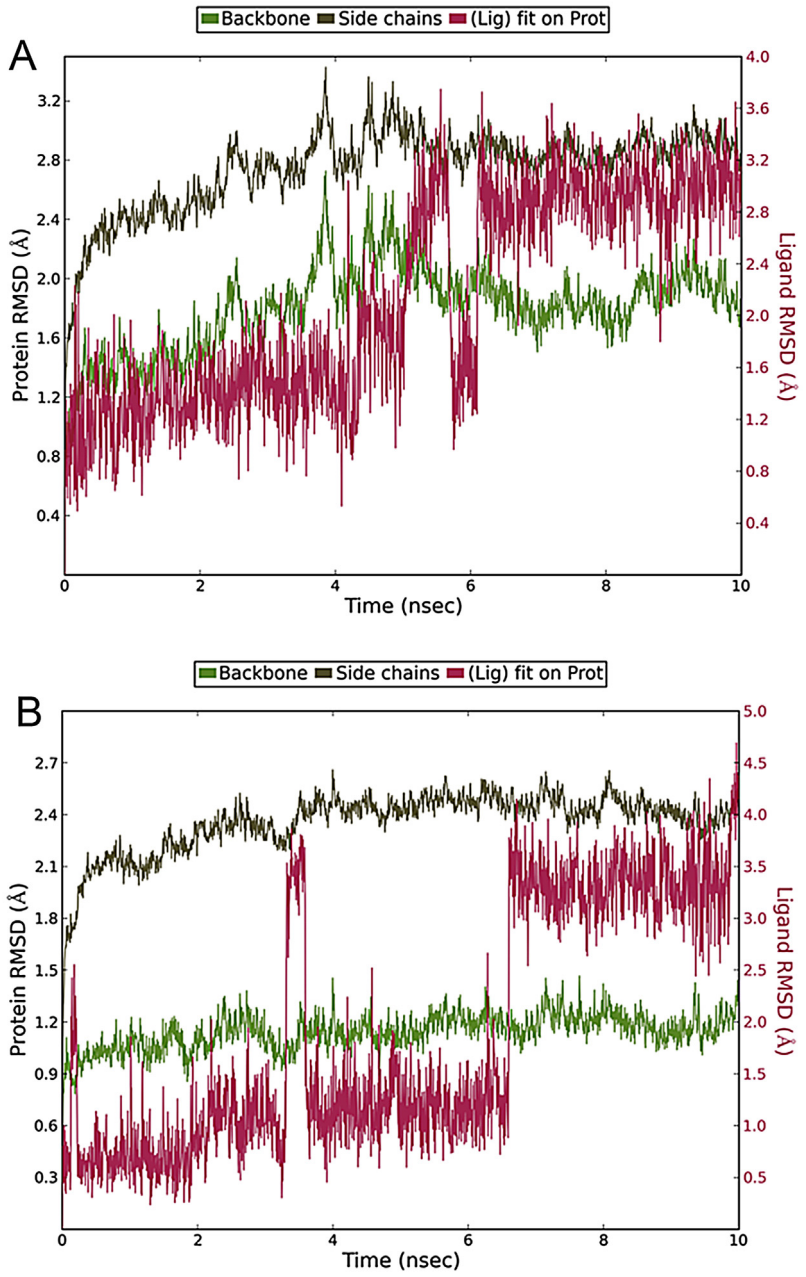

Fig. 5. Event analysis graphs with RMSD. (A) PLA-1ERE docked complex (B) PLA-1E3K docked complex.

backbone of ligand-1ERE complexes are more than the ligand-1E3 K complex. And the histogram bar diagram (Fig. 7) depicts the amino acid residues involved for ligand-protein interactions throughout the MD simulation. Fig. 7A (ligand-estrogen receptor complex) displays many prominent hydrophobic interactions (Ala 350, Leu 384, Leu 391 and Phe 404) which are only maintained their interaction above 10% of the total simulation time. But, ligand-progesterone receptor complex (Fig. 7B) shows that there is a prominent H-bond with Leu 718 along with many hydrophobic interactions (Leu 715, Leu 718, Met 756, Leu 794 and Met 801) which are maintained their interaction above 10% of the total simulation time. Ultimately the MD study shows the reproducible interaction with the docking study where similar interactions were established between the ligand-protein interactions (Fig. 8). Both the docking and dynamics studies also suggest that for the binding with the ligand (Piperolactam A) with both progesterone and estrogen receptor, the hydrophobic interaction is very much effective than other interaction.

