ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Issue 2, 2016

Online - 2455-3891
Print - 0974-2441

Research Article

IN SILICO MOLECULAR DOCKING STUDIES ON THE PHYTOCONSTITUENTS OF *CADABA FRUTICOSA* (L.) DRUCE FOR ITS FERTILITY ACTIVITY

MURUGESAN AMUDHA*, SHANMUGAM RANI

Department of Pharmacy, Annamalai University, Annamalai Nagar, Cuddalore - 608 002, Tamil Nadu, India.
Email: amudhapharma3@gmail.com

Received: 29 January 2015, Revised and Accepted: 05 May 2015

ABSTRACT

Objective: To study the effectiveness of phytocomponents from *Cadaba fruticosa* (L.) Druce against CYP 17 using computational molecular docking studies. The characterization of polycystic ovarian syndrome is hyperinsulinemia, menstural irregularities, long-term metabolic disturbances, and hyperandrogenism. Adrenal and ovarian androgen synthesis are induced by an enzyme, CYP 17 (P450c 17α). Due to increase in the activity of this enzyme, hyperandrogenism mainly occurs. By inhibiting the enzymatic activity, excess androgen synthesis can be prevented in ovarian theca cells. Literature study has reported that the gas chromatography mass spectrometry analysis on the ethanolic extract of the aerial parts of *C. fruticosa* possesses 20 compounds.

Methods: Molecular docking analysis was performed for the reported 20 compounds against CYP 17 using Schrodinger Glide software. These results were compared with the docked score of fertility inducing drug clomiphene citrate.

Results: All 20 compounds have exhibited moderate to potent inhibition with a range of −3.3 to −7.9. Androstan-3-one, 17-hydroxy-2, 4-dimethyl and 3-Trifluoroacetoxypentadecane have showed potent inhibition with the docking score of −7.9 and −6.8, respectively.

Conclusion: The results revealed out that the compounds present in *C. fruticosa* can reduce the activity of CYP 17. This study throws a light on establishing the novel drug for infertility through experimental procedures.

Keywords: *Cadaba fruticosa*, Glide-score, Infertility, *In silico*, CYP 17, Polycystic ovarian syndrome.

INTRODUCTION

In worldwide, up to 15% of reproductive-aged couples were affected by infertility [1]. Anovulation and fallopian tube obstruction are the two major factors of female infertility [2]. The most common endocrine disorder is polycystic ovarian syndrome (PCOS) which is characterized by hyperinsulinemia, hyperandrogenism, menstrual irregularities, and long-term metabolic disturbances in female [3]. Women with PCOS are frequently affected by diabetes, metabolic syndrome and obesity which further subjected to increased risk of infertility due to the ovulatory dysfunction that is associated with adverse reproductive outcomes [4]. Mainly 5-10% of women in the reproductive age are affected by PCOS. PCOS is well acknowledged to be a state of genetic disorder. The most common symptom of PCOS is hypersecretion of androgen. Most of the women with PCO have an elevated level of androgen. Specific gene and its loci for PCOS as follows:

- Gene CYP 11A1 contains coding and promoter regions for the translation of protein, P450 SCC
- Promoter region of CYP 17 encodes a specific androgen regulating protein, P450 17α
- Gene which encoding the enzyme, leptin plays a vital role in reproductive function and obesity.

The steroidogenic enzyme, CYP 17 functions as hydroxylation and lyase. It is present in the zona reticularis and zona fasciculata of the respective gonad tissues and adrenal cortex. In the first step of enzymatic activity, hydroxylation of pregnenolone and progesterone at the C17 position occurs resulting in the formation of 17-hydroxypregnenolone and 17-hydroxyprogesterone. During the second step of enzymatic action, C17-C20 bond of 17-hydroxypregnenolone and 17-hydroxyprogesterone are cleaved to generate dehydroepiandrosterone and androstenedione respectively [5]. Increase in CYP 17 activity in adrenal and ovarian sites induces hyperandrogenism in PCOS. In PCOS patients, gene that encodes CYP 17 has been overexpressed and androgen has been converted more efficiently to testosterone than normal theca cells [6]. The main side effects of the commercial synthetic drug, clomiphene citrate used for infertility are congenital heart disease and congenital malformation [7]. Thus, naturally extracted compounds from the medicinal plants have been preferred and developed as a potent therapeutic agent to treat infertility disorders. Easily accessible, scanty availability and lesser side effects must be the targeted role in identifying medicines from plants for all disease related problems.

