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Original Article

Molecular docking on the phytoconstituents of *Cordia Retusa* (Vahl) masam for its anti-infertility activity

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ABSTRACT

To find out the efficacy of phytoconstituents in *Cordia retusa* (Vahl.) Masam for its inhibition action against CYP17 using computational molecular docking studies. Clinically, Poly Cystic Ovarian Syndrome (PCOS) is characterized by menstural irregularities, hyperinsulinaemia, hyperandrogenism and long term metabolic disturbances. CYP 17 (P450c 17 α) is a key enzyme for adrenal and ovarian androgen synthesis. Hyperandrogenism in PCOS is due to increased activity of P450c 17 α. The inhibition of this enzyme can help to prevent excess androgen synthesis in theca cells. Previous reports revealed that the presence of fourteen compounds in ethanolic extract of aerial parts of *C. retusa* by using GC-MS analysis. Here molecular docking studies were performed for all fourteen compounds along with commercially known fertility drug Clomifene citrate against P450c 17 α using Schrodinger Glide software. All the compounds showed moderate to potent inhibition at a range of -3.4 to -7.8. Especially, compounds such as 1-Iodo-2-methylundecane and 2(1H)Naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl) were found to be potent with a docking score of -7.8 and -6.6 respectively. The results revealed that the ability of compounds present in *C. retusa* can diminish the action of P450c 17 α, which effected in reducing the hypersecretion of androgen. This study will help to design and develop more potent natural compounds for PCOS.

Keywords: G- score, In silico, P450c 17 α, PCOS.

INTRODUCTION

It is appraised that, between 70 and 80 million couples suffer from infertility worldwide and most of those are residents of developing countries [1]. Failure to ovulate and obstruction of fallopian tube are the two major causes of infertility in women [2]. Poly Cystic Ovarian Syndrome (PCOS) is the most common endocrine disorder in women which is characterized by hyperandrogenism, menstrual irregularities and hyperinsulinaemia and long term metabolic disturbances [3]. PCOS affects 5% to 10% of women of reproductive age. PCOS is now well accepted to be a condition with a genetic basis. Hypersecretion of androgens is now known to be the most common feature of PCOS. High prevalence of elevated androgens has been found among women with PCO. The candidate gene and loci screened for PCOS were [4],

- ✓ Coding and promoter regions of CYP 11A1 which encodes P450 SCC
- ✓ Promoter region of CYP 17, the gene encoding P45017α, a specific androgen regulating gene
- ✓ Coding region of leptin which is known to have a significant role in obesity and reproductive function

CYP17 is a steroidogenic enzyme located in the zona fasciculata and zona reticularis of the adrenal cortex and gonad tissues and which has dual functions – hydroxylation and as a lyase. The first enzymic activity offers hydroxylation of pregnenolone and progesterone at the C17 position to generate 17-hydroxypregnenolone and 17-hydroxyprogesterone, while the second enzymic activity cleaves the C17-C20 bond of 17-hydroxypregnenolone and 17-hydroxyprogesterone to form dehydroepiandrosterone and androstenedione respectively [5]. It was reported that treatment of infertile women with Clomiphene citrate leads to congenital malformation; congenital heart disease, which might creates a big problem in treatment of infertility [6]. Now a day's, modern people more and more prefer drugs of natural origin mostly from plant origin due to abundant accessibility and fewer side effects.

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The plant *Cordia retusa* (Vahl) Masam belongs to Boraginaceae occurs widely in eastern and south-eastern Asia from Japan, Taiwan, China, Indochina and India. Traditionally paliyar tribes in Madurai district of Tamil Nadu (India) used the sap of leaves internally for three days prior to and later than the menstrual period for three to four months to intensify fertility [7]. Previous reports revealed that the presence of fourteen compounds in ethanolic extract of aerial parts of *C. retusa* by using GC-MS analysis [8]. In investigation of novel active compounds from plant basis and to access the potent therapeutic properties with least side effects, applications of advanced systems like in silico techniques are generally used. It plays a vital role in designing and development of drug of interest. Molecular docking studies are generally applied in modern drug design process to recognize the protein ligand interactions.

Hence our work is framed to find out the efficacy of phytoconstituents present in ethanolic extract of aerial parts of *C. retusa* to diminish the action of P45017α. For that, molecular docking studies were performed for all fourteen compounds along with commercially known fertility inducing drug Clomiphene citrate.

MATERIAL AND METHODS

Molecular Modeling Studies: Molecular modeling studies have been carried out using GLIDE software v5.5 developed by Schrödinger running on Red Hat Enterprise Linux 5 workstation. Maestro v9.5 Graphical User Interface (GUI) workspace was used for all the steps involved in ligand preparation, protein preparation and HTVS (High Throughput Virtual Screening).