3.4. ADMET analysis

The molecular structure of the promising ligands (PLA, Compound 15, Compound 18) were uploaded to Molinspiration, admetSAR and OSIRIS Properties calculator to calculate various properties such as drug likeliness and ADMET properties. All these potential compounds followed Lipinski's rule of five without any violation. The rule states that most "drug likely" molecules will have octanol-water partition coefficient (log $P \leq 5$), molecular weight (nON $\leq$ 500 KDa), number of H-bond donors (nOHNH ≤ 5), number of H-bond acceptors ($\leq$10), molecular refractivity (40–130) as tabulated in Table 3. In ADMET analysis, different pharmacokinetic and pharmacodynamic parameters are evaluated

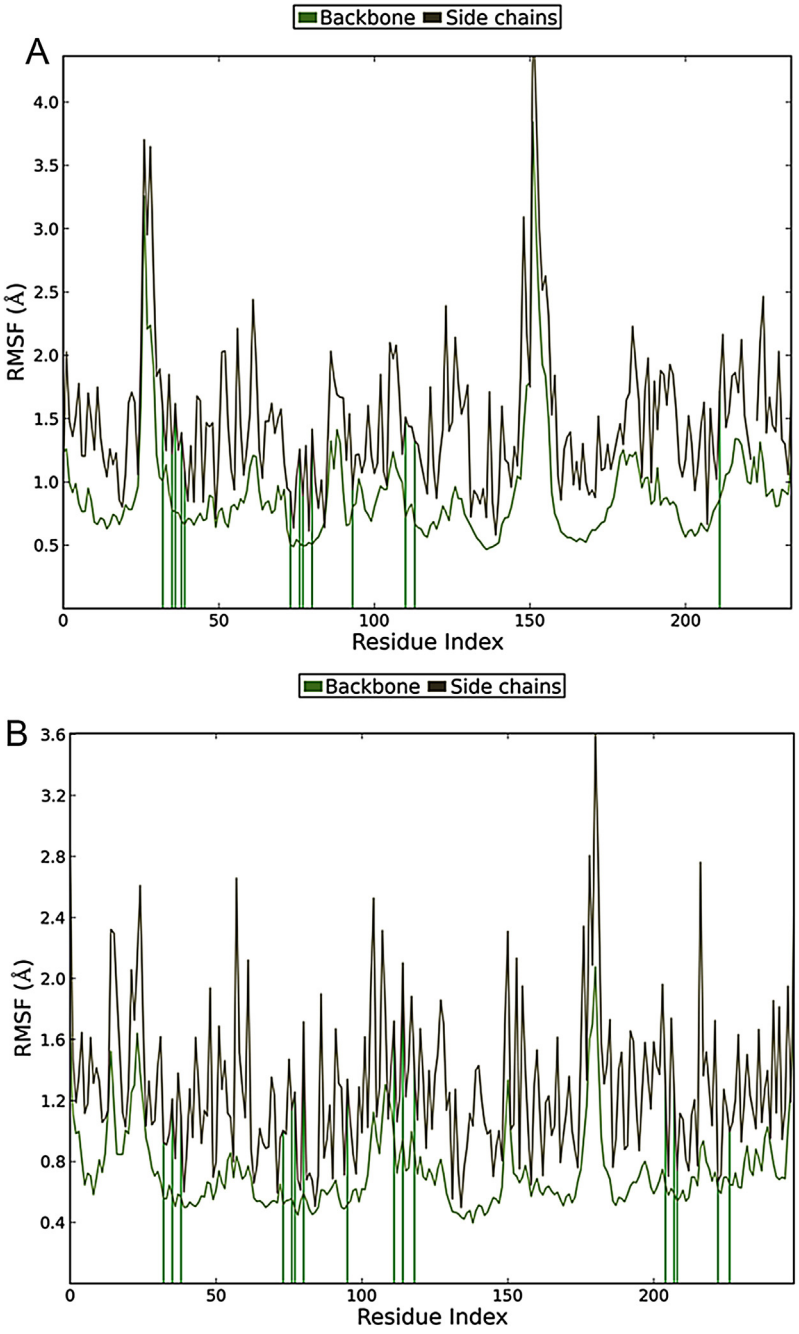

Fig. 6. RMSF calculations with backbone and side chains. (A) PLA-1ERE docked complex (B) PLA-1E3K docked complex.

such as aqueous solubility (Cheng and Merz, 2003), human intestinal absorption (Egan et al., 2000), blood brain barrier penetration, Caco-2 permeability (Hubatsch et al., 2007), cytochrome P450 inhibition (Susnow and Dixon, 2003), cytochrome P (CYP) inhibitory promiscuity (Lynch and Price, 2007), renal organic cation transportation, human ether-a-go-go-related genes inhibition (Du et al., 2011), rat acute toxicity, fish toxicity, *Tetrahymena pyriformis* toxicity (Yoshioka et al., 1985) and AMES toxicity (Jena et al., 2002).