The plant *Cadaba fruticosa* (L) Druce belongs to Capparidaceae occurs widely in Angola, Cameroon, Congo, Egypt, Ethiopia, India, Kenya, Niger, Saudi Arabia, Senegal, and Somalia. It also presents in tropical and sub-tropical regions of Asia, Australia, and Africa. In India, the plant is widely distributed in Punjab, central and western India, Gujarat, Tamil Nadu, Visakhapatnam and Karnataka [8]. Root and leaves are used as deobstruent, emmenagogue and uterine obstructions [9]. Literature studies have reported that the gas chromatography mass spectrometry (GC-MS) analysis of ethanolic extract from the aerial parts of *C. fruticosa* showed the presence of 20 compounds [10]. In modern drug design process, molecular docking studies are generally applied to identify the protein ligand interactions. Exploitation of natural active compounds which has potent therapeutic properties with least side effects using *in silico* docking analysis is necessary to manage female reproductive disorders. The framework of this study is mainly on finding out the binding effectiveness of phytoconstituents present in the ethanolic extract of aerial parts of *C. fruticosa* against CYP 17 using molecular docking studies. Furthermore, the results obtained were compared with the docking score of known fertility inducing drug clomiphene citrate against CYP 17. This study provides a suitable platform for novel drug development for PCOS.

METHODS

Molecular modeling studies

Glide software v5.5 exploited by Schrödinger running on Red Hat Enterprise Linux 5 workstation was used for molecular docking studies. Maestro v9.5 graphical user interface workspace was employed during protein preparation, ligand preparation and high throughput virtual screening.

Ligand preparation

LigPrep module of v2.3 from Schrödinger Suite 2013 was applied to draw the structure of ligands. LigPrep follows optimized potential liquid simulations for All Atoms force fields for energy minimization (OPLS-AA) [11].

Protein preparation

PDB database was used to retrieve the raw X-ray crystal structure of CYP 17 (PDB: 3RUK), but the raw structure could not be applied directly for molecular docking studies. Since the raw structure describes only heavy atoms, cofactors, waters, metal ions and multimeric and it fails to provide information on topologies, bond orders, or formal atomic charges. Hence, raw PDB structure has been altered using protein preparation wizard present in Glide software for the convenience of docking process. Protein preparation method also follows OPLS-AA force fields for energy minimization.

Docking protocol

Extra precision (XP) mode of Glide program was used for all docking calculations. The binding site is defined in terms of two concentric cubes such as bounding box and enclosing box. The former box includes the center of any acceptable ligand pose, and the latter one encloses all ligand atoms of an acceptable pose. Various energy grids for binding site were calculated and stored. Acceptable pose for enclosing box was <0.5 Å with a root mean square deviation and <1.3 Å with maximum atomic displacement, which were eliminated as redundant to intensify the diversity in the retained ligand poses. Atoms in proteins and ligands with absolute partial charges ≤0.15 (scale factor of 0.8) and 0.25 (scale factor of 1.0) electrons, respectively, has been applied as a scale factor by the concept of van der Waals radii. During the initial phase of docking calculation, the maximum poses generated from the variables were fixed to 5000 and the best variable which set the number of poses per ligand that enters the energy minimization was set to 1000. The dielectric constant of 4.0 and 1000 steps of conjugate gradient was applied in the energy minimization protocol. At the ending of each docking calculation, utmost 100 poses per ligand were generated. Using Glide scores (G-score) function, the best docked structure was chosen. E-model is also one of the scoring functions used by Glide, which is inferred from the combination of the G-score, van der Waals and Coulombic interactions, and the strain energy of the ligand [12].