Ligand Preparation: The ligands used in this study were prepared using LigPrep module of v2.3 of Schrödinger Suite 2013. LigPrep follows OPLS-AA (Optimized Potential Liquid Simulations for All Atoms) force fields for energy minimization [9].

Protein Preparation: The X-ray crystal structure of CYP 17 (P450c 17 α) (PDB: 3RUK) was retrieved from PDB database as raw could not be suitable for molecular docking studies. A typical PDB structure consists only of heavy atoms, cofactors, waters, metal ions and can be of multimeric. These structures do not have the information about bond orders, topologies or formal atomic charges. So, the raw PDB structure should be prepared in a suitable manner for docking. Then Protein Preparation Wizard of GLIDE software was used to process and prepare the protein. This also follows the Optimized Potential for Liquid Simulations-All Atoms (OPLS-AA) force fields for energy minimization.

Docking Protocol: All docking calculations were performed using the ‘‘Extra Precision’’ (XP) mode of GLIDE program. The binding site, for which the various energy grids were calculated and stored, is defined in terms of two concentric cubes: the bounding box, which must include the center of any acceptable ligand pose, and the enclosing box, which must enclose all ligand atoms of an acceptable pose, with a Root Mean Square Deviation (RMSD) of less than 0.5 Å and a maximum atomic displacement of less than 1.3 Å were eradicated as redundant in order to increase diversity in the retained ligand poses. The scale factor for van der Waals radii was applied to those atoms with absolute partial charges less than or equal to 0.15 (scale factor of 0.8) and 0.25 (scale factor of 1.0) electrons for protein and ligand, respectively. The max keep variable which sets the maximum number of poses generated during the initial phase of the docking calculation were set to 5000 and keep the best variable which sets the number of poses per ligand that enters the energy minimization was set to 1000. Energy minimization protocol comprises dielectric constant of 4.0 and 1000 steps of conjugate gradient. Upon completion of each docking calculation, at most 100 poses per ligand were invoked. The best docked structure was selected using a GLIDE score (G-score) function. Another scoring function used by GLIDE is E-model, which itself deduced from a combination of the G-score, Coulombic, Vander Waals and the strain energy of the ligand [10].

RESULTS AND DISCUSSION

GLIDE receptor grid was generated to determine the size of the active site. The most probable orientation of the ligands in the binding pocket is identified and a scoring function is used to quantify the strength of the interaction a molecule can make in a particular orientation. In order to afford better correlation between good poses and good scores, the GLIDE XP precision was favored over the standard mode.

The docking analysis was done for the ligands such with the target protein CYP 17 (P450c 17 α) using the docking software GLIDE and the docked images are shown (Fig 1a-3c). The structures docked by GLIDE are generally ranked according to the GLIDE Scoring Function (more negative). Here the scoring function of GLIDE docking program is presented in the G-score form. The most clear-cut method of evaluating the accuracy of a docking procedure is to determine how closely the lowest energy pose (binding conformation)

predicted by the object scoring function. In the current study, Extra Precision GLIDE docking procedure was validated by removing the inhibitor compound with CYP 17 (P450c 17 α) protein which has been analyzed from the GLIDE energy, H-bonds and G-score. Totally fourteen compounds were detected from ethanolic extract of *C. retusa* by using GC-MS analysis. To learn the molecular basis of interaction and affinity of binding of ligand analogues to CYP 17 (P450c 17 α) protein, all the ligands were docked into the active site of CYP 17 (P450c 17 α). Clomiphene citrate, a synthetic fertility drug is considered as reference ligand for this docking study. The docking result of these ligands is given in Table 1.The interaction energy includes van der Waals energy, intermolecular hydrogen bonding as well as electrostatic energy was determined for each minimized complex. The docking score using GLIDE varied from -3.4 to -7.8 against CYP 17 (P450c 17 α). The GLIDE Score for a standard Clomiphene citrate docked with CYP 17 (P450c 17 α) was found to be -6.3. This proves that chemical constituent present in the plant could be potential drug of choice for anti-infertility activity and new drug development. The GLIDE score can be used as a semi-quantitative descriptor for the ability of ligands to bind to a specific conformation of the protein receptor. Generally speaking, for low GLIDE score, better ligand affinity to the receptor may be expected. Especially, compounds 1-Iodo-2-methyl undecane and 2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl) were found to be potent with a docking score of -7.8 and -6.6 respectively. It was reviewed that the compound 1-Iodo-2-methylundecane is recommended to be an iodo compound and it might act as an antimicrobial and it improves sexual activities and 2(1H)Naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)- is recommended to be a ketone and act as an anti inflammatory. (8). Once the docking studies were completed for all the 15 molecules, the docking simulations resulted in a very closely related crystallographic structure which supports this study. We found a very good agreement between the localization of the inhibitor upon docking and from the crystal structure of the protein. Conformational analysis of different docked complexes also shows that residues ASP 487, SER 488, LEU 473 and LYS 490 for CYP 17 (P450c 17 α) protein plays important role in this receptor's activity. Docking studies completed by GLIDE has accorded that above inhibitors fit into the binding pocket of the CYP 17 (P450c 17 α) protein. From the results, we may observe that the intermolecular hydrogen bonding and lipophilic interactions between the ligand and the receptor are very important for successful docking.