ADMET analyses over three compounds with promising activities have been performed to evaluate if they could cause any adverse effect to human. Interestingly, all the compounds showed some of both toxic and non-toxic effects (Table 2). For example, all the compounds revealed low aqueous solubility suggesting lower bioavailability and tendency to cross blood brain barrier which can induce contraindication on the central nervous system (CNS). In addition, the molecules also displayed AMES toxicity suggesting they might be mutagenic on use. However, on the other hand, all the compounds showed good intestinal absorption and cell permeability (Caco-2 permeability of PLA revealed 1.31). The compounds are also suggested to be non-inhibitor of P-glycoprotein indicating uninterrupted passage of drug through the cell membrane. Most notably, a set of isoforms of CYP450 such as 2C9, 2D6, 2C19, and 3A4 have been found non-inhibited by the toxicological prediction; only 1A2 is inhibited by the prediction. The cytochrome P450 superfamily shows a significant role in metabolizing the drug and its clearance in the liver. Therefore, the inhibition of cytochrome P450 isoforms might affect the drug metabolism and elevate the toxicity level (Susnow and Dixon, 2003; Lynch and Price, 2007). Since CYP450 is mostly

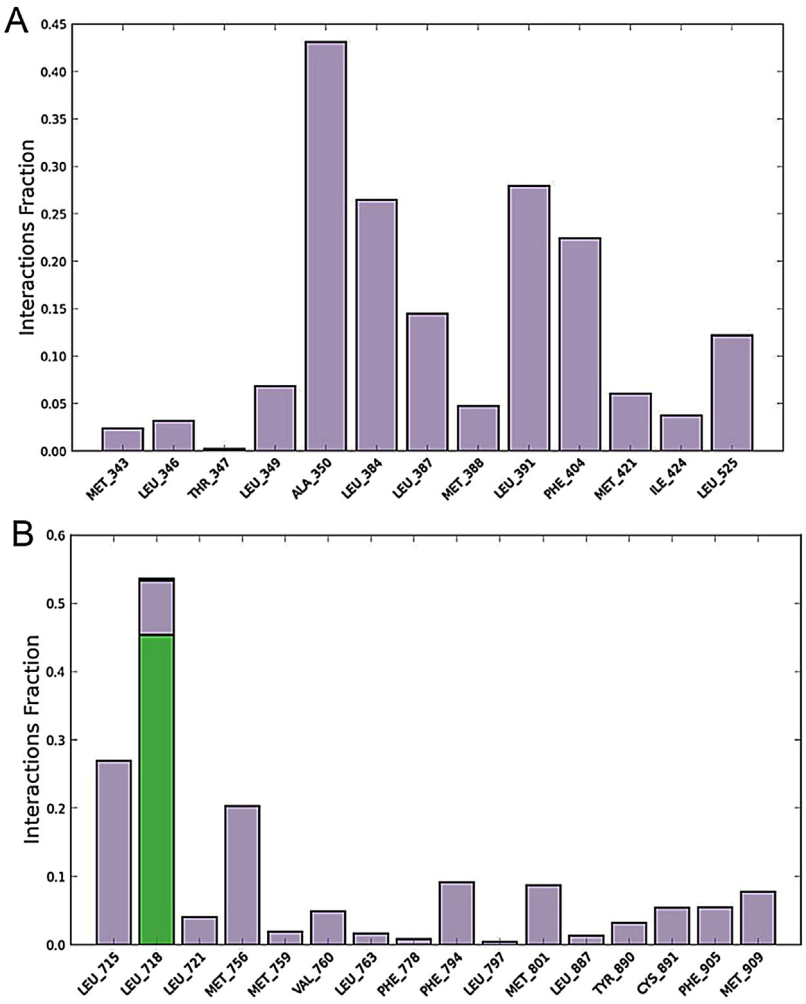

Fig. 7. Major binding residues interacting with PLA revealed as per MD simulation. (A) PLA-1ERE docked complex (B) PLA-1E3K docked complex.