RESULTS AND DISCUSSION

The size of the active site was determined by generating the Glide receptor grid. The most feasible orientation of the ligands in the binding pocket is predicted, and the strength of the interaction in the particular orientation was quantified from a scoring function. Glide XP precision was preferred over standard mode mainly to determine the better correlation between good poses and good scores. Docking analysis was performed between ligands and the target protein CYP 17 using Glide software and the docked images were illustrated in Fig. 1-3. The more negative value on the scoring function indicates better docking, and the docking of ligand and protein were ranked according to the corresponding Glide scoring function. The scoring function of Glide docking program was exhibited in the form of G-score. G-score indicates the binding ability of a ligand to the specific conformation of the protein receptor. The accuracy of a docking procedure is determined from the object scoring function which was predicted even in lowest energy pose (binding conformation). In this study, the validation of Glide XP docking procedure was done by eliminating the inhibitor compound with the protein, CYP 17 that was evaluated from the Glide energy, H-bonds, and G-score. 20 molecules were identified through ethanolic extract from the aerial parts of *C. fruticosa* using GC-MS analysis [9]. Molecular interaction and binding affinity of ligand analogs with protein, CYP 17

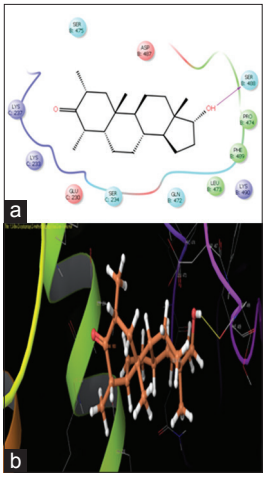

Fig. 1: (a) Ligand interaction of androstan-3-one, 17-hydroxy-2,4-dimethyl with CYP 17, (b) glide docking image of androstan-3-one, 17-hydroxy-2,4-dimethyl with CYP 17

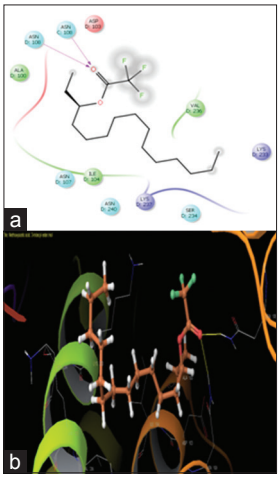

Fig. 2: (a) Ligand interaction of 3-trifluoroacetoxypentadecane with CYP 17, (b) glide docking image of 3-trifluoroacetoxypentadecane with CYP 17

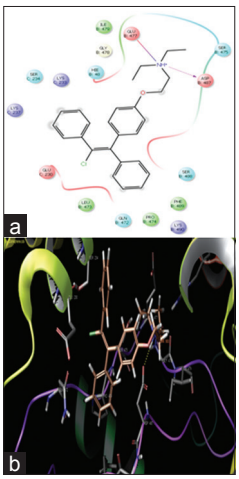

Fig. 3: (a) Ligand interaction of clomiphene citrate with CYP 17, (b) glide docking image of clomiphene citrate with CYP 17

Table 1: Glide scores for the phytoconstituents from ethanolic extract of aerial parts of *C. fruticosa*

S. No.	Name of the compound	G-score
1	2-Tridecen-1-ol	−3.5
2	Pyrrolidine, 1,1'-methylenebis	−5.1
3	1,6-Anhydro-3,4-dideoxy-á-D-manno-hexapyranose	−4.6
4	Phytol	−3.8
5	5,10-Dioxatricyclo[7.1.0.0 (4,6)]decane	−3.7
6	Azonia-5-hexene-1-ol, N, N-dimethyl-, carbamate ester	−4.1
7	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	−3.9
8	Octane, 1,1'-oxybis	−3.8
9	Octadecane, 1-(ethenyloxy)-	−4.2
10	1,2-15,16-Diepoxyhexadecane	−3.4
11	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	−4.5
12	Methoxyacetic acid, 3-tridecyl ester	−6.1
13	3-trifluoroacetoxypentadecane	−6.8
14	Bicyclo[3.3.1]nonan-9-one, 1,2,4-trimethyl-3-nitro-,(2-endo, 3-exo, 4-exo)-	−4.6
15	Heptadecane, 2,6,10,15-tetramethyl-	−3.3
16	(1-Ethyl-3,7-dimethylocta-2,6-dienylthio) benzene	−3.9
17	Z, Z, Z-4,6,9-Nonadecatriene	−4.8
18	1,3-Bis-(2-cyclopropyl, 2-methylcyclopropyl)-but-2-en-1-one	−3.7
19	1-Naphthalenepropanol, à-ethyldecahydro-5-(hydroxymethyl)-à,5,8a-trimethyl-2-methylene-, [1S- [1à(S*),4aá,5à,8aà]]-	−5.1
20	Androstan-3-one, 17-hydroxy-2,4-dimethyl	−7.9
21	Clomiphene citrate	−6.3