CONCLUSION

Virtual screening methods are extensively used in drug discovery process to reduce the time spent on their search as well as expenditure. The approach utilized in this study resulted in identifying compounds 1-Iodo-2-methyl undecane and 2(1H) Naphthalenone, 3,5,6,7,8,8a- hexahydro-4,8a-dimethyl-6-(1-methylethenyl) with high binding affinity towards CYP 17 (P450c 17 α). The docked pose of compound 1-Iodo-2-methyl undecane and 2(1H) Naphthalenone, 3,5,6,7,8,8a- hexahydro-4,8a-dimethyl-6-(1-methylethenyl) revealed more number of H-bond interactions than the co crystallized ligand. Hence, this work states the importance of molecules from various plant sources as docking agents. Further, work can be extended to study the receptor-ligand interactions experimentally and evaluation of their biological activity would help in specific isolation and effective treatment of PCOS which is associated with female infertility disorders.

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Table 1: GLIDE SCORE FOR THE PHYTOCONSTITUENTS OF AERIAL PARTS OF ETHANOLIC EXTRACT OF *CORDIA RETUSA.*

S. No	Name of the compound	G- Score
1	1,6-Octadiene, 3,7-dimethyl-, (S)-	-3.5
2	trans-2-Undecen-1-ol	-5.1
3	1,14-Tetradecanediol	-4.6
4	Phytol	-3.8
5	1-Iodo-2-methylundecane	-7.8
6	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	-3.7
7	Octadecane, 1-(ethenyloxy)-	-4.1
8	2-[(2-methoxyphenyl)-(5-methyl-2-furyl)-methyl]-5-methyl-furan	-3.9

9	Squalene	-3.8
10	Vitamin E	-4.2
11	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methyl ethenyl)-	-6.6
12	α-Amyrin	-3.4
13	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3á,4à,5à)-	-4.5
14	d-Norandrostane (5à,14à)	-6.1
15	Standard (Clomiphene citrate)	-6.3