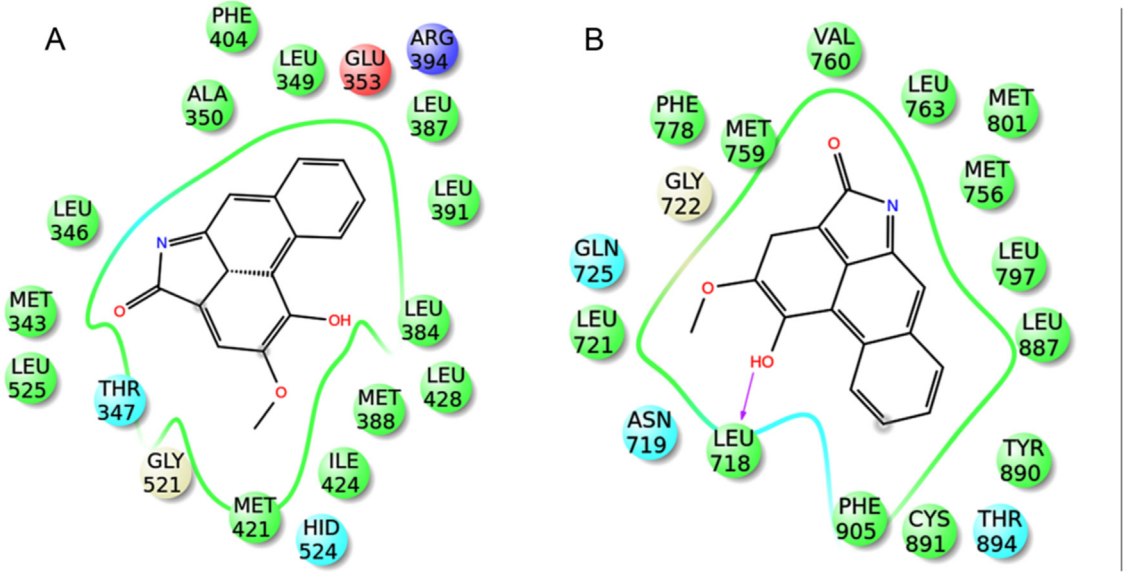

Fig. 8. Binding site mapping *via* MD simulation (A) PLA-1ERE docked complex (B) PLA-1E3K docked complex.

Table 2
The ADMET analysis of Piperolactams obtained from *Piper betle* root by admetSAR.

Compound No	1	2	3	4
Aqueous solubility (LogS)	−3.2807	−3.2807	−3.2123	−3.3027
Blood-Brain Barrier	BBB+	BBB+	BBB+	BBB+
Human Intestinal Absorption	HIA+	HIA+	HIA+	HIA+
Caco-2 Permeability (LogPapp, cm/s)	1.3100	1.3100	1.6716	0.9602
P-glycoprotein Substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
P-glycoprotein Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
Renal Organic Cation Transporter	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2C9 Substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 2D6 Substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 3A4 Substrate	Substrate	Substrate	Substrate	Non-substrate
CYP450 1A2 Inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor
CYP450 2C9 Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2D6 Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2C19 Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 3A4 Inhibitor	Non-inhibitor	Non-inhibitor	Inhibitor	Non-inhibitor
CYP Inhibitory Promiscuity	High CYP Inhibitory Promiscuity	High CYP Inhibitory Promiscuity	High CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity
Human Ether-a-go-go-Related Gene Inhibition	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
AMES Toxicity	AMES toxic	AMES toxic	AMES toxic	AMES toxic
Carcinogens	Non-carcinogens	Non-carcinogens	Non-carcinogens	Non-carcinogens
Honey Bee Toxicity	Low HBT	Low HBT	Low HBT	Low HBT
Biodegradation	Not ready biodegradable	Not ready biodegradable	Not ready biodegradable	Not ready biodegradable
RAT, LD50, mol/kg	2.5785	2.5785	2.4753	2.6154
Fish Toxicity, pLC50 mol/kg	1.2171	1.2171	0.8681	1.1103
Tetrahymena Pyriformis Toxicity pIGC50, ug/L	0.6799	0.6799	0.0959	0.3228

Abbreviations: ADMET, absorption, distribution, metabolism, and excretion-toxicity; BBB, blood–brain barrier penetration; CYP, cytochrome P; RAT, rat acute toxicity; pLC50, lethal concentration, 50%; pIGC50, blood glucose.