C. fruticosa: Cadaba fruticosa

were studied by docking every ligand into the active site of the protein, and the docking results of the ligands were tabulated in Table 1. For each minimized complex, the different interaction energies such as van der Waals energy, intermolecular hydrogen bonding and electrostatic energy were reckoned. G-score for 20 ligands using Glide was in the range of −3.3 to −7.9 against CYP 17. G-score for the known fertility drug, clomiphene citrate docked against CYP 17 was -6.3. Compounds from *C. fruticosa* such as Androstan-3-one, 17-hydroxy-2, 4-dimethyl, and 3-trifluoroacetoxypentadecane have a docking score of −7.9 and −6.8, respectively. This suggested that the both compounds have the highest binding ability upon docking against the crystal structure of the protein. The residues ASN108, SER 488 present in the protein, CYP 17 have a significant role in receptor binding activity with ligands which was revealed from the conformational analysis of different docked complexes. Docking studies using Glide software have proved that the above inhibitors fit into the binding pocket of the protein, CYP 17. Althrough the results, it was observed that successful docking has been correlated with good intermolecular hydrogen bonding and liphophilic interactions between the ligand and the receptor. From this study, it was inferred that the phytoconstituents exist in the *C. fruticosa* could be promising alternatives for the development of fertility drugs.

CONCLUSION

During drug discovery, effective screening procedures could be applied to reduce cost and time. In this study one of the effective methods, molecular docking by Glide software has been used to analyze the binding ability between 20 compounds with CYP 17. Compounds such as Androstan-3-one, 17-hydroxy-2, 4-dimethyl, and 3-Trifluoroacetoxypentadecane have good docked pose with the protein by analyzing its G-score. This showed the importance of molecules from different herbals as docking agents. From this investigation, initial screening on identifying the potential fertility drugs from *C. fruticosa* has been developed for PCOS. This study throws a light on establishing the novel drug for infertility through experimental procedures.

ACKNOWLEDGMENT

The author AM was grateful to the University Grant Commission, New Delhi, India for providing UGC-BSR fellowship.

REFERENCES

1. Bull World Health Organ 2010;88:881-2. Available from: http://www.karger.com/Article/FullText/343058. [Last accessed on 2012 Jun].
2. Amudha M, Rani S, Kannan K, Manavalan R. An updated overview on causes, diagnosis and management of infertility. Int J Pharm Sci Rev Res 2013;18(1):155-64.
3. Kamel HH. Role of phyto-oestrogens in ovulation induction in women with polycystic ovarian syndrome. Eur J Obstet Gynecol Reprod Biol 2013;168:60-3.
4. Apridonidze T, Essah PA, Iuorno MJ, Nestler JE. Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2005;90:1929-35.
5. Akhtar MK, Kelly SL, Kaderbhai MA. Cytochrome b(5) modulation of 17{alpha} hydroxylase and 17-20 lyase (CYP17) activities in steroidgenesis. J Endocrinol 2005;187:267-74.
6. Swetha R. Study of serum lipoprotein and lipid profile in polycystic ovarian syndrome. (Doctoral Dissertation). Rajiv Gandhi University of Health Sciences, Bangalore, Karnataka, India; 2013.
7. Tulandi T, Martin J, Al-Fadhli R, Kabli N, Forman R, Hitkari J, *et al.* Congenital malformations among 911 newborns conceived after infertility treatment with letrozole or clomiphene citrate. Fertil Steril 2006;85:1761-5.
8. Telrandhe UB, Uplanchiwar V. Phyto-pharmacological perspective of *Cadaba farinosa* forsk. Am J Phytomed Clin Ther 2013;1(1):11-22.
9. Khare CP. Indian Medicinal Plants - An Illustrated Dictionary. New York: Springer; 2007. p. 107.
10. Amudha M, Rani S. Assessing the bioactive constituents of *Cadaba fruticosa* (L.) Druce through GC-MS. Int J Pharm Pharm Sci 2014;6(2):383-5.
11. Halgren TA. Identifying and characterizing binding sites and assessing drug gability. J Chem Inf Model 2009;49:377-89.
12. Parasuraman P, Suresh R, Premnath D. Balancing anti-amyloid and anti-cholinesterase capacity in a single chemical entity: *In silico* drug design. Int J Pharm Pharm Sci 2014;6(2):571.