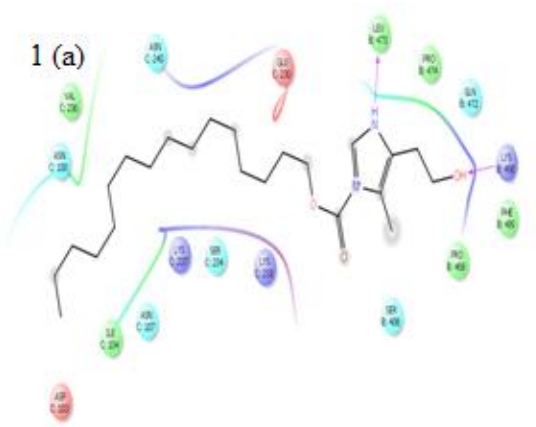

Fig 1 (a): Ligand interaction of 1-Iodo-2-methylundecane with CYP 17

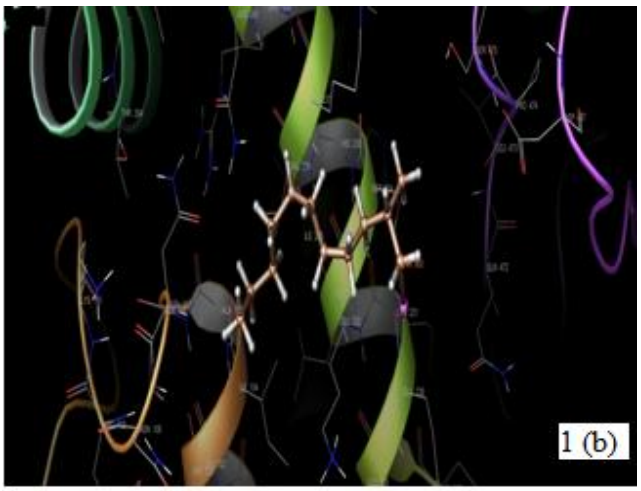

Fig 1 (b): Glide docking image of 1-Iodo-2-methylundecane with CYP17

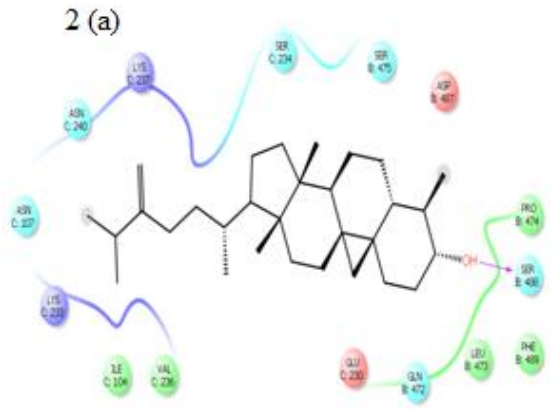

Fig 2 (a): Ligand interaction of 2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6- (1-methylethenyl)with CYP 17

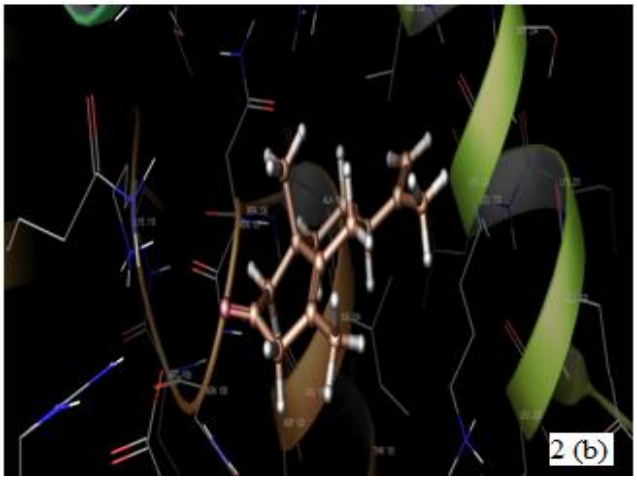

Fig 2 (b): Glide docking image of 2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl- 6-(1-methylethenyl)with CYP 17

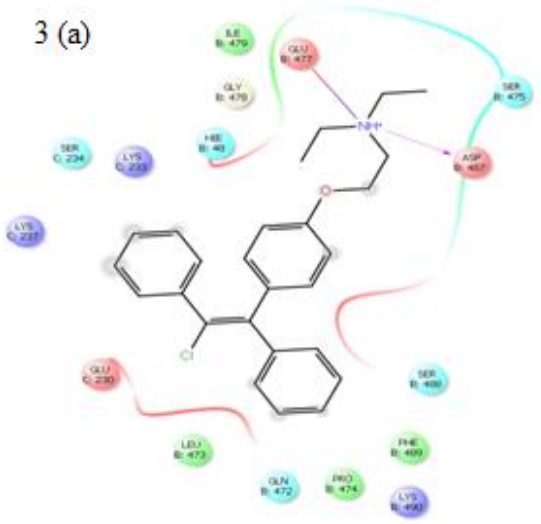

Fig 3 (a): Ligand interaction of clomiphene with CYP 17

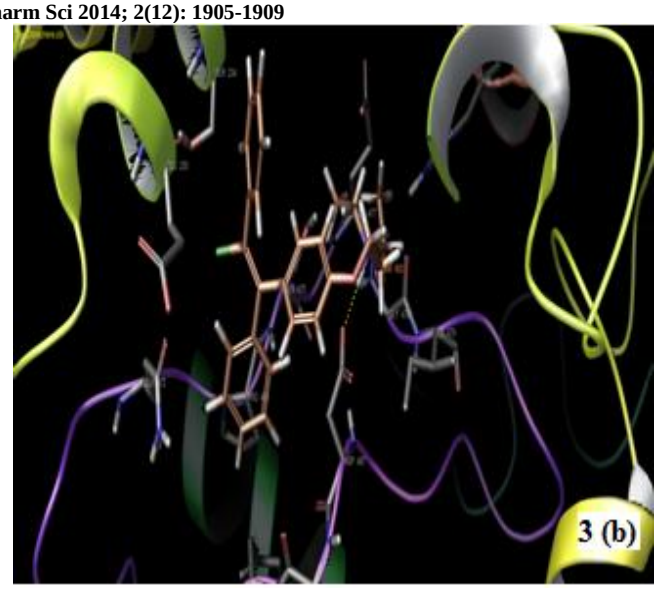

Fig 3 (b): Glide docking image of clomiphene with CYP 17

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