Table 3
Drug likeliness prediction of Piperolactam A (compound 1) from OSIRIS Property Explorer and molinspiration softwares.

OSIRIS Property Explorer	molinspiration
Clog P = 2.75	milogP = 3.403
Solubility = − 4.98	TPSA = 62.324
Molecular Weight = 265	Natoms = 20
TPSA = 58.56	MW = 265.268
Drug Likeness = 0.77	nON = 4
Drug Score = 0.13	nONNH = 2
	nviolations = 0
	nrotb = 1
	Volume = 226.135

unaffected by the study molecules, chances of liver toxicity are less likely with these agents. The structures do not also interfere with the human ether-a-go-go-related gene indicating free of cardiac toxicity. The compounds also proved to be eco-friendly with low honey bee toxicity and fish toxicity. Thus it is likely that the compounds will produce low toxicity and more drug likeliness when administered to human being.

Since PLA has been undertaken as the key compound in this study, validity of Lipinski's rule of five was verified by OSIRIS Property Explorer and Molinspiration. Both OSIRIS Property Explorer and Molinspiration revealed that logP of PLA is 2.75–3.40 where molecular weight is 265 (<500). nON and nOHNH of the compound being less than 10 and 5 respectively (Table 3), the compound PLA passes Lipinski's rule of five without any violation. It suggests the drug likeliness of the compound under investigation.

3.5. Nuclear receptor binding affinity

We have checked the probable binding affinity of PLA against other nuclear receptors such as androgen receptor (AR); glucocorticoid receptor (GR); liver X receptors α (LXR α) and β (LXR β); mineralocorticoid receptor (MR); peroxisome proliferator activated receptors α (PPAR α), β/δ (PPAR β), and γ (PPAR γ); retinoid X receptor α (RXR α); and thyroid receptors α (TR α) and β (TR β). The results showed that piperolactam A has low probability of binding affinity for glucocorticoid receptor (GR); liver X receptors α (LXR α) and β (LXR β); mineralocorticoid receptor (MR); peroxisome proliferator activated receptors α β/δ and γ; retinoid X receptor α (RXR α); and thyroid receptors β (TR β) while medium probability of binding affinity for thyroid receptors α (TR α) (Table 4).

For further confirmation of our results, we have undertaken two model nuclear receptors hERG and CYP450 and analyzed the PLA binding site together with binding affinity through our docking. We have taken the homology modelled structure of hERG based on MthK channel (PDB id: 1LNQ) as described earlier (Imai et al., 2009). Our docking analysis showed nine poses of piperolactam may bind to hERG channel (Table S1) where the binding domain within the large tetrameric hERG transmembrane protein has been shown in supplementary Fig. S7. The detailed introspection to binding (supplementary Fig. S8) revealed that first seven poses are not close to the residue Tyr 652 and Phe 656 (shown in green color). It is said that Tyr 652 and Phe 656 are the key aromatic residues for the π-related or hydrophobic interactions important in hERG binding. Pose 8 (pink color) and 9 (navy blue color) are more than 4.2 Å and 5.1 Å away from Tyr 652 and Phe 656 and have weak binding affinity of 6.24 μm from our docking analysis (supplementary Fig. S8). In order to confirm further that our lead molecule is not binding to hERG channel we have analysed piperolactam by pred hERG tool (http://labmol.farmacia.ufg.br/predherg). The prediction is based on a large database of hERG binding compounds and also validated according to the OECD (the organization for economic and co-operation and development) principles. The analysis clearly said that piperolactam have more than 80% probability to be a non-binder to HERG channel (supplementary Fig. S9)

Table 4
Binding affinity revealed with other nuclear receptor as performed in Endocrine Disruptome (http://endocrinedisruptome.ki.si/).

Receptor	Binding affinity (Kcal/ mol)
Androgen receptor (anabolic)	−8.7[a]
Estrogen receptor β (anabolic)	−9.2[a]
Androgen receptor (AR)	−8.2[b]
Estrogen receptor α (Anabolic)	−7.9[b]
Estrogen receptor (β)	−8.9[b]
Glucocorticoid receptor (GR)	−8.9[d]
GR (Anabolic)	−8.4[c]
Liver X receptors α (LXR α)	−9.2[d]
Liver X receptors β (LXR β)	−10.3
Glucocorticoid receptor (GR, anabolic)	−8.4[d]
Peroxisome proliferator activated receptors α (PPAR α)	−8.5[d]
(PPAR β)	−8.5[d]
(PPAR γ)	−8.7[d]
Retinoid X receptor α (RXR α)	−8.5[d]
Thyroid receptors α (TR α)	−7.5[c]
Thyroid receptors β (TR β)	−7.2[d]

[a] High probability of binding.
[b] Moderately high probability of binding.
[c] Moderately low probability of binding.
[d] Low probability of binding.

For docking against human CYP450, six variants of CYP450 have been considered and subjected to docking interactions namely CYP3A4, CYP2D6, CYP2C19, CYP2C9, CYP1A2 (supplementary Figs. S10–S14 respectively) and CYP2A6. The docking analyses exhibited that PLA has lower binding affinity towards any CYP450 variant compared to that of standard inbound ligand (Table S2). Even, PLA revealed no binding with CYP2A6 by docking analysis. Altogether, CYP450 has been found to bear weaker interactions with PLA thus having lesser chance to be inhibited by the latter.

However, the result is encouraging in general at the lead finding stage and further lead optimization may be needed in order to avoid the thyroid related adverse effects.

Since this work is the study of PLA for investigating its potential as contraceptive agent; with a view to design several leads from the same, *de novo* ligand design has been undertaken. The three compounds obtained have been demonstrated in Fig. 9. The structure A and B are developed from the docked structure of ligand with PDB: 1E3K, and also the structure C is developed from PDB: 1ERE.

4. Conclusion

This study has been an *in-silico* approach to investigate the potential of PLA as a contraceptive agent. The docking scores of PLA revealed more binding affinity towards relevant receptors than standard antagonists such as Rohitukine and OrgC. The molecular dynamics simulation of the PLA-receptor docked complex reconfirmed the binding site of the ligand while *de novo* ligand design suggested potential derivatives of the ligand exerting anti-fertility potential. The compound showed more hydrogen bonding towards estrogen and progesterone receptor than the standards. Moreover, the ADMET analysis revealed that PLA successfully passed Lipinsky's Rule of Five and showed negligible toxicity such as cardiac toxicity, nuclear receptor toxicity, liver toxicity and

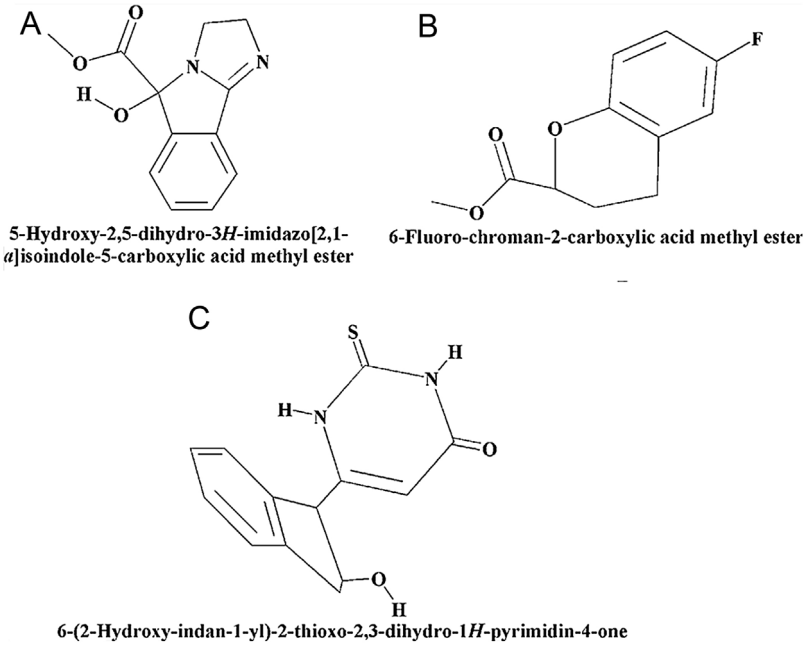

Fig. 9. *De novo* ligand design to elucidate other potential leads for estrogen, progesterone binding. The structure **A** and **B** are developed from the docked structure of ligand with PDB: 1E3K, and also the structure **C** is developed from PDB: 1ERE.

environmental toxicity. Thus this study suggests that PLA encompasses enough potential to be revealed as a non-steroidal contraceptive agent and be successfully employed alone or in combination with other therapeutic agents.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.compbiolchem.2017.01.004.